PART 1: SYNTHESIS OF HEPARIN OLIGOSACCHARIDES AND MIMETICS; PART 2: DEVELOPMENT TOWARDS GANGLIOSIDE BASED ANTICANCER VACCINES.

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Chemistry - Doctor of Philosophy

2016

ABSTRACT

PART 1: SYNTHESIS OF HEPARIN OLIGOSACCHARIDES AND MIMETICS; PART 2: DEVELOPMENT TOWARDS GANGLIOSIDE BASED ANTICANCER VACCINES.

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Heparin and heparan sulfate are implicated in cell signaling and a host of other biological processes. The main issue in evaluating these interactions is the sheer number of possible oligosaccharides and the fact that chemical synthesis of pure oligosaccharides continues to be long and arduous. Use of the natural sulfotransferase enzymes can allow the divergent synthesis of multiple sulfation patterns from a single backbone, greatly simplifying the synthesis of a library of heparin and heparan sulfate oligosaccharides. Herein a single hexasaccharide backbone has been elaborated into 7 different sulfation patterns utilizing both chemical and enzymatic sulfation. These along with other oligosaccharides have been used to make a carbohydrate microarray to evaluate binding with FGF-2. Another route utilizing heparin mimetics was also explored.

Part 2 entails the development of anticancer vaccine based on the GM2 tumor-associated carbohydrate. GM2 was chemically synthesized and conjugated to the virus-like Qβ particle. Conjugation using copper-catalyzed azide-alkyne cycloaddition efficiently linked GM2 but the resulting product only produced significant antibodies against the triazole ring formed by coupling. Switching to a thiourea linker produced a construct that elicited a strong immune response including IgG antibodies that could bind GM2-positive tumor cells and were found to be effective in complement dependant cytotoxicity.

ACKNOWLEDGEMENTS

I need to start with thanking Professor Xuefei Huang. He has been extremely helpful in allowing me to gain experience in almost all aspects of higher education from writing AHA grants to sending me to UNC Chapel Hill to work with our collaborator Prof. Jian Liu. This continues, even as I finish my dissertation from Angola, with quick feedback and helpful suggestions. Aside from Prof. Huang, many faculty members at Michigan State have been paragons of aid. Professor Walker graciously agreed to be my second reader with only a months warning. Professor Jackson has not only joined my committee on short notice, but written a few letters of recommendation. Of course Professor Spence may have sacrificed the most, accepting my invitation to join a committee for an organic student, if the groans I hear from my students at Trine University are to be believed.

Support staff at have also played integral roles in my success at MSU. Dr. Dan Holmes and Kermit Johnson assisted me in running and tweaking experiments on the 900 MHz NMR, along with setting up the exceedingly useful 2-D TOCSY pulse sequence up for the automated NMRs. Professor Dan Jones and Lijun Chen in the Mass Spectrometry facility were always willing to lend a hand finding and evaluating conditions for ionizing tricky sugar molecules. It wasn't just the faculty and staff at MSU that made it great, students and postdocs were always willing to lend a hand or thought.

Huang Group members were from disparate areas of the globe but all shared a passion for our research. Post docs Keisuke, Zhaojun, Gopi, Mo, and especially Medha were knowledgeable and generous with their time and talents. My fellow graduate students were no slouches either. Bo and Gilbert were great for spitballing synthesis problems while Hovig, Herbert, and Phil were exceptional for biological and ELISA questions. Without naming everyone there was no lack of

collaboration and help in the Huang group. Each person had at least one reaction or technique that they could teach everyone, without gripe or grumble. Of course I have to mention Zhen Wang, who started my project and left behind the scaffold for me to build upon. It was not just colleagues and coworkers who assisted my journey, but also family.

My parents, Don and Julie, and grandparents, Earlene and Bernard, were rocks of support for as long as I can remember. While none had higher than a high school education they were the greatest cheerleaders a student could find. Whether it was a box of cookies or blue jeans, my mother could not be help herself from sending support. And lastly, to ensure as long a life as possible, I must thank my wife, Emily, and children, Amelia and Fiona. Emily has had to endure numerous years with little quality time or contact, but has been supportive through it all. So I'll have to return the favor as she finishes up her Master's degree this coming spring.

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KEY TO ABBREVIATIONS

2-NAP	2-naphthyl
2-OST	2-*O*-sulfotransferase
3-OST	3-*O*-sulfotransferase
6-OST	6-*O*-sulfotransferase
AcOH	Acetic acid
Ac_2O	Acetic anhydride
Ac	Acetyl
AgOTf	Silver triflate
All	Allyl
ATIII	Antithrombin III
AZMB	2-(azidomethyl)benzoyl
BAIB	Iodobenzene diacetate
Bn	Benzyl
BnBr	Benzyl bromide
BRSM	Based on recovered starting material
BSA	Bovine serum albumin
BSP	1-benzenesulfinylpiperidine
Bz	Benzoyl
$C_{5\text{-epi}}$	C_5-epimerase
CAN	Ceric ammonium nitrate
cat.	Catalytic
Cbz	Benzyloxycarbonyl

conc.	Concentrated
COSY	Correlation Spectroscopy
CSA	Camphorsulfonic Acid
CuAAC	Cu(I)-catalyzed azide-alkyne cycloaddition
Cy5	Cyanine dye
d	Day
DBU	1,8-diazabicyclo[5.4.0]undece-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-dichloro-5,6-dicyanobenzoquinone
DIAD	Diisopropylazodicarboxylate
DIC	*N*,*N*'-diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMTST	dimethyl(methylthio)sulfonium triflate
DMSO	Dimethyl sulfoxide
EDC. HCl	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide Hydrochloride
ELISA	Enzyme-linked immunosorbent assay
Et	Ethyl
EtOAc	Ethyl acetate
Et_3N	Triethylamine
FGF	Fibroblast growth factor
FITC	Fluorescein isothiocyanate

Fmoc	Fluorenylmethoxycarbonyl
FPLC	Fast protein liquid chromatography
GAG	Glycosaminoglycan
GalN	Galactosamine
GalNAc	*N*-acetylgalactosamine
D-GlcA	D-glucuronic acid
D-GlcN	D—glucosamine
D-GlcNAc	*N*-acetyl-D-glucosamine
GM2	Ganglioside monosialic two
GM3	Ganglioside monosialic three
h	Hour
HDTC	Hydrazine dithiocarbonate
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HOBT	Hydroxybenzotriazole
HPLC	Higher performance liquid chromatography
HRMS	High Resolution Mass Spectrometry
HS	Heparan sulfate
HSQC	Heteronuclear single quantum coherence
L-IdoA	L-iduronic acid
IgG	Immunoglobulin G
IgM	Immunoglobulin M
KDa	Kilo-Dalton

KfiA	*N*-acetyl-glucosaminyl transferase
KLH	Keyhole limpet hemocyanin
Lev	Levulinoyl
MALDI	Matrix-assisted laser desorption/ionization
Me	Methyl
MeOH	Methanol
MES	Morpholine-4-ethanesulfonic acid
MP	4-methoxyphenyl
MPEG	Monomethyl polyethylene glycol
Ms	Mesyl
MS	Mass spectrometry
NaHMDS	Sodium hexamethyldisilazide
NBS	*N*-bromosuccinimide
NDST	*N*-deacetylase/*N*-sulfotransferase
NHS	N-Hydroxysuccinimide ester
NIS	*N*-iodoxuccinimide
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect Spectroscopy
NST	*N*-sulfotransferase
OAc	Acetate
OD	Optical density
O-HEP	Oxyheparin
PAPs	3’-Phosphoadenosine-5’-phosphosulfate
PBS	Phosphate-buffered saline

PEG	Polyethyleneglycol
Ph	Phenyl
Piv	Pivaloyl
PMB	*p*-methoxybenzyl ether
PmHS2	Heparan synthase-2
pTolSCl	*p*-toluenesulfenyl chloride
pTolSOTf	*p*-toluenesulfenyl triflate
pyr	Pyridine
RO-Hep	Reduced oxyheparin
RPM	Revolutions per minute
RRV	Relative reactivity value
rt	Room temperature
sat.	Saturated
sec	Second
SEM	2-(trimethylsilyl)ethoxymethyl
TACA	Tumor-associated carbohydrate antigen
TBAI	Tetrabutylammonium iodide
TBAF	Tetrabutylammonium fluoride
TBDMS	*t*-butyldimethylsilyl
TBDMSOTf	*t*-butyldimethylsilyl triflate
TBDPS	*t*-butyldiphenylsilyl
TBS	*t*-butyldimethylsilyl
*t*Bu	*t*-butyl

TCA	Trichloroacetyl
TCE	2,2,2-trichloroethyl
TDS	Dimethylthexylsilyl
TEA	Triethylamine
TEMPO	2,2,6,6-tetramethylpiperidin-1-oxyl
Tf	Trifluoromethylsulfonyl (triflate)
Tf_2O	Triflic anhydride
TFA	Trifluoroacetic acid
Th	T helper cell
THF	Tetrahydrofuran
THPTA	tri-(3-hydroxypropyltriazolylmethyl)amine
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TMSOTf	Trimethylsilyl triflate
Tn	*N*-acetylgalactosamine linked to protein
Tol	Tolyl
TOCSY	Total correlation spectroscopy
p-TsOH	*p*-toluenesulfonic acid
Troc	2,2,2-tricholoroethoxycarbonyl
TTBP	2,4,6-tri-*t*-butylpyrimidine
UDP	Uridine 5'-diphosphate
USP	United States Pharmacopeia
VLP	Virus-like particle

Chapter 1 – Strategies in Synthesis of Heparin/Heparan Sulfate Oligosaccharides

1.1 Introduction

Heparin, first isolated in 1917, was found to be highly effective as an anticoagulant, and within two decades it was being used clinically.[1] Besides their anticoagulation activities, heparin and the related heparan sulfate (HS) play important roles in a wide range of biological functions such as cell differentiation, viral infection, and cancer metastasis.[2]

Heparin is a member of the glycosaminoglycan (GAG) family, which ranges from the unsulfated polymer hyaluronan to chondroitin and dermatan sulfates, and to the most complex examples, heparin and HS.[3] Heparin and HS share the basic disaccharide components, composed of D-glucosamine (GlcN) α-(1→4)-linked to a uronic acid (Figure 1.1a). The GlcN component has a high degree of variability, as its O-6 and O-3 positions can be free or sulfated, and the amino group can be sulfated, acylated, or unmodified. The uronic acid can be either D-glucuronic acid (D-GlcA) or its C-5 epimer, L-iduronic acid (L-IdoA), both of which can be sulfated at the O-2 position.

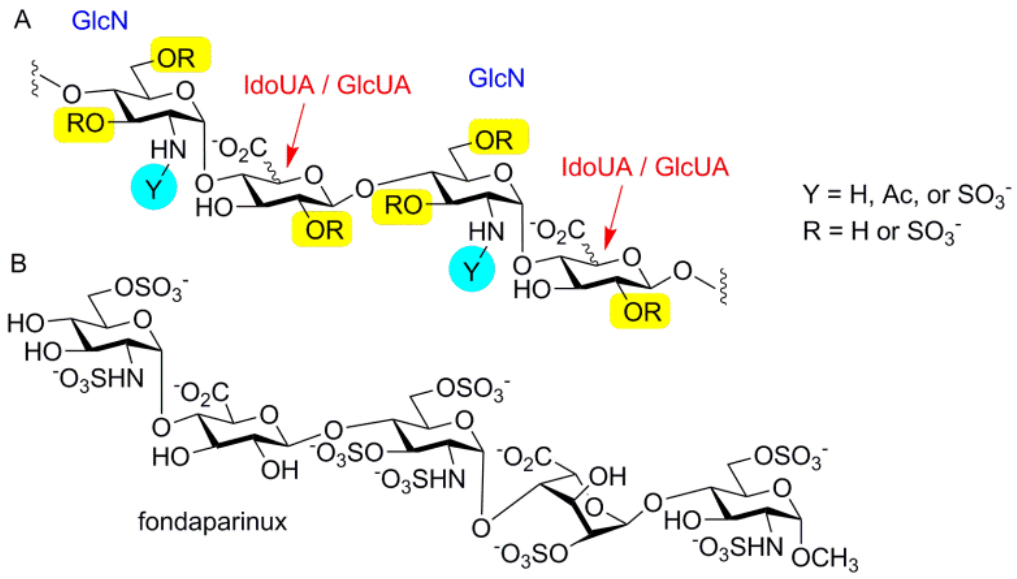


Figure 1.1: (A) Structures of heparin/HS; and (b) Structure of fondaparinux (Arixtra®). (Note Idose and iduronic acid are presented in the $^1C_4$ conformation following the tradition of the field.)

Heparin and HS are differentiated by their tissue location and their detailed structures. Heparin has a higher degree of sulfation, with around 2.7 sulfate groups per disaccharide unit, and about 90% of its uronic acid as L-IdoA. Heparin is selectively synthesized in mast cells, whereas HS is omnipresent on cell surfaces and in the extracellular matrix as part of the proteoglycan complex.[4] More prevalent and heterogeneous, HS has on average 1 sulfate group per disaccharide, but it includes areas of high sulfation and swaths of unsulfated disaccharides.[5] The backbone sequence of HS is also more varied, in that the uronic acid residue is around 40% L-IdoA, with the major entity being D-GlcA.[2a, 6]

Although the naturally occurring heparin/HS is an exceedingly heterogeneous mixture, its interactions with biological receptors can be highly specific, as is evident from its binding to antithrombin III (ATIII).[7] Thorough structural analysis has demonstrated that the oligosaccharide sequence in heparin responsible for ATIII binding is a rare pentasaccharide fragment that is

sulfated at O-3 in the middle GlcN component.[1c, 7-8] Removal of this O-3 sulfate group diminished its antithrombin affinity 10,000 fold.[9] The understanding of this structure activity relationship led to the development of the drug fondaparinux (trade name: Arixtra, Figure 1.1b), a fully synthetic pentasaccharide approved by the US Food and Drug Administration for the treatment of deep-vein thrombosis.[9a]

Despite the success in establishing the ATIII binding-site, the heterogeneities of heparin and HS from natural sources present a major challenge in obtaining sufficient quantities of pure materials for the determination of detailed structure activity relationships. To overcome this limitation, a frequently employed strategy has involved chemical modification of natural heparin and HS. However, this approach can give a complex mixture of many partially modified products from incomplete reactions.[10] Thus, the synthesis of pure and homogenous oligosaccharide sequences of the parent heparin and HS polysaccharides becomes crucial in facilitating biological studies. Commercially, Arixtra has been prepared by pure chemical synthesis, which is an impressive accomplishment considering there are over 50 synthetic steps, and synthetic routes are still being improved.[11] For Arixtra synthesis and other synthetic work prior to 2000, the reader should refer to several excellent reviews.[9a, 12] This article focuses on the advancement of heparin and HS component synthesis since 2000.

1.2 Challenges in Synthesis of Oligosaccharides of Heparin and HS

The synthesis of heparin/HS oligosaccharides presents a major challenge. Multiple factors must be considered for a successful synthetic design. These include (a) synthetic access to L-iduronic acid and L-idose; (b) the choice of uronic acid or the corresponding pyranoside as building blocks; (c) formation of the 1,2-*cis* linkage from the GlcN donor; (d) suitable

protecting-group strategy to install sulfate groups at desired locations; and (e) methods used for elongation of the backbone sequence.

L-IdoA and the corresponding idopyranosides are not available from natural sources in large quantities and must be synthesized. There has been much research in order to access L-IdoA and its derivatives efficiently. Many approaches start from the commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**1**), followed by the inversion of the configuration at C-5 through formation of an L-*ido* epoxide as in compound **3** (Figure 1.2a).[13] Other routes employing compound **1** involve oxidation of the 5-hydroxyl group to aldehyde **4** through a three-step process, followed by stereoselective addition of a cyano group (Figure 2b) or elimination of the primary hydroxyl group with subsequent hydroboration to invert the stereochemistry at C-5 (compound **6** in Figure 1.2c).[14]

Alternative routes to L-IdoA have been reported.[13c, 14-15] As an example, Seeberger and coworkers have spearheaded research in the *de novo* synthesis of L-IdoA. Early work from their laboratory started from L-arabinose, but the low selectivity in the Mukaiyama aldol reaction with aldehyde **7** resulted in a low overall yield (6%) (Figure 1.3a).[15c] Starting from D-xylose and switching the aldol reaction to a more-selective cyanation furnished the L-IdoA building block **11** in 24% overall yield (Figure 1.3b).[15b] However, despite the many routes developed towards the preparation of L-IdoA or L-idose,[13c, 14-15] the synthesis of heparin/HS oligosaccharides remains difficult as long as 8-12 synthetic steps are required for the preparation of a single monosaccharide building block.

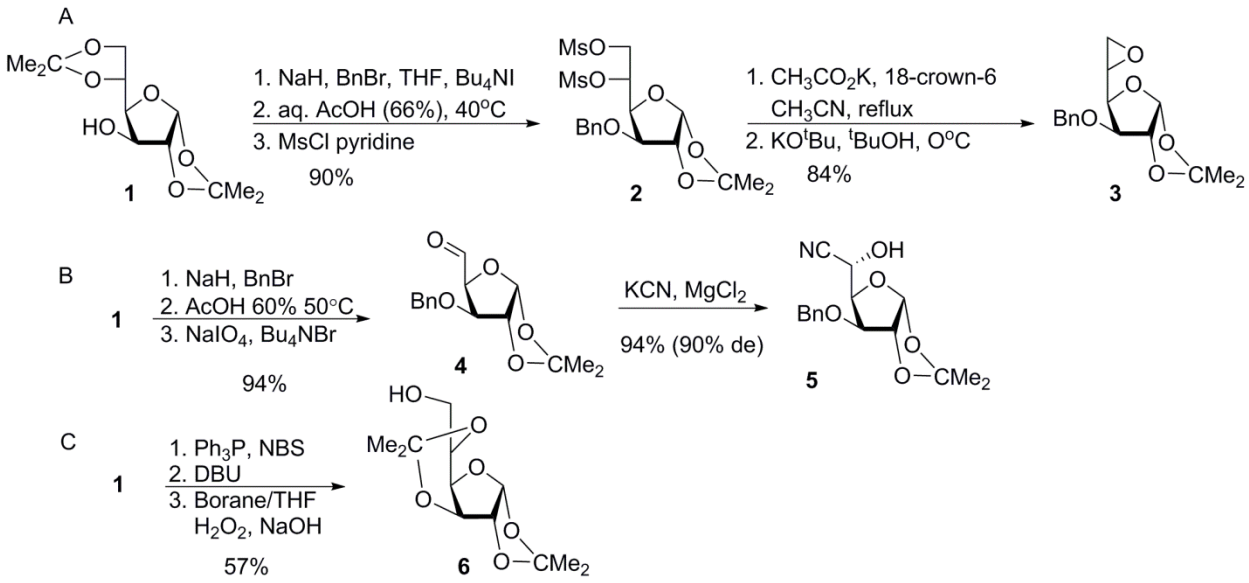


Figure 1.2: Various routes for inverting D-glucose to L-idose derivatives.

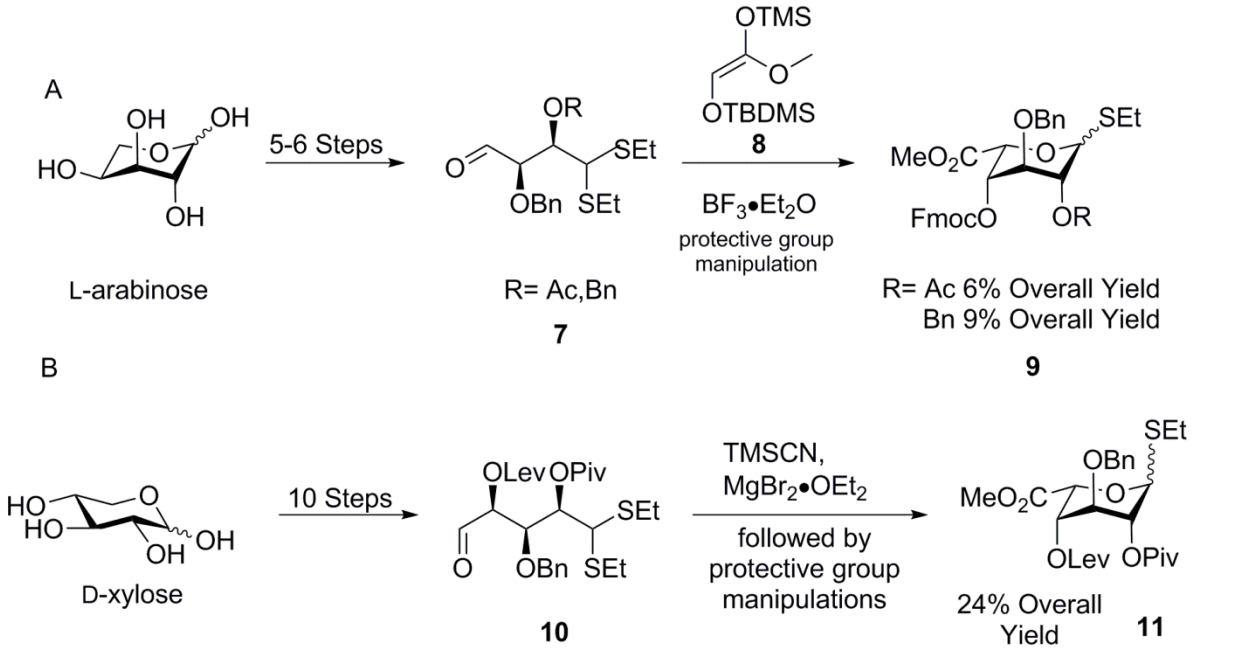


Figure 1.3: Recent routes to monosaccharide precursors of L-IdoA.

As glycosyl donors based on uronic acids can potentially be epimerized during their preparation, and they are typically less reactive than the corresponding glycopyranosides, the latter are commonly used as surrogate glycosyl donors. However, this approach requires adjustment of the oxidation state on the oligosaccharide after its assembly. As the size of the

oligosaccharide increases, high-yielding oxidation can become very difficult.[16] Early syntheses relied on the Jones oxidation or the use of similar chromium reagents, which are toxic and frequently give low yields of the desired products (Figure 1.4a).[13b, 17] This problem was subsequently overcome by using the mild TEMPO-mediated oxidations, which are typically effected with a co-oxidant such as NaOCl[18] or iodobenzene diacetate (BAIB, Figure 1.4b),[19] and this can be followed by Pinnick oxidation to achieve high yields (Figure 1.4c).[16, 20] Alternatively, glycopyranosides could be used to prepare disaccharide intermediates as precursors for longer oligosaccharides by taking advantage of the high anomeric reactivity of the pyranoside donors. Adjustment of the oxidation state can then be performed on the disaccharide through oxidation at C-6 of the non-reducing end to the uronic acid, thus avoiding a late-stage oxidation of the more-valuable larger oligosaccharides (Figure 1.4d).[21]

The monosaccharide glucuronic and iduronic acids, suitably derivatized, can be used directly as donors. Sinaÿ's synthesis of the ATIII-binding pentasaccharide used uronic acid-based glycosyl bromide donors, which gave glycosylation yields typically around 50%.[13a, 13b] The availability of newer glycosylation methods, and an understanding of the effects of protecting groups on anomeric reactivities, have potentially circumvented this issue.[22] Bonnaffé and coworkers synthesized the disaccharide building block **22** in 75% yield by using the bromide donor **20** (Figure 1.5a). The yield was increased to 91% employing the trichloroacetimidate donor **23** (Figure 1.5b).[15e, 23] The resultant disaccharide was then used in a highly convergent manner to afford a dodecasaccharide derivative that was used for the synthesis of an HS proteoglycan analogue (see Figure 1.18).[24]

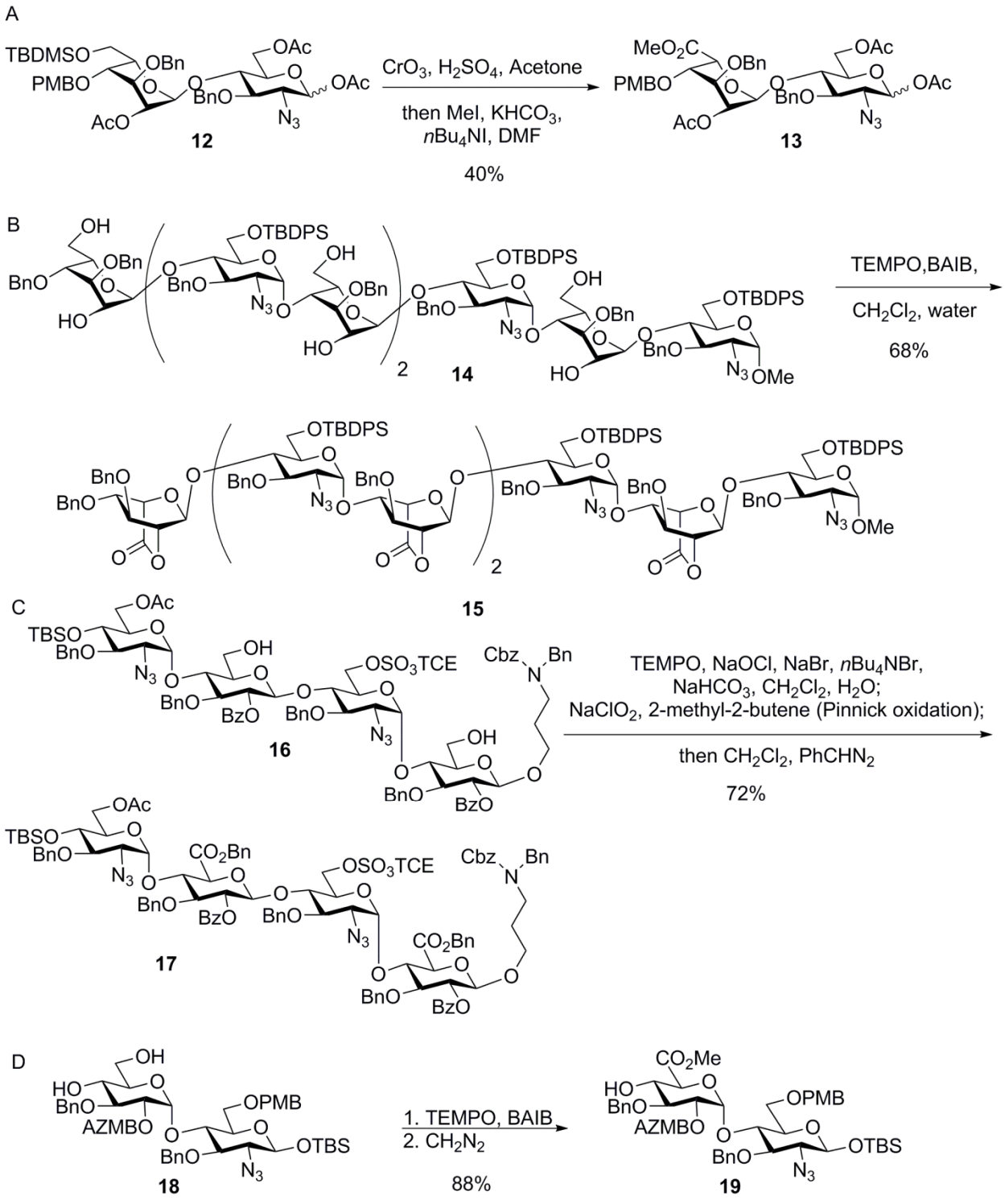


Figure 1.4: Conversion of glycopyranosides into uronic acids in synthesis of Heparin/HS oligosaccharides.

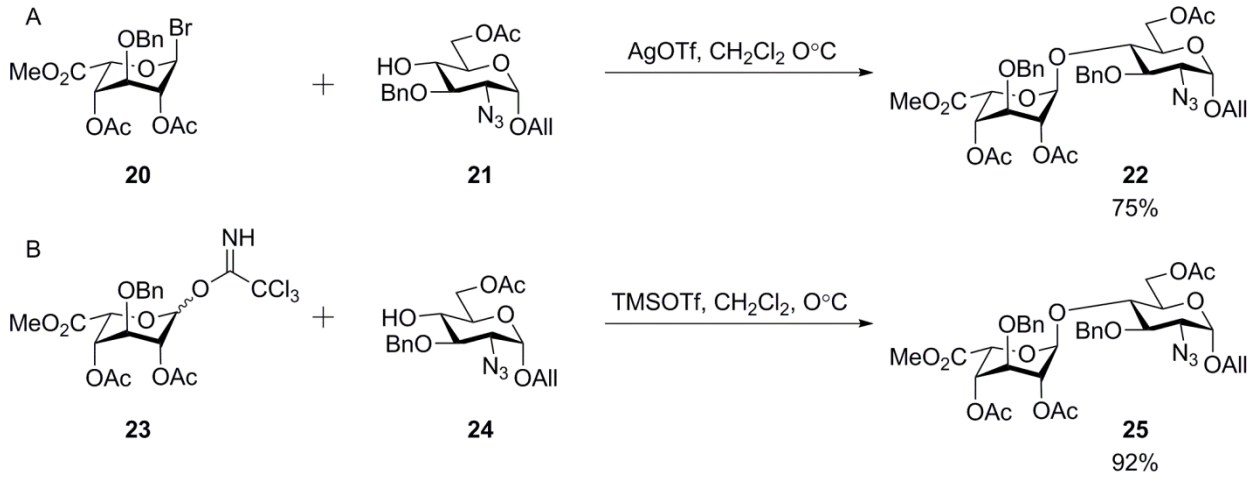


Figure 1.5: Comparison of glycosyl bromide and trichloroacetimidate donors in glycosylation.

Stereochemical control is a crucial issue in the synthesis of heparin/HS components. The 1,2-trans linkage from the uronic acid to glucosamine is usually achieved through use of a participating group at the 2-position of the uronic acid. However, formation of the 1,2-cis linkage from the glucosamine donor can be difficult to control. The azido group, as a non-participating functionality, is widely employed as a precursor for the nitrogen atom at C-2 of glucosamine.[25] Such 2-azido glucosamine precursors can lead to the thermodynamically more-stable α glycosides.[12e] This route generally provides high stereoselectivities in reactions with L-idosyl acceptors. However, for D-glucuronic acid-based acceptors, anomeric mixtures often result from the glycosylation, and this requires fine tuning of protecting groups to achieve high stereoselectivities.[20b, 26] For example, substituting the 4-benzyl ether in donor **26** by a 4-*t*-butyldimethylsilyl ether (donor **29**) led to formation of the α-linked disaccharide **28b** exclusively (Figures 1.6a and 1.6b).[20b] Bulky protecting groups at O-6 of the glucosamine component have also been explored to decrease the proportion of β anomer formed.[19a] In addition to the protecting groups, the conformation of the acceptor can play an important role in determining the stereochemical outcome of the glycosylation. While glycosylation of pentenyl glycoside **31** with

trichloroacetimidate **30** gave the disaccharide derivative **32** with an α:β ratio of 3:1 (Figure 1.6c), locking the glucuronic acid component into the $^{1}C_{4}$ conformation (**33**) led to exclusive α selectivity (Figure 1.6d).[27] However, caution needs to be taken in extrapolating these results to the assembly of larger oligosaccharides.

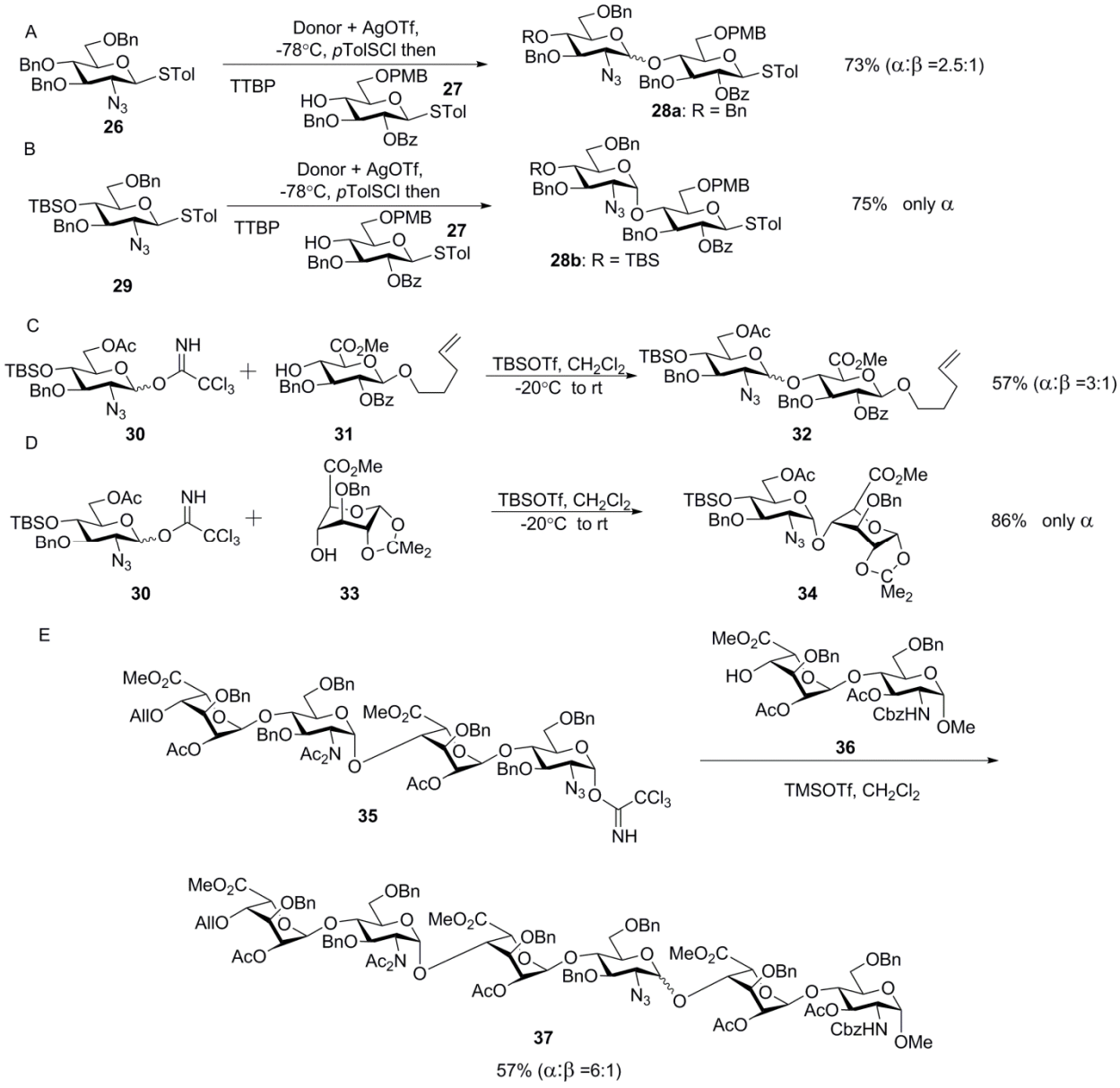


Figure 1.6: Strategies for enhancing steroselectivity in glycosylation.

For example, glycosylation of the L-idosyl-configured disaccharide derivative **36** by tetrasaccharide donor **35** led to hexasaccharide **37** as an inseparable anomeric mixture (Figure 1.6e).[28] The stereochemical outcome of the glycosylation reaction needs to be investigated individually, especially in the formation of large oligosaccharides.

In addition to their roles in dictating stereochemistry, protecting groups are widely used to control the location of sulfate groups. With the high level of functionality in heparin/HS oligosaccharides, and the large number of protecting groups employed, syntheses must be suitably designed to prevent the premature removal of a protecting group.

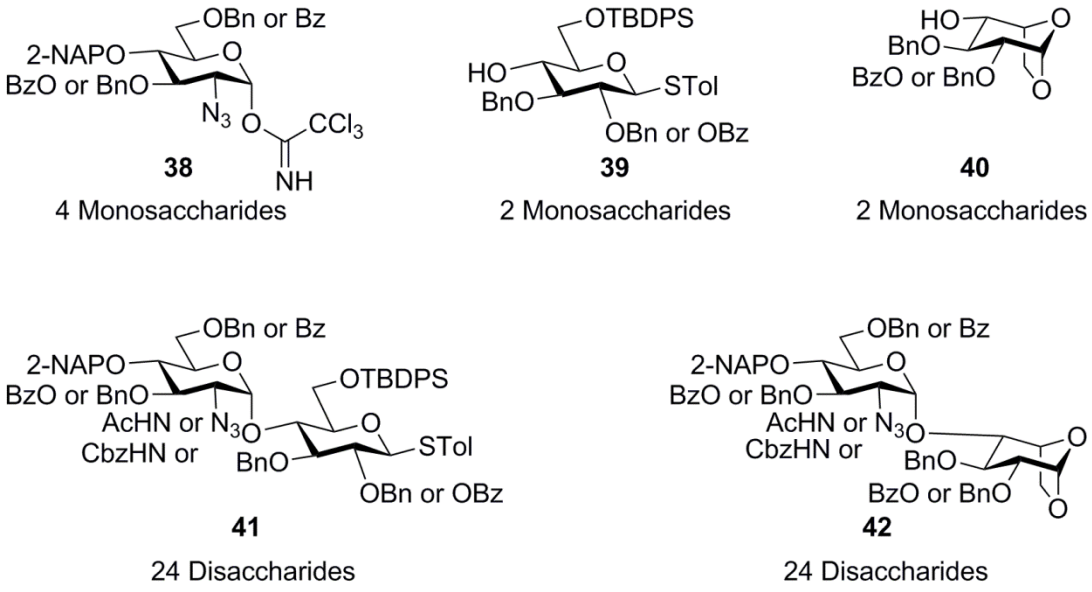


Figure 1.7: Synthesis of all potential heparin/HS disaccharides from eight monosaccharide precursors.

To establish protecting groups suitable for regioselective sulfation, the Hung group explored the possibility of synthesizing all 48 possible heparin/HS disaccharide structures (disaccharide derivatives **41** and **42**), starting from eight monosaccharide building-blocks (**38-40**) that are strategically protected.[29] The benzoyl group was used to protect those hydroxyl groups to be sulfated, and benzyl ethers were employed as persistent protecting groups for hydroxyl groups

that would remain free in the final oligosaccharide products. The TBDPS substituent temporarily masked the primary hydroxyl group on compound **39** to permit subsequent oxidation to glucuronic acid. The azido group could be selectively reduced by Staudinger reduction and then either acetylated or sulfated, while the benzyloxycarbonylamino (Cbz) group could be deprotected to generate the free amine upon the final hydrogenolysis step. This panel of 48 disaccharide derivatives (compounds **41** and **42**) will be very useful for the assembly of heparin/HS libraries (Figure 1.7).

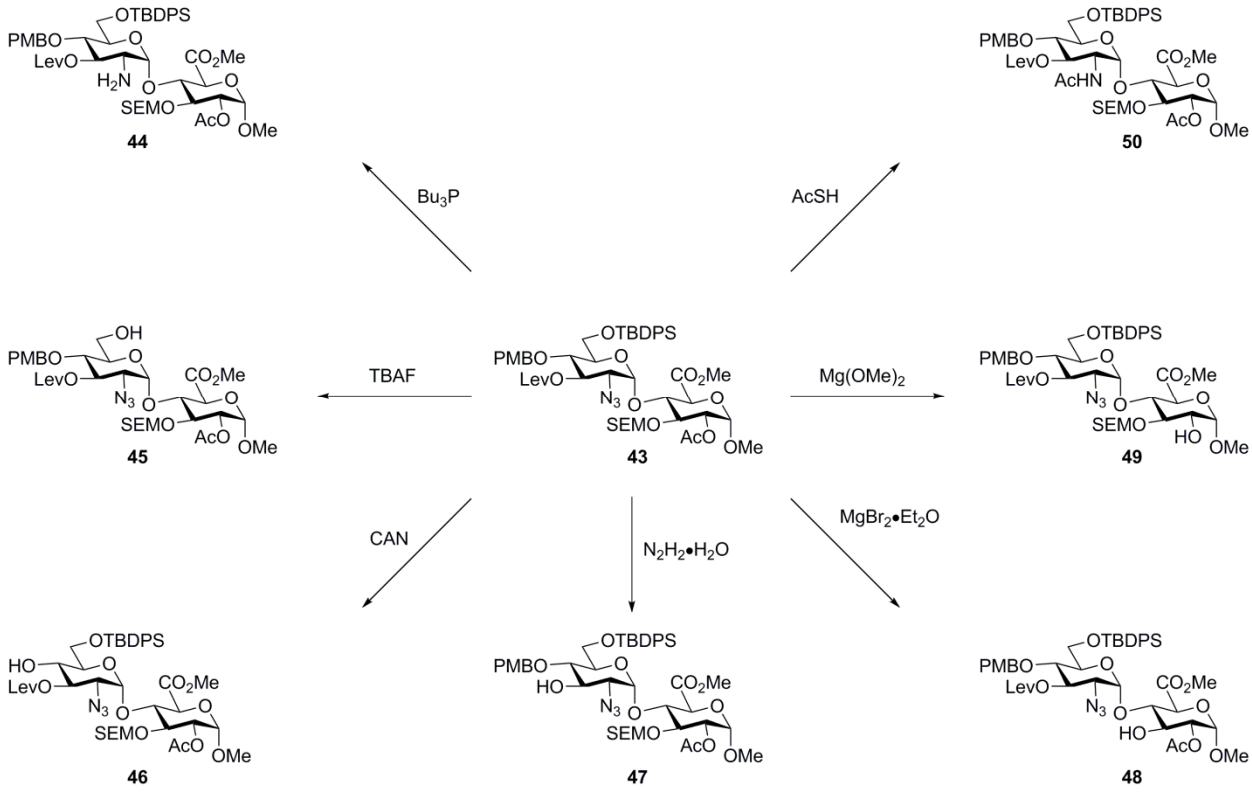


Figure 1.8: Disaccharide derivative **43** can be orthogonally deprotected for sulfation at various locations.

Instead of preparing multiple monosaccharides, Wei and coworkers synthesized the glucuronic acid-containing HS disaccharide **43** having each hydroxyl group orthogonally protected (Figure 1.8). Each protecting group could be removed selectively without affecting others. The newly liberated hydroxyl group was sulfated and other protecting groups were then

removed to ensure that the sulfate groups were stable under each set of deprotection conditions.[30] This strategy allowed the divergent synthesis of multiple sulfation patterns from a single backbone, but required more synthetic steps to remove the various protecting groups remaining after sulfation. As the biologically active heparin/HS domains typically are pentasaccharides or longer, these protecting-group strategies need to be extended to the synthesis of longer oligosaccharides.

Sulfate groups have traditionally been installed after assembly of the oligosaccharide backbone. However, late-stage sulfation, especially with larger oligosaccharides, can be quite capricious and challenging. Low yields[31] and incomplete reactions[24, 32] are common. As an alternative, the sulfate groups can be installed on building blocks as protected esters prior to glycosylation. Numerous sulfate esters have been developed[33] and Huang and coworkers investigated the utility of 2,2,2-trichloroethyl (TCE) sulfates[20a] as developed by the Taylor group. TCE sulfates are stable to common transformations encountered in oligosaccharide synthesis, and the deprotection conditions are very mild.[34] An additional benefit of using TCE was that both sulfated and unsulfated building blocks can be derived from a common intermediate, thus increasing the efficiency of the overall process. For example, deprotection of the primary *O*-acetyl group in disaccharide derivative **51** followed by treatment with the sulfuryl imidazolium salt **52** provided the sulfate ester **53** (Figure 1.9a).[20a] The disaccharide derivative **51** was also used for conversion into the non-sulfated acceptor **57** (Figure 1.9b). The presence of sulfate ester groups in the building blocks did not significantly affect the glycosylation yield, as reaction of the sulfate ester donor **54** with the acceptor **57** gave tetrasaccharide derivative **58** in 82% yield (Figure 1.9c). The sulfate ester-containing tetrasaccharide **59** also functioned as a competent acceptor, as it underwent glycosylation by donor **54** in 70% yield (Figure 1.9d). The

tetrasaccharide **59** was successfully deprotected, giving rise to the HS tetrasaccharide component **61** (Figure 1.9e), demonstrating the compatibility of TCE sulfate esters in the synthesis of heparin/HS oligosaccharides.

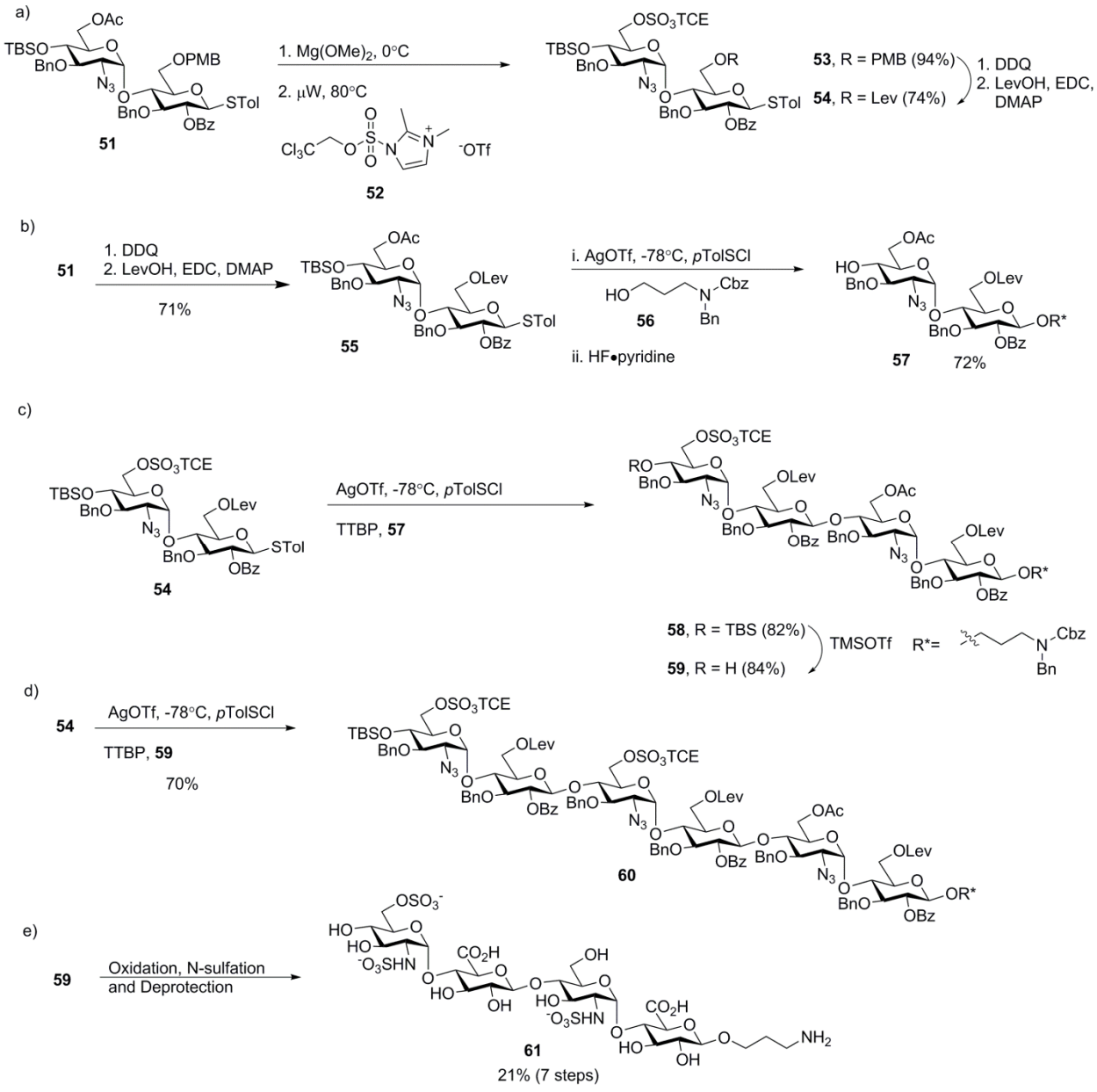


Figure 1.9: Evaluation of TCE sulfate ester-containing donors and acceptors in glycosylation reactions.

With the foregoing general understanding of synthesis of heparin/HS components, the following sections focus on the recent development of strategies to form and extend the

heparin/HS oligosaccharide backbone. The discussions are grouped according to the strategy utilized.

1.3 Linear Synthesis in Solution Phase

The linear approach is one of the earliest strategies in oligosaccharide synthesis, and is the route employed by Nature to produce heparin/HS.[3] Chemical glycosylation involves the activation of a donor, followed by nucleophilic attack of the activated donor on the acceptor to form a new glycosidic linkage. In the linear approach towards heparin/HS oligosaccharides, the protecting group on the 4-hydroxyl group at the non-reducing end of the newly formed disaccharide is selectively removed, leading to a new acceptor, which undergoes further glycosylation, extending the chain from the reducing end to the non-reducing end, and producing the heparin/HS oligosaccharide backbone (Figure 1.10).

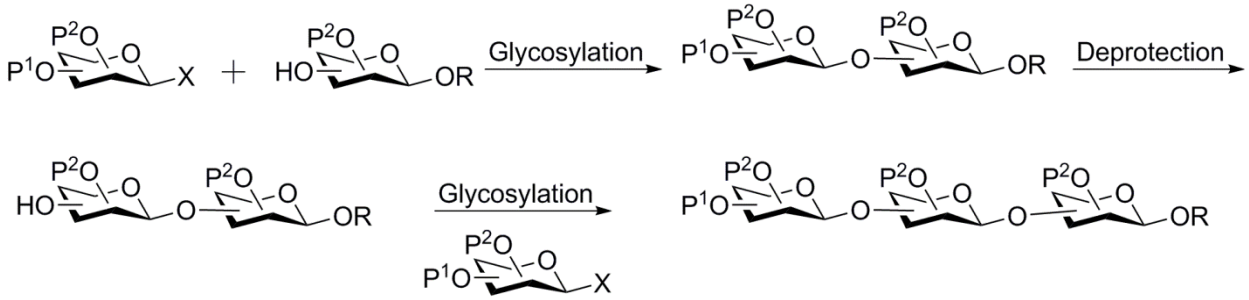


Figure 1.10: Linear synthesis of oligosaccharides from the reducing end.

Overall, the number of synthetic steps in linear synthesis is high because of the number of oligosaccharide intermediates generated and the deprotection step required after each glycosylation. Linear synthesis of oligosaccharides is therefore performed mainly for preparing shorter oligosaccharide sequences but with modular synthetic strategy has been used to synthesize upwards of dodecasaccharides by Gardiner and coworkers.[35] The Fügedi group used

the linear strategy to synthesize HS trisaccharides considered to be responsible for interactions of HS with the fibroblast growth factors FGF-1 and FGF-2.[13c, 36] Glycosylation of acceptor **63** with thioglycoside **62,** using the thiophilic promoter dimethyl(methylthio)sulfonium triflate (DMTST), followed by removal of the chloroacetyl protecting-group with hydrazine dithiocarbonate (HDTC) furnished the disaccharide acceptor **64** (Figure 1.11). A second round of DMTST-mediated glycosylation using donor **65** produced the trisaccharide **66**. After removal of the benzoyl, the 4-methoxyphenyl (MPh), and the *t*-butyl group, sulfation of the newly liberated hydroxyl groups was performed with the sulfur trioxide-pyridine complex in DMF. Hydrogenation and selective *N*-sulfation with the sulfur trioxide-trimethylamine complex under basic conditions furnished the final product **70**.[37] Following the same reaction sequence, except for reversing the steps of MPh-group removal and *O*-sulfation, generated the trisaccharide **71**, which bore sulfation patterns different from those in compound **70** (Figure 1.11).

Boons and coworkers used the linear approach to synthesize a trisaccharide, using the monosaccharide building blocks **72** and **73**.[18a] Glycosylation of vinyl donor **72a** with acceptor **73** was followed by removal of the *p*-methoxybenzyl (PMB) group at the non-reducing end by TFA, which generated the disaccharide acceptor **75** (Figure 1.12). The trichloroacetimidate donor **72b** was found to be superior to the corresponding vinyl donor **72a**. The monosaccharide derivative **73** was benzoylated to produce vinyl donor **74**, which glycosylated the acceptor **75** to furnish trisaccharide **76**. Deprotection of **76** led to the unsulfated HS trisaccharide derivative **78**. The synthesis, while linear, could be modified into a modular active-latent approach, as discussed in section III.

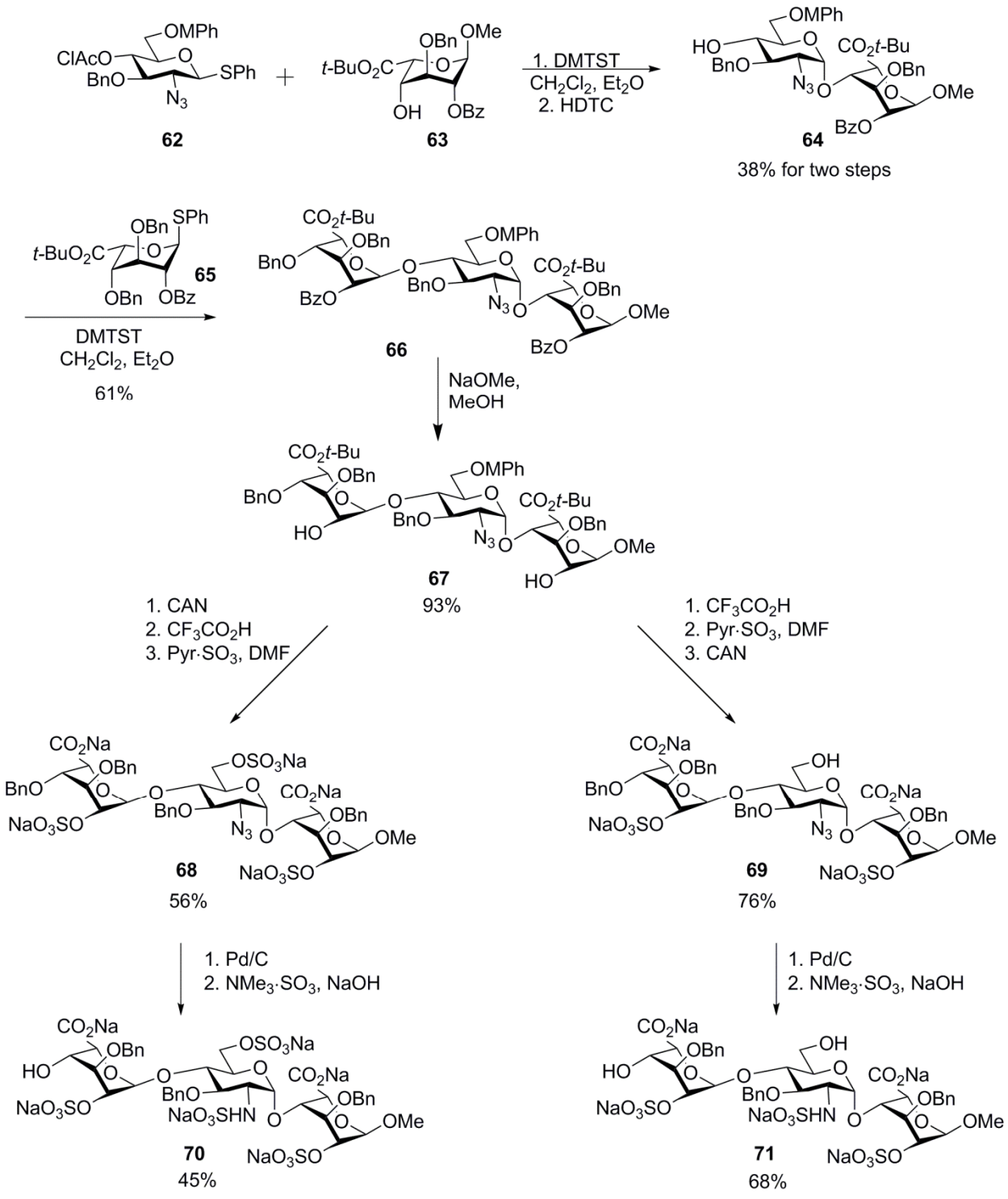


Figure 1.11: A linear synthesis of the heparin/HS trisaccharides responsible for binding with the fibroblast growth factors FGF-1 and FGF-2.

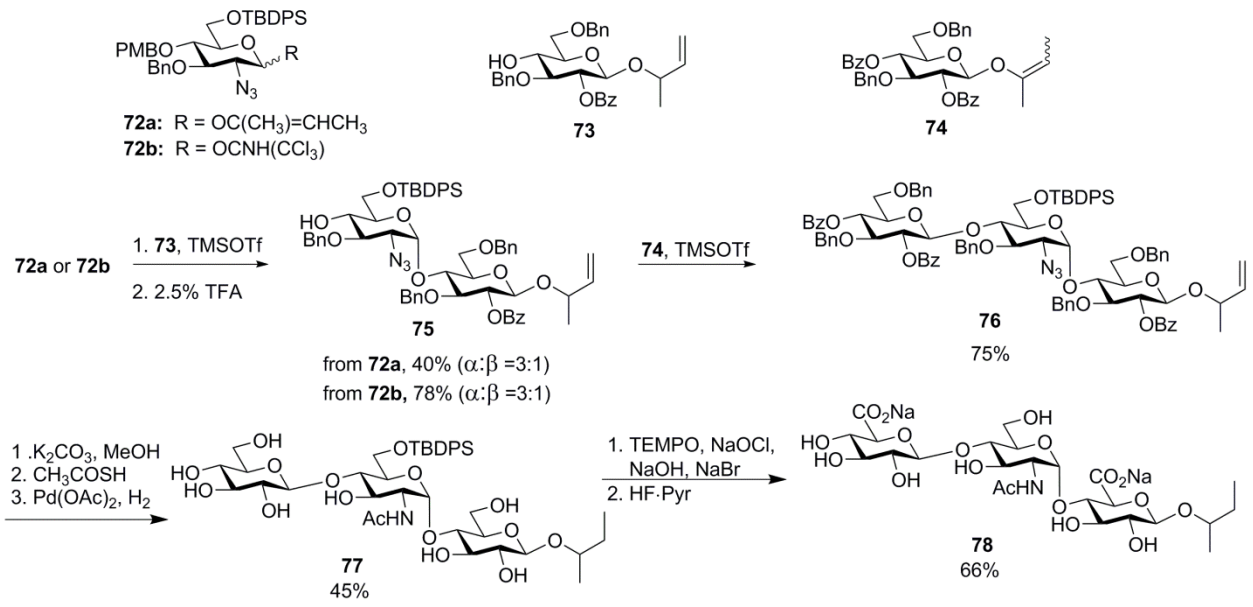


Figure 1.12: Linear synthesis and late-stage oxidation to generate trisaccharide **78**.

1.4 Linear Synthesis using Polymer Support

The Holy Grail in oligosaccharide synthesis would be the availability of a general and fully automated system having the synthetic efficiency of the established automated systems for peptide synthesis. Towards this goal, the Seeberger group has adapted an automated peptide synthesizer for the synthesis of complex oligosaccharides.[38] Thus far, the automated synthesis of heparin/HS oligosaccharides has not been achieved, because of the difficulties in translating solution-phase synthesis to high-yielding polymer-supported synthesis.

To determine the influence of polymer supports on the synthesis of heparin/HS components, Martin-Lomas and co-workers evaluated the use of various polymer supports and linkers on the glycosylation process.[39] Through a succinic acid linker, disaccharide derivative **79** was grafted onto the polystyrene resin Argogel^{TM}, which was then further functionalized, leading to the polymer-bound acceptor **82** (Figure 1.13). Glycosylation of disaccharide derivative **82,**

mediated by TMSOTf, was performed using 3.7 equivalents of the disaccharide donor **80,** and

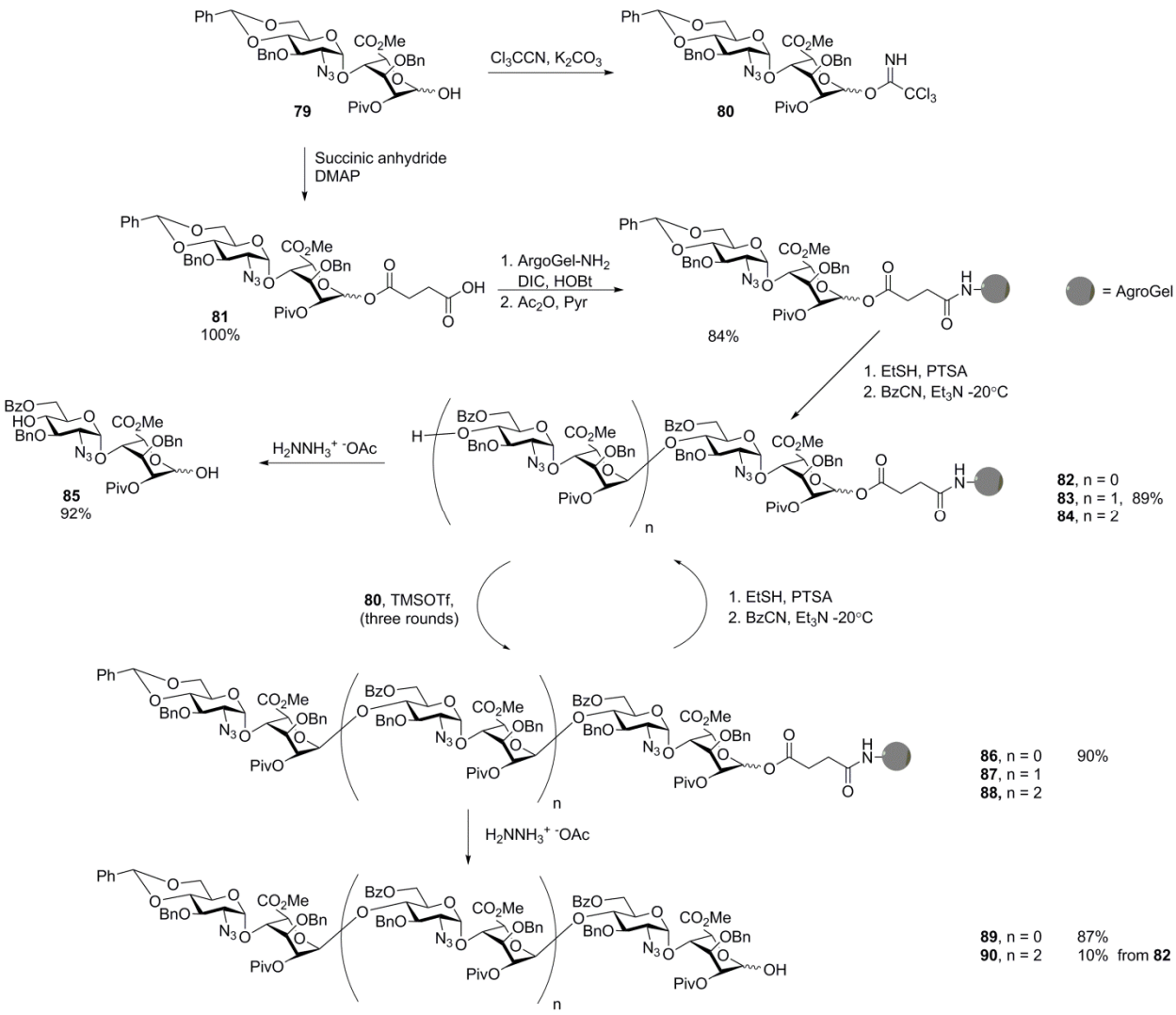


Figure 1.13: Solid-supported synthesis of heparin oligomers.

the excess of activated donor and reagent were removed after the reaction by multiple washes of the resin. As the reactivity of the polymer- bound acceptor was low, the glycosylation reaction was repeated two more times. The resin was then treated with hydrazine to cleave off the product, affording tetrasaccharide derivative **83** in 89% yield. This method was further extended to the synthesis of octasaccharide derivative **90** through successive iterations of deprotection and glycosylation. However, the overall yield of the octasaccharide was very low (~ 10%),

presumably because of the low reactivity of the larger glycosyl acceptor caused by steric hindrance posed by the insoluble polymer.

To increase the flexibility of the polymer, a water-soluble polymer, monomethyl polyethylene glycol (MPEG), was tested as a support. The succinylated disaccharide derivative **81**was linked to MPEG in a manner similar to that employed with Argogel^{TM} (Figure 1.14). Following TMSOTf-catalyzed glycosylation with the imidate donor **80**, the polymer was precipitated with diethyl ether and isolated by filtration. The glycosylation reaction was repeated three more times and the tetrasaccharide derivative **92**, released from the polymer support by hydrazinolysis, was obtained in 20% overall yield from the polymer-bound disaccharide derivative **91**. The authors proposed that the yield differences in using MPEG vs Argogel could be attributed to the inefficiency of MPEG precipitation, as small losses compounded could become significant over multiple steps of manipulation.

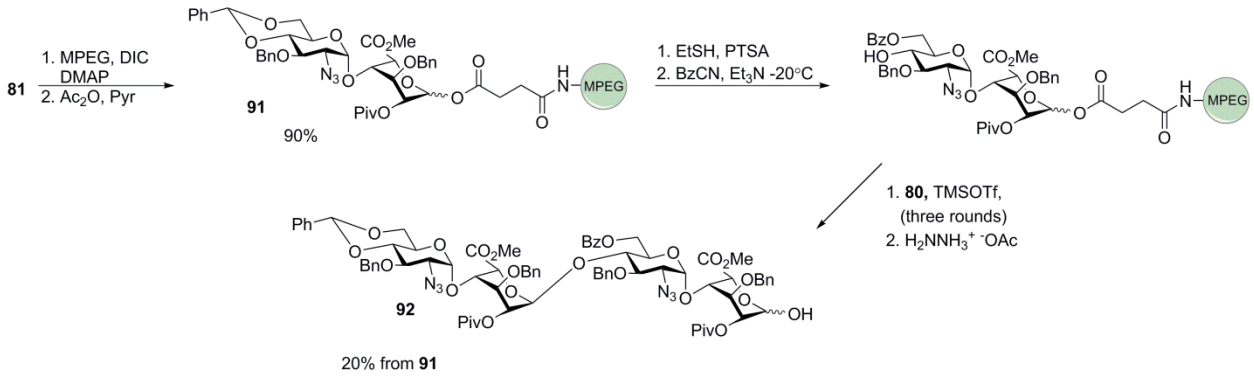


Figure 1.14: Use of the water-soluble polymer MPEG in synthesis of heparin/HS oligosaccharides.

One complication in using the anomeric position to link with the polymer is the production of anomeric mixtures upon release from the polymer. To avoid this, the 6-position of the glucosamine precursor was tested as the site of attachment to MPEG. However, conjugation to the polymer was only 40% effective, and with three rounds of glycosylation and subsequent

detachment of the polymer, only 36% of the desired tetrasaccharide was obtained.[39b] The carboxylate position of iduronic acid was next evaluated as the site of attachment. This position was ideal for attachment of the polymer as it avoided blocking a potential sulfation site, and cleavage from the polymer support could afford an anomerically pure product. The disaccharide acceptor **93** was bound to the MPEG polymer through the carboxylate site of its iduronic residue, and this was subjected to glycosylation by disaccharide donor **80**. Each backbone-elongation cycle consisted of four rounds of glycosylation (Figure 1.15). After each glycosylation, to avoid the loss of desired product through incomplete MPEG precipitation, the non-consumed acceptor was scavenged by carboxylic acid-functionalized, insoluble Merrifield resins, which were removed by simple filtration. Through this procedure, hexasaccharide product **94** was isolated in 37% overall yield from disaccharide derivative **93**. One more round of elongation gave the octasaccharide derivative **95** in 26% overall yield from compound **93.**[39b] Based on yields of product obtained, this route was more efficient than previous MPEG-supported synthesis.

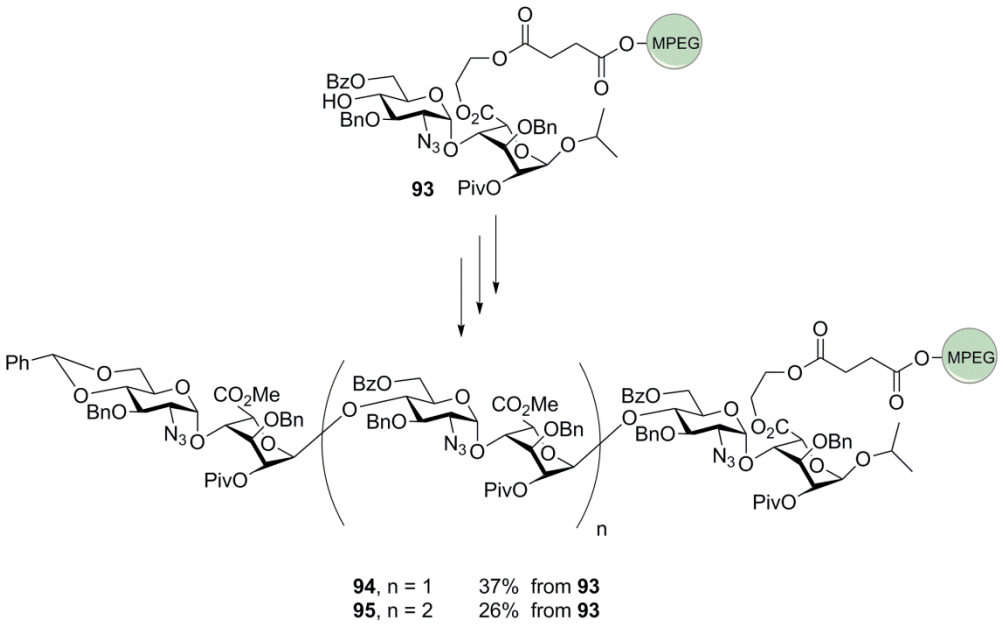


Figure 1.15: Soluble polymers anchored through the carboxylate group of the iduronic component.

In addition to the succinic acid linker, the Martin-Lomas group designed a novel linker that immobilized an idose-based acceptor (**97**) onto MPEG (Figure 1.16). Five iterations of glycosylation of **97** by the imidate **96**, followed by deprotection, gave the disaccharide derivative **99** in 82% yield. Further elongation of the chain by imidate **96** produced the polymer-bound

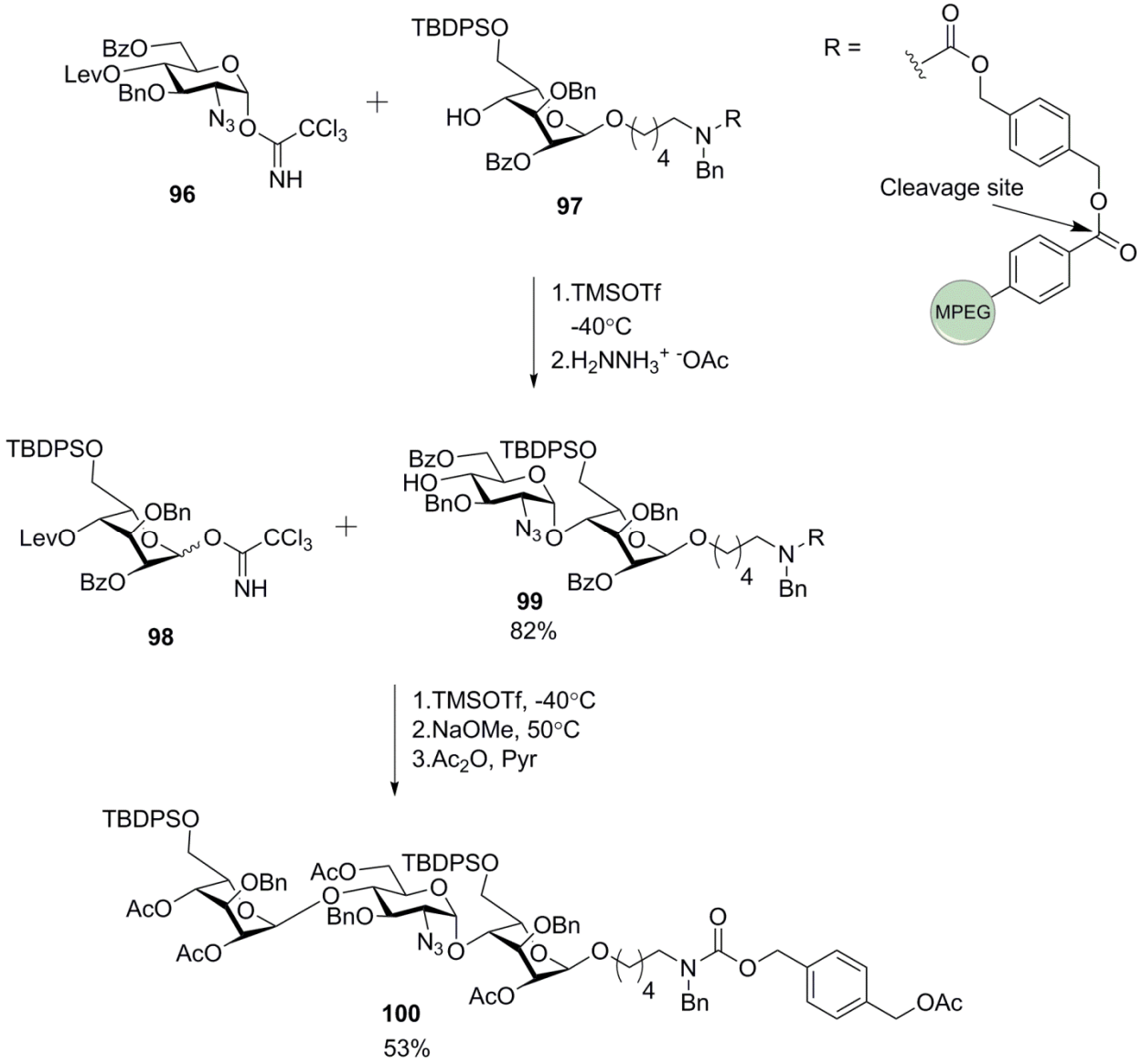


Figure 1.16: Protected amino linker used in conjunction with monosaccharide building blocks used in solid-supported synthesis of heparin/HS oligosaccharides.

trisaccharide, from which the polymer was cleaved off under basic conditions to yield oligosaccharide **100** bearing a protected amino group in the linker, in 53% yield. The free amino group could be released by hydrogenolysis, which is useful for bioconjugation or immobilization of the oligosaccharides onto glycan microarrays.

In summary, the yields of heparin/HS oligosaccharide by glycosylation through polymer-supported synthesis decrease drastically as the chains grow longer. This is a serious challenge to any efforts at automation. Novel chemistry needs to be developed to significantly enhance the glycosylation yields on polymer support without resorting to the use of large excesses of donors. Until this becomes reality, solution-phase synthesis remains the preferred method for preparing complex heparin/HS oligosaccharides.

1.5 Active-Latent Glycosylation Strategy

The active-latent strategy is a solution-based method that builds oligosaccharides from the non-reducing end to the reducing end. In this approach, the acceptor carries a latent aglycon, which is inert to the conditions of glycosyl-donor activation. After glycosylation, the resultant oligosaccharide is transformed into an active donor for further chain elongation (Figure 1.17).

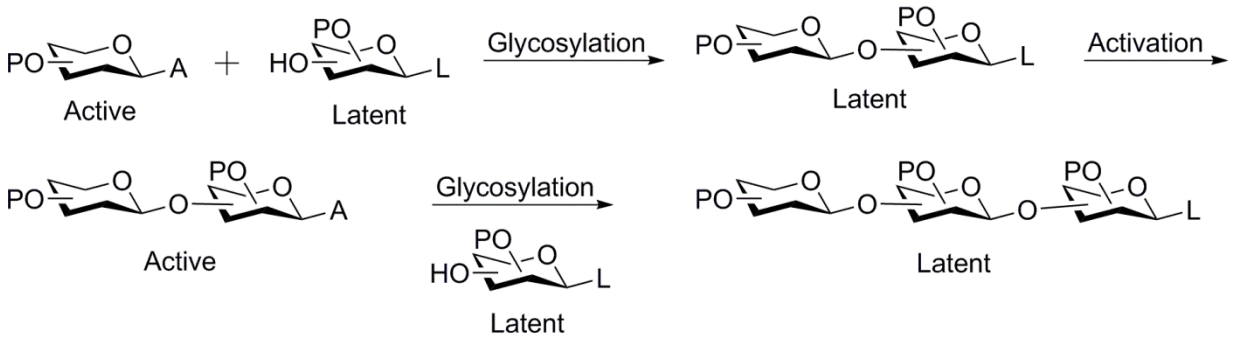


Figure 1.17: The active-latent glycosylation strategy.

Allyl glycosides are used widely for the active-latent glycosylation strategy. Inert to many of the conditions used for donor activation, allyl glycosides can be readily transformed into

vinyl glycosides, which serve directly as active glycosyl donors in Lewis acid-catalyzed glycosylations. However, the vinyl donors typically give low yields in glycosylation reactions (see Figure 1.12).[40] To circumvent this problem, the vinyl aglycon can be cleaved to generate the hemiacetal, which can then be transformed into trichloroacetimidate donors, which are much more reactive.[40-41]

The Bonnaffé group developed an impressive synthesis of a heparin dodecamer by the active-latent strategy, using the allyl glycoside and glycosyl trichloroacetimidate combination.[24] To improve the overall synthetic efficiencies, a PMB group was employed to protect the 4-position at the non-reducing end of the oligosaccharide intermediate. This substituent could be removed selectively to expose a free hydroxyl group for further elongation of the chain. In this synthesis, the PMB-derivatized latent allyl disaccharide **103** was first transformed into a trichloroacetimidate donor, **105,** and the allyl disaccharide acceptor **104** (Figure 1.18a).[41a] Glycosylation of acceptor **104** by imidate **105** generated the latent allyl tetrasaccharide, which was then modified to an active trichloroacetimidate donor **106** (Figure 1.18b). The reaction of **106** with tetrasaccharide **107,** followed by removal of the PMB group at the non-reducing end, and another round of glycosylation, furnished the dodecasaccharide **108** in 45% overall yield from the acceptor **107**. After completion of the backbone, deprotection and sulfation were performed. *O*-Deacetylation by potassium carbonate, and reduction with 1,3-propanedithiol, followed by simultaneous *O*- and *N*-sulfation with the sulfur trioxide--pyridine complex gave the sulfated dodecamer. The simultaneous sulfation with pyridine--SO_3 of the hydroxyl and amino groups did not proceed to completion. A second round of sulfation with pyridine--SO_3 in basified water was necessary to complete the sulfation.[24, 32] Hydrolysis of the methyl esters, followed by hydrogenolysis, gave the fully deptrotected dodecamer **109**, which is the longest heparin

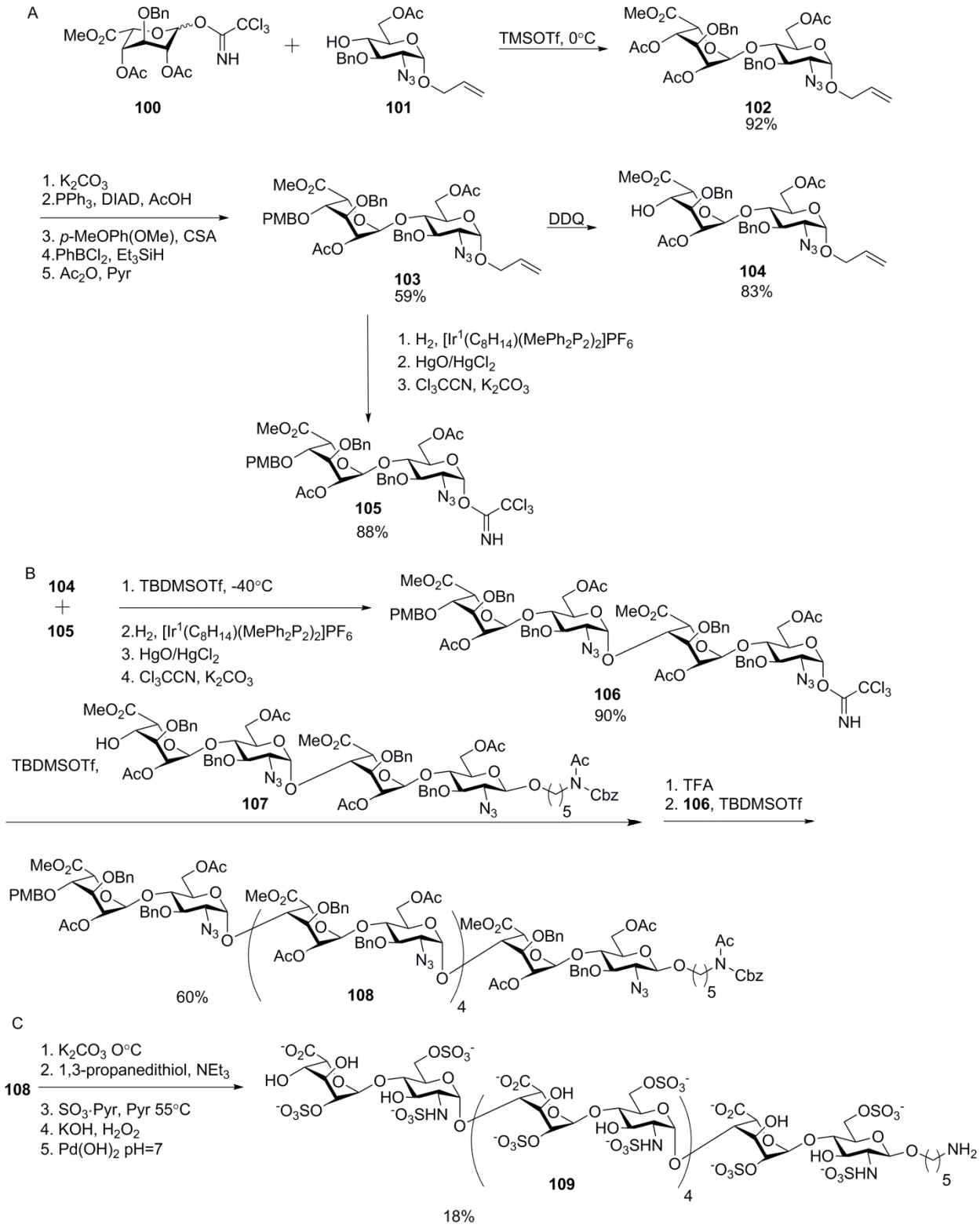


Figure 1.18. Active-latent synthesis of dodecamer **109.**

oligosaccharide yet prepared by chemical synthesis excluding recent heparin related oligomers synthesized by Gardiner and coworkers[24]

As two synthetic steps are needed to cleave the allyl groups necessitating the use of expensive transition-metal reagents and toxic mercury salts, silyl protecting-groups provide attractive alternatives for masking the anomeric position for the active--latent strategy. Many successful syntheses have used a variety of silyl ethers, such as dimethylthexylsilyl (TDS), *t*-butyldimethylsilyl (TBDMS), and trimethylsilyl (TMS).[15a, 27b, 32, 39c, 42] As an example, glycosylation of the monosaccharide acceptor **111** by the trichloroacetimidate donor **110,** followed by acetylation, generated the latent disaccharide derivative **112** (Figure 1.19a). Removal of the anomeric TDS group from **112,** followed by formation of the trichloroacetimidate converted compound **112** into the active donor **113** (Figure 1.19b).[42c] The acceptor **114** was prepared by acid-mediated removal of the 4,6-benzylidene acetal from disaccharide derivative **112** and selective benzoylation of the primary hydroxyl group. Glycosylation by donor **113** of acceptor **114** furnished the tetrasaccharide acceptor **115**. The overall yield was only 40% because of the low glycosylation yield, with 44% of the starting disaccharide derivative **114** being recovered. Hexasaccharide derivative **117** was prepared by the reaction of tetrasaccharide derivative **115** with disaccharide donor **116**. With the removal of its anomeric TDS group, the hexasaccharide derivative **117** was transformed into an active trichloroacetimidate donor **118**, which upon reaction with methanol afforded the methyl glycoside **119** (Figure 1.19d).

Besides being compatible with the trichloroacetimidate donors, the silyl protecting group is robust and has also been applied with thioglycosides under various activating systems.[15a, 21] The Boons group used this strategy to prepare disaccharide building-blocks for their heparin/HS

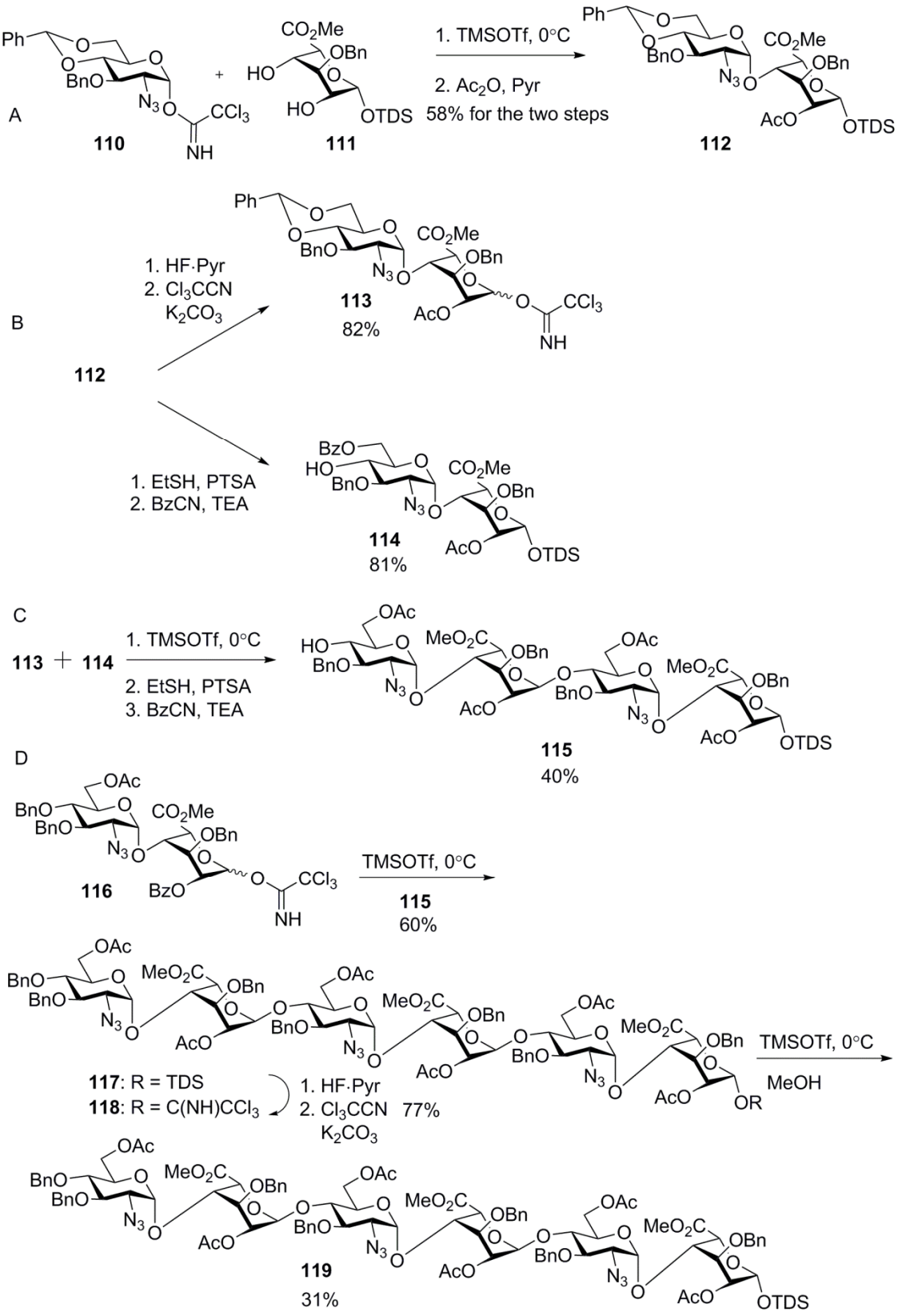


Figure 1.19: Active-latent synthesis with silyl protecting groups.

oligosaccharide synthesis (Figure 1.20).[21a] Glycosylation of the TDS-protected acceptor **121** by the thioglycosyl donor **120** formed the latent disaccharide **122**. After oxidation and protecting group manipulation, the TDS group in **122** was removed and the resulting hemiacetal was converted into the trichloroacetimidate disaccharide donor **123**. Eight disaccharide building-blocks were prepared in this manner, and were used to construct a panel of eleven heparin/HS tetrasaccharides and one hexasaccharide having different backbone structures and sulfation patterns. These tetrasaccharides were used to probe the important structural features of HS for inhibiting β-secretase, a protease considered to be involved in the development of Alzheimer's disease.

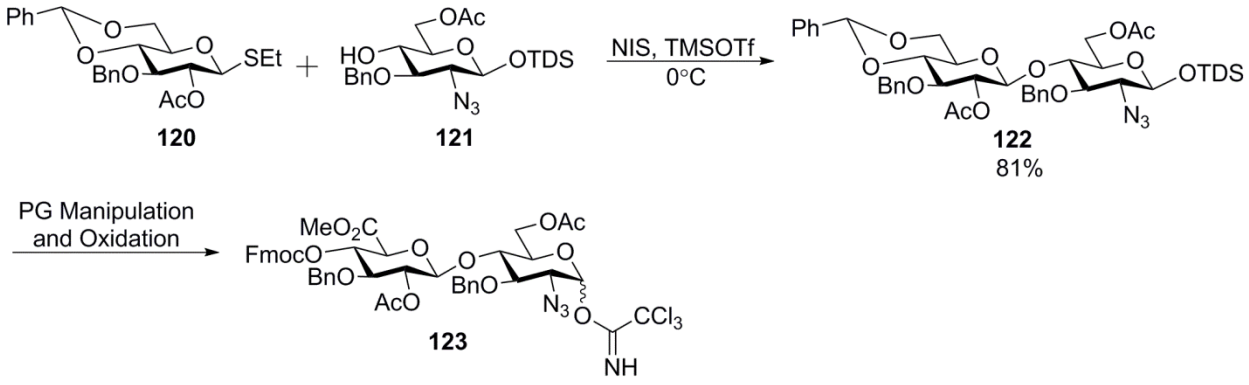


Figure 1.20: Synthesis of one of the eight disaccharide building blocks used by Boons and coworkers to prepare a library of heparin oligosaccharides.

In addition to allyl and silyl groups, other functionalities, including isopropylidene acetals[14c, 28, 31] and 1,6-anhydro sugars, have been used to mask the anomeric position of the latent glycosyl donors. The 1,6-anhydro sugars are advantageous to use as they do not require another selectively removable protecting-group for the anomeric position. Hung and coworkers developed rapid routes of access to such 1,6-anhydro-L-idose building-blocks as compound **125**.[14c, 14d] Glycosylation of anhydro derivative **125** by the glycosyl trichloroacetimidate donor

124 furnished disaccharide **126** (72% yield, α : β = 5.5 :1, Figure 1.21a). To activate this latent disaccharide, the 1,6-anhydro ring of the α anomer of **126** was cleaved by $Cu(OTf)_2$-catalyzed acetolysis, and the newly installed acetyl group at O-6 was exchanged for the more selectively cleavable levulinoyl ester, followed by formation of the trichloroacetimidate (Figure 1.21b). The resulting disaccharide donor was condensed with the glucosaminide precursor **128,** generating trisaccharide derivative **129**. The 2-naphthyl-substituted trisaccharide **129** was selectively deprotected to expose the 4-hydroxyl group at the non-reducing end, where it was glycosylated

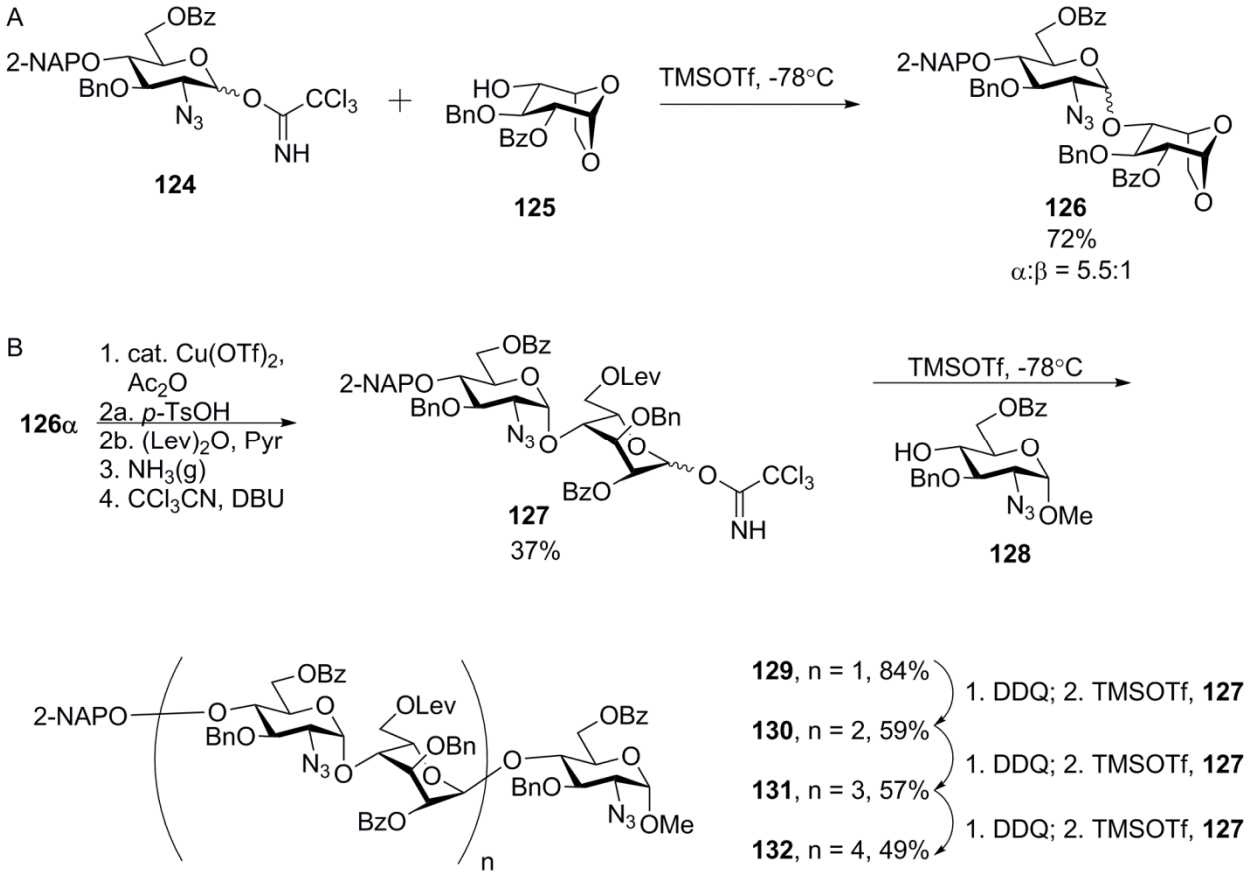


Figure 1.21: The use of the 1,6 anhydro sugars in latent-active strategy.

by the disaccharide donor **127**. Repetition of these deprotection and glycosylation sequences two more times led to formation of the HS nonasaccharide derivative **132**.[31] The active-latent

strategy, coupled with the use of a selectively removable protecting-group, such as 2-naphthyl, at the 4-hydroxyl group at the non-reducing end, provides additional versatility in comparison to the linear strategy, as oligosaccharides can be built up from both the non-reducing and reducing end. However, multiple synthetic manipulations are still needed on the oligosaccharide intermediates to activate the latent donor.

1.6 Selective Activation

To decrease the number of steps required for modification of intermediates, as encountered in the latent-active strategy, the selective-activation method utilizes donors and acceptors having different types of activable aglycons. Upon selective activation of the donor and glycosylation of the acceptor, the resulting disaccharide can be used directly as a donor under a new set of activation conditions, without the need for manipulation of the intermediate (Figure 1.22). The most common pairs of glycosyl building-blocks in selective-activation methods are glycosyl trichloroacetimidates and thioglycosides, since thioglycosides are stable under the acidic conditions encountered in trichloroacetimidate activation.[19a, 42c, 43] The selective-activation method can often be combined with the active-latent strategy within a single synthetic operation.

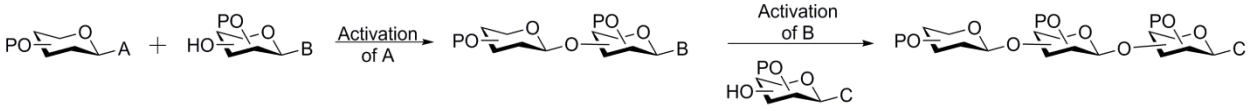


Figure 1.22: Glycosylation strategy employing selective activation.

In the preparation of two heparin/HS tetrasaccharides, Yu and coworkers used the active-latent approach to produce disaccharide building-blocks and selective activation for extension of the backbone. Glycosylation of the 1,6-anhydro acceptor **134** by the ethyl 1-thio-L-idoside donor **133** was performed with NIS and AgOTf (Figure 1.23).[27b, 42c] To convert disaccharide derivative

135 into an active donor, the anhydro ring was opened, followed by protecting-group adjustment, an oxidative manipulation at C-6, and formation of the trichloroacetimidate disaccharide donor **136**. Donor **136** was selectively activated by TMSOTf, with the thioglycoside **137** serving as acceptor, leading to the trisaccharide derivative **138**. Without further synthetic manipulation, trisaccharide **138** was activated by the thiophilic promoter 1-benzenesulfinylpiperidine (BSP) and Tf_2O, which glycosylated monosaccharide **139** to produce tetrasaccharide derivative **140**.

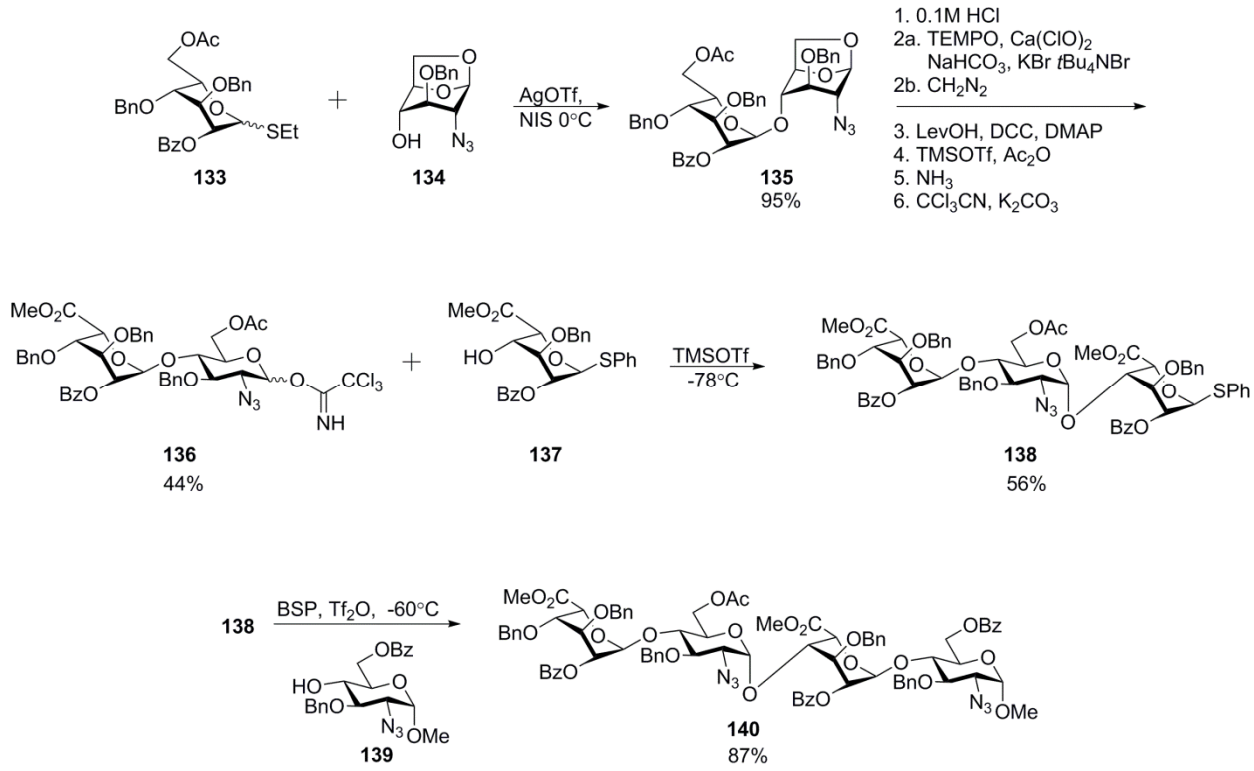


Figure 1.23: Synthesis utilizing the selective activation of trichloroacetimidate donors in the presence of thioglycoside acceptors.

Instead of glycosyl trichloroacetimidates and thioglycosides, van der Marel and coworkers explored the utility of free glycoses (glycosyl hemiacetals) and thioglycosides in a selective glycosylation approach towards the pentasaccharide derivative **148,** a fully protected precursor of the heparin backbone. The hemiacetal **141** was selectively activated in the absence of the glycosylthio acceptor **142,** utilizing the pre-activation strategy, where the donor was

treated with the diphenyl sulfoxide and Tf_2O promoter-system developed by the Gin laboratory.[44] Upon complete activation, the acceptor **142** was added to the reaction mixture to yield disaccharide derivative **143** (Figure 1.24). To extend the chain, the 1,6-anhydro acceptor **134** was glycosylated with disaccharide derivative **143**, producing trisaccharide derivative **144** as

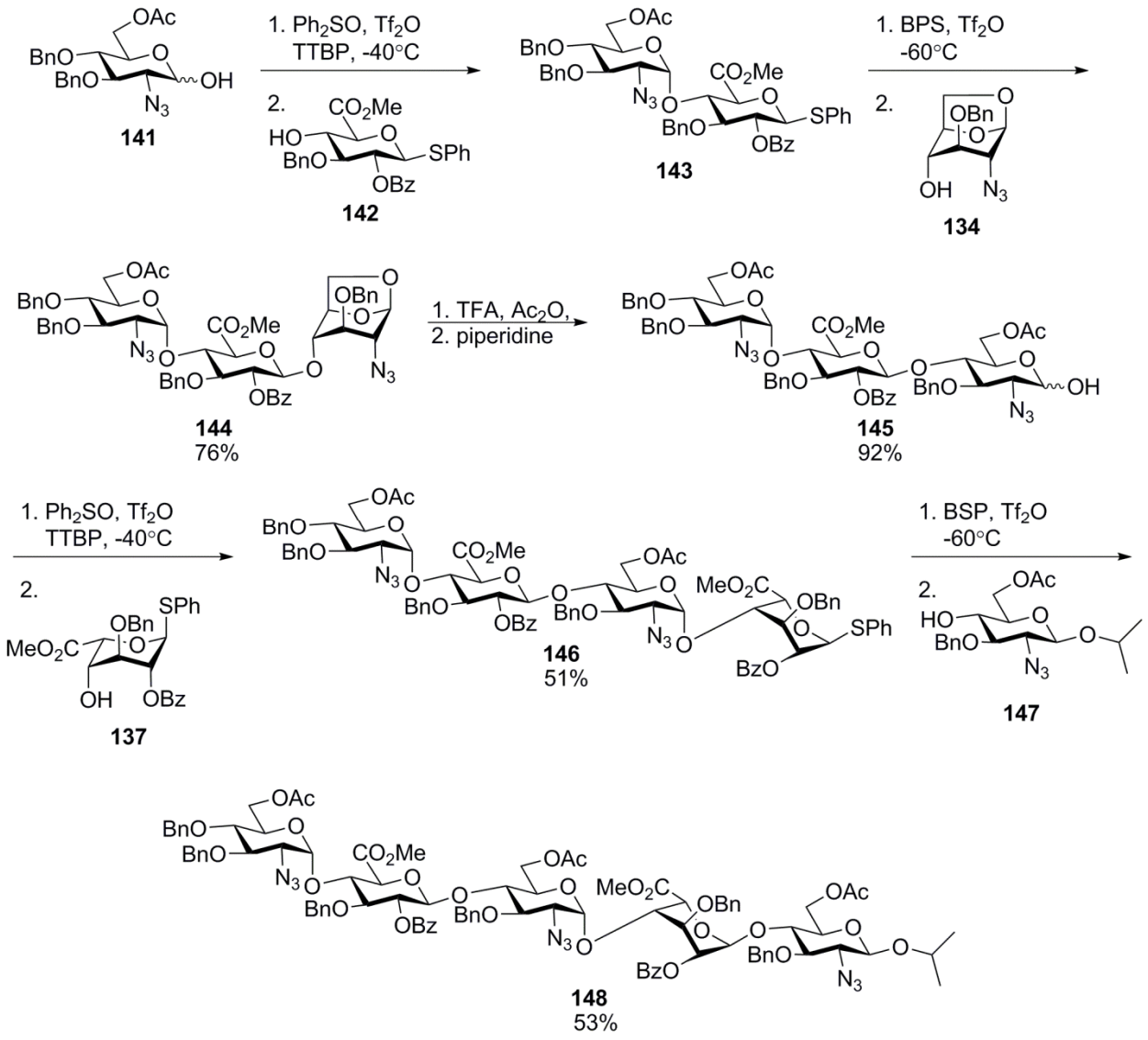


Figure 1.24: Congruent use of hemiacetals and thioglycosides.

a latent donor. The 1,6-anhydro bridge was then opened under acidic conditions to create a trisaccharide hemiacetal donor **145**, which after selective activation was coupled to the glycosylthio acceptor **137**. The resultant tetrasaccharide thioglycoside **146** reacted with the

reducing-end acceptor **147** to complete the synthesis.[26] Aided by the 1,6-anhydro ring as a masked hemiacetal, thioglycosides and glycosyl hemiacetals proved to be very effective partners for the selective glycosylation approach. Only one manipulation of an intermediate aglycon was required for preparation of the pentasaccharide derivative **148**.

Although the selective-activation strategy improves synthetic efficiency, it requires two different types of glycosyl donor. To simplify the overall synthetic design, it is desirable for a single type of glycosyl donor to be employed throughout the synthesis, avoiding the need for modification of the aglycon leaving-group of the intermediate oligosaccharides. Towards this goal, two chemoselective strategies have been developed. These are the reactivity-based, armed--disarmed method and the reactivity-independent, pre-activation-based method.

1.7 Reactivity-Based Chemoselective Glycosylation

In reactivity-based, armed-disarmed glycosylation strategy, glycosyl building-blocks, typically thioglycosides, are designed to have different reactivities at the anomeric position. When a mixture of a more-reactive donor (armed) and an acceptor having lower anomeric reactivity (disarmed) is subjected to a limiting amount of promoter, the more-reactive, armed donor is activated preferentially, and this donor glycosylates the acceptor (Figure 1.25). The resulting oligosaccharide can function directly as a donor by using the same conditions for further glycosylation with a thioglycoside acceptor that has even lower anomeric reactivity. With suitable design, the anomeric reactivities of various building-blocks can be sufficiently different so as to enable multiple glycosylation reactions sequentially in one vessel without the need for purification of the oligosaccharide intermediates.

To achieve the required differentiation of anomeric reactivity, the electronic property or conformational rigidity of the donor can be tuned by strategically placing suitable protecting

groups on the glycon ring[45] or by modification of the aglycon.[46] Reactivities can be quantified as relative-reactivity values (RRVs), with the reactivity of *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside towards methanol acceptor being set as 1.0.[45c]

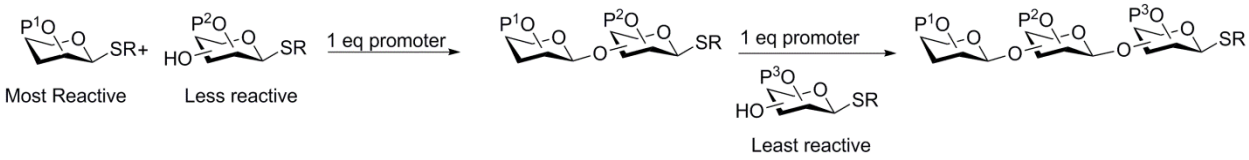


Figure 1.25: The armed-disarmed strategy for chemoselective glycosylation relies on differences in anomeric reactivities of the building blocks.

Wong and coworkers conducted the synthesis of heparin/HS oligosaccharides via the reactivity-based approach. Four monosaccharide building-blocks (**149-152)** were prepared and their RRVs measured (Figure 1.26a).[47] As the glucoside building block **151** was 30 times more reactive than the acceptor glucosamine precursor **150**, chemoselective activation of **151** was achieved in preference to **150,** leading to disaccharide derivative **153** in excellent (89%) yield (Figure 1.26b). Manipulation of protecting groups and oxidation at C-6 of the glucosyl component furnished the new disaccharide building-block **154** having a RRV of 18.3. Chemoselective glycosylation of **154** by the azidoglucose donor **149** (RRV = 53.7) was therefore feasible (Figure 1.26c) to give the corresponding trisaccharide derivative. The latter was coupled to O-4 of the disaccharide acceptor **155,** and more promoter was added to the reaction mixture. This led to the formation in one vessel of the fully protected HS pentasaccharide precursor **156** in 20% overall yield for the two steps. The modest net yield was most probably attributable to the small reactivity differential between donor **149** and disaccharide **154**.

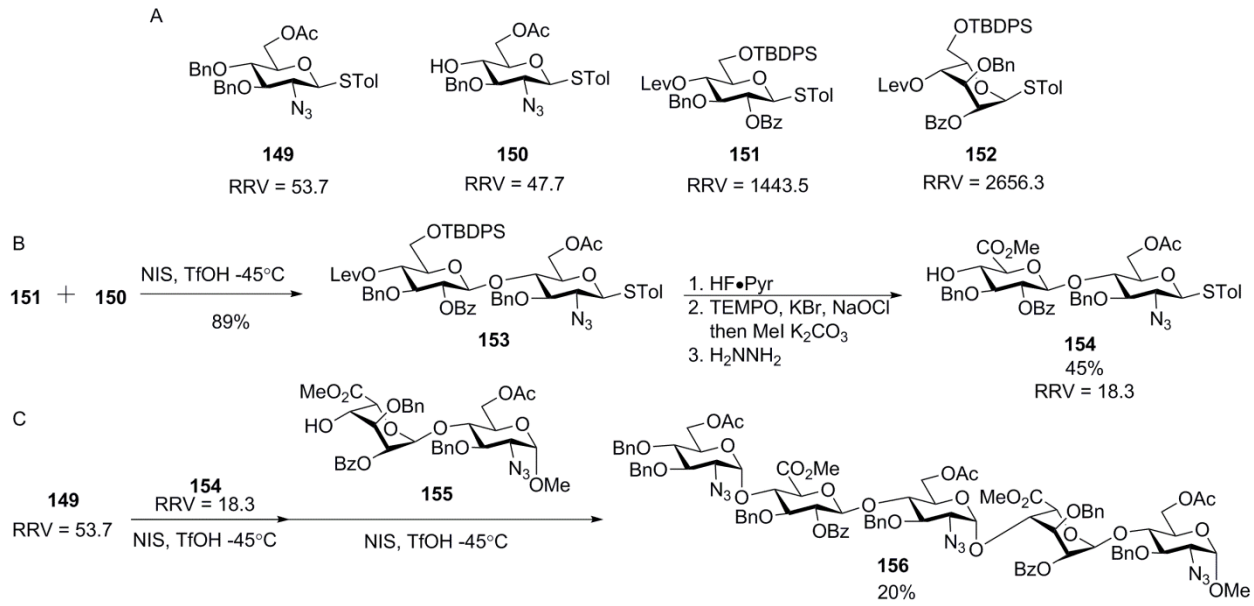


Figure 1.26: (A) Monosaccharide building blocks used in Wong's synthesis of heparin components; (B) preparation of disaccharide building block **154**; (C) one-pot synthesis of heparin pentasaccharide precursor **156** by the armed-disarmed strategy.

The RRVs provide general guidance towards the selection of building blocks. However, the RRVs are quantified with reference to methanol as the acceptor, and these values can change according to the structure of the acceptor and the reaction conditions.[48] Accordingly, caution needs to be exercised in relying solely on RRVs to predict the outcome of a reaction. Furthermore, applying the reactivity-based method to the synthesis of longer heparin/HS oligosaccharides could be challenging because the polymeric nature of heparin/HS would require the same glycosyl units to have greatly differing reactivities according to their location in the backbone. The building blocks at the non-reducing end should have higher reactivities than those situated towards the reducing end. This challenge can be overcome by the reactivity-independent, pre-activation-based chemoselective strategy for glycosylation.

1.8 Reactivity-Independent, Pre-Activation-Based, Chemoselective Glycosylation

The aforementioned glycosylation strategies rely on differences in anomeric reactivity. The acceptor either cannot be activated, as in the case of linear, active-latent, and selective activation methods, or has a much lower reactivity than the donor in the armed-disarmed reactivity-based approach. The underlying cause for this is the fact that the glycosyl donor and acceptor are both present in the reaction mixture when the promoter is added. Thus, the anomeric reactivities of donors and acceptors must be differentiated to achieve selective activation of the donor. To overcome this limitation, the pre-activation strategy was developed, wherein the donor is activated by a promoter to generate a reactive intermediate in the absence of an acceptor (Figure 1.27). The acceptor is then added to react with the reactive intermediate and form a new glycosidic bond. Activation of the donor in the absence of the acceptor allows the acceptor to carry the same aglycon group as the donor, negating the need for reactivity tuning. The prerequisite for pre-activation is that the promoter used must be in stoichiometric amount to avoid activation of the acceptor or product, and any side-products from activation of the donor must not be nucleophilic. Several types of glycosyl donor have been used in the pre-activation figure, and these include hemiacetals,[44] glycals,[49] selenoglycosides,[50] and thioglycosides.[51]

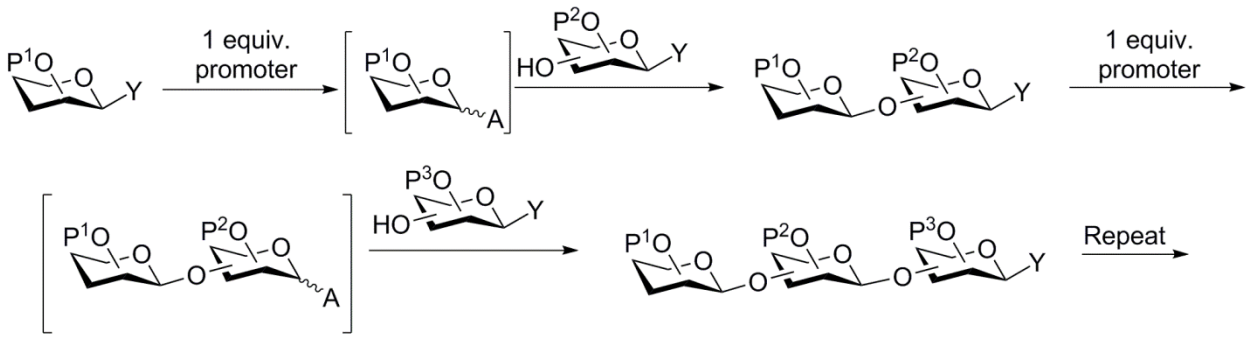


Figure 1.27: Pre-activation-based strategy for glycosylation.

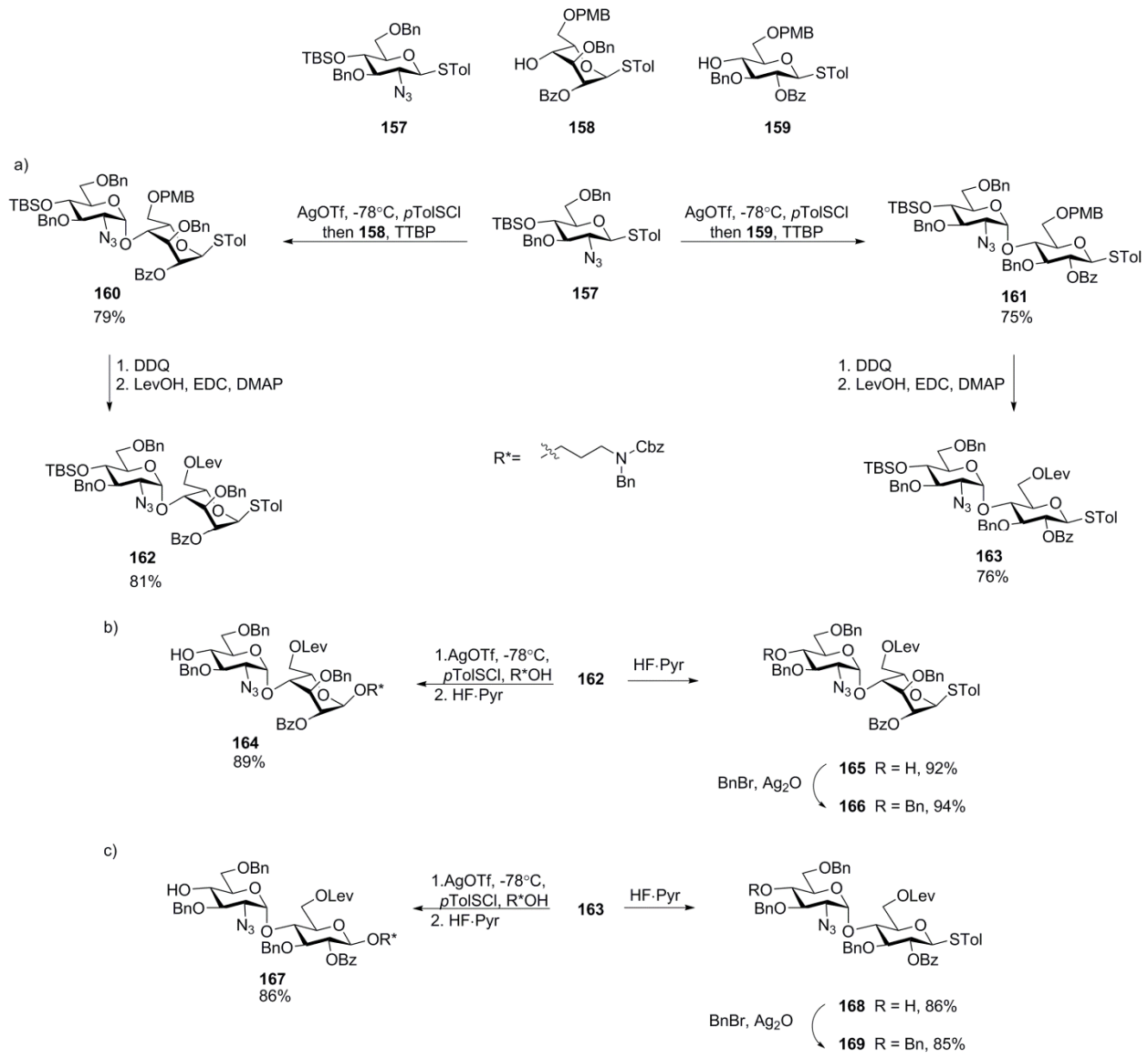


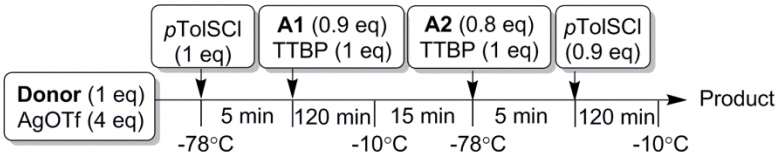
Figure 1.28: Divergent synthesis of the building blocks needed for the assembly of a hexasaccharide library.

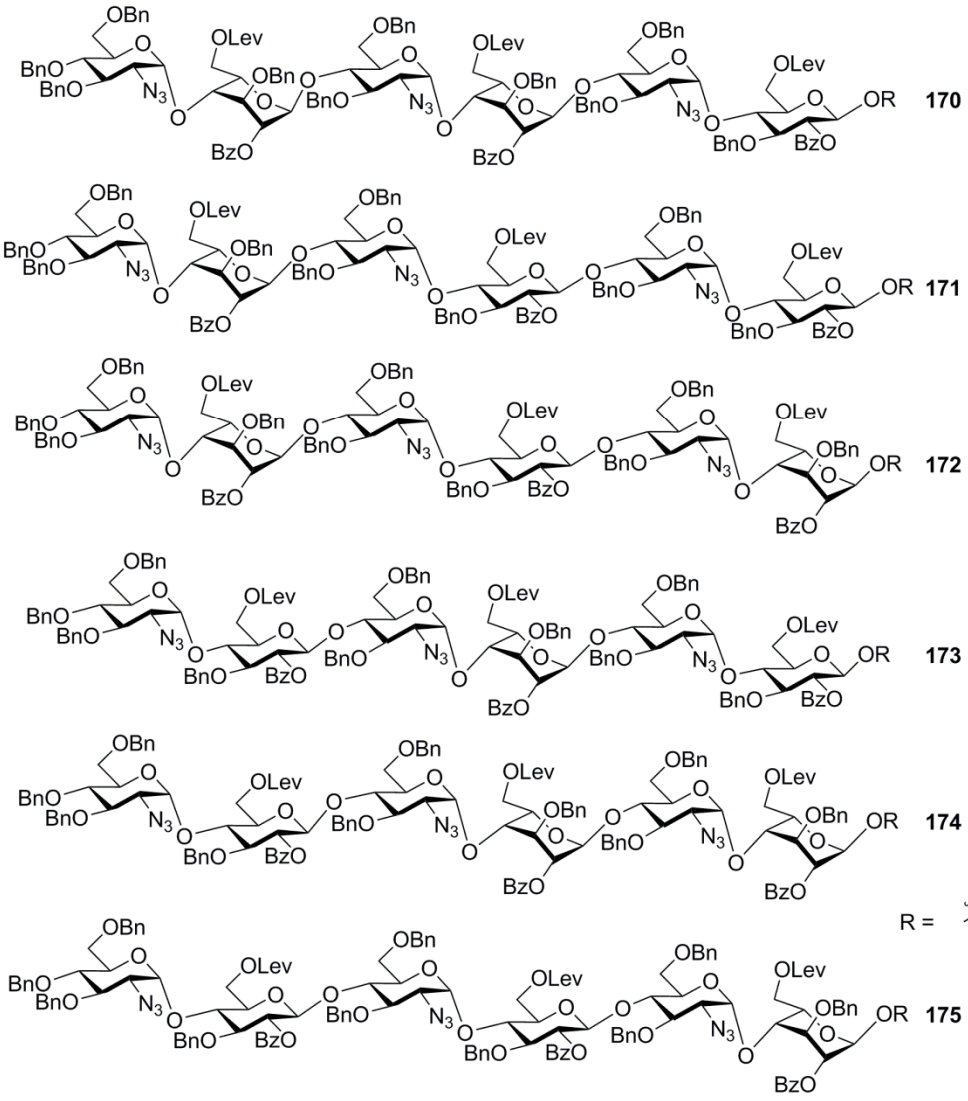
Huang and coworkers synthesized a library of twelve heparin/HS hexasaccharides by the reactivity-independent, pre-activation-based strategy. This synthesis employed thioglycoside modules and the powerful promoter *p*-toluene sulfenyl triflate (*p*TolSOTf), which was generated *in situ* from *p*-toluenesulfenyl chloride (*p*TolSCl) and AgOTf.[51b] To simplify the preparation of building blocks, a divergent approach was designed. Starting from three monosaccharide

building-blocks, two disaccharide derivatives (**162** and **163)** were prepared (Figure 1.28a). These compounds were then divergently modified, leading to six disaccharide modules (**164** to **169,** Figures 1.28b and 1.28c).[20b] To assemble the hexasaccharide, disaccharide donor **166** was pre-activated with *p*TolSCl and AgOTf at -78 °C (Table 1.1). Upon complete activation, the bifunctional 1-thioglycoside acceptor **165** was added to the reaction mixture. The reactive intermediate generated through activation of the donor glycosylated the acceptor **165**, producing a tetrasaccharide. As this tetrasaccharide product already bore an arylthio aglycon, it was activated directly with another equivalent of the promoter, and allowed to react with acceptor **167** in the same reaction flask. Hexasaccharide **170** was obtained from this reaction in 54% yield in less than five hours. Since this synthesis did not require adjustment of the aglycon structure or purification of the intermediate tetrasaccharide, the efficiency of the glycosidic assembly was greatly enhanced.

As the pre-activation method does not require the glycosyl donor to have higher anomeric reactivities than the glycosyl acceptor, the disaccharide building-blocks **164**-**169** could be used in a combinatorial fashion to prepare a library of oligosaccharides (Table 1.1).[20b] For example, substituting compound **165** by **168** and then following the same reaction scheme as in the preparation of hexasaccharide **170**, hexasaccharide derivative **171** was formed in 59% yield in a one-pot process. By mixing the disaccharide building-blocks **164-169**, six hexasaccharides having systematically varied and precisely controlled backbone structures were produced in 50--62% yields within a few hours (Table 1.1). These hexasaccharides were then deprotected and subsequently sulfated, creating a set of 12 heparin/HS hexasaccharides, which were used to decipher structure-activity relationships in the binding of fibroblast growth factor-2 to heparin.

Table 1.1: One-pot preparation of heparin/HS hexasaccharides.





Donor	Acceptor 1	Acceptor 2	Product	Yield (%)
166	**165**	**167**	**170**	**54**
166	**168**	**167**	**171**	**59**
166	**168**	**164**	**172**	**58**
169	**165**	**167**	**173**	**62**
169	**165**	**164**	**174**	**57**
169	**168**	**164**	**175**	**50**

In summary, as discussed up to this point, chemical synthesis has been the major path for access to synthetically pure heparin/HS oligomers. Given the length and difficulties in chemical synthesis, several groups have begun to explore the potential of enzymatic synthesis and its integration with chemical methods.

1.9 Chemoenzymatic Synthesis

In Nature, the biosynthesis of heparin/HS is performed by multiple enzymes in the Golgi apparatus. Assembly of the HS backbone by glycosyltransferases is followed by such enzymatic modifications as *N*-deacetylase/*N*-sulfotransferase (NDST) for removal of the *N*-acetyl group and subsequent *N*-sulfation, C_5-epimerase for isomerization of the uronic acid, and three types of *O*-sulfotransferases, namely 2-OST for sulfating *O*-2 of IdoA, 3-OST for sulfating *O*-3 of GlcN, and 6-OST for sulfating *O*-6 of GlcN. The enzymatic modification of the HS backbone is typically incomplete, and thus leading to a wide range of structural variations in naturally occurring heparin and HS.

In order to develop a laboratory synthesis of a pentasaccharide exhibiting strong binding with ATIII, the Rosenberg group explored the enzymatic approach.[52] The backbone of their oligosaccharide was obtained from "heparosan," a polysaccharide from the *E. coli* K5 capsule composed of disaccharide repeating-units of [→4)-α-D-GlcNAc-(1→4)-β-D-GlcA-(1→]. The synthesis of pentasaccharide **178** started with *N*-sulfation of the "heparosan" by incubation with NDST2 and the sulfate-group donor 3'-phospho-5'-adenylyl sulfate (PAPS, Figure 1.29). Following *N*-sulfation, the polymer was depolymerized by heparitinase, and hexasaccharide **176** was isolated by HPLC from the resulting mixture. Sequential epimerization and *O*-2 sulfation of hexasaccharide **176** by C_5-epimerase and 2-OST1, followed by sulfation at *O*-6 provided the

sulfated hexasaccharide **177**. Removal of the unsaturated uronic acid by $\Delta^{4,5}$-glycosiduronase with subsequent 3-OST-catalyzed sulfation at *O*-3 produced pentasaccharide **178**, a compound having anticoagulant activity. While this synthesis was groundbreaking, the product **178** was isolated in only microgram quantity and with an overall yield of 1.1%.[52] The

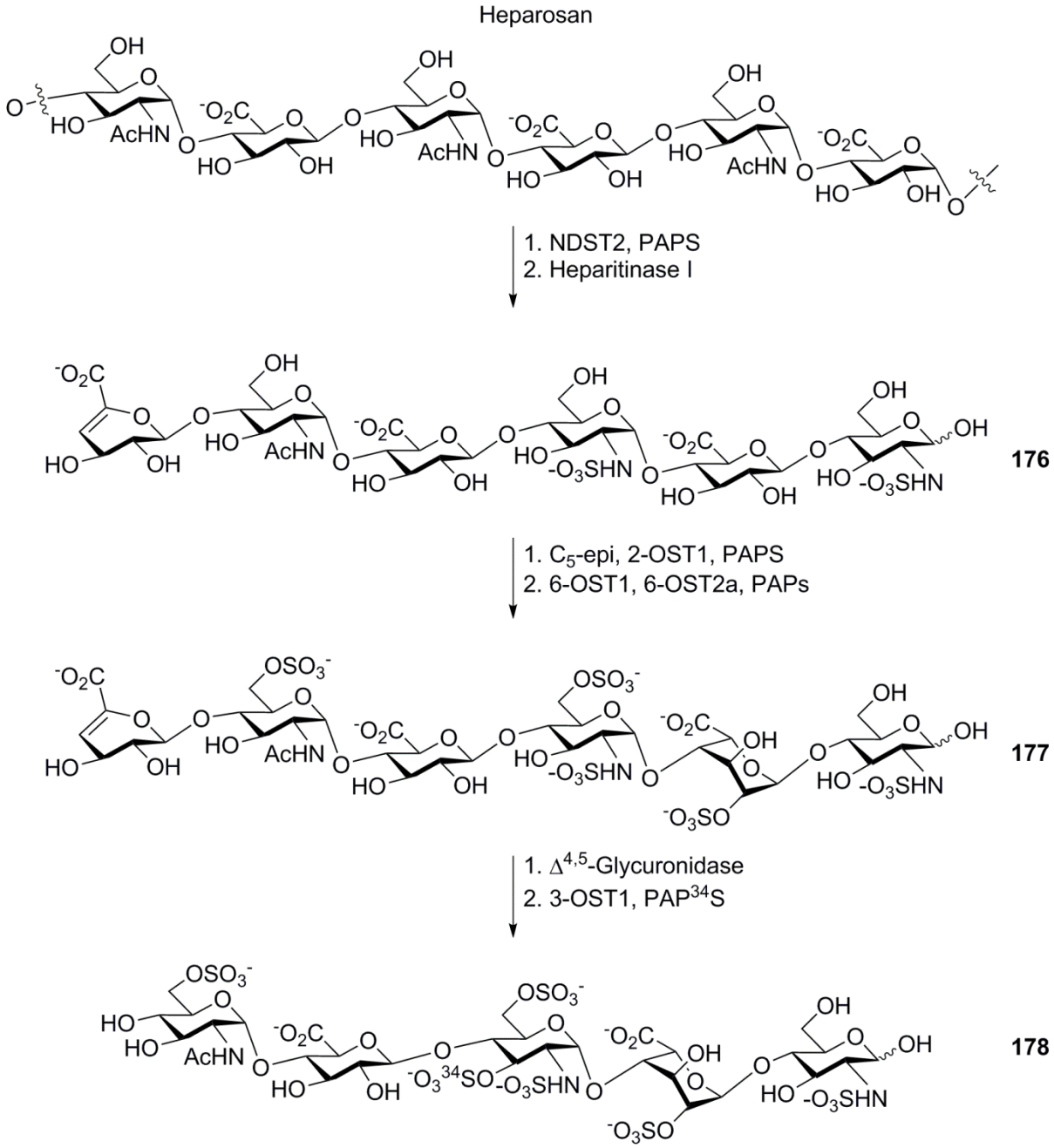


Figure 1.29: Enzymatic synthesis of pentasaccharide **178** from "heparosan."

low yield was presumably due to the difficulties in purification, particularly in the isolation of hexasaccharide **176** from the complex mixture that arose from cleavage by heparitinase.[53] Another obstacle was the low yields of the enzymes expressed from a baculovirus system.

The Liu and Linhardt groups took a different approach for the chemoenzymatic synthesis of heparin/HS oligosaccharides. Instead of relying on the difficult isolation of hexasaccharide **176** from the complex mixture of degradation products resulting from the action of heparitinase on "heparosan", they obtained gram quantities of disaccharide **179** through the complete digestion of "heparosan" by nitrous acid.[53b, 54] To elongate the chain, two bacterial glycosyltransferases, heparan synthase-2 (pmHS2)[55] and the *N*-acetylglucosaminyltransferase of *Escherichia coli* (KfiA),[56] were used to transfer GlcA and GlcNAc respectively (Figure 1.30). All of the enzymes for backbone modification, including C_5-epimerase, NDST2, and the *O*-sulfotransferases were expressed in large quantities in the *E. coli* system. The conversion of the *N*-acetyl group to *N*-sulfate is difficult because of the stability of the acetamido group and the low activity of the *N*-deacetylase. To overcome this, Liu, Linhardt and coworkers took advantage of the broad substrate-specificity of KfiA by incorporating *N*-trifluoroacetyl-protected glucosamine (GlcNTFA) into the backbone where *N*-sulfation is desired.[57] Treatment of disaccharide **179** with the glycosyl donor UDP-GlcNTFA and the transferase KfiA, followed by UDP-GlcA and transferase pmHS2 provided tetrasaccharide **180** in 75% yield. An additional round of elongation with both monosaccharides, followed by removal of the TFA protecting groups with triethylamine, and subsequent *N*-sulfation by *N*-sulfotransferase (NST) furnished the *N*-sulfated hexasaccharide **181**. Following the addition of another GlcNTFA group, epimerization and sulfation at *O*-2 were performed in one flask with 2-OST and C_5-epimerase to

yield the heptasaccharide **182**. The location of enzymatic modification was controlled by the substrate

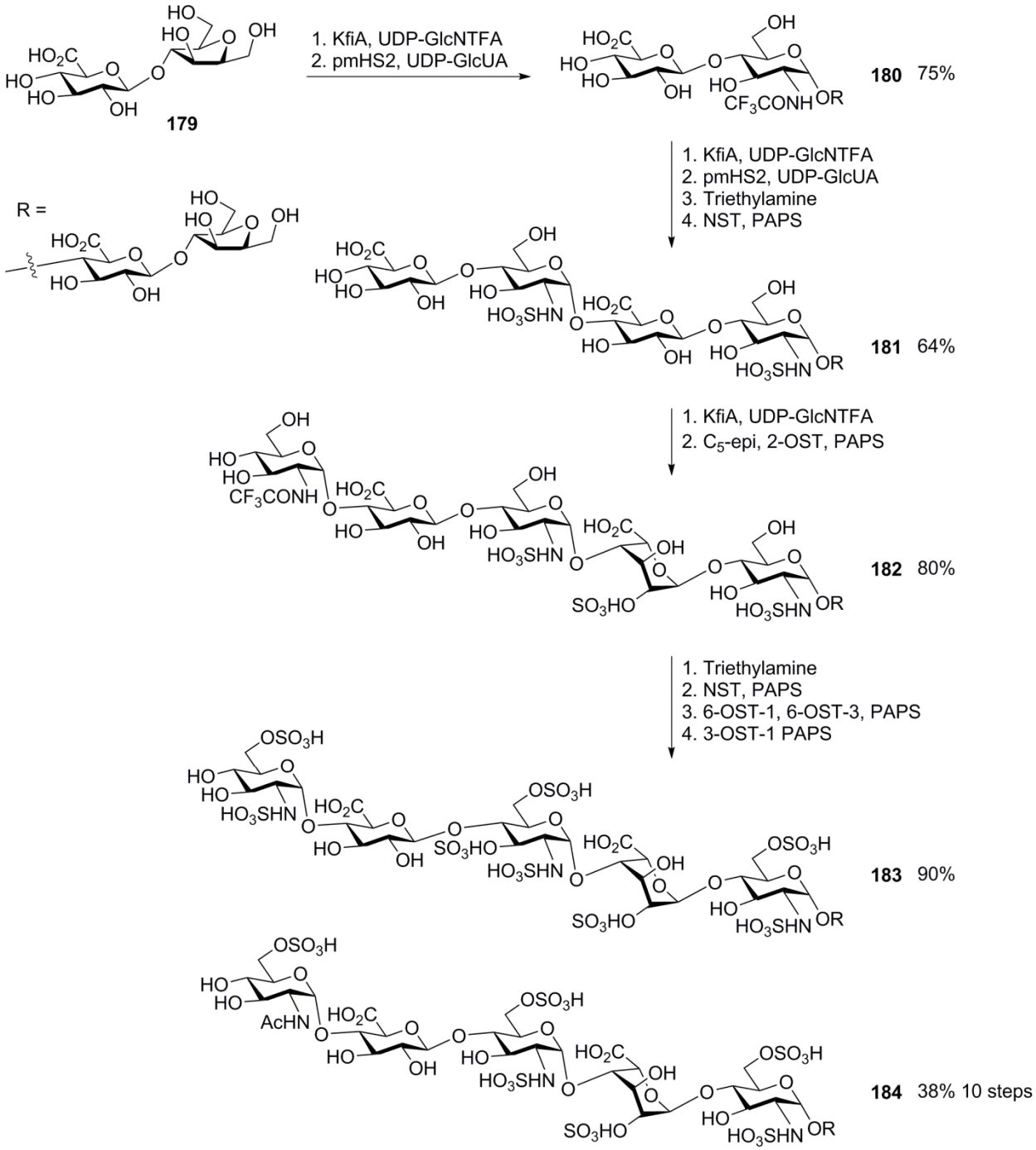


Figure 1.30: The chemoenzymatic synthesis of heparin heptasaccharides **183** and **184**.

structure. As the C_5-epimerase causes GlcA to be modified only when flanked by *N*-sulfated glucosamine groups, the GlcA component closer to the reducing end in **181** alone was epimerized and O-2 sulfated. The last TFA protecting group in **182** was removed with triethylamine and the product was incubated with NST and PAPS, then PAPS, 6-OST-1, 6-OST-3, and finally PAPS, and 3-OST1 in sequential reactions to provide the final heptasaccharide **183,** which had anticoagulant activity similar to that of the FDA-approved pentasaccharide fondaparinux.[57a] In an analogous manner, 49 mg of the heptasaccharide **184** was prepared with an overall yield of 38% from the disaccharide **179**. This work has laid a great foundation for future gram-scale preparation of heparin/HS oligosaccharides.[57b] Extending the chemoenzymatic strategy to preparation of fondaparinux will provide an attractive alternative complementing the current complex chemical synthesis of this important molecule.

1.10 Future Outlook

The past decade has seen tremendous advancements in the production of heparin/HS oligosaccharides. In addition to the more traditional target-oriented synthesis, efforts are being directed toward generating an array of oligosaccharides having diverse patterns of sulfation. In chemical synthesis, multiple strategies have been developed to expedite the glyco-assembly process. Methods are now available for access to tens of oligosaccharides to construct a sample library. However, challenges remain in decreasing the number of synthetic steps required for preparation of building blocks, as well as for establishing a robust method to perform multiple sulfations simultaneously. The enzymatic synthesis of compound **184** at the 49 mg scale is an impressive accomplishment. The substrate specificities of the enzymes may possibly limit the total number of structures that can be generated. Ongoing research has suggested that enzymatic modification can be integrated with chemical synthesis.[58] The combination of the regiospecificity

of enzymatic reactions with the flexibility of chemical synthesis can significantly expand our overall synthetic capability, which in turn can greatly aid in the efforts to decipher the exciting biological functions of heparin and HS.

Rather than focus on generating a wide array of oligosaccharides Gardiner and coworkers have focused on synthesizing large oligosaccharides. Utilizing a simple linear approach with the use of a tetrasaccharide building block they have pushed the boundaries of oligosaccharide synthesis. Tetrasaccharide **185** utilizes many of the strategies previously described (Section 1.2). Through 9 rounds of coupling and 4-*O* deprotection they managed to prepare a 40-mer heparin-related oligosaccharide(**199**, Figure 1.31).[59] The key issue found was the deprotection of the 4-*O* position for chain elongation.[35] They previously reported issues removing the commonly used *p*-methoxyl benzyl ether and found success utilizing the trichloro-acetyl (TCA) protecting group that was removed with an 87% yield from a 36-mer oligomer. Glycosylations were also achieved in high yield utilizing thioglycosides even producing the 40-mer (**199**) in 64% yield while requiring 1.5 equivalents of donor. The synthesis of **199** is the longest sugar yet produced and **200** is by far the longest heparin related oligomer.

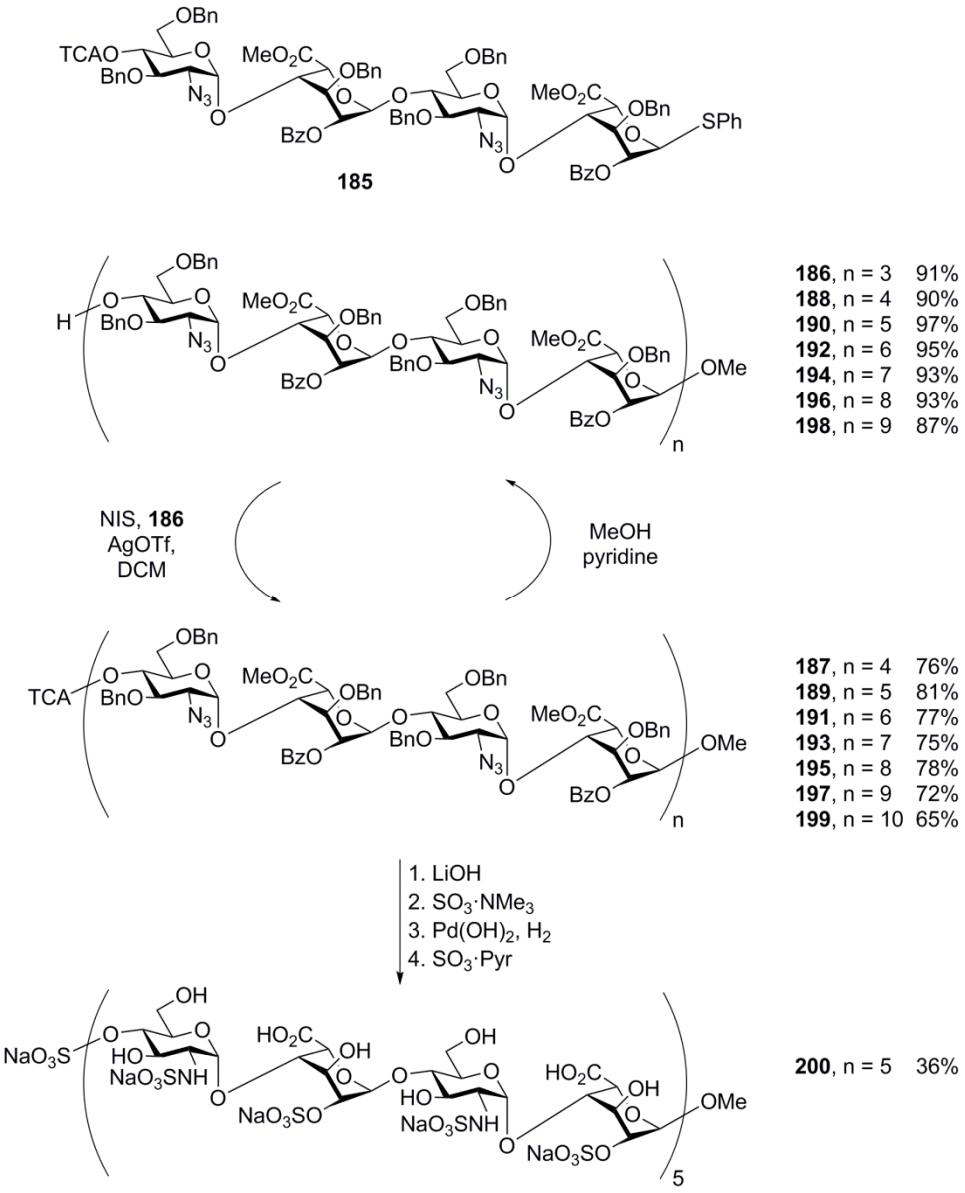


Figure 1.31. Syntheis of 20-mer heparin related oligomer.

Chapter 2 – Synthesis and evaluation of a heparin microarray

2.1 Synthetic Design and Building Block Preparation

The first step in any synthetic plan is to develop an appropriate strategy to reach the desired goal. Many major problems are averted by proper design but there are always unknown factors that inevitably crop up during a synthesis. Heparin/HS synthesis has known issues that have been detailed in Chapter 1. The retrosynthetic analysis in Figure **2.1** illustrates how several of these issues were addressed. Sites of sulfation, the 2-*O* position of idose and the 6-*O* position of glucosamine were protected with base labile groups that could be removed simultaneously and subsequently sulfated. The benzoyl on the 2-*O* position has a dual function as a neighboring group participator that will help form the needed 1,2 trans glycosidic linkage. To form the

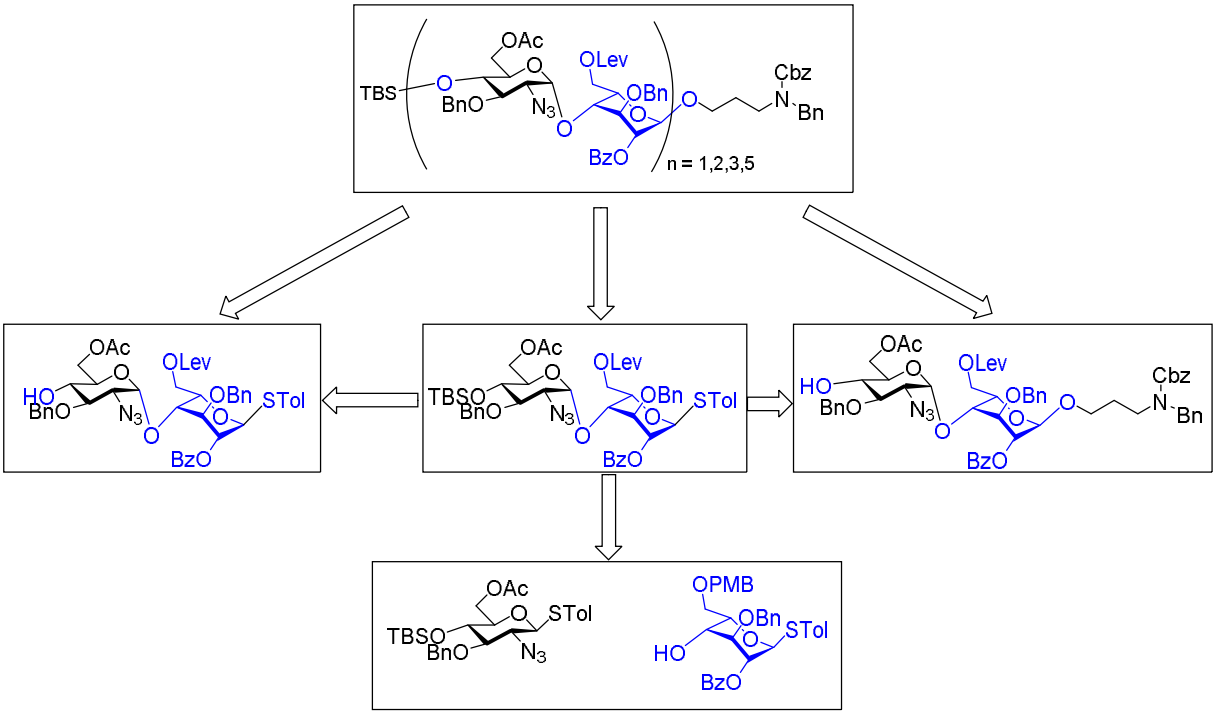


Figure 2.1. Retrosynthetic analysis of heparin/HS oligosaccharides.

needed 1,2 cis linkage, the amine group is protected as an azide, a non-participating neighboring group. To avoid the issue of iduronic acid epimerizing, it is used as a levulinoyl ester protected idoside that can be selective removed and oxidized.[28] The issue of access to iduronic acid will be discussed with the preparation of building blocks.

Accessing L-iduronic acid in large enough quantities is difficult as it is not commercially available and has to be synthesized. This involves two main steps, inversion of the C-5 position and oxidation. Oxidation from the L-idose to L-iduronic acid was performed after glycosylation to avoid epimerization and due to the known low reactivity of L-iduronic acids in glycosylation.[18a] L-idose was prepared starting with commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. The first step was protection of the free hydroxyl as a benzyl ether with sodium hydride and BnBr. Removal of the isopropylidene on hydroxyls 5-*O* and 6-*O*, followed by dimesylation provided compound **1** in 84% yield for the three steps. C-5 inversion was accomplished by displacement of the primary mesylate on C-6 with acetate and subsequent cleavage of that acetate and epoxide formation provided compound **2** having the inverted L-idose configuration. The furanose ring was rearranged to the more stable pyranose by opening the epoxide with sulfuric acid, which simultaneously cleaved the isopropylidene. Global acetylation of the resulting product furnished compound **3** in 74% yield. Due to their ease of selective activation and great stability, **3** was converted into thioglycoside **4** using boron trifluoride etherate and thiotoluene. The reaction provided a 2:1 ratio of **4α** and **4β** and to simplify analysis only the alpha anomer was subsequently used. Removal of the acetates and protection of the 4,6-diol with *p*-methoxybenzylidene left the commonly sulfated 2-*O* position free. This was protected as a base labile benzoyl ester forming compound **5** in 70%. The benzoyl ester served a second purpose as through neighboring group participation it can help form 1,2 trans glycosidic

bonds. The last remaining step was to open the 4-*O* position for glycosylation reactions and this was done by treating the *p*-methoxybenzylidene with trifluoroacetic acid and sodium cyanoborohydride producing the two regioisomers **6** and **7** in 72% and 17% yields respectively. 8 grams of the desired building block **6** was prepared thus access to L-idose was not an issue.

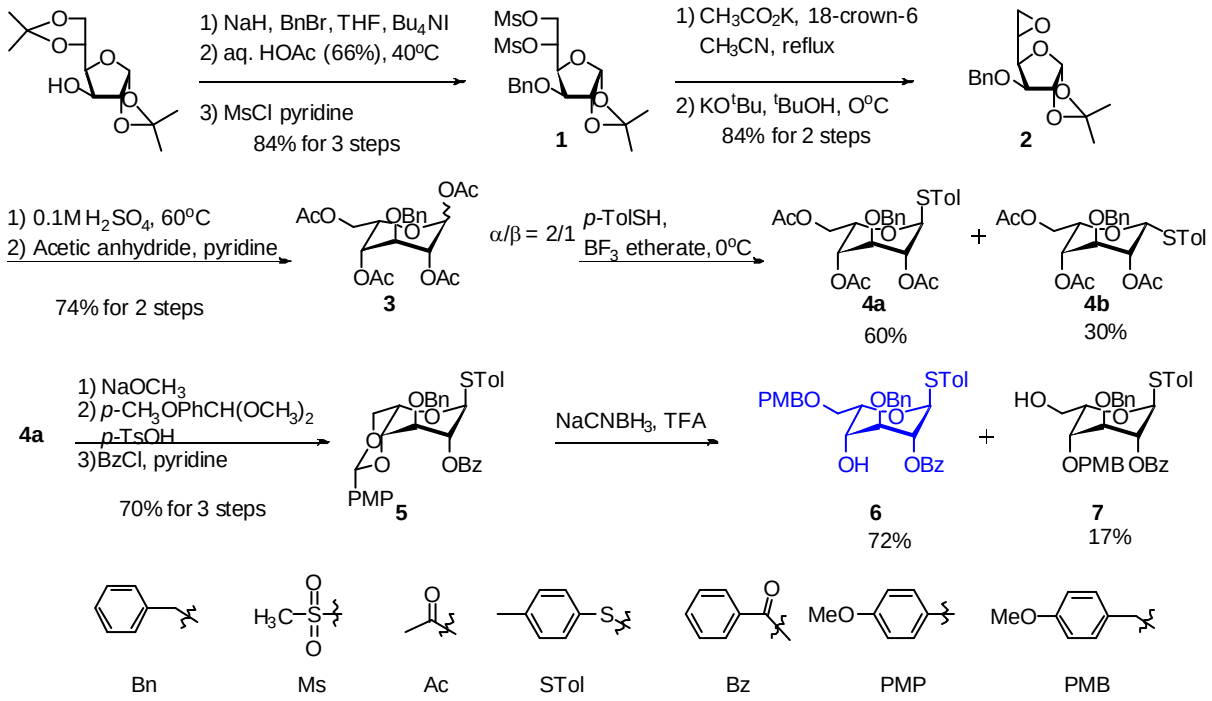


Figure 2.2: Synthesis of L-idose building block **6.**

Procuring the other building block, glucosamine, was more straightforward as glucosamine hydrochloride is commercially available. For more selectivity during thioglycoside formation the amine was protected with the bulky Troc group by treatment with trichloroethyl chloroformate and global acetylation forming **8**. Installation of the thiotoluene with boron trifluoride etherate produced 67% of the β anomer **9**. Small amounts of the α anomer were produced but they were not isolated. Removal of the Troc with zinc dust and deacetylation provided **10** in 90% yield. The amine was protected as an azide, allowing selective reduction by dithiol or Staudinger conditions. The non-participatory nature of the azide also helped to form

the 1,2 cis glycosidic bond in **15**. Installation of the 4,6-benzylidene in **11**, allowed protection of the seldom sulfated 3-*O* position with a benzyl ether. Removal of the benzylidene and acetylation of the primary hydroxyl at low temperature produced **12**. The remaining 4-*O* position was protected with the silyl TBS, **13**, as this could be removed selectively to allow for chain elongation. **12** could also be protected with a benzyl ether on the 4-*O* position to terminate the oligosaccharide, **14**. Numerous conditions were tried for benzylation. The most common conditions for base sensitive substrates, benzyl 2,2,2 trichloroacetimidate and an acid catalyst, failed completely. Numerous solvent systems were attempted with a triflic acid catalyst but each one resulted in degradation by TLC. **14** could be accessed with BnBr and a variety of basic catalysts. While NaHMDS gave a 50% yield in DMF, NaH was found to be more effective if monitored closely, providing **14** in 74% yield or 91% BRSM. With the monosaccharide building blocks in hand, the disaccharide building blocks could be assembled for the modular synthesis of longer heparin oligosaccharides.

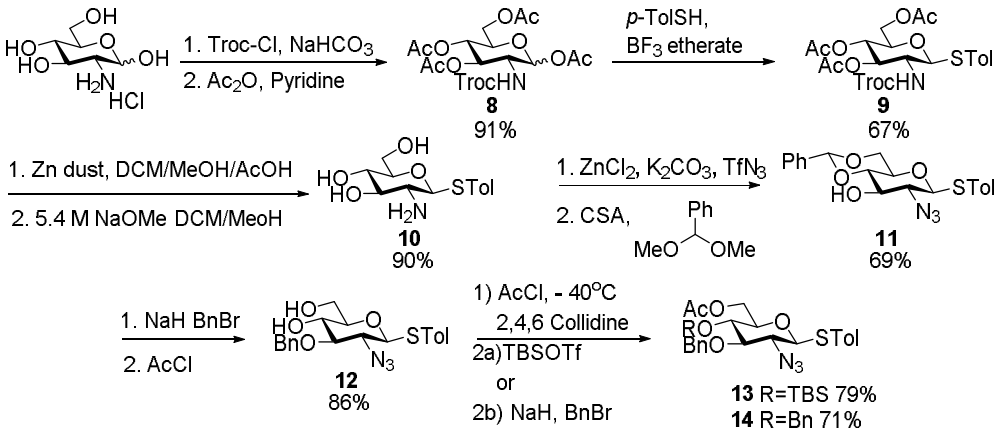


Figure 2.3: Synthesis of glucosamine building blocks **13** and **14**.

Preparation of the disaccharides needed was done from monosaccharides **6** and **13** in seven steps. Pre-activation of **13** with *in situ* generated *p*-toluene sulfenyl triflate, followed by addition of **6** led to the formation of disaccharide **15** in 85% yield of only the desired α product.

A cyclic side-product, **16**, was formed in high yield following the general procedure of glycosylation. Running the reaction at -78°C for longer times or increasing the amount of base, TTBP, did not improve the results. The only solution found to stop the formation of **16** was to keep the solvent mixture at a minimum concentration of 50% diethyl ether. Due to the nucleophilicity of the 6-*O* imparted by the PMB group, the PMB had to be replaced before **15** could be used as a donor otherwise **16** would form in high yields.[20b] Replacing PMB with another orthogonal protecting group, levulinoyl, was done in two steps. DDQ was used to remove the PMB producing **17.** The newly liberated hydroxyl was protected as a levulinoyl ester with levulinic acid, DMAP, and EDC hydrochloride yielding **18** in 88% for the two steps.

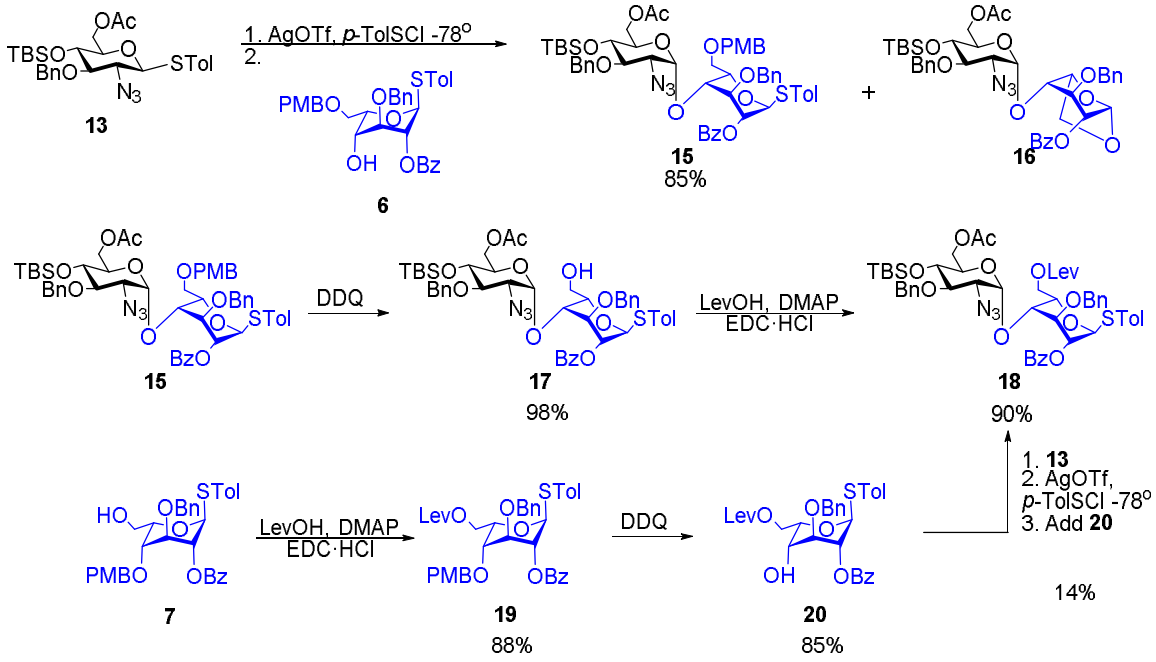


Figure 2.4: Preparation of key disaccharide **18** by two routes.

The issues of preparing disaccharide **18** led to the development of an alternative route. The problematic PMB protecting group in **6** was removed immediately after glycosylation and replaced with a levulinoyl ester. The logical solution would then be to replace the PMB with the levulinoyl ester before glycosylation. Two steps were required and would hopefully improve

glycosylation yield. To attempt this route the side product **7** which carried a free 6-OH was protected with a levulinoyl ester providing **19** in 88% yield. The PMB was then removed with DDQ to yield acceptor **20** (85%). Activation of **13** followed by addition of **20** provided the vital building block **18,** unfortunately only in 14% yield. Several reactions were run to attempt to improve the yield but all were as dismal as the first. In each of the reactions, acceptor **20** could be isolated after the reaction, presumably due to the low nucleophilicity imparted by the electron withdrawing levulinoyl ester. Since an efficient route to produce **18** was known, the alternative route with acceptor **20** was abandoned.

Disaccharide **18** was a key building block as it was transformed into all the needed disaccharide building blocks in just four steps. The three building blocks needed were at the reducing end, **24**, an internal or elongation disaccharide, **21**, and finally a capping disaccharide, **22**. The reducing end disaccharide **24** contained an amino linker, for coupling to the microarray. It was synthesized in two steps by first activating **18** in the presence of N-(benzyl)-benzyloxycarbonyl-3-aminopropanol. Removal of the TBS group on **23** by HF·pyridine furnished acceptor **24** in 77% yield for the two steps (Figure 2.5a). For the internal disaccharide, removal of the TBS group by HF·pyridine provided **21** in 98% yield. Protecting the newly freed hydroxyl in **21** as a benzyl ether was not straightforward. One route to disaccharide **22** was by using monosaccharide **14** already carrying a benzyl protecting group. Unfortunately coupling **14** with acceptor **6** resulted in low yields despite repeated attempts with a typical yield of 36% for **25** (Figure 2.5b). The conditions used to produce monosaccharide **14**, NaH and BnBr in DMF, yielded only 40% of **22**. This was not an acceptable yield for a key building block and other bases were evaluated. The mild base Ag_2O gave the best yield, 83%, of the final capping

disaccharide **22**. With the disaccharides in hand, heparin/HS oligosaccharides up to a decasaccharide were readily synthesized.

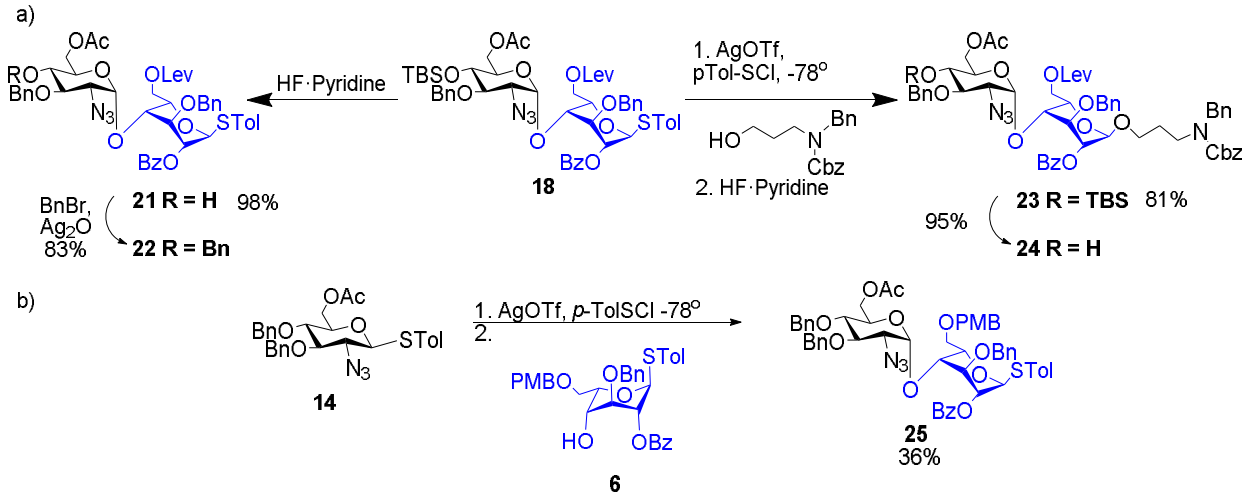


Figure 2.5. Preparation of disaccharide building blocks.

The modular strategy using the disaccharide building blocks allowed oligosaccharides as large as decasaccharides to be produced. Oligosaccharides **27-31** were made quickly and in reasonable yields. Disaccharide **26** was synthesized by benzylating the internal disaccharide **21** in 65% yield. The pre-activation method of glycosylation meant no protecting group manipulation between glycosylations. Activating **20** followed by addition of either **21** or **24** furnished the tetrasaccharides **27** and **28**, in 63% and 67% yield respectively**.** While tetrasaccharide **28** was capped at the non-reducing end and could not be further elongated, **27** was activated in the presence of **24** producing hexasaccharide **31** in 85% yield**.** The use of the TBS protecting group on **18** was flexible as it allowed a glycosylation product to be transformed into an acceptor. Coupling **18** and **21** yielded tetrasaccharide **29** in 81% yield. Activating **29** followed by addition of **24** provided hexasaccharide **30** in 72% yield. Compound **30** was transformed into an acceptor by removing the silyl protecting group with HF·pyridine producing

the hydroxyl containing **32** in 80% yield. Activating tetrasaccharide **29** in the presence of **32** furnished decasaccharide **33** in a respectable 61% yield.

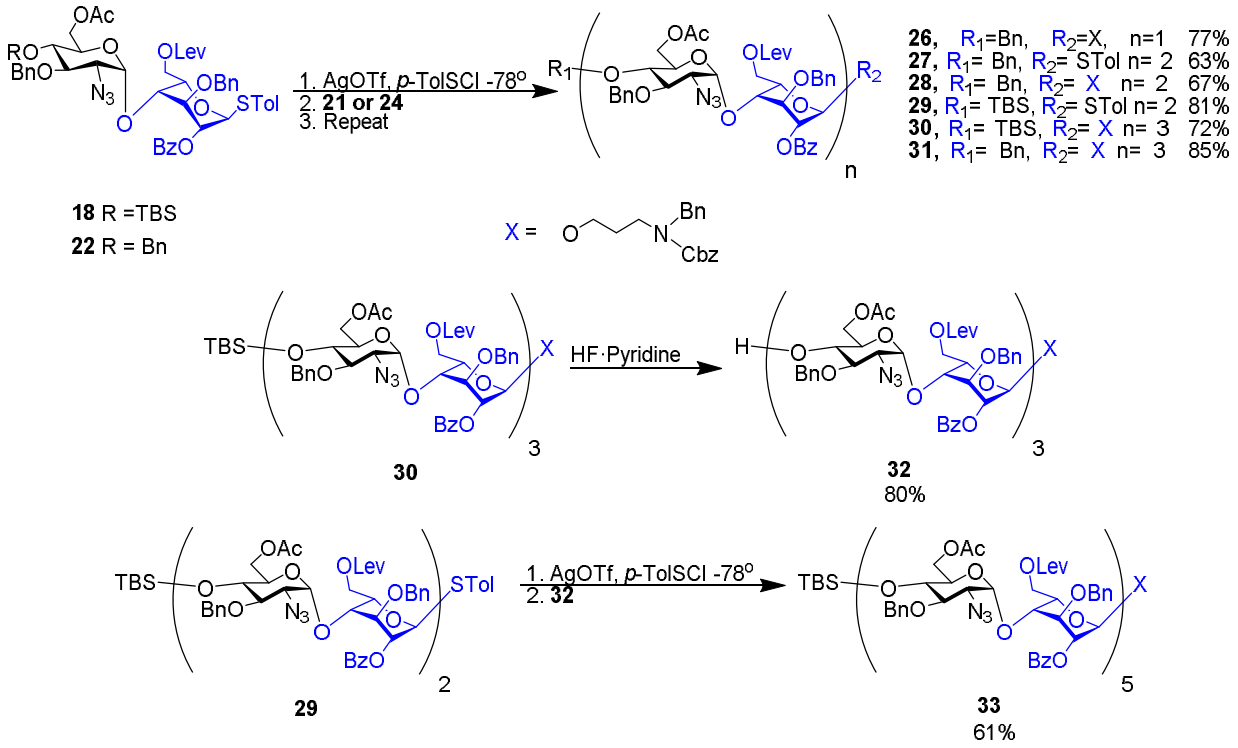


Figure 2.6. Assembly of the oligosaccharide backbones **26-31**.

Accessing oligosaccharides **26-31** illustrates that several common problems with heparin /HS synthesis have been dealt with. Procuring sufficient idose was not an issue nor was stereochemical control of glycosylation. All yields for glycosylation were at or above 60% and selectively formed the desired α-bond. The stereochemistry of glycosylation products were verified by analyzing their $^{1}J_{H1C1}$ coupling value. The alpha configuration was confirmed by $^{1}J_{H1C1}$ being near 170 Hz.[60] With the backbones in hand all that remained was to oxidize the idose moieties to iduronic acid, install the requisite sulfates, and fully deprotect the oligosaccharides.

2.2 Deprotection and Chemical Sulfation

The first step after assembly of the heparin oligosaccharides was to convert the idose residues to iduronic acids. This was done after glycosylation to avoid issues of low reactivity and poor stereochemical control. To prepare for oxidation the idose, its 6-*O* position had to be deprotected by removal of the levulinoyl esters with hydrazine, **34-38**. Oxidation was first performed using a two step oxidation technique developed in the Huang lab.[61] Oxidation under phase-transfer conditions using catalytic TEMPO and co-oxidant sodium hypochlorite followed by Pinnick Oxidation conditions provided the crude carboxylates. Oligosaccharides with free carboxylates/hydroxyls have broad peaks and are difficult to elute from silica gel. Benzyl esters were installed to ease purification and characterization. These conditions yielded disaccharide, **39**, and tetrasaccharide, **40**, in relatively good yields but yields dropped for longer oligosaccharides. It was found that using TEMPO with the stoichiometric oxidant BAIB, bis(acetoxy) iodobenzene, was higher yielding. Hexasaccharide **42** was produced in 90% yield compared to a 60% yield of **41** from the two-step method. Utilizing BAIB also greatly simplified the oxidation reaction. The two step oxidation required numerous freshly made solutions and hinged upon having fresh sodium hypochlorite. BAIB facilitated oxidation only required that the three solvents and the two oxidants stirred overnight with the substrate. With newly oxidized substrates **39-43**, deprotection and sulfation remained.

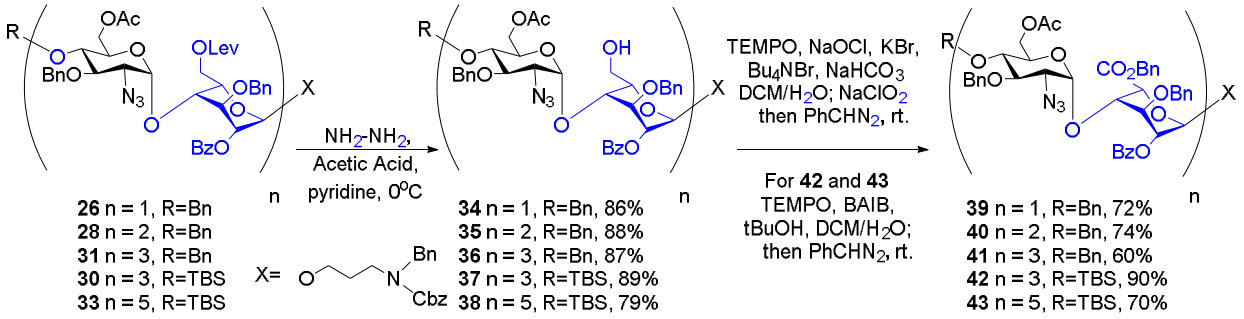


Figure 2.7. Deprotection and oxidation of idose to iduronic acid.

Chemical sulfation is generally non-selective so only the desired sites of sulfation should be unprotected. To reduce synthetic steps, the 2-*O* and 6-*O* positions for sulfation were protected with base labile ester groups. Saponification of these groups by lithium hydroxide, hydrogen peroxide, and potassium hydroxide freed the sites of O-sulfation. The azido groups were converted to amines by Staudinger reduction. With all sites deprotected, a two-step sulfation strategy was used. O-sulfation was performed by reaction with sulfur trioxide triethylamine complex in DMF and was followed by *N*-sulfation with sulfur trioxide·pyridine in pyridine. After sulfation, a global debenzylation by hydrogenation over Pearlman's catalyst would provide the fully deprotected and sulfated oligosaccharides. Disaccharide **44** and tetrasaccharide **45** were prepared in good yields, 77% and 63%, over the 5 steps. However, this procedure failed to yield hexasaccharide **46**. While saponification and reduction of hexasaccharide **41** were successful, *O*-sulfation was incomplete and not reproducible. Using extended reaction times, excess reagent, or higher temperatures only led to degradation of the hexasaccharide backbone. After exhausting all routes for sulfating the backbone with free hydroxyl and amine groups an alternative route was attempted. As previous groups have performed sulfation on long oligosaccharides, up to a dodecamer, and a similar strategy was adopted.

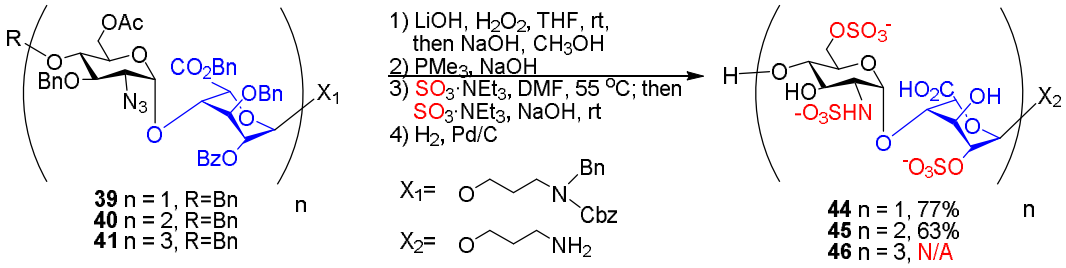


Figure 2.8. Deprotection and chemical sulfation of heparin oligosaccharides **44**-**46**.

The key to sulfating longer oligosaccharides lies in other groups present on the oligosaccharide, the azidos and carboxylates. Previously in our group, and others, the iduronic acid carboxylate group has not been of high concern. It was typically deprotected along with the base labile groups prior to sulfation.[19a, 20b, 27a, 47] The work of Bonnaffé and coworkers was intriguing as they successfully sulfated a dodecasaccharide. Their strategy kept the carboxylate protected as a methyl ester during sulfation.[21a, 24] The methyl ester, rather than benzyl, was used to simplify characterization of the product as deprotection of the base labile groups by methanolysis would leave the methyl esters unchanged. The other potential issue was with the azido group. Leaving the azido group protected would allow for *O*-sulfation followed by reduction and subsequent *N*-sulfation. These alternative routes also required little alteration of the synthetic plan. Oxidation of **37** by TEMPO/BAIB followed by treatment of methyl iodide and potassium carbonate furnished the methyl ester containing **47** in 77% yield. Deprotection of the base labile groups by sodium methoxide furnished **48** in 91% yield. With **48** in hand, sulfation with protected amines could be attempted.

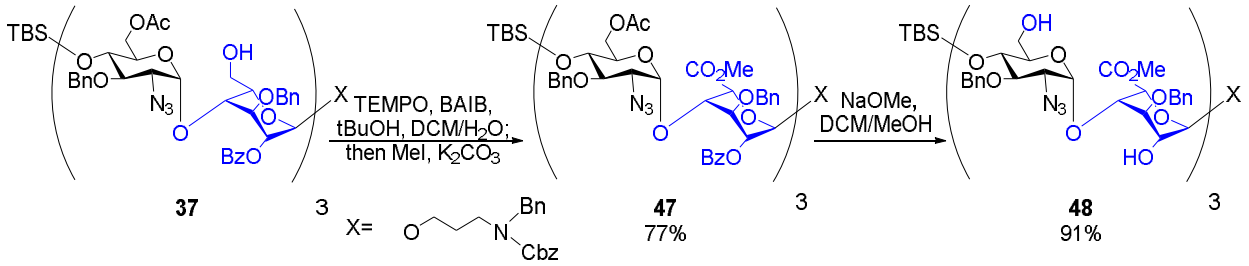


Figure 2.9. Preparation of methyl ester containing hexasaccharide **48** for sulfation.

Sulfation of **48** was attempted under a range of conditions but all led to incomplete conversion. The use of sulfur trioxide complexes in DMF or pyridine furnished a range of partially sulfated products. The conditions used by Bonnaffé and coworkers, extended reaction times at 55 °C, also lead to incomplete sulfation. This led to an examination of the sulfating agent, sulfur trioxide pyridine. As commercial sulfur trioxide complexes can contain considerable acidic impurities, it was washed sequentially by water, methanol, and DCM, then dried under vacuum overnight. Even using the washed agent, sulfation of **48** was incomplete. As retaining the azido groups was found to not be the solution, **48** was treated with 1,3 dithiopropane to reduce them to amines. Staudinger reduction was not used due to the issues of backbone cleavage of ester containing heparin oligosaccharides.[20a] The lengthy reduction, 96 hours and 120 equivalents of 1,3 dithiopropane, provided the starting material for sulfation trials on protected carboxylates, **50**.

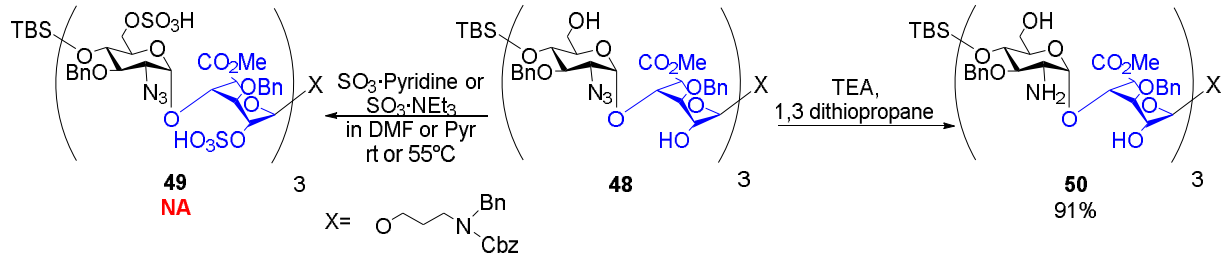


Figure 2.10. Sulfation route exploration of **48** bearing protected carboxylates and amines.

Sulfation trials of **50** provided extremely interesting results. Typical sulfation conditions utilizing five equivalents of sulfating agent per free group produced partially sulfated oligomers,

even at 55°C for three days. The synthesis deviated from previous groups as they had run sulfation on larger scale, 50 to 100 milligrams of substrate and 200 milligrams of sulfating agent in just one or two milliliters of solvent. Due to the effort and time used to synthesize the hexasacccharide backbones sulfation trials were done on just 1-5 milligrams of substrate. This causes a wide discepency in the concentration between Bonnaffé's and our synthesis. For a comparable concentration, the sulfation reaction was run for 24 hours at 55°C in just 200 µL of pyridine with 20 milligrams of sulfur trioxide triethyl amine complex. These conditions provided the fully sulfated product **51.** The product was purified by elution from a Sephadex LH-20 column, preparatory TLC, and conversion to the sodium salt by eluting from a Dowex resin. To ensure the issue had been sulfating agent concentration, and to avoid the issues of running reactions with mere µLs of solvent, **49** was sulfated in 1 mL of pyridine with 100 milligrams of sulfur trioxide pyridine. The reaction again provided the fully sulfated product **51**. It was found that the 100 mg/mL concentration of sulfating agent was optimum for both O and N sulfation, irrespective of substrate concentration. This was not a panacea however as sulfation in DMF failed. The conditions were also unsuccessful in sulfating the hexasaccharide still containing azido groups, **48**. But with the fully sulfated hexasaccharide **51** all that remained was deprotection to provide the chemical sulfated backbones for the carbohydrate microarray.

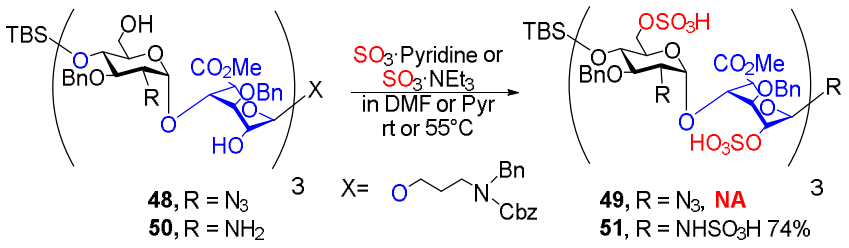


Figure 2.11. Successful chemical sulfation of hexasaccharide **50**.

Where the silyl protecting group on **51** was kept to increase glycosylation yields and synthetic flexibility it was soon found to be a major headache for removal. Three deprotection steps, i.e., desilylation, hydrogenation and saponification separated **51** from being a useful heparin oligosaccharide. Numerous attempts were made to remove the TBS protecting group on **51**. As *N*-sulfates are notoriously acid sensitive, tetra-*n*-butyl ammonium fluoride (TBAF) was tested first to remove the TBS group. High concentrations, extended reaction times, and heating to 60°C only managed to convert **51** from a sodium salt to a tetra-n-butyl ammonium salt, but left the TBS intact. As the TBS had been removed by HF·pyridine before as in conversion of **30** to **32**, it was employed next. Unfortunately after several days, HF only partially cleaved the TBS group. It also cleaved numerous sulfates from the molecule. Running the reaction at lower temperatures only elongated the reaction time but did not alter the results. Deprotection of the silyl group was also attempted at each step. **51** was debenzylated by hydrogenation over Pearlman's catalyst followed by desilylation, which was unsuccessful either through use of TBAF or soluble fluoride salts such as NaF or KF, Figure 2.12. Desilylation was also attempted as the final step. Treatment of **51** with hydrogenation followed by saponification left only the TBS. No method attempted fully removed the silyl protecting group. KF was completely ineffective and the addition of 18-crown-6 made no difference.[62] With no other viable options, the synthetic plan had to be altered before sulfation to remove the silyl protecting group.

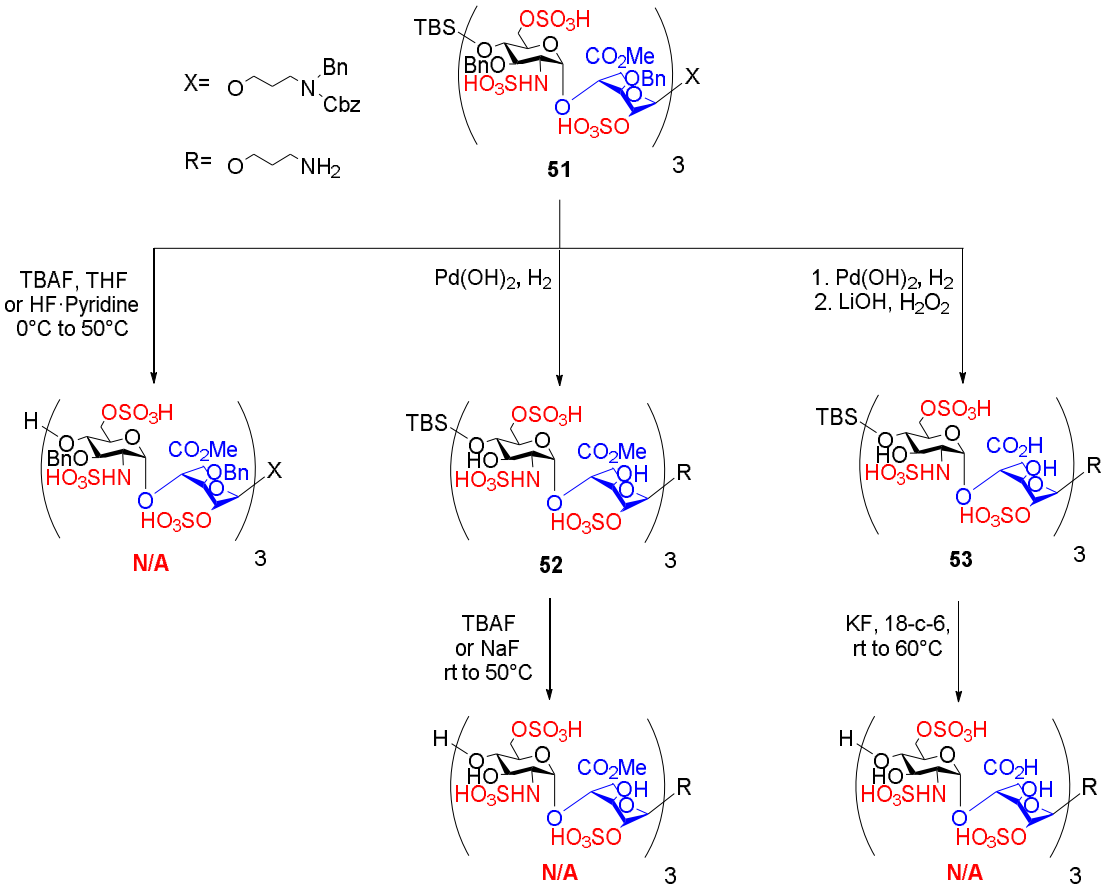


Figure 2.12. Attempts at the desilylation of sulfated hexasaccharides.

The troublesome TBS had to be replaced with a more easily removed protecting group. As newly oxidized **47**, Figure 2.9, was the last protected compound before sulfation, trials started there. Removal of the TBS was done by treatment with HF·pyridine leading to **54** in 94% yield. To keep deprotection to only two steps, a benzyl ether was installed. All prior conditions used to install benzyl ethers were attempted on **54** but gave mixtures. The optimum conditions for benzylating disaccharide **21**, BnBr and Ag_2O were unsuccessful. Believing the issue to be decreased reactivity of the hexasaccharide hydroxyl compared to the disaccharide, TBAI was added to help catalyze the reaction. Using one equivalent of TBAI, twenty equivalents of Ag_2O and forty equivalents of BnBr provided a 52% yield of **55** and 62% BRSM. The reaction was only run for one hour as running the reaction longer only led to an increase in the amount of side

products produced. Pure DCM was found to be optimum as reactions done in other solvents such as THF or acetonitrile provided no products. Even solvent mixtures containing DCM only led to complex mixtures of products. With the benzylated product **55,** sulfation and deprotection could again be attempted. Treatment of **55** with sodium methoxide provided **56** in 80% yield. Reduction with 1,3 dithiopropane provided the backbone for sulfation, **57**.

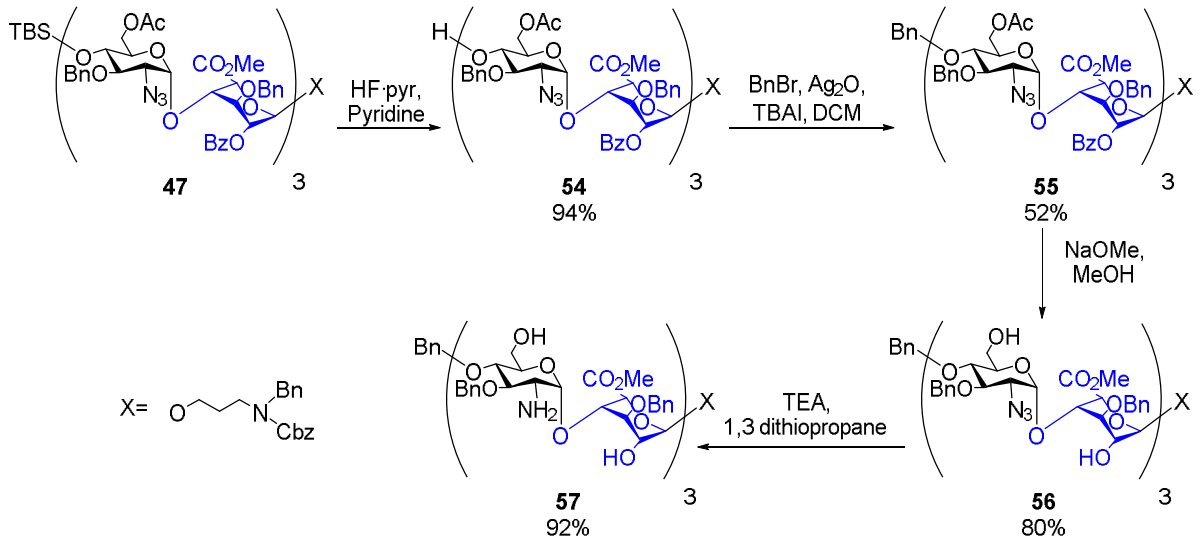


Figure 2.13. Replacement of silyl protection and preparation of sulfation scaffold **57**.

Exploring the sulfating conditions of **57** led to a novel discovery. Using the high concentration of 100 mg/mL of sulfating agent led to complete sulfation. Deprotection was much more straightforward as hydrogenation over Pearlman's catalyst and saponification of the methyl esters provided the fully sulfated and deprotected product **46** in 66% over the three steps. Sulfation reactions on **57** were run using lower concentrations to evaluate the minimum concentration of sulfur trioxide pyridine that would provide the desired products. It was discovered that at a concentration 20 mg/mL of sulfur trioxide pyridine, a single product containing six sulfate groups was produced. As hexasaccharide **57** bore six free hydroxyl groups, it was thought that the product was selectively *O*-sulfated. This was examined by treating the product with acetylation conditions followed by hydrogenation and saponification. Any esters

formed by acetylation would be cleaved while amides would be retained. The final product **58**, *O*-sulfated and *N*-acetylated, was synthesized in 47% over the 4 steps from **57**. The ability to selectively perform *O*-sulfation was also employed to synthesize a hexasaccharide bearing unmodified amines. After sulfation, hydrogenation and saponification provided **59** in 76% yield over three steps.

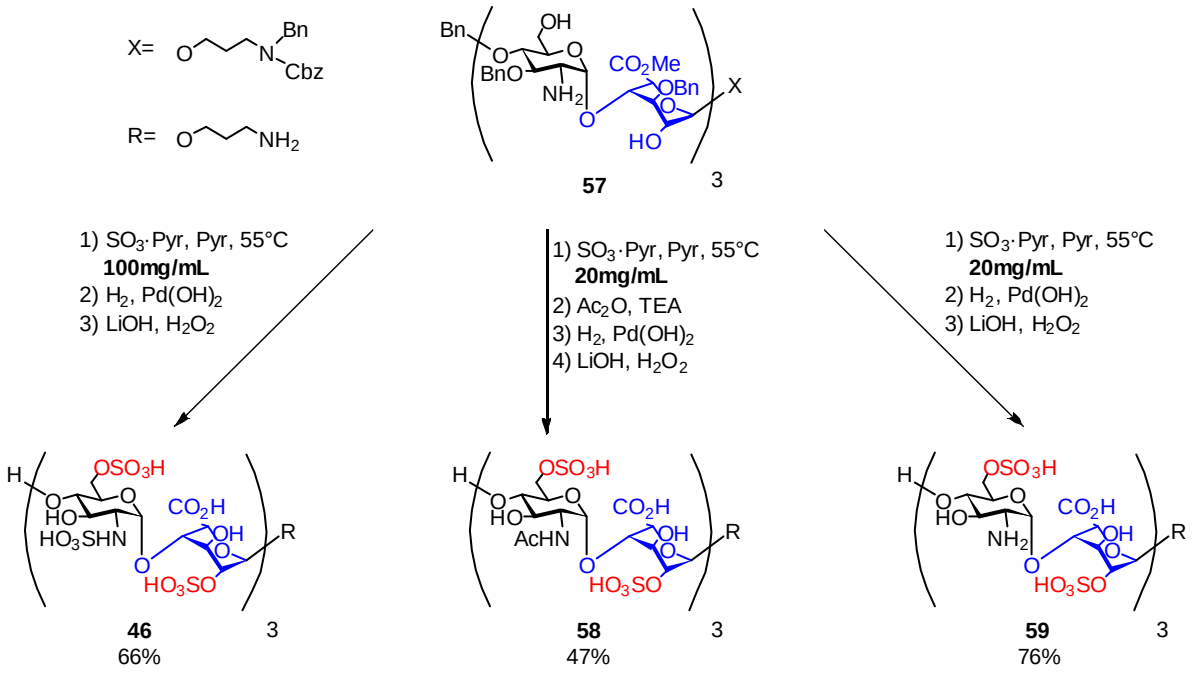


Figure 2.14. Chemo-selective sulfation leading to heparin hexasaccharides **46, 58, 59**.

The selectivity of the sulfation reaction implied by mass spectrometry was verified by NMR. The easiest peaks used for comparison were the anomeric protons and carbons of the glucosamine residues. The anomeric carbons resonating around 100 ppm are well separated from other carbons in the molecule. In addition, the anomeric proton is highly deshielded and the glucosamine anomeric has a much stronger J_{H1H2} coupling constant, being in the $^4C_1$ conformation, than the iduronic acid in the $^1C_4$. As reported in literature, heparin oligomers carrying *N*-acetyl groups and *O*-sulfates generally have glucosamine H-1 around 5.14 ppm and C-1 at 96.8 ppm. **58**'s glucosamine anomeric protons were at 5.05 ppm and C-1 was at 93.5 ppm.

This was in direct contrast to oligomers carrying free amines that have H-1 and C-1 of glucosamine at 5.40 ppm and 93.7 ppm respectively. **59** compared well to this with its H-1 and C-1 for glucosamine at 5.31 ppm and 91.00 ppm. The fully sulfated **46** has H-1 and C-1 of 5.32/5.21 ppm and 99.7/97.9 ppm also fits with the naturally derived oligomers carrying 2-*O*, 6-*O*, and *N*-sulfation having glucosamine H-1 and C-1 of 5.42 ppm and 99.5 ppm respectively.[63]

The discovery of the selective chemical sulfation conditions was a welcome addition to the divergent synthetic plan of assembling a heparin library. Many previous examples of selective chemical sulfation employ alternative solvents and sulfation over two steps.[21a, 42a] Selective sulfation allows for **57** to be elaborated into three distinct hexasaccharides without the need for extensive protecting group manipulation or multiple sulfation steps. This simplifies the overall strategy while reducing difficult late stage reactions to provide a diverse array of products from a small number of backbones. To further diversify the library of sulfated products, enzymatic sulfation was employed.

2.3 Enzymatic Sulfation

To increase the sequence diversity that can be generated from a single backbone, sulfotransferase enzymes were employed. The synthetic plan had to account for the limitations of the enzymes used in heparin/HS biosynthesis, Figure 2.15.[53a] Epimerization of glucuronic acid occurs after *N*-sulfation. Thus the glucosaminyl *N*-deacetylase/*N*-sulfotransferase (NDST) would be inactive against the backbones previously prepared as they contain iduronic acid residues.[64] As *N*-sulfation is a requirement for *O*-sulfotransferase enzyme activity, they had to be installed chemically in order to utilize enzymatic sulfation.

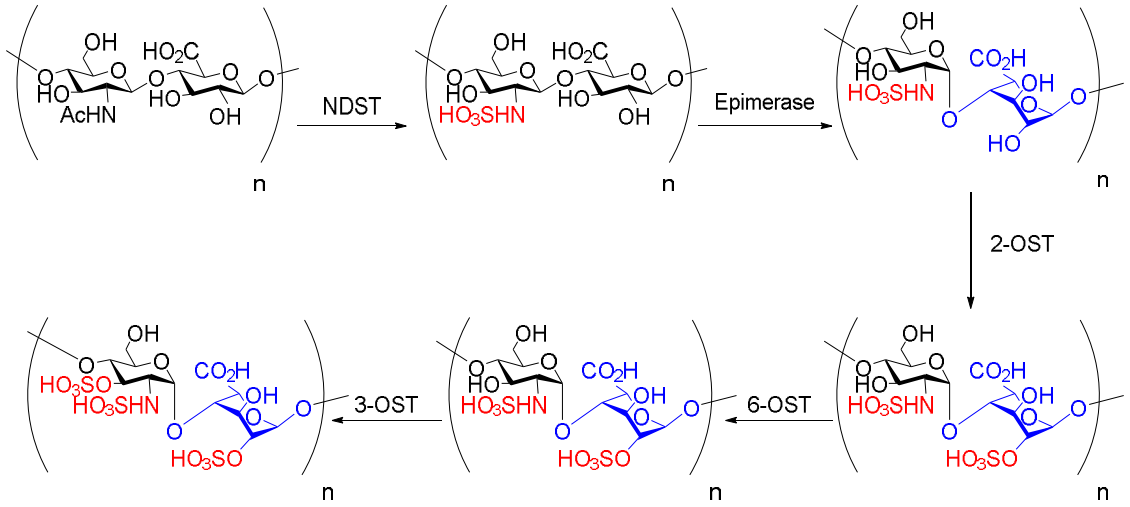


Figure 2.15. Biosynthetic pathway of heparin/HS synthesis.

Preparation of the enzymatic starting material started with compound **42**. To prepare the starting material for the *O*-sulfotransferases, only *N*-sulfation was needed. It was known that the TBS protecting group had caused many issues with chemical sulfation so the first step was removing it with HF·pyridine furnishing **60** in 87% yield. Saponification of the base labile groups furnished **61** in 85% yield. Reduction of the azides by Staudinger reduction provided **62** in 97% yield. With both hydroxyls and amines deprotected, selective *N*-sulfation was needed. Under basic conditions, pH=9.5, sulfur trioxide triethylamine sulfated the three free amines on **62**. Global debenzylation then provided the fully deprotected and *N*-sulfated **63** in 70% yield over the two steps, Figure 2.16.[21a] With the starting material in hand, modifications with the natural sulfotransferase enzymes were evaluated.

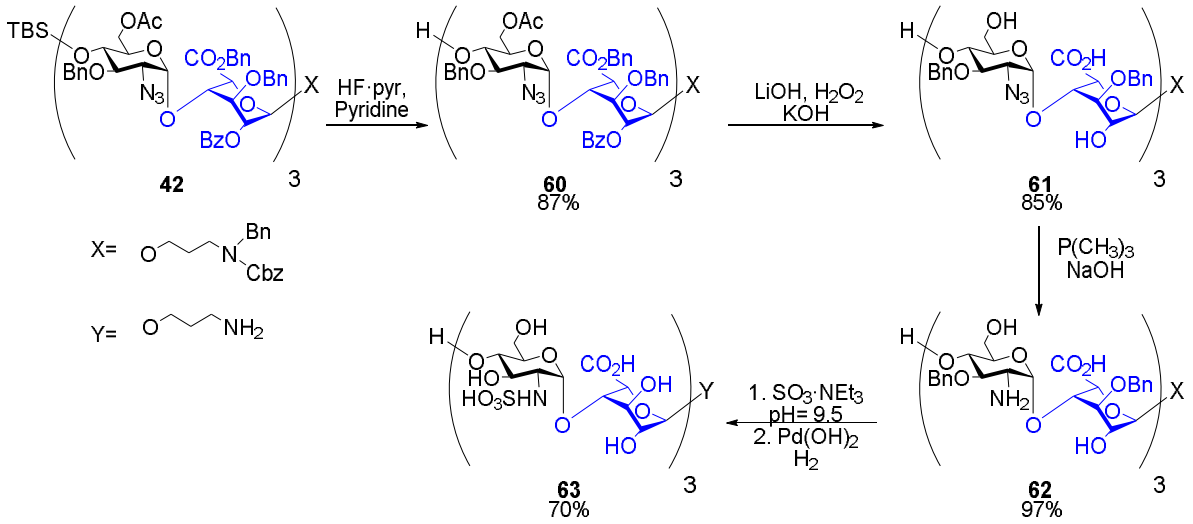


Figure 2.16. Preparation of *N*-sulfated heparin for enzymatic sulfation.

With the assistance and expertise of Prof. Jian Liu's lab at UNC Chapel Hill, the sulfotransferase enzymes were expressed in *E. coli* and purified by FPLC. 2-OST-1 was expressed well, providing 40 mg of enzyme per liter of bacteria. For purification, it was labeled with a maltose binding protein tag and purified with an amylose column on FPLC. 6-OST-1 and 3-OST were less efficient only providing around 5 - 10 mg per liter of bacteria respectively. Both enzymes were labeled with a polyhistidine tag allowing for purification using a Ni-Sepharose column. The concentration of purified enzymes was analyzed by Nanodrop spectrophotometer. Activity was confirmed by trial runs with K-5 capsular polysaccharide and radioactive PAPs (^{35}S labeled 3'-phosphoadenosine-5'-phosphosulfate). After digestion by heparinase I, II, and III, the resulting disaccharides were separated by HPLC and compared with commercially available standards. This provided the location of sulfation. The degree of sulfation was ascertained by radioactive counts of the disaccharides and then compared with previous batches of enzymes. With active enzymes in hand, their specificity towards **61** could be examined.

The first trials were performed with 2-*O*-sulfotransferase (2-OST) as it is the first enzyme active after C_5-epimerase converts glucuronic acid to iduronic acid. Using the conditions

suggested by Liu and coworkers, **63** was incubated with 2-OST and the sulfate donor PAPs in 5 mL of a 50 mM MES (morpholine-4-ethanesulfonic acid) solution with 0.5% v/v Triton X-100 for 24 hours at 37 °C. The reaction was purified on a 0.75 cm x 200 cm Biogel P-2 column and found to be a mixture containing oligosaccharides with 1 or 2 added sulfate groups. The mixture was subjected to the same conditions again and the resulting product carried 2 sulfates as shown in Figure 2.17A. It was found that full sulfation could be affected in one pot by adding another portion of enzyme and PAPs after 24 hours, and incubating for another 24 hours. Unfortunately the MS of the second and third trial runs illustrated one glaring issue with the reaction conditions, Figure 2.17B. As Triton X is a polyethylene glycol surfactant, the numerous peaks separated by 44 m/z was indicative of contamination. Each fraction coming off the column contained Triton X and the fractions containing sugar were identifiable by TLC (stain 1,3 dihydroxynaphthalene) but not usable for MS or NMR. Triton X suppressed any other ions in MS. This column contamination came after just two purification cycles. Even after extensive washing over 2 months, Triton X continued to elute from the column. New reaction conditions would have to be found in order to procure pure enzymatic sulfation products.

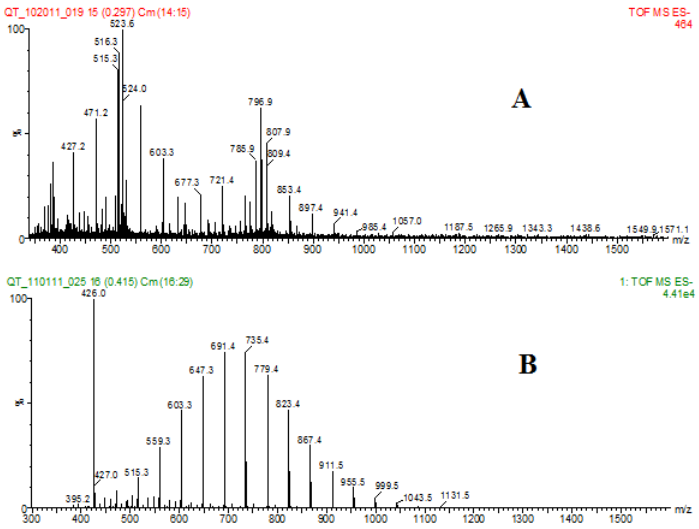


Figure 2.17. Early 2-OST Trials with Triton X-100.

Finding successful enzymatic reaction conditions was made difficult by long purification time and the special columns used. After loading the long P-2 column the product would not elute for 2-3 days and the column would then have to be washed for 2 weeks before another reaction could be purified. The columns were specially made by Scott Bankroff in the MSU Glassblowing shop and extremely fragile (0.75 cm x 200 cm) as a fellow lab member found when they accidentally broke one. Once packed with the P-2 gel, the gel could not be removed. If the P-2 gel became contaminated with Triton X the entire column was scrapped. New conditions had to be found that were compatible with the workup and purification.

Two routes were attempted in finding new successful sulfation conditions; removing Triton X during workup but before running the column, and running the reaction without Triton X. A quick test reaction under the same conditions without Triton X failed. After this illustrated the necessity of Triton X, its removal was attempted after the reaction. The reaction was ran with Triton X and concentrated down. The resulting viscous material was diluted with water and washed five times with 5 mL portions of diethyl ether, ethyl acetate, and DCM. MS was taken of all four fractions and all four contained Triton X. The fraction containing water still contained the oligosaccharide, by TLC analysis, but also Triton X. The reaction was tried with a half the concentration of Triton X (0.5% v/v) but it also failed. Being unable to remove the Triton X after the reaction without ruining a column the only option left was to leave it out of the equation.

Without the surfactant, enzymatic sulfation failed at the concentrations of 0.1 mg/mL of PAPs and 0.1 mg/mL of substrate. It was even attempted with fresh PAPs and 2-OST sent from Prof. Liu, but this also failed. The next trials focused on modifying the concentration of reactants to try and find successful conditions. As the surfactant likely helped with solubility, it was thought that without it the reactions had to be more dilute. Diluting the reaction from 6 to 12 mL

and keeping the concentration of enzyme and PAPs constant at 0.1 mg/mL led to no product. Thus, substrate concentration was not an issue. This only left the sulfate donor PAPs and the 2-OST as the offending participant. The reaction was run with a lower concentration of PAPs (0.05 mg/mL) in a total volume of 25 mL, 20 mM MES. This led to a partially sulfated product, Figure 2.18. Running the reaction again led to doubly sulfated product **64.** While this provided the product running the reaction twice would take 1 month with just one column. This led to the final reaction conditions. The reaction was run at with 0.05 mg/mL of PAPs in a volume of 12.5 mL for 24 hours at 37°C. It was then diluted to 25 mL and enzyme and PAPs were added to keep their concentrations constant. This led to sulfated product **64** in one step.

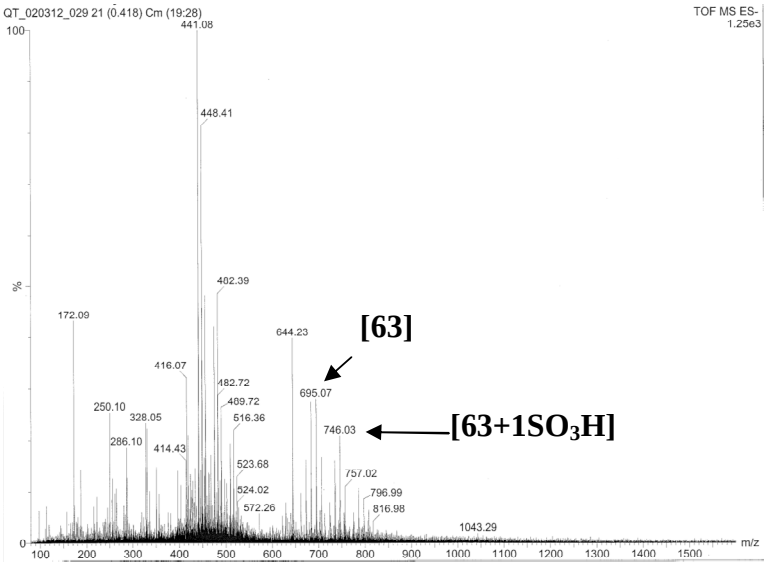


Figure 2.18. Partially Sulfated **63**

The starting material **63** carried three 2-*O* positions but 2-OST only added two sulfates. Normally disaccharide analysis on digested oligosaccharides is done to identify sulfation patterns but non-destructive techniques were desired. Thus to identify the locations of the newly added sulfates, numerous NMR experiments were performed including 1D and 2D TOCSY, COSY, and NOESY. As sulfation causes deshielding of neighboring protons, residues carrying additional sulfates would be more deshielded than their non-sulfated precursors. Comparisons

between the starting material and the product can be useful. The two additional sulfates on **63** were placed on the non-reducing and internal disaccharides units as shown in Figure 2.19 based on NMR analysis discussed below.

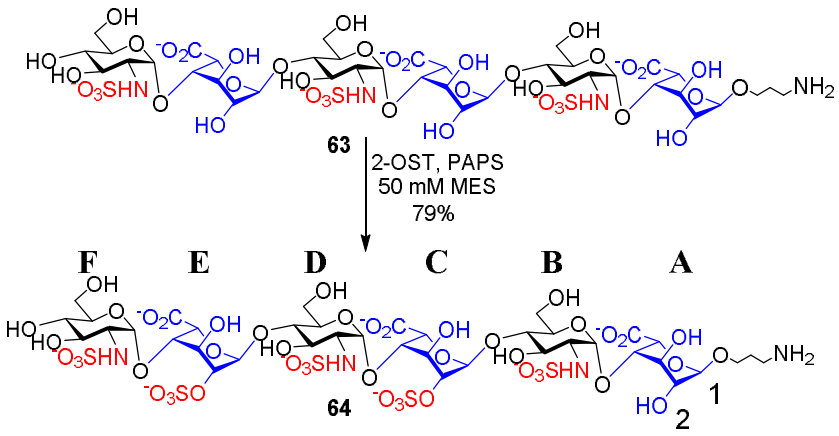


Figure 2.19. Enzymatic Sulfation of **63** by 2-OST**.**

The relatively shielded linker protons worked as a great handle on elucidating the structure of **64**. The reducing end iduronic acid was found by NOESY correlations from the well seperated linker protons at 3.00 ppm and H-5 of iduronic acid labeled **A,** Figure 2.19. The NMR of **64** can be compared to **63,** which only has *N*-sulfation. The sulfated iduronic acids should be more deshielded in **64.** H-1A is relatively unchanged, 4.78 ppm for **64** and 4.83 ppm for **63**. H-2A is almost exactly the same with 3.58 ppm vs 3.59 ppm for **64** and **63** respectively. While iduronic acid A is relatively unchanged, the other two are quite different. H-1C,F in **63** were at 4.90 ppm but those shifted up to 5.17 ppm and 5.15 ppm in **64.** H-2 for C/F was similarly affected going from 3.64 ppm in **63** to 4.22 ppm in **64.** The 0.27 ppm shift for H-1C/F and the 0.58 ppm shift of H-2 C/F is from the addition of sulfates added by 2-OST which is also confirmed by comparing with reported data.[63] Yates and coworkers report H-1 and H-2 for iduronic acid residues not carrying 2-*O*-sulfation at 4.95 and 3.74 ppm, **64A** is comparable at 4.78 and 3.59 ppm. Sulfation moved iduronic acid protons downfield to 5.26 for H-1 and 4.35

for H-2. This fits with **64C** and **64F** having H-1s of 5.17 and 5.15 ppm and H-2s of 4.22 ppm. This confirms **64** having been sulfated on the non-reducing and internal disaccharides.

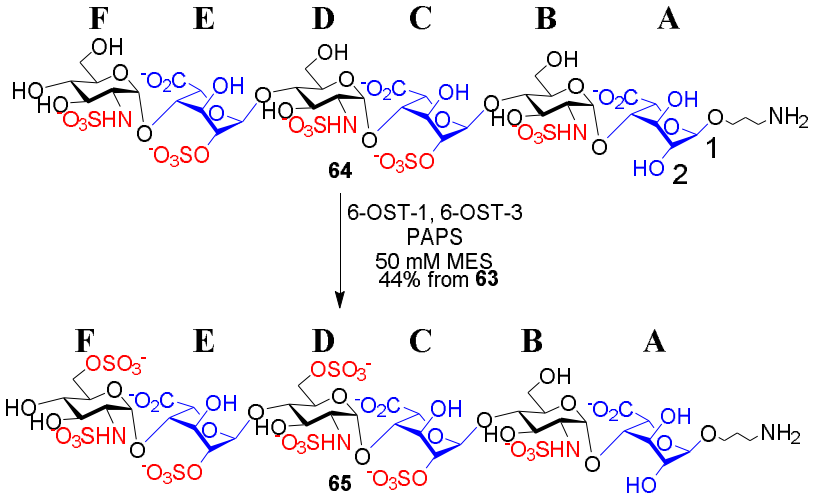


Figure 2.20. Sulfation of **64** by 6-OST.

With reproducible sulfation conditions found, the other major sulfotransferase enzyme, 6-OST), could be evaluated. Taking **64** and subjecting it to the same conditions but with 6-OST-1/6-OST-3 resulted in product **65**, Figure 2.20. The product carries an additional two sulfate groups and repeated attempts at sulfation only led to the continued isolation of **65**. As 6-OST modification occurs after 2-OST in the body, it was immediately thought that the non-reducing end and internal disaccharides were modified. This was confirmed by NMR analysis. The linker protons were again used to find H-1A and through 2D TOCSY the rest of iduronic acid A was assigned. NOESY correlations between H-3A and H-4A and an H-1 at 5.20 ppm lead it to be assigned as H-1B. With the first two residues found they were compared with those of **63**. The H-6 and H-6' protons of all glucosamines in **63** were between 3.55 and 3.72 ppm. In **65** only glucosamine B still had H-6 and H-6' in that range, 3.55-3.72 ppm, whereas H-6 and H-6' for C and E had been deshielded to 4.30 ppm and 4.15 ppm respectively. This correlates with the reported values of 4.23-4.42 ppm for H-6's on glucosamine carrying 6-*O* sulfates.[63]

Lastly the effect of order of sulfation was tested, Figure 2.21. When **63** was treated with 6-OST and PAPs, the product **66**, carried an additional 3 sulfates, implicating sulfation of the three open 6-*O* positions. As **65** only carried two 6-*O*-sulfates, it was believed that performing 2-*O* sulfation on **66** could lead to another product. Unfortunately reacting **66** with 2-OST and PAPs only led to the recovery of **66** with no further modifications. This was unsurprising as previous experiments published by Lui and coworkers reported extremely low yields performing 2-*O* sulfation after 6-*O* sulfation.[57a]

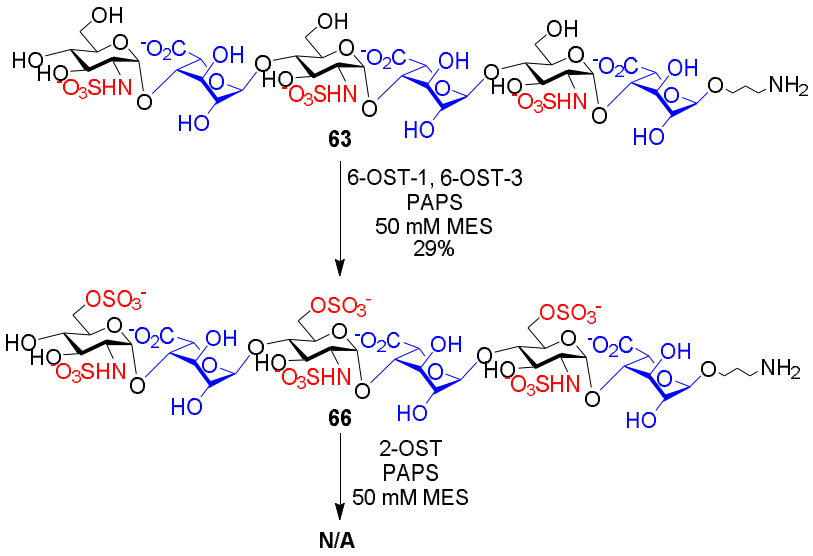


Figure 2.21. Effects of using 6-OST before 2-OST.

The enzymatically sulfated hexasaccharides **64-66** greatly enhanced the variety of the heparin/heparan sulfate library. They were produced from just one backbone and required no modifications or protecting group manipulations. If they were to be produced chemically, they would require multiple disaccharide building blocks with individualized protecting groups. This would be compounded by the differentiation in **64** and **65,** where the sulfation pattern is not uniform. By using one building block, **18**, and elaborating its oligomer with the sulfotransferase enzymes, the overall synthetic efficiency was improved. With all the sulfated oligosaccharides in

hand, they could be used to investigate biological requirements of various sulfation patterns and lengths.

2.4 Carbohydrate Microarray

Given the small amounts made, to get the most use of the carbohydrate library, a microarray was utilized. Each spot on the microarray only required nL of solution, allowing numerous microarrays to be prepared from the amount of oligosaccharides produced. Microarray technology also allowed a large number of moieties to be screened against a particular glycan binding protein at one time. This would greatly expedite the analysis.[65] The main decision in making the microarray was how to attach the carbohydrate library to the slide.

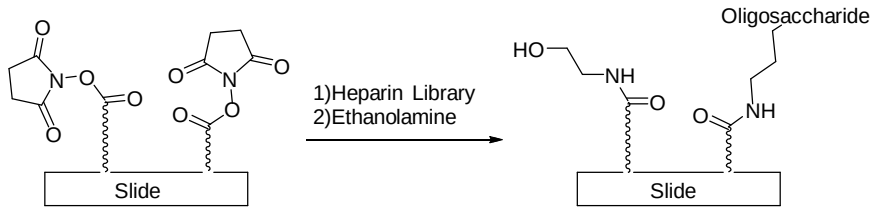


Figure 2.22. Attachment of Heparin Library to Microarray Slide.

Of all the methods available to covalently attach analytes, *N*-hydroxysuccinimide (NHS) functionalized slides were chosen. With the library carrying amino linkers, it was the simplest and is well established.[65] The displacement of the NHS by the oligosaccharide's amine also introduced as little modification to the library as possible, Figure 2.22. With assistance from Jeff Landgraf in MSU's Genomics core, oligosaccharide solutions were printed in quadruplicate onto NHS coated slides from SurModics and incubated overnight at 75% humidity. The high humidity slowed the evaporation of the spots and allowed time for the coupling reaction to occur. After washing, the un-reacted NHS sites were quenched by incubating them with ethanolamine at

50°C. With the glycoarray slides in hand the protein of interest, Fibroblast Growth Factor 2 (FGF-2), could be evaluated.

Heparin is known to play a role in regulating FGF-2, a protein of interest in angiogenesis, cell proliferation/differentiation, and tumor development.[66] Regulation is done through direct interaction between the two species, an ideal interaction to probe with the microarray.[66a, 66b] The library containing varying degrees of sulfation, would test the requirements of FGF-2/heparin binding. As controls, both commercial heparin (sodium salt 18 kDa) and chondroitin sulfate A (sodium salt 50 kDa) were printed along with the oligosaccharide library. The prepared slides were analyzed in a way reminiscent of a sandwich enzyme-linked immunosorbent assay (ELISA). The oligosaccharide coated slides were incubated with human FGF-2 solution that bound with any desirable oligosaccharides. With FGF-2 now bound to the slide, rabbit anti human FGF-2 was added, to bind the immobilized FGF-2. The interaction was visualized by adding a fluorescently labeled protein, Cy5 anti-rabbit IgG, to cap the anti FGF-2 antibody. The resulting slides were analyzed on an Agilent G2565AA array scanner and an interesting result procured.

The first slides from SurModics gave only background signal. Even the positive control heparin produced no signal above background, Figure 2.23. The issue could have been with any part of process from fabrication of the microarray to the analysis. To rule out low signal, trials were done with increasing amounts of protein and antibodies but this only served to increase background noise, Figure 2.23. The slides received were checked, and though they were sealed and stored correctly, the company had sent slides past their expiration date. NHS coated slides can degrade over time, so new slides were ordered from three companies, Surmodics, Schott, and

Xantec. A further positive control was added by printing a solution of the anti FGF-2 alongside the oligosaccharides to verify the effectiveness of the ELISA protocol.

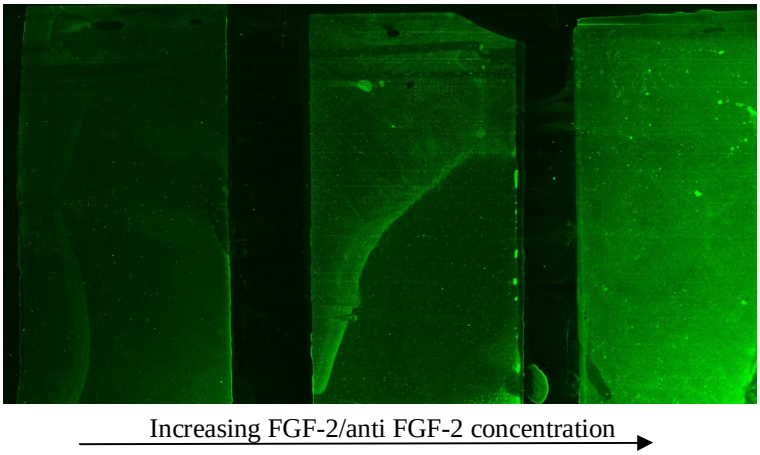


Figure 2.23. Microarray trials with SurModics NHS slides.

With numerous positive controls the results of further trials were unchanged. Slides from both Surmodics and Schott showed no signal for any spot. Slides from Xantec told a different story however, Figure 2.24A. Though the spots seem haphazard there are several rows visible, **A**. The randomness of the spots was found to be caused by an issue with the slide holder on the robotic printer. New slides were printed and aside from the high background symmetrical and well spaced spots can be seen, Figure 2.24B. The issue was not with the procedure but with either the coupling of oligosaccharides or their presentation on the slide surface. All three companies touted the 3-D presentation of molecules allowing for high loading and low background but only Xantec's were successful and the cause was not determined.

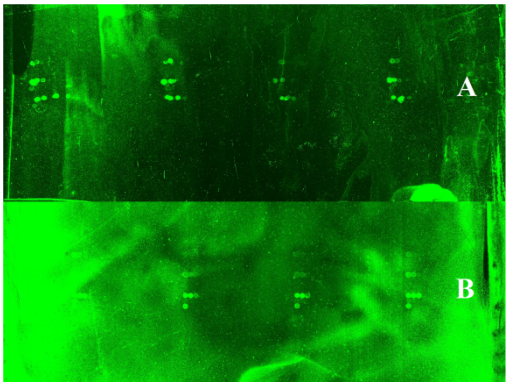


Figure 2.24. a) Early results with Xantec slides illustrating issues with plotter. b) Showing the adjusted plotter but issues with high background fluorescence.

The success of coupling between the oligosaccharide library and the Xantec slides allowed for optimization of the array analysis. The main issue was the high background seen in Figure 2.24B. This could have been caused by several factors including the nonspecific binding of proteins to the slide, extreme amounts of Cy5 labelled antibody, or even excess excitation laser power. Non-specific binding was reduced by having each solution contain 1% bovine serum albumin (BSA). Arrays analyzed without BSA had extremely high background noise, Figure 2.24B. Improvements then focused on the excitation laser. A microarray could be scanned numerous times with excitation power from 1% to 100%. Where Figure 2.24B used 100% power for the laser (633 nm helium-neon), the five arrays seen in Figure 2.25 were excited using 60% power. This was found to give the best signal while keeping background noise at a minimum.

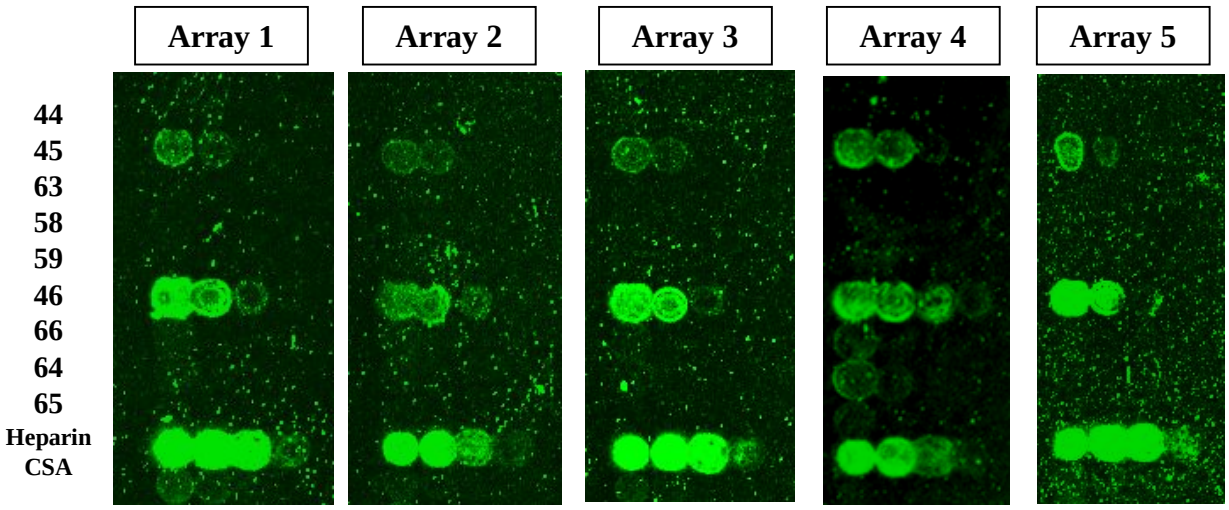


Figure 2.25. FGF-2 binding of five arrays of the oligosaccharide library.

The arrays in Figure 2.25 illustrate the utility of the microarray method. With conditions in hand, a heparin binding protein was analyzed in a single day against the prepared heparin library. The five displayed arrays also show the reproducibility of results. While the arrays were run on different slides and on different days they gave similar results. Unfortunately the visual nature of Figure 2.25 did not provide concrete information to compare the strength of binding. To do that, ImageJ, software available for free at http://rsb.info.nih.gov/ij/, was used to quantify the microarray data, Figure 2.26.

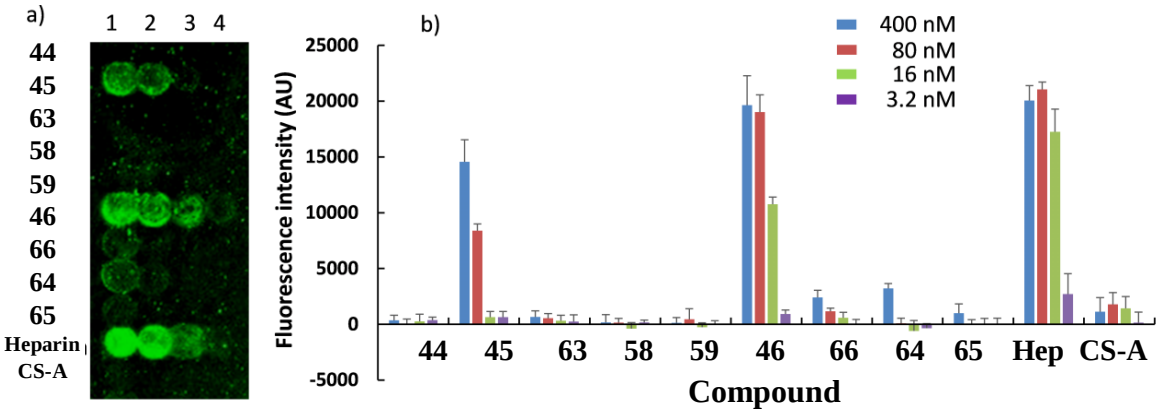


Figure 2.26. a) A representative microarray image. b) Quantification of microarray data of Figure 2.25 using ImageJ.

The results shown in Figure 2.26 reinforced what is known about FGF-2 and heparin binding. The two factors that are known to influence binding, chain length/structure and sulfation, were confirmed. A minimum chain length for binding was reported to be a tetrasaccharide.[67] This was confirmed by the data shown. Tetrasaccharide **45** produced strong binding while disaccharide **44**, carrying the same sulfation pattern, illustrated little to no binding. Hexasaccharide **46** bound well enough to produce almost as much signal as the bound heparin polymer. Binding to a disaccharide has been reported but utilized higher concentrations, up to 2 mM.[32] Chain length was not the only determing factor in FGF-2 binding, sulfation was also integral to binding.

The binding interactions from sulfation were not due to random electrostatic interactions. The sulfated chondroitin sulfate A had minimal binding in comparison to the strong signal given by bound heparin. As chondroitin sulfate is mainly glucuronic acid backbone structure must play a large role in FGF-2 binding, not just net negative charges. The less than fully sulfated hexasaccharides (**58**-**59**, **63**-**66**) interacted weakly with FGF-2. Though they have multiple sulfations their binding is greatly decreased in comparison to **46**, bearing full 2-*O*, 6-*O*, and *N*-sulfation. The absence of *N*-sulfation, **59**, or 2-*O* sulfation, **66**, led to a precipitous drop in binding. Perhaps the most interesting finding was from **65** as it included the structure of tetrasaccharide **45** but bound weakly. The presence of the nearby disaccharide not bearing *O*-sulfation was unfavorable to FGF-2 binding. This effect could be amplified by the shorter oligomer chain as the heparin polymer has to include similar regions between low and high sulfation but still binds effectively. Preliminary studies with FGF-2 have shown the utility of the library and microarray analysis to use small amounts of pure oligosaccharides to probe carbohydrate-protein interactions and help guide future studies.

2.5 Conclusions

A series of oligosaccharide backbones up to a decasaccharide were synthesized efficiently from a common disaccharide building block with only one hangup, the PMB group. The PMB protecting group that caused issues with glycosylation was found to be tamed by the careful monitoring of diethyl ether not acid/base. Issues with deprotection and chemical sulfation led to several strategy changes late in a formerly well planned synthesis. While the problem with cyclization caused by PMB was troublesome, one product could still be isolated. Accessing pure sulfated oligosaccharides took much longer. Numerous alterations of the two step sulfation strategy were unsuccessful and not reproducible. After consultation with Prof. Bonnaffé, keeping the carboxylates protected led to pure sulfation products and a novel find. Chemo-selective sulfation by altering sulfating agent concentration was discovered. Where research groups in the past have relied on protecting groups and multistep sulfation, the only requirement for the newly found method was concentration. Having produced pure sulfated products only a few deprotection steps remained but a new problem was found shortly thereafter.

Using TBS as a non-reducing end protecting group increased the flexibility of the synthetic plan. Utilizing this allowed the production of decasaccharide **33** in just two steps from products **29** and **30**. The early stage flexibility of TBS was favored as oligosaccharide donors containing TBS gave better yields than those carrying Bn, Figure 2.5. The issue arose when it came time to remove TBS. The only conditions that could remove it also led to loss of multiple sulfates. Replacing the TBS with Bn before sulfation simplified the deprotection sequence and allowed the isolation of pure sulfated products. Unfortunately no conditions were found to successfully benzylate the decasaccharide backbone. For future syntheses using a benzylated donor for the final glycosylation would solve this issue as disaccharide **22** can be synthesized

efficiently. Solving all of these issues allowed the isolation of four different chemically sulfated hexasaccharides from a single backbone along with shorter oligomers. The divergence of our strategy was further elaborated by the use of enzymatic sulfation.

The sulfotransferase enzymes 2-OST and 6-OST allowed the modification of one hexasacharide into three additional products with only three synthetic steps. While enzymatic conditions were found to be fickle, dilute conditions were discovered that reproducibly sulfated backbone **63**. It has been known that 2-OST acts before 6-OST *in vivo* and this was confirmed by enzymatic trials where 2-OST left the backbone carrying 6-*O*-sulfates unmodified. The activity of 6-OST was also impacted by the presence of 2-*O*-sulfates. It was found that 6-OST could fully sulfate the backbone only carrying *N*-sulfates but was more selective on the backbone already containing 2-*O*-sulfates. 6-OST readily sulfated disaccharide units already containing 2-*O*-sulfates but did not act on units not bearing 2-*O*-sulfated, even after multiple reactions. This is in agreement with Lindahl and coworkers who showed 6-OST selectivity was affected by the presence of 2-*O*-sulfates depending on concentration.[68] Overall the utility and breadth of the synthesis was greatly improved by enzymatic sulfation, providing oligosaccharides that otherwise would require specific and lengthy chemical syntheses and are not accessible through strickly enzymatic routes.

The synthetic plan culminated in utilizing the newly produced oligosaccharides to probe biological interactions of heparin/HS through the use of a microarray. Preliminary studies with FGF-2 confirmed the ease of the method and previously shown requirements for Heparin-FGF-2 binding. As numerous synthetic/enzymatic issues were solved, further backbones can be synthesized with increasing ease and be easily screened against any desired glycan binding protein.

2.6 Experimental Section

2.61 General Experimental Procedures.

All reactions were performed under a nitrogen atmosphere with anhydrous solvents. Solvents were dried using a solvent purification system. Glycosylation reactions were performed with 4Å molecular sieves that were flamed dried under high vacuum. Chemicals used were reagent grade unless noted. Reactions were visualized by UV light (254 nm) and by staining with either $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL), 5% H_2SO_4 in EtOH, or for deprotected oligosaccharides 0.2 g 1,3-dihyroxynaphthalene in 50 mL of 5% H_2SO_4 in EtOH. Flash chromatography was performed on silica gel 601 (230-400 Mesh). NMR spectra were referenced using residual $CHCl_3$ (δ ^{1}H-NMR 7.26 ppm ^{13}C-NMR 77.0 ppm). Peak and coupling constants assignments are based on ^{1}H-NMR, ^{1}H-^{1}H gCOSY, ^{1}H and ^{1}H-^{1}H TOCSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C gHMQC/HSQC, ^{1}H-^{13}C gHMBC.

2.62 Characterization of anomeric stereochemistry.

The stereo-chemistries of newly formed glycosidic bonds for idose and glucosamine were determined by $^3J_{H1,H2}$ through ^{1}H-NMR and/or $^1JC_1,H_1$ through gHMQC 2-D NMR (without 1H decoupling). Smaller $^3J_{H1,H2}$ (3 Hz) indicate α linkages and larger $^3J_{H1,H2}$ (7 Hz or larger) indicate β linkages. $^1J_{C1,H1}$ couplings around 170 Hz suggest α linkages while constants around 160 Hz imply β linkages.

2.63. General procedure for pre-activation based glycosylation.

A solution of donor (60 µmol) and freshly activated 4Å molecular sieves (200 mg) in CH_2Cl_2 was stirred at room temperature for 30 min, and then cooled to -78 °C. Once cooled, AgOTf (31 mg, 120 µmol) dissolved in Et_2O was added directly to the solution making sure the solution did not touch the walls of the flask. After 5 minutes, orange colored *p*-TolSCl (9.5 µL, 60 µmol) was added via a microsyringe directly to the flask as the reaction temperature was lower than the freezing point of *p*-TolSCl and it would freeze to the walls of the flask. The color of *p*-TolSCl disappeared rapidly, indicating the consumption of *p*-TolSCl. After the donor was completely consumed as verified by TLC analysis (about 5 min at -78 °C), a solution of acceptor (54 µmol) in CH_2Cl_2 (1 mL) along with 1 equivalent of TTBP (2,4,5-tri-*tert*-butylpyrimidine) was slowly added dropwise along the walls of the flask. This was done to allow the acceptor solution to cool before mixing with the activated donor. The reaction mixture was warmed to 0 °C under stirring in around 2 h. The mixture was diluted with CH_2Cl_2 and filtered through Celite. After washing the Celite with CH_2Cl_2 until all organic compounds were removed as verified by TLC, the CH_2Cl_2 fractions were combined and washed twice with a saturated aqueous solution of $NaHCO_3$ (20 mL), and twice with water (10 mL). The organic layer was collected and dried over Na_2SO_4. After removal of the solvent the product was purified by silica gel flash chromatography unless noted.

2.64 General procedure for TBS removal.

The TBS containing oligosaccharide (0.54 mmol) was transferred to a 50 mL plastic centrifuge tube by three portions of 3.33 mL of pyridine. While stirring the pyridine solution was

cooled to 0 °C. 5 mL of HF·pyridine was then added dropwise to the stirring solution. The reaction was then allowed to warm to room temperature and kept overnight. After verifying the reaction was complete by TLC, the reaction was diluted with DCM and washed sequentially by sat. $CuSO_4$, sat. $NaHCO_3$, and 10% HCl. The organic layer was dried over Na_2SO_4, concentrated, and purified by silica gel flash chromatography.

2.65 General procedure for benzylation.

The oligosaccharide to be protected (15 µmol) was dissolved in 5 mL of DCM. To this solution was added TBAI (1 eq), benzyl bromide (40 eq), and freshly prepared Ag_2O (20 eq). The reaction was stirred at room temperature until TLC indicated the reaction was no longer progressing (30 min). The reaction was quenched by diluting with DCM and filtering through Celite to remove Ag_2O. The reaction was concentrated and purified by silica gel chromatography.

2.66 General procedure for removal of levulinoyl esters.

A solution of the oligosaccharide containing Lev esters (56 µmol) in 2.4 mL of pyridine and 1.6 mL of glacial acetic acid was cooled to 0°C. To this was added 27 µL of hydrazine hydrate (560 µmol or 5 equivalents per Lev ester). The reaction was stirred at 0 °C for 3 hours or until TLC shows the reaction complete. To quench the reaction, 1 mL of acetone was added and the reaction was stirred at room temperature for 30 min. The reaction mixture was then diluted with ethyl acetate and washed with 25 mL of each of the following solutions; sat. $NaHCO_3$, 10% HCl, H_2O, and brine. The resulting organic layer was then dried over Na_2SO_4, concentrated and purified by silica gel flash chromatography.

2.67 General procedure for oxidation of 6-OH.

The desired compound to be oxidized (45 µmol) was dissolved in a solution of 2 mL DCM, 2 mL *t*BuOH, and 0.5 mL H_2O. To this solution was added TEMPO (26.5 µmol or 0.3 eq per 6-OH) followed by BAIB (221 µmol or 2.5 eq per 6-OH). The reaction was then stirred at room temperature overnight. After ensuring the reaction was complete by TLC(1% Acetic Acid in Ethyl Acetate), the reaction was quenched by addition of 2 mL of $Na_2S_2O_3$ solution and allowed to stir at room temperature for 15 min. The mixture was then diluted with 10 mL DCM and 3 mL H_2O and separated. The aqueous layer was acidified with 1 M HCl solution and extracted three times with DCM. The organic layers were combined, dried over Na_2SO_4 and concentrated. The crude product could then be protected as a benzyl or methyl ester.

2.68 General procedure for benzyl ester formation after oxidation.

The crude product from oxidation was dissolved in 5 mL of DCM. To this was added phenyl diazomethane until a deep red color persisted.[69] The reaction was allowed to stir overnight. After TLC indicated the reaction was complete, the mixture was concentrated and purified by column chromatography.

2.69 General procedure for methyl ester formation after oxidation.

The crude product from oxidation was dissolved in DMF (2 mL for 15 µmol). To this solution was added K_2CO_3 (5 eq per COOH) followed by CH_3I (2.5 eq per COOH) and the reaction was allowed to stir overnight at room temperature. After verifying the reaction was complete by TLC, the reaction was diluted with ethyl acetate and water. The mixture was then

washed with 0.1 M HCl followed by sat. $NaHCO_3$, dried over Na_2SO_4, concentrated, and purified by flash silica gel chromatography.

2.610 General procedure for saponification.

The mixture of compound (for 100 mg of compound, 1 equiv), THF (2.5 mL), and 1 M LiOH (13 equiv per COOBn) was cooled to 0 °C, followed by addition of H_2O_2 (150 equiv per COOBn, 30%). The mixture was stirred at room temperature for 16 h and then methanol (6 mL) and 3 M potassium hydroxide (80 equiv per COOBn) were added to the solution. The mixture was stirred for another 24 h, then acidified with 10% HCl, and concentrated to dryness. The resulting residue was purified by quickly passing through a short silica gel column (4:1, DCM:MeOH).

2.611 General procedure for transesterification.

The methyl ester containing oligosaccharide (10 µmol) was dissolved in 2 mL of dry DCM and 2 mL of dry methanol. The two solvents were dried over 4Å molecular sieves for 24 hours. A sodium methoxide solution was made by adding sodium to a portion of dried methanol. This fresh sodium methoxide solution was added to the oligosaccharide solution until the pH reached 12. The reaction was maintained at pH=12 and stirred at room temperature for 2 hours. After the reaction was confirmed complete by TLC, it was quenched to pH=7 by a 1 M acetic acid solution in dry methanol. The quenched reaction was concentrated and purified by silica gel chromatography.

2.612 General procedure for Staudinger reduction.

1 M PMe_3 solution in THF (5 equiv per N3), 0.1 M aqueous solution of NaOH (3 equiv per N_3), and H_2O (2 mL) were added consecutively to a solution of azide-containing compound (for 50 mg of compound, 1 equiv) in THF (7 mL). The mixture was stirred at room temperature overnight and neutralized with 0.1 M HCl until pH=7. The mixture was concentrated to dryness and the resulting residue was purified Sephadex LH-20 (50/50 DCM/MeOH).

2.613 General procedure for 1,3-dithiopropane mediated azide reduction.

The starting oligosaccharide was dissolved in dry MeOH (dried over 4Å molecular sieves) and protected from light. To this solution triethylamine (6.66 eq per N_3) and 1,3-dithiopropane (6.66 eq per N_3) were added and the reaction was stirred at room temperature for 24 hours. After one day another portion of triethylamine and 1,3-dithiopropane (6.66 eq per N_3 of each) were added and the reaction was stirred for another 72 hours. The reaction was diluted with a 1:1 mixture of DCM:MeOH and was layered onto a Sephadex LH-20 column and eluted with 1:1 DCM:MeOH.

2.614 General procedure for O-sulfation.

The mixture of OH-containing compound (for 5 mg of compound, 1 equiv), DMF (1 mL dried over 4Å molecular sieves), and $SO_3 \cdot NEt_3$ (5 equiv per OH) was stirred at 55 °C for 24 h. The mixture was quenched by adding NEt_3 (0.2 mL) and then diluted with DCM/MeOH (1 mL:1 mL). The resulting solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with DCM/MeOH (1:1)

2.615 General procedure for N-sulfation.

A mixture of NH_2-containing compound (for 5 mg of compound, 1 equiv), pyridine (1 mL dried over 4Å molecular sieves), Et_3N (0.2 mL), and SO_3·Pyridine (5 equiv per NH_2) was stirred at room temperature for 3 h. The mixture was diluted with DCM/MeOH (1 mL/1 mL) and the resulting solution was layered on the top of a Sephadex LH-20 chromatography column that was eluted with DCM/MeOH (1/1).

2.616 General procedure for global debenzylation.

A mixture of the Bn-containing compound (for 6 mg of compound, 1 equiv), MeOH/H_2O (4 mL/2 mL), and $Pd(OH)_2$ on carbon (100 mg) was stirred under H_2 at room temperature overnight and then filtered. The filtrate was concentrated to dryness under vacuum and then diluted with H_2O (15 mL). The aqueous phase was further washed with CH_2Cl_2 (3x5 mL) and EtOAc (3x5 mL) and then the aqueous phase was dried under vacuum. The crude product was further purified by size exclusion chromatography (G-15) and for final compounds then eluted from a column of Dowex 50WX4-Na^+ to convert the compound into the sodium salt form.

2.617 General procedure for selective O-sulfation.

A compound (8 mg or 4 µmol) containing both free OH and NH_2 groups was dissolved in 1 mL of dry pyridine (dried over 4Å molecular sieves). To this mixture was added 20 mg of SO_3·pyridine. The sulfating agent had been previously washed with H_2O, MeOH, DCM and dried under vacuum. The reaction was protected from light and stirred for 24 hours at 55 °C. The reaction was diluted with 1:1 DCM:MeOH and eluted from a Sephadex LH-20 column ensuring

all pyridine was removed. The fractions containing sugar were concentrated and further purified by prep TLC (3:1:1 EtOAc:MeOH:H_2O 1% AcOH).

2.617 General procedure for simultaneous O,N-sulfation.

A compound (8 mg or 4 μmol) containing both free OH and NH_2 groups was dissolved in 1 mL of dry pyridine (dried over 4Å molecular sieves). To this mixture was added 100 mg of SO_3·pyridine. The sulfating agent had been previously washed with H_2O, MeOH, DCM and dried under vacuum. The reaction was protected from light and stirred for 24 hours at 55 °C. The reaction was diluted with 1:1 DCM:MeOH and eluted from a Sephadex LH-20 column ensuring all pyridine was removed. The fractions containing sugar were concentrated and further purified by prep TLC (3:1:1 EtOAc:MeOH:H_2O 1% AcOH).

2.618 General procedure for methyl ester saponification.

The compound to be saponified was dissolved in H_2O (1 mL for 5 mg) and 1 M LiOH (15 equiv per ester) was added and the mixture was cooled to 0 °C. This was followed by addition of H_2O_2 (150 equiv per ester, 30%) and the reaction was allowed to warm to rt and stir overnight. The reaction was neutralized with 1 M AcOH and eluted from a Sephadex G-15 column with H_2O. To simplify mass spec, the product was then eluted from a column of Dowex 50WX4-Na^+ to convert the compound into the sodium salt form.

2.619 General procedure for enzymatic sulfation.

The oligosaccharide to be sulfated (500 μg or 0.4 μmol) was mixed with 1 mg of the needed enzyme(s) in 12.5 mL of solution. This solution had a concentration of 20 mM MES and

0.05 mg/mL of PAPS. This reaction was stirred at 37°C overnight. Another 1 mg of the needed enzyme(s) was added and the reaction was diluted to 25 mL keeping the concentration of MES at 20 mM and PAPS at 0.05 mg/mL respectively. After another 24 hours at 37 °C the reaction was stopped. It was concentrated by utilizing a Q-Sepharose Fast Flow column. The mixture was passed through the column, which was then washed with 20 mL of 25 mM NaOAc. The product was then eluted from the column with a solution of 1M NaCl and 25 mM NaOAc. The product eluted within the first 2 mL and the column was further washed with 10 mL of the elution solution and 25 mL of the 25 mM NaOAc solution. The fractions containing sugar were lyophilized and loaded onto a P-2 column (2m x 0.75cm diameter) with 1 mL of 0.1 M NH_4HCO_3. Additional NH_4HCO_3 was added until the loading solution was neutralized. An indicator, 5 µl of Phenol red, was added to monitor the column, and the product was eluted with 0.1M NH_4HCO_3. Tubes containing product had to be lyophilized at least 3 times to remove any residual NH_4HCO_3 to allow for mass spec analysis.

2.620 General procedure for microarray preparation.

All solutions were prepared with nanopure water. Recombinant human basic Fibroblast Growth Factor (FGF-2) and rabbit anti-human FGF-2 were purchased from PeproTech (Rocky Hill, NJ) and Cy5 conjugated goat ant-rabbit IgG (H+L) was purchased from Life Technologies (Grand Island, NY). NHS coated slides (SL HCX) were purchased from Xantec Bioanalytics GmbH (Germany). Microarrays were produced using a PixSys 5500 robotic printer (Cartesian Technologies Inc., California). Oligosaccharides were dissolved in 50 mM sodium phosphate buffer (pH=9) and mechanically printed onto the NHS coated slides at 50% relative humidity and room temperature. After printing, slides were incubated at 75% humidity and room temperature

overnight. Oligosaccharides were printed in four concentrations (400 nM, 80 nM, 16 nM, and 3.2 nM), and each spot was replicated four times. Two natural sources were printed alongside the synthesized oligosaccharides. Heparin (HP, sodium salt, av 18 kDa, 177 USP unit/mg) and Chondroitin sulfate A (sodium salt from bovine trachea av 50 kDa) were both purchased from Sigma Aldrich and printed in the same concentration as synthesized oligosaccharides using their average molecular weights. Slides were washed three times with water. To quench un-reacted NHS groups, the slides were then incubated in a pre-heated 50 °C solution of 100 nM ethanolamine in sodium phosphate buffer (50 mM, pH=9) for one hour. After quenching, the slides were washed three times with water, dried by centrifugation (2000 RPM for 2 min), and stored in a dessicator at -5 °C until use. For all protein incubations, Lifterslips from Thermo Scientific were used in concert with 20 µL of solution. Analysis of slides was done on an Agilent G2565AA Array Scanner.

2.621 General procedure for microarray binding assay.

Slides to be used were warmed to rt before removing from dessicator. Protein solutions were prepared by diluting stock solutions to concentrations of 8 µg/mL with PBS buffer (10 mM pH=7.5) containing 1% BSA. An assay was run as follows. Slides were incubated with 20 µL of FGF-2 solution (placed between Lifterslip and slide) and incubated in a microarray cassette at room temperature protected from light for 1 hour. After one hour the slide was washed once with a solution of PBS (10 mM pH= 7.5) with 1% Tween-20 and 0.1% BSA and twice with water. The slide was dried by centrifugation then incubated with 20 µL of rabbit anti-Human FGF-2 for one hour as done previously. The slide was then washed in the same way and finally incubated with 20 µL of the secondary antibody Cy5 goat anti-rabbit IgG for 1 hour and washed. After

drying by centrifugation, the slide was imaged on an Agilent G2565AA Array Scanner. The intensities of the bands were quantified using ImageJ software.

3-*O*-Benzyl-1,2-*O*-isopropylidene-5,6-dimesyl-α-D-glucofuranose (1) Compound **1** was prepared in three steps by dissolving of diacetone D-glucose (25 g, 96 mmol) in 250 mL of THF and cooling it to 0 °C. Then NaH (60% in mineral oil, 4.8 g, 1.25 eq) was added in portions. After evolution of H_2 ceased, benzyl bromide (12.5 mL, 105 mmol) and tetrabutyl ammonium iodide (0.25 g, 0.68 mmol) were added in portions. The reaction was stirred at room temperature overnight. Water was added to the reaction slowly and the organic solvents were removed under vacuum. The resulting water mixture was extracted three times with ethyl acetate, dried over Na_2SO_4, concentrated, and a silica gel column was run (8:1 hexane-EtOAc) furnishing the product as an orange oil. The orange oil was dissolved in 66% acetic acid (150 mL), heated to 40 °C and stirred for 6 hours. After this time, the solvent was removed under high vacuum. The residue was then dissolved in DCM and washed with sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated. A silica column (1:1 hexane-EtOAc) was run to purify the product. Finally mesylation was done by dissolving the previous product in pyridine (105 mL) and cooling it to 0 °C. Mesyl chloride (16.6 mL, 0.21 mol, 1.2 eq per OH) was added dropwise and the reaction was stirred at 4 °C for 16 hours. The reaction mixture was then poured into 50°C water (500 mL) and then cooled to recrystallize the product which was filtered and dried under vacuum. The yield of **1** was 37.6 grams (80.5 mmol) or 84% over three steps. ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.42 – 7.30 (5 H, m), 5.90 (1 H, d, *J* 3.6), 5.27 (1 H, ddd, *J* 7.6, 5.7, 2.1), 4.70 (1 H, dd, *J* 11.9, 2.1), 4.68 (1 H, d, *J* 10.9), 4.63 (1 H, d, *J* 10.9), 4.62 (1 H, d, *J* 3.7), 4.46 (1 H, dd, *J* 11.9, 5.7), 4.42 (1 H, dd, *J* 7.4, 3.2), 4.15 (1 H, d, *J* 3.1), 3.11 (3 H, s), 3.03 (3 H, s), 1.51 (3 H,

s), 1.33 (3 H, s). This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[13b]

5,6-Anhydro-3-*O*-benzyl-1,2,-*O*-isopropylidene-β-L-idofuranose (2) Compound two was prepared from **1** in two steps. **1** (10 g, 21.4 mmol) was dissolved in acetonitrile (200 mL). To this was added potassium acetate (20 g, 0.20 mol, 9.5 eq) and 18-crown-6 (0.62 g, 2.3 mmol, 0.11 eq). This mixture was refluxed for 24 hours and then cooled and filtered. The resulting solution was concentrated then recrystallized from EtOAc and hexane providing of the product (8.9 g, 20.5 mmol) in 96% yield. The crystalline product was dissolved in a mixture of tert-butanol (45 mL) and DCM (90 mL). The mixture was cooled to 0 °C and potassium tert-butoxide (4.64 g, 41 mmol, 2 eq) was added. The reaction was stirred at 0 °C for 16 hours then diluted with DCM and washed with water. After concentration a 2:1 hexane-EtOAc column was run providing **2** (5.2 g, 17.8 mmol, 87% yield.) ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.39 – 7.27 (5 H, m), 5.99 (1 H, d, *J* 3.7), 4.73 (1 H, d, *J* 12.2), 4.63 (1 H, d, *J* 3.8), 4.51 (1 H, d, *J* 12.2), 3.96 (1 H, d, *J* 3.5), 3.80 (1 H, dd, *J* 6.1, 3.5), 3.29 – 3.24 (1 H, m), 2.78 – 2.73 (1 H, m), 2.53 (1 H, ddd, *J* 4.9, 2.7, 0.9), 1.44 (3 H, s), 1.31 (3 H, s). This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[13b]

1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-α/β-D-idopyranoside (3) Compound **3** was prepared in two steps. **2** (5.5 g, 18.8 mmol) was dissolved in 0.1 M H_2SO_4 (50 mL) and stirred at 60 °C for 18 hours. To quench the reaction, $Ba(OH)_2 \cdot 8H_2O$ (1.514 g, 4.8 mmol) was added. The produced $BaSO_4$ was removed by filtration and the solvent removed under vacuum. The resulting residue was dried by co-evaporation three times with toluene. The crude residue was dissolved pyridine (35 mL) and cooled to -15 °C. To this was added acetic anhydride (17.6 mL, 188 mmol, 10 eq) and the reaction was stirred at 0 °C for 16 hours. To quench the reaction, dimethylaminopyridine

(DMAP) (2.3 g, 18.8 mmol, 1 eq) was added and it was stirred at room temperature for 3 hours. After addition of MeOH the reaction was concentrated and a 3:1 toluene-acetone column was run to isolate **3** (6.1 g, 14 mmol, 74% over two steps).

***p*-Tolyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-α-L-idopyranoside (4)** Compound **3** (2.75 g, 6.37 mmol) was dissolved in DCM (30 mL) and cooled to 0 °C and 4-methylbenzenethiol (0.918 g, 7.4 mmol, 1.2 eq) was added. The mixture was stirred for 30 min at 0 °C after which chilled $BF_3 \cdot Et_2O$ (2.38 mL, 19 mmol, 3 eq) was added dropwise. After reacting for 2.5 hours the reaction was diluted with DCM and quenched with triethylamine. The resulting mixture was washed with sat. $NaHCO_3$ and brine, then dried and a gradient column was run starting with 15% EtOAc in hexane and ending with 30% EtOAc in hexane. **4** (1.89 g, 3.82 mmol, 60%) was isolated along with the β anomer (0.95 g, 1.9 mmol, 30%). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.46 – 7.32 (7 H, m, Ar), 7.13 – 7.09 (2 H, m, Ar), 5.43 (1 H, d, *J* 0.5), 5.18 – 5.16 (1 H, m), 5.05 – 5.00 (1 H, m), 4.91 – 4.88 (1 H, m), 4.85 (1 H, d, *J* 11.8), 4.71 (1 H, d, *J* 11.9), 4.27 (1 H, dd, *J* 11.5, 7.8), 4.20 (1 H, dd, *J* 11.5, 5.0), 3.78 (1 H, td, *J* 2.7, 1.3), 2.33 (3 H, s,$SPhCH_3$), 2.10 (3 H, s, Ac), 2.07 (3 H, s, Ac), 2.06 (3 H, s, Ac). This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[47]

***p*-Tolyl 3-*O*-benzyl-4,6-*O*-*p*-methoxybenzylidene-1-thio-α-L-idopyranoside (5)** Compound **4 (**3.49 g, 6.95 mmol), was dissolved in MeOH (30 mL) and DCM(15 mL). To this mixture, freshly prepared 5.4 M NaOH (1.2 mL, 20.9 mmol) was added and the reaction was stirred overnight. After the reaction was complete by TLC (1:1 hexanes-EtOAc) the reaction was quenched with Amberlite resin to pH=7 and the resin was filtered off. The solvents were removed and the resulting residue was dried by co-evaporation with toluene twice. The resulting residue was dissolved in anhydrous DMF (20 mL) to which was added camphorsulfonic acid

(0.325 g, 1.4 mmol) and *p*-anisaldehyde dimethyl acetal (1.43 mL, 8.34 mmol). The reaction was allowed to stir overnight and monitored by TLC (1:1 hexane-EtOAc). After the reaction was complete the reaction was quenched to pH=7 with triethylamine, concentrated, and a gradient column run starting with 5% ethyl acetate in hexanes ending with 25% of ethyl acetate isolating the product (2.9 g, 5.9 mmol, 85% over two steps.). The product was dissolved in DCM (30 mL) to which DMAP (2.2 g, 17.7 mmol) and benzoyl chloride (0.82 mL, 7.08 mmol) were added. The reaction was stirred at room temperature overnight and monitored by TLC (6:1:1 hexane-EtOAc-DCM). To quench the reaction methanol was added and the solvents were evaporated. A column (6:1:1 hexane-EtOAc-DCM) was run and **5** was isolated (2.9 g, 4.8 mmol, 82%). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.03 – 7.97 (2 H, m), 7.54 – 7.32 (10 H, m), 7.28 – 7.24 (2 H, m), 7.16 – 7.09 (2 H, m), 6.83 – 6.77 (2 H, m), 5.77 (1 H, s), 5.56 (1 H, s), 5.55 – 5.53 (1 H, m), 5.00 (1 H, d, *J* 11.8), 4.72 (1 H, d, *J* 11.8), 4.53 (1 H, d, *J* 1.3), 4.38 (1 H, dd, *J* 12.6, 1.4), 4.20 (1 H, dd, *J* 12.6, 1.9), 4.11 (1 H, s), 3.93 (1 H, d, *J* 0.9), 3.82 (3 H, s), 2.34 (3 H, s). This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[20b]

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio-α-L-idopyranoside (6)**

Compound **5** (1.8 g, 3.1 mmol) was dissolved in DMF (30 mL) and cooled to 0 °C. To this was added sodium cyanoborohydride (1.55 g, 24.7 mmol) and trifluoroacetic acid (2.4 mL, 31 mmol). The reaction was allowed to warm to room temperature and react for 36 hours. Reaction was quenched by adding sodium bicarbonate and diluting with EtOAc. The solution was then washed with saturated $NaHCO_3$ and water. After drying over sodium sulfate the solvents were removed and a silica gel column was run (5:1:1 hexane-EtOAc-DCM) to provide **6** (1.34 g, 2.6 mmol, 72%)^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.02 (2 H, dd, *J* 8.4, 1.2), 7.63 – 7.56 (1 H, m),

7.51 – 7.27 (11 H, m), 7.06 (2 H, d, *J* 8.3), 6.93 – 6.88 (2 H, m), 5.56 (1 H, s), 5.52 (1 H, dt, *J* 2.4, 1.0), 5.00 (1 H, t, *J* 4.9), 4.93 (1 H, d, *J* 11.8), 4.69 (1 H, d, *J* 11.9), 4.56 (2 H, q, *J* 11.5), 3.90 (1 H, td, *J* 2.9, 1.3), 3.88 – 3.77 (6 H, m), 2.85 (1 H, d, *J* 9.7), 2.33 (3 H, s). It also provided **7** (0.31 g, 0.51 mmol), 17%), ***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-*p*-methoxybenzyl-1-thio-α-L-idopyranoside (7)** ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.02 – 7.98 (2 H, m), 7.55 – 7.31 (10 H, m), 7.14 – 6.99 (4 H, m), 6.79 – 6.74 (2 H, m), 5.54 (1 H, m), 5.50 – 5.45 (1 H, m), 4.94 (1 H, d, *J* 11.9), 4.72 (1 H, ddd, *J* 6.4, 4.2, 2.1), 4.67 (1 H, d, *J* 12.0), 4.44 (1 H, d, *J* 11.3), 4.26 (1 H, d, *J* 11.3), 4.03 – 3.99 (1 H, m), 3.98 (1 H, dd, *J* 3.6, 2.6), 3.80 (3 H, s), 3.79 – 3.73 (1 H, ddd, *J* 11.6, 9.6, 4.3), 3.56 (1 H, t, *J* 2.4), 2.33 (3 H, s), 2.09 (1 H, dd, *J* 9.6, 2.9). The data for both **6** and **7** compare favorable with data published previously.[20b]

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)-α/β-D-glucopyranoside (**8**). Glucosamine hydrochloride (10.0 g, 46.3 mmol) was dissolved in water (100 mL) along with $NaHCO_3$ (11.6 g, 140 mmol) and cooled to 0 °C. To this 2,2,2-trichloroethyl chloroformate (7.7 mL, 57 mmol) was added dropwise over 1 hour. The reaction was then allowed to warm to rt and run for 2 hours. The white precipitate was filtered and dried under vacuum overnight. The crude product, 16.85 g, was dissolved in pyridine (60 mL) and cooled to 0 °C. Acetic Anhydride (32.6 mL, 0.344 mol, 8 eq) was added over 30 min and the reaction was allowed to warm to rt and run overnight. The reaction was cooled to 0 °C and quenched with EtOH (30 mL). The mixture was concentrated under vacuum then diluted with EtOAc and was sequentially with sat. $NaHCO_3$, 10% HCl, water, and finally brine. The organic phase was dried over Na_2SO_4 and concentrated giving **8** (21.6 g, 42.2 mmol, 91% over two steps.) ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 6.24 (1 H, d, *J* 3.7), 5.29 (1 H, dd, *J* 14.7, 5.7), 5.20 (2 H, t, *J* 9.9), 4.84 (1 H, dd, *J* 12.6??, 6.5), 4.63 (1 H, d, *J* 12.1), 4.28 (1 H, dd, *J* 12.5, 4.0), 4.21 (1

H, ddd, *J* 10.9, 9.5, 3.7), 4.07 (1 H, dd, *J* 12.5, 2.3), 4.04 – 4.00 (1 H, m). This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[70]

***p*-Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloro-ethoxycarbonylamino)-1-thio-β-D-glucopyranoside** (**9**). Compound **9** was prepared from **8** (13.3 g, 26 mmol) which was dissolved in DCM (140 mL)along with *p*-toluenethiol (3.8 g, 31 mmol, 1.2 eq) and cooled to 0 °C. $BF_3 \cdot OEt_2$ (140 mL, 78 mmol, 3 eq) was added slowly and the reaction was allowed to react at 0 °C for 3 hours. The reaction was warmed to rt and reacted overnight. The reaction was then diluted with DCM and washed sequentially with 1 M NaOH, water, and brine. The reaction was then dried over Na_2SO_4 and concentrated. The crude product was recrystallized in EtOAc-hexanes providing **9** (10.2 g, 17.8 mmol, 67% yield). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.43 (1 H, d, *J* 8.1), 7.16 – 7.12 (1 H, m), 5.29 (1 H, t, *J* 9.8), 5.13 (1 H, d, *J* 8.8), 5.03 (1 H, t, *J* 9.7), 4.84 – 4.79 (1 H, m), 4.74 (1 H, d, *J* 12.1), 4.25 (1 H, dd, *J* 12.2, 5.1), 4.18 (1 H, dd, *J* 12.2, 2.4), 3.71 (1 H, ddd, *J* 9.8, 5.1, 2.3), 3.65 (1 H, dd, *J* 19.3, 9.8), 2.37 (2 H, s), 2.10 (2 H, s), 2.03 (2 H, s), 2.02 (1 H, s).The data collected concurs with that previously reported.[45c]

***p*-Tolyl 2-amino-2-deoxy-1-thio-β-D-glucopyranoside** (**10**). Compound **10** was prepared in two steps from **9**. **9** (4.85 g, 8.27 mmol) was dissolved in methanol (20 mL), acetic acid (10 mL), DCM (10 mL) and cooled 0°C. Once cooled zinc dust (8.00 g, 0.124 mol) was added in portions and the reaction was allowed to warm to rt and stir for 2 hours. The zinc was removed by filtering through celite, and the mixture was dried under vacuum. The resulting residue was diluted with DCM and washed with sat. $NaHCO_3$ until the solution pH=7 then dried over Na_2SO_4 and concentrated. The crude product was immediately dissolved in a mixture of methanol (10 mL) and DCM (10 mL). To this mixture freshly prepared 5.4 M NaOMe (7.65 mL)

was added dropwise. The reaction was allowed to stir overnight at rt and was quenched with conc. HCl until pH=7 and concentrated under vacuum. Purification of the residue by silica gel column (4:1, DCM-MeOH) provided **10** (2.1 g, 7.5 mmol 90% yield). ^{1}H-NMR δ_H (500 MHz, CD_3OD) 7.55 – 7.51 (2 H, m), 7.21 – 7.17 (2 H, m), 4.66 (1 H, d, *J* 10.2), 3.89 (1 H, dd, *J* 12.1, 2.1), 3.70 (1 H, dd, *J* 12.1, 5.4), 3.45 (1 H, dd, *J* 10.2, 8.5), 3.36 (1 H, s), 3.33 (2 H, m), 2.79 (1 H, t, *J* 10.2), 2.34 (3 H, s). The data collected concurs with that previously reported.[20b]

***p*-Tolyl 2-azido-4,6-*O*-benzylidine-2-deoxy-1-thio-β-D-glucopyranoside (11).** Compound **10** (3.53 g, 12.3 mmol) was dissolved in methanol (40 mL) and water (10 mL). To this solution was added K_2CO_3 (5.1 g, 37 mmol) and $ZnCl_2$ (5.1 g, 2.5 mmol). Once the solution had stirred for 10 min, a solution of freshly prepared triflic azide in DCM (25 mL) was added (prepared from 4.83 g of NaN_3 and 6.24 mL of triflic anhydride) dropwise.[71] The reaction was allowed to stir overnight and was quenched with conc. HCl to pH = 6 and diluted with 4:1 DCM:MeOH and filtered through celite and concentrated. The crude product was taken to the next step without further purification. The residue was dissolved in acetonitrile (40 mL) to which benzyaldehyde dimethyl acetal (2.80 mL, 18.5 mmol) was added. This mixture was cooled to 0 °C followed by the addition of camphorsulfonic acid (0.86 g, 3.7 mmol). The reaction was allowed to warm to rt and stir for 4 hours. The reaction was quenched with triethylamine and diluted with EtOAc. The mixture was then washed with sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated. A 2:1 hexane-EtOAc column furnished **11** (3.4 g, 8.5 mmol, 69% over two steps). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.50 – 7.35 (1 H, m), 7.19 – 7.16 (1 H, m), 5.52 (1 H, s), 4.46 (1 H, d, *J* 10.1), 4.37 (1 H, dd, *J* 10.6, 4.7), 3.75 (2 H, m), 3.45 (1 H, t, *J* 9.1), 3.41 (1 H, dt, *J* 9.4, 4.3), 3.31 (1 H, dd, *J* 10.1, 9.1), 2.80 (1 H, s), 2.38 (1 H, s). The data collected concurs with that previously reported.[20b]

***p*-Tolyl *2*-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (12).** Compound **11** (5.47 g, 13.7 mmol) was dissolved in DMF (60 mL) along with benzyl bromide (1.96 mL, 16.4 mmol) and tetrabutyl ammonium iodide (0.51 g, 1.3 mmol). This was cooled to 0 °C after which (60% in Oil, 1.10 g, 27.4 mmol) was added. This was allowed to warm to rt and stir for 2 hours after which the reaction was diluted with EtOAc and washed with sat. $NaHCO_3$, water, and brine. The quenched mixture was concentrated and recrystallized hexane-EtOAc to give ***p*-Tolyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidine-2-deoxy-1-thio-β-D-glucopyranoside** (6.3 g, 12.9 mmol, 94%). The intermediate was then dissolved in DCM (100 mL) and MeOH (100 mL) and cooled to 0 °C for 30 min. After this acetyl chloride (2.7 mL, 38 mmol) was added dropwise and the reaction was allowed to warm to rt and stir for 6 hours. The reaction was quenched by addition of solid $NaHCO_3$ and diluted with DCM. The mixture was washed with sat. $NaHCO_3$, water, and brine, followed by drying over Na_2SO_4. After concentration a silica gel column, 1:1 hexane-EtOAc, was run providing **12** (4.73 g, 11.8 mmol, 86% over two steps.) ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.46 (1 H, d, *J* 8.0), 7.40 – 7.30 (3 H, m), 7.15 (1 H, d, *J* 8.2), 4.94 (1 H, d, *J* 11.2), 4.78 (1 H, d, *J* 11.2), 4.42 (1 H, d, *J* 9.8), 3.90 – 3.84 (1 H, m), 3.76 (1 H, ddd, *J* 11.8, 6.7, 5.0), 3.54 (1 H, td, *J* 9.0, 3.5), 3.35 (1 H, t, *J* 9.0), 3.32 – 3.26 (1 H, m), 2.36 (2 H, s). The data collected concurs with that previously reported.[20b, 47]

***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-1-thio-β-D-glucopyranoside (13).** Compound **12** (4.0 g, 9.9 mmol) was dissolved in DCM (80 mL) and cooled to -40 °C. 2,4,6 trimethylpyridine (4.0 mL, 36 mmol) was added and after the reaction was stirred for 20 min acetyl chloride (0.71 mL, 9.9 mmol) was added dropwise at -40 °C. The reaction was subsequently chilled to -60°C and stirred for 2 hours then warmed to -30 °C. At this point the reaction was diluted with EtOAc and washed with 1 M HCl, sat. $NaHCO_3$, and brine

then dried over Na_2SO_4. A silica gel column (3:2 hexane-EtOAc) provided ***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside** (4.23 g, 9.54 mmol), which was used to make both **13** and **14.** ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.52 – 7.47 (1 H, m), 7.41 – 7.30 (3 H, m), 7.17 – 7.10 (1 H, m), 4.93 (1 H, d, *J* 11.0, Bn), 4.81 (1 H, d, *J* 11.0, Bn), 4.46 (1 H, dd, *J* 12.2, 4.1, H-6a), 4.39 (1 H, d, *J* 10.0, H-1), 4.32 (1 H, dd, *J* 12.2, 2.0, H-6b), 3.40 (3 H, m, H-3, H-4, H-5), 3.30 – 3.25 (1 H, m. H-2), 2.69 (1 H, d, *J* 2.9, OH), 2.37 (1 H, s, $SPhCH_3$), 2.13 (1 H, s, $COCH_3$). The data collected concurs with that previously reported.[47] **p-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside** (2.85 g, 6.42 mmol) was dissolved in DCM (90 mL) along with 2,6 lutidine (1.5 mL, 13 mmol) and cooled to -40 °C. After 15 min, TBSOTf (2.22 mL, 9.64 mmol) was added dropwise. The reaction was allowed to warm to rt and stir overnight. The reaction was diluted with DCM and washed with 1 M HCl, water, and brine then concentrated. A gradient hexane-EtOAc column starting at 8% EtOAc and ending at 15% EtOAc was run and furnished **13** (2.92 g, 5.25 mmol, 79% over two steps from **12**). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.52 – 7.48 (2 H, m), 7.36 (4 H, d, *J* 4.5), 7.33 – 7.28 (1 H, m), 7.15 (2 H, d, *J* 7.9), 4.91 (1 H, d, *J* 11.0, Bn), 4.79 (1 H, d, *J* 11.0, Bn), 4.50 (1 H, dd, *J* 11.8, 2.3, H-6a), 4.42 (1 H, d, *J* 9.9, H-1), 4.08 (1 H, dd, *J* 11.8, 5.6, H-6b), 3.61 – 3.54 (1 H, m, H-4), 3.46 (1 H, ddd, *J* 9.4, 5.6, 2.3, H-5), 3.36 – 3.25 (2 H, m, H-2, H-3), 2.38 (3 H, s, $SPhCH_3$), 2.11 (3 H, s, $COCH_3$), 0.92 (9 H, s, $(CH_3)_3CSi$), 0.05 (3 H, s, CH_3Si), 0.04 (3 H, s, CH_3Si). This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[20b]

***p*-Tolyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl--2-deoxy-1-thio-β-D-glucopyranoside (14).** *p*-Tolyl 6-*O*-Acetyl-2-azido-3-*O*-benzyl-2-deoxy-1-thio-β-D-glucopyranoside (1.05 g, 2.37 mmol) and benzyl bromide (0.85 mL, 7.1 mmol) were dissolved in DMF (40 mL) and cooled to -

60 °C. Once cooled, NaH (60% in Oil, 95 mg, 2.4 mmol) was added and the reaction was allowed to warm to 0 °C and stir for 3 hours. The reaction was quenched by addition of sat. NH_4Cl solution then diluted with EtOAc. After washing with water, sat. $NaHCO_3$, and brine the mixture was dried over Na_2SO_4 and concentrated. A 4:1 hexane-EtOAc column afforded **14** (0.934 g, 1.75 mmol, 74% yield) along with starting material (0.20 g, 0.44 mmol, giving 91% BRSM). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.53 – 7.48 (2 H, m), 7.39 – 7.31 (8 H, m), 7.31 – 7.27 (3 H, m), 7.18 – 7.13 (2 H, m), 4.92 (1 H, d, *J* 10.5, Bn), 4.85 (2 H, d, *J* 10.5, Bn), 4.59 (1 H, d, *J* 10.9, Bn), 4.42 (1 H, dd, *J* 11.9, 2.1, H-6a), 4.37 (1 H, d, *J* 10.1, H-1), 4.20 (1 H, dd, *J* 11.9, 4.9, H-6b), 3.57 – 3.50 (2 H, m, H-3, H-5), 3.49 – 3.44 (1 H, m, H-4), 3.31 (1 H, t, *J* 10.1, H-2), 2.38 (3 H, s, $SPhCH_3$), 2.09 – 2.06 (3 H, m, $COCH_3$). This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[47]

***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-tert-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio-α-L-idopyranoside** (**15**). Compound **15** was synthesized from donor **13** (0.45 g, 0.81 mmol) and acceptor **6** (0.53 g, 0.90 mmol) providing **15** (0.72 g, 0.69 mmol, 85% yield) after a 1:1 hexane-EtOAc silica column. This was done following the general procedure of a single step glycosylation with one alteration. The reaction solution must be around 50% Et_2O after addition of the acceptor. Any decrease in the concentration of Et_2O leads to formation of a cyclic side product **16**. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.14 (2 H, dd, *J* 8.4, 1.3), 7.52 – 7.22 (15 H, m), 7.15 (2 H, d, *J* 6.9), 7.07 – 7.03 (2 H, m), 6.93 – 6.89 (2 H, m), 5.58 (1 H, s, H-1), 5.38 (1 H, t, *J* 2.1, H-2), 5.00 – 4.94 (2 H, m,H-5,Bn), 4.76 (1 H, d, *J* 11.7,Bn), 4.69 (1 H, d, *J* 3.7, H-1'), 4.57 – 4.49 (2 H, m, Bn), 4.31 – 4.22 (2 H, m, H-6a', Bn), 4.18 (1 H, t, *J* 3.2, H-3), 4.08 (1 H, d, *J* 11.3, Bn), 4.06 – 4.02 (1 H, m, H-6b'), 3.87 – 3.80 (5 H, m, $PhOCH_3$, H-5',H-6a) 3.77 (1 H, dd, *J*

10.2, 5.0, H-6b), 3.71 (1 H, s, H-4), 3.55 (1 H, t, *J* 9.1, H-4'), 3.40 – 3.35 (1 H, m, H-3'), 3.29 (1 H, dd, *J* 10.2, 3.7, H-2'), 2.33 (3 H, s, S-Ph-CH_3), 2.06 (3 H, s, Ac), 0.90 (9 H, s, $(CH_3)_3Si$), -0.00 (3 H, s CH_3Si), -0.10 (3 H, s, CH_3Si). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 170.83, 165.92, 159.47, 138.04, 137.81, 137.64, 133.44, 132.73, 131.97, 130.41, 130.25, 130.07, 129.85, 129.51, 128.70, 128.64, 128.35, 128.30, 128.16, 127.54, 127.26, 114.04, 98.70, 86.67, 80.78, 75.23, 74.80, 73.17, 72.90, 72.24, 71.59, 71.19, 70.41, 69.44, 67.58, 64.88, 63.20, 55.50, 26.15, 21.35, 21.07, 18.23, -3.53, -4.74. gHSQCAD (without ^1H decoupling) $^1J_{C1'H1'}$ = 171Hz $^1J_{C1H1}$ = 167.5 Hz. HRMS $[M+Li]^+$ $C_{56}H_{67}LiN_3O_{12}SSi^+$ calcd. 1040.4369, obsd. 1040.4373

***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-Benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-Benzyl-1-thio-α-L-idopyranoside (17).** Compound **15** (1.98 g, 1.89 mmol) was dissolved in DCM (144 mL) and water (16 mL) and cooled to 0 °C after which DDQ (0.645 g, 2.84 mmol) was added and the reaction was allowed to warm to rt and stir overnight. The reaction was then diluted with DCM and washed with sat. $NaHCO_3$ and water until the wash was colorless. After concentrating a 4:1:1 hexane-DCM-EtOAc silica gel column proved **17** (1.70 g, 1.83 mmol, 98% yield)**.** ^1H-NMR δ_H (500 MHz, $CDCl_3$) 8.07 (2 H, dd, *J* 7.1, 1.3), 7.45 – 7.12 (14 H, m), 7.06 (2 H, d, *J* 7.9), 7.00 (2 H, d, *J* 7.5), 5.52 (1 H, s, H-1), 5.31 (1 H, d, *J* 1.3, H-2), 4.92 (1 H, d, *J* 11.8, Bn), 4.78 (1 H, t, *J* 6.8, H-5), 4.69 (1 H, d, *J* 11.8, Bn), 4.48 (1 H, d, *J* 1.8, H-1'), 4.33 (1 H, dd, *J* 11.8, 1.7), 4.10 (1 H, s, H-3), 4.08 – 3.99 (2 H, m), 3.91 (1 H, dd, *J* 11.8, 6.6), 3.88 – 3.78 (2 H, m, H-6a, H-4'), 3.74 (2 H, d, *J* 11.2, H-6b), 3.62 (1 H, s, H-4), 3.39 – 3.31 (1 H, m, H-3'), 3.19 (2 H, d, *J* 4.8, H-2'), 2.26 (3 H, s, S-Ph-CH_3), 2.02 (3 H, s, Ac), 0.80 (9 H, s, $(CH_3)_3Si$), -0.08 (3 H, s, CH_3Si), -0.21 (3 H, s, CH_3Si). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 170.59, 165.61, 137.86, 137.72, 137.32, 133.22, 132.41, 131.70, 130.15, 129.83, 129.79, 128.53, 128.43, 128.32, 128.05, 128.03, 127.26, 126.91, 99.49, 86.49, 80.56,

76.17, 74.42, 72.56, 71.82, 71.29, 71.24, 69.93, 67.96, 64.64, 63.40, 61.51, 25.93, 25.88, 21.12, 20.74, 17.96, 14.21, -3.81, -4.82. HRMS $[M+H]^+$ $C_{48}H_{60}N_3O_{11}SSi^+$ calcd. 914.3712, obsd. 914.3800

***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-Benzyl-6-levulinoyl-1-thio-α-L-idopyranoside (18).** **17** (1.70 g, 1.85 mmol) was dissolved in DCM (100 mL) followed by the addition of levulinillic acid (0.266 mL, 2.60 mmol), EDC·HCl (0.569 g, 2.97 mmol), and DMAP (23 mg, 0.19 mmol). The reaction was allowed to stir at rt overnight. The completed reaction was diluted with DCM, washed with sat. $NaHCO_3$, and dried over Na_2SO_4. Concentrating the sample and a running a 2:1 hexane-EtOAc silica gel column provided **18** (1.7 g, 1.7 mmol, 92% yield)**.** ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.13 (2 H, d, *J* 7.3), 7.48 (4 H, d, *J* 8.0), 7.45 – 7.20 (9 H, m), 7.13 (2 H, d, *J* 7.8), 7.09 (2 H, d, *J* 7.7), 5.58 (1 H, s, H-1), 5.35 (1 H, s, H-2), 4.97 (1 H, d, *J* 11.7, Bn), 4.95 (1H, m, H-5), 4.76 (1 H, d, *J* 11.7, Bn), 4.56 (1 H, d, *J* 3.3, H-1'), 4.45 – 4.39 (1 H, m, H-6a), 4.35 (2 H, d, *J* 11.9, H-6b, H-6a'), 4.16 (2 H, d, *J* 11.4, Bn, H-3), 4.03 (1 H, dd, *J* 12.0, 5.3, H-6b'), 3.90 (1 H, d, *J* 11.5, Bn), 3.82 (1 H, m, *J* 9.7, H-5'), 3.63 (1 H, s, H-4), 3.48 (1 H, t, *J* 8.5, H-4'), 3.32 – 3.23 (2 H, m, H-2', H-3'), 2.79 – 2.67 (2 H, m, CH_2 Lev), 2.64 – 2.53 (2 H, m, CH_2 Lev), 2.33 (3 H, s, $SPhCH_3$), 2.16 (3 H, s), 2.04 (3 H, s), 0.86 (9 H, s, $(CH_3)_3Si$), -0.03 (3 H, s, CH_3Si), -0.14 (3 H, s, CH_3Si).^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 206.21, 172.25, 170.63, 165.60, 137.76, 137.62, 137.20, 133.24, 131.99, 131.92, 130.04, 129.77, 129.69, 128.51, 128.45, 128.33, 128.07, 128.01, 127.24, 126.93, 99.17, 86.32, 80.55, 75.90, 74.42, 72.60, 71.62, 71.22, 70.99, 69.69, 66.21, 64.65, 63.83, 62.98, 60.33, 37.84, 29.76, 27.82, 25.91, 21.11, 21.01, 20.76, 17.97, 14.22, -3.80, -4.92. gHSQCAD (without ^{1}H decoupling) $^1J_{C1'H1'}$ = 171.5Hz $^1J_{C1H1}$ = 168 Hz. HRMS $[M+H]^+$ $C_{53}H_{66}N_3O_{11}SSi^+$ calcd. 1012.4080, obsd. 1012.4083

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-4-*O*-*p*-methoxybenzyl-1-thio-α-L-idopyranoside (19)** Compound **7** (0.615 g 0.880 mmol) was dissolved in DCM (15 mL) followed by the addition of levulinilic acid (0.15 mL, 1.4 mmol), EDC·HCl (314mg, 1.64 mmol), and DMAP (13 mg, 0.11 mmol) and the reaction was allowed to stir overnight at rt. The reaction was diluted with DCM, washed with sat. $NaHCO_3$ and dried over Na_2SO_4. After concentrating a 3:1:1 hexane-DCM-EtOAc silica gel column was run providing **19 (**0.63 g, 0.88 mmol), 88%. ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.04 – 7.98 (2 H, m), 7.56 – 7.31 (10 H, m), 7.15 – 7.02 (4 H, m), 6.79 – 6.73 (2 H, m), 5.55 (1 H, s, H-1), 5.49 – 5.44 (1 H, m, H-2), 4.93 (1 H, d, *J* 12.1, Bn), 4.90 (1 H, m, H-5), 4.67 (1 H, d, *J* 12.0, Bn), 4.46 – 4.41 (2 H, m, Bn, H-6), 4.33 (1 H, dd, *J* 11.5, 4.6, H-6'), 4.28 (1 H, d, *J* 11.2, Bn), 3.97 (1 H, td, *J* 3.0, 1.0, H-3), 3.80 (3 H, s, Ph-OCH_3), 3.51 (1 H, t, *J* 2.3, H-4), 2.73 (2 H, t, *J* 6.8, CH_2-Lev), 2.57 (2 H, t, *J* 6.8, CH_2-Lev), 2.34 (3 H, s, S-Ph-CH_3), 2.17 (3 H, s, $COCH_3$). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 206.36, 172.42, 165.63, 159.29, 137.44, 137.37, 133.18, 132.28, 131.95, 131.93, 130.00, 129.62, 129.56, 129.46, 128.52, 128.31, 128.00, 127.98, 113.71, 86.11, 77.28, 77.03, 76.77, 76.74, 72.98, 72.38, 71.78, 70.47, 69.09, 66.35, 63.84, 55.24, 37.92, 29.80, 27.90, 21.08. HRMS $[M+Na]^+$ $C_{40}H_{42}NaO_9S^+$ calcd. 721.2442, obsd. 721.2444

***p*-Tolyl 2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside (20) 19** (0.63 g, 0.89 mmol) was dissolved in DCM (27 mL) and water (3 mL) and cooled to 0 °C. At this point DDQ (0.33 g, 1.4 mmol) was added and the reaction was allowed to warm to rt and stir overnight. The reaction was then diluted with DCM and washed with water and sat. $NaHCO_3$ until colorless. The mixture was then concentrated and purified by silica gel, 1:1 hexane-EtOAc providing **20** (0.44 g of, 0.76 mmol 85%). ^{1}H-NMR δ_H (600 MHz, $CDCl_3$) 8.00 – 7.94 (2 H, m), 7.61 – 7.29 (10 H, m), 7.16 – 7.09 (2 H, m), 5.52 (1 H, s, H-1), 5.50 (1 H, dd, *J* 2.6, 1.0, H-2),

4.99 (1 H, dd, *J* 7.8, 4.5. H-5), 4.91 (1 H, d, *J* 11.8, Bn), 4.67 (1 H, d, *J* 11.8, Bn), 4.40 (1 H, dd, *J* 11.8, 7.9, H-6), 4.36 (1 H, dd, *J* 11.7, 4.6, H-6'), 3.88 (1 H, dd, *J* 4.8, 2.6, H-3), 3.78 – 3.73 (1 H, m, H-4), 2.73 (2 H, t, *J* 6.7, CH_2-Lev), 2.62 – 2.59 (2 H, m, CH_2-Lev), 2.57 (1 H, d, *J* 11.6, OH), 2.32 (3 H, s, S-Ph-CH_3), 2.16 (3 H, s, $COCH_3$). ^{13}C-NMR δ_C (151 MHz, $CDCl_3$) 206.58, 172.73, 165.09, 138.08, 137.39, 133.94, 132.52, 132.29, 129.97, 129.90, 129.24, 128.90, 128.76, 128.26, 128.06, 87.10, 74.04, 72.61, 70.07, 67.49, 66.76, 64.12, 38.14, 30.03, 28.10, 21.34. HRMS $[M+H]^+$ $C_{32}H_{35}O_8S^+$ calcd. 579.2047, obsd. 579.2046

***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl -2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside (21). 18** (0.30 g, 0.33 mmol) was dissolved in pyridine (5 mL) and cooled to 0 °C. To this was added dropwise HF·pyridine (2.5 mL) and the reaction was allowed to warm to rt and stir overnight. Reaction was quenched by diluting with DCM and washing sequentially with sat. $CuSO_4$, sat. $NaHCO_3$, and finally 10% HCl. The mixture was dried over Na_2SO_4, concentrated and purified by a silica gel column (2:1 hexane-EtOAc) providing **21** (0.26 g, 0.32 mmol, 98% yield). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.20 – 8.17 (2 H, m), 7.53 – 7.25 (13 H, m), 7.19 (2 H, dd, *J* 9.1, 2.6), 7.15 (2 H, d, *J* 8.1), 5.60 (1 H, s, H-1), 5.41 (1 H, s, H-2), 5.02 – 4.97 (2 H, m, *J* 11.7, Bn, H-5), 4.78 (1 H, d, *J* 11.7, Bn), 4.60 (1 H, d, *J* 3.8, H-1'), 4.50 (1 H, dd, *J* 12.4, 4.4, H-6a'), 4.42 (1 H, dd, *J* 11.6, 8.0, H-6a), 4.37 (1 H, d, *J* 10.6, Bn), 4.34 (1 H, dd, *J* 11.6, 4.4, H-6b), 4.22 (1 H, dt, *J* 6.0, 3.0, H-6b'), 4.19 (1 H, t, *J* 2.4, H-3), 4.07 (1 H, d, *J* 10.6, Bn), 3.86 (1 H, ddd, *J* 10.0, 4.2, 2.0, H-5'), 3.67 (1 H, t, *J* 2.3, H-4), 3.47 (1 H, dd, *J* 10.1, 9.0, H-3'), 3.35 (1 H, t, *J* 9.5, H-4'), 3.24 (1 H, dd, *J* 10.1, 3.8, H-2'), 3.02 (1H, br, OH), 2.75 (2 H, t, *J* 6.6, CH_2 Lev), 2.60 (2 H, t, *J* 6.6, CH_2 Lev), 2.36 (3 H, s, S-Ph-CH_3), 2.17 (3 H, s, CH_3, Lev), 2.08 (3 H, s, Ac).^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 206.68, 172.46, 171.85, 165.69, 137.77, 137.68, 137.21, 133.32, 132.23, 131.83, 129.94, 129.86, 129.74,

128.60, 128.48, 128.46, 128.33, 128.26, 128.12, 128.10, 127.94, 98.88, 86.37, 80.14, 75.34, 75.08, 72.64, 71.34, 71.16, 70.54, 69.53, 66.07, 63.82, 63.26, 62.95, 37.88, 29.84, 27.81, 21.16, 20.78, 14.23. HRMS $[M+Na]^+$ $C_{47}H_{51}N_3NaO_{13}S^+$ calcd. 920.3040, obsd. 920.3034

***p*-Tolyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl -2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside (22). 21** (0.290 g, 0.323 mmol) was dissolved in DMF (10 mL) along with benzyl bromide (116 µl, 0.969 mmol) and cooled to -40 °C. To this was added NaH (60% in mineral oil, 13 mg, 0.32 mmol) and the reaction was stirred for 30 min at -40 °C. The reaction was quenched by addition of sat. NH_4Cl solution then diluted with EtOAc and washed with water and sat. $NaHCO_3$. The mixture was then dried over Na_2SO_4 and concentrated. A silica gel column 2:1 hexane-EtOAc provided **22** (0.26 g, 0.26 mmol, 83% yield). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.15 (2 H, dd, *J* 7.4, 0.7), 7.49 (4 H, t, *J* 7.8), 7.43 – 7.22 (15 H, m), 7.14 (4 H, t, *J* 7.5), 5.57 (1 H, s), 5.39 (1 H, s), 4.99 (1 H, d, *J* 11.7), 4.97 – 4.93 (1 H, m), 4.78 (1 H, d, *J* 11.8), 4.73 (1 H, d, *J* 10.9), 4.57 (1 H, d, *J* 3.8), 4.50 (1 H, d, *J* 10.8), 4.42 (1 H, dd, *J* 11.5, 8.1), 4.33 (1 H, dd, *J* 11.6, 4.5), 4.29 (1 H, d, *J* 12.4), 4.26 – 4.20 (2 H, m), 4.17 (1 H, s), 3.98 – 3.92 (2 H, m), 3.64 (1 H, s), 3.56 (1 H, t, *J* 9.6), 3.38 (1 H, t, *J* 9.5), 3.29 (1 H, dd, *J* 10.2, 3.7), 2.72 (2 H, q, *J* 6.4), 2.59 (2 H, td, *J* 6.8, 2.6), 2.35 (3 H, s), 2.17 (3 H, s), 2.02 (3 H, s, *J* 0.8). δ_C (151 MHz, $CDCl_3$) 172.50, 165.85, 137.92, 137.62, 137.44, 133.40, 132.36, 132.11, 130.19, 130.02, 129.91, 128.82, 128.69, 128.65, 128.58, 128.48, 128.26, 128.17, 128.05, 99.38, 86.63, 80.94, 77.83, 76.17, 75.26, 75.17, 72.79, 70.57, 69.73, 66.24, 64.12, 63.98, 62.93, 38.07, 30.00, 28.01, 21.33, 20.95. gHSQCAD (without ^{1}H decoupling) $^1J_{C1'H1'}$ = 170.4Hz $^1J_{C1H1}$ = 168 Hz HRMS $[M+Na]^+$ $C_{54}H_{57}N_3NaO_{13}S^+$ calcd. 1010.3504, obsd. 1010.3507

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl 2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-**

levulinoyl-α-L-idopyranoside (23). Compound **23** was prepared by glycosylation of donor **18** (307 mg, 0.303 mmol) with *N*-(Benzyl)-benzyloxycarbonyl-3-amino-1-propanol (118 mg, 0.394 mmol) in 81% yield following the procedure for glycosylation. Purification was done by silica gel chromatography (2 fractions 2:1 hexane-EtOAC then 1:1 hexane-EtOAC) providing **23 (**290 mg, 0.244 mmol). ^{1}H-NMR δ_{H} (500 MHz, $CDCl_3$) 8.14 (2 H, d, *J* 7.2), 7.52 – 7.08 (23 H, m), 5.17 (2 H, d, *J* 8.5, CH_2-Cbz), 5.11 (1 H, s, H-2), 4.96 (1 H, d, H-1), 4.88 – 4.81 (1 H, m, Bn), 4.74 (1 H, d, *J* 11.4, Bn), 4.67 (1 H, d, *J* 3.5, H-1'), 4.56 – 4.40 (4 H, m, H-5, H-6a, CH_2-Bn), 4.40 – 4.34 (2 H, m, H-6a', Bn), 4.30 (1 H, s, H-6b), 4.14 (1 H, d, *J* 9.0, Bn), 4.11 – 4.04 (2 H, m, H-3, H-6a'), 3.89 – 3.75 (2 H, m, H-5', H-Linker), 3.68 (1 H, s, H-4), 3.57 – 3.46 (2 H, m, H-4', H-Linker), 3.45 – 3.32 (3 H, m, *J* 10.1, H-3', CH_2-Linker), 3.27 (1 H, dd, *J* 10.1, 3.5, H-2'), 2.74 (2 H, s, CH_2 Lev), 2.60 (2 H, d, *J* 16.9, CH_2 Lev), 2.17 (3 H, s, CH_3 Lev), 2.06 (3 H, s, Ac), 1.89 (2 H, d, *J* 21.0, CH_2-Linker), 0.90 (9 H, s, $(CH_3)_{3c}$Si), 0.01 (1 H, s, CH_3Si), -0.08 (2 H, s, CH_3Si). ^{13}C-NMR δ_{C} (125 MHz, $CDCl_3$) 206.17, 172.29, 170.65, 165.62, 156.65, 156.11, 137.96, 137.79, 137.64, 133.23, 129.96, 129.75, 128.51, 128.48, 128.33, 128.09, 128.06, 127.90, 127.85, 127.78, 127.30, 127.03, 98.60, 98.33, 80.50, 75.03, 74.63, 72.46, 72.29, 71.53, 71.08, 68.93, 67.13, 65.67, 64.49, 63.64, 62.99, 60.36, 50.77, 44.87, 43.86, 37.77, 29.79, 28.35, 27.77, 25.92, 21.04, 20.79, 18.00, 14.22, -3.77, -4.91. HRMS $[M+H]^+$ $C_{64}H_{79}N_4O_{16}Si^+$ calcd. 1187.5255, obsd. 1187.5254

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside (24).** Compound **24** was prepared from **23** (550 mg, 0.46 mmol) by the general procedure for TBS removal followed by silica gel chromatography (1:1 hexane-EtOAc) to yield 95% of compound **24** (470 mg, 0.44 mmol). ^{1}H-NMR δ_{H} (500 MHz, $CDCl_3$) 8.17 (2 H, d, *J* 7.1), 7.51 (1

H, t, *J* 7.3), 7.45 (2 H, t, *J* 7.5), 7.41 – 7.14 (20 H, m), 5.22 – 5.12 (3 H, m, H-2, CH_2-CBz), 4.96 (1 H, d, H-1), 4.85 (1 H, t, *J* 10.6, Bn), 4.77 – 4.72 (1 H, m, Bn), 4.71 (1 H, d, *J* 3.5, H-1'), 4.56 – 4.45 (4 H, m, H-6a, H-6a', 2Bn), 4.44 – 4,37(2 H, d, *J* 9.5, H-5, Bn), 4.32 – 4.22 (3 H, m, H-6b', H-6b, Bn), 4.10 (1 H, s, *J* 10.4, H-3), 3.86 (1 H, s, H-5'), 3.79 (1 H, s, H-Linker), 3.71 (1 H, s, H-4), 3.59 (1 H, t, *J* 9.5, H-3'), 3.56 – 3.32 (4 H, m, H-4', H-Linker, CH_2-Linker), 3.24 (1 H, dd, *J* 10.1, 3.5, H-2'), 2.73 (2 H, s, CH_2 Lev), 2.58 (2 H, d, *J* 13.9, CH_2 Lev), 2.17 (3 H, s, CH_3 Lev), 2.07 (3 H, s, Ac), 1.89 (2 H, d, *J* 23.2, CH_2-Linker). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 206.57, 172.45, 171.66, 165.63, 156.69, 156.14, 137.92, 137.78, 137.65, 136.74, 133.27, 129.89, 129.83, 128.61, 128.56, 128.53, 128.45, 128.33, 128.20, 128.10, 128.01, 127.92, 127.86, 127.78, 127.47, 127.29, 98.36, 98.27, 80.05, 75.14, 74.51, 72.43, 72.31, 71.24, 70.70, 68.80, 67.17, 65.61, 63.60, 63.21, 63.02, 60.40, 50.97, 50.78, 44.90, 43.89, 37.81, 30.89, 29.79, 28.37, 27.78, 21.04, 20.75, 14.21. gHMQC (without ^{1}H decoupling) $^1J_{C1'H1'}$ = 171.5 Hz $^1J_{C1H1}$ = 170 Hz. HRMS $[M+H]^+$ $C_{58}H_{65}N_4O_{16}^+$ calcd. 1073.4390, obsd. 1073.4394

***p*-Tolyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxybenzyl-1-thio-α-L-idopyranoside** (**25**). Compound **25** was prepared from donor **14** (57 mg, 0.10 mmol) and acceptor **6** (64 mg, 0.11 mmol) following the general for glycosylation. After a 6:1:1 hexane-DCM-EtOAc silica gel column this afforded **25** (40 mg, 38 µmol, 36% yield,). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.15 – 8.10 (2 H, m), 7.49 – 7.45 (4 H, m), 7.40 – 7.30 (12 H, m), 7.27 – 7.24 (4 H, m), 7.20 – 7.16 (2 H, m), 7.07 – 7.04 (2 H, m), 6.89 – 6.85 (2 H, m), 5.55 (1 H, dd, *J* 1.4, 0.6), 5.40 (1 H, t, *J* 2.2), 4.95 (1 H, d, *J* 11.8), 4.92 (1 H, dd, *J* 6.1, 3.8), 4.77 (1 H, d, *J* 4.7), 4.74 (1 H, d, *J* 3.8), 4.72 (1 H, d, *J* 3.6), 4.51 (3 H, t, *J* 5.4), 4.31 (1 H, d, *J* 10.5), 4.20 – 4.13 (4 H, m), 3.96 (1 H, dt, *J* 10.0, 3.4), 3.81 (3 H, s), 3.78 (2 H, d, *J* 6.3), 3.74 (1 H, t, *J* 2.8), 3.63 (1 H, dd, *J* 10.1, 8.9), 3.41 (1 H, dd, *J* 9.9, 9.0), 3.30 (1 H,

dd, *J* 10.2, 3.6), 2.33 (3 H, s), 2.01 (3 H, s). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 170.74, 165.88, 159.44, 137.76, 132.66, 130.43, 130.08, 129.86, 129.55, 129.26, 128.80, 128.73, 128.62, 128.32, 128.28, 128.18, 128.12, 125.53, 114.00, 98.77, 86.66, 80.92, 77.94, 77.50, 77.24, 76.99, 75.30, 75.27, 75.25, 73.23, 72.84, 72.37, 70.28, 69.25, 67.39, 64.27, 62.98, 55.49, 21.34, 21.02. $[M+H]^+$ $C_{57}H_{60}N_3O_{12}S^+$ calcd. 1010.3892, obsd. 1010.3889

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside (26).** Compound **26** was prepared by dissolving **24** (47 mg, 47 µmol) in DCM (5 mL) along with benzyl bromide (17 µl, 140 µmol). To this was added Ag_2O (88 mg, 380 µmol) and the reaction was protected from light and stirred overnight. The reaction was filtered through celite and evaporated. A silica gel column (3:2 hexane-EtOAc) provied **26** (39 mg, 33 µmol, 71% yield). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.17 – 8.13 (2 H, m), 7.46 – 7.13 (28 H, m), 5.16 (2 H, s, 2Bn), 5.11 (1 H, s, H-2), 4.93 (1 H, d, H-1), 4.85 (1 H, s)p, 4.77 (1 H, d, *J* 10.8, Bn), 4.75 – 4.70 (1 H, m), 4.65 (1 H, d, *J* 3.5, H-1'), 4.56 – 4.44 (4 H, m, Bn), 4.39 (2 H, d, *J* 10.5, Bn), 4.27 (3 H, m, H-6a'), 4.13 (1 H, d, *J* 10.4, Bn), 4.08 (1 H, s, H-3), 3.95 (1 H, ddd, *J* 9.7, 4.2, 1.8, H-5'), 3.79 (1 H, m), 3.66 (2 H, m, H-4), 3.58 (1 H, dd, *J* 11.5, 5.8), 3.51 – 3.45 (1 H, m), 3.44 – 3.38 (2 H, m, H-4'), 3.38 – 3.32 (1 H, m), 3.29 (1 H, dd, *J* 10.2, 3.7, H-2'), 2.71 (2 H, s, CH_2 Lev), 2.60 (2 H, s, CH_2 Lev), 2.16 (3 H, s, Lev CH_3), 2.03 (3 H, s, Ac), 1.94 – 1.81 (2 H, m, Linker CH_2). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 206.27, 172.30, 170.62, 165.64, 137.72, 137.44, 137.26, 133.21, 132.16, 131.93, 130.00, 129.84, 129.72, 128.63, 128.50, 128.46, 128.38, 128.29, 128.07, 127.97, 127.85, 99.19, 86.44, 80.75, 77.66, 75.97, 75.06, 74.97, 72.60, 71.42, 70.39, 69.55, 66.06, 63.94, 63.78, 62.75, 37.87, 29.80, 27.82, 21.13, 20.75, 14.23. HRMS $[M+Na]^+$ $C_{65}H_{70}N_4NaO_{16}^+$ calcd. 1185.4679, obsd. 1185.4670

***p*-Tolyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside (27).** Compound **27** was prepared in 63% yield from donor **22** (68 mg, 68 µmol) and acceptor **21** (81 mg, 90 µmol) using the general glycosylation procedure. A silica gel column (2:1:1 hexane-DCM-EtOAc) provided **27** (70 mg, 43 µmol). ^{1}H-NMR δ_{H} (500 MHz, $CDCl_3$) 8.20 – 8.15 (2 H, m), 8.14 – 8.09 (2 H, m), 7.54 – 7.24 (34 H, m), 7.16 (4 H, d, *J* 7.5), 5.60 (1 H, s, H-1A), 5.40 (1 H, d, *J* 1.6, H-2A), 5.17 – 5.12 (1 H, m, H-2C), 5.10 (1 H, d, *J* 3.5, H-1C), 5.00 (1 H, d, *J* 11.7, Bn), 4.97 – 4.94 (1 H, m, H-5A), 4.84 (1 H, d, *J* 11.3, Bn), 4.82 – 4.73 (3 H, m, H-1D, 2-Bn), 4.59 – 4.51 (4 H, m, H-1B, 3-Bn), 4.42 – 4.33 (5 H, m, H-5C, H-6aA, H-6aB, H-6aD, Bn), 4.32-4.25 (4 H, m, H-6bA, H-6bB, H-6bD, Bn), 4.20 – 4.13 (2 H, m, H-3A, H-6aC), 4.07 (2 H, dd, *J* 10.2, 4.1, H-3C, H-6bC), 3.87 (2 H, d, *J* 8.9, H-5B, H-5D), 3.79 – 3.67 (4 H, m, H-3B, H-3D, H-4C, Bn), 3.64 (1 H, s, H-4A), 3.54 – 3.43 (2 H, m, H-4B, H-4D), 3.32 – 3.26 (2 H, m, H-2B, H-2D), 2.79 – 2.43 (8 H, m, 4-CH_2 Lev), 2.37 (1 H, s, S-Ph-CH_3), 2.17 (1 H, s, CH_3 Lev), 2.12 (1 H, s, CH_3 Lev), 2.06 (1 H, s, Ac), 2.01 (1 H, s, Ac). ^{13}C-NMR δ_{C} (125 MHz, $CDCl_3$) 206.26, 206.24, 172.20, 170.66, 170.54, 165.69, 165.40, 137.74, 137.70, 137.52, 137.35, 137.25, 133.37, 133.30, 132.14, 131.91, 129.92, 129.84, 129.72, 129.61, 128.64, 128.57, 128.46, 128.40, 128.37, 128.27, 128.23, 128.18, 128.11, 128.06, 128.03, 127.98, 127.93, 127.56, 98.87, 98.77, 97.79, 86.40, 80.44, 79.15, 77.68, 75.69, 75.19, 74.82, 74.73, 74.25, 73.42, 72.62, 71.33, 70.25, 70.17, 69.57, 67.66, 66.01, 63.93, 63.71, 63.56, 62.57, 62.16, 60.39, 37.89, 37.75, 29.78, 29.77, 27.80, 27.77, 21.13, 20.74, 20.73. gHMQC (without ^{1}H decoupling) $^{1}J_{C1AH1A}$ = 169 Hz $^{1}J_{C1BH1B}$ = 171.5 Hz $^{1}J_{C1CH1C}$ = 160 Hz $^{1}J_{C1DH1D}$ = 175 Hz HRMS $[M+H]^+$ $C_{94}H_{101}N_6O_{26}S^+$ calcd. 1762.6514, obsd. 1762.6505

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside (28)** Compound **28** was prepared in 77% yield from donor **22** (89 mg, 89 µmol) and acceptor **24** (61 mg, 59 µmol). This furnished **28** (87 mg, 45 µmol) after a silica gel column (1 fraction 2:1:1 then 1:1:1 hexane-DCM-EtOAc). ^{1}H-NMR δ_H (600 MHz, $CDCl_3$) 8.13 (2 H, d, *J* 8.3), 8.08 (2 H, d, *J* 8.4), 7.49 – 7.11 (44 H, m), 5.15 (2 H, d, *J* 11.1), 5.12 (1 H, t, *J* 3.9), 5.08 (2 H, d, *J* 3.4), 4.92 (1 H, d br), 4.82 (2 H, d, *J* 11.3), 4.76 (2 H, dd, *J* 11.0, 3.3), 4.73-4.68 (2 H, m, *J* 3.6), 4.63 (2 H, d, *J* 9.6), 4.53 – 4.43 (4 H, m), 4.38 – 4.31 (5 H, m), 4.29 (1 H, d, *J* 10.6), 4.26 – 4.21 (3 H, m), 4.18 (1 H, s), 4.07 – 4.01 (3 H, m), 3.95 (1 H, d, *J* 10.1), 3.86 – 3.82 (2 H, m), 3.74 (1 H, s, CH-Linker), 3.69 (2 H, dt, *J* 11.8, 5.7), 3.66 (1 H, d, *J* 3.7), 3.62 (1 H, s), 3.57 (1 H, t, *J* 9.5), 3.43 (2 H, m, *J* 9.4, CH-Linker), 3.37 (2 H, d, Linker), 3.25 (2 H, dt, *J* 10.1, 3.1), 2.68 (2 H, s, Lev), 2.59 (2 H, t, *J* 6.7, Lev), 2.57 – 2.40 (4 H, m, Lev), 2.13 (3 H, s, CH_3 Lev), 2.10 (3 H, s, CH_3 Lev), 2.03 (3 H, s, Ac), 1.98 (3 H, s, Ac), 1.86 (2 H, d, Linker).^{13}C-NMR δ_C (150 MHz, $CDCl_3$) 206.46, 172.41, 170.86, 170.76, 165.64, 137.96, 137.71, 137.53, 133.51, 130.03, 130.01, 129.84, 128.84, 128.78, 128.73, 128.66, 128.64, 128.61, 128.53, 128.44, 128.39, 128.33, 128.27, 128.26, 128.19, 128.13, 128.05, 127.79, 127.50, 98.98, 98.51, 98.05, 80.67, 79.31, 77.87, 75.46, 75.39, 75.09, 75.03, 73.56, 72.47, 70.38, 67.68, 67.37, 65.62, 64.06, 63.93, 62.78, 62.47, 37.97, 30.00, 27.97, 20.97, 20.95. gHMQC (without ^{1}H decoupling) $^{1}J_{C1AH1A}$ = 174.6 Hz $^{1}J_{C1B*H1B*}$ = 169.8 Hz $^{1}J_{C1C*H1C*}$ = 176.4 Hz $^{1}J_{C1D*H1D*}$ = 172.2 Hz. HRMS $[M+H]^+$ $C_{105}H_{114}N_7O_{29}^+$ calcd. 1937.7689, obsd. 1937.7681

***p*-Tolyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-**

acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside (29). Compound **29** was synthesized according to the general procedure of glycosylation with donor **18** (200 mg, 197 µmol) and acceptor **21** (177 mg, 197 µmol) and purified by silica gel chromatography (1:1 hexane-EtOAc) providing **29** (285 mg, 160 µmol, 81% yield). ^{1}H-NMRδ_H (500 MHz, $CDCl_3$) 8.31 – 8.26 (2 H, m), 8.22 (2 H, t, *J* 9.0), 7.66 – 7.25 (32 H, m), 5.70 (1 H, s, H-1A), 5.51 (1 H, s, H-2A), 5.28 (1 H, t, *J* 4.1, H-2C), 5.21 (1 H, d, *J* 3.0, H-1C), 5.10 (1 H, d, *J* 11.7, Bn), 5.08 – 5.02 (1 H, m, H-5A), 4.95 (1 H, d, *J* 2.4, H-1D), 4.91 (3 H, t, *J* 11.0, 3Bn), 4.69 (1 H, d, *J* 3.4, H-1B), 4.65 (2 H, t, *J* 9.9, Bn), 4.58 – 4.51 (2 H, m, Bn), 4.50 – 4.42 (4 H, m, H-6aA), 4.42 – 4.34 (2 H, m, H-6bA, H-6aD), 4.30 – 4.27 (1 H, m, H-3A), 4.26 – 4.16 (3 H, m, H-3C, H-6bD), 3.98 (1 H, d, *J* 8.9, H-5B), 3.88 (3 H, m, *J* 10.3, Bn, H-5D), 3.81 (1 H, t, *J* 9.4, H-4B), 3.75 (1 H, s, H-4A), 3.71 (1 H, t, *J* 9.0, H-4D), 3.62 (2 H, dt, *J* 12.2, 9.5, H-3B, H-3D), 3.39 (2 H, td, *J* 9.8, 2.8, H-2B, H-2D), 2.90 – 2.60 (8 H, m, 4-CH_2 Lev), 2.48 (3 H, s, S-Ph-CH_3), 2.28 (3 H, s, CH_3 Lev), 2.26 (3 H, s, CH_3 Lev), 2.16 (3 H, s, Ac), 2.14 (3 H, s, Ac), 1.03 (9 H, s $(CH_3)_3Si$), 0.15 (3 H, s, CH_3Si), 0.08 (3 H, s, CH_3Si).^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 206.21, 172.17, 172.16, 170.64, 170.58, 165.66, 165.37, 137.82, 137.70, 137.32, 137.26, 133.38, 133.33, 132.15, 131.90, 129.90, 129.84, 129.71, 129.55, 128.66, 128.58, 128.45, 128.40, 128.29, 128.26, 128.15, 128.10, 128.05, 128.01, 127.98, 127.54, 127.41, 127.13, 98.94, 98.63, 97.72, 86.39, 80.29, 79.04, 75.77, 75.54, 74.78, 74.72, 74.57, 73.57, 72.62, 71.49, 71.41, 71.09, 70.78, 70.27, 69.55, 68.21, 66.02, 64.22, 63.91, 63.52, 62.85, 62.36, 62.01, 37.89, 37.79, 29.78, 29.77, 27.81, 25.93, 21.12, 20.74, 20.71, 18.04, -3.74, -4.94. gHMQC (without ^{1}H decoupling) $^{1}J_{C1AH1A}$ = 168 Hz $^{1}J_{C1BH1B}$ = 171.5 Hz $^{1}J_{C1CH1C}$ = 169.5 Hz $^{1}J_{C1DH1D}$ = 173 Hz. HRMS $[M+H]^+$ $C_{93}H_{109}N_6O_{26}SSi^+$ calcd. 1786.6910, obsd. 1786.6910

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside (30)** Compound **30** was synthesized in 72% yield from donor **29** (346 mg, 190 µmol) and acceptor **24** (208 mg, 190 µmol) following the general procedure for glycosylation and was purified by silica gel flash chromatography (1:1:1 hexane-DCM-EtOAc). This furnished **30** (384 mg, 137 µmol). ^{1}H-NMR δ_H (600 MHz, $CDCl_3$) 8.09 (2 H, d, *J* 7.1), 8.06 (4 H, ddd, *J* 8.5, 2.2, 1.3), 7.50 – 7.07 (49 H, m), 5.15 – 5.09 (4 H, m), 5.06 (1 H, d, *J* 3.9), 5.03 (1 H, s), 5.02 (1 H, d, *J* 4.0), 4.88 (1 H, d), 4.81 – 4.70 (8 H, m), 4.68 (1 H, s), 4.58 (2 H, dd, *J* 12.9, 7.0), 4.49 – 4.39 (3 H, m), 4.39 – 4.27 (9 H, m), 4.26 – 4.22 (2 H, m), 4.20 – 4.12 (4 H, m), 4.08 – 3.96 (5 H, m), 3.90 (1 H, d, *J* 10.2), 3.81 – 3.76 (1 H, m), 3.75 – 3.67 (5 H, m), 3.67 – 3.63 (2 H, m), 3.62 – 3.56 (2 H, m), 3.54 (1 H, d, *J* 9.6), 3.51 (1 H, d, *J* 10.1)3.44 (1 H, dd, *J* 10.0, 8.6), 3.41 – 3.26 (3 H, m), 3.24 – 3.19 (3 H, m), 2.69 – 2.34 (12 H, m, 4-CH_2 Lev), 2.10 (3 H, s, CH_3 Lev), 2.09 (3 H, s, CH_3 Lev), 2.07 (3 H, s, CH_3 Lev), 1.98 (3 H, s, Ac), 1.97 (3 H, s, Ac), 1.97 (3 H, s, Ac), 1.88 – 1.76 (2 H, m), 0.86 (9 H, s, $(CH_3)_3Si$), -0.03 (3 H, s, CH_3Si), -0.10 (3 H, s, CH_3Si). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 206.49, 172.47, 172.43, 172.39, 170.87, 170.82, 165.87, 165.67, 165.65, 138.11, 138.02, 137.91, 137.57, 137.48, 133.65, 130.07, 130.04, 129.78, 129.75, 128.87, 128.84, 128.75, 128.63, 128.59, 128.55, 128.39, 128.36, 128.34, 128.29, 128.25, 128.15, 128.08, 127.86, 127.78, 127.64, 127.52, 127.32, 98.84, 98.59, 98.06, 97.97, 80.56, 79.23, 78.99, 75.63, 75.25, 75.08, 75.00, 74.77, 74.48, 73.69, 72.46, 71.74, 71.32, 70.72, 70.40, 68.89, 68.17, 67.38, 65.55, 64.47, 64.04, 63.90, 63.62, 63.10, 62.40, 62.23, 38.02,

37.99, 30.03, 30.01, 28.04, 28.00, 26.17, 21.27, 21.00, 18.27, 14.47, -3.49, -4.69. gHMQC (without ^{1}H decoupling) $^{1}J_{C1AH1A}$ = 170.4 Hz $^{1}J_{C1B*H1B*}$ = 169.2 Hz $^{1}J_{C1C*H1C*}$ = 168.4 Hz $^{1}J_{C1D*H1D*}$ = 171.6 Hz $^{1}J_{C1E*H1E*}$ = 172.8 Hz $^{1}J_{C1F*H1F*}$ = 168.6 Hz. HRMS $[M+H]^{+}$ $C_{144}H_{165}N_{10}O_{42}Si^{+}$ calcd. 2735.0880, obsd. 2735.0883

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside (31)** Compound **31** was synthesized from donor **27** (105 mg, 59.6 µmol) and acceptor **24** (50 mg, 46 µmol). A 1:1:2 hexane-DCM-EtOAc column provided **31** (110 mg, 39 µmol, 85% yield). ^{1}H-NMR δ_{H} (600 MHz, $CDCl_3$) 8.11 (2 H, d, *J* 7.2), 8.08 (4 H, d, *J* 7.1), 7.51 – 7.10 (56 H, m), 5.17 – 5.10 (4 H, m, H-2C, H-2E, 2Bn), 5.07 (3 H, m, *J* 3.7, H-1C*, H-1E*, H-2A), 4.90 (1 H, m, H-1A), 4.83 – 4.71 (8 H, m, H-1F*), 4.70 (1 H, s), 4.67 (1 H, d, *J* 3.7, H-1D*), 4.60 (2 H, dd, *J* 10.7, 7.0, H-1B*, Bn), 4.52 – 4.42 (4 H, m), 4.37 – 4.29 (7 H, m), 4.27 (2 H, d, *J* 10.3), 4.24 – 4.14 (6 H, m, H-6bB, H-6aD), 4.02 (5 H, m, H-3C, H-3E, H-5F), 3.93 (1 H, d, *J* 10.1, Bn), 3.85 – 3.80 (2 H, m, H-5B, H-5D), 3.77 – 3.72 (3 H, m, H Linker, H-3F), 3.70 – 3.59 (6 H, m, H-3B, H-4C, H-3D, H-4E, H-4F), 3.54 (1 H, t, *J* 9.5, H-4B), 3.46 (1 H, s, H Linker), 3.44 – 3.40 (1 H, t, *J* 9.5, H-4D), 3.36 (2 H, m, CH_2 Linker), 3.25 (3 H, dt, *J* 10.1, 3.2, H-2B, H-2D, H-2F), 2.71 – 2.37 (12 H, m, 6 CH_2 Lev), 2.13 (6 H, s, CH_3 Lev), 2.09 (6 H, s, 2 CH_3 Lev), 2.01 (6 H, s, 2 Ac), 1.97 (3 H, s, Ac), 1.91-1.78 (2 H, m, CH_2 Linker).^{13}C-NMRδ_{C} (125 MHz, $CDCl_3$) 206.25, 206.22, 172.19, 172.14, 171.09, 170.63, 170.58, 170.53, 165.64, 165.45, 165.42, 137.82, 137.68, 137.51, 137.32, 137.27, 133.34, 129.81, 129.63, 129.54, 128.66, 128.62, 128.59, 128.57, 128.51,

128.45, 128.39, 128.34, 128.32, 128.22, 128.18, 128.12, 128.10, 128.08, 128.05, 128.03, 128.02, 128.00, 127.96, 127.92, 127.83, 127.62, 127.54, 127.30, 98.76, 98.37, 98.30, 97.90, 97.75, 80.48, 78.99, 78.85, 77.68, 75.42, 75.25, 75.18, 75.02, 74.87, 74.78, 74.60, 73.93, 73.50, 73.34, 72.27, 70.19, 70.12, 70.00, 68.77, 67.84, 67.44, 67.15, 65.43, 63.82, 63.74, 63.34, 62.57, 62.25, 61.99, 60.38, 37.80, 37.74, 29.77, 27.78, 27.76, 21.05, 20.74, 20.73, 14.22. gHMQC (without ^{1}H decoupling) $^{1}J_{C1AH1A}$ = 170 Hz $^{1}J_{C1B*H1B*}$ = 172 Hz $^{1}J_{C1C*H1C*}$ = 174 Hz $^{1}J_{C1D*H1D*}$ = 175.5 Hz $^{1}J_{C1E*H1E*}$ = 174 Hz$^{1}J_{C1F*H1F*}$ = 170 Hz. HRMS $[M+Na]^{+}$ $C_{145}H_{156}N_{10}NaO_{42}^{+}$ calcd. 2733.0304, obsd. 2733.0308

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside (32)** Compound **32** was prepared from **30** (210 mg, 76 μmol) by dissolving it in pyridine (5 mL) and cooling it to 0 °C. HF·pyridine (1.5 mL) was added dropwise to the reaction and it was allowed to warm to rt and stir overnight. The reaction was diluted with DCM and washed with sat. $CuSO_4$, 10% HCl, sat. $NaHCO_3$, dried over Na_2SO_4 and concentrated. A 1:1:3 hexane-DCM-EtOAc column provided **32** (160 mg, 61 μmol, 80% yield). ^{1}H-NMR δ_H (600 MHz, $CDCl_3$) 8.09 (6 H, m,), 7.54 – 7.07 (49 H, m), 5.12 (4 H, dt, *J* 8.0, 4.6), 5.05 (3 H, dd, *J* 12.0, 3.7), 4.89 (1 H, m), 4.84 – 4.65 (9 H, m), 4.59 (3 H, dd, *J* 12.0, 6.8), 4.50 – 4.39 (4 H, m), 4.37 – 4.24 (8 H, m), 4.23 – 4.09 (5 H, m), 4.06 – 3.97 (5 H, m), 3.92 (1 H, d, *J* 10.1), 3.81 (1 H, d, *J* 8.3), 3.79 – 3.70 (4 H, m), 3.70 – 3.65 (3 H, m), 3.63 – 3.57 (3 H, m), 3.53 (1 H, t, *J* 9.5), 3.50 – 3.26 (4 H, m), 3.23 (2 H, dt, *J* 10.0, 3.4), 3.19 (1 H, dd, *J* 10.1, 3.6), 2.99 (1 H, s, br), 2.70 – 2.35 (12 H, m),

2.11 (3 H, s), 2.10 (3 H, s), 2.08 (3 H, s), 2.02 (3 H, s,), 2.00 (6 H, s), 1.83 (2 H, d). ^{13}C-NMR δ_C (150 MHz, $CDCl_3$) 206.93, 206.50, 172.50, 172.42, 172.34, 171.89, 170.87, 170.84, 165.84, 165.68, 165.61, 138.04, 137.99, 137.93, 137.86, 137.51, 137.45, 133.63, 133.58, 133.51, 130.03, 129.99, 129.71, 128.83, 128.81, 128.78, 128.72, 128.71, 128.59, 128.59, 128.57, 128.55, 128.51, 128.33, 128.28, 128.27, 128.24, 128.20, 128.10, 128.03, 127.77, 127.73, 127.47, 98.65, 98.55, 98.48, 97.99, 97.93, 79.95, 79.18, 78.96, 77.47, 77.26, 77.05, 75.57, 75.40, 75.34, 75.18, 75.05, 74.82, 74.50, 74.30, 74.01, 73.68, 73.55, 72.45, 71.39, 70.87, 70.55, 70.49, 70.36, 70.29, 68.93, 67.80, 67.34, 65.59, 64.00, 63.86, 63.55, 63.26, 62.97, 62.53, 62.41, 62.31, 62.19, 38.02, 37.98, 30.00, 29.98, 27.96, 20.93. HRMS $[M+Na]^+$ $C_{138}H_{150}N_{10}NaO_{42}^+$ calcd. 2642.9835, obsd. 2642.9838

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-1-thio-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside (33).** Compound **33** was synthesized according the general procedure for glycosylation from donor **30** (68 mg, 38 µmol) and **32** (100 mg, 38 µmol)**.** After a silica gel chromatography (1:1:2 hexane-DCM-EtOAc) **33** (100 mg, 24 µmol, 61% yield) was isolated. ^{1}H-NMR δ_H (600 MHz, $CDCl_3$) 8.11 – 8.09 (2 H, m), 8.09 – 8.05 (8 H, m), 7.51 – 7.09 (75 H, m), 5.12 (6 H, dd, *J* 8.9, 4.4), 5.09 – 5.01 (5 H, m), 4.89 (1 H, m), 4.83 – 4.65 (17 H, m),

4.60 (1 H, d, *J* 3.8), 4.57 (1 H, d, *J* 10.2), 4.51 – 4.22 (20 H, m), 4.22 – 4.13 (8 H, m), 4.07 – 3.96 (10 H, m), 3.91 (1 H, d, *J* 10.3), 3.80 (1 H, d, *J* 8.5), 3.71 (8 H, m), 3.68 – 3.64 (4 H, m), 3.60 (4 H, m), 3.54 (2 H, m), 3.49 – 3.43 (1 H, m),p 3.44 – 3.27 (3 H, m), 3.26 – 3.20 (5 H, m), 2.70 – 2.35 (20 H, m), 2.11 (3 H, s), 2.10 (3 H, s), 2.07 (9 H, s), 1.99 (3 H, s, Ac), 1.98 (12 H, m, 3 Ac), 0.89 – 0.84 (9 H, s, $(CH_3)_3Si$), -0.01 (3 H, s, CH_3Si), -0.08 (3 H, s, CH_3Si). ^{13}C-NMRδ_C (150 MHz, $CDCl_3$) 208.90, 208.86, 174.80, 174.76, 173.71, 173.26, 173.22, 173.20, 168.26, 168.05, 140.47, 140.40, 140.29, 139.95, 139.89, 139.86, 136.03, 132.44, 132.15, 132.11, 131.62, 131.27, 131.22, 131.13, 131.02, 131.00, 130.98, 130.95, 130.93, 130.77, 130.72, 130.68, 130.66, 130.53, 130.45, 130.24, 130.20, 130.15, 130.03, 129.90, 129.72, 101.21, 101.00, 100.45, 100.41, 100.35, 82.94, 81.60, 81.37, 78.05, 77.99, 77.63, 77.47, 77.39, 77.17, 77.12, 76.99, 76.11, 75.11, 74.87, 74.12, 73.69, 73.18, 73.03, 72.76, 71.35, 70.63, 70.45, 69.76, 68.01, 66.85, 66.42, 66.25, 65.96, 65.47, 64.81, 64.73, 64.56, 62.99, 40.41, 40.39, 32.41, 32.39, 30.42, 30.38, 28.54, 23.66, 23.38, 23.35, 23.34, 20.65, 16.84, -1.12, -2.32. $^1J_{C1AH1A}$ = 172.8 Hz $^1J_{C1B*H1B*}$ = 173.4 Hz $^1J_{C1C*H1C*}$ = 169.8 Hz $^1J_{C1D*H1D*}$ = 172.2 Hz $^1J_{C1E*H1E*}$ = 170.4 Hz $^1J_{C1F*H1F*}$ = 175.2 Hz $^1J_{C1G*H1g*}$ = 170.4 Hz $^1J_{C1H*H1H*}$ = 175.2 Hz $^1J_{C1I*H1I*}$ = 170.4 Hz $^1J_{C1J*H1J*}$ = 175.2 Hz [Maldi-TOF] $[M+K]^+$ $C_{224}H_{250}KN_{16}O_{68}Si^+$ calcd. 4320.61, obsd. 4322.18

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside (34).**

Compound **34** was prepared by treating compound **26** (70 mg, 59 µmol) with the procedure for levulinoyl ester removal. After purification by a 2:1 hexane-EtOAc silica gel column, **34** was isolated (53 mg, 51 µmol, 86% yield). ^{1}H-NMRδ_H (500 MHz, $CDCl_3$) 8.18 – 8.15 (2 H, m), 7.46 – 7.23 (26 H, m), 7.19 – 7.14 (2 H, m), 5.23 – 5.06 (3 H, m), 5.00 – 4.82 (2 H, m), 4.78 (1 H, d, *J* 10.9), 4.73 (1 H, d, *J* 11.4), 4.62 (1 H, d, *J* 18.0), 4.55 – 4.35 (5 H, m), 4.31 (1 H, dd, *J* 12.0, 2.1),

4.26 – 4.14 (1 H, m), 4.13 – 4.04 (2 H, m), 3.99 (1 H, s), 3.92 – 3.62 (5 H, m), 3.61 – 3.23 (5 H, m), 2.55 (1 H, s), 2.06 (3 H, s, *J* 4.0), 1.97 – 1.80 (2 H, m).^{13}C-NMRδ_C (125 MHz, $CDCl_3$) 170.81, 165.80, 138.04, 137.67, 133.38, 130.32, 130.03, 128.78, 128.70, 128.63, 128.51, 128.34, 128.24, 128.23, 128.18, 128.12, 128.10, 128.08, 128.06, 128.04, 127.55, 98.68, 81.03, 78.11, 75.36, 75.34, 72.29, 70.49, 67.50, 64.08, 63.28, 20.97.HRMS $[M+H]^+$ $C_{60}H_{65}N_4O_{14}^+$ calcd. 1065.4492, obsd. 1065.4484.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside** (**35**) Compound **28** (75 mg, 39 μmol) was treated according to the procedure to remove levulinoyl protecting groups to furnish **35** (59 mg, 34 μmol, 88% yield) after silica gel chromatography (1:1:2 hexane-DCM-EtOAc). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.09 (4 H, ddd, *J* 8.6, 5.8, 4.0), 7.44 – 7.16 (38 H, m), 7.16 – 7.10 (3 H, m), 5.18 – 5.04 (4 H, m), 5.01 (1 H, d, *J* 1.9), 4.99 – 4.89 (1 H, m), 4.88 – 4.79 (2 H, m), 4.73 (2 H, d, *J* 11.2), 4.71 – 4.65 (2 H, m), 4.59 – 4.52 (1 H, m), 4.51 – 4.41 (4 H, m), 4.41 – 4.24 (4 H, m), 4.23 – 4.07 (5 H, m), 4.04 (2 H, t, *J* 3.2), 3.93 (2 H, s), 3.82 (2 H, ddd, *J* 10.1, 4.5, 2.3), 3.79 – 3.69 (3 H, m), 3.65 (2 H, dd, *J* 10.0, 9.0), 3.60 – 3.35 (6 H, m), 3.35 – 3.18 (4 H, m), 2.53 (1 H, s), 2.01 (3 H, s), 1.98 (3 H, s), 1.93 – 1.78 (2 H, m).^{13}C-NMRδ_C (125 MHz, $CDCl_3$) 170.57, 170.47, 165.78, 165.70, 137.82, 137.75, 137.49, 137.48, 137.36, 133.31, 133.16, 130.04, 129.84, 129.72, 129.67, 128.61, 128.59, 128.56, 128.56, 128.51, 128.48, 128.44, 128.33, 128.31, 128.16, 128.16, 128.12, 128.09, 127.97, 127.94, 127.92, 127.83, 127.34, 98.15, 97.92, 80.61, 79.34, 77.73, 75.19, 75.15, 75.00, 74.00, 72.93, 72.09, 70.32, 69.97, 67.30, 64.10, 63.77, 62.74, 62.65, 61.21, 20.77, 20.75.HRMS $[M+H]^+$ $C_{95}H_{102}N_7O_{25}^+$ calcd. 1741.6953, obsd. 1741.6951.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside** (**36**) The levulinoyl esters of **31** (175 mg, 64.6 μmol) were removed according to the general procedure, forming **36** (135 mg, 55.9 μmol , 87% yield) after silica gel chromatography (1:1:2 hexane-DCM-EtOAc).^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.13 – 8.05 (6 H, m), 7.44 – 7.09 (54 H, m), 5.12 (3 H, dd, *J* 8.2, 5.5), 5.09 (2 H, d, *J* 2.5), 5.04 – 4.89 (3 H, m), 4.85 (3 H, m), 4.76 – 4.62 (6 H, m), 4.60 – 4.51 (1 H, m), 4.46 (5 H, m), 4.40 – 4.28 (3 H, m), 4.27 – 4.06 (9 H, m), 4.06 – 3.99 (3 H, m), 3.93 (2 H, s), 3.88 – 3.69 (7 H, m), 3.68 – 3.61 (2 H, m), 3.60 – 3.50 (5 H, m), 3.50 – 3.42 (3 H, m), 3.40 – 3.36 (1 H, m), 3.35 – 3.15 (6 H, m), 2.53 (1 H, s), 2.00 (6 H, s, *J* 0.5), 1.97 (3 H, s), 1.93 – 1.77 (2 H, m). ^{13}C-NMRδ_C (125 MHz, $CDCl_3$) 170.56, 170.47, 165.79, 165.73, 137.82, 137.48, 137.45, 137.41, 137.35, 133.37, 133.30, 133.17, 130.04, 129.82, 129.80, 129.71, 129.70, 129.61, 128.60, 128.56, 128.55, 128.50, 128.47, 128.45, 128.43, 128.41, 128.40, 128.39, 128.37, 128.33, 128.31, 128.20, 128.18, 128.15, 128.14, 128.12, 128.10, 128.02, 128.00, 127.97, 127.95, 127.93, 127.93, 127.91, 127.90, 127.89, 127.83, 127.80, 127.33, 98.15, 97.91, 97.84, 97.31, 80.63, 79.35, 79.23, 77.73, 75.17, 75.15, 73.98, 73.75, 73.22, 73.14, 72.94, 72.85, 72.09, 70.02, 69.96, 69.04, 67.71, 67.29, 64.11, 63.98, 63.78, 62.73, 62.65, 62.36, 61.25, 61.15, 21.05, 20.81, 20.76.HRMS $[M+H]^+$ $C_{130}H_{139}N_{10}O_{36}^+$ calcd. 2416.9382, obsd. 2416.9380.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-**

***O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside** (**37**). Treating **30** (300 mg, 110 μmol) to remove levulinoyl esters using the general procedure provided **37** (239 mg, 95.7 μmol, 87% yield) after silica gel chromatography (1 fraction 1:1:2 hexane-DCM-EtOAC then 1:1:3 hexane-DCM-EtOAC). ^{1}H-NMR δ_{H} (600 MHz, $CDCl_3$) 8.15 – 8.09 (6 H, m), 7.48 – 7.12 (49 H, m), 5.19 – 5.12 (4 H, m), 5.09 (1 H, d, *J* 14.8), 5.07 – 5.04 (2 H, m), 5.03 – 4.91 (1 H, m), 4.90 – 4.80 (3 H, m), 4.72 (5 H, ddd, *J* 12.7, 11.5, 7.3), 4.57 (1 H, d, *J* 21.3), 4.53 – 4.27 (9 H, m), 4.27 – 4.19 (3 H, m), 4.19 – 4.13 (3 H, m), 4.07 – 4.03 (3 H, m), 4.02 – 3.73 (9 H, m), 3.72 – 3.47 (11 H, m), 3.45 – 3.40 (1 H, m), 3.29 (4 H, ddd, *J* 36.3, 10.2, 3.7), 3.23 (2 H, dd, *J* 11.3, 5.3), 2.03 (3 H, s), 2.02 (3 H, s), 2.01 (3 H, s), 1.95 – 1.80 (2 H, m), 0.87 (9 H, s), -0.01 (3 H, s), -0.09 (3 H, s). ^{13}C-NMRδ_{C} (150 MHz, $CDCl_3$) 170.85, 170.68, 165.99, 165.93, 138.03, 137.99, 137.97, 137.63, 137.61, 137.59, 133.57, 133.39, 133.38, 130.26, 130.01, 129.97, 129.93, 129.89, 129.87, 129.82, 128.82, 128.77, 128.74, 128.73, 128.71, 128.67, 128.63, 128.59, 128.57, 128.51, 128.47, 128.46, 128.46, 128.44, 128.42, 128.39, 128.34, 128.31, 128.27, 128.27, 128.23, 128.23, 128.18, 128.15, 128.11, 128.07, 128.02, 127.60, 127.58, 127.55, 127.54, 127.53, 127.51, 127.49, 127.48, 127.47, 127.45, 127.26, 99.14, 98.69, 98.22, 98.09, 97.56, 80.68, 79.55, 79.54, 79.45, 75.35, 75.24, 74.95, 74.20, 74.04, 73.57, 73.42, 73.40, 73.13, 72.29, 71.55, 71.26, 70.55, 70.23, 69.50, 69.08, 68.25, 68.03, 67.50, 66.42, 65.69, 64.53, 64.34, 64.34, 64.32, 64.17, 63.17, 62.87, 62.61, 62.09, 61.53, 61.35, 60.60, , 26.12, 21.26, 21.03, 21.01, 20.97, 18.21, 14.44, -3.53, -4.70. HRMS $[M+H]^+$ $C_{129}H_{147}N_{10}O_{36}Si^+$ calcd. 2440.9777, obsd. 2440.9768.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-**

***O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-a-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside** (**38**). **33** (0.14 g, 34 µmol) was treated with the general procedure for levulinoyl ester removal providing **38** (0.10 g, 27 µmol, 79% yield) after a 1:1:3 hexane-DCM-EtOAc silica gel column. ^{1}H-NMR δ_{H} (500 MHz, $CDCl_3$) 8.17 – 8.10 (10 H, m), 7.52 – 7.13 (75 H, m), 5.22 – 5.10 (7 H, m), 5.09 – 5.02 (4 H, m), 4.94 – 4.86 (5 H, m), 4.81 – 4.71 (7 H, m), 4.71 – 4.67 (2 H, m), 4.63 – 4.58 (1 H, m), 4.55 – 4.44 (6 H, m), 4.40 – 4.13 (20 H, m), 4.10 – 3.96 (8 H, m), 3.85 – 3.75 (9 H, m), 3.75 – 3.70 (2 H, m), 3.65 – 3.42 (16 H, m), 3.38 – 3.32 (5 H, m), 3.29 (2 H, dd, *J* 10.1, 3.8), 3.26 – 3.18 (4 H, m), 2.07 (3 H, s), 2.06 (3 H, s), 2.05 (3 H, s), 2.04 (3 H, s), 2.04 (3 H, s), 1.94 – 1.83 (2 H, m), 0.90 (9 H, s), 0.02 (3 H, s), -0.06 (3 H, s). ^{13}C-NMRδ_{C} (125 MHz, $CDCl_3$) 170.61, 170.43, 165.82, 165.81, 165.78, 165.71, 137.83, 137.82, 137.76, 137.45, 137.42, 137.41, 137.38, 137.36, 133.34, 133.17, 130.05, 129.80, 129.78, 129.71, 129.68, 129.67, 129.66, 129.64, 129.62, 128.57, 128.55, 128.50, 128.47, 128.45, 128.40, 128.39, 128.36, 128.33, 128.30, 128.20, 128.16, 128.12, 128.08, 128.05, 128.03, 128.01, 127.96, 127.93, 127.87, 127.83, 127.37, 127.32, 127.29, 127.29, 127.05, 97.99, 97.93, 97.92, 97.91, 97.85, 97.33, 80.46, 79.34, 79.26, 75.12, 75.04, 75.03, 74.73, 73.99, 73.81, 73.71, 73.69, 73.68, 73.67, 73.18, 73.15, 73.14, 73.13, 73.12, 72.92, 72.86, 72.10, 71.35, 71.05, 70.37, 70.07, 70.03, 69.32, 69.31, 69.31, 69.29, 69.13, 69.13, 67.62, 67.61, 67.60, 67.60, 67.29, 64.32, 64.01, 62.95, 62.65, 62.37, 61.32, 61.20, 61.16, 29.70, 25.90, 20.80, 20.77, 20.74, 18.00, -3.76, -4.93.MALDI $[M+Na]^+$ $C_{199}H_{220}N_{16}NaO_{58}Si^+$ calcd. 3814.45, obsd. 3815.53.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate** (**39**) Compound **34** (27 mg, 25 µmol) was oxidized and protected as a benzyl ester according to the procedures for oxidation and benzyl ester formation furnishing **39** (21 mg, 18 µmol, 72% yield) after silica gel chromatography (2:1 hexane-EtOAc). ^{1}H-NMRδ_H (500 MHz, $CDCl_3$) 8.11 (2 H, d, *J* 7.8), 7.57 – 7.03 (33 H, m), 5.33 – 5.30 (1 H, m, Bn), 5.28 (1 H, d, *J* 12.2, Bn), 5.21 – 5.08 (5 H, m, H-1, Bn), 4.91 – 4.78 (2 H, m, H-4), 4.78 – 4.68 (3 H, m, H-1'), 4.55 (1 H, d, *J* 10.7, Bn), 4.45 (3 H, dd, *J* 12.3, 1.9, H-6a'), 4.28 (1 H, d, *J* 12.4, H-6b'), 4.16 (1 H, s, H-2), 4.14 – 3.99 (3 H, m, H-3, H-5'), 3.79 (1 H, d, H Linker), 3.60 – 3.54 (1 H, m, H-3'), 3.53 – 3.44 (2 H, m, H-4', H Linker), 3.33 (2 H, d, *J* 27.1, CH_2 Linker), 3.19 (1 H, dd, *J* 10.2, 3.4, H-2'), 2.01 (3 H, s, *J* 1.3, Ac), 1.83 (2 H, d, *J* 29.8, CH_2 Linker). HRMS $[M+H]^+$ $C_{67}H_{69}N_4O_{15}^+$ calcd. 1169.4754, obsd. 1169.4760

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate** (**40**). Compound **40** was prepared from **35** (59 mg, 34 µmol) by using the general procedures for oxidation and benzyl ester formation and was isolated after silica gel chromatography (3:2 hexane-EtOAc). **40** (49 mg, 25 µmol, 74%) ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.16 – 8.10 (4 H, m), 7.57 – 7.09 (51 H, m), 5.55 (1 H, d, *J* 5.0), 5.21 (1 H, t, *J* 5.2), 5.17 – 5.13 (4 H, m), 5.11 – 5.03 (4 H, m), 4.93 (1 H, d, *J* 3.6), 4.89 – 4.80 (2 H, m), 4.80 – 4.68 (5 H, m), 4.66 (1 H, d, *J* 4.4), 4.63 (1 H, d, *J* 3.6), 4.55 (1 H, d, *J* 10.7), 4.45 (1 H, s), 4.43 – 4.37 (3 H, m), 4.37 – 4.30 (4 H, m), 4.25 (1 H, dd, *J* 12.2, 3.1), 4.17 – 4.11 (2 H, m), 4.05 (1 H, dd, *J* 5.8, 4.6), 3.97 (3 H, m), 3.84 – 3.79 (1 H, m), 3.71 (1

H, d, *J* 10.1), 3.63 – 3.58 (1 H, m), 3.54 – 3.44 (3 H, m), 3.37 – 3.25 (2 H, m), 3.21 (1 H, dd, *J* 10.2, 3.6), 3.17 (1 H, dd, *J* 10.2, 3.6), 2.11 (3 H, s), 1.99 (3 H, s), 1.84 (2 H, m). HRMS $[M+H]^+$ $C_{109}H_{110}N_7O_{27}^+$ calcd. 1949.7478, obsd. 1949.7470

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate** (**41**) Compound **36** (63 mg, 26 μmol) was oxidized and resulting carboxylates protected as benzyl esters according to general procedures to provide **41** (43 mg, 23 μmol, 90% yield) after silica gel purification (3:2 hexane-EtOAc) (**39**) ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.19 – 8.07 (6 H, m), 7.59 – 7.05 (69 H, m), 5.54 (1 H, d, *J* 4.9), 5.49 (1 H, d, *J* 5.3), 5.20 (2 H, dd, *J* 10.9, 5.4), 5.13 (4 H, dd, *J* 12.2, 2.8), 5.06 (4 H, dd, *J* 14.6, 12.4), 5.00 (2 H, s), 4.93 (1 H, d, *J* 3.5), 4.86 (1 H, d, *J* 3.8), 4.84 – 4.75 (5 H, m), 4.73 (3 H, d, *J* 13.6), 4.69 – 4.61 (4 H, m), 4.55 (1 H, d, *J* 10.7), 4.48 (1 H, d, *J* 4.9), 4.43 (2 H, d, *J* 12.8), 4.36 (3 H, t, *J* 9.6), 4.31 (4 H, dd, *J* 10.5, 4.4), 4.24 (2 H, dd, *J* 12.5, 3.7), 4.20 – 4.08 (5 H, m), 4.07 – 4.04 (1 H, m), 4.00 – 3.94 (3 H, m), 3.91 (1 H, d, *J* 10.0), 3.86 (2 H, d, *J* 5.1), 3.79 – 3.74 (1 H, m), 3.69 (2 H, d, *J* 9.9), 3.61 (1 H, t, *J* 9.5), 3.55 – 3.48 (2 H, m), 3.44 – 3.38 (2 H, m), 3.38 – 3.25 (2 H, m), 3.21 (2 H, ddd, *J* 10.1, 6.4, 3.7), 3.13 (1 H, dd, *J* 10.2, 3.6), 2.09 (3 H, s, *J* 6.9), 2.04 (3 H, s), 1.98 (3 H, s, *J* 6.4), 1.86 – 1.73 (2 H, m). MALDI $[M+Li]^+$ $C_{151}H_{150}LiN_{10}O_{39}^+$ calcd. 2735.03, obsd. 2735.36

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-**

α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate (42) Compound **37** (27 mg, 11 μmol) was oxidized by TEMPO/BAIB and the resulting carboxylates were protected as benzyl esters according to the general procedures to produce compound **42** (27 mg, 10 μmol, 90% yield) after silica gel chromatography (9:1 toluene-acetone). (**42**)^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.30 – 8.22 (6 H, m), 7.74 – 7.23 (64 H, m), 5.69 (1 H, d, *J* 5.3), 5.62 (1 H, d, *J* 5.4), 5.39 – 5.32 (3 H, m), 5.31 – 5.11 (10 H, m), 5.03 – 4.77 (11 H, m), 4.63 – 4.36 (11 H, m), 4.34 – 4.30 (2 H, m), 4.27 – 4.20 (4 H, m), 4.10 (2 H, t, *J* 5.6), 4.05 (1 H, d, *J* 9.9), 4.02 – 3.98 (3 H, m), 3.93 – 3.81 (3 H, m), 3.78 (1 H, t, *J* 9.1), 3.66 – 3.61 (1 H, m), 3.61 – 3.53 (3 H, m), 3.53 – 3.41 (2 H, m), 3.38 (1 H, dd, *J* 10.2, 3.7), 3.33 (1 H, dd, *J* 10.2, 3.7), 3.28 (1 H, dd, *J* 10.2, 3.6), 2.20 (4 H, s,), 2.18 (3 H, s,), 2.14 (3 H, s), 2.00 – 1.87 (2 H, m), 1.04 (9 H, s), 0.17 (3 H, s), 0.10 (3 H, s). MALDI $[M+Na]^+$ $C_{129}H_{146}N_{10}NaO_{36}Si^+$ calcd. 2775.04, obsd. 2775.26.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate (43)** Compound **38** (72 mg, 19 μmol) was treated with the

general procedure for TEMPO/BAIB oxidation followed by benzyl ester formation providing **43** (57 mg, 13 µmol, 70% yield) after a silica gel column (1 fraction 3:2 then 1:1 hexane-EtOAc). ^{1}H-NMR δ_{H} (600 MHz, $CDCl_3$) 8.12 – 8.03 (10 H, m), 7.55 – 7.03 (100 H, m), 5.50 (1 H, d, *J* 5.5), 5.42 (3 H, dd, *J* 11.0, 5.5), 5.20 – 4.91 (20 H, m), 4.83 – 4.78 (3 H, m), 4.77 – 4.57 (15 H, m), 4.53 (2 H, t, *J* 11.0), 4.44 (2 H, dd, *J* 7.2, 5.1), 4.42 – 4.34 (6 H, m), 4.33 – 4.17 (7 H, m), 4.16 – 4.11 (4 H, m), 4.09 (1 H, s), 4.08 – 4.01 (7 H, m), 3.93 – 3.88 (4 H, m), 3.87 – 3.69 (10 H, m), 3.66 (1 H, d, *J* 10.7), 3.60 (1 H, t, *J* 9.0), 3.47 – 3.34 (6 H, m), 3.32 – 3.22 (1 H, m), 3.20 (1 H, dd, *J* 10.3, 3.5), 3.17 – 3.06 (3 H, m), 2.01 (3 H, s), 1.98 (3 H, s), 1.96 (9 H, s), 1.80 – 1.70 (2 H, m), 0.85 (9 H, s), -0.02 (3 H, s), -0.09 (3 H, s). MALDI $[M+Na]^+$ $C_{234}H_{240}N_{16}NaO_{63}Si^+$ calcd. 4334.5797, obsd. 4335.8.

3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate salt (**44**) Treatment of **39** (21 mg, 18µmol) sequentially with the general procedures for saponification, Staudinger reduction, O-sulfation, N-sulfation, and finally global debenzylation provided **44** (9 mg, 14 µmol, 77% over 5 steps). ^{1}H-NMR δ_{H} (600 MHz, D_2O) 5.29 (1 H, d, *J* 3.6), 4.99 (1 H, d, *J* 3.1), 4.37 (1 H, d, *J* 2.8), 4.24 – 4.20 (1 H, m), 4.16 – 4.11 (1 H, m), 4.11 – 4.05 (2 H, m), 4.00 – 3.95 (1 H, m), 3.88 – 3.76 (2 H, m), 3.62 – 3.55 (1 H, m), 3.52 (1 H, dd, *J* 10.1, 9.3), 3.44 (1 H, dd, *J* 10.1, 9.3), 3.13 (1 H, dd, *J* 10.3, 3.5), 3.06 – 3.02 (2 H, m), 1.91 – 1.85 (2 H, m). HRMS $[M]^+$ $C_{15}H_{25}N_2O_{20}Na_4S_3^+$ calcd. 740.9748 obsd. 740.9734

3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate salt (45) Compound **45** was prepared from **40** (10 mg, 3.7 µmol) by treating it with the general procedures for saponification,

Staudinger reduction, O-sulfation, N-sulfation, and finally global debenzylation. These provided **45** (3 mg, 2.3 µmol, 63% over three steps). ^{1}H-NMR δ_H (500 MHz, D_2O) 5.35 (1 H, d, *J* 3.1), 5.28 (1 H, d, *J* 3.2), 5.09 (1 H, s), 4.92 (1 H, m), 4.38 (1 H, d, *J* 3.0), 4.29 – 4.18 (3 H, m), 4.18 – 4.13 (2 H, m), 4.06 (3 H, m), 4.03 – 3.95 (2 H, m), 3.95 – 3.81 (3 H, m), 3.73 – 3.56 (3 H, m), 3.55 – 3.50 (1 H, m), 3.45 (1 H, t, *J* 9.4), 3.23 (1 H, d, *J* 0.7), 3.16 (1 H, dd, *J* 10.5, 2.7), 3.12 (1 H, dd, *J* 9.8, 3.8), 3.09 – 3.00 (2 H, m), 1.92 – 1.84 (3 H, m). HRMS $[M]^{2-}$ $C_{27}H_{41}N_3O_{39}Na_3S_6^{3-}$ calcd. 430.6450 obsd. 430.6460

3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate salt (**46**). Compound **46** was prepared from **57** in three steps. Simultaneous O,N sulfation was performed on **57** (5 mg, 2.5 µmol) by dissolving it in pyridine (1 mL) and adding washed SO_3·pyridine (100 mg, 0.63 mol). The reaction was heated to 55 °C and stirred for 24 hours. The reaction was allowed to cool to rt and was quenched by addition of TEA (200 µL) and MeOH (400 µL) and allowed to stir for 1 hour then diluted with 1:1 DCM-MeOH and eluted from a Sephadex LH-20 column to remove pyridine. The crude product was then purified by Prep-TLC (3:1:1 EtOAc-MeOH-H_2O with 1% AcOH). The product was then treated with the conditions for global debenzylation. Briefly, it was dissolved in MeOH (1 mL) and water (0.5 mL) to which was added $Pd(OH)_2$ (45 mg, 0.32mol) and it was stirred under an H_2 atmosphere for 24 hours. After workup the methyl esters were saponified providing **46** (3 mg, 1.7 µmol, 66% yield over three steps.). (**46**)^{1}H-NMR δ_H (500 MHz, D_2O) 5.38 – 5.26 (2 H, m), 5.21 (1 H, d, *J* 3.3), 5.13 (1 H, d, *J* 3.7), 5.10 (1 H, s), 5.07 – 5.02 (1 H, m), 4.83 (1 H, d, *J* 3.1),

4.38 (1 H, m), 4.29 – 4.16 (8 H, m), 4.14 – 4.11 (1 H, m), 4.08 (1 H, s), 4.04 (1 H, s), 3.88 – 3.83 (1 H, m), 3.80 – 3.73 (7 H, m), 3.72 – 3.65 (2 H, m), 3.61 (3 H, t, *J* 9.4), 3.55 – 3.50 (2 H, m), 3.49 – 3.43 (2 H, m), 3.16 (2 H, dd, *J* 10.3, 3.2), 3.12 (1 H, dd, *J* 10.2, 3.3), 3.09 – 3.05 (1 H, m), 1.97 – 1.86 (2 H, m). HRMS $[M]^{4-}$ $C_{39}H_{60}N_4Na_2O_{58}S_9^{4-}$ calcd. 461.4793, obsd. 461.4626.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate (47)** Compound **47** was prepared from **37** (30 mg, 12 μmol) using the general procedure for oxidation and methyl ester formation. **47** (24 mg, 9.2 μmol, 77%) after silica gel chromatography (3:2 hexane-EtOAc). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.21 – 8.07 (6 H, m), 7.61 – 7.05 (45 H, m), 5.57 (1 H, d, *J* 5.9), 5.47 (1 H, d, *J* 5.7), 5.24 (1 H, t, *J* 6.3), 5.19 (1 H, t, *J* 6.1), 5.15 (2 H, s), 5.07 (3 H, d, *J* 3.5), 4.92 (1 H, d, *J* 3.6), 4.87 – 4.76 (5 H, m), 4.73 (4 H, dd, *J* 10.8, 7.3), 4.69 – 4.60 (4 H, m), 4.51 – 4.33 (7 H, m), 4.27 (3 H, m), 4.19 – 4.13 (2 H, m), 4.12 – 4.05 (4 H, m), 3.98 (1 H, s), 3.96 – 3.92 (1 H, m), 3.90 (2 H, d, *J* 9.1), 3.87 – 3.83 (1 H, m), 3.79 (4 H, dd, *J* 10.7, 7.4), 3.71 (3 H, d, *J* 5.4), 3.70 – 3.65 (1 H, m), 3.61 – 3.55 (4 H, m), 3.53 – 3.49 (1 H, m), 3.47 – 3.41 (4 H, m), 3.33 – 3.26 (3 H, m), 3.21 (1 H, dd, *J* 10.2, 3.5), 2.12 (3 H, s), 2.11 (3 H, s), 2.03 (3 H, s), 1.82 (2 H, m), 0.92 (9 H, s), 0.03 (3 H, s), -0.00 (3 H, s). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 170.67, 170.64, 170.56, 169.71, 169.54, 165.55, 165.16, 165.13, 137.78, 137.67, 137.34, 137.30, 133.70, 133.57, 133.45, 129.99, 129.90, 129.85, 129.19, 129.08, 128.78, 128.72, 128.52, 128.45, 128.36, 128.32, 128.25, 128.01, 127.98, 127.92, 127.88, 127.84, 127.76, 127.71, 127.56, 127.45, 127.16, 99.00, 98.72, 98.66, 98.12, 98.00, 80.20, 78.23,

78.06, 76.48, 76.19, 75.96, 74.95, 74.89, 74.71, 74.58, 74.47, 74.37, 74.12, 72.64, 72.23, 71.95, 71.60, 71.39, 71.22, 71.09, 70.64, 69.74, 69.60, 67.95, 67.46, 67.18, 63.85, 63.35, 63.02, 62.38, 61.63, 52.11, 51.90, 51.69, 25.87, 20.81, 20.78, 17.98, -3.68, -5.07. HRMS $[M+H]^+$ $C_{132}H_{147}N_{10}O_{39}Si^+$ calcd. 2524.9624, obsd. 2524.9628.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate (48)**. Compound **47** (24 mg, 9.5 µmol) was dissolved in dry MeOH (2 mL dried over 4Å sieves overnight) and dry DCM (0.5 mL). To this solution was added freshly prepared NaOMe dropwise until pH = 12. The NaOMe was prepared from the addition of solid Na metal to dry MeOH. The reaction was stirred at rt for 2 hours and quenched by addition of 1 M AcOH in dry MeOH solution until pH = 7. The neutral solution was concentrated and **46** (18 mg, 8.6 µmol, 91% yield) was isolated by a 19:1 DCM-MeOH silica gel column. ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.45 – 7.09 (40 H, m), 5.28 (1 H, s), 5.25 (1 H, d, *J* 1.7), 5.17 (2 H, s), 5.04 – 4.95 (4 H, m), 4.90 – 4.78 (5 H, m), 4.78 – 4.68 (5 H, m), 4.62 (2 H, dd, *J* 11.2, 2.7), 4.60 – 4.49 (3 H, m), 4.49 – 4.43 (2 H, m), 4.15 (1 H, s, *J* 7.0), 4.04 (2 H, d, *J* 2.9), 3.96 – 3.83 (6 H, m), 3.83 – 3.70 (10 H, m), 3.68 – 3.48 (14 H, m), 3.46 (1 H, dd, *J* 9.8, 3.7), 3.42 (2 H, s), 3.40 – 3.35 (2 H, m), 1.89 – 1.80 (2 H, m), 0.87 (9 H, s), 0.07 (3 H, s), -0.03 (3 H, s). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 169.57, 169.41, 137.79, 137.59, 137.04, 137.01, 128.72, 128.69, 128.54, 128.45, 128.39, 128.35, 128.27, 128.26, 128.22, 128.12, 127.96, 127.84, 127.67, 127.44, 127.33, 127.24, 127.23, 127.07, 101.76, 100.92, 100.88, 95.61, 95.45, 80.79, 79.27, 79.08, 75.54, 75.09, 75.03, 74.18, 74.03, 73.44, 73.11, 72.98, 72.81, 72.75, 72.56,

72.51, 72.16, 71.80, 70.43, 68.14, 67.86, 67.66, 67.44, 67.25, 66.20, 64.35, 64.18, 64.04, 61.26, 61.20, 61.04, 52.40, 52.13, 52.07, 25.82, 17.89, -3.84, -4.82.HRMS [M+H] $C_{105}H_{129}N_{10}O_{33}Si^{+}$ calcd. 2086.8521, obsd. 2086.8518

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-amino-3-*O*-benzyl-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate (50).** Compound **50** was prepared by dissolving **48** (8 mg, 3.8 µmol) in MeOH (0.500 mL) and protecting from light 1,3 propane dithiol (10 µL, 76.7 µmol) and TEA (11 µL, 77 µmol) were added and the reaction was stirred for 24 hours at rt. At this point another portion 1,3 propane dithiol (10 µL) and TEA (11 µL) were added and the reaction was stirred another 72 hours at rt. The reaction was then diluted with 1:1 DCM-MeOH and eluted from a Sephadex LH-20 column. This furnished compound **50** (7 mg, 3.5 µmol, 91% yield) . HRMS $[M+3H]^{+3}$ $C_{105}H_{137}N_4O_{33}Si^{+3}$ calcd. 670.2984, obsd. 670.2978.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-sulfoamino-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-6-*O*-sulfonate-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-sulfonate-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-sulfoamino-3-*O*-benzyl-6-*O*-sulfonate-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-sulfonate-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-sulfoamino-3-*O*-benzyl-6-*O*-sulfonate-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-sulfonate-3-*O*-benzyl-α-L-idopyranosyluronate (51). 50** (5 mg, 2.5 µmol) was dissolved in pyridine (1 mL dried over 4Å sieves) along with washed SO_3·pyridine (100 mg, 6.28 µmol). The SO_3·pyridine was washed sequentially with water,

MeOH, and DCM then dried under vacuum before use. The reaction was stirred at 55 °C for 24 hours. To quench the reaction MeOH (100 uL) and TEA (100 uL) were added and the reaction was stirred at rt for 1 hour. The reaction was the diluted with 1:1 DCM-MeOH and eluted from a Sephadex LH-20 column. The fractions containing sugar were collected and purified by prep-TLC. 3:1:1 EtOAc-MeOH-H_2O with 1% AcOH. This provided compound **51** (5 mg, 1.9 µmol, 75%). HRMS $[M-8H+6Na]^{-2}$ $C_{105}H_{126}N_4Na_6O_{60}S_9Si^{-2}$ calcd. 1428.6809, obsd. 1428.6824

3-Aminopropyl 2-sulfoamino-4-*O*-*tert*-butyldimethylsilyl-6-*O*-sulfonate-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-sulfoamino-6-*O*-sulfonate-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-sulfoamino-6-*O*-sulfonate-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-sulfonate-α-L-idopyranosyluronate (52). **51** (10 mg, 3.7 µmol) was dissolved in MeOH (1 mL) and water (0.500 mL) to which $Pd(OH)_2$ (30 mg, 0.21 mmol) were added. The reaction was stirred under an H_2 atmosphere for 24 hours after which another 30 mg of $Pd(OH)_2$ were added and the reaction was stirred another 24 hours. The reaction was filtered and concentrated. It was then diluted with 10 mL of water and washed with 5 mL of DCM three times and 5 mL of EtOAc three times. This was then concentrated and eluted from a Sephadex G-15 column with water. This provided **52** (6 mg, 3.2 µmol, 86% yield). $C_{48}H_{78}N_4Na_5O_{58}S_9Si^{-3}$ calcd. 689.6679, obsd. 689.6672

3-Aminopropyl 2-deoxy-2-sulfoamino-4-*O*-*tert*-butyldimethylsilyl-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate salt (53). Treatment of **52** (1.5 mg, 0.8 µmol)

with the procedure for saponification followed by elution from a Sephadex G-15 column provided **53** (1 mg, 0.5 µmol, 68% yield). $C_{45}H_{69}N_4Na_7O_{58}S_9Si^{-4}$ calcd. 517.4784, obsd. 517.4783

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate (54) 47** (211 mg, 83.6 µmol) was dissolved in pyridine (5 mL) and cooled to 0 °C. HF·pyridine (2.5 mL) was added dropwise and the reaction was allowed to warm to rt and stir overnight. The reaction was then diluted with DCM and washed sequentially with sat. $CuSO_4$, 10% HCl, sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated. A 1:1 hexane-EtOAc silica gel column provided **54** (179 mg, 78.6 mmol, a 94% yield). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.14 (6 H, m), 7.60 – 7.09 (47 H, m), 5.52 (1 H, d, *J* 5.3), 5.47 (1 H, d, *J* 5.5), 5.24 – 5.21 (1 H, m), 5.21 – 5.17 (1 H, m), 5.15 (2 H, s), 5.07 (2 H, s), 5.01 (1 H, d, *J* 3.5), 4.92 (1 H, d, *J* 3.6), 4.84 – 4.61 (11 H, m), 4.59 – 4.53 (2 H, m), 4.48 – 4.39 (5 H, m), 4.32 – 4.23 (3 H, m), 4.21 – 4.06 (7 H, m), 4.00 – 3.84 (6 H, m), 3.83 – 3.76 (3 H, m), 3.65 (3 H, s), 3.62 – 3.55 (5 H, m), 3.51 – 3.41 (6 H, m), 3.31 (2 H, dd, *J* 10.3, 3.6), 3.22 (2 H, ddd, *J* 10.3, 3.5, 1.6), 2.94 (1 H, s), 2.11 (3 H, s), 2.10 (2 H, s), 2.08 (2 H, s), 1.84 (2 H, s). ^{13}C-NMR δ_C (125 MHz, $CDCl_3$) 171.89, 170.75, 170.65, 169.58, 169.50, 165.55, 165.18, 137.80, 137.73, 137.70, 137.32, 137.24, 133.69, 133.55, 133.44, 129.99, 129.91, 129.58, 129.22, 129.13, 128.83, 128.71, 128.56, 128.51, 128.39, 128.37, 128.32, 128.21, 128.08, 128.06, 128.02, 127.99, 127.97, 127.95, 127.88, 127.71, 127.67, 127.44, 127.26, 99.27, 98.96, 98.61, 98.22, 98.06, 78.94, 78.26, 78.22, 76.24, 76.03, 75.92,

75.66, 75.00, 74.89, 74.67, 74.62, 74.57, 74.29, 74.14, 72.69, 72.25, 71.43, 71.11, 70.97, 70.41, 69.75, 69.63, 68.02, 67.50, 67.19, 63.38, 63.08, 62.88, 62.48, 61.74, 61.66, 60.38, 52.11, 52.01, 51.70, 29.69, 20.80, 20.78, 20.77. HRMS $[M+H]^+$ $C_{126}H_{133}N_{10}O_{39}^+$ calcd. 2410.8759, obsd. 2410.8751.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate (55).** Compound **55** was prepared from **54** (18 mg, 7.5 µmol) by first dissolving it in DCM (3 mL). To this was added benzyl bromide (36 µL, 300 µmol), tetrabutyl ammonium iodide (3 mg, 8 µmol) and finally Ag_2O (35 mg, 150 µmol). This was protected from light and stirred at rt and after careful TLC monitoring (1:1 hexane-EtOAc) was stopped after 30 min. The reaction was filtered through celite and concentrated and a 3:2 hexane-EtOAc silica gel column provided **55** (10 mg, 3.9 µmol, 52% yield) and 3 mg of **54** which gave a 64% yield based on recovered starting material. ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.11 (6 H, ddd, *J* 8.3, 7.5, 4.2), 7.58 – 7.07 (49 H, m), 5.49 (1 H, d, *J* 4.8), 5.45 (1 H, d, *J* 5.5), 5.17 (2 H, ddd, *J* 8.8, 5.9, 2.5), 5.13 (2 H, d, *J* 3.0), 5.04 (2 H, s), 4.96 (1 H, d, *J* 3.5), 4.89 (1 H, d, *J* 3.6), 4.83 – 4.75 (6 H, m), 4.72 (3 H, m), 4.66 (2 H, dd, *J* 6.2, 4.2), 4.57 (1 H, d, *J* 10.9), 4.48 (1 H, d, *J* 10.6), 4.41 (5 H, m), 4.32 – 4.18 (6 H, m), 4.16 – 4.11 (2 H, m), 4.06 (3 H, ddd, *J* 8.0, 5.8, 2.7), 3.98 – 3.92 (3 H, m), 3.88 (2 H, t, *J* 9.5), 3.81 – 3.72 (4 H, m), 3.69 – 3.62 (4 H, m), 3.61 – 3.54 (4 H, m), 3.54 – 3.49 (1 H, m), 3.48 – 3.42 (5 H, m), 3.37 – 3.26 (3 H, m), 3.24 (1 H, dd, *J* 10.3, 3.5), 3.20 (1 H,

dd, *J* 10.2, 3.6), 2.12 – 2.06 (6 H, m), 1.99 – 1.95 (3 H, m), 1.85 – 1.76 (2 H, m). HRMS $[M+H]^+$ $C_{133}H_{139}N_{10}O_{39}^+$ calcd. 2500.9229, obsd. 2500.9230.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate (56) 55** (12 mg, 4.8 μmol) was dissolved in DCM (2 mL) and MeOH (2 mL, dried over 4Å sieves). The mixture was stirred at rt and 1 M NaOMe was added until pH = 12. The react was stirred at rt for 2 hours until the reaction was complete. The reaction was quenched by addition of 1 M AcOH (in anhydrous MeOH) until pH = 7. The reaction was concentrated and eluted from a Sephadex LH-20 column (1:1 DCM-MeOH) yielding **56** (8 mg, 3.8 μmol, 80% yield). ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 7.46 – 7.07 (45 H, m), 5.28 (1 H, s), 5.25 (1 H, d, *J* 1.6), 5.17 (2 H, s), 5.04 – 4.95 (4 H, m), 4.91 – 4.85 (1 H, m), 4.85 – 4.78 (5 H, m), 4.77 – 4.70 (4 H, m), 4.66 – 4.61 (3 H, m), 4.60 – 4.50 (3 H, m), 4.50 – 4.43 (2 H, m), 4.15 (1 H, s), 4.04 (2 H, t, *J* 6.1), 3.94 (1 H, t, *J* 9.6), 3.91 – 3.83 (5 H, m), 3.83 – 3.71 (10 H, m), 3.71 – 3.59 (5 H, m), 3.59 – 3.47 (8 H, m), 3.46 (3 H, s), 3.43 (3 H, s), 3.41 – 3.34 (2 H, m), 1.88 – 1.78 (2 H, m). HRMS $[M+H]^+$ $C_{106}H_{120}N_{10}O_{33}^+$ calcd. 2061.8053, obsd. 2061.8058

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-amino-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-methyl 3-*O*-benzyl-α-L-idopyranosyluronate (57).** Compound **57** was prepared by dissolving **56** (18 mg, 8.7 μmol) in MeOH (2 mL) and protecting it from light. To this was added 1,3 propanedithiol (25 μL, 180

μmol) and TEA (25 μL, 180 μmol). The reaction was stirred at rt for 24 hours. At this point a further 25 μL of 1,3 propanedithiol and 25 μL of TEA were added and the reaction as stirred an additional 72 hours. The reaction was then diluted with 1:1 DCM-MeOH and eluted from a Sephadex LH-20 column providing **57** (16 mg, 8 μmol, 92% yield). HRMS $[M+H]^+$ $C_{106}H_{127}N_4O_{33}^+$ calcd. 1984.8411, obsd. 1984.8417.

3-Aminopropyl 2-deoxy-2-acetamido-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-acetamido-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-acetamido-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate salt (58) Compound **58** was prepared from **57** in four steps. First **57** (5 mg, 2.5 μmol) was dissolved in pyridine (1 mL, dried over 4Å sieves) to which was added SO_3·pyridine (20 mg, 130 μmol). The reaction as stirred for 24 hours at 55 °C after which it was allowed to cool to rt and was quenched by addition of TEA (200 μL) and MeOH (400 μL) then stirred for 1 hour. After quenching the mixture was diluted with 1:1 DCM-MeOH and eluted from a Sephadex LH-20 column to remove pyridine. The crude product was then purified by Prep-TLC (3:1:1 EtOAc-MeOH-H_2O with 1% AcOH). The selectively O-sulfated product was acetylated by was dissolving it in methanol (1 mL). To this was added TEA (15 μL, 105 μmol) and acetic anhydride (3 μL , 32 μmol, 10 eq per NH_2). This was stirred at rt for 5 hours and was diluted with 1:1 DCM:MeOH and eluted from a Sephadex LH-20 column. The product of acetylation was further treated with global debenzylation and methyl ester saponification conditions to produce **58** (2 mg, 1.2 μmol, 47% yield over 4 steps from **57**). (**58**) ^{1}H-NMR δ_H (500 MHz, D_2O) 5.09 (2 H, s), 5.05 (3 H, m, *J* 4.1), 5.00 (1 H, s), 4.83 (2 H, s), 4.43 (1 H, s), 4.28 – 4.18 (10 H, m), 4.14 (2 H, d, *J* 10.9), 4.00 – 3.90 (8 H, m), 3.89 (1 H, d, *J* 3.5), 3.88 – 3.80

(1 H, m), 3.71 – 3.58 (6 H, m), 3.48 (1 H, m), 3.14 – 3.05 (2 H, m), 2.81 (1 H, s), 2.62 (1 H, s), 1.98 – 1.95 (9 H, m). HRMS $[M]^{3-}$ $C_{45}H_{66}N_4Na_3O_{52}S_6^{3-}$ calcd. 585.0226, obsd. 585.0218.

3-Aminopropyl 2-deoxy-2-amino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-amino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-amino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate salt (59). Compound **59** was prepared from compound **57** in 3 steps. First **57** (5 mg, 2.5 μmol) was dissolved in pyridine (1 mL, dried over 4Å sieves) to which was added SO_3·pyridine (20 mg, 130 μmol). The reaction as stirred for 24 hours at 55°C after which it was allowed to cool to rt and was quenched by addition of TEA (200 μL) and MeOH (400 μL) then stirred for 1 hour. After quenching the mixture was diluted with 1:1 DCM-MeOH and eluted from a Sephadex LH-20 column to remove pyridine. The crude product was then purified by Prep-TLC (3:1:1 EtOAc-MeOH-H_2O with 1% AcOH). The selectively O-sulfated product was then treated with the general procedures for global debenzylation and methyl ester saponification and after a Sephadex G-15 column provided **59** (3 mg, 1.9 μmol, 76% yield over three steps).(**59**) ^{1}H-NMR δ_H (500 MHz, D_2O) 5.37 – 5.29 (3 H, m), 5.14 (2 H, d, *J* 11.1), 5.05 (1 H, s), 4.84 (2 H, dd, *J* 7.4, 1.3), 4.46 (1 H, d, *J* 1.5), 4.31 – 4.25 (5 H, m), 4.21 (4 H, s), 4.18 – 4.12 (2 H, m), 4.11 – 4.07 (3 H, m), 3.97 – 3.91 (2 H, m), 3.90 – 3.85 (3 H, m), 3.84 – 3.75 (3 H, m), 3.74 – 3.68 (2 H, m), 3.66 – 3.61 (1 H, m), 3.48 (1 H, t, *J* 9.7), 3.35 – 3.25 (3 H, m), 3.12 – 3.03 (2 H, m), 1.96 – 1.87 (2 H, m). HRMS $[M]^{3-}$ $C_{39}H_{63}N_4O_{49}S_6^{3-}$ calcd. 521.0301, obsd. 521.0304.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-**

(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate (60). Compound **60** was prepared by dissolving **42** (24 mg, 8.7 μmol) pyridine (2 mL) and cooling it to 0 °C. Once cooled HF·pyridine (0.75 mL) was added dropwise and the reaction was allowed to warm to rt and stirred for 24 hours. The mixture was then diluted with DCM and washed with sat. $CuSO_4$, 10% HCl, sat. $NaHCO_3$, and dried over Na_2SO_4. After concentration and a 3:2 hexane-EtOAc column, **60** (20 mg, 7.6 μmol, 87% yield) was isolated. ^{1}H-NMR δ_H (600 MHz, $CDCl_3$) 8.16 – 8.04 (6 H, m), 7.57 – 7.06 (64 H, m), 5.51 (1 H, d, *J* 5.3), 5.46 (1 H, d, *J* 5.4), 5.20 (1 H, t, *J* 5.3), 5.18 (1 H, t, *J* 5.6), 5.10 (4 H, m), 5.07 – 4.99 (4 H, m), 4.98 (2 H, s), 4.91 (1 H, d, *J* 3.6), 4.85 (1 H, d, *J* 3.7), 4.77 – 4.70 (5 H, m), 4.68 – 4.60 (4 H, m), 4.49 (1 H, d, *J* 11.1), 4.48 – 4.43 (2 H, m), 4.43 – 4.30 (5 H, m), 4.30 – 4.21 (3 H, m), 4.17 – 4.13 (2 H, m), 4.09 – 4.03 (4 H, m), 3.97 – 3.91 (2 H, m), 3.90 – 3.81 (4 H, m), 3.77 – 3.64 (3 H, m), 3.50 – 3.42 (3 H, m), 3.42 – 3.37 (2 H, m), 3.34 – 3.22 (2 H, m), 3.19 (1 H, dd, *J* 10.2, 3.7), 3.15 (1 H, dd, *J* 10.1, 3.6), 3.11 (1 H, dd, *J* 10.2, 3.5), 2.75 (1 H, s,), 2.06 (3 H, s), 2.03 (3 H, s), 2.01 (3 H, s), 1.83 – 1.70 (2 H, m). ^{13}C-NMR δ_C (150 MHz, $CDCl_3$) 172.02, 171.00, 170.77, 169.19, 169.01, 165.64, 165.41, 138.13, 138.07, 138.00, 137.73, 137.46, 137.39, 136.93, 135.37, 135.31, 135.04, 133.85, 133.80, 130.23, 130.19, 130.12, 129.76, 129.46, 129.39, 129.04, 128.98, 128.91, 128.87, 128.84, 128.81, 128.78, 128.72, 128.71, 128.66, 128.61, 128.59, 128.58, 128.51, 128.39, 128.34, 128.30, 128.21, 128.17, 128.13, 128.05, 127.82, 127.60, 127.44, 100.14, 99.47, 99.24, 98.61, 98.50, 79.35, 78.48, 78.44, 76.23, 75.99, 75.28, 75.10, 74.95, 74.45, 74.30, 73.40, 72.58, 71.82, 71.65, 71.39, 71.33, 70.68, 70.15, 69.94, 68.08, 67.87, 67.43, 67.22, 63.73, 63.36, 63.07, 62.65, 62.07, 61.85, 21.25, 14.42. MALDI-MS $[M+Na]^+$ $C_{144}H_{144}NaN_{10}NaO_{39}^+$ calcd. 2659.95, obsd. 2659.99.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)- 3-*O*-benzyl-α-L-idopyranosyluronate (61). 60** (50 mg, 19 µmol) was dissolved in THF (2.5 mL) to which 1 M LiOH (0.74 mL, 0.74 mmol, 13 eq per CO_2Bn) was added. This mixture was cooled to -5 °C followed by the addition of H_2O_2 solution (minimum 30% H_2O_2, 0.87 mL, 8.5 mmol, 150 eq per CO_2Bn). This was allowed to warm to rt and stir for 16 hours after which MeOH (6 mL) and 3M KOH (1.5 mL, 4.5 mmol) were added. The reaction was then allowed to stir an additional 24 hours. To quench the reaction was acidified with 10% HCl and concentrated to dryness. The resulting solid was purified by eluting from a Sephadex LH-20 column (1:1 DCM-MeOH). This provided **61** (31 mg, 16 µmol, 85% yield). MALDI-MS $[M+K]^+$ $C_{96}H_{108}KN_{10}O_{33}^+$ calcd. 1967.67, obsd. 1967.65.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-2-amino-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)- 3-*O*-benzyl-α-L-idopyranosyluronate (62). 61** (31 mg, 16 µmol) was dissolved in THF (7 mL) to which 1 M trimethylphosphine (0.241 mL, 241 µmol), 0.10 M NaOH (1.25 mL, 125 µmol), and water (2 mL) were added. The reaction was allowed to stir overnight until TLC (7:1 DCM-MeOH) showed the reaction was complete. The reaction was then neutralized with 0.10 M HCl and concentrated to dryness. The crude mixture was then eluted from a Sephadex LH-20 column (1:1 DCM-MeOH) which provided **62** (29 mg, 16 µmol, 98% yield). MALDI-MS $[M+Na]^+$ $C_{96}H_{114}N_4NaO_{33}^+$ calcd. 1874.73, obsd. 1874.42.

3-Aminopropyl 2-deoxy-2-sulfoamino-α-D-glucopyranosyl-(1→4)-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-α-D-glucopyranosyl-(1→4)-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-α-D-glucopyranosyl-(1→4)-α-L-idopyranosyluronate salt (63) Compound **63** was prepared from **62**. Briefly **62** (4.3 mg, 2.3 µmol) was dissolved in MeOH (0.5 mL) and cooled to 0 °C. The pH of the solution was brought to 9.5 by addition of 1 M NaOH. Once pH was equal to 9.5, $SO_3 \cdot NEt_3$ complex (6.3 mg, 45 µmol) was added and pH was maintained at 9.5 by addition of 1 M NaOH as needed. The reaction was allowed to warm to rt and stirred overnight. By TLC (3:1:1 EtOAc-MeOH-H_2O 1% AcOH) the reaction was incomplete so an additional portion of sulfur trioxide triethylamine (2.5 mg, 18 µmol) was added and the reaction was stirred an additional 12 hours. The reaction was diluted with 1:1 DCM-MeOH and eluted from a Sephadex LH-20 column with the same mixture. The product from sulfation was dissolved in MeOH (2 ml) and water (1 mL) to which $Pd(OH)_2$ (50 mg, 360 µmol) was added. The reaction was stirred under a H_2 atmosphere overnight. The reaction was then filtered, concentrated and diluted with 10 mL of water. The solution was then washed with 5 mL of DCM three times and 5 mL of EtOAc three times. The reaction was then concentrated and eluted from a Sephadex G-15 column providing **63** (2 mg, 1.6 µmol, 70% yield over two steps). ^{1}H-NMR δ_H (900 MHz, D_2O) 5.26 (1 H, d, *J* 3.5, H-1F), 5.25 (1 H, d, *J* 3.5, H-1D), 5.20 (1 H, d, *J* 3.6, H-1B), 4.87 (4 H, d, *J* 7.9, H-1C, H-1E, H-5C, H-5E), 4.80 (1 H, s, H-1A), 4.44 (1 H, s, H-5A), 4.07 (1 H, t, *J* 2.9, H-3A), 4.04 (1 H, t, H-3C, H-3E), 4.02 (1H, t, 3.98 – 3.94 (2 H, m, H-4C, H-4E), 3.93 (1 H, s, H-4A), 3.78 (1 H, ddd, *J* 10.0, 7.9, 4.7, H-Linker), 3.72 – 3.55 (16 H, m, H-2A, H-4B, H-5B, H-6aB, H-6bB, H-2C, H-4D, H-5D, H-6aD, H-6bD, H-2E, H-5F, H-6aF, H-6bF, H-Linker), 3.55 – 3.52 (1 H, m, H-3D), 3.49 (1 H, t, *J* 9.8, H-3F), 3.38 (1 H, t, *J* 9.6, H-4F), 3.14 – 3.10 (2 H, m, H-2B, H-2D), 3.09 (1 H, dd, *J* 10.4, 3.5, H-2F),

3.03 (2 H, m, CH_2-Linker), 1.92 – 1.84 (2 H, m, CH_2-Linker). HRMS $[M]^{3-}$ $C_{39}H_{63}N_4O_{40}S_3^{3-}$ calcd. 441.0732, obsd. 441.0717

3-Aminopropyl 2-deoxy-2-sulfoamino-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-α-D-glucopyranosyl-(1→4)- α-L-idopyranosyluronate salt (64) 63 (500 µg, 0.38 µmol) was incubated with 2-OST (1.00 mg) and the phosphate donor PAPs (2.5 µmol) in 20 mM MES (morpholine-4-ethanesulfonic acid) solution with a total volume of 12.5 mL for 24 hours at 37 °C. At this point another 1.00 mg of 2-OST was added along with 2.5 µmol of PAPs and the reaction was diluted to 25 mL keeping the solution concentration at 20 mM MES. The reaction was then stirred overnight at 37 °C. The reaction was then concentrated using a Q-Sepharose Fast Flow column (GE 17-0510-10). The mixture was passed through the column which was then washed with 20 mL of 25 mM NaOAc solution. The product was eluted from the column with 10 mL of a 1.0 M NaCl and 25 mM NaOAc solution. The product was in the first 2 mL of eluent which was collected and lyophilized to concentrate. The resulting residue was dissolved in 0.1 M ammonium bicarbonate solution and to monitor the purification 10 µL of phenol red solution was added to the crude mixture and it was eluted from a 0.75 cm x 200 cm Biogel P-2 column. The elution buffer was 0.1 M ammonium bicarbonate. Tubes containing oligosaccharide (TLC, stain 1,3 dihydroxynaphthalene) were lyophilized three times to remove ammonium bicarbonate to allow for MS analysis. Four reactions combined produced **64** (1.8 mg, 1.2 µmol, 79% yield). ^{1}H-NMR δ_H (900 MHz, D_2O) 5.22 (1 H, d, *J* 3.6, H-1F*), 5.21 – 5.17 (2 H, d, *J* 3.4, H-1B, H-1D*), 5.17-5.15 (2 H, m, H-1C*, H-1E*), 5.15 (1 H, s), 4.78 (1 H, s, H-1A), 4.77 – 4.74 (2 H, m, H-5B, H-5D, H-6aB, H-6aD), 4.36 – 4.34 (1 H, m, H-5A), 4.22 (2 H, s, H-2C, H-2E), 4.13 (2 H,

dd, *J* 7.7, 4.2, H-3C, H-3E), 4.06 – 4.04 (1 H, m, H-3A), 3.93 (3 H, d, *J* 10.2, H-4A, H-4C, H-4E), 3.81 – 3.65 (10 H, m, H-5B, H-5D), 3.62 – 3.57 (4 H, m, H-2A, H-3F), 3.56 – 3.53 (2H, t, *J* 9.8, H-3B, H-3D) 3.37 – 3.33 (2 H, t, *J* 9.8, H-4B, H-4D), 3.13 (1 H, dd, *J* 10.3, 3.1, H-2F), 3.12 – 3.08 (2 H, m, H-2B, H-2D), 3.04 – 3.00 (2 H, m), 1.91 – 1.86 (2 H, m). HRMS $[M]^{3-}$ $C_{39}H_{59}N_4Na_4O_{46}S_5^{3-}$ calcd. 523.6870, obsd. 523.6890.

3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-2-*O*-sulfonate-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-α-D-glucopyranosyl-(1→4)- α-L-idopyranosyluronate salt (65) Compound **65** was prepared from compound **64** (460 µg, 0.31 µmol) using the procedure for enzymatic sulfation. Utilizing enzymes 6-OST-1 and 6-OST-3 provided **65** (276 µg, 0.17 µmol, 44% yield from compound **63**. **(65)** ^{1}H-NMR δ_H (900 MHz, D_2O) 5.30 (1 H, d, *J* 3.1, H-1F), 5.22 (1 H, d, *J* 3.8, H-1D), 5.20 (1 H, d, *J* 3.3, H-1B), 5.18 (1 H, s, H-1C*), 5.14 (1 H, s, H-1E*), 4.78 (1 H, d, *J* 2.2, H-1A), 4.77 (1H, s, H-5C) 4.74 (1 H, s, H-5E), 4.35 (1 H, d, *J* 2.1, H-5A), 4.30 (2 H, d, *J* 11.3, H-6D, H-6F), 4.26 – 4.21 (2 H, m, H-2C, H-2E), 4.15 (2 H, d, *J* 10.6, H-6'D, H-6'F), 4.13 – 4.08 (2 H, m, H-3C, H-3E), 4.06 – 4.04 (1 H, m, H-3A), 3.99 (1 H, d, *J* 2.3, H-4E), 3.96 – 3.94 (1 H, m, H-4C), 3.93 – 3.92 (1 H, m, H-4A), 3.91 – 3.87 (2 H, m, H-5D, H-5F), 3.78 (2 H, m, H-5B, H-Linker), 3.73 – 3.71 (2 H, m, H-4B, H-6aB), 3.70 – 3.66 (1 H, m, H-4D), 3.63 – 3.56 (5 H, m, H-2A, H-3B, H-3D, H-6bB, H-Linker), 3.53 (1 H, t, *J* 9.9, H-3F), 3.46 (1 H, t, *J* 9.6, H-4F), 3.17 (1 H, dd, *J* 10.3, 3.0, H-2D), 3.13 (1 H, dd, *J* 10.4, 3.3, H-2F), 3.10 (1 H, dd, *J* 9.5, 3.3, H-2B), 3.05 – 3.00 (2 H, m, CH_2-Linker), 1.91 – 1.86 (2 H, m, CH_2-Linker). HRMS $[M]^{3-}$ $C_{39}H_{59}N_4Na_4O_{52}S_7^{3-}$ calcd. 576.9916, obsd. 576.9904.

3-Aminopropyl 2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-α-L-idopyranosyluronate-(1→4)-2-deoxy-2-sulfoamino-6-*O*-sulfonate-α-D-glucopyranosyl-(1→4)-α-L-idopyranosyluronate salt (66) Compound **66** was prepared from **63** using the general procedure for enzymatic sulfation with 6-OST-1 and 6-OST-3 as the enzymes. This produced **66** (170 µg, 0.11 µmol, 29% yield). ^{1}H-NMR δ_H (900 MHz, D_2O) 5.23 (1 H, d, *J* 3.6), 5.22 (1 H, d, *J* 3.7), 5.20 (1 H, d, *J* 3.5), 4.98 (1 H, s), 4.95 (1 H, s), 4.80 – 4.75 (2 H, m), 4.36 (1 H, s), 4.26 – 4.20 (3 H, m), 4.11 – 4.07 (2 H, m), 4.07 – 4.03 (2 H, m), 4.03 – 4.01 (1 H, m), 3.97 – 3.93 (2 H, m), 3.93 – 3.88 (2 H, m), 3.83 – 3.80 (1 H, m), 3.80 – 3.75 (2 H, m), 3.74 – 3.69 (3 H, m), 3.65 – 3.62 (2 H, m), 3.62 – 3.54 (5 H, m), 3.54 – 3.51 (1 H, m), 3.47 – 3.44 (1 H, m), 3.15 – 3.09 (3 H, m), 3.05 – 3.00 (2 H, m), 1.90 – 1.85 (2 H, m).HRMS $[M]^{3-}$ $C_{39}H_{60}N_4Na_3O_{49}S_6^{3-}$ calcd. 543.0120, obsd. 543.0130.

Chapter 3 – Heparin Mimics: Head to Tail Oligomers

3.1 Background

Heparin's use as an anticoagulant is widely known but its other biological interactions, specifically with FGF-2 and heparanase are of great interest. FGF-2 is known for its angiogenic nature and heparin/HS is required for activation.[72] Heparin/HS can also inhibit FGF-2 by binding but not inducing dimerization of the protein.[73] Heparanase is an enzyme overexpressed by most cancer cells that cleaves heparin/HS from cell surfaces and the extracellular matrix. It is a vital player in the angiogenesis and metastasis mechanism.[74] Release of heparin/HS by heparanase can activate FGF-2 increasing angiogenesis and cell invasion. Inhibition of either FGF-2 or heparanase can be favored by specific sulfation patterns, acetylation of the amino groups, and chemical modification. A special type of heparin mimic produced by glycol splitting of native heparin/HS has been found to be excellent at binding FGF-2 and heparanase but lacking in anticoagulant activity.[75] The effect is pronounced enough that one glycol-split mixture is currently in Phase I trials for myeloma.[76]

Glycol-split derivatives were first used to produce nonanticoagulant heparin. Figure 3.1 illustrates the pathway to produce glycol split oligosaccharides. Desulfation of the 2-*O* position is performed by dissolving the heparin in a basic solution, commonly 0.1 M NaOH and pH = 11.5, followed by freezing and lyophilizing. This removes the sulfation by elimination to form the epoxide. The epoxide can be opened to the more stable galacturonic acid conformer by heating a neutral solution to 60 °C or converted back to iduronic acid by lyophilization with higher concentration of base, pH = 12.5.[77] The uronic acids not carrying 2-*O* sulfates contain the only vicinal diol in the molecule, which upon treatment with sodium periodate in the dark provides the aldehyde containing cleaved product known as oxyheparins (O-HEP). The reactive

aldehydes can be reduced with sodium borohydride to produce the reduced oxyheparins (RO-HEP). The cleavage and reduction reactions are commonly carried out in one pot.[78] The produced oligosaccharides show a great reduction of anticoagulant activity but other affinities were unchanged or increased including those to FGF-2 and heparanase likely caused by increased flexibility.[75] The elimination of anticoagulant activity is vital for any potential therapeutic applications. Heparin can cause serious side effects such as bleeding and thrombocytopenia. For applications targeting FGF-2 or heparanase there would be need for antithrombic effect.[79]

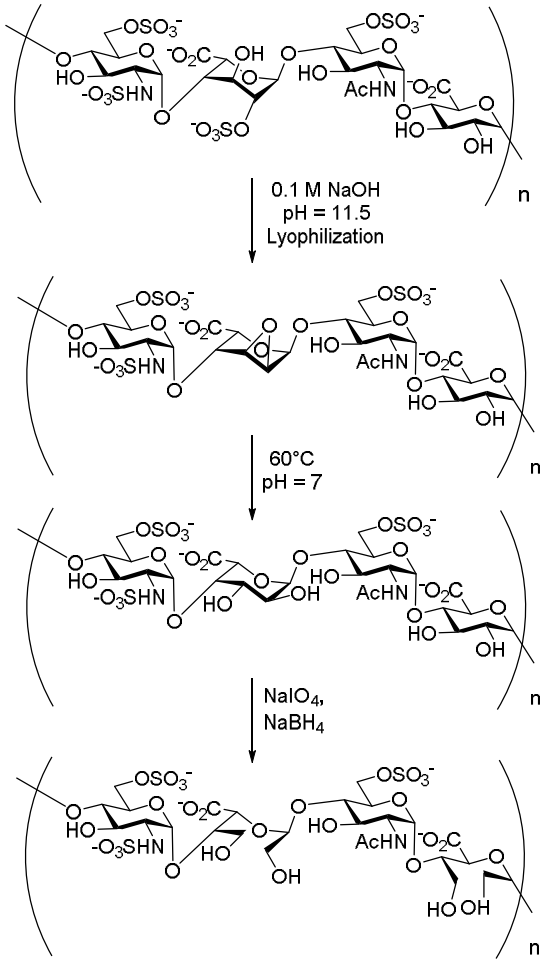


Figure 3.1. General route to glycol split heparins. Note not all uronic acids are split.

Glycol-split heparins have similar limitations as other naturally derived oligosaccharides, i.e., the heterogeneity. They are already varied before undergoing further chemical reactions that provide further heterogenous products. The large mixtures of oligomers were used by Casu and co-workers, who explored the requirements of sulfation of glycol-split heparins for heparanase inhibition. They found that as long as the glycol-split polysaccharides carried 2-*O* or 6-*O* sulfation, heparanase was strongly inhibited. Backbone structure also played a role as those with iduronic acid residues strongly inhibited heparanase but a polymer without it was a much weaker inhibitor. The final alteration, amine functionality, was found to be variable. The best binding came from polymers carrying at least 50% acetylated amines. While some general information can be gained, detailed structure activity relationship is much more difficult to obtain. The oligosaccharides used that were 6-*O* desulfated still carried from 23%-29% 6-*O* sulfation and had lost 15% of their 2-*O* sulfates. The common practice for deciphering the heterogenous products is to use ^{1}H-NMR of the anomeric protons to find the mole fraction of the different uronic acids and the percentage that have been split.[79a] To fully investigate the requirements of flexible heparin mimics with heparanase and FGF-2, access to pure synthetic materials is needed.

3.2 Building Block Synthesis

Glycol-split oligosaccharides contain areas of high sulfation separated by areas of low sulfation and high flexibility.[79b] The previously prepared heparin oligomers are lacking a highly flexible yet low charged portion and would be poor mimics of glycol-split heparins. They would also be ill-suited for glycol splitting as they do not contain glucuronic acid. To imitate glycol-split heparin the sulfated oligosaccharides need to be connected by a long flexible linker, producing a single structure with both key elements, areas of high sulfation separated by low sulfation/high flexibility. The building blocks shown in Figure 3.2 fufill those requirements and

can be easily acquired from oligosaccharides synthesized previously. Each contains a tetrasaccharide group for binding that will be separated by long flexible linkers, much like the glycol-split oligosaccharides. The reducing end still contains an amino group, protected with TFA to allow for selective coupling. It will be made by adding to the amino propyl linker already present on all the oligosaccharides used in Chapter 2. The non-reducing end carries a ketone group to allow for coupling through a reductive amination reaction.

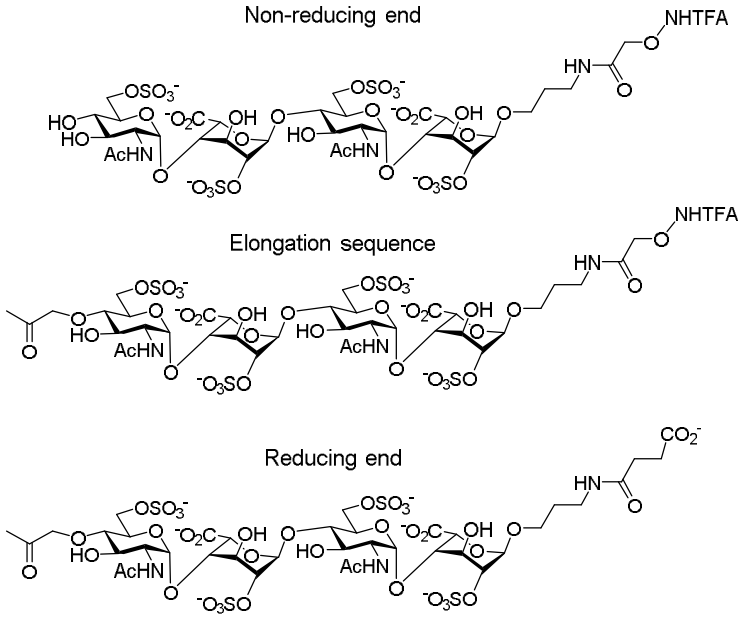


Figure 3.2. The three building blocks for synthetic glycol split heparin mimics.

Synthesis of the building blocks started with disaccharide building blocks previously utilized. The glycosylation of **18** and **24,** from Chapter 2, provided **67** in 75% yield (Figure 3.3). To minimize the steps required after installation of the ketone linker, conversion of the idose to iduronic acid was performed first. Removal of the levulinoyl esters from **67** with hydrazine provided **68** in 89% yield. Oxidation of the newly generated hydroxyl groups by TEMPO/BAIB followed by protection of the carboxylates as benzyl esters furnished **69** in 85% yield. The non-reducing end TBS was removed with HF·pyridine giving **70** in 91% yield. With **70** in hand the ketone functionality could be installed on the reducing end and elongation building blocks.

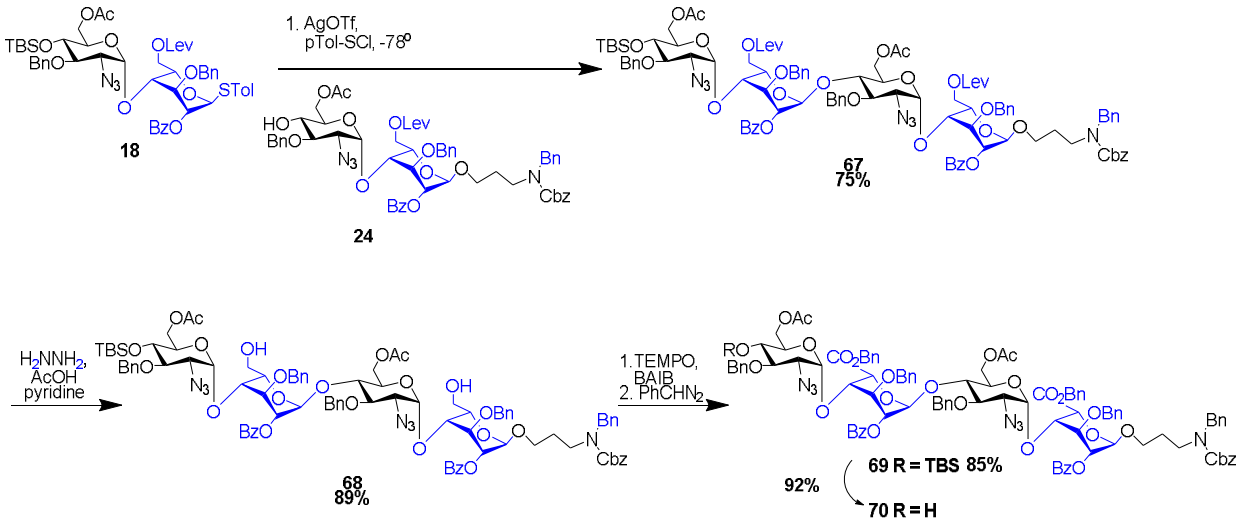


Figure 3.3. Synthesis of key building block **6** for heparin mimic.

Installation of the ketone functionality was first attempted under basic conditions with chloroacetone.[80] In order to keep as conditions as mild as possible, the first base used was the weak and non-nucleophilic 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU). This failed to produce **71**. TBAI was added as a catalyst to the reaction to increase the reactivity of chloroacetone. Alkylation of **70** with TBAI and DBU only led to the isolation of the starting material. Increasing the strength of the base, by using K_2CO_3 or NaHMDS, also failed to facilitate the alkylation of **70**. Even silver oxide, which facilitated the benzylation of a hexasaccharide, Figure 2.14, failed to produce **71**. Getting nowhere with chloroacetone, the substrate was changed to 3-chloro-2-methyl-prop-1-ene, as this had been successfully installed on a protected oligosaccharide.[81] It could also be converted to a ketone through a reaction shown to be safe on deprotected heparin, ozonolysis.[82] Unfortunately all attempts with 3-chloro-2-methyl-prop-1-ene failed to produce an alkylated product.

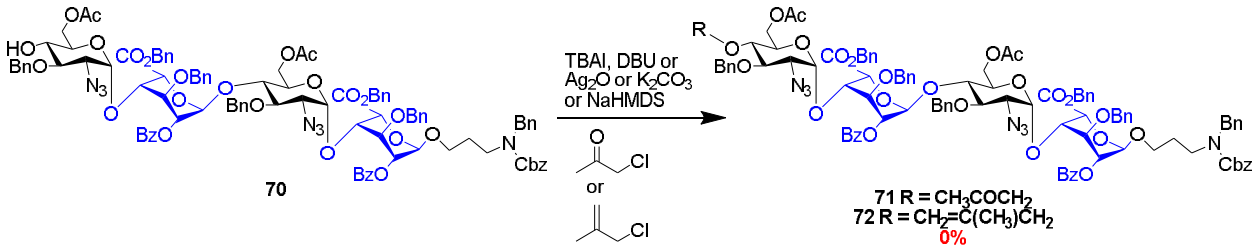


Figure 3.4. Attempted alkylation of **6** with chloroacetone and chloropropene.

3.3 Conclusions

The inability to install the ketone functionality on **70** could have been caused by poor nucleophilicity. The installation of the carboxylates greatly reduces the reactivity of the oligosaccharide and as detailed in **Chapter 1.2b**. Reactivity was also seen to decrease for longer oligosaccharides. This was confirmed recently by another member of the Huang group who continued this project. Installation of the ketone before glycosylation, on a monosaccharide building block was achieved. The resulting building block was then successfully used in glycosylation. Installation of the ketone building block provided a different, but ultimately successful route to viable building blocks. These results likely confirm that the failure to procure **71** or **72** was due to the unreactivity of the oxidized compound **70.** It will be interesting to see how the project comes to fruition with the future work by Huang Group members.

3.4 Experimental Section

3.41 General Experimental Procedures.

All reactions were performed under a nitrogen atmosphere with anhydrous solvents. Solvents were dried using a solvent purification system. Glycosylation reactions were performed with 4Å molecular sieves that were flamed dried under high vacuum. Chemicals used were reagent grade unless noted. Reactions were visualized by UV light (254 nm) and by staining with either $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL) or 5% H_2SO_4 in EtOH. Flash chromatography was performed on silica gel 601 (230-400 Mesh). NMR spectra were referenced using residual $CHCl_3$ (δ ^{1}H-NMR 7.26 PPM ^{13}C-NMR 77.0 PPM). Peak and coupling constants assignments are based on ^{1}H-NMR, ^{1}H-^{1}H gCOSY, ^{1}H and ^{1}H-^{1}H TOCSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C gHMQC/HSQC, ^{1}H-^{13}C gHMBC.

3.42 Characterization of anomeric stereochemistry.

The stereochemistries of newly formed glycosidic bonds for idose and glucosamine were determined by $^3J_{H1,H2}$ through ^{1}H-NMR and/or $^1J_{C1,H1}$ through gHMQC 2-D NMR (without 1H decoupling). Smaller $^3J_{H1,H2}$ (3 Hz) indicate α linkages and larger $^3J_{H1,H2}$ (7 Hz or larger) indicate β linkages. $^1J_{C1,H1}$ couplings around 170 Hz suggest α linkages while constants around 160 Hz imply β linkages.

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-levulinoyl-α-L-idopyranoside (67)**

Compound **67** was synthesized in 75% yield. Donor **18** (403 mg, (400 µmol), acceptor **24** (403 mg, 376 µmol), and 1.5 grams of 4Å molecular sieves were dissolved in DCM (40 mL) and stirred at rt for 30 min. The mixture was cooled to -78 °C and AgOTf (212 mg, 830 µmol) dissolved in DCM-EtOH (10 mL, 1:1) was added directly into the solution which was allowed to stir for 10 mins. Then p-TolSCl (72 µL) was added and the reaction was allowed to warm to rt over 3 hours. The reaction was filtered through celite to remove sieves, washed with $NaHCO_3$, dried over Na_2SO_4 and concentrated. After a silica gel column (1 fraction 11:10 hexane-EtOAc then 1:1 hexane-EtOAc) **67** (548 mg, 280 µmol, 75% yield) was isolated. ^{1}H-NMR δ_H (500 MHz, $CDCl_3$) 8.13 (4 H, m), 7.36 (36 H, m), 5.17 (3 H, d, *J* 4.2), 5.13 (2 H, m), 4.94 (1 H, m), 4.75 (8 H, m), 4.51 (3 H, t, *J* 12.1), 4.39 (7 H, m), 4.24 (2 H, m), 4.11 (4 H, m), 3.98 (1 H, d, *J* 10.2), 3.86 (1 H, m), 3.74 (3 H, m), 3.66 (1 H, s), 3.60 (2 H, td, *J* 9.6, 5.0), 3.51 (2 H, t, *J* 9.3), 3.36 (3 H, m), 3.28 (2 H, m), 2.69 (4 H, m), 2.52 (4 H, m), 2.14 (6 H, s), 2.05 (3 H, s), 2.02 (3 H, s), 1.89 (2 H, m), 0.92 (9 H, s), 0.04 (3 H, s), -0.03 (3 H, s). gHMQC (without ^{1}H decoupling) $^1J_{C1AH1A}$ = 171 Hz $^1J_{C1BH1B}$ = 173.5 Hz $^1J_{C1CH1C}$ = 170 Hz $^1J_{C1DH1D}$ = 171.5 Hz. HRMS $[M+H]^+$ $C_{107}H_{122}N_7O_{29}Si^+$ calcd. 1960.8012, obsd. 1960.8007

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranoside (68)** Compound **68** was prepared by dissolving **67** (99 mg, 56 µmol) in a mixture of pyridine (2.4 mL) and acetic acid (1.6 mL). This was cooled to 0 °C after which hydrazine hydrate (27 µL, 560 µmol) was added. The reaction was stirred at 0 °C for 3 hours after which TLC showed the reaction had gone to completion so acetone (2 mL) was added to quench and the reaction was stirred at rt for 30 min. The reaction was diluted with

EtOAc and washed with sat. $NaHCO_3$, 10% HCl, and brine. The organic layer was dried over Na_2SO_4 and concentrated. A silica gel column (1:1:1.5 hexane-DCM-EtOAc) was run providing **68** (79 mg, 50 µmol, 89% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.18 – 8.08 (4 H, m), 7.66 – 6.89 (36 H, m), 5.21 – 5.14 (3 H, m), 5.12 (1 H, s), 5.07 (1 H, s), 5.02 – 4.93 (1 H, m), 4.89 (1 H, d, *J* = 11.2), 4.90 – 4.81 (2 H, m), 4.76 (1 H, d, *J* = 11.2), 4.75 – 4.69 (2 H, m), 4.62 – 4.55 (1 H, m), 4.54 – 4.47 (2 H, m), 4.45 (1 H, d, *J* = 11.2 Hz), 4.39 (1 H, d, *J* = 10.5), 4.35 – 4.28 (3 H, m), 4.27 – 4.19 (2 H, m), 4.16 (1 H, s), 4.09 – 4.04 (2 H, m), 4.02 (1 H, dd, *J* = 12.1, 5.0), 3.98 (2 H, s), 3.90 – 3.81 (2 H, m), 3.80 – 3.64 (5 H, m), 3.63 – 3.56 (3 H, m), 3.57 – 3.52 (2 H, m), 3.46 – 3.42 (1 H, m), 3.33 (2 H, dd, *J* = 10.1, 3.1), 3.27 (2 H, dd, *J* = 10.1, 3.8), 2.05 (3 H,s), 2.03 (3 H, s), 1.91 – 1.82 (2 H, m), 0.88 (9 H, s), 0.01 (3 H, s), -0.07 (3 H, s). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.62, 170.43, 165.76, 165.67, 137.80, 137.74, 137.42, 137.36, 133.35, 133.15, 130.02, 129.80, 129.70, 129.62, 128.54, 128.51, 128.46, 128.44, 128.32, 128.28, 128.14, 128.11, 128.07, 127.95, 127.92, 127.91, 127.85, 127.83, 127.37, 127.31, 127.26, 127.25, 127.24, 127.04, 98.48, 98.03, 97.95, 80.43, 79.35, 77.25, 75.05, 74.72, 73.86, 72.97, 72.07, 71.33, 71.02, 70.30, 67.27, 64.34, 64.28, 64.09, 64.06, 62.94, 62.66, 61.28, 60.37, 30.65, 25.88, 21.04, 20.81, 20.72, 19.12, 17.98, 14.20, 13.71, -3.78, -4.94. HRMS $[M+H]^+$ $C_{94}H_{110}N_7O_{25}Si^+$ calcd. 1764.7276, obsd. 1764.7284

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-Acetyl-2-azido-3-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate** (**69**). **68** (79 mg, 44 µmol) was dissolved in a mixture of DCM (2 mL), *t*-butanol (2 mL), and water (0.5 mL). To this was added TEMPO (4 mg, 27 µmol) and BAIB (71 mg, 220 µmol). The reaction

was stirred at rt for 24 hours. To quench the reaction 2 mL of sat. $Na_2S_2O_3$ were added and the reaction was stirred at rt for 15 mins. The mixture was diluted with DCM and water and separated. The water layer was acidified with 1 M HCl and extracted three times with DCM. The organic layers were collected, dried over Na_2SO_4, and concentrated. The crude mixture was dissolved in 5 mL of DCM to which was added phenyldiazomethane until the red color persisted. After 1 hour additional phenyldiazomethane was added and the reaction was allowed to stir overnight. The reaction was concentrated (only on small scale do not perform on large scale) and purified by a 3:1 hexane-EtOAc column providing **69** (75 mg, 37 mmol, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 – 8.08 (4 H, m), 7.73 – 6.87 (46 H, m), 5.56 (1 H, s), 5.22 (2 H, d, *J* = 11.7), 5.14 (3 H, d, *J* = 10.8), 5.07 (4 H, m), 5.00 (1 H, s), 4.88 – 4.80 (2 H, m), 4.78 (2 H, s), 4.75 – 4.63 (4 H, m), 4.48 – 4.36 (7 H, m), 4.33 (1 H, d, *J* = 12.5), 4.20 – 4.15 (1 H, m), 4.13 (1 H, d, *J* = 3.2), 4.08 (2H, d, *J* = 8.1), 3.99 – 3.91 (2 H, m), 3.86 (1 H, t, *J* = 9.3), 3.83 – 3.79 (1 H, m), 3.75 (1 H, d, *J* = 10.5), 3.65 (1H, t, *J* = 8.9), 3.49 – 3.40 (3 H, m), 3.37 – 3.27 (2 H, m), 3.24 (1 H, d, *J* = 10.3), 3.16 (1 H, d, *J* = 10.3), 2.09 (3 H, s), 2.01 (3 H, s), 1.86 – 1.73 (2 H, m), 0.90 (9 H, s), 0.01 (3 H, s), -0.06 (3 H, s). HRMS $[M+H]^+$ $C_{108}H_{118}N_7O_{27}Si^+$ calcd. 1972.7800, obsd. 1972.7806

***N*-(Benzyl)-benzyloxycarbonyl-3-aminopropyl 6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate-(1→4)-6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-benzyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate** (**70**). **69** (75 mg, 38 μmol) was dissolved in pyridine (5 mL) and transferred to a plastic centrifuge tube and cooled to 0 °C. To this was added dropwise HF·pyridine (2.5 mL). The reaction was allowed to stir and warm to rt overnight. The reaction was then diluted with DCM and washed with sat. $CuSO_4$, 10% HCl,

sat. $NaHCO_3$, and dried over Na_2SO_4. After concentrating and a silica gel column (2 fractions 3:2 hexane-EtOAc then 1:1 hexane-EtOAc) **70** (65 mg, 35 µmol 92% yield) was isolated. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (4 H, dt, *J* = 18.1, 9.2), 7.58 – 7.08 (46 H, m), 5.55 (1 H, d, *J* = 5.4), 5.24 (1 H, t, *J* = 5.3), 5.15 (4 H, dd, *J* = 12.1, 4.0), 5.07 (4 H, dd, *J* = 12.0, 10.5), 4.94 (1 H, d, *J* = 3.7), 4.80 (2 H, s), 4.70 (1 H, d, *J* = 9.8), 4.65 (2 H, dd, *J* = 8.1, 4.2), 4.49 (2 H, m), 4.45 – 4.33 (6 H, m), 4.19 – 4.16 (1 H, m), 4.15 – 4.13 (2 H, m), 4.11 (1 H, d, *J* = 8.1), 4.10 – 4.07 (1 H, m), 3.96 (2 H, m), 3.89 – 3.83 (2 H, m), 3.82 – 3.66 (3 H, m), 3.53 – 3.45 (3 H, m), 3.45 – 3.39 (2 H, m), 3.36 – 3.25 (2 H, m), 3.19 (1 H, t, *J* = 3.5), 3.17 (1 H, t, *J* = 3.4), 2.76 (1 H, d, *J* = 4.6), 2.13 (3 H, s), 2.06 (3 H, s), 1.80 (2 H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.82, 171.11, 170.82, 168.99, 165.44, 165.26, 137.92, 137.81, 137.53, 137.22, 136.73, 135.15, 134.89, 133.55, 130.07, 129.91, 129.59, 129.29, 128.83, 128.78, 128.69, 128.67, 128.63, 128.58, 128.51, 128.49, 128.45, 128.44, 128.41, 128.31, 128.17, 128.14, 128.10, 127.96, 127.95, 127.87, 127.85, 127.79, 127.49, 127.24, 99.93, 99.40, 99.12, 98.35, 79.12, 78.46, 77.25, 76.13, 75.82, 74.95, 74.87, 74.45, 74.13, 73.13, 72.36, 71.54, 71.20, 71.11, 70.48, 69.90, 67.90, 67.67, 67.21, 67.17, 63.58, 62.87, 62.42, 61.82, 60.38, 21.04, 20.86, 20.77, 14.21. HRMS $[M+H]^+$ $C_{102}H_{103}N_7O_{27}^+$ calcd. 1858.6935, obsd. 1858.6940

Chapter 4 – Chemical Synthesis of GM2 Glycans, Bioconjugation with Bacteriophage Qβ, and the Induction of Anticancer Antibodies

4.1 Introduction

Aberrant glycosylation is a hallmark of many human cancers.[83] Tumor-associated carbohydrate antigens (TACAs) are attractive targets for anti-tumor vaccines due to their high levels of expression in tumor cells.[84] However, the development of an effective carbohydrate-based antitumor vaccine is extremely challenging. In nature, TACAs are often expressed as a heterogeneous mixture. As a result, it is difficult to obtain sufficient quantities of TACAs in conjugatable forms through isolation. In addition, there are concerns of highly active trace contaminants present in isolated samples. Thus, synthesis becomes critical to produce these complex molecules.[85]

In addition to the challenge of accessing TACAs, the immunological obstacle to a successful vaccine is that TACAs are T cell independent B cell antigens.[84] When administered alone, they generally produce low titers of low affinity IgM antibodies, which do not persist for a long time. To induce high affinity IgG antibodies, a typical approach is to conjugate TACAs with carriers containing helper T (Th) cell epitopes, which include immunogenic proteins,[85b, 86] peptides,[84b, 87] multiple antigenic glycopeptides,[88] nanoparticles,[89] polymers[89c, 90] and polysaccharides.[91] Recently, we have demonstrated that self-assembled virus like particles (VLPs) could deliver a TACA, the Tn antigen to the immune system and generate powerful antibody responses.[92] The induced antibodies bound strongly with Tn expressing tumor cells, resulting in tumor cell death and protection of immunized mice from tumor development.[92a]

Building on the success of VLP-Tn studies, we become interested in testing whether the VLP platform could potently induce antibody responses against another important family of

TACAs, that is, the gangliosides,[83c] as represented by GM2. GM2 contains a sialic acid terminated branched tetrasaccharide linked to a ceramide chain. GM2 is expressed on the surfaces of a wide range of human cancers, which include cancer cells of neuroectodermal origin (melanoma, sarcoma and neuroblastoma) as well as epithelial cancers such as breast and prostate cancers.[84c, 93] The wide expression of GM2 on multiple types of cancer renders it an intriguing target for developing a potentially "universal" anticancer vaccine. In addition, clinical studies have shown that elevated levels of anti-GM2 IgM antibodies are strongly associated with prolonged survival of melanoma patients.[94] Both passive administration of anti-GM2 monoclonal antibodies[95] and active immunity gained through vaccination[94a, 96] could lead to favorable prognosis, such as tumor regression or longer disease-free intervals. These clinical outcomes have inspired the drive towards GM2-based anticancer vaccines.[94a, 97]

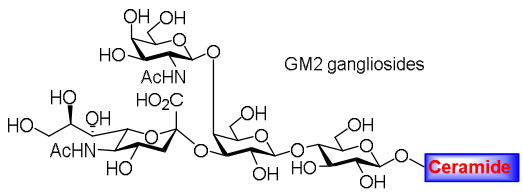


Figure 4.1. The GM2 family of gangliosides.

The generation of antibodies is a highly complex process. Many structural features of the construct can significantly impact the results of antibody responses. Livingston and coworkers showed that the anti-GM2 antibody titers were highly dependent upon the carrier moiety of the vaccine construct.[98] The Lo-Man group demonstrated that GM2 coupled with a Th epitope through the copper catalyzed azide alkyne cycloaddition (CuAAC) reaction gave good titers of anti-GM2 antibodies.[97c] Yet, when the same Th cell peptide was conjugated with two GM2 molecules, despite the higher valency, it failed to elicit detectable levels of IgM or IgG antibodies in mice even after repeated immunizations. Thus, the structure of a vaccine construct needs to be carefully designed and evaluated. Herein, we report our results on using synthetic

GM2 antigens to conjugate with the VLP bacteriophage Qβ for the induction of anti-tumor antibodies.

4.2 Results and Discussion

Prior anti-GM2 vaccine studies have primarily utilized GM2 glycan extracted from mammalian tissues[94a, 97a] or prepared through enzymatic synthesis.[97b, 97c, 99] Chemical synthesis can bestow flexibility in functionalizing the antigen for immunological investigations. Although GM2 glycans have been chemically synthesized previously,[100] with the need for stereoselective sialylation and formation of branched glycans, its preparation in a conjugatable form is not a trivial task. Our synthetic target was the GM2 tetrasaccharide **73**, bearing a reducing end free amine, which was prepared by regioselective sialylation of the lactosyl diol acceptor **74** by sialyl donor **75**, followed by glycosylation of the 4'-OH by galactosamine (GalN) donor **76** (Figure 4.2).

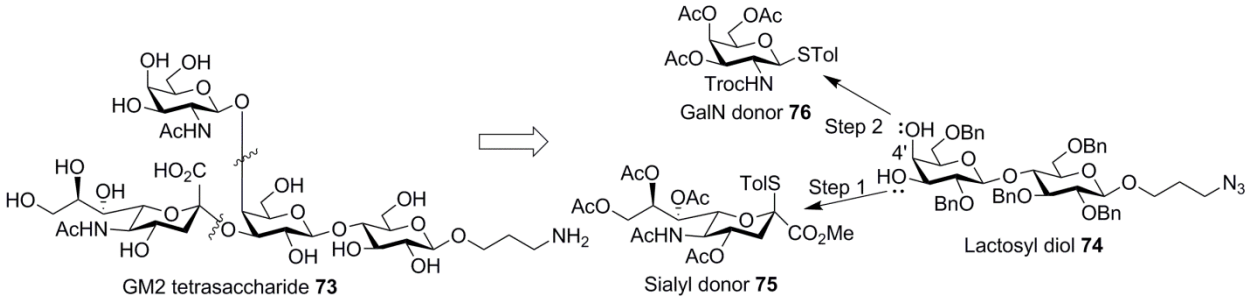


Figure 4.2. Retrosynthetic analysis of GM2 tetrasaccharide **73**.

Our synthesis commenced with lactoside **77**,[101] which was derived from D-lactose and subsequently transformed to diol **74** through protective group manipulations (Figure 4.2a). Sialylation of acceptor **74** was performed with thiosialoside donor **75**. Initial coupling of **74** and **75** was mediated using *N*-iodosuccinimide (NIS) and triflic acid as the promoter, which gave the desired α-sialoside **78** in 42% yield, along with 8% of the β-anomer. The stereochemistry of the

newly formed glycosyl linkage of **78** was assigned based on the 3-bond coupling constant between C_1 and H_{3ax} of sialic acid ($^3J_{C1,H3ax}$ = 8 Hz) as well as that between H-7 and H-8 of sialic acid ($^3J_{H\text{-}7,H\text{-}8}$ = 7.9 Hz).[102] Regioselectivity was confirmed by the correlation between C_2 of sialic acid with $H_{3'}$ of the lactose unit in the HMBC NMR spectrum. In order to improve the sialylation yield, various reaction conditions were examined. Whereas changing the solvent, reaction time, or temperature did not lead to significant enhancement, the combination of *p*-TolSCl/AgOTf[51b, 103] as the promoter system improved the yield of **78** to 65% with no evidence of the β-anomer. Recently, modified sialyl donors with groups such as 4-*O*,5-*N* oxazolidinone, and 5-*N*-trifluoroacetyl have been shown to give high yields and stereoselectivities in sialylation reactions.[104] Donor **75** has the advantage that no additional synthetic steps were needed to adjust the protective groups on C-5 of sialic acid, while achieving good yield and stereoselectivity. With trisaccharide **78** in hand, its glycosylation by the GalN donor **76** was carried out by using the *p*-TolSCl/AgOTf promoter system producing the protected GM_2 **79** in 63% yield with the new glycosidic bond being exclusively β ($^1J_{H1,C1\ of\ GalN}$ = 161.4 Hz, $^3J_{H1,H2\ of\ GalN}$ = 8.8 Hz).[105]

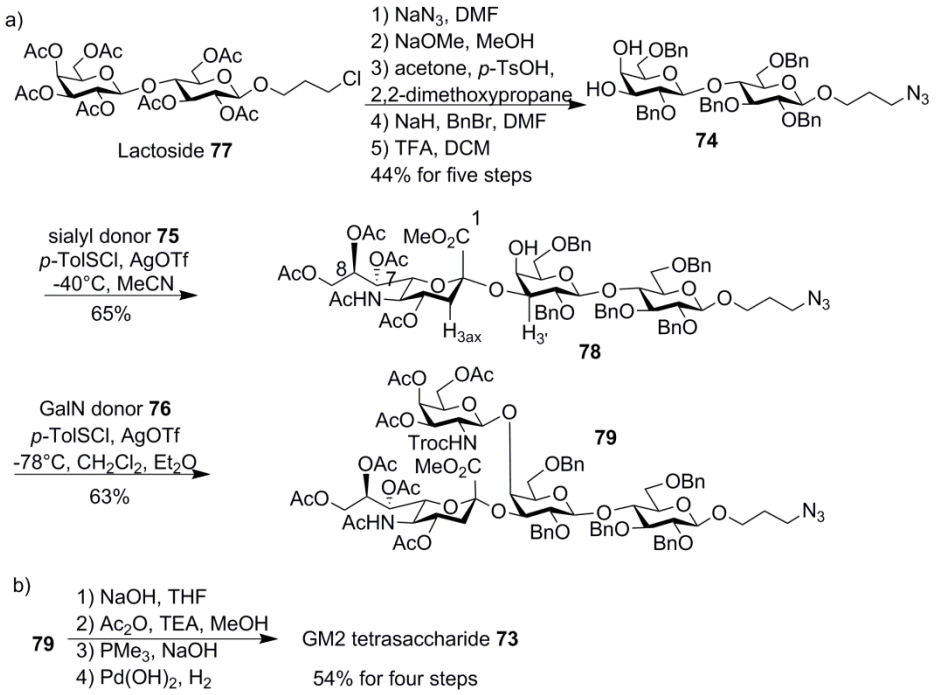


Figure 4.3. Synthesis of GM2 tetrasaccharide **73**.

Compound **79** was deprotected in four steps, starting from the hydrolysis of *O*-acetyl groups concomitant with Troc removal (Figure 4.2b). The newly freed amino group on GalN was selectively acetylated with acetic anhydride in methanol. Finally Staudinger reduction of the azido group and global debenzylation with Pearlman's catalyst provided the fully deprotected GM2 tetrasaccharide **73** in 54% yield over the four deprotection steps.

With the GM2 glycan in hand, we prepared GM2 conjugate vaccine with the VLP bacteriophage Qβ as the carrier, as we have previously shown that Qβ is superior to several other VLP platforms in boosting anti-Tn immunity.[92b] Our initial approach for bioconjugation utilized the CuAAC reaction, due to its high reaction rate, mild reaction condition, and bio-orthogonal nature.[106] GM2 **73** was treated with the activated ester **80** to attach an azide moiety to the reducing end) for bioconjugation (GM2 **81**, 77% yield; Figure 4.4a). Subsequently, **81** was coupled with the alkyne functionalized Qβ **82** under CuAAC condition, which introduced 237

copies of GM2 antigen to each Qβ capsid (Figure 4.3b). The remaining free alkyne groups on Qβ were capped with 3-azidopropan-1-ol **84** to afford Qβ-GM2 **85**.

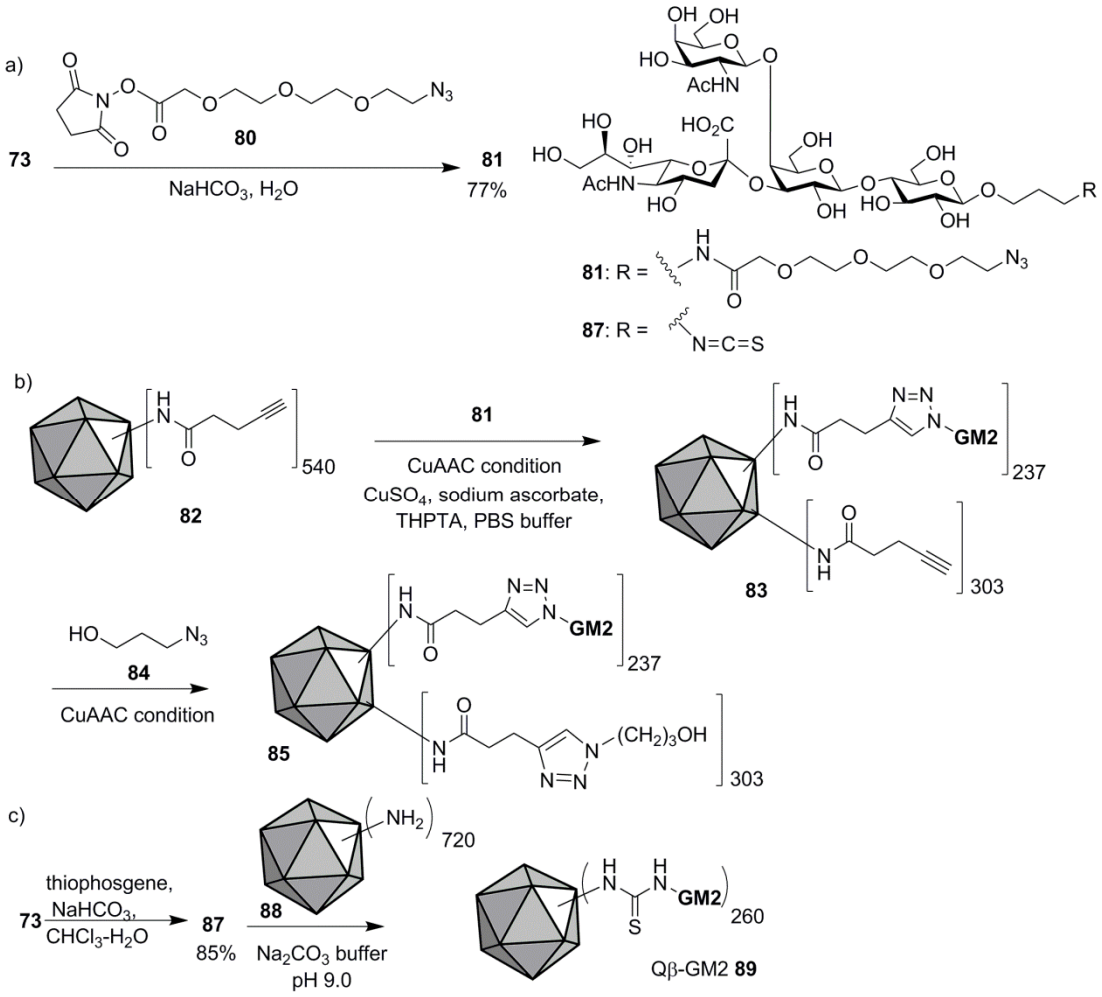


Figure 4.4 Synthesis of GM2-QB conjugates.

Next, the ability of Qβ-GM2 **85** to generate anti-GM2 antibodies was evaluated. C57BL/6 mice were immunized subcutaneously with three biweekly injections of Qβ-GM2 **85**, and sera from these mice were collected one week after the final boost injection. The control group of mice received the unconjugated Qβ only. For enzyme linked immunosorbent assay (ELISA) analysis of serum antibodies, a bovine serum albumin (BSA) conjugate of GM2 (BSA-GM2 **86**) was prepared through reductive amination with glutaraldehyde,[107] with an average of

11 GM2 glycans coupled to BSA. ELISA analysis showed no significant binding to BSA-GM2 **86** by any post-immune sera compared to the control sera from mice immunized with Qβ only. To test serum binding with GM2 expressed in its native environment, that is, on tumor cell surface, flow cytometry analysis of all sera were performed. None of the sera were able to bind with GM2-positive human lymphoma Jurkat cells even at a relatively high concentration (1:10 dilution). These results demonstrated that Qβ-GM2 **85** was unable to elicit high titers of anti-GM2 antibodies *in vivo*.

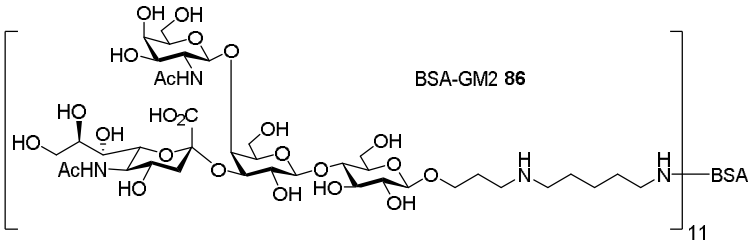


Figure 4.5. BSA-linked GM2 construct.

To better understand Qβ-GM2 **85** vaccine, the epitope profiles of antibodies generated were screened by ELISA. BSA conjugates to structural components of GM2-*N*-acetyl galactosamine (GalNAc),[92c] lactose, GM3, as well as BSA-triazole[92a] were synthesized and immobilized onto ELISA plates. Although there were some IgG bindings to BSA-GalNAc, BSA-GM3 and BSA-GM2, the binding to BSA-triazole was significantly stronger (Figure 4.7). This suggests that the triazole linker is the dominant epitope among the components analyzed.

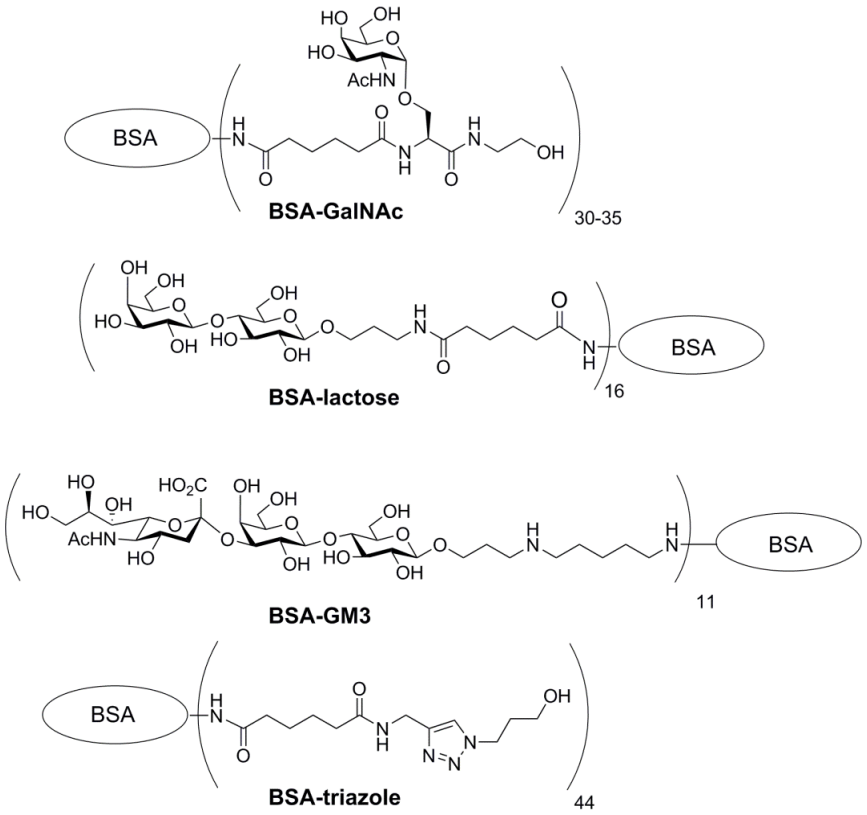


Figure 4.6. Various BSA constructs used to test serum binding specificity.

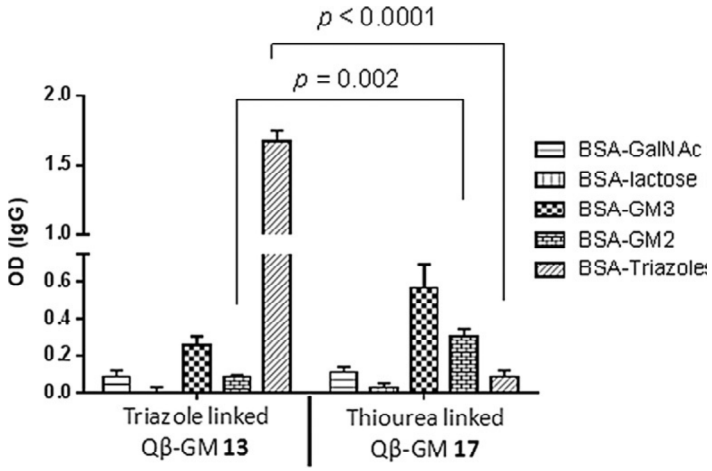


Figure 4.7. ELISA analysis of the epitope profiles of post-immune sera from mice immunized with triazole linked Qβ-GM2 conjugate **85** and thiourea linked Qβ-GM2 **89** respectively. For 13, the anti-triazole antibody level was significantly higher than other types of antibodies, such as anti-GM2 or anti-GM3 antibodies (p>0.0001). Qβ-GM2 **89** induced significantly higher anti-GM2 antibodies (p = 0.002) but much lower levels of anti-triazole antibodies (< 0.0001) than did **85**. Sera from each group were analyzed at 1600 fold dilution. The average optical density value and SEM were shown. Statistics were performed by Student's t-test.

To avoid the antibody responses to the triazole linker, alternative strategies were explored. Previously, we showed that reducing the number of triazoles on the Qβ by removing the triazole used to cap the unreacted alkynes did not lead to enhanced anti-glycan responses.[108] Therefore, other members of the group including Zhaojun Yin, Claire Baniel, and Sherif Ramadan, utilized another bioconjugation approach to ligate GM2 to Qβ. Their work is included to provide a completed picture. Treatment of GM2 **73** with thiophosgene converted the amine group to isothiocyanate[109] in 85% yield (Figure 4.4c). The resulting GM2 **87** was incubated with the wild-type Qβ particle **88** at pH = 8.5 to afford the Qβ-GM2 conjugate **89**. This reaction proceeded smoothly, introducing an average of 220 copies of GM2 per Qβ particle (Figure 4.4c).

With Qβ-GM2 **17** in hand, mice were immunized. In contrast to Qβ-GM2 **85**, ELISA analysis of post-immune sera showed good anti-GM2 IgG and IgM antibody responses, with IgG as the main antibody type (Figure 4.8a). The subclasses of IgG antibodies were also determined. The levels of IgG2 antibodies (IgG2b and IgG2) were much higher than those of IgG1 and IgG3, suggesting a more Th1-weighted immune response (Figure 4.8b).[110] This is likely due to the ability of Qβ to encapsulate single stranded *E. coli* RNA in the interior, which are potent agonists of Toll like receptors **7** and **8** for immune-potentiation favoring a Th1 response.[111] The antibodies elicited by Qβ-GM2 **89** could bind with multiple types of GM2 positive tumor cells, as determined by flow cytometry (Figures 4.8c and d), whereas sera from the control mice receiving Qβ or the pre-immunized mice did not show any tumor cell recognition.

The epitope profiles of antibodies induced by Qβ-GM2 **89** were analyzed by ELISA (Figure 4.7). The antibodies exhibited strongest binding to BSA-GM3, but the recognition of BSA-GalNAc and BSA-lactose was much weaker. This suggests that the sialic acid motif contains the major recognition sites of GM2. This observation is consistent with a literature

report in which the removal of sialic acid from GM2 abrogated the binding by anti-GM2 polyclonal antibodies.[97c]

To assess the therapeutic potential of anti-GM2 antibodies, we evaluated the complement-dependent cytotoxicity against tumor cells. The classical pathway of complement activation is triggered by multivalent binding between C1 complex and Fc region of antibodies.[112] Compared to other IgG subclasses, the IgG2 antibodies in mice have the strongest abilities to initiate the complement cascade.[113] As shown in Figure 4.8E, the antibodies induced by Qβ-GM2 **89** were able to efficiently kill GM2-positive Jurkat cells by the complement mechanism.

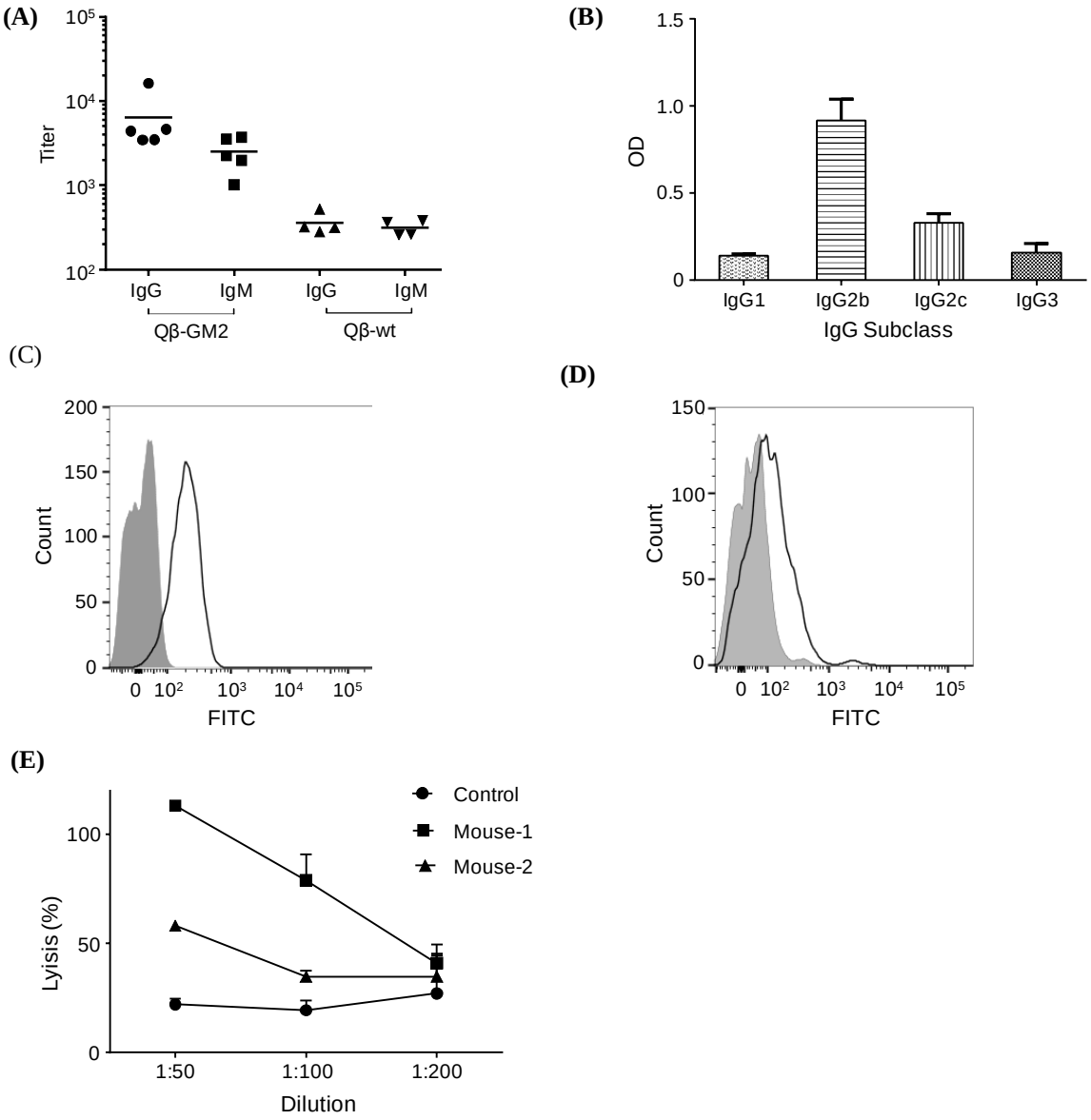


Figure 4.8. Immunological evaluation of Qβ-GM2 conjugate vaccine **89**. (A) IgM and IgG titer of anti-GM2 antibodies tested by ELISA. Sera from mice immunized with wild type Qβ particle were tested as control; (B) The levels of anti-GM2 IgG subclasses as determined by ELISA. Sera were tested at 1:1000 dilution. (C) Binding of GM2-expressing Jurkat cells and (D) MCF-7 cells with representative mouse sera diluted at 1:20. Grey filled: pre-immune sera and sera from mice immunized with Qβ only; solid line: day 35 sera from a mouse immunized with Qβ-GM2 **89**; (E) complement-dependent toxicity against Jurkat cells measured by LDH assay. Sera from two mice immunized with Qβ-GM2 **89** are shown (mouse 1: ■, mouse 2: ▲). Pre-immune serum was utilized as a control (●). Sera from mice immunized with Qβ gave similar results as the pre-immune sera.

The CuAAC reaction and the triazole linker have been commonly used in carbohydrate-based vaccines.[92b, 92c, 114] In our recent studies on Qβ-Tn conjugates, we observed that the triazole linked Qβ-Tn failed to induce antibodies capable of recognizing Tn expressed on tumor cell TA3HA, which was attributed to the possible hindrance of Tn-specific B cell binding to the vaccine construct by anti-triazole antibodies.[92a] The inability of the triazole-containing Qβ-GM2 **85** to generate anti-GM2 antibodies was consistent with the Qβ-Tn results, suggesting that the detrimental effect of triazole on anti-TACA immunity was not restricted to a small antigen such as Tn, which contains only a monosaccharide *N*-acetyl galactosamine linked with serine or threonine. Although the exact reasons for the suppressive effect of triazole on anti-GM2 antibody responses need further investigations, these results indicate that caution should be taken in applying CuAAc chemistry in future glycan-based vaccine design.

Compared to GM2 vaccine candidates reported to date,[94a, 97] the Qβ-GM2 **89** elicited similar total titers of anti-GM2 IgG antibodies and bindings to GM2-positive tumor cells. Conjugates such as KLH-GM2 produced more IgG1 and IgG3 in human patients.[98] Qβ-GM2 **89** elicited higher titers of IgG2, which can be potentially advantageous for future clinical applications, as mouse IgG2s have been recognized as the most efficient IgG subclass to induce effector functions against tumor cells.[115]

In conclusion, we have established an efficient chemical synthesis of GM2 glycans. The synthetic approach can bestow flexibilities to prepare GM2 derivatives such as GM2 lactones[116] in the future to further enhance the immunogenecity of the antigen. In order to develop a GM2-based vaccine, our first generation approach utilized the CuAAC reaction linking 237 copies of GM2 onto a VLP carrier protein-bacteriophage Qβ. However, no significant anti-GM2 antibodies were generated over control. To overcome this obstacle, isothiocyanate chemistry was

employed introducing GM2 glycan onto Qβ. The resulting Qβ-GM2 conjugate, **89**, was able to induce high titers of anti-GM2 antibodies, in particular IgG2 antibodies. The antibodies produced were capable of binding GM2-expressing tumor cells and exhibited complement-dependent cytotoxicity, lysing the tumor cells. Therefore, these results demonstrate that bacteriophage Qβ can be an effective vaccine platform for a GM2-based vaccine. Studies are ongoing to optimize the GM2 antigen structure as well as the vaccine construct, to further enhance the vaccine efficacy.

4.3 Experimental Section

4.31 General Experimental Procedures.

All reactions were performed under a nitrogen atmosphere with anhydrous solvents. Solvents were dried using a solvent purification system. Glycosylation reactions were performed with 4Å molecular sieves that were flamed dried under high vacuum. Chemicals used were reagent grade unless noted. Reactions were visualized by UV light (254 nm) and by staining with either $Ce(NH_4)_2(NO_3)_6$ (0.5 g) and $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (24.0 g) in 6% H_2SO_4 (500 mL), 5% H_2SO_4 in EtOH, or for deprotected oligosaccharides 0.2 g 1,3-dihydroxynaphthalene in 50 mL of 5% H_2SO_4 in EtOH. Flash chromatography was performed on silica gel 601 (230-400 Mesh). NMR spectra were referenced using residual $CHCl_3$ (δ ^{1}H-NMR 7.26 ppm ^{13}C-NMR 77.0 ppm). Peak and coupling constants assignments are based on ^{1}H-NMR, ^{1}H-^{1}H gCOSY, ^{1}H and ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C gHMQC.

4.32 Characterization of Anomeric Stereochemistry

The stereo-chemistries of newly formed glycosidic bonds for idose and glucosamine were determined by $^{3}J_{H1,H2}$ through ^{1}H-NMR and/or $^{1}J_{C1,H1}$ through gHMQC 2-D NMR (without 1H decoupling). Smaller $^{3}J_{H1,H2}$ (3 Hz) indicate α linkages and larger $^{3}J_{H1,H2}$ (7 Hz or larger) indicate β linkages. $^{1}J_{C1,H1}$ couplings around 170 Hz suggest α linkages while constants around 160 Hz imply β linkages. For Sialic acid anomeric configuration was done by analysis of $J_{H\text{-}7,H\text{-}8}$.[117] The β anomer of acetylated sialic acid normally has a small $J_{H\text{-}7,H\text{-}8}$ value, 2.4-2.6 Hz, while the α anomer has much larger coupling value of 6.2-8.2 Hz.

Methyl 5-acetamindo-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-p-tolyl-2-thio-D-β-galacto-2-nonulopyranosylonate (75). Compound **75** was prepared from sialic acid in 3 steps. First sialic acid (5.00 g, 16.2 mmol) were dissolved in dry methanol (25 mL) to which 2 grams of Dowex-50-hydrogen resin was added. The mixture was heated to 60 °C for 2 hours after which it was filtered and concentrated. The concentrate and DMAP (187 mg, 1.6 mmol) was then dissolved in pyridine (50 mL) and cooled to 0 °C. To this mixture was added acetic anhydride (30.0 mL, 272 mmol) and the reaction was allowed to warm to rt and stir for two hours. The solvents were evaporated and the residue dissolved in DCM. The solution was then washed sequentially with 10% HCl, sat. $NaHCO_3$, brine, and then dried over Na_2SO_4. The crude product was taken with no further purification. The acetylated product was dissolved in DCM (30 mL) to which of *p*-toluene thiol (2.41 g, 19.4 mmol) was added. This mixture was cooled to 0 °C after which $BF_3{\cdot}Et_2O$ (10 mL, 81 mmol) was added dropwise. The reaction was stirred overnight and was quenched by diluting with DCM and addition of sat. $NaHCO_3$. The organic layer was separated and dried over Na_2SO_4 and concentrated. The crude product was recrystallized from EtOAc/hexanes providing **75** (6.91 g, 11.6 mmol, 71% yield over 3 steps). The product was

found to be a 9:1 ratio of β:α. by analysis of $J_{H\text{-}7,H\text{-}8}$ as the major component of **75** has a coupling of 2.4 Hz, within the expected range of β-sialic acid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 – 7.31 (2 H, m,), 7.18 – 7.12 (2 H, m), 5.47 (1H, t, *J* = 2.4, H-7), 5.39 (1 H, ddd, *J* = 11.6, 10.5, 4.8, H-4), 4.99 (1 H, dt, *J* = 8.5, 2.4, H-8), 4.62 (1 H, dd, *J* = 10.5, 2.4, H-6), 4.51 (1 H, dd, *J* = 12.3, 2.4, H-9), 4.14 (1 H, q, *J* = 10.5 Hz, H-5), 4.04 (1 H, dd, *J* = 12.3, 8.5, H-9'), 3.62 (3 H, s), 2.66 (1 H, dd, *J* = 13.8, 4.8, H-3), 2.35 (3 H, s), 2.12 (3 H, s), 2.09 (3 H, s), 2.08 – 2.06 (1 H, m, H-3'), 2.05 (3 H, s), 1.98 (3 H, s), 1.92 (3 H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.22, 171.13, 170.46, 170.42, 168.49, 140.39, 136.45, 130.10, 125.44, 89.05, 73.33, 73.21, 69.26, 69.07, 62.90, 52.81, 49.78, 37.58, 23.44, 21.55, 21.31, 21.09, 20.98, 20.93. HRMS $M+H^+$ $C_{20}H_{29}NO_{12}$ Calc. 475.1684 Obsv. 475.1687

***p*-Tolyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (76).** Compound **76** was prepared from galactosamine hydrochloride in three steps. First galactosamine hydrochloride (2.0 g, 9.3 mmol) was dissolved in methanol (100 mL) to which sodium methoxide (0.50 g, 9.3 mmol) was added. The mixture was stirred for 30 minutes at rt and then 2,2,2-trichloroethyl chloroformate (1.3 mL, 9.3 mmol) was added dropwise along with TEA (200 µL). The reaction was stirred at rt for two hours and then concentrated. The residue was dissolved in pyridine (10 mL) and cooled to 0 °C. Once cooled acetic anhydride (5.0 mL, 53 mmol) and DMAP (113 mg, 9.3 mmol) were added and the reaction was allowed to warm to rt and stir overnight. The reaction was diluted with EtOAc and washed with 5% $CuSO_4$ and brine then dried over Na_2SO_4 and concentrated. The compound was purified by a silica gel column (hexane-EtOAc 3:1). The per-acetylated compound (3.15 g, 6 mmol) was dissolved in DCM (75 mL) along with *p*-toluenethiol (1.1 g, 9 mmol) and cooled to 0 °C. $BF_3 \cdot Et_2O$ (2.3 mL, 18 mmol) was added dropwise and the reaction was allowed to stir at 0

°C for 2 hours and warm to rt and stir for 2 hours. The mixture was diluted with DCM (125 mL) and washed with sat. $NaHCO_3$, water then dried over Na_2SO_4 and concentrated. A 3:1 hexane-EtOAC column provided **76** (1.8 g, 3.8 mmol, 41% yield over three steps). ^{1}H NMR (500 MHz, $CDCl_3$) 7.44 (2 H, d, *J* = 8.1), 7.13 (2 H, d, *J* = 7.8), 5.39 (1 H, d, *J* = 2.8 H-1), 5.21 – 5.15 (1 H, m, H-2), 4.84 (1 H, d, *J* = 10.3, H-4), 4.80 (1 H, d, *J* = 12.2), 4.74 (1 H, d, *J* = 12.1), 4.19 (1 H, dd, *J* = 11.2, 7.0, H-6), 4.12 (1 H, td, *J* = 7.0, 2.9, H-6'), 3.92 (2 H, m, H-3, H-5), 2.35 (3 H, s, $SPhCH_3$), 2.13 (3 H, s, $COCH_3$), 2.05 (3 H, s, $COCH_3$), 1.99 (3 H, s, $COCH_3$). ^{13}C NMR (125 MHz, $CDCl_3$) 260.78, 170.63, 170.53, 170.38, 154.18, 138.70, 133.39, 129.92, 128.85, 87.77, 74.71, 74.63, 71.15, 67.14, 61.93, 51.54, 21.40, 20.92, 20.89, 20.86. $^3JH_1,H_2$ = 2.8 Hz HRMS $M+H^+$ $C_{15}H_{20}Cl_3NO_9$ Calc. 463.0198 Obsv. 463.0205

3-Chloropropyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (77). Compound **77** was prepared in two steps from lactose. First sodium acetate (6.25 g, 76 mmol) was dissolved in acetic anhydride (50 mL, 0.53 mol). This was heated to reflux, around 150 °C. Lactose monohydrate (25 g, 69 mmol) was added in portions and the mixture was refluxed for an additional 20 minutes then cooled without solidifying. The mixture was then poured into water (300 mL) and triturated. The resulting oil was washed and triturated three times with 250 mL portions of water. The resulting product was then recrystallized from ethanol (250 mL) with stirring providing per-acetylated lactose (30.35 g, 44.8 mmol). This product (10.0 g, 14.7 mmol) was dissolved DCM (200 mL) along with 1-chloro-3-propanol (1.5 mL, 18 mmol) and cooled to 0 °C. $BF_3{\cdot}Et_2O$ (5.6 mL, 44 mmol) was added dropwise and the reaction was warmed to rt over 2 hours then allowed to stir an additional 3 hours. The reaction was diluted with DCM and washed with sat. $NaHCO_3$. The organic layer was then dried over Na_2SO_4 and concentrated. A 1:1 hexane-EtOAc column provided **77** (6.4 g, 9

mmol, 40% yield over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) 5.44 (1 H, d, *J* = 3.4), 5.30 (1 H, t, *J* = 9.5), 5.21 (1 H, dd, *J* = 10.1, 8.0), 5.05 (1 H ddd, *J* = 10.4, 3.4, 0.5), 4.98 (1 H dd, *J* = 9.2, 8.3), 4.61 – 4.56 (3 H, m), 4.25 – 4.15 (3 H, m), 4.07 – 4.02 (1 H, m), 3.97 (1 H, t, *J* = 6.8), 3.89 (1 H, t, *J* = 9.4), 3.80 – 3.75 (1 H, m), 3.72 (1 H, d, *J* = 9.7), 3.69 (2 H, t, *J* = 6.2), 2.25 (3 H, s), 2.22 (3 H, s), 2.18 – 2.12 (14 H, m), 2.06 (3 H, s). HRMS $M+H^+$ $C_{29}H_{42}ClO_{18}$ Calc. 713.2054 Obsv. 713.2052 This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[101]

3-Azidopropyl 3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (90). **77** (11.8 g, 16.6 mmol) was dissolved in DMF (40 mL) along with sodium azide (10.8 g, 166 mmol). The reaction was heated to 80 °C and stirred overnight. The reaction was allowed to cool to rt before being diluted with EtOAc (300 mL). The mixture was then washed with brine three times. The organic layer was dried over Na_2SO_4 and concentrated. The product was taken with no further purification and dissolved in methanol (300 mL) to which NaOMe (0.894 g, 16.5 mmol) was added. The reaction was stirred overnight at rt. The progress of the reaction was monitored by MS. The reaction was then neutralized with Amberlite IR 120 resin, filtered, and concentrated. The crude product was taken on without purification and dissolved in acetone (150 mL) that had been dried over 4Å sieves. To this mixture was added 2,2-dimethoxy propane (30.8 mL, 0.25 mmol) and p-toluenesulfonic acid (315 mg, 1.6 mmol). The reaction was then stirred at rt overnight. The reaction was neutralized by addition of TEA and concentrated. The crude solid was dissolved in a 10:1 mixture of methanol-water and refluxed for 3 hours. After cooling to rt the solvents were removed and a 8:1 DCM-MeOH column was run providing **90** (4.16 g, 8.9 mmol, 54% over three steps). ^{1}H NMR (600 MHz, $CDCl_3$) 4.17 (1 H, dd, *J* = 8.1, 3.7), 4.12 (1 H, dd, *J* = 7.8, 3.8), 3.98 – 3.88 (7 H, m), 3.81 – 3.74

(2 H, m), 3.72 – 3.68 (1 H, m), 3.68 – 3.66 (2 H, m), 3.63 – 3.59 (1 H, m), 3.51 – 3.46 (1 H, m), 3.44 – 3.36 (2 H, m), 3.35 – 3.31 (1 H, m), 3.28 – 3.25 (2 H, m), 3.24 – 3.22 (1 H, m), 3.17 – 3.12 (1 H, m), 1.74 – 1.69 (2 H, m), 1.33 (3 H, s), 1.17 – 1.15 (3 H, s). HRMS $M+H^+$ $C_{18}H_{32}N_3O_{11}$ Calc. 466.2031 Obsv. 466.2024 This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[101]

3-Azidopropyl 2,6-di-*O*-benzyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (91). 90 (1.96 g, 4.2 mmol) was dissolved in DMF (20 mL) to which NaH (1.68 g, 42 mmol) was added. After stirring for 30 minutes benzyl bromide (5.0 mL, 42 mmol) was slowly added and the reaction was stirred at rt overnight. The reaction was diluted with EtOAc (200 mL) then washed with water, sat. NH_4Cl, and brine. After washing the solution was dried over Na_2SO_4 and concentrated. A column (two fractions 4:1 hexane-EtOAc then 3:1 hexane-EtOAc) provided **91** (3.42 g, 3.7 mmol, 89% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.36 – 7.17 (25 H, m), 4.91 (1 H, d, *J* = 10.5), 4.79 (1 H, d, *J* = 11.1), 4.76 (1 H, d, *J* = 11.8), 4.70 (2 H, m), 4.64 (1 H, d, *J* = 11.8), 4.54 (1 H, d, *J* = 12.1), 4.48 (1 H, d, *J* = 12.0), 4.39 (1 H, d, *J* = 12.1), 4.37 (1 H, d, *J* = 8.0), 4.34 (1 H, d, *J* = 7.8), 4.29 (1 H, d, *J* = 12.0 Hz), 4.08 (1 H, dd, *J* = 5.6, 1.6), 4.01 – 3.99 (1 H, m), 3.95 (1 H, dd, *J* = 9.9, 5.8), 3.94 – 3.91 (1 H, m), 3.78 (1 H, dd, *J* = 10.9, 4.3), 3.69 (1 H, dd, *J* = 11.0, 1.8), 3.67 – 3.64 (2 H, m), 3.59 (1 H, ddd, *J* = 10.0, 7.1, 5.4), 3.54 (1 H, t, *J* = 9.1 Hz), 3.51 (1 H, dd, *J* = 12.6, 9.0), 3.38 – 3.36 (3 H, m), 3.34 (1 H, dd, *J* = 6.9, 5.6), 3.32 (1 H, dd, *J* = 7.2, 6.1), 1.91 – 1.81 (2 H, m), 1.38 (s, 3H), 1.33 (s, 3H). HRMS $M+H^+$ $C_{53}H_{62}N_3O_{11}$ Calc. 916.4384 Obsv. 916.4380. This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[101]

3-Azidopropyl 2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (74). 91 (3.41 g, 3.7 mmol) was dissolved in DCM (50 mL) and cooled to 0 °C. After 15 minutes, trifluoroacetic acid (5.0 mL, 74 mmol) of was added dropwise followed by water (5 mL). The reaction was allowed to warm to rt and stir overnight. The mixture was then diluted with DCM and washed with water, sat. $NaHCO_3$, dried over Na_2SO_4 and concentrated. A 1:1 hexane-EtOAc column was run and provided **74** (2.97 g, 3.38 mmol, 91% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.22 (25 H, m), 5.00 (1 H, d, *J* = 11.0), 4.85 (1 H, d, *J* = 11.1), 4.81 (2 H, d, *J* = 11.1), 4.78 (1 H, d, *J* = 10.9), 4.75 (1 H, d, *J* = 11.0), 4.68 (1 H, d, *J* = 11.6), 4.61 (1 H, d, *J* = 12.2), 4.45 (3 H, dt, *J* = 7.8, 4.0), 4.40 (1 H, d, *J* = 12.1), 4.38 (1 H, d, *J* = 7.8), 4.01 (1 H, t, *J* = 10.2), 3.99 (1 H, dd, *J* = 5.9, 4.1), 3.97 – 3.96 (1 H, m), 3.82 (1 H, dd, *J* = 10.9, 4.1), 3.75 (1 H, dd, *J* = 11.0, 1.8), 3.65 – 3.60 (2 H, m), 3.59 (1 H, d, *J* = 9.0), 3.51 (1 H, dd, *J* = 9.9, 5.0), 3.45 – 3.38 (6 H, m), 3.38 – 3.35 (1 H, m), 2.49 – 2.47 (1 H, m), 2.41 – 2.39 (1 H, m), 1.94 – 1.86 (2 H, m). HRMS M+H$^+$ $C_{50}H_{58}N_3O_{11}$ Calc. 876.4066 Obsv. 876.4068. This compound has been previously prepared and comparison of ^{1}H-NMR with reported literature confirmed the structure.[101]

3-Azidopropyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**78**). Compound **78** was prepared from donor **75** and lactose acceptor **74**. Donor **75** (1.40 g, 2.34 mmol) was dissolved in acetonitrile (30 mL) along with **74** (1.03 g, 1.17 mmol) and 3 g of 4Å molecular sieves. This was stirred at rt for 30 minutes then cooled to -40 °C. Silver triflate (1.21 g, 4.7 mmol) of dissolved in acetonitrile (5 mL) was added and the reaction was stirred for 10 min at -40 °C. Then promoter, *p*-toluenesulfenyl chloride (338 µL, 2.34 mmol) was added directly into the solution. The reaction was allowed to

slowly warm to -10 °C over three hours. When the reaction appeared to be complete it was diluted with DCM and filtered through celite. The mixture was washed with sat. $NaHCO_3$ and concentrated. After a gradient column, starting at 3:1 toluene-Acetone and increasing 5% each fraction, **78** (1.03 g, 0.76 mmol, 65% yield) was isolated. Also isolated was the β linked anomer (0.21 g, 0.15 mmol, 13% yield). ^{1}H NMR (500 MHz, $CDCl_3$) 7.48 – 7.14 (30 H, m), 5.43 (1 H, ddd, *J* = 7.9, 4.4, 1.8, H-8''), 5.34 (1 H, dd, *J* = 7.9, 2.1, H-7''), 5.26 (1 H, d, *J* = 10.0), 5.02 (1 H, d, *J* = 10.7), 4.92 – 4.86 (1 H, m, H-4''), 4.86 – 4.81 (1 H, m), 4.81 – 4.74 (3 H, m), 4.70 (1 H, d, *J* = 11.7), 4.60 (1 H, d, *J* = 7.7, H-1'), 4.52 (2 H, q, *J* = 12.2), 4.47 (1 H, d, *J* = 12.0), 4.38 (1 H, d, *J* = 6.2), 4.36 – 4.31 (2 H, m, H-9a''), 4.13 (1 H, t, *J* = 10.3, H-5''), 4.08 (1 H, dd, *J* = 9.0, 3.8, H-3'), 4.05 – 3.95 (4 H, m, H-6''), 3.88 – 3.84 (1 H, m), 3.78 (3 H, s, CO_2Me), 3.75 – 3.68 (3 H, m), 3.65 – 3.58 (2 H, m), 3.57 (2 H, d, *J* = 8.9 Hz, H-2'), 3.54 – 3.49 (2 H, m), 3.44 – 3.35 (5 H, m), 2.76 – 2.72 (1 H, m), 2.54 (1 H, ddd, *J* = 8.8, 6.8, 2.7, H-3eq), 2.11 (3 H, s), 2.06 (1 H, m, H-3ax), 2.04 (3 H, s), 2.00 (3 H, s), 1.92 (2 H, s), 1.90 – 1.85 (5 H, m, H-3'). ^{13}C NMR (125 MHz, $CDCl_3$) 171.03, 170.80, 170.52, 170.24, 170.15, 168.61, 139.33, 139.19, 138.86, 138.69, 138.66, 129.26, 128.54, 128.50, 128.45, 128.39, 128.36, 128.28, 128.14, 127.84, 127.81, 127.74, 127.71, 127.54, 127.43, 103.71, 102.55, 98.68, 83.21, 82.06, 78.68, 77.58, 77.32, 77.07, 76.62, 76.60, 75.62, 75.35, 75.23, 75.18, 73.56, 73.31, 73.01, 72.77, 69.38, 69.13, 68.72, 68.64, 68.16, 67.47, 66.68, 62.54, 53.27, 49.49, 48.59, 36.72, 29.52, 23.39, 21.36, 21.06, 20.96, 20.78. $^1J_{C1BH1B}$ = 163 Hz $^1J_{C1AH1A}$ = 162.5 Hz $J_{H\text{-}7,H\text{-}8}$ = 7.9 Hz. HRMS M+NH_4^+ $C_{70}H_{88}N_5O_{23}$ Calc. 1366.5865 Obsv. 1366.5867

3-Azidopropyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→4)-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-2,6-di-*O*-benzyl-β-D-

galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**79**). Compound **79** was prepared from **76** (120 mg, 0.205 mmol) and acceptor **78** (230 mg, 0.170 mmol). The two oligosaccharides were dissolved in DCM (10 mL) along with 0.9 g of freshly activated 4Å molecular sieves for 30 minutes at rt. The mixture was then cooled to -78 °C followed by the addition of silver triflate (105 mg, 0.409 mmol) dissolved in diethyl ether (2 mL). After stirring for 10 minutes *p*-toluenesulfenyl chloride (29.5 µL, 0.205 mmol) was added directly to the solution and was observed to disappear. The reaction was allowed to warm to -20 °C over 2.5 hours and was then diluted with DCM. The mixture was then washed with sat. $NaHCO_3$, dried over Na_2SO_4, and concentrated. A gradient toluene-acetone column (4:1, then 3:1, then 7:3 etc.) provided **79** (194 mg, 0.107 mmol, 63% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 – 7.47 (2 H, m), 7.43 – 7.09 (23 H, m), 6.14 (1 H, d, *J* = 9.0, NHTroc), 5.44 – 5.35 (1 H, m), 5.27 – 5.14 (5 H, m), 5.11 (1 H, d, *J* = 12.3), 5.03 (1 H, d, *J* = 8.8, H-1’’’), 4.92 (1 H, d, *J* = 9.6), 4.88 – 4.80 (2 H, m), 4.79 – 4.66 (3 H, m), 4.63 (1 H, d, *J* = 11.8), 4.58 – 4.46 (2 H, m), 4.44 (1 H, d, *J* = 7.5, H-1’) 4.41 – 4.36 (1 H, m), 4.34 (1 H, d, *J* = 7.8, H-1), 4.33 – 4.22 (4 H, m), 4.21 – 4.15 (1 H, m, H-2’’’), 4.07 – 3.88 (11 H, m), 3.81 (1 H, dd, *J* = 7.5, 9.6, H-3’’), 3.79 – 3.69 (3 H, m), 3.65 – 3.57 (1 H, m), 3.56 – 3.53 (1 H, m), 3.51 (1 H, dd, *J* = 7.5, 9.6, H-2’), 3.42 – 3.32 (6 H, m), 2.19 – 2.12 (3 H, m), 2.12 – 2.09 (3 H, m), 1.98 – 1.92 (12 H, m), 1.89 (3 H, s), 1.88 – 1.84 (5 H, m). ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.24, 173.04, 172.94, 172.87, 172.28, 172.16, 171.19, 157.15, 141.35, 141.14, 140.99, 140.76, 131.96, 131.01, 130.96, 130.95, 130.90, 130.88, 130.83, 130.72, 130.54, 130.46, 130.26, 130.18, 130.13, 130.07, 130.02, 129.97, 129.78, 106.13, 104.81, 104.43, 101.90, 85.44, 84.34, 81.56, 81.00, 78.83, 78.67, 77.90, 77.80, 77.75, 76.88, 76.09, 75.96, 75.72, 74.84, 74.30, 72.60, 71.86, 71.16, 70.89, 70.22, 69.87, 69.15, 69.10, 64.70, 63.84, 56.17, 55.83, 55.58, 51.71, 50.96, 37.63, 31.90, 25.80, 23.67, 23.49, 23.43, 23.33, 23.26, 23.14, 23.09. $^1J_{C1B-}$

$_{H1B}$ = 161.4 Hz $^{1}J_{C1B-H1B}$ = 163.2 Hz $^{1}J_{C1C-H1C}$ = 162.6 Hz $^{3}J_{H-1'''*H2'''}$ = 8.8 Hz. HRMS M+Na^{+} $C_{85}H_{102}Cl_3N_5NaO_{32}$ Calc. 1832.5466 Obsv. 1832.5457

3-Azidopropyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylic acid-(2→3)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**73**) Compound **73** was prepared from **79** in four deprotection steps. **79** (60 mg, 33 µmol) was dissolved in THF (25 mL) to which 1 M NaOH (0.7 mL) was added and the reaction was stirred at 50 °C overnight. The reaction was confirmed complete by crude MS so the solvents were evaporated and the solid was dissolved in DCM (50 mL) and washed with brine. The DCM was dried over Na_2SO_4 and concentrated. The crude product was dissolved in MeOH (5 mL) to which TEA (46 µL, 330 µmol) and acetic anhydride (31 µL, 330 µmol) were added. The reaction was then stirred at rt overnight. After confirmation of the reaction completion by crude MS the reaction was quenched with a few drops of water and diluted with EtOAc. The solution was then washed with sat. $NaHCO_3$, dried over Na_2SO_4 and concentrated. The crude product was layer onto a Sephadex LH-20 column and eluted with 1:1 DCM-MeOH solution and the acetylated product (34 mg, 25 µmol) was isolated. This was dissolved in THF (5 mL) to which 1 M trimethyl phosphine (124 µl, 124 µmol, in THF) was added followed by 0.1 M NaOH (620 µL, 62 µmol). The reaction was stirred at rt overnight and was quenched by the addition of 0.1 M HCl until pH = 7. The mixture was then concentrated and the solid was dissolved in a mixture of 1:1 DCM-MeOH (2 mL) and eluted from a Sephadex LH-20 column. The reduced product (30 mg, 22 µmol), was dissolved in MeOH (2 mL) and water (1 mL). To this solution was added $Pd(OH)_2$ (250 mg, 1.8 mmol). The atmosphere was removed by vacuum and replaced by H_2 and stirred overnight. The reaction was filtered through cotton followed by a 0.22 µm syringe filter to remove the catalyst then concentrated. The solid was

dissolved in water (2 mL) and eluted from a Sephadex G-15 column providing **73** (16 mg, 18 mmol, 54% yield over four steps). ^{1}H NMR (500 MHz, D_2O) δ 4.57 (1 H, d, *J* = 8.6, H-1’’’), 4.40 – 4.34 (2 H, m, H-1, H-1’), 3.99 (1 H, dd, *J* = 9.8, 1.5, H-3’), 3.97 – 3.92 (1 H, m), 3.86 – 3.80 (2 H, m), 3.77 (2 H, dd, *J* = 13.1, 3.5, H-2’’), 3.73 – 3.69 (2 H, m), 3.69 – 3.59 (9 H, m, H-4sia), 3.57 – 3.53 (2 H, m, H-3, H-3’’), 3.48 (4 H, dd, *J* = 11.7, 4.4, H-3), 3.46 – 3.42 (2 H, m), 3.39 (2 H, dd, *J* = 11.7, 6.6), 3.32 (1 H, d, *J* = 10.0), 3.22 – 3.18 (2 H, m, H-2, H-2’), 2.68 (1 H, t, *J* = 6), 2.52 – 2.48 (1 H, dd, *J* = 12.6, 4.5, H-3sia), 1.87 (3 H, s), 1.86 (3 H, s), 1.80 – 1.73 (1 H, m, H-3sia). HRMS $[M-H]^-$ $C_{34}H_{58}N_3O_{24}$ Calc 892.3416 Obsv. 892.3416

11-Azido-3,6,9-trioxaundecanoic acid NHS ester (80). 11-Azido-3,6,9-trioxaundecanoic acid (25 mg, 0.11 mmol) was dissolved in DCM (5 mL) to which was added DCC (27 mg, 0.13 mmol) and NHS (14 mg, 0.12 mmol). This was stirred overnight and then filtered and concentrated. Crude NMR showed the product **80** so it was taken without further purification. ^{1}H NMR (500 MHz, CD_3OD) δ 3.94 – 3.91 (2 H, m), 3.85 – 3.81 (5 H, m), 3.61 – 3.56 (1 H, m), 3.54 – 3.51 (2 H, m), 3.00 – 2.97 (4 H, m), 2.17 – 2.13 (1 H, m), 2.02 – 1.95 (1 H, m), 1.89 – 1.82 (3 H, m), 1.76 – 1.72 (1 H, m), 1.55 – 1.44 (1 H, m), 1.38 – 1.32 (1 H, m), 1.31 – 1.21 (1 H, m).

GM2 derivative (81)

80 (35 mg, 106 µmol) and $NaHCO_3$ (20 mg, 240 µmol) were placed in a round bottom. **1** (20 mg, 22 µmol) was dissolved in water (2 mL) and added to the round bottom. The reaction was sonicated to dissolve all the materials. The reaction stirred at rt for 90 minutes. The reaction was then layered onto a Sephadex G-15 column and eluted with water. **81** (19 mg, 82 µmol, 77% yield. ^{1}H NMR (500 MHz, D_2O) δ 4.63 (1 H, d, *J* = 8.6, H-1’’), 4.42 (1 H, d, *J* = 7.8, H-1’), 4.37 (1 H, d, *J* = 8.1, H-1), 4.04 (1 H, dd, *J* = 9.8, 2.9), 4.01 (1 H, d, *J* = 2.9), 3.97 (2 H, s), 3.89 – 3.48 35 H, (m, H-3, H-4, H-2’’, H-3’’, H-4sia), 3.41 – 3.39 (2 H, m), 3.29 – 3.24 (3 H, m), 3.23 – 3.17 (1 H, m, H-2), 2.59 – 2.53 (1 H, m, H-3sia), 1.93 (3 H, s), 1.91 (3 H, s), 1.82 (1 H, dd, *J* = 15.2, 8.8, H-3’sia), 1.76 (2 H, m). ^{13}C NMR (125 MHz, D_2O) δ 174.96, 174.76, 174.01, 172.40, 102.70, 102.56, 102.02, 101.57, 78.61, 77.12, 74.70, 74.66, 74.32, 74.28, 73.97, 73.02, 72.72,

72.21, 71.24, 70.27, 69.96, 69.62, 69.57, 69.50, 69.46, 69.35, 69.18, 68.64, 67.96, 67.76, 67.73, 62.78, 61.10, 60.50, 52.29, 51.55, 50.11, 36.90, 35.92, 28.44, 22.56, 22.00. MS $[M]^-$ $C_{42}H_{71}N_6O_{28}$ calcd. 1107.4, obsd. 1107.4.

GM2 Derivative (87)

73 (5 mg, 5.6 µmol) was dissolved in 500 µl $NaHCO_3$ solution (10 mg/mL), then 750 µl chloroform containing thiophosgene (1.67 µl, 21.8 µmol) was added, and the mixture was stirred vigorously at room temperature. When the starting material **73** disappeared, the reaction mixture was diluted with water (5 mL), and the aqueous layer was extracted twice with chloroform (2 mL) to remove excess thiophosgene. The aqueous layer was collected and lyophilized, and the crude mixture was used directly for next step of conjugation without purification. ^{1}H NMR (500 MHz, D_2O) 4.57 (1 H, d, *J* = 8.7), 4.31-4.37 (2 H, m), 3.95-3.99 (2 H, m), 3.27-3.89 (25 H, m), 3.27-3.34 (2 H, m), 3.13-3.21 (2 H, m), 2.49 (2 H, dd, *J* = 4.51, 12.7), 1.86 (3 H, s), 1.85 (3 H, s), 1.74-1.81 (2 H, m). HRMS $[M-H]^-$ $C_{35}H_{56}N_3O_{24}S$ Calc 934.2974 Obsv. 934.2963

4.33 Bioconjugation of GM2 Antigen to Qβ Particle

Centrifugal filter units of 100,000 molecular weight cut-off (MWCO) were purchased from EMD Millipore. For protein liquid chromatography GE ÄKTA Explorer (Amersham Pharmacia) on a Superose-6 column was used. *Microfluidic capillary* gel *electrophoresis was performed with* Bioanalyzer 2100 Protein 80 microfluidics chip (Agilent Technologies). Continuous 10-40% sucrose gradients were prepared with a Biocomp GradientMaster and visualized with a Piston Gradient Fractionator (BioComp Instruments, Inc.,Fredericton, NB, Canada). For MALDI-TOF MS analysis, each viral sample (10 µL, 1 mg/mL) was denaturated and cleaned using Cleanup C18 Pipette Tips (Agilent Technologies). The mixture (0.6 µL) and

matrix solution (0.6 μL, saturated sinapic acid in 50% acetonitrile, 0.1% trifluoroacetic acid) was spotted on a MALDI plate, air-dried, and analyzed by MALDI-TOF mass spectrometry (AB SCIEX Voyager DE Pro MALDI-TOF). Protein concentration was measured using the Coomasie Plus Protein Reagent (Pierce) with bovine serum albumin as standard.

Qβ-GM2 (85): Qβ-alkyne **82**[36] (5.56 mg/mL in 0.1 M phosphate buffer, 2.6 mL, approx. 4.06 μmol of alkyne group), 1xPBS (3.21 mL), GM2-azide **81** (20 mM in DMSO, 0.304 mL, 6.09 μmol, 6 equiv per CP, which is approximately 1.50 equiv per alkyne) premixed Cu-ligand solution [$CuSO_4$, 50 mM in water, 0.244 mL, 12.2 μmol, 3 equiv per alkyne + THPTA ligand, 50 mM in water, 0.406 mL, 20.3 μmol, 5 equiv per alkyne), aminoguanidine (100 mM in water, 1.01 mL, 0.10 mmol, 25 equiv per alkyne), and sodium ascorbate (100 mM in water, 1.01 mL, 0.10 mmol, 25 equiv per alkyne). Final concentrations: Qβ particles, 2.50 mg/mL; alkyne groups on the particles, 0.70 mM; GM2-azide, 1.05 mM; Cu, 2.1 mM; THPTA ligand, 3.5 mM; aminoguanidine, 17.5 mM; sodium ascorbate, 17.5 mM. The reaction tube was sealed in a larger closed glass vial, and agitated gently by slow tumbling at room temperature for 16 hours. The VLP conjugates were purified by continuous 10-40% sucrose gradients and concentrated using centrifugal filter (MWCO: 100 kDa, 4 mL). The particles (1.5 mL, 6.87 mg/mL, 71% recovery) were composed of >95% intact particles, as determined by analytical size-exclusion fast protein liquid chromatography (FPLC) on a Superose-6 column. The GM2 content of the conjugates was determined by microfluidic electrophoresis using a Bioanalyzer 2100 Protein 80 microfluidics chip. The average GM2 loading (237 GM2 per Qβ) was determined from the ratio of the integration of the Bioanalyzer electrophoretic peaks and was confirmed by MALDI-TOF.

BSA-GM2 (86). 73 (2 mg, 10 μmol) was dissolved in 1 M sodium acetate solution (1 mL, pH 7.5) and mixed with a solution of BSA (10 mg, 0.15 μmol, in 1 M sodium acetate) and

the pH was adjusted to 8. The reaction was stirred at rt for 10 min and the development of a yellow color indicated the completion of the reaction. Then a 0.1 M sodium borohydride solution (200 µL, 20 µmol) was added to reduce the formed imines and the reaction became clear and the reaction was allowed to stir at r.t. for one hour. The mixture was then layered onto a Sephadex G-15 column and eluted with water. The average GM2 loading (11 per BSA) was determined by MALDI-TOF.[119]

Qβ-GM2 (89). A solution of Qβ-WT (4 mg, 0.28 µmol subunit, 1.1 µmol reactive amine) in 0.1 M sodium borate pH 8.5 (0.16 mL) was added GM2-ITC **87** (4 mg, 2.7 µmol, 4.8 equiv.). The reaction was spun gently on a rotisserie shaker at rt for 10 h. The reaction was diluted to 4 mL with 0.1 M potassium phosphate pH 7.0, then was transferred to an Amicon Ultra 100 kDa MW-cut-off device and was purified by centrifugal filtration against 0.1 M potassium phosphate (3 x 4 mL). The total protein concentration was determined by Bradford assay against BSA standards. Percent protein recovery was 75 %. The extent of particle modification was determined by ESI and by electrophoretic analysis. Particle stability following bioconjugation is shown by FPLC.

APPENDIX

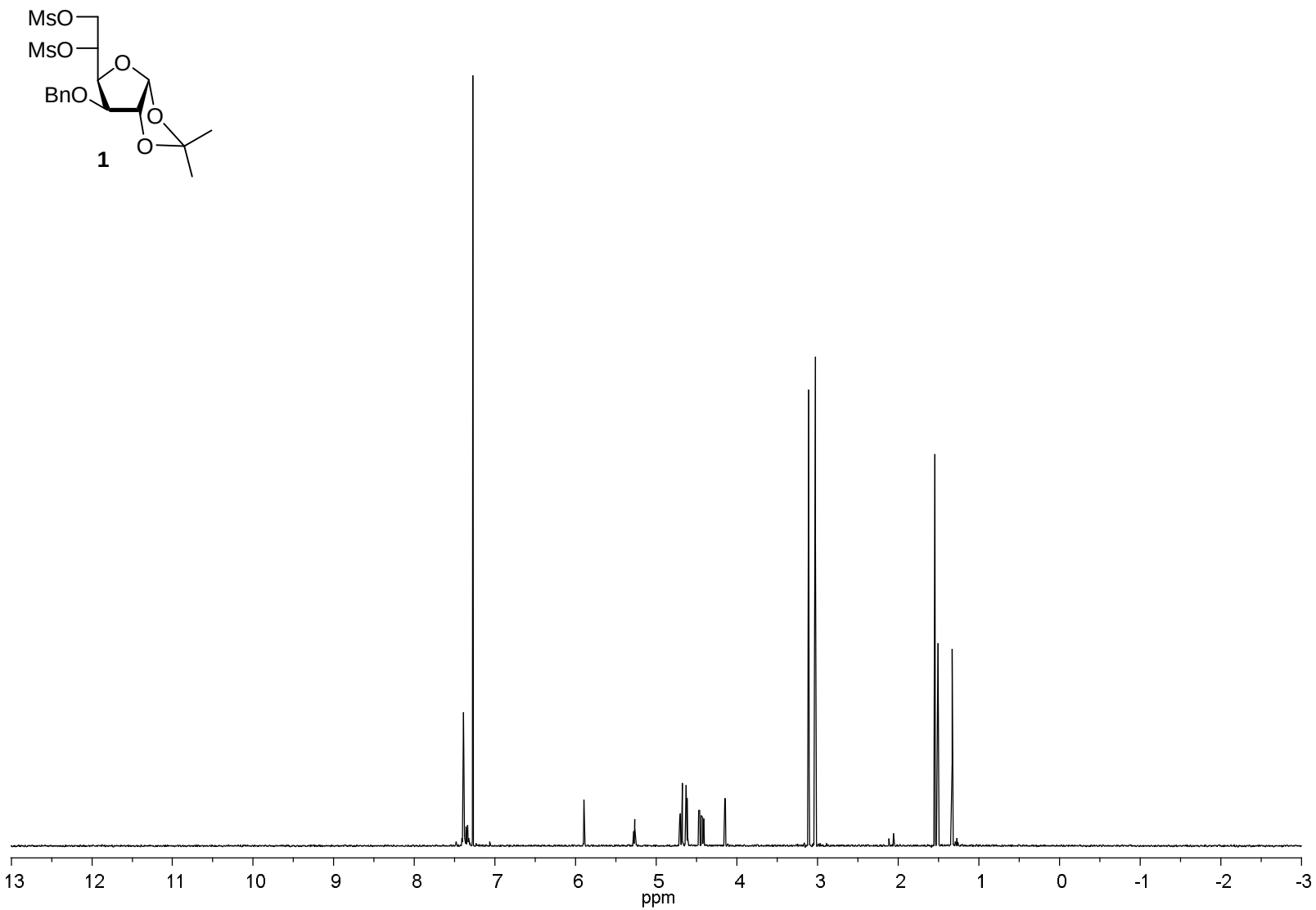


Figure 4.9. 500 MHz, $CDCl_3$ ^1H NMR of **1**

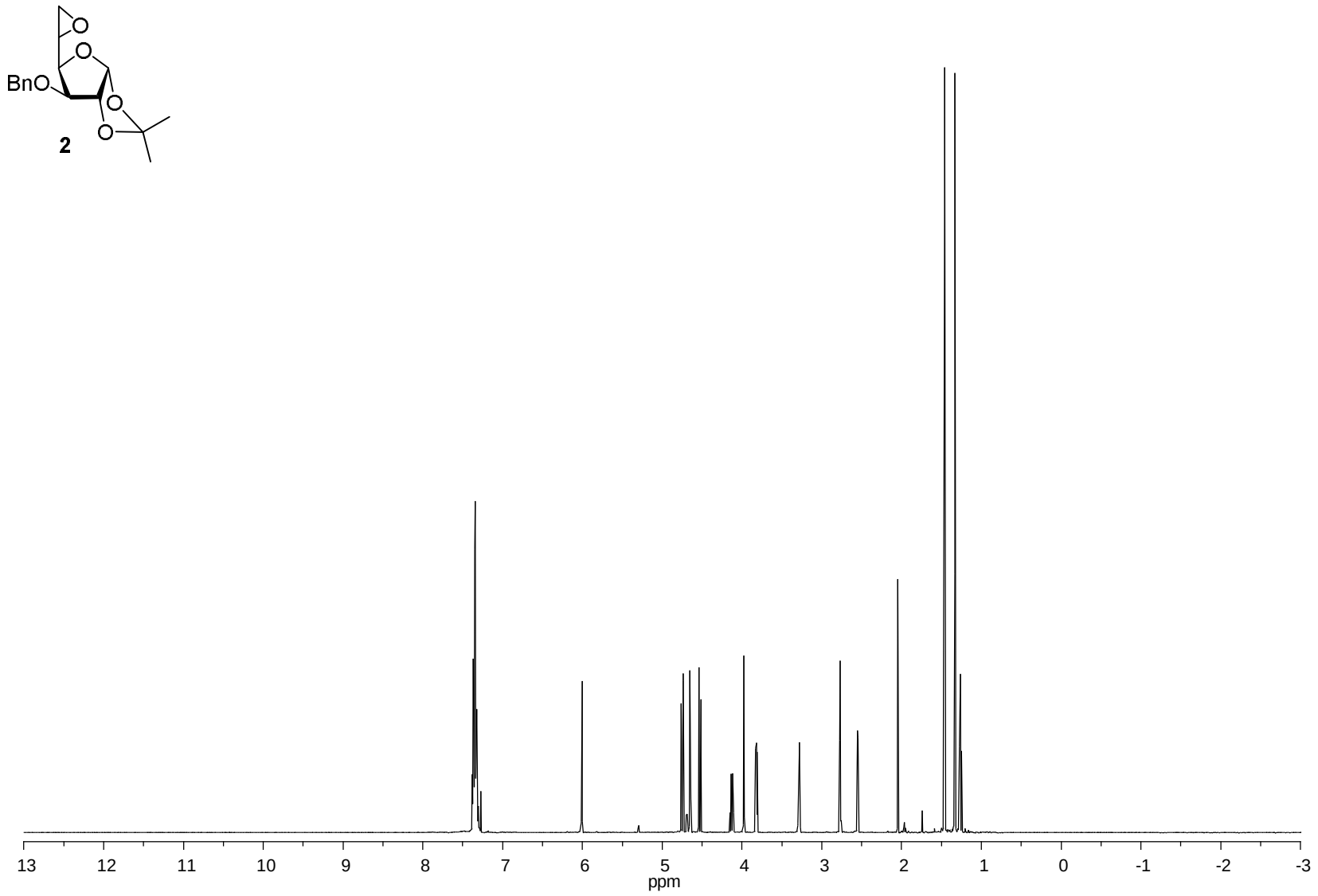


Figure 4.10. 500 MHz, $CDCl_3$ ^1H NMR of **2**

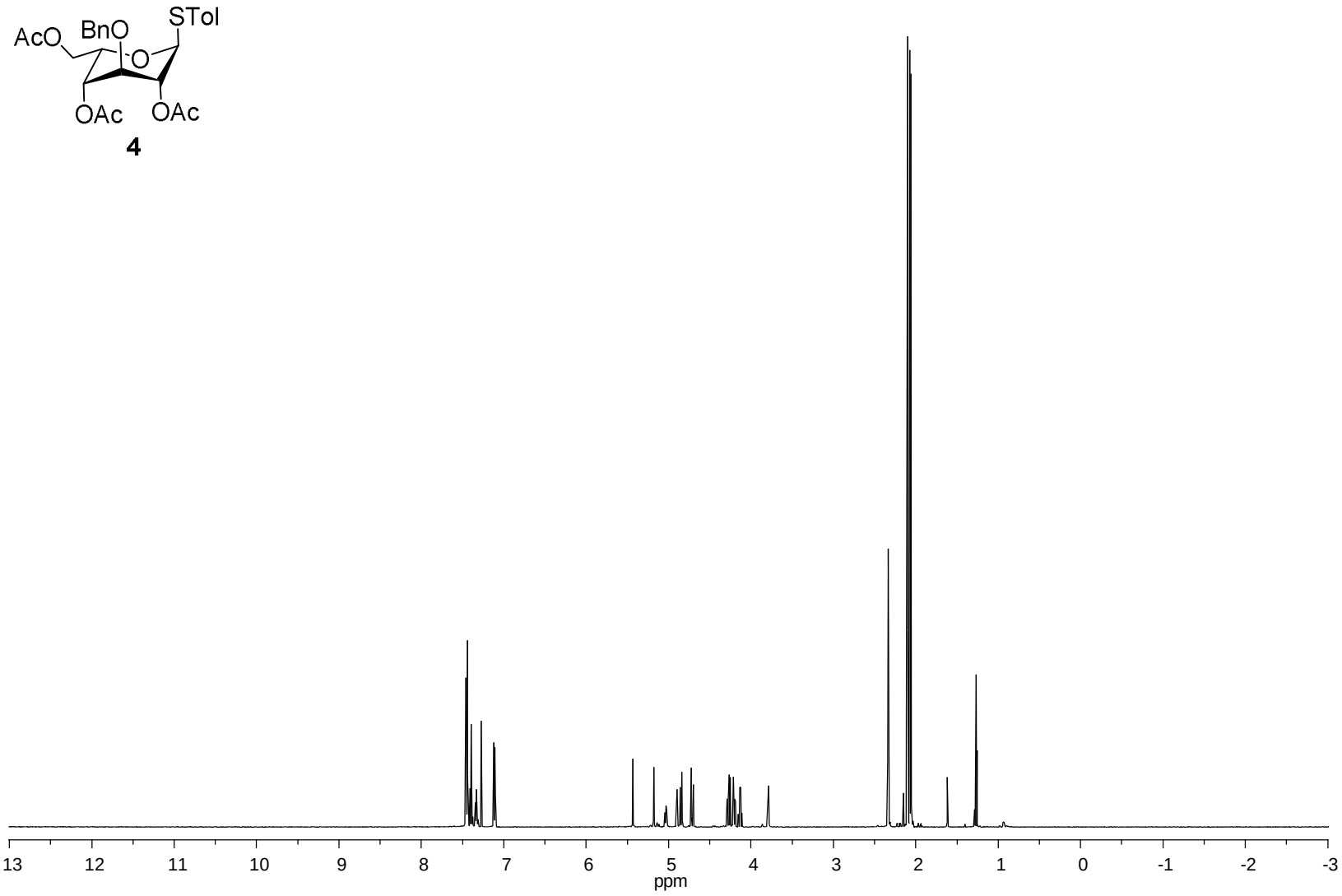


Figure 4.11. 500 MHz, $CDCl_3$ ^1H NMR of **4**

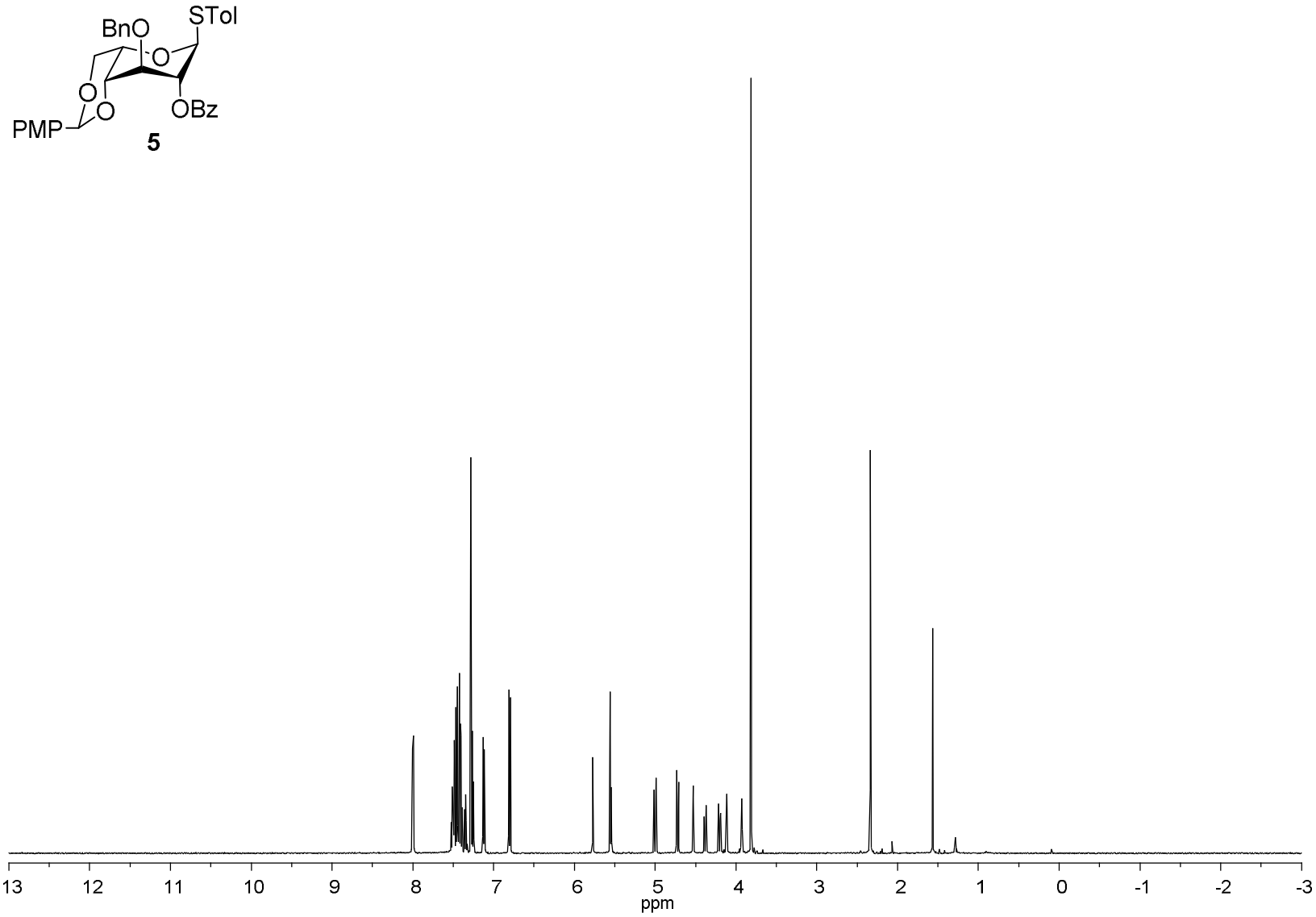
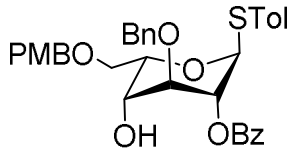


Figure 4.12. 500 MHz, $CDCl_3$ ^1H NMR of **5**



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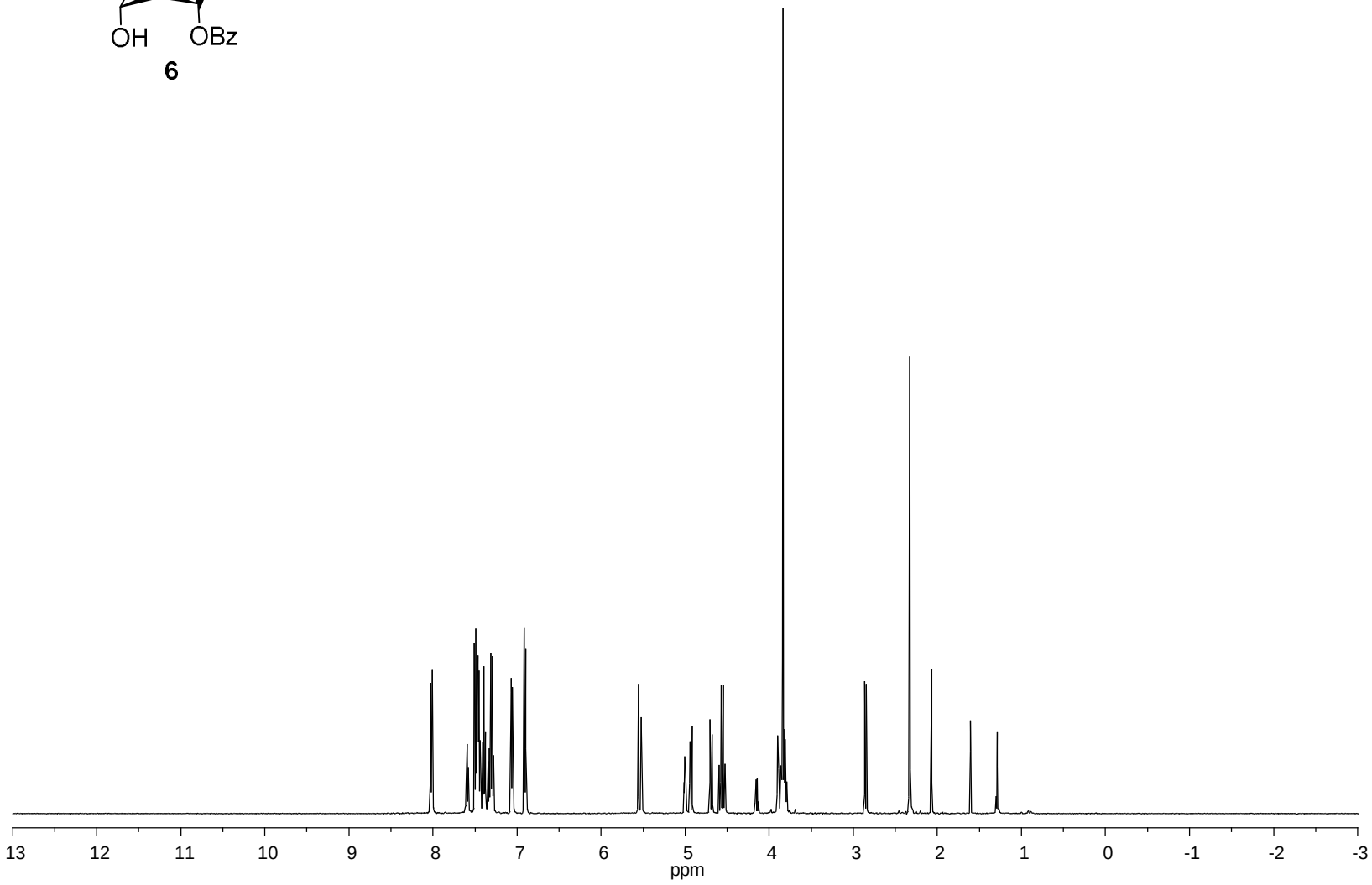


Figure 4.13. 500 MHz, $CDCl_3$ ^1H NMR of **6**

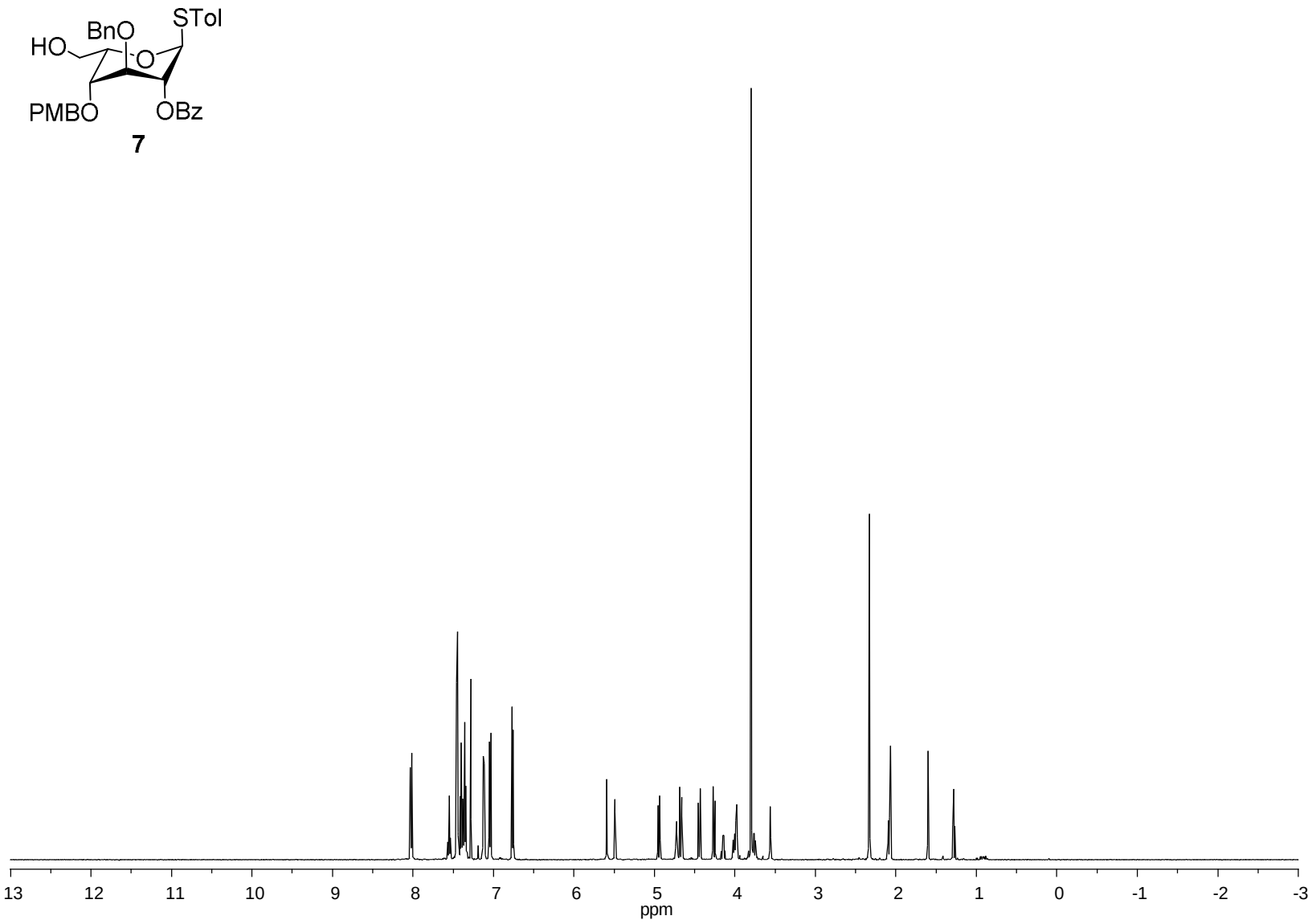
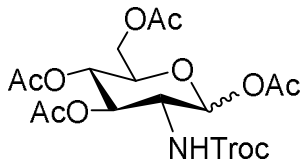


Figure 4.14. 500 MHz, $CDCl_3$ ^1H NMR of **7**



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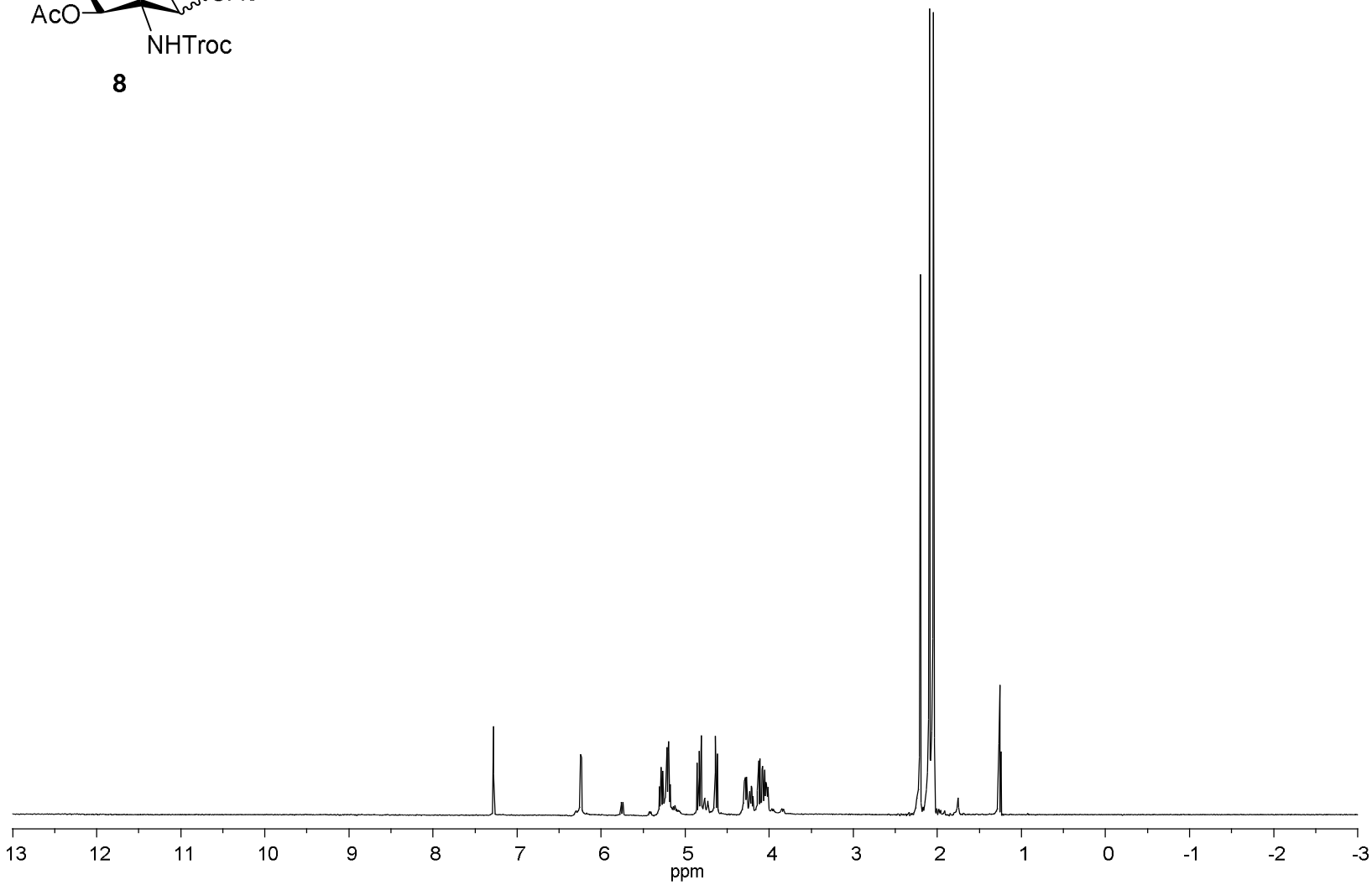


Figure 4.15. 500 MHz, $CDCl_3$ ^1H NMR of **8**

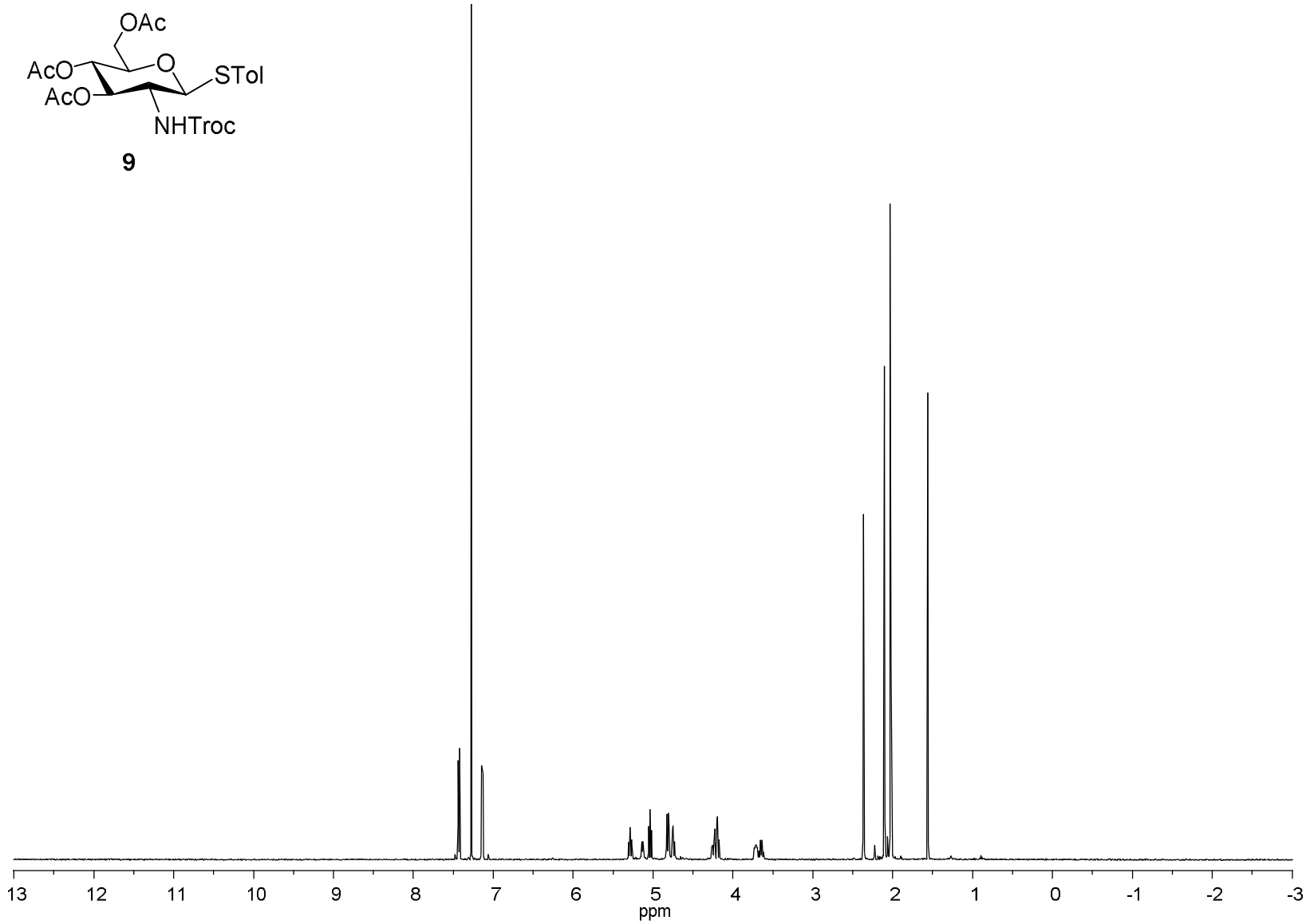
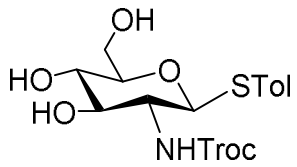


Figure 4.16. 500 MHz, $CDCl_3$ ^1H NMR of **9**



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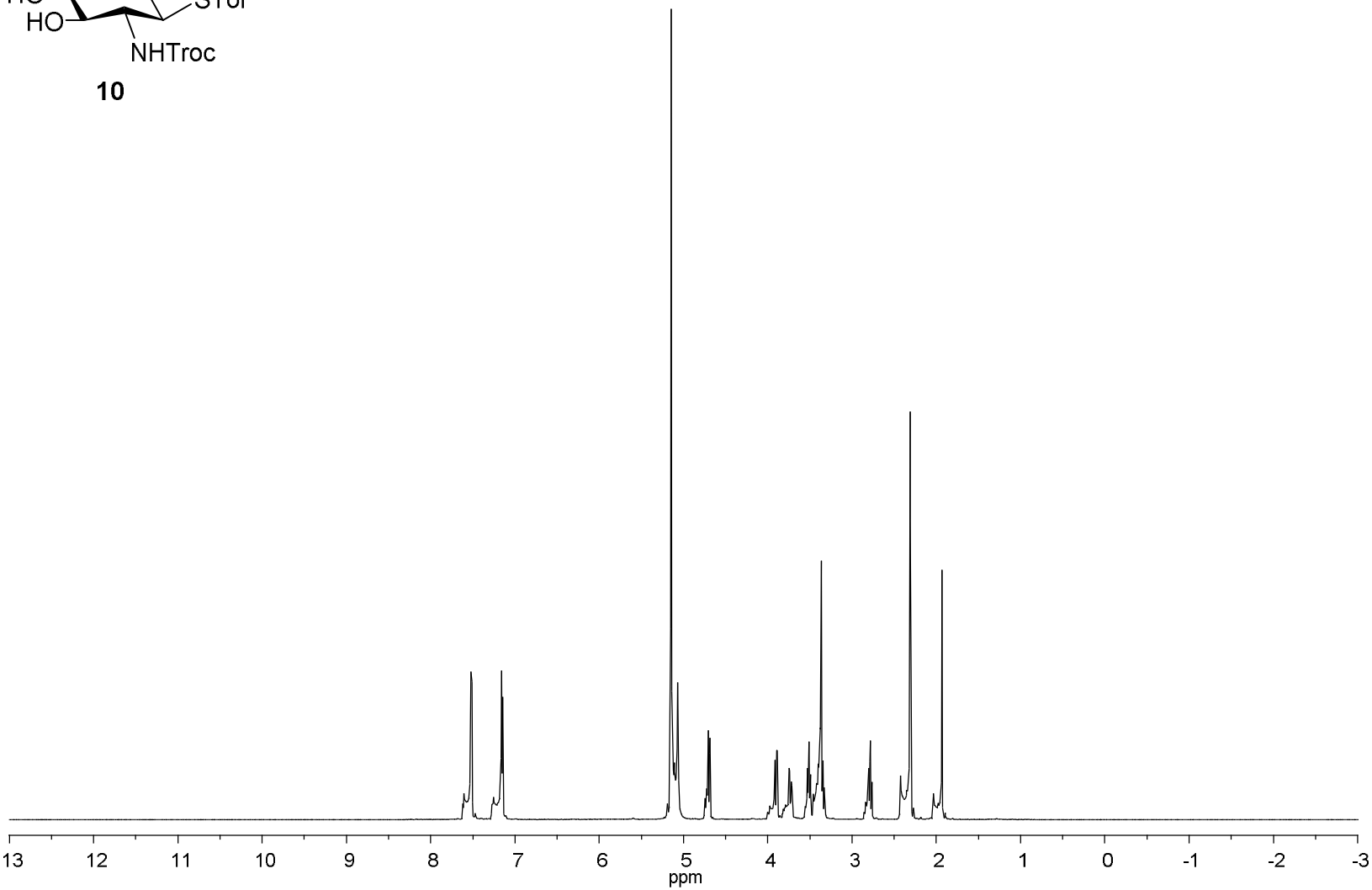
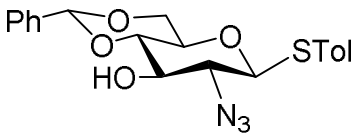


Figure 4.17. 500 MHz, $CDCl_3$ ^1H NMR of **10**



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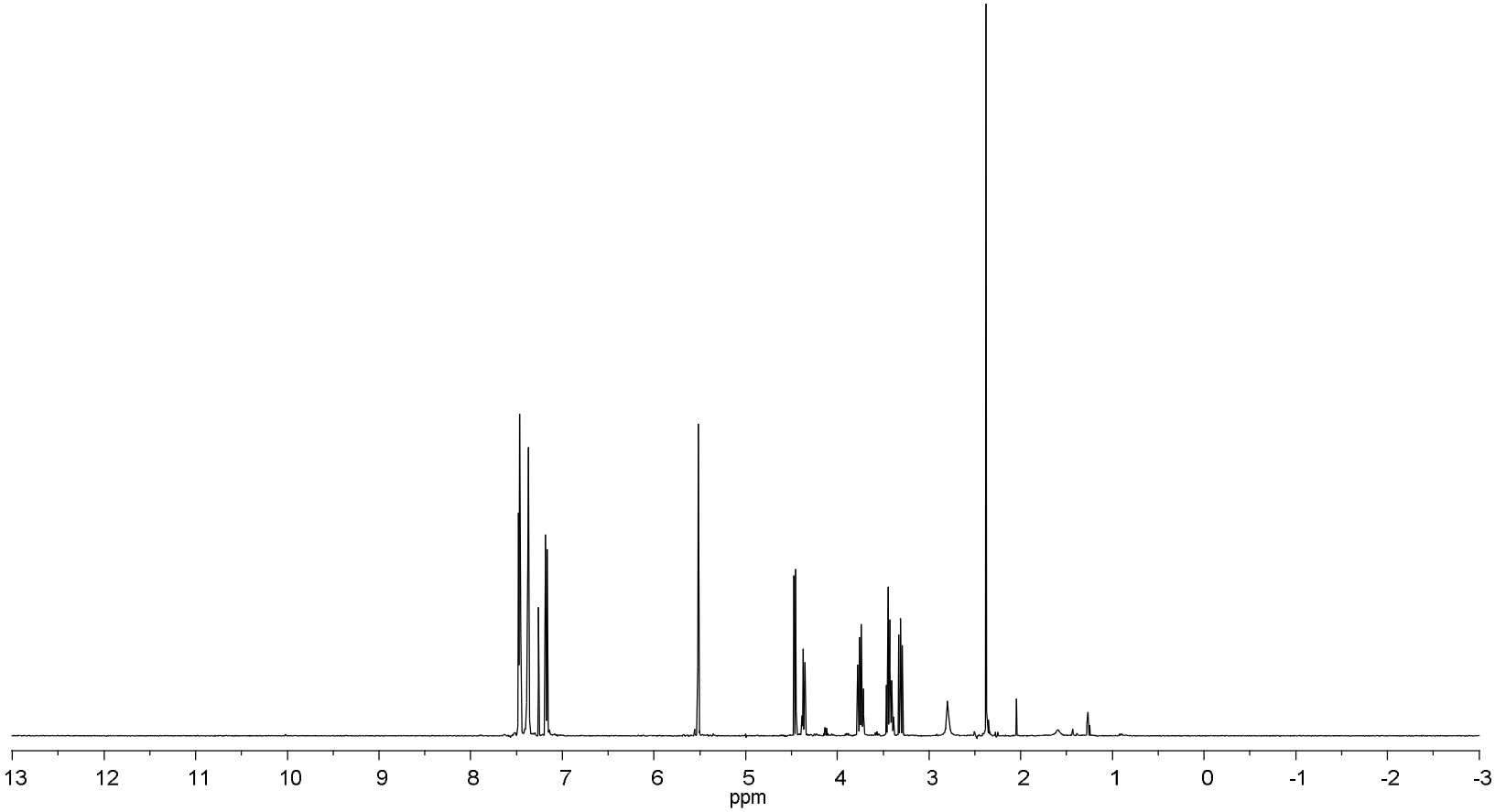


Figure 4.18. 500 MHz, $CDCl_3$ ^1H NMR of **11**

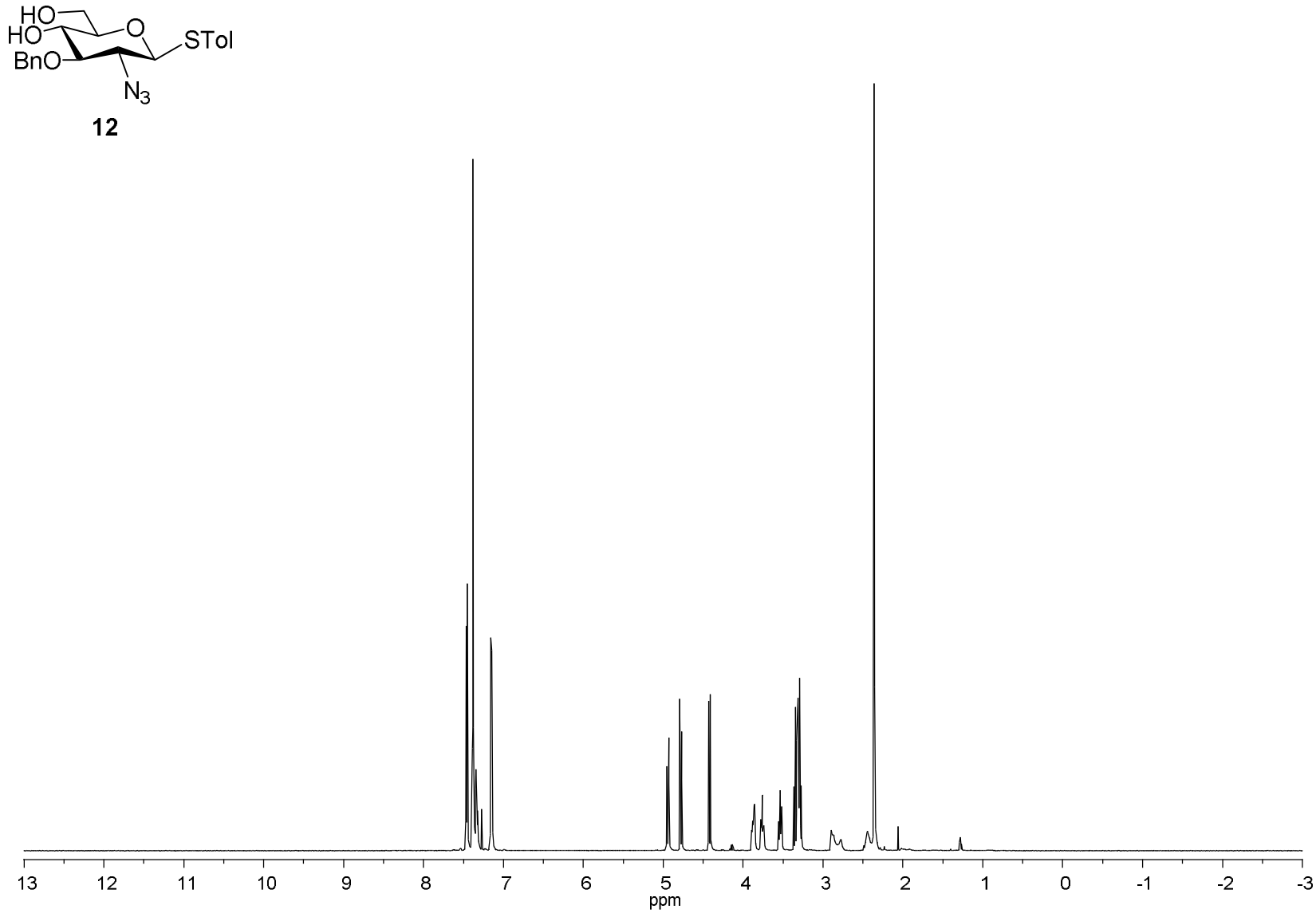


Figure 4.19. 500 MHz, $CDCl_3$ ^1H NMR of **12**

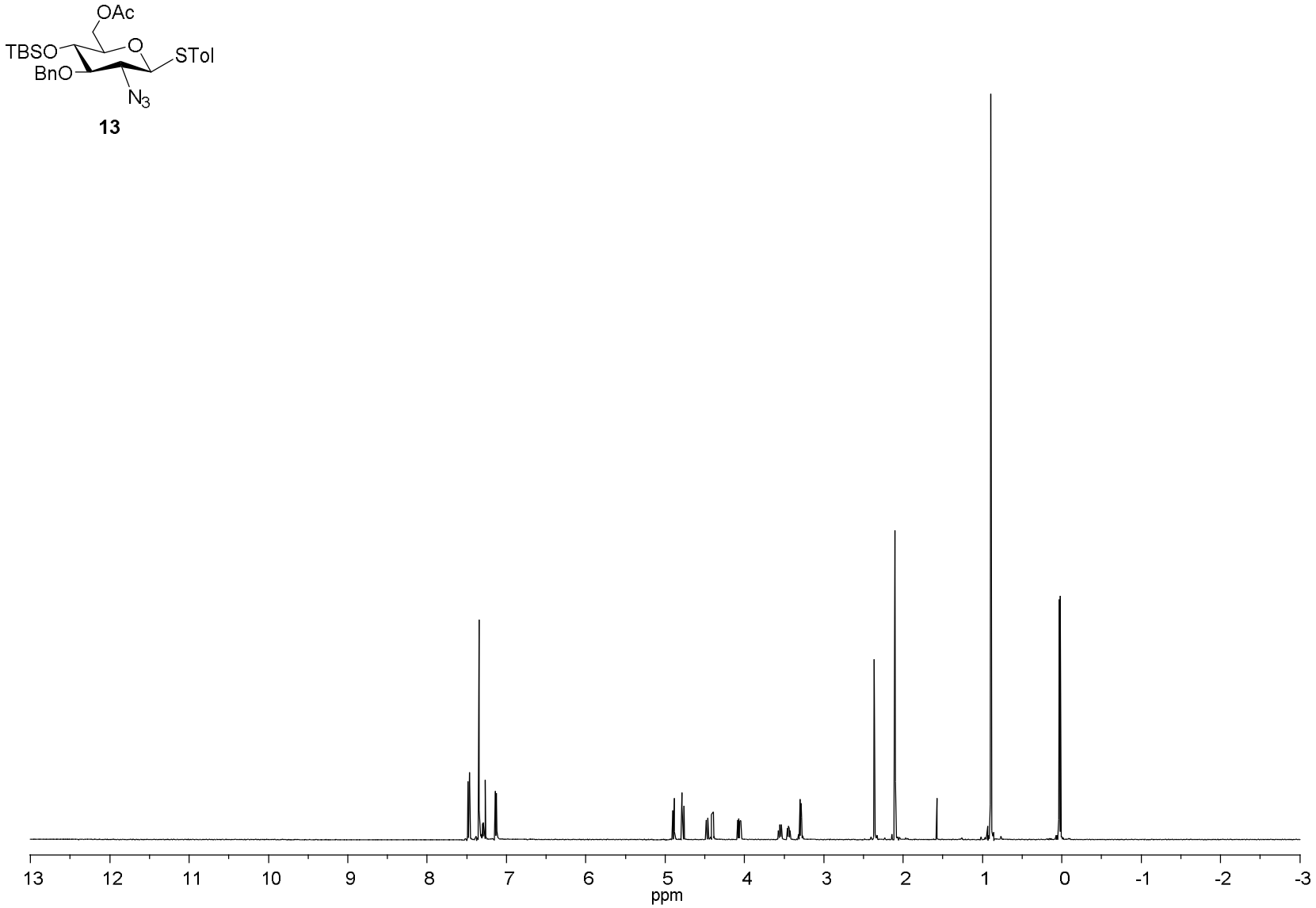


Figure 4.20. 500 MHz, $CDCl_3$ ^1H NMR of **13**

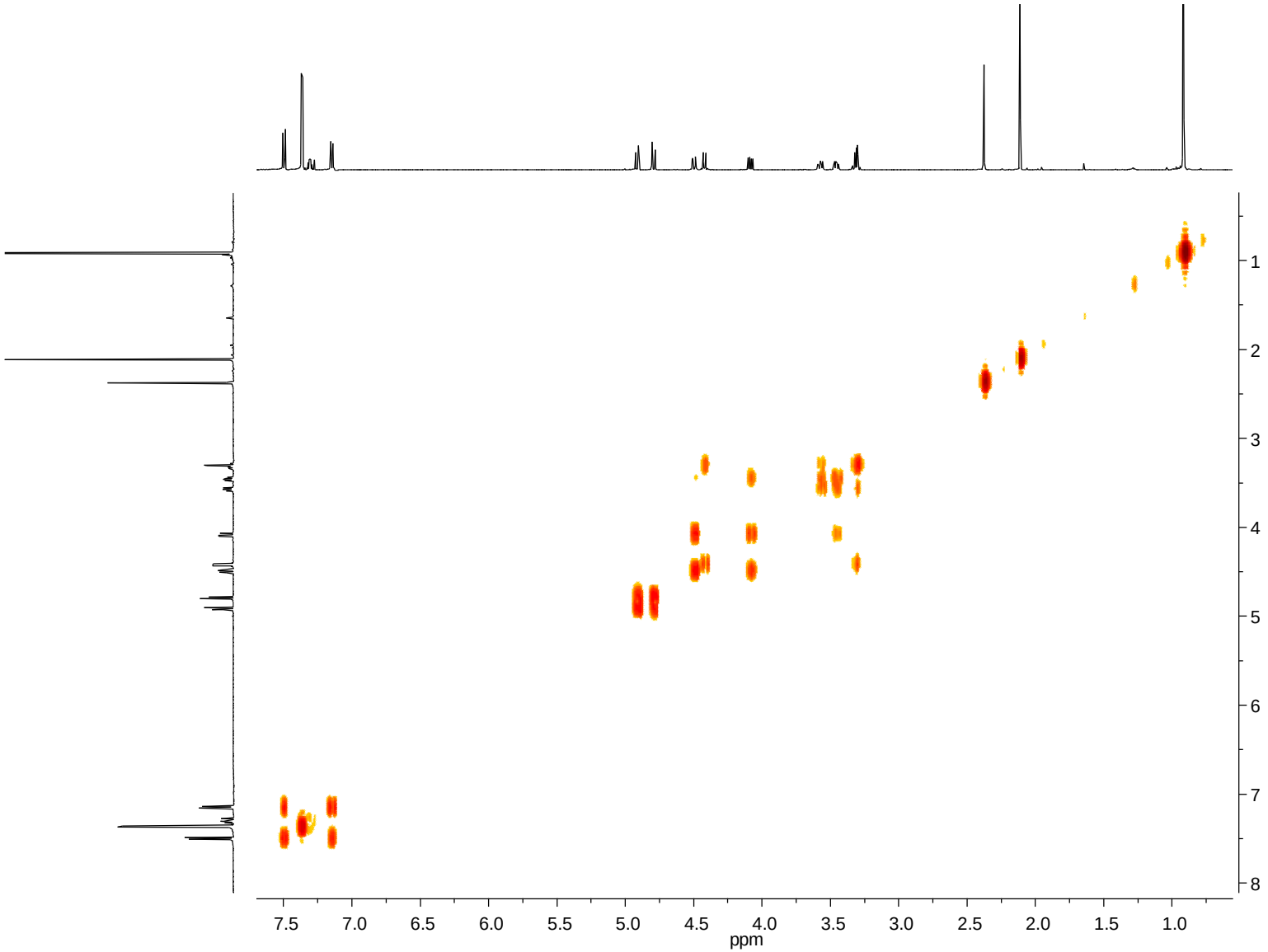
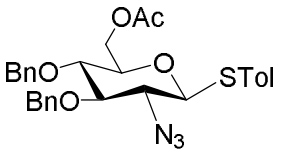


Figure 4.21. 500 MHz, $CDCl_3$ ^{1}H-^{1}H gCOSYof **13**



14

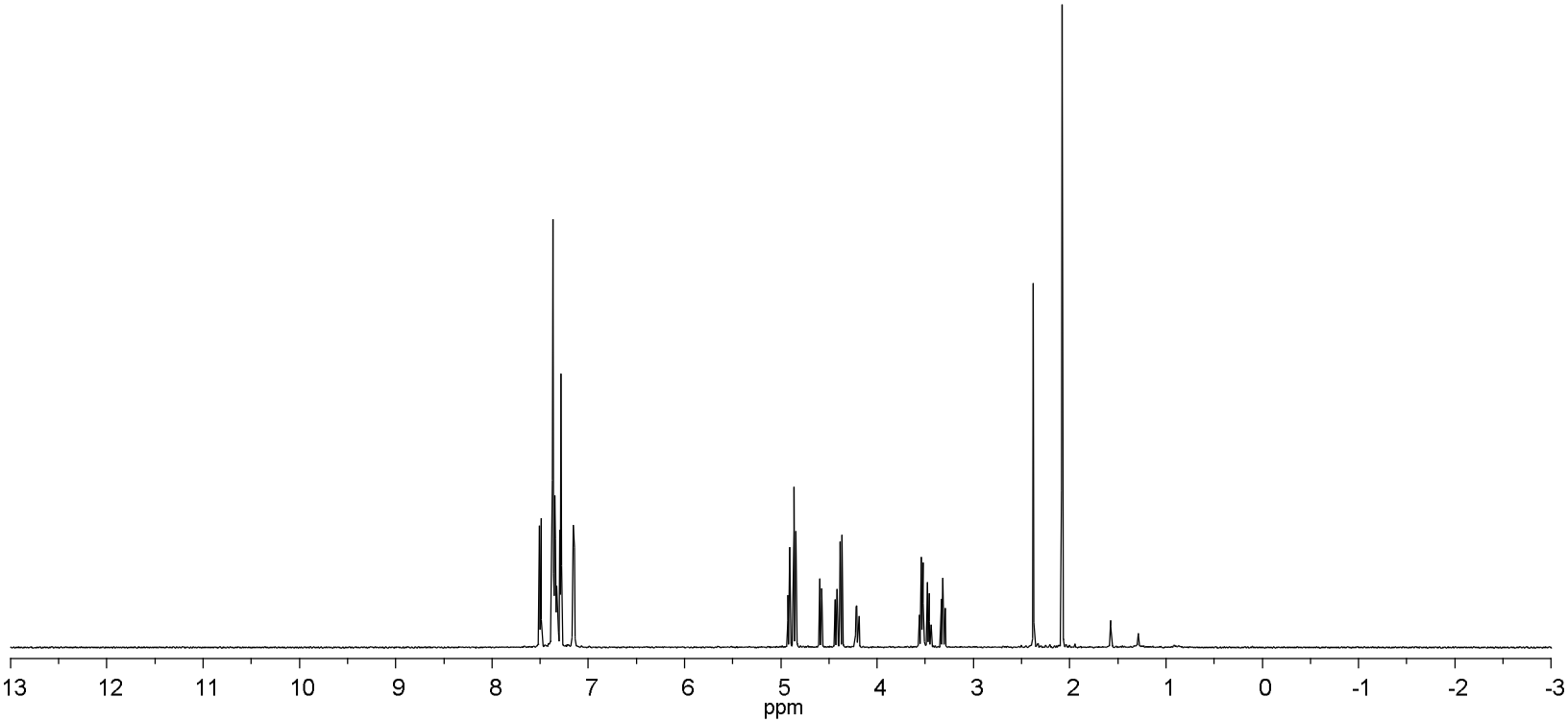
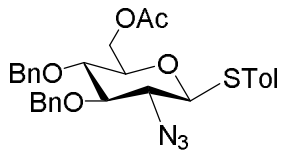


Figure 4.22. 500 MHz, $CDCl_3$ ^1H NMR of **14**



14

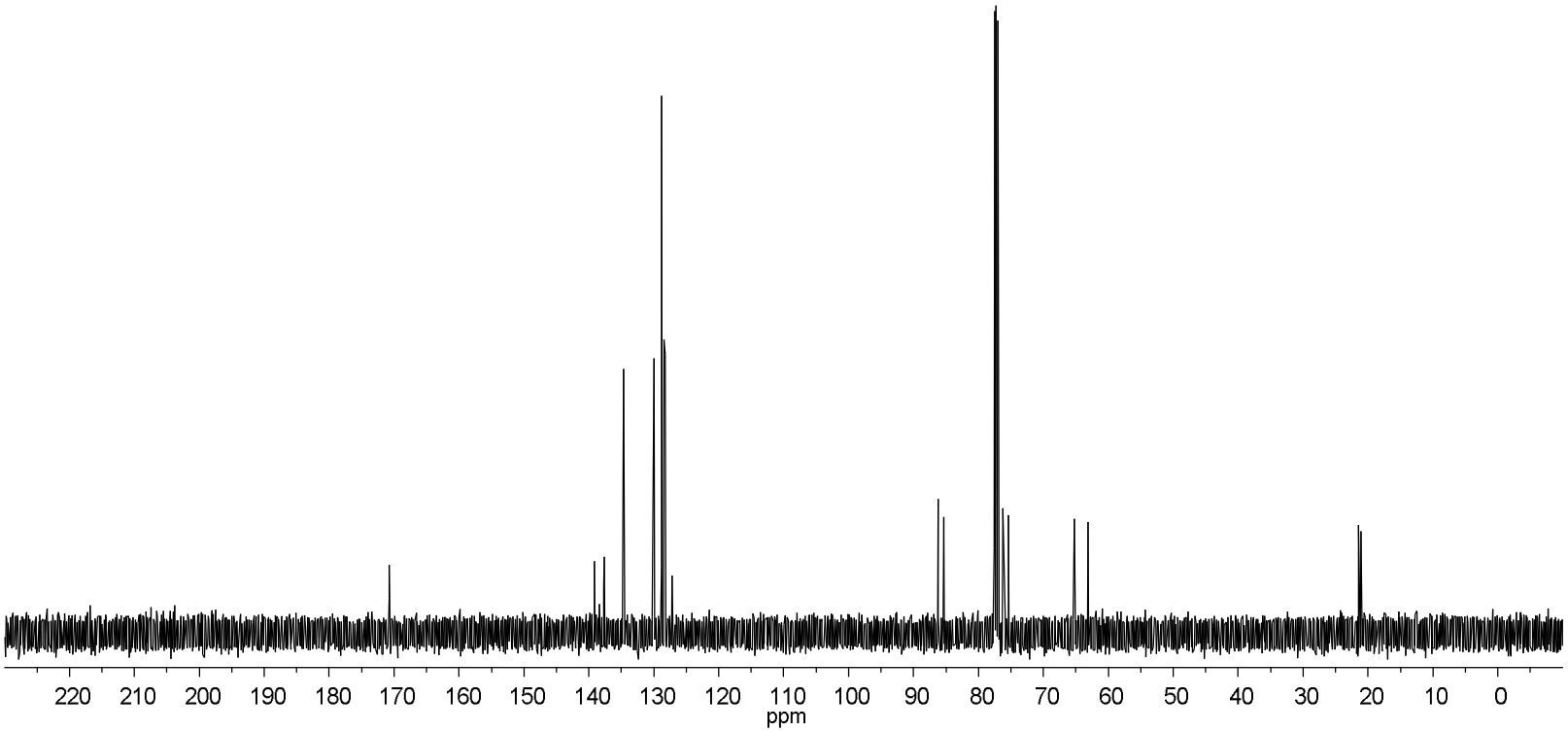


Figure 4.23. 125 MHz, $CDCl_3$ ^{13}C NMR of **14**

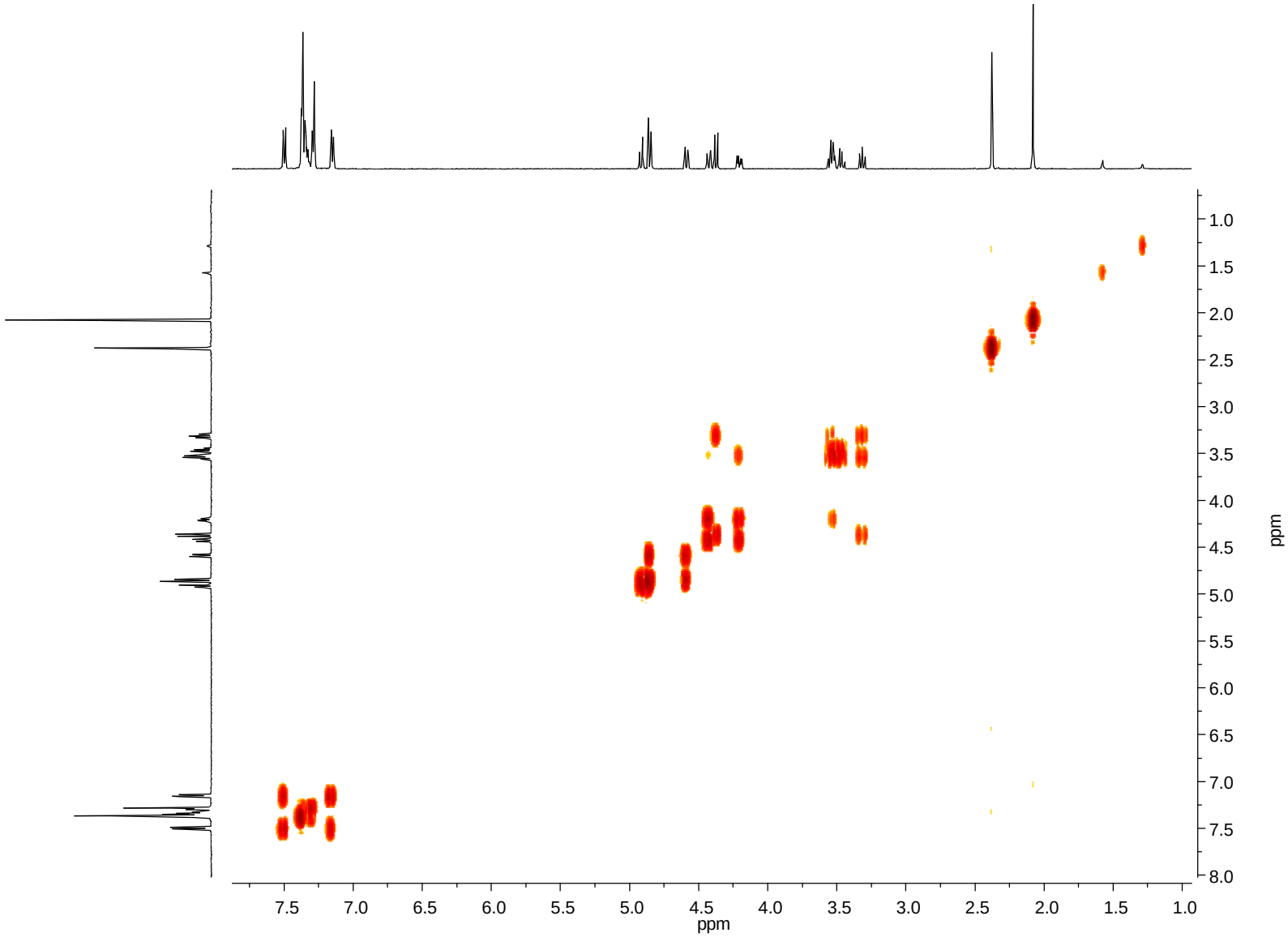


Figure 4.24. 500 MHz, $CDCl_3$ ^1H-^1H gCOSYof **14**

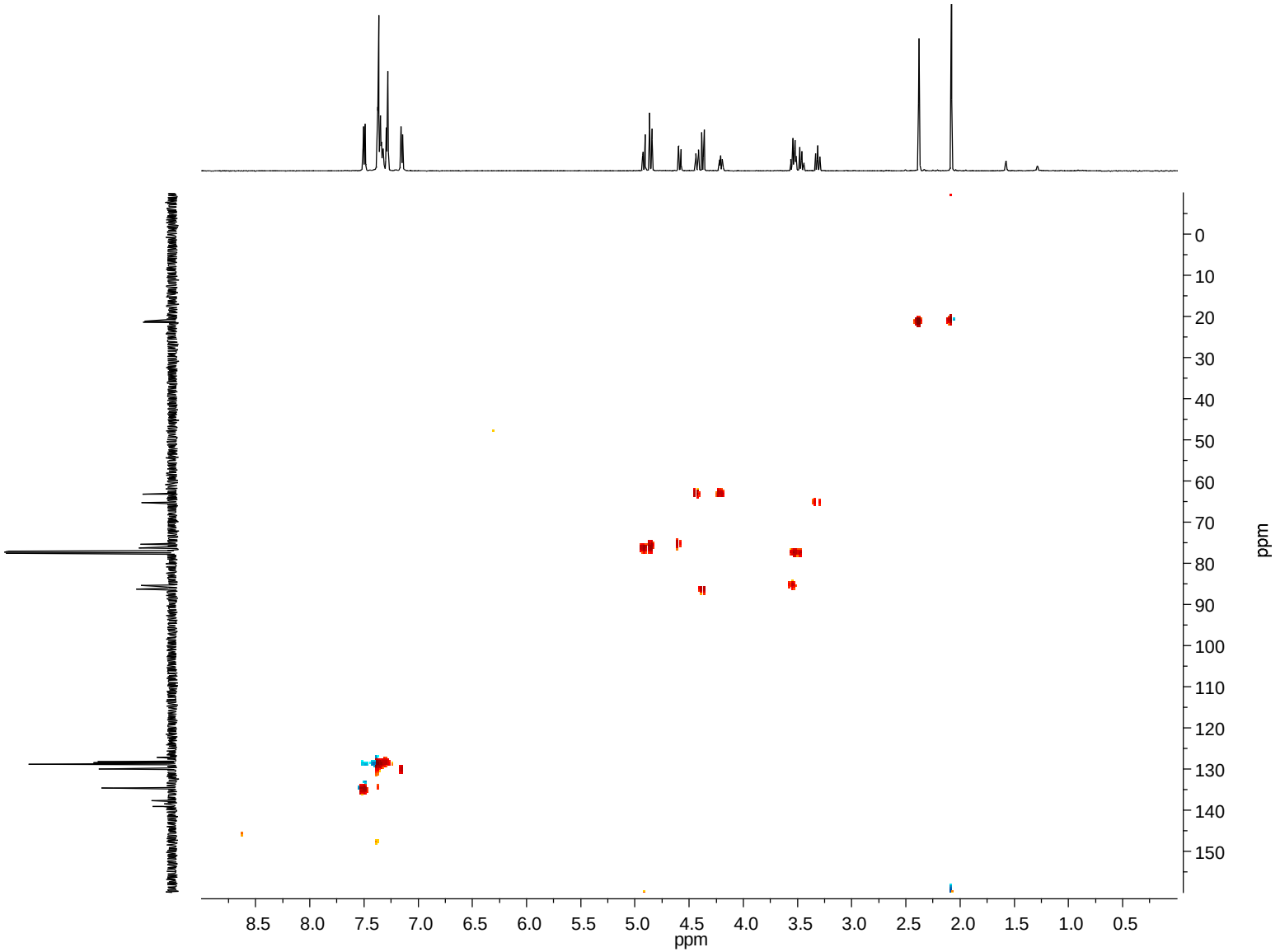


Figure 4.25. 500 MHz, $CDCl_3$ gHMQC of **14**

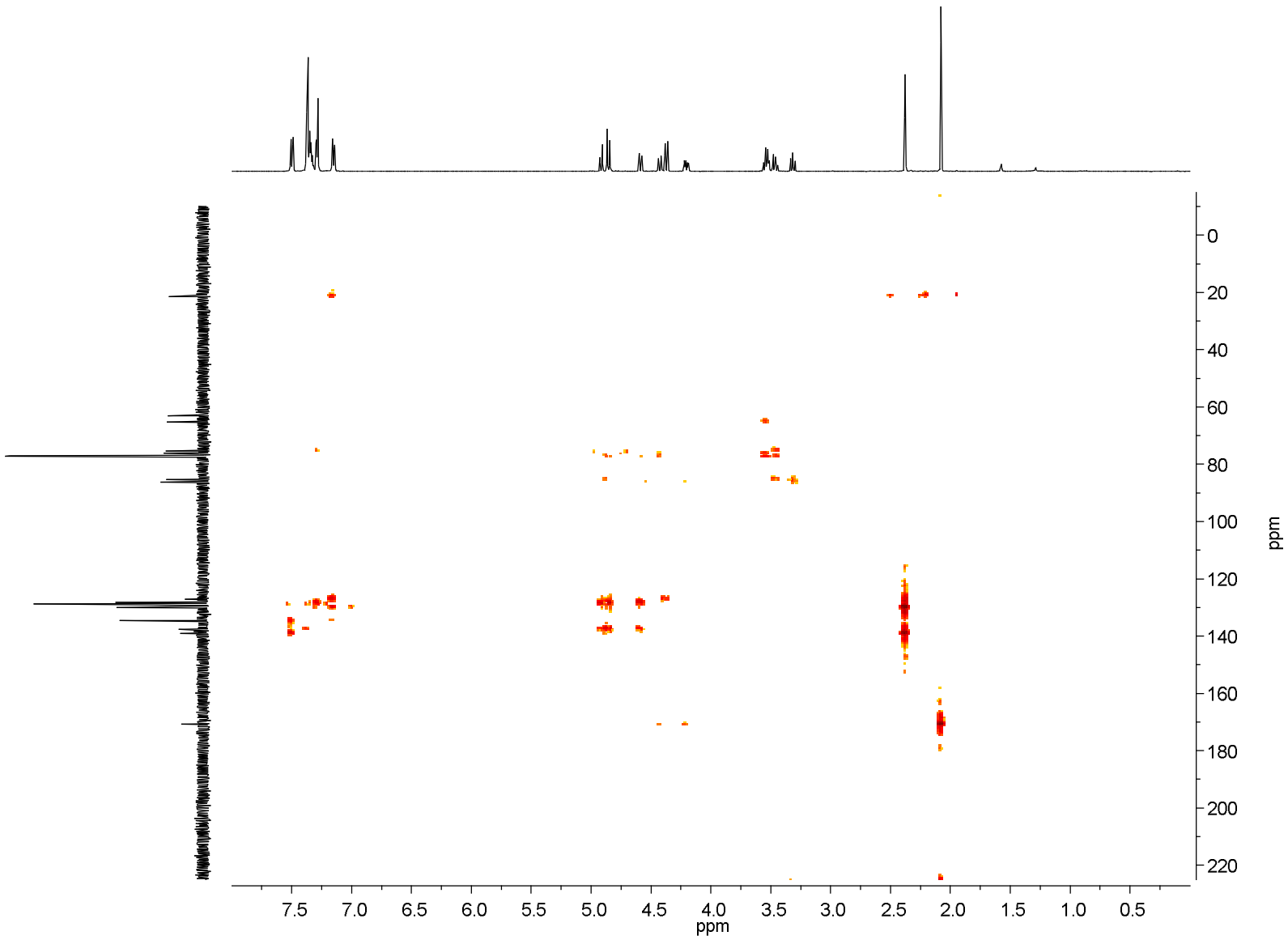


Figure 4.26. 500 MHz, $CDCl_3$ gHMBC of **14**

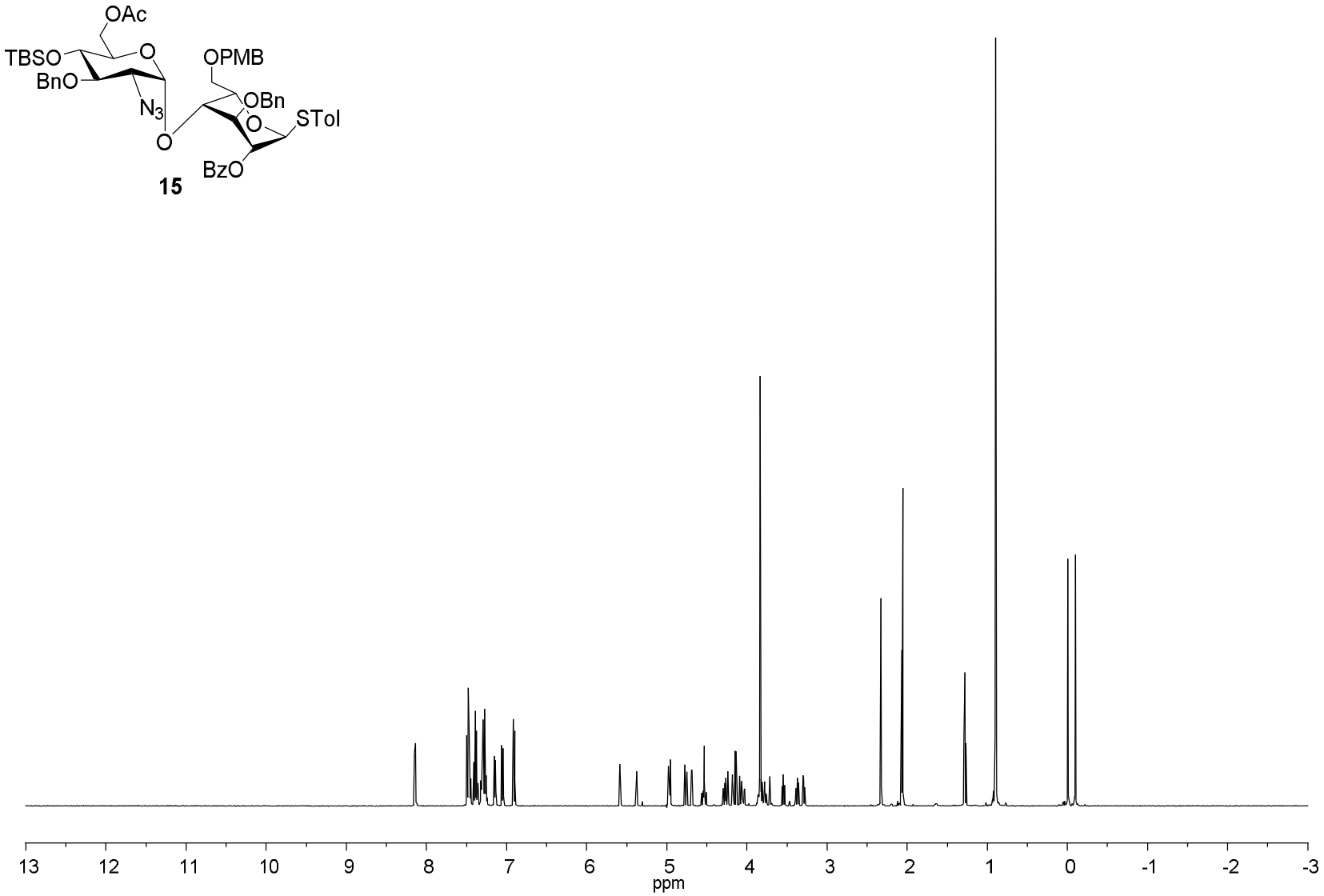


Figure 4.27. 500 MHz, $CDCl_3$ ^1H NMR of **15**

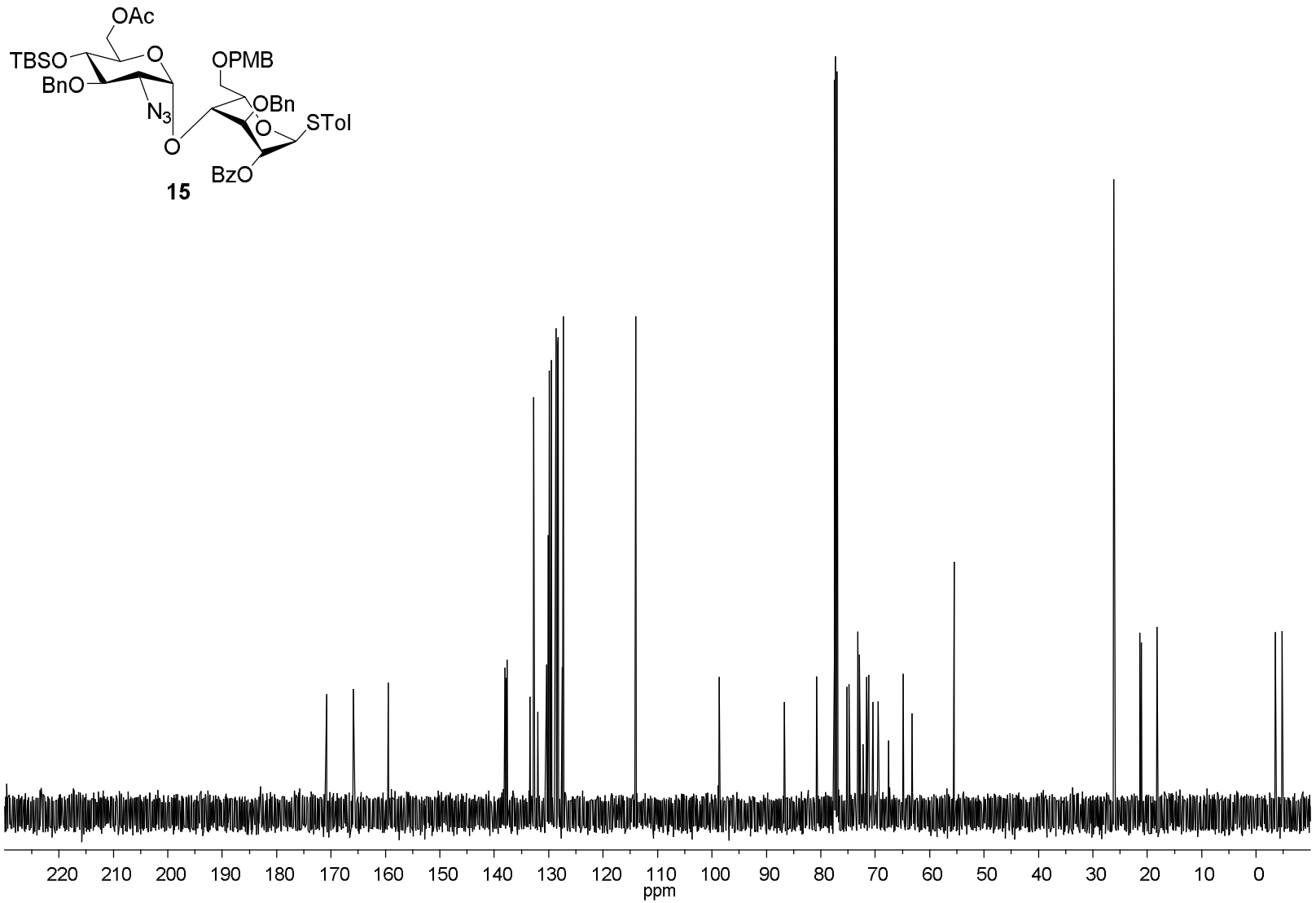


Figure 4.28. 125 MHz, $CDCl_3$ ^{13}C NMR of **15**

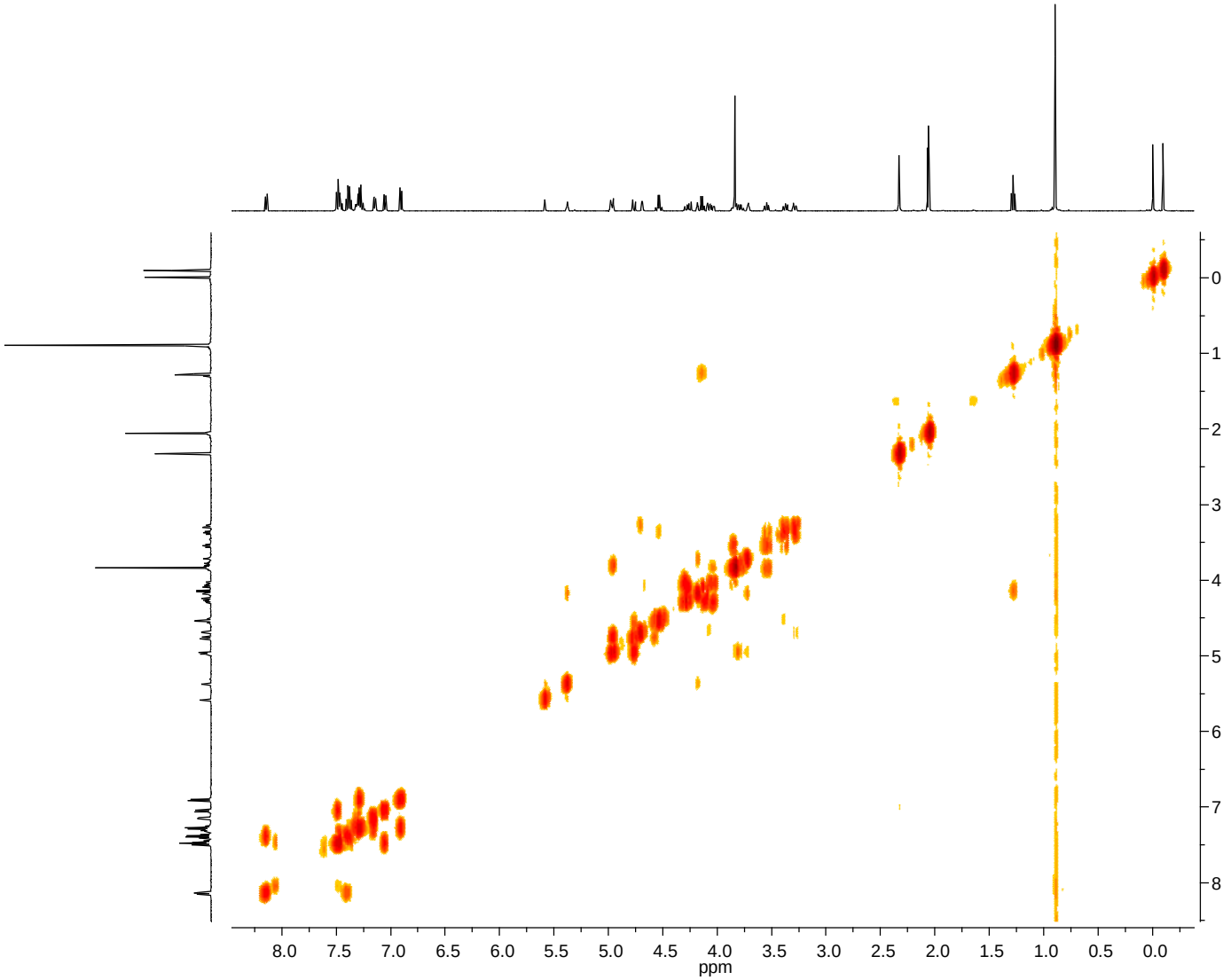


Figure 4.29. 500 MHz, $CDCl_3$ ^{1}H-^{1}H gCOSYof **15**

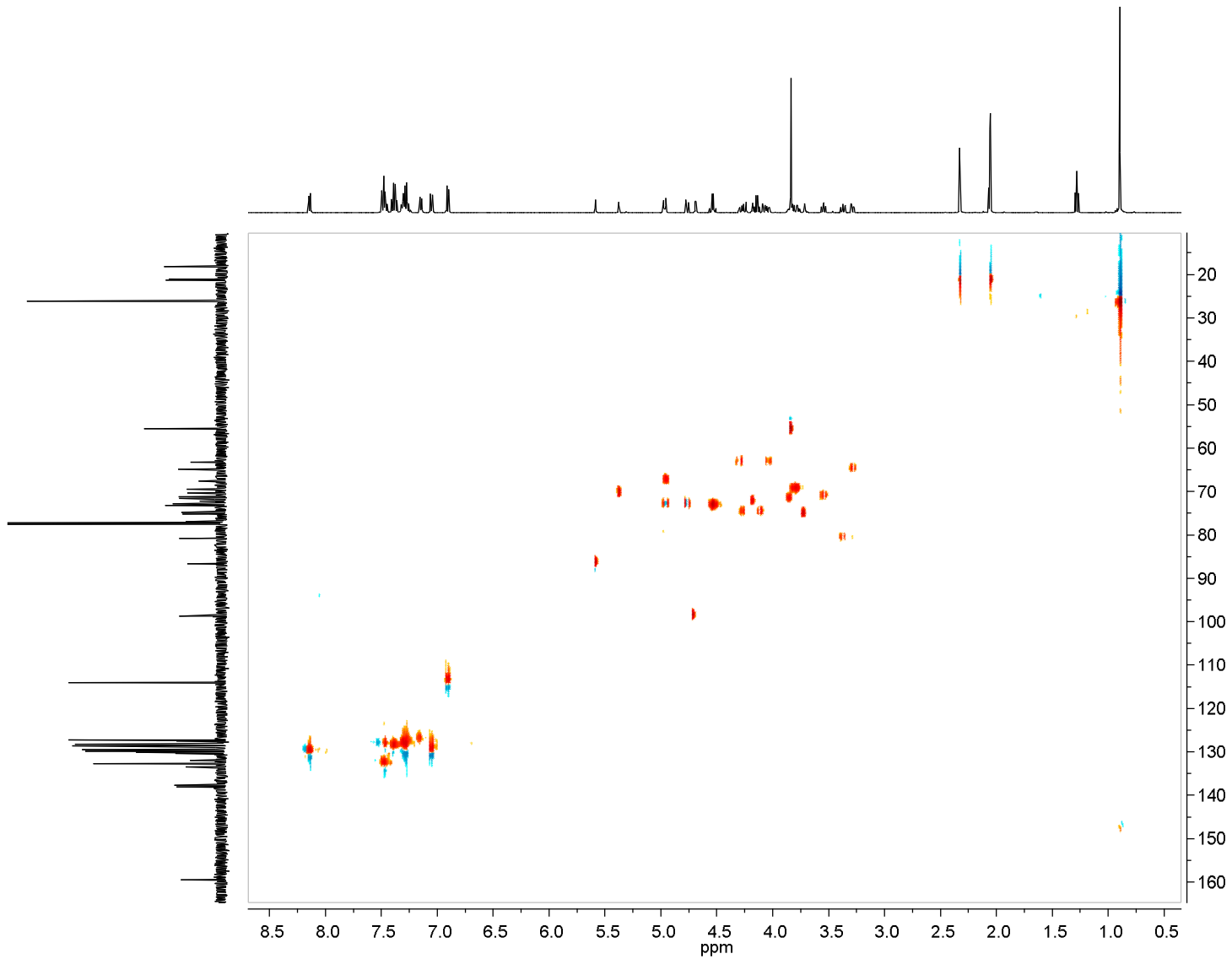


Figure 4.30. 500 MHz, $CDCl_3$ gHMQC of **15**

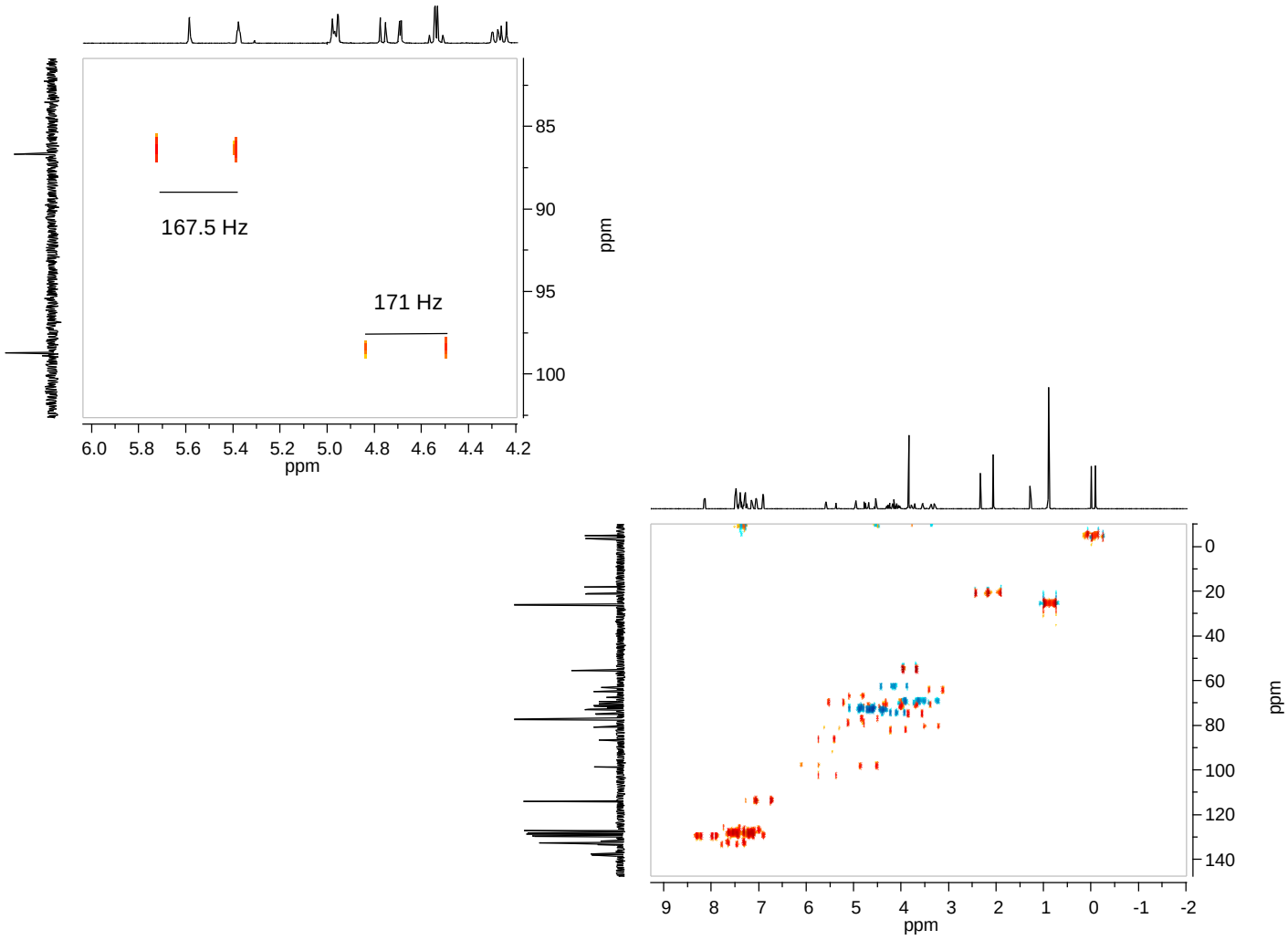


Figure 4.31. 500 MHz, $CDCl_3$, gHSQCAD (without ^1H Decoupling) of **15**

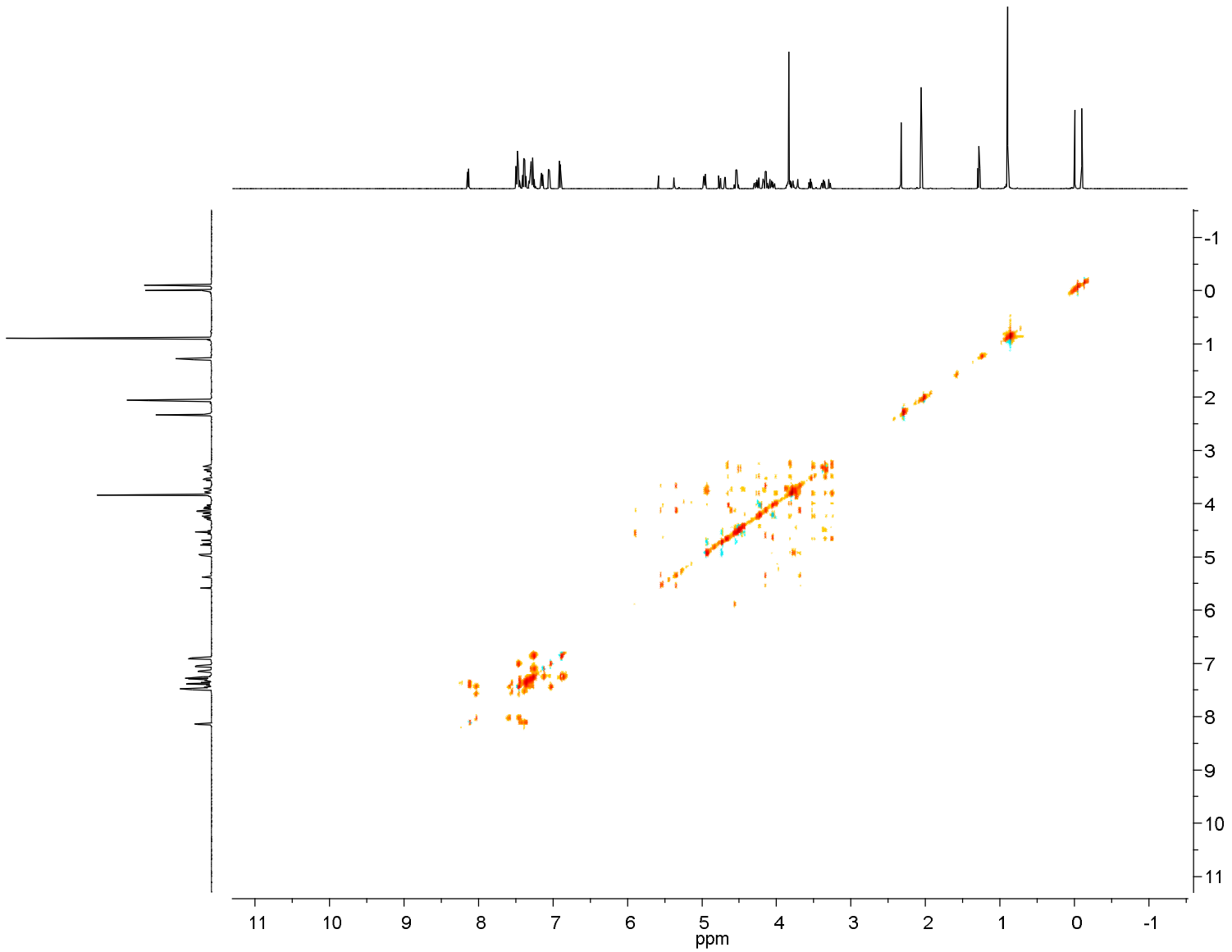
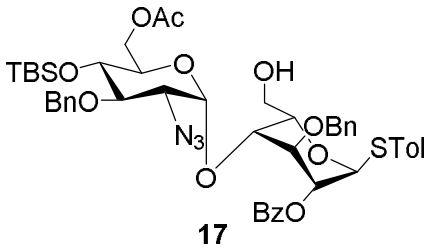


Figure 4.32. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **15**



17

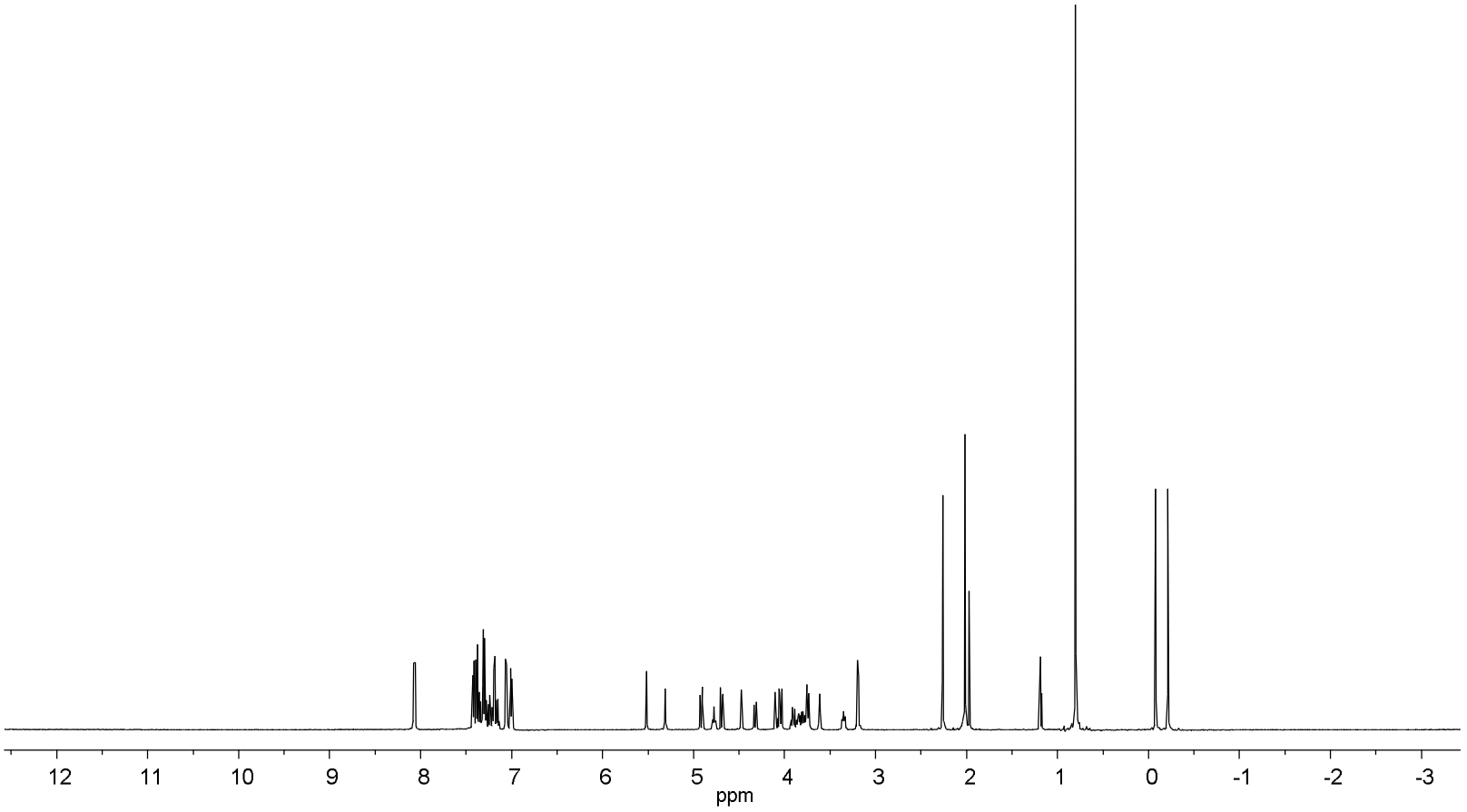


Figure 4.33. 500 MHz, $CDCl_3$, ^1H NMR of **17**

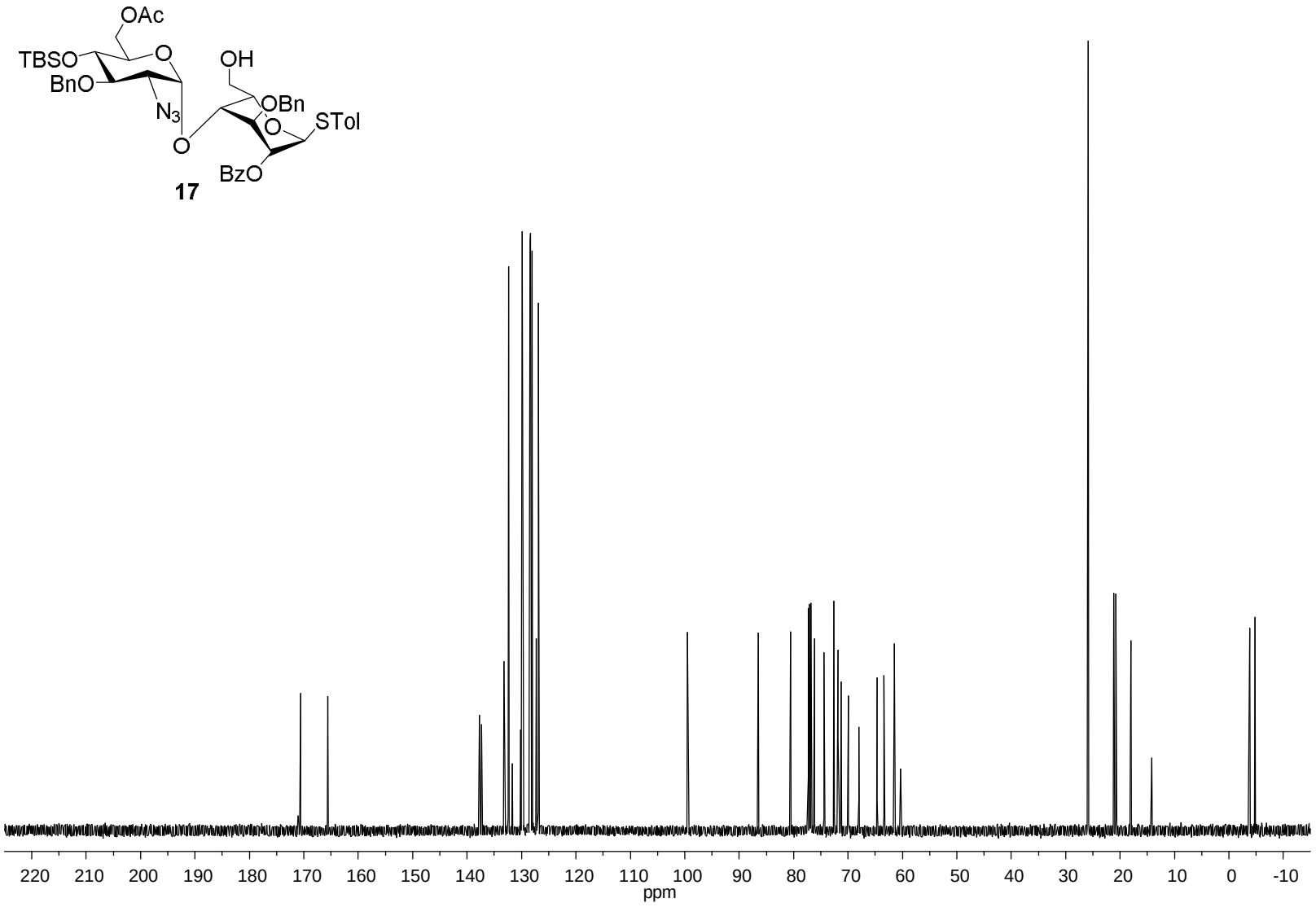


Figure 4.34. 125 MHz, $CDCl_3$, ^{13}C NMR of **17**

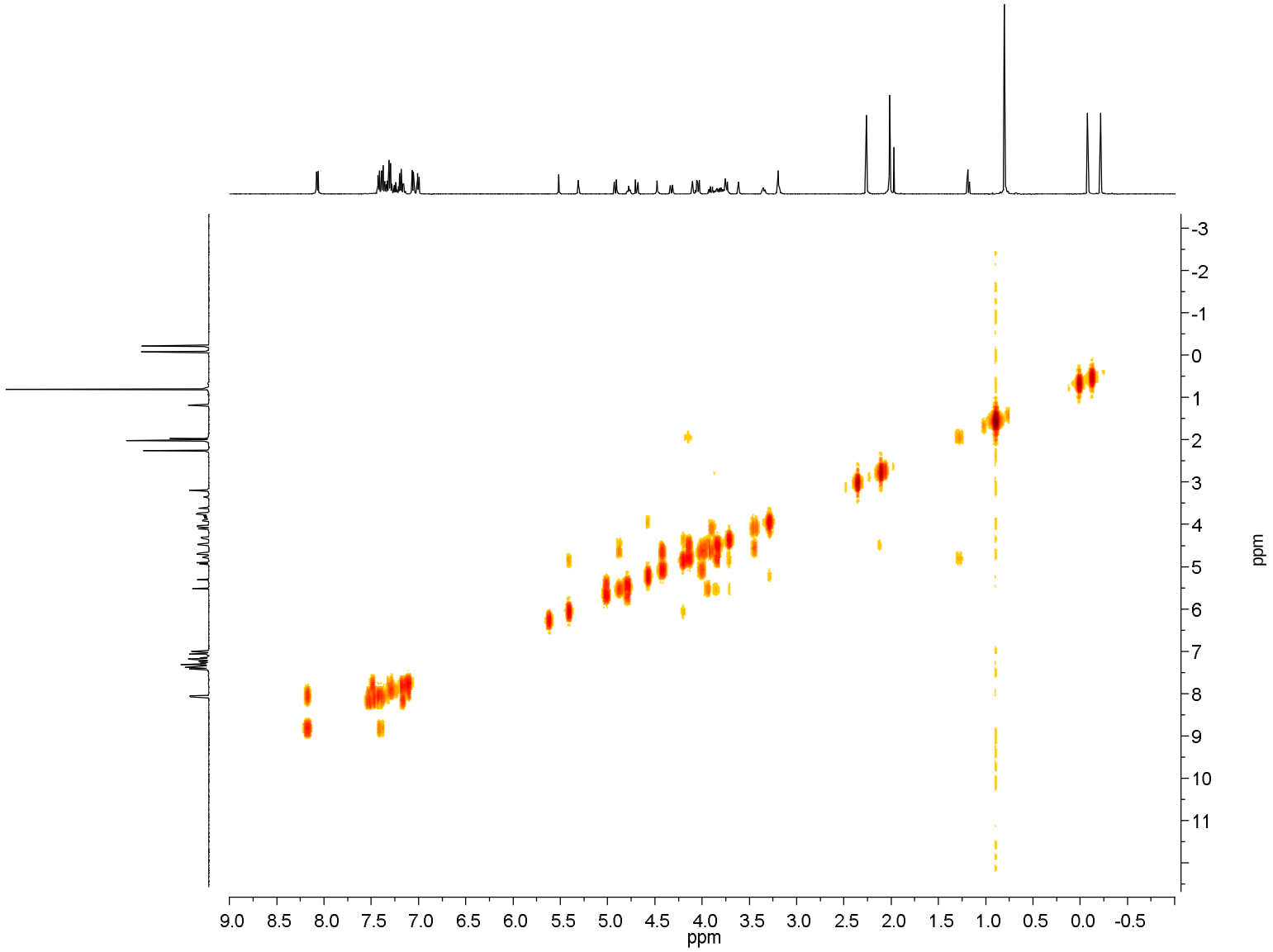
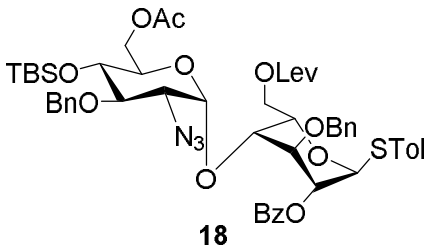


Figure 4.35. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **17**



18

Figure 4.36. 500 MHz, $CDCl_3$, ^1H NMR of **18**

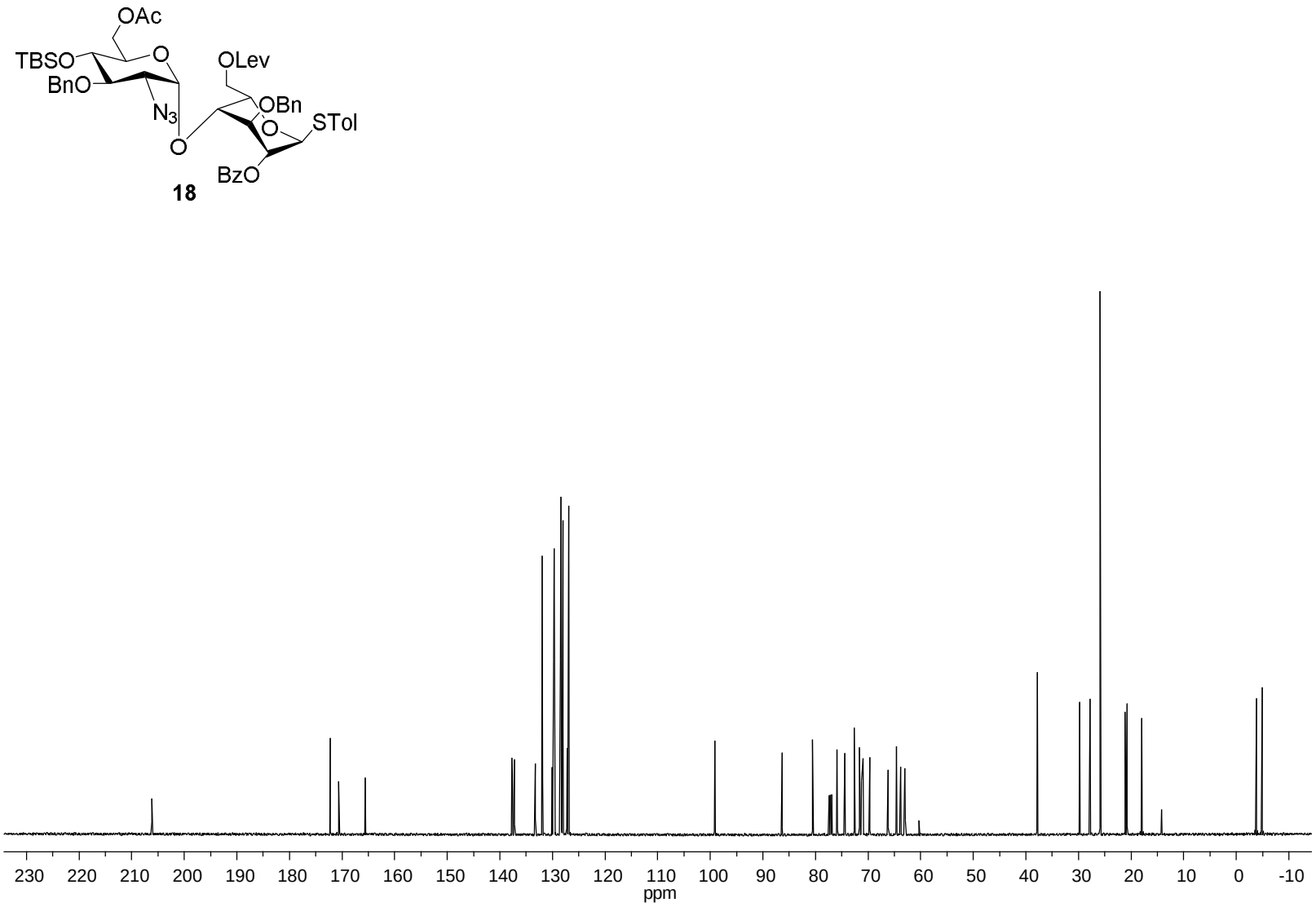


Figure 4.37. 125 MHz, $CDCl_3$, ^{13}C NMR of **18**

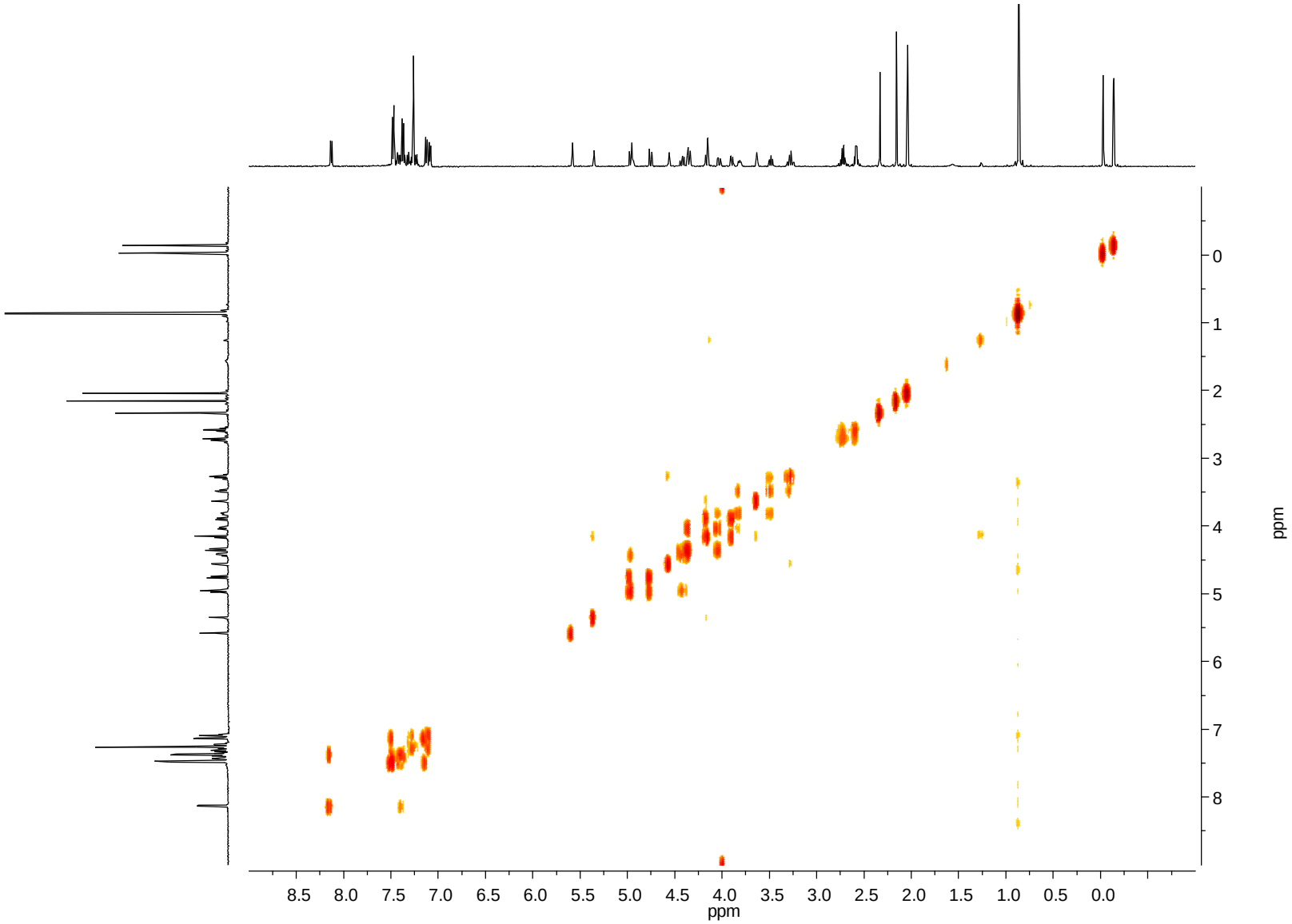


Figure 4.38. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **18**

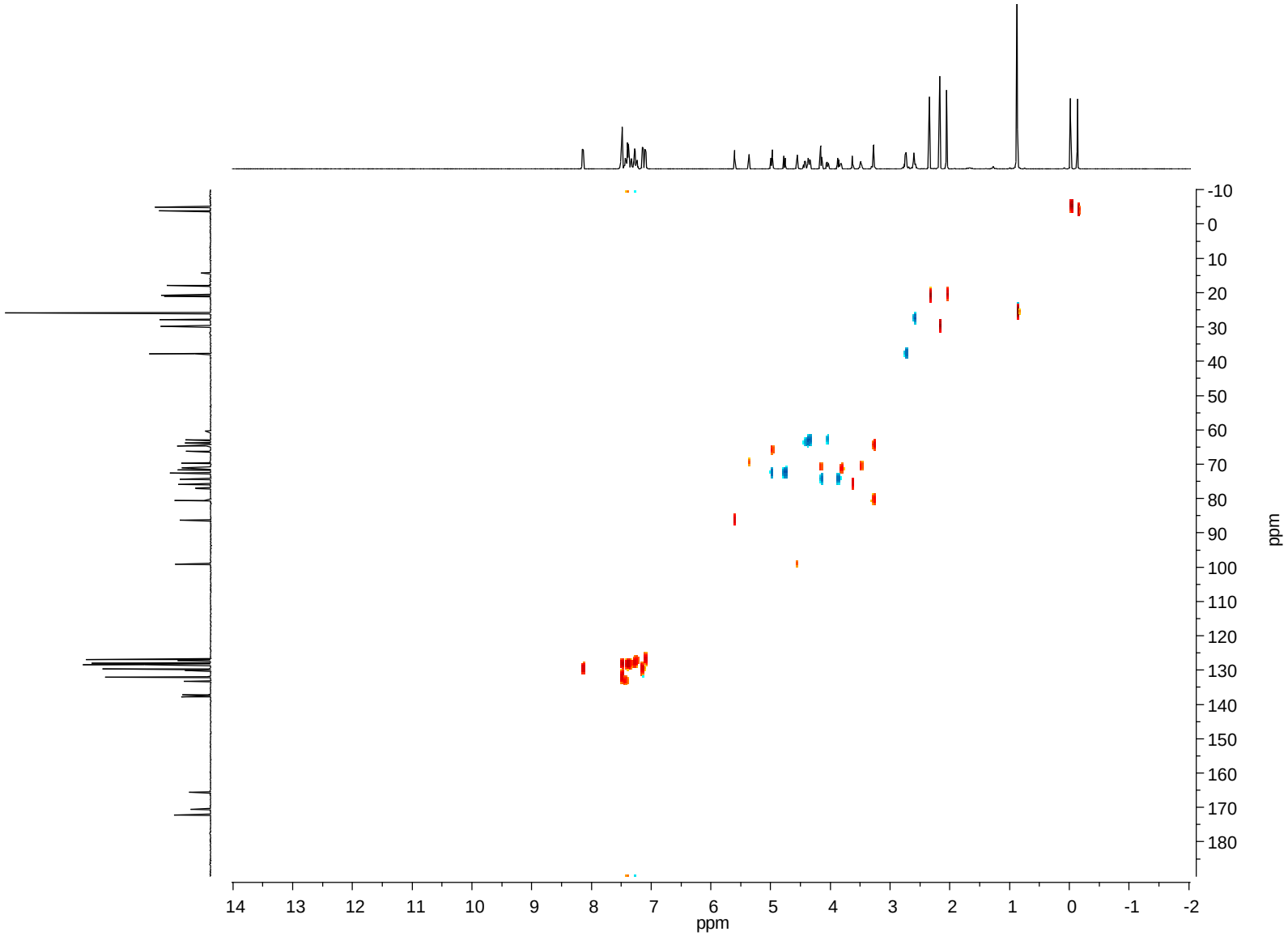


Figure 4.39. 500 MHz, $CDCl_3$, gHSQCAD of **18**

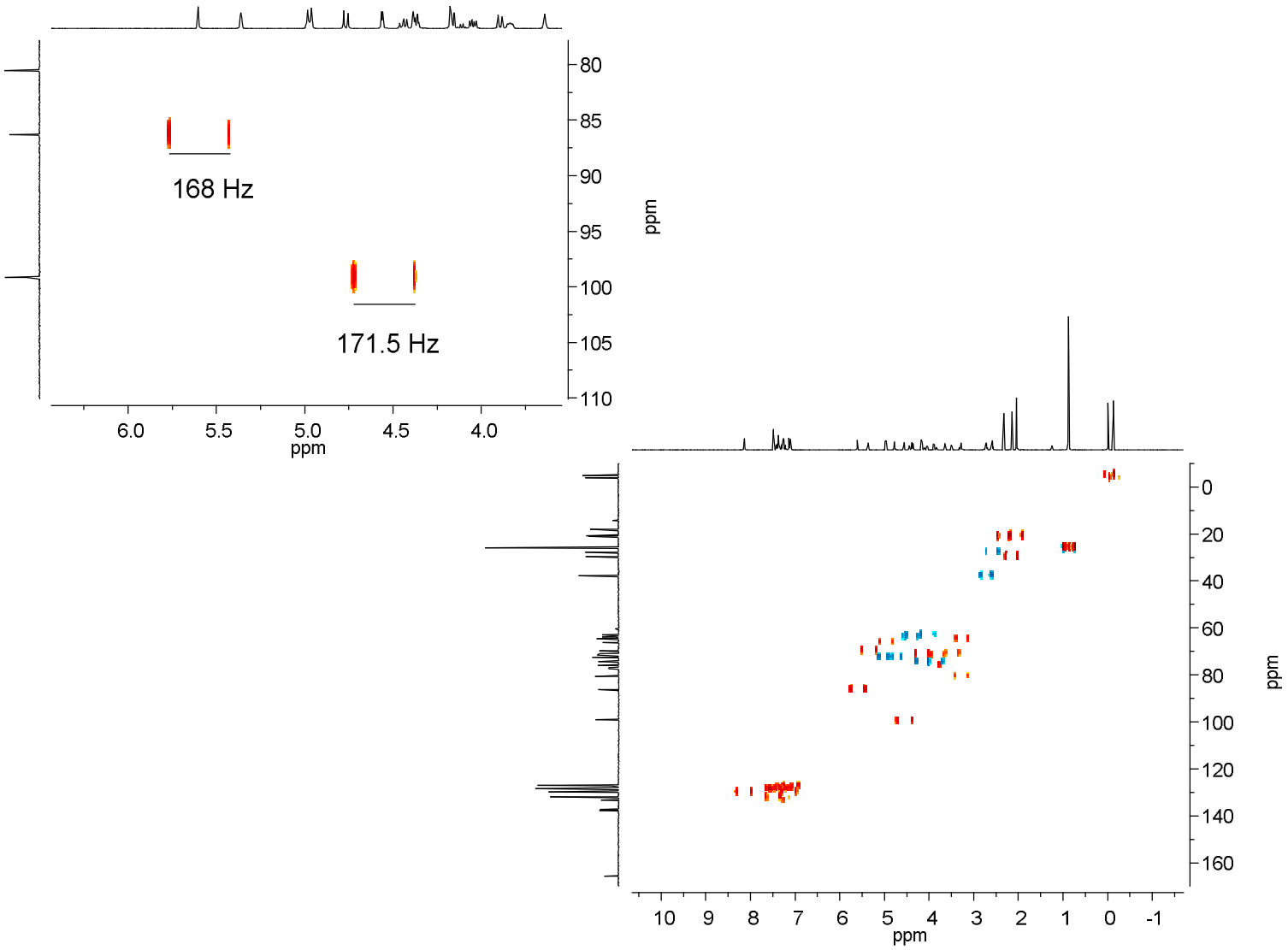


Figure 4.40. 500 MHz, $CDCl_3$, gHSQCAD (without ^1H Decoupling) of **18**

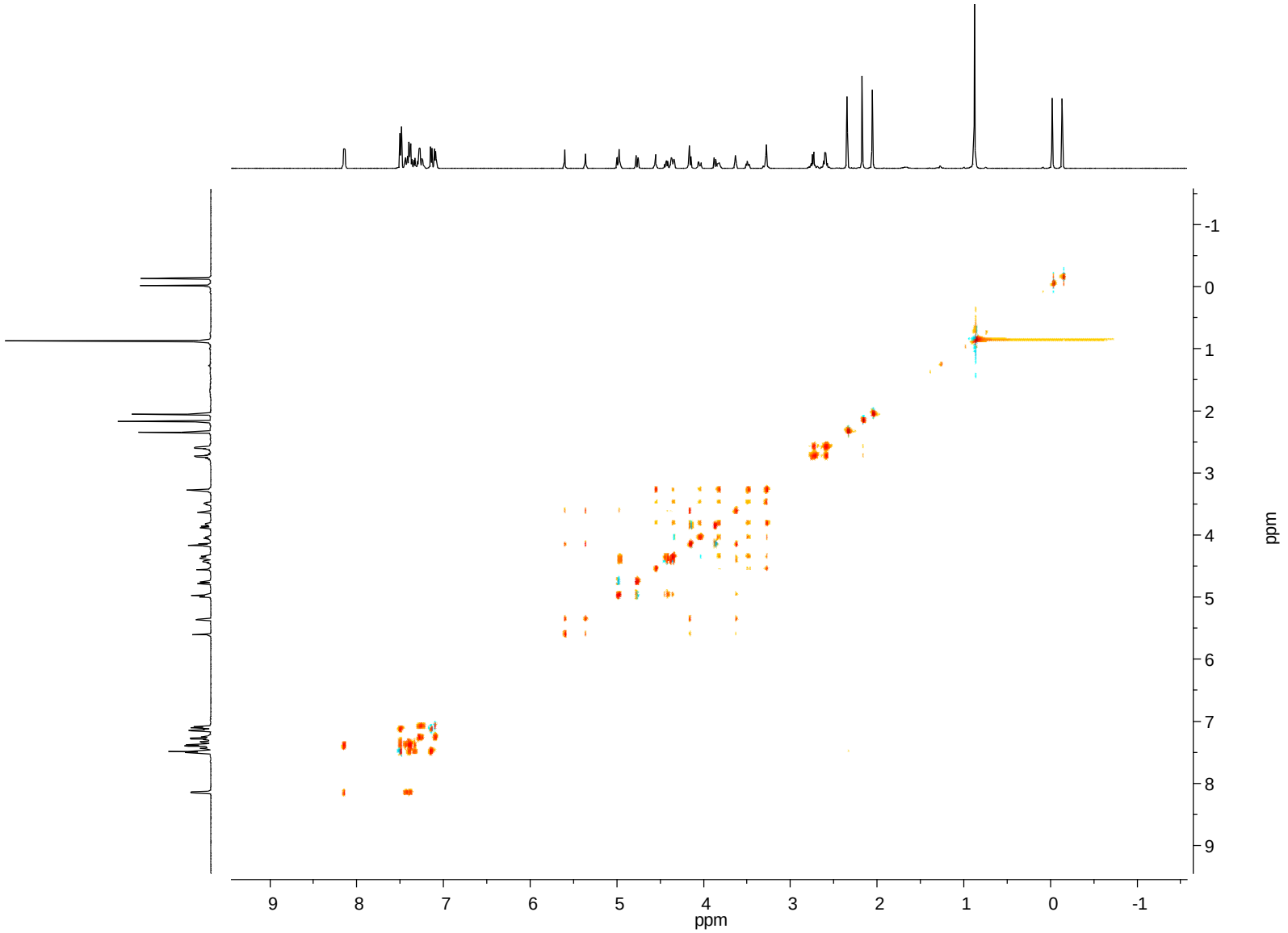


Figure 4.41. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **18**

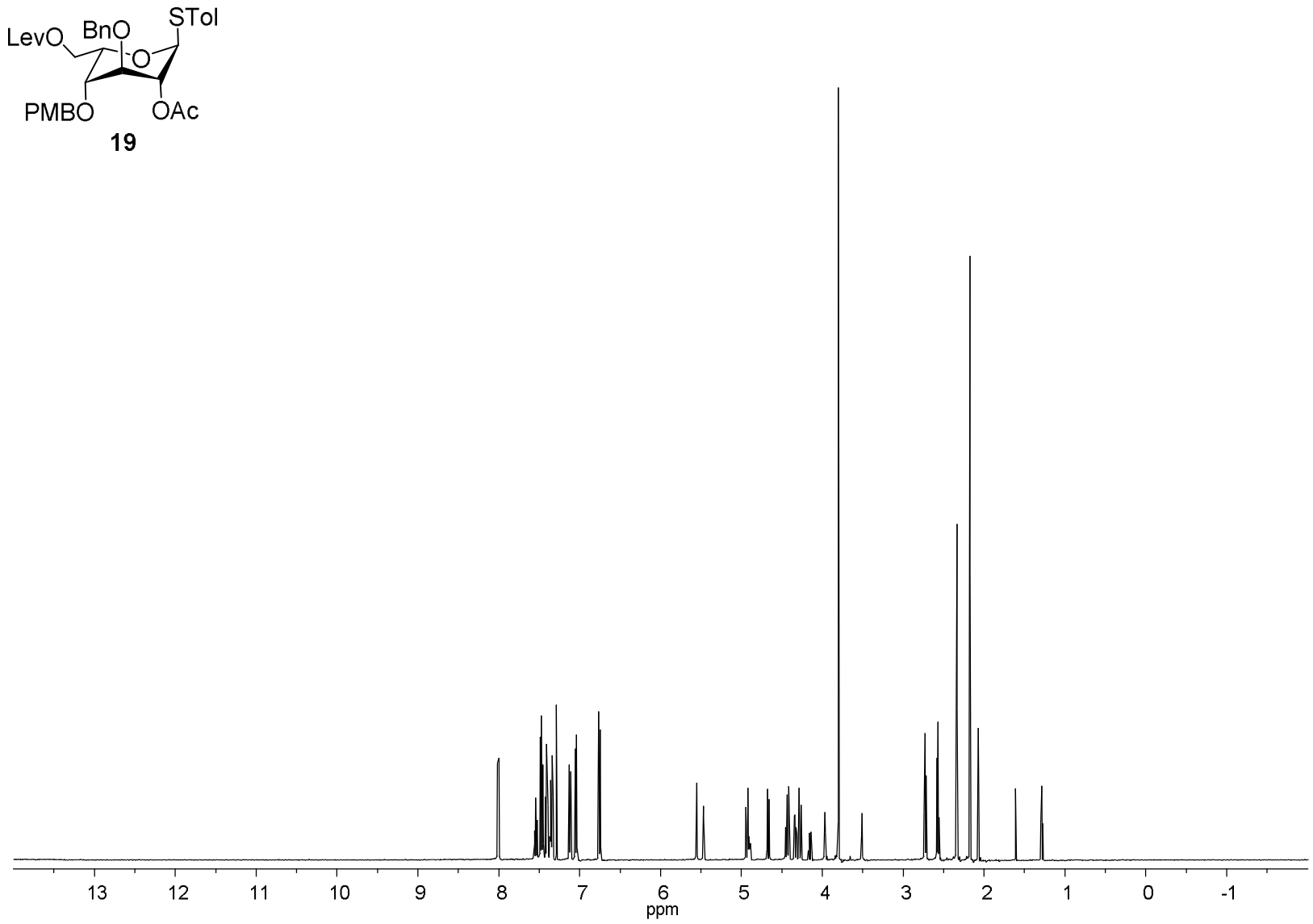


Figure 4.42. 600 MHz, $CDCl_3$, ^1H NMR of **19**

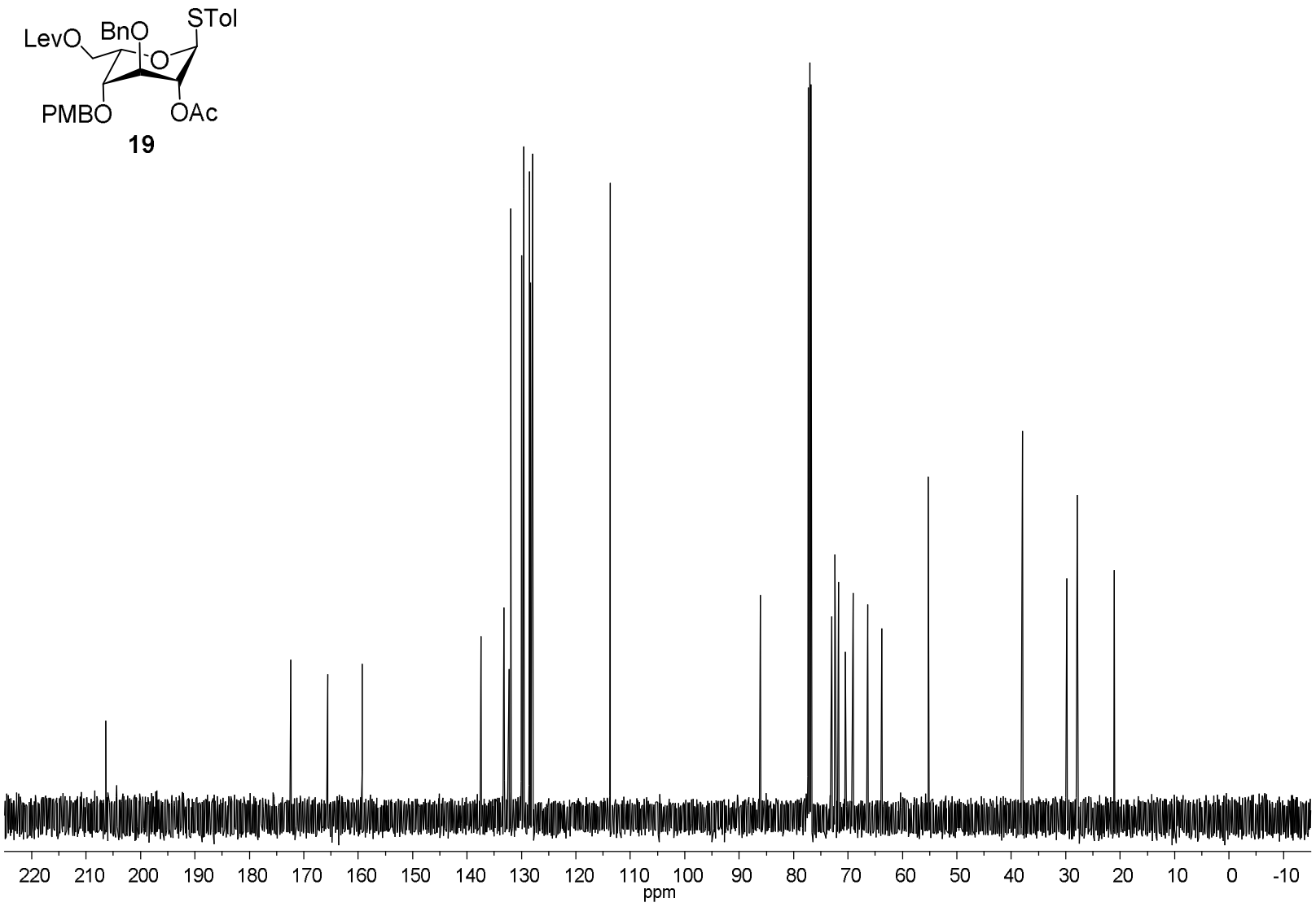


Figure 4.43. 150 MHz, $CDCl_3$, ^{13}C NMR of **19**

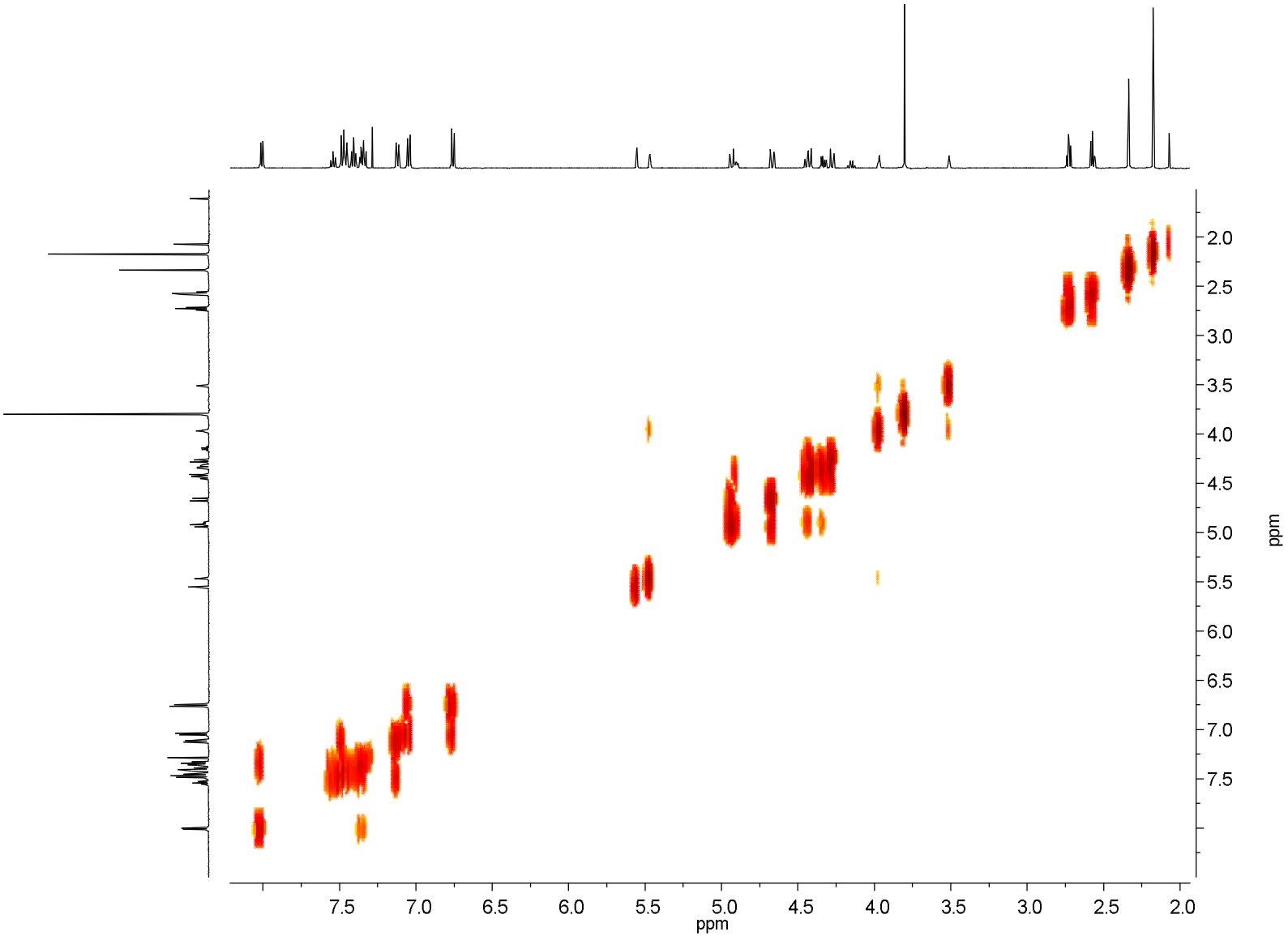
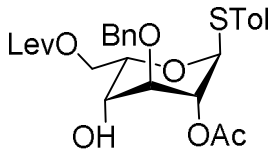


Figure 4.44. 600 MHz, $CDCl_3$ ^{1}H-^{1}H gCOSY of **19**



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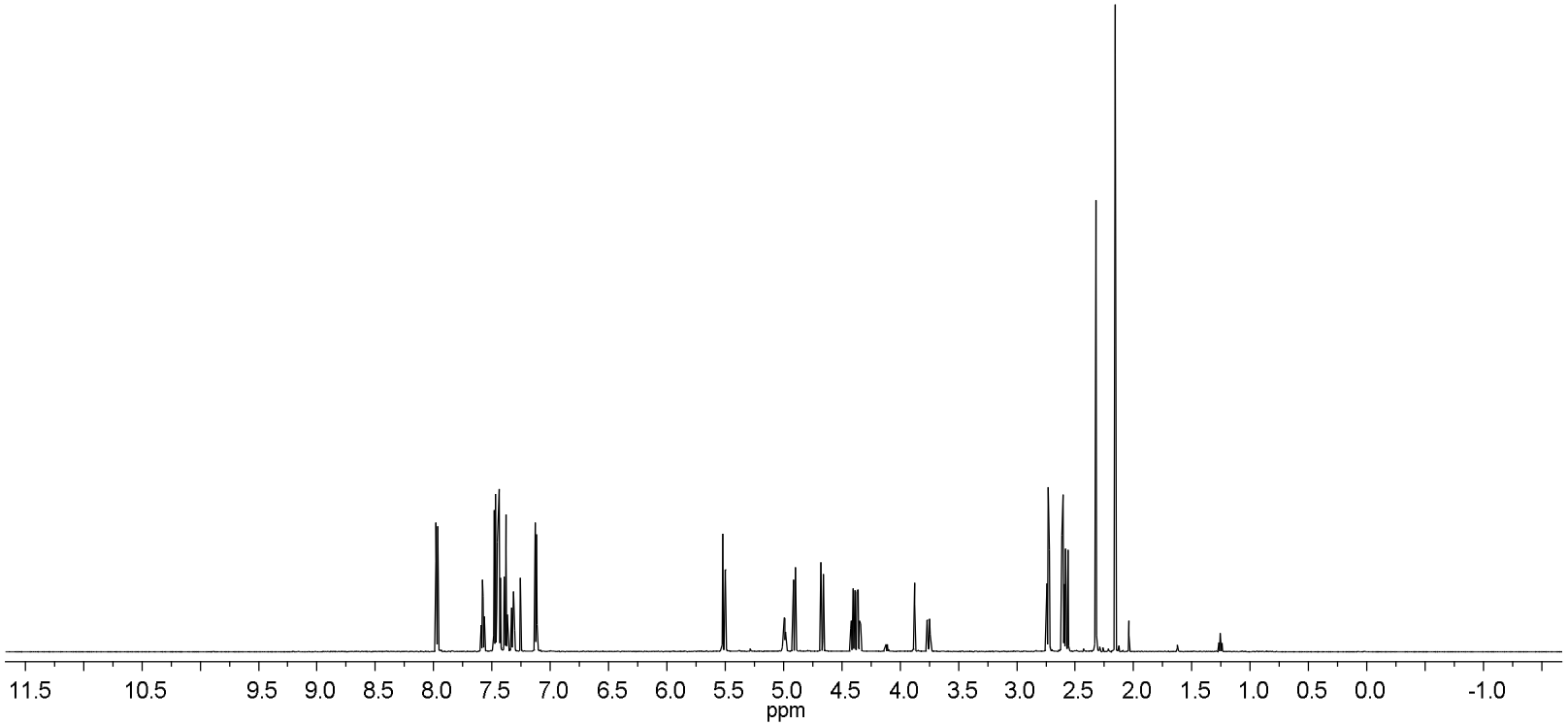
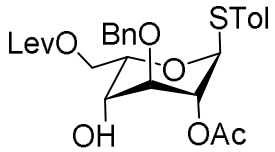


Figure 4.45. 600 MHz, $CDCl_3$, ^1H NMR of **20**



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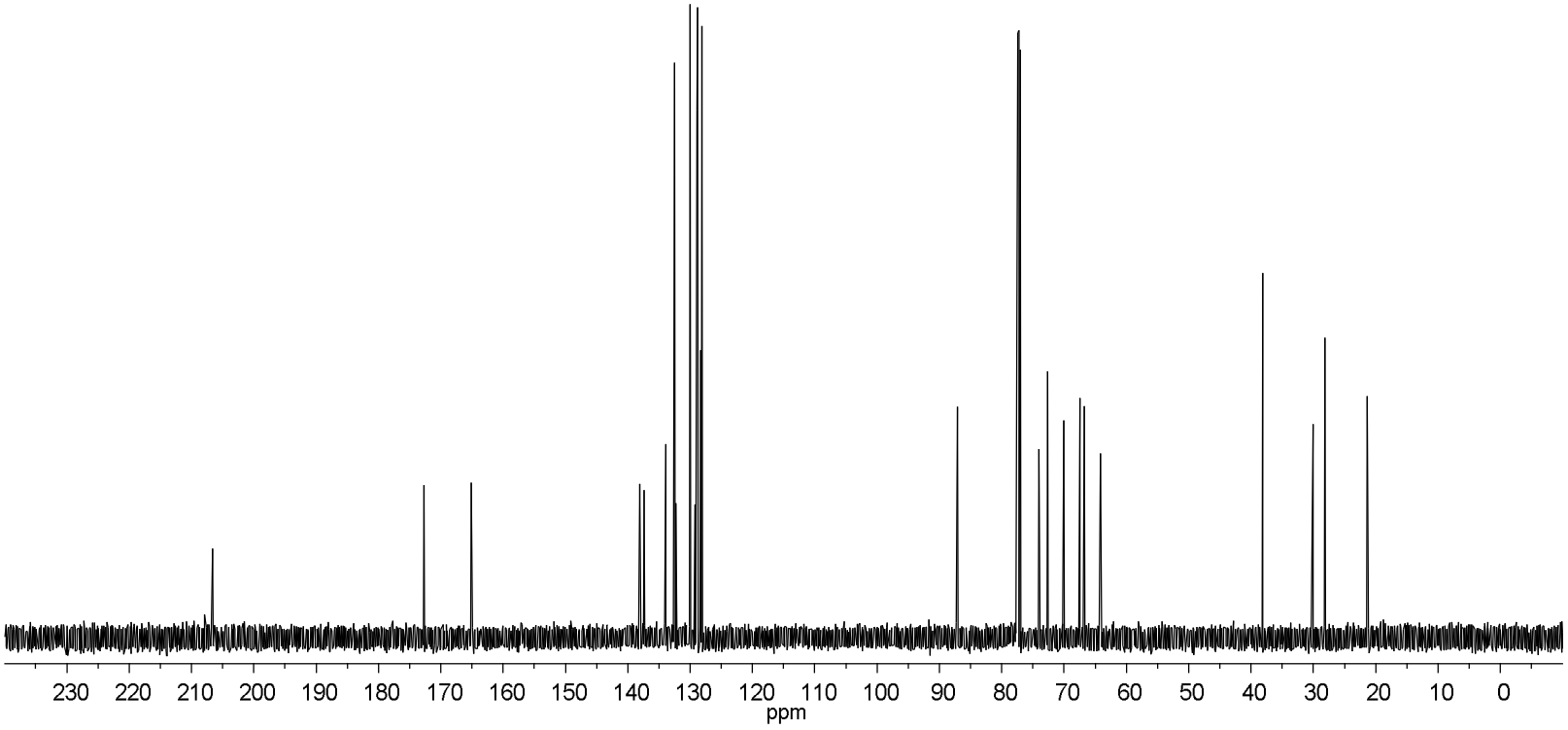


Figure 4.46. 150 MHz, $CDCl_3$, ^{13}C NMR of **20**

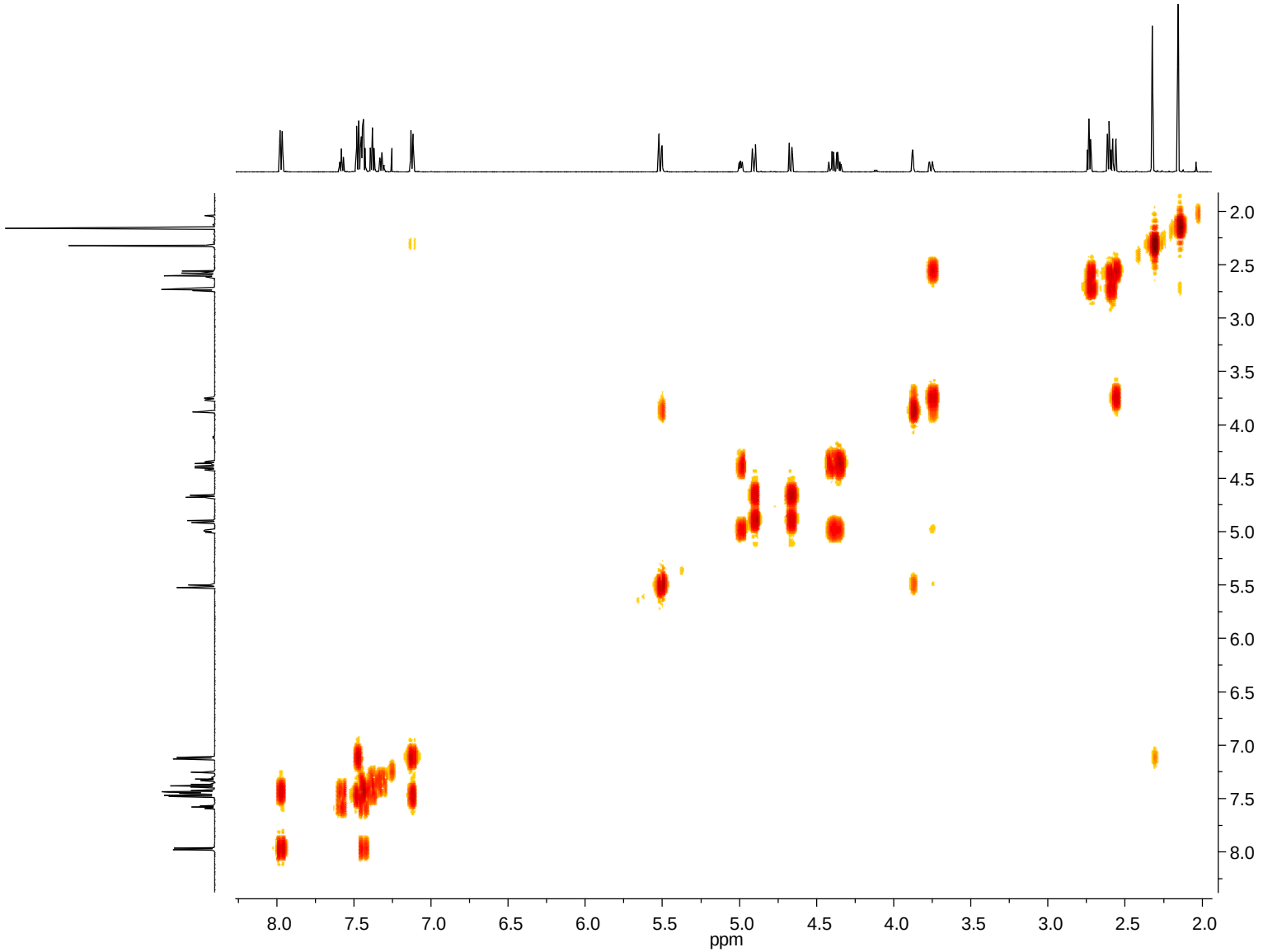


Figure 4.47. 600 MHz, $CDCl_3$ ^{1}H-^{1}H gCOSY of **20**

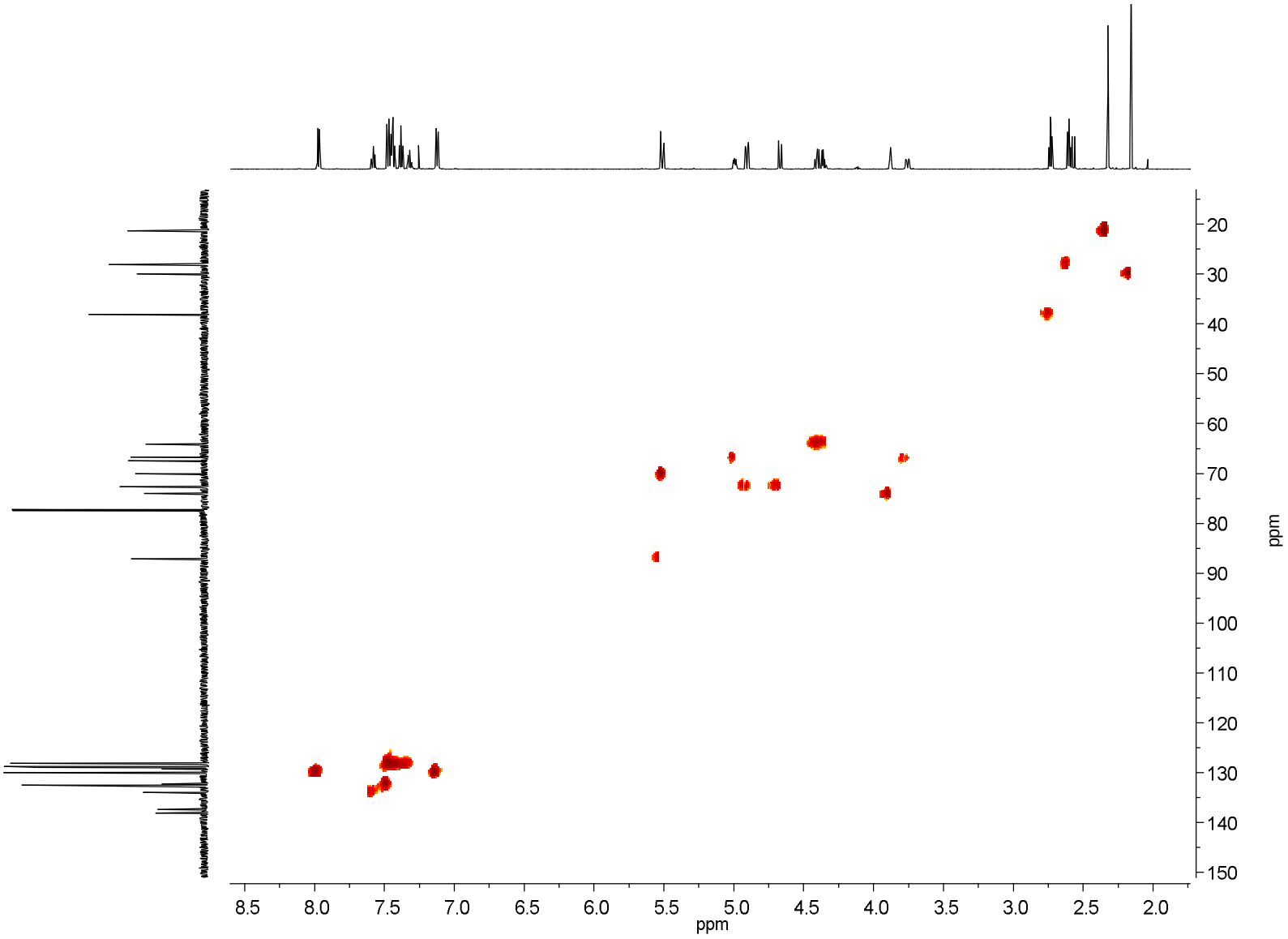


Figure 4.48. 600 MHz, $CDCl_3$, HMQC of **20**

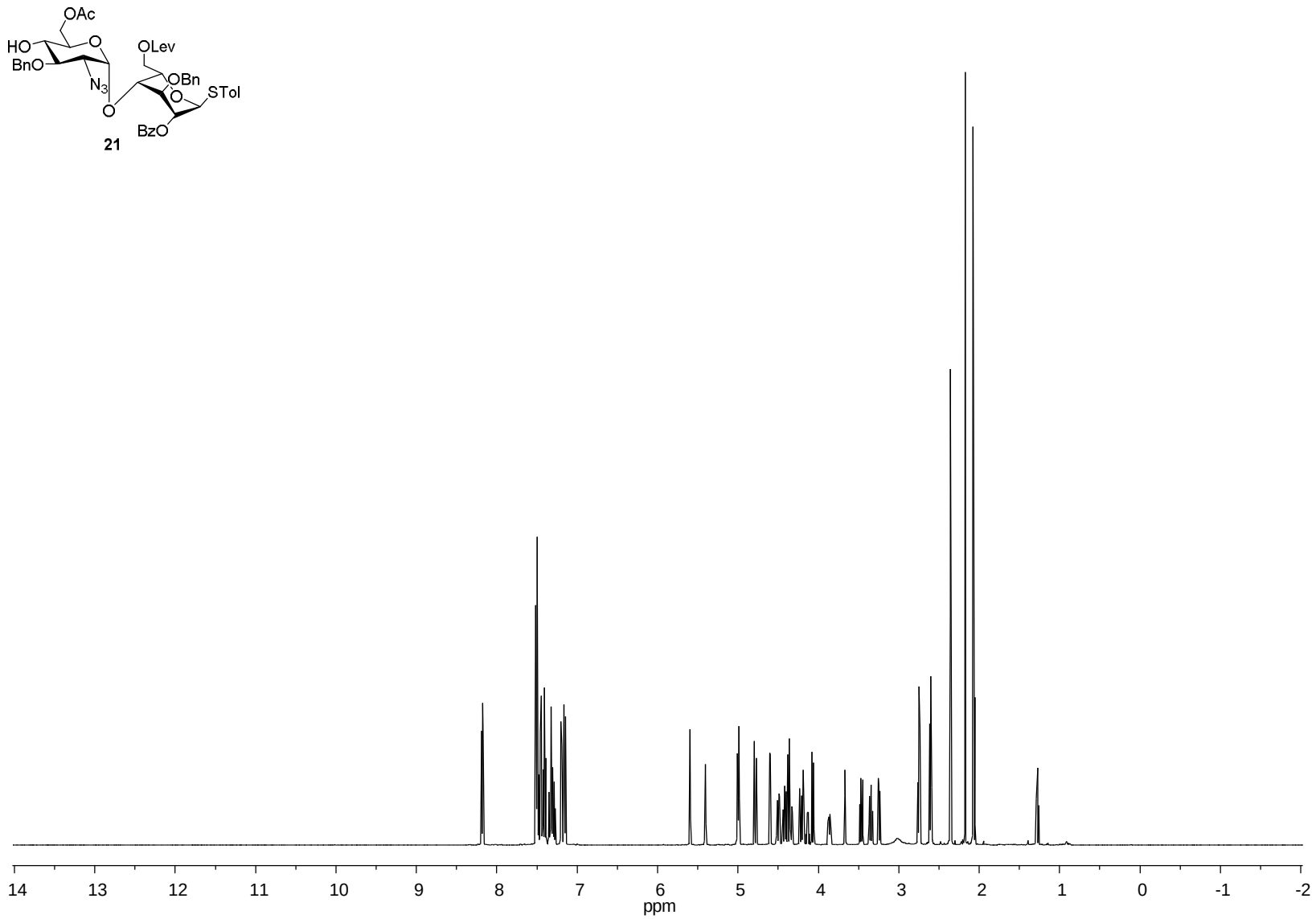


Figure 4.49. 500 MHz, $CDCl_3$, ^1H NMR of **21**

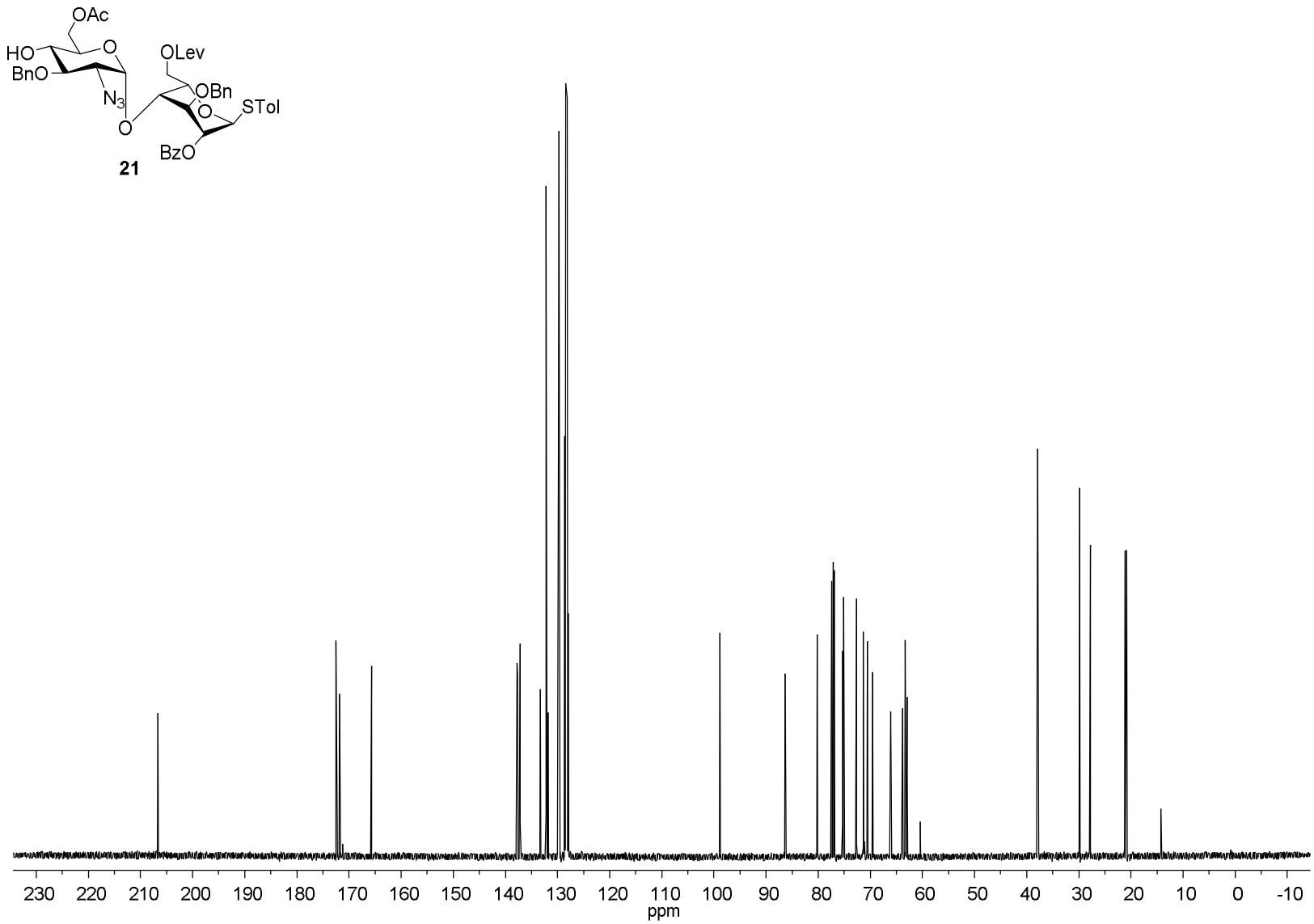


Figure 4.50. 125 MHz, $CDCl_3$, ^{13}C NMR of **21**

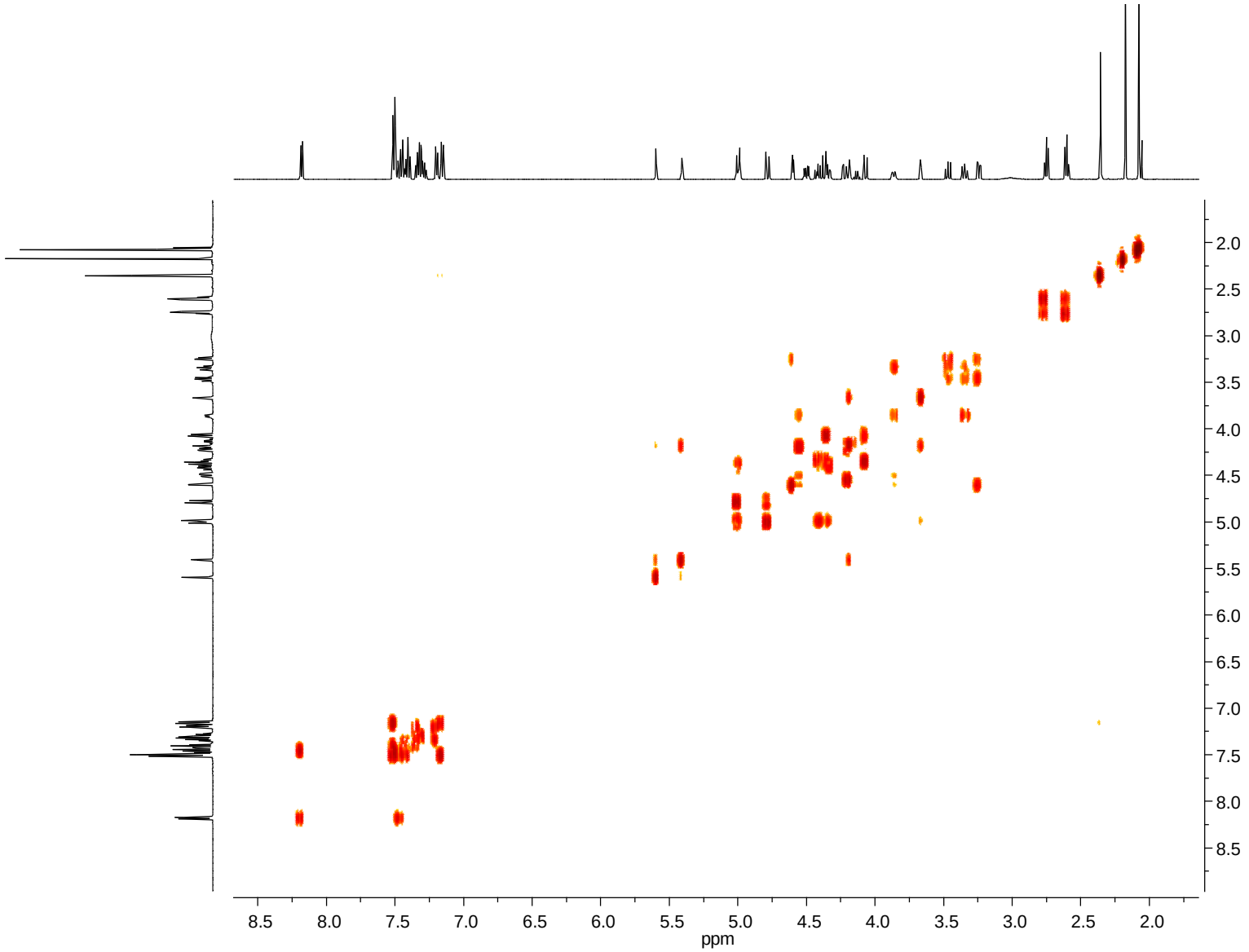


Figure 4.51. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **21**

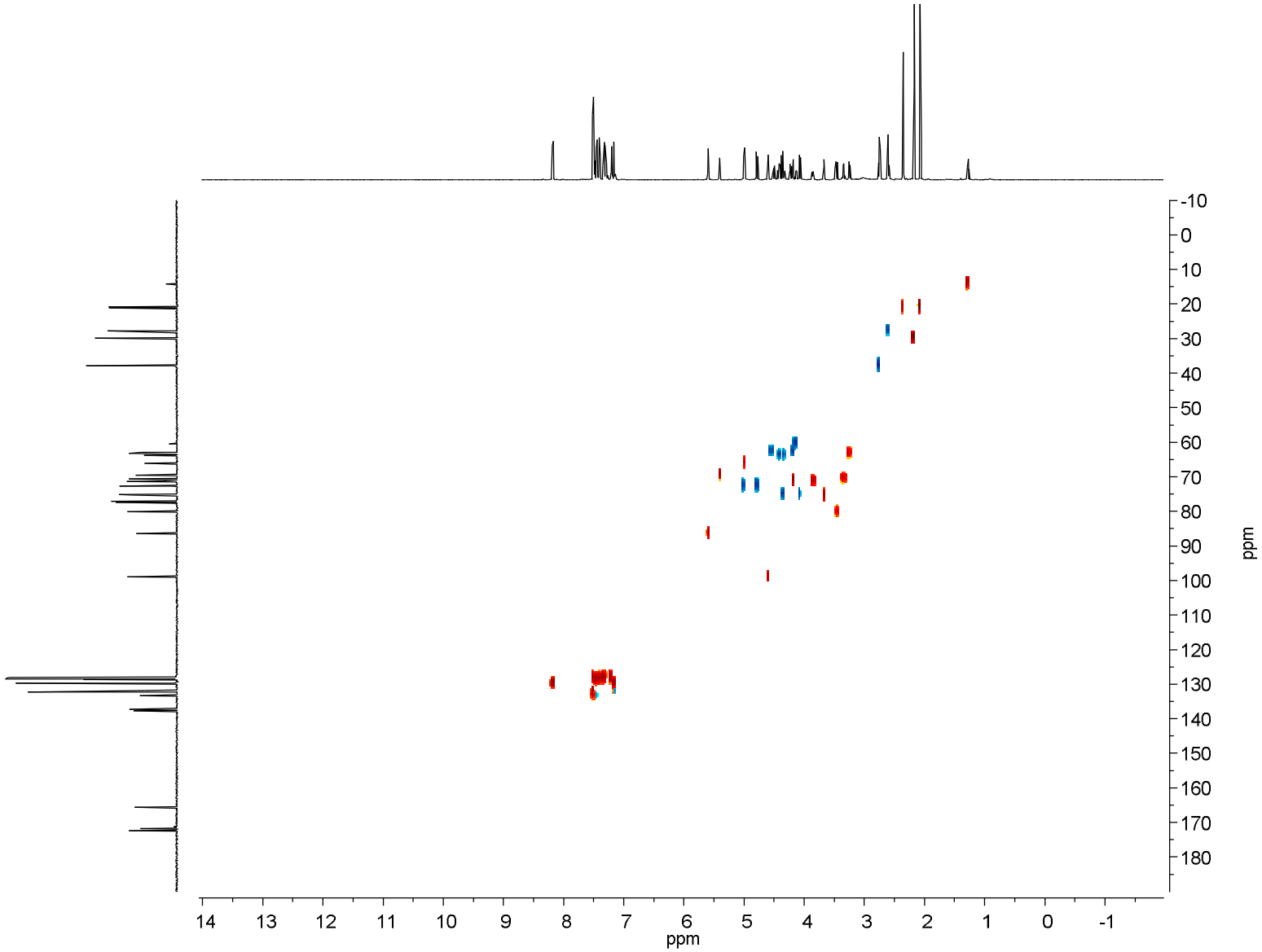


Figure 4.52. 500 MHz, $CDCl_3$, gHSQCAD of **21**

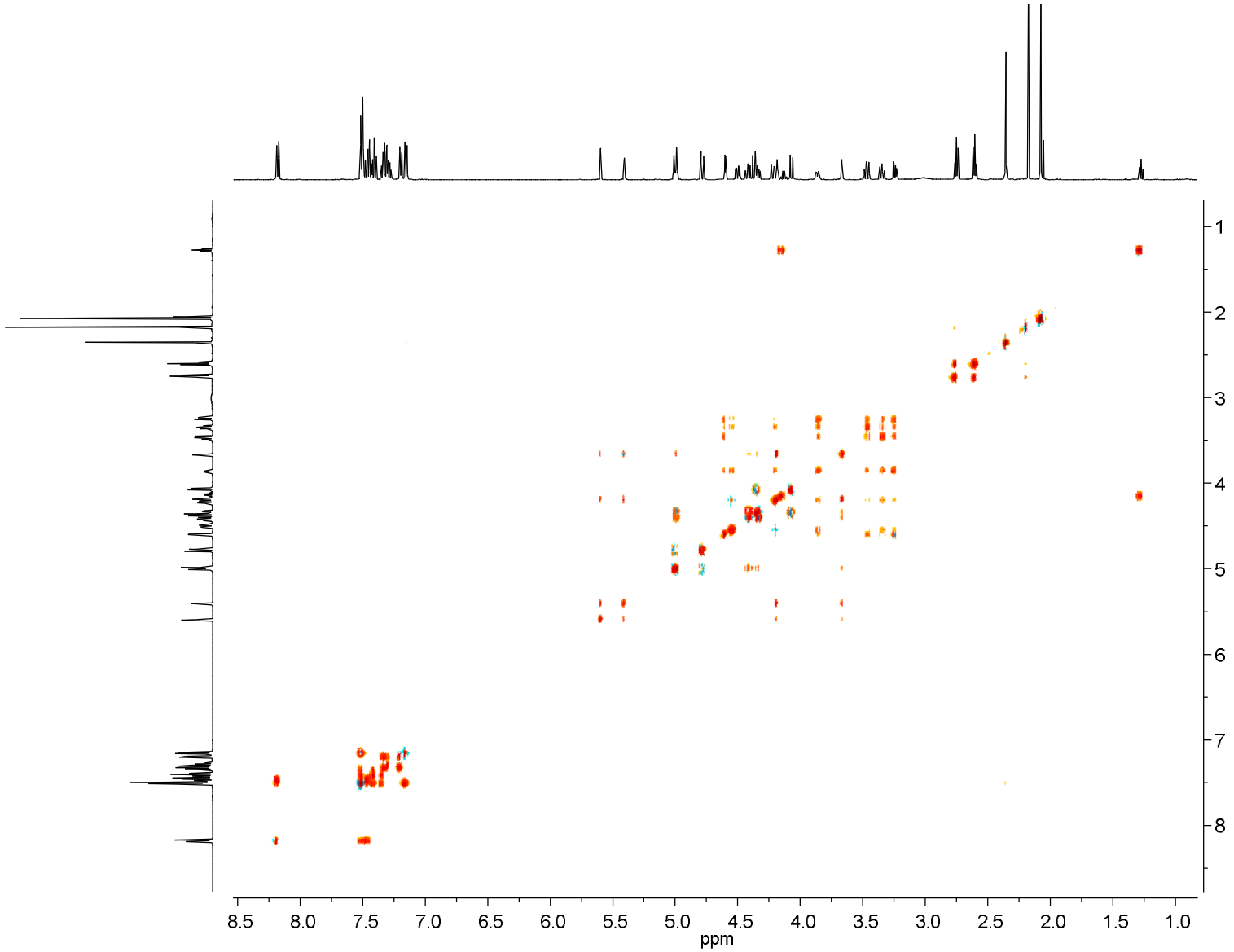


Figure 4.53. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **21**

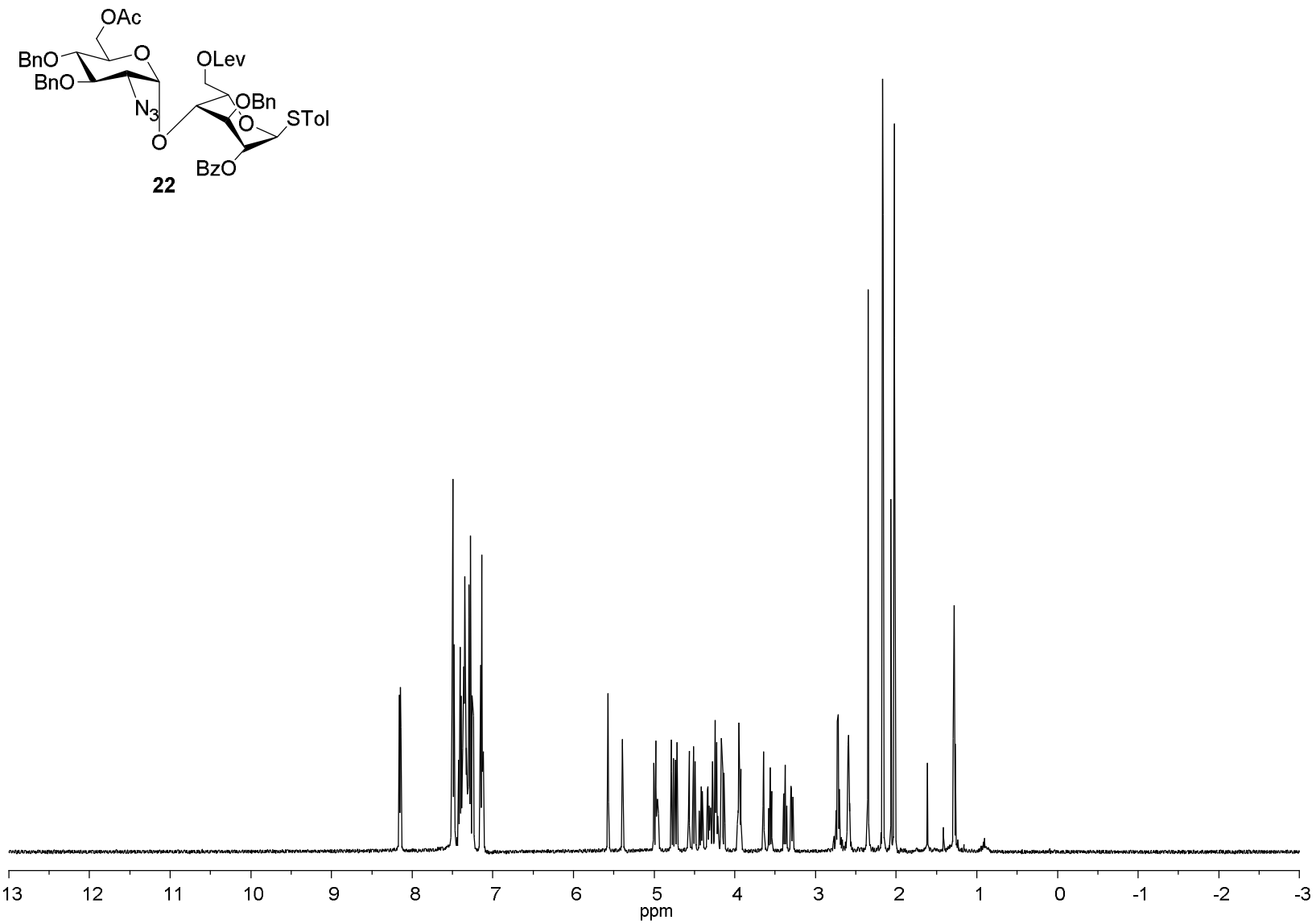


Figure 4.54. 600 MHz, $CDCl_3$, ^1H NMR of **22**

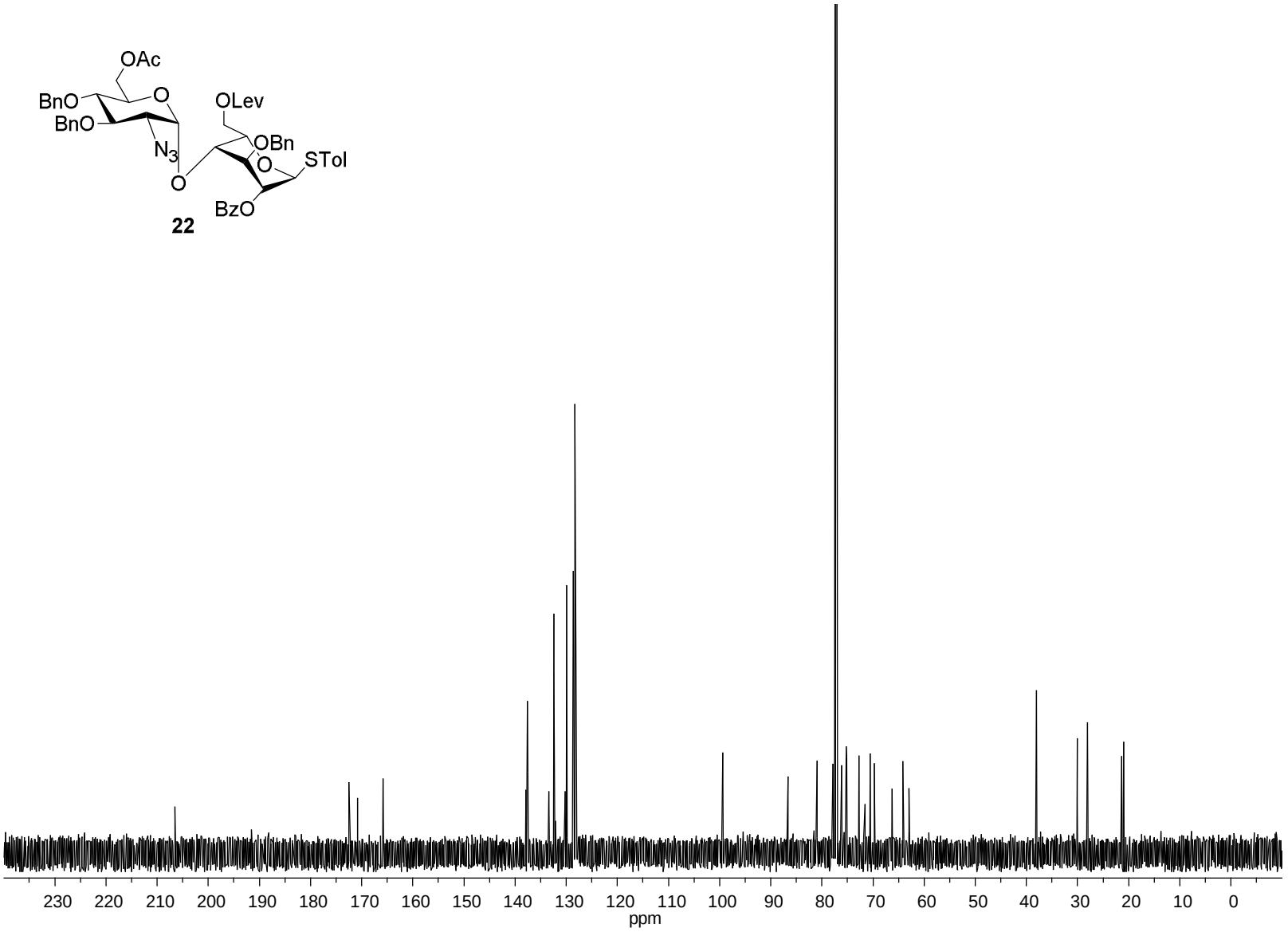


Figure 4.55. 150 MHz, $CDCl_3$, ^{13}C NMR of **22**

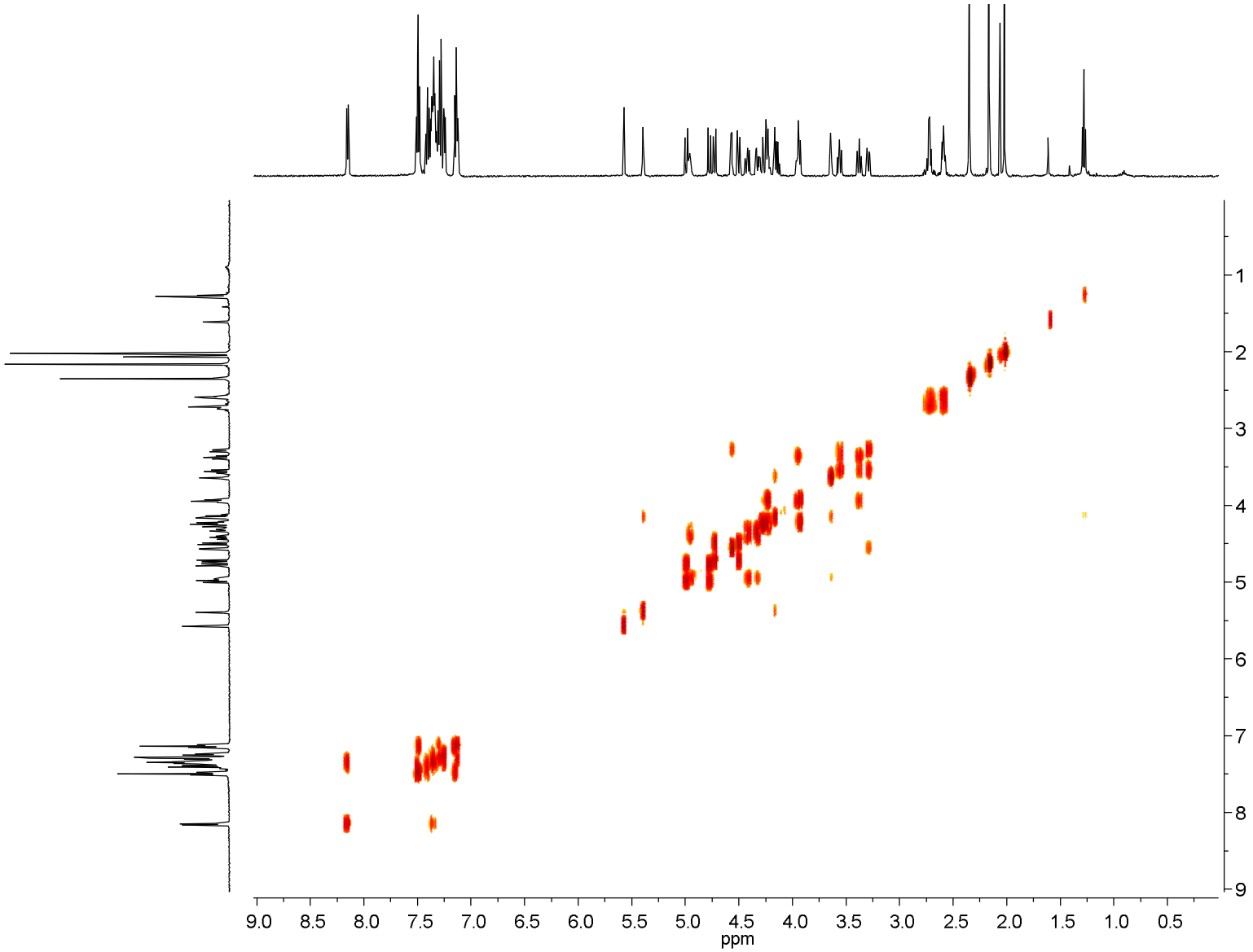


Figure 4.56. 600 MHz, $CDCl_3$, ^1H-^1H gCOSY of **22**

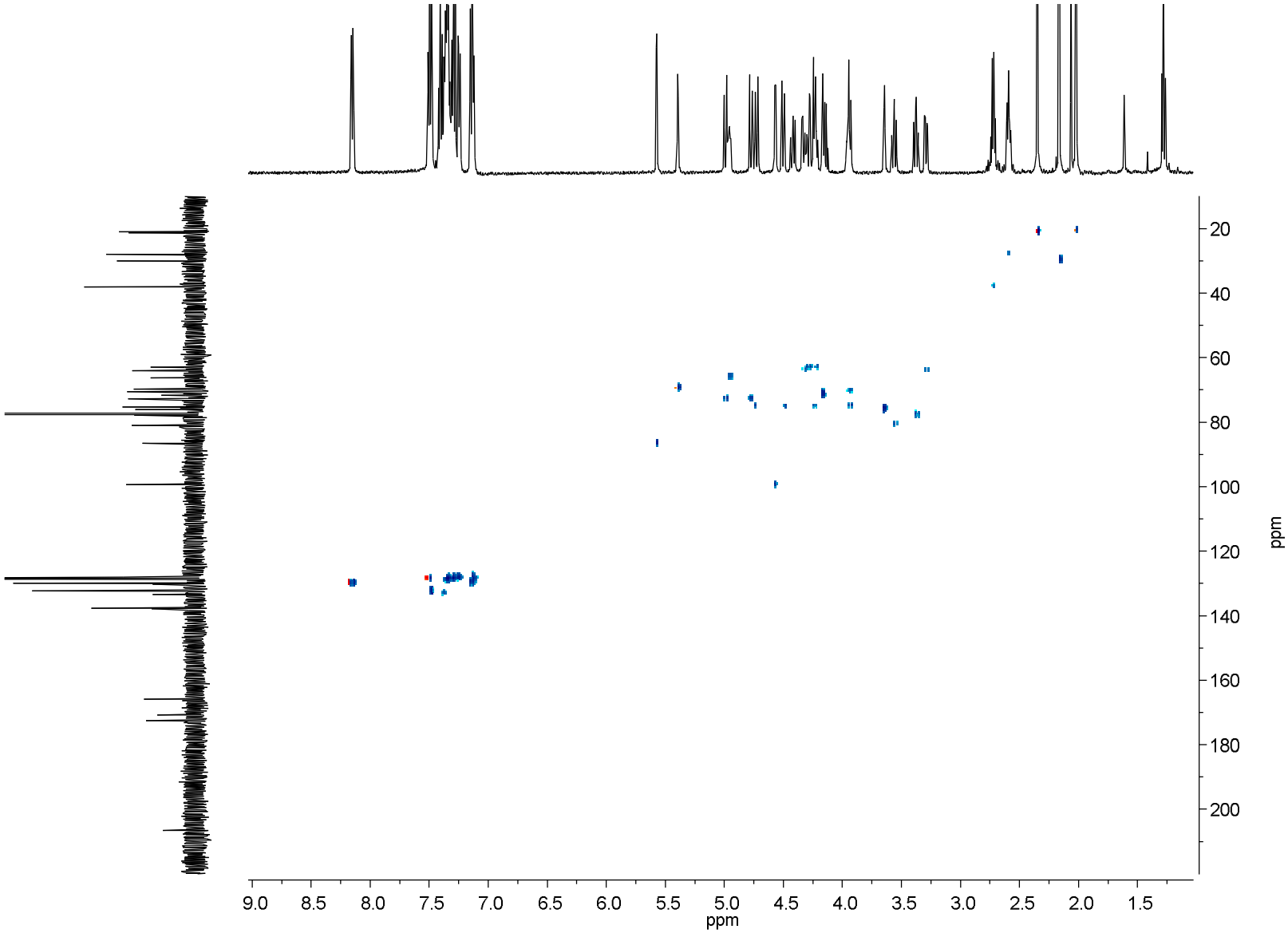


Figure 4.57. 600 MHz, $CDCl_3$, gHMQC of **22**

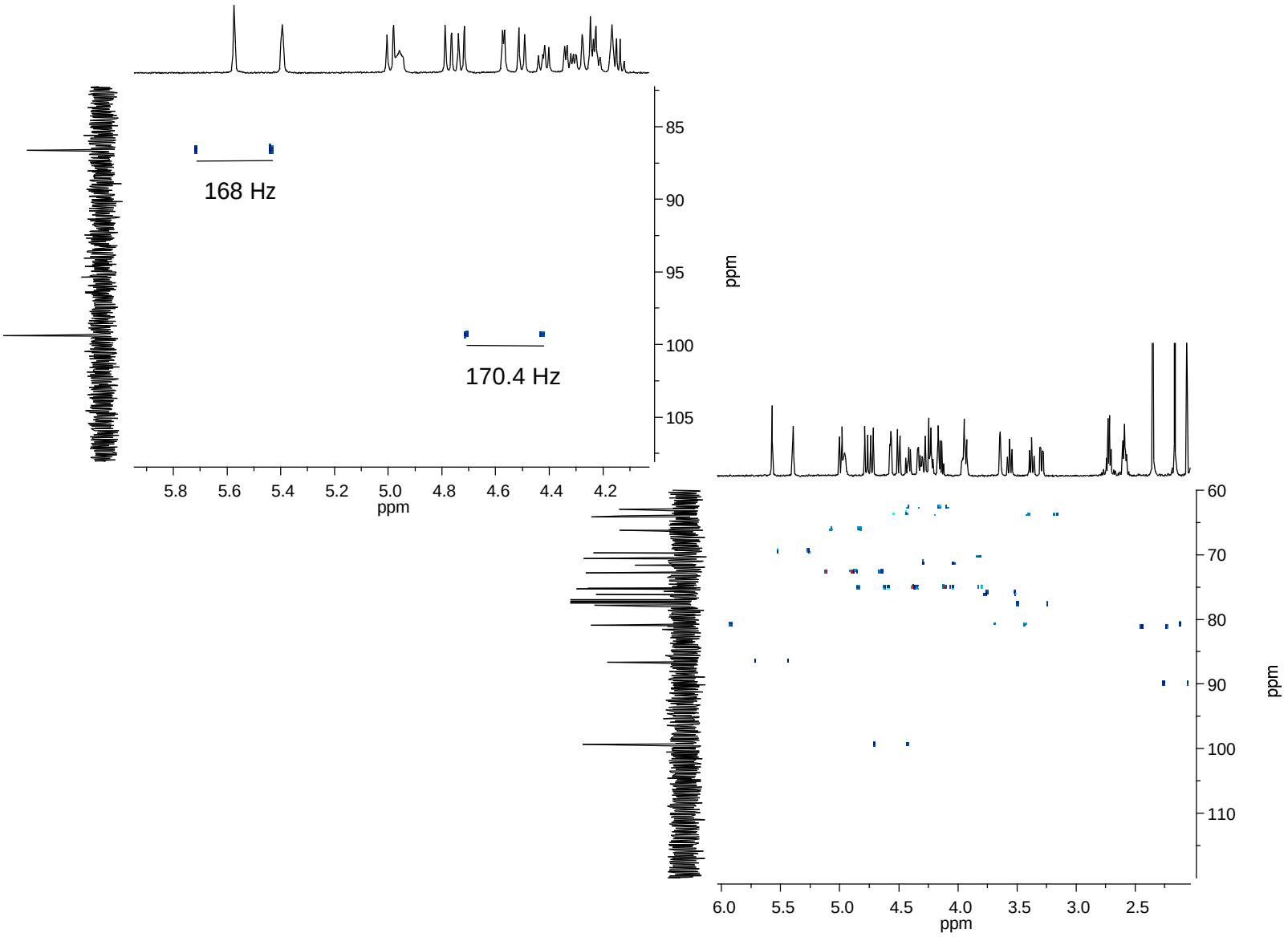
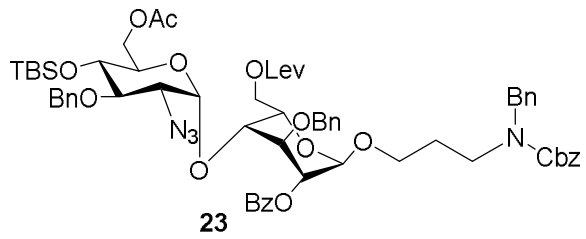


Figure 4.58. 500 MHz, $CDCl_3$, gHMQC (without ^1H Decoupling) of **22**



23

Figure 4.59. 500 MHz, $CDCl_3$, ^1H NMR of **23**

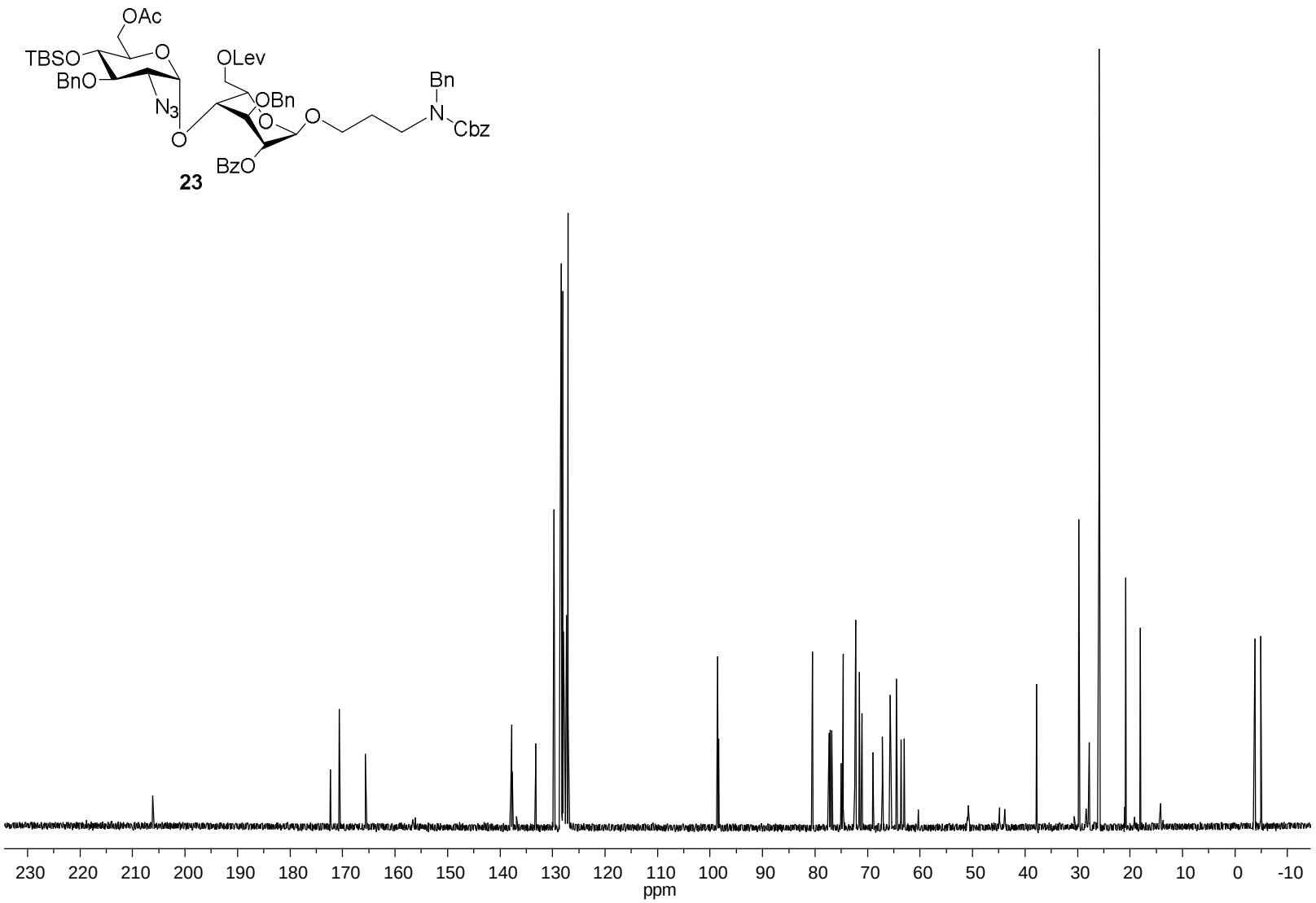


Figure 4.60. 125 MHz, $CDCl_3$, ^{13}C NMR of **23**

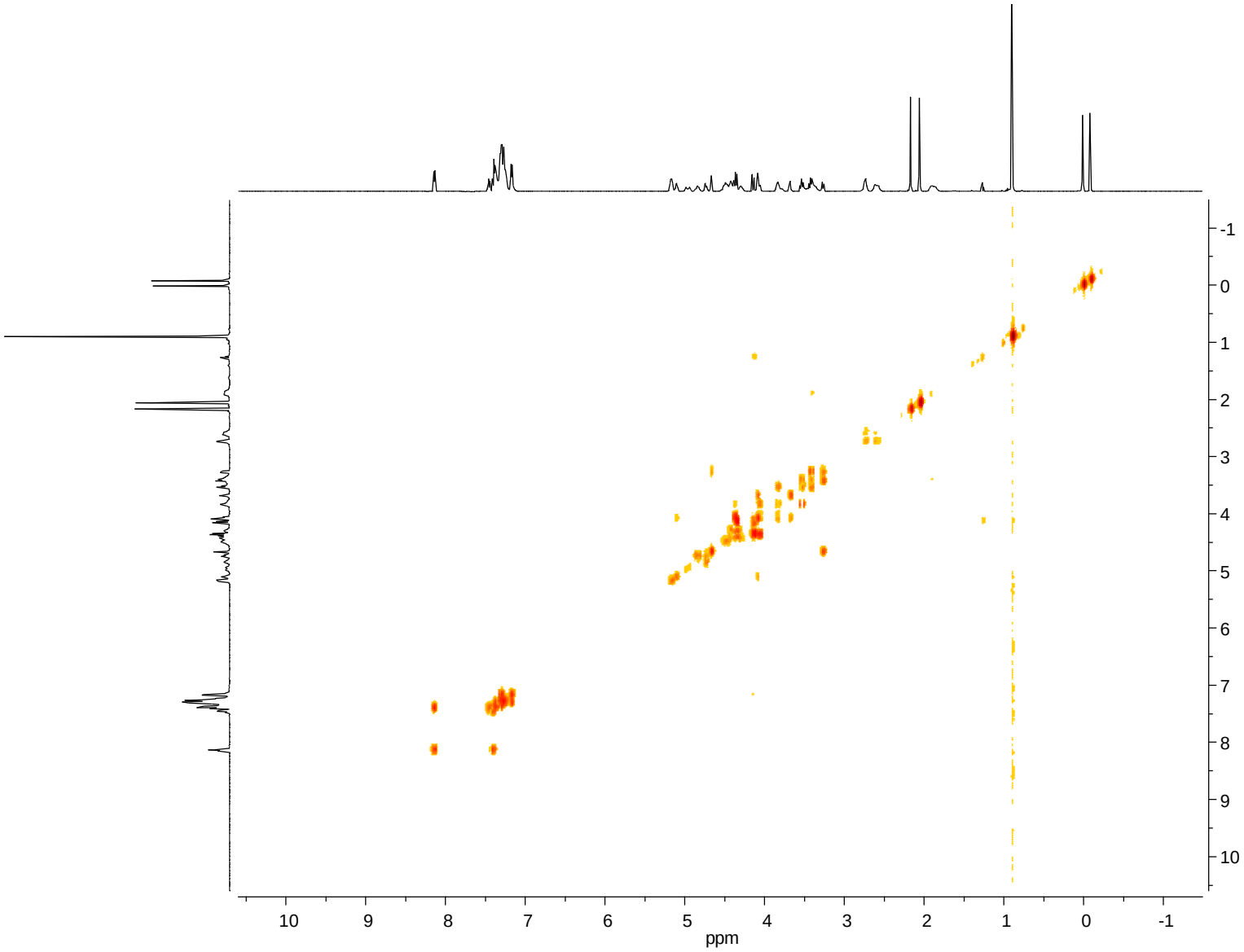


Figure 4.61. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **23**

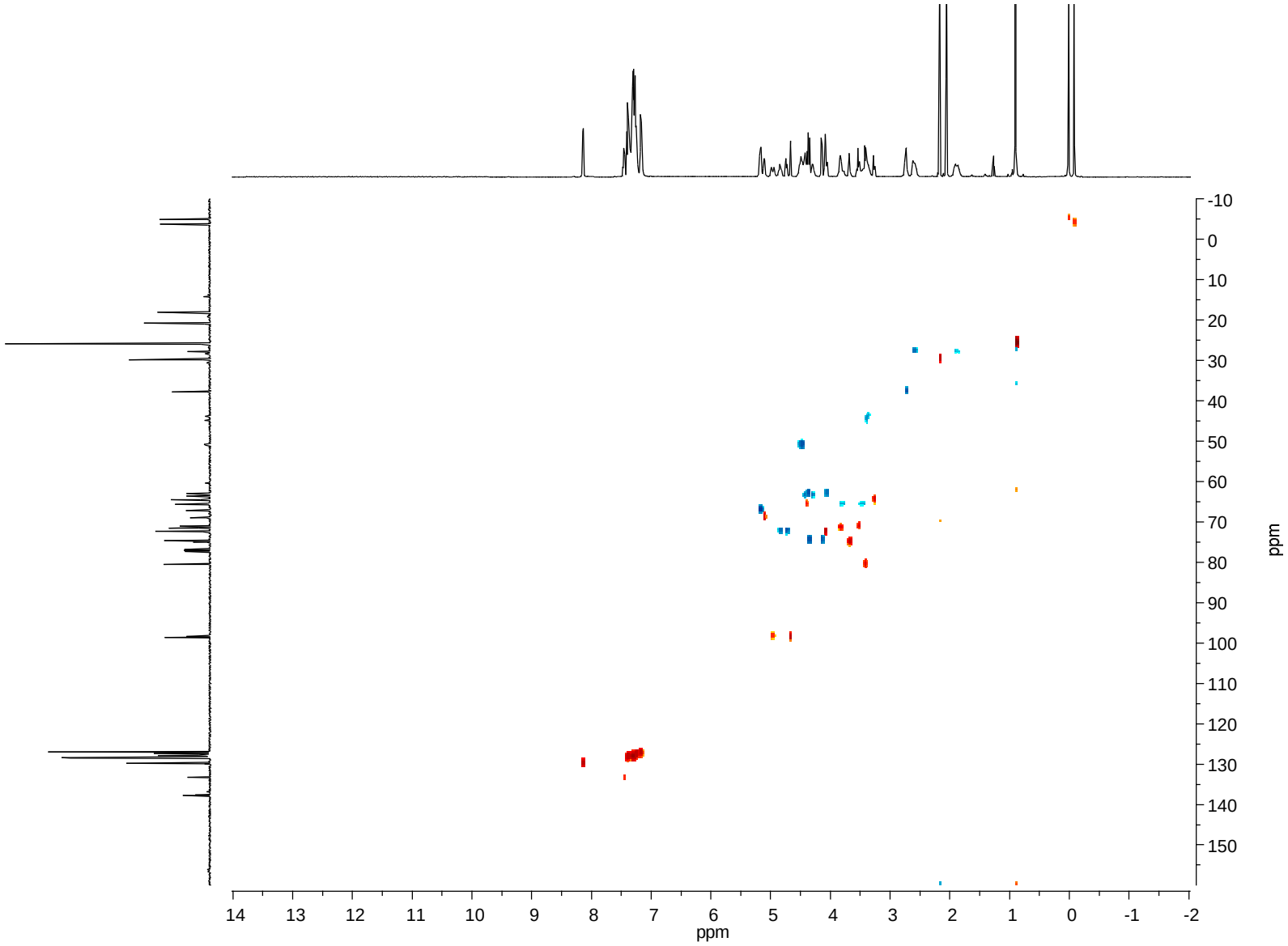


Figure 4.62. 500 MHz, $CDCl_3$, gHSQCAD of **23**

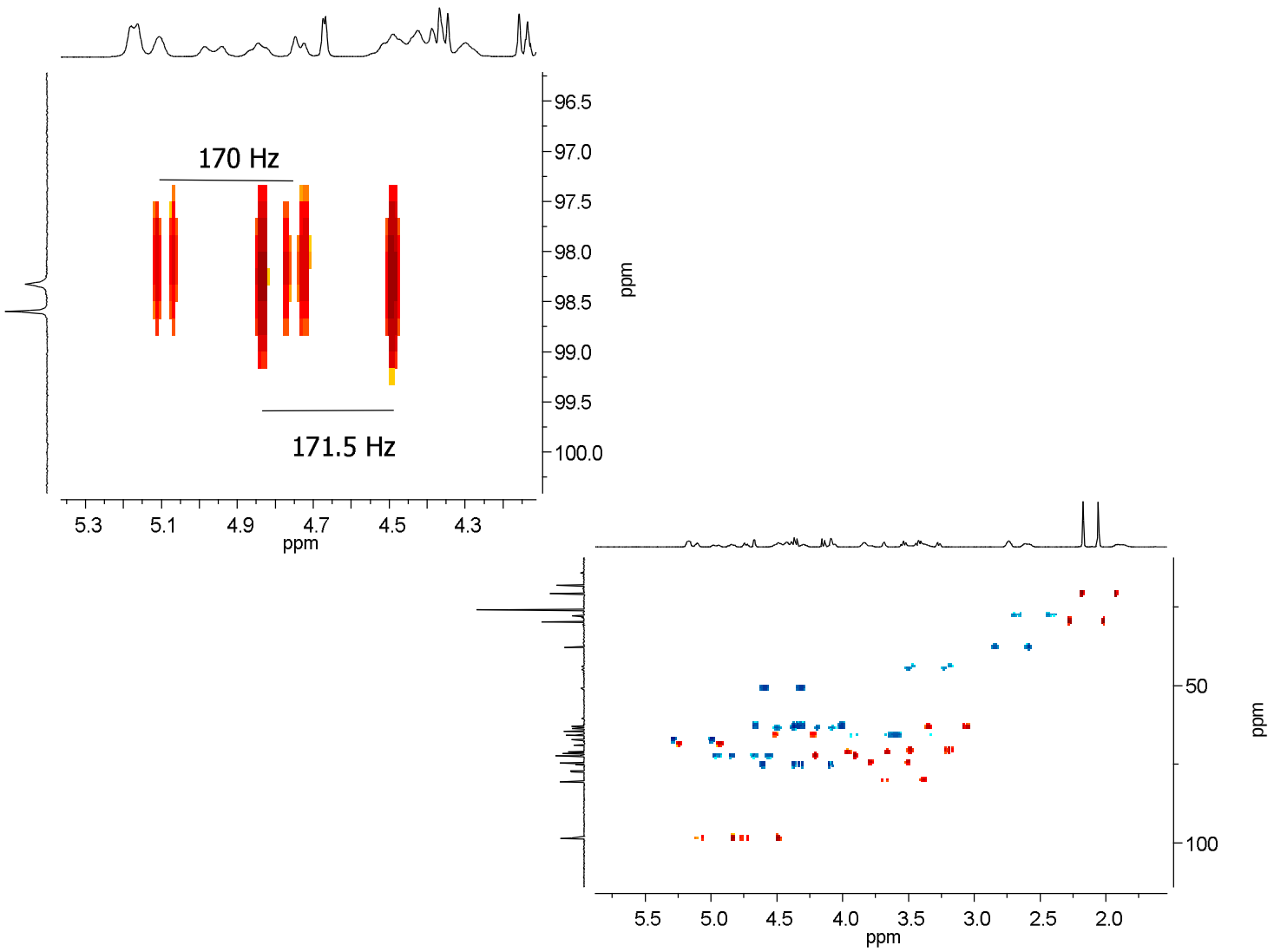


Figure 4.63. 500 MHz, $CDCl_3$, gHSQCAD (without ^1H Decoupling) of **23**

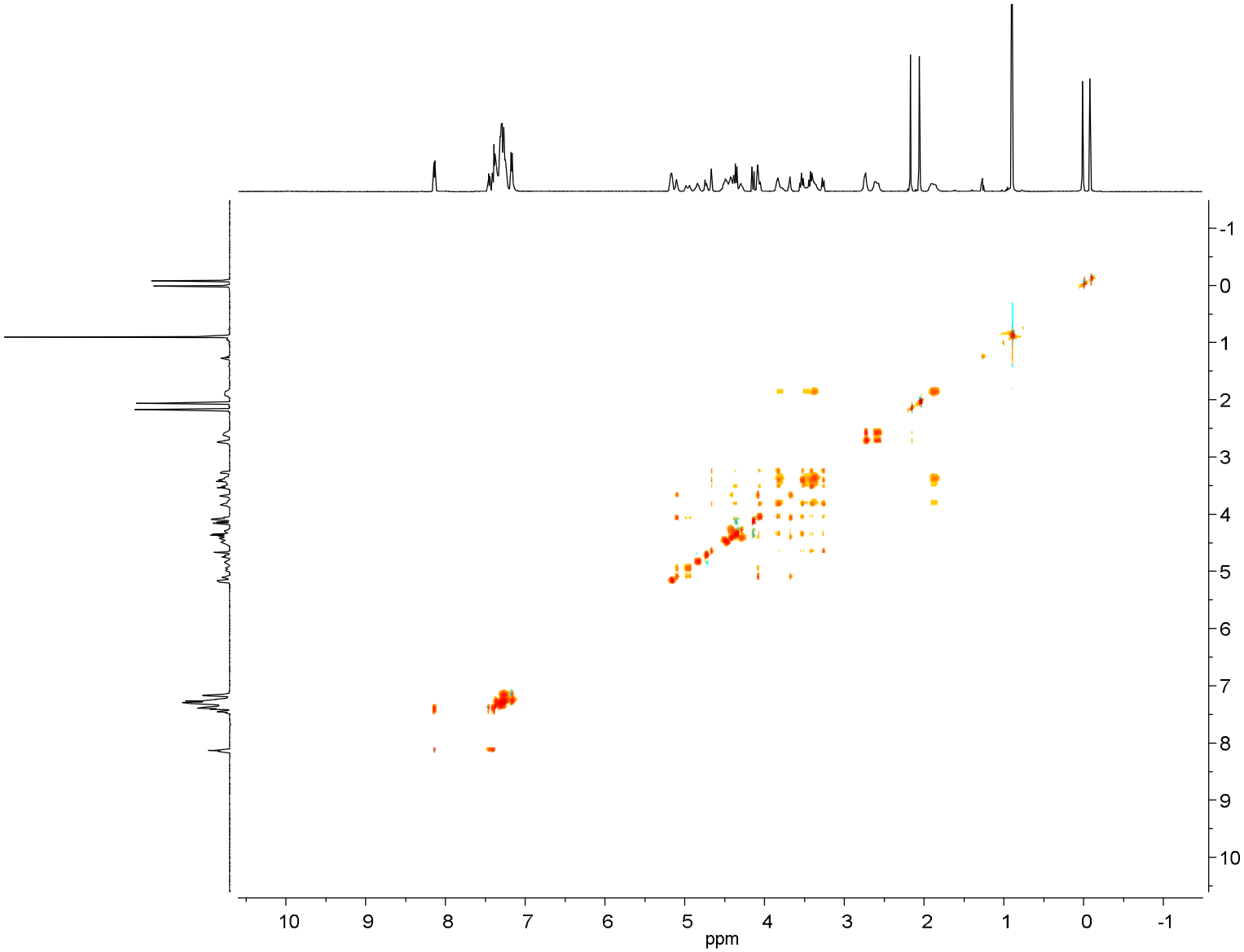


Figure 4.64. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **23**

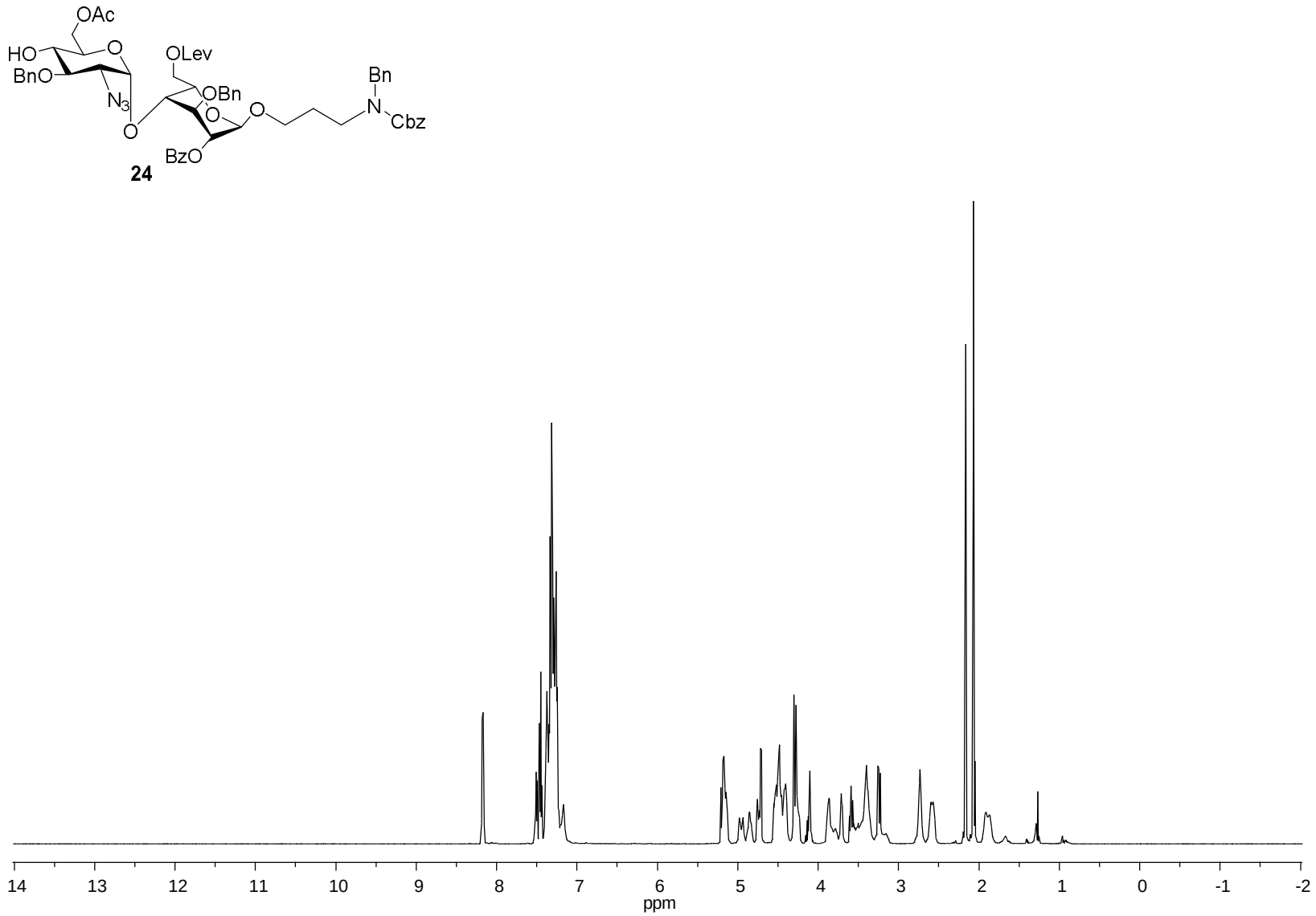
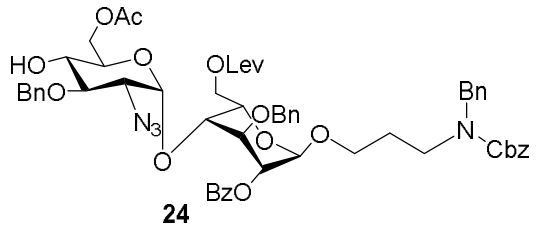


Figure 4.65. 500 MHz, $CDCl_3$, ^1H NMR of **24**



24

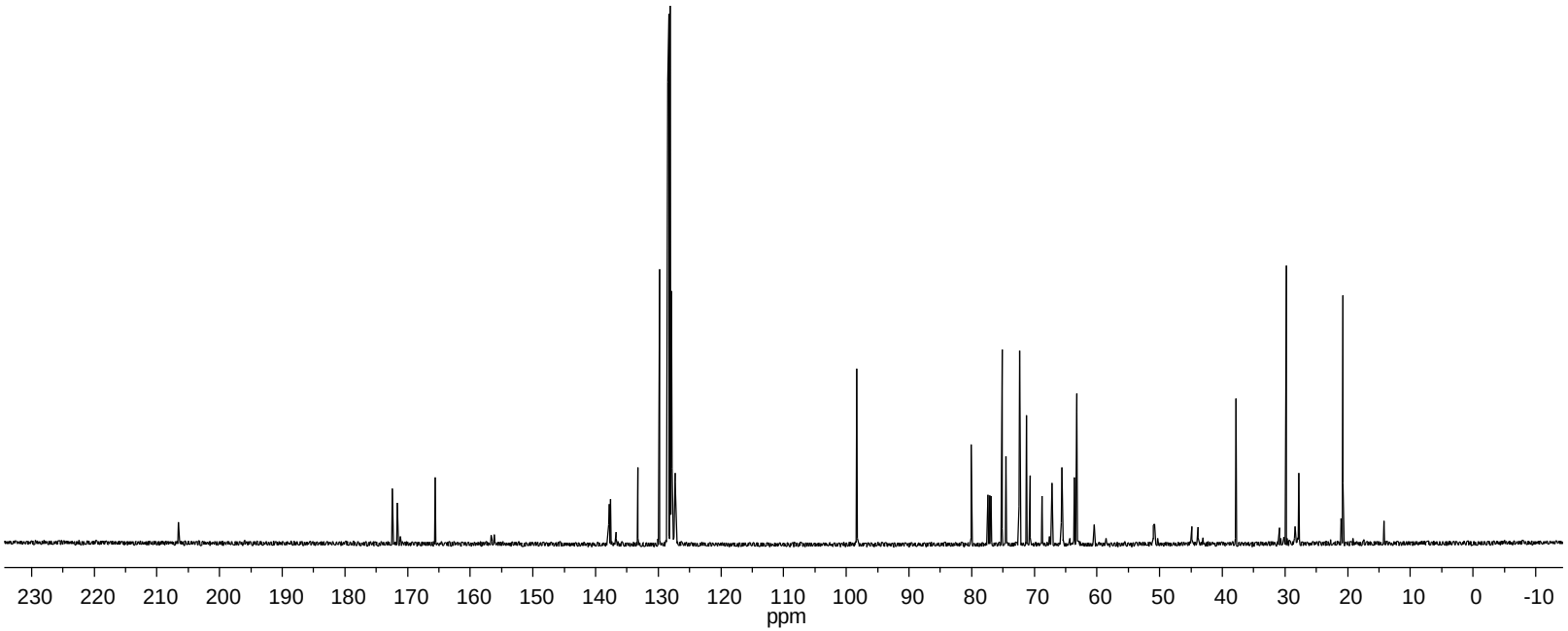


Figure 4.66. 125 MHz, $CDCl_3$, ^{13}C NMR of **24**

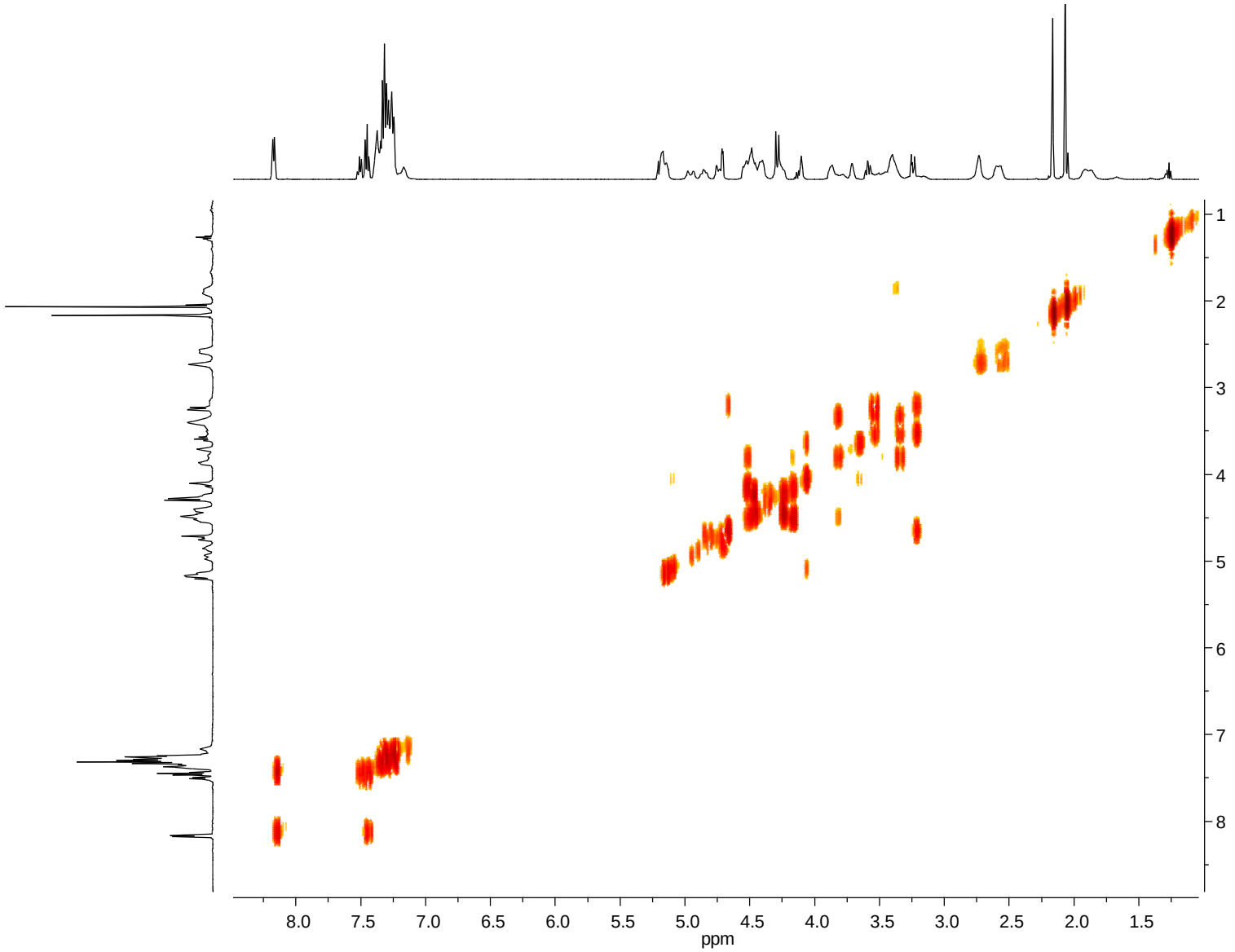


Figure 4.67. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **24**

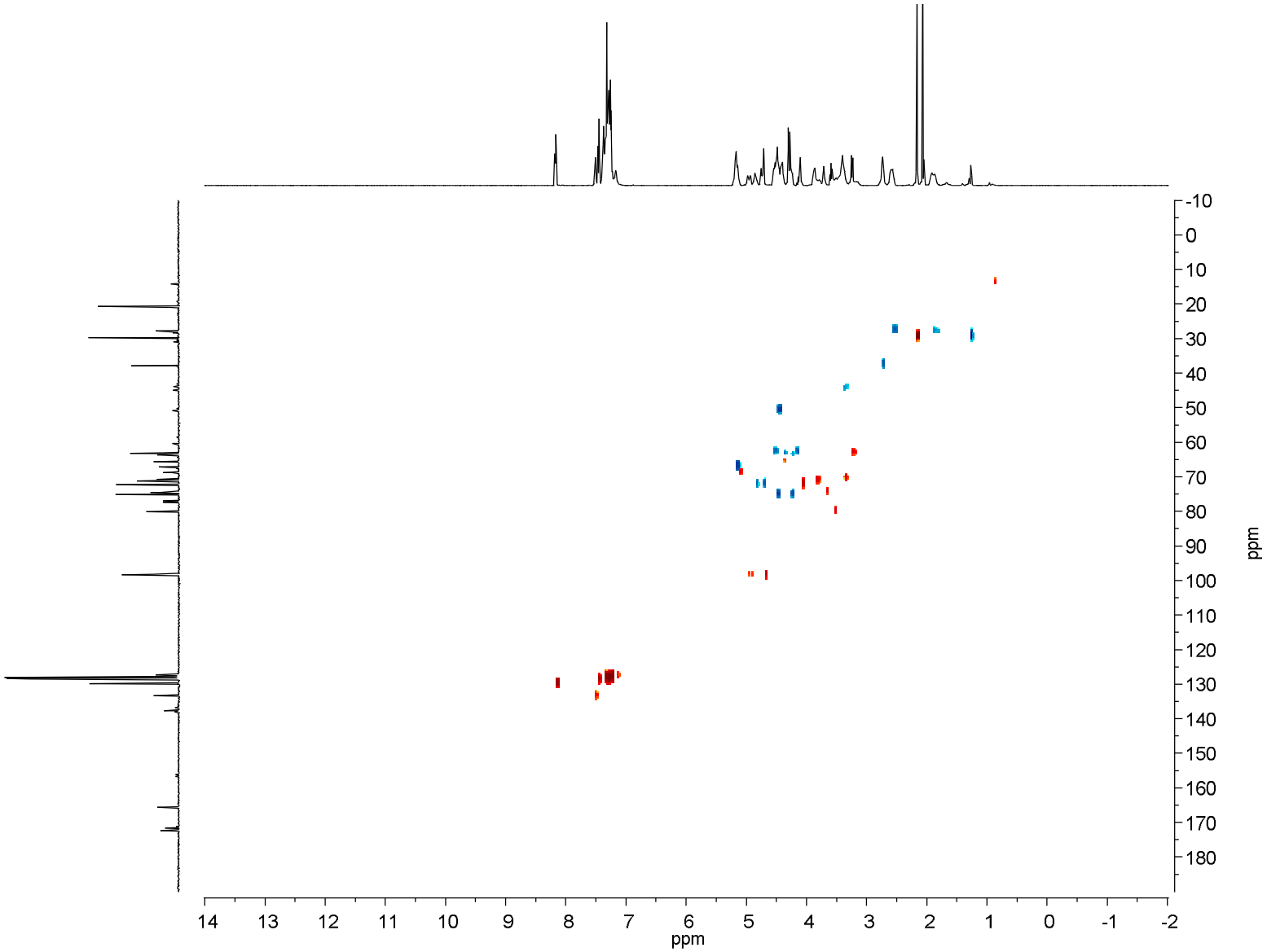


Figure 4.68. 500 MHz, $CDCl_3$, gHSQCAD of **24**

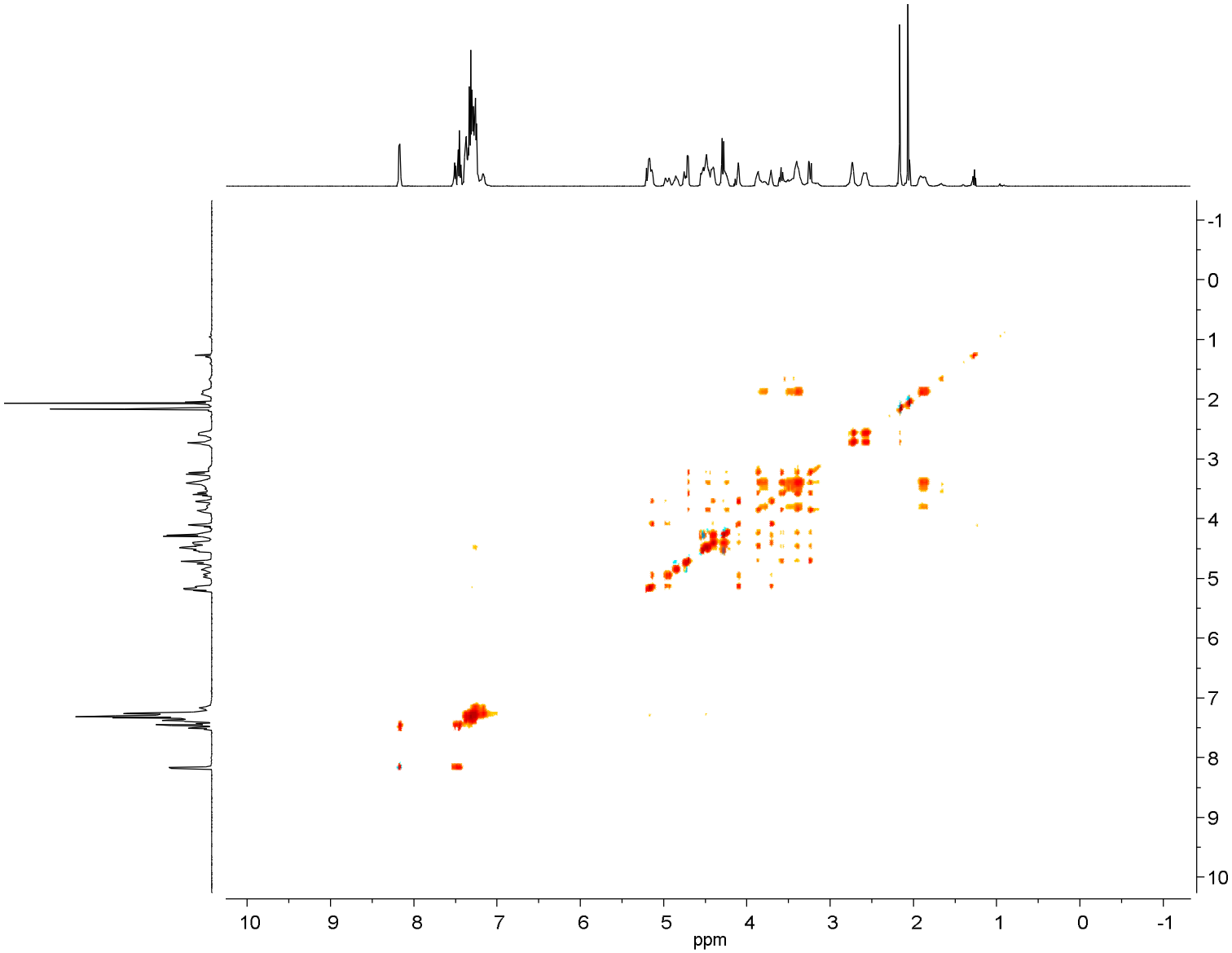
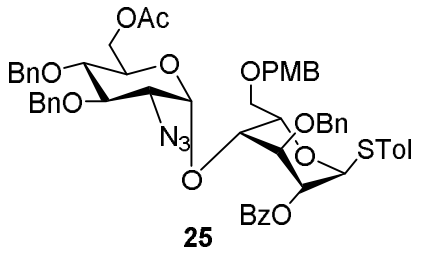


Figure 4.69. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **24**



25

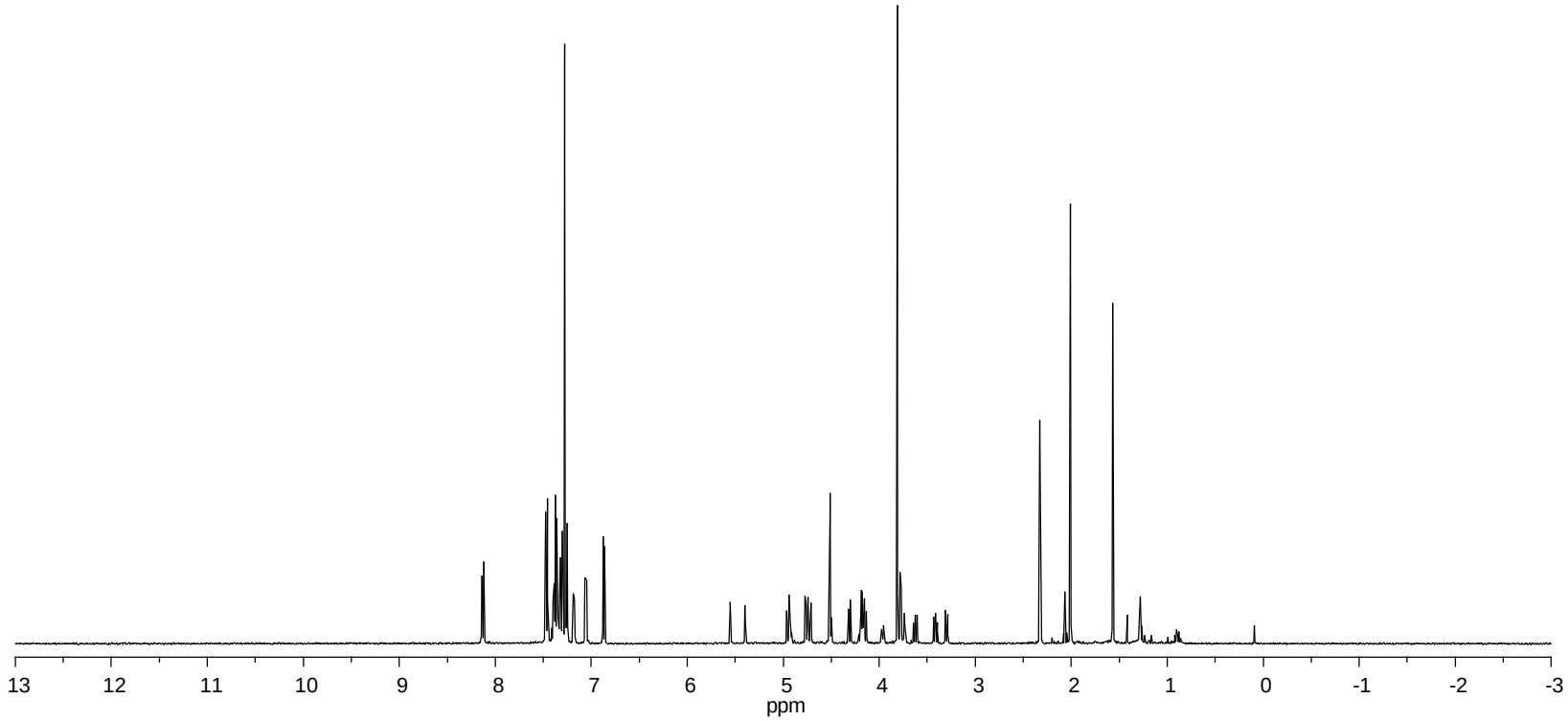


Figure 4.70. 500 MHz, $CDCl_3$, ^1H NMR of **25**

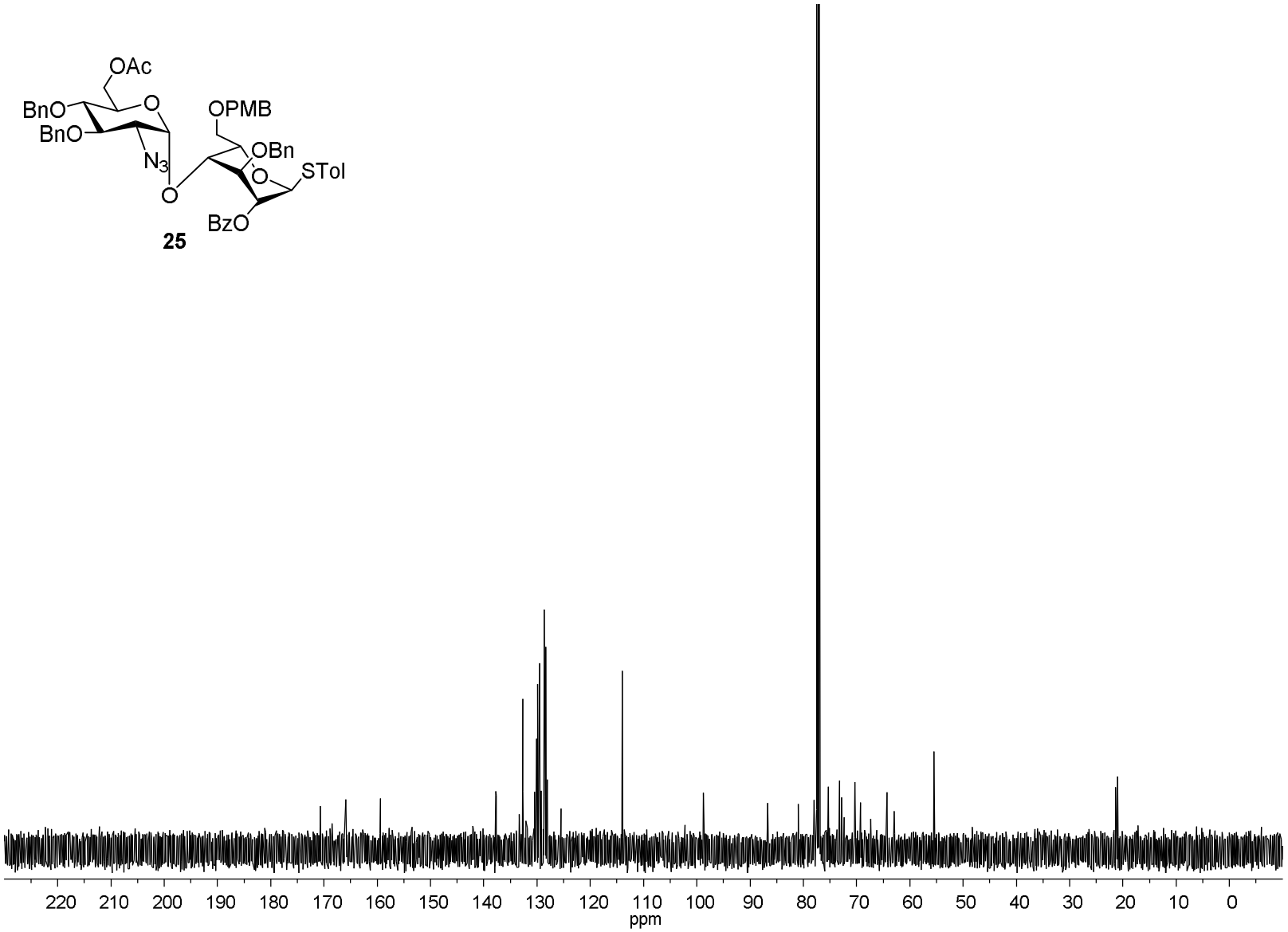


Figure 4.71. 125 MHz, $CDCl_3$, ^{13}C NMR of **25**

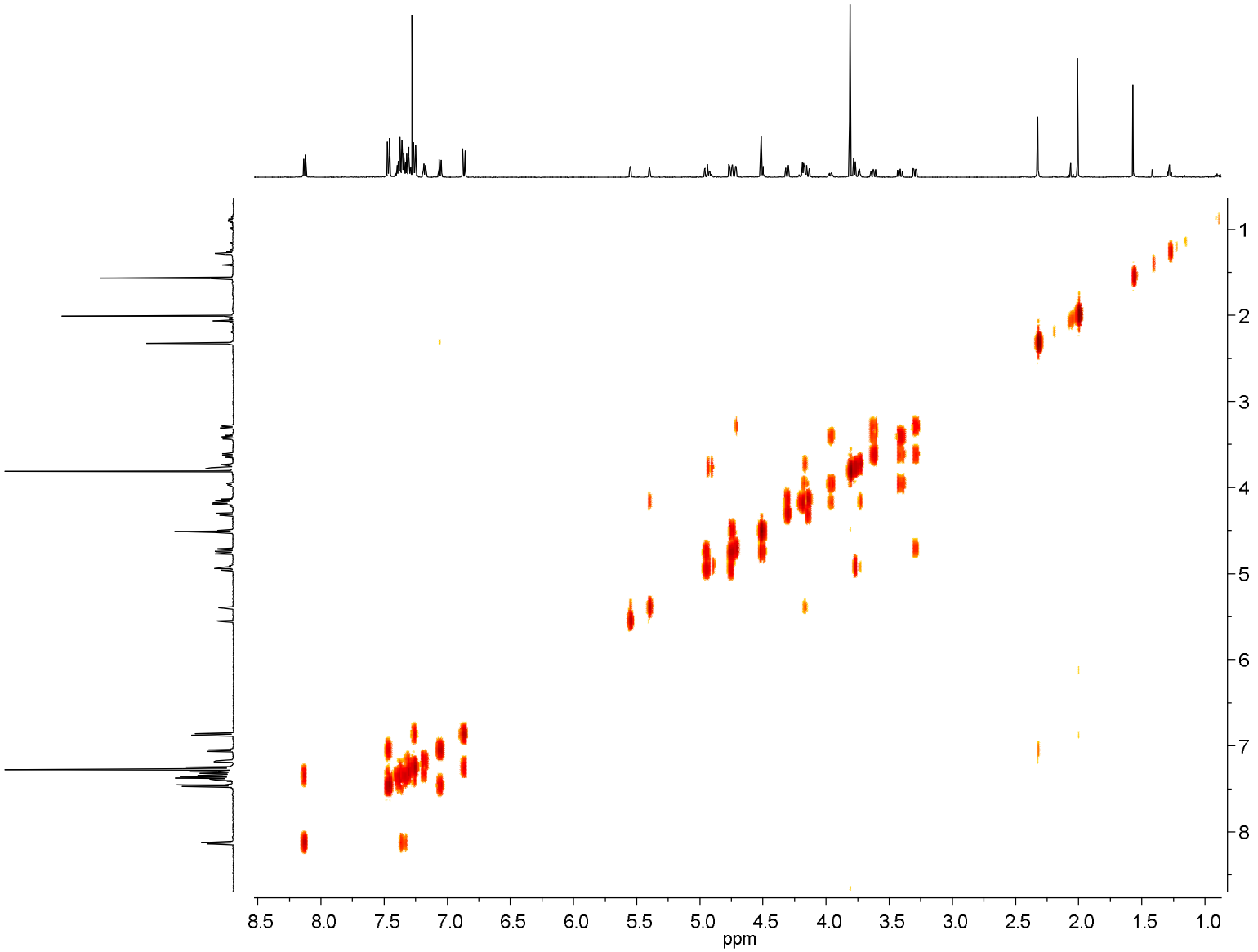


Figure 4.72. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **25**

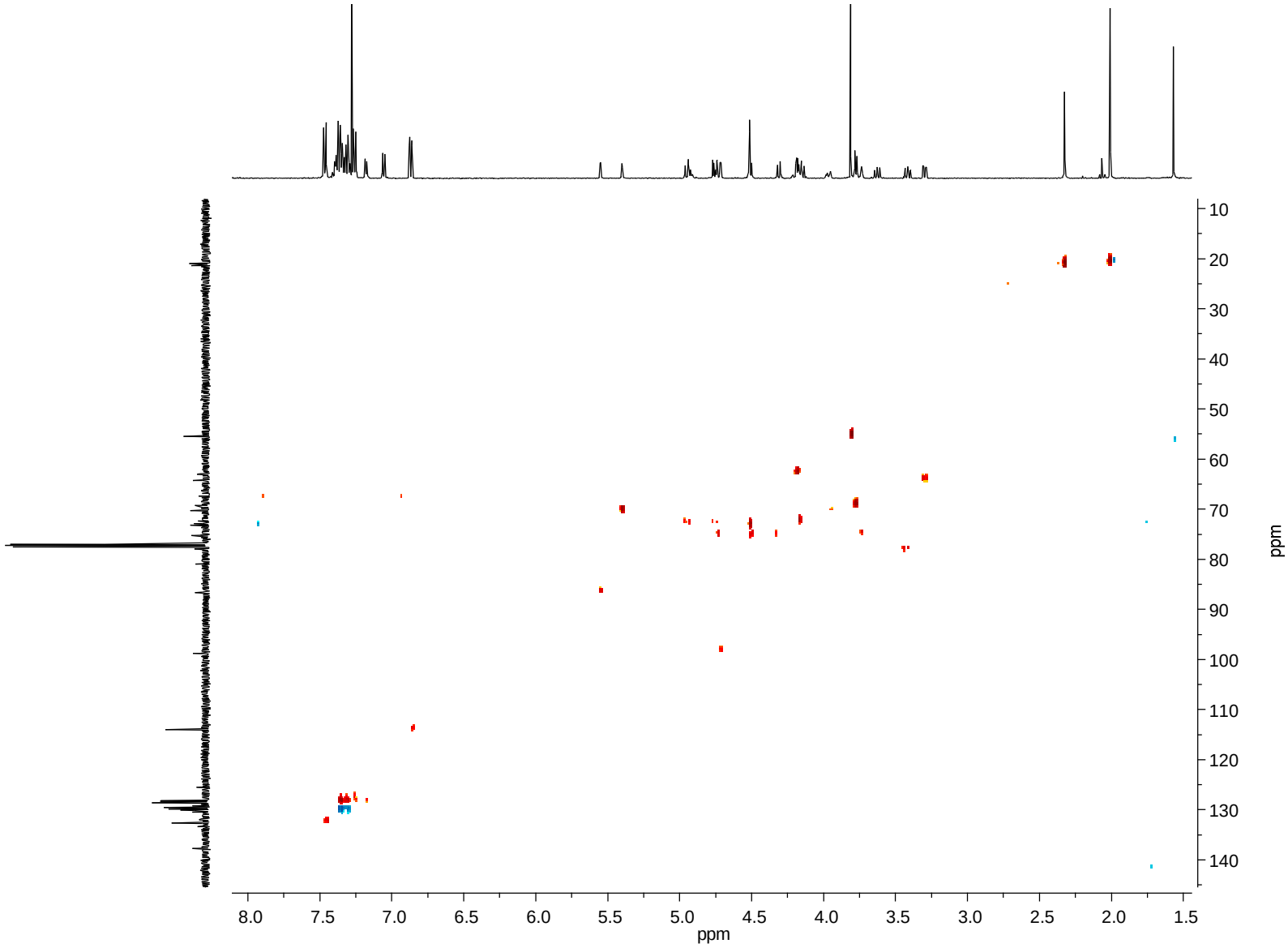


Figure 4.73. 500 MHz, $CDCl_3$, gHMQC of **25**

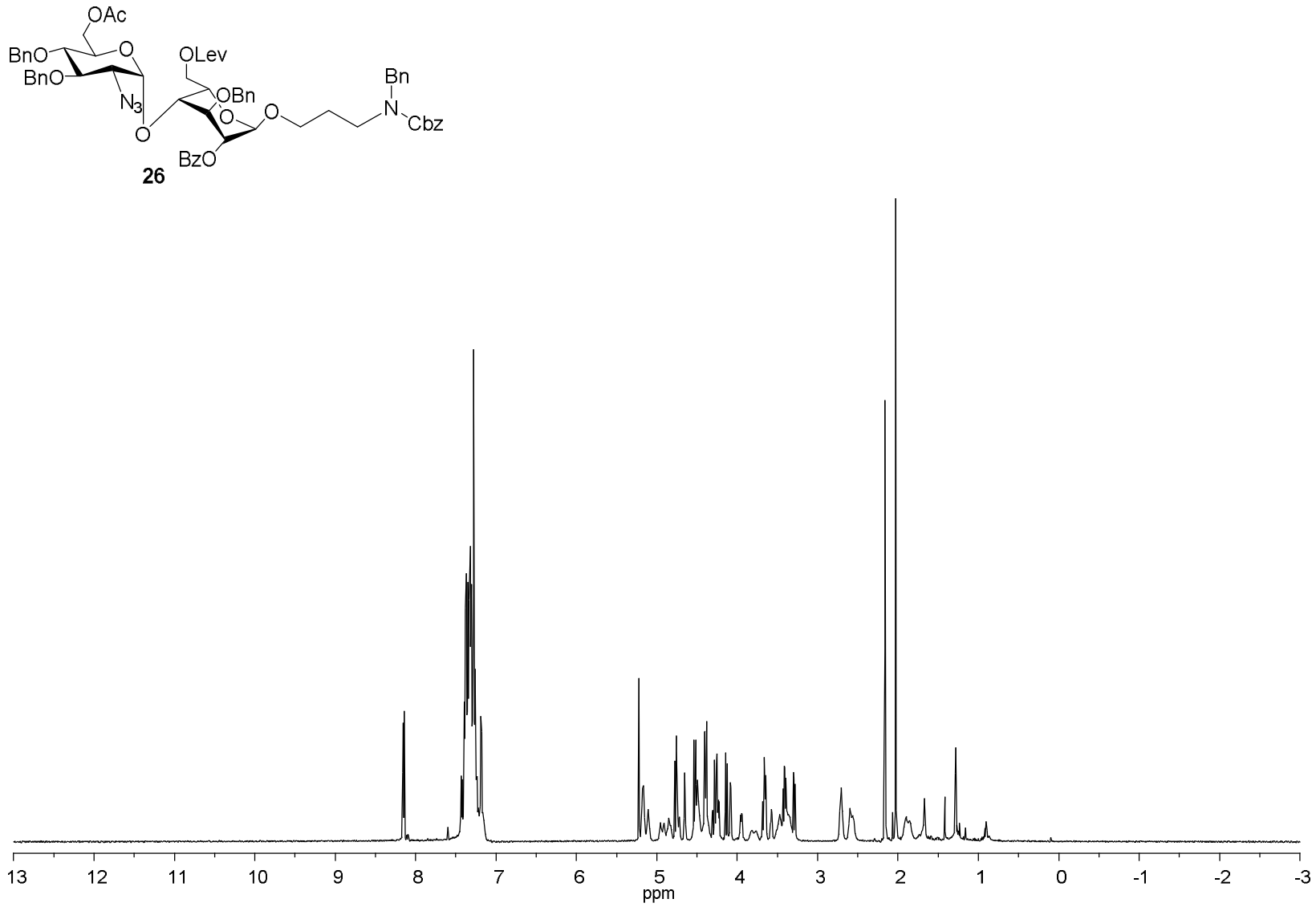


Figure 4.74. 500 MHz, $CDCl_3$, ^1H NMR of **26**

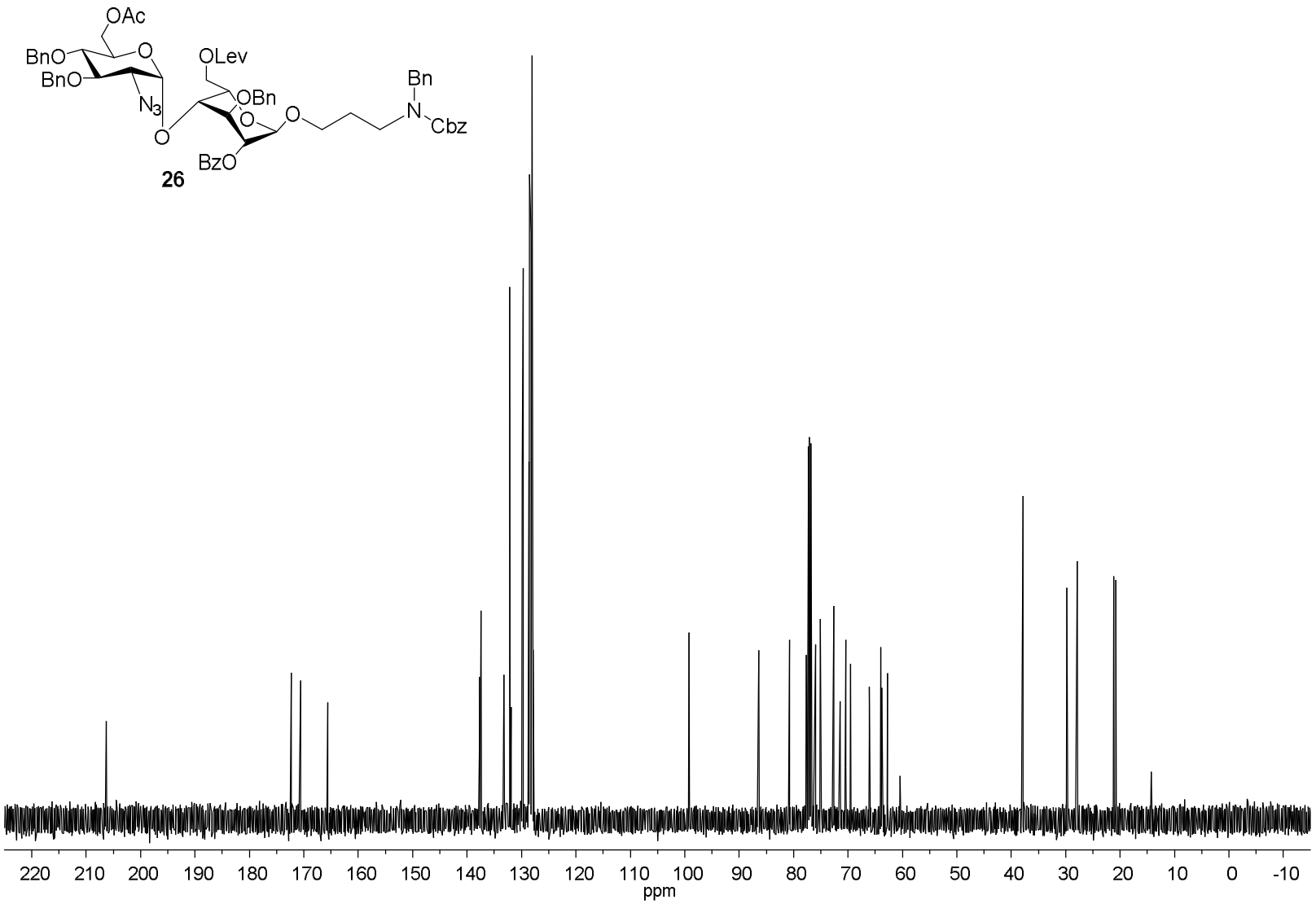


Figure 4.75. 125 MHz, $CDCl_3$, ^{13}C NMR of **26**

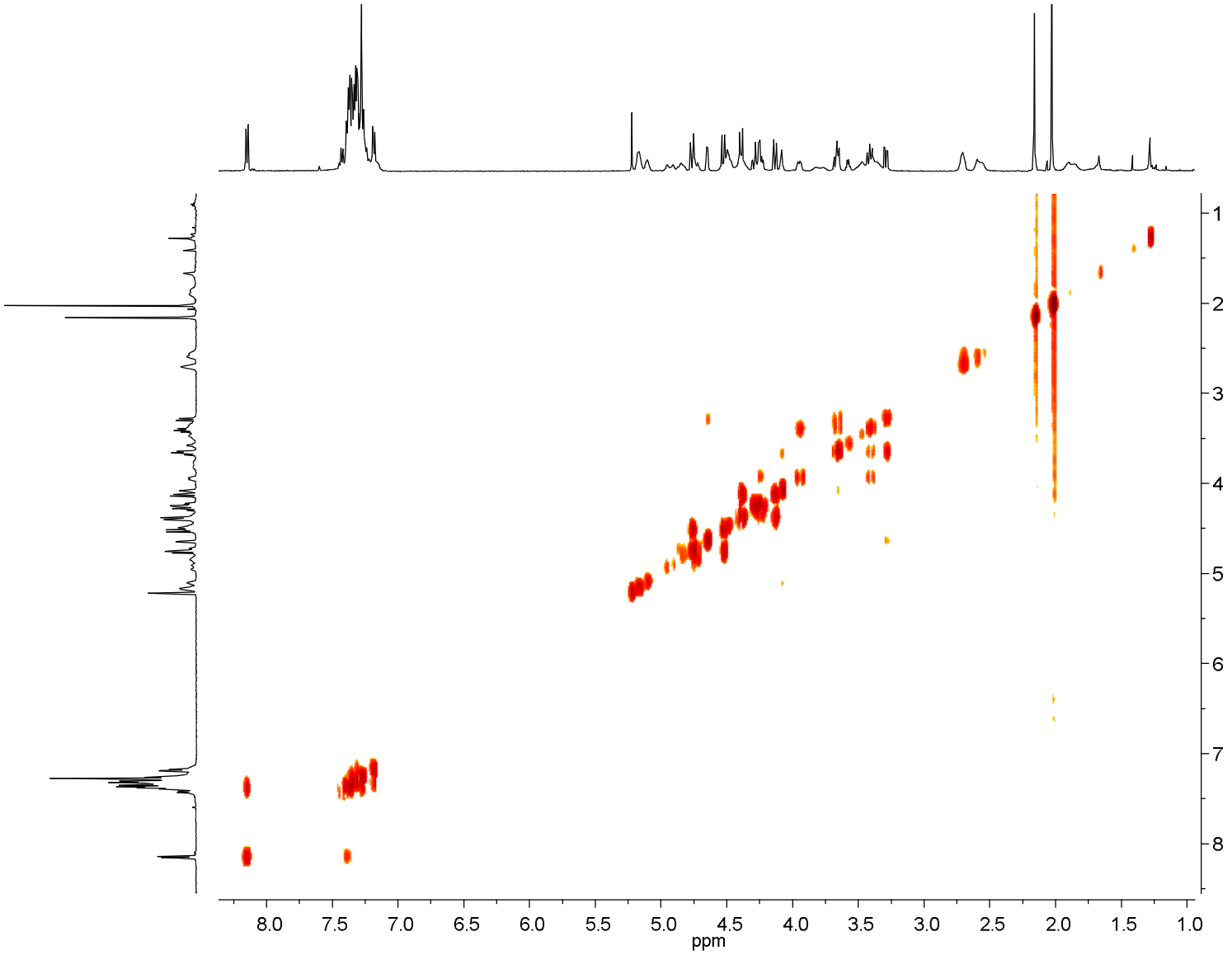
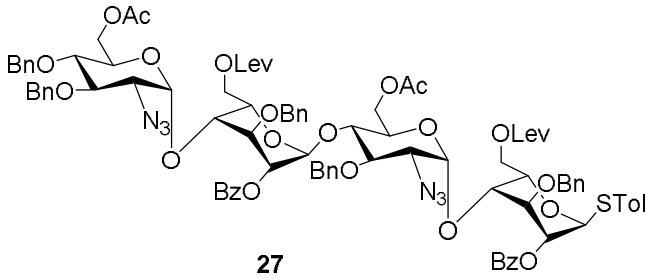


Figure 4.76. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **26**



27

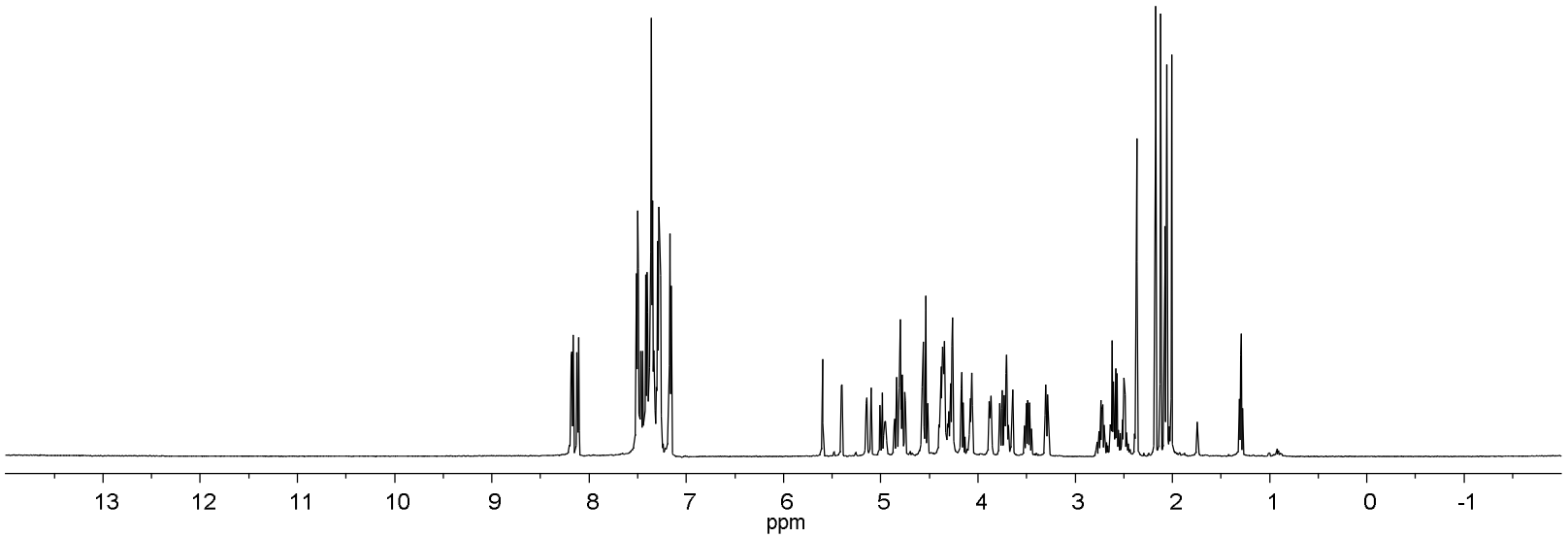


Figure 4.77. 500 MHz, $CDCl_3$, ^1H NMR of **27**

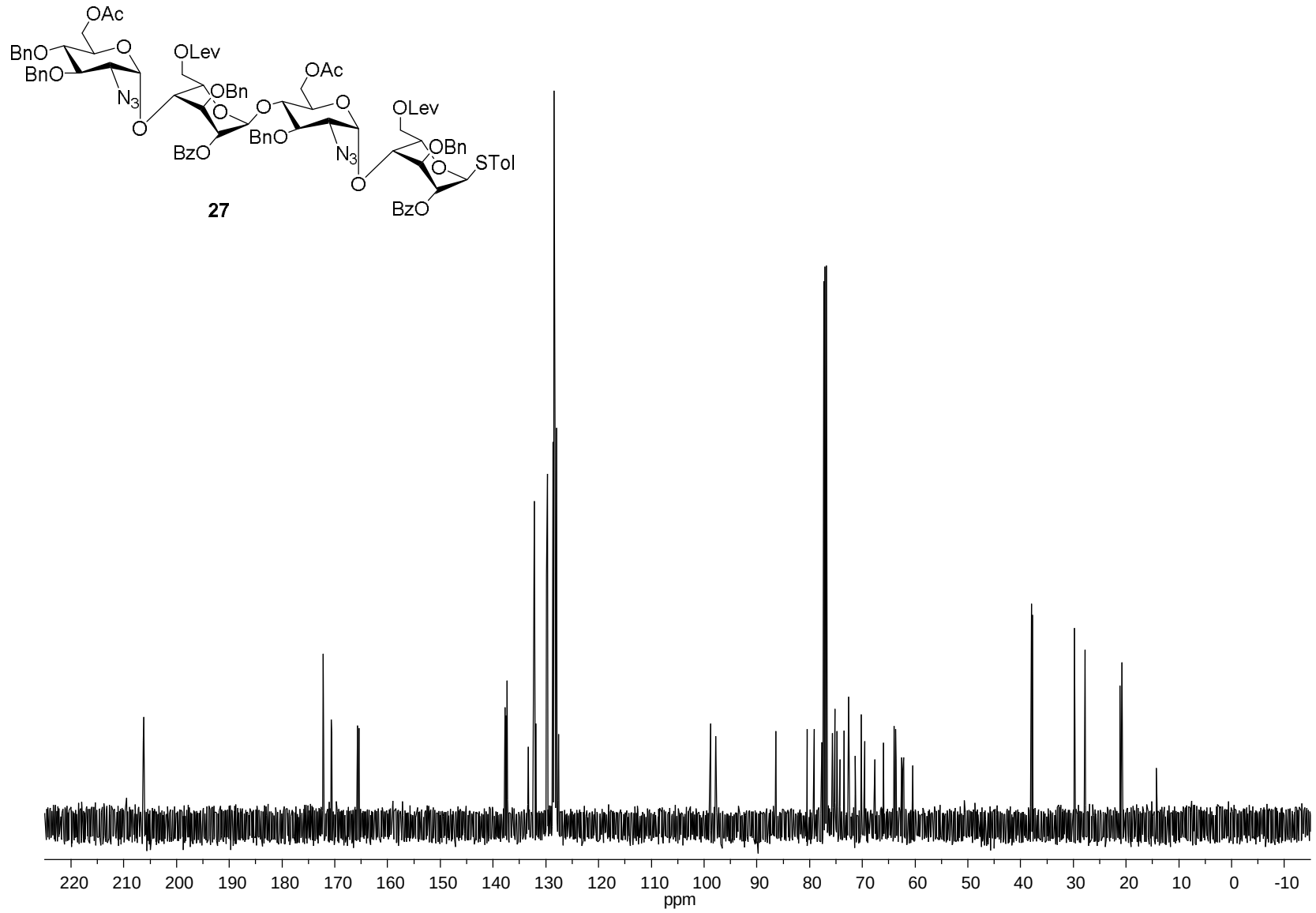


Figure 4.78. 125 MHz, $CDCl_3$, ^{13}C NMR of **27**

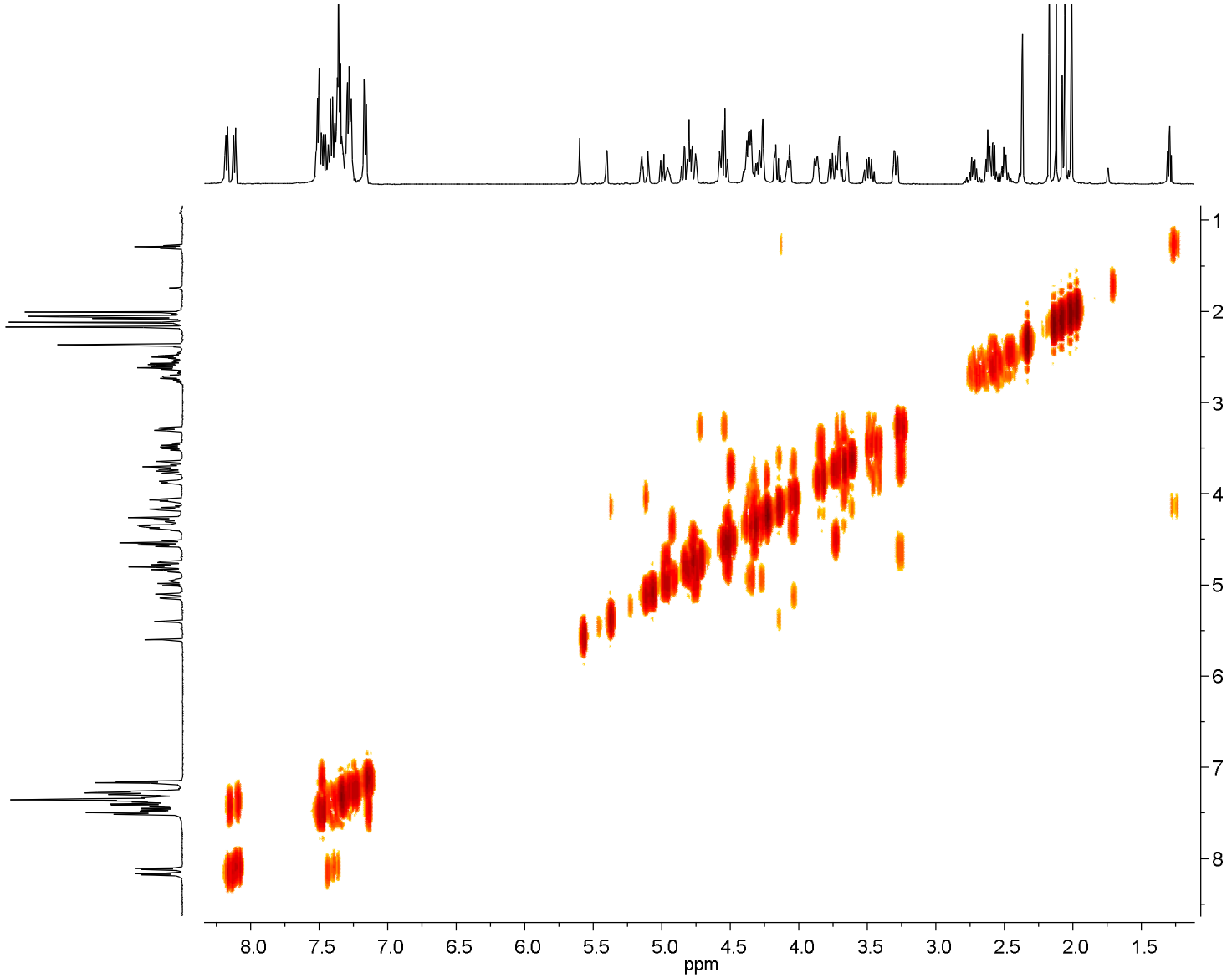


Figure 4.79. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **27**

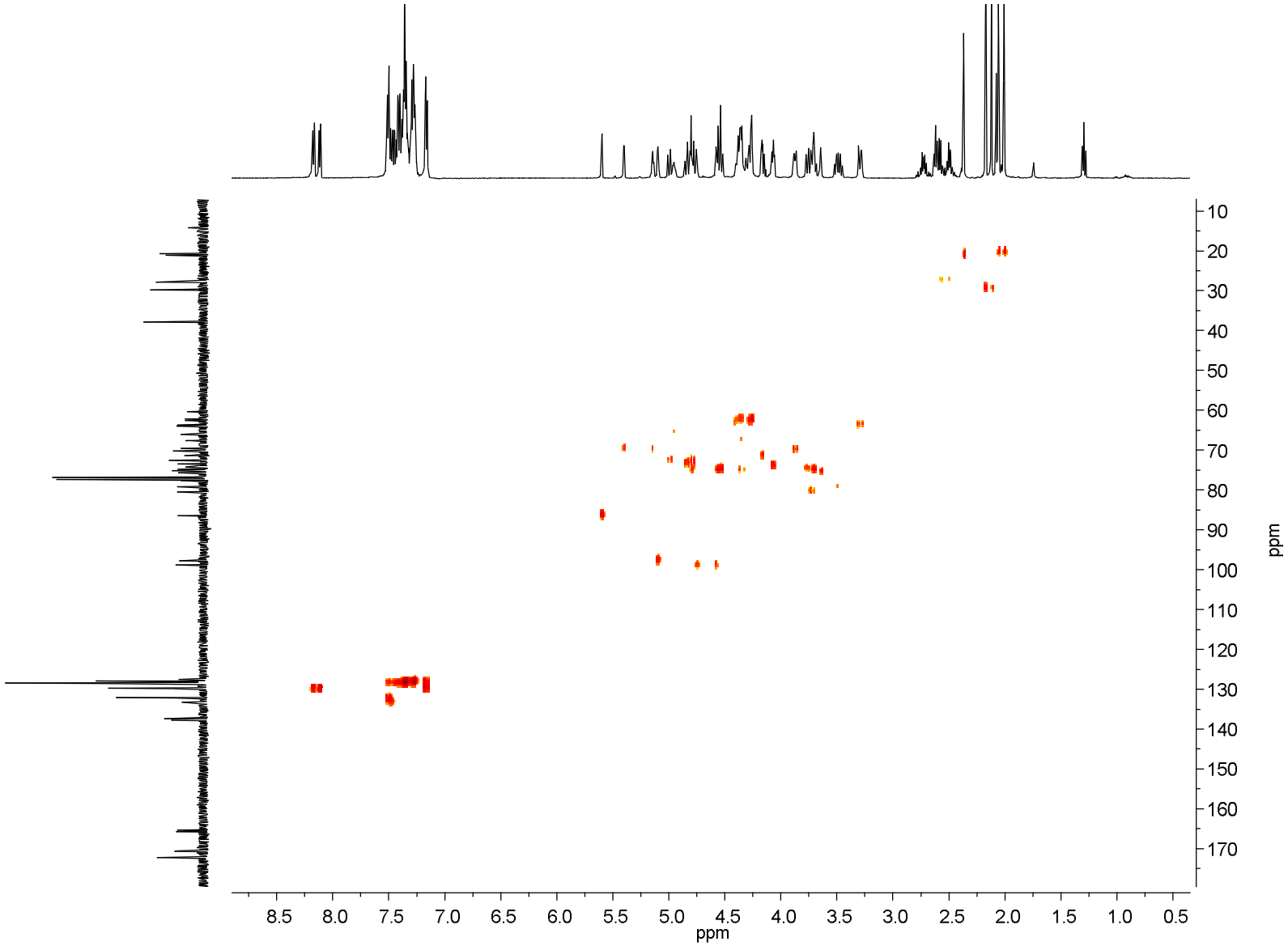


Figure 4.80. 500 MHz, $CDCl_3$, HMQC of **27**

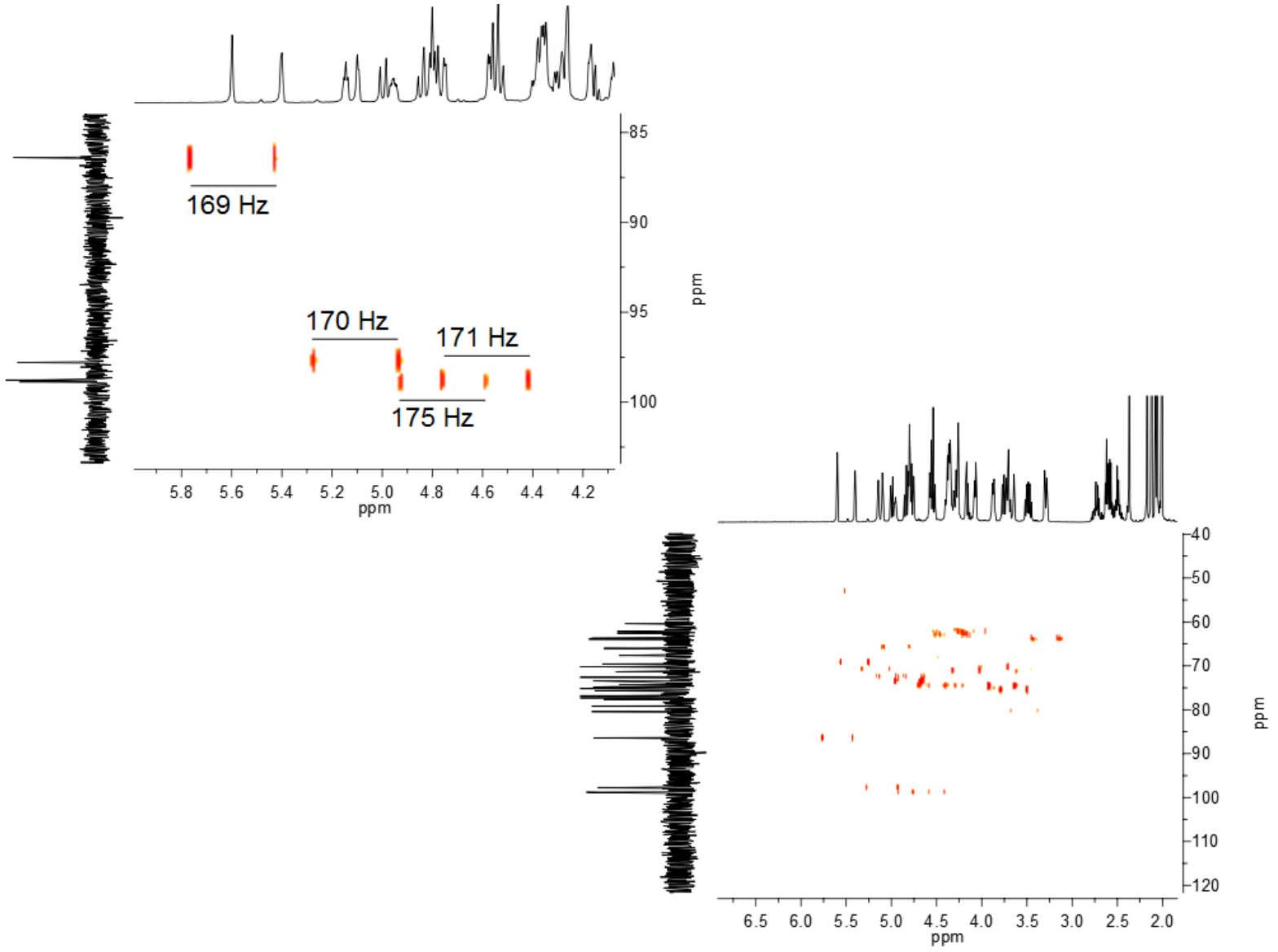
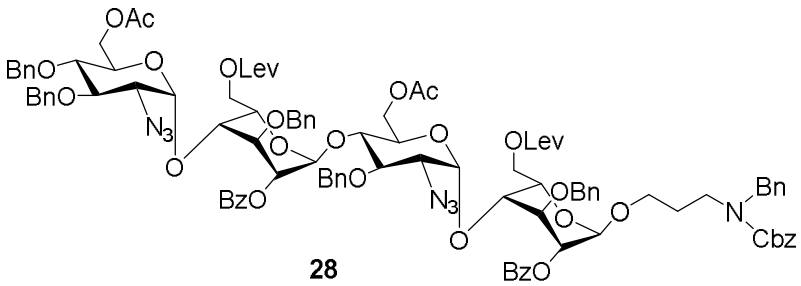


Figure 4.81. 500 MHz, $CDCl_3$, gHMQC (without ^1H Decoupling) of **27**



28

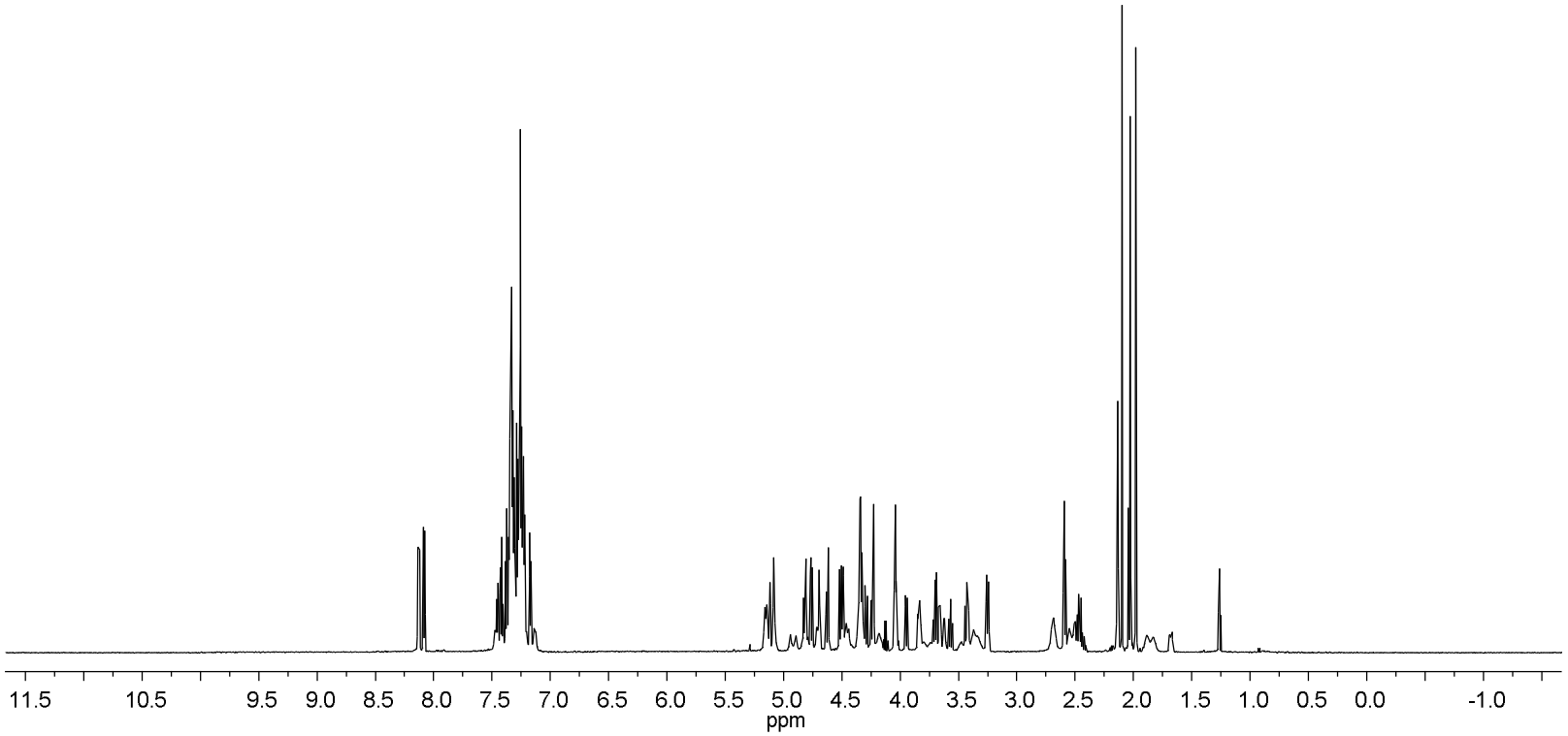


Figure 4.82. 600 MHz, $CDCl_3$, ^1H NMR of **28**

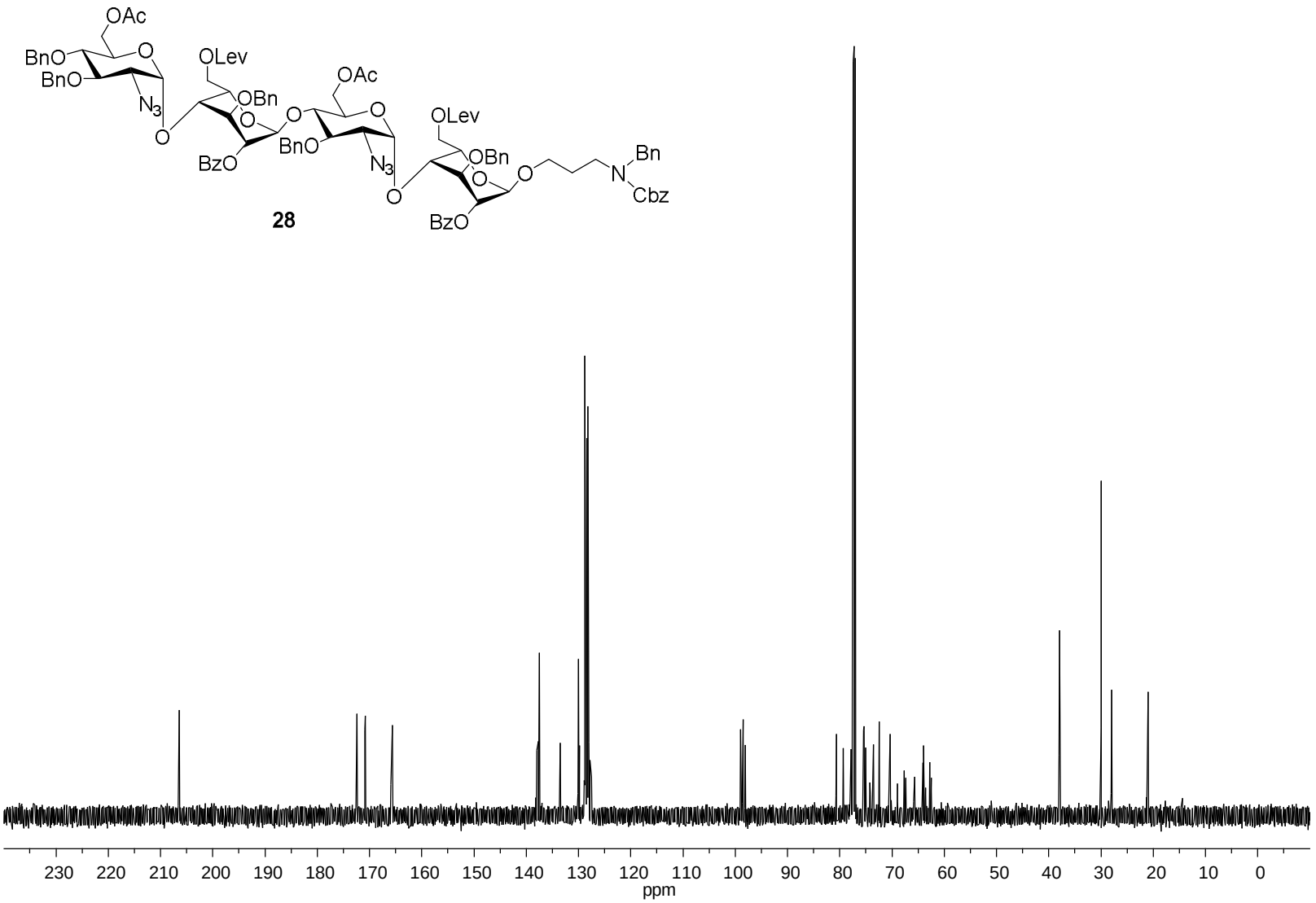


Figure 4.83. 150 MHz, $CDCl_3$, ^{13}C NMR of **28**

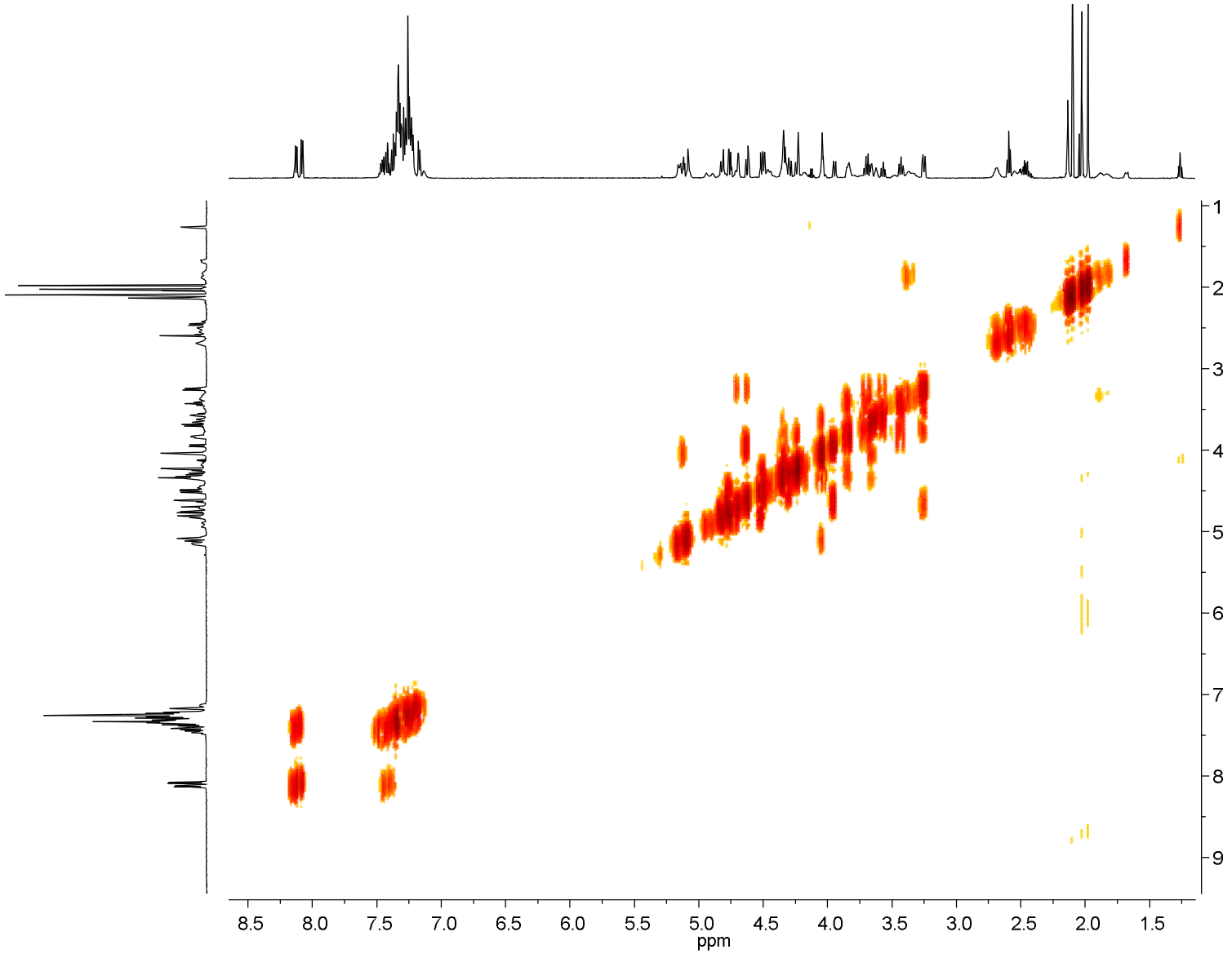


Figure 4.84. 600 MHz, $CDCl_3$, ^1H-^1H gCOSY of **28**

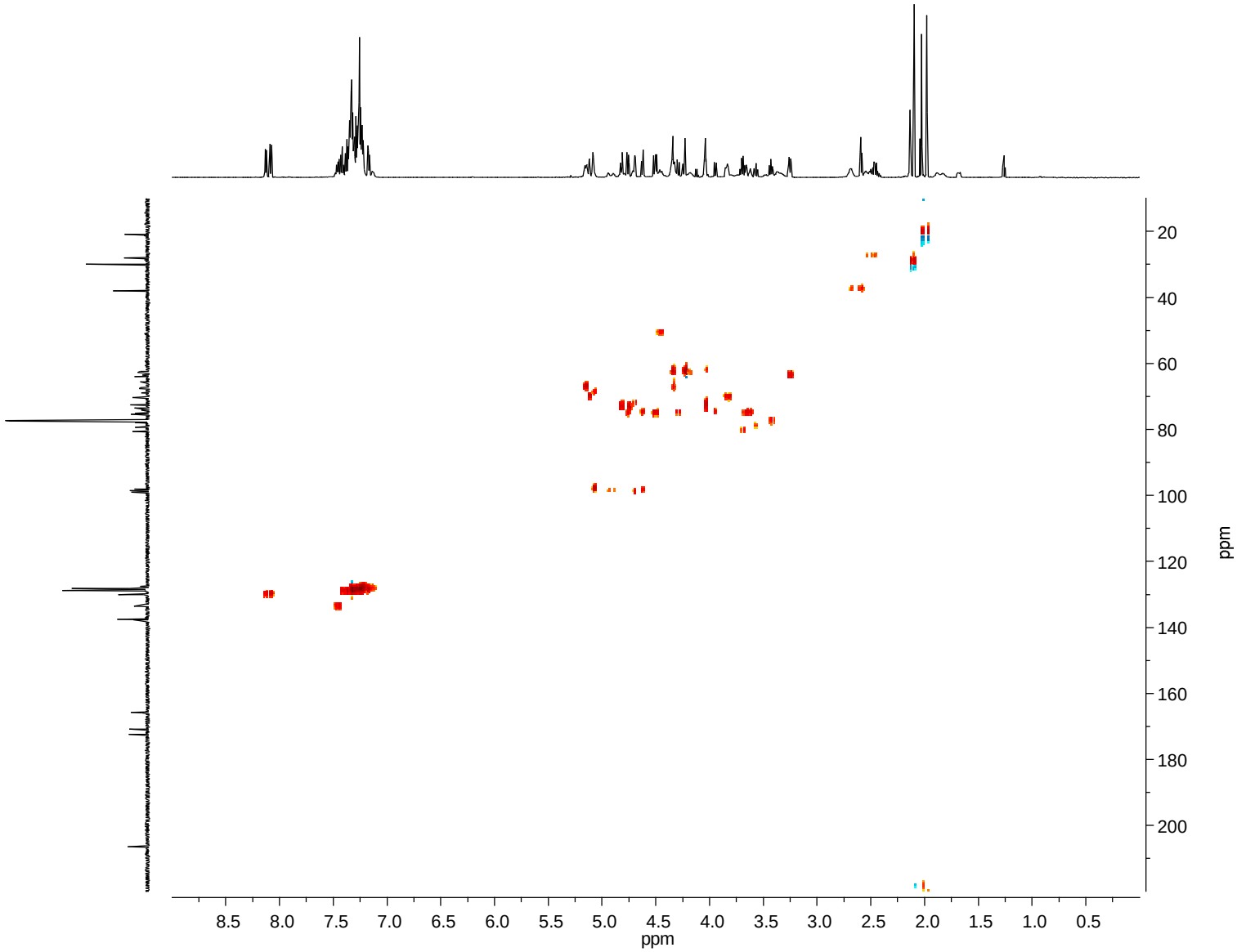


Figure 4.85. 600 MHz, $CDCl_3$, HMQC of **28**

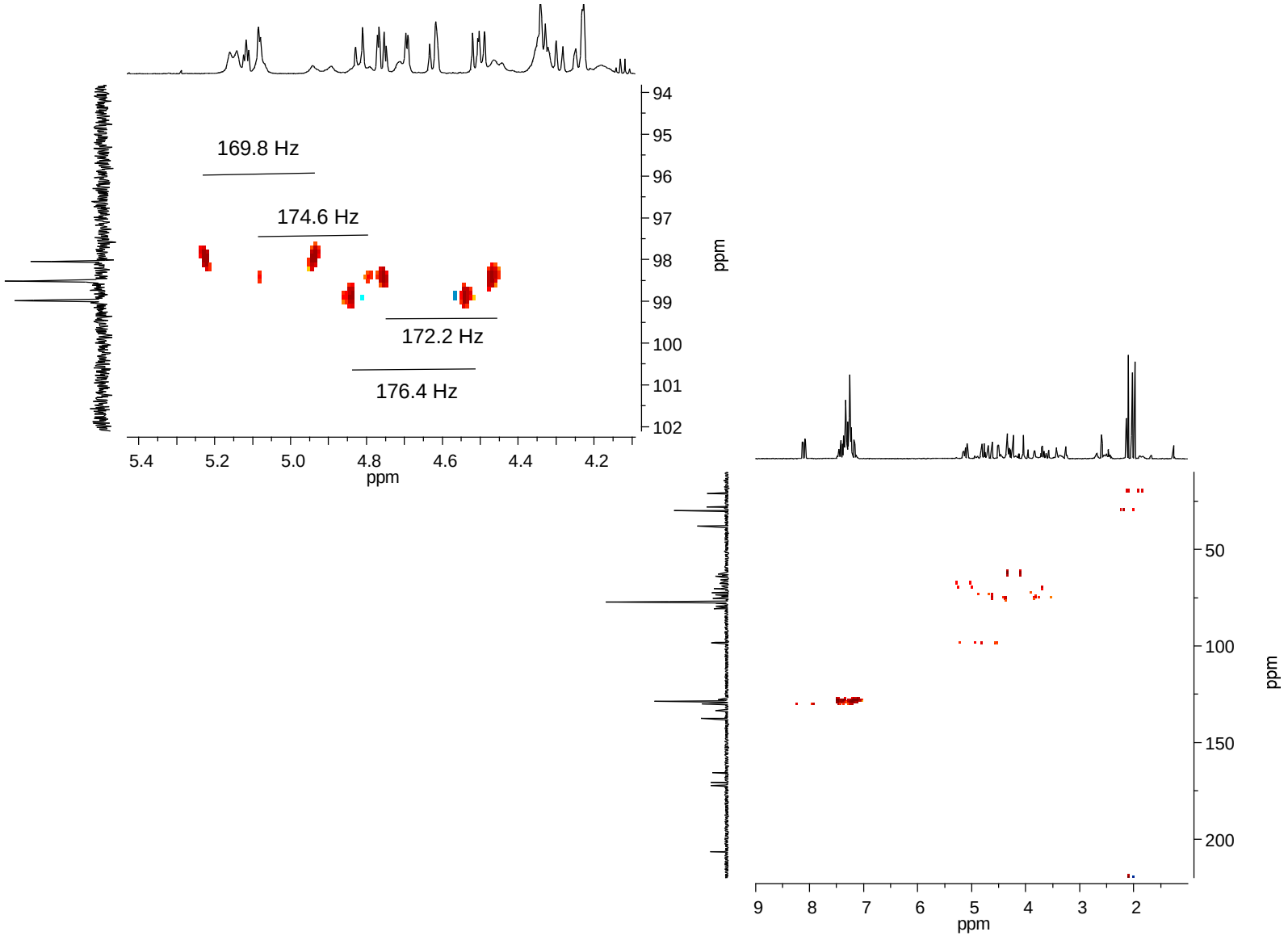


Figure 4.86. 600 MHz, $CDCl_3$, gHMQC (without ^1H Decoupling) of **28**

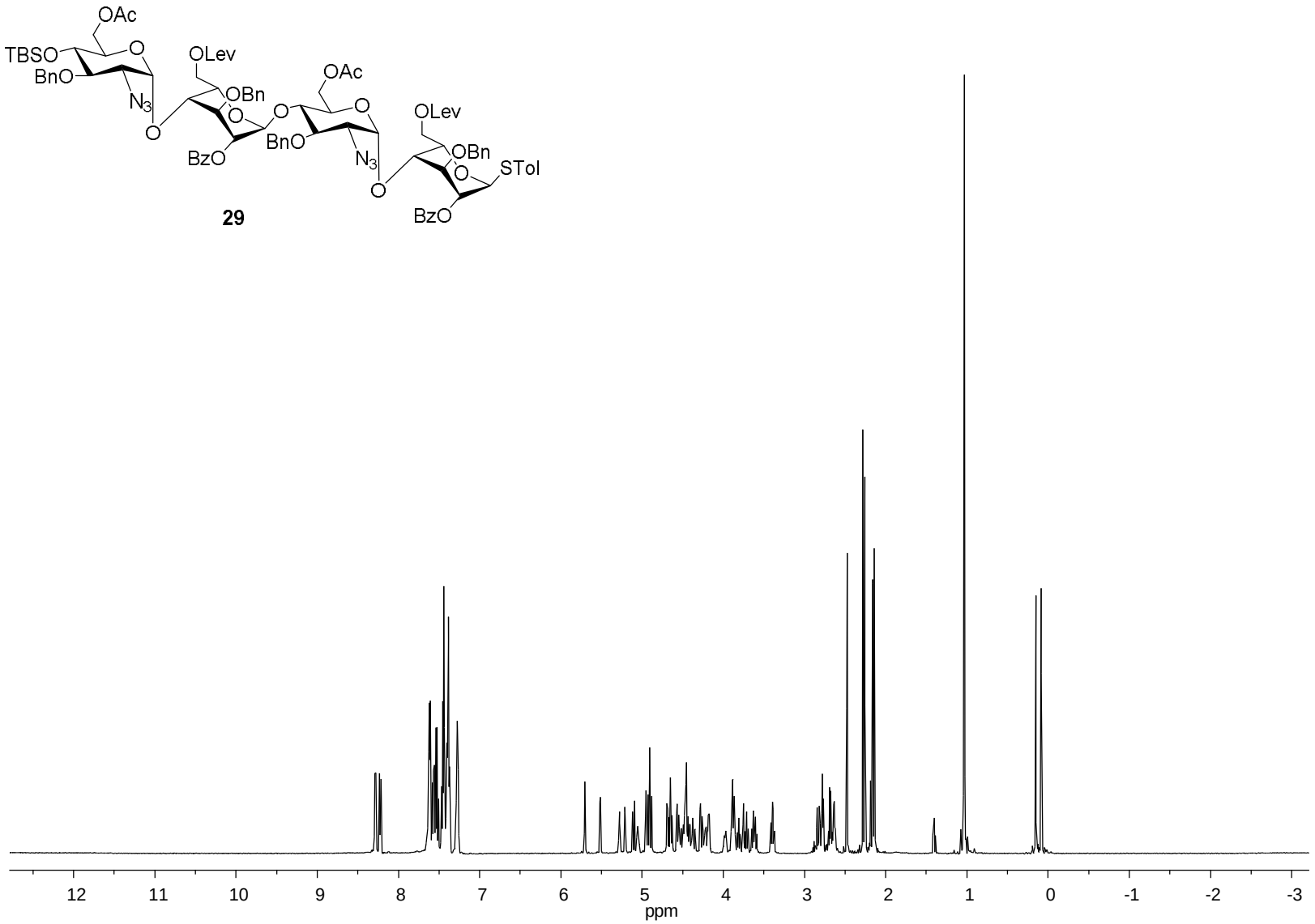


Figure 4.87. 500 MHz, $CDCl_3$, ^1H NMR of **29**

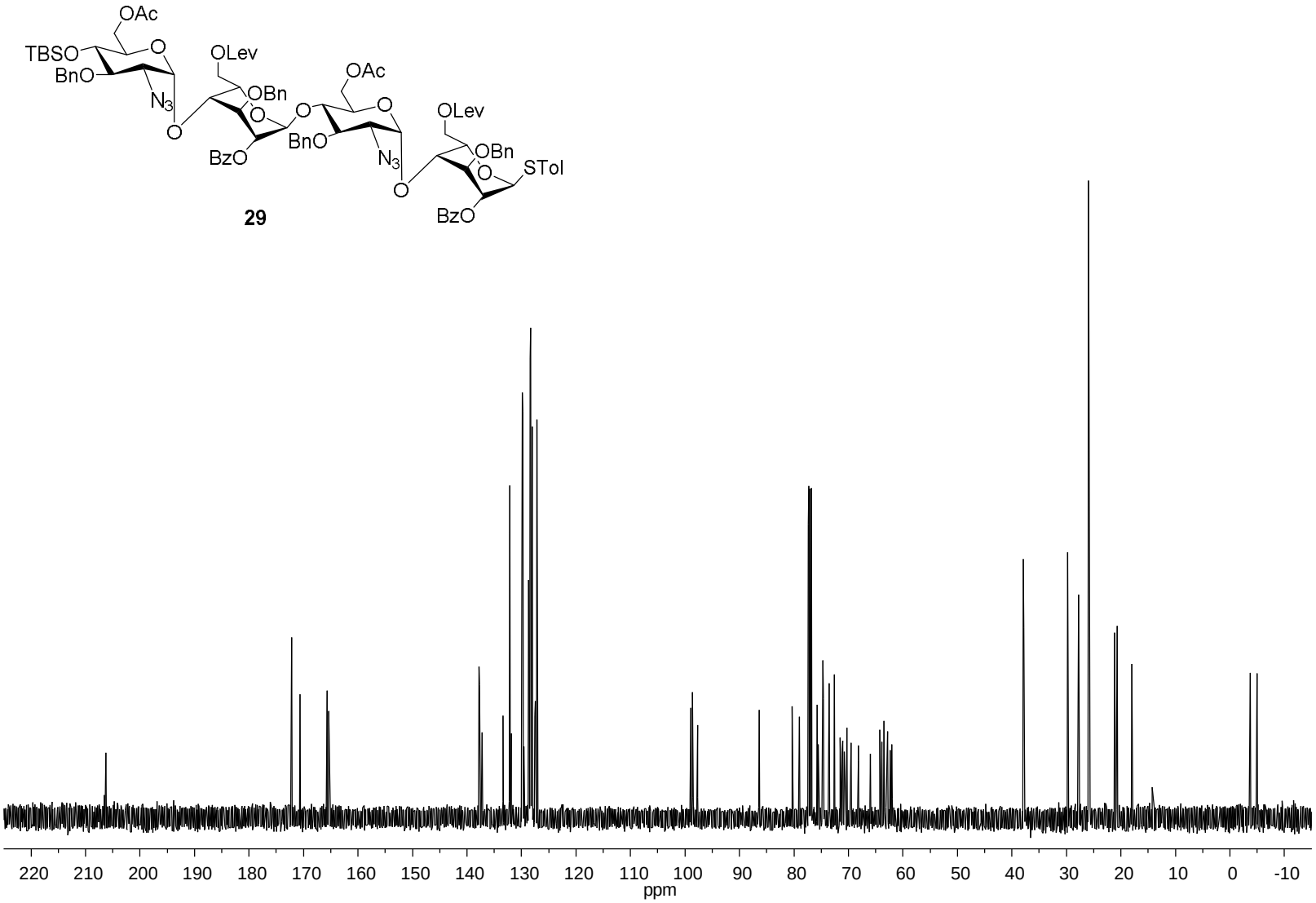


Figure 4.88. 125 MHz, $CDCl_3$, ^{13}C NMR of **29**

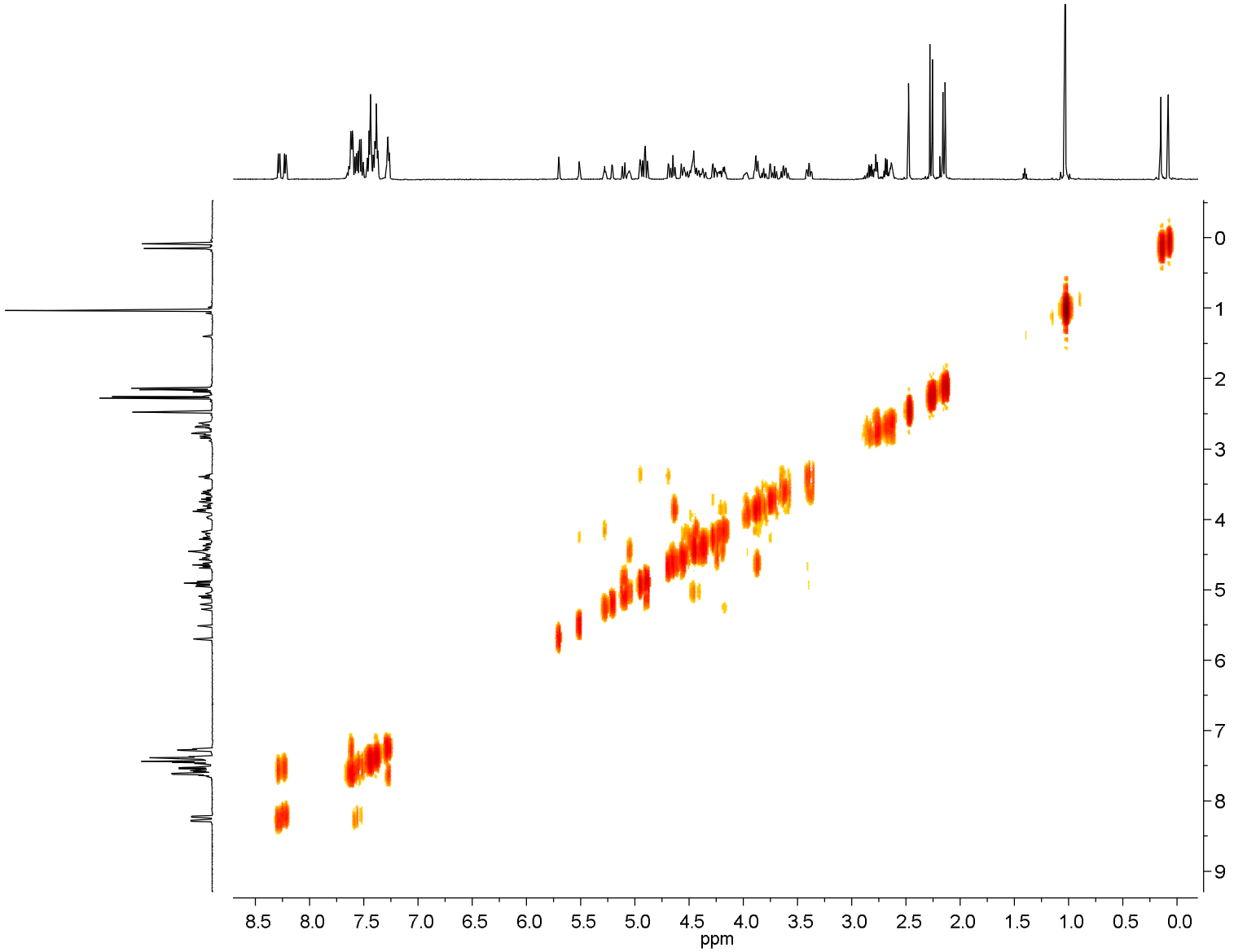


Figure 4.89. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **29**

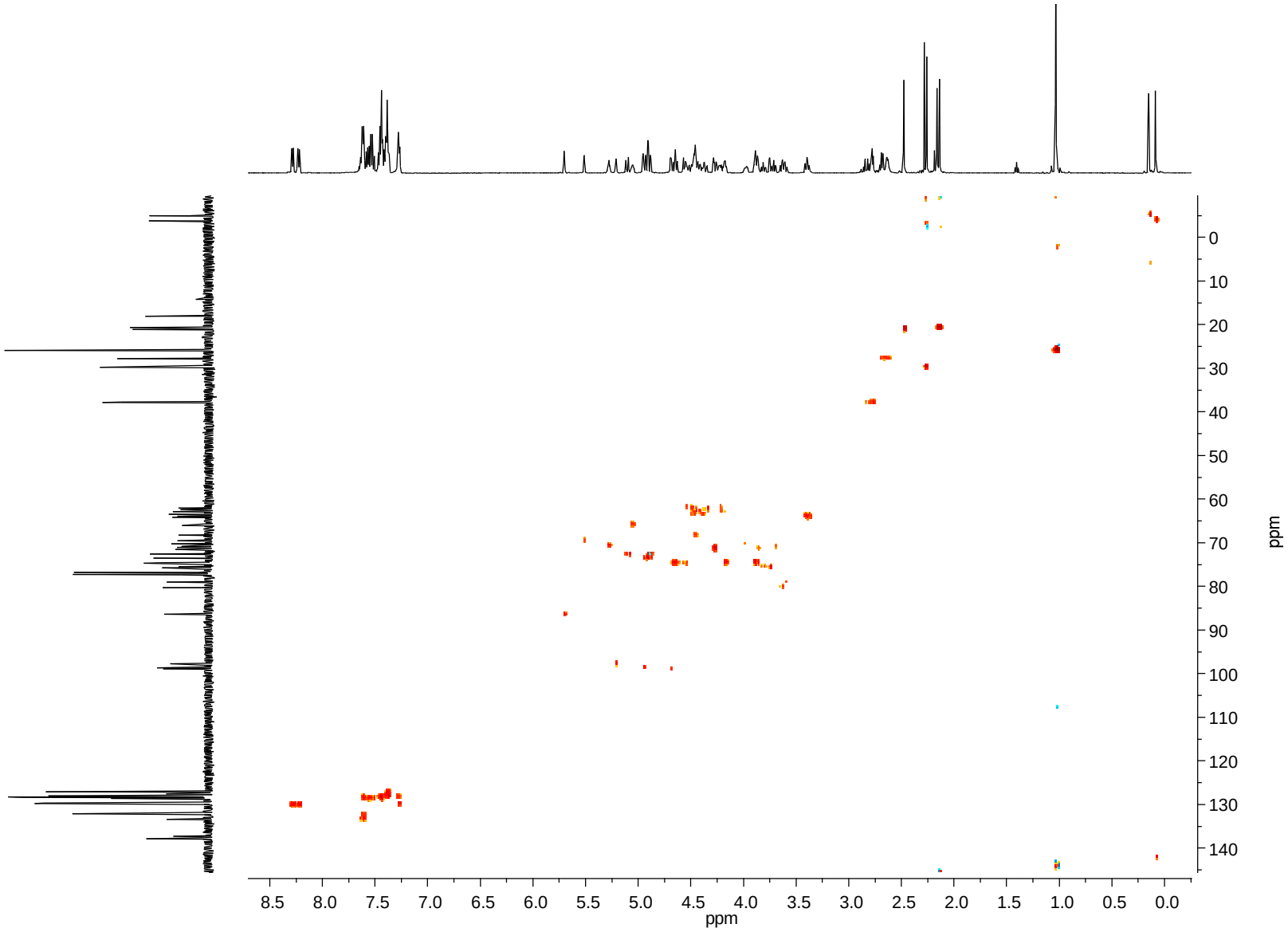


Figure 4.90. 500 MHz, $CDCl_3$, HMQC of **29**

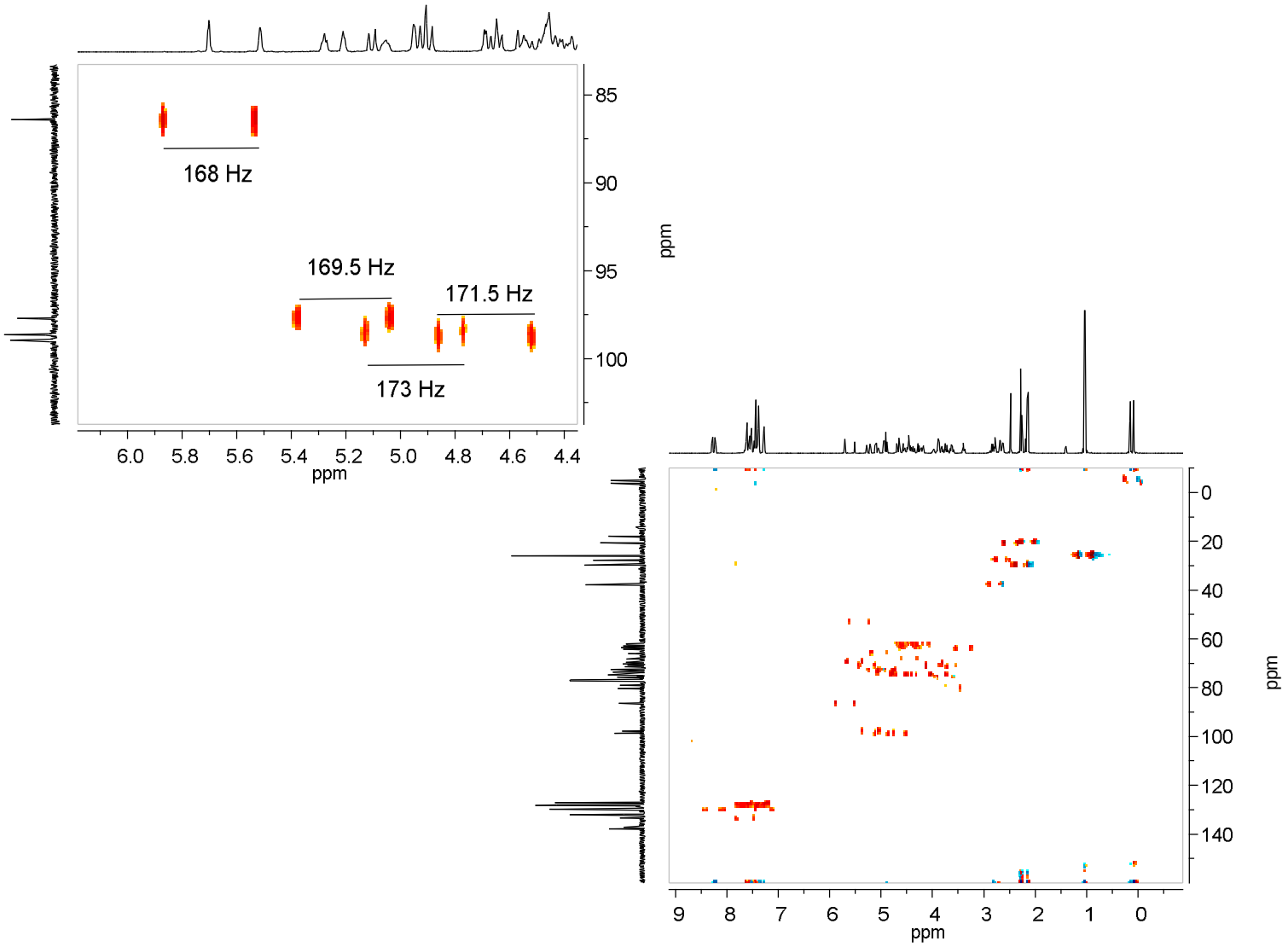


Figure 4.91. 500 MHz, $CDCl_3$, gHMQC (without ^1H Decoupling) of **29**

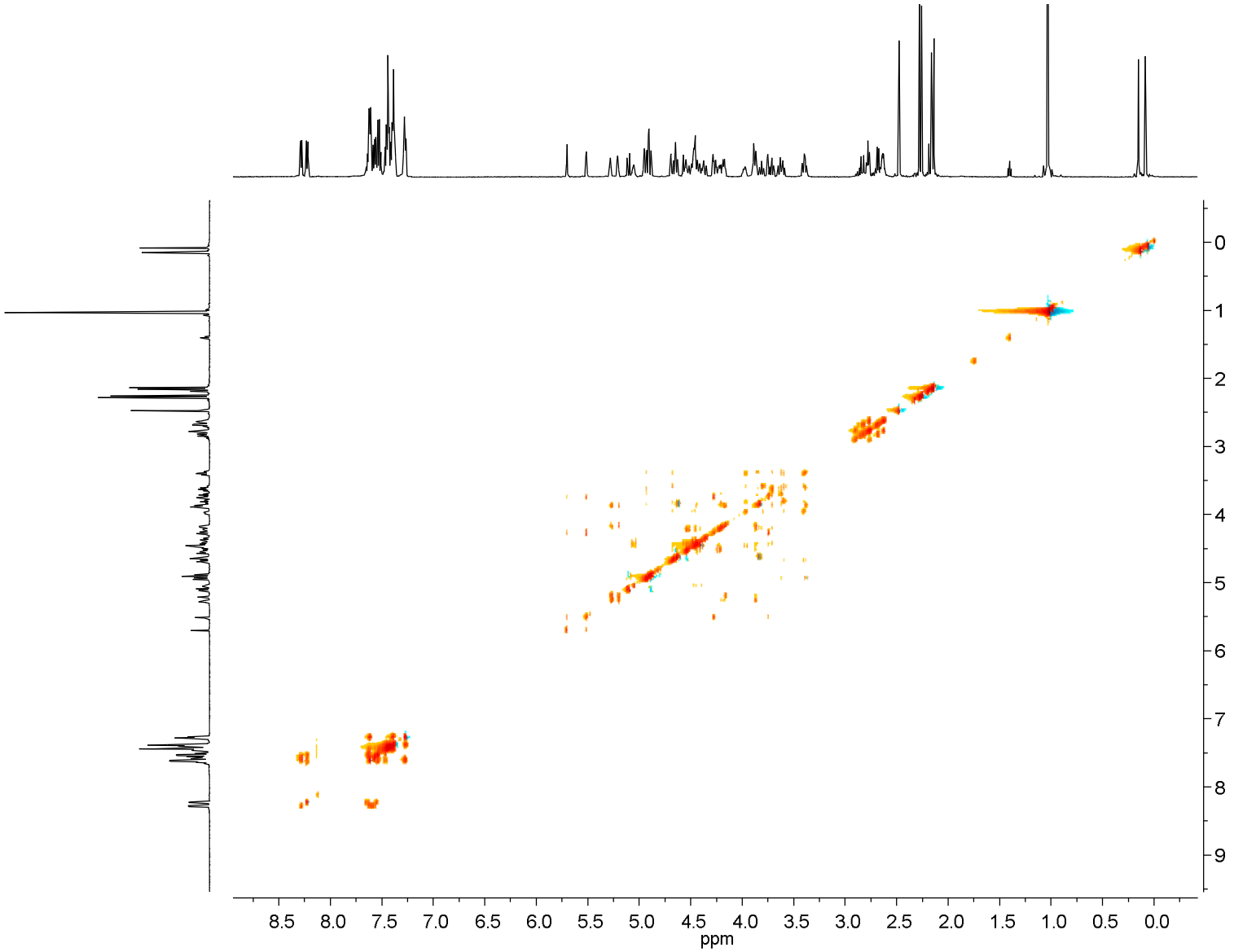


Figure 4.92. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **29**

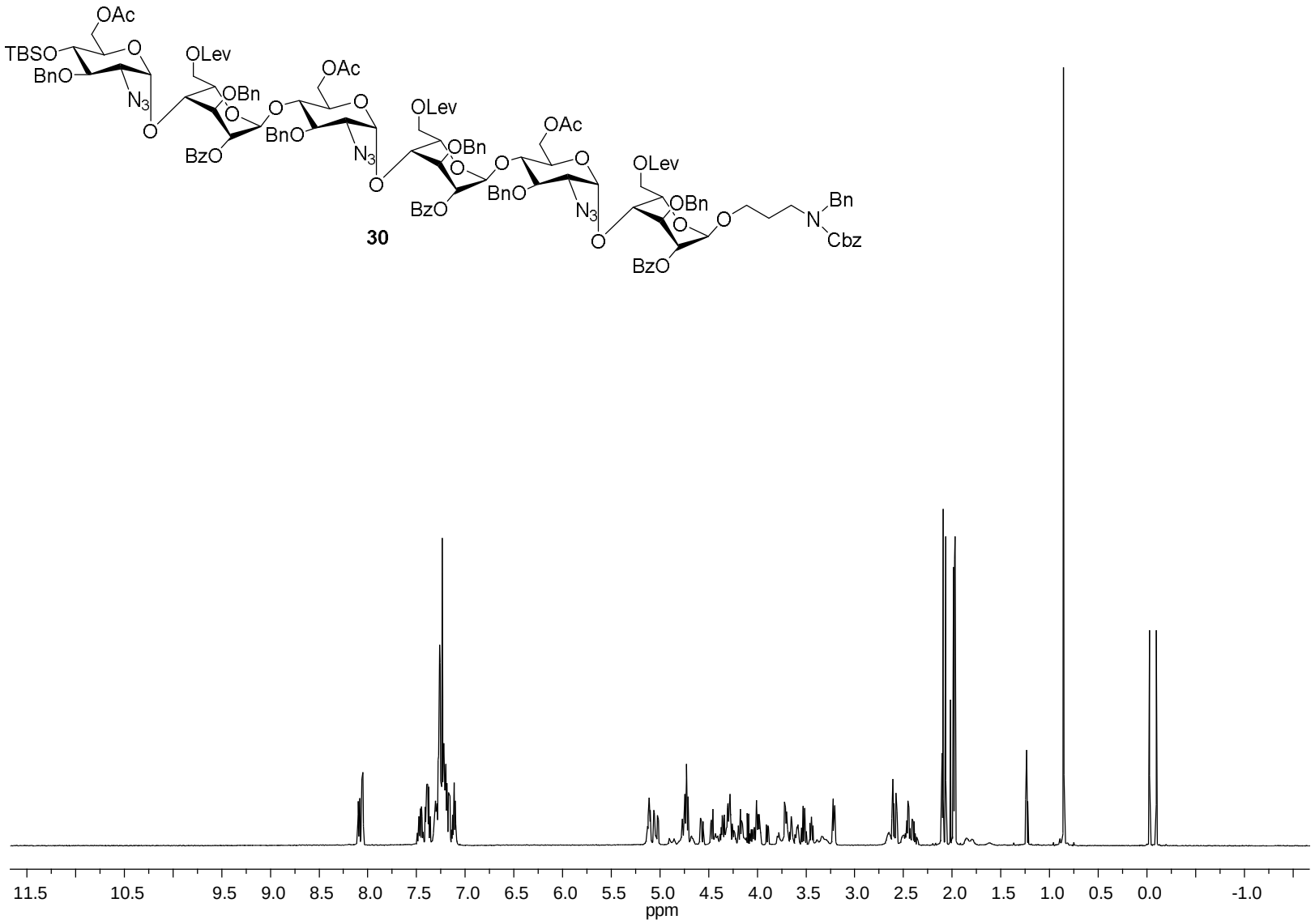


Figure 4.93. 600 MHz, $CDCl_3$, ^1H NMR of **30**

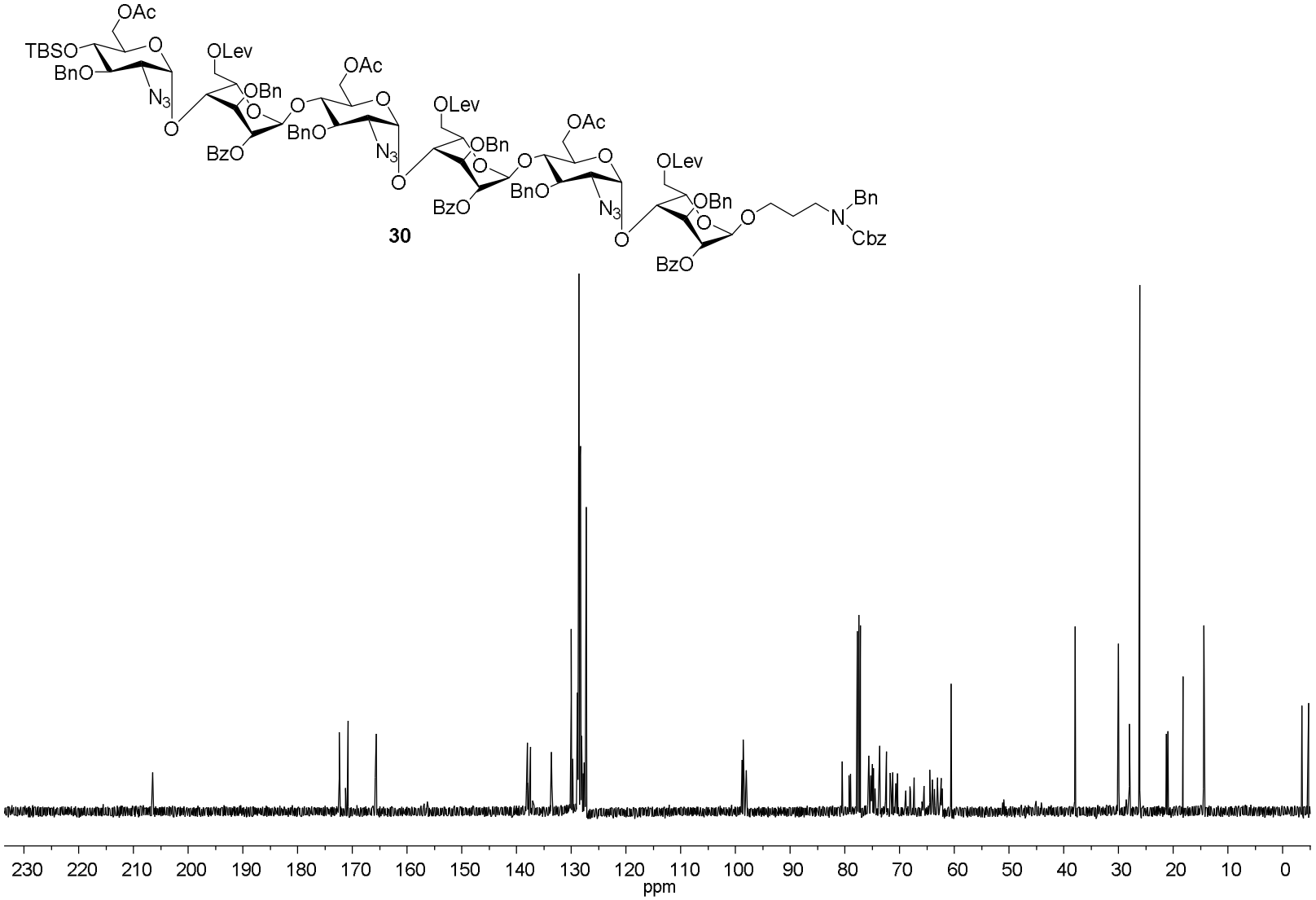


Figure 4.94. 150 MHz, $CDCl_3$, ^{13}C NMR of **30**

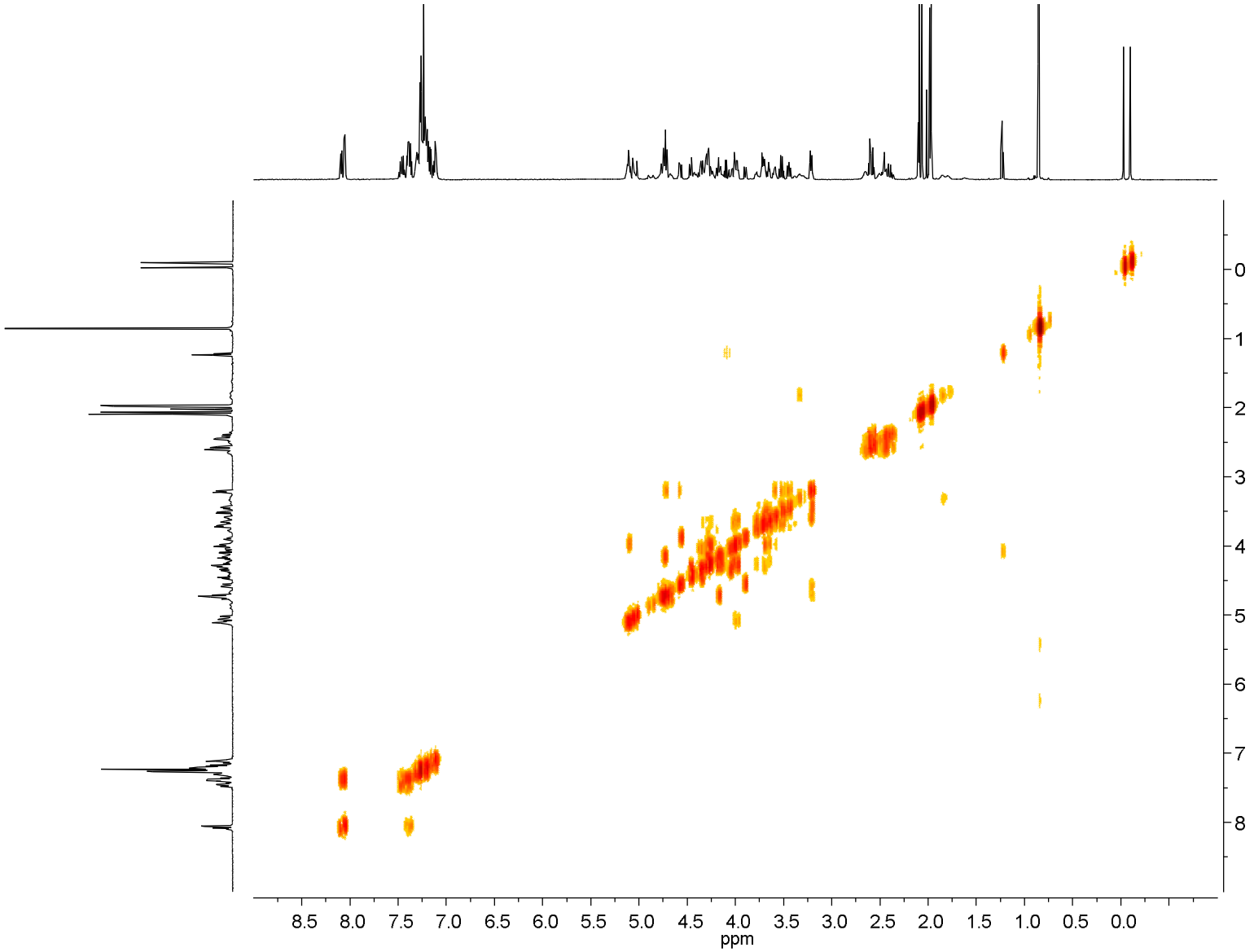


Figure 4.95. 600 MHz, $CDCl_3$, ^1H-^1H gCOSY of **30**

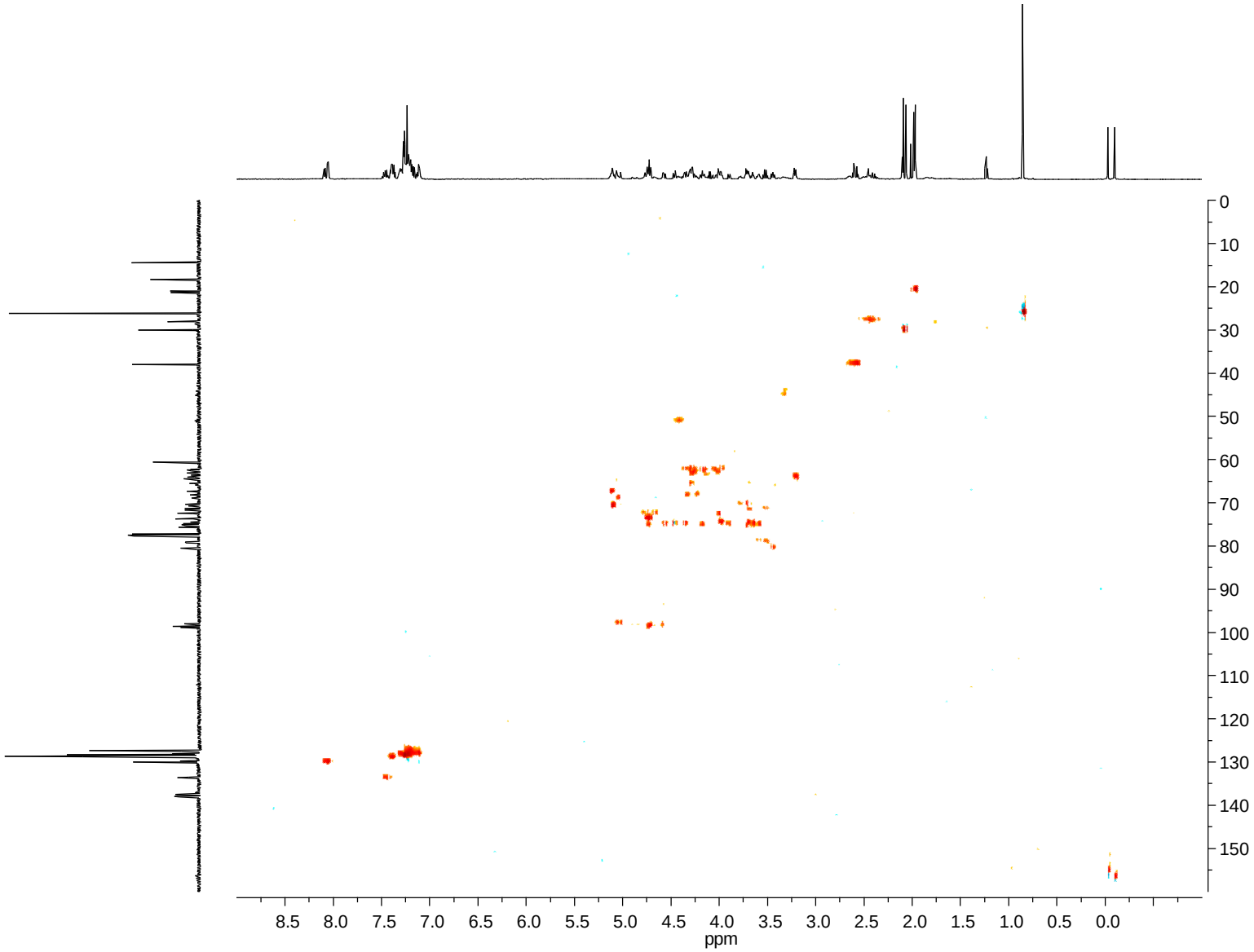


Figure 4.96. 600 MHz, $CDCl_3$, gHMQC of **30**

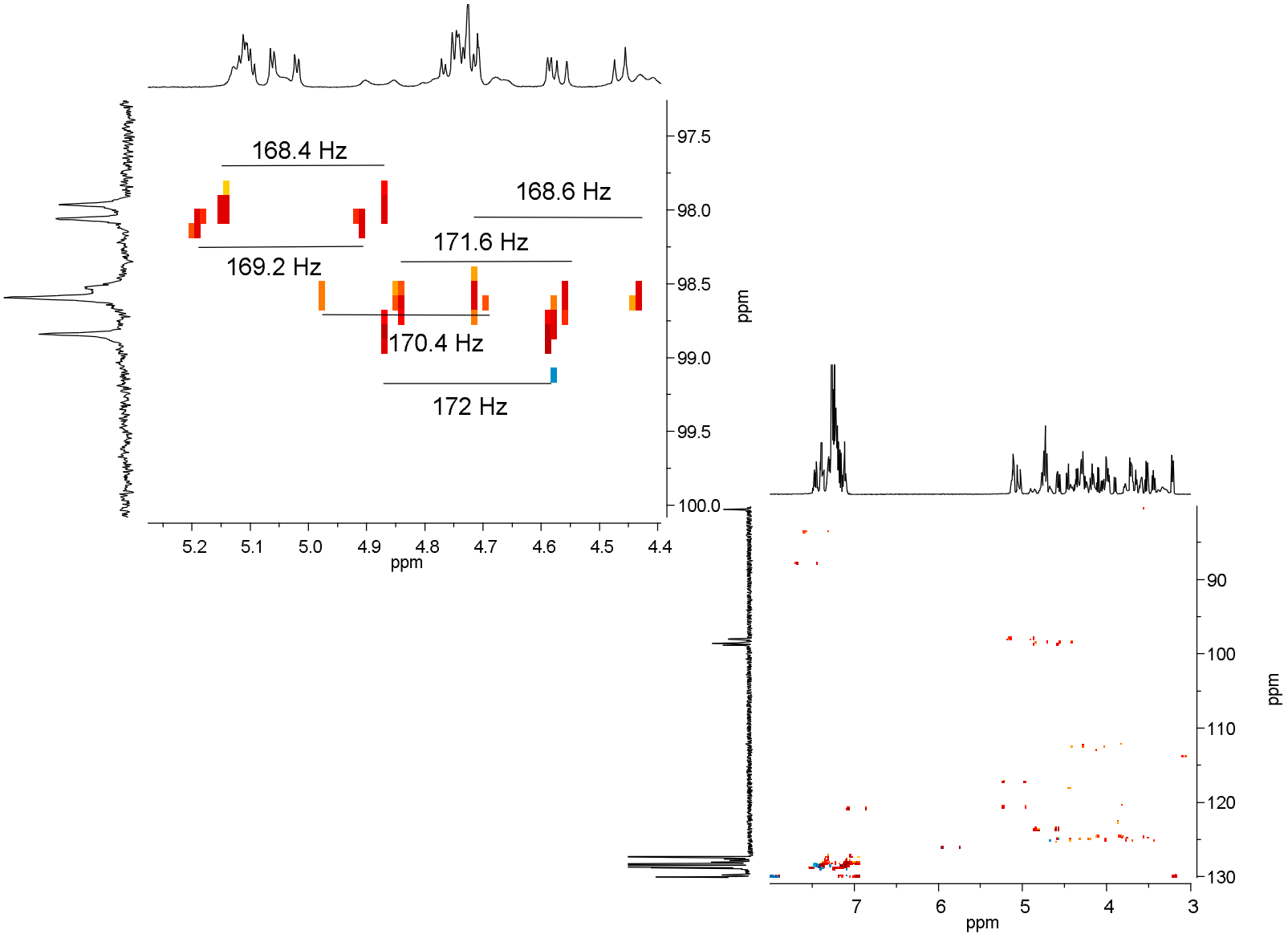


Figure 4.97. 600 MHz, $CDCl_3$, gHMQC (without ^1H Decoupling) of **30**

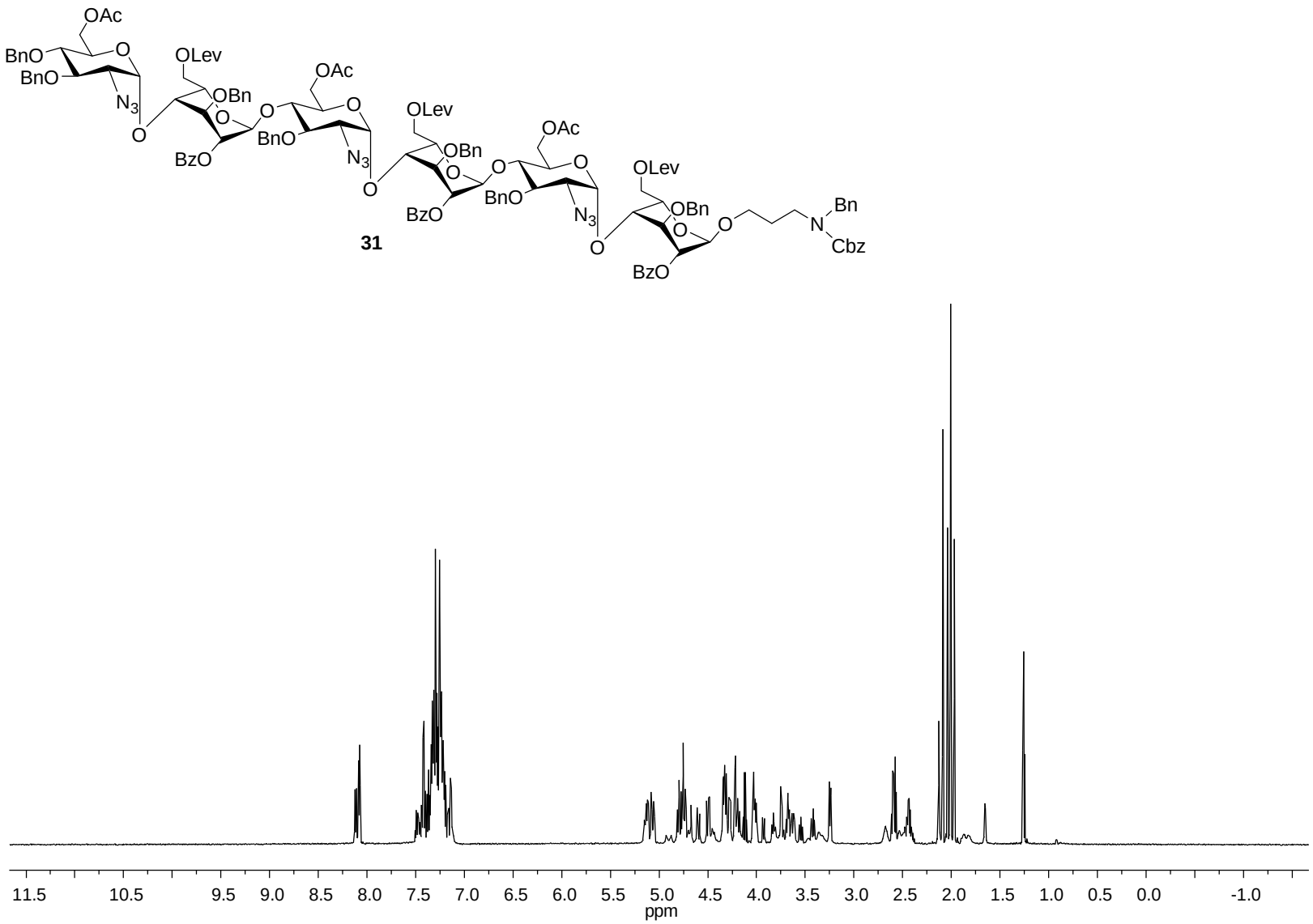


Figure 4.98. 500 MHz, $CDCl_3$, ^1H NMR of **31**

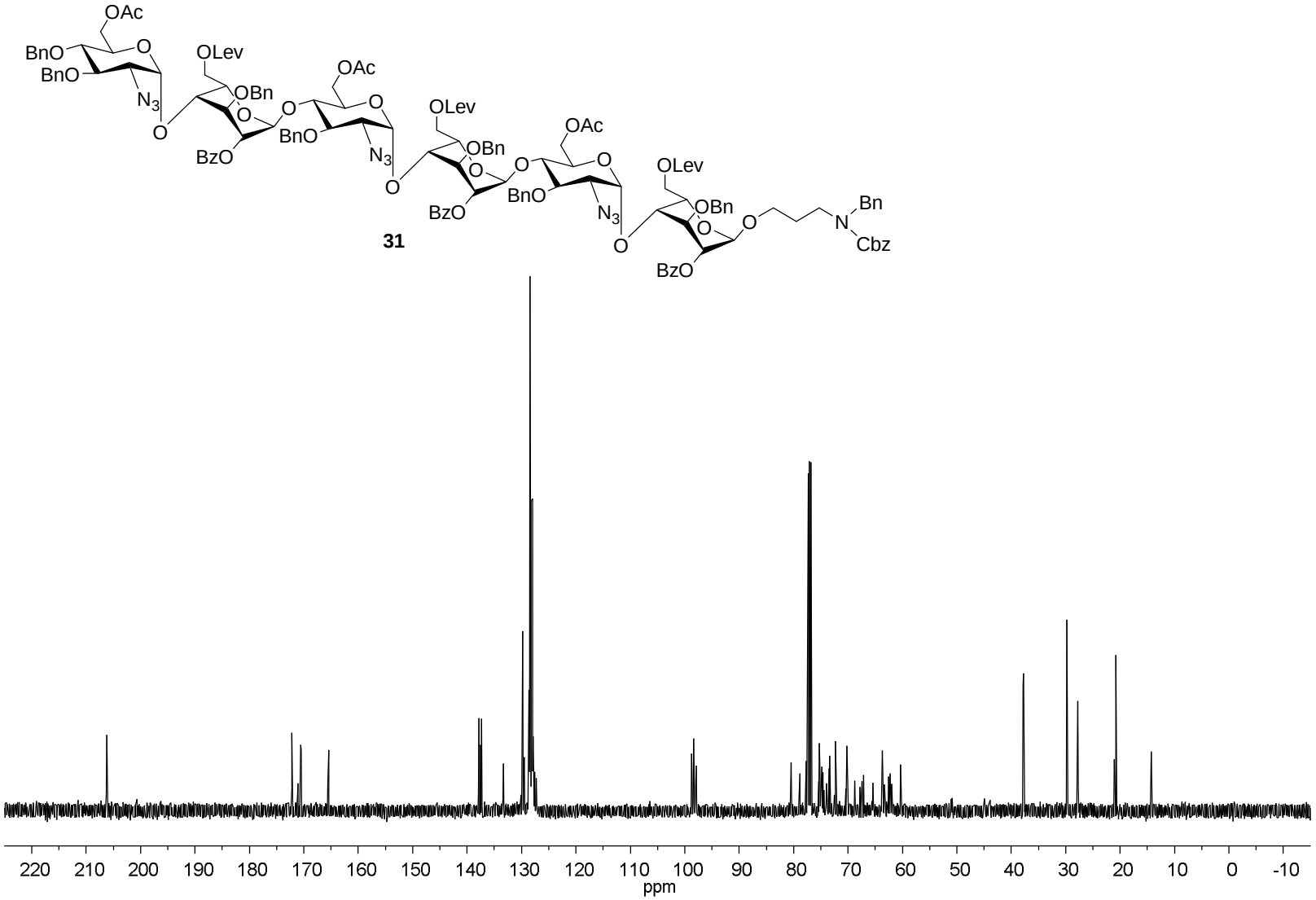


Figure 4.99. 125 MHz, $CDCl_3$, ^{13}C NMR of **31**

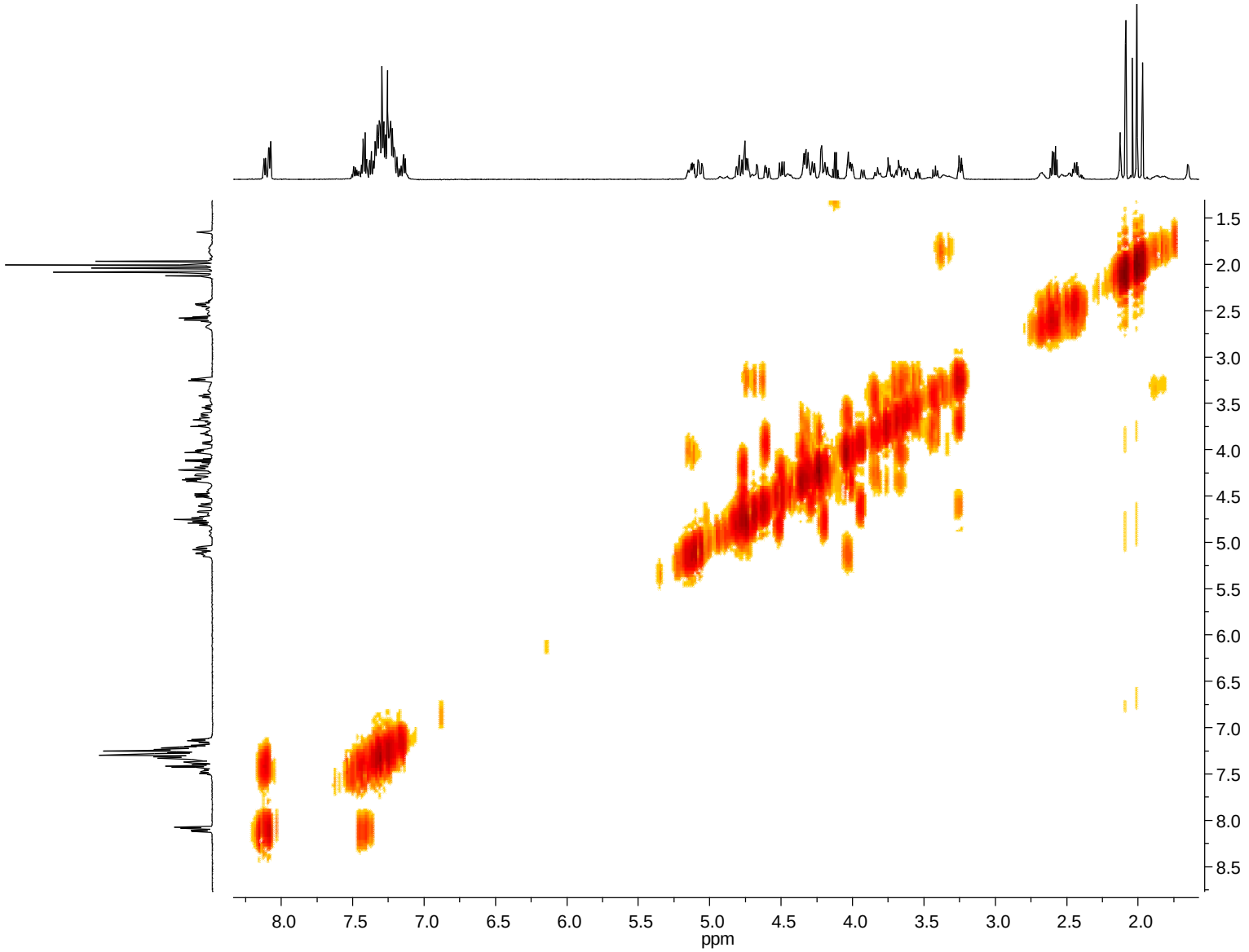


Figure 4.100. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **31**

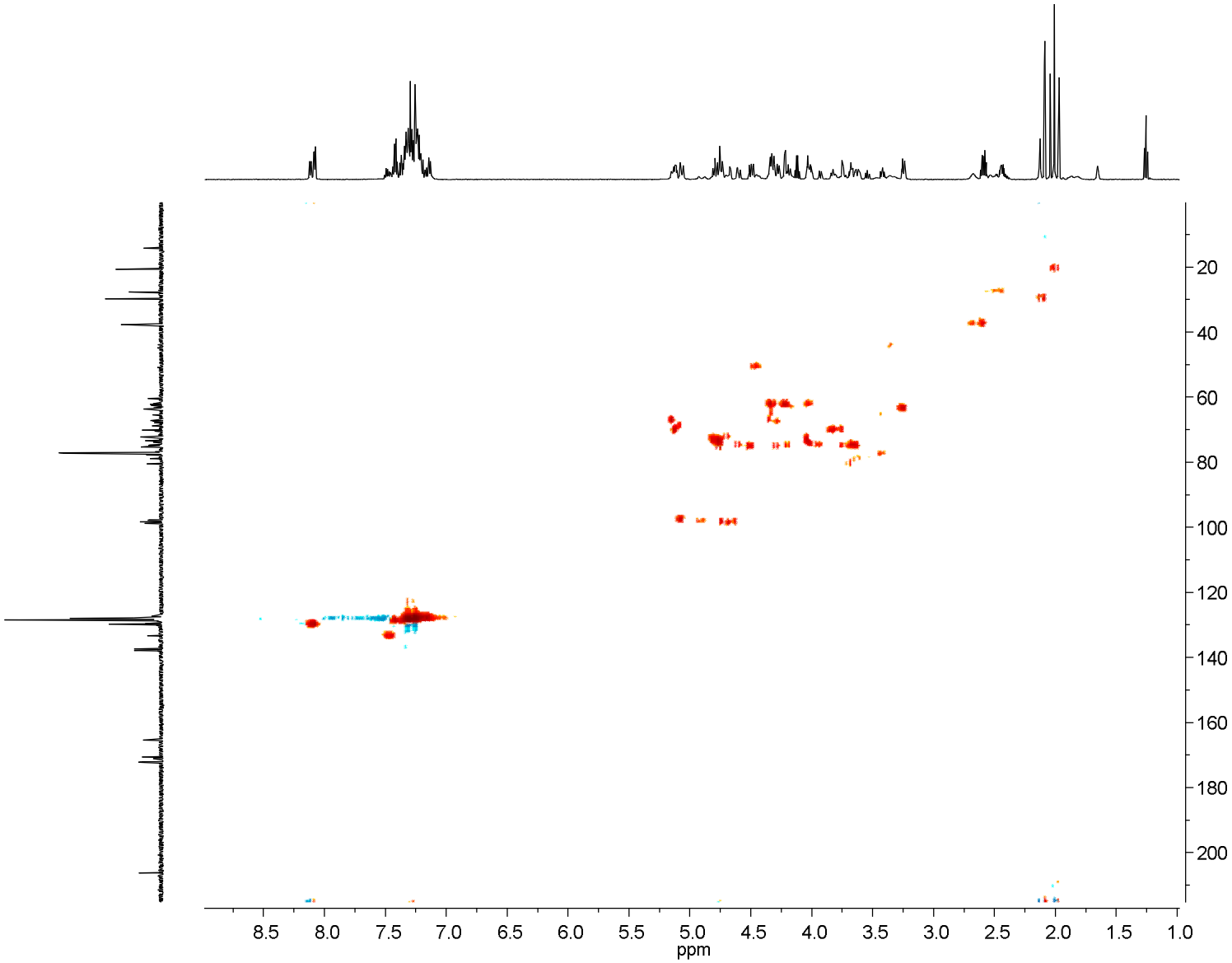


Figure 4.101. 500 MHz, $CDCl_3$, gHMQC of **31**

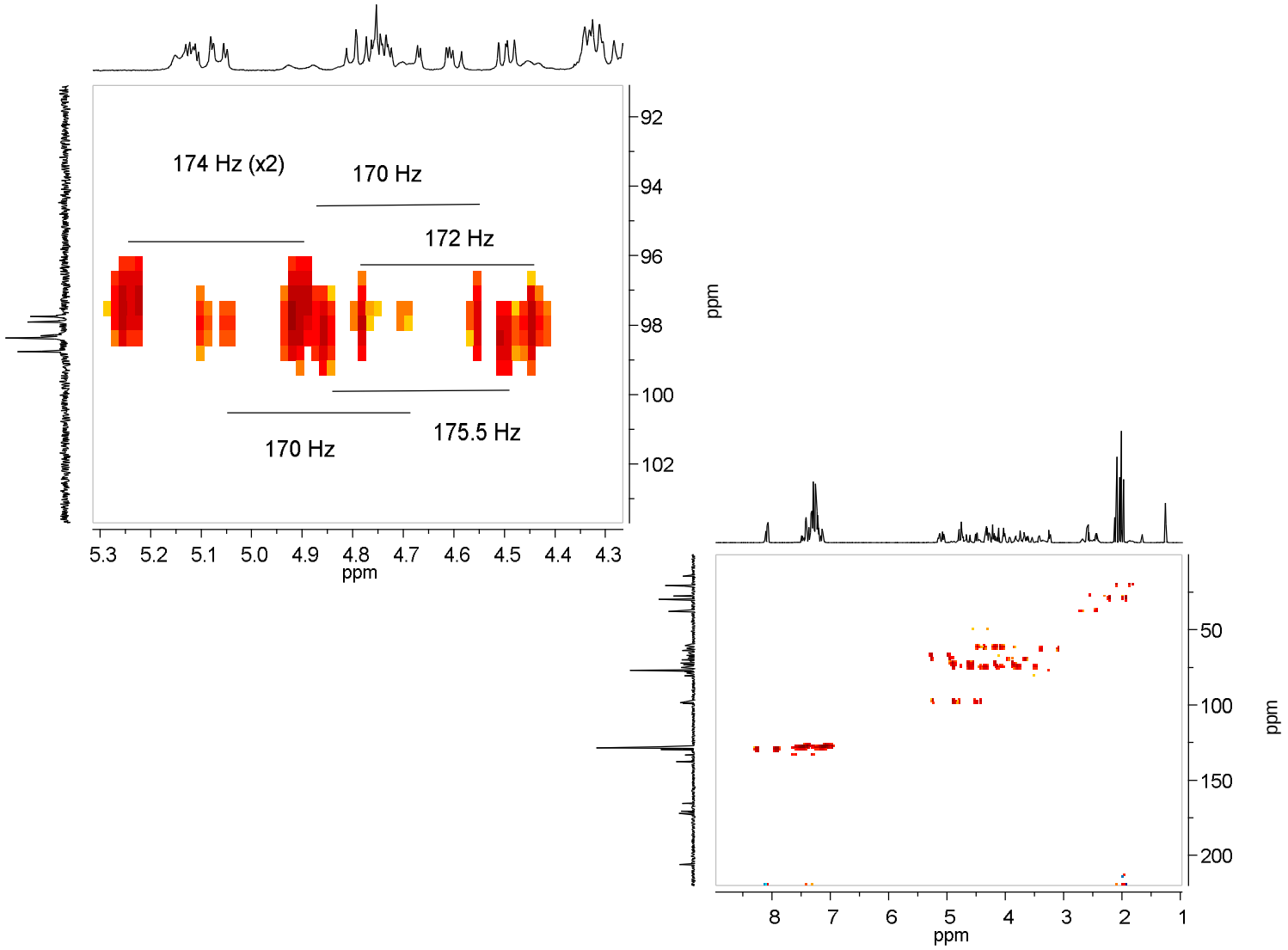
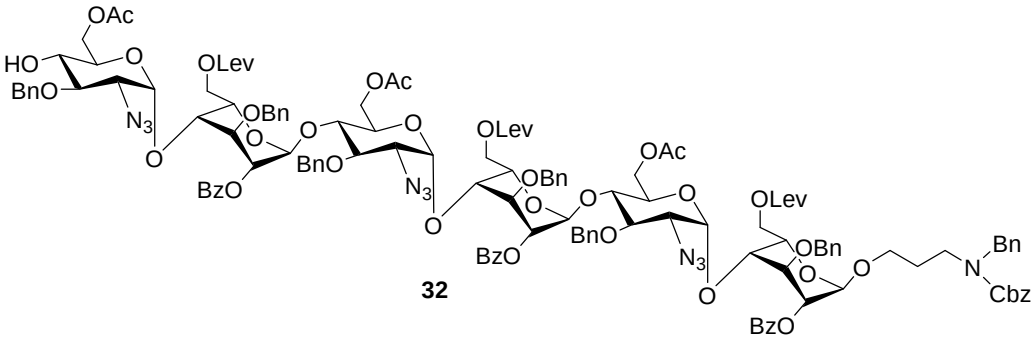


Figure 4.102. 500 MHz, $CDCl_3$, gHMQC (without ^1H Decoupling) of **31**



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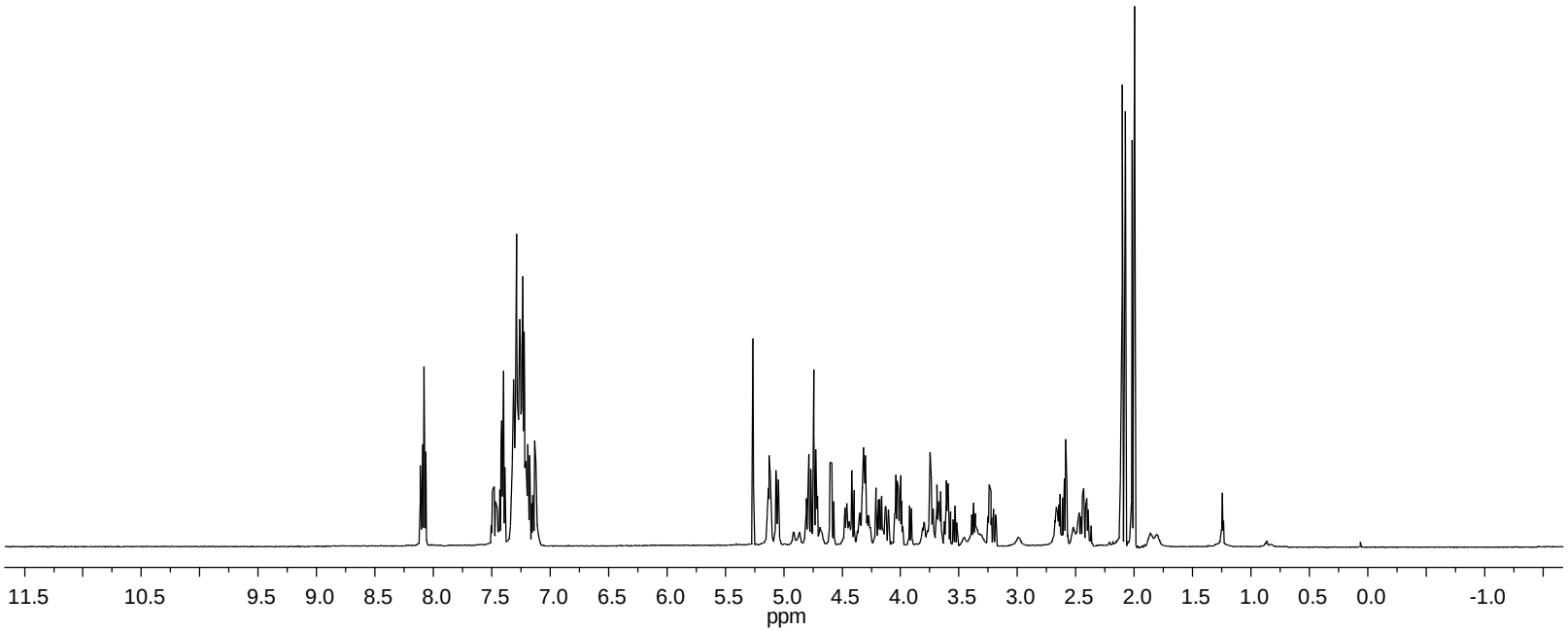


Figure 4.103. 600 MHz, $CDCl_3$, ^1H NMR of **32**

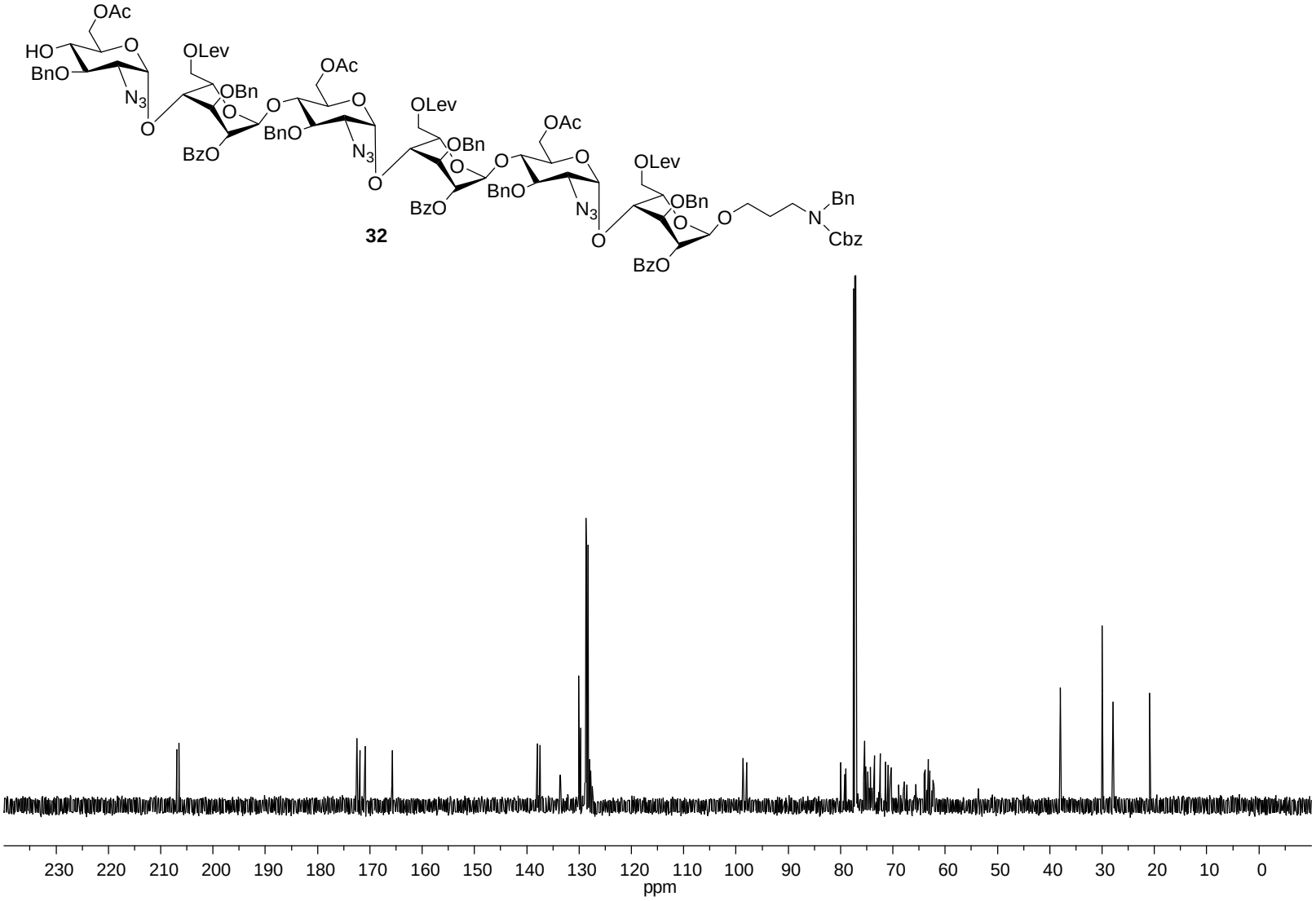


Figure 4.104. 150 MHz, $CDCl_3$, ^{13}C NMR of **32**

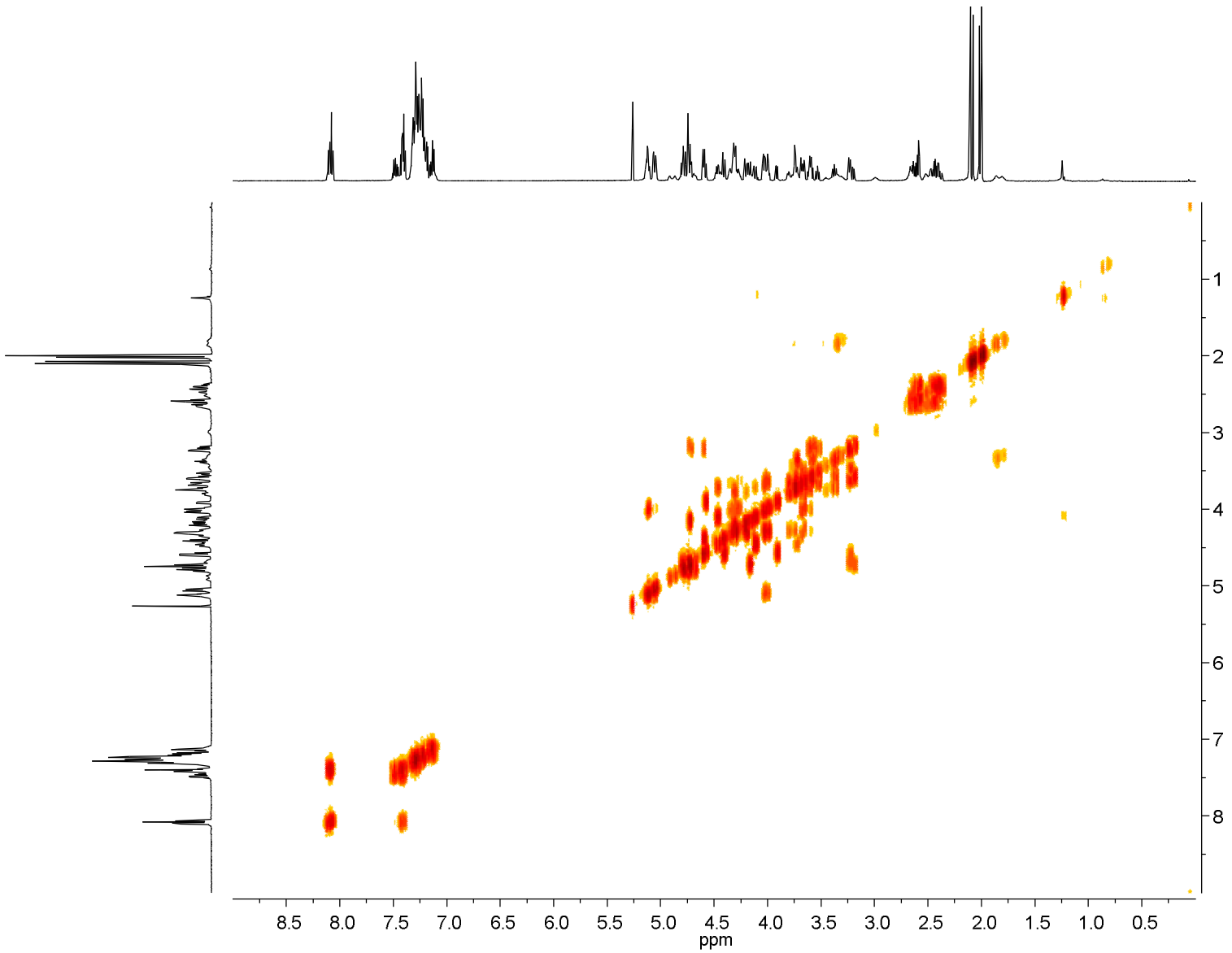


Figure 4.105. 600 MHz, $CDCl_3$, ^1H-^1H gCOSY of **32**

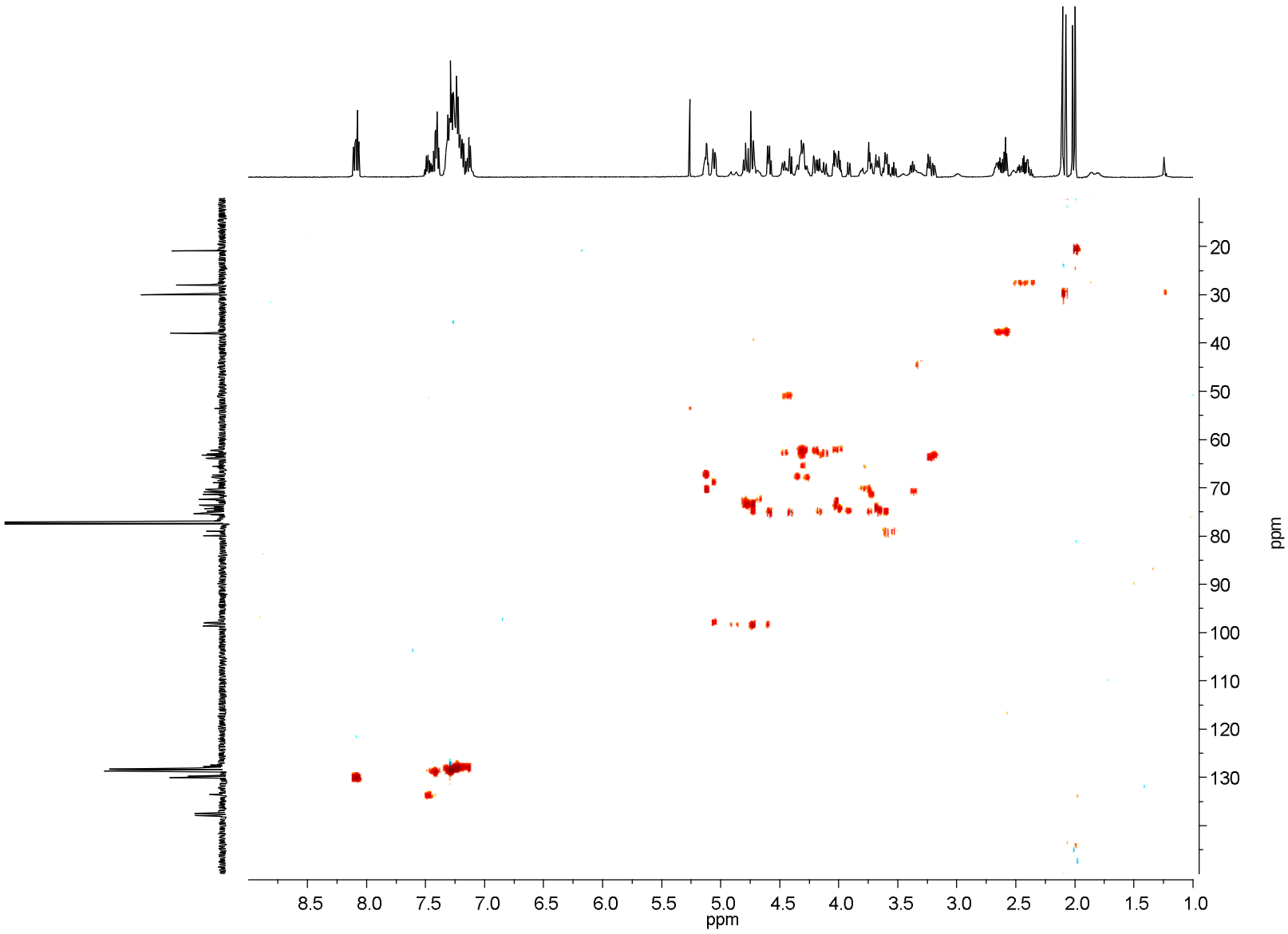
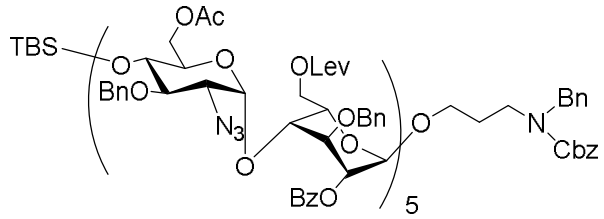


Figure 4.106. 600 MHz, $CDCl_3$, gHMQC of **32**



33

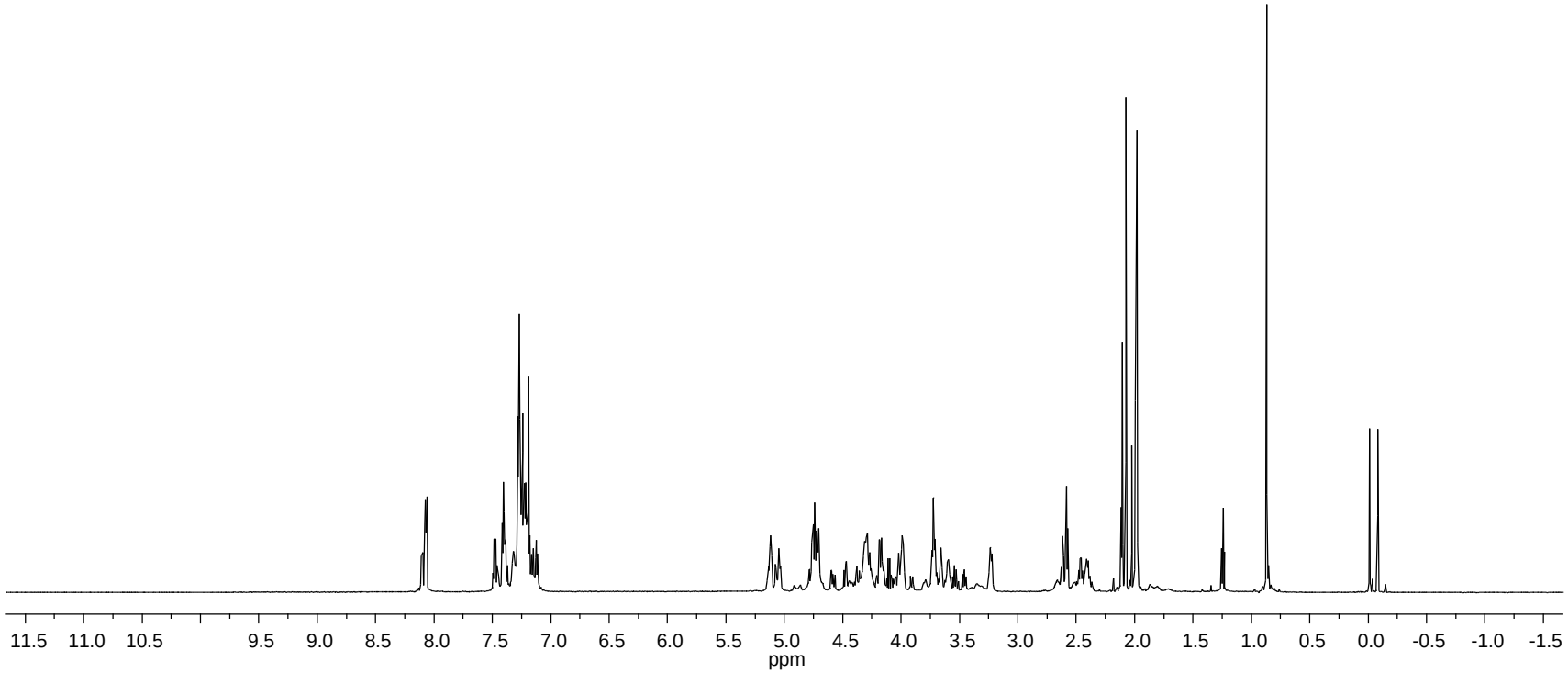


Figure 4.107. 600 MHz, $CDCl_3$, ^1H NMR of **33**

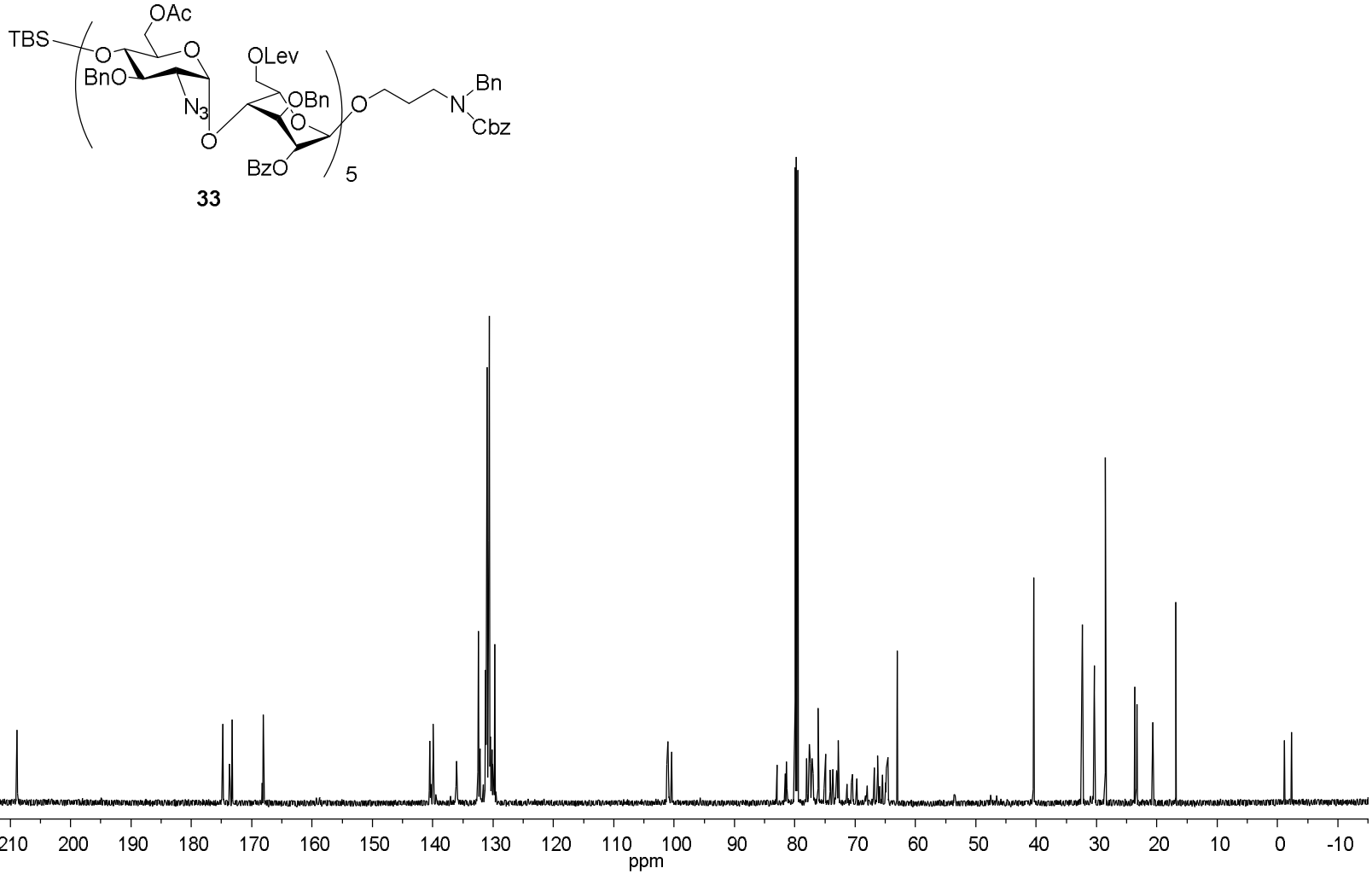


Figure 4.108. 150 MHz, $CDCl_3$, ^{13}C NMR of **33**

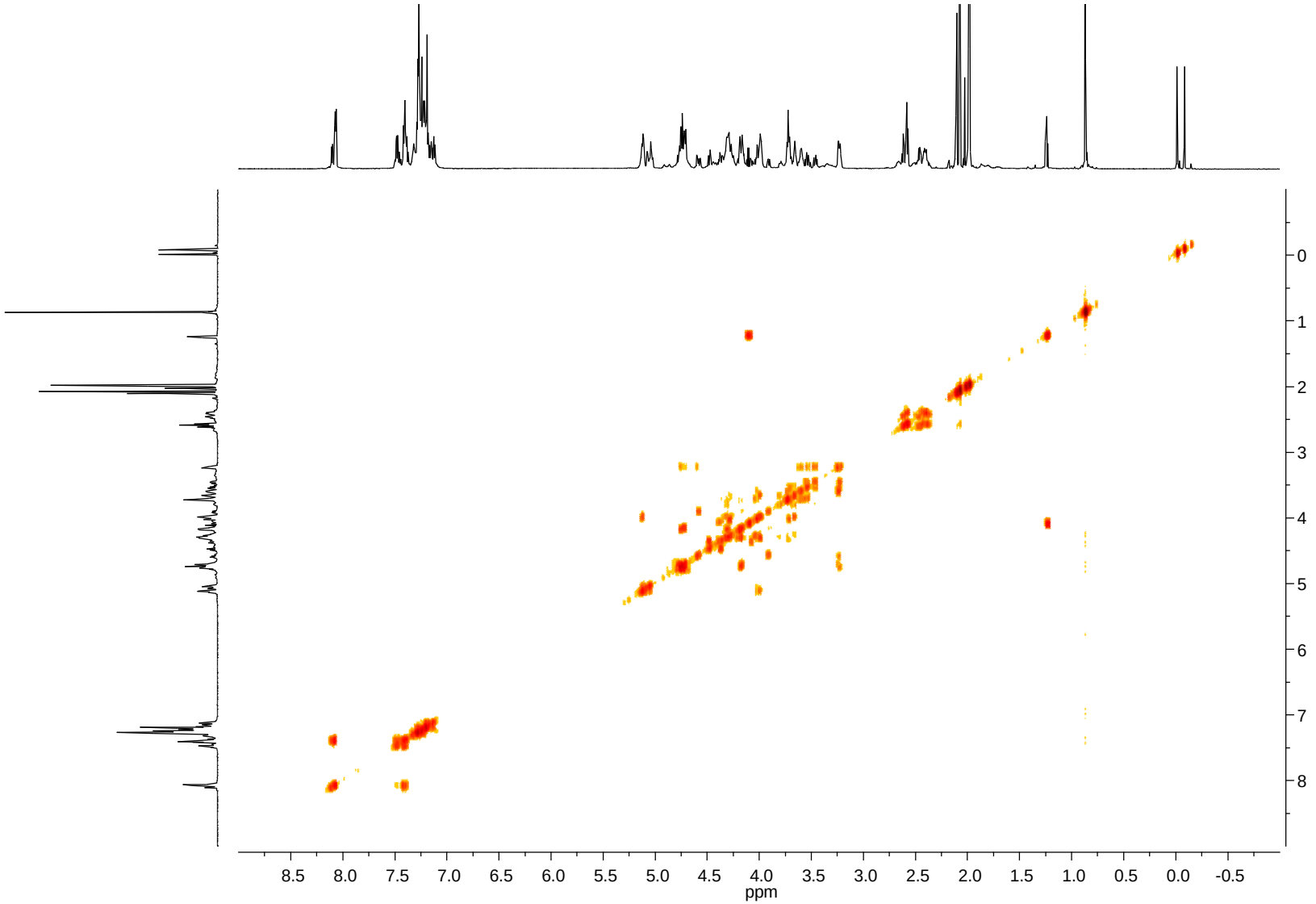


Figure 4.109. 600 MHz, $CDCl_3$, ^1H-^1H gCOSY of **33**

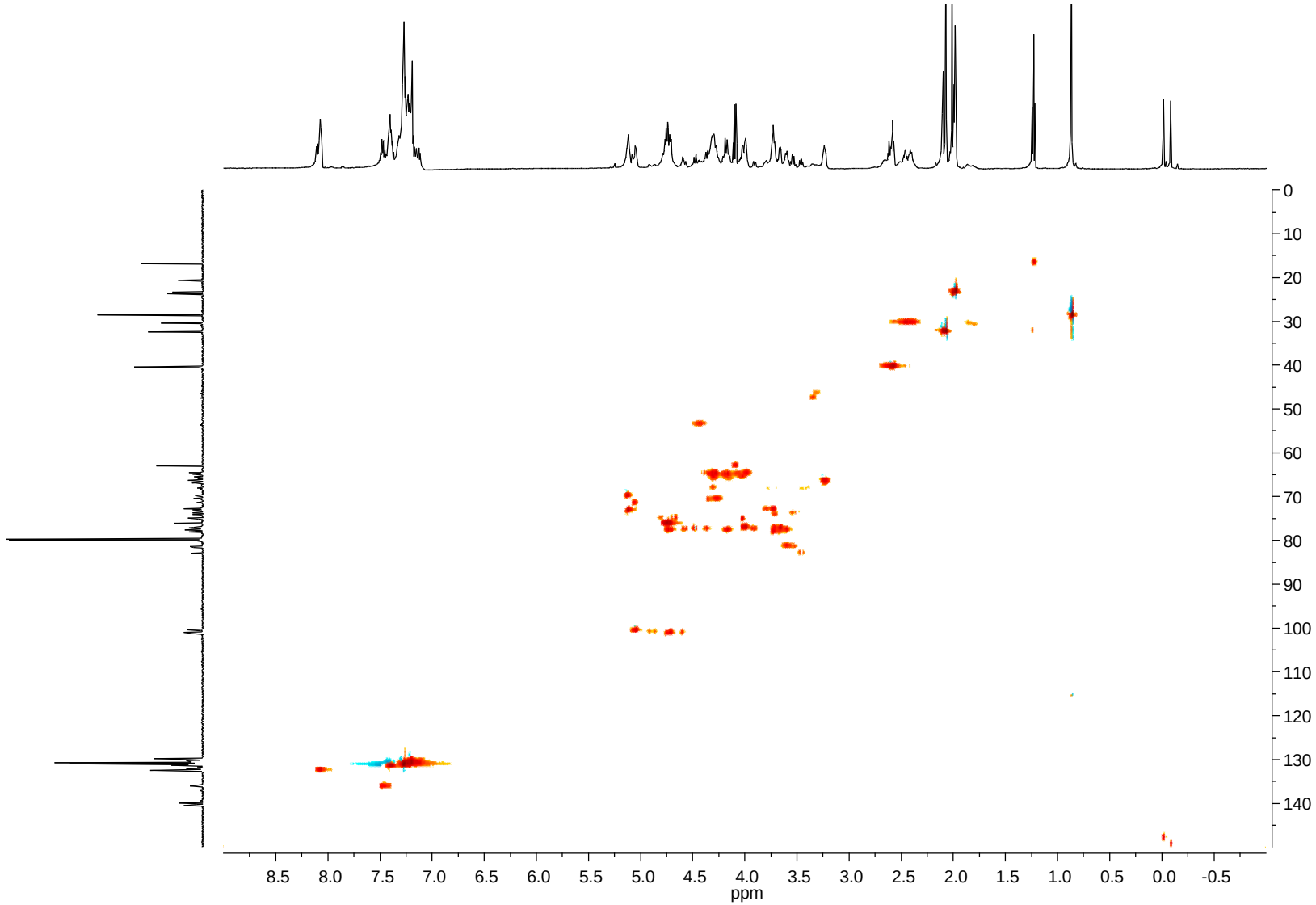


Figure 4.110. 600 MHz, $CDCl_3$, gHMQC of **33**

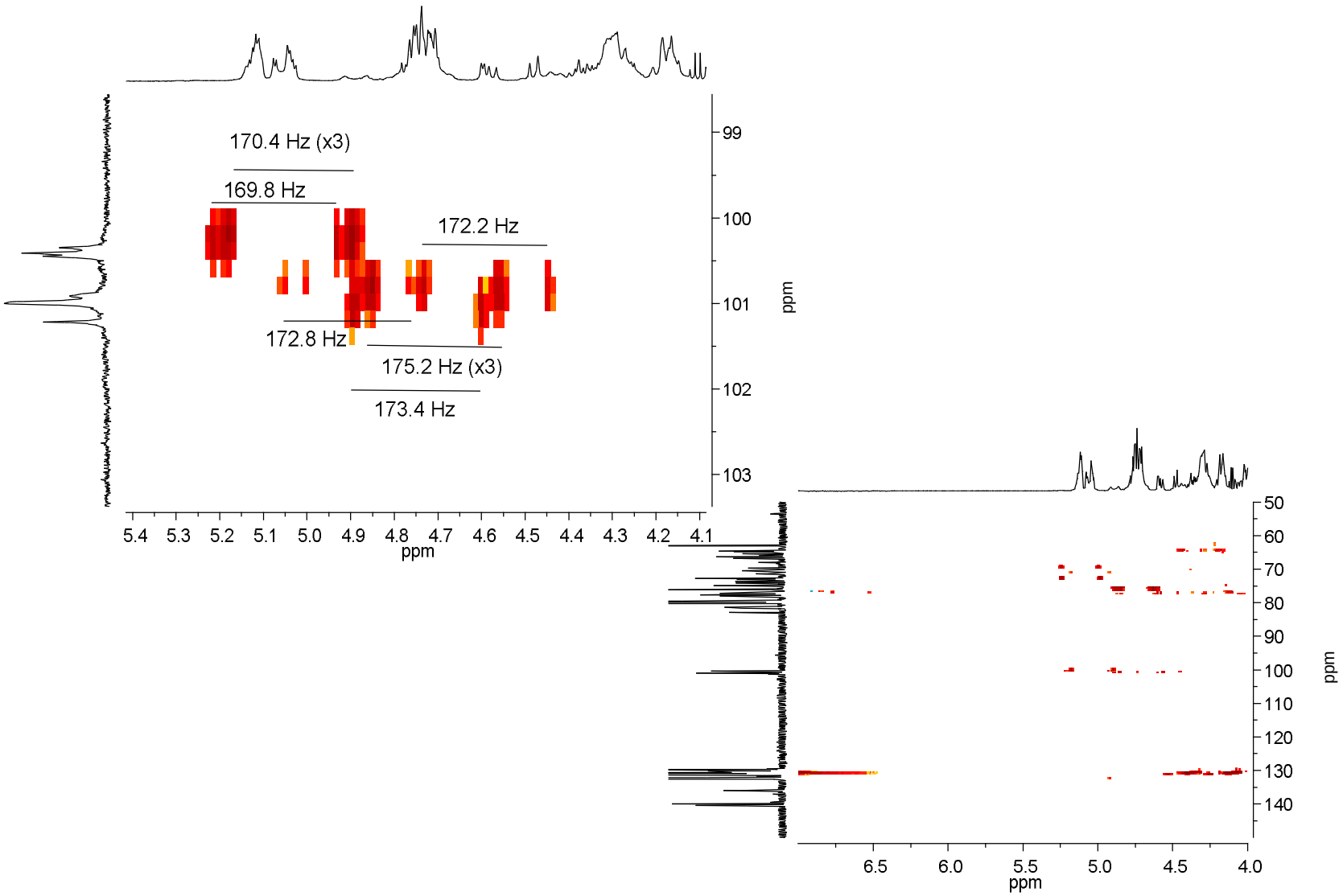


Figure 4.111. 600 MHz, $CDCl_3$, gHMQC (without ^1H Decoupling) of **33**

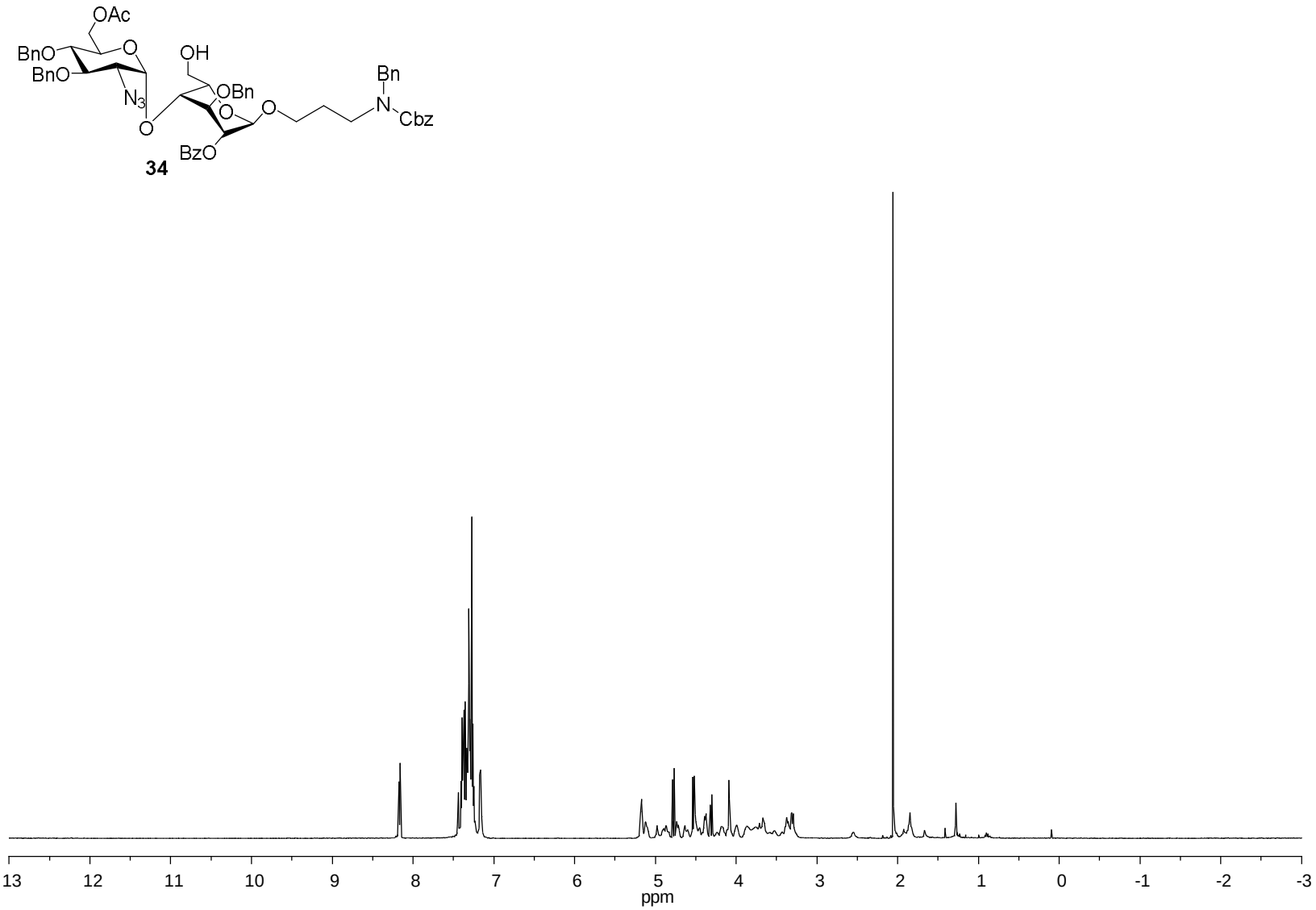


Figure 4.112. 500 MHz, $CDCl_3$, ^1H NMR of **34**

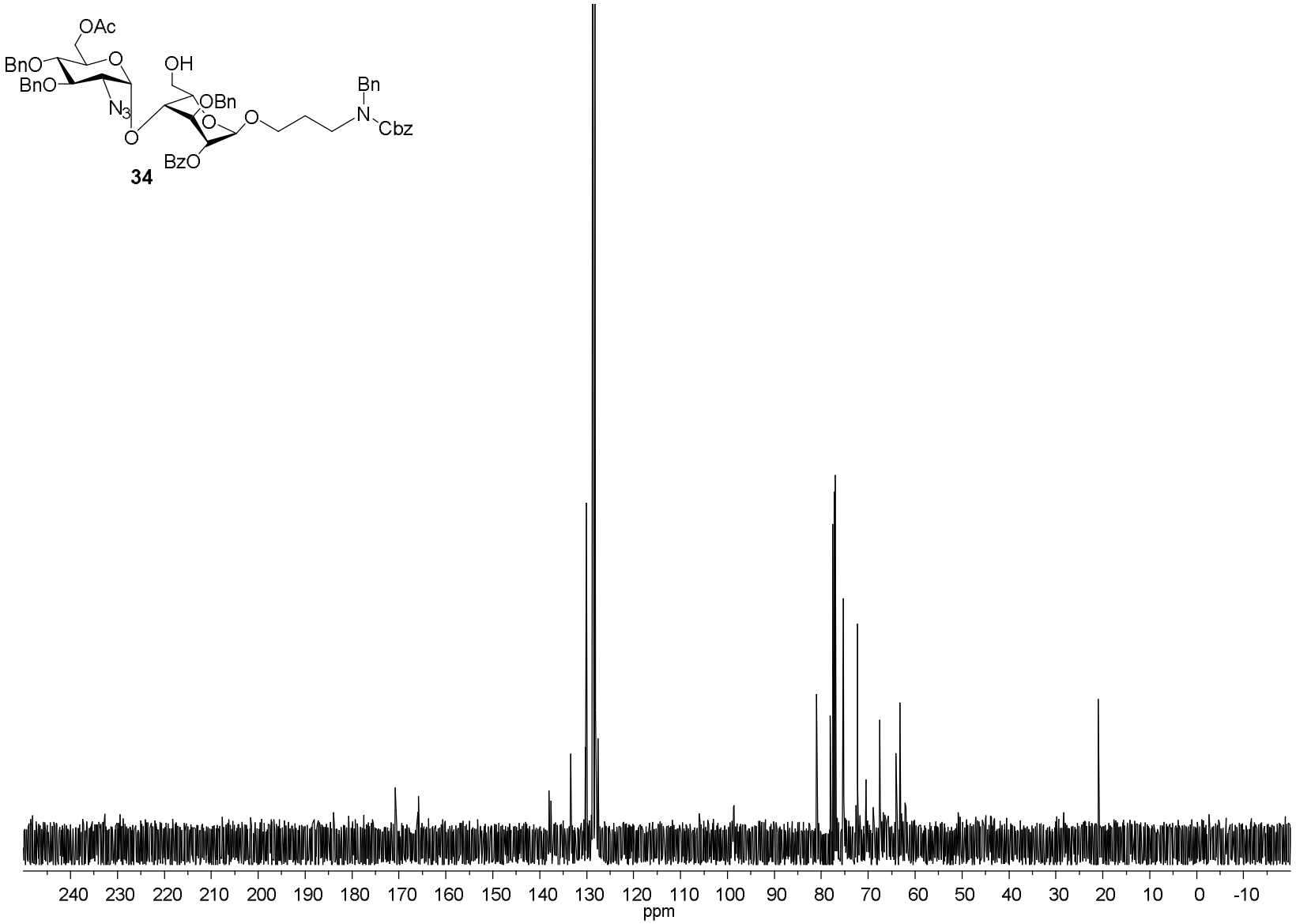


Figure 4.113. 125 MHz, $CDCl_3$, ^{13}C NMR of **34**

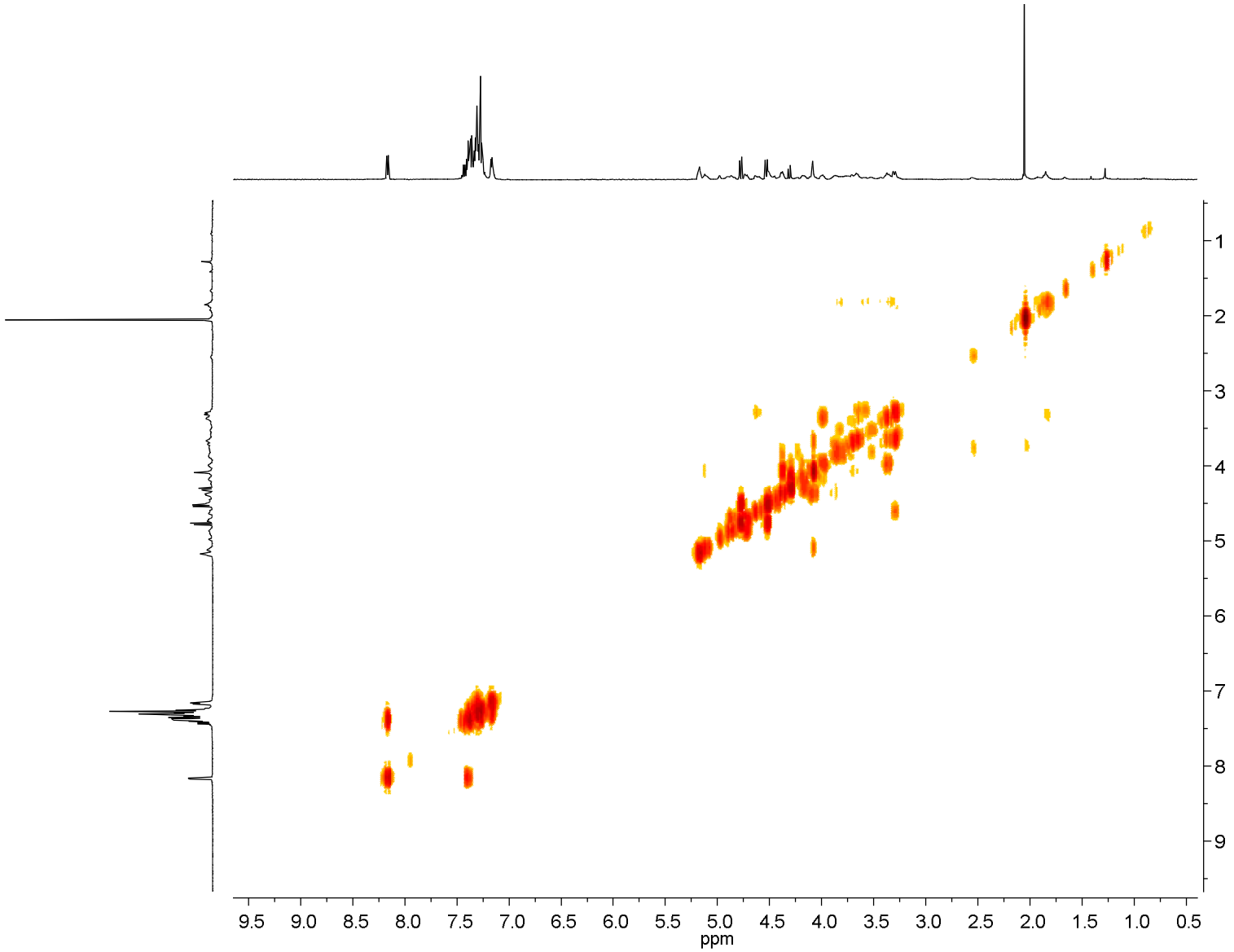
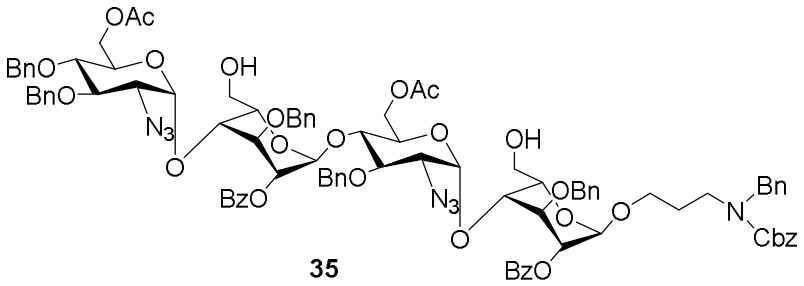


Figure 4.114. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **34**



35

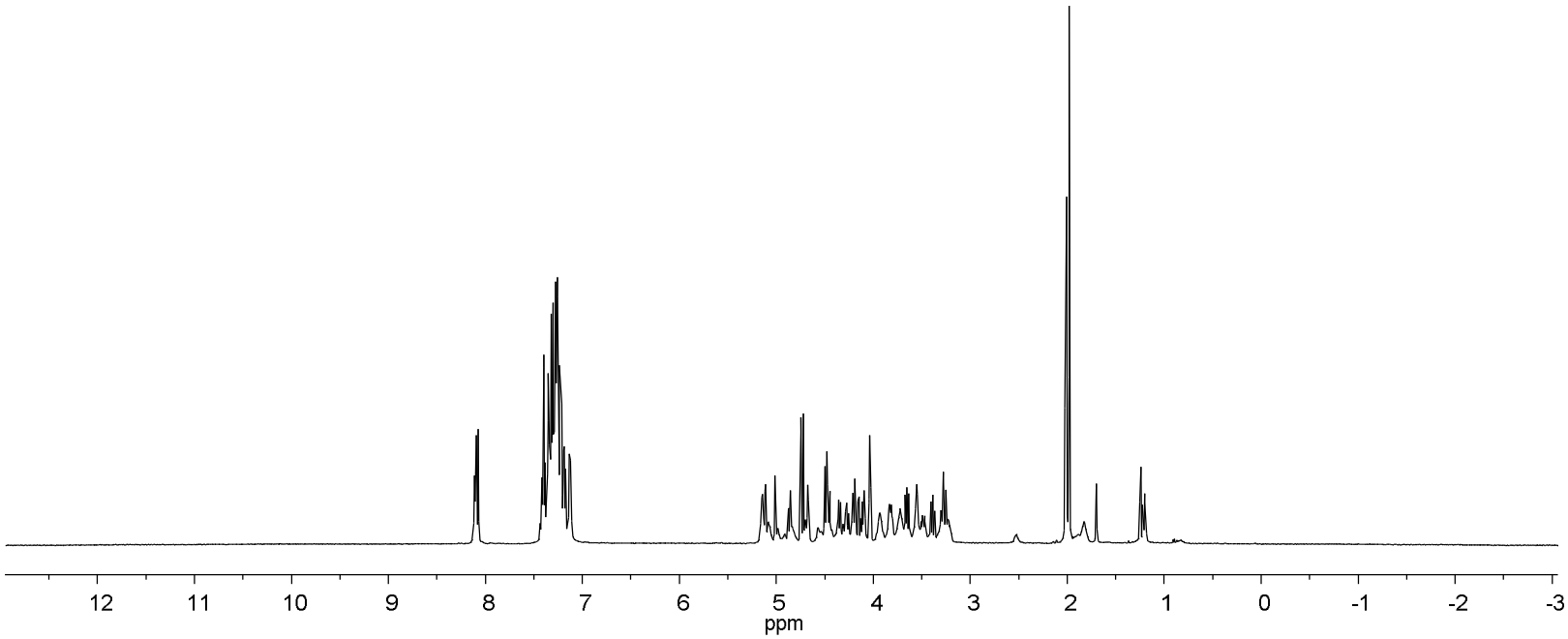
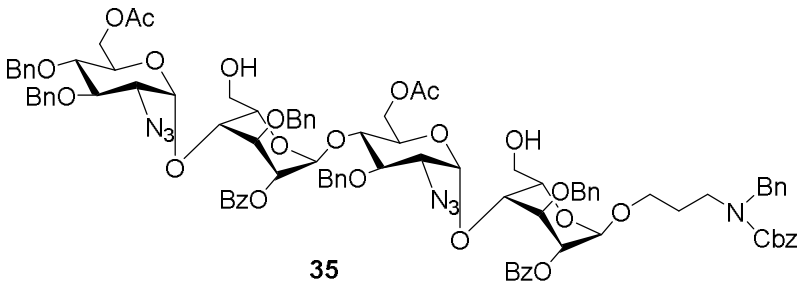


Figure 4.115. 500 MHz, $CDCl_3$, ^1H NMR of **35**



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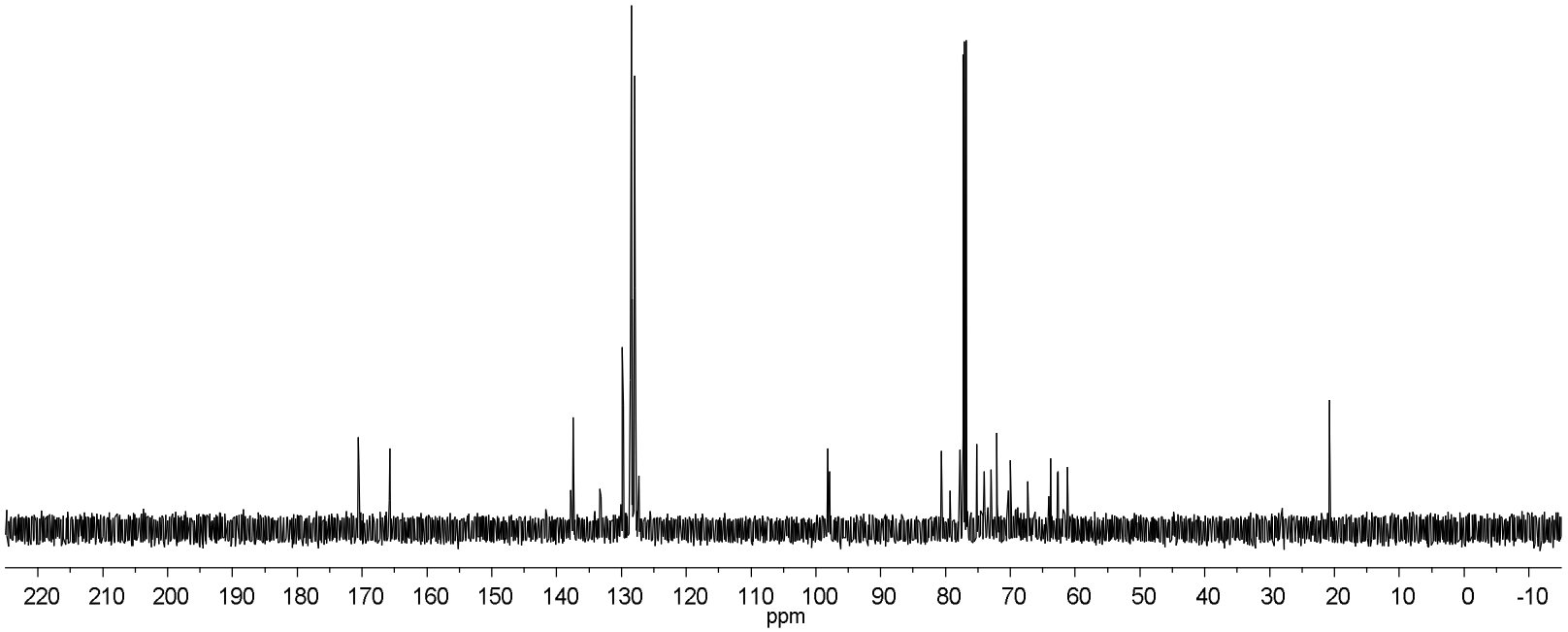


Figure 4.116. 125 MHz, $CDCl_3$, ^{13}C NMR of **35**

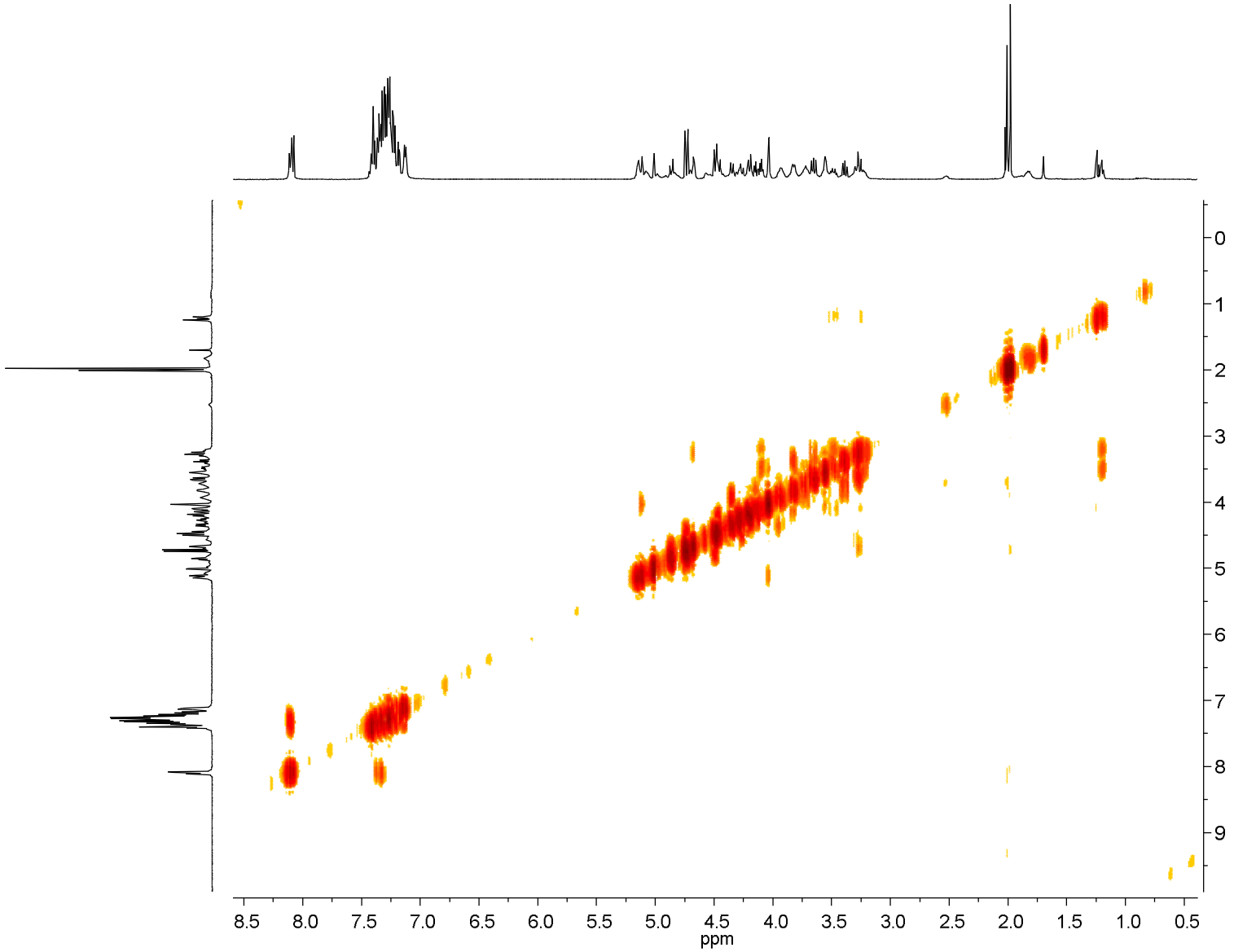


Figure 4.117. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **35**

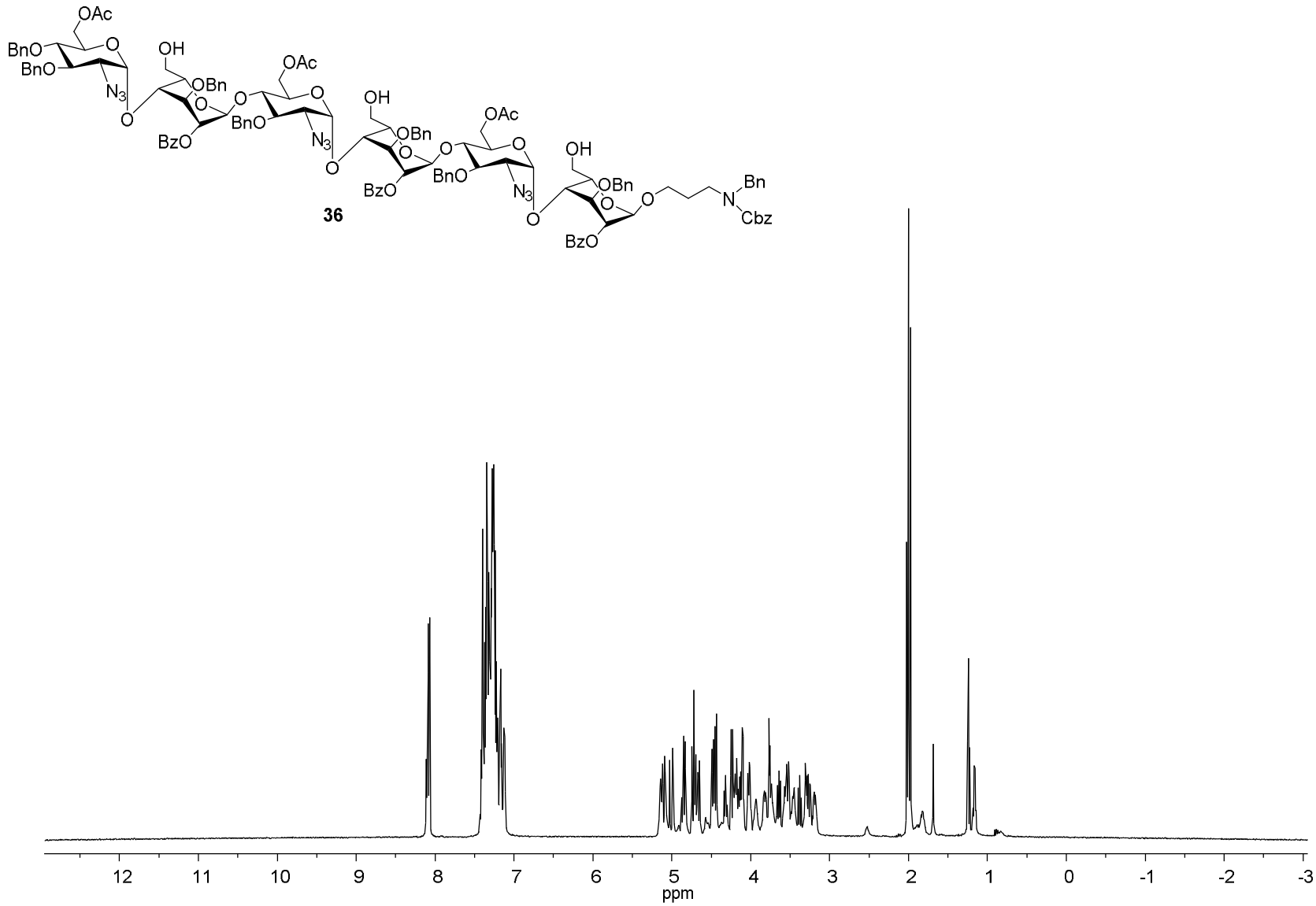


Figure 4.118. 500 MHz, $CDCl_3$, ^1H NMR of **36**

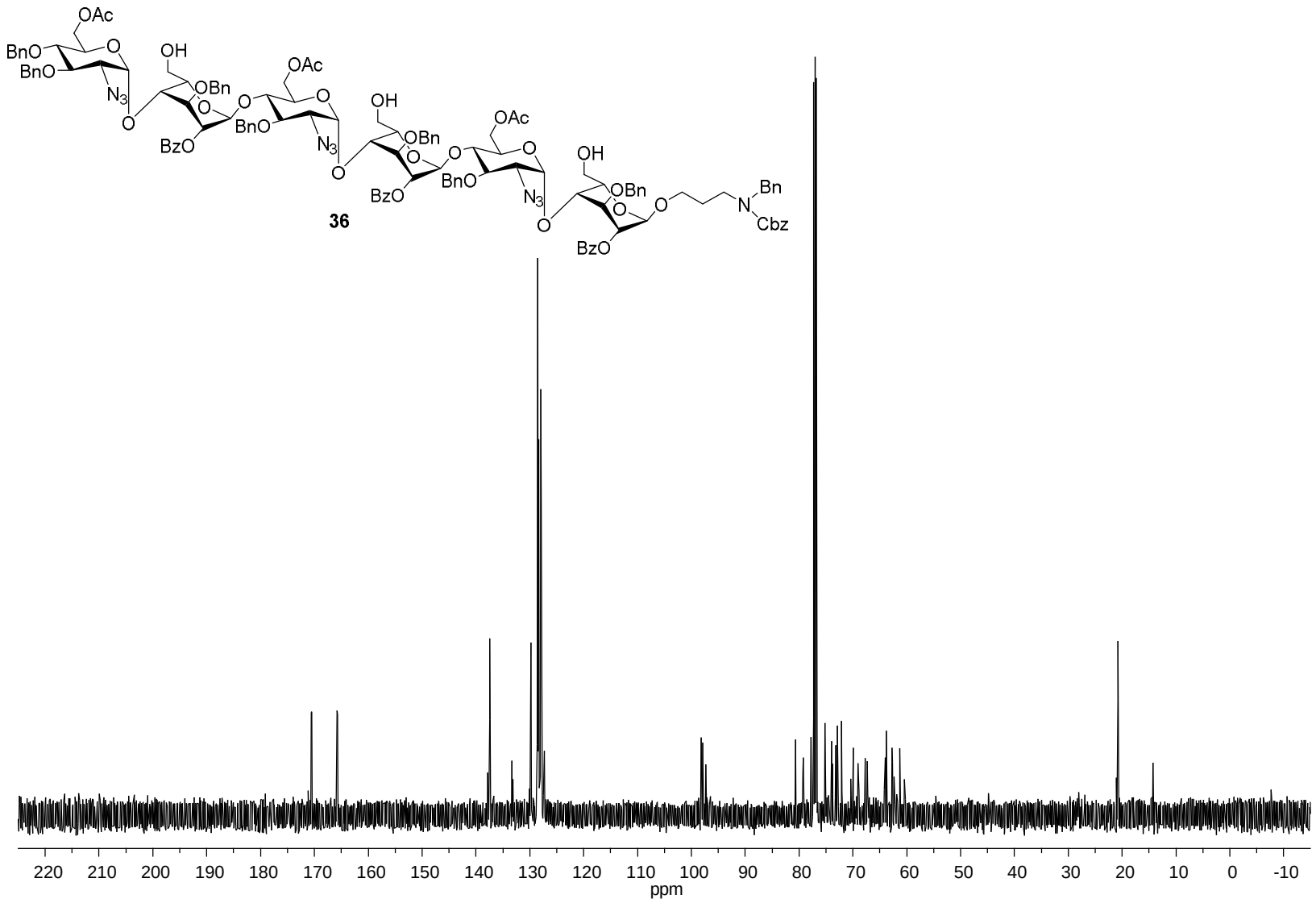


Figure 4.119. 125 MHz, $CDCl_3$, ^{13}C NMR of **36**

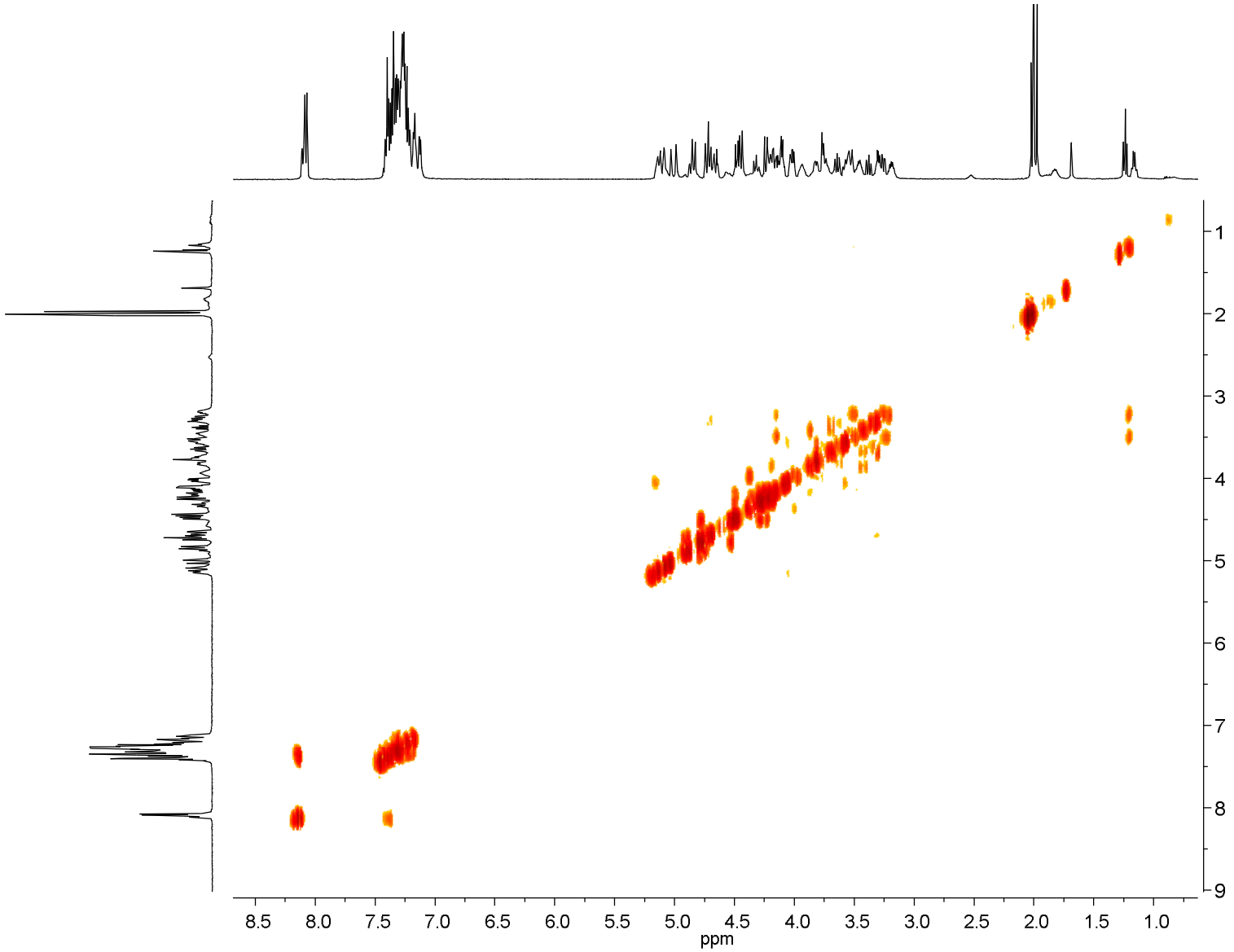


Figure 4.120. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **36**

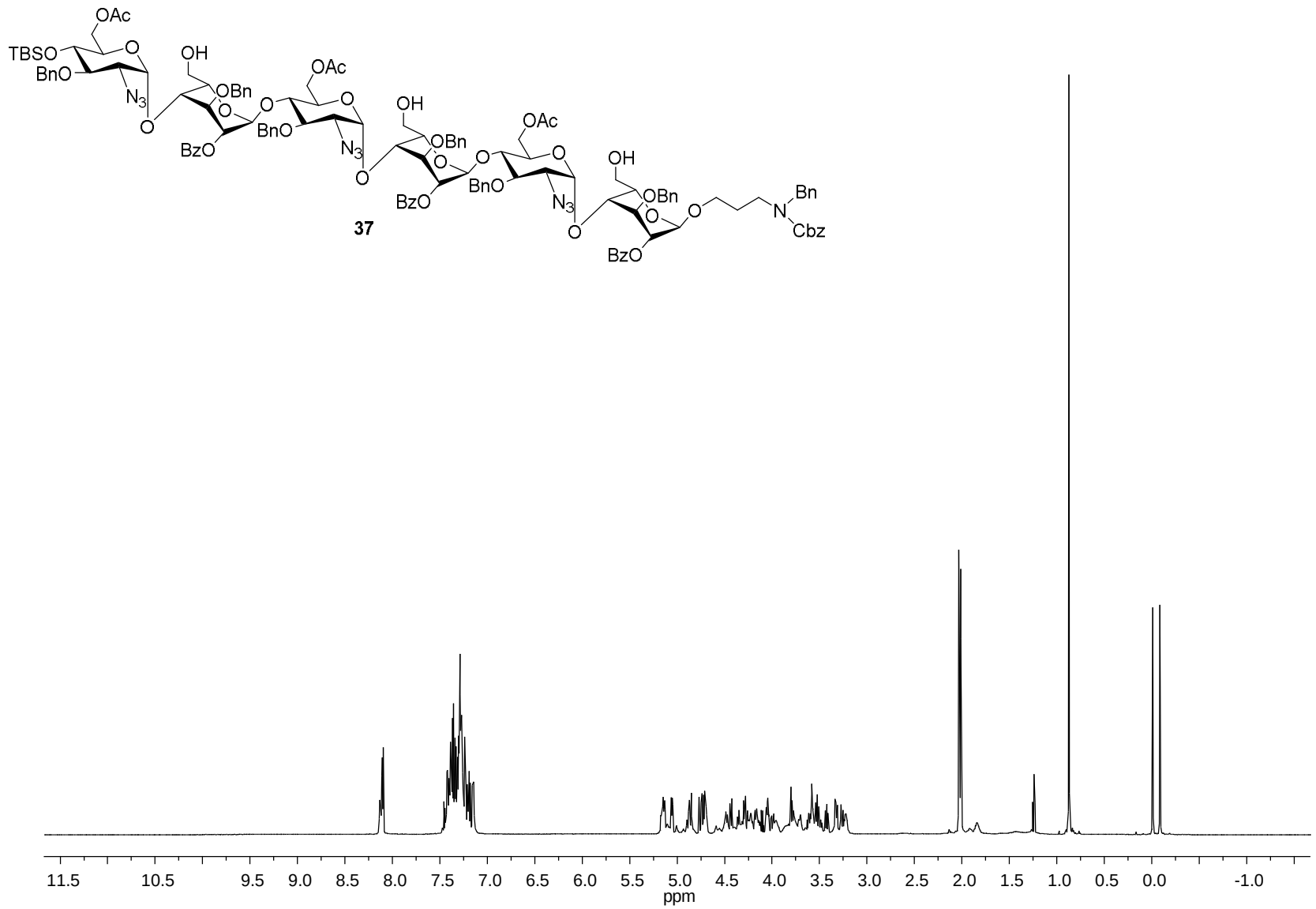


Figure 4.121. 600 MHz, $CDCl_3$, ^1H NMR of **37**

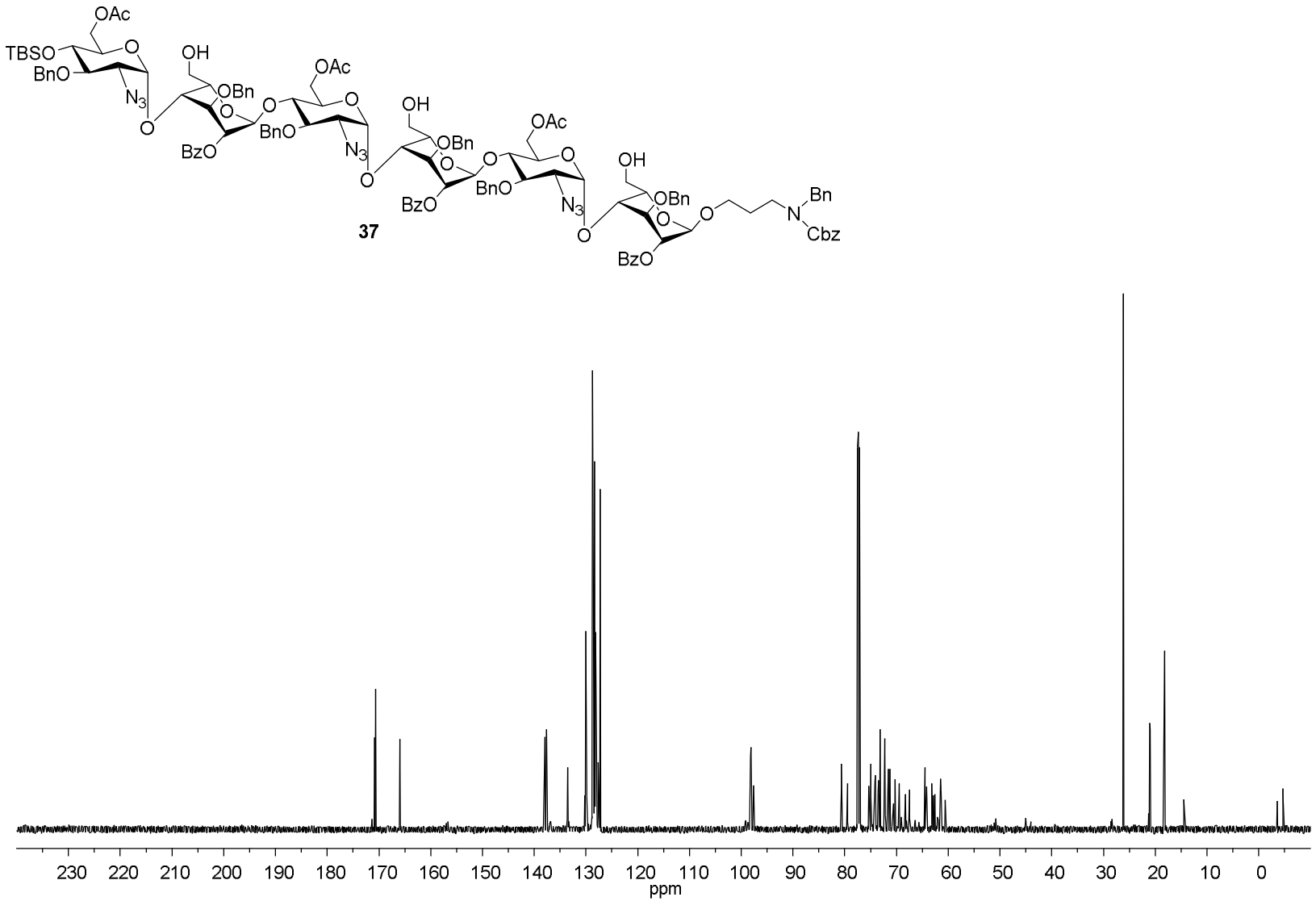


Figure 4.122. 150 MHz, $CDCl_3$, ^{13}C NMR of **37**

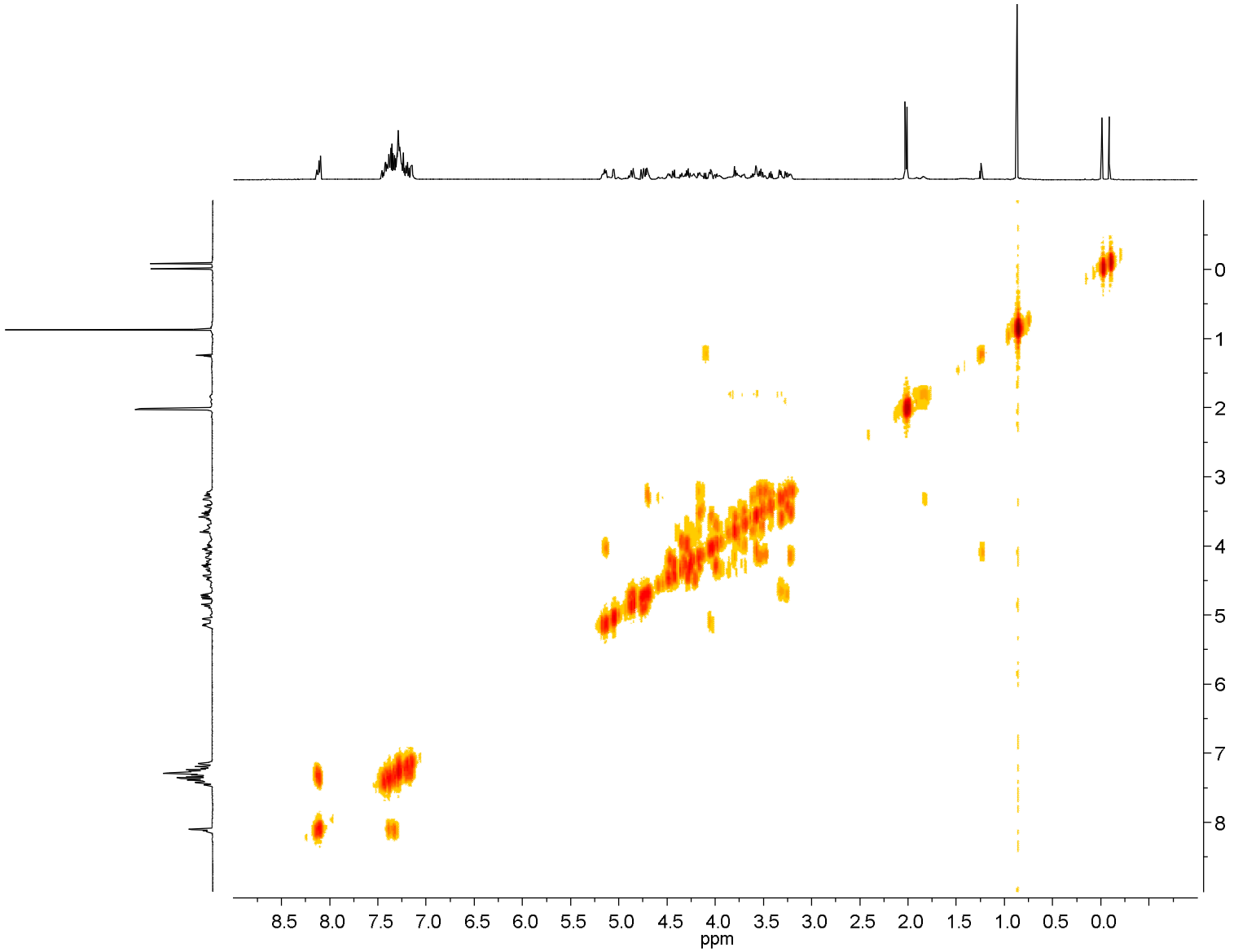
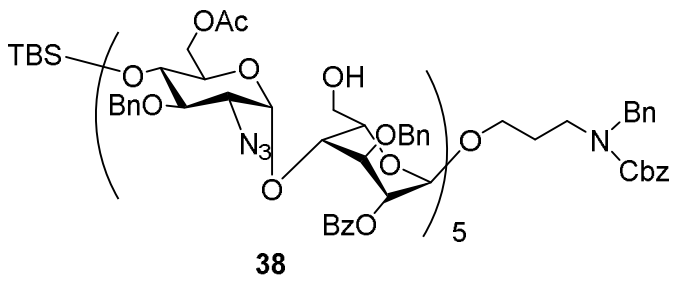


Figure 4.123. 600 MHz, $CDCl_3$, ^1H-^1H gCOSY of **37**



38

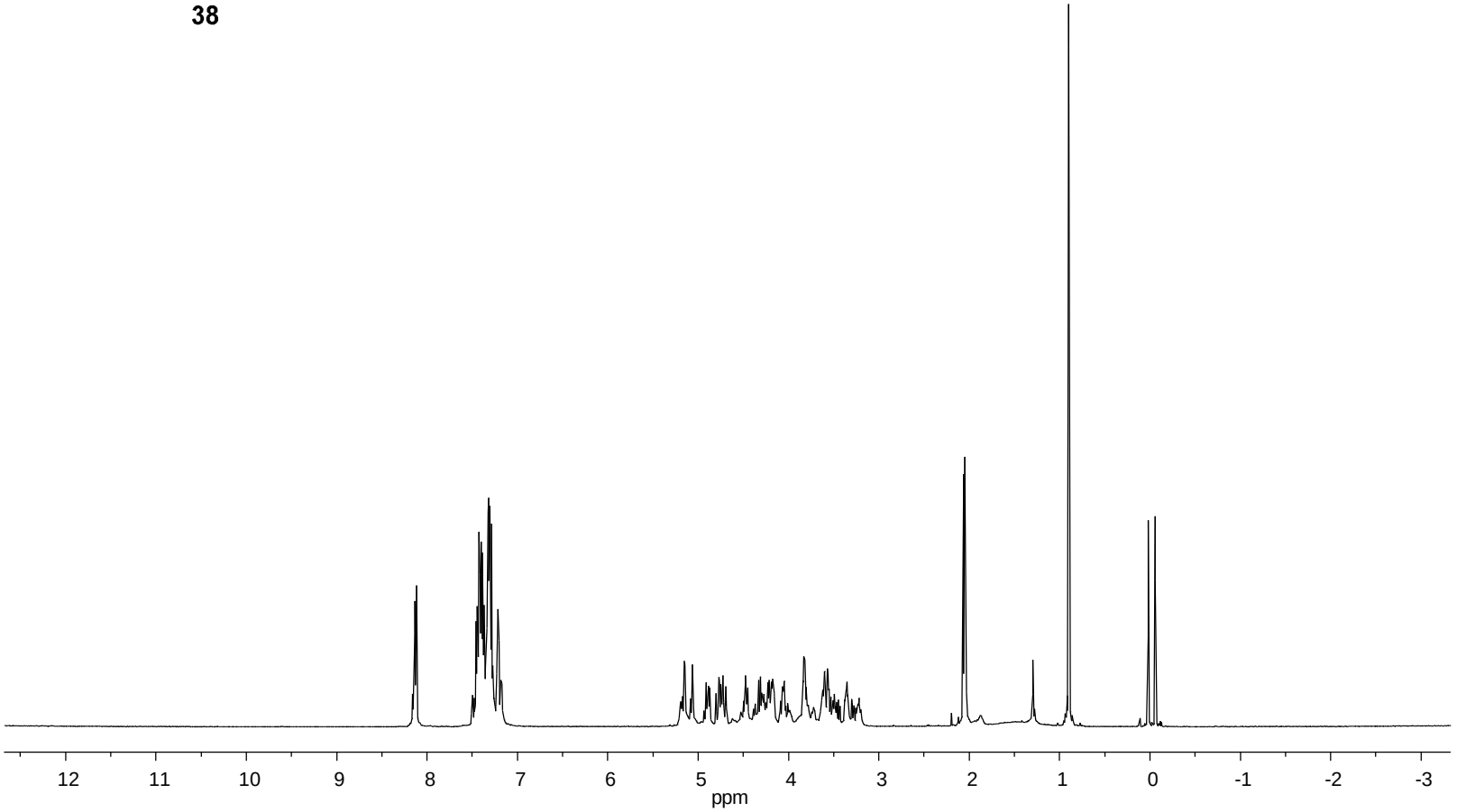


Figure 4.124. 500 MHz, $CDCl_3$, ^1H NMR of **38**

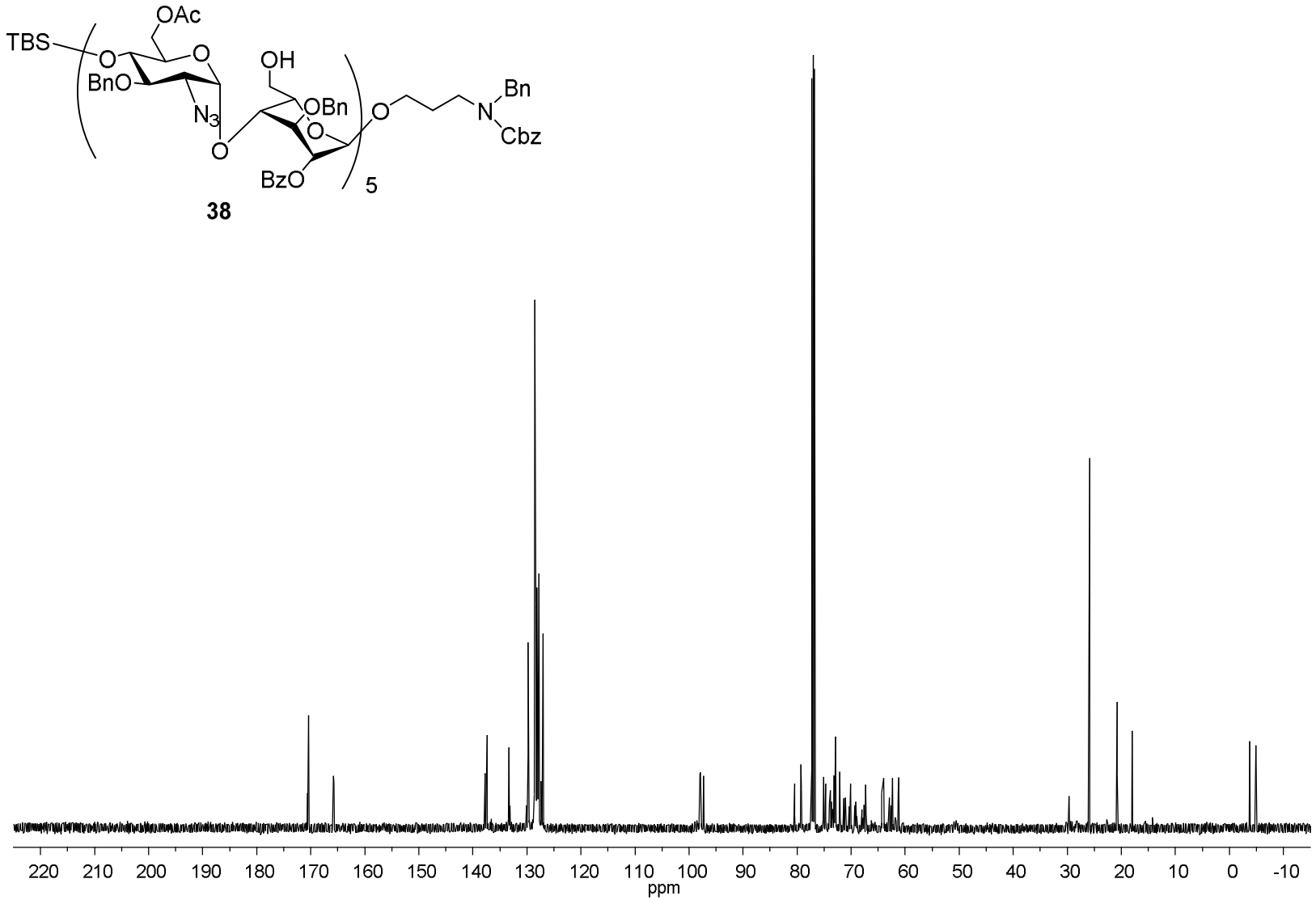


Figure 4.125. 150 MHz, $CDCl_3$, ^{13}C NMR of **38**

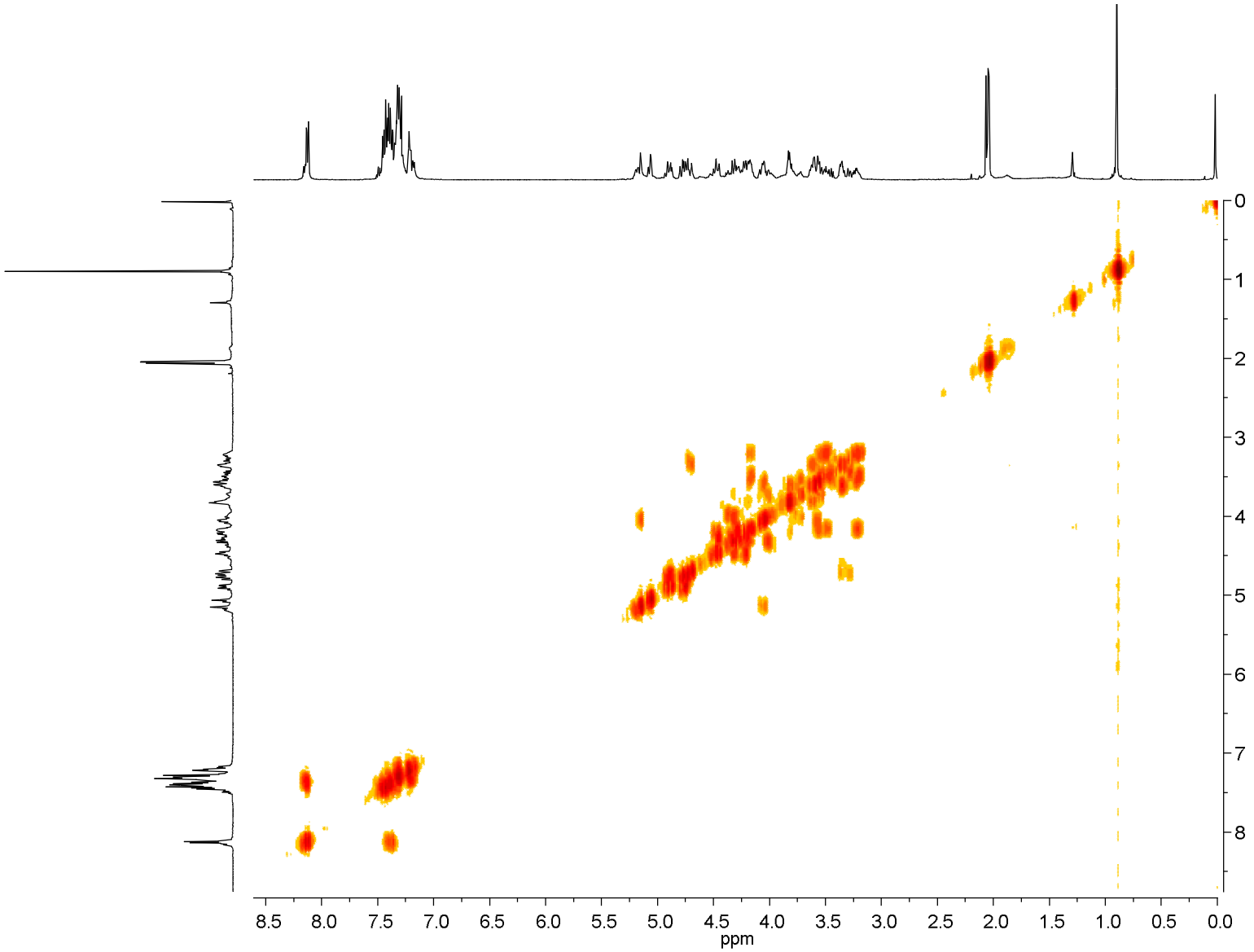
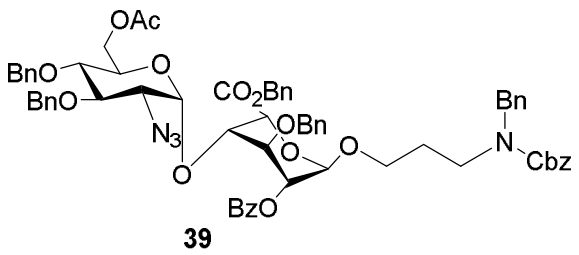


Figure 4.126. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **38**



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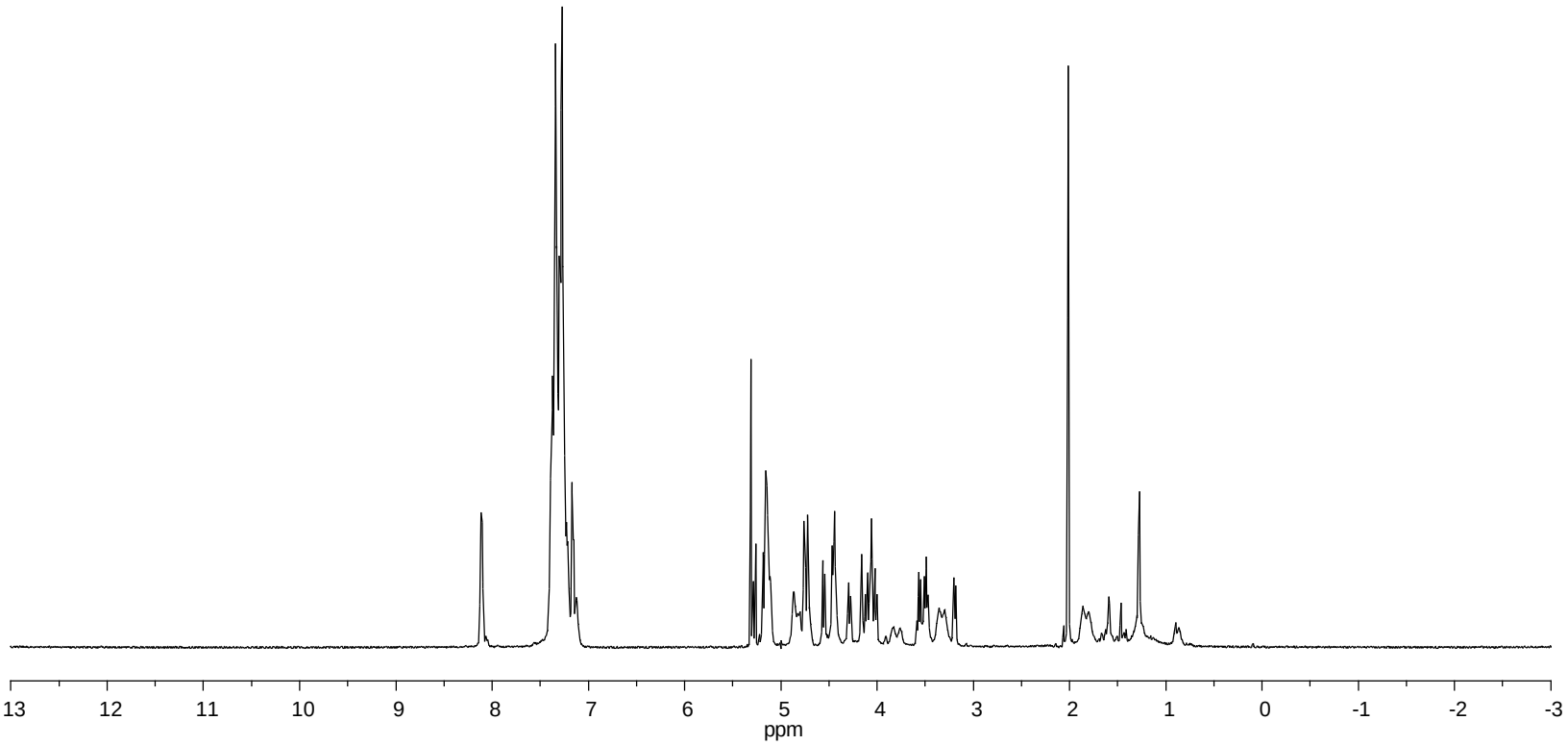


Figure 4.127. 500 MHz, $CDCl_3$, ^1H NMR of **39**

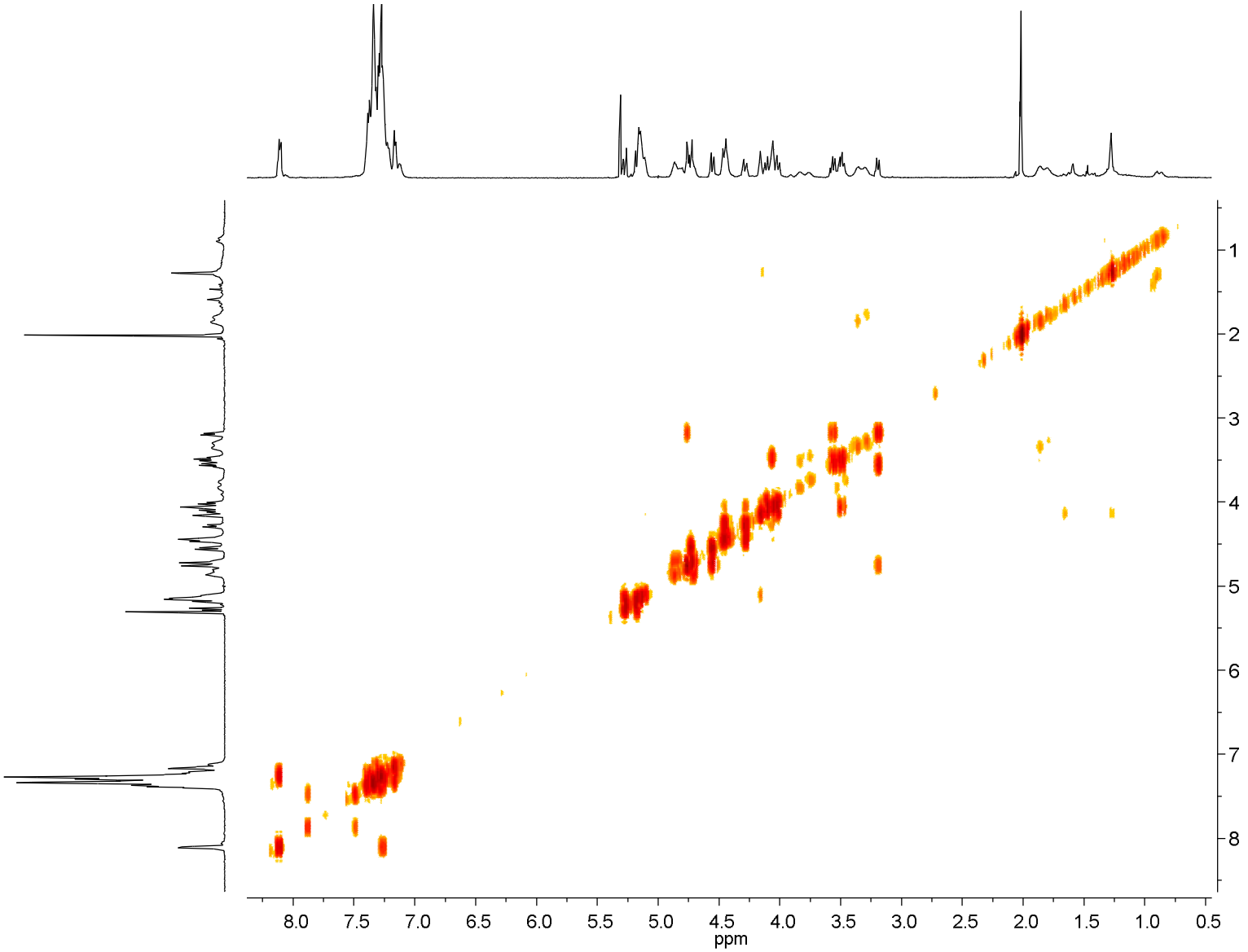
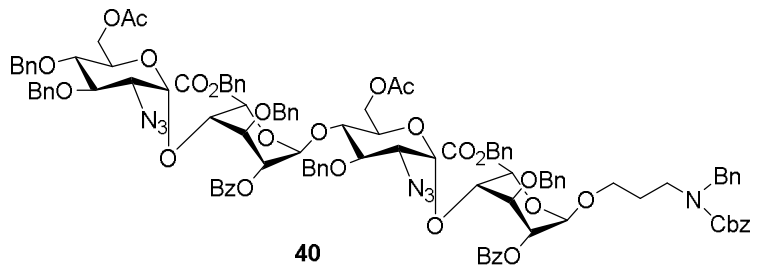


Figure 4.128. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **39**



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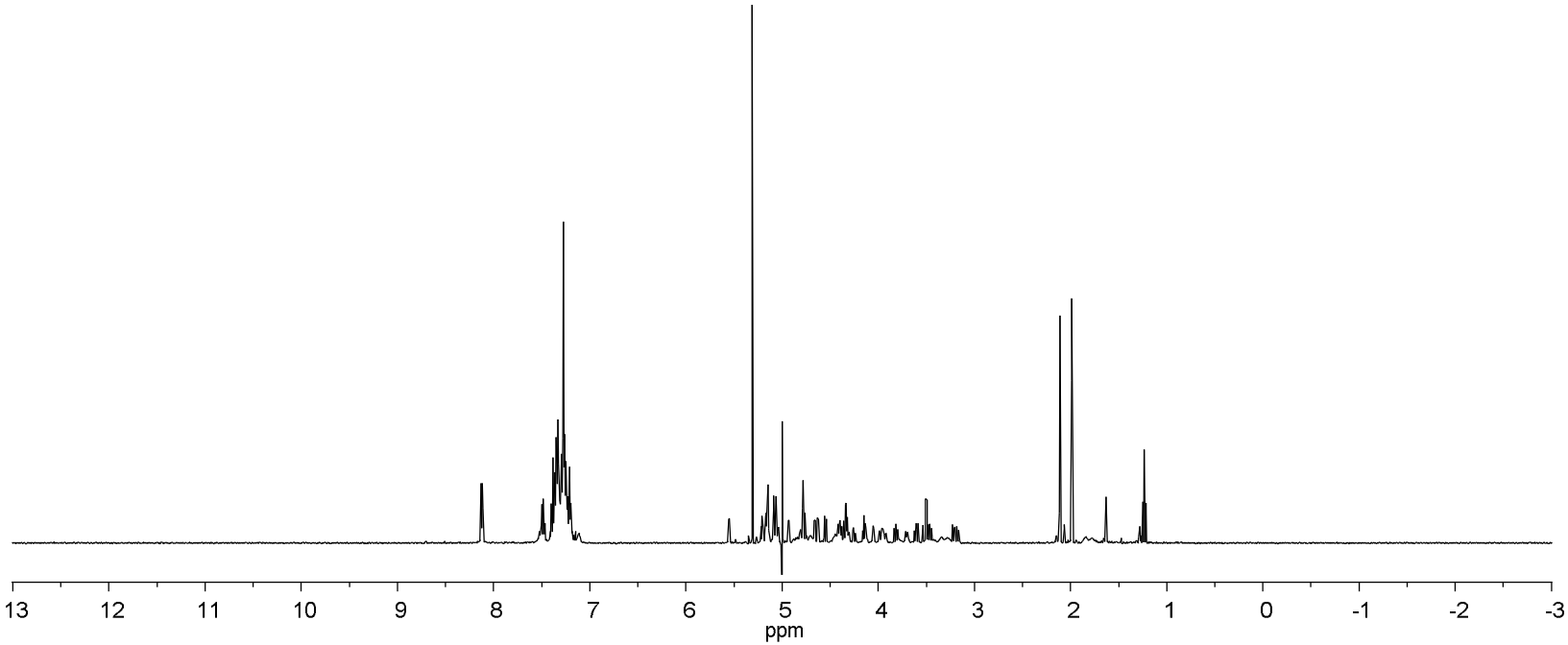
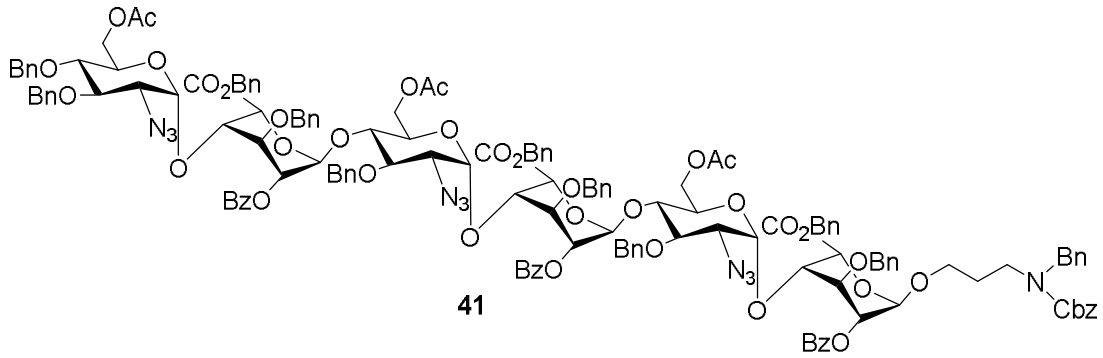


Figure 4.129. 500 MHz, $CDCl_3$, ^1H NMR of **40**



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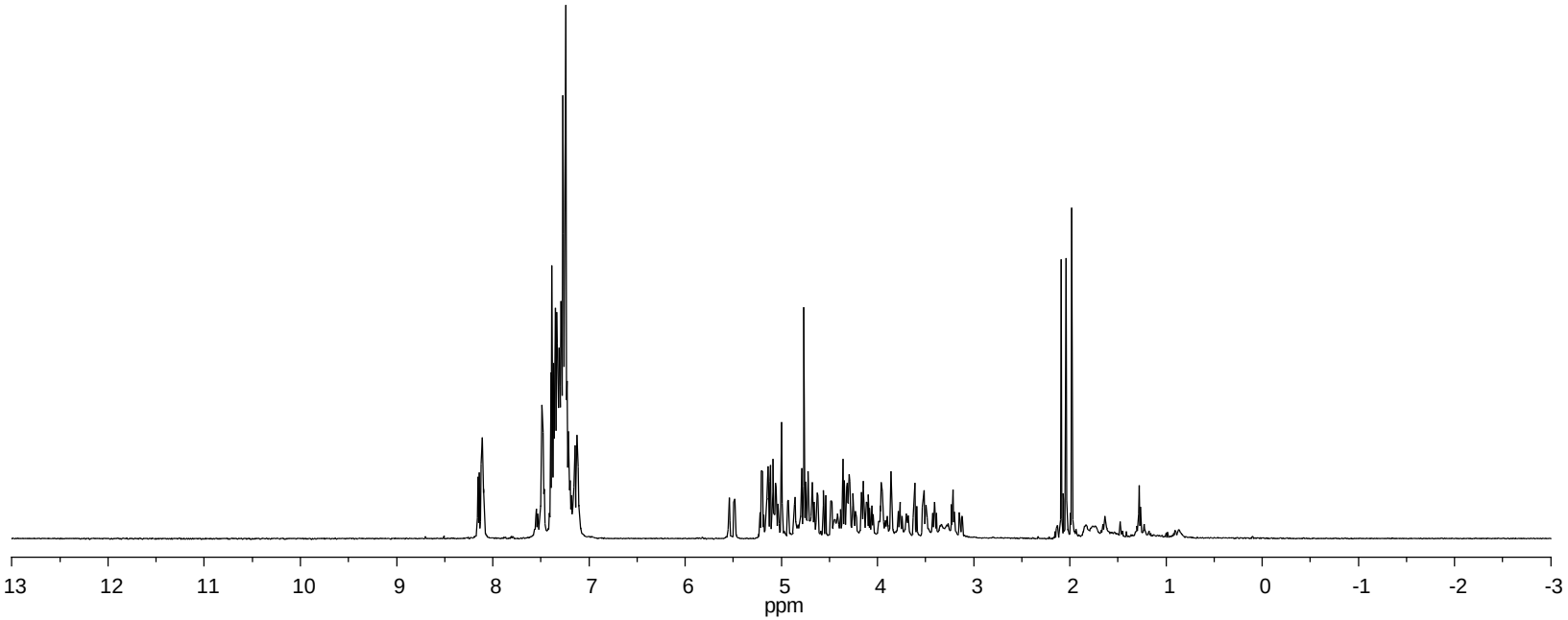


Figure 4.130. 500 MHz, $CDCl_3$, ^1H NMR of **41**

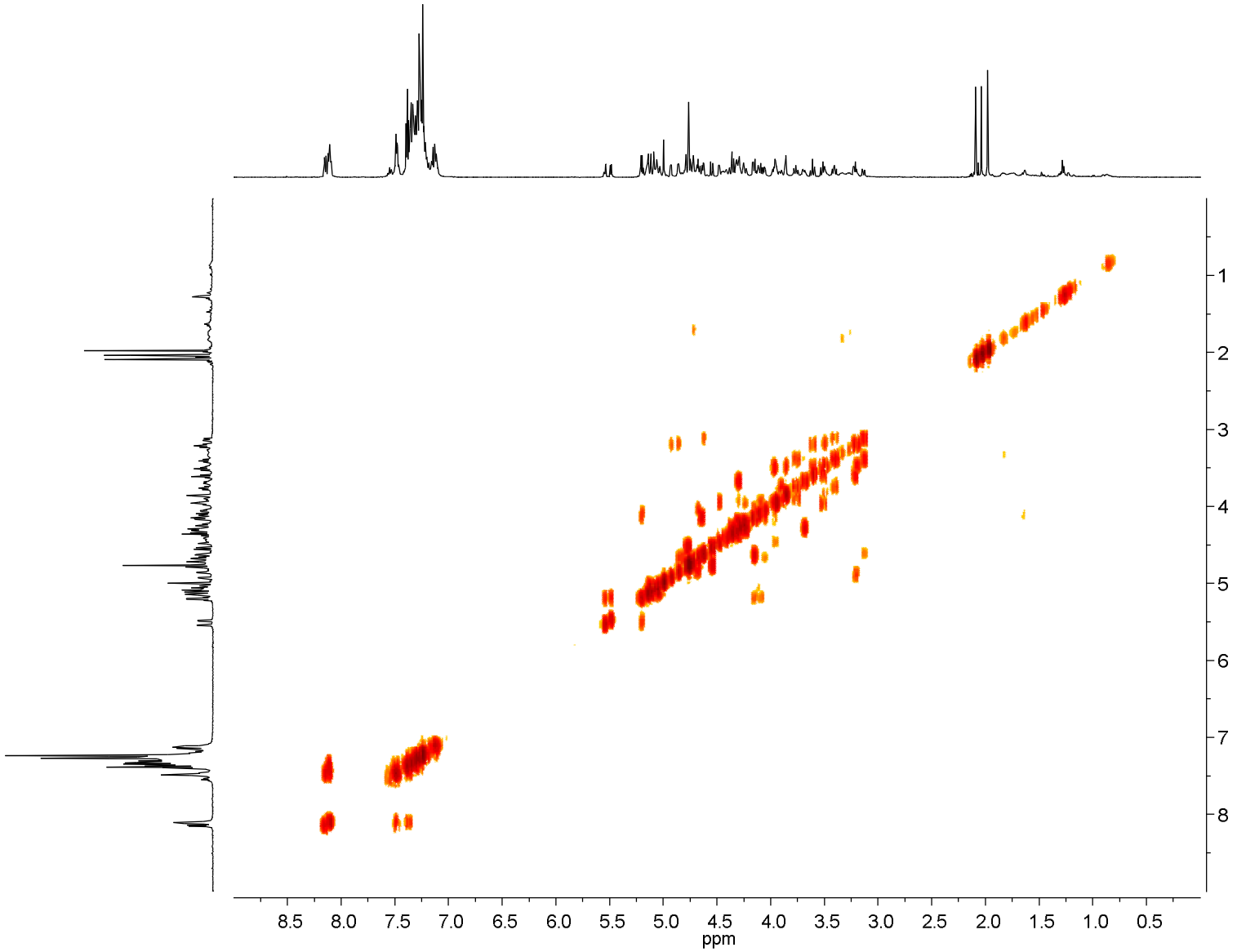


Figure 4.131. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **41**

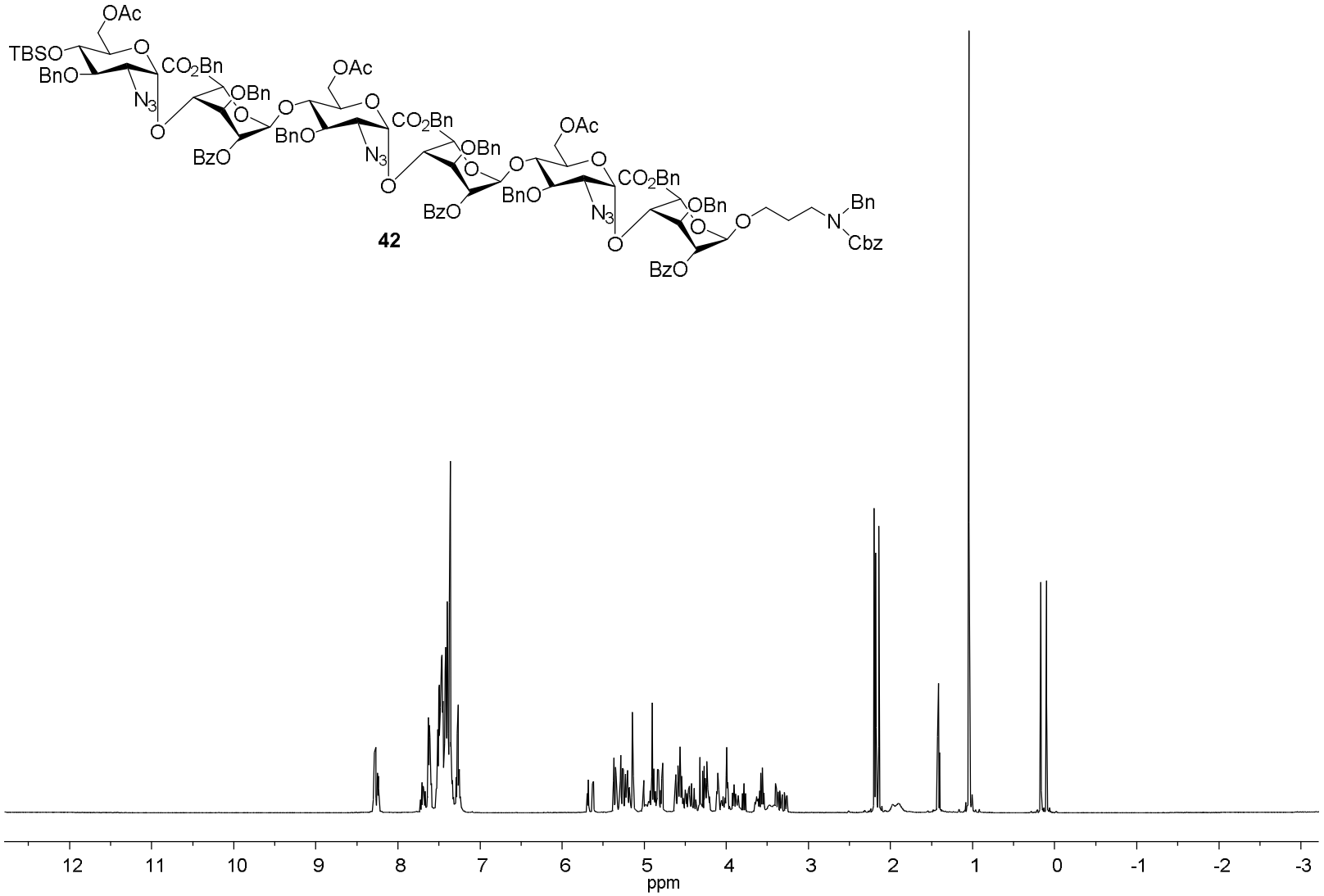


Figure 4.132. 500 MHz, $CDCl_3$, ^1H NMR of **42**

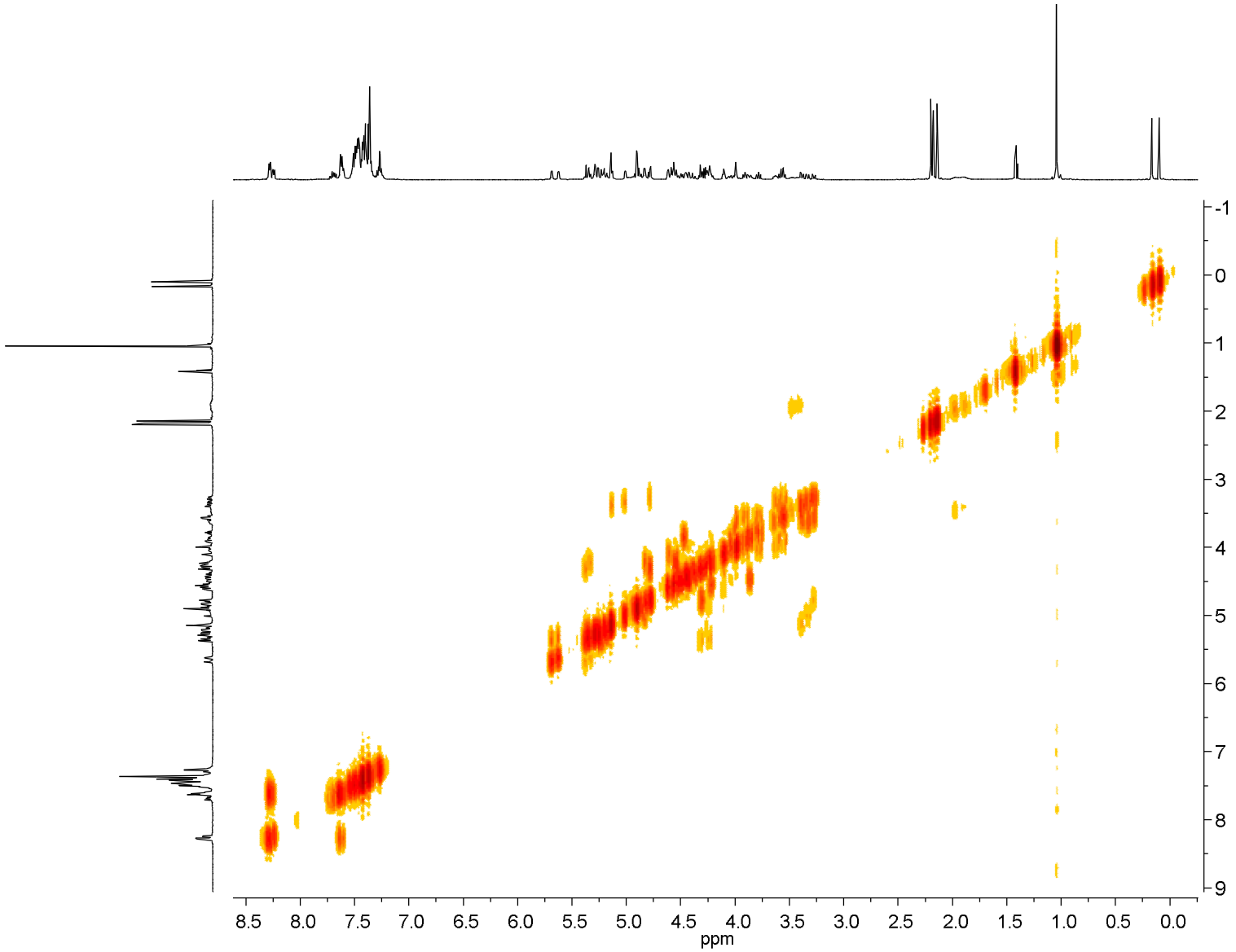
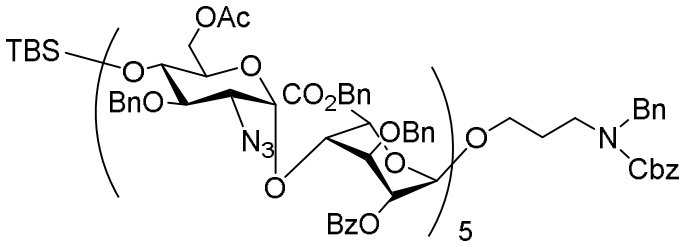


Figure 4.133. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **42**



43

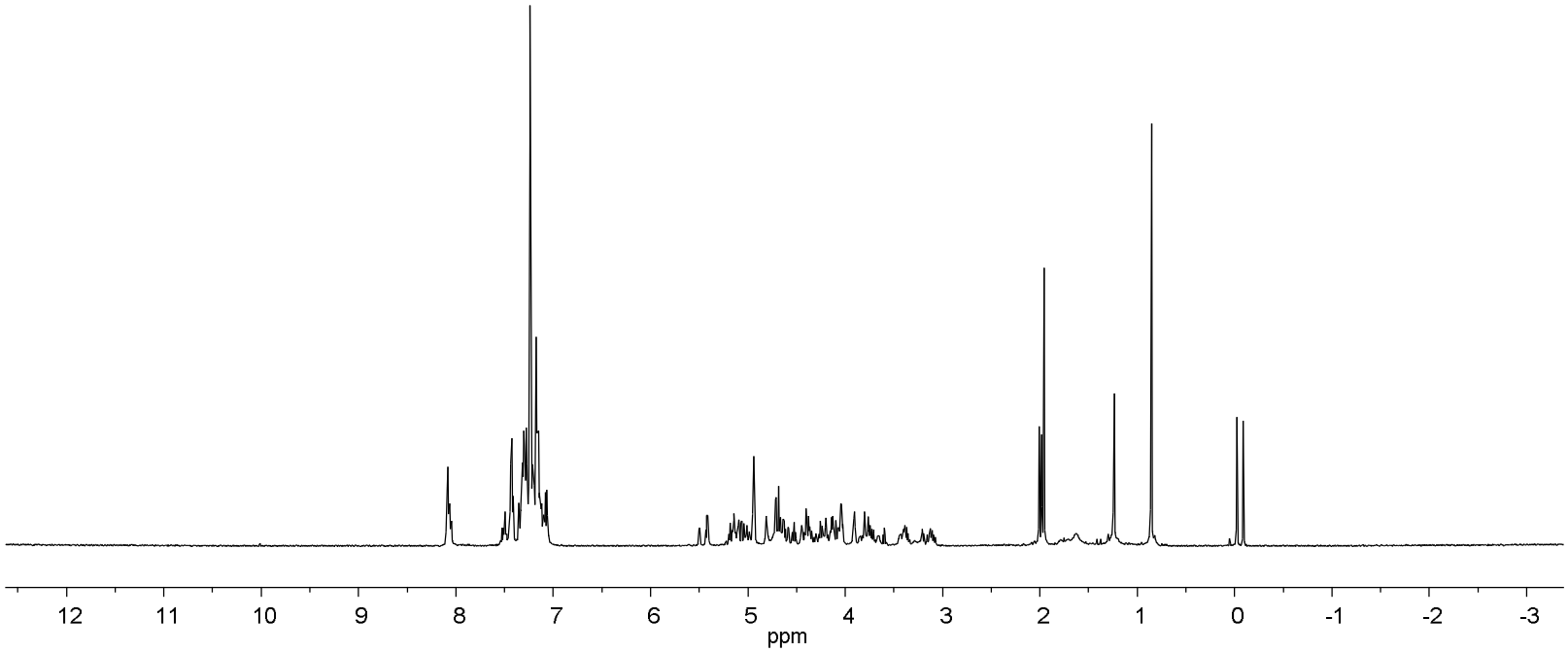


Figure 4.134. 500 MHz, $CDCl_3$, ^1H NMR of **43**

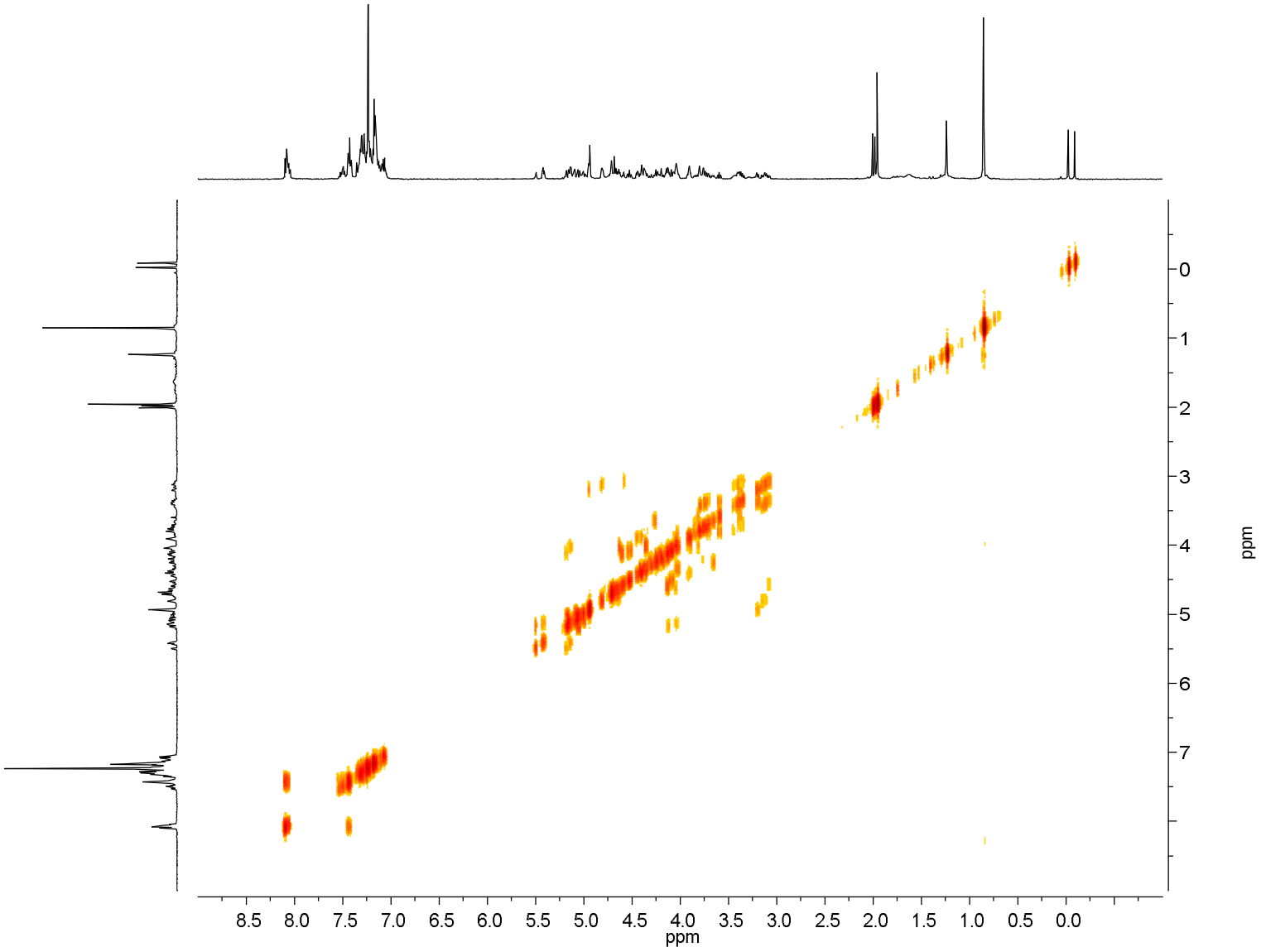
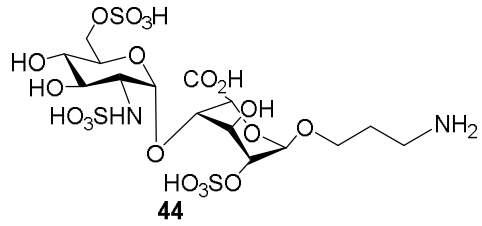


Figure 4.135. 500 MHz, $CDCl_3$, gCOSY of **43**



44

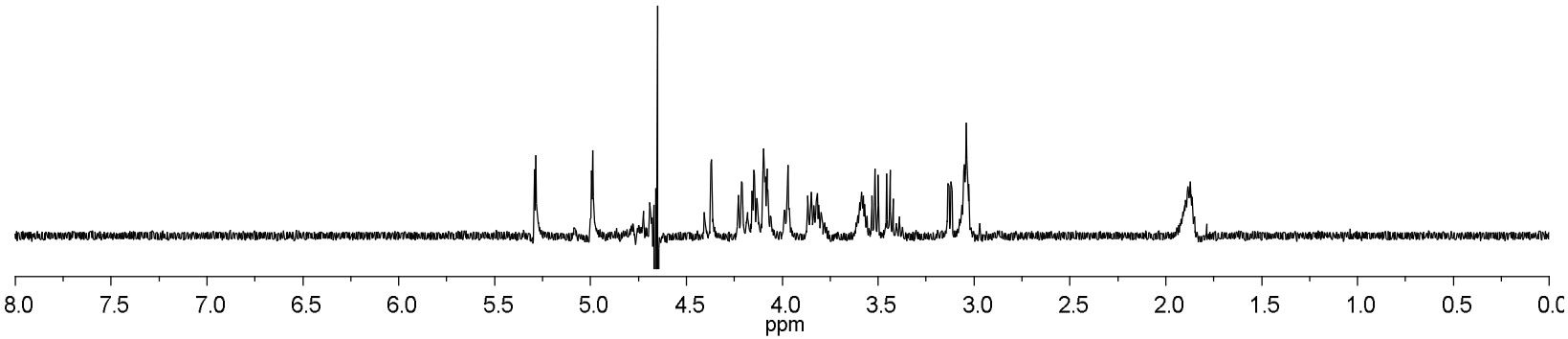


Figure 4.136. 600 MHz, D_2O, ^1H NMR of **44**

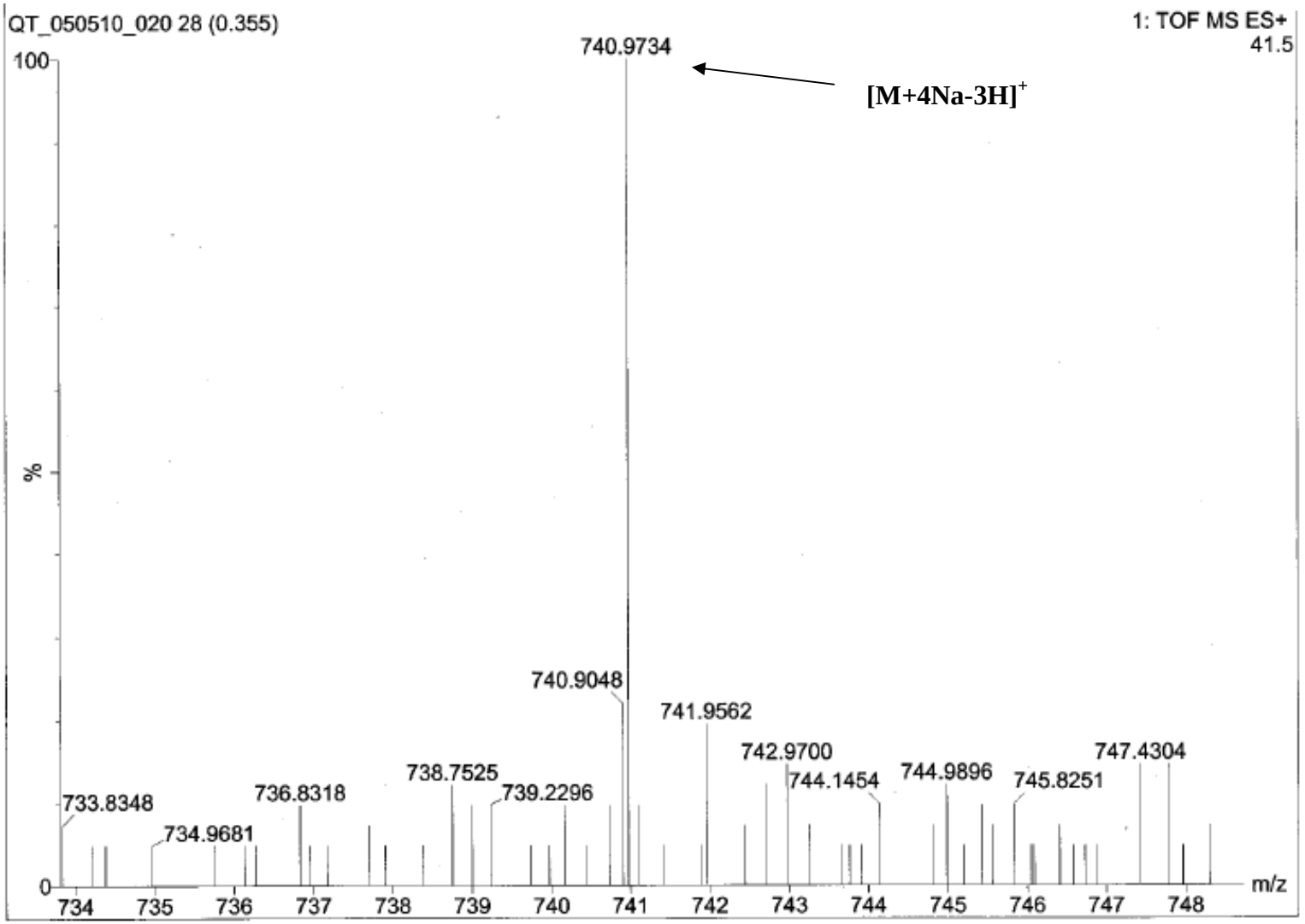


Figure 4.137. ESI-MS of **44**

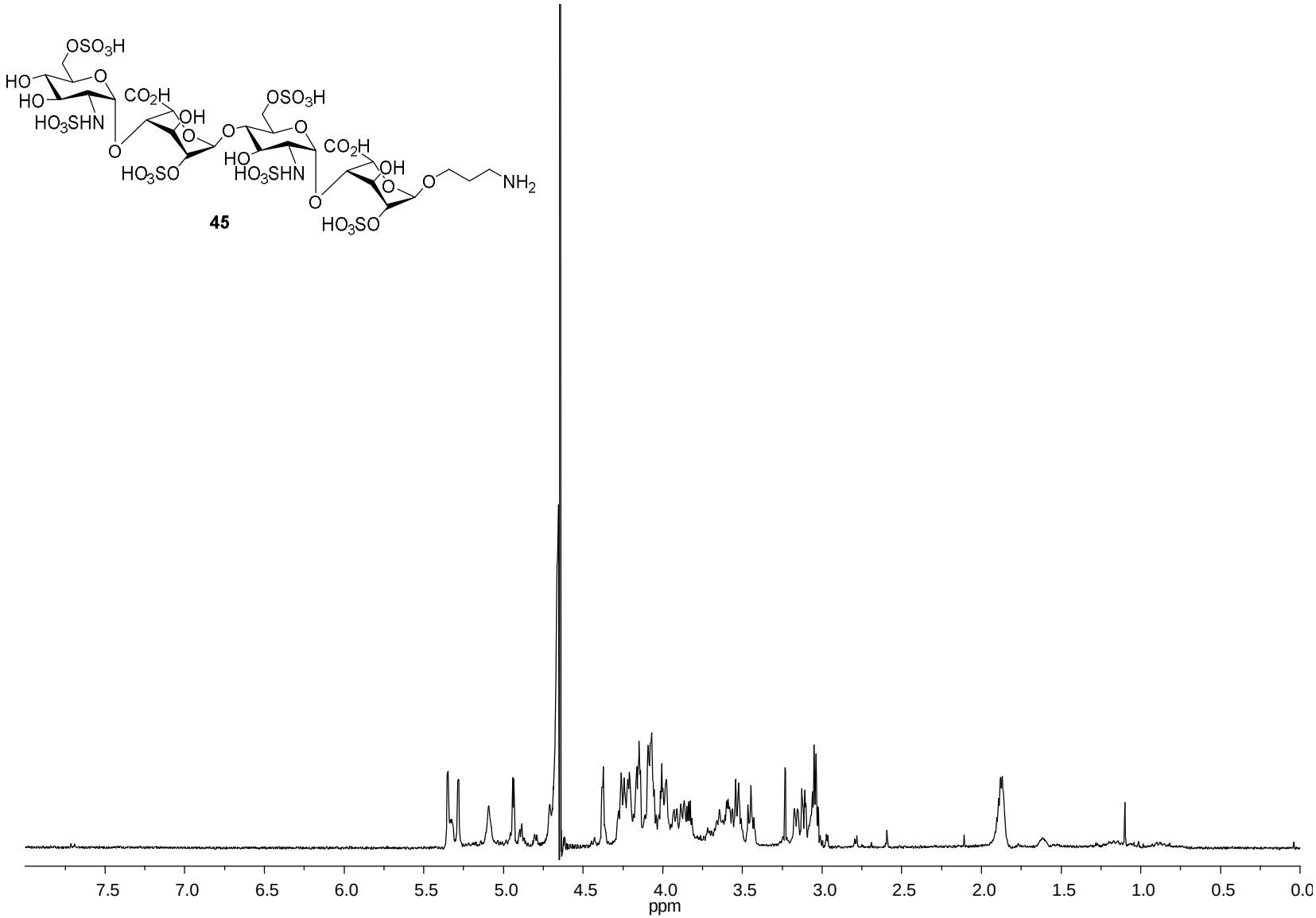


Figure 4.138. 500 MHz, D_2O, ^1H NMR of **45**

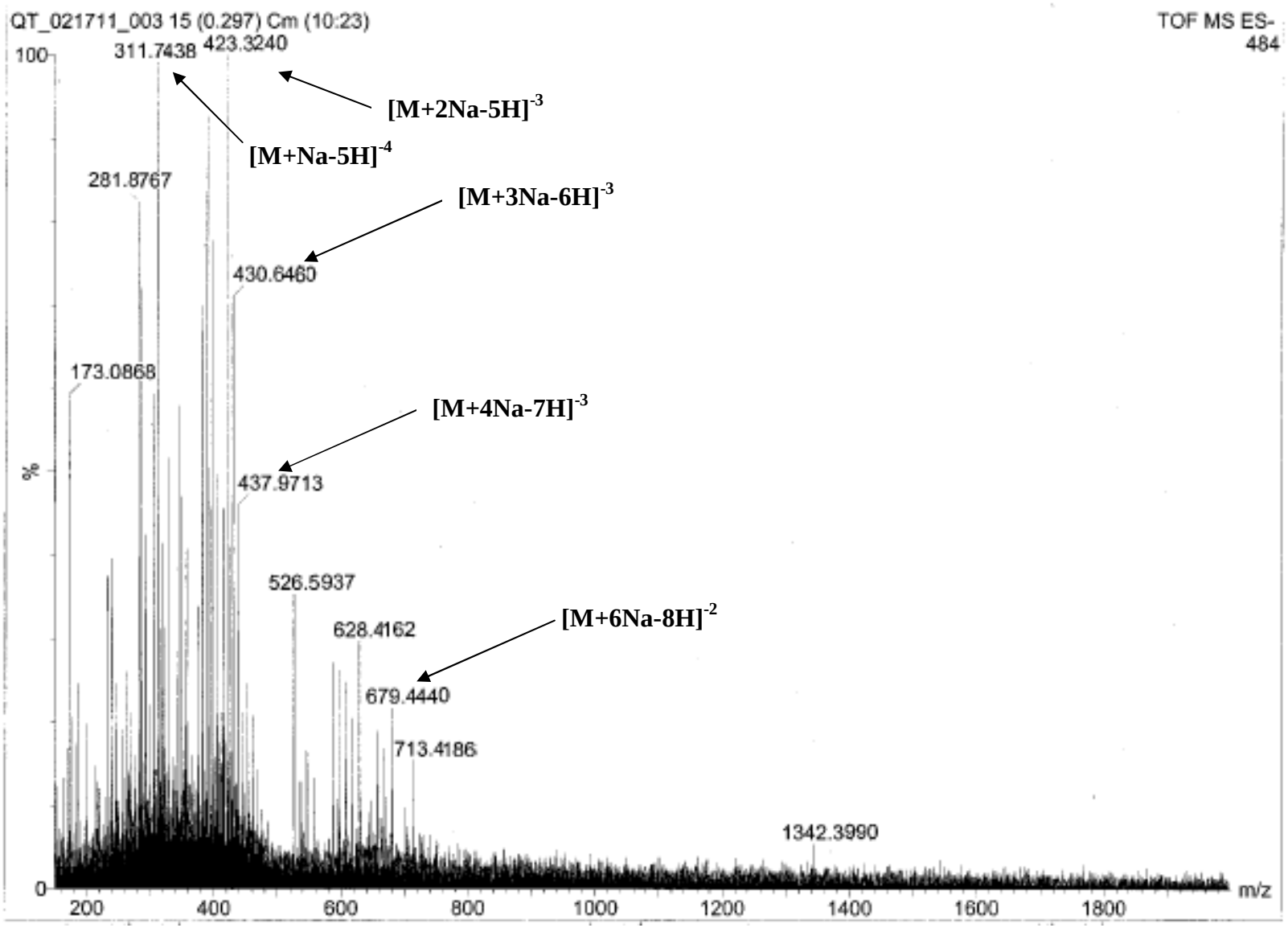


Figure 4.139. ESI-MS of **45**

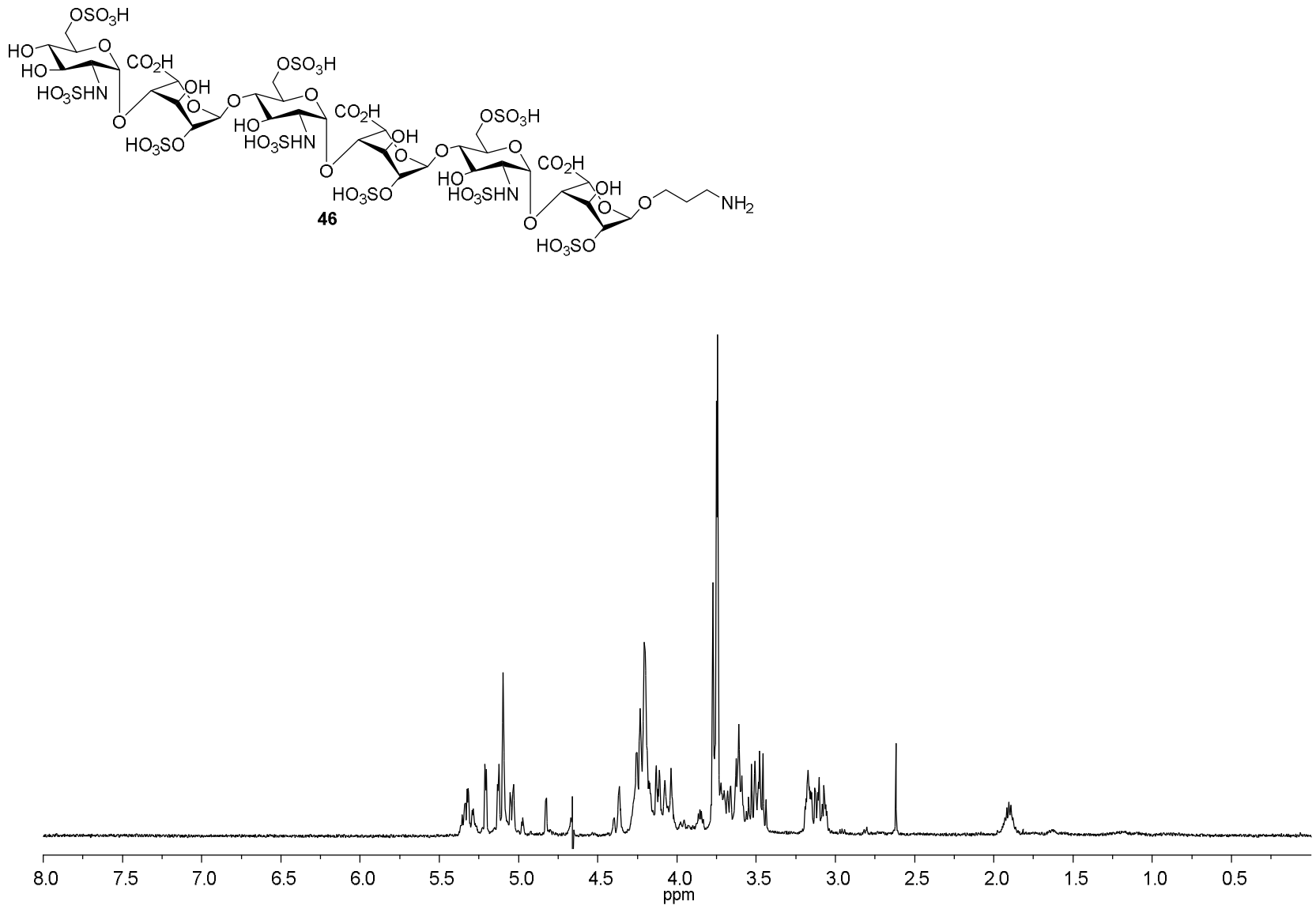


Figure 4.140. 500 MHz, D_2O, ^1H NMR of **46**

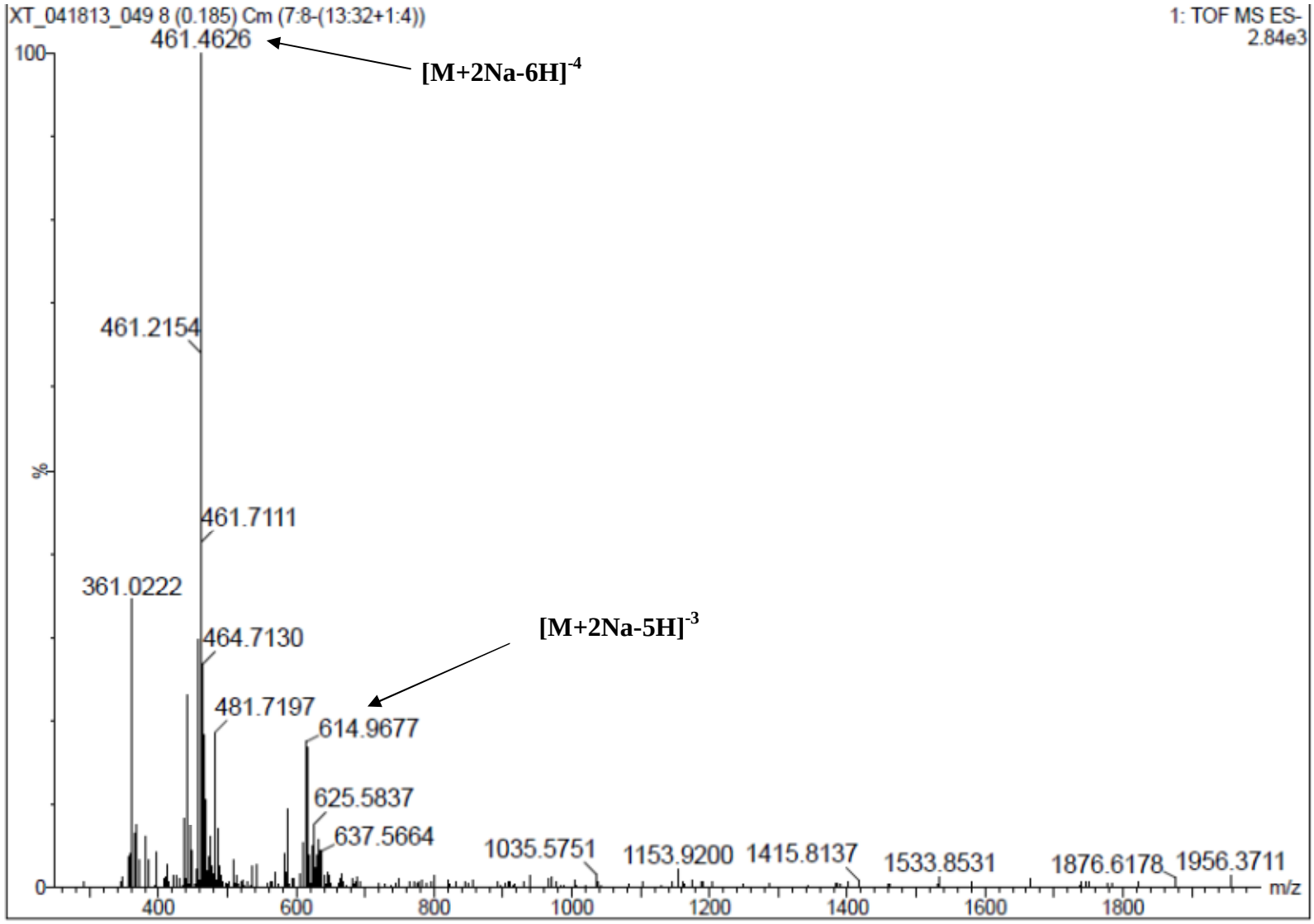


Figure 4.141. ESI-MS of **46**

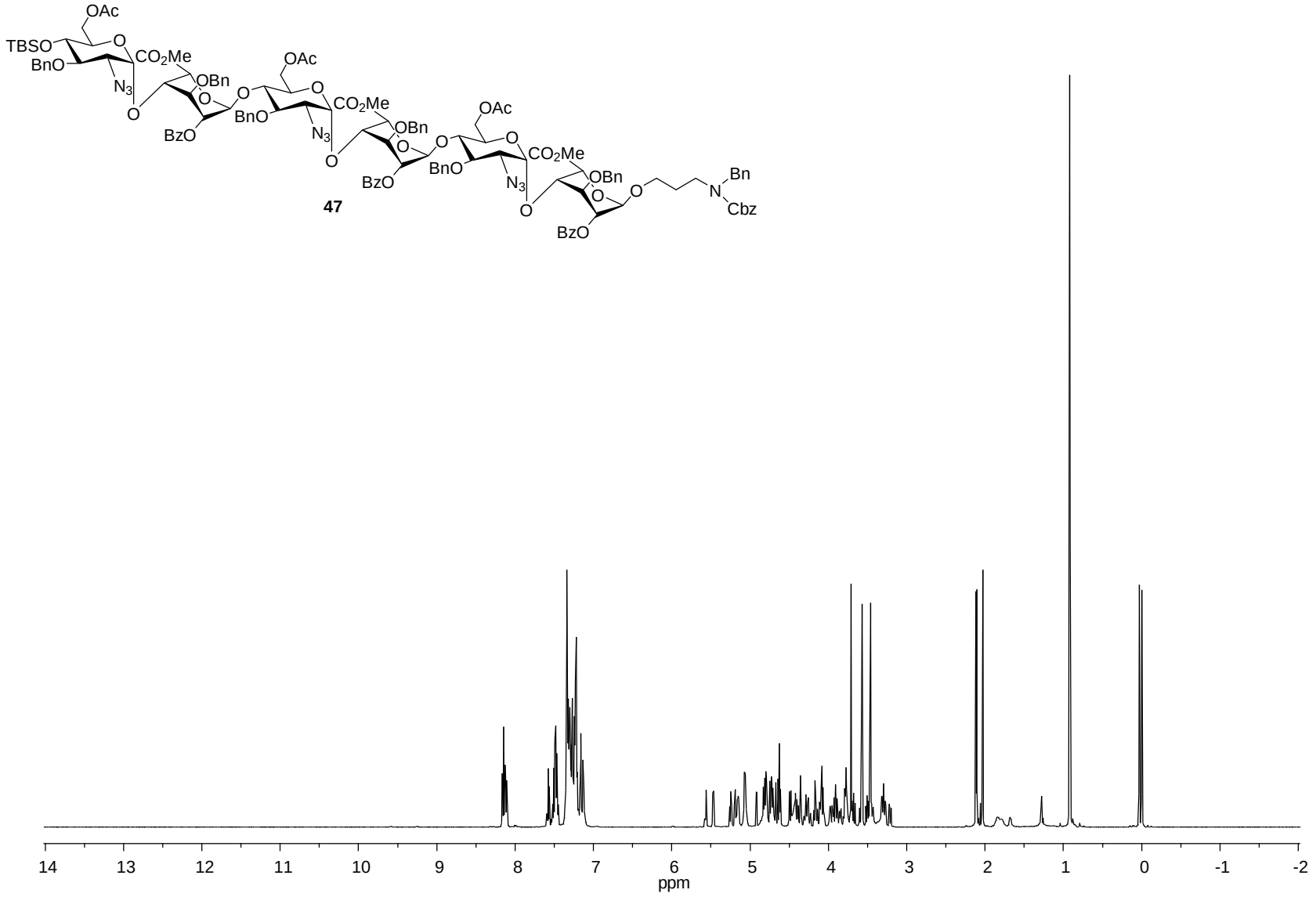


Figure 4.142. 500 MHz, $CDCl_3$, ^1H NMR of **47**

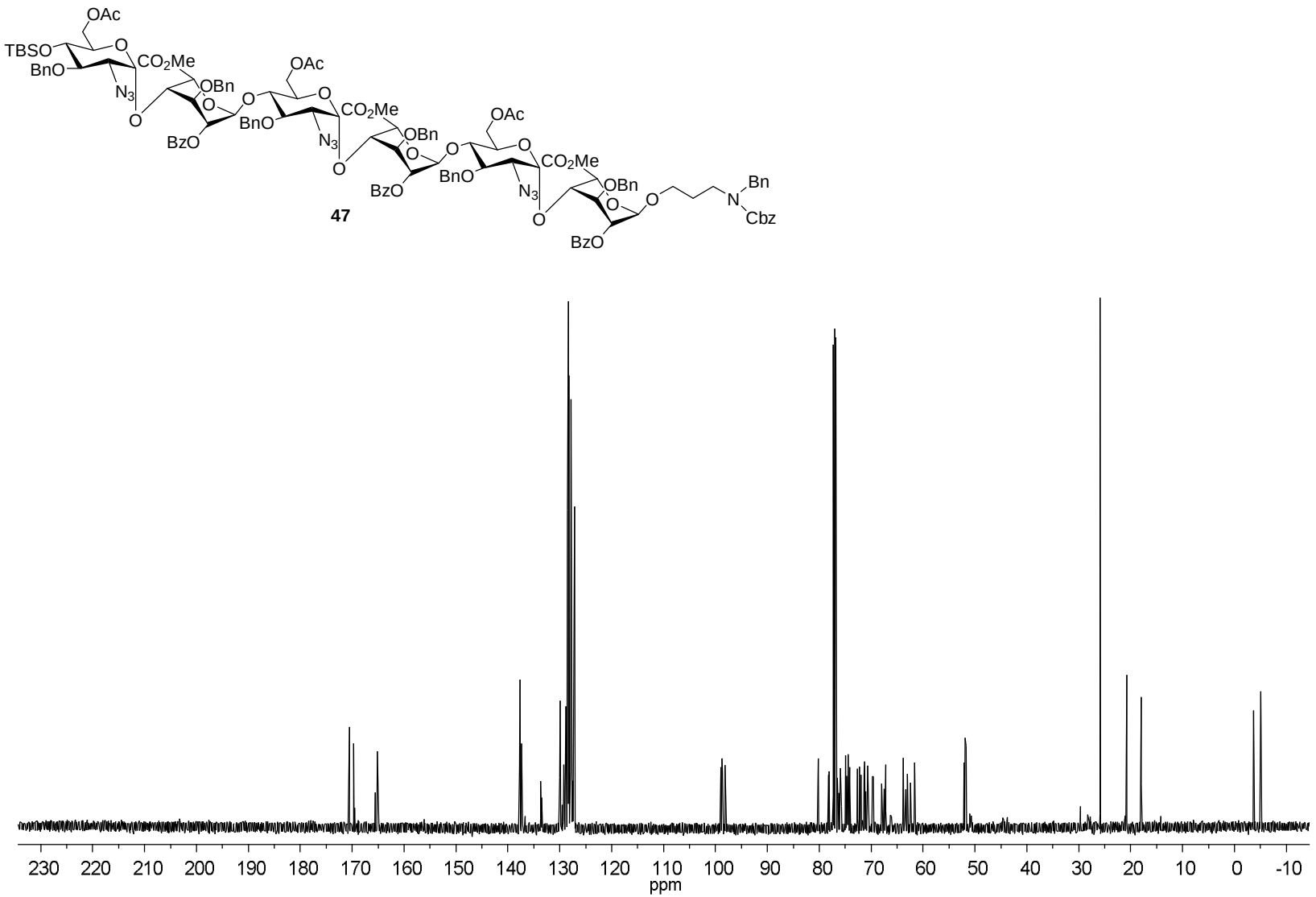


Figure 4.143. 125 MHz, $CDCl_3$, ^{13}C NMR of **47**

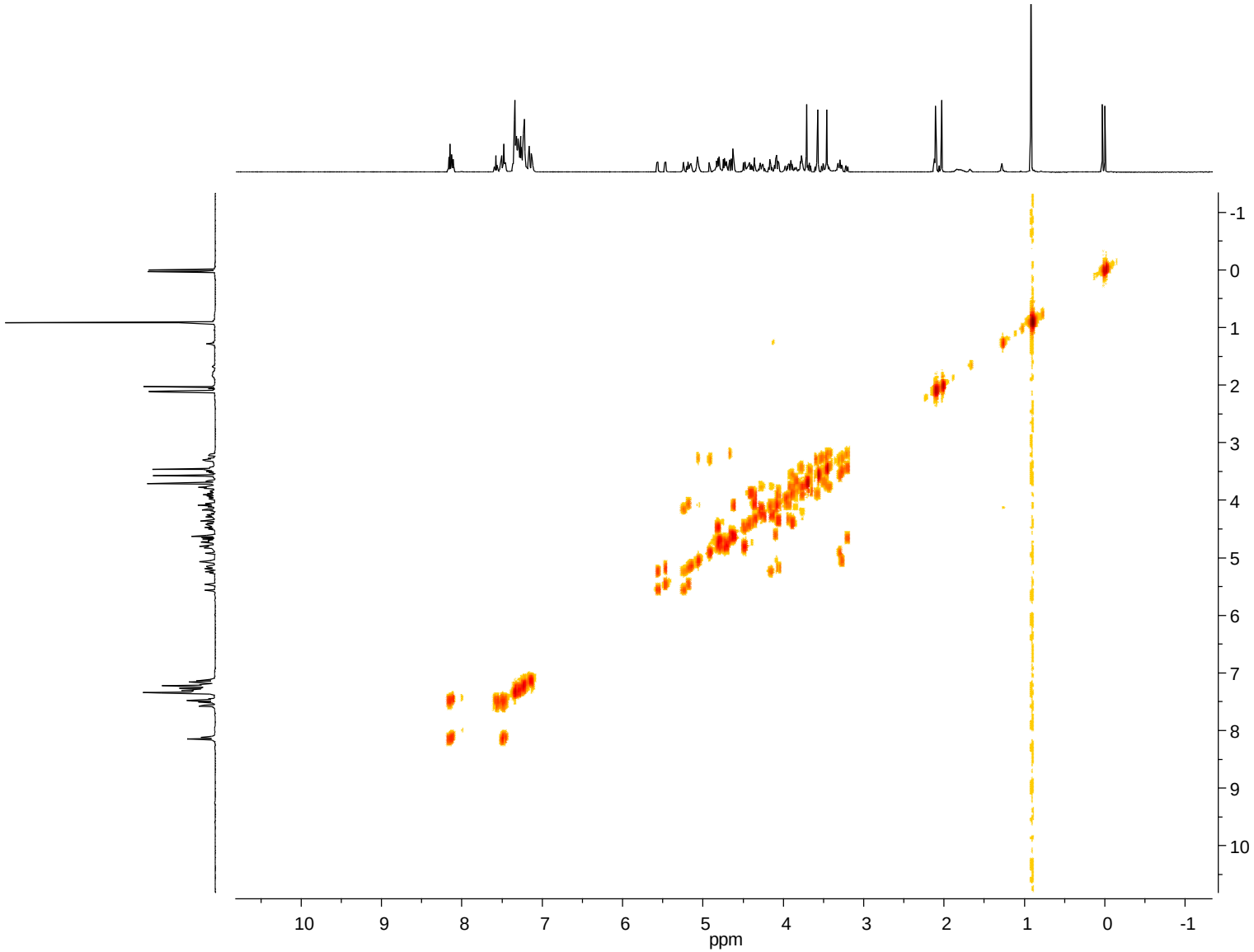


Figure 4.144. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **47**

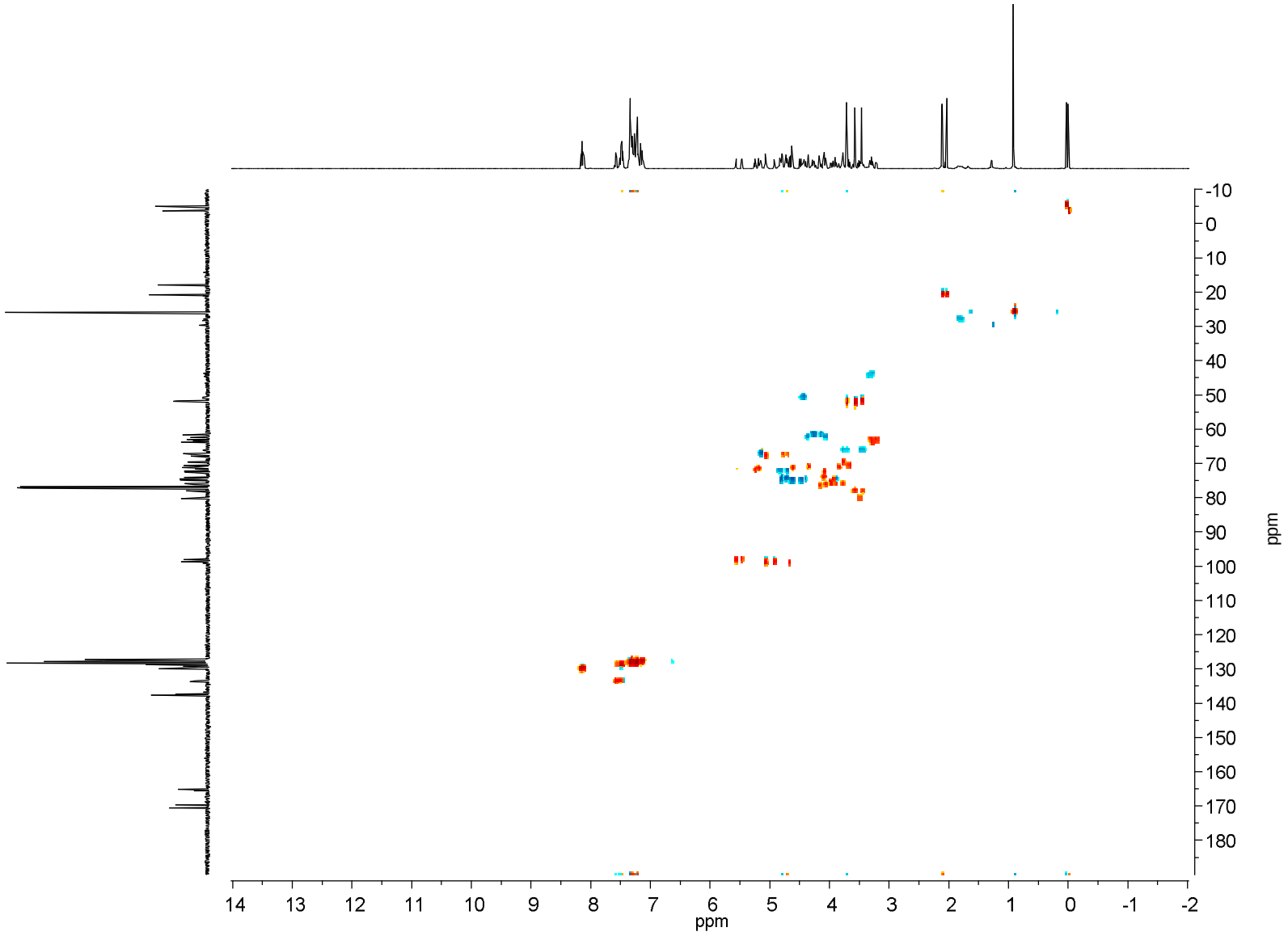


Figure 4.145. 500 MHz, $CDCl_3$, gHSQCAD of **47**

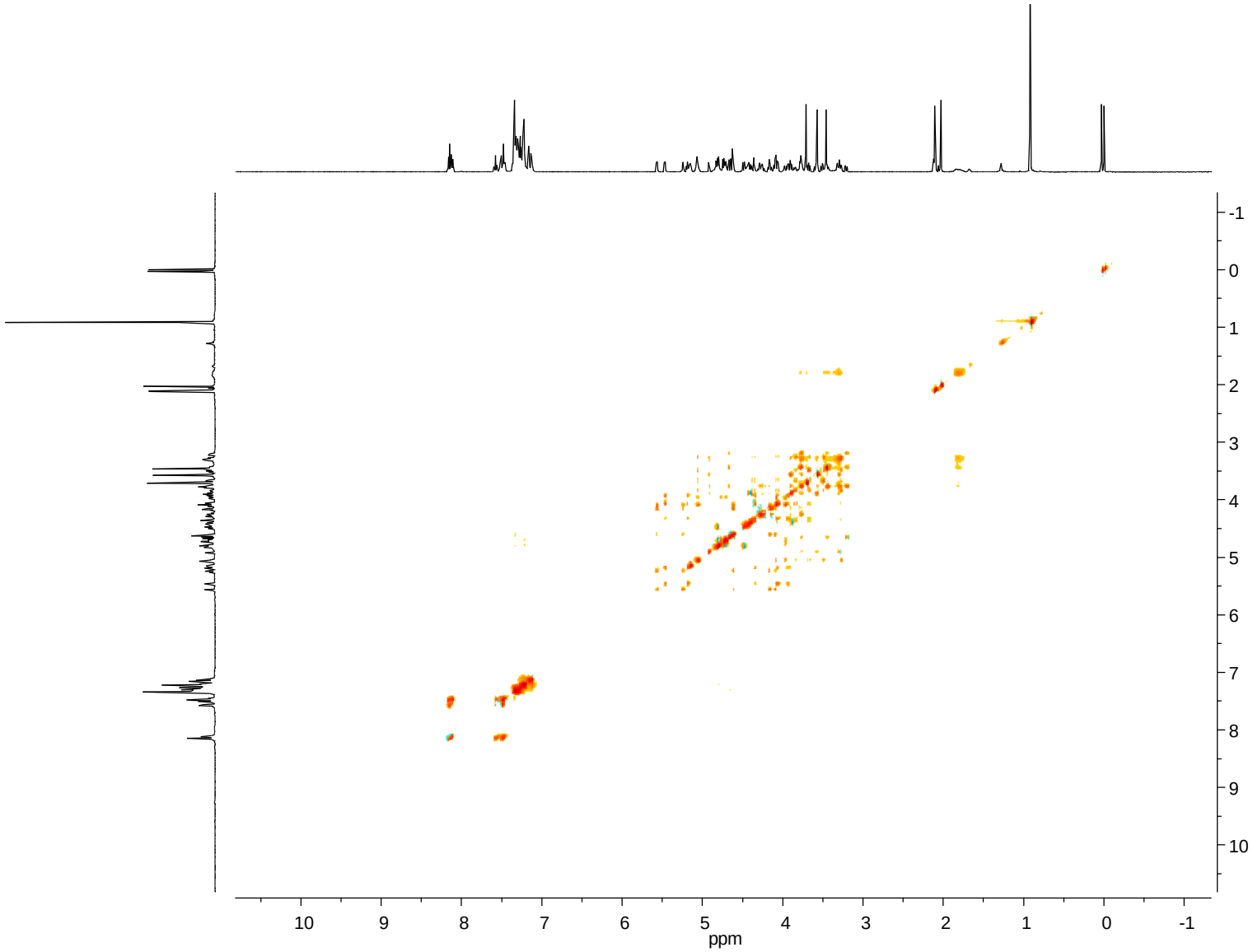


Figure 4.146. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **47**

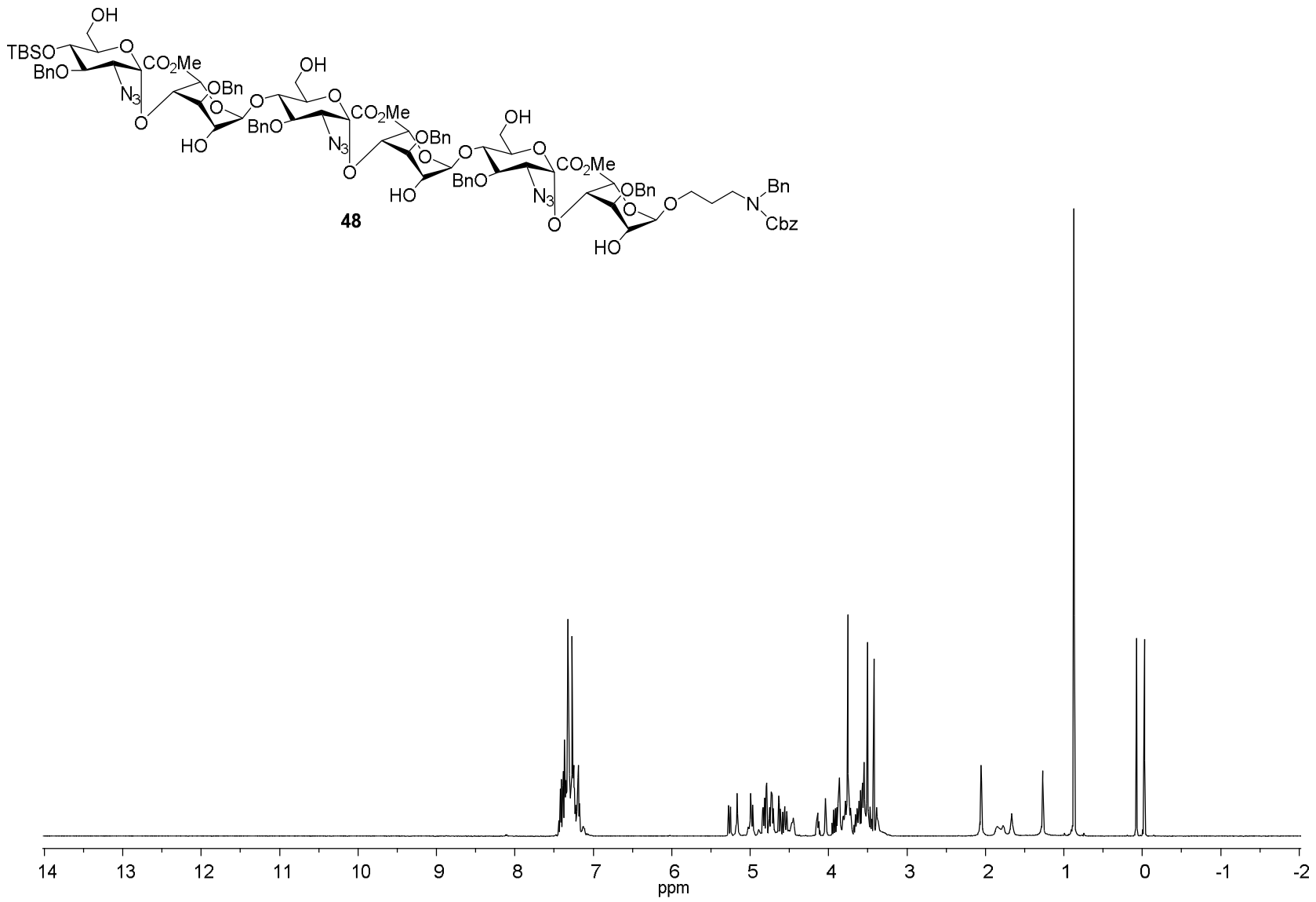


Figure 4.147. 500 MHz, $CDCl_3$, ^1H NMR of **48**

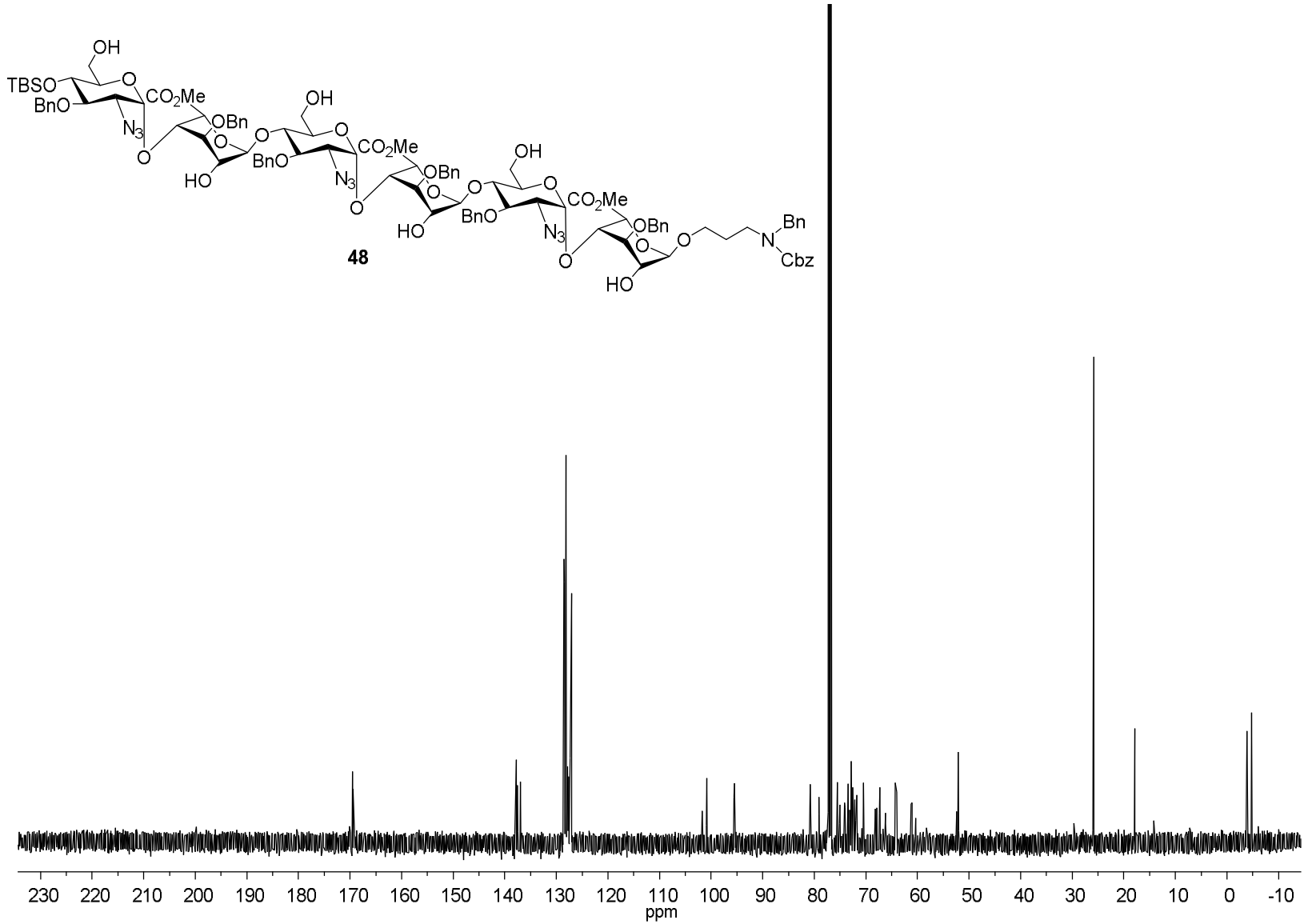


Figure 4.148. 125 MHz, $CDCl_3$, ^{13}C NMR of **48**

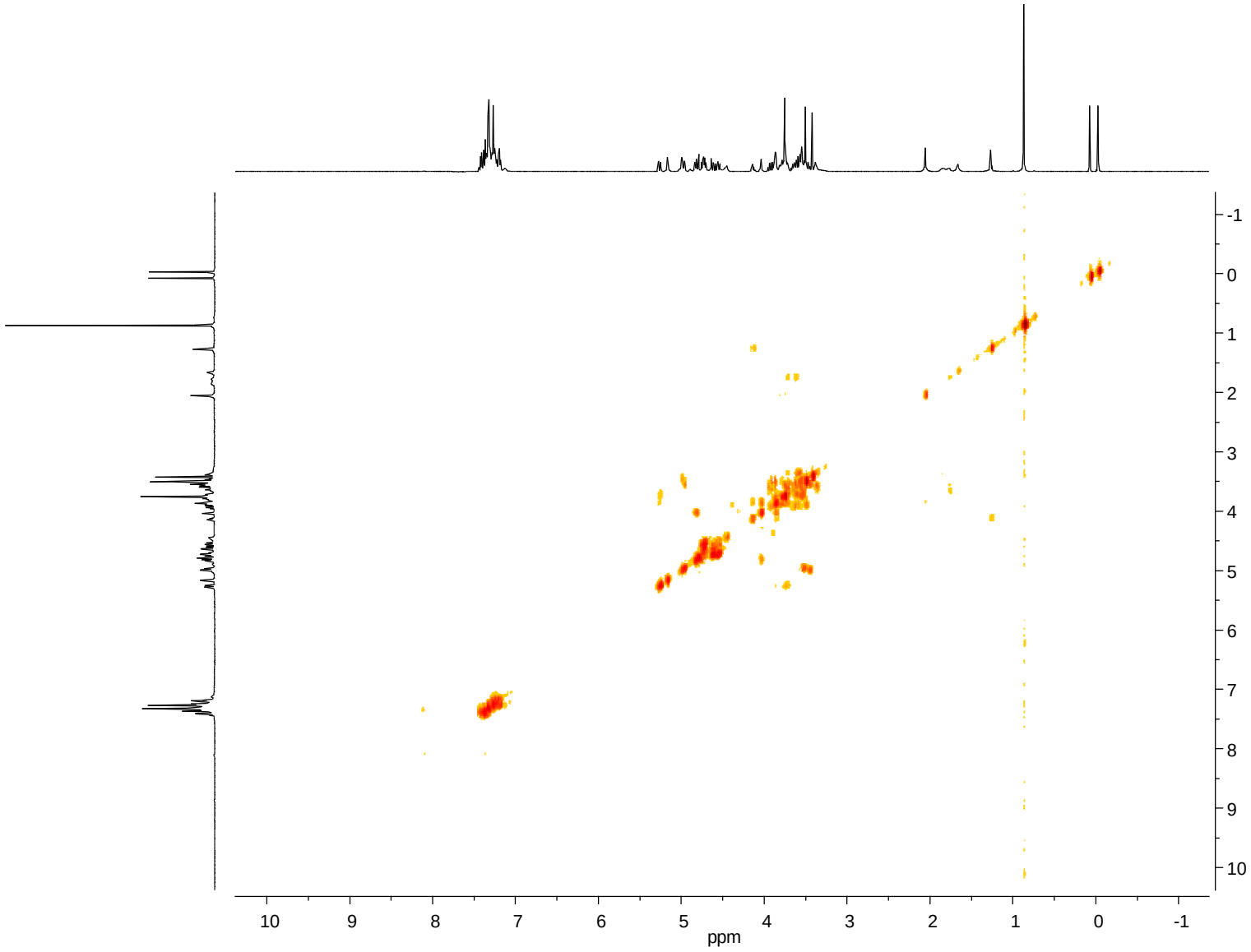


Figure 4.149. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **48**

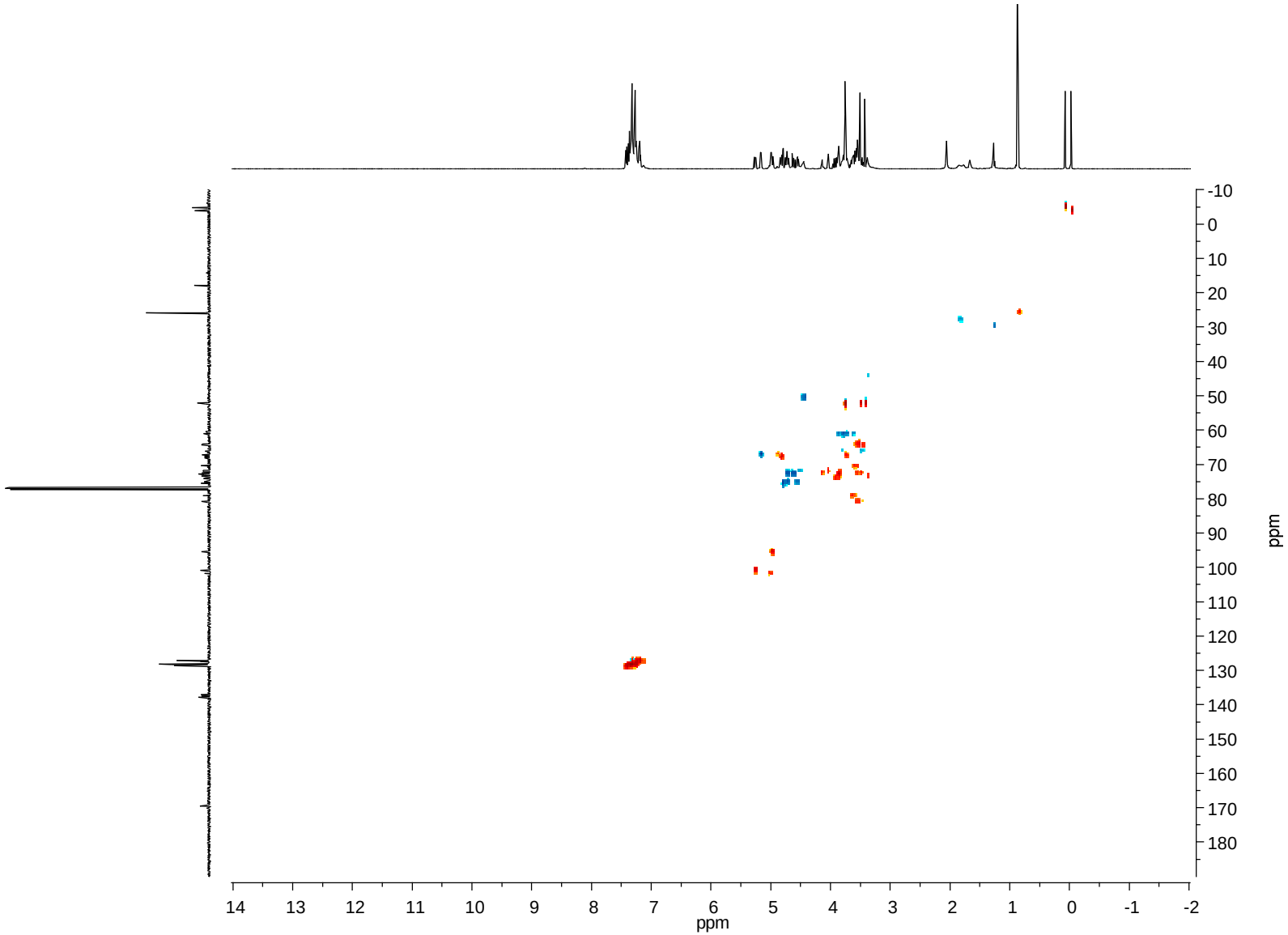


Figure 4.150. 500 MHz, $CDCl_3$, gHSQCAD of **48**

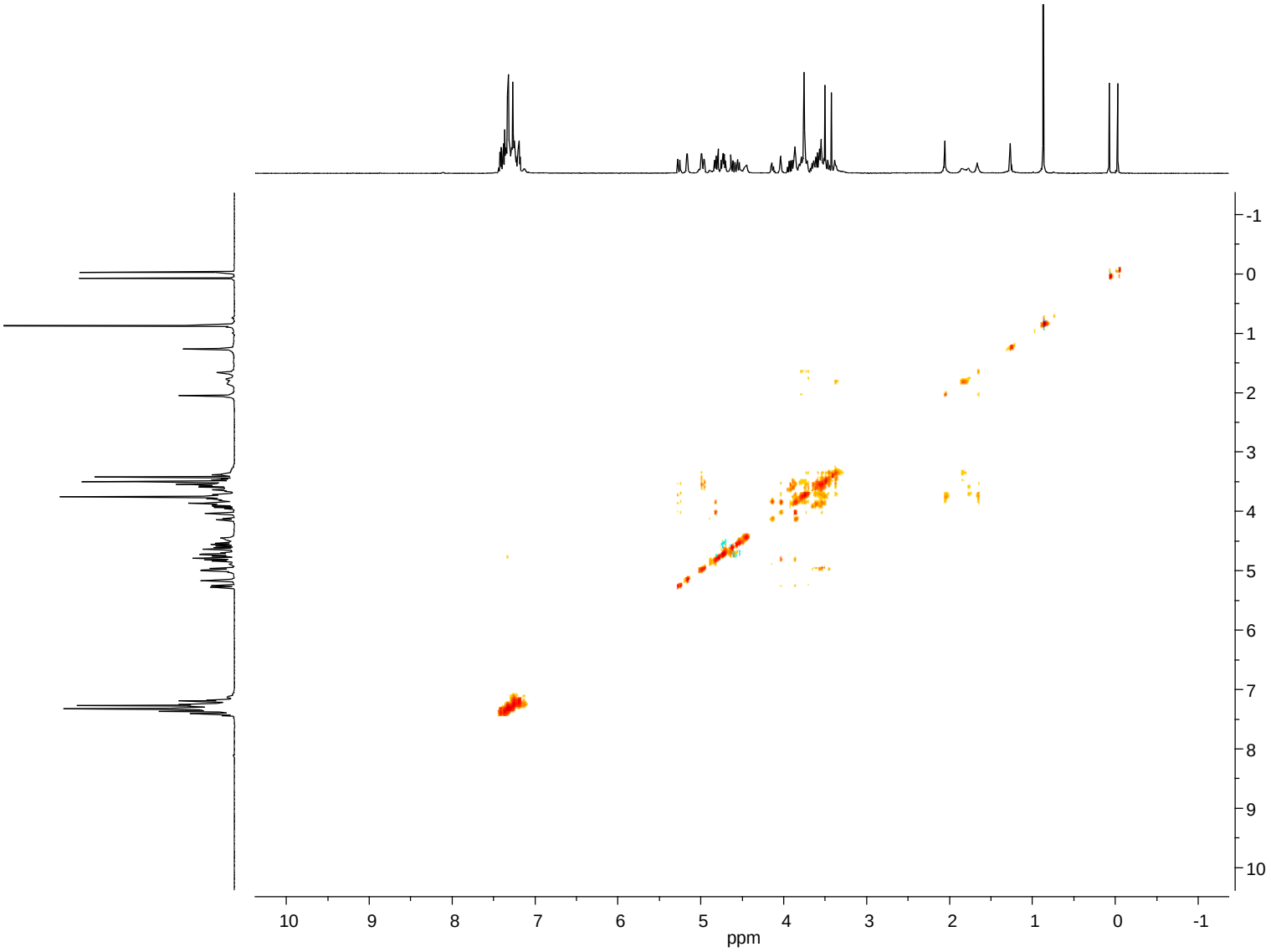


Figure 4.151. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **48**

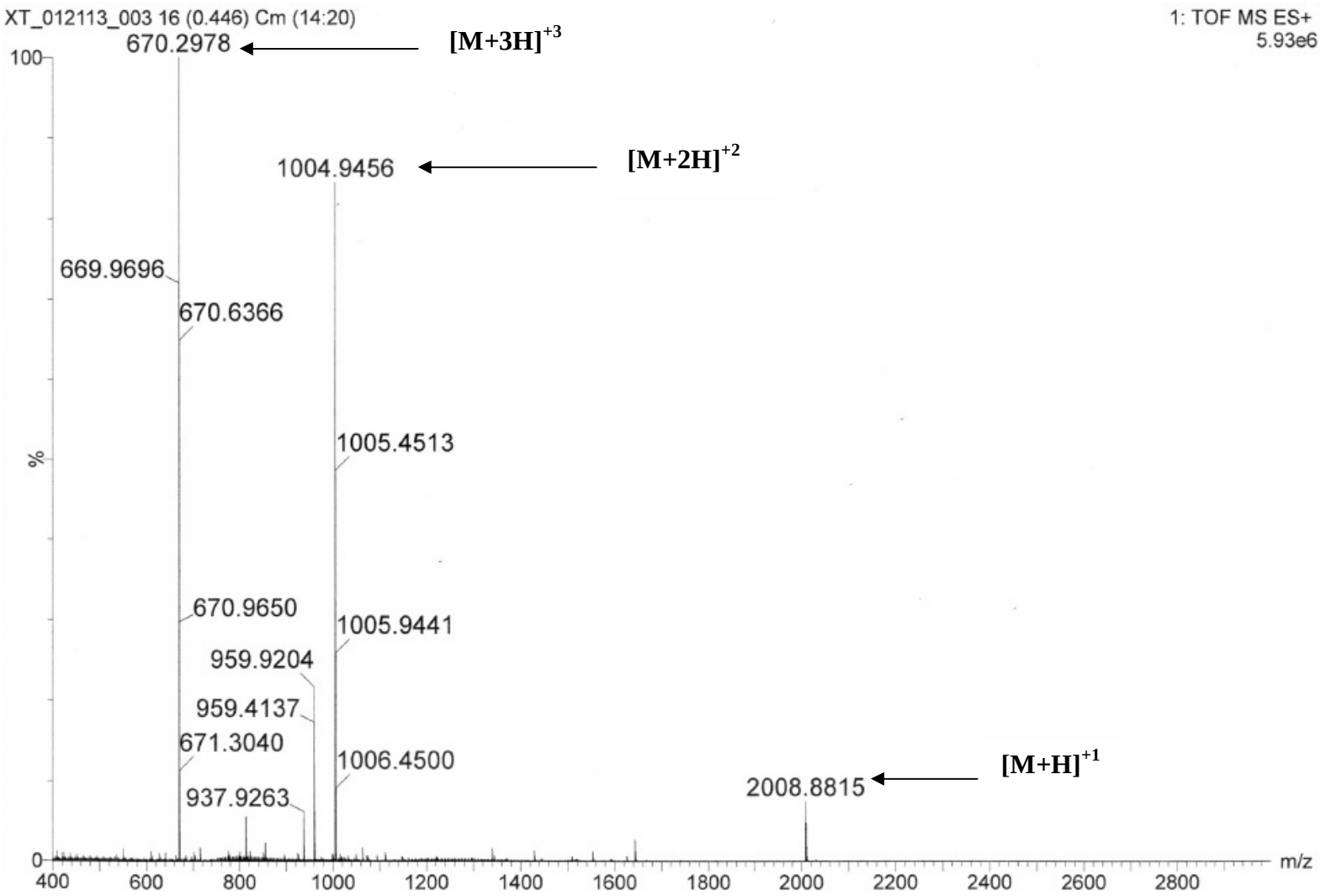


Figure 4.152. ESI-MS of **50**

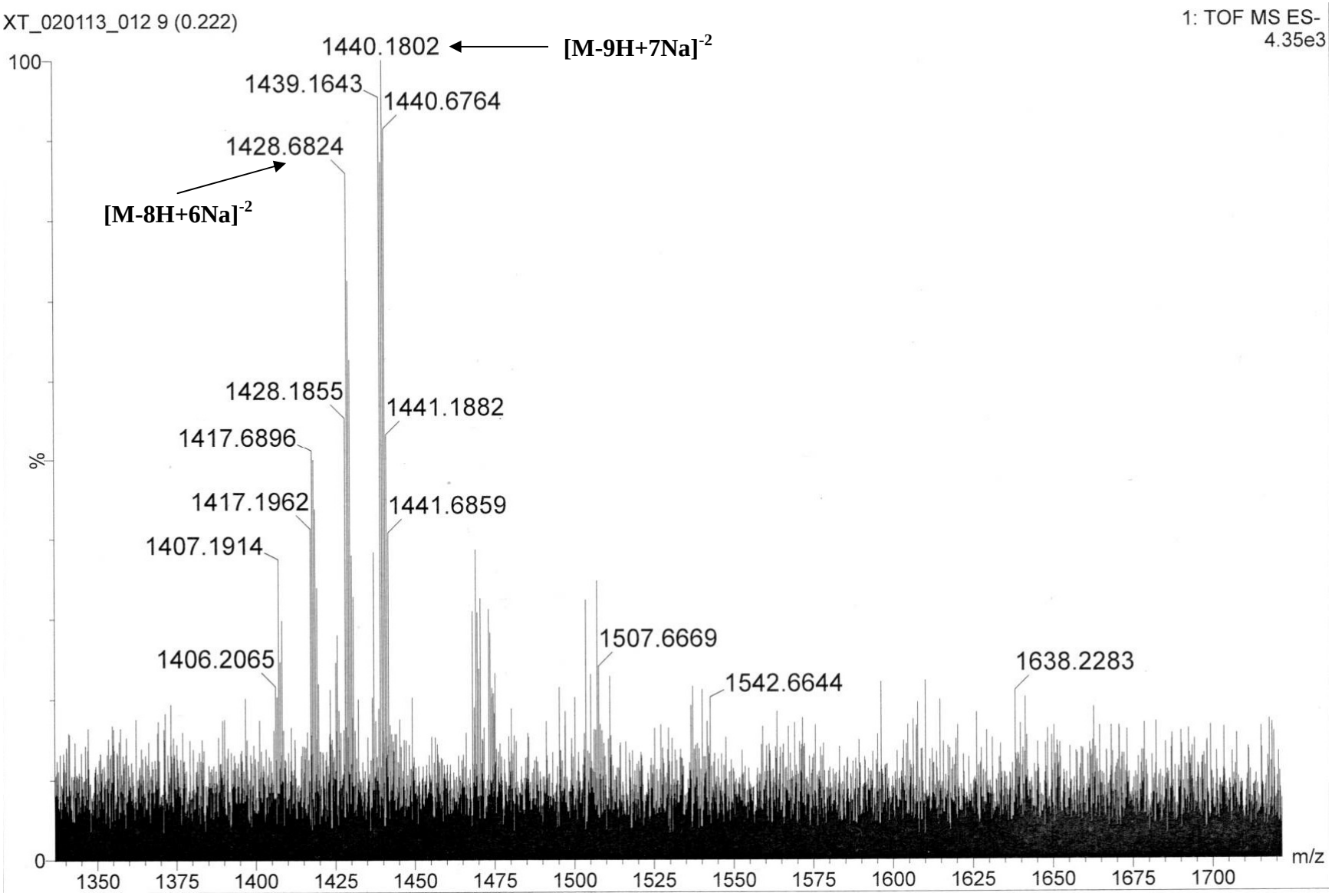


Figure 4.153. ESI-MS of **51**

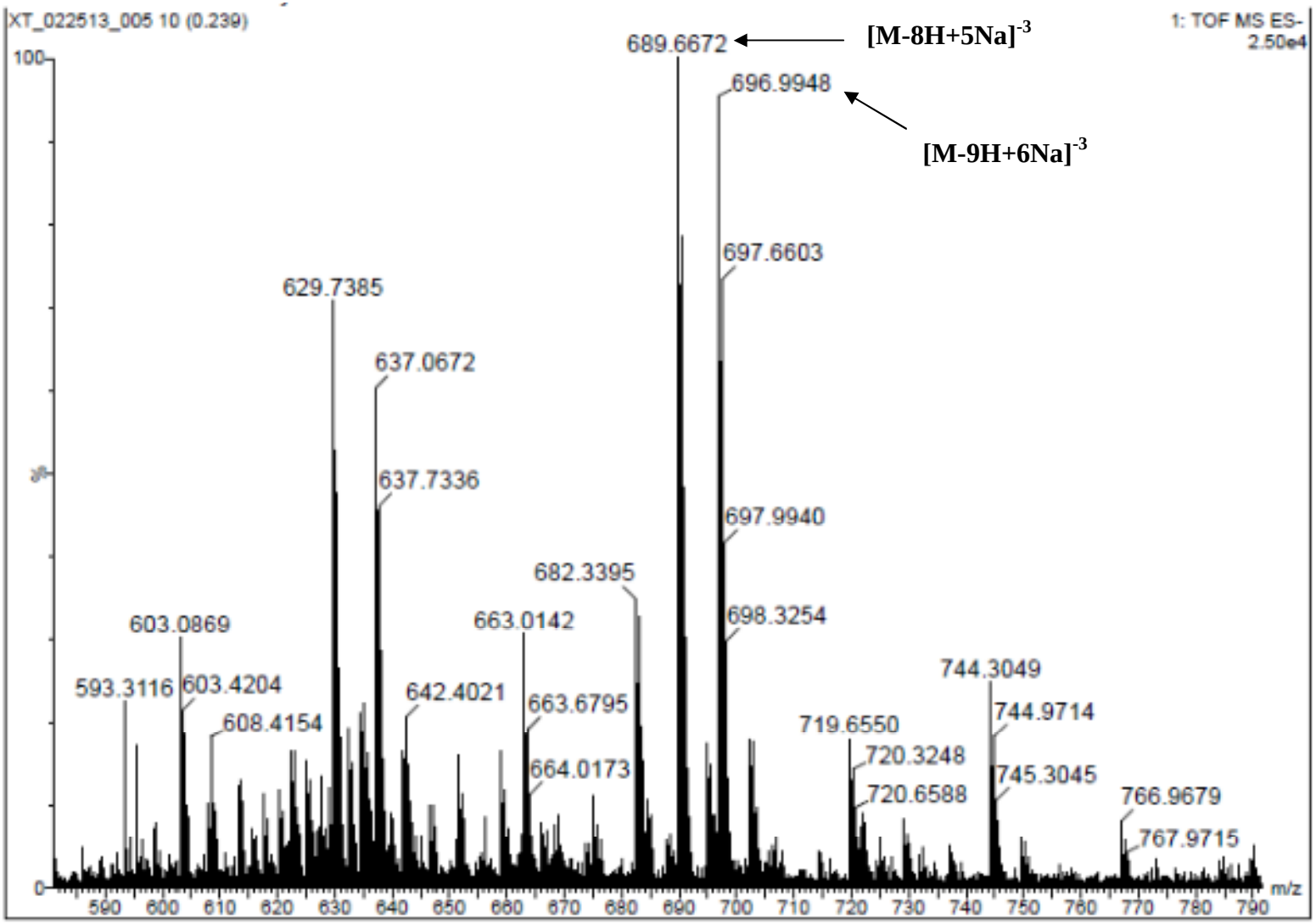


Figure 4.154. ESI-MS of **52**

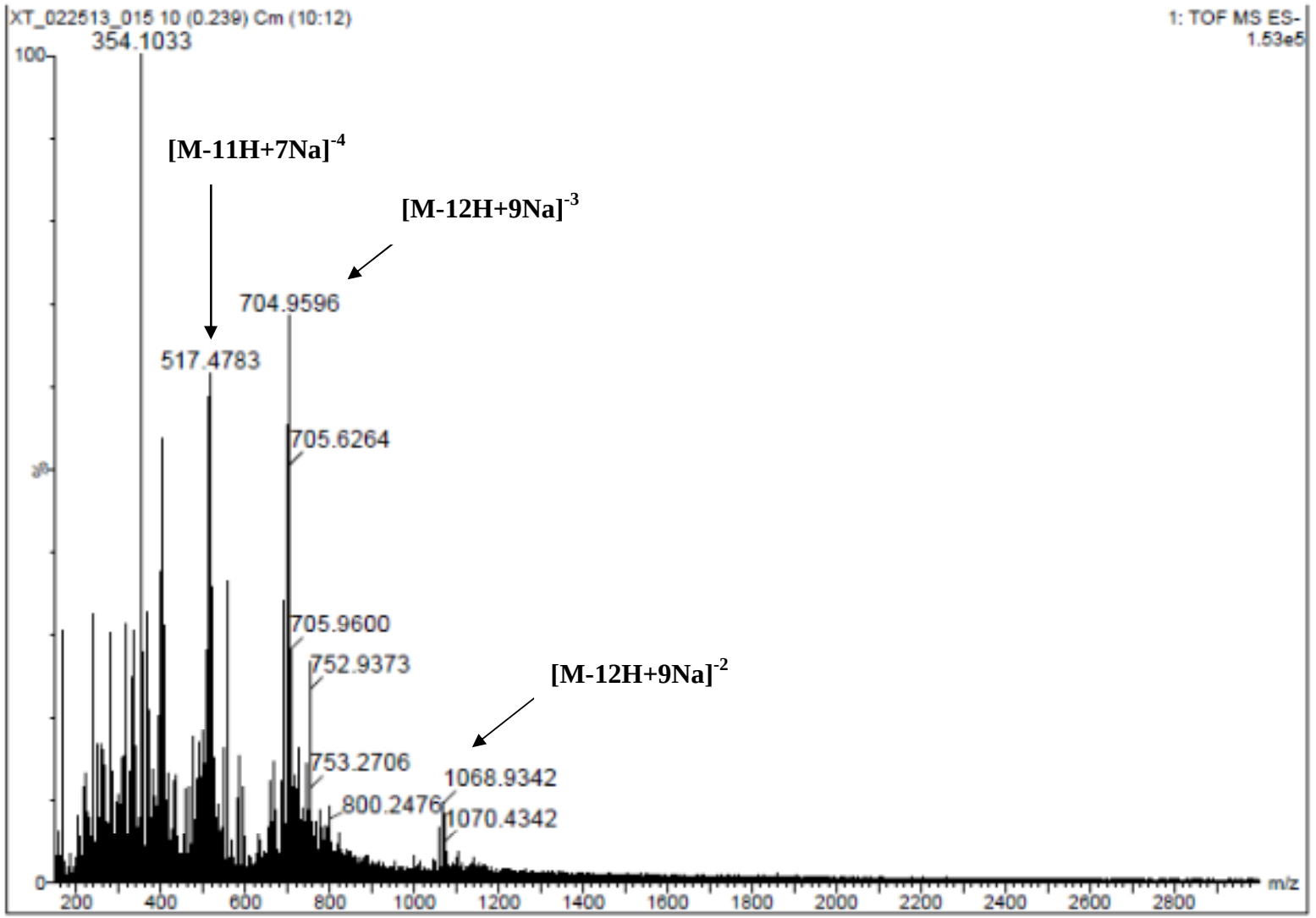


Figure 4.155. ESI-MS of **53**

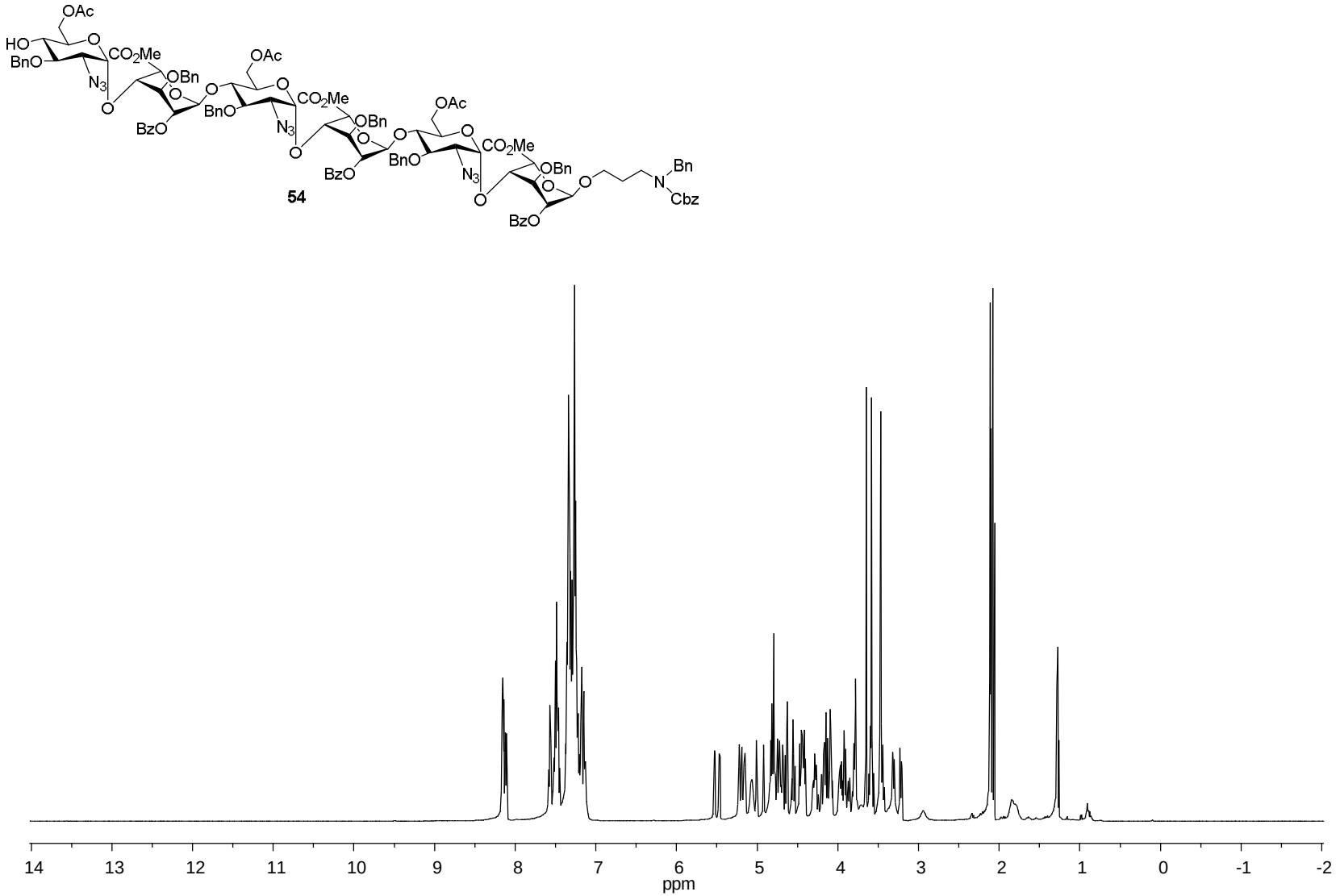


Figure 4.156. 500 MHz, $CDCl_3$, ^1H NMR of **54**

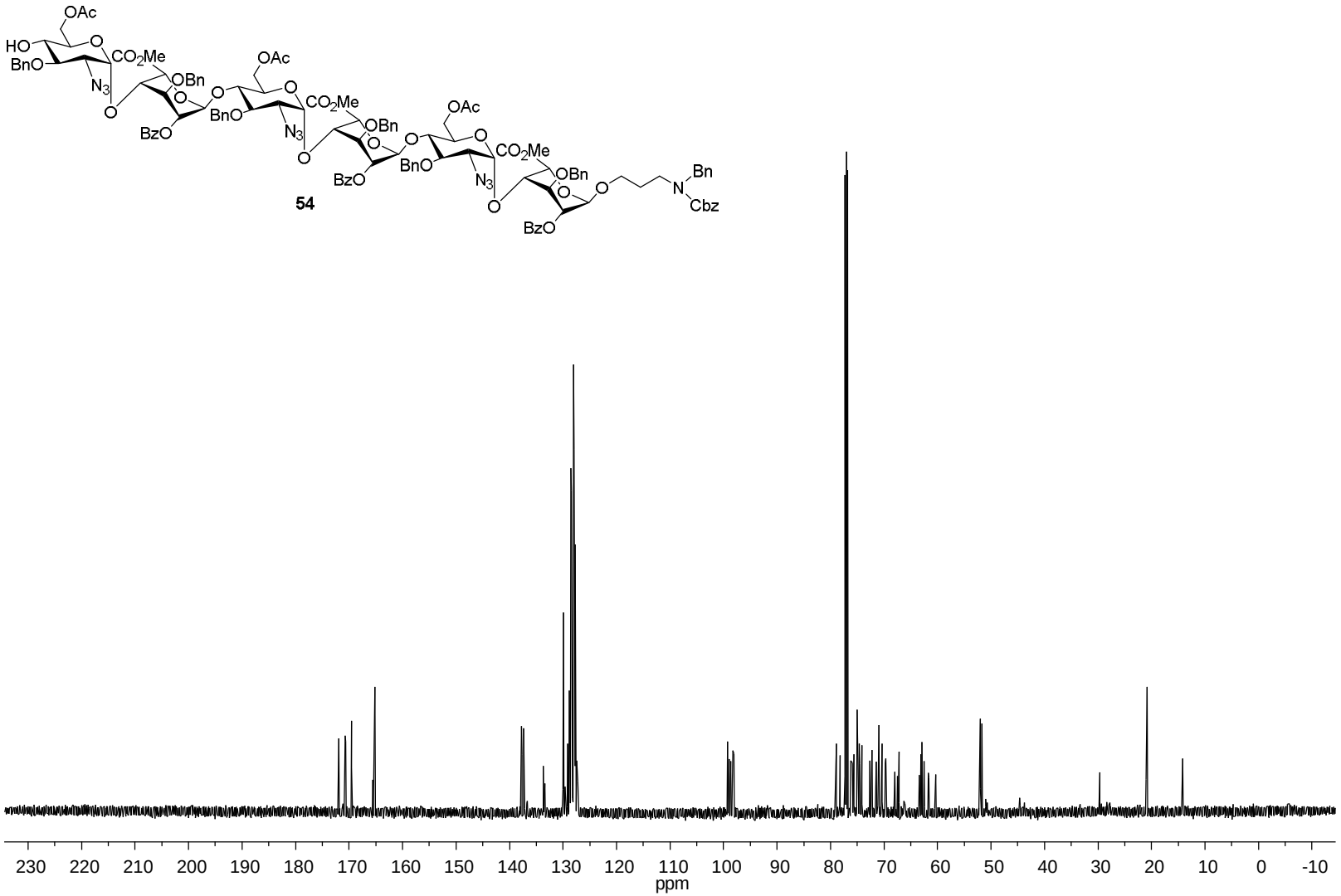


Figure 4.157. 125 MHz, $CDCl_3$, ^{13}C NMR of **54**

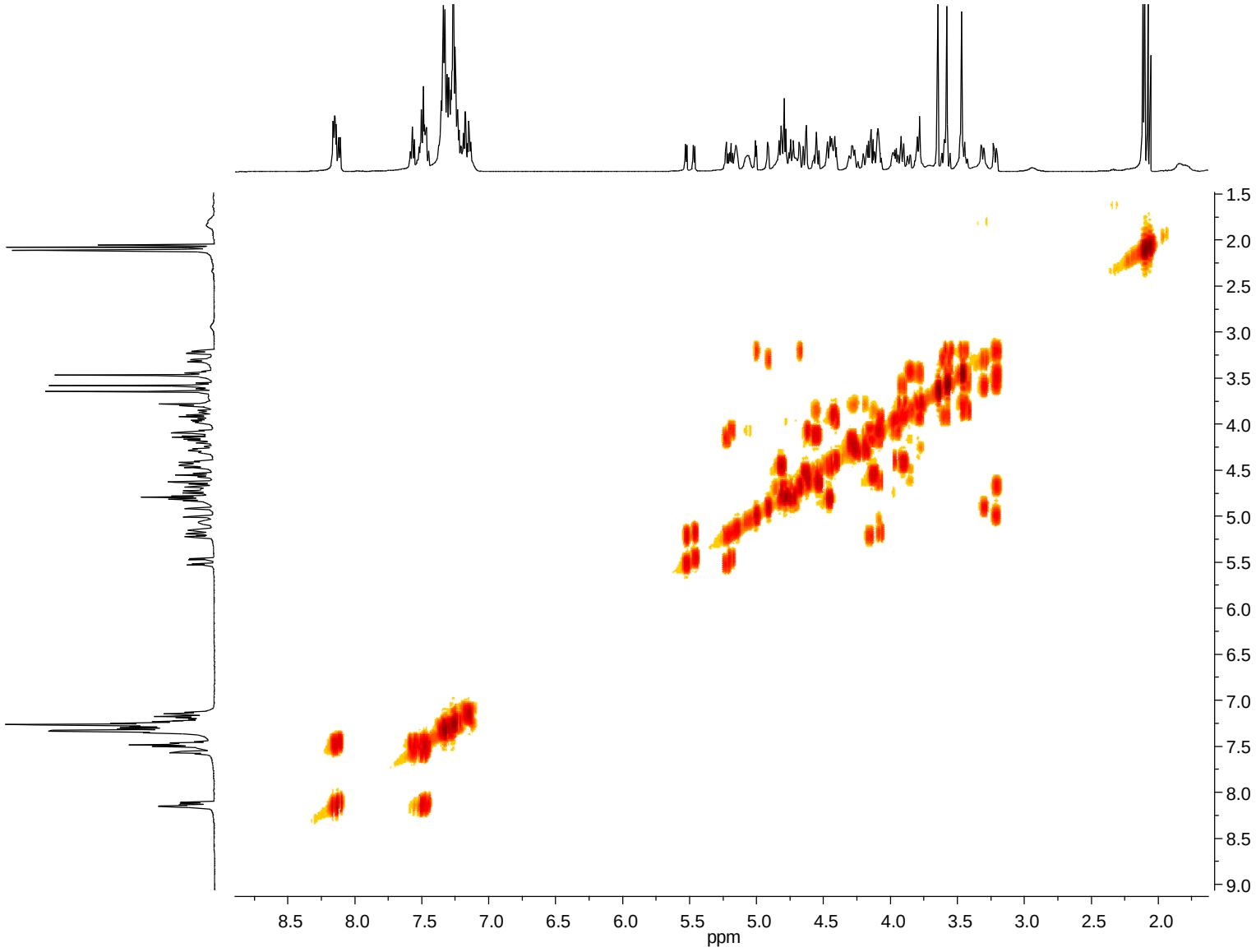


Figure 4.158. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **54**

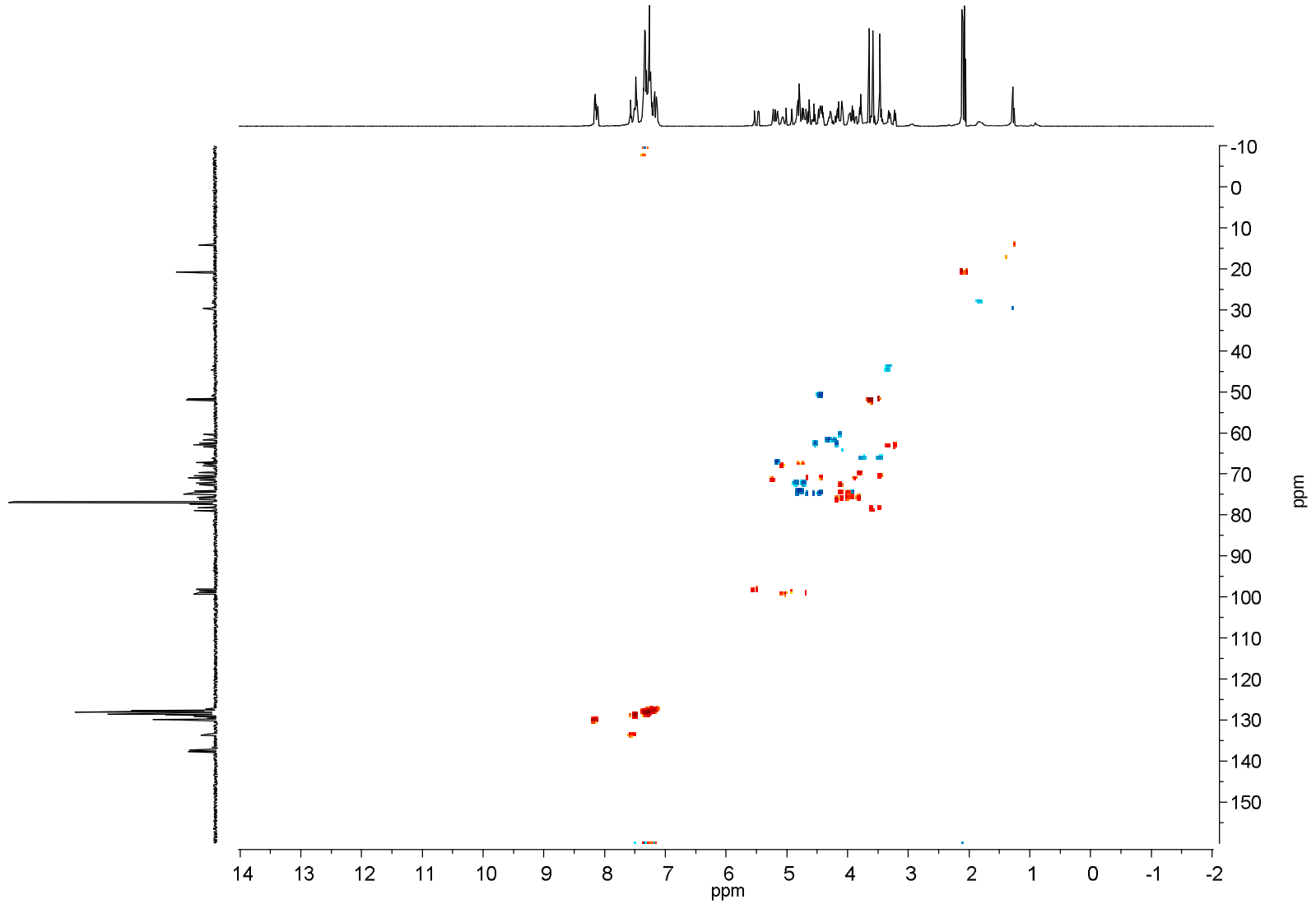


Figure 4.159. 500 MHz, $CDCl_3$, gHSQCAD of **54**

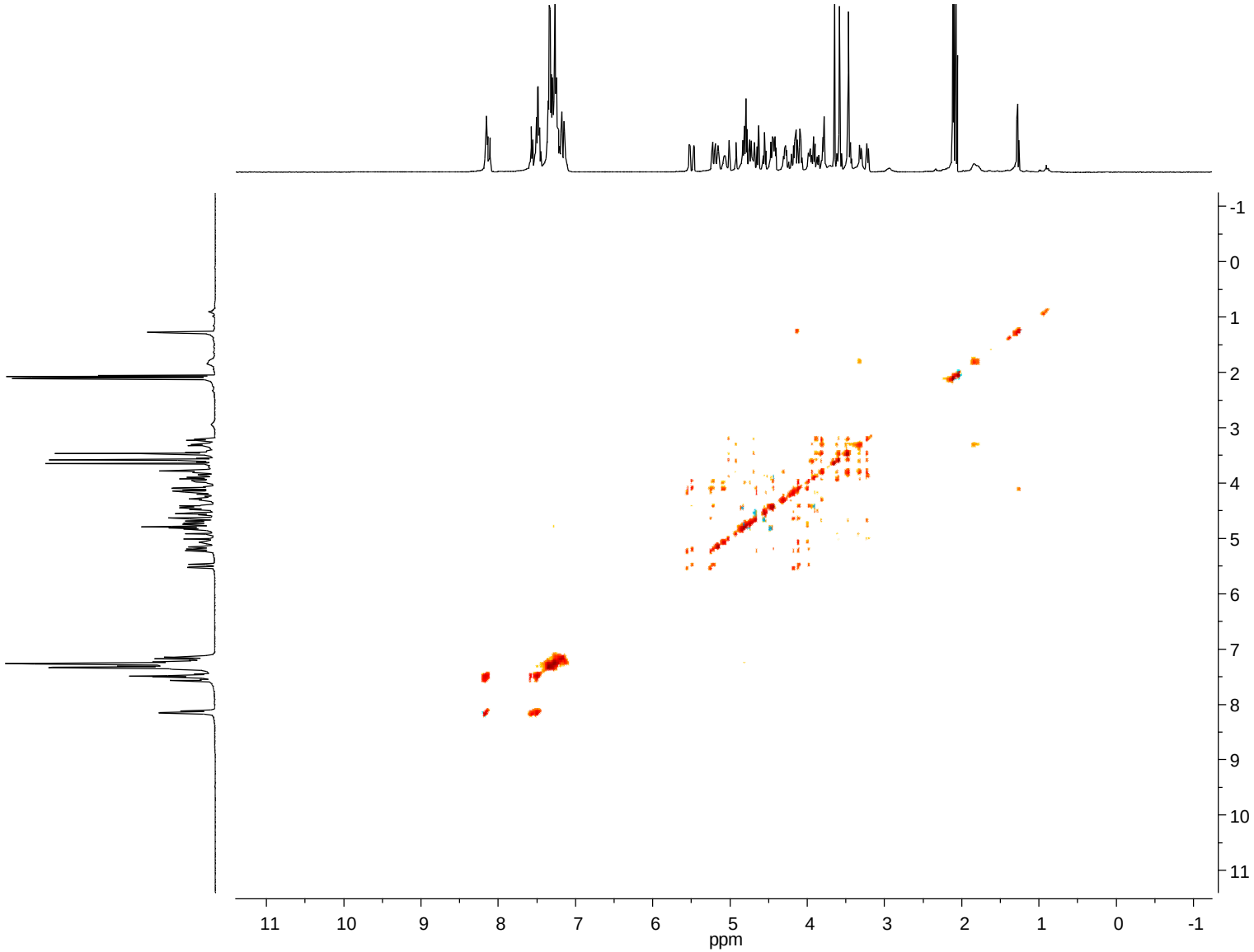
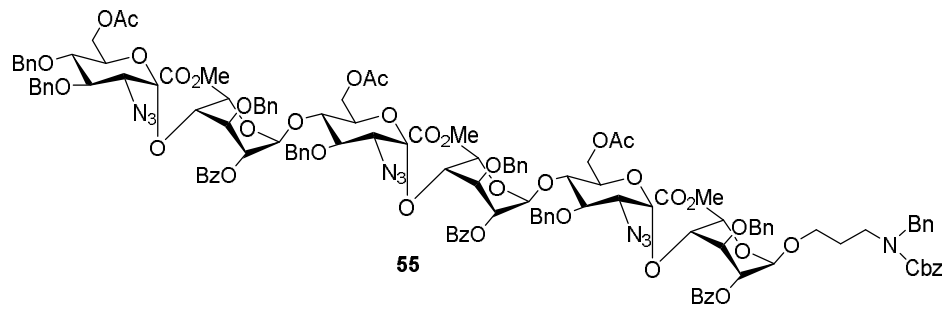


Figure 4.160. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **54**



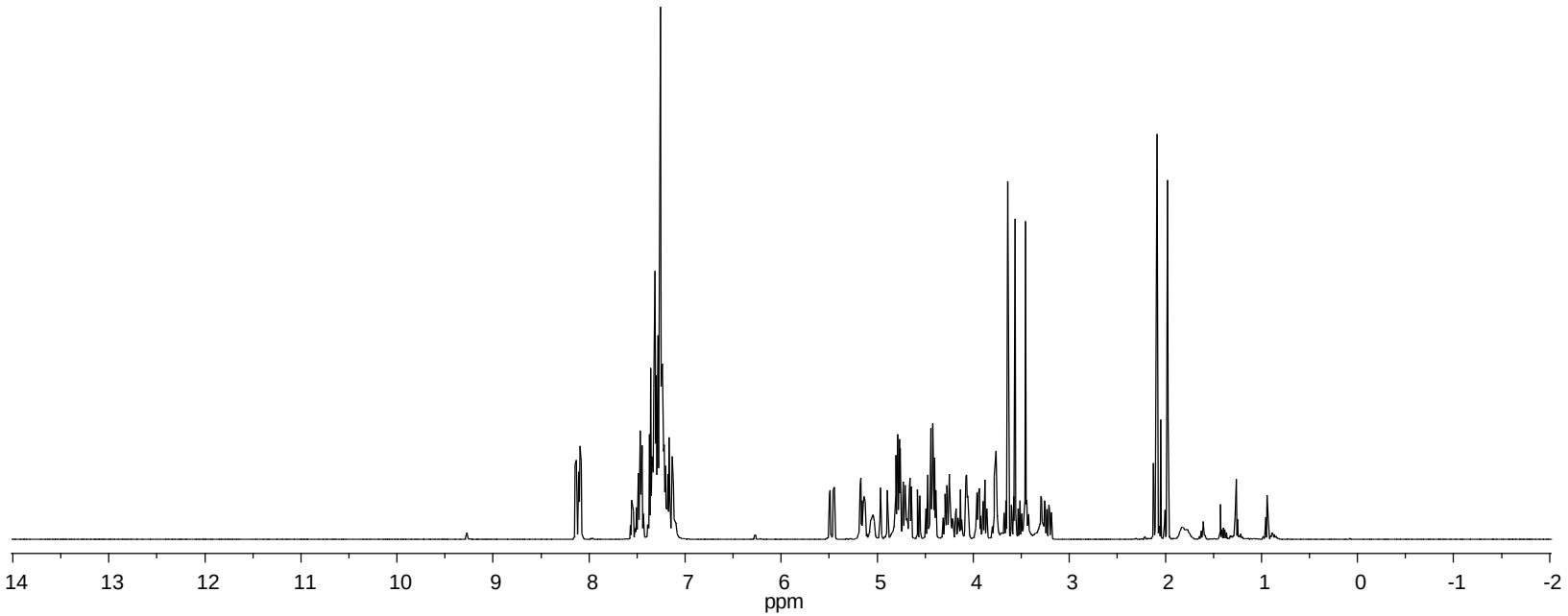


Figure 4.161. 500 MHz, $CDCl_3$, ^1H NMR of **55**

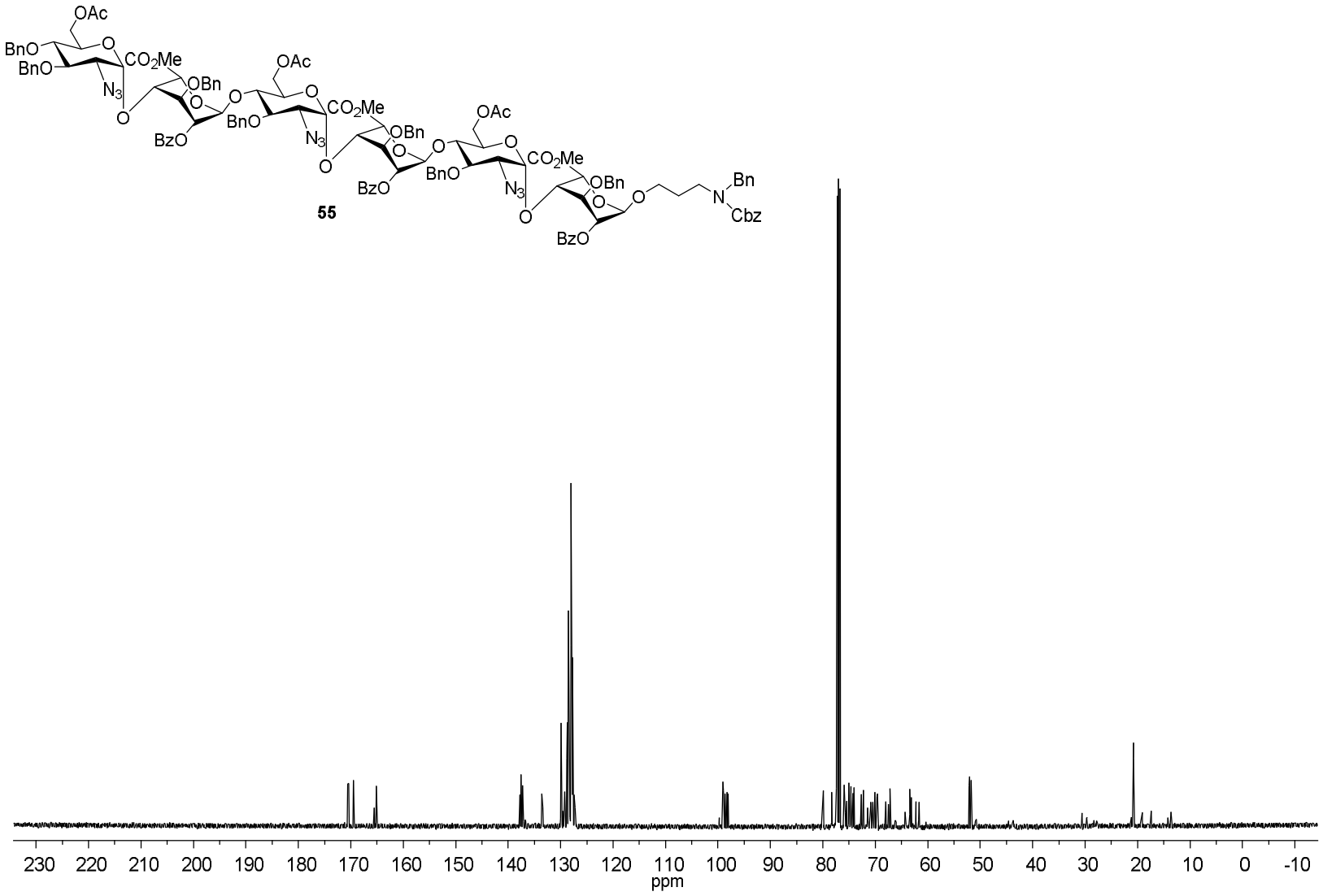


Figure 4.162. 125 MHz, $CDCl_3$, ^{13}C NMR of **55**

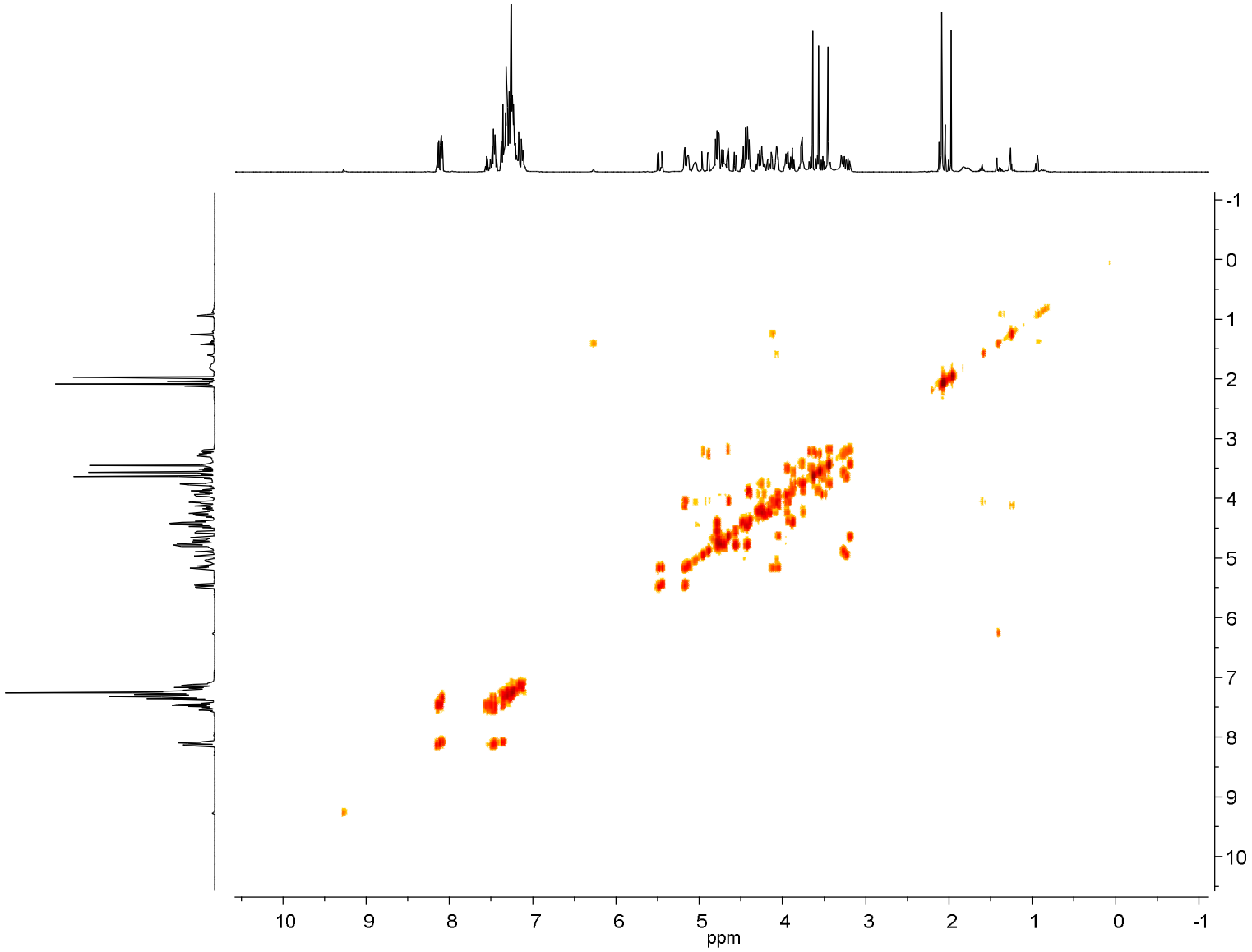


Figure 4.163. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **55**

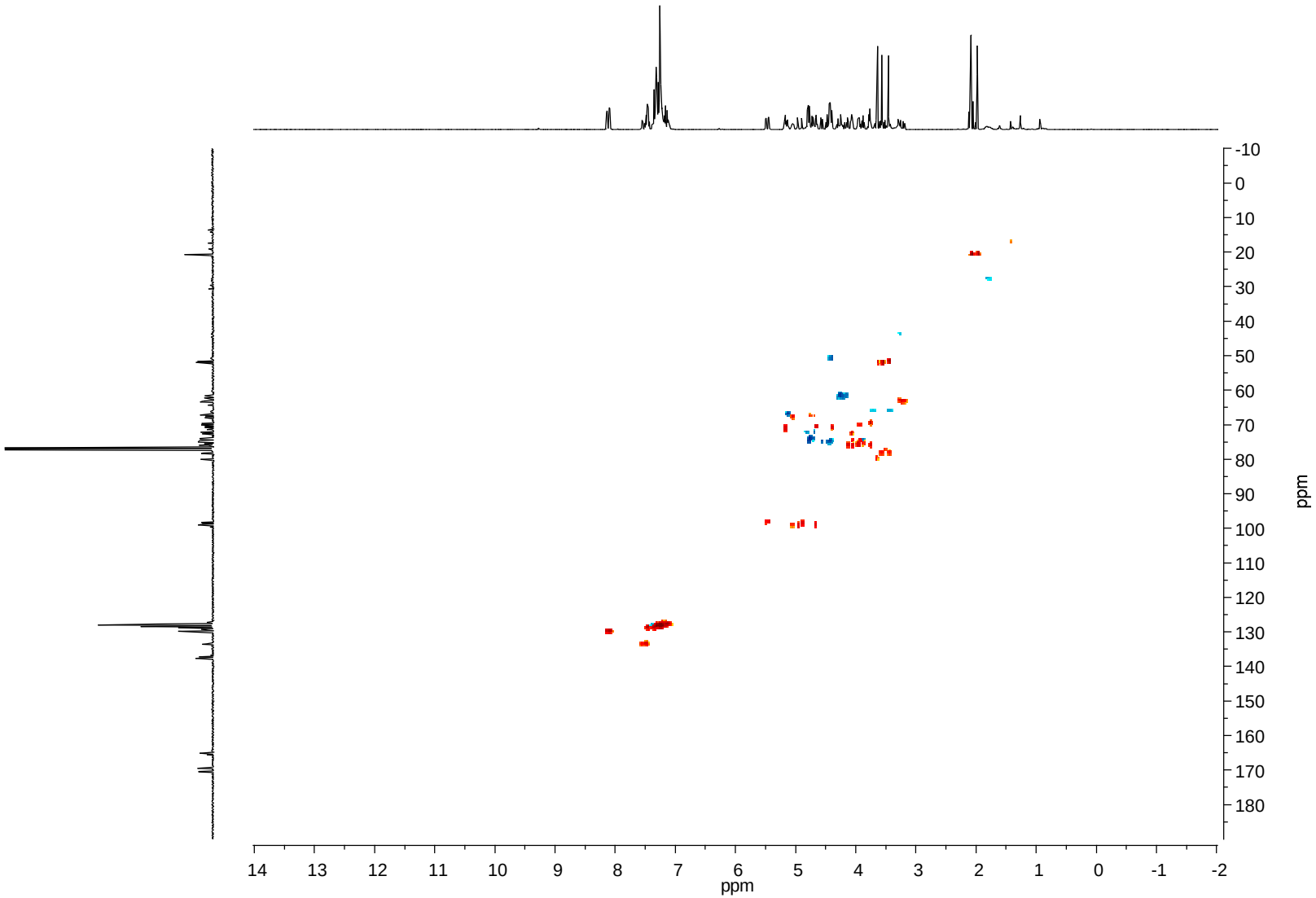


Figure 4.164 500 MHz, $CDCl_3$, gHSQCAD of **55**

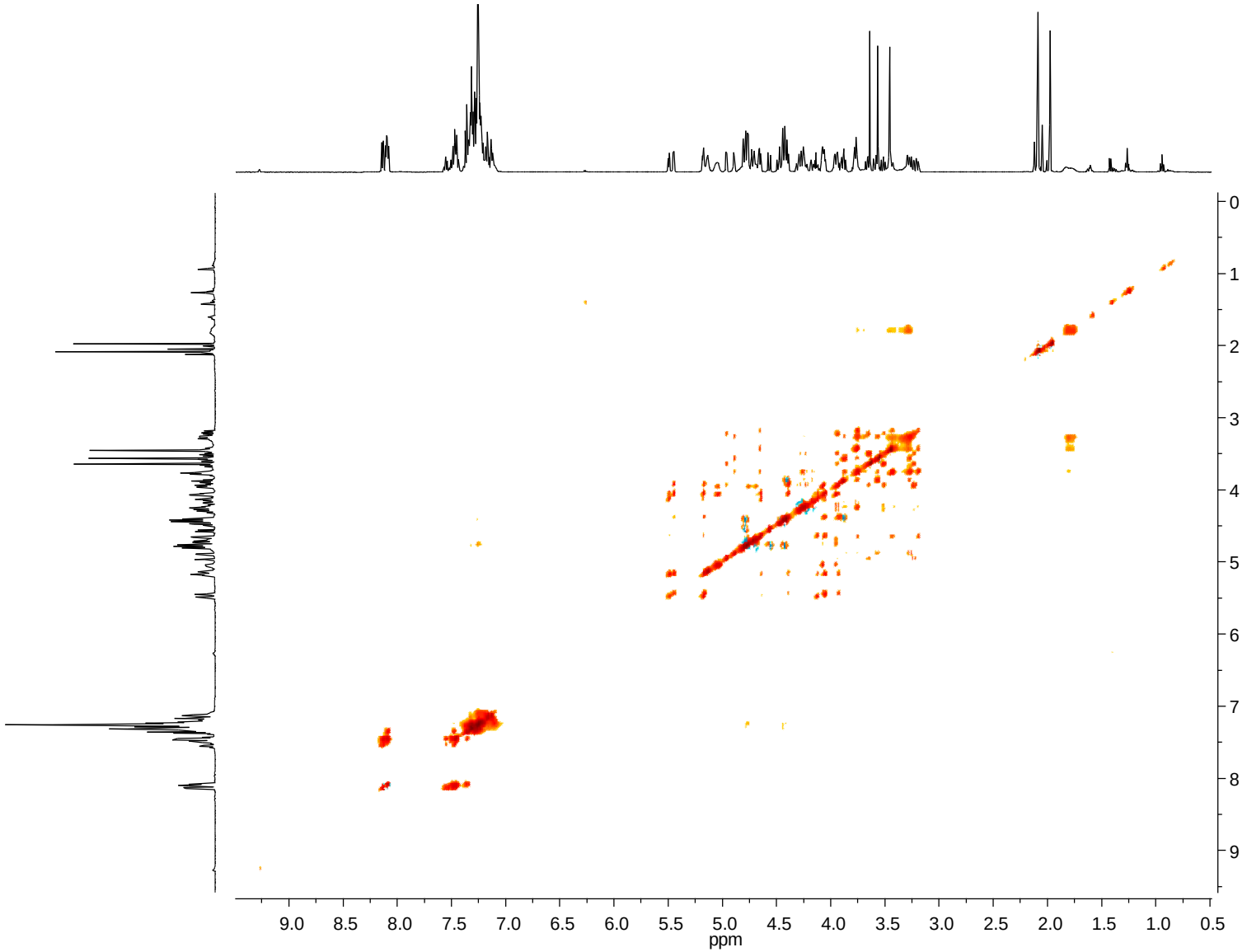
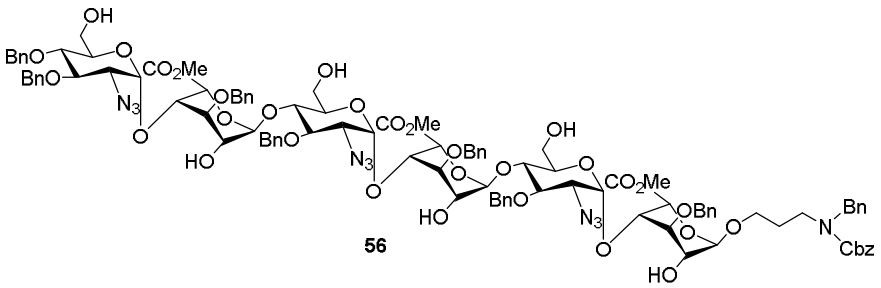


Figure 4.165. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **55**



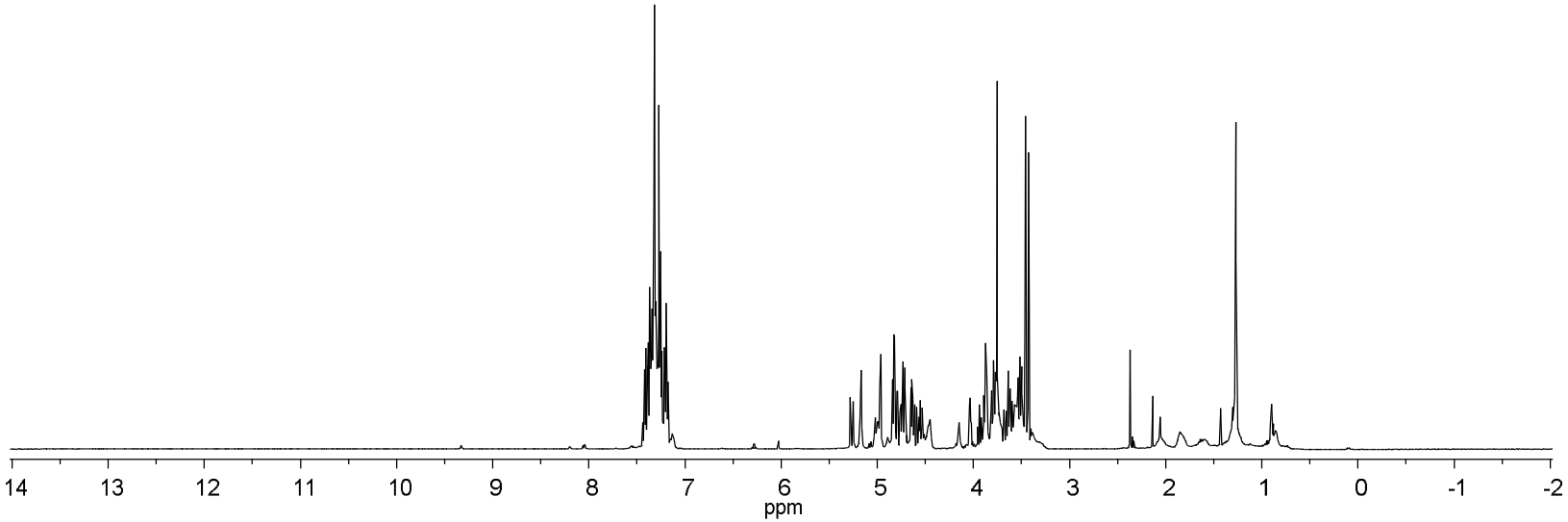


Figure 4.166. 500 MHz, $CDCl_3$, ^1H NMR of **56**

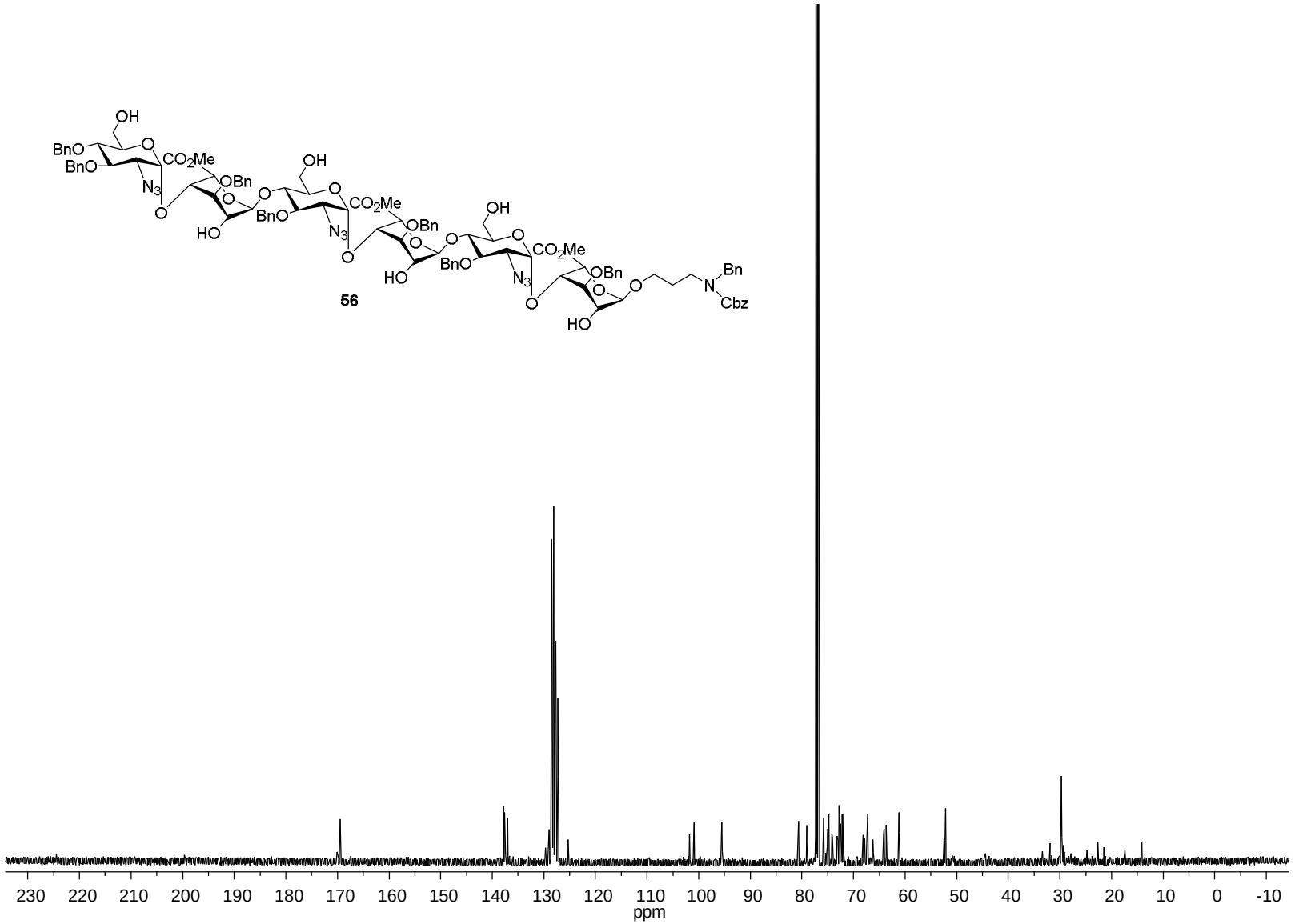


Figure 4.167. 125 MHz, $CDCl_3$, ^{13}C NMR of **56**

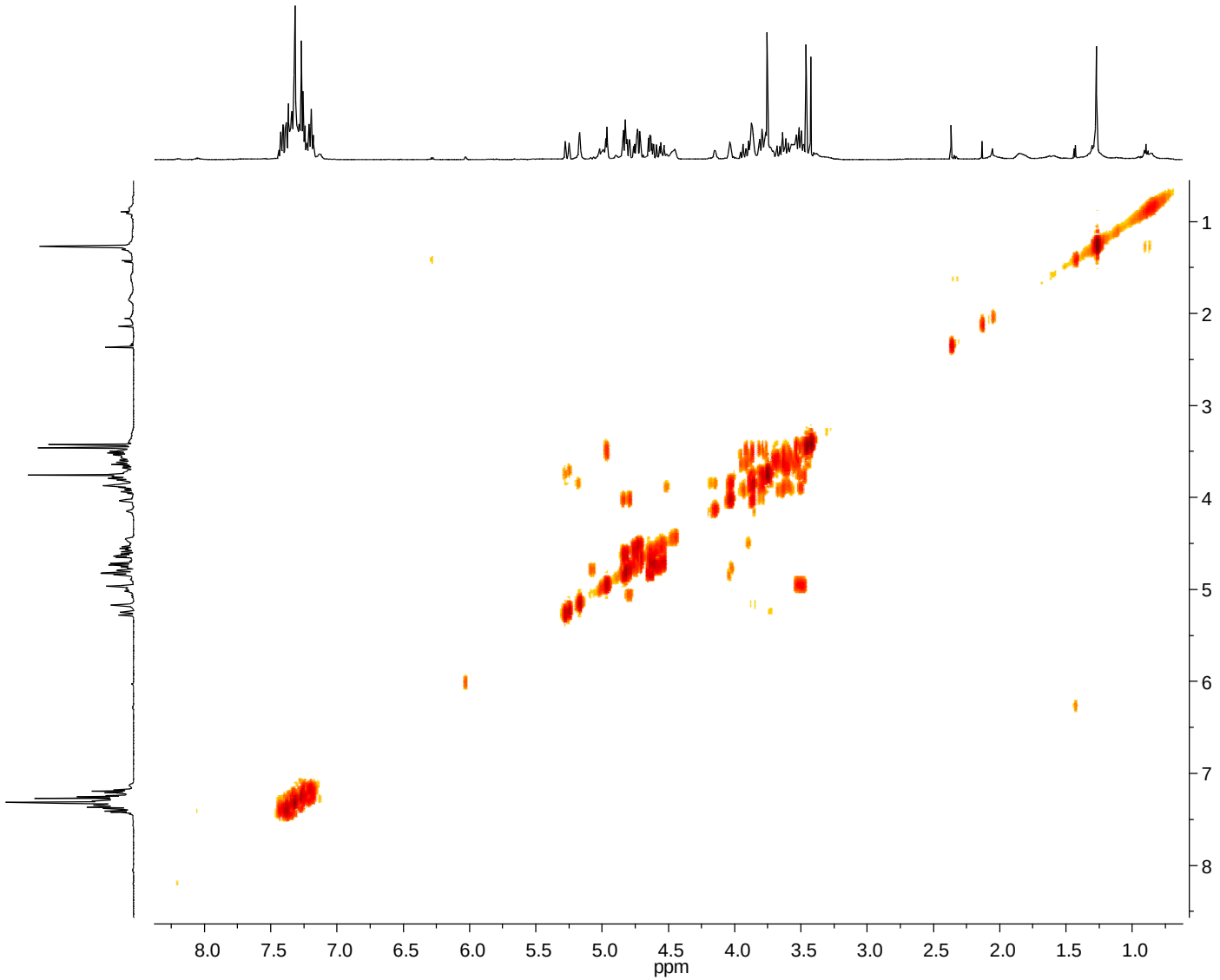


Figure 4.168. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY NMR of **56**

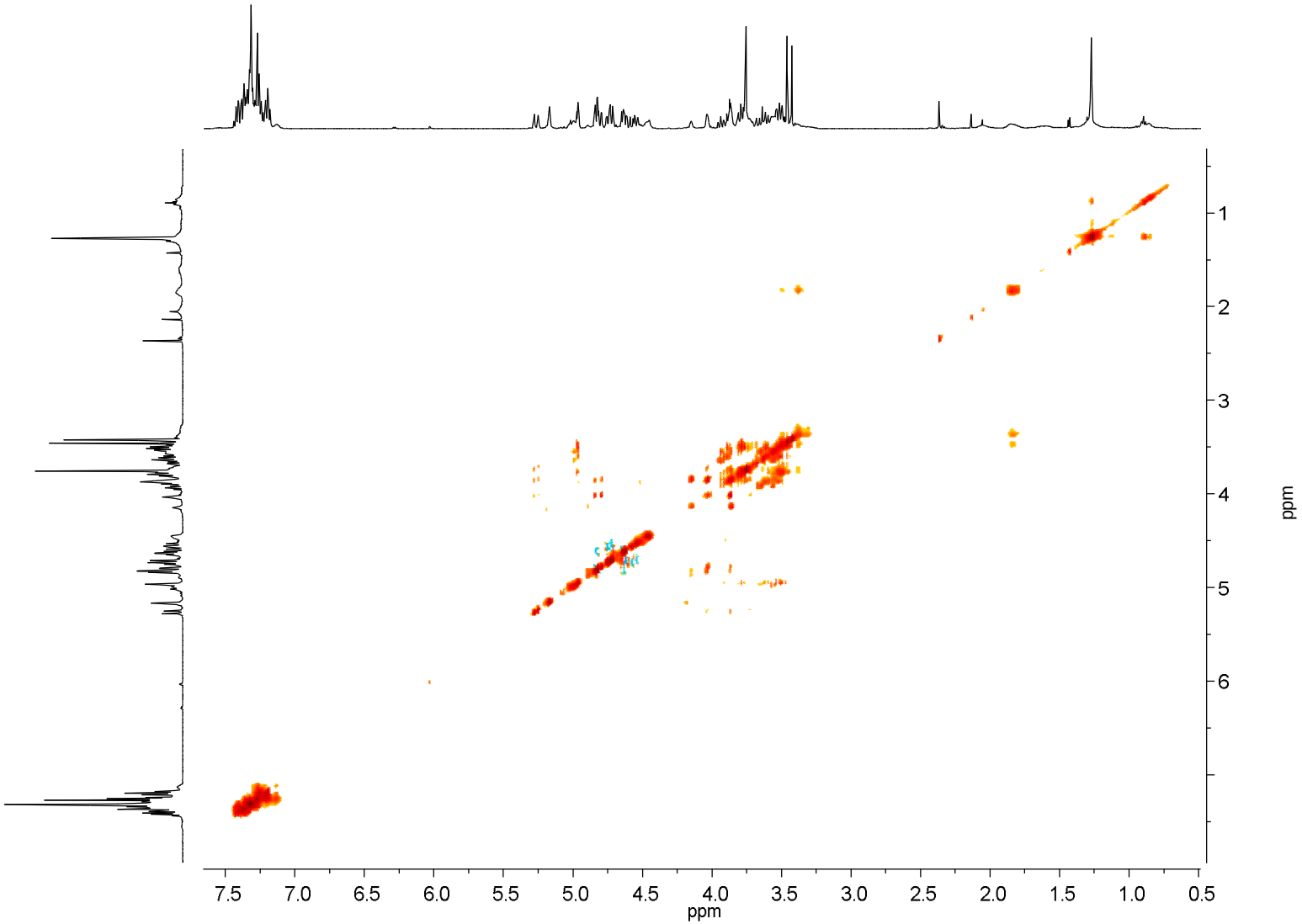


Figure 4.169. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY NMR of **56**

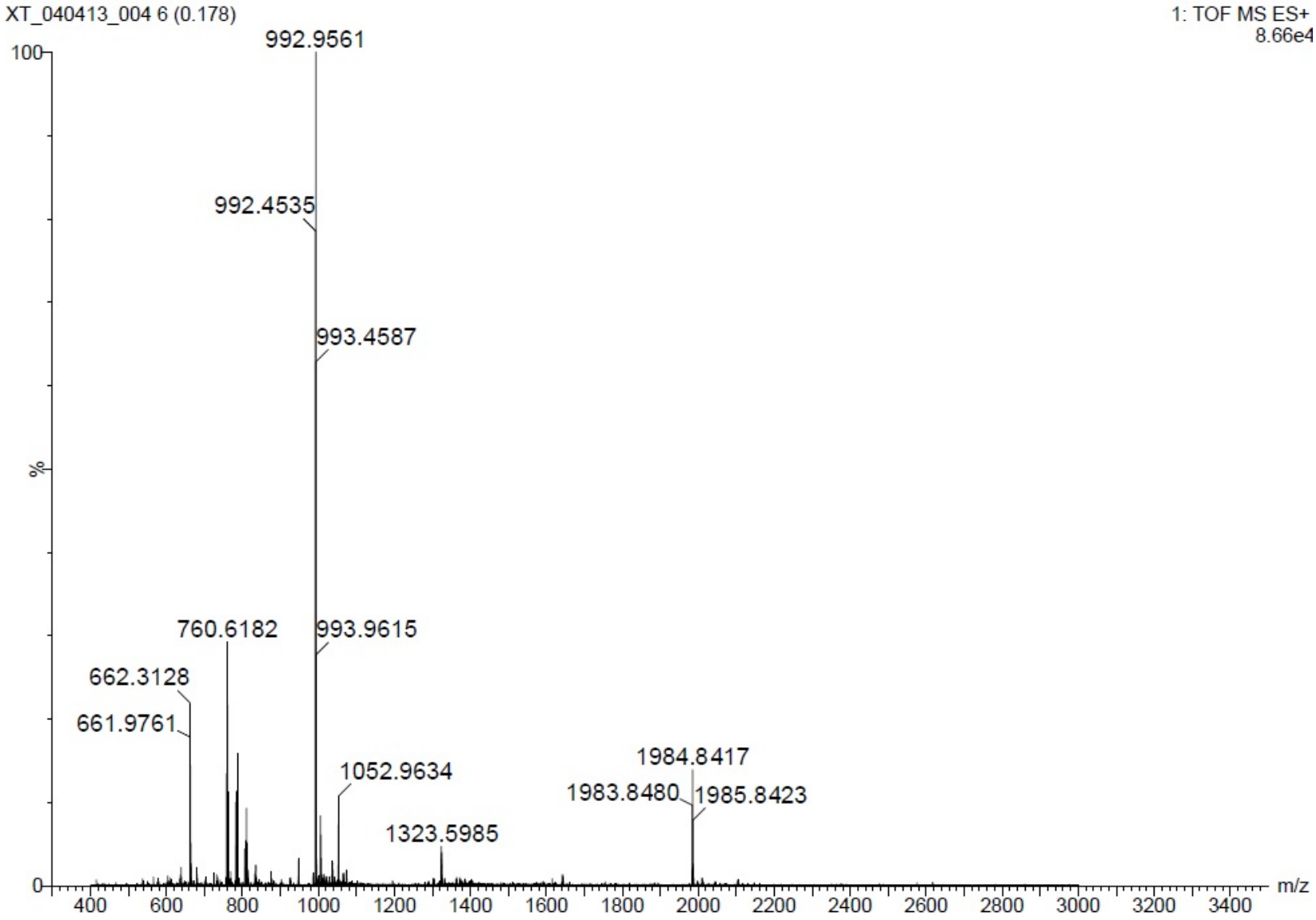
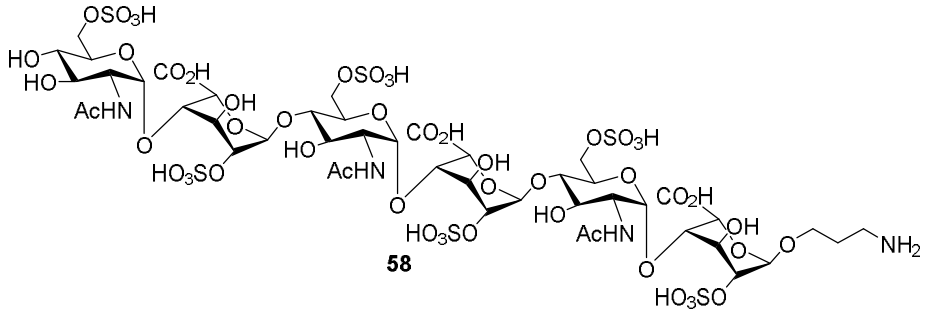


Figure 4.170. ESI-MS of **57**



58

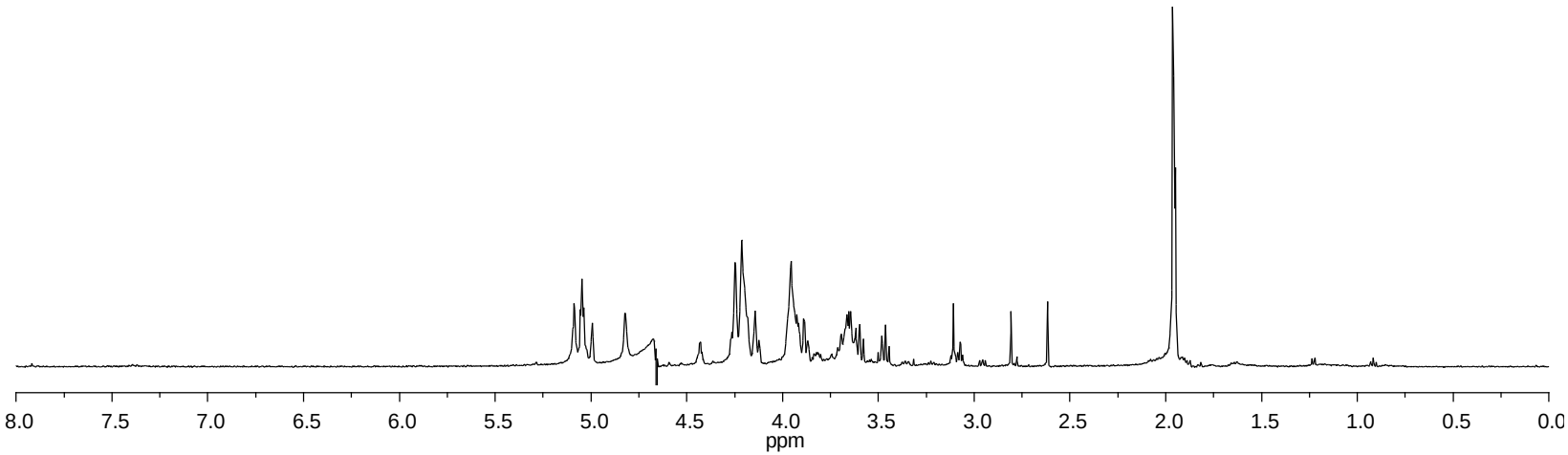


Figure 4.171. 500 MHz, D_2O, ^1H NMR of **58**

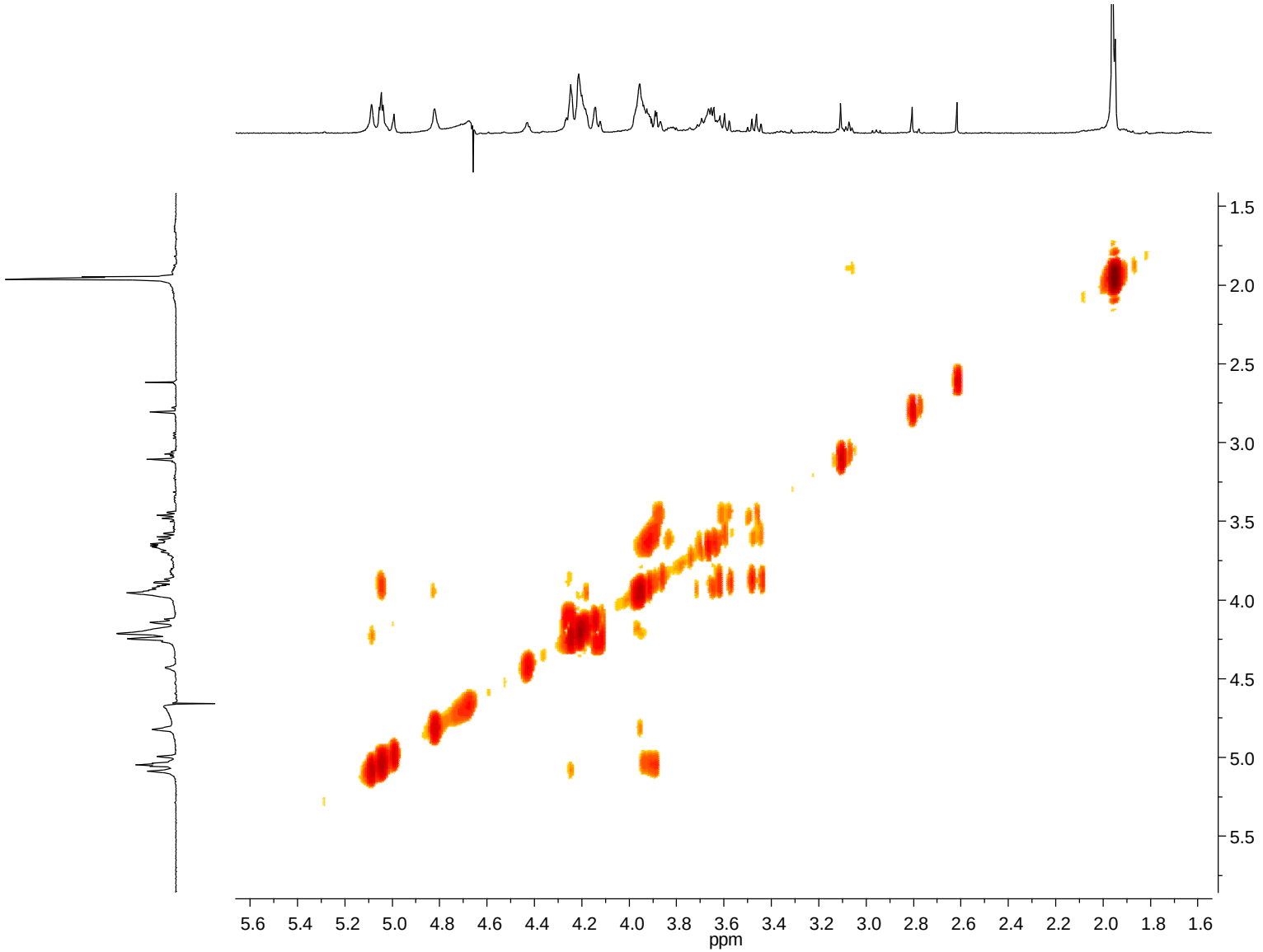


Figure 4.172. 500 MHz, D_2O, ^{1}H-^{1}H gCOSY NMR of **58**

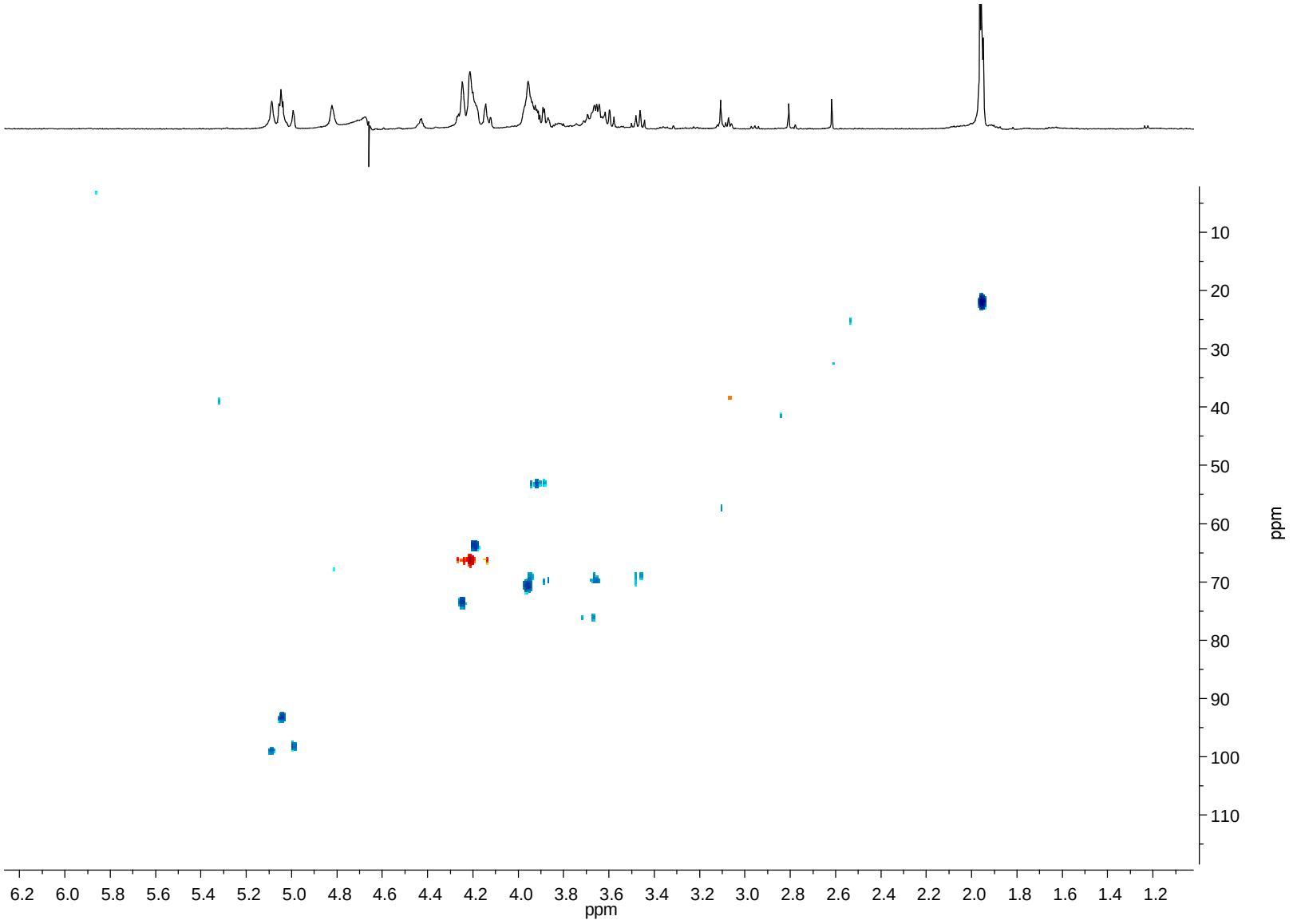


Figure 4.173. 500 MHz, D_2O, gHSQCAD of **58**

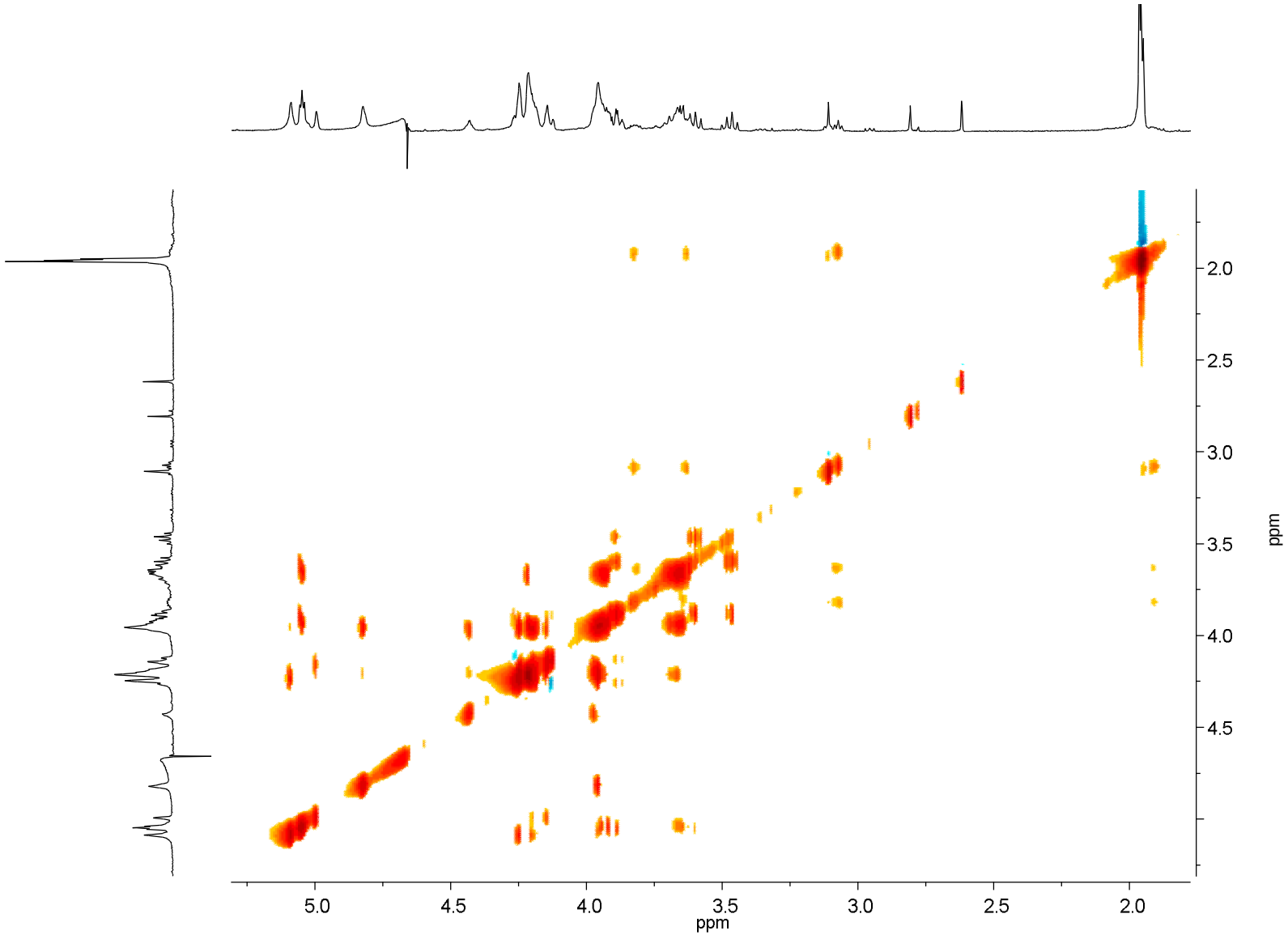


Figure 4.174. 500 MHz, D_2O, ^{1}H-^{1}H TOCSY of **58**

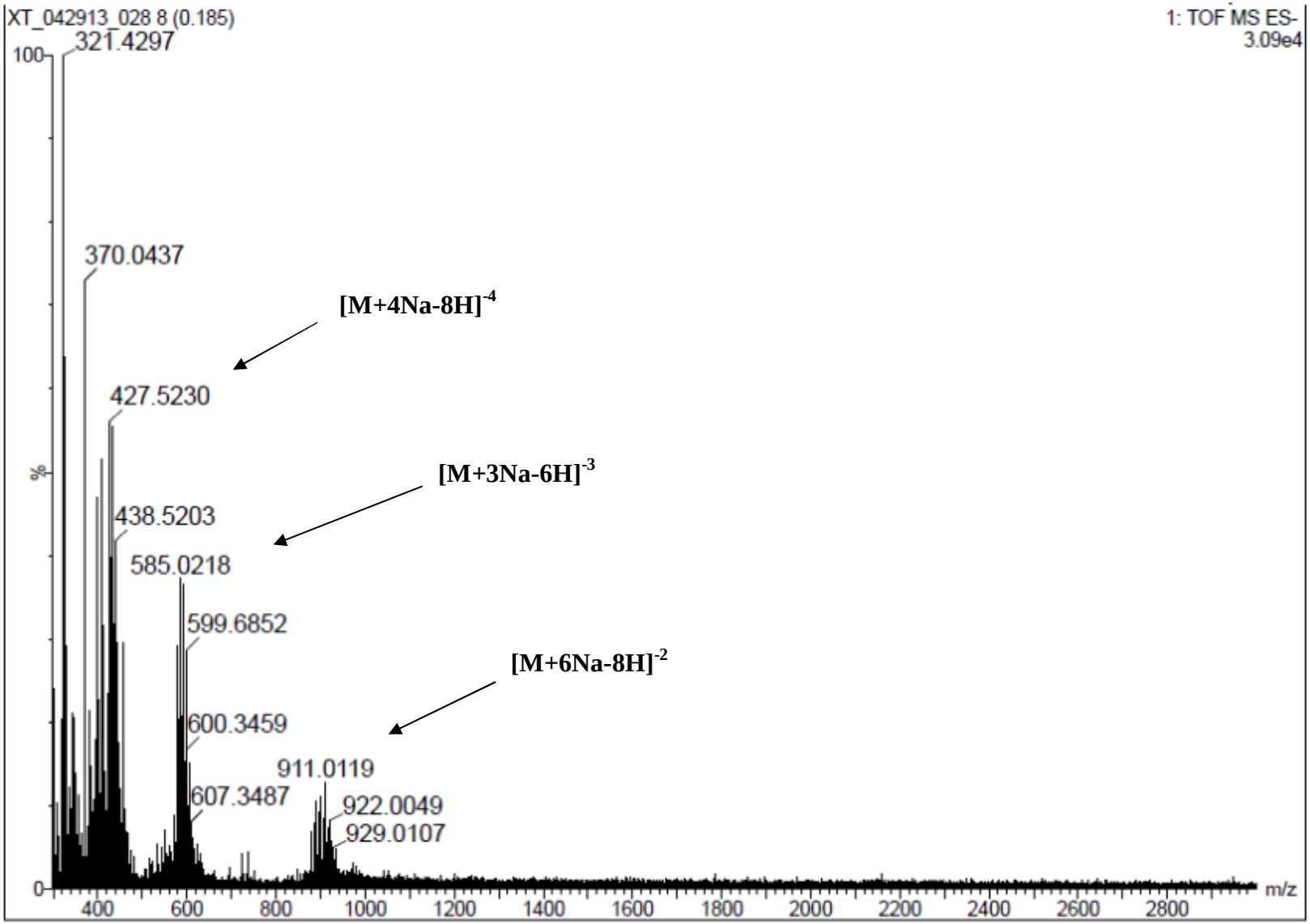
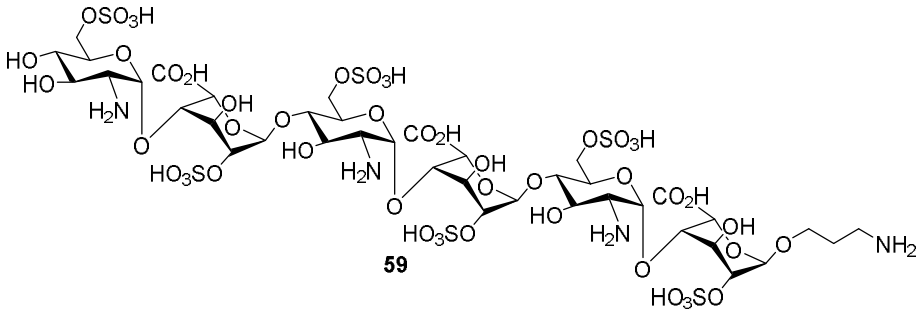


Figure 4.175. ESI-MS of **58**



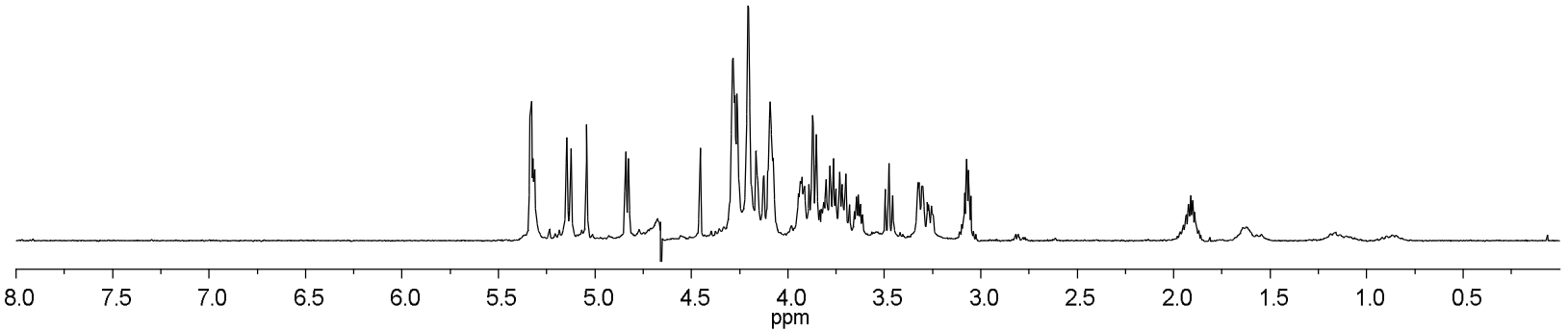


Figure 4.176. 500 MHz, D_2O, ^1H NMR of **59**

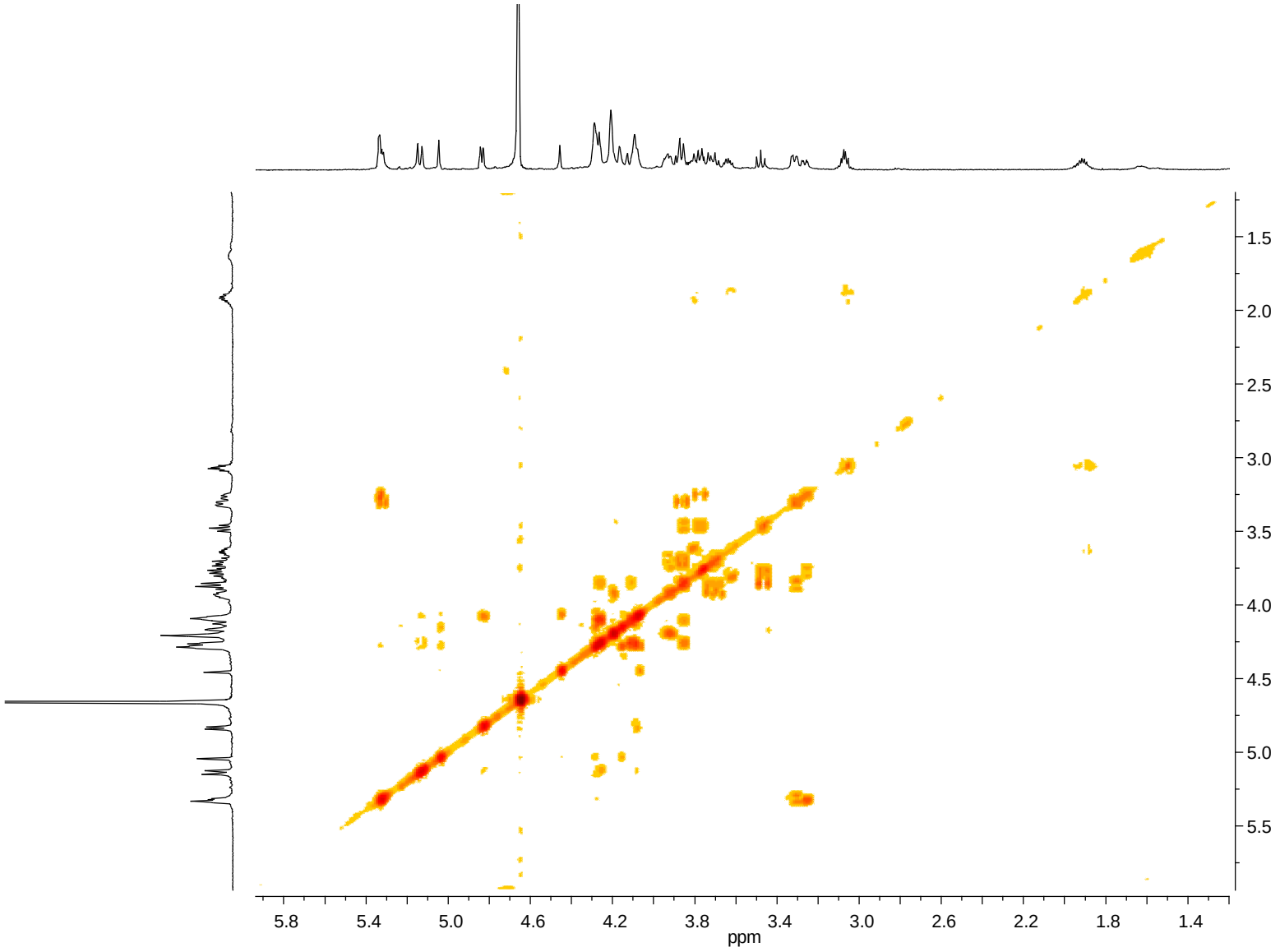


Figure 4.177. 500 MHz, D_2O, ^{1}H-^{1}H gCOSY NMR of **59**

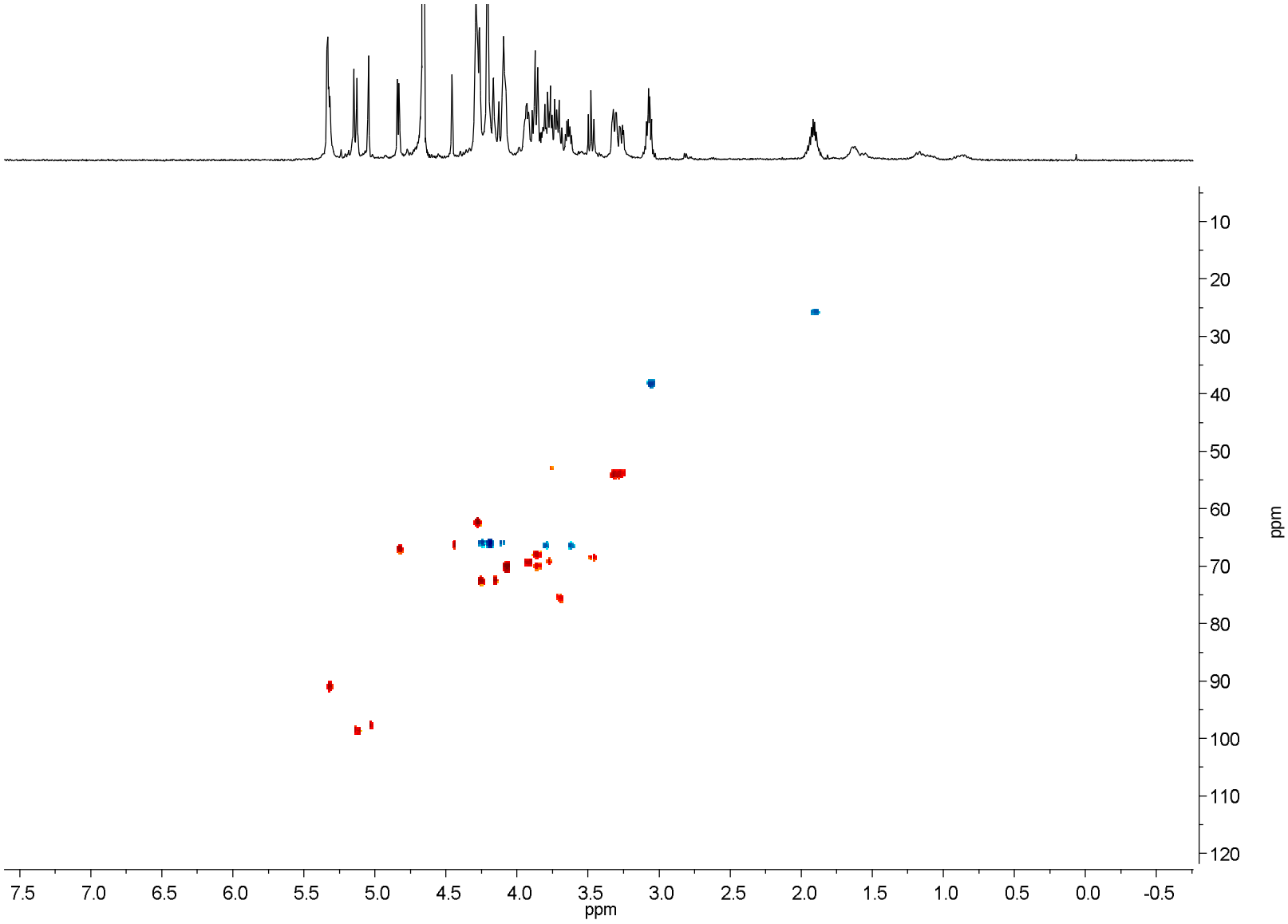


Figure 4.178. 500 MHz, D_2O, gHSQCAD of **59**

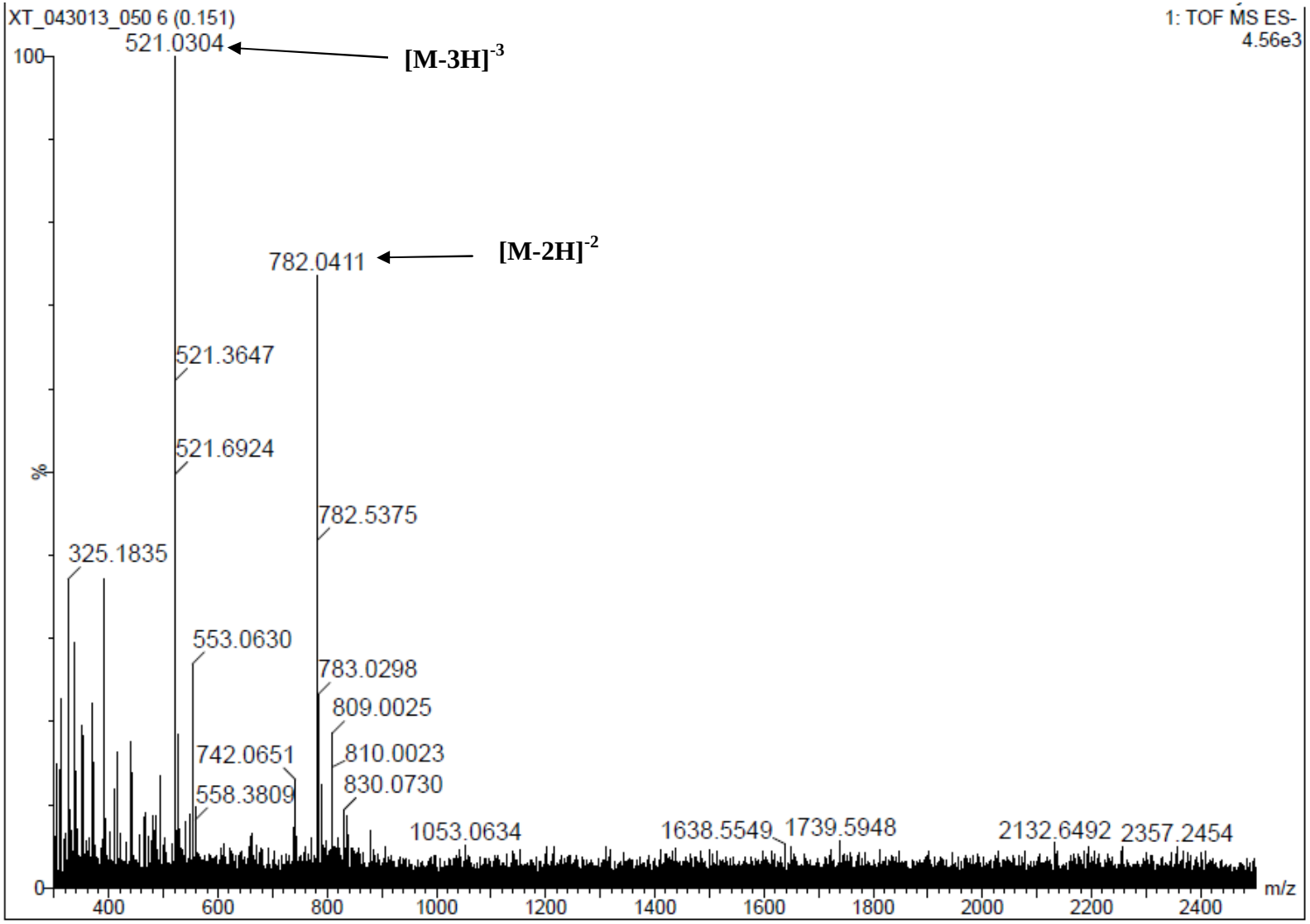


Figure 4.179. ESI-MS of **59**

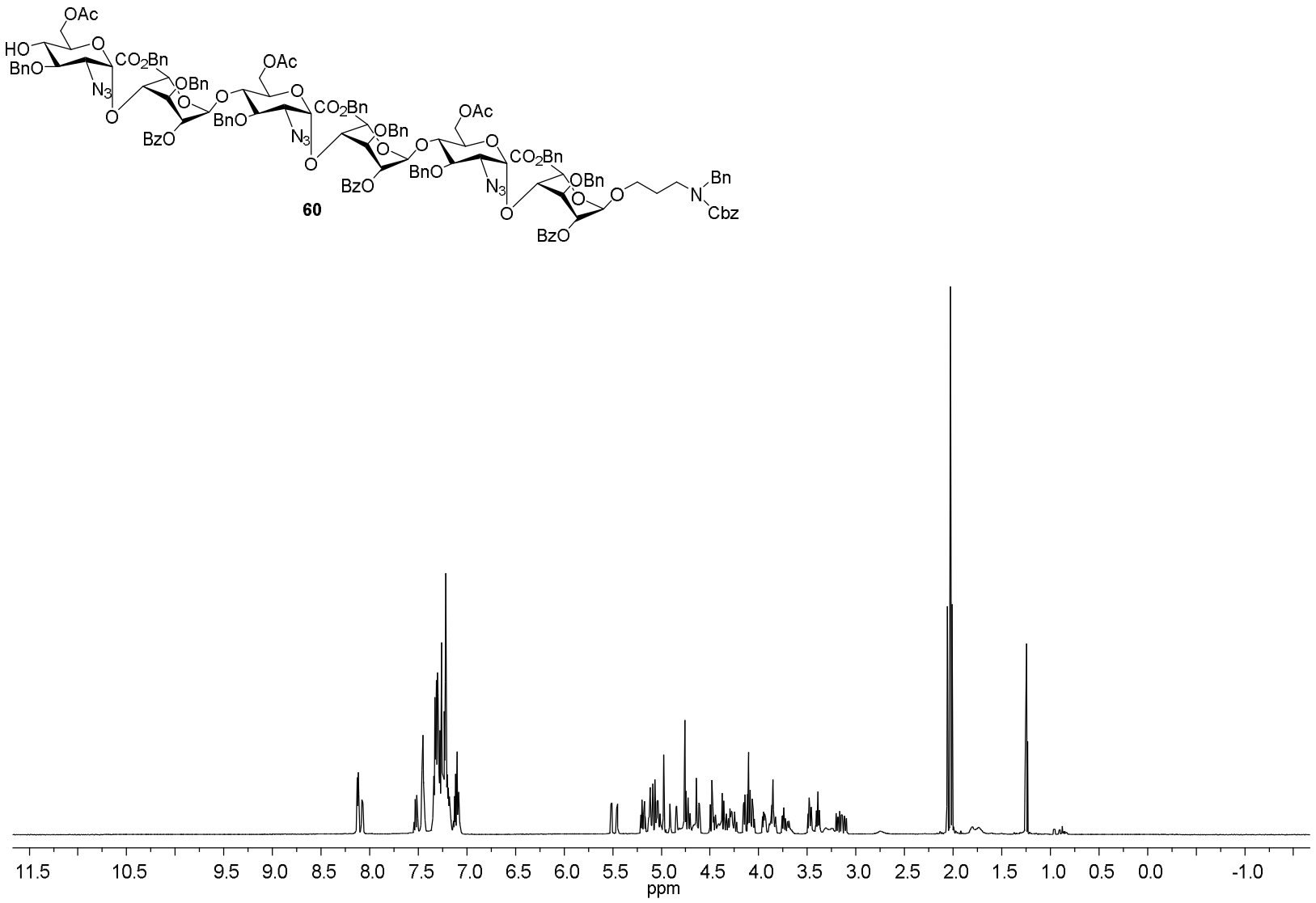


Figure 4.180. 600 MHz, $CDCl_3$, ^1H NMR of **60**

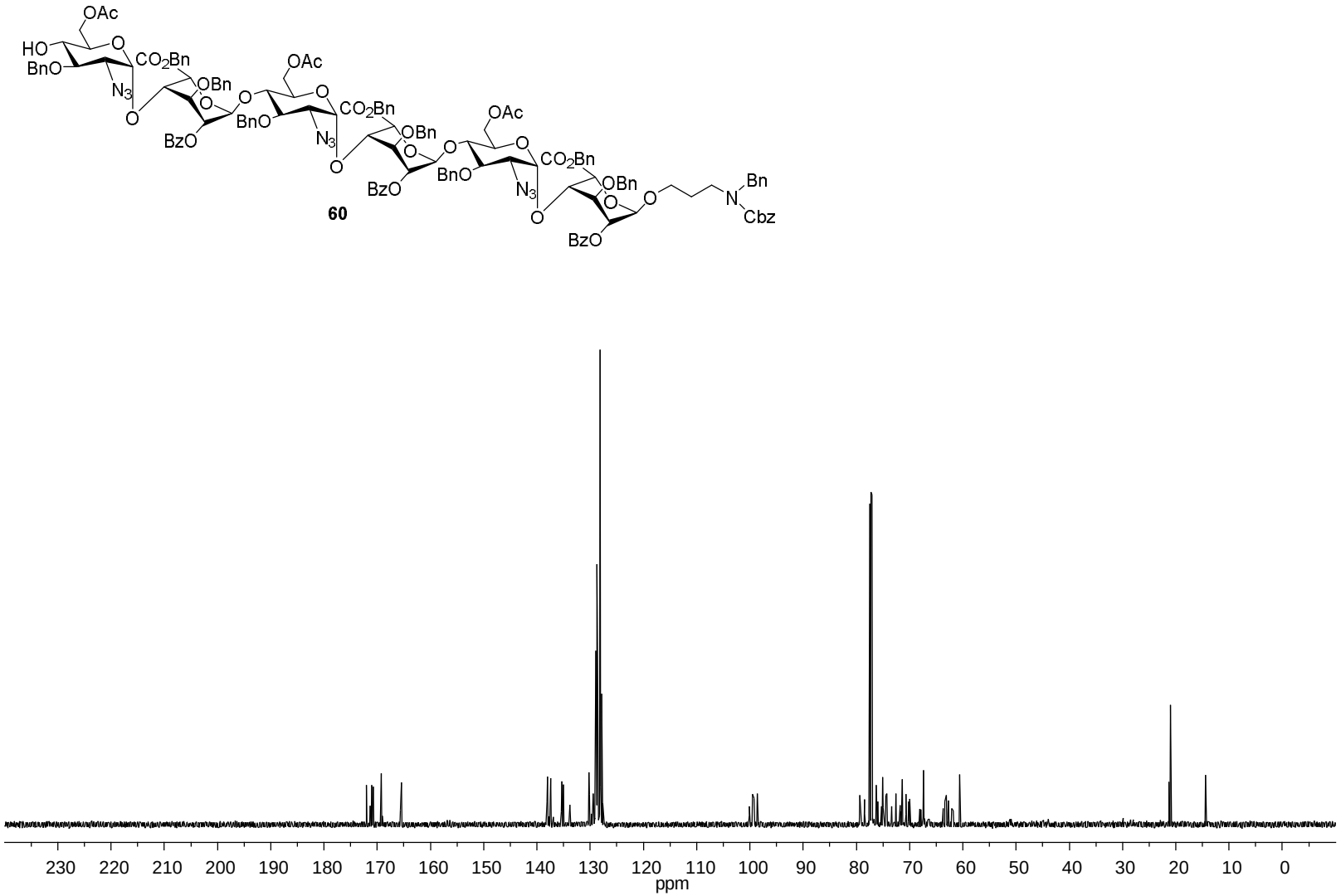


Figure 4.181. 150 MHz, $CDCl_3$, ^{13}C NMR of **60**

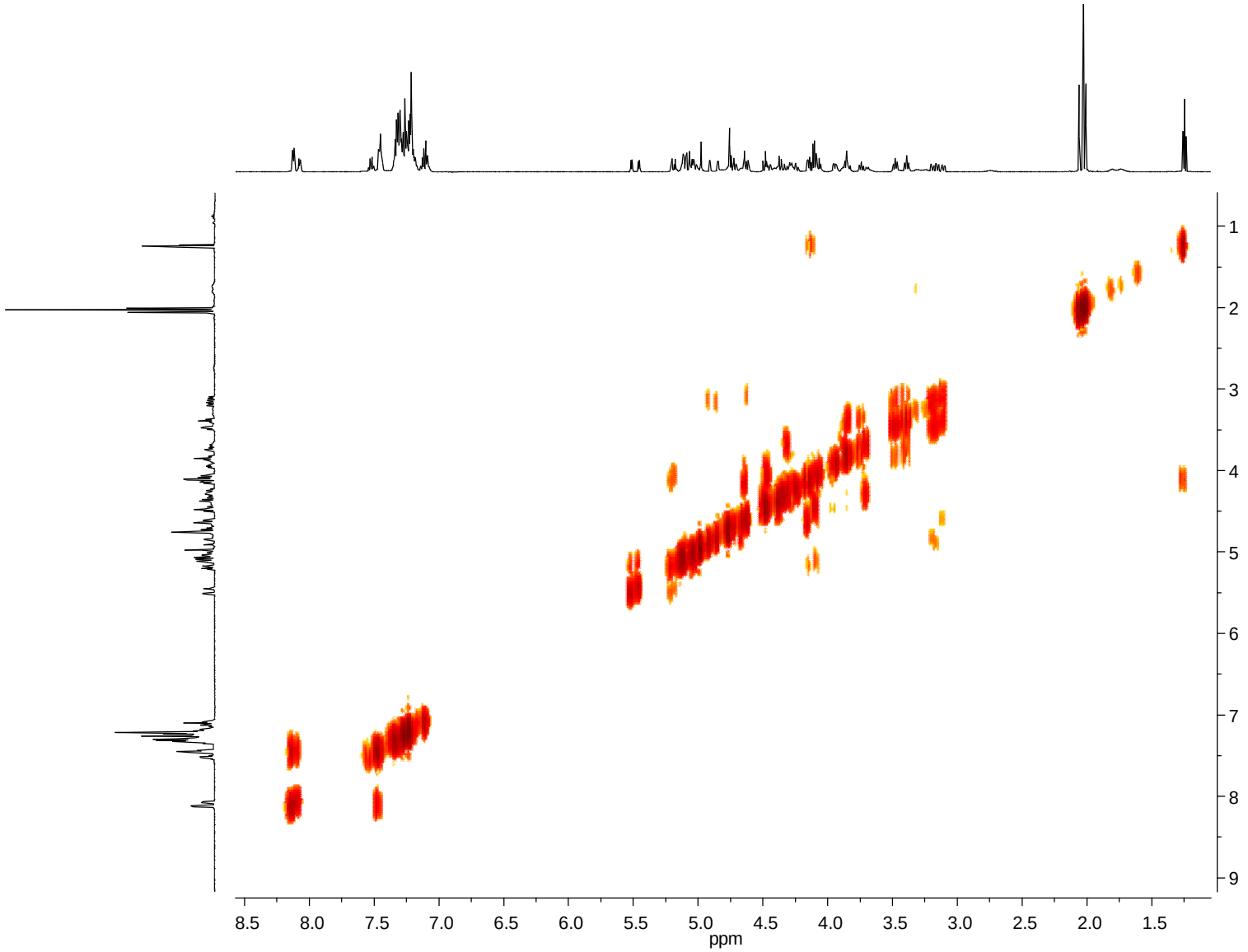


Figure 4.182. 600 MHz, $CDCl_3$, ^1H-^1H gCOSY of **60**

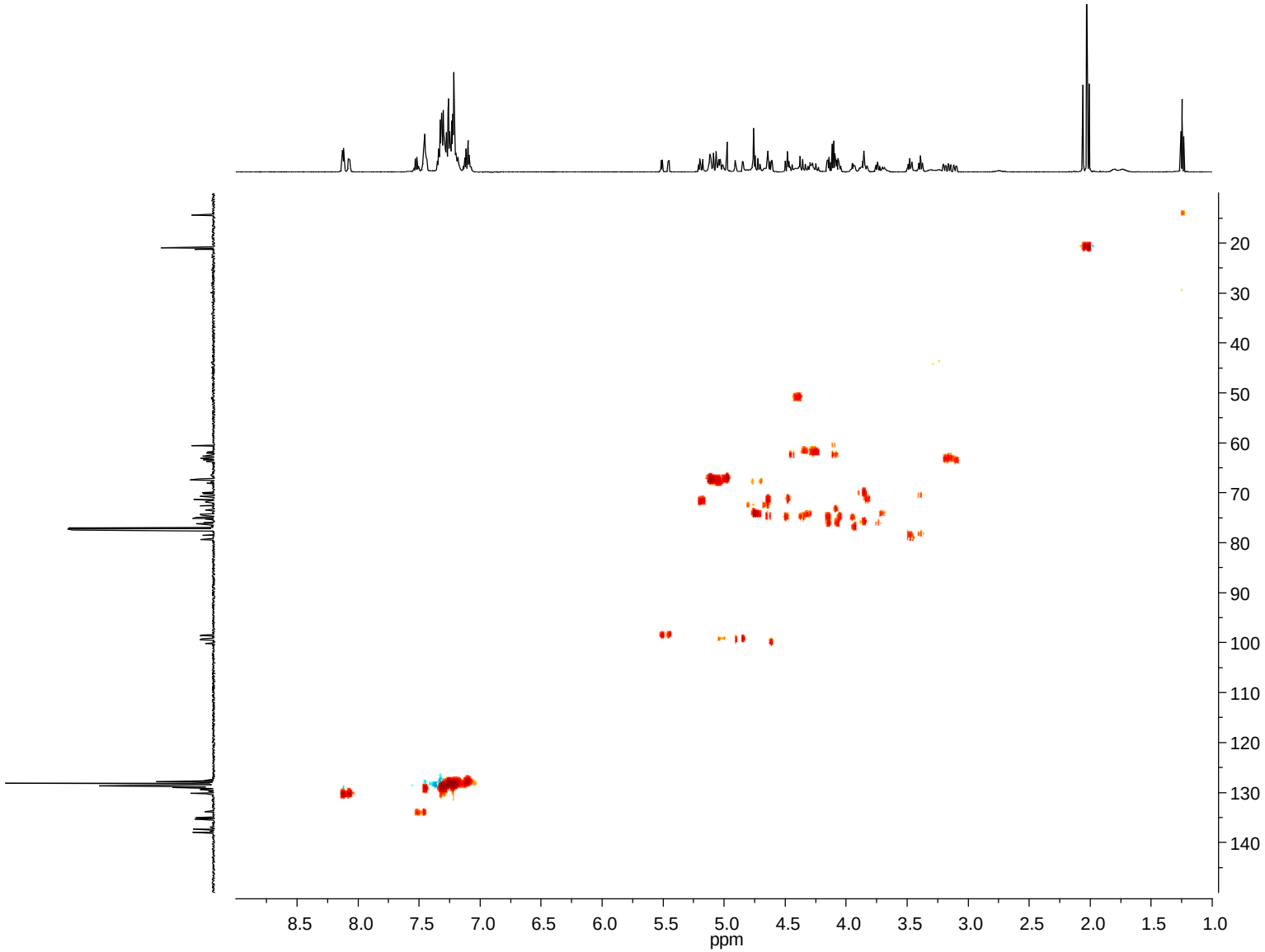


Figure 4.183. 600 MHz, $CDCl_3$, gHMQC of **60**

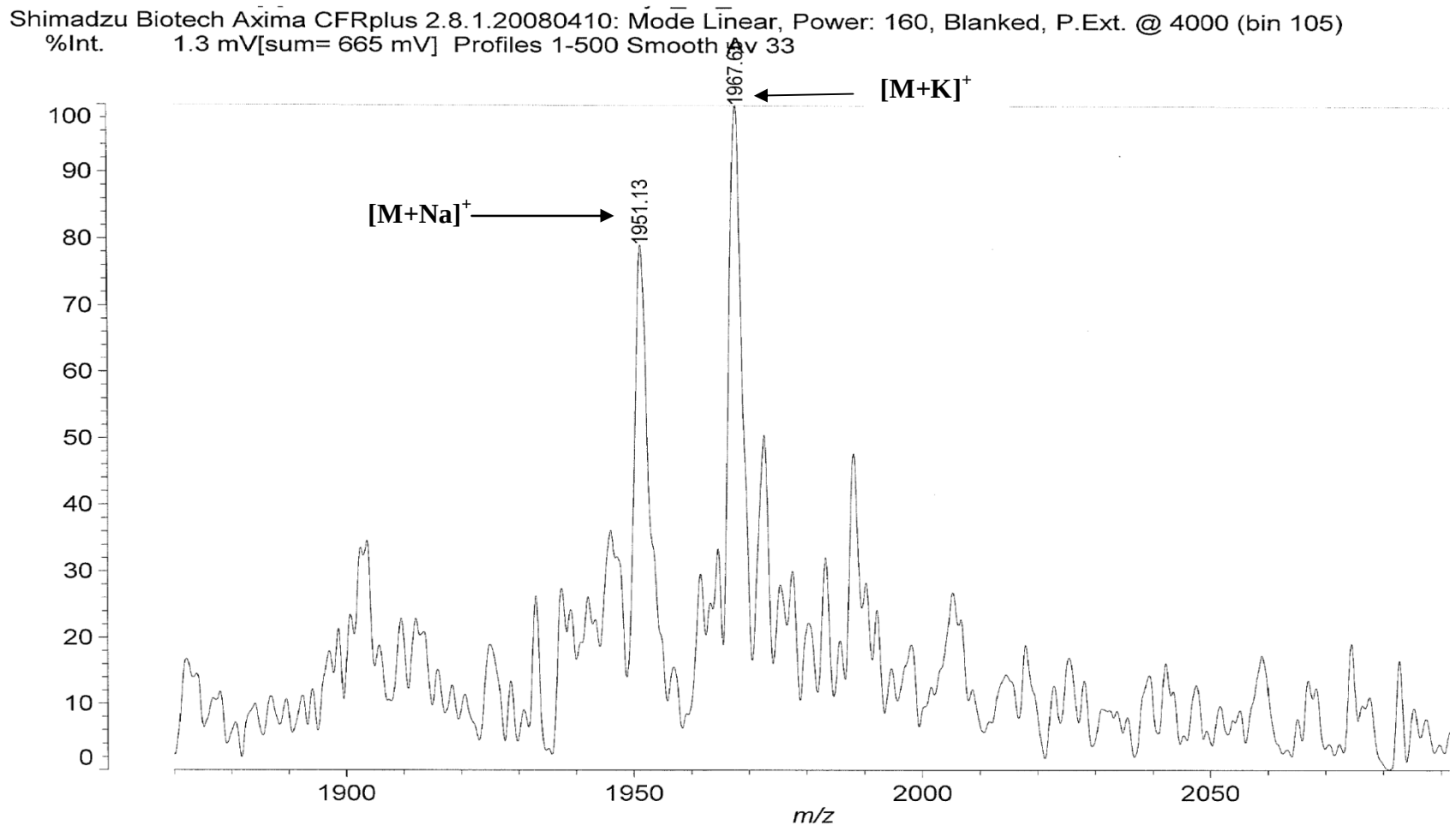


Figure 4.184. MALDI-MS of **61**

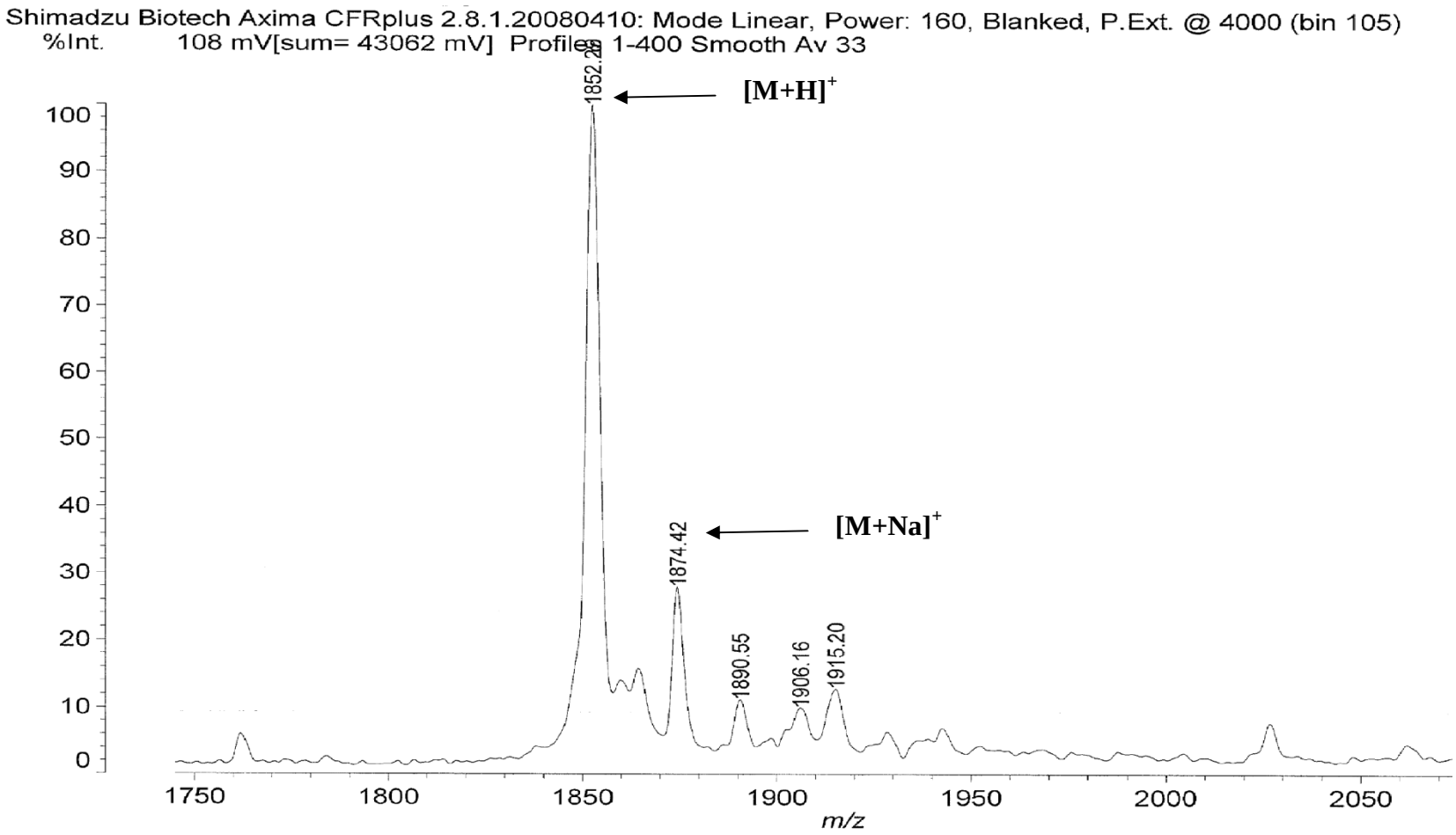


Figure 4.185. MALDI-MS of **62**

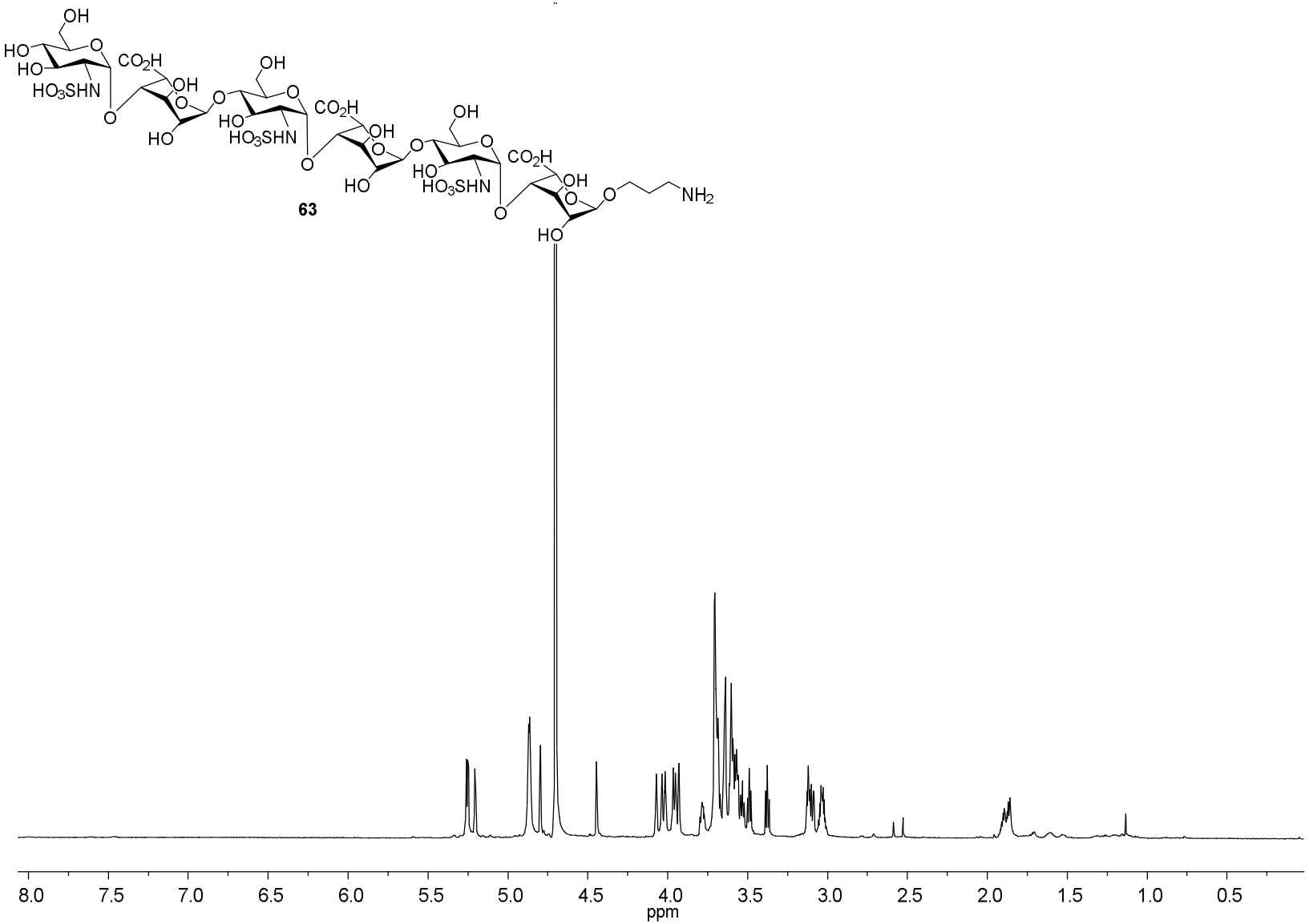


Figure 4.186. 900 MHz, D_2O, ^1H NMR of **63**

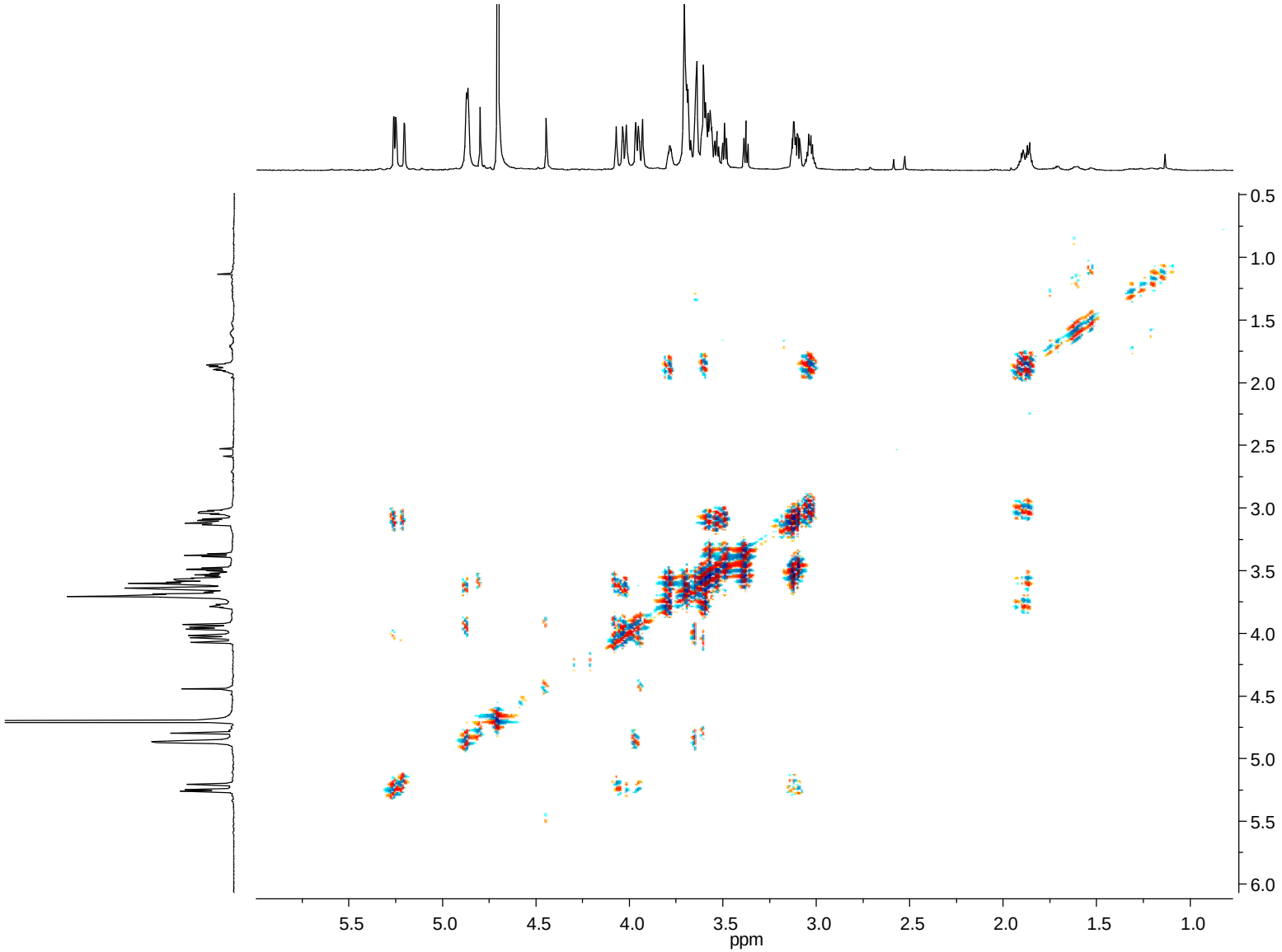


Figure 4.187. 900 MHz, D_2O, ^{1}H-^{1}H gCOSY of **63**

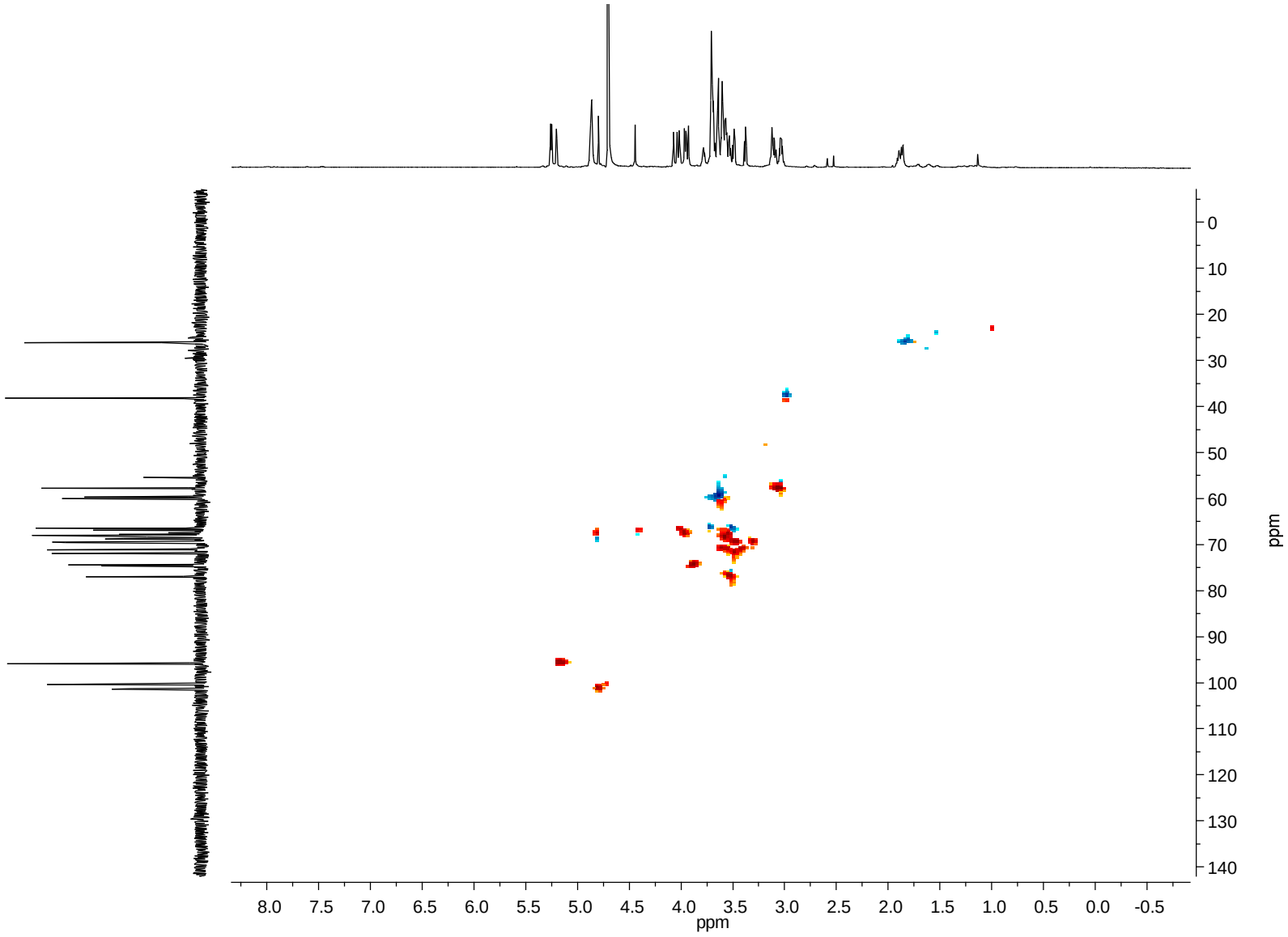


Figure 4.188. 900 MHz, D_2O, gHSQC of **63**

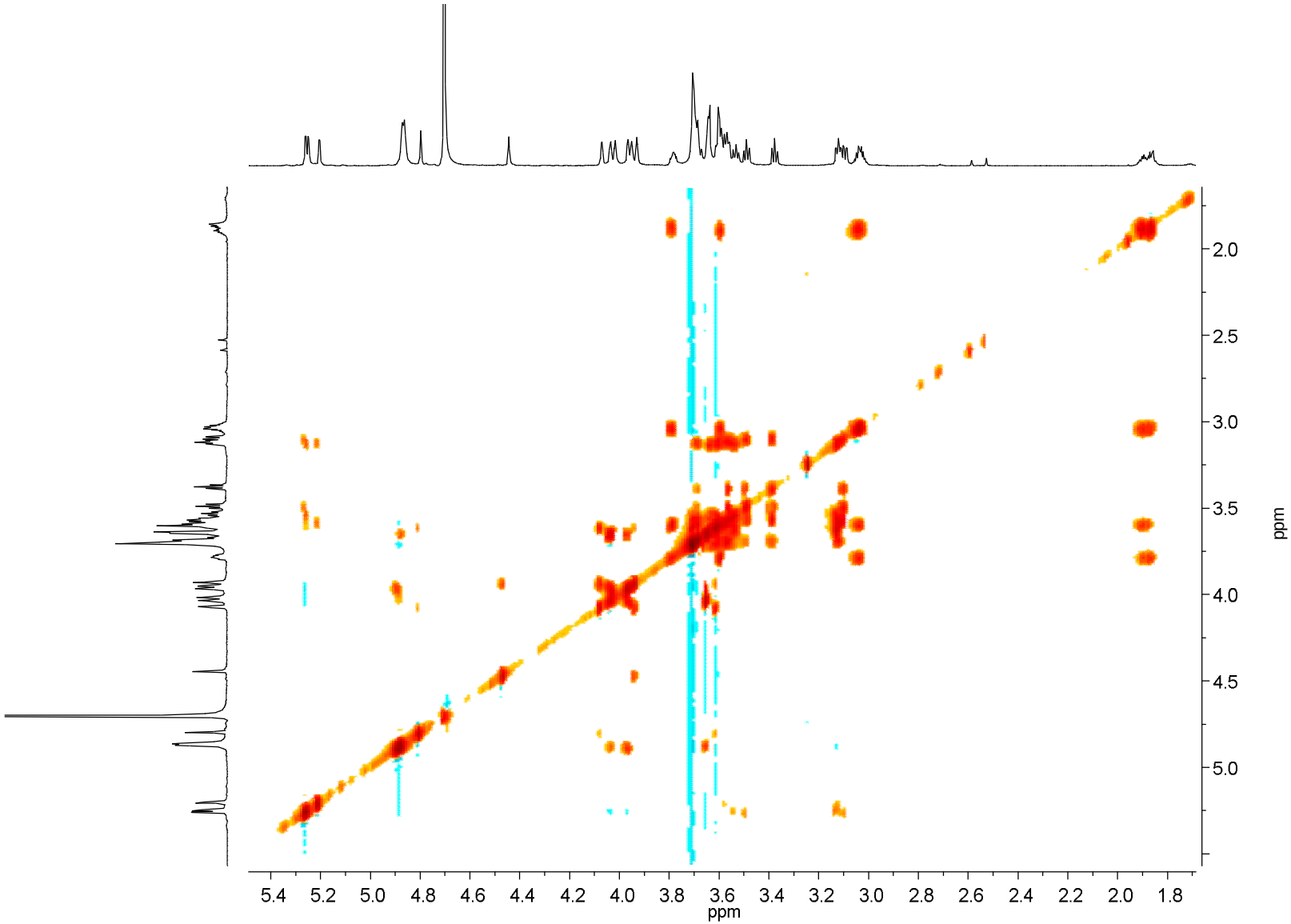


Figure 4.189. 900 MHz, D_2O, ^1H-^1H TOCSY of **63**

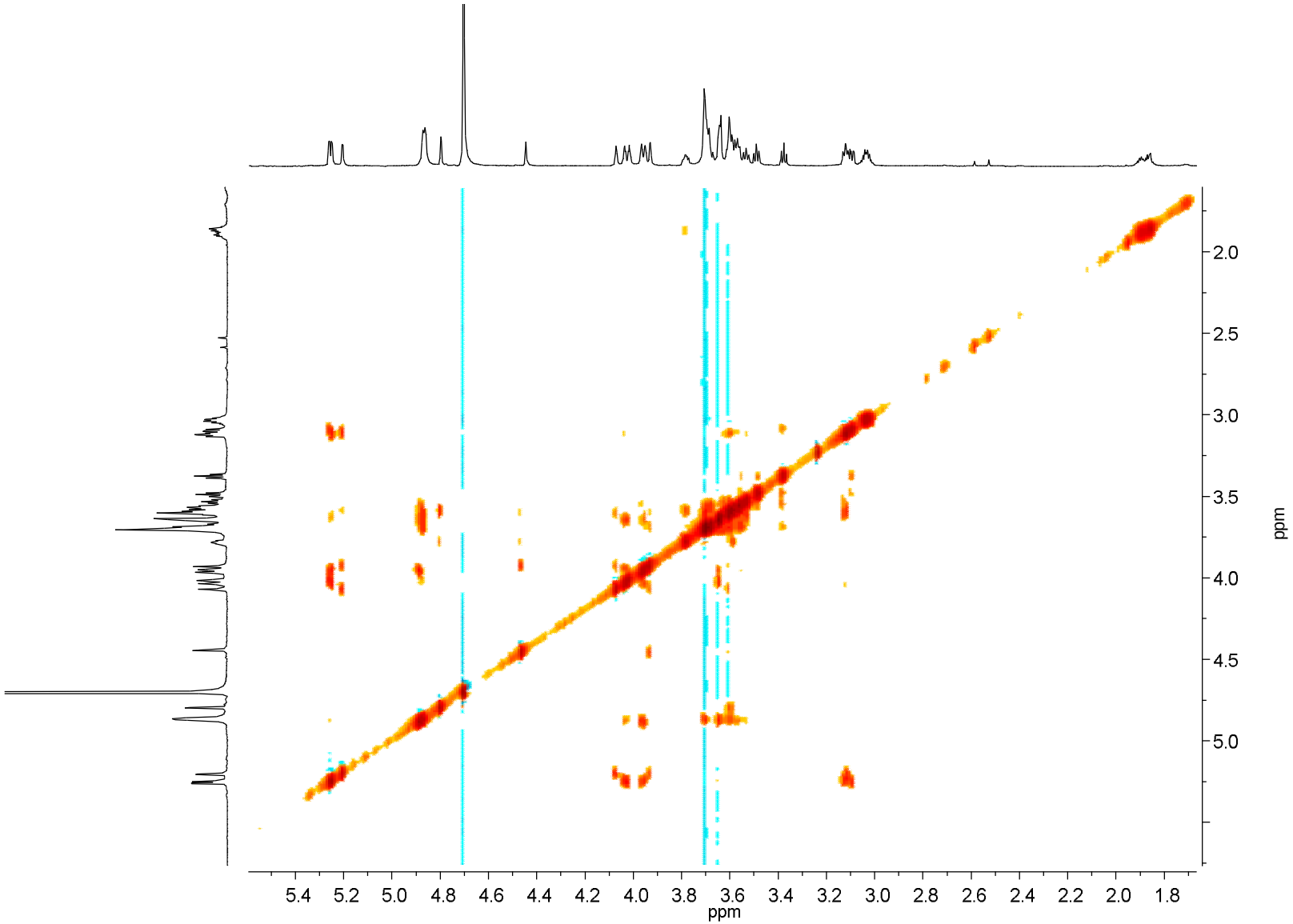


Figure 4.190. 900 MHz, D_2O, ^1H-^1H NOESY of **63**

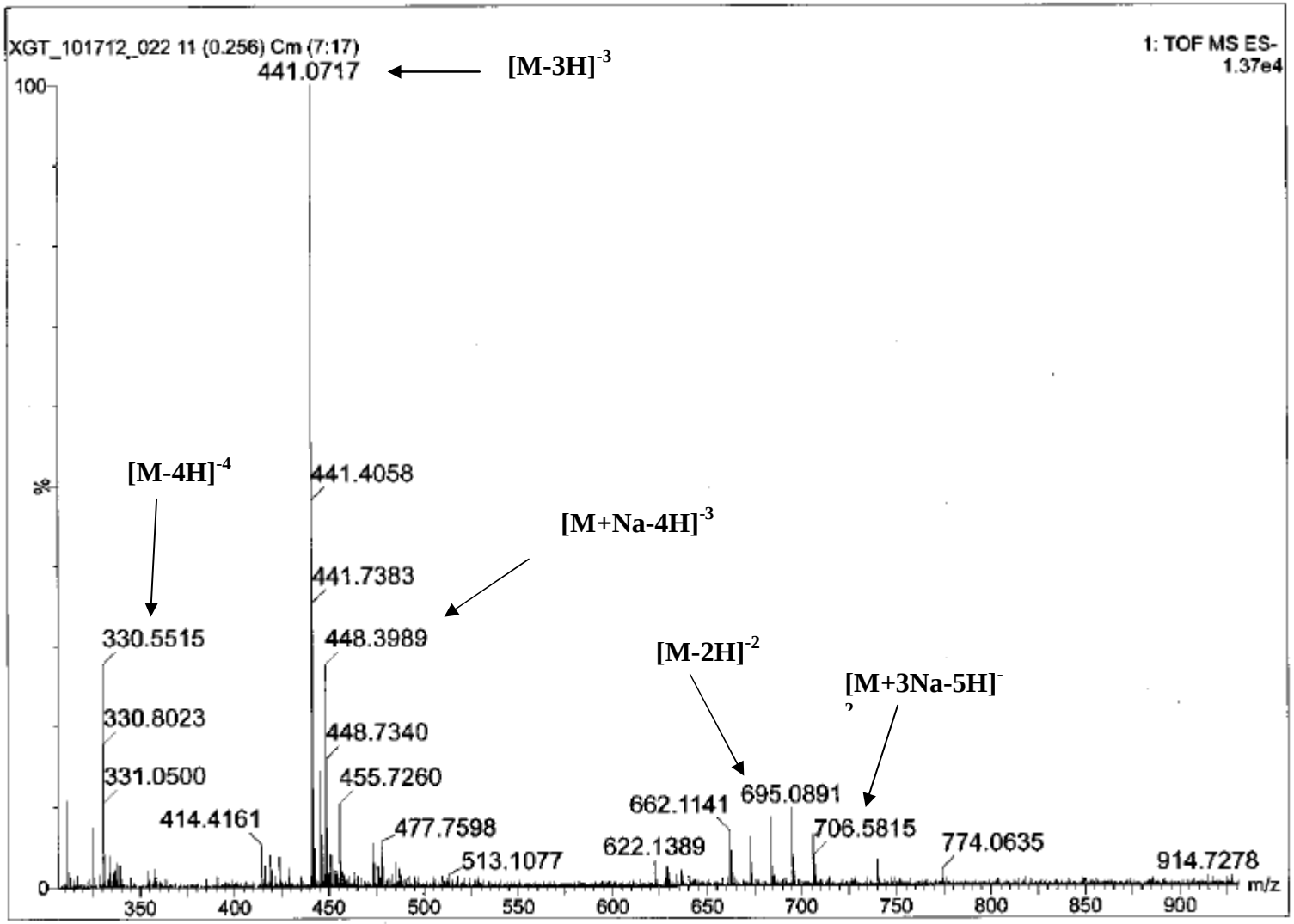


Figure 4.191. ESI-MS of **63**

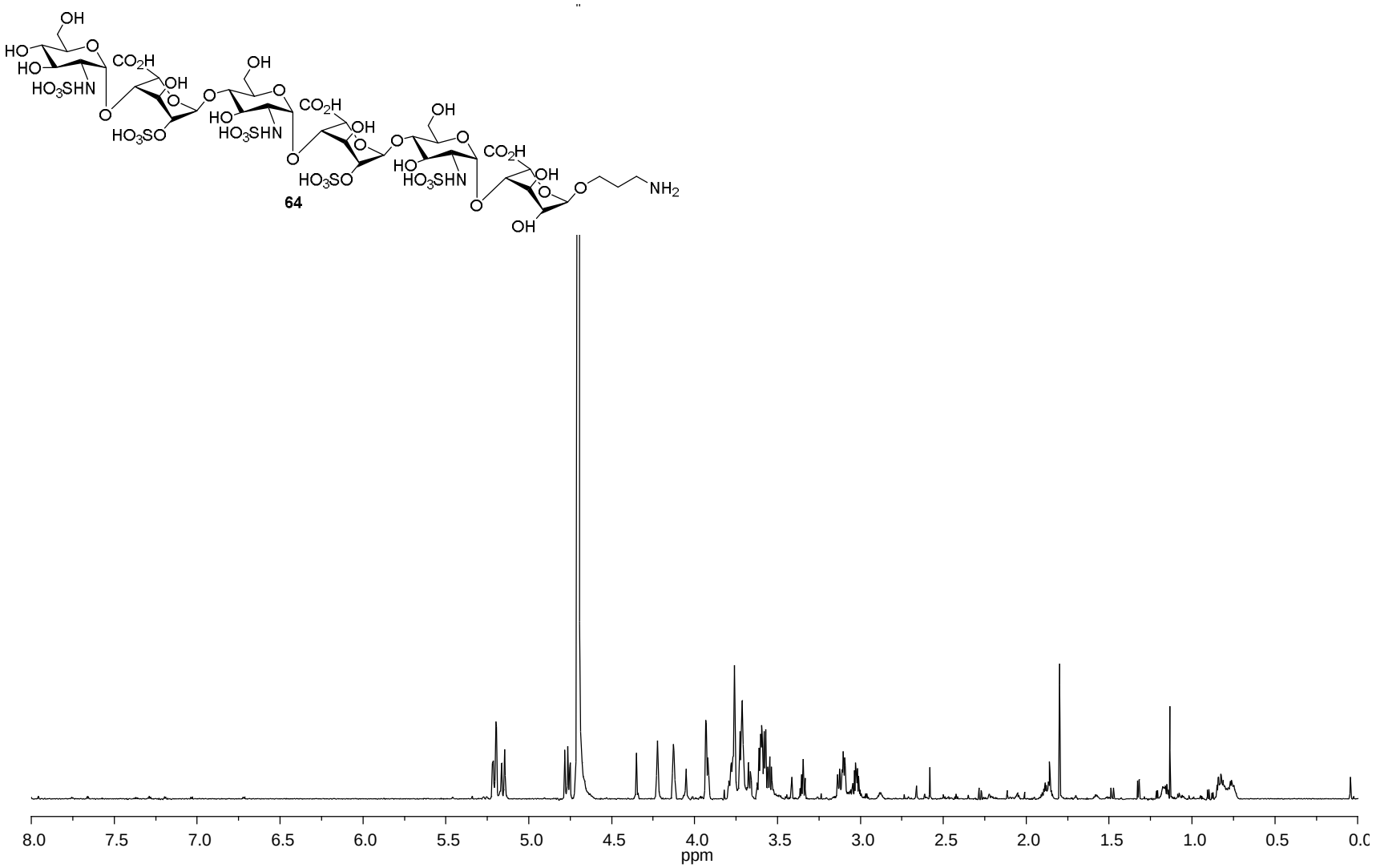


Figure 4.192. 900 MHz, D_2O, ^1H NMR of **64**

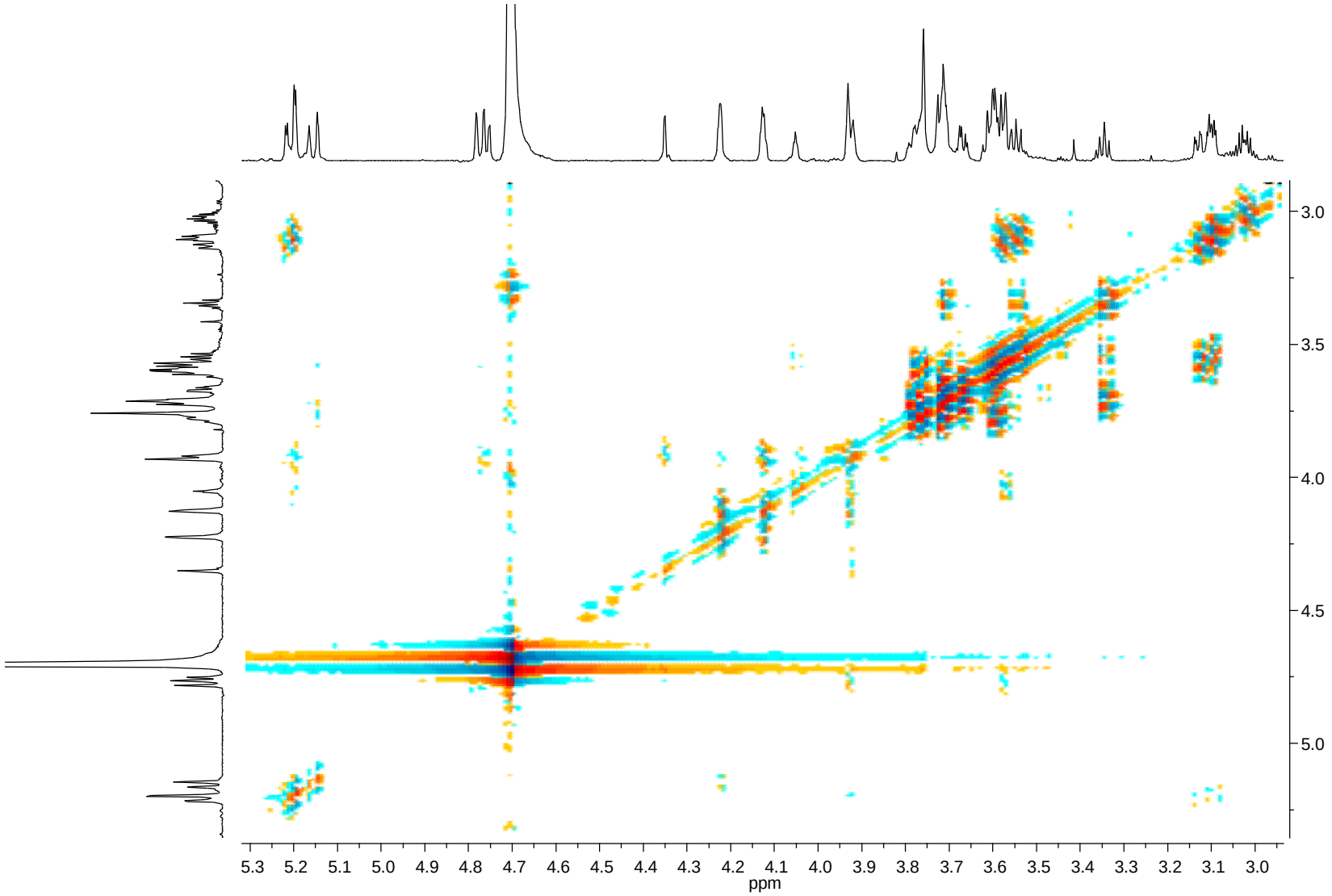


Figure 4.193. 900 MHz, D_2O, ^{1}H-^{1}H gCOSY of **64**

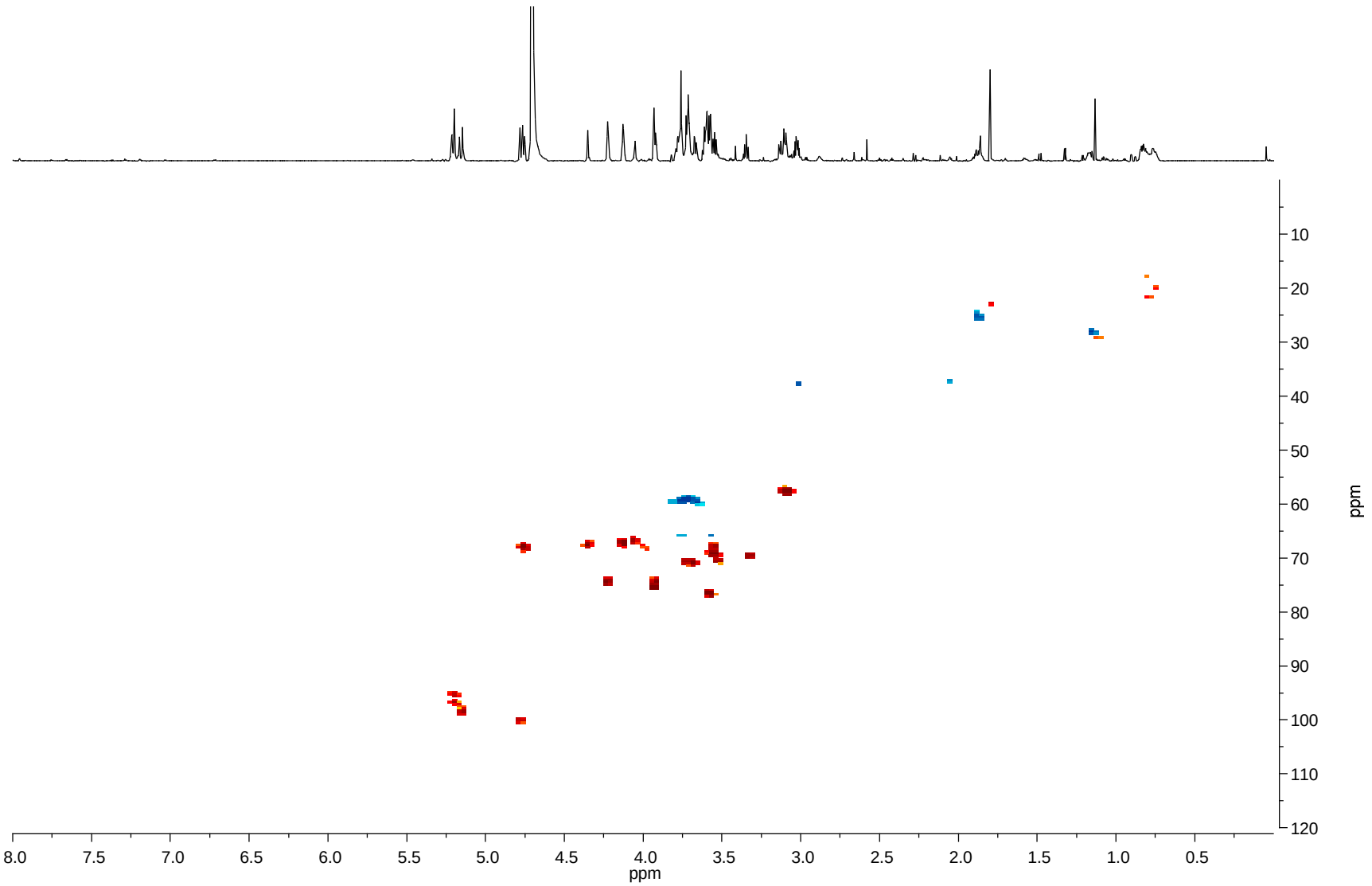


Figure 4.194. 900 MHz, D_2O, gHSQC of **64**

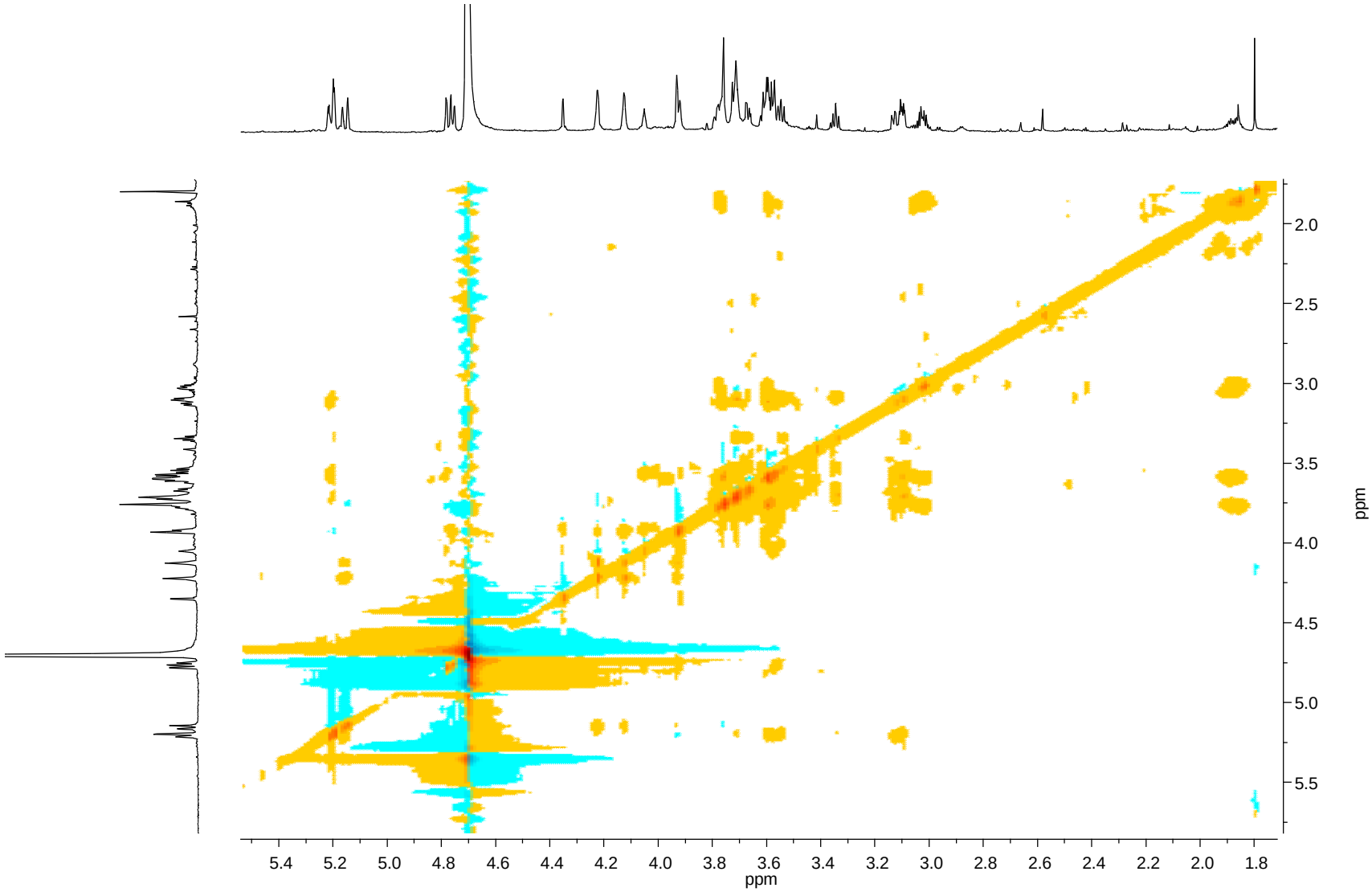


Figure 4.195. 900 MHz, D_2O, ^{1}H-^{1}H TOCSY of **64**

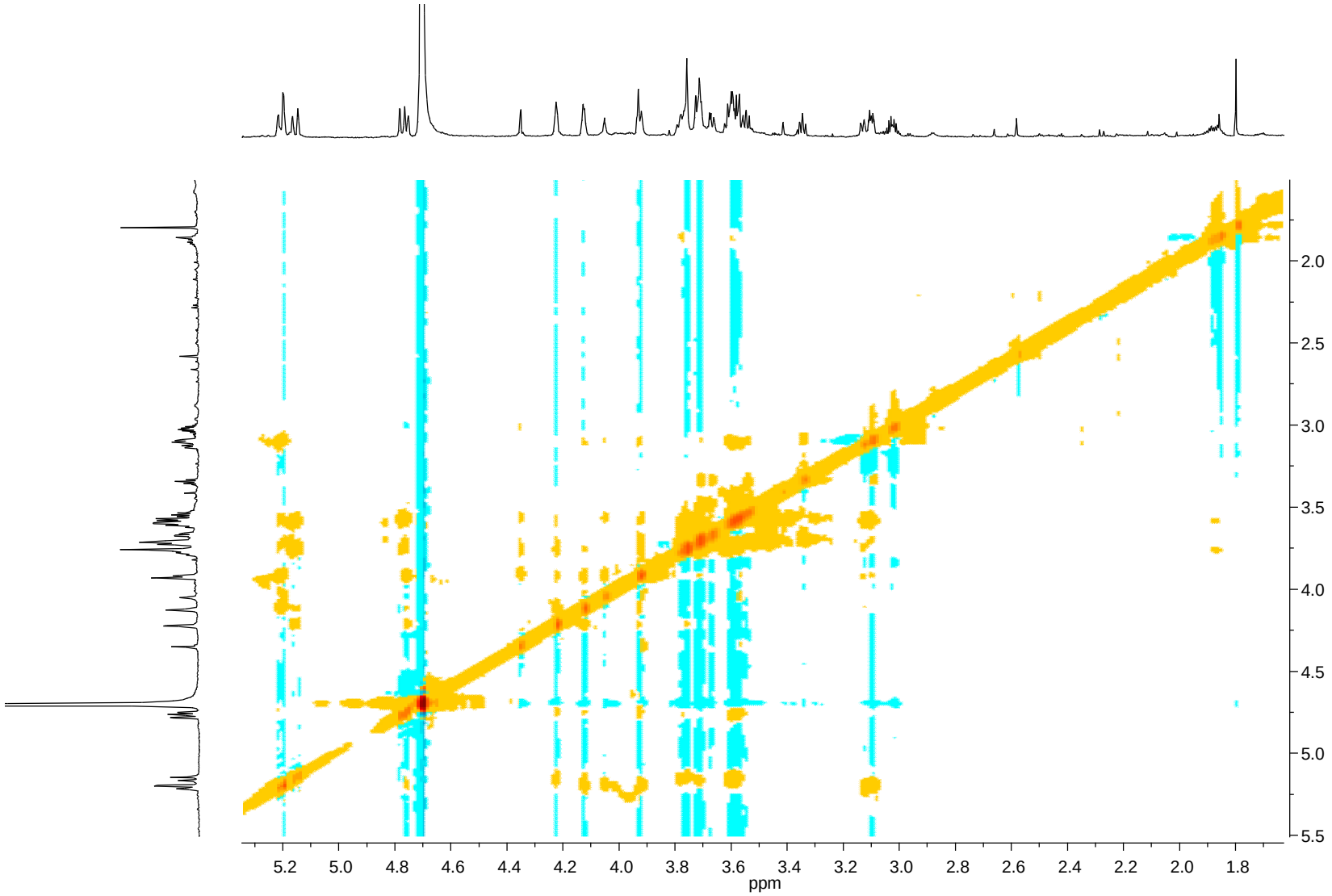


Figure 4.196. 900 MHz, D_2O, ^1H-^1H NOESY of **64**

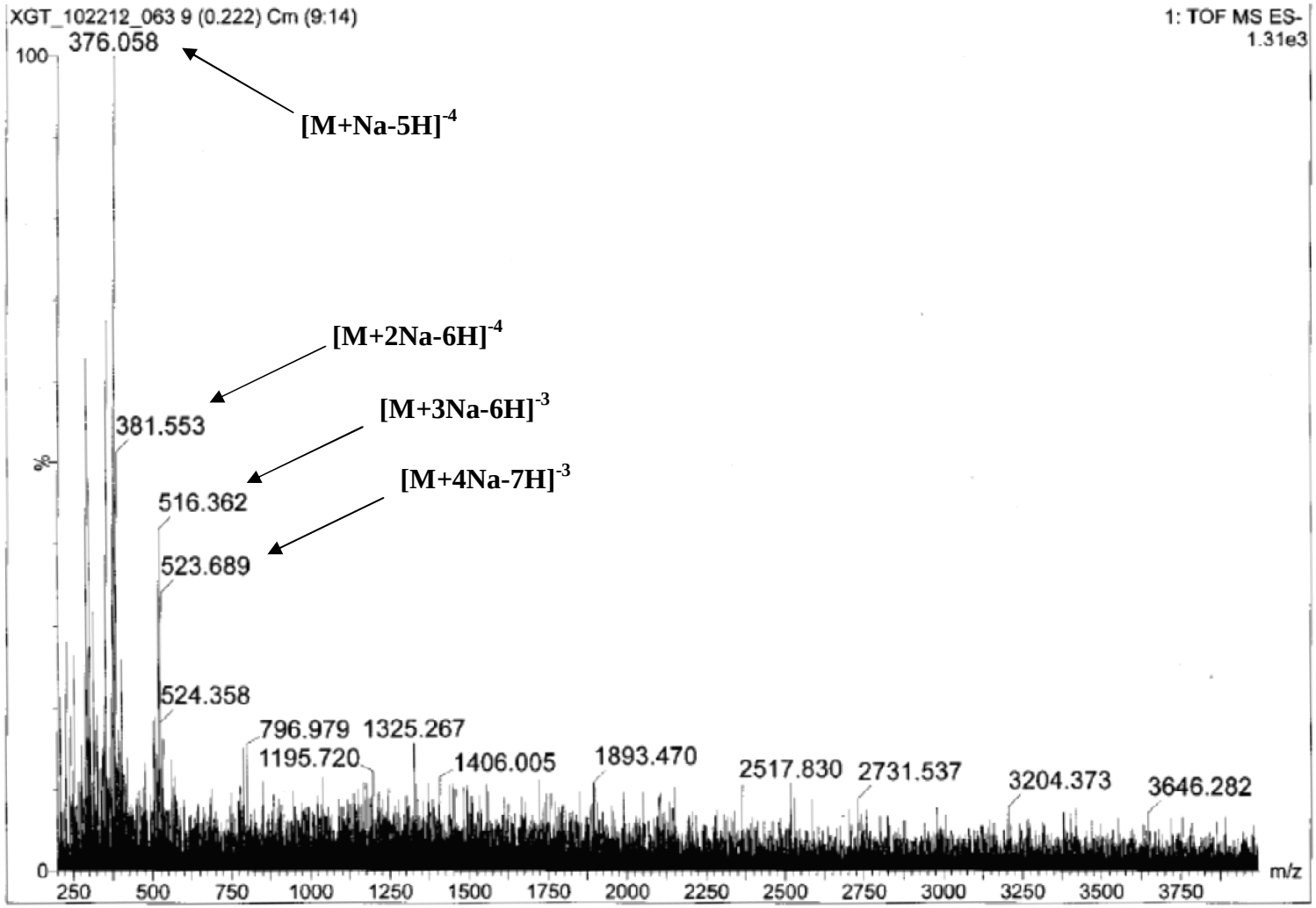


Figure 4.197. ESI-MS of **64**

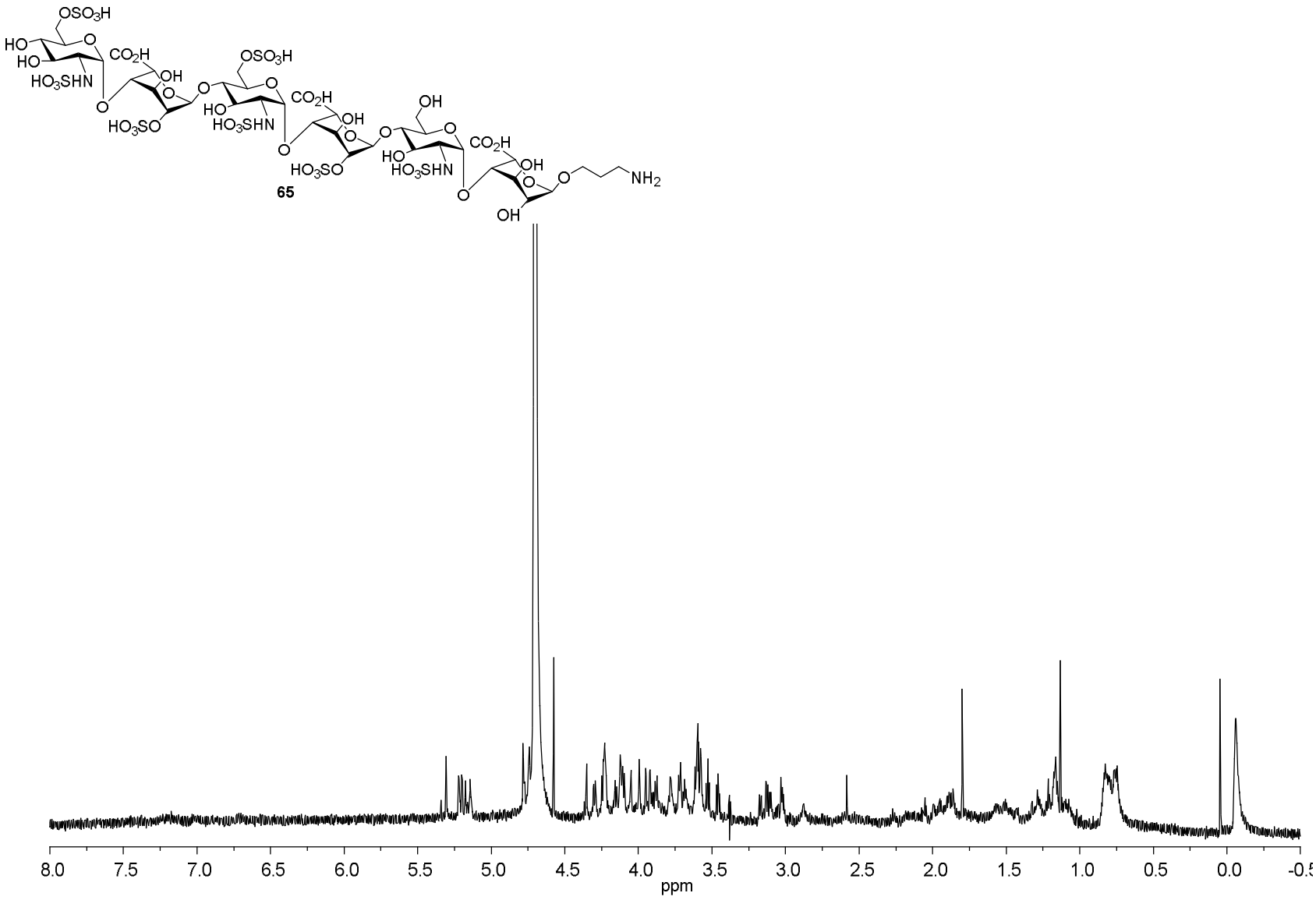


Figure 4.198. 900 MHz, D_2O, ^1H NMR of **65**

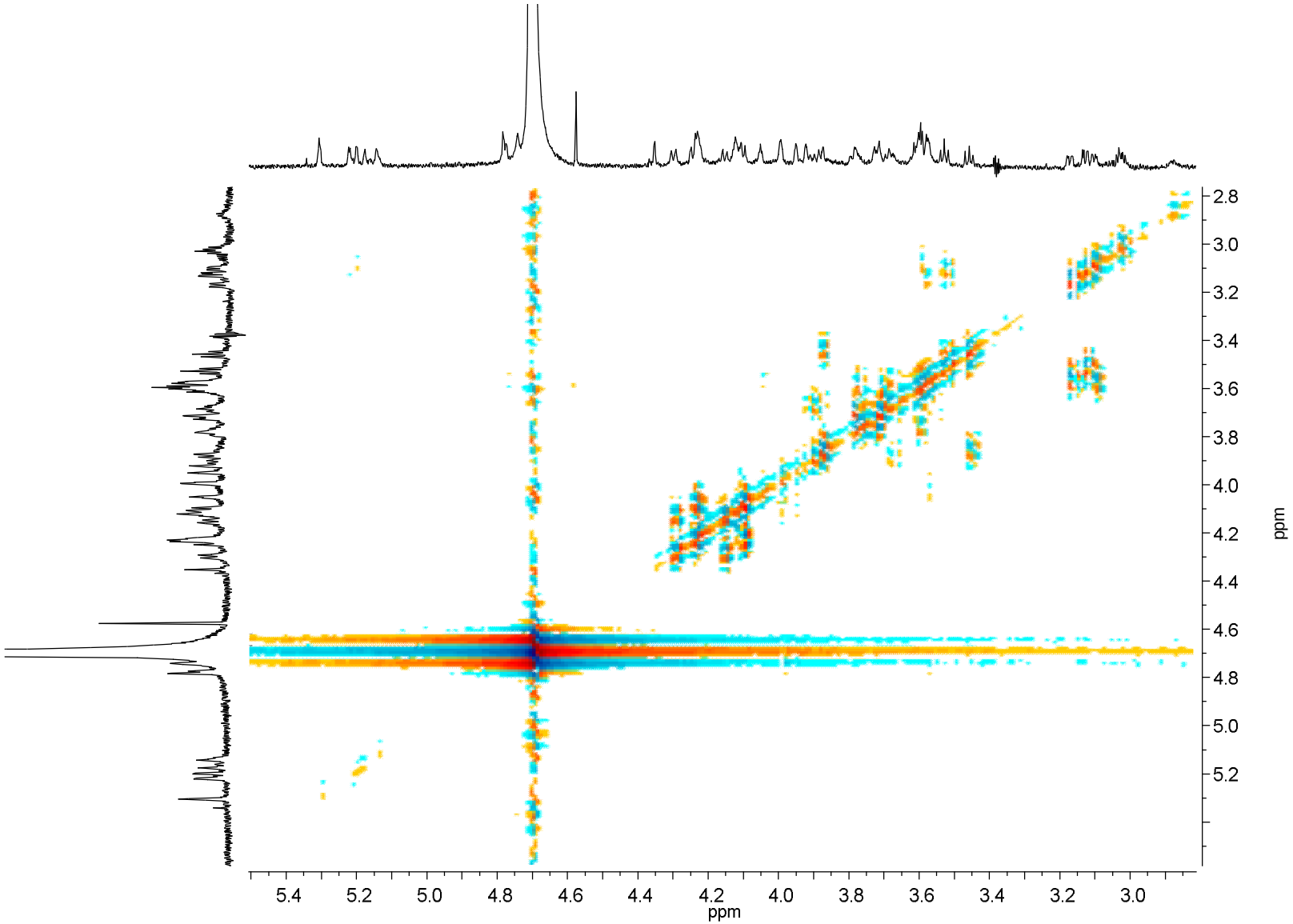


Figure 4.199. 900 MHz, D_2O, ^{1}H-^{1}H gCOSY of **65**

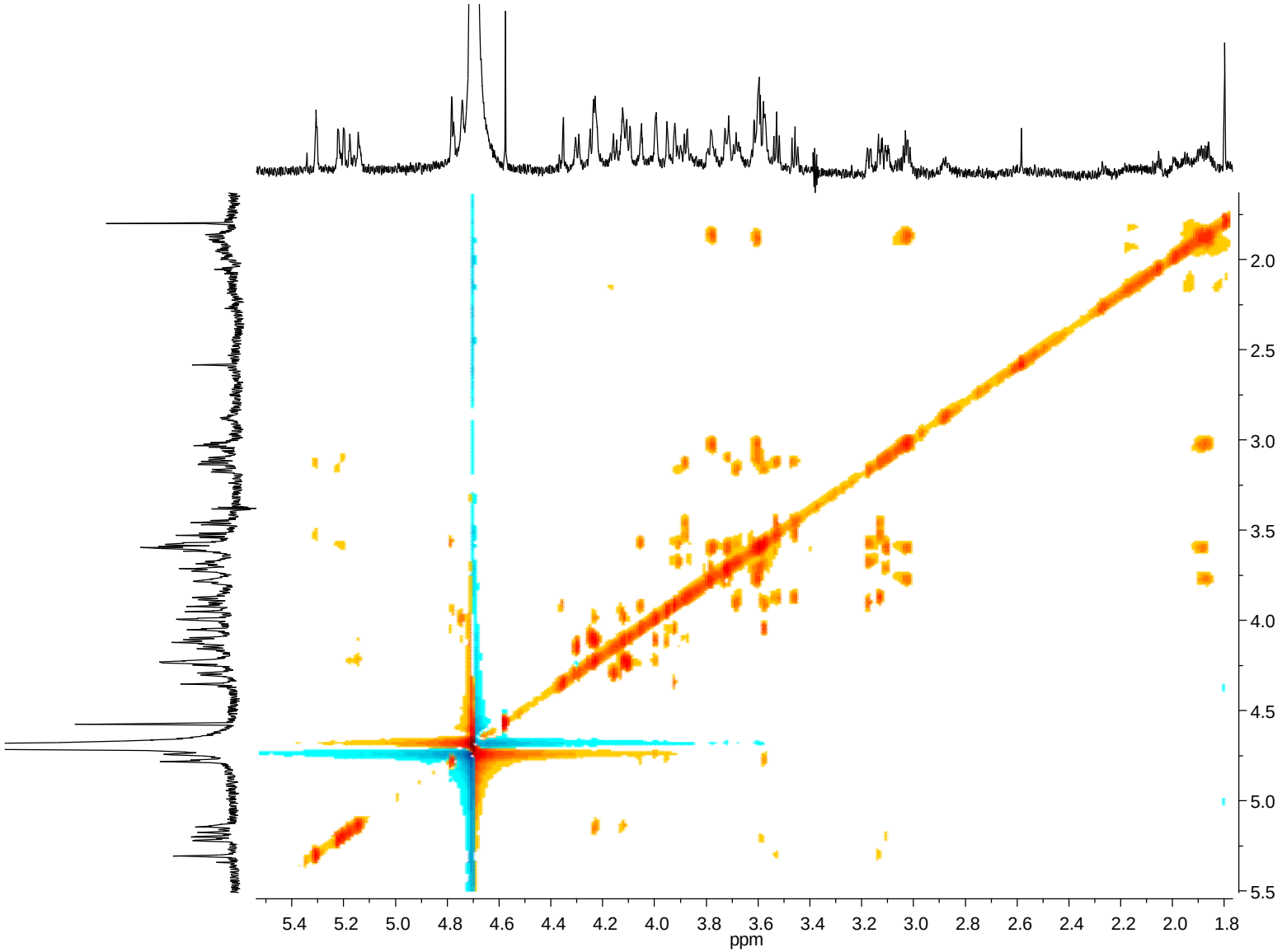


Figure 4.200. 900 MHz, D_2O, ^{1}H-^{1}H TOCSY of **65**

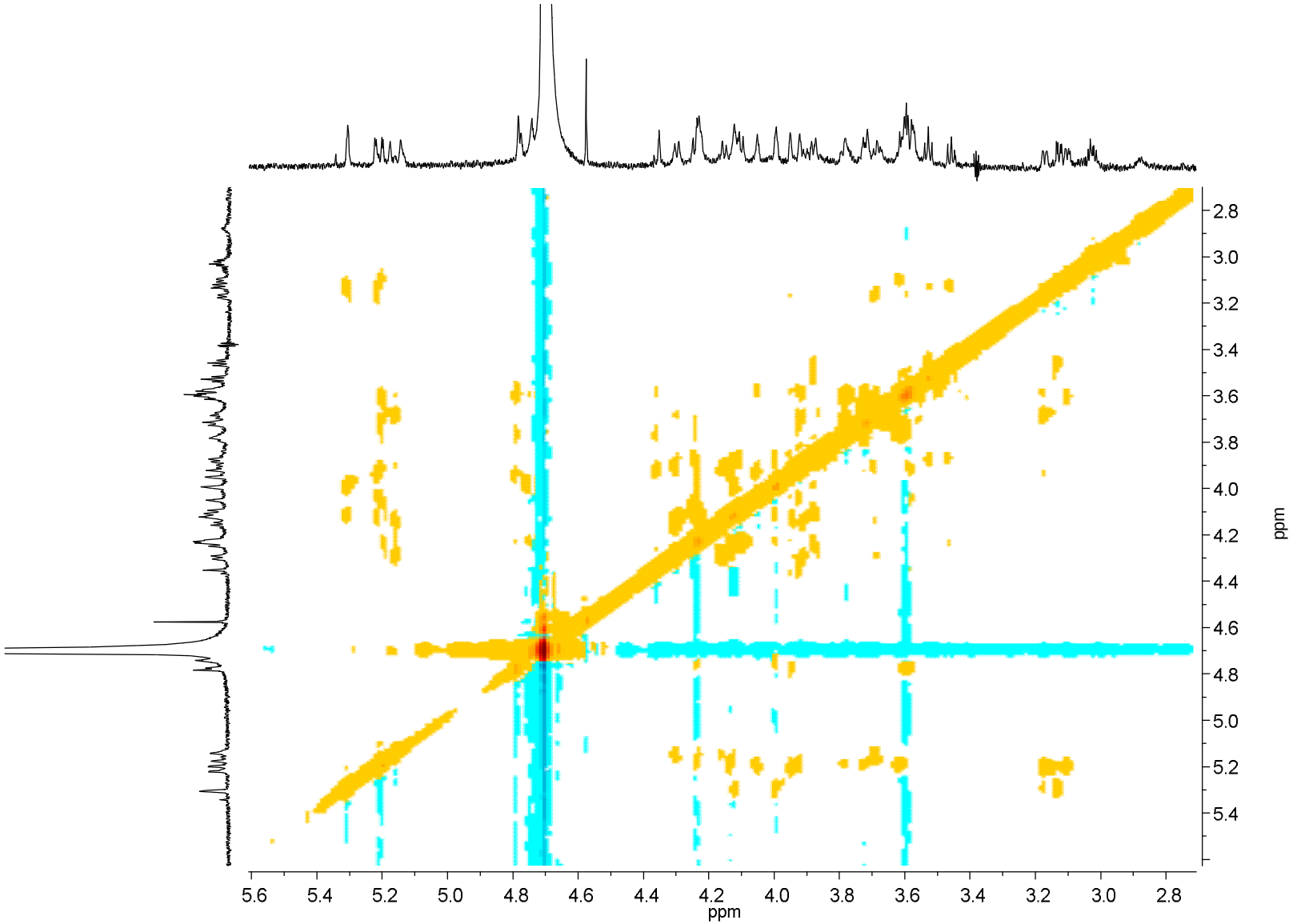


Figure 4.201. 900 MHz, D_2O, ^1H-^1H NOESY of **65**

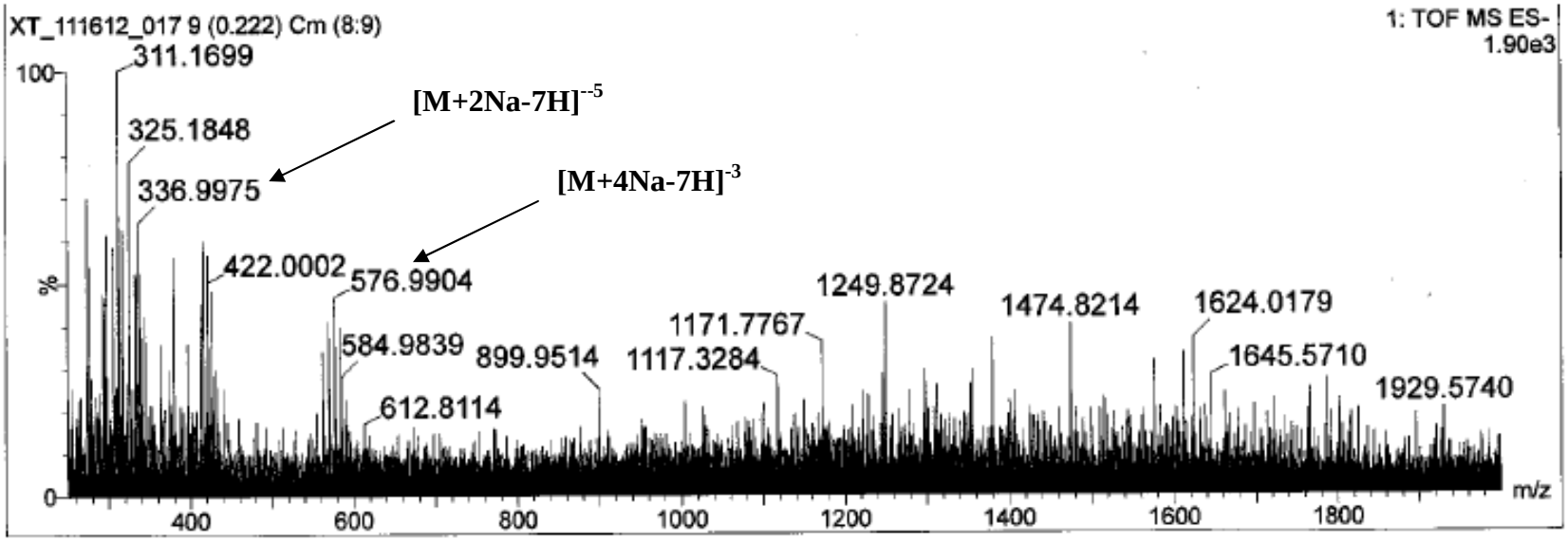


Figure 4.202. ESI-MS of **65**

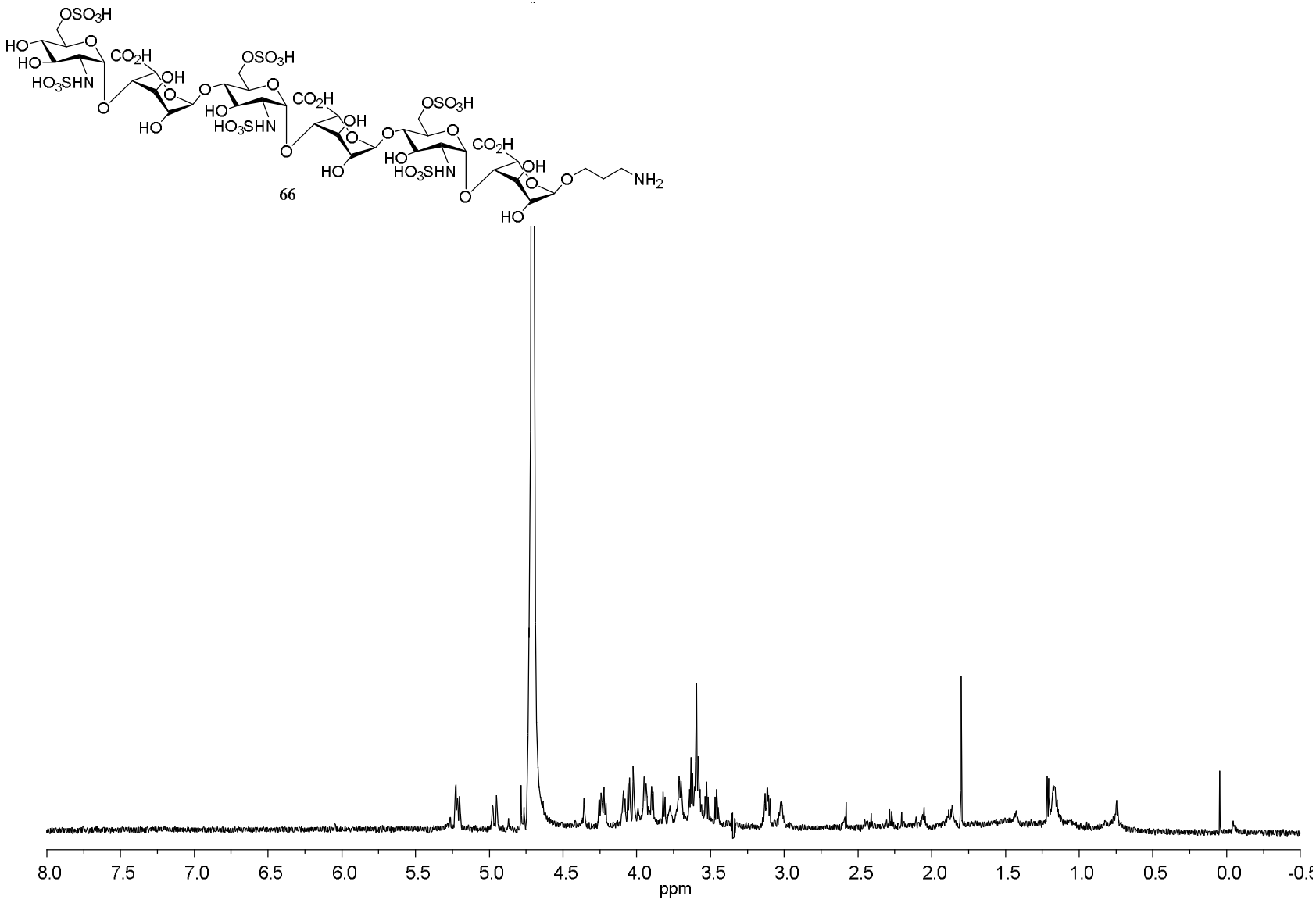


Figure 4.203. 900 MHz, D_2O, ^1H NMR of **66**

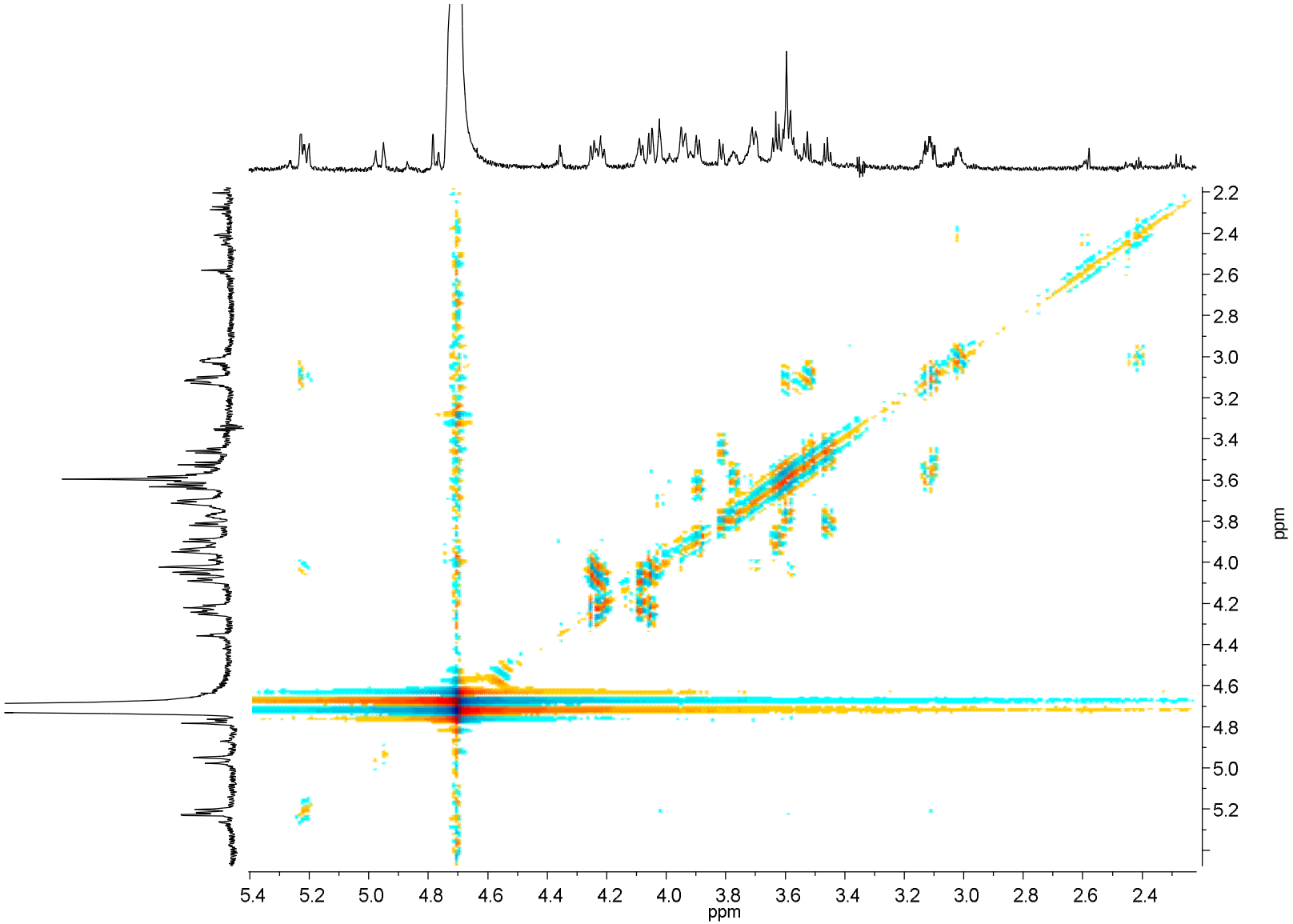


Figure 4.204. 900 MHz, D_2O, ^{1}H-^{1}H gCOSY of **66**

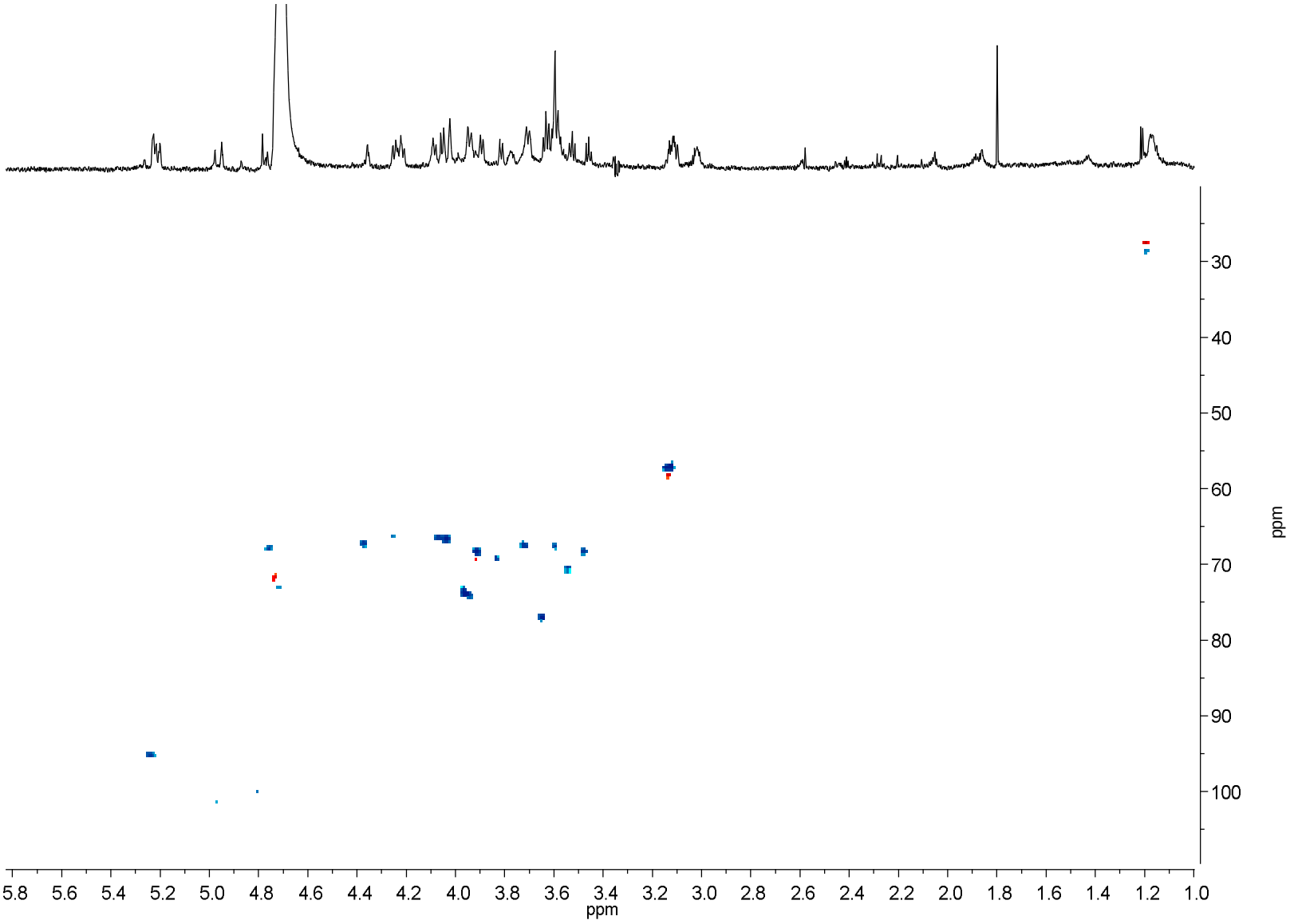


Figure 4.205. 900 MHz, D_2O, gHSQC of **66**

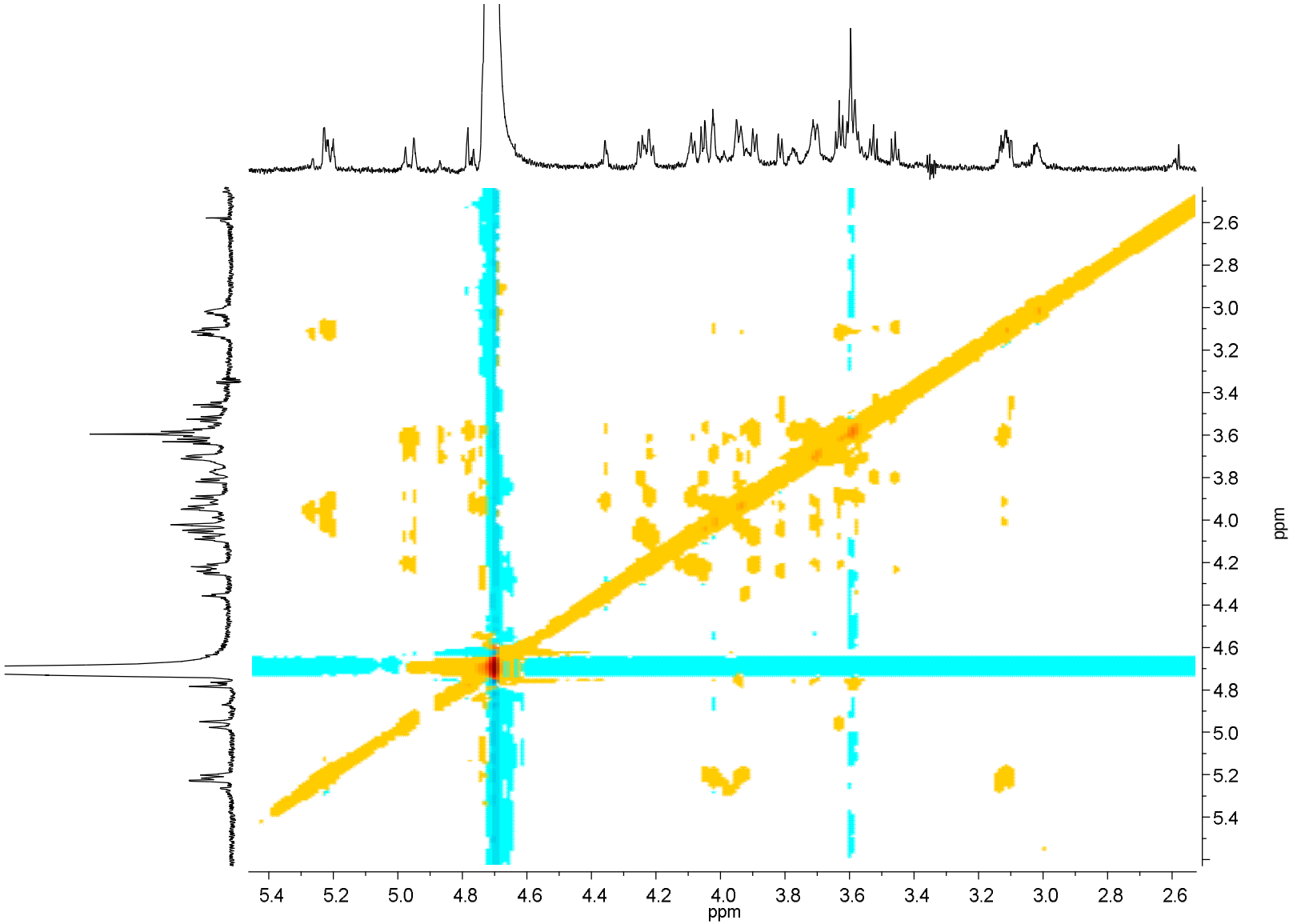


Figure 4.206. 900 MHz, D_2O, ^1H-^1H NOESY of **66**

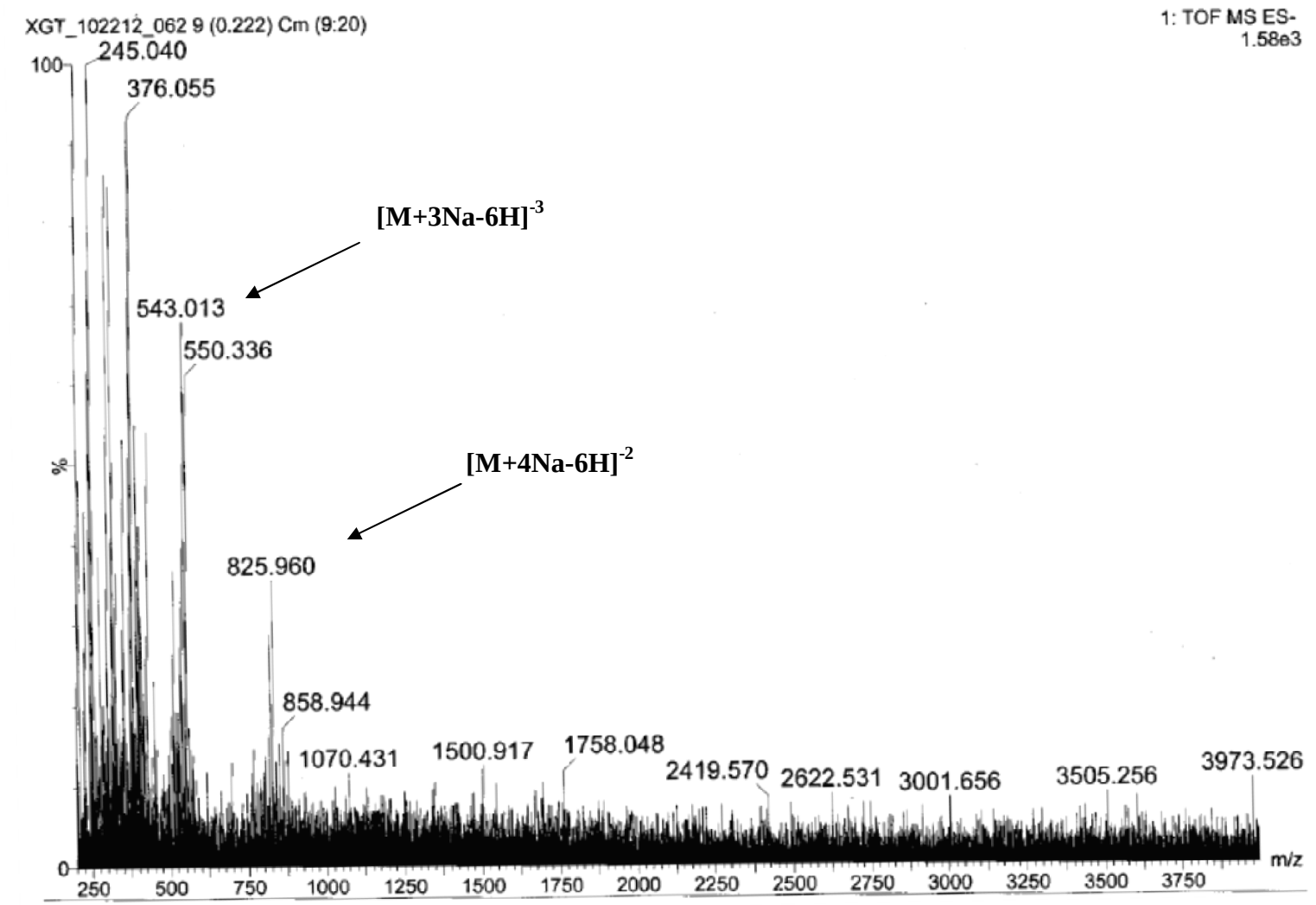


Figure 4.207. ESI-MS of **66**

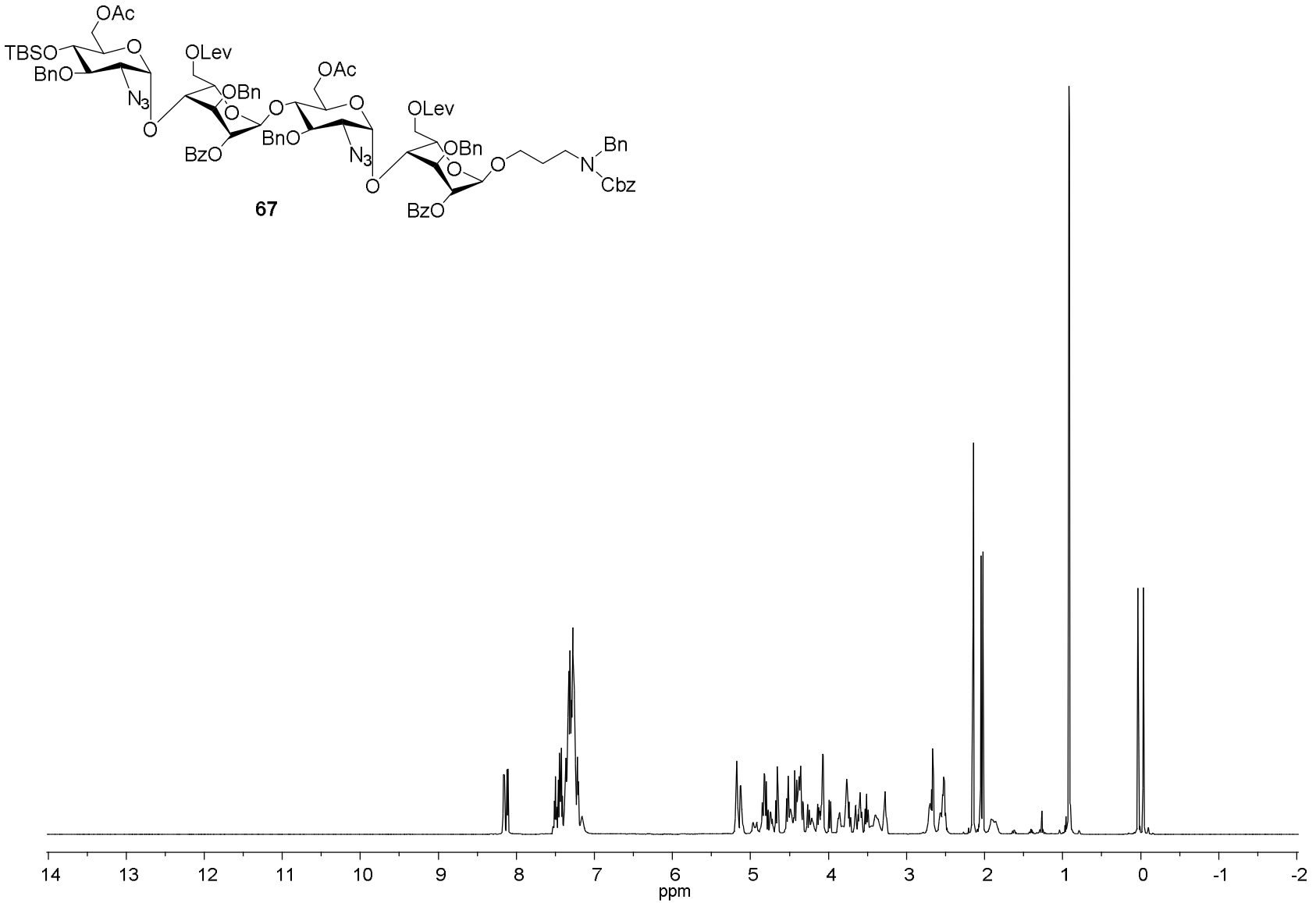


Figure 4.208. 500 MHz, $CDCl_3$, ^1H NMR of **67**

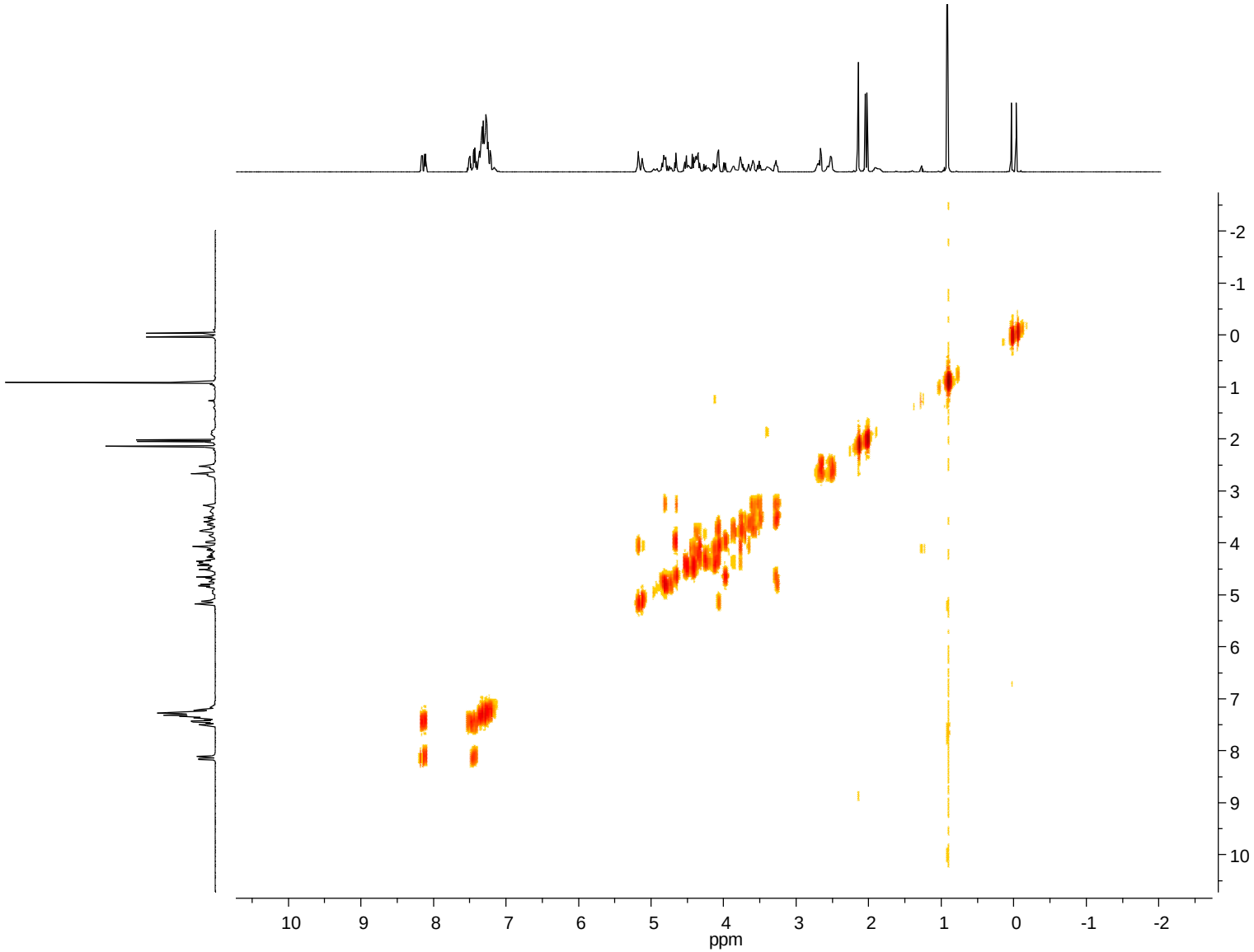


Figure 4.209. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **67**

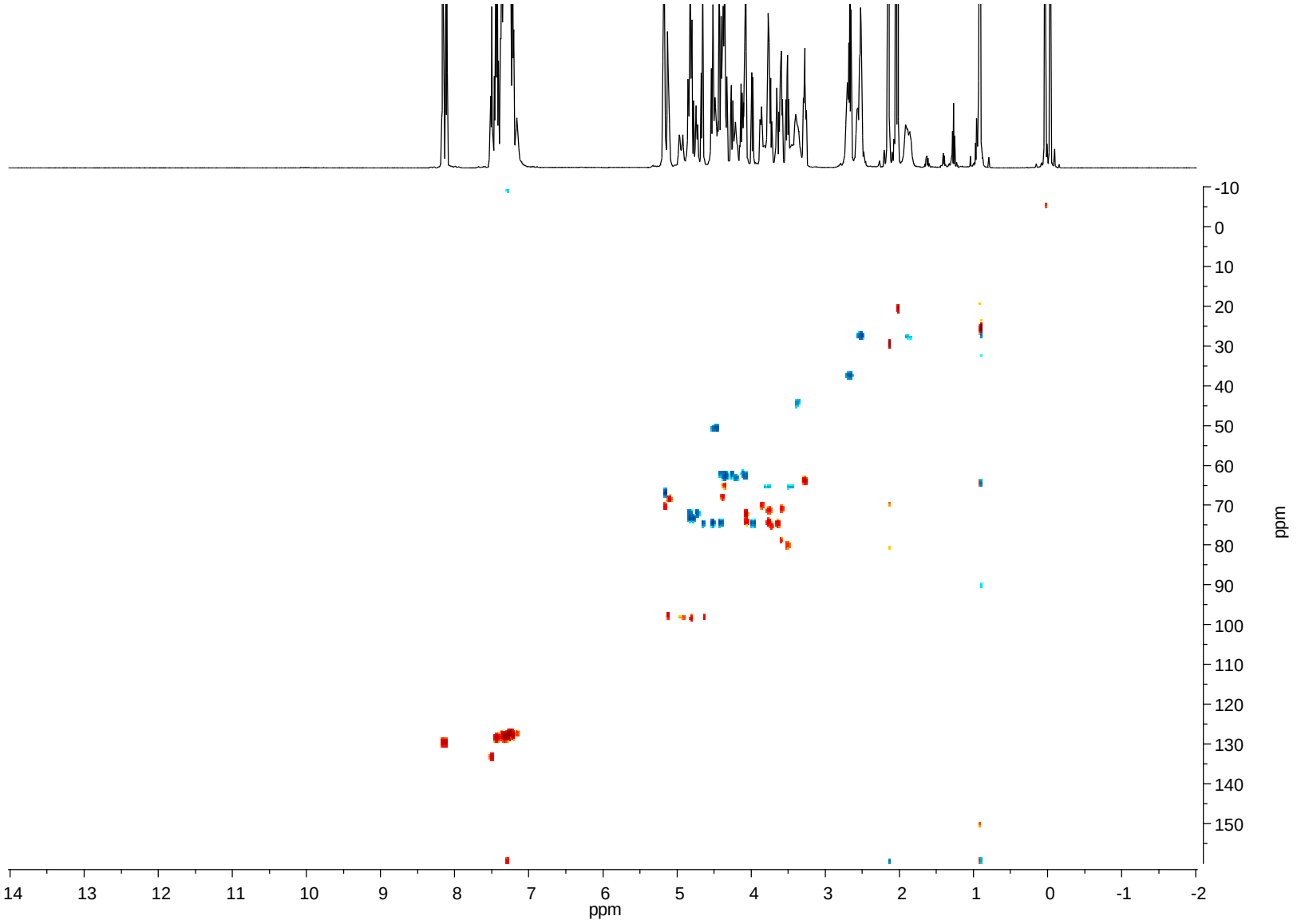


Figure 4.210. 500 MHz, $CDCl_3$, gHSQC of **67**

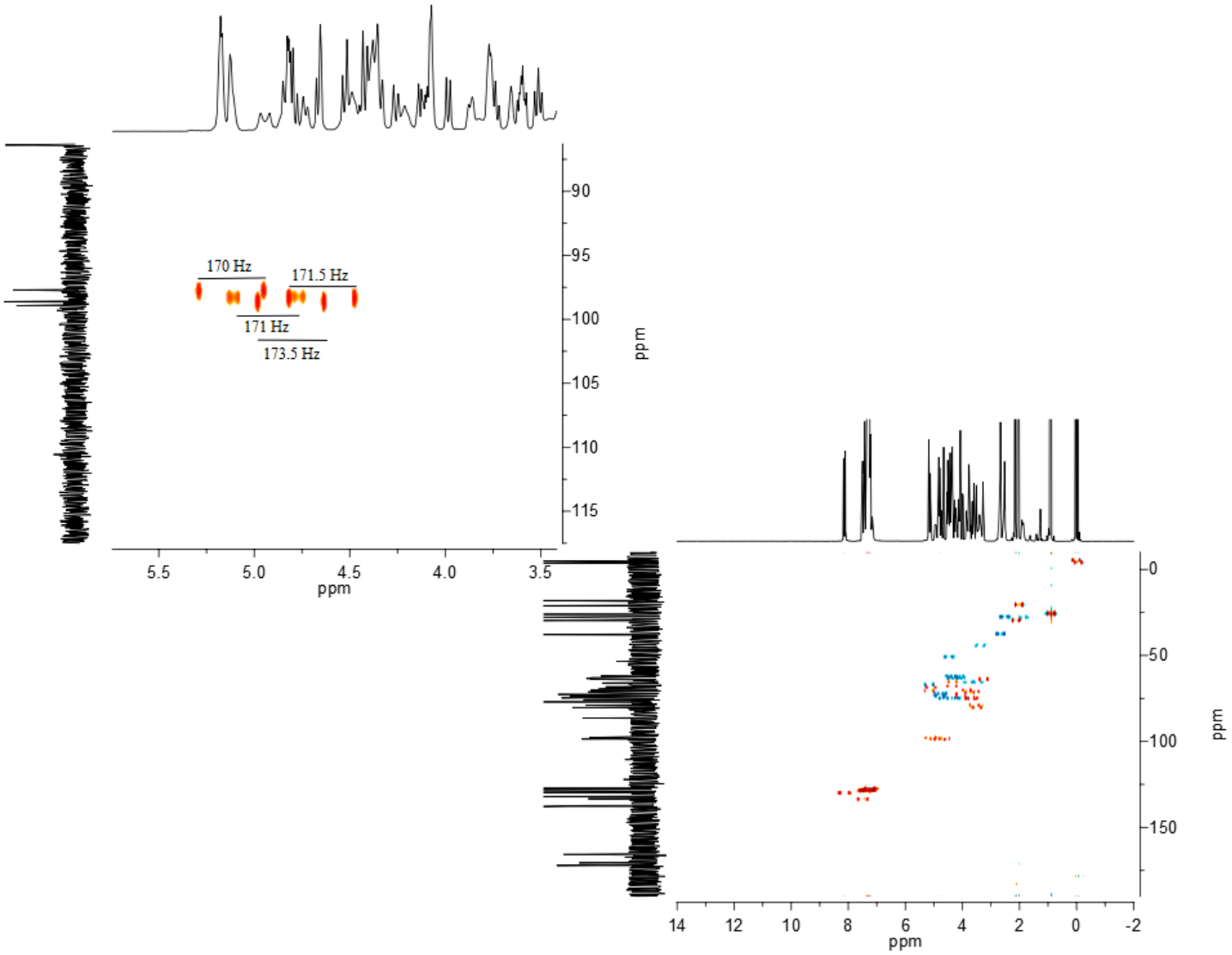


Figure 4.211. 500 MHz, $CDCl_3$, gHSQC (without ^1H Decoupling) of **67**

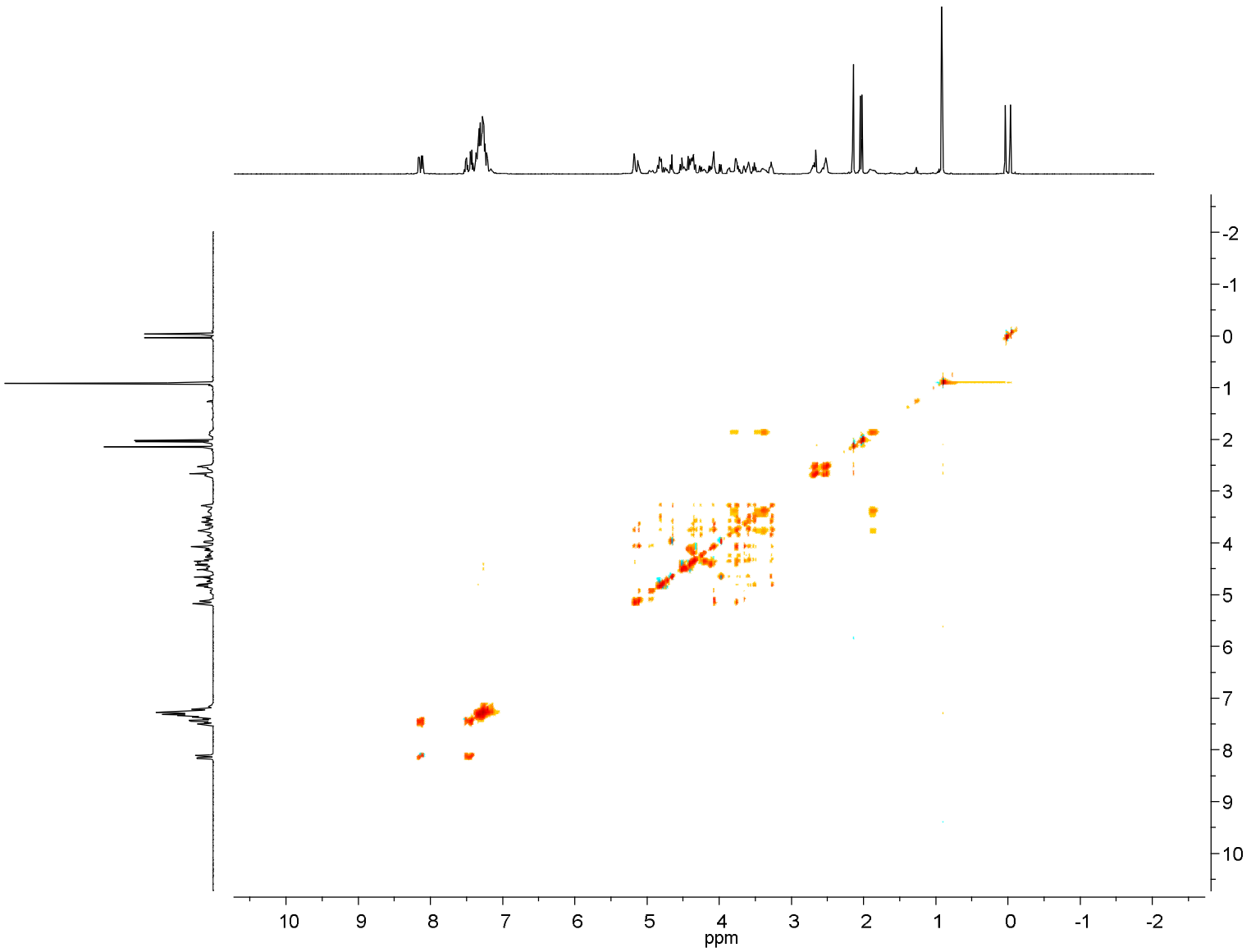


Figure 4.212. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **67**

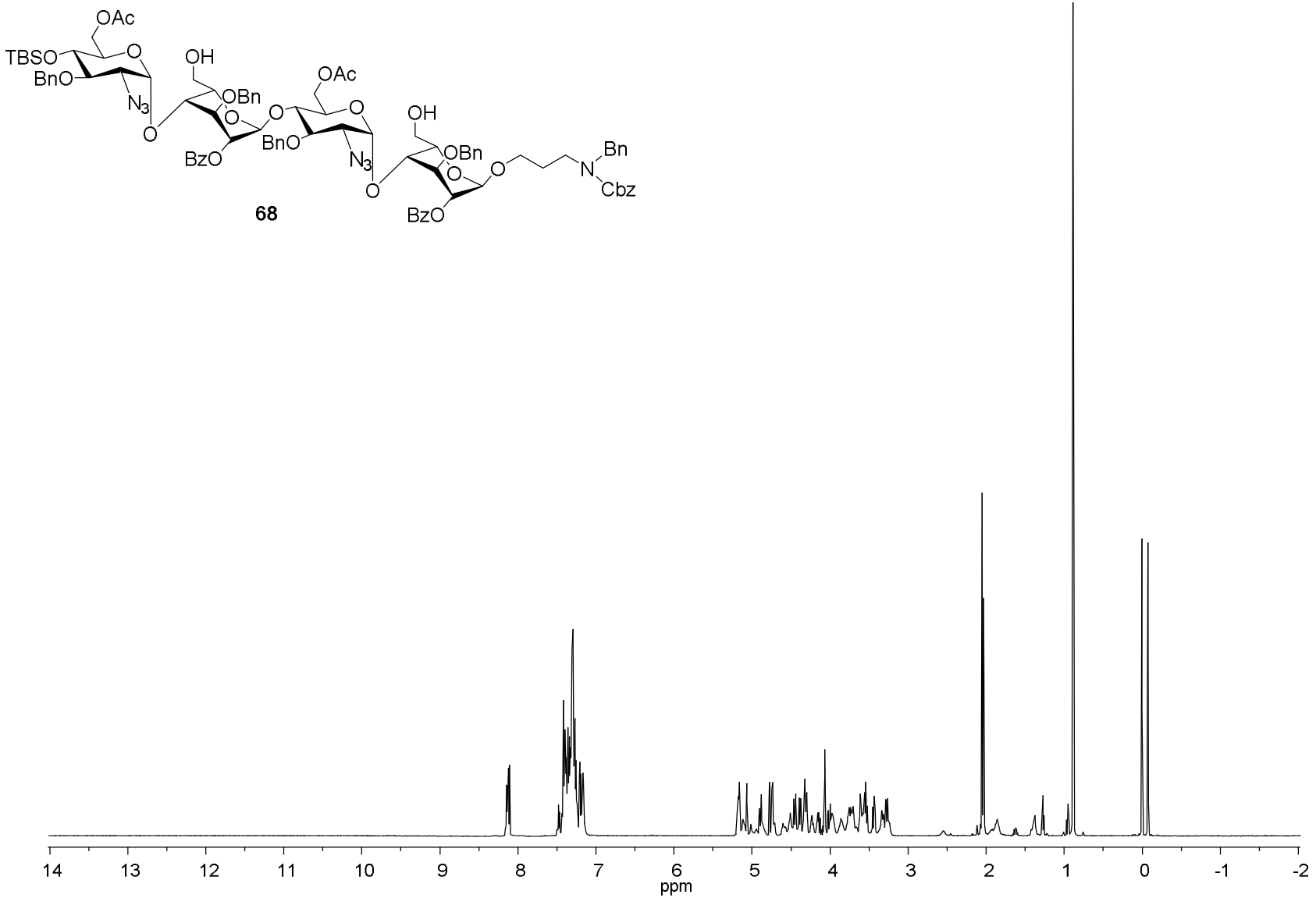


Figure 4.213. 500 MHz, $CDCl_3$, ^1H NMR of **68**

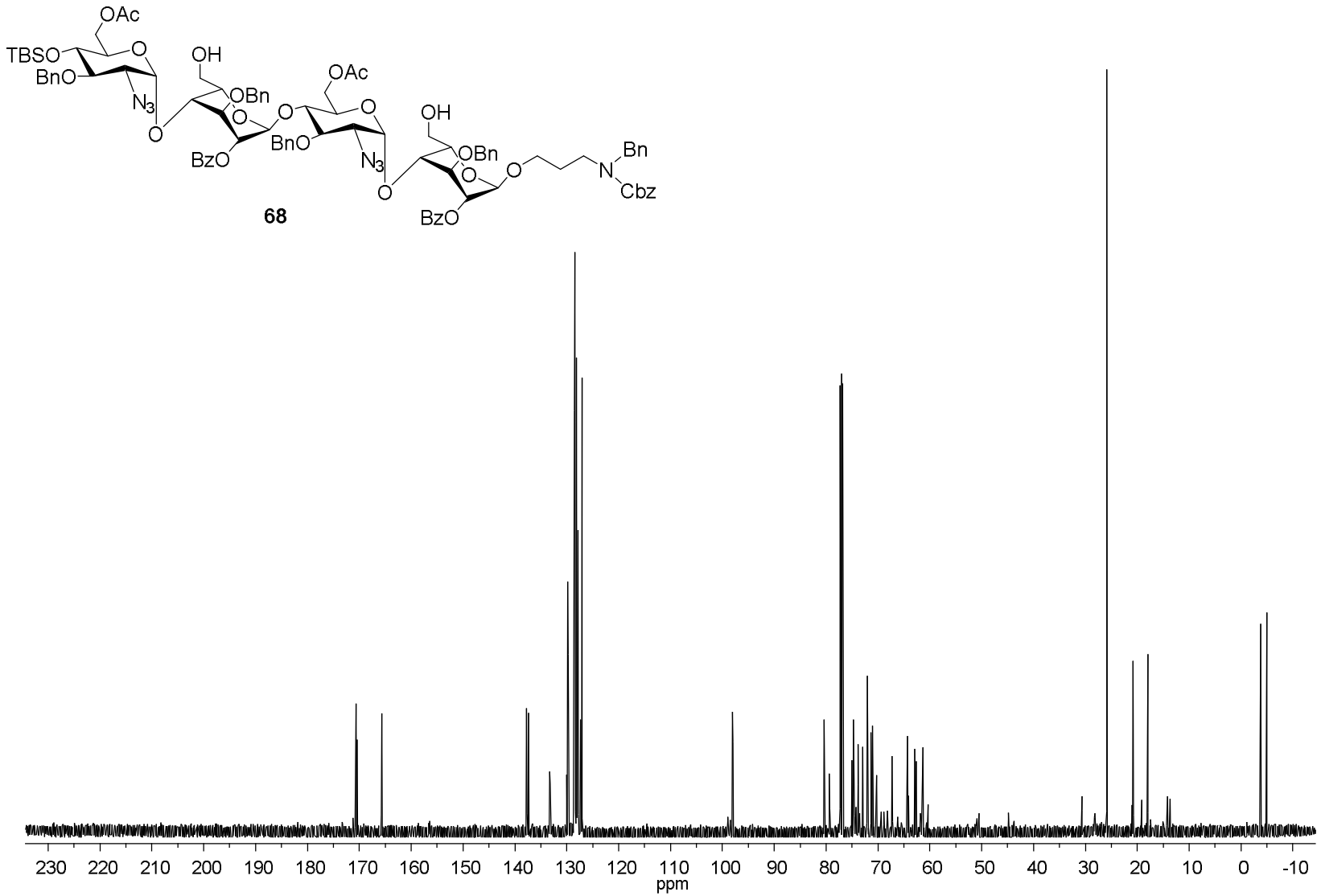


Figure 4.214. 125 MHz, $CDCl_3$, ^{13}C NMR of **68**

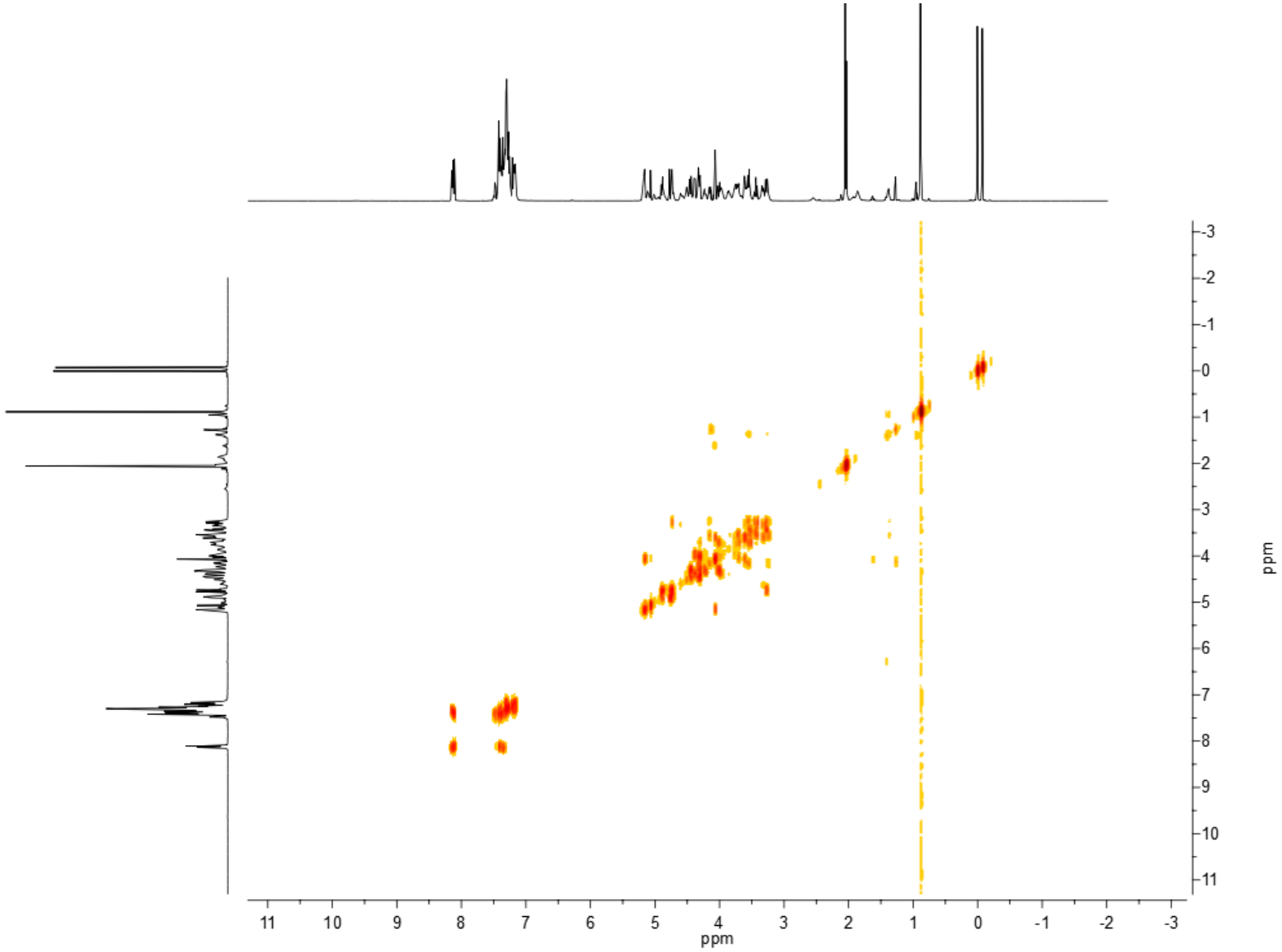


Figure 4.215. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **68**

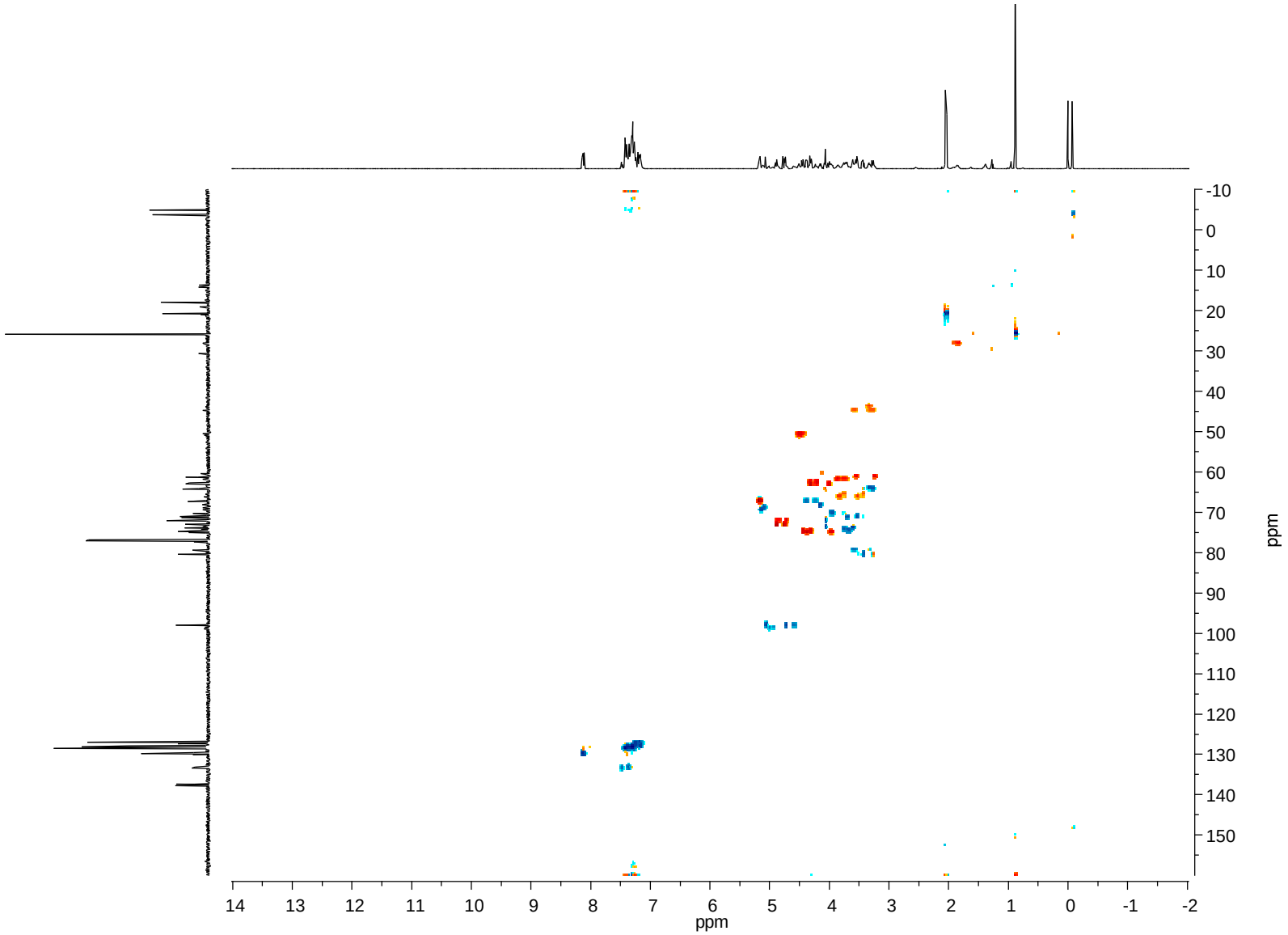


Figure 4.216. 500 MHz, $CDCl_3$, gHSQC of **68**

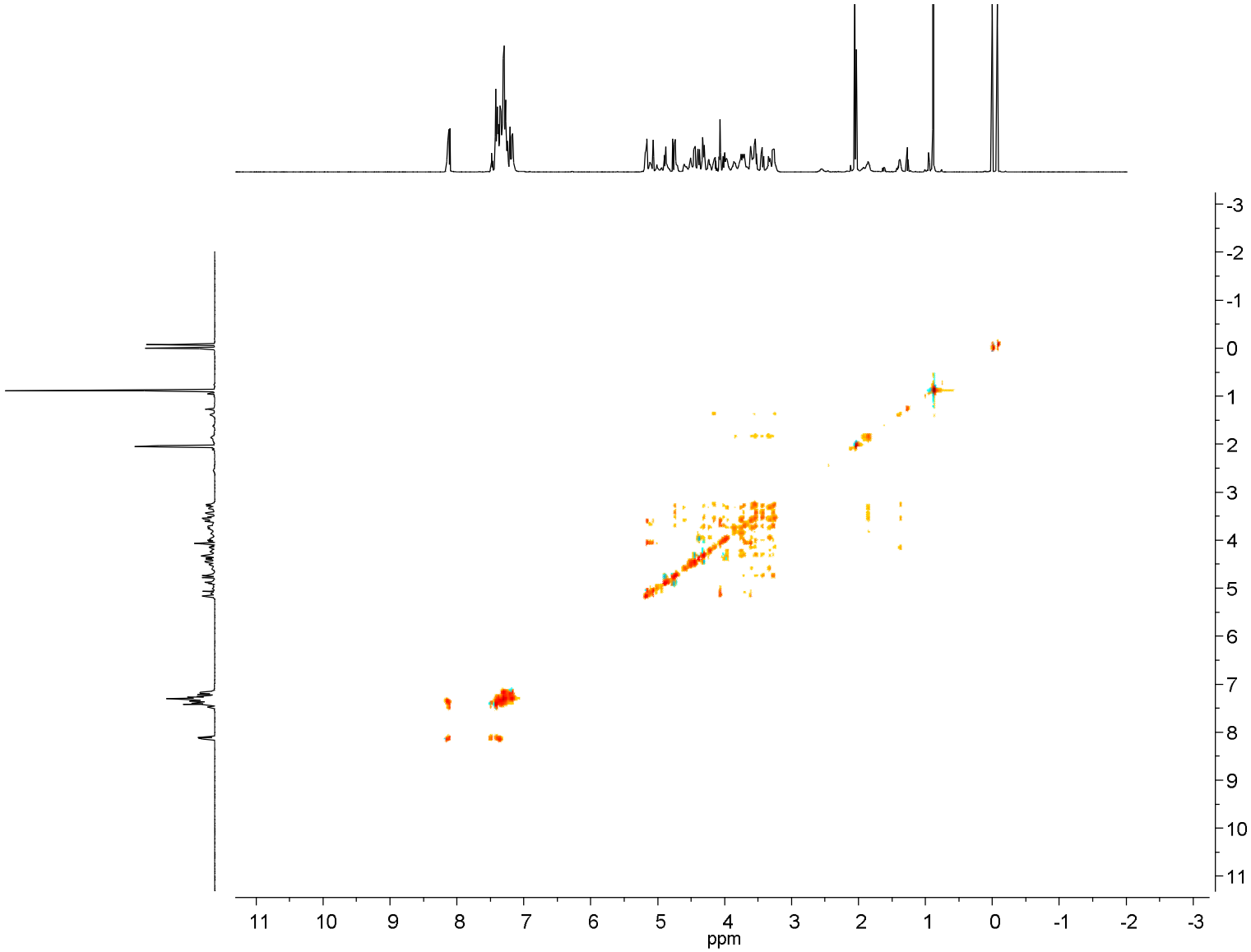


Figure 4.217. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **68**

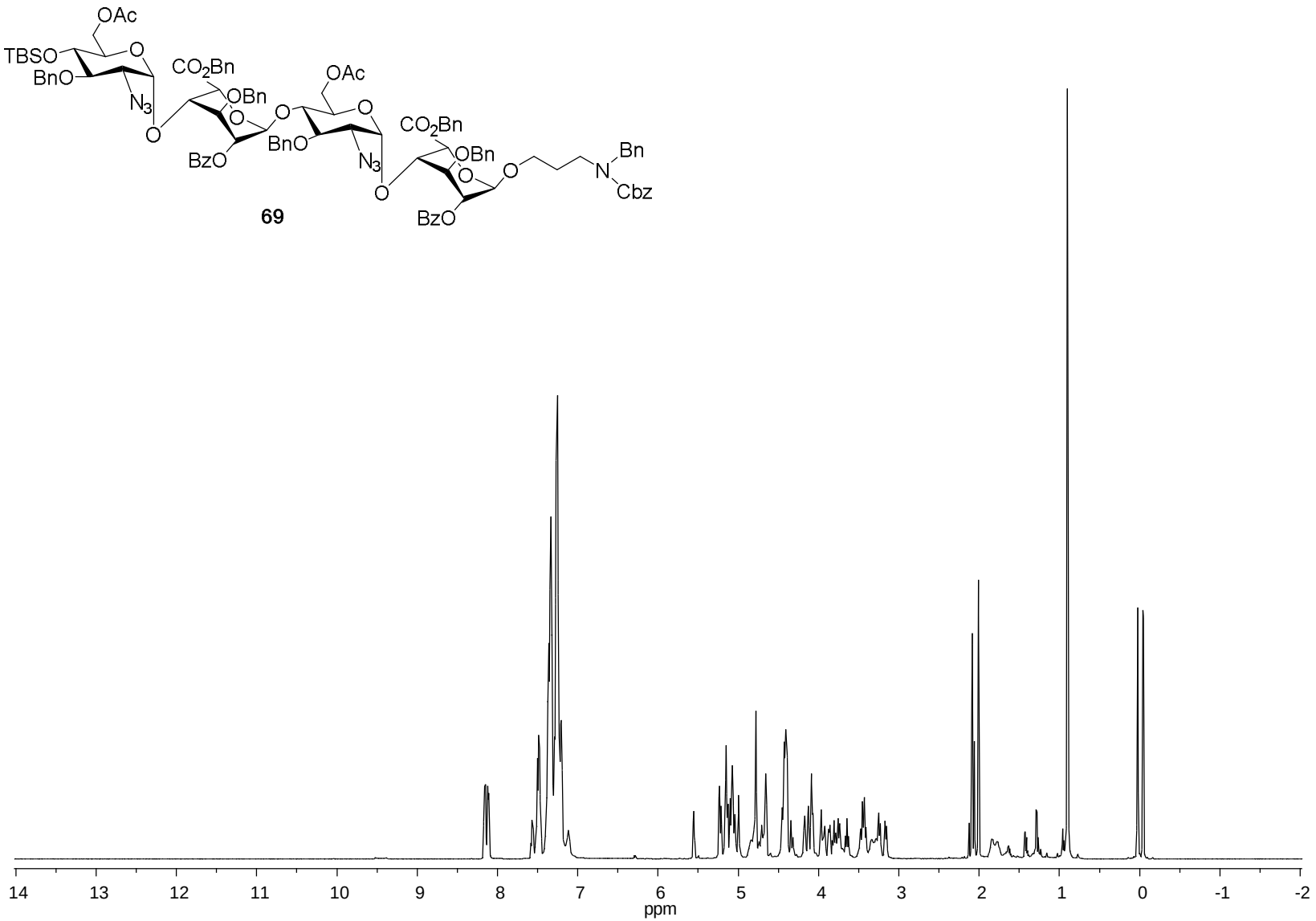


Figure 4.218. 500 MHz, $CDCl_3$, ^1H NMR of **69**

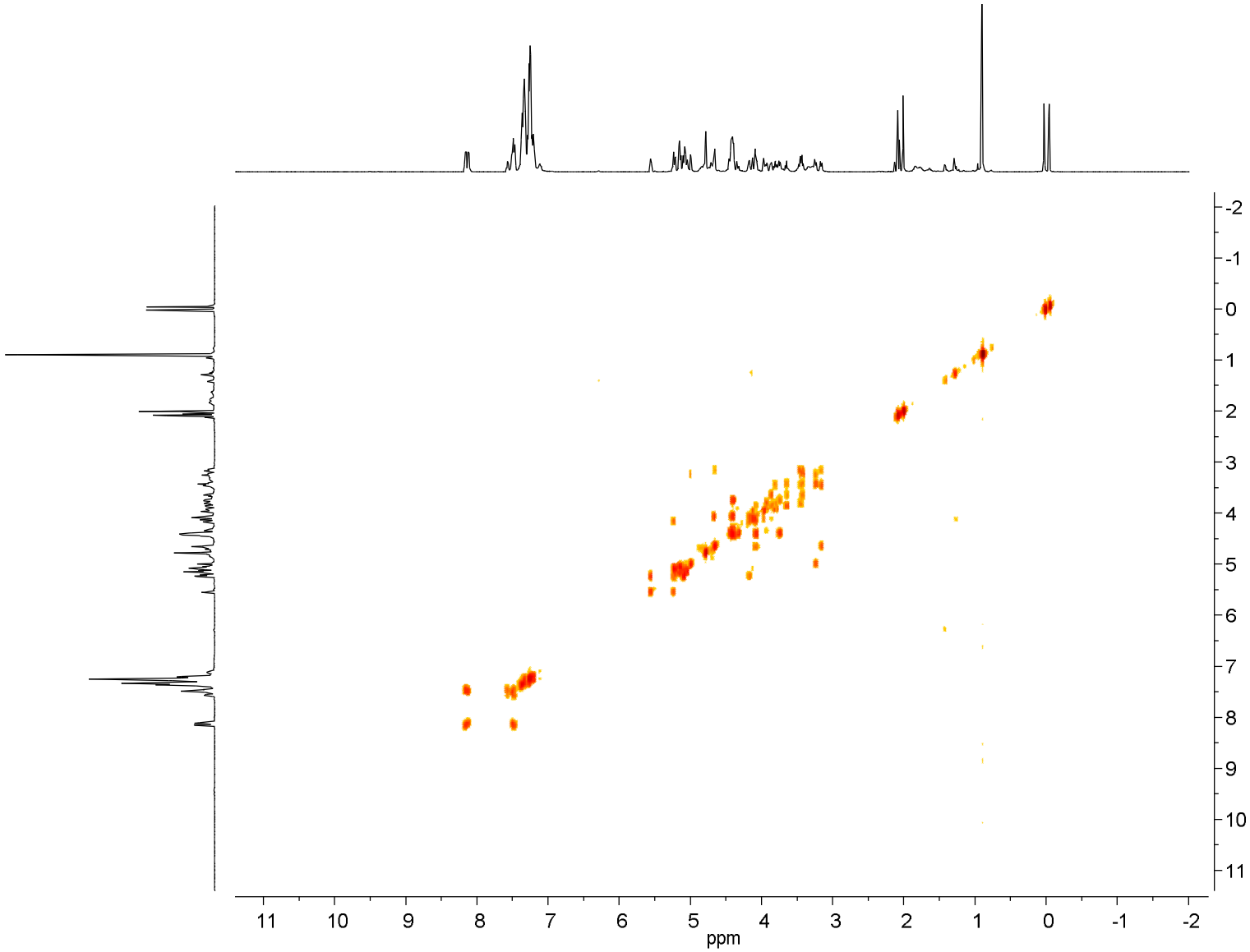


Figure 4.219. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **69**

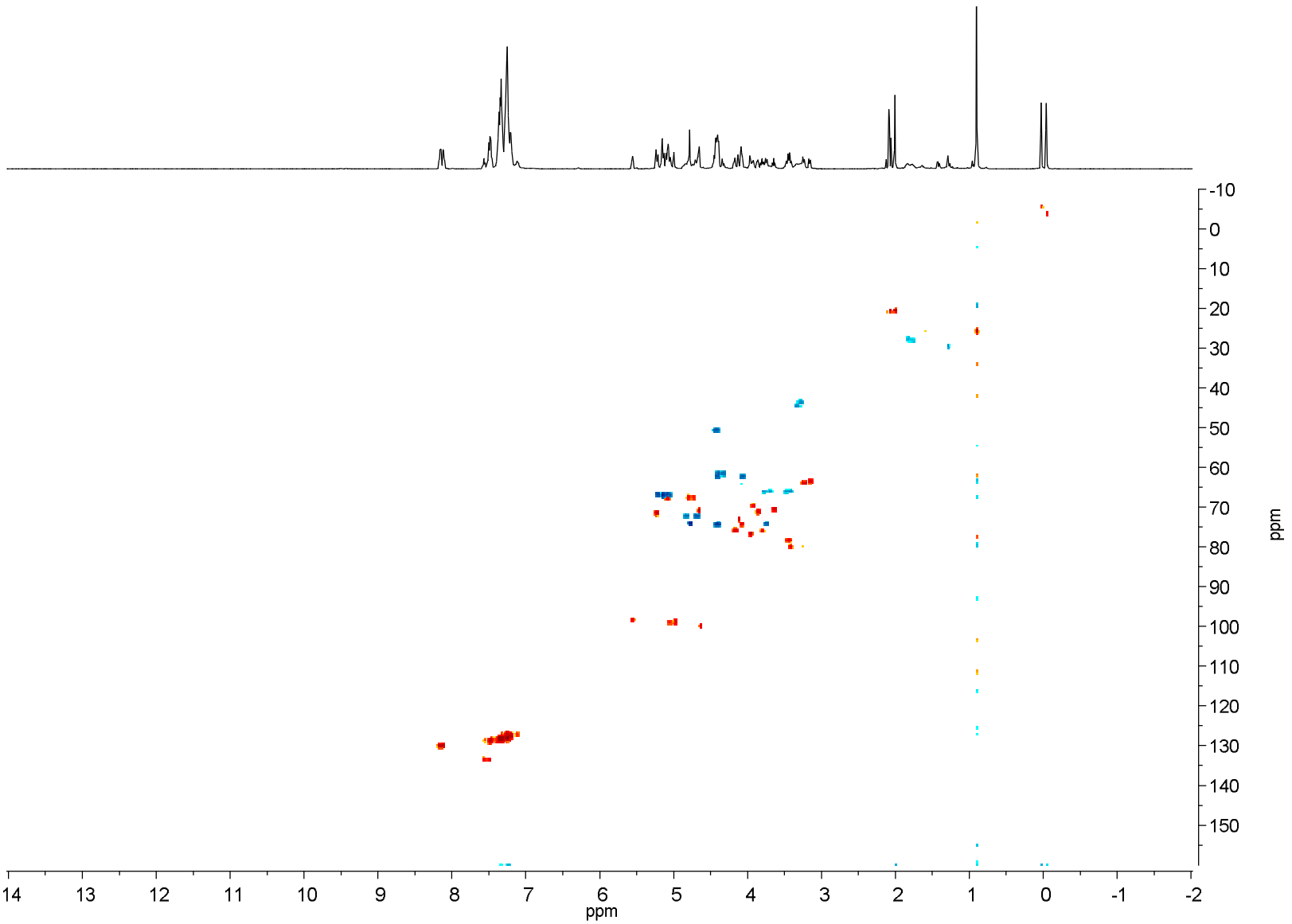


Figure 4.220. 500 MHz, $CDCl_3$, gHSQC of **69**

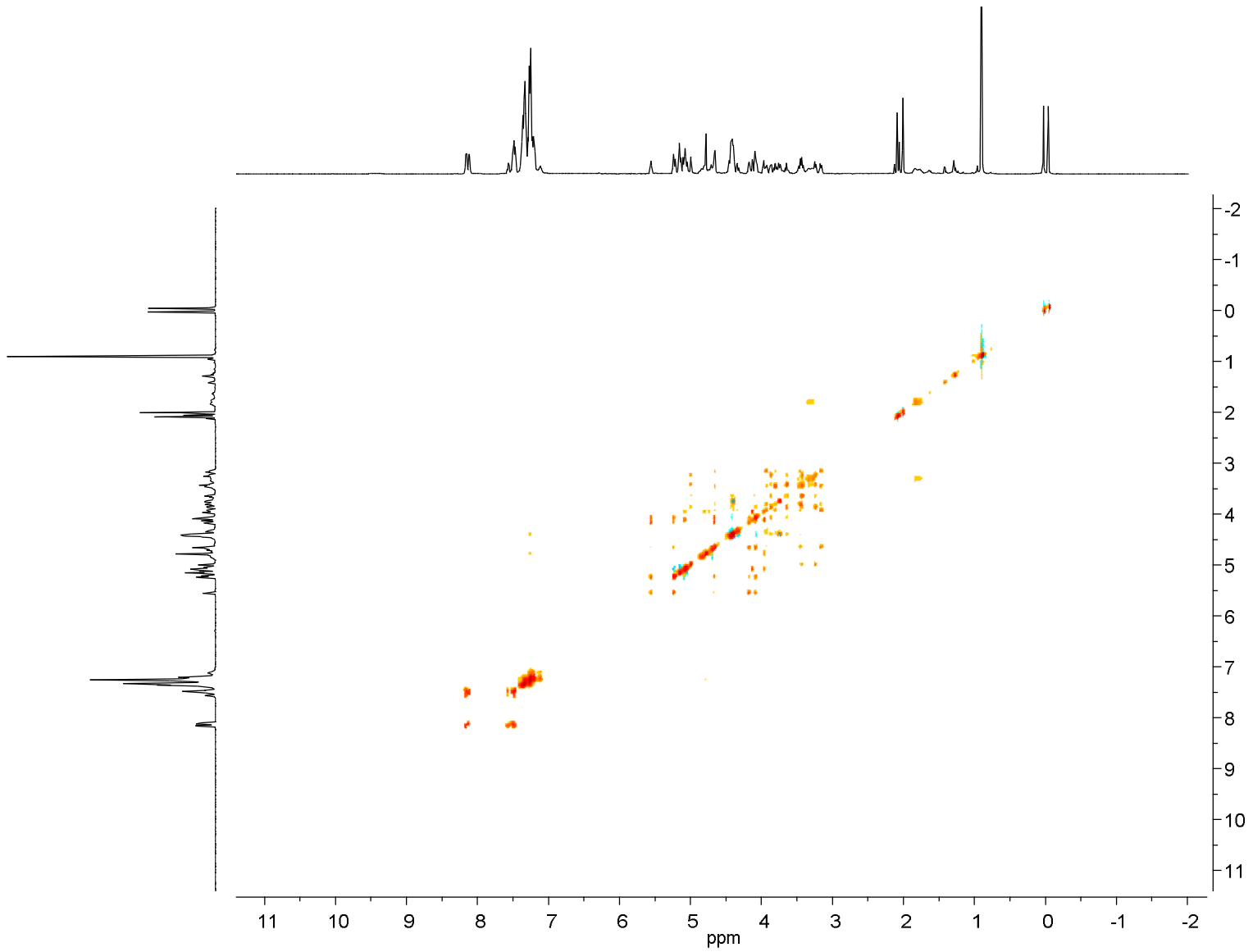


Figure 4.221. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **69**

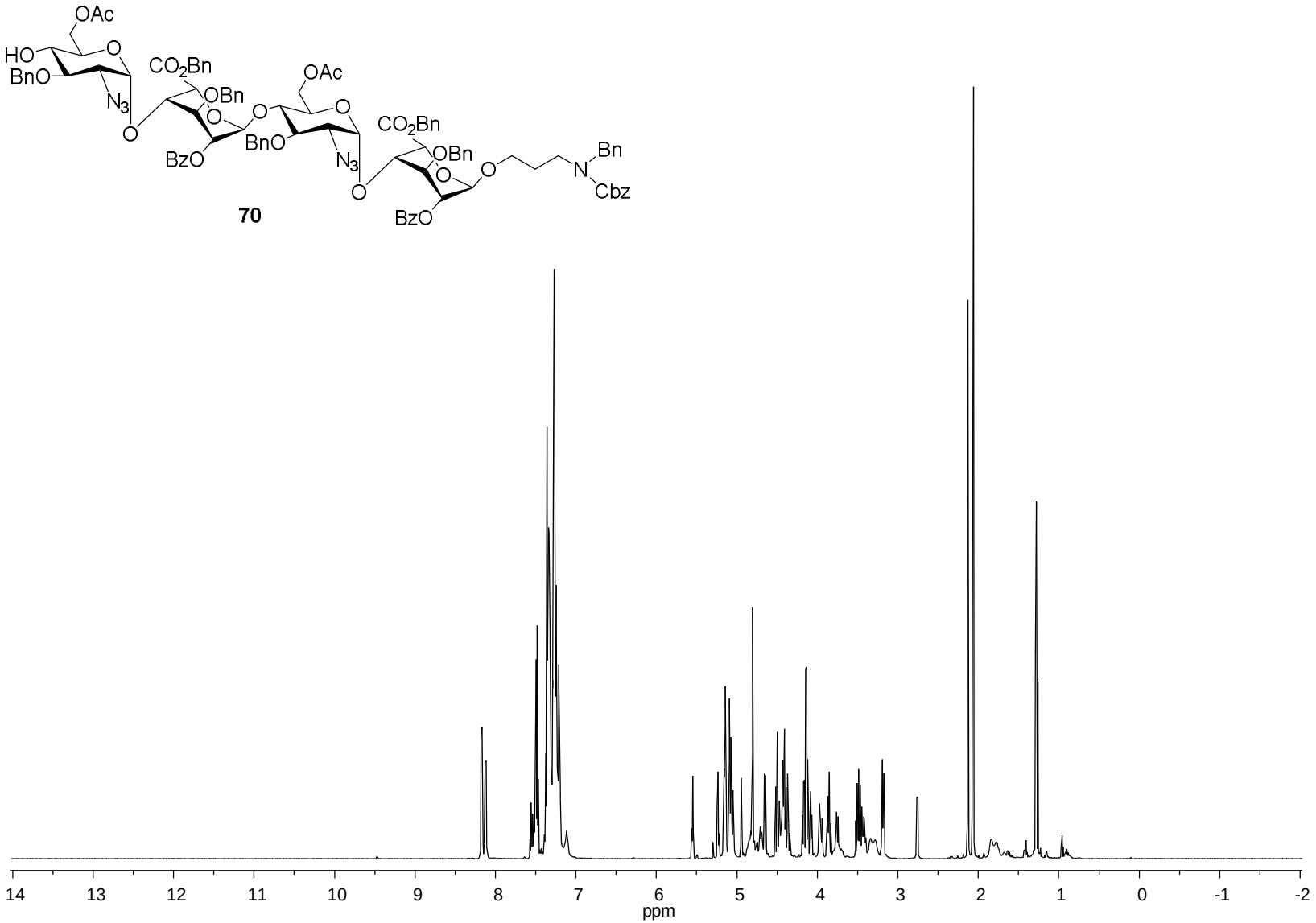


Figure 4.222. 500 MHz, $CDCl_3$, ^1H NMR of **70**

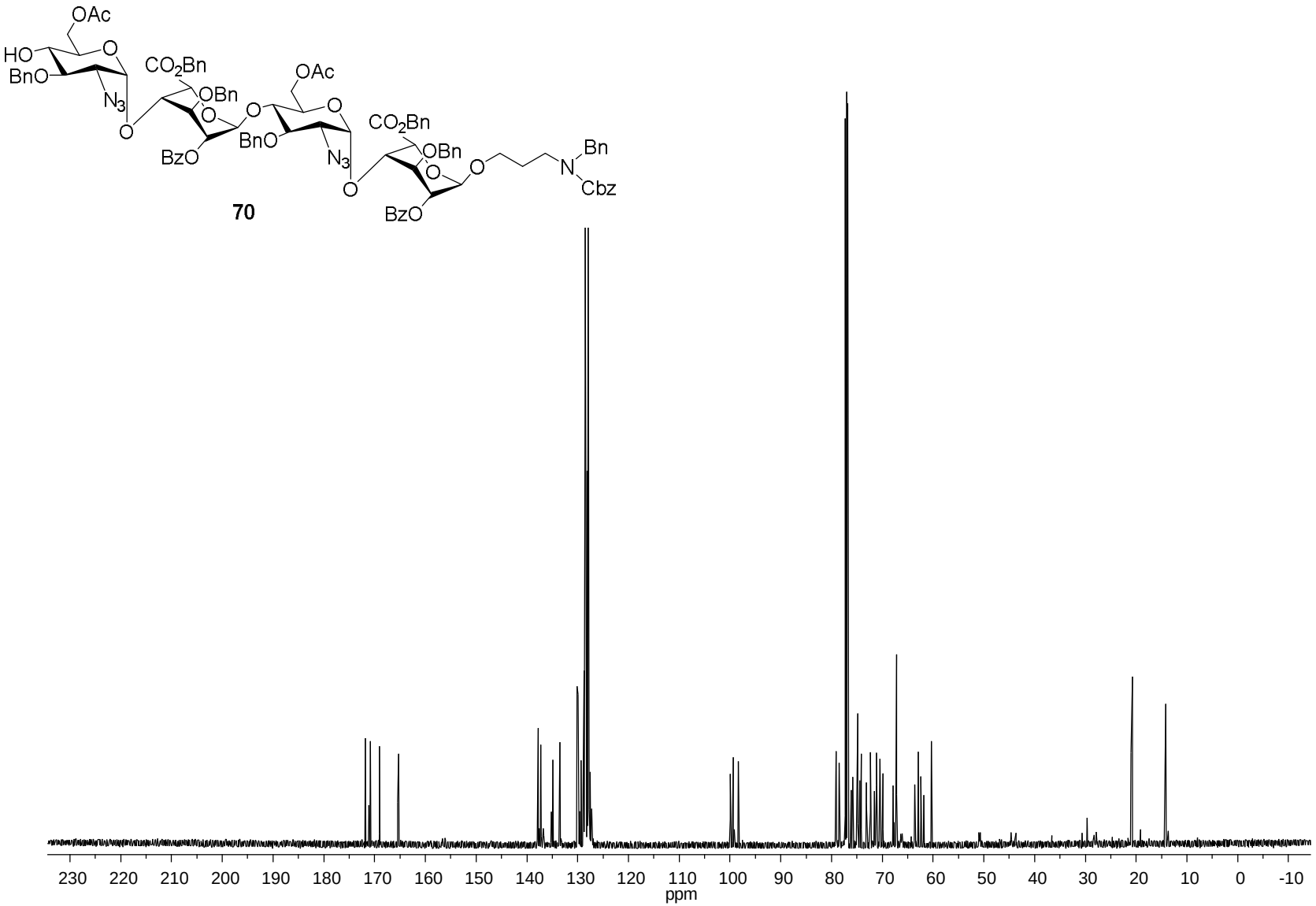


Figure 4.223. 125 MHz, $CDCl_3$, ^{13}C NMR of **70**

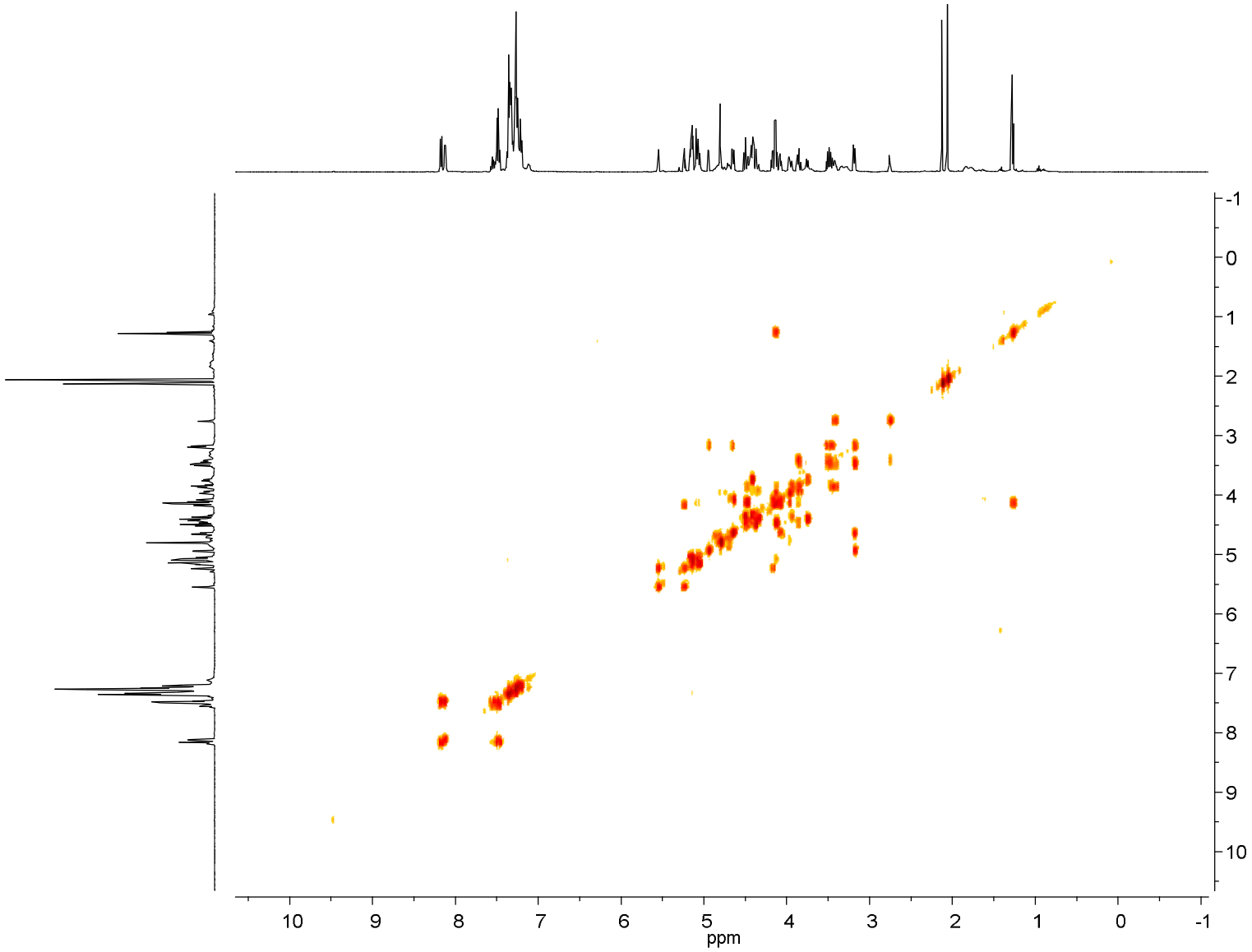


Figure 4.224. 500 MHz, $CDCl_3$, ^1H-^1H gCOSY of **70**

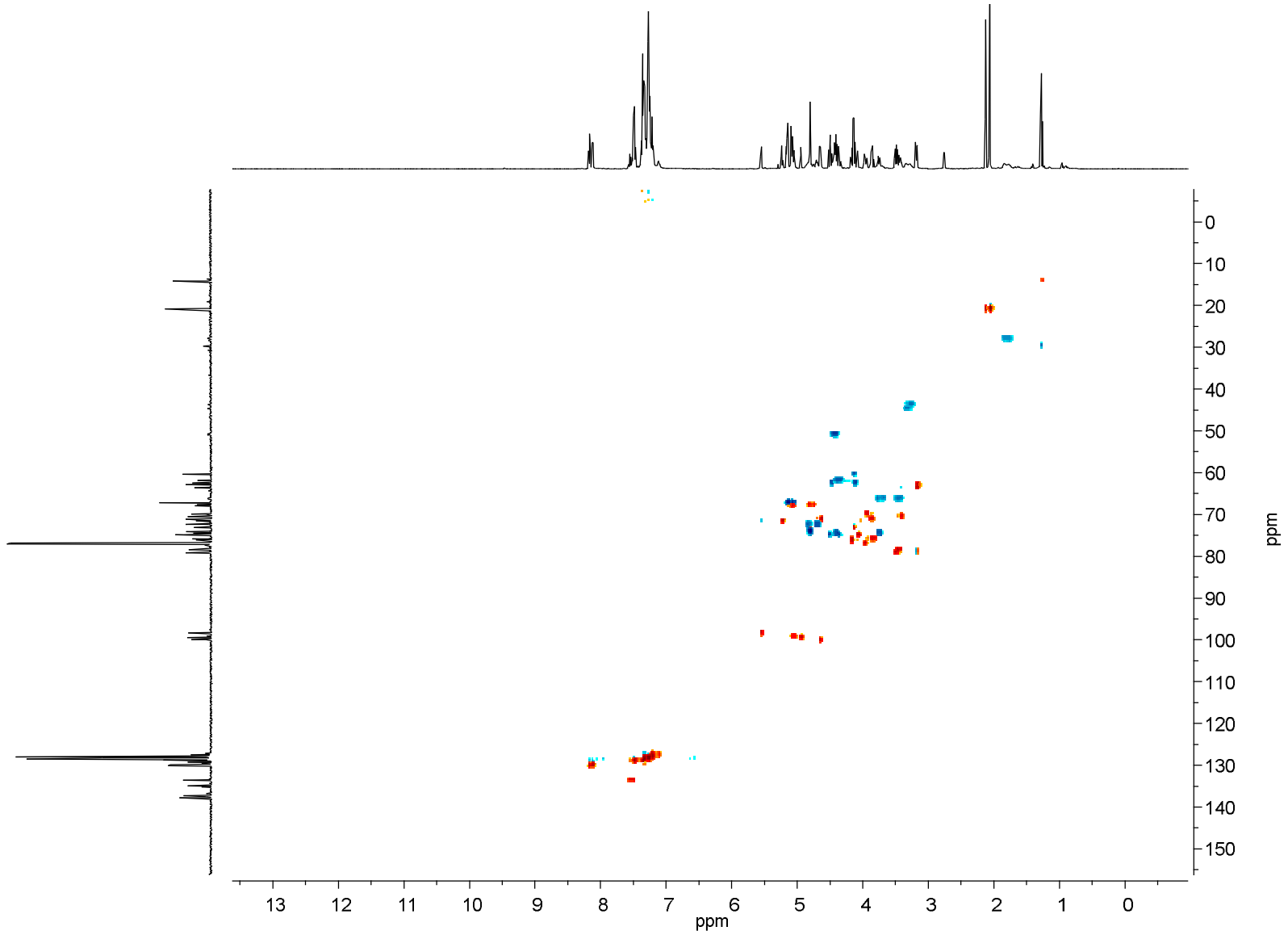


Figure 4.225. 500 MHz, $CDCl_3$, gHSQC of **70**

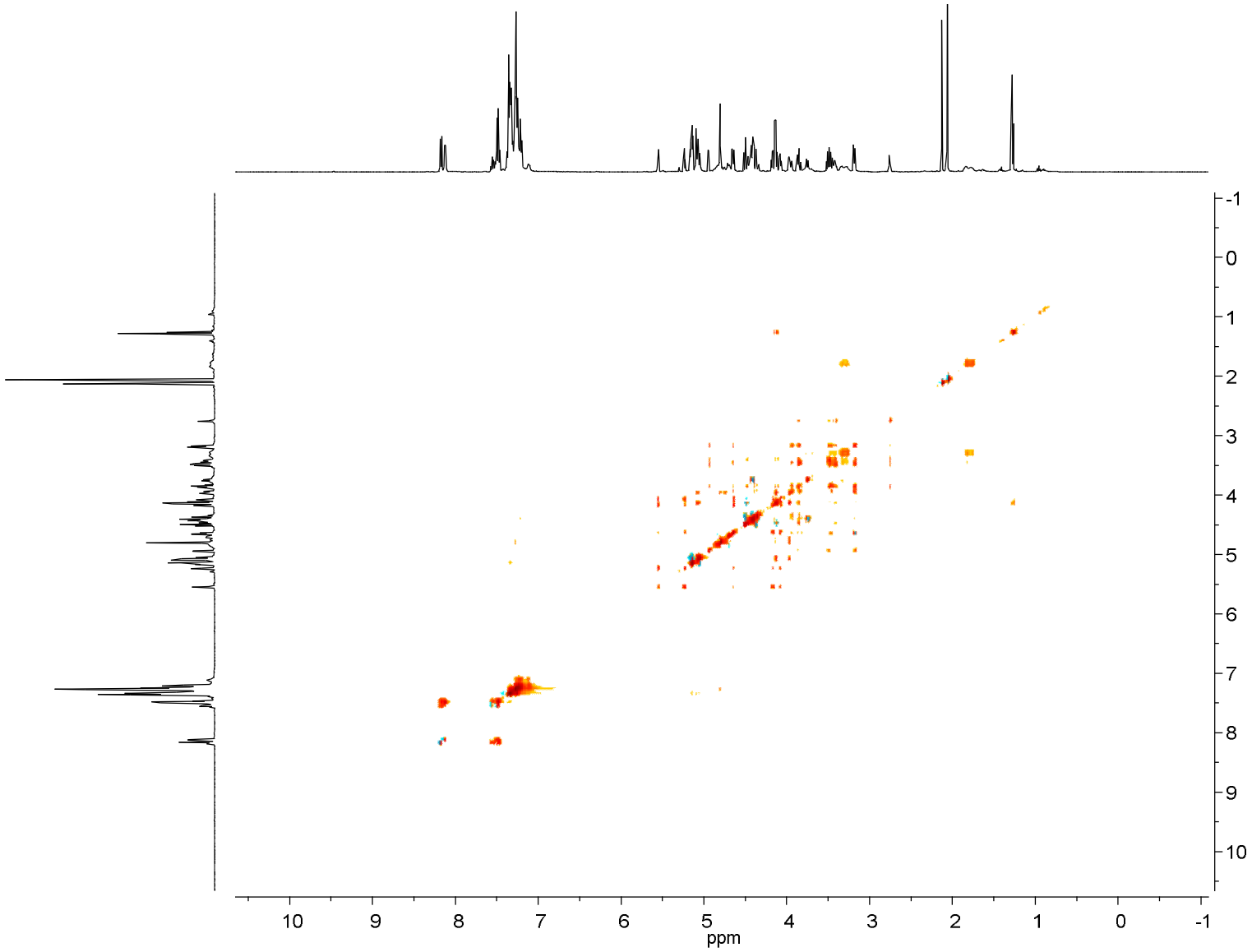
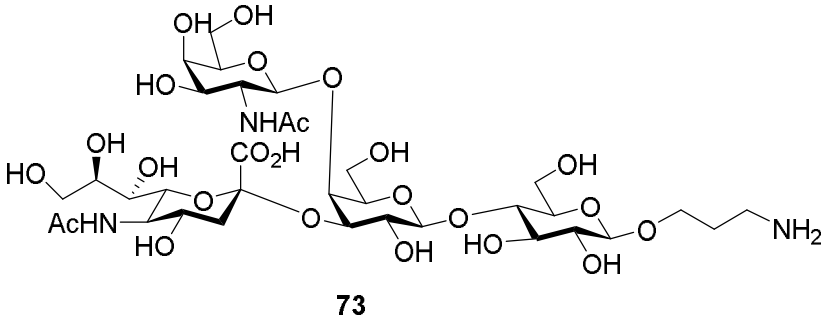


Figure 4.226. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of **70**



73

Figure 4.227. 500 MHz, D_2O, ^1H NMR of compound **73**

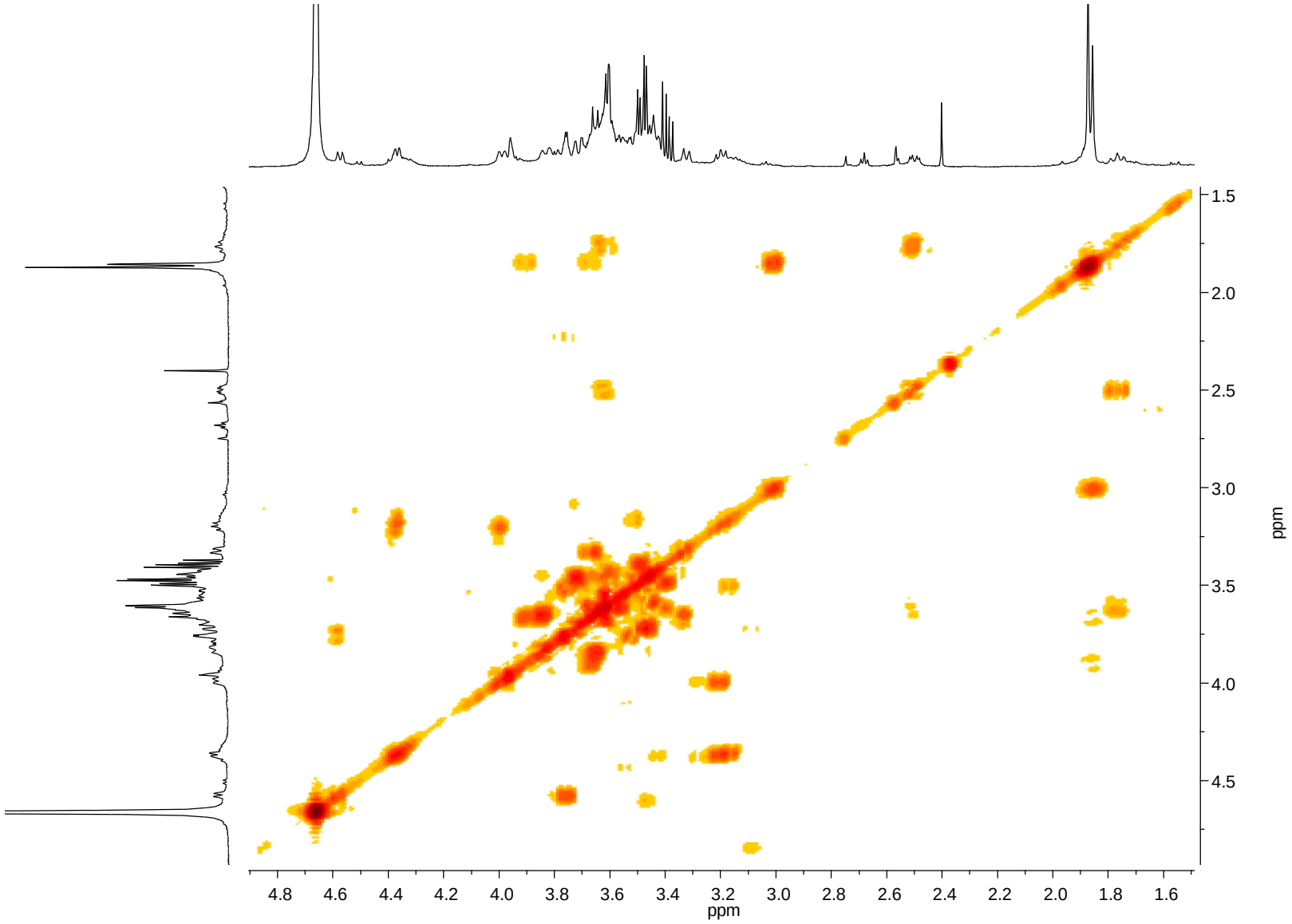


Figure 4.228. 500 MHz, D_2O, ^1H-^1H COSY of compound **73**

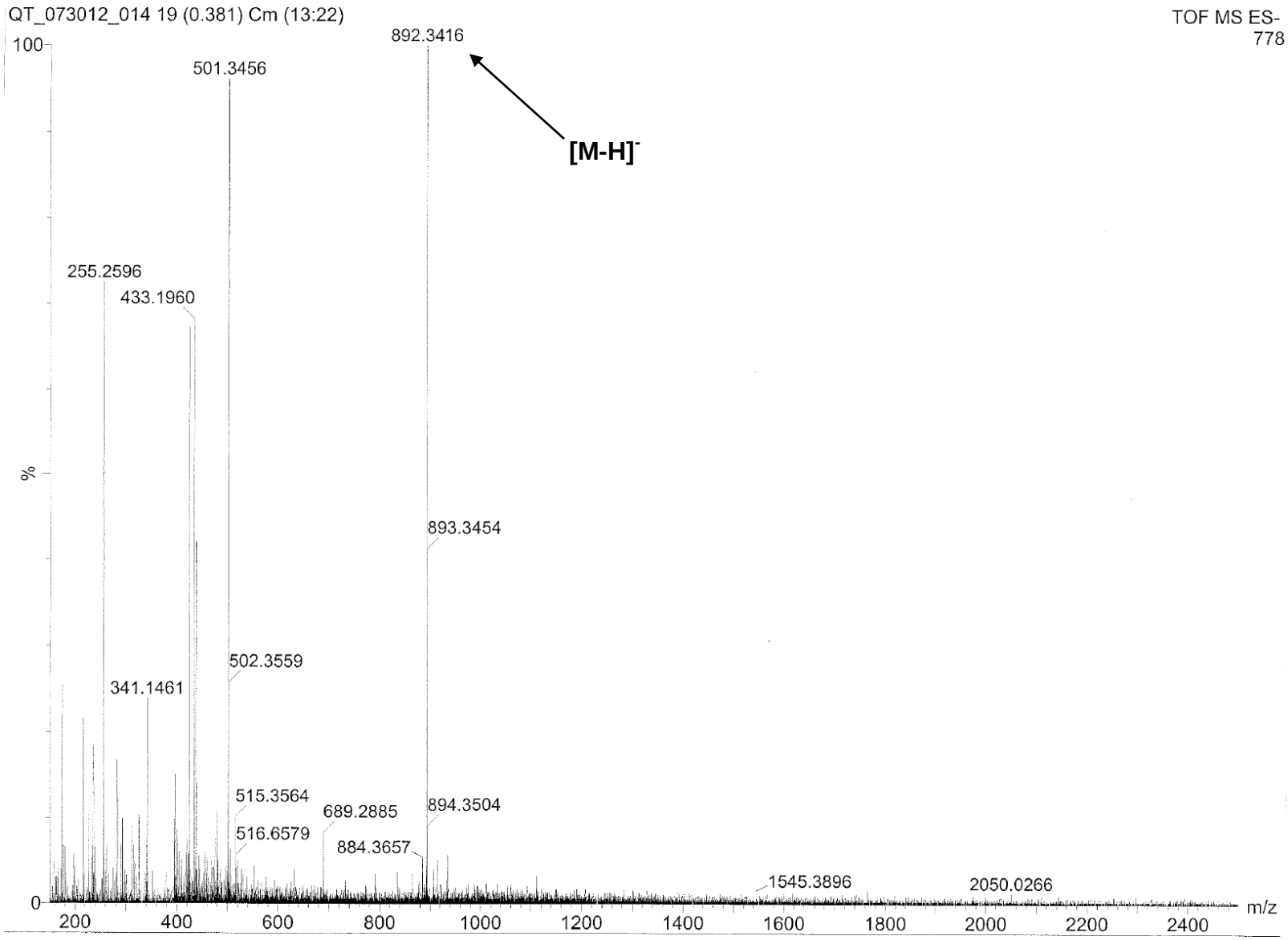
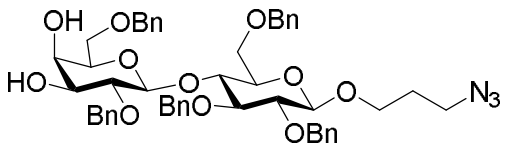


Figure 4.229. ESI-MS of compound **73**



74

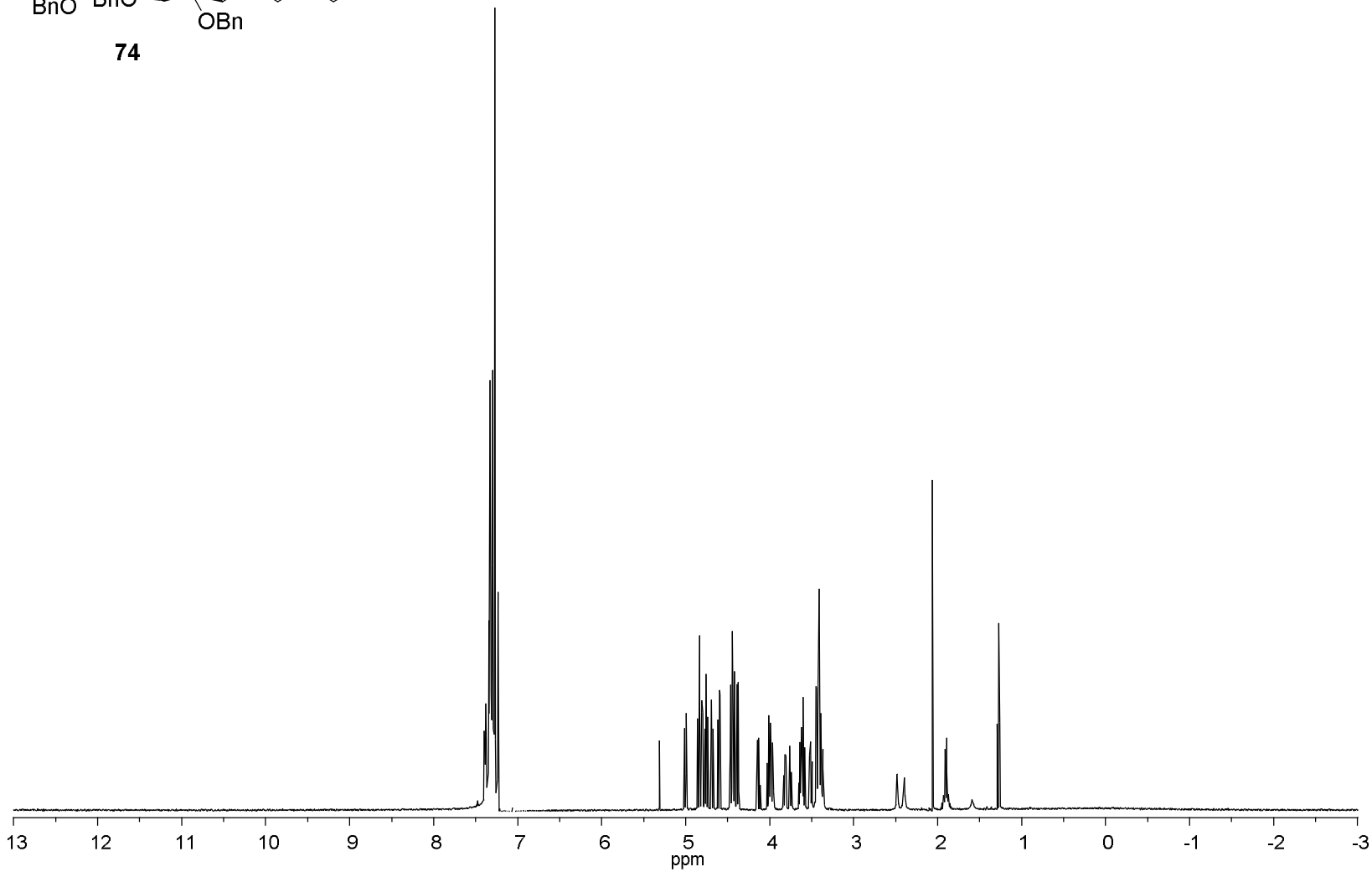


Figure 4.230. 500 MHz, $CDCl_3$, ^1H NMR of compound **74**

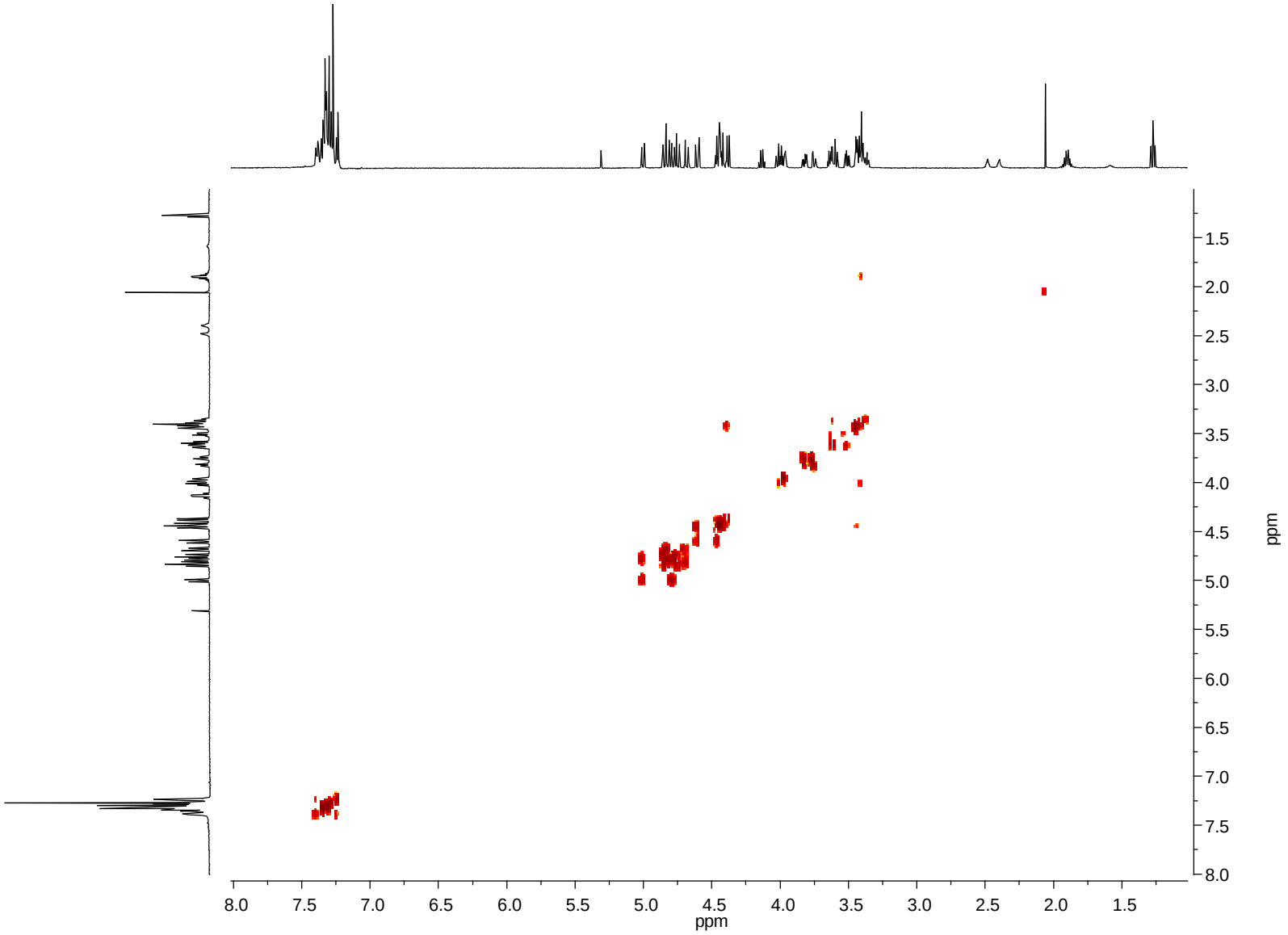
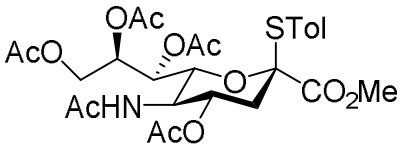


Figure 4.231. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **74**



75

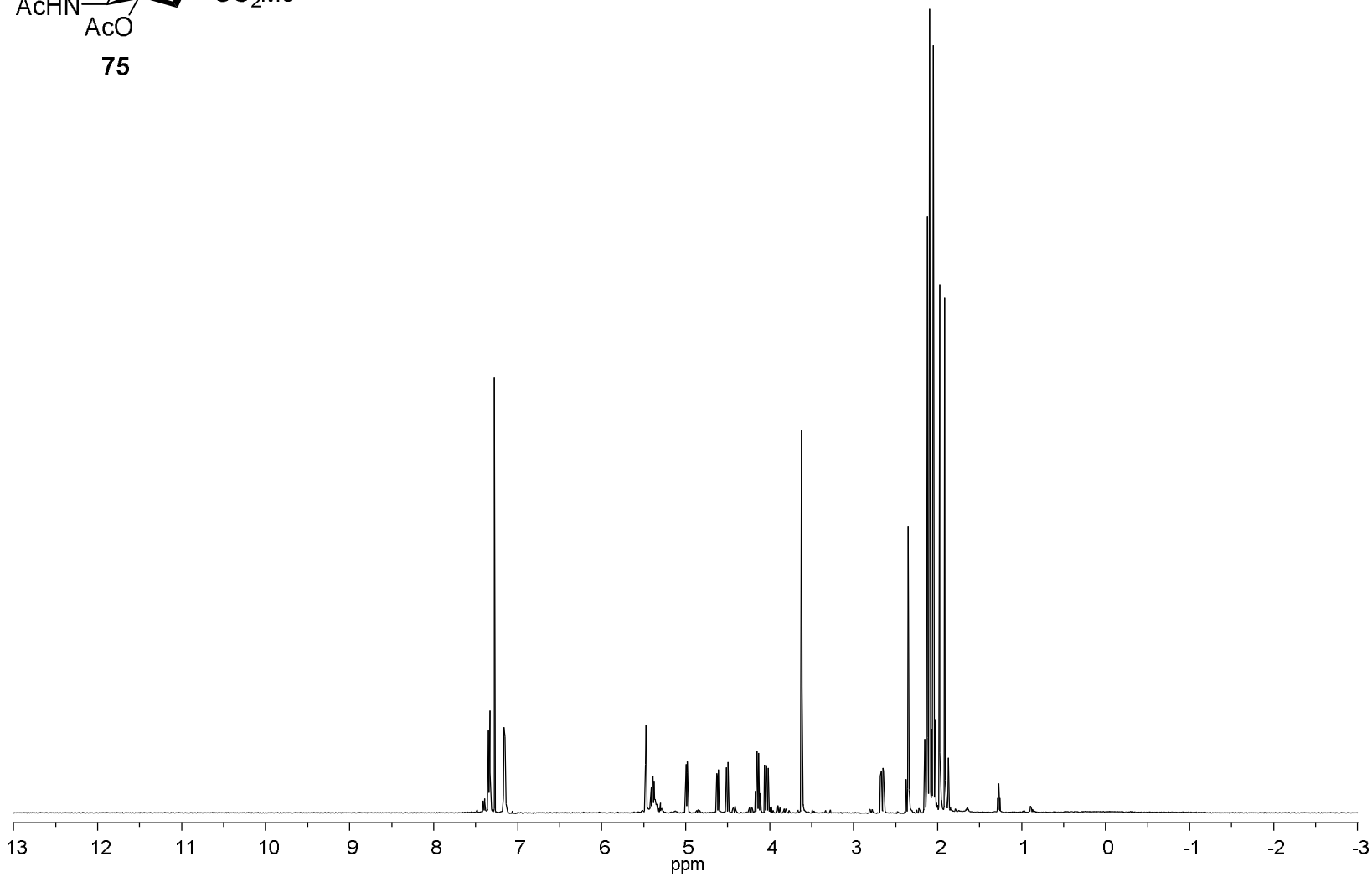
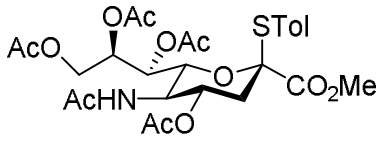


Figure 4.232. 500 MHz, $CDCl_3$, ^1H NMR of compound **75**



75

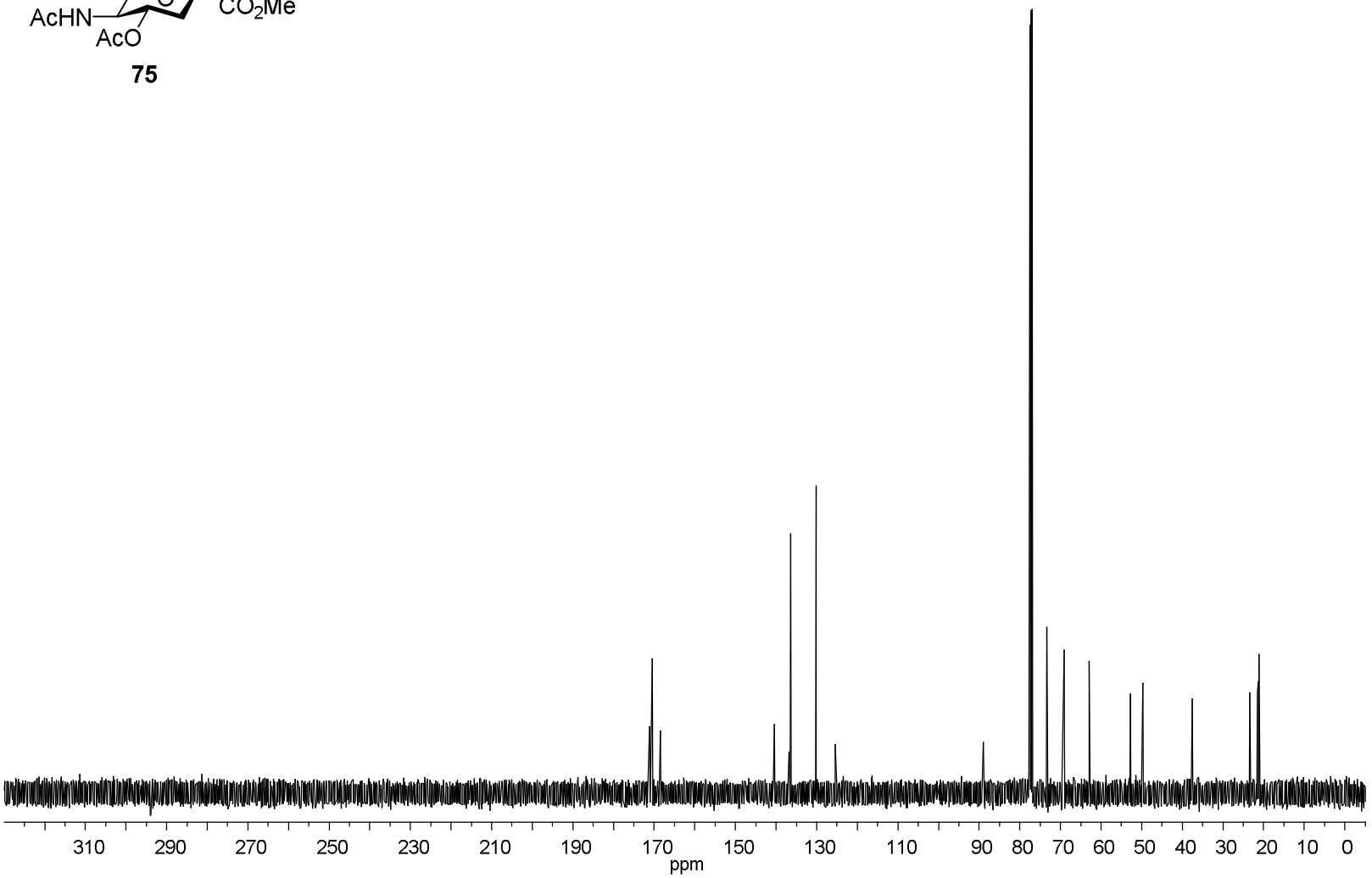


Figure 4.233. 125 MHz, $CDCl_3$, ^{13}C NMR of compound **75**

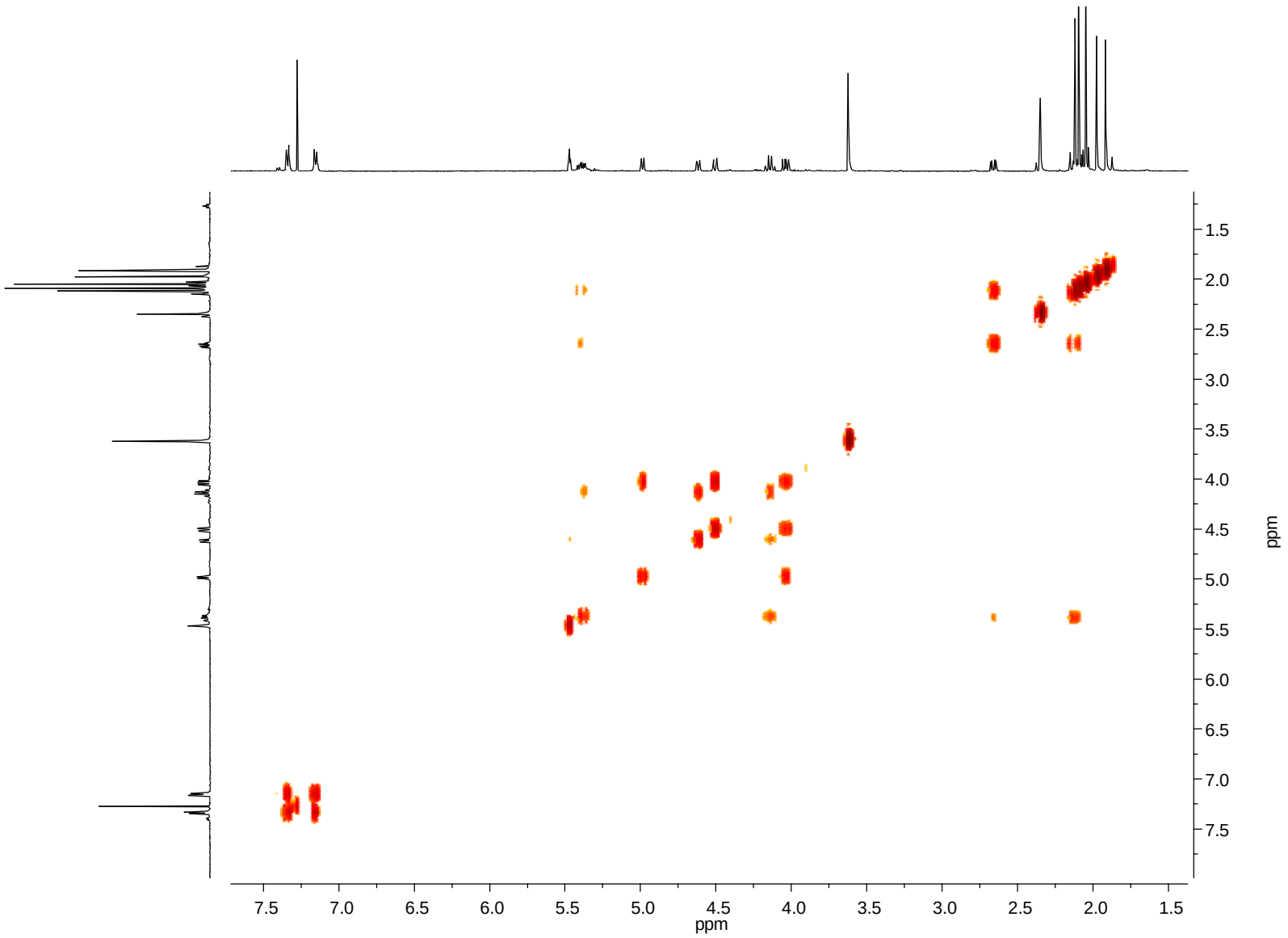


Figure 4.234. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **75**

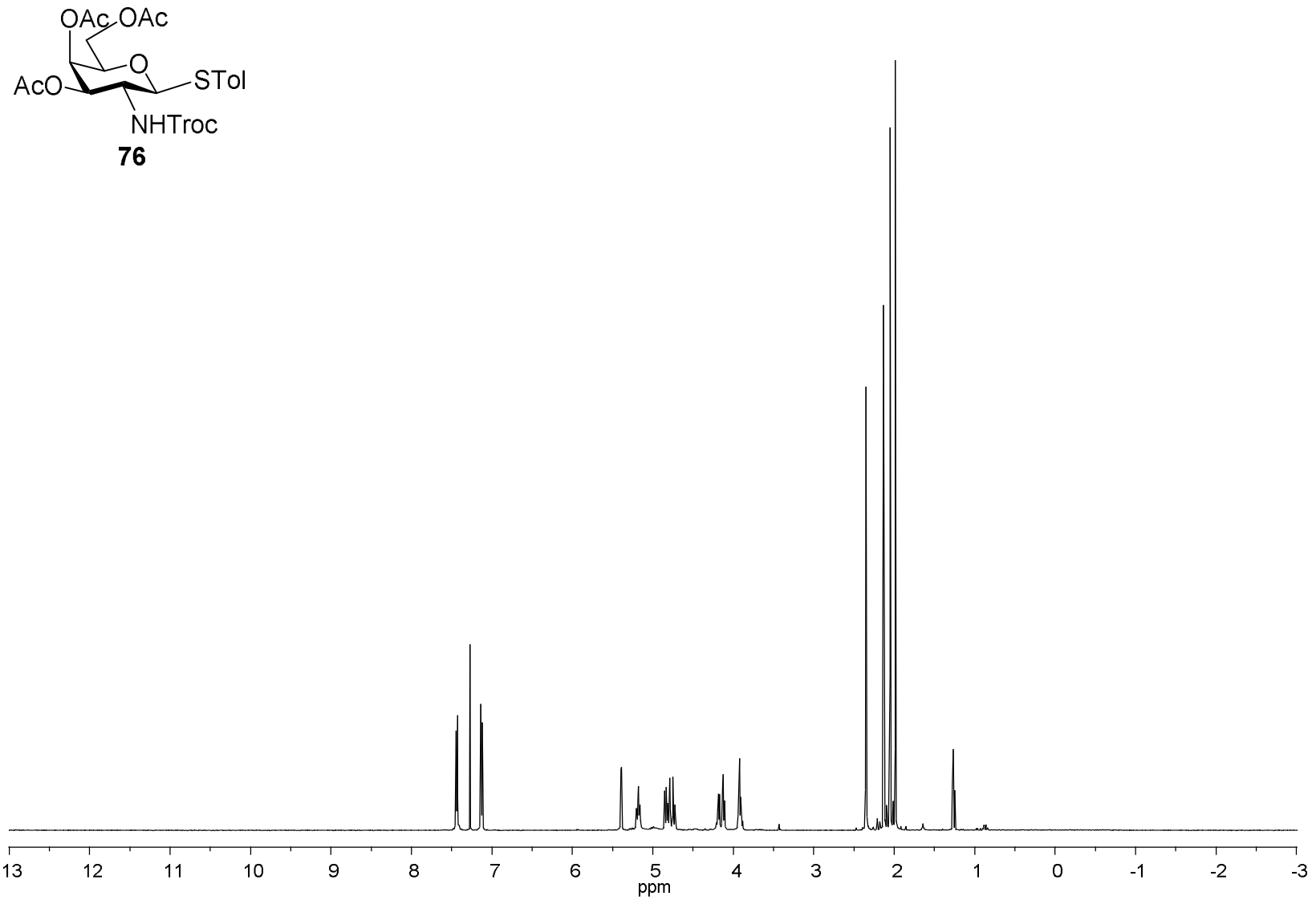


Figure 4.235. 500 MHz, $CDCl_3$, ^1H NMR of compound **76**

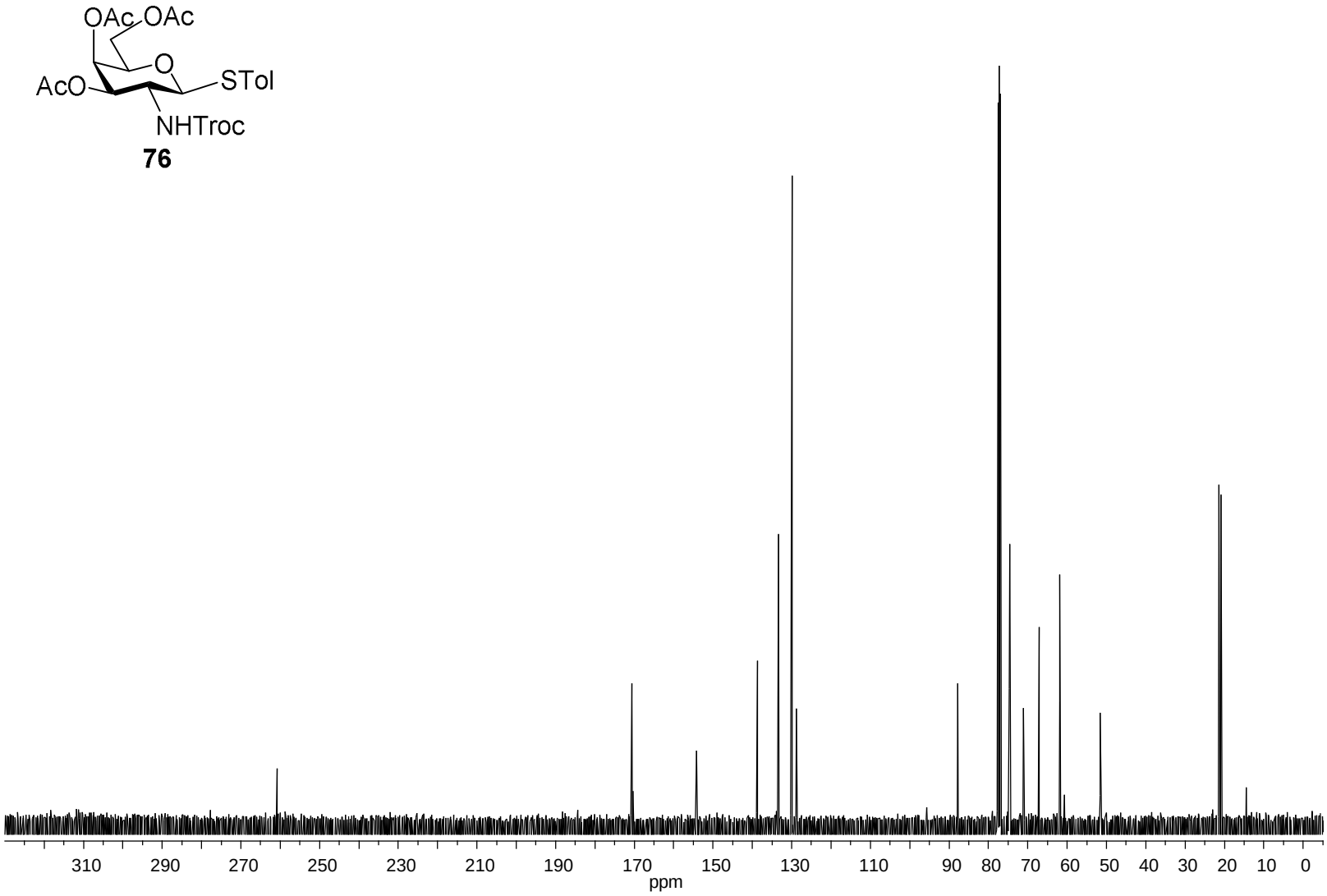


Figure 4.236. 125 MHz, $CDCl_3$, ^{13}C NMR of compound **76**

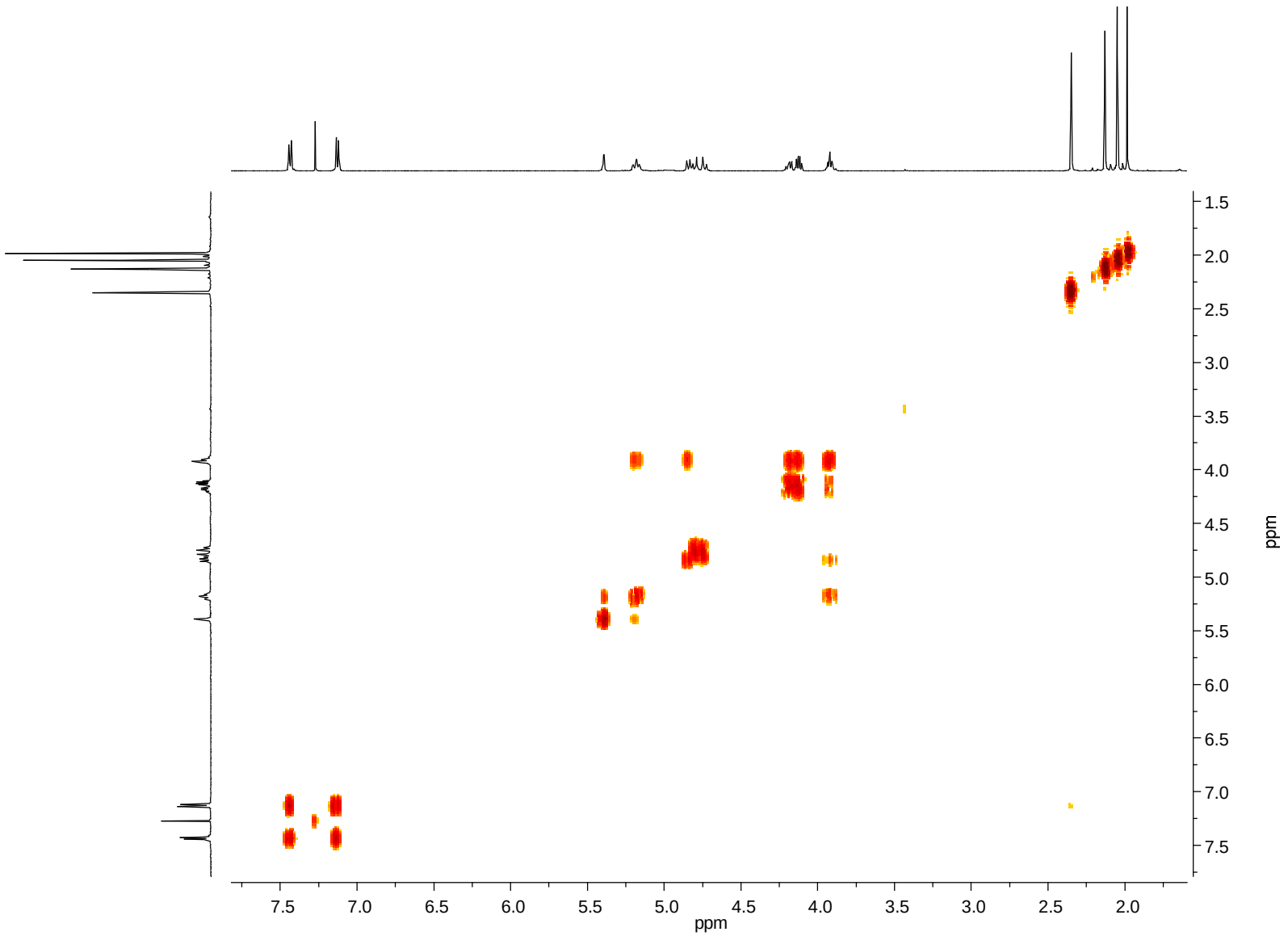
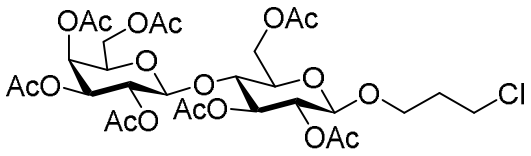


Figure 4.237. 500 MHz, CDCl3, 1H-1H COSY of compound **76**



77

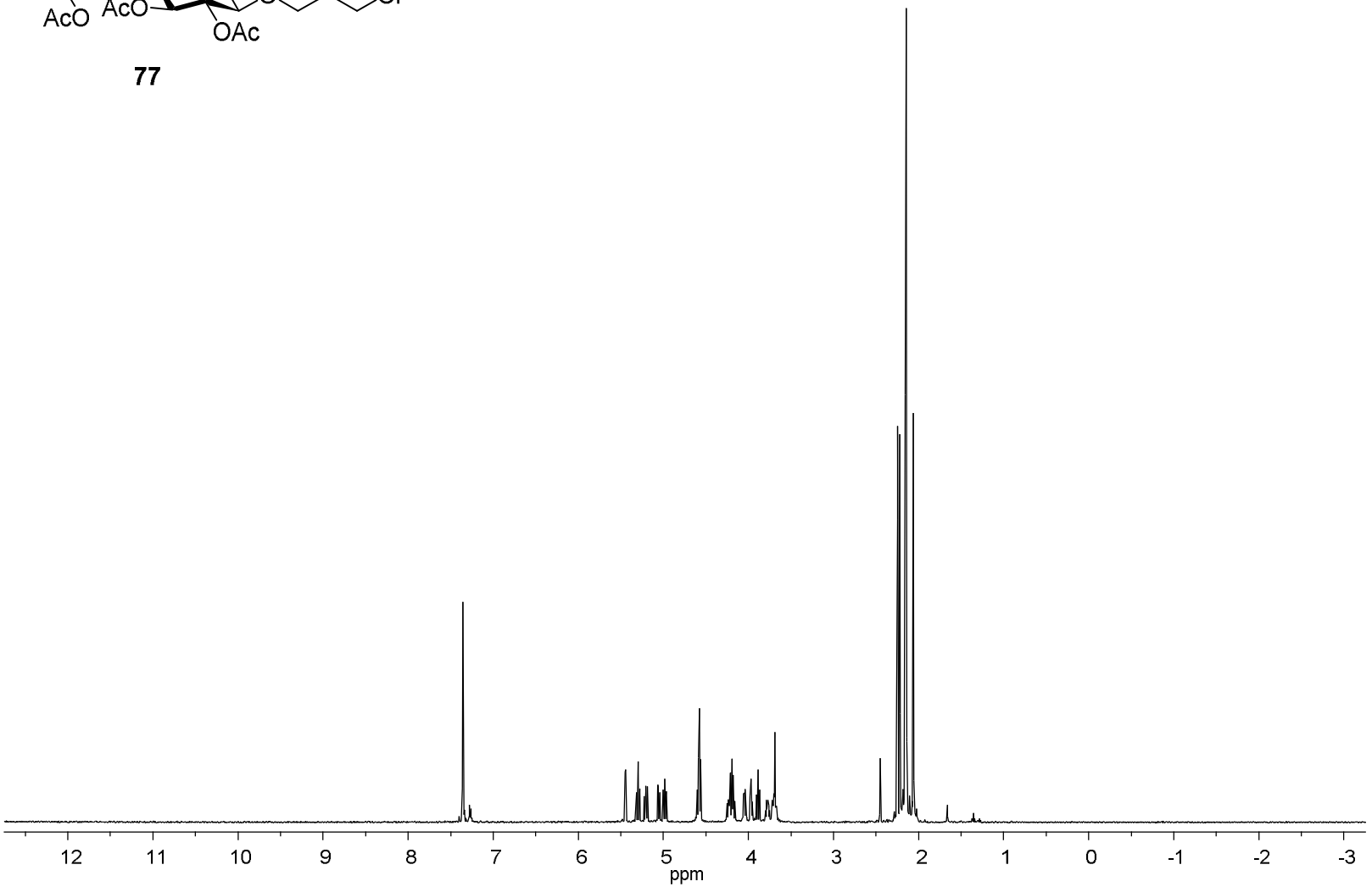


Figure 4.238. 500 MHz, $CDCl_3$, ^1H NMR of compound **77**

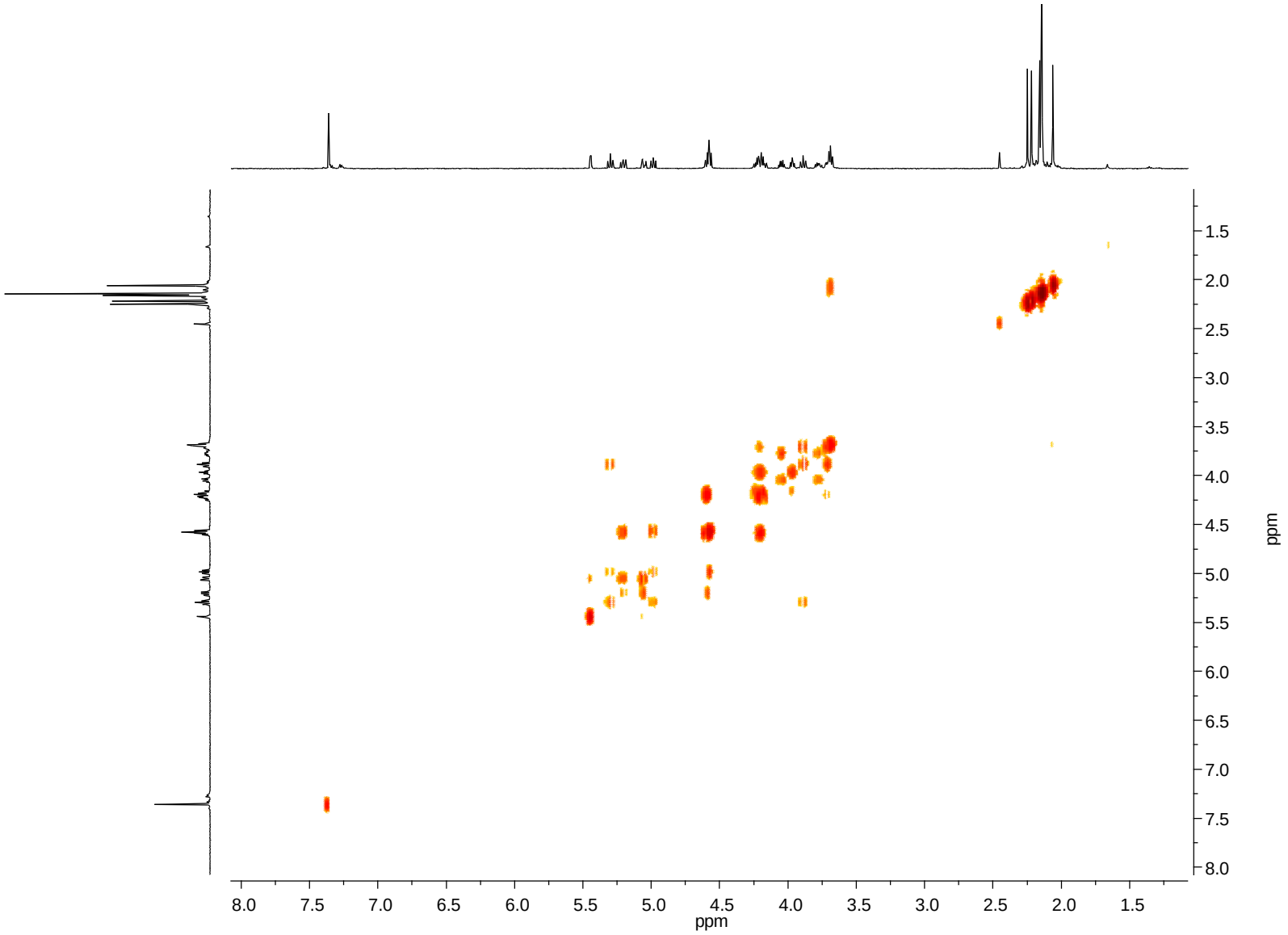


Figure 4.239. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **77**

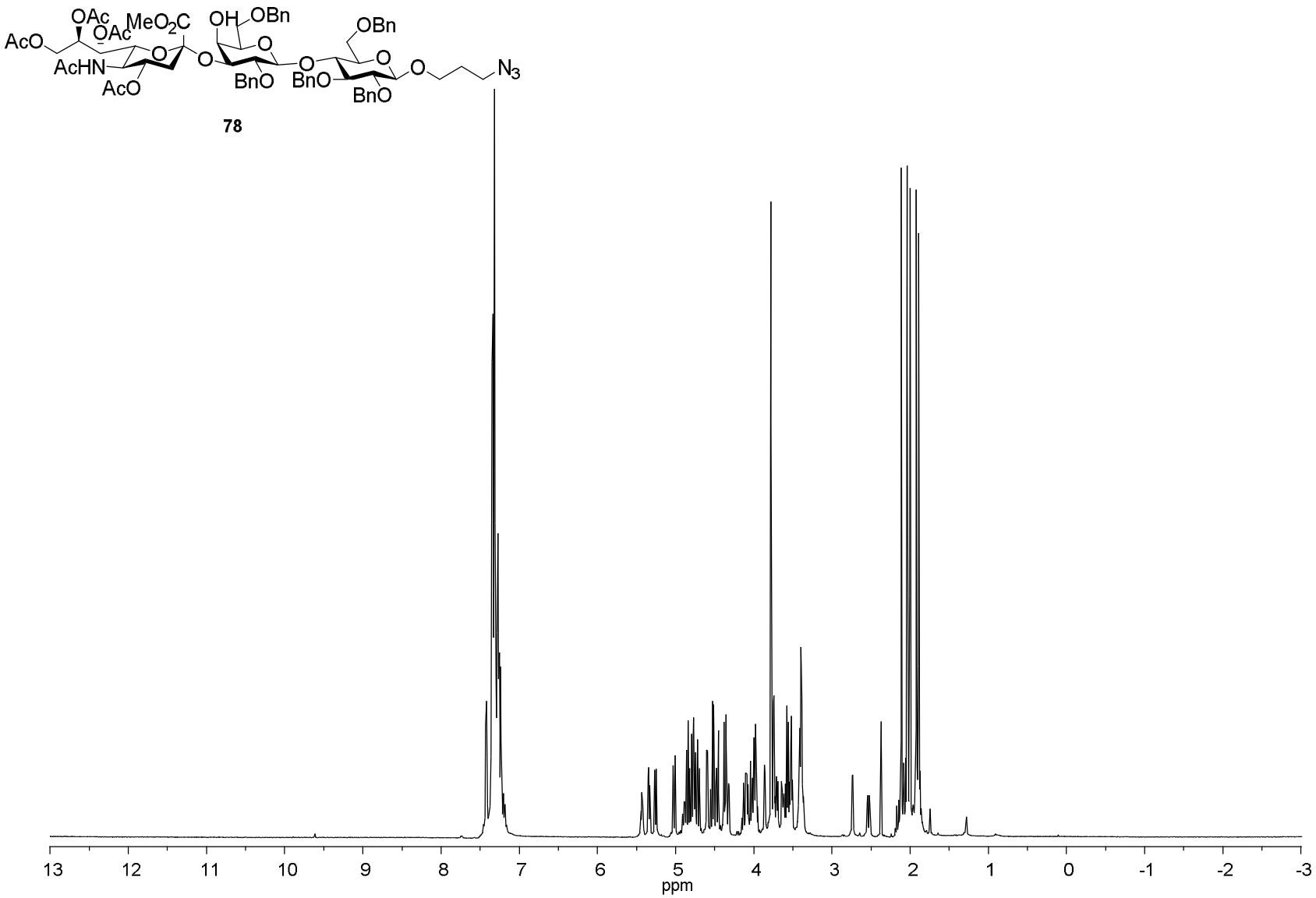
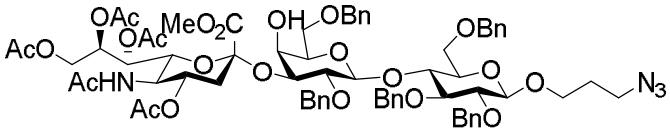


Figure 4.240. 500 MHz, $CDCl_3$, ^1H NMR of compound **78**



78

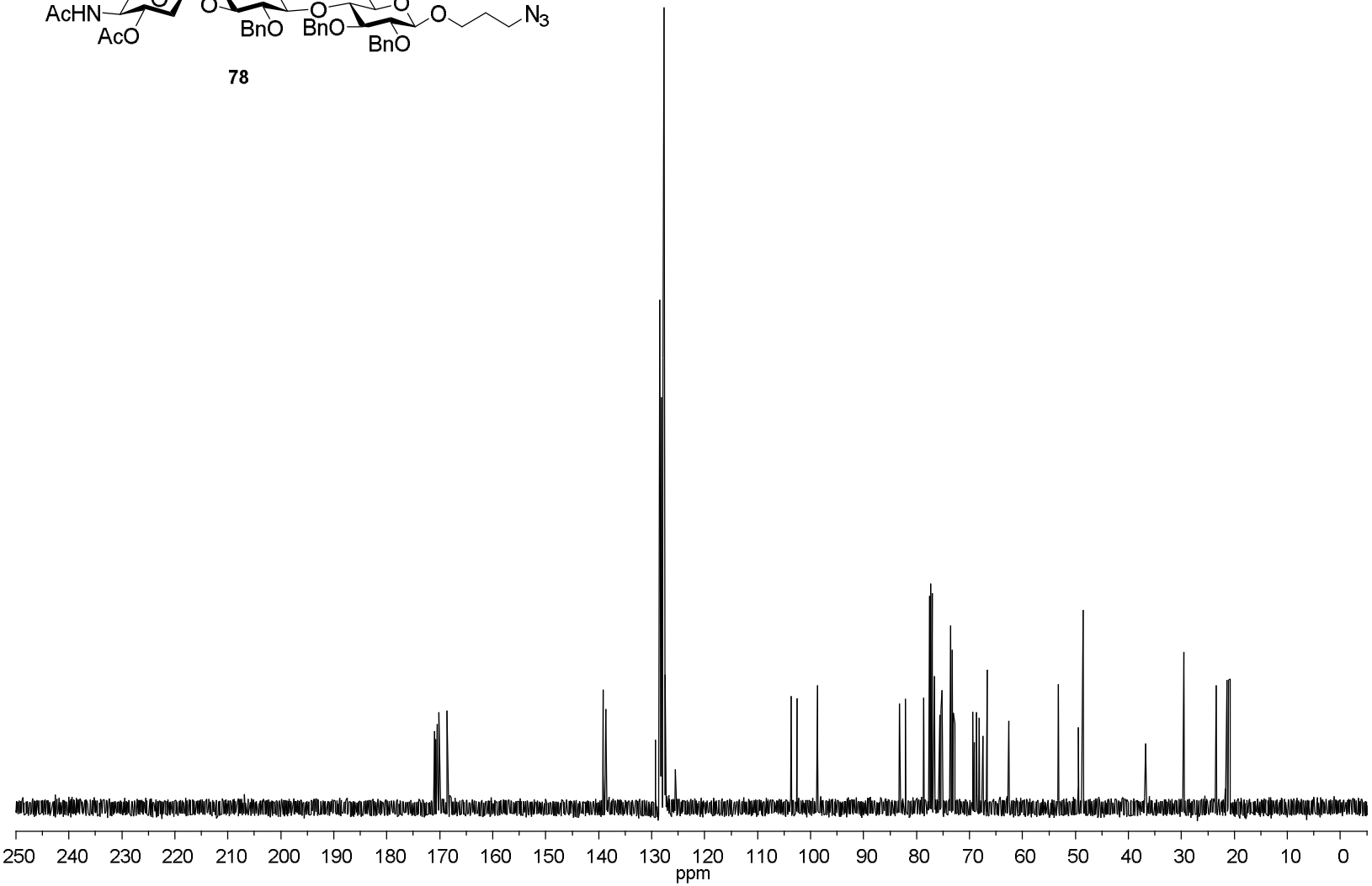


Figure 4.241. 125 MHz, $CDCl_3$, ^{13}C NMR of compound **78**

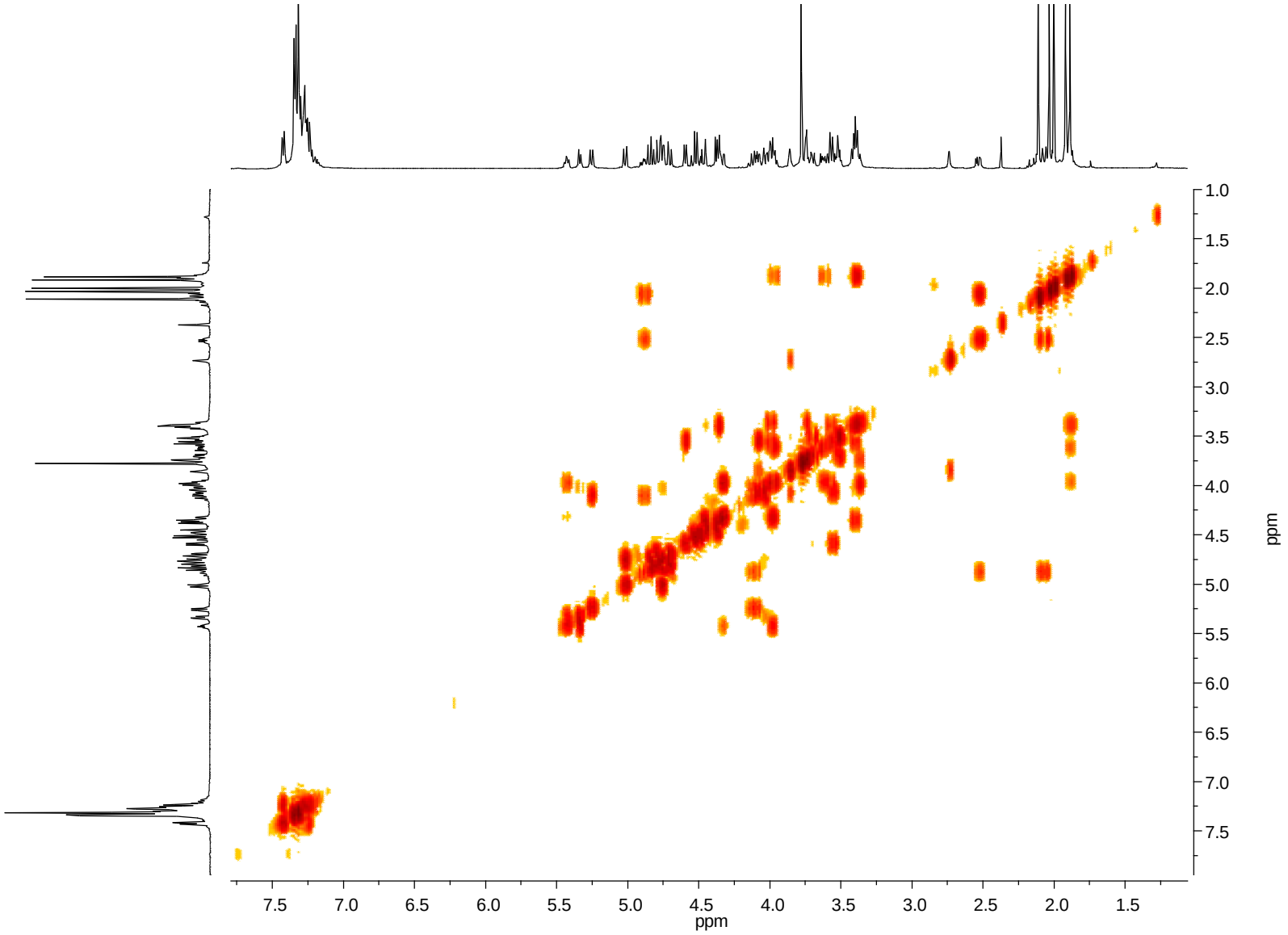


Figure 4.242. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **78**

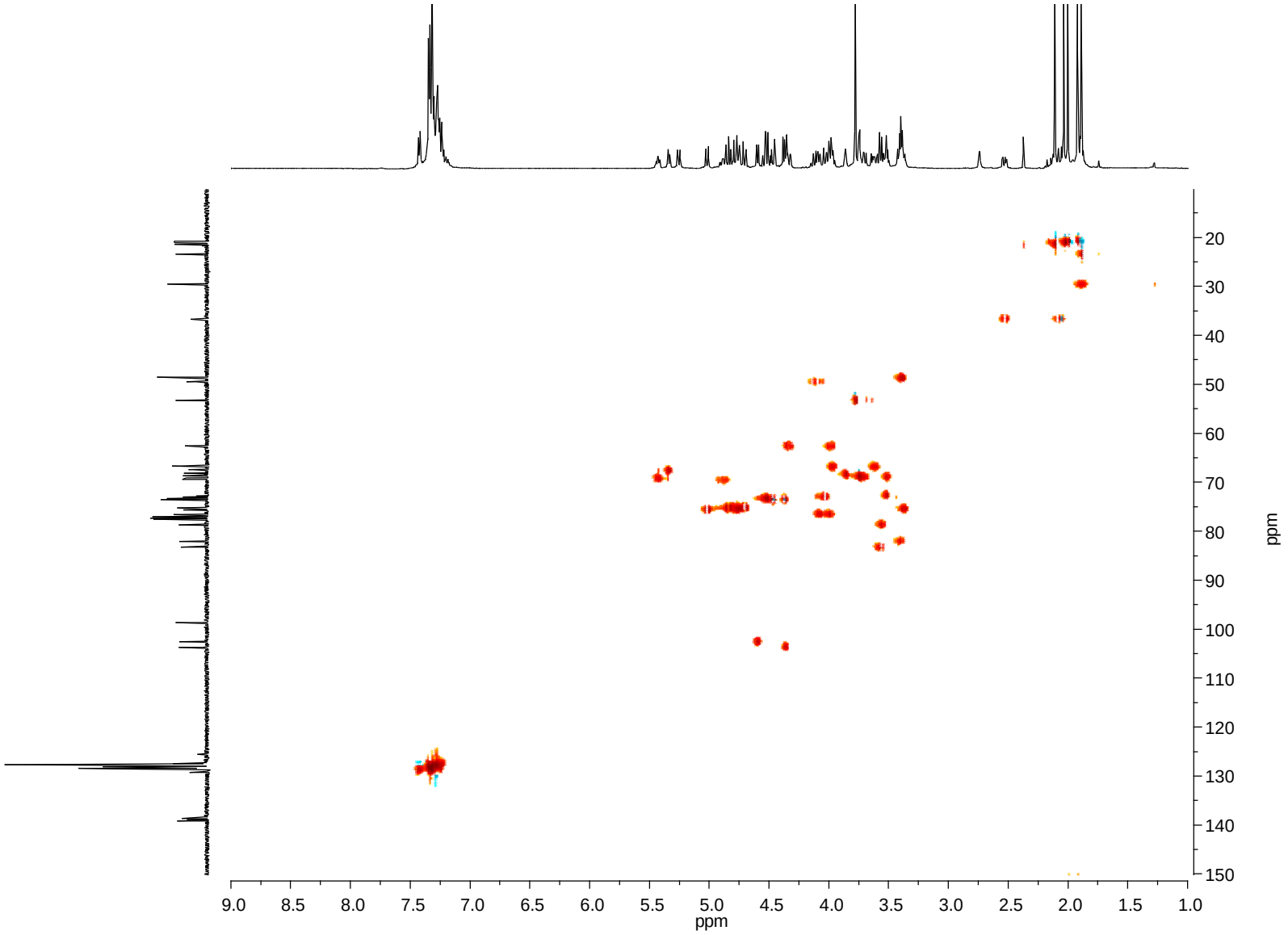


Figure 4.243. 500 MHz, $CDCl_3$, ^{1}H-^{13}C HMQC of compound **78**

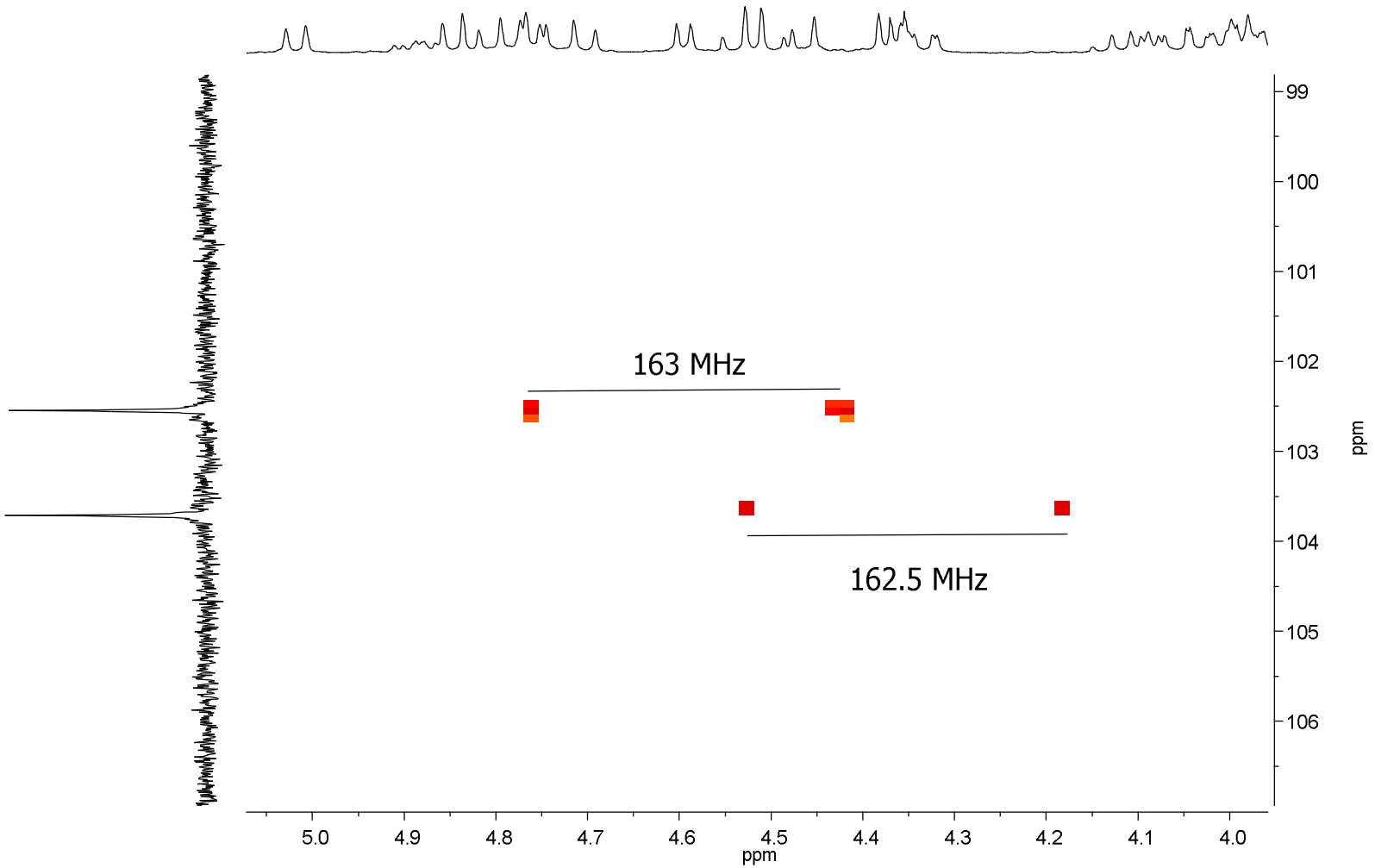


Figure 4.244. 500 MHz, $CDCl_3$, ^{1}H-^{13}C HMQC (without ^{1}H Decoupling) of compound **78**

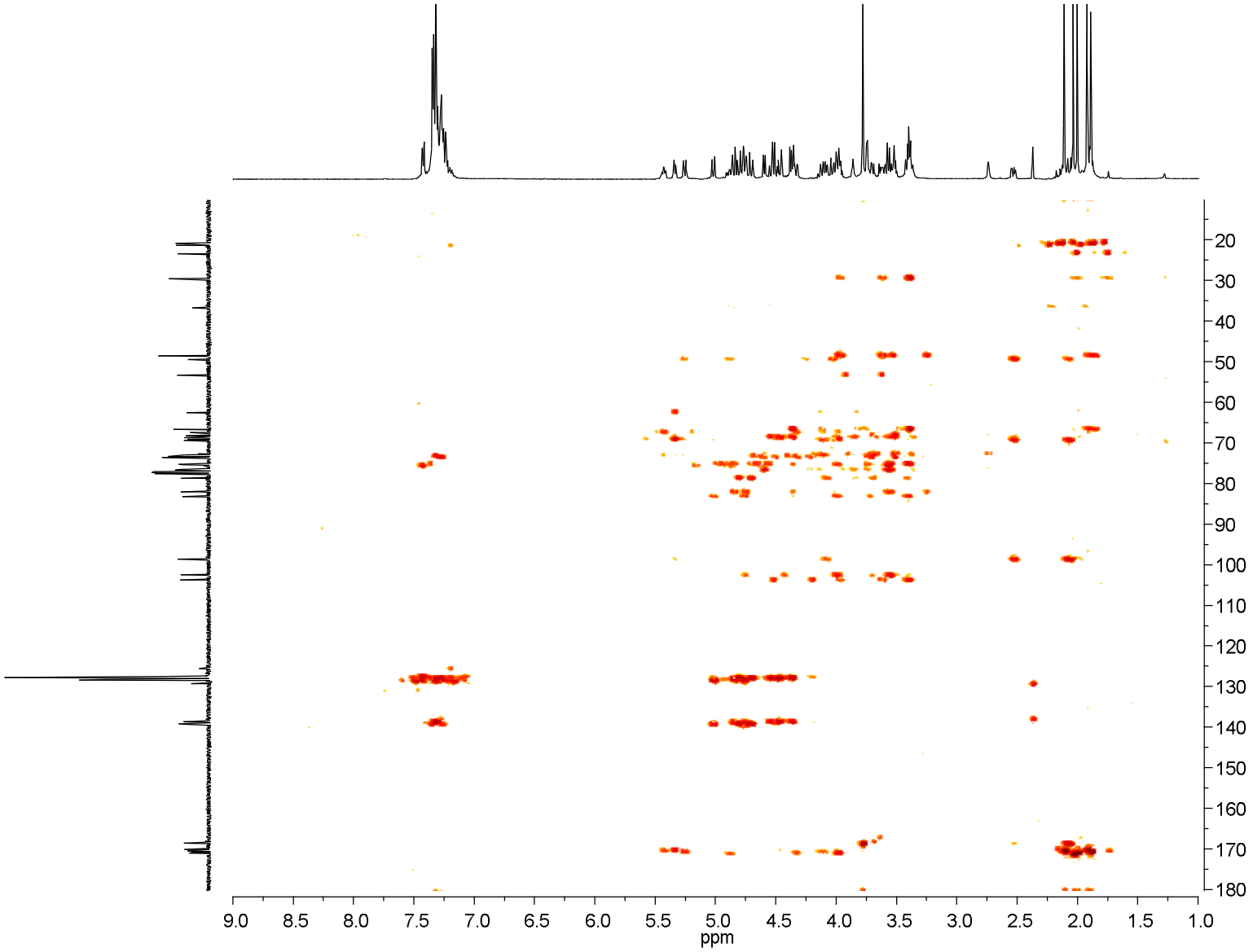


Figure 4.245. 500 MHz, $CDCl_3$, ^{1}H-^{13}C HMBC of compound **78**

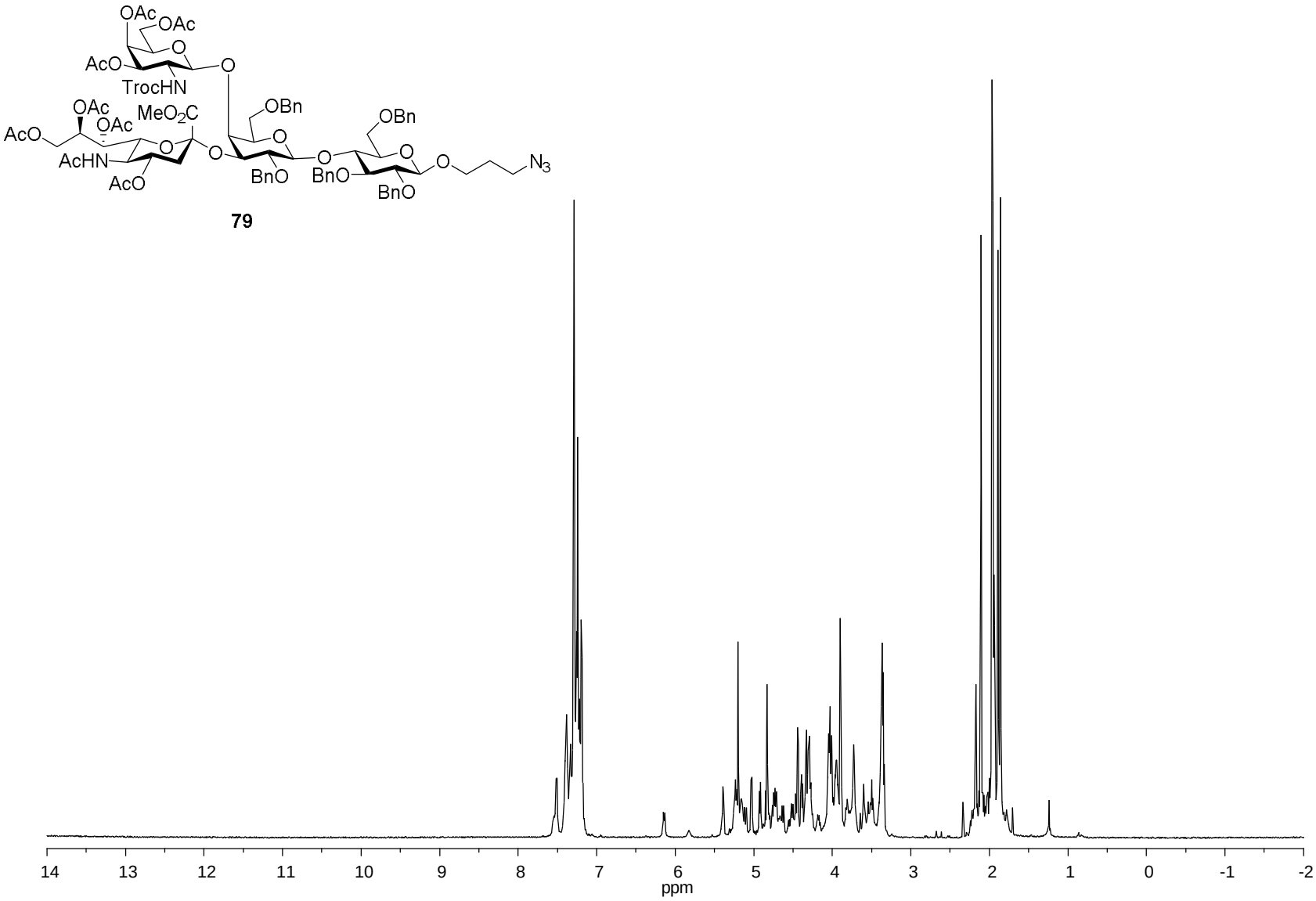
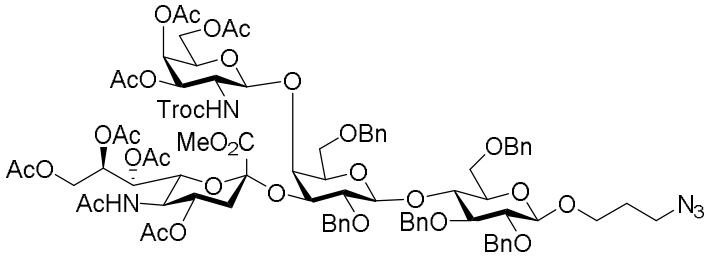


Figure 4.246. 500 MHz, $CDCl_3$, ^1H NMR of compound **79**



79

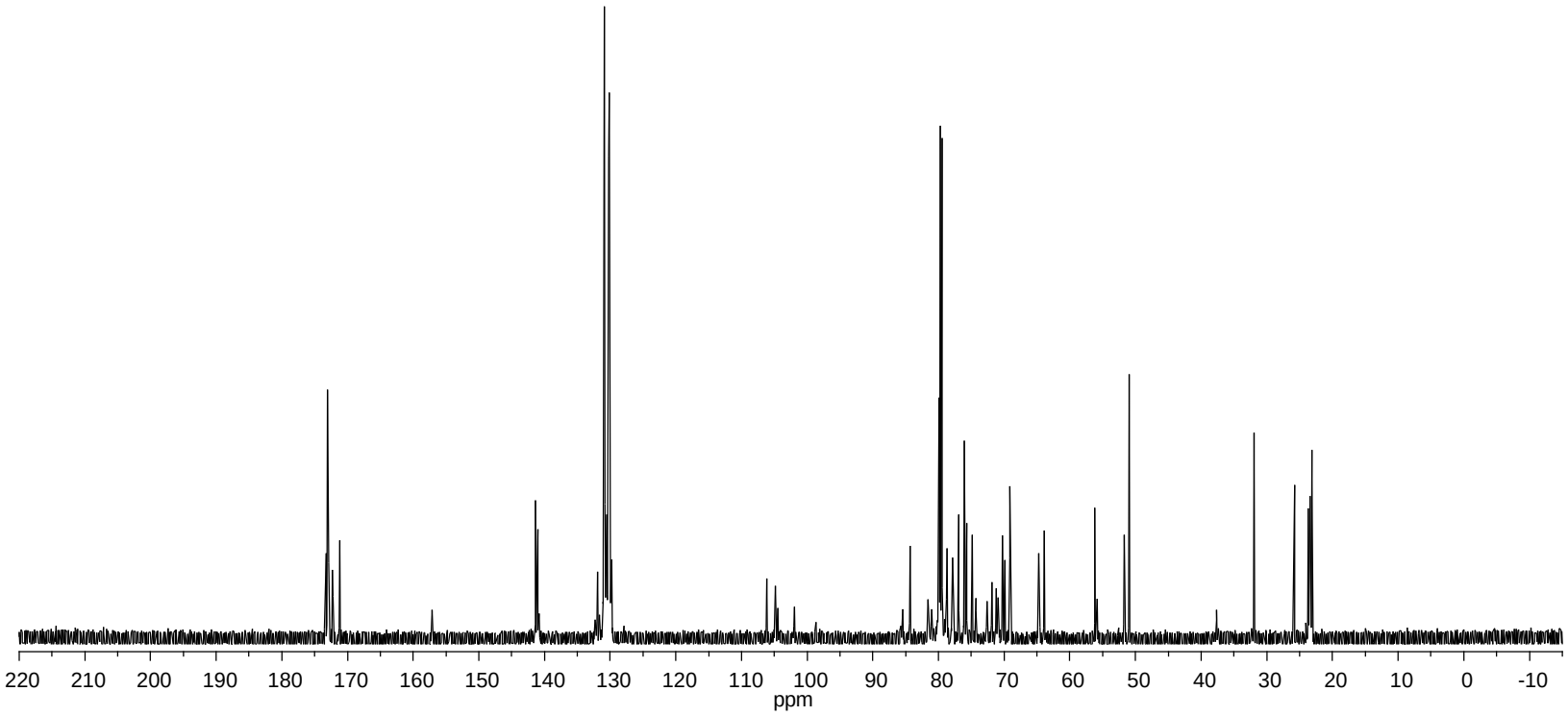


Figure 4.247. 125 MHz, $CDCl_3$, ^{13}C NMR of compound **79**

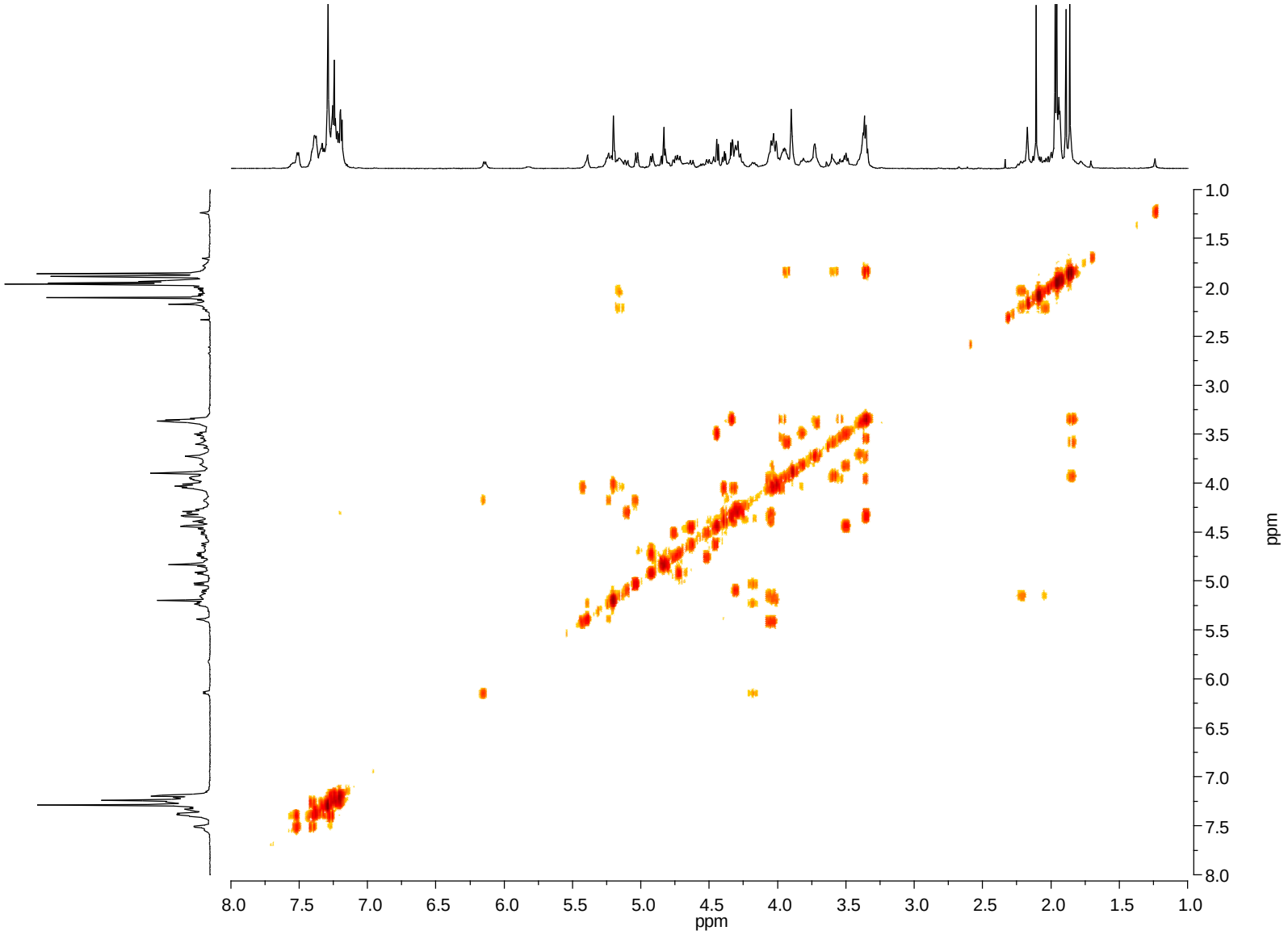


Figure 4.248. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **79**

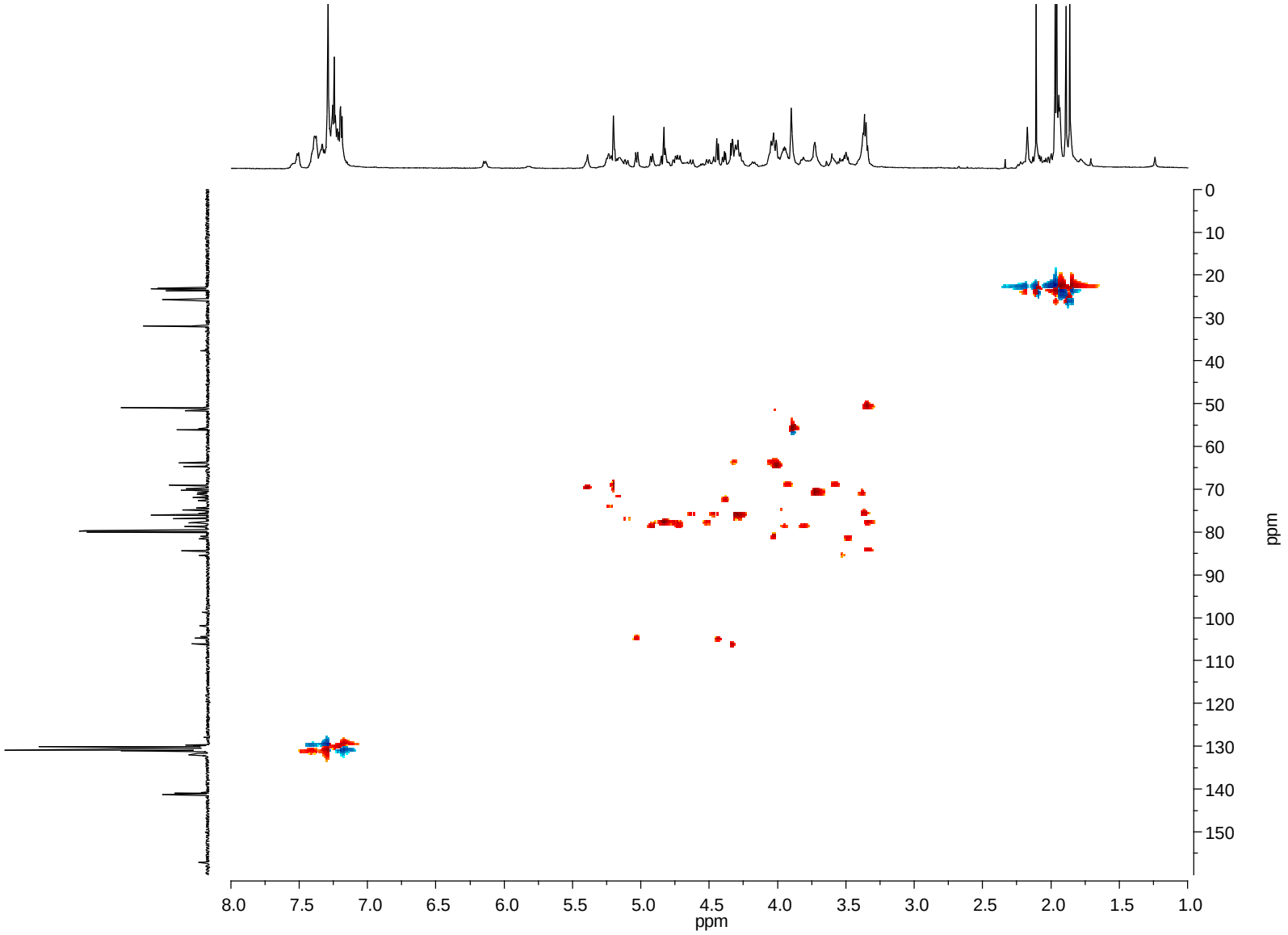


Figure 4.249. 500 MHz, $CDCl_3$, ^{1}H-^{13}C HMQC of compound **79**

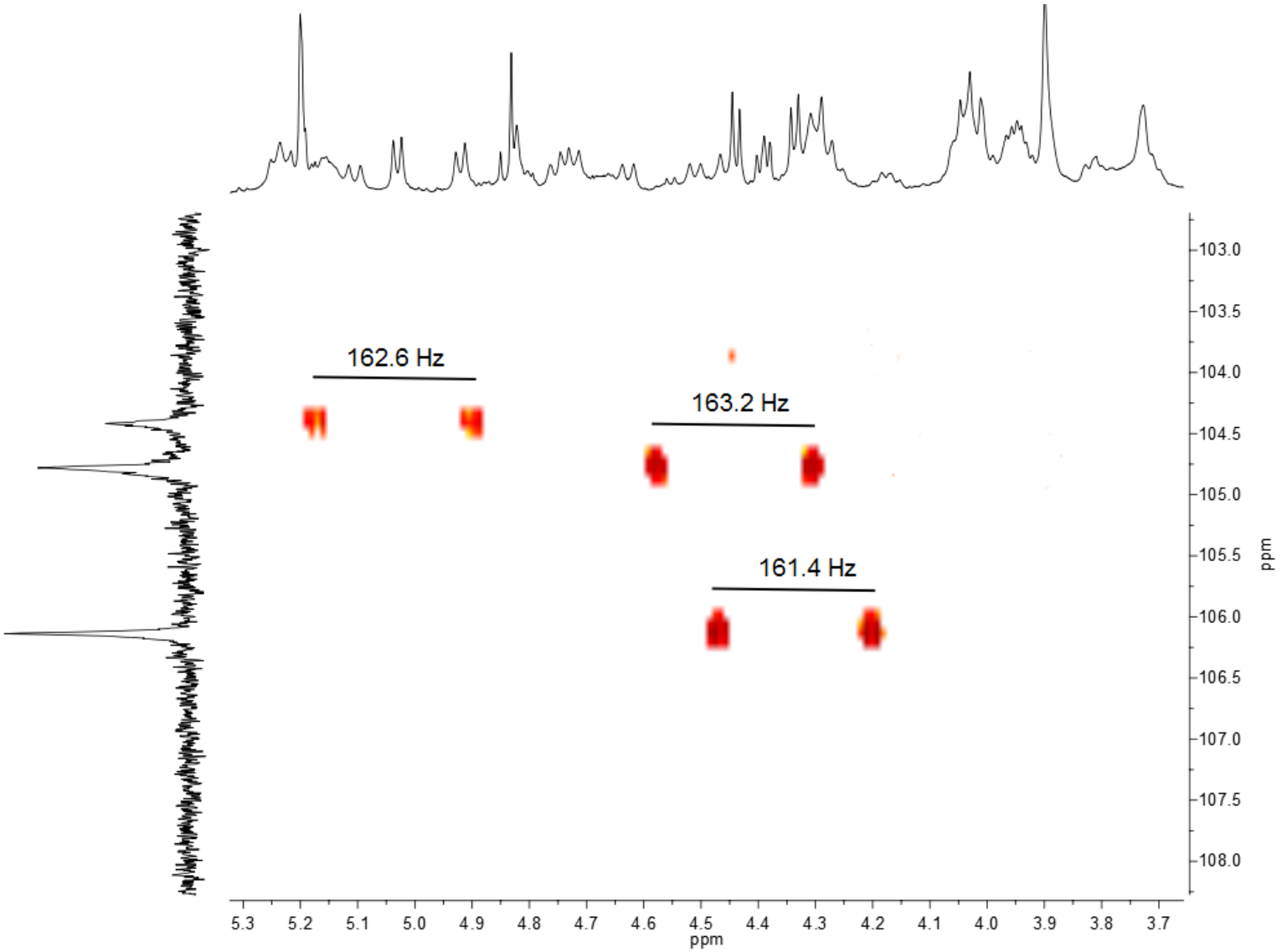
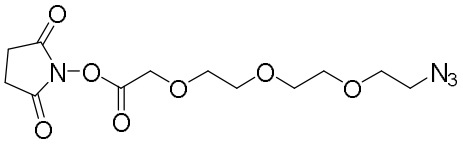


Figure 4.250. 500 MHz, $CDCl_3$, ^{1}H-^{13}C HMQC (without ^{1}H Decoupling) of compound **79**



80

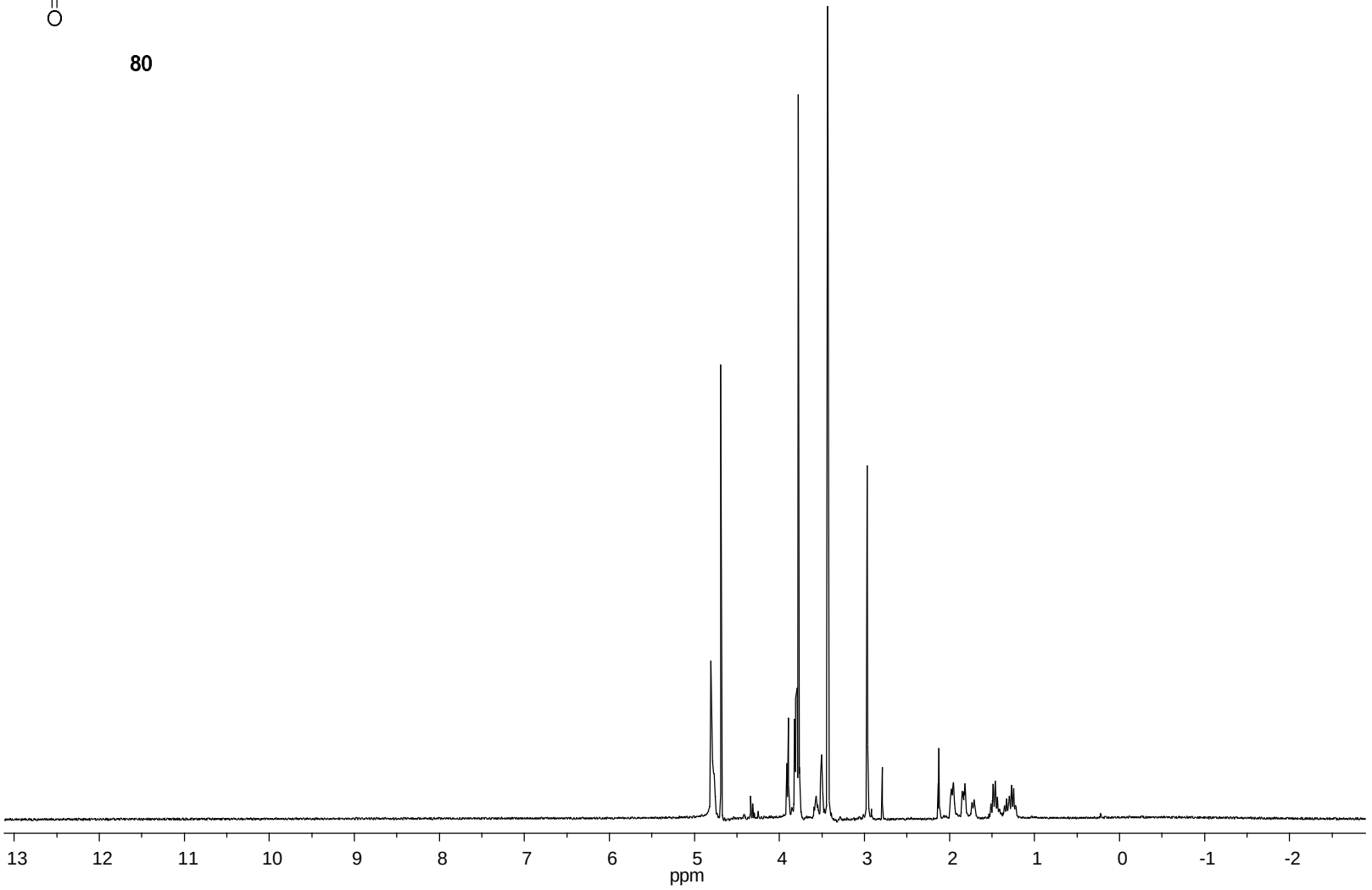


Figure 4.251. 500 MHz, CD_3OD, ^1H NMR of compound **80**

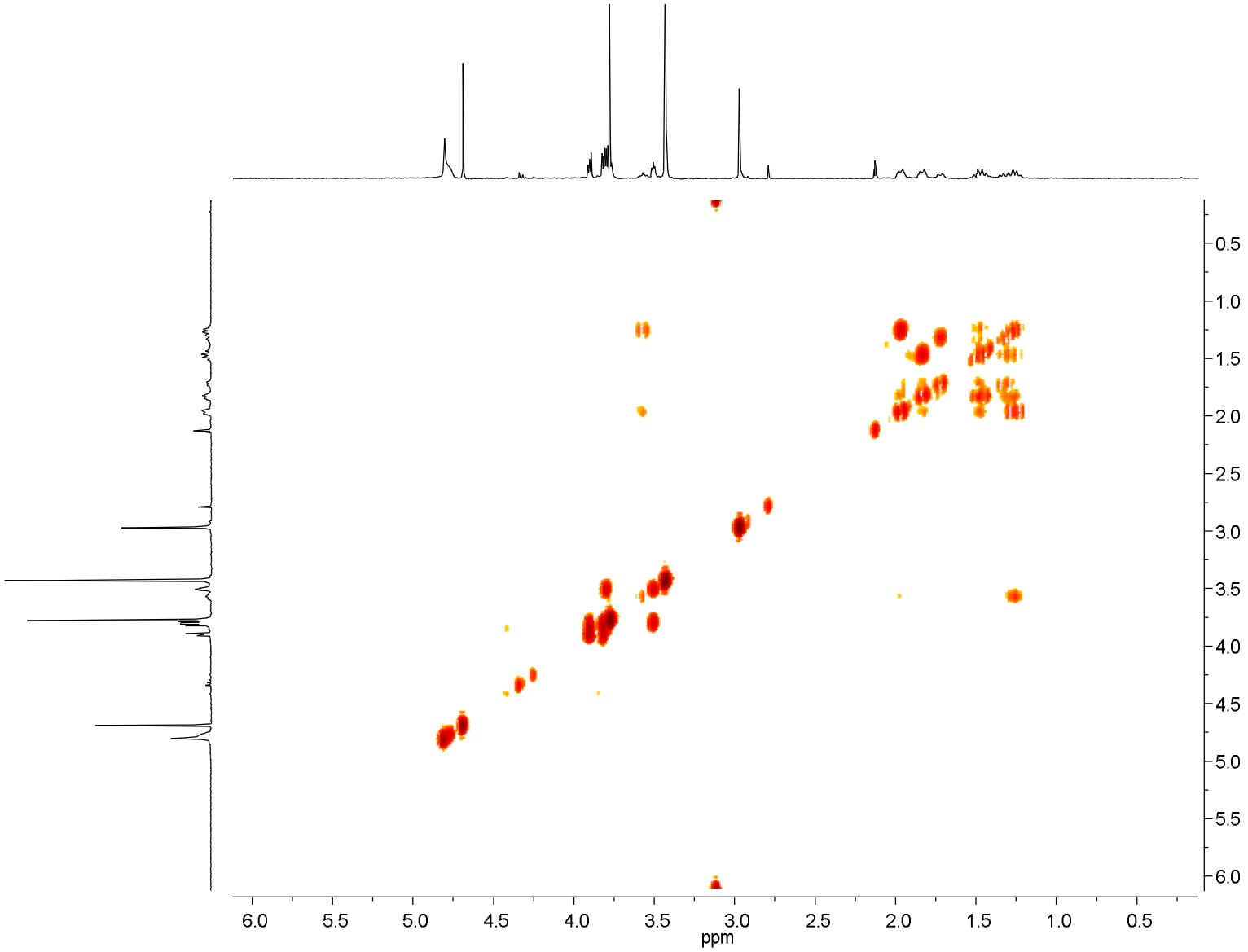
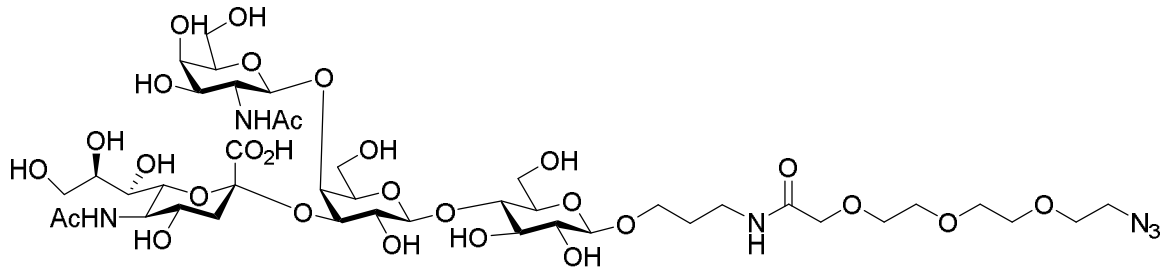


Figure 4.252. 500 MHz, CD_3OD, ^1H-^1H COSY of compound **80**



81

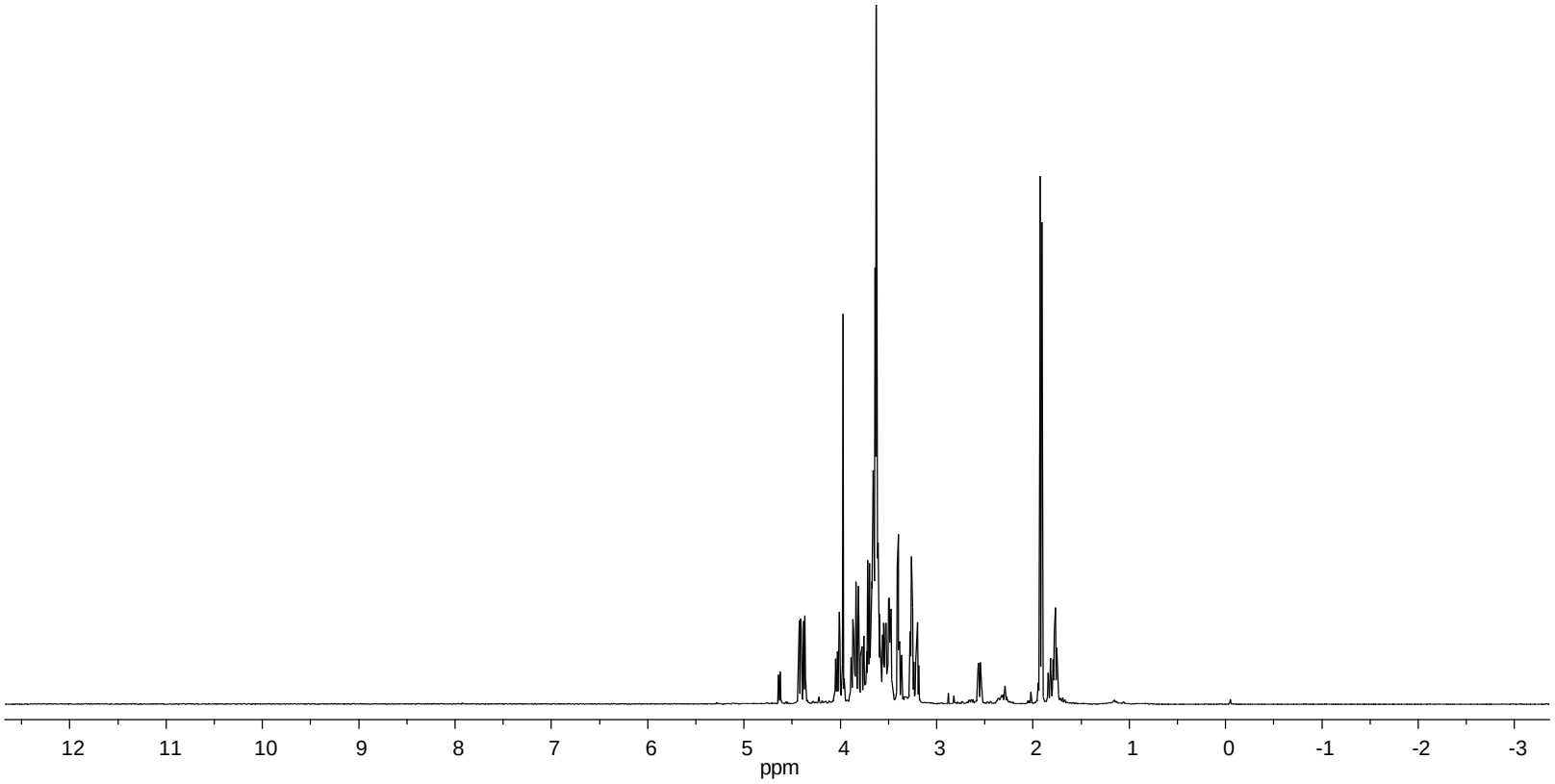
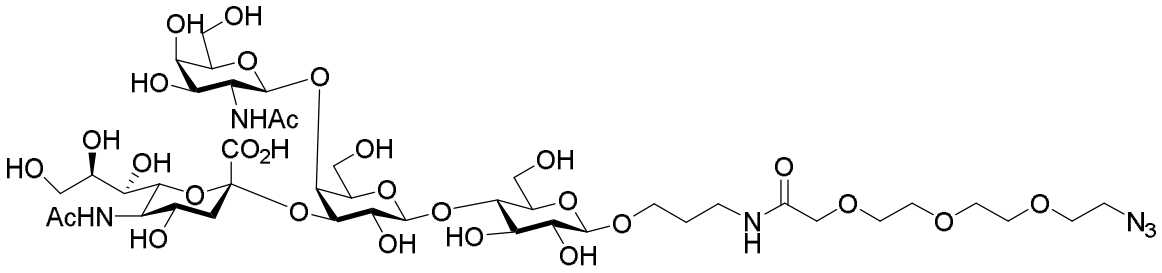


Figure 4.253. 500 MHz, $CDCl_3$, ^1H NMR of compound **81**



81

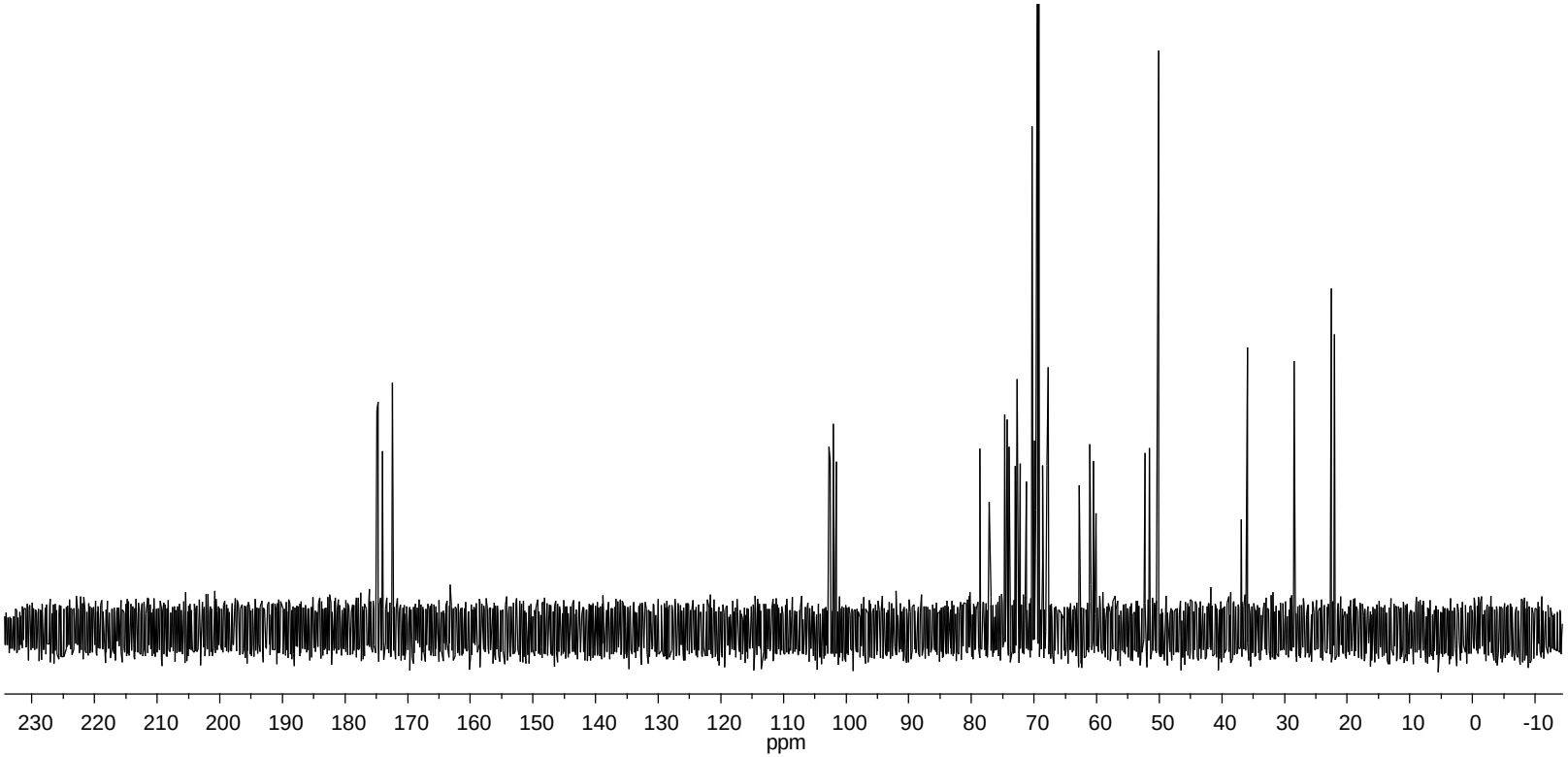


Figure 4.254. 125 MHz, $CDCl_3$, ^{13}C NMR of compound **81**

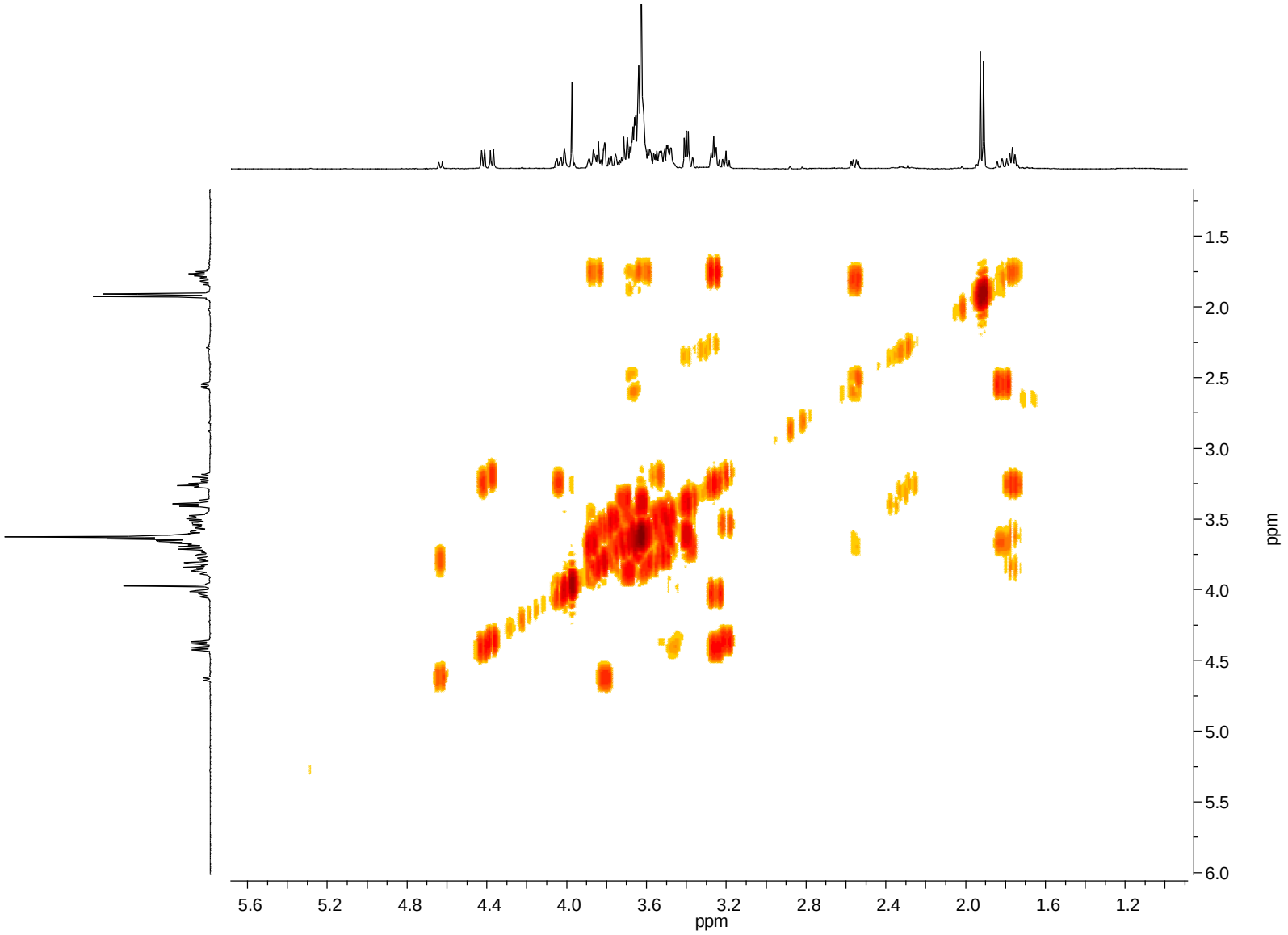


Figure 4.255. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **81**

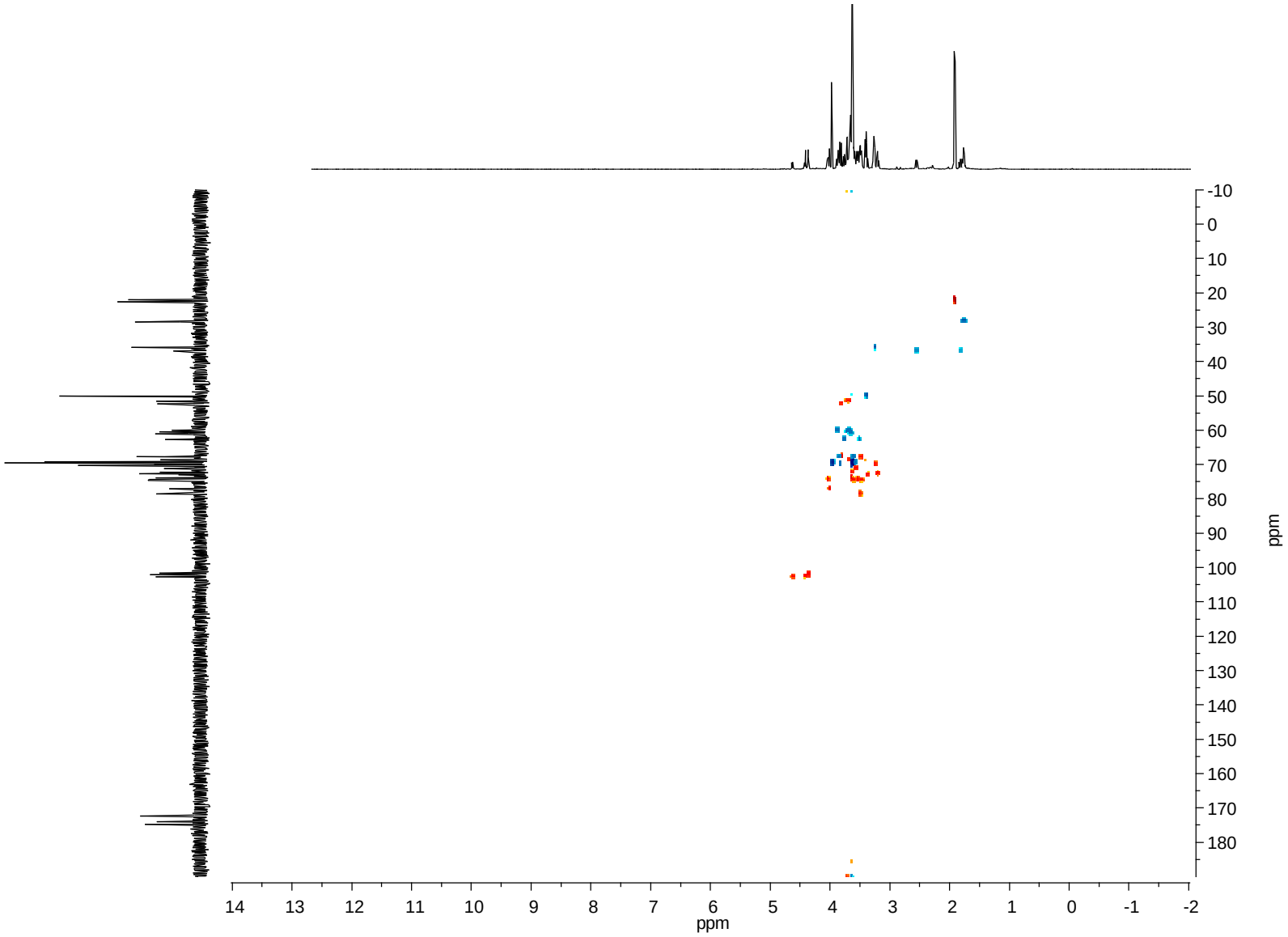


Figure 4.256.B 500 MHz, $CDCl_3$, ^{1}H-^{13}C HMQC of compound **81**

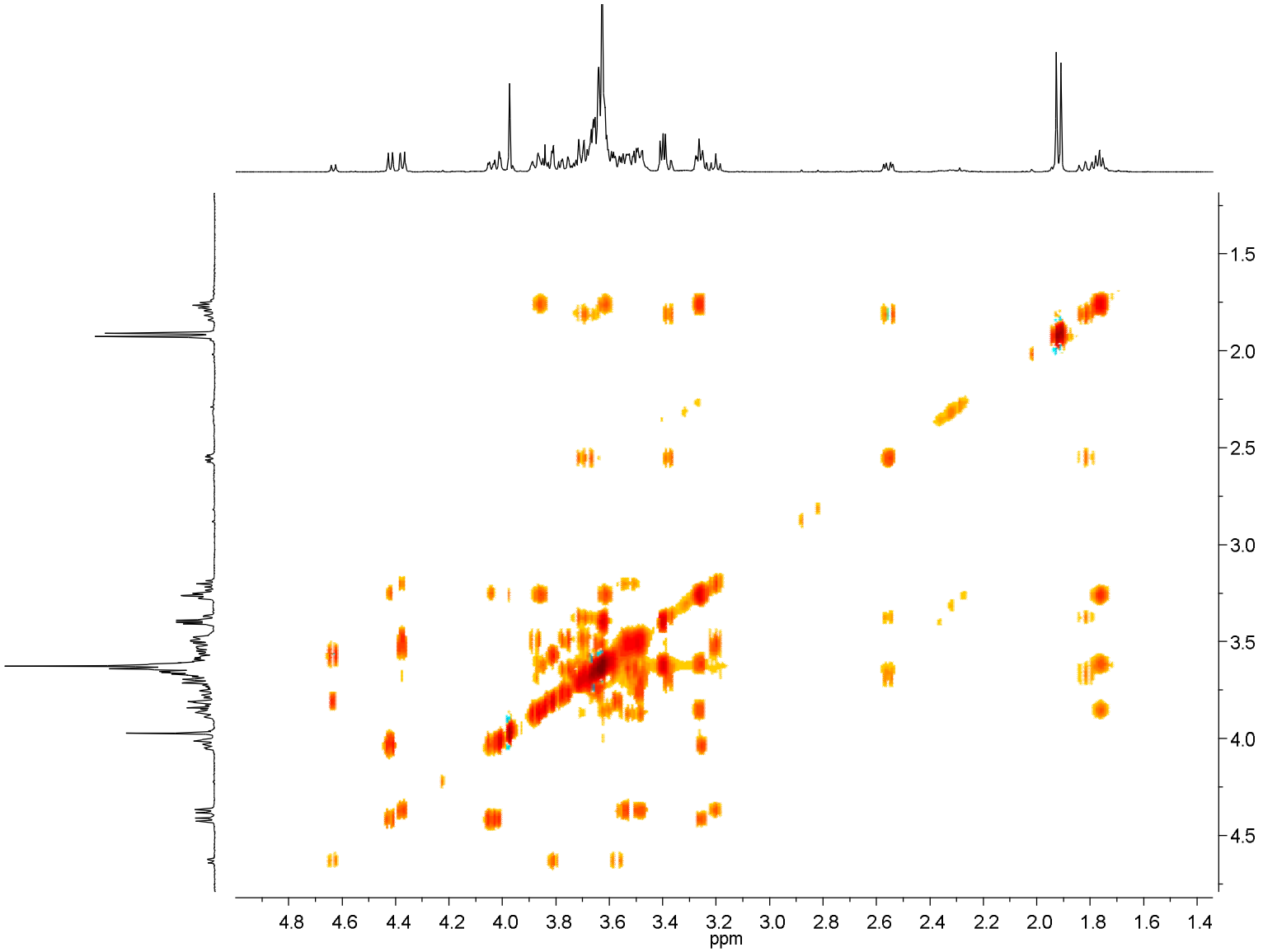


Figure 4.257. 500 MHz, $CDCl_3$, ^1H-^1H TOCSY of compound **81**

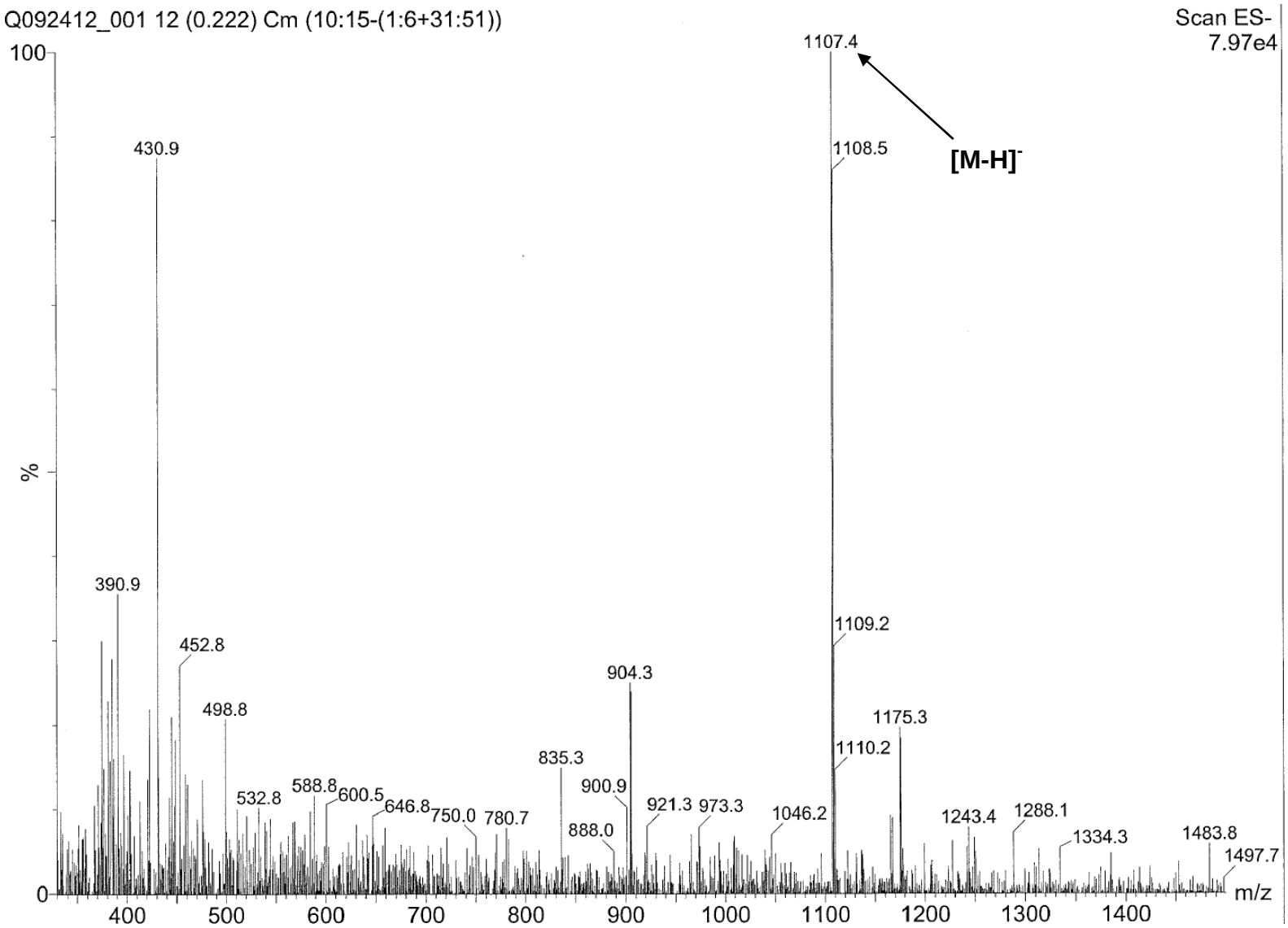


Figure 4.258. ESI-MS of compound **81**

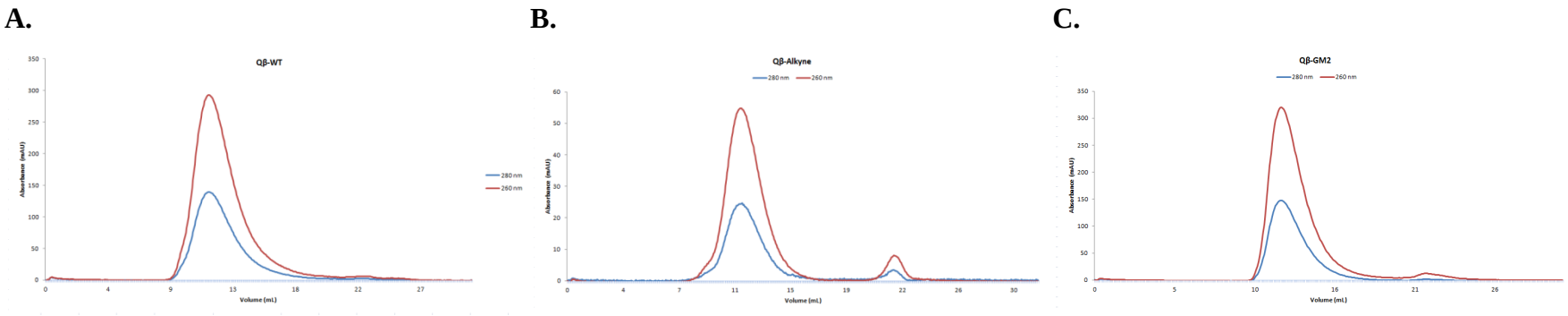


Figure 4.259. FPLC traces of Qβ-conjugates: (A) Qβ-WT, (B) Qβ-alkyne, (C) Qβ-GM2 **85**.

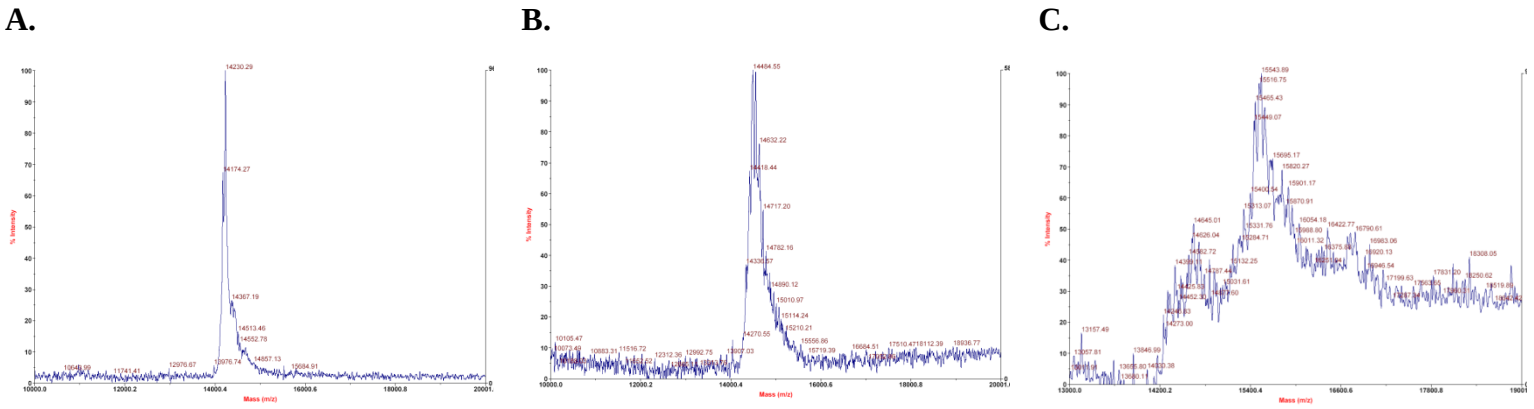


Figure 4.260. MALDI mass spectrometry of the following particles. (A) Qβ-WT, (B) Qβ-alkyne, (C) Qβ-GM2 **85**.

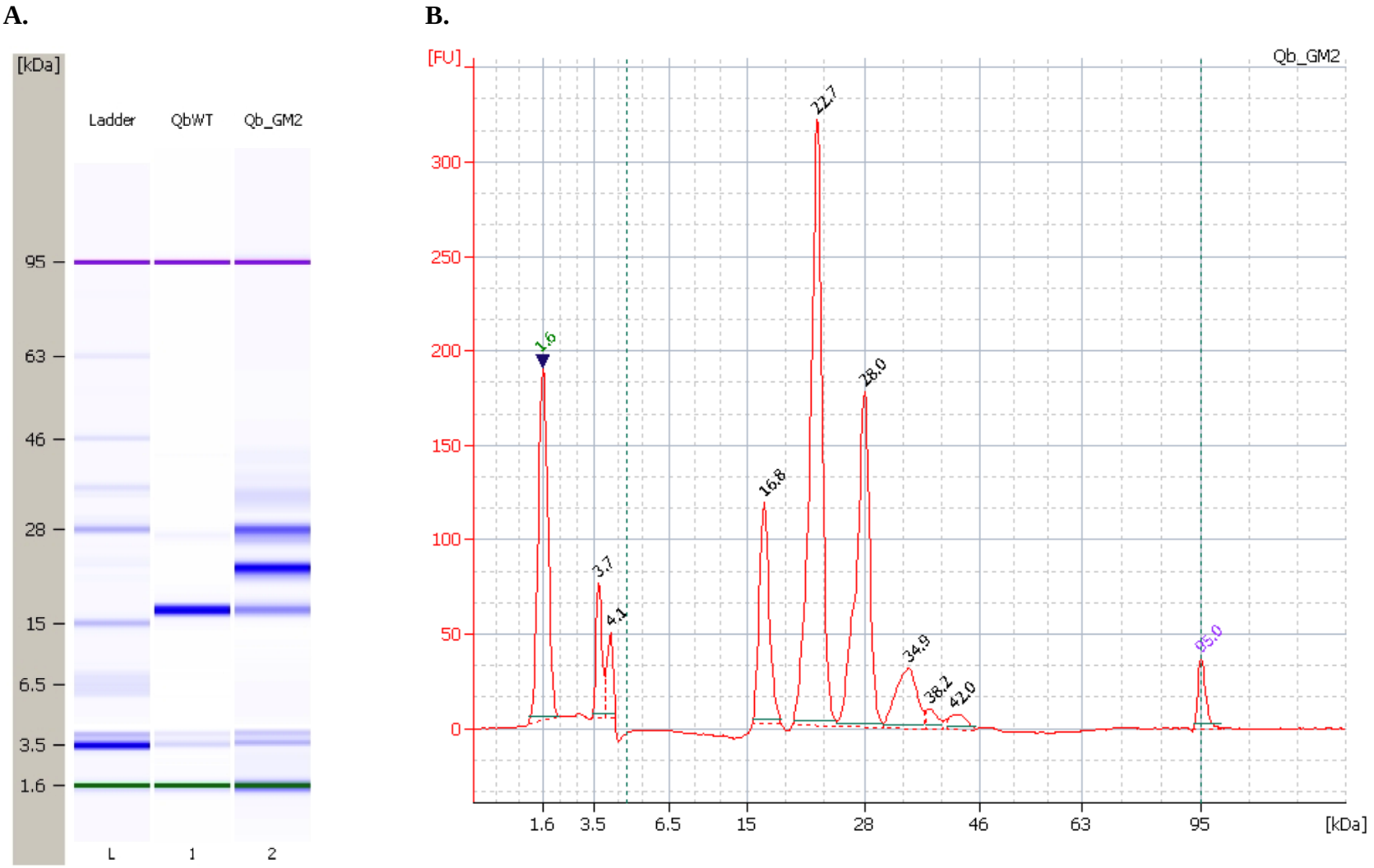


Figure 4.261. Electrophoretic analysis (A) and Qβ-conjugates loading determination (B) of Qβ-WT, Qβ-alkyne, and Qβ-GM2 **85**.

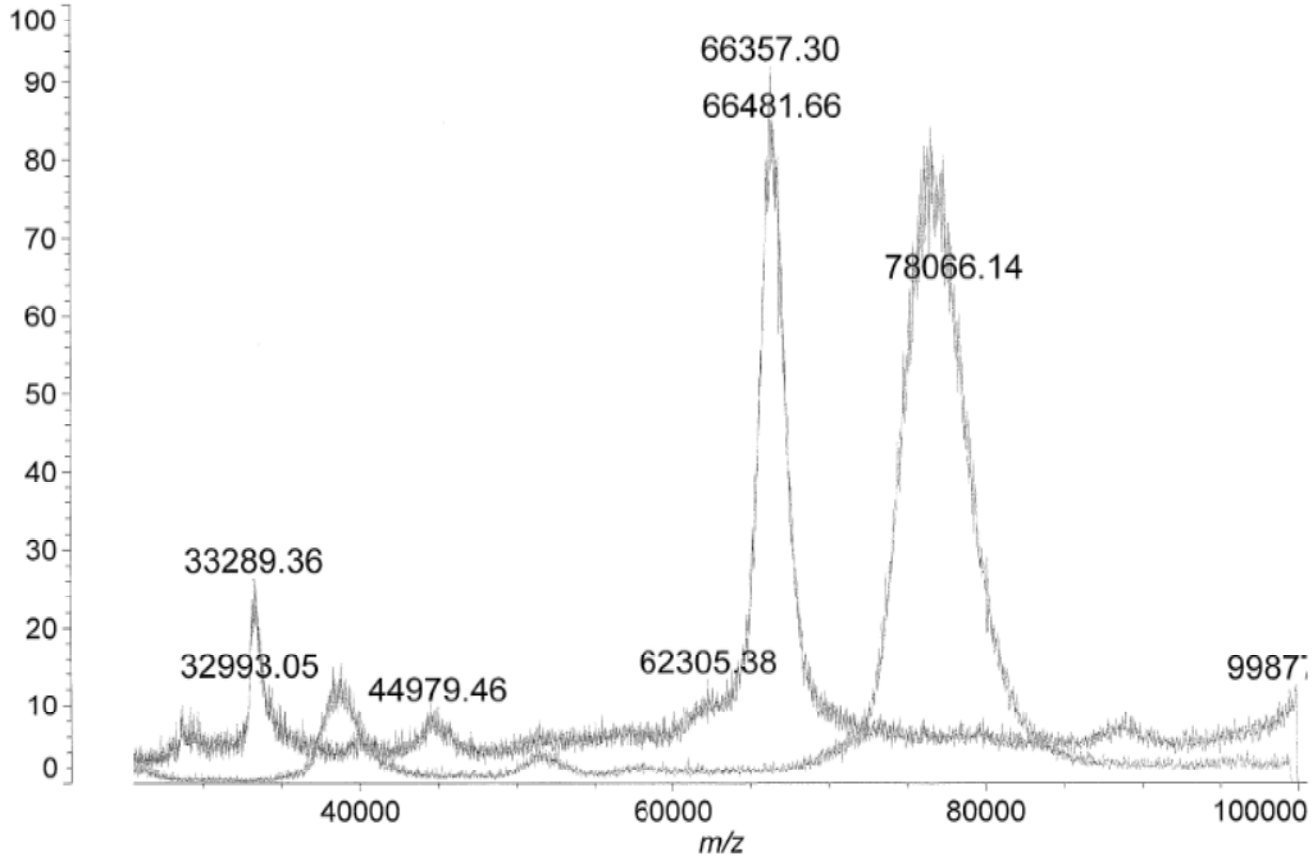


Figure 4.262. MALDI-TOF of BSA and BSA-GM2 (**86**).

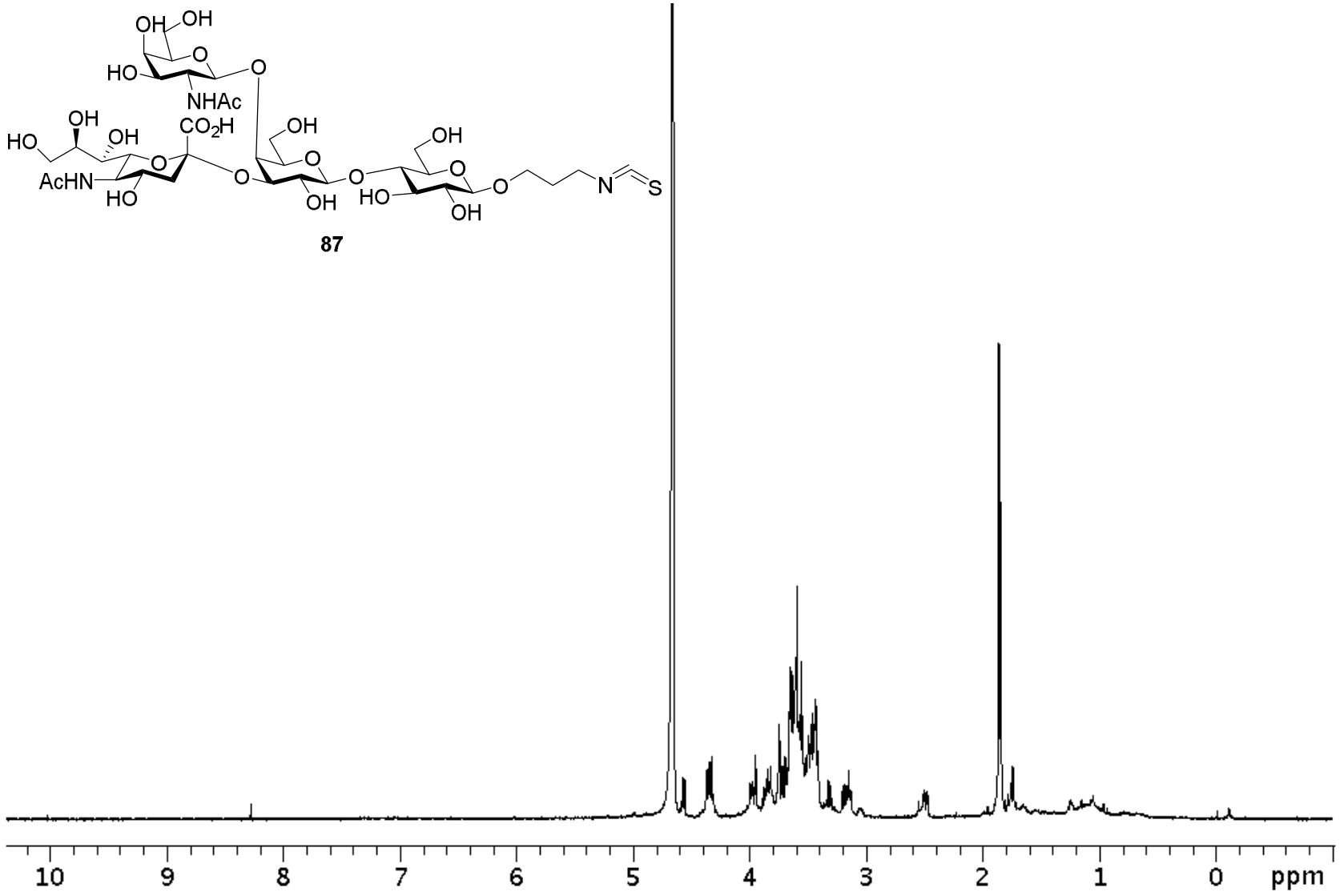


Figure 4.263. 500 MHz, D_2O, ^1H NMR of compound **87**

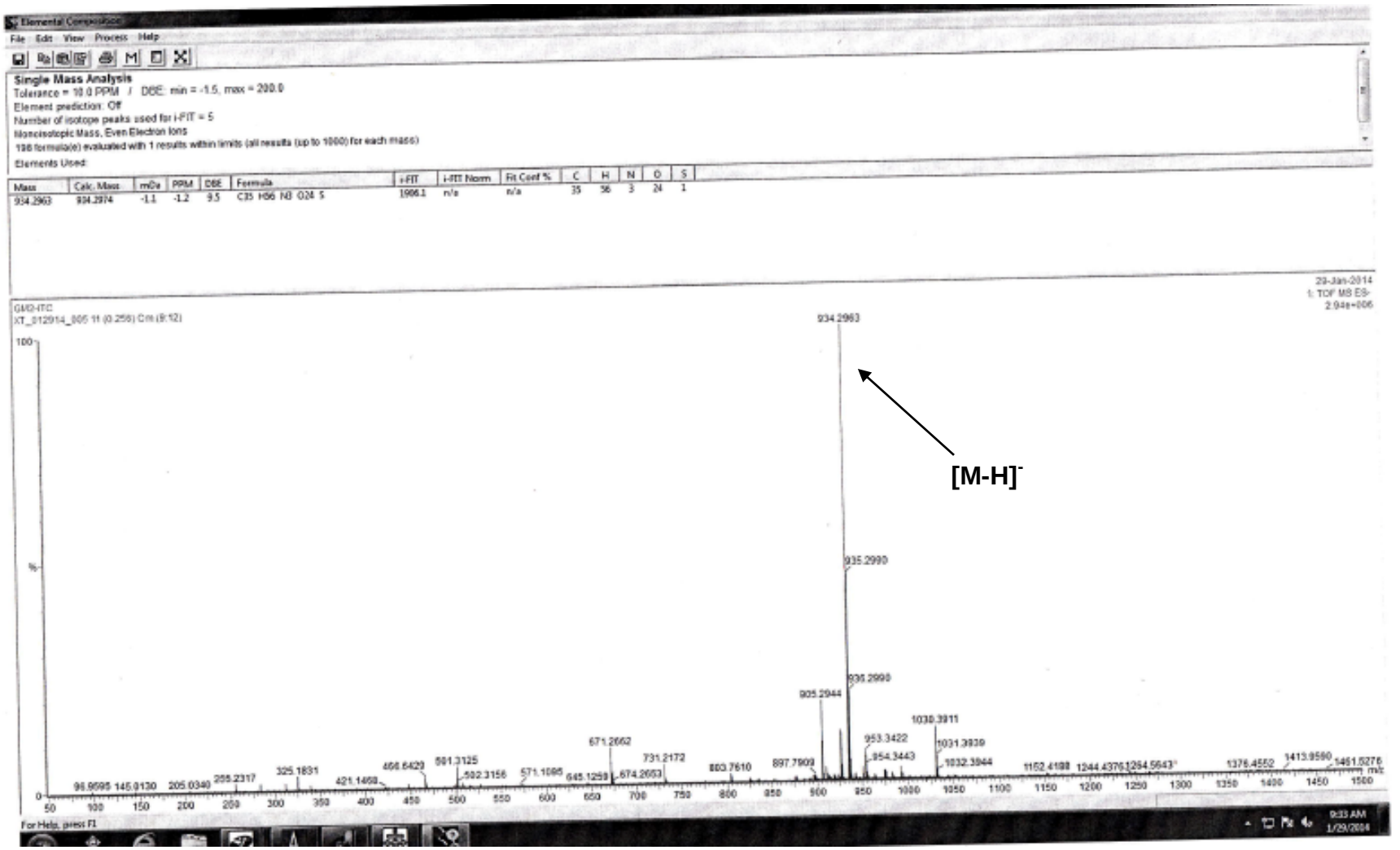


Figure 4.264. HR ESI-MS of compound **87**

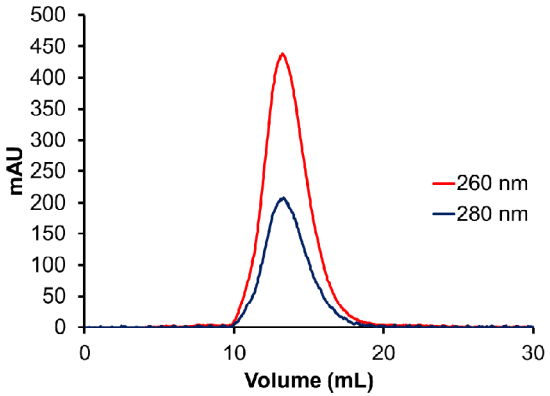


Figure 4.265. FPLC analysis of Qβ-GM2 **89** conjugate

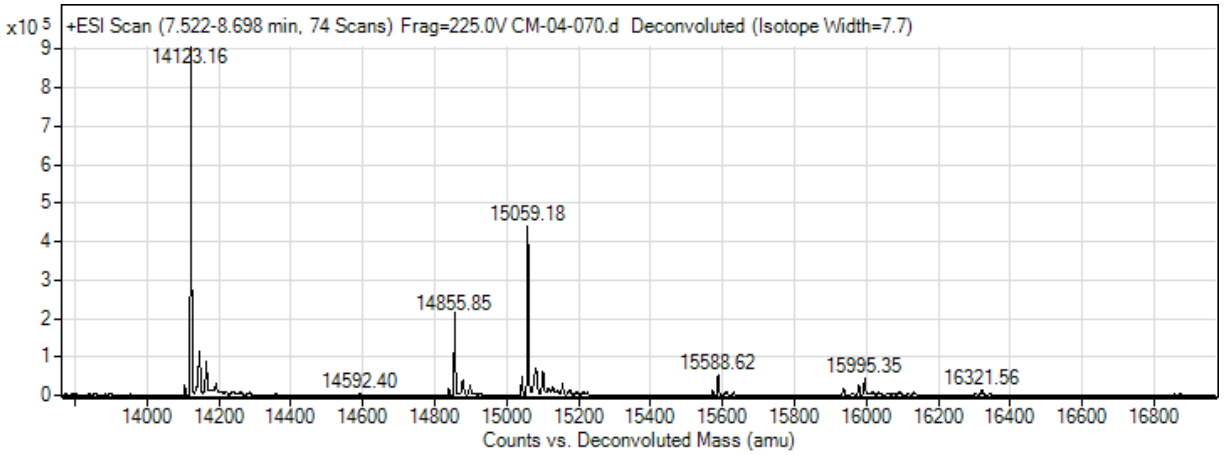


Figure 4.266. ESI-TOF HRMS spectra obtained Qβ-GM2 **89** conjugate

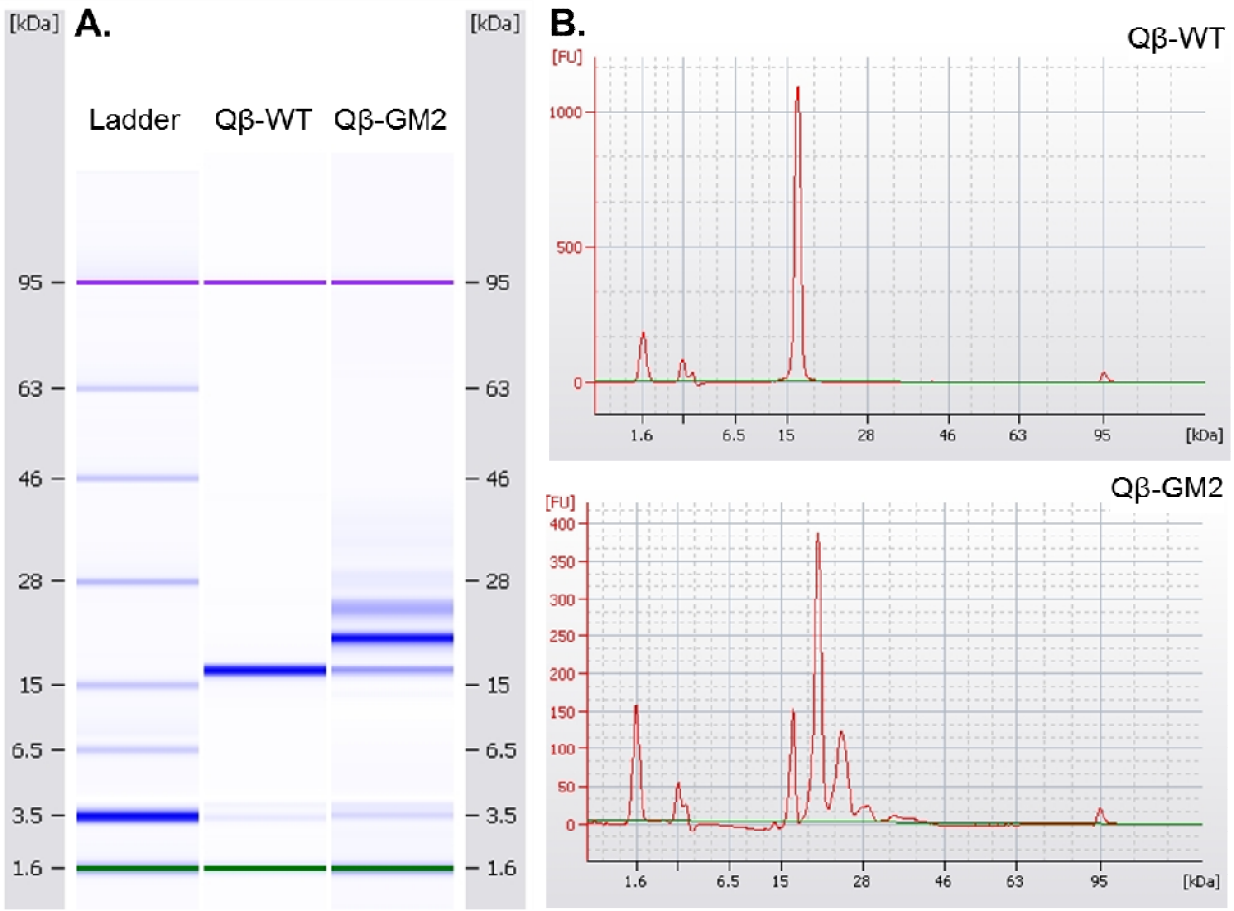
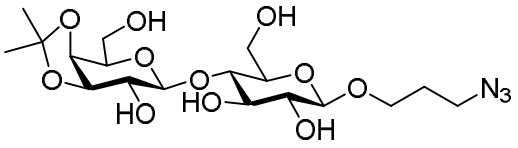


Figure 4.267. Electrophoretic analysis of Qβ-GM2 **89** conjugate. (A) gel results and (B) electropherogram



90

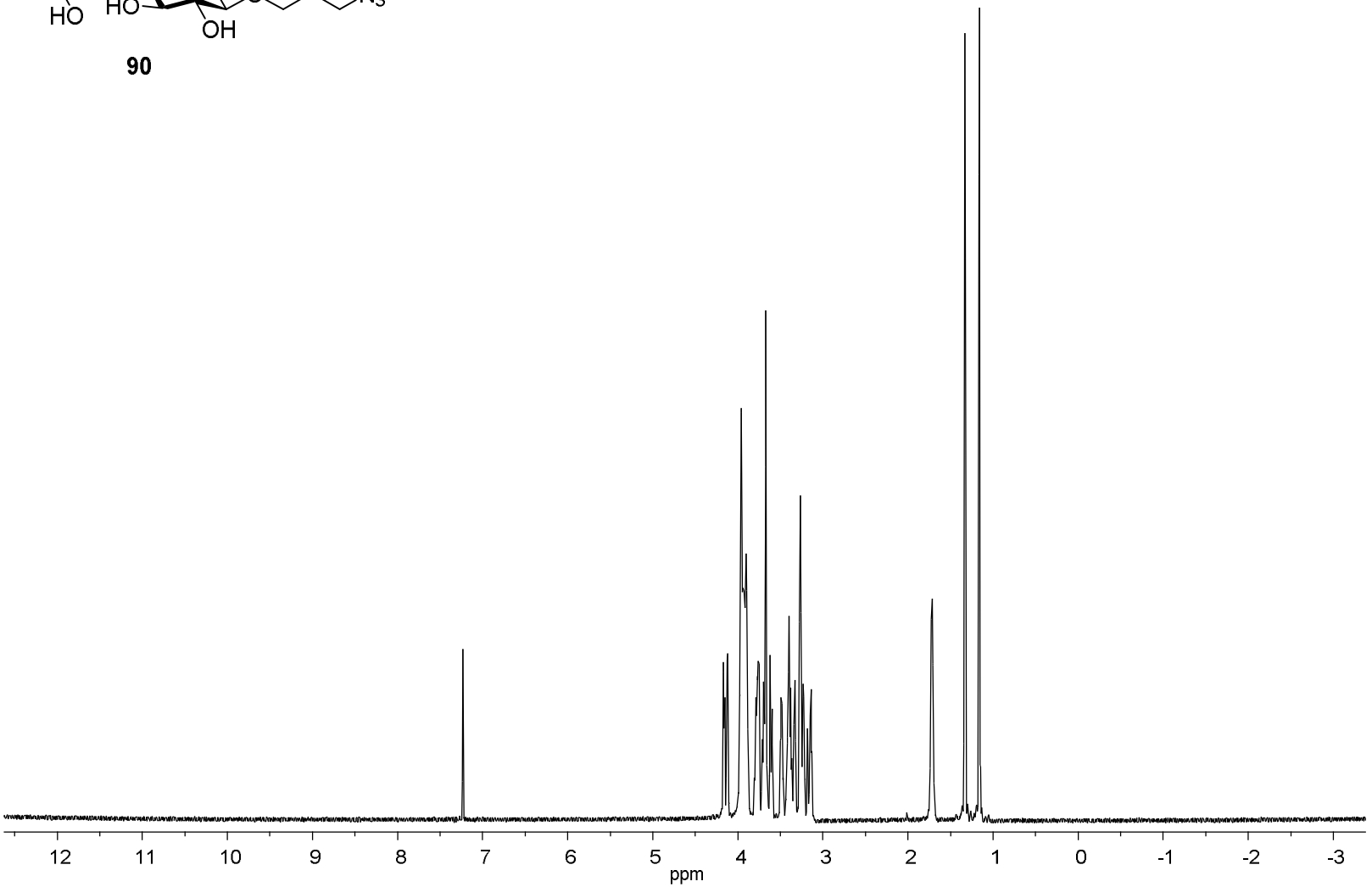


Figure 4.268. 500 MHz, $CDCl_3$, ^1H NMR of compound **90**

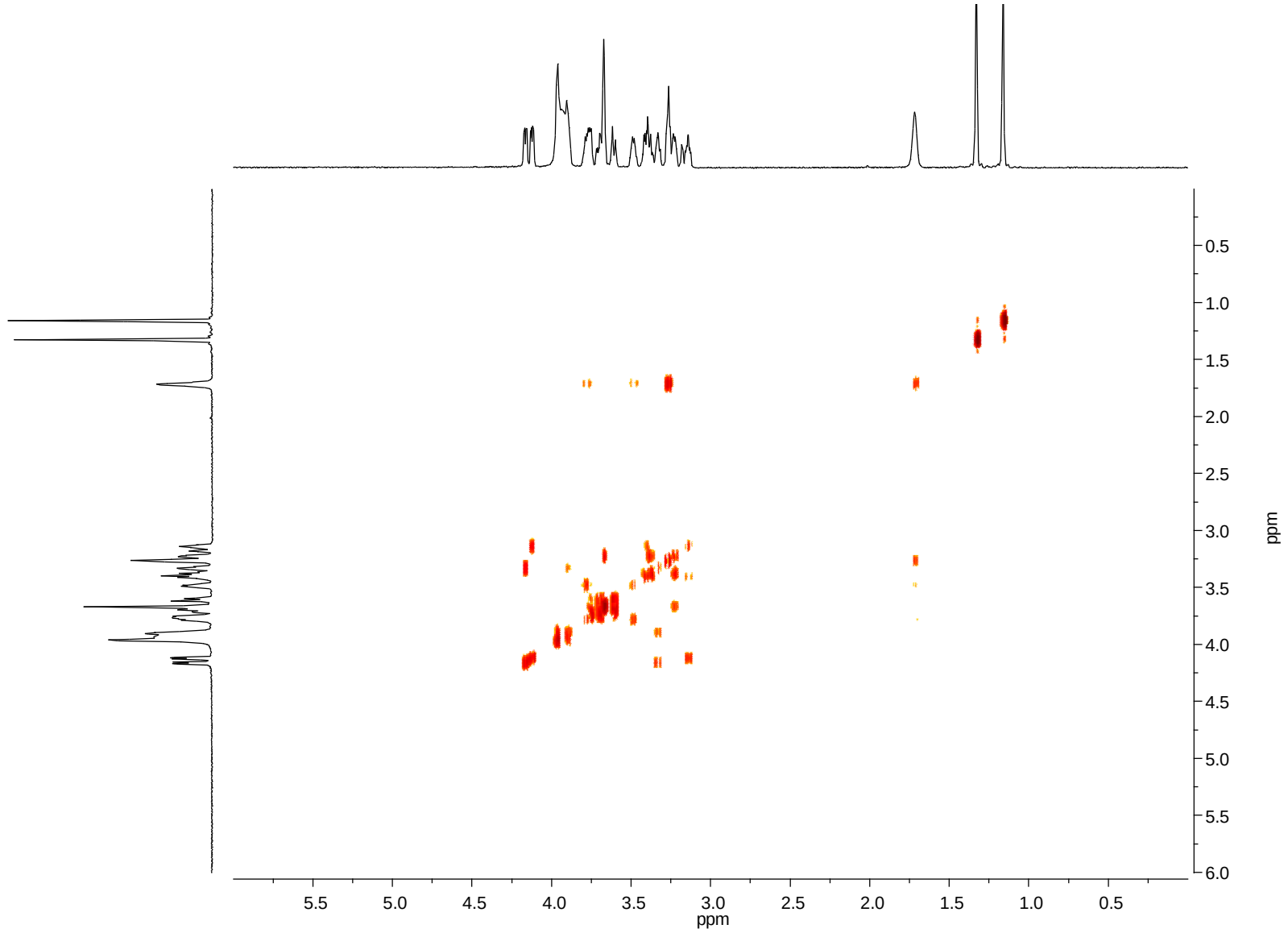
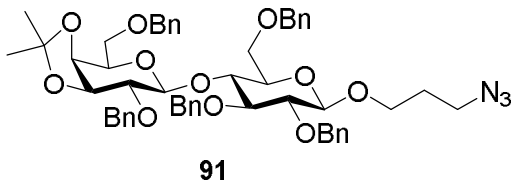


Figure 4.269. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **90**



91

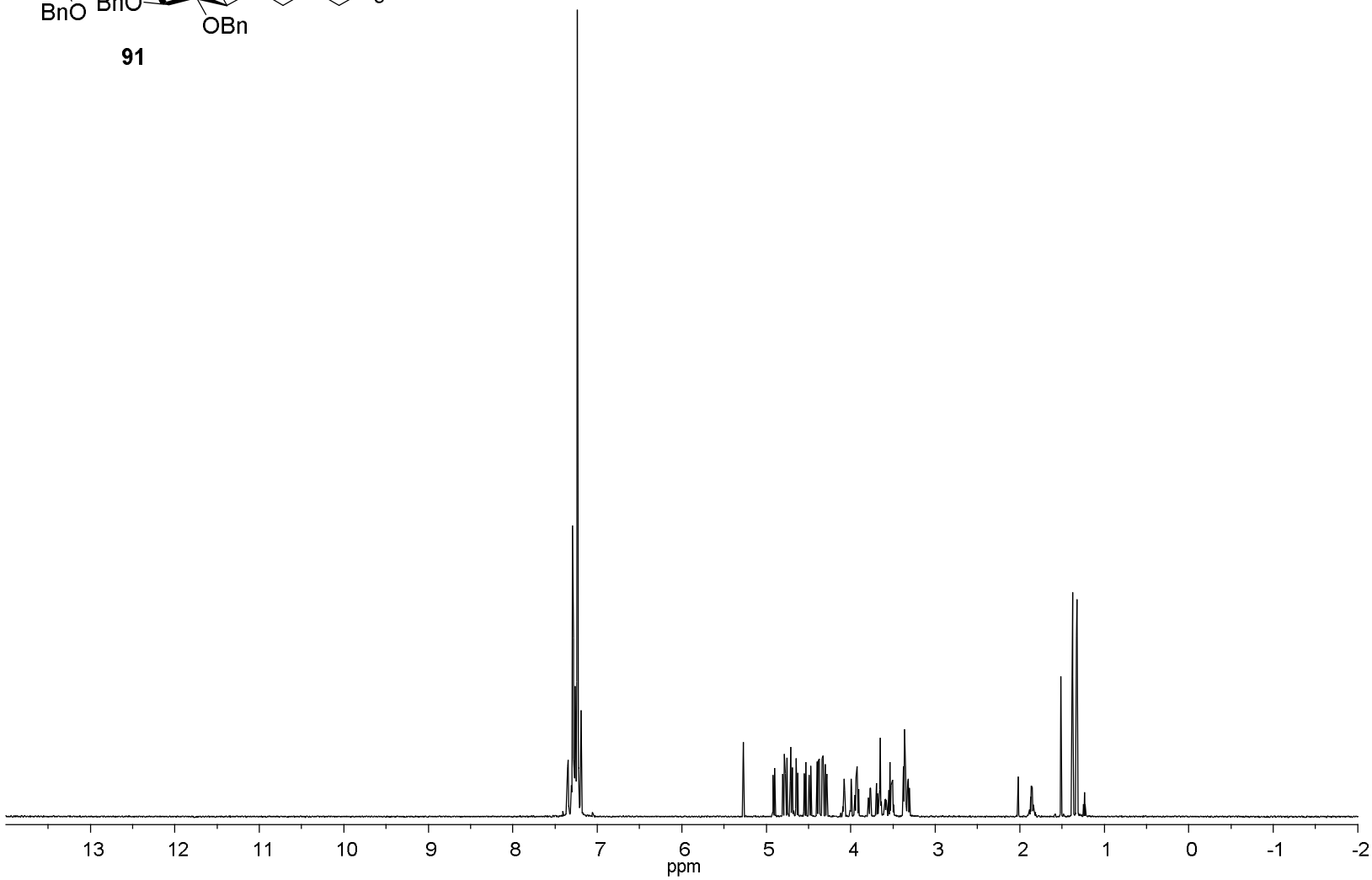


Figure 4.270. 500 MHz, $CDCl_3$, ^1H NMR of compound **91**

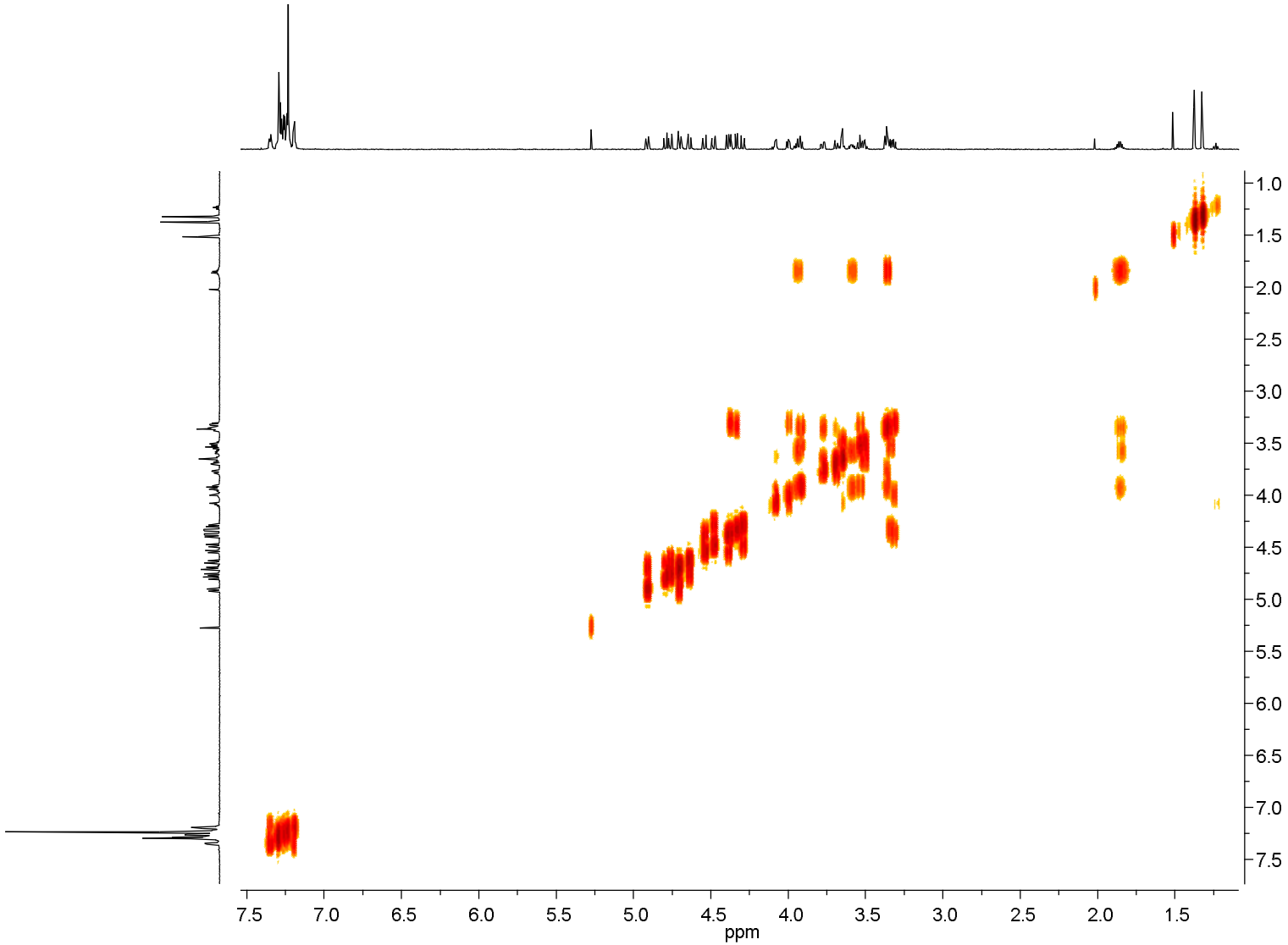


Figure 4.271. 500 MHz, $CDCl_3$, ^1H-^1H COSY of compound **91**

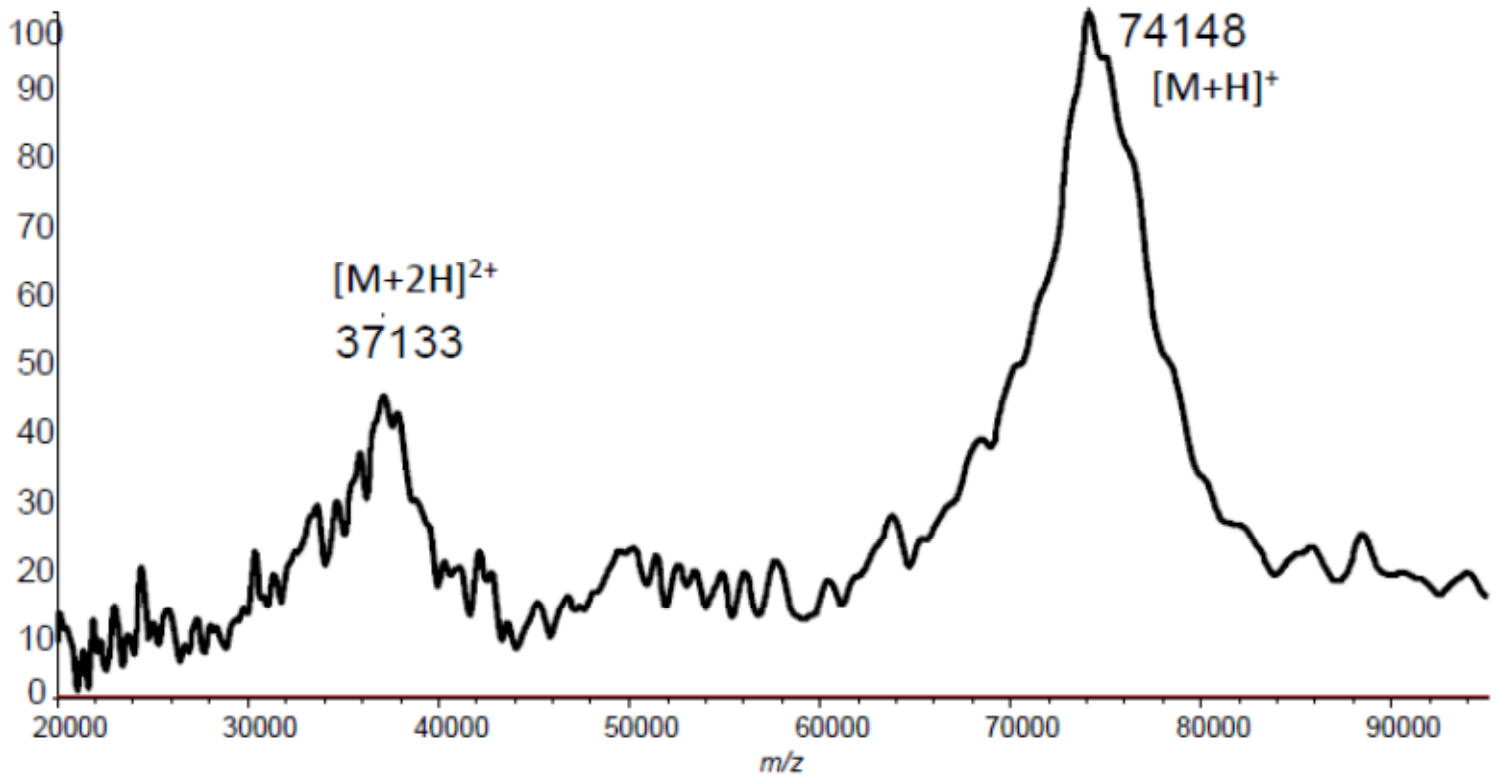


Figure 4.272. MALDI-TOF of BSA-lactose.

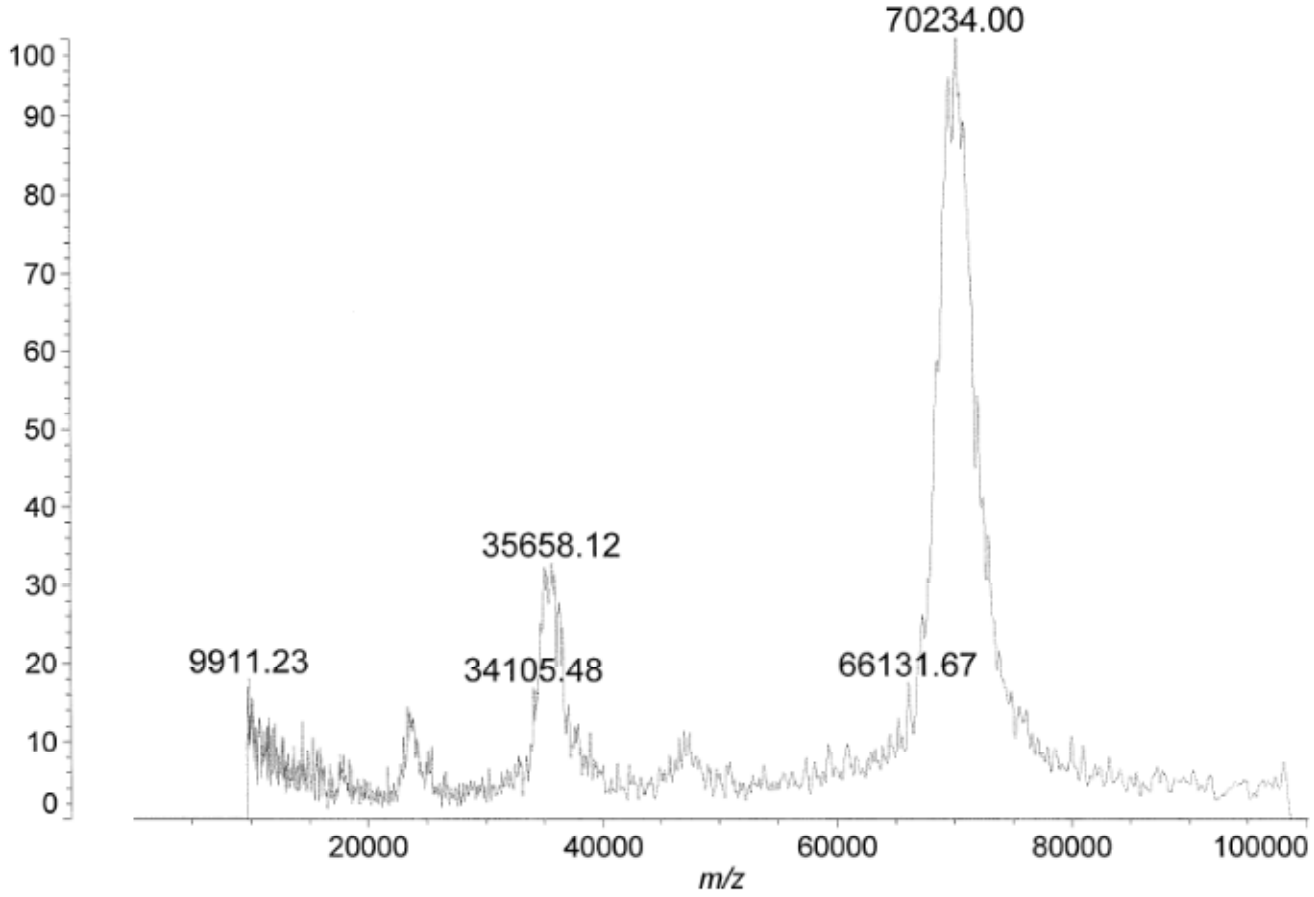


Figure 4.273. MALDI-TOF of BSA-GM3.

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