Modification of Polysialic Acid towards Scaffolds for Tissue Engineering and Synthesis of Sialic Acid Derivatives

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Meinen Eltern

Abstract

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Key words: tissue engineering, polysialic acid, sialic acid, 3D-scaffold

Tissue engineering is a powerful tool in the field of regenerative medicine. It provides the opportunity to generate artificial organs and tissues. More and more attention is drawing to this topic due to the lack of organ donors. A challenging technology in this field is the synthesis of suitable scaffold materials which mimic the extracellular matrix of the required tissue. An ideal scaffold should provide an optimal environment for cell adhesion, proliferation, and growth.

Three dimensional scaffolds based on natural occurring polysaccharides fulfill these requirements. In the past, various scaffolds based on polysaccharides such as chitosan, alginate, and hyaluronate were used for tissue engineering. The aim of this study was to synthesise novel 3D-scaffolds based on α-2,8-polysialic acid (polySia) for applications in nerve regeneration. PolySia is a promising candidate for tissue engineering due to its poor immunogenicity, non-cytotoxicity, and selective enzymatic degradability. To form three dimensional water insoluble networks, polySia was decorated with different functional groups. PolySia hydrogels were successfully prepared using three crosslinking strategies including Cu-catalyzed and metal-free “click” chemistry (A), photopolymerization (B), or hydrazone crosslinking (C). The synthesized polySia hydrogels were investigated regarding their biocompatibility and biodegradability. Hydrogels were degradable by endosialidase endoNF, when the degree of derivatization was adjusted under 50%. In order to improve the cell-scaffold interactions, the polySia scaffolds were successfully immobilized with cyclic RGD peptides.

Besides the derivatization of polysaccharides, their monomeric unit, neuraminic acid was also modified with various functionalities such as azido-, alkyno-, methacryl-, and pentenyl-groups. To get functional polymers, the modified monosaccharides should be enzymatically polymerized in further investigations.

Zusammenfassung

Yi Su

Modifikation von Polysialinsäure zur Synthese neuer Gerüstmaterialien für das Tissue Engineering und Synthese Sialinsäure Derivate

Schlagwörter: Tissue Engineering, Polysialinsäure, Sialinsäure, 3D-Gerüstmaterial

Tissue Engineering ist ein zunehmend bedeutsames Feld im Bereich der regenerativen Medizin, da es die Herstellung künstlicher Organe und Gewebe ermöglicht. Aufgrund einer geringen Verfügbarkeit von Spenderorganen, gewinnt das Tissue Engineering mehr und mehr Aufmerksamkeit. Eine anspruchsvolle Aufgabe in diesem Bereich ist die Synthese von geeigneten Gerüstmaterialien, die die extrazelluläre Matrix des entsprechenden Gewebes imitieren. Ein ideales Gerüstmaterial bietet eine geeignete Umgebung für Zell-adhäsion, -Proliferation und -Wachstum.

Dreidimensionale Gerüstmaterialien basierend auf natürlich vorkommenden Polysacchariden erfüllen diese Anforderungen. In der Vergangenheit, werden verschiedene Gerüstmaterialien, die auf den Polysacchariden wie Chitosan, Alginat und Hyaluronat basieren, für das Tissue Engineering eingesetzt. Ziel der vorliegenden Arbeit war die Synthese von neuartigen 3D-Gerüsten basierend auf α-2,8-Polysialsäure (PolySia) für die Anwendungen in der Nervenregeneration. PolySia ist ein besonders viel versprechendes Polymers aufgrund seiner begünstigten Immunogenität, Nicht-Zytotoxizität und enzymatischen Abbaubarkeit. Um dreidimensionale, wasserunlösliche Netzwerke herzustellen, wurde das Polysaccharid mit verschiedenen funktionellen Gruppen modifiziert. Die polySia Hydrogele wurden unter Verwendungen von drei Vernetzensstrategien erfolgreich hergestellt: Cu-katalysierte und Metall-freie "click“ Chemie (A), Photopolymerisation (B), oder Hydrazon-basierte Quervernetzung (C). Die synthetisierten PolySia Hydrogele wurden hinsichtlich ihrer biologischen Abbaubarkeit untersucht. Wenn die PolySia in Derivatisierungsgraden unter 50% funktionalisiert wurden, die PolySia Hydrogele waren enzymatisch abbaubar gegenüber der Endosialidase EndoNF Um die Adhäsion von Zellen auf PolySia Hydrogels zu erhöhen, wurden die Hydrogels auch mit zyklischen RGD Peptide erfolgreich funktionalisiert.

Neben der Derivatisierung der Polysaccharide wurde auch dessen Monomer Neuraminäure mit verschiedenen Funktionalitäten wie Azid-, Alkin-, Methacryl-, und Pentenyl-Gruppen modifiziert. Um die funktionalisierten Polymere zu erhalten, sollten die modifizierten Monosaccharide in folgende Experimenten enzymatisch polymerisiert.

Abbreviations

abs	absolute
Ac	acetyl
APS	ammonium persulpahte
ADH	adipic dihydrazide
BCP	bromocresol purple
Bn	benzoyl
BPB	bromophenol blue
Bz	benzoyl
c	conzentration
Cy	cyclohexane
CAMs	cell adhesion molecules
CMP	cytidinmonophosphate
CuAAC	Cu-catalyzed azide-alkyne cycloaddition
d	day
DAPI	4´,6-diamidino-2-phenyindole
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
DCU	*N*,*N´*-Dicyclohexylurea
DMF	dimethylformamide
DMAP	4-(dimethylamino)-pyridine
DMEM	dulbecco´s modified Eagles medium
DMSO	dimethylsulfoxide
DS	degree of substitution
DTPA	diethylenetriaminepentaacetic acid
E. coli	*Escherichia coli*
ECM	extracellular matrix
EDC	*N*-(3-dimethylaminopropyl-)-*N´*-ethyl-carbodiimide
EDTA	ethylendiamintetraacetic acid
EndoN	Endo-*N*-acetylneuraminidase
EE	ethylacetat
eq	equivalent
ESI	electrospray-ionisation
Et	ethyl
FCS	fetal calf serum
h	hour
HMQC	heteronuclear multiple quantum coherence
HPLC	high pressure liquid chromatography
HSQC	heteronuclear single quantum coherence
IR	infrared spectroscopy
Neu5Ac	*N*-acetylneuramin acid

Me	methyl
min	minute
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NCAM	neural cell adhesion molecule
NSF	nation science Foundation
NHS	*N*-hydroxylsucinimide
NMR	nulear magnetic resonance
PAGE	polyarylamide gel electrophoresis
PBS	phosphate buffered saline
PE	petrol ether
PEG-diald	poly(ethylene glycol)-propiondialdehyde
ph	phenyl
PLL	poly-L-lysine
polySia	polysialic acid
PSGP	polysialoglycoprotein
PVA	polyvinyl alcohol
q	quartet
RGD	arginine-glycine-aspartic acid
R_f	retention factor
RuAAC	Ru-catalyzed azide-alkyne cycloaddition
SDS	sodiumdodecysulphate
SPAAC	strain-promoted alkyne-azide cycloaddition
t	triplett
t_R	time of retention
TBA	tetrabutylammunia
TB	trypan blue
TFA	trifluoro acetic acid
TEMED	tetramethylethylendiamine
THF	tetrahydrofuran
TLC	thin-layer-chromatography
UDP	uridindiphosphate
v	valume
V	valter
w	weight
XC	xylenecyanol
δ	chemical shift

1 Introduction Background

1.1 General aspects of tissue engineering

The term “Tissue Engineering” was initially defined at the first National Science Foundation (NSF) sponsored meeting in 1988 as “application of the principles and methods of engineering and life sciences towards fundamental understanding of structure-function relationship in normal and pathological mammalian tissues and the development of biological substitutes for the repair or regeneration of tissue or organ function.”[1] The commonly applied definition of tissue engineering was given by Langer and Vacanti as “an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain, or improve tissue".[2] Tissue engineering has also been defined by Macarthur and Oreffo as "understanding the principles of tissue growth and applying this to produce functional replacement tissue for clinical use." [3]

Tissue engineering in the nervous system facilitates the controlled application and organization of neural cells to perform appropriate diagnostic and therapeutic tasks in the nervous system.[4] This is a novel strategy for the therapy of human neurodegenerative diseases such as Parkinson´s disease. Over the last decades the investigations of tissue engineering in the nervous system focussed on the following three areas: [5](i) “The functional replacement of missing neuroactive components; (ii) The rescue or regeneration of degenerated neural tissue; (iii) The building of intelligent neural cell-based biosensors and simple *in vitro* neural circuits.”

Tissue engineering is a combination of cells, biomaterials, and signalling molecules such as growth factor. Cells are harvested and dissociated from the donor tissue including nerve, liver, pancreas, cartilage, and bone. Embryonic or adult stem or precursor cells are used as well. Biomaterials are scaffold substrates in which cells are attached, cultured, and followed by implantation at the desired site of the functional tissue. Growth factors were naturally occurring proteins capable of stimulating cellular proliferation and cellular differentiation. They are important for the regulation a variety of cellular processes.

The basic principles of tissue engineering are illustrated in Figure 1. At first, different cells (osteoblasten, chondrozyten, hepatozyten, enterozyten, and urotheliale cells) are isolated from

[1] R. Skalak, C. F. Fox, *Tissue engineering. Granlibakken, Lake Tahoe: Proc workshop*, New York Liss, **1988**, 26–29.
[2] R. Langer, J. P. Vacanti, *Science* **1993**, *260*, 920-926.
[3] B. D. Macarthur, R. O. C. Oreffo, *Nature* **2005**, *433*, 19.
[4] R. Bellamkonda, P. Aebischer, *The Biomedical Engineering Handbook, Second Edition*. CRC Press LLC, **2000**.
[5] R. Bellamkonda, P. Aebischer, *Biotechnol. Bioeng*. **1994**, *43*, 543-554.

the patient's body and expanded in a Petri dish *in vitro*. Once enough cells are available, they can be seeded on a polymeric scaffold material and cultured *in vitro* in a bioreactor or incubator under appropriate conditions. When the construct is matured enough it can be implanted in the area of defect in the patient's body.

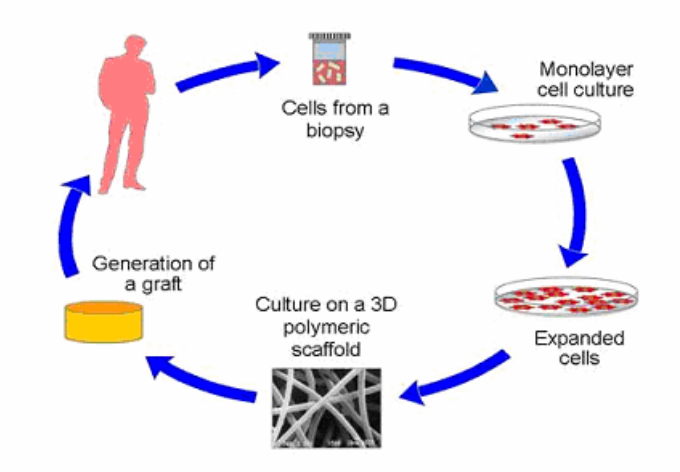


Figure 1. Schematic illustration of the basic principles of tissue engineering.[6]

1.2 Biodegradable scaffold matrices for tissue engineering

Scaffolds used in tissue engineering should have biological and physical properties which are compatible with the physiological conditions *in vitro* and *in vivo*. The major function of the scaffolds is to provide a temporary support to tissue structures. The scaffolds are commonly made from biodegradable polymers. In this context, biodegradability means that the polymer can be digested into less complex intermediates by enzymes and the end products can be easily removed by solubilisation or simple hydrolysis from the implantion site.[7] The degradation rate of the biopolymers depends on crystallinity, molecular weight, hydrophobicity, and location in the body. Additionally, the process conditions for the formation of the scaffolds also play a critical role for the biodegradability.

Ideally the scaffolds should regulate cell adhesion, proliferation, expression of a specific phenotype and extracellular matrix deposition in a predictable and controlled fashion. An ideal biodegradable polymer for a particular application should be configured possessing the following properties:

- The scaffold should have an appropriate mechanical strength to mimic *in vivo* conditions.
- The polymer does not invoke an inflammatory or toxic response.

[6] Image from http://web.iitd.ac.in/~textile/highlights/fol8/01.htm
[7] Y. Tabata, *J. R. Soc. Interface* **2009**, *6*, 311-324.

- The polymer is metabolised in the body after fulfilling its purpose, leaving no trace (bioabsorbable).
- The polymer is easily processable into the final 3D form. It should be either porous or compatible with a range of extremely additives (starch, salt) to create porosity.
- The polymer demonstrates acceptable shelf life and is easily sterilized.

Biodegradable polymers can be basically classified into four types.[8] They are (i) natural polymers, (ii) synthetic polymers, (iii) composites, and (iv) hydrogels. Natural polymers used for tissue engineering applications include collagen, alginate, agarose, chitosan, fibrin, and hyaluronic acid. Synthetic polymers compared to natural polymers offer many advantages. They are more flexible, more predictable, and processable into different size and shapes. Furthermore, the physical and chemical properties of synthetic polymers can be modified and their mechanical and degradation characteristics can be altered by changing the chemical composition of the macromolecular precursor. Composites are polymers prepared from two or more materials to produce a better scaffold taking the advantages from each of the materials independently. Hydrogels are three-dimensional highly hydrated polymeric networks, which swell in water without dissolving. Most of the naturally occurring polymers can be synthetically modified to form hydrogels such as collagen, alginate, agarose[9] Due to the natural origin these hydrogels have macromolecular properties similar to the natural extracellular matrix (ECM). Hydrogels made of nature polymers often have high tissue compatibility and are suitable for many tissue engineering applications.[10] Hydrogels are usually made of hydrophilic polymers, which are crosslinked by various interactions including chemical bonds, hydrogen bonding, ionic interactions, and hydrophobic interactions. Crosslinking can be achieved either through physical (UV irradiation, freeze drying and heating) and chemical means such as ionic crosslinking in the presence of divalent ions or by chemical crosslinkers such as diepoxyoctane and glutaraldehyde. Besides the standard chemical crosslinking methods some novel approaches like hydrazone crosslinking and “click” chemistry[11] have been used to construct 3D hydrogels for tissue engineering.

1.3 Scaffold materials based on polysaccharides

The selection of the scaffold material plays a key role in the design and development of a particular tissue engineering product. In addition, the process conditions and additives can also

[8] H. Y. Cheung, K. T. Lau, T. P. Lu, D. Hui, *Composites. Part B* **2007**, *38*, 291-300.
[9] N. A. Peppas, P. Bures, W. Leobandung, H. Ichikawa, *Eur. J. Pharm. Biopharm.* **2000**, *50*, 27-46.
[10] A. Montembault, C. Viton, A. Domard, *Biomaterials* **2005**, *14*, 1633-1643.
[11] S. A. Krovi, D. D. Smith, S. B. T. Nguyen, *Chem. Commun*. **2010**, *46*, 5277-5279.

affect the mechanical performance of biodegradable polymers. Polysaccharides, the widest distributed naturally occurring biopolymer, possess excellent properties including non-toxicity and good water solubility.[12] Furthermore, polysaccharides have a broad variety of chemical structures such as linear chains or various degrees of branching. Besides hydroxyl groups they often contain functional groups such as amino and carboxyl groups. These groups can be easily modified or functionalized with other groups. The naturally occurring polymers are often biodegradable and biocompatible. Therefore, polysaccharides are suitable macromolecular precursors for the synthesis of 3D-scaffolds in tissue engineering.

1.3.1 Scaffold materials from Alginate and Hyaluronate

Alginic acid **1** (also called algin or alginate) is one of the most versatile natural materials known to form 3D-hydrogels. Alginate is a linear unbranched anionic polysaccharide which is widely distributed in the cell walls of brown algae. Alginate consists of 1,4-linked ß-D-manuronic acid (M) and α-L-guluronic acid (G) with a molecular mass ranging from 10,000 up to 600.000 Da (*Figure 2*).

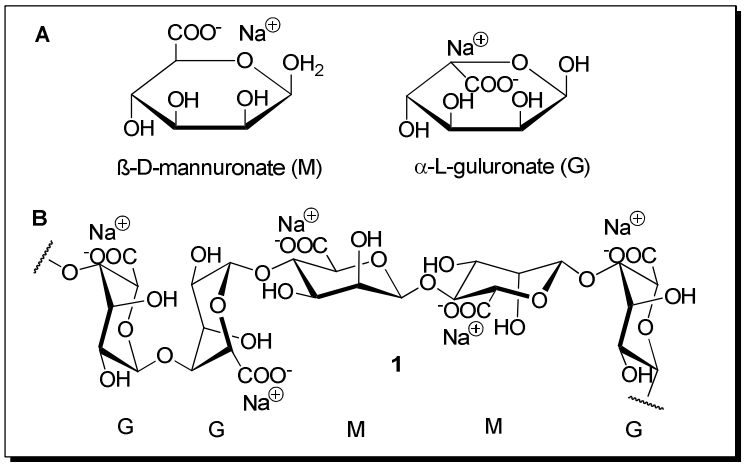


Figure 2. Structural date for alginates: A) The monomers of alginate; B) The alginate chain.

The hydrogel based on alginate has reversible gelling properties in aqueous solutions due to the ionic interactions of the carboxylic acid moieties on the sugar residues with divalent cations like calcium, barium, and magnesium.[13] Due to limitation in the control of mechanically properties, swelling ratios, and degradation profiles the ionic crosslinked alginate hydrogels are limited to the applications in tissue engineering. The investigation of photo-crosslinked alginate hydrogels has gained increased attention.

[12] M. A. Barbosa, P. L. Granja, C. C. Barrias, I. F. Amaral, *ITBM-RBM* **2005**, *26*, 212-217.
[13] T. Covillo, P. Matricardi, C. Marianecci, F. Alhaique, *J. Controlled Release* **2007**, *119*, 5-24.

Hyaluronic acid **2** (also called hyaluronan, or hyaluronate) is also a naturally occurring linear polysaccharide comprised of *ß*-1,4-linked D-glucuronic acid (*ß*-1,3) *N*-acetyl-D-glucosamine disaccharide units (*Figure 3*). This polyanionic polymer can consist of 25,000 disaccharide units in length and has a range of naturally occurring molecular sizes from 1000,000 to 10.000.000 Da. It can be found in connective, epithelial, and neural tissues of all higher animals.

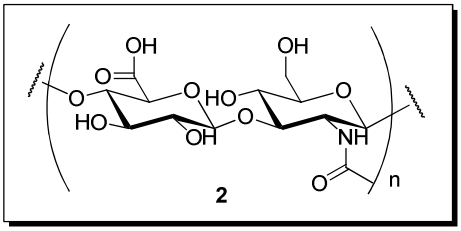


Figure 3. Structure of hyaluronate **2** composing of D-glucuronic acid and *N*-acetyl-D-glucosamine.

Hyaluronate is enzymatically degraded by hyaluronidase and is completely resorbable through multiple metabolic pathways.[14] Therefore, hyaluronate is an ideal candidate for tissue engineering and for modulation of wound healing.[15]

1.3.2 PolySia as a biomaterial for neural tissue engineering

Polysialic acid **3** (polySia) is a homopolymer of 8-200 sialic acid units. The most common structure of polySia is the negatively charged homopolymer of α-2,8-linked neuraminic acid residues (*Figure 4*).

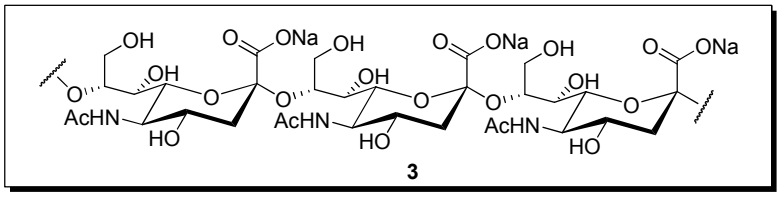


Figure 4. Structure of *α*-2,8-linked polSia **3** (polySia (sodium salt)).

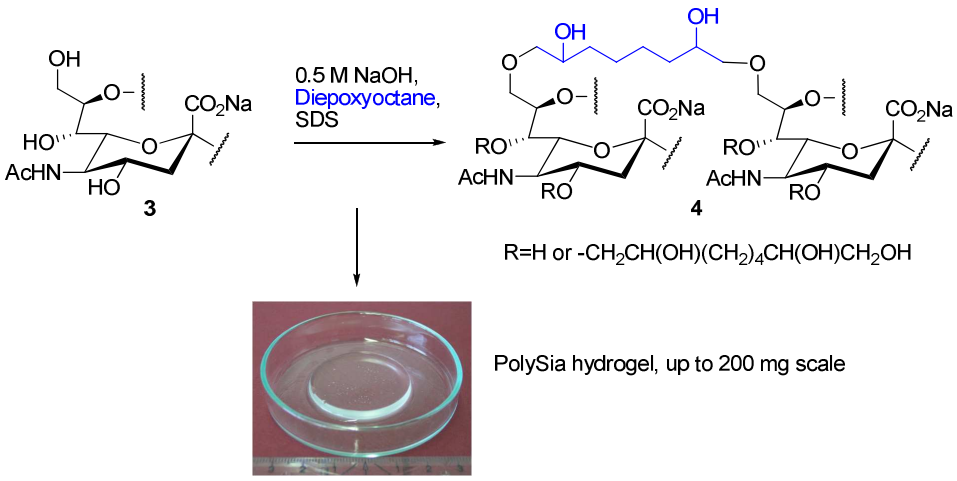
The structure of polySia was analyzed by Sato and co-workers using different methods including methylation analysis, NMR, and mild acid hydrolysis-TLC. They described the conformation of polySia as a helical structure in which the *N*-acetyl residue of the C-5 position faces the outer spacer of the helix.[16]

[14] P. A. Band, *Portland Press Ltd* **1998**, 33-42.
[15] J. B. Leach, K. A. Bivens, C. W. Patrick Jr, C. E. Schmidt, *Biotechnol. Bioeng*. **2003**, *82*, 579-589.
[16] C. Sato, K. Kitajima, *Experimental Glycoscience* **2008**, Part 1, Section II, 77-81.

PolySia **3** was first identified in the glycocalyx of neuroinvasive bacteria. PolySia conjugates in vertebrates are for example the polysialoglycoprotein (PSGP) in fish egg, in the neural cell adhesion molecule (NCAM) in brain, a voltage-gated sodium channel in eel and rat brain, CD36 in human milk, and neuropilin-2 in human lymphocytes.[17] In humans polySia is expressed in embryonic brains during neural differentiation and mostly disappears in adult brains.

PolySia on NCAM possesses antiadhesive effects on the cell-cell/extracellular matrix interaction due to its bulky polyanionic nature.[18] It is also involved in tumor progression and differentiation.[19,20] In nervous system, polySia plays an important part in cell migration, axonal guidance, synapse formation, and functional plasticity via not only homophilic binding of NCAM but also heterophilic binding of other cell adhesion molecule (CAMs) in a tissue- and stage-specific manner.[21]

For neural tissue engineering, polySia **3** was used for the synthesis of 3D-scaffold **4** which was completely degradable and presented good biological properties like perfect cytocompatibility and no cytotoxicity by viability assay on cultured Schwann cells.[22] In a previous investigation, a biocompatible scaffold material on the basis of polySia has been developed for nerve regeneration. For the synthesis of the scaffold material diepoxyoctane was used as a hydrophobic cross-linking agent to increase the molecular weight of the polymer and decrease its hydrophilicity resulting in a flexible network structure (*Scheme 1*).



Scheme 1. A 3D-scaffold based on the polySia use of diepoxyoctane as crosslinker**.**

[17] Y. Kanato, K. Kitajima, C. Sato, *Glycobiology* **2008**, *18*, 1044-1053.
[18] L. Bonfanti, *Prog. Neurobiol*. **2006**, *80*, 129-164.
[19] J. L. Bruse´s, U. Rutishauser, *Biochimie* **2001**, *83*, 635-643.
[20] U. Rutishauser, *Nat. Rev. Neurosic*. **2008**, *9*, 26-35.
[21] J. L. Bruses, U. Rutishauser, *Glycobiology* **2000**, 116-128.
[22] Y. Haile, S. Berski, G. Dräger, A, Nobre, K. Stummeyer, R. Gerardy-Schahn, C. Grothe, *Biomaterials* **2008**, *29*, 1880-1891.

The analysis of neural and glial cell viability on the scaffold material and the degradability of the hydrogel by phage born endosialidase demonstrated that the novel biomaterial is well suitable for neural tissue engineering.[23]

1.4 Strategies for 3D-scaffold formation

Since 3D-scaffolds were introduced as novel materials possibly suitable for a variety of applications in the field of tissue engineering, they have become a fast-developing and exciting research field. In last decade, various crosslinking strategies were based on chemical reaction or physical interactions exploited for the design of polymeric networks. The often applied chemical crosslinking approaches included photopolymerization, hydrazone crosslinking, "click" chemistry, and other chemical reactions.

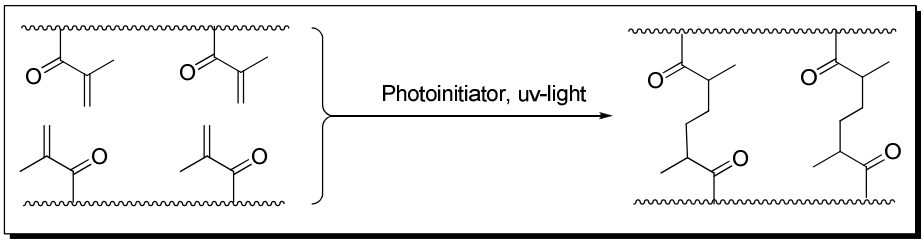
1.4.1 Hydrogel formation via photocrosslinking

Photopolymerization is used to convert a liquid monomer or macromer to a hydrogel by free radical polymerization at room temperature or under physiological conditions. As an exciting new technique, photopolymerization has obtained considerable attention in the area of tissue engineering in recent years. Up to date, photopolymerization techniques are applied in many different research areas. For example, photopolymerized hydrogels have been investigated for a number of biomaterial applications including prevention of thrombosis, post-operative adhesion formation, drug delivery, coatings for biosensors, and for cell transplantation.[24] Furthermore, photopolymerization was used in the fabrication of electronic and printing materials as well as for coating and surface modifications.[25]

Many photopolymerizable macromolecular hydrogel precursors like PEG acrylate derivatives,[26] PEG methacrylate derivatives,[27] polyvinyl alcohol (PVA) derivatives,[28] and dextran methacrylate[29] were developed. The common principle is illustrated in Scheme 2. The macromolecular precursor is initially functionalized with methacrylate group. The modified macromolecular precursor is dissolved in a solution containing a suitable photoinitiator, which is a light-sensitive compound used to create free radicals. The solution is then irradiated by UV-

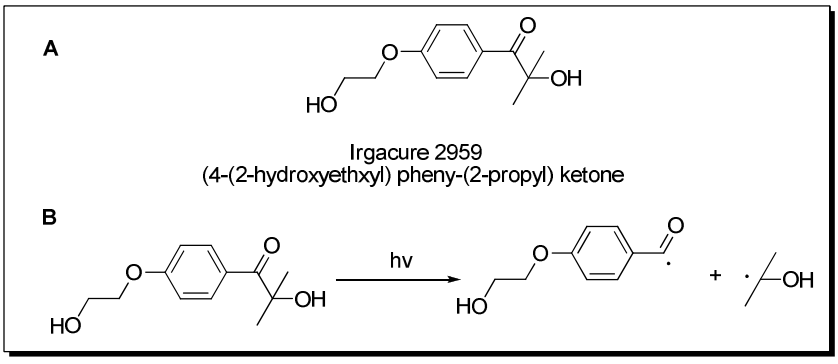
[23] S. Berski, J. V. Bergeijk, D. Schwarzer, Y. Stark, C. Kasper, T. Scheper, C. Grothe, R. Gerardy-Schahn, A. Kirschning, G. Dräger, *Biomacromolecules* **2008**, *9*, 2353-2359.
[24] K. T. Nguyen, J. L. West, *Biomaterials* **2002**, *23*, 4307-4314.
[25] A. B. Scranton, C. N. Bowman, *ACS Publishers, New Orleans* **1996**.
[26] A. S. Sawhney, C. P. Pathak, J. A. Hubbell, *Macromolecules* **1993**, *26*, 581-587.
[27] J. Elisseeff, W. Mclntosh, J. K. Anseth, S. Riley, P. Ragan, R. Langer, *J. Biomed. Mater. Res.* **2000**, *51*, 164-171.
[28] P. Martens, K. S. Anseth, *Polymer* **2000**, *41*, 7715-7722.
[29] S. H. Kim, C. C. Chu, *J. Biomed. Mater. Res.* **2000**, *49*, 517-527.

light at a wave length of 245 nm to form the crosslinked hydrogel. The mechanical properties of the hydrogel such as swelling, compression and creep compliance can be easily controlled by changing the degree of methacrylate modification.[30]



Scheme 2. Schematic illustration of 3D-hydrogel formation from methacrylate modified macromolecular precursor using photopolymerization.

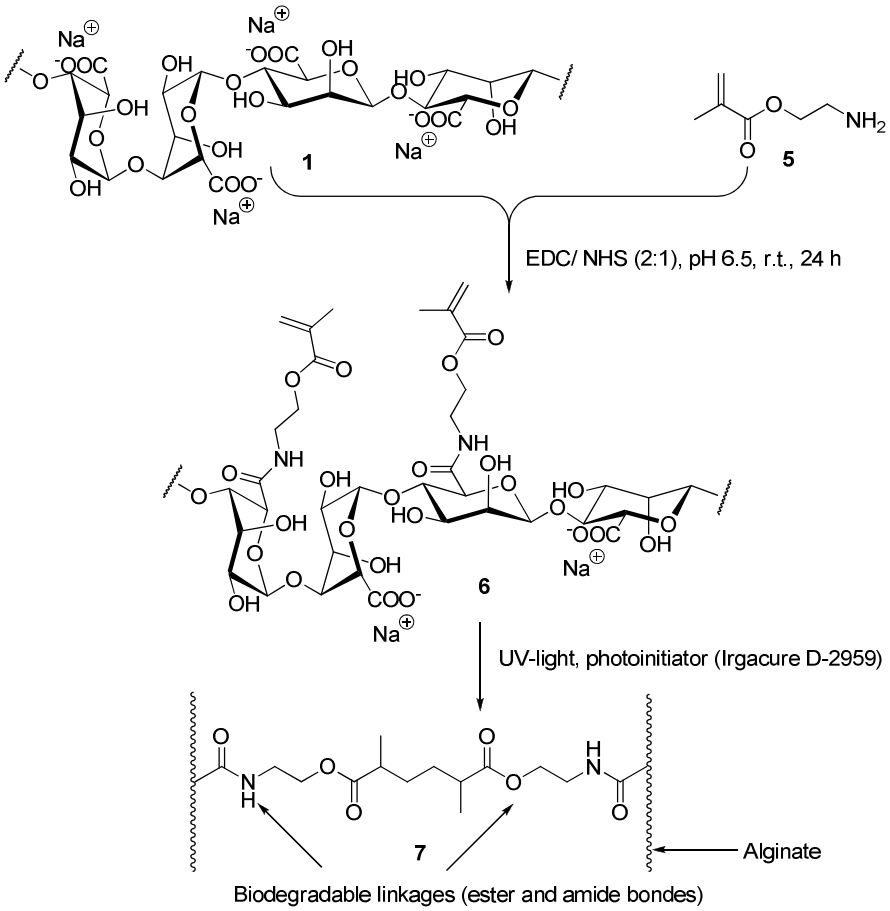
For the photoinitiator, many factors should be considered such as its biocompatibility, solubility in water and cytotoxicity. Photopolymerized hydrogels were often used in *in vivo* system. Therefore, the photoinitiator should be nontoxic and very efficient in low concentration. According to the mechanism involved in photolysis, photoinitiators are classified in two types include cationic and radical photoinitiators. Cationic photoinitiators are problematic for tissue engineering application since they generate protonic acids. Most photoinitiators used for tissue engineering applications are radical photoinitiators. As one example, Irgacure 2959 is commonly used as photoinitiator to form hydrogels for tissue engineering due to its high solubility in water and its minimal toxicity compared to other photoinitiators. Upon UV-irradiation Irgacure 2959 undergoes a homolytic cleavage resulting in two radicals (*Scheme 3*).[31]



Scheme 3. The chemical structure of Irgacure 2959 (**A**) and creating of radical by photocleavage (**B**).

[30] A. I. V. D. Bulcke, B. Bogdanov, N. D. Rooze, E. H. Schacht, M. Cornelissen, H. Berghmans, *Biomacromolecules* **2000**, *1*, 31-38.
[31] B. D. Fairbanks, M. P. Schwartz, C. N. Bowman, K. S. Anseth, *Biomaterials* **2009**, *30*, 6702-6707.

A novel photocrosslinked alginate hydrogel was developed by Jeon and co-workers.[32] They also characterized the procedure of the polymerization using ^{1}H-NMR analysis. Sodium alginate **1** has been functionalized with methacrylate groups by reacting low molecular weight alginate with 2-amionethyl methacrylate **5** in presence of *N*-hydroxy succinimide (NHS) and EDC to give methacrylated alginate **6**. The 3D alginate hydrogel **7** was obtained by irradiation using UV-light with 0.05% (w/v) photoinitiator (*Scheme 4*).



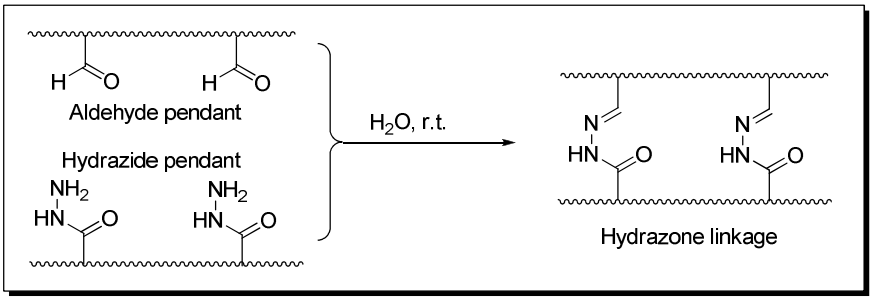
Scheme 4. Synthesis of alginate hydrogel based on methacrylated alginate using photopolymerization.

The photocrosslinked alginate hydrogels showed low cytotoxicity and excellent cytocompatibility. The hydrogels have broad biomedical applications, including use in cell transplantation and drug delivery. This gelling methodology can also be used for the synthesis of biodegradable 3D-scaffold materials for tissue engineering based on many other naturally occurring polymers and synthetic polymers.[33] For example, Smeds and co-workers have reported on a viscoelastic gels from two methacrylate modified polysaccharides alginate and hyaluronate using photopolymerization.[34]

[32] O. Jeon, K. H. Bouhadir, J. M. Mansour, E. Alsberg, *Biomaterials* **2009**, *30*, 2724-2734.
[33] Q. P. Hou, P. A. D. Bank, K. M. Shakesheff, *J. Mater. Chem.* **2004**, *14*, 1915-1923.
[34] K. A. Smeds, M. W. Grindstaff, *J. Biomed. Mater. Res.* **2001**, *54*, 115-121.

1.4.2 Hydrogel formation via hydrazone crosslinking

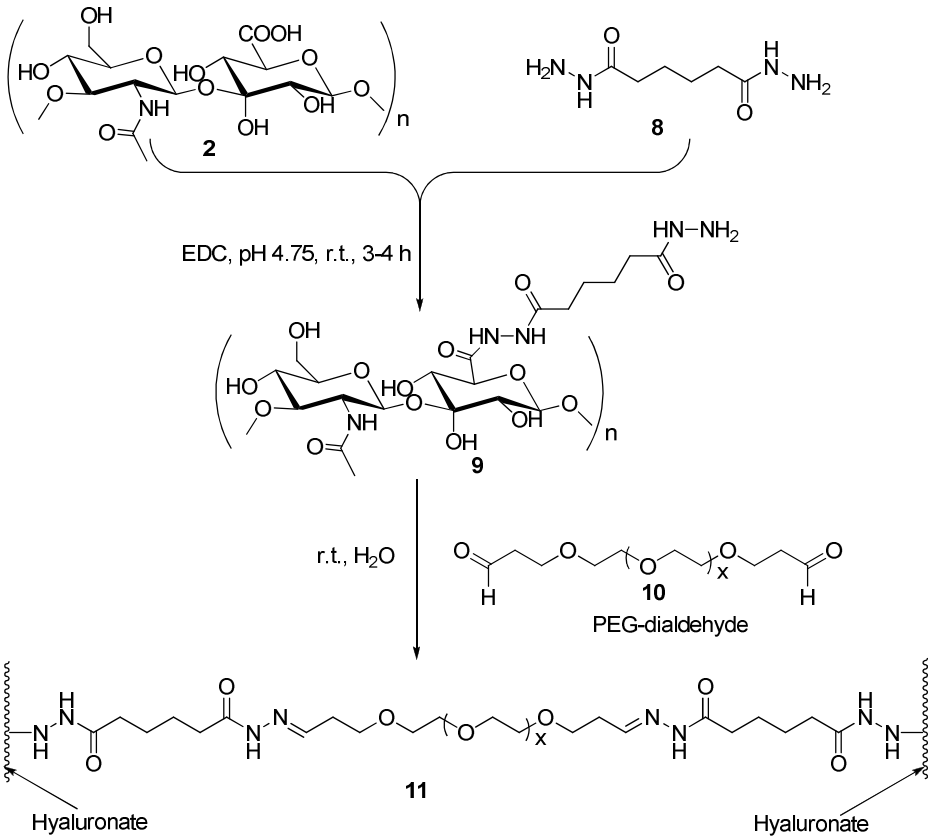
Hydrazone crosslinking provides also a powerful method for the chemical hydrogel formation as well as site-specific crosslinking of biomolecules. Carbonyl-groups (aldehydes and ketones) react with hydrazides and amines at slightly acidic aqueous conditions (pH = 5-7) to form hydrazones and water as by-product. This method can be used for *in situ* gelling scaffold materials including hyaluronate, alginate, and dextran hydrogels.[35] Aldehydo- and hydrazido-functional groups were introduced to the macromolecular precursors, and then simply mixed in aqueous solution to create 3D-hydrogel network (*Scheme 5*).



Scheme 5. Schematic illustration of 3D-hydrogel formation from aldehydo- and hydrazido-modified macromolecular precursor using hydrazone crosslinking.

Ossipov and co-workers described the development of an injectable hydrogel based on hydrazido- and aldehyde-modified polyvinyl alcohol (PVA) precursors. The hydrogel was formed *in situ* after injection. They demonstrated that the hydrazone crosslinking chemistry is a biocompatible method thus it can potentially be used for cells and tissues.[36] Luo and co-workers developed crosslinked hyaluronate hydrogel films for drug delivery using a macromolecular crosslinker poly(ethylene glycol)-propiondialdehyde **8** (PEG-diald) under neutral aqueous conditions.[37] For their applications, the hyaluronate **2** was functionalized with hydrazide groups. Under aqueous acidic conditions, the carboxylic acid group of the glucuronic acid reacted with the adipic dihydrazide **9** (ADH) in the presence of coupling reagent EDC resulting to desired hydrazide-modified product **10**. The 3D hyaluronate hydrogel **11** was formed by simply mixing the modified hyaluronate and PEG-dialdehyde in water (*Scheme 6*). The resulted hydrogel film has significant potential to act as a delivery vehicle for sustained release of drugs at wound sites.

[35] Y. Luo, J. B. Kobler, J. T. Heaton, X. Q. Jia, S. M. Zeitels, R. langer, *J. Biomed. Mater. Res. B* **2010**, *93*, 386-393.
[36] D. A. Ossipov, K. Brännvall, K. Forsberg-Nilsson, J. Hilborn, *J. Appl. Polym. Sci.* **2007**, *106*, 60-70.
[37] Y. Luo, K. R. Kirker, G. D. Prestwich. *J. Controlled Release* **2000**, *69*, 169-184.



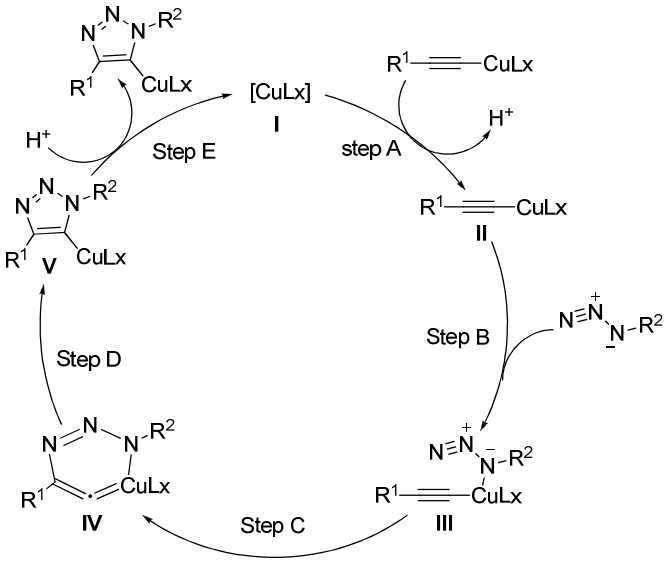
Scheme 6. Synthesis of hyaluronate hydrogel based on hydrazide modified alginate using hydrazone crosslinking.

1.4.3 Application of "click" chemistry in the field of polymer science

The [2+3] dipolar cycloaddition between an azide and an acyclic alkyne was developed by Huisgen.[38] The reaction requires heat to overcome the activation barrier to deform the alkyne bond angle and form the triazole. It often produces mixtures of two regioisomers when using asymmetric alkynes.[39] In 2001, Sharpless developed a Cu-catalyzed version of this reaction using terminal alkynes as substrates allowing the selective synthesis of 1,4-disubstituted regioisomers and introduced the name "click" reaction.[40] By definition "click" reactions are hemoselective high yielding with little or no by-products, and can be carried out under various reaction conditions.[41]

The mechanism of the Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction was discussed by Fokin (*Scheme 7*). [42]

[38] R. Huisgen, *Angew. Chem. Int. Ed. Engl*. **1963**, *2*, 565-598.
[39] J. C. Jewett, C. R. Bertozzi, *Chem. Soc. Rev*. **2010**, 39, 1272-1279.
[40] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed*. **2002**, *41*, 2596-2599.
[41] M. G. Finn, V. V. Fokin, *Chem. Soc. Rev*. **2010**, *39*, 1231-1232.
[42] P. Wu, V. V. Folkin. *Aldrichimica ACTA* **2007**, *40*, 7-17.



Scheme 7. The proposed mechanism of the1,3-dipolar cycloaddition of azides and alkynes.[42]

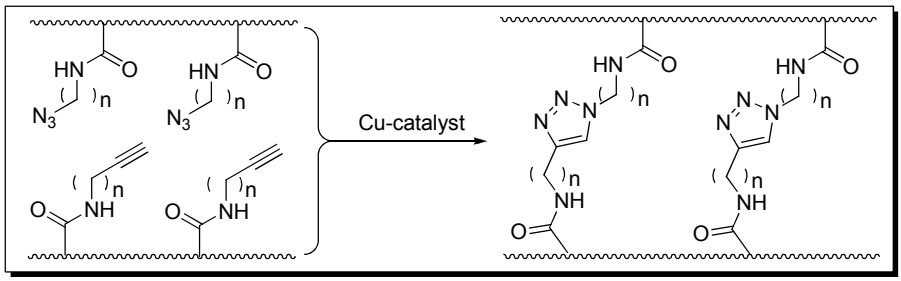
In the first step, Cu(I) coordinates to the alkyne forming the copper acetylide **II**. In water, this step has been calculated to be exothermic by 11.7 kcal/mol. Then the azide group binds to copper giving intermediate **III** which is converted to the unusual 6-membered copper metallacycle **IV**. The reaction is endothermic by 12.6 kcal/mol with a calculated barrier of 18.7 kcal/mol. Compound **IV** is not stable therefore it leads to the triazolyl-copper derivative **V**. Protonolysis of **V** releases the final triazole and closes the catalytic cycle.[43,44]

The active Cu(I) catalyst can be generated from Cu(I) salts like CuBr and CuI. In cases where the Cu(I) species are not stable enough the Cu(I) catalyst can be generated from Cu(II) salts using sodium ascorbate as the reducing reagent. Besides the Cu-catalyst, Ruthenium can be used as catalyst for "click" reaction termed as RuAAC as well. In contrast to the CuAAC the RuAAC leads to 1,5-disubstituted 1,2,3-triazoles and can be used with internal alkynes to provide fully substituted 1,2,3-triazoles.[45] Besides the two species Ni(II), Pt(II) and Pd(II) can also be used in azide-alkyne 1,3-dipolar cycloaddition.

The metal catalyzed azide/alkyne "click" reaction has experienced wide application in polymer, material, and surface science.[46] In the area of tissue engineering the "click" reaction was used for the formation of 3D-hydrogel which was used as material for cell growth. The polymers were modified with azide and alkyne residues, respectively. When the two solutions

[43] A. Ulman, *Chem. Rev.* **1996**, *96*, 1533-1554.
[44] R. K. Smith, S. M. Reed, P. A. Lewis, J. D. Monnell, R. S. Clegg, K. F. Kelly, L. A. Bumm, J. E. Hutchison, P. S. Weiss, *J. Phys. Chem. B* **2001**, *105*, 1119-1122.
[45] B. C. Boren, S. Narayan, L.K. Rasmussen, L. Zhang, H. Zhao, Z. Lin, G. Jia, V. V. Fokin, *J. Am. Chem. Soc.* **2008**, *130*, 8923-8930.
[46] W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Commun.* **2007**, *28*, 15-54.

were mixed together a 3D-hydrogel was obtained through the formation of the triazole connection in the presence of a Cu-catalyst (*Scheme 8*).



Scheme 8. Schematic illustration of 3D-hydrogel formation from azido- and alkyno-modified macromolecular precursor using Cu-catalyzed "click" cycloaddition.

However, the CuAAC has its limitations in living systems due to the potential toxicity of the copper catalyst.[47] For example, the copper catalyst may induce oligonucleotide[48] and polysaccharide[49] degradation. In order to overcome this drawback, Bertozzi and co-workers developed an alternative approach without metal-catalyst which was a strain-promoted [3+2] cycloaddition between azides and different strained cyclooctyne derivatives (**12-15**, *Figure 5*).[50] They named this metal-free "click" reaction a strain-promoted alkyne-azide cycloaddition (SPAAC).[51] This method was widely used in the field of ligation processes like bioorthogonal labelling in living system.

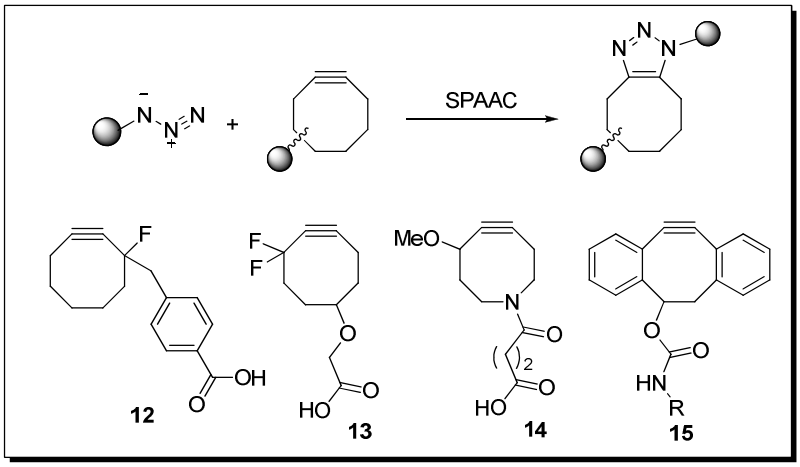
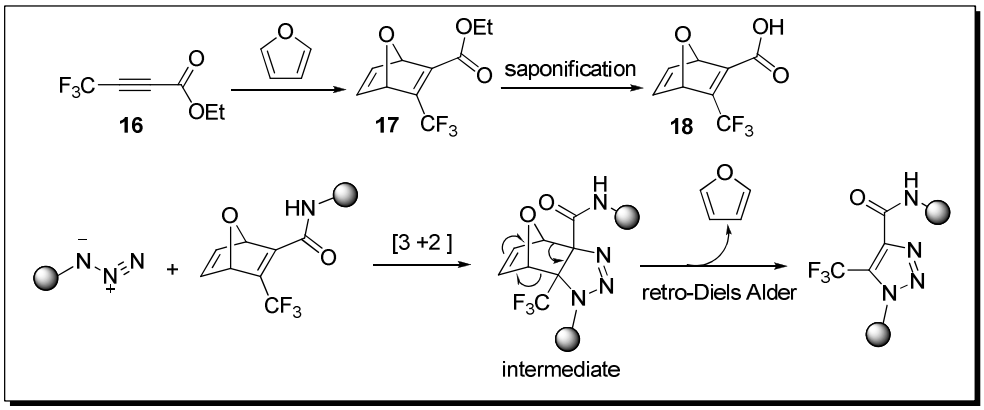


Figure 5. Strain-promoted alkyne-azide cycloaddition (SPAAC) and structures of the most commonly employed clickable cyclooctynes.

[47] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057-3064.
[48] J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639-3642.
[49] E. Lallana, E. Fernandez-Megia, R. Riguera, *J. Am. Chem. Soc.* **2009**, *131*, 5748-5750.
[50] a) N. J. Agard, J. M. Maskin, J. A. Prescher, A. Lo, C. R. Bertozzi, *ACS Chem. Biol*. **2006**, *1*, 644-648;
b) J. C. Jewett, C. R. Bertozzi, *Chem. Soc. Rev.* **2010**, *39*, 1272-1279.
[51] N. J. Agard, J. A. Prescher, C. R. Bertozzi, *J. Am. Chem. Soc*. **2004**, *126*, 15046-15047.

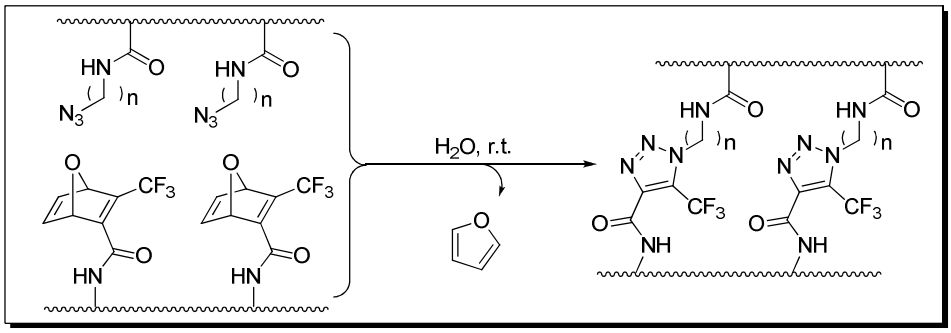
The metal-free "click" chemistry is hampered by the limited commercial availability and lengthy synthetic routes to prepare the most common cyclooctynes. Most of reaction strategies require more than eight synthetic steps while the yields are usually very low.[52] As alkyne surrogate an activated oxanorbornadiene was developed by Rutjes and co-workers.[53] The oxabridged bicyclic system **18** can be prepared by a Diels-Alder reaction from substituted propionate **16** and furan in high yield. Firstly, the oxanorbornadiene reacts with an azide in a [3+2] cycloaddition to give an intermediate which gives a stable 1,2,3-triazole linkage (*Scheme 9*). This method was a combination of ring strain and electron deficiency. The strong electron withdrawing effect of the trifluoromethyl substituent results in an activation of the adjacent double bond which is more reactive than a common triple bond.



Scheme 9. Synthesis of oxanorbornadiene and the metal-free "click" reaction of oxanorbornadiene with azido to form a triazole linkage.

This method was used as an important tool for bioconjugation like labelling of peptide and proteins in living systems. For example, the cycloaddition of an azide functionalized cyclic RGD-containing peptide (*see chapter 1.5*) and diethylenetriaminepentaacetic acid (DTPA)-functionalized oxanorbornadiene resulted in the formation of the desired RGD-CF_3-triazole-DTPA conjugate which showed high affinity for integrin receptor $a_v ß_3$ and favourable pharmacokinetics.[54] Up to date there is no report in which the method is used in the area of polymer chemistry. In this dissertation the oxanorbornadienyl-linker will be used to achieve 3D-scaffold material formation. The azido- and oxanorbornadienyl-system modified macromolecular precursors are crosslinked each other via the CF_3-triazole linkage to give a network structure (*Scheme 10*).

[52] J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl, F. L. van Delft, *Angew. Chem. Int. Ed*. **2010**, 4*9*, 9422-9425.
[53] S. S. van Berkel, A. J. Dirks, M. F. Debets, F. L. van Delft, J. J. L. M. Cornelissen, F. P. J. T. Rutjes, *ChemBioChem* **2007**, *8*, 1504-1508.
[54] S. S. van Berkel, A. J. Dirks, S. A. Meeuwissen, D. L. L. Pingen, O. C. Boerman, P. Laverman, F. L. van Delft, J. J. L. M. Cornelissen, F. P. J. T. Rutjes, *Chembiochem* **2008**, *9*, 1805-1815.



Scheme 10. Schematic illustration of 3D-hydrogel formation from azido- and oxanorbornadienyl-modified macromolecular precursors using metal-free "click" cycloaddition.

1.5 Significance of RGD peptides for tissue engineering

The interactions of cells and extracellular matrix are vital for tissue engineering. This interaction can be facilitated by the adhesion of integrin receptors and ECM which is called receptor–mediated cell binding (*Figure 6*, ***A***). Integrins are a class of cell surface receptors that mediate the interconnection between a cell and the surrounding tissues such as other cells or ECM. A few known integrins recognize and bind to a trimer RGD peptide sequence which is also present in ECM compound and can mediate adhesion of many cell types including neurons.[55,54] The RGD peptide sequence was first discovered in fibronectin by Pierschbacher & Ruoslahti.[56] This tripeptide motif can also be found in other ECM proteins such as fibrinogen, prothrombin, tenascin, thrombospondin, vitronectin, von Willebrand factor, Adenovirus penton base protein, bone sialoprotein, collagen, decorsin and disintegrins.[57] Many cyclic RGD peptides and peptide templates have a greater stability and selectivity over linear peptides and were designed and synthesized for many different purposes. *Cyclo* (Arg-Gly-Asp-D-Phe-Lys) is one of the various cyclic RGD peptides raised our interest and is an integral part of this thesis. According to the structure-activity relationship (SAR) investigations by Kessler [58] the Arg(R)-Gly(G)-Asp(D) sequence plays a crucial role for the adhesion process of cells and ECM as a common receptor recognition motif. The hydrophobic aromatic amino acid with D-conformation (D-Phe) in position 4 also increases the activity for some integrins like the $\alpha_v\beta_3$ receptor. The amino acid in position 5 (Lys) does not significantly influence the activity and selectivity of the RGD-sequence. The lysine residue can act as spacer or anchors for

[55] U. Hersel, C. Dahmen, H. Kessler, *Biomaterials* **2003**, *24*, 4385-4415.
[56] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30-33.
[57] a) P. C. Brooks, *Eur. J. Cancer*, *32a* **1996**, 2423-2429;
b) E. F. Plow, T. A. Haas, L. Zhang, J. Loftus, J. W. Smith, *J. Biol. Chem.* **2000**, *275*, 21785-21788.
[58] R. Haubner, R. Gratias, B. Diefenbach, S.L. Goodman, A. Jonczyk, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 7461-7472.

diverse surfaces.[59] The **ε**–amino group of the lysine residue can react with carboxyl or aldehyde groups. The amino group is frequently modified into an azide moiety for conjugation with other groups like alkynyl group via "click" reaction (*Figure 6, B*).[60]

Many scaffolds immobilized with RGD peptides were developed in purpose to enhance the interaction of cells and scaffold. For example, Lee and co-workers synthesized a type of RGD peptide immobilized hyaluronate hydrogel and used this material for brain tissue engineering. The RGD peptide modified hydrogel can promote neurite extension.[61] Cohen and co-workers designed a RGD peptide immobilized alginate scaffold for cardiac tissue engineering. The modified scaffold contributed to the formation of functional cardiac muscle tissue and to a better preservation of the regenerated tissue in culture.[62]

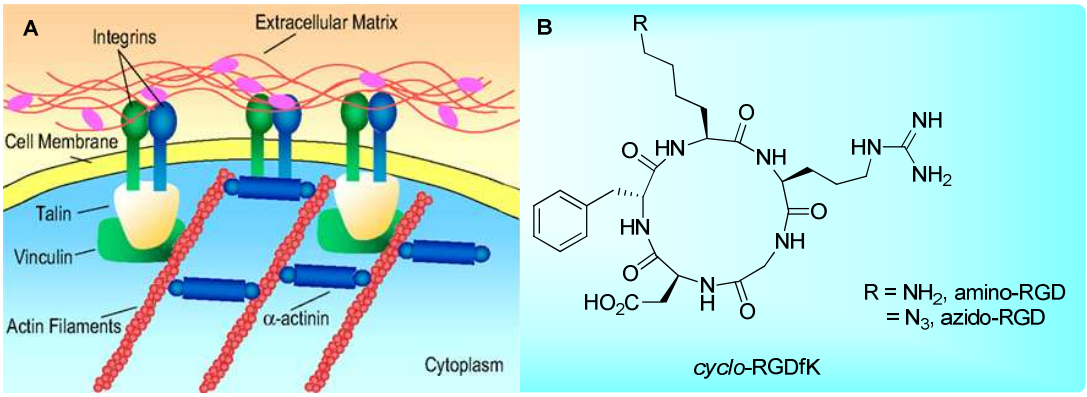


Figure 6. The effect of RGD peptidefor tissue engineering. A) Receptor-mediated cell and ECM interaction; B) *cyclo*-RGD peptide.

1.6 Sialic acids and their analogues

Sialic acid, the monomeric unit of polySia, is an essential component of sialoglycosphingolipids, which are widespread in living systems, and as part of oligosaccharide chains of numerous glycoproteins and glycolipids. They can be found in some microorganisms too. Most of them were located in cellular secretions and on the outer surface of cells.[63] Since the first discovery of sialic acids in vertebrates by Blix and Klenk in 1941, an extensive effort has been focused on the identification of their structure and biological roles.[64] More than 50 natural derivatives have been identified, the most common are *O*-acylated derivatives of

[59] S. Petersen, J. M. Alonso, A. Specht, P. Duodu, Maurice, M. Goeldner, A. del Campo, *Angew. Chem. Int. Ed.* **2008**, *47*, 3192-3195.
[60] J. Paleček, G. Dräger, A. Kirsching, *Synthesis* **2011**, 653-661.
[61] F. Z. Cui, W. M. Tai, S. P. Hou, Q. Y. Xu, I. S. Lee, *J. Mater. Sci. Mater Med*. **2006**, *17*, 1393-1401.
[62] M. Shachar, O. Tsur-Gang, T. Dvir, J. Leor, S. Cohen, *Acta Biomaterialia*. **2011**, *7*, 152-162.
[63] J. P. Kamerling, G. J. Boons, Y. C. Lee, A. Suzuki, N. Taniguchi, A. G. J. Voragen (Eds): *Comprehensive Glycoscience-From Chemistry to Systems Biology* **2007**, Vol. 1-4, Oxford, Elsevier.
[64] R. Schauer, *Sialic Acids: Chemistry, Metabolism and Function* **1982**, *Vol. 10*, Springer-Verlag, New York City.

5-acetamido-3,5-dideoxy-D-glycero-D-galacto-2-nonulosonic acid called *N*-acetylneuraminic acid **19** (or named neuraminic acid).[65] The 9-carbon monosaccharide contains an anomeric carboxylate, a deoxygenated methylene C-3 ring carbon, and an oligohydroxalated side chain at C-6. Other structurally similar sialic acids are *N*-glycolylneuraminic acid **20** [66] and ketodeoxy-nonulosonic acid **21**, which is a potential oncofetal antigen (*Figure 7*).[67]

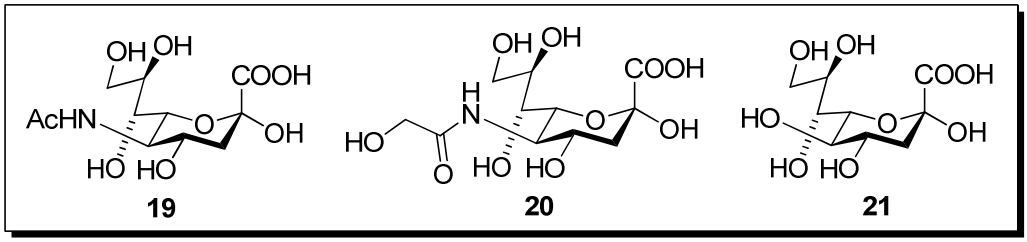


Figure 7. The most common sialic acids: *N*-acetylneuraminic acid (**19**), *N*-glycolylneuraminic acid (**20**), and ketodeoxy-nonulosonic acid (**21**).

The biological activities of sialic acids are widely discussed in the literature. The main roles of sialic acid in vivo are classified by Maru [68] as follows: (i) "endowment of negative charge on cellular membranes as glycoconjugates, (ii) determination of the macromolecular structures of certain glycoproteins, (iii) information transfer between cells and (iv) recognition of specified glycoconjugates and cells based on specific bio-activities." Most recently, sialic acids regulators of molecular and cellular interactions was described by Schauer.[67]

Due to their unique biological role, their densely functionalization, and the stereochemistry-rich frameworks sialic acids have elicited increasing attention in different fields. Sialic acids and their analogues have been widely used as probes or inhibitors in biological and medical research, some of the sialic acid analogues have achieved commercial success. Examples for commercially successful sialic acid analogues are Relenza and Tamiflu which are used for the treatment of influenza.[69] During the last decade, many neuraminic acids based novel pharmaceutical agents and diagnostic reagents for influenza viruses were investigated in medical fields.

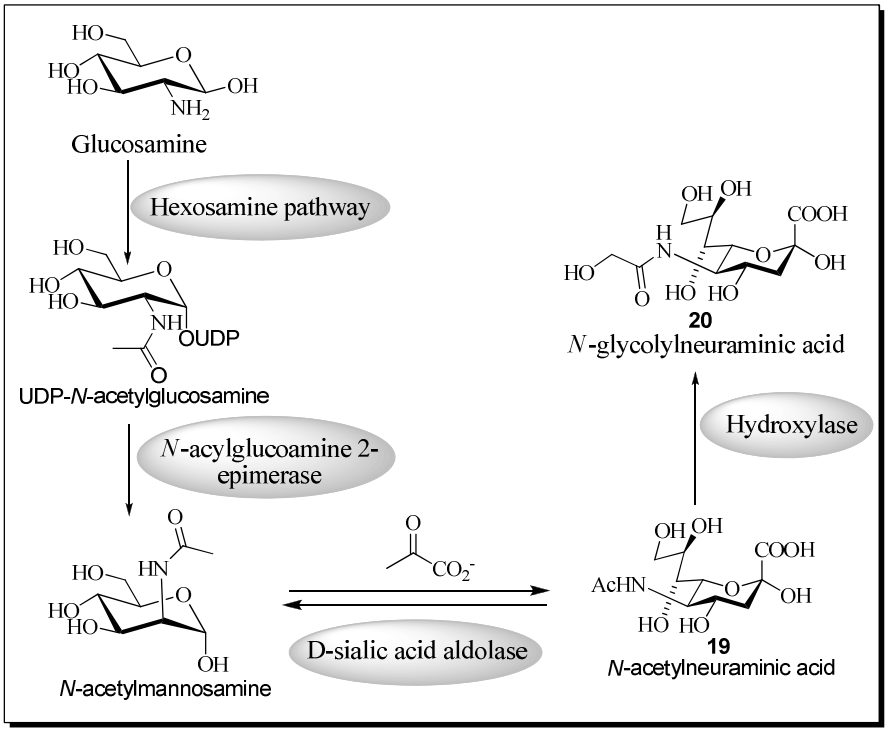
N-acetylneuraminic acid is the most ubiquitous sialic acid and is the biosynthetic precursor for all other sialic acids. The limited natural sources of sialic acids make them unsuitable for

[65] M. J. Kiefel, Von M. Itzstein, *Chem. Rev*. **2002**, 102, 471-490.
[66] R. Schauer, *Current Opinion in Structural Biology* **2009**, *19*, 1-8.
[67] E. A. Voight, C. Rein, S. D. Burke, *J. Org. Chem*. **2002**, *67*, 8489-8499.
[68] I. Maru, J. Ohnishi, Y, Ohta, Y. Tsukada, *J. BioSci. Bioeng*. **2002**, *93*, 258-265.
[69] a) M. von Itzstein, W. Y. Wu, G. B. Kok, M. S. Pegg, J. C. Dyason, B. Jin, P. T. Van, M. L. Smythe, H. F. White, S. W. Oliver, *Nature* **1993**, *363*, 418-423;
b) C. U. Kim, W. Lew, M. A. Williams, H. Liu, L. Zhang, S. Swaminathan, N. Bischofberger, M. S. Chen, D. B. Mendel, C. Y. Tai, W. G. Laver, R. C. Stevens, *J. Am. Chem. Soc*. **1997**, *119*, 681-690.

large–scale applications. Recently, several biological and chemical methods for the synthesis of sialic acids have been developed, allowing a large scale synthesis of sialic acid.

1.6.1 Biosynthesis of neuraminic acids and analogues

In the past two decades the biosynthetic pathway of sialic acids in mammalian cells has been intensively investigated for metabolic glycoengineering by glycobiologists. Most recently a detailed description of the sialic acid biosynthetic pathway was given by Yarema which is briefly illustrated in Scheme 11.[70] The pathway begins with glucosamine which is phosphorylated into active glucosamine-6-phosphate and converted into the nucleotide sugar UDP-*N*-acetyl-glucosamine through the hexosamine pathway. Subsequently, the UDP-*N*-acetyl-glucosamine is converted into *N*-acetyl-D-mannosamine.[71] This process is catalyzed by *N*-acylglucosamine 2-epimerase. *N*-acetyl-mannosamine is the dedicated metabolic precursor for the sialic acid biosynthetic pathway. Then *N*-acetylmannosamine reacts with pyruvate to form *N*-acetylneuraminic acid catalyzed by D-sialic acid aldolase. In some bacterial cells the *N*-acetylneuraminic acid can be converted into the sialic acid analogue *N*-glycolylneuraminic acid.



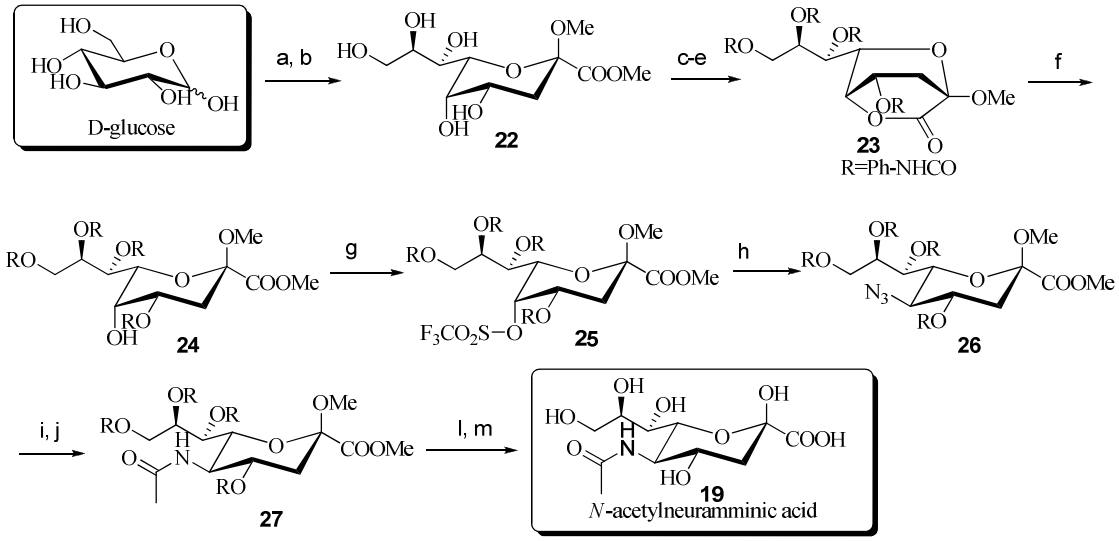
Scheme 11. Metabolic pathway of *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid.

[70] J. Du, M. A. Meledeo, Z. Y. Wang, H. S. Khanna, V. D. P. Paruchuri, K. Yarema, *Glycobiology* **2009**, *19*, 1382-1401.
[71] J. A. Hanover, *FASEB J*. **2001**, *15*, 1865-1876.

Before the above-mentioned pathway of mammalian sialic acid metabolism was established various approaches for the enzymatic synthesis of neuraminic acid were reported.[55] For example, neuraminic acid was synthesized from *N*-acetylmannosamine and pyruvate catalyzed by *N*-acetylneuraminiate lyase [EC 4.1.3.3].[72] Due to the use of expensive starting materials, this method is of limited use. After the discovery of the epimerization of *N*-acetylglucose and *N*-acetylmannose catalyzed by *N*-acylglucosamine 2-epimerase [EC 5.1.3.1] inexpensive *N*-acetylglucose was established as starting material in the synthesis of neuraminic acid through the coupling of the two enzymes in a membrane reactor.[73]

1.6.2 Chemical synthesis of neuraminic acid

Numerous chemical approaches were developed to produce neuraminic acid. However, most of these procedures can not deliver satisfactory amount of sialic acids from an economically point of view. Shiba and co-workers developed a novel approach to generate neuraminic acid with the correct stereochemistry using inexpensive D-glucose as starting material.[74] The synthetic route is shown in Scheme 12.



Scheme 12. Synthesis of sialic acid through aldol condensation of glucose with oxalacetic acid. Conditions: a) Oxalacetic acid, sodium borate buffer (pH = 11.0), RT, 72 h; b) HCl, MeOH; c) H_2O, $Ba(OH)_2$; d) DCC, pyridine; e) Ph-NCO, pyridine, 35.1% over 3 steps; f) HCl, MeOH, 57.8%; g) $(CF_3SO_3)_2O$, pyridine, CH_2Cl_2, 92.8%; h) $(CH_3(CH_2)_3)_4NN_3$, 92.3%; i) H_2/Pd-C; j) $(CH_3CO)_2O$, DMAP, 35% over 2 steps; k) NaOH; l) Amberlyst 15 (H form), 62.3% over 2 steps.

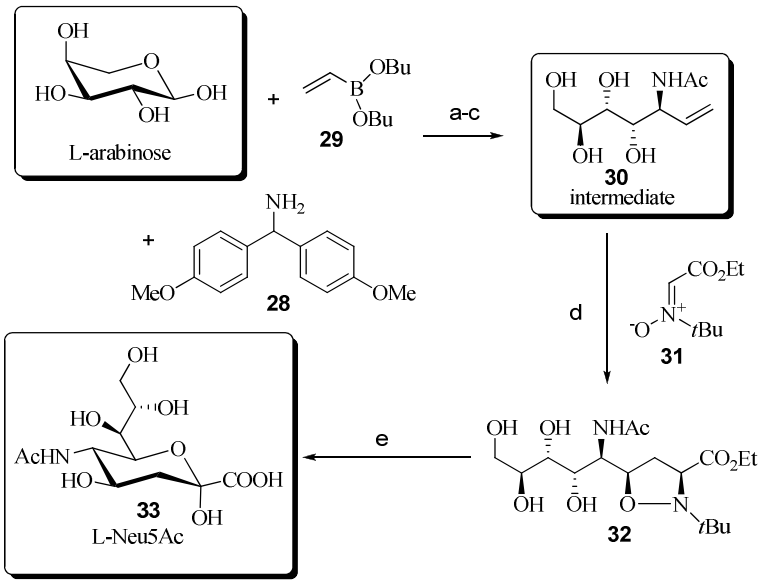
[72] M. J. Kim, W. J. Hennen, H. M. Sweers, C. H. Wong, *J. Am. Chem. Soc.* **1988**, *110*, 6481-6484.
[73] U. Kragl, D. Gygax, O. Ghisalba, C. Wandrey, *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 827-828.
[74] T. Yamamoto, T. Teshima, K. Inami, T. Shiba. *Tetrahedron Lett.* **1992**, *33*, 325-328.

The synthesis starts with the coupling of D-glucose and oxalacetic acid in alkaline solution and decarboxylated under acidic conditions, followed the treatment with acid to give desired pyranose compound **22**. After liberation of the carboxyl group, *δ*-hydroxy ester **22** was lactonized selectively with the axial C5-hydroxyl group. Simultaneous protection of hydroxyl groups was achieved with phenyl isocyanate. Through the one-pot reaction, the key intermediate lactone **23** was provided. Lactone **23** was treated with acid to convert to 5-hydroxy methyl ester **24**. The hydroxyl group at C-5 was sulfonylated to give methyl ester **25**. Subsequently, an azide group was introduced to C5 to afford **26**. Afterward, the azide group was reduced to amine group and acetylated resulting in protected neuraminic acid **27**. After deprotection, the desired *N*-acetylneuraminic acid **19** was obtained.

1.6.3 Syntheses of *N*-acetylneuraminic acid derivatives

Many procedures were optimized for the chemical synthesis of sialic acids and its derivatives. Some of these were not cost effective due to the long synthetic routes and low overall yields. A novel and interesting route was successfully developed by Wong and co-workers for the synthesis of L-*N*-acetylneuraminic acid.[75] The fairly straightforward synthetic route is depicted in scheme 13 with L-arabinose as starting material. A Petasis reaction served to form the imine, which was treated with the dibutyl vinyl boronic acid ester **29** to subsequently afford the acetamide intermediate **30**. Then, the vinyl group of **30** reacted with *N*-*tert*-butyl nitrone **31** by means of a 1,3-dipolar cycloaddition to afford the nine–carbon sugar **32** containing a five-membered ring as a 10:1 mixture of diastereomers, the desired 4R-isomer being the major product. After deprotonation and ring-opening in one step, the desired product L-*N*-acetylneuraminic acid **33** was obtained. This route has also been used for the synthesis of D-*N*-acetylneuraminic acid using D-arabinose as chiral starting material, as well as other sialic acid derivatives using various aldoses or their substituted derivatives. This methodology is advantageous compared to other methods due to the simple procedure, good stereoselectivity and the avoidance of protection groups.

[75] Z. Y. Hong, L. Liu, C. C. Hsu, C. H. Wong, *Angew. Chem. Int. Ed*. **2006**, *45*, 7417-7421.



Scheme 13. Three-step synthesis of L-*N*-acetylneuraminic acid. Conditions: a) EtOH/H_2O (4:1), 50 °C, 3 d; b) TFA, 50 °C; c) Ac_2O, MeOH, 55% over 3 steps, > 99% de; d) dioxane, 30°C, 90%; e) NaOMe in MeOH followed by H_2O, 60%.

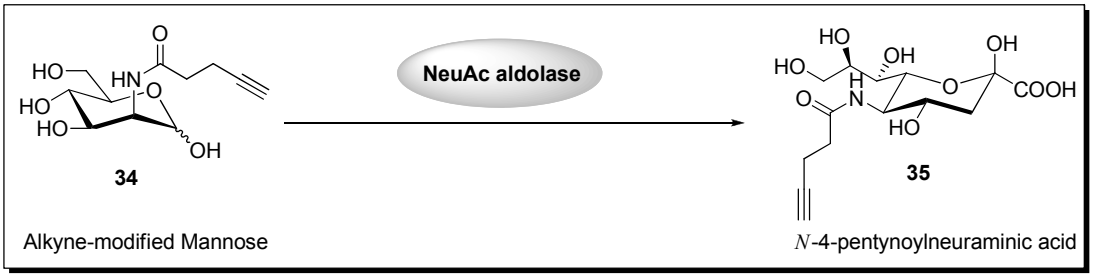
Another approach to sialic acid derivatives uses *N*-acetylmannose which can be modified to yield various analogues. These derivatives reacted with pyruvate to give desired neuraminic acid derivatives via a chemoenzymatic approach. Additionally, neuraminic acid itself can be used as starting material for the synthesis of many derivatives. In theory every position on the nine-carbon backbone of neuraminic acid can be derivatized for such purpose. A recent review by Bennet described synthetic strategies developed for the synthesis of sialic acid and its derivatives at each position on the carbon skeleton.[76]

The carboxylate moiety at C-1 is responsible for binding to numerous sialic acid specific proteins and is partially responsible for many of the biological functions of sialosides. An evaluation of the construction of the C-1 carboxyl group to enzyme binding and receptor interaction can be conducted by different functional groups. One example is the replacement by a phosphonate group in order to investigate the effects of another negatively charged functional group.[77] Usually, C-2 and C-3 were decorated by various functionalities in order to investigate the actual structural information of the neuraminic acid or impact on binding to various enzymes.[78] The synthesis of C4-modified sialic acid analogues has been of interest as influenza sialidase inhibitors like Tamiflu and Relenza.[70] Tamiflu is a sialic acid analogue, in which among other variations at the C-4 position the hydroxyl group was substituted with an amino

[76] I. Hemeon, A. J. Bennet, *Synthesis* **2007**, *13*, 1899-1926.
[77] A. Vasella, R. Wyler, *Helv. Chim. Acta* **1991**, *74*, 451-463.
[78] R. Gantt, S. Millner, S. B. Binkley, *Biochemistry*. **1964**, *3*, 1952-1960.

group. Relenza is a sialic acid analogue with a guanidinyl group in place of a hydroxyl group at C4-position.

For various purposes, the acetamido group at C-5 was substituted by different functional groups. The acetamido group can be hydrolysed to an amino group under acidic or basic conditions. Thereafter new substituents can be introduced to give a variety of *N*-alkyl amides.[79] Using this method Guo and co-workers synthesized numerous *N*-derivatives of neuraminic acid including *N*-propionyl, *N*-butanoyl, *N*-isobutanoyl, *N*-phenylacetyl, *N*-benzoyl, and *N*-trifluoropropanoyl derivatives to investigate the properties of the important *N*-acetyl-D-neuraminic acid aldolase.[80] Elofsson and co-workers also designed and synthesized a series of *N*-acyl-modified sialic acids which were used as inhibitors in place of multivalent sialic acid for adenoviruses which cause epidemic Keratoconjunctivitis.[81] They synthesized various *N*-acyl functionalized sialic acids from pre-treated neuraminic acid and different acyl groups. However, the isolation of completely deprotected products after hydrolysis of the methyl ester appeared to be problematic. This can be explained by the instability of sodium *N*-acetylneuraminate under thermal alkaline conditions whereby the sialic acid ring structure is degraded.[82] Bertozzi and Wu synthesized an *N*-alkyno-modified neuraminic acid analogue using a combination of chemical and enzymatic methods obtaining a good yield.[83] Alkyno-modified mannose derivative **34** [84] reacted with sodium pyruvate in buffer (pH 7.4) to give the desired *N*-4-pentynoylneuraminic acid **35** (*Scheme 14*).



Scheme 14. Chemoenzymatic synthesis of *N*-functionalized neuraminic acid derivatives. Conditions: sodium pyruvate, 1% NaN_3, KH_2PO_4 (pH = 7.4), 89%.

[79] a) R. Roy, C. A. Laferrière, *Can. J. Chem*. **1990**, *68*, 2045-2054;
b) H. Tanaka, M. Adachi, T. Takahashi, *Chem. Eur. J*. **2005**, *11*, 849-862;
c) Y. B. Pan, P. Chefalo, N. Nagy, C. Harding, Z. W, Guo, *J. Med. Chem*. **2005**, *48*, 875-883.
[80] Y. B. Pan, T. Ayani, J. Nadas, S. M. Wen, Z. W. Guo, *Carbohydr. Res*. **2004**, *339*, 2091-2100.
[81] S. Johansson, E. Nilsson, W. X. Qian, D. Guilligay, T. Crepin, S. Cusack, N. Arnberg, M. Elofsson, *J. Med. Chem*. **2009**, *52*, 3666-3678.
[82] K. Saito, K. Sugai, K. Fujikura, N. Yamada, M. Goto, *Carbohydr. Res*. **1989**, *185*, 307-314.
[83] P. V. Chang, X. Chen, C. Smyrnoitis, A. Xenakis, T. S. Hu, C. R. Bertozzi, P. Wu, *Angew. Chem. Int. Ed.* **2009**, *48*, 4030-4033.
[84] T. L. Hsu, S. R. Hanson, K. Kishikawa, S. K. Wang, M. Sawa, C. H. Wong, *Proc. Natr. Acad. Sci. USA* **2007**, *104*, 2614-2619.

Structural variations at the oligohydroxylated side chain were prepared by Zbiral and coworkers. They performed an array of useful approaches to synthesis of stereo and deoxy analogues of neuraminic acid.[85] Various epimers of neuraminic acid including 7-epi-, 8-epi-, and 7,8-bis(epi)-*N*-acetylneuraminic acids [86] and deoxy analogues like 7-deoxy-, 8-deoxy-, 9-deoxy-*N*-acetylneuraminic acids [87] were synthesized and used as inhibitors or substrates of sialidase to investigate the profile of enzyme-substrate-interactions, their behaviours towards CMP-sialate synthetase as well as structure information about neuraminic acid.[88]

[85] B. P. Bandgar, M. Hartmann, W. Schmid, E. Zbiral, *Liebigs Ann. Chem*. **1990**, 1185-1195.
[86] M. Hartmann, E. Zbiral, *Monatsh. Chem.* **1989**, *120*, 899-906.
[87] a) M. Salunke, M. Hartmann, W. Schmid, E. Zbiral, *Liebigs Ann. Chem*. **1988**, 187-189;
b) E. Zbiral, H. H. Brandstetter, E. P. Schreiner, *Monatsh. Chem.* **1988**, *119*, 127-141.
[88] E. Schreiner, R. Christian, E. Zbiral, *Liebigs Ann. Chem*. **1990**, 93-97.

2 Aims and Objectives

Tissue engineering aims to develop functional substitutes for damaged or diseased tissues through constructs of living cells, bioactive molecules, and 3D porous scaffolds, which support cell attachment, proliferation, and differentiation. As a significant branch, the nervous system presents a challenge to the field of tissue engineering because some diseases such as Parkinson´s or injured nerves or reconnection of served neural pathway may require regeneration of nervous tissue. One of the important tasks of tissue engineering is the synthesis of a biocompatible and nontoxic 3D-scaffold. Since polySia is present in the mammalian neural system as integral part of NCAM, this polysaccharide is an ideal starting material for the formation of a novel 3D-scaffold for nerve tissue engineering.

The natural occurring polySia such as colominic acid, which was isolated from capsules of Escherichia coli K1 (K1-polySia), is highly soluble in water. This limits the application in nerve tissue engineering. This work focuses on the chemical modifications of natural polySia, which is decorated with functional groups and subsequently transformed to water insoluble 3D-scaffolds using different polymerization methodologies. The synthesized 3D-scaffolds will be first tested *in vitro*. In this context, the cell viability will be evaluated by incubation of cell lines such as PC12 and HepG2 on the scaffolds. These two cell lines are ideal *in vitro* model systems for the biological evaluation of synthetic scaffold materials. Successively the application of the 3D-scaffold material for nerve tissue engineering is planed. Here the degradation of the scaffolds should be investigated (*Figure 8*).

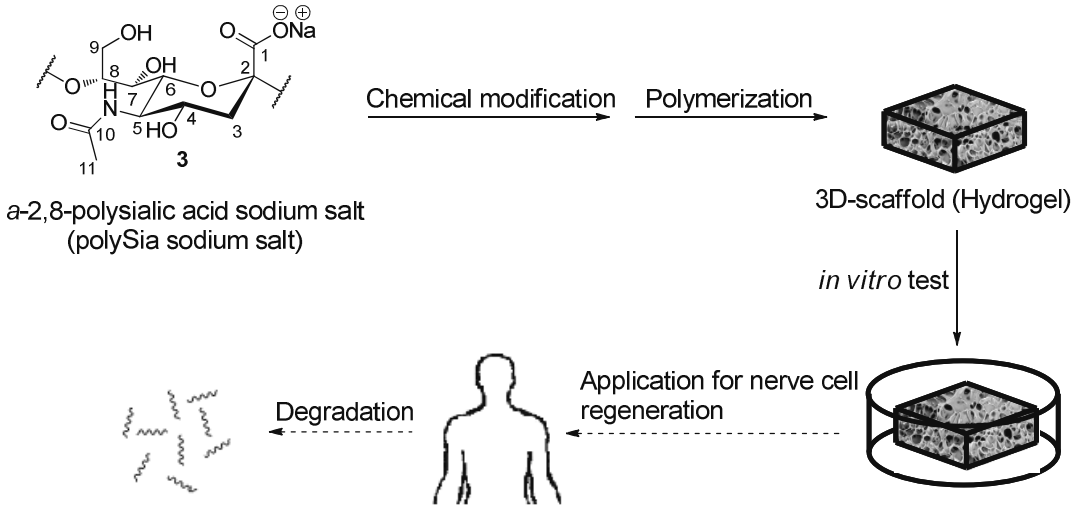
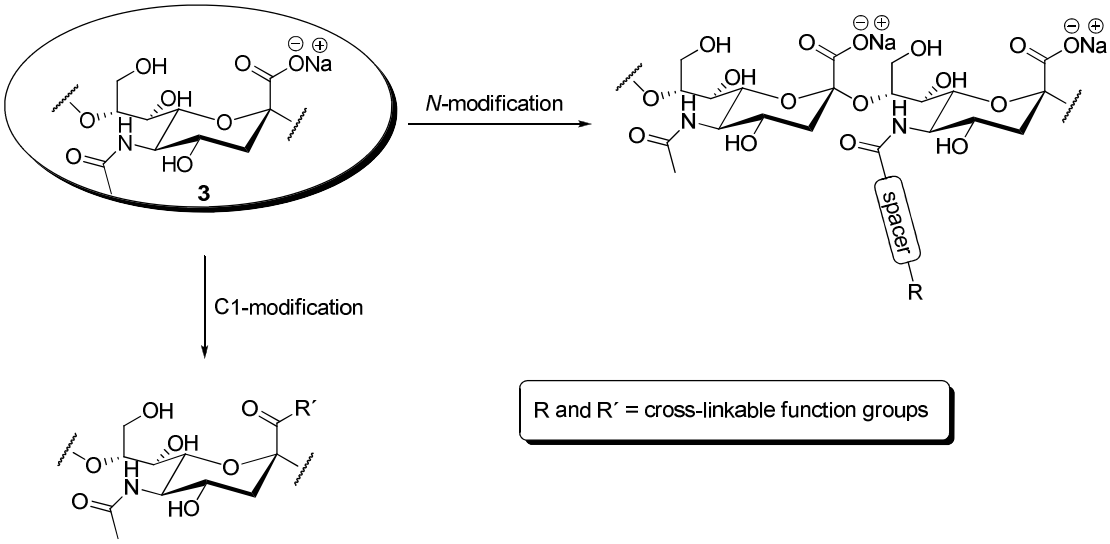


Figure 8. Schematic description of the aims and objectives.

An additional part of this work is the synthesis of various neuraminic acid derivatives from commercially available *N*-acetylneuraminic acid. The synthesized neuraminic acid derivatives will be provided for biological applications like the enzymatic synthesis of modified polySia.

2.1 Synthesis of 3D-scaffold based on modified polySia

In the previous study, Berski has been developed a stable 3D-hydrogel based on polySia.[89] For the formation of the insoluble hydrogel diepoxyoctane was used as a hydrophobic cross-linking agent connecting the hydroxyl groups to form a 3D-network. However, the crosslinking was not well defined since a high degree of the linker reacted with water giving "dead ends". In the continuing study of this project, a selective crosslinking between polysaccharide chains will be attempted to achieve the 3D-scaffolds. For this purpose polySia (sodium salt) **3** will be decorated with different functional groups including azido-, alkyno-, methacrylate-, aldehydo-, and hydrazido-group, respectively (*Scheme 15*). Then the modified polymers can be inter-connected to form stable networks through crosslinking reaction between the functional groups. In this work three different strategies will be used to accomplish the formation of 3D-scaffolds, namely "click" chemistry, photocrosslinking, and hydrazone crosslinking.

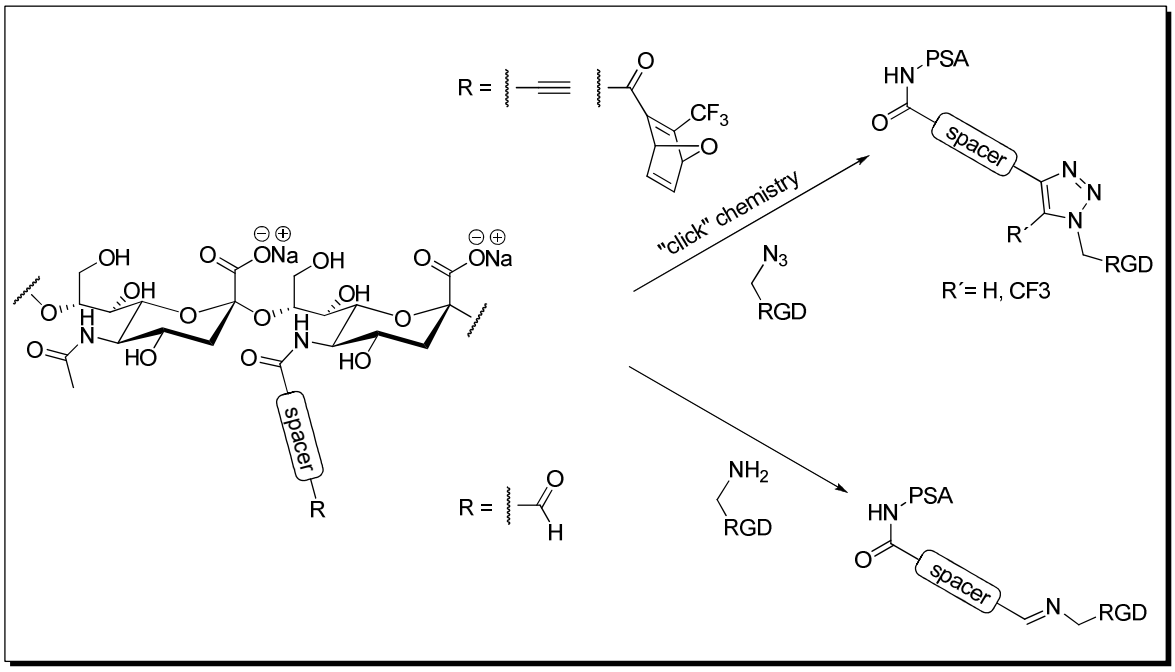


Scheme 15. Modification plan of polySia with crosslinkable functional groups.

For the "click" chemistry strategy, polySia will be modified with azido- and alkyno-functionalities respectively. The different modified polySia will be synthesized following previously reported methods from commercial polySia (sodium salt **3**).[89] The crosslinking reaction occurs in the presence of a Cu catalyst. Considering the metal catalyst has a potential

[89] S. Berski. PhD Thesis, 2008, Gottfried Wilhelm Leibniz University of Hannover.

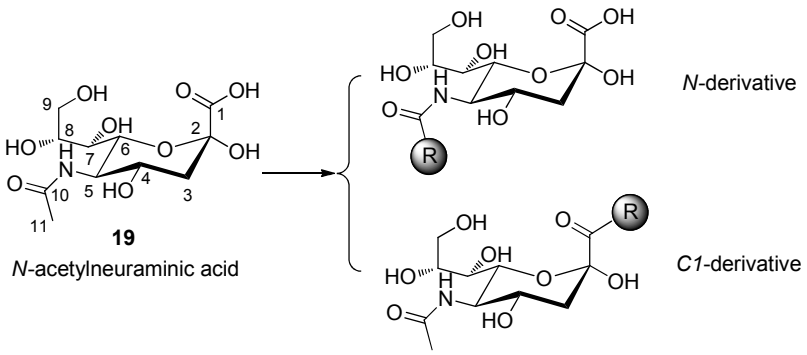
toxicity which is problematic in later *in vivo* tests for nerve tissue engineering, metal-free "click" chemistry is desirable for this approach. Therefore, the alkyno-analogue oxanorbornadiene, which has a bicyclic system and a trifluoromethyl group, will be prepared according to a known procedure. For the second strategy, methacrylate was selected as photoactive linker, which was attached through a new amide bond to the polymer. For hydrazone crosslinking, polySia will be decorated with aldehyde- and hydrazido-groups, respectively. The aldehyde-group can be introduced via the following method. At first, the polymer is modified with a 1,2-diol functionality and then oxidized with sodium periodate. The hydrazido containing polySia will be prepared by aminolysis of polySia methyl ester or of polySia lactone by hydrazide monohydrate. All of the functional groups will be linked to the polymer through amide bonds at C-5 position except the hydrazido at C-1. The degree of derivatization will be controlled in range of 25-80% considering their degradability. Furthermore, improvement of the interaction between cells and synthesized cell matrix is anticipated. Hence cyclic RGD peptide as described earlier is used. Binding of the RGD peptide to the polySia will be attempted by means of "click" chemistry or imine formation (*Scheme 16*). The reaction conditions should not alter the structure and conformation of the RGD peptide. The RGD peptide modified polySia will be used for formation of 3D-scaffolds together with azido-/hydrazido-modified polySia.



Scheme 16. Synthesis plan of RGD-modified polySia.

2.2 Synthesis of neuraminic acid derivatives

The second major target of the project is the synthesis of different neuraminic acid derivatives including C1- and *N*-derivative from commercial *N*-acetylneuraminic acid **19** (*Scheme 17*).



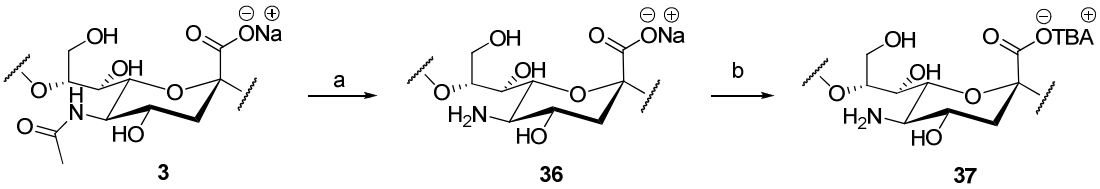
Scheme 17. Synthesis plan for the purposed C1- and *N*- derivatives from *N*-acetylneuraminic acid.

The synthesized derivatives will be provided to collaboration partner for the enzymatic synthesis of polySia derivatives. Moreover, these neuraminic acid derivatives can also be applied for other biomedical purposes.

3 Description and Discussion

3.1 Modification of polySia and preparation of 3D-scaffolds

In this work, three innovative crosslinking strategies were applied to achieve a series of appropriate structured 3D-scaffolds material from commercially available α-2,8-polysialic acid (polySia). The strategies used are "click" chemistry mediated crosslinking, photo-initiated crosslinking, and hydrazone crosslinking. This requires that the starting material was modified with the corresponding crosslinkable functional groups. The necessary groups can be attached to the polymer chains via amide bonds at the C-5 and the C-1 positions to yield *N*- and C1-modified polySia. Afterwards these modifications were used to form flexible and stable 3D-scaffolds material under mild conditions. Regarding the *N*-modification of polySia, polySia (sodium salt) **3** was deacetylated to form *γ*-amino polySia (sodium salt) **36**. This allowed to introduce new functional groups by attaching various side chains to the free amino group of the polymer via amide bonds. However, the deacetylated polySia (sodium salt) **36** was not soluble in any organic solvent and therefore reactions in non-aqueous solvent were limited. To overcome this problem, **36** was converted into *γ*-amino polySia tetrabutylammonium (TBA) salt **37** through a dialysis process. TBA salt **37** can be dissolved in polar organic solvents such as pyridine, DMF, and DMSO (*Scheme18*).



3 36 37

Scheme 18. Synthesis of *γ*-amino polySia **36** and **37**. Conditions: a) NaOH, EtOH/H_2O, reflux, 16 h, 80-99%; b) Dialysis against TBA-Bromide (0.5 M), 24 h, then dialysis against distilled H_2O.

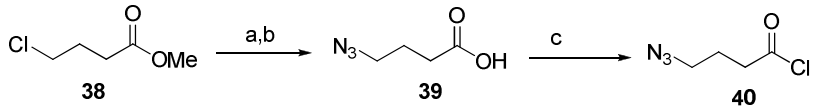
3.1.1 Formation of polySia hydrogels using "click" chemistry

In order to generate 3D-scaffolds via "click" crosslinking, polySia derivatives with "clickable" azido, alkyno or alkyno-analogues (as Rutjes´oxanorbornadiene) moieties were prepared from *γ*-amino polySia (TBA salt) **37**.

3.1.1.1 Synthesis of azido- and alkyno-modified polySia

For Cu-catalyzed generation of polySia hydrogel by multiple "click" reactions, the azido- and alkyno-modified polySia **41** and **44** derivatives were prepared according to Berski.[89] For the

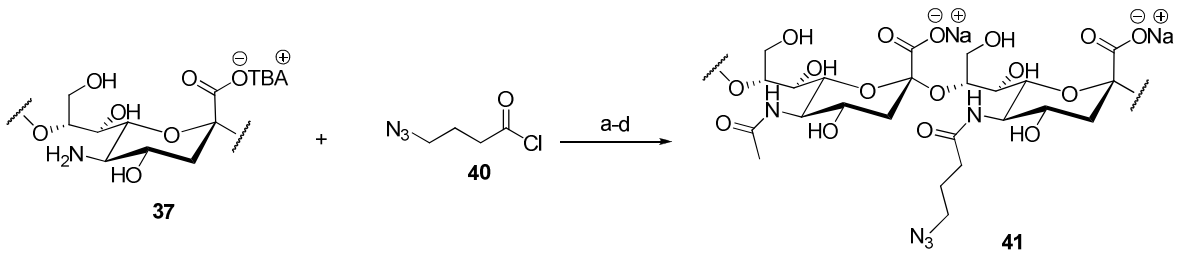
synthesis of azido-polySia, azido butyric acid chloride (**40**) was prepared. The acid chloride **40** was synthesized from 4-chloro butyryl methyl ester (**38**) over 3 steps.[90] At first, ester **38** was transferred into the azide via S_N2 substitution in the presence of a catalytic amount of sodium iodide. Then, the methyl ester was treated with base to give carboxylic acid **39**, which was finally converted into desired 4-azidobutyryl chloride **40** (*Scheme 19*).



Scheme 19. Synthesis of 4-azidobutyryl chloride **40**. Conditions: a) DMF, NaI (cat.), NaN_3, 60 °C, 24 h; b) H_2O, NaOH (1 M), r.t., 4 h; c) Thionyl chloride, 60 °C, 1 h, then 80 °C, 1 h. Quantitative conversion over three steps.

Product **40** was identified by IR- analysis. Introduction of azido-functionality (2096 cm^{-1}), and transformation of carboxylic acid (1709 cm^{-1}) into acid chloride (1790 cm^{-1}) were clearly detected.

PolySia derivative **41** was gained from *γ*-amino polySia (TBA salt) **37** and azido acid chloride **40** under basic conditions. Anhydrous pyridine as basic organic solvent was suitable for a successful reaction. By changing the equivalents of acid chloride the product was obtained with different derivatization degrees in a range of 25-90 %. The unreacted amino groups were protected as *N*-acetyl group by treatment with acetic anhydride, which was important for the enzymatic degradation process. Undesired ester-bonds were cleaved by final basic treatment. After dialysis the desired product **41** was obtained (*Scheme 20*). The degree of derivatization could be evaluated by ^{1}H-NMR.



Scheme 20. Synthesis of *N*-4-azidobutytyl polySia **41**. Conditions: a) Pyridine, r.t., 24 h; b) Acetic anhydride, r.t., 8 h; c) NaOH (1 M), r.t., 8 h; d) Dialysis against NaCl (pH = 9), 2 d, then against distilled H_2O, 2 d, 92%.

Azido-polySia derivative could be identified qualitatively by IR-measurement at 2096 cm^{-1} (*Figure 9*).

[90] a) B. Carboni, M. Vaultier, R. Carrié, *Tetrahedron* **1987**, *43*, 1799-1810;
b) M. Rothe, T. Toth, *Chem. Ber.* **1966**, *99*, 3820-3829.

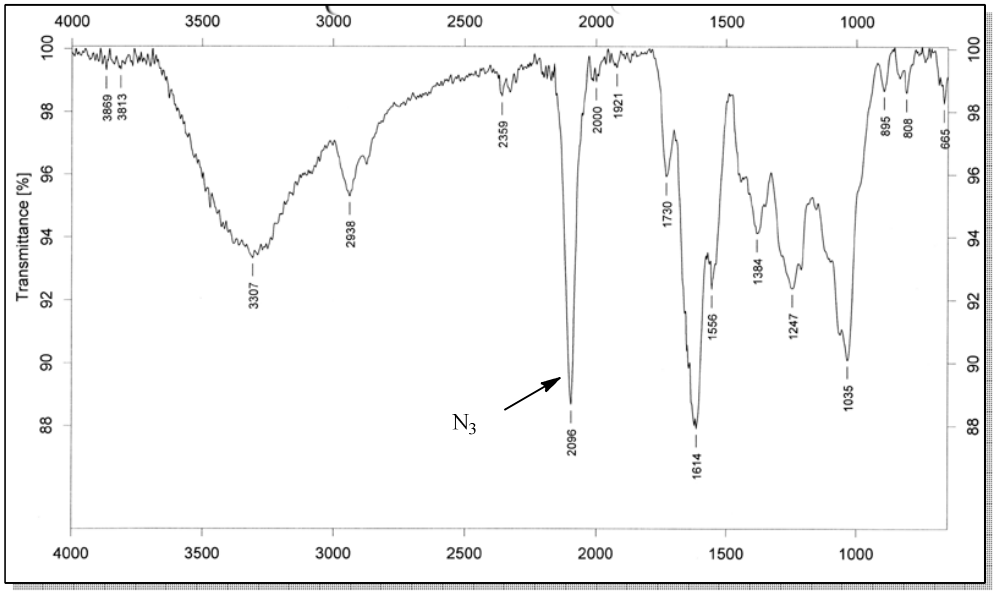
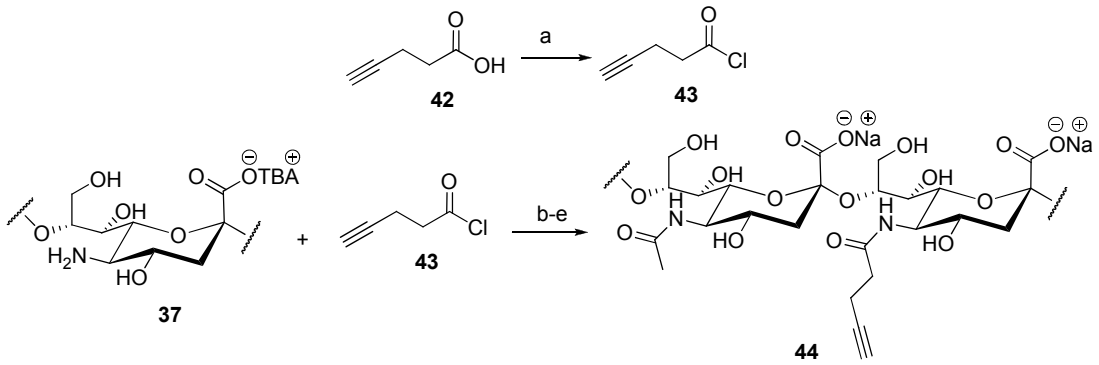


Figure 9. IR-spectra of azido-modified polySia **41**.

PolySia derivative **44**, which was decorated with an alkyno-group, was synthesized from 4-pentynoic chloride (**43**) and *γ*-amino polySia TBA salt **37** using the similar process as described before for the synthesis of polySia **41**. 4-Pentynoic chloride (**43**) was prepared from commercial available 4-pentinoic acid (**42**) by treatment with thionyl chloride (*Scheme 21*).[91]



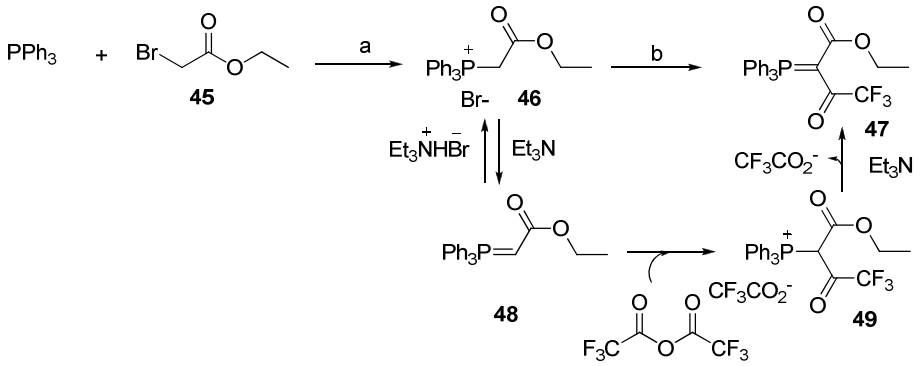
Scheme 21. Synthesis of *N*-(4-pentynoyl) polySia **44**. Conditions: a) Thionyl chloride, 60 °C, 1 h, then 80 °C, 1 h; b) Pyridine, r.t., 24 h; c) Acetic anhydride, RT, 8 h; d) NaOH (1 M), r.t., 8 h; e) Dialysis against NaCl (pH = 9), 2 d, then against distilled H_2O, 2 d, 56%.

3.1.1.2 Synthesis of oxanorbornadienyl-modified polySia

In an alternative approach, polySia hydrogels shall be prepared via metal-free "click" chemistry. Therefore, oxanorbornadienyl-substituted polySia **52** was synthesized from *γ*-amino polySia (sodium salt) **36** or (TBA salt) **37** and oxanorbornadienyl acids **53**. The oxanorbornadienyl-system was prepared according to modified literature procedures. The (carbethoxy-

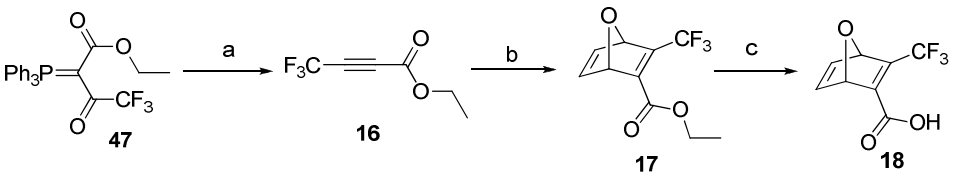
[91] R. S. Atkinson, M. J. Grimshire, *J. Chem. Soc. Perkin Trans* **1986**, *1*, 1215-1224.

methyl)-triphenylphosphonium bromide (**46**) was prepared from triphenylphosphine and ethyl 2-bromoacetate (**45**). Subsequently, the phosphonium salt **46** was treated with triethylamine to give in monosubstituted methylenephosphorane **48** in a reversible step. Acylation of phosphorane **48** with trifluoroacetic anhydride afforded phosphonium salt **49**, which was converted into the desired phosphorane **47** in the presence of base. This transformation was achieved in a one-pot reaction (*Scheme 22*). [92]



Scheme 22. Preparation of (α-acymethylene)phosphorane **47**. Conditions: a) Toluene, r.t., 48 h; b) THF, Et_3N, 0 °C, 15 min; Trifluoroacetic anhydride (over 15 min, dropwise), 0°C, 1 h, 100%.

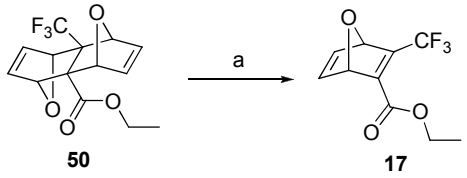
Phosphorane **47** was converted into ethyl-2-flurobut-2-ynoate (**16**) by pyrolysis at 150-210 °C (14 mbar).[93] As by-product triphenylphosphine oxide was produced. The oxanorbornadienyl-ester **17** was obtained from the acetylene **16** and furan via Diels-Alder-reaction.[94] The literature procedure for this step described that acetylene **16** and furan (1.2 eq.) were heated in dichloromethane under argon atmosphere at 40 °C for 4 d. After further optimization, the reaction was carried out under microwave conditions at 60 °C in 30 min and without any decrease of yield. Different equivalents of furan were tested for the Diels-Alder-reaction. It was found that 1.5 equivalent of furan afforded best results. Ester **17** was treated with base to obtain oxanorbornadienyl carboxylic acid **18** (*Scheme 23*).



Scheme 23. Synthesis of oxanorbornadienyl carboxylic acid **18**. Conditions: a) 150-210 °C, 14 mbar, 2 h, 86%; b) Furan (1.5-2.0 eqval.), microwave oven, 60 °C, 0.5 h, 70%; c) H_2O/THF (1:1), NaOH (1 M), r.t., 2 h, >90%.

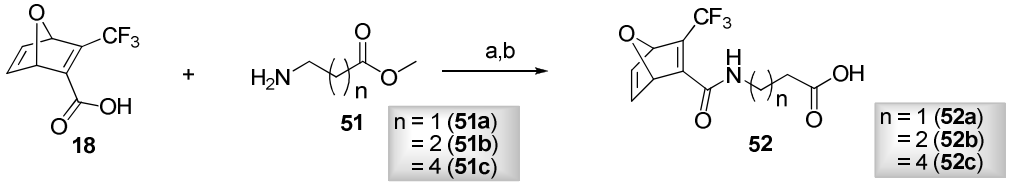
[92] B. C. Hamper, *J. Org. Chem.* **1988**, *53*, 5558-5562.
[93] Y. Z. Huang, Y. C. Shen, W. Ding, J. Zheng, *Tetrahedron Lett.* **1981**, *22*, 5283-5284.
[94] M. G. Barlow, N. N. E. Suliman, A. E. Tipping. *J. Fluorine Chem.* **1995**, *70*, 59-69.

In this thesis, various double cycloaddition by-products were observed like *endo, exo*-isomer and *exo, exo*-isomer, which were also described in detail in the literature.[95] In this case, the obtained by-products could be easily transferred into the desired product via retro-Diels-Alder reaction. For example, the *endo, exo*-isomer **50** was heated in dioxane in a microwave oven at 100 °C for 30 min to give the desired product **17** with practically complete conversion (*Scheme 24*).



Scheme 24. Conversion of *endo, exo*-isomer **50** to oxanorbornadienyl-ester **17**. Condition: a) 1,4-dioxane microwave oven, 100 °C, 0.5 h, 100% (determined by ^{1}H-NMR).

Carboxylic acid **18** was directly tested for the coupling with the free amino group of *γ*-amino polySia (TBA salt) **37**. However, the coupling reaction failed. A possible reason was the steric hindrance of the ring systems. Therefore, a suitable spacer was introduced for the coupling of both components. The spacer could be connected to oxanorbornadienyl carboxylic acid **18** via ester or amide bond formation. Different amino esters **51** were coupled to carboxylic acid **18** catalyzed by peptide coupling reagent. After treatment with base, the elongated oxanorbornadienyl carboxylic acids **52a-c** were prepared successfully in good to moderate yield over two steps (*Scheme 25*).



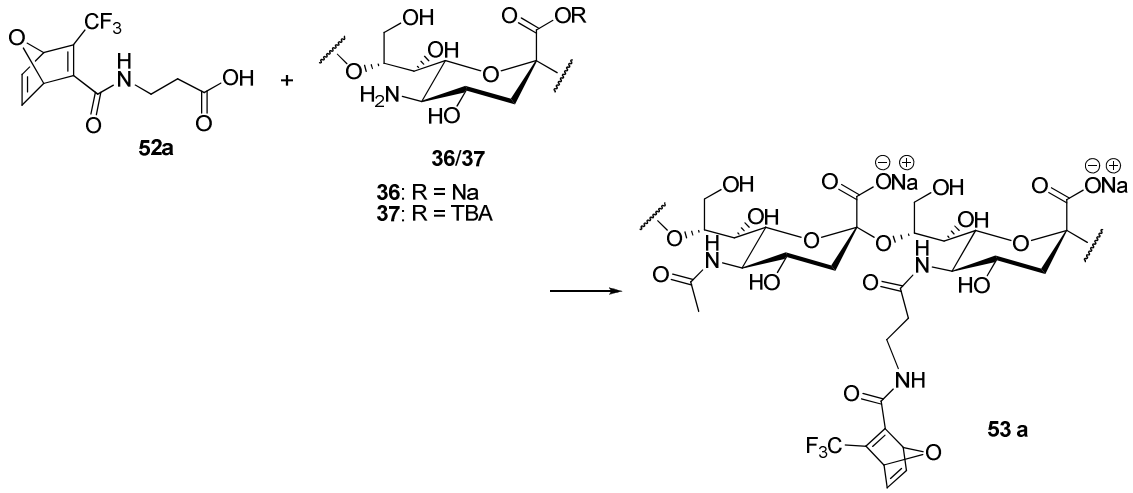
Scheme 25. Synthesis of elongated oxanorbornadienyl carboxylic acids **52**. Conditions: a) EDC·HCl, DMAP, DCM, 0 °C→r.t., 24 h, 57-70%; b) THF/H_2O, LiOH (0.5 M), 2 h, 90-95%.

Regarding the coupling between the deacetylated polySia and carboxylic acids **52a-c**, different methods were tested. The carboxylic acids **52a-c** were directly attached to the amino group of the polySia in presence of coupling reagents, or the carboxylic acids **52a-c** were first achieved as NHS active esters by treating with NHS, which will be described in chapter 3.2.2. Considering the solubility of the polymer in different solvents, the *γ*-amino polySia (sodium

[95] A. Nezis, J. Fayn, A. Cambon. *J. Fluorine Chem.* **1991**, *53*, 285-295.

salt) **36** or (TBA salt) **37** were used for the different reaction conditions. It was found that the use of NHS-esters or coupling reagents did not show significant difference. The degree of derivatization could be raised up to 25 % (*entries 1 and 4, Table 1*). Variation of the reaction conditions could not improve the degree of derivatization.

Table 1. Synthesis of oxanorbornadienyl-group containing polySia derivative **53**.

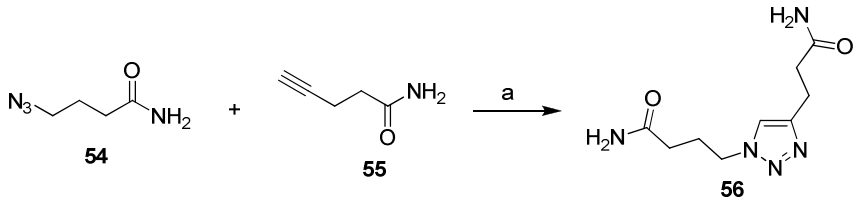


Entry	PolySia (**36** or **37**)	Coupling method (Solvent, coupling reagent)	Degree of derivatization[a]
1	**36**	H_2O, ECD·HCl, HOBt, DIPEA	25 %
2	**36**	H_2O/dioxane, Et_3N, NHS-ester of **52a**	5-10 %
3	**37**	DMF, ECD·HCl, HOBt, DIPEA	5-10 %
4	**37**	DMF, DIPEA, NHS-ester of **52a**	25 %
5	**37**	DMF, ECD·HCl, DMAP	5-10 %

[a] The degree of derivatization was determined by ^{1}H-NMR.

3.1.1.3 Modulation of "click" reaction

Prior to the polymerization of modified polySia derivatives via "click" reaction, model reactions of copper-catalyzed and metal-free "click" reactions were established. 4-Azidobutanamide (**54**) and pent-4-ynamide (**55**) were prepared from the corresponding acid chlorides with treatment of ammonia. The fact, that both reactants showed good water solubility is important, because this is an essential requirement for the final hydrogelation process. The reaction of 4-azidobutanamide (**54**) and pent-4-ynamide (**55**) afforded the 1,2,3-triazole formation via [3+2] dipolar cycloaddition in the presence of Cu-catalyst. Under these conditions, the reaction yielded selectively 1,4-triazole **56** (*Scheme 26*).



Scheme 26. Modulation of copper-catalyzed "click" reaction. Condition: a) H_2O, $CuSO_4$ (2 mol%), Na-ascorbate (3 mol%), r.t., 48 h, 80 %.

The hydrogen of the triazole ring presented a unique proton signal by ^{1}H-NMR analysis. The expected proton signal was found at 7.70 ppm which indicated that the "click" cyclization process was successful (*Figure 10*).

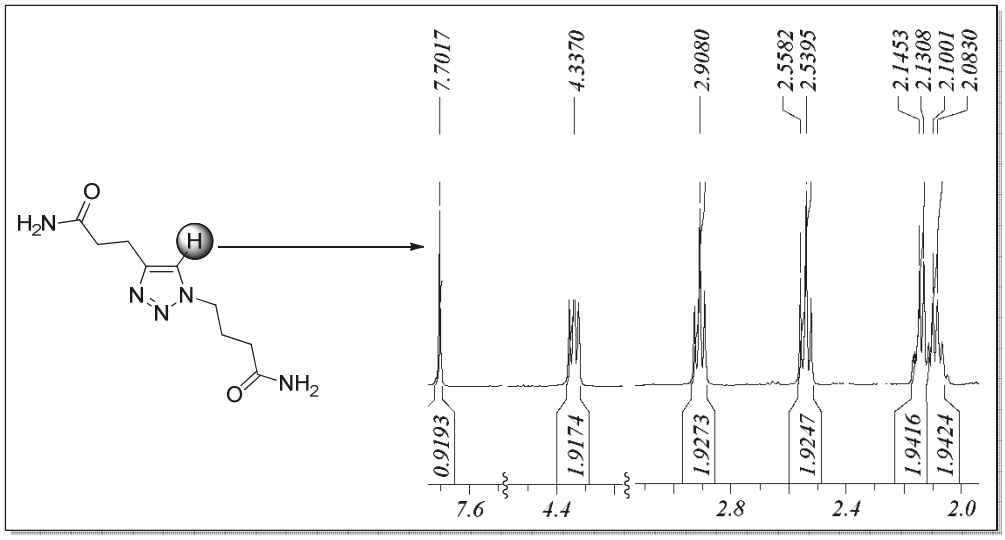
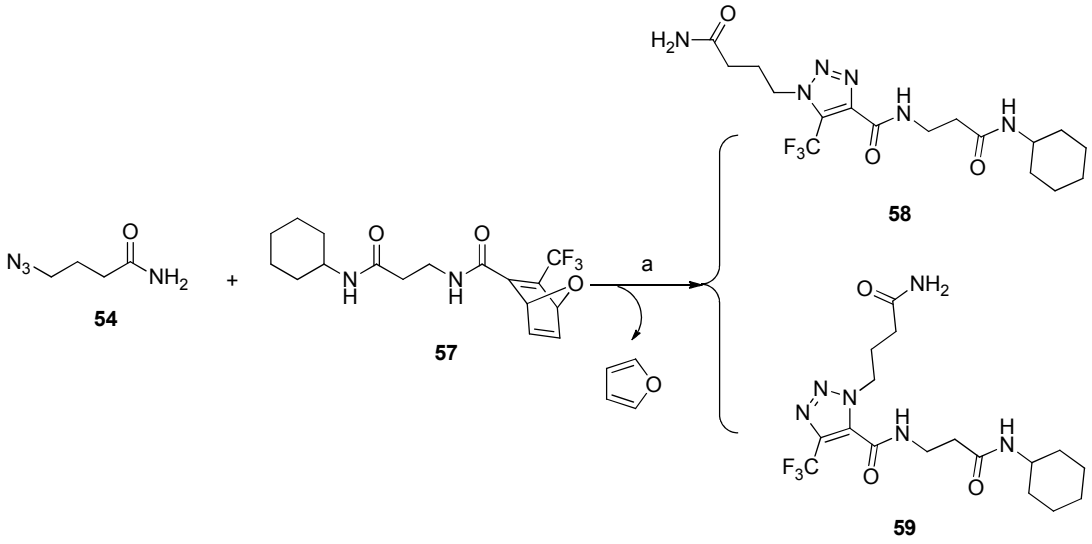


Figure 10. ^{1}H-NMR spectra for copper-catalyzed "click" model reaction.

In comparison to the copper-catalyzed "click" reaction, a similar model reaction for the metal-free "click" reaction was established from 4-azidobutanamide (**54**) and oxanorbornadienyl-species **57,** which was prepared from **52a** and cyclohexane amine. The cyclohexane moiety simulated the pyranose ring system of polySia. Both molecules were connected via [3+2] cycloaddition retro-Diels-Alder reaction which led to the formation of stable 1,4,5-tri-substituted 1,2,3-triazoles **58**, **59** and furan as by-product (*Scheme 27*).



Scheme 27. Model reaction for metal-free "click" chemistry. Condition: a) H_2O/MeOH, r.t., 24 h, 80%, mixture of **58** and **59**).

Final products **58** and **59** were analyzed by ^{19}F-NMR analysis, which is a very useful method, since ^{19}F is a sensitive nucleus and yields sharp signals. Analyses of ^{19}F-NMR spectra showed very clear results for the reaction (*Figure 11*). The ^{19}F signal of reactant was found at -61.6 ppm, while isomer **58** which was obtained as major product and appeared at -58.0 ppm and the isomer **59** appeared at -59.5 ppm. The ratio of both products **58** and **59** can be easily determined by integration of ^{19}F-signals. The results are useful to confirm the connection between azido- and oxanorbornadienyl-modified polySia derivatives, as well as polySia derivative immobilized with RGD peptide.

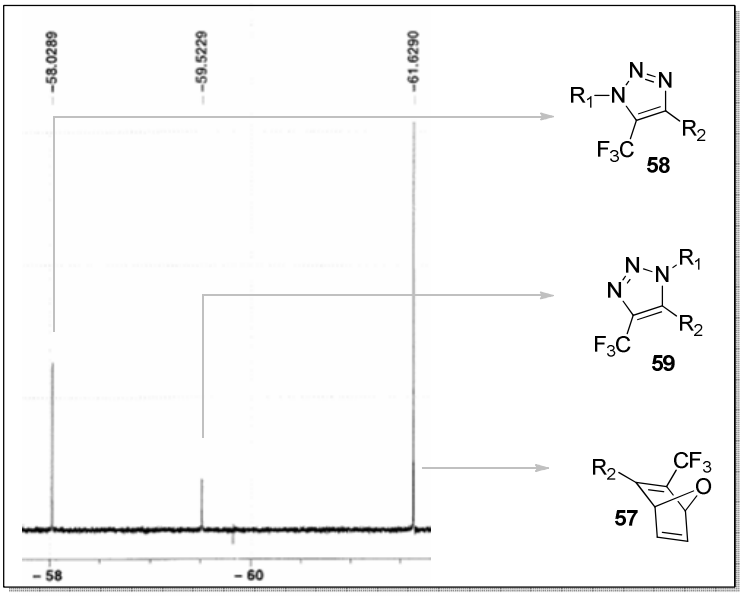
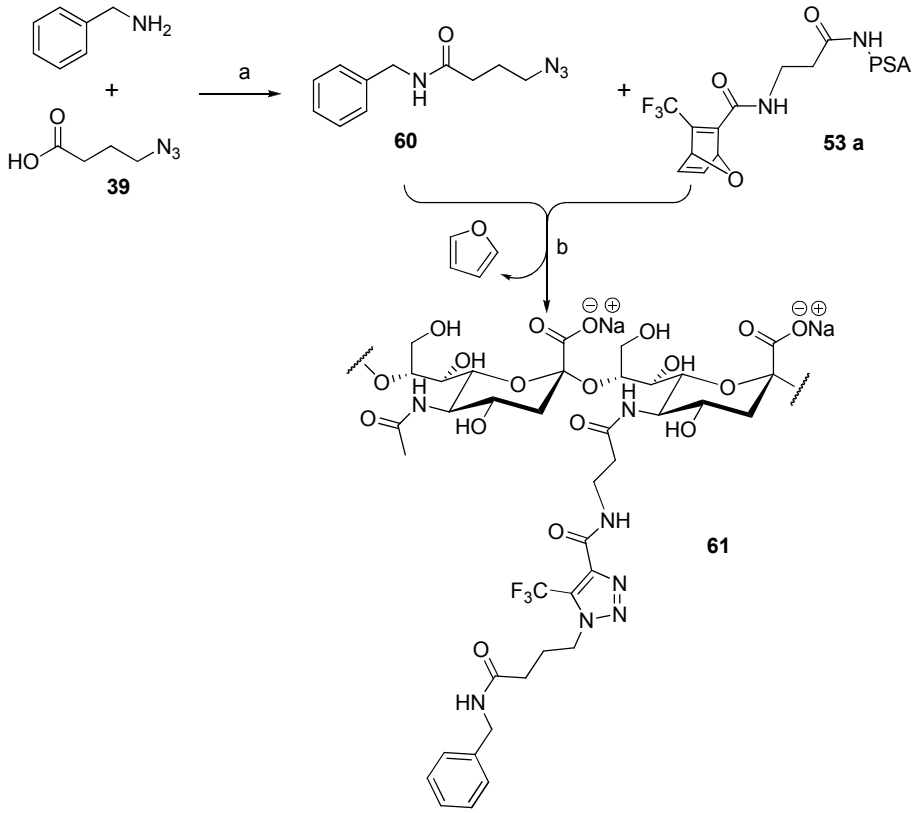


Figure 11. ^{19}F-NMR analysis for model reaction of metal-free "click" chemistry.

Besides the model reaction of metal-free "click" chemistry between the above-mentioned two small molecules, a model "click" reaction for polySia derivatives was also established. Generally, it is difficult to identify a successful "click" reaction of polymers using ^{1}H-NMR due to overlap of signals. Therefore, a reagent with an aromatic-ring system was prepared for modulating of metal-free "click" reaction of polySia, since no signal of polySia disappeared in the area between 7 and 8 ppm. At first, the 4-azido-*N*-benzylbutanamide (**60**) was prepared from benzylamine and 4-azidobutanoic acid (**39**) under using peptide coupling reagents. Then, the 4-azido-*N*-benzylbutanamide (**60**) was coupled to polySia **53a** to afford the "click" product **61** under mild conditions (*Scheme 28*).



Scheme 28. Metal-free "click" reaction of oxanorbornadienyl-modified polySia **53a**. Conditions: a) CH_2Cl_2, r.t., EDC·HCl, DMAP, 16 h, 90%; b) MeOH/H_2O (1:9), r.t., 48 h, 100% conversion (^{1}H-NMR).

"Click" product **61** was easily confirmed by ^{1}H-NMR analysis (*Figure 12*). Comparing the proton signals of the starting material and desired product, the two characteristic proton signals of the oxanorbornadienyl-system completely disappeared after the reaction. Instead, formation of aromatic proton signals could be observed in the area of 7 to 8 ppm of the product spectra. The degree of derivatization of the polymer could be evaluated by integration of the signals. At high field, the proton signals of the side chains were also changed obviously.

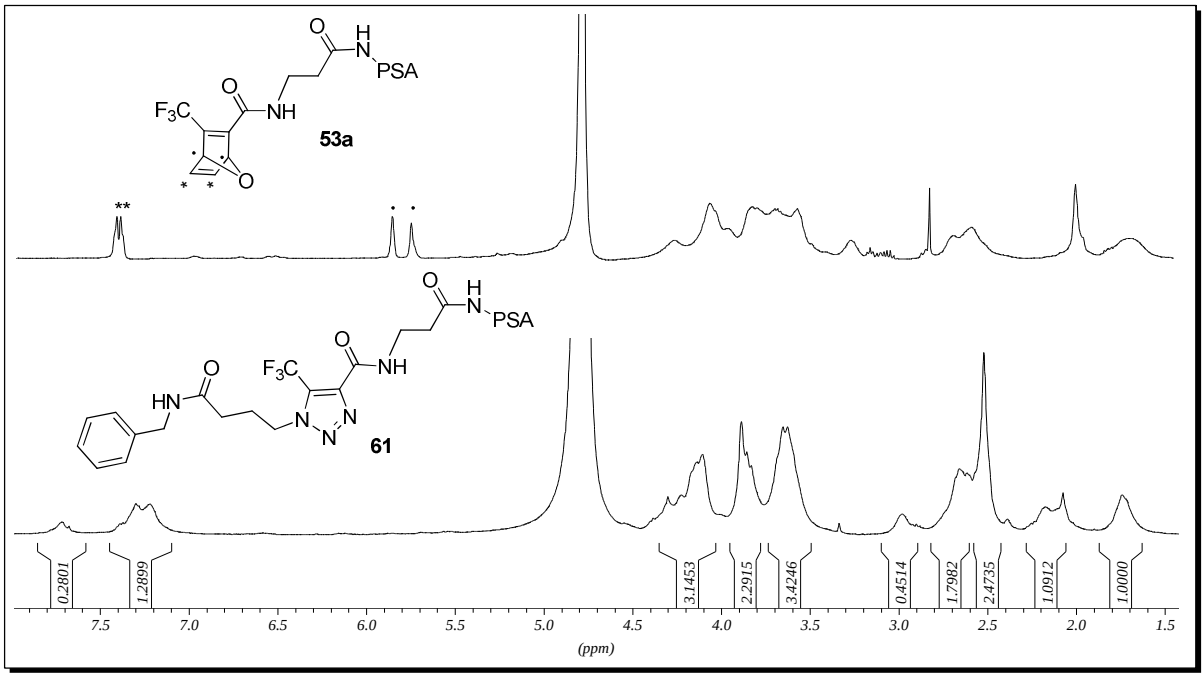


Figure 12. [1]H-NMR spectroscopic analysis of the metal-free "click" product **61** from oxanorbornadienyl-polySia **53a** with 4-azido-*N*-benzylbutanamide (**60**).

3.1.1.4 Synthesis of polySia hydrogels using "click" chemistry

For the preparation of 3D-scaffolds using Cu-catalyzed "click" reaction, azido- and alkyno-functionalized polySia derivatives (**41** and **44**) were dissolved in aqueous solution. As catalyst, copper sulphate and sodium ascorbate were added. After intensive mixing, a flexible hydrogel was slowly formed at room temperature (*Figure 13*). For the evaluation of the swelling behaviour, the hydrogel was placed in 50 mM phosphate buffer (pH = 7.2) and allowed to equilibrate at 37 °C for 24 h. The diameter of the hydrogel was measured. After equilibration, the hydrogel size raised about a factor of 1.5.

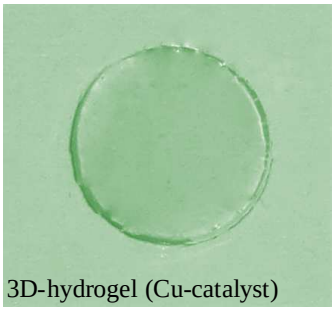
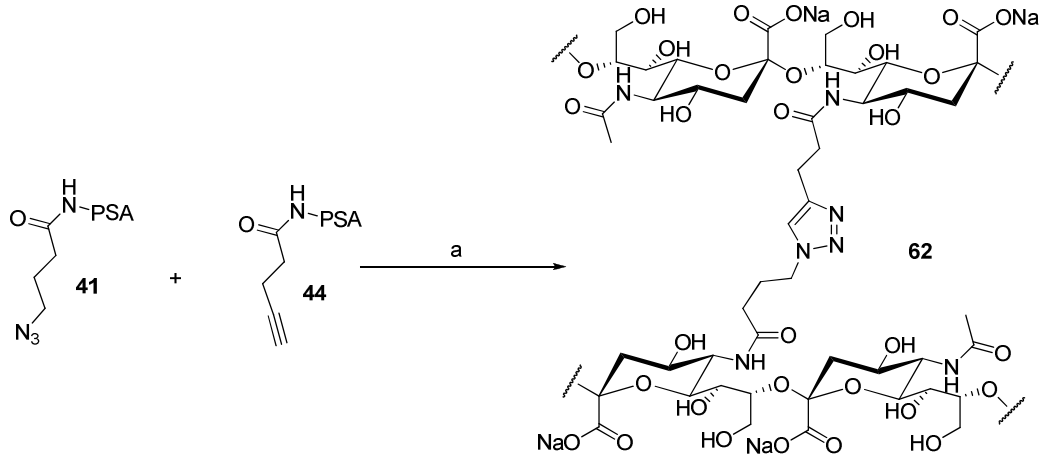


Figure 13. 3D-Hydogel based on azido-/alkyno-modified polySia **41** and **44** via Cu-catalyzed "click" reaction. Condition: *N*-azido-polySia **41** (5 mg, 60% derivatization) and *N*-alkyno-polySia **44** (5 mg, 60% derivatization), dissolved in 80 µL H_2O, addition of $CuSO_4$ (8 µL, 0.1 M) and Na-ascorbate (12 µL, 0.1 M), r.t., 24 h. (Ø = 0.9 cm, thickness, 0.1 cm).

In general, the gelating process was depended on the amount of Cu-catalyst, the degree of derivatization of polySia, and the length of the used polySia chain (*Table 2*). The hydrogels were formed in a very short time when more than 8% Cu-catalyst was used (*entries 1 and 2*). In case of less then 1% of Cu-catalyst, gel formation was impossible (*entry 6*). Apparently,

gel formation is faster from polymers with a higher molar mass. When polySia with a molecular mass of 45.000 Da (typical colominic acid has a molecular mass of 15.000 Da) was used, stable hydrogels could be formed using a low degree of derivatization (10%) and a low catalyst loading (1%) (*entry 8*).

Table 2. Results of the formation of polySia hydrogels based on azido- and alkyno-modified polySia **41** and **44** using copper catalyzed "click" chemistry.



Entry	Alkyno-polySia		Azido-polySia		Catalyst[a]		Time	Result
	DS [c]	weight	DS	weight	A	B		
1	60%	10 mg	60%	10 mg	15%	10%	10 s	Hydrogel
2	60%	10 mg	60%	10 mg	12%	8%	30 min	Hydrogel
3	60%	10 mg	60%	10 mg	8%	6%	24 h	Hydrogel
4	60%	10 mg	60%	10 mg	3%	2%	48 h	Hydrogel
5	80%	10 mg	25%	25 mg	3%	2%	5 d	Hydrogel
6	25%	10 mg	25%	10 mg	3%	2%	7 d	Hydrogel
7	80%	10 mg	80%	10 mg	1.5%	1%	7 d	Liquid
8	60%	10 mg	10%[b]	10 mg	1.5%	1%	7 d	Hydrogel

[a] Cu-catalyst (mol%) **A**: Na-ascorbate (0.1 M), **B**: $CuSO_4$(0.1 M); [b] The polySia was provided by Institute of Technical Chemistry of Leibniz University of Hannover, about 150 monomer units per chain; [c] DS: the degree of derivatization.

For further analysis, hydrogels were placed in an aqueous solution containing 0.1 M trifluoroacetic acid and heated at 80 °C for 1 h. The hydrolyzed product was directly analyzed by UPLC-MS spectrometry (*Figure 14*). The desired mass m/z = 724.25 of the degraded polySia for "click" product was found. This result indicated that the Cu-catalyzed "click" reaction of the polySia **41** and **44** was successful. Besides the "click" product, other hydrolyzed products of polySia were also found (*Table 3*).

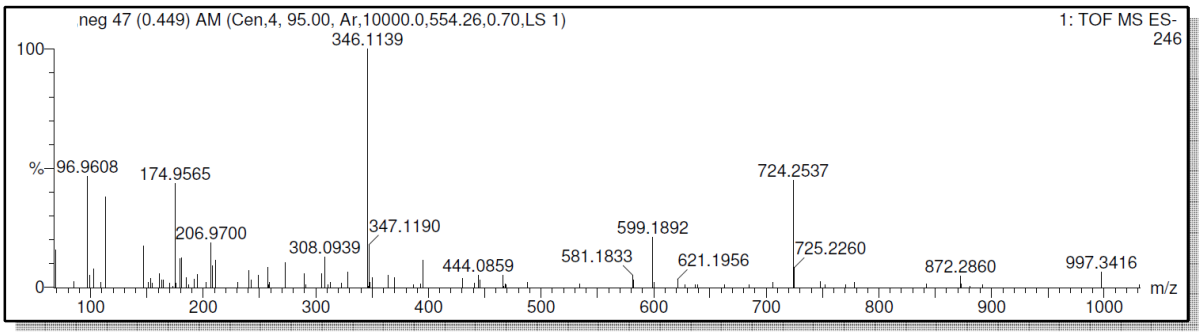
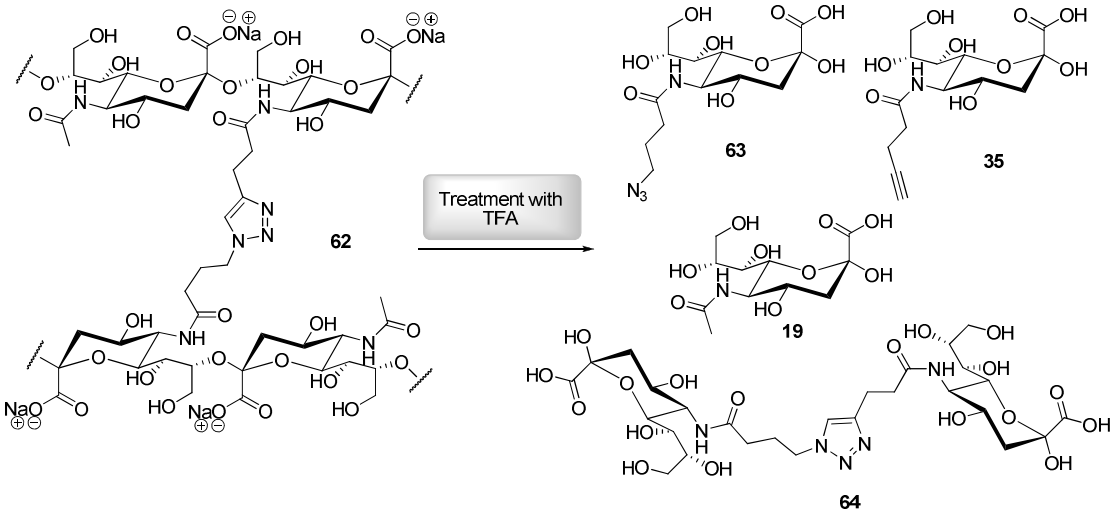


Figure 14. UPLC-MS spectra of hydrolyzed products the "click" reaction between polySia **41** and **44**.

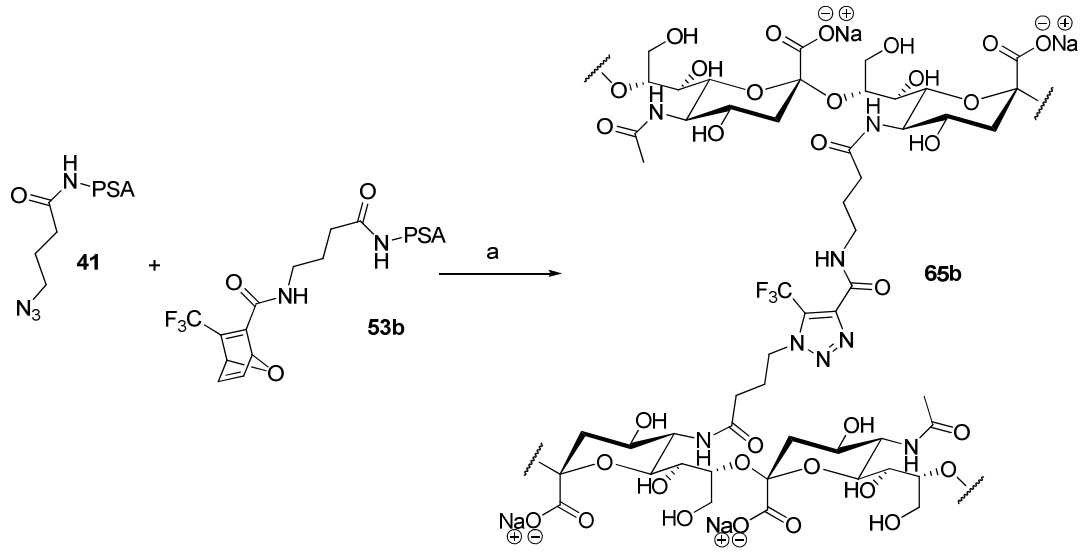
Table 3. Chemical hydrolysis of hydrogels based on azido-/alkyno-modified polySia formed by Cu-catalyzed "click" reaction. Condition: a) 0.1 M TFA, 80 °C, 1 h.



Molecule	Formula [M-H]	Calcd. Mass (ES-)	Found mass (ES-)
63	$C_{13}H_{21}N_4O_9$	377.1309	377.1320
35	$C_{14}H_{20}NO_9$	346.1138	346.1151
19	$C_{11}H_{18}NO_9$	308.0982	308.0997
Dimer (**64**)	$C_{27}H_{42}N_5O_{18}$	724.2525	724.2537

PolySia hydrogels were also successful synthesized form *N*-azido-polySia **41** and oxanorbornadienyl-polySia **53** by metal-free "click" chemistry mediated crosslinking. The functionalized polySia derivatives were dissolved in aqueous solution without any catalyst. The hydrogel formation was also dependent on the concentration of the starting materials, the derivatization degree, and the reaction time (*Table 4*). High concentration of starting material resulted in the formation of stable hydrogels in a short time (*entry 1*). However, the mechanical properties of the formed hydrogel were not sufficient. The hydrogel was easily broken into small pieces. On other side, very low concentration of starting material led to larger gelating times or failing of gel formation (*entries 6 and 7*). When starting material had a too low degree of substitution, hydrogel formation was impossible (*entries 11 and 12*).

Table 4. 3D-Hydrogel based on polySia **41** and **53b** using metal-free "click" chemistry.



Entry	Starting material		H_2O (µL)	Time for gelation)
	5 (DS)[a] 10 mg	**15** (DS) 10 mg		
1	25%	25%	90	5 h
2	25%	25%	120	11 h
3	25%	25%	150	20 h
4	25%	25%	180	36 h
5	25%	25%	200	3 d
6	25%	25%	210	4 d
7	25%	25%	240	Liquid (5 d)
8	80%	25%	200	36 h
9	60%	25%	200	36 h
10	60%	20%	200	48 h
11	60%	10%	100	Liquid (5 d)
12	60%	7%	50	Liquid (5 d)

[a] DS: the degree of derivatization

The optimal conditions for hydrogel formation were: *N*-azido-polySia **41** with a degree of derivatization 25%, *N*-oxanorbornadienyl-polySia **53b** with a degree of derivatization 25%, and in a concentration of 50 mg/mL per component (*entry 5, Table 4*). Under this conditions, a suitable polySia hydrogel was formed (*Figure 15*), since it was stable and relative flexible at room temperature. In comparison to the Cu-catalyzed "click" chemistry mediated polySia hydrogels a similar swelling behaviour was obtained. Furthermore, the hydrogel showed good enzymatic degradation properties due to relatively high degree of acetyl residues present.

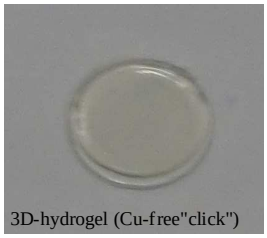


Figure 15. 3D-Hydrogel based on modified polySia using metal-free "click" chemistry. Gelation conditions: Azido-polySia (10 mg, DS: 25%) and oxanorbornadienyl-polySia (10 mg, DS:25%) were dissolved in 200 µL H_2O and kept at room temperature for 3 days.

The hydrogel was treated with 0.1 M trifluoroacetic acid under heating at 80 °C for 1 h. The hydrolyzed products were analyzed by UPLC-MS spectrometry, directly. The desired mass m/z 849.26 for the metal-free "click" reaction mediated dimer **67** was found (*Figure 16*).

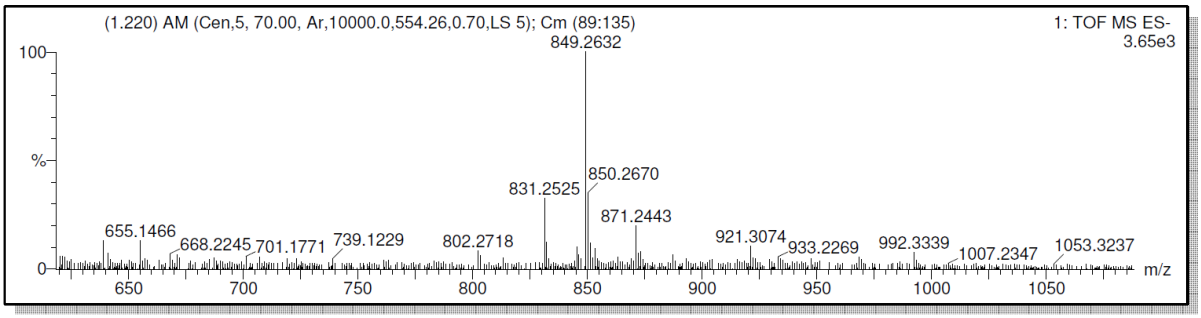
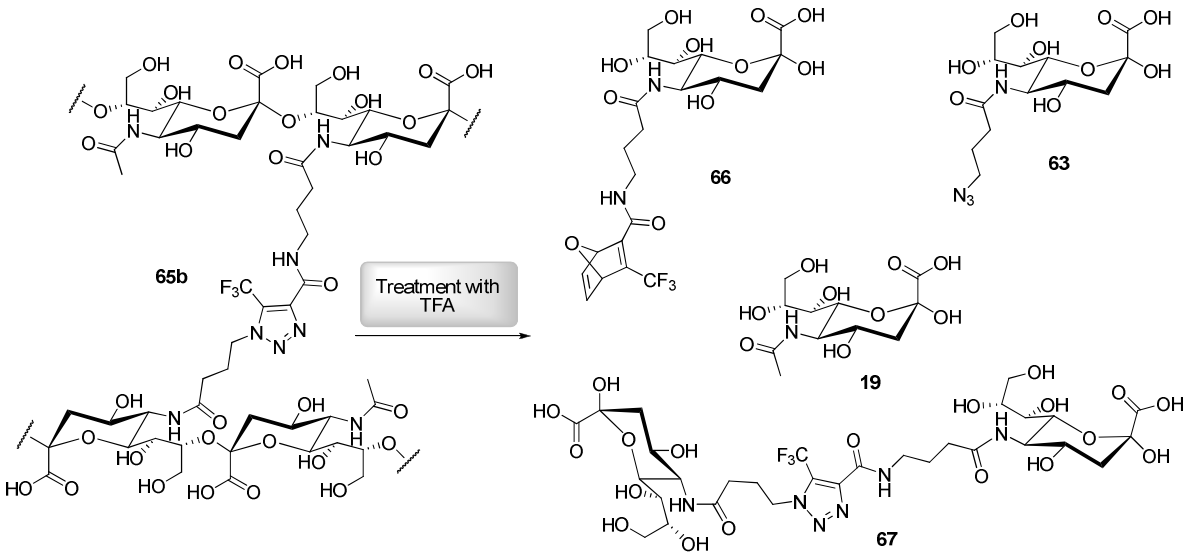


Figure 16. UPLC-MS spectra of hydrolyzed products of the "click" reaction between polySia **41** and **53b**.

N-Acetylneuraminic acid **19** (m/z = 308.09) and the unreacted monomer **63** (m/z = 377.13) were also found besides the dimer product **67** (*Table 5*). The oxanorbornadienyl-modified neuraminic acid **66** was not found. This might be due to the fact that the oxanorbornadienyl-system is not stable under thermal acidic conditions.

Table 5. Chemical hydrolysis of the hydrogel based on azido-/oxanorbornadienyl-modified polySia using metal-free "click" reaction and UPLC-MS analyse. Condition: a) 0.1 M TFA, 80 °C, 1 h.



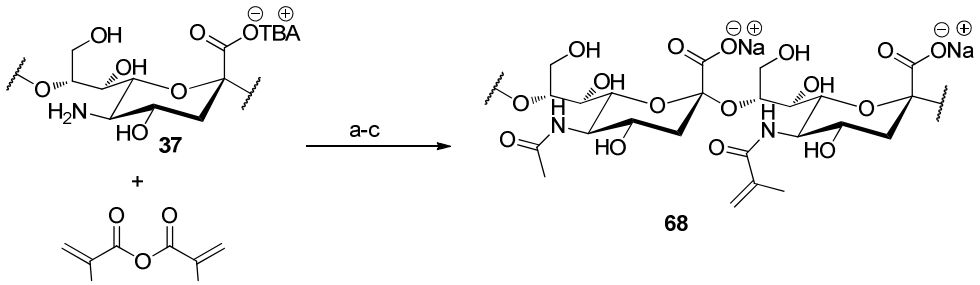
Molecule	Formula [M-H]	Calcd. Mass (ES-)	Found mass (ES-)
63	$C_{13}H_{21}N_4O_9$	377.1309	377.1302
66	$C_{21}H_{27}N_2O_{11}F_3$	539.1489	decomposed
19	$C_{11}H_{18}NO_9$	308.0982	308.0988
Dimer (**67**)	$C_{30}H_{44}N_6O_{19}F_3$	849.2613	849.2638

3.1.2 Formation of polySia hydrogels using photopolymerization

As an alternative to the synthesis of azido- and alkyno-modified polySia, which form hydrogels through 1,3 dipolar cycloadditions, polySia will also be decorated with methacrylate or acrylate groups. The methacrylated polySia will be photopolymerized to create crosslinked networks.

3.1.2.1 Synthesis of *N*-methacryl-polySia

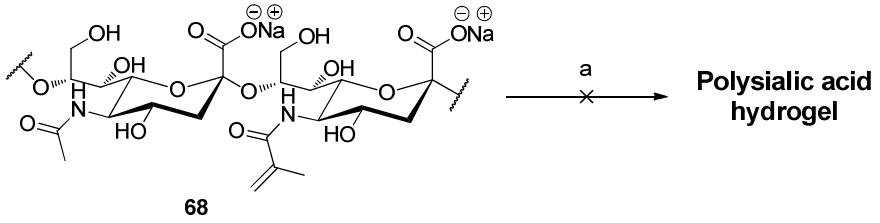
Methacrylated polySia **68** was synthesized starting with γ-amino polySia (TBA-salt) **37** and commercially available methacrylic anhydride (*Scheme 29*). The remaining free amino groups were reacetylated by treating with acetic anhydride. Under these conditions, unprotected hydroxyl groups were also partially acylated to form esters, which can lead to lower water solubility of the polymer. Therefore, the ester bonds were cleaved by treatment with sodium hydroxide solution (0.5 M). The degree of methacrylation could be adjusted in a range between 10% and 80% by varying the amount of methacrylic anhydride.



Scheme 29. Synthesis of *N*-methacryl-polySia **68** from *γ*-amino polySia (TBA-salt) **37** and methacrylic anhydride. Conditions: a) Anhydrous pyridine, 0 °C→r.t., 24 h; b) Ac_2O, r.t., 8 h; c) NaOH (0.5 M), r.t., 8 h, over 3 steps, 80% yield and the degree of derivatization 80%.

Methacrylate modified polySia **68** was treated under photo-crosslinking conditions to form hydrogels. *N*-methacryl-polySia **68** was dissolved in an aqueous solution of photoinitiator Irgacure 2959 and irradiated with UV-light at 320 nm. Irgacure 2959 is light-sensitive and therefore the photoinitiator solution was freshly prepared before use. The reaction conditions were varied by changing the concentration of photoinitiator and starting material **68**, exposure time, or the degree of derivatization of polySia **68**. Unfortunately, the expected hydrogel was not formed under the applied conditions (*Table 6*).

Table 6. Conditions for the attempted photopolymerization of *N*-methacryl-polySia **68**.

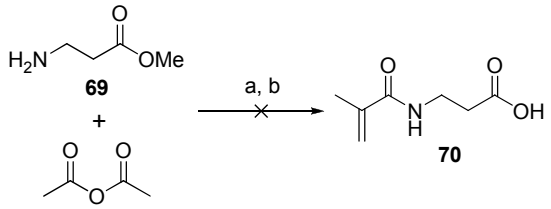


DS of 68[a]	**Irgacure 2959** [w/v]	**Conz. of 68**[b] [mg/ µL]	**Exposure time** [min]	**Result**
10%	0.5%	10 mg/100 µL	10 min	no hydrogel
10%	1.0%	10 mg/80 µL	10 min	no hydrogel
10%	2.0%	10 mg/100 µL	20 min	no hydrogel
80%	1.0%	10 mg/80 µL	10 min	no hydrogel
80%	2.0%	10 mg/100 µL	10 min	no hydrogel
80%	3.0%	10 mg/80 µL	20 min	no hydrogel
80%	5.0%	10mg/80 µL	60 min	no hydrogel

[a] DS: degree of derivatization; [b] Conz.: distilled water as solvent.

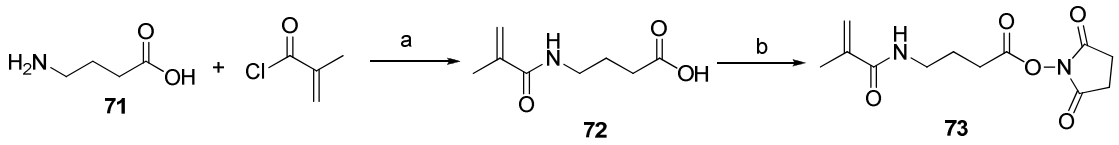
The formation of the hydrogel from *N*-methacryl-polySia **68** might be hindered by the steric repulsion caused by the polySia backbone. In order to overcome this problem, an extension spacer was introduced between the functional methacrylate group and the polySia backbone.

Therefore, ß-alanine methylester (**69**) was reacted with methacrylic anhydride to form 3-methacrylamidopropanoic acid (**70**) (*Scheme 30*). However, the desired product **70** was not obtained.



Scheme 30. Preparation of 3-methacrylamidopropanoic acid (**70**) from *ß*-alanine methylester and methacrylic anhydride. Condition: a) Pyridine, r.t., 24 h; b) H_2O, NaOH (1 M), r.t., no product.

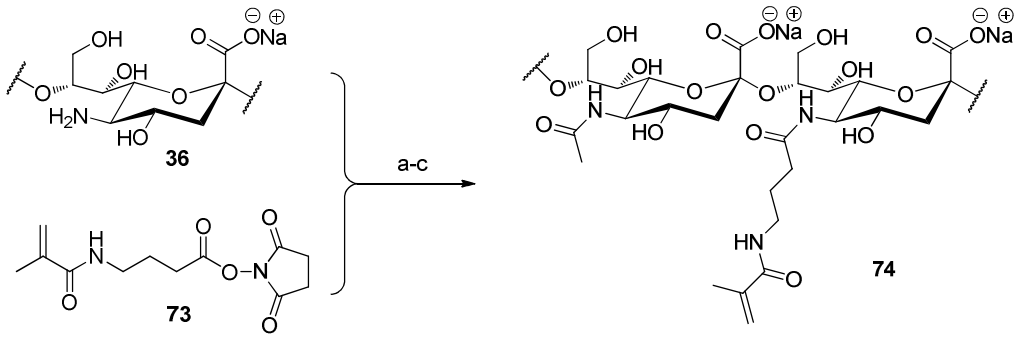
Following a literature known procedure carboxylic acid **72** was prepared from γ-aminobutanoic acid (**71**) and methacrylic acid chloride.[96] Subsequently, carboxylic acid **72** was treated with NHS in the presence of DCC to give NHS activated ester **73** (*Scheme 31*).



Scheme 31. Preparation of NHS-ester **73**. Conditions: a) NaOH, dioxane/H_2O, -10 °C, 1 h, 61%; b) THF, DCC, 0 °C→r.t., 24 h, 60%.

To synthesize *N*-methacryl-polySia **74** with an extended spacer, NHS-ester **73** reacted with *γ*-amino-polySia (sodium salt) **36** at room temperature under basic conditions, followed by reacetylation of the remaining free amino groups and liberation of all alcohol groups. The desired *N*-methacryl-polySia **74** with extension spacer was obtained with a degree of derivatization of 45% (*Scheme 32*).

[96] J. Drobník, J. Kopeček, J. Labskỳ, P. Rejmanová, J. Exner, V. Saudek, J. Kálal, *Macromol. Chem.* **1976**, *177*, 2833-2848.



Scheme 32. Synthesis of *N*-methacryl-polySia **74** from *γ*-amino-polySia (sodium salt) **36** and NHS-ester **73**. Conditions: a) Dioxane/H_2O, DIPEA, 0 °C→r.t., 24 h; b) Ac_2O, r.t., 8 h; c) NaOH (0.5 M), r.t., 8 h; over 3 steps, approx. 80% yield and the degree of derivatization approx. 45%.

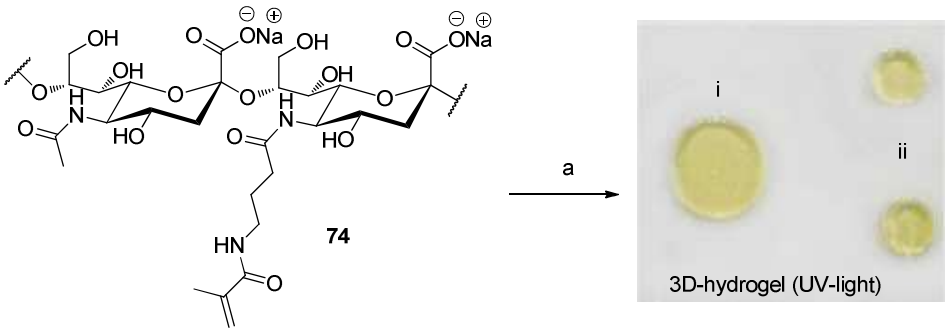
3.1.2.2 Synthesis of 3D-hydrogels using photocrosslinking

N-Methacryl-polySia **74** was used to form 3D-scaffolds.Therefore, polySia-derivative **74** was dissolved in an aqueous solution of photoinitiator, and irradiated with UV-light at 320 nm. Hydrogel formation depends on parameters such as the concentration of photoinitiator and starting material, exposure time, as well as the degree of derivatization of polySia **74** (*Table 7*). When the concentration of photoinitiator was lower than 2% (w/v), hydrogels did not form. The methacrylated polySia with a low degree of derivatization (< 10%) could also not generate a network structure. But, under appropriate conditions like 25% methacrylated starting material, using 2% (w/v) photoinitiator, and 10 min exposure with UV-light led to the formation of stable and flexible hydrogels in variable sizes (*Scheme 33*).

Table 7. Conditions for the photopolymerization of *N*-methacryl-polySia **74**.

DS of 74[a]	**Irgacure 2959** [w/v]	**Conz. of 74**[b] [mg/ µL]	**Exposure time** [min]	**Result**
10%	0.5%	10 mg/80 µL	10 min	no hydrogel
10%	1.0%	10 mg/80 µL	10 min	no hydrogel
10%	2.0%	10 mg/80 µL	20 min	hydrogel
20%	1.0%	10 mg/100 µL	10 min	no hydrogel
20%	2.0%	10 mg/100 µL	10 min	hydrogel
25%	2.0%	10 mg/80 µL	10 min	hydrogel
45%	0.5%	10 mg/100 µL	20 min	no hydrogel
45%	1.0%	80 mg/100 µL	20 min	no hydrogel
45%	2.0%	10 mg/80 µL	10 min	hydrogel

[a] DS: degree of derivatization; [b] Conz.: distilled water as solvent.



Scheme 33. 3D-Hydrogels based on *N*-methacryl-polySia **74** using photocrosslinking. Conditions: For gel picture (i: ø = 0.6 cm; ii: ø = 0.3 cm). The hydrogels were prepared in Culture Well Silicone Gaskests (*BioCat*, Germany).

The formed hydrogel was equilibrated in PBS-buffer to evaluate the swelling behaviour. By comparing the diameter of the swollen gel with the original gel size from the *N*-methacryl-polySia **74**, it was found that the swelling ratio was inverse proportional to the degree of derivatization. For example, the size of a hydrogel formed from polySia **74** with 10% of methacrylate groups increased about 10 times after swelling. Notably, the hydrogels from 20% modified polySia swelled only 1.5 to 2 times. This indicated that materials with more methacrylate groups can create higher crosslinked gels with lower swelling capacity. Also, polySia hydrogel produced from highly substituted starting material was more stable than the low cross-linked hydrogel.

The polymerization reaction could be analyzed by ^{1}H-NMR spectroscopy. To achieve this, photopolymerization was performed using the *N*-methacryl-polySia **74** in very low concentration of 20 mg/mL. After UV-light irradiation, the polymer solution remained in liquid state. The sample was dried and directly measured by ^{1}H-NMR spectroscopy without removing the photoinitiator. Comparing to the proton signals of starting material with the formed product, the proton signals of the double bond of the methacrylate group at 5.68 and 5.32 ppm completely disappeared after irradiation. Meanwhile, the methyl group of the methacrylate shifted to higher fields (*Figure 17*).

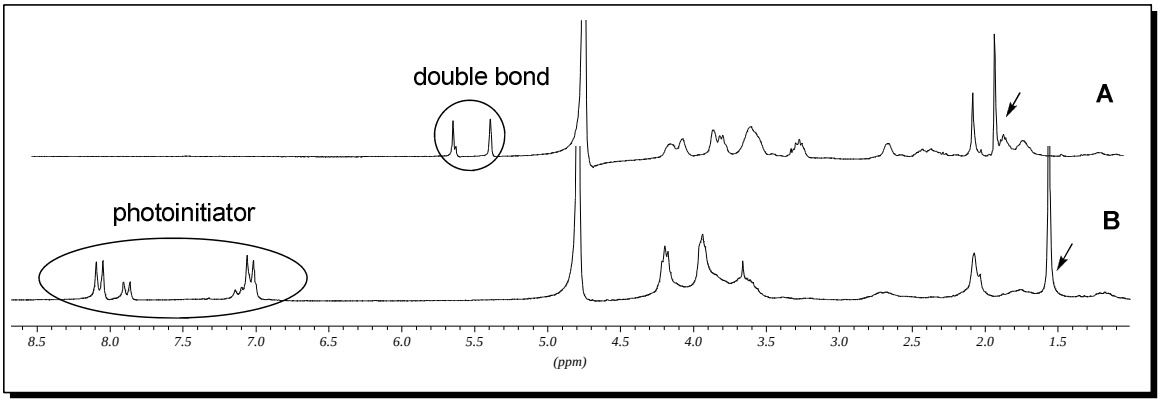


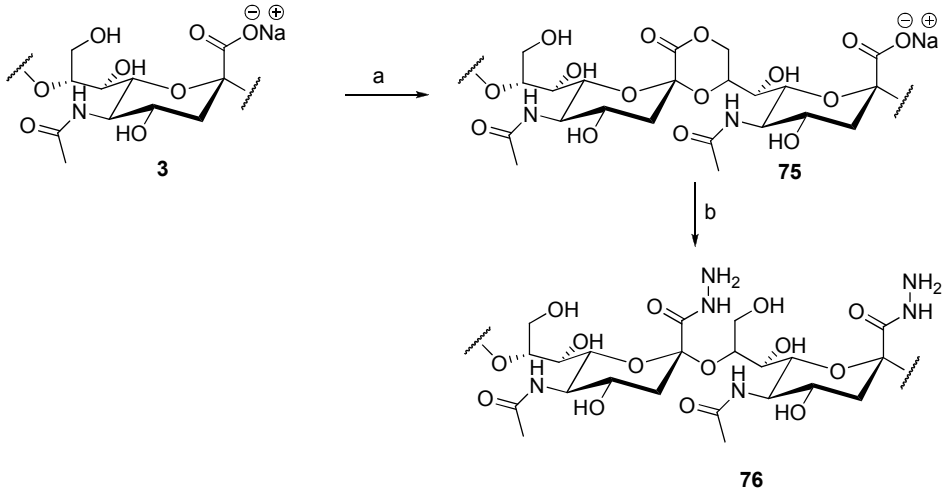
Figure 17. ^{1}H-NMR spectra of the *N*-methacryl-polySia **74** in D_2O (**A**), and photocrosslinked polySia containing photoinitiator in D_2O (**B**).

3.1.3 Formation 3D-hydrogels using hydrazone crosslinking

The third approach for the preparation of polySia hydrogels was based on hydrazone crosslinking. For this purpose, polySia was functionalized with aldehydo- and hydrazido-groups, respectively. After mixing both components, a 3D–scaffold was formed without using any catalyst or additive.

3.1.3.1 Synthesis of hydrazido-modified polySia

The carboxyl group at C-1 can react under classic carbodiimide conditions with the hydroxyl group at C-9 of the neighboring sialic acid to form in polySia lactone **75** in quantitative yield.[97] The lactone **75** could be aminolyzed with hydrazine monohydrate to form C-1 hydrazido-polySia **76** (*Scheme 34*).



Scheme 34. Synthesis of C-1 hydrazido-polySia **76**. Conditions: a) EDC·HCl, H_2O, pH 4.7; b) H_2O, N_2H_4 (64-65%), r.t., 2 h, over two steps 78%.

[97] M. R. Lifely, A. S. Gilbert, C. Moreno, *Carbohydr. Res.* **1981**, *94*, 193-203.

Formation of polySia hydrazide **76** could be confirmed by ^{13}C-NMR spectroscopy. The ^{13}C-NMR spectra showed distinct carbon-signals for the starting material **3**, PolySia lactone **75** and C-1 hydrazido-polySia **76**. Signal changes at three positions C-1, C-2, and C-9 could be easily observed, since the carbon atoms have different chemical environment (*Figure 18*). The C-1 signal of polySia **3** at 173.8 ppm shifted to 166.1 ppm in polySia lactone **75** as well as C-1 hydrazido-polySia **76**. The C-2 and C-9 signals of polySia **3** and C-1 hydrazido-polySia **76** had approximate same chemical shifts at 101.3 and 60.6 ppm, respectively. C-2 and C-9 of polySia lactone **75** moved to 96.4 and 66.7 ppm. Meanwhile, the spectra also demonstrated that the conversions of the both steps were quantitative.

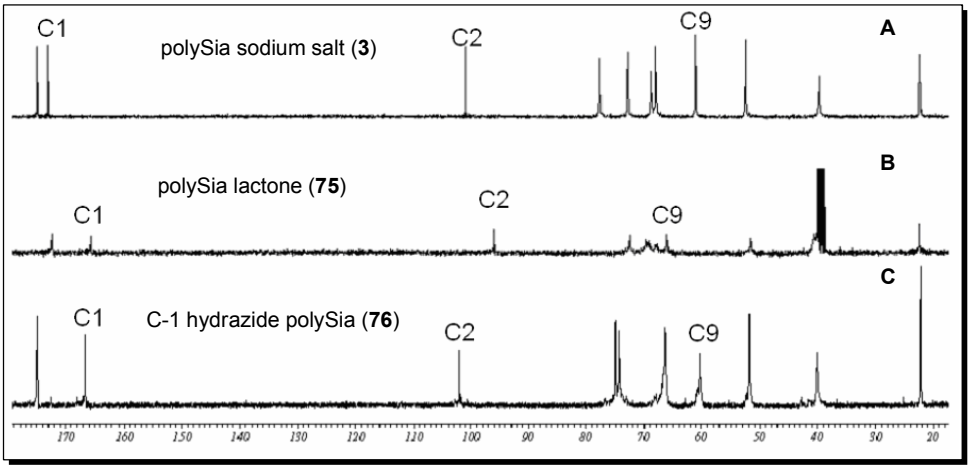
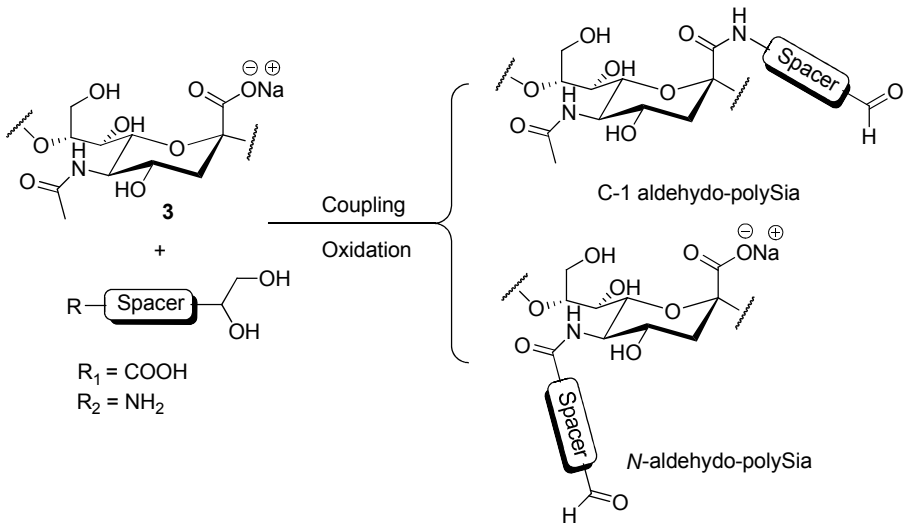


Figure 18. ^{13}C-NMR spectra of polySia (sodium salt) **3** in D_2O (**A**), polySia lactone **75** in DMSO (**B**), and hydrazido-polySia **76** in D_2O (**C**).

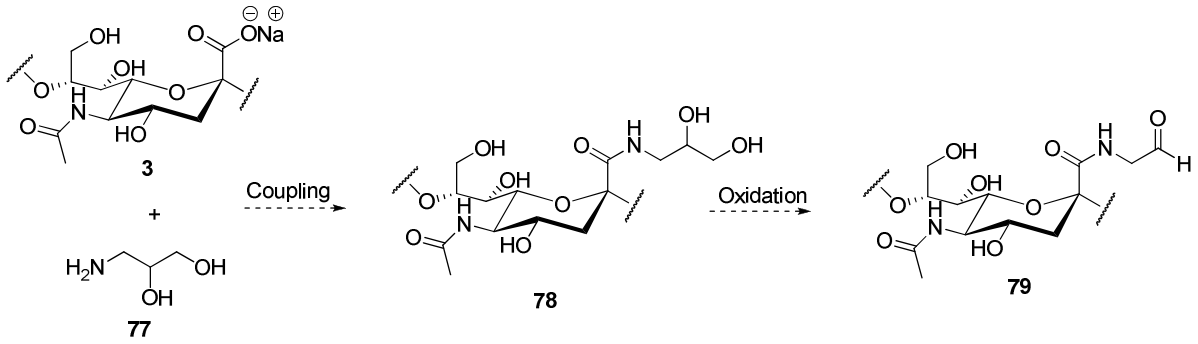
3.1.3.2 Synthesis of aldehydo-modified polySia

Introduction of an aldehyde group into polySia can be achieved at positions C-1 or C-5 of the polySia molecule via amide bond formation. Aldehyde modified polySia could be synthesized in two steps. Firstly, a vicinal diol group was introduced into polySia **3** using carbodiimide chemistry. Subsequently, the 1,2-diol moiety could be oxidized with sodium periodate to give desired aldehyde group (*Scheme 35*).



Scheme 35. Strategies for the synthesis of *N*-aldehydo-functionalized polySia derivatives.

C-1 aldehydo-modified polySia derivative **79** was planed to be generated by oxidizing precursor **78**, which could be obtained from the polySia sodium salt **10** and a commercially available 3-aminopropane-1,2-diol (**77**) in the presence of coupling reagents (*Scheme 36*).



Scheme 36. Synthesis plan for the formation of C-1 aldehydo-modified polySia derivative **79**.

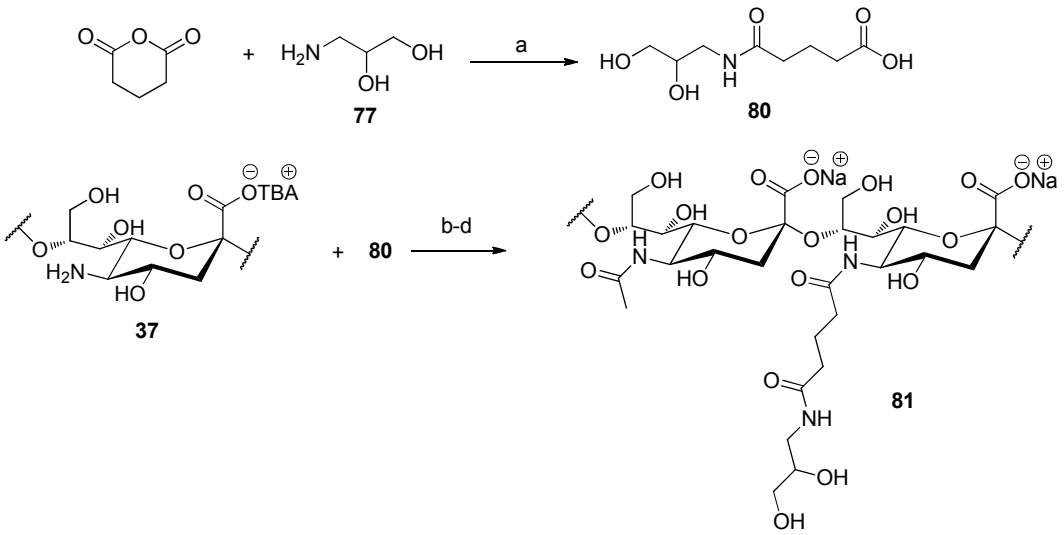
The coupling of polySia **3** and the 3-aminopropane-1,2-diol (**77**) was investigated by using different coupling conditions by varying solvents and coupling reagents (*Table 8*). Unfortunately, formation of the coupling product **78** was not observed.

Table 8. The reaction conditions for the synthesis of C-1 vicinal diol modified polySia derivative **78**.

Entry	Solvent[a]	Coupling reagent	Result
1	H_2O	ECD·HCl, DIPEA, HOBt	no product
2	DMF	ECD·HCl, DMAP	no product
3	DMF	ECD·HCl, DIPEA, HOBt	no product
4	DMSO	ECD·HCl, DMAP	no product
3	DMSO	ECD·HCl, DIPEA, HOBt	no product

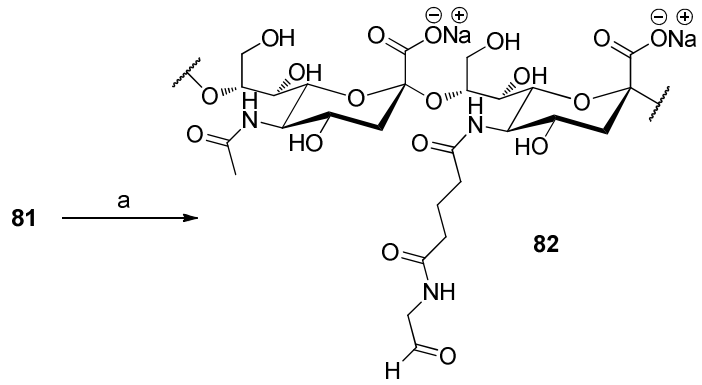
[a] For the reactions in DMF and DMSO, polySia (TBA-salt) was used.

A second approach towards *N*-aldehydo-modified polySia was also carried out in two steps. At first, building block **80** containing the required carboxyl group and 1,2-diol moiety was prepared from glutaric anhydride and 3-aminopropane-1,2-diol (**77**) in excellent yield. Carboxylic acid **80** was successfully coupled to the free amino group of *γ*-amino polySia **37** using carbodiimide chemistry (*Scheme 37*). ^{1}H-NMR spectra showed that approximately of 40% of all amino groups in the polySia chain were successfully decorated with diol **80** (*Figure 19*, ***A***).



Scheme 37. Synthesis of 1,2-diol-modified polySia **81**. Conditions: a) Pyridine, r.t., 16 h, quantitative; b) EDC·HCl, HOBt, DIPEA, DMF, r.t., 24 h; c) Acetic anhydride, r.t., 8 h; d) NaOH (1 M), r.t., 8 h; e) Dialysis against NaCl, r.t., 2 d, then against distilled H_2O, r.t., 2 d, the degree of derivatization approx. 40%.

The second step was the conversion of the 1,2-diol group into the corresponding aldehyde group without risking the decomposition of other groups of polymer. The 1,2-diol-functionalized polySia **81** was oxidized with sodium periodate at 0 °C for 30 min in dark to yield *N*-aldehydo-polySia **82** (*Scheme 38*).



Scheme 38. Conversion of 1,2-diol-functionalized polySia into *N*-aldehydo-modified polySia **82**. Condition: a) $NaIO_4$, H_2O, 0 °C, 30 min, light exclusion, quantitative.

Formation of functionalized polySia derivatives **81** and **82** were confirmed by ^{1}H-NMR-analysis. The ^{1}H-NMR spectra of the 1,2-diol-modified polySia **81** (Figure **A**) and oxidized product **81** (*Figure 19* ***B***) are shown in Figure 18. In aqueous media, the aldehyde group of **81** was hydrated. Two proton signals [$H^{1'}$ and $H^{2'}$, *Figure 19* ***A***] of polySia **81** at 3.35 and 3.20 ppm disappeared in product **82** spectra. Instead of these, the signal at 3.30 ppm was assigned as CH_2 protons [H^2 and $H^{2'}$, *Figure 19* ***B***] adjacent to the hydrated aldehyde group and the signal at 5.10 ppm was assigned the dominating $CH(OH)_2$ signal [H^1-hydrate, *Figure 19* ***B***]. By comparing the two spectra, the conversion from 1,2-diol into its aldehyde was almost quantitatively and no decomposition could be observed.

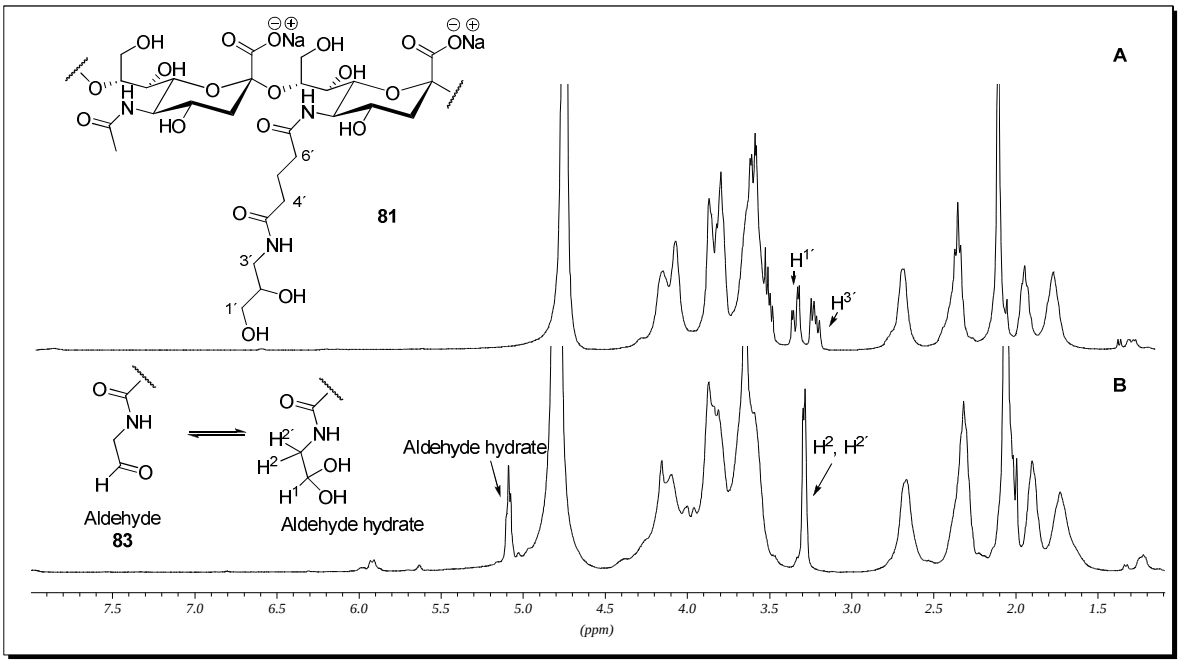
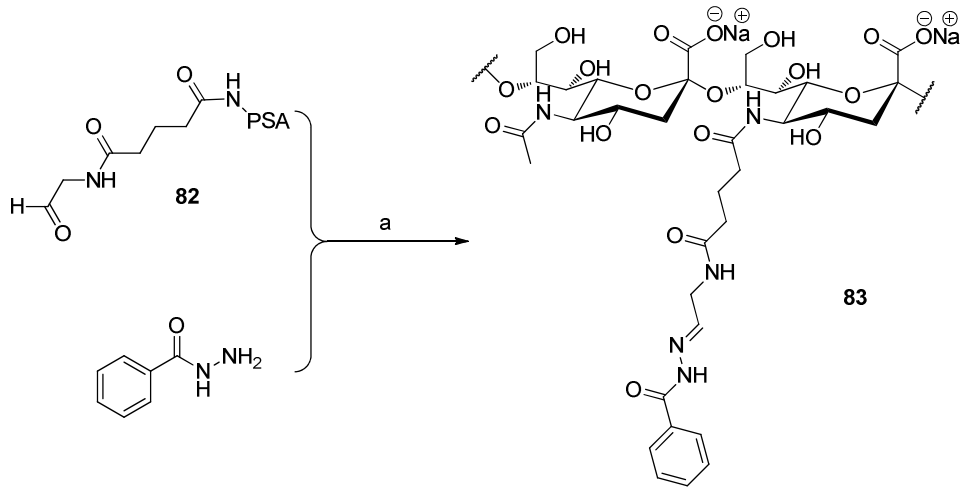


Figure 19. ^{1}H-NMR spectra of the 1,2-diol-modified polySia **81** in D_2O (**A**) and *N*-aldehydo-polySia **82** in D_2O (**B**).

3.1.3.3 Synthesis of polySia hydrogel using hydrazone crosslinking

To investigate the hydrazone formation from hydrazido- and aldehydo-functionalized polySia **76** and **82**, a model reaction between aldehydo-functionalized polySia derivative **82** and commercially available benzoyl hydrazide was studied (*Scheme 39*). The hydrazone was formed between the two components in water at room temperature. After removal of excess benzoyl hydrazide, characteristic proton signals of the aromatic system **83** appeared and the signals for the aldehyde hydrate lost intensity in the ^{1}H-NMR spectra. Notably, a model reaction of the C-1 hydrazido-polySia **76** and benzaldehyde was also tried under same conditions, but the expected hydrazone formation was not observed.



Scheme 39. Model reaction of the *N*-aldehydo-polySia **82** and benzoyl hydrazide. Condition: a) H_2O, r.t., 24 h; b) Dialysis against distilled H_2O, r.t., 2 d, 50%, conversion rate 50%.

Figure 20 shows the ^{1}H-NMR spectra of polySia **83** (**A**) and the product of the model reaction between benzoyl hydrazide and polySia **83** (**B**). The success of the reaction was confirmed by the appearance of the characteristic signals at 7.4 to 7.8 ppm belonging to the aromatic system. The proton signals at 5.10 and 3.30 ppm did not completely disappear but their signals were reduced significantly to about 50% (**B**).

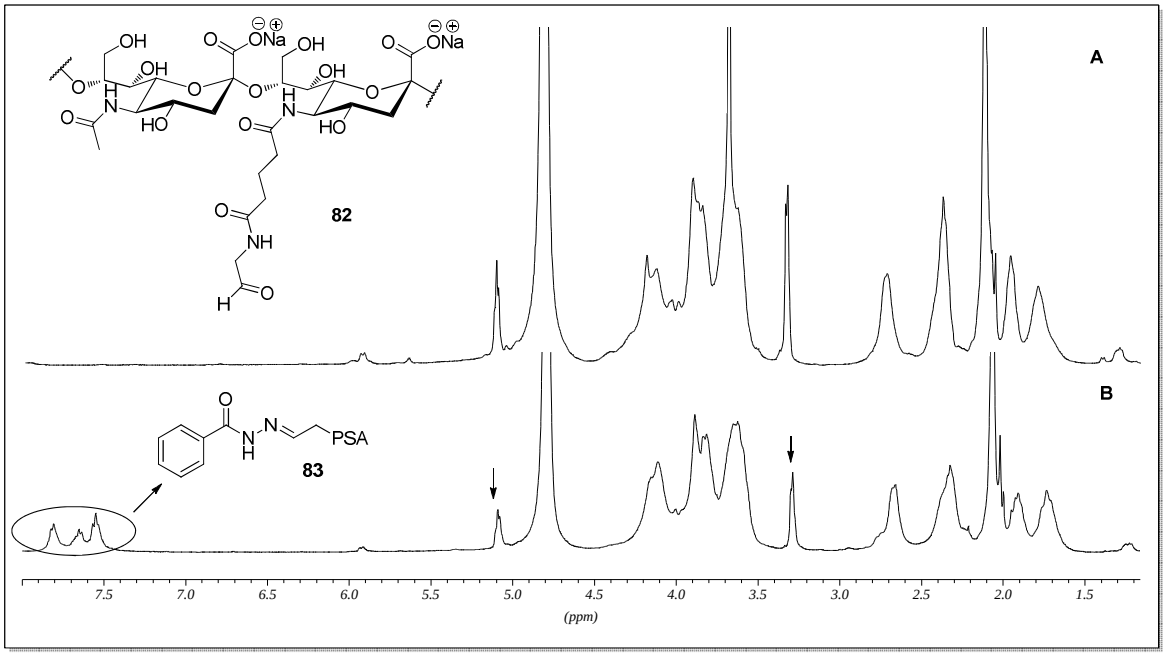
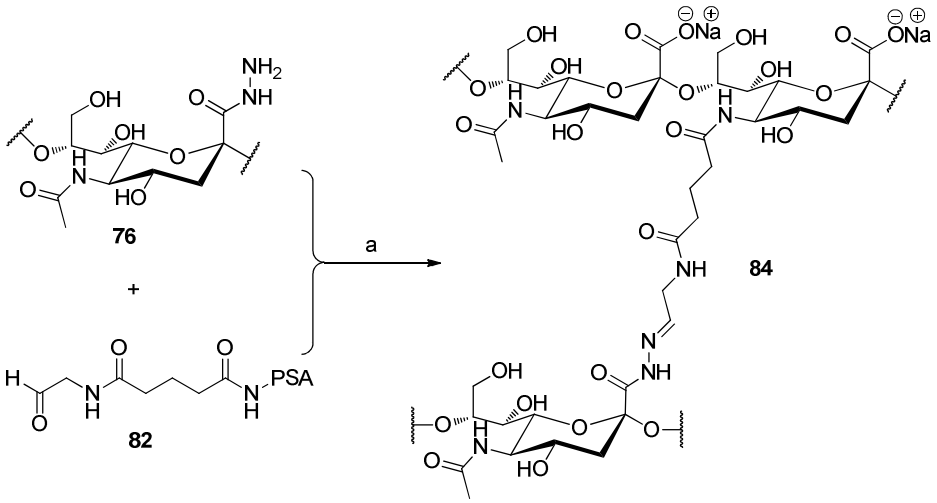


Figure 20. ^{1}H-NMR analysis of the model reaction of *N*-aldehydo-polySia **82** and benzoyl hydrazide. *N*-aldehyde polySia **82** in D_2O (**A**); hydrazone product **83** in D_2O (**B**)

Equal amounts of C-1 hydrazido-polySia **76** and *N*-aldehydo-polySia **82** were mixed in aqueous media in order to form a 3D-scaffold mediated through hydrazone crosslinkage between the terminal hydrazido- and aldehyde-groups of polySia derivatives (*Scheme 40*).



Scheme 40. Crosslinking reaction of C-1 hydrazido-polySia **76** and *N*-aldehydo-polySia **82** formation 3D-scaffolds Condition: a) in H_2O, r.t., several minutes.

After mixing of the two components in water, a hydrogel was formed within a short period of time. However, the crosslinked hydrogel was not stable. Within several hours, the hydrogel turned liquid again (*Figure 21, a*). This might be due to the short chain length of polySia. To overcome this problem, hydrogel blended from polySia and high molecular weight polysaccharides such as alginate and hyaluronate were investigated. Therefore, C-1 hydrazido-polySia **76** and aldehyde functionalized alginate and hyaluronate as well as *N*-aldehydo-polySia **82** with hydrazide functionalized alginate and hyaluronate were investigated to form composite scaffolds.[98] Chemically stable and flexible hydrogel networks were obtained from the *N*-aldehydo-functionalized polySia **82** and hydrazide functionalized alginate or hyaluronate (*Figure 21, b and c*). Still, the C-1 hydrazido-polySia **76** did not react with aldehydo-functionalized alginate or hyaluronate to generate a stable hydrogel networks.

[98] Aldehydo- and hydrazido-functionalized alginate and hyaluronate were prepared by L. Möller and A. Krause, as part of their phD thesis (Institute of Organic Chemistry, Gottfried Wilhelm Leibniz University of Hannover).

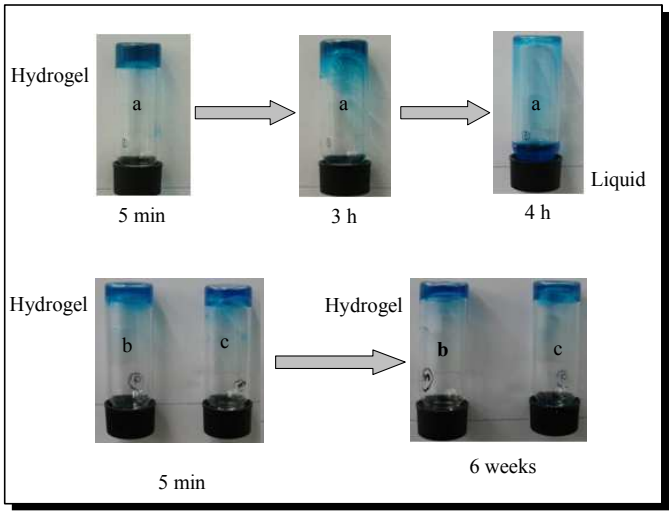


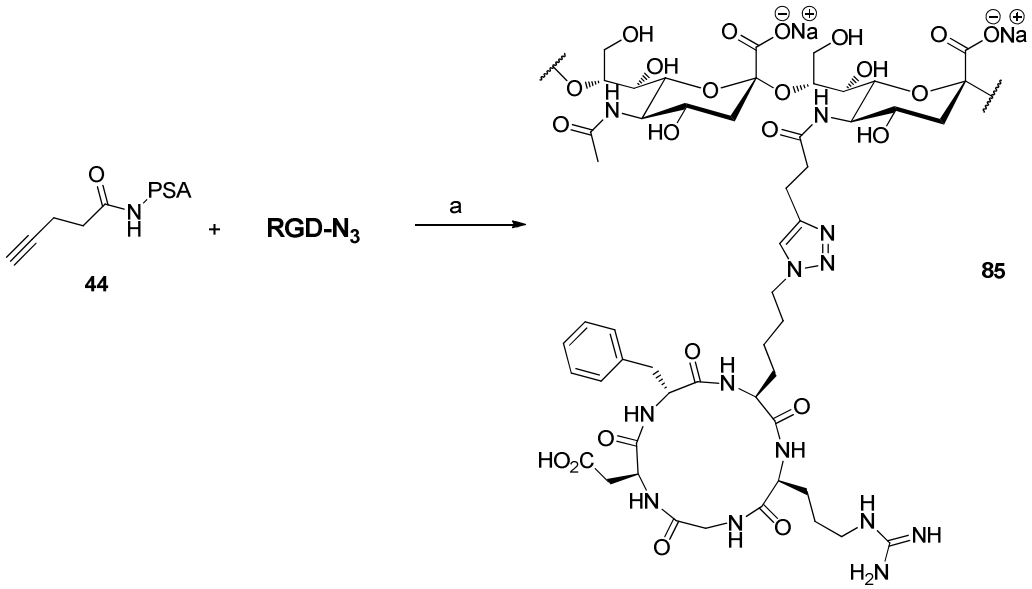
Figure 21. Hydrogel formation using hydrazone crosslinking: Hydrogel from C-1 hydrazido- and *N*-aldehydo-functionalized polySia **76** and **82** (a); Hydrogels from *N*-aldehydo-polySia **82** blended with hydrazido-functionalized alginate (b) and hyaluronate (c).

3.1.4 Decoration of polySia hydrogels with RGD peptide

In order to improve the interaction between cells and the polySia hydrogels, RGD peptides [99] were immobilized on the hydrogels. The ligation was enabled through different approaches such as coupling of azido-RGD peptide with alkyno- or oxanorbornadienyl-functionalized polySia **44**, **53** using "click" chemistry as well as imine-mediated coupling of amino-RGD peptide with aldehydo-functionalized polySia derivative **82**. Afterwards, the RGD peptide decorated polySia was used to form hydrogels.

3.1.4.1 Modification of polySia with RGD peptide using "click" chemistry

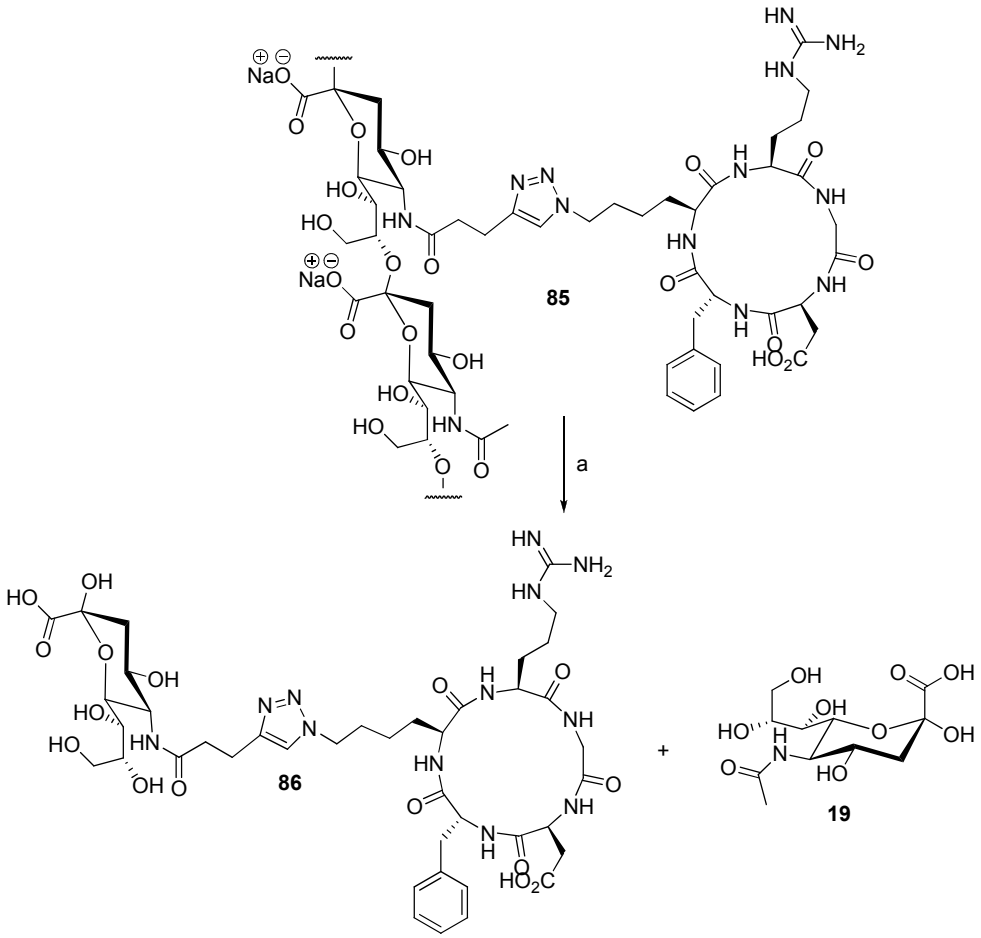
The reaction of *N*-(4-pentynoyl)-polySia **44** and azido-RGD peptide was performed in aqueous media containing copper sulphate as catalyst, which was reduced by sodium ascorbate *in situ* (*Scheme 41*). The coupling process under microwave irradiation condition proceeded quantitatively with respect to azido-RGD peptide, while no conversion could be observed by classical oil bath heating.



Scheme 41. Synthesis of RGD peptide modified polySia **85** from *N*-(4-pentynoyl)-polySia **44** and azido-RGD peptide using Cu-catalyzed "click" chemistry. Condition: a) H_2O, $CuSO_4$ (8 mol%), Na-ascorbate (12 mol%), MW, 40 °C, 30 min.

In order to confirm that the "click" reaction occurred, the crude product was purified and treated with trifluoroacetic acid (0.1 M) at 80 °C for 1 h (*Scheme 42*). The hydrolyzed products were analyzed using UPLC-MS as well as LC-MS/MS spectroscopy.

[99] Azido- and amino-RGD peptides were synthesized by J. Paleček (Institute of Organic Chemistry, Gottfried Wilhelm Leibniz University of Hannover).



Scheme 42. Hydrolysis of azido-RGD peptide modified polySia **85** with trifluoroacetic acid. Condition: a) TFA (0.1 M), 80 °C, 1 h.

Figure 22 shows the mass spectra of polySia modified with azido-RGD peptide using Cu-catalyzed "click" chemistry. Due to the observed peak at m/z = 975.4186, which belongs to sialic acid-RGD conjugate **86**, the ligation was proven.

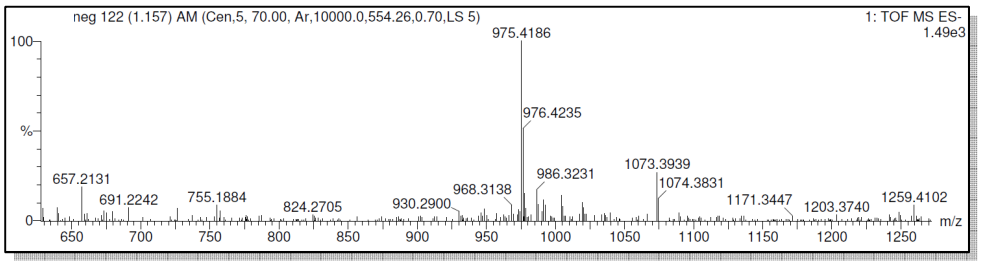


Figure 22. UPLC-MS spectra of the "click"-linked product **86**. (calcd for $C_{41}H_{59}N_{12}O_{16}$ $[M-H]^-$: 975.4172; found: 975.4186).

Further analysis of the fragmentation pattern of the "click" product **86**, the mass m/z = 975 was further investigated with LC-MS/MS spectrometry. Different fragment ions were obsered as shown in figure 23.

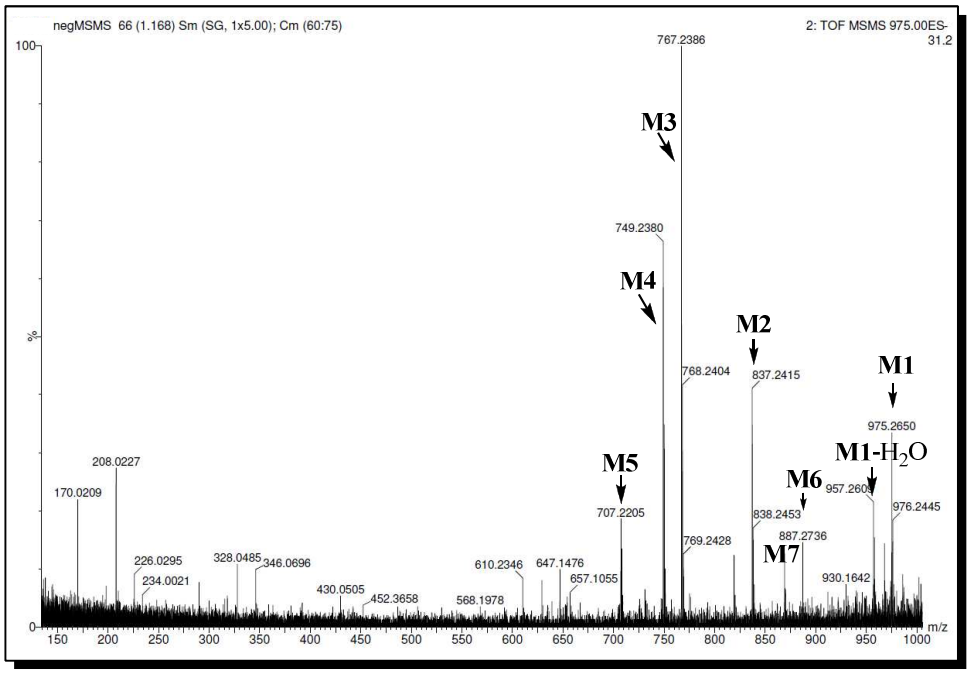
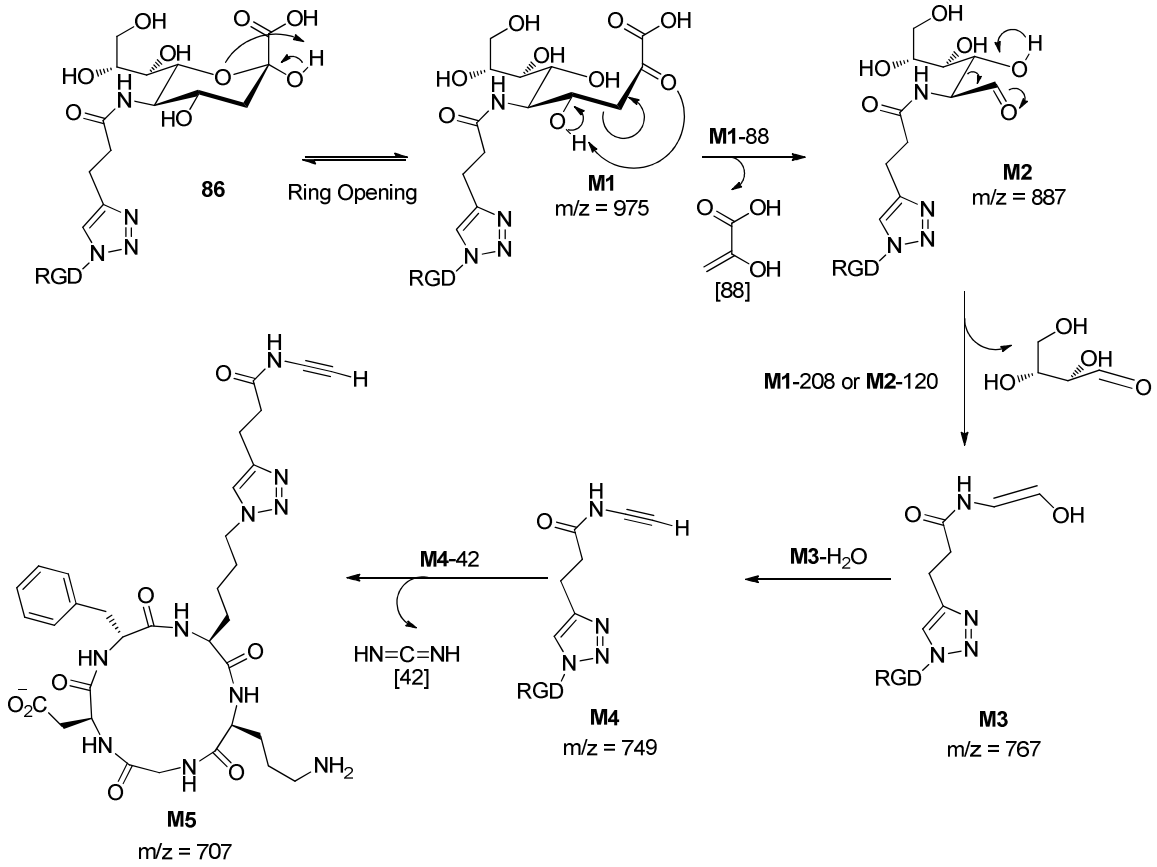


Figure 23. LC-MS/MS spectra of "click" product **86**.

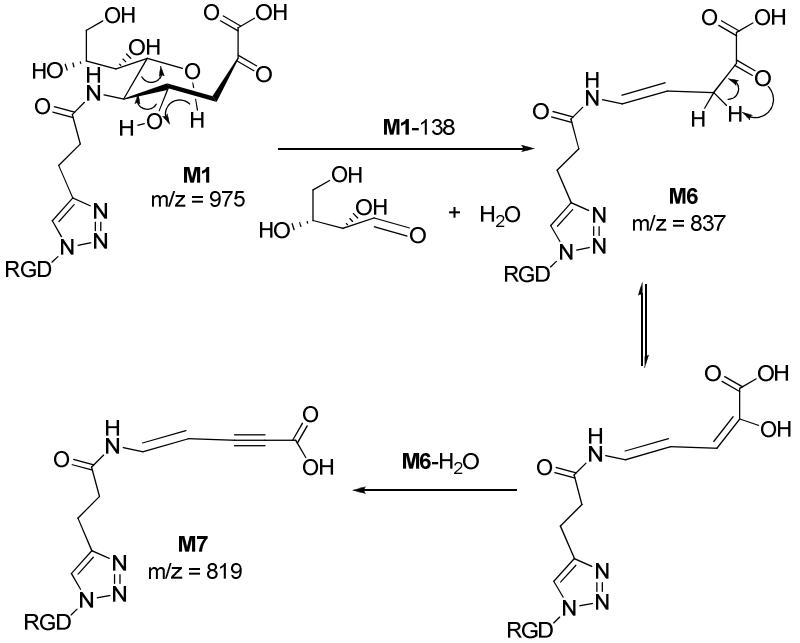
According to previous reports,[100] the proposed fragmentation mechanisms corresponds to the ions as is illustrated in Scheme 43. At first, the anomer hydroxyl group was deprotonated leading to ring opening fragmentation (**[**M-H**]**$^-$: m/z = 975), which was cleaved a water molecule to give charged ion [**M1**-H_2O]$^-$ (m/z = 957). Subsequently, via a retro-aldol rearrangement mechanism it afforded fragment ion **M2** ([**M1**-88]$^-$) with a terminal aldehyde (m/z = 887). The molecule was further cleaved to give enol **M3** ([**M2**-88]$^-$) at m/z = 767. The enol was dehydrated to generate ion **M4** ([**M3**-H_2O]$^-$: m/z = 749). The [**M4**-42]$^-$ ion at m/z = 707 was regarded to be the guanidine group of the RGD moiety losing a methane diimine to form fragment ion **M5** (Scheme 43A).

[100] a) M. van der Ham, B. H. C. M. T. Prinsen, J. G. M. Huijimans, N. G. G. M. Abeling, B. Dorland, R. Berger, T. J. de Koning, M. G. M. de Sain-van der Velden, *J. Chromatogr. B* **2007**, *848*, 251-257.
b) J. A. Carrol, D. Willard, C. B. Lebrilla, *Anal. Chim. Acta* **1995**, *307*,431-447.
c) O. M. Saad, J. A. Leary, *J. Am. Soc. Mass Spectrom* **2004**, *15*, 1274-1287.
d) S. P. Galuska, H. Geyer, C. Bleckmann, R. C. Röhrich, K. Maßes, A. K. Berfeld, M. Mühlenhoff, R. Geyer, *Anal. Chem*. **2010**, *82*, 2059-2066.
e) T. Minamisawa, K. Suzuki, J. Hirabayashi, *Anal. Chem*. **2006**, *78*, 891-900.



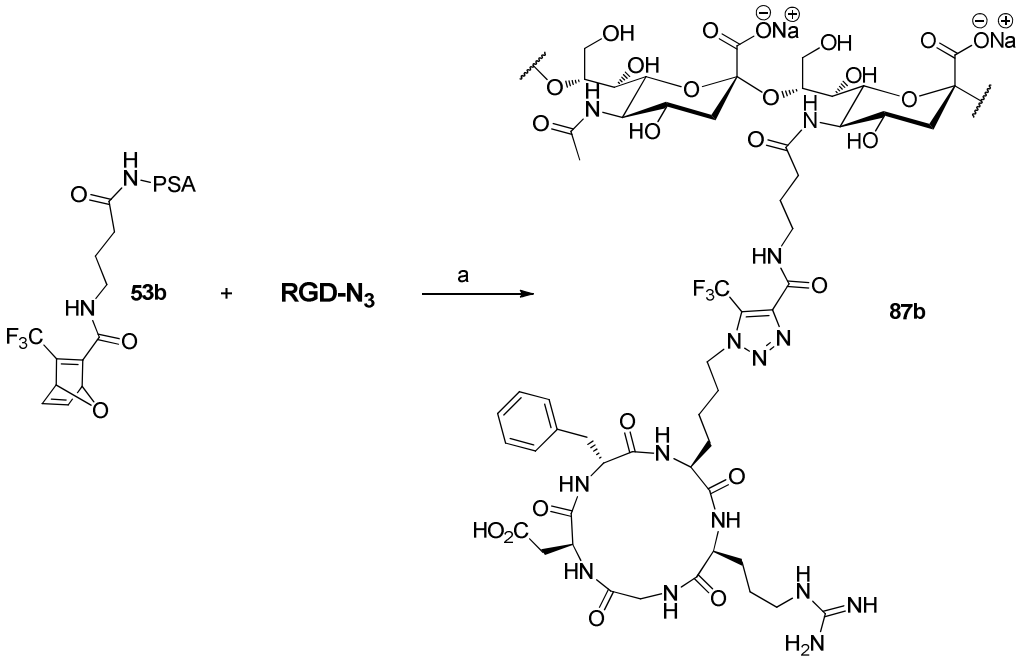
Scheme 43A. Proposed fragmentation of "click" product **86** according to LC-MS/MS.

The [**M1**-138]$^-$ ion at m/z = 837 might be created from the **M1** ion via a retro-aldol rearrangement and cleavage of a water molecule in ketone form **M6**. Due to keto-enol tautomerism, it generated the enol form, which could be further dehydrated to give fragment **M7** ([**M6**-H_2O]$^-$) at m/z = 819 (Scheme 43B).



Scheme 43B. Proposed fragmentation pattern of "click" product **86** according to LC-MS/MS.

The second approach towards RGD-modified polySia used metal-free "click" conditions for the coupling of oxanorbornadienyl-functionalized polySia **53b** and azido-RGD peptide. The "click" reaction was carried out in water under mild conditions to give "click" conjugate **87b** (*Scheme 44*).



Scheme 44. Synthesis of RGD peptide modified polySia **87b** from oxanorbornadienyl-functionalized polySia **53 b** and azido-RGD peptide using metal-free "click" chemistry. Conditions: a) H_2O, r.t., 2 d.

In contrast to the Cu-catalyzed "click" reaction of *N*-alkyno-polySia **44** and RGD peptide, the process of metal-free "click" reaction could be monitored using ^{19}F-NMR analysis (see model reactions in chapter 3.1.1.3). Figure 24 shows the ^{19}F-NMR spectra of polySia **53a** and "click" product **87a**. The CF_3 signal of the oxanorbornadienyl system of polySia **53a** could be observed at -62.3 ppm (*left*). After the "click" reaction, this signal shifted to -58.2 ppm (*right*).

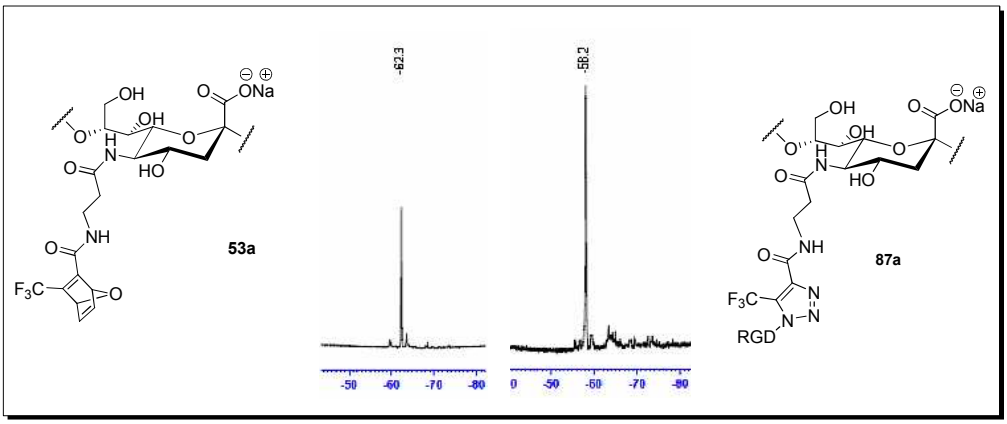
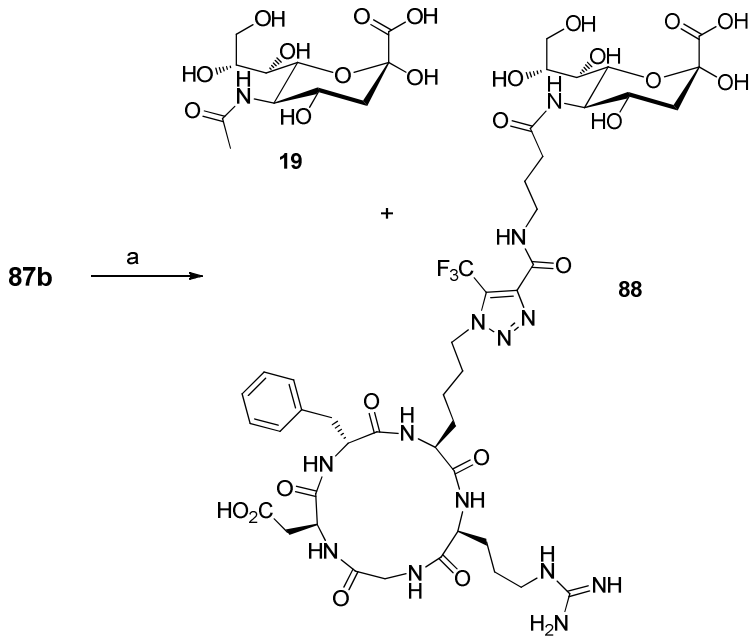


Figure 24. ^{19}F-NMR spectra of oxanorbornadienyl-functionalized polySia **53a** (left), and azido-RGD peptide modified polySia **87a**.

Further proofs for the successful "click" reaction towards RGD peptide modified polySia **87b** were obtained from the UPLC-MS and LC-MS/MS analysis of its hydrolyzed products *(Scheme 45*). In this way, two different units including *N*-Acetylneuraminic acid **19** and the "click" product **88** containing a CF_3-trazole linkage could be observed.



Scheme 45. Hydrolysis of azido-RGD peptide modified polySia **87b** with trifluoroacetic acid. Condition: a) TFA (0.1 M), 80 °C, 1 h.

Both hydrolyzation products **19** and **88** were detected by UPLC-MS spectroscopy. Figure 25 shows the "click" product **88** with m/z = 1100.4280.

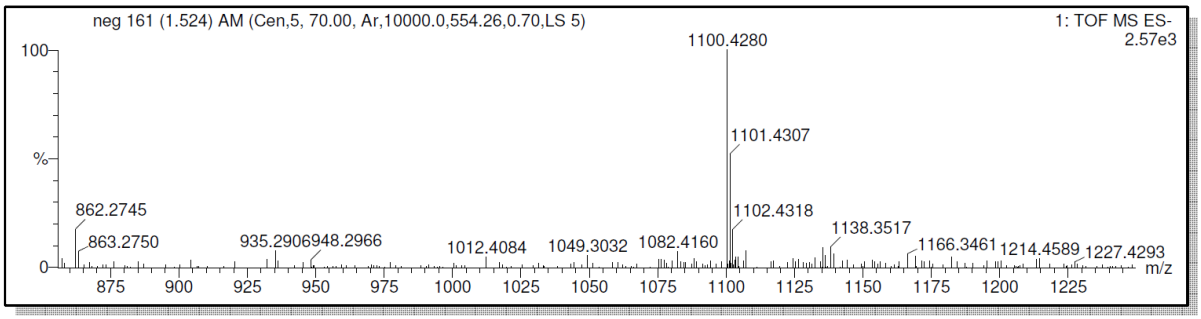


Figure 25. UPLC-MS spectra of the "click"-linked product **88**. (calcd for $C_{44}H_{61}N_{13}O_{17}F_3$ $[M-H]^-$: 1100.4260; found 1100.4280).

The "click" conjugate **88** was further analyzed with LC-MS/MS spectrometry (*Figure 26*). Similar to the Cu-catalyzed "click" reaction, alle corresponding fragments ions were found such as $[\mathbf{M}\text{-}H_2O]^-$ at m/z = 1082, $[\mathbf{M}\text{-}88]^-$ at m/z = 1012, $[\mathbf{M}\text{-}138]^-$ at m/z = 962, $[\mathbf{M}\text{-}208]^-$ at m/z = 892, $[\mathbf{M}\text{-}208\text{-}H_2O]^-$ at m/z = 874, and $[\mathbf{M}\text{-}208\text{-}H_2O\text{-}42]^-$ at m/z = 832.

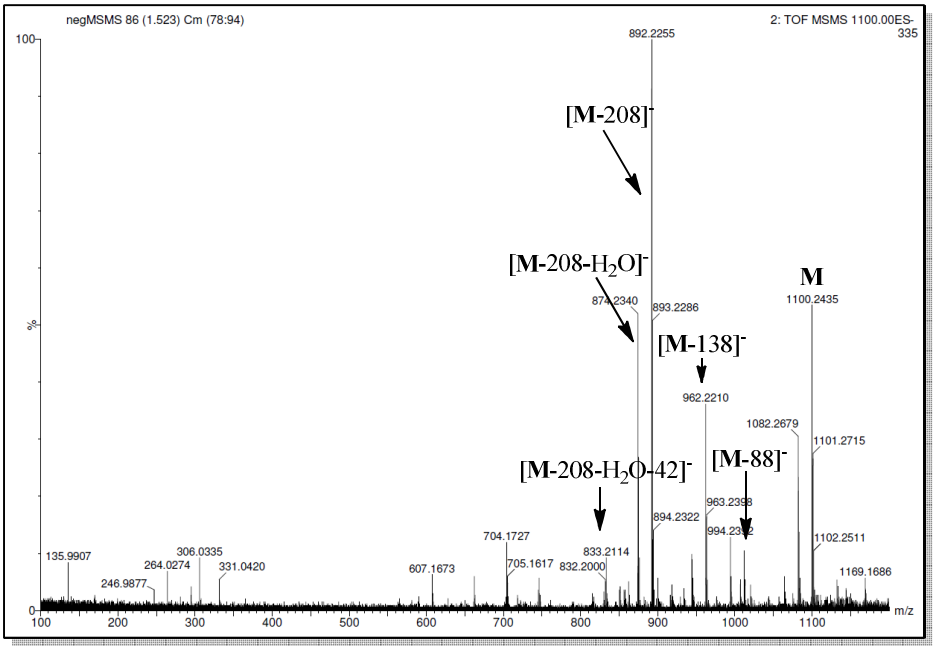
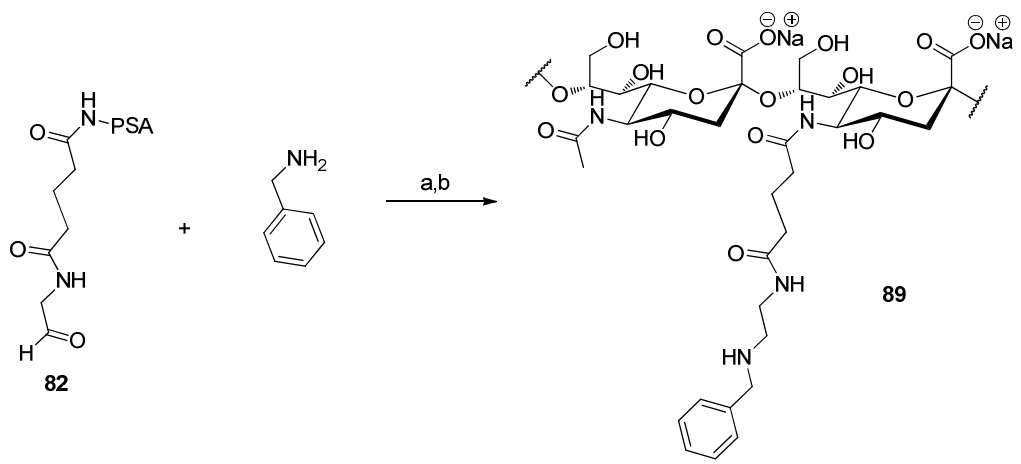


Figure 26. LC-MS/MS spectra of "click" product **88**.

3.1.4.2 Modification of polySia with RGD peptide using imine crosslinking

At first, a model reaction for the cross-coupling of amino-RGD peptide with *N*-aldehydo-polySia **80** was performed using commercially available benzylamine instead of amino-RGD peptide. The imine bond was further reduced to the corresponding amine **89** by treatment with sodium cyanoborohydride to improve the hydrolytic stability of the new material (*Scheme 46*).



Scheme 46. Model reaction for the cross-coupling of amino-RGD peptide and *N*-aldehydo-polySia **80** using benzyl amine instead of RGD peptide. Conditions: a) H_2O, r.t., 24 h; b) $NaCNBH_3$ (0.1 eq.), r.t., 16 h, quantitative conversion.

The reductive amination product was analyzed using ^{1}H-NMR spectroscopy. The characteristic proton signals of the aromatic system could be observed between 7.30 and 7.50 ppm. The CH_2 proton signals at 3.30 ppm adjacent to the aldehyde group and the C<u>H</u>$(OH)_2$ proton sig-

nal at 5.10 ppm, which were assigned to the polySia **82** (*Figure 27**A***) disappeared completely in the product spectra (*Figure 27**B***).

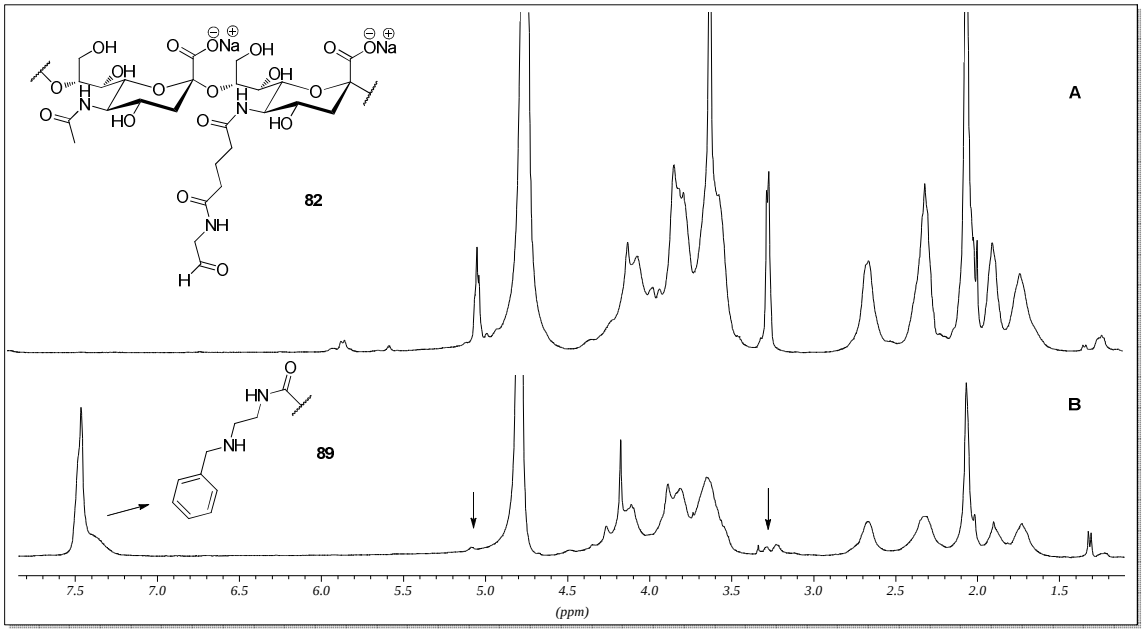
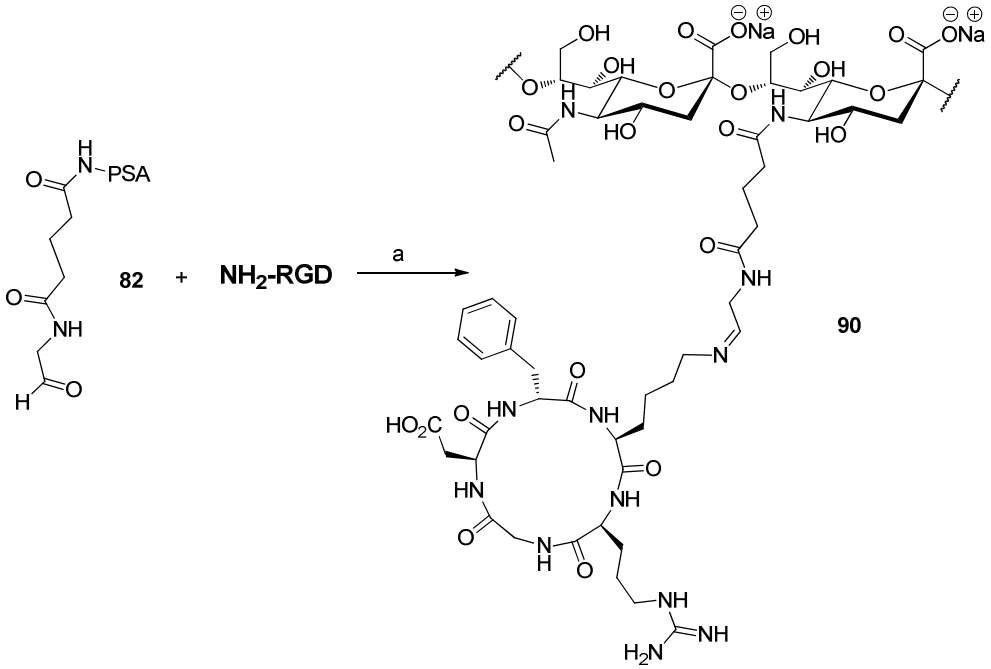


Figure 27. ^{1}H-NMR spectra of *N*-aldehydo-PolySia **82** in D_2O (**A**) and benzylamino-polySia **89** in D_2O (**B**).

RGD peptide modified polySia **90** was generated from *N*-aldehydo-polySia **82** and amino-RGD peptide (*Scheme 47*). The reaction was carried out under very mild aqueous condition without any catalyst. The solubility of RGD peptide played an important role for this process, since amino-RGD peptide was not very soluble in water. In contrast, addition of small amounts of organic solvents such as MeOH or DMF improved the solubility.



Scheme 47. Cross-coupling of *N*-aldehydo-polySia **82** and amino-RGD peptide. Condition: a) H_2O (1% MeOH), r.t., 3 d.

The cross-coupling product **90** was investigated by ^{1}H-NMR analysis (*Figure 28*). Compared to the ^{1}H-NMR spectra of the starting material, characteristic signals between 7.20 and 7.40 ppm appeared, which could be assigned to the aromatic protons of RGD peptide. Some weak signals at 3.20 and 1.50 ppm could be identified as RGD peptide proton signals.

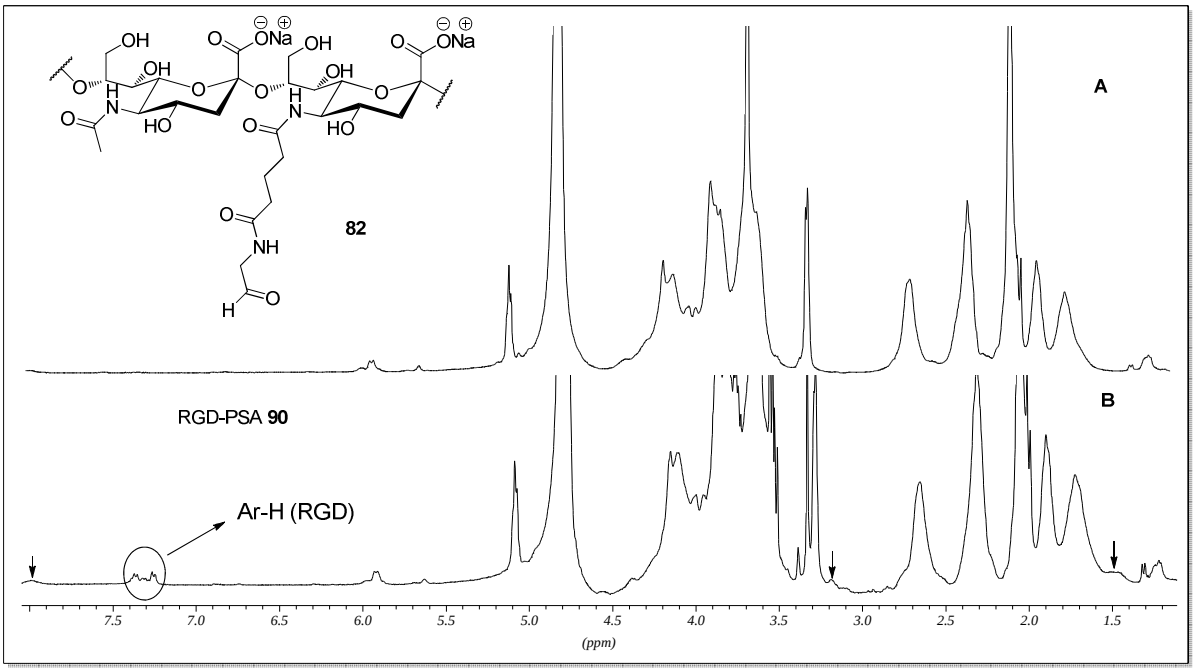
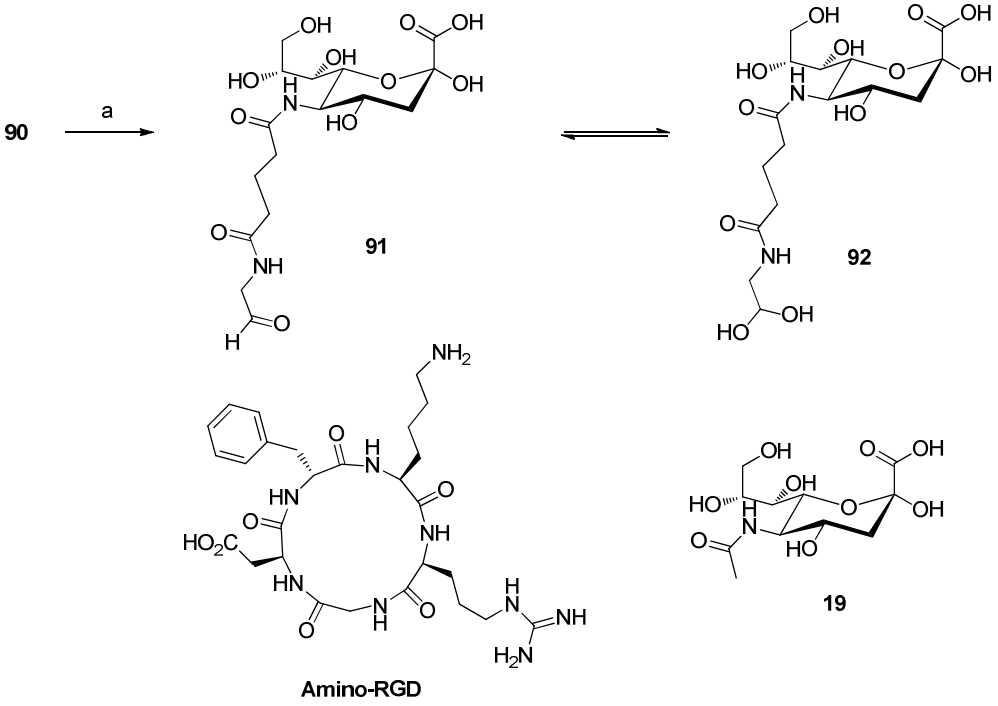


Figure 28. ^{1}H-NMR spectra *N*-aldehydo-polySia **82** in D_2O (**A**) and RGD peptide modified polySia **90** in D_2O (**B**).

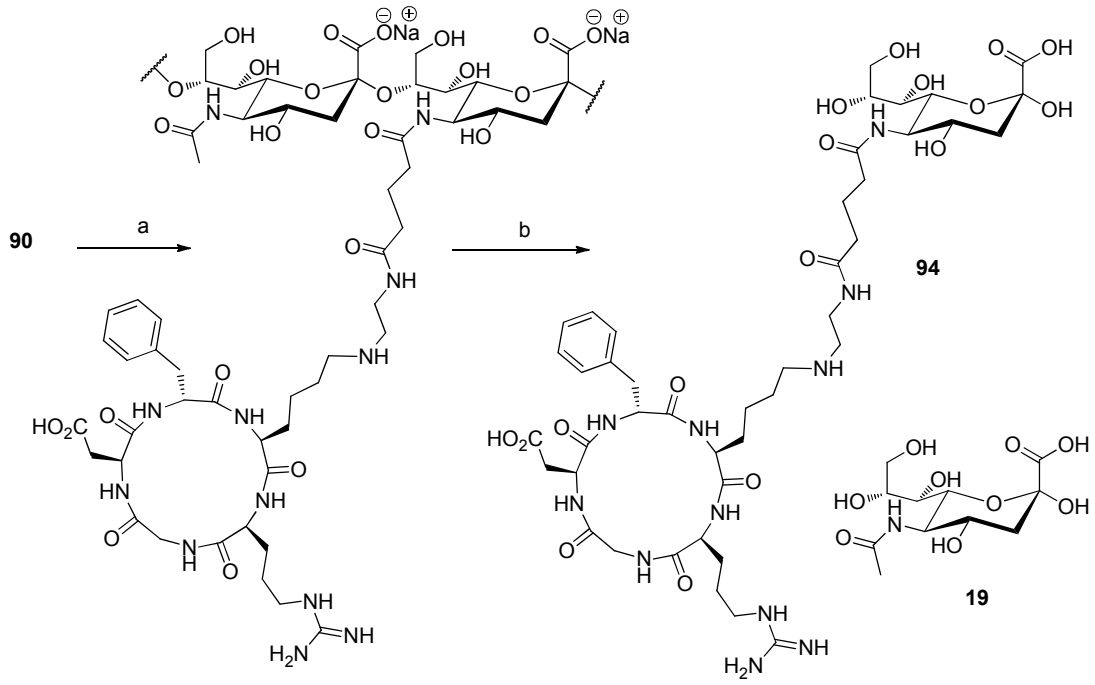
The cross-coupling product **90** was further analyzed by applying UPLC-MS spectrometry. The polymer was treated with 0.1 M trifluoroacetic acid at 80 °C to hydrolyze the glycosidic bonds. The mass of monomers of aldehydo-modified polySia **91**, and hydrated aldehyde **92** were detected as well as neuraminic acid **19** and amino-RGD peptide (*Table 9*).

Table 9. Chemical hydrolysis of RGD-modified polySia **90.** Conditions: a) TFA (0.1 M), 80 °C, 1 h.



Molecule	Formula [M-H]	Calcd. Mass (ES-)	Found mass (ES-)
91	$C_{16}H_{25}N_2O_{11}$	421.1458	421.1474
92	$C_{16}H_{27}N_2O_{12}$	439.1564	439.1572
19	$C_{11}H_{18}NO_9$	308.0982	308.0992
Amino-RGD	$C_{27}H_{40}N_9O_7$	602.3051	602.3052

Since the imine crosslinkage is not stable under acidic conditions, the mass of the coupling product between sialic acid and RGD peptide could not be found in the UPLC-MS spectra. Therefore, the polymer **90** was reduced with sodium cyanoborohydride to give amine **93** and then treated with trifluoroacetic acid (*Scheme 48*). Afterwards, the hydrolyzed products were analyzed by UPLC-MS (*Figure 29*). The mass of the cross-coupling product **94** (m/z = 1008.4614) was detected. Thus, the polySia-RGD ligation was successfully performed.



Scheme 48. Reduction and hydrolysis of amino-RGD modified polySia **90**. Conditions: a) H_2O, $NaCNBH_3$ (0.1 eq), r.t., 8 h; b) TFA (0.1 M), 80 °C, 1 h.

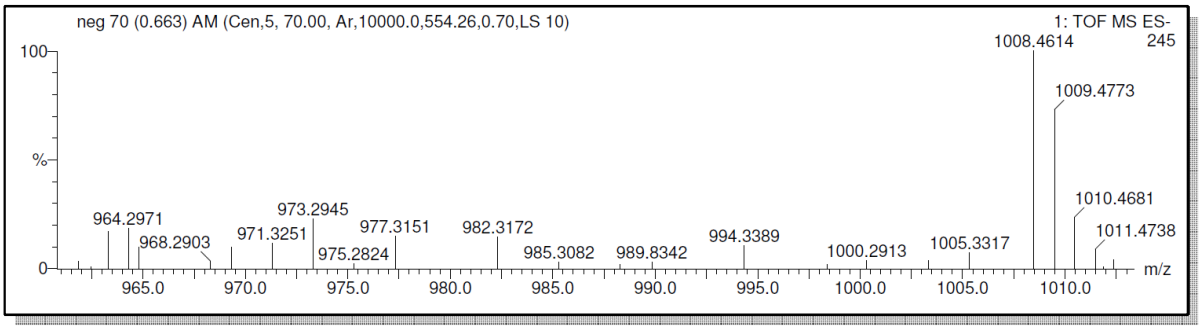


Figure 29. UPLC-MS spectra of the "click"-linked product **94**. (calcd for $C_{43}H_{66}N_{11}O_{17}$ $[M-H]^-$: 1008.4638; found 1008.4614).

Additionally, the cross-coupling product **94** was further analyzed by LC-MS/MS (*Figure 30*). Good agreement with the "click" chemistry mediated coupling of RGD peptide and polySia, all charateristical fragmentation ions were found. ($[\mathbf{M}\text{-}H_2O]^-$ at m/z = 992, $[\mathbf{M}\text{-}88]^-$ at m/z = 920, $[\mathbf{M}\text{-}138]^-$ at m/z = 870, $[\mathbf{M}\text{-}208]^-$ at m/z = 800, $[\mathbf{M}\text{-}208\text{-}H_2O]^-$ at m/z = 782, and $[\mathbf{M}\text{-}208\text{-}H_2O\text{-}42]^-$ at m/z = 740).

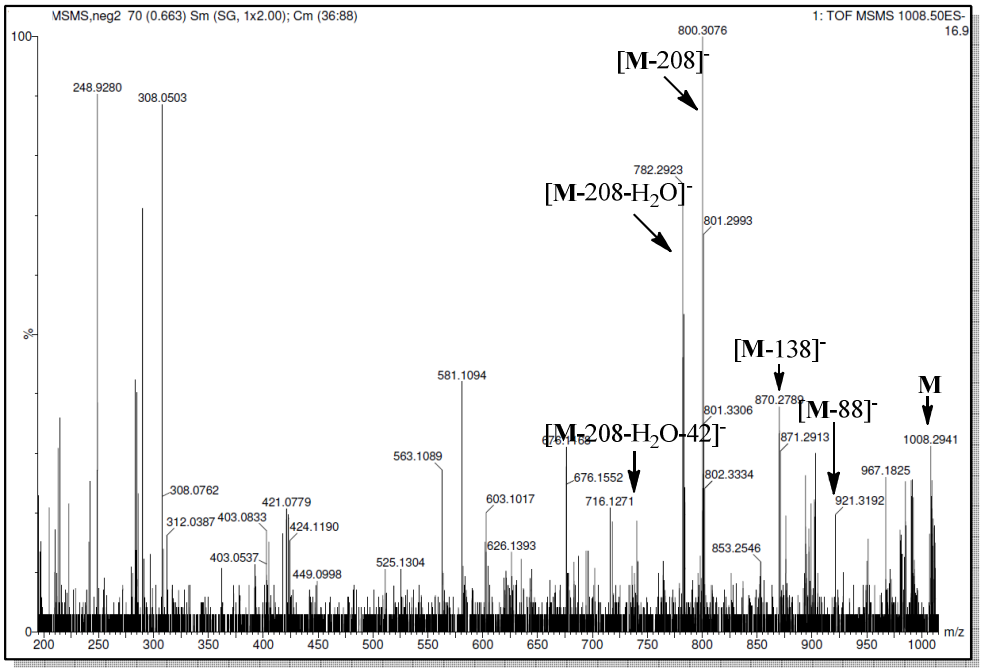


Figure 30. LC-MS/MS analysis of the cross-coupling product **94**.

3.1.5 Biological evaluation of the polySia hydrogels

3.1.5.1 Enzymatic degradation of the polySia hydrogels

A crucial property of an ideal scaffold for tissue engineering is that the scaffold should be biodegradable after fulfilling its purpose. PolySia can be enzymatically hydrolyzed through cleavage of the glycosidic bonds by a specific phage-borned endosialidase [101] (EndoNF, *Figure 31*).

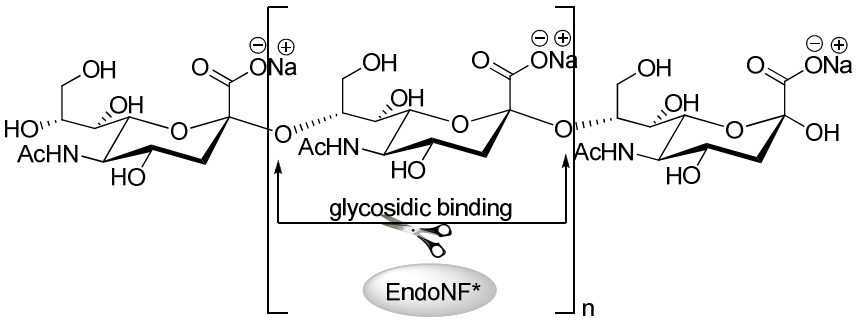


Figure 31. Enzymatic hydrolysis of α-2,8-polySia by phase-borned EndoNF.

Previous studies demonstrated that various functionalized polySia derivatives as well as polySia hydrogels using diepoxyoctane as a hydrophobic cross-linking agent could be degraded in phosphate-buffer (pH = 7.4) containing endosialidase.[89] It was carried out that the degree of derivatization plays a very important role for the degradability of this biomaterial. For example, when more than 60% the *N*-acetyl group of polySia was substituted with other substituents, the polySia derivatives could not be hydrolyzed by the endosialidase. Therefore, for the synthesis of new generation polySia hydrogels, the derivatization degree should be adjusted under 60%.

To study the enzymatic degradability of the polySia hydrogels synthesized in this work, the hydrogels were equilibrated with phosphate buffer (pH = 7.4) and treated with endosialidase. The degradation speed depended on many factors including activity of the enzyme, degree of derivatization of the modified polySia as well as the crosslinking method (*Table 10*). The hydrogel from *N*-azido-polySia **41** (60% derivatization) and alkyno-polySia **44** (60% derivatization), which was prepared using copper catalyzed “click” chemistry, was not completely degraded by endosialidase within 20 days (*entry 1*). Hydrogels generated from less decorated polySia can be easily degraded by endosialidase (*entries 2-5*). Hydrogel based on 40% *N*-methacryl-polySia **74** was partially degraded by endosialidase (*entry 6*).

[101] K. Stummeyer, A. Dickmanns, M. Mühlenhoff, R. Gerardy-Schahn, R. Ficner, *Nat. Struct. Mol. Biol*. **2005**, *12*, 90-96.

Table 10. Enzymatic degradation of polySia hydrogels.

Entry	PolySia A	PolySia B	Approach	Results
1[a]	**41** (60%)[b]	**44** (60%)	Cu-catalyzed "click"	20 d, hydrogel
2[a]	**41** (25%)	**44** (25%)	Cu-catalyzed "click"	3 d, liquid
3	**41** (25%)	**53a** (25%)	Metal free "click"	3 d, liquid
4	**41** (60%)	**53b** (25%)	Metal free "click"	20 d, liquid
5[c]	**74** (25%)		Photopolymerization	5 d, liquid
6[c]	**74** (40%)		Photopolymerization	30, hydrogel

[a]The polySia hydrogels were formed in the presence of $CuSO_4$ (4 mol%) and Na-ascorbate (6 mol%); [b]The percentage 60%, 40% , and 25% were the degree of derivatization of polySia; [c]The polySia hydrogels were formed in the presence of photoinitiator Irgacure 2959 (2% w/v).

The enzymatic degradation process could be analyzed using gel electrophoresis (PAGE). Here polySia hydrogel, which was generated from *N*-azido-polySia **41** (25% derivatization) and *N*-oxanorbornadienyl-polySia **53a** (25% derivatization) using metal-free "click" chemistry, was treated with endosialidase. The degraded products were analyzed by electrophoresis using polyarylamide gel and followed by silver straining method (*Figure 32*). Within 24 hours, only the surface of the hydrogels was degraded by the enzyme. After 72 hours, most of them were completely degraded.

Figure 32. Electrophoresis of the degraded polySia hydrogel by EndoNF. (Control: polySia hydrogel without treatment with EndoNF, Size Marker: TB: trypan blue; XC: xylencyanol; BPB: bromophenol blue; BCP: bromocresol purple. Their mobility matched the numbers of sialyl residues: TB = 117; XC = 52; DPB = 19; and BCP = 11.5. The size marker should be signed on a paper after alcian blue staining, because they could not be enhanced by silver staining).

3.1.5.2 Cytotoxic evaluation of the polySia hydrogels

The 3D-scaffolds based on modified polySia derivatives were evaluated by measuring the cell variability and proliferation behaviour of mammalian cells seeded on the different polySia hydrogels.[102] In this case, a human hepatocellular carcinoma cell line (HepG2) was selected as an *in vitro* model system for the biological evaluation of polySia hydrogels. The HepG2 cells were cultured on the polySia hydrogels under suitable conditions for one or two weeks. The viability of the cells was measured by the MTT assay, which is widely used as previously described. [103]

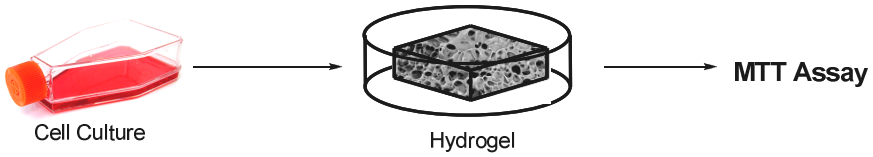
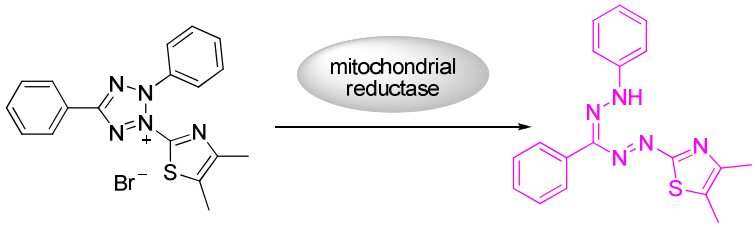


Figure 33. Cytotoxicity evaluation of polySia scaffolds by MTT assay.

The MTT assay is a colorimetric method for measuring the activity of a reductase enzyme like mitochondrial dehydrogenase, which can reduce the yellow tetrazolium salt MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to give a formazan dye with a purple color (*Scheme 49*). Only in living cells the mitochondrial dehydrogenase is active. The absorbance of the colored solution can be quantified precisely on a multiwell scanning spectrophotometer (ELISA reader). A main application of this assay allows to assess the viability (cell counting) and the proliferation of cells (cell culture assays). It can also be used to determine the cytotoxicity of potential medicinal agents and toxic materials, since those agents would stimulate or inhibit cell viability and growth.[104]



Scheme 49. Chemical reaction illustration of the MTT assay.

The results presented in Figure 34 showed that the viability of the HepG2 cells on hydrogels based on modified polySia **41** (60% derivatization) and **44** (60% derivatization) was slightly

[102] The experiments were carried by S. Böhm, Institute of Technical Chemistry, Gottfried Wilhelm Leibniz University of Hannover
[103] a) T. F. Slater, B. Sawyer, U. Straeuli, Biochim. *Biophys. Acta* **1963**, *77*, 383-393;
b) F. Denizot, R. Lang, *J. Immunol. Methods* **1986**, *89*, 271-277.
[104] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55-63.

better than that in culture medium. It can be concluded that this novel type of polySia hydrogel is not cytotoxic.

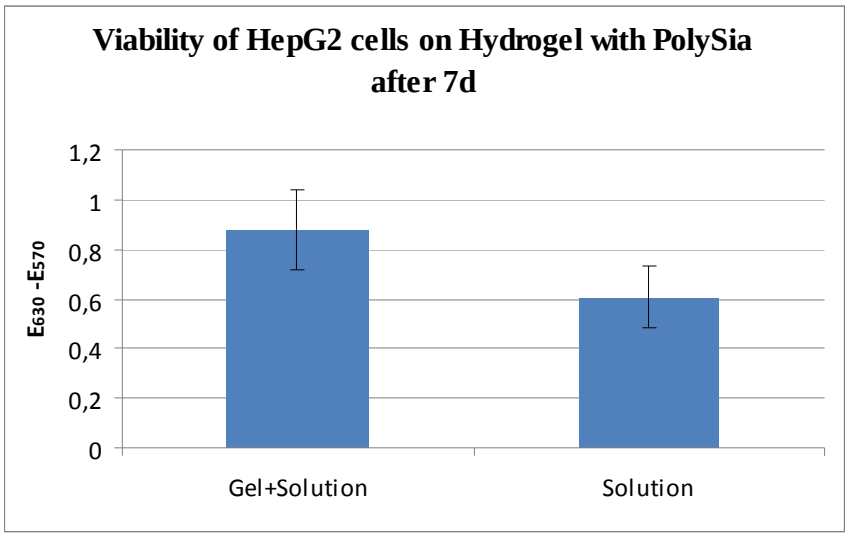


Figure 34. Viability behaviour of HepG2 cells on the polySia hydrogel. Cells on the hydrogel (left column) and cells in culture medium (right column). (The hydrogels were prepared from azido-/alkyno-modified polySia **41** and **44** using Cu-catalyzed "click" reaction).

Because the cells could not be observed by normal phase contrast microscopy, they were stained with 4´,6-diamidino-2-phenyindole (DAPI) (*Figure 35*). The DAPI molecules bond to DNA molecules in the cell nucleus, and then the cells are visible in fluorescence microscopy. Comparing to the MTT assay, DAPI strain method could not differentiate between living and dead cells.

A: Phase contrast B: DAPI

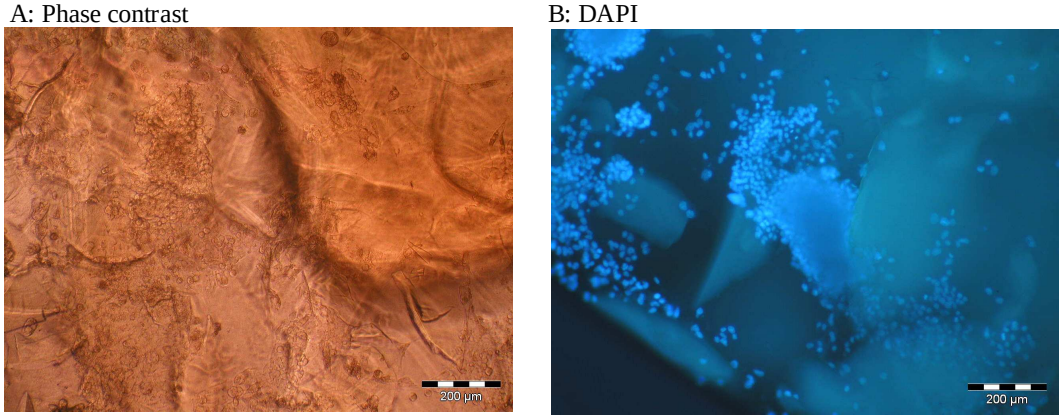


Figure 35. Cultivation of HepG2 cell line on the polySia hydrogel (the hydrogels were prepared from azido-/alkyno-modified polySia using Cu-catalyzed "click" reaction).

Besides the hydrogels formed from azido-/alkyno-modified polySia, all of the other polySia hydrogels prepared, including those with RGD peptides, were investigated using HepG2 cell

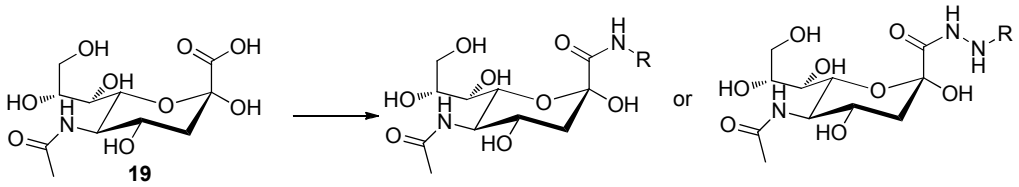
cultivation. Unfortunately, the current results did not show significant improvement of the cellular adhesion onto the scaffolds.

3.2 Synthesis of neuraminic acid derivatives

Two positions of neuraminic acid **19** could be modified with various functional groups like alkyno-, azido-, methacrylate or acrylate. At first, C-1 carboxyl group could be modified with the functional groups to give corresponding esters or amides. Generally, ester bonds are not stable under basic conditions so that amides are favoured due their stability and versatility. An alternative position for modification is C-5. The acetyl group can also be replaced by various functional groups.

3.2.1 Synthesis of neuraminic acids derivatized at C-1

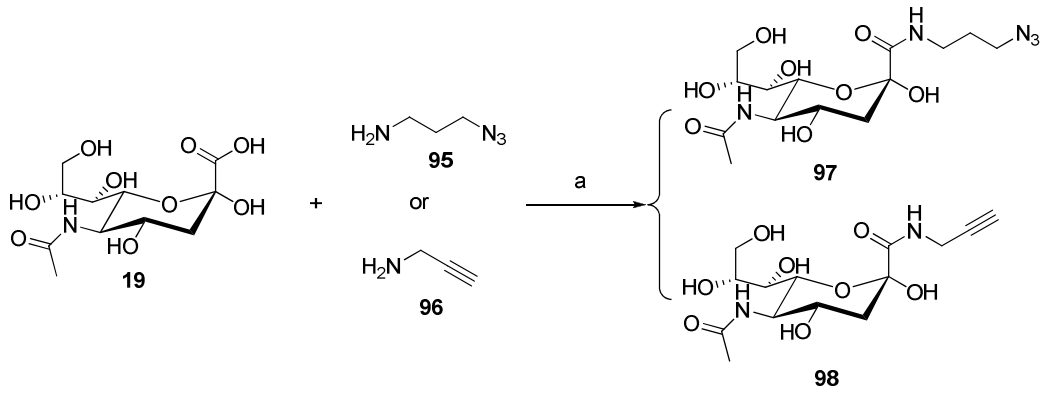
For the C-1 modification of neuraminic acid, two strategies were applied. The first approach used the carboxyl group to attach different amines containing functional groups like alkyno and azido. In an alternative approach, the carboxyl group was first converted into a hydrazide. The hydrazide moiety was then reacted with functionalized aldehydes to give modified neuraminic acids (*Scheme 50*).



Scheme 50. Synthetic approach to C-1 modification of neuraminic acid.

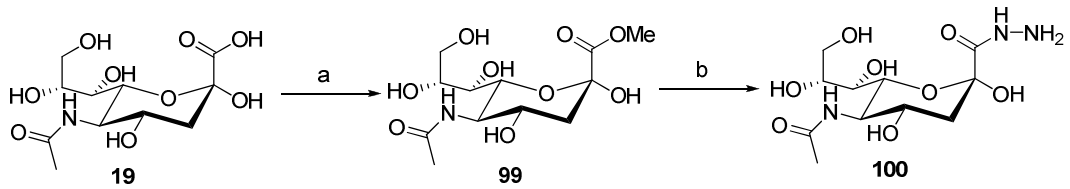
According to the first approach, C-1 azido-modified neuraminic acid **97** was synthesized from neuraminic acid **19** and 3-azidopropan-1-amine (**95**), which was prepared from 3-aminopropyl bromide according to a literature method.[105] The coupling reaction was established using carbodiimide chemistry. Under identical conditions, the commercially available propargylamine (**96**) could be coupled with neuraminic acid **19** to afford the C-1 alkyno-modified neuraminic acid **98**. The reactions were achieved in moderate yields without the necessity of installing any protecting groups (*Scheme 51*).

[105] B. Carboni, A. Benalil, M. Vaultier, *J. Org. Chem.* **1993**, *58*, 3736-4741.



Scheme 51. Synthesis of the C-1 derivatives of neuraminic acid **97** and **98**. Conditions a) DAMP, EDC·HCl, anhydrous DMF, r.t., 36 h, 60-70%.

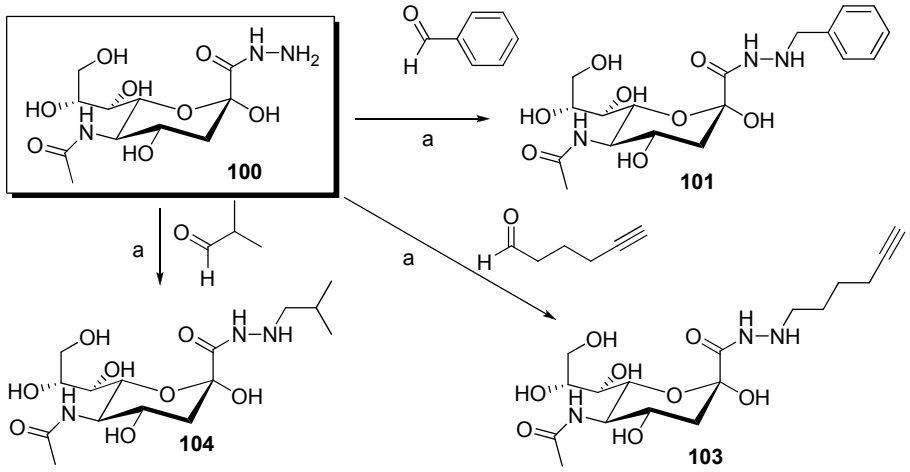
For the preparation of the C-1 hydrazido-neuraminic acid **100**, methyl ester of neuraminic acid **99** was firstly prepared according to a literature known procedure from neuraminic acid **19** and methanol in the presence of catalytic amounts of trifluoroacetic acid.[106] The obtained ester was treated with an excess of hydrazine monohydrate to give C-1 hydrazido-neuraminic acid **100** in 95% yield (*Scheme 52*).[107]



Scheme 52. Synthesis of C-1 hydrazido-neuraminic acid **100**. Condition: a) TFA, MeOH, r.t., 24 h, quantitative; b) H_2N-NH_2·H_2O, H_2O, r.t., 2 h, 95%.

In order to investigate the reaction between C-1 hydrazido-neuraminic acid **100** and different aldehydes, hydrazide **100** was coupled with benzaldehydo-, isobutyl- aldehyde-, and 5-hexynal.[108] Generally, the hydrazone linkage was not stable. Therefore, the hydrazone crosslinked products were reduced with sodium cyanoborohydride. The products were analysed by UPLC-MS spectrometry, and showed good rates of conversion. However, HPLC chromatography of substituted neuraminic acid **101** gave only 7% of the product as a mixture of α and ß anomers. Other products could also not be isolated.

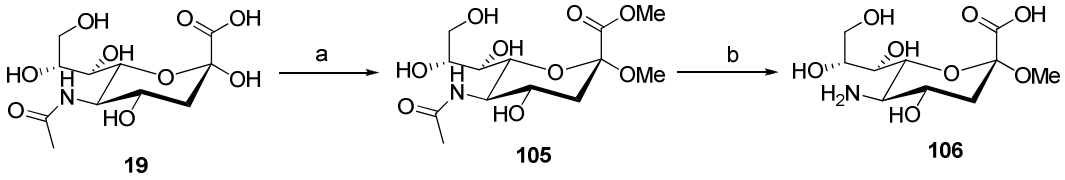
[106] B. P. Bandgar, M. Hartmann, W. Schmid, E. Zbiral, *Liebigs Ann. Chem.* **1990**, 1185-1195.
[107] J. Becher, I. Seidel, W. Plass, D. Klemm, *Tetrahedron* **2006**, *62*, 5675-5681.
[108] T. Böttcher, S. A. Sieber, *Angew. Chem. Int. Ed.* **2008**, *47*, 4600–4603.



Scheme 53. C-1 Modification of neuraminic acid via hydrazone linkage. Conditions: a) MeOH/H_2O, 0 °C→r.t., 8 h, and then $NaCNBH_3$ (0.1 eq.), r.t., 8 h.

3.2.2 Synthesis of neuraminic acids derivatized at the nitrogen

To introduce various substituents at position C-5 of the neuraminic acid **19**, the *N*-acetyl group was firstly deacetylated to give a free amino group. Furthermore, it turned out to be necessary to protect the anomeric hydroxyl group, requiring a two step approach to γ-amino methoxyneuraminic acid **106**. At first, the neuraminic acid **19** was heated in anhydrous methanol in the presence of Amberlite IR 120 (H^+-form) to give the methyl ester of methoxyneuraminic acid **105**. Subsequently, the ester was treated with sodium hydroxide to give γ-amino methoxyneuraminic acid **106** in a good yield (*Scheme 54*).

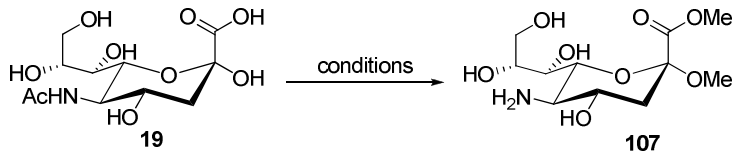


Scheme 54. Synthesis of γ-amino methoxyneuraminic acid **106**. Conditions: a) Amberlite 120 (H^+-form), dry MeOH, reflux, 16 h, >90%; b) NaOH, H_2O/EtOH, reflux, 16 h, 85%.

γ-Amino methoxyneuraminic acid **106** was used as starting material to prepare of *N*-acyl-modified neuraminic acid derivatives. Due to the very high polarity, the neuraminic acid **106** turned out to be insoluble in any organic solvent. In aqueous media, neuraminic acid **106** was coupled with different carboxylic acids such as pentinoic acid, pent-4-ynoic acid, and 4-azido butanoic acid but only very low or no conversion was obtained.

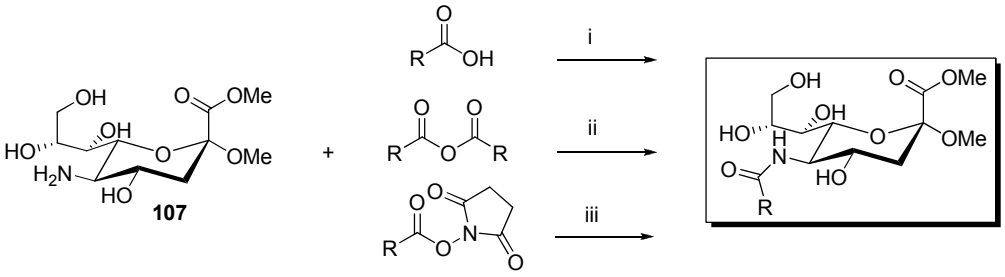
Therefore, γ-amino methoxyneuraminic acid methylester **107** was prepared according to a preciously reported one-pot procedure.[109] Here, the carboxyl group was protected with methyl ester to improve the solubility in organic solvents. Different conditions were tried to prepare deacetylated γ-amino methoxyneuraminic acid methyl ester **107** (*Table 11*). The starting material was heated in the microwave oven in methanol containing 0.6-1.25 M hydrochloric acid (*entry 1*). The conversion was only moderate. Changing the concentration of the acid or extension of reaction time did not improve of conversion. The use of acetyl chloride instead of hydrochloric acid resulted in a little improvement of the yield (*entry 2*). The reactions under pressure in sealed tube or autoclave leaded to higher yields (*entries 3 and 4*).

Table 11. Synthesis of γ-amino methoxyneuraminic acid methyl ester **107**.



Entry	Condition (solvent, method, temperature, time)	Result
1	HCl (0.9-1.25 M), MeOH, microwave oven, 120 °C, 30 min	30%
2	CH_3COCl, MeOH, microwave oven, 120 °C, 30 min	50%
3	CH_3COCl, MeOH, sealed tube (pressure), 100 °C, 3 h	80%
4	CH_3COCl, MeOH, autoclave, 100 °C, 3 h	80%

To obtain *N*-derivatives of neuraminic acid, three strategies were applied starting with the γ-amino methoxyneuraminic acid methyl ester **107** (*Scheme 55*). At first, the amino group of neuraminic acid **107** was reacted with different carboxylic acids in presence of coupling reagents. In a second approach, anhydrides were used instead of carboxylic acids to react with the amino sugar without activation reagents. Finally, the amino sugar was reacted with active NHS-esters under basic conditions.



Scheme 55. Synthesis of *N*-acyl-modified neuraminic acid derivatives. Conditions: i) EDC·HCl, DIPEA, DMF, r.t., 24 h; ii) Pyridine, r.t., 24 h; iii) DMF, DIPEA, 0→40 °C, 24 h.

[109] N. E. Byramoca, *Carbohydr. Res.* **1982**, *237*, 161-175.

Comparing the results obtained from the three approaches, the third strategy gave best yields. The active esters were prepared from the corresponding carboxylic acids and NHS according to literature known procedures (*Table 12*).[110]

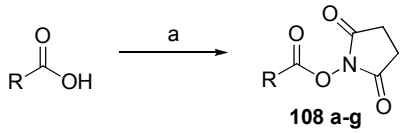
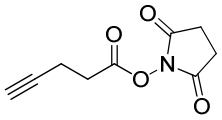
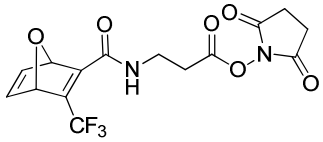
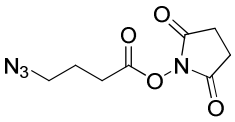
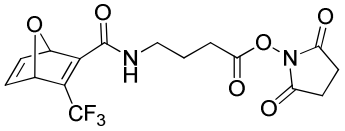
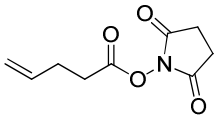
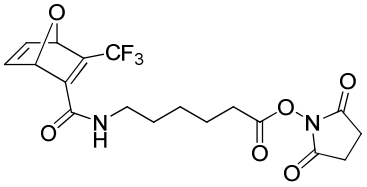
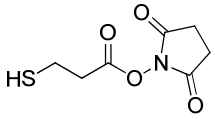


Table 12. Preparation of NHS active esters **108a-g**. Conditions: a) NHS, EDC·HCl, dry CH_2Cl_2 or THF, 0→4 °C, 16 h.

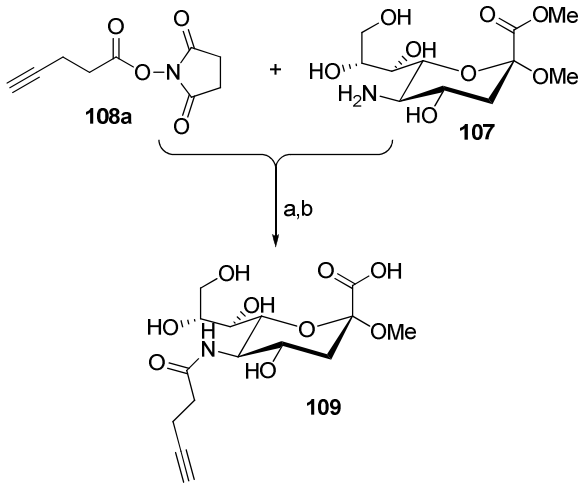
N-hydroxysuccinimide esters (NHS-esters)	
 **108a**	 **108e**
 **108b**	 **108f**
 **108c**	 **108g**
 **108d**	

NHS active esters **108a**,**c**,**d** were prepared from commercial available carboxylic acids. Esters containing azido-group **108b** or oxanorbornadienyl-linker **108e-g** groups were prepared from the carboxylic acids, whose syntheses were described in chapter 3.1.1.2.

The coupling reaction of the 4-pentynoyl NHS-ester **108a** and the γ-amino methyl ester of methoxyneuraminic acid **107** were carried out in anhydrous DMF under basic conditions. Subsequently, the crude products were treated with sodium hydroxide to liberate the C-1 car-

[110] a) K. Shimizu, K. Nakayama, M. Akiyama, *Bull. Chem. Soc. Jpn*. **1984**, *57*, 2456-2462;
b) H. G. Batz, J. Koldehoff, *Makromol. Chem.* **1976**, *177*, 683-689.

boxylic group. To remove the sodium salt, the reaction mixture was treated with Amberlyst 15 (H^+-form).



Scheme 56. Synthesis of *N*-alkyno-methoxyneuraminic acid **109**. Condition: a) DIPEA, dry DMF, r.t.→40 °C, 24 h; b) NaOH (0.5 M), 0 °C→r.t., 30 min.

Cleavage of the glycosidic bond was not easily accomplished. In the early 1960s, Karkas and Chargaff had discussed this reaction in detail.[111] The pH value plays a very important role in the removal of the glycosidic methoxy group. Between pH 5 and 7, methoxysialic acid was not hydrolyzed in appreciable amounts. Moderate cleavage took place at pH = 4. The optimal condition for the hydrolysis of the glycosidic bond was at pH = 3, since the cleavage was completed in a relatively short time, without the destruction of sialic acid. At low pH values, cleavage occurs more rapidly. However, the free sialic acid evidently is degraded further. They found that the formation of a hydrogen bond between the hydrogen of the carboxyl and the glycosidic oxygen has an intramolecular catalytic effect. As a result formation of the 5-membered ring can increase the rate of the reaction overall and avoid the risk of decomposition of the ring structure (*Figure 36*).

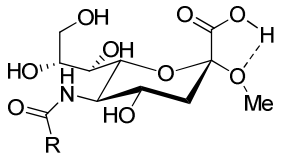


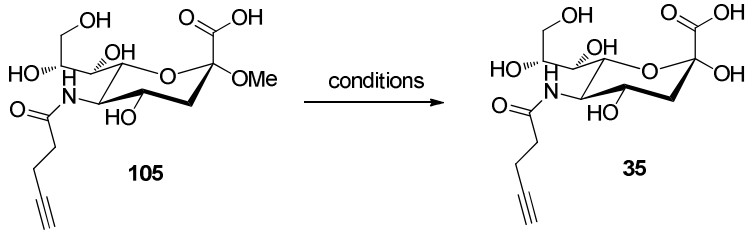
Figure 36. Formation of 5-membered ring under acidic conditions during the cleavage of the methyl glycoside.

In this work, different conditions were tested to cleave the glycosidic bond of alkyno-modified methoxyneuraminic acid **109** (*Table 13*). Under strong acidic conditions (*entries 1-3*), no conversion could be observed. The reactions catalyzed by ion exchange resin

[111] J. D. Karkas, E. Chargaff, *J. Biol. Chem*. **1964**, *239*, 949-957.

Dowex 50 (H^+-form) or Amberlite 120 (H^+-form) in the presence of 0.025 M hydrochloric acid as hydrogen source leaded to little conversion (*entries 5-7*). With the ion exchange resin Amberlyst 15 (H^+-form) best results were obtained. The conversion was almost complete (*entries 8 and 9*). The reaction was also tested under microwave condition at 100 °C. The cleavage occurred more rapidly, but decomposition of the material was observed.

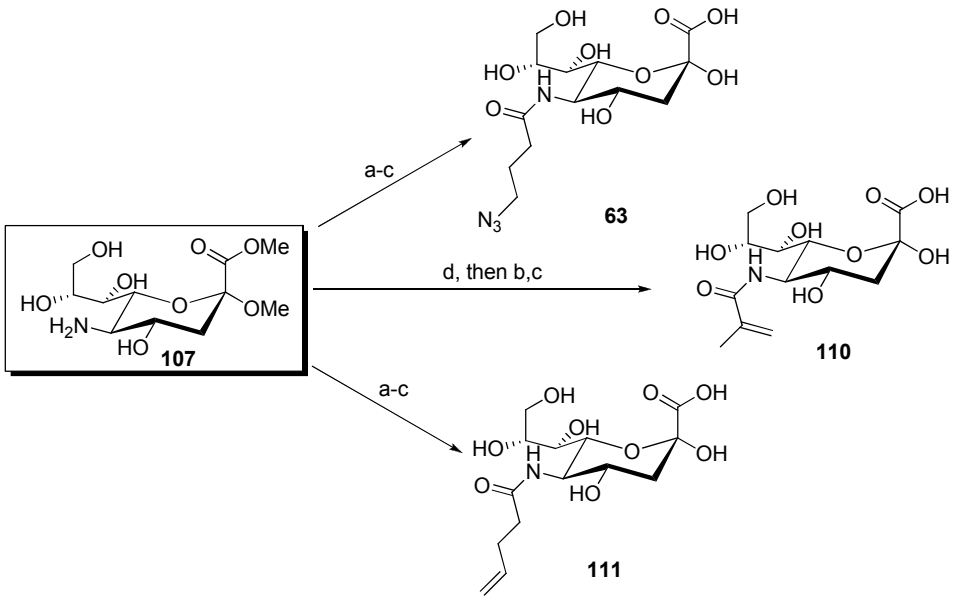
Table 13. Demethoxylation of *N*-4-pentynoyl-methoxyneuraminic acid **105**.



Entry	Conditions	Result (ESI)[a]
1	AcOH:H_2O (4:1) reflux 2 h	SM
2	1 M HCl, reflux, 2 h	SM
3	0.1 M TFA, 80 °C, 2 h	SM
4	0.025 M HCl, Dowex 50 (H^+-form), 80 °C, 2 h	SM
5	0.025 M HCl, Dowex 50 (H^+-form), MW, 100 °C, 2 h	SM + P (trace)
6	0.025 M HCl, Amberlite 120 (H^+-form), 80 °C, 2 h	SM + P (trace)
7	0.025 M HCl, Amberlite 120 (H^+-form), 100 °C, 2 h	SM + P (trace)
8	0.025 M HCl, Amberlyst 15 (H^+-form), 80 °C, 2h	P
9	0.025 M HCl, Amberlyst 15 (H^+-form), MW, 100 °C, 10 min	SM (trace) + P

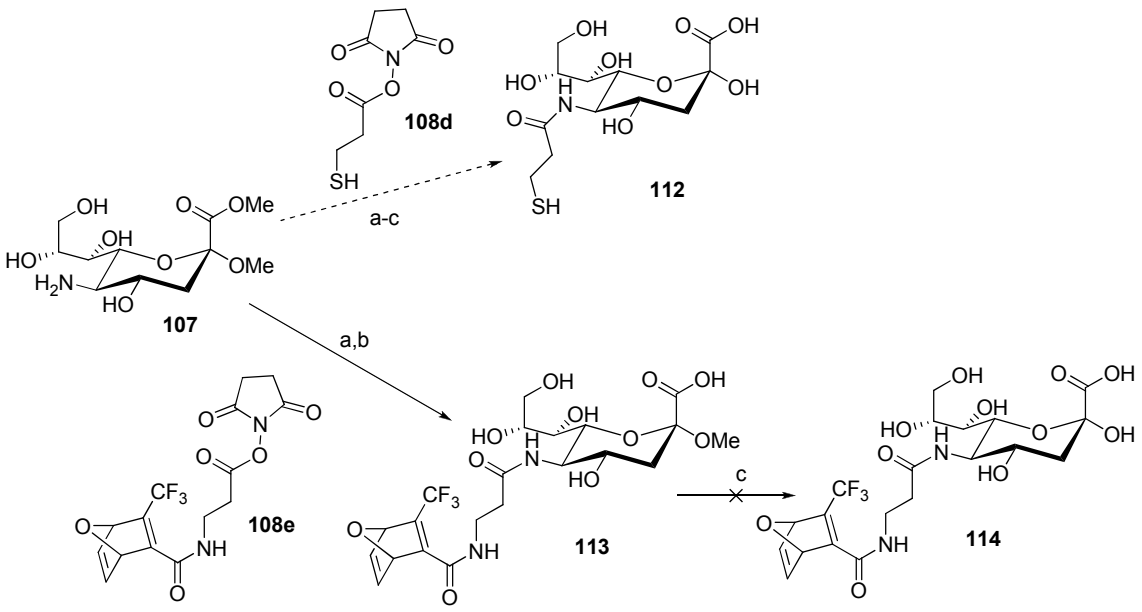
[a] SM: starting material, P: product

According to the optimized procedure, the following *N*-modified neuraminic acid derivatives were obtained, which were *N*-4-azidobutytyl-neuraminic acid **63**, *N*-methacryl-neuraminic acid **110** and *N*-pent-4-enoyl-neuraminic acid **111** (*Scheme 57*).



Scheme 57. Synthesis of *N*-functionalized neuraminic acid derivatives with azido-butanoyl group **63**, methacryl-group **106** and pent-4-enoyl group **107**. Conditions: a) NHS-ester, DMF, DIPEA, 40 °C, 16 h; b) NaOH (0.5 M), r.t., 2 h; c) HCl (0.0125 M), Amberlyst 15 (H^+-form), 80 °C, 2 h, 60-70% over 3 steps; d) Pyridine, methacrylic anhydride, 0→r.t., 24 h, then the same steps b and c, over 3 steps 70%.

Additionally, the two NHS-esters **108d**/**108e**, which contained thiol- and oxanorbornadienyl-functional groups, were coupled the neuraminic acid **107** to synthesis of new neuraminic acid derivatives (*Scheme 58*). Although the thiol-functionalized product **112** could be detected by UPLC-MS, it could not be isolated. The oxanorbornadienyl-modified neuraminic acid derivative **114** was not obtained from glycoside **113** because the oxanorbornadienyl-system decomposed under the demethoxylation conditions.



Scheme 58. Synthesis of *N*-functionalized neuraminic acid derivatives with thiol-group **112** and oxanorbornadienyl-group **114**. Conditions: a) NHS-ester, DMF, DIPEA, 40 °C, 16 h; b) NaOH (0.5 M), r.t., 2 h; c) HCl (0.0125 M), Amberlyst 15 (H^+-form), 80 °C, 2 h, no pure desired product was isolated.

4 Conclusion and Outlook

In this thesis two general projects were investigated. The first part focused on the decoration of the α-2,8-linked polySia with various functional groups. The different functionalized polySia batches were used as macromolecular precursor to form 3D-hydrogel networks, which serve as scaffolds in the field of tissue engineering. Furthermore, polySia was successfully decorated with RGD peptides via different covalent linkages in order to improve the interactions of cells and scaffold. The second project aimed at the synthesis of various neuraminic acid analogues. The neuraminic acid plays a crucial role in many biological events and was widely investigated for applications in novel pharmaceutical agents, inhibitors for neuraminic acid aldolase, and other biological applications. The neuraminic acid derivatives were transferred to Hannover Medical School for further biological applications. These *N*-acyl-modified sialic acids will be used as starting material for the enzymatic synthesis of polySia derivatives and as substrates to study the polySia synthetase involved in this polymerization.

4.1 Modification of polySia and generation of novel scaffolds for tissue engineering

Commercially available colominic acid sodium salt and the polySia provided by the Institute of Technical Chemistry at the Leibniz university Hannover (TCI, Uni-Hannover) were used as starting material for the synthesis of polySia derivatives. Two major types of novel polySia derivatives were successfully synthesized in this work (*Figure 37*). The first class aimed at the polySia decoration at position C-1. The polySia lactone was synthesized and further converted into polySia hydrazide **80**, which can be used for hydrogel preparation by cross-linking with aldehydes. The second class consists of *N*-derivatives of polySia by replacing the acetyl-group at C-5 with various functional groups. Azido- and alkyno-functionalized polySia (**41** and **44**) were synthesized from deacetylated polySia (TBA-salt) **37** using the corresponding acid chlorides. In order to perform metal-free “click” chemistry, oxanorbornadienyl-modified polySia **53** was synthesized as alkyno-analogue. *N*-methacryl-polySia **74** was prepared from deacetylated polySia **37**. The formation of aldehydo-functionalized polySia **82** was achieved by a building block, which contained a terminal carboxyl group as well as a 1,2-diol moiety. The building block was coupled to the amino group of polySia using carbodiimide chemistry. Afterwards, the 1,2-diol moiety was oxidized with sodium periodate to give the *N*-aldehydo-polySia **82**.

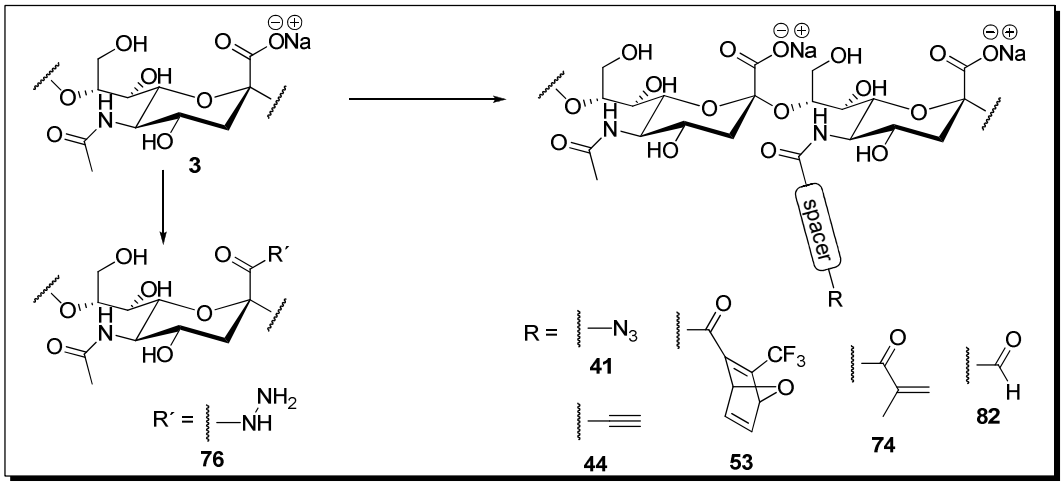


Figure 37. PolySia derivatives decorated with functional groups.

The degree of substitution has significant influence on the properties of the formed polySia derivatives such as hydrogel formation, enzymatic degradability, and stability of hydrogel. Thus, degree of derivatization was adjusted in a range of 25-60%. Additionally, the *N*-acetyl group plays a crucial role in the process of enzymatic degradation and had to be reconstructed for each derivative by reacetylation of the remaining amino groups with acetic anhydride.

The modified polySia derivatives were used to prepare hydrogels, which were applied as 3D-scaffolds for neural tissue engineering. PolySia hydrogels were formed via different crosslinking methods (*Figure 38*). Azido- and alkyno-decorated polySia derivatives (**41** and **44**) were used as "clickable" macromolecular precursors and in the presence of Cu-catalyst, triazoles were formed which led to crosslinkage and therefore hydrogel formation (**A**). Since copper is potentially cytotoxic for organisms, the metal-free "click" linkage is more favoured for the generation of polySia hydrogels. Oxanorbornadienyl-modified polySia **53** reacted with azido-modified polySia **41** to create a network structure based on a CF_3-triazole linckage in the absence of any catalyst (**B**). Photoinitiator induced radical crosslinking is also an appropriate approach for preparation of hydrogels. *N*-Methacryl-polySia **74** was irradiated with UV-light in the presence of Irgacure 2959 to give flexible polySia hydrogels (**C**). Besides the crosslinking approaches, molecular weight of polySia plays a crucial role by the Gelation process. Comparing to the commercial polySia (molecular mass: 15.000 Da), the polySia from TCI with a molecular mass of 45.000 Da could easily lead to hydrogel formation, even though it was less functionalized.

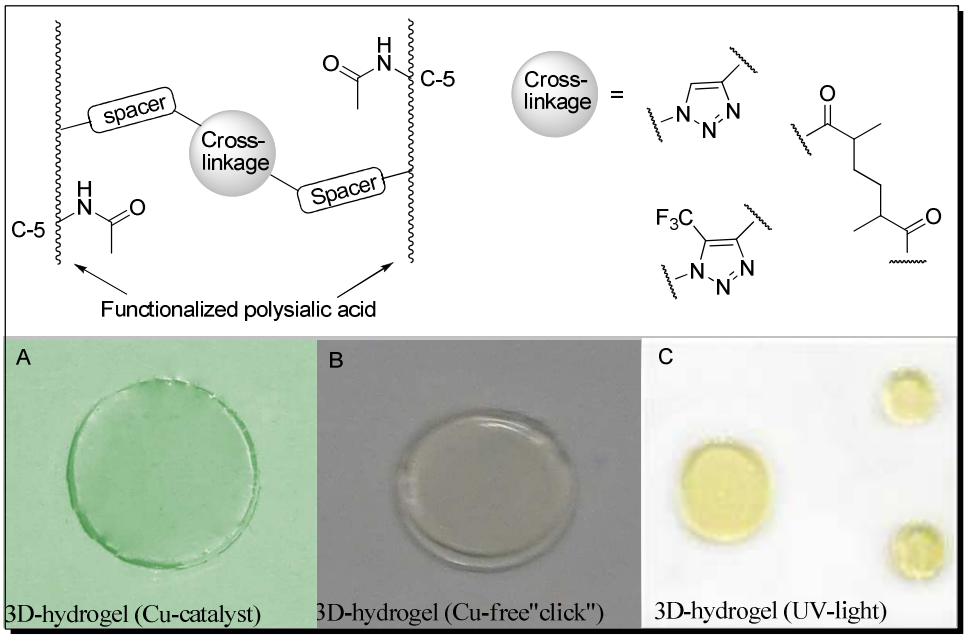


Figure 38. PolySia hydrogels based on different crosslinking techniques: Cu-catalyzed "click" reaction (**A**), metal-free "click" reaction (**B**), and photo-induced radical polymerization (**C**).

An alternative method for the synthesis of 3D-scaffolds is the formation of hydrazones. Therefore, hydrazido- and aldehydo-functionalized polySia (**76** and **80**) were prepared. Based on the hydrazone formation a hydrogel was formed within a few minutes. The hydrazones turned out to be labile towards hydrolysis and the hydrogel liquefied within several hours. Nevertheless, blends of aldehydo-modified polySia and hydrazido-decorated alginate or hyaluronate can generate very stable and flexible hydrogels.

In addition, biological properties of polySia hydrogels were evaluated. The biodegradability was studied by enzymatic degradation experiments. PolySia hydrogels were treated with EndoNF for 3 to 30 days. The rates of degradation especially depend on the degree of derivatization of the starting materials. Cytotoxicity and biocompatibility was evaluated using the human hepatocellular carcinoma cell line (HepG2) as *in vitro* model. The results demonstrated that polySia hydrogels caused no cytotoxicity, but also did not afford significant increase of cell adhesion.

In order to improve cell adhesion, polySia was decorated with unique RGD peptides via "click" reaction or imine formation (*Figure 39*). PolySia hydrogels decorated with RGD peptides were also tested in cell cultures. Unfortunately, the current results did not present significant improvement of cell adhesion.

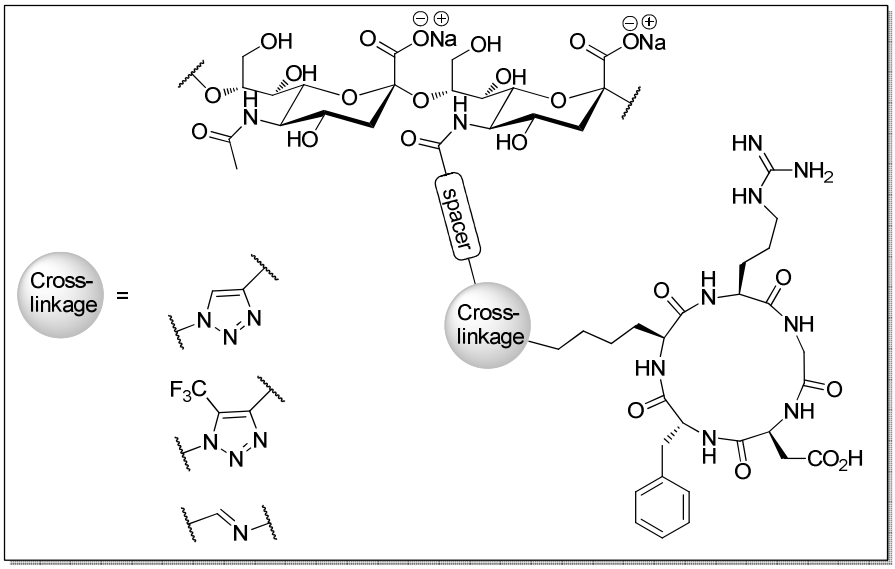


Figure 39. RGD peptide modified polySia derivatives afforded by different crosslinking strategies.

4.2 Sythesis of *N*-neuraminic acid derivatives

In this project, neuraminic acid was successfully modified at two positions (C-1 and C-5) with various functional groups (*Figure 40*). C-1 Modification was accomplished via coupling of neuraminic acid **19** and 3-azidopropan-1-amine or propargylamine to give products **97** and **98**, respectively. *N*-Acyl derivatizations were established from various substituents and deacetylated neuraminic acid. At first, the functionalized carboxylic acids were transferred into their corresponding NHS-esters. Subsequently, the NHS-esters were coupled to the amino group of neuraminic acid to afford different neuraminic acid derivatives (**35**, **63** and **111**). Methacryl-modified neuraminic acid **110** was synthesized directly from methacrylic anhydride and deacetylated neuraminic acid under mild conditions.

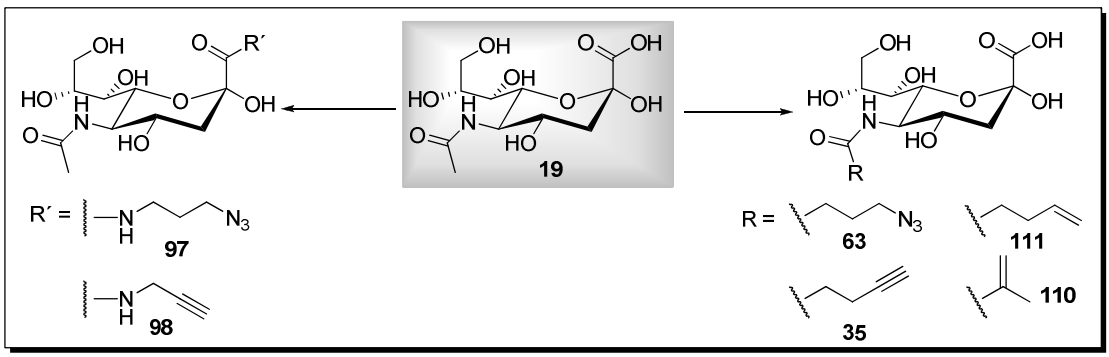


Figure 40. Neuraminic acid derivatives decorated with various functional groups.

4.3 Outlook

For the optimization of polySia hydrogels, the polySia can be modified with an appropriate substitution degree in a range of 40-50%. Accompanied polySia hydrogels should have better stability and degradability. Additional coating of polySia hydrogels with collagen (I/II) or poly-L-lysine (PLL) should be tested to improve cell adhesion. Another optimization possibility is the combination of polySia with other macromolecular precursor like alginate or hyaluronate to give blended scaffolds with combined properties. PolySia is favoured due to its good biodegradability and chemical versatility. Alignate and hyaluronate can generate improved mechanically stable hydrogels due to their high-molecular weight. For example, 3D-scaffolds could be prepared form aldehydo- or RGD peptide decorated polySia and alginate or hyaluronate hydrazide (*Figure 41*).

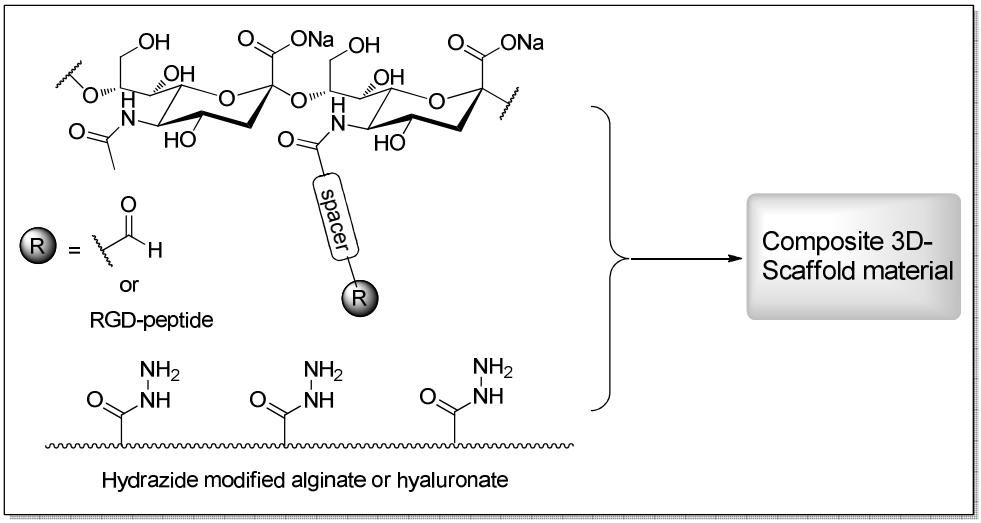


Figure 41. Composite 3D-scaffolds from alginate or hyaluronate hydrazide with aldehydo- or RGD peptide modified polySia.

As a final goal of this project, polySia hydrogels with optimal properties will be applied as cell scaffolds for the investigation of neural cell regeneration. The obtained neuraminic acid derivatives will be probed for the synthesis of different polySia derivatives or used for other biological applications.

5 Experimental Parts

5.1 General methods

Reactions

Unless otherwise stated, all experiments were performed under room atmosphere and temperature. All reactions involving air- or/and moisture-sensitive reagents were carried out under nitrogen or argon atmosphere. The glassware was heated in vacum before use.

Solvent and reagents

All solvents were dried with conventional methods. Unless otherwise stated, all chemicals were obtained from commercial sources and used without further purification.

Thin layer and silica gel chromatography

Analytical thin layer chromatography (TLC) was performed using precoated silica gel 60 F-254 plates (layer thickness 0.25 mm) from Merk. Spots were visualized with (UV) irradiation at 254 nm or 366 nm or staining with $KMnO_4$. Preparative thin layer chromatography (Prep-TLC) was performed on *Merck* precoated silica gel 60 F-254 plates (layer thickness 1.00 mm) with concentration zone and visualization by UV irradiation at 254 nm or 366 nm. Flash column chromatography was performed on silica gel *Acros* (0.035-0.070 mm, pore diameter ca. 6 nm) with slight overpressure.

Ion exchange chromatography and HPLC

Ion exchange chromatography was performed with an AG®1-X2 resin (50-110 mesh, chloride-form) purchased from *BIO-RAD*. The resin is sold in the chloride form so it had to be converted to the OH^- form. The resin is loaded into a column and converted the resin to the OH^- form by washing it with 5 to10 bed volumes of 1 to 2 M sodium hydroxide. Subsequently, the resin is washed with distilled water until the pH is near neutral. Finally, the resin is eluted with 5 bed volumes of 1 to 2 M formic acid and rinse off the excess formic acid with distilled water.

For *reversed phase* HPLC applications, bidistilled water as well as commercially available HPLC-quality methanol were used as elution solvents (formic acid was added as additive in 0.1%v/v). Preparative HPLC was performed with a VARIAN HPLC system (pump prepstar model 218, variable wavelength detector Prostar (λ_{max} = 248 nm) equipped with a mass detector (MICROMASS ZMD ESI-Quad-Spectrometer) on a C-18 (TRENTEC, Reprosil-Pur 120

C18 AQ 5 µm, 250 mm * Ø 25 mm) HPLC column. The applied elution program and corresponding retentions time (t_R) were given in the experimental section.

NMR-spectroscopy

^{1}H-NMR spectra were recorded on a Bruker DPX-200 (200 MHz) and AM-400 (400 Hz) spectrometer. Chemical shifts are reported in ppm (parts per million) relative to internal tetramethylsilane (δ = 0.00), chloroform (δ = 7.26), Methanol (δ = 3.31), dimethylsulphoxide (δ = 2.50), acetone (δ = 2.05) and deuterium oxide (δ = 4.79). Coupling constants are given in Herz (Hz). Multipilicities are described by using the following abbreviations: s = singulet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

^{13}C-NMR spectra were recorded on a Bruker AM-400 (100 Hz) spectrometer. Chemical shifts are reported in ppm (parts per million) relative to internal standards such as chloroform (δ = 77.0), Methanol (δ = 49.0), dimethylsulphoxide (δ = 39.7), acetone (δ = 29.8). Multiplicities refer to the resonance in the off-resonance spectra and was elucidated using the distortionless enhancement by polarization transfer (DEPT) spectral editing technique, with secondary pulses at 90° and 135°. For the degree of substitution are described by using the following abbreviations: s = singlet (quaternary carbon), d = doublet (tertiary carbon), t = triplet (secondary carbon), q = quartet (primary carbon). Complete assignments of structures were performed employing a combination of homo- and heteronuclear correlation experiments (H, H-COSY, HMBC, and HSQC).

Mass spectrometry

Mass spectra were obtained on a micromass LCT with a Lock-Spray dualo ion source coupled with a Waters Alliance 2695 HPLC (injection in a “loop” mode without chromatographic separation). Alternatively measurement was carried out using an Acquity-UPLC (Column: BEH C18 1.7 µm, [SN 0473711315545], elution solvent A: water + 0.1%[v/v] formic acid; Elution solvent B: MeOH + 0.1%[v/v]; Flow rate: 0.4 mL/min; Gradient: {t[min]/B[%]}: {0/5}{2.5/95}{6.5/95}{6.6/5} {8/5}) coupled with a Q-Tof premier mass spectrometer.

Infraredspectroscopy

Infrared (IR) spectra were recorded with a Bruker vector 22 FT-IR spectrophotometer (Golden Gate ATR unit).

Polarimeter

Optical rotations were measured with a Perkin-Elmer model 341 polarimeter at 589 nm (sodium D-line) and given concentration in g per 100 mL.

Melting point

Melting points were determined with a SRS OptiMelt apparatus and were not corrected.

CHN-analysis

All combustion analyses (CHN) were measured on a Elementar Vario EL (Version F, Analyse System, Germany): Due to the hygroscopic properties of polySia and polySia based materials, the measured and theoretical carbon and nitrogen content differ as shown for commercial α-2,8-polySia (lyophilized sodium salt, 98% purity, obtained from Nacali Tesque, Inc., Japan):

$C_{measured}$ = 34.02% $C_{theorical}$ = 45.21%

$N_{measured}$ = 3.63% $N_{theorical}$ = 4.79%

The quotient C/N was used to draw conclusions from the measured data in this way, the water content of the polymer was not taken into account.

$C/N_{measured}$ = 9.36 $C/N_{theorical}$ = 9.44

The theoretical C/N quotient was corrected by the degree of derivatization measured by integration of ^{1}H-NMR spectra.

Dialysis

PolySia products were purified with a dialyse membrane Visking (Carl Roth, Germany, Karsruhe). Molecular weight cut off (MWCO) 14000 g/mol and diameter 21.5 mm.

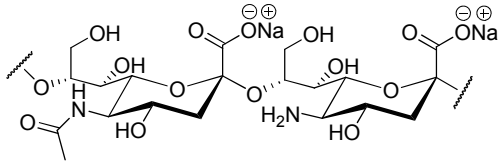
Microwave oven

Reactions under microwave irradiation conditions were performed with a microwave oven Discover (CEM, USA). The processes were controlled with software “synergy”.

5.2 Synthesis of polySia derivatives

5.2.1 Experiments towards azido- and alkyno-modified polySia

γ-Amino polySia (sodium salt) 36[89]



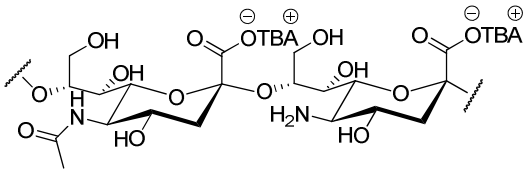
PolySia (sodium salt) **3** (3 g, 9.6 mmol) was dissolved in 200 mL solvent, which was a mixture of NaOH (7.5 g), H_2O (12 mL), and EtOH (188 mL). The reaction mixture was refluxed and stirred over night. Afterwards, the reaction mixture was neutralized with HCl (0.5 M). EtOH was evaporated under reduced pressure and the crude product was desalted by dialysis against distilled H_2O. The solution was lyophilized to afford γ-amino polySia (sodium salt) **36** as a colorless, amorphous solid (2.4 g, 8.3 mmol, 91%).

^{1}H-NMR (400 MHz, D_2O) δ: 4.20-4.08 (3H, H-4, H-6 & H-9_a), 3.75-3.64 (1H, H-8), 3.63-3.56 (1H, H-9_b), 3.55-3.45 (1H, H-7), 2.80 (0.92H, H-5), 2.63 (1H, H-3_{eq}), 2.09 (0.24H, NHCOC$\underline{H}_3$), 1.68 (1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 173.8 (s, C-1), 101.3 (s, C-2), 77.6 (d, C-8), 75.1 (d, C-6), 69.9 (d, C-7), 69.6 (d, C-4), 61.4 (t, C-9), 53.5 (d, C-5), 39.9 (t, C-3), 22.4 (q, NHCO$\underline{C}H_3$) ppm.

Degree of derivatization: 92% (According to integrations of H-5 at 2.80 ppm and NHCOC$\underline{H}_3$).

γ-Amino polySia (TBA salt) 37



γ-Amino polySia (sodium salt) **36** (2.3 g, 8.5 mmol) was dialyzed against an aqueous solution of TBA bromide (500 mL, 0.25 M) at room temperature for 24 h. To remove of excess TBA bromide, the product was dialyzed against demineralized H_2O (pH = 10, adjusted with TBAOH) at room temperature for 24 h. The aqueous layers were concentrated and lyophilized

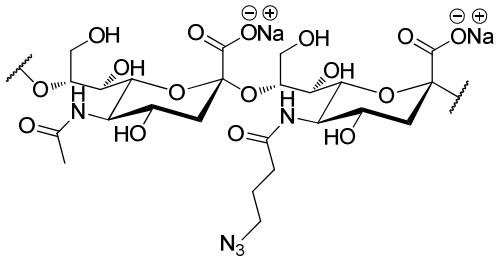
to afford γ-amino polySia (TBA) salt **37** as a colorless amorphous solid (3.3 g, 6.8 mmol, 80%).

^{1}H-NMR (400 MHz, D_2O) δ: 4.20-4.04 (3H, H-4, H-6 & H-9_a), 3.72-3.65 (1H, H-8), 3.64-3.58 (1H, H-9_b), 3.57-3.42 (1H, H-7), 3.18 (6.8H, $N^+(C\underline{H}_2CH_2CH_2CH_3)_4$), 2.85 (0.98H, H-5), 2.62 (1H, H-3_{eq}), 2.09 (0.06H, $NHCOC\underline{H}_3$), 1.68 (1H, H-3_{ax}), 1.63 (6.8H, $N^+(CH_2C\underline{H}_2CH_2CH_3)_4$), 1.34 (6.8H, $N^+(CH_2CH_2C\underline{H}_2CH_3)_4$), 0.93 (10.2H, $N^+(CH_2CH_2CH_2C\underline{H}_3)_4$) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 173.7 (s, C -1), 101.1 (s, C-2), 77.4 (d, C-8), 74.8 (d, C-6), 69.6 (d, C-7), 69.0 (d, C-4), 61.2 (t, C-9), 58.8 ($N^+(\underline{C}H_2CH_2CH_2CH_3)_4$), 53.5 (d, C-5), 39.4 (t, C-3), 23.1 (t, $N^+(CH_2\underline{C}H_2CH_2CH_3)_4$), 19.1 (t, $N^+(CH_2CH_2\underline{C}H_2CH_3)_4$), 12.8 (q, $N^+(CH_2CH_2CH_2\underline{C}H_3)_4$) ppm.

For free amino group (degree of derivatization): 98% (According to integrations of H-5 at 2.85 ppm and $NHCOC\underline{H}_3$); For TBA group (degree of derivatization): 85% (According to integrations of H_{TBA}).

***N*-(4-Azidobutyryl)-polySia 41**[89]



γ-Amino polySia (TBA salt) **37** (50 mg, 0.102 mmol) was dissolved in anhydrous pyridine (2 mL) and cooled to 0 °C. Subsequently, 4-azidobutyric chloride (**40**) (70 µL, 0.5 mmol), which was prepared according to known procedure was added and the reaction mixture was stirred at room temperature over night. To reacetylate the remaining free amino-groups the reaction mixture was cooled to 0 °C and acetic anhydride (1 mL) was added. After stirring at room temperature for 8 h, the solvent was evaporated under reduced pressure and treated with NaOH (3 mL, 1 M). After stirring for 8 h, the mixture was neutralized with HCl (1 M) and dialyzed against aqueous NaCl solution (0.5 M) for 2 days (pH = 9) and against distilled H_2O for 2 days. The aqueous layers were concentrated and lyophilized to give the product **41** as a colorless, amorphous solid (36 mg, 0.094 mmol, 92%).

^{1}H-NMR (400 MHz, D_2O) δ: 4.20-4.01 (2H, H-4 & H-6), 3.91-3.81 (2H, H-5 & H-9_a), 3.72-3.58 (3H, H-7, H-8 & H-9_b), 3.41 (1.86H, $NHCOCH_2CH_2C\underline{H}_2N_3$), 2.63 (1H, H-$3_{eq}$), 2.42

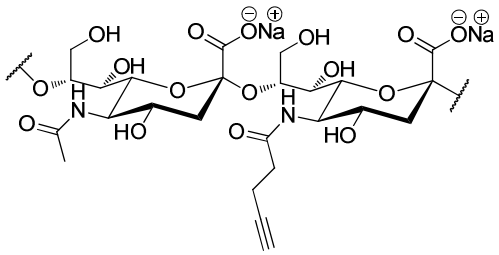
(1.86H, NHCOC$\underline{H}_2$CH$_2$CH$_2$N$_3$), 2.09 (0.21H, NHCOC$\underline{H}_3$), 1.91 (1.86H, NHCOCH$_2$C$\underline{H}_2$CH$_2$N$_3$), 1.75 (1H, H-3$_{ax}$) ppm.

^{13}C-NMR (100 MHz, D$_2$O) δ: 176.6 (s, NH$\underline{C}$OCH$_2$CH$_2$CH$_2$N$_3$), 173.2 (s, C-1), 101.1(s, C-2), 77.5 (d, C-8), 73.2 (d, C-6), 69.2 (d, C-7), 68.3 (d, C-4), 61.2 (t, C-9), 52.3 (d, C-5), 50.4 (t, NHCOCH$_2$CH$_2$$\underline{C}H_2N_3$), 40.0 (t, C-3), 33.12 (t, NHCO$\underline{C}$H$_2$CH$_2$CH$_2$N$_3$), 24.5 (t, NHCOCH$_2$$\underline{C}H_2CH_2N_3$) ppm.

Combustion analysis: C/N = 3.22 (theoretical: C/N = 2.98).

Degree of derivatization: 93% (According to integrations of C$\underline{H}_2$ at 3.41 ppm and NHCOC$\underline{H}_3$).

***N*-(4-Pentynoyl)-polySia 44** [89]



γ-Amino polySia (TBA salt) **37** (55 mg, 0.1 mmol) was dissolved in anhydrous pyridine (2 mL) and cooled to 0 °C. After addition of 4-pentynoic acid chloride (**43**) (55 µl, 0.5 mmol), which was prepared according to reported procedure, the reaction mixture was slowly warmed up to room temperature and stirred over night. The reacetylation and purification processes were carried out according to the synthesis of *N*-(4-azidobutyryl)-polySia **41**. The desired *N*-(4-pentynoyl)-polySia **44** was afforded as a colorless amorphous solid (20 mg, 0.056 mmol, 56%)

^{1}H-NMR (400 MHz, D$_2$O) δ: 4.20-4.05 (2H, H-4 & H-6), 3.91-3.78 (2H, H-5 & H-9$_a$), 3.72-3.52 (3H, H-7, H-8 & H-9$_b$), 2.65 (1H, H-3$_{eq}$), 2.50 (2.4H, 2xC$\underline{H}_2$), 2.40 (0.6H, CC$\underline{H}$), 2.09 (1.2H, NHCOC$\underline{H}_3$), 1.75 (1H, H-3$_{ax}$) ppm.

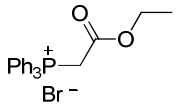
^{13}C-NMR (100 MHz, D$_2$O) δ: 175.4 (s, NH$\underline{C}$OCH$_2$CH$_2$CCH), 173.2 (s, C-1), 101.1(s, C-2), 84.0 (s, NHCOCH$_2$CH$_2$$\underline{C}$CH), 77.6 (d, C-8), 73.2 (d, C-6), 70.1 (d, NHCOCH$_2$CH$_2$C$\underline{C}$H), 69.2 (d, C-7), 68.3 (d, C-4), 61.2 (t, C-9), 52.3 (d, C-5), 40.0 (t, C-3), 34.6 (t, NHCO$\underline{C}$H$_2$CH$_2$CCH), 14.3 (t, NHCOCH$_2$$\underline{C}H_2$CCH) ppm.

Combustion analysis: C/N = 11.01 (theoretical: C/N = 11.44).

Degree of derivatization: 60% (According to integrations of C<u>H</u>$_2$ at 2.50 ppm and NHCOC<u>H</u>$_3$).

5.2.2 Experiments towards oxanorbornadienyl-modified polySia

Ethoxycarbonylmethyl-triphenyl-phosphonium bromide (46)



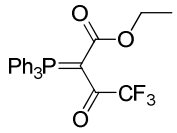
A mixture of bromoacetic acid ethyl ester **45** (28.5 mL, 256.2 mmol), triphenylphosphine (61.10 g, 232.94 mmol) and catalytic amounts of KI in anhydrous toluene (500 mL) was stirred at room temperature for 48 h. After filtration the residue was washed twice with toluene. The colorless solid was dried under reduced pressure to afford the desired product **46** (232 g, 100%).

^{1}H-NMR (400 Hz, $CDCl_3$) δ: 7.53-7.56 (m, 6H, Ar-<u>H</u>), 7.69-7.68 (m, 3H, Ar-H), 7.67-7.64 (m, 6H, Ar-H), 5.58 (d. *J* = 13.6 Hz, 2H, $Ph_3P^+C\underline{H}_2$), 4.02 (q, *J* = 7.16 Hz, $C\underline{H}_2CH_3$), 1.06 (t, *J* = 7.16 Hz, 3H, $C\underline{H}_3$) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 164.7 (s, <u>C</u>O), 135.3 (d, Ar-C), 134.2 (d, Ar-C), 130.3 (d, Ar-C), [118.7, 117.8] (d, Ar-C-P), 63.0 (t, $\underline{C}H_2$), [33.7-33.2] (d, $Ph_3P^+\underline{C}H_2$), 13.73 (q, $\underline{C}H_3$) ppm

Melting point: 152.0-154.5 °C.

Trifluoro-3-oxo-2-(triphenyl-phosphanylidene)-butyric acid ethyl ester (47)



Phosphonium salt **46** (20 g, 46.6 mmol) was suspended in anhydrous THF (50 mL) and cooled to 0 °C. Afterwards, the reaction mixture was treated with Et_3N (14.3 mL). After stirring for 15 min, trifluoroacetic anhydride (7.90 mL, 55.91 mmol) was added dropwise to the reaction mixture and stirring was continued for 1 h. The mixture was filtered and the residue was washed three times with cold THF. The filtrate was concentrated under reduced pressure to afford a yellow oily residue. Cold distilled H_2O (50 mL) was added to the residue and filtered to give the desired product **47** which was dried under reduced pressure (20.62 g, 46.0 mmol, quantitative).

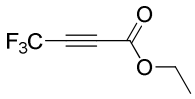
^{1}H-NMR (400 Hz, D_2O,) δ: 7.53-7.56 (m, 6H, Ar-H), 7.49-7.51 (m, 3H, Ar-H), 7.45-7.48 (m, 6H, Ar-H), 3.82 (q. *J* = 7.16 Hz, 2H, C<u>H</u>$_2$), 0.88 (t, *J* = 7.16 Hz, 3H, C<u>H</u>$_3$) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 165.9 (s, <u>C</u>O), 165.8 (s, <u>C</u>O), [132.6, 132.5, 129.1, 128.9] (<u>C</u>$_{ph}$), [124.8, 123.8] (d, P<u>C</u>COCF$_3$), [119.6, 119.5, 116.8, 116.6] (q, <u>C</u>F$_3$), 60.0 (t, <u>C</u>H$_2$), 13.73 (q, <u>C</u>H$_3$) ppm.

ESI: calcd for $C_{24}H_{21}O_3F_3P$ $[M+Na]^+$: 445.1180; found: 445.1185.

Melting point: 129-130 °C.

Trifluoro-butynoic acid ethyl ester (16)

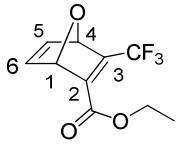


Phosphorane **47** (20.6 g, 46.36 mmol) was heated to 180-230 °C at 14 mbar for 40 min. The desired product **16** was collected at -78°C as a yellow oil (6.39 g, 38.48 mmol, 83%).

^{1}H-NMR (400 Hz, $CDCl_3$) δ: 4.32 (q. *J* = 7.16 Hz, 2H, C<u>H</u>$_2$), 1.35 (t, *J* = 7.16 Hz, 3H, C<u>H</u>$_3$) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 150.9 (s, <u>C</u>OCH$_2$CH$_3$), [117.3, 114.7, 112.1, 109.5] (q, <u>C</u>F$_3$), [75.6, 75.5] (d, <u>C</u>CCF$_3$), [70.2-69.6] (q, C<u>C</u>CF$_3$), 63.5 (t, <u>C</u>H$_2$), 13.97 (q, <u>C</u>H$_3$) ppm.

Oxanorbornadienyl ethyl ester 17



A mixture of trifluoro-butynoic acid ethyl ester **16** (2 g, 12 mmol) and furan (1.8 mL, 24 mmol) was heated under microwave irradiation conditions at 60 °C for 30 min. The resulting mixture was concentrated under reduced pressure. The crude mixture was purified by column chromatography (CH_2Cl_2) resulting in the desired product **17** as a slightly reddish oil (2.20 g, 9.36 mmol, 78%).

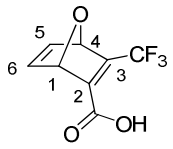
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.30 (dd, *J* = 5.3, 1.9 Hz, 1H, 6-H), 7.19 (dd, *J* = 5.3, 1.9 Hz, 1H, 5-H), 5.69 (m, 1H, 4-H), 5.65 (m, 1H, 1-H), 4.27 (m□2H, C<u>H</u>$_2$), 1.32 (t, *J* = 7.16 Hz, 3H, C<u>H</u>$_3$) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.9 (s, $\underline{C}O$), [151.8-151.2] (q, C-3), 151.16 (d, C-2), 144.0 (d, C-6), 142.8 (d, C-5), [125.7, 123.1, 120.5, 117.8] (q, $\underline{C}F_3$), 85.3 (d, C-1), 84.1 (d, C-4), 61.9 (t, $\underline{C}H_2$), 14.0 (q, $\underline{C}H_3$) ppm.

R_f = 0.63 (CH_2Cl_2).

ESI: calcd for $C_{10}H_{10}O_3F_3$ $[M+H]^+$: 234.0506; found: 234.0510.

Oxanorbornadienyl carboxylic acid 18



Oxanorbornadienyl ethyl ester **17** (2 g, 8.55 mmol) was dissolved in a mixture solvent of THF (15 mL) and water (100 mL). The reaction mixture was cooled to 0 °C. To the mixture was added NaOH (30 mL, 1 M) and stirred at 0 °C for 30 min, and stirring was continued at room temperature for 2 h. The reaction was tested by thin-layer chromatography. After complete conversion, EtOAc (20 mL) was added. The layers were separated and the aqueous layer was acidified to pH = 3 with HCl (1 M). The aqueous layers were extracted with MTB ether (3x30 mL). The organic layers were dried over $MgSO_4$ and evaporated to dryness. The desired oxanorbornadienyl carboxylic acid **18** was obtained as a colorless solid (1.6 g, 7.7 mmol, 90%).

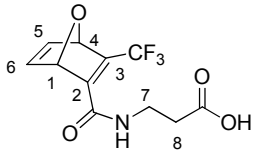
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.31 (dd, *J* = 5.1, 1.9 Hz, 1H, 6-H), 7.22 (dd, *J* = 5.1, 1.9 Hz, 1H, 5-H), 5.66 (s, 1H, 4-H), 5.55 (s, 1H, 1-H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 175.2 (s, $\underline{C}OOH$), 165.3 (s, NH$\underline{C}O$), 156. (s, C-2), 143.7 (s, C-3), 143.1 (d, C-6), 142.1 (d, C-5), [128.0, 125.5, 122.7, 119.4] (q, $\underline{C}F_3$), 87.3 (d, C-1), 84.6 (d, C-4) ppm.

ESI: calcd for $C_8H_4O_3F_3$ $[M-H]^-$: 205.0113; Found 205.0110.

Melting point: 107.5-109.5 °C.

Oxanorbornadienyl propanoic acid 52a



Oxanorbornadienyl carboxylic acid **18** (618 mg, 3 mmol), ß-alanine methyl ester hydrochloride (460.61 mg, 3.3 mmol) and DMAP (733.02 mg, 6 mmol) were dissolved in anhydrous CH_2Cl_2 (6 mL) and cooled to 0 °C. Subsequently, EDC·HCl (573.45 mg, 3.3 mmol) was added. After 30 min the reaction mixture was warmed up to room temperature and stirred for 16 h. The reaction was hydrolyzed with HCl (6 mL, 1 M) and the layers were separated. The organic layer was washed with saturated NH_4Cl solution as well as saturated NaOH solution. The aqueous layers were extracted with EtOAc (3x10 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH = 9:1) as a colorless solid. The saponification process was performed as described for the acid **18**. After work up the desired product **52a** was obtained as a colorless solid (432.4 mg, 1.56 mmol, 52% over 2 steps)

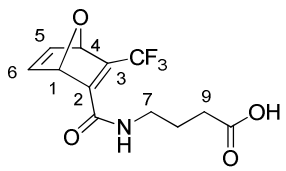
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.31 (dd, *J* = 5.1, 1.7 Hz, 1H, 6-H), 7.22 (dd, *J* = 5.1, 1.7 Hz, 1H, 5-H), 5.66 (s, 1H, 4-H), 5.55 (s, 1H, 1-H), 3.53 (m, 2H, 7-H), 2.55 (t, *J* = 6.8 Hz, 2H, 8-H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 175.2 (s, COOH), 165.3 (s, NHCO), 156. (s, C-2), 143.7 (s, C-3), 143.1 (d, C-6), 142.1 (d, C-5), [128.0, 125.5, 122.7, 119.4] (q, CF_3), 87.3 (d, C-1), 84.6 (d, C-4), 36.7 (t, C-7), 34.5 (t, C-8) ppm.

ESI: calcd for $C_{11}H_{10}O_4NF_3$ $[M-H]^-$: 276.0484; found 276.0490.

R_f = 0.48 (CH_2Cl_2/MeOH = 9:1).
Melting point: 145.5-149.0 °C.

Oxanorbornadienyl butanoic acid 52b



Preparation of methyl 4-aminobutanoate: 4-aminobutanoic acid (10.3 g, 0.1 mmol) was dissolved in anhydrous methanol (40 mL) and cooled to 0 °C. To the solution was added dropwise thionyl chloride (22 ml, 0.3 mol) over 15 min. The resulting solution was then

heated to reflux over night. The solvent and excess of thionyl chloride were then removed under reduce pressure. The solid product was used for the next step without further purification.

Oxanorbornadienyl butanoic acid **52b** was obtained from oxanorbornadienyl carboxylic acid **18** and methyl 4-aminobutanoate in 60% yield according to the procedure as described for **52a**.

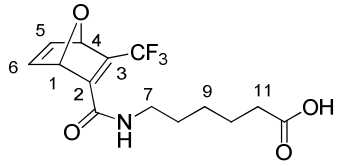
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.31 (dd, *J* = 5.1, 1.7 Hz, 1H, 6-H), 7.22 (dd, *J* = 5.1, 1.7 Hz, 1H, 5-H), 5.66 (s, 1H, 4-H), 5.55 (s, 1H, 1-H), 3.58 (m, 2H, 7-H), 2.55 (t, *J* = 6.8 Hz, 2H, 9-H), 2.04 (m, 2H, 8-H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.6 (s, COOH), 163.7 (s, NHCO), 154.1 (s, C-2), 143.7 (s, C-3), 143.1 (d, C-6), 142.1 (d, C-5), [126.4, 123.7, 121.1, 118.4] (q, CF_3), 86.0 (d, C-1), 83.6 (d, C-4), 35.1 (t, C-7), 32.9 (t, C-9), 25.5 (t, C-8) ppm.

ESI: calcd for $C_{12}H_{11}O_4NF_3$ $[M-H]^-$: 290.0640; found: 290.0641.

Melting point: 145.0-148.0 °C.

Oxanorbornadienyl hexanoic acid 52c

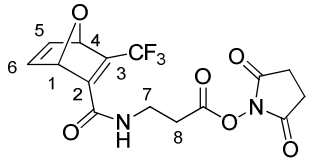


Oxanorbornadienyl hexanoic acid **52c** was obtained from oxanorbornadienyl carboxylic acid **18** and methyl 6-aminonate hydrochloride in 70% yield according to the procedure as is described for **52a**.

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.30 (dd, *J* = 5.3, 1.9 Hz, 1H, 6-H), 7.19 (dd, *J* = 5.3, 1.9 Hz, 1H, 5-H), 5.66 (m, 1H, 4-H), 5.55 (m, 1H, 1-H), 3.57 (m, 1H, 7-H), 2.61 (t, *J* = 7.2 Hz, 2H, 11-H), 1.77 (m, 2H, 10-H), 1.58 (m, 2H, 8-H), 1.47 (m, 2H, 9-H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 173.6 (s, COOH), 163.7 (s, CONH), [154.8-154.1] (d, C-2), 143.7 (d, C-6), 143.1 (q, C-3), 142.1 (d, C-5), [126.4, 123.7, 121.1, 118.4] (q, CF_3), 86.0 (d, C-1), 83.6 (d, C-4), 38.7 (t, C-7), 31.0 (t, C-11), 28.6 (t, C-8), 25.6 (t, C-9), 24.2 (t, C-10) ppm.

Oxanorbornadienyl NHS-ester 108e



Oxanorbornadienyl propanoic acid **52a** (1.28 g, 4 mmol) was dissolved in CH_2Cl_2, and cooled to 0 °C. DCC (0.92 g, 4.8 mmol), *N*-hydroxysuccinimide (0.55 g, 4.8 mmol) were added to the solution, respectively. The reaction mixture was stirred at 0 °C for 1 h and continued at room temperature for 24 h. After filtering of the precipitate, the solvent of the filtrate was removed under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/MeOH = 9:1) to give desired active oxanorbornadienyl NHS-ester **108e** (0.99 g, 2.4 mmol, 60%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.31 (dd, *J* = 5.3, 1.9 Hz, 1H, 6-H), 7.13 (dd, *J* = 5.3, 1.9 Hz, 1H, 5-H), 6.64 (b, N<u>H</u>CO), 5.63 (m, 2H, 4-H, 1-H), 3.75 (q, 2H, *J* = 6.2 Hz, 7-H), 2.91 (t, 2H, *J* = 6.2 Hz, 8-H), 2.85 (s, 4H, 2xC<u>H</u>$_{2NHS}$) ppm.

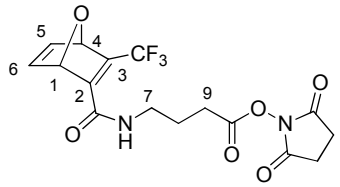
^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.1 (s, <u>C</u>O$_{NHS}$), 167.4 (s, <u>C</u>OON), 162.7 (s, NH<u>C</u>O), [153.7-153.8] (d, C-2), [144.1-143.8] (q, C-3), 143.7 (d, C-6), 142.1 (d, C-5), [126.4, 123.7, 121.1, 118.4] (q, <u>C</u>F$_3$), 86.0 (d, C-1), 83.6 (d, C-4), 35.1 (t, C-7), 32.9 (t, C-8), 25.7 (t, <u>C</u>H$_{2NHS}$) ppm.

R_f = 0.5 (CH_2Cl_2/MeOH = 9:1).

ESI: calcd for $C_{16}H_{16}N_2O_6F_3$ $[M+H]^+$: 375.0804; found: 375.0816.

Melting point: 131.5-134.0 °C.

Oxanorbornadienyl NHS-ester 108f



Oxanorbornadienyl NHS-ester **108f** was obtained from oxanorbornadienyl butanoic acid **52b** and *N*-hydroxyl succinimide in 65% yield according to the procedure as described for **108e**.

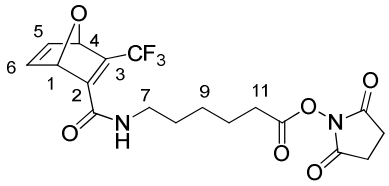
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.32 (dd, *J* = 5.1, 1.7 Hz, 1H, 6-H), 7.14 (dd, *J* = 5.1, 1.7 Hz, 1H, 5-H), 6.26 (b, N<u>H</u>CO), 5.63 (s, 1H, 4-H), 5.63 (s, 1H, 1-H), 3.49 (m, 2H, 7-H), 2.84 (s, 4H□2xC<u>H</u>$_{2NHS}$), 2.62 (t, *J* = 7.2 Hz, 2H, 9-H), 2.04 (m, 2H, 8-H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.1 (s, $\underline{C}O_{NHS}$), 168.4 (s, $\underline{C}OONH$), 162.9 (s, $NH\underline{C}O$), 154.4 (s, C-2), 143.7 (d, C-6) 143.1 (s, C-3), 142.1 (d, C-5), [126.4, 123.7, 121.1, 118.4] (q, $\underline{C}F_3$), 86.0 (d, C-1), 83.6 (d, C-4), 38.7 (t, C-7), 28.7 (t, C-9), 25.7 (t, $\underline{C}H_{2NHS}$), 24.5 (t, C-8) ppm.

ESI: calcd for $C_{16}H_{16}N_2O_6F_3$ $[M+H]^+$: 389.0960; found: 389.0951.

Melting point: 133.5-137.0 °C.

Oxanorbornadienyl NHS-ester 108g



Oxanorbornadienyl NHS-ester **108g** was obtained from oxanorbornadienyl hexanoic acid **52c** and *N*-hydroxyl succinimide in 56% yield according to the procedure as described for **108e**.

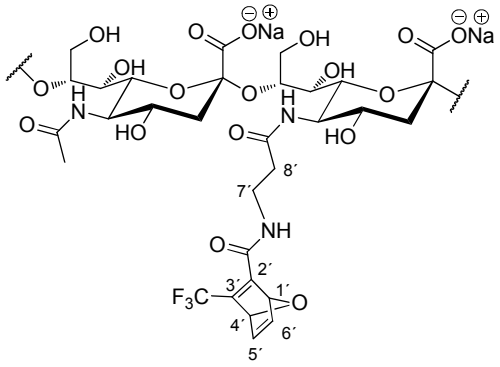
^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.32 (dd, *J* = 5.5, 2.1 Hz, 1H, 6-H), 7.12 (dd, *J* = 5.5, 2.1 Hz, 1H, 5-H), 6.13 (b, $N\underline{H}CO$), 5.62 (s, 1H, 4-H), 5.59 (s, 1H, 1-H), 3.36 (m, 2H, 7-H), 2.82 (s, 4H, 2x$C\underline{H}_{2NHS}$), 2.61 (t, *J* = 7.2 Hz, 2H, 11-H), 1.77 (m, 2H, 10-H), 1.58 (m, 2H, 8-H), 1.47 (m, 2H, 9-H) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 169.4 (s, $\underline{C}O_{NHS}$), 168.5 (s, $\underline{C}OON$), 162.4 (s, $NH\underline{C}O$), 154.4 (s, C-2), 143.8 (d, C-6), 143.1 (q, C-3), 141.9 (d, C-5), [126.4, 123.8, 121.1, 118.4] (q, $\underline{C}F_3$), 86.0 (d, C-1), 83.6 (d, C-4), 39.4 (t, C-7), 31.0 (t, C-11), 28.6 (t, C-8), 25.7 (t, $\underline{C}H_{2NHS}$), 25.6 (t, C-9), 24.2 (t, C-10) ppm.

ESI: calcd for $C_{18}H_{19}N_2O_6F_3Na$ $[M+Na]^+$: 439.1093; found 439.1111.

Melting point: 147.5-149.0 °C.

***N*-Oxanorbornadienyl-polySia 53a**



Method A:

γ-Amino polySia (sodium salt) **36** (500 mg, 1.84 mmol) was dissolved in demineralized H_2O (40 mL) and stirred at 5 °C. Subsequently, oxanorbornadienyl propanoic acid **52a** (2.6 g, 9.2 mmol), HOBt (1.24 g, 9.2 mmol), DIPEA (1.9 mL, 11.04 mmol) and EDC·HCl (1.76 g, 9.2 mmol) were added. Thereafter, the reaction mixture was stirred at room temperature for 24 h. To reacetylate the remaining free amino groups the reaction mixture was cooled to 0 °C and acetic anhydride (1 mL) was added. After stirring at room temperature for 8 h, the mixture was dried under reduced pressure and treated with NaOH solution (3 mL, 1 M). After stirring for 8 h, the mixture was neutralized with HCl (1 M) and dialyzed against aqueous NaCl solution (0.5 M) for 2 days (pH = 9) and against distilled H_2O for 2 days. The aqueous layers were concentrated and lyophilized to give the desired product **53a** as a colorless amorphous solid (470 mg, 1.29 mmol, 70%).

Methode B:

γ-Amino polySia (TBA salt) **37** (460 mg, 0.94 mmol) was dissolved in anhydrous DMF (3 mL) and cooled to 0 °C. Subsequently, NHS-ester **108e** (449.2 mg, 1.2 mmol) and DIPEA (261 µL, 1.5 mmol) was added. At first, the reaction mixture was stirred at room temperature for 24 h, and then the mixture was slowly warmed up to 40 °C for 8 h. The solvent was removed under reduced pressure. The residue was dissolved in water and dialyzed against aqueous NaCl (0.5 M) for 2 days and against distilled H_2O for 2 days, the aqueous layers were pooled and lyophilized giving a colorless solid.

To reacetylate the remaining amino groups, the residue was dissolved in Na_2CO_3 solution (40 mL, 5%w/v) and cooled to 0 °C. Subsequently, acetic anhydride (2 mL) was slowly added and the reaction mixture was stirred at room temperature for 4 h. Another portion of Na_2CO_3 solution (1.0 g) and acetic anhydride (2 mL) were added to the solution. After stirring under the same condition for 8 h, NaOH solution (10 mL, 1 M) was added to the mixture and stirred

for additional 8 h. The mixture was neutralized with HCl (1 M) and dialyzed against H_2O (pH = 9) for 2 day at room temperature. The aqueous layers were concentrated and lyophilized giving product **53a** as an amorphous solid (218 mg, 0.60 mmol, 65%).

^{1}H-NMR (400 MHz, D_2O) δ: 7.34 (0.25H, H-6´), 7.26 (0.25H, H-5´), 5.84 (0.25H, H-4´), 5.73 (0.25H, H-1´), 4.35-4.10 (2H, H-4 & H-6), 3.90-3.58 (5.5H, H-5, H-9_a, H-8, H-7, H-9_b, H-7´), 2.75-2.45 (1.5H, H-8´ & H-3_{eq}), 2.07 (1H, NHCOC$\underline{H}_3$), 1.75 (1H, H-3_{ax}) ppm.

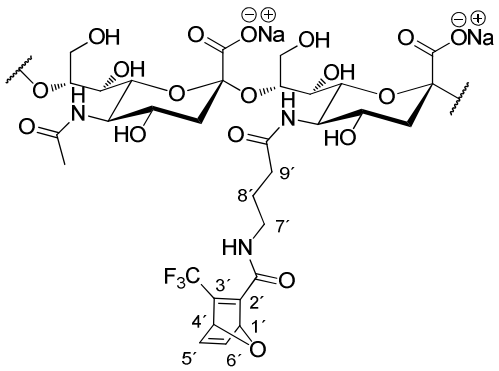
^{13}C-NMR (100 MHz, D_2O) δ: [174.9, 173.8, 173.2] (s, 4xC$\underline{O}$), 165.0 (s, C-1), 153.7 (q, C-2´), 143.7 (d, C-3´), 142.9 (d, C-6´), 142.8 (d, C-5´) [120.5–123.2] (q, $\underline{C}F_3$), 101.0 (s, C-2), 85.6 (d, C-1´), 83.0 (d, C-4´), 77.3 (d, C-8), 73.5 (d, C-6), 70.1 (d, C-7), 69.2 (d, C-4), 61.3 (t, C-9), 52.4 (d, C-5), 39.9 (t, C-3), 35.4 (t, C-7´), 34.7 (t, C-8´), 22.4 (d, NCO$\underline{C}H_3$) ppm.

Combustion analysis: C/N = 7.16 (theoretical: C/N = 8.40).

Degree of derivatization: 25% (According to integrations of C$\underline{H}$ at 7.34 ppm).

^{19}F-NMR δ: -63.37 ppm.

***N*-Oxanorbornadienyl-polySia 53b**



N-Oxanorbornadienyl-polySia **53b** was obtained from γ-amino polySia (TBA salt) **37** and oxanorbornadienyl NHS-ester **108f** according to the synthesis of *N*-oxanorbornadienyl-polySia **53a** using method B in 60 % yield.

^{1}H-NMR (400 MHz, D_2O) δ: 7.31 (0.25H, H-6´), 7.25 (0.25H, H-5´), 5.84 (0.25H, H-4´), 5.73 (0.25H, H-1´), 4.25-4.02 (2H, H-4 & H-6), 3.90-3.75 (4H, H-5, H-9_a),* 3.70-3.60 (4H, H-8, H-9_b, H-7, H-7´),* 2.75-2.45 (1.5H, H-8´ & H-3_{eq}), 2.07 (2H, NHCOC$\underline{H}_3$), 1.75 (1H, H-3_{ax}) ppm.

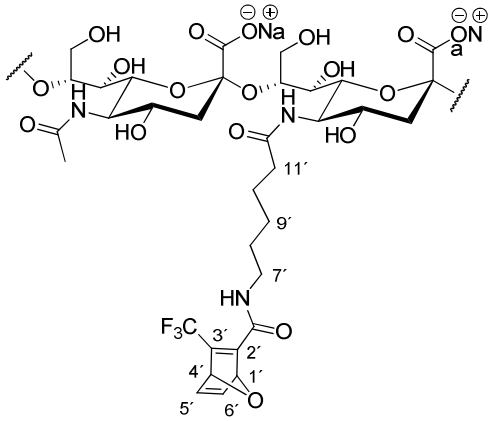
^{13}C-NMR (100 MHz, D_2O) δ: 176.8 (s, PSA-NH$\underline{C}$OR), 173.2 (s, NH$\underline{C}$O-R), 169.5 (s, N$\underline{C}$OCH$_3$), 164.9 (s, C-1), 153.8 (s, C-2´), 142.9 (q, C-6´), 140.6 (s, C-3´), 139.9 (d, C-5´), [120.5–123.2] (q, $\underline{C}F_3$), 101.1 (s, C-2), 85.6 (d, C-1´), 83.0 (d, C-4´), 77.6 (d, C-8), 73.1 (d,

C-6), 69.3 (d, C-7), 69.5 (d, C-4), 62.4 (t, C-9), 52.4 (d, C-5), 40.0 (t, C-3), 38.9 (t, C-7´), 28.7 (t, C-9´), 24.5 (t, C-8´), 22.4 (q, NHCO<u>C</u>H$_3$) ppm.

*EDC could not be completely removed.

Degree of derivatization: 25% (According to integrations of C<u>H</u> at 7.31 ppm).

N-Oxanorbornadienyl-polySia 53c



Synthesis of *N*-oxanorbornadienyl-polySia **53c** was performed according to the synthesis of **52a** using method B. However, after the reacetylation, it was found that the integrations of the special proton signals of the oxanorbornadienyl-system were reduced by NMR-measurement.

^{1}H-NMR (400 MHz, D_2O) δ: 7.35 (0.27H, H-6´), 7.34 (0.27H, H-5´), 5.84 (0.27H, H-4´), 5.71 (0.27H, H-1´), 4.20-4.05 (2H, H-4 & H-6), 3.90-3.78 (2H, H-5 & H-9$_a$), 3.70-3.51 (3H, H-8, H-7 & H-9$_b$), 3.33 (0.54H, H-7´), 2.68 (0.54H, H-11´), 2.34 (1H, H-3$_{eq}$), 2.07 (0.1H, NHCOC<u>H</u>$_3$), 1.73 (0.54H, H-10´), 1.62-1.55 (1.54H, H-3ax, H-8´)*, 1.32 (0.27H, H-9´) ppm. [*Before reacetylation*]

^{13}C-NMR (100 MHz, D_2O) δ: [177.8, 175.0, 173.2] (s, 3<u>C</u>O), 173.2 (s, N<u>C</u>OCH$_3$), 169.5 (s, C-1), 153.8 (s, C-2´), 145.8 (s, C-3´), 143.8 (d, C-6´), 142.0 (d, C-5´), [120.5–123.2] (q, <u>C</u>F$_3$), 101.1 (s, C-2), 85.6 (d, C-1´), 83.0 (d, C-4´), 77.3 (d, C-8), 73.5 (d, C-6), 70.1 (d, C-7), 69.2 (d, C-4), 61.3 (t, C-9), 52.4 (d, C-5), 39.6 (t, C-3), 39.4 (t, C-7´) , 35.8 (t, C-11´), 27.8 (t, C-8´), 25.5 (t, C-9´), 24.8 (t, C-11´) ppm. [*Before reacetylation*]

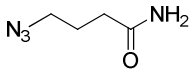
*Overlapped by polySia backbone.

Degree of derivatization: = 27% (According to integrations of C<u>H</u> at 7.35 ppm).

5.2.3 Experiments towards the synthesis of polySia hydrogels using "click" reaction

Model reaction of the Cu-catalyzed "click" chemistry

4-Azidobutanamide (54)

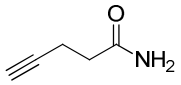


4-Azidobutyric chloride **40** (280 mg, 1.9 mmol) was dissolved in THF (1 mL) and cooled to -78 °C. Ammonia (0.8 mL, 25% in methanol) was added, and the reaction mixture was slowly warmed up to room temperature and stirred over night. After removing of the solvent the reaction mixture was dissolved in H_2O and extracted with EtOAc (3x10 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Recrystallization in CH_2Cl_2/hexane gave the desired product **54** as a colorless solid (205 mg, 1.60 mmol, 84%).

^{1}H-NMR (400 Hz, D_2O) δ: 3.36 (t, *J* = 6.8 Hz, 2H, C$\underline{H_2}N_3$), 2.37 (t, *J* = 7.5 Hz, 2H, C$\underline{H_2}$CO), 1.92 (m, 2H, C$\underline{H_2}CH_2$CO) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 178.9 (s, $\underline{C}$O), 50.4 (t, $\underline{C}H_2N_3$), 32.2 (t, $CH_2\underline{C}H_2$CO), 24.4 (t, $\underline{C}H_2CH_2$CO) ppm.
Melting point: 71.3-72.5°C.

Pent-4-ynamide (55)



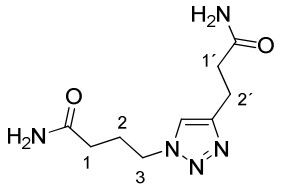
A solution of pentynoic acid (**42)** (2 g, 20 mmol) in anhydrous THF (50 mmL) was cooled to 0 °C. Et_3N (2.1 mL, 20 mmol) and isobutyl chloroformate (IBCF) (2.58 mL 20 mmol) were added subsequently. After the addition was completed, the resulting suspension was stirred at 0°C for 30 min. Afterwards, the reaction was treated with condensed ammonia (2.1 mL, 26% in water) and stirred at -78 °C for 1 h. The reaction mixture was warmed up to room temperature and stirred for additional 11 h (ammonia was evaporated). At the end of this period the resulting mixture was diluted with saturated NaCl solution (20 mL) and extracted with EtOAc (3x10 mL). The organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Then, the crude product was purified by column chromatography

(EE/PE = 1:1) to afford **55** as a colorless solid (0.42 g, 4.4 mmol, 22%).[1]*H-NMR data were identical with those given in literature.*[112]

^{1}H-NMR (200 Hz, $CDCl_3$) δ: 5.74 (b, N$\underline{H}_2$, 2H),) 2,51 (m, 4H, 2xC$\underline{H}_2$), 2.01 (t, *J* = 2.4 Hz, 1H, C$\underline{C}$H) ppm.

Melting point: 112.5-113.0 °C.

4-(4-(3-Amino-3-oxopropyl)-1H-1,2,3-triazol-1-yl)butanamide (56)



As a model reaction for the copper-catalyzed "click" reaction, 4-azidobutanamide (**54**) (128.12 mg, 1.0 mmol) and Pent-4-ynamide (**55**) (97 mg, 1.0 mmol) were dissolved in demineralized H_2O (1 mL). An aqueous $CuSO_4$ solution (100 µL, 0.1 mM) as well as aqueous Na-ascorbate solution (150 µL, 0.1 mM) were added and the reaction mixture was stirred at room temperature for 2 days. Then, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (CH_2Cl_2 = 5:1) resulting in the desired product **56** as a colorless solid (180 mg, 0.8 mmol, 80%).

^{1}H-NMR (400 Hz, D_2O) δ: 7.79 (s, 1H, C$\underline{H}_{triazole}$), 4.42 (t, *J* = 6.5 Hz, 2H, 3-H), 2.99 (t, *J* = 6.5 Hz, 2H, 2´-H), 2.62 (t, *J* = 7.5 Hz, 2H, 1´H), 2.22 (q, *J* = 7.5 Hz, 2H, 1-H), 2.17 (m, 2H, 2-H) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: [178.0, 177.9] (s, 2x$\underline{C}$O), 146.5 (s, $\underline{C}_{triazole}$), 123.3 (d, $\underline{C}_{triazole}$), 49.4 (t, C-3), 34.4 (t, C-1´), 31.5 (t, C-1), 25.4 (t, C-2´), 20.8 (t, C-2) ppm.

ESI: calcd for $C_9H_{15}O_2N_5Na$ $[M+Na]^+$: 248.1123; found: 248.1123.

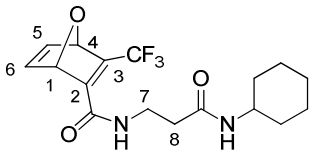
R_f = 0.57 (CH_2Cl_2 = 5:1).

Melting point: decomposition > 130 °C.

[112] P. A. Jacobi, H. L. Brielmann, S. I. Hauck, *J. Org. Chem.* 1996, 61, 5013-5023.

Model reaction of the metal-free "click" chemistry

Alkyno-analogue 57



NHS-ester **108e** (37.4 mg, 0.1 mmol) was dissolved in a mixture of dioxane/H_2O = 1:1 (1 mL) and stirred at room temperature over night. Subsequently, cyclohexylamine (12.57 µL, 0.11 mmol) and Et_3N (13.9 µL, 1.2 mmol) were added and stirring was continued at room temperature for 24 h. The aqueous layers were extracted with EtOAc (3x10 mL). The organic layer was dried over $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. Recrystallization from EE/PE (2:1) gave the desired product **57** as a colorless solid (35.6 mg, 0.9 mmol, 90%).

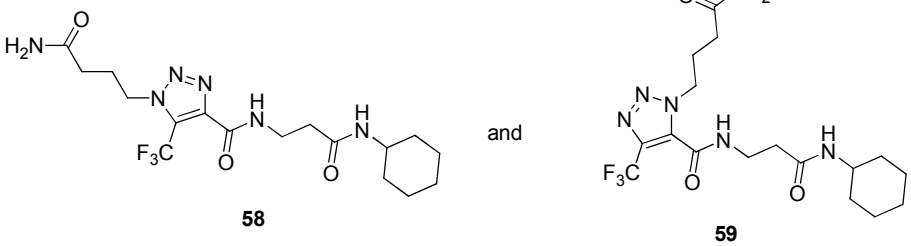
^{1}H-NMR (400 Hz, $CDCl_3$) δ: 7.30 (dd, *J* = 5.3, 2.1 Hz, 1H, 6-H), 7.19 (dd, *J* = 5.3, 2.1 Hz, 1H, 5-H), 6.8 (bs, 1H, N<u>H</u>), 5.63 (m, 1H, 4-H), 5.60 (m, 1H, 1-H), 5.33 (bs, 1H, N<u>H</u>), 3.76 (m, 1H, NC<u>H</u>$_{Cy}$), 3.6 (m, 2H, H-7), 2.38 (m, 2H, H-8), 1.08-2.40 (m, 10H, C<u>H</u>$_{2cy}$) ppm.

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.0 (s, <u>C</u>ON$_{cy}$), 163.7 (s, <u>C</u>O), [154.8-154.1] (q, C-2), 143.7 (d, C-6), 143.1(q, C-3), 142.1 (d, C-5), [126.4, 123.7, 121.1, 118.4] (q, <u>C</u>F$_3$), 86.2 (d, C-1), 83.7 (d, C-4), 48.5 (d, C-7), 33.3 (t, C-8), [35.7, 35.3, 25.6, 24.9] (C_{cy}) ppm.

ESI: cald for $C_{17}H_{21}O_3N_2F_3Na$ $[M+Na]^+$: 381.1402; found: 381.1397.

Melting point: 165.0-166.0 °C.

"Click" product: CF_3-1,2,3-triazole 58 and 59



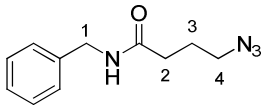
58 **59**

4-Azidobutanamide (**54**) (6.8 mg, 1 mmol) and alkyno-analogue **57** (36 mg, 1 mmol) were dissolved in MeOH/H_2O = 1:1 (200 µL). The reaction mixture was stirred at room temperature for 2 days. Then, the solvent was removed under reduced pressure and the crude product was purified by column chromatography (CH_2Cl_2/MeOH = 5:1). The "click" product was obtained as an inseparable mixture of regioisomers (**58**:**59** = 4:1, 23.5 mg, 0.8 mmol, 80%).

The reaction mixture was analyzed by ^{19}F-NMR: -58.03 ppm (**58**), -59.5 ppm (**59**), -61.3 ppm (**57**).

Melting point: 70.5-71.5 °C.

4-Azido-*N*-benzylbutanamide (60)



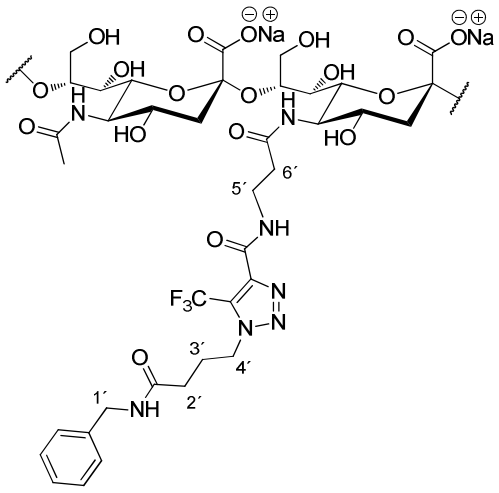
Benzylamine (0.5 ml, 5.0 mmol) and 4-azidobutanoic acid (**39**) (0.65 g, 5.0 mmol) were dissolved in anhydrous CH_2Cl_2 (20 mL) and cooled to 5 °C. Subsequently, DMAP (0.92 g, 7.5 mmol) and EDC·HCl (1.15 g, 6.0 mmol) were added to the reaction mixture were added. The reaction mixture was warmed up to room temperature and stirred for 24 h. The reaction mixture was hydrolyzed with HCl (10 mL, 1 M), layers were separated, and the organic layer was washed with saturated NH_4Cl solution and saturated NaCl solution. The combined aqueous layers were extracted with EtOAc (3x20 mL), dried over $MgSO_4$, filtrated, and the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography (CH_2Cl_2/MeOH = 5:1) to give the desired product **60** as a colorless solid (0.98 g, 4.5 mmol, 90%).

^{1}H-NMR (400 MHz, MeOD) δ: 7.23-7.28 (m, 5H, H_{ar}), 4.36 (s, 2H, H-1), 3.32 (t, *J* = 6.8 Hz, H-4), 2.32 (t, 2H, *J* = 6.8 Hz, H-4), 1.88 (m, 2H, H-3) ppm.

^{13}C-NMR (100 MHz, MeOD) δ: 174.8 (s, NH<u>C</u>O), 140.0 (s, Ar-C), 129.5 (d, Ar-C), 128.5 (d, Ar-C), 128.2 (d, Ar-C), 51.8 (t, C-4), 44.1 (t, C-1), 33.9 (t, C-2), 26.1 (t, C-3) ppm.
R_f = 0.3 (CH_2Cl_2/MeOH = 5:1).

ESI: calcd for $C_9H_{15}O_2N_5Na$ $[M+H]^+$: 219.1246; found: 219.1254.

Benzylbutanamido-polySia 61



N-Oxanorbornadienyl-polySia **53a** (40 mg, 0.11 mmol) was dissolved in demineralized H_2O (5 mL), and cooled to 0 °C. A solution of 4-azido-*N*-benzylbutanamide (**60**) (40 mg, excess) in MeOH (1 mL) was added and the reaction mixture was stirred at room temperature for 2 days. Afterwards, the reaction mixture was dialyzed against demineralized H_2O for 2 days. Product **61** was obtained after lyophilization as a colorless, amorphous solid (32 mg, ca. 80%).

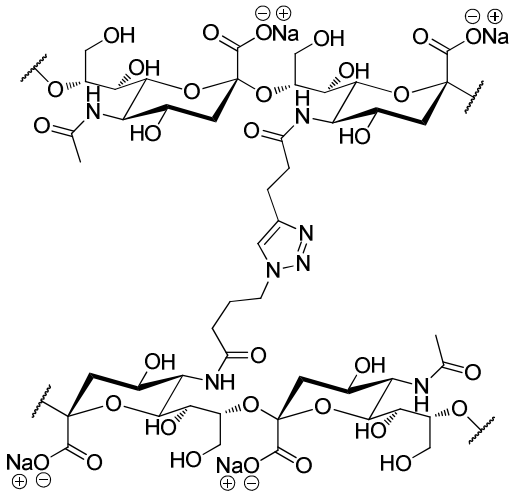
^{1}H-NMR (400 MHz, D_2O) δ: 7.71 (0.25H, Ar-H), 7.34 (1.0H, Ar-H), 4.43 (0.5H, 1´-H), 4.20-4.05 (2H, H-4 & H-6), 3.95-3.78 (2H, H-5 & H-9$_a$), 3.75-3.45 (3H, H-7, H-8 & H-9$_b$), 3.05-2.91 (0.5H, H-3´), 2.71-2.25 (2.5H, H-1´, H-5´, H-2´ & H-3$_{eq}$), 2.07 (1H, NHCOC<u>H</u>$_3$), 1.75 (1H, H-3$_{ax}$) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 175.4 (s, NH<u>C</u>O-R), 173.2 (s, NH<u>C</u>O-R), 173.2 (s, NH<u>C</u>OCH$_3$), 168.4 (s, C-1), 153.7 (q, <u>C</u>CF$_3$), 147.0 (s, C$_{triazole}$), 146.7 (s, C$_{triazole}$), [128.7, 127.9, 123.4] (d, Ar-C), 123.2 (q, <u>C</u>F$_3$), 101.1 (s, C-2), 77.3 (d, C-8), 73.5 (d, C-6), 70.1 (d, C-7), 69.3 (d, C-4), 61.3 (C-9), 52.4 (d, C-5), 49.4 (t, C-4´), 43.0 (t, C-1´), 39.9 (t, C-3), 35.1 (t, C-2´), 34.6 (t, C-5´), 32.3 (t, C-6´), 25.5 (t, C-3´), 20.5 (d, NHCO<u>C</u>H$_3$) ppm.

Degree of derivatization: 25% (According to integrations of C<u>H</u> at 7.71 ppm).

Hydrogel formation based of modified polySia using "click" chemisty

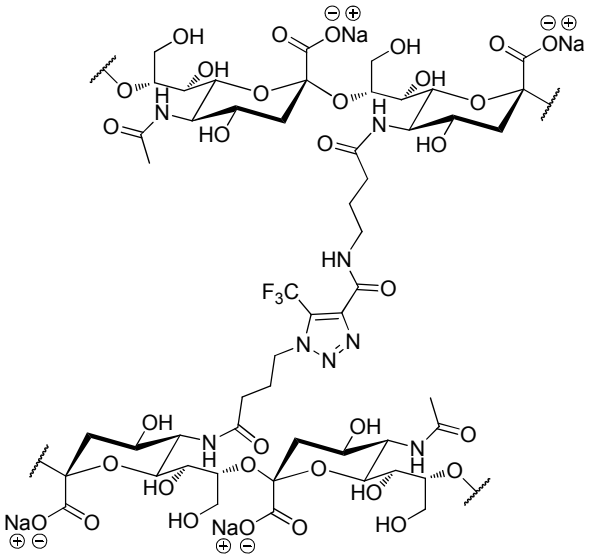
A) PolySia hydrogel formation using Cu-catalyzed "click" chemistry



N-(4-Azidobutyryl)-polySia **41** (10 mg, ds = 60% and *N*-(4-pentynoyl)-polySia **44** (10 mg, ds = 60%) were dissolved in demineralized H_2O (160 µl). An aqueous $CuSO_4$ solution (16 µL, 0.1 M) as well as a Na-ascorbate solution (24 µL, 0.1 M) were added. After vigorous shaking, the reaction mixture was kept at room temperature for 2-3 days. A stable hydrogel was formed.

In order to analyse the "click" reaction, the polySia hydrogel (20 mg) was hydrolyzed by immerging it in an acidic aqueous solvent (1.0 mL, 0.1 M TFA). The heterogeneous system was heated at 80 °C for 1 h and the sample was analyzed directly by QPLC-MS measurement (see chapter 3.1.1.4).

B) PolySia hydrogels formation using metal-free “click” chemistry

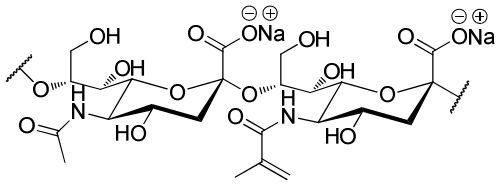


N-(4-Azidobutyryl)-polySia **41** (10 mg, ds = 25-60%) and *N*-oxanorbornadienyl-polySia **53b** (10 mg, ds = 20-25%) were dissolved in demineralized H_2O (200 µl). After short time vigorous shaking the reaction mixture was incubated at room temperature for 2-3 days. A stable hydrogel was formed.

For confirming the outcome of the “click” coupling reaction, the polySia hydrogel (20 mg) was immerged in an acidic aqueous solvent (1.0 mL, 0.1 M TFA) and heated at 80 °C for 1 h. The sample was analyzed directly by QPLC-MS measurement.

5.2.4 Experiments towards *N*-methacryl-polySia

_N_-methacryl-polySia 68



The γ-amino polySia (TBA salt) **37** (53.7 mg, 0.109 mmol) was dissolved in anhydrous pyridine (2 mL) and cooled to 0 °C. After dropwise addition of methacrylic anhydride (75.60 µL, 0.5 mmol) the mixture was slowly warmed up to room temperature and stirred over night. The reacetylation and purification processes were carried out according to the synthesis of *N*-(4-azidobutyryl)-polySia **41**. Product **68** was afforded as a colorless, amorphous solid (32.39 mg, 0.092 mmol, 92%).

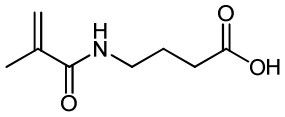
^{1}H-NMR (400 MHz, D_2O) δ: 5.80 (0.7H, $CH_3CC\underline{H}_{2cis}$), 5.50 (0.7H, $CH_3CC\underline{H}_{2trans}$), 4.21-4.05 (2H, H-4 & H-6), 4.04-3.95 (1H, H-5), 3.94-3.55 (4H, H-9_a, H-8, H-7 & H-9_b), 2.72 (1H, H-3_{eq}), 2.10 (0.9H, $NHCOC\underline{H}_3$), 1.97 (2.1H, $C\underline{H}_3CCH_2$), 1.81 (1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 175.0 (s, $NH\underline{C}OCH_3$), 172.9 (s, C-1), 138.9 (s, $CH_3\underline{C}CH_2$), 121.7 (t, $CH_3C\underline{C}H_2$), 101.0 (s, C-2), 77.1 (d, C-8), 72.5 (d, C-6), 69.9 (d, C-7), 68.7 (d, C-4), 61.2 (t, C-9), 53.5 (d, C-5), 39.9 (t, C-3), 22.3 (d, $NHCO\underline{C}H_3$), 17.9 (q, $\underline{C}H_3CCH_2$) ppm.

Combustion analysis: C/N = 10.50 (theoretical: C/N = 10.74).

Degree of derivatization: 70% (According to integrations of C$\underline{H}$ at 5.80 ppm).

4-Methacrylamidobutanoic acid (72)

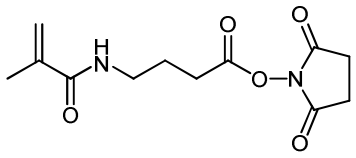


To a mixture of ω-amino butanoic acid (**71**) (4 g, 38.78 mmol) and sodium hydroxide (1.55 g, 38.78 mmol) in demineralized H_2O (2 mL), a small amounts of hydroquinone was added and cooled to -10 °C. Under stirring sodium hydroxide (1.55 g, 38.78 mmol) in demineralized H_2O (2 mL) and methacryloyl chloride (4.36 mL, 38.8 mmol) in dioxane (2 mL) was added over a period of 10 min. After stirring for 1 h at the same temperature the reaction mixture was acidified to pH = 3 with hydrochloride acid. The crude product was extracted with EtOAc and washed with distilled H_2O. The organic layer was concentrated under reduced pressure to

afford a faint purple oily product **72** (4.6 g, 26.8 mmol, 70%). *^{1}H-NMR data were identical with those given in literature.*[110b]

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.69 (m, 1 H, $CC\underline{H}_{2cis}$), 5.33 (m, 1H, $CC\underline{H}_{2trans}$), 3.38 (q, *J* = 6.9 Hz, 2H, $NHC\underline{H}_2$), 2.43 (t, *J* = 6.9 Hz, 2H, $C\underline{H}_2CO$), 1.94 (m, 3H, $C\underline{H}_3$), 1.89 (t, *J* = 6.9 Hz, 2H, $NHCH_2C\underline{H}_2$) ppm.

2,5-Dioxopyrrolidin-1-yl-4-methacrylamidobutanoate (73)

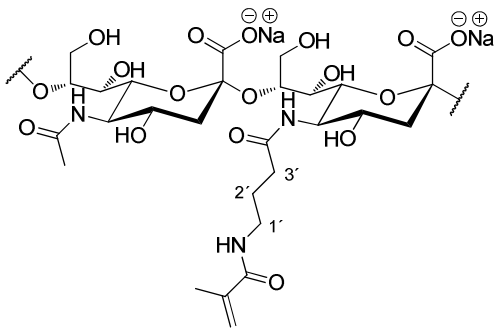


N-Hydroxysuccinimide (0.51 g, 3.3 mol) and 4-methacrylamidobutanoic acid (**72**) (0.38 g, 3 mmol) were dissolved in anhydrous THF (2 mL) at 5 °C. To the reaction mixture a solution of DCC (0.68 g, 3.3 mmol) in anhydrous CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was warmed up to room temperature and stirring continued for 12 h. Precipitated DCU was removed by filtration and the filtrate was evaporated to give an oily residue. The residue was diluted in acetonitrile (2 mL) and kept over night at 4 °C to remove additional DCU. After filtration the filtrate was concentrated to give a colorless oil. Recrystallization in CH_2Cl_2/hexane (1:2) at 4 °C gave the product **73** as a colorless solid (0.44 g, 1.64 mmol, 50%). *^{1}H-NMR data were identical with those given in literature.*[110b]

^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.68 (s, 1H, $COCCH_3C\underline{H}_{2cis}$), 5.32 (s, 1H, $COCCH_3C\underline{H}_{2trans}$), 3.41 (m, 2H, $CONHC\underline{H}_2$), 2.83 (s, 4H, $2xC\underline{H}_{2NHS}$), 2.63 (t, *J* = 6.9 Hz, 2H, $C\underline{H}_2CONHS$), 2.04 (m, 2H, $NHCH_2C\underline{H}_2CH_2CO$), 1.96 (t, 3H, $C\underline{H}_3$) ppm.

Melting point: 96-99 °C.

***N*-Methacryl-polySia 74**



γ-Amino polySia (sodium salt) **36** (330 mg, 0.82 mmol) was dissolved in 8 mL H_2O/dioxane (1:1) and cooled to 0 °C. To the mixture the NHS-ester **73** (375 mg, 1.4 mmol) was added and

the reaction mixture was stirred at room temperature over night. The reacetylation and purification processes were carried out according to the synthesis of *N*-(4-azidobutyryl)-polySia **41**. Product **74** was collected as a colorless solid (212 mg, 85%).

^{1}H-NMR (400 MHz, D_2O) δ: 5.61 (0.45H, CC$\underline{H}_{2cis}$), 5.38 (0.45H, CC$\underline{H}_{2trans}$), 4.18-3.95 (2H, H-4 & H-6), 3.82-3.71 (2H, H-5 & H-9_a), 3.61-3.45 (3H, H-8, H-7 & H-9_b), 3.25-3.15 (0.9H, H-1´), 2.65-2.55 (0.9H, H-3´), 2.35 (1H, H-3_{eq}), 2.06 (1.0H, NHCOC$\underline{H}_3$), 1.85 (1.35H, C$\underline{H}_3$CH_2), 1.80-1.71 (0.9H, H-2´), 1.65 (1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: [176.9, 175.0, 173.4, 173.1] (s, 4x$\underline{C}$O), 171.9 (C-1), 138.9 (s, $CH_3\underline{C}CH_2$), 120.9 (t, $CH_3C\underline{C}H_2$), 101.0 (s, C-2), 77.1 (d, C-8), 72.5 (d, C-6), 69.9 (d, C-7), 68.7 (d, C-4), 61.2 (t, C-9), 52.4 (d, C-5), 39.9 (t, C-3), 38.9 (t, C-1´), 33.2 (t, C-3´), 24.6 (t, C-4´), 22.3 (d, NHCO$\underline{C}H_3$), 17.9 (q, $\underline{C}H_3CCH_2$) ppm.

Combustion analysis: C/N = 8.22 (theoretical: C/N = 8.55).

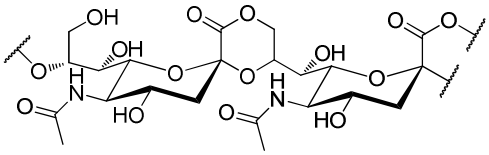
Degree of derivatization: 45% (According to integrations of C$\underline{H}$ at 5.61 ppm).

PolySia hydrogels formation using photopolymerization reaction

Photoinitiator Irgacure 2958 (20 mg) was dissolved in demineralized H_2O (1 mL) and warmed up to 60 °C for about 10 min. The photoinitiator solution (2%w/v) was freshly prepared to avoid inactivation by photo exposure. *N*-methacryl-polySia **74** (20 mg) was dissolved in photoinitiator solution (200 µL), and transferred into culture well silicone gasket. The polymerization was completed by UV-light irradiation at 320 nm for about 5 min. The obtained hydrogel was equilibrated in phosphate buffer solution (pH = 5.1) and could be stored for some weeks at room temperature.

5.2.5 Experiments towards the hydrazido- and aldehydo-modified polySia

PolySia lactone 75



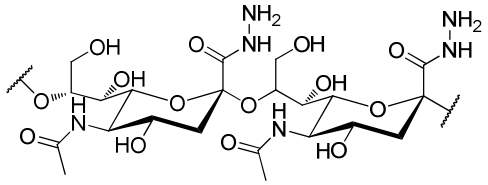
PolySia (sodium salt) **3** (0.5 g, 1.6 mmol) was dissolved in demineralized H_2O (20 mL) containing EDC·HCl (2.5 g, 13 mmol). After adjustment to pH = 4 with aqueous HCl (1 M) the solution was stirred at room temperature for 4 h. The yellow precipitate was filtrated and further purified by repeated ultrasonic-assisted suspension in a mixture of acetone (10 mL, twice) and CH_2Cl_2 (10 mL, seven cycles) followed by centrifugation and drying under reduced pressure to yield a colorless, amorphous solid **75** (401 mg, 1.44 mmol, 92%).

^{1}H-NMR: according to starting material **3**.

^{13}C-NMR (100 MHz, DMSO-d_6) δ: 172.7 (s, NH<u>C</u>OCH_3), 165.7 (s, C-1), 96.0 (s, C-2), 72.6 (d, C-8), 69.8 (d, C-6), 69.3 (d, C-7), 67.9 (d, C-4), 66.2 (t, C-9), 51.7 (d, C-5), 40.7 (t, C-3), 22.6 (q, NHCO<u>C</u>H_3) ppm.

Degree of derivatization: 100%.

PolySia hydrazide 76



PolySia lactone **75** (360 mg, 1.30 mmol) was suspended in demineralized H_2O (15 mL). After dropwise addition of hydrazine monohydrate (96 µL), the reaction mixture was stirred for 2 h at room temperature. The crude product was dialyzed against distilled H_2O for 2 d and lyophilized to yield a colorless, amorphous solid **76** (330 mg, 1.02 mmol, 78%).

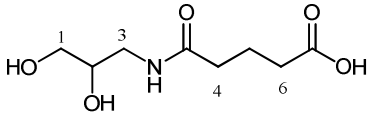
^{1}H-NMR: according to starting material **3**.

^{13}C NMR (100 MHz; D_2O) δ: 170.7(s, NH<u>C</u>OCH_3), 166.0 (s, C-1), 101.3 (s, C-2), 74.9 (d, C-8), 74.4 (d, C-6), 66.6 (d, C-7), 65.5 (d, C-4), 60.0 (t, C-9), 52.5 (d, C-5), 40.1 (t, C-3), 23.0 (q, NHCO<u>C</u>H_3).

Combustion analysis: C/N = 3.37 (theoretical: C/N = 3.14).

Degree of derivatization: 100%.

Preparation of 5-(2,3-dihydroxypropylamino)-5-oxopentanoic acid (80)



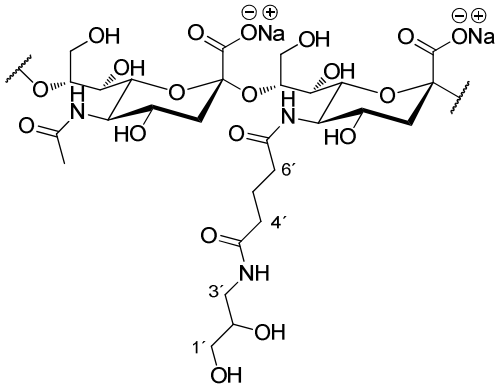
A solution of glutaric anhydride (1.60 g, 14 mmol) and 3-amino-1,2-propandiol (**77**) (1.41 g, 15 mmol) in anhydrous pyridine (10 mL) was stirred at room temperature for 12 h. The pyridine was removed under reduced pressure to give a colorless solution. The solution was diluted in H_2O (10 mL) and extracted with CH_2Cl_2 (5x10 mL) in order to eliminate the water insoluble compound. The aqueous layer was finally concentrated to yield product **80** as a colorless oil (2.34 g, 11.4 mmol, 82%).

^{1}H-NMR (400 MHz, D_2O) δ: 3.75-3.70 (m, 1H, H-2), 3.52 (dd, *J* = 12.0, 4.1 Hz, 1H, H-1_a), 3.43 (dd, *J* = 11.2, 6.5 Hz, 1H, H-3_a), 3.26 (dd, *J* = 14.0, 4.8 Hz, 1H, H-1_b), 3.43 (dd, *J* = 11.2, 7.2 Hz, 1H, H-3_b), 2.32 (t, *J* = 3.4 Hz, 2H, H-4), 2.24 (t, *J* = 7,5 Hz, 2H, H-5), 1.80 (tt, *J* = 7.5 Hz, 3.4 Hz, 2H, H-6) ppm

^{13}C-NMR (100 MHz, D_2O) δ: 178.5 (s, COOH), 176.1 (s, NHCO), 70.1 (t, C-2), 63.2 (t, C-1), 41.6 (t, C-3), 34.7 (t, C-4), 33.1 (t, C-6), 20.1 (t, C-5) ppm.

ESI: calcd for $C_8H_{14}O_5$ $[M-H]^-$: 204.0872; found: 204.0865.

***N*-(1,2-diol) polySia 81**



γ-Amino polySia (TBA salt) **37** (1.10 g, 2.2 mmol) was dissolved in anhydrous pyridine (20 mL) and cooled to 0 °C. Carboxylic acid **80** (861 mg, 4.2 mmol) and DIPEA (980 µL, 6.3 mmol) were added to the reaction mixture and stirred at room temperature over night. The solvent was removed under reduced pressure and the obtained residue was resolved in distilled H_2O and dialyzed against NaCl solution (0.5 M) (pH = 9) for 2 days and against distilled H_2O for 2 days at room temperature. The layers were concentrated and lyophilized to result a colorless amorphous solid.

The reacetylation was done according to **53b**. Product **81** was collected as a colorless amorphous solid (714.0 mg, 1.9 mmol, 87%).

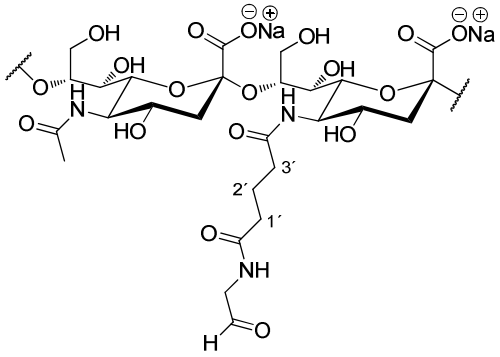
^{1}H-NMR (400 MHz, D_2O) δ: 4.21-4.05 (2H, H-4 & H-6), 3.90-3.71 (2H, H-5 & H-9_a), 3.70-3.42 (3H, H-8, H-7 & H-9_b) 3.48-3.58 (0.8H, H-$1'_a$ & H-$3'_a$)*, 3.34 (0.4H, H-$1'_b$), 3.22 (0.4H, H-$3'_b$), 2.66 (1H, H-3_{eq}), 2.33 (1.6H, H-4´ & H-6´), 2.07 (1.8H, NHCOC$\underline{H}_3$), 1.90 (0.8H, H-5´), 1.72 (1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: [176.6, 176.4, 174.9, 173.2] (s, 4xC$\underline{O}$), 100.9 (C-2), 77.8 (d, C-8), 73.1 (d, C-6), 70.2 (d, C-2´), 69.0 (d, C-7), 68.3 (d, C-4), 63.3 (t, C-1´), 61.2 (t, C-9), 52.4 (d, C-5), 41.7 (t, C-3´), 39.8 (t, C-3), 35.1 (t, C-4´), 34.9 (t, C-6´), 22.5 (q, NHCOC$\underline{H}_3$), 21.5 (t, C-5´) ppm.

*Overlapped by polySia backbone and H-2´ (3.75 ppm) was also overlapped by polySia backbone.

Degree of derivatization: 40% (According to integrations of C$\underline{H}$ at 3.34 ppm and NHCOC$\underline{H}_3$).

***N*-Aldehydo-polySia 82**



PolySia **81** (713 mg, 1.9 mmol) was dissolved in demineralized H_2O (10 mL) and cooled to 0 °C. An aqueous solution of $NaIO_4$ (5 g in 70 mL water) was added to the reaction mixture in the dark ([$NaIO_4$]: 0.3 M) and the reaction mixture was stirred at 5 °C for 15 min. To stop the reaction, ethylene glycol (25 mL) was added to the solution. The solution was dialyzed against distilled H_2O at room temperature for 4 days. After lyophilization a colorless, amorphous solid **82** was obtained (470 mg, 1.3 mmol, 68%).

^{1}H-NMR (400 MHz, D_2O) δ: 5.10 (0.4H, C$\underline{H}$$(OH)_2$)*, 4.25-4.09 (2H, H-4 & H-6), 3.95-3.70 (2H, H-5 & H-9_a), 3.69-3.51 (3H, H-8, H-7, H-9_b), 3.29 (0.8H, C$\underline{H}_2$CHO), 2.66 (1H, H-3_{eq}), 2.31 (1.6H, H-1´ & H-3´), 2.07 (1.8H, NHCOC$\underline{H}_3$), 1.90 (0.8H, H-2´), 1.73 (1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: [176.6, 176.4, 174.9, 173.0] (s, 4x<u>C</u>O), 100.9 (C-2), 88.2 (t, <u>C</u>H_2CHO), 77.7 (d, C-8), 73.1 (d, C-6), 69.0 (d, C-7), 68.2 (d, C-4), 61.2 (t, C-9), 52.4 (d, C-5), 39.8 (t, C-3), 35.0 (t, C-1´), 34.8 (t, C-3´), 22.4 (q, NHCOC<u>H</u>$_3$), 21.5 (t, C-2´) ppm.

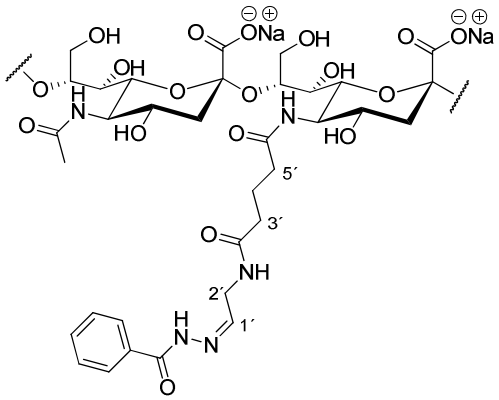
*Hydrated aldehyde in D_2O.

Combustion analysis: C/N = 8.91 (theoretical: C/N = 8.43).

Degree of derivatization: = 40% (According to integrations of C<u>H</u> at 5.10 ppm).

Model reaction: hydrazone and imine formation of *N*-aldehydo-polySia 82

Benzyhrazone-polySia 83



N-Aldehydo-polySia **82** (37 mg, 0.1 mmol) and benzhydrazide (13 mg, 0.1 mmol) were dissolved in demineralized H_2O (2 mL) and stirred at room temperature for 2 days. Then, the solution was dialyzed against distilled H_2O (pH = 9) at room temperature for 2 days. The aqueous layers were concentrated and lyophilized to yield a colorless, amorphous solid **83** (26 mg, 0.067 mmol, 67%).

^{1}H-NMR (400 MHz, D_2O) δ: 7.71-7.46 (1H, Ar-H), 4.99 (0.2H, C<u>H</u>$(OH)_2$)*, 4.25-4.09 (2H, H-4 & H-6), 3.91-3.70 (2.6H, H-5, H-9$_a$, H-1´ & H-2´), 3.69-3.51 (3H, H-8, H-7, H-9$_b$), 3.29 (0.4H, C<u>H</u>$_2$CHO)*, 2.57 (1H, H-3$_{eq}$), 2.24 (1.6H, H-3´ & H-5´), 1.98 (1.8H, NHCOC<u>H</u>$_3$), 1.82 (0.8H, H-4´), 1.64 (1H, H-3$_{ax}$) ppm.

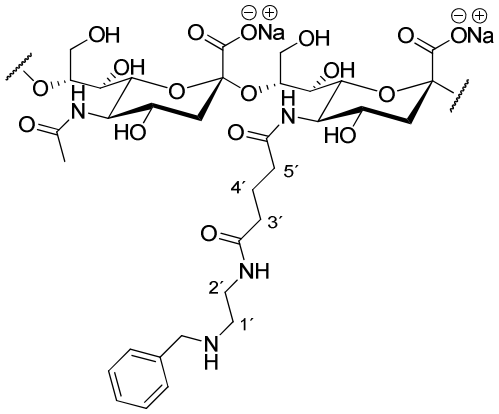
^{13}C-NMR (100 MHz, D_2O) δ: [176.6, 176.4, 174.9, 173.0] (s, 4x<u>C</u>O), 150.5 (d, C-1´), [132.8, 128.9, 128.8, 127.5] (Ar-C), 101.0 (s, C-2), 88.2 (t, <u>C</u>H_2CHO)*, 77.7 (d, C-8), 73.1 (d, C-6), 69.1 (d, C-7), 68.4 (d, C-4), 61.2 (t, C-9), 52.4 (d, C-5), 43.1 (t, C-2´), 39.9 (t, C-3), 35.0 (t, C-3´), 34.8 (t, C-5´), 22.4 (q, NHCOC<u>H</u>$_3$), 21.5 (t, C-4´) ppm.

* Belongs to non-reacted *N*-aldehydo-polySia **82**.

Combustion analysis: C/N = 7.22 (theoretical: C/N = 8.27).

Degree of derivatization: 20% of polySia **83** and 20% of non-reacted *N*-aldehydo-polySia **82** (According to integrations of CH at 4.99 and 3.29 ppm).

Benzylamino-polySia 89



N-Aldehydo-polySia **82** (37 mg, 0.1 mmol) was dissolved in demineralized H_2O (2 mL). Benzylamine (17 µL, 0.1 mmol) and a catalytic amount of $NaCNBH_3$ were added to the solution. The reaction was stirred at room temperature for 2 days. The mixture was dialyzed against distilled H_2O (pH = 9) at room temperature for 2 day. The aqueous layer was concentrated and lyophilized to yield a colorless, amorphous solid **89** (29 mg, 0.078 mmol, 78%).

^{1}H-NMR (400 MHz, D_2O) δ: 7.46 (2H, Ar-H), 4.15-3.95 (2.8H, H-4, H-6 & C$\underline{H}_2$Ph), 3.95-3.70 (2.8H, H-5, H-9$_a$ & H-2´), 3.69-3.51 (3.8H, H-8, H-7, H-9$_b$ & H-1´), 2.66 (1H, H-3$_{eq}$), 2.31 (1.6H, H-3´ & H-5´), 2.07 (1.8H, NHCOC$\underline{H}_3$), 1.90 (0.8H, H-4´), 1.72 (1H, H-3$_{ax}$) ppm.

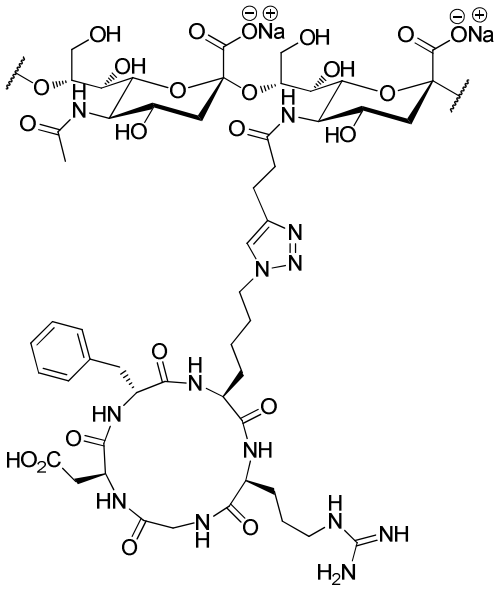
^{13}C-NMR (100 MHz, D_2O) δ: [176.6, 176.4, 174.9, 173.0] (s, 4xC$\underline{O}$), [129.8, 129.3, 129.2, 128.8] (Ar-C), 100.9 (C-2), 77.6 (d, C-8), 73.1 (d, C-6), 69.1 (d, C-7), 68.4 (d, C-4), 68.2 (t, C$\underline{H}_2$Ph), 68.0 (t, C-1´), 61.2 (t, C-9), 52.4 (d, C-5), 43.1 (t, C-2´), 39.8 (t, C-3), 35.0 (t, C-3´), 34.7 (t, C-5´), 22.4 (q, NHCOC$\underline{H}_3$), 21.5 (t, C-4´) ppm.

*Overlapped by polySia backbone.

Degree of derivatization: 40% (According to integrations of Ar-H at 7.46 ppm).

5.2.6 Experiments towards the synthesis of RGD peptide modified polySia

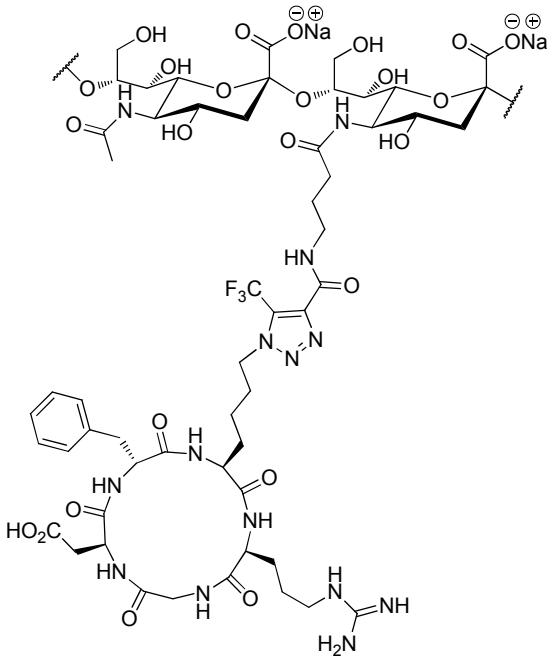
A) Preparation of RGD-polySia 85 via Cu-catalyzed "click" reaction



N-(4-Pentynoyl)-polySia **44** (5 mg, 0.016 mmol) and azido-RGD (2 mg, 0.003 mmol) were dissolved in demineralized H_2O (1 mL). Aqueous $CuSO_4$ solution (40 µL, 0.1 M) and Na-ascorbate solution (60 µL, 0.1 M) were added to the reaction mixture. The reaction was heated under microwave irradiation conditions up to 40 °C for 30 min. Subsequently, TFA (9.7 µL, 0.1 mmol) was added and the mixture was heated up to 80 °C for 1 h. The hydrolyzed products were directly analyzed by using UPLC-MS and LC-MS/MS.

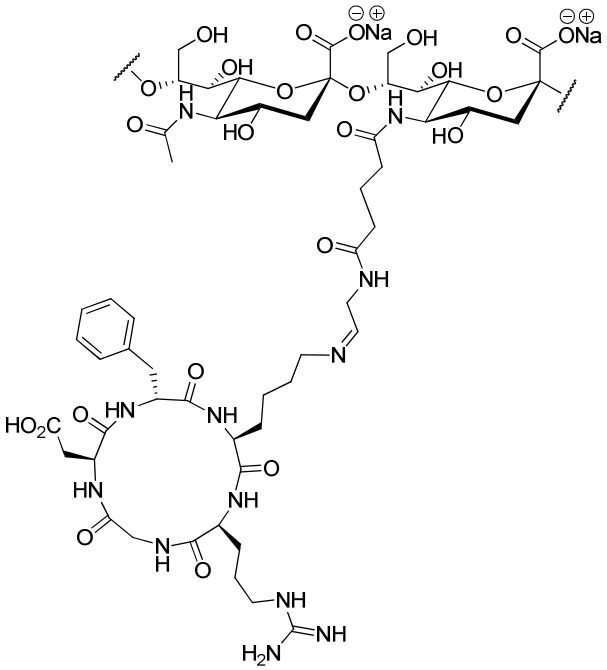
B) Preparation of RGD-polySia (87b) via metal-free “click” reaction

Metal-free “click” reaction mediate RGD-polySia 87b



N-Oxanorbornadienyl-polySia **53b** (5 mg, 0.015 mmol) and azido-RGD (2 mg, 0.003 mmol) were dissolved in demineralized H_2O (1 mL). The reaction was heated under microwave irradiation conditions up to 40 °C for 60 min. Subsequently, TFA (9.7 µL, 0.1 mmol) was added and the reaction mixture was heated up to 80 °C for 1 h. The hydrolyzed products were directly analyzed by using UPLC-MS and LC-MS/MS.

C) Preparation of RGD-polySia 90 via imine-bond formation



N-aldehydo-polySia **82** (26 mg, 0.07 mmol) was dissolved in demineralized H_2O (5 mL) and cooled to 0 °C. Amino-RGD peptide (5 mg, 0.008 mmol) and one drop of MeOH was added to give of better solubility. The mixture was stirred at room temperature for 24 h. The mixture were dialyzed against distilled H_2O at room temperature (pH = 9) for 2 days. The aqueous lays were concentrated and lyophilized to obtain product **90** as a colorless solid (24 mg).

Reduction of imine to amine, RGD-polySia **90** (2 mg) was dissolved in demineralized H_2O (1 mL). A catalytic amount of $NaCNBH_3$ was added to the solution. The reaction was stirred at room temperature over night. Subsequently, TFA (9.7 µL, 0.1 mmol) was added. The reaction was heated up to 80 °C for 1 h. The hydrolyzed products were directly analyzed by using UPLC-MS and LC-MS/MS.

5.3 Biological evaluation of polySia hydrogels

5.3.1 Experiments on the enzymatic degradation of polySia hydrogels

Enzyme degradation procedure

PolySia hydrogels were prepared via copper-catalyzed and metal-free "click" reaction as well as photopolymerization. Hydrogels (100 mg, dry material about 10 mg) were equilibrated in phosphate buffer (1 mL, pH = 7.2) at 37 °C for 3-4 h. The buffer solution was carefully removed and fresh buffer (400 µL) was added to the gel samples. Subsequently, 10 µL enzyme (EndoNF) were added and all samples were incubated at 37 °C in a period of 2-30 days. In order to analyse degradation process 20 µL samples were collected regularly. Cold EtOH (20 µL) was added to the collected samples to stop the enzyme reaction. Before analysis, the analytic samples were cooled down to -18 °C.

Analysis by electrophoresis

In order to analyse the enzymatic degradation of the polySia hydrogels, a polyarylamide gel electrophoresis (PAGE) method was used.

Buffers and solutions

a. 50 % acrylamide stock solution
- 49.6 g acrylamide
- 0.4 g bisacrylamide
- Filled with water to 100 mL

b. 10 fold TBE-buffer
- 0.98 M tris base
- 0.98 M boric acid
- 0.02 M EDTE

c. Size marker
- 0.05% trypan blue
- 0.02% xylenecyanol
- 0.02% bromophenol blue
- 0.02% phenol red
- 0.02% bromocresol purple

d. Loading buffer
- 2 M sucrose
- in 10 fold TBE-buffer

Preparation of 25% polyacrylamide gel: 50% acylamide stock solution (15 mL), 10-fold TBE-buffer (3 mL) and water (12 mL) were mixed well. To the mixture 125 µl of 10% ammonium persulphate solution and 25 µL of *N,N,N´,N´*-tetramethylethylenediamine were added. The mixture (15 mL) was subjected electrophoresis chamber. After complete polymerization, the gel was pre-electrophorized at 10 V/cm for 1 h at 4 °C. The samples were mixed with a solu-

tion of sucrose (2 M in TBE-buffer) and TBE-buffer (10-fold) in a ratio of 1:1. Electrophoresis was performed at 6 V/cm for 12-14 h at 4 °C. Then, the gel was dyed with an alcian blue silver staining method.

Alcian blue silver stain

Buffers and solutions

a. Fixing solution
40% ethanol
5% acetic acid

b. Periodic acid solution
0.7 g periodic acid
100 mM fixing solvent (a)

c. Silver nitrate solution
1.6 mL ammonia (25%)
1.0 mL sodium hydroxide (2 M)
Filled with water to 100 mL
Addition of silver nitrate (0.6 g)

d. Formaldehyde developer solution
100 mL 240 µM zitronic acid
135 µL formaldehyde (37%)

f. Dry solution
10% glycerine
20% ethanol

Alcian blue (0.5 g) was dissolved in distilled water (50 mL) under stirring at 40 °C for 15 min. After filtration, the gel was stained for 30 min and washed with distilled H_2O until polysaccharides were able to detect. Afterwards, the gel was treated with a fixing solution for 60 min, periodic acid solution for 5 min, and intense washing with distilled water for 3 times (3x15 min). The gel was incubated in silver nitrate solution for 10 min and washed with distilled waster 3 times (3x10 min). After treatment with formaldehyde developer solution for 1 to 3 min, the staining process was ended by addition a solution of 5% acetic acid in water. The dyed gel was treated with dry solution for 30 to 60 min, covered with cleophane film and dried at 45 °C.

5.3.2 Experiments on the cytotoxic evaluation of polySia hydrogels

Preparation of cell cultures (HepG2 cell line)

All cell culture procedures were done in a flow hood. Materials were autoclaved or sterilized by using isopropyl alcohol. Solutions and media were autoclaved or sterile filtered. Cells were cultured in dulbecco´s modified Eagles medium (DMEM) containing 10% fetal calf serum (FCS), Penicillin (100 U/ml) and Streptomyin (100 µg/mL) and were incubated at 37 °C with 5% CO_2 in a humidified atmosphere. First, hydrogels formed in 96 Well plates were sterilized

by exposure to UV-light for 1 h. Cells were then seeded on the polySia hydrogels (3000 cells /wells) and cultivated for 14 days in culture medium (200 µL).

MTT Assay

Samples were covered in MTT solution (100 µL, 50 mg/ml in distilled water) and incubated for 4 h at 37 °C and 5% CO_2 before adding SDS solution (90 µL, 5%w/v in 0.01 M HCl). After incubation over night at 37 °C and 5% CO_2 the samples were homogenized by pipetting. The samples (100 µL) were moved to a 96 well plate and absorption (570-630 nm) was measured on a plate reader.

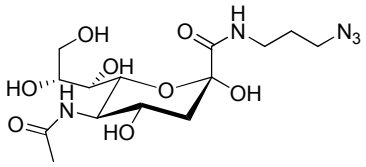
DAPI staining

Cells were fixed with ethanol (95%). Therefore, medium was removed from the samples, cells were rinsed twice with PBS-buffer and incubated in ice cold ethanol at 4 °C for 1 h. Afterwards, cells were rinsed twice with PBS-buffer, and the samples were covered in 4´,6-diamidino-2-phenyindole (DAPI) solution and incubated at 37 °C for 15 min in the dark before rinsed twice and covered in PBS-buffer. Cells were visualized under the microscope at a wavelength of 460 nm.

5.4 Synthesis of neuraminic aicd derivatives

5.4.1 Experiments towards the synthesis of neuraminic acid C-1 derivatives

C-1 Azido-neuraminic acid 97



N-Acetylneuraminic acid (100 mg, 0.32 mmol) was dissolved in anhydrous DMF (5 mL) and cooled to 0 °C. Subsequently, 3-azido-1-aminopropane (**95**) (50 mg, 0.5 mmol), EDC·HCl (96 mg, 0.5 mmol), and DMAP (62 mg 0.5 mmol) were added. The reaction was stirred at room temperature for 36 h. The solvent was removed under reduced pressure. The residue was dissolved in H_2O (5 mL) and the aqueous solution was washed with EtOAc (3x5 mL) to remove water insoluble compound. The product was obtained by *reversed phase* HPLC purification. (HPLC program: H_2O/MeOH (%) 100:0, 10 min, 50:50, 20 min, t_R = 9-10 min: α-anomer, t_R = 14-15 min: ß-anomer, flow rate 16 mL min^{-1}) (70 mg, 0.18 mmol, 56%).

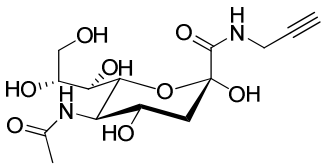
For ß-anomer:

^{1}H-NMR (400 MHz, D_2O) δ: 4.04-4.06 (m, 2H, H-4 & H-6), 3.90-3.93 (appt, *J* = 10.2 Hz, 1H, H-5), 3.81-85 (dd, *J* = 12.0, 2.7 Hz, 1H, H-9_a), 3.72-3.76 (dd, *J* = 12.0, 9.0 Hz, 1H, H-8), 3.64 (dd, *J* = 9.0, 2.7 Hz, 1H, H-9_b), 3.57 (dd, *J* = 12.0, 6.5 Hz, 1H, H-7), 3.37 (t, *J* = 6.8 Hz, 2H, $CH_2CH_2C\underline{H}_2N_3$), 3.31 (t, *J* = 6.8 Hz, 2H, $C\underline{H}_2CH_2CH_2N_3$), 2.31 (dd, *J* = 12.8, 4.8 Hz, 1H, H-3_{eq}), 2.04 (s, 3H, $NHCOC\underline{H}_3$), 1.81 (quintet, 2H, $CH_2C\underline{H}_2CH_2N_3$), 1.68 (dd, *J* = 12.8, 11.6 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 174.8 (s, $NH\underline{C}OCH_3$), 172.6 (s, C-1), 95.5 (s, C-2), 70.4 (d, C-6), 70.0 (d, C-8), 68.2 (d, C-7), 66.7 (d, C-4), 63.1 (t, C-9), 52.8 (d, C-5), 48.7 (t, $CH_2CH_2\underline{C}H_2N_3$), 39.5 (t, C-3), 36.7 (t, $\underline{C}H_2CH_2CH_2N_3$), 27.4 (t, $CH_2\underline{C}H_2CH_2N_3$), 22.0 (q, $NHCO\underline{C}H_3$) ppm.

ESI: calcd for $C_{14}H_{26}O_8N_5$ $[M+H]^+$: 392.1781; found: 392.1776.

C-1 Alkyno-neuraminic acid 98



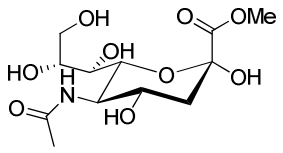
N-Acetylneuraminic acid (100 mg, 0.32 mmol) was dissolved in anhydrous DMF (5 mL) and cooled to 0 °C. Subsequently, propargyl amine (**86)** (21 mg 0.37 mmol), EDC·HCl (96 mg, 0.5 mmol), and DIPEA (62 mg 0.5 mmol) were added. The reaction was stirred at room temperature for 36 h. The solvent was removed under reduced pressure and the residue was dissolved in H_2O (5 mL). The aqueous solution was washed with EtOAc (3x5 mL) to remove water insoluble impurities. The product was obtained by *reversed phase* HPLC purification (HPLC program: H_2O/MeOH (%) 100:0, 5 min, 50:50, 20 min, t_R = 10 min: α-anomer, t_R = 14-17 min: ß-anomer, flow rate 16 mL min^{-1}) (65 mg, 0.19 mmol, 59%).

^{1}H-NMR (400 MHz, D_2O) δ: 4.04-4.07 (m, 2H, H-4 & H-6), 3.99-4.0 (m, 2H, C$\underline{H_2}$CCH), 3.90-3.93 (appt, *J* = 10.2 Hz, 1H, H-5), 3.81-3.85 (dd, *J* = 12.0, 2.7 Hz, 1H, H-9_a), 3.72-3.76 (m, 1H, H-8), 3.63 (dd, *J* = 12.0, 1.0 Hz, 1H, H-9_b), 3.57 (dd, *J* = 12.0, 6.5 Hz, 1H, H-7), 2.60 (s, 1H, CH_2CC$\underline{H}$), 2.32 (dd, *J* = 12.8, 4.8 Hz, 1H, H-3_{eq}), 2.04 (s, 3H, NHCOC$\underline{H_3}$), 1.68 (dd, *J* = 12.8, 11.3 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 174.8 (s, NH$\underline{C}$OCH_3), 172.5 (s, C-1), 95.4 (s, C-2), 79.1 (s, $CH_2\underline{C}$CH), 71.8 (d, $CH_2C\underline{C}$H), 70.4 (d, C-6), 70.0 (d, C-8), 68.2 (d, C-7), 66.7 (d, C-4), 63.1 (t, C-9), 52.0 (d, C-5), 39.5 (t, C-3), 28.7 (t, $\underline{C}H_2$CCH), 22.0 (q, NHCO$\underline{C}H_3$) ppm.

ESI: calcd for $C_{14}H_{23}O_2N_8$ $[M+H]^+$: 347.1298; found: 347.1311.

Neuraminic acid methyl ester 99



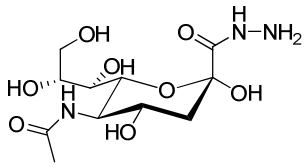
N-Acetylneuraminic acid **19** (1.6 g, 5.15 mmol) was dissolved anhydrous MeOH (100 mL) and stirred at room temperature. A catalytic amount of TFA (400 µL) was added and the reaction was stirred for 48 h under the same conditions. Following the solvent was removed under reduced pressure. After complete drying, product **99** was obtained as a colorles amorphous solid (1.48 g, 4.82 mmol, 94%).

^{1}H-NMR (400 MHz, CD_3OD) δ: 4.06-3.98 (m, 2H, H-4 & H-6), 3.83-3.79 (m, 2H, H-8, H-9_a), 3.82 (s, 3H, $COOCH_3$), 3.72-3.68 (m, H-8), 3.67 (dd, *J* = 12.0, 1.0 Hz, 1H, H-9_b), 3.52 (d, *J* = 12.9 Hz, 1H, H-7), 2.36 (dd, *J* = 12.8, 4.9 Hz, 1H, H-3_{eq}), 2.03(s, 3H, $NHCOCH_3$), 1.66 (dd, *J* = 12.8, 11.3 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, CD_3OD) δ: 175.1 (s, $NHCOCH_3$), 171.8 (s, C-1), 96.7 (s, C-2), 72.1 (d, C-6), 71.7 (d, C-8), 70.2 (d, C-7), 67.9 (d, C-4), 64.8 (t, C-9), 54.3 (d, C-5), 53.1 (q, $COOCH_3$), 40.7 (t, C-3), 22.6 (q, $NHCOCH_3$) ppm.

ESI: calcd for $C_{11}H_{22}N_3O_8$ $[M+Na]^+$: 324.1294; found: 324.1289.

C-1 Hydrazido-neuraminic acid 100



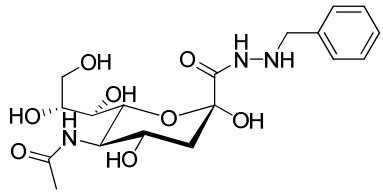
Neuraminic acid methyl ester **99** (300 mg, 1.07 mmol) was suspended in distilled H_2O (10 mL) and stirred at room temperature. Hydrazine monohydrate (88 µL, 65%, 1.18 mmol) was added to the suspension. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure. The desired product **100** was obtained as a colorless, amorphous solid (290 mg, 0.90 mmol, 84%)

^{1}H-NMR (400 MHz, D_2O) δ: 4.02 (m, 1H, H-4), 3.92-3.78 (m, 4H, H-8, H-5, H-9_a, H-6), 3.67 (dd, *J* = 12.0, 6.0 Hz, 1H, H-9_b), 3.52 (d, *J* = 12.9 Hz, 1H, H-7), 2.36 (dd, *J* = 12.8, 4.9 Hz, 1H, H-3_{eq}), 2.03 (s, 3H, $NHCOCH_3$), 1.66 (dd, *J* = 12.8 Hz, 11.3 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 173.8 (s, $NHCOCH_3$), 169.9 (s, C-1), 99.4 (s, C-2), 71. 3 (d, C-6), 70.4 (d, C-8), 69.2 (d, C-7), 66.7 (d, C-4), 60.3 (t, C-9), 52.8 (d, C-5), 40.6 (t, C-3), 21.8 (q, $NHCOCH_3$) ppm.

ESI: cald for $C_{11}H_{22}N_3O_8$ $[M+H]^+$: 324.1407; found: 324.1400.

C-1 Benzylhydrazido-neuraminic acid 101



Hydrazido-neuraminic acid **100** (100 mg, 0.31 mmol) was dissolved in distilled H_2O (5 mL) and stirred at room temperature. Benzaldehyde (35 µL, 0.34 mmol) was diluted in MeOH (1 mL) and slowly added to the reaction mixture. Subsequently, $NaCNBH_3$ (19.5 mg, 0.31 mmol) was added and the reaction was stirred under the same conditions for 16 h. The solvent was removed by evaporation under reduced pressure. The product was obtained by *reversed phase* HPLC purification (HPLC program: H_2O/MeOH (%) 100:0, 10 min, 70:30, 20 min, 50:50, 30 min, t_R = 50-55 min, a mixture of α and ß anomers, flow rate 16 mL min^{-1}). Two anomeric products were obtained at same time (10 mg, 0.02 mmol, 7 %, α/ß 2:1 from ^{1}H-NMR).

For ß anomer

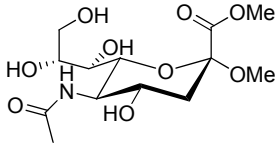
^{1}H-NMR (400 MHz, D_2O) δ: 7.78 (d, *J* = 6.2 Hz, 2H, Ar-H), 7,51 (3H, m, Ar-H), 4.02 (m, 1H, H-4), 3.92-3.78 (m, 4H, H-8, H-5, H-9_a & H-6), 3.67 (dd, *J* = 12.0, 6.0 Hz, 1H, H-9_b), 3.60 (m, 2H, C$\underline{H}_2$Ph) 3.52 (d, *J* = 12.9 Hz, 1H, H-7), 2.42 (dd, *J* = 12.8, 4.9 Hz, 1H, H-3_{eq}), 2.03(s, 3H, NHCOC$\underline{H}_3$), 1.79 (dd, *J* = 12.8 Hz, 11.3 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 174.9 (s, NH$\underline{C}$OCH_3), 169.1 (s, C-1), [132.7, 131.5, 129.0, 127.8] (Ar-C), 95.5 (s, C-2), 70.6 (d, C-6), 70.3 (d, C-8), 70.1 (d, C-7), 70.0 (t, NHNH$\underline{C}H_2$Ph), 68.1 (d, C-4), 66.6 (t, C-9), 63.2 ($\underline{C}H_2$Ar), 52.1 (d, C-5), 39.7 (t, C-3), 22.0 (q, NHCO$\underline{C}H_3$) ppm.

ESI: calcd for $C_{18}H_{27}N_3O_8Na$ $[M+Na]^+$: 436.1696; found: 436.1685.

5.4.2 Experiments towards the synthesis of neuraminic acid *N*-derivatives

Methoxyneuraminic acid methyl ester 105



An ion exchange resin Amberlite IR-120 (H^+-form) was applied for preparation of methylglycoside. At first, the resin was pre-treated by washing with MeOH and H_2O until the filtrate became clear. After completely drying the resin could be used for the reaction.

Dried neuraminic acid **19** (1 g, 3.2 mmol) was dissolved in anhydrous MeOH, which contained fresh pre-treated Amberlite IR-120 (H^+-form,1.7 g) and orthoformic acid methyl ester (0.71 mL, 6.4 mmol). The reaction was stirred and refluxed for 24 h. After cooling to room temperature, the resin was filtrated and washed with MeOH. The solvent was removed under reduce pressure. The crude product was purified by column chromatography (CH_2Cl_2/MeOH = 4:1). Both anomers were isolated whereas the major product was obtained as was ß-anomer (0.86 g, 2.56 mmol, 80%).

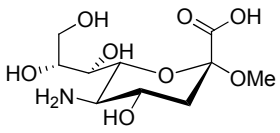
^{1}H-NMR (400 MHz, CD_3OD) δ: 4.03-3.97 (m, 1H, H-4), 3.92-3.78 (m, 4H, H-8, H-5, H-9_a & H-6), 3.82 (s, 3H, COOC$\underline{H}_3$), 3.67 (dd, *J* = 12.0, 6.0 Hz, 1H, H-9_b), 3.49 (d, *J* = 12.9 Hz, 1H, H-7), 3.28 (s, 3H, OC$\underline{H}_3$), 2.36 (dd, *J* = 12.8, 4.9 Hz, 1H, H-3_{eq}), 2.03(s, 3H, NHCOC$\underline{H}_3$), 1.66 (dd, *J* = 12.8, 11.5 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, CD_3OD) δ: 174.8 (s, NH$\underline{C}$OCH_3), 170.9 (s, C-1), 100.4 (s, C-2), 72.3 (d, C-6), 71.4 (d, C-8), 70.1 (d, C-7), 66.7 (d, C-4), 65.3 (t, C-9), 53.8 (d, C-5), 53.1 (q, COO$\underline{C}H_3$), 51.6 (q, O$\underline{C}H_3$), 41.6 (t, C-3), 22.7 (q, NHCO$\underline{C}H_3$) ppm.

R_f = 0.2 for ß-anomer (CH_2Cl_2/MeOH = 4:1).

ESI: calcd for $C_{13}H_{24}O_9N$ $[M+H]^+$: 338.1451; found: 338.1445.

***N*-Amino methoxyneuraminic acid 106**



Methoxyneuraminic acid methyl ester **105** (1.6 g, 4.7 mmol) was dissolved in a solvent containing NaOH (7.5 g), demineralized H_2O (12 mL) and EtOH (188 mL) and heated under re-

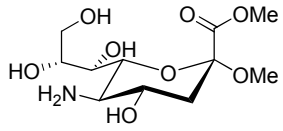
fluxing conditions over 24 h. The reaction was allowed to cool to room temperature. The reaction mixture was acidified (pH = 3) with Amberlyst 15 (H^+-form), filtered and the filtrate was concentrated and lyophilized. The crude product was recrystallized from MeOH/CH_2Cl_2 and afforded a colorless amorphous solid (0.8 g, 2.8 mmol, 60%)

^{1}H-NMR (400 MHz, CD_3OD) δ: 4.15 (dt, *J* = 10.5, 5.0 Hz, 1H, H-4), 4.10 (d, *J* = 10.2 Hz, 1H, H-6), 3.99-3.95 (m, 1H, H-9_a), 3.86 (dt, *J* = 12.1, 2.6 Hz, 1H, H-8), 3.75(d, *J* = 5.1 Hz. 1H, H-9_b), 3.68 (d, *J* = 8.5 Hz, 1H, C-7), 3.29 (t, *J* = 10.5 Hz, 1H, H-5), 3.18 (s, 3H, OCH_3), 2.38 (dd, *J* = 13.1, 5.0 Hz, 1H, H-3_{eq}), 1.68 (dd, *J* = 13.1, 11.5 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, CD_3OD) δ: 174.6 (s, C-1), 100.3 (s, C-2), 69.6 (d, C-6), 68.7 (d, C-8), 67.9 (d, C-7), 65.12 (d, C-4), 63.1 (t, C-9), 52.5 (d, C-5), 50.7 (q, O<u>C</u>H_3), 39.8 (t, C-3) ppm.

ESI: calcd for $C_{10}H_{18}O_8N$ $[M-H]^-$: 280.1032; found: 280.1044.

N-Amino methoxyneuraminic acid methyl ester 107



N-Acetylneraminic acid **19** (241 mg, 0.75 mmol) was dissolved in a solution of a freshly prepared solution of acetyl chloride (1.2 mL) in anhydrous MeOH (15 mL) at 0 °C and then heated in a sealed tube at 100 °C for 3 h. The solution was concentrated and the solvent was evaporated to afford the crude product. The crude product was used without further purification. For analyzing the sample, the brown crude product was dissolved in distilled H_2O and added a little active coal to eliminate colored substances. After filtration through Celite^{TM}, the aqueous solution was lyophilized to obtain a colorless solid. The solid was dissolved in a small amount of MeOH and then precipitated in Et_2O. Product **107** was obtained as a colorless solid (150 mg, 0.51 mmol, 68%).

^{1}H-NMR (400 MHz, D_2O) δ: 4.14-4.16 (m, 2H, H-4 & H-6), 3.92-3.95 (appt, *J* = 10.2 Hz, 1H, H-9_a), 3.83 (s, 3H, CO_2C<u>H_3</u>), 3.81-3.85 (dd, *J* = 12.0, 2.7 Hz, 1H, H-8), 3.76 (d, *J* = 10.2 Hz, 1H, H-9_b), 3.63 (dd, *J* = 9.0, 1.0 Hz, 1H, H-7), 3.35 (dd, *J* = 12.0, 6.5 Hz, 1H, H-5), 3.31 (s, 3H, OC<u>H_3</u>), 2.43 (dd, *J* = 12.8, 4.8 Hz, 1H, H-3_{eq}), 1.82 (dd, *J* = 12.8, 11.6 Hz, 1H, H-3_{ax}) ppm.

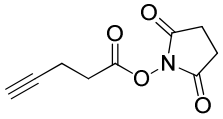
^{13}C-NMR (100 MHz, D_2O) δ: 169.8 (s, C-1), 99.1 (s, C-2), 69.5 (d, C-6), 69.1 (d, C-8), 67.6 (d, C-7), 64.6 (d, C-4), 62.9 (t, C-9), 53.6 (q, COO<u>C</u>H_3) 52.1 (d, C-5), 51.3 (q, O<u>C</u>H_3), 39.2 (t, C-3) ppm.

ESI: calcd for $C_{11}H_{22}O_8N$ $[M+H]^+$: 296.1345; found: 296.1336.

<u>Synthesis of NHS-esters 108a-c</u>

The corresponding carboxylic acid was dissolved in anhydrous THF (or CH_2Cl_2) and cooled to 0 °C. Peptide coupling reagent DCC (1.1 eq.) and *N*-hydroxysuccinimide (1.1 eq.) were added to the resulting solution. The reaction mixture was stirred at room temperature over-night and was filtered to separate precipitated the DCU. In order to remove additional DCU the filtrate was evaporated to give a residue, which was diluted with EtOAc.

4-Pentynoyl NHS-ester 108a



4-Pentynoyl NHS-ester **108a** was obtained from 4-pentynoic acid (**42**) and *N*-hydroxysuccinimide as a colorless solid in a yield of 80%.

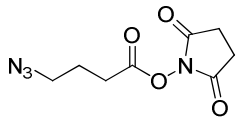
^{1}H-NMR (400 MHz, CD_3Cl) δ: 2.89 (t, 2H, *J* = 7.0 Hz, C<u>H</u>$_2$CH_2CCH), 2.84 (s, 4H, COC<u>H</u>$_2$C<u>H</u>$_2$CO), 2.61 (dt, 2H, *J* = 7.0, 2.7 Hz, CH_2C<u>H</u>$_2$CCH), 2.05 (t, *J* = 2.7 Hz, CH_2CH_2C<u>C</u>H) ppm.

^{13}C-NMR (100 MHz, CD_3Cl) δ: 169.1(s, <u>C</u>OCH_2CH_2<u>C</u>O), 167.2 (s, <u>C</u>OCH_2CH_2CCH), 81.0 (s, CH_2CH_2<u>C</u>CH), 70.2 (d, CH_2CH_2C<u>C</u>H), 30.4 (t, <u>C</u>H_2CH_2CCH), 25.7 (t, CO<u>C</u>H_2<u>C</u>H_2CO), 14.2 (t, CH_2<u>C</u>H_2CCH) ppm.

ESI: calcd for $C_9H_{10}O_4N$ $[M+H]^+$:196.0610; found: 196.0621.

Melting point: 80.3-81.5 °C

4-Azidobutanoyl NHS-ester 108b



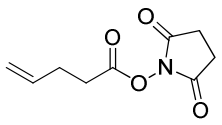
4-Azidobutanoyl NHS-ester **108b** was obtained from 4-azidobutyric acid (**39**) and *N*-hydroxysuccinimide as a colorless oil in a yield of 60%.

^{1}H-NMR (400 MHz, CD_3Cl) δ: 3.42 (t, *J* = 6.5 Hz, 2H, $CH_2CH_2C\underline{H}_2N_3$), 2.48 (s, 4H, $COC\underline{H}_2C\underline{H}_2CO$), 2.70 (t, *J* = 7.2 Hz, 2H, $C\underline{H}_2CH_2CH_2N_3$), 1.98 (tt, *J* = 6.5, 7.2 Hz, 2H, $CH_2C\underline{H}_2CH_2N_3$) ppm.

^{13}C-NMR (100 MHz, CD_3Cl) δ: 169.3 (s, $\underline{C}OCH_2CH_2\underline{C}O$), 168.0 (s, $\underline{C}OCH_2CH_2CH_2N_3$), 50.0 (t, $COCH_2CH_2\underline{C}H_2N_3$), 28.3 (t, $CO\underline{C}H_2CH_2CH_2N_3$), 25.6 (t, $CO\underline{C}H_2\underline{C}H_2CO$), 24.1 (t, $COCH_2\underline{C}H_2CH_2N_3$) ppm.

ESI: calcd for $C_8H_{11}O_4N_4$ $[M+H]^+$: 227.0780; found: 227.0785.

4-Pentenoyl NHS-ester 108c



4-Pentenoyl NHS-ester **108c** was obtained from 4-pentenoic acid and *N*-hydroxysuccinimide as a colorless solid in a yield of 70%.

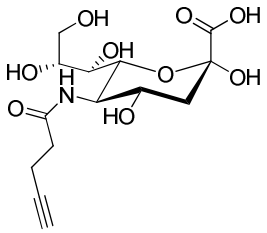
^{1}H-NMR (400 MHz, CD_3Cl) δ: 5.85 (m, 1H, $CH_2CH_2C\underline{H}CH_2$), 5.15 (dd, *J* = 10.2, 1.0 Hz, 1H, $CH_2CH_2CHC\underline{H}_{2cis}$), 5.09 (dd, *J* = 10.2, 4.8 Hz, 1H, $CH_2CH_2CHC\underline{H}_{2tran}$), 2.83 (s, 4H, $COC\underline{H}_2C\underline{H}_2CO$), 2.71 (t, *J* = 7.5 Hz, 2H, $COC\underline{H}_2$), 2.48 (dt, *J* = 6.8, 7.5 Hz, 2H, $COCH_2C\underline{H}_2$) ppm.

^{13}C-NMR (100 MHz, CD_3Cl) δ: 169.2 (s, $\underline{C}OCH_2CH_2\underline{C}O$), 168.2 (s, $\underline{C}OCH_2CH_2CHCH_2$), 135.3 (d, $COCH_2CH_2\underline{C}HCH_2$), 116.8 (t, $COCH_2CH_2CH\underline{C}H_2$), 30.4 (t, $CO\underline{C}H_2CH_2CHCH_2$), 28.5 (t, $COCH_2\underline{C}H_2CHCH_2$), 25.7 (t, $CO\underline{C}H_2\underline{C}H_2CO$) ppm.

Melting point: 47.5-49.5 °C.

ESI: calcd for $C_9H_{12}O_4N$ $[M+H]^+$: 198.0766; found: 198.0778.

***N*-4-Pentynoyl-neuraminic acid 35**



Method A:

N-Amino methoxyneuraminic acid methyl ester **107** (100 mg, 0.34 mmol) was dissolved in anhydrous DMF (2 mL) and cooled to 0 °C. NHS-ester **108a** (132.2 mg, 0.68 mmol) and DIPEA (291 µL, 1.7 mmol) were added to the mixture, subsequently. The reaction mixture was warmed up to 40 °C, and stirred for 24 h. After removal of the solvent in vacuum, the residue was re-dissolved in distilled H_2O (1 mL) and cooled to 0 °C, followed by addition of NaOH solution (1 mL, 1 M). The reaction mixture was stirred at room temperature for 30 min. To stop the reaction, Amberlyst 15 (H^+-form) was added and adjusted to pH = 3.0. After filtrating of the Amberlyst, the aqueous phase was concentrated and lyophilized to furnish a colorless solid. The solid was dissolved in an aqueous solution containing HCl (0.025 M), followed by addition of Amberlyst 15 (H^+-form, 1 g). The mixture was stirred at 80 °C for 2 h, and separated by ion exchange chromatography (*BIO-Rad* GX2 $HCOO^-$) using the program: [Double distilled water (20 mL), 1 M HCOOH (20 mL), 2 M HCOOH (20 mL), and 2.5 M HCOOH (20 mL)]. Solvent was removed under reduced pressure and lyophilized to give *N*-4-pentynoyl-neuraminic acid **35** (75 mg, 0.22 mmol, 64%). For analysis, the sample was recrystallized from ethanol and diethyl ether.

Method B:

A solution of *N*-amino methoxyneuraminic acid methyl ester **107** (150 mg, 0.51 mmol) in anhydrous pyridine (4 mL) was cooled to 4 °C. Subsequently, 4-pentynoic acid (**42**) (49.9 mg, 0.51 mg) and DCC (157.8 mg, 0.75 mmol) were added to the solution. The reaction mixture was placed at 4 °C over night. Pyridine was azeotropically removed under reduced pressure by addition of absolute toluene. The dry residue was dissolved in a mixture of water and Et_2O (20 mL, 1:1). Precipitated DCU was removed by filtration. The aqueous solution was extracted with Et_2O (10x10 mL) to eliminate unreacted reagents. The aqueous layers were collected and lyophilized to give a slightly brown solid. The solid was dissolved in MeOH (1 mL) and was precipitated in Et_2O (20 mL). After centrifugation, a colorless solid was ob-

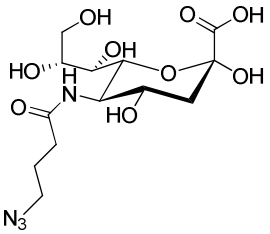
tained. Deprotection steps were carried out according to method **A**. However, this approach afforded reduced yield in comparison to method **A**.

^{1}H-NMR (400 MHz, D_2O) δ: 4.04-4.07 (m, 2H, H-4 & H-6), 3.93-3.97 (appt, *J* = 10.2 Hz, 1H, H-5), 3.83 (dd, *J* = 12.0, 2.7 Hz, 1H, H-9_a), 3.72-3.74 (m, 1H, H-8), 3.66 (dd, *J* = 12.0, 1.0 Hz, 1H, H-9_b), 3.58 (dd, *J* = 12.0, 6.5 Hz, 1H, H-7), 2.50 (m, 4H, $C\underline{H}_2C\underline{H}_2CCH$), 2.38 (s, 1H, $CH_2CH_2CC\underline{H}$), 2.29 (dd, *J* = 12.8, 4.8 Hz, 1H, H-3_{eq}), 1.86 (dd, *J* = 12.8, 11.5 Hz, 1H, H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 175.4 (s, NH$\underline{C}$OR), 173.7 (s, C-1), 95.5 (s, C-2), 83.4 (s, $CH_2CH_2\underline{C}CH$), 70.3 (d, $CH_2CH_2C\underline{C}H$), 70.3 (d, C-8), 70.2 (d, C-6), 68.2 (d, C-7), 66.7 (d, C-4), 63.1 (t, C-9), 52.0 (d, C-5), 38.9 (t, C-3), 34.7 (t, $\underline{C}H_2CH_2CCH$), 14.5 (t, $CH_2\underline{C}H_2CCH$) ppm.

ESI: calcd for $C_{14}H_{20}O_9N$ $[M-H]^-$: 346.1138; found: 346.1155.

N-4-Azidobutanoyl-neuraminic acid 63



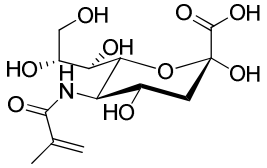
N-Amino methoxyneuraminic acid methyl ester **107** (100 mg, 0.34 mmol) was dissolved in anhydrous DMF (2 mL) and cooled to 0 °C. NHS-ester **108b** (153.8 mg, 0.68 mmol) and DIPEA (291 µL, 1.7 mmol) were added to the mixture, subsequently. The reaction mixture was warmed up to 40 °C, and stirred for 24 h. Deprotection steps were carried out according to the synthesis of *N*-4-pentynoyl-neuraminic acid **35**. Lyophilization afforded *N*-4-azidobutytyl-neuraminic acid **63** (75 mg, 0.20 mmol, 58%) as a colorless solid.

^{1}H-NMR (400 MHz, D_2O) δ: 4.08-4.02 (m, 2H, H-4 & H-6), 3.95-3.90 (appt, *J* = 10.2 Hz, 1H, H-5), 3.83 (dd, *J* = 11.9, 2.4 Hz, 1H, H-9_a), 3.73 (dt, *J* = 6.5, 2.4 Hz, 1H, H-8), 3.60 (dd, *J* = 12.0, 6.5 Hz, 1H, H-7), 3.53 (dd, *J* = 11.9, 1.0 Hz, 1H, H-9_b), 3.37 (t, *J* = 6.5 Hz, 2H, $CH_2CH_2C\underline{H}_2N_3$), 2.39 (t, *J* = 7.5 Hz, 2H, $C\underline{H}_2CH_2CH_2N_3$), 2.29 (dd, *J* = 12.8, 4.8 Hz, 1H, H-3_{eq}), 1.83-1.90 (m, 3H, $CH_2C\underline{H}_2CH_2N_3$ and H-3_{ax}) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 176.5 (s, NH<u>C</u>OR), 173.5 (s, C-1), 95.2 (s, C-2), 70.4 (d, C-6), 70.1 (d, C-8), 68.3 (d, C-7), 66.6 (d, C-4), 63.1 (t, C-9), 51.9 (d, C-5), 50.4 (t, $CH_2CH_2\underline{C}H_2N_3$), 38.7 (t, C-3), 33.0 (t, $\underline{C}H_2CH_2CH_2N_3$), 24.5 (t, $CH_2\underline{C}H_2CH_2N_3$) ppm.

ESI: calcd for $C_{13}H_{21}O_9N_4$ $[M-H]^-$: 377.1309; found: 377.1293.

N-Methacryl-neuraminic acid 110



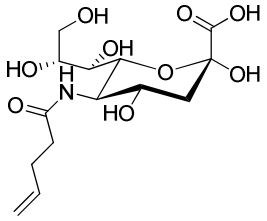
N-Amino methoxyneuraminic acid methyl ester **107** (100 mg, 0.34 mmol) was dissolved in anhydrous pyridine (2 mL) and cooled to 0 °C. After dropwise addition of methacrylic anhydride (256 µL, 0.5 mmol) the mixture was slowly warmed up to room temperature and stirred over night. Deprotection steps were carried out according to the synthesis of *N*-4-pentynoyl-neuraminic acid **35**. After work up, *N*-methacryl-neuraminic acid **110** was obtained as a colorless solid (70 mg, 0.21 mmol, 61%).

^{1}H-NMR (400 MHz, D_2O) δ: 5.70 (s, 1H, COC(CH_3)C<u>H</u>$_{2cis}$), 5.47 (s, 1H, COC(CH_3)C<u>H</u>$_{2trans}$), 4.08-4.02 (m, 2H, H-4 & H-6), 3.90 (t, *J* = 10.1 Hz, 1H, H-5), 3.82 (dd, *J* = 12.0, 2.7 Hz, 1H, H-9$_a$), 3.73 (dt, *J* = 6.5, 2.4 Hz, 1H, H-8), 3.58 (dd, *J* = 12.0, 6.5 Hz, 1H, H-7), 3.54 (dd, *J* = 12.0, 1.0 Hz, 1H, H-9$_b$), 2.32 (dd, *J* = 13.0, 4.8 Hz, 1H, H-3$_{eq}$), 1.94 (s, COC(C<u>H</u>$_3$)CH_2), 1.89 (dd, *J* = 13.0, 11.5 Hz, 1H, H-3$_{ax}$) ppm.

^{13}C-NMR (100 MHz, D_2O) δ: 172.8 (s, NH<u>C</u>OR), 171.0 (s, C-1), 139.0 (s, CO<u>C</u>(CH_3)CH_2), 121.1 (t, COC(CH_3)<u>C</u>H_2), 95.3 (s, C-2), 70.3 (d, C-8), 70.2 (d, C-6), 68.3 (d, C-7), 66.4 (d, C-4), 63.2 (t, C-9), 52.2 (d, C-5), 38.7 (t, C-3), 17.7 (q, COC(<u>C</u>H_3)CH_2) ppm.

ESI: calcd for $C_{13}H_{20}O_9N$ $[M-H]^-$: 334.1138; found: 334.1149.

***N*-4-Pentenoyl-neuraminic acid 111**



N-Amino methoxyneuraminic acid methyl ester **107** (100 mg, 0.34 mmol) was dissolved in anhydrous DMF (2 mL) and cooled to 0 °C. NHS-ester **108d** (101 mg, 0.51 mmol) and DIPEA (291 µL, 1.7 mmol) were added to the mixture, subsequently. The reaction mixture was stirred for 24 h at 40 °C. Deprotection steps were archieved according to the synthesis of *N*-4-pentynoyl-neuraminic acid **35**. After work up product **111** was collected as a colorless solid (80 mg, 0.24 mmol, 67%).

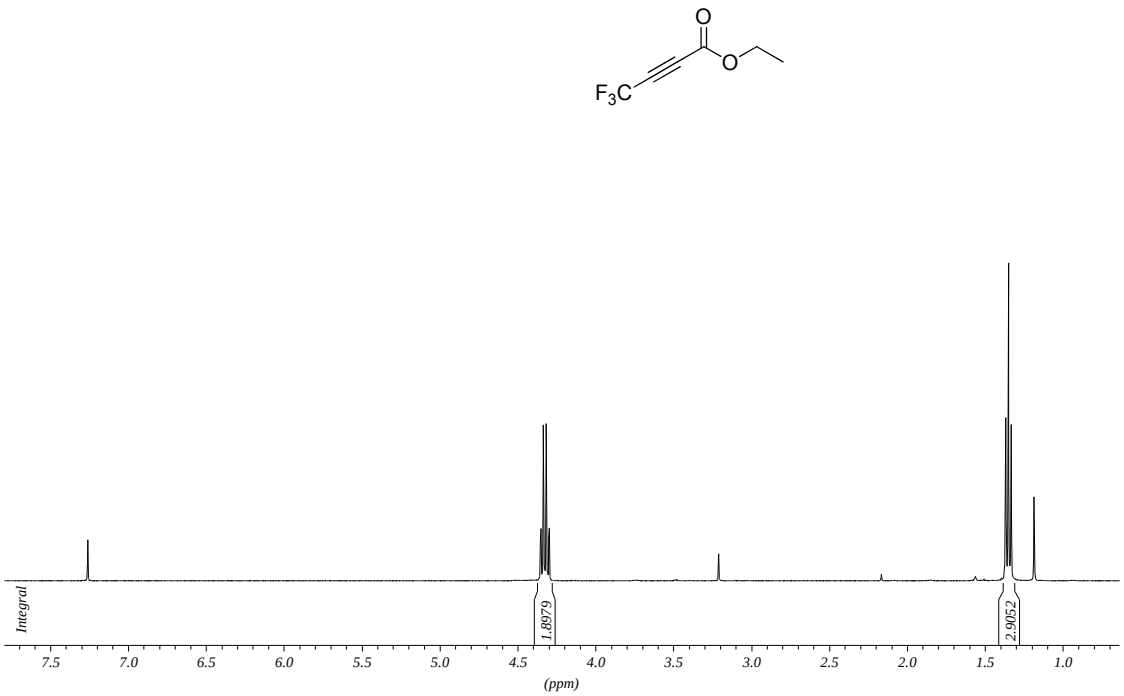
^{1}H-NMR (400 MHz, D_2O) δ: 5.85 (m, 1H, $CH_2CH_2C\underline{H}CH_2$), 5.06 (dd, *J* = 17.2, 1.7 Hz, 1H, $CH_2CH_2CHC\underline{H}_{2cis}$), 5.06 (dd, *J* = 17.2, 1.2 Hz, 1H, $CH_2CH_2CHC\underline{H}_{2trans}$), 4.08-4.02 (m, 2H, H-4 & H-6), 3.87-3.93 (t, *J* = 10.2 Hz, 1H, H-5), 3.83 (dd, *J* = 12.0, 2.7 Hz, 1H, H-9_a), 3.73 (dt, *J* = 6.5, 2.4 Hz, 1H, H-8), 3.59 (dd, *J* = 12.0, 1.0 Hz, 1H, H-9_b), 3.55 (dd, *J* = 12.0, 6.5 Hz, 1H, H-7), 2.39 (m, 4H, $C\underline{H}_2C\underline{H}_2CHCH_2$), 2.29 (dd, *J* = 12.9, 4.8 Hz, 1H, H-3_{eq}), 1.88 (dd, *J* = 12.9, 11.5 Hz, 1H, H-3_{ax}) ppm.

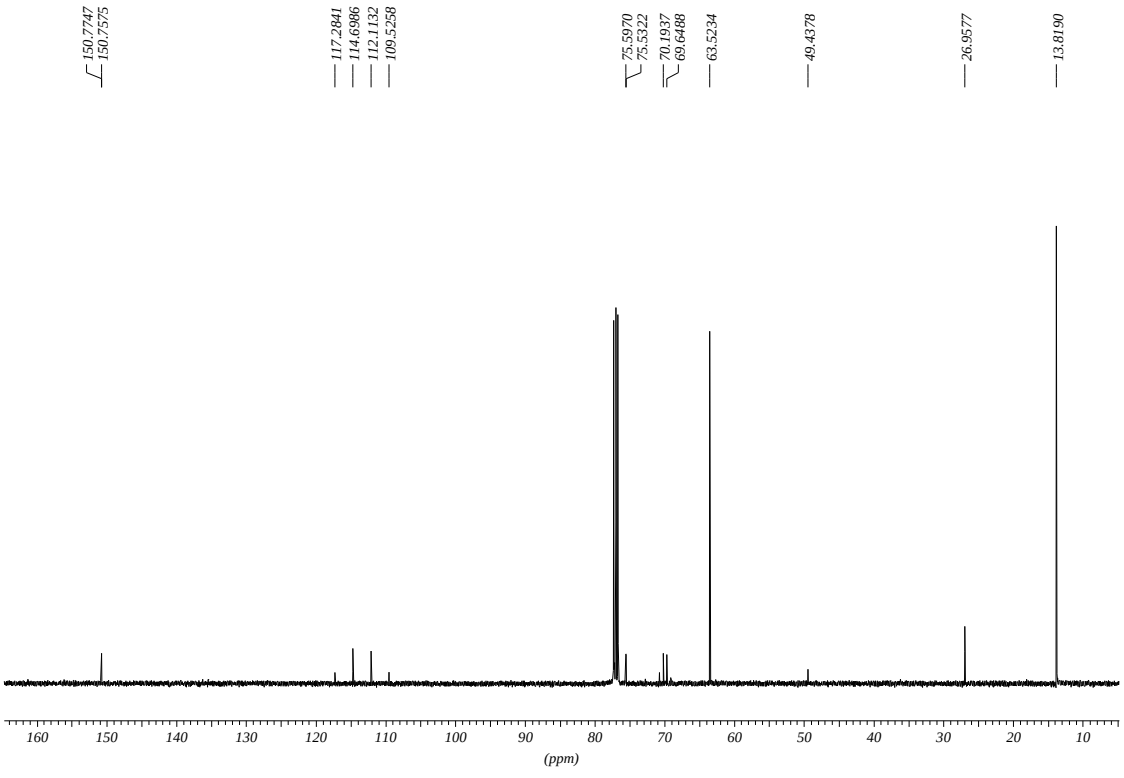
^{13}C-NMR (100 MHz, D_2O) δ: 176.9 (s, NH$\underline{C}$OR), 171.0 (s, C-1), 137.0 (d, $CH_2CH_2\underline{C}HCH_2$), 115.8 (t, $CH_2CH_2CH\underline{C}H_2$), 95.2 (s, C-2), 70.4 (d, C-8), 70.2 (d, C-6), 68.3 (d, C-7), 66.5 (d, C-4), 63.2 (t, C-9), 51.9 (d, C-5), 38.7 (t, C-3), 35.2 (t, $\underline{C}H_2CH_2CHCH_2$), 27.4 (t, $CH_2\underline{C}H_2CHCH_2$) ppm.

ESI: calcd for $C_{14}H_{22}O_9N$ $[M-H]^-$: 348.1295; found: 348.1283.

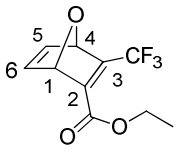
6 Spectra

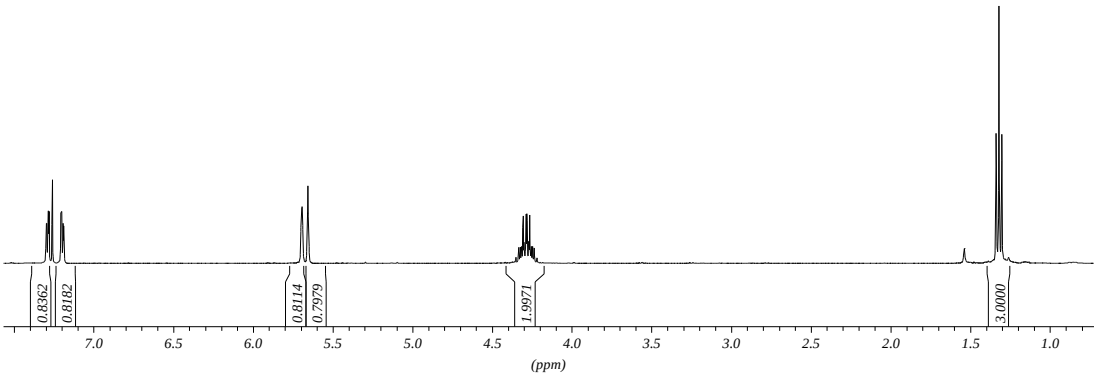
Trifluoro-butynoic acid ethyl ester (**16**)

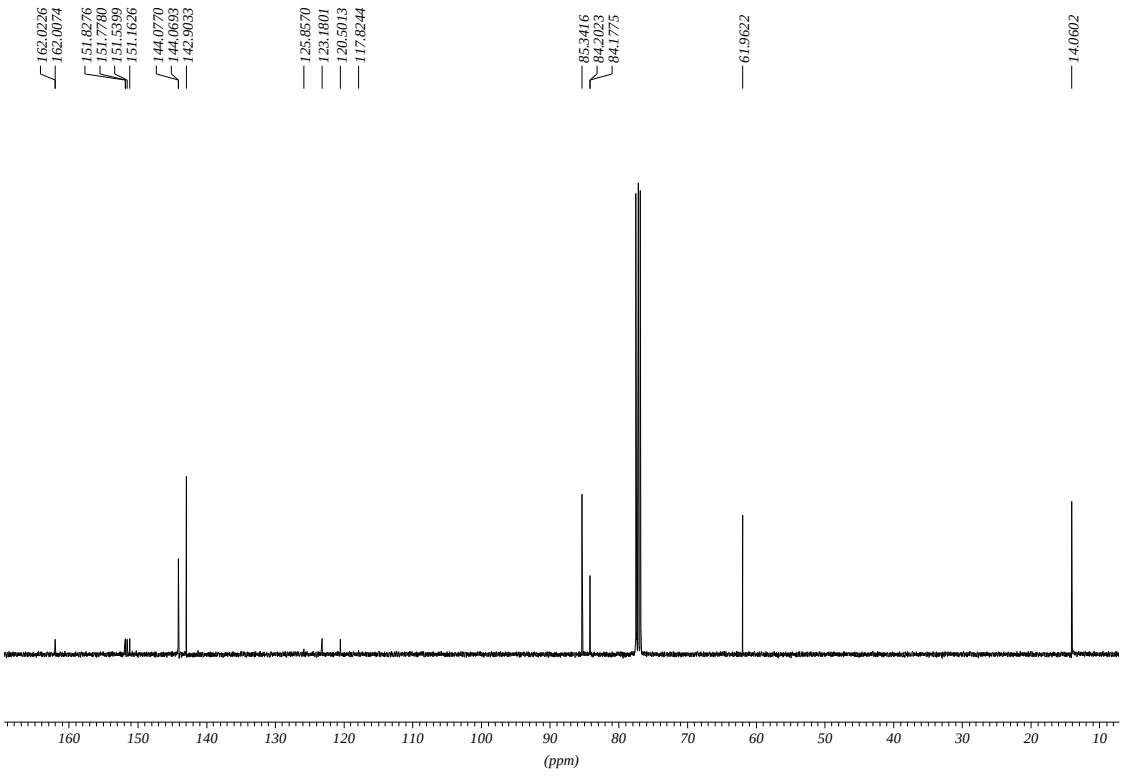




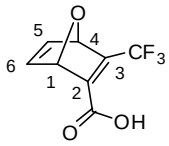
Oxanorbornadienyl ethyl ester **17**

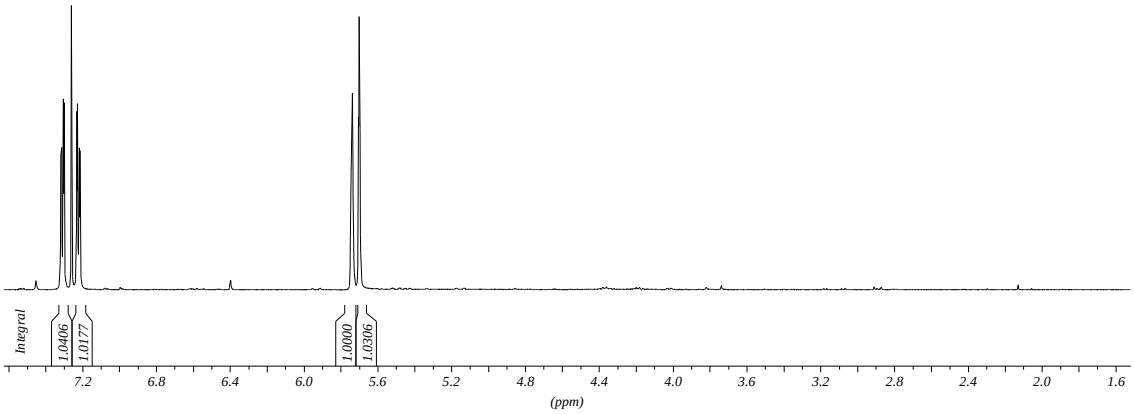


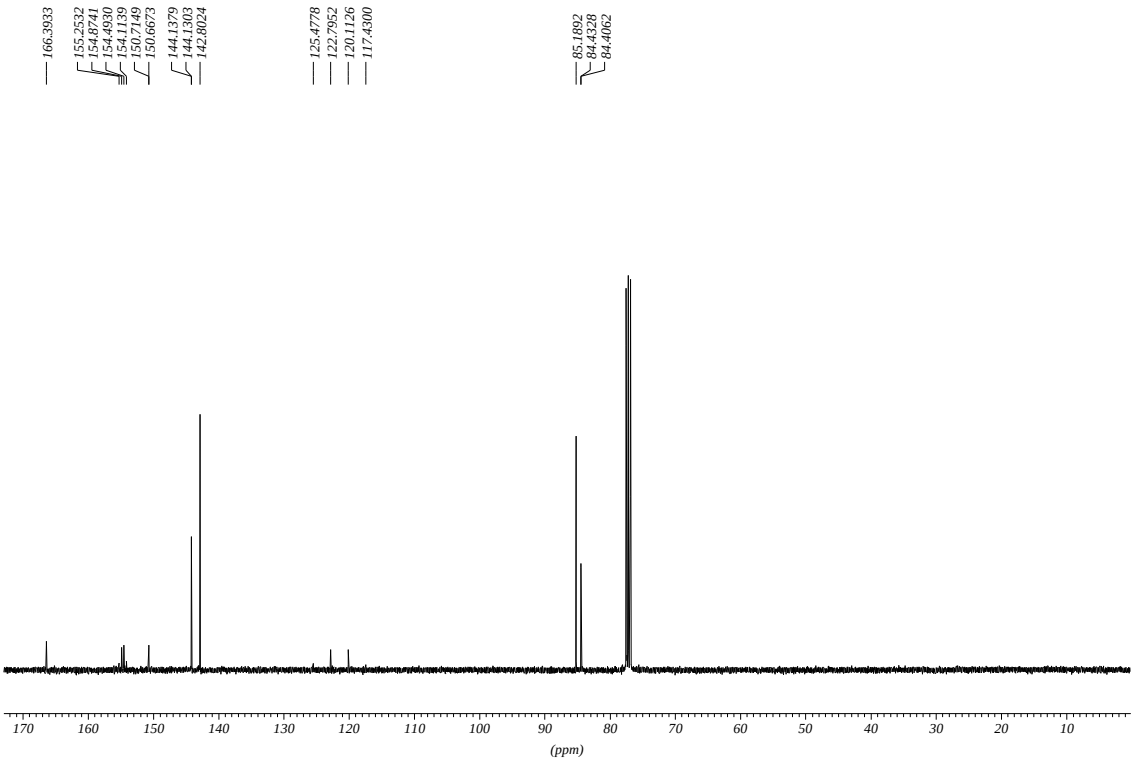




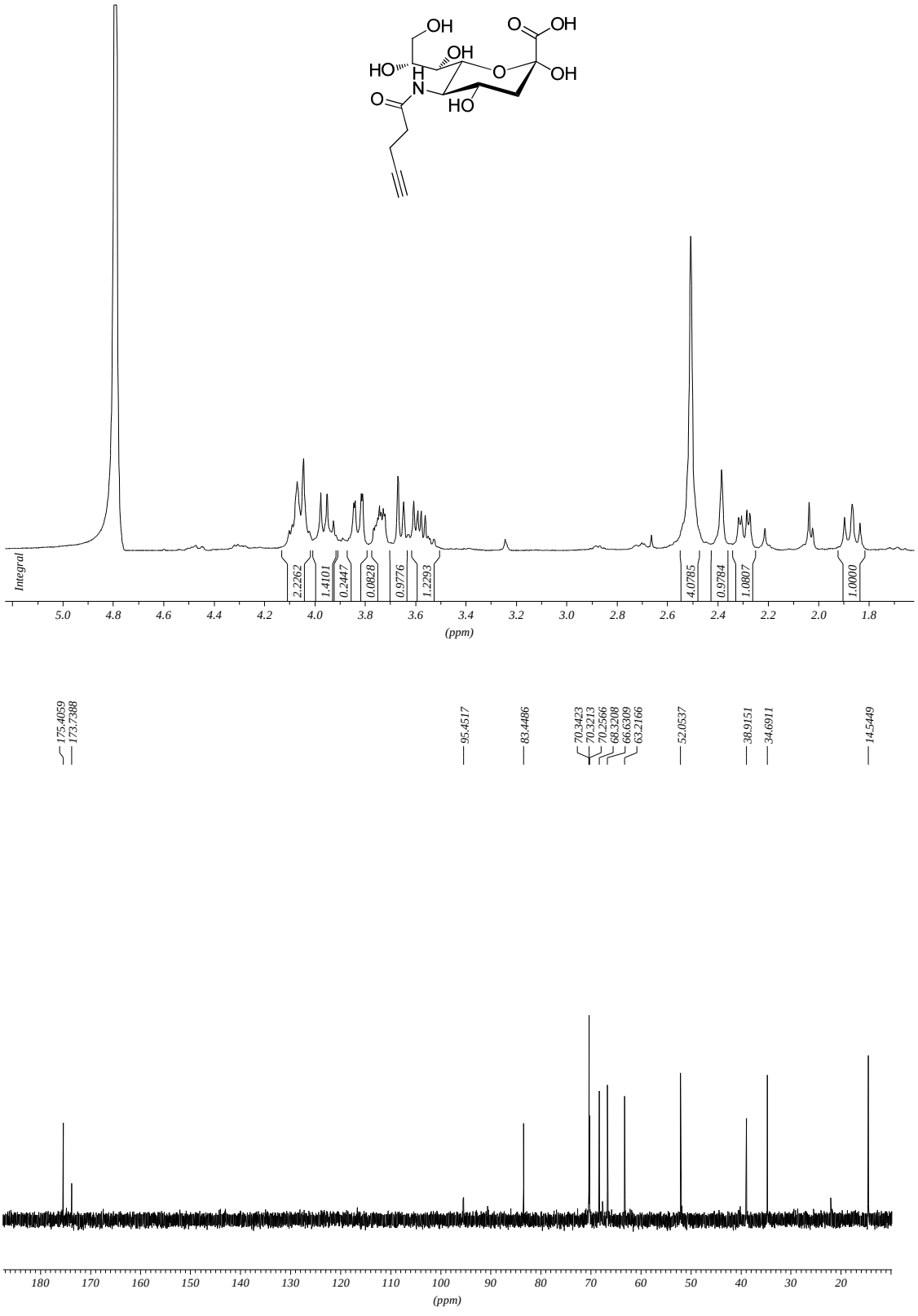
Oxanorbornadienyl carboxylic acid **18**



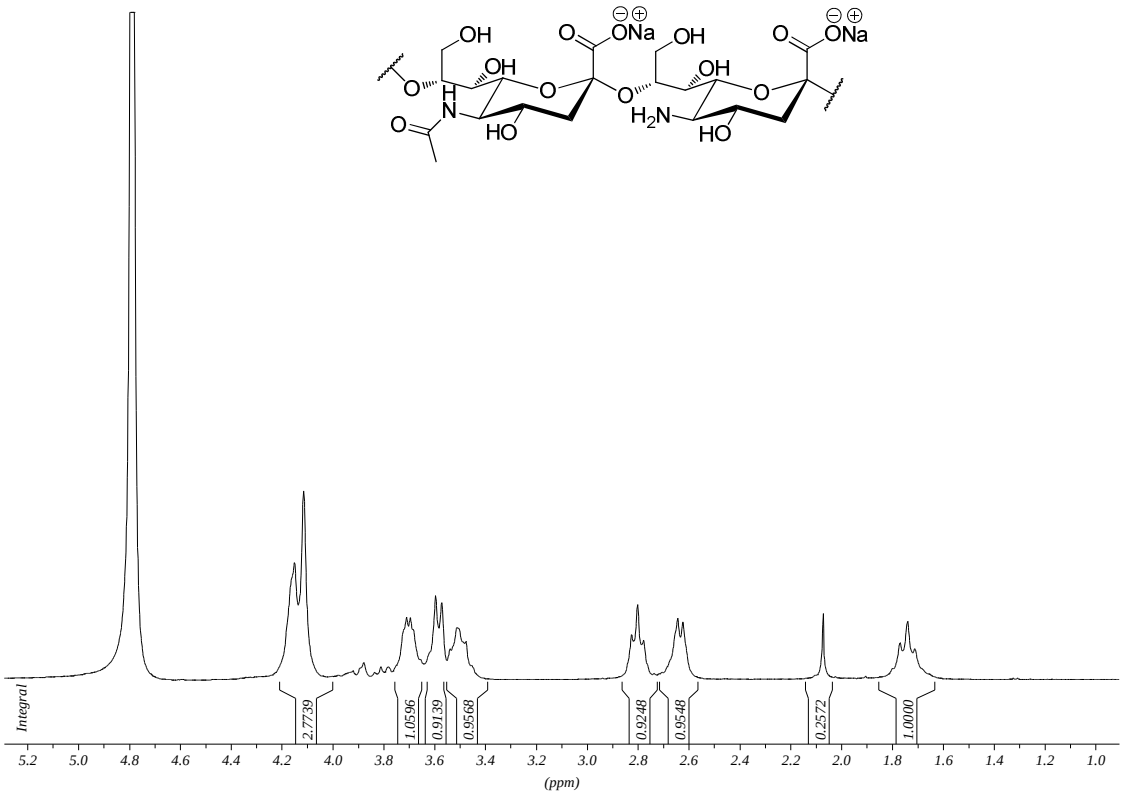


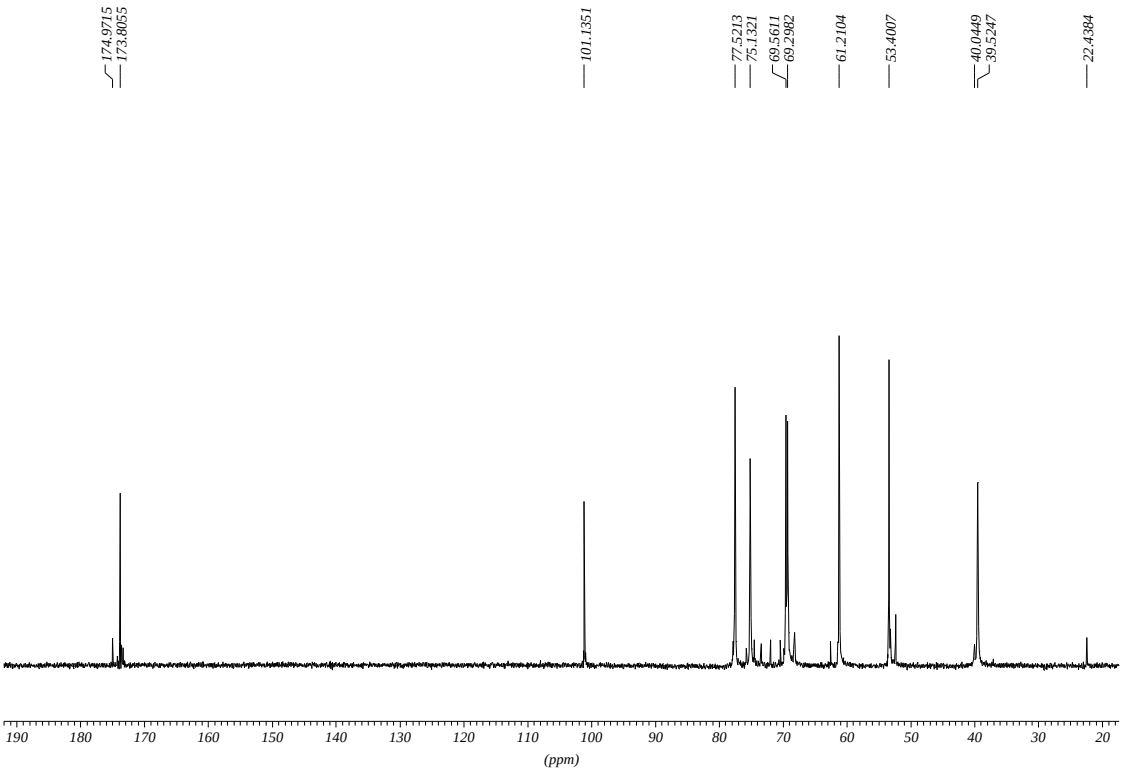


N-4-Pentynoyl-neuraminic acid **35**

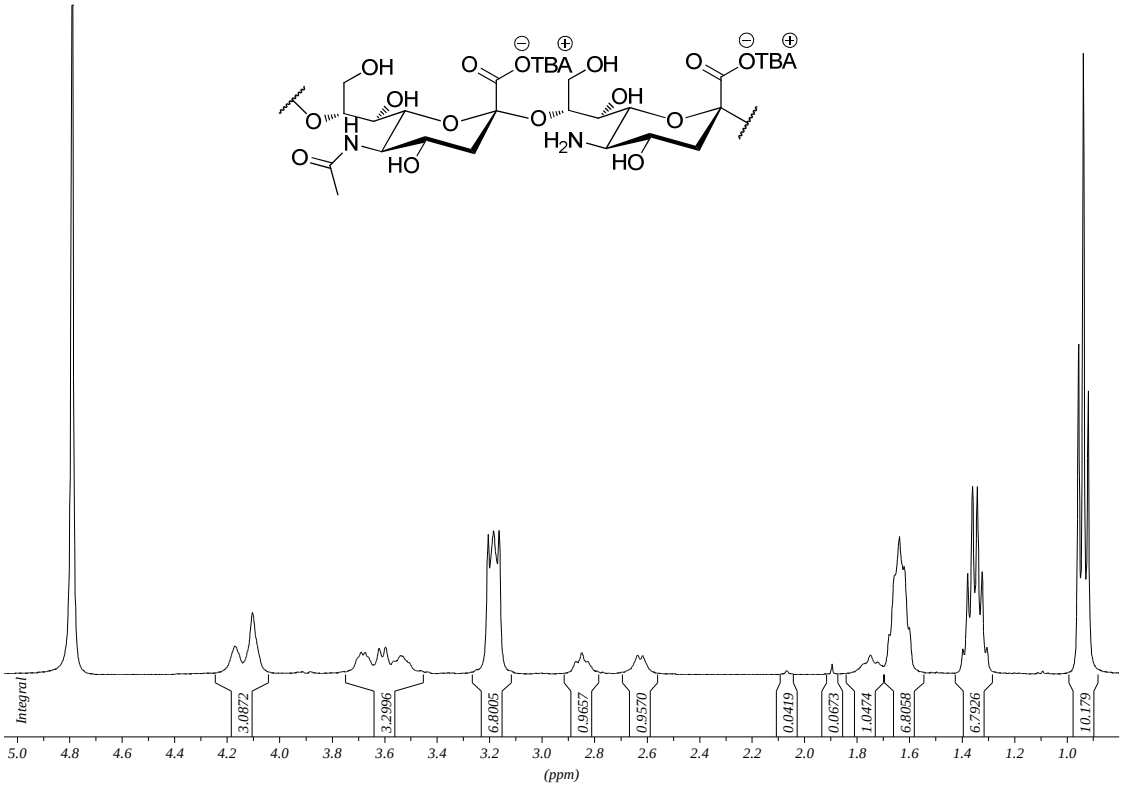


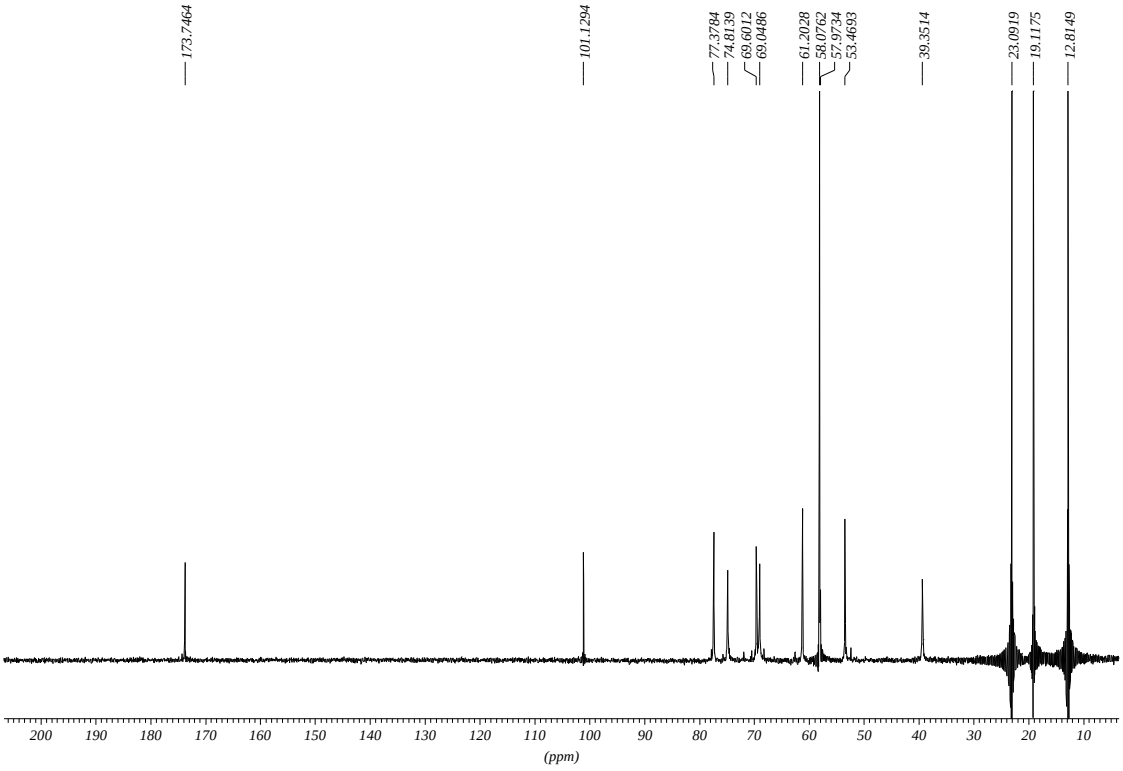
γ-Amino polySia (sodium salt) **36**



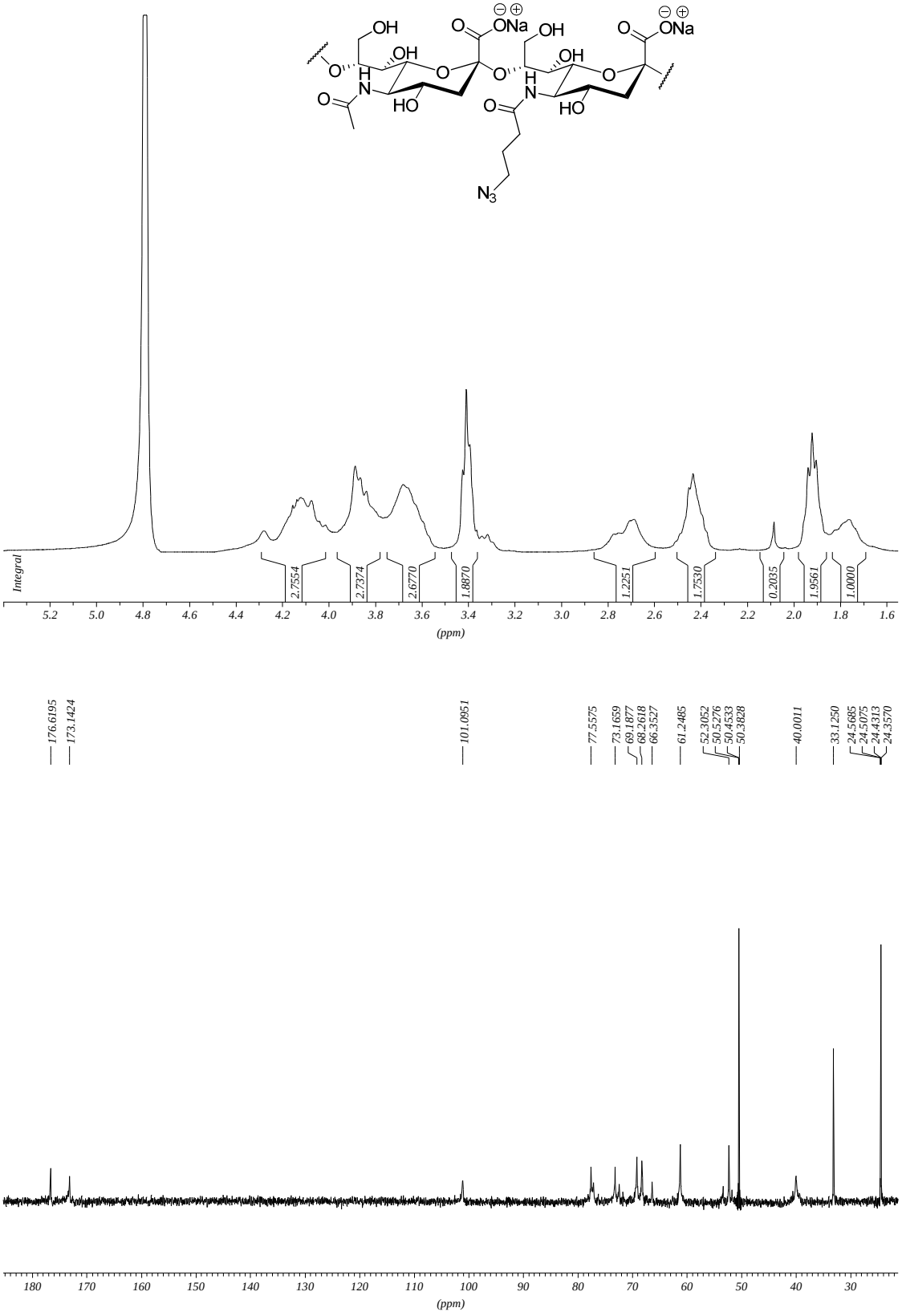


γ-Amino polySia (TBA salt) **37**

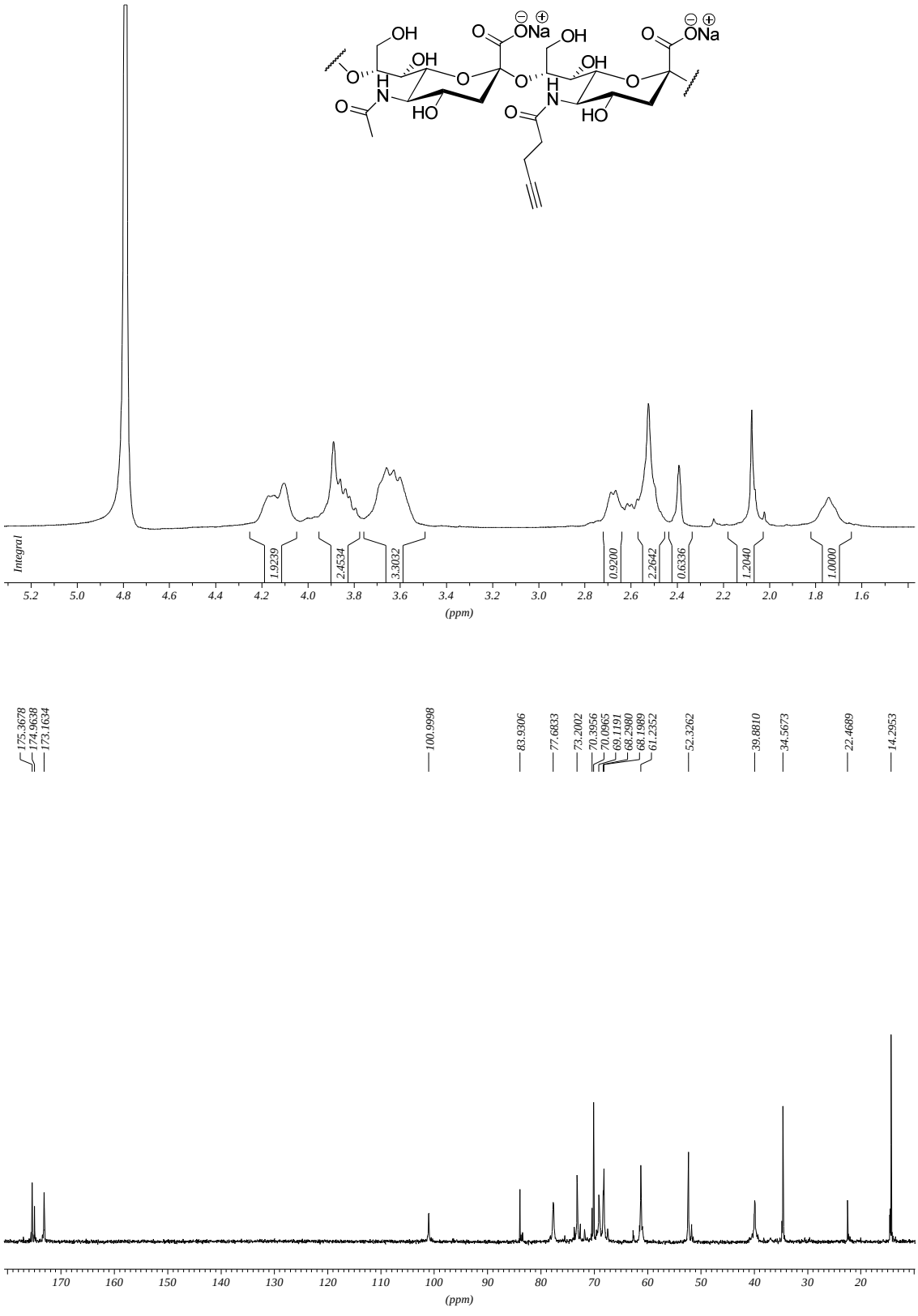




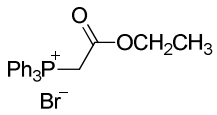
N-(4-Azidobutyryl)-polySia **41**

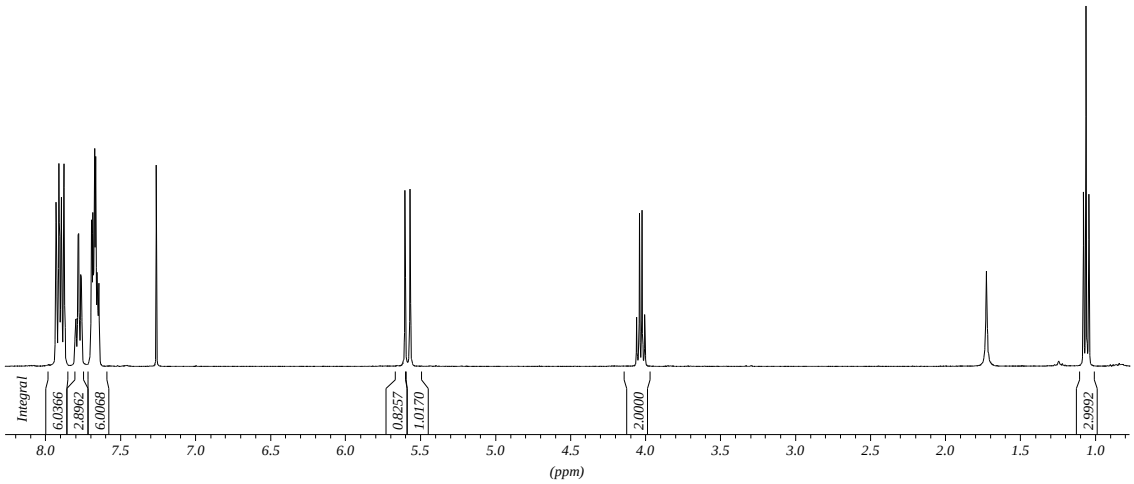


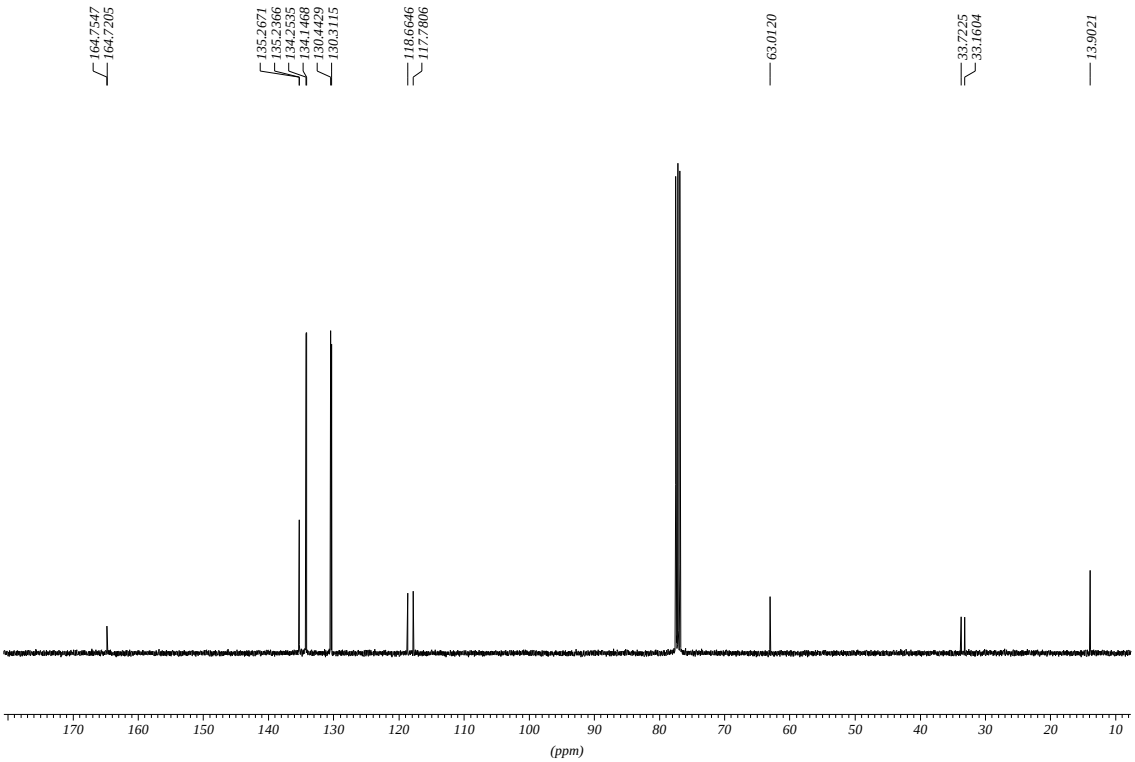
N-(4-Pentynoyl)-polySia **44**



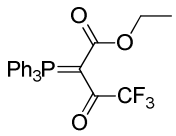
Ethoxycarbonylmethyl-triphenyl-phosphonium bromide (**46**)

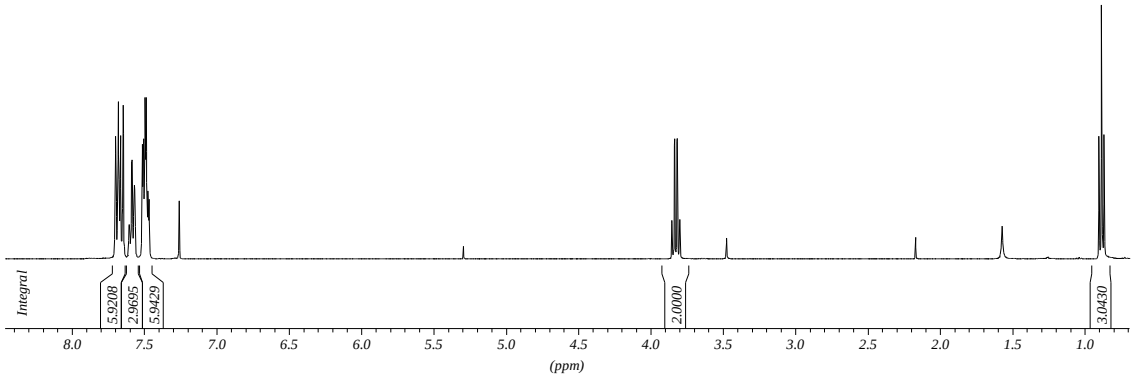


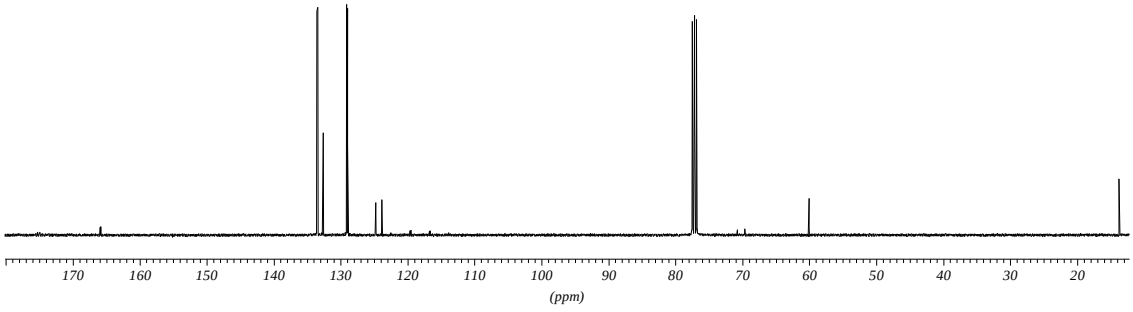




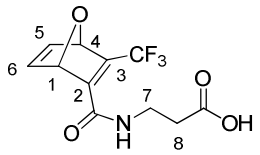
Trifluoro-3-oxo-2-(triphenyl-phosphanylidene)-butyric acid ethyl ester (**47**)

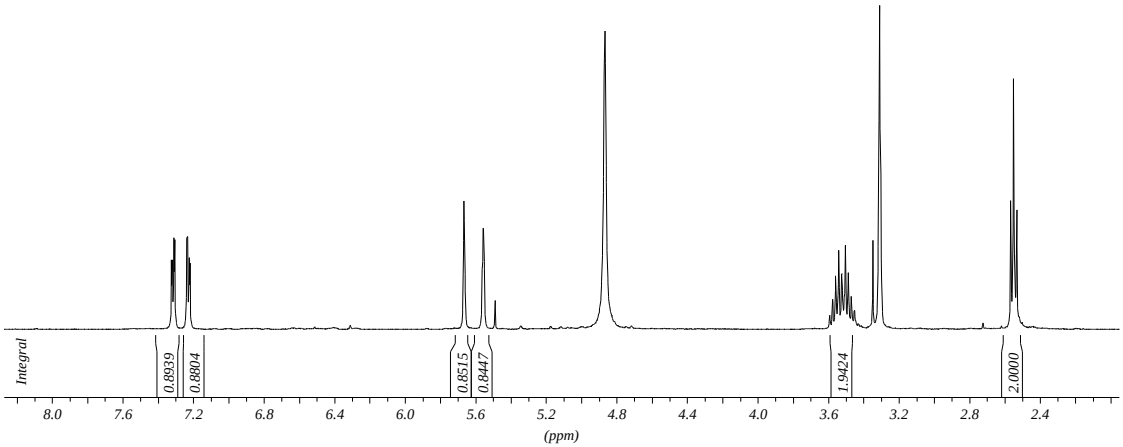


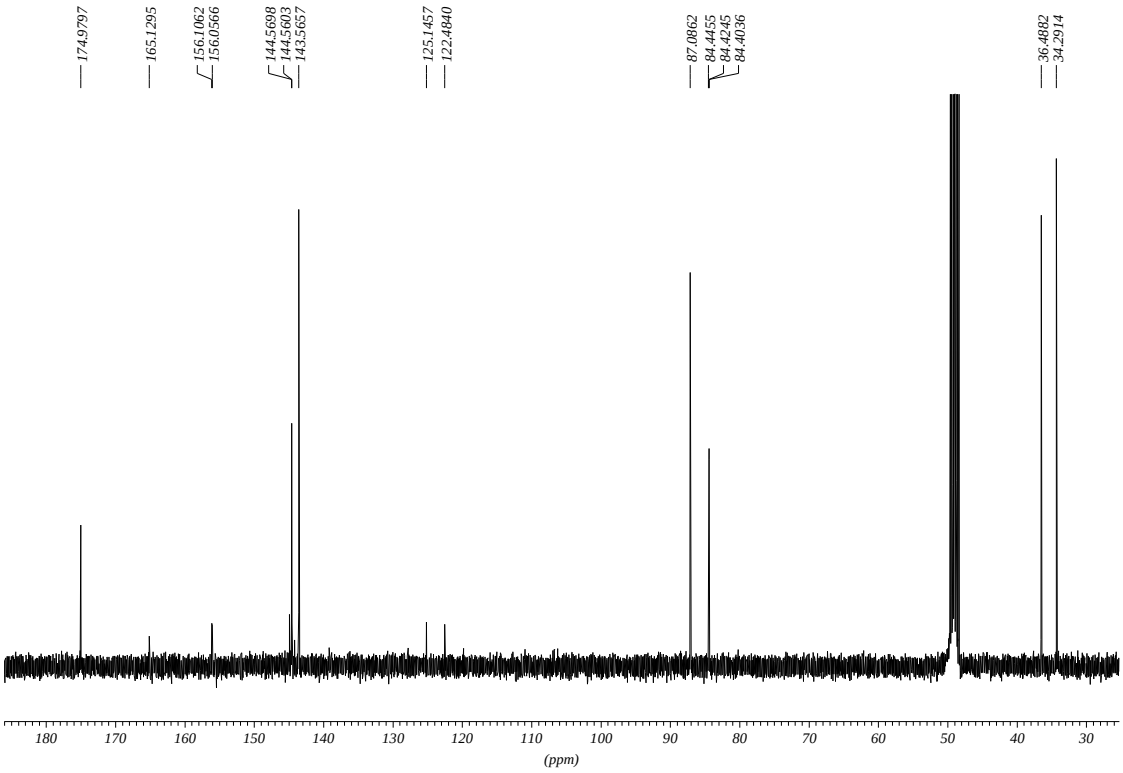




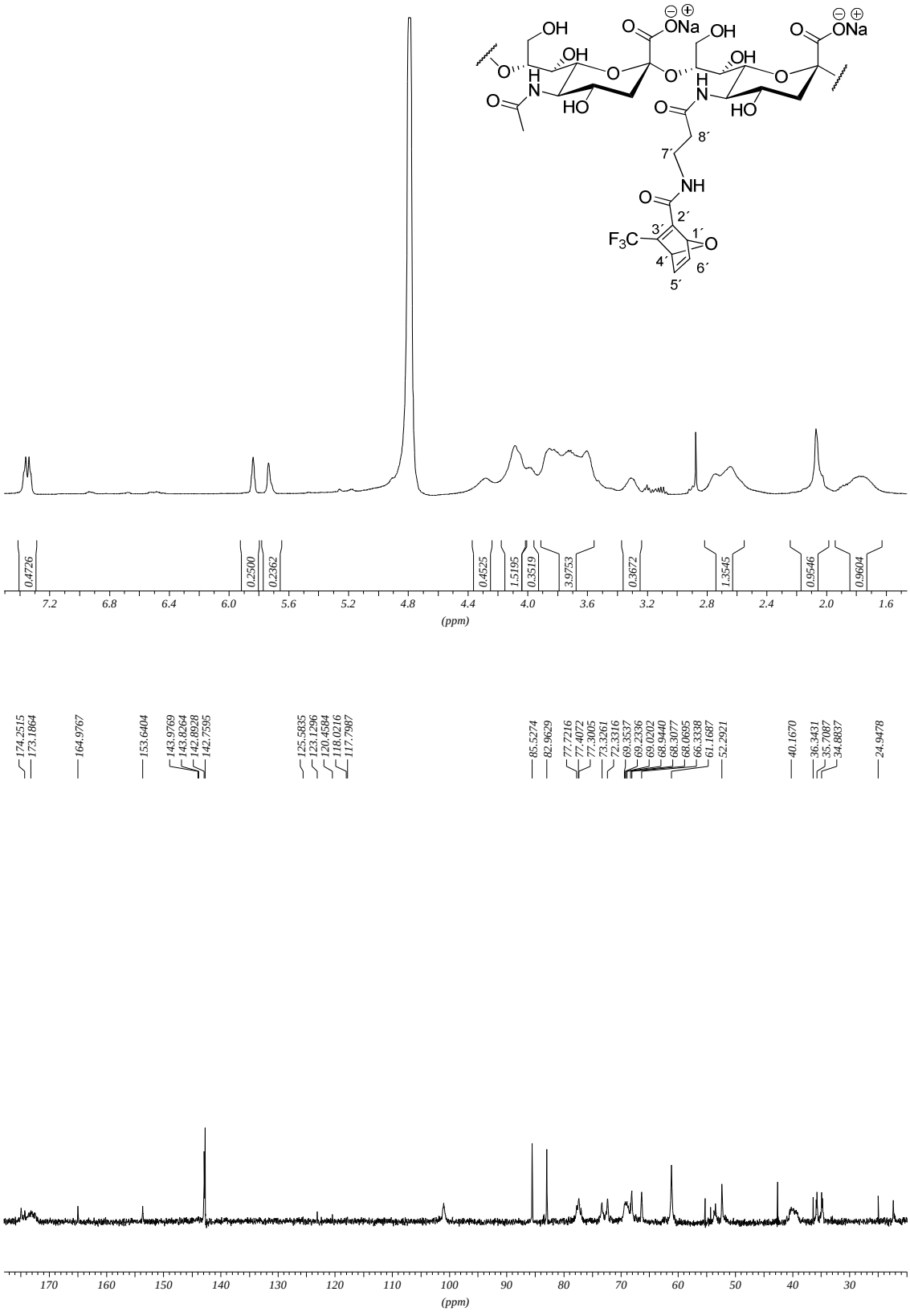
Oxanorbornadienyl propanoic acid **52a**



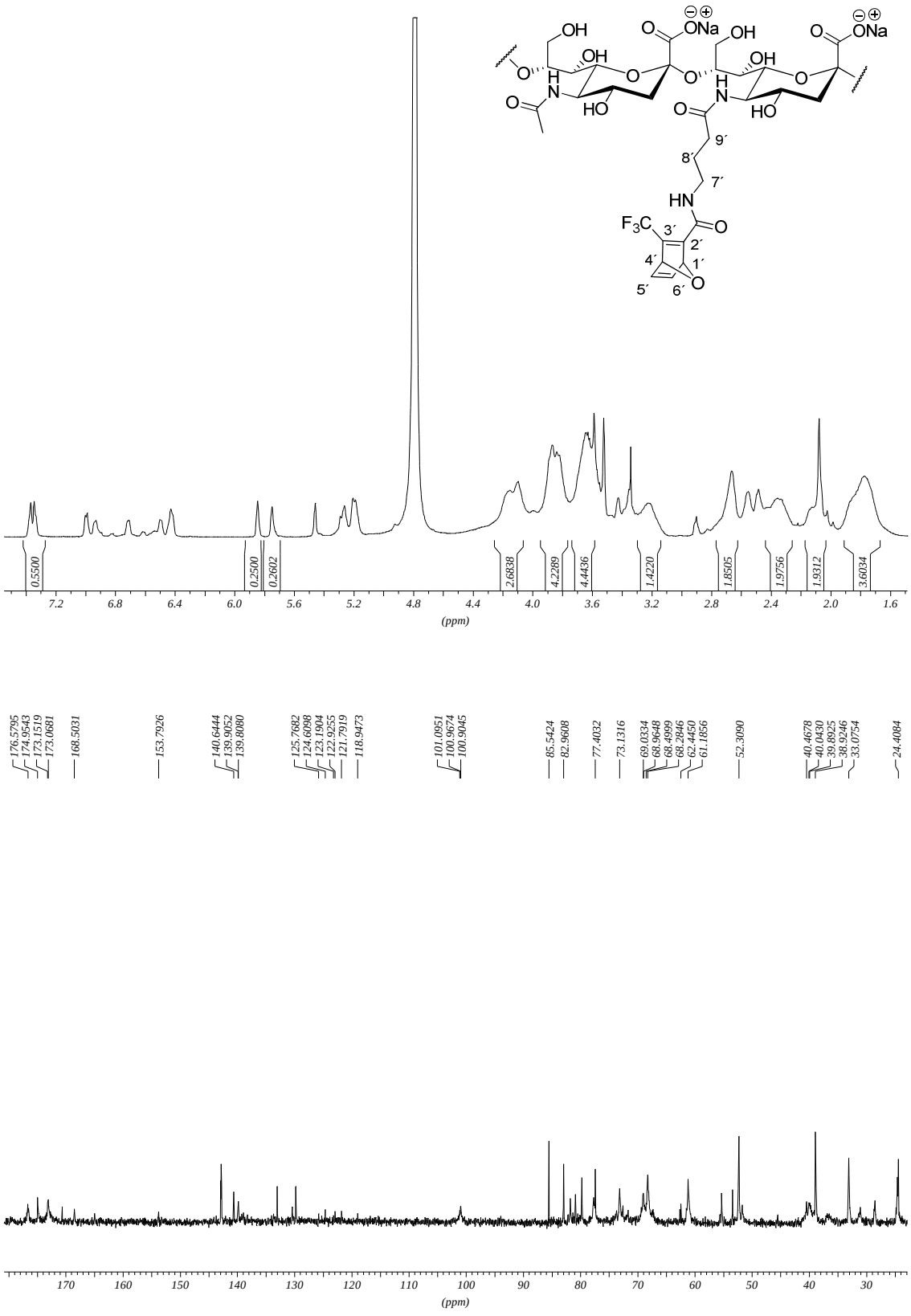




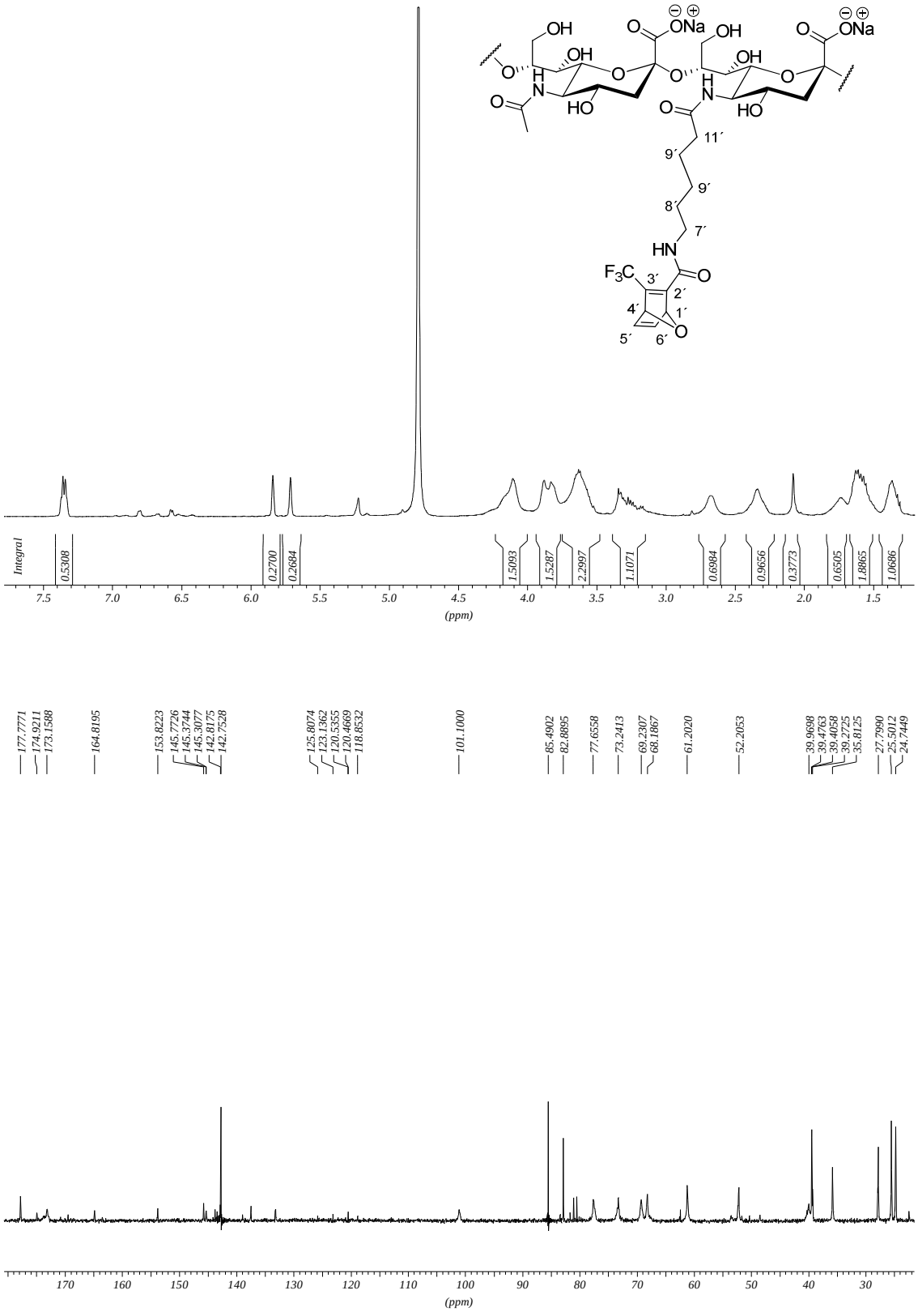
N-Oxanorbornadienyl-polySia **53a**



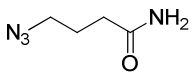
N-Oxanorbornadienyl-polySia **53b**

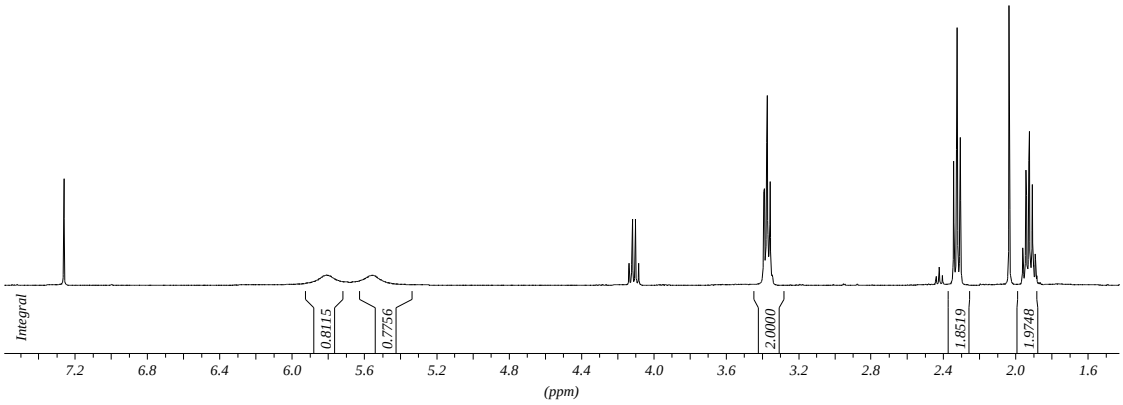


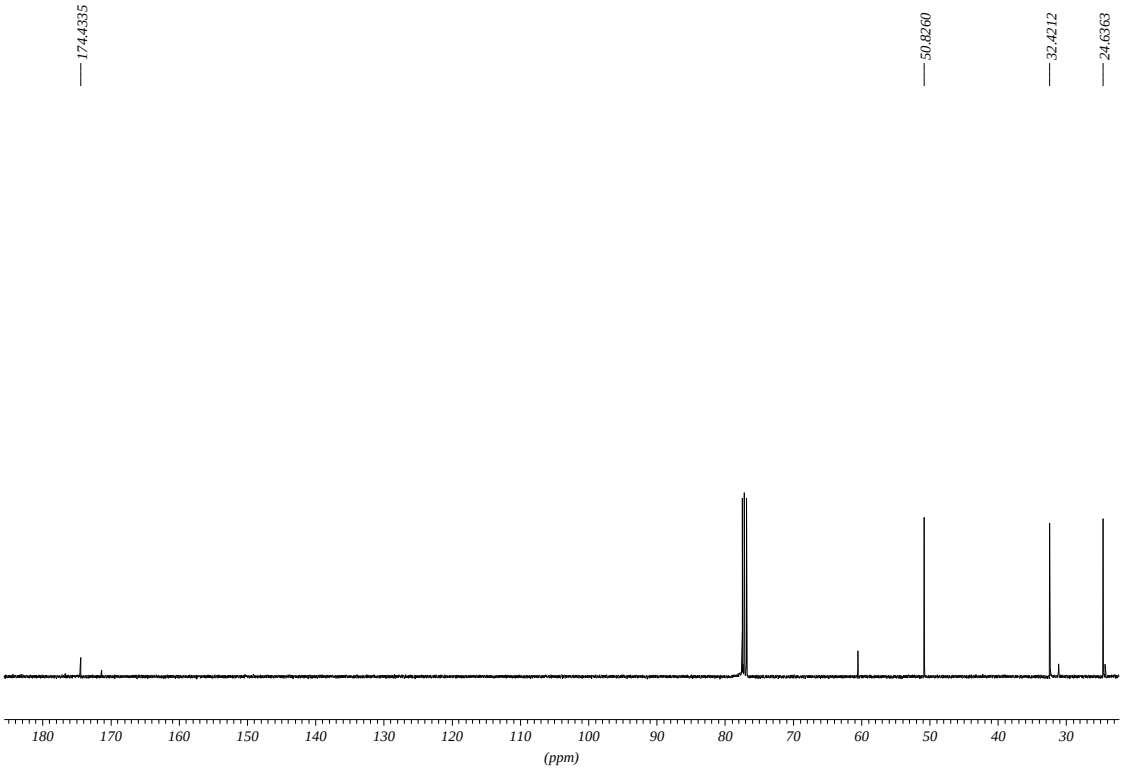
N-Oxanorbornadienyl-polySia **53c** (*Before reacetylation*)



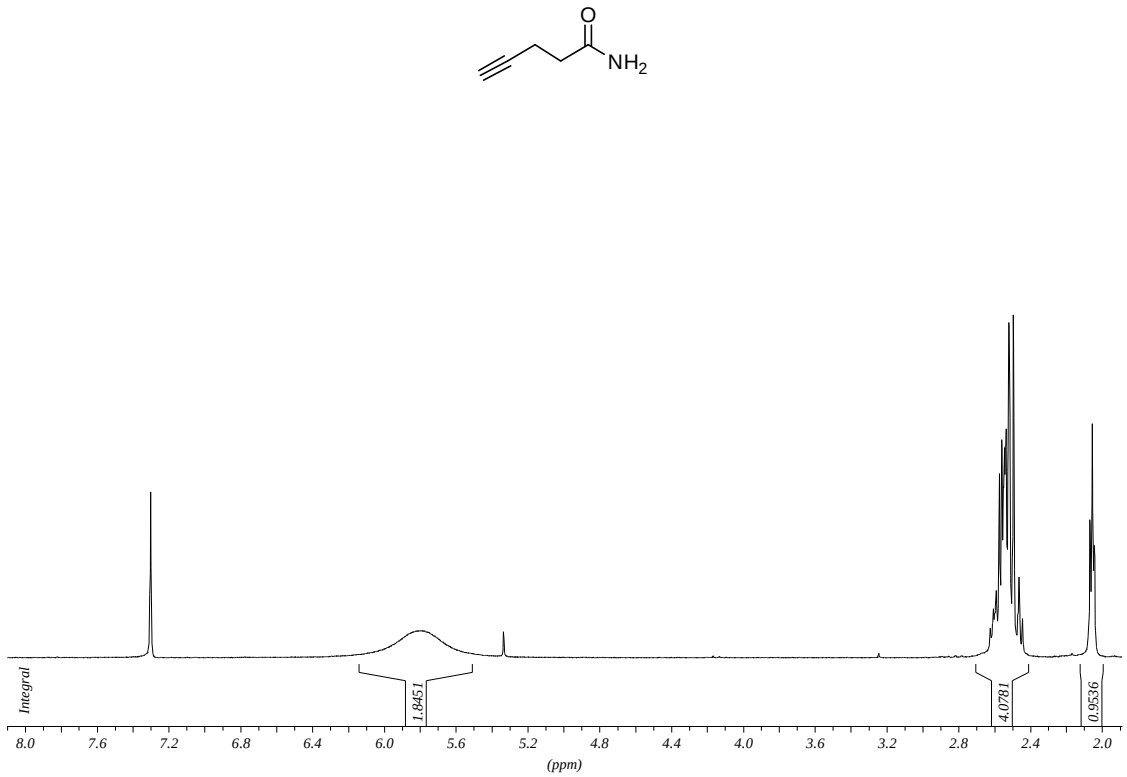
4-Azidobutanamide (**54**)



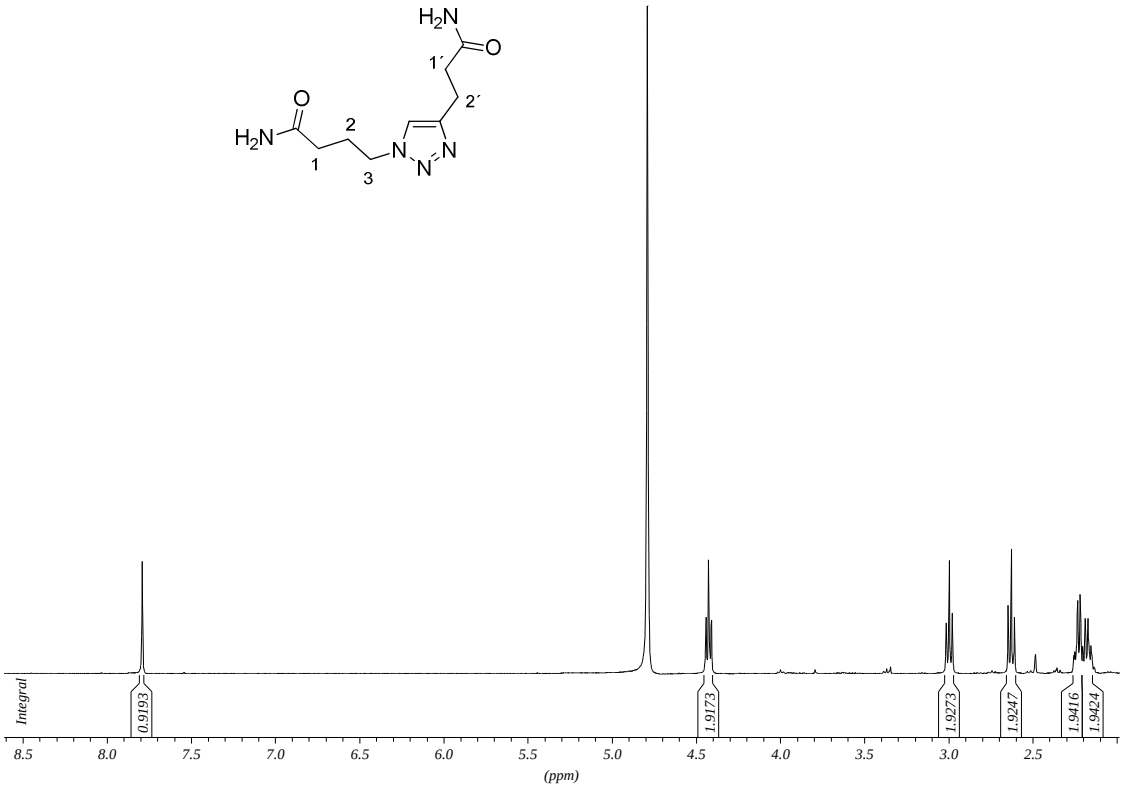


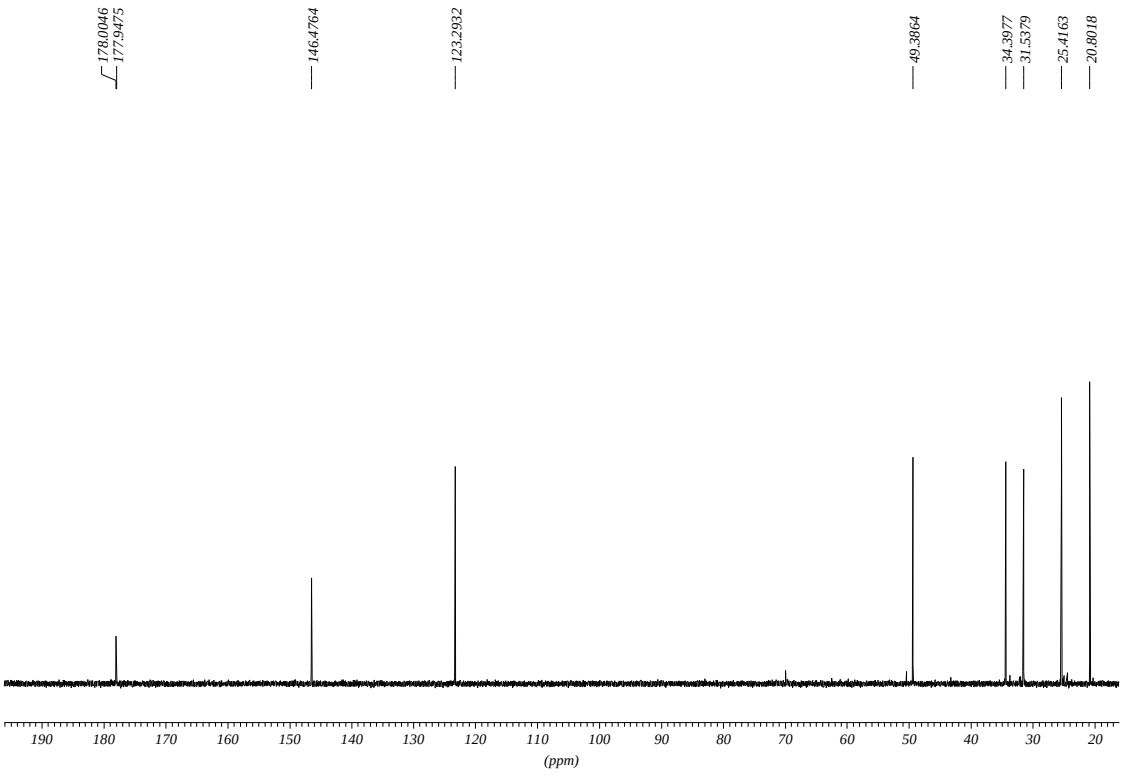


Pent-4-ynamide (**55**)

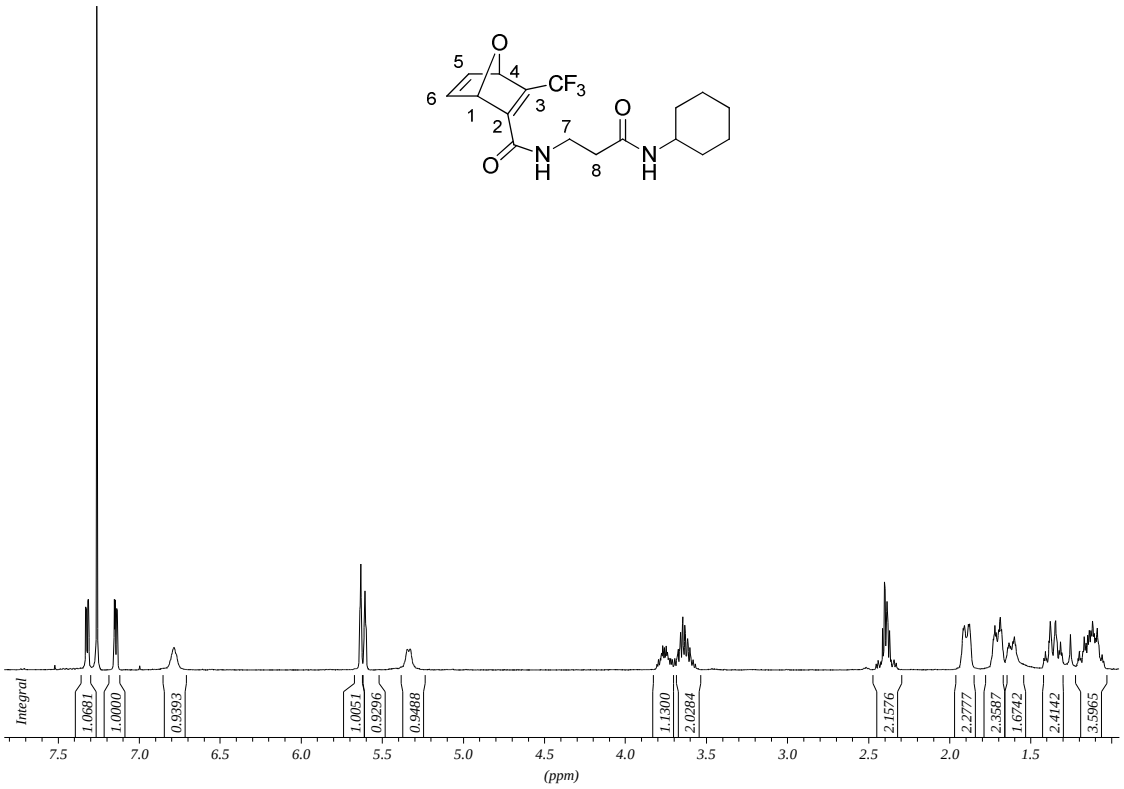


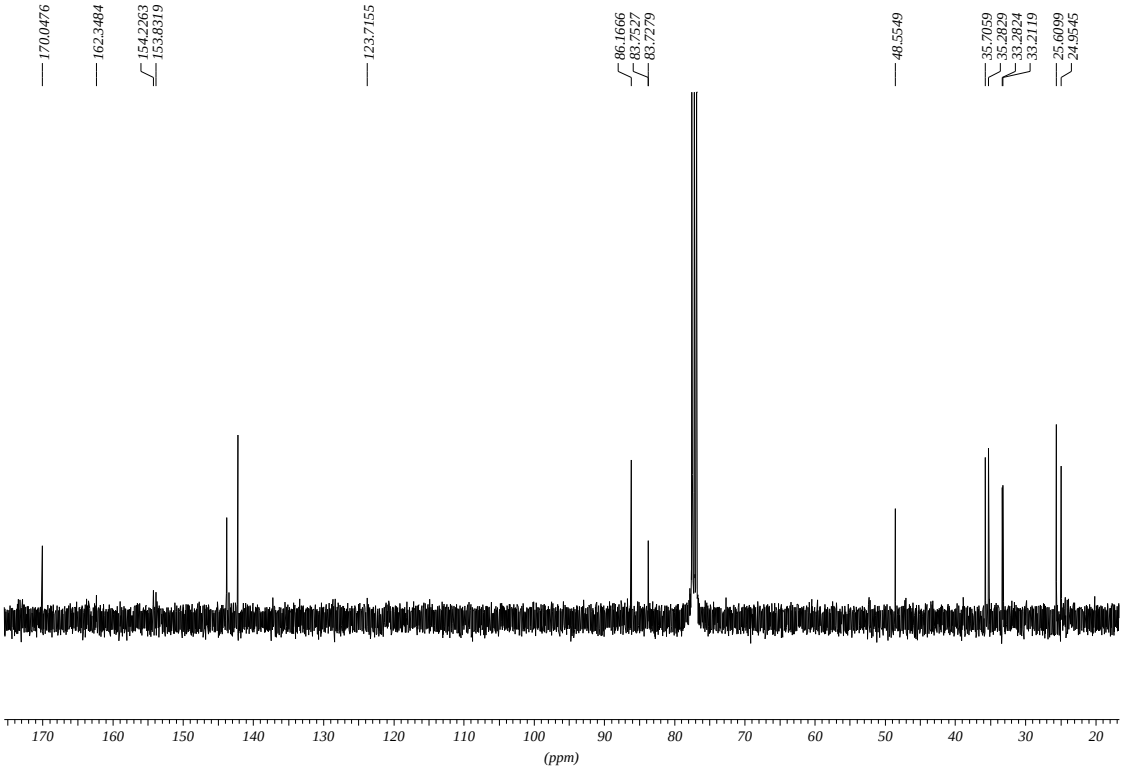
4-(4-(3-Amino-3-oxopropyl)-1H-1,2,3-triazol-1-yl)butanamide (**56**)



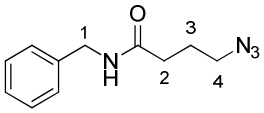


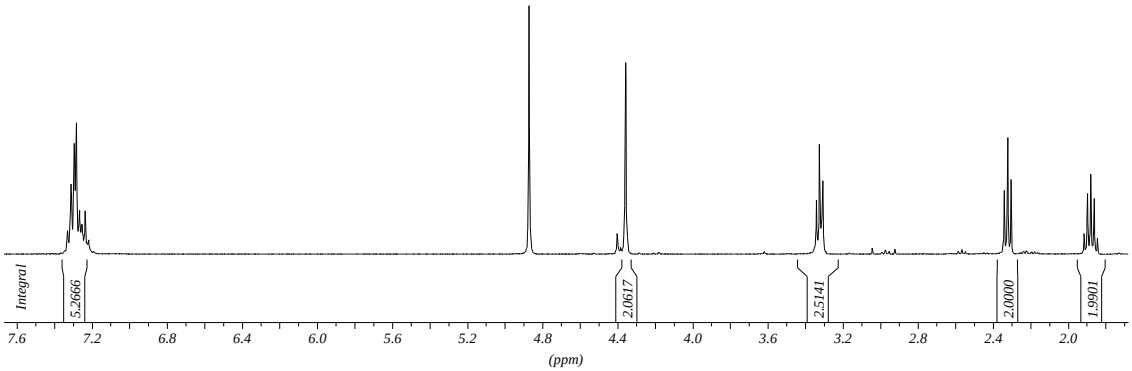
Alkyno-analogue **57**

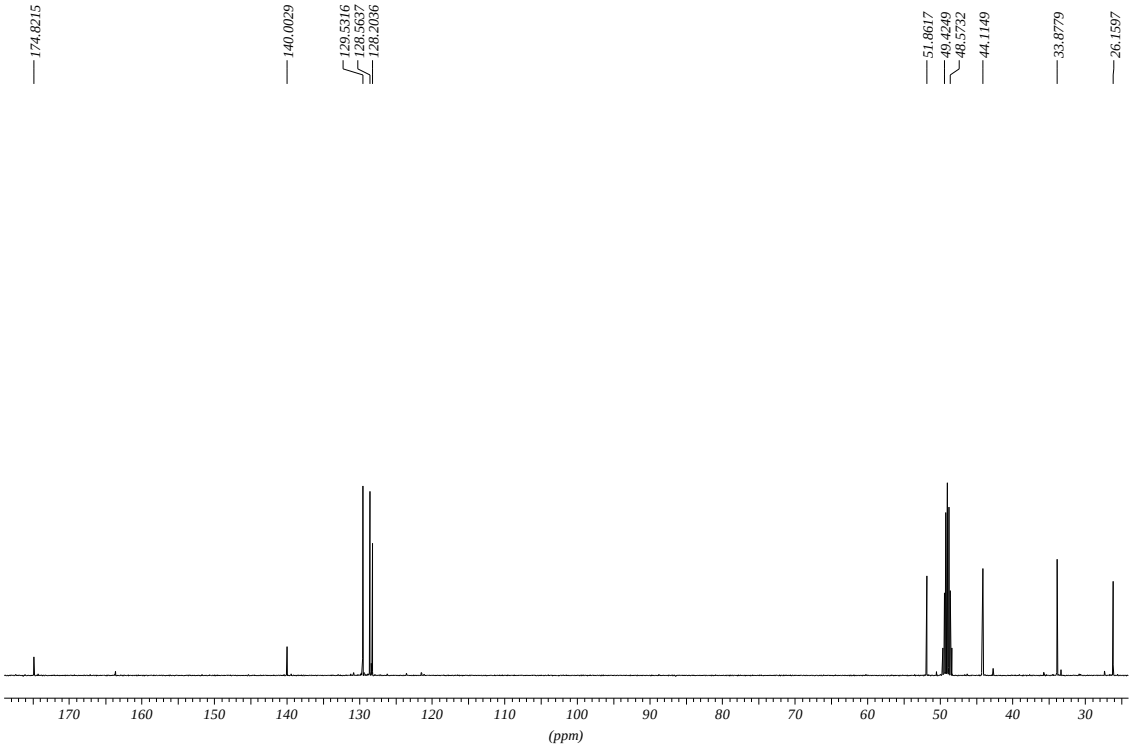




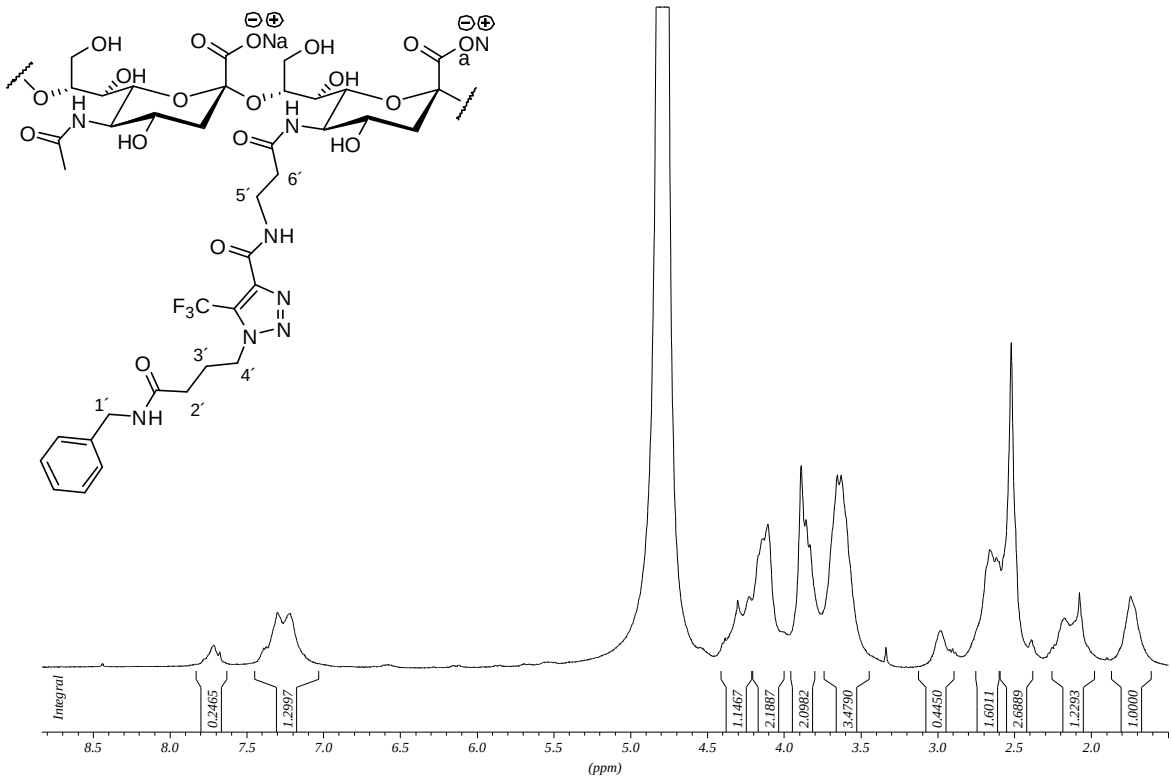
4-Azido-*N*-benzylbutanamide (**60**)

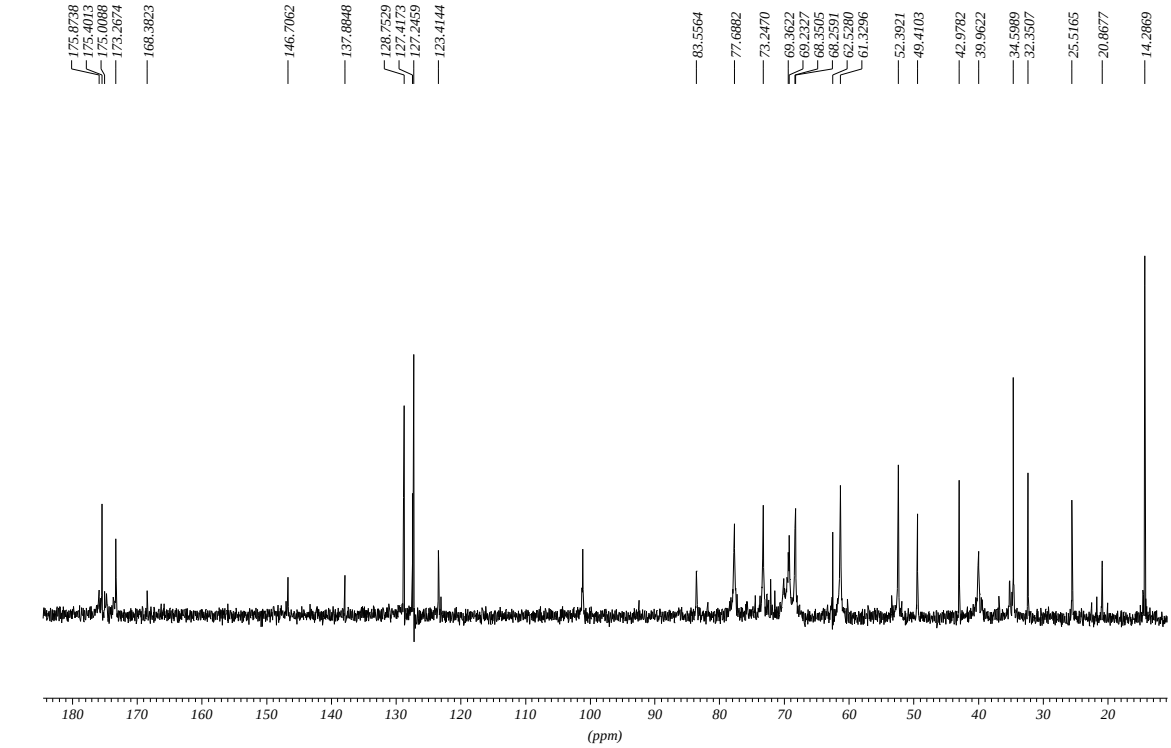




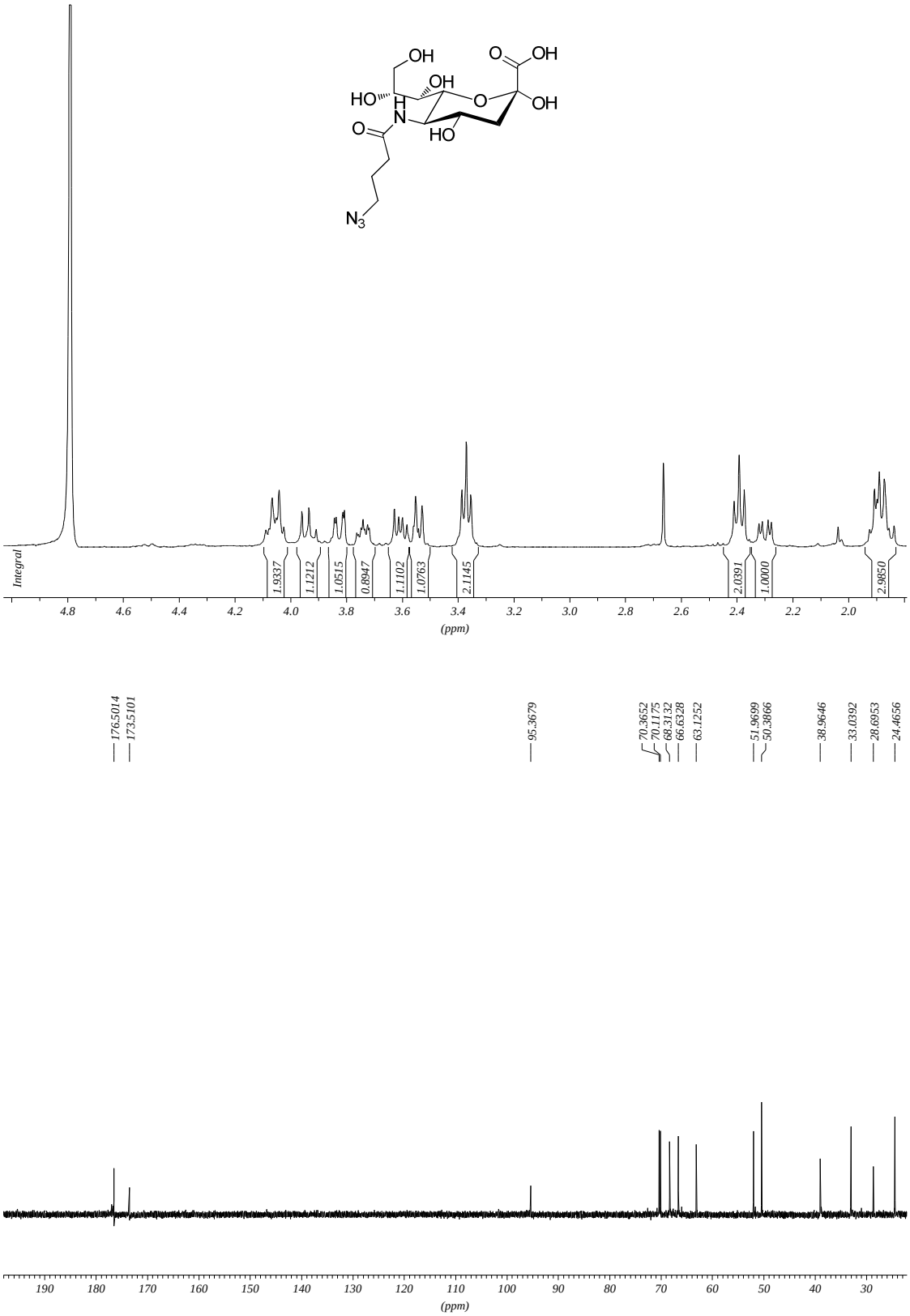


N-Benzylbutanamido-polySia **61**

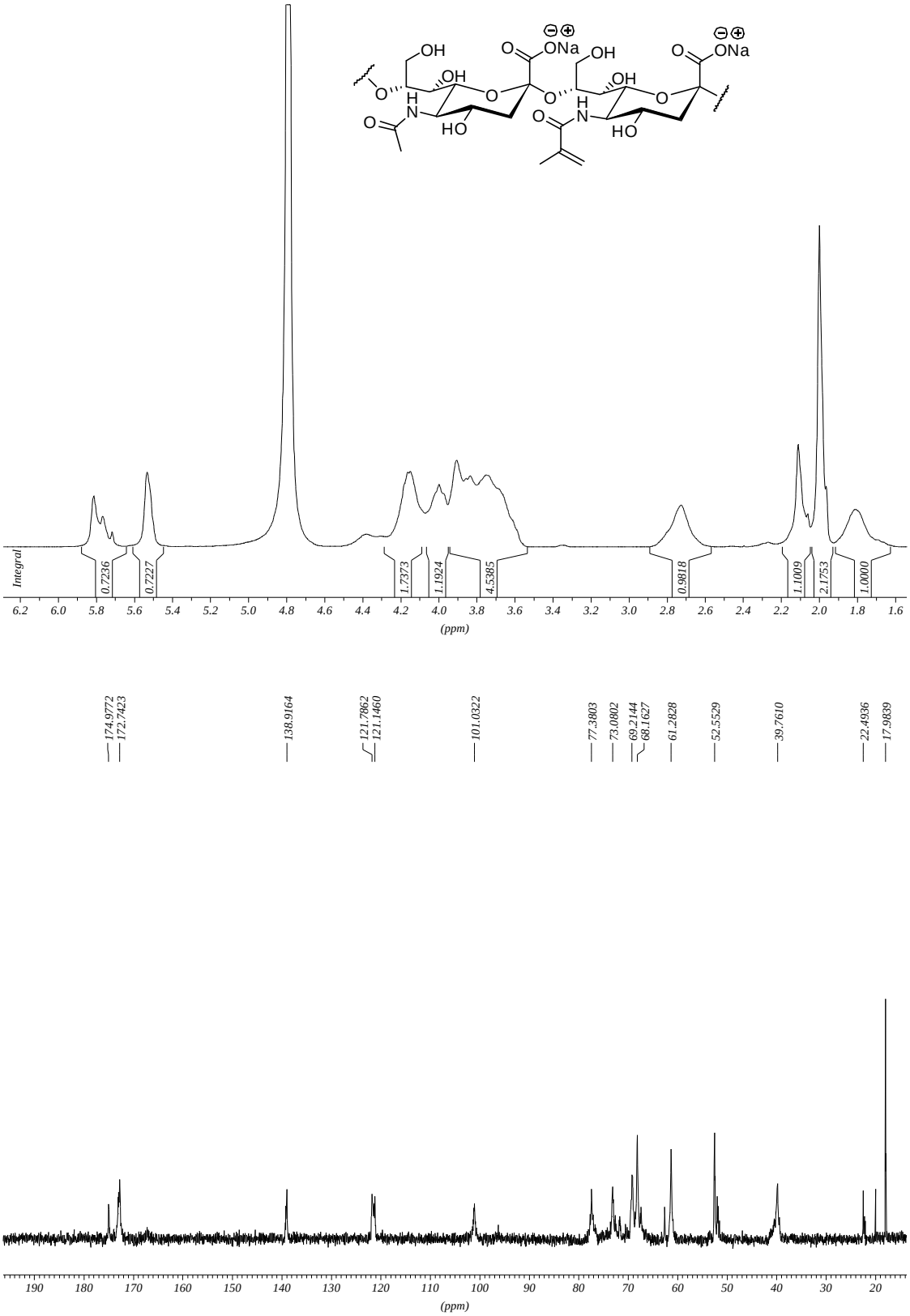




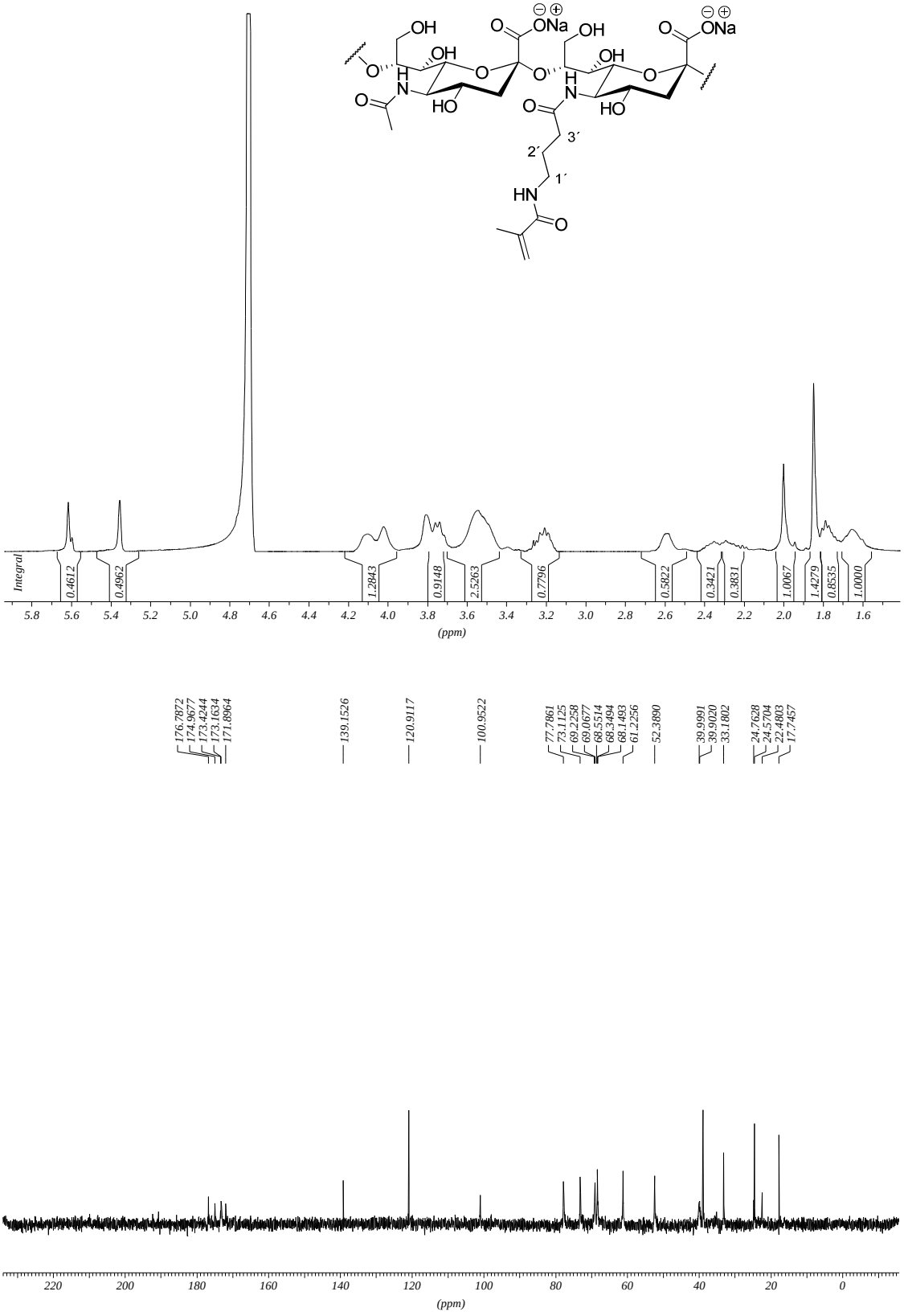
N-4-Azidobutanoyl-neuraminic acid **63**



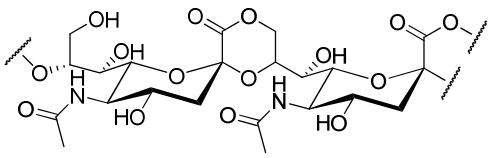
N-Methacryl-polySia **68**

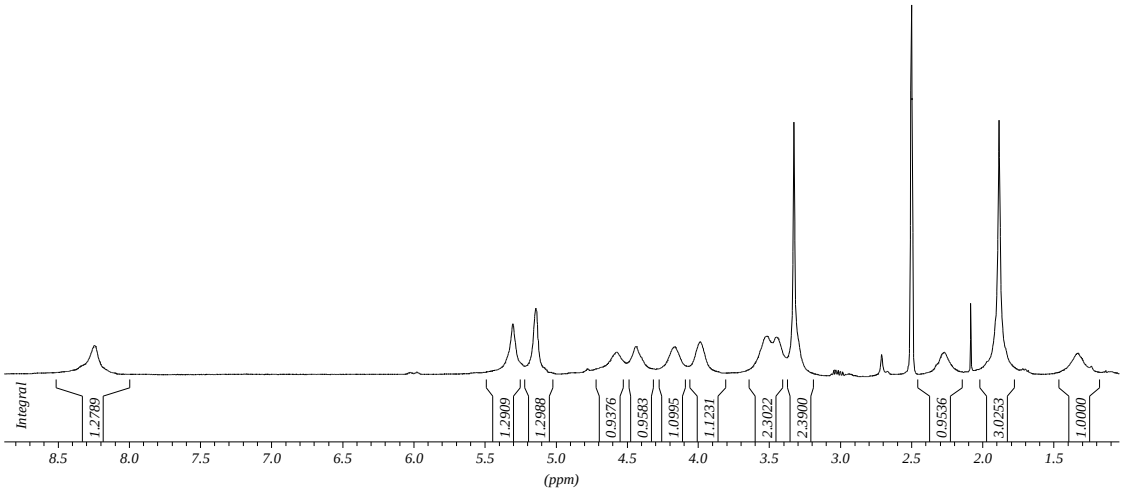


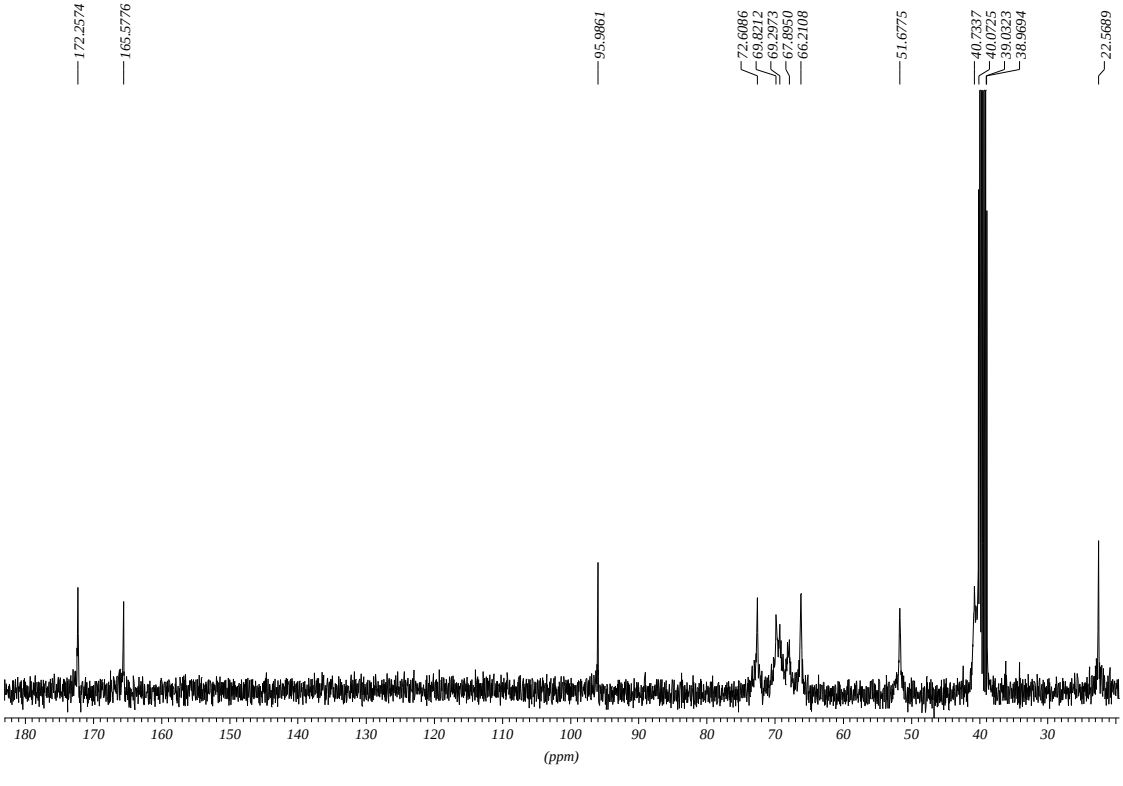
N-Methacryl-polySia **74**



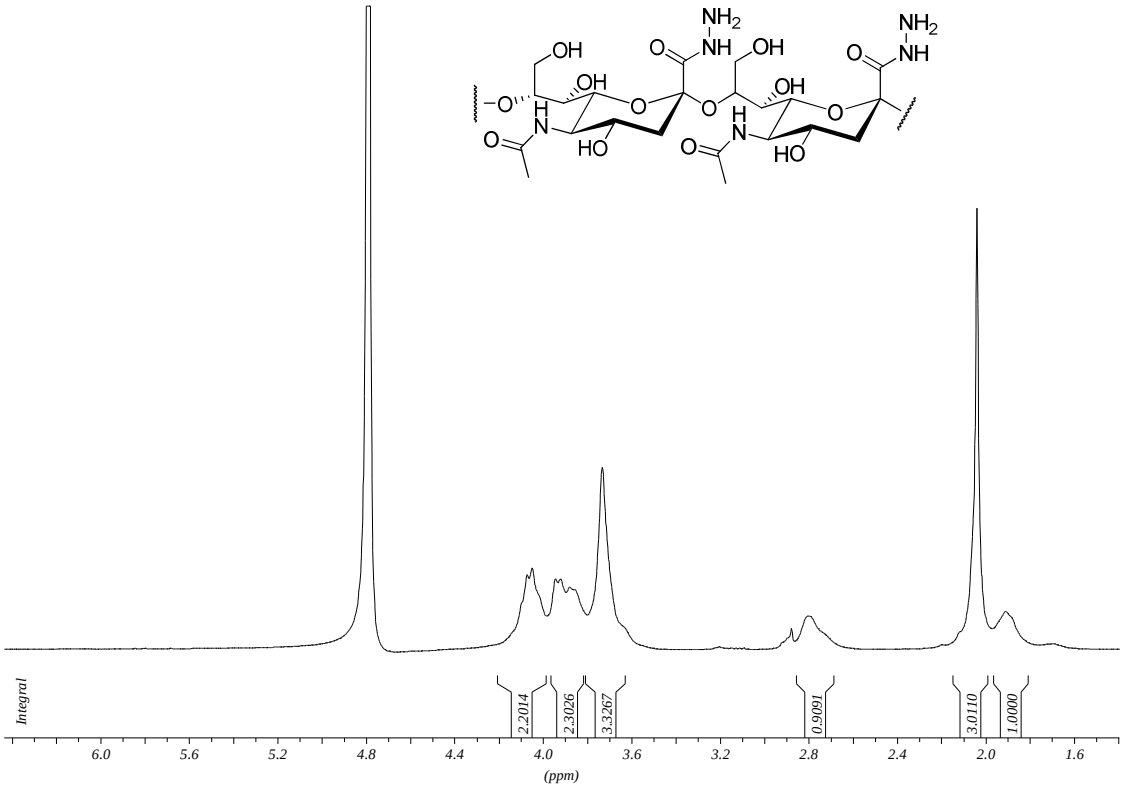
PolySia lactone **75**

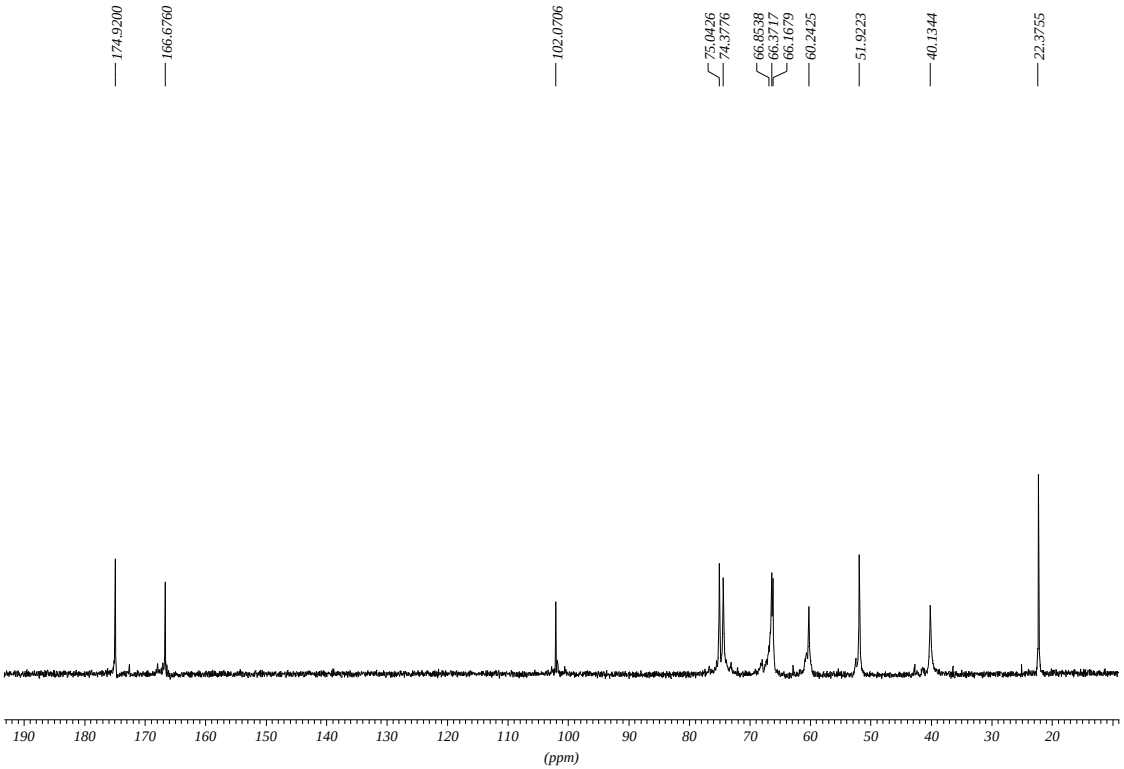




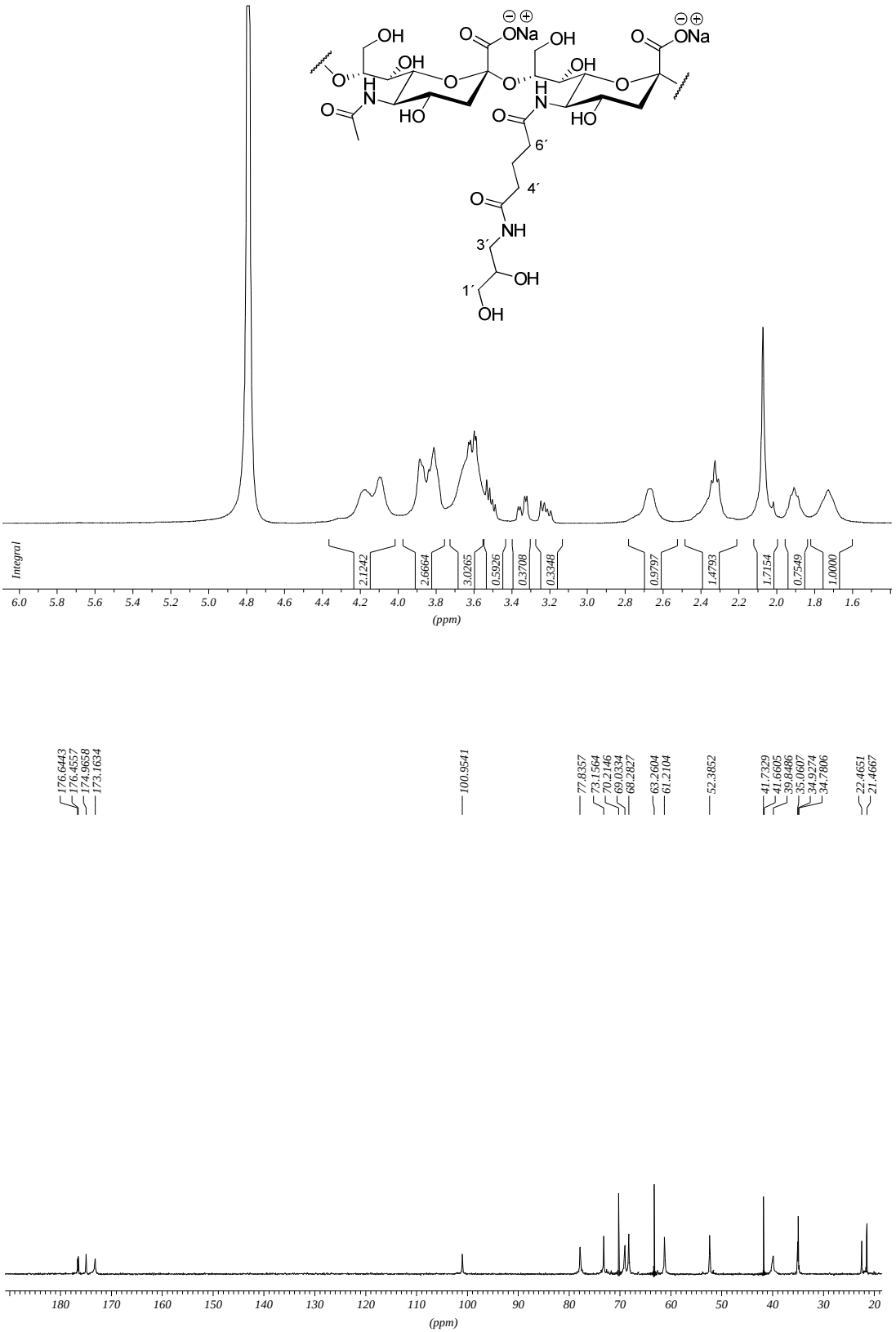


PolySia hydrazide **76**

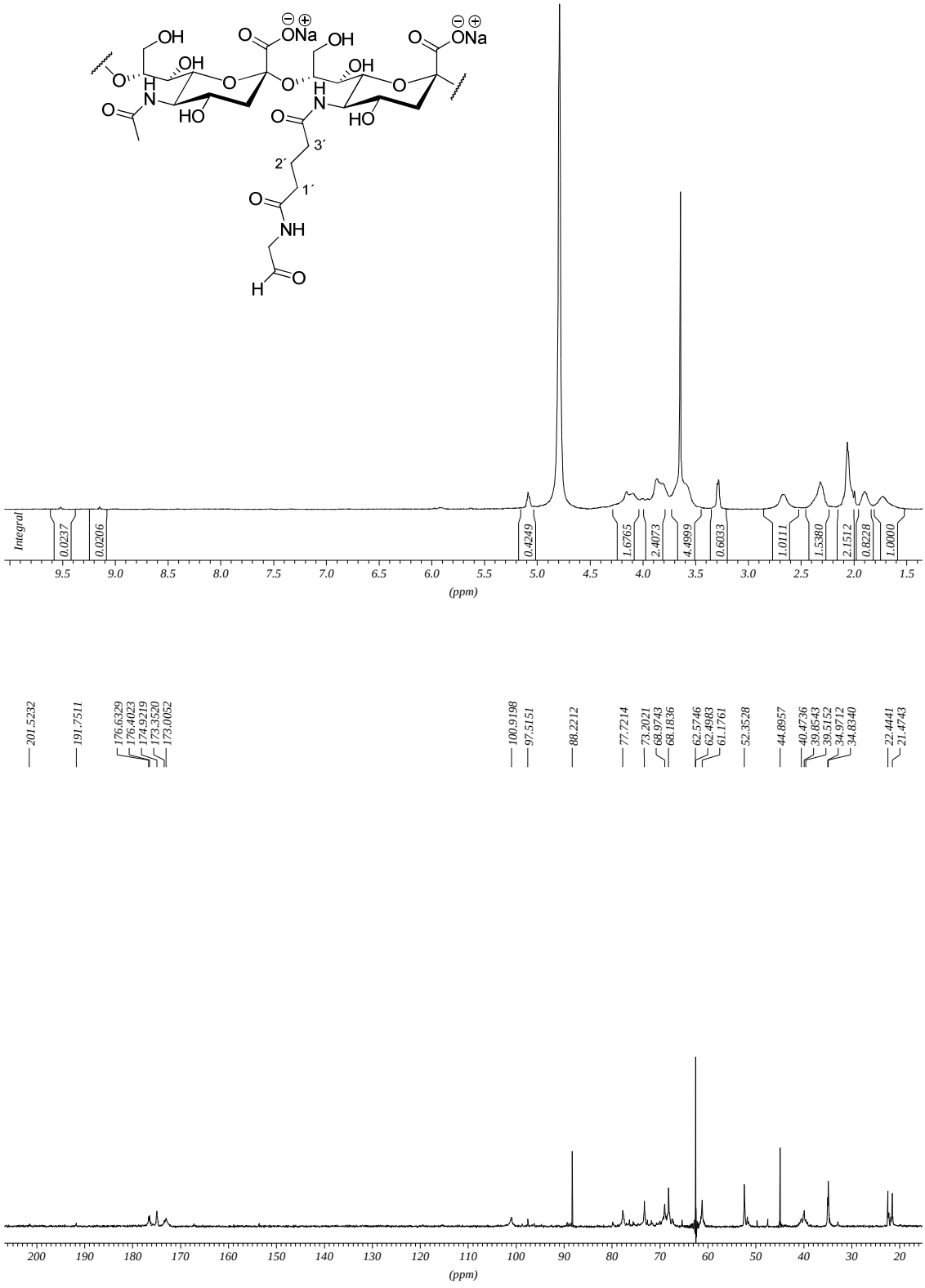




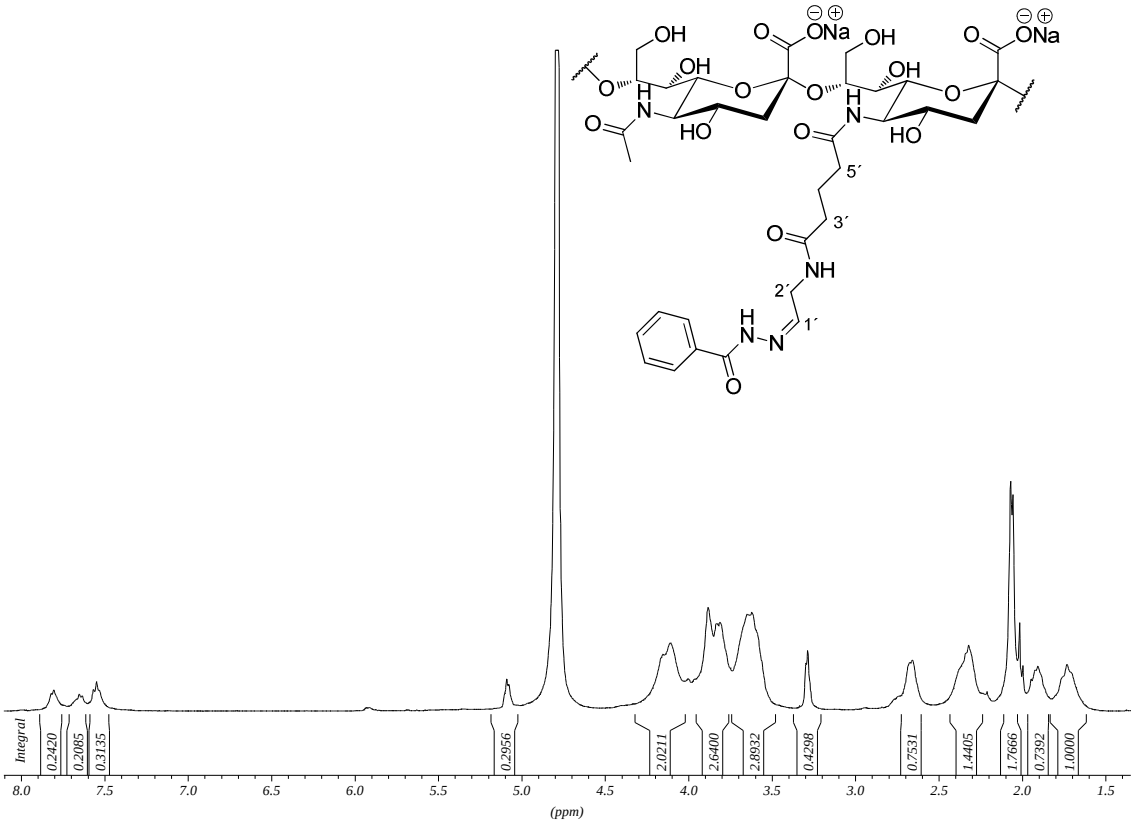
N-(1,2-diol) polySia **81**

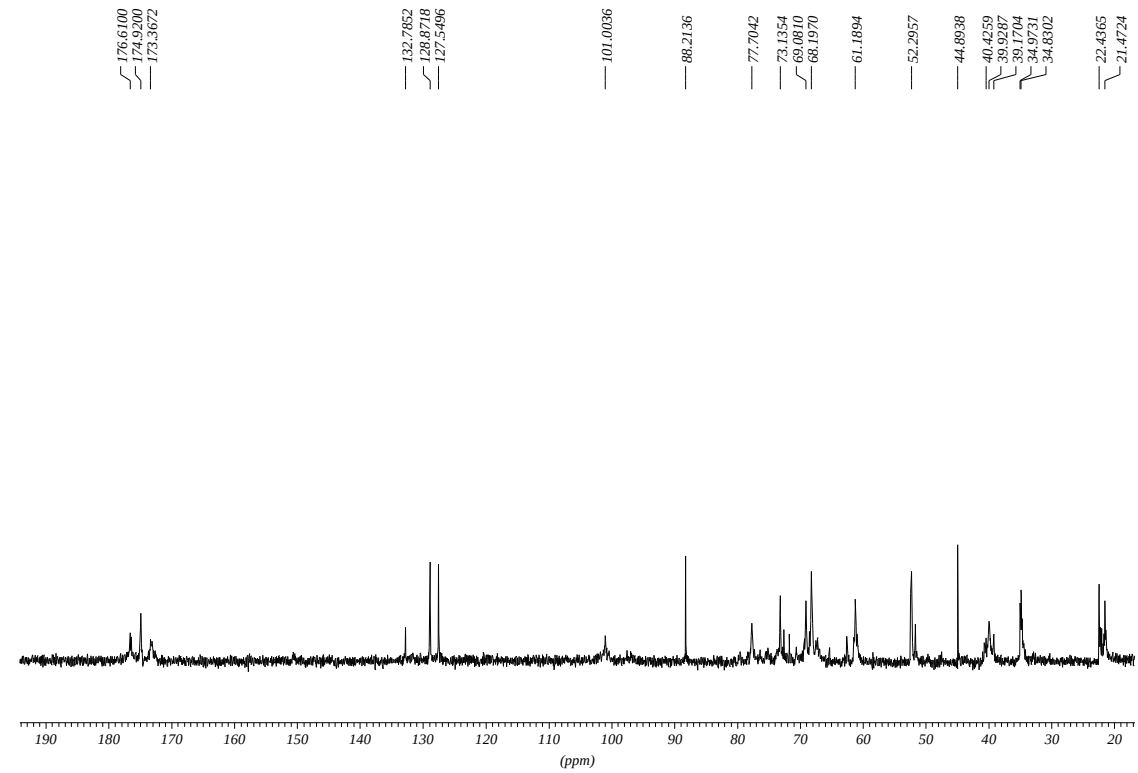


N-Aldehydo-polySia **82**

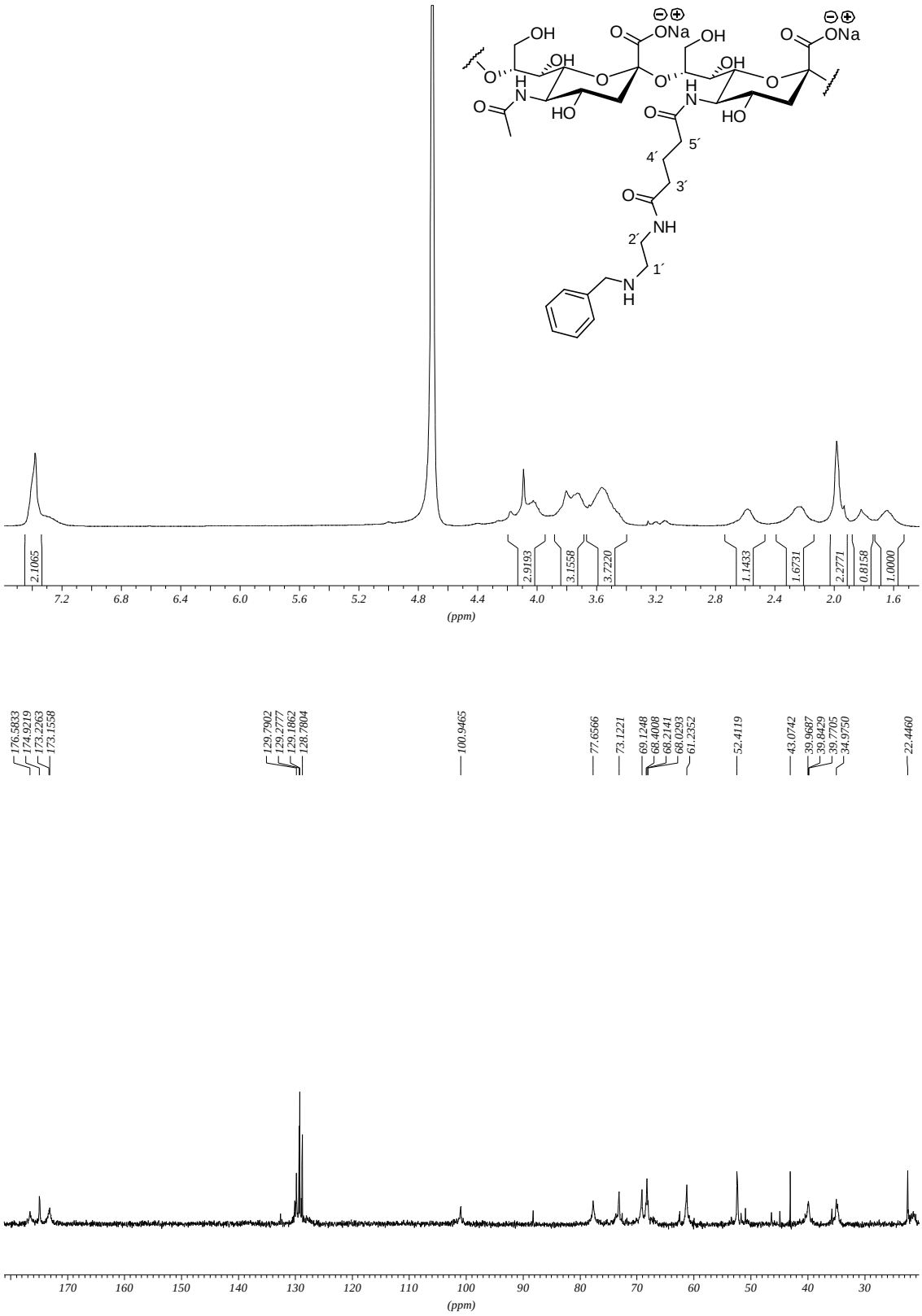


Benzhydrazone-polySia **83**

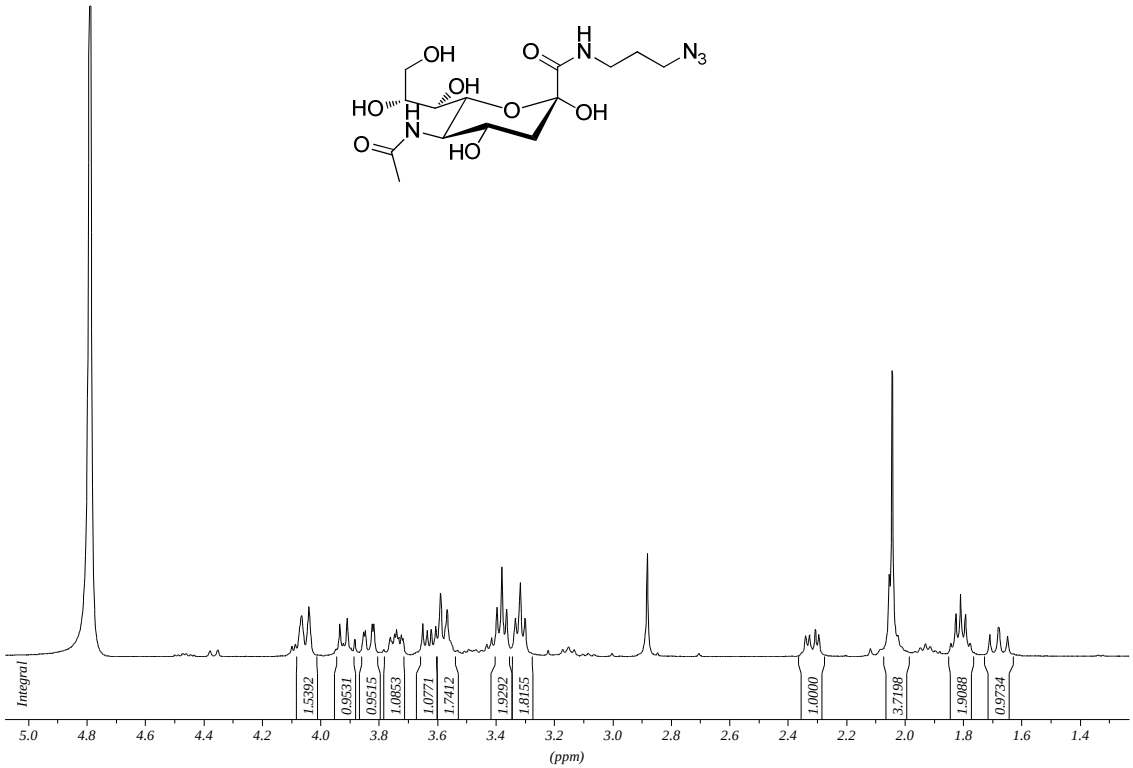


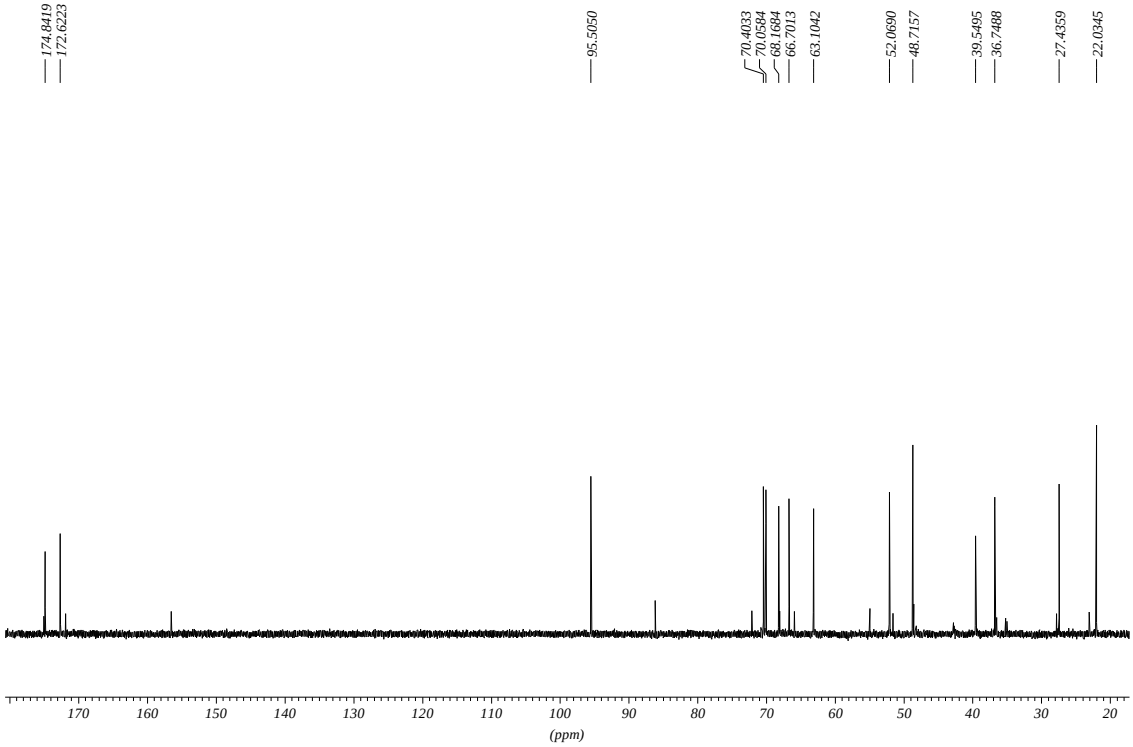


Benzylamino-polySia **89**

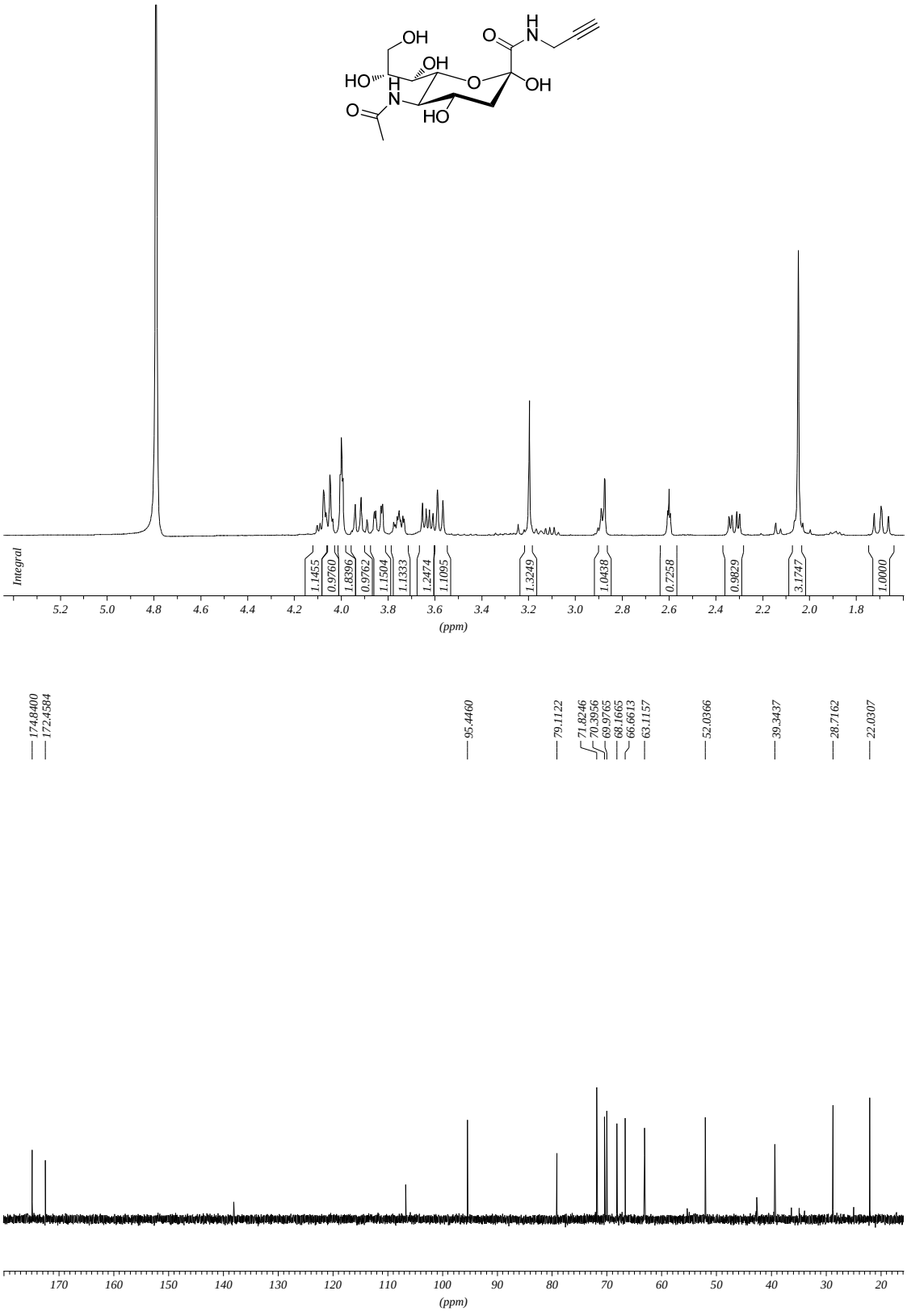


C-1 Azido-neuraminic acid **97**

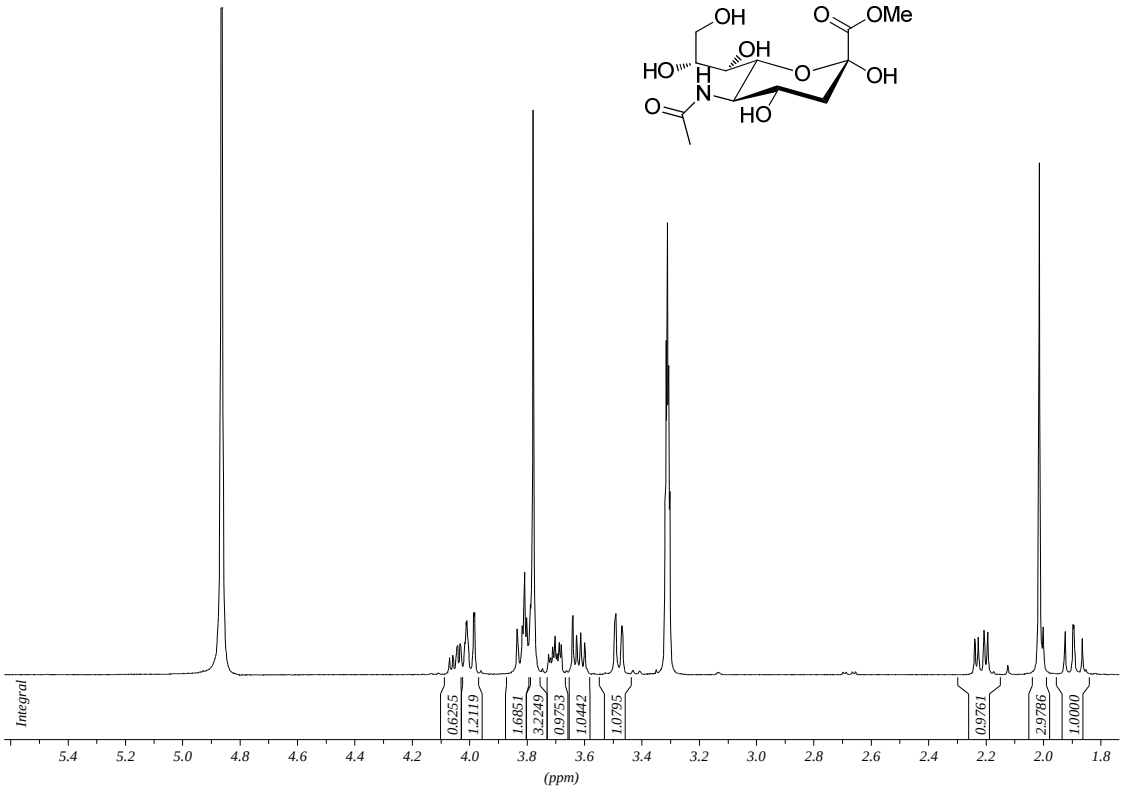


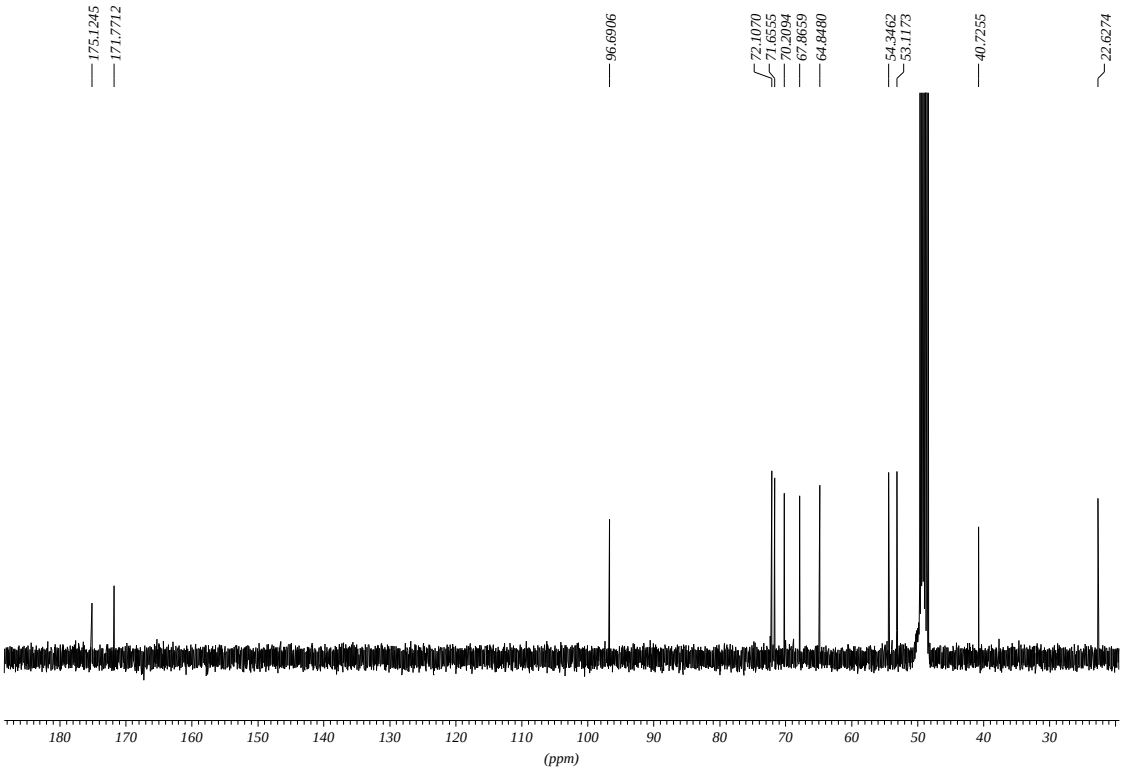


C-1 Alkyno-neuraminic acid **98**

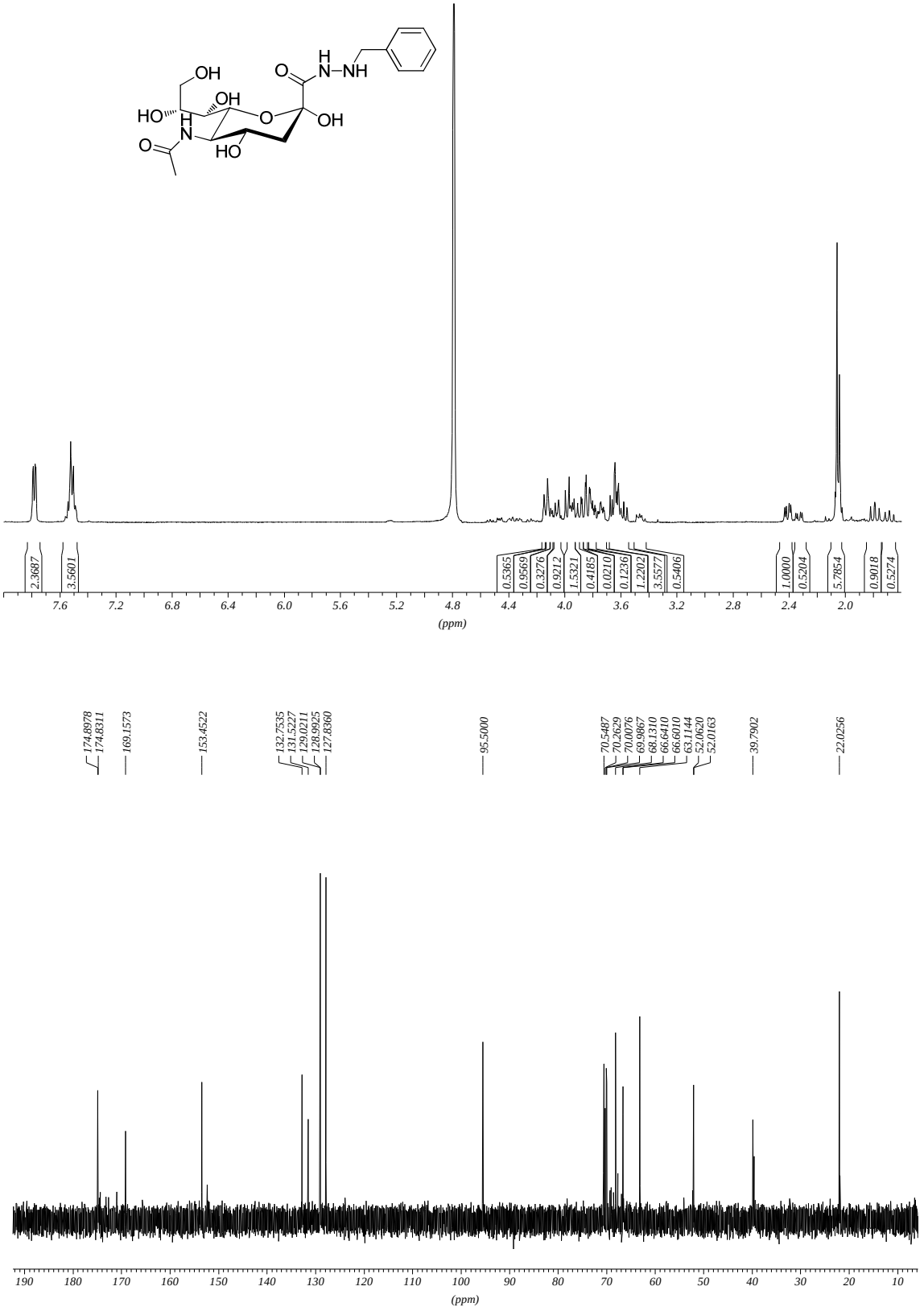


Neuraminic acid methyl ester **99**

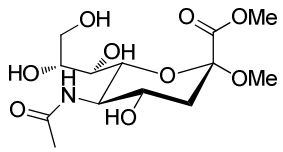


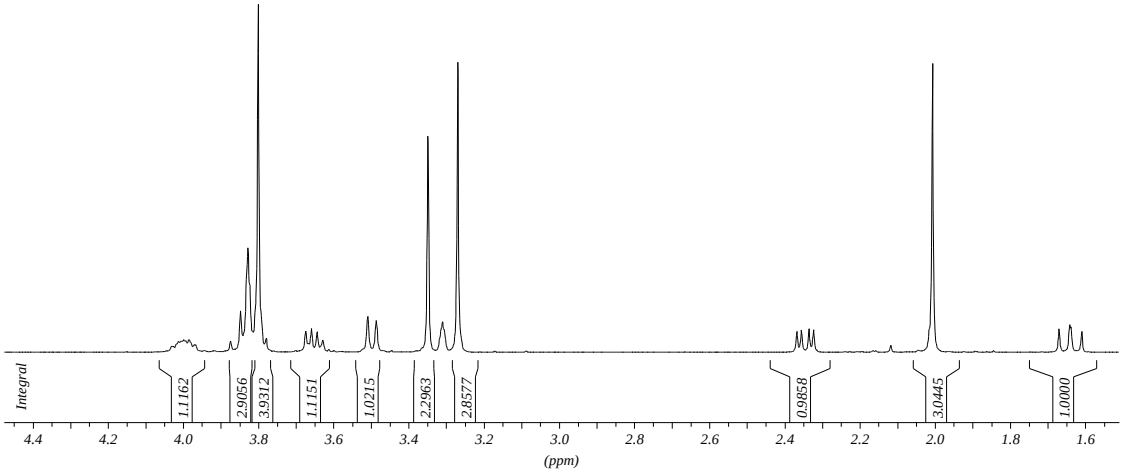


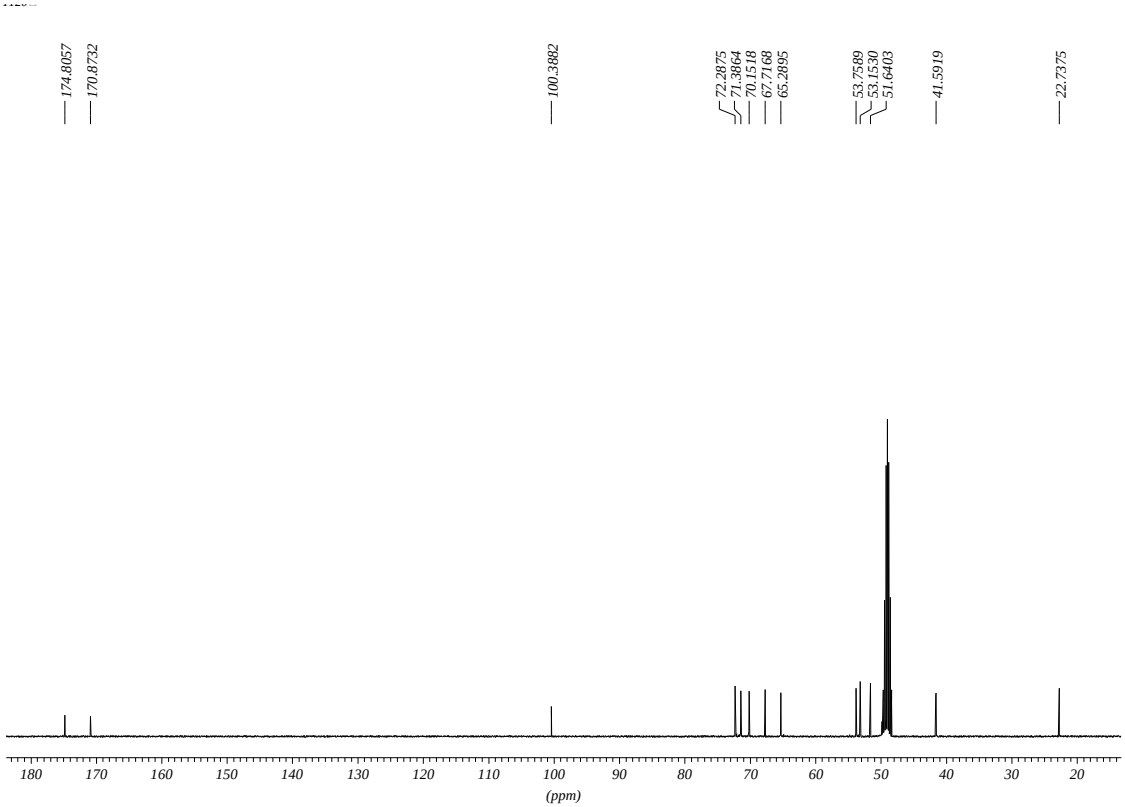
C-1 Benzylhydrazido-neuraminic acid **101**



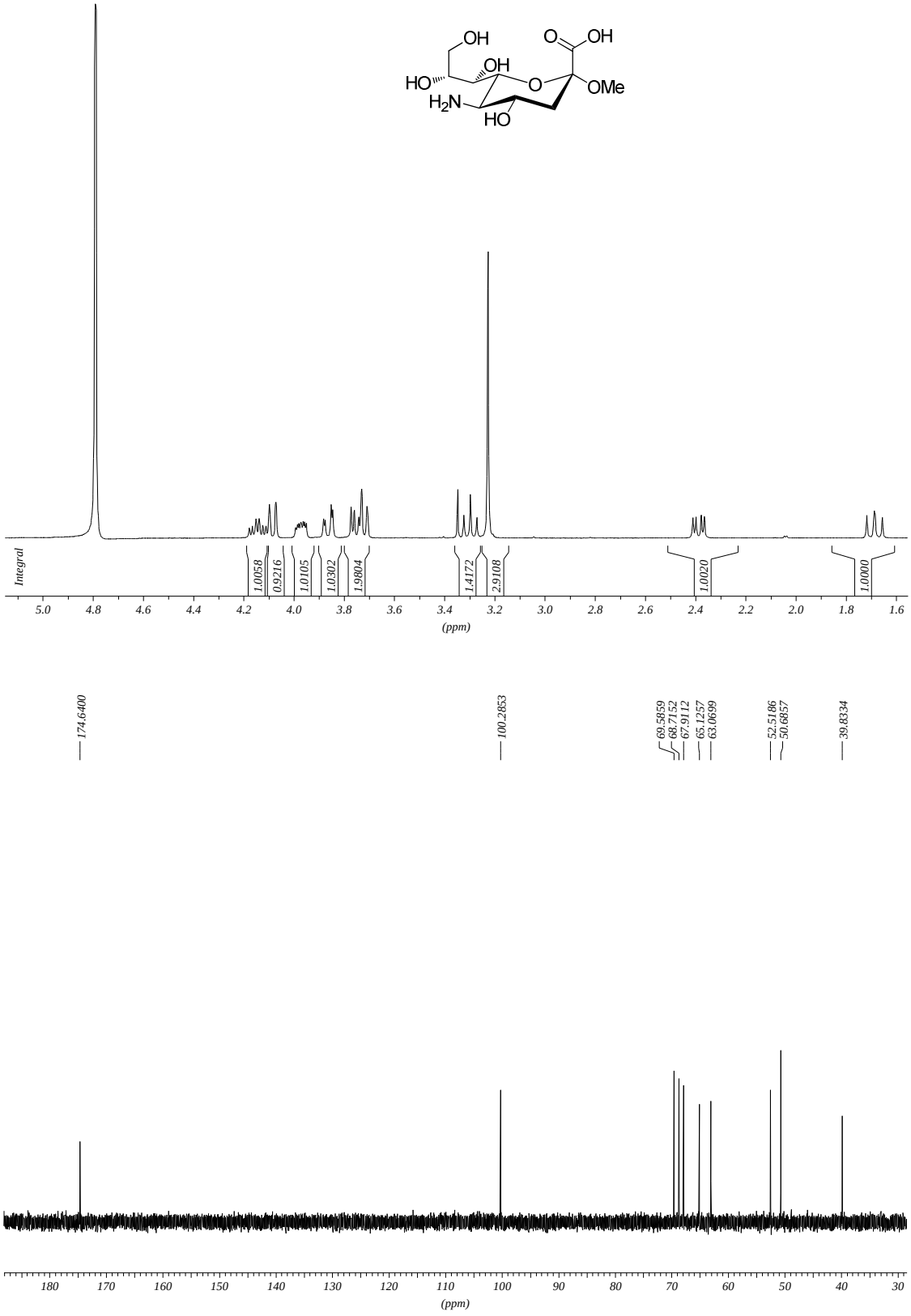
Methoxyneuraminic acid methyl ester **105**



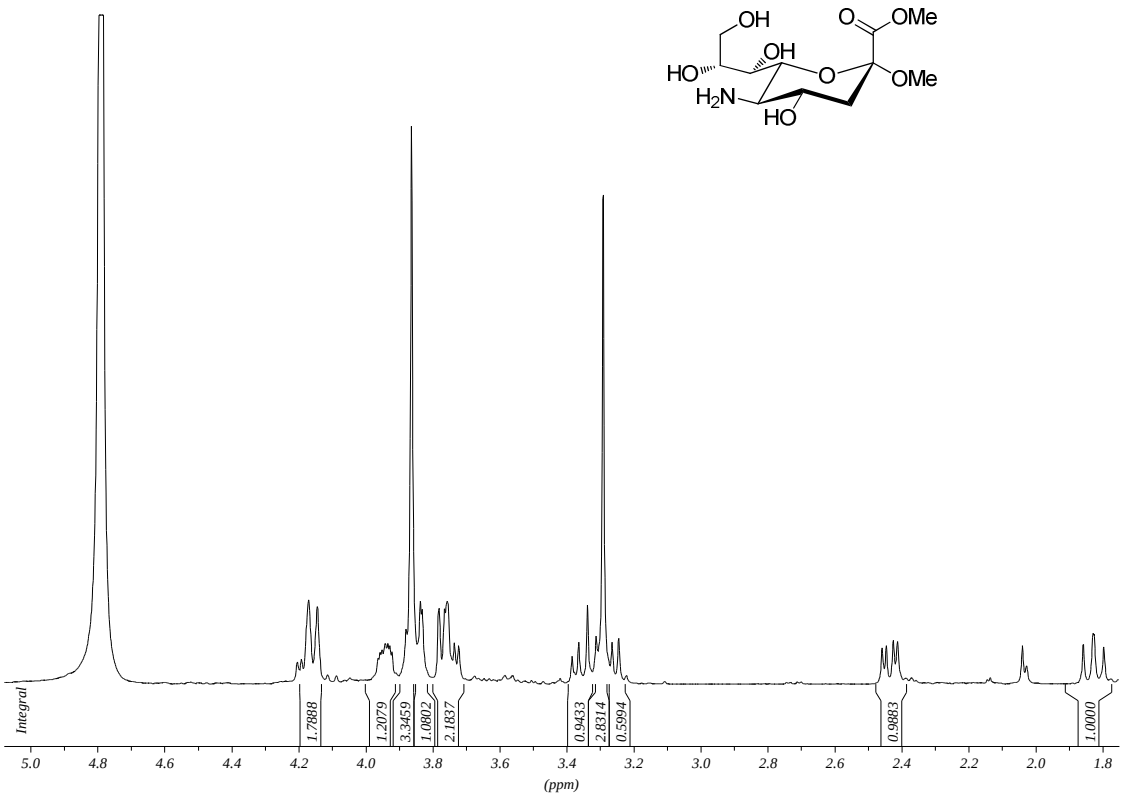


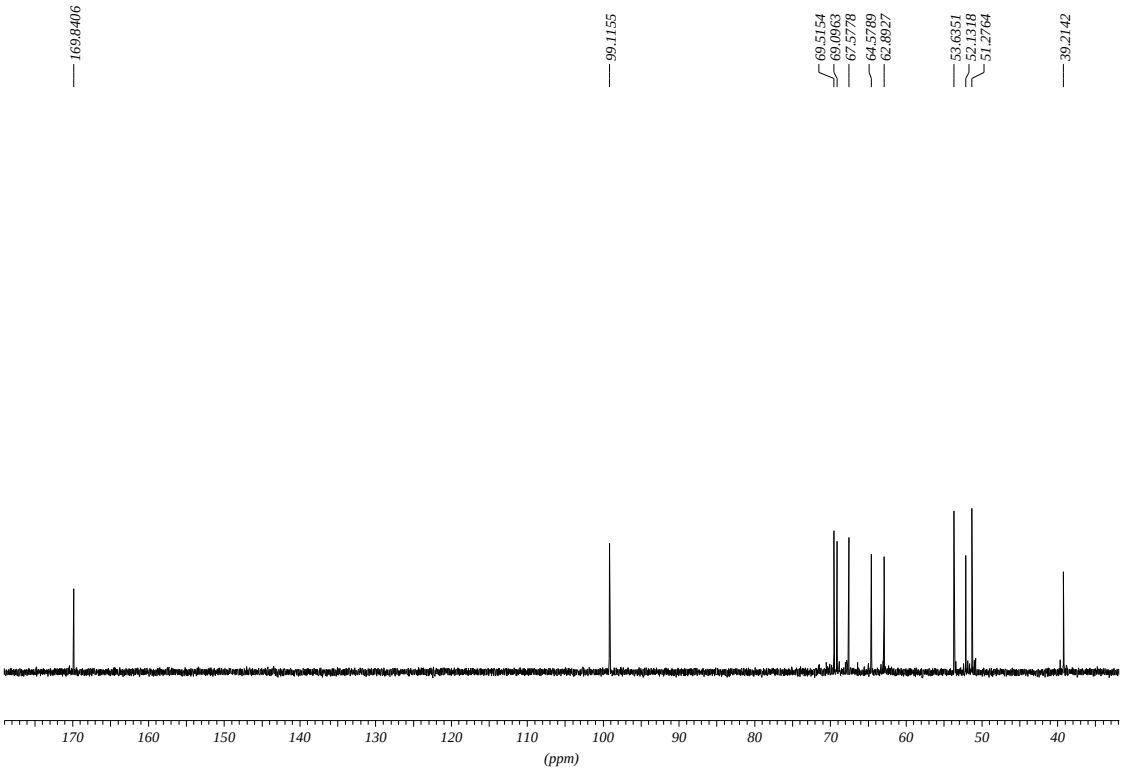


N-Amino methoxyneuraminic acid **106**

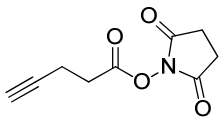


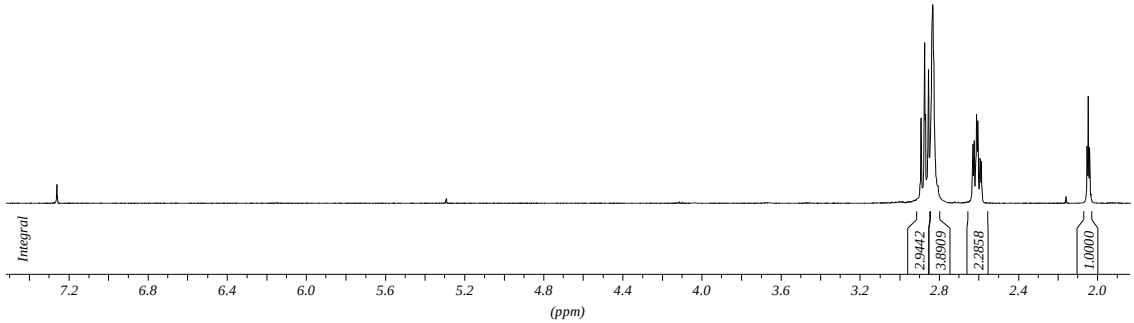
N-Amino methoxyneuraminic acid methyl ester **107**

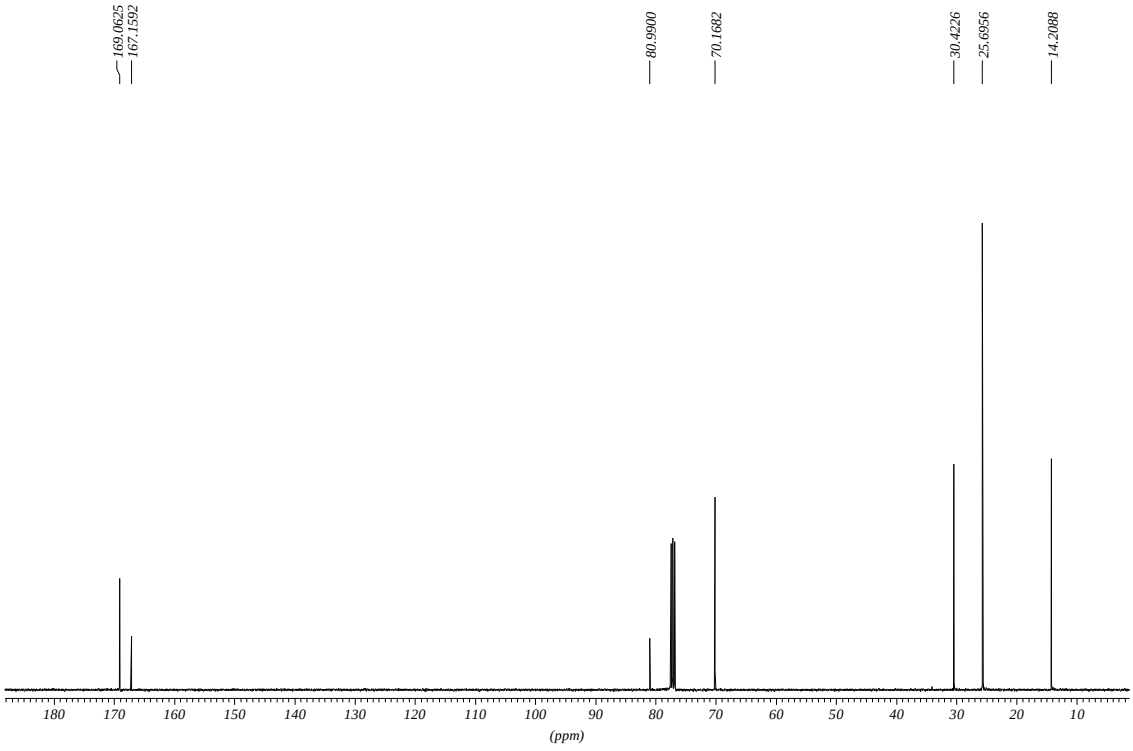




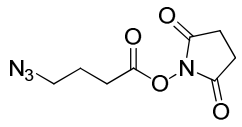
4-Pentynoyl NHS-ester **108a**

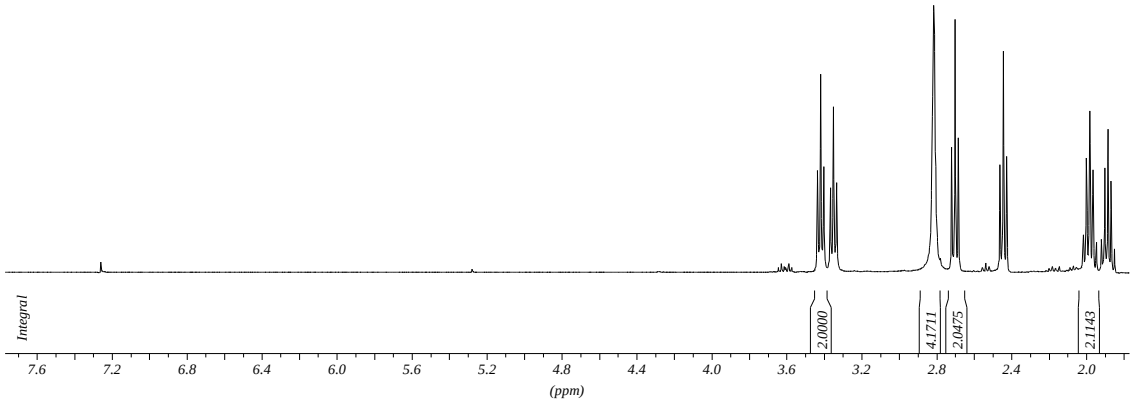


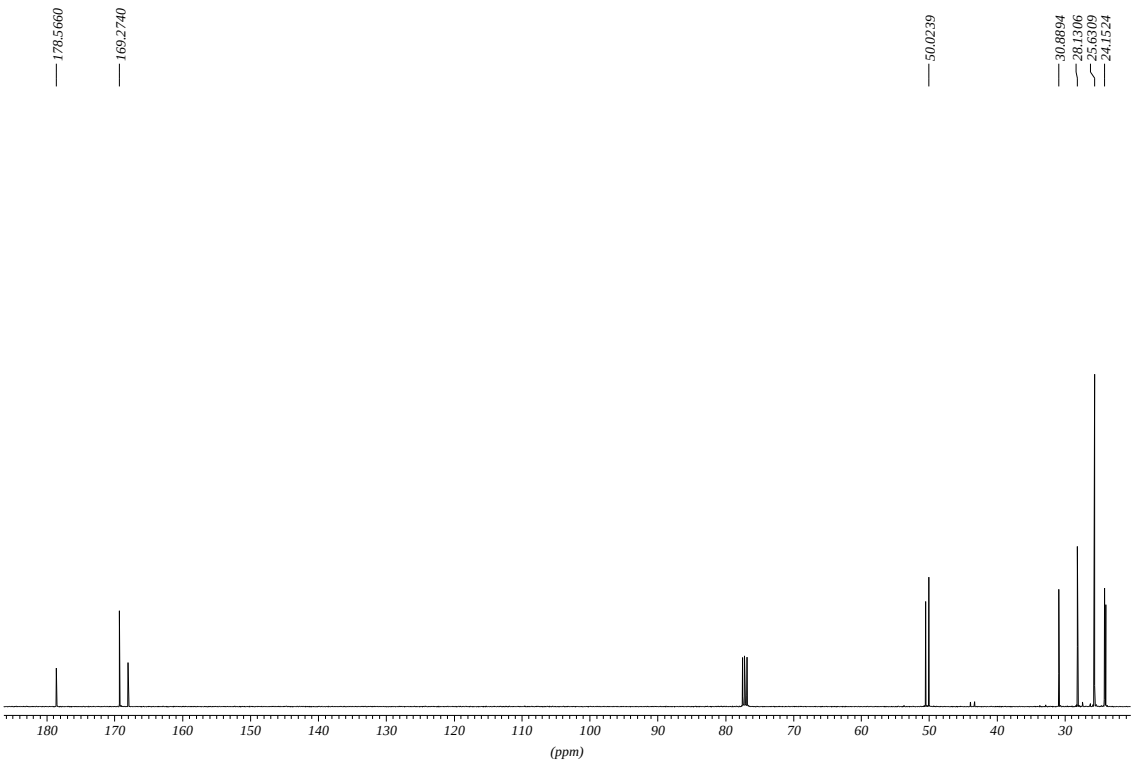




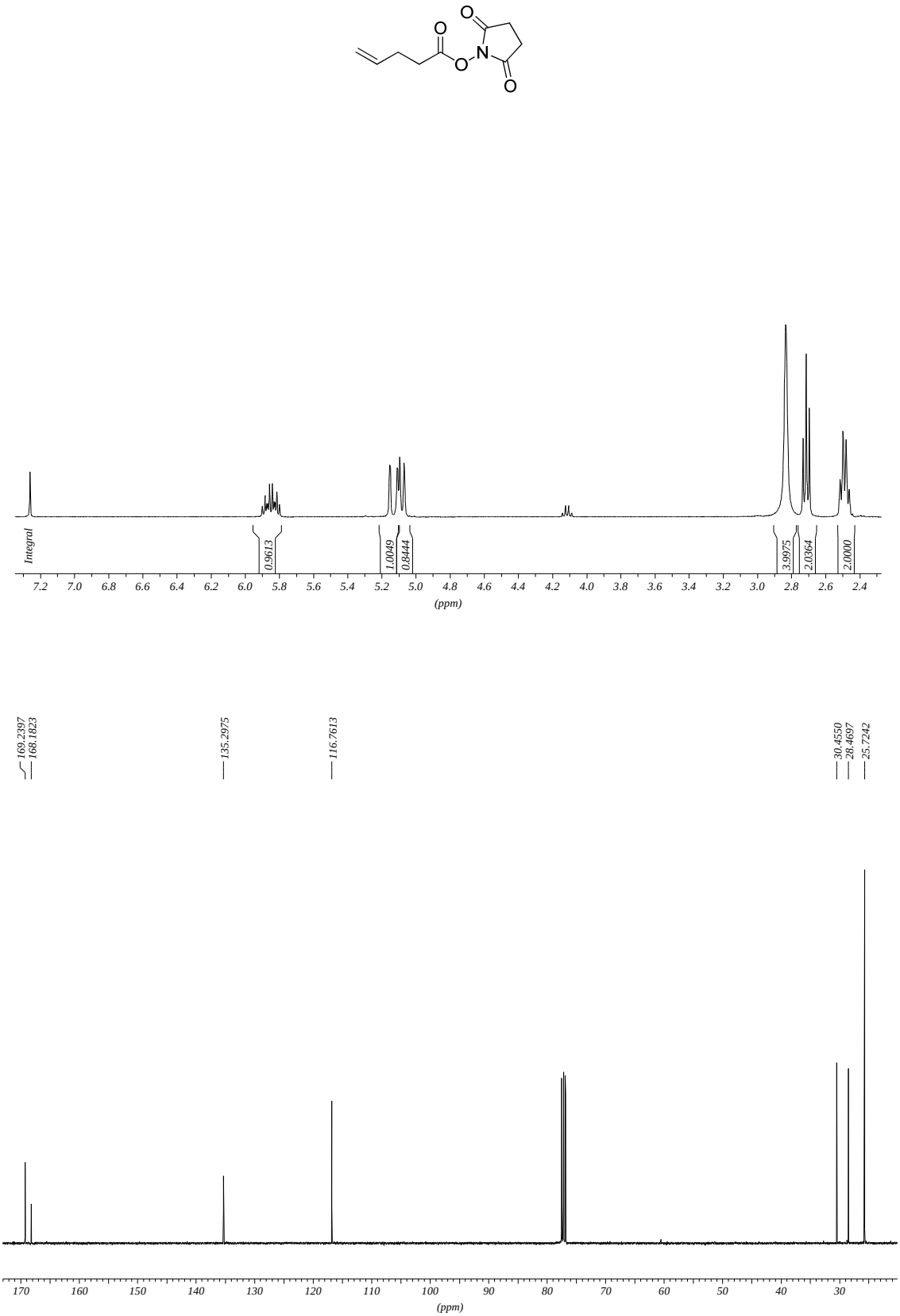
4-Azidobutanoyl NHS-ester **108b**



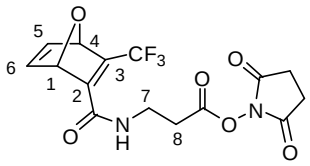


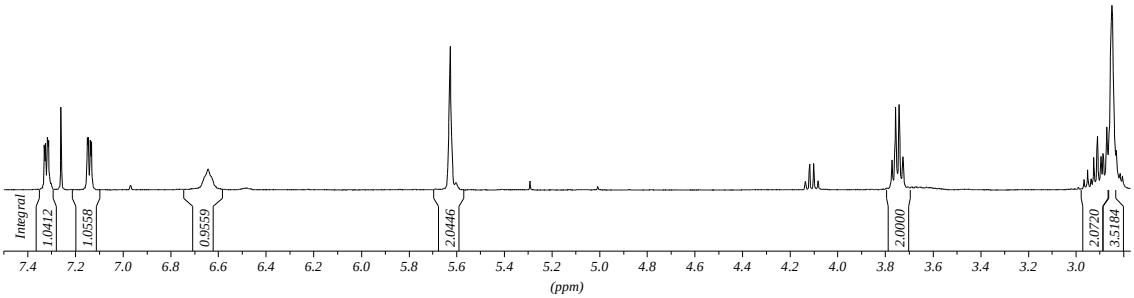


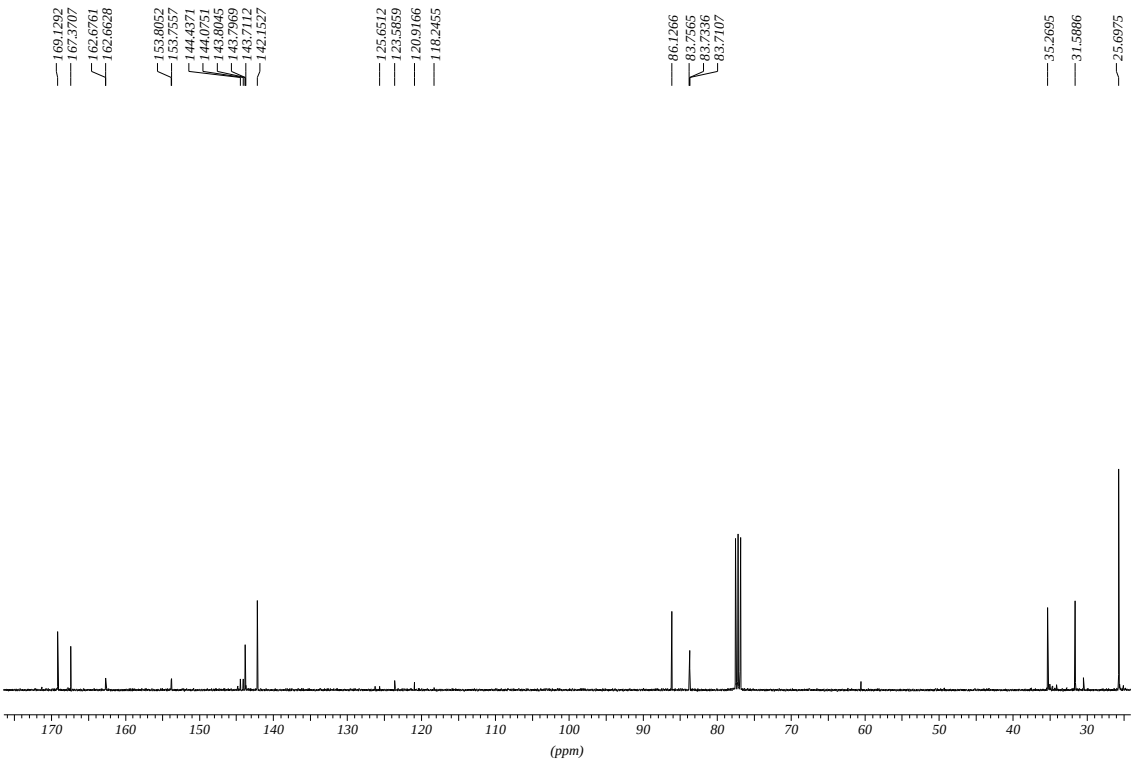
4-Pentenoyl NHS-ester **108c**



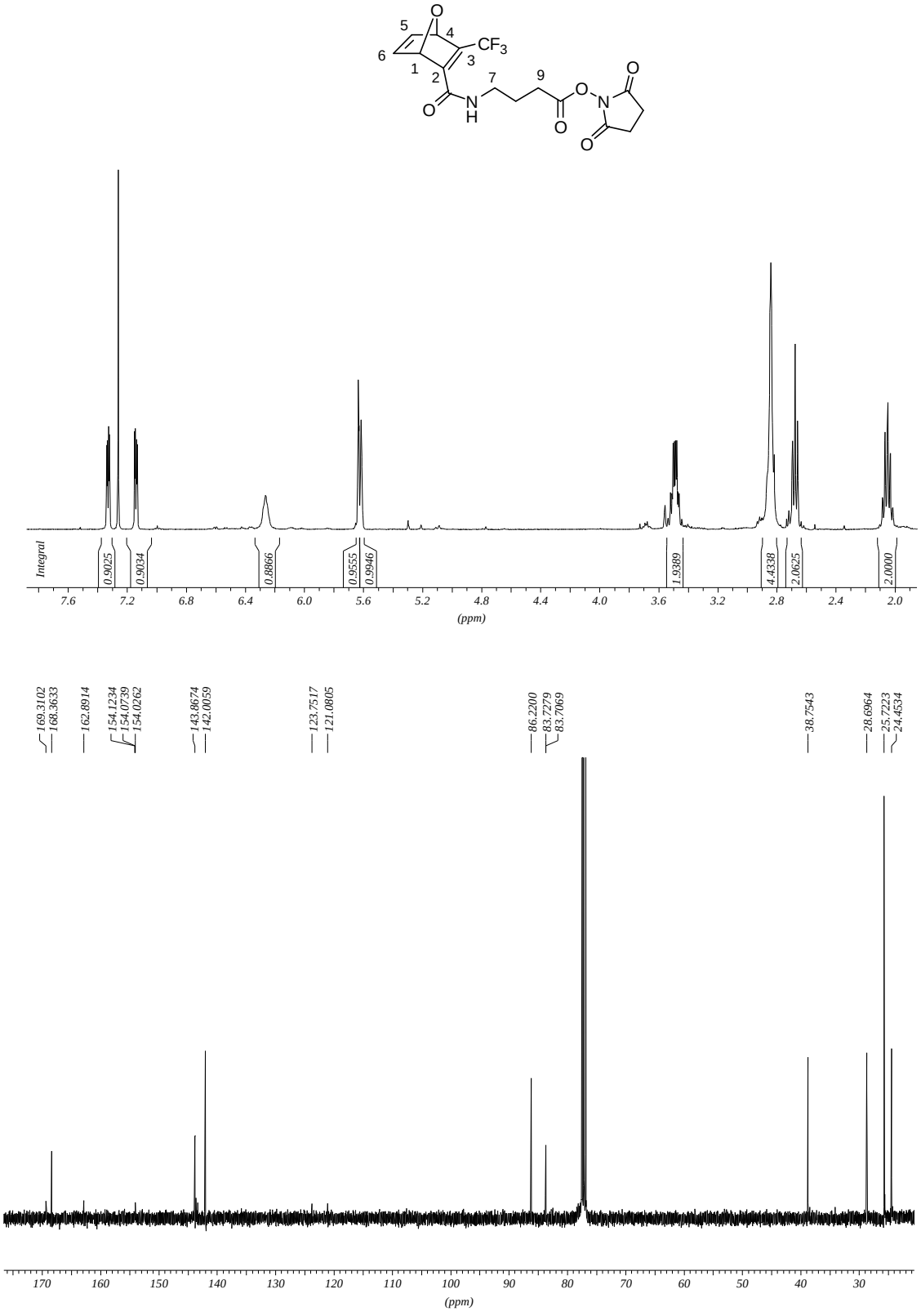
Oxanorbornadienyl NHS-ester **108e**



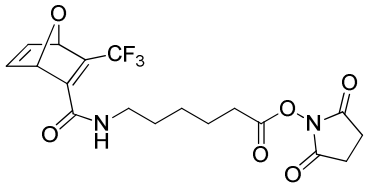


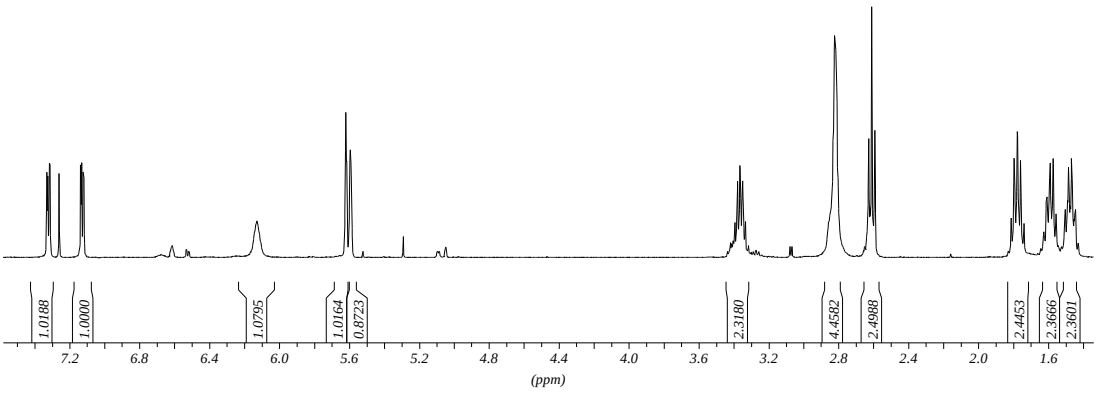


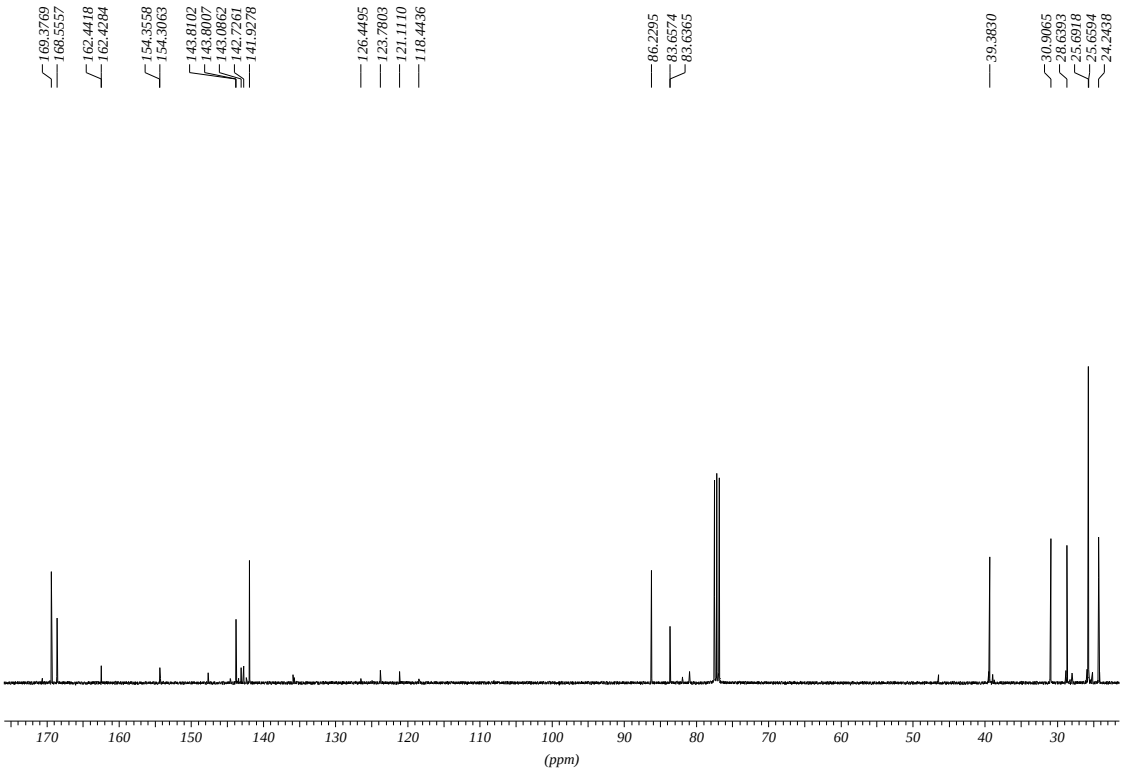
Oxanorbornadienyl NHS-ester **108f**



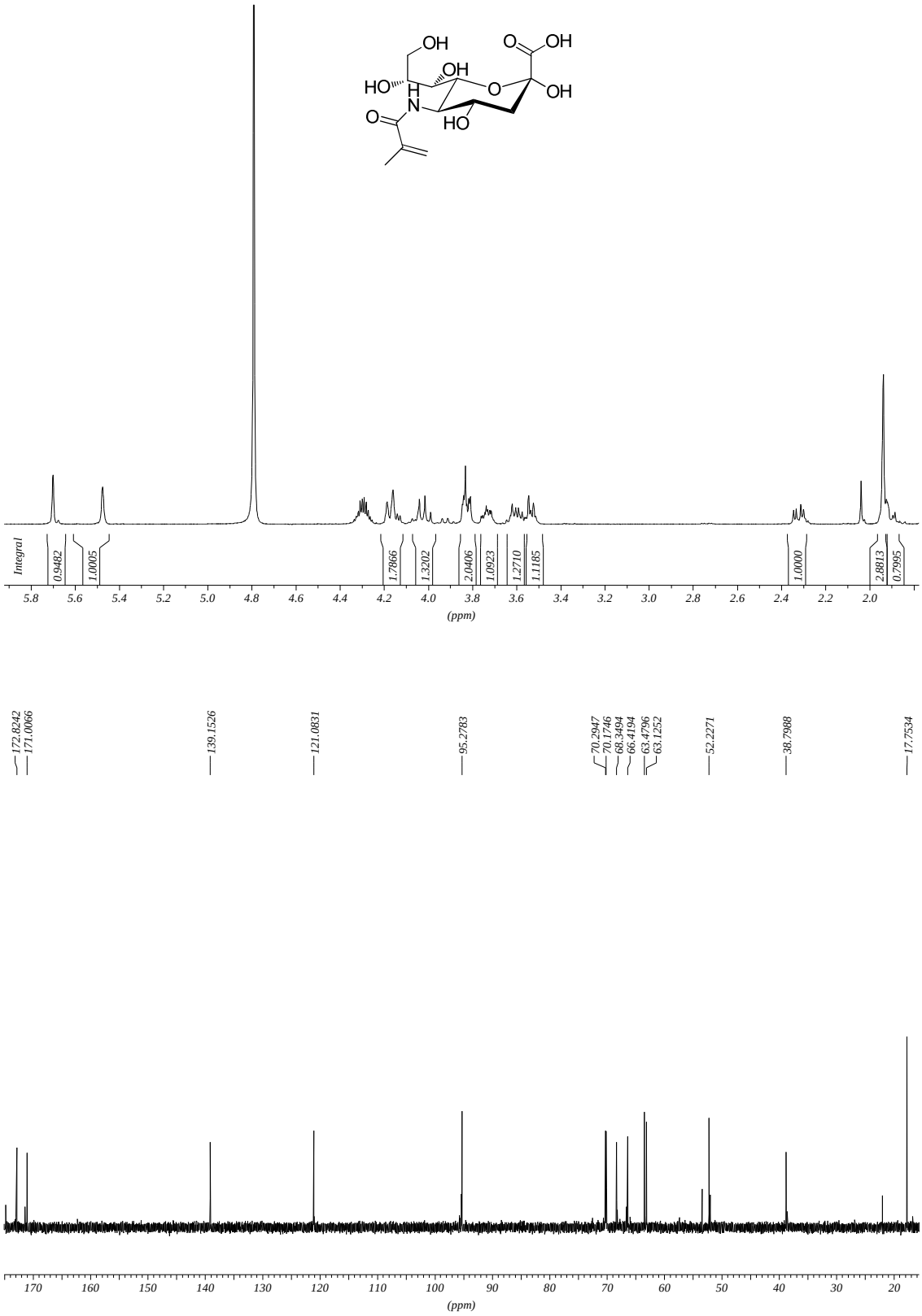
Oxanorbornadienyl NHS-ester **108g**



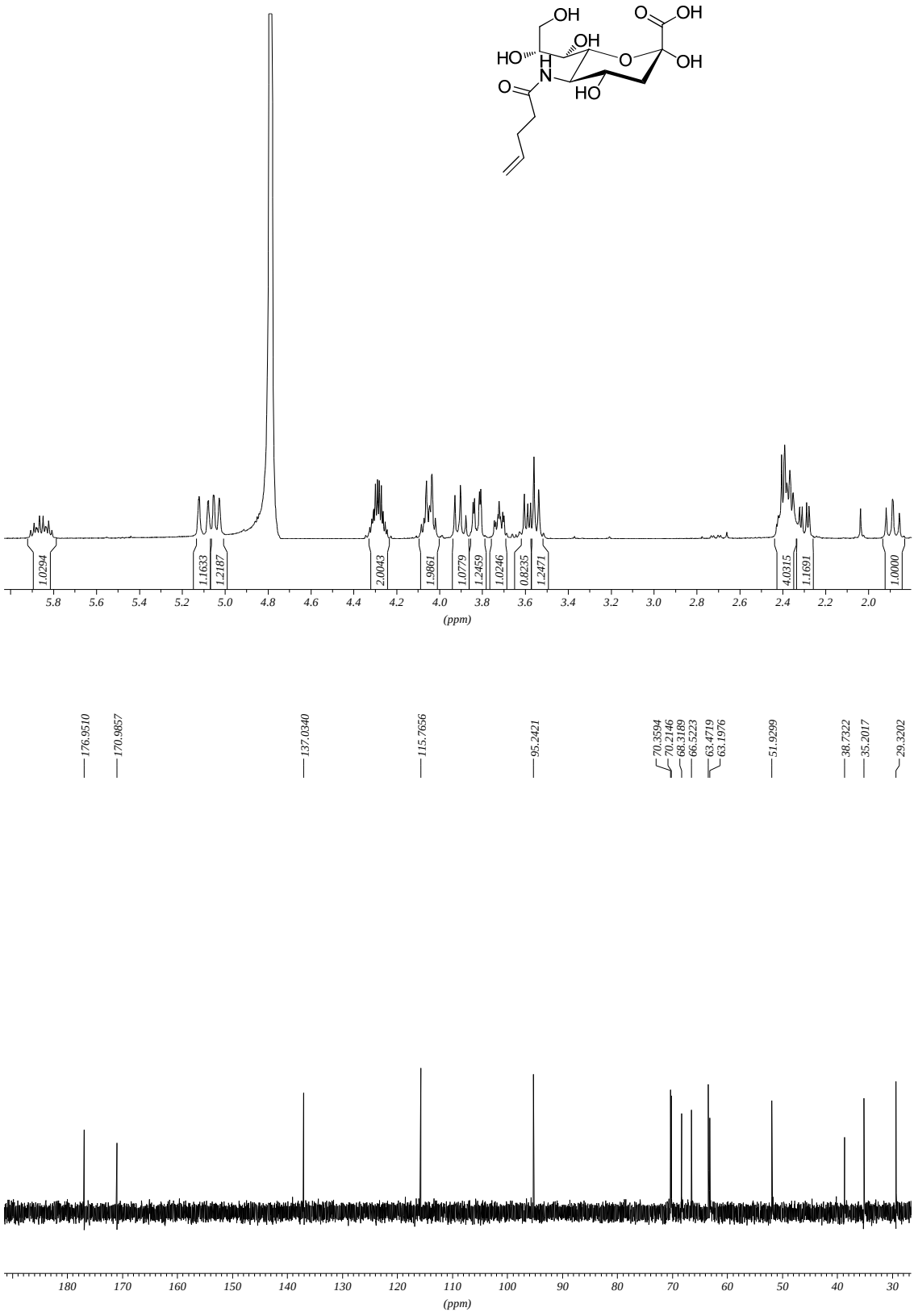




N-Methacryl-neuraminic acid **110**



N-4-Pentenoyl-neuraminic acid **111**



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Akademischer Werdegang

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