Synthetic optimization of cystobactamid analogs as antibiotics

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Kurzfassung

Trotz einer Vielzahl klinisch verfügbarer Antibiotika nimmt die Verbreitung von multiresistenten Bakterienstämmen gegen ganze Wirkstoffklassen rapide zu[1],[2]. Um diese alarmierende Entwicklung zu verlangsamen, ist es außerordentlich wichtig neue Antibiotikaklassen zu entdecken. Cystobactamide stellen eine solch neuartige Antibiotikaklasse dar. Sie sind Naturstoffe und werden von *Cystobacter sp*. aus der Ordnung der Myxobakterien produziert. Cystobactamide inhibieren Gyrase und Topoisomerase IV als Schlüsselenzyme in der Replikation von bakterieller DNA. Das Wirkspektrum umfasst gramnegative und grampositive Bakterien[3]. Eine Totalsynthese wurde etabliert und führte zur Entwicklung einer ersten Struktur-Wirkungs-Beziehung und der Leitstruktur CN-DM 861[4],[5].

Basierend auf der etablierten Synthese wurden im Rahmen dieser Arbeit neue Analoga hergestellt. Die Verwendung neuer Methoden, z.B. für die Peptidkopplungen oder zum Aufbau eines tetrasubstituierten Ring D Aromaten, führten zu verbesserten Ausbeuten und erlaubten den Zugang zu neuen Strukturelementen. Das Einbringen eines Alkins in die Seitenkette der zentralen Aminosäure führte zu einer Verbindung mit erhöhter Potenz gegen *A. baumannii* und *S. aureus*. Weiterhin zeigte sie Aktivität gegen multiresistente *S. aureus* und *Acinetobacter* Stämme, sowie Vancomycin-resistente *Enterococci*. Der zusätzliche Austausch eines aromatischen Ringsystems durch ein Bicyclo[1.1.1]pentan führte zu erhöhter Löslichkeit und erweitertem Wirkspektrum gegen *K. pneumoniae*, *S. marcescens* und *Proteus* Stämme.

Die Struktur-Wirkungs-Beziehung wurde erweitert. Erste Erkenntnisse über die räumliche Orientierung von aktiven Cystobactamiden zeigten die Notwendigkeit eines linearen und starren AB Systems. An der zentralen Aminosäure konnte die L-Konfiguration als bevorzugte Konfiguration identifiziert werden. Zusätzlich wurden Cystobactamide mit photoreaktiven und bioorthogonalen Gruppen entwickelt. Diese erlaubten Experimente zur Untersuchung der Bindetasche und die Suche nach sekundären Targets.

Insgesamt wurden mehr als 50 neue Cystobactamide in einem Maßstab von bis zu 800 mg synthetisiert. Über die Kombination des Alkins in der Seitenkette mit einem verbrückten Alkansystem als Benzen Bioisoster konnten zwei Analoga mit verbesserter Aktivität, Löslichkeit, erweitertem Wirkspektrum und niedrigeren Resistenzraten entdeckt werden. Die so gesammelten Informationen führten zu neuen, optimierten Strukturmotiven für die weitere Entwicklung von prä-klinischen Kandidaten. Diese Resultate zeigen, dass Cystobactamide vielversprechende Moleküle für die Entwicklung eines Antibiotikums gegen multiresistente Bakterien sind.

Schlagworte: Cystobactamide, medizinische Chemie, Naturprodukt

Abstract

Although there are many different types of antibiotic scaffolds in clinical use, the prevalence of multiresistant strains against whole drug classes increases rapidly[1],[2]. It is of utmost importance to seek for novel chemical scaffolds to overcome this alarming situation. Cystobactamids are a new class of antibiotics, which occur in *Cystobacter sp.* from the order of myxobacteria. Cystobactamids inhibit gyrase and topoisomerase IV as key enzymes in the replication of bacterial DNA. They show broad-spectrum activity against Gram-negative and Gram-positive bacteria[3]. A synthetic route was established that allowed for the synthesis of derivatives and led to a first structure-activity relationship and the lead compound CN-DM 861[4],[5].

Based on the established synthesis, novel analogues were produced. New synthetic methods, including novel peptide couplings and the formation of the tetrasubstituted ring D, were introduced to increase the overall yield and to gain access to new building blocks. A highly active central amino acid analogue with an alkyne group was found and showed enhanced potency against *A. baumannii* and *S. aureus*. Furthermore, resistant breaking properties against previously unsusceptible vancomycin-resistant *Enterococci*, *S. aureus* and *Acinetobacter* strains were discovered. The additional exchange of an aromatic system for a bicyclo[1.1.1]pentane allowed for a coverage of *K. pneumoniae*, *S. marcescens* and *Proteus* strains while increasing the solubility significantly.

The structure-activity relationship was extended and revealed basic topological requirements for antibiotic activity like the necessity of a linear and rigid AB system. A preference for a L-configuration at the central amino acid was found. Additionally, the development of cystobactamids with photoreactive and bioorthogonal groups was carried out. This enabled experiments for the investigation of the binding site and secondary targets.

Overall, more than 50 cystobactamids were synthesized on a scale of up to 800 mg. Through combination of the alkyne side chain and the introduction of a bridged alkane system as benzene bioisostere, optimized analogues with superior activity, spectrum, solubility and low resistance were found. The new-found data helped to identify new optimized motifs for the development of pre-clinical drug candidates. These results show the potential of cystobactamids to tackle the rise of multiresistant bacteria. Nevertheless, subsequent studies are required to obtain a pre-clinical drug candidate.

Keywords: Cystobactamids, medicinal chemistry, natural product

Table of content

Abbreviations

ADC = aminodeoxychorismate

Allyl Br = allyl bromide

ATP = adenosine triphosphate

BEP = 2-bromo-1-ethyl-pyridinium tetrafluoroborate

BSA = bovine serum albumin

Bipy = 2,2'-Bipyridine

CFU = colony forming unit

CHO = Chinese hamster ovary

CIP = Ciprofloxacin

CuAAC = Copper(I)-catalyzed Azide-Alkyne Cycloaddition

cUTI = complicated urinary tract infection

DCM = dichloromethane

DEA = diethylamine

DIPEA = diisopropylethylamine

DMA = dimethylamine

DMAP = 4-dimethylaminopyridine

DMF = N,N-Dimethylformamide

DMP = Dess-Martin periodinane

DMSO = dimethyl sulfoxide

DNA = deoxyribonucleic acid

DPPA = diphenylphosphoryl azide

EDG = electron donating group

EE = ethyl acetate

EEDQ = 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline

ESI = electrospray ionization

Et = ethyl

EWG = electron withdrawing group

FDA = Food and drug administration (USA)

FDAA = 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide

Fmoc = fluorenylmethoxycarbonyl

h = hours

HATU = 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate

HBA = hydrogen bond acceptor

HBD = hydrogen bond donor

HepG2 = Hepatocellular carcinoma cell line

HOAt = 1-hydroxy-7-azabenzotriazole

HRMS = high resolution mass spectrometry

i.v. = intravenous

i-PrMgCl*LiCl = isopropylmagnesium chloride lithium chloride complex

IC_{50} = half maximal inhibitory concentration

IIDQ **=** 1-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline

K.p. = *Klebsiella pneumoniae*

LCMS = liquid chromatography coupled to mass spectrometry

$M+H^+$ = protonated molecular ion

$M-H^+$ = deprotonated molecular ion

MIC = minimal inhibitory concentration

MOM = methoxymethyl

MRGN = multidrug resistant Gram-negative bacteria

MRSA = methicillin resistant *staphylococcus aureus*

MS = mass spectrometry

MSSA = methicillin susceptiple *staphylococcus aureus*

n.d. = not determined

NMR = nuclear magnetic resonance

NOESY = Nuclear Overhauser Enhancement Spectroscopy

P-gp = P-glycoprotein 1

P.a. = *Pseudomonas aeruginosa*

PABA = para-aminobenzoic acid

PE = petroleum ether

PEP = phosphoenolpyruvate

pTSA = *p*-toluenesulfonic acid

Pyr = pyridine

SAR = structure-activity relationship

RP HPLC = reversed phase high-performance liquid chromatography

Rt = room temperature

T3P = propylphosphonic anhydride

t-Bu = *tert*-butyl

TBAF = tetra-*n*-butylammonium fluoride

TBME = *tert*-butyl methyl ether

TBTA = tris((1-benzyl-4-triazolyl)methyl)amine

TEA = triethylamine

Tf_2O = trifluoromethanesulfonic anhydride

TFA = trifluoroacetic acid

TFFH = **t**etramethylfluoroformamidinium hexafluorophosphate

THF = tetrahydrofuran

TI IV = topoisomerase IV

TIPS = triisopropyl silane

TMD = transmembrane domains

TMHI = 1,1,1-trimethylhydrazinium iodide

TMS = trimethylsilyl

UTI = urinary tract infection

VRE = vancomycin resistant *enterococci*

WT = wild type

y = yield

1 Introduction

1.1 History of antibiotics

Although the existence of bacteria was known since their discovery by Antonie van Leeuwenhoek in 1676, their function was mainly unknown[6]. It was not until the first half of the 1800s that Ignaz Semmelweis and Oliver Holmes discovered a correlation between unwashed hands and the transmission of the Puerperal Fever. Semmelweis concluded that physicians that were involved in autopsies of infected patients carried "cadaverous particles" responsible for the infection and death of other patients. By ordering physicians to wash their hands with hypochlorite before going to the patients, the transmission of the Puerperal Fever dropped significantly[7]. The use of disinfectants is still applied today.

The first breakthrough in the development of antibiotics was achieved by Paul Ehrlich in the late 1800s. Based on his research with bacteria, he proposed his so-called side-chain theory that suggests that antigens of bacteria bind to pre-existing side chains on human cells. This interaction triggers the secretion of the human side chains ultimately leading to the neutralization of the antigen[8]. With Ehrlichs interest in dyes and his theory in hand, he tried to find chemical molecules that specifically bind to microbes without binding to human cells. By this interaction with the microbe, he proposed an antimicrobial effect. His research ultimately led to the dye derived molecules Salvarsan and Neosalvarsan (Figure 1) that were used against syphilis and the sleeping sickness[9]. The first antibiotics were born.

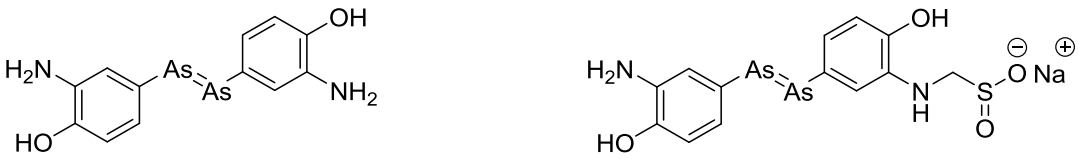


Figure 1: Proposed structures of Salvarsan and Neosalvarsan by Ehrlich. More recent studies suggest a pentameric structure. Formula adapted from[10].

Inspired by the work of Ehrlich on dyes, Gerhard Domagk found Prontosil in the early 1930s (Figure 2) – an antibiotic sulfonamide dye and precursor of the sulfonamide drugs we still use today. For his development of Prontosil he received the Nobel prize in Physiology or Medicine 1939[11]. Although Alexander Fleming already discovered the antibiotic properties of *Penicillium* cultures in 1929[12], it took more than ten years until the potential of the active ingredient penicillin was unlocked (Figure 2). In collaboration with Norman

Heatley and Ernst Chain, Howard Florey was able to purify enough penicillin to treat pre-infected mice successfully in 1939. Clinical tests started soon after[13]. The class of β-lactam antibiotics was born.

Figure 2: Structure of penicillin G (benzylpenicillin) and prontosil. Formula adapted from[14].

The development of new antibiotics grew of interest and a variety of different antibiotics was found. In the so-called "golden era of antibiotics" that lasted until the 1960s, most of the antibiotic classes we still use today were found (Figure 3). Amongst them are the classes of the β-lactams, quinolones, diaminopyrimidines, macrolides, tetracyclines, aminoglycosides and glycopeptides to only name a few[15].

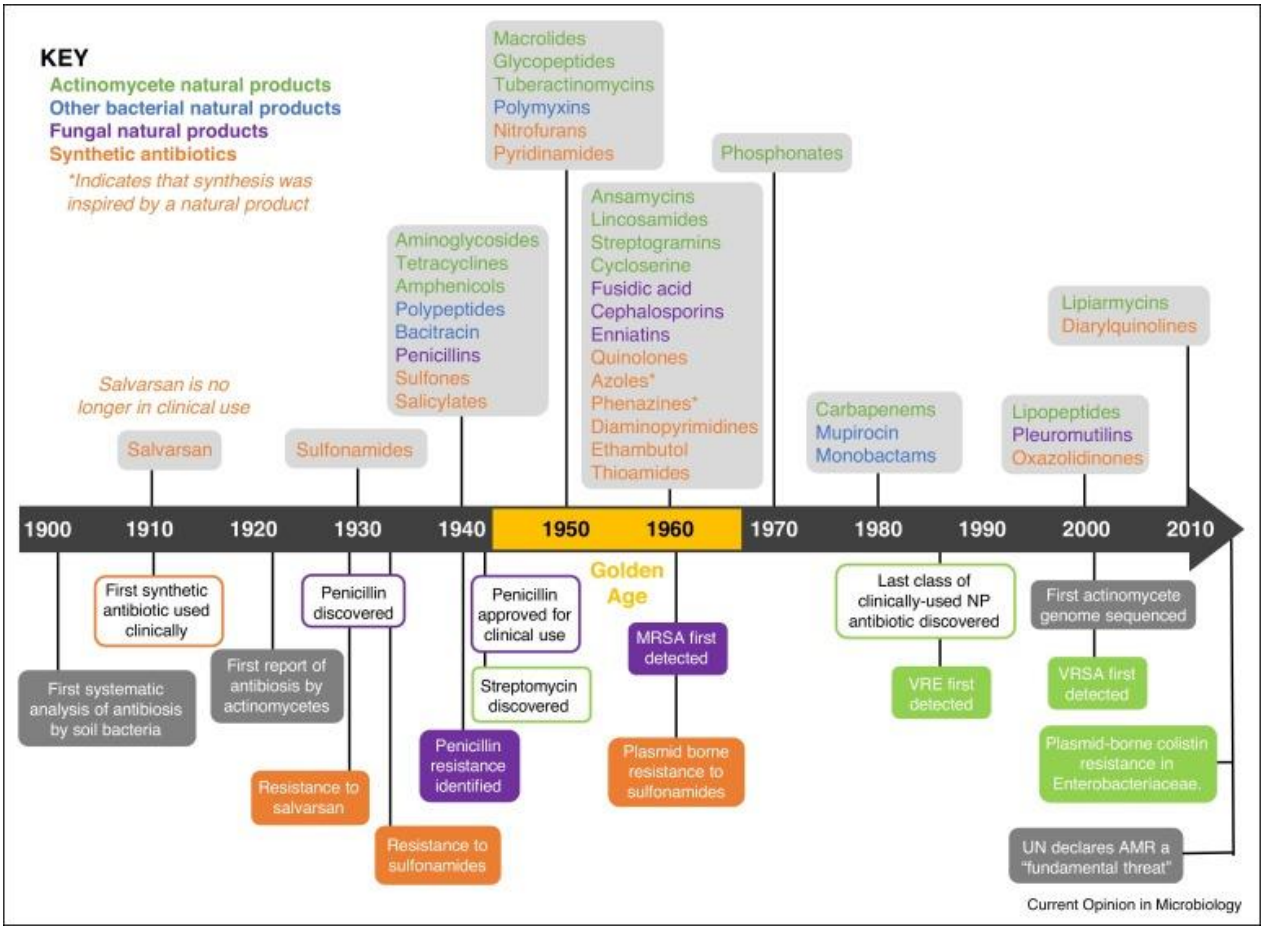


Figure 3: Discovery of antibiotic classes and their origin including date of resistance. Image taken from[16].

Most of the antibiotics that were discovered up until now were originating from nature[16]. Efforts in medicinal chemistry ultimately led to optimized structures with higher and broader activity. Nevertheless, the extensive use of antibiotics and the ability of microorganisms to adapt to their surroundings lead to the development of resistances against whole classes[17]. Surprisingly, penicillin resistant strains were already known in 1940, 3 years prior to its commercial use[18].

1.2 Antibiotic resistance

In the following, general mechanisms of antibiotic resistances are discussed. The development of an antibiotic resistance is based on one or more of the following biochemical processes (Figure 4)[19]:

1. Increased efflux
2. Membrane alterations
3. Metabolic inactivation
4. Target modification and bypass
5. Sequestration of the antibiotic
6. Target overproduction
7. Production of repair enzymes

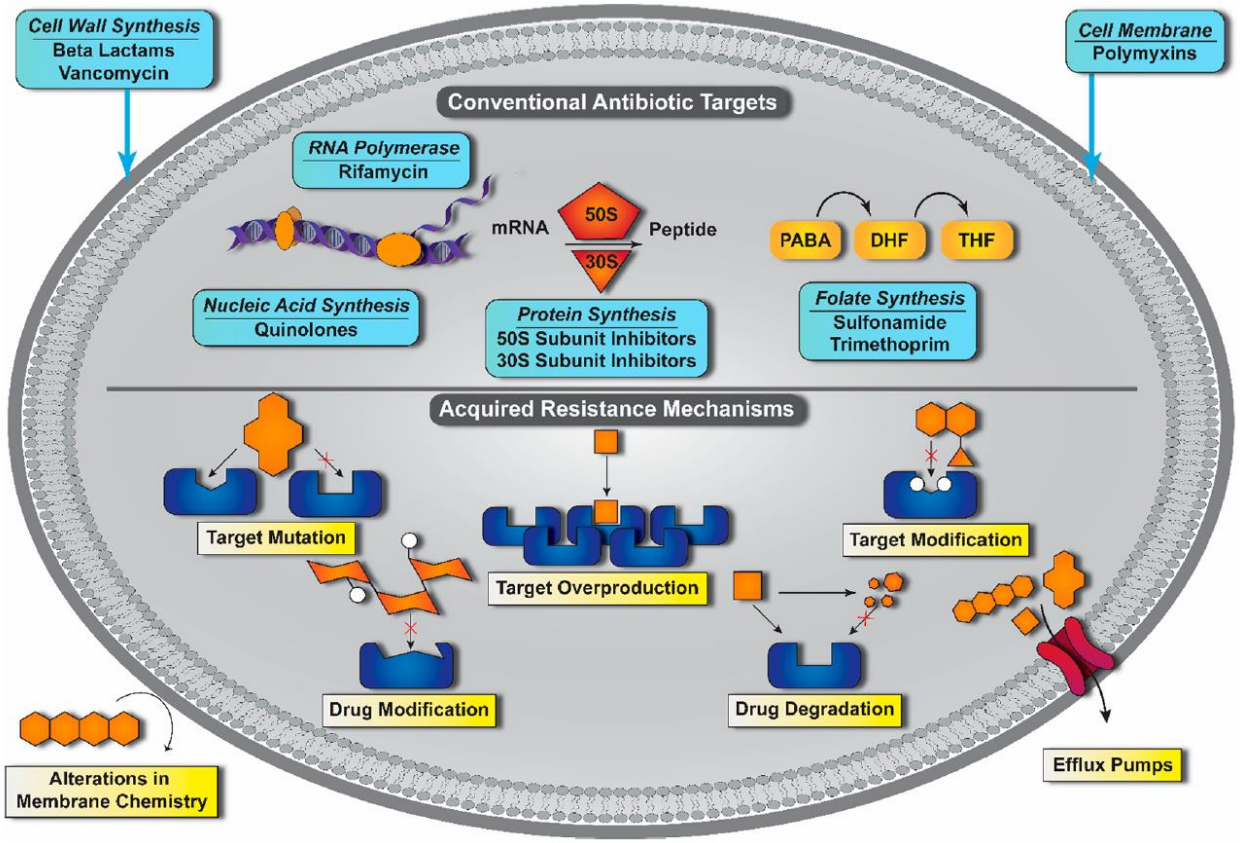


Figure 4: Overview of antibiotic targets and resistance mechanisms. Image taken from[20].

Efflux is one of the major mechanisms for the resistance of Gram-negative bacteria. Pumps transport antibiotics, metabolites or quorum sensing molecules from inside of the bacteria into the environment. Antibiotics that possess a target within the cell are less likely to reach their target. An efflux pump can be a one-component system or consist of several components. For instance, *P. aeruginosa* exhibits many

multidrug resistant pumps (MDR) with MexAB-OprM as the most important one[21]. This transporter allows for the efflux of tetracycline, chloramphenicol, quinolones and β-lactams, ultimately leading to a lower susceptibility to those drugs[22],[23].

Changes in the membrane of a bacteria can prevent or decrease the penetration of the antibiotic. While Gram-positive bacteria possess a thick peptidoglycane layer over the membrane, Gram-negative bacteria have an additional outer membrane (Figure 5)[24]. This membrane is a lipid-protein bilayer and consists of proteins, phospholipids and lipopolysaccharides (LPS). Compared to the peptidoglycane layer, molecules are less likely to diffuse through the outer membrane. Therefore, Gram-negative bacteria rely on the use of integrated porins and transporters that allow the influx of small hydrophilic compounds[25]. More hydrophilic β-lactams like amoxicillin make use of those porins while more hydrophobic macrolides can penetrate through the lipid bilayer directly[26]. Changes or the elimination of specific porins can reduce or prevent the penetration of the antibiotic. An alteration in the LPS can lead to a decreased penetration for lipophilic antibiotics. By decreasing negatively charged residues in the LPS system, antibiotic cationic peptides are significantly less active[27].

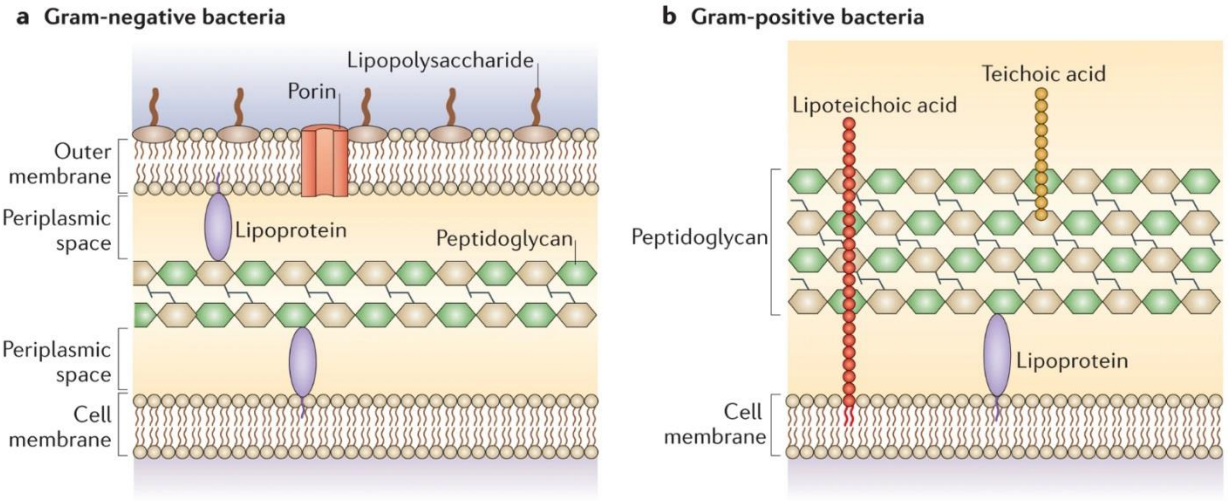


Figure 5: Cell wall structure of Gram-positive and Gram-negative bacteria. Image taken from[28].

The most famous example for a resistance through metabolic inactivation concerns the β-lactam antibiotics. The hydrolysis of β-lactams is catalyzed by β-lactamases (Figure 6). Depending on their structure and class, their inactivation includes penicillins, cephalosporins, monobactams and carbapenems. A subdivision in serine-β-lactamases (SBLs), metallo-β-lactamases (MBLs) or by their amino acid sequence is possible. The clinically relevant extended-spectrum β-lactamases (ESBL) hydrolyze narrow

and broad-spectrum penicillins and cephalosporins. Several serine β-lactamase inhibitors are available to overcome the resistance. So far, no inhibitor against MBL is marketed[29].

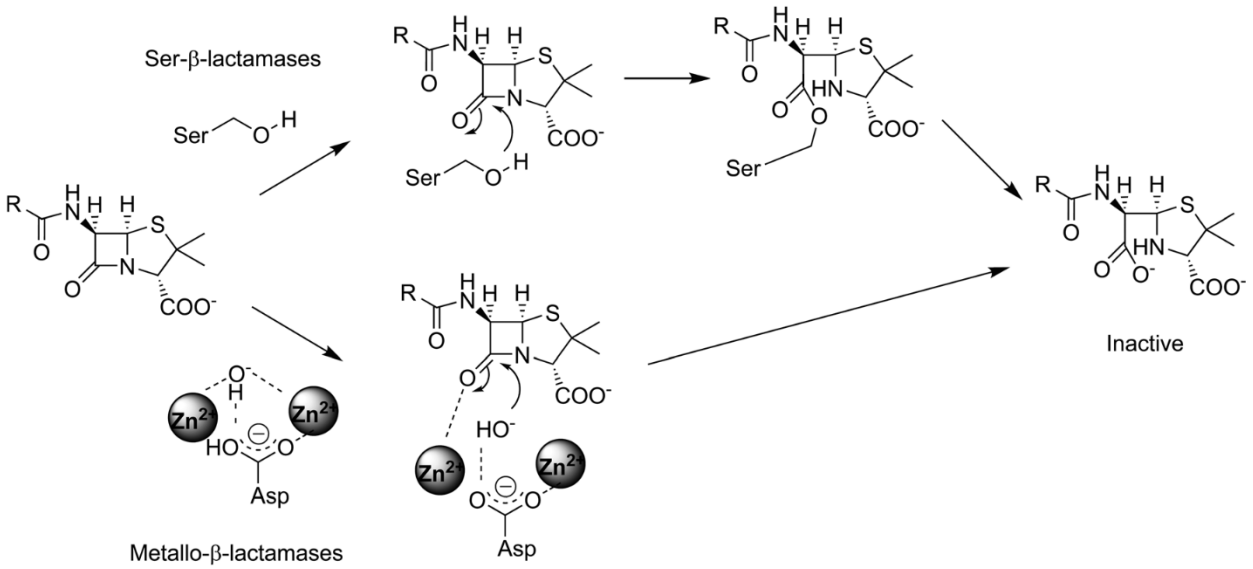


Figure 6: Simplified mechanism for hydrolysis of penicillins by beta-lactamases. Image adapted from[30].

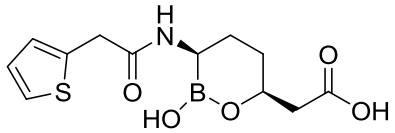
Metabolic modifications can occur on aminoglycosides. Enzymes like aminoglycoside acetyl transferase (AAC), phosphotransferase (APH) and adenyltransferase (ANT) modify the aminoglycoside to inactive metabolites[31].

Alterations in the binding site of a target can influence the activity significantly. This resistance mechanism occurs for nearly every antibiotic class. The changes can be the substitution of amino acids, an enzymatic modification and the replacement or bypass of the target[32]. The exchange of the amino acids in the S83L and D87N mutations in *E. coli* gyrA increase the MIC of ciprofloxacin by more than 15-fold. Combinations of several mutations can further increase the resistance[33]. Enzymatic modifications can be found in macrolide-resistant bacteria. By methylation of an adenine residue in the binding pocket, by methyltransferases encoded in *erm* genes, a resistance is gained. The interaction of the macrolide with its target is decreased, ultimately leading to lower activity[34]. A resistance via target bypass is carried out when the microorganism loses the target entirely. By exchanging the target for a different structure with the same function, the bacterium gains resistance. The exchange of the penicillin-binding protein PBP2 for the novel PBP2a in *S. aureus* leads to a methicillin-resistant *S. aureus* (MRSA)[35].

In contrast to the target bypass, bacteria can evolve to produce new targets and sequestrate the antibiotic. This new target is of low interest for the function of the bacteria but prevents the drug from reaching the biologically relevant target. *S. aureus* with the Δ*agrA* mutation in the quorum sensing system release membrane phospholipids upon contact with daptomycin. Normally, daptomycin embeds into the bacterial membrane ultimately leading to ion leakage and death of the bacteria. By release of the phospholipids the interaction with the cell membrane is reduced[36],[37]. A similar effect can be gained by the overproduction of the target. Through higher concentrations of the target, more drug must be applied to inhibit all of them. This overproduction was observed for dihydrofolate reductase (DHFR) in *E. coli*. The several hundredfold more abundant targets lead to a resistance against the DHFR inhibitor trimethoprim[38]. Other resistance mechanisms include the expression of repair enzymes to prevent the cellular damage of the antibiotic. This resistance was observed in *E. coli* for the quinolone antibiotics[39].

1.3 Antibiotic pipeline

In the "golden era of antibiotics" between 1940 and 1962, over 20 classes of antibiotics were marketed. Now, the amount of new antibiotics is not sufficient to overcome the increasing occurrence of antibiotic resistance, especially against Gram-negative bacteria. In the last 20 years only two novel broad-spectrum classes were introduced, the lipopeptides and the oxazolidinones. Both are solely active against Gram-positive bacteria. The quinolones from the 1960s are still the most recent class with activity against Gram-negative bacteria. A majority of novel antibiotics are analogues of marketed classes with benefits in spectrum or activity[40]. To exemplify this, from 2017 to now 12 new antibiotics and antibiotic combinations were approved by the FDA. Vaborbactam is the only structure with a novel scaffold, but also without antibiotic activity itself (Figure 7). The compound acts as novel β-lactamase inhibitor and prevents the inactivation of β-lactam antibiotics[41].



Vaborbactam

Figure 7: Structure of vaborbactam. Structure adapted from[42].

Many pharmaceutical companies stopped the development of antibiotics as result of the low success and low financial benefit, leaving the field to academia and smaller companies[43]. As for the end of 2019, 19 compounds with new pharmacophores were in clinical trials (Figure 8)[44]. In the following, the most advanced clinical candidates with novel scaffolds and mechanisms are discussed.

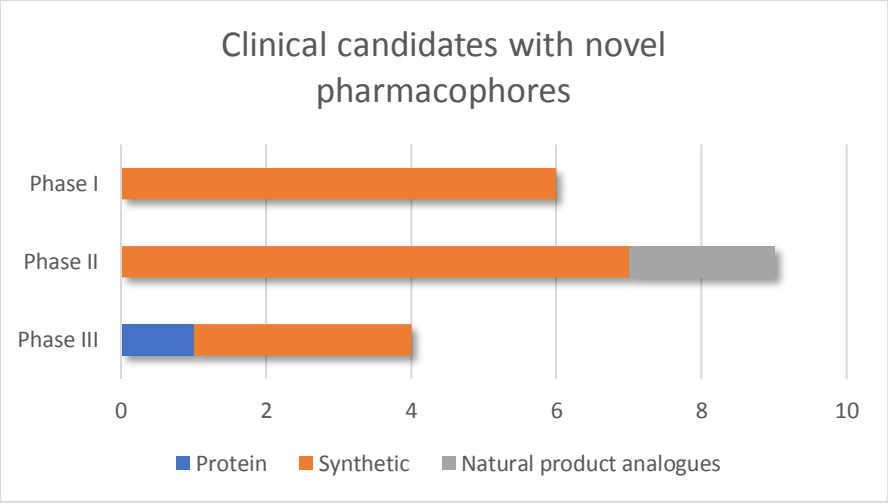


Figure 8: Status of clinical antibiotic candidates with novel pharmacophores (Oct. 2019). Image adapted from[44].

Gepotidacin is a novel gyrase inhibitor developed by GlaxoSmithKline (GSK) with a novel triazaacenaphthylene structure (Figure 9). Both gepotidacin and the quinolones share a similar binding pocket in GyrA of *S. aureus* gyrase but interact in a different manner. The binding of one of the two inhibits the interaction of the other[45]. Gepotidacin introduces single strand breaks of the DNA and differs from the quinolones that introduce double strand breaks. Only one molecule of gepotidacin interacts with the target instead of two for the quinolones. Gepotidacin shows activity against quinolone resistant strains[45]. The orally applicable drug successfully passed phase II studies for uncomplicated urogenital infections[46].

Zoliflodacin is the second novel gyrase inhibitor in the pipeline and the first member of the spiropyrimidinetrione class (Figure 9). It was originally developed by AstraZeneca. The spectrum of coverage includes Gram-positive and some Gram-negative bacteria like *Neisseria gonorrhoeae*. Zoliflodacin shows activity against MRSA, VRE and penicillin resistant *Streptococci*. The potency is independent from a resistance against quinolones[47]. Zoliflodacin introduces double strand breaks in the DNA. In contrast to the quinolones the double strand breaks are less likely to religate[48]. The binding pocket in GyrA of *S. aureus* is distinct from the quinolones and does not require the interaction with Mg^{2+}[49]. The drug successfully passed the phase II studies for uncomplicated gonorrhea infections and is currently in phase III studies[50].

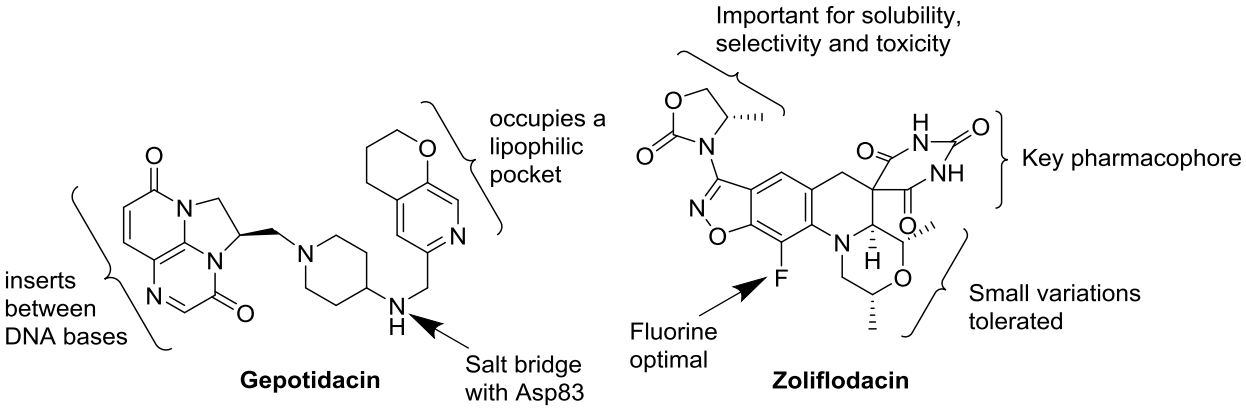


Figure 9: Structure and SAR of the novel gyrase inhibitors gepotidacin and zoliflodacin. Image adapted from[45],[49].

Brilacidin is a defensine mimetic that was originally developed by PolyMedix (Figure 10). Defensins are proteins of the innate immune system and take part in the first immune response against microorganisms. Similar to daptomycin, brilacidin kills bacteria through depolarization of the bacterial cell membrane. The compound shows broad-spectrum activity including MRSA and VRE with higher activity against Gram-positive bacteria[51]. In a phase IIa study against acute bacterial skin and skin structure infections (ABSSSIs) caused by MRSA brilacidin exhibited similar effectivity to daptomycin. The study compared single dose or a three days treatment of brilacidin with a seven days treatment with daptomycin[52].

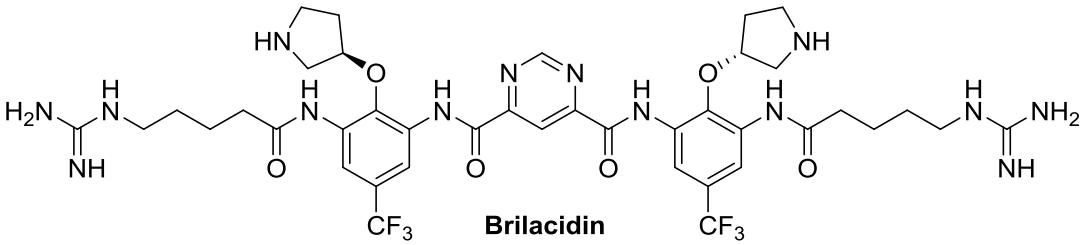


Figure 10: Structure of the defensine mimetic brilacidin. Structure adapted from[53].

Afabicin is an enoyl-acyl carrier protein reductase (FabI) inhibitor that inhibits the fatty acid synthesis in *Staphylococcus* ssp. specifically (Figure 11)[54]. By inhibition of FabI the bacteria are not able to synthesize fatty acids for the assembly of the membrane[55]. Afabicin was developed by Debiopharm and can be administered i.v. or orally. To enhance the solubility the antibiotic is applied as prodrug and must be converted to the active afabicin desphosphono. In a phase II study against ABSSSI, afabicin showed non-inferiority to a combination of vancomycin and linezolid[54],[56].

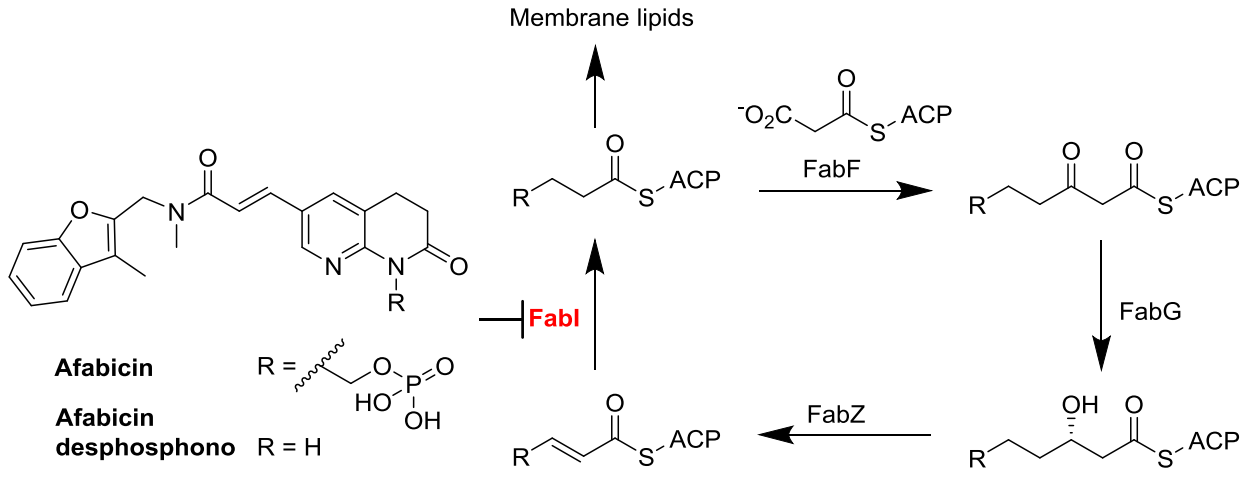


Figure 11: Structure of afabicin and afabicine desphosphino (left). Simplified fatty acid biosynthesis pathway in S. aureus (right) with the acyl carrier protein (ACP). Images adaptet from[57],[56].

Another very specific antibiotic is the novel bisimidazole ridinilazole of Summit Therapeutics (Figure 12). The antibiotic is highly active against the Gram-positive *Clostridioides difficile*. Because of its high specificity, the influence of the gut microbiota is diminished. Other *Clostridioides* species are less susceptible. An exact mechanism of action is not known. It is assumed that the molecule inhibits the cell division without influencing the duplication of the DNA. Additionally, a treatment with ridinilazole decreased the production of the *C. difficile* toxins A and B significantly[58]. A phase II study with vancomycin as competitor showed a non-inferiority with similar adverse effect profiles[59].

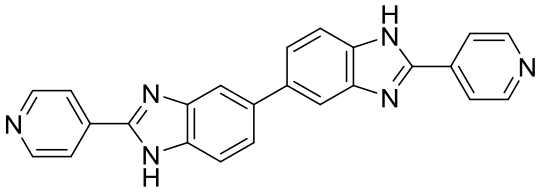


Figure 12: Structure of ridinilazole. Structure adapted from[44].

With OPC-167832 and macozinone two new clinical candidates against *Mycobacterium tuberculosis* are in the pipeline (Figure 13). Both inhibit the decaprenylphosphoryl-β-d-ribose 2'-oxidase (DprE1) essential for the cell wall biosynthesis[60],[61]. The new drugs are currently in phase II studies. With BTZ-043, another antitubercular drug with structural similarities to macozinone entered clinical trials[62].

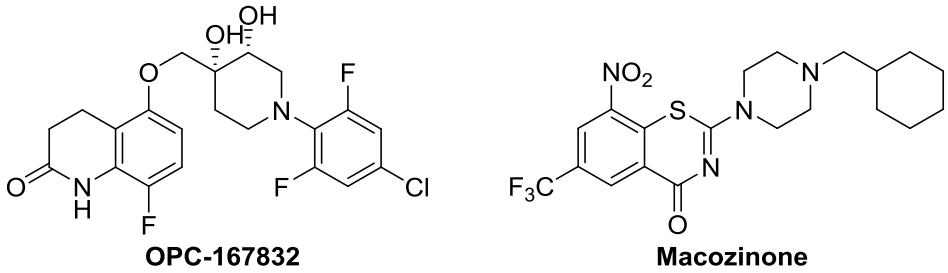


Figure 13: Novel candidates against tuberculosis. Structures adapted from[61].

1.3.1 Urinary Tract Infections (UTIs)

Urinary Tract Infections (UTIs) are among the most common bacterial infections and include all infections of the kidneys, ureters, urethra and the bladder. They are divided into uncomplicated and complicated Urinary Tract Infections (cUTIs)[63]. Uncomplicated cases are usually less severe, well treatable and sometimes benign. cUTIs on the other hand have a higher risk for life-threatening infections, reoccurrence and chronical progression. Risk factors like diabetes, immune deficiency or urinary obstruction are usually absent in uncomplicated UTIs but common in cUTIs[64]. If untreated, a cUTI can result in a systemic inflammation, organ dysfunction, systemic shock and death[65]. Resistances are common in UTIs and predominantly observed for Gram-negative pathogens and *Enterococci*. The percentage of resistant Gram-negatives lies between 5 – 80 %, depending on the antibiotic class, with increasing prevalence of resistant microorganisms[64].

The most abundant pathogen in UTIs is the uropathogenic *E. coli* (UPEC) followed by *K. pneumoniae* and *Enterococci*. *Staphylococcus saprophyticus* can only be found in uncomplicated UTIs. Other relevant bacteria are shown in Figure 14[66].

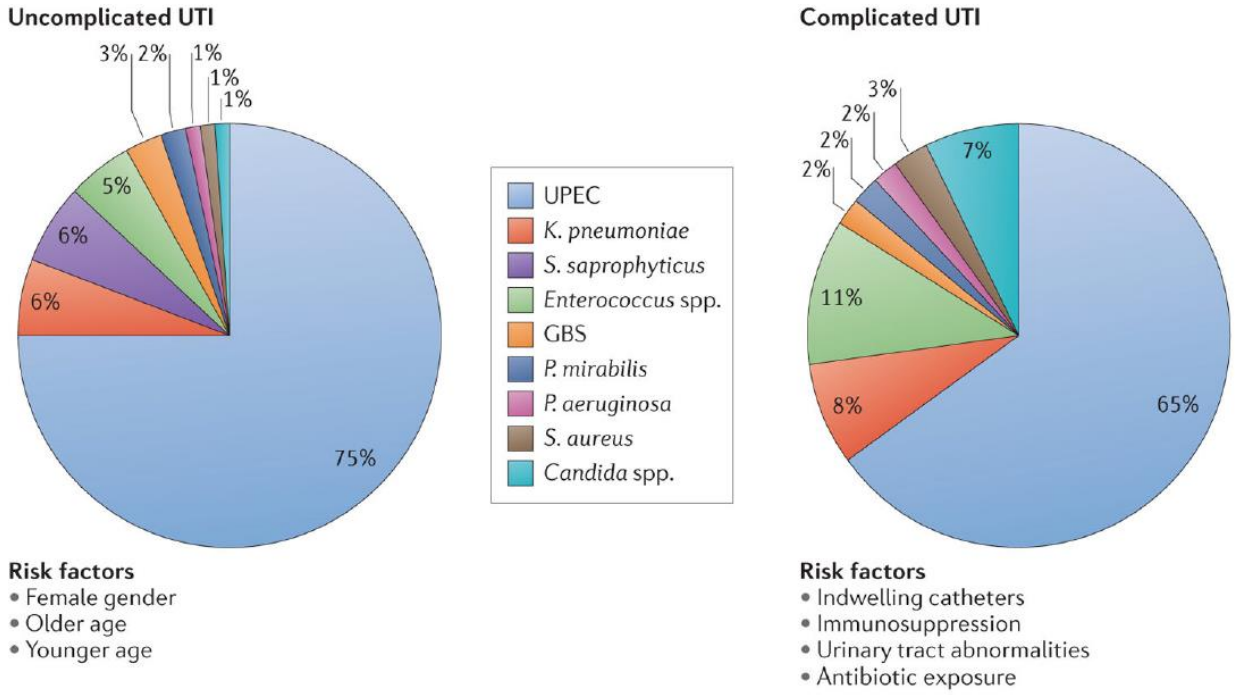


Figure 14: Causes for UTIs. Uropathogenic E. coli (UPEC) is the main reason for UTIs. Other pathogens like group B Streptococci (GBS) are of lower significance. Image taken from[66].

For the development of a drug against UTIs, the activity against *E. coli*, *Klebsiella*, *Enterococci* and *Proteus* strains is mandatory. Additionally, an activity against less common strains in cUTIs like *S. aureus, P. mirabilis* and *P. aeruginosa* amongst others is desired. In total, the antibiotic has to cover a broad-spectrum including Gram-positive and -negative bacteria. Due to the rising development of resistances, there is a high demand for novel antibiotics. With gepotidacin, a new drug candidate with novel scaffold is in development.

1.4 Topoisomerases

The first discovery of topoisomerases was in 1971, when Jim Wang observed the loss of negatively superhelical turns on closed DNA, when treated with the purified *E. coli* protein ω[67]. One year later another topoisomerase was found. While protein ω relaxed negatively supercoiled DNA, the newfound topoisomerase was able to relax positively supercoiled DNA[68]. The discovery of other topoisomerases revealed proteins that were able to introduce supercoils, like the DNA gyrase[69].

Until today, several different topoisomerases are known and can be classified into type I and type II topoisomerases according to their reaction mechanism with the DNA. While type I topoisomerases cleave one strand of the DNA, type II topoisomerases lead to double strand breaks[70]. All topoisomerases induce the strand break of DNA by an attack of the tyrosyl phenol at the phosphodiester bond connecting two backbone sugars (Figure 15)[71].

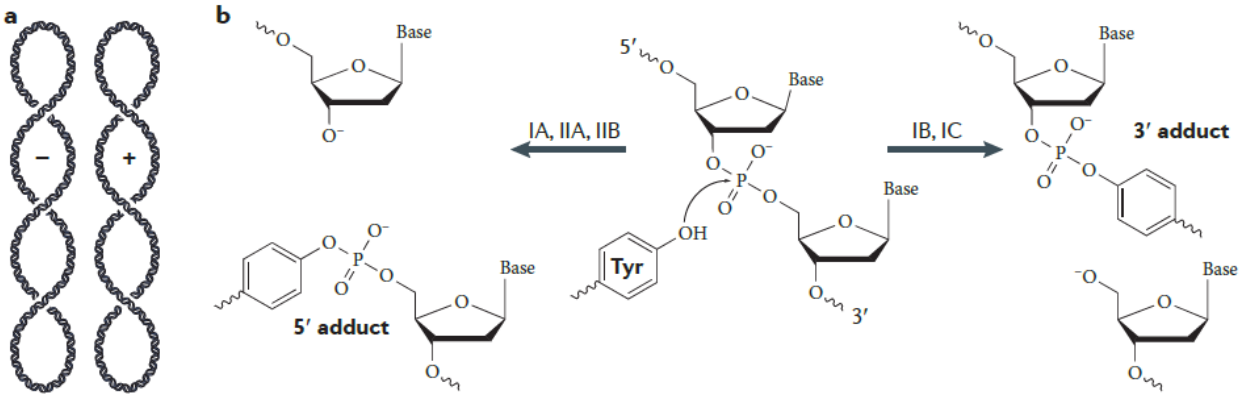


Figure 15: Simplified mechanism of the DNA strand break. Depending on the topoisomerase subfamily 5' adducts or 3' adducts can be formed. Image taken from[71].

Topoisomerases fulfill essential functions and are crucial for DNA compaction, replication, transcription, recombination and repair[72]. As cystobactamids are known to inhibit gyrase and topoisomerase IV that belong to the bacterial type IIa topoisomerases, other topoisomerases are not covered in the following[73],[3].

1.4.1 Bacterial Type IIa topoisomerases

The two members of the bacterial type IIa topoisomerases, namely topoisomerase IV and gyrase, are heterotetramers consisting of two subunits. While topoisomerase IV is comprised of the two subunits ParC and ParE forming a $ParC_2ParE_2$ complex, the analogue complex of gyrase is formed by GyrA and GyrB[74]. Compared to bacteria, humans only carry one topoisomerase of the type IIa class, DNA topoisomerase II. This enzyme is a homodimer and its α- and β-subunits share a homology of 77 % to each other[75].

As mentioned before, type IIa topoisomerases induce double strand breaks in the DNA. Gyrase is the only topoisomerase known to introduce negative supercoils responsible for the negative supercoils in the bacterial DNA[76]. Topoisomerase IV on the other hand cannot introduce negative supercoils, but relaxes positive supercoils and negative supercoils to some extent[77]. The type IIa reaction cycle starts with the interaction of the so-called G segment of the DNA with the center of the enzyme at the GyrA or the ParC subunit (Figure 16). Upon binding of two ATP molecules at GyrB or ParE the T segment of the same DNA strand binds to the enzyme. Segment G gets cleaved and the T segment gets transferred through the broken strand. The reconnection of the G segment and the release of the product completes the reaction [78],[79].

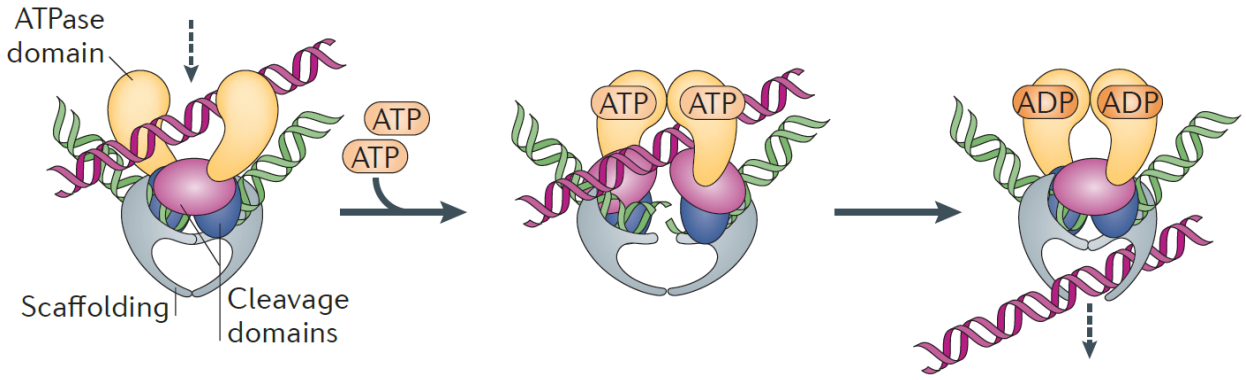


Figure 16: Schematic representation of the catalytic reaction of DNA with type IIa topoisomerases. Segment G (green) is bound to the DNA binding subunit (gray) of GyrA (gyrase) or ParC (TI IV). Segment T (rose) gets transferred through the broken strand of segment G. After the reannealing of segment G, the catalytic cycle is completed. The reaction requires ATP as energy source bound to the ATP binding subunit (yellow) of GyrB (gyrase) or ParE (TI IV). Image taken from[71].

The importance of gyrase in the regulation of DNA related processes can be visualized by its possibility to participate in various reactions with DNA including supercoiling, relaxation, catenation, decatenation and unknotting[80]. In bacteria a negative supercoiling is required for the DNA replication and the opening of the double helix by promoters[81],[82]. Topoisomerase IV is mainly involved in the DNA relaxation,

decatenation and the chromosome segregation[83],[84]. Although gyrase and topoisomerase IV show a significant amount of homology, their similarity cannot be reflected by their physiological function in bacteria[85].

Inhibitors of bacterial type IIa topoisomerases are bacteriostatic or bactericidal depending on their exact binding site on gyrase. So far, two major classes of inhibitors were used clinically: the aminocoumarines and the quinolones (Figure 17). While the former were withdrawn from the market, because of superior alternatives and toxicity, the latter are still used clinically[86],[87]. Aminocoumarines act as competitive inhibitors at the ATP binding site of GyrB exhibiting a bacteriostatic effect[88],[89]. Quinolones on the other hand are bactericidal inhibitors at the DNA binding GyrA subunit of gyrase. Compared to the aminocoumarines, the antimicrobial effect does not come from the inhibition of gyrase alone, but the introduction of permanent double strand breaks in the DNA. Quinolones lead to a reversible formation of a ternary complex between the cleaved DNA, the enzyme and the drug. Upon collision with tracking enzymes, like polymerases or helicases, the double strand break becomes permanent and can ultimately lead to cell death[79],[90].

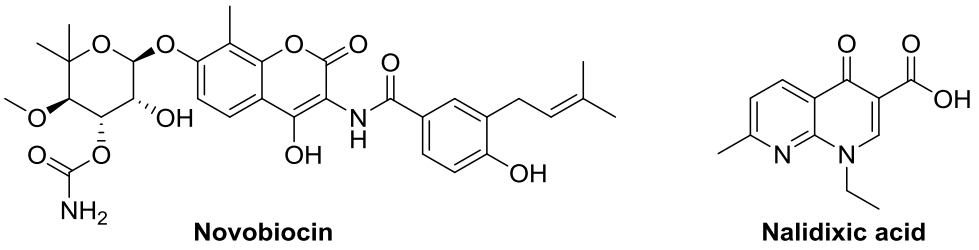


Figure 17: Structure of the aminocoumarine novobiocine and nalidixic acid as first member of the quinolone antibiotics. Structures adapted from[80].

To illustrate the interaction of the quinolones with gyrase, the binding of moxifloxacin with gyrase of *S. aureus* is described (Figure 18).

Both the carboxylate and the ketone interact with the Mg^{2+} ion in the binding pocket. Together with four additional water molecules, an octahedral complex is formed. Two of the water molecules form hydrogen bonds with the DNA bases below and above the quinolone. Another water molecule is stabilized by Ser84 and Glu88[91].

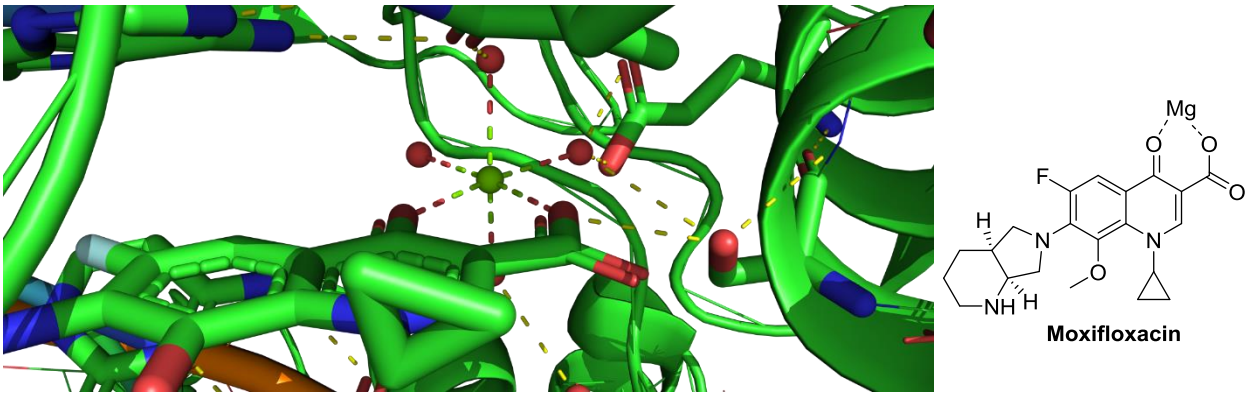


Figure 18: Interaction of moxifloxacin with S. aureus gyrase (left). Structure of moxifloxacin (right). Images adapted from PDB 5CDQ[91]. Red = oxygen, green = carbon, blue = nitrogen, white = hydrogen. 3D structure generated by PyMOL.

The 4-quinolone system with the β-keto acid is of crucial importance for the binding. To prevent the tautomerization to the 4-hydroxyquinoline, the nitrogen is always alkylated[92]. The fluorine in position 6 is significantly improving the activity. Due to the high benefit of this fluorine all newer quinolones possess this residue[93]. They are often referred to as the class of fluoroquinolones. Heterocyclic systems in position 7 fine-tune the activity against Gram-positive and Gram-negative bacteria[93].

1.5 Albicidines and cystobactamids

1.5.1 Albicidin

The first appearance of albicidin in literature was in 1985, when Robert Birch and Suresh Patil discovered the antibiotic properties of an isolated substance from *Xanthomonas albilineans*, a sugarcane pathogen. Although the structure of the substance was not elucidated, its influence on the replication of DNA was discovered[94]. Similar to the antibiotic class of the quinolones, it was found that albicidin inhibits gyrase and topoisomerase IV of *E. coli.*[95]. The structure was resolved 20 years after its discovery (Figure 19)[96]. A total synthesis for albicidin was published in the same year[97].

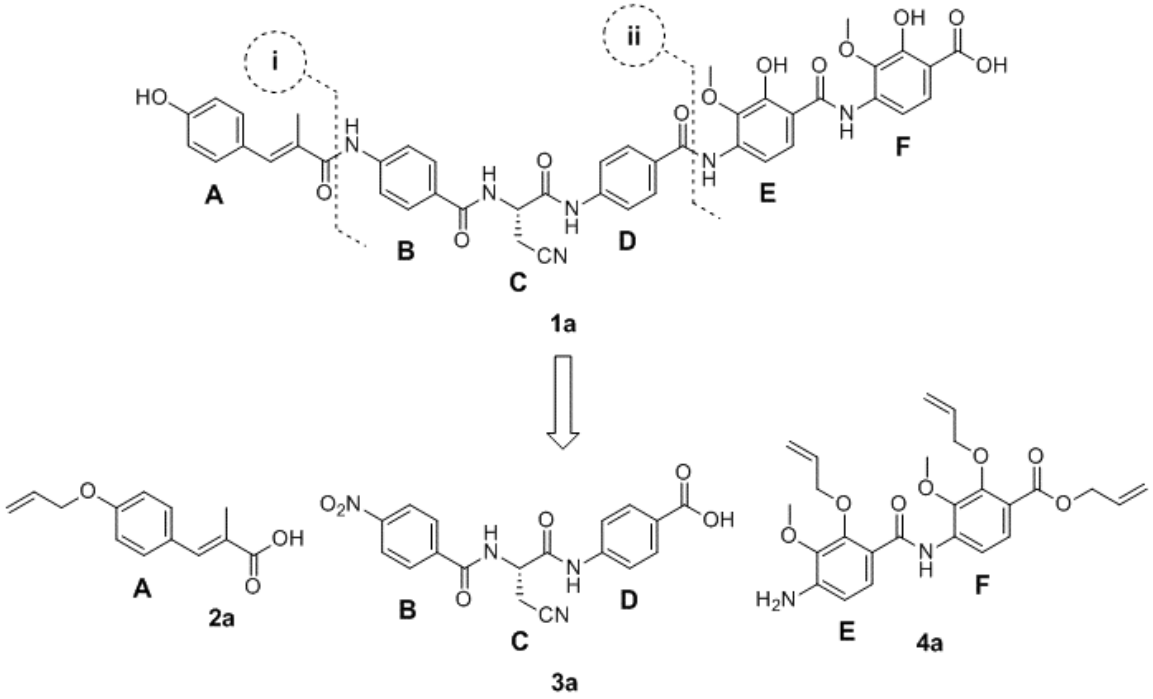


*Figure 19: Retrosynthetic scheme for albicidin (**1a**) from the six building blocks **A-F**. Fragment **2a**, **3a** and **4a** are coupled at **i** and **ii**. Image adapted from[97].*

The biosynthesis of albicidin is carried out by a polyketide synthase-nonribosomal-peptide synthase hybrid expressed by the *alb* gene cluster. The production of albicidin by *Xanthomonas albilineans* cultures is very low with approximately 10 µg/L[98]. A carbamoylated albicidin was discovered in *X. albilineans* and confirmed to be a product of an O-carbamoyl transferase expressed from the *alb* cluster (Figure 20). This was confirmed by a knockout of the Δalb15 gene in *X. albilineans* and the resulting absence of the carbamoylated albicidin. By synthesis of the carbamoylated albicidin a MIC improvement of around 50 % against *Salmonella typhimurium* and *E. coli K12* was found[98].

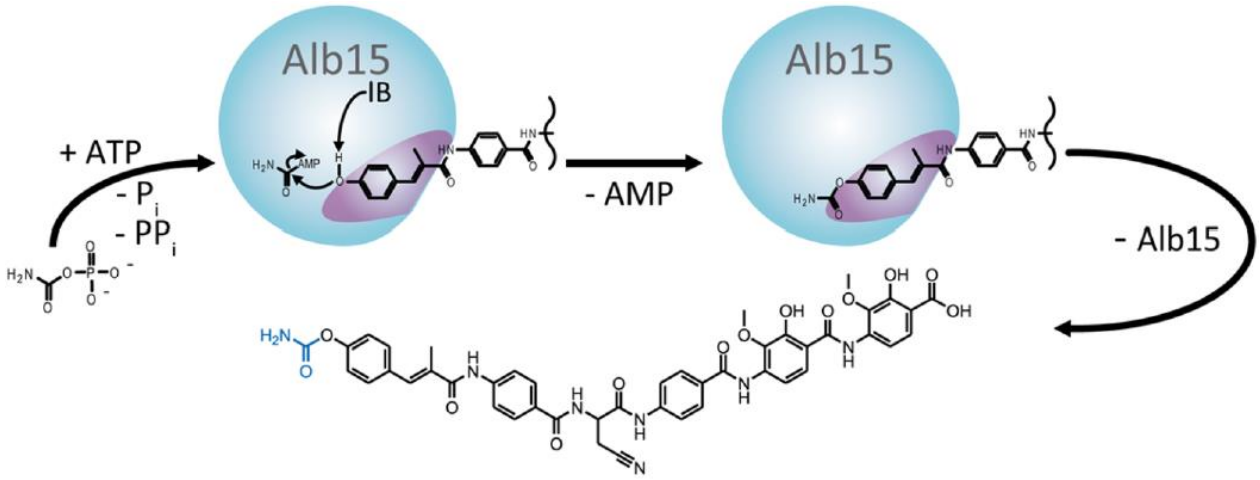


Figure 20: Proposed carbamoylation of albicidin after nonribosomal-peptide synthesis. Image taken from[98].

First structure-activity relationship (SAR) studies on the albicidines were carried out at the central amino acid, where the β-cyanoalanine was exchanged for other amino acids. In total, five different amino acids were used (Figure 21). Although the IC_{50} values on gyrase showed similar activities for all derivatives, the biological activity differed significantly. Good activity was observed for the α-aminoisobutyric acid (Aib) and threonine (Thr). The introduction of aspartic acid (Asp) or lysine (Lys) led to a loss of activity[99].

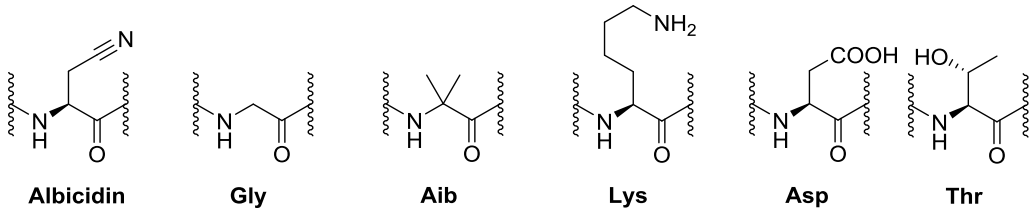


Table 2. Antibacterial activity of albicidin, apramycin, and the newly synthesized compounds.

Strain	MIC [μg mL^{-1}][a]						
	Albicidin	Gly	Aib	Lys	Asp	Thr	Apramycin
B. subtilis[b]	0.25	1	0.5	>4	>4	0.5	1
E. coli[c]	0.062	0.5	0.25	>4	>4	0.062	>4
M. luteus[d]	2	8	1	>64	>64	1	16
M. phlei[e]	2	8	2	>64	>64	2	4

[a] Values are the mean of two replicates; see the Supporting Information for details. [b] *B. subtilis* DSM 10. [c] *E. coli* DSM 1116. [d] *M. luteus* DSM 1790. [e] *M. phlei* DSM 750.

Figure 21: Structures and antibacterial activity of albicidines with modified central amino acid. Images adapted from[99].

Further SAR studies were carried out at ring N-terminal part (ring A) of the albicidin oligopeptide. In total 16 derivatives were synthesized and biologically tested (Figure 22). The substitution of the *p*-OH by -CF_3 or -F was well tolerated and showed comparable activity to albicidin[100]. Those well tolerated functional groups share the common feature to act as hydrogen-bond acceptor (HBA), although this feature is less pronounced for fluorine. The lack of the *p*-OH decreased the activity by approximately 50 %. All other modifications led to decreased activity or a loss thereof. Especially the reduction of the rigid cinnamomic acid linker to an alkane chain was associated with a big loss in potency. Omitting any unsaturated system as N-terminal part resulted in a total loss of activity[100].

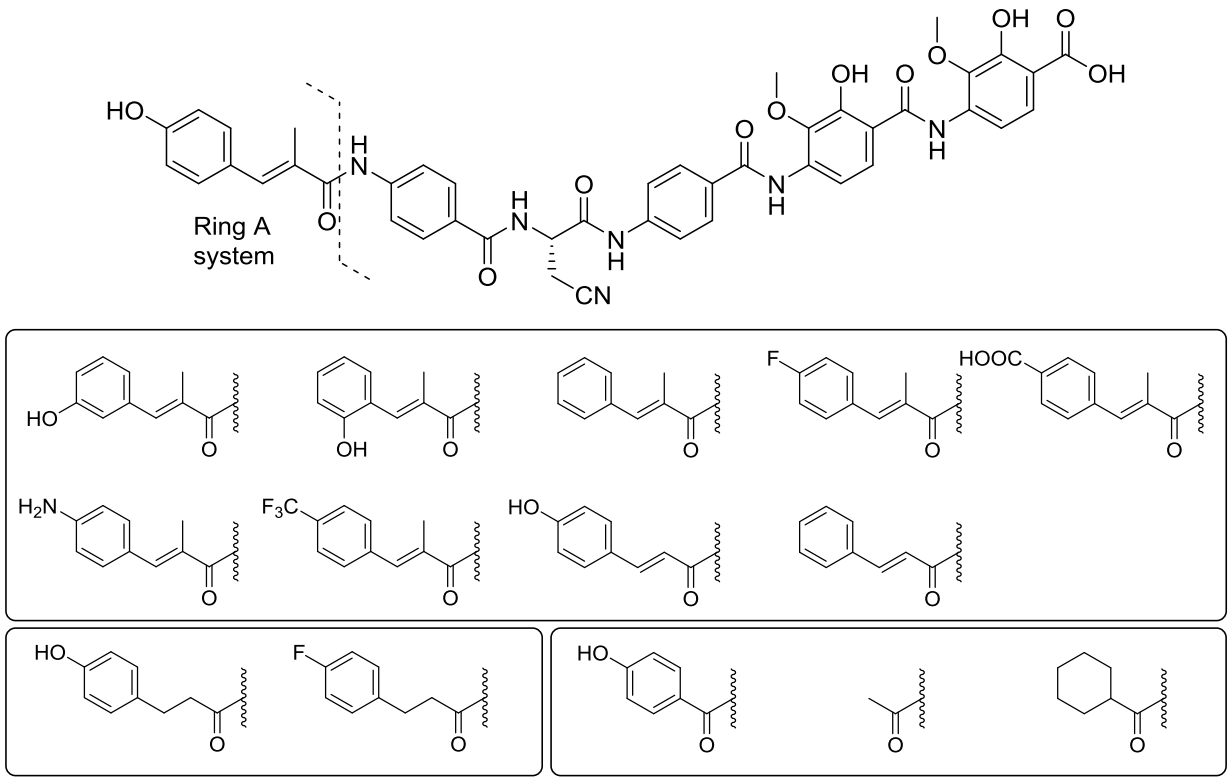


Figure 22: Ring A derivatives of albicidin. Image adapted from[100].

To not be affected by the antibiotic it produces, *X. albilineans* carries several modifications that make it unsusceptible to albicidin. The major target gyrase, with its subunits GyrA and GyrB, differs from the *E. coli* homologue through the substitution of amino acids and several insertions. Those changes result in a 20- to 25-fold higher resistance compared to the gyrase of *E. coli* against albicidin and ciprofloxacin[101]. Other mutations include the resistance genes AlbF and AlbG. The former encodes for a DHA14 drug efflux pump with 14 transmembrane domains (TMDs) with closest similarity to the efflux proteins of antibiotic-producing *actinomycetes*. The expression of DHA14 in *E. coli* leads to a decrease in activity by 3000-fold[102]. AlbG is located in the gene cluster responsible for the biosynthesis of albicidin. Upon expression, the

product, a DNA-interacting protein, increases the resistance of *E. coli* by six-fold, while the activity of ciprofloxacin is not influenced[103].

The spontaneous development of resistance of *E. coli* was found simultaneously to the first isolation of albicidin from *X. albilineans*. No cross-resistance to other gyrase inhibiting drugs was observed[94]. Alterations in tsx, a gene for the expression of an outer-membrane protein, can lead to a resistance of *E. coli* against albicidin. Tsx encodes proteins responsible for the uptake of nucleosides and albicidin through the membrane. By this mutation in tsx, a 100-fold amount of albicidin was necessary to block the DNA replication[104].

Other resistance mechanisms against albicidin were found in *Alcaligenes denitrificans* and *Klebsiella oxytoca*. The former produces the resistance protein AlbB and the later AlbA. The binding of albicidin to AlbA induces the cyclization of the β-cyanoalanine with the adjacent amide. The resulting amidine hydrolyses to the imide with significantly reduced activity[105]. As *K. oxytoca* can be pathogenic, a resistance through AlbA is of high significance[106],[107]. Albicidin can also be irreversibly inactivated by hydrolysis catalyzed by the serine endopeptidase AlbD (Figure 23). AlbD is expressed by *Pantoea dispersa* and hydrolyses the peptide bond between S1 and S1'. Studies with different fragments of albicidin showed that the hydrolase does not require the full structure. The residues S2-S4 were not necessary for the hydrolysis. Furthermore, leaving out S4 and S3 or additionally S2 accelerated the hydrolysis compared to the full-length albicidin. The use of a D-β-cyanoalanine (D-Cya) as central amino acid instead of the casual L-β-cyanoalanine decreased the cleavage rate[108].

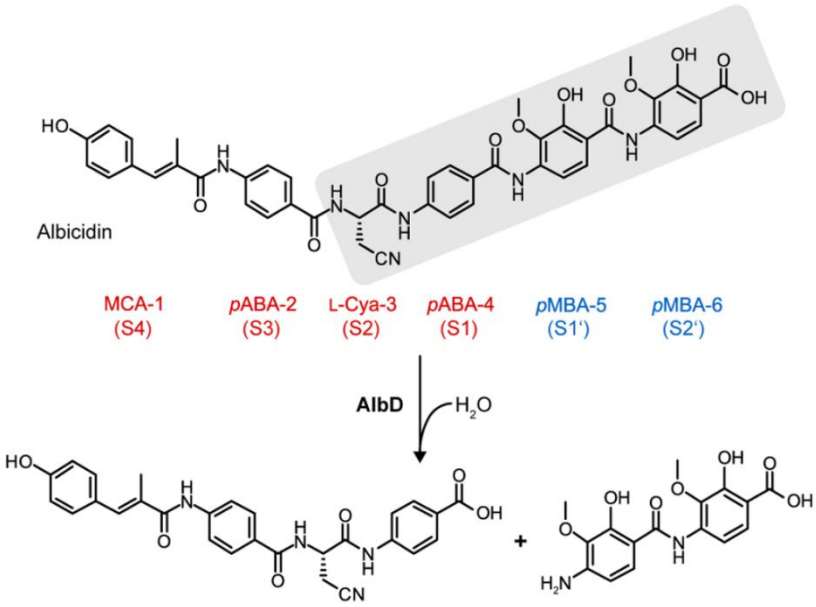
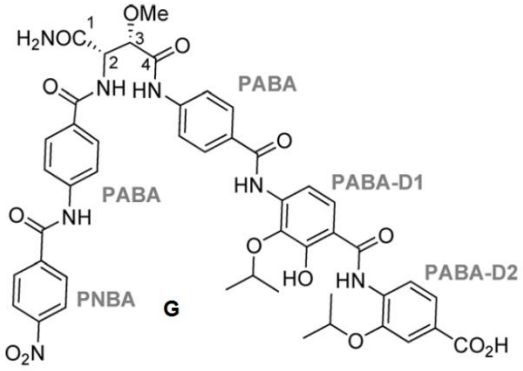


Figure 23: Hydrolysis of albicidin catalyzed by AlbD. Image taken from[108].

1.5.2 Cystobactamids

Cystobactamids were first discovered and isolated from *Cystobacter* sp. in 2014 (Figure 24). Like albicidin, gyrase and topoisomerase IV were found to be targeted by this new class of antibiotics. Cystobactamid 919-2 was able to inhibit gyrase independent of an excess of ATP, which ruled out a competitive inhibition at the ATP binding site of the GyrB subunit. It also showed the formation of linearized plasmids in a plasmid linearization assay just like the quinolone antibiotics. This hinted to a binding at the GyrA subunit of gyrase. While a GyrA S83L or D87G mutations lead to a significant decrease in activity by 30-60-fold for the quinolone antibiotic ciprofloxacin, cystobactamid 919-2 was influenced by a lesser extent of two- to seven-fold. This confirmed that the binding site is located on the GyrA subunit, as mutations would not influence the activity of the cystobactamids otherwise. An overlapping binding pocket to the quinolones was presumed[3].



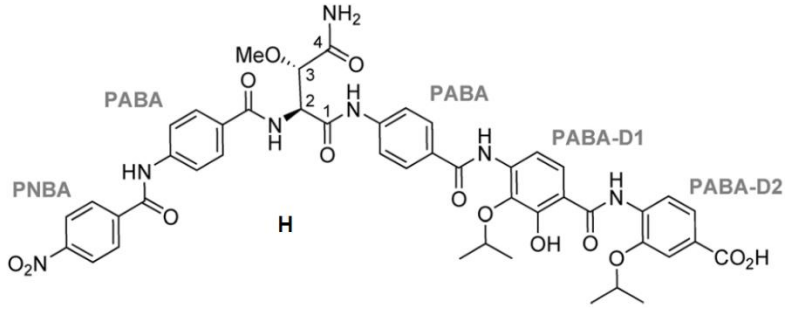


Table 1: Minimum inhibitory concentrations (MICs in μg mL^{-1}) of **G**, **H** and Cp.

	G	**H**	Cp
Acinetobacter baumannii	> 59	7.4	0.2–0.4
Escherichia coli	14.7–29.4	0.9	0.013
Enterococcus faecalis	3.7–7.4	0.1	0.8
Staphylococcus aureus	32.5	0.1	0.05–0.1
Streptococcus pneumoniae	14.7	0.1	0.8–1.6

*Figure 24: The structures and activities of cystobactamid 919-1 (**G**) and cystobactamid 919-2 (**H**). PABA = para-aminobenzoic acid. Image adapted from[3].*

Another source for cystobactamid 919-2 is *Corallococcus coralloides* M23, which was also able to produce the cystobactamid derivatives coralmycin A and B. The configuration at the central amino acid was studied and the stereochemistry of a (2*S*,3*R*)-β-methoxyasparagine was assigned[109].

Both albicidin and cystobactamids share a very similar scaffold that consists of substituted and unsubstituted *para*-aminobenzoic acid (PABA) elements. Instead of the C-terminal 4-nitrobenzoic acid in the cystobactamids, albicidin has a residue similar to p-coumaric acid. Another difference is given by the central amino acid. Cystobactamids have a methoxylated asparagine while albicidin has a β-cyanoalanine. It was suggested that the formation of β-cyanoalanine was originating from asparagine[96]. In the following, only the biosynthesis of cystobactamids is covered.

1.5.2.1 Biosynthesis of para-aminobenzoic acid (PABA)

The first part of the synthesis of PABA is carried out via the shikimate pathway. This pathway includes seven biochemical transformations and ends with the formation of chorismate. Further transformations can lead to aromatic amino acids and other aromatic secondary metabolites, but intermediates of the pathway may also be substrates for other biochemical reactions. The shikimate pathway can only be carried out by microorganisms and plants[110]. Therefore, animals need to ingest products of the shikimate pathway, for example via food. Relevant products for humans that require the shikimate pathway, are for instance the essential amino acids L-phenylalanine and L-tryptophan as well as folic acid (Figure 25)[111],[112].

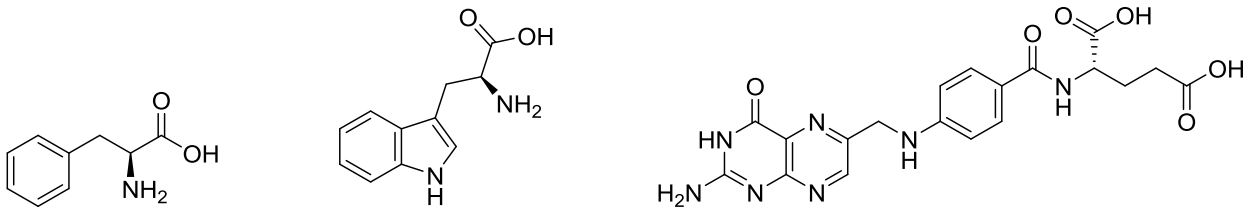


Figure 25: Structures of molecules that require the products of the shikimate pathway for their biosynthesis. Structures adapted from[113].

The shikimate pathway starts with phosphoenolpyruvate (PEP) and D-erythrose-4-phosphate (E4P), which are both found in the metabolism of carbohydrates. After the merging of both starting materials and cyclization, the majority of the remaining pathway consists of the dehydration of the cycle[110]. As mentioned before, chorismate is the final product. The conversion of chorismate to PABA is carried out by an aminodeoxychorismate (ADC) synthase and ADC lyase (Figure 26)[3]. The mechanism includes two addition-elimination reactions followed by an aromatization to PABA.

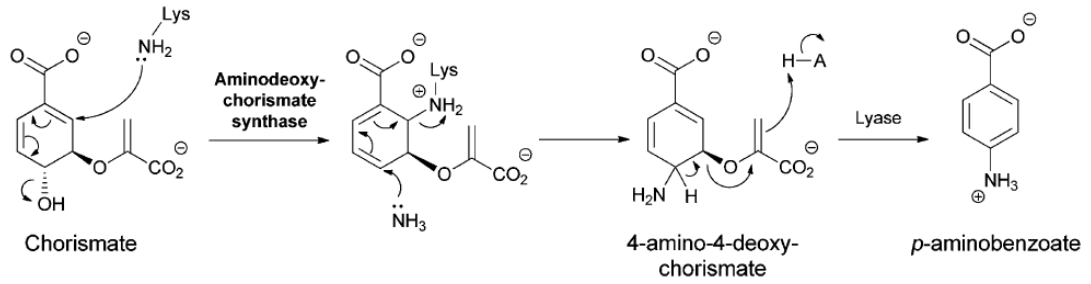


Figure 26: Simplified biosynthesis of PABA from chorismate. Image taken from[114].

1.5.2.2 Assembling of the cystobactamids

Like albicidin, cystobactamids were assumed to be a product of the nonribosomal peptide synthetase (NRPS)[3]. The proposed biosynthesis is shown in Figure 27.

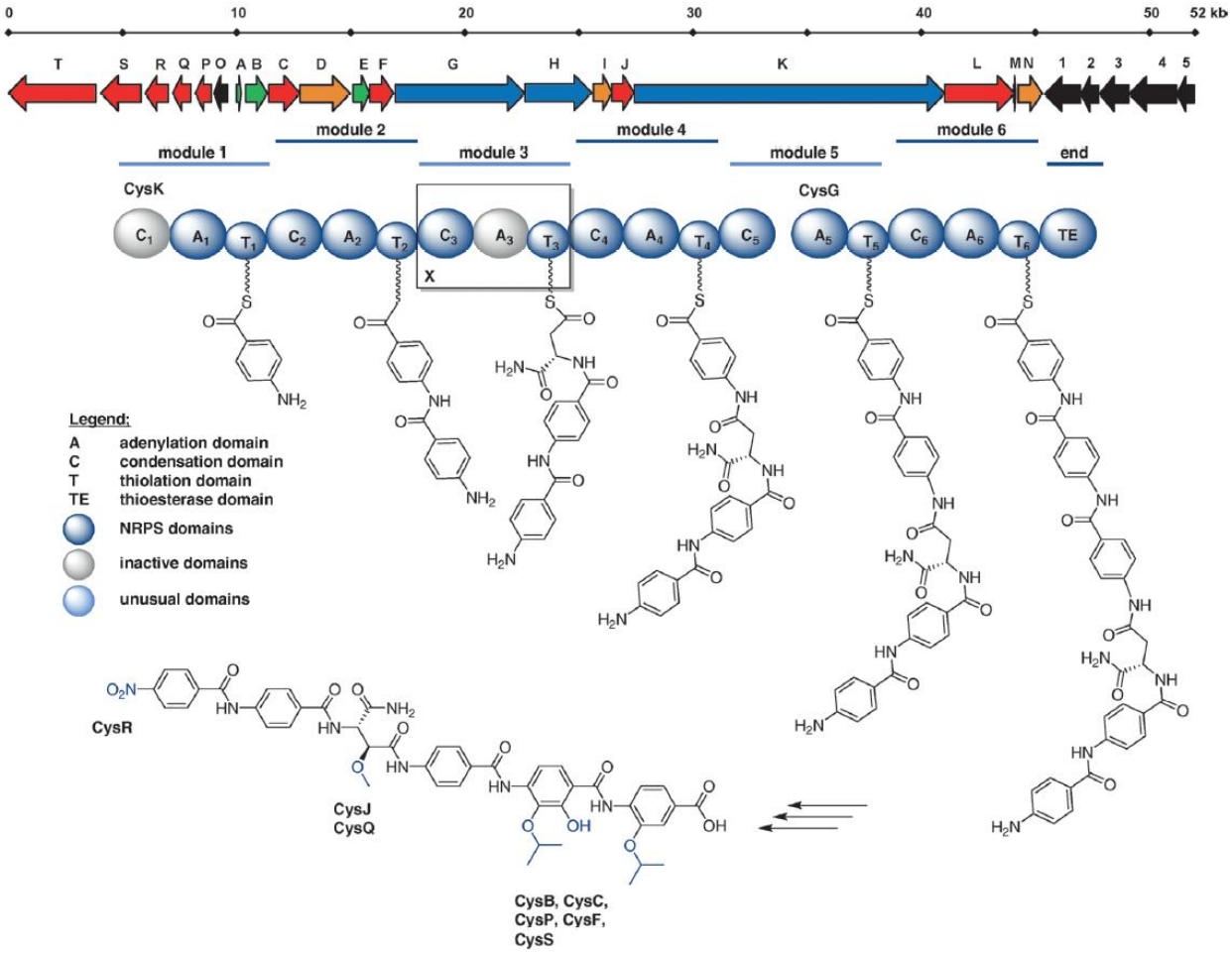


Figure 27: Proposed synthetic pathway for the biosynthesis of cystobactamids. Image taken from[3].

Other modifications, like the oxidation of the N-terminal amine, the attachment of the alkoxy ethers and the oxidation to the 2-hydroxy PABA, are carried out by tailoring enzymes. It is uncertain, whether the modifications are carried out during the assembly or after its completion[3].

The formation of the branched isopropyl ether requires the cobalamin-dependent radical S-adenosylmethionine methyltransferase CysS. The proposed mechanism starts with the dissociation of methionine from S-adenosylmethionine under oxidation of the iron-sulfur cluster (Figure 28)[115]. The formed adenosyl radical abstracts a hydrogen from the methoxy group and a radical substitution with methyl cobalamin takes place while cobalt gets reduced. After reduction of the iron-sulfur cluster and the regeneration of methyl cobalamin, the reaction can be repeated[115].

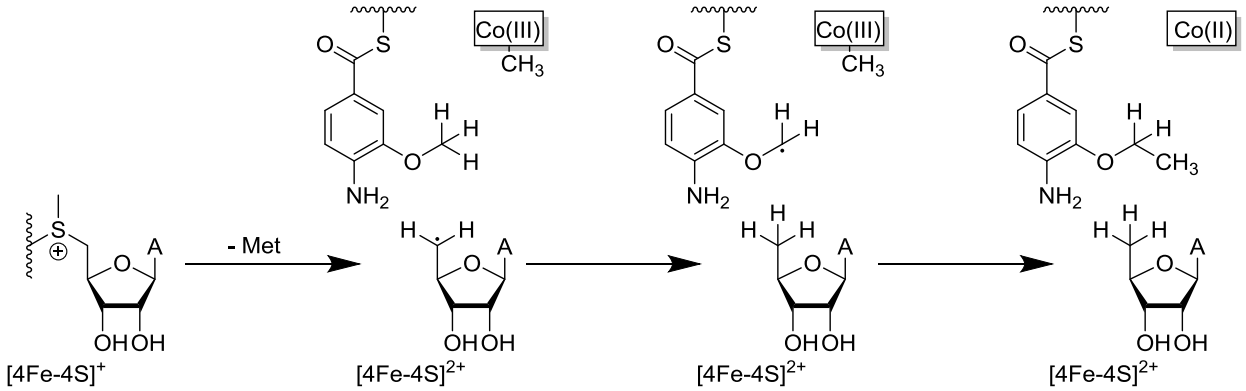


Figure 28: Simplified mechanism of the radical methyl transfer by CysS from the methoxy ether to the ethoxy ether. Additional methyl transfers can be carried out after regeneration of the reactants. Image adapted from[115].

The first SAR study was carried out by isolation of novel analogues from fermenting *Myxococcus sp.* SBCy9270 (Figure 29). The biochemical modifications were carried out at the central amino acid and the two C-terminal PABA elements. Variations at the central amino acid included residues found in cystobactamid 919-1, cystobactamid 919-2 and albicidin[4].

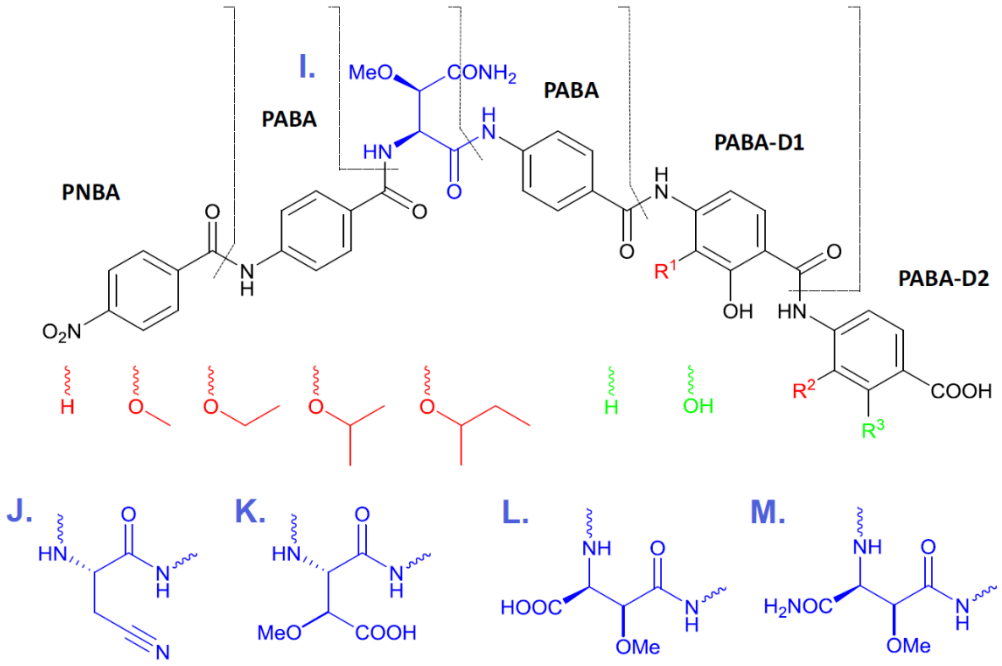


Figure 29: Structural motives in the new cystobactamid derivatives. The motives I, J and M can be found in cystobactamid 919-2, albicidin and cystobactamid 919-1 respectively. Image taken from[4].

A biological testing on a broad variation of bacteria identified cystobactamid 861-2 as most promising derivative for further investigations and optimizations (Figure 30). The total synthesis of cystobactamid 861-2 was carried out[4].

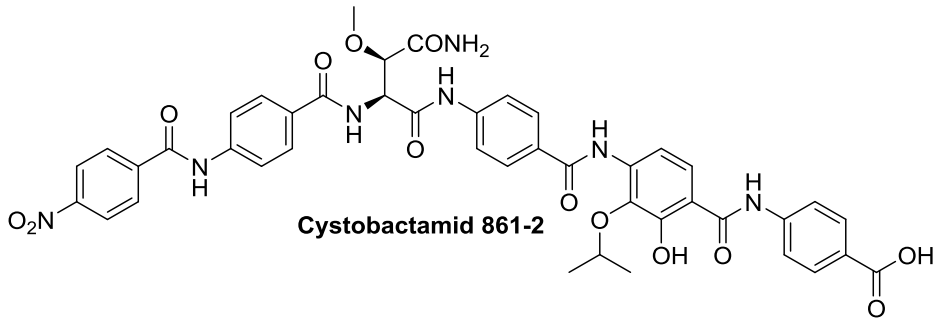


Figure 30: Structure of cystobactamid 861-2. Formula adapted from[4].

1.5.2.3 Chemical synthesis of cystobactamids

The first total synthesis of cystobactamid 919-2 confirmed the stereochemistry of the isolated structure. Like in the synthesis of albicidin, a retrosynthesis with three fragments was used (Figure 31). The reaction makes use of a cyclic anhydride to activate the central amino acid for the amide coupling with the aniline[116].

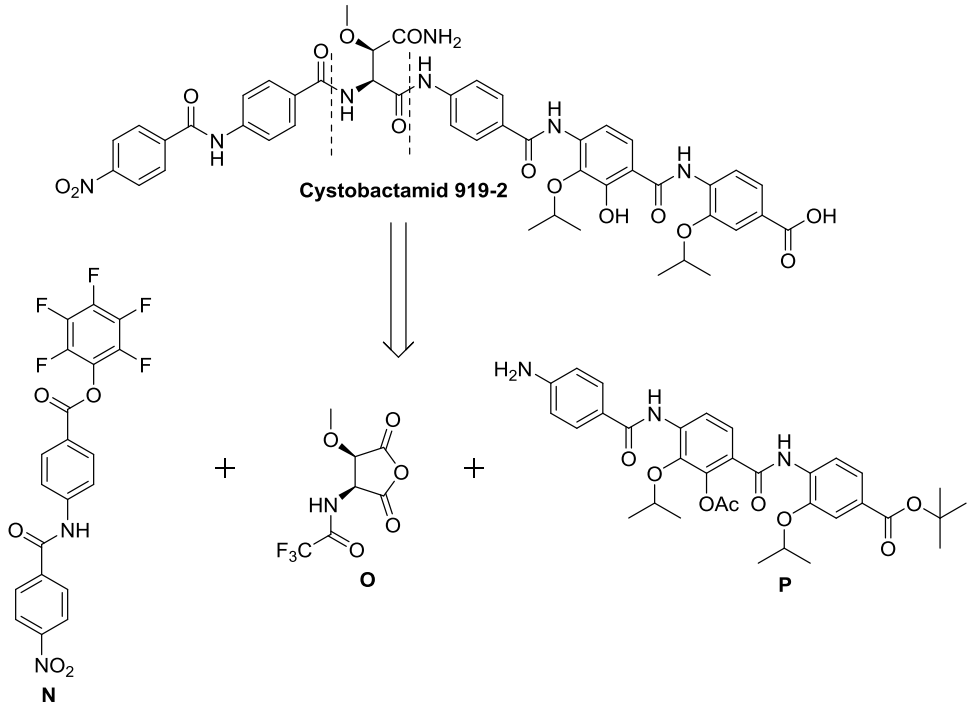


*Figure 31: Retrosynthesis of cystobactamid 919-2 by the three fragments **N**, **O** and **P**. Image adapted from[116].*

The stereoselective synthesis of the central amino acid started with the cyclic sulfite formation from L-tartaric acid ester (Figure 32). By treatment with sodium azide, the desired stereoisomer was formed[117]. The following methylation and reduction led to the desired amino acid. The anhydride **O** was obtained by acid treatment and acylation of the amine by trifluoroacetic anhydride[116].

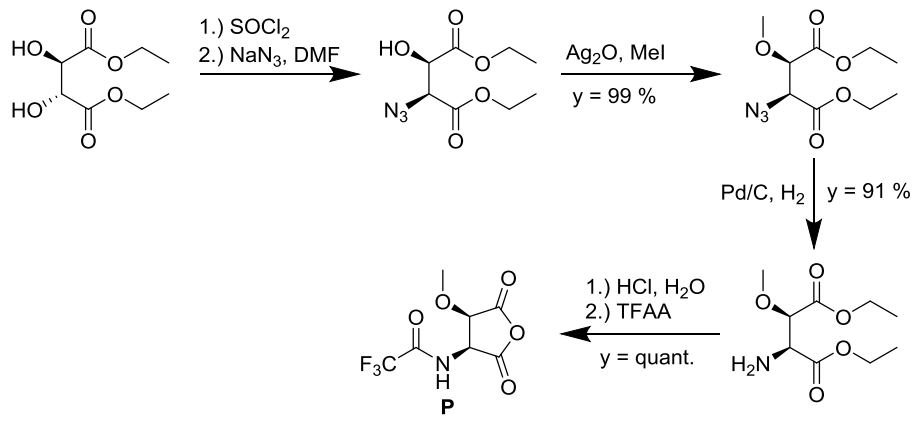


Figure 32: Stereoselective synthesis of the amino acid anhydride. Image adapted from[116].

Unfortunately, the regioselectivity in the coupling is in favor for position 4 (Figure 33). After esterification and aminolysis, the desired intermediate can be obtained. The final product was isolated after amide coupling with **N** and deprotection of the *tert*-butyl ester with TFA[116].

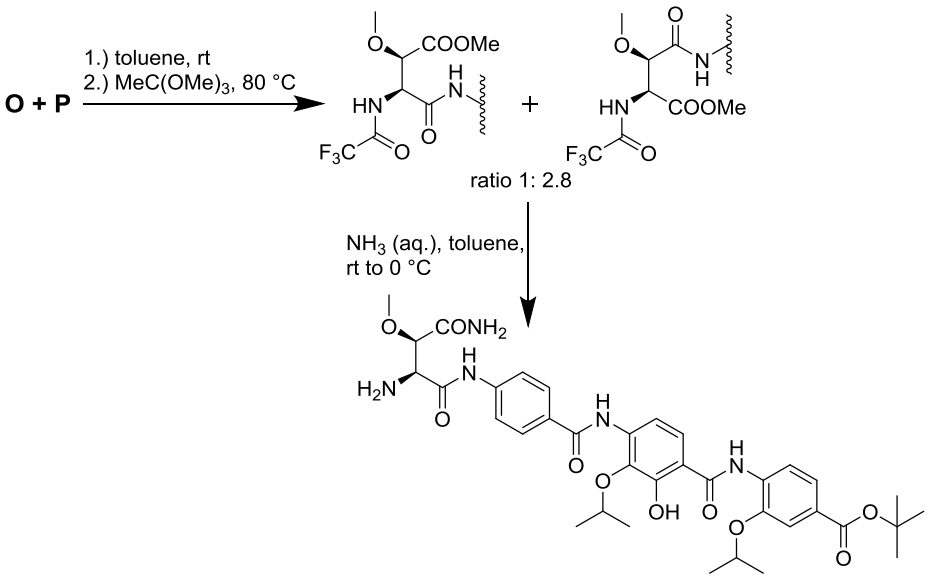


Figure 33: Amide coupling and esterification followed by aminolysis with ammonia. Image adapted from[116].

The synthesis of 861-2 was carried out in a similar fashion but applied a different retrosynthesis with disconnections between the aromatic amides (Figure 34)[4].

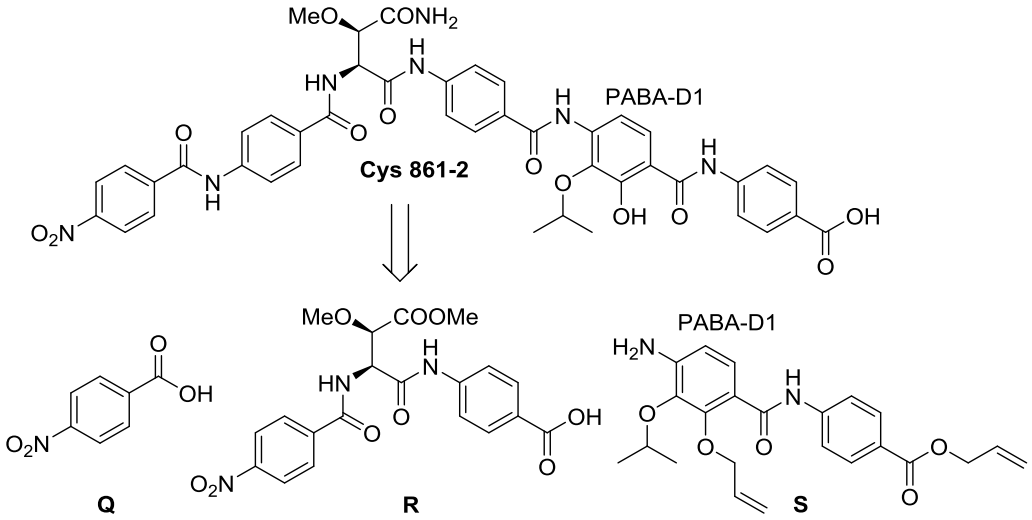


Figure 34: Retrosynthesis of cystobactamid 861-2. Image adapted from[4].

An asymmetric dihydroxylation of methyl cinnamate furnished a similar intermediate to the L-tartaric acid (Figure 35). After the introduction of the azide, the carboxylic acid was obtained by a ruthenium-catalyzed oxidation of the phenyl system. Instead of the anhydride formation, the carboxylic acid was directly used in an amide coupling. The azide was reduced and directly used in the amide coupling as well[4].

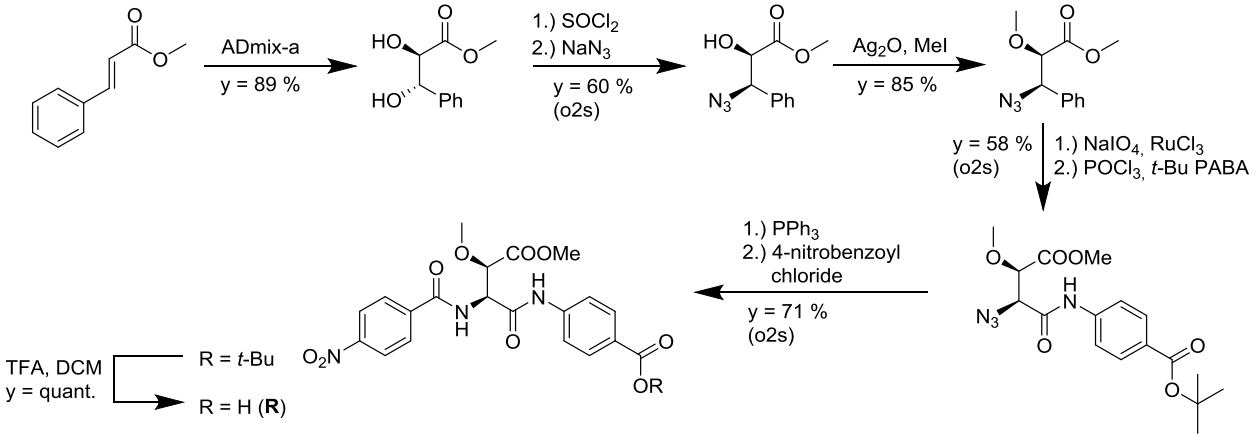


*Figure 35: Synthesis of the central fragment **R**. Image adapted from[4].*

Fragment **S** was connected by amide coupling. After reduction and aminolysis, a coupling with **Q** was carried out. By allyl deprotection cystobactamid 861-2 was obtained[4].

A newer route for the synthesis of the central amino acid was established (Figure 36). Similar to the synthesis of **O**, the diethyl (2S,3R)-2-amino-3-methoxysuccinate intermediate was formed. In the following steps, both esters were hydrolyzed and the acid was neutralized by propylene oxide. The esterification with methanol, catalyzed by *in situ* generation of HCl from acetyl chloride, led to the selective methyl ester formation adjacent to the methoxy group. An amine protection with di-*tert*-butyl dicarbonate and the subsequent conversion of the methyl ester to the amide generated the desired central amino acid. The method allowed for a multigram scale synthesis[182].

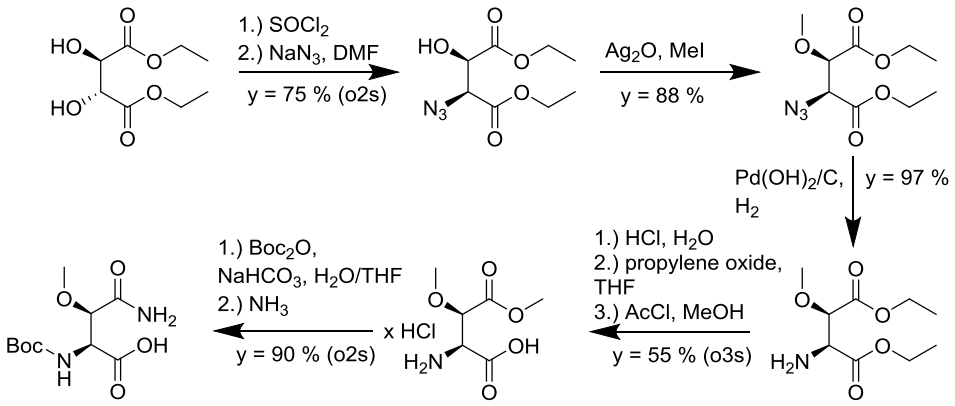


Figure 36: Stereoselective synthesis of the central amino acid. Image adapted from[182].

The synthesis of cystobactamid 919-2 and 861-2 required the tetrasubstituted aromatic system PABA-D1 in **S**. Both syntheses were only of small difference. The synthetic pathway for fragment **S** in cystobactamid 861-2 is shown in Figure 37.

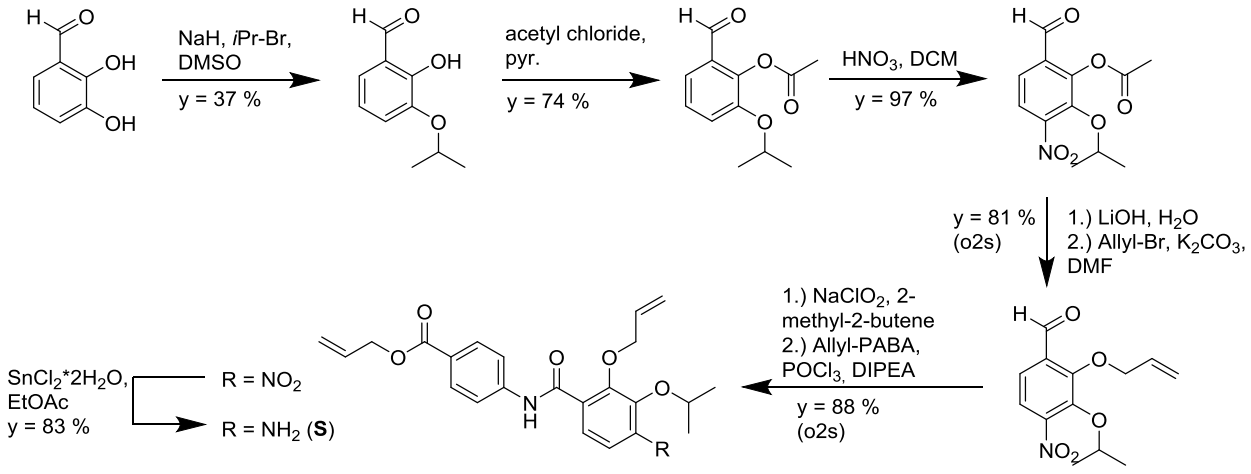


*Figure 37: Synthesis of the tetrasubstituted PABA-D1 element and the subsequent build-up of fragment **S**. Image adapted from[4].*

The synthetic pathway to cystobactamid 861-2 was applied in the synthesis of novel cystobactamid analogues. First chemical modifications were mainly carried out at the N-terminal aromatic system. A simplification at the central amino acid and the substitution of the nitro group for a nitrile yielded the lead compound CN-DM 861 (Figure 38). A SAR was established (Figure 39).

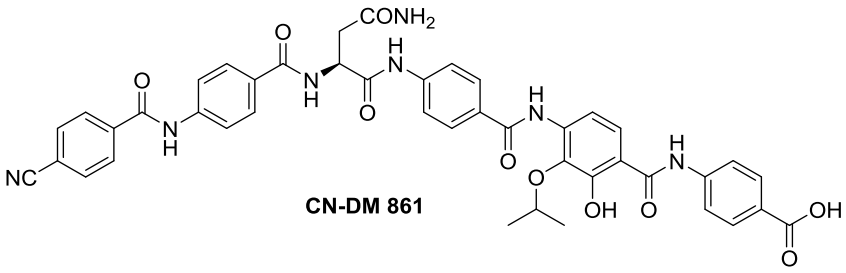


Figure 38: Structure of CN-DM 861. Structure adapted from[5].

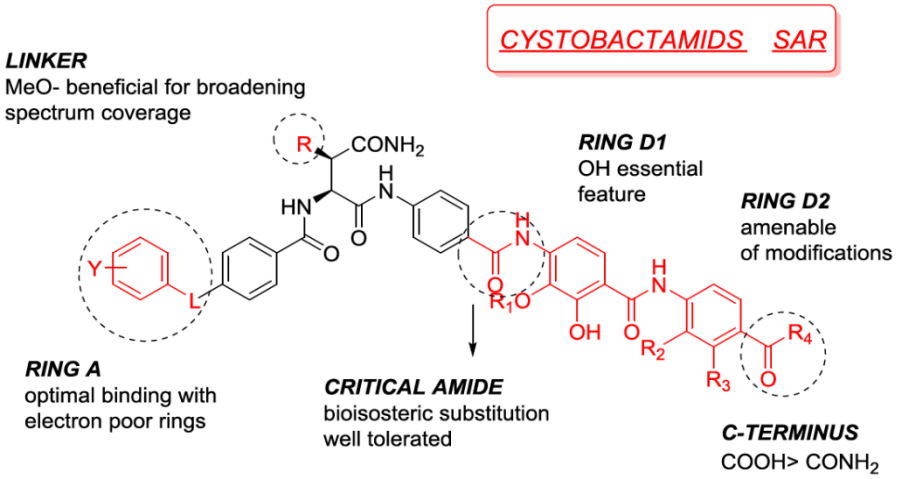


Figure 39: SAR from the Ph.D. thesis of Giambattista Testolin. Image taken from[118].

Like albicidin, AlbA-containing *K. pneumoniae* are significantly less susceptible to cystobactamids. Compared to albicidin, the treatment with cystobactamids did not induce the expression of AlbA and decreased the bacterial growth. A metabolic inactivation by AlbA was not observed[105]. Cystobactamids can be hydrolyzed by the serine endopeptidase AlbD from *Pantoea dispersa*. This hydrolysis can be prevented by the introduction of a triazole bioisostere at the cleaved position (Figure 40). So far AlbD has not been found in clinically relevant bacteria[5].

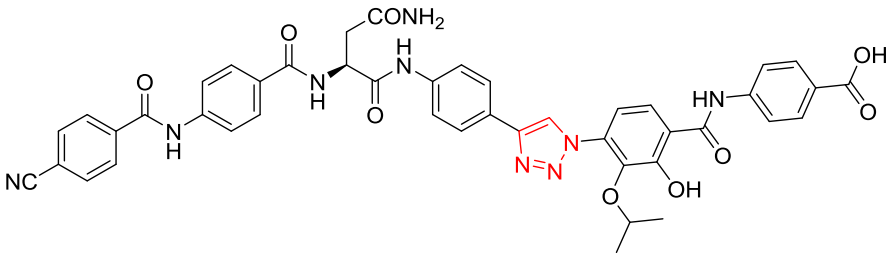


Figure 40: AlbD stable cystobactamid analogue. Structure taken from[118].

1.6 Target identification and photoaffinity probes

The target identification for a given compound usually starts with the so-called target deconvolution. This process allows to narrow down the number of targets. For the full confirmation, a validation of the target is required by applying a second method. All deconvolution methods take advantage of the interaction between the compound and the target and either detect altered biochemical processes as result of the binding or the binding itself. The former can be determined by changes in genetic expression, protein expression and results thereof[119]. Mutations, knockouts or the suppression of the gene expression by siRNA can help to understand the influenced biochemical pathways[120]. Alterations in a biochemical pathways can lead to detectable metabolites that are not found or of fewer quantity in the wild type in absence of the drug[121],[122].

The affinity chromatography is the most commonly used deconvolution method that can detect the binding of a compound to its target. The workflow can be seen in Figure 41. In the first step, the cell lysate of the investigated cell is incubated with the compound of interest. Upon binding of the compound to the target, the complex can be isolated. This isolation requires the attachment of the ligand to a solid support or a tag, like biotin, that interacts with a solid supported protein[123]. The biotinylated compound, with the attached target, binds to avidin or streptavidin with very high affinity and selectivity. Proteins that are not bound to the biotinylated ligand or the solid support can be removed in a washing step[124].

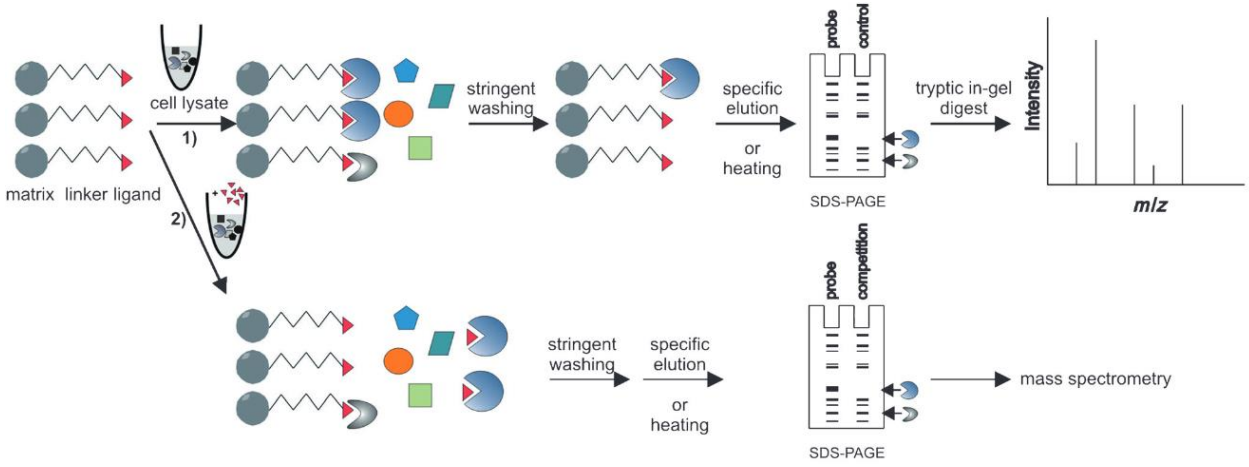


Figure 41: Principle of an affinity-based target fishing approach with (2) or without (1) a competitor. Image taken from[128].

To analyze the protein-compound complex of biotinylated ligands, the strong interaction of avidin or streptavidin with the biotinylated ligand has to be broken under harsh conditions. Instead of the solid support in the affinity chromatography streptavidin-coated beads can be used for the purification of the target-compound complex. This allows for the denaturation and release of streptavidin from the target-compound complex by heating the beads in 1% sodium dodecyl sulfate (SDS) solution[125]. A separation of the isolated proteins can be carried out by SDS-PAGE[126]. The analysis of the protein is performed by mass spectroscopy after the digestion of the protein[127].

Photoaffinity probes are analogues of the investigated compound that can covalently interact with the target upon photoactivation[129]. Those compounds can either be used on their own or in combination with a tag that allows for the isolation of the compound-target adduct[130]. The most frequently used photoaffinity probes contain azides, diaziridines and benzophenones (Figure 42). Upon irradiation with light of short wavelength, those groups form highly reactive nitrenes, carbenes or diradicals[131].

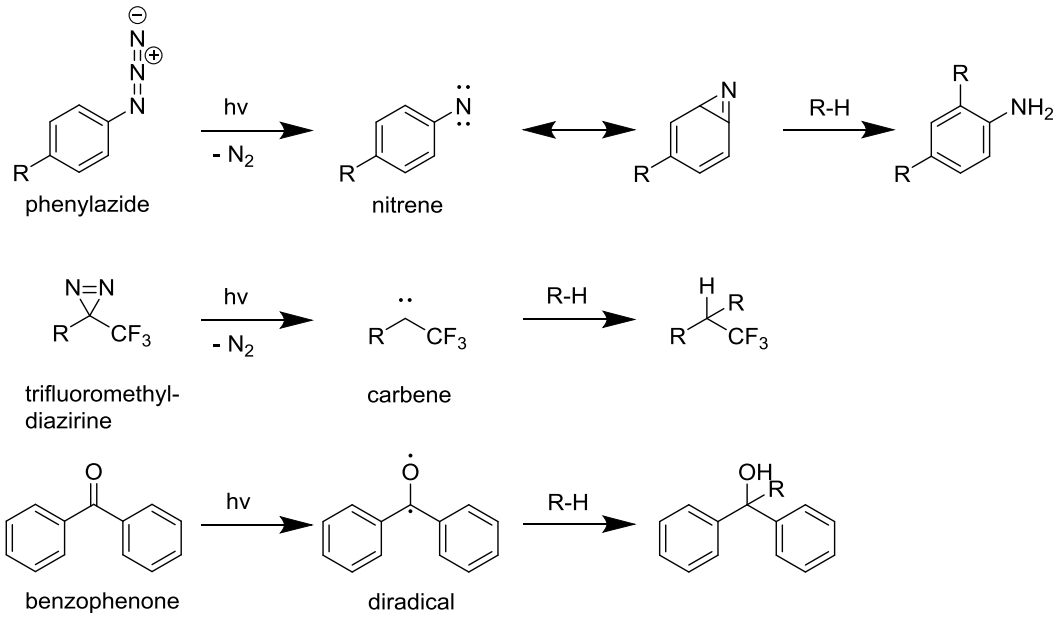


Figure 42: Examples for reaction of photoaffinity groups. Image adapted from[132].

A digestion of the compound-target adducts followed by mass spectroscopy gives insights into the binding site. The identification of the digested compound-target fragments can be facilitated by the addition of stable isotopes, tags or fluorescence groups[133].

The combination of a photoaffinity probe and a tagged compound can allow the simultaneous identification of targets and off-targets as well as the identification of the binding site. A crucial requirement for the design of a photoaffinity or a tagged probe is the tolerance of the pharmacophore for the introduced functional group[134]. If the probe shows no or significantly less activity, it is likely to bind to the target with lower affinity. One possibility to circumvent this is to connect the tag to the probe after the covalent bond was formed (Figure 43)[135].

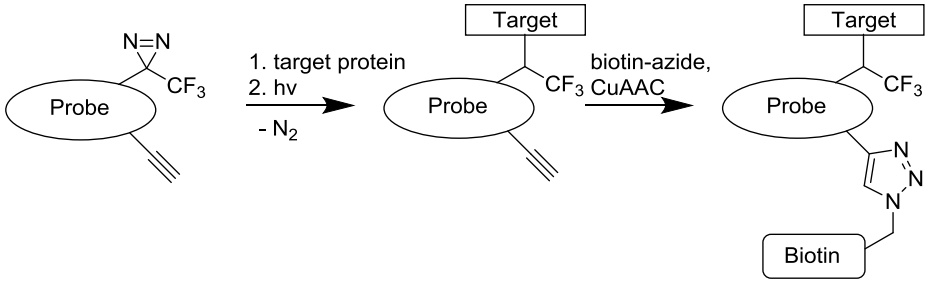


Figure 43: Addition of a biotin tag after photoactivation and reaction with the target. Image adapted from[135].

For the attachment of the tag, a reaction has to be chosen that allows the attachment under physiological conditions and in presence of various electrophiles and nucleophiles. Performable reactions under those conditions are called bioorthogonal reactions[136]. Bioorthogonal reactions include the Staudinger ligation, copper-catalyzed azide-alkyne cycloadditions (CuAAC), strain promoted azide-alkyne cycloadditions and the tetrazine ligation (Figure 44). Due to the toxicity of Cu(I) salts, the CuAAC cannot always be applied[137].

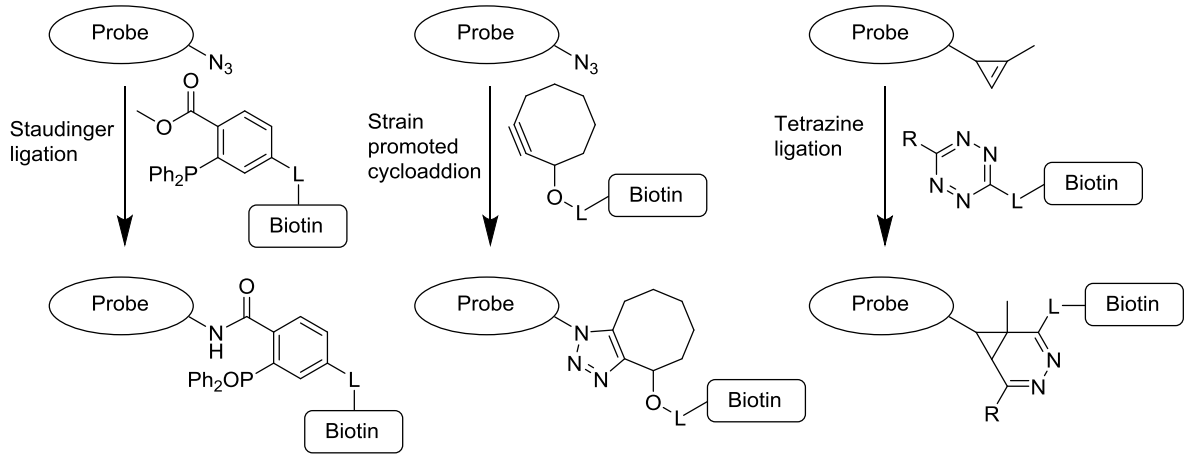


Figure 44: Bioorthogonal reactions. Image adapted from[132].

2 Aim of the thesis

The aim of the thesis is the synthesis and optimization of cystobactamids with not only superior antibiotic activity to the natural products and the lead structure CN-DM 861, but also suitable pharmacokinetic and physicochemical properties. With the main goal to develop a drug against cUTIs (complicated Urinary Tract Infections) the activity against *E. coli*, *Klebsiella* and *Enterococci* strains is mandatory. Additionally, an activity against less common strains in cUTIs like *S. aureus, P. mirabilis* and *P. aeruginosa* amongst others is desired[66]. If the development of a drug against cUTIs cannot be achieved, the development of a highly active drug against *A. baumannii* was set as secondary goal.

To achieve this outcome, a general structure-activity relationship is necessary, as it elucidates the function of the employed functional groups. If known, the functional group can be exchanged for a suitable bioisoster that not only preserves or enhances the activity, but aids towards more druglike compounds.

The first goal was to synthesize ring B derivatives of cystobactamids and continue at the central amino acid and other parts of the structure (Figure 45). By combination of different beneficial structures, a synergetic gain or loss in spectrum and activity can be obtained.

The low solubility and high plasma protein binding of former cystobactamids is an issue that can lead to decreased *in vivo* activity[138]. A rational modification of the cystobactamids to tackle the low solubility and enhance hydrophilicity is necessary.

During the development, more pronounced requirements like a low frequency of resistance (FoR), sufficient metabolic stability and low toxicity were introduced to qualify a compound for *in vivo* tests. To allow for *in vivo* tests and in-depth profiling, a scale-up of the synthesis needed to be established and conducted.

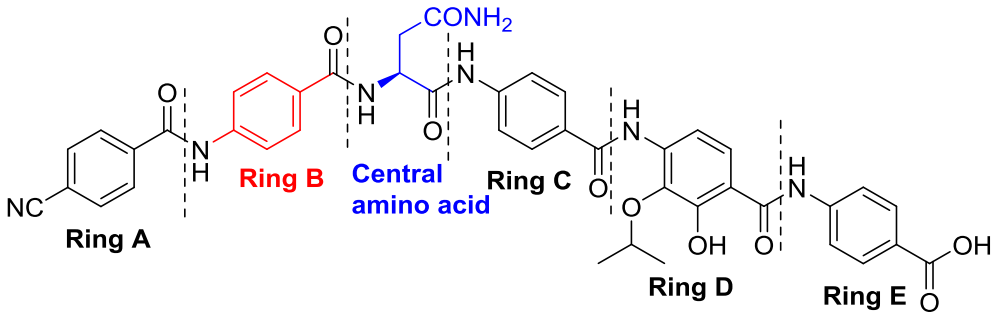


Figure 45: Main positions for the synthetic modification of the lead structure.

To provide novelty and exploit the chemical space, the introduction of yet unused structures is desired. A deviation from the structurally similar albicidin allows for patentability without interfering with the development of albicidin analogues.

3 Concept

3.1 Design considerations

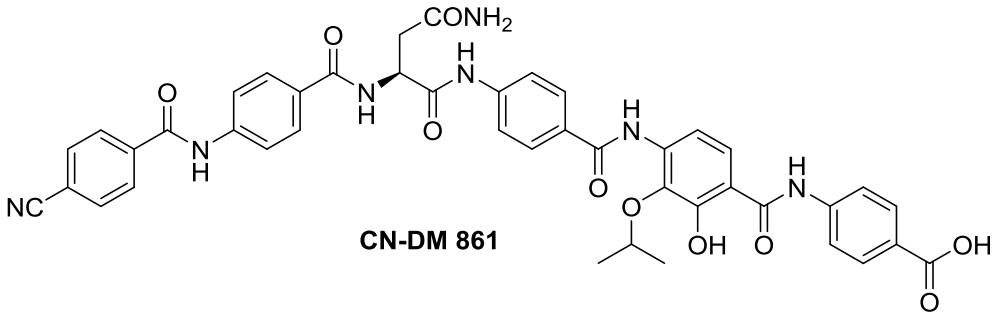


Figure 46: Structure of the lead structure CN-DM 861. Structure adapted from[5].

With CN-DM 861 as lead structure, a basic framework for further modifications was provided (Figure 46). New derivatives were generated in a ligand-based drug design. Once enough data about the bioactive conformation and the target will be generated, a computer-aided or a structure-based optimization can be carried out. In the following, the overall utility of common drug optimization strategies was evaluated for the design of novel cystobactamid analogues.

3.1.1 Simplification

A simplification of a given structure directly reduces the synthetic effort necessary to produce the desired compound. Additionally, different "rules of the thumb" include the molecular weight or related criteria to estimate pharmacokinetic parameters[139],[140],[141],[142]. A high molecular weight is generally associated with undesirable properties[143],[144],[145]. Unfortunately, cystobactamids only allow for very small simplifications. The methoxy residue at the central amino acid in Cys 861-2 is not required. This simplification in combination with the substitution of the N-terminal nitro for a cyano group led to the lead structure CN-DM 861 (Figure 47). Simplifications on other parts of the molecule were not tolerated. Leaving out ring A or E as terminal ring systems was known to lead to a complete loss of activity. Additionally, the elimination of the terminal nitrile or modifications at the terminal carboxylic acid resulted in a loss of activity[5]. From the information given, it was concluded that the terminal carboxylic acid and the cyano group of the lead structure CN-DM 861 were groups of crucial importance for the pharmacophore[118]. Therefore, the simplification strategy was not applied in the design for new cystobactamid analogues.

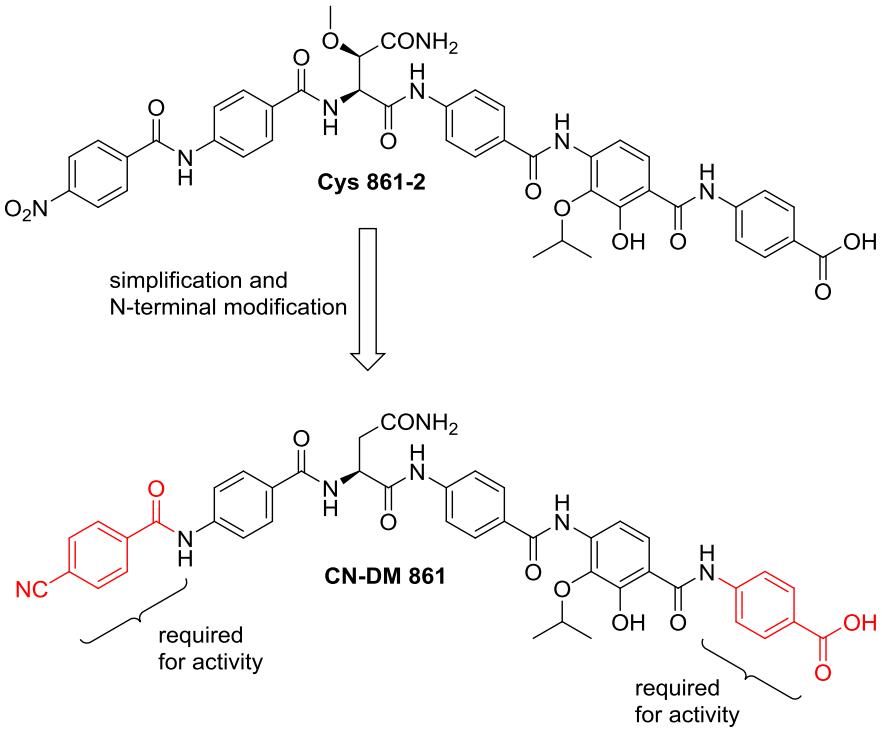


Figure 47: Optimization of Cys 861-2 to CN-DM 861 with the required terminal systems (red). Adapted from[118].

If novel binding sites are found that compensate for the omission of another pharmacophore function, the simplification can be a valuable tool to enhance pharmacokinetic properties.

3.1.2 Desolvation

An optimization via desolvation takes advantage of the fact that water molecules at hydrophobic surfaces show higher order and lower entropy than bulk water molecules[146]. Through interaction of two hydrophobic molecules, like the drug and the target, those highly ordered water molecules can easily be displaced due to their weak interaction with the hydrophobic surface[147]. This entropy driven process enhances the binding affinity[148]. On the contrary, strongly bound water molecules are considered to be hard to remove[149]. The desolvation strategy introduces hydrophobic residues at specific regions of the ligand and facilitates an energetically more favourable displacement of water[150].

In case of the cystobactamids, it was assumed that not every amide bond is crucial for binding but capable of interacting with the surrounding water. The introduction of a hydrophobic residue instead of an amide can lead to an increased affinity, since water can be displaced more easily. Nevertheless, the low hydrophilicity of the cystobactamids restricted the extensive use of this attempt. To compensate for the low hydrophilicity, solubilizing residues were intended to be attached to positions that were not involved in binding and can be solvated without a loss of activity. The strategy of desolvation as well as the introduction of solubilizing residues required the previous investigation of the SAR. Such investigations were mainly carried out by the introduction of isosteres and will be mentioned in chapter 3.1.4.

3.1.3 Structure extension and addition of new residues

A structure extension and the addition of new residues can be carried out to enhance and exploit additional interactions with the target. The extension can either be carried out by linking or merging of two different ligands with each other or by adding new residues to a single ligand in a growing manner[151],[152]. Thus, the growing approach is the only approach that does not require an additional ligand[153].

Considering the complex synthesis and the physicochemical properties of cystobactamids, the growing strategy was mainly carried out by smaller residues. This included the addition of various functional groups like halogens, alkanes, amines or ethers. Several different substituted PABA analogues were commercially available and allowed for a fast modification at the aromatic systems. Bigger residues were introduced to investigate the binding pocket of gyrase. Linking and merging were not applicable due to the lack of information about the binding pocket.

3.1.4 Isosteres

Isosteres are molecules or molecular residues with similar features, like size, shape or pK_a, and can be classified into two broad categories: classical and non-classical isosteres[154]. If an isostere shows similar chemical, physical and biological activity to its progenitor, it is called a bioisoster[155]. By introducing isosteres with altered interaction capabilities, the function of the modified position can be determined. The exchange can also eliminate metabolically susceptible groups or enhance the solubility[156].

The cystobactamid framework offers a lot of space for the implementation of isosteres. The five aromatic systems allow an introduction of aromatic and non-aromatic isosteres (Figure 48). The peptide bonds could be exchanged for other rigid or non-rigid isosteres including alkenes, alkynes, triazoles, amidines, trifluoroethylamines to only name a few. Once the functions of the terminal carboxylic acid and the asparagine side chain are known, an exchange for other substituents is possible. The use of isosteres was first limited to positions that are chemically easy to modify. This restricted complex modifications at the central amino acid and ring D at the beginning, as both modifications demanded for a new synthetic route.

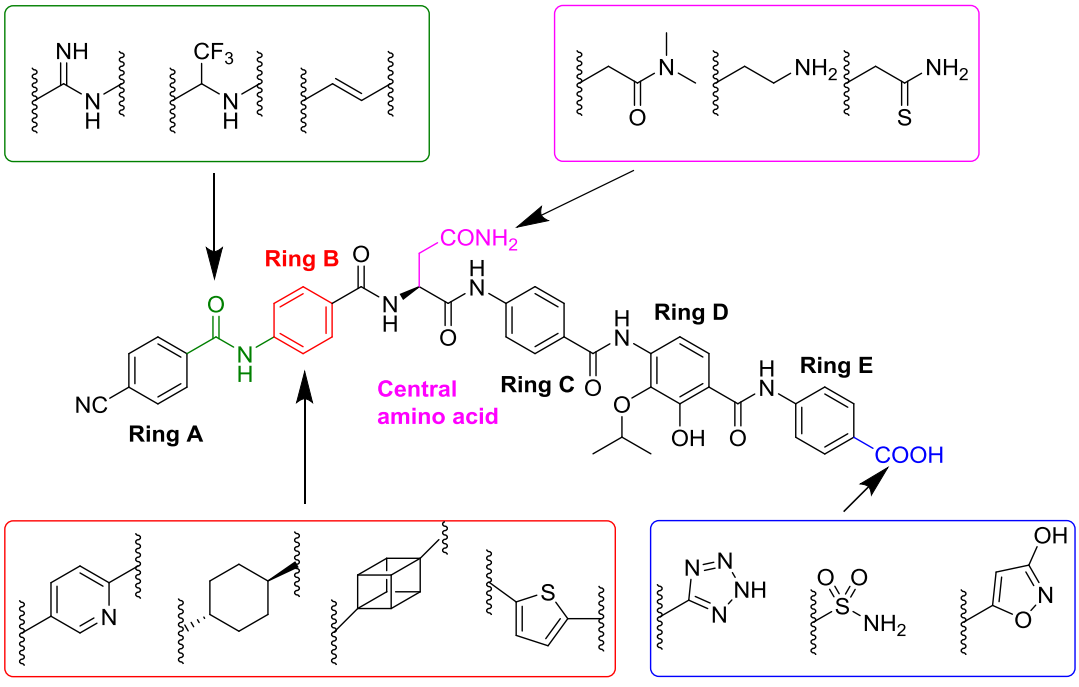


Figure 48: Examples for potential bioisosteres including isosteres for the peptide bonds (green), the aromatic systems (red), the side chain of the central amino acid (pink) and the terminal carboxylic acid (blue).

Some modification at ring A, E and the linker between ring A and B have already been carried out[5]. Additionally, two amide bioisosteres were found at different positions[5]. They will be discussed in the next subchapter.

3.1.5 Rigidification

A prior rigidification of a ligand in its active conformation enhances the binding affinity to a target by decreasing the loss of freedom and entropy during the binding process[157],[147]. Since less conformations can be obtained, the selectivity for the target increases[158]. Additionally, less flexible molecules tend to show higher oral bioavailability[141].

The rigidification of the cystobactamids can be a valuable tool for the determination of their bioactive conformation. The fragment connecting amides can be present as different conformational isomers with the *cis* and the *trans* isomers as the most stable ones[159]. If the amides are indeed *cis* or *trans* configurated, every amide bears two rotatable bonds. A rigidification of these bonds can restrict the number of possible conformers and elucidate the bioactive conformation (Figure 49). The modification of important residues during rigidification can diminish their interaction with the target. This must be considered for the interpretation of the biological data. Rigidifications can also be performed at the central amino acid or by connecting two cycles with each other.

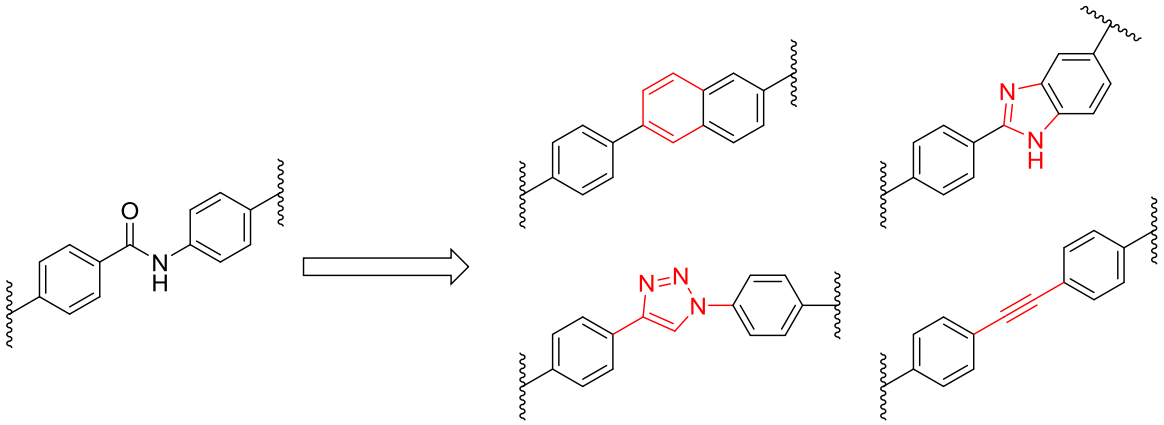


Figure 49: Examples of rigidified amide surrogates (red) resembling a trans-like topology.

Some rigidification were already carried out (Figure 50)[5]. It was shown that the amides between ring A and B as well as between ring C and D are preferably *trans* configurated. The former was concluded by the

high activity of an alkyne at this position and the inactivity of the respective triazole. The amide between ring C and D was substituted by a triazole resembling the *trans* amide. Although showing decreased activity, the triazole at this position was tolerated[5].

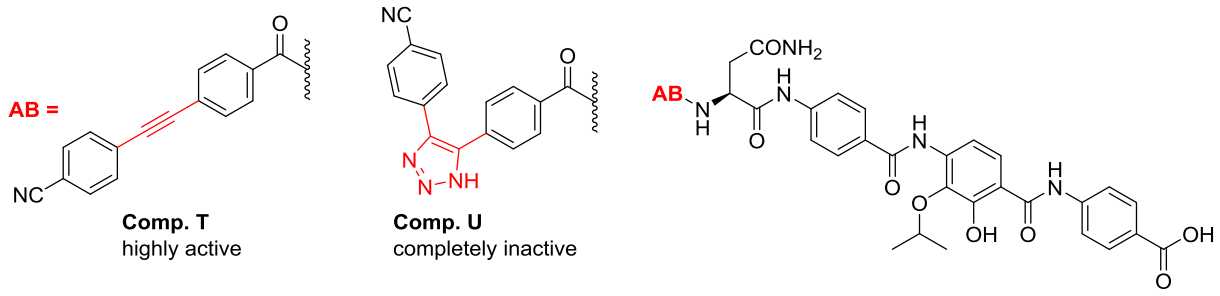


*Figure 50: Structures of the compounds **T** and **U**. Image adapted from[5].*

Another possibility to stabilize the active conformation is the addition of any substituent on a position close to an amide in order to inhibit the free rotation of the adjacent bond. This restriction can either be carried out by sterical hinderance, electrostatic or H-bond interactions. One can assume that the free phenol at ring D interacts with the neighbouring oxygen of the amide in the full length cystobactamid stabilizing a coplanar structure between ring D and the amide[160]. A conformational analysis of the tripeptide fragment supports the thesis, but also an interaction between the phenol with the NH of the amide is possible (Figure 51). NMR studies via NOESY observed both conformations at elevated temperatures of 37 °C[161].

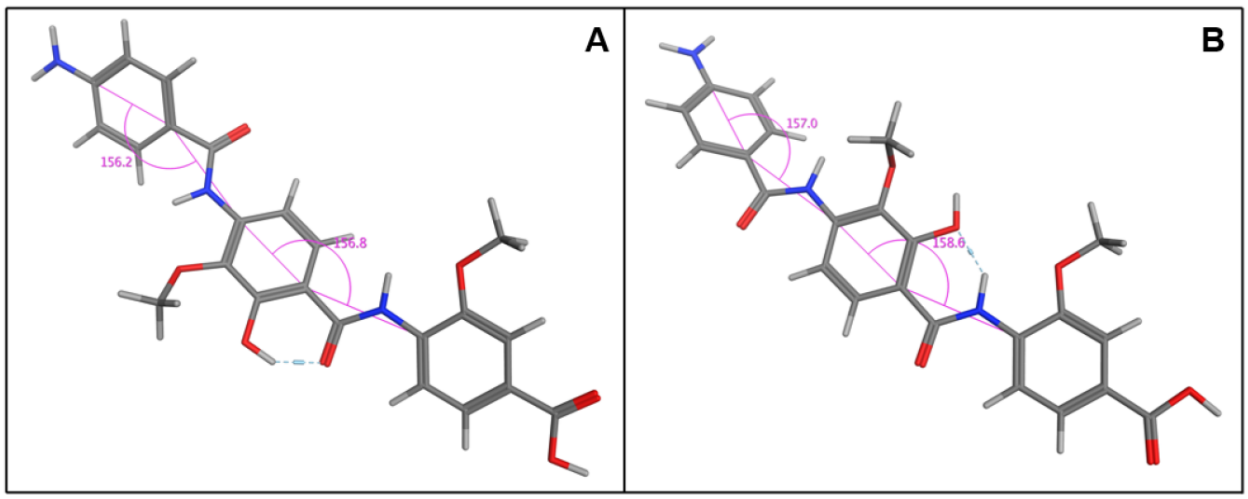


Figure 51: Conformational analysis of the tripeptide fragment and the interaction of the phenol with the adjacent amide. Conformer B can only be found at elevated temperature (predicted ΔE = 0.5 kcal/mol). Image taken from[161].

Although the rigidification of the cystobactamids can be a tempting method to enhance activity, one of its advantages, the increase in selectivity, can also be its disadvantage. Cystobactamids are inhibitors of gyrase and topoisomerase IV[3]. A rigidification of the molecule might stabilize the bioactive conformation for gyrase while abolishing the possibility to adopt the bioactive conformation for topoisomerase IV and vice versa. Additionally, alterations in the peptide sequence of gyrase between different bacteria are known. While the conserved catalytic region of GyrA is mostly retained, the GyrA protein of *S. aureus* exhibits only 50 % identity with GyrA of *E. coli*[162]. Therefore, any kind of modifications might lead to a "niche activity" against one specific bacterial strain. Nevertheless, the rigidification of different bonds is of high importance for the elucidation of the SAR.

3.1.6 Disruption of the crystal packing

To enhance the solubility of a given structure, two general attempts can be applied. The first one attaches a hydrophilic group that helps to solubilize the drug[163],[164],[165],[166]. The second one decreases the inter- and intramolecular interactions within the crystal structure. This disruption of the crystal packing reduces the required energy to remove a molecule from its crystal during the dissolution process[167]. In contrast to the addition of solubilizing groups, the disruption of the crystal lattice does not necessarily lead to more hydrophilic compounds, if one considers the logP as metric[168].

The cystobactamid scaffold offers several positions that can be optimized to disrupt the crystal structure. The exchange of any of the five aromatic systems for saturated systems, like cubane, cyclohexyl or bicyclic isosteres, decreases the possibility for intermolecular π-π stacking. If tolerated at one position, the modification can be carried out at other positions as well. Considering intramolecular hydrogen bonds, targeting the amide between ring D and E can have a very high influence on solubility. As mentioned in chapter 3.1.5, studies have observed two preferred conformers with a coplanar structure. Both conformers are stabilized via an intramolecular hydrogen bond, which further decreases the solubility of the cystobactamid. A deprotonation of the phenol can lead to the twist of the amide and its interaction with the amide NH (Figure 52).

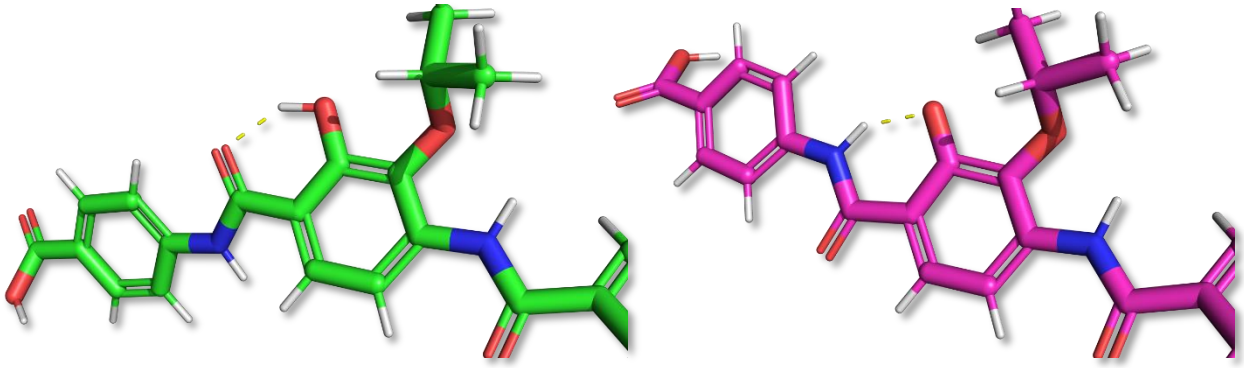


Figure 52: Intramolecular hydrogen bonding between the protonated cystobactamid (green) and the deprotonated cystobactamid (magenta). Red = oxygen, blue = nitrogen, white = hydrogen. Image created by PyMOL[169].

Other potential hydrogen bonds can occur at the central amino acid. Similar to the intramolecular hydrogen bonds within asparagine, the primary amide in the side chain can form hydrogen bonds with the amide backbone of the cystobactamids (Figure 53)[170]. While intramolecular hydrogen bonds to six-membered pseudocycles occur often, five-membered and especially seven-membered pseudocycles are less common[160].

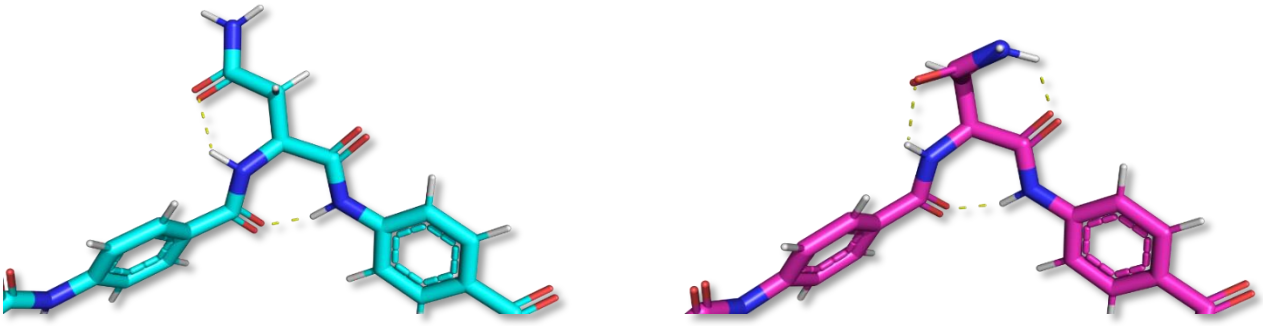


Figure 53: Examples for hydrogen bonds at the central amino acid. Red = oxygen, blue = nitrogen, white = hydrogen. Image created by PyMOL[169].

A disruption of the intramolecular hydrogen bonds can be carried out by alkylation of the amides or the exchange of the amide for bioisosteres. Other possibilities include the introduction of a different linker system. By the elimination of any intramolecular hydrogen bond, the molecule becomes more flexible. It cannot be ruled out that the intramolecular hydrogen bonds are of crucial importance for the stabilization of the bioactive conformation.

3.1.7 Avoidance of toxicophores

Toxicophores are functional groups or formations thereof that are generally non-toxic but may lead to highly reactive species during the metabolism[171],[172]. A meta-analysis compared the ratio of toxic metabolites to the abundance of metabolism at the associated functional group. The most abundant biotransformations were oxidations of sp^3, sp^2 and sp carbons, alcohols and aldehydes as well as conjugations. Nevertheless, the most frequent toxic metabolites resulted from the oxidation of quinones and analogues, sp^2 and sp carbons, amines, hydroxylamines and sulfur[173].

Cystobactamids contain several toxicophores. Nitro groups, like in Cys 919-2, are prone to reductions. The nitro radical anion, formed by the first electron transfer, can oxidize DNA. Furthermore, the hydroxylamine can be acetylated, generating a good leaving group that allows for nucleophilic substitutions[174]. The exchange of the nitro group by the cyano group solved this potential issue. A formation of the hydroxylamine is also possible from anilines, in rare cases also from secondary amides[174],[175]. The aromatic system at ring D contains a potential 1,2-benzoquinone imine and 1,2-benzoquinone after dealkylation (Figure 54). Both substituents are important for activity and cannot be left out[118]. Some glucuronidations lead to the toxification of carboxylic acids. An acyl migration and ring opening of the ester can lead to a highly reactive aldehyde. The glucuronic ester can also transacylate other nucleophiles directly[176].

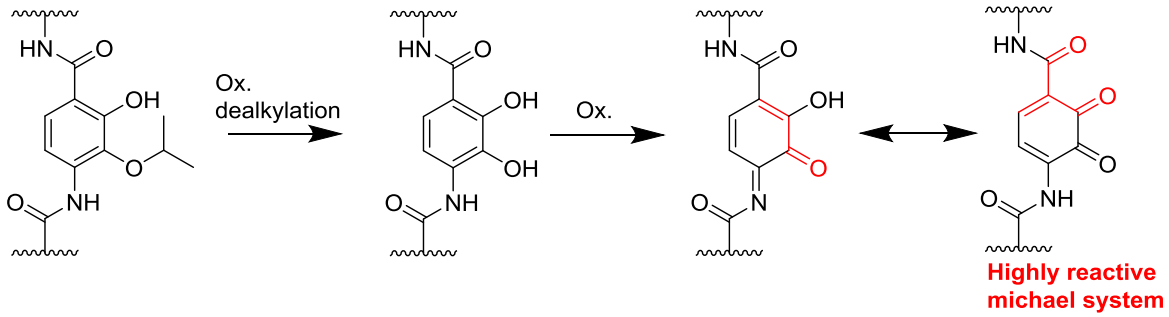


Figure 54: Potential toxification of ring D by oxidation to a quinone or quinone-like system.

In the development of novel cystobactamids, potential toxicophores should be avoided, if possible. Although the inversion of the connecting amides is an easy task to investigate their function, this modification should be restricted to one position. Besides the N-terminal amide, every other position leads to a metabolically and chemically unstable 1,4-dianilide (Figure 55). Therefore, modifications like this should be avoided.

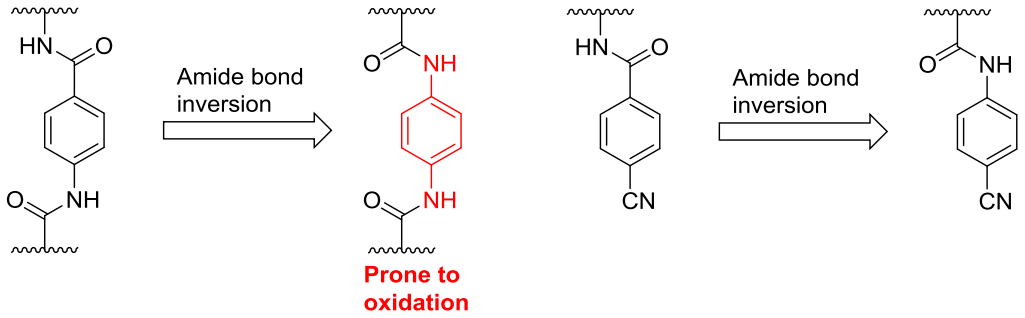


Figure 55: Inversion of an amide bond at a PABA motive to the potential toxicophore (red). A rotation at non-PABA elements might be of lower impact (right).

Phenols should only be introduced in a limited amount and preferably in position two of the PABA systems, to prevent the formation of a quinone imine. The addition of nitro, anilines, thiols, hydrazines, aldehydes as well as other unselective and highly reactive groups must be avoided. Chlorine and fluorine are preferably to be used for the introduction of halogens. The use of unsaturated non-aromatic systems, like alkynes, should be reduced to a minimum, unless for the development of mixed fishing- and photoaffinity probes. If a glucuronidation of the carboxylic acid leads to toxic metabolites, an exchange for a bioisostere must be considered. Essentially, the development of the SAR must not be inhibited by the avoidance of potential toxicophores. If necessary, an analysis of the cystobactamid metabolites can guide the development of less toxic analogues.

4 Results and discussion

4.1 Synthesis of ring B analogues

First modifications were carried out on ring B. The retrosynthesis led to two fragments and a commercially available central amino acid (Figure 56). Analogous to the established synthesis, trityl and Fmoc were chosen as protecting groups for the central amino acid to allow a selective deprotection of the amine after the amide coupling[5]. For the protection of fragment CDE an allyl ether and a *t*-Bu ester were chosen.

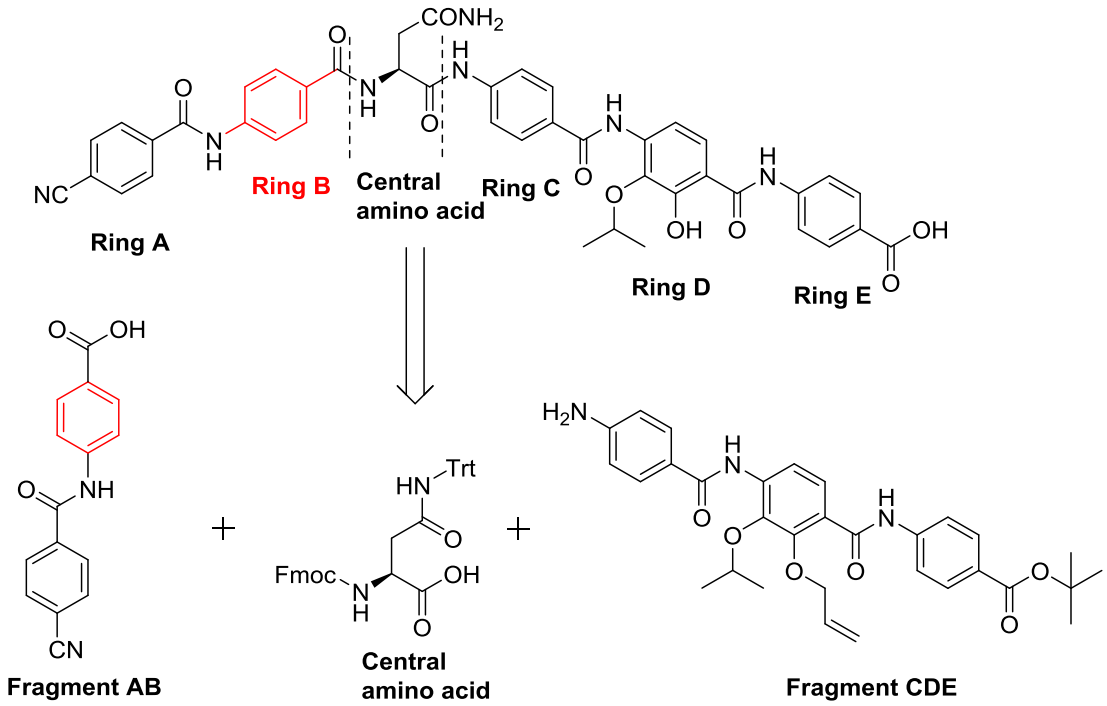


Figure 56: Retrosynthesis of cystobactamids with variable Fragment AB derivatives.

The retrosynthetic scheme was also applied for the synthesis of novel ring A and central amino acid derivatives. The synthesis of ring A analogues was carried out to validate the previously proposed SAR [118].

4.1.1 Fragment AB synthesis

Strategy a) – acid chloride and protected PABA analogues

Starting from the commercially available methyl ester or allyl ester of PABA, an amide coupling was carried out. Compared to aliphatic amines, it was presumed that anilines react rather slowly with activated carboxylic acids. To prevent an incomplete conversion and long reaction times, the carboxylic acid was converted to an acid chloride before the coupling. The amide coupling was carried out in dry dichloromethane (DCM) with triethylamine (TEA) as base[177].

Surprisingly, the use of triethylamine was not suitable for the coupling of electron poor anilines and led to a double acylation as major product (Figure 57). The exchange of triethylamine for the less basic pyridine prevented the side reaction and yielded the desired product.

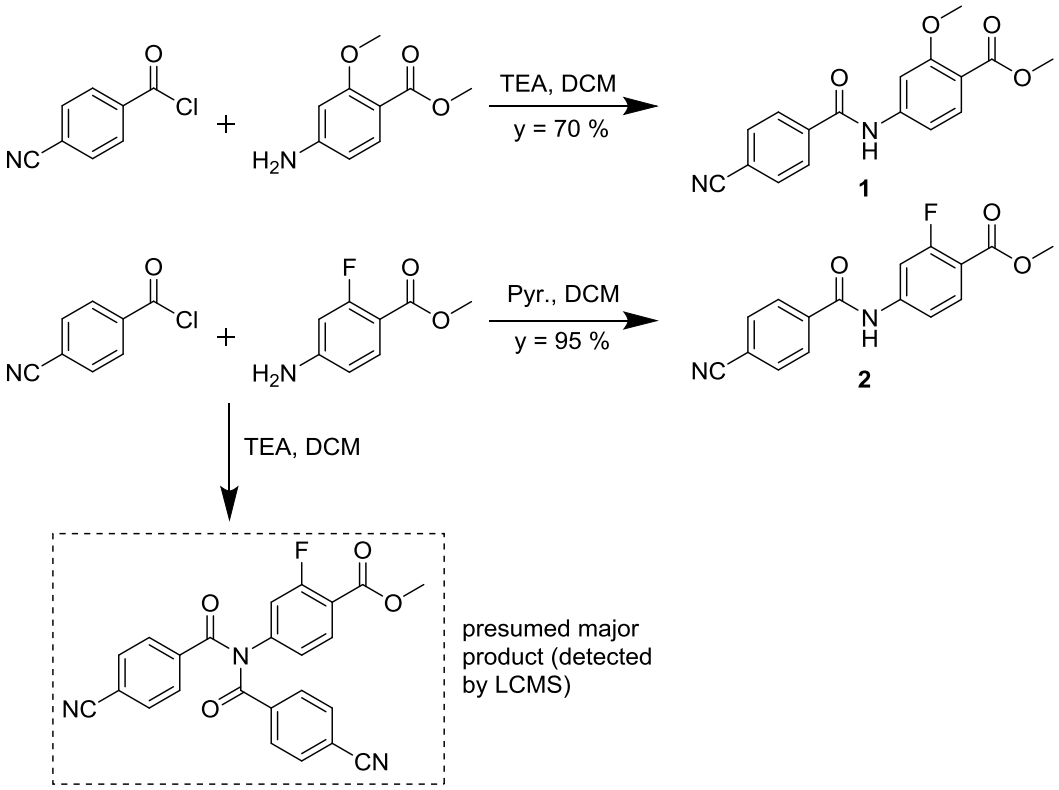


Figure 57: Influence of pyridine and triethylamine on the outcome of the amide coupling for electron poor aromatic systems.

The deprotection of the methyl ester was carried out with LiOH in THF/water in 48 % to 82 % yield[178]. For electron rich ring B derivatives, a deprotection via LiI in refluxing ethyl acetate (EE) or pyridine was used to prevent the hydrolysis of the nitrile (Figure 58)[179],[180].

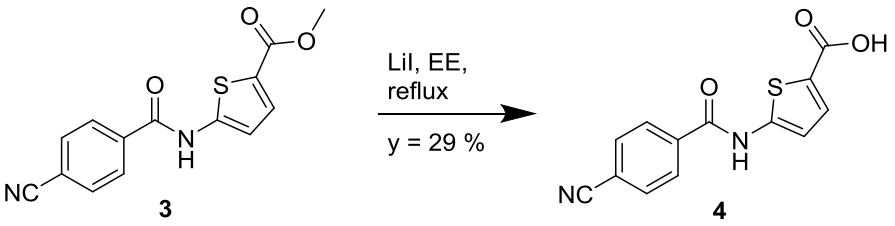


Figure 58: Deprotection of the methyl ester by LiI in refluxing ethyl acetate.

In some cases, the use of trimethyltin hydroxide was superior to LiI[181]. The yields were generally fair with 45 % to 66 %. The deprotection of allyl esters was achieved by tetrakis(triphenylphosphine)palladium(0) with phenylsilane as scavenger in yields between 30 % and 62 % (Figure 59)[4].

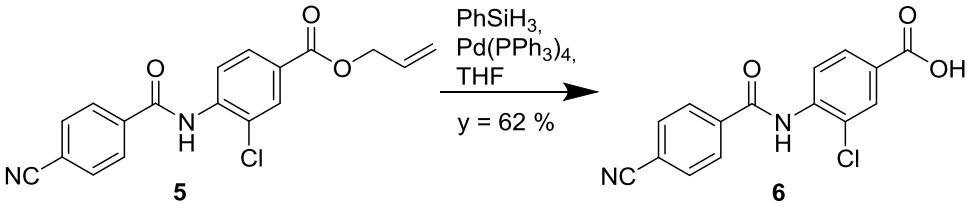


Figure 59: Palladium catalyzed allyl deprotection.

Modified ring A analogues were coupled with the *tert*-butyl protected PABA. The subsequent deprotection with trifluoroacetic acid (TFA) in DCM was quantitative (Figure 60)[182].

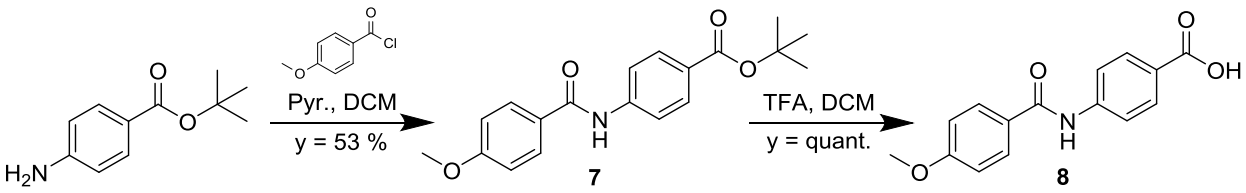


*Figure 60: Synthesis of the AB fragment **8**.*

Strategy b) – Schotten-Baumann

For building blocks that were not available as esters, an amide coupling under Schotten-Baumann conditions was carried out. Analogue to strategy a), the carboxylic acid was converted to an acid chloride beforehand. The coupling was carried out in a biphasic emulsion out of THF and saturated sodium bicarbonate (Figure 61)[183].

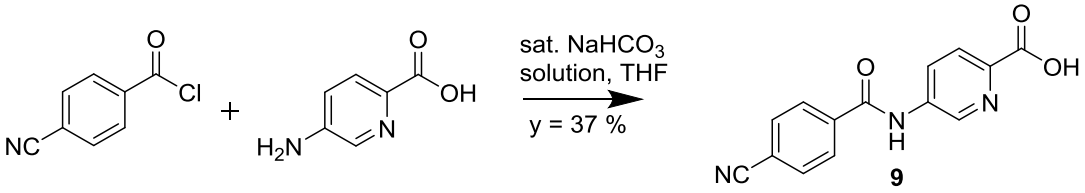


Figure 61: Amide coupling under Schotten-Baumann conditions.

The Schotten-Baumann conditions were restricted to lipophilic aromatic systems and completely failed for highly hydrophilic building blocks. Hydrophilic acid chlorides were immediately quenched in the aqueous phase to the respective carboxylic acid.

Strategy c) – Amide coupling with HATU

An amide coupling via 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) was used, if neither the amide coupling via acid chloride nor the Schotten-Baumann conditions were suitable (Figure 62)[184].

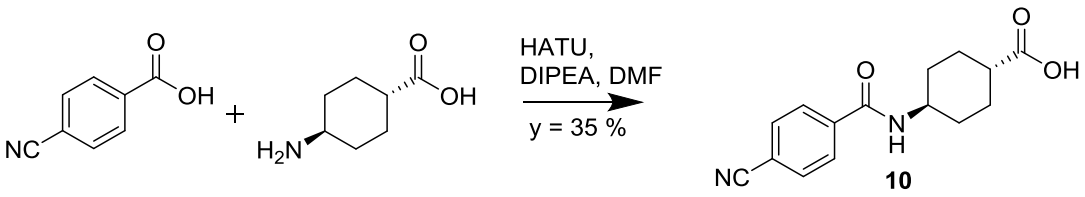


Figure 62: Amide coupling via HATU.

The amide coupling with HATU was generally avoided, because the work-up and the purification of the compound was more tedious than with other amide coupling procedures. Additionally, electron-poor ring B derivatives were not reactive enough for the amide coupling with the HOAt active ester.

4.1.2 Fragment CDE synthesis

The assembly of fragment CDE was carried out following the established synthetic route[118]. In the first step, the catechol system was deprotonated twice by an excess of sodium hydride. This allowed for the selective isopropylation of the less acidic phenol in position three in 49 % yield (Figure 63). In the subsequent step the phenol in position two was acetylated to decrease the directing influence of the substituent.

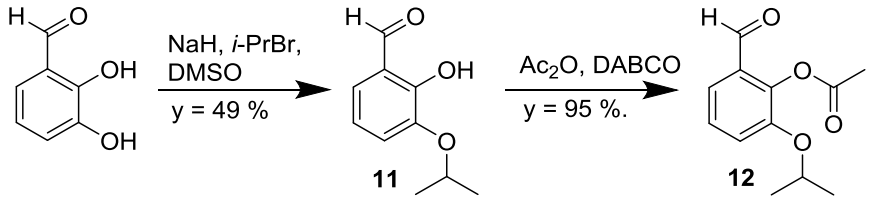


Figure 63: Isopropylation and acetylation of the catechol system.

The nitration led to the desired tetrasubstituted intermediate as major product and the 6-nitro regioisomer at approximately 15 % to 20 % (Figure 64). A chromatographic separation was not possible at this step. After the hydrolysis of the acetate and the subsequent allyl protection, **13** was obtained by chromatography with a yield of 63 % over three steps[118].

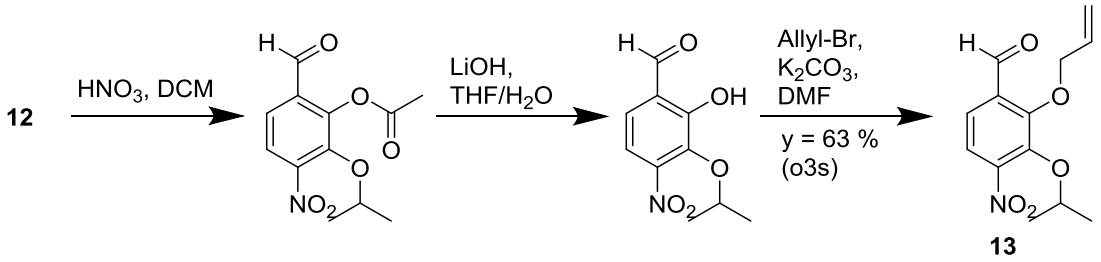


Figure 64: Build-up of the tetrasubstituted ring D system.

A Pinnick oxidation provided the carboxylic acid, which was directly used for the amide coupling with *tert*-butyl protected PABA (Figure 65)[118].

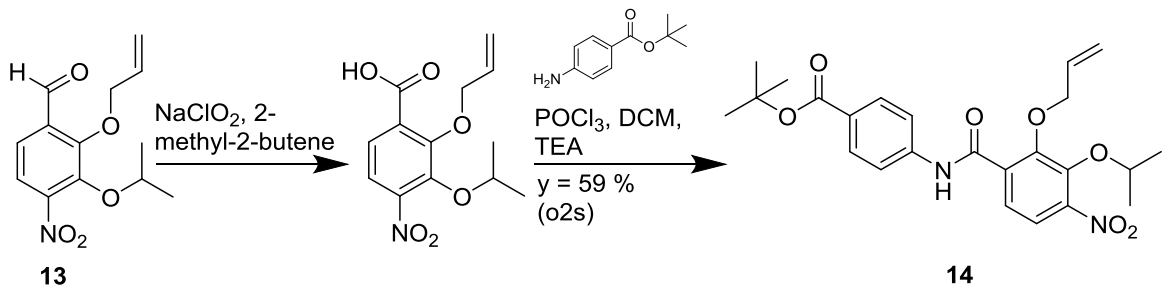


Figure 65: Pinnick oxidation and amide coupling to the DE system.

The reduction of the nitro group was achieved by zinc dust and acetic acid in a THF/ethanol mixture in a yield of 93 % (Figure 66). The aniline **15** was coupled with 4-nitrobenzoic acid in DCM and pyridine followed by the reduction with zinc dust and acetic acid to provide the CDE fragment **16**[182].

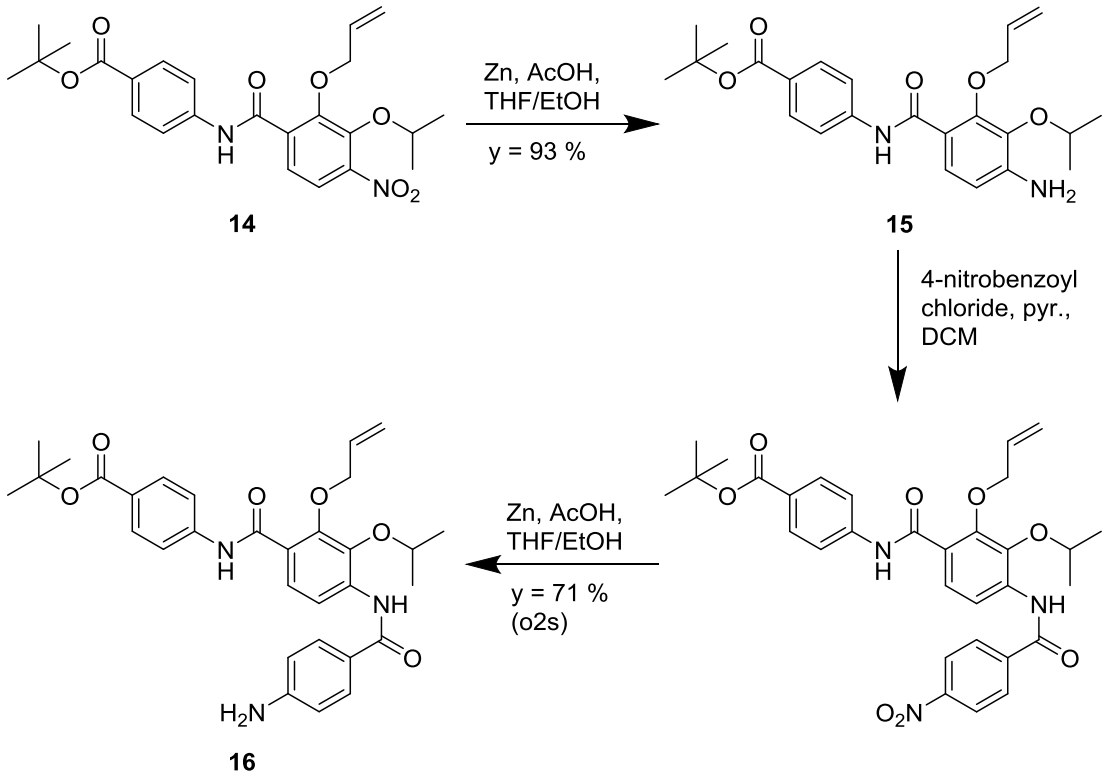


Figure 66: Final steps in the CDE fragment synthesis.

4.1.3 Assembling and global deprotection

The coupling between fragment CDE and the central amino acid was carried out with phosphoryl chloride in DCM. To reduce the risk of racemization, pyridine was used instead of triethylamine (Figure 67). Unfortunately, these conditions resulted in a von Braun amide degradation for the majority of the product. The desired product was gained in a poor yield of 24 %, while the nitrile was obtained at 45 % yield. The amide coupling with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in chloroform led to 69 % yield at minimum risk of racemization[182].

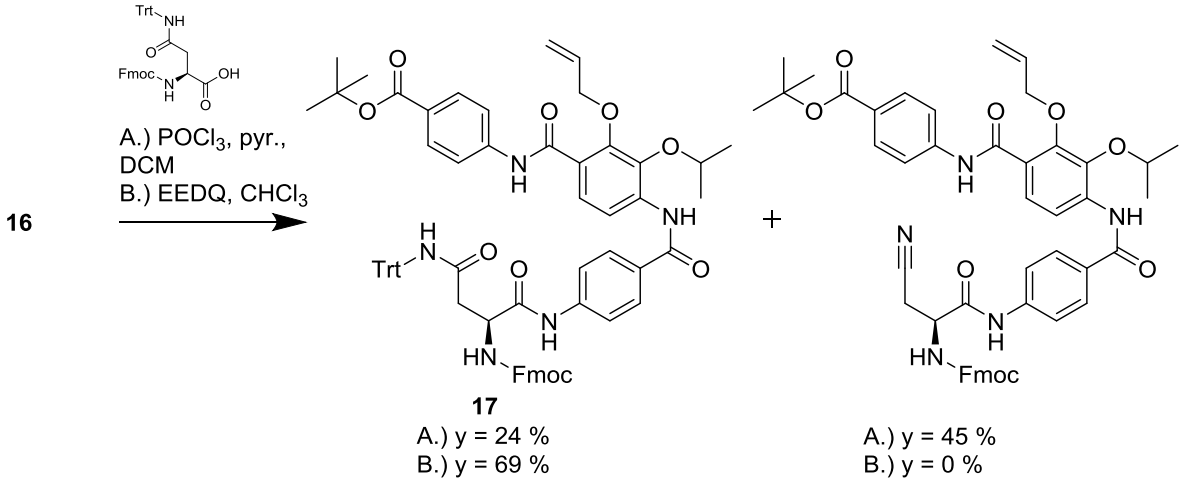


Figure 67: Amide coupling of fragment CDE and the central amino acid with $POCl_3$ or EEDQ.

Fmoc was cleaved off by diethylamine (DEA) in acetonitrile and directly used in the subsequent amide coupling with the respective AB fragment (Figure 68). The allyl deprotection was achieved by tetrakis(triphenylphosphine)palladium(0) and phenylsilane as scavenger[5].

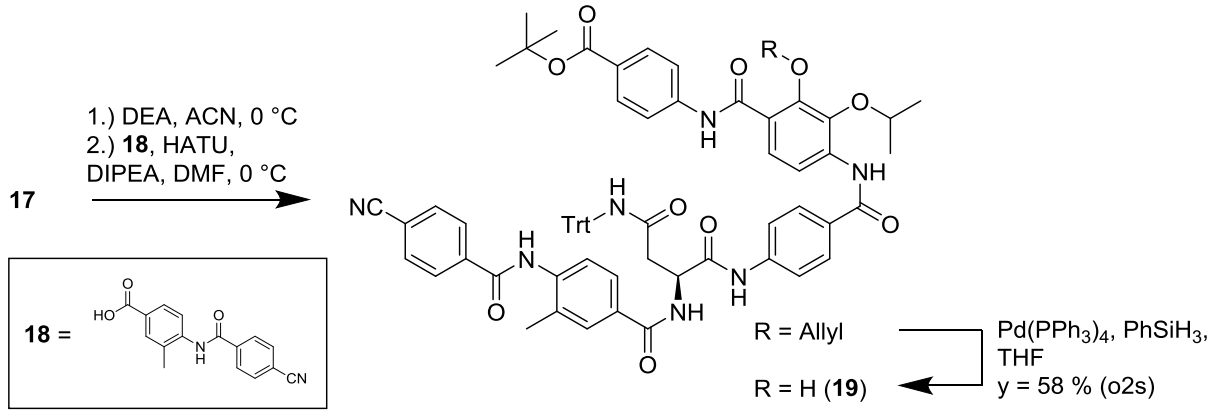


Figure 68: Amide coupling and allyl deprotection.

It was later discovered that those conditions led to the reduction of unsaturated non-aromatic systems. Aniline was applied instead of phenylsilane to circumvent potential reductions[182].

The last deprotection cleaved of the trityl and the *tert*-butyl group simultaneously (Figure 69). The scavenger triisopropyl silane (TIPS) was used[5].

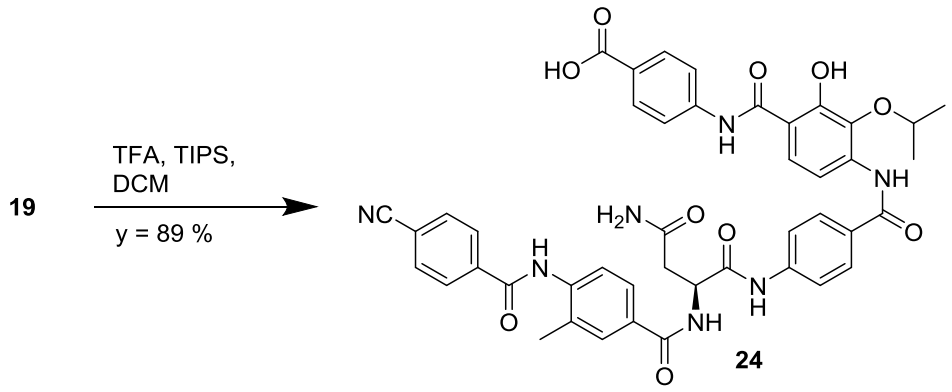


*Figure 69: Last deprotection step to the final compound **24**.*

The final purification was carried out by basic RP HPLC with a 10 mM ammonium bicarbonate solution and acetonitrile. Through lyophilization the excess of ammonium bicarbonate decomposed to water, ammonia and carbon dioxide. An extended drying process was able to remove the ammonia from the product salt and yielded the free phenol and the free carboxylic acid, which was proven by ^{1}H-NMR.

4.2 Ring B derivatives

4.2.1 First generation of ring B derivatives

The first target molecules were chosen based on commercially available building blocks with the intention to synthesize a set of molecules with different electronic, steric and structural properties. Similar to the Topliss Scheme, each generation was meant to decide the design of the following generation as the SAR gets elucidated (Figure 70).

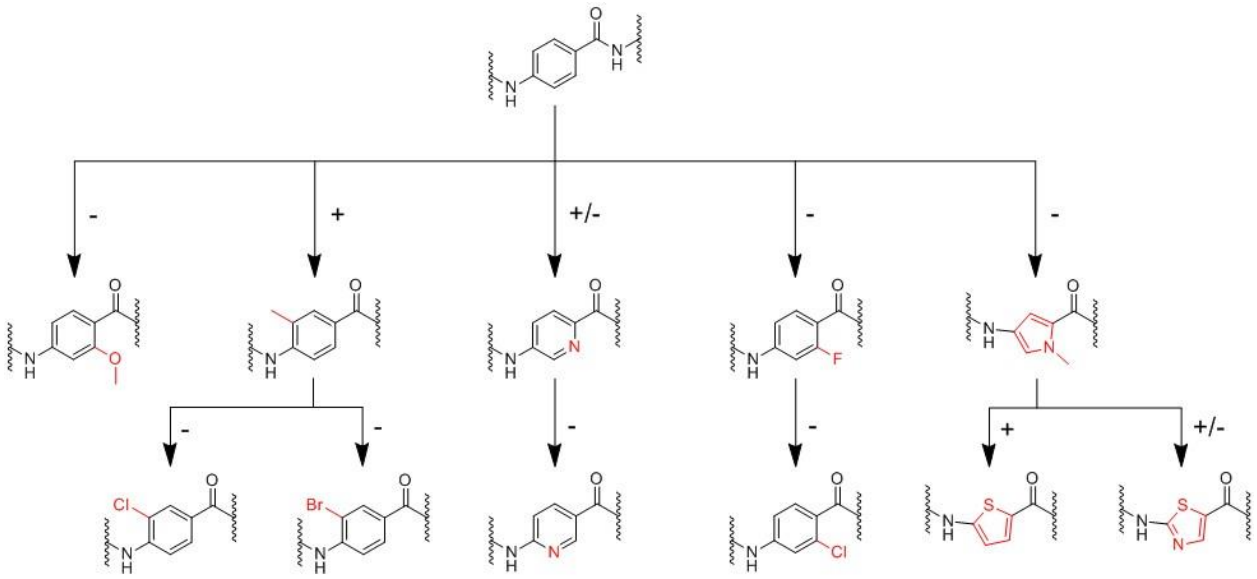
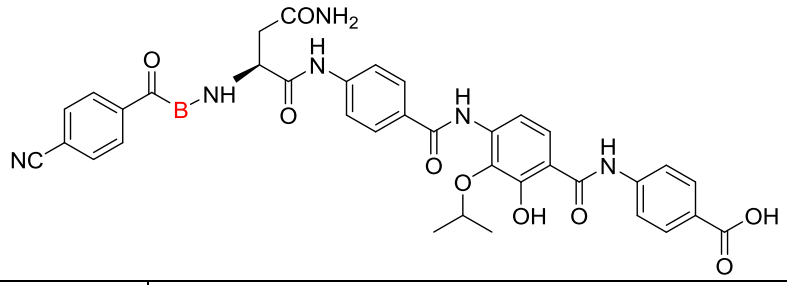
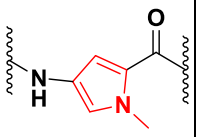
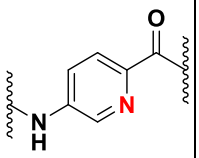
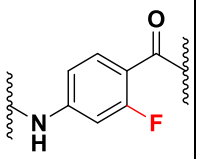
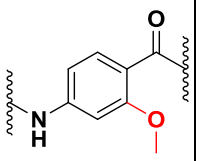
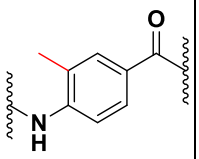


Figure 70: Topliss Scheme for the first and second generation of ring B derivatives. +/- indicate an overall improvement or deterioration with respect to the former generation.

The first generation was solely comprised of aromatic systems. The compounds **23** and **24** were mainly synthesized for their electronic and steric properties and represented electron-rich aromatic systems (Table 1). Their bulky substituent was presumed to hinder the rotation of the adjacent amide bond to some extent. Compound **21** and **22** were chosen as electron deficient systems. The five-membered ring in compound **20** was used to investigate the importance of the ring A orientation, as the pyrrole is no longer capable to connect ring A and the central amino acid in an angle of 180 °. A 3-aminobenzoic acid as ring B was not included and already known to be inactive[118]. The biological activity is depicted in Table 1.

Table 1: MIC and IC_{50} values for the first generation of ring B derivatives compared to CN-DM 861, Cys 861-2 and CIP (ciprofloxacin).



		MIC [µg/mL]					IC_{50} [µM]
Compound	System for **B**	*E. coli* WT (DSM1116)	*E. coli* ΔTolC	*S. aureus* Newman	*P. aeruginosa* WT (Pa14)	Pa14 Δ*mexA B*	*E. coli* gyrase
20		32	2	>64	>64	64	n.d.
21		<0.03	<0.03	0.25	4	<0.03	0.09
22		<0.03	<0.03	0.125	>64	<0.03	n.d.
23		32	0.125	>64	>64	8	2.90
24		<0.03	<0.03	0.06	1	<0.03	0.09
CIP		0.013	<0.03	0.01	0.025	0.006	0.18
861-2		2	1	0.125	4	1	n.d.
CN-DM 861		<0.03	<0.03	0.05	1	0.25	0.13

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186].

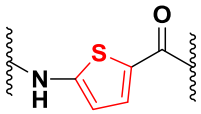
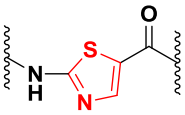
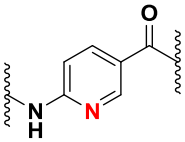
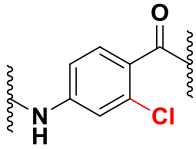
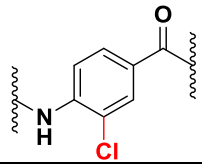
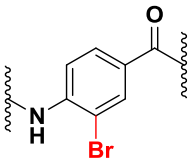
To determine the overall benefit of the applied modification, the lead structure CN-DM 861 was added as reference compound. Compound **24** showed very good activity with higher activity than CN-DM 861 against the MexAB deficient *P. aeruginosa* strain. The picolinic analogue **21** had slightly decreased, but mostly retained activity. Both the picolinic analogue **21** and the 3-Me analogue **24** showed improved IC_{50} values on the *E. coli* gyrase. As the MIC values were not determined at levels below 0.03 µl/ml, the enhanced activity could not be reflected by the MIC values.

The results showed the possibility for a substituent at position three of the aromatic system, while the sterically more demanding methoxy substituent in **23** at position two was not tolerated. Smaller substituents, like the fluorine in **22**, were tolerated, but decreased activity against the *P. aeruginosa* wild type. The five-membered pyrrole analogue **20** was not tolerated. As the properties of the methylated pyrrole differ significantly from the other residues, further analogues were necessary to disqualify the use of five-membered aromatic systems as ring B.

4.2.2 Second generation of ring B derivatives

The 3-methyl analogue **24** was used as starting point for the next generation of ring B analogues. Additionally, the nicotinic acid analogue **27** was added to investigate, whether the nitrogen is also tolerated in position three. Two other five-membered aromatic ring B derivatives were included to allow an interpretation of the decreased activity seen on the methylated pyrrole **20**. The biological activities and the structures of the second generation are shown in Table 2.

Table 2: MIC and IC_{50} values for the second generation of ring B derivatives compared to CN-DM 861, Cys 861-2 and CIP (ciprofloxacin).

		MIC [µg/mL]					IC_{50} [µM]
Compound	System for **B**	*E. coli* WT (DSM1116)	*E. coli* ΔTolC	*S. aureus* Newman	*P. aeruginosa* WT (Pa14)	Pa14Δ *mexAB*	*E. coli* gyrase
25		0.5	<0.03	>64	>64	>64	n.d.
26		>64	<0.03	>64	>64	>64	n.d.
27		0.25	<0.03	>64	>64	0.25	n.d.
28		<0.03	<0.03	0.5	>64	0.5	0.15
29		0.125	<0.03	<0.03	>64	0.06	0.24
30		<0.03	<0.03	0.03	>64	0.25	0.21
CIP		0.05	0.003	0.16	0.1	0.5	0.18
861-2		0.25	<0.03	0.25	1	0.125	n.d.
CN-DM 861		<0.03	<0.03	0.25	4	0.125	0.13

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186].

As demonstrated by the results of **25** and **26**, five-membered aromatic systems were not tolerated. Like the methylated pyrrole analogue, some activity against *E. coli* was retained. Five-membered aromatic systems were therefore discarded. The nicotinic acid analogue **27** was not tolerated. An exchange of the methyl in position three for the chlorine (**29**) and more favorable for the bromine (**30**) were well tolerated but resulted in a lack of activity for the *P. aeruginosa* wild type. A shift of chlorine to position two in **28** led to decreased activity against *S. aureus* and the *P. aeruginosa* mexAB deficient strain and showed the same inactivity against the *P. aeruginosa* wild type as **29**. The methyl derivative **24** was still used for the design of the next generations of ring B derivatives.

4.2.3 Third generation of ring B derivatives

Most of the other ring B derivatives are successors of **24**. Their structures are depicted in Table 3. By swapping the methyl of **24** to position two of the aromatic system, **38** was obtained. The ethyl analogue **42** was synthesized to investigate the tolerance for bigger residues at position three, while **40** was synthesized to investigate whether another methyl was tolerated. An ethinyl group was introduced in **41** to evaluate if position three was suitable for a "click"-reaction, necessary for target fishing experiments. To test a completely electron deficient compound, a tetra fluorinated derivative **39** was included. Compound **36** was originally planned to possess an alkyne instead of the aromatic system but was reduced during the last deprotection steps by phenyl silane.

A *trans*-cyclohexyl ring system was chosen for **37** to resemble the planar structure of an aromatic system without its π-character (Figure 71). With a calculated distance of 3.0 angstrom between the substituted positions, cyclohexane shows only low difference to the phenyl system with 2.8 angstrom[169]. As both substituents are *trans* to each other in the predominantly occurring chair conformation of cyclohexane, the cyclohexane can either bear them in axial or equatorial position (Figure 72)[187]. The latter is energetically preferred[188].

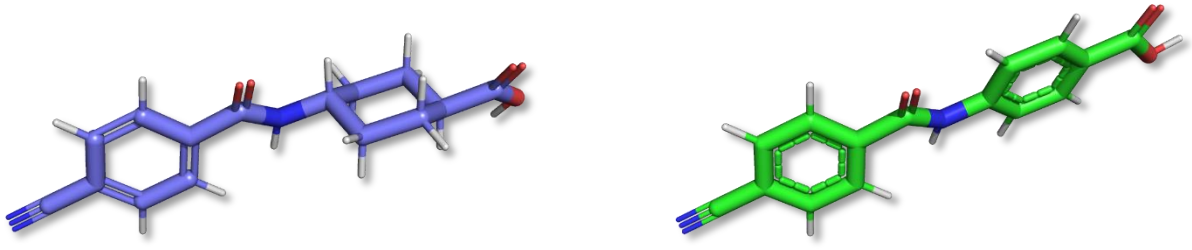


Figure 71: Comparison of the trans-cyclohexyl fragment AB (blue) and the unaltered AB fragment (green). Red = oxygen, dark blue = nitrogen, white = hydrogen. Image created by overlay of both fragments in PyMOL[169].

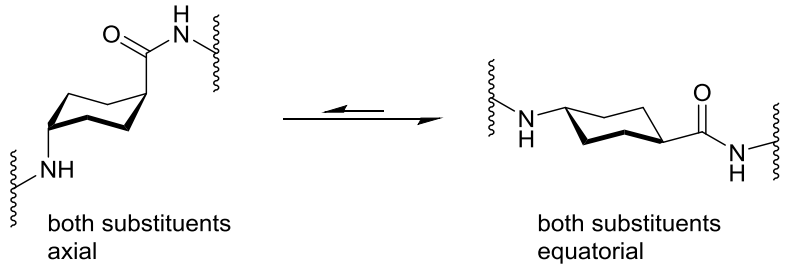


Figure 72: Ring flip of the axial to the energetically favored equatorial substituted cyclohexane.

The 3-ethyl PABA building block was commercially not available and had to be synthesized by hydrolysis of the respective nitrile (Figure 73). The crude product was directly used in a Schotten-Baumann amide coupling. The overall yield was poor with 21 % over two steps.

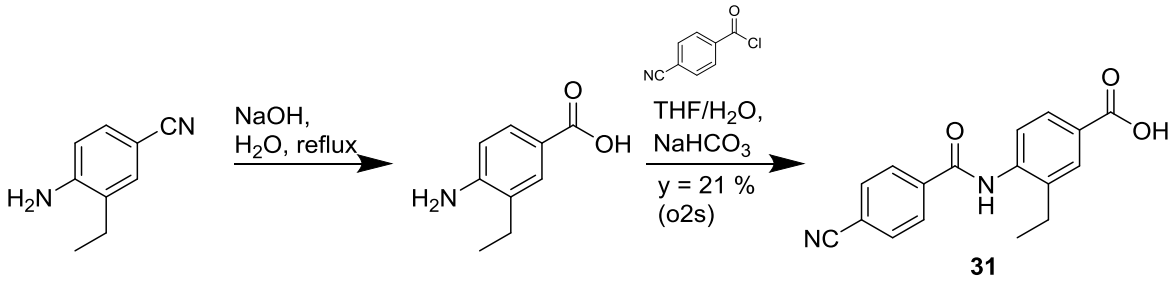


Figure 73: Synthesis of the 3-ethyl fragment AB.

An 3-ethinyl PABA element **32** was synthesized by a Sonogashira cross coupling and deprotection in 38 % yield (Figure 74)[189]. The desired fragment AB **33** was obtained after amide coupling and hydrolysis at standard conditions.

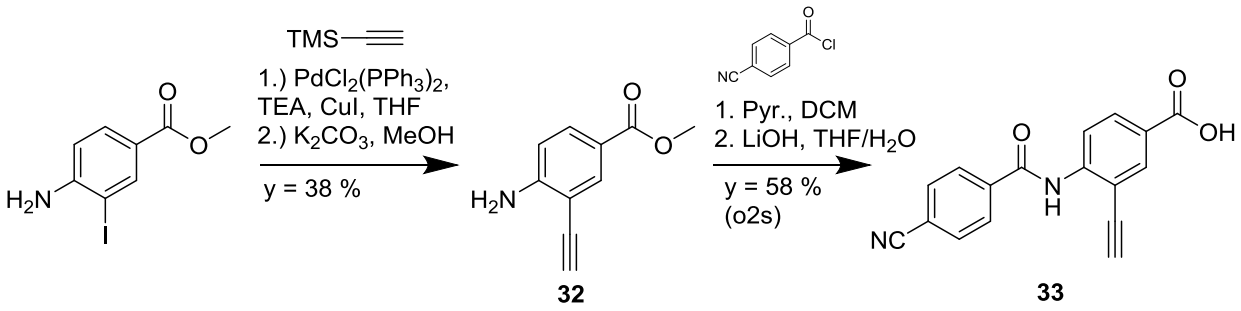


Figure 74: Synthesis of the 3-ethinyl fragment AB.

For the introduction of an alkyne instead of the aromatic system, a carboxylation of N-Boc propargylamine was carried out. A subsequent Boc deprotection provided the carboxylated propargylamine. This synthetic pathway was discarded due to the high water solubility of the products and low yields.

A shorter synthetic pathway was applied (Figure 75). Propargylamine was coupled to the 4-cyanobenzoylchloride in quantitative yield. The carboxylation of the alkyne was carried out with two equivalents of lithium bis(trimethylsilyl) amide (LiHMDS) and treatment with sublimated CO_2 in acceptable 46 % yield. Unfortunately, the palladium-catalyzed allyl deprotection with phenylsilane reduced the alkyne to an alkene during the global deprotection.

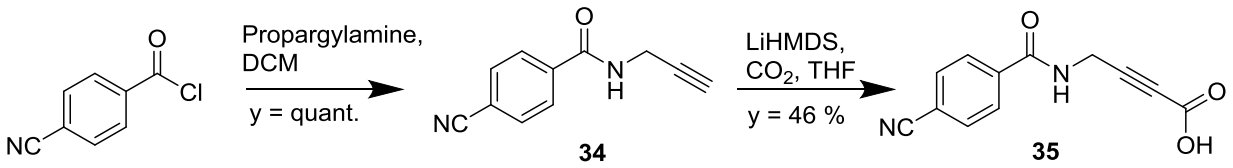
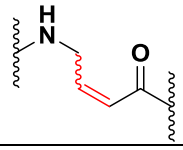
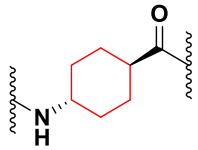
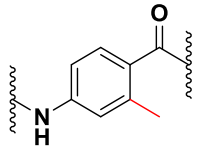
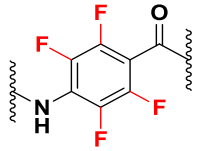
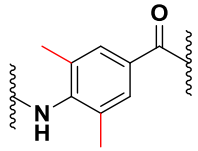
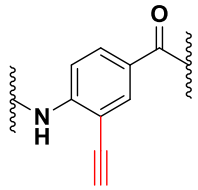
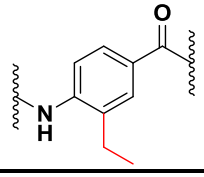


Figure 75: Synthesis of the alkyne B analogue.

The biological activities of the ring B analogues are illustrated in Table 3.

Table 3: MIC and IC_{50} values for the third generation of ring B derivatives compared to CN-DM 861 and CIP (ciprofloxacin).

		MIC [µg/mL]					IC_{50} [µM]
Compound	System for **B**	*E. coli* WT (DSM1116)	*E. coli* ΔTolC	*S. aureus* Newman	*P. aeruginosa* WT (Pa14)	Pa14Δ *mexAB*	*E. coli* gyrase
36		16	<0.03	>64	>64	8	n.d.
37		2	4	4	>64	>64	1.96
38		0.5	<0.03	2	>64	>64	n.d.
39		1	1	1	4	>64	n.d.
40		1	2	4	32	32	n.d.
41		0.125	0.06	2	>64	8	1.55
42		0.125	0.25	2	>64	4	n.d.
CIP		0.013	0.05	0.2	0.05	0.01	0.18
CN-DM 861		<0.03	0.06	0.25	4	1	0.13

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186].

Compound **36** was mostly inactive and comparable to the five-membered aromatic systems. Bigger substituents at position three, like in the ethinyl **41** or the ethyl derivative **42** were not beneficial. Nevertheless, the ethinyl in position three was a suitable residue for the development of a mixed photoaffinity/target-fishing probe, as the activity against *E. coli* was still high. The target-fishing experiments were performed in an *E. coli* lysate. Therefore, an activity against other strains was not required. A shift of the methyl from position three to position two in **38** eliminated the activity against *P. aeruginosa* completely and decreased the activity against the other strains as well.

The tetrafluorinated analogue **39** showed low activity against all stains. Surprisingly, the general benefit of electron withdrawing substituents at ring B did not apply to this compound and seemed to be reversed. The compound showed better activity against the *P. aeruginosa* wild type strain than the ΔmexAB deficient one. The non-aromatic cyclohexyl **37** was poorly active against the tested strains.

4.2.4 Biological activity of ring B derivatives on an extended panel of pathogenic bacteria

To determine the activity spectrum of the synthesized compounds, the most promising ring B analogues were tested on an extended panel against various Gram-positive and -negative bacterial strains with clinical relevance. As the main goal was the development of a broad-spectrum antibiotic for cUTIs, an activity against *E. coli*, *K. pneumoniae*, *Enterococcus spp.* and *Proteus* strains was mandatory. Additionally, the second goal was to cover *A. baumannii*.

Besides the *E. coli* wild type, a quinolone resistant strain with mutations at the positions S83L and S87G of gyrA was included[190]. Additionally, the marR deficient stain allows the investigation of multidrug efflux proteins and porins on the antimicrobial activity of cystobactamids[191]. The activity against two *P. aeruginosa* strains with extended-spectrum β-lactamases was tested. All other bacteria represent wild-type strains.

Ciprofloxacin and the lead structure CN-DM 861 were used as competitors to compare activity and spectrum. In total seven novel B derivatives were tested (Figure 76). The results are depicted in Table 4.

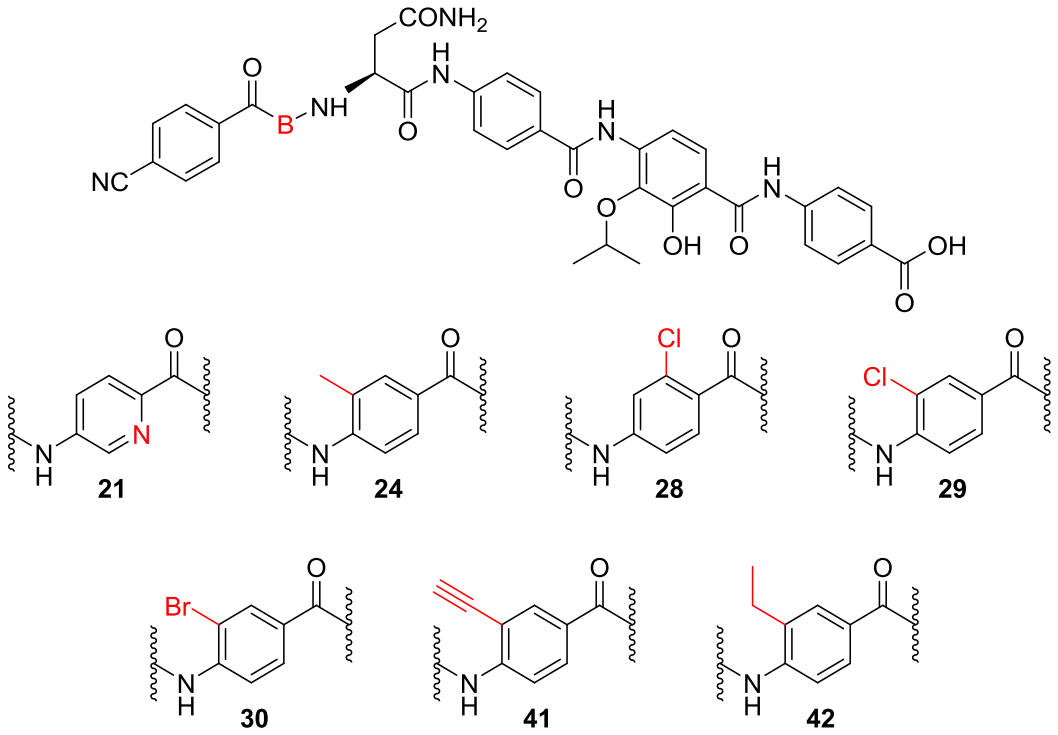


Figure 76: Structure of the ring B derivatives tested in an extended panel of pathogenic bacteria.

Table 4: Antibiotic activities of selected ring B analogues on an extended panel of pathogenic bacteria with CN-DM 861 and CIP (ciprofloxacin) as reference.

	MIC (µg/mL)								
	CN-DM 861	**CIP**	**21**	**24**	**28**	**29**	**30**	**41**	**42**
***E. faecalis* ATCC-29212**	0.5	1.6	1	0.25	0.5	0.06	<0.03	0.5	1
***S. epidermidis* DSM-28765**	0.06	0.4	<0.03	<0.03	0.06	<0.03	<0.03	0.5	0.5
***A. baumannii* DSM-30008**	0.5	0.4	4-16	>64	>64	0.06	0.06	0.25	4
***E. coli* DSM-1116**	<0.03	0.01	<0.03	<0.03	<0.03	0.06	<0.03	0.06	0.06
***E. coli* WT**	2	0.03	<0.03	<0.03	<0.03	<0.03	<0.03	0.5	<0.03
***E. coli* WT-3 [*gyrA*(S83L,D87G)]**	0.125	0.8	0.25	0.25	0.5	1	<0.03	0.25	0.5
***E. coli* WT-III [*marR*Δ74bp]**	0.125	0.05	0.125	0.125	0.125	<0.03	0.06	0.125	0.5
***E. aerogenes* DSM-30053**	0.125	0.1	0.5	0.5	0.5	0.125	0.125	0.25	1
***E. cloacae* DSM-30054**	64	0.8	>64	>64	>64	>64	>64	1	>64
***P. aeruginosa* ESBL1**	4	6.4	>64	>64	>64	>64	>64	>64	8
***P. aeruginosa* ESBL2**	1	0.16	>64	0.5	64	64	32	64	2
***K. pneumoniae* DSM-30104**	>64	0.4	1	>64	>64	>64	>64	0.5	>64
***C. freundii* DSM-30039**	<0.03	0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
***S. marcescens* DSM-30121**	>64	0.4	>64	<0.03	>64	>64	4	0.25	<0.03
***P. vulgaris* DSM-2140**	1	0.05	0.5	1	0.5	<0.03	0.125	0.5	0.5
***P. mirabilis* DSM-2140**	64	0.02	4	1	64	16	16	>64	0.5
***S. pneumoniae* DSM-20566**	0.06	0.8	<0.03	<0.03	<0.03	<0.03	<0.03	0.125	0.125
***S. aureus* ATCC-29213**	2	0.4	>64	64	>64	>64	0.125	0.4	>64

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185].

None of the novel ring B derivatives showed sufficient broad-spectrum activity against the desired strains. Although **24** showed significantly better activity than CN-DM 861 on the small panel, it lacked activity against *A. baumannii* and *S. aureus*. On the other hand, it showed very high potency against *Serratia marcescens*. Amongst the halogenated analogues the 3-Br (**30**) showed the best broad-spectrum activity with improvements against *E. faecalis*, *A. baumannii*, *S. marcescens*, *P. vulgaris* and *S. aureus* but no activity against *P. aeruginosa*. As already observed on the small panel, the 2-Cl analogue **28** showed inferior activity compared to the 3-Cl analogue **29**. The picolinic acid derivative **21** as well as the 3-ethinyl in **41** were the only derivatives with activity against *K. pneumoniae*. The latter was also the only compound with activity against *E. cloacae* and had the overall broadest activity.

4.3 Ring A derivatives and AB-linker analogues

Compared to the synthesis of the ring B derivatives, the ring A derivatives and the linker analogues were synthesized to expand or validate the SAR at the time. The SAR at the beginning of the Ph.D. thesis stated the importance of an electron poor aromatic system at ring A and its connection to ring B with a rigid spacer. Although the best cystobactamids carry a nitro or a cyano residue as electron withdrawing group (EWG) at ring A, other functionalities like a lactone or a fluorine were tolerated with slightly decreased activity, while the strong electron withdrawing -CF_3 was not. Furthermore, the addition of electron withdrawing or electron donating groups (EDG) to ring A was not showing consistent improvements or deteriorations in activity[118]. From the information given, it was hypothesized that the *para*-substituent at ring A might act as an HBA. Presumably, the low activity of the *para*-aniline might be the result of the geometric orientation as HBA and the delocalization of the free electron pair within the π-system (Figure 77).

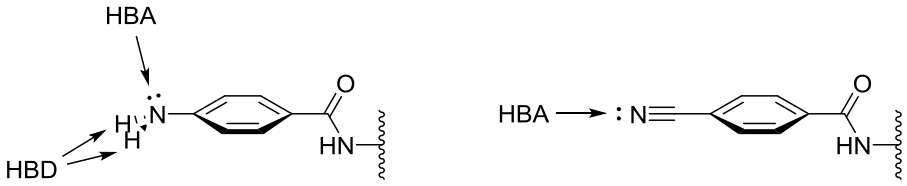


Figure 77: Simplified orientations of the HBAs and HBDs in the terminal 4-amino and the 4-cyano system.

In total three analogues were synthesized to validate the original hypothesis (Figure 78).

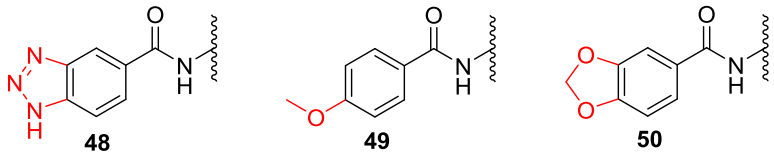


Figure 78: Structure of novel ring A analogues with at least one HBA attached (red).

49 and **50** are similar in their orientation of the HBA in the *para* position, while the benzotriazole in **48** is significantly different. The slightly acidic proton can be shifted between the different nitrogen atoms leading to three possible tautomers with the 1*H* and the 3*H* tautomers as the most stable ones (Figure 79)[192],[193]. With a pKa similar to phenol and the possibility to act as a HBA and a HBD, benzotriazoles can be used as isosteres for their respective phenols[194].

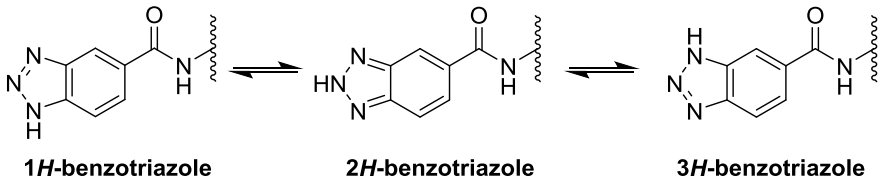
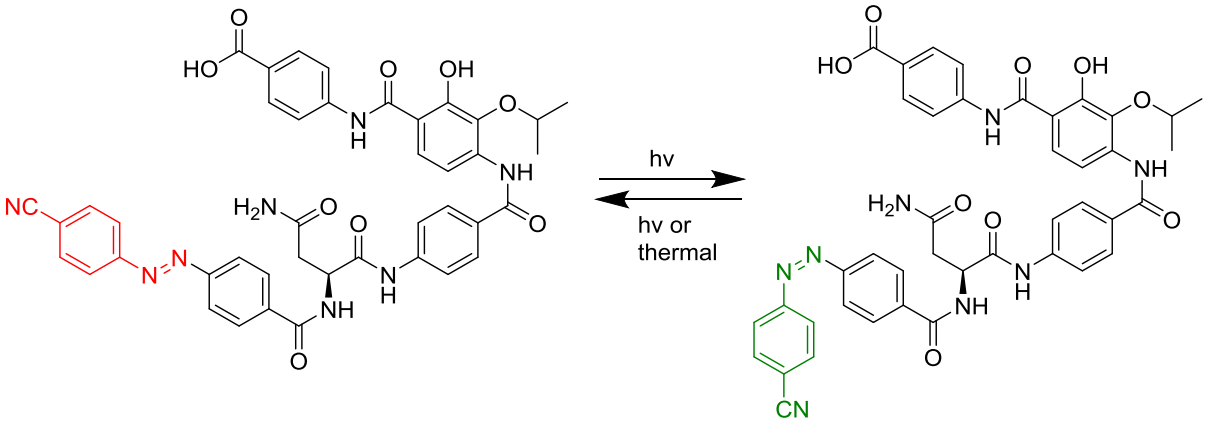


Figure 79: The three possible tautomers of benzotriazole. Image adapted from[193].

Previous modifications at the cystobactamids included the introduction of an azo isostere instead of the amide (Figure 80). A compound with an azo group between ring A and B can be switched from its *trans* to its *cis* isomer by light[195]. Investigations on the biological activity of the respective *cis* and *trans* isomers revealed that the *cis* isomer exhibited higher activity against *E. coli* and *E. coli* gyrase[118].



	MIC E. Coli [µg/ml]	*EC gyrase IC_{50} [µM]*
trans	0.64	0.5
cis	0.45	0.1

Figure 80: Biological activity of the cis- and the trans-isomer and their conversion to each other. Image adapted from[118].

From this observation it was assumed that the binding pocket of gyrase offers space adjacent to ring A that can be exploited via structure extension (Figure 81).

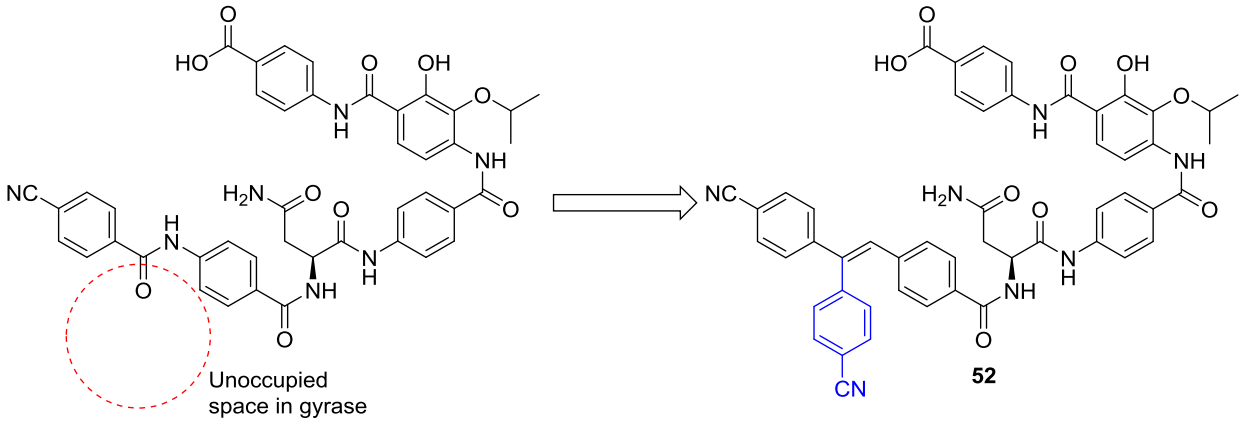


Figure 81: Proposed position for a structure extension (red) and its occupation by another aromatic system (blue).

The synthesis of the stilbene-like structure was carried out by a Horner-Wadsworth-Emmons reaction of the ring B phosphonate and the benzophenone (Figure 82)[196]. The benzophenone was prepared from its respective alcohol by oxidation with Dess-Martin periodinane (DMP)[197]. The phosphonate was obtained by a Michaelis-Arbuzov reaction of the benzyl bromide with trimethyl phosphite[198].

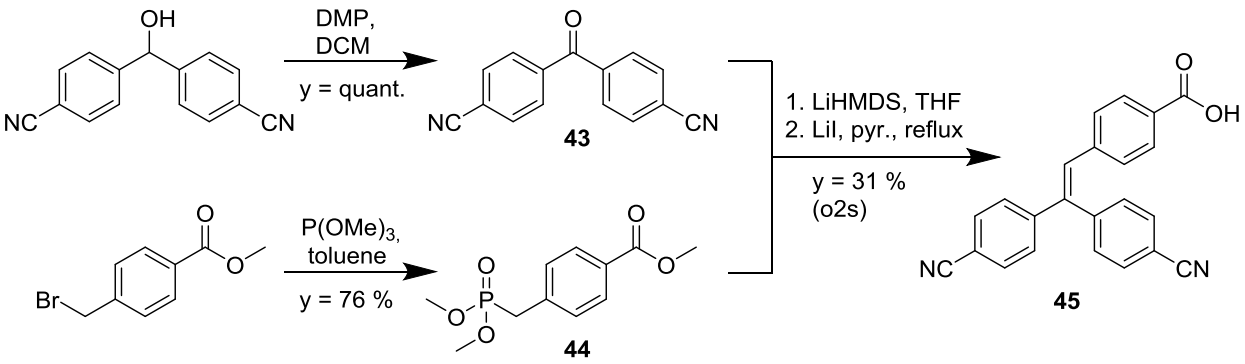


*Figure 82: Synthesis of the stilbene-like derivative **45**.*

Another option to make use of this position is the addition of solubilizing groups. The retrosynthesis led to three possibilities to synthesize stilbene analogues with altered systems adjacent to the terminal ring system (Figure 83). While the cross-coupling methods allows the synthesis of one desired diastereomer, the aldol reaction is less specific in its outcome. Nevertheless, this pathway was applied for the synthesis of a novel compound mentioned in chapter 4.6.1.

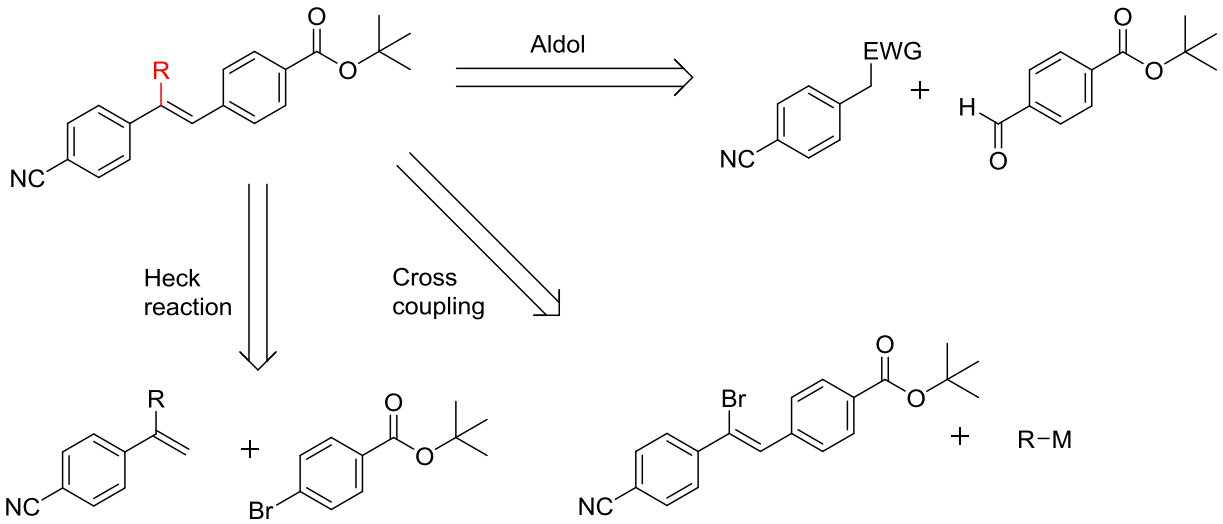


Figure 83: Retrosynthesis of stilbene-like fragment AB analogues with R as the desired residue and M as metal or functional group required for the cross coupling.

As styrene is prone to auto-polymerization, the alternative synthesis via the brominated stilbene was carried out (Figure 84)[199]. The synthesis included the first step of the Corey-Fuchs reaction, a Wittig reaction with carbon tetrabromide[200]. The following Suzuki coupling with 4-cyanophenylboronic acid gave the desired intermediate for following reactions[201]. Unfortunately, the transmetalation with *i*-PrMgCl*LiCl led to the decomposition of the starting material.

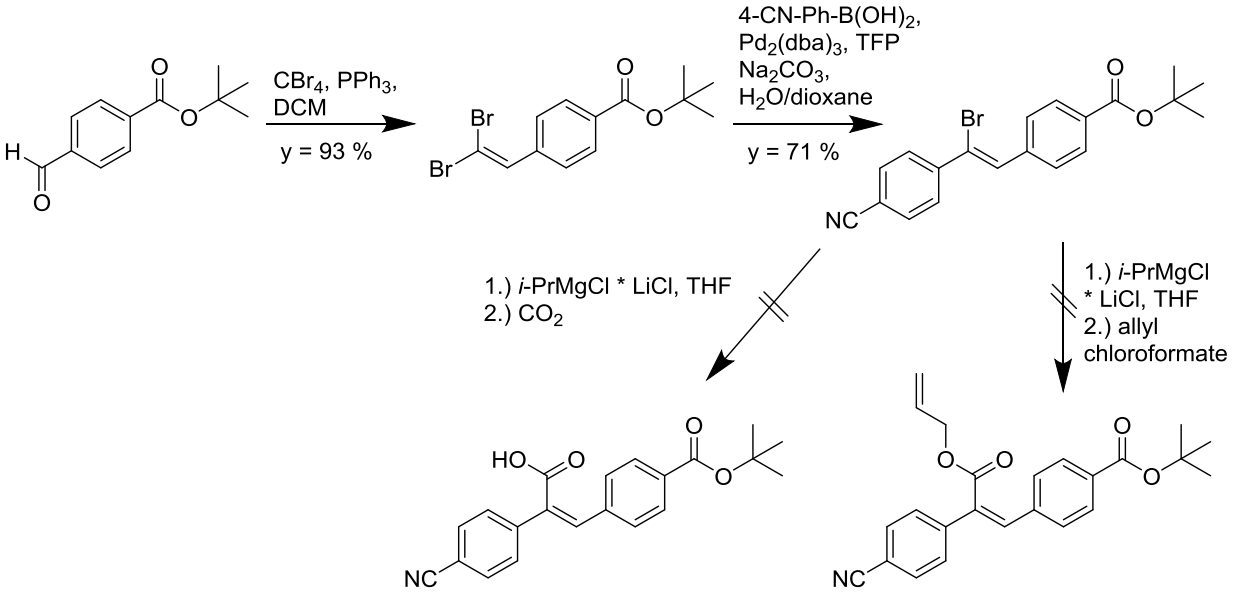


Figure 84: Strategy for the synthesis of carboxylated stilbene analogues.

Compared to albicidin, the cystobactamids are shorter at their N-terminal position. Given that both natural products bind to the same targets, an extension of the N-terminal position might be beneficial (Figure 85).

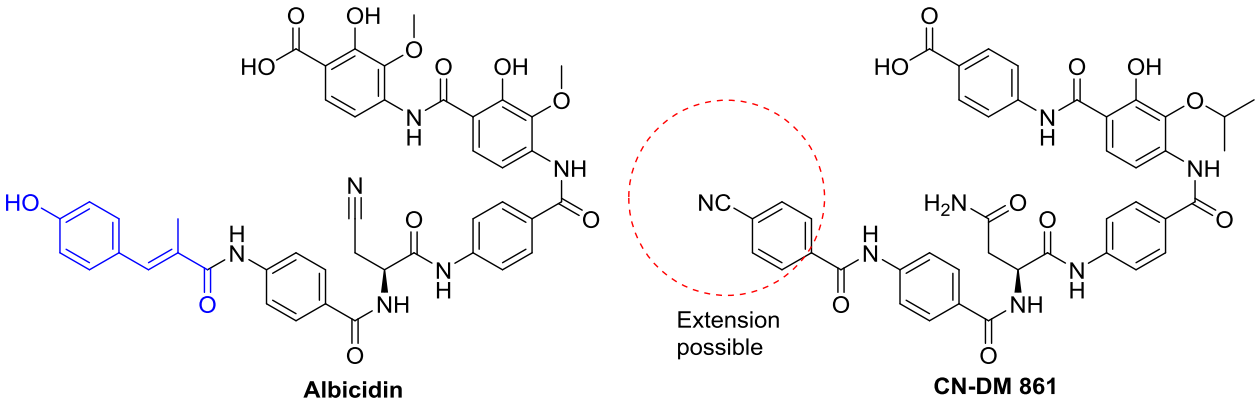


Figure 85: Extended p-coumaric acid motive in albicidin (blue). Proposed position for a structure extension in CN-DM 861 (red).

In accordance with the proposed importance of a HBA in *para* position of the terminal system, a morpholine was introduced, leading to compound **51** (Figure 86). The 4-morpholinobenzoic acid building block was commercially available and applied under standard conditions.

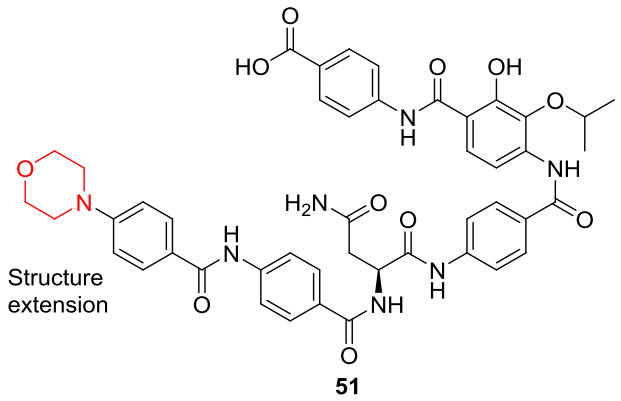


*Figure 86: Structure of the extended cystobactamid **51**.*

An introduction of an amide isostere at the AlbD cleaving site between ring C and D was planned. A substitution of the amide by a trifluoroethylamine isostere was attempted. To find a general method, the experiments were first performed at the connection between ring A and B. Due to the electron withdrawing effect of the CF_3 group, the amine loses its function as HBA and offers amide-like interaction possibilities[202]. The first synthetic route involved the synthesis of the trifluoroethylalcohol from the aldehyde at 33 % yield (Figure 87)[203]. A subsequent triflation and substitution was unsuccessful.

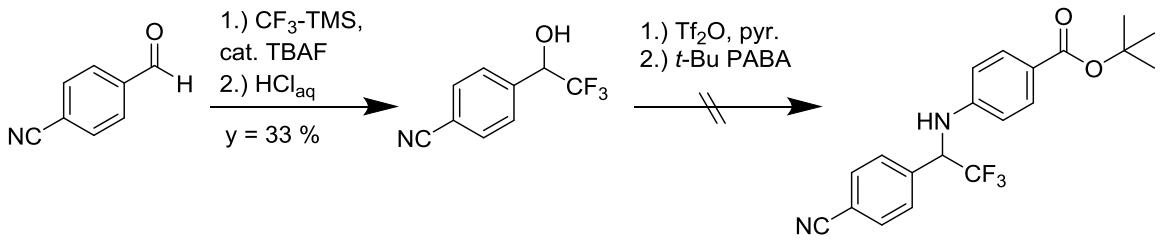


Figure 87: Attempt for the synthesis of a trifluoroethylamine analogue.

It was assumed that the aniline is not nucleophilic enough for the substitution. This was also observed during the attempts to synthesize amidine linkers between the ring C and D systems (Figure 88). Several methods via thioimidates and imidates were tried. Reactions with aniline either led to no conversion or an elimination to the nitrile.

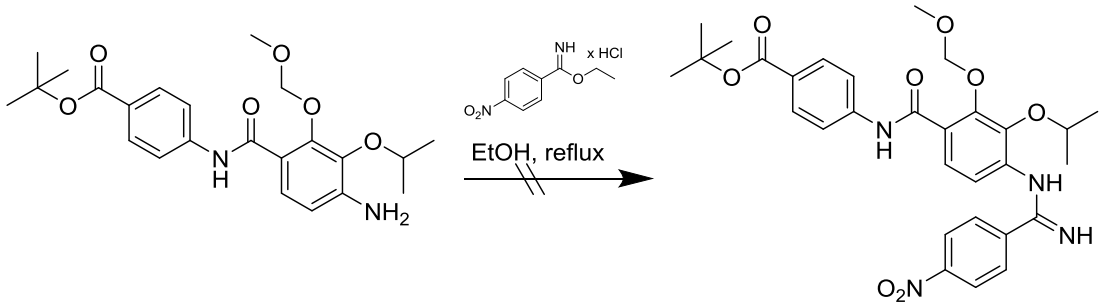


Figure 88: Synthetic approach for the synthesis of an amidine between rind C and D.

Another attempt was carried out by addition of a Grignard reagent to a preformed hemiaminal ether (Figure 89)[204]. After synthesis and isolation of the hemiaminal ether, the reaction with the Grignard reagent failed.

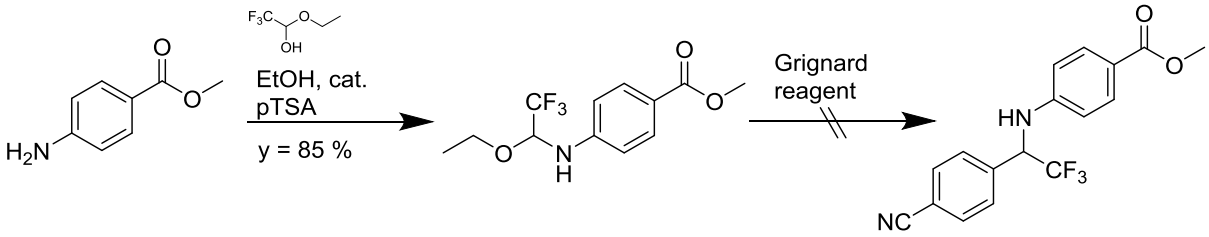


Figure 89: Synthetic pathway to the trifluoroethylamine via Grignard reagents.

The hemiaminal ether was utilized in a palladium catalyzed addition of arylboronic acid but failed as well (Figure 90)[205]. Further attempts to synthesize trifluoroethylamines or amidines were discarded. An altered strategy for the synthesis of the trifluoroethylamine was required.

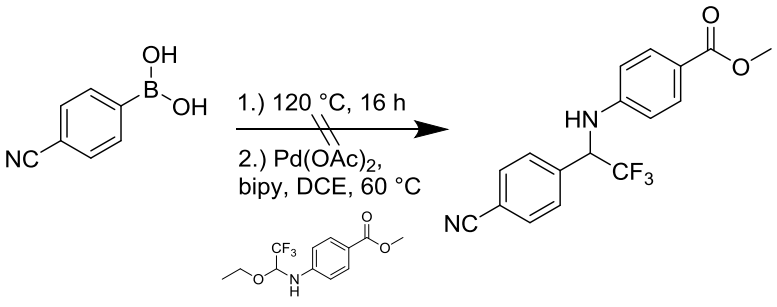


Figure 90: Attempt to synthesize the trifluoroethylamine via a palladium catalyzed addition.

To investigate, whether other AB connections were tolerated, a sulfonamide was inserted between ring A and B (Figure 91). The desired intermediate was obtained by a reaction of the respective sulfonylchloride with *tert*-butyl PABA and subsequent deprotection with TFA[206].

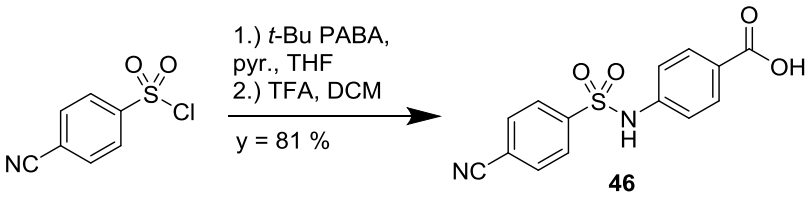
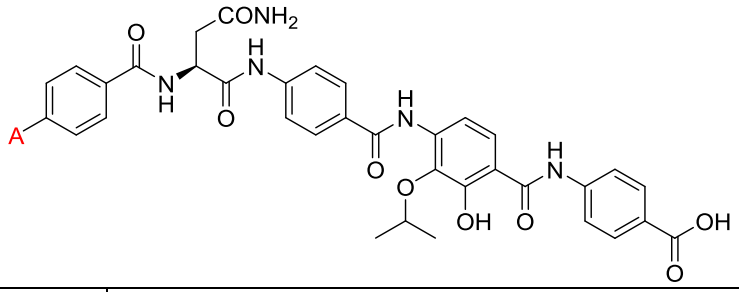
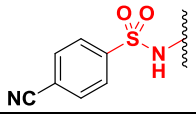
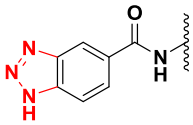
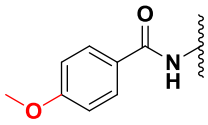
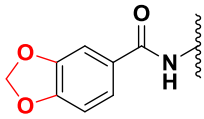
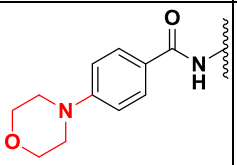
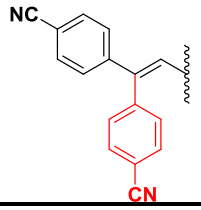


Figure 91: Synthesis of the sulfonamide.

The A and AB-linker analogues were tested against a panel of Gram-positive and -negative bacteria (Table 5).

Table 5: MIC and IC_{50} values for cystobactamids with modified ring A or AB-linker systems. The derivatives were compared to CN-DM 861 and CIP (ciprofloxacin).



Comp.	Structure of **A**	MIC [µg/mL]					IC_{50} [µM]
		E. coli WT (DSM1116)	*E. coli* ΔTolC	*S. aureus* Newman	*P. aeruginosa* WT (Pa14)	Pa14Δ *mexAB*	*E. coli* gyrase
47		>64	>64	>64	>64	>64	>25
48		8	8	>64	>64	>64	2.33
49		<0.03	<0.03	0.25	>64	0.125	3.66
50		<0.03	<0.03	>64	>64	<0.03	0.98
51		<0.03*	<0.03*	>64	>64	0.5	1.15
52		4	0.25	2	>64	>64	0.24
CIP		≤0.05	≤0.05	0.06 – 0.2	0.05 – 1	0.01 – 0.5	0.18
CN-DM 861		<0.03	≤0.06	0.125 – 0.5	0.1 – 4	0.04 – 1	0.13

*MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. Only compound **49** and **50** were tested in the same batch for the MIC determination, therefore a span in activity was given for the references.*

** values against E. coli BW25113 and E. coli ΔacrB. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186].*

The sulfonamide **47** was not tolerated and entirely inactive. This might be the result of the higher polarity or an overall change in topology. Previous studies observed a decrease in activity for more polar and less rigid linker systems between ring A and B[5]. Derivatives **49** and **50** showed very good activity against *E. coli* and the ΔmexAB deficient *P. aeruginosa* strain, but no activity against the wild type and no activity against *S. aureus* for compound **50**. Both analogues confirm the hypothesis of the necessary HBA in para position of ring A and disprove the need for an electron poor aromatic system.

The extension strategy carried out in **52** showed that gyrase offers space for branched substituent. With an IC_{50} value of 0.24 µM, the inhibitory effect against the *E. coli* gyrase was mainly retained. At the same time the antimicrobial activity droped significantly. Surprisingly, the compound showed a 15-fold lower IC_{50} value compared to compound **49** with very high activity against *E. coli*. This observation indicated that the IC_{50} value on *E. coli* gyrase does not necessarily correlate with the antimicrobial activity, even against *E. coli*. Presumably factors like penetration, efflux, solubility or the activity against other targets were responsible for the poor activity of **52**, but contribute to the higher activity of **49**.

The benzotriazole **48** was only showing low antimicrobial activity but a lower IC_{50} value against *E. coli* gyrase than **49**. The morpholine analogue **51** was equally well tolerated as **50**, showing the possibility for a N-terminal extension.

None of the synthesized analogues showed improved activity compared to CN-DM 861.

4.4 Altered central amino acid

Modifications at the central amino acid were carried out using the same retrosynthesis as mentioned in chapter 4.1. Commercially available Fmoc protected amino acids were chosen to allow a selective deprotection of the amine while retaining the allyl and the *tert*-butyl protecting groups. Only non-proteinogenic amino acids were used. It was assumed that the highly abundant proteinogenic amino acids would have been found in natural cystobactamids, if they were beneficial for activity. Basic amino acid chains were either applied with an Alloc or a Boc protecting group in the side chain to allow a simultaneous deprotection with allyl or *tert*-butyl.

Most of the amino acids were commercially available. The *N*,*N*-dimethylated asparagine analogue required the amide coupling of the protected aspartic acid with dimethylamine (DMA) (Figure 92).

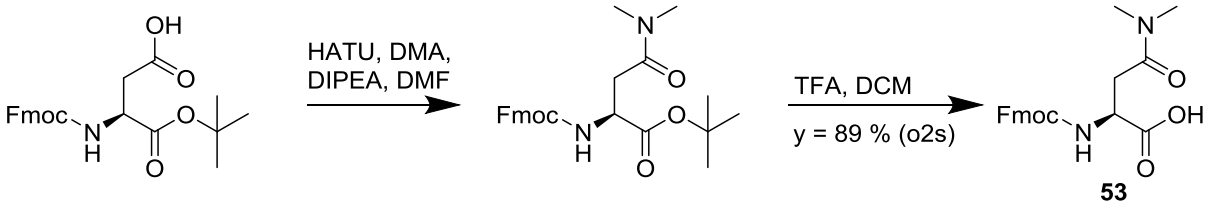


*Figure 92: Synthesis of N,N-Dimethylasparagine **53**.*

A synthesis of amino acid analogues was carried out by a stereoselective O'Donnell amino acid synthesis, starting from the imine of *tert*-butyl glycine with benzophenone (Figure 93). The cinchonidine derived catalyst in this reaction allows for a stereoselective alkylation. By interaction of the enolate with the quaternary ammonium salt, one side of the enolate is blocked by the bulky residues of the cinchonidine, enabling the selective addition of the electrophile at the opposite side. The subsequent hydrolysis of the imine obtained the desired *tert*-butyl protected amino acid[211]. After coupling to the AB fragment, the *t*-Bu protected AB amino acid fragment was obtained. Unfortunately, the amide coupling of the *t*-Bu deprotected central amino acid to fragment CDE failed. The attempt to determine the enantiomeric ratio off the deprotected carboxylic via Marfey's Reagent was unsuccessful. Presumably, the acidic conditions for the hydrolysis were not tolerated by the alkyne side chain.

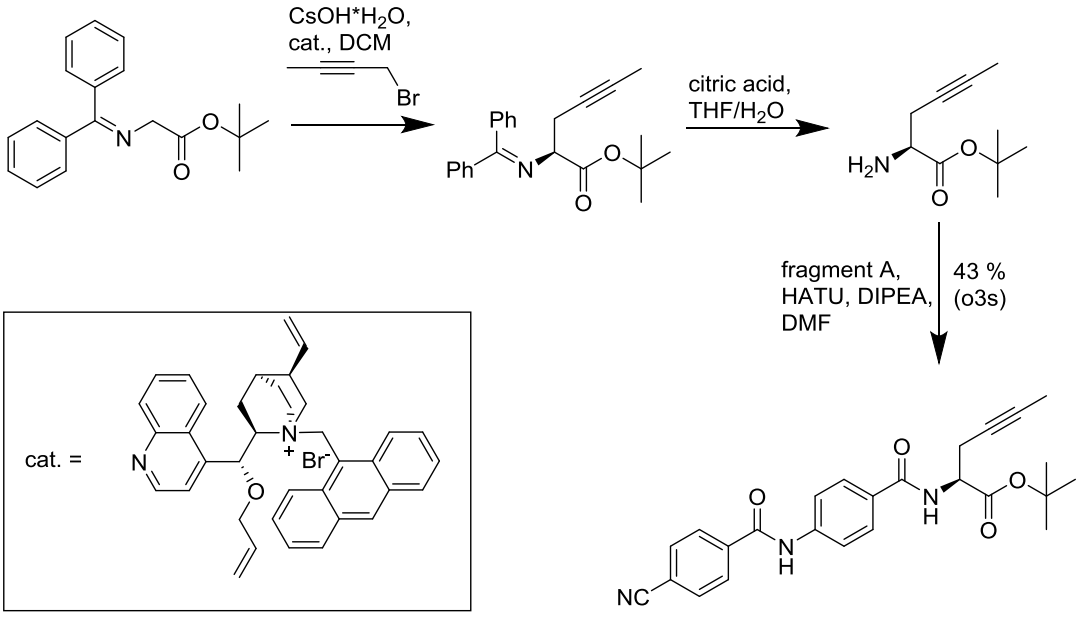


Figure 93: Stereoselective synthesis of amino acid derivatives.

In total three methods were used in the amide coupling between the central amino acid and fragment CDE. In literature, a preformation of the acid chloride from the amino acid was shown to lead to a highly reactive species without significant racemization[207]. The acid chloride was coupled with the aniline of fragment CDE in 60 % to 74 % yield (Figure 94). Pyridine was used to decrease the racemization during the amide coupling. A preactivation with TFFH to the acid fluoride was not sufficient for the amide coupling.

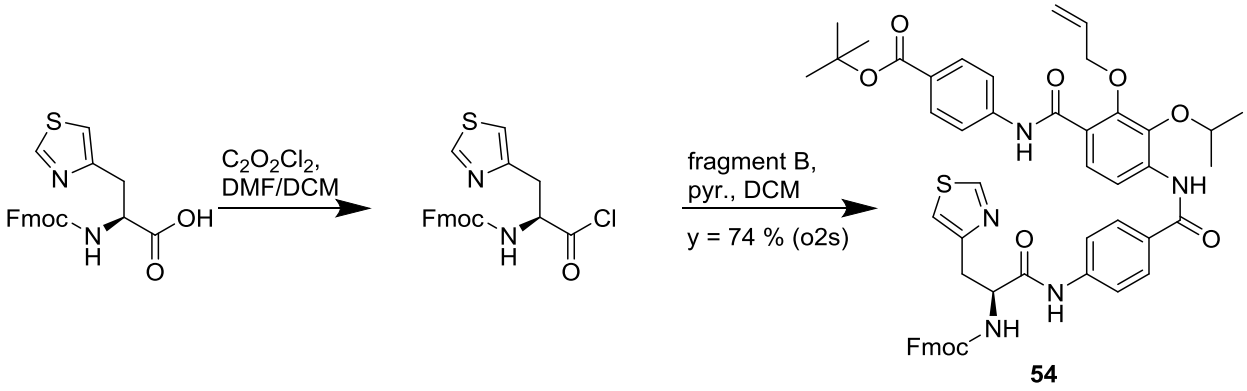


Figure 94: Activation of the central amino acid with oxalyl chloride and subsequent use in the amide coupling.

The coupling reagents 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) or isobutyl 1,2-dihydro-2-isobutoxy-1-quinolinecarboxylate (IIDQ) were used, if the preformation of the acid chloride was not feasible (Figure 95). A disadvantage of this method was the formation of the ethyl or isobutyl esters as byproduct that were hardly separable from the product via chromatography[208].

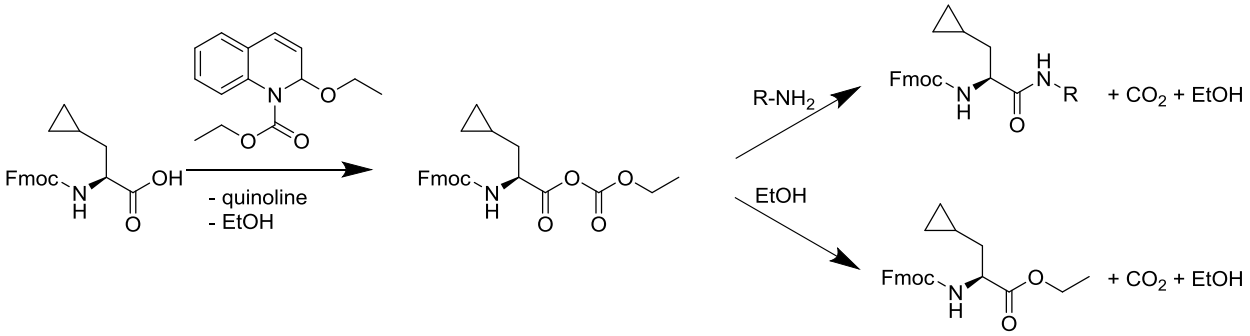


Figure 95: Example for the activation of an amino acid by EEDQ. The nucleophilic substitution can either lead to the desired amide or the ester byproduct. Image adapted from[209].

A purification via RP flash or RP HPLC after the Fmoc deprotection with diethylamine (DEA) was necessary to obtain a clean product (Figure 96). For the complete consumption of fragment CDE an excess of the amino acid was necessary, as the activated ester partially reacts with the released alcohol during the coupling process. The yields ranged from 19 % to 67 % over two steps, depending on the properties and size of the amino acid side chain.

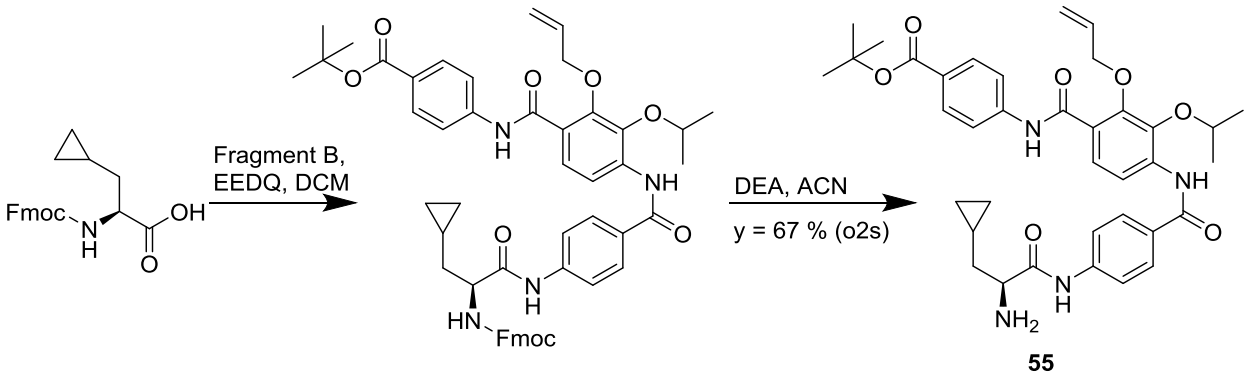


*Figure 96: Amide coupling via EEDQ and subsequent deprotection of the Fmoc protecting group exemplified at the synthesis of **55**.*

For the coupling of very electron-poor fragment CDE analogues, the use of propanephosphonic acid anhydride (T3P) was required (Figure 97). The method allows a coupling with various anilines in good to excellent yields with low racemization, when pyridine is used[210]. For a fragment CDE analogue with a terminal 5-aminopicolinic acid, the other coupling methods were insufficient for the amide formation. Other standard coupling reagents, like HATU or 2-bromo-1-ethylpyridinium tetrafluoroborate (BEP), did not lead to any amide bond formation.

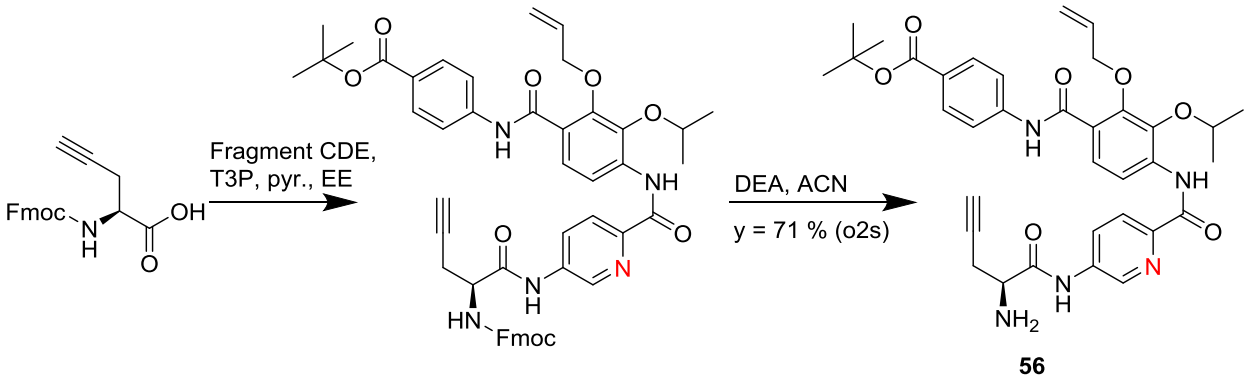


Figure 97: Amide coupling via T3P and subsequent deprotection of the Fmoc protecting group.

The formation of the full length cystobactamid was carried out under the conditions described in chapter 4.1.3. Only minor alterations concerning the global deprotection were implemented. Novel central amino acids with double or triple bonds were treated with aniline instead of phenylsilane in the allyl deprotection step. This prevented potential reductions that were observed for fragment AB analogues with similar moieties. Additionally, the use of triisopropyl silane in the *tert*-butyl deprotection step was left out. With the lack of the trityl group, a scavenger was not required. To investigate the degree of racemization during the three different amide couplings to the central amino acid, the three different cystobactamid analogues were investigated after derivatization with Marfey's Reagent. This is described in chapter 4.8.

4.4.1 First generation of altered central amino acids

The first derivatives were chosen from a variety of unnatural amino acids with different interaction possibilities. The scaffold of the cystobactamids and albicidin mostly differ at the N-terminus and the central amino acid. Both side chains of the amino acid carry a HBA. A dimethylated analogue was synthesized to eliminate the HBD properties and determine whether a HBA is sufficient in the side chain (Figure 98).

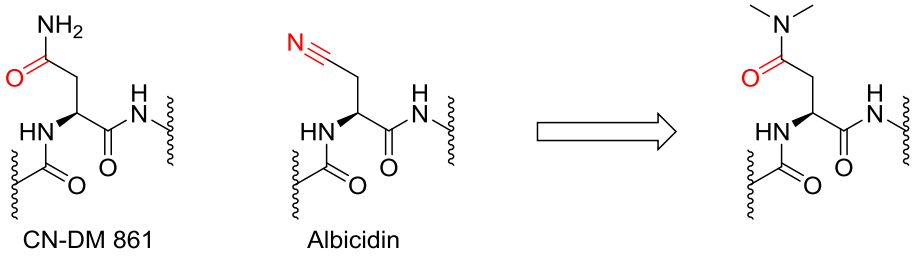


Figure 98: Both CN-DM 861 and albicidin share a HBA (red) in the side chain. The demethylated asparagine is devoid of the HBD but still carries a HBA.

To investigate the bioactive conformation of the cystobactamids, the amino acid was rigidified. The six-membered (*S*)-picolinic acid was chosen to imitate the predicted pseudo-cycle resulting from an intramolecular hydrogen bond (Figure 99). To determine the importance of the configuration, the respective (*R*)-enantiomer was also synthesized.

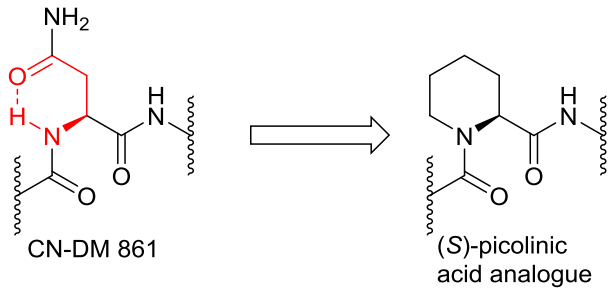


Figure 99: Rigidification of the central amino acid similar to the predicted pseudo cycle (red).

To include an aromatic non-proteinogenic amino acid, a thiazole analogue of histidine was included that lacks a HBD but still retains the HBA property (Figure 100). Additionally, a tautomerization cannot occur at the thiazole system.

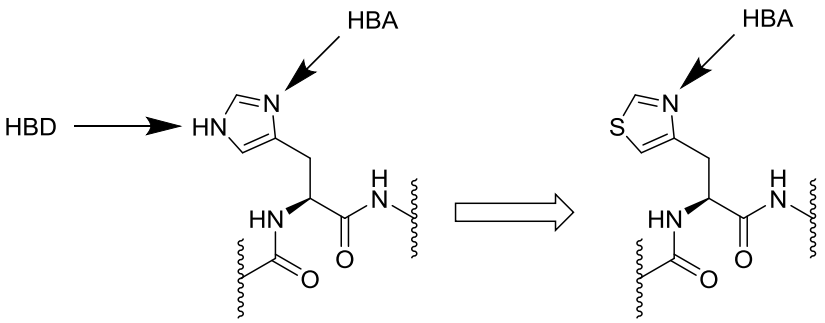
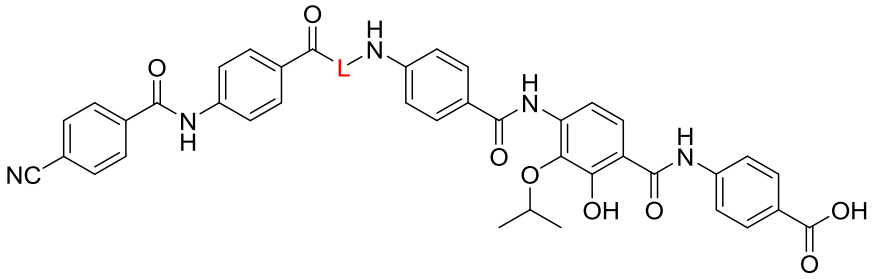


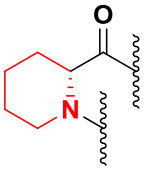
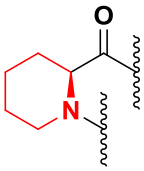
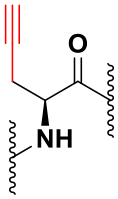
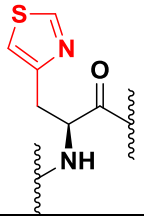
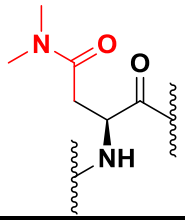
Figure 100: Comparison of a thiazole and imidazole side chain at the central amino acid.

The introduction of an alkyne was carried out to subsequently introduce the triazole as amide isostere via a “click”-reaction. Furthermore, the alkyne can be used for fishing experiments and for the elucidation of the SAR.

The cystobactamids with altered amino acids were tested on a small MIC panel and compared with the lead structure CN-DM 861 (Table 6).

Table 6: MIC and IC_{50} values for the first generation of cystobactamids with modified central amino acid compared to CN-DM 861 and CIP (ciprofloxacin).



		MIC [µg/mL]					IC_{50} [µM]
Compound	System for **L**	*E. coli* WT (DSM1116)	*E. coli* ΔTolC	*S. aureus* Newman	*P. aeruginosa* WT (Pa14)	Pa14Δ *mexAB*	*E. coli* gyrase
57		1	<0.03	0.5	64	4	1.07
58		0.5	<0.03	0.25	64	2	0.18
59		<0.03	<0.03	<0.03	0.5	<0.03	0.23
60		0.125	<0.03	0.125	>64	0.25	0.34
61		<0.03	<0.03	0.125	>64	0.5	1.47
CIP		<0.03	<0.03	0.06	1	0.03	0.18
CN-DM 861		0.013	0.003	0.16	0.1	0.04	0.13

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186].

The results of **58** compared to **57** proved the benefit of a (*S*)-configurated amino acid. **57** shows a nearly six-fold lower IC_{50}, but only twice the biological activity. The lower IC_{50} value suggests that the rigidification stabilized the bioactive conformation in *E. coli* gyrase. As drawback both rigidified compounds have decreased activity against the *P. aeruginosa* strains and the *E. coli* wild type.

The dimethylation of asparagine **61** abolished the activity against the *P. aeruginosa* wild strain and decreased the activity on the ΔmexAB strain as well as the IC_{50} value on gyrase. This could either mean that the additional methyl residues were sterically too demanding or that the side chain was not interacting with the target or targets as HBA. Surprisingly, side chains with a π-system, like the thiazole in **60** and the alkyne in **59** showed low IC_{50} values. While **60** lacks the activity against the *P. aeruginosa* wild type, **59** had a broader activity with even higher potency against *S. aureus* than CN-DM 861.

4.4.2 Second generation of amino acids

To further probe the interactions between the side chain and the targets, modifications were carried out to investigate the benefit of a π-system or a HBD in the side chain. For this cause the alkyne, a π-system and weak HBD, was exchanged for the respective alkene (**63**) and alkane (**64**, **65**). Additionally, the triazole **66** was obtained via a "click"-reaction with sodium azide (Figure 101). Both the triazole and the alkyne share the same features as π-system and HBD. It was assumed that the triazole is predominately found as 2*H* tautomer[212].

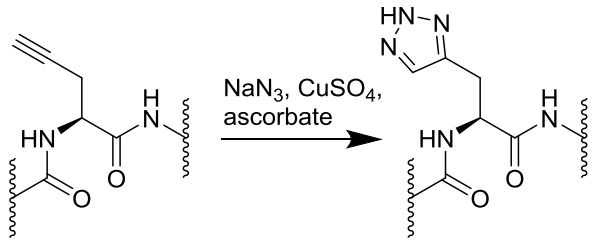
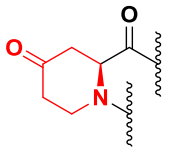
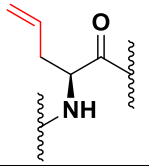
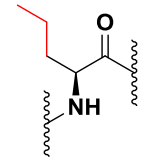
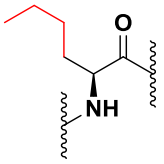
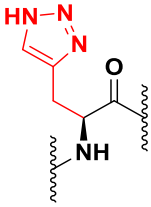


Figure 101: Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC) of the alkyne to the triazole.

To imitate the predicted conformation, stabilized by an intramolecular H-bond within CN-DM 861 better than **58**, the 4-keto analogue **62**, with an additional sp^2 center, was synthesized. The new analogues were tested on a small MIC panel of Gram-positive and -negative bacteria and were compared with the lead structure CN-DM 861 as well as **59** (Table 7).

Table 7: MIC and IC_{50} values for the second generation of cystobactamids with modified central amino acid compared to CN-DM 861, **59** and CIP (ciprofloxacin).

		MIC [µg/mL]					IC_{50} [µM]
Compound	System for **L**	*E. coli WT* (BW25113)	*E. coli ΔacrB*	*S. aureus* Newman	*P. aeruginosa* WT (Pa14)	Pa14Δ *mexAB*	*E. coli* gyrase
62		<0.03	<0.03	1	16	1	0.86
63		<0.03	0.125	<0.03	0.5	0.125	0.49
64		<0.03	<0.03	<0.03	>64	0.5	0.28
65		<0.03	<0.03	<0.03	>64	4	0.45
66		<0.03	<0.03	<0.03	1	<0.03	0.33
CIP		0.005	0.0013	0.08 – 0.1	0.05	0.025	0.18
59		<0.03	<0.03	<0.03	1	0.03	0.23
CN-DM 861		<0.03	<0.03	0.06 – 0.5	0.5 – 4	0.06 – 0.25	0.13

*MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. Compound **63** and **65** were tested in a different batch for the MIC determination, therefore a span in activity was given for the references. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186]. The* E. coli *wildtype strain was changed and the* acrB *deficient strain substituted* E. coli *ΔtolC. Both tolC and acrB are components of the multidrug efflux pump system AcrAB-TolC[213]. For the function of the AcrAB-TolC efflux pump every component is required[214]. ΔTolC strains show signs of membrane stress and lower membrane integrity[215],[216]. The deletion of AcrAB does not influence the membrane permeability of drugs through the outer membrane[217].* E. coli *ΔacrB was chosen as more resilient strain without impairments within the membrane.*

Compared to the alkyne **59**, the alkene **63** and the alkane **64** showed slightly decreased activities. While **63** retained activity against the *P. aeruginosa* wild type, **64** lacked activity entirely, despite its lower IC_{50} value. The elongation of the alkane side chain in **65** decreased the potency against the *P. aeruginosa* strains and increased the IC_{50} value against *E. coli* gyrase. These results confirmed the hypothesis that a π-system in the side chain is beneficial for broad-spectrum coverage. The hypothesis was not sufficient to explain the high activity of CN-DM 861 entirely, as the dimethylated analogue **61** showed decreased activity. Nevertheless, it explained the function of the cyanoalanine in albicidin.

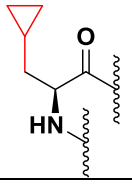
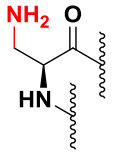
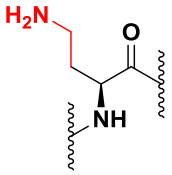
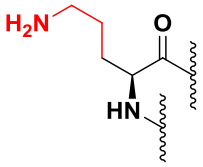
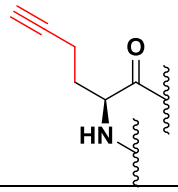
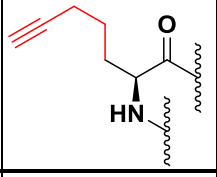
The clear decline in potency from the highly beneficial alkyne **59** over the alkene **63** to the alkane **64** also indicated that a HBD in the side chain might be beneficial. Not only would this explain the difference in activity of CN-DM 861 and **61**, but also the high activity of the triazole **66**. Both **59** and **66** share the feature of a π-system as well as a HBD in the side chain. The triazole compound **66** was highly active with the same efficiency and spectrum as the respective alkyne **59**. Both compounds showed improved antibacterial properties compared to CN-DM 861. It was assumed that the amide in the side chain of CN-DM 861 also acts as HBD.

The rigidified derivative **62** with the additional ketone as HBA was showing high activity against *E. coli*, but only mediocre to low potency against the other strains. Overall, no significantly improved antibiotic potency was observed, compared to **59**.

4.4.3 Third generation

The third generation focused on the investigation of an optimal orientation and positioning of the functional group in the side chain. For this purpose, **71** and **72**, elongated analogues of the highly active alkyne derivative **59**, were synthesized. Primary amines were introduced into the side chain (**68** – **70**) to influence solubility and simultaneously allow for the interaction as HBD. At physiological pH, the primary amines were protonated. Similar to **71** and **72**, different side chain lengths were introduced to investigate the optimal position of the amine. As the alkene in **63** was tolerated very well, a cyclopropyl analogue was synthesized. Cyclopropyl residues share similarities to π-systems and can act as isosteres for unsaturated systems[218]. The biological results and structures are depicted in Table 8.

Table 8: MIC and IC_{50} values for the third generation of cystobactamids with modified central amino acid compared to CN-DM 861, **59** and CIP (ciprofloxacin).

Compound	System for **L**	MIC [µg/mL]					IC_{50} [µM]
		E. coli WT (BW25113)	*E. coli* Δ*acrB*	*S. aureus* Newman	*P. aeruginosa* WT (Pa14)	Pa14Δ *mexAB*	*E. coli* gyrase
67		<0.03	<0.03	1	0.5	0.25	n.d.
68		<0.03	<0.03	<0.03	0.25	0.25	0.86
69		0.125	0.125	16	4	1	n.d.
70		<0.03	<0.03	>64	>64	2	0.63
71		<0.03	<0.03	<0.03	32	2	0.21
72		<0.03	<0.03	<0.03	64	2	0.23
CIP		0.005	0.0013	0.08 – 0.1	0.05	0.025	0.18
59		<0.03	<0.03	<0.03	0.5	<0.03	0.23
CN-DM 861		<0.03	<0.03	0.06 – 0.5	0.5 – 4	0.06 – 0.25	0.13

*MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. Compound **67** and **69** as well as **71** and **72** were tested in a separate batch for the MIC determination, therefore a span in activity was given for the references. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186].*

The 2,3-diaminopropionic acid in compound **68** showed very broad activity against all strains and was the first compound not to be influenced by MexAB. As this compound lacked the π-system in the side chain, it was not able to interact in the same fashion as an unsaturated system. At physiological pH, the amino side chain was protonated, generating a stronger HBD and a cation. As π-systems, HBDs and cations share the capability to interact with other π-systems, we hypothesized that the side chain interacts with an aromatic system of some kind. Unfortunately, this assumption did not apply to the interaction with *E. coli* gyrase, as low IC_{50} values were found for compounds lacking those functionalities. This did not exclude the possibility of this interaction in other targets or species. Nevertheless, it validated the hypothesis that a HBD in the side chain was beneficial for activity.

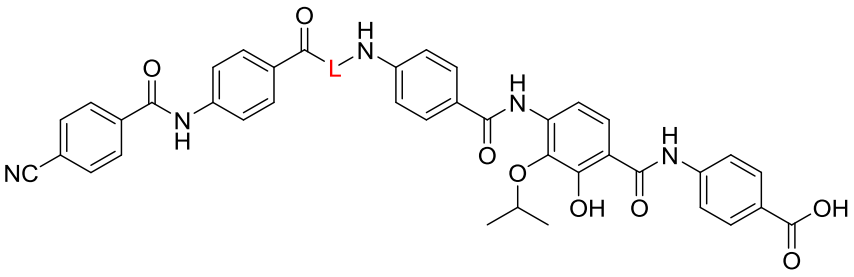
As confirmed by the three analogues with the amino side chain **68** to **70**, zwitterionic cystobactamids were tolerated. Nevertheless, the elongation of the amino side chain from **68** to **69** and **70** decreased the activity against *S. aureus* and *P. aeruginosa* significantly. The same trend was observed with the alkyne side chain in **59**. For **59**, a homologation to **71** and **72** decreased the potency against *P. aeruginosa*, although no reduced activity against *S. aureus* was observed. Therefore, the β-position of the central amino acid was the optimal position for the amine and the alkyne in the side chain.

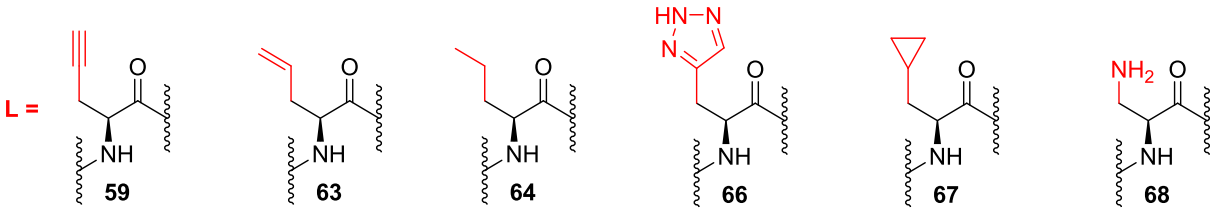
The potency of the cyclopropyl derivative **67** was in between the alkene **63** and the alkane **64**, featuring properties of both analogues.

4.4.4 Biological activity of amino acid derivatives on an extended panel of pathogenic bacteria

Similar to the ring B analogues, the most promising amino acid analogues were tested on an extended panel against various Gram-positive and -negative bacterial strains with clinical relevance. The results are shown in Table 9.

Table 9: Antibiotic activities of selected cystobactamid analogues with modified central amino acids on an extended panel of pathogenic bacteria. CN-DM 861 and CIP (ciprofloxacin) were added as references.





	MIC (µg/mL)							
	CN-DM 861	**CIP**	**59**	**63**	**64**	**66**	**67**	**68**
***E. faecalis* ATCC-29212**	0.5	0.8	<0.03	<0.03	<0.03	<0.03	<0.03	0.5
***S. epidermidis* DSM-28765**	<0.06	0.2 – 0.32	<0.03	<0.03	<0.03	<0.03	<0.03	0.06
***A. baumannii* DSM-30008**	0.5	0.2 – 0.32	<0.03	<0.03	<0.03	0.125	<0.03	0.125
***E. coli* DSM-1116**	<0.03	0.01	<0.03	<0.03	0.125	<0.03	<0.03	<0.03
***E. coli* WT**	<0.03	0.025	0.125	0.5	1	0.25	0.25	<0.03
***E. coli* WT-3 [*gyrA*(S83L,D87G)]**	0.06 – 0.125	0.32 – 0.8	<0.03	0.125	0.25	0.25	1	0.06
***E. coli* WT-III [*marR*Δ74bp]**	0.125	0.05 – 0.1	0.06	<0.03	0.25	1	0.125	<0.03
***E. aerogenes* DSM-30053**	0.25 – 0.5	0.08 – 0.1	0.5	16	>64	>64	>64	0.5
***E. cloacae* DSM-30054**	0.25 – 1	0.1 – 0.5	0.5	4	0.125	1	0.5	0.125
***P. aeruginosa* ESBL1**	64	3.2 – 6.4	64	>64	>64	32	>64	>64
***P. aeruginosa* ESBL2**	1	0.1 – 0.4	0.5	2	>64	16	>64	64
***K. pneumoniae* DSM-30104**	0.25 – >64	0.01 – 0.1	>64	>64	>64	0.25	>64	>64
***C. freundii* DSM-30039**	<0.03 – 0.125	<0.03	<0.03	<0.03	0.06	0.06	<0.03	<0.03
***S. marcescens* DSM-30121**	64	0.2	>64	>64	>64	64	>64	64
***P. vulgaris* DSM-2140**	0.25 – 0.5	<0.06	0.125	0.06	0.25	0.06	0.25	0.25
***P. mirabilis* DSM-2140**	32 – 64	0.02	64	64	0.25	32	0.125	1
***S. pneumoniae* DSM-20566**	<0.03 – 0.125	0.8	<0.03	<0.03	<0.03	<0.03	<0.03	0.06
***S. aureus* ATCC-29213**	0.25 – 1	0.4 – 0.8	<0.03	<0.03	<0.03	<0.03	<0.03	0.25

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The MIC determination was carried out in two batches. A range of activities is given for the references.

The reference CN-DM 861 showed varying MIC values against *K. pneumoniae* reaching from 0.25 to >64 µg/mL that did not allow for an interpretation of the activity against this strain. None of the compounds were active against *S. marcescens* and the *P. aeruginosa* ESBL1 strain. **59**, **66** and **68** were the derivatives with the broadest spectrum coverage. Especially the alkyne in **59** provided significant improvements compared to CN-DM 861, with enhanced activity against *E. faecalis*, *A. baumannii* and *S. aureus* without drawbacks against other bacterial strains. **68** shared a very similar activity pattern with CN-DM 861 with exceeded potency against *P. mirabilis* but insufficient activity against *P. aeruginosa* ESBL2. The triazole **66** was lacking activity against *E. aerogenes*, but mostly retained the activity against other strains with improvements against *S. aureus*. The derivatives **59, 63**, **64** and **67** provided excellent activity against *A. baumannii*, thereby addressing the secondary goal of the project.

Most of the introduced side chains were assumed to have lower water solubility and higher plasma-protein binding compared to CN-DM 861. Both properties are unfavorable for the *in vivo* application. Because of the superiority of the alkyne **59**, the derivative was tested against multidrug-resistant clinical strains at the Universitätsklinikum Homburg (Table 10).

Table 10: Antibiotic activities of CN-DM 861, **59** and CIP (ciprofloxacin) against susceptible and multiresistant bacteria.

Genus	Species	Median MIC (µg/mL)			Resistance phenotype
		CN-DM 861	**59**	**CIP**	
Enterococcus 17 strains	*Not specified*	64	1	64	17x VRE
Staphylococcus 10 strains	*S. aureus*	4-8	0.125	6.4 – >6.4	5x MRSA, 5x MSSA
Klebsiella 5 strains	*K. oxytoca*	0.5	0.125	0.0125	2x non-resistant, 2x 2MRGN, 1x 4MRGN
Klebsiella 5 strains	*K. pneumoniae*	2	8	>6.4	1x non-resistant, 1x 2MRGN, 1x 3MRGN, 2x 4MRGN
Pseudomonas 11 strains	*P. aeruginosa*	>64	8	3.2	5x 3MRGN, 6x 4MRGN
Acinetobacter 8 strains	*A. johnsinii, A. lwoffii, A. ursingii* and *A. baumannii*	4	0.06 – 0.125	0.1 – 0.2	4x non-resistant, 4x 3MRGN

MIC values determined by Katarina Cirnski at the Universitätsklinikum Homburg[185].

The results against the clinical strains make clear that **59** surpassed the activity of CN-DM 861 against most of the tested multiresistant strains. The only exception was *K. pneumoniae* with a higher susceptibility for CN-DM 861. Surprisingly, the opposite was observed for *K. oxytoca*. In case of *S. aureus* and *Enterococci*, **59** was neither influenced by a resistance against CN-DM 861 nor a resistance against ciprofloxacin. A high activity of **59** was also shown against several different *Acinetobacter* strains that were less susceptible to CN-DM 861. Especially the coverage of *Enterococci* and *Acinetobacter* strains was of high importance for the desired indication.

For the first time, a superiority against ciprofloxacin was determined for several multiresistant strains. Despite the potential *in vivo* instability and undesired physicochemical properties, the alkyne moiety in the central amino acid was utilized for the design of novel compounds.

4.5 Fragment CDE modifications

As mentioned in chapter 3.1.5, the phenol at ring D interacts with the adjacent amide via a hydrogen bond. In the protonated state it was shown that the preferred conformer interacts with the oxygen of the amide (Figure 102)[161]. In the deprotonated state, the phenolate can interact with the NH of the adjacent amide.

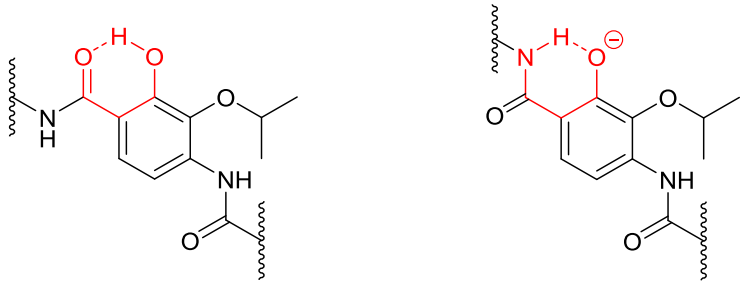


Figure 102: Observed hydrogen bonding of the phenol (left) and predicted hydrogen bonding of the phenolate (right).

The synthesis of biphenyl systems was envisaged to disrupt the crystal packing and enhance the solubility by eliminating these intramolecular interactions. Additionally, the phenol acts as conformational block, restricting both rings to adopt a coplanar conformation (Figure 103)[219].

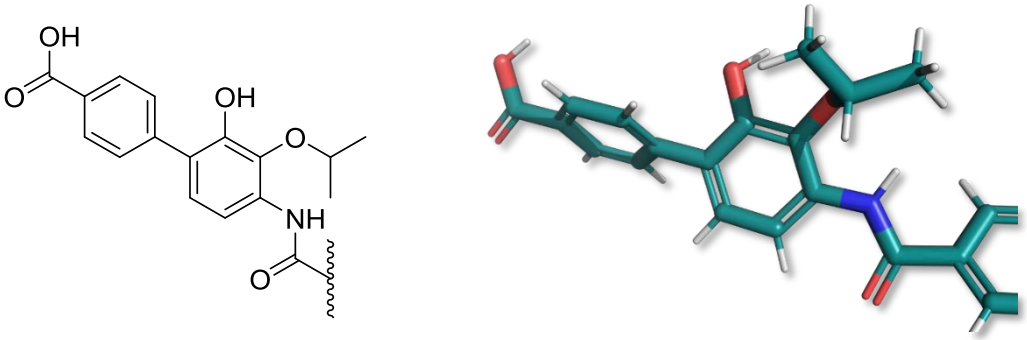


Figure 103: Biphenyl system between ring D and E. Ring D and E are twisted to prevent a steric clash with the phenol. Red = oxygen, blue = nitrogen, white = hydrogen. Image created by PyMOL[169].

The synthesis of the biphenylic system required a new strategy for the synthesis of ring D and the connection of ring D with ring E. The retrosynthetic analysis of the biphenyl led to a disconnection between both aromatic systems (Figure 104).

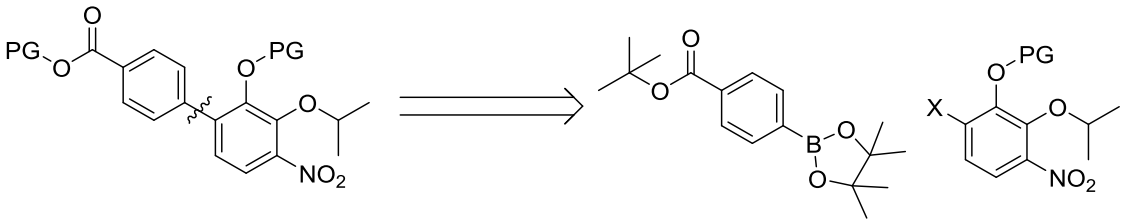


Figure 104: Retrosynthesis of the biphenyl system. PG = protecting group.

A Suzuki coupling was chosen for the formation of the C-C bond, as the boronic acid ester was commercially available and the coupling tolerates various functionalities. The synthesis of ring D required the introduction of a halogen or group like mesyl or tosyl for the oxidative addition of palladium. This group is not only important as leaving group, but also as directing group in the synthesis of the fragment. For this purpose, bromine was chosen.

The first step of the ring D synthesis started with an *ortho*-selective bromination of 2-isopropoxyphenol, mediated by *tert*-butylamine (Figure 105)[220]. The phenol was acetylated to decrease the overall electron density and its directing effect at the aromatic system. In the following nitration, only one regioisomer was formed. Both the isopropoxy and the bromine directed in *ortho* and *para*, but the electron withdrawing effect of bromine suppressed the electrophilic aromatic substitution adjacent to it. After hydrolysis and purification, a methoxymethyl (MOM) protecting group was attached in quantitative yields[221]. The benefit of the MOM protection, compared to the allyl protecting group, is its stability in palladium catalyzed cross couplings. Additionally, the MOM and the *tert*-butyl group can be cleaved off simultaneously under acidic conditions. In this manner, an additional deallylation step was not required.

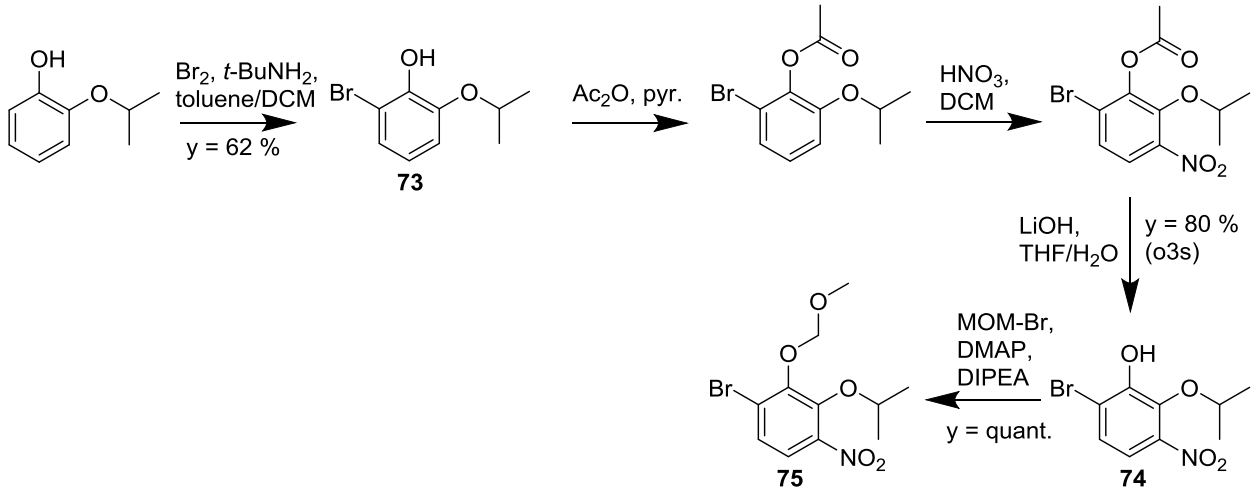


Figure 105: Synthesis of the tetrasubstituted ring D system.

The new synthetic route was probed for its potential to replace the established synthesis of fragment DE, as it required less purifications and offered higher yields and potentially higher flexibility. The bromide was thought to be used in a lithium-halogen exchange or as Grignard reagent to carboxylate ring D directly or to add the species to an isocyanate to form the desired amide between ring D and E (Figure 106).

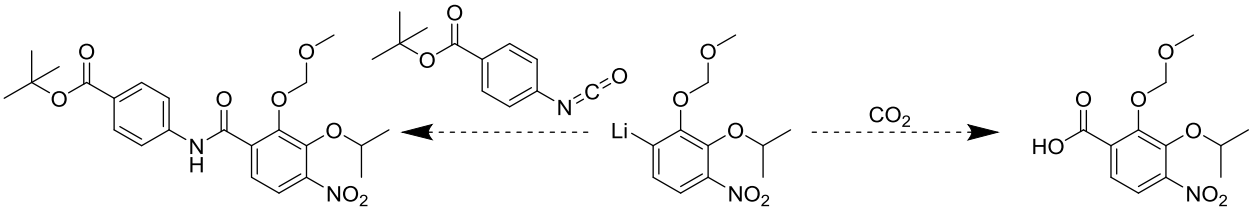


Figure 106: Alternative pathway to synthesize the conventional D and DE fragment via the lithium species.

The lithium-halogen exchange with *n*-butyllithium failed. It was assumed that *n*-butyllithium was not tolerated by the nitro group. Side reactions of organo-lithium reagents and Grignard reagents with nitro groups are known[222],[223]. Surprisingly, the stabilizing effect of the MOM group was not sufficient to prevent a side reaction. Without the possibility of a lithium-halogen exchange or a metal-insertion, the conversion to the carboxylic acid or the amide was not feasible. This also prevented the synthesis of boronic acids and esters from **75** (Figure 107). A Miyaura borylation as alternative synthesis to the boronic ester failed. Also, a stannylation with hexamethyldistannane and hexabutyldistannane was unsuccessful.

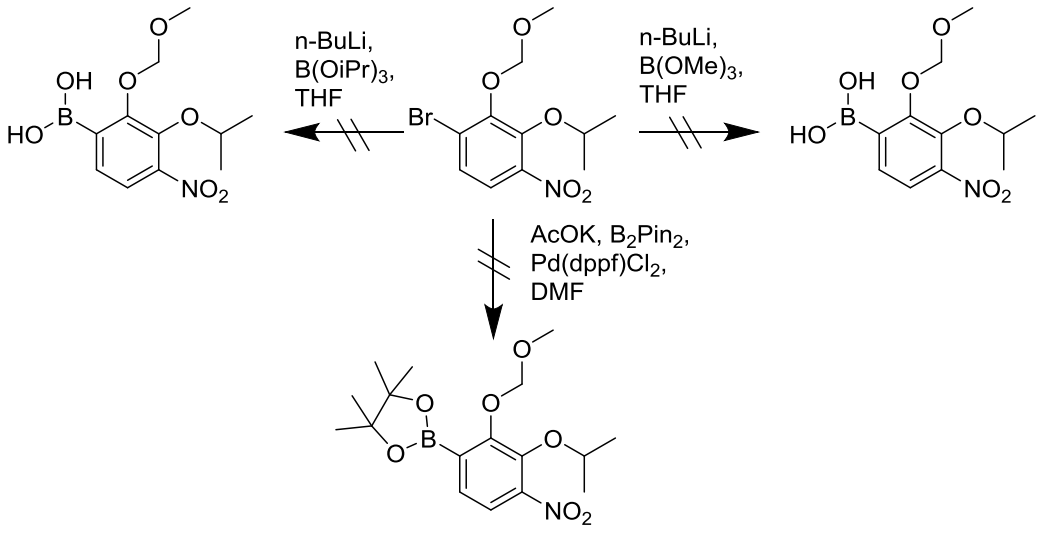


Figure 107: Attempts to synthesize boronic acids and boronic esters from the bromide analogue.

The boronic acids, boronic acid esters and stannanes were important for the synthesis of planar DE systems. Compared to the biphenyl, isoquinoline and quinoline analogues exhibit a rigid conformation due to an intramolecular hydrogen bond (Figure 108). By this interaction, isoquinoline is forced into a conformation similar to the unaltered DE system in which the phenol interacts with the amide oxygen. The quinoline resembles the conformation of the phenol or phenolate with the NH of the amide. Those rigidifications come at the cost of a decreased solubility but allow for the determination of the active conformation.

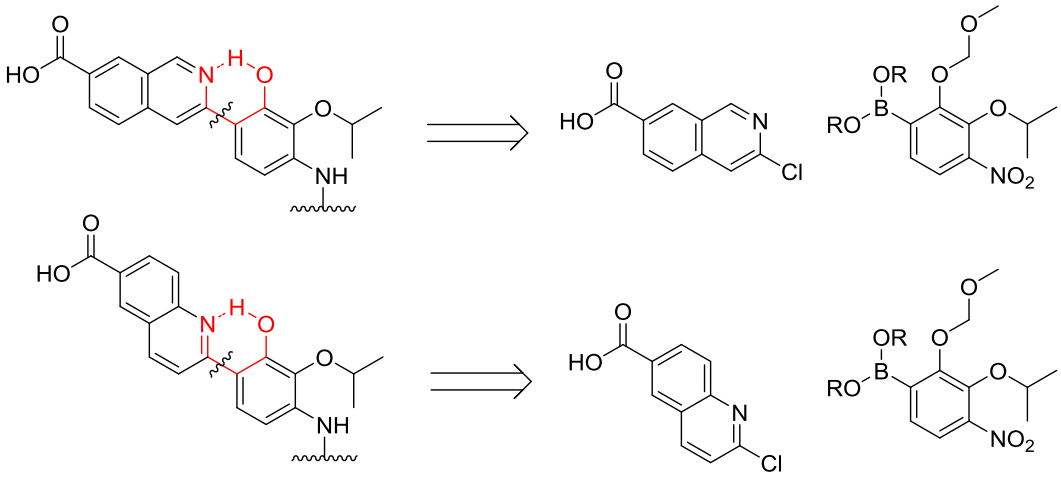


Figure 108: Rigid isoquinoline and quinoline analogues and their retrosynthesis. Intramolecular hydrogen bonds (red) stabilize a coplanar conformation.

As the synthesis of both the quinoline and the isoquinoline required a new strategy, the synthesis of a quinazoline system was prepended. The quinazoline system can adopt both conformers but might show preference for one. The synthesis started with the reduction of the nitro group of methyl 3-formyl-4-nitrobenzoate in 61 % yield (Figure 109). The cyclization with ammonium acetate and simultaneous oxidation under air led to the desired quinazoline system in 42 % yield[224]. The reduction of **77** with zinc opened the quinazoline system. To avoid this side reaction sodium dithionite was used[225]. Due to the formation of other side products, the desired product was obtained in only 14 % yield.

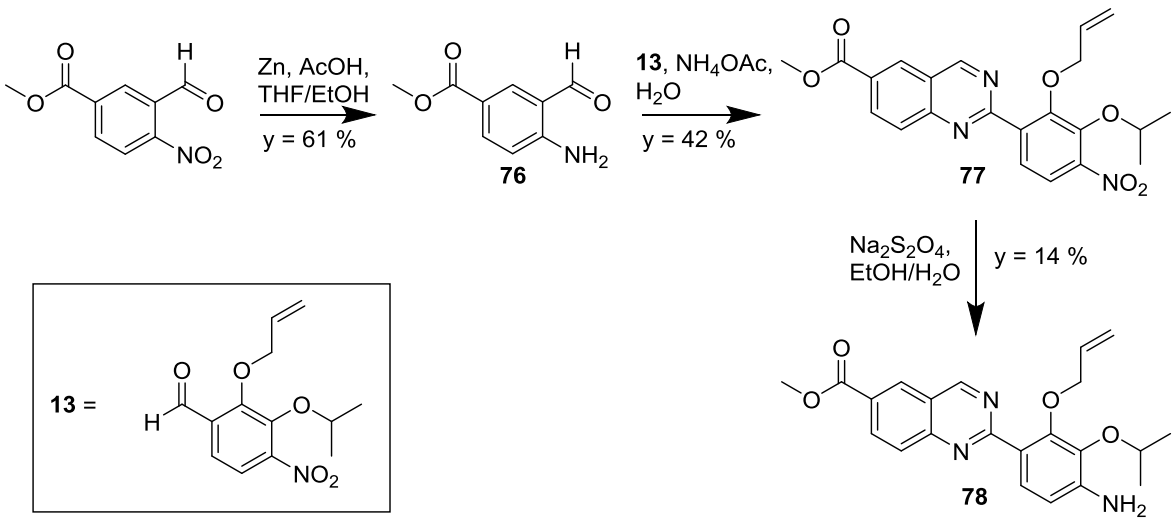


Figure 109: Synthesis of the quinazoline DE fragment.

Because of the susceptibility of the quinazoline for side reactions during the reduction, the fully functionalized A-C fragment **80** was prepared for coupling (Figure 110). This convergent route assured that no other reduction steps were required. For the synthesis of **80**, the standard procedures for the connection of the central amino acid to fragment CDE and fragment AB were applied.

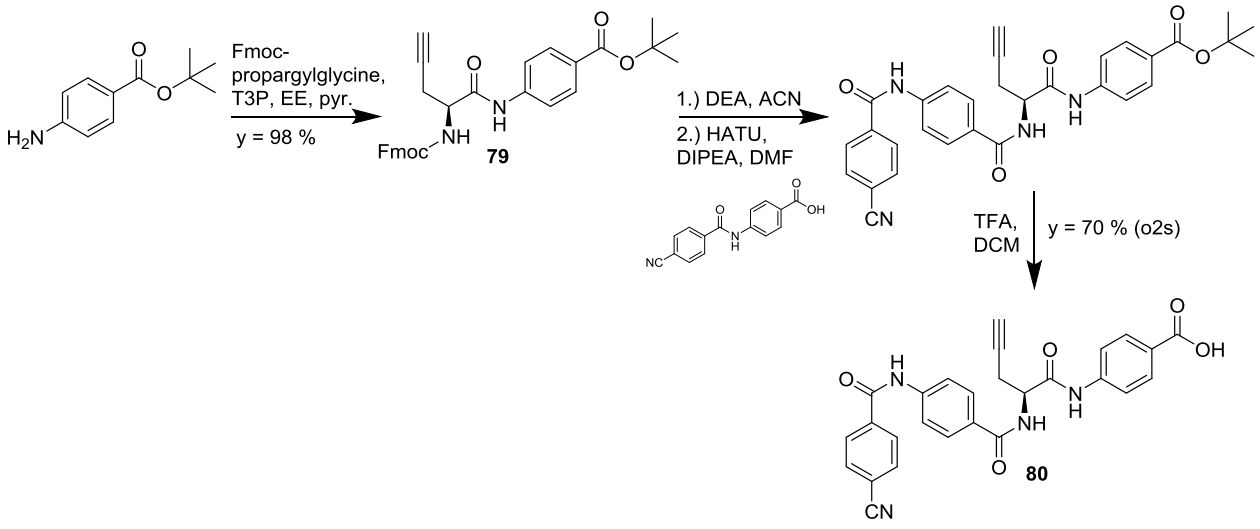


Figure 110: Synthesis of the A-C fragment for the amide coupling with novel DE derivatives.

The amide coupling was carried out with $POCl_3$, as other methods led to insufficient conversion. The allyl group was deprotected under standard conditions (Figure 111). The hydrolysis of the ester with trimethyltin hydroxide failed and a classic hydrolysis with lithium hydroxide was required to obtain the final product **90**.

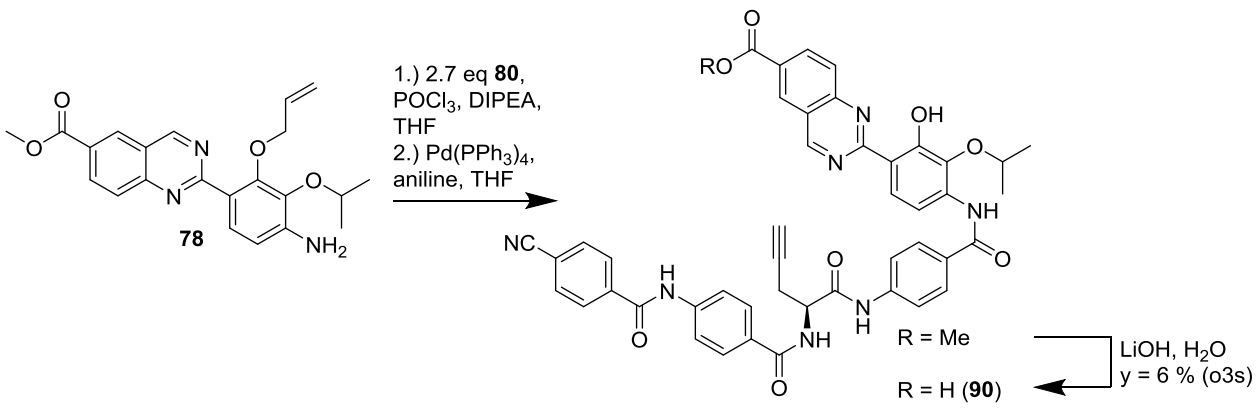


*Figure 111: Amide coupling and global deprotection to the quinazoline analogue **90**.*

As mentioned in the beginning of the chapter, a biphenyl DE system was envisaged to enhance solubility. Nevertheless, the omission of the amide connection led to a shorter DE system. To allow for a similar alignment of the terminal carboxylic acid, a *trans*-cinnamic acid as ring E was introduced (Figure 112). The elongated biphenyl system was obtained by a Suzuki coupling of the MOM-protected ring D bromide **75** with (E)-3-(4-boronophenyl)acrylic acid[226]. Following the protection of the carboxylic acid with allyl bromide, the biphenyl **81** was obtained in 39 % yield over two steps. The reduction with zinc and acetic acid led to the free aniline and unidentifiable side products. To minimize the number of subsequent reduction steps, the crude product was directly coupled with the A-C fragment **80** via BEP. After global deprotection, the product **88** was obtained in 4 % yield over four steps.

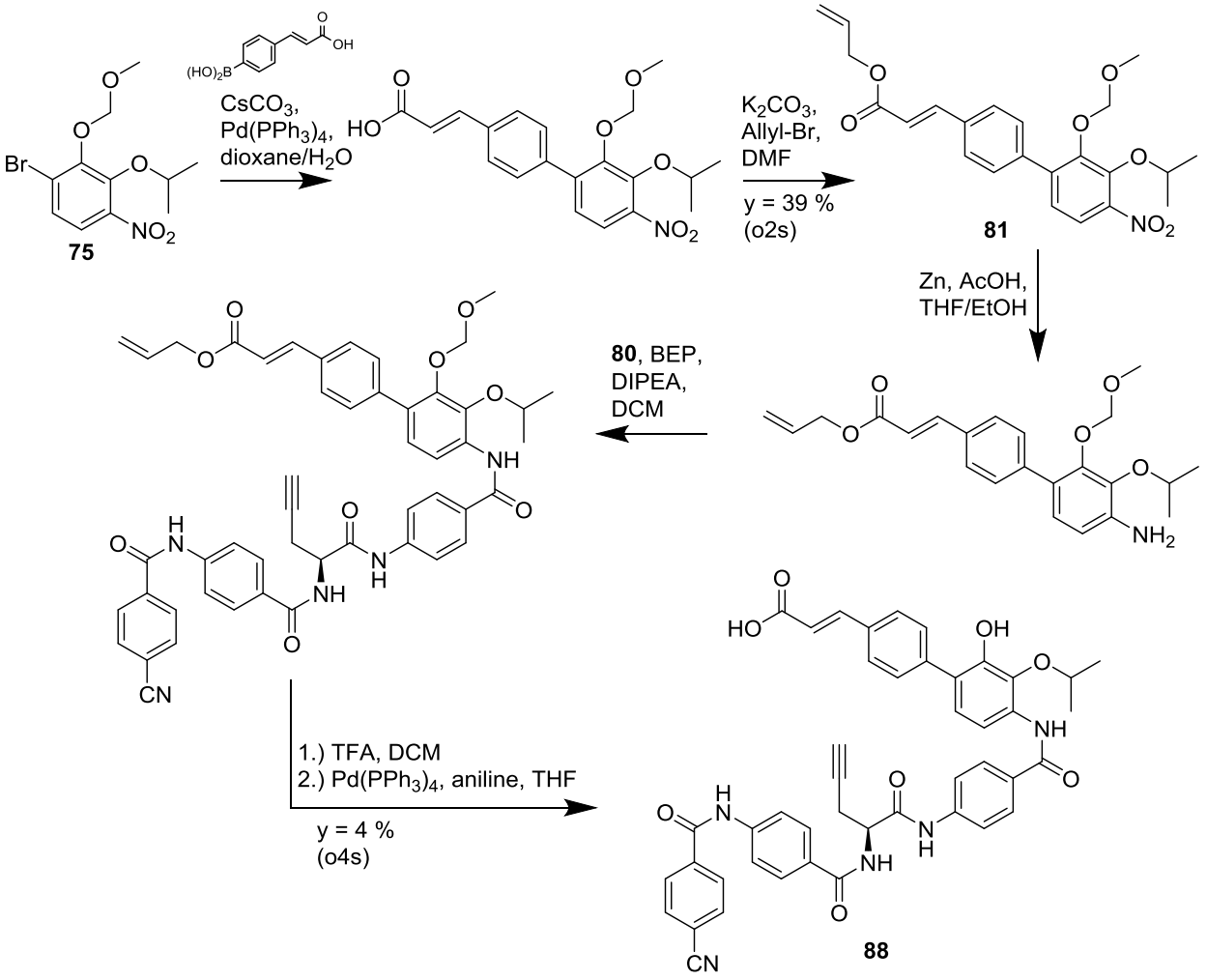


*Figure 112: Synthesis of the cinnamic acid derivative **88**.*

The synthesis of the shorter biphenyl system targeted the complete CDE fragment to avoid the low yielding amide coupling between the A-C fragment and the DE system (Figure 113). The biphenyl system was introduced by a Suzuki coupling of the brominated ring D system with 4-(*tert*-butoxycarbonyl)-phenylboronic acid pinacol ester and was simultaneously hydrolyzed[227]. The MOM protection followed by the nitro reduction gave the biphenyl **83** in quantitative yield. The desired CDE fragment **84** was obtained after amide coupling with 4-nitro benzoyl chloride followed by a nitro reduction with zinc. The remaining steps to the full length cystobactamid **89** were carried out under standard conditions.

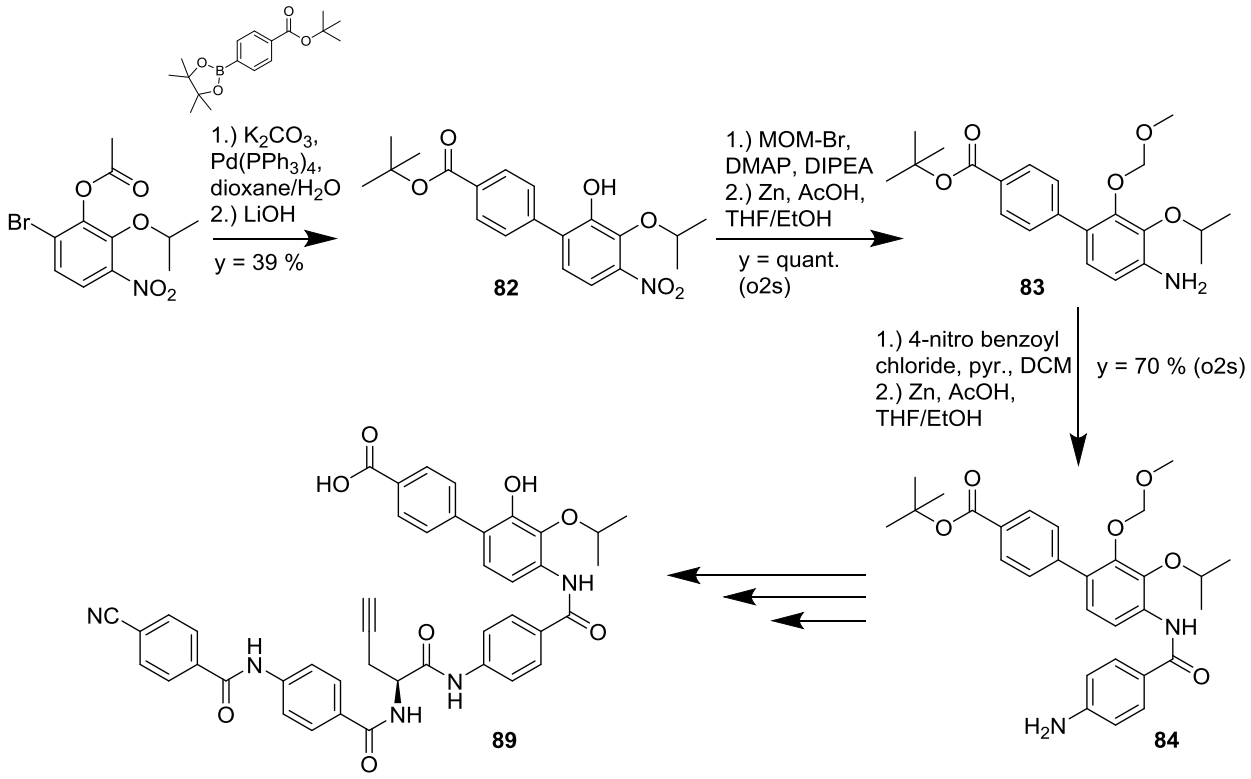


*Figure 113: Synthesis of the biphenyl compound **89**.*

To investigate whether isosteres of the ring D system were tolerated, a benzimidazole analogue was designed. The rationale behind this isostere was that the nitrogen atoms of benzimidazole adopt the same orientation and function as the oxygen atoms of the D ring in the natural product (Figure 114). The benzimidazole exists in two tautomeric forms. Both tautomers can interact with the adjacent amide similarly to the phenol or the phenolate.

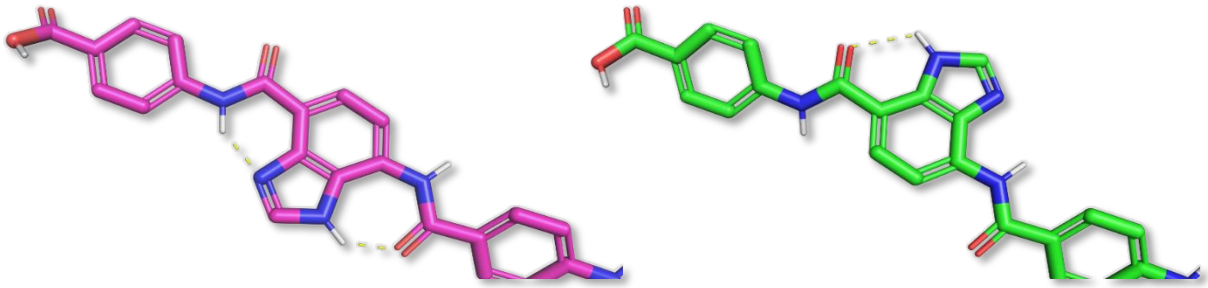


Figure 114: Intramolecular hydrogen bonds of a D benzimidazole, serving as ring D isostere, with the adjacent amide. Red = oxygen, blue = nitrogen, white = hydrogen. Image generated by PyMOL[169].

To allow for a regioselective protection of the benzimidazole, the first synthetic pathway involved the formation of a diamine system by nucleophilic substitution of fluorine (Figure 115). This synthetic pathway failed, as the attempts to oxidize the benzylic position were unsuccessful.

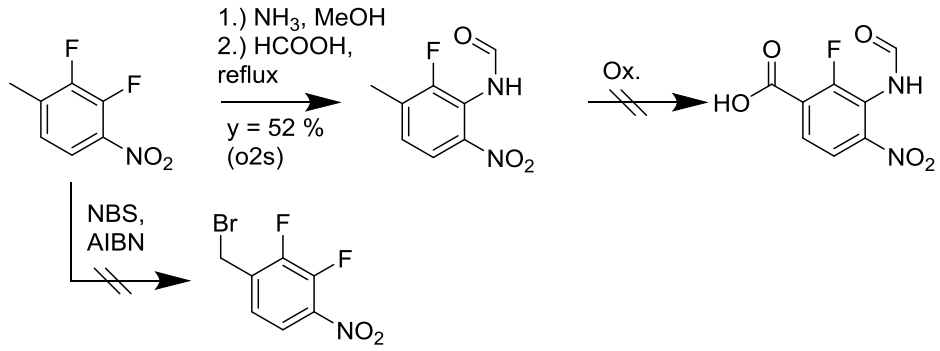


Figure 115: Attempts for the synthesis of the benzimidazole system.

An alternative route via a vicarious aromatic substitution with trimethylhydrazinium iodide (TMHI) was carried out to get to the desired diamine (Figure 116)[228]. The condensation with trimethyl orthoformate yielded only one tautomer, as seen by NMR analysis. It was assumed that the intramolecular hydrogen bond between the NH and the nitro group stabilizes the tautomer **86**. An allyl protection and hydrolysis of the nitrile yielded two regioisomers with free carboxylic acid in 67 % yield.

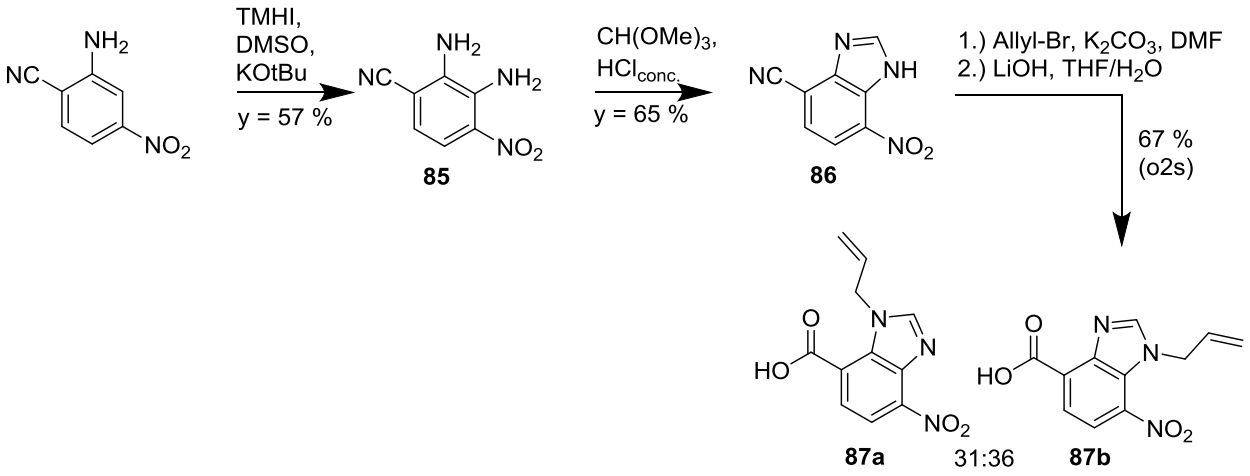


Figure 116: Synthesis of the benzimidazole analogue.

The remaining steps to fragment CDE were carried out with **87a** by the standard procedures (Figure 117). Unfortunately, the amide coupling with the central amino acid failed and gave only traces of the desired product. The other regioisomer **87b** was converted to the respective DE analogue. The coupling to the PABA elongated central amino acid failed.

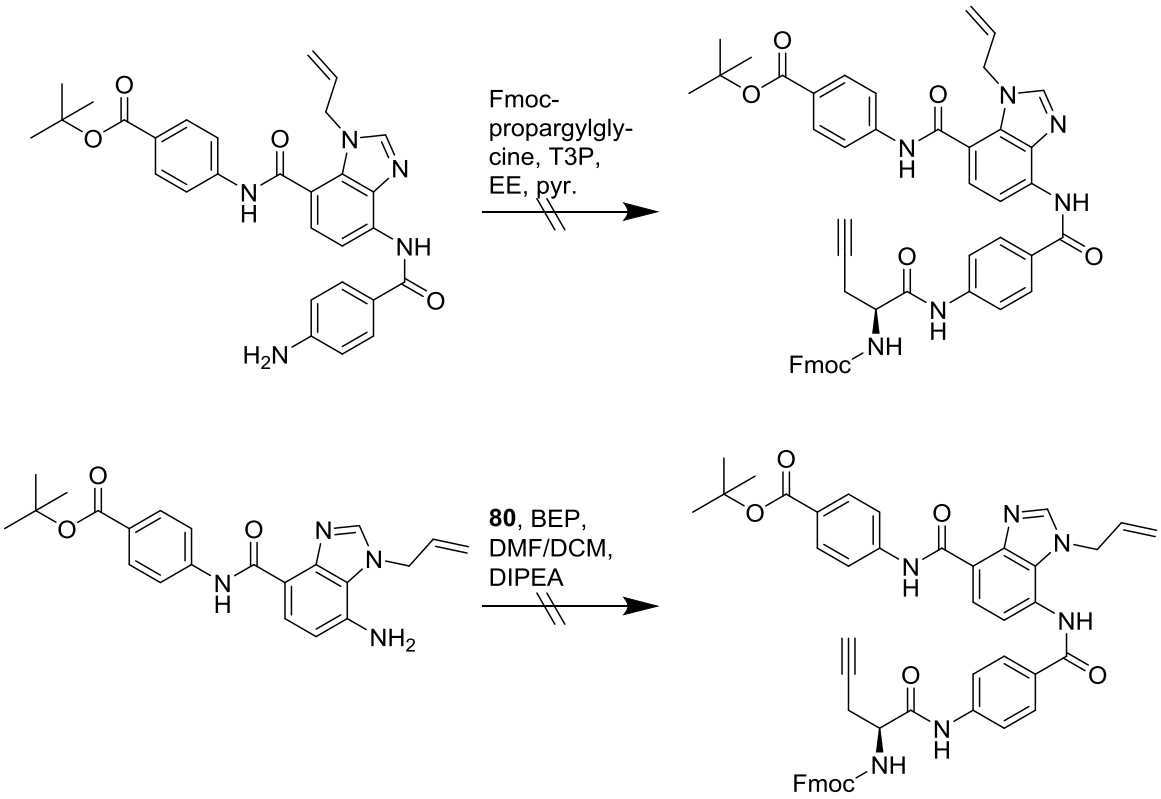
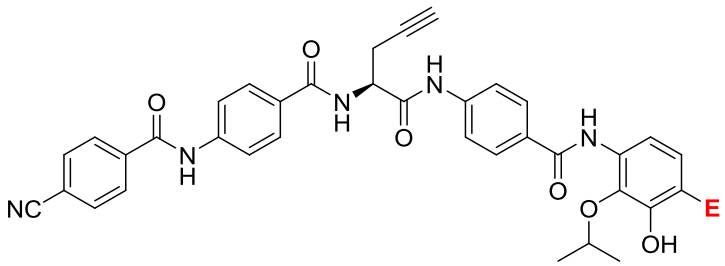


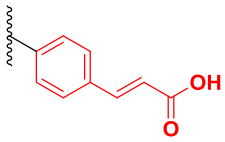
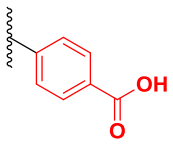
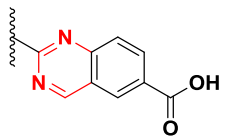
Figure 117: Attempts for the amide coupling of the D benzimidazole building blocks.

It was assumed that the benzimidazole itself showed higher nucleophilicity than the aniline and led to the benzimidazolium salt. Surprisingly, an excess of the carboxylic acid and the other coupling reagents was not able to facilitate the reaction. An alternative strategy for the synthesis of the benzimidazole analogue was required. Presumably, the introduction of an electron-withdrawing protecting group at the benzimidazole, like Boc or Alloc, would decrease its nucleophilicity.

The biological activity of the directly connected DE analogues is depicted in Table 11.

Table 11: Antibiotic activities of cystobactamid analogues with directly connected DE systems in comparison to CIP (ciprofloxacin).



		MIC [µg/mL]					IC_{50} [µM]
Compound	System for **E**	*E. coli* WT (MG1655)	*E. coli CH448 (S83L, QnrS)*	*S. a.* ATCC-29213	*P. aeruginosa* WT (Pa14)	Pa14*Δ mexAB*	*E. coli* gyrase
88		>64	>64	>64	>64	>64	0.37
89		>64*	<0.03*	>64*	>64	>64	n.d.
90		>64	>64	>64	>64	>64	n.d.
CIP		0.01 – 0.125	>6.4	0.4	0.2	0.05 – 0.1	0.18

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186]. *** 89** *was tested against E. coli* BW25113, *E. coli ΔacrB* and S. aureus Newman instead of the mentioned strains. *The MIC determination was carried out in three batches. A range of activity was given for the reference.*

The direct connection of ring D to E with a biphenyl system was not tolerated and led to a complete loss of activity. Surprisingly, the IC_{50} value of **89** indicated that the compound is still highly active against *E. coli* gyrase. The phenols in **88** and **89** acted as conformational block and prevented a coplanar structure of both aromatic systems[219]. In contrast, the loss of activity for **90** suggested that the lack of coplanarity in **88** and **89** was not the only reason for their inactivity. However, the relevance of **90** for the SAR was ambiguous, as a deprotonation of the phenol in **90** leads to a twisted conformation similar to **88** and **89**, whereas the neutral **90** was predicted to be planar.

4.6 Combination of structural changes

To determine whether compound **59** was qualified as a preclinical candidate, the *in vivo* activity in mice was investigated. Due to the elimination of the amide side chain and the introduction of the alkyne, unfavorable physicochemical properties and low metabolic stability were likely. The cytotoxicity assay performed by Katharina Rox indicated a low cytotoxicity against the HepG2 and CHO cell lines with IC_{50}'s of 30 µM and > 100 µM, respectively[138]. Additionally, the plasma stability in mice was sufficient with around 80 % of the residual compound remaining after four hours[138]. *In vivo* experiments in mice were carried out with ciprofloxacin, CN-861 and the lead structure CN-DM 861 as competitors. **59** was dosed up to 80 mg/kg per day. At the highest dose, a bacteriostatic effect was reached, corresponding to a reduction of colony forming units (CFU) by two to three log units. However, **59** was outperformed by CN-861 and CN-DM 861 (Figure 118)[229].

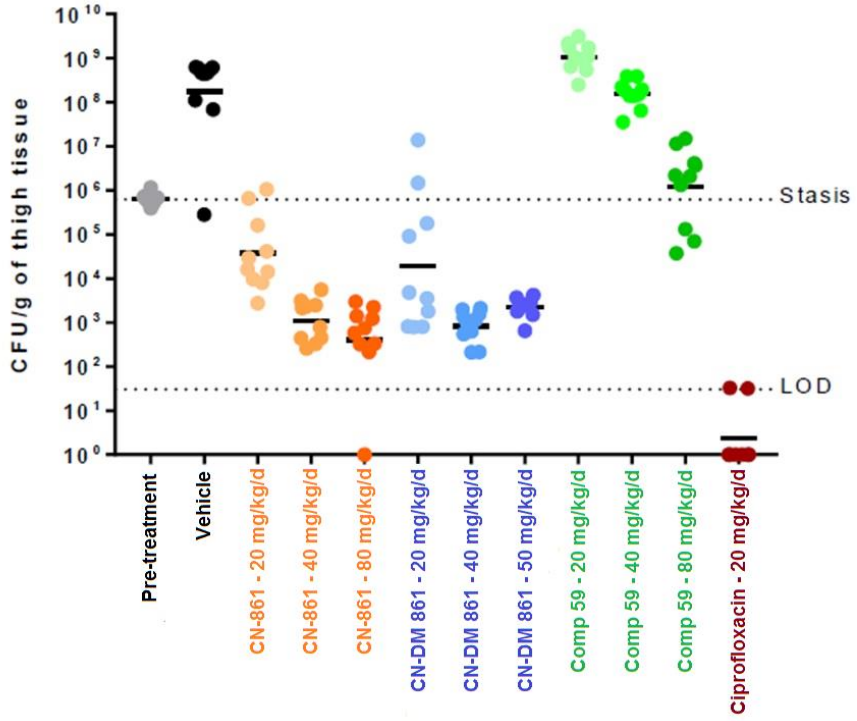


Figure 118: In vivo efficacy of cystobactamids in a thigh infection mouse model (E. coli). Dosing q6h. The experiment was carried out by Evotec[229].

A subsequent investigation of the poorly active **59** revealed a six-fold decreased microsomal stability compared to the lead structure. A MIC determination in presence of 6 % BSA revealed a shift in activity of 256-fold for all cystobactamids while the activity of ciprofloxacin was only shifted by four-fold[230].

As expected, the solubility of **59** was decreased and below the detection limit (Table 12)[230]. The combined information suggested that an improvement of the metabolic stability and the physicochemical properties were crucial for the development of a drug candidate.

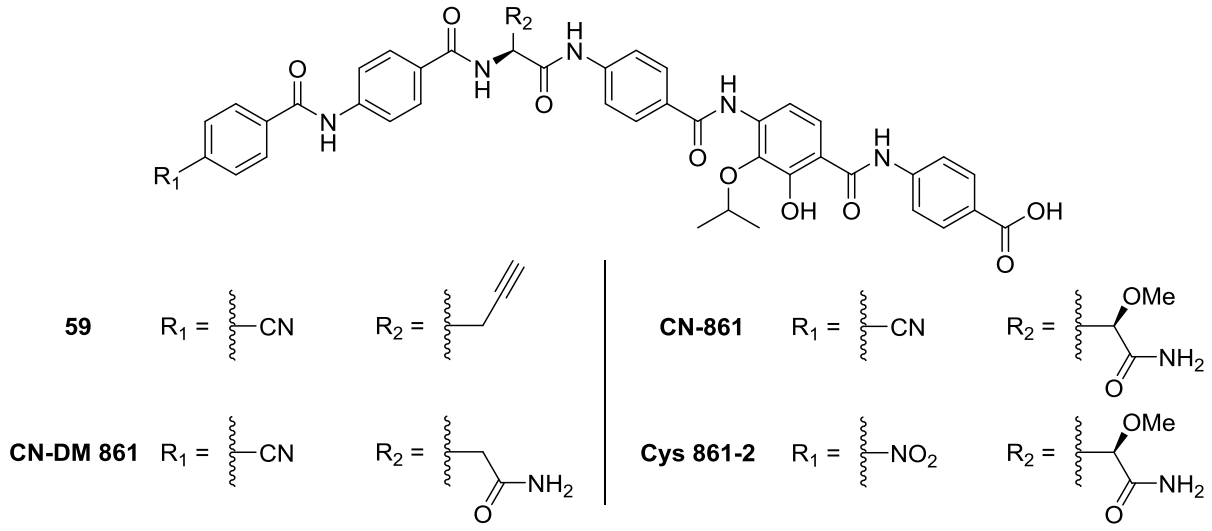


Table 12: Aqueous solubility of **59**, CN-DM 861, CN-861 and Cys 861-2 at different pH values.

Compound	$LogD_{7.4}$	Aqueous solubility (µg/ml)		
		pH 7.4	**pH 9**	**pH 5**
59	2.26	BDL	BDL	BDL
CN-DM-861	1.49	<1	56	<1
CN-861	1.56	10	956	n.d.
Cys 861-2	1.76	<1	>1000	n.d.

BDL = below detection limit. Values determined by Evotec[230].

4.6.1 Cystobactamids with two structural alterations

To improve the physicochemical properties of **59**, an introduction of a solubilizing group or the disturbance of the crystal packing can be applied. So far, the addition of substituents was not beneficial for activity at most of the aromatic systems. A disturbance of the crystal packing by a biphenyl system was also not tolerated. The triazole **66** and the amine **68** in the side chain were well tolerated. In case of the latter, a zwitterionic compound is generated.

Another possibility to disturb the crystal packing is the introduction of sp^3 centers, as non-aromatic residues do not participate in π-π interactions. Analogues of the *trans*-cyclohexyl moiety, present in **37**, were chosen. Although this modification of ring B was not beneficial for activity, it showed moderate activity against *E. coli* and *S. aureus*. Instead of this rather flexible moiety, [1.1.1]bicyclopentane and cubane systems were integrated (Figure 119). In terms of calculated distance, the [1.1.1]bicyclopentane with 1.9 angstrom is significantly shorter than the cubane with 2.6 angstrom or benzene with 2.8 angstrom[169]. Nevertheless, they share high similarity with the topology of phenyl and provide a high rigidity that can contribute to the activity. A combination with the alkyne side chain, as in **59,** or the amine, as in **68**, can counteract the loss of activity by the aliphatic system, if both residues influence the activity independently.

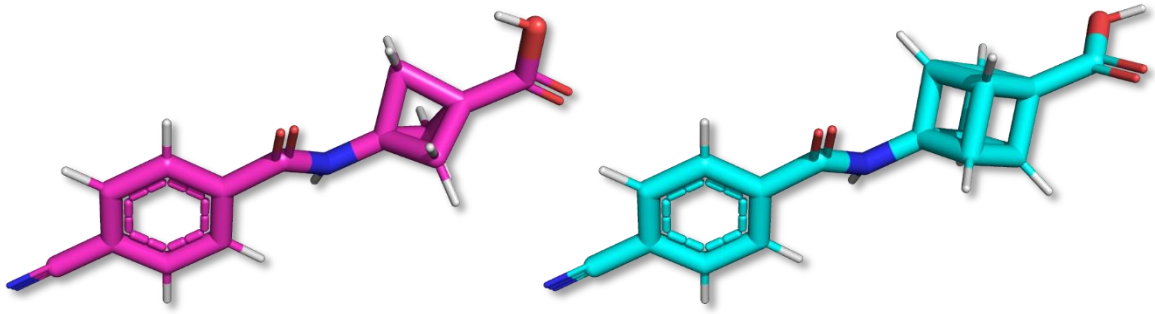


Figure 119: Structure of fragment AB analogues with [1.1.1]bicyclopentane (magenta) and cubane (cyan). Red = oxygen, blue = nitrogen, white = hydrogen. Image created by PyMOL[169].

The methyl ester of the [1.1.1]bicyclopentane building block was commercially available and allowed the application of the standard amide coupling with the acid chloride of ring A (Figure 120). In the subsequent deprotection of the methyl ester, trimethyltin hydroxide was superior to lithium iodide with a yield of 66 %.

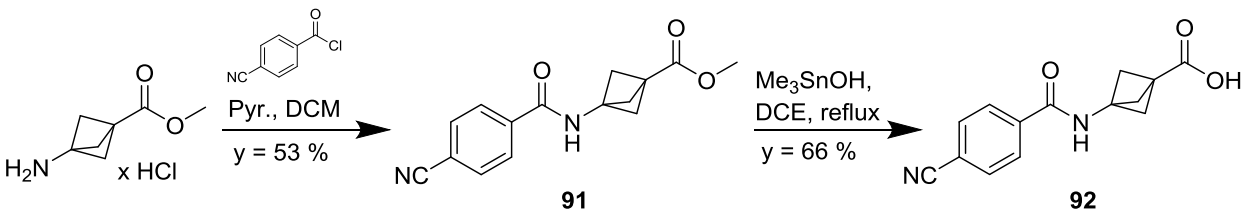


*Figure 120: Synthesis of the [1.1.1]bicyclopentane AB fragment **92**.*

The AB fragment **92** was coupled to the L-propargylglycine and the L-2,3-diaminopropionic acid building block and subsequently deprotected, according to the described procedures from chapter 4.1.3. The synthesis for the L-propargylglycine building block is depicted in Figure 121. The established route allowed the synthesis of **99** on an 800 mg scale.

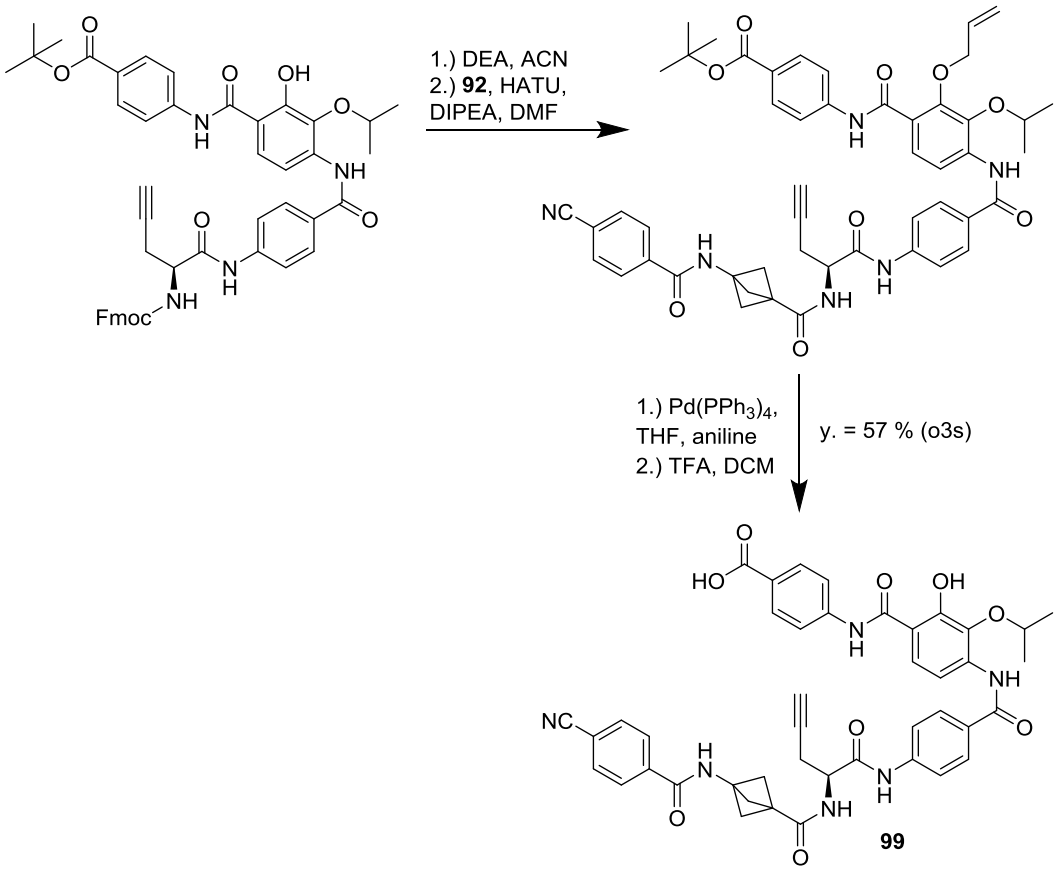


*Figure 121: Synthesis of the [1.1.1]bicyclopentane analogue **99**.*

The desired cubane building block was not commercially available and had to be synthesized from the carboxylic acid by a published synthesis (Figure 122)[231]. In the first step a Curtius rearrangement with diphenylphosphoryl azide (DPPA) in refluxing *tert*-butanol led to the Boc-protected amine. The Boc-protecting group was cleaved off by HCl generated *in situ* by addition of acetyl chloride to methanol leading to **93**. The resulting hydrochloride was coupled with ring A and deprotected with trimethyltin hydroxide like the [1.1.1]bicyclopentane **92**.

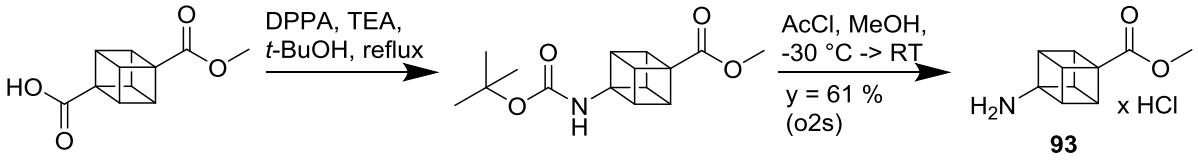


Figure 122: Synthesis of the cubane building block.

Cubanes rearrange to cuneanes under palladium(II) and silver(I) catalysis (Figure 123)[232]. As the allyl deprotection requires the use of palladium, the allyl protection group was removed from the amino acid CDE fragment beforehand. After amide coupling and deprotection, the cubane derivative **100** was obtained.

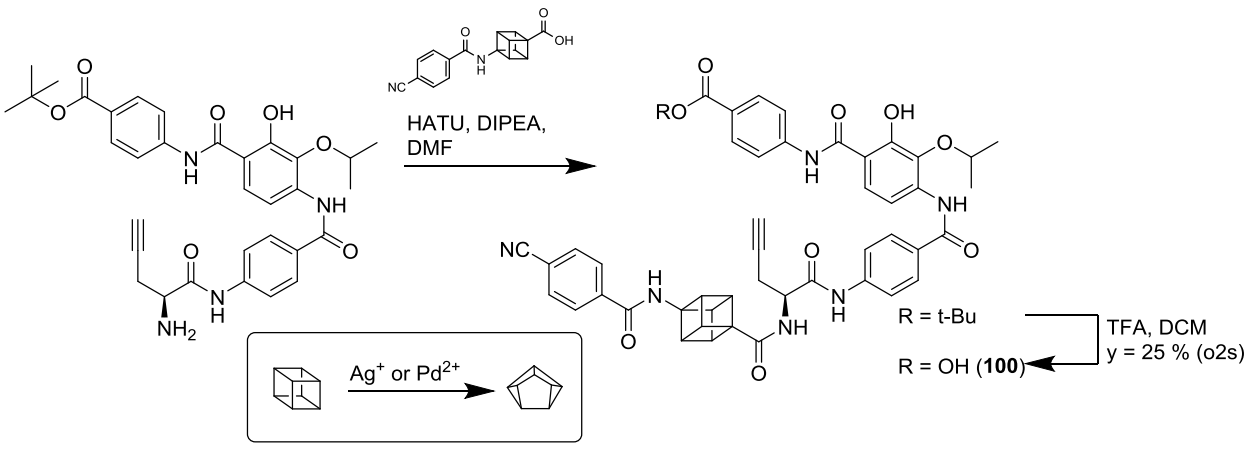


Figure 123: Amide coupling and deprotection of the cubane analogue. The circled image shows the isomerisation of cubane under silver(I) or palladium(II) catalysis. Image adapted from[232].

Since the cystobactamid scaffold only tolerates very small substituents at ring A and B, the attachment of solubilizing groups at the AB system is rather difficult. However, as demonstrated by the stilbene **52**, *E. coli* gyrase offers space for the addition of a residue adjacent to ring A (Figure 124).

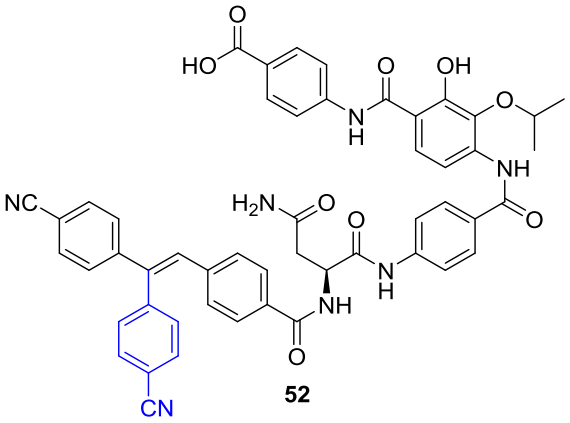


*Figure 124: Structure of the stilbene compound **52**.*

Due to the tolerance for another aryl system adjacent to ring A, it was presumed that this position can be exploited for the introduction of a solubilizing group. The synthesis started with the conversion of 4-cyanophenylacetic acid to the respective acid chloride and esterification with allyl alcohol to give **94** (Figure 125). After aldol condensation with *tert*-butyl 4-formylbenzoate and deprotection, the AB fragment **95** was obtained.

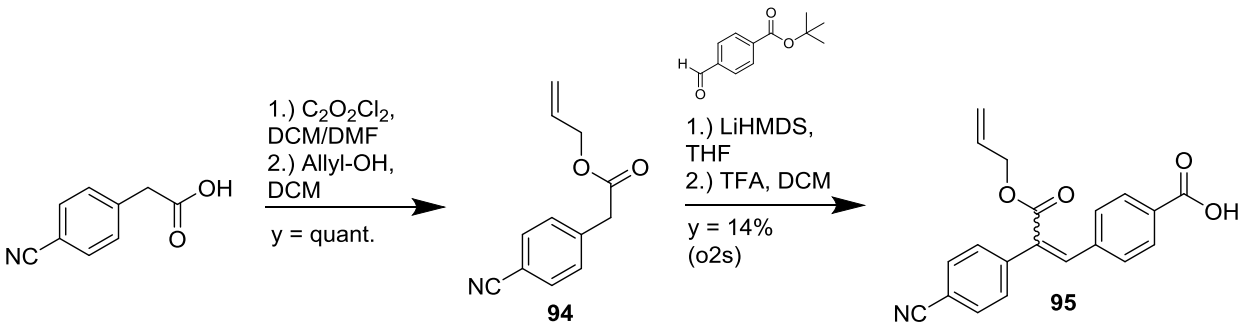


Figure 125: Synthesis of the allyl protected carboxy stilbene analogue.

As former findings indicated a high benefit of a HBA in position 4 of ring A, it was assumed that an enhanced electron density at this function might be beneficial for binding and activity. To circumvent the unfavorable introduction of new substituents, the amide between ring A and B was inversed (Figure 126). From the SAR of ring B, it was expected that the derivative shows low activity against *P. aeruginosa* wild type because of the electron poor aromatic system. If tolerated, this inversed amide can be of great value for the synthesis of [1.1.1]bicyclopentane and cubane analogues, as the amines are made from the respective carboxylic acid. Additionally, both analogues do not exhibit an aromatic system and might not be influenced by the electron withdrawing effect of the inversed amide. Like **95**, the AB fragment **97** was coupled with the L-propargylglycine fragment according to the described procedures in chapter 4.1.3.

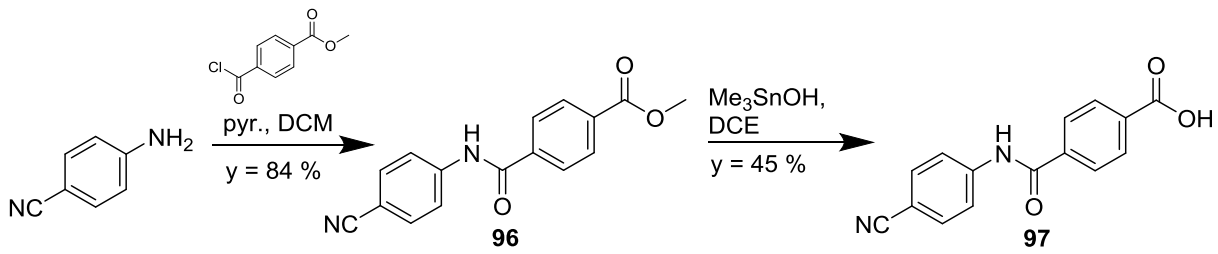


Figure 126: Synthesis of the building block with an inversed amide bond.

Synthetic efforts of Danny Solga at the Leibniz University Hanover were showing a beneficial influence of a ring C pyridine. The compound DS158 was showing superior activity against *P. mirabilis*, *A. baumannii* and *E. cloacae*[185]. To investigate a synergetic effect with other central amino acids, the 5-aminopicolinic acid was introduced as ring C system (Figure 127). The CDE fragment was coupled with the L-propargyl-glycine, that was also present in **59**, and converted to the full length cystobactamid **103** under standard procedures.

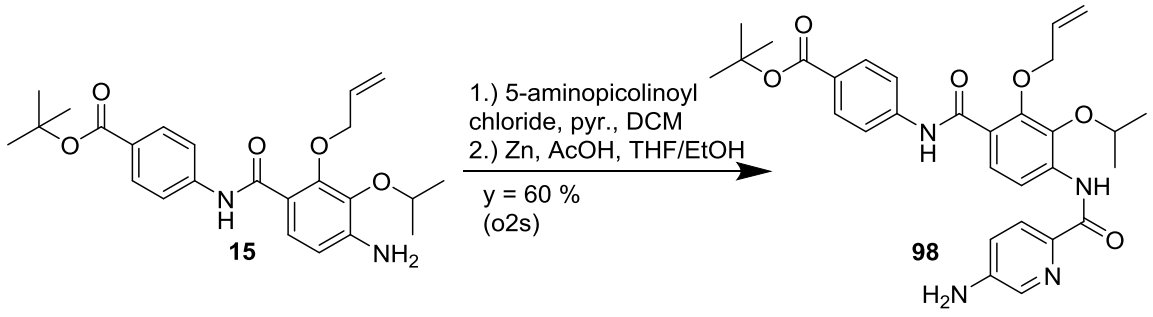


Figure 127: Introduction of 5-aminopicolinic acid into the CDE fragment.

A fraction of **103** was utilized in a “click”-reaction to the triazole **104** catalyzed by tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA) (Figure 128).

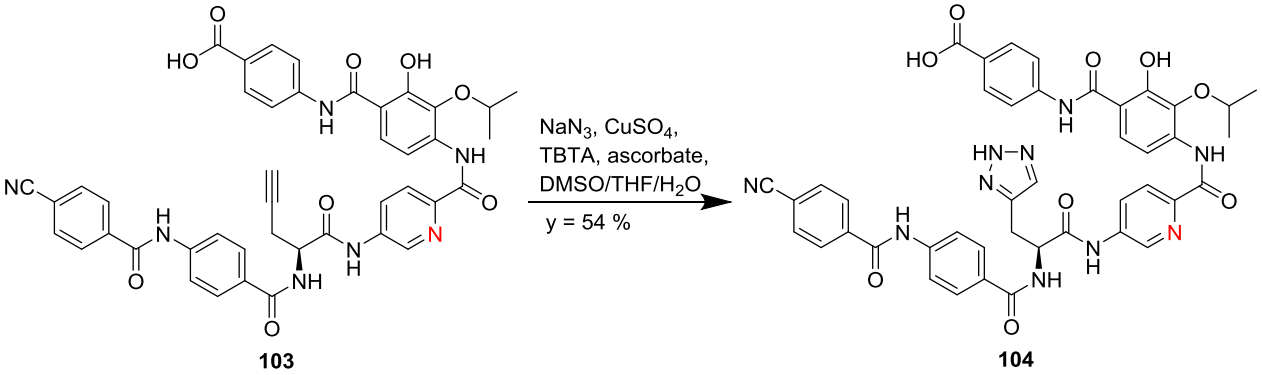
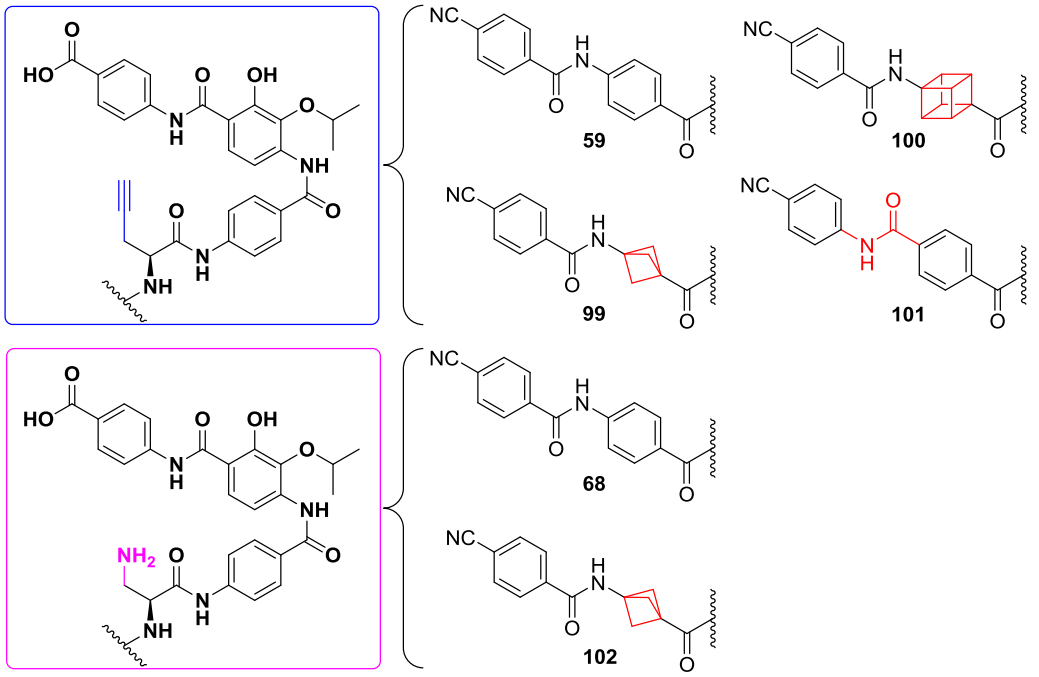


*Figure 128: Copper(I)-catalyzed azide-alkyne cycloaddition of **103** to the triazole **104**.*

The cystobactamid analogues with two structural alterations were directly tested on the extended panel of pathogenic bacteria (Table 13 to Table 15).

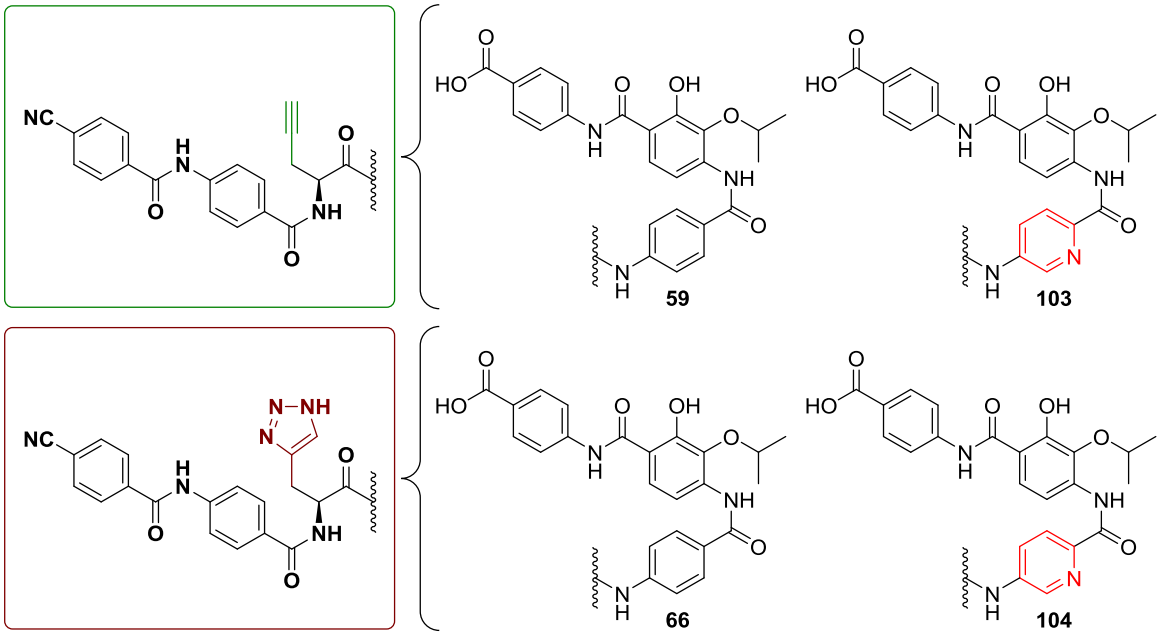
Table 13: Antibiotic activities of doubly modified analogues on an extended panel of pathogenic bacteria. CN-DM 861 and CIP (ciprofloxacin) were added as competitors.



	MIC (µg/mL)							
	CN-DM 861	**CIP**	**59**	**99**	**100**	**101**	**68**	**102**
***E. coli* MG1655/K12**	0.02	0.01	0.05	0.08	0.25	<0.03	≤ 0.03	1
***E. coli* LM705 (S83L, D87N, S80I, Δ*marR*, Δ*acrR*)**	0.25	>6.4	0.2	1	0.5	0.25-0.125	0.5	8
***E. coli* CH448 (S83L, QnrS)**	0.02	>6.4	0.04	0.16	0.25	<0.03	≤ 0.03	0.5
***K. pneumoniae* DSM-30104**	1	0.16	0.5	1	2	2	0.06	2
***K. pneumoniae* CIP-104298**	4	0.025	8	0.25	2	0.125	0.25	4
***K. pneumoniae* KP10581 (*waaC*::Tn30)**	4	>6.4	0.25	1	1	0.125	0.125	16
***A. baumannii* DSM-30008**	1	0.2	0.02	1	1	<0.03	0.5	8
***A. baumannii* ATCC BAA-1710**	>64	>6.4	0.25	0.5	n.d.	n.d.	2	n.d.
***P. mirabilis* DSM-4479**	64	0.05	8	0.064	0.125	4	1	1
***P. mirabilis* ATCC BAA-2081**	64	>6.4	4	0.06	0.5	2	2	0.5
***P. vulgaris* DSM-2140**	0.5	0.006	0.125	≤0.03	0.125	0.0625	0.125	1
***C. freundii* DSM-30039**	0.125	0.01	0.06	0.06	0.25	0.0625	≤0.03	1
***S. marcescens* DSM-30121**	64	0.2	64	≤0.03	4	8	0.5	1
***E. cloacae* DSM-30054**	0.5	0.25	0.5	4	16	>64	0.06	2
***P. aeruginosa* PA14**	32	0.125	8-16	16	8	>64	>64	64
P. aeruginosa* PA14Δ*mexAB	2	0.02	0.5	1	0.5	1	1	4
***S. aureus* ATCC-29213**	2	0.4	0.02	0.06	0.125	<0.03	0.25	8

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185].

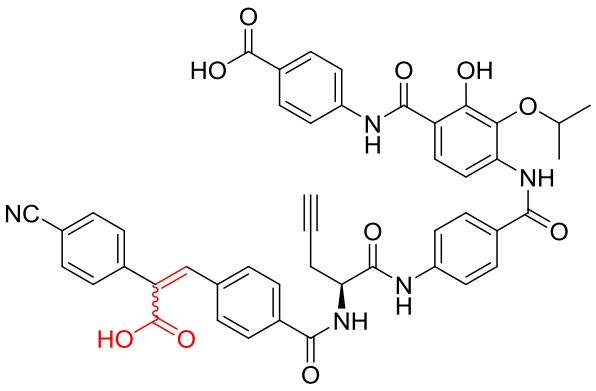
Table 14: Antibiotic activities of doubly modified analogues on an extended panel of pathogenic bacteria with CIP (ciprofloxacin) as competitor.



	MIC (µg/mL)				
	CIP	**59***	**103**	**66**	**104**
***E. coli* AG100*/K12**	0.001	0.0005	<0.03	<0.03	<0.03
***E. coli* LM705 (S83L, D87N, S80I, Δ*marR*, Δ*acrR*)**	>6.4	0.2	<0.03	0.25	<0.03
***E. coli* CH448 (S83L, QnrS)**	>6.4	0.04	<0.03	<0.03	<0.03
***K. pneumoniae* DSM-30104**	0.16	0.5	4	0.25	>64
***K. pneumoniae* CIP-104298**	0.025	8	8	4	>64
***K. pneumoniae* KP10581 (*waaC*::Tn30)**	>6.4	0.25	0.5	1	2
***P. mirabilis* DSM-4479**	0.01	8	1	16	32
***P. mirabilis* ATCC BAA-2081**	>6.4	4	4	16	8
***P. aeruginosa* PA14**	0.1	8-16	4-16	8	>64
P. aeruginosa* PA14Δ*mexAB	0.025	0.5	0.5	0.5	0.25
***A. baumannii* DSM-30008**	0.16	0.02	<0.03	2	0.5
***A. baumannii* ATCC BAA-1710**	>6.4	0.25	<0.03	1	4
***P. vulgaris* DSM-2140**	0.006	0.125	0.06	<0.03	<0.03
***C. freundii* DSM-30039**	0.0125	0.06	<0.03	<0.03	<0.03
***S. marcescens* DSM-30121**	0.1	64	>64	4	>64
***E. cloacae* DSM-30054**	0.5	0.5	0.25	0.5	0.25
***S. aureus* ATCC-29213**	0.1	0.02	<0.03	<0.03	<0.03

*MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. *MIC determination of **59** in a separate batch.*

Table 15: Antibiotic activities of **105** on the extended panel of pathogenic bacteria with CN-DM 861, CIP (ciprofloxacin) **59** and **66** as competitors.



105

	MIC (µg/mL)				
	CN-DM 861	**CIP**	**59**	**66**	**105**
***E. faecalis* ATCC-29212**	0.5	0.8	<0.03	<0.03	32
***S. epidermidis* DSM-28765**	<0.03	0.2	<0.03	<0.03	>64
***A. baumannii* DSM-30008**	0.5	0.2	<0.03	0.125	64
***E. coli* DSM-1116**	<0.03	0.0125	0.06	<0.03	>64
***E. coli* WT**	<0.03	0.025	0.125	0.25	>64
***E. coli* WT-3 [*gyrA*(S83L,D87G)]**	0.125	0.8	0.125	0.25	>64
***E. coli* WT-III [*marR*Δ74bp]**	0.125	0.1	0.06	1	>64
***E. aerogenes* DSM-30053**	0.5	0.08	1	>64	>64
***E. cloacae* DSM-30054**	1	0.5	0.5	1	>64
***P. aeruginosa* ESBL1**	n.d.	3.2	64	32	>64
***P. aeruginosa* ESBL2**	n.d.	0.4	4	16	>64
***K. pneumoniae* DSM-30104**	0.25	0.1	n.d.	0.25	>64
***C. freundii* DSM-30039**	0.125	0.0125	0.06	0.06	32
***S. marcescens* DSM-30121**	64	0.2	64	64	>64
***P. vulgaris* DSM-2140**	0.5	0.0125	0.125	0.06	>64
***P. mirabilis* DSM-2140**	32	0.025	8	32	>64
***S. pneumoniae* DSM-20566**	0.125	0.8	<0.03	<0.03	0.5
***S. aureus* ATCC-29213**	1	0.8	<0.03	<0.03	>64

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185].

The combination of the [1.1.1]bicyclopentane and the alkyne in **99** was well tolerated. Although slightly less active against *E. cloacae* and one of the two *A. baumannii* strains, the overall activity of **59** was retained. The modification allowed the coverage of *S. marcescens* and depicted less fluctuations against the *K. pneumoniae* strains. Compared to **99**, the cubane analogue **100** was of similar activity with minor disadvantages. In line with the observed SAR at this position, the bigger size of the cubane might contribute to its decreased antibiotic properties. The combination of the [1.1.1]bicyclopentane and the amine side chain in **102** was significantly worse than its aromatic analogue **68**. The carboxy stilbene analogue **105** was completely inactive.

The inversed amide **101** was well tolerated with similar activity to **59**. As expected, this modification led to inactivity against the *P. aeruginosa* wild type. Enhanced activity was found against one *K. pneumoniae* strain and *S. marcescens* at the cost of the inactivity against *E. cloacae*. As mentioned before, a combination with the cubane or the [1.1.1]bicyclopentane might reduce these flaws.

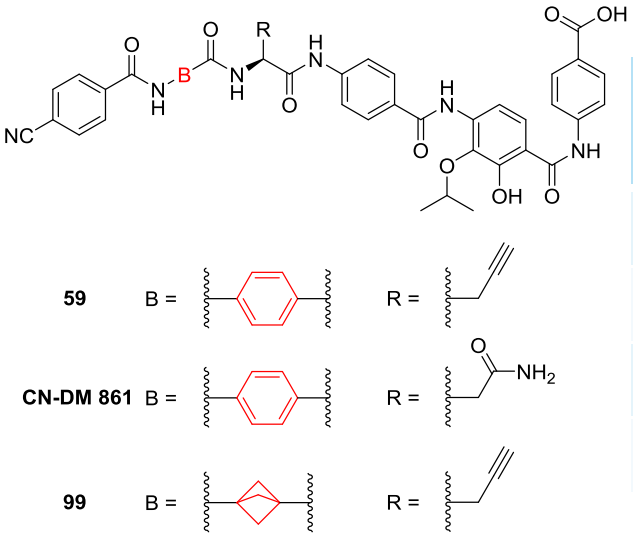
The introduction of the pyridine system was not beneficial. While the combination with the alkyne **103** retained activity, a combination with the triazole **104** was significantly worse. The expected benefit against *P. mirabilis*, *A. baumannii* and *E. cloacae* was not gained, as both analogues mostly retained their activity. For **101** the pyridine led to a loss of activity against two *K. pneumoniae* strains and the *P. aeruginosa* wild type. This observation demonstrated that the central amino acid and ring C contribute to the antibiotic activity in a dependent manner. Therefore, the addition of a beneficial group in one cystobactamid is not necessarily beneficial for another cystobactamid. As the influence of the [1.1.1]bicyclopentane motif in **99** on activity was significantly higher than in **102**, this effect might be present throughout the whole cystobactamid scaffold. It is likely that the pyridine further decreases the solubility of the system. Former studies on fragment CDE suggested that pyridine is capable of an interaction with the adjacent amide[161].

The good and broad activity of **99,** combined with the assumed benefits in solubility, set the starting point for an extended investigation. This included *in vitro* tests for toxicity, solubility, metabolic stability and frequency of resistance.

4.6.1.1 Extended biological profiling

The introduction of the [1.1.1]bicyclopentane in **99** was highly beneficial for solubility. The $logD_{7.4}$ of the compound was between CN-DM 861 and **59** (Table 16)[230]. Nevertheless, the solubility at pH 7.4 and pH 9 exceeded the values of CN-DM 861 significantly[230]. This proved that the disturbance of the crystal lattice was a suitable method for the improvement of the cystobactamid solubility. Conversely, it also illustrated that the aromatic systems were the major contributors to the low solubility.

Table 16: Thermodynamic solubility in water for **59**, CN-DM 861 and **99** at different pH values.



Compound	$LogD_{7.4}$	Aqueous Solubility (µg/ml)		
		pH 7.4	**pH 9**	**pH 5**
59	2.26	BDL	BDL	BDL
CN-DM-861	1.49	<1	56	<1
99	1.72	901	869	n.d.

Values determined by Evotec[230].

To allow for a reasonable antibiotic therapy, a low frequency of resistance is mandatory. If the frequency of resistance (FoR) is too high, resistant organisms may emerge during the first drug regimen, endangering the success of further treatments. As cut-off for the maximal FoR, a value of 10^{-7} was set. All values above this were defined as too high. The FoR was determined for **99** on various strains at four- and eight-times the MIC (Table 17). Ciprofloxacin was added as competitor.

The FoR values for **99** were not sufficient at four-times MIC for all bacterial strains, especially for the clinically relevant *K. pneumoniae*, *P. mirabilis* and *A. baumannii*. At eight-times the MIC the FoR was sufficient for all strains except one *K. pneumoniae* strain[229]. It should be noted that those values have to be viewed in conjunction with the MIC values. Even though ciprofloxacin shows good FoR for the *E. coli* CH448 variant, a four-times MIC means a concentration of 128 µg/ml. The required dose to obtain such a high concentration at the infected tissue during therapy is most likely associated with severe side effects. The same might be true for **99** and *K. pneumoniae* ATCC-13883.

Table 17: Frequency of resistance of various bacterial strains upon exposure with **99** and CIP (ciprofloxacin).

	MIC (µg/mL)		FoR at 4x MIC		FoR at 8x MIC	
	99	**CIP**	**99**	**CIP**	**99**	**CIP**
***E. coli* ATCC-25922**	0.06	0.01	$< 3.8\times10^{-10}$	5.2×10^{-9}	$< 3.8\times10^{-10}$	$< 3.8\times10^{-10}$
***E. coli* CH448 (S83L. QnrS)**	0.06	32	2.9×10^{-7}	$< 3.7\times10^{-10}$	1.6×10^{-9}	$< 3.7\times10^{-10}$
***K. pneumoniae* ATCC-13883 (DSM30104)**	4	0.06	2×10^{-8}	$< 2\times10^{-10}$	4×10^{-9}	$< 2\times10^{-10}$
***K. pneumoniae* ATCC-43816**	1	0.06	1×10^{-8}	$\leq 4\times10^{-10}$	1×10^{-8}	$\leq 4\times10^{-10}$
***K. pneumoniae* CIP-105705**	1	0.06	7.9×10^{-7}	9.6×10^{-7}	2.5×10^{-7}	3.2×10^{-9}
***K. pneumoniae* R-1525 (QnrA1)**	1	>64	1.3×10^{-7}	n.d.	4.0×10^{-8}	n.d.
***P. mirabilis* ATCC-BAA-2081**	0.06	>8	1.4×10^{-6}	n.d.	7.3×10^{-9}	n.d.
***A. baumannii* DSM-30008**	0.5	0.25	$< 7.9\times10^{-10}$	8.6×10^{-8}	$< 7.9\times10^{-10}$	$< 7.9\times10^{-10}$
***A. baumannii* ATCC-BAA-747**	0.25	0.5	Lawn	4.6×10^{-8}	3.2×10^{-8}	$< 9.0\times10^{-10}$
***P. aeruginosa* CIP-107309**	0.25	0.125	2.1×10^{-8}	Lawn	9.2×10^{-9}	5.4×10^{-8}
***S. aureus* ATCC-29213**	0.5	1	$< 1.8\times10^{-10}$	$< 1.8\times10^{-10}$	$< 1.8\times10^{-10}$	$< 1.8\times10^{-10}$
***E. faecalis* ATCC-51299 (DSM12956)**	0.125	0.25	$< 1.4\times10^{-10}$	$< 1.4\times10^{-10}$	$< 1.4\times10^{-10}$	$< 1.4\times10^{-10}$

Values determined by Evotec[229].

First mice *in vivo* experiments of **99** were performed by Katharina Rox at HZI. No compound was detected in lung, kidney or muscle tissues but in broncheo-alveolar lavage fluid (BALF) and epithelial linage fluid (ELF)[138]. Similar to **59**, **99** was dosed up to 80 mg/kg in an *in vivo* mouse thigh infection model with *E. Coli* and *K. pneumoniae*. For *E. Coli* a bacteriostatic effect was obtained at the highest dose. This corresponded to a reduction of CFUs by five log units. Therefore, **99** was slightly better than **59**. Against *K. pneumoniae* no efficacy was observed[229].

As **99** still carries the central amino acid with an alkyne side chain, a susceptibility to metabolic inactivation is likely. This has been observed for **59** with the same side chain before.

4.6.2 Other cystobactamids with novel moieties

To obtain cystobactamids with higher structural variety compared to the basic scaffold, some novel AB fragments were introduced. Similar to the biphenyl modification at the DE system of **88** and **89**, a direct connection of ring A and B was carried out. To retain the overall length and orientation of the system, the amide between both aromatic systems was integrated into a naphthyl system. This either resulted into a ring A or a ring B naphthyl system. Through this modification, the AB system became more rigid, as it lost one rotable bond between both aromatic systems. As the elimination of an aromatic system, like the [1.1.1]bicyclopentane in **99**, led to more soluble analogues, it was assumed that the extension of either ring A or ring B to a naphthyl system was unbeneficial for the solubility. The removal of the connecting amide reduced the capability of this position to interact with water, ultimately causing a desolvation. Again, this modification was assumed to decrease the solubility.

For the synthesis of the A naphthyl building block, 6-hydroxy-2-naphthonitrile was converted to the triflate followed by a Suzuki coupling (Figure 129)[235],[236]. The deprotection with TFA gave the desired AB fragment **108**.

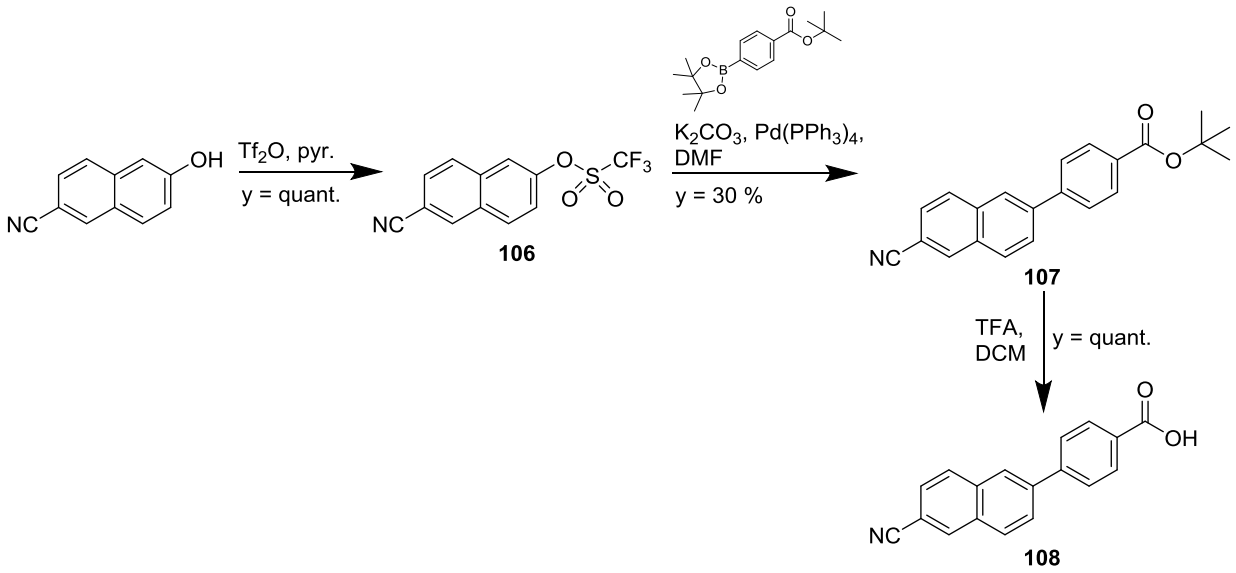


Figure 129: Synthesis of the A naphthyl building block.

The synthesis of the B naphthyl building block was carried out by a Suzuki coupling with 4-cyanophenylboronic acid (Figure 130)[238].

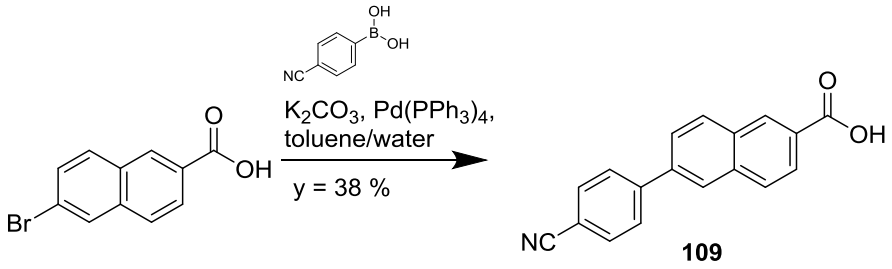


Figure 130: Synthesis of the B naphthyl building block.

Former results indicated that gyrase tolerates aromatic residues in proximity to ring A. Therefore, a ferrocene system was introduced at ring A (Figure 131). The AB fragment was synthesized from the ferrocenecarboxylic acid under standard procedures.

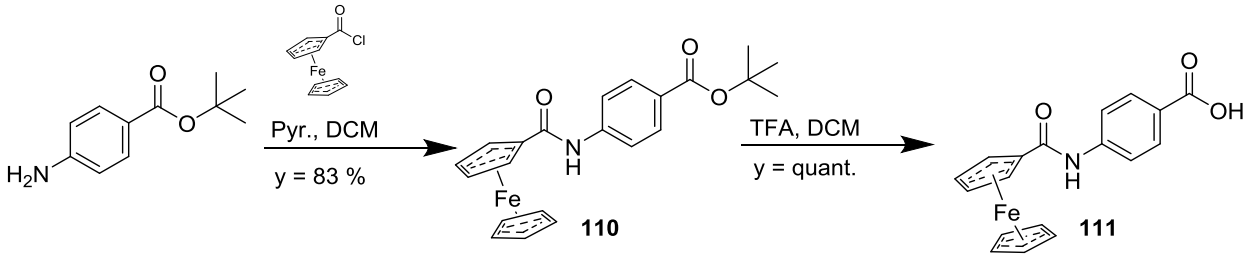


Figure 131: Synthesis of the ferrocene AB system.

Ferrocene is unique in its barrel-shaped structure with two aromatic systems. This arrangement allows for the exploitation of a new hydrophobic cavity that cannot be filled by other π-aromatic systems. Although the first ferrocenyl drugs were synthesized in the 1960s, the antianemic drug ferrocerone was the only ferrocene analogue that was ever marketed. Nevertheless, the antimalarial ferroquine and the cytostatic class of ferrocifens are promising drug candidates and currently under investigation[233].

The AB Fragments **108**, **109** and **111** were coupled to the L-propargylglycine fragment according to the described procedures in chapter 4.1.3. The three novel analogues **112**, **113** and **114** were obtained (Figure 132).

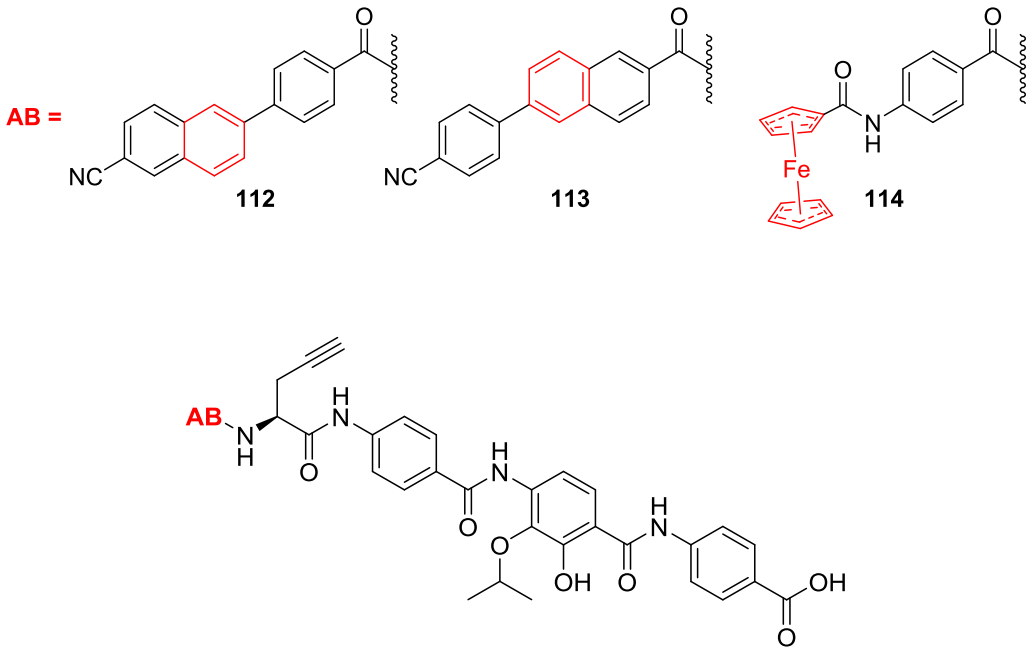


Figure 132: Structure of the novel AB modified cystobactamids.

The three analogues were tested on the extended panel of pathogenic bacteria and compared to ciprofloxacin and CN-DM 861 (Table 18).

Table 18: Antibiotic activities of the novel analogues compared to CIP (ciprofloxacin) and CN-DM 861.

	MIC (µg/mL)				
	CN-DM 861	**CIP**	**112**	**113**	**114**
***E. coli* MG1655/K12**	0.01	0.01	<0.03	0.125	>64
***E. coli* LM705 (S83L, D87N, S80I, Δ*marR*, Δ*acrR*)**	0.2 - 0.25	>6.4	1	1	>64
***E. coli* CH448 (S83L, QnrS)**	0.02 - 0.04	>6.4	1	0.125	>64
***K. pneumoniae* DSM-30104**	0.5 - 1	0.16 - 0.2	>64	>64	>64
***K. pneumoniae* CIP-104298**	4 - >64	0.02 - 0.05	>64	>64	>64
***K. pneumoniae* KP10581 (*waaC*::Tn30)**	1 - 2	>6.4	0.25	0.06	32
***A. baumannii* DSM-30008**	0.5 - 1	0.16 - 0.2	0.5	0.5	16
***A. baumannii* ATCC BAA-1710**	>64	>6.4	0.125	0.5	n.d.
***P. mirabilis* DSM-4479**	32	0.013 - 0.05	0.5	>64	>64
***P. mirabilis* ATCC BAA-2081**	32 - 64	> 6.4	0.5	64	>64
***P. vulgaris* DSM-2140**	0.5	0.006 - 0.01	2	1	4
***C. freundii* DSM-30039**	0.125	0.01	8	2	>64
***S. marcescens* DSM-30121**	64	0.025 - 0.2	>64	>64	>64
***E. cloacae* DSM-30054**	1	0.125 - 0.25	>64	>64	>64
***P. aeruginosa* PA14**	4-32	0.1 - 0.2	8	>64	>64
P. aeruginosa* PA14Δ*mexAB	1	0.02 - 0.1	32	8	>64
***S. aureus* ATCC-29213**	1	0.2 - 0.4	2	0.25	>64

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The MIC determinations were carried out in separate batches. A range of activity is shown for the references.

The introduction of a naphthyl system led to the inactivity against several strains. In comparison, **112** was superior to **113** with lower MIC values for *P. mirabilis*. The ferrocene **114** was inactive against every strain. As **114** lacks the crucial HBA, it is uncertain, if a ferrocene was tolerated. To evaluate the tolerance for a ferrocene system at ring A, an analogue with an HBA, like a nitrile, would be required.

4.7 Photoaffinity probes and covalent inhibitors

Although various analogues were synthesized so far and a SAR was established, the exact binding mode and binding site was still unclear. Additionally, a discrepancy between the IC_{50} on gyrase and the MIC values was observed for several analogues. The IC_{50} describes the ligand concentration that blocks 50 % of its target activity[234]. With gyrase as main target, a correlation of the IC_{50} on *E. coli* gyrase and the MIC of *E. coli* was assumed, but not observed. Although the cell penetration or resistance mechanisms can contribute to this discrepancy, the "fishing" for other targets were a valuable method to explain these differences. Additionally, the elucidation of the binding site allows for a computer-aided or structure-based optimization. As described in chapter 1.6, the ligand requires a reactive functional group that forms a covalent bond with the target, as well as a tag for the subsequent isolation. Through the synthesis of novel fragment AB and central amino acid analogues, a general understanding of tolerated groups was gained. The overall goal in the design of the photoaffinity probes was not to receive a highly active and optimized structure. A mediocre to good IC_{50} on *E. coli* gyrase was sufficient proof that the compound bound to gyrase. Antibiotic activity was not required, as the target identification method was carried out in lysated cells. Figure 133 shows a simplified SAR for the design of photoaffinity probes.

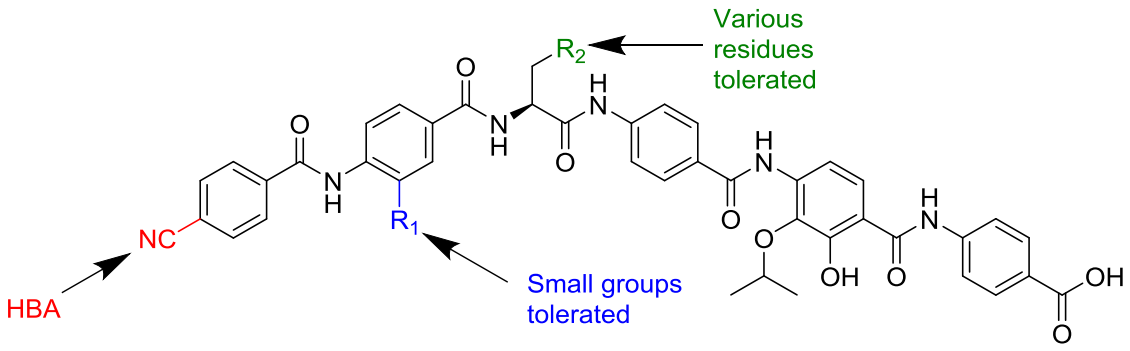


Figure 133: SAR of the cystobactamids relevant for to the development of photoaffinity probes.

The compounds **41** and **59** have already proven to be good starting points for the synthesis of photoaffinity probes (Figure 134). Both derivatives show good to high activity and sufficient IC_{50} values on *E. coli* gyrase. The alkyne enabled the attachment of a biotin tag, required for the isolation step. As bigger residues decreased the activity significantly, the attachment of the biotin tag was meant to be carried out after photoactivation and formation of the covalent bond.

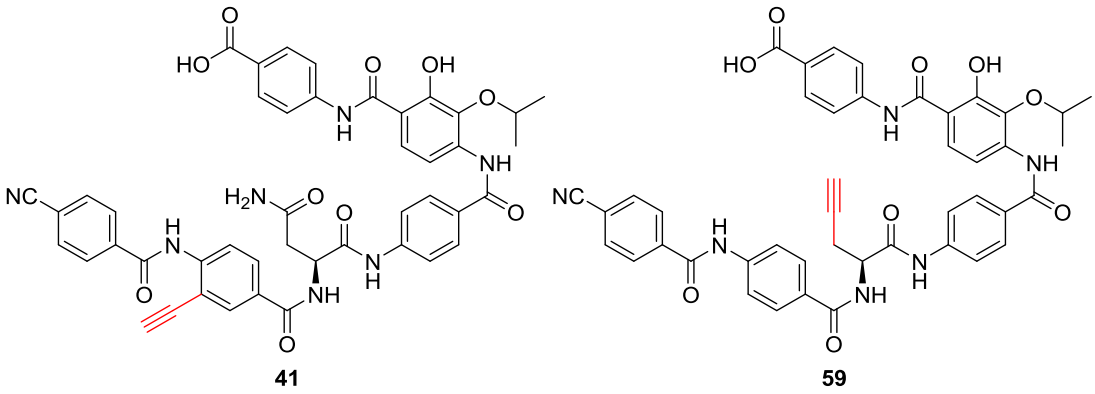


*Figure 134: Starting point for the synthesis of photoaffinity probes: The compounds **41** and **59**.*

Two other positions can be exploited for the addition of a photoreactive group. The cyano group can be substituted by another HBA, while the central amino acid tolerates various substituents. An azide was considered to be an appropriate substituent in exchange for the cyano. For the central amino acid, L-photo-leucine and L-photo-methionine were commercially available (Figure 135). Because of its smaller size, L-photo-leucine was chosen.

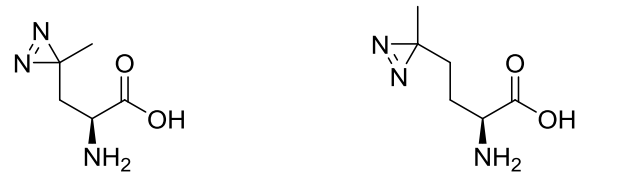


Figure 135: Structures of two photoreactive amino acids. Structures adapted from[237].

As the Fmoc protected L-photo-leucine was commercially not available, the Boc-protected amino acid was used in the amide coupling (Figure 136). The deprotection with TFA cleaved off the *tert*-butyl and the Boc protecting group. By preactivation of **33** with HATU, the activation of the other carboxylic acid was prevented. The subsequent deprotection provided the desired fishing probe **115**.

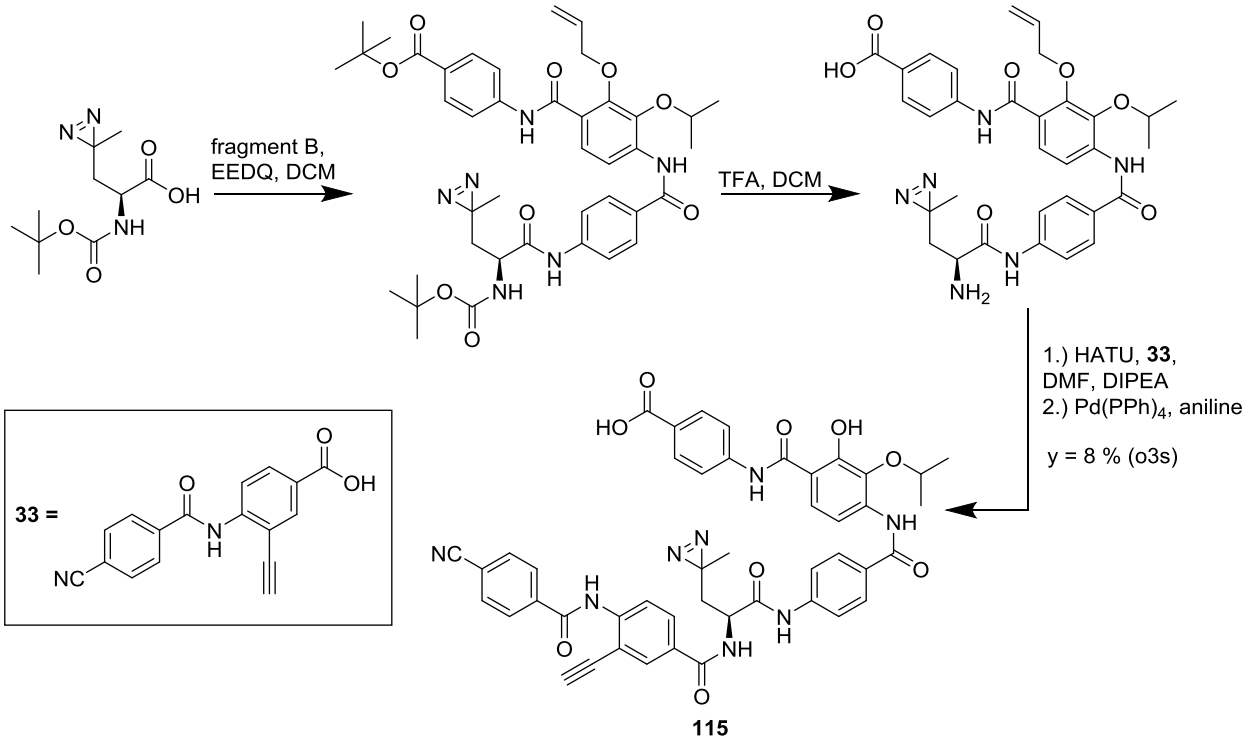


*Figure 136: Synthesis of the diazirine photoaffinity probe **115**.*

The introduction of the phenylazide into the cystobactamid scaffold was more difficult. Azides are known to decompose under acidic conditions, thereby excluding MOM and *tert*-butyl protecting groups[239]. In presence of phosphines, azides participate in a Staudinger reduction or ligation. This prohibits the established palladium catalyzed allyl deprotection. To circumvent those side reactions fragment AB was introduced without the utilization of any protecting group on its coupling partner. As for **33**, the preactivation decreased side reactions. The AB fragment was partially coupled to the free phenol at ring D. A hydrolysis was required to cleave the phenol ester.

Another possibility to generate a covalent bond with the target is to introduce a reactive group that does not require photoactivation. This group must be reactive enough to bind to the target, but stable enough for the synthesis. Inspired by the ketoamide in boceprevir, a trifluoromethyl ketone was chosen[240]. Through the electron withdrawing properties of the fluorines, this group is highly electrophilic and can react with alcohols or thiols of the target reversibly (Figure 137)[241],[242]. The group was introduced at the N-terminal position instead of the nitrile to retain the HBA and allow for the covalent interaction with a nucleophile.

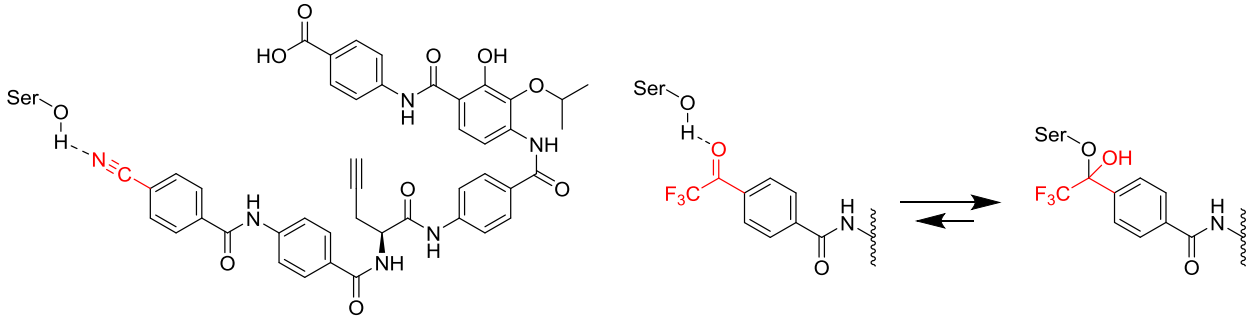


*Figure 137: Potential interaction of **59** with serine (left) and its covalent interaction with the trifluoromethyl ketone (right).*

Both the photoaffinity probes and the covalent inhibitor require a tight interaction with the target. Surrounding water can quench the photoaffinity probe after activation[131],[134]. In a similar way, the covalent inhibitor cannot form a bond with the target. The covalent inhibitor **117** was tested against a small panel of bacteria (Table 19).

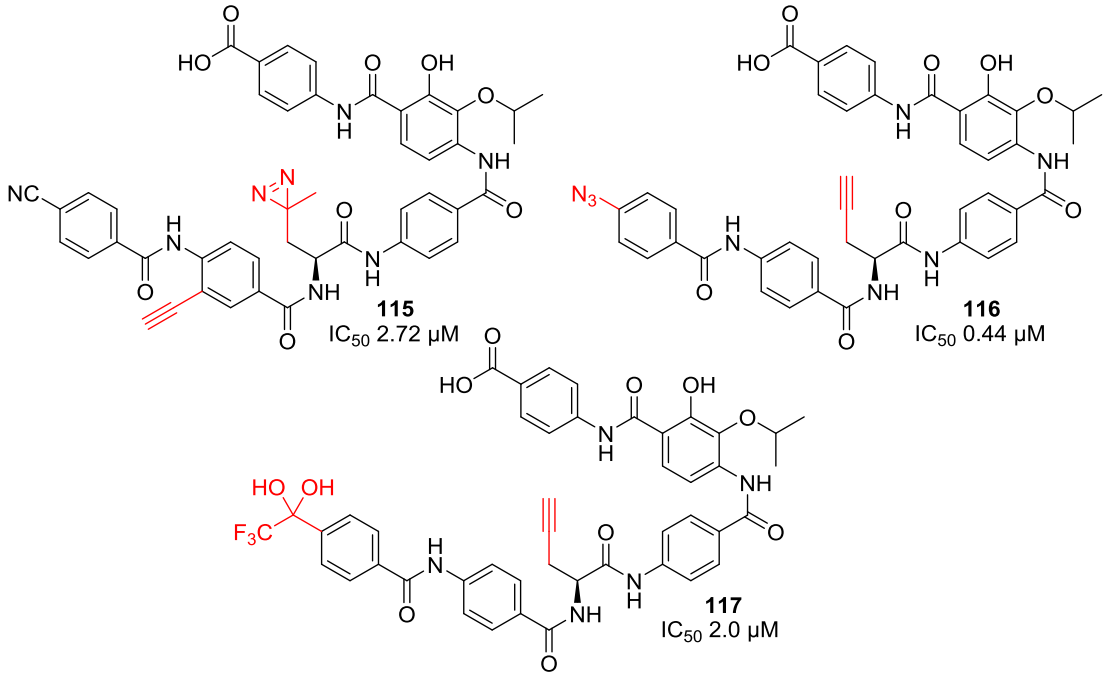


Figure 138: Structures and IC_{50} values on E. coli gyrase for the photoaffinity probes and the covalent inhibitor.

Table 19: Antibiotic activities of the **117** compared to CIP (ciprofloxacin) and **59**.

	MIC [µg/mL]					IC_{50} [µM] *E. coli* gyrase
Compound	*E. coli* BW25113	*E. coli ΔacrB*	*S. aureus* Newman	*P. aeruginosa* Pa14	Pa14*ΔmexAB*	
117	0.5	<0.03	4	>64	32	2.0
CIP	0.005	0.0013	0.1	0.05	0.025	0.18
59	<0.03	<0.03	<0.03	0.5	<0.03	0.23

MIC values determined by Katarina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS)[185]. The IC_{50} values on E. coli gyrase were determined by Jana Krull at the HZI[186].

The biological experiments for target identification and binding site elucidation are still ongoing for **115** and **116**. For **117**, the mediocre IC_{50} value of the covalent inhibitor indicated that either no covalent bond with gyrase was formed, or the reversible reaction was in favor for the starting materials. Therefore, the compound was excluded for further biological experiments. It is possible that the terminal HBA does not interact with the binding pocket directly but interacts with the target via a water mediated hydrogen bond. This would also decrease the possibility of **116** to form a covalent bond with gyrase after its photoactivation. In conclusion, the SAR enabled the successful synthesis of functional photoaffinity and covalent probes.

4.8 Marfey analysis

To determine the degree of racemization during the synthesis of the cystobactamids, three compounds and a reference compound were analysed by Marfey analysis. For the synthesis of the cystobactamid analogues, three different methods were applied for the coupling of the chiral amino acid to the CDE fragment: the reaction via T3P, EEDQ and the acid chloride. Amide coupling reactions of chiral α-amino acids are known to be susceptible to racemization. The degree of racemization depends on the substrates and the coupling reagents[245]. Therefore, one representative was chosen for each coupling method. The detailed procedure is described in chapter 5.2.1.2.

For the analysis, the cystobactamid was hydrolyzed by 4 M HCl to cleave the amide bonds and release the chiral amino acid. After removal of HCl, a 1 % solution of 1-fluoro-2-4-dinitrophenyl-5-L-alanine amide (FDAA) in acetone and saturated $NaHCO_3$ solution were added. The free amine of the chiral amino acid reacted with the FDAA to form one diastereomer. If the chiral amino acid racemized during the synthesis of the cystobactamid or the hydrolysis, another diastereomer was formed (Figure 139). Both diastereomers show different physical properties and can be quantified by LCMS analysis[246].

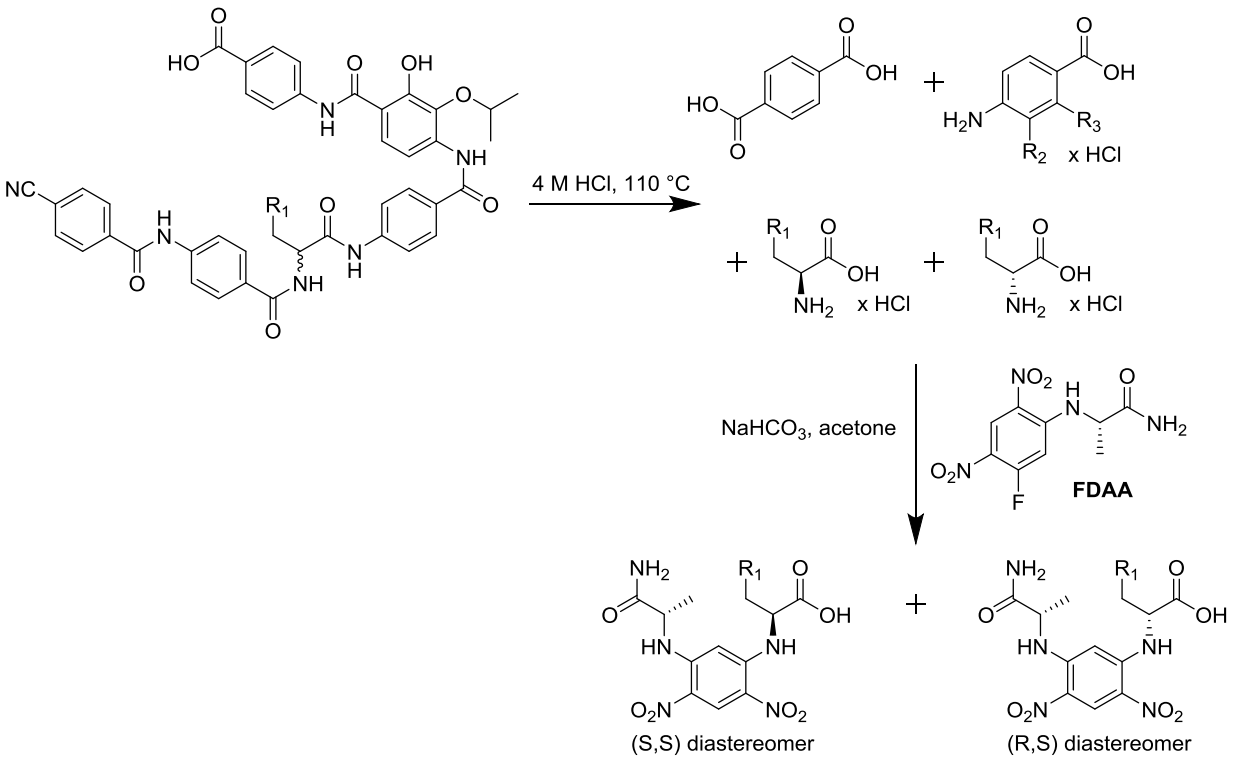
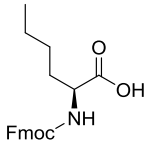
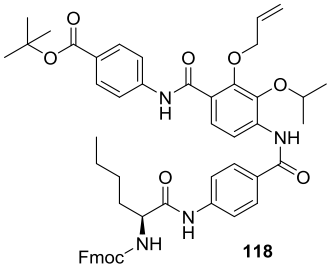
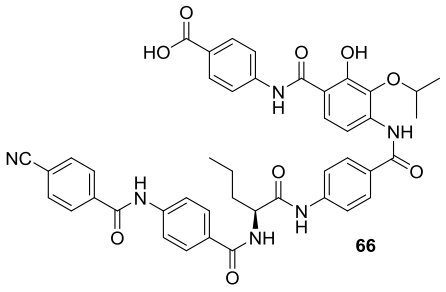
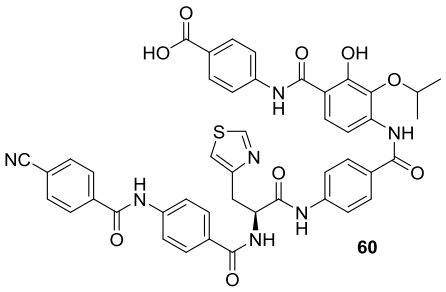


Figure 139: Marfey analysis of the cystobactamids.

The Fmoc-protected L-norleucine was chosen as reference for the analysis. All full-length cystobactamids, obtained by the T3P method, either carried an alkyne or a triazole in the amino acid side chain. During the hydrolysis of the former, several chlorinated side products were detected by LCMS. For the respective triazole, the hydrolysis led to the consumption of the starting material, but no conversion to the unprotected central amino acid was observed. Therefore, the degree of racemization for the T3P method was investigated after amide coupling of Fmoc-protected L-norleucine to the CDE fragment (**118**). The procedure is described in chapter 5.2.1.2.1. The results of the Marfey analysis are visualized in Table 20.

Table 20: Marfey analysis of three full-length cystobactamids obtained by three different amide coupling methods.

Molecule	Coupling method	% (*S*,*S*) diastereomer	enantiomeric excess
	None	>99.5	>99 %
	T3P	>99.5	>99 %
	EEDQ	94.5	89 %
	Acid chloride	85.0	70 %

No racemization was observed for the Fmoc protected L-norleucine even after amide coupling to **118** by T3P. This verified that the amide coupling with T3P was suitable for the synthesis of cystobactamid analogues. A direct comparison to the other compounds was not feasible, as the subsequent reactions to the full-length cystobactamid were not carried out.

Minor racemization was observed for **66**, obtained by the amide coupling with EEDQ. The synthesis of **60** via the acid chloride underwent the highest degree of racemization. Amide couplings with histidine are prone to racemization, because one of the nitrogens at the imidazole can participate in the reaction or lead to a deprotonation in α-position to the activated acid[247]. As the central amino acid in **60** shares high similarity to histidine, a higher degree of racemization was expected.

A previous Marfey analysis of a full-length cystobactamid with the central asparagine showed the (*S*,*S*) diastereomer in 91.2 %, reflecting an enantiomeric excess of 82.4 %. Surprisingly, the analysis of the protected asparagine itself resulted in a partial racemization with only 95.8 % of the (*S*,*S*) diasteromer and an enantiomeric excess of 91.6 %. These results indicated that the analytical procedure itself led to partial inversion of the configuration[118]. As this was not observed for Fmoc protected L-norleucine, the degree of inversion must be substrate dependent. The detected amount of (*S*,*S*) diastereomers reflected the sum of racemization from synthesis and Marfey analysis and might be lower for the applied synthesis itself.

4.9 Structure-activity relationship

For the determination of the structure-activity relationship, the broad-spectrum coverage was the most important metric. The IC_{50} on *E. coli* gyrase was a secondary metric to investigate the binding pocket of gyrase. As the IC_{50} values did not correlate with the antibiotic activity, both values were evaluated separately.

Modifications at ring A verified the importance of a HBA in *para* position with a cyano group as preferred substituent. A direct interaction with nucleophilic groups in the binding pocket is not likely. Elongations of the system were possible but were not beneficial so far. The orientation of the HBA was of crucial importance and needed to be straight for optimal activity. *E. coli* gyrase tolerated small elongations in *para* position and branched residues adjacent to the ring A system. Both modifications were not beneficial for the overall activity. The electronic properties of ring A were of low significance. An inversion of the amide between ring A and B was well tolerated.

Investigations at aromatic ring B systems revealed that electron withdrawing systems lose their activity against the *P. aeruginosa* 14 wild type, while retaining activity at the ΔmexAB deficient variant. The potency against *E. coli* and *S. aureus* was either retained or enhanced. The picolinic acid was the only exception from this observation and was not entirely inactive against the *P. aeruginosa* 14 wild type. It is possible that the electron-poor systems exhibited higher affinity for the mexAB efflux system and were transported more effectively.

A rigid and linear B system was mandatory for broad spectrum activity. Five-membered aromatic systems showed significantly less activity than six-membered systems. With the high importance of the straight HBA at ring A, topological changes at the B systems influenced the orientation of the ring A and the HBA significantly. Such changes were not tolerated. *E. coli* was less influenced by topological changes than other strains. Substituents in position two of the aromatic system were unfavorable for activity. The loss in activity was mainly correlating with the size of the substituent and the electronic properties as secondary influence. The order of activity was: H > F > Cl > CH_3 > OCH_3. The attachment of small substituents in position three was tolerated. Modifications at this position were superior to modifications at position two and showed niche activity against some bacterial strains. Larger substituents, like ethyl, were not tolerated. The niche activity can be of importance for a combination with other modifications. A non-substituted PABA was generally superior to a substituted one.

An aromatic system for B was not mandatory. Rigid systems like [1.1.1]bicyclopentane or cubane were well tolerated and beneficial for solubility and spectrum in combination with the alkyne side chain in the central amino acid. Those combinations were less prone to resistance development. The cubane was less active than the [1.1.1]bicyclopentane. This observation supported the findings at the aromatic ring B systems, as the former were sterically more demanding.

The central amino acid tolerated various substituents and did not require an amide in the side chain. L-amino acids were preferred to the less active D-amino acids. A rigidification to a six-membered system stabilized the bioactive conformation for *E. coli* gyrase. The rigidification was linked to a decreased antimicrobial activity. It is possible that the bioactive conformation was distinct from the permeable conformation. Additionally, rigidifications are known to enhance selectivity. A decreased activity against other targets besides gyrase is likely.

A HBD and a π-system in the side chain enhanced activity and spectrum. The orientation of the HBD and the π-system was crucial for spectrum and activity. The optimal position for such functions was in position three of the amino acid. Central amino acids with elongated side chains were less potent. A HBA was not required in the side chain. Residues with the highest broad-spectrum activity were: Alkyne, triazole, amide and amine. The alkyne was superior to other substituents *in vitro* with higher activity, spectrum and resistance breaking properties. The modification was prone to metabolism. However, as amine side chains are tolerated, the bacterial membrane allowed for the passage of zwitterionic compounds.

Modifications at multiple positions of the cystobactamids influenced the activity in a dependent manner. This means that combination of two beneficial substituents not always led to a synergistic gain in activity and spectrum. Combinations with the alkyne as central amino acid were the most active ones so far.

The SAR of ring A and B (Fragment AB) and the central amino acid is described in Figure 140.

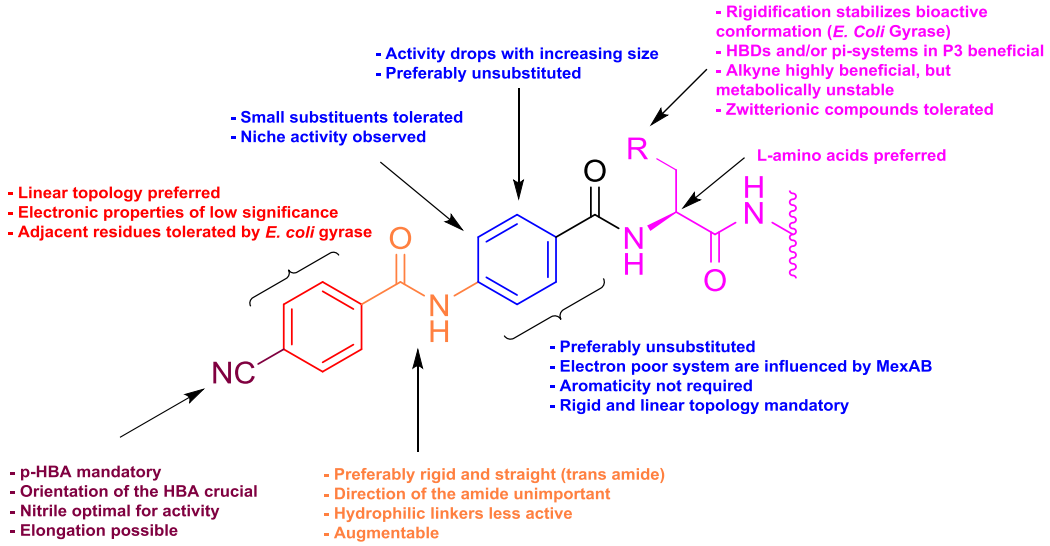


Figure 140: Detailed SAR of fragment AB and the central amino acid combined with the SAR of[5].

Only few modifications were carried out at the other parts of the structure. The introduction of a picolinic acid for ring C was tolerated but did not contribute to higher activity. As this was only observed in combination with the triazole and alkyne side chain at the central amino acids, other combinations might be beneficial. A direct connection of ring D and E was not tolerated. Presumably, ring D and the amide need to be coplanar for activity. It is uncertain, if ring E must be coplanar to ring D or the amide. In combination with the biological data from previous findings the general structure-activity relationship can was extended (Figure 141).

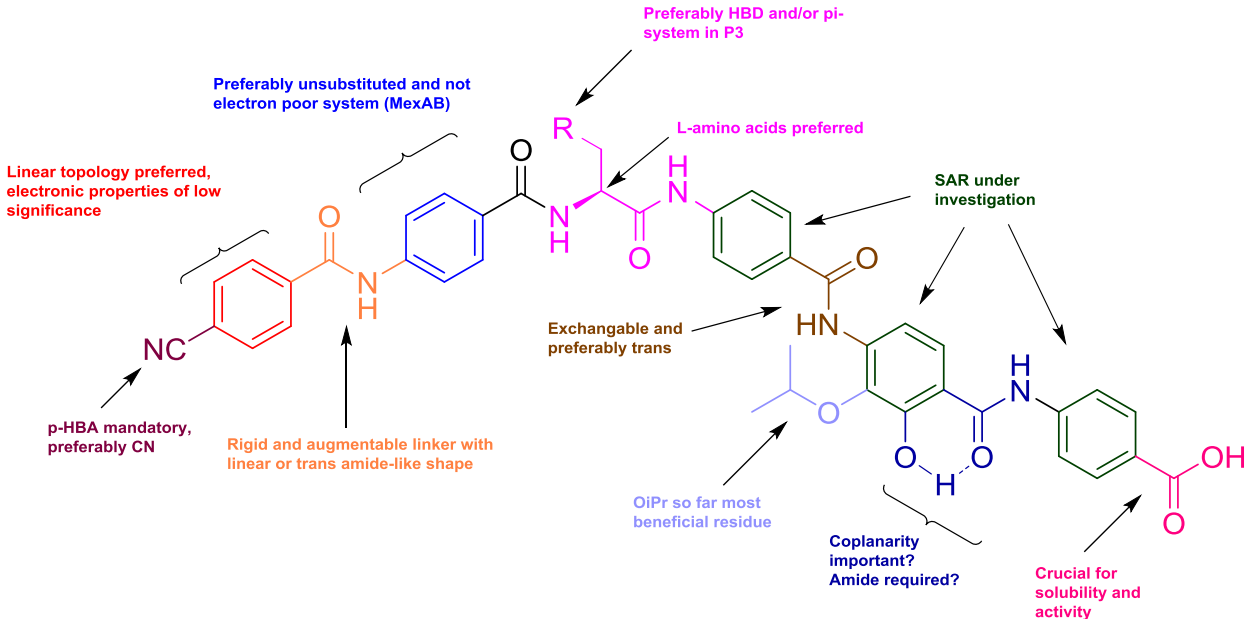


Figure 141: Simplified SAR of the full length cystobactamid combined with[5].

4.10 Outlook

The presented results illustrate that modifications at several different positions on the cystobactamids can lead to improved compounds. The majority of the novel compounds were generated by modifications at ring A, B and the central amino acid while other positions were investigated to a lesser extent (Figure 142). This is partially caused by the high synthetic effort to gain novel C, D or E analogues. Especially the late stage amide coupling to ring D and ring E is known to be highly inefficient and needs to be improved.

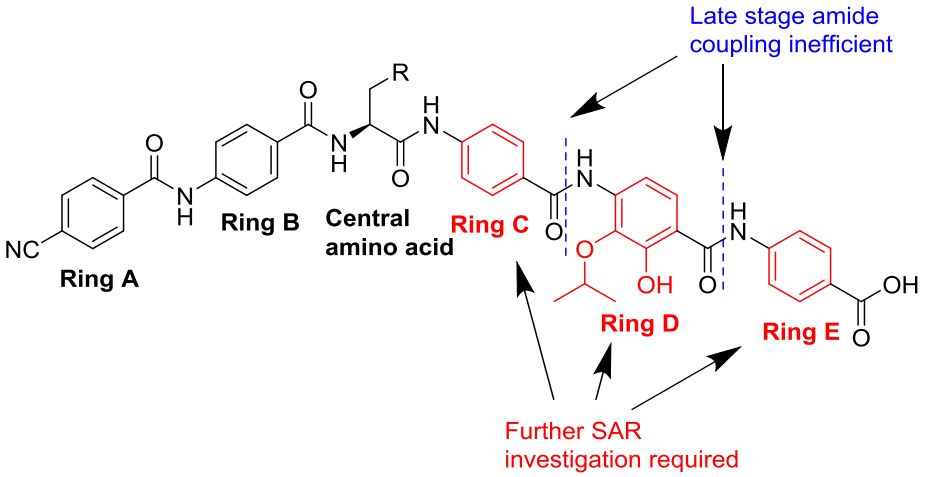


Figure 142: Less investigated ring systems (red) and problematic late stage coupling sites (blue).

Other strategies and approaches should be considered:

- The high influence of rigidified bridged systems on the solubility can be extended to other positions besides ring B. So far, this approach was the most efficient method to obtain water soluble cystobactamids and should be further exploited.
- A general optimization of the biological exposure time is required to increase the *in vivo* efficacy. This might be achieved by the introduction of new side chains at the central amino acid, but also amide bioisosteres and other alterations to increase the metabolic stability.
- As two modifications at the cystobactamids act in a dependent manner, combinations of several beneficial and maybe even unbeneficial residues are required.
- The low correlation between the IC_{50} values on *E. coli* gyrase and the MIC values on *E. coli* should be investigated. It is possible that the bacterial species and the cystobactamid structure determine, if the main target is either gyrase or topoisomerase IV. This was observed within the class of quinolones[243],[244]. If this is the case, a correlation of the IC_{50} values on topoisomerase IV

might be observable. Additionally, the penetration and distribution of the cystobactamids in the cell and its compartments should be investigated. This rules out a misinterpretation of the determined IC_{50} values and their correlation to the antimicrobial activity.

- The generation of other photoaffinity probes can improve the understanding of the binding site.
- New and uncommon residues should be introduced to obtain analogues with higher variety.
- Rigidifications within the molecule should be carried out to enhance potency and determine the bioactive conformation.

4.11 Conclusion

The primary objective of my research was to improve the cystobactamids in their activity and physicochemical properties. A focus was set on the optimization towards a preclinical drug candidate against cUTIs and *Acinetobacter baumannii*. This required the extended spectrum activity against Gram-negative and Gram-positive bacteria.

During my research, the established synthesis was optimized by new synthetic conditions and methods. A novel pathway was introduced to build-up benzimidazole building blocks at ring D. Additionally, a brominated D ring analogue was synthesized. In combination with a new MOM protecting group at the phenol of ring D, the building block was utilized for cross coupling reactions with other systems. The novel protecting group was proven to be suitable for all subsequent reactions under standard conditions and simplified the final deprotection by one step.

The SAR was extended at ring B and the central amino acid. New information about the function of the *para*-cyano residue at ring A were gained. General requirements in topology were found to obtain antibacterial compounds.

In total, more than 50 cystobactamid derivatives were synthesized with an overall yield up to 4.7 %. The longest linear sequence had 16 steps. Novel cystobactamids with enhanced activity against *K. pneumoniae*, *A. baumannii*, *P. mirabilis*, *P. vulgaris*, *S. marcescens* and *S. aureus* were synthesized. The antibiotic coverage included multi-resistant bacterial strains with resistances against the former frontrunner CN-DM 861 and ciprofloxacin. For the first time, a cystobactamid with acceptable solubility and *in vitro* activity superior to ciprofloxacin was generated. This was achieved through the introduction of an alkyne group into the central amino acid and exchange of the aromatic system at ring B for a bicyclo[1.1.1]-pentane. The high solubility of this analogue verified that the aromatic systems were the main cause for the low solubility of other cystobactamids. Therefore, the disturbance of the crystal lattice was found to be a suitable method for the optimization of novel analogues. Although the new derivative was highly active *in vitro*, the *in vivo* activity was rather low and might be the result of metabolic instability due to the alkyne.

Overall, two photoaffinity probes were synthesized that allowed for the attachment of a purification tag and subsequent isolation. Those probes can be applied to reveal valuable information about the binding site at the targets as well as potential off-targets.

To conclude, the synthesis of new cystobactamids with enhanced spectrum against cUTI relevant strains and *Acinetobacter baumannii* was accomplished in this thesis. The low solubility of the scaffold was successfully tackled. The carried-out modifications and the new SAR guide the design for upcoming derivates. As soon as the problem for low *in vivo* activity will be solved, the development of preclinical candidates comes within reach.

5 Experimental part

5.1 Biology

The MIC assays were carried out by Katharina Cirnski at the Helmholtz Institute for Pharmaceutical Research (HIPS). The test compounds were diluted to a 5 mg/ml solution in DMSO-d_6 for the assay. The methods are described in the following.

5.1.1 Minimal inhibitory concentration (MIC) – Method 1

All microorganisms were handled according to standard procedures. The microorganisms were obtained from the German Collection of Microorganisms and Cell Cultures (*Deutsche Sammlung für Mikroorganismen und Zellkulturen*, DSMZ), the American Type Culture Collection (ATCC) or were part of the internal strain collection. The cystobactamids were prepared as DMSO stock solutions with a concentration of 5 mg/ml. The Minimum inhibitory concentrations (MIC) were determined by standard procedures[3]. Single colonies of the bacterial strains were suspended in Müller-Hinton broth and grown overnight at appropriate temperature. On the following day, the bacterial count was adjusted by dilution to achieve a final inoculum of approximately 5 x 10^5 – 1 x 10^6 CFU/ml in cation-adjusted Müller-Hinton broth. Serial dilutions of the tested cystobactamids and reference antibiotics (0.03 µg/mL – 64 µg/ml) were prepared in sterile 96-well plates and the bacterial suspension was added. The microorganisms were grown overnight at 30 °C under shaking conditions. The growth inhibition was assessed visually. The determined MIC value represented the lowest concentration of antibiotic at which there was no visible growth. This determination method was applied for the testing of most of the compounds (Table 1 – 9, 15 and 19).

Bacterial strains used in this MIC determination method were:

- *Escherichia coli* DSM-1116
- *Escherichia coli* DSM-26863 (tolC3)
- *Escherichia coli* WT
- *Escherichia coli* WT-3 [*gyrA*(S83L,D87G)]
- *Escherichia coli* WT-III [*marR*Δ74bp]
- *Escherichia coli* BW25113
- *Escherichia coli ΔacrB*
- *Staphylococcus aureus* Newman

- *Staphylococcus aureus* ATCC-29213
- *Klebsiella pneumoniae* DSM-30104
- *Acinetobacter baumannii* DSM-30008
- *Pseudomonas aeruginosa* Pa14
- *Pseudomonas aeruginosa* Pa14*ΔmexAB*
- *Pseudomonas aeruginosa* DSM-24600 (ESBL)
- *Pseudomonas aeruginosa* DSM-46316 (ESBL)
- *Enterobacter aerogenes* DSM-30053
- *Enterobacter cloacae* DSM-30054
- *Enterococcus faecalis* ATCC-29212
- *Staphylococcus epidermidis* DSM-28765
- *Streptococcus pneumoniae* DSM-20566
- *Citrobacter freundii* DSM-30039
- *Serratia marcescens* DSM-30121
- *Proteus vulgaris* DSM-2140
- *Proteus mirabilis* DSM-4479

5.1.2 Minimal inhibitory concentration (MIC) – Method 2

All microorganisms were handled according to standard procedures. The microorganisms were obtained from the German Collection of Microorganisms and Cell Cultures (*Deutsche Sammlung für Mikroorganismen und Zellkulturen*, DSMZ), the American Type Culture Collection (ATCC), Evotec or were part of the internal strain collection. The cystobactamids were prepared as DMSO stock solutions with a concentration of 5 mg/ml. The Minimum inhibitory concentrations (MIC) were determined by standard procedures[248]. The bacterial cultures were streaked out on Müller-Hinton agar and grown overnight at appropriate temperature. The following day, three to four isolated colonies were collected with a sterile cotton swab and resuspended in saline solution to obtain turbidity equal to McFarland Standard 0.5. Serial dilutions of the tested cystobactamids and reference antibiotics (0.03 µg/mL – 64 µg/ml) were prepared in cation-adjusted Müller-Hinton broth in sterile 96-well plates and the bacterial suspension was added. The growth inhibition was assessed after 16 – 20 hours or after satisfactory growth of the controls was observed at appropriate temperature under static conditions. The determined MIC value represented the lowest concentration of antibiotic at which there was no visible growth. This determination method was applied for the testing of compounds (Table 11, 13, 14 and 18).

Bacterial strains used in this MIC determination method were:

- *Escherichia coli* MG1655
- *Escherichia coli* LM705 (S83L, D87N, S80I, Δ*marR*, Δ*acrR*)
- *Escherichia coli* CH448 (S83L, QnrS)
- *Escherichia coli* ATCC-25922
- *Staphylococcus aureus* ATCC-29213
- *Klebsiella pneumoniae* DSM-30104
- *Klebsiella pneumoniae* CIP-104298
- *Klebsiella pneumoniae* KP10581 (*waaC*::Tn30)
- *Klebsiella pneumoniae* ATCC 43816
- *Klebsiella pneumoniae* CIP-105705
- *Klebsiella pneumoniae* R1525 (QnrA1)
- *Acinetobacter baumannii* DSM-30008
- *Acinetobacter baumannii* ATCC BAA-1710
- *Acinetobacter baumannii* ATCC-BAA-747
- *Pseudomonas aeruginosa* Pa14
- *Pseudomonas aeruginosa* Pa14*ΔmexAB*
- *Pseudomonas aeruginosa* DSM-24600 (ESBL)
- *Pseudomonas aeruginosa* DSM-46316 (ESBL)
- *Pseudomonas aeruginosa* CIP-107309
- *Enterobacter cloacae* DSM-30054
- *Enterobacter aerogenes* DSM-30053
- *Enterococcus faecalis* DSM-12956 (VRE; vanB-type)
- *Enterococcus faecalis* ATCC-51299
- *Proteus mirabilis* DSM-4479
- *Proteus mirabilis* ATCC BAA-2081
- *Proteus vulgaris* DSM-2140
- *Citrobacter freundii* DSM-30039
- *Serratia marcescens* DSM-30121

5.1.3 DNA supercoiling assay

The supercoiling assay for the determination if IC_{50} values on *E. coli* gyrase was carried out in two steps by Jana Krull at the Helmholtz centre for Infection Research. The test compounds were diluted to a 0.75 mM for the assay. The method is described in the following.

5.1.3.1 DNA relaxation

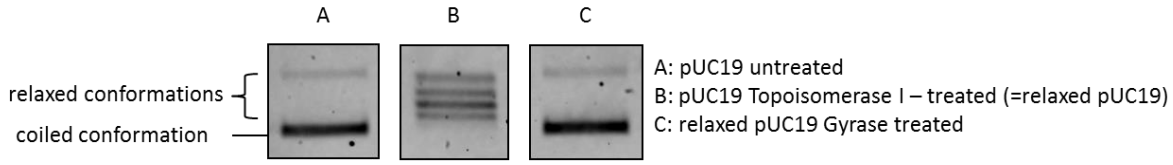
In two separate reactions 25 µl (1 µg/µl) purified circular pUC19 plasmid was mixed with 13.5 µl H_2O, 0.5 µl Topoisomerase I (6.5 U) and 50 µl topoisomerase buffer (250 mM Tris [pH 7.5], 250 mM KCl, 50 mM $MgCl_2$, 2.5 mM DTT, 0.5 mM EDTA and 150 µg/ml BSA). The mixture was incubated for 90 minutes at 37 °C. Both reactions were combined, and the relaxed plasmid DNA was purified by spin-columns according to the vendor's manual. The concentration was determined by measurement of the optical density at 600 nm and adjusted to 50 ng/µl.

5.1.3.2 DNA supercoiling assay

The 0.75 mM test compound solution was diluted to obtain final concentrations of 25 µM, 8.33 µM, 2.78 µM, 0.93 µM, 0.31 µM, 0.1 µM and 0.03 µM. For a singular determination 7.9 µl H_2O, 3 µl DNA gyrase buffer (Inspiralis) and 0.1 µl *E. coli* gyrase (5 U/µl; Inspiralis) were added to a 0.2 ml test tube and a control tube. 1 µl of the compound dilution series was added to the test tube. Both tubes were vortexed. 1 µl relaxed plasmid DNA (50 ng) was diluted with 2 µl water, added to each tube and vortexed. The mixtures were incubated for 30 min at 37 °C and the reaction stopped by increased temperature at 60 °C for 10 minutes.

5.1.3.3 Agarose gel electrophoresis

3 µl Agarose gel loading buffer was added to each sample and the control. 15 µl of the sample was loaded to the 0.8 % agarose gel. The gel was run at 25 minutes at 100 V and subsequently stained with ethidium bromide (1 µg/ml) for 5 minutes. The ethidium bromide fluorescence was documented under a UV lamp. The gel was divided into lanes. The intensity of the staining was assessed by Image Lab 5.0 (BioRad). By densitometric analysis, each lane was analysed for bands corresponding to the coiled pUC19 DNA. The intensity of the bands was determined by relative intensity to the untreated control. A graph was generated with the concentration of the test sample at the X-axis and the relative intensity of the pUC19 DNA band at the Y-axis. The IC_{50} values were calculated by non-linear regression by graph pad prism[249].



5.2 Chemistry

5.2.1 Material and methods

Commercially available solvents, reagents and reactants were used without previous quality control or purification. All reactions were carried out with oven dried glass ware and stir bars. Non-volatile reactants and reagents were dried under high vacuum before use. If mentioned in the procedure, reactions were carried out under nitrogen or argon gas.

Bruker Advance-III HD 500 MHz and Bruker Advance-III HD 700 MHz spectrometer were used to measure NMR spectra. The chemical shifts for ^{1}H, ^{13}C and ^{19}F spectra are reported in ppm. The solvent residual peak was set as reference according to the publication of Fulmer et al.[250]. ^{19}F spectra lack an internal reference. One dimensional ^{13}C were measured with ^{1}H decoupling. Multiplicities are specified with following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, hept./sept. = septet, m = multiplet, br = broad signal and combinations thereof. ^{1}H and ^{13}C signals were assigned to their respective position by two dimensional experiments for final molecules. Such experiments included heteronuclear single-quantum correlation spectroscopy (HSQC), heteronuclear multiple-bond correlation spectroscopy (HMBC) and correlation spectroscopy (COSY).

LCMS reaction controls were carried out by Agilent 1260 Infinity II LC connected to an Agilent 6130 (quadrupole MS) in ESI mode by a Phenomenex Gemini NX-C18 (50 mm x 2 mm, 3 µm) column. The gradient went from 0 % – 100 % acetonitrile in water with 0.1 % formic acid over three minutes at a flow rate of 1.5 ml/min.

High resolution mass spectra were measured at a Bruker maXis HD spectrometer in positive or negative ESI mode.

Thin-layer chromatography analytics were run on pre-coated silica gel 60 F_{254} plates (Merck). The sample was detected by UV light at 254 nm or 366 nm. Non-UV-absorbent samples were stained by a cerium-ammonium-molybdate, potassium permanganate or ninhydrin. To allow for the detection of Boc-protected amines trifluoroacetic acid was added to the ninhydrin solution.

Chromatographic seperations by flash chromatography were carried out by Grace Reveleris X2 (Büchi) with FlashPure EcoFlex cartridges (Büchi). A Pure C-850 FlashPrep (Büchi) with FlashPure EcoFlex cartridges (Büchi) was utilized for reversed phase flash chromatography. For manual columns silica gel 60 0.04-0.063 mm; 230-400 mesh (Macherey-Nagel) was used. Purifications by reversed phase high performance

liquid chromatography (RP HPLC) were performed by a Thermo Scientific Dionex UltiMate 3000 system with a Phenomenex Luna C18 column (250 mm x 21.2 mm, 5 µm) column under basic (10 mM NH_4HCO_3) or acidic (0.1 % formic acid or acetic acid) conditions.

A Christ Alpha 1-4 LCSbasic was used for the lyophilization of the products after purification by HPLC.

5.2.1.1 RP HPLC purification

Three methods were used for purification by RP HPLC:

1. 10 % – 80 % acetonitrile to 10 mM NH_4HCO_3 in water over 40 minutes. The sample was dissolved in 0.8 ml THF and 0.8 ml 10 mM NH_4HCO_3. If not fully dissolved, a few drops of 1 M NaOH and DMSO were added. The solution was filtered through a syringe filter (Chromafil PET, 15 mm diameter, 0.45 µm pore size) before injection.
2. 10 % – 90 % acetonitrile in water with 0.1 % formic acid over 50 minutes. The sample was dissolved in 0.8 ml. If not fully dissolved, a few drops of DMSO were added. The solution was filtered through a syringe filter (Chromafil PET, 15 mm diameter, 0.45 µm pore size) before injection.
3. 10 % – 90 % acetonitrile in water with 0.1 % acetic acid over 50 minutes. The sample was dissolved in 0.8 ml. If not fully dissolved, a few drops of DMSO were added. The solution was filtered through a syringe filter (Chromafil PET, 15 mm diameter, 0.45 µm pore size) before injection.

The fractions were analysed by LCMS. The desired fraction was lyophilized.

5.2.1.2 Marfey analysis

The procedure for the Marfe analysis was adapted from the literature[246].

5 µmol (1 eq) of the investigated full-length cystobactamid was added to a vial. 1.0 ml 4 M HCl (4 mmol, 800 eq) was added and the mixture was stirred at 110 °C for 6 h. The solvent was concentrated, frozen and lyophilized. The residue was neutralized with 0.1 ml saturated $NaHCO_3$. 2.0 mg Marfey's Reagent (7.4 µmol, 1.5 eq) in 200 µL acetone (approx. 1 % solution) was added and the mixture was stirred 1 hour at 40 °C.

A sample of the mixture was analysed by LCMS with a Thermo Scientific Ultimate 3000 RS LC connected to a Phenomenex Kinetex C18 (150 mm x 2.1 mm, 1.7 µm) column. For the mass detection a Bruker maXis HD UHD-TOF in ESI mode was used. The gradient went from 1 % to 100 % acetonitrile in water with 0.1 % formic acid over 20 minutes at a flow rate of 300 µl/min.

The fraction of the (S,S) diastereomer was given in percent and was calculated from the relative abundance of the larger signal after MS single-ion extraction at the calculated mass ± 0.05 Da deviation.

5.2.1.2.1 Synthesis of *tert-butyl (S)-4-(4-(4-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)hexanamido)-benzamido)-2-(allyloxy)-3-isopropoxybenzamido)benzoate* (118)

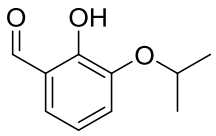
1.7 mg (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)hexanoic acid (4.8 µmol, 1.0 eq) and 4.0 mg tert-butyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-isopropoxybenzamido)benzoate (7.3 µmol, 1.5 eq) were added to a dry flask and were further dried under high vacuum. 1.2 µl dry pyridine (14.9 µmol, 3.1 eq) and 0.1 ml dry ethyl acetate were added under nitrogen atmosphere. The reaction mixture was cooled down to 0 °C. 5 µl T3P solution (50 wt % in ethyl acetate, 8.4 µmol, 1.8 eq) was added while keeping the temperature below 0 °C. The reaction was stirred at 0 °C and controlled over LCMS. After completion, the reaction was quenched with 1 ml 1 M HCl and 3 ml brine and extracted with 3 x 2 ml ethyl acetate. The combined organic phases were concentrated under reduced pressure and the residue was dried at high vacuum. The crude product was directly used for the Marfey analysis described in 5.2.1.2.

5.2.2 Experimental Procedures

5.2.2.1 Synthesis Fragment CDE

The reported experimental data of fragment CDE was mainly adapted from the established synthesis[4]. Previously synthesized intermediates and adapted experimental procedures from literature have a quotation at the end of the molecule name.

2-Hydroxy-3-isopropoxybenzaldehyde (**11**)[4]



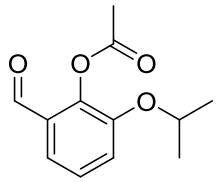
10.0 g 2,3-dihydroxybenzaldehyde (72.5 mmol, 1 eq) and 145 mL dry DMSO were added into a dry flask under nitrogen atmosphere. 5.8 g sodium hydride (60% suspension, 144.9 mmol, 2 eq) was added slowly in small portions. After the mixture was stirred for 1 hour, it was cooled to 0 °C and 7.40 mL 2-bromopropane (9694 mg, 78.8 mmol, 1.1 eq) was added. The mixture was stirred for another 24 hours and another 1.45 g NaH (60 % suspension in mineral oil, 36.2 mmol, 0.5 eq) and 1.35 mL 2-bromopropane (1769 mg, 14.4 mmol, 0.2 eq) were added. The mixture was stirred for 20 hours. After the reaction was completed, the reaction was quenched with 80 ml 1 M HCl and 70 ml brine. The solution was extracted with 5 x 60 ml diethyl ether. The organic phases were combined and washed four times with a mixture of 40 ml brine and 10 ml 6 M HCl, before the solvent was removed at reduced pressure. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate). The product was a yellow oil.

Yield:	6,403 mg (49 %)
Rf:	0.6 (PE:EE 3:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.96 (s, 1H, Ar-OH), 9.92 (s, 1H, CHO), 7.19 (dd, 1H, Ar-H, J = 1.5 Hz, 7.8 Hz), 7.14 (dd, 1H, Ar-H, J = 1.5 Hz, 8.0 Hz), 6.94 (t, 1H, Ar-H, J = 7.9 Hz), 4.59 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.39 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 196.4 (CHO), 152.95 (C_{Ar}-OH), 146.4 (C_{Ar}-O), 125.2 (C_{Ar}-H), 122.7 (C_{Ar}-H), 121.3 (C_{Ar}), 119.5 (C_{Ar}-H), 72.1 $CH(Me)_2$), 22.0 (CH_3)

2-Formyl-6-isopropoxyphenyl acetate(**12**)[4]



6.38 g 2-hydroxy-3-isopropyloxybenzaldehyde (35.4 mmol, 1 eq), 7.95 g DABCO (70.9 mmol, 2 eq) and 60 ml of dry DMF were added in a dry flask and stirred for 15 minutes at 0 °C. 5.7 ml of acetic anhydride (6167 mg, 60.4 mmol, 1.7 eq) was added to the stirring solution slowly. After 5 minutes, the reaction was allowed to heat up to room temperature. The reaction was controlled via TLC with petroleum ether:EE (3:1). After the reaction was completed, the solvent was carefully evaporated under reduced pressure. The crude product was dissolved in 60 ml of ethyl acetate. 60 ml 1 M HCl and 20 ml Water were added to wash the organic phase and the organic phase was separated and stored. The water phase was extracted with ethyl acetate (4 x 40 ml). The combined organic phases were washed with 2 x 60 ml brine. The organic phases were concentrated under reduced pressure and dried under high vacuum.

Yield: 7,482 mg (95 %)

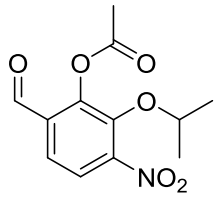
Rf: 0.5 (PE:EE 3:1)

^{1}H NMR (500 MHz, CDCl3, 300 K): δ = 10.14 (s, 1H, CHO), 7.44 (dd, 1H, Ar-H, J = 1.5 Hz, 7.7 Hz), 7.30 (t, 1H, Ar-H, J = 8.0 Hz), 7.21 (dd, 1H, Ar-H, J = 1.4 Hz, 8.2 Hz), 4.56 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.39 (s, 3H, $COCH_3$), 1.34 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 188.9 (CHO), 168.8 (COO), 150.3 (C_{Ar}), 143.0 (C_{Ar}), 129.6 (C_{Ar}), 126.7 (C_{Ar}-H), 121.2 (C_{Ar}-H), 120.7 (C_{Ar}-H), 72.0 ($CH(Me)_2$), 22.1 (CH_3), 20.6 (CH_3)

HRMS (ESI) calculated 223.0970 [M+H$^+$], 223.0964 found.

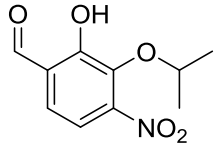
6-Formyl-2-isopropoxy-3-nitrophenyl acetate[4]



To a dry flask 20.71 ml fuming nitric acid (497 mmol, 17 eq) was added and cooled down to – 40 °C. 6.482 g 2-acetoxy-3-isopropyloxybenzaldehyde (29.2 mmol, 1 eq) was dissolved in 42 ml dry DCM and the solution was slowly added to the cooled mixture over 1.5 hours, while stirring vigorously. The mixture was kept at – 40 °C. After completion, the reaction mixture was poured into 150 ml of ice water. The organic phase was separated and stored. The water phase was extracted with 4 x 50 ml of DCM. The organic phases were combined, and the solvent was evaporated under reduced pressure. The crude oil was used in further reactions.

Yield: 7,571 mg (crude)

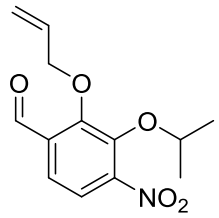
2-Hydroxy-3-isopropoxy-4-nitrobenzaldehyde[4]



7.565 g crude 2-acetoxy-3-isopropyloxy-4-nitrobenzaldehyde (28.3 mmol, 1 eq) was dissolved in a mixture of 50 ml water and 50 ml THF. 3.39 g of Lithium hydroxide (141.5 mmol, 5 eq) was added to the stirring solution. After completion, the reaction was acidified with 150 ml of 1 M HCl and extracted with 4 x 200 ml of ethyl acetate. The organic fractions were combined and the solvent was removed under reduced pressure.

Yield: 6,246 mg (crude)

2-(Allyloxy)-3-isopropoxy-4-nitrobenzaldehyde (**13**)[4]



6.24 g 2-hydroxy-3-isopropoxy-4-nitrobenzaldehyde (27.7 mmol, 1 eq) and 7.66 g potassium carbonate (55.5 mmol, 2 eq) were added to a dry flask and further dried under high vacuum. The flask was flushed with nitrogen. 65 ml dry DMF and 3.56 ml allyl bromide (4.98 g, 41.1 mmol, 1.5 eq) were added under nitrogen atmosphere. The reaction was stirred overnight and controlled via TLC. The solvent was carefully removed under reduced pressure. A mixture of 200 ml Water and 133 ml 1 M HCl was added to the residue and it was extracted with 6 x 200 ml ethyl acetate. The combined organic phases were removed under reduced pressure and purified by flash chromatography (petroleum ether/ethyl acetate).

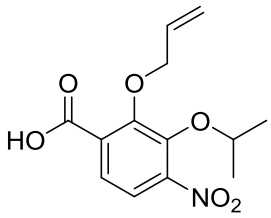
Yield: 4,887 mg (63 % - over 3 steps)

Rf: 0.2 (PE:EE 19:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.39 (s, 1H, CHO), 7.64 (d, 1H, Ar-H, J = 8.5 Hz), 7.50 (dd, 1H, Ar-H, J = 0.7 Hz, 8.5 Hz), 6.05 (ddt, 1H, CH=, J = 6.1 Hz, 10.3 Hz, 16.5 Hz), 5.40 (dq, 1H, $=CH_2$, J = 1.4 Hz, 17.1 Hz), 5.33 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.3 Hz), 4.74 – 4.64 (m, 3H, CH_2 & $CH(Me)_2$), 1.32 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 188.7 (CHO), 156.4 (C_{Ar}), 150.0 (C_{Ar}), 145.2 (C_{Ar}), 133.1 (C_{Ar}), 132.1 (CH=), 122.5 (C_{Ar}-H), 120.4 (CH_2=), 119.6 (C_{Ar}-H), 78.4 ($CH(Me)_2$), 75.8 (OCH_2), 22.5 (CH_3)

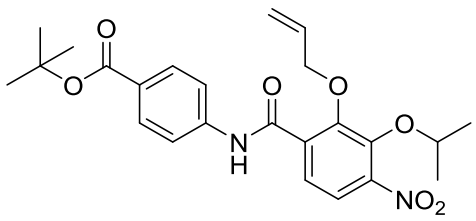
2-(Allyloxy)-3-isopropoxy-4-nitrobenzoic acid[4]



790 mg 2-(allyloxy)-3-isopropoxy-4-nitrobenzaldehyde (3.0 mmol, 1 eq), 22 ml *tert*-butanol and 15 ml of a 2 M solution of 2-methyl-2-butene in THF (30 mmol, 10 eq) were added to a flask and the flask was sealed with a septum and a nitrogen filled ballon. The solution was cooled down to 0 °C. 350 mg sodium chlorite (3.9 mmol, 1.3 eq) was dissolved in 3.3 ml of a 1 N sodium dihydrogen phosphate solution and was added to the reaction dropwise while stirring. After 10 minutes, the reaction mixture was allowed to reach room temperature. The reaction was controlled via TLC. The reaction was carefully quenched with a solution of 751 mg sodium sulfite (6.0 mmol, 2 eq) in 12 ml water. The solvent was partially removed at reduced pressure. 39 ml 1 M HCl was added to the residue and it was extracted with 6 x 40 ml ethyl acetate. The organic fractions were combined and the solvent was removed under reduced pressure. The crude product was used without further purification.

Yield: 959 mg (crude)

tert-Butyl 4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (**14**)[4]



477 mg *tert*-butyl-4-aminobenzoate (2.5 mmol, 1 eq) and 834 mg 2-(allyloxy)-3-isopropoxy-4-nitrobenzoic acid (3.0 mmol, 1.2 eq) were added to a dry flask and further dried under high vacuum. 0.69 ml dry triethylamine (501 mg, 5.0 mmol, 2 eq) and 41 ml dry DCM were added under nitrogen atmosphere. The reaction was cooled to 0 °C and 0.28 ml phosphoryl chloride (460.6 mg, 3.0 mmol, 1.2 eq) was added to the stirring mixture. After completion, the reaction was quenched with saturated $NaHCO_3$ solution and the solvent was partially removed. 60 ml water was added to the residue and extracted with 3 x 60 ml ethyl acetate. The combined organic phases were washed with 120 ml 1 M HCl and 120 ml brine. The crude product was purified by flash chromatography with a mixture of ethyl acetate and petroleum ether. The product was a beige solid.

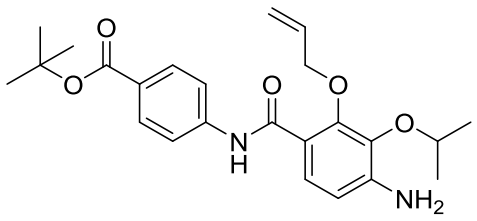
Yield: 666 mg (59 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.13 (s, 1H, CONH), 8.09 (d, 1H, Ar-H, J = 8.8 Hz), 8.00 (d, 2H, Ar-H, J = 8.7 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 7.63 (d, 1H, Ar-H, J = 8.8 Hz), 6.12 (ddt, 1H, CH=, J = 6.1 Hz, 10.4 Hz, 16.5 Hz), 5.50 (dq, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.43 (dd, 1H, $=CH_2$, J = 1.0 Hz, 10.3 Hz), 4.78 (d, 2H, OCH_2, J = 6.1 Hz), 4.66 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 1.6 (s, 9H, $(CH_3)_3$) 1.36 (d, 6H, CH_3, J = 6.2 Hz)

^{13}C-NMR (176 MHz, $CDCl_3$, 300 K): δ (ppm) = 165.4 (COO), 161.2 (CONH), 151.6 (C_{Ar}), 148.3 (C_{Ar}), 144.8 (C_{Ar}), 141.5 (C_{Ar}), 131.7 (CH=), 130.9 (C_{Ar}-H), 130.8 (C_{Ar}), 128.2 (C_{Ar}), 126.4 (C_{Ar}-H), 121.2 ($=CH_2$), 120.2 (C_{Ar}), 119.4 (C_{Ar}-H), 81.2 ($C(CH_3)_3$), 78.9 ($CH(Me)_2$), 75.9 (OCH_2), 28.2 ($(CH_3)_3$), 22.4 ($(CH_3)_2$)

HRMS (ESI) calculated 457.1975 [M+H$^+$], 457.1969 found.

tert-Butyl 4-(2-(allyloxy)-4-amino-3-isopropoxybenzamido)benzoate (**15**)[4]



0.66 g *tert*-butyl-4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (1.45 mmol, 1 eq) was dissolved in 6.6 ml THF and 5.2 ml ethanol. 1.33 ml acetic acid (1395 mg, 23.3 mmol, 16.1 eq) was added to the stirring mixture and the mixture was cooled to 0 °C. 1.42 g zinc dust (21.7 mmol, 15.0 eq) was added in small portions. The mixture was allowed to reach room temperature and was stirred for 3 hours. The zinc dust was filtered off. 80 ml saturated $NaHCO_3$ solution was added to the solution and it was extracted with 3 x 60 ml ethyl acetate. The solvent of the combined organic phases was evaporated under reduced pressure. The product was a yellow solid.

Yield: 571 mg (93 %)

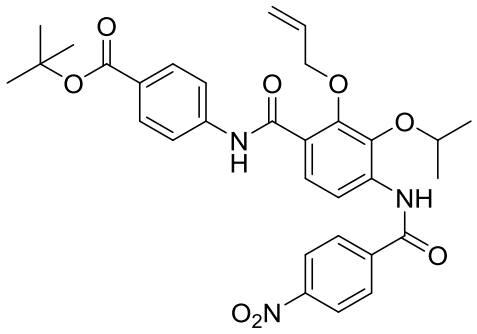
Rf: 0.67 (PE:EE 3:1)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.22 (s, 1H, CONH), 7.86 (d, 2H, Ar-H, J = 8.8 Hz), 7.76 (d, 2H, Ar-H, J = 8.8 Hz), 7.39 (d, 1H, Ar-H, J = 8.6 Hz), 6.55 (d, 1H, Ar-H, J = 8.6 Hz), 6.12 – 6.03 (m, 1H, CH=), 5.58 (br s, 2H, NH_2), 5.45 (dq, 1H, $=CH_2$, J = 1.6 Hz, 17.2 Hz), 5.27 (ddd, 1H, $=CH_2$, J = 1.2 Hz, 2.9 Hz, 10.5 Hz), 4.61 (d, 2H, OCH_2, J = 5.6 Hz), 4.46 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.54 (s, 9H, $C(CH_3)_3$), 1.26 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 164.6 (COO), 163.8 (CONH), 150.7 (C_{Ar}), 147.8 (C_{Ar}), 143.2 (C_{Ar}), 135.3 (C_{Ar}), 133.5 (OCH_2), 130.1 (C_{Ar}-H), 126.1 (C_{Ar}-H), 125.5 (C_{Ar}), 118.6 (C_{Ar}-H), 118.2 ($=CH_2$), 114.5 (C_{Ar}), 109.0 (C_{Ar}-H), 80.2 ($C(Me)_3$), 74.4 ($CH(Me)_2$), 73.7 (OCH_2), 27.9 ($(CH_3)_3$), 22.2 ($(CH_3)_2$)

HRMS (ESI) calculated 427.2233 [M+H$^+$], 427.2229 found.

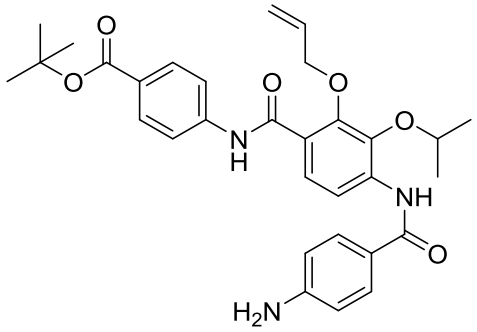
tert-Butyl 4-(2-(allyloxy)-3-isopropoxy-4-(4-nitrobenzamido)benzamido)benzoate[182]



465 mg *tert*-butyl 4-(2-(allyloxy)-4-amino-3-isopropyloxybenzamido)benzoate (1.1 mmol, 1 eq) and 304 mg 4-nitrobenzoyl chloride (1.6 mmol, 1.5 eq) were added to a dry flask under nitrogen atmosphere. 9 ml of dry DCM was added and the mixture was cooled down to 0° C. 0.35 ml of dry pyridine (344 mg, 4.3 mmol, 4 eq) was slowly added to the stirring mixture. The reaction was allowed to get to room temperature and stirred for 2 hours. The reaction was quenched with 9 ml 1 M $NaHSO_4$ and 28 ml water. The organic phase was separated and stored while the water phase was extracted with 4 x 18 ml DCM. The organic phases were combined and washed with 2 x 30 ml saturated $NaHCO_3$ solution. The solvent was evaporated under reduced pressure. The crude product was used without further purification.

Yield: 693 mg (crude)

tert-Butyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-isopropoxybenzamido)benzoate (**16**)[182]



693 mg crude *tert*-butyl-4-(2-(allyloxy)-3-isopropoxy-4-nitrobenzamido)benzoate (1.2 mmol, 1 eq) was dissolved in 5.7 ml THF and 4.8 ml ethanol. 1.1 ml acetic acid (1154 mg, 19.2 mmol, 16.0 eq) was added to the stirring mixture and the mixture was cooled to 0 °C. 1.18 g zinc dust (18.1 mmol, 15.0 eq) was added in small portions. The mixture was allowed to reach room temperature and was stirred for 3 hours. The zinc dust was filtered off. 65 ml saturated $NaHCO_3$ solution was added and the aqueous phase was extracted with 3 x 50 ml ethyl acetate. The solvent of the combined organic phases was evaporated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate). The product was a yellow solid.

Yield: 425 mg (71 % - over 2 steps)

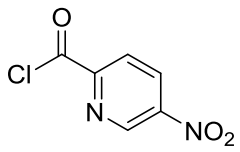
Rf: 0.33 (PE:EE 1:1)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.49 (s, 1H, CONH), 9.05 (s, 1H, CONH), 7.95 (d, 1H, Ar-H, J = 8.7 Hz), 7.89 (d, 2H, Ar-H, J = 8.7 Hz), 7.83 (d, 2H, Ar-H, J = 8.6 Hz), 7.69 (d, 2H, Ar-H, J = 8.6 Hz), 7.40 (d, 1H, Ar-H, J = 8.6 Hz), 6.63 (d, 2H, Ar-H, J = 8.7 Hz), 6.07 – 5.98 (m, 1H, CH=), 5.88 (br s, 2H, NH_2), 5.38 (dq, 1H, =CH_2, J = 1.6 Hz, 17.2 Hz), 5.21 (ddd, 1H, =CH_2, J = 1.3 Hz, 3.0 Hz, 10.5 Hz), 4.60 (d, 2H, OCH_2, J = 5.5 Hz), 4.53 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.55 (s, 9H, $(CH_3)_3$), 1.28 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 164.6 (C=O), 164.5 (C=O), 164.4 (C=O), 152.6 (C_{Ar}-NH_2), 149.3 (C_{Ar}-O), 143.0 (C_{Ar}-NH), 141.2 (C_{Ar}-O), 136.3 (C_{Ar}-NH), 133.6 (CH=), 130.1 (C_{Ar}-H), 129.0 (C_{Ar}-H), 126.0 (C_{Ar}), 125.8 (C_{Ar}), 123.9 (C_{Ar}-H), 119.9 (C_{Ar}), 118.8 (C_{Ar}-H), 117.8 (=CH_2), 117.4 (C_{Ar}-H), 112.9 (C_{Ar}-H), 80.3 ($C(Me)_3$), 76.1 ($CH(Me)_2$), 74.3 (CH_2), 27.8 (($CH_3)_3$), 22.4 (($CH_3)_2$)

HRMS (ESI) calculated 546.2604 [M+H$^+$], 546.2604 found.

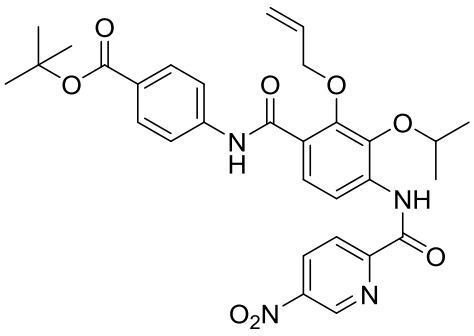
5-Nitropicolinoyl chloride



100.0 mg 5-nitropicolinic acid (0.59 mmol, 1 eq), 1.5 ml dry DCM and one drop of dry DMF were added to a dry flask under nitrogen atmosphere. The mixture was cooled to 0 °C and 0.08 ml oxalyl chloride (118.4 mg, 0.93 mmol, 1.6 eq) was slowly added to the stirring mixture. The reaction stirred for 3.5 hours. After completion, the solvent was removed under reduced pressure. The faint rose solid was dried under high vacuum. The crude product was used without further purification.

Yield: 108.0 mg (crude)

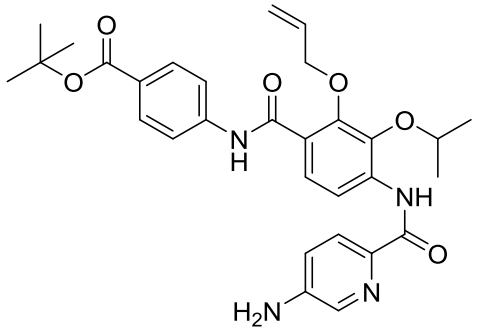
tert-Butyl 4-(2-(allyloxy)-3-isopropoxy-4-(5-nitropicolinamido)benzamido)benzoate[182]



230 mg *tert*-butyl 4-(2-(allyloxy)-4-amino-3-isopropyloxybenzamido)benzoate (0.54 mmol, 1 eq) and 108 mg 5-nitropicolinoyl chloride (0.58 mmol, 1.1 eq) were added to a dry flask under nitrogen atmosphere. 4.0 ml of dry DCM was added and the mixture was cooled down to 0° C. 0.13 ml of dry pyridine (128 mg, 1.61 mmol, 3.0 eq) was slowly added to the stirring mixture. The reaction was allowed to reach room temperature and controlled over LCMS. After completion, the reaction was quenched with 5 ml 1 M HCl and 10 ml brine. The aqueous phase was extracted with 3 x 5 ml ethyl acetate. The organic solvent was removed under reduced pressure and the crude product was used for further reactions.

Yield: 310.0 mg (crude)

tert-Butyl 4-(2-(allyloxy)-4-(5-aminopicolinamido)-3-isopropoxybenzamido)benzoate (**98**)[182]



310 mg crude *tert*-butyl 4-(2-(allyloxy)-3-isopropoxy-4-(5-nitropicolinamido)benzamido)benzoate (0.54 mmol, 1 eq) was dissolved in 2.4 ml THF and 2.0 ml ethanol and cooled down to 0 °C. 528.0 mg zinc dust (8.07 mmol, 15.0 eq) was added. 0.48 ml acetic acid (504 mg, 8.4 mmol, 15.6 eq) was added to the stirring mixture over the timespan of 30 minutes and the mixture was allowed to reach room temperature. The reaction was controlled over LCMS. After completion, the zinc dust was filtered off. 30 ml saturated $NaHCO_3$ solution was added to the solution and the aqueous phase was extracted with 3 x 18 ml ethyl acetate. The organic phases were combined and the solvent was removed under reduced pressure. The crude product was purified by chromatography (petroleum ether/ ethyl acetate).

Yield: 178.0 mg (60 % - over 2 steps)

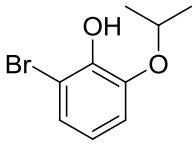
Rf: 0.15 (PE:EE 3:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.65 (s, 1H, CONH), 10.25 (s, 1H, CONH), 8.54 (d, 1H, Ar-H, J = 8.9 Hz), 8.09 – 8.04 (m, 3H, Ar-H), 7.98 (d, 2H, Ar-H, J = 8.8 Hz), 7.74 (d, 2H, Ar-H, J = 8.8 Hz), 7.08 (dd, 1H, Ar-H, J = 2.8 Hz, 8.4 Hz), 6.15 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (dd, 1H, $=CH_2$, J = 1.4 Hz, 17.1 Hz), 5.39 (dd, 1H, $=CH_2$, J = 1.2 Hz, 10.4 Hz), 4.73 (d, 2H, OCH_2, J = 5.9 Hz), 4.65 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.14 (br s, 2H, NH_2), 1.60 (s, 9H, $(CH_3)_3$), 1.42 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 165.6 (COO), 163.0 (CONH), 162.8 (CONH), 149.9 (C_{Ar}-O), 145.6 (C_{Ar}-NH_2), 142.5 (C_{Ar}-NH), 140.2 (C_{Ar}), 139.5 (C_{Ar}-O), 138.2 (C_{Ar}-NH), 135.4 (C_{Ar}-H), 132.6 (CH=), 130.8 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.3 (C_{Ar}), 123.9 (C_{Ar}-H), 121.3 (C_{Ar}-H), 121.0 (C_{Ar}), 120.0 ($=CH_2$), 119.1 (C_{Ar}-H), 115.4 (C_{Ar}-H), 80.9 ($C(Me)_3$), 75.0 (OCH_2), 28.4 ($(CH_3)_3$), 22.7 ($(CH_3)_2$)

HRMS (ESI) calculated 547.2557 [$M+H^+$], 547.2552 found.

2-Bromo-6-isopropoxyphenol (**73**)[220]



2.8 ml dry *tert*-butylamine (1949 mg, 26.6 mmol, 2 eq) and 26.5 ml dry toluene were added to a dry flask under nitrogen atmosphere. The mixture was cooled down to - 30 °C. 0.68 ml bromine (2110 mg, 13.2 mmol, 1 eq) was slowly added under nitrogen atmosphere and the reaction mixture was stirred for 30 minutes at - 30 °C. Afterwards the reaction was cooled down to - 78 °C. 1.95 ml 2-isopropoxyphenol (2009 mg, 13.2 mmol, 1 eq) dissolved in 2 ml dry DCM was slowly added. The reaction was allowed to warm up to room temperature over 5 hours. After completion, 40 ml water and 10 ml diethyl ether were added. 5 ml 1 M HCl was added and the organic phase was separated. The aqueous phase was extracted with 3 x 10 ml diethyl ether. The organic phase was washed with 15 ml 1 M HCl and saturated Na_2SO_3 solution. The solvent was removed under reduced pressure. The product was isolated by vacuum distillation (bp 113 °C at 14.7 mbar). The product was a clear colorless oil.

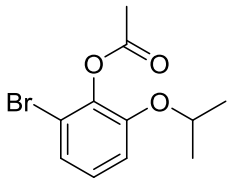
Yield: 1,898 mg (62 %)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.08 (dd, 1H, Ar-H, J = 1.4 Hz, 8.2 Hz), 6.82 – 6.80 (m, 1H- Ar-H), 6.71 (t, 1H, Ar-H, J = 8.2 Hz), 6.01 (s, 1H, Ar-OH), 4.58 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.37 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 145.5 (C_{Ar}), 144.4 (C_{Ar}), 125.0 (C_{Ar}-H), 120.6 (C_{Ar}-H), 112.6 (C_{Ar}-H), 108.4 (C_{Ar}), 72.5 ($CH(Me)_2$), 22.3 ($(CH_3)_2$)

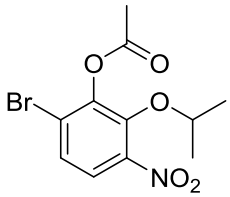
HRMS (ESI) calculated 228.9864/230.9844 [M-H$^+$], 228.9870/230.9850 found.

2-Bromo-6-isopropoxyphenyl acetate[4]



5.0 g 2-bromo-6-isopropoxyphenol (21.6 mmol, 1 eq) was added to a dry flask under nitrogen atmosphere. 10 ml dry pyridine (9.82 g, 124.1 mmol, 5.7 eq) and 4.0 ml acetic anhydride (4328 mg, 42.4 mmol, 2.0 eq) were added to the stirring mixture under nitrogen atmosphere at 0 °C. The reaction was slowly allowed to reach room temperature. After 2 hours, 23 ml 6M HCl and 47 ml brine was added. The aqueous phase was extracted 3 times with 25 ml diethyl ether. The organic phase was washed 2 times with 20 ml brine. The organic phase was removed under reduced pressure. The crude product was used without further purification.

6-Bromo-2-isopropoxy-3-nitrophenyl acetate[4]



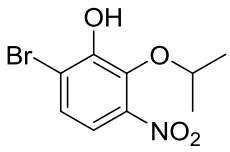
To a dry flask 10.0 ml fuming nitric acid (15.1 g, 240.0 mmol, 13.4 eq) was added and cooled down to - 40 °C. 4.91 g crude 2-bromo-6-isopropoxyphenyl acetate (18.0 mmol, 1 eq) was dissolved in 30 ml dry DCM and the solution was slowly added to the cooled mixture, while stirring vigorously. The mixture was kept at - 40 °C and controlled over TLC. After completion, the reaction was quenched with 150 ml of cold water. The aqueous phase was extracted with 3 x 50 ml DCM. The solvent was removed under reduced pressure. The crude product was used without further purification.

Yield: 5,717.0 mg (crude)

Rf: 0.5 (PE:EE 9:1)

HRMS (ESI) calculated 317.9977/319.9957 [M+H$^+$], 317.9971/319.9952 found.

6-Bromo-2-isopropoxy-3-nitrophenol (**74**)[4]



5.72 g crude 6-bromo-2-isopropoxy-3-nitrophenyl acetate (18.0 mmol, 1 eq) was dissolved in 20 ml THF and 40 ml water. 920 mg lithium hydroxide (38.4 mmol, 2.1 eq) was added to the stirring mixture. After completion, the THF was partially removed under reduced pressure. The reaction was acidified by 6 M HCl and 40 ml brine was added. The aqueous phase was extracted with 3 x 30 ml ethyl acetate. The combined organic fractions were combined. The crude product was purified by chromatography (petroleum ether/ ethyl acetate + 2% AcOH 5:1). The product was a pale-yellow oil.

Yield: 4,762.4 mg (80 % - over 3 steps)

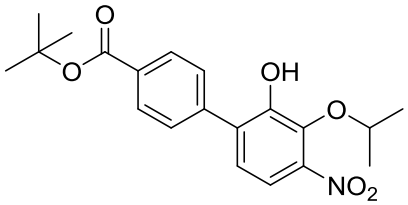
Rf: 0.33 (PE:EE + 2 % AcOH 5:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.38 (d, 1H, Ar-H, J = 9.0 Hz), 7.34 (d, 1H, Ar-H, J = 9.0 Hz), 6.27 (s, 1H, Ar-OH), 4.46 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.37 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 149.0 (C_{Ar}-OH), 142.6 (C_{Ar}-NO_2), 139.5 (C_{Ar}-O), 127.2 (C_{Ar}-H), 117.1 (C_{Ar}-H), 114.8 (C_{Ar}-Br), 80.0 ($CH(Me)_2$), 22.6 ($(CH_3)_2$)

HRMS (ESI) calculated 273.9715/275.9694 [M-H^+], 273.9721/275.9701 found.

tert-Butyl 2'-hydroxy-3'-isopropoxy-4'-nitro-[1,1'-biphenyl]-4-carboxylate (**82**)[227]



200 mg 6-bromo-2-isopropoxy-3-nitrophenyl acetate (0.63 mmol, 1 eq), 192.0 mg *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (0.63 mmol, 1 eq), 36.0 mg Tetrakis(triphenylphosphine)-palladium(0) (0.03 mmol, 0.05 eq) and 261 mg potassium carbonate (1.89 mmol, 3.0 eq) were added to a flask under nitrogen atmosphere. 6.9 ml 1,4-dioxane and 2.3 ml water were added and the reaction mixture was degassed with nitrogen. The reaction flask was sealed and stirred at 100 °C for 2 hours. After completion, 5 ml 1 M HCl and 15 ml brine were added to the mixture. The aqueous phase was extracted

with 3 x 8 ml ethyl acetate. The solvent was removed under reduced pressure. The crude residue was dissolved in 1 ml THF and 1 ml water and 75.0 mg lithium hydroxide (3.13 mmol, 5 eq) were added. The reaction was controlled over LCMS. After completion, the reaction was quenched with 5 ml 1 M HCl and 15 ml brine and extracted with 3 x 8 ml of ethyl acetate. The organic solvent was removed under reduced pressure and the crude product was purified by chromatography (petroleum ether/ ethyl acetate). The product was a yellow solid.

Yield: 70.4 mg (30 %)

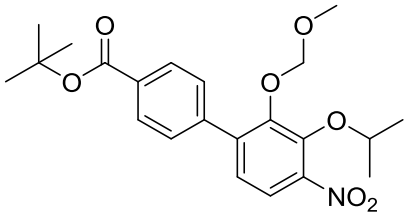
Rf: 0.5 (PE:EE 9:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.08 (d, 2H, Ar-H, J = 8.4 Hz), 7.66 (d, 2H, Ar-H, J = 8.4 Hz), 7.58 (d, 1H, Ar-H, J = 8.7 Hz), 7.20 (d, 1H, Ar-H, J = 8.7 Hz), 6.41 – 6-39 (m, 1H, Ar-OH), 4.39 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.61 (s, 9H, $(CH_3)_3$), 1.40 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 165.5 (COO), 148.7 (C_{Ar}-OH), 141.8 (C_{Ar}-NO_2), 140.0 (C_{Ar}), 139.3 (C_{Ar}-O), 132.4 (C_{Ar}), 132.0 (C_{Ar}), 129.7 (C_{Ar}-H), 129.1 (C_{Ar}-H), 124.8 (C_{Ar}-H), 116.9 (C_{Ar}-H), 81.4 ($C(Me)_3$), 80.1 ($CH(Me)_2$), 28.4 (($CH_3)_3$), 22.7 (($CH_3)_2$)

HRMS (ESI) calculated 372.1447 [M-H^+], 372.1453 found.

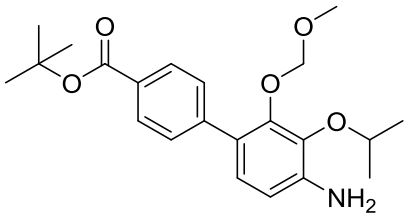
tert-Butyl 3'-isopropoxy-2'-(methoxymethoxy)-4'-nitro-[1,1'-biphenyl]-4-carboxylate[221]



70.0 mg *tert*-butyl 2'-hydroxy-3'-isopropoxy-4'-nitro-[1,1'-biphenyl]-4-carboxylate (0.19 mmol, 1 eq) and 8.0 mg DMAP (0.07 mmol, 0.35 eq) were added to a dry flask and further dried under high vacuum. 4.2 ml dry DCM, 1 ml dry THF and 66.0 µl DIPEA (49.0 mg, 0.38 mmol, 2 eq) were added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 17.0 µL MOM-Br (90 % technical grade, 0.19 mmol, 1 eq) was slowly added to the stirring mixture under nitrogen atmosphere. The reaction was allowed to reach room temperature and stirred for 3 hours. After completion, 1 ml 1 M HCl and 4 ml water were added. The aqueous phase was extracted three times with 2 ml diethyl ether. The combined organic phases were concentrated under reduced pressure and dried under high vaccum. The crude product was a yellow solid and used without further purification.

Yield: 78.5 mg (crude)

tert-Butyl 4'-amino-3'-isopropoxy-2'-(methoxymethoxy)-[1,1'-biphenyl]-4-carboxylate (**83**)[4]



75.0 mg crude *tert*-butyl 3'-isopropoxy-2'-(methoxymethoxy)-4'-nitro-[1,1'-biphenyl]-4-carboxylate (0.18 mmol, 1 eq) was dissolved in 0.8 ml THF and 0.65 ml ethanol and cooled down to 0 °C. 176 mg zinc dust (2.7 mmol, 15.0 eq) was added. 0.16 ml acetic acid (168 mg, 2.8 mmol, 15.5 eq) was added to the stirring mixture over 30 minutes and the mixture was allowed to reach room temperature. After completion, the zinc dust was filtered off. 10 ml saturated $NaHCO_3$ solution was added and the aqueous phase was extracted with 3 x 6 ml ethyl acetate. The combined organic phases were concentrated under reduced pressure and dried under high vacuum. The product was an orange to brown oil.

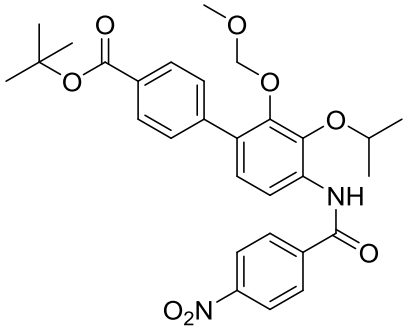
Yield: 74.6 mg (quantitative over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.98 (d, 2H, Ar-H, J = 8.5 Hz), 7.56 (d, 2H, Ar-H, J = 8.5 Hz), 6.90 (d, 1H, Ar-H, J = 8.3 Hz), 6.58 (d, 1H, Ar-H, J = 8.3 Hz), 4.88 (s, 2H, OCH_2O), 4.61 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 3.91 (br s, 2H, NH_2), 3.01 (s, 3H, OCH_3), 1.61 (s, 9H, $(CH_3)_3$), 1.35 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 166.1 (COO), 148.1 (C_{Ar}-O), 143.6 (C_{Ar}), 142.3 (C_{Ar}-NH_2), 138.1 (C_{Ar}-O), 129.8 (C_{Ar}), 129.4 (C_{Ar}-H), 129.3 (C_{Ar}-H), 126.0 (C_{Ar}), 125.5 (C_{Ar}-H), 111.6 (C_{Ar}-H), 98.8 (OCH_2O), 81.0 ($C(Me)_3$), 75.2 ($CH(Me)_2$), 57.3 (OCH_3), 28.4 ($(CH_3)_3$), 22.9 ($(CH_3)_2$)

HRMS (ESI) calculated 388.2124 [$M+H^+$], 388.2118 found.

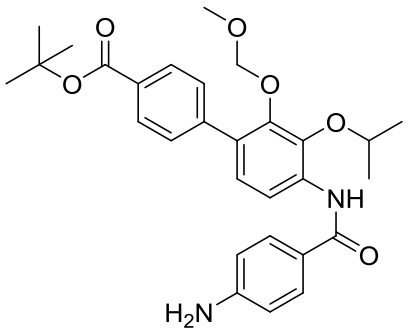
tert-Butyl 3'-isopropoxy-2'-(methoxymethoxy)-4'-(4-nitrobenzamido)-[1,1'-biphenyl]-4-carboxylate[182]



73.0 mg *tert*-butyl 4'-amino-3'-isopropoxy-2'-(methoxymethoxy)-[1,1'-biphenyl]-4-carboxylate (0.19 mmol, 1 eq) and 53.0 mg 4-nitrobenzoyl chloride were added to a dry flask under nitrogen atmosphere. 1.6 ml dry DCM was added and the mixture was cooled down to 0 °C. 61.0 µl dry pyridine (60 mg, 0.73 mmol, 4 eq) was slowly added to the stirring mixture. After completion, the reaction was quenched with 2 ml 1M HCl and 8 ml brine. The organic phase was extracted with 4 x 4 ml ethyl acetate. The organic phases were combined and washed with 2 x 5 ml saturated $NaHCO_3$ solution. The solvent was evaporated under reduced pressure. The crude product was used without further purification.

Yield: 102.3 mg (crude)

tert-Butyl 4'-(4-aminobenzamido)-3'-isopropoxy-2'-(methoxymethoxy)-[1,1'-biphenyl]-4-carboxylate (**84**)[182]



102 mg crude *tert*-butyl 3'-isopropoxy-2'-(methoxymethoxy)-4'-(4-nitrobenzamido)-[1,1'-biphenyl]-4-carboxylate (0.2 mmol, 1 eq) and 187 mg zinc dust (2.86 mmol, 15.0 eq) were added to 0.9 ml THF and 0.7 ml ethanol. The mixture was cooled down to 0 °C. 0.17 ml acetic acid (178.3 mg, 3.0 mmol, 15.6 eq) was slowly added to the stirring mixture at 0 °C over 1 hour. The mixture was allowed to reach room temperature. After completion, the zinc dust was filtered off. 10 ml saturated $NaHCO_3$ solution was added and the aqueous phase was extracted with 3 x 8 ml ethyl acetate. The organic solvent was removed under reduced pressure. The crude product was purified by chromatography (petroleum ether/ ethyl acetate).

Yield: 66.8 mg (70 % over 2 steps)

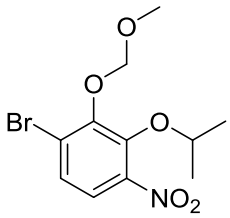
Rf: 0.12 (PE:EE 3:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.59 (s, 1H, CONH), 8.38 (d, 1H, Ar-H, J = 8.6 Hz), 8.02 (d, 2H, Ar-H, J = 8.6 Hz), 7.75 (d, 2H, Ar-H, J = 8.7 Hz), 7.60 (d, 2H, Ar-H, J = 8.5 Hz), 7.13 (d, 1H, Ar-H, J = 8.6 Hz), 6.74 (d, 2H, Ar-H, J = 8.7 Hz), 4.86 (s, 2H, OCH_2O), 4.76 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.03 (s, 3H, OCH_3), 1.62 (s, 9H, $(CH_3)_3$), 1.38 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 166.0 (COO), 164.9 (CONH), 150.1 (C_{Ar}), 147.0 (C_{Ar}), 142.8 (C_{Ar}), 139.8 (C_{Ar}), 134.1 (C_{Ar}), 130.9 (C_{Ar}), 130.5 (C_{Ar}), 129.6 (C_{Ar}-H), 129.4 (C_{Ar}-H), 129.0 (C_{Ar}-H), 125.7 (C_{Ar}-H), 124.5 (C_{Ar}), 115.8 (C_{Ar}-H), 114.5 (C_{Ar}-H), 99.0 (OCH_2O), 81.1 ($C(Me)_3$), 76.2 ($CH(Me)_2$), 57.5 (OCH_3), 28.4 (($CH_3)_3$), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 507.2495 [M+H$^+$], 507.2490 found.

1-Bromo-3-isopropoxy-2-(methoxymethoxy)-4-nitrobenzene (**75**)[221]



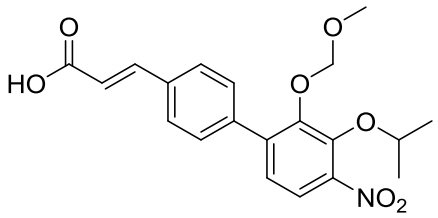
520 mg 6-bromo-2-isopropoxy-3-nitrophenol (1.88 mmol, 1 eq) and 80.0 mg DMAP (0.65 mmol, 0.35 eq) were added to a dry flask and further dried under high vaccum. 16 ml dry DCM, 4 ml dry THF and 0.66 ml DIPEA (490 mg, 3.8 mmol, 2.0 eq) were added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 0.18 ml MOM-Br (90 % technical grade, 2.0 mmol, 1.05 eq) was slowly added to the stirring mixture under nitrogen atmosphere. The reaction was kept at 0 °C. After completion, 6 ml 1 M HCl and 30 ml water were added. The aqueous phase was extracted 3 times with 10 ml TBME. The combined organic solvents were washed with 2 x 10 ml brine. The organic solvent was removed under reduced pressure. The product was a yellow oil.

Yield: 605.6 mg (quantitative)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.45 (d, 1H, Ar-H, J = 8.9 Hz), 7.40 (d, 1H, Ar-H, J = 8.9 Hz), 5.24 (s, 2H, CH_2), 4.59 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.67 (s, 3H, OCH_3), 1.30 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 149.9 (C_{Ar}), 145.7 (C_{Ar}), 145.4 (C_{Ar}), 127.6 (C_{Ar}-H), 123.6 (C_{Ar}), 120.7 (C_{Ar}-H), 99.6 (CH_2), 78.7 ($CH(Me)_2$), 58.7 (OCH_3), 22.4 ($(CH_3)_2$)

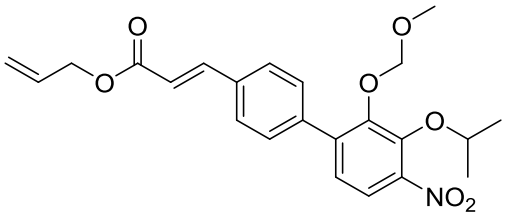
(*E*)-3-(3'-Isopropoxy-2'-(methoxymethoxy)-4'-nitro-[1,1'-biphenyl]-4-yl)acrylic acid[226]



150 mg 1-bromo-3-isopropoxy-2-(methoxymethoxy)-4-nitrobenzene (0.47 mmol, 1 eq), 135 mg (*E*)-3-(4-boronophenyl)acrylic acid (0.7 mmol, 1.5 eq) and 225 mg caesium carbonate (0.7 mmol, 1.5 eq) were added to a dry and pressure stable vial and further dried under high vacuum. 4.0 ml 1,4-dioxane and 0.8 ml water were added and the mixture was degassed with nitrogen. 27.0 mg Tetrakis-(triphenylphosphine)palladium(0) (0.02 mmol, 0.05 eq) was added under nitrogen atmosphere. The vial was sealed and the mixture was refluxed overnight. After completion, 1 ml 1 M HCl was added and the solvent was removed under reduced pressure. The crude product was purified by RP flash chromatography.

Yield: 74.6 mg (crude)

Allyl (*E*)-3-(3'-isopropoxy-2'-(methoxymethoxy)-4'-nitro-[1,1'-biphenyl]-4-yl)acrylate (**81**)[100]



74.6 mg crude (*E*)-3-(3'-isopropoxy-2'-(methoxymethoxy)-4'-nitro-[1,1'-biphenyl]-4-yl)acrylic acid (0.19 mmol, 1 eq) and 53.0 mg potassium carbonate (0.38 mmol, 2 eq) were added to a dry flask and further dried under high vacuum. The flask was flushed with nitrogen. 0.5 ml dry DMF and 25.0 µl allyl bromide (35.0 mg, 0.29 mmol, 1.5 eq) were added under nitrogen atmosphere. The reaction was stirred overnight and controlled via TLC. After completion, a mixture of 15 ml brine and 2 ml 1 M HCl were added to the residue and it was extracted with 6 x 3 ml ethyl acetate. The product was purified by flash chromatography (petroleum ether/ethyl acetate).

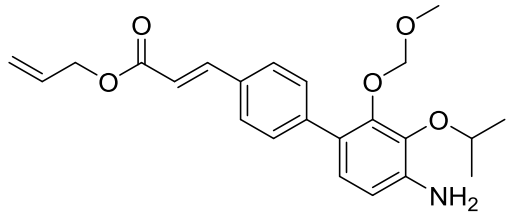
Yield: 78.7 mg (39 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.75 (d, 1H, CH=, J = 16.0 Hz), 7.62 – 7.60 (m, 3H, Ar-H), 7.57 (d, 2H, Ar-H, J = 8.4 Hz), 7.16 (d, 1H, Ar-H, J = 8.5 Hz), 6.53 (d, 1H, CH=, J = 16.0 Hz), 6.01 (ddt, 1H, CH=, J = 5.7 Hz, 10.4 Hz, 17.2 Hz), 5.39 (dq, 1H, $=CH_2$, J = 1.5 Hz, 17.2 Hz), 5.29 (dq, 1H, $=CH_2$, J = 1.3 Hz, 10.4 Hz), 4.95 (s, 2H, OCH_2O), 4.73 (dt, 2H, OCH_2, J = 1.4 Hz, 5.7 Hz), 4.68 (q, 1H, $CH(Me)_2$, J = 5.7 Hz), 2.96 (s, 3H, OCH_3), 1.35 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 166.6 (COO), 149.4 (C_{Ar}), 145.4 (C_{Ar}), 144.3 (CH=), 141.1 (C_{Ar}), 139.0 (C_{Ar}), 134.3 (C_{Ar}), 132.4 (CH=), 130.3 (C_{Ar}-H), 128.2 (C_{Ar}-H), 124.7 (C_{Ar}-H), 120.3 (CH=), 118.8 (C_{Ar}-H), 118.6 ($=CH_2$), 99.4 (OCH_2O), 78.2 ($CH(Me)_2$), 65.5 (OCH_2), 57.4 (OCH_3), 22.5 ($(CH_3)_2$)

HRMS (ESI) calculated 428.1709 [$M+H^+$], 428.1702 found.

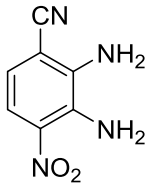
Allyl (*E*)-3-(4'-amino-3'-isopropoxy-2'-(methoxymethoxy)-[1,1'-biphenyl]-4-yl)acrylate[4]



78.0 mg allyl (*E*)-3-(3'-isopropoxy-2'-(methoxymethoxy)-4'-nitro-[1,1'-biphenyl]-4-yl)acrylate (0.18 mmol, 1 eq) was dissolved in 1.5 ml THF and cooled down to 0 °C. 165 mg zinc dust (2.52 mmol, 13.8 eq) was added. 0.15 ml acetic acid (157 mg, 2.6 mmol, 14.4 eq) was added to the stirring mixture over the timespan of 30 minutes and the mixture was allowed to reach room temperature. After completion, the zinc dust was filtered off. 10 ml saturated $NaHCO_3$ solution was added and the aqueous phase was extracted with 3 x 6 ml ethyl acetate. The solvent of the combined organic phases was evaporated under reduced pressure. The crude product was used without further purification.

Yield: 73.0 mg (crude)

2,3-Diamino-4-nitrobenzonitrile (**85**)[251]



200 mg 2-amino-4-nitrobenzonitrile (1.23 mmol, 1 eq) and 248 mg 1,1,1-trimethylhydrazinium iodide (1.23 mmol, 1 eq) were dissolved in 10.0 ml dry DMSO under nitrogen atmosphere. 420 mg potassium *tert*-butoxide (3.74 mmol, 3.05 eq) was added portionwise under nitrogen atmosphere. The reaction mixture was stirred overnight. After completion, the reaction was cooled to 0 °C and quenched with 3 M HCl to a pH of 3. The aqueous phase was extracted with 3 x 20 ml DCM. The organic solvent was removed under reduced pressure. The crude product was purified by chromatography (petroleum ether/ethyl acetate). The product was a dark red solid.

Yield: 123.9 mg (57 %)

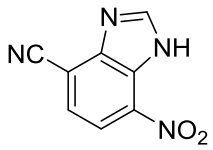
Rf: 0.5 (PE:EE 2:1)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 7.46 (br s, 2H, NH_2), 7.28 (d, 1H, Ar-H, J = 9.2 Hz), 6.69 (d, 1H, Ar-H, J = 9.2 Hz), 6.42 (br s, 2H, NH_2)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 142.9 (C_{Ar}), 135.1 (C_{Ar}), 131.3 (C_{Ar}), 117.5 (CN), 116.4 (C_{Ar}-H), 112.1 (C_{Ar}-H), 94.5 (C_{Ar})

HRMS (ESI) calculated 179.0569 [M+H$^+$], 179.0566 found.

7-Nitro-1-benzo[*d*]imidazole-4-carbonitrile (**86**)[252]



100 mg 2,3-diamino-4-nitrobenzonitrile (0.56 mmol, 1 eq) was added to a microwave vial. 1.11 ml trimethoxymethane (1.07 g, 10 mmol, 18 eq) and a few drops of concentrated hydrochloric acid were added and the vial was sealed. The reaction was stirred at 80 °C overnight. After completion, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (DCM:MeOH). The product was an orange brown solid.

Yield: 68.3 mg (65 %)

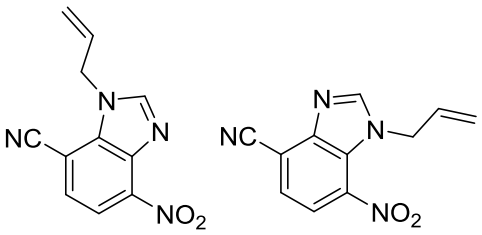
Rf: 0.26 (DCM:MeOH 19:1)

^{1}H-NMR (500 MHz, THF-d_8, 300 K): δ (ppm) = 13.03 (br s, 1H, NH), 8.51 (s, 1H, Ar-H), 8.25 (d, 1H, Ar-H, J = 8.4 Hz), 7.80 (d, 1H, Ar-H, J = 8.4 Hz)

^{13}C-NMR (126 MHz, THF-d_8, 300 K): δ (ppm) = 148.7 (C_{Ar}), 147.2 (C_{Ar}-H), 136.8 (C_{Ar}), 128.8 (C_{Ar}), 126.4 (C_{Ar}-H), 119.1 (C_{Ar}-H), 115.7 (CN), 110.3 (C_{Ar})

HRMS (ESI) calculated 189.0413 [M+H$^+$], 189.0407 found.

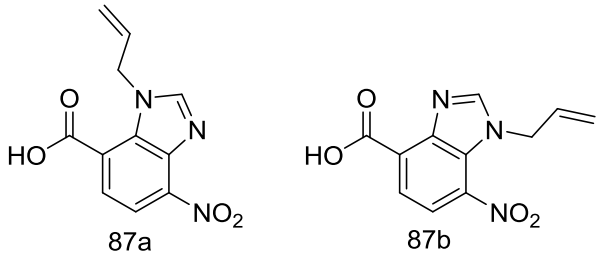
1-Allyl-4-nitro-1*H*-benzo[*d*]imidazole-7-carbonitrile & 1-Allyl-7-nitro-1*H*-benzo[*d*]imidazole-4-carbonitrile[253]



63 mg 7-nitro-1*H*-benzo[*d*]imidazole-4-carbonitrile (0.33 mmol, 1 eq) and 93 mg potassium carbonate (0.67 mmol, 2 eq) were added to a dry flask and further dried under high vacuum. The flask was flushed with nitrogen. 0.35 ml dry DMF and 35 µl allyl bromide (49 mg, 0.40 mmol, 1.2 eq) were added under nitrogen atmosphere. The reaction was stirred overnight and controlled over LCMS. After completion, the solid was filtered off and washed with DCM. The solvent was carefully removed and coevaporated with *n*-heptane. The crude product was used in the next reaction without purification.

Yield: 76.4 mg (crude)

1-Allyl-4-nitro-1*H*-benzo[*d*]imidazole-7-carboxylic acid (**87a**) & 1-Allyl-7-nitro-1*H*-benzo[*d*]imidazole-4-carboxylic acid (**87b**)



76.4 mg of a crude mixture containing 1-allyl-4-nitro-1*H*-benzo[*d*]imidazole-7-carbonitrile and 1-allyl-7-nitro-1*H*-benzo[*d*]imidazole-4-carbonitrile (0.33 mmol, 1 eq), 1.0 ml THF and 1.0 ml water were added to a flask. 48 mg lithium hydroxide (2.0 mmol, 6.0 eq) was added and the mixture was refluxed overnight. The reaction was controlled over LCMS. After completion, the crude mixture was acidified with 1 M HCl and the solvent was removed under reduced pressure. The crude product was purified by RP flash.

Yield: 102a - 25.7 mg (31 % over 2 steps)

102b - 29.9 mg (36 % over 2 steps)

55.6 mg in total (67 % in total over 2 steps)

87a.

^{1}H-NMR (500 MHz, THF-d_8, 300 K): δ (ppm) = 8.28 (s, 1H, Ar-H), 7.94 (d, 1H, Ar-H, J = 8.3 Hz), 7.87 (d, 1H, Ar-H, J = 8.3 Hz), 5.95 (ddt, 1H, CH=, J = 5.3 Hz, 10.5 Hz, 17.1 Hz), 5.27 (d, 2H, NCH_2, J = 5.3 Hz), 5.12 (dd, 1H, $=CH_2$, J = 1.3 Hz, 10.4 Hz), 4.90 (dd, 1H, $=CH_2$, J = 1.4 Hz, 17.2 Hz)

^{13}C-NMR (126 MHz, THF-d_8, 300 K): δ (ppm) = 166.7 (COOH), 149.9 (C_{Ar}-H), 143.1 (C_{Ar}), 140.0 (C_{Ar}), 135.1 (C_{Ar}) 134.5 (CH=), 125.1 (C_{Ar}-H), 123.4 (C_{Ar}), 117.4 (C_{Ar}-H), 117.4 ($=CH_2$), 50.5 (NCH_2)

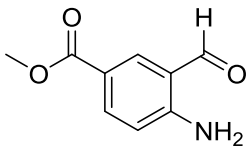
87b.

^{1}H-NMR (500 MHz, THF-d_8, 300 K): δ (ppm) = 8.56 (s, 1H, Ar-H), 8.09 (br s, 2H, Ar-H), 6.03 – 5.95 (m, 1H, CH=), 5.20 – 5.15 (m, 3H, $=CH_2$ & NCH_2), 4.96 – 4.91 (m, 1H, $=CH_2$)

^{13}C-NMR (126 MHz, THF-d_8, 300 K): δ (ppm) = 163.9 (COOH), 148.9 (C_{Ar}-H), 146.9 (C_{Ar}), 139.9 (C_{Ar}), 133.8 (CH=), 126.3 (C_{Ar}), 126.1 (C_{Ar}), 125.0 (C_{Ar}-H), 121.0 (C_{Ar}-H), 118.2 ($=CH_2$), 51.1 (NCH_2)

HRMS (ESI) for 87a calculated 248.0671 [$M+H^+$], 248.0666 found.

Methyl 4-amino-3-formylbenzoate (76)



300 mg methyl 3-formyl-4-nitrobenzoate (1.4 mmol, 1 eq) was dissolved in 6.2 ml THF and 5.0 ml ethanol and cooled down to 0 °C. 1.38 g zinc dust (21.1 mmol, 15 eq) was added. 1.15 ml acetic acid (1.21 g, 20.1 mmol, 14.0 eq) was added to the stirring mixture over 30 minutes and the mixture was allowed to reach room temperature. After completion, the zinc dust was filtered off. 45 ml saturated $NaHCO_3$ solution was added and the aqueous phase was extracted with 3 x 25 ml ethyl acetate. The combined organic phases were removed under reduced pressure and the crude product was purified by chromatography (petroleum ether/ ethyl acetate). The product was a yellow solid.

Yield: 156.1 mg (61 %)

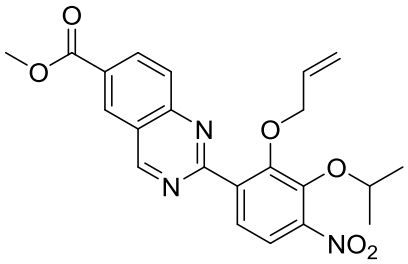
Rf: 0.33 (PE:EE 4:1)

^{1}H-NMR (700 MHz, MeOH-d_4, 300 K): δ (ppm) = 9.85 (s, 1H, CHO), 8.21 (d, 1H, Ar-H, J = 2.1 Hz), 7.86 (dd, 1H, Ar-H, J = 2.1 Hz, 8.8 Hz), 6.76 (d, 1H, Ar-H, J = 8.8 Hz), 3.86 (s, 3H, OCH_3)

^{13}C-NMR (176 MHz, MeOH-d_4, 300 K): δ (ppm) = 195.1 (CHO), 168.0 (COO), 155.6 (C_{Ar}), 140.0 (C_{Ar}-H), 136.5 (C_{Ar}-H), 118.6 (C_{Ar}), 118.0 (C_{Ar}), 116.8 (C_{Ar}-H), 52.2 (OCH_3)

HRMS (ESI) calculated 180.0661 [M+H$^+$], 180.0655 found.

Methyl 2-(2-(allyloxy)-3-isopropoxy-4-nitrophenyl)quinazoline-6-carboxylate (**77**)[224]



50 mg methyl 4-amino-3-formylbenzoate (0.28 mmol, 1 eq) and 93 mg 2-(allyloxy)-3-isopropoxy-4-nitrobenzaldehyde (0.35 mmol, 1.26 eq) were added to a flask. 5.0 ml water and 220 mg ammonium acetate (2.85 mmol, 10 eq) were added and the mixture was heated up to 75 °C. After completion, the solid was filtered off and dissolved in ethyl acetate. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate).

Yield: 49.3 mg (42 %)

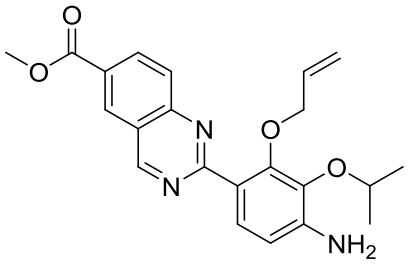
Rf: 0.7 (PE:EE 2:1)

^{1}H-NMR (500 MHz, Aceton-d_6, 300 K): δ (ppm) = 9.87 (d, 1H, Ar-H, J = 0.8 Hz), 8.89 (dd, 1H, Ar-H, J = 0.6 Hz, 1.9 Hz), 8.55 (dd, 1H, Ar-H, J = 1.9 Hz, 8.8 Hz), 8.17 (dt, 1H, Ar-H, J = 0.7 Hz, 8.8 Hz), 7.85 (d, 1H, Ar-H, J = 8.6 Hz), 7.73 (d, 1H, Ar-H, J = 8.6 Hz), 6.08 (ddt, 1H, CH=, J = 5.6 Hz, 10.5 Hz, 17.2 Hz), 5.34 (dq, 1H, $=CH_2$, J = 1.7 Hz, 17.2 Hz), 5.16 (dq, 1H, $=CH_2$, J = 1.3 Hz, 10.5 Hz), 4.83 – 4.76 (m, 3H, OCH_2 & $CH(Me)_2$), 4.01 (s, 3H, OCH_3), 1.30 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, Aceton-d_6, 300 K): δ (ppm) = 166.2 (COO), 163.1 (C_{Ar}-H), 162.7 (C_{Ar}), 153.4 (C_{Ar}), 153.0 (C_{Ar}), 139.3 (C_{Ar}), 134.9 (C_{Ar}-H or CH=), 134.5 (C_{Ar}-H or CH=), 131.3 (C_{Ar}-H), 130.6 (C_{Ar}), 129.8 (C_{Ar}-H), 127.1 (C_{Ar}-H), 123.7 (C_{Ar}), 119.8 (C_{Ar}-H), 117.7 ($=CH_2$), 78.0 ($CH(Me)_2$), 75.5 (OCH_2), 53.0 (OCH_3), 22.6 ($(CH_3)_2$)

HRMS (ESI) calculated 424.1509 [$M+H^+$], 424.1503 found.

Methyl 2-(2-(allyloxy)-4-amino-3-isopropoxyphenyl)quinazoline-6-carboxylate (**78**)[225]



212 mg methyl 2-(2-(allyloxy)-3-isopropoxy-4-nitrophenyl)quinazoline-6-carboxylate (0.5 mmol, 1 eq) was dissolved in 3.0 ml ethanol and cooled down to 0 °C. The reaction was sealed with a septum. 436 mg sodium dithionite (2.5 mmol, 5 eq) was dissolved in 1.2 ml water and added dropwise while maintaining 0 °C. The reaction was allowed to reach room temperature. After 3 h, another 436 mg sodium dithionite (2.5 mmol, 5 eq) was added. The reaction was stirred overnight. After completion, the solvent was removed under reduced pressure. The crude product was purified by RP flash chromatography.

Yield: 28.0 mg (14 %)

^{1}H-NMR (500 MHz, MeOH-d_4, 300 K): δ (ppm) = 8.18 (d, 1H, Ar-H, J = 1.8 Hz), 8.08 (dd, 1H, Ar-H, J = 1.9 Hz, 8.4 Hz), 7.38 (d, 1H, Ar-H, J = 8.7 Hz), 7.30 (d, 1H, Ar-H, J = 8.4 Hz), 6.68 (d, 1H, Ar-H, J = 8.7 Hz), 6.03 (ddt, 1H, CH=, J = 6.1 Hz, 10.4 Hz, 16.8 Hz), 5.64 (s, 1H, Ar-H), 5.27 (dq, 1H, =CH_2, J = 1.4 Hz, 17.2 Hz), 5.16 – 5.13 (m, 1H, =CH_2), 4.74 – 4.67 (m, 3H, $CH(Me)_2$ & OCH_2), 4.53 – 4.48 (m, 1H, OCH_2), 3.92 (s, 3H, OCH_3), 1.43 (d, 3H, $(CH_3)_2$, J = 6.2 Hz), 1.26 (d, 3H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, MeOH-d_4, 300 K): δ (ppm) = 188.4 (COOMe), 176.4 (C_{Ar}), 171.9 (C_{Ar}), 171.0 (C_{Ar}), 153.8 (C_{Ar}), 153.4 (C_{Ar}), 149.9 (C_{Ar}-H), 147.3 (C_{Ar}-H), 146.8 (C_{Ar}-H), 143.8 (C_{Ar}), 142.4 (C_{Ar}-H), 132.2 (=CH_2), 131.6 (C_{Ar}), 130.4 (C_{Ar}-H), 122.6 (C_{Ar}), 118.8 (C_{Ar}-H), 81.4 (C_{aliph}-H), 80.7 (OCH_2), 72.3 (C_{aliph}-H), 53.5 (C_{aliph}-H), 18.6 (C_{aliph}-H), 17.5 (C_{aliph}-H)

HRMS (ESI) calculated 394.1767 [M+H^+], 394.1760 found.

5.2.2.2 Synthesis Fragment AB

The amide coupling and deprotection methods were standardized. Adapted experimental procedures from the literature are quoted in the listing of the general procedures. Citations at the end of the molecule name are added, if methods other than the general procedures were applied.

4.2.2.2.1 General procedures – amide coupling

The amide coupling between ring A and B was carried out by one of three general procedures depending on the electronic properties of ring A and B. The three general procedures for the amide coupling are:

A.) Acid chloride formation via oxalyl chloride and subsequent coupling with triethylamine [A1] or pyridine [A2][177].
B.) Acid chloride formation via oxalyl chloride and subsequent coupling under Schotten-Baumann conditions in THF and saturated sodium bicarbonate solution[183].
C.) Coupling via HATU and DIPEA[184].

A.) Amide coupling via acid chloride

0.68 mmol of the desired benzoic acid (1 eq), 2 ml dry DCM and one drop of dry DMF were added to a dry flask under nitrogen atmosphere. The mixture was cooled to 0 °C and 0.08 ml oxalyl chloride (0.93 mmol, 1.4 eq) was slowly added to the stirring mixture. The reaction was controlled over TLC with a part of crude product in methanol and petroleum ether: ethyl acetate as solvent mixture. The solvent was evaporated under reduced pressure. The crude product was dried under high vacuum and used without further purifications.

0.6 mmol of the desired amine (1.0 eq) and 0.63 mmol of the desired benzoyl chloride (1.04 eq) were added to a dry flask and further dried under high vacuum. 4.0 ml dry DCM was added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 0.20 ml dry triethylamine [A1] (1.84 mmol, 3.1 eq) or 0.15 ml dry pyridine [A2] (1.86 mmol, 3.1 eq) were slowly added to the stirring mixture while maintaining 0 °C. After 15 minutes, the reaction was allowed to reach room temperature. The reaction was stirred overnight and controlled over TLC. After the reaction was completed, 5 ml 1 M HCl and 5 ml water were added to the mixture. The organic phase was separated and stored. The aqueous phase was extracted with 3 x 8 ml of ethyl acetate and all organic phases were combined. The organic phase was washed with 3 x 10 ml saturated $NaHCO_3$ solution. The organic phase was concentrated under reduced pressure and dried under high vacuum.

B.) Amide coupling under Schotten-Baumann conditions

0.68 mmol of the desired benzoic acid (1 eq), 2 ml dry DCM and one drop of dry DMF were added to a dry flask under nitrogen atmosphere. The mixture was cooled to 0 °C and 0.08 ml oxalyl chloride (0.93 mmol, 1.4 eq) was slowly added to the stirring mixture. The reaction was controlled over TLC with a part of crude product in methanol and petroleum ether: ethyl acetate as solvent mixture. The solvent was evaporated under reduced pressure. The crude product was dried under high vacuum and used without further purifications.

0.6 mmol of the desired amine (1 eq) was dissolved in 0.8 ml THF and 0.9 ml saturated $NaHCO_3$ solution (1 mmol, 1.7 eq). The solution was cooled down to 0 °C and 0.63 mmol of the desired benzoyl chloride (1.04 eq) was added to the stirring solution and the mixture was allowed to reach room temperature. The reaction was stirred overnight. After the reaction was completed, the mixture was acidified by 1 M HCl. The precipitate was filtered off and washed with 0.1 M HCl and methanol. The solid residue was dried under reduced pressure and further dried under high vacuum.

C.) Amide coupling with HATU

0.33 mmol of the desired benzoic acid (1 eq) and 0.33 mmol HATU (1 eq) were added to a dry flask and further dried under high vacuum. 2 ml dry DMF and 0.18 ml DIPEA (1.0 mmol, 3.1 eq) were added to the flask under nitrogen atmosphere and the mixture was stirred for 30 minutes. 0.33 mmol of the desired amine (1 eq) was added under nitrogen atmosphere. The reaction was controlled over TLC or LCMS.

After completion, an individual workup and purification was carried out depending on the utilized starting materials. The procedure is described in the respective section of the product.

4.2.2.2.2 General procedures – Fragment AB deprotection

The deprotection of the Fragment AB ester was carried out by on of the following general procedures:

D.) Hydrolysis of the ester via lithium hydroxide in a mixture of water and THF[178].
E.) Palladium catalyzed deprotection of allyl esters with phenysilane as scavenger[4].
F.) Deprotection of methyl esters via lithium iodide in dry ethyl acetate [F1][179] or dry pyridine [F2][180] under reflux.
G.) Deprotection of methyl esters via trimethyltin hydroxide in dichloroethane under reflux[181].
H.) Deprotection of *tert*-butyl esters via trifluoroacetic acid in DCM[182].

D.) Hydrolysis via lithium hydroxide

0.19 mmol of the desired ester (1 eq) was dissolved in 0.9 ml water and 0.6 ml THF. 0.76 mmol lithium hydroxide (4 eq) was added to the mixture. The reaction was controlled over TLC. After completion, the mixture was acidified with 1 M HCl. The solid was filtered off and washed with 1 M HCl. The solid residue was added to a flask and the remaining solvent was evaporated under reduced pressure.

E.) Allyl deprotection with palladium and phenylsilane

0.16 mmol of the desired allyl ester (1 eq), 0.05 mmol phenylsilane (3 eq) and 4 ml dry THF were added to a dry flask under nitrogen atmosphere. 0.02 mmol Tetrakis(triphenylphosphine)palladium(0) (0.1 eq) was added and the mixture was stirred overnight at room temperature. The reaction was controlled over TLC. After the starting material vanished completely, the solvent was removed under reduced pressure. The residue was dissolved in 4 ml 0.1 M NaOH and washed with 2 x 4 ml DCM. The aqueous phase was acidified to pH 1 with 6 M HCl and the precipitate was filtered off. The precipitate was washed with 1 M HCl and dried under high vacuum.

F1.) Deprotection of methyl esters via lithium iodide in ethyl acetate

0.32 mmol of the desired methyl ester (1 eq) was dissolved in 2 ml dry ethyl acetate. 3.2 mmol lithium iodide (10 eq) was added and the mixture was heated up to 110 °C in a sealed glass tube and stirred overnight. After completion, the mixture was cooled down to room temperature. The solid residue was filtered off and washed with ethyl acetate and 0.1 M HCl. The solid residue was dried under reduced pressure.

F2.) Deprotection of methyl esters via lithium iodide in pyridine

0.17 mmol of the desired methyl ester (1 eq) and 1.02 mmol lithium iodide (6 eq) were added to a dry and pressure stable glass tube. 0.42 ml dry pyridine was added under nitrogen atmosphere. The mixture was heated up to 110 °C and stirred overnight. After completion, the solvent was removed under reduced pressure. The solid residue was dissolved in 4 ml 0.1 M HCl and extracted with 3 x 3 ml ethyl acetate. The combined organic phases were evaporated under reduced pressure.

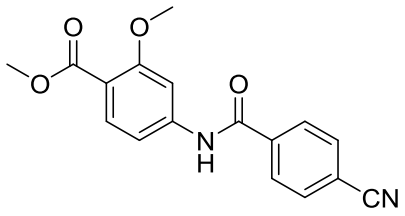
G.) Deprotection of methyl esters via trimethyl tin hydroxide in DCE

1.11 mmol of the desired methyl ester (1 eq) was dissolved in 2.5 ml 1,2-dichloroethane. 3.33 mmol trimethyltin hydroxide (3 eq) was added and the mixture was heated up to 80 °C in a sealed pressure stable vial. After completion, the solvent was removed under reduced pressure. The crude solid was washed with 2 M HCl and dried under reduced pressure.

H.) Deprotection of tert-butyl esters via trifluoroacetic acid in DCM

0.18 mmol of the desired *tert*-butyl ester (1 eq) was dissolved in 2.5 ml DCM under nitrogen atmosphere. 3.9 mmol TFA (22 eq) was added to the stirring mixture under nitrogen. After completion, the solvent was removed under reduced pressure. The excess of TFA was removed by coevaporation with DCM.

Methyl 4-(4-cyanobenzamido)-2-methoxybenzoate (**1**)



The aniline (1.1 mmol) was coupled with benzoic acid using procedure A1. The crude product was of sufficient purity for further reactions.

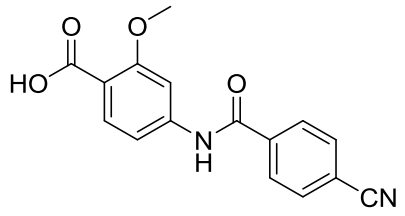
Yield: 240.0 mg (70 %)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.99 (d, 2H, Ar-H, J = 8.3 Hz), 7.87 (d, 1H, Ar-H, J = 8.4 Hz), 7.81 (d, 2H, Ar-H, J = 8.3 Hz), 7.73 (d, 1H, Ar-H, J = 1.6 Hz), 7.00 (dd, 1H, Ar-H, J = 1.8 Hz, 8.5 Hz), 3.95 (OCH_3), 3.89 (OCH_3)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 166.1 (COO), 164.2 (CONH), 160.7 (C_{Ar}), 142.5 (C_{Ar}), 138.5 (C_{Ar}), 133.1 (C_{Ar}), 132.9 (C_{Ar}), 127.9 (C_{Ar}), 117.9 (CN), 116.1 (C_{Ar}), 116.0 (C_{Ar}), 110.8 (C_{Ar}), 103.7 (C_{Ar}), 56.3 (CH_3-O), 52.2 (CH_3-COO)

HRMS (ESI) calculated 311.1032 [$M+H^+$], 311.1039 found.

4-(4-cyanobenzamido)-2-methoxybenzoic acid



The methyl ester (0.32 mmol) was hydrolyzed using procedure D. The crude product was of sufficient purity for further reactions.

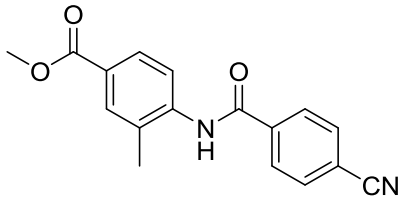
Yield: 63.0 mg (66 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.38 (br s, 1H, COOH), 10.68 (s, 1H, NHCO), 8.11 (d, 2H, Ar-H, J = 8.7 Hz), 8.05 (d, 2H, Ar-H, J = 8.6 Hz), 7.73 (d, 1H, Ar-H, J = 8.5 Hz), 7.65 (d, 1H, Ar-H, J = 1.9 Hz), 7.46 (dd, 1H, Ar-H, J = 1.9 Hz, 8.5 Hz), 3.82 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.5 (COOH), 164.6 (CONH), 159.2 (C_{Ar}), 143.5 (C_{Ar}), 138.6 (C_{Ar}), 132.5 (C_{Ar}-H), 132.1 (C_{Ar}-H), 128.6 (C_{Ar}-H), 118.3 (CN), 115.8 (C_{Ar}), 114.1 (C_{Ar}), 111.4 (C_{Ar}-H), 103.8 (C_{Ar}-H), 55.6 (CH_3O)

HRMS (ESI) calculated 297.0875 [M+H$^+$], 297.0876 found.

Methyl 4-(4-cyanobenzamido)-3-methylbenzoate



The aniline (3.0 mmol) was coupled with benzoic acid using procedure A1. The crude product was of sufficient purity for further reactions.

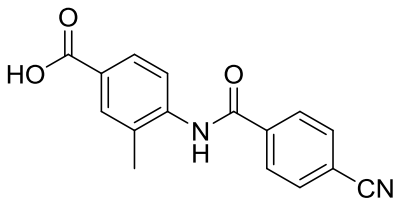
Yield: 893.3 mg (quantitative)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.22 (d, 1H, Ar-H, J = 8.5 Hz), 8.00 – 7-94 (m, 3H, Ar-H), 7.83 (d, 2H, Ar-H, J = 8.6 Hz), 7.77 (d, 1H, Ar-H, J = 8.7 Hz), 3.92 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 166.6 (CONH), 163.8 (C=O), 139.5 (C_{Ar}), 138.5 (C_{Ar}), 132.8 (C_{Ar}-H), 132.5 (C_{Ar}-H), 132.0 (C_{Ar}-H), 129.3 (C_{Ar}-H), 128.8 (C_{Ar}-H), 127.8 (C_{Ar}-H), 127.8 (C_{Ar}), 127.5 (C_{Ar}), 126,7 (C_{Ar}), 121.4 (C_{Ar}-H), 115.9 (C_{Ar}), 52.1 (CH_3), 17.7 (CH_3O)

HRMS (ESI) calculated 295.1083 [M+H$^+$], 295.1078 found.

4-(4-cyanobenzamido)-3-methylbenzoic acid (**18**)



The methyl ester (0.34 mmol) was hydrolyzed using procedure D. The crude product was of sufficient purity for further reactions.

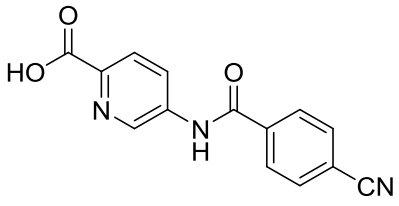
Yield: 71.0 mg (75 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.88 (br s, 1H, COOH), 10.23 (s, 1H, CONH), 8.13 (d, 2H, Ar-H, J = 8.6 Hz), 8.04 (d, 2H, Ar-H, J = 8.6 Hz), 7.87 (d, 1H, Ar-H, J = 1.5 Hz), 7.80 (dd, 1H, Ar-H, J = 1.9 Hz, 8.2 Hz), 7.57 (d, 1H, Ar-H, J = 8.3 Hz), 2.31 (s, 3H, CH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.0 (COOH), 164.1 (CONH), 140.2 (C_{Ar}), 138.4 (C_{Ar}), 133.2 (C_{Ar}), 132.6 (C_{Ar}-H), 131.5 (C_{Ar}-H), 128.6 (C_{Ar}), 128.1 (C_{Ar}), 127.3 (C_{Ar}), 125.8 (C_{Ar}-H), 125.8 (C_{Ar}), 118.3 (C_{Ar}), 114.0 (C_{Ar}), 114.0 (C_{Ar}), 17.9 (CH_3)

HRMS (ESI) calculated 281.0926 [M+H$^+$], 281.0921 found.

5-(4-cyanobenzamido)picolinic acid (**9**)



The aniline (1.45 mmol) was coupled with benzoic acid using procedure B. The crude product was of sufficient purity for further reactions.

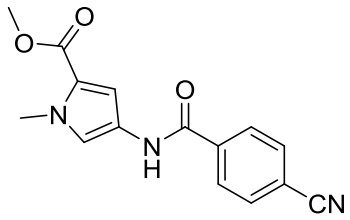
Yield: 144.0 mg (37 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.04 (br s, 1H, COOH), 10.97 (s, 1H, CONH), 9.04 (d, 1H, Ar-H, J = 2.0 Hz), 8.40 (dd, 1H, Ar-H, J = 2.5 Hz, 8.6 Hz), 8.15 (d, 2H, Ar-H, J = 8.6 Hz), 8.10 (d, 1H, Ar-H, J = 8.6 Hz), 8.07 (d, 2H, Ar-H, J = 8.6 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 165.7 (CONH), 164.9 (COO), 143.2 (C_{Ar}) 141.2 (C_{Ar}-H), 138.3 (C_{Ar}), 138.0 (C_{Ar}), 132.6 (C_{Ar}-H), 128.7 (C_{Ar}-H), 127.1 (C_{Ar}-H), 125.3 (C_{Ar}-H), 118.2 (CN), 114.4 (C_{Ar})

HRMS (ESI) calculated 268.0722 [M+H$^+$], 268.0716 found.

Methyl 4-(4-cyanobenzamido)-1-methyl-1*H*-pyrrole-2-carboxylate



The amine (1.05 mmol) was coupled with benzoic acid using procedure A1. The crude product was purified by flash chromatography with petroleum ether and ethyl acetate.

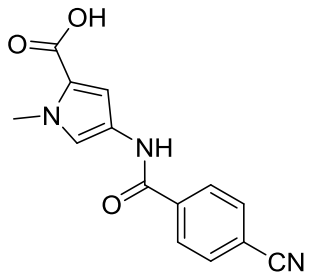
Yield:	212.0 mg (71 %)
Rf:	0.33 (PE:EE 2:1)

^{1}H-NMR (500 MHz, DMSO-d6, 300 K): δ (ppm) = 10.59 (s, 1H, CONH), 8.08 (d, 2H, Ar-H, J = 8.6 Hz), 8.01 (d, 2H, Ar-H, J = 8.6 Hz), 7.54 (d, 1H, Ar-H, J = 2.0 Hz), 6.95 (d, 1H, Ar-H, J = 2.0 Hz), 3.87 (s, 3H, NCH_3), 3.75 (s, 3H, CH_3O)

^{13}C-NMR (176 MHz, DMSO-d6, 300 K): δ (ppm) = 162.1 (CONH), 160.7 (COO), 138.3 (C_{Ar}-CO), 132.5 (C_{Ar}-H), 128.2 (C_{Ar}-H),122.4 (N-C_{Ar}) 121.2 (N-C_{Ar}-H), 118.9 (N-C_{Ar}), 118.3 (CN), 113.6 (C_{Ar}), 108.5 (C_{Ar}-H), 51.1 (CH_3-O), 36.3 (CH_3-N)

HRMS (ESI) calculated 284.1035 [M+H$^+$], 284.1031 found.

4-(4-cyanobenzamido)-1-methyl-1*H*-pyrrole-2-carboxylic acid



The methyl ester (0.35 mmol) was deprotected using procedure F1. The product was of sufficient purity for further reactions.

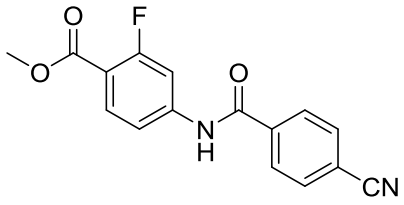
Yield: 37.0 mg (39 %)

^{1}H-NMR (500 MHz, MeOH-d_4, 300 K): δ (ppm) = 8.04 (d, 2H, Ar-H, J = 8.6 Hz), 7.85 (d, 2H, Ar-H, J = 8.6 Hz), 7.26 (d, 1H, Ar-H, J = 2.0 Hz), 6.78 (d, 1H, Ar-H, J = 2.1 Hz), 3.91 (s, 3H, NCH_3)

^{13}C-NMR (176 MHz, MeOH-d_4, 300 K): δ (ppm) = 170.0 (COOH), 165.1 (CONH), 140.2 (C_{Ar}), 133.5 (C_{Ar}), 129.3 (C_{Ar}), 129.1 (C_{Ar}), 129.0 (C_{Ar}), 128.7 (C_{Ar}), 128.2 (C_{Ar}), 122.4 (C_{Ar}), 119.2 (CN), 115.7 (C_{Ar}), 107.8 (C_{Ar}), 37.0 (NCH_3)

HRMS (ESI) calculated 270.0879 [M+H$^+$], 270.0874 found.

Methyl 4-(4-cyanobenzamido)-2-fluorobenzoate (**2**)



The aniline (0.59 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a yellow solid.

Yield: 168.0 mg (95 %)

Rf: 0.94 (PE:EE 1:1)

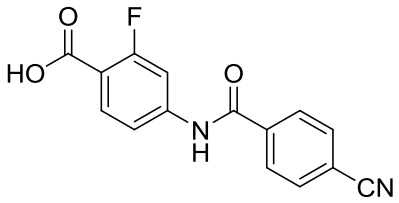
^{1}H-NMR (700 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.00 – 7.97 (m, 3H, Ar-H), 7.94 (br s, 1H, CONH), 7.83 (d, 2H, Ar-H, J = 8.4 Hz), 7.77 (dd, 1H, Ar-H, J = 2.0 Hz, 12.5 Hz), 7.32 (dd, 1H, Ar-H, J = 2.0 Hz, 8.6 Hz), 3.94 (s, 3H, OCH_3)

^{13}C-NMR (176 MHz, $CDCl_3$, 300 K): δ (ppm) = 164.5 (d, COO, J = 3.9 Hz), 164.0 (CONH), 162.8 (d, C-F, J = 260.2 Hz), 142.8 (d, C_{Ar}, J = 11.5 Hz), 138.1 (C_{Ar}), 133.3 (C_{Ar}-H), 133.0 (C_{Ar}-H), 128.0 (C_{Ar}-H), 117.8 (C_{Ar}), 116.3 (C_{Ar}), 114.9 (d, C_{Ar}-H, J = 3.3 Hz), 108.4 (d, C_{Ar}-H, J = 28.3 Hz), 52.3 (OCH_3)

^{19}F-NMR (659 MHz, $CDCl_3$, 300 K): δ (ppm) = - 105.7 (dd, Ar-F, J = 8.0 Hz, 12.6 Hz)

HRMS (ESI) calculated 299.0832 [M+H$^+$], 299.083 found.

4-(4-cyanobenzamido)-2-fluorobenzoic acid



The methyl ester (0.17 mmol) was hydrolyzed using procedure D. The crude product was of sufficient purity for further reactions.

Yield: 23.0 mg (48 %)

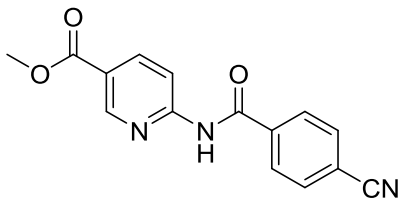
^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.06 (br s, 1H, COOH), 10.89 (s, 1H, CONH), 8.11 (d, 2H, Ar-H, J = 8.7 Hz), 8.06 (d, 2H, Ar-H, J = 8.6 Hz), 7.90 (t, 1H, Ar-H, J = 8.6 Hz), 7.83 (dd, 1H, Ar-H, J = 2.0 Hz, 13.5 Hz), 7.64 (dd, 1H, Ar-H, J = 2.0 Hz, 8.7 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 164.9 (COOH), 164.6 (CONH), 160.5 (C_{Ar}-F), 156.0 (C_{Ar}), 138.3 (C_{Ar}), 132.7 (C_{Ar}-H), 132.6 (C_{Ar}-H), 128.7 (C_{Ar}-H), 118.3 (CN), 115.5 (d, C_{Ar}-H, J = 2.5 Hz), 114.3 (C_{Ar}), 107.5 (d, C_{Ar}-H, J = 27.8 Hz)

^{19}F{^{1}H}-NMR (470 MHz, $CDCl_3$, 300 K): δ (ppm) = - 108.0 (Ar-F)

HRMS (ESI) calculated 285.0675 [M+H$^+$], 285.0672 found.

Methyl 6-(4-cyanobenzamido)nicotinate



The aniline (0.66 mmol) was coupled with benzoic acid using procedure A2. The crude product was purified by flash chromatography with DCM and MeOH.

Yield: 52.0 mg (28 %)

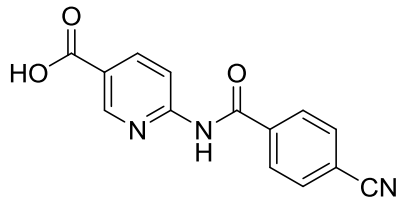
Rf: 0.32 (DCM:MeOH 10:1)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 11.52 (br s, 1H, CONH), 8.93 (d, 1H, Ar-H, J = 2.2 Hz), 8.38 (dd, 1H, Ar-H, J = 2.3 Hz, 8.8 Hz), 8.35 (d, 1H, Ar-H, J = 8.8 Hz), 8.16 (d, 2H, Ar-H, J = 8.3 Hz), 8.01 (d, 2H, Ar-H, J = 8.3 Hz), 3.88 (s, 3H, OCH_3)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 165.4 (CONH), 164.8 (COO), 155.2 (C_{Ar}-NH), 149.4 (C_{Ar}-H), 139.4 (C_{Ar}-H), 137.8 (C_{Ar}), 132.4 (C_{Ar}-H), 129.0 (C_{Ar}-H), 121.5 (C_{Ar}), 118.2 (CN), 114.4 (C_{Ar}), 113.8 (C_{Ar}-H), 52.2 (OCH_3)

HRMS (ESI) calculated 282.0879 [M+H$^+$], 282.0882 found.

6-(4-cyanobenzamido)-nicotinic acid



The methyl ester (0.18 mmol) was hydrolyzed using procedure D. The crude product was of sufficient purity for further reactions.

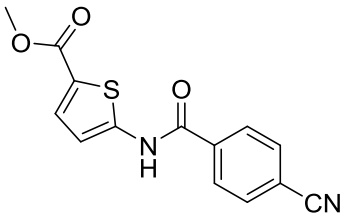
Yield: 31 mg (65 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.24 (br s, 1H, COOH), 11.47 (s, 1H, CONH), 8.90 (dd, 1H, Ar-H, J = 1.0 Hz, 2.1 Hz), 8.34-8.32 (m, 2H, Ar-H), 8.16 (d, 2H, Ar-H, J = 8.7 Hz), 8.01 (d, 2H, Ar-H, J = 8.6 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 165.7 (CONH), 165.1 (COOH), 154.7 (C_{Ar}), 149.4 (C_{Ar}-H), 139.3 (C_{Ar}-H), 137.7 (C_{Ar}), 132.2 (C_{Ar}-H), 128.8 (C_{Ar}-H), 122.4 (C_{Ar}), 118.0 (CN), 114.2 (C_{Ar}), 113.5 (C_{Ar}-H)

HRMS (ESI) calculated 268.0722 [M+H$^+$], 268.0717 found.

Methyl 5-(4-cyanobenzamido)thiophene-2-carboxylate (**3**)



The aniline (0.64 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions.

Yield: 151 mg (83 %)

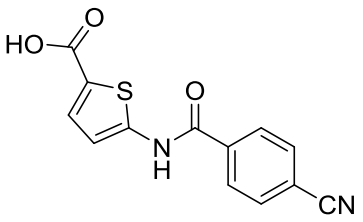
Rf: 0.8 (PE:EE 1:2)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.30 (s, 1H, CONH), 8.18 (d, 2H, Ar-H, J = 8.7 Hz), 8.08 (d, 2H, Ar-H, J = 8.6 Hz), 7.67 (d, 1H, Ar-H, J = 4.2 Hz), 7.01 (d, 1H, Ar-H, J = 4.2 Hz), 3.80 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 162.7 (CONH), 162.6 (COO), 146.1 (C_{Ar}), 136.3 (C_{Ar}), 132.7 (C_{Ar}-H), 132.0 (C_{Ar}-H), 128.7 (C_{Ar}-H), 122.7 (C_{Ar}), 118.2 (CN), 114.6 (C_{Ar}), 113.7(C_{Ar}-H), 51.8 (OCH_3)

HRMS (ESI) calculated 287.0490 [M+H$^+$], 287.0486 found.

5-(4-cyanobenzamido)-thiophene-2-carboxylic acid (**4**)



The methyl ester (0.51 mmol) was deprotected using procedure F1. The crude product was of sufficient purity for further reactions. The product was an aquamarine solid.

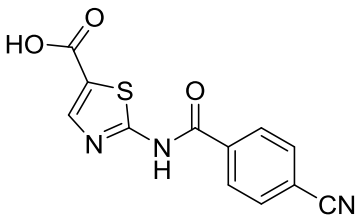
Yield: 40.5 mg (29 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.72 (br s, 1H, COOH), 12.16 (s, 1H, CONH), 8.16 (d, 2H, Ar-H, J = 8.6 Hz), 8.07 (d, 2H, Ar-H, J = 8.6 Hz), 7.59 (d, 1H, Ar-H, J = 4.1 Hz), 6.97 (d, 1H, Ar-H, J = 4.2 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 163.6 (COOH), 162.6 (CONH), 145.6 (C_{Ar}), 136.5 (C_{Ar}), 132.7 (C_{Ar}-H), 131.4 (C_{Ar}-H), 128.7 (C_{Ar}-H), 124.6 (C_{Ar}), 118.2 (CN), 114.5 (C_{Ar}), 113.5 (C_{Ar}-H)

HRMS (ESI) calculated 273.0334 [M+H^{+}], 273.0326 found.

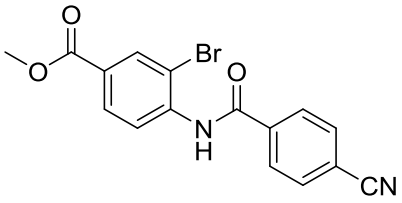
2-(cyanobenzamido)thiazole-5-carboxylic acid



The aniline (0.7 mmol) was coupled with benzoic acid using procedure C. The crude product was washed with HCl and methanol to decrease the amount if impurities. A crude product was obtained and used in further reactions.

Yield: 61.0 mg (crude)

Methyl 3-bromo-4-(4-cyanobenzamido)benzoate



The aniline (0.22 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a white solid.

Yield: 76.0 mg (97 %)

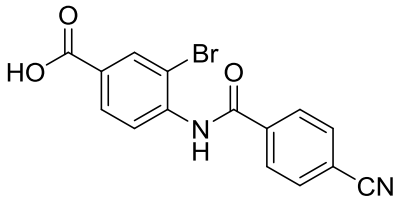
Rf: 0.68 (PE:EE + 2 % AcOH 2:1)

^{1}H-NMR (500 MHz, Aceton-d_6, 300 K): δ (ppm) = 9.35 (br s, 1H, CONH), 8.35 (d, 1H, Ar-H, J = 8.5 Hz), 8.26 (d, 1H, Ar-H, J = 1.9 Hz), 8.23 (d, 2H, Ar-H, J = 8.7 Hz), 8.07 (dd, 1H, Ar-H, J = 1.9 Hz, 8.5 Hz), 8.01 (d, 2H, Ar-H, J = 8.7 Hz), 3.91 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, Aceton-d_6, 300 K): δ (ppm) = 165.7 (C=O), 165.0 (C=O), 141.1 (C_{Ar}), 139.2 (C_{Ar}), 134.5 (C_{Ar}-H), 133.7 (C_{Ar}-H), 130.3 (C_{Ar}-H), 129.4 (C_{Ar}-H), 129.0 (C_{Ar}), 124.9 (C_{Ar}-H), 118.7 (CN), 116.6 (C_{Ar}), 116.5 (C_{Ar}), 52.8 (OCH_3)

HRMS (ESI) calculated 356.9875/358.9854 [M-H$^+$], 356.9881/358.9861 found.

3-Bromo-4-(4-cyanobenzamido)benzoic acid



The methyl ester (0.19 mmol) was hydrolyzed using procedure D. The crude product was of sufficient purity for further reactions. The product was a white to rosa-red solid.

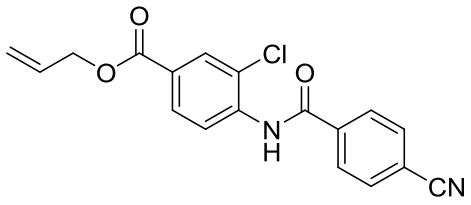
Yield: 54.9 mg (82 %)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.34 (br s, 1H, COOH), 10.44 (s, 1H, CONH), 8.19 (d, 1H, Ar-H, J = 1.9 Hz), 8.14 (d, 2H, Ar-H, J = 8.6 Hz), 8.06 (d, 2H, Ar-H, J = 8.6 Hz), 7.98 (dd, 1H, Ar-H, J = 1.9 Hz, 8.3 Hz), 7.76 (d, 1H, Ar-H, J = 8.3 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 165.7 (CO), 164.1 (CO), 140.0 (C_{Ar}), 137.7 (C_{Ar}), 133.5 (C_{Ar}-H), 132.7 (C_{Ar}-H), 129.1 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.1 (C_{Ar}-H), 124.5 (C_{Ar}), 119.6 (C_{Ar}), 118.2 (CN), 114.3 (C_{Ar})

HRMS (ESI) calculated 344.9875/346.9854 [M+H$^+$], 344.9866/346.9847 found.

Allyl 3-chloro-4-(4-cyanobenzamido)benzoate (**5**)



The aniline (0.19 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a white yellowish solid.

Yield: 58.3 mg (91 %)

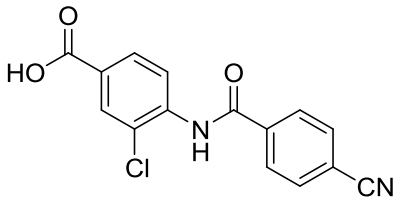
Rf: 0.75 (PE:EE + 2 % AcOH 2:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.69 (d, 1H, Ar-H, J = 8.7 Hz), 8.61 (s, 1H, CONH), 8.16 (d, 1H, Ar-H, J = 1.9 Hz), 8.07 (dd, 1H, Ar-H, J = 1.9 Hz, 8.7 Hz), 8.04 (d, 2H, Ar-H, J = 8.5 Hz), 7.86 (d, 2H, Ar-H, J = 8.5 Hz), 6.06 (ddt, 1H, =CH, J = 5.8 Hz, 10.6 Hz, 16.2 Hz), 5.43 (dd, 1H, $=CH_2$, J = 1.4 Hz, 17.2 Hz), 5.32 (dd, 1H, $=CH_2$, J = 1.2 Hz, 10.4 Hz), 4.85 (d, 2H, OCH_2, J = 5.7 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 164.5 (C=O), 163.5 (C=O), 138.0 (C_{Ar}), 137.9 (C_{Ar}), 132.9 (C_{Ar}-H), 131.9 (=CH), 130.5 (C_{Ar}-H), 129.7 (C_{Ar}-H), 127.8 (C_{Ar}-H), 126.9 (C_{Ar}), 122.7 (C_{Ar}), 120.5 (C_{Ar}-H), 118.7 ($=CH_2$), 117.7 (CN), 116.2 (C_{Ar}), 66.9 (OCH_2)

HRMS (ESI) calculated 341.0693 [$M+H^+$], 341.0686 found.

3-Chloro-4-(4-cyanobenzamido)benzoic acid (6)



The ester (0.16 mmol) was deprotected using procedure E. The crude product was of sufficient purity for further reactions. The product was a white solid.

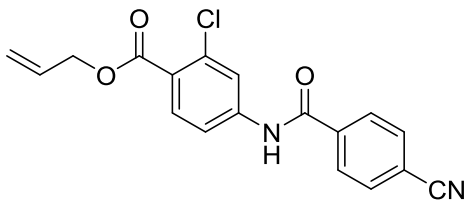
Yield: 29.7 mg (62 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.29 (br s, 1H, COOH), 10.47 (s, 1H, CONH), 8.14 (d, 2H, Ar-H, J = 8.6 Hz), 8.05 (d, 2H, Ar-H, J = 8.6 Hz), 8.03 (d, 1H, Ar-H, J = 1.9 Hz), 7.95 (dd, 1H, Ar-H, J = 1.9 Hz, 8.3 Hz), 7.82 (d, 1H, Ar-H, J = 8.4 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 165.7 (C=O), 164.2 (C=O), 138.6 (C_{Ar}), 137.7 (C_{Ar}), 132.6 (C_{Ar}-H), 130.3 (C_{Ar}-H), 129.7 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 127.5 (C_{Ar}-H), 118.2 (CN), 114.3 (C_{Ar})

HRMS (ESI) calculated 299.0223 [$M-H^+$], 299.0229 found.

Allyl 2-chloro-4-(4-cyanobenzamido)benzoate



The aniline (0.19 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a white yellowish solid.

Yield: 61.0 mg (95 %)

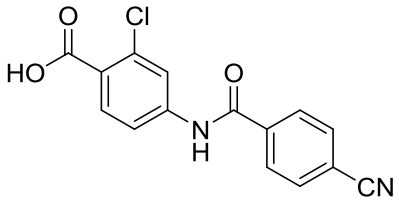
Rf: 0.57 (PE:EE + 2 % AcOH 2:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.09 (br s, 1H, CONH), 7.99 (d, 2H, Ar-H, J = 8.5 Hz), 7.95 (d, 1H, Ar-H, J = 8.6 Hz), 7.88 (d, 1H, Ar-H, J = 2.1 Hz), 7.80 (d, 2H, Ar-H, J = 8.6 Hz), 7.63 (dd, 1H, Ar-H, J = 2.2 Hz, 8.6 Hz), 6.04 (ddt, 1H, CH=, J = 5.7 Hz, 10.5 Hz, 17.2 Hz), 5.44 (dq, 1H, $=CH_2$, J = 1.5 Hz, 17.2 Hz), 5.31 (ddd, 1H, $=CH_2$, J = 1.2 Hz, 2.5 Hz, 10.5 Hz), 4.83 (dt, 2H, OCH_2, J = 1.4 Hz, 5.7 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 164.5 (C=O), 164.0 (C=O), 141.1 (C_{Ar}), 138.0 (C_{Ar}), 135.4 (C_{Ar}), 132.8 (C_{Ar}-H), 132.7 (C_{Ar}-H), 131.8 (=CH), 127.9 (C_{Ar}), 125.6 (C_{Ar}), 122.0 (C_{Ar}-H), 118.8 ($=CH_2$), 117.7 (CN), 117.6 (C_{Ar}-H), 116.0 (C_{Ar}), 66.1 (OCH_2)

HRMS (ESI) calculated 341.0693 [$M+H^+$], 341.0686 found.

2-Chloro-4-(4-cyanobenzamido)benzoic acid



The ester (0.16 mmol) was deprotected using procedure E. The crude product was of sufficient purity for further reactions. The product was a white solid.

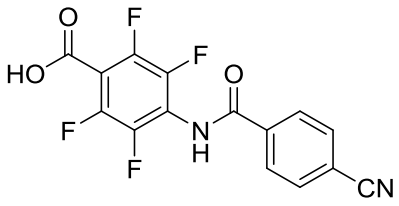
Yield: 15.0 mg (30 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.19 (br s, 1H, COOH), 10.82 (s, 1H, CONH), 8.11 (d, 2H, Ar-H, J = 8.5 Hz), 8.07 – 8.04 (m, 3H, Ar-H), 7.90 (d, 1H, Ar-H, J = 8.6 Hz), 7.82 (dd, 1H, Ar-H, J = 2.0 Hz, 8.6 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 165.9 (C=O), 164.8 (C=O), 142.4 (C_{Ar}), 138.3 (C_{Ar}), 132.6 (C_{Ar}-H), 132.3 (C_{Ar}-H), 128.7 (C_{Ar}-H), 125.4 (C_{Ar}), 121.4 (C_{Ar}-H), 118.2 (CN), 118.2 (C_{Ar}), 114.3 (C_{Ar})

HRMS (ESI) calculated 299.0223 [M-H$^+$], 299.0229 found.

4-(4-cyanobenzamido)-2,3,5,6-tetrafluorobenzoic acid



The aniline (0.20 mmol) was coupled with benzoic acid using procedure A2. The crude product was dissolved in 10 ml DCM and extracted with 3 x 5 ml 1 M NaOH. The aqueous phase was acidified with 6 M HCl. The precipitate was extracted with 3 x 10 ml ethyl acetate and the solvent was removed under reduced pressure. The crude product was of sufficient purity for further reactions. The product was a faint yellow solid.

Yield: 19.0 mg (28 %)

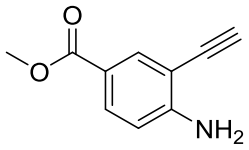
^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 11.07 (s, 1H, CONH), 8.16 (d, 2H, Ar-H, J =8.3 Hz), 8.07 (d, 2H, Ar-H, J = 8.5 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 164.0 (C=O), 160.0 (C=O), 144.6 (C_{Ar}), 143.8 (d, C_{Ar}-F, J = 252.3 Hz), 142.3 (dd, C_{Ar}-F, J = 14.4 Hz, 249.2 Hz), 136.2 (C_{Ar}), 132.8 (C_{Ar}-H), 128.9 (C_{Ar}-H), 127.9 (d, C_{Ar}, J = 51.6 Hz), 118.1 (CN), 114.9 (C_{Ar})

^{19}F-NMR (470 MHz, DMSO-d_6, 300 K) δ (ppm) = - 141.52 (d, Ar-F, J = 12.4 Hz), - 143.8 (dd, Ar-F, J = 22.9 Hz)

HRMS (ESI) calculated 339.0393 [M+H$^+$], 339.0386 found.

Methyl 4-amino-3-ethynylbenzoate (**32**)[189]



15.2 mg Bis(triphenylphosphine)palladium(II) chloride (0.02 mmol, 0.06 eq), 4.2 mg copper(I) iodine (0.02 mmol, 0.06 eq) and 100.0 mg methyl 4-amino-3-iodobenzoate (0.36 mmol, 1 eq) were added to a dry flask under nitrogen atmosphere and further dried under high vacuum. The flask was flushed with nitrogen and 0.9 ml dry THF and 0.14 ml triethylamine (101.6 mg, 1.0 mmol, 2.8 eq) were added under nitrogen atmosphere. The solvent was degassed by bubbling nitrogen through the mixture. 65 µl ethynyltrimethylsilane (46 mg, 0.47 mmol, 1.3 eq) were added to the stirring mixture under nitrogen atmosphere. The reaction was stirred at room temperature and controlled over LCMS. After completion, the mixture was filtered through celite. The solvent was removed under reduced pressure. The crude product was dissolved in 2 ml MeOH. 75 mg potassium carbonate (0.54 mmol, 1.5 eq) was added and the mixture was stirred for 2 h at room temperature. The solvent was removed under reduced pressure. The residue was dissolved in 2 ml water and extracted with 3 x 4 ml diethyl ether. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate). The product was a red brown solid.

Yield: 24.2 mg (38 %)

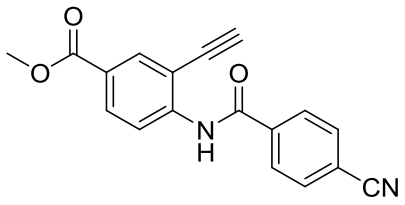
Rf: 0.29 (PE:EE 3:1)

^{1}H-NMR (500 MHz, Aceton-d_6, 300 K): δ (ppm) = 7.89 (d, 1H, Ar-H, J = 2.0 Hz), 7.74 (dd, 1H, Ar-H, J = 2.1 Hz, 8.6 Hz), 6.82 (d, 1H, Ar-H, J = 8.7 Hz), 5.77 (br s, 2H, NH_2), 3.95 (s, 1H, ≡CH), 3.79 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, Aceton-d_6, 300 K): δ (ppm) = 165.6 (COO), 153.6 (C_{Ar}), 134.2 (C_{Ar}-H), 131.3 (C_{Ar}-H), 117.7 (C_{Ar}), 113.0 (C_{Ar}-H), 104.9 (C_{Ar}), 83.6 (C≡C), 79.3 (C≡C), 50.8 (OCH_3)

HRMS (ESI) calculated 176.0712 [M+H$^+$], 176.0704 found.

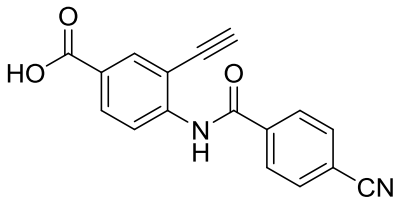
Methyl 4-(4-cyanobenzamido)-3-ethynylbenzoate



The aniline (0.13 mmol) was coupled with benzoic acid using procedure A2. The red brown crude product was used in further reactions.

Yield: 37.9 mg (crude)

4-(4-cyanobenzamido)-3-ethynylbenzoic acid (**33**)



The ester (0.12 mmol) was hydrolyzed using procedure D. The crude product was of sufficient purity for further reactions. The product was a faint orange brown solid.

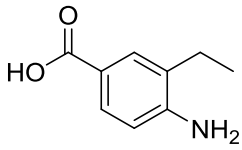
Yield: 22.1 mg (58 % over 2 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.18 (br s, 1H, COOH), 10.32 (s, 1H, CONH), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.07 – 8.03 (m, 3H, Ar-H), 8.04 (s, 1H, Ar-H), 8.01 (dd, 1H, Ar-H, J = 2.0 Hz, 8.4 Hz), 7.89 (d, 1H, Ar-H, J = 8.5 Hz), 4.59 (s, 1H, ≡CH)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.1 (C=O), 164.1 (C=O), 142.7 (C_{Ar}), 137.9 (C_{Ar}), 133.5 (C_{Ar}-H), 132.7 (C_{Ar}-H), 130.4 (C_{Ar}-H), 128.5 (C_{Ar}-H), 127.9 (C_{Ar}), 124.8 (C_{Ar}-H), 118.2 (CN), 117.0 (C_{Ar}), 114.4 (C_{Ar}), 87.1 (Ar-C≡), 79.2 (≡CH)

HRMS (ESI) calculated 291.077 [M+H^{+}], 291.0776 found.

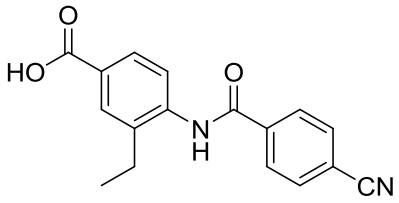
4-Amino-3-ethylbenzoic acid[254]



100 mg 4-amino-3-ethylbenzonitrile (0.68 mmol, 1 eq) was added to a solution of 96.0 mg sodium hydroxide (2.4 mmol, 3.5 eq) in 2 ml water and heated up to 70 °C. The reaction was controlled over TLC. After completion, the aqueous phase was acidified with 1 M HCl to pH 4 and extracted with 5 x 3 ml ethyl acetate. The organic phase was evaporated under reduced pressure. The crude product was a faint red solid.

Yield: 113.0 mg (crude)

4-(4-cyanobenzamido)-3-ethylbenzoic acid (**31**)



The aniline (0.61 mmol) was coupled with benzoic acid using procedure B. The crude product was of sufficient purity for further reactions. The product was a white solid.

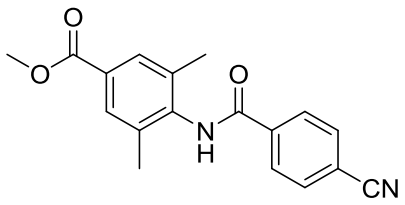
Yield: 42.0 mg (21 % over 2 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.97 (br s, 1H, COOH), 10.29 (s, 1H, CONH), 8.12 (d, 2H, Ar-H, J = 8.4 Hz), 8.05 (d, 2H, Ar-H, J = 8.4 Hz), 7.89 (d, 1H, Ar-H, J = 1.9 Hz), 7.82 (dd, 1H, Ar-H, J = 1.9 Hz, 8.2 Hz), 7.52 (d, 1H, Ar-H, J = 8.2 Hz), 2.70 (q, 2H, CH_2, J = 7.5 Hz), 1.15 (t, 3H, CH_3, J = 7.6 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.1 (C=O), 164.5 (C=O), 139.6 (C_{Ar}), 139.3 (C_{Ar}), 138.3 (C_{Ar}), 132.7 (C_{Ar}-H), 129.7 (C_{Ar}-H), 128.7 (C_{Ar}), 128.6 (C_{Ar}-H), 127.3 (C_{Ar}-H), 127.0 (C_{Ar}-H), 118.4 (CN), 114.1 (C_{Ar}), 23.8 (CH_2), 13.9 (CH_3)

HRMS (ESI) calculated 295.1083 [M+H$^+$], 295.1078 found.

Methyl 4-(4-cyanobenzamido)-3,5-dimethylbenzoate



The aniline (0.56 mmol) was coupled with benzoic acid using procedure A1. The crude product was of sufficient purity for further reactions. The product was a dark orange solid.

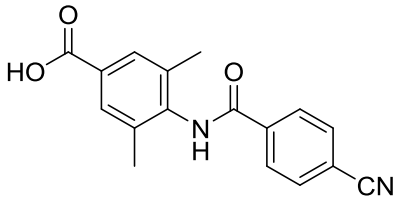
Yield: 154.0 mg (90 %)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.03 (d, 2H, Ar-H, J = 8.2 Hz), 7.83 – 7.79 (m, 4H, Ar-H), 3.92 (s, 3H, OCH_3), 2.31 (s, 6H, CH_3)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 166.9 (C=O), 164.0 (C=O), 138.0 (C_{Ar}), 137.7 (C_{Ar}), 135.7 (C_{Ar}), 132.9 (C_{Ar}-H), 129.8 (C_{Ar}-H), 129.3 (C_{Ar}), 128.1 (C_{Ar}-H), 118.0 (CN), 115.9 (C_{Ar}), 52.4 (OCH_3), 18.7 (CH_3)

HRMS (ESI) calculated 309.1239 [M+H$^+$], 309.1235 found.

4-(4-cyanobenzamido)-3,5-dimethylbenzoic acid



The ester (0.32 mmol) was deprotected using procedure F1. The crude product was of sufficient purity for further reactions.

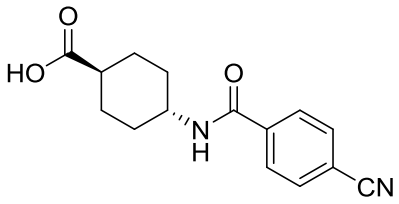
Yield: 27.8 mg (29 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.89 (s, 1H, COOH), 10.19 (s, 1H, CONH), 8.15 (d, 2H, Ar-H, J = 8.6 Hz), 8.04 (d,2H, Ar-H, J = 8.5 Hz), 7.72 (s, 2H, Ar-H), 2.24 (s, 6H, CH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.1 (C=O), 163.6 (C=O), 139.1 (C_{Ar}), 138.0 (C_{Ar}), 135.9 (C_{Ar}), 132.6 (C_{Ar}-H), 129.1 (C_{Ar}), 128.8 (C_{Ar}-H), 128.4 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 18.0 (CH_3)

HRMS (ESI) calculated 295.1083 [M+H$^+$], 295.1075 found.

(1*s*,4*s*)-4-(4-cyanobenzamido)cyclohexane-1-carboxylic acid (**10**)



The aniline (0.56 mmol) was coupled with benzoic acid using procedure C. After completion, the solvent was removed under reduced pressure and purified by RP HPLC. The product was a white solid.

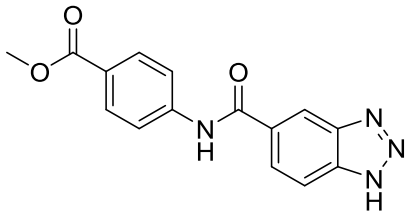
Yield: 53.2 mg (35 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.08 (br s, 1H, COOH), 8.50 (d, 1H, CONH, J = 7.8 Hz), 7.98 (d, 2H, Ar-H, J = 8.6 Hz), 7.94 (d, 2H, Ar-H, J = 8.6 Hz), 3.73 (dtt, 1H, CH, J = 3.4 Hz, 6.8 Hz, 14.0 Hz), 2.20 – 2.13 (m, 1H, CH), 1.98 – 1.93 (m, 2H, CH_2), 1.92 – 1.86 (m, 2H, CH_2), 1.45 – 1.31 (m, 4H, CH_2)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 176.3 (COOH), 164.0 (CONH), 138.7 (C_{Ar}), 132.3 (C_{Ar}-H), 128.1 (C_{Ar}-H), 118.3 (CN), 113.4 (C_{Ar}), 48.1 (CH), 41.7 (CH), 31.1 (CH_2), 27.7 (CH_2)

HRMS (ESI) calculated 273.1239 [M+H$^+$], 273.1234 found.

Methyl 4-(benzo[*d*][1,2,3]triazole-5-carboxamido)benzoate

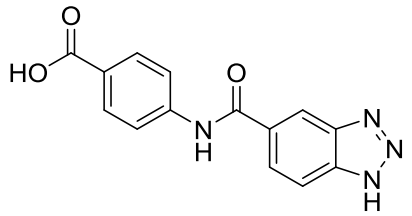


The aniline (0.33 mmol) was coupled with benzoic acid using procedure C without pre-activation of the acid. The crude product was quenched with 5 ml 0.1 M HCl and extracted with 3 x 4 ml ethyl acetate. The combined organic phases were washed with 0.1 M HCl and brine. The crude product was purified by flash chromatography with DCM. After removal of the solvent, the solid was washed with 0.05 M NaH_2PO_4 and 0.1 M HCl and dried under reduced pressure. The product was a white solid.

Yield: 48.0 mg (crude)

Rf: 0.79 (DCM:MeOH 10:1)

4-(benzo[*d*][1,2,3]triazole-5-carboxamido)benzoic acid



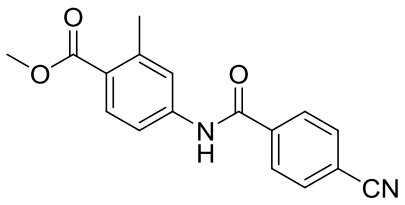
The methyl ester (0.14 mmol) was hydrolyzed using procedure D without the use of THF as solvent. After completion, the crude product was neutralized with 1 M HCl. The crude product was purified by RP HPLC.

Yield: 22.9 mg (60 %)

^{1}H-NMR (500 MHz, THF-d_8, 300 K): δ (ppm) = 9.81 (s, 0.5 H, NH), 8.56 (s, 1H, Ar-H), 8.05 (dd, 1H, Ar-H, J = 1.3 Hz, 8.6 Hz), 8.00 (d, 2H, Ar-H, J = 8.7 Hz), 7.91 (dd, 2H, Ar-H, J = 1.6 Hz, 8.8 Hz), 7.87 (d, 1H, Ar-H, J = 8.6 Hz)

^{13}C-NMR (126 MHz, THF-d_8, 300 K): δ (ppm) = 167.1 (COOH), 165.9 (CONH), 165.9 (CONH), 144.2 (C_{Ar}-NH), 131.3 (C_{Ar}-H), 126.5 (C_{Ar}-H), 126.3 (C_{Ar}-H), 119.7 (C_{Ar}-H), 119.6 (C_{Ar}-H), 119.3 (C_{Ar}), 117.1 (C_{Ar}-H), 114.8 (C_{Ar}), 112.5 (C_{Ar})

Methyl 4-(4-cyanobenzamido)-2-methylbenzoate



The aniline (0.61 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a brownish solid.

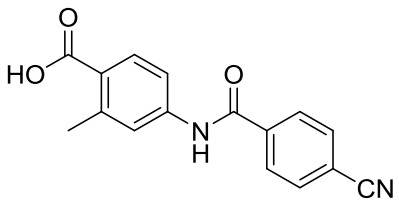
Yield: 156.4 mg (88 %)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.00 – 7.96 (m, 3H, Ar-H), 7.91 – 7.86 (br s, 1H, CONH), 7.81 (d, 2H, Ar-H, J = 7.8 Hz), 7.57 (dd, 1H, Ar-H, J = 2.0 Hz, 8.6 Hz), 7.54 (s, 1H, Ar-H), 3.89 (s, 3H, OCH_3), 2.64 (s, 3H, CH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) =167.4 (C=O), 164.0 (C=O), 142.5 (C_{Ar}), 140.5 (C_{Ar}), 138.6 (C_{Ar}), 132.9 (C_{Ar}-H), 132.4 (C_{Ar}-H), 127.9 (C_{Ar}-H), 126.0 (C_{Ar}), 122.6 (C_{Ar}-H), 117.9 (CN), 117.0 (C_{Ar}-H),115.9 (C_{Ar}), 52.0 (OCH_3), 22.3 (CH_3)

HRMS (ESI) calculated 295.1083 [$M+H^+$], 295.1079 found.

4-(4-cyanobenzamido)-2-methylbenzoic acid



The ester (0.17 mmol) was deprotected using procedure F2. The crude product was of sufficient purity for further reactions. The product was a white solid.

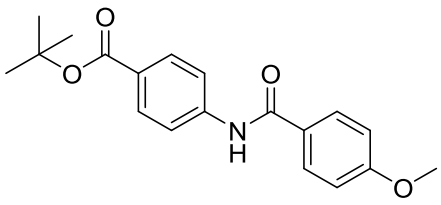
Yield: 18.9 mg (40 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.64 (br s, 1H, COOH), 10.63 (s, 1H, CONH), 8.11 (d, 2H, Ar-H, J = 8.4 Hz), 8.04 (d, 2H, Ar-H, J = 8.4 Hz), 7.88 (d, 1H, Ar-H, J = 9.4 Hz), 7.72 (m, 2H, Ar-H), 2.54 (s, 3H, CH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 168,0 (C=O), 164.5 (C=O), 141.8 (C_{Ar}), 140.5 (C_{Ar}), 138.7 (C_{Ar}), 132.5 (C_{Ar}-H), 131.6 (C_{Ar}-H), 128.6 (C_{Ar}-H), 125.4 (C_{Ar}), 122.5 (C_{Ar}-H), 118.3 (CN), 117.2 (C_{Ar}-H), 114.0 (C_{Ar}), 21.8 (CH_3)

HRMS (ESI) calculated 281.0926 [M+H$^+$], 281.0923 found.

tert-Butyl 4-(4-methoxybenzamido)benzoate (**7**)



The aniline (0.36 mmol) was coupled with benzoic acid using procedure A2. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate). The product was an orange solid.

Yield: 62.6 mg (53 %)

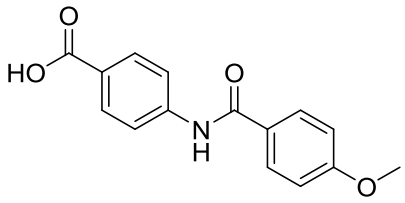
Rf: 0.07 (PE:EE 8:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.99 (d, 2H, Ar-H, J = 8.7 Hz), 7.85 (d, 2H, Ar-H, J = 8.8 Hz), 7.70 (d, 2H, Ar-H, J = 8.7 Hz), 6.98 (d, 2H, Ar-H, J = 8.8 Hz), 3.88 (s, 3H, OCH_3), 1.60 (s, 9H, $(CH_3)_3$)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 165.5 (C=O), 165.3 (C=O), 162.9 (C_{Ar}), 142.0 (C_{Ar}), 130.8 (C_{Ar}-H), 129.1 (C_{Ar}-H), 127.7 (C_{Ar}), 126.9 (C_{Ar}), 119.1 (C_{Ar}-H), 114.3 (C_{Ar}-H), 81.0 ($C(Me)_3$), 55.7 (OCH_3), 28.4 ($(CH_3)_3$)

HRMS (ESI) calculated 328.1549 [M+H$^+$], 328.1552 found.

4-(4-methoxybenzamido)benzoic acid (**8**)



The *tert*-butyl ester (0.18 mmol) was deprotected using procedure H. The crude product was of sufficient purity for further reactions. The product was a yellow orange solid.

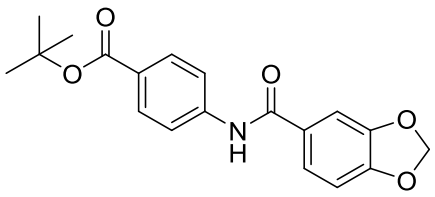
Yield: 51 mg (quantitative)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.69 (br s, 1H, COOH), 10.35 (s, 1H, CONH), 7.98 (d, 2H, Ar-H, J = 8.9 Hz), 7.92 – 7.90 (m, 4H, Ar-H), 7.08 (d, 2H, Ar-H, J = 8.9 Hz), 3.85 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.0 (C=O), 165.2 (C=O), 162.1 (C_{Ar}), 143.5 (C_{Ar}), 130.2 (C_{Ar}-H), 129.8 (C_{Ar}-H), 126.6 (C_{Ar}), 125.2 (C_{Ar}), 119.4 (C_{Ar}-H), 113.7 (C_{Ar}-H), 55.5 (OCH_3)

HRMS (ESI) calculated 272.0923 [M+H$^+$], 272.0917 found.

tert-Butyl 4-(benzo[*d*][1,3]dioxole-5-carboxamido)benzoate

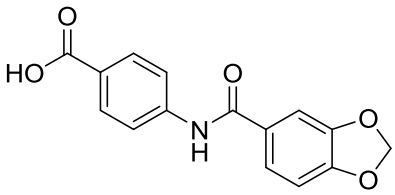


The aniline (0.52 mmol) was coupled with benzoic acid using procedure C. The crude product was purified by chromatography (petroleum ether/ ethyl acetate) and directly used without further purification.

Yield: 94.3 mg (crude)

Rf: 0.22 (PE:EE 8:1)

4-(benzo[*d*][1,3]dioxole-5-carboxamido)benzoic acid



The *tert*-butyl ester (0.15 mmol) was deprotected using procedure H. The crude product was of sufficient purity for further reactions. The product was a beige solid.

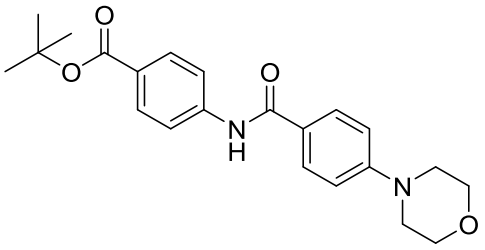
Yield: 42.4 mg (54 % over 2 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.70 (br s, 1H, COOH), 10.32 (s, 1H, CONH), 7.92 (d, 2H, Ar-H, J = 9.0 Hz), 7.89 (d, 2H, Ar-H, J = 9.0 Hz), 7.59 (dd, 1H, Ar-H, J = 1.8 Hz, 8.2 Hz), 7.52 (d, 1H, Ar-H, J = 1.8 Hz), 7.07 (d, 1H, Ar-H, J = 8.2 Hz), 6.14 (s, 2H, CH_2)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.9 (C=O), 164.9 (C=O), 150.3 (C_{Ar}), 147.4 (C_{Ar}), 143.4 (C_{Ar}), 130.2 (C_{Ar}-H), 128.3 (C_{Ar}), 125.3 (C_{Ar}), 123.1 (C_{Ar}-H), 119.4 (C_{Ar}-H), 108.0 (C_{Ar}-H), 107.8 (C_{Ar}-H), 101.9 (CH_2)

HRMS (ESI) calculated 286.0715 [M+H$^+$], 286.0711 found.

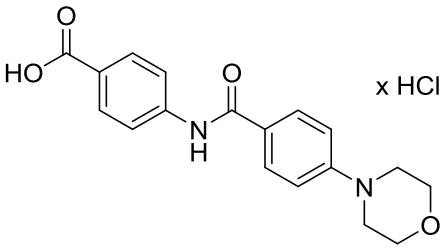
tert-Butyl 4-(4-morpholinobenzamido)benzoate



The aniline (0.52 mmol) was coupled with benzoic acid using procedure A2. The crude product was purified by RP flash and directly used for further reactions.

Yield: 18.4 mg (crude)

4-(4-morpholinobenzamido)benzoic acid hydrochloride



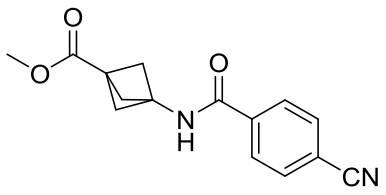
The *tert*-butyl ester (0.05 mmol) was deprotected using procedure H. After coevaporation with DCM, 4 M HCl in dioxane was added. The solvent was removed under reduced pressure and the product was dried under high vaccum. The product was a beige solid.

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.24 (s, 1H, CONH), 7.93 – 7.89 (m, 6H, Ar-H), 7.04 (d, 2H, Ar-H, J = 9.1 Hz), 3.77 – 3.73 (m, 4H, $(CH_2)_2O$), 3.29 – 3.25 (m, 4H, $(CH_2)_2N$)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.0 (C=O), 165.3 (C=O), 153.3 (C_{Ar}-N), 143.7 (C_{Ar}-N), 130.1 (C_{Ar}-H), 129.3 (C_{Ar}-H), 124.9 (C_{Ar}), 123.6 (C_{Ar}), 119.3 (C_{Ar}-H), 113.3 (C_{Ar}-H), 65.9 (CH_2), 47.2 (CH_2)

HRMS (ESI) calculated 327.1345 [M+H$^+$], 327.1339 found.

Methyl 3-(4-cyanobenzamido)bicyclo[1.1.1]pentane-1-carboxylate (**91**)



The amine (0.45 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a slightly yellow solid.

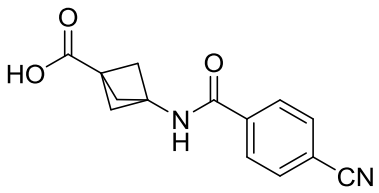
Yield: 65.0 mg (53 %)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 9.35 (s, 1H, CONH), 7.98 (d, 2H, Ar-H, J = 8.7 Hz), 7.95 (d, 2H, Ar-H, J = 8.7 Hz), 3.63 (s, 3H, OCH_3), 2.34 (s, 6H, CH_2)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 169.3 (C=O), 165.2 (C=O), 137.9 (C_{Ar}), 132.4 (C_{Ar}-H), 128.0 (C_{Ar}-H), 118.3 (CN), 113.8 (C_{Ar}), 53.9 (CH_2), 51.6 (OCH_3), 45.8 (C_{aliph}), 35.8 (C_{aliph})

HRMS (ESI) calculated 271.1083 [M+H$^+$], 271.1078 found.

3-(4-cyanobenzamido)bicyclo[1.1.1]pentane-1-carboxylic acid (**92**)



The methyl ester (1.11 mmol) was deprotected using procedure G. The crude product was of sufficient purity for further reactions. The product was a white solid.

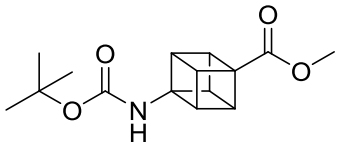
Yield: 189.5 mg (66 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.45 (s, 1H, COOH), 9.32 (s, 1H, CONH), 7.98 (d, 2H, Ar-H, J = 8.7 Hz), 7.95 (d, 2H, Ar-H, J = 8.7 Hz), 2.29 (s, 6H, CH_2)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.6 (C=O), 165.2 (C=O), 138.0 (C_{Ar}), 132.4 (C_{Ar}-H), 128.0 (C_{Ar}-H), 118.3 (CN), 113.7 (C_{Ar}), 53.7 (CH_2), 45.6 (C_{aliph}), 36.1 (C_{aliph})

HRMS (ESI) calculated 257.0926 [M+H$^+$], 257.0918 found.

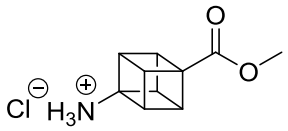
Methyl (1*s*,2*R*,3*s*,4*r*,5*S*,6*r*,7*R*,8*S*)-4-((*tert*-butoxycarbonyl)amino)cubane-1-carboxylate[255]



400 mg 4-methoxycarbonylcubanecarboxylic acid (1.94 mmol, 1.0 eq) was dissolved in 8 ml dry *tert*-butanol under nitrogen atmosphere. 0.27 ml dry triethylamine (196.0 mg, 1.94 mmol, 1.0 eq) was added and the mixture was stirred until clear. 0.42 ml DPPA (536 mg, 1.95 mmol, 1 eq) was added and the reaction was refluxed for 2 days. The solvent was removed under reduced pressure. The product was purified by flash chromatography (petroleum ether/ethyl acetate). The solvent was removed under reduced pressure. The crude product was directly used without further purification.

Rf: 0.39 (PE:EE 3:1)

Methyl (1*s*,2*R*,3*s*,4*r*,5*S*,6*r*,7*R*,8*S*)-4-aminocubane-1-carboxylate hydrochloride (**93**)[255]



400 mg crude methyl (1*s*,2*R*,3*s*,4*r*,5*S*,6*r*,7*R*,8*S*)-4-((*tert*-butoxycarbonyl)amino)cubane-1-carboxylate (1.94 mmol, 1.0 eq) was suspended in 10 ml dry methanol under nitrogen atmosphere and cooled down to - 40 °C. 4.3 ml acetyl chloride (4.75 g, 60.5 mmol, 42 eq) was slowly added to the mixture while stirring vigorously. The reaction was allowed to reach room temperature and controlled over TLC with ninhydrin as staining reagent. After completion, the solvent was removed under reduced pressure. The crude product was washed with a mixture of cold diethyl ether and acetone (4:1) and dried under reduced pressure.

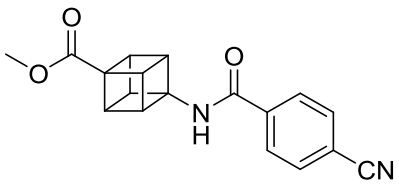
Yield: 250.8 mg (61 % over 2 steps)

^{1}H-NMR (500 MHz, CD_3OD, 300 K): δ (ppm) = 4.20 (s, 6H, CH), 3.71 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, CD_3OD, 300 K): δ (ppm) = 173.3 (COO), 66.1 (C_{quart}), 57.9 (C_{quart}), 52.2 (OCH_3), 46.3 (C_{aliph}-H)

HRMS (ESI) calculated 178.0868 [M+H$^+$], 178.0863 found.

Methyl (1*s*,2*R*,3*s*,4*r*,5*S*,6*r*,7*R*,8*S*)-4-(4-cyanobenzamido)cubane-1-carboxylate



The amine (0.37 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a white solid.

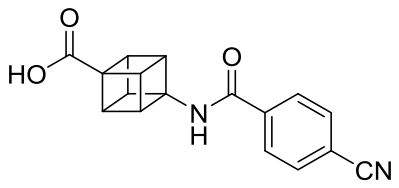
Yield: 53.5 mg (47 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 9.48 (s, 1H, CONH), 8.02 (d, 2H, Ar-H, J = 8.6 Hz), 7.98 (d, 2H, Ar-H, J = 8.6 Hz), 4.16 (m, 3H, CH), 4.12 (m, 3H, CH), 3.63 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.7 (C=O), 164.0 (C=O), 137.5 (C_{Ar}), 132.5 (C_{Ar}-H), 128.1 (C_{Ar}-H), 118.3 (CN), 113.8 (C_{Ar}), 66.6 (C_{quart}), 55.0 (C_{quart}), 51.2 (OCH_3), 49.6 (C_{aliph}-H), 44.3 (C_{aliph}-H)

HRMS (ESI) calculated 307.1083 [M+H$^+$], 307.1078 found.

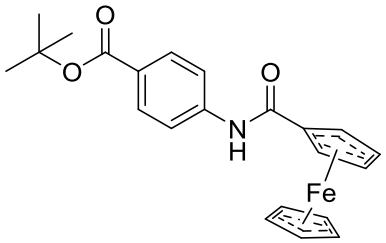
(1*s*,2*R*,3*s*,4*r*,5*S*,6*r*,7*R*,8*S*)-4-(4-cyanobenzamido)cubane-1-carboxylic acid



The methyl ester (0.14mmol) was deprotected using procedure G. The crude was directly used in further reactions.

Yield: 17.4 mg (crude)

tert-Butyl 4-(ferrocenecarboxamido)benzoate (**110**)



The amine (0.52 mmol) was coupled with benzoic acid using procedure A2. The crude product was purified by flash chromatography with petroleum ether and ethyl acetate. The product was an orange to red solid.

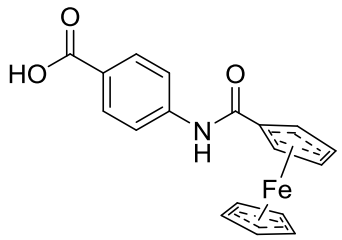
Yield: 173.9 mg (83 %)

^{1}H-NMR (700 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.96 (br s, 2H, Ar-H), 7.61 (br s, 2H, Ar-H), 5.17 (br s, 2H, Cp-H), 4.78 (br s, 2H, Cp-H), 4.64 (br s, 4H, Cp-H), 1.59 (s, 9H, $(CH_3)_3$)

^{13}C-NMR (176 MHz, $CDCl_3$, 300 K) = 169.0 (C=O), 165.5 (C=O), 141.8 (C_{Ar}-NH), 130.9 (C_{Ar}-H), 127.4 (C_{Ar}), 119.2 (C_{Ar}-H), 81.0 ($C(Me)_3$), 77.4 (C_{Cp}-H), 75.2 (C_{Cp}), 73.4 (C_{Cp}-H), 71.7 (C_{Cp}), 28.4 (($CH_3)_3$)

HRMS (ESI) calculated 406.1106 [M+H$^+$], 406.1097 found.

4-(ferrocenecarboxamido)benzoic acid (**111**)



The *tert*-butyl ester (0.41 mmol) was deprotected using procedure H. The crude product was of sufficient purity for further reactions. The product was a brown to red solid.

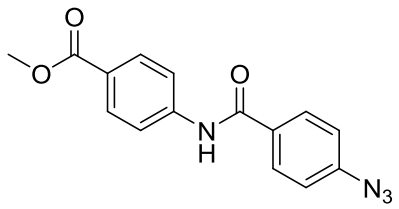
Yield: 154.5 mg (quantitative)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.66 (br s, 1H, COOH), 9.67 (s, 1H, CONH), 7.91 (d, 2H, Ar-H, J = 8.7 Hz), 7.86 (d, 2H, Ar-H, J = 8.7 Hz), 5.04 (br s, 2H, Cp-H), 4.48 (br s, 2H, Cp-H), 4.22 (br s, 4H, Cp-H)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K) = 168.5 (C=O), 166.8 (C=O), 143.2 (C_{Ar}-NH), 130.0 (C_{Ar}-H), 124.7 (C_{Ar}), 119.1 (C_{Ar}-H), 75.7 (C_{Cp}), 70.6 (C_{Cp}-H), 69.4 (C_{Cp}-H), 68.6 (C_{Cp}-H)

HRMS (ESI) calculated 350.048 [M+H$^+$], 350.071 found.

Methyl 4-(4-azidobenzamido)benzoate

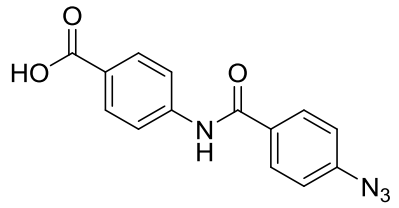


The aniline (0.53 mmol) was coupled with benzoic acid using procedure C. The crude product was purified by flash chromatography with petroleum ether and ethyl acetate. The product was directly used in the next reaction.

Yield: 53.1 mg (crude)

Rf: 0.59 (PE:EE 3:1)

4-(4-azidobenzamido)benzoic acid



The crude methyl ester (0.14 mmol) was hydrolyzed using procedure D. The crude product was of sufficient purity for further reactions.

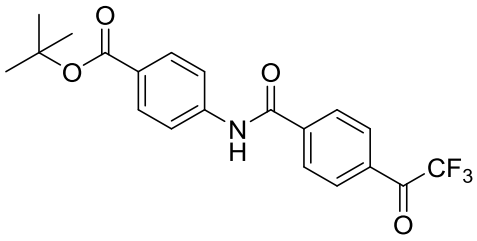
Yield: 20.6 mg (14 % over 2 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.73 (br s, 1H, COOH), 10.52 (s, 1H, CONH), 8.04 (d, 2H, Ar-H, J = 8.6 Hz), 7.93 (d, 2H, Ar-H, J = 8.8 Hz), 7.91 (d, 2H, Ar-H, J = 8.9 Hz), 7.29 (d, 2H, Ar-H, J = 8.6 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.9 (C=O), 164.9 (C=O), 143.3 (C_{Ar}-N), 143.0 (C_{Ar}-N), 131.0 (C_{Ar}), 130.2 (C_{Ar}-H), 129.8 (C_{Ar}-H), 125.5 (C_{Ar}), 119.5 (C_{Ar}-H), 119.1 (C_{Ar}-H)

HRMS (ESI) calculated 283.0831 [M+H$^+$], 283.0825 found.

tert-Butyl 4-(4-(2,2,2-trifluoroacetyl)benzamido)benzoate

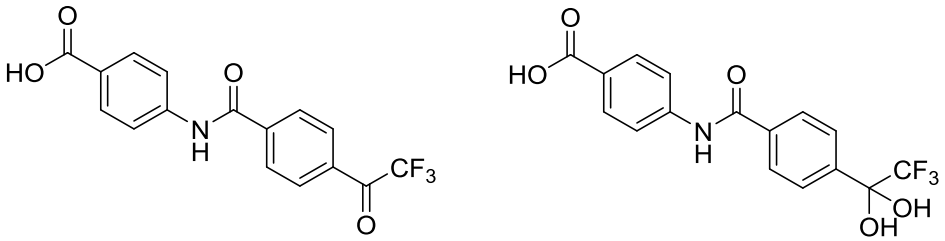


The aniline (0.46 mmol) was coupled with benzoic acid using procedure C. The crude product was purified by chromatography using (petroleum ether/ ethyl acetate 1:2). The crude product was directly used in the next reaction.

Yield: 64.3 mg (crude)

Rf: 0.22 (PE:EE 1:2)

4-(4-(2,2,2-trifluoroacetyl)benzamido)benzoic acid and 4-(4-(2,2,2-trifluoro-1,1-dihydroxyethyl)-benzamido)benzoic acid



The *tert*-butyl ester (0.15 mmol) was deprotected using procedure H. The crude product was of sufficient purity for further reactions. The product was a beige solid and contained a mixture of the ketone and the hydrate.

Yield: 48.8 mg (31 - 33 % over 2 steps)

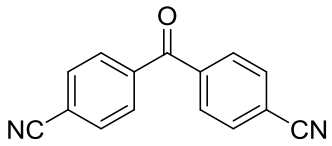
^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.77 (br s, 1H, COOH), 10.81 (s, 0.6 H, CONH), 10.58 (s, 0.4 H, CONH), 8.19 (s, 2.5H, C_{Ar}-H), 7.99 – 7.90 (m, 5H, C_{Ar}-H), 7.77 – 7.72 (m, 1.5 H, $(OH)_2$)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 179.5 (q, C=O, J = 34.4 Hz), 166.9 (COOH), 166.9 (COOH), 165.7 (CONH), 164.8 (CONH), 142.6 (d, C_{Ar}, J = 154.6 Hz), 141.8 (d, C_{Ar}, J = 255.2 Hz), 135.4 (C_{Ar}), 131.6 (C_{Ar}), 130.3 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.9 (d, C_{Ar}-H, J = 1.3 Hz), 128.8 (C_{Ar}), 127.5 (C_{Ar}-H), 127.3 (C_{Ar}-H), 126.0 (C_{Ar}), 125.6 (C_{Ar}), 119.6 (C_{Ar}-H), 119.4 (C_{Ar}-H), 116.3 (d, CF_3, J = 291.5 Hz), 92.4 (d, $C(OH)_2$, J = 31.4 Hz)

^{19}F{^{1}H}-NMR (470 MHz, DMSO-d_6, 300 K) δ (ppm) = - 70.8 (s, CF_3), - 82.7 (s, CF_3)

HRMS (ESI) calculated 338.0640 [M+H^+] and 356.0746 [$M_{hydrate}$+H^+], 338.0635 and 356.0740 found.

4,4'-Carbonyldibenzonitrile (**43**)[197]



100 mg 4,4'-(hydroxymethylene)dibenzonitrile (0.43 mmol, 1 eq) was dissolved in 1.5 ml dry DCM in a dry flask under nitrogen atmosphere and cooled down to 0 °C. 2 ml 0.3 M Dess-Martin periodinane in DCM (0.6 mmol, 1.4 eq) was added to the stirring solution. The reaction was stirred at 0 °C and controlled over TLC. After completion, the reaction was quenched with a solution of 675 mg sodium thiosulfate (4.27 mmol, 10 eq) in 4 ml water and 4 ml saturated $NaHCO_3$ solution. It was stirred for another 10 minutes. The organic phase was separated and stored. The water phase was extracted with 3 x 10 ml of ethyl acetate. The organic layers were combined and washed with brine. The solvent was removed under reduced pressure. The product was a slightly yellow solid.

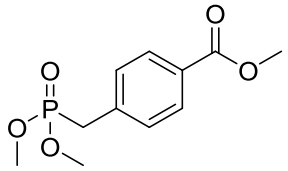
Yield: 125 mg (quantitative)

Rf: 0.44 (PE:EE 3:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.88 (d, 4H, Ar-H, J = 8.5 Hz), 7.83 (d, 4H, Ar-H, J = 8.5 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 193.6 (CO), 139.9 (C_{Ar}), 132.6 (C_{Ar}-H), 130.4 (C_{Ar}-H), 117.8 (CN), 116.8 (C_{Ar})

Methyl 4-((dimethyloxyphosporyl)methyl)benzoate (**44**)[198]



100 mg methyl 4-(bromomethyl)benzoate (0.44 mmol, 1 eq) and 0.26 ml trimethyl phosphite (274 mg, 2.2 mmol, 5 eq) were added to a flask. 6 ml toluene was added and the reaction was refluxed under nitrogen atmosphere overnight. The mixture was allowed to reach room temperature and the solvent was evaporated under reduced pressure. The excess of trimethyl phosphite was coevaporated with toluene and the product was dried under high vacuum. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate). The product is a colorless oil.

Yield: 85.4 mg (76 %)

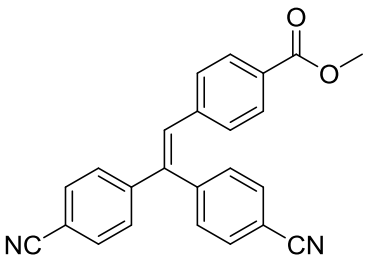
Rf: 0.1 (PE:EE 1:2)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.99 (d, 2H, Ar-H, J = 7.7 Hz), 7.37 (dd, 2H, Ar-H, J = 2.5 Hz, 8.4 Hz), 3.91 (s, 3H, OMe), 3.68 (d, 6H, $P(OMe)_2$, J = 10.9 Hz), 3.22 (d, 2H, PCH_2, J = 22.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 166.8 (COO), 136.7 (d, C_{Ar}, J = 9.3 Hz), 129.9 (d, C_{Ar}-H, J = 2.9 Hz), 129.7 (d, C_{Ar}-H, J = 6.6 Hz), 128.9 (d, C_{Ar}, J = 3.4 Hz), 53.0 (d, $P(OCH_3)_2$, J = 6.9 Hz), 52.1 (OCH_3), 33.1 (d, PCH_2, J = 137.8 Hz)

HRMS (ESI) calculated 259.0735 [$M+H^+$], 259.0732 found.

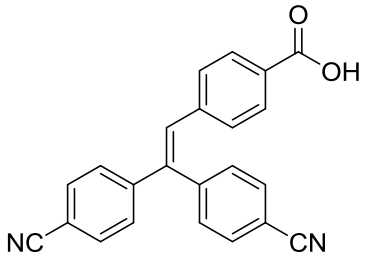
Methyl 4-(2,2-bis(4-cyanophenyl)vinyl)benzoate[196]



50 mg methyl 4-((dimethoxyphosphoryl)methyl)benzoate (0.19 mmol, 1 eq) was added to a dry flask and further dried under high vacuum. 1.4 ml of dry THF was added under nitrogen atmosphere and the solution was cooled down to - 78 °C. 0.194 ml LiHMDS (1 M in THF, 0.19 mmol, 1 eq) was slowly added while stirring vigorously and maintaining - 78 °C. The mixture was warmed up to 0 °C and stirred for 15 minutes before it was cooled down to - 78 °C. 45.0 mg 4,4'-carbonyldibenzonitrile (0.19 mmol, 1 eq) dissolved in 1.3 ml dry THF was slowly added to the mixture. The reaction was allowed to reach room temperature and was stirred for 14 hours. The solvent was removed under reduced pressure and major byproducs were removed by column chromatography using ethyl acetate and petroleum ether as solvents. The crude product was used without further purification.

Rf: 0.17 (PE:EE 6:1)

4-(2,2-bis(4-cyanophenyl)vinyl)benzoic acid (**45**)[180]



45.2 mg crude methyl 4-(2,2-bis(4-cyanophenyl)vinyl)benzoate (0.12 mmol, 1 eq) and 49.8 mg lithium iodide (0.37 mmol, 3 eq) were added to a dry and pressure stable sealed glass tube. 0.3 ml dry pyridine was added under nitrogen atmosphere and the tube was sealed. The mixture was heated up to 110 °C and stirred overnight. After completion, the solvent was removed under reduced pressure. The solid residue was dissolved in 10 ml saturated $NaHCO_3$ solution and a small amount of NaOH and washed with 2 x 4 ml DCM. The aqueous layer was acidified with 1 M HCl and extracted with 4 x 5 ml ethyl acetate. The organic phases were combined and the solvent was removed under reduced pressure. The crude product was purified by RP HPLC.

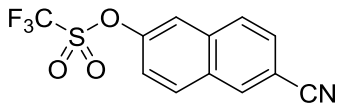
Yield: 31.2 mg (31 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.93 (d, 2H, Ar-H, J = 8.5 Hz), 7.67 (d, 2H, Ar-H, J = 5.7 Hz), 7.65 (d, 2H, Ar-H, J = 5.8 Hz), 7.38 (d, 2H, Ar-H, J = 8.7 Hz), 7.29 (d, 2H, Ar-H, J = 8.5 Hz), 7.16 (s, 1H, =CH), 7.11 (d, 2H, Ar-H, J = 8.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 170.5 (COOH), 146.2 (C_{Ar}), 143.7 (C_{Ar}), 141.9 (C=), 141.2 (C_{Ar}), 133.0 (C_{Ar}-H), 132.6 (C_{Ar}-H), 131.4 (=CH), 131.3 (C_{Ar}-H), 130.4 (C_{Ar}-H), 129.8 (C_{Ar}-H), 128.5 (C_{Ar}), 128.4 (C_{Ar}-H), 118.6 (CN), 118.5 (CN), 112.5 (C_{Ar}), 112.2 (C_{Ar})

HRMS (ESI) calculated 351.1134 [M+H$^+$], 351.1128 found.

6-Cyanonaphthalen-2-yl trifluoromethanesulfonate (106)[235]



150 mg 6-hydroxy-2-naphthonitrile (0.89 mmol, 1 eq) was dissolved in 1.0 ml dry pyridine (981.9 mg, 12.4 mmol, 14 eq) under nitrogen atmosphere and cooled down to 0 °C. 0.165 ml triflic anhydride (275 mg, 0.97 mmol, 1.1 eq) was slowly added to the stirring solution under nitrogen atmosphere while keeping 0 °C. The reaction mixture was slowly allowed to reach room temperature and was stirred overnight. After completion, 25 ml 1 M HCl was added. The aqueous phase was extracted 3 times with 10 ml ethyl acetate. The combined organic phases were washed with 10 ml 1 M HCl, 10 ml saturated $NaHCO_3$ solution and 10 ml brine. The organic solvent was removed under reduced pressure. The product was an orange solid.

Yield: 274.6 mg (quantitative)

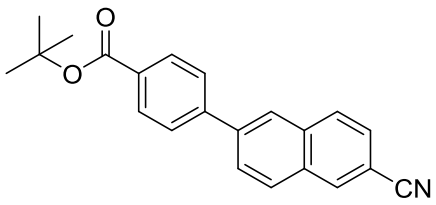
^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.31 – 8.29 (m, 1H, Ar-H), 8.02 (d, 1H, Ar-H, J = 9.1 Hz), 7.98 (d, 1H, Ar-H, J = 8.6 Hz), 7.83 (d, 1H, Ar-H, J = 2.4 Hz), 7.73 (dd, 1H, Ar-H, J = 1.6 Hz, 8.5 Hz), 7.52 (dd, 1H, Ar-H, J = 2.5 Hz, 9.0 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 149.1 (C_{Ar}-O), 135.0 (C_{Ar}), 134.1 (C_{Ar}-H), 131.5 (C_{Ar}-H), 131.4 (C_{Ar}), 129.6 (C_{Ar}-H), 128.3 (C_{Ar}-H), 121.8 (C_{Ar}-H), 119.7 (C_{Ar}-H), 118.9 (SO_3CF_3, J = 321.0 Hz), 118.5 (CN), 111.2 (C_{Ar})

^{19}F{^{1}H}-NMR (470 MHz, $CDCl_3$, 300 K): δ (ppm) = - 72.7 (SO_3CF_3)

HRMS (ESI) calculated 302.0099 [M+H$^+$], 302.0093 found.

tert-Butyl 4-(6-cyanonaphthalene-2-yl)benzoate (**107**)[236]



100 mg 6-cyanonaphthalen-2-yl trifluoromethanesulfonate (0.33 mmol, 1 eq), 110.0 mg *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (0.36 mmol, 1.1 eq) and 83 mg potassium carbonate (0.60 mmol, 1.8 eq) were added to dry flask under nitrogen atmosphere. 3.4 ml dry DMF and 40 mg Tetrakis(triphenylphosphine)palladium(0) (0.03 mmol, 0.1 eq) were added under nitrogen atmosphere and the mixture was degassed with nitrogen. The mixture was refluxed overnight. After completion, the mixture was cooled down to room temperature. 15 ml ethyl acetate was added and the organic phase was washed 3 times with a mixture of 1 ml 0.5 M HCl and 4 ml brine. The product was purified by flash chromatography using petroleum ether and ethyl acetate as solvent mixture. The product was a slightly yellow solid.

Yield: 32.6 mg (30 %)

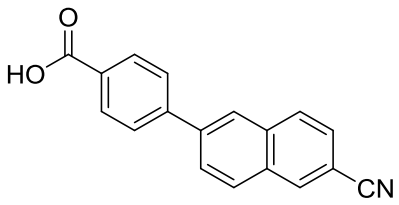
Rf: 0.54 (PE:EE 9:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.28 – 8.26 (m, 1H, Ar-H), 8.14 – 8.10 (m, 3H, Ar-H), 7.99 (dd, 2H, Ar-H, J = 1.9 Hz, 8.5 Hz), 7.88 (dd, 1H, Ar-H, J = 1.8 Hz, 8.6 Hz), 7.76 (d, 2H, Ar-H, J = 8.6 Hz), 7.66 (dd, 1H, Ar-H, J = 1.6 Hz, 8.5 Hz), 1.63 (s, 9H, $(CH_3)_3$)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 165.6 (COO), 144.0 (C_{Ar}), 140.9 (C_{Ar}), 135.0 (C_{Ar}), 134.1 (C_{Ar}-H), 131.8 (C_{Ar}), 131.8 (C_{Ar}), 130.3 (C_{Ar}-H), 129.7 (C_{Ar}-H), 129.3 (C_{Ar}-H), 127.4 (C_{Ar}-H), 127.4 (C_{Ar}-H), 127.2 (C_{Ar}-H), 126.4 (C_{Ar}-H), 119.3 (CN), 109.8 (C_{Ar}), 81.5 ($C(Me)_3$), 28.4 ($(CH_3)_3$)

HRMS (ESI) calculated 330.1494 [$M+H^+$], 330.1490 found.

4-(6-cyanonaphthalene-2-yl)benzoic acid (**108**)[182]



27.6 mg *tert*-butyl 4-(6-cyanonaphthalene-2-yl)benzoate (0.08 mmol, 1 eq) was dissolved in 1.0 ml dry DCM under nitrogen atmosphere. 0.16 ml TFA (238.2 mg, 2.1 mmol, 25.0 eq) was added to the stirring mixture. The reaction was controlled over LCMS. After completion, the solvent was removed under reduced pressure. The excess of TFA was removed by coevaporation with DCM. The product was a white solid.

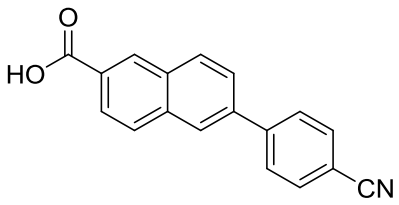
Yield: 25.0 mg (quantitative)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.06 (br s, 1H, COOH), 8.63 (s, 1H, Ar-H), 8.48 – 8.46 (m, 1H, Ar-H), 8.22 – 8.18 (m, 2H, Ar-H), 8.11 – 8.07 (m, 3H, Ar-H), 8.00 (d, 2H, Ar-H, J = 8.6 Hz), 7.84 (dd, 1H, Ar-H, J = 1.6 Hz, 8.5 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.0 (COOH), 143.2 (C_{Ar}), 139.4 (C_{Ar}), 134.6 (C_{Ar}), 134.1 (C_{Ar}-H), 131.4 (C_{Ar}), 130.3 (C_{Ar}), 130.1 (C_{Ar}-H), 129.8 (C_{Ar}-H), 129.4 (C_{Ar}-H), 127.4 (C_{Ar}-H), 126.9 (C_{Ar}-H), 126.7 (C_{Ar}-H), 126.1 (C_{Ar}-H), 119.1 (CN), 108.7 (C_{Ar})

HRMS (ESI) calculated 274.0868 [M+H$^+$], 274.0863 found.

6-(4-cyanophenyl)-2-naphthoic acid (**109**)[238]



100 mg 6-bromo-2-naphthoic acid (0.40 mmol, 1 eq), 62.0 mg (4-cyanophenyl)boronic acid and 110 mg potassium carbonate (0.80 mmol, 2.0 eq) were added to dry flask under nitrogen atmosphere. 2.0 ml toluene, 0.4 ml water and 23.0 mg Tetrakis(triphenylphosphine)palladium(0) (0.02 mmol, 0.05 eq) were added and the mixture was degassed with nitrogen. The mixture was refluxed overnight. After completion, the product was filtered off and washed with 1 M HCl and diethyl ether. The product was dried under reduced pressure and purified by flash chromatography (petroleum ether/ ethyl acetate + 2% AcOH). The product was a white solid.

Yield: 41.7 mg (38 %)

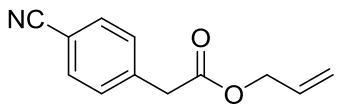
Rf: 0.29 (PE:EE + 2% AcOH 1:1)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.14 (br s, 1H, COOH), 8.65 (d, 1H, Ar-H, J = 0.7 Hz), 8.43 (d, 1H, Ar-H, J = 1.3 Hz), 8.25 (d, 1H, Ar-H, J = 8.8 Hz), 8.11 (d, 1H, Ar-H, J = 8.8 Hz), 8.07 (d, 2H, Ar-H, J = 8.6 Hz), 8.03 (dd, 1H, Ar-H, J = 1.6 Hz, 8.6 Hz), 8.00 (m, 3H, Ar-H)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 167.3 (COOH), 144.0 (C_{Ar}), 137.7 (C_{Ar}), 135.1 (C_{Ar}), 133.0 (C_{Ar}-H), 131.9 (C_{Ar}), 130.3 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.8 (C_{Ar}-H), 128.0 (C_{Ar}-H), 126.2 (C_{Ar}-H), 125.9 (C_{Ar}-H), 125.6 (C_{Ar}-H), 118.8 (CN), 110.5 (C_{Ar})

HRMS (ESI) calculated 274.0868 [M+H$^+$], 274.0862 found.

Allyl 2-(4-cyanophenyl)acetate (**94**)



100 mg 2-(4-cyanophenyl)acetic acid (0.62 mmol, 1 eq) was added to a small flask and further dried under high vacuum. 1 ml dry DCM and a drop DMF was added under nitrogen atmosphere. The mixture was cooled down to 0 °C and 0.08 ml oxalyl chloride (0.93 mmol, 1.5 eq) was added to the stirring mixture. After 1 hour, the solvent was evaporated under reduced pressure. 1 ml dry DCM was added to the residue under nitrogen atmosphere and the solution was cooled down to 0 °C. 0.2 ml allyl alcohol (170.8 mg, 2.94 mmol, 4.7 eq) was added under nitrogen atmosphere to the stirring solution. After completion, the solvent was removed under reduced pressure. 2 ml saturated $NaHCO_3$ solution and 6 ml brine were added to the residue and the product was extracted with 3 x 4 ml ethyl acetate. The solvent was removed under reduced pressure and the product was dried under high vacuum. The product was a yellow orange solid.

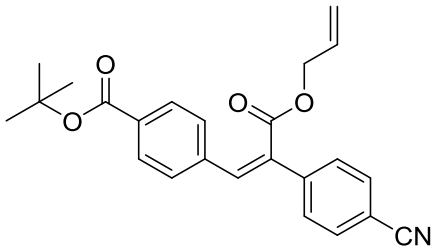
Yield: 133.5 mg (quantitative)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 7.63 (d, 2H, Ar-H, J = 8.4 Hz), 7.41 (d, 2H, Ar-H, J = 8.5 Hz), 5.89 (ddt, 1H, CH=, J = 5.8 Hz, 10.4 Hz, 17.2 Hz), 5.28 (dq, 1H, =CH_2, J = 1.5 Hz, 17.2 Hz), 5.24 (dq, 1H, =CH_2, J = 1.2 Hz, 10.4 Hz), 4.61 (dt, 2H, OCH_2, J = 1.4 Hz, 5.8 Hz), 3.71 (s, 2H, CH_2)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.1 (COO), 139.3 (C_{Ar}), 132.5 (CH=) 131.8 (C_{Ar}-H), 130.3 (C_{Ar}-H), 118.9 (=CH_2), 118.8 (CN), 111.4 (C_{Ar}), 66.0 (OCH_2), 41.4 (CH_2)

HRMS (ESI) calculated 202.0868 [M+H$^+$], 202.0863 found.

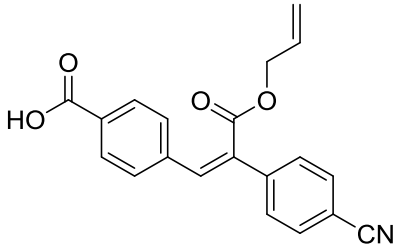
tert-butyl 4-(3-(allyloxy)-2-(4-cyanophenyl)-3-oxoprop-1-en-1-yl)benzoate[256]



40.0 mg allyl 2-(4-cyanophenyl)acetate (0.2 mmol, 1 eq) was added to a dry flask under nitrogen atmosphere. 1 ml dry THF was added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 0.2 ml 1 M LiHMDS (0.2 mmol, 1 eq) was slowly added to the stirring mixture under nitrogen atmosphere and it was stirred for 30 minutes at 0 °C. The reaction was cooled down to - 78 °C. 41.0 mg *tert*-butyl 4-formylbenzoate (0.2 mmol, 1 eq) dissolved in 0.5 ml dry THF was slowly added to the reaction at - 78 °C and the reaction was slowly allowed to reach room temperature. The reaction was controlled over LCMS and quenched with saturated NH_4Cl solution after completion. The solvent was removed under reduced pressure. The remaining solvent was removed over lyophilization. The crude product was directly used without further purification.

Yield: 77.0 mg (crude)

4-(3-(allyloxy)-2-(4-cyanophenyl)-3-oxoprop-1-en-1-yl)benzoic acid (**95**)[182]



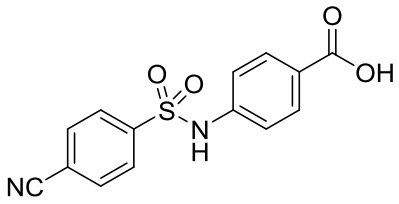
77.0 mg crude *tert*-butyl 4-(3-(allyloxy)-2-(4-cyanophenyl)-3-oxoprop-1-en-1-yl)benzoate (0.2 mmol, 1 eq) was dissolved in 2.5 ml dry DCM under nitrogen atmosphere. 0.4 ml TFA (595.6 mg, 5.2 mmol, 26.4 eq) was added to the stirring mixture. The reaction was controlled over LCMS. After completion, the solvent was removed under reduced pressure. The excess of TFA was removed by coevaporation with DCM. 4 ml 1 M HCl and 16 ml brine was added to the residue. The aqueous phase was extracted with 3 x 5 ml ethyl acetate. The combined organic phases were concentrated under reduced pressure. The product was purified via RP HPLC. The product was a yellow solid.

Yield: 9.0 mg (14 % over 2 steps)

^{1}H-NMR (500 MHz, THF-d_8, 300 K): δ (ppm) = 11.40 (br s, 1H, COOH), 7.95 (s, 1H, =CH), 7.79 (d, 2H, Ar-H, J = 8.4 Hz), 7.66 (d, 2H, Ar-H, J = 8.3 Hz), 7.35 (d, 2H, Ar-H, J = 8.4 Hz), 7.11 (d, 2H, Ar-H, J = 8.3 Hz), 5.90 (ddt, 1H, CH=, J = 5.5 Hz, 10.8 Hz, 17.1 Hz), 5.22 (dq, 1H, =CH_2, J = 1.6 Hz, 17.2 Hz), 5.13 (ddd, 1H, =CH_2, J = 1.3 Hz, 2.8 Hz, 10.5 Hz), 4.65 (dt, 2H, OCH_2, J = 1.4 Hz, 5.5 Hz)

^{13}C-NMR (126 MHz, THF-d_8, 300 K): δ (ppm) = 166.9 (C=O), 166.2 (C=O), 141.5 (C_{Ar}), 141.0 (=CH), 139.3 (C_{Ar}), 134.2 (C_{Ar}-H), 133.4 (C_{Ar}-H), 133.0 (C_{Ar}-H), 131.9 (C_{Ar}-H), 131.0 (C_{Ar}-H), 130.4 (C_{Ar}-H), 125.9 (C_{Ar}), 119.0 (CN), 118.0 (=CH_2), 66.5 (OCH_2)

4-((4-cyanophenyl)sulfonamido)benzoic acid (**46**)[206]



100 mg *tert*-butyl 4-aminobenzoate (0.52 mmol, 1 eq) and 104 mg 4-cyanobenzenesulfonyl chloride (0.52 mmol, 1 eq) were added to a dry flask and further dried under high vacuum. 5 ml dry THF and 0.13 ml pyridine (127.7 mg, 1.61 mmol, 3.1 eq) were added under nitrogen atmosphere. The reaction was stirred overnight and controlled by TLC. After completion, the reaction was quenched with ice and 2 ml 1 M HCl. The solvent was partially removed under reduced pressure and the residue was diluted with 20 ml ethyl acetate and 20 ml water. The aqueous phase was separated. The organic phase was washed with 2 x 8 ml saturated $NaHCO_3$ solution. The organic phase was removed under reduced pressure. The crude residue was cooled down to 0 °C. 4 ml dry DCM and 0.8 ml TFA (1191 mg, 10.45 mmol, 20.2 eq) were added. The reaction was stirred for 1.5 h and controlled over TLC. After completion, the solvent was removed under reduced pressure and the residue was dissolved in 30 ml ethyl acetate. The organic phase was extracted with 3 x 10 ml saturated $NaHCO_3$ solution. The aqueous phases were combined and acidified with HCl to pH 1. The acidic phase was extracted with 4 x 20 ml ethyl acetate. The combined organic phases were concentrated under reduced pressure. The product was dried under high vacuum. The product was a rose red colored solid.

Yield: 126.0 mg (81 %)

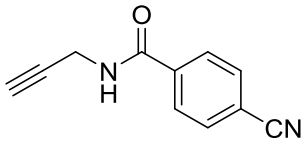
Rf Intermediate: 0.29 (PE:EE 3:1)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.78 (s, 1H, COOH), 11.04 (s, 1H, SO_2NH), 8.06 (d, 2H, Ar-H, J = 8.7 Hz), 7.96 (d, 2H, Ar-H, J = 8.7 Hz), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.20 (d,2H, Ar-H, J = 8.8 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 166.6 (COOH), 143.2 (C_{Ar}), 141.2 (C_{Ar}), 133.7 (C_{Ar}-H), 130.9 (C_{Ar}-H), 127.4 (C_{Ar}-H), 126.2 (C_{Ar}), 118.7 (C_{Ar}-H), 117.5 (CN), 115.7 (C_{Ar})

HRMS (ESI) calculated 303.0440 [M+H$^+$], 303.0432 found.

4-Cyano-N-(prop-2-yn-1-yl)benzamide (**34**)



145 mg 4-cyanobenzoyl chloride (0.88 mmol, 1.0 eq) was added to a dry flask and further dried under high vacuum. 2.0 ml dry DCM was added under nitrogen atmosphere and the mixture was cooled to 0 °C. 0.17 ml propargylamine (146 mg, 2.65 mmol, 3 eq) was slowly added to the stirring mixture while maintaining 0 °C. After 15 minutes, the reaction was allowed to reach room temperature. The reaction was stirred overnight. After the reaction was completed, 5 ml 1 M HCl and 5 ml Water were added to the mixture. The organic phase was separated and stored. The aqueous phase was extracted with 3 x 4 ml of ethyl acetate and all organic phases were combined. The organic phase was washed with brine. The organic phase was concentrated under reduced pressure. The product was dried under high vacuum. The product was a yellow solid.

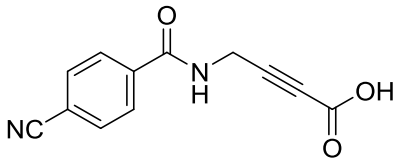
Yield: 167.8 mg (quantitative)

^{1}H-NMR (500 MHz, Aceton-d_6, 300 K): δ (ppm) = 8.36 (s, 1H, CONH), 8.09 (d, 2H, Ar-H, J = 8.5 Hz), 7.90 (d, 2H, Ar-H, J = 8.7 Hz), 4.22 – 4.20 (m, 2H, CH_2), 2.70 (t, 1H, ≡CH, J = 2.6 Hz)

^{13}C-NMR (126 MHz, Aceton-d_6, 300 K): δ (ppm) = 165.6 (CONH), 139.1 (C_{Ar}), 133.3 (C_{Ar}-H), 129.0 (C_{Ar}-H), 118.8 (CN), 115.6 (C_{Ar}), 81.0 (C≡), 72.2 (≡CH), 29.7 (CH_2)

HRMS (ESI) calculated 185.0715 [M+H$^+$], 185.0712 found.

4-(4-cyanobenzamido)-but-2-ynoic acid (**35**)[257]



1.15 ml LiHMDS (1M in THF, 1.15 mmol, 2.12 eq) and 11 ml dry THF were added to a dry flask under nitrogen atmosphere at - 78 °C. 100 mg 4-cyano-N-(prop-2-yn-1-yl)benzamide (0.54 mmol, 1 eq) dissolved in 1.0 ml dry THF was slowly added and stirred for 0.5 h while maintaining - 78 °C. CO_2 was bubbled through the solution for 2 h at - 78 °C. The reaction was quenched with saturated NH_4Cl solution and warmed up to room temperature. The solvent was partially removed under reduced pressure. The residue was dissolved in 1 M NaOH and washed with DCM. The aqueous phase was acidified with 6 M HCl. The precipitate was filtered off and washed with 0.1 M HCl. The solid product was dried under high vacuum.

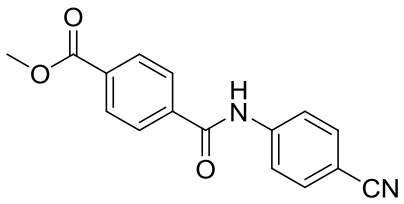
Yield: 56.6 mg (46 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.68 (s, 1H, COOH), 9.33 (t, 1H, CONH, J = 5.4 Hz), 8.02 (d, 2H, Ar-H, J = 8.7 Hz), 7.99 (d, 2H, Ar-H, J = 8.6 Hz), 4.28 (d, 2H, CH_2, J = 5.5 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 164.8 (C=O), 153.9 (C=O), 137.4 (C_{Ar}-H), 132.6 (C_{Ar}-H), 128.2 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 83.8 (C≡), 74.9 (C≡), 28.7 (CH_2)

HRMS (ESI) calculated 229.0613 [M+H$^+$], 229.0613 found.

Methyl 4-((4-cyanophenyl)carbamoyl)benzoate (**96**)



The aniline (0.52 mmol) was coupled with benzoic acid using procedure A2. The crude product was of sufficient purity for further reactions. The product was a brown solid.

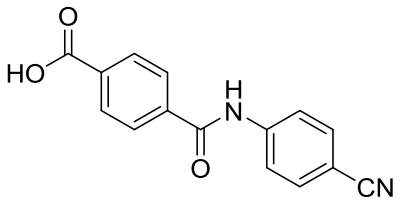
Yield: 121.2 mg (84 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.82 (br s, 1H, CONH), 8.13 – 8.06 (m, 4H, Ar-H), 7.99 (d, 2H, Ar-H, J = 8.8 Hz), 7.84 (d, 2H, Ar-H, J = 8.8 Hz), 3.90 (s, 3H, OCH_3)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K) = 165.6 (C=O), 165.3 (C=O), 143.2 (C_{Ar}), 138.4 (C_{Ar}), 133.2 (C_{Ar}-H), 132.4 (C_{Ar}), 129.2 (C_{Ar}-H), 128.3 (C_{Ar}-H), 120.3 (C_{Ar}-H), 119.0 (CN), 105.7 (C_{Ar}), 52.5 (OCH_3)

HRMS (ESI) calculated 281.0926 [M+H$^+$], 281.0921 found.

4-((4-cyanophenyl)carbamoyl)benzoic acid (**97**)



The methyl ester (0.39 mmol) was deprotected using procedure G. The crude product was of sufficient purity for further reactions. The product was a beige solid.

Yield: 47.2 mg (45 %)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 13.30 (br s, 1H, COOH), 10.79 (s, 1H, CONH), 8.09 (d, 2H, Ar-H, J = 8.6 Hz), 8.05 (d, 2H, Ar-H, J = 8.6 Hz), 8.00 (d, 2H, Ar-H, J = 8.9 Hz), 7.84 (d, 2H, Ar-H, J = 8.8 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K) = 166.7 (C=O), 165.5 (C=O), 143.2 (C_{Ar}), 138.1 (C_{Ar}), 133.7 (C_{Ar}), 133.2 (C_{Ar}-H), 129.4 (C_{Ar}-H), 128.1 (C_{Ar}-H), 120.3 (C_{Ar}-H), 119.0 (CN), 105.6 (C_{Ar})

HRMS (ESI) calculated 267.077 [M+H$^+$], 267.0764 found.

5.2.2.3 Assembly of central amino acid and fragment CDE

The amide coupling between fragment CDE and the central amino acid was carried out by one of three general procedures. Adapted experimental procedures from the literature are quoted in the listing of the general procedures. Citations at the end of the molecule name are added, if other methods were applied.

5.2.2.3.1 General procedures – Coupling between central amino acid and fragment CDE

The amide coupling between the central amino acid and fragment CDE was carried out by three general procedures. Suitable coupling conditions were determined by the attached functional groups at the central amino acid and their tolerance for the chosen conditions. The three general procedures were:

I.) Acid chloride formation via oxalyl chloride and subsequent coupling with pyridine in DCM[207].
J.) Amide coupling with EEDQ [J1] or IIDQ [J2] followed by deprotection with diethylamine[182],[5].
K.) Activation with T3P and amide coupling with pyridine in ethyl acetate[210].

I.) Acid chloride formation and subsequent coupling

0.11 mmol of the desired Fmoc-protected amino acid (1 eq), 0.4 dry DCM and one drop of dry DMF were added to a dry flask under nitrogen atmosphere. The mixture was cooled to 0 °C and 17 µl oxalyl chloride (0.2 mmol, 1.4 eq) was slowly added to the stirring mixture. The reaction was controlled by quenching a sample in methanol and running a TLC with petroleum ether and ethyl acetate. After completion, the solvent was evaporated under reduced pressure. The crude product was dried under high vacuum overnight and directly used without further purification.

0.09 mmol of fragment CDE (1 eq) and 0.14 mmol of the preformed acid chloride (1.5 eq) were added to a dry flask and further dried under high vacuum. 1.0 ml dry DCM was added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 23 µl pyridine (0.29 mmol, 3.1 eq) was added under nitrogen atmosphere and the solution was kept at 0 °C for the whole reaction. The reaction was controlled over LCMS. After completion, the reaction was quenched with 2 ml 1 M HCl and 4 ml water. The aqueous phase was extracted with 3 x 4 ml of ethyl acetate. The organic phases were combined and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography with petroleum ether and ethyl acetate.

J.) Amide coupling with EEDQ or IIDQ

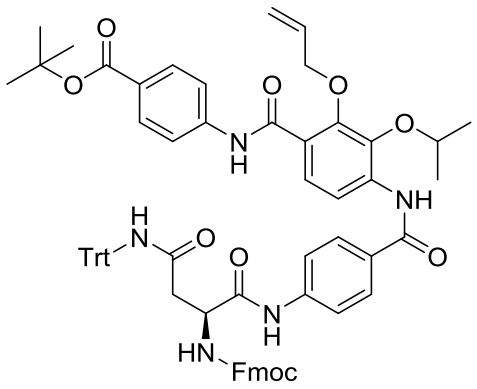
0.09 mmol of fragment CDE (1 eq) and 0.14 mmol of the desired amino acid (1.5 eq) were added to a dry flask and were further dried under high vacuum. 0.15 ml dry DCM was added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 33.0 mg EEDQ [J1] or 40 µl IIDQ [J2] (0.13 mmol, 1.5 eq) dissolved in 0.15 ml dry DCM was added to the stirring solution. The reaction was kept at 0 °C for 30 minutes and slowly allowed to reach room temperature afterwards. The reaction was stirred overnight and controlled over LCMS. The reaction was quenched with 2 ml 1 M HCl and 6 ml brine and extracted with 3 x 3 ml DCM. The combined organic phases were washed with brine and the solvent was removed under reduced pressure. The crude product was used in the next reaction.

0.09 mmol (1 eq) of the crude product was dissolved in 0.8 ml acetonitrile and 0.3 ml diethylamine (2.9 mmol, 31.5 eq) at 0 °C and stirred for 1 hour. The reaction was controlled over LCMS. The solvent was evaporated under reduced pressure and coevaporated with acetonitrile 3 times. The crude product was purified by RP flash or RP HPLC with acetonitrile and water mixed with 0.1 % HCOOH.

K.) Amide coupling with T3P

0.18 mmol of fragment CDE (1 eq) and 0.27 mmol of the desired amino acid (1.5 eq) were added to a dry flask and further dried under high vacuum. 50 µl dry pyridine (0.62 mmol, 3.4 eq) and 0.4 ml dry ethyl acetate were added under nitrogen atmosphere. The reaction mixture was cooled down to 0 °C. 0.25 ml T3P solution (50 wt % in ethyl acetate, 0.42 mmol, 2.3 eq) was added very slowly while keeping the temperature below 0 °C. The reaction was stirred at 0 °C overnight and controlled over LCMS. After completion, the reaction was quenched with 4 ml 1 M HCl and 12 ml brine and extracted with 3 x 6 ml ethyl acetate. The combined organic phases were washed with saturated $NaHCO_3$ solution and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography with petroleum ether and ethyl acetate or used without further purification.

tert-Butyl (*S*)-4-(4-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-oxo-4-(tritylamino)butanamido)-benzamido)-2-(allyloxy)-3-isopropyloxybenzamido)benzoate (**17**)[182]



175 mg N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4-trityl-L-asparagine (0.29 mmol, 1.6 eq) and 100 mg *tert*-butyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-isopropyloxybenzamido)benzoate (0.18 mmol, 1 eq) were added to a dry flask and further dried at high vacuum. 0.4 ml dry chloroform was added under nitrogen atmosphere and the solution was cooled down to 0 °C. 70 mg EEDQ (0.28 mmol, 1.54 eq) dissolved in 0.3 ml dry chloroform was added to the stirring solution. The reaction was allowed to reach room temperature and controlled over LCMS. The reaction was quenched with 10 ml 0.1 M HCl and 2 ml brine and extracted with 4 x 5 ml DCM. The combined organic phases were washed with brine. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate). The product was a off-white solid.

Yield: 140.5 mg (69 %)

Rf: 0.59 (PE:EE 1:1)

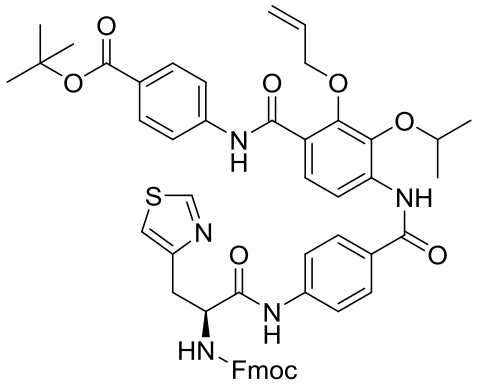
^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.18 (s, 1H, CONH), 9.15 (br s, 1H, CONH), 8.76 (s, 1H, CONH), 8.52 (d, 1H, CONH, J = 9.0 Hz), 8.08 (d, 1H, Ar-H, J = 8.9 Hz), 7.99 (d, 2H, Ar-H, J = 8.7 Hz), 7.88 (d, 2H, Ar-H, J = 8.7 Hz), 7.78 (d, 2H, Ar-H, J = 7.4 Hz), 7.74 (d, 2H, Ar-H, J = 8.8 Hz), 7.59 (d, 2H, Ar-H, J = 7.5 Hz), 7.56 (d, 2H, Ar-H, J = 8.7 Hz), 7.40 (t, 2H, Ar-H, J = 7.1 Hz), 7.33 – 7.28 (m, 2H, Ar-H), 7.25-7.22 (m, 9H, Ar-H), 7.21 – 7.15 (m, 6H, Ar-H), 6.93 (br s, 1H, CONH), 6.53 – 6.45 (m, 1H, CONH), 6.15 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.50 (dq, 1H, $=CH_2$, J = 1.4 Hz, 17.1 Hz), 5.41 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 4.78 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 4.73-4.69 (m, 3H, CH & OCH_2), 4.52 – 4.42 (m, 2H, OCH_2), 4.22 (t, 1H, CH, J = 6.8 Hz), 3.20 (d, 1H, CH_2, J = 15.1 Hz), 2.71 (dd, 1H, CH_2, J = 7.0 Hz, 16.1 Hz), 1.60 (s, 9H, $(CH_3)_3$), 1.40 (dd, 6H, $(CH_3)_2$, J = 1.8 Hz, 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 170.7 (CONH), 169.2 (CONH), 165.4 (COO), 164.2 (CONH), 162.6 (CONH), 156.3 (OCONH), 149.3 (C_{Ar}-O), 143.9 (C_{Ar}), 143.6 (C_{Ar}), 142.2 (C_{Ar}),141.3 (C_{Ar}), 141.0 (C_{Ar}), 138.9 (C_{Ar}), 137.6 (C_{Ar}), 132.2 (CH=), 130.7 (C_{Ar}-H), 129.9 (C_{Ar}), 128.6 (C_{Ar}-H), 128.1 (C_{Ar}-H), 128.0 (C_{Ar}-H),

127.8 (C_{Ar}-H), 127.5 (C_{Ar}-H), 127.3 (C_{Ar}), 127.3 (C_{Ar}), 127.1 (C_{Ar}-H), 125.0 (C_{Ar}-H), 121.5 (C_{Ar}), 120.1 (C_{Ar}-H), 120.0 (=CH_2), 119.8 (C_{Ar}-H), 119.0 (C_{Ar}-H), 115.7 (C_{Ar}-H), 80.8 ($C(Me)_3$), 76.6 ($CH(Me)_2$), 74.9 (OCH_2), 71.2 ($C(Ph)_3$), 67.3 (CH_2), 51.9 (CH-N), 47.1 (CH), 38.8 (CH_2), 28.2 (($CH_3)_3$), 22.8 (($CH_3)_2$)

HRMS (ESI) calculated 1124.481 [$M+H^+$], 1124.4818 found.

tert-Butyl (*S*)-4-(4-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(thiazol-4-yl)propanamido)-benzamido)-2-(allyloxy)-3-isopropyloxybenzamido)benzoate (**54**)



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure I. The product was a yellow orange solid.

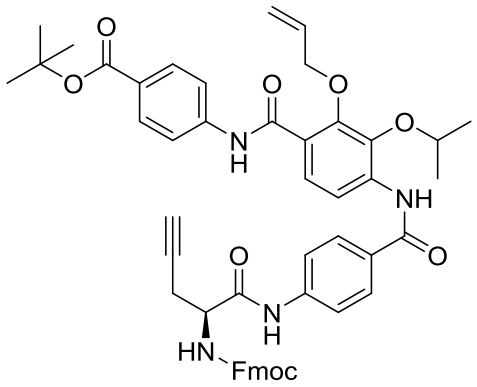
Yield: 62.2 mg (74 %)

^{1}H-NMR (700 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.53 (s, 1H, CONH), 8.88 (s, 1H, N=CH-S), 8.73 (s, 1H, CONH), 8.49 (d, 1H, Ar-H, J = 8.9 Hz), 8.06 (d, 1H, Ar-H, J = 8.9 Hz), 7.98 (d, 2H, Ar-H, J = 8.7 Hz), 7.87 (d, 2H, Ar-H, J = 8.7 Hz), 7.79 – 7.75 (m, 2H, Ar-H), 7.73 (d, 2H, Ar-H, J = 8.7 Hz), 7.69 (d, 2H, Ar-H, J = 7.9 Hz), 7.61 – 7.57 (m, 2H, Ar-H), 7.43 – 7.38 (m, 2H, Ar-H), 7.33 – 7.28 (m, 2H, Ar-H), 7.20 (s, 1H, C=CH-S), 6.51 (d, 1H, NHCH, J = 5.6 Hz), 6.14 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (dd, 1H, =CH_2, J = 1.3 Hz, 17.1 Hz), 5.40 (dd, 1H, =CH_2, J = 1.1 Hz, 10.4 Hz), 4.77 – 4.72 (m, 2H, NHCH & CHNH), 4.69 (d, 2H, $ArOCH_2$, J = 5.9 Hz), 4.49 – 4.39 (m, 2H, CH_2O), 4.23 (t, 1H, CH, J = 6.6 Hz), 3.46 – 3.34 (m, 2H, CH_2), 1.60 (s, 9H, $(CH_3)_3$), 1.38 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, $CDCl_3$, 300 K): δ (ppm) = 169.6 (CONH), 165.6 (COO), 164.4 (CONH), 162.8 (CONH), 156.7 (OCONH), 153.6 (N=CH-S), 152.6 (C_{Ar}), 149.4 (C_{Ar}-O), 143.8 (C_{Ar}), 142.3 (C_{Ar}-NH), 141.5 (C_{Ar}), 141.5 141.5 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.7 (C_{Ar}-NH), 132.3 (CH=), 130.8 (C_{Ar}), 130.0 (C_{Ar}), 128.2 (C_{Ar}-H), 128.0 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}), 127.3 (C_{Ar}-H), 125.2 (C_{Ar}-H), 121.7 (C_{Ar}), 120.2 (C_{Ar}-H), 120.2 (=CH_2), 119.8 (C_{Ar}-H), 119.2 (C_{Ar}-H), 116.5 (=CH-S), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 ($ArOCH_2$), 67.5 (CH_2O), 55.3 (CHNH), 47.3 (CH), 33.4 (CH_2), 28.4 (($CH_3)_3$), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 922.3486 [M+H$^+$], 922.3456 found.

tert-Butyl (*S*)-4-(4-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynamido)benzamido)-2-(allyloxy)-3-isopropyloxybenzamido)benzoate



Fragment CDE (2.75 mmol) was coupled with the central amino acid using procedure K. The product was a slightly yellow solid.

Yield: 1,604.5 mg (68 %)

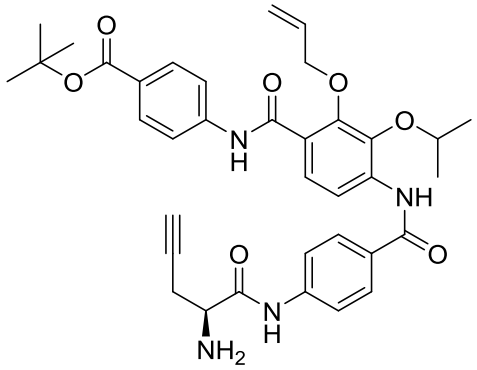
Rf: 0.29 (PE:EE 2:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.16 (s, 1H, CONH), 8.74 (s, 1H, CONH), 8.49 (d, 1H, Ar-H, J = 8.9 Hz), 8.07 (d, 1H, Ar-H, J = 8.9 Hz), 7.98 (d, 2H, Ar-H, J = 8.8 Hz), 7.89 (d, 2H, Ar-H, J = 8.7 Hz), 7.77 (d, 2H, Ar-H, J = 7.5 Hz), 7.73 (d, 2H, Ar-H, J = 8.8 Hz), 7.69 (d, 2H, Ar-H, J = 8.8 Hz), 7.59 (d, 2H, Ar-H, J = 7.4 Hz), 7.40 (t, 2H, Ar-H, J = 7.5 Hz), 7.30 (t, 2H, Ar-H, J = 7.4 Hz), 6.14 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 17.1 Hz), 5.64 – 5.57 (m, 1H, NHCH), 5.49 (dq, 1H, $=CH_2$, J = 1.4 Hz, 17.1 Hz), 5.41 (dq, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 4.75 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 4.69 (dt, 2H, $ArOCH_2$, J = 1.2 Hz, 5.9 Hz), 4.57 – 4.46 (m, 3H, CH_2 & CHNH), 4.25 (t, 1H, CH, J = 6.7 Hz), 2.94 – 2.68 (m, 2H, $CHCH_2$), 2.17 (t, 1H, ≡CH, J = 2.6 Hz), 1.60 (s, 9H, $(CH_3)_3$), 1.38 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 168.5 (CONH), 165.6 (COO), 164.3 (CONH), 162.8 (CONH), 156.7 (OCONH), 149.4 (C_{Ar}-O), 143.6 (C_{Ar}), 142.3 (C_{Ar}-NH), 141.5 (C_{Ar}), 141.0 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.6 (C_{Ar}-NH), 132.3 (CH=), 130.8 (C_{Ar}-H), 130.4 (C_{Ar}), 128.3 (C_{Ar}-H), 128.0 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.5 (C_{Ar}), 127.3 (C_{Ar}-H), 125.1 (C_{Ar}-H), 121.7 (C_{Ar}), 120.3 (C_{Ar}-H), 120.2 (CH_2=), 120.0 (C_{Ar}-H), 119.2 (C_{Ar}-H), 115.8 (C_{Ar}-H), 81.0 ($C(Me)_3$), 79.0 (C≡), 76.9 ($CH(Me)_2$), 75.1 ($ArOCH_2$), 72.5 (≡CH), 67.7 (CH_2O), 54.2 (CHNH), 47.2 (CH), 29.8 (CH_2), 28.4 ($(CH_3)_3$), 23.0 ($(CH_3)_2$)

HRMS (ESI) calculated 863.3656 [M+H$^+$], 863.3667 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-aminopent-4-ynamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.55 mmol) was coupled with the central amino acid using procedure K and the deprotection of J. The product was a yellow glass.

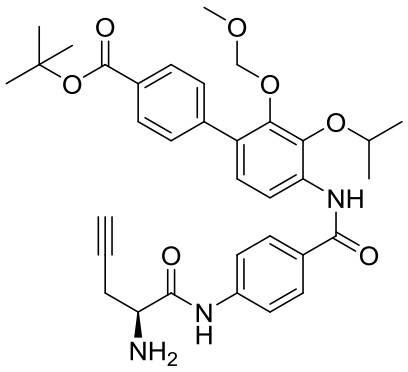
Yield: 318.0 mg (90 % over 2 steps)

^{1}H-NMR (700 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.96 (br s, 1H, CONH), 8.74 (s, 1H, CONH), 8.47 (d, 1H, Ar-H, J = 8.9 Hz), 8.04 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.88 (d, 2H, Ar-H, J = 8.6 Hz), 7.80 (d, 2H, Ar-H, J = 8.4 Hz), 7.72 (d, 2H, Ar-H, J = 8.7 Hz), 6.13 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (ddd, 1H, $=CH_2$, J = 1.3 Hz, 2.7 Hz, 17.1 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 4.74 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.69 (d, 2H, OCH_2, J = 5.9 Hz), 2.89 – 2.76 (m, 2H, CH_2), 2.11 (br s, 1H, ≡CH), 1.59 (s, 9H, $(CH_3)_3$), 1.37 (dd, 6H, $(CH_3)_2$, J = 1.4 Hz, 6.1 Hz)

^{13}C-NMR (176 MHz, $CDCl_3$, 300 K): δ (ppm) = 170.9 (CONH), 165.5 (COO), 164.4 (CONH), 162.8 (CONH), 149.4 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 141.3 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.8 (C_{Ar}-NH), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.9 (C_{Ar}), 128.3 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}), 121.7 (C_{Ar}), 120.2 ($=CH_2$), 119.5 (C_{Ar}-H), 119.2 (C_{Ar}-H), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 79.5 (C≡), 76.9 ($CH(Me)_2$), 75.1 (OCH_2), 72.2 (≡CH), 53.8 (CHNH), 28.4 (($CH_3)_3$), 24.5 (CH_2), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 641.2975 [$M+H^+$], 641.2972 found.

tert-Butyl (*S*)-4'-(4-(2-aminopent-4-ynamido)benzamido)-3'-isopropoxy-2'-(methoxymethoxy)-[1,1'-biphenyl]-4-carboxylate



Fragment CDE (0.11 mmol) was coupled with the central amino acid using procedure J1. The product was a slightly yellow solid.

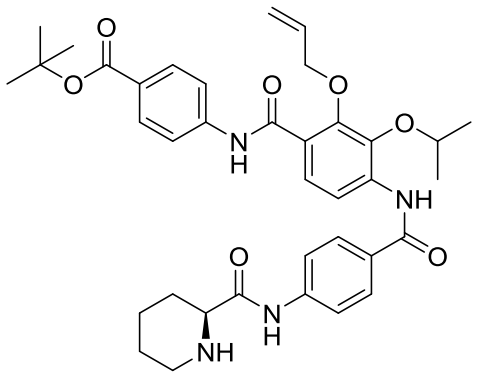
Yield: 42.8 mg (63 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 9.79 (s, 1H, CONH), 8.69 (s, 1H, CONH), 8.38 (d, 1H, Ar-H, J = 8.6 Hz), 8.02 (d, 2H, Ar-H, J = 8.5 Hz), 7.91 (d, 2H, Ar-H, J = 8.7 Hz), 7.78 (d, 2H, Ar-H, J = 8.7 Hz), 7.61 (d, 2H, Ar-H, J = 8.5 Hz), 7.14 (d, 1H, Ar-H, J = 8.6 Hz), 4.86 (s, 2H, OCH_2O), 4.78 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.67 (dd, 1H, CHN, J = 4.5 Hz, 7.4 Hz), 3.03 (s, 3H, OCH_3), 2.80 (dddd, 2H, CH_2, J = 2.7 Hz, 5.9 Hz, 10.1 Hz, 17.0 Hz), 2.09 (t, 1H, ≡CH, J = 2.6 Hz), 1.62 (s, 9H, $(CH_3)_3$), 1.38 (dd, 6H, $(CH_3)_2$, J = 1.6 Hz, 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 171.4 (CONH), 165.9 (COO), 164.3 (CONH), 147.0 (C_{Ar}), 142.7 (C_{Ar}), 141.0 (C_{Ar}), 139.9 (C_{Ar}), 133.7 (C_{Ar}), 131.4 (C_{Ar}), 130.6 (C_{Ar}), 130.4 (C_{Ar}) 129.6 (C_{Ar}-H), 129.4 (C_{Ar}-H), 128.2 (C_{Ar}-H) 125.7 (C_{Ar}-H), 119.4 (C_{Ar}-H), 115.8 (C_{Ar}-H), 99.0 (OCH_2O), 81.2 ($C(Me)_3$), 80.1 (C≡), 76.3 ($CH(Me)_2$), 71.8 (≡CH), 57.5 (OCH_3), 54.1 (CHN), 28.4 ($(CH_3)_3$), 24.9 (CH_2), 23.0 ($(CH_3)_2$)

HRMS (ESI) calculated 602.2866 [$M+H^+$], 602.2863 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(piperidine-2-carboxamido)benzamido)benzamido)-benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure I and the deprotection of J. The product was a yellowish solid.

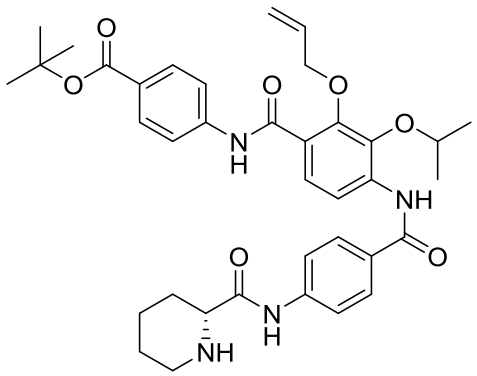
Yield: 23.6 mg (39 % over 2 steps)

^{1}H-NMR (700 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.47 (s, 1H, CONH), 8.73 (s, 1H, CONH), 8.46 (d, 1H, Ar-H, J = 8.9 Hz), 8.04 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.86 (d, 2H, Ar-H, J = 8.7 Hz), 7.76 (d, 2H, Ar-H, J = 8.7 Hz), 7.72 (d, 2H, Ar-H, J = 8.7 Hz), 6.14 (ddt, 1H, CH=, J = 5.9 Hz, 10.5 Hz, 16.3 Hz), 5.49 (d, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 4.74 (quart., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.69 (d, 2H, OCH_2, J = 5.9 Hz), 3.52 (d, 1H, CH-N, J = 7.8 Hz), 3.12 (d, 1H, CH_2-N, J = 12.1 Hz), 2.81 (t, 1H, CH_2-N, J = 11.1 Hz), 2.06 (dd, 1H, CH_2-CH, J = 2.9 Hz, 13.0 Hz), 1.85 – 1.81 (m, 1H, CH_2), 1.67 – 1.63 (m, 2H, CH_2-CH & CH_2), 1.59 (s, 9 H, $(CH_3)_3$), 1.53 – 1.49 (m, 2H, CH_2 & CH_2), 1.37 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, $CDCl_3$, 300 K): δ (ppm) = 172.0 (CONH), 165.5 (COO), 164.4 (CONH), 162.8 (CONH), 149.4 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.7 (C_{Ar}-NH), 132.2 (CH=), 130.8 (C_{Ar}-H), 129.6 (C_{Ar}), 128.2 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.1 ($=CH_2$), 119.5 (C_{Ar}-H), 119.2 (C_{Ar}-H), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 ($ArOCH_2$), 60.1 (CH-N), 45.4 (CH_2-N), 29.2 (CH_2), 28.4 (($CH_3)_3$), 25.5 (CH_2), 23.5 (CH_2), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 657.3288 [$M+H^+$], 657.3293 found.

tert-Butyl (*R*)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(piperidine-2-carboxamido)benzamido)benzamido)-benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure I and the deprotection of J. The product was a white solid.

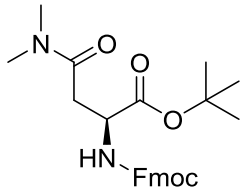
Yield: 10.6 mg (18 % over 2 steps)

^{1}H-NMR (700 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.36 (s, 1H, CONH), 8.74 (s, 1H, CONH), 8.48 (d, 1H, Ar-H, J = 8.9 Hz), 8.05 (d, 1H, Ar-H, J = 8.9 Hz), 7.98 (d, 2H, Ar-H, J = 8.7 Hz), 7.88 (d, 2H, Ar-H, J = 8.7 Hz), 7.77 (d, 2H, Ar-H, J = 8.7 Hz), 7.73 (d, 2H, Ar-H, J = 8.8 Hz), 6.14 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (dd, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.0 Hz, 10.4 Hz), 4.75 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.69 (d, 2H, $ArOCH_2$, J = 5.9 Hz), 3.47 (d, 1H, CH-N, J = 7.4 Hz), 3.10 (d, 1H, CH_2-N, J = 12.1 Hz), 2.83 – 2.77 (m, 1H, CH_2-N), 2.05 (dd, 1H, CH_2-CH, J = 3.3 Hz, 13.1 Hz), 1.86 – 1.80 (m, 1H, CH_2), 1.67 – 1.63 (m, 2H, CH_2-CH & CH_2), 1.60 (s, 9 H, $(CH_3)_3$), 1.53 – 1.49 (m, 2H, CH_2 & CH_2), 1.38 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, $CDCl_3$, 300 K): δ (ppm) = 172.3 (CONH), 165.6 (COO), 164.4 (CONH), 162.8 (CONH), 149.4 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.7 (C_{Ar}-NH), 132.2 (CH=), 130.8 (C_{Ar}-H), 129.7 (C_{Ar}), 128.3 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.2 ($=CH_2$), 119.4 (C_{Ar}-H), 119.2 (C_{Ar}-H), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 ($ArOCH_2$), 60.3 (CH-N), 45.5 (CH_2-N), 29.4 (CH_2), 28.4 (($CH_3)_3$), 25.7 (CH_2), 23.6 (CH_2), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 657.3288 [$M+H^+$], 657.3281 found.

tert-Butyl N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4,N^4-dimethyl-L-asparagine



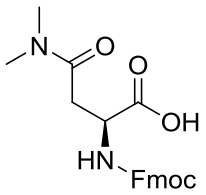
200 mg (S)-3-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-4-(*tert*-butoxy)-4-oxobutanoic acid (0.49 mmol, 1 eq) and 185 mg HATU (0.49 mmol, 1.0 eq) were added to a dry flask and further dried under high vacuum. The flask was cooled to 0 °C. 3.5 ml dry DMF and 0.09 ml DIPEA (66.8 mg, 0.52 mmol, 1.1 eq) were added under nitrogen atmosphere. The reaction was stirred for 30 minutes at 0 °C. 0.27 ml 2 M dimethylamine in THF (0.54 mmol, 1.1 eq) was added to the stirring solution. The mixture was stirred at 0 °C for the whole reaction. The reaction was controlled over TLC. After completion, 8 ml of 0.1 M HCl and 6 ml brine were added. The aqueous layer was extracted with 3 x 5 ml of ethyl acetate. The organic phases were combined and washed with 2 x 5 ml brine. The crude product was purified by chromatography (petroleum ether/ ethyl acetate) and used directly in the next reaction.

Yield: 238.6 mg (crude)

Rf: 0.13 (PE:EE 3:1)

HRMS (ESI) calculated 439.2233 [M+H$^+$], 439.2223 found.

N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4,N^4-dimethyl-L-asparagine (**53**)



238 mg *tert*-butyl N^2-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^4,N^4-dimethyl-L-asparagine (0.54 mmol, 1 eq) was dissolved in 6 ml dry DCM under nitrogen atmosphere. 0.77 ml TFA (1147 mg, 10.1 mmol, 22.0 eq) was added to the stirring mixture. The reaction was controlled over LCMS. After completion, the solvent was removed under reduced pressure. The excess of TFA was removed by coevaporation with DCM.

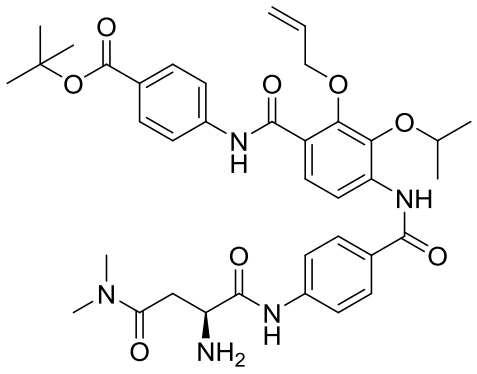
Yield: 166.3 mg (89 % over 2 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 7.89 (d, 2H, Ar-H, J = 7.5 Hz), 7.71 (d, 2H, Ar-H, J = 7.5 Hz), 7.42 (t, 2H, Ar-H, J = 7.5 Hz), 7.36 (d, 1H, NHCH, J = 8.4 Hz), 7.33 (t, 2H, Ar-H, J = 7.4 Hz), 4.42 – 4.37 (m, 1H, CHNH), 4.29 (dd, 2H, CH_2O, J = 3.0 Hz, 7.0 Hz), 4.22 (t, 1H, $CHCH_2$, J = 7.0 Hz), 2.94 (s, 3H, NCH_3), 2.82 (s, 3H, NCH_3), 2.77 (dd, 1H, CH_2, J = 7.3 Hz, 16.4 Hz), 2.72 – 2.69 (m, 1H, CH_2)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 173.2 (C=O), 169.2 (C=O), 155.8 (OCONH), 143.8 (C_{Ar}), 140.7 (C_{Ar}), 127.6 (C_{Ar}-H), 127.1 (C_{Ar}-H), 125.3 (C_{Ar}-H), 120.1 (C_{Ar}-H), 65.7 (CH_2O), 50.5 (CH/CH_3), 46.6 (CH/CH_3), 38.3 (CH/CH_3), 36.6 (CH/CH_3), 34.9 (CH/CH_3), 34.6 (CH_2)

HRMS (ESI) calculated 383.1607 [M+H$^+$], 383.1601 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-amino-4-(dimethylamino)-4-oxobutanamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure I and the deprotection of J. The product was a slightly yellow solid.

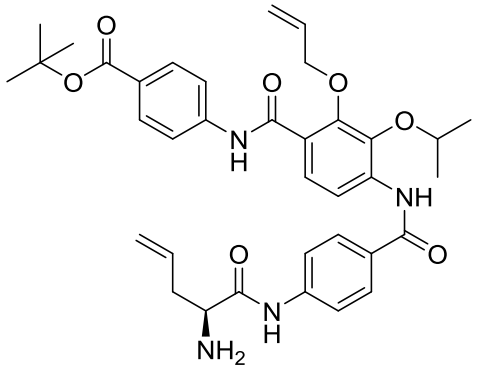
Yield: 18.0 mg (29 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.16 (s, 1H, CONH), 8.73 (s, 1H, CONH), 8.48 (d, 1H, Ar-H, J = 8.9 Hz), 8.05 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 (d, 2H, Ar-H, J = 8.8 Hz), 7.79 (d, 2H, Ar-H, J = 8.5 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 6.14 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (ddd, 1H, $=CH_2$, J = 1.4 Hz, 2.8 Hz, 17.1 Hz), 5.40 (ddd, 1H, $=CH_2$, J = 1.0 Hz, 2.1 Hz, 10.4 Hz), 4.74 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 4.69 (d, 2H, $ArOCH_2$, J = 5.9 Hz), 3.97 (s, 1H, CHN), 3.05 – 2.87 (m, 8H, NCH_3 & NCH_3 & CH_2), 1.59 (s, 9H, $(CH_3)_3$), 1.37 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 172.4 (C=O), 170.8 (C=O), 165.5 (COO), 164.5 (CONH), 162.8 (CONH), 149.4 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.8 (C_{Ar}-NH), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.6 (C_{Ar}), 128.2 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.1 ($=CH_2$), 119.4 (C_{Ar}-H), 119.1 (C_{Ar}-H), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 ($ArOCH_2$), 52.5 (CH-N), 37.5 (CH_2), 37.3 (NCH_3), 35.6 (NCH_3), 28.4 (($CH_3)_3$), 22.9 (($CH_3)_2$)

HRMS (ESI) calculated 688.3346 [M+H$^+$], 688.3345 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-aminopent-4-enamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure I and the deprotection of J. The product was a yellow to orange solid.

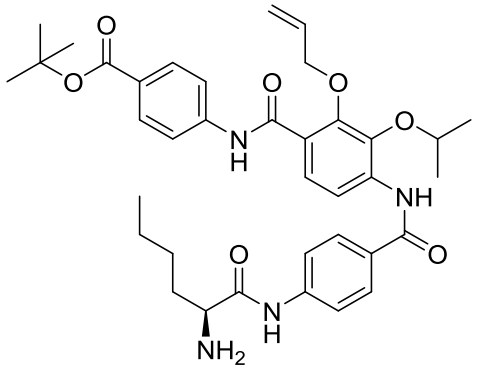
Yield: 32.2 mg (51 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.85 (s, 1H, CONH), 8.75 (s, 1H, CONH), 8.49 (d, 1H, Ar-H, J = 8.9 Hz), 8.05 (d, 1H, Ar-H, J = 8.9 Hz), 7.98 (d, 2H, Ar-H, J = 8.8 Hz), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.79 (d, 2H, Ar-H, J = 8.8 Hz), 7.73 (d, 2H, Ar-H, J = 8.8 Hz), 6.14 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.84 – 5.74 (m, 1H, CH=), 5.49 (dq, 1H, $=CH_2$, J = 1.4 Hz, 17.1 Hz), 5.40 (ddd, 1H, $=CH_2$, J = 1.0 Hz, 2.2 Hz, 10.4 Hz), 5.22 – 5.18 (m, 2H, $=CH_2$), 4.75 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.69 (d, 2H, OCH_2, J = 5.9 Hz), 3.59 (dd, 1H, CHNH, J = 3.5 Hz, 8.3 Hz), 2.75 – 2.67 (m, 1H, CH_2), 2.45 – 2.38 (m, 1H, CH_2), 1.59 (s, 9H, $(CH_3)_3$), 1.38 (dd, 6H, $(CH_3)_2$, J = 1.7 Hz, 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 173.1 (CONH), 165.5 (COO), 164.4 (CONH), 162.8 (CONH), 149.4 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 141.5 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.8 (C_{Ar}-NH), 134.0 (CH=), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.7 (C_{Ar}), 128.3 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.2 ($=CH_2$), 119.6 ($=CH_2$), 119.6 (C_{Ar}-H), 119.3 (C_{Ar}-H), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 (OCH_2), 54.5 (CHNH), 39.3 (CH_2), 28.4 (($CH_3)_3$), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 643.3132 [$M+H^+$], 643.3126 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-aminohexanamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure I and the deprotection of J. The product was a slightly yellow solid.

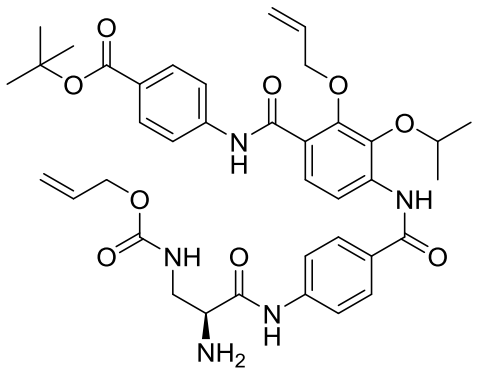
Yield: 19.1 mg (30 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.98 (s, 1H, CONH), 8.74 (s, 1H, CONH), 8.46 (d, 1H, Ar-H, J = 8.9 Hz), 8.03 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.86 (d, 2H, Ar-H, J = 8.4 Hz), 7.78 (d, 2H, Ar-H, J = 8.4 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 6.13 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (dd, 1H, $=CH_2$, J = 1.4 Hz, 17.2 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 4.74 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.69 (d, 2H, OCH_2, J = 5.8 Hz), 3.75 – 3.63 (m, 1H, CHNH), 2.03 – 1.94 (m, 1H, CH_2), 1.73 – 1.63 (m, 1H, CH_2), 1.59 (s, 9H, $(CH_3)_3$), 1.45 – 1.39 (m, 2H, CH_2), 1.39 – 1.33 (m, 8H, CH_2 & $(CH_3)_2$), 0.91 (t, 3H, CH_3, J = 7.0 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 173.0 (CONH), 165.6 (COO), 165.5 (CONH), 162.8 (CONH), 149.5 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.7 (C_{Ar}-NH), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.7 (C_{Ar}), 128.3 (C_{Ar}-H), 127.5 (C_{Ar}-H), 127.5 (C_{Ar}), 121.7 (C_{Ar}), 120.2 ($=CH_2$), 119.5 (C_{Ar}-H), 119.2 (C_{Ar}-H), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 (OCH_2), 55.6 (CHNH), 34.1 (CH_2), 28.4 ($(CH_3)_3$), 27.9 (CH_2), 22.9 ($(CH_3)_2$), 22.6 (CH_2), 14.0 (CH_3)

HRMS (ESI) calculated 659.3445 [$M+H^+$], 659.3439 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(3-(((allyloxy)carbonyl)amino)-2-aminopropanamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure I and the deprotection of J. The product was a slightly yellow solid.

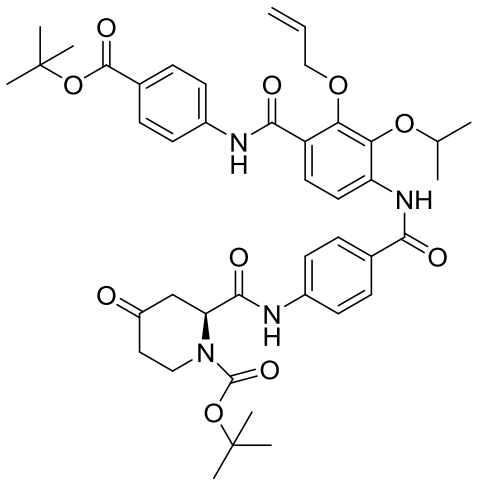
Yield: 14.4 mg (21 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.16 (s, 1H, CONH), 9.99 (br s, 1H, CONH), 8.74 (s, 1H, CONH), 8.46 (d, 1H, Ar-H, J = 8.9 Hz), 8.04 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.88 (d, 2H, Ar-H, J = 8.6 Hz), 7.77 (d, 2H, Ar-H, J = 8.6 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 6.13 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.89 (ddd, 1H, CH=, J = 5.7 Hz, 11.0 Hz, 16.1 Hz), 5.49 (dd, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 5.29 (dd, 1H, $=CH_2$, J = 1.5 Hz, 17.2 Hz), 5.21 (dd, 1H, $=CH_2$, J = 1.2 Hz, 10.4 Hz), 4.74 (quart., 1H, $CH(Me)_3$, J = 6.2 Hz), 4.69 (d, 2H, OCH_2, J = 5.9 Hz), 4.57 (d, 2H, OCH_2, J = 4.7 Hz), 3.78 – 3.72 (m, 1H, CHNH), 3.71 – 3.65 (m, 2H, CH_2NH), 1.59 (s, 9H, $(CH_3)_3$), 1.37 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 171.4 (CONH), 165.6 (COO), 164.4 (CONH), 162.8 (CONH), 157.8 (OCONH), 149.4 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 141.2 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 137.7 (C_{Ar}-NH), 132.6 (CH=), 132.3 (CH=), 130.8 (C_{Ar}-H), 130.0 (C_{Ar}), 128.3 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.5 (C_{Ar}), 121.7 (C_{Ar}), 120.2 ($=CH_2$), 119.5 (C_{Ar}-H), 119.2 (C_{Ar}-H), 118.2 ($=CH_2$), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 (OCH_2), 66.2 (OCH_2), 56.4 (CHNH), 44.7 (CH_2NH), 28.4 ($(CH_3)_3$), 23.0 ($(CH_3)_2$)

HRMS (ESI) calculated 716.3296 [$M+H^+$], 716.3289 found.

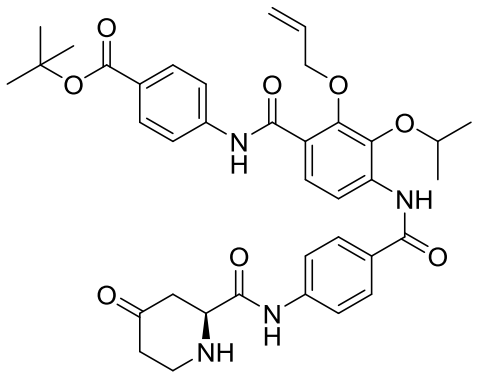
tert-Butyl (*S*)-2-((4-((3-(allyloxy)-4-((4-(*tert*-butoxycarbonyl)phenyl)carbamoyl)-2-isopropoxyphenyl)-carbamoyl)phenyl)carbamoyl)-4-oxopiperidine-1-carboxylate[4]



50 mg *tert*-butyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-isopropyloxybenzamido)benzoate (0.09 mmol, 1 eq) and 26.8 mg (S)-1-(*tert*-butoxycarbonyl)-4-oxopiperidine-2-carboxylic acid (0.11 mmol, 1.2 eq) were added to a dry flask and further dried under high vacuum. 0.64 ml dry DCM and 38 µl triethylamine (27.6 mg, 0.27 mmol, 3 eq) were added under nitrogen atmosphere and the solution was cooled down to 0 °C. 10.0 µl phosphoryl chloride (16.5 mg, 0.11 mmol, 1.2 eq) was slowly added and the mixture was kept at 0 °C. The reaction was controlled over LCMS. After completion, the reaction was quenched with 4 ml water and 1 ml 1 M HCl. The aqueous phase was extracted with 3 x 4 ml of DCM. The organic phases were combined and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate) and directly used without further purification.

Yield: 106.1 mg (crude)

tert-Butyl (*S*)-4-(2-(allyloxy)-3-isopropoxy-4-(4-(4-oxopiperidine-2-carboxamido)benzamido)benzamido)-benzoate[258]

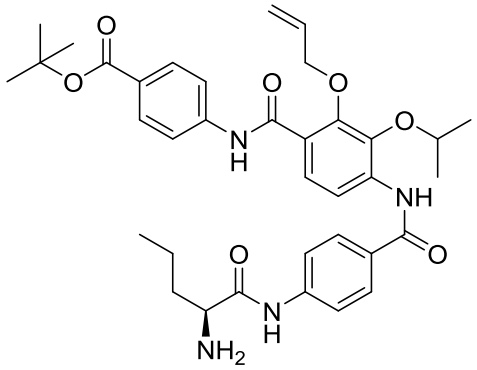


70 mg crude *tert*-butyl (S)-2-((4-((3-(allyloxy)-4-((4-(tertbutoxycarbonyl)phenyl)carbamoyl)-2-isopropoxyphenyl)carbamoyl)phenyl)carbamoyl)-4-oxopiperidine-1-carboxylate (0.09 mmol, 1 eq) was dissolved in 0.4 ml *tert*-butyl acetate (3.0 mmol, 32.6 eq) and 0.1 ml dry DCM under nitrogen atmosphere. 16 µl trifluoromethanesulfonic acid (27.1 mg, 0.18 mmol, 2.0 eq) was slowly added to the stirring solution under nitrogen atmosphere. The reaction was controlled over LCMS. After completion, the reaction was quenched with Na_2CO_3 and 4 ml water. The aqueous phase was extracted with 3 x 2 ml ethyl acetate. The solvent was removed under reduced pressure and the residue was purified by RP HPLC.

Yield: 9.5 mg (crude)

HRMS (ESI) calculated 671.3081 [$M+H^+$], 671.3075 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-aminopentanamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure J1. The product was a yellowish solid.

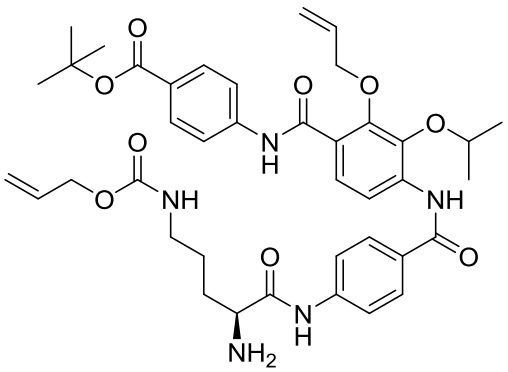
Yield: 26.8 mg (42 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.29 (br s, 1H, CONH), 10.15 (s, 1H, CONH), 8.72 (s, 1H, CONH), 8.37 (d, 1H, Ar-H, J = 8.1 Hz), 7.97 – 7.92 (m, 3H, Ar-H), 7.82 – 7.73 (m, 4H, Ar-H), 7.69 (d, 2H, Ar-H, J = 8.6 Hz), 6.10 (dq, 1H, CH=, J = 5.8 Hz, 10.9 Hz), 5.46 (d, 1H, $=CH_2$, J = 17.1 Hz), 5.38 (d, 1H, $=CH_2$, J = 10.4 Hz), 4.74 – 4.61 (m, 3H, $CH(Me)_2$ & OCH_2), 4.20 – 4.02 (m, 1H, $CHNH_2$), 2.02 – 1.90 (m, 1H, CH_2CHN), 1.86 – 1.74 (m, 1H, CH_2CHN), 1.58 (s, 9H, $(CH_3)_3$), 1.52 – 1.40 (m, 2H, CH_2CH_3), 1.33 (d, 6H, $(CH_3)_2$), J = 5.6 Hz), 0.93 – 0.86 (m, 3H, CH_3)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 170.6 (C=O), 165.3 (C=O), 164.5 (C=O), 162.7 (C=O), 149.3 (C_{Ar}), 142.0 (C_{Ar}), 141.4 (C_{Ar}), 139.1 (C_{Ar}), 137.4 (C_{Ar}), 132.1 (CH=), 130.6 (C_{Ar}-H), 129.6 (C_{Ar}), 128.0 (C_{Ar}-H), 127.4 (C_{Ar}), 127.2 (C_{Ar}-H), 121.6 (C_{Ar}), 120.0 ($=CH_2$), 119.7 (C_{Ar}-H), 119.1 (C_{Ar}-H), 115.7 (C_{Ar}-H), 80.8 ($C(Me)_3$), 76.7 ($CH(Me)_2$), 74.9 (OCH_2), 54.8 ($CHNH_2$), 35.1 (CH_2CHN), 28.2 (($CH_3)_3$), 22.7 (($CH_3)_2$), 18.5 (CH_2CH_3), 13.7 (CH_3)

HRMS (ESI) calculated 645.3288 [$M+H^+$], 645.3284 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(5-(((allyloxy)carbonyl)amino)-2-aminopentanamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure J2. The product was a yellow solid.

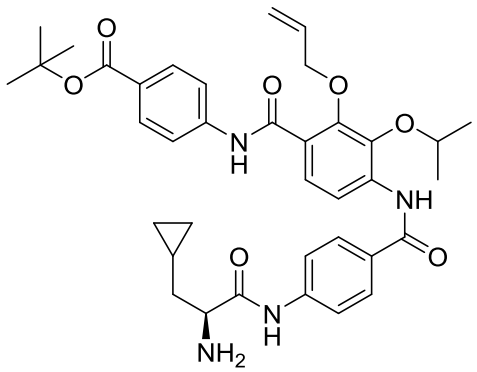
Yield: 13.8 mg (19 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.14 (br s, 1H, CONH), 8.72 (s, 1H, CONH), 8.39 (d, 1H, Ar-H, J = 8.7 Hz), 7.96 (m, 3H, Ar-H), 7.84 – 7.74 (m, 4H, Ar-H), 7.70 (d, 2H, Ar-H, J = 8.7 Hz), 6.16 – 6.07 (m, 1H, CH=), 5.85 (ddd, 1H, CH=, J = 5.5 Hz, 10.7 Hz, 22.5 Hz), 5.47 (dd, 1H, $=CH_2$, J = 1.1 Hz, 17.1 Hz), 5.39 (d, 1H, $=CH_2$, J = 10.4 Hz), 5.25 (d, 1H, $=CH_2$, J = 17.2 Hz), 5.15 (d, 1H, $=CH_2$, J = 10.3 Hz), 4.74 – 4.64 (m. 3H, $CH(Me)_2$ & OCH_2), 4.53 (d, 2H, OCH_2, J = 5.3 Hz), 4.25 – 4.15 (m, 1H, $CHNH_2$), 3.36 – 3.24 (m, 1H, CH_2NH), 3.22 – 3.09 (m, 1H, CH_2NH), 2.12 – 1.99 (m, 1H, CH_2CH), 1.98 – 1.86 (m, 1H, CH_2CH), 1.82 – 1.65 (m, 2H, CH_2), 1.59 (s, 9H, $(CH_3)_3$), 1.35 (d, 6H, $(CH_3)_2$), J = 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 165.5 (C=O), 164.5 (C=O), 162.8 (C=O), 157.4 (NHCOO), 149.5 (C_{Ar}), 142.2 (C_{Ar}-NH), 141.5 (C_{Ar}-NH), 139.3 (C_{Ar}), 137.5 (C_{Ar}-NH), 132.7 (CH=), 132.3 (CH=), 130.8 (C_{Ar}-H), 130.0 (C_{Ar}), 128.2 (C_{Ar}-H), 127.5 (C_{Ar}), 127.4 (C_{Ar}-H), 121.8 (C_{Ar}), 120.1 ($=CH_2$), 119.8 (C_{Ar}-H), 119.2 (C_{Ar}-H), 117.9 ($=CH_2$), 115.8 (C_{Ar}-H), 81.0 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 (OCH_2), 66.0 (OCH_2), 54.1 ($CHNH_2$), 53.6 (CH_2), 39.9 (CH_2), 28.4 (($CH_3)_3$), 26.1 (CH_2), 22.9 (($CH_3)_2$)

HRMS (ESI) calculated 744.3609 [$M+H^+$], 744.3603 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-amino-3-cyclopropylpropanamido)benzamido)-3-isopropoxybenzamido)benzoate (**55**)



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure J1. The product was a yellow solid.

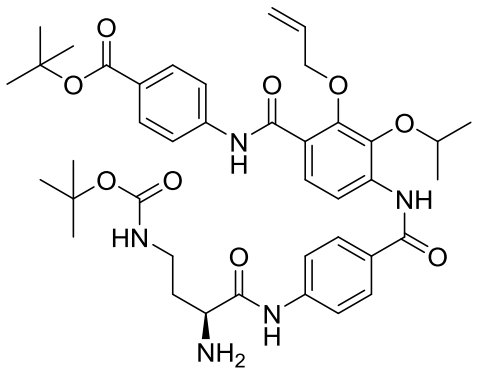
Yield: 43.3 mg (67 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.16 (s, 1H, CONH), 9.97 (br s, 1H, CONH), 8.74 (s, 1H, CONH), 8.46 (d, 1H, Ar-H, J = 8.9 Hz), 8.03 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.87 (d, 2H, Ar-H, J = 8.5 Hz), 7.78 (d, 2H, Ar-H, J = 8.3 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 6.13 (ddt, 1H, CH=, J = 5.9 Hz, 10.5 Hz, 16.3 Hz), 5.48 (dd, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.39 (dd, 1H, $=CH_2$, J = 1.0 Hz, 10.4 Hz), 4.74 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.68 (d, 2H, OCH_2, J = 5.9 Hz), 3.78 – 3.66 (m, 1H, CH), 1.88 – 1.80 (m, 1H, CH_2), 1.67 – 1.61 (m, 1H, CH_2), 1.59 (s, 9H, $(CH_3)_3$), 1.37 (d, 6H, $(CH_3)_2$, J = 6.1 Hz), 0.83 – 0.74 (m, 1H, $CH_{cyclopr}$), 0.58 – 0.44 (m, 2H, CH_2-CH_2), 0.20 – 0.10 (m, CH_2-CH_2)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 173.2 (C=O), 165.5 (C=O), 164.5 (C=O), 162.8 (C=O), 149.4 (C_{Ar}), 142.3 (C_{Ar}), 141.6 (C_{Ar}), 139.1 (C_{Ar}), 137.7 (C_{Ar}), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.6 (C_{Ar}), 128.2 (C_{Ar}-H), 127.5 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.1 ($=CH_2$), 119.4 (C_{Ar}-H), 119.1 (C_{Ar}-H), 115.7 (C_{Ar}-H), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.0 (OCH_2), 56.3 (CHN), 39.1 (CH_2), 28.4 ($(CH_3)_3$), 22.9 ($(CH_3)_2$), 7.6 ($CH_{cyclopr}$), 4.9 (CH_2), 3.9 (CH_2)

HRMS (ESI) calculated 657.3288 [$M+H^+$], 657.3283 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-amino-4-((*tert*-butoxycarbonyl)amino)butanamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure J1. The product was a yellow solid.

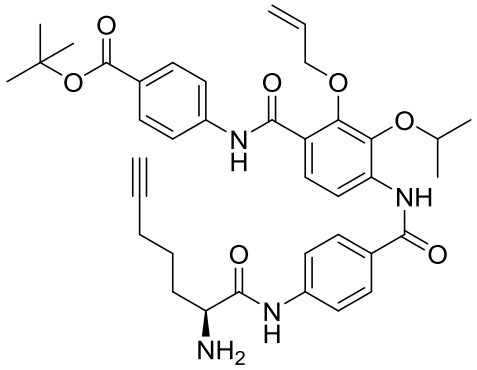
Yield: 37.7 mg (52 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.16 (s, 1H, CONH), 10.11 (br s, 1H, CONH), 8.74 (s, 1H, CONH), 8.48 (d, 1H, Ar-H, J = 8.9 Hz), 8.05 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.8 Hz), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.80 (d, 2H, Ar-H, J = 8.6 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 6.13 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (dd, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.0 Hz, 10.4 Hz), 4.89 (t, 1H, $NHCH_2$, J = 6.3 Hz), 4.74 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 4.69 (d, 2H, OCH_2, J = 5.9 Hz), 3.60 – 3.48 (m, 1H, $CHNH_2$), 3.37 – 3.29 (d, 2H, CH_2, J = 6.0 Hz), 1.83 – 1.74 (m, 1H, CH_2), 1.59 (s, 9H, $(CH_3)_3$), 1.42 (s, 9H, $(CH_3)_3$), 1.38 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 173.7 (C=O), 165.5 (C=O), 164.4 (C=O), 162.8 (C=O), 157.0 (NHCOO), 149.4 (C_{Ar}), 142.3 (C_{Ar}), 141.7 (C_{Ar}), 139.1 (C_{Ar}), 137.8 (C_{Ar}), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.6 (C_{Ar}), 128.2 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.1 ($=CH_2$), 119.4 (C_{Ar}-H), 119.1 (C_{Ar}-H), 115.8 (C_{Ar}-H), 80.9 ($C(Me)_3$), 80.0 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.1 (OCH_2), 53.2 (CHN), 37.1 (CH_2), 36.1 (CH_2), 28.5 (($CH_3)_3$), 28.4 (($CH_3)_3$), 22.9 (($CH_3)_2$)

HRMS (ESI) calculated 746.3765 [$M+H^+$], 746.3759 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-aminohept-6-ynamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure J1. The product was a yellow solid.

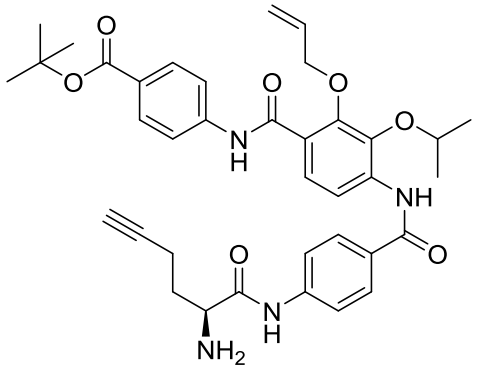
Yield: 41.2 mg (63 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.88 (br s, 1H, CONH), 8.74 (s, 1H, CONH), 8.46 (d, 1H, Ar-H, J = 8.9 Hz), 8.04 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.87 (d, 2H, Ar-H, J = 8.7 Hz), 7.77 (d, 2H, Ar-H, J = 8.6 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 6.13 (ddt, 1H, CH=, 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (dd, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 4.74 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.69 (d, 2H, OCH_2, J = 5.9 Hz), 3.66 – 3.59 (m, 1H, CHN), 2.29 – 2.24 (m, 2H, $CH_2C{\equiv}$), 2.13 – 2.04 (m, 1H, CH_2CH), 1.98 (t, 1H, ≡CH, J = 2.6 Hz), 1.82 – 1.73 (m, 1H, CH_2CH), 1.73 – 1.65 (m, 2H, CH_2), 1.59 (s, 9H, $(CH_3)_3$), 1.37 (dd, 6H, $(CH_3)_2$, J = 0.9 Hz, 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 173.0 (C=O), 165.5 (C=O), 164.5 (C=O), 162.8 (C=O), 149.4 (C_{Ar}), 142.3 (C_{Ar}), 141.5 (C_{Ar}), 139.1 (C_{Ar}), 137.7 (C_{Ar}), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.7 (C_{Ar}), 128.3 (C_{Ar}-H), 127.5 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.1 ($=CH_2$), 119.4 (C_{Ar}-H), 119.2 (C_{Ar}-H), 115.8 (C_{Ar}-H), 83.7 (C≡), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.0 (OCH_2), 69.3 (≡CH), 55.2 (CHN), 33.9 (CH_2), 28.4 (($CH_3)_3$), 24.8 (CH_2), 22.9 (($CH_3)_2$), 18.4 (CH_2)

HRMS (ESI) calculated 669.3288 [$M+H^+$], 669.3283 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-aminohex-5-ynamido)benzamido)-3-isopropoxybenzamido)benzoate



Fragment CDE (0.09 mmol) was coupled with the central amino acid using procedure J1. The product was a yellow solid.

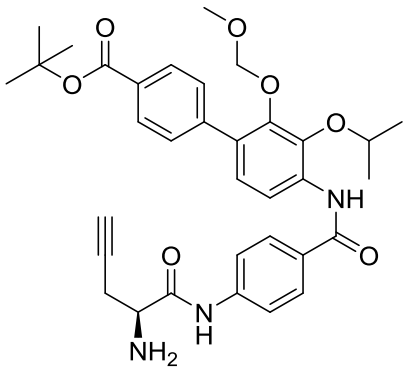
Yield: 34.9 mg (56 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.17 (s, 1H, CONH), 9.88 (br s, 1H, CONH), 8.74 (s, 1H, CONH), 8.46 (d, 1H, Ar-H, J = 8.9 Hz), 8.03 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.87 (d, 2H, Ar-H, J = 8.8 Hz), 7.77 (d, 2H, Ar-H, J = 8.7 Hz), 7.72 (d, 2H, Ar-H, J = 8.8 Hz), 6.13 (ddt, 1H, CH=, J = 5.9 Hz, 10.4 Hz, 16.3 Hz), 5.49 (dd, 1H, $=CH_2$, J = 1.3 Hz, 17.1 Hz), 5.40 (dd, 1H, $=CH_2$, J = 1.1 Hz, 10.4 Hz), 4.74 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.69 (d, 2H, OCH_2, J = 5.9 Hz), 3.71 (dd, 1H, CHN, J = 4.4 Hz, 8.4 Hz), 2.44 – 2.39 (m, 2H, $CH_2C\equiv$), 2.26 (ddd, 1H, CH_2CH, J = 4.5 Hz, 7.3 Hz, 11.8 Hz), 2.03 (t, 1H, ≡CH, J = 2.6 Hz), 1.81 (td, 1H, CH_2CH, J = 6.5 Hz, 14.8 Hz), 1.59 (s, 9H, $(CH_3)_3$), 1.37 (dd, 6H, $(CH_3)_2$, J = 1.2 Hz, 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 172.8 (C=O), 165.5 (C=O), 164.4 (C=O), 162.8 (C=O), 149.4 (C_{Ar}), 142.3 (C_{Ar}), 141.5 (C_{Ar}), 139.1 (C_{Ar}), 137.7 (C_{Ar}), 132.3 (CH=), 130.8 (C_{Ar}-H), 129.7 (C_{Ar}), 128.2 (C_{Ar}-H), 127.5 (C_{Ar}-H), 127.4 (C_{Ar}), 121.6 (C_{Ar}), 120.1 ($=CH_2$), 119.4 (C_{Ar}-H), 119.1 (C_{Ar}-H), 115.7 (C_{Ar}-H), 83.1 (C≡), 80.9 ($C(Me)_3$), 76.9 ($CH(Me)_2$), 75.0 (OCH_2), 70.1 (≡CH), 55.1 (CHN), 33.0 (CH_2), 28.4 (($CH_3)_3$), 22.9 (($CH_3)_2$), 15.7 (CH_2)

HRMS (ESI) calculated 655.3132 [$M+H^+$], 655.3126 found.

tert-Butyl (*S*)-4'-(4-(2-aminopent-4-ynamido)benzamido)-3'-isopropoxy-2'-(methoxymethoxy)-[1,1'-biphenyl]-4-carboxylate



Fragment CDE (0.11 mmol) was coupled with the central amino acid using procedure J1. The product was a slightly yellow solid.

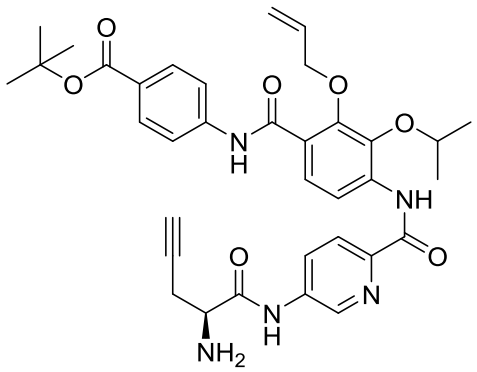
Yield: 42.8 mg (63 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 9.79 (s, 1H, CONH), 8.69 (s, 1H, CONH), 8.38 (d, 1H, Ar-H, J = 8.6 Hz), 8.02 (d, 2H, Ar-H, J = 8.5 Hz), 7.91 (d, 2H, Ar-H, J = 8.7 Hz), 7.78 (d, 2H, Ar-H, J = 8.7 Hz), 7.61 (d, 2H, Ar-H, J = 8.5 Hz), 7.14 (d, 1H, Ar-H, J = 8.6 Hz), 4.86 (s, 2H, OCH_2O), 4.78 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.67 (dd, 1H, CHN, J = 4.5 Hz, 7.4 Hz), 3.03 (s, 3H, OCH_3), 2.80 (dddd, 2H, CH_2, J = 2.7 Hz, 5.9 Hz, 10.1 Hz, 17.0 Hz), 2.09 (t, 1H, ≡CH, J = 2.6 Hz), 1.62 (s, 9H, $(CH_3)_3$), 1.38 (dd, 6H, $(CH_3)_2$, J = 1.6 Hz, 6.2 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 171.4 (CONH), 165.9 (COO), 164.3 (CONH), 147.0 (C_{Ar}), 142.7 (C_{Ar}), 141.0 (C_{Ar}), 139.9 (C_{Ar}), 133.7 (C_{Ar}), 131.4 (C_{Ar}), 130.6 (C_{Ar}), 130.4 (C_{Ar}) 129.6 (C_{Ar}-H), 129.4 (C_{Ar}-H), 128.2 (C_{Ar}-H) 125.7 (C_{Ar}-H), 119.4 (C_{Ar}-H), 115.8 (C_{Ar}-H), 99.0 (OCH_2O), 81.2 ($C(Me)_3$), 80.1 (C≡), 76.3 ($CH(Me)_2$), 71.8 (≡CH), 57.5 (OCH_3), 54.1 (CHN), 28.4 ($(CH_3)_3$), 24.9 (CH_2), 23.0 ($(CH_3)_2$)

HRMS (ESI) calculated 602.2866 [$M+H^+$], 602.2863 found.

tert-Butyl (*S*)-4-(2-(allyloxy)-4-(5-(2-aminopent-4-ynamido)picolinamido)-3-isopropoxybenzamido)-benzoate (**56**)



Fragment CDE (0.18 mmol) was coupled with the central amino acid using procedure K and the deprotection of J. The product was a off-white solid.

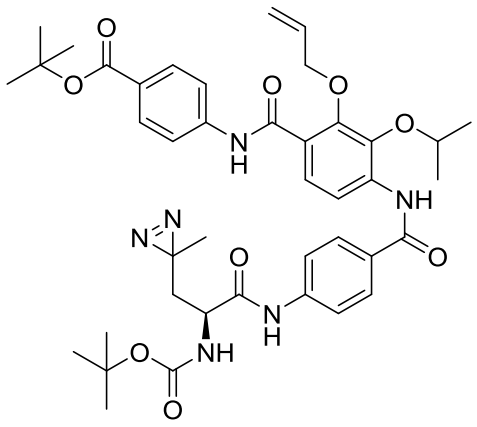
Yield: 99.5 mg (71 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 10.72 (d, 1H, CONH, J = 2.6 Hz), 10.23 (s, 1H, CONH), 9.96 (br s, 1H, CONH), 8.91 (s, 1H, Ar-H), 8.50 – 8.46 (m, 1H, Ar-H), 8.23 – 8.19 (m, 1H, Ar-H), 8.18 – 8.14 (m, 1H, Ar-H), 8.03 – 7.99 (m, 1H, Ar-H), 7.97 – 7.93 (m, 2H, Ar-H), 7.73 – 7.69 (m, 2H, Ar-H), 6.19 – 6.10 (m, 1H, CH=), 5.52 – 5.46 (m, 1H, $=CH_2$), 5.41 – 5.37 (m, 1H, $=CH_2$), 4.73 (d, 2H, OCH_2, J = 5.9 Hz), 4.68 – 4.61 (m, 1H, $CH(Me)_2$), 3.71 – 3.66 (m, 1H, CHNH), 2.83 – 2.73 (m, 2H, CH_2), 2.10 – 2.08 (m, 1H, ≡CH), 1.58 – 1.56 (m, 9H, $(CH_3)_3$), 1.42 – 1.38 (m, 6H, $(CH_3)_2$)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 171.8 (CONH), 165.5 (COO), 162.9 (CONH), 161.9 (CONH), 149.8 (C_{Ar}-O), 145.0 (C_{Ar}), 142.3 (C_{Ar}-NH), 139.5 (C_{Ar}-O), 139.4 (C_{Ar}-H), 137.7 (C_{Ar}-NH), 137.2 (C_{Ar}-NH), 132.4 (CH=), 130.7 (C_{Ar}-H), 127.4 (C_{Ar}-H), 127.3 (C_{Ar}), 127.0 (C_{Ar}-H), 123.2 (C_{Ar}-H), 121.3 (C_{Ar}), 120.0 ($=CH_2$), 119.1 (C_{Ar}-H), 115.3 (C_{Ar}-H), 80.9 ($C(Me)_3$), 79.6 (C≡), 76.6 ($CH(Me)_2$), 74.9 (OCH_2), 72.0 (≡CH), 53.8 (CHNH), 28.3 ($(CH_3)_3$), 24.6 (CH_2), 22.7 ($(CH_3)_2$)

HRMS (ESI) calculated 642.2928 [$M+H^+$], 642.2923 found.

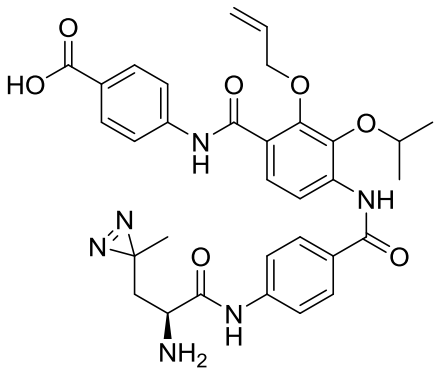
tert-Butyl (*S*)-4-(2-(allyloxy)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-3-(3-methyl-3*H*-diazirin-3-yl)propanamido)benzamido)-3-isopropoxybenzamido)benzoate[228]



33.0 mg (S)-2-((*tert*-butoxycarbonyl)amino)-3-(3-methyl-3H-diazirin-3-yl)propanoic acid (0.14 mmol, 1.5 eq) and 50 mg *tert*-butyl 4-(2-(allyloxy)-4-(4-aminobenzamido)-3-isopropyloxybenzamido)benzoate (0.09 mmol, 1.0 eq) were added to a dry flask and were further dried under high vacuum. 0.15 ml dry DCM was added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 34.0 mg EEDQ (0.14 mmol, 1.5 eq) dissolved in 0.15 ml dry DCM was added to the stirring solution. The reaction was stirred at 0 °C for 30 minutes and slowly allowed to reach room temperature afterwards. The reaction was stirred overnight and controlled over LCMS. The reaction was quenched with 2 ml 1 M HCl and 6 ml brine and extracted with 3 x 3 ml DCM. The combined organic phases were washed with brine and the solvent was removed under reduced pressure. The crude product was used in further reactions.

Yield: 75.9 mg (crude)

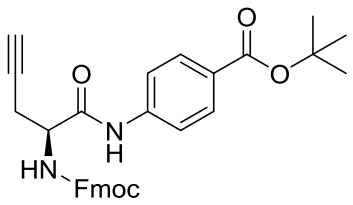
(*S*)-4-(2-(allyloxy)-4-(4-(2-amino-3-(3-methyl-3*H*-diazirin-3-yl)propanamido)benzamido)-3-isopropoxybenzamido)benzoic acid



70 mg crude *tert*-butyl (S)-4-(2-(allyloxy)-4-(4-(2-((*tert*-butoxycarbonyl)amino)-3-(3-methyl-3H-diazirin-3-yl)propanamido)benzamido)-3-isopropoxybenzamido)benzoate (0.09 mmol, 1 eq) was dissolved in 0.5 ml dry DCM under nitrogen atmosphere. 0.3 ml TFA (444.0 mg, 3.9 mmol, 43 eq) was added at 0 °C under nitrogen atmosphere to the stirring mixture. After completion, the solvent was evaporated under reduced pressure and coevaporated with DCM 3 times. The crude product was purified via RP HPLC.

Yield: 23.6 mg (crude)

tert-Butyl (*S*)-4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynamido)benzoate (**79**)[210]



500 mg (S)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynoic acid (1.5 mmol, 1.4 eq) and 200 mg *tert*-butyl 4-aminobenzoate (1.0 mmol, 1.0 eq) were added to a dry flask and were further dried at high vacuum. 0.25 ml dry pyridine (246 mg, 3.1 mmol, 3.0 eq) and 10 ml dry ethyl acetate were added under nitrogen atmosphere. The reaction mixture was cooled down to 0 °C. 1.4 ml T3P solution (50 wt % in ethyl acetate, 2.4 mmol, 2.3 eq) was added very slowly while keeping the temperature below 0 °C. The reaction was stirred at 0 °C for 4 h and controlled over LCMS. After completion, the reaction was quenched with 10 ml 1 M HCl and 30 ml brine and extracted with 3 x 10 ml ethyl acetate. The combined organic phases were washed with brine and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/ ethyl acetate). The product was a colorless glass.

Yield: 519.3 mg (98 %)

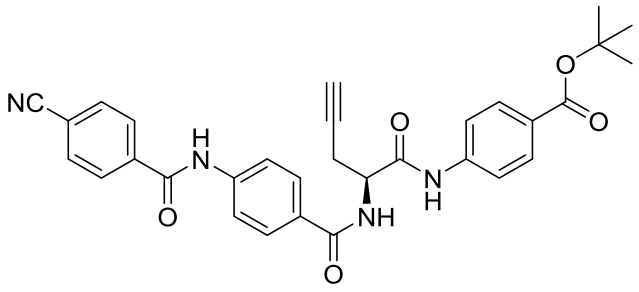
Rf: 0.11 (PE:EE 5:1)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 8.30 (br s, 1H, CONH), 7.95 (d, 2H, Ar-H, J = 8.8 Hz), 7.77 (d, 2H, Ar-H, J = 7.6 Hz), 7.58 (d, 2H, Ar-H, J = 7.5 Hz), 7.55 (d, 2H, Ar-H, J = 8.8 Hz), 7.40 (t, 2H, Ar-H, J = 7.5 Hz), 7.30 (t, 2H, Ar-H, J = 7.3 Hz), 5.57 (br s, 1H, NHCH), 4.54 – 4.43 (m, 3H, CH_2O & CHNH), 4.24 (t, 1H, CH, J = 6.7 Hz), 2.95 – 2.81 (m, 1H, CH_2), 2.74 – 2.65 (m, 1H, CH_2), 2.15 (t, 1H, ≡CH, J = 2.6 Hz), 1.59 (s, 9H, $(CH_3)_3$)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 168.1 (C=O), 165.3 (C=O), 143.7 (C=O), 143.6 (C_{Ar}), 141.5 (C_{Ar}), 140.9 (C_{Ar}), 130.8 (C_{Ar}-H), 128.3 (C_{Ar}), 128.0 (C_{Ar}-H), 127.3 (C_{Ar}-H), 125.1 (C_{Ar}-H), 120.3 (C_{Ar}-H), 119.2 (C_{Ar}-H), 81.2 ($C(Me)_3$), 72.5 (≡CH), 67.6 (CH_2O), 54.2 (CHNH), 47.3 (CH), 28.4 ($(CH_3)_3$), 22.1 (CH_2)

HRMS (ESI) calculated 511.2233 [M+H$^+$], 511.2228 found.

tert-Butyl (*S*)-4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzoate[5]

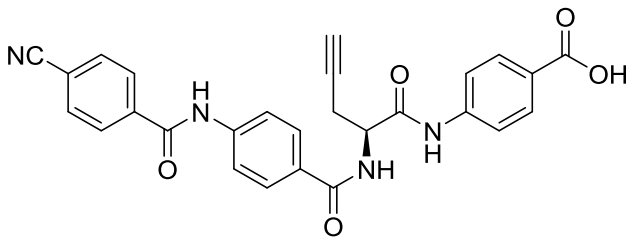


510 mg *tert*-butyl (S)-4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynamido)benzoate (1.0 mmol, 1 eq) was dissolved in 1 ml acetonitrile and 1.5 ml diethylamine (1061 mg, 14.5 mmol, 14.5 eq) at 0 °C and stirred for 1 hour. The solvent was evaporated under reduced pressure. 1 ml acetonitrile was added to the residue and the solvent was removed again. This was repeated twice. The product was dried under high vacuum overnight. The residue was stored under nitrogen atmosphere [1].

300 mg 4-(4-cyanobenzamido)benzoic acid (1.1 mmol, 1.1 eq) and 430 mg HATU (1.1 mmol, 1.1 eq) were added to [1] in a dry flask and further dried under high vacuum. 3.0 ml dry DMF and 0.35 ml dry DIPEA (260 mg, 2.0 eq) were added under nitrogen atmosphere and stirred at 0 °C. The reaction was controlled over LCMS. After completion, the reaction was quenched with 3 ml of 1 M HCl and 15 ml brine. The inorganic layer was extracted with 4 x 6 ml of ethyl acetate. The organic phases were combined and washed with brine. The crude product was purified by chromatography (petroleum ether/ ethyl acetate). The product was direcly used in the next reaction.

Yield: 515.9 mg (crude)

(*S*)-4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzoic acid (**80**)[5]



516 mg crude *tert*-butyl (S)-4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzoate (0.96 mmol, 1 eq) was added to a dry flask and further dried under high vacuum. 6.4 ml dry DCM was added under nitrogen atmosphere and the solution was cooled down to 0 °C. 3.9 ml trifluoroacetic acid (5.77 g, 50.6 mmol, 53 eq) was added under nitrogen atmosphere. The solution was stirred for 3 hours at 0 °C and controlled over LCMS. After completion, the solvent was removed under reduced pressure. The residue was dissolved in DCM and the solvent was removed again. This was repeated twice. The crude product was purified by RP chromatography. The product was a white solid.

Yield: 334.0 mg (70 % over 2 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.72 (br s, 1H, COOH), 10.70 (s, 1H, CONH), 10.55 (s, 1H, CONH), 8.75 (d, 1H, NHCH, J = 7.5 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.04 (d, 2H, Ar-H, J = 8.5 Hz), 7.95 (d, 2H, Ar-H, J = 8.8 Hz), 7.92 – 7.87 (m, 4H, Ar-H), 7.75 (d, 2H, Ar-H, J = 8.8 Hz), 4.78 (dd, 1H, CHNH, J = 7.7 Hz, 14.7 Hz), 2.92 (t, 1H, ≡H, J = 2.6 Hz), 2.82 – 2.71 (dddd, 2H, CH_2, J = 2.6 Hz, 7.4 Hz, 11.1 Hz, 16.8 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.7 (CONH), 166.9 (COOH), 165.9 (CONH), 164.5 (CONH), 142.8 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 138.7 (C_{Ar}), 132.5 (C_{Ar}-H), 130.4 (C_{Ar}-H), 128.9 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 125.4 (C_{Ar}), 119.5 (C_{Ar}-H), 118.7 (C_{Ar}-H), 118.3 (CN), 114.1 (C_{Ar}), 80.6 (C≡), 73.2 (≡CH), 53.5 (CHNH), 21.4 (CH_2)

HRMS (ESI) calculated 481.1512 [M+H$^+$], 481.1507 found.

5.2.2.4 Amide coupling with fragment AB and global deprotection

The amide coupling between the central amino acid and fragment AB as well as the following global deprotection steps were standardized. The procedures were adapted from established syntheses[5],[182]. Adapted experimental procedures from the literature are quoted in the listing of the general procedures. Citations at the end of the molecule name were added, if methods other than the general procedures were applied.

5.2.2.4.1 General procedures – Amide coupling and global deprotection

The amide coupling with the central amino acid was either carried out in one pot after Fmoc deprotection or with the free amino group. The allyl deprotection was carried out with phenylsilane or aniline as scavenger. Triisopropylsilane was used as a scavenger, if a simultaneous deprotection of Trt was required. The four general procedures were:

L.) Fmoc deprotection and subsequent amide coupling[5].
M.) Amide coupling of free amines[182].
N.) Allyl deprotection with phenylsilane [N1] or aniline [N2] as scavenger[5],[182].
O.) Deprotection with TFA [O1] without or with triisopropylsilane [O2][5].

L.) Fmoc deprotection and amide coupling of Fmoc protected central amino acids with fragment AB

68.0 µmol of the desired Fmoc protected central amino acid (1 eq) was dissolved in 0.4 ml acetonitrile and 105 µl diethylamine (1 mmol, 15.1 eq) at 0 °C and stirred for 1 hour. The solvent was evaporated under reduced pressure. 1 ml acetonitrile was added to the residue and the solvent was removed under reduced pressure again. This was repeated twice. The crude residue was dried under high vacuum overnight [1].

82.6 µmol of the desired fragment AB (1.2 eq) and 78.9 µmol HATU (1.2 eq) were added to a separate flask and dried under high vacuum. 0.5 ml dry DMF and 38 µl DIPEA (3 eq) were added under nitrogen atmosphere and the reaction was stirred for 30 minutes. The solution was added to the residue [1] and stirred at 0 °C. The reaction was controlled over LCMS. After completion, the reaction was quenched with 8 ml of 0.1 M HCl and 4 ml brine. The inorganic layer was extracted with 3 x 6 ml of ethyl acetate. The organic phases were combined and washed with 2 x 5 ml brine. The solvent was removed under reduced pressure. The crude product was used without further purification.

M.) Amide coupling of the fragment AB with the central amino acid

65.5 µmol of the desired central amino acid (1 eq), 79.0 µmol HATU (1.2 eq) and 79.0 µmol of the desired fragment AB (1.2 eq) were added to a dry flask and further dried under high vacuum. 0.4 ml dry DMF and 35 µl DIPEA (3.1 eq) were added under nitrogen atmosphere at 0 °C. The solution was stirred at 0 °C and controlled over LCMS. After completion, the reaction was quenched with 6 ml of 0.1 M HCl and 10 ml brine. The inorganic layer was extracted with 3 x 4 ml of ethyl acetate. The organic phases were combined and washed with 2 x 4 ml brine. The crude product was used without further purification.

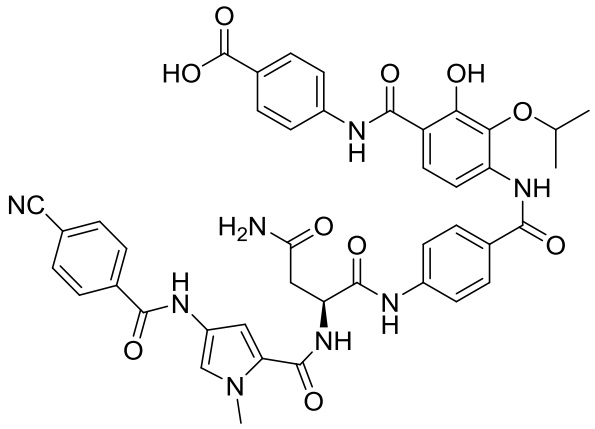
N.) Allyl deprotection

65.5 µmol of the desired allyl protected cystobactamid and 198 µmol phenylsilane [N1] or aniline [N2] (3.0 eq) were added to a dry flask under nitrogen atmosphere. 1.2 ml dry THF and 6.5 µmol Tetrakis(triphenylphosphine)palladium(0) (0.1 eq) were added and the mixture was stirred for 3 hours at room temperature. The reaction was controlled over LCMS. After completion, the solvent was removed under reduced pressure. 3 ml 0.1 M HCl and 10 ml brine were added to the residue and extracted with 3 x 4 ml ethyl acetate. The combined organic phases purified by flash chromatography with petroleum ether and ethyl acetate mixed with 2 % acetic acid.

O.) Deprotection with TFA

65.5 µmol of the desired *tert*-butyl protected cystobactamid (1 eq) was added to a dry flask and further dried under high vacuum. 0.5 ml dry DCM was added under nitrogen atmosphere and the solution was cooled down to 0 °C. 0.24 ml of trifluoracetic acid [O1] (3.1 mmol, 54 eq) or 0.24 ml of trifluoracetic acid (3.1 mmol, 53.9 eq) and 40 µl triisopropylsilane (195 µmol, 3 eq) [O2] were added under nitrogen atmosphere. The solution was stirred for 3 hours at 0 °C and controlled over LCMS. After completion, the solvent was removed under reduced pressure. The residue was coevaporated with DCM twice. The crude product was purified by reversed phase HPLC.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-1-methyl-1H-pyrrol-2-carboxamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**20**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a slightly yellow solid.

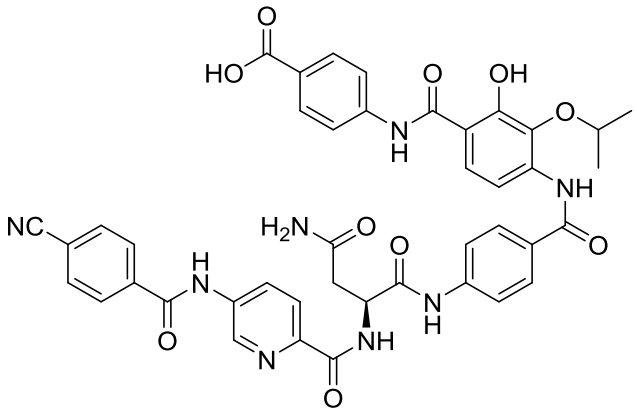
Yield: 4.3 mg (29 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.61 (s, 1H, CONH), 10.40 (s, 1H, CONH), 9.13 (br s, 1H, CONH), 8.33 (d, 1H, NHCH, J = 7.5 Hz), 8.11 (d, 2H, Ar-H, J = 8.4 Hz), 8.02 (d, 2H, Ar-H, J = 8.5 Hz), 7.93 – 7.88 (m, 4H, Ar-H), 7.81 (d, 4H, Ar-H, J = 8.7 Hz), 7.70 – 7.60 (m, 1H, Ar-H), 7.40 (s, 1H, $CONH_2$), 7.36 (d, 1H, Ar-H, J = 1.8 Hz), 7.06 (d, 1H, Ar-H, J = 1.8 Hz), 7.00 (s, 1H, $CONH_2$), 4.86 – 4.75 m, 2H, CHNH & $CH(Me)_2$), 3.84 (s, 3H, NCH_3), 2.70 – 2.62 (m, 2H, CH_2), 1.23 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.5 ($CONH_2$), 170.9 (CONH), 167.9 (CONH), 167.1 (COO), 163.7 (CONH), 162.0 (CONH), 161.1 (CONH), 142.3 (C_{Ar}-NH), 138.5 (C_{Ar}), 137.0 (C_{Ar}), 132.5 (C_{Ar}-H), 130.3 (C_{Ar}-H), 128.8 (C_{Ar}), 128.2 (C_{Ar}-H), 128.0 (C_{Ar}-H), 123.3 (C_{Ar}-H), 122.6 (C_{Ar}), 121.8 (C_{Ar}), 119.4 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.7 (C_{Ar}-H), 118.4 (CN), 113.5 (C_{Ar}), 105.1 (C_{Ar}-H), 51.1 (CHNH), 36.2 (CH_2), 22.5 ((CH_3)$_2$)

HRMS (ESI) calculated 815.2789 [M+H$^+$], 815.2784 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-picolinamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**21**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a slightly yellow solid.

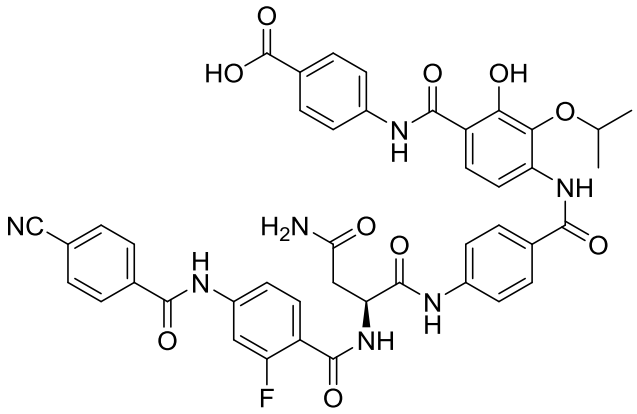
Yield: 10.6 mg (70 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.98 (s, 1H, CONH), 10.44 (s, 1H, CONH), 9.26 (s, 1H, CONH), 9.04 (d, 1H, Ar-H, J = 2.5 Hz), 8.95 (d, 1H, NHCH, J = 8.0 Hz), 8.43 (dd, 1H, Ar-H, J = 2.4 Hz, 8.6 Hz), 8.16 (d, 2H, Ar-H, J = 8.5 Hz), 8.10 (d, 1H, Ar-H, J = 8.5 Hz), 8.07 (d, 2H, Ar-H, J = 8.5 Hz), 7.95 – 7.91 (m, 4H, Ar-H), 7.83 (d, 2H, Ar-H, J = 8.6 Hz), 7.80 (d, 2H, Ar-H, J = 8.8 Hz), 7.76 – 7.70 (m, 1H, Ar-H), 7.56 – 7.51 (m, 2H, Ar-H & $CONH_2$), 7.00 (s, 1H, $CONH_2$), 4.94 (dd, 1H, CHNH, J = 7.6 Hz, 13.0 Hz), 4.70 – 4.62 (m, 1H, $CH(Me)_2$), 2.76 (ddd, 2H, CH_2, J = 6.4 Hz, 15.3 Hz, 20.5 Hz), 1.24 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.4 ($CONH_2$), 170.1 (CONH), 168.2 (CONH), 167.0 (COO), 164.9 (CONH), 163.9 (CONH), 163.4 (CONH), 144.6 (C_{Ar}), 142.2 (C_{Ar}-NH), 140.3 (C_{Ar}-H), 138.1 (C_{Ar}), 136.8 (C_{Ar}), 132.6 (C_{Ar}-H), 130.3 (C_{Ar}-H), 128.8 (C_{Ar}-H), 128.1 (C_{Ar}-H), 127.8 (C_{Ar}-H), 123.1 (C_{Ar}-H), 122.5 (C_{Ar}-H), 120.1 (C_{Ar}-H), 119.1 (C_{Ar}-H), 118.3 (CN), 114.4 (C_{Ar}), 73.7 ($CH(Me)_2$), 51.0 (CHNH), 37.0 (CH_2), 22.4 (($CH_3)_2$)

HRMS (ESI) calculated 813.2633 [M+H^+], 813.2627 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-2-fluorobenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**22**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a beige solid.

Yield: 6.5 mg (42 % over 3 steps)

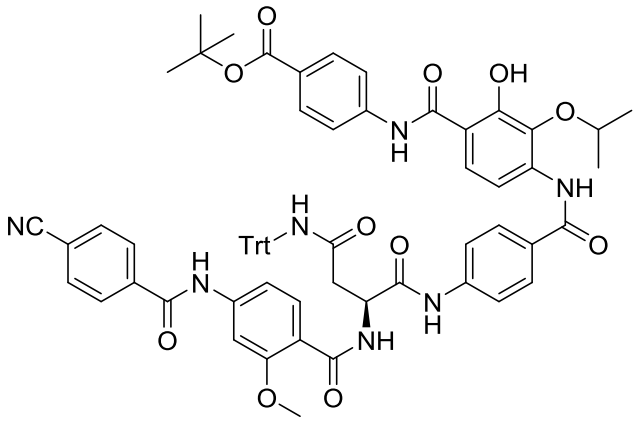
^{1}H-NMR (500 MHz, $DMSO_6$, 300 K): δ (ppm) = 10.86 (s, 1H, CONH), 10.44 (s, 1H, CONH), 9.34 (s, 1H, CONH), 8.55 – 8.50 (m, 1H, NHCH), 8.13 (d, 2H, Ar-H, J = 8.4 Hz), 8.06 (d, 2H, Ar-H, J = 8.4 Hz), 7.97 – 7.93 (m, 4H, Ar-H), 7.89 – 7.76 (m, 7H, Ar-H), 7.65 (dd, 1H, Ar-H, J = 1.7 Hz, 8.7 Hz), 7.63 – 7.59 (m, 1H, Ar-H), 7.48 (s, 1H, $CONH_2$), 7.02 (s, 1H, $CONH_2$), 4.91 (dd, 1H, CHNH, J = 6.7 Hz, 6.9 Hz), 4.65 – 4.56 (m, 1H, $CH(Me)_2$), 2.72 – 2.68 (m, 2H, CH_2), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3 ($CONH_2$), 170.2 (CONH), 168.3 (CONH), 166.9 (COO), 164.7 (CONH), 164.1 (CONH), 162.8 (CONH), 159.8 (C_{Ar}-F, J = 247.9 Hz), 142.9 (C_{Ar}-H, J = 11.4 Hz), 142.3 (C_{Ar}-NH), 138.3 (C_{Ar}-NH), 132.6 (C_{Ar}-H), 131.2 (C_{Ar}-H, J = 3.4 Hz), 130.3 (C_{Ar}-H), 128.7 (C_{Ar}-H), 128.6 (C_{Ar}), 128.2 (C_{Ar}-H), 123.0 (C_{Ar}-H), 120.4 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 117.4 (C_{Ar}, J = 13.2 Hz), 115.7 (C_{Ar}-H), 114.3 (C_{Ar}), 107.1 (C_{Ar}-H, J = 28.5 Hz), 74.3 ($CH(Me)_2$), 51.5 (CHNH), 36.7 (CH_2), 22.4 (($CH_3)_2$)

^{19}F{^{1}H}-NMR (470 MHz, DMSO-d_6, 300 K): δ (ppm) = - 73.4 (CF_3COOH), - 110.8 (C_{Ar}-F)

HRMS (ESI) calculated 830.2586 [M+H^+], 830.2580 found.

tert-Butyl (*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)-2-methoxybenzamido)-4-oxo-4-(tritylamino)-butanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoate



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1. The product was a slightly yellow solid.

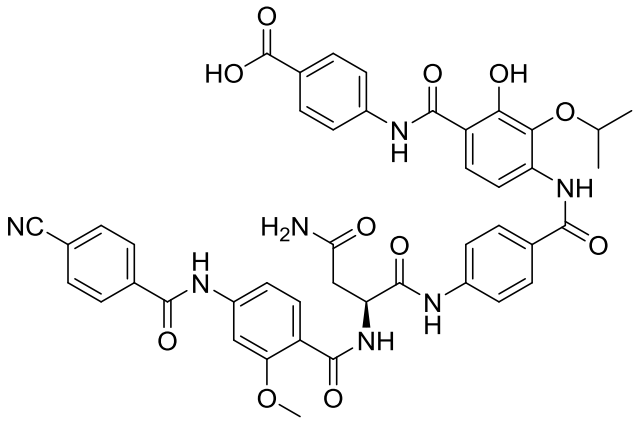
Yield: 9.4 mg (46 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 12.21 (br s, 1H, Ar-OH), 9.65 – 9.59 (m, 2H, CONH), 8.88 (s, 1H, CONH), 8.20 – 8.14 (m, 3H, Ar-H & CONH), 8.01 (d, 2H, Ar-H, J = 8.7 Hz), 7.98 (d, 2H, Ar-H, J = 8.1 Hz), 7.83 (d, 2H, Ar-H, J = 8.6 Hz), 7.81 – 7.78 (m, 3H, Ar-H), 7.68 (d, 2H, Ar-H, J = 8.7 Hz), 7.60 (d, 2H, Ar-H, J = 8.4 Hz), 7.34 (d, 1H, Ar-H, J = 9.1 Hz), 7.25 – 7.21 (m, 12H, Ar-H), 6.97 (d, 1H, Ar-H, J = 8.1 Hz), 5.13 – 5.07 (m, 1H, CHNH), 4.87 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.69 (s, 3H, OCH_3), 3.29 (d, 1H, CH_2, J = 15.1 Hz), 2.71 (dd, 1H, CH_2, J = 6.0 Hz, 15.2 Hz), 1.60 (s, 9H, $(CH_3)_3$), 1.37 (dd, 6H, $(CH_3)_2$, J = 3.2 Hz, 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 170.6 (CONH), 169.8 (CONH), 168.4 (CONH), 165.7 (CONH), 165.2 (COO), 164.6 (CONH), 164.1 (CONH), 159.0 (C_{Ar}-O-Me), 154.8 (C_{Ar}-OH), 144.2 (C_{Ar}), 142.2 (C_{Ar}), 141.5 (C_{Ar}), 140.6 (C_{Ar}), 138.4 (C_{Ar}), 137.6 (C_{Ar}), 134.6 (C_{Ar}-O), 132.8 (C_{Ar}-H), 132.7 (C_{Ar}-H), 130.7 (C_{Ar}-H), 129.6 (C_{Ar}), 128.7 (C_{Ar}-H), 128.3 (C_{Ar}), 128.0 (C_{Ar}-H), 127.8 (C_{Ar}-H), 127.1 (C_{Ar}-H), 120.9 (C_{Ar}-H), 119.9 (C_{Ar}-H), 119.8 (C_{Ar}), 117.7 (-CN), 116.5 (C_{Ar}), 115.8 (C_{Ar}), 111.7 (C_{Ar}-H), 110.8 (C_{Ar}), 109.7 (C_{Ar}-H), 103.1 (C_{Ar}-H), 81.1 ($C(Me)_3$), 75.3 ($CH(Me)_2$), 70.9 ($C(Ph)_3$), 56.0 (Ar-CH_3), 51.3 (CHNH), 37.7 (CH_2), 28.2 (($CH_3)_3$), 22.9 (($CH_3)_2$), 17.8 (CH_3)

HRMS (ESI) calculated 1140.4507 [M+H^+], 1140.4507 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-2-methoxybenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**23**)



The *tert*-butyl and trityl-protected cystobactamid (8.2 µmol) was deprotected by procedure O2. The product was a slightly yellow solid.

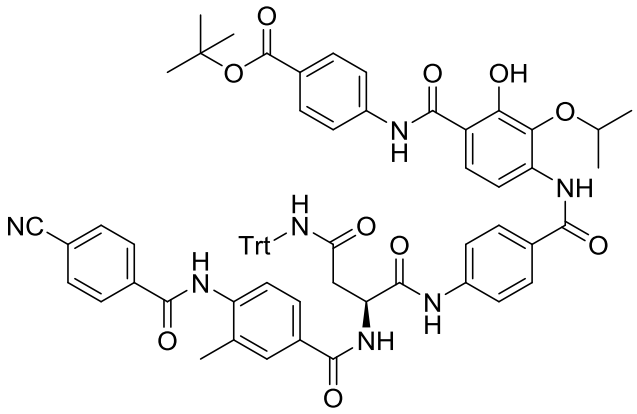
Yield: 3.4 mg (47 %)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.75 (br s, 1H, COOH), 12.29 (br s, 1H, Ar-OH), 10.72 (s, 1H, CONH), 10.43 (s, 1H, CONH), 9.35 (br s, 1H, CONH), 8.91 (d, 1H, NHCH, J = 6.8 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 7.97 – 7.93 (m, 5H, Ar-H), 7.85 (d, 2H, Ar-H, J = 8.7 Hz), 7.82 – 7.79 (m, 3H, Ar-H), 7.76 (d, 1H, Ar-H, J = 1.8 Hz), 7.69 – 7.61 (m, 1H, Ar-H), 7.58 (br s, 1H, $CONH_2$), 7.51 (dd, 1H, Ar-H, J = 1.8 Hz, 8.7 Hz), 7.07 (br s, 1H, $CONH_2$), 4.87 (dd, 1H, CHNH, J = 6.7 Hz, 12.6 Hz), 4.59 (br s, 1H, $CH(Me)_2$), 3.97 (s, 3H, OCH_3), 2.69 (ddd, 2H, CH_2, J = 6.3 Hz, 15.0 Hz, 20.3 Hz), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.5 ($CONH_2$), 170.3 (CONH), 168.4 (CONH), 166.9 (COOH), 164.6 (CONH), 164.1 (CONH), 163.8 (CONH), 157.9 (C_{Ar}-OMe), 143.1 (C_{Ar}-NH), 142.4 (C_{Ar}-NH), 138.6 (C_{Ar}), 136.5 (C_{Ar}), 132.5 (C_{Ar}-H), 131.8 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}), 128.2 (C_{Ar}-H), 122.9 (C_{Ar}-H), 120.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 116.5 (C_{Ar}), 114.1 (C_{Ar}), 112.1 (C_{Ar}-H), 103.5 (C_{Ar}-H), 74.5 ($CH(Me)_2$), 55.9 (OCH_3), 51.6 (CHNH), 37.1 (CH_2), 22.4 ($(CH_3)_2$)

HRMS (ESI) calculated 842.2780 [M+H$^+$], 842.2780 found.

tert-Butyl (*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)-3-methylbenzamido)-4-oxo-4-(tritylamino)butanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoate (**19**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1. The product was a slightly yellow solid.

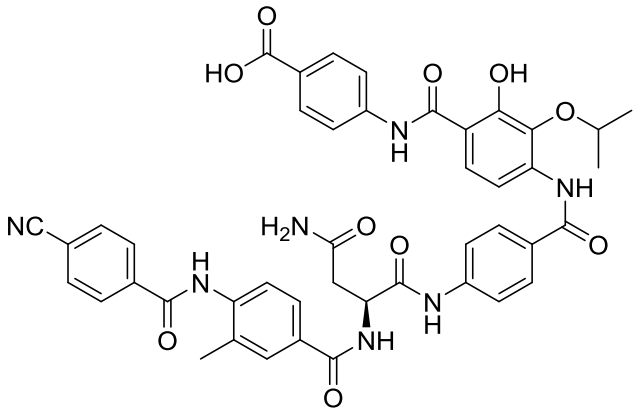
Yield: 11.6 mg (58 %)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 12.23 (br s, 1H, Ar-OH), 9.65 (s, 1H, CONH), 8.87 (s, 1H, CONH), 8.30 (s, 1H, CONH), 8.12 (d, 1H, CONH, J = 6.4 Hz), 8.10 (d, 1H, Ar-H, J = 8.9 Hz), 8.05 (d, 1H, Ar-H, J = 7.6 Hz), 8.01 – 7.97 (m, 4H, Ar-H), 7.90 (s, 1H, CONH), 7.82 (d, 2H, Ar-H, J = 8.6 Hz), 7.80 (d, 2H, Ar-H, J = 8.3 Hz), 7.69 – 7.66 (m, 3H, Ar-H), 7.63 (d, 1H, Ar-H, J = 8.4 Hz), 7.57 (d, 2H, Ar-H, J = 8.6 Hz), 7.33 (d, 1H, Ar-H, J = 9.1 Hz), 7.29 – 7.22 (m, 15H, Ar-H), 5.06 (dd, 1H, CH-N, J = 6.7 Hz, 9.8 Hz), 4.86 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.29 (d, 1H, CH_2, J = 13.2 Hz), 2.75 (dd, 1H, CH_2, J = 7.0 Hz, 15.6 Hz), 2.34 (s, 3H, CH_3), 1.60 (s, 9H, $(CH_3)_3$), 1.36 (t, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, $CDCl_3$, 300 K): δ (ppm) = 171.1 (CONH), 169.4 (CONH), 168.5 (CONH), 167.2 (CONH), 165.2 (COO), 164.5 (CONH), 163.9 (CONH), 154.8 (C_{Ar}-OH), 144.0 (C_{Ar}), 141.3 (C_{Ar}), 140.7 (C_{Ar}), 138.9 (C_{Ar}), 138.4 (C_{Ar}), 137.5 (C_{Ar}), 134.5 (C_{Ar}-O), 132.8 (C_{Ar}-H), 130.6 (C_{Ar}-H), 129.9 (C_{Ar}-H), 129.8 (C_{Ar}), 129.6 (C_{Ar}), 128.9 (C_{Ar}), 128.6 (C_{Ar}-H), 128.3 (C_{Ar}), 128.1 (C_{Ar}-H), 128.0 (C_{Ar}-H), 127.9 (C_{Ar}-H), 127.3 (C_{Ar}-H), 126.2 (C_{Ar}-H), 122.4 (C_{Ar}-H), 121.0 (C_{Ar}-H), 120.0 (C_{Ar}-H), 119.8 (C_{Ar}-H), 117.8 (-CN), 115.8 (C_{Ar}), 110.9 (C_{Ar}), 109.7 (C_{Ar}-H), 81.1 ($C(Me)_3$), 75.3 ($CH(Me)_2$), 71.2 ($C(Ph)_3$), 51.1 (CHNH), 37.8 (CH_2), 28.2 ($(CH_3)_3$), 22.9 ($(CH_3)_2$), 17.8 (CH_3)

HRMS (ESI) calculated 1124.4558 [$M+H^+$], 1124.4549 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-3-methylbenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**24**)



The *tert*-butyl and trityl-protected cystobactamid (10.3 µmol) was deprotected by procedure O2. The product was a slightly yellow solid.

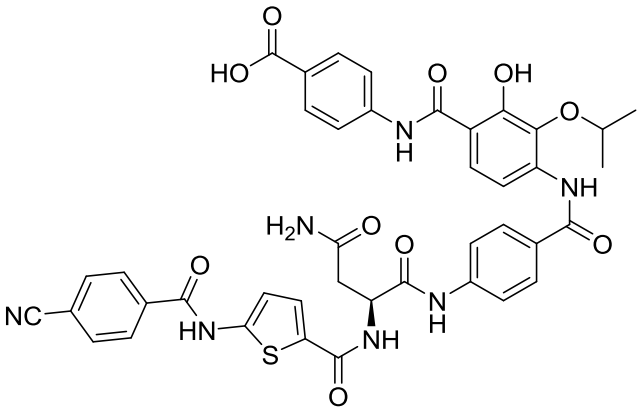
Yield: 7.9 mg (89 %)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.55 (br s, 1H, CONH), 10.45 (s, 1H, CONH), 10.24 (s, 1H, CONH), 9.17 (s, 1H, CONH), 8.71 (d, 1H, NHCH, J = 7.3 Hz), 8.14 (d, 2H, Ar-H, J = 8.3 Hz), 8.04 (d, 2H, Ar-H, J = 8.4 Hz), 7.93 – 7.89 (m, 4H, Ar-H), 7.84 – 7.80 (m, 4H, Ar-H), 7.76 (dd, 1H, Ar-H, J = 1.6 Hz, 8.3 Hz), 7.67 (d, 1H, Ar-H, J = 7.6 Hz), 7.52 (d, 1H, Ar-H, J = 8.3 Hz), 7.46 – 7.39 (m, 2H, Ar-H & $CONH_2$), 6.99 (s, 1H, $CONH_2$), 4.93 (dd, 1H, CHNH, J = 7.3 Hz, 14.0 Hz), 4.75 (br s, 1H, $CH(Me)_2$), 2.71 – 2.67 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 1.23 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3 ($CONH_2$), 170.6 (CONH), 168.0 (CONH), 167.1 (COO), 166.0 (CONH), 164.1 (CONH), 163.7 (CONH), 142.3 (C_{Ar}-NH), 138.8 (C_{Ar}-NH), 138.4 (C_{Ar}), 136.9 (C_{Ar}), 133.1 (C_{Ar}-Me), 132.6 (C_{Ar}-H), 131.4 (C_{Ar}), 130.3 (C_{Ar}-H), 129.8 (C_{Ar}-H), 128.8 (C_{Ar}), 128.6 (C_{Ar}-H), 128.0 (C_{Ar}-H), 125.8 (C_{Ar}-H), 125.5 (C_{Ar}-H), 123.2 (C_{Ar}-H), 119.6 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 72.9 ($CH(Me)_2$), 51.6 (CHNH), 36.8 (CH_2), 22.5 (($CH_3)_2$), 18.0 (CH_3)

HRMS (ESI) calculated 826.2837 [M+H$^+$], 826.2832 found.

(*S*)-4-(4-(4-(4-amino-2-(5-(4-cyanobenzamido)thiophene-2-carboxamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**25**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a yellowish solid.

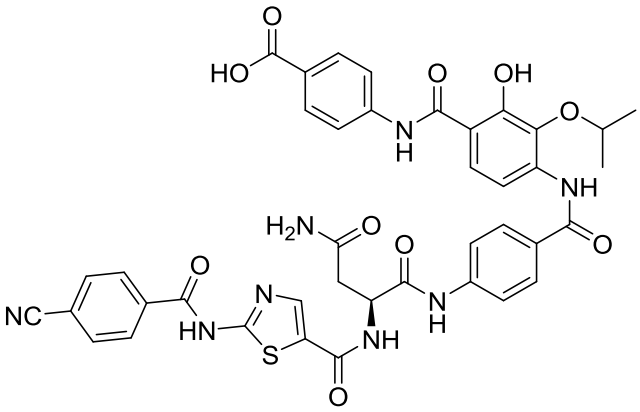
Yield: 6.9 mg (46 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.56 (br s, 1H, OH), 10.46 (s, 1H, CONH), 8.88 (s, 1H, CONH), 8.36 (s, 1H, CONH), 8.20 (d, 2H, Ar-H, J = 8.3 Hz), 7.92 (d, 2H, Ar-H, J = 7.6 Hz), 7.85 – 7.80 (m, 6H, Ar-H), 7.67 (d, 2H, Ar-H, J = 8.3 Hz), 7.63 (d, 1H, Ar-H, J = 3.8 Hz), 7.45 (d, 1H, Ar-H, J = 8.7 Hz), 7.43 (s, 1H, $CONH_2$), 7.10 (d, 1H, Ar-H, J = 8.7 Hz), 6.71 (br s, 1H, Ar-H), 5.00 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.87 (dd, 1H, CHNH, J = 7.3 Hz, 14.2 Hz), 2.67 – 2.64 (m, 2H, CH_2), 1.20 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.4 ($CONH_2$), 170.9 (CONH), 169.1 (COO), 167.1 (CONH), 165.2 (C_{Ar}-O), 163.8 (CONH), 163.2 (CONH), 162.6 (CONH), 143.2 (C_{Ar}-NH), 142.1 (C_{Ar}-NH), 137.6 (C_{Ar}-O), 134.2 (C_{Ar}-NH), 132.0 (C_{Ar}-H), 130.0 (C_{Ar}-H), 129.4 (C_{Ar}), 128.6 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.4 (C_{Ar}-H), 124.2 (C_{Ar}), 123.7 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.8 (CN), 117.9 (C_{Ar}-H), 115.8 (C_{Ar}), 112.8 (C_{Ar}-H), 112.3 (C_{Ar}), 101.1 (C_{Ar}-H), 70.5 ($CH(Me)_2$), 51.4 (CHNH), 37.0 (CH_2), 22.7 ($(CH_3)_2$)

HRMS (ESI) calculated 818.2244 [M+H$^+$], 818.2241 found.

(*S*)-4-(4-(4-(4-amino-2-(2-(4-cyanobenzamido)thiazole-5-carboxamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**26**)



The Fmoc protected amino acid (26.7 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a yellow solid.

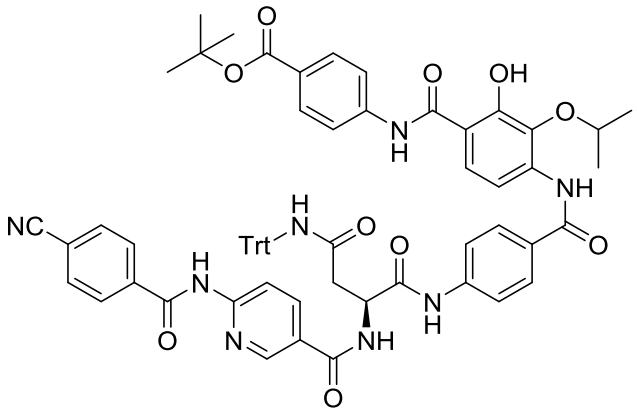
Yield: 10.3 mg (45 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.44 (br s, 1H, OH), 10.42 (s, 1H, CONH), 8.88 (s, 1H, CONH), 8.27 (d, 2H, Ar-H, J = 8.2 Hz), 8.19 (d, 1H, NHCH, J = 7.4 Hz), 8.01 (s, 1H, Ar-H), 7.85 – 7.79 (m, 8H, Ar-H), 7.62 (d, 2H, Ar- H, J = 8.5 Hz), 7.45 (d, 1H, Ar-H, J = 8.9 Hz), 7.42 (s, 1H, $CONH_2$), 7.09 (d, 1H, Ar-H, J = 8.8 Hz), 6.96 (s, 1H, $CONH_2$), 5.00 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.85 (quart., 1H, CHNH, J = 7.2 Hz), 2.64 (d, 2H, CH_2, J = 7.0 Hz), 1.20 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 173.6 (C_{Ar}-NH), 171.5 ($CONH_2$), 170.9 (CONH), 169.9 (COO), 168.4 (CONH), 167.0 (CONH), 165.3 (C_{Ar}-O), 163.2 (CONH), 162.5 (CONH), 144.4 (C_{Ar}), 142.6 (C_{Ar}-NH), 142.1 (C_{Ar}-NH), 142.0 (C_{Ar}-H), 137.6 (C_{Ar}-O), 134.1 (C_{Ar}-NH), 131.7 (C_{Ar}-H), 129.9 (C_{Ar}-H), 129.4 (C_{Ar}), 128.7 (C_{Ar}-H), 127.6 (C_{Ar}-H), 124.2 (C_{Ar}), 123.7 (C_{Ar}-H), 121.2 (C_{Ar}), 119.2 (CN), 119.0 (C_{Ar}-H), 117.8 (C_{Ar}-H), 115.9 (C_{Ar}), 111.4 (C_{Ar}), 100.9 (C_{Ar}-H), 70.4 ($CH(Me)_2$), 51.3 (CHNH), 37.1 (CH_2), 22.7 (($CH_3)_2$)

HRMS (ESI) calculated 819.2197 [M+H$^+$], 819.2192 found.

tert-Butyl (*S*)-4-(4-(4-(2-(6-(4-cyanobenzamido)-nicotinamido)-4-oxo-4-(tritylamino)butanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoate



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1. The product was a white solid.

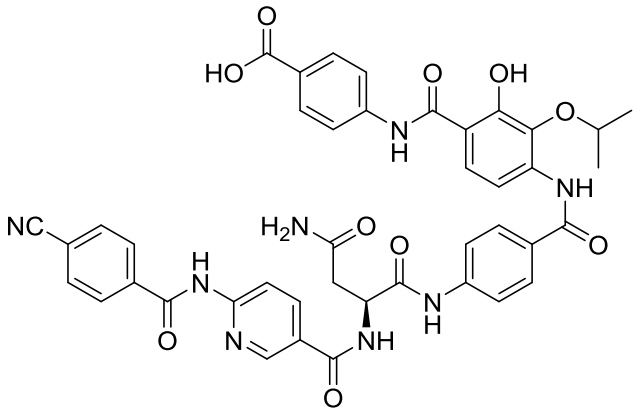
Yield: 14.0 mg (71 % over 2 steps)

^{1}H-NMR (700 MHz, Aceton-d_6, 300 K): δ (ppm) = 10.16 (br s, 1H, Ar-OH), 9.93 (s, 1H, CONH), 9.85 (s, 1H, CONH), 9.00 (s, 1H, CONH), 8.55 (d, 1H, Ar-H, J = 1.8 Hz), 8.43 (d, 1H, CONH, J = 7.7 Hz), 8.38 (d, 1H, Ar-H, J = 8.7 Hz), 8.29 – 8.25 (m, 4H, Ar-H & CONH), 8.08 (d, 1H, Ar-H, J = 9.0 Hz), 7.98 (d, 2H, Ar-H, J = 8.8 Hz), 7.95 (d, 2H, Ar-H, J = 8.5 Hz), 7.92 (d, 2H, Ar-H, J = 8.7 Hz), 7.88 (d, 2H, Ar-H, J = 8.8 Hz), 7.81 (d, 2H, Ar-H, J = 8.7 Hz), 7.78 (d, 1H, Ar-H, J = 9.1 Hz), 7.30 – 7.28 (m, 6H, Ar-H), 7.24 – 7.18 (m, 9H, Ar-H), 5.14 (dd, 1H, CHNH, J = 7.4 Hz, 13.4 Hz), 4.84 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 3.10 (ddd, 2H, CH_2, J = 6.6 Hz, 15.7 Hz, 21.4 Hz), 1.59 (s, 9H, $(CH_3)_3$), 1.38 (dd, 6H, $(CH_3)_2$, J = 3.0 Hz, 6.1 Hz)

^{13}C-NMR (176 MHz, Aceton-d_6, 300 K): δ (ppm) = 171.0 (CONH), 170.8 (CONH), 170.3 (COO), 165.6 (CONH), 164.9 (CONH), 155.9 (C_{Ar}-OH), 155.1 (C_{Ar}-NH), 149.0 (C_{Ar}-H), 145.9 (C_{Ar}), 143.4 (C_{Ar}-NH), 142.9 (C_{Ar}-NH), 139.1 (C_{Ar}), 138.8 (C_{Ar}-NH), 138.4 (C_{Ar}-H), 135.5 (C_{Ar}-O), 133.3 (C_{Ar}-H), 131.0 (C_{Ar}-H), 130.2 (C_{Ar}), 129.9 (C_{Ar}-H), 129.7 (C_{Ar}-H), 128.9 (C_{Ar}-H), 128.8 (C_{Ar}), 128.5 (C_{Ar}-H), 127.6 (C_{Ar}-H), 126.7 (C_{Ar}), 123.1 (C_{Ar}-H), 121.4 (C_{Ar}-H), 120.3 (C_{Ar}-H), 118.8 (-CN), 116.3 (C_{Ar}-CN), 114.1 (C_{Ar}-H), 111.8 (C_{Ar}), 110.5 (C_{Ar}-H), 81.1 ($C(Me)_3$), 75.8 ($CH(Me)_2$), 71.4 ($C(Ph)_3$), 52.8 (CHNH), 39.0 (CH_2), 28.4 (($CH_3)_3$), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 1111.4354 [M+H^+], 1111.4346 found.

(*S*)-4-(4-(4-(4-amino-2-(6-(4-cyanobenzamido)-nicotinamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**27**)



The *tert*-butyl and trityl-protected cystobactamid (12.6 µmol) was deprotected by procedure O2. The product was a yellow solid.

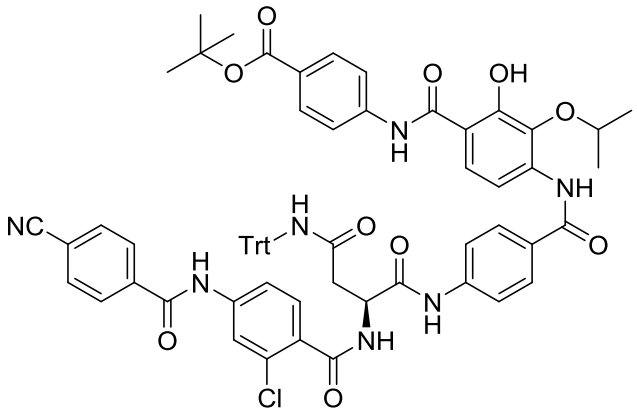
Yield: 10.8 mg (quantitative)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 11.42 (s, 1H, CONH), 10.48 (s, 1H, CONH), 8.95 (d, 1H, NHCH, J = 7.2 Hz), 8.92 (s, 1H, Ar-H), 8.35 (d, 1H, Ar-H, J = 8.6 Hz), 8.30 (d, 1H, Ar-H, J = 8.8 Hz), 8.17 (d, 2H, Ar-H, J = 8.2 Hz), 8.01 (d, 2H, Ar-H, J = 8.3 Hz), 7.87 – 7.84 (m, 4H, Ar-H), 7.81 (d, 2H, Ar-H, J = 8.5 Hz), 7.77 (d, 2H, Ar-H, J = 8.0 Hz), 7.51 – 7.47 (m, 1H, Ar-H), 7.42 (s, 1H, $CONH_2$), 7.18 – 7.13 (m, 1H, Ar-H), 6.99 (s, 1H, $CONH_2$), 4.98 – 4.94 (m, 2H, $CH(CH_3)_2$ & CHNH), 2.71 (qd, 2H, CH_2, J = 7.1 Hz, 15.5 Hz), 1.20 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.2 ($CONH_2$), 170.5 (CONH), 167.4 (COO), 165.1 (CONH), 164.4 (CONH), 163.3 (CONH), 153.9 (C_{Ar}-NH), 147.8 (C_{Ar}-H), 145.0 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 138.0 (C_{Ar}), 137.8 (C_{Ar}-H), 137.5 (C_{Ar}-O), 134.6 (C_{Ar}-NH), 132.4 (C_{Ar}-H), 130.4 (C_{Ar}-H), 129.4 (C_{Ar}), 129.0 (C_{Ar}-H), 127.7 (C_{Ar}-H), 125.6 (C_{Ar}), 123.6 (C_{Ar}-H), 119.1 (C_{Ar}-H), 118.5 (C_{Ar}-H), 118.2 (CN), 115.3 (C_{Ar}), 114.3 (C_{Ar}), 113.5 (C_{Ar}-H), 102.0 (C_{Ar}-H), 70.9 ($CH(Me)_2$), 51.6 (CHNH), 36.8 (CH_2), 22.6 ($(CH_3)_2$)

HRMS (ESI) calculated 813.2633 [M+H$^+$], 813.2628 found.

tert-Butyl (*S*)-4-(4-(4-(2-(2-chloro-4-(4-cyanobenzamido)benzamido)-4-oxo-4-(tritylamino)butanamido)-benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoate



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1. The product was a white solid.

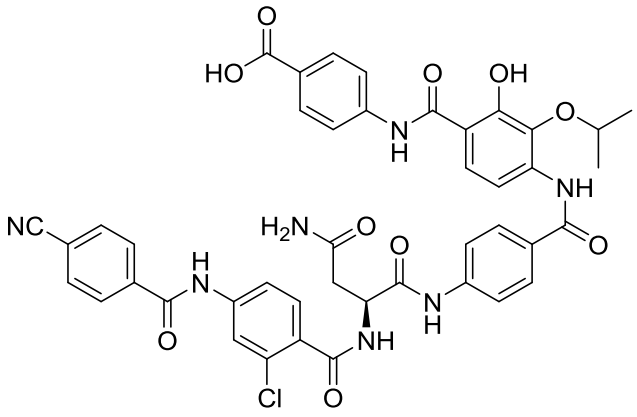
Yield: 12.2 mg (60 % over 2 steps)

^{1}H-NMR (700 MHz, Aceton-d_6, 300 K): δ (ppm) = 12.65 (br s, 1H, Ar-OH), 10.02 (s, 1H, CONH), 9.75 (s, 1H, CONH), 9.04 (s, 1H, CONH), 8.28 (s, 1H, CONH), 8.18 (d, 2H, Ar-H, J = 8.3 Hz), 8.15 (d, 1H, CONH, J = 7.7 Hz), 8.12 – 8.09 (m, 2H, Ar-H), 7.99 – 7.97 (m, 4H, Ar-H), 7.95 (dd, 2H, Ar-H, J = 2.3 Hz, 8.3 Hz), 7.90 (d, 2H, Ar-H, J = 8.6 Hz), 7.83 – 7.80 (m, 3H, Ar-H), 7.78 (d, 1H, Ar-H, J = 8.5 Hz), 7.61 (dd, 1H, Ar-H, J = 2.2 Hz, 8.4 Hz), 7.29 (d, 6H, Ar-H, J = 7.4 Hz), 7.24 (t, 3H, Ar-H, J = 7.5 Hz), 7.22 – 7.19 (m, 3H, Ar-H), 5.10 (q, 1H, CH-N, J = 6.6 Hz), 4.86 (hept, $CH(Me)_2$, J = 6.0 Hz), 3.26 (dd, 1H, CH_2, J = 5.8 Hz, 16.1 Hz), 3.07 (dd, 1H, CH_2, J = 6.6 Hz, 16.1 Hz), 1.59 (s, 9H, $(CH_3)_3$), 1.38 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, Aceton-d_6, 300 K): δ (ppm) = 171.0 (CONH), 170.7 (CONH), 170.3 (CONH), 166.9 (CONH), 165.7 (COO), 165.4 (CONH), 165.0 (CONH), 156.0 (C_{Ar}-OH), 145.9 (C_{Ar}), 143.4 (C_{Ar}-NH), 142.9 (C_{Ar}-NH), 142.5 (C_{Ar}-NH), 139.6 (C_{Ar}), 138.8 (C_{Ar}-NH), 135.5 (C_{Ar}-O), 133.4 (C_{Ar}-H), 132.8 (C_{Ar}), 132.3 (C_{Ar}-Cl), 131.5 (C_{Ar}-H), 131.0 (C_{Ar}-H), 130.3 (C_{Ar}), 129.9 (C_{Ar}-H), 129.5 (C_{Ar}-H), 129.0 (C_{Ar}-H), 128.8 (C_{Ar}), 128.5 (C_{Ar}-H), 127.6 (C_{Ar}-H), 123.1 (C_{Ar}-H), 121.9 (C_{Ar}-H), 121.5 (C_{Ar}-H), 120.4 (C_{Ar}-H), 119.2 (C_{Ar}-H), 118.8 (CN), 116.1 (C_{Ar}), 111.9 (C_{Ar}), 110.5 (C_{Ar}-H), 81.3 ($C(Me)_3$), 75.8 ($CH(Me)_2$), 71.4 ($C(Ph)_3$), 52.6 (CHN), 38.9 (CH_2), 28.4 (($CH_3)_3$), 23.0 (($CH_3)_2$)

HRMS (ESI) calculated 1144.4012 [M+H$^+$], 1144.4004 found.

(*S*)-4-(4-(4-(4-amino-2-(2-chloro-4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**28**)



The *tert*-butyl and trityl-protected cystobactamid (10.7 µmol) was deprotected by procedure O2. The product was a slightly yellow solid.

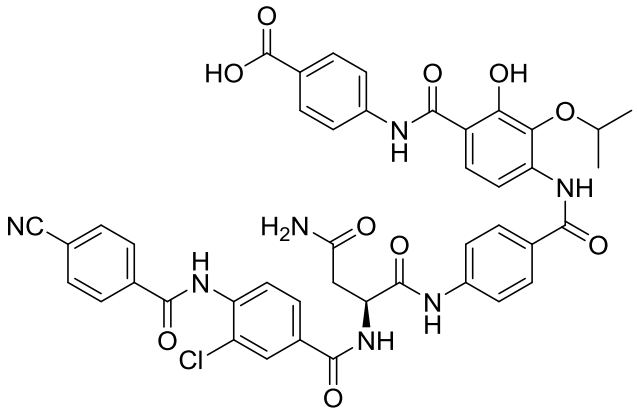
Yield: 7.9 mg (84 %)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.70 (br s, 1H, OH), 10.79 (s, 1H, CONH), 10.46 (s, 1H, CONH), 8.90 (s, 1H, CONH), 8.81 (d, 1H, NHCH, J = 6.8 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.3 Hz), 8.02 (d, 1H, Ar-H, J = 1.7 Hz), 7.86 (d, 2H, Ar-H, J = 8.5 Hz), 7.84 – 7.81 (m, 4H, Ar-H), 7.77 (dd, 1H, Ar-H, J = 1.7 Hz, 8.5 Hz), 7.72 (d, 2H, Ar-H, J = 8.2 Hz), 7.55 (d, 1H, Ar-H, J = 8.4 Hz), 7.46 (d, 1H, Ar-H, J = 8.7 Hz), 7.42 (s, 1H, $CONH_2$), 7.12 (d, 1H, Ar-H, J = 8.7 Hz), 6.98 (s, 1H, $CONH_2$), 5.00 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.91 (dd, 1H, CHNH, J = 7.3 Hz, 14.1 Hz), 2.66 (ddd, 1H, CH_2, J = 7.0 Hz, 15.4 Hz, 23.1 Hz), 1.21 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.1 ($CONH_2$), 170.1 (CONH), 168.1 (COO), 167.2 (CONH), 165.8 (CONH), 165.2 (C_{Ar}-O), 164.5 (CONH), 163.2 (CONH), 144.4 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 140.8 (C_{Ar}-NH), 138.3 (C_{Ar}), 137.6 (C_{Ar}-O), 134.4 (C_{Ar}-NH), 132.5 (C_{Ar}-H), 131.1 (C_{Ar}), 130.5 (C_{Ar}-Cl), 130.2 (C_{Ar}-H), 129.9 (C_{Ar}-H), 129.5 (C_{Ar}), 128.6 (C_{Ar}-H), 127.6 (C_{Ar}-H), 124.2 (C_{Ar}), 123.7 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (C_{Ar}-H), 118.1 (C_{Ar}-H), 118.1 (CN), 115.6 (C_{Ar}), 114.2 (C_{Ar}), 101.2 (C_{Ar}-H), 70.5 ($CH(Me)_2$), 51.4 (CHNH), 36.8 (CH_2), 22.7 (($CH_3)_2$)

HRMS (ESI) calculated 846.2290 [M+H$^+$], 846.2285 found.

(*S*)-4-(4-(4-(4-amino-2-(3-chloro-4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**29**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

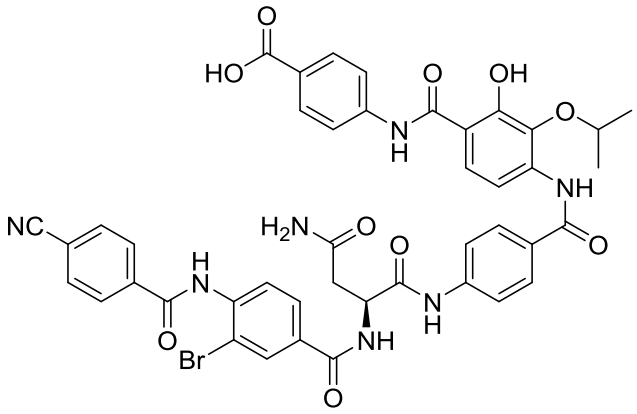
Yield: 8.0 mg (51 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.48 (s, 1H, CONH), 10.47 (CONH), 9.35 (s, 1H, CONH), 8.91 (d, 1H, NHCH, J = 7.2 Hz), 8.15 (d, 2H, Ar-H, J = 8.4 Hz) 8.11 (d, 1H, Ar-H, J = 1.9 Hz), 8.06 (d, 2H, Ar-H, J = 8.4 Hz), 7.96 – 7.93 (m, 4H, Ar-H), 7.92 (dd, 1H, Ar-H, J = 1.9 Hz, 8.4 Hz), 7.85 (d, 2H, Ar-H, J = 8.7 Hz), 7.83 – 7.80 (m, 3H, Ar-H), 7.78 (d, 1H, Ar-H, J = 8.4 Hz), 7.67 – 7.61 (m, 1H, Ar-H), 7.40 (s, 1H, $CONH_2$), 7.00 (s, 1H, $CONH_2$), 4.94 (dd, 1H, CHNH, J = 7.4 Hz, 13.9 Hz), 4.59 (br s, 1H, $CH(Me)_2$), 2.70 (qd, 2H, CH_2, J = 7.1 Hz, 15.5 Hz), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.1 ($CONH_2$), 170.5 (CONH), 168.4 (CONH), 166.9 (COO), 164.6 (CONH), 164.2 (CONH), 164.1 (CONH), 142.4 (C_{Ar}-NH), 142.3 (C_{Ar}-NH), 137.7 (C_{Ar}), 136.8 (C_{Ar}-NH), 136.5 (C_{Ar}-O), 132.8 (C_{Ar}), 132.6 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.8 (C_{Ar}-Cl), 128.7 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}), 128.2 (C_{Ar}-H), 127.7 (C_{Ar}-H), 126.9 (C_{Ar}-H), 126.0 (C_{Ar}), 122.9 (C_{Ar}-H), 120.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.2 (CN), 114.3 (C_{Ar}), 112.7 (C_{Ar}), 74.4 ($CH(Me)_2$), 51.7 (CHNH), 36.7 (CH_2), 22.3 (($CH_3)_2$)

HRMS (ESI) calculated 846.2290 [M+H$^+$], 846.2285 found.

(*S*)-4-(4-(4-(4-amino-2-(3-bromo-4-(4-cyanobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**30**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

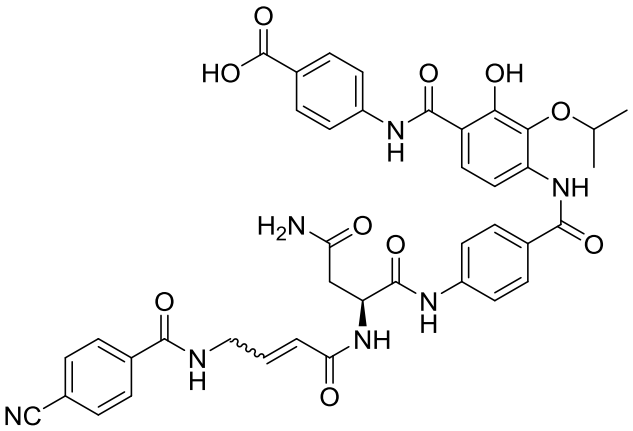
Yield: 7.6 mg (46 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.47 (br s, 1H, CONH), 10.45 (s, 1H, CONH), 9.26 (s, 1H, OH), 8.92 (d, 1H, NHCH, J = 7.2 Hz), 8.26 (d, 1H, Ar-H, J = 1.9 Hz), 8.15 (d, 2H, Ar-H, J = 8.4 Hz), 8.06 (d, 2H, Ar-H, J = 8.5 Hz), 7.96 – 7.91 (m, 6H, Ar-H), 7.84 – 7.81 (m, 4H, Ar-H), 7.76 – 7.72 (m, 2H, Ar-H), 7.60 – 7.50 (m, 1H, Ar-H), 7.40 (1H, s, $CONH_2$), 6.99 (1H, s, $CONH_2$), 4.94 (dd, 1H, CHNH, J = 7.4 Hz, 13.9 Hz), 4.71 – 4.62 (m, 1H, $CH(Me)_2$), 2.70 (qd, 2H, CH_2, J = 7.1 Hz, 15.5 Hz), 1.25 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.1 ($CONH_2$), 170.5 (CONH), 168.2 (CONH), 167.0 (COO), 164.5 (CONH), 164.1 (CONH), 163.9 (CONH), 157.6 (C_{Ar}-O), 142.3 (C_{Ar}-NH), 138.8 (C_{Ar}-NH), 137.8 (C_{Ar}), 136.7 (C_{Ar}-O), 133.3 (C_{Ar}), 132.7 (C_{Ar}-H), 131.8 (C_{Ar}-H), 130.3 (C_{Ar}-H), 128.7 (C_{Ar}), 128.6 (C_{Ar}-H), 128.2 (C_{Ar}-H), 128.1 (C_{Ar}-H), 127.5 (C_{Ar}-H), 123.1 (C_{Ar}-H), 120.1 (C_{Ar}-H), 119.8 (C_{Ar}-Br), 119.0 (C_{Ar}-H), 118.3 (CN), 114.3 (C_{Ar}), 73.8 ($CH(Me)_2$), 51.7 (CHNH), 36.7 (CH_2), 22.4 ($(CH_3)_2$)

HRMS (ESI) calculated 890.1785/892.1765 [M+H$^+$], 890.1777/892.1765 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)but-2-enamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**36**)



The Fmoc deprotected amino acid (18.9 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

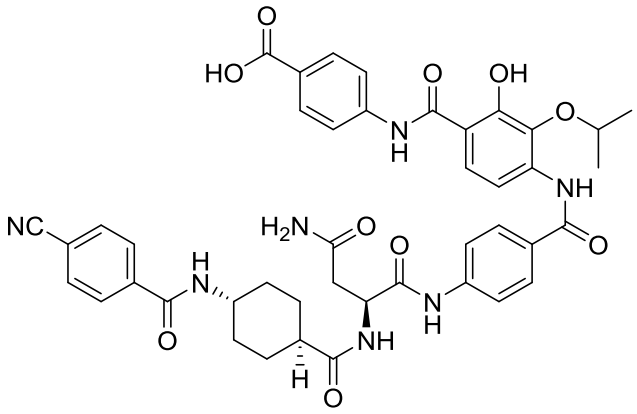
Yield: 5.7 mg (37 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.53 (br s, 1H, CONH), 10.43 (s, 1H, CONH), 9.04 (t, 1H, $NHCH_2$, J = 5.6 Hz), 8.52 (d, 1H, NHCH, J = 7.5 Hz), 8.00 (d, 2H, Ar-H, J = 8.5 Hz), 7.96 (d, 2H, Ar-H, J = 8.5 Hz), 7.89 – 7.86 (m, 4H, Ar-H), 7.82 – 7.78 (m, 4H, Ar-H), 7.61 – 7.54 (m, 1H, Ar-H), 7.37 (s, 1H, $CONH_2$), 6.95 (s, 1H, $CONH_2$), 6.04 – 5.97 (m, 2H, CH= & =CH), 4.91 – 4.82 (m, 1H, $CH(Me)_2$), 4.79 (dd, 1H, CHNH, J = 7.6 Hz, 13.9 Hz), 4.50 (t, 2H, CH_2CH=, J = 4.7 Hz), 2.65 – 2.51 (m, 2H, CH_2), 1.22 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.9 ($CONH_2$), 170.5 (CONH), 167.7 (CONH), 167.1 (COO), 165.3 (CONH), 164.8 (CONH), 163.5 (CONH), 142.2 (CH=), 142.1 (C_{Ar}-NH), 138.3 (C_{Ar}), 137.2 (C_{Ar}), 133.2 (C_{Ar}), 132.5 (C_{Ar}-H), 130.4 (C_{Ar}-H), 129.1 (C_{Ar}), 128.1 (C_{Ar}-H), 127.8 (C_{Ar}-H), 123.4 (C_{Ar}-H), 122.8 (=CH-CONH), 119.0 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 113.6 (C_{Ar}), 50.7 (CHNH), 38.5 (CH_2NH), 37.0 (CH_2), 22.6 (($CH_3)_2$)

HRMS (ESI) calculated 776.2680 [M+H$^+$], 776.2674 found.

4-(4-(4-((S)-4-amino-2-((1r,4S)-4-(4-cyanobenzamido)cyclohexan-1-carboxamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**37**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

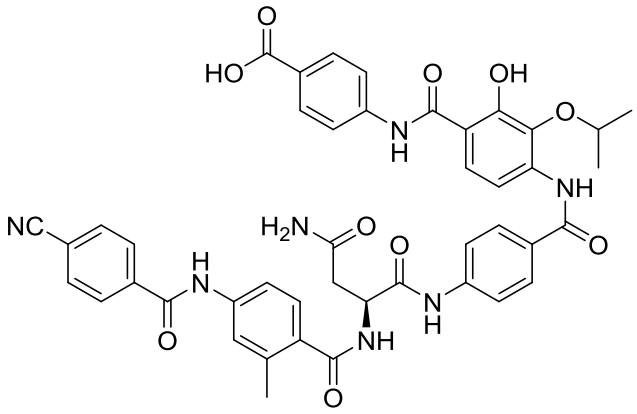
Yield: 5.9 mg (39 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.35 (br s, 1H, CONH), 10.38 (s, 1H, CONH), 8.87 (s, 1H, CONH), 8.50 (d, 1H, NHCH, J = 7.9 Hz), 8.24 (d, 1H, NHCH, J = 7.5 Hz), 7.99 (d, 2H, Ar-H, J = 8.5 Hz), 7.94 (d, 2H, Ar-H, J = 8.4 Hz), 7.83 (d, 2H, Ar-H, J = 8.8 Hz), 7.80 – 7.77 (m, 4H, Ar-H), 7.57 (d, 2H, Ar-H, J = 8.5 Hz), 7.45 (d, 1H, Ar-H, J = 8.7 Hz), 7.39 (s, 1H, $CONH_2$), 7.08 (d, 1H, Ar-H, J = 8.7 Hz), 6.92 (s, 1H, $CONH_2$), 5.02 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.70 (dd, 1H, CHNH, J = 7.6 Hz, 14.1 Hz), 3.74 (tdt, 1H, CHNH, J = 4.1 Hz, 7.9 Hz, 11.6 Hz), 2.59 (dd, 1H, CH_2, J = 6.1 Hz, 15.1 Hz), 2.21 (tt, 1H, CHNH, J = 3.3 Hz, 11.9 Hz), 1.90 (d, 2H, CH_2, J = 11.9 Hz), 1.86 – 1.78 (m, 2H, CH_2), 1.44 (qd, 2H, CH_2, J = 2.7 Hz, 13.0 Hz), 1.39 – 1.32 (m, 2H, CH_2), 1.20 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 174.9 (CONH), 171.1 ($CONH_2$), 170.6 (CONH), 170.3 (COO), 166.9 (CONH), 165.3 (C_{Ar}-O), 164.0 (CONH), 163.1 (CONH), 142.0 (C_{Ar}-NH), 141.8 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.5 (C_{Ar}-O), 134.0 (C_{Ar}-NH), 132.3 (C_{Ar}-H), 129.7 (C_{Ar}-H), 129.5 (C_{Ar}), 128.1 (C_{Ar}-H), 127.6 (C_{Ar}-H), 124.2 (C_{Ar}), 123.7 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.4 (CN), 117.6 (C_{Ar}-H), 116.0 (C_{Ar}), 113.4 (C_{Ar}), 100.6 (C_{Ar}-H), 70.3 ($CH(Me)_2$), 50.8 (CHNH), 48.2 (CHNH(CO)), 42.8 (CH), 37.2 (CH_2), 31.4 (CH_2), 31.3 (CH_2), 28.3 (CH_2), 28.0 (CH_2), 22.7($(CH_3)_2$)

HRMS (ESI) calculated 818.3150 [M+H$^+$], 818.3143 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-2-methylbenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (38)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

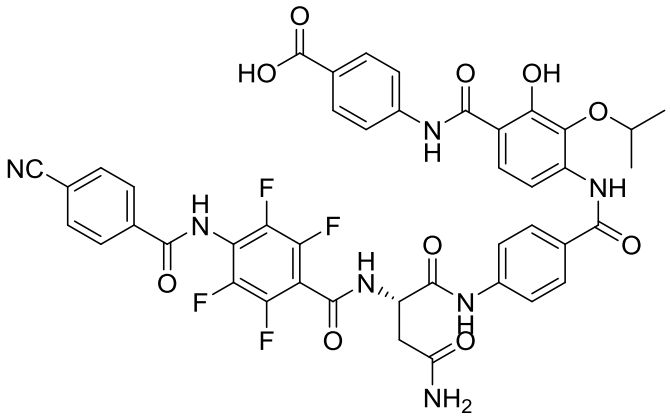
Yield: 4.1 mg (27 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.82 (br s, 1H, CONH), 10.55 (s, 1H, CONH), 10.44 (s, 1H, CONH), 8.90 (s, 1H, CONH), 8.53 (d, 1H, NHCH, J = 7.4 Hz), 8.12 (d, 2H, Ar-H, J = 8.4 Hz), 8.04 (d, 2H, Ar-H, J = 8.4 Hz), 7.86 (d, 2H, Ar-H, J = 8.8 Hz), 7.84 – 7.81 (m, 4H, Ar-H), 7.74 (d, 2H, Ar-H, J = 8.4 Hz), 7.68 – 7.66 (m, 2H, Ar-H), 7.46 – 7.43 (m, 2H, Ar-H), 7.39 (s, 1H, $CONH_2$), 7.12 (d, 1H, Ar-H, J = 8.7 Hz), 6.98 (s, 1H, $CONH_2$), 5.01 (dt, 1H, $CH(Me)_2$, J = 6.0 Hz, 12.2 Hz), 4.89 (dd, 1H, CHNH, J = 7.6 Hz, 13.7 Hz), 2.65 (ddd, 2H, CH_2, J = 7.0 Hz, 15.3 Hz, 23.7 Hz), 1.20 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.2 ($CONH_2$), 170.5 (CONH), 168.6 (CONH), 167.6 (COO), 167.3 (CONH), 165.3 (C_{Ar}-O), 164.2 (CONH), 163.2 (CONH), 145.0 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 139.7 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.6 (C_{Ar}-O), 136.7 (C_{Ar}-Me), 134.4 (C_{Ar}-NH), 132.5 (C_{Ar}-H), 131.9 (C_{Ar}), 130.3 (C_{Ar}-H), 129.4 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.3 (C_{Ar}-H), 127.7 (C_{Ar}-H), 123.7 (C_{Ar}-H), 122.0 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 118.3 (C_{Ar}-H), 117.1 (C_{Ar}-H), 115.5 (C_{Ar}), 114.0 (C_{Ar}), 101.2 (C_{Ar}-H), 70.6 ($CH(Me)_2$), 51.4 (CHNH), 36.8 (CH_2), 22.7 ($(CH_3)_2$), 19.9 (Ar-CH_3)

HRMS (ESI) calculated 826.2837 [M+H^+], 826.2832 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-2,3,5,6-tetrafluorobenzamido)-4-oxobutanamido)-benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**39**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

Yield: 4.3 mg (26 % over 3 steps)

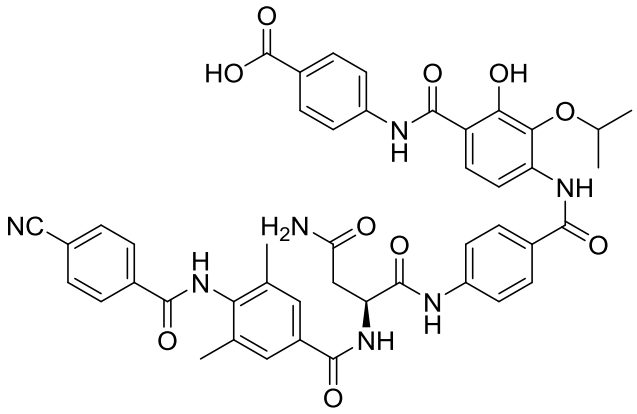
^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.57 (s, 1H, CONH), 9.39 (d, 1H, NHCH, J = 7.4 Hz), 8.16 (d, 2H, Ar-H, J = 8.5 Hz), 8.07 (d, 2H, Ar-H, J = 8.5 Hz), 7.94 – 7.89 (m, 4H, Ar-H), 7.84 – 7.80 (m, 4H, Ar-H), 7.70 – 7.63 (m, 1H, Ar-H), 7.50 – 7.40 (m, Ar-H & $CONH_2$), 7.00 (s, 1H, $CONH_2$), 4.96 (dd., 1H, CHNH, J = 7.4 Hz, 14.1 Hz), 4.80 – 4.70 (m, 1H, $CH(Me)_2$), 2.66 (ddd, 1H, CH_2, J = 7.4 Hz, 15.7 Hz, 23.5 Hz), 1.24 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.5 (C=O), 169.4 (C=O), 167.9 (C=O), 167.0 (C=O), 164.1 (C=O), 163.7 (C=O), 157.0 (C=O), 143.6 (C_{Ar}), 142.7 (C_{Ar}-F), 142.1 (C_{Ar}), 141.3 (C_{Ar}-F), 136.9 (C_{Ar}), 136.3 (C_{Ar}), 132.8 (C_{Ar}-H), 130.3 (C_{Ar}-H), 129.0 (C_{Ar}), 128.8 (C_{Ar}-H), 128.1 (C_{Ar}-H), 127.5 (C_{Ar}-H), 123.2 (C_{Ar}-H), 119.6 (C_{Ar}), 119.0 (C_{Ar}-H), 118.1 (CN), 116.5 (C_{Ar}), 114.8 (C_{Ar}), 51.5 (CHNH), 37.0 (CH_2), 22.5 ((CH_3)$_2$)

^{19}F{^{1}H}-NMR (658 MHz, $CDCl_3$, 300 K): δ (ppm) = - 142.59 (d, Ar-F, J = 15.1 Hz), - 144.55 (d, Ar-F, J = 14.3 Hz)

HRMS (ESI) calculated 884.2303 [M+H$^+$], 884.2298 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-3,5-dimethylbenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**40**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

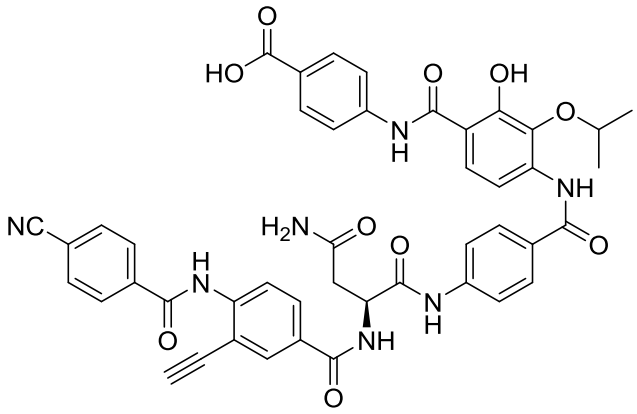
Yield: 9.0 mg (58 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.74 (br s, 1H, CONH), 10.46 (s, 1H, CONH), 10.18 (s, 1H, CONH), 8.90 (s, 1H, CONH), 8.76 – 8.72 (m, 1H, NHCH), 8.16 (d, 2H, Ar-H, J = 8.5 Hz), 8.04 (d, 2H, Ar-H, J = 8.4 Hz), 7.86 – 7.83 (m, 4H, Ar-H), 7.81 (d, 2H, Ar-H, J = 8.5 Hz), 7.75 (d, 2H, Ar-H, J = 8.3 Hz), 7.68 (s, 2H, Ar-H), 7.46 (d, 1H, Ar-H, J = 8.7 Hz), 7.42 (br s, 1H, $CONH_2$), 7.13 (d, 1H, Ar-H, J = 8.7 Hz), 6.98 (s, 1H, $CONH_2$), 4.99 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.93 (dd, 1H, CHNH, J = 7.4 Hz, 14.0 Hz), 2.72 – 2.68 (m, 2H, CH_2), 2.25 (s, 6H, $(CH_3)_2$), 1.20 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3 ($CONH_2$), 170.6 (CONH), 167.6 (COO), 167.3 (CONH), 166.1 (CONH), 165.1 (C_{Ar}-O), 163.6 (CONH), 163.2 (CONH), 144.9 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 138.1 (C_{Ar}), 137.7 (C_{Ar}-NH), 137.6 (C_{Ar}-O), 135.5 (C_{Ar}-Me), 134.4 (C_{Ar}-NH), 132.6 (C_{Ar}-H), 132.3 (C_{Ar}), 130.4 (C_{Ar}-H), 129.4 (C_{Ar}), 128.4 (C_{Ar}-H), 127.6 (C_{Ar}-H), 127.1 (C_{Ar}-H), 124.2 (C_{Ar}), 123.7 (C_{Ar}-H), 119.1 (C_{Ar}-H), 118.3 (C_{Ar}-H), 118.3 (CN), 115.5 (C_{Ar}), 114.0 (C_{Ar}), 101.5 (C_{Ar}-H), 70.6 ($CH(Me)_2$), 51.7 (CHNH), 36.8 (CH_2), 22.7 (($CH_3)_2$), 18.1 (Ar-CH_3)

HRMS (ESI) calculated 840.2993 [M+H^+], 840.2988 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-3-ethynylbenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**41**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a slightly yellow solid.

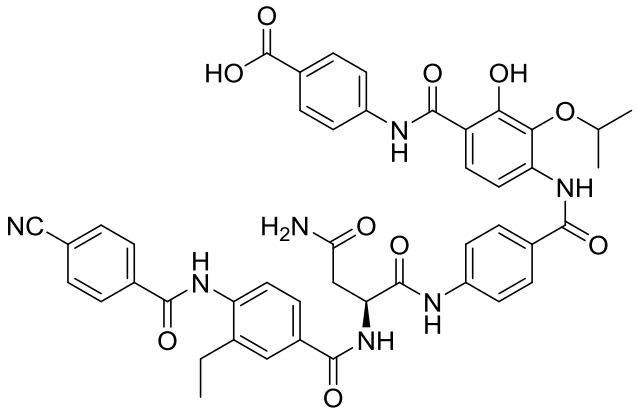
Yield: 2.7 mg (18 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.46 (s, 1H, CONH), 10.33 (s, 1H, CONH), 8.91 – 8.90 (m, 2H, CONH & NHCH), 8.16 – 8.13 (m, 3H, Ar-H), 8.05 (d, 2H, Ar-H, J = 8.3 Hz), 7.98 (dd, 1H, Ar-H, J = 1.8 Hz, 8.5 Hz), 7.87 – 7.82 (m, 5H, Ar-H), 7.81 (d, 2H, Ar-H, J = 8.8 Hz), 7.76 (d, 2H, Ar-H, J = 8.6 Hz), 7.47 (d, 1H, Ar-H, J = 8.7 Hz), 7.41 (s, 1H, $CONH_2$), 7.14 (d, 1H, Ar-H, J = 8.6 Hz), 6.98 (s, 1H, $CONH_2$), 4.98 (hept., 1H, $CH(Me)_2$, J = 5.9 Hz), 4.93 (dd, 1H, CHNH, J = 7.4 Hz, 13.9 Hz), 4.56 (s, 1H, ≡CH), 2.73 – 2.67 (m, 2H, CH_2), 1.20 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.2 ($CONH_2$), 170.5 (CONH), 167.5 (COO), 167.4 (CONH), 164.9 (CONH), 164.9 (C_{Ar}-O), 164.0 (CONH), 163.3 (CONH), 145.0 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.5 (C_{Ar}-NH), 138.0 (C_{Ar}), 137.5 (C_{Ar}-O), 134.5 (C_{Ar}-NH), 132.7 (C_{Ar}-H), 131.8 (C_{Ar}-H), 131.0 (C_{Ar}), 130.4 (C_{Ar}-H), 129.4 (C_{Ar}), 128.8 (C_{Ar}-H), 128.5 (C_{Ar}-H), 127.6 (C_{Ar}-H), 124.9 (C_{Ar}-H), 124.2 (C_{Ar}), 123.7 (C_{Ar}-H), 119.1 (C_{Ar}-H), 118.4 (C_{Ar}-H), 118.2 (CN), 117.0 (C_{Ar}-Alkyne), 115.4 (C_{Ar}), 114.3 (C_{Ar}), 101.7 (C_{Ar}-H), 86.7 (≡CH), 79.6 (-C≡), 70.7 ($CH(Me)_2$), 51.7 (CHNH), 36.8 (CH_2), 22.6 ($(CH_3)_2$)

HRMS (ESI) calculated 836.2680 [M+H$^+$], 836.2675 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)-3-ethylbenzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**42**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

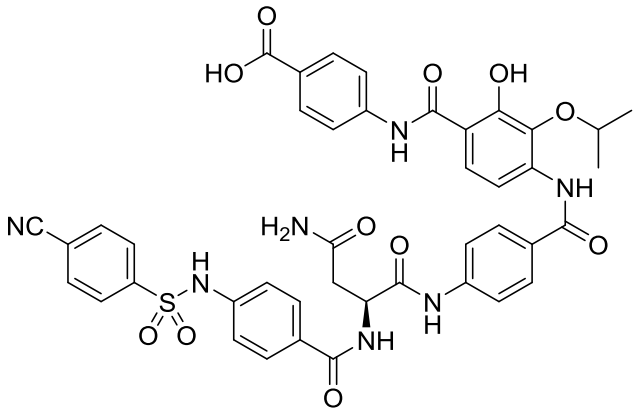
Yield: 8.8 mg (57 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.47 (s, 1H, CONH), 10.26 (s, 1H, CONH), 8.91 (s, 1H, CONH), 8.80 (m, 1H, NHCH), 8.14 (d, 2H, Ar-H, J = 8.3 Hz), 8.04 (d, 2H, Ar-H, J = 8.4 Hz), 7.86 – 7.84 (m, 5H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.7 Hz), 7.78 (dd, 1H, Ar-H, J = 1.8 Hz, 8.3 Hz), 7.75 (d, 2H, Ar-H, J = 8.6 Hz), 7.49 – 7.45 (m, 2H, Ar-H), 7.42 (s, 1H, $CONH_2$), 7.13 (d, 1H, Ar-H, J = 8.6 Hz), 6.99 (s, 1H, $CONH_2$), 4.99 (hept., 1H, $CH(Me)_2$, J = 6.0 Hz), 4.94 (dd, 1H, CHNH, J = 7.4 Hz, 14.0 Hz), 2.71 – 2.69 (m, 4H, CH_2 & $ArCH_2Me$), 1.20 (d, 6H, $(CH_3)_2$, J = 6.2 Hz), 1.18 (t, 3H, CH_3, J = 7.5 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3 ($CONH_2$), 170.6 (CONH), 167.6 (COO), 167.3 (CONH), 166.0 (CONH), 165.0 (C_{Ar}-O), 164.4 (CONH), 163.2 (CONH), 145.0 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 139.2 (C_{Ar}-CH_2), 138.4 (C_{Ar}), 138.2 (C_{Ar}-NH), 137.5 (C_{Ar}-O), 134.4 (C_{Ar}-NH), 132.6 (C_{Ar}-H), 132.0 (C_{Ar}), 130.4 (C_{Ar}-H), 129.4 (C_{Ar}), 128.5 (C_{Ar}-H), 128.0 (C_{Ar}-H), 127.6 (C_{Ar}-H), 126.8 (C_{Ar}-H), 125.5 (C_{Ar}-H), 124.2 (C_{Ar}), 123.7 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (C_{Ar}-H), 118.3 (CN), 115.4 (C_{Ar}), 114.0 (C_{Ar}), 101.5 (C_{Ar}-H), 70.7 ($CH(Me)_2$), 51.7 (CHNH), 36.8 (CH_2), 23.9 ($ArCH_2Me$), 22.6 (($CH_3)_2$), 14.0 (CH_3)

HRMS (ESI) calculated 840.2993 [M+H^+], 840.2989 found.

(*S*)-4-(4-(4-(4-amino-2-(4-((4-cyanophenyl)sulfonamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**47**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

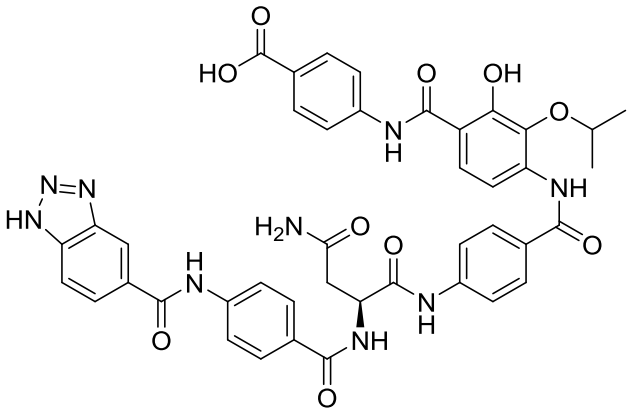
Yield: 8.3 mg (53 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.35 (s, 1H, CONH), 9.03 (s, 1H, OH), 8.39 (d, 1H, NHCH, J = 5.6 Hz), 7.93 (d, 2H, Ar-H, J = 8.2 Hz), 7.89 – 7.74 (m, 6H, Ar-H), 7.80 – 7.76 (m, 4H, Ar-H), 7.64 (d, 2H, Ar-H, J = 8.4 Hz), 7.56 (d, 1H, Ar-H, J = 6.9 Hz), 7.36 (s, 1H, $CONH_2$), 7.31 – 7.25 (m, 1H, Ar-H), 6.99 (d, 2H, Ar-H, J = 8.0 Hz), 6.94 (s, 1H, $CONH_2$), 4.90 – 4.82 (m, 2H, $CH(CH_3)_2$ & CHNH), 2.66 – 2.59 (m, 2H, CH_2), 1.21 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3 ($CONH_2$), 170.8 (CONH), 167.7 (CONH), 167.2 (COO), 166.1 (CONH), 163.5 (CONH), 147.5 (C_{Ar}-SO_2), 144.4 (C_{Ar}-NH), 142.1 (C_{Ar}-NH), 137.2 (C_{Ar}-O), 135.1 (C_{Ar}-NH), 132.9 (C_{Ar}-H), 130.4 (C_{Ar}-H), 129.0 (C_{Ar}), 128.5 (C_{Ar}-H), 127.8 (C_{Ar}-H), 127.1 (C_{Ar}-H), 124.2 (C_{Ar}), 123.4 (C_{Ar}-H), 119.2 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.0 (CN), 114.6 (C_{Ar}-H), 113.6 (C_{Ar}), 71.8 ($CH(Me)_2$), 51.5 (CHN), 36.8 (CH_2), 22.5 ($(CH_3)_2$)

HRMS (ESI) calculated 848.2350 [M+H$^+$], 848.2345 found.

(S)-4-(4-(4-(2-(4-(benzo[d][1,2,3]triazole-5-carboxamido)benzamido)-4-amino-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**48**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a slightly yellow solid.

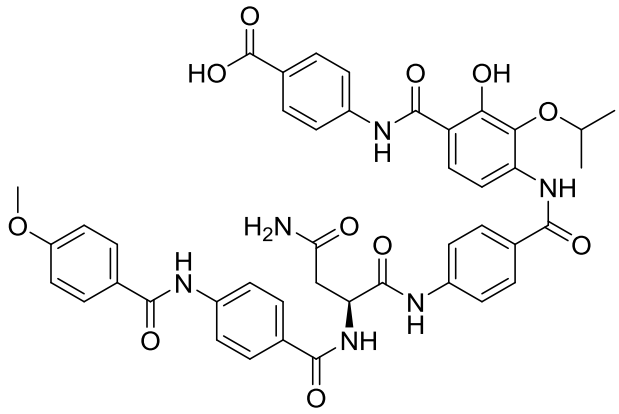
Yield: 6.2 mg (41 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.35 (s, 1H, CONH), 10.47 (s, 1H, CONH), 10.30 (s, 1H, CONH), 8.88 (s, 1H, CONH), 8.69 (d, 1H, NHCH, J = 6.3 Hz), 8.44 (s, 1H, Ar-H), 7.96 (d, 2H, Ar-H, J = 8.8 Hz), 7.90 (d, 2H, Ar-H, J = 8.7 Hz), 7.84 (d, 2H, Ar-H, J = 8.9 Hz), 7.82 (d, 2H, Ar-H, J = 9.0 Hz), 7.78 (d, 2H, Ar-H, J = 8.2 Hz), 7.66 (d, 1H, Ar-H, J = 8.5 Hz), 7.58 (d, 2H, Ar-H, J = 8.4 Hz), 7.55 (dd, 1H, Ar-H, J = 1.5 Hz, 8.5 Hz), 7.45 – 7.43 (m, 2H, Ar-H & $CONH_2$), 7.09 (d, 1H, Ar-H, J = 8.7 Hz), 6.99 (s, 1H, $CONH_2$), 5.02 (dt, 1H, $CH(Me)_2$, J = 6.2 Hz, 12.4 Hz), 4.92 (dd, 1H, CHNH, J = 7.1 Hz, 14.2 Hz), 2.69 (d, 2H, CH_2, J = 7.0 Hz), 1.20 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.4 ($CONH_2$), 170.7 (CONH), 170.4 (COO), 167.3 (CONH), 166.9 (CONH), 165.9 (CONH), 165.3 (C_{Ar}-O), 163.2 (CONH), 147.3 (C_{Ar}-N), 144.9 (C_{Ar}-N), 142.9 (C_{Ar}-NH), 141.8 (C_{Ar}-NH), 137.6 (C_{Ar}-O), 134.0 (C_{Ar}-NH), 133.4 (C_{Ar}), 129.7 (C_{Ar}-H), 129.5 (C_{Ar}), 128.2 (C_{Ar}-H), 128.0 (C_{Ar}), 127.6 (C_{Ar}-H), 125.0 (C_{Ar}), 123.7 (C_{Ar}-H), 119.1 (C_{Ar}-H), 119.1 (C_{Ar}-H), 119.0 (C_{Ar}-H), 117.6 (C_{Ar}-H), 116.7 (C_{Ar}-H), 116.0 (C_{Ar}), 115.3 (C_{Ar}-H), 100.7 (C_{Ar}-H), 70.4 ($CH(Me)_2$), 51.7 (CHNH), 36.9 (CH_2), 22.7 (($CH_3)_2$)

HRMS (ESI) calculated 828.2742 [$M+H^+$], 828.2737 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-methoxybenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**49**)



The Fmoc deprotected amino acid (18.9 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

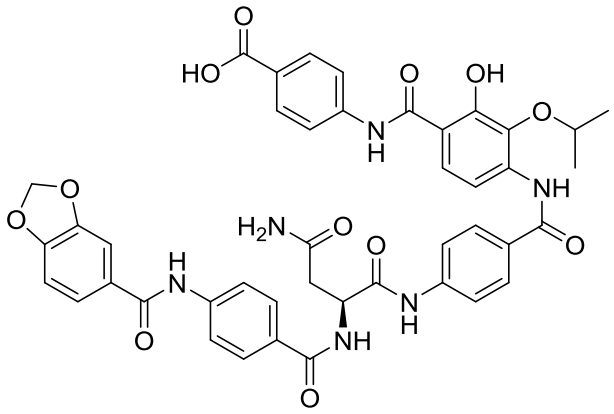
Yield: 6.4 mg (40 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.83 (br s, 1H, COOH), 12.29 (s, 1H, Ar-OH), 10.61 (s, 1H, CONH), 10.48 (s, 1H, CONH), 10.32 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.65 (d, 1H, NHCH, J = 7.3 Hz), 7.99 (d, 2H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.95 (d, 2H, Ar-H, J = 8.9 Hz), 7.92 – 7.88 (m, 4H, Ar-H), 7.87 – 7.85 (m, 3H, Ar-H & Ar-H), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.70 (d, 1H, Ar-H, J = 8.9 Hz), 7.41 (s, 1H, $CONH_2$), 7.08 (d, 2H, Ar-H, J = 8.9 Hz), 6.99 (s, 1H, $CONH_2$), 4.92 (quart., 1H, CHNH, J = 7.2 Hz), 4.54 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 3.85 (s, 3H, OCH_3), 2.69 (d, 2H, CH_2, J = 7.1 Hz), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3 ($CONH_2$), 170.8 (CONH), 168.5 (CONH), 166.9 (COO), 165.9 (CONH), 165.1 (CONH), 164.2 (CONH), 162.1 (C_{Ar}-OMe), 154.1 (C_{Ar}-O), 142.5 (C_{Ar}-NH), 142.3 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 137.1 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 130.2 (C_{Ar}-H), 129.7 (C_{Ar}-H), 128.4 (C_{Ar}), 128.3 (C_{Ar}-H), 128.3 (C_{Ar}), 128.2 (C_{Ar}-H), 126.6 (C_{Ar}), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.3 (C_{Ar}-H), 118.9 (C_{Ar}-H), 113.7 (C_{Ar}-H), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 55.5 (CH_3O), 51.7 (CHNH), 36.8 (CH_2), 22.3 (($CH_3)_2$)

HRMS (ESI) calculated 817.2833 [M+H$^+$], 817.2828 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(benzo[*d*][1,3]dioxole-5-carboxamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**50**)



The Fmoc deprotected amino acid (18.9 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

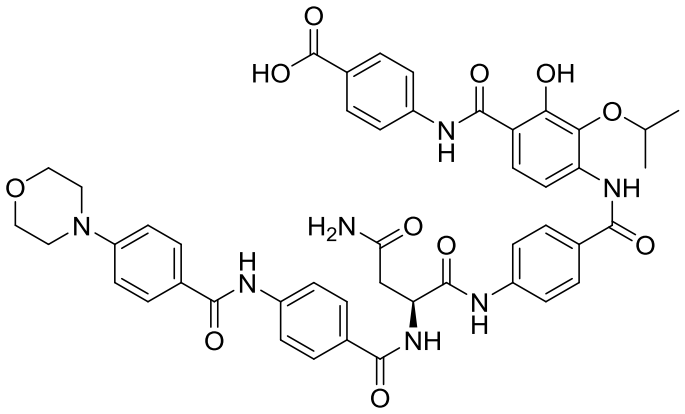
Yield: 6.6 mg (41 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.35 (s, 1H, CONH), 10.53 (s, 1H, CONH), 10.30 (s, 1H, CONH), 8.87 (s, 1H, CONH), 8.81 (d, 1H, NHCH, J = 6.0 Hz), 7.91 (d, 2H, Ar-H, J = 8.9 Hz), 7.87 (d, 2H, Ar-H, J = 8.9 Hz), 7.84 – 7.81 (m, 4H, Ar-H), 7.80 (d, 2H, Ar-H, J = 8.4 Hz), 7.61 (dd, 1H, Ar-H, J = 1.8 Hz, 8.2 Hz), 7.58 (d, 2H, Ar-H, J = 8.5 Hz), 7.54 (d, 1H, Ar-H, J = 1.8 Hz), 7.48 (s, 1H, $CONH_2$), 7.45 (d, 1H, Ar-H, J = 8.7 Hz), 7.08 (d, 1H, Ar-H, J = 8.7 Hz), 7.07 (d, 1H, Ar-H, J = 8.2 Hz), 6.98 (s, 1H, $CONH_2$), 6.14 (s, 2H, OCH_2O), 5.01 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 4.91 (dd, 1H, CHNH, J = 7.6 Hz, 13.9 Hz), 2.70 (dq, 2H, CH_2, J = 7.1 Hz, 15.2 Hz), 1.20 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.4 (CONH), 170.8 (CONH), 170.5 (COO), 166.9 (CONH), 165.8 (CONH), 165.3 (C_{Ar}-O), 164.8 (CONH), 163.2 (CONH), 150.2 (C_{Ar}-O), 147.4 (C_{Ar}-O), 142.1 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.8 (C_{Ar}-NH), 137.6 (C_{Ar}-O), 134.0 (C_{Ar}-NH), 133.3 (C_{Ar}), 129.7 (C_{Ar}-H), 129.5 (C_{Ar}), 128.6 (C_{Ar}), 128.4 (C_{Ar}), 128.3 (C_{Ar}-H), 127.6 (C_{Ar}-H), 123.7 (C_{Ar}-H), 123.1 (C_{Ar}-H), 119.3 (C_{Ar}-H), 119.1 (C_{Ar}-H), 117.6 (C_{Ar}-H), 116.0 (C_{Ar}), 108.0 (C_{Ar}-H), 107.8 (C_{Ar}-H), 101.9 (OCH_2O), 100.7 (C_{Ar}-H), 70.4 ($CH(Me)_2$), 51.8 (CHNH), 36.9 (CH_2), 22.7 ($(CH_3)_2$)

HRMS (ESI) calculated 831.2626 [M+H$^+$], 831.2621 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-morpholinobenzamido)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**51**)



The Fmoc deprotected amino acid (18.9 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

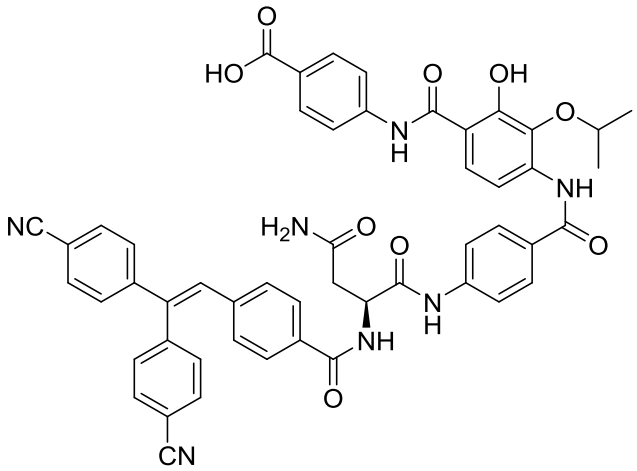
Yield: 2.7 mg (16 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.45 (s, 1H, CONH), 10.18 (s, 1H, CONH), 9.38 (s, 1H, CONH), 8.62 (d, 1H, NHCH, J = 7.3 Hz), 7.96 (d, 2H, Ar-H, J = 8.7 Hz), 7.95 (d, 2H, Ar-H, J = 8.9 Hz), 7.92 (d, 2H, Ar-H, J = 8.9 Hz), 7.90 – 7.88 (m, 4H, Ar-H), 7.86 – 7.83 (m, 4H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.7 Hz), 7.68 (d, 1H, Ar-H, J = 8.7 Hz), 7.40 (s, 1H, $CONH_2$), 7.04 (d, 2H, Ar-H, J = 9.0 Hz), 6.99 (s, 1H, $CONH_2$), 4.92 (quart., 1H, CHNH, J = 7.1 Hz), 4.55 (dt, 1H, $CH(Me)_2$, J = 6.0 Hz, 12.1 Hz), 3.76 – 3.74 (m, 4H, $O(CH_2)_2$), 3.28 – 3.25 (m, 4H, $N(CH_2)_2$), 2.69 (d, 2H, CH_2, J = 7.6 Hz), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.3 ($CONH_2$), 170.8 (CONH), 168.5 (CONH), 166.9 (COO), 165.9 (CONH), 165.2 (CONH), 164.2 (CONH), 153.4 (C_{Ar}-N), 142.5 (C_{Ar}-NH), 142.1 (C_{Ar}-NH), 137.0 (C_{Ar}-NH), 136.4 (C_{Ar}-O), 130.2 (C_{Ar}-H), 129.2 (C_{Ar}-H), 128.3 (C_{Ar}-H), 128.2 (C_{Ar}), 128.2 (C_{Ar}-H), 126.2 (C_{Ar}), 123.7 (C_{Ar}), 122.9 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.2 (C_{Ar}-H), 118.9 (C_{Ar}-H), 113.3 (C_{Ar}-H), 112.5 (C_{Ar}), 112.0 (C_{Ar}-H), 74.7 ($CH(Me)_2$), 65.9 ($O(CH_2)_2$), 51.6 (CHNH), 47.2 ($N(CH_2)_2$), 36.8 (CH_2), 22.3 ($(CH_3)_2$)

HRMS (ESI) calculated 872.3255 [M+H$^+$], 872.3251 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(2,2-bis(4-cyanophenyl)vinyl)benzamido)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**52**)



The Fmoc protected amino acid (17.8 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O2. The product was a white solid.

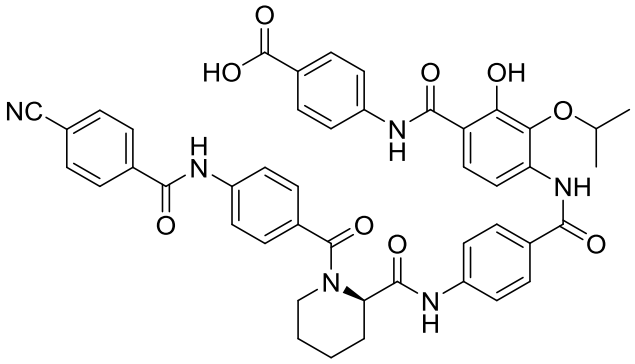
Yield: 4.8 mg (29 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.42 (s, 1H, CONH), 9.24 (br s, 1H, CONH), 8.67 (d, 1H, NHCH, J = 7.2 Hz), 7.94 – 7.90 (m, 6H, Ar-H), 7.85 (d, 2H, Ar-H, J = 8.5 Hz), 7.83 (d, 2H, Ar-H, J = 8.5 Hz), 7.79 (d, 2H, Ar-H, J = 8.7 Hz), 7.73 (d, 3H, Ar-H, J = 8.4 Hz), 7.50 (d, 2H, Ar-H, J = 8.4 Hz), 7.46 (s, 1H, CH=), 7.38 (d, 2H, Ar-H, J = 8.2 Hz), 7.36 (s, 1H, $CONH_2$), 7.12 (d, 2H, Ar-H, J = 8.4 Hz), 6.96 (s, 1H, $CONH_2$), 4.88 (dd, 1H, CHNH, J = 7.2 Hz, 14.0 Hz), 4.74 – 4.62 (br s, 1H, $CH(Me)_2$), 2.67 – 2.64 (m, 2H, CH_2), 1.24 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.2 ($CONH_2$), 170.2 (CONH), 168.1 (CONH), 167.0 (COO), 165.7 (CONH), 163.9 (CONH), 145.6 (C_{Ar}), 143.9 (C_{Ar}), 142.3 (C_{Ar}-NH), 140.2 (C=), 138.8 (C_{Ar}), 136.7 (C_{Ar}), 133.1 (C_{Ar}-H), 132.8 (C_{Ar}), 132.5 (C_{Ar}-H), 131.2 (CH=), 131.2 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.4 (C_{Ar}-H), 128.1 (C_{Ar}-H), 128.0 (C_{Ar}-H), 127.5 (C_{Ar}-H), 125.1 (C_{Ar}), 123.1 (C_{Ar}-H), 120.0 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.7 (CN), 118.6 (CN), 110.9 (C_{Ar}), 110.4 (C_{Ar}), 74.4 ($CH(Me)_2$), 51.6 (CHNH), 36.7 (CH_2), 22.4 ($(CH_3)_2$)

HRMS (ESI) calculated 896.3044 [M+H$^+$], 896.3039 found.

(*R*)-4-(4-(4-(1-(4-(4-cyanobenzamido)benzoyl)piperidine-2-carboxamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**57**)



The Fmoc deprotected amino acid (15.2 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O1. The product was a beige solid.

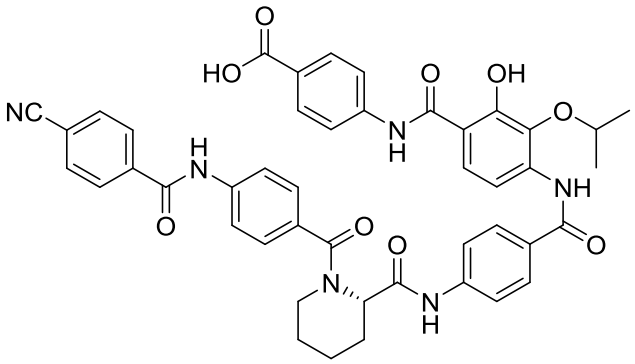
Yield: 7.0 mg (57 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.66 (s, 1H, CONH), 10.61 (s, 1H, CONH), 10.38 (br s, 1H, CONH), 9.42 (s, 1H, CONH), 8.11 (d, 2H, Ar-H, J = 7.7 Hz), 8.04 (d, 2H, Ar-H, J = 8.2 Hz), 7.98 – 7.96 (m, 4H, Ar-H), 7.89 – 7.84 (m, 5H, Ar-H), 7.84 – 7.80 (m, 2H, Ar-H), 7.71 (d, 1H, Ar-H, J = 8.8 Hz), 7.50 – 7.43 (m, 2H, Ar-H), 5.24 (br s, 1H, CHNH), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.25 – 2.20 (m, 1H, CH_2CH), 1.90 – 1.81 (m, 1H, CH_2CH), 1.70 (dd, 1H, CH_2, J = 3.1 Hz, 9.4 Hz), 1.50 – 1.43 (m, 2H, CH_2), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz), 1.25 – 1.21 (m, 3H, CH_2)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.6 (CON), 168.5 (CONH), 166.9 (COOH), 164.4 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.4 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 139.9 (C_{Ar}-NH), 138.8 (C_{Ar}-NH), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 127.8 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 120.0 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 53.1 (CHN), 45.7 (CH_2N), 28.7 (CH_2), 27.3 (CH_2CH), 22.3 (($CH_3)_2$), 20.2 (CH_2)

HRMS (ESI) calculated 809.2935 [M+H$^+$], 809.2929 found.

(*S*)-4-(4-(4-(1-(4-(4-cyanobenzamido)benzoyl)piperidine-2-carboxamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**58**)



The Fmoc deprotected amino acid (35.5 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O1. The product was a white solid.

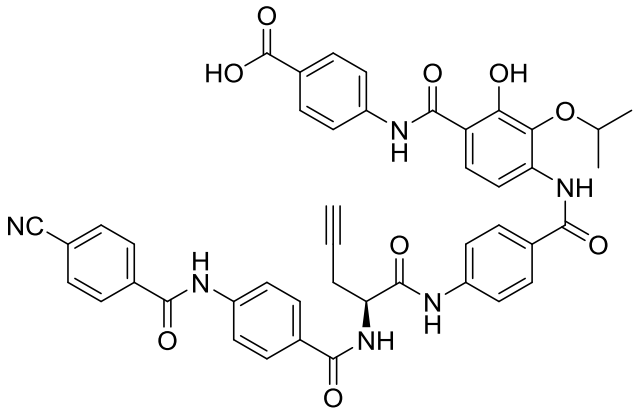
Yield: 9.0 mg (31 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.65 (s, 1H, CONH), 10.61 (s, 1H, CONH), 10.38 (s, 1H, CONH), 9.42 (s, 1H, CONH), 8.11 (d, 2H, Ar-H, J = 7.6 Hz), 8.04 (d, 2H, Ar-H, J = 8.2 Hz), 7.99 – 7.96 (m, 4H, Ar-H), 7.89 – 7.85 (m, 5H, Ar-H), 7.84 – 7.79 (m, 2H, Ar-H), 7.71 (d, 1H, Ar-H, J = 8.8 Hz), 7.50 – 7.43 (m, 1H, Ar-H), 5.24 (br s, 1H, CHNH), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.67 – 3.53 (m, 2H, CH_2N), 2.26 – 2.17 (m, 1H, CH_2CH), 1.90 – 1.82 (m, 1H, CH_2CH), 1.70 (dd, 1H, CH_2, J = 3.2 Hz, 9.3 Hz), 1.52 – 1.42 (m, 2H, CH_2), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz), 1.25 – 1.21 (m, 2H, CH_2)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.6 (CON), 168.5 (CONH), 166.9 (COOH), 164.4 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.4 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 139.9 (C_{Ar}-NH), 138.8 (C_{Ar}-NH), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 127.8 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 120.0 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 53.1 (CHN), 45.7 (CH_2N), 28.7 (CH_2), 27.3 (CH_2CH), 22.3 (($CH_3)_2$), 20.2 (CH_2)

HRMS (ESI) calculated 809.2935 [M+H$^+$], 809.2930 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**59**)



The Fmoc protected amino acid (54.5 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N2 and O1. The product was a yellowish solid.

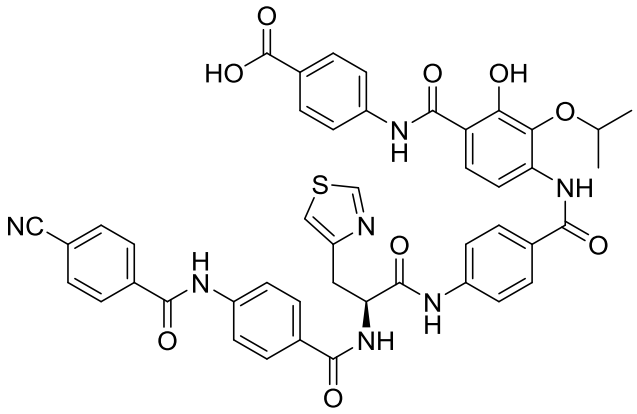
Yield: 9.7 mg (23 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.80 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.71 (s, 1H, CONH), 10.58 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.77 (d, 1H, NHCH, J = 7.5 Hz), 8.13 (d, 2H, Ar-H, J = 8.4 Hz), 8.05 (d, 2H, Ar-H, J = 8.4 Hz), 7.98 – 7.95 (m, 6H, Ar-H), 7.90 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.83 (d, 2H, Ar-H, J = 8.8 Hz), 7.70 (d, 1H, Ar-H, J = 8.8 Hz), 4.81 (dd, 1H, CHNH, J = 7.6 Hz, 14.7 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.94 (t, 1H, ≡CH, J = 2.6 Hz), 2.79 (dddd, 2H, CH_2, J = 2.6 Hz, 7.4 Hz, 11.1 Hz, 16.8 Hz), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.7 (CONH), 168.5 (CONH), 166.9 (COOH), 166.0 (CONH), 164.5 (CONH), 164.2 (CONH), 154.2 (C_{Ar}-OH), 142.2 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.4 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.9 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}-H), 126.2 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.1 (C_{Ar}-H), 80.6 (-C≡), 74.8 ($CH(Me)_2$), 73.2 (≡CH), 53.5 (CHNH), 22.3 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 793.2622 [$M+H^+$], 793.2617 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)-3-(thiazol-4-yl)propanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**60**)



The Fmoc protected amino acid (67.5 µmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N1 and O1. The product was a white solid.

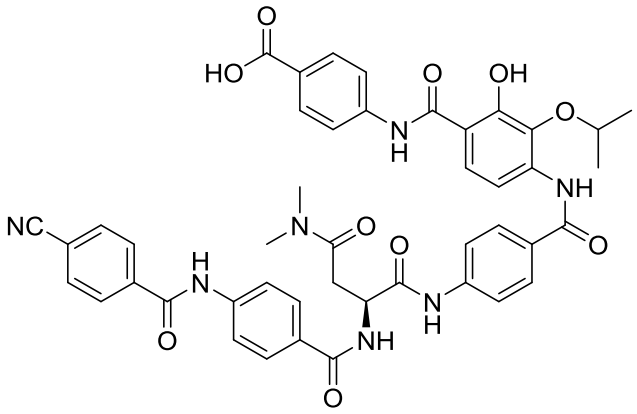
Yield: 9.1 mg (16 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.81 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.69 (s, 1H, CONH), 10.62 (br s, 1H, CONH), 10.56 (s, 1H, CONH), 9.40 (s, 1H, CONH), 9.06 (d, 1H, S-CH=N, J = 1.9 Hz), 8.73 (d, 1H, NHCH, J = 7.6 Hz), 8.12 (d, 2H, Ar-H, J = 8.5 Hz), 8.04 (d, 2H, Ar-H, J = 8.5 Hz), 7.98 – 7.94 (m, 4H, Ar-H), 7.90 – 7.87 (m, 4H, Ar-H), 7.87 – 7.84 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 1H, Ar-H, J = 8.8 Hz), 7.49 (d, 1H, S-CH=, J = 1.9 Hz), 5.02 (dd, 1H, CHNH, J = 7.8 Hz, 14.5 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.40 – 3.34 (m, 2H, CH_2), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.8 (CONH), 168.5 (CONH), 166.9 (COOH), 165.9 (CONH), 164.5 (CONH), 164.2 (CONH), 154.2 (C_{Ar}-OH), 153.8 (S-CH=N), 153.1 (C_{Ar}), 142.4 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.1 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}), 128.3 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 115.9 (C_{Ar}-H), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.1 (C_{Ar}-H), 74.8 ($CH(Me)_2$), 54.3 (CHNH), 32.9 (CH_2), 22.3 ($(CH_3)_2$)

HRMS (ESI) calculated 852.2452 [$M+H^+$], 852.2448 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)-4-(dimethylamino)-4-oxobutanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**61**)



The Fmoc deprotected amino acid (25.6 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O1. The product was a white solid.

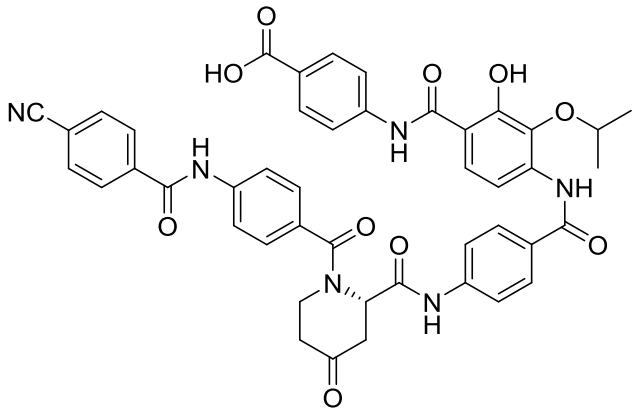
Yield: 7.3 mg (34 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.83 (br s, 1H, COOH), 12.29 (s, 1H, Ar-OH), 10.70 (s, 1H, CONH), 10.60 (s, 1H, CONH), 10.46 (s, 1H, CONH), 9.39 (s, 1H, CONH), 8.62 (d, 1H, NHCH, J = 7.1 Hz), 8.13 (d, 2H, Ar-H, J = 8.4 Hz), 8.04 (d, 2H, Ar-H, J = 8.4 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.95 (d, 2H, Ar-H, J = 8.8 Hz), 7.93 (d, 2H, Ar-H, J = 8.8 Hz), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.83 (d, 2H, Ar-H, J = 8.7 Hz), 7.70 (d, 1H, Ar-H, J = 8.8 Hz), 5.00 (quart., 1H, CHNH, J = 6.9 Hz), 4.54 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.01 (s, 3H, NCH_3), 2.95 – 2.88 (m, 2H, CH_2), 2.85 (s, 3H, NCH_3), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.8 (CONH), 169.2 ($CON(Me)_2$), 168.5 (CONH), 166.9 (COOH), 165.7 (CONH), 164.4 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.7 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.1 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.2 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.3 (C_{Ar}-H), 128.2 (C_{Ar}), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.9 (C_{Ar}-H), 118.3 (-CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 51.6 (CHNH), 36.6 (NCH_3), 34.9 (NCH_3), 34.5 (CH_2), 22.3 ($(CH_3)_2$)

HRMS (ESI) calculated 840.2993 [M+H$^+$], 840.2988 found.

(*S*)-4-(4-(4-(1-(4-(4-cyanobenzamido)benzoyl)-4-oxopiperidine-2-carboxamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**62**)



The Boc-deprotected amino acid (14.0 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a white solid.

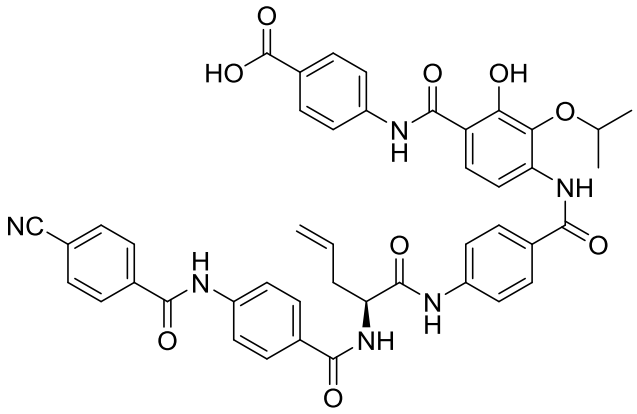
Yield: 2.7 mg (25 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.29 (s, 1H, Ar-OH), 10.68 (s, 1H, CONH), 10.60 (s, 1H, CONH), 10.58 (1H, CONH), 9.43 (s, 1H, CONH), 8.12 (d, 2H, Ar-H, J = 8.3 Hz), 8.05 (d, 2H, Ar-H, J = 8.4 Hz), 7.99 – 7.96 (m, 4H, Ar-H), 7.89 (d, 2H, Ar-H, J = 8.1 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.81 – 7.77 (m, 2H, Ar-H), 7.70 (d, 1H, Ar-H, J = 8.8 Hz), 7.55 (d, 2H, Ar-H, J = 6.8 Hz), 5.15 (br s, 1H, CHN), 4.54 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.99 – 3.91 (m, 1H, CH_2N), 3.88 – 3.83 (m, 1H, CH_2N), 3.09 (dd, 1H, CH_2CH, J = 6.9 Hz, 15.8 Hz), 2.75 (d, 1H, CH_2CH, J = 12.5 Hz), 2.54 (dd, 2H, CH_2CO, J = 4.5 Hz, 6.4 Hz), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 206.3 (C=O), 170.1 (CONH), 170.0 (CON), 168.5 (CONH), 166.9 (COOH), 164.5 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH),142.0 (C_{Ar}-NH), 140.4 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.4 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.7 (C_{Ar}), 130.2 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.1 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.9 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.5 (C_{Ar}), 112.3 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 55.0 (CHN), 43.4 (CH_2N), 41.1 (CH_2CH), 39.5 (CH_2CO), 22.3 (($CH_3)_2$)

HRMS (ESI) calculated 823.2728 [M+H$^+$], 823.2722 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-enamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**63**)



The Fmoc deprotected amino acid (49.8 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a beige solid.

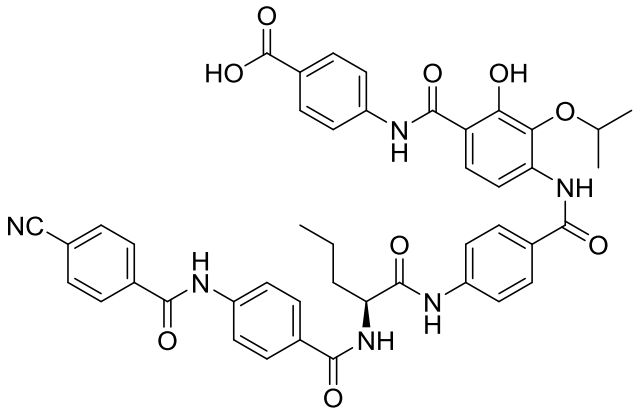
Yield: 12.8 mg (35 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.70 (s, 1H, CONH), 10.60 (s, 1H, CONH), 10.50 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.62 (d, 1H, NHCH, J = 7.5 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 7.98 – 7.95 (m, 6H, Ar-H), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.85 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.7 Hz), 7.71 (d, 1H, Ar-H, J = 8.8 Hz), 5.89 (ddt, 1H, CH=, J = 6.9 Hz, 10.2 Hz, 17.1 Hz), 5.20 (dd, 1H, =CH_2, J = 1.8 Hz, 17.2 Hz), 5.09 (dd, 1H, =CH_2, J = 1.9 Hz, 10.2 Hz), 4.70 (quart., 1H, CHNH, J = 7.5 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.62 (t, 2H, CH_2, J = 7.1 Hz), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.0 (CONH), 168.5 (CONH), 166.9 (COOH), 166.0 (CONH), 164.4 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.3 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 134.3 (CH=), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.1 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.8 (C_{Ar}-H), 118.3 (CN), 117.8 (=CH_2), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.1 (C_{Ar}-H), 74.8 ($CH(Me)_2$), 54.1 (CHNH), 35.7 (CH_2), 22.3 (($CH_3)_2$)

HRMS (ESI) calculated 795.2779 [M+H$^+$], 795.2774 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)pentanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**64**)



The Fmoc deprotected amino acid (40.3 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a beige solid.

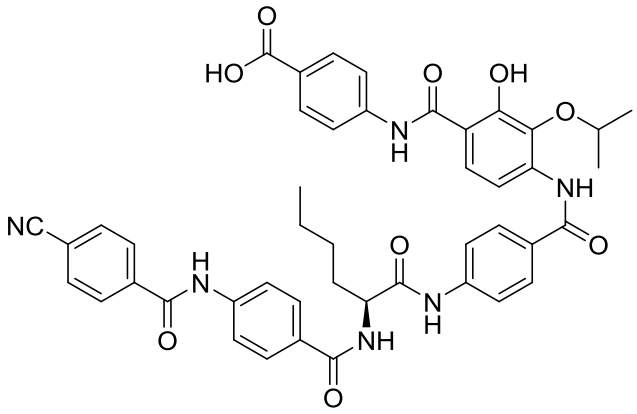
Yield: 14.0 mg (47 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.69 (s, 1H, CONH), 10.60 (s, 1H, CONH), 10.47 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.57 (d, 1H, NHCH, J = 7.4 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.4 Hz), 7.98 – 7.95 (m, 6H, Ar-H), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.85 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 1H, Ar-H, J = 8.9 Hz), 4.61 (dd, 1H, CHNH, J = 7.4 Hz, 14.7 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.87 – 1.78 (m, 2H, CH_2CH), 1.55 – 1.48 (m, 1H, CH_2CH_3), 1.43 – 1.36 (m, 1H, CH_2CH_3), 1.27 (dd, 6H, $(CH_3)_2$, J = 0.9 Hz, 6.1 Hz), 0.95 (t, 3H, CH_3, J = 7.4 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.9 (CONH), 168.5 (CONH), 166.9 (COOH), 166.1 (CONH), 164.4 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.5 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.5 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.1 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.2 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.3 (C_{Ar}), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.8 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 54.4 (CHNH), 33.5 (CH_2CH), 22.3 ($(CH_3)_2$), 19.2 (CH_2CH_3), 13.7 (CH_3)

HRMS (ESI) calculated 797.2935 [M+H$^+$], 797.2929 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)hexanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**65**)



The Fmoc deprotected amino acid (33.2 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a beige solid.

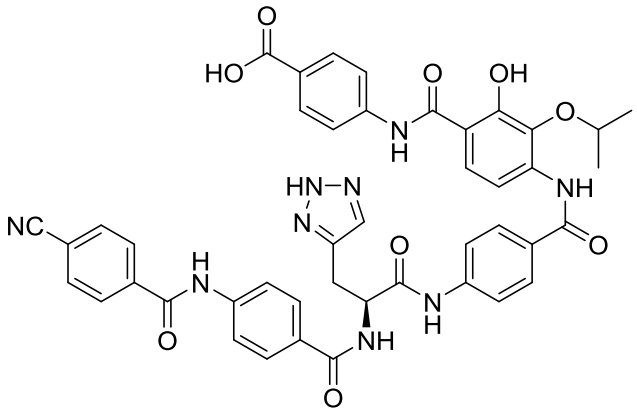
Yield: 10.9 mg (50 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.69 (s, 1H, CONH), 10.60 (s, 1H, CONH), 10.47 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.57 (d, 1H, NHCH, J = 7.4 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 7.98 – 7.95 (m, 6H, Ar-H), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 1H, Ar-H, J = 8.8 Hz), 4.59 (dd, 1H, CHNH, J = 7.3 Hz, 14.7 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.84 (dd, 2H, -CH_2-CH, J = 7.3 Hz, 14.5 Hz), 1.50 – 1.44 (m, 1H, -CH_2-), 1.38 – 1.33 (m, 3H, -CH_2- & -CH_2-Me), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz), 0.90 (t, 3H, -CH_3, J = 7.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.9 (CONH), 168.5 (CONH), 166.9 (COOH), 166.1 (CONH), 164.4 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.5 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.5 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.1 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.2 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.3 (C_{Ar}), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.8 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 54.7 (CHNH), 31.2 (-CH_2-CH), 28.1 (-CH_2-), 22.3 (($CH_3)_2$), 21.9 (-CH_2-Me), 13.9 (-CH_3)

HRMS (ESI) calculated 811.3092 [M+H$^+$], 811.3086 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)-3-(1,2,3-triazol-5-yl)propanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid[259] (66)



7.4 mg (S)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (9.3 µmol, 1 eq), 0.15 mg copper(II) sulfate (0.9 µmol, 0.1 eq), 1.1 mg sodium ascorbate (5.6 µmol, 0.6 eq) and 2.0 mg TBTA (3.7 µmol, 0.4 eq) were added to a dry flask and further dried under high vacuum. 0.2 ml DMSO and 0.1 mg THF were added under nitrogen atmosphere. 6.1 mg sodium azide was dissolved in 1.0 ml water and 0.1 ml of the solution was added to the mixture (9.3 µmol, 1 eq) under nitrogen atmosphere. The reaction was stirred at room temperature and controlled over LCMS. After completion, the crude product was purified by RP HPLC. The product was a white solid.

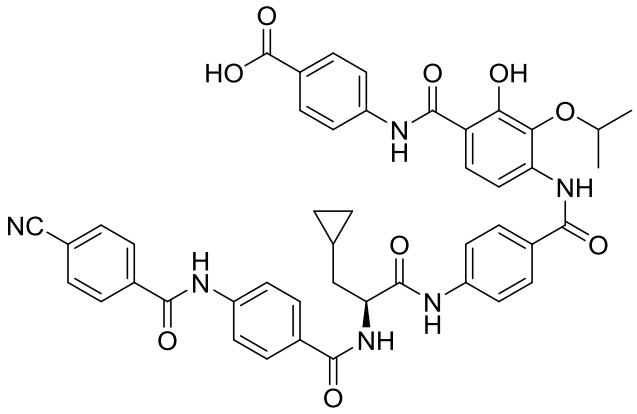
Yield: 3.8 mg (49 %)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.00 (br s, 0.3 H, N-NH), 14.64 (s, 0.6 H, N-NH), 12.82 (br s, 1H, COOH), 12.29 (s, 1H, Ar-OH), 10.70 (s, 1H, CONH), 10.60 (s, 1H, CONH), 10.55 (s, 1H, CONH), 9.41 (s, 1H, CONH), 8.77 (d, 1H, NHCH, J = 7.5 Hz), 8.13 (d, 2H, Ar-H, J = 8.4 Hz), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 7.98 – 7.95 (m, 4H, Ar-H), 7.92 (d, 2H, Ar-H, J = 8.8 Hz), 7.88 (d, 2H, Ar-H, J = 8.7 Hz), 7.87 – 7.85 (m, 3H, Ar-H), 7.81 (d, 2H, Ar-H, J = 8.6 Hz), 7.71 (d, 1H, Ar-H, J = 8.8 Hz), 7.65 (s, 0.6 H, CH=N), 4.93 (dd, 1H, CHNH, J = 7.7 Hz, 14.3 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.0 Hz), 3.30 – 3.21 (m, 2H, CH_2CH), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.6 (CONH), 168.5 (CONH), 166.9 (COOH), 166.0 (CONH), 164.5 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 143.3 (C=N), 142.3 (C_{Ar}-NH),142.0 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.9 (CH=N),132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.0 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.9 ($CH(Me)_2$), 54.3 (CHNH), 27.5 (CH_2CH), 22.3 (($CH_3)_2$)

HRMS (ESI) calculated 836.2792 [M+H$^+$], 836.2788 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)-3-cyclopropylpropanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**67**)



The Fmoc deprotected amino acid (65.5 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a faint yellow solid.

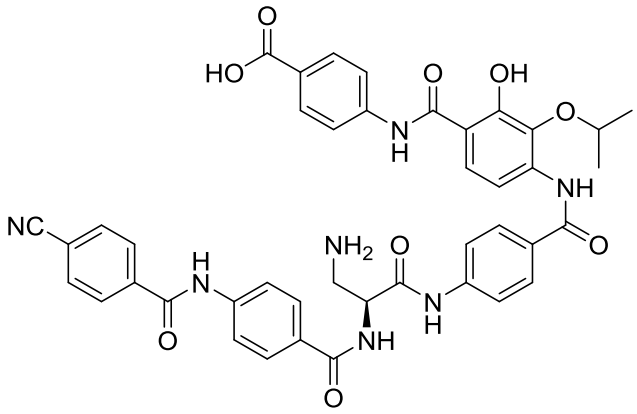
Yield: 20.4 mg (41 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.80 (br s, 1H, COOH), 12.31 (br s, 1H, Ar-OH), 10.69 (s, 1H, CONH), 10.65 (br s, 1H, CONH), 10.48 (s, 1H, CONH), 9.38 (s, 1H, CONH), 8.61 (d, 1H, NHCH, J = 7.4 Hz), 8.13 (d, 2H, Ar-H, J = 8.6 Hz), 8.04 (d, 2H, Ar-H, J = 8.5 Hz), 7.99 – 7.94 (m, 6H, Ar-H), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.72 (d, 1H, Ar-H, J = 8.8 Hz), 4.70 (dd, 1H, CHNH, J = 7.9 Hz, 14.0 Hz), 4.56 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 1.98 – 1.90 (m, 1H, CH_2), 1.62 – 1.55 (m, 1H, CH_2), 1.27 (dd, 6H, $(CH_3)_2$, J = 1.0 Hz, 6.1 Hz), 0.93 – 0.86 (m, 1H, $CH_{Cyclopr}$), 0.50 – 0.44 (m, 1H, CH_2-CH_2), 0.43 – 0.36 (m, 1H, CH_2-CH_2), 0.23 (td, 1H, CH_2-CH_2, J = 4.6 Hz, 8.9 Hz), 0.16 (td, 1H, CH_2-CH_2, J = 4.7 Hz, 9.0 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.7 (CONH), 168.5 (CONH), 166.9 (COOH), 166.1 (CONH), 164.4 (CONH), 164.2 (CONH), 154.2 (C_{Ar}-OH), 142.5 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.5 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.3 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.3 (C_{Ar}), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.8 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 74.8 ($CH(Me)_2$), 55.3 (CHNH), 36.3 (CH_2), 22.3 (($CH_3)_2$), 8.1 ($CH_{Cyclopr}$), 4.6 (CH_2), 4.0 (CH_2)

HRMS (ESI) calculated 809.2935 [M+H$^+$], 809.2929 found.

(*S*)-4-(4-(4-(3-amino-2-(4-(4-cyanobenzamido)benzamido)propanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**68**)



The Fmoc deprotected and Alloc protected amino acid (19.8 µmol) was coupled with fragment AB using procedure M. After deprotection of the *tert*-butyl ester using procedure O1, the crude product was purified by RP HPLC. The product was obtained by deprotection with procedure N1 followed by RP HPLC purification. The product was a white solid.

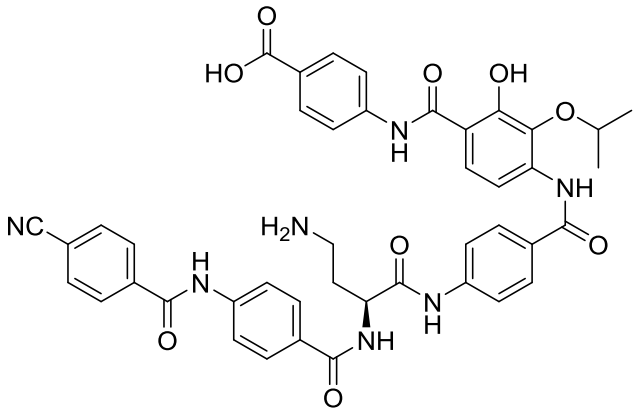
Yield: 2.2 mg (15 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.73 (s, 1H, CONH), 9.31 (s, 1H, CONH), 8.83 (d, 1H, NHCH, J = 6.9 Hz), 8.13 (d, 2H, Ar-H, J = 8.4 Hz), 8.05 (d, 2H, Ar-H, J = 8.4 Hz), 7.99 (d, 2H, Ar-H, J = 8.7 Hz), 7.97 – 7.94 (m, 4H, Ar-H), 7.92 (d, 2H, Ar-H, J = 8.8 Hz), 7.84 – 7.81 (m, 4H, Ar-H), 7.77 (d, 1H, Ar-H, J = 7.5 Hz), 7.59 – 7.52 (m, 1H, Ar-H), 4.90 (d, 1H, CHNH, J = 5.2 Hz), 4.68 – 4.59 (m, 1H, $CH(Me)_2$), 3.21 (dd, 1H, CH_2CH, J = 8.2 Hz, 13.0 Hz), 1.25 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 168.4 (CONH), 168.2 (CONH), 167.0 (COO), 166.5 (CONH), 164.5 (CONH), 164.0 (CONH), 141.9 (C_{Ar}-NH), 138.6 (C_{Ar}), 136.7 (C_{Ar}), 132.5 (C_{Ar}-H), 130.3 (C_{Ar}-H), 129.1 (C_{Ar}), 128.9 (C_{Ar}), 128.6 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.2 (C_{Ar}-H), 123.0 (C_{Ar}-H), 120.2 (C_{Ar}-H), 119.5 (C_{Ar}-H), 119.4 (C_{Ar}-H), 118.3 (CN), 114.1 (C_{Ar}), 52.7 (CHNH), 40.0 (CH_2CH), 22.4 (($CH_3)_2$)

HRMS (ESI) calculated 784.2731 [M+H$^+$], 784.2727 found.

(*S*)-4-(4-(4-(4-amino-2-(4-(4-cyanobenzamido)benzamido)butanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**69**)



The Fmoc deprotected amino acid (49.6 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N1 and O1. The product was a slightly beige solid.

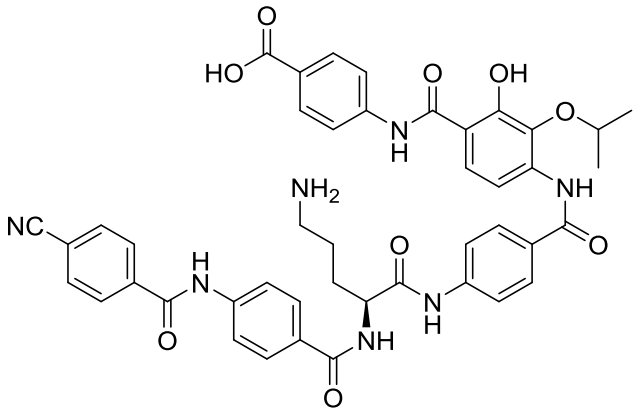
Yield: 19.1 mg (51 % over 3 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.73 (s, 1H, CONH), 10.52 (br s, 1H, CONH), 9.22 (s, 1H, CONH), 8.81 (d, 1H, NHCH, J = 6.7 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.04 (d, 2H, Ar-H, J = 8.5 Hz), 7.98 (d, 2H, Ar-H, J = 8.7 Hz), 7.96 – 7.89 (m, 6H, Ar-H), 7.84 – 7.80 (m, 4H, Ar-H), 7.72 (d, 1H, Ar-H, J = 8.8 Hz), 7.49 (d, 1H, Ar-H, J = 8.5 Hz), 4.76 – 4.67 (m, 2H, $CH(Me)_2$ & CHNH), 3.00 – 2.93 (m, 2H, CH_2N), 2.25 – 2.19 (m, 1H, CH_2CH), 2.17 – 2.09 (m, 1H, CH_2CH), 1.24 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.4 (CONH), 168.0 (CONH), 167.2 (COO), 166.3 (CONH), 164.5 (CONH), 163.8 (CONH), 158.0 (C_{Ar}-OH), 143.0 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 138.6 (C_{Ar}), 136.9 (C_{Ar}-O), 136.0 (C_{Ar}-NH), 132.5 (C_{Ar}-H), 130.3 (C_{Ar}-H), 129.0 (C_{Ar}), 128.9 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}-H), 128.1 (C_{Ar}-H), 125.6 (C_{Ar}), 123.2 (C_{Ar}-H), 119.8 (C_{Ar}-H), 119.5 (C_{Ar}-H), 119.1 (C_{Ar}-H), 118.3 (CN), 114.1 (C_{Ar}), 113.7 (C_{Ar}), 73.3 ($CH(Me)_2$), 52.2 (CHNH), 36.4 (CH_2N), 29.4 (CH_2CH), 22.4 ($(CH_3)_2$)

HRMS (ESI) calculated 798.2888 [M+H$^+$], 798.2884 found.

(*S*)-4-(4-(4-(5-amino-2-(4-(4-cyanobenzamido)benzamido)pentanamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**70**)



The Fmoc deprotected amino acid (17.5 µmol) was coupled with fragment AB using procedure M. After deprotection of the *tert*-butyl ester using procedure O1, the crude product was purified by RP HPLC. The product was obtained by deprotection with procedure N1 followed by RP HPLC purification. The product was a white solid.

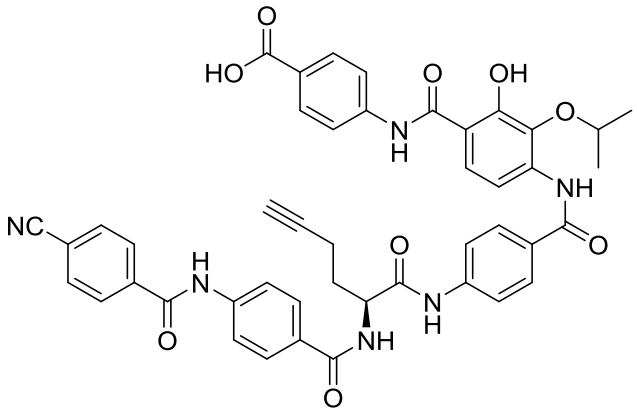
Yield: 6.4 mg (48 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 10.72 (s, 1H, CONH), 10.53 (s, 1H, CONH), 9.08 (br s, 1H, CONH), 8.67 (d, 1H, NHCH, J = 7.6 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.4 Hz), 7.98 (d, 2H, Ar-H, J = 8.8 Hz), 7.92 – 7.88 (m, 6H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.7 Hz), 7.79 (d, 2H, Ar-H, J = 8.6 Hz), 7.62 – 7.58 (m, 1H, Ar-H), 7.36 – 7.30 (m, 1H, Ar-H), 4.84 (br s, 1H, $CH(Me)_2$), 4.66 (dd, 1H, CHNH, J = 7.9 Hz, 14.2 Hz), 2.86 (t, 2H, CH_2N, J = 7.7 Hz), 1.95 – 1.85 (m, 2H, CH_2CH), 1.77 – 1.64 (m, 2H, CH_2), 1.22 (dd, 6H, $(CH_3)_2$, J = 2.5 Hz, 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.2 (CONH), 167.8 (CONH), 167.2 (COO), 166.1 (CONH), 164.5 (CONH), 163.6 (CONH), 144.1 (C_{Ar}), 142.0 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.1 (C_{Ar}), 135.4 (C_{Ar}), 132.5 (C_{Ar}-H), 130.4 (C_{Ar}-H), 129.2 (C_{Ar}), 129.1 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}-H), 128.0 (C_{Ar}), 128.0 (C_{Ar}-H), 123.4 (C_{Ar}-H), 119.5 (C_{Ar}-H), 119.2 (C_{Ar}-H), 118.9 (C_{Ar}-H), 118.3 (CN), 114.1 (C_{Ar}), 72.1 ($CH(Me)_2$), 53.8 (CHNH), 38.7 (CH_2N), 28.5 (CH_2CH), 24.0 (CH_2), 22.5 (($CH_3)_2$)

HRMS (ESI) calculated 812.3044 [$M+H^+$], 812.3040 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)hex-5-ynamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**71**)



The Fmoc deprotected amino acid (51.9 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a beige solid.

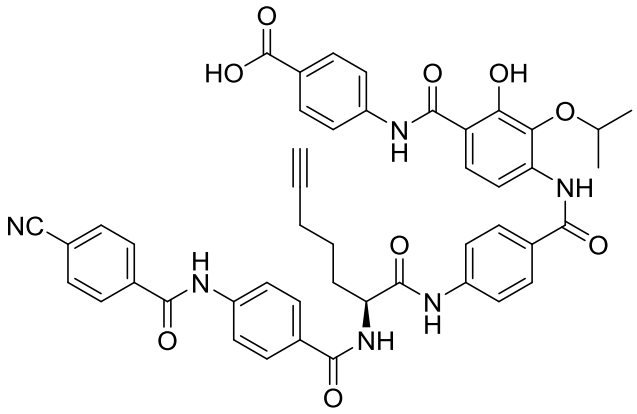
Yield: 21.4 mg (55 % over 3 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.81 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.69 (s, 1H, CONH), 10.60 (s, 1H, CONH), 10.47 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.63 (d, 1H, NHCH, J = 7.4 Hz), 8.13 (d, 2H, Ar-H, J = 8.6 Hz), 8.04 (d, 2H, Ar-H, J = 8.4 Hz), 7.99 – 7.95 (m, 6H, Ar-H), 7.90 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 1H, Ar-H, J = 8.9 Hz), 4.67 (dd, 1H, CHNH, J = 7.4 Hz, 14.5 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.85 (t, 1H, ≡CH, J = 2.6 Hz), 2.43 – 2.30 (m, 2H, CH_2C≡), 2.07 (dd, 2H, CH_2, J = 7.6 Hz, 14.7 Hz), 1.27 (dd, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.1 (CONH), 168.5 (CONH), 166.9 (COOH), 166.3 (CONH), 164.4 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.4 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.1 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}-H), 128.4 (C_{Ar}), 128.3 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.1 (C_{Ar}-H), 83.4 (C≡), 74.9 ($CH(Me)_2$),71.8 (≡CH), 53.9 (CHNH), 30.3 (CH_2), 22.3 (($CH_3)_2$), 15.2 (CH_2C≡)

HRMS (ESI) calculated 807.2779 [M+H$^+$], 807.2773 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)hept-6-ynamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**72**)



The Fmoc deprotected amino acid (55.3 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a beige solid.

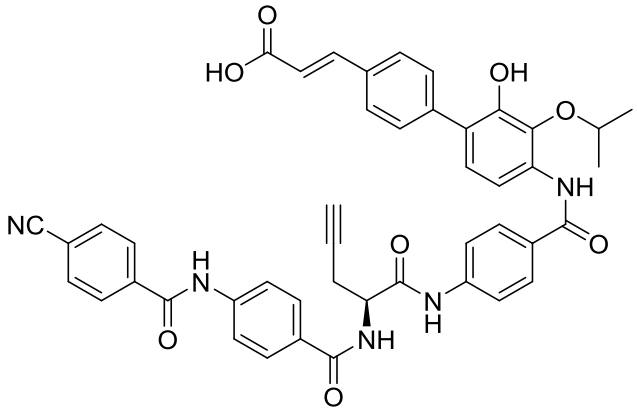
Yield: 13.6 mg (29 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.30 (s, 1H, Ar-OH), 10.70 (s, 1H, CONH), 10.61 (s, 1H, CONH), 10.49 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.62 (d, 1H, NHCH, J = 7.3 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 7.99 – 7.95 (m, 6H, Ar-H), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 1H, Ar-H, J = 8.8 Hz), 4.61 (dd, 1H, CHNH, J = 7.3 Hz, 14.6 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.81 (t, 1H, ≡CH, J = 2.6 Hz), 2.26 – 2.23 (m, 2H, CH_2C≡), 1.97 – 1.89 (m, 2H, CH_2CH), 1.71 – 1.65 (m, 1H, CH_2), 1.58 – 1.52 (m, 1H, CH_2), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.6 (CONH), 168.5 (CONH), 166.9 (COOH), 166.1 (CONH), 164.5 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.4 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 138.7 (C_{Ar}), 137.1 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.1 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.8 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 84.2 (C≡), 74.9 ($CH(Me)_2$), 71.6 (≡CH), 54.3 (CHNH), 30.7 (CH_2CH), 25.0 (CH_2), 22.3 (($CH_3)_2$), 17.6 (CH_2C≡)

HRMS (ESI) calculated 821.2935 [M+H$^+$], 821.2929 found.

(*S*,*E*)-3-(4'-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzamido)-2'-hydroxy-3'-isopropoxy-[1,1'-biphenyl]-4-yl)acrylic acid[260](**88**)



100 mg (S)-4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzoic acid (0.21 mmol, 2.1 eq), 40 mg allyl (E)-3-(4'-amino-3'-isopropoxy-2'-(methoxymethoxy)-[1,1'-biphenyl]-4-yl)acrylate (0.1 mmol, 1.0 eq) and 80.0 mg BEP (0.29 mmol, 2.9 eq) were added to a dry flask and further dried at high vacuum. 0.4 ml dry DCM was added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 90 µl dry DIPEA (66.8 mg, 0.52 mmol, 5.1 eq) was added to the stirring mixture under nitrogen atmosphere and the reaction was slowly warmed up to room temperature. The reaction was controlled over LCMS. After completion, the solvent was removed under reduced pressure. The crude product was mixed with DCM and the precipitate was filtered off. The solid was washed with DCM and the combined organic phases were concentrated under reduced pressure and dried under high vacuum. The crude product was deprotected by procedure O1 and purified by chromatography (petroleum ether/ ethyl acetate + 2 % AcOH). The product was obtained by deprotection with procedure N2 followed by RP HPLC. The product was a faint yellow solid.

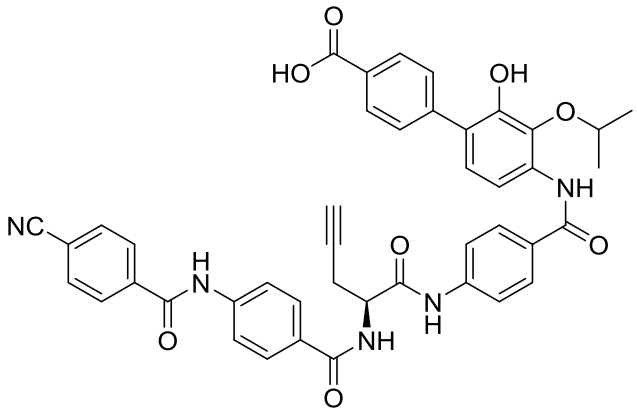
Yield: 3.2 mg (4 % over 4 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.35 (br s, 1H, COOH), 10.71 (s, 1H, CONH), 10.54 (s, 1H, CONH), 9.53 (s, 1H, CONH), 8.80 (s, 1H, Ar-OH), 8.76 (d, 1H, NHCH, J = 7.5 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 8.00 – 7.95 (m, 4H, Ar-H), 7.90 (d, 2H, Ar-H, J = 8.8 Hz), 7.80 (d, 2H, Ar-H, J = 8.8 Hz), 7.73 (d, 2H, Ar-H, J = 8.4 Hz), 7.65 – 7.61 (m, 3H, Ar-H & =CH), 7.32 (d, 1H, Ar-H, J = 8.4 Hz), 7.09 (d, 1H, Ar-H, J = 8.4 Hz), 6.55 (d, 1H, =CH, J = 16.0 Hz), 4.80 (dd, 1H, CHNH, J = 7.7 Hz, 14.6 Hz), 4.29 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 2.93 (t, 1H, ≡CH, J = 2.6 Hz), 2.79 (dddd, 2H, CH_2, J = 2.6 Hz, 7.4 Hz, 11.2 Hz, 16.8 Hz), 1.24 (d, 12H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.6 (CONH), 167.7 (COOH), 166.0 (CONH), 164.5 (CONH), 164.3 (CONH), 147.8 (C_{Ar}-OH), 143.7 (=CH), 141.8 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 140.1 (C_{Ar}), 139.1 (C_{Ar}-O), 138.7 (C_{Ar}), 132.5 (C_{Ar}-H), 132.5 (C_{Ar}), 131.8 (C_{Ar}-NH), 129.4 (C_{Ar}-H), 129.0 (C_{Ar}), 129.0 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.0 (C_{Ar}-H), 125.3 (C_{Ar}), 124.2 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.8 (C_{Ar}-H), 118.7 (=CH), 118.3 (CN), 116.3 (C_{Ar}-H), 114.1 (C_{Ar}), 80.7 (-C≡), 75.3 ($CH(Me)_2$), 73.2 (≡CH), 53.5 (CHNH), 22.0 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 776.2720 [M+H$^+$], 776.2714 found.

(*S*)-4'-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzamido)-2'-hydroxy-3'-isopropoxy-[1,1'-biphenyl]-4-carboxylic acid (**89**)



The Fmoc deprotected amino acid (0.63 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure O1. The product was a white solid.

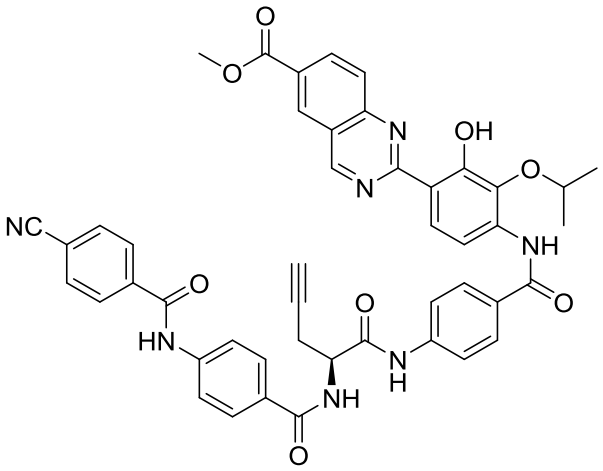
Yield: 16.4 mg (37 % over 2 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.85 (br s, 1H, COOH), 10.71 (s, 1H, CONH), 10.55 (s, 1H, CONH), 9.55 (s, 1H, CONH), 8.86 (s, 1H, Ar-OH), 8.77 (d, 1H, NHCH, J = 7.5 Hz), 8.13 (d, 2H, Ar-H, J = 8.4 Hz), 8.05 (d, 2H, Ar-H, J = 8.2 Hz), 8.00 – 7.95 (m, 6H, Ar-H), 7.91 (d, 2H, Ar-H, J = 8.8 Hz), 7.80 (d, 2H, Ar-H, J = 8.7 Hz), 7.71 (d, 1H, Ar-H, J = 8.3 Hz), 7.34 (d, 1H, Ar-H, J = 8.4 Hz), 7.11 (d, 1H, Ar-H, J = 8.4 Hz), 4.81 (dd, 1H, CHNH, J = 7.7 Hz, 14.7 Hz), 4.29 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.94 (t, 1H, ≡CH, J = 2.4 Hz), 2.79 (dddd, 2H, CH_2, J = 2.5 Hz, 7.4 Hz, 11.0 Hz, 16.8 Hz), 1.24 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.7 (CONH), 167.3 (COOH), 166.0 (CONH), 164.5 (CONH), 164.3 (CONH), 147.9 (C_{Ar}-OH), 142.7 (C_{Ar}), 141.9 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 139.1 (C_{Ar}-O), 138.7 (C_{Ar}), 132.5 (C_{Ar}-H), 132.1 (C_{Ar}-NH), 129.1 (C_{Ar}-H), 129.1 (C_{Ar}-H), 129.0 (C_{Ar}), 128.8 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}-H), 128.4 (C_{Ar}-H), 125.0 (C_{Ar}), 124.4 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.8 (C_{Ar}-H), 118.3 (CN), 116.3 (C_{Ar}-H), 114.1 (C_{Ar}), 80.7 (-C≡), 75.3 ($CH(Me)_2$), 73.2 (≡CH), 53.5 (CHNH), 22.0 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 750.2564 [M+H$^+$], 750.2557 found.

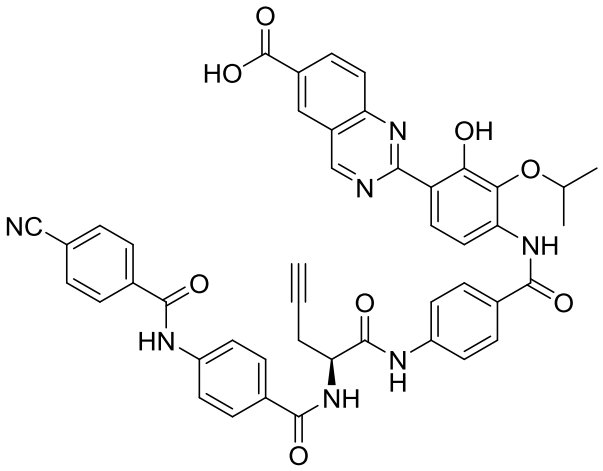
Methyl (*S*)-2-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxyphenyl)quinazoline-6-carboxylate[4]



90 mg (S)-4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzoic acid (187.3 µmol, 2.7 eq), 27.6 mg methyl 2-(2-(allyloxy)-4-amino-3-isopropoxyphenyl)quinazoline-6-carboxylate (70.2 µmol, 1 eq) were added to a dry vial and further dried under reduced pressure. 0.7 ml dry THF and 98.0 µl dry DIPEA (72.7 mg, 0.56 mmol, 8.0 eq) were added under nitrogen atmosphere and the mixture was cooled down to 0 °C. 17.0 µl phosphoryl chloride (28.0 mg , 182.4 µmol, 2.6 eq) was dissolved in 0.15 ml dry DCM and added very slowly under nitrogen atmosphere. The reaction was kept at 0 °C and controlled over LCMS. After completion, 1 ml saturated NH_4Cl solution and 19 ml brine were added. The aqueous phase was extracted with 3 x 10 ml ethyl acetate. The solvent was removed under reduced pressure. 2.2 ml dry THF and 35.0 µl aniline (35.8 mg, 0.38 mmol, 3.0 eq) were added under nitrogen atmosphere. 10.0 mg Tetrakis(triphenylphosphine)palladium(0) (8.7 µmol, 0.07 eq) was added to the mixture and it was stirred for 3 hours at room temperature. The reaction was controlled over LCMS. After completion, the crude product was purified by RP flash chromatography. The product was directly used in further reactions.

Yield: 13.7 mg (crude)

(*S*)-2-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxyphenyl)quinazoline-6-carboxylic acid (**90**)



13.7 mg crude methyl (S)-2-(4-(4-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxyphenyl)quinazoline-6-carboxylate (16.8 µmol, 1 eq) was suspended in 0.1 ml water and 0.05 ml THF. 1.5 mg lithium hydroxide (62.6 µmol, 3.7 eq) was added and the mixture. The reaction was controlled via LCMS. After completion, the crude product was purified by RP HPLC. The product was a yellow solid

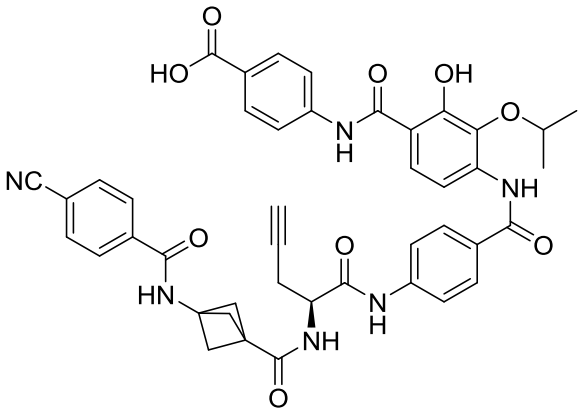
Yield: 3.5 mg (6 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 14.07 (s, 1H, Ar-OH), 10.72 (br s, 1H, CONH), 10.61 (br s, 1H, CONH), 9.92 (br s, 1H, Ar-H), 9.36 (br s, 1H, CONH), 8.83 (br s, 1H, Ar-H), 8.80 (d, 1H, NHCH, J = 7.6 Hz), 8.48 (dd, 1H, Ar-H, J = 1.5 Hz, 8.8 Hz), 8.37 (d, 1H, Ar-H, J = 9.0 Hz), 8.18 (d, 1H, Ar-H, J = 8.6 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.2 Hz), 7.98 – 7.96 (m, 4H, Ar-H), 7.90 (d, 2H, Ar-H, J = 8.8 Hz), 7.85 – 7.81 (m, 3H, Ar-H), 4.81 (dd, CHNH, J = 7.7 Hz, 14.7 Hz), 4.72 – 4.66 (m, 1H, $CH(Me)_2$), 2.93 (t, 1H, ≡CH, J = 2.6 Hz), 2.79 (dddd, 2H, CH_2, J = 2.6 Hz, 7.4 Hz, 11.2 Hz, 16.8 Hz), 1.32 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (156 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.7 (CONH), 166.4 (COO), 166.0 (CONH), 164.5 (CONH), 164.1 (CONH), 163.2 (C_{Ar}-H), 162.0 (C_{Ar}), 154.1 (C_{Ar}-OH), 149.0 (C_{Ar}), 142.2 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 138.7 (C_{Ar}), 136.6 (C_{Ar}), 136.0 (C_{Ar}-O), 135.1 (C_{Ar}-H), 132.5 (C_{Ar}-H), 130.6 (C_{Ar}-H), 129.6 (C_{Ar}), 128.7 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 128.3 (C_{Ar}-H), 126.6 (C_{Ar}-H), 124.2 (C_{Ar}-H), 122.2 (C_{Ar}), 119.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 115.6 (C_{Ar}), 114.0 (C_{Ar}), 112.1 (C_{Ar}-H), 80.7 (C≡), 74.6 ($CH(Me)_2$), 73.2 (≡CH), 53.3 (CHNH), 22.5 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 802.2625 [M+H$^+$], 802.2619 found.

(*S*)-4-(4-(4-(2-(3-(4-cyanobenzamido)bicyclo[1.1.1]pentane-1-carboxamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**99**)



The Fmoc protected amino acid (1.84 mmol) was deprotected and coupled with fragment AB using procedure L. The product was obtained by deprotection with procedure N2 and O1. The product was a white solid.

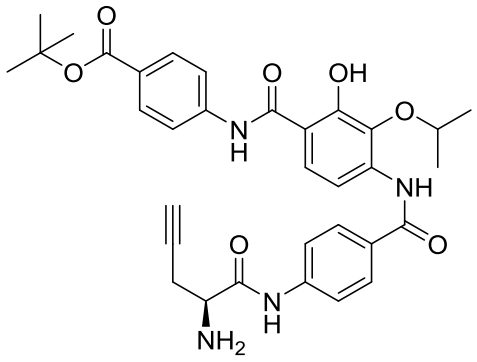
Yield: 818.8 mg (57 % over 3 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.81 (br s, 1H, COOH), 12.29 (s, 1H, Ar-OH), 10.60 (s, 1H, CONH), 10.52 (s, 1H, CONH), 9.40 (s, 1H, CONH), 9.30 (s, 1H, CONH), 8.21 (d, 1H, NHCH, J = 7.8 Hz), 8.00 (d, 2H, Ar-H, J = 8.7 Hz), 7.98 – 7.94 (m, 6H, Ar-H), 7.87 – 7.84 (m, 3H, Ar-H), 7.80 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 2H, Ar-H, J = 8.9 Hz), 4.62 (dd, 1H, CHNH, J = 7.7 Hz, 14.8 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 2.92 (t, 1H, ≡CH, J = 2.6 Hz), 2.65 (dddd, 2H, CH_2C≡, J = 2.6 Hz, 7.4 Hz, 10.6 Hz, 16.7 Hz), 2.32 (s, 6H, $(CH_3)_2$), 1.27 (d, 6H, $(CH_3)_2$, 6.2 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.4 (CONH), 168.7 (CONH), 168.5 (CONH), 166.9 (COOH), 165.2 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.1 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 138.1 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.4 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.6 (C_{Ar}), 128.4 (C_{Ar}-H), 128.1 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 118.9 (C_{Ar}-H), 118.3 (CN), 113.7 (C_{Ar}), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 80.3 (C≡), 74.9 ($CH(Me)_2$), 73.2 (≡CH), 53.6 (CH_2), 52.6 (CHNH), 45.3 (C_{aliph}-NH), 37.3 (C_{aliph}-CONH), 22.3 (($CH_3)_2$), 21.4 (CH_2C≡)

HRMS (ESI) calculated 783.2779 [M+H$^+$], 783.2774 found.

tert-Butyl (*S*)-4-(4-(4-(2-aminopent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoate



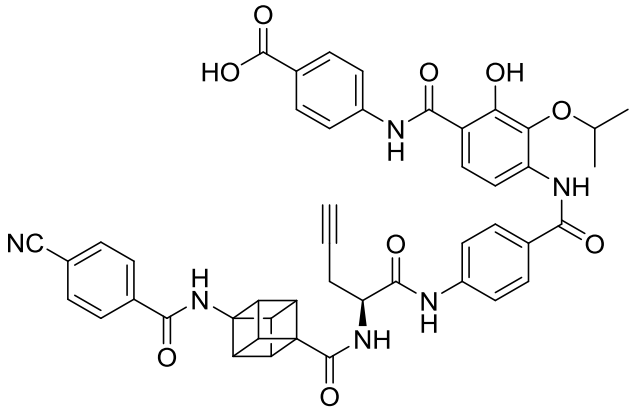
70 mg *tert*-butyl (S)-4-(4-(4-(2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoate (81.1 µmol, 1 eq) was deprotected by procedure N2 without purification. The crude product was dissolved in 0.8 ml acetonitrile and 0.3 ml diethylamine (2.9 mmol, 35.8 eq) at 0 °C and stirred for 1 hour. The reaction was controlled by LCMS. The solvent was removed under reduced pressure and coevaporated with acetonitrile 3 times. The crude product was purified by RP HPLC with acetonitrile and water mixed with 0.1 % HCOOH.

Yield: 31.9 mg (66 % over 2 steps)

^{1}H-NMR (500 MHz, $CDCl_3$, 300 K): δ (ppm) = 9.82 (br s, 1H, CONH), 8.86 (s, 1H, CONH), 8.58 (s, 1H, CONH), 8.08 (d, 1H, Ar-H, J = 8.9 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.84 (d, 2H, Ar-H, J = 8.7 Hz), 7.74 (d, 2H, Ar-H, J = 8.7 Hz), 7.69 (d, 2H, Ar-H, J = 8.8 Hz), 7.45 (d, 1H, Ar-H, J = 9.1 Hz), 4.85 (hept., 1H, $CH(Me)_2$, J = 6.2 Hz), 2.78 (dddd, 2H, CH_2, J = 2.5 Hz, 5.8 Hz, 9.8 Hz, 17.0 Hz), 2.09 (t, 1H, ≡CH, J = 2.6 Hz), 1.59 (s, 9H, $(CH_3)_3$), 1.34 (d, 6H, $(CH_3)_2$)

HRMS (ESI) calculated 601.2662 [M+H$^+$], 601.2657 found.

4-(4-(4-((*S*)-2-((1r,2R,3R,4R,5S,6S,7S,8R)-4-(4-cyanobenzamido)cubane-1-carboxamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**100**)



17.0 mg (1s,2R,3s,4r,5S,6r,7R,8S)-4-(4-cyanobenzamido)cubane-1-carboxylic acid (58.2 µmol, 1.1 eq) was added to a dry flask and further dried under high vacuum. 0.4 ml of dry DMF, 27.0 µl DIPEA (20.0 mg, 3.0 eq) and 22.0 mg HATU (57.9 µmol, 1.1 eq) were added under nitrogen atmosphere. The solution was stirred for 30 minutes. The solution was refered as [1]. 31.0 mg *tert*-butyl (S)-4-(4-(4-(2-aminopent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoate (51.6 µmol, 1 eq) was dissolved in 0.2 ml dry DMF under nitrogen atmosphere and cooled down to 0 °C. Solution [1] was added and the mixture was stirred at 0 °C. The reaction was controlled over LCMS. After completion, the reaction was quenched with a drop of aniline. 1 ml 1 M HCl and 9 ml brine were added and the aqueous phase was extracted with 3 x 4 ml ethyl acetate. The crude product was purified by chromatography with PE:EE + 2 % acetic acid. The product was obtained by deprotection with procedure O1 followed by RP HPLC. The product was a white solid.

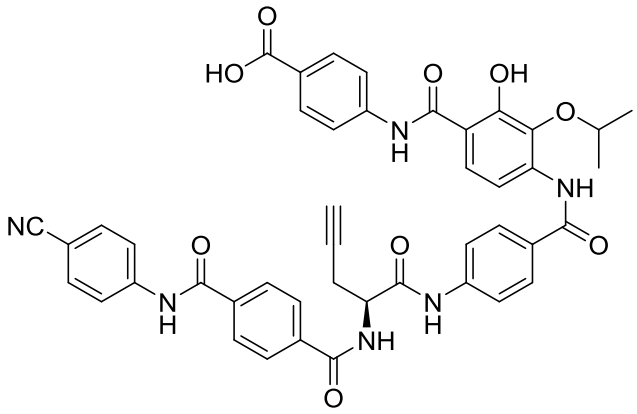
Yield: 10.6 mg (25 % over 2 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.80 (br s, 1H, COOH), 12.30 (br s, 1H, Ar-OH), 10.62 (br s, 1H, CONH), 10.50 (s, 1H, CONH), 9.45 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.15 (d, 1H, NHCH, J = 7.8 Hz), 8.04 (d, 2H, Ar-H, J = 8.6 Hz), 8.00 – 7.94 (m, 6H, Ar-H), 7.88 – 7.84 (m, 3H, Ar-H), 7.81 (d, 2H, Ar-H, J = 8.8 Hz), 7.71 (d, 1H, Ar-H, J = 8.9 Hz), 4.65 (dd, 1H, CHNH, J = 7.8 Hz, 14.8 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.15 – 4.09 (m, 6H, C_{cub}-H), 2.92 (t, 1H, ≡CH, J = 2.6 Hz), 2.66 (dddd, 2H, CH_2, J = 2.6 Hz, 7.3 Hz, 10.7 Hz, 16.7 Hz), 1.27 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.4 (CONH), 169.6 (CONH), 168.5 (CONH), 166.9 (COOH), 164.2 (CONH), 163.9 (CONH), 154.1 (C_{Ar}-OH), 142.1 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 137.6 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.5 (C_{Ar}), 128.4 (C_{Ar}-H), 128.1 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.3 (CN), 113.7 (C_{Ar}), 112.4 (C_{Ar}), 112.1 (C_{Ar}-H), 80.5 (-C≡), 74.8 ($CH(Me)_2$), 73.2 (≡CH), 66.4 (C_{cub}-NH), 56.8 (C_{cub}), 52.4 (CHNH), 49.3 (C_{cub}-H), 44.4 (C_{cub}-H), 22.3 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 819.2779 [M+H$^+$], 819.2771 found.

(*S*)-4-(4-(4-(2-(4-((4-cyanophenyl)carbamoyl)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**101**)



The Fmoc deprotected amino acid (46.8 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a white solid.

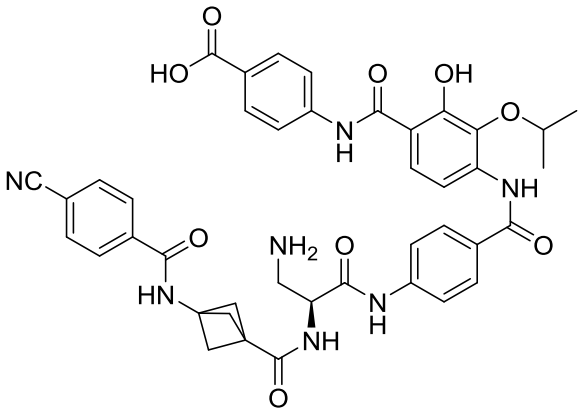
Yield: 14.0 mg (38 % over 3 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.80 (br s, 1H, COOH), 12.30 (br s, 1H, Ar-OH), 10.76 (s, 1H, CONH), 10.63 – 10.59 (m, 2H, CONH & CONH), 9.41 (br s, 1H, CONH), 9.05 (d, 1H, NHCH, J = 7.5 Hz), 8.08 (s, 4H, Ar-H), 8.01 (d, 2H, Ar-H, J = 8.9 Hz), 7.99 – 7.95 (m, 4H, Ar-H), 7.88 – 7.81 (m, 7H, Ar-H), 7.71 (d, 1H, Ar-H, J = 8.9 Hz), 4.84 (dd, 1H, CHNH, J = 7.8 Hz, 14.6 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.95 (t, 1H, ≡CH, J = 2.6 Hz), 2.88 – 2.75 (m, 2H, CH_2), 1.27 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.5 (CONH), 168.5 (CONH), 166.9 (COOH), 165.8 (CONH), 165.5 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 143.3 (C_{Ar}-NH), 142.1 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 137.0 (C_{Ar}-NH), 136.8 (C_{Ar}), 136.6 (C_{Ar}), 136.3 (C_{Ar}-O), 133.2 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.6 (C_{Ar}), 128.4 (C_{Ar}-H), 127.9 (C_{Ar}-H), 127.7 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 120.3 (C_{Ar}-H), 119.0 (CN), 119.0 (C_{Ar}-H), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 105.6 (C_{Ar}), 80.5 (C≡), 74.9 ($CH(Me)_2$), 73.2 (≡CH), 53.6 (CHNH), 22.3 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 793.2622 [M+H$^+$], 793.2615 found.

(*S*)-4-(4-(4-(3-amino-2-(3-(4-cyanobenzamido)bicyclo[1.1.1]pentane-1-carboxamido)propanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**102**)



The Fmoc deprotected and Alloc protected amino acid (106.6 µmol) was coupled with fragment AB using procedure M. After deprotection of the *tert*-butyl ester using procedure O1, the crude product was purified by RP HPLC. The product was obtained by deprotection with procedure N1 followed by RP HPLC purification. The product was a beige solid.

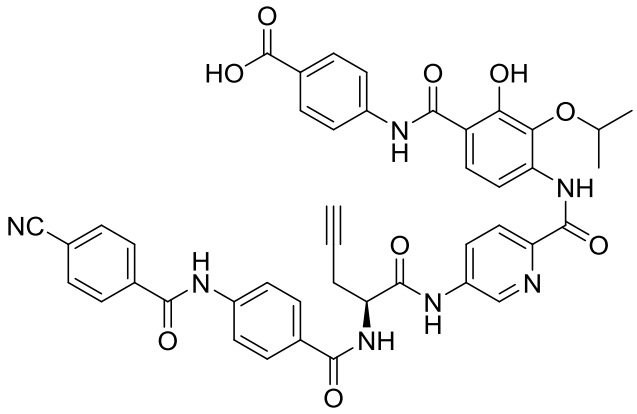
Yield: 36.4 mg (44 % over 3 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 9.32 (s, 1H, CONH), 9.17 (s, 1H, CONH), 8.42 – 8.26 (m, 1H, NHCH), 8.00 (d, 2H, Ar-H, J = 8.3 Hz), 7.97 – 7.89 (m, 6H, Ar-H), 7.85 – 7.78 (m, 4H, Ar-H), 7.69 (d, 1H, Ar-H, J = 8.5 Hz), 7.45 (d, 1H, Ar-H, J = 8.2 Hz), 4.75 (hept., 1H, $CH(Me)_2$, J = 5.9 Hz), 4.70 – 4.60 (m, 1H, CHNH), 3.28 – 3.00 (m, 2H, CH_2), 2.34 (s, 6H, $(CH_2)_3$), 1.24 (d, 6H, $(CH_3)_2$, J = 5.8 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.2 (CONH), 168.6 (CONH), 167.8 (COO), 167.5 (CONH), 165.2 (CONH), 163.7 (CONH), 143.1 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 138.1 (C_{Ar}), 136.9 (C_{Ar}-O), 135.8 (C_{Ar}-NH), 132.4 (C_{Ar}-H), 130.2 (C_{Ar}-H), 129.1 (C_{Ar}), 128.1 (C_{Ar}-H), 128.0 (C_{Ar}-H), 127.3 (C_{Ar}), 127.0 (C_{Ar}), 123.3 (C_{Ar}-H), 119.6 (C_{Ar}-H), 119.2 (C_{Ar}-H), 118.3 (CN), 114.0 (C_{Ar}), 113.7 (C_{Ar}), 107.5 (C_{Ar}-H), 73.0 ($CH(Me)_2$), 53.6 ($(CH_2)_3$), 45.4 (C_{quat}-NH), 37.5 (C_{quat}), 22.5 ($(CH_3)_2$)

HRMS (ESI) calculated 774.2888 [M+H$^+$], 774.2884 found.

(*S*)-4-(4-(5-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)picolinamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**103**)



The Fmoc deprotected amino acid (154.3 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a brown to beige solid.

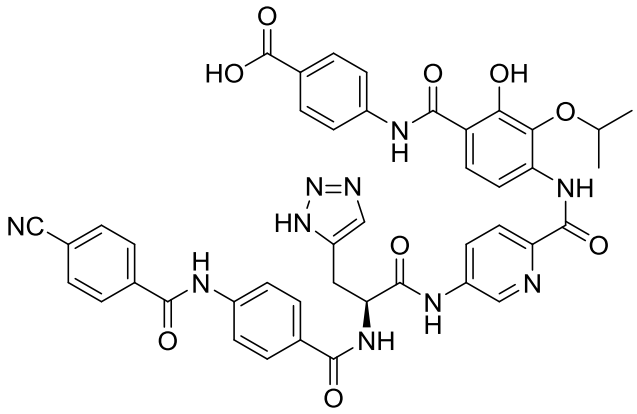
Yield: 62.4 mg (51 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.45 (br s, 1H, Ar-OH), 10.87 (br s, 1H, CONH), 10.75 (s, 1H, CONH), 10.71 (s, 1H, CONH), 10.61 (br s, 1H, CONH), 9.01 (d, 1H, Ar-H, J = 2.3 Hz), 8.84 (d, 1H, NHCH, J = 7.4 Hz), 8.33 (dd, 1H, Ar-H, J = 2.3 Hz, 8.6 Hz), 8.20 (d, 1H, Ar-H, J = 8.6 Hz), 8.13 (d, 2H, Ar-H, J = 8.5 Hz), 8.11 (d, 1H, Ar-H, J = 8.9 Hz), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 7.98 – 7.96 (m, 4H, Ar-H), 7.92 – 7.89 (m, 3H, Ar-H), 7.86 (d, 2H, Ar-H, J = 8.7 Hz), 4.81 (dd, 1H, CHNH, J = 7.4 Hz, 14.7 Hz), 4.68 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.93 (t, 1H, ≡CH, J = 2.6 Hz), 2.81 (dddd, 2H, CH_2, J = 2.5 Hz, 7.4 Hz, 11.0 Hz, 16.8 Hz), 1.34 (t, 6H, $(CH_3)_2$, J = 5.8 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.3 (CONH), 168.7 (CONH), 166.9 (COOH), 166.0 (CONH), 164.5 (CONH), 161.3 (CONH), 154.2 (C_{Ar}-OH), 143.5 (C_{Ar}), 141.9 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 139.5 (C_{Ar}-H), 138.7 (C_{Ar}-NH), 136.8 (C_{Ar}-NH), 134.0 (C_{Ar}-O), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.8 (C_{Ar}), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}-H), 127.3 (C_{Ar}-H), 126.3 (C_{Ar}), 123.5 (C_{Ar}-H), 122.9 (C_{Ar}-H), 120.8 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.3 (CN), 114.1 (C_{Ar}), 108.3 (C_{Ar}-H), 80.5 (C≡), 74.8 ($CH(Me)_2$), 73.3 (≡CH), 53.5 (CHNH), 22.3 (($CH_3)_2$), 22.3 (($CH_3)_2$), 21.2 (CH_2)

HRMS (ESI) calculated 794.2575 [M+H$^+$], 794.2570 found.

(*S*)-4-(4-(5-(2-(4-(4-cyanobenzamido)benzamido)-3-(1,2,3-triazol-5-yl)propanamido)picolinamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**104**)[259]



20.0 mg (S)-4-(4-(5-(2-(4-(4-cyanobenzamido)benzamido)pent-4-ynamido)picolinamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (25.2 µmol, 1 eq), 0.6 mg copper(II) sulfate pentahydrate (2.4 µmol, 0.1 eq), 3.0 mg sodium ascorbate (15.1 µmol, 0.6 eq) and 5.4 mg TBTA (10.2 µmol, 0.4 eq) were added to a dry flask and further dried under high vacuum. 0.3 ml DMSO and 0.1 mg THF were added under nitrogen atmosphere. 4.0 mg sodium azide (30.8 µmol, 1.2 eq) was dissolved in 0.2 ml water and 0.1 ml of the solution was added to the mixtureunder nitrogen atmosphere. The reaction was stirred at room temperature and controlled over LCMS. After completion, the crude product was purified by RP HPLC. The product was a brown to beige solid.

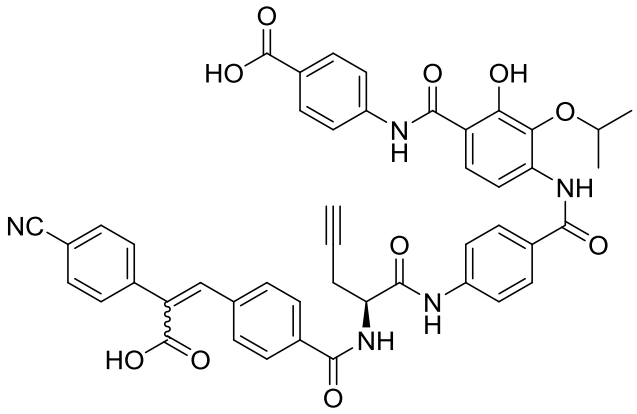
Yield: 11.4 mg (54 %)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 15.01 (br s, 0.3 H N-NH), 14.65 (br s, 0.6 H, N-NH), 12.82 (br s, 1H, COOH), 12.45 (s, 1H, Ar-OH), 10.82 (br s, 1H, CONH), 10.75 (s, 1H, CONH), 10.70 (s, 1H, CONH), 10.60 (br s, 1H, CONH), 9.00 (d, 1H, Ar-H, J = 2.4 Hz), 8.85 – 8.78 (m, 1H, NHCH), 8.31 (dd, 1H, Ar-H, J = 1.5 Hz, 8.6 Hz), 8.19 (d, 1H, Ar-H, J = 8.5 Hz), 8.14 – 8.10 (m, 3H, Ar-H), 8.05 (d, 2H, Ar-H, J = 8.5 Hz), 7.97 (d, 2H, Ar-H, J = 8.7 Hz), 7.94 – 7.90 (m, 3H, Ar-H), 7.89 (d, 2H, Ar-H, J = 8.8 Hz), 7.86 (d, 2H, Ar-H, J = 8.8 Hz), 7.66 (br s, 0.6H, CH=N), 4.95 – 4.90 (m, 1H, CHNH), 4.68 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 3.35 – 3.23 (m, CH_2), 1.35 (t, 6H, $(CH_3)_2$, J = 6.4 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 171.2 (CONH), 168.7 (CONH), 166.9 (COOH), 166.0 (CONH), 164.5 (CONH), 161.3 (CONH), 154.1 (C_{Ar}-OH), 143.4 (C_{Ar}), 143.2 (C_{Ar}), 141.9 (C_{Ar}-NH), 141.7 (C_{Ar}-NH), 139.5 (C_{Ar}-H), 138.8 (C_{Ar}-NH), 138.7 (C_{Ar}), 136.8 (C_{Ar}-NH), 134.0 (C_{Ar}-O), 133.0 (CH=N), 132.5 (C_{Ar}-H), 130.2 (C_{Ar}-H), 128.9 (C_{Ar}), 128.6 (C_{Ar}-H), 128.4 (C_{Ar}-H), 127.3 (C_{Ar}-H), 126.3 (C_{Ar}), 123.5 (C_{Ar}-H), 122.9 (C_{Ar}-H), 120.8 (C_{Ar}-H), 119.5 (C_{Ar}-H), 118.4 (CN), 114.1 (C_{Ar}), 111.6 (C_{Ar}), 108.4 (C_{Ar}-H), 74.8 ($CH(CH_3)_2$), 54.3 (CHNH), 27.3 (CH_2), 22.3 (($CH_3)_2$), 22.3 (($CH_3)_2$)

HRMS (ESI) calculated 837.2745 [M+H$^+$], 837.2737 found.

(*S*)-4-(4-(4-(2-(4-(2-carboxy-2-(4-cyanophenyl)vinyl)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**105**)



The Fmoc deprotected amino acid (23.4 µmol) was coupled with fragment AB using procedure M. After deprotection of the *tert*-butyl ester using procedure O1, the crude product was purified by RP HPLC. The product was obtained by deprotection with procedure N1 followed by RP HPLC purification. The product was a white solid.

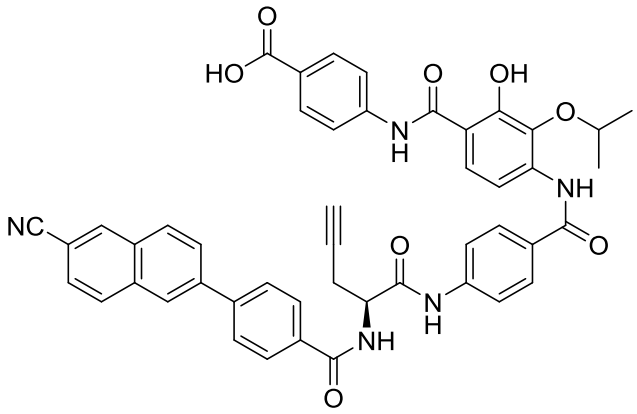
Yield: 2.2 mg (11 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 2H, COOH), 12.29 (br s, 1H, Ar-OH), 10.62 (s, 1H, CONH), 10.53 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.82 (d, 1H, NHCH, J = 7.5 Hz), 7.79 (d, 2H, Ar-H, J = 8.8 Hz), 7.95 (d, 2H, Ar-H, J = 8.8 Hz), 7.91 (s, 1H, =CH), 7.86 – 7.84 (m, 5H, Ar-H), 7.79 (d, 2H, Ar-H, J = 8.8 Hz), 7.76 (d, 2H, Ar-H, J = 8.5 Hz), 7.70 (d, 1H, Ar-H, J = 8.9 Hz), 7.41 (d, 2H, Ar-H, J = 8.5 Hz), 7.15 (d, 2H, Ar-H, J = 8.5 Hz), 4.75 (dd, 1H, CHNH, J = 7.8 Hz, 14.6 Hz), 4.54 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.91 (t, 1H, ≡CH, J = 2.6 Hz), 2.79 – 2.68 (m, 2H, CH_2), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.5 (CONH), 168.5 (CONH), 167.3 (COOH), 166.9 (COOH), 165.8 (CONH), 164.4 (CONH), 154.2 (C_{Ar}-OH), 142.1 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.3 (C_{Ar}), 139.3 (=CH-), 137.0 (C_{Ar}-NH), 136.4 (C_{Ar}-O), 133.8 (C_{Ar}), 133.5 (C=) 132.4 (C_{Ar}-H), 130.9 (C_{Ar}-H), 130.2 (C_{Ar}-H), 130.0 (C_{Ar}-H), 128.6 (C_{Ar}), 128.4 (C_{Ar}-H), 127.6 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.8 (CN), 112.5 (C_{Ar}), 112.2 (C_{Ar}-H), 110.5 (C_{Ar}), 80.6 (C≡), 74.9 ($CH(Me)_2$), 73.2 (≡CH), 53.5 (CHNH), 22.3 ($(CH_3)_2$), 21.3 (CH_2)

HRMS (ESI) calculated 820.2619 [$M+H^+$], 820.2614 found.

(*S*)-4-(4-(4-(2-(4-(6-cyanonaphthalen-2-yl)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**112**)



The Fmoc deprotected amino acid (54.6 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a slightly yellow solid.

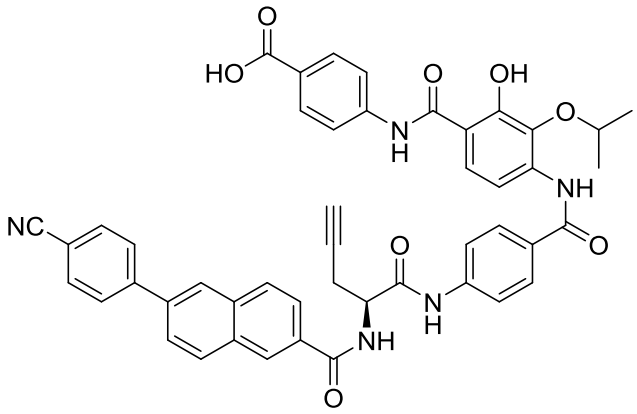
Yield: 21.0 mg (46 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (s, 1H, COOH), 12.31 (br s, 1H, Ar-OH), 10.61 (br s, 2H, CONH & CONH), 9.41 (s, 1H, CONH), 8.98 (d, 1H, NHCH, J = 7.5 Hz), 8.63 (s, 1H, Ar-H), 8.48 (s, 1H, Ar-H), 8.21 (d, 1H, Ar-H, J = 8.7 Hz), 8.19 (d, 1H, Ar-H, J = 8.8 Hz), 8.12 – 8.09 (m, 3H, Ar-H), 8.02 (d, 2H, Ar-H, J = 8.5 Hz), 7.99 – 7.96 (m, 4H, Ar-H), 7.88 – 7.82 (m, 6H, Ar-H), 7.71 (d, 1H, Ar-H, J = 8.9 Hz), 4.86 (dd, 1H, CHNH, J = 7.6 Hz, 14.7 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.96 (t, 1H, ≡CH, J = 2.6 Hz), 2.88 – 2.78 (m, 2H, CH_2), 1.27 (d, 6H, $(CH_3)_2$, J = 6.2 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.6 (CONH), 168.5 (CONH), 166.9 (COOH), 166.1 (CONH), 164.2 (CONH), 154.2 (C_{Ar}-OH), 142.2 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 139.5 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 134.7 (C_{Ar}), 134.1 (C_{Ar}-H), 133.2 (C_{Ar}), 131.4 (C_{Ar}), 130.2 (C_{Ar}-H), 129.8 (C_{Ar}-H), 129.3 (C_{Ar}-H), 128.6 (C_{Ar}), 128.5 (C_{Ar}-H), 128.4 (C_{Ar}-H), 127.1 (C_{Ar}-H), 126.9 (C_{Ar}-H), 126.8 (C_{Ar}-H), 126.3 (C_{Ar}), 125.9 (C_{Ar}-H), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.2 (CN), 119.0 (C_{Ar}-H), 112.4 (C_{Ar}), 112.2 (C_{Ar}-H), 108.6 (C_{Ar}), 80.6 (-C≡), 74.6 ($CH(Me)_2$), 73.2 (≡CH), 53.6 (CHNH), 22.3 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 800.2720 [M+H$^+$], 800.2714 found.

(*S*)-4-(4-(4-(2-(6-(4-cyanophenyl)-2-naphthamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**113**)



The Fmoc deprotected amino acid (54.6 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a slightly yellow solid.

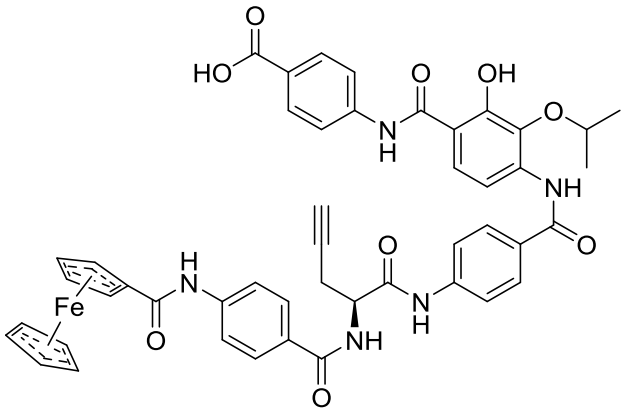
Yield: 10.2 mg (25 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.76 (br s, 1H, COOH), 12.31 (br s, 1H, Ar-OH), 10.87 (br s, 1H, CONH), 10.64 (s, 1H, CONH), 9.38 (s, 1H, CONH), 9.07 (d, 1H, NHCH, J = 7.5 Hz), 8.61 (s, 1H, Ar-H), 8.43 (s, 1H, Ar-H), 8.19 (d, 1H, Ar-H, J = 8.7 Hz), 8.13 (d, 1H, Ar-H, J = 8.7 Hz), 8.08 (d, 2H, Ar-H, J = 8.4 Hz), 8.06 (dd, 1H, Ar-H, J = 1.6 Hz, 8.6 Hz), 8.02 – 7.99 (m, 3H, Ar-H), 7.98 – 7.95 (m, 4H, Ar-H), 7.86 – 7.81 (m, 5H, Ar-H), 7.66 (d, 1H, Ar-H, J = 8.4 Hz), 4.89 (dd, 1H, CHNH, J = 7.6 Hz, 14.7 Hz), 4.61 – 4.55 (m, 1H, $CH(Me)_2$), 2.96 (t, 1H, ≡CH, J = 2.6 Hz), 2.89 – 2.79 (m, 2H, CH_2), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.6 (CONH), 168.4 (CONH), 166.9 (COOH), 166.5 (CONH), 164.1 (CONH), 144.1 (C_{Ar}), 142.2 (C_{Ar}-NH), 142.2 (C_{Ar}-NH), 137.2 (C_{Ar}), 136.8 (C_{Ar}-NH), 136.4 (C_{Ar}-O), 134.5 (C_{Ar}), 133.0 (C_{Ar}-H), 131.9 (C_{Ar}), 131.7 (C_{Ar}), 130.2 (C_{Ar}-H), 129.9 (C_{Ar}-H), 128.7 (C_{Ar}), 128.6 (C_{Ar}-H), 128.3 (C_{Ar}-H), 128.0 (C_{Ar}-H), 127.7 (C_{Ar}-H), 126.2 (C_{Ar}-H), 125.7 (C_{Ar}-H), 125.1 (C_{Ar}-H), 122.9 (C_{Ar}-H), 120.5 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.9 (CN), 112.7 (C_{Ar}), 110.4 (C_{Ar}), 80.6 (-C≡), 74.6 ($CH(Me)_2$), 73.3 (≡CH), 53.6 (CHNH), 22.3 ($(CH_3)_2$), 21.5 (CH_2)

HRMS (ESI) calculated 800.2720 [M+H$^+$], 800.2713 found.

(*S*)-4-(4-(4-(2-(4-(ferrocenecarboxamido)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropyloxybenzamido)benzoic acid (**114**)



The Fmoc deprotected amino acid (46.8 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a red to orange solid.

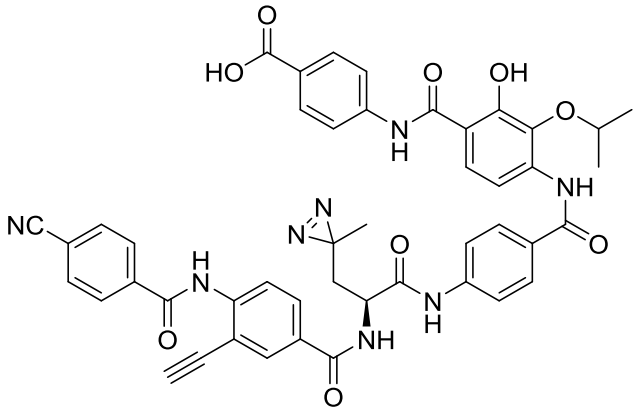
Yield: 17.0 mg (42 % over 3 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.80 (br s, 1H, COOH), 12.30 (br s, 1H, Ar-OH), 10.66 (br s, 1H, CONH), 10.57 (s, 1H, CONH), 9.64 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.70 (d, 1H, NHCH, J = 7.5 Hz), 7.99 – 7.95 (m, 4H, Ar-H), 7.93 (d, 2H, Ar-H, J = 8.8 Hz), 7.88 – 7.81 (m, 7H, Ar-H), 7.70 (d, 1H, Ar-H, J = 8.8 Hz), 5.04 (br s, 2H, Cp-H), 4.81 (dd, CHNH, J = 7.6 Hz, 14.6 Hz), 4.56 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 4.48 (br s, 2H, Cp-H), 4.23 (s, 5H, Cp-H), 2.93 (t, 1H, ≡CH, J = 2.4 Hz), 2.85 – 2.72 (m, 2H, CH_2), 1.27 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.7 (CONH), 168.6 (CONH), 168.5 (CONH), 166.9 (COOH), 166.1 (CONH), 164.2 (CONH), 154.2 (C_{Ar}-OH), 142.3 (C_{Ar}-NH), 142.2 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 130.2 (C_{Ar}-H), 128.5 (C_{Ar}), 128.4 (C_{Ar}-H), 128.3 (C_{Ar}-H), 127.9 (C_{Ar}), 126.2 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.2 (C_{Ar}-H), 119.0 (C_{Ar}-H), 112.5 (C_{Ar}), 112.0 (C_{Ar}-H), 80.6 (C≡), 76.0 (C_{Cp}), 74.8 ($CH(Me)_2$), 73.1 (≡CH), 70.7 (C_{Cp}-H), 69.5 (C_{Cp}-H), 68.7 (C_{Cp}-H), 53.4 (CHNH), 22.3 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 876.2332 [M+H$^+$], 876.2315 found.

(*S*)-4-(4-(4-(2-(4-(4-cyanobenzamido)-3-ethynylbenzamido)-3-(3-methyl-3H-diazirin-3-yl)propanamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**115**)



13.0 mg 4-(4-cyanobenzamido)-3-ethynylbenzoic acid (44.8 µmol, 1.2 eq) and 16.0 mg HATU (42.0 µmol, 1.1 eq) were added to a dry flask and further dried under high vacuum. 0.15 ml of dry DMF and 20.0 µl DIPEA (14.8 mg, 3 eq) were added under nitrogen atmosphere. The solution was stirred for 30 minutes. The solution was refered as [1]. 23.6 mg (S)-4-(2-(allyloxy)-4-(4-(2-amino-3-(3-methyl-3H-diazirin-3-yl)propanamido)benzamido)-3-isopropoxybenzamido)benzoic acid (38.4 µmol, 1 eq) was dissolved in 0.15 ml dry DMF under nitrogen atmosphere and cooled down to 0° C. Solution [1] was added and the mixture was stirred at 0 °C. After 2 h, 4.5 mg Tetrakis(triphenylphosphine)palladium(0) (3.9 µmol, 0.1 eq) and 15 µl aniline were added under nitrogen atmosphere. The reaction was controlled over LCMS. After completion, the crude mixture was purified by RP HPLC. The product was a slightly yellow solid.

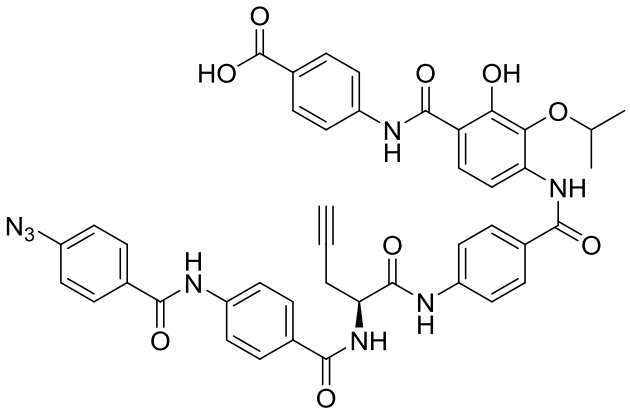
Yield: 5.8 mg (8 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.82 (br s, 1H, COOH), 12.29 (br s, 1H, Ar-OH), 10.62 (br s, 1H, CONH), 10.55 (s, 1H, CONH), 10.35 (s, 1H, CONH), 9.41 (s, 1H, CONH), 8.90 (d, 1H, NHCH, J = 7.8 Hz), 8.19 (d, 1H, Ar-H, J = 2.1 Hz), 8.15 (d, 2H, Ar-H, J = 8.4 Hz), 8.06 (d, 2H, Ar-H, J = 8.5 Hz), 8.02 (dd, 1H, Ar-H, J = 2.0 Hz, 8.5 Hz), 7.98 – 7.95 (m, 4H, Ar-H), 7.87 – 7.84 (m, 4H, Ar-H), 7.81 (d, 2H, Ar-H, J = 8.8 Hz), 7.70 (d, 2H, Ar-H, J = 8.8 Hz), 4.59 (s, 1H, ≡CH), 4.57 – 4.51 (m, 2H, CHNH & $CH(Me)_2$), 2.07 – 1.94 (m, 2H, CH_2), 1.26 (d, 6H, $(CH_3)_2$, J = 5.4 Hz), 1.12 (s, 3H, CH_3)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 170.2 (CONH), 168.5 (CONH), 166.9 (COOH), 165.1 (CONH), 164.2 (CONH), 164.1 (CONH), 154.2 (C_{Ar}-OH), 142.2 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.6 (C_{Ar}-NH), 138.0 (C_{Ar}), 137.0 (C_{Ar}-NH), 136.4 (C_{Ar}-O), 132.7 (C_{Ar}-H), 131.9 (C_{Ar}-H), 130.9 (C_{Ar}), 130.2 (C_{Ar}-H), 128.9 (C_{Ar}-H), 128.6 (C_{Ar}-H), 128.5 (C_{Ar}-H), 128.4 (C_{Ar}-H), 126.3 (C_{Ar}), 125.0 (C_{Ar}-H), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.0 (C_{Ar}-H), 118.2 (CN), 117.1 (C_{Ar}), 114.3 (C_{Ar}), 112.5 (C_{Ar}), 112.2 (C_{Ar}-H), 86.8 (≡CH), 79.6 (C≡), 74.8 ($CH(Me)_2$), 50.5 (CHNH), 35.7 (CH_2), 24.6 (C(N=N)), 22.3 ($(CH_3)_2$), 19.8 (CH_3)

HRMS (ESI) calculated 847.2840 [$M+H^+$], 847.2833 found.

(*S*)-4-(4-(4-(2-(4-(4-azidobenzamido)benzamido)pent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (**116**)



7.0 mg 4-(4-azidobenzamido)benzoic acid (24.8 µmol, 1.2 eq) was added to a dry flask and further dried under high vacuum. 0.2 ml dry DMF, 11.0 µl DIPEA (8.2 mg, 3.1 eq) and 9.4 mg HATU (24.7 µmol, 1.2 eq) were added under nitrogen atmosphere. The solution was stirred for 30 minutes. The solution was refered as [1].

11.2 mg (S)-4-(4-(4-(2-aminopent-4-ynamido)benzamido)-2-hydroxy-3-isopropoxybenzamido)benzoic acid (20.6 µmol, 1 eq) was dissolved in 0.1 ml dry DMF under nitrogen atmosphere and cooled down to 0 °C. Solution [1] was added and the mixture was stirred at 0 °C. The reaction was controlled over LCMS. After completion, the solvent was removed under reduced pressure. 0.2 ml THF and 0.1 ml 10 mM ammonium hydrogen carbonate were added and the mixture was stirred overnight. The crude product was purified by RP HPLC.

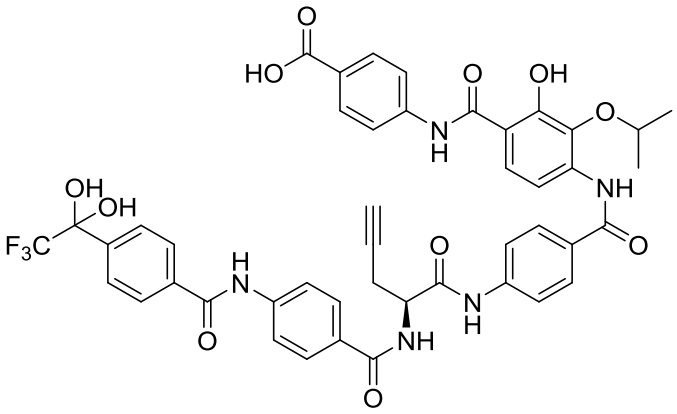
Yield: 1.0 mg (2 % over 3 steps)

^{1}H-NMR (700 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.80 (s, 1H, COOH), 12.29 (s, 1H, Ar-OH), 10.58 (s, 1H, CONH), 10.47 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.74 (d, 1H, NHCH, J = 7.5 Hz), 8.05 (d, 2H, Ar-H, J = 8.6 Hz), 7.97 – 7.93 (m, 6H, Ar-H), 7.90 (d, 2H, Ar-H, J = 8.8 Hz), 7.85 (d, 2H, Ar-H, J = 8.7 Hz), 7.82 (d, 2H, Ar-H, J = 8.8 Hz), 7.69 (d, 1H, Ar-H, J = 7.8 Hz), 7.29 (d, 2H, Ar-H, J = 8.6 Hz), 4.80 (quart. 1H, CHNH, J = 7.6 Hz), 4.56 (m, 1H, $CH(Me)_2$), 2.93 (t, 1H, ≡CH, J = 2.6 Hz), 2.78 (m, 2H, CH_2), 1.26 (d, 6H, $(CH_3)_2$, J = 6.1 Hz)

^{13}C-NMR (176 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.7 (CONH), 168.5 (CONH), 166.9 (COOH), 166.0 (CONH), 164.7 (CONH), 164.2 (CONH), 151.5 (C_{Ar}), 143.0 (C_{Ar}), 142.2 (C_{Ar}), 142.1 (C_{Ar}), 139.2 (C_{Ar}), 136.4 (C_{Ar}), 131.0 (C_{Ar}), 130.2 (C_{Ar}-H), 129.8 (C_{Ar}-H), 128.5 (C_{Ar}), 128.4 (C_{Ar}-H), 128.0 (C_{Ar}), 124.9 (C_{Ar}-H), 122.8 (C_{Ar}), 120.7 (C_{Ar}), 119.4 (C_{Ar}-H), 119.1 (C_{Ar}-H), 119.0 (C_{Ar}-H), 80.7 (C≡), 73.2 (≡CH), 53.5 (CHNH), 22.3 ($(CH_3)_2$), 21.4 (CH_2)

HRMS (ESI) calculated 809.2683 [M+H$^+$], 809.2678 found.

(*S*)-4-(2-hydroxy-3-isopropoxy-4-(4-(2-(4-(4-(2,2,2-trifluoro-1,1-dihydroxyethyl)benzamido)benzamido)pent-4-ynamido)benzamido)benzamido)benzoic acid (**117**)



The Fmoc deprotected amino acid (21.9 µmol) was coupled with fragment AB using procedure M. The product was obtained by deprotection with procedure N2 and O1. The product was a slightly yellow solid.

Yield: 5.5 mg (28 % over 3 steps)

^{1}H-NMR (500 MHz, DMSO-d_6, 300 K): δ (ppm) = 12.79 (s, 1H, COOH), 12.29 (s, 1H, Ar-OH), 10.59 (s, 1H, CONH), 10.57 (s, 1H, CONH), 10.52 (s, 1H, CONH), 9.40 (s, 1H, CONH), 8.73 (d, 1H, NHCH, J = 7.5 Hz), 7.99 (d, 2H, Ar-H, J = 8.5 Hz), 7.98 – 7.94 (m, 6H, Ar-H), 7.91 (d, 2H, Ar-H, J = 8.9 Hz), 7.87 – 7.84 (m, 3H, Ar-H), 7.83 (d, 2H, Ar-H, J = 8.8 Hz), 7.76 (d, 1H, Ar-H, J = 8.3 Hz), 7.73 – 7.70 (m, 3H, Ar-H & $C(OH)_2$), 4.81 (dd, 1H, CHNH, J = 7.6 Hz, 14.6 Hz), 4.55 (hept., 1H, $CH(Me)_2$, J = 6.1 Hz), 2.93 (t, 1H, ≡CH, J = 2.6 Hz), 2.85 – 2.73 (m, 2H, CH_2C≡), 1.27 (d, 6H, $(CH_3)_2$, 6.2 Hz)

^{13}C-NMR (126 MHz, DMSO-d_6, 300 K): δ (ppm) = 169.7 (CONH), 168.5 (CONH), 166.8 (COOH), 166.0 (CONH), 165.5 (CONH), 164.2 (CONH), 154.1 (C_{Ar}-OH), 142.2 (C_{Ar}), 142.1 (C_{Ar}-NH), 142.0 (C_{Ar}-NH), 141.9 (C_{Ar}-NH), 137.0 (C_{Ar}-NH), 136.3 (C_{Ar}-O), 135.4 (C_{Ar}), 130.2 (C_{Ar}-H), 128.6 (C_{Ar}), 128.5 (C_{Ar}), 128.4 (C_{Ar}-H), 128.3 (C_{Ar}-H), 127.4 (C_{Ar}-H), 127.2 (C_{Ar}-H), 126.3 (C_{Ar}), 122.8 (C_{Ar}-H), 120.7 (C_{Ar}-H), 119.3 (C_{Ar}-H), 119.0 (C_{Ar}-H), 112.4 (C_{Ar}), 112.1 (C_{Ar}-H), 92.3 ($C(OH)_2$, J = 31.5 Hz), 80.6 (C≡), 74.8 ($CH(Me)_2$), 73.1 (≡CH), 53.4 (CHNH), 22.3 (($CH_3)_2$), 21.4 (CH_2)

^{19}F{^{1}H}-NMR (470 MHz, DMSO-d_6, 300 K) δ (ppm) = - 82.69 (CF_3)

HRMS (ESI) calculated 882.2598 [M+H^+], 882.2592 found.

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7 Supplementary information

The NMR spectra can be found in a separated file.

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