Université de Strasbourg (UDS)

Institut de Science et d'Ingénierie Supramoléculaires (ISIS)

Laboratoire de Chimie Organique et Bioorganique

THESE DE DOCTORAT

Kuo-Ting HUANG

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Novel Strategies for Carbohydrate Synthesis and Assemblies

Directeur

Professor Nicolas WINSSINGER

Jury

Professor Philippe COMPAIN	*University of Strasbourg, France*
Professor Stephane VINCENT	*University of Namur, Belgium*
Professor Helma WENNEMERS	*University of Basel, Switzerland*

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French Abstract

La première partie de la thèse est consacrée à de nouvelles méthodes de synthèse. Dans un premier temps, nous avons évalué une nouvelle méthode d'activation des thioglycans. Le réactif IPy2BF4 s'est avéré comme étant une des meilleures sources de I^+ (iodonium) dans un nombre de réactions cyclisantes. Ce réactif est disponible commercialement mais n'a jamais été évalué dans le contexte de réactions de glycosylations malgré de nombreux précédant par rapport à l'utilisation d'électrophile pour activer les thioglycans. Par comparaison à la méthode classique (NIS/TfOH), nous avons démontrer que ce réactif à une réactivité unique en particulier dans le contrôle anomérique avec des donneurs activés. De plus, ce reactif permet une préparation facile de fluoroglycan. Dans un deuxième temps, nous avons étudié des approches synthèse de novo d'oligosaccharides. Basée sur la condensation itérative de sous-unités α-oxyaldéhyde catalysé par la proline, nous nous sommes intéressés à la synthèse d'oligosaccharides, en particulier avec une substitution de l'oxygène anomeric pour un carbone. Dans un troisième temps, nous avons étudié une réaction de ligation pour les glycans inspirée par des développements parallèles en chimie peptidique.

Dans la seconde partie, les travaux ce sont centrés sur les oligomères de carbohydrates fonctionnalisés. Basé sur l'expérience de notre groupe dans le domaine des peptides d'acides nucléiques (PNAs), nous voulions étudier la conjugaison de carbohydrates avec des PNAs. A cette fin, nous avons étudié trois approches, couplage hydrazone glycal, des couplages de cycloaddition (click), et couplages chimiosélectifs entre un chloroacetamide et glycosyl thiol. Ces deux dernières approches ce sont avérées robustes donnant des conjugués PNA-glycan avec de très bon rendements. Afin d'émuler la fonction glycans complexes par autoassemblage de fragments de glycan-PNAs sur des brins ADN, nous avons évalué l'affinité de plus de 30 complexes pour l'anticorps 2G12. Ces études nous ont permis de démontrer qu'il était en effet possible de mimer des interactions oligomeriques en programment la distance entre les fragments. Suite à ces résultats, nous avons synthétisé une chimiothèque de glycans encodée par des PNAs afin d'optimiser l'affinité pour le DC-SIGN, un récepteur de cellules dendritiques impliqué dans les réponses immunitaires. Nous avons aussi étendu l'utilité des ces conjugués glycan-PNA en démontrant qu'il peuvent être autoassemblé dans un format micrarray pour cribler des protéines reconnaissant les carbohydrates telles que les lectins.

Abbreviations

Ac	acetyl
AIBN	2,2'-Azobisisobutyronitrile
All	allyl
Bn	benzyl
Bz	benzoyl
ConA	concanavalin A
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-dicyclohexylcarbodiimide
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	*N,N*- dimethylformamide
DMTST	dimethyl(methylthio)sulfonium triflate
TBS	*tert*-butyldimethylsilyl
TBAHS	*tetra*-n-butylammonium hydrogen sulfate
DTBMP	2,6-di-tert-butyl-4-methylpyridine
EtSNPhth	*N*-(ethylthio)phthalimide
Fmoc	9-fluorenylmethoxycarbonyl
Fuc	fucose
Gal	galactose
GalNAc	*N*-acetylgalactose amine
Glc	glucose
IDCP	iodonium dicollidine perchlorate
IPy2BF4	bis(pyridine)iodonium tetrafluoroborate
Man	mannose
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
Ph	phenyl
PTSA	*p*-toluene sulfonic acid
Pyr	pyridine
TFA	trifluoroacetic acid
Tf_2O	trifluoromethanesulfonic anhydride
TfOH	trifluoromethanesulfonic acid
Tm	melting temperatures
TMSI	trimethylsilyl iodide
Troc	2,2,2-trichloroethoxycarbonyl
TTBP	2,4,6-tri-tert-butylpyrimidine

1. Summary

Carbohydrate plays a variety of function in living organisms beyond their energetic role (Fig.1), most notably in cellular recognition, cellular communication and protein regulation. The prominent place occupied by glycoscience and glycobiology in the scientific landscape attest to the essential roles of carbohydrates in living organisms.[1-5] A longstanding challenge in glycoscience has been access to complex glycoconjugates as a means to study their structure-function. This thesis addresses this challenge by investigating novel strategies for carbohydrate synthesis and assemblies. The first aspect was to exploit a powerful iodination reaction in glycosylation reactions with thioglycoside donors offering alternative outcome with "armed donors" than traditional activation method and hence potentially useful in one pot glycosylations. The second was to explore glycoconjugation methods in analogy to the native chemical ligation for protein synthesis. The third aspect of my thesis was to explore *de novo* oligosaccharides synthesis using Proline catalysis. The last topic was to investigate the self-assembly of PNA-encoded oligosaccharides onto DNA template to emulate large carbohydrate complexes. This latter topic required the development of efficient methods to couple carbohydrates to PNAs.

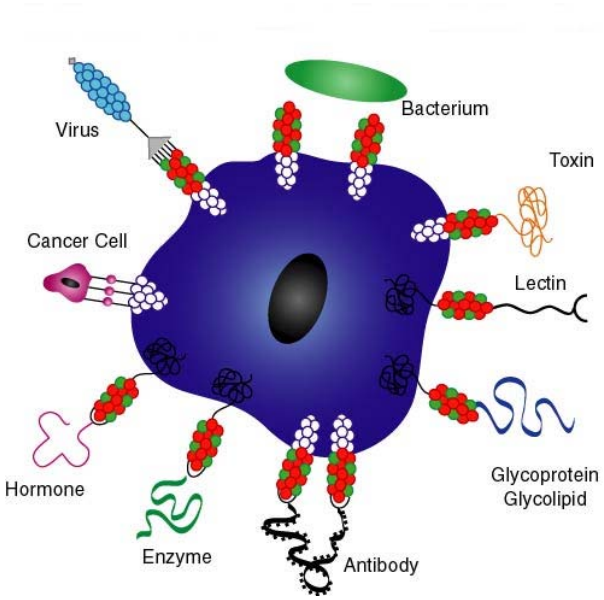

Figure 1 Cell-surface carbohydrates involved in molecular recognition. (http://www.scripps.edu/chem/wong/)

1.1 Novel Synthesis Methods

1.1.1 IPy_2BF_4-mediated glycosylation and glycosyl fluoride formation

Thioglycosides are attractive glycosyl donors in glycosylation reaction. A series of thiophilic reagents have been indicated to promote glycosylation[6] (Table 1.1.1.1). The advantage of thioglycosides is their resistance towards the majority of protecting group manipulations and their compatibility with other glycosylation methods. Bis (pyridine)iodonium tetrafluoroborate (Ipy_2BF_4) **1** was the reagent first tested as the powerful source of iodonium ion (Fig. 1.1.1.1). Its superior reactivity has been exemplified by the diversity of chemistry and it has become commercially available from many supplier.[7-12]

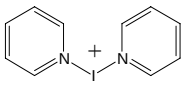

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Figure 1.1.1.1 IPy_2BF_4 bis(pyridine)iodonium tetrafluoroborate.

Table 1.1.1.1: Thiophilic reagent as a promoter in glycosylation

Thiophilic reagent	Ref.
(NBS) N-bromosuccinamide	[13]
(DMTST) Dimethyl(thiomethyl)sulfonium trifluoromethane suldonate	[14]
MeSOTf	[15]
(IDCP) Iodonium dicollidine perchlorate	[16]
NIS/TfOH	[17,18]
(BSP) Benzenesulfinyl piperidine/Tf_2O	[19]
N-(phenylthio)-ε-caprolactam/Tf_2O	[20]
Ph_2SO/Tf_2O	[21]
NIS/$TrB(C_6F_5)_4$	[22]

We demonstrated that the iodonium reagent Ipy_2BF_4 could be used to convert thioglycosides **2** to glycosyl fluorides **4** and also used as a promoter of glycosylation **3**

(Fig. 1.1.1.2). Compared to traditional activation methods such as NIS/TfOH, the activation gave the opposite stereochemical outcome in glycosylation with "armed donors". It thus provided a unique reactivity profile which can be useful in one-pot glycosylation.[23]

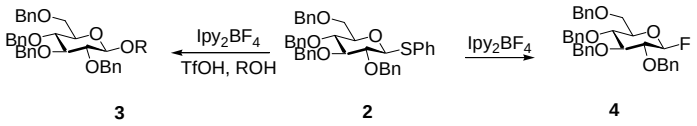


Figure 1.1.1.2 Glycosylation and glycosyl fluoride formation.

1.1.2 Ligation for the preparation of glycoconjugates

The native chemical ligation developed by Kent and coworkers.[24] has had a significant impact on our capacity to access proteins by chemical synthesis and in particular enables the incorporation of unnatural amino acids. A technology which would allow the coupling of unprotected carbohydrates to generate larger oligosaccahrides or link carbohydrates to proteins under mild condition could develop new visions in our access to complex carbohydrates and glycoconjugates. By the recent development of a new peptide ligation technology from Bode and coworkers[25] , we were inspired to ask whether similar reactivity could not be harnessed for a glycoconjugation reaction (Fig. 1.1.2.1).

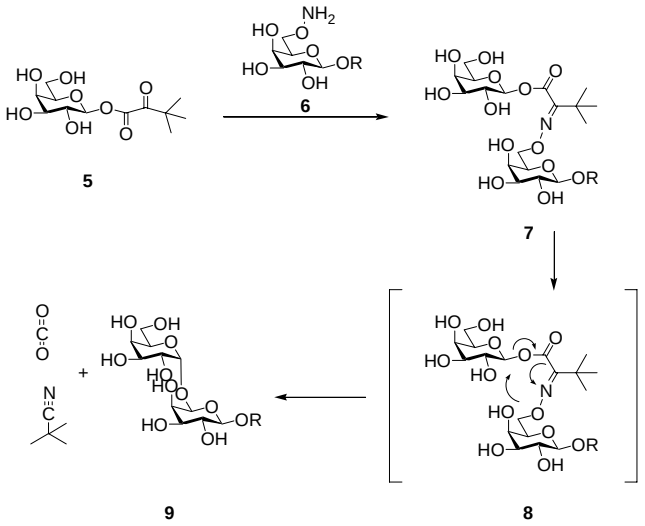
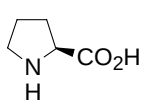


Figure 1.1.2.1 Chemoselective ligation by decarboxylative condensations for the carbohydrate.

1.1.3 Organocatalysis

Following the pioneering work of Hajos–Parrish–Eder–Sauer–Wiechert in the 70's, there has been a outstanding revival in the use of proline since its role in the catalysis of aldol reaction has been shown to be much more general than previously anticipated (Fig. 1.1.3.1). List and coworkers[26-27] reported the intermolecular aldol addition reaction of acetone to various aldehydes catalyzed by proline.



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1. Stable in air and water
2. Simple to use
3. Non-toxic
4. Inexpensive
5. Available from biological materials
6. Both enantioseries available

Figure 1.1.3.1 The advantages of using proline as an organocatalyst.

MacMillan and coworkers demonstrated the two-step synthesis of carbohydrates by selective aldol reaction (Fig. 1.1.3.2).[28] The dimerization of α-oxyaldehydes **14** catalyzed by L-proline, followed by Lewis acid catalyzed a tandem Mukaiyama aldol addition/cyclization step. They could obtain a variety of stereoisomers **17** such as glucose, allose, and mannose, in high yield and good stereochemical purity, simply by changing the solvent and Lewis acid.

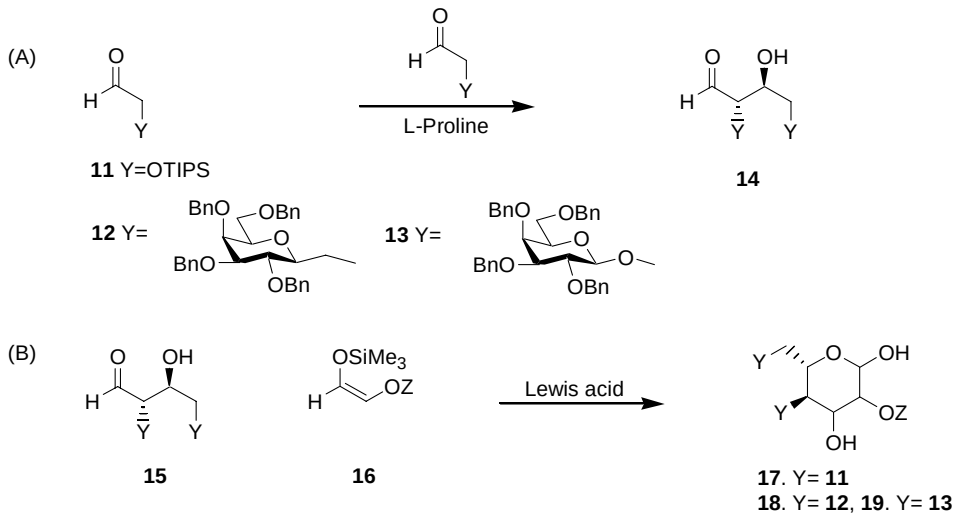

Figure 1.1.3.2 (A) Proline-catalyzed dimerization. (B) Mukaiyama aldol-carbohydrate cyclization.

Following this concept, we investigated whether this strategy could be adopted to synthesize oligosaccharides by the condensation of glycosylated α-oxyaldehydes. Our particular interest was the apparent suitability of this technology to access C- or S- glycosides which can be laborious to access from native glycosides.

1.2 Functional Carbohydrate Oligomers: Chemical modification of carbohydrate for conjugation

1.2.1 Synthetic multivalent carbohydrate ligands

Multivalent protein-carbohydrate interactions mediate many important biological processes and provide another hierarchical level to control their function (Fig. 1.2.1.1).[29]

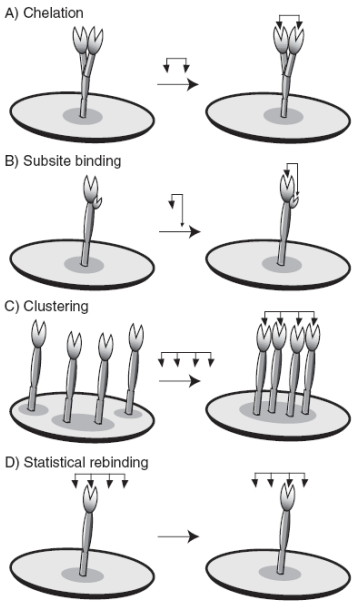

Figure 1.2.1.1 Receptor binding mechanisms to multivalent ligands.

A number of studies have shown that it is possible to dramatically increase affinity to carbohydrate-binding proteins by capitalizing on these multimeric interactions using scaffolds which provides a distance that matches the spacing and geometry between the receptors. For instance, a decamer of the shiga toxin ligand was shown to have a million fold enhancement in affinity compared to the monomeric ligand.[30] However, modulating the geometry of multimeric ligand through chemical synthesis of various scaffolds to display them can be tedious. Based on experience of our laboratory in peptide-nucleic acid (PNA) encoding,[31] We investigated the assembly of PNA-encoded carbohydrates into olimeric structures with controlled topology based on their hybridization to a DNA template (Fig.1.2.1.2). Thus, robust chemical technologies to link carbohydrates to PNA were required. Upon the increasing number of biologically-relevant carbohydrates becoming commercially available, the ideal technology would enable the coupling of a fully deprotected carbohydrate to the PNA with minimum manipulations.

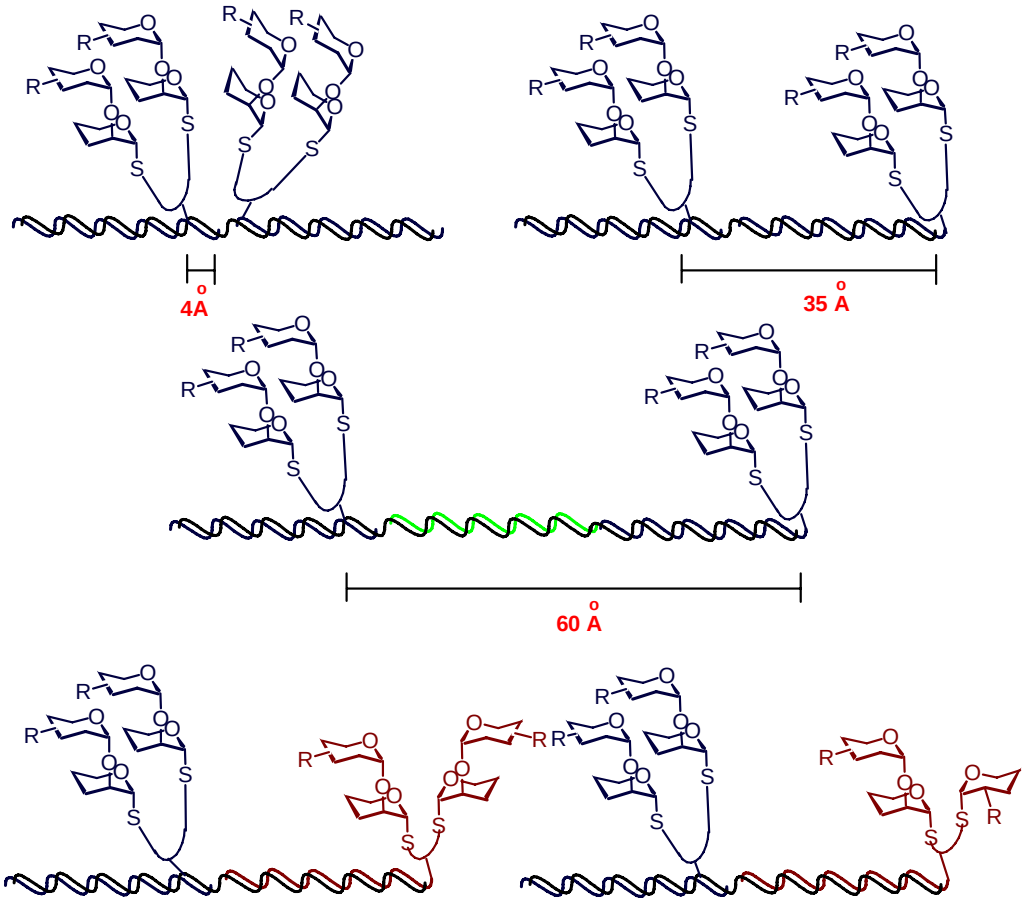

Figure 1.2.1.2 Homodimers and heterodimers with controlled distances.

1.2.2 Oxime and hydrazone formation ligation of carbohydrates to PNA

Chemoselective ligation allows the use of aqueous environments and non-protected substrates to provide rapid access to complex glycoconjugates.[32] The best chemoselective ligations: (a) are fast, high yielding; (b) do not require a large excess of reagent, or employ excess reagents that are easily removable; (c) take place in friendly solvent; and (d) are irreversible under physiological conditions.[33] Molecules containing carbon–nitrogen double bonds are widespread in both chemical and biological contexts. Oxime **23** and hydrazone **24** possess greater intrinsic hydrolytic stability than imines (Fig. 1.2.2.1).[33,34] A number of strategies for constructing glycopeptides by oxime and/or hydrazone bonds have been proposed.[35-37] Many of these reports involve the ligation of aminooxy- or hydrazine-substituted peptides with reducing sugars.

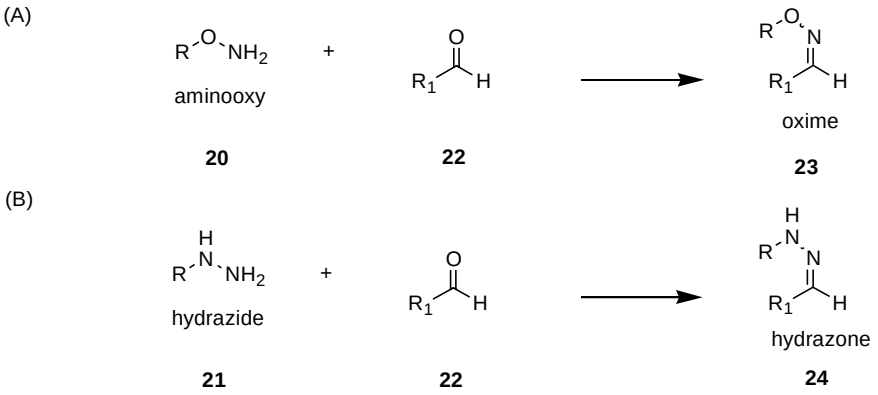

Figure 1.2.2.1 Chemoselective ligation reactions (A) oxime (B) hydrazone.

Most of sugar immobilization strategies require the availability of modified glycans whose synthesis is mostly time-consuming and difficult.[38] We attempted to apply the oxime formation **26** or hydrazone formation **27** to attach non-protected sugars to PNA tags (Fig. 1.2.2.2) to construct self-assembled PNA-encoded carbohydrate microarray. However, while moderate yield could be obtained, this method was deemed unsuitable for our purpose and we turned our attention to alternatives.

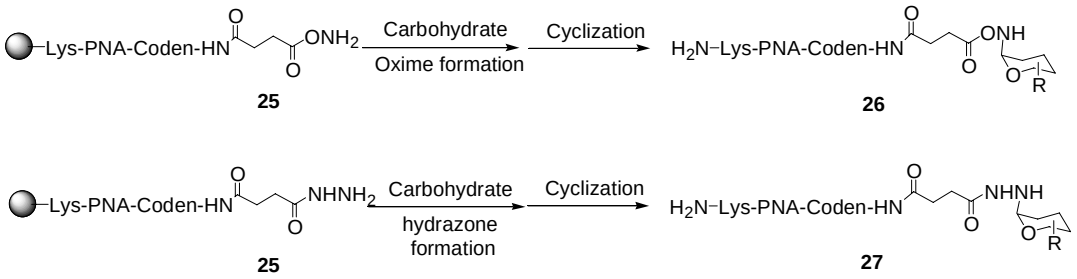

Figure 1.2.2.2 Synthesis of carbohydrate-PNA conjugates.

1.2.3 Chemoselective ligation

Chemoselective ligation refers to the covalent coupling of unprotected biomolecules that contain uniquely reactive functional group.[39,40] Thiolates are excellent nucleophiles, and since other nucleophiles such as amines are protonated at neutral condition. Thiols have been used widely in chemoselective ligation for carbohydrate-containing structures.[41] Grandjean and coworkers[42-44] have used chemoselective ligation to assemble molecules that contain a cluster of mannosides conjugated to a peptidic antigen. Several technologies exist to obtain carbohydrate bearing a thiol group at the anomeric position or appended to a spacer linked to the reducing-end of the carbohydrates. While the one pot procedure indicated by Davis and coworkers is most appealing, problems of solubility and low yields for larger carbohydrates led us to explore and optimize alternatives. The most practical method was found to peracetylate the carbohydrate and treated it with TMSI[45-47] followed by KSAc to obtain the anomeric thioester carbohydrates. In the case of aminosugars, it was found preferable to treat the carbohydrate with neat AcCl[48-50] followed by KSAc. This methodology was used to obtain 16 different carbohydrates (Fig.1.2.3.1). While the anomeric thiol is ultimately required for coupling to the PNA, the thioester obtained was found sufficiently labile under the coupling condition (basic methanol) and was hydrolyzed *in situ* thus affording excellent coupling yields.

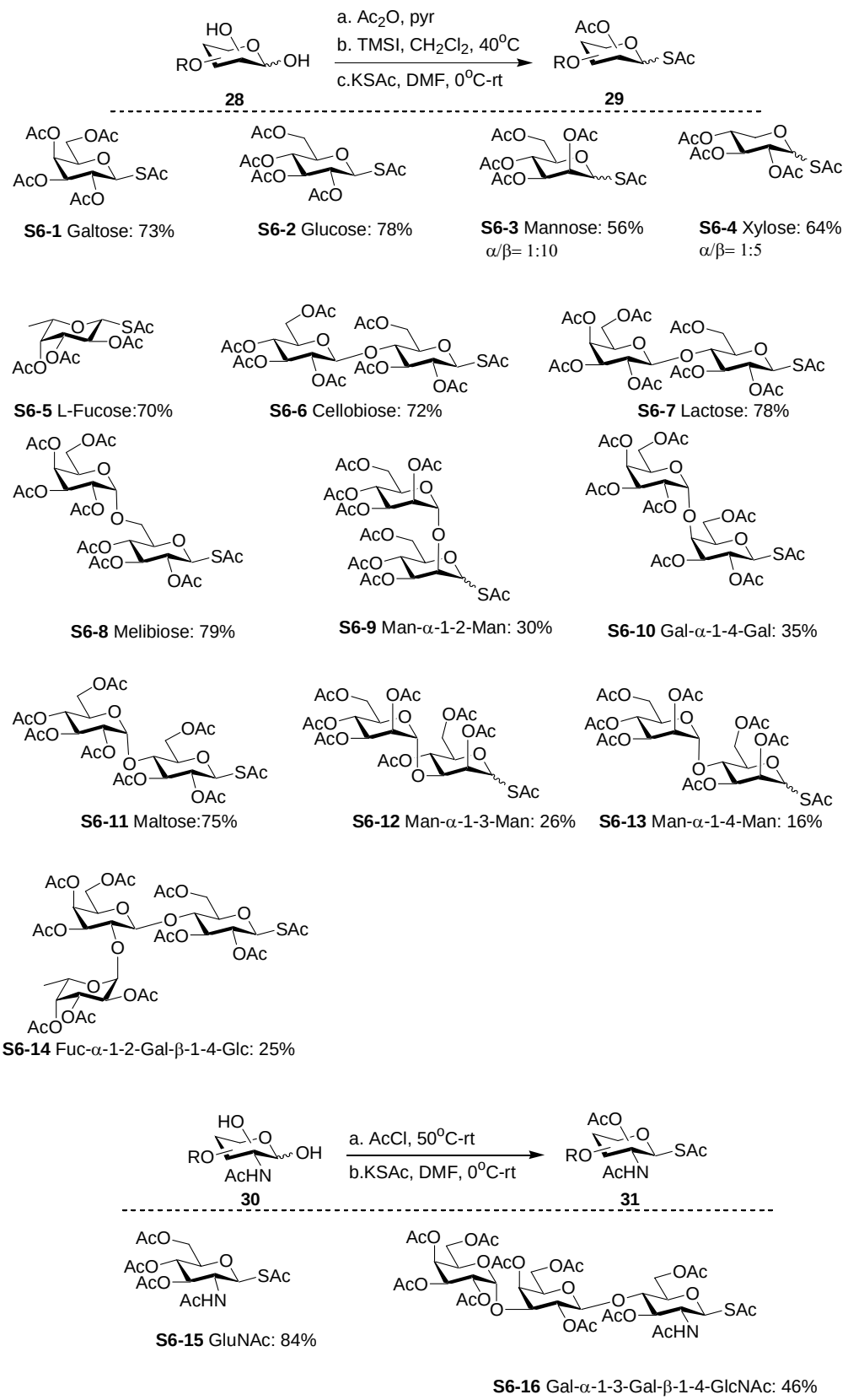

Figure 1.2.3.1 Carbohydrate libraries of thioglycoside.

Alternatively, carbohydrates bearing an allyl group at the anomeric position (Fig.1.2.3.2), readily introduced by trans-acetalization or standard glycosylation procedures could be derivatized with a thiol under free radical procedure (AcSH, AIBN) **33**.[51] In order to further extend the repertoire of reaction enabling the coupling of carbohydrates to PNAs, the allyl group was also oxidatively processed to obtain carboxylic acids **34**[51-53] which can be coupled to the PNAs under standard amide-bond forming reaction in excellent yield. Both of these procedures were used successfully for the preparation of PNA-glycan conjugates. Last but not least, inspired by the publication of Shoda and coworkers[54], we investigated the chemoselective conversion of lactols to glycosyl azide (Fig.1.2.3.3), the latter could be coupled to PNA via click cycloadditions.[55,56]

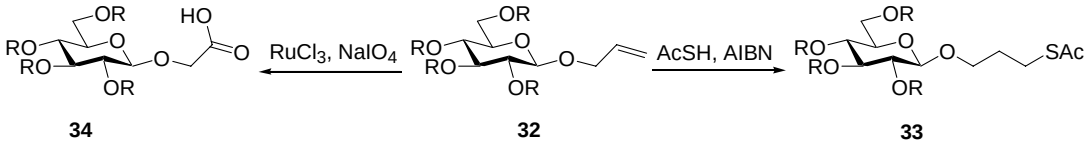

Figure 1.2.3.2 Ridical elongation and Oxidation of allylglycoside.

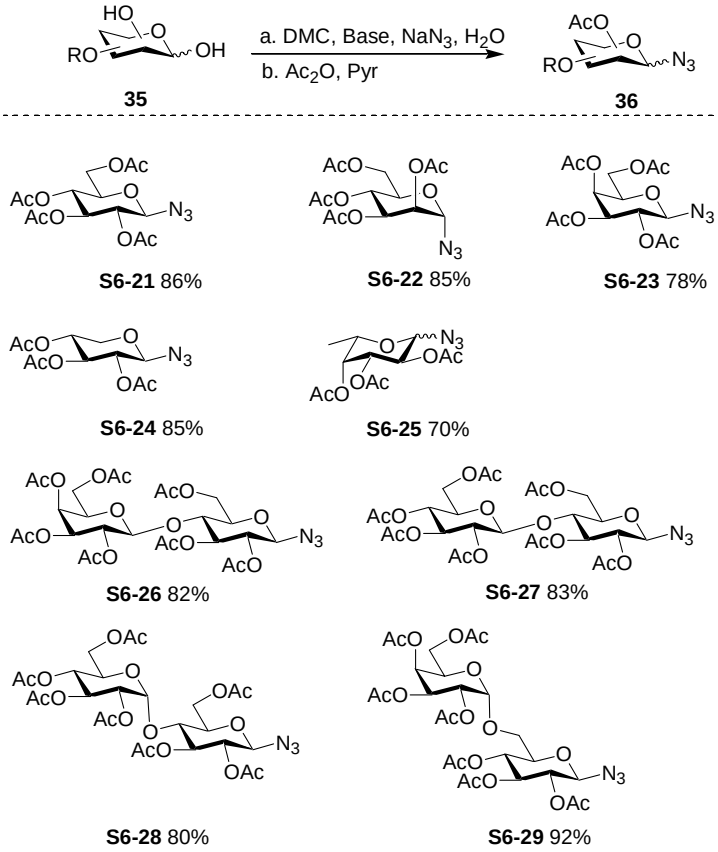

Figure 1.2.3.3 Carbohydrate libraries of azidesugar.

1.2.4 PNA-encoded display of carbohydrates: Mimicking the gp120 epitope of anti-HIV antibody 2G12 – A proof of principle for the utility of self assembled glycans

The crystal structure of 2G12 with a high-mannose nonasaccharide coating gp120 showed that 2G12 assembled into an interlocked dimer resulting in two additional binding sites at its dimerization interface (Fig. 1.2.4.1).[57,58] This finding not only provided a rational for the high affinity of the antibody for its target (HIV's glycoprotein 120 - gp120) by virtue of the highly cooperative binding mode but also for its selectivity for gp120 bearing high-mannose carbohydrates *vs* the host. Indeed, the 2G12 antibody displays appropriately spaced binding sites that match the spacing of these structures on the viral surface. Noticeably, the crystalographic information suggests that only the terminal mannoses are involved in the interaction with the antibody.

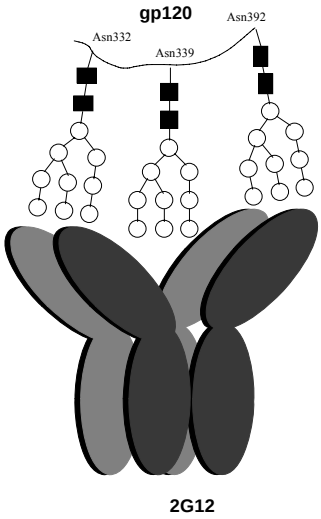

Figure 1.2.4.1 Proposed binding mode of 2G12 dimer with gp120.

We asked if this recognition could be emulated by displaying the required mannose disaccharide at appropriate spacing using PNA-encoded display (Fig. 1.2.4.2). [59]

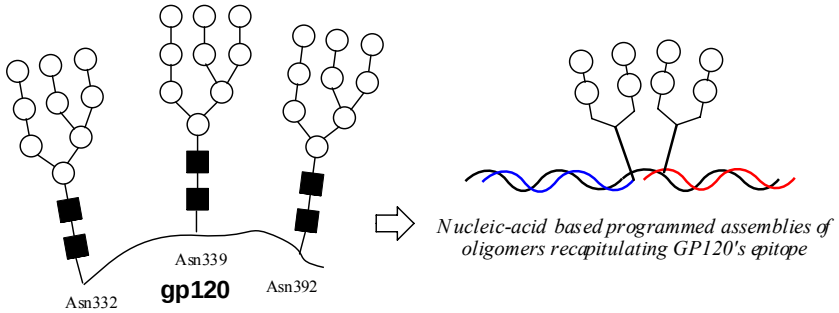

Figure 1.2.4.2 Mannose disaccharide at appropriate spacing using PNA-encoded display.

To this end, a series of different carbohydrates we linked to PNAs, via the thiol chemistry, with different spacing between them and hybridized onto DNA templates with different geometry. A pilot library of over 30 assemblies was tested for their affinity to 2G12 by surface plasmon resonance (SPR). A significant correlation between the geometry of the display and the affinity was observed with the best complex showing an affinity in the low micromolar range. It is critical to note the nonasaccharide did not show measurable affinities under these conditions suggesting that this supramolecular assembly could emulate complex carbohydrates topologies. Based on this result, a library of 625 PNA carbohydrates bearing modification on the carbohydrate through click cycloaddition was prepared and is currently being tested (Fig. 1.2.4.3).

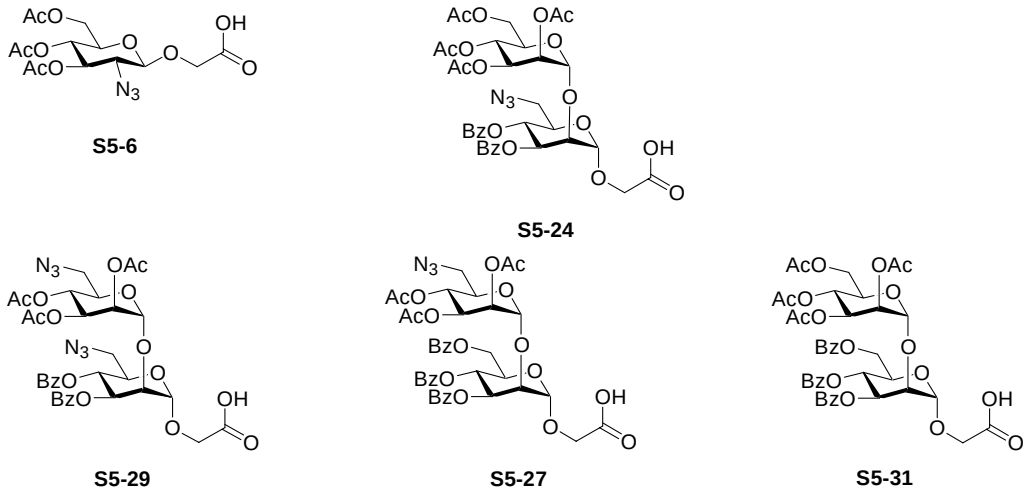

Figure 1.2.4.3 Synthesis of Azidodimannose building block for a library of 625 PNA-encode carbohydrates targeting 2G12.

1.2.5 Self-Assembled PNA-encoded carbohydrate microarray

In the post-genomic era, surface-based glycomic tools in high throughput formats gradually became crucial for probing carbohydrate-protein interactions. Recognizing the specificity of cell-surface carbohydrates interaction is critical for both the discovery of new roles for carbohydrates in cell biology and the development of new therapeutics and high-sensitivity diagnostics. Microarray systems have been extensively applied in almost every field of biological research to clinical diagnostics since large libraries of compounds can be quickly screened and only small quantities of material are required. The development of carbohydrate microarray enabled the high-sensitivity and high-throughput analysis of carbohydrate-protein interactions and

contributed to a significant progress in glycomics.[60-69] To date, a number of chemical pathways have been developed to derivative glass surfaces to immobilize oligosaccharides, proteins and small molecules in the microarray format.[31] However, access to suitably derivatized carbohydrates to immobilize on an array remains laborious and a limiting factor in the broad implementation of this technology. The largest glycan arrays available nowadays are prepared by the consortium for functional glycomics and contain under 500 glycans (http://www.functionalglycomics.org/). We suggested that a broad diversity of glycan could be accessed by combinatorial assembly of smaller glycan libraries in a microarray format (Fig. 1.2.5.1). To this end, a library of 50 different PNA-glycan conjugates was assembled into a microarray of 625 unique combinations.

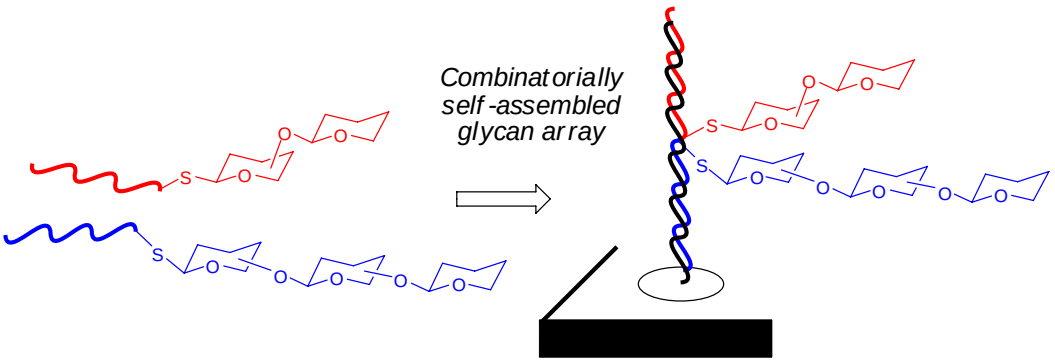

Figure 1.2.5.1 Self-assembled carbohydrate microarray.

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2. Glycosylation and Glycosyl Fluoride Formation

2.1 Introduction

2.1.1 Thioglycosides

The success of glycoside bond-forming reactions in the complex carbohydrate synthesis remains fickly. Although a number of powerful glycosylation technologies have already been developed, the success of complex synthesis often depends on the different strategies of these various methods and there is certainly a need for novel methods and new reagents to promote glycosylation.[1-3] Thioglycosides are frequently used as glycosyl donors in glycoside synthesis. They are also useful intermediates for the preparation of glycosyl fluorides,[4] sulfoxides and sulfones, which are used as glycosyl donors for the glycosylation.[5-7] Thioglycosides are attractive glycosyl donors as the anomeric thioether is stable to most protecting group manipulation and they are compatible with a number of other glycosylation methods. [8]

There are some references for the preparation of thioglycosides. The most useful protocol for the thioglycosides preparation is the treatment of glycosyl acetates with various alkyl or aryl thiols in the presence of Lewis acid.[9-13] Another synthetic pathway for the preparation of alkyl thioglycosides uses *S*-glycosyl isothiouronium derivatives generated from glycosyl halide.[14-17] Thioglycosides have been activated with other halogen promoter systems; for example, Nicolaou and co-workers indicated a mild and general method by the treatment of *N*-bromosuccinimide (NBS) as a promoter for the synthesis of *O*-glycosides.[18] Thioglycosides can also be activated by a series of thiophilic reagents to promote glycosylation reaction (Fig. 2.1.1.1). Van Boom and co-workers,[17] explored the use of a stoichiometric amount or an excess of *N*-iodosuccinimide (NIS) in the presence of catalytic amount of trifluoromethanesulfonic acid (TfOH) as a promoter to activate the iodonium ion. The use of *N*-iodosuccinimide-silver trifluoromethanesulfonate (NIS/AgOTf) was reported soon by Fraser-Reid and co-workers.[19] The effiency of the iodonium promoter system has been proven by many successful applications, and many changes have been developed. For instance, Van Boom and co-workers reported an efficient thioglycoside-mediated formation of α-glycosidic linkages promoted by iodonium dicollidine perchlorate (IDCP).[20] Mukaiyama and co-workers introduced stereoselective glycosylation of thioglycosides promoted by respective combinations of *N*-iodosuccinimide (NIS) or *N*-bromosuccinimide (NBS) and trityl tetrakis(pentafluorophenyl)borate $TrB(C_6F_5)_4$.[21] Ellervik and co-workers applied iodine monochloride-silver trifluoromethanesulfonate (ICl/AgOTf) as a convenient and efficient promoter for the activation of thioglycosides.[22] High-yielding

sialylation with this promoter system were also elaborated.[23]

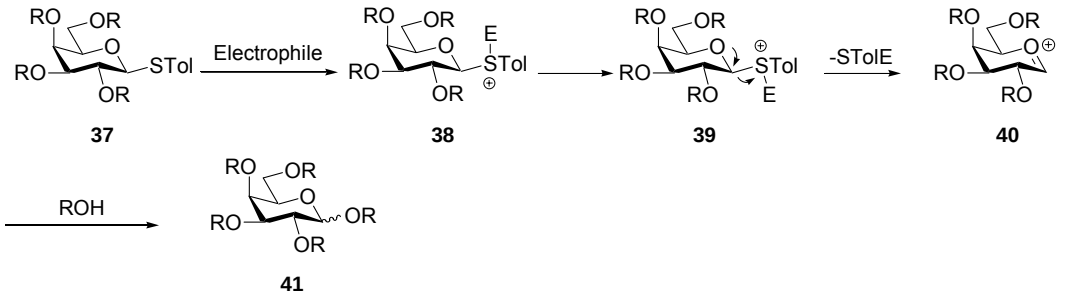


Figure 2.1.1.1 Activation of thioglycosides for glycosylations by electrophilic reagents.

Other thiophilic reagents have also been expolred (Fig. 2.1.1.2). Dimethyl(methylthio)sulfonium triflate (DMTST) **42** is a powerful alkylsulfenylating agent that demonstrated to be efficient in a wide range of glycosylation reactions.[24,25] DMTST was prepared from dimethyl disulfide and methyl triflate. According to the introduction of DMTST, based on the same principle, including methylsulfenyl triflate (MeSOTf) **43**,[26] and phenylsulfenyl triflate (PhSOTf) **44**[27,28] have been developed. MeSOTf was usually prepared *in situ* from methanesulfenyl bromide and silver triflate. Methanesulfenyl bromide in turn was synthesized from dimethyl disulfide and bromine. PhSOTf is prepared *in situ* by reacting benezenesulfenyl chloride with silver triflate. Benezenesulfenyl chloride was synthesized by reacting phenyl thioacetate with SO_2Cl_2. It also can be conveniently prepared by treatment of thiophenol or diphenyl disulfide with Cl_2 or SO_2Cl_2.

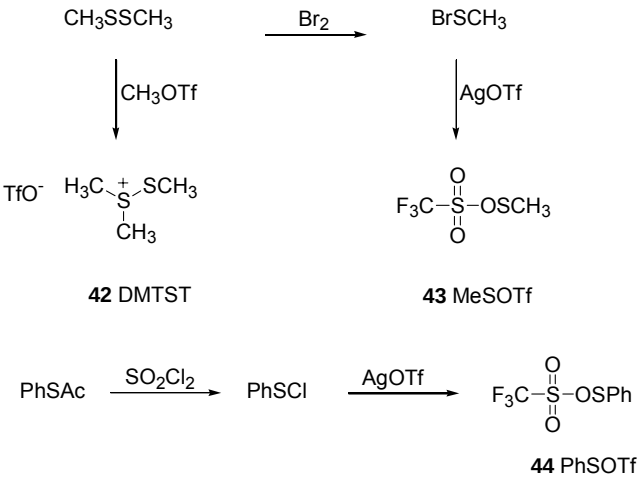
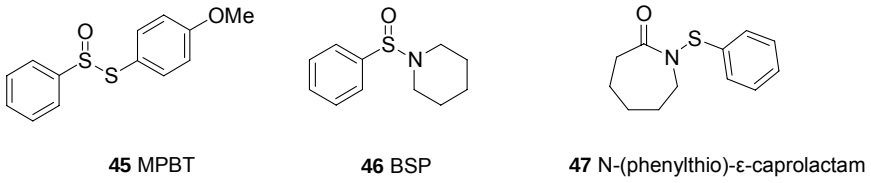


Figure 2.1.1.2 Synthesis of organosulfur activating reagents.

Sulfenamide type activators in combination with Lewis acids such as PhSNPhth-TMSOTf,[29] EtSNPhth-TrB$(C_6F_5)_4$,[30] and *N*-(phenylthio)-ε-caprolactam-Tf_2O **47**[31] systems have also been reported. Sulfinates in combination with triflic anhydride (Tf_2O) have also been used as thioglycoside activators. These sulfinates include *S*-(4-methoxyphenyl)benzenethiosulfinate (MPBT) **45**,[32] benzenesulfinyl-piperidine (BSP) **46** (Fig. 2.1.1.3),[33] diphenyl sulfoxide.[34] Another powerful system, namely Me_2S_2/Tf_2O, was developed for the activation of thioglycosides.[35]

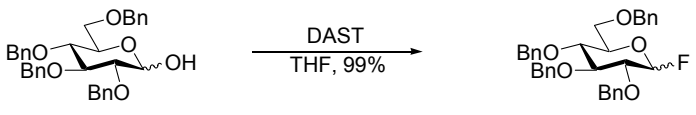


45 MPBT **46** BSP **47** N-(phenylthio)-ε-caprolactam

Figure 2.1.1.3 Structures of promoters for thioglycosides.

2.1.2 Glycosyl fluoride

In 1981 Mukaiyama and co-workers,[36] reported the use of glycosyl fluoride as a glycosyl donor. In these examples, α-glucosides were obtained with good stereoselectivities when glucosyl fluoride and various glycosyl acceptors were treated with a promoter generated from stannous chloride ($SnCl_2$) and silver perchlorate ($AgClO_4$) in diethyl ether. After the above mentioned system was reported, the fluorides became widely recognized as useful glycosyl donors. The significant advantage of glycosyl fluoride as a glycosyl donor is its higher thermal and chemical stability relative to glycosyl chlorides, glucosyl bromides, and glycosyl iodides. It is possible to purify glycosyl fluorides by column chromatography on silica gel. Many approachs for the preparation of glycosyl fluorides have been developed by using various fluorinating reagents.[37-39] Hydrogen fluoride-pyridine (HF/pyridine) has been applied to fluorination of sugars.[40,41] Diethylaminosulfur trifluoride (DAST) has been adopted as an excellent reagent for replacement of free hydroxyl group with fluorine. [42] Treatment of the benzylated D-glucose derivative **48** with DAST in THF at low temperature produces the fluoride **49** in excellent yield and high β selectivity (Fig. 2.1.2.1).



48 **49** α:β = 1:7.7

Figure 2.1.2.1 Synthesis of glycosyl fluoride.

The direct conversion of thioglycosides into glycosyl fluorides can be achieved by concomitant use of Diethylaminosulfur trifluoride and *N*-bromosuccinimide (DAST/NBS).[43] Glycosyl fluorides have found use in the synthesis of a wide range of complex natural products. In Nicolaou's synthesis of avermectin B1a (Fig. 2.1.2.2), the glycosyl fluoride **51** was prepared from the thioglycoside **50** by reaction with NBS and DAST. Glycosylation of **52** with **51** created the disaccharide thioglycoside **53** (α anomer exclusively), which was activated as the fluoride **54**. Glycosylation of the aglycone was accomplished in a stereoselective manner, and the resulting **55** was deprotected to give avermectin B1a **56**. Alternatively, Selectfluor [(1-chloromethyl-4-fluoro- 1,4-diazoniabicyclo-[2.2.2]-octane bis(tetrafluoroborate) **58** has also been reported as an electrophilic fluorinating reagent in carbohydrate chemistry.[44] The transformation of 1-hydroxy sugars to glycosyl fluorides was performed with a mixture of Selectflour and dimethyl sulfide (Fig. 2.1.2.3). In addition, Selectfluor can also be used for the conversion of thioglycosides to glycosyl fluorides (Fig. 2.1.2.4). Tetrabutylammonium fluoride (TBAF) is used for the preparation of glycosyl fluorides from 1,2-anhydro-α-D-hexopyranose derivatives.[45]

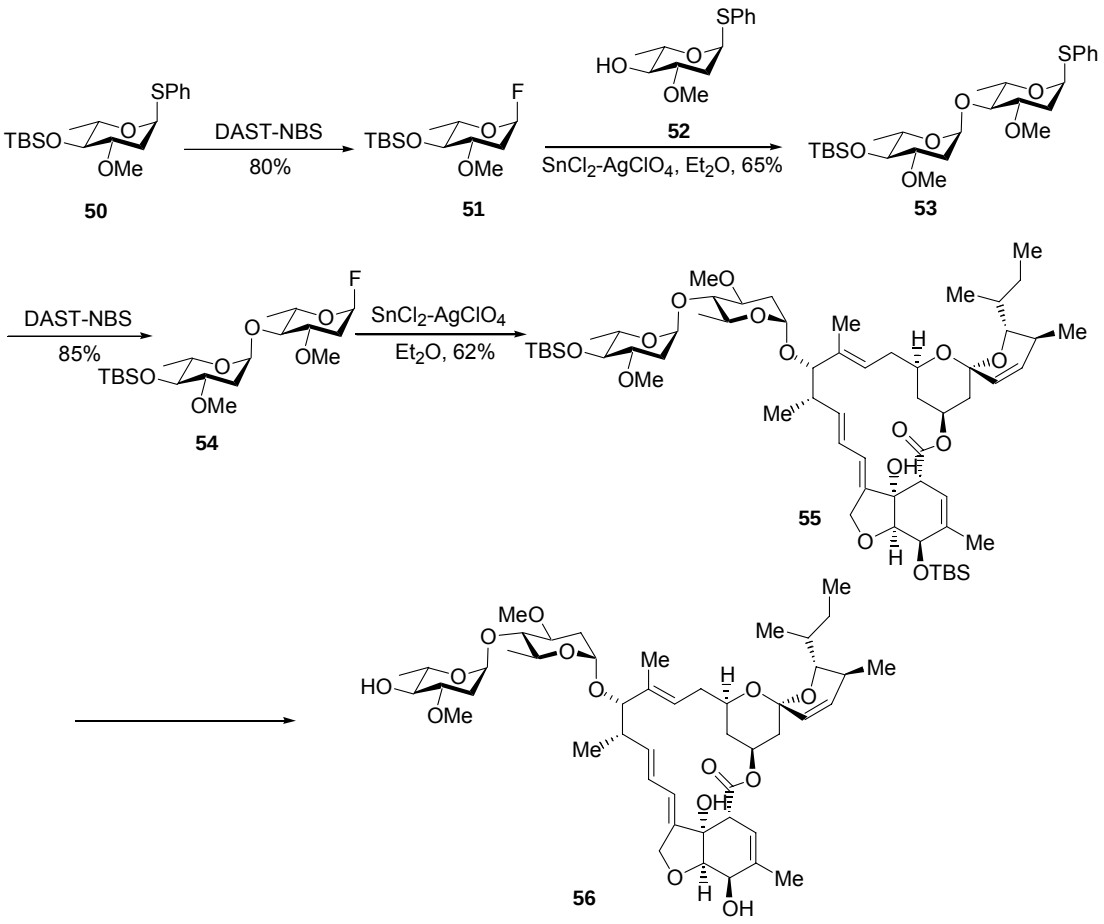


Figure 2.1.2.2 Synthesis of avermectin B1a.

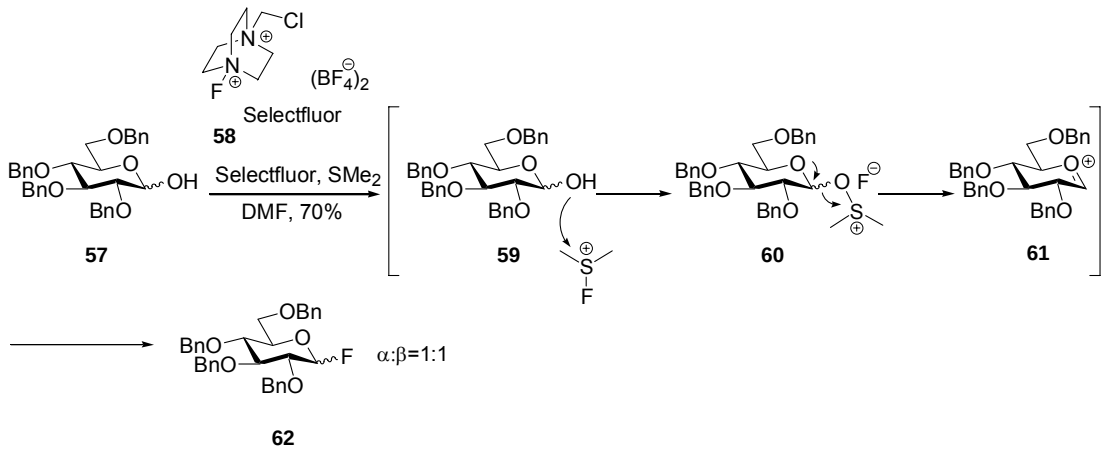


Figure 2.1.2.3 Synthesis of glycosyl fluorides using Selectfluor.

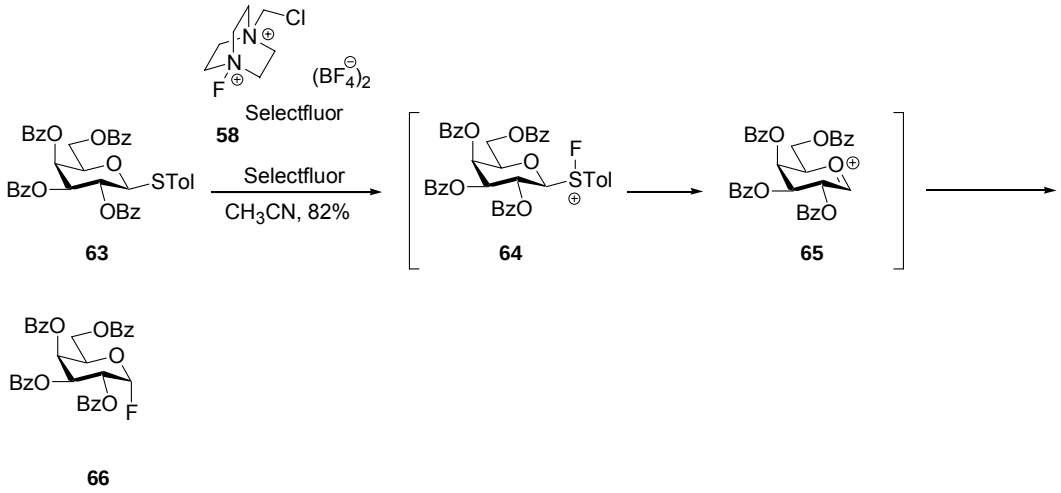


Figure 2.1.2.4 Synthesis of glycosyl fluorides using Selectfluor.

2.1.3 Bis (pyridine) iodonium tetrafluoroborate (IPy_2BF_4)

The bis (pyridinium) iodonium tetrafluoroborate (IPy_2BF_4) is a stable and solid reagent, it acts as a mild source of iodonium ions towards different types of unsaturated compounds. Initial efforts were directed of the development of a methology that can be useful to accomplish vinical iodofunctionalization of an alkene (Fig. 2.1.3.1).[46] Therefore, when the reagent is mixed in CH_2Cl_2 with an alkene and a nucleophile the corresponding products derived from the addition of an iodine atom and the nucleophile across the double bond are formed. Generally speaking, an acid is required to neutralize the supply of pyridine molecules from the iodinating reagent, avoiding the formation of adducts, such as **71**, that incorporate pyridine as a

nucleophile through a competition process for the capture of the intermediate iodonium ion. Tetrafluoroboric acid is very useful due to the low nucleophilic character of BF_4^- counteranion. When simply the reagent, the acid and the alkene are mixed at low temperature, a clean, regioselective and stereoselective iodofluorination takes place **72**.

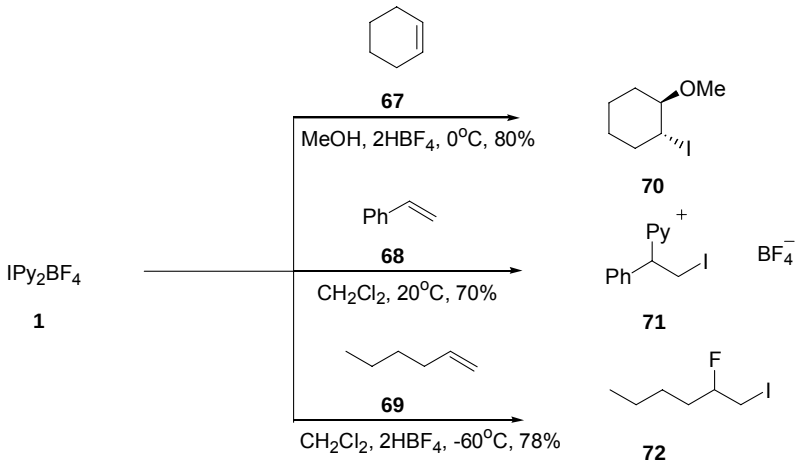


Figure 2.1.3.1 Vinical iodofunctionalization of an alkene.

Based on the iodinating reagent Bis(pyridinium) iodonium tetrafluoroborate (IPy_2BF_4), Barluenga and coworkers presented a powerful intramolecular addition of aniline and of several of its *N*-derivatives to alkynes, which relies on a simple iodination process to direct assembly of 3-Iodoindole cores (Fig. 2.1.3.2).[47]

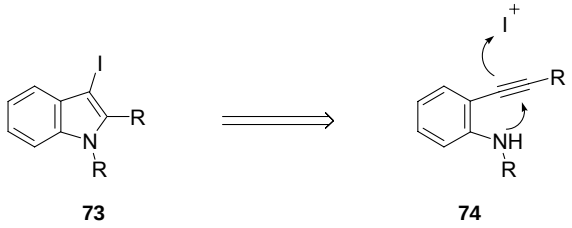


Figure 2.1.3.2 Synthesis of 3-iodoindole from the iodocyclization of 2-alkynylaniline

The iodoarylation of alkenes and alkynes **75** is an efficient tool for the modular assembly of benzofused heterocycles **76** with quick generation of diversity (Fig. 2.1.3.3).[48]

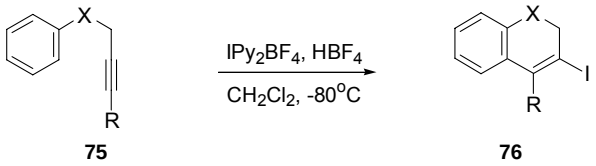
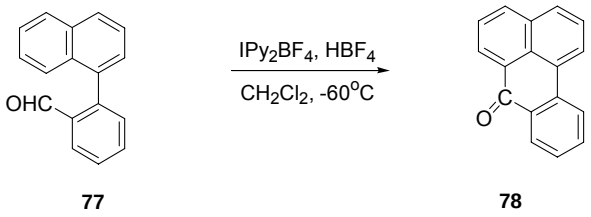


Figure 2.1.3.3 Iodoarylation reactions for the ring-closing step.

Synthesis of benzocyclic ketones through direct intramolecular arylation of aldehydes promoted by reaction with IPy_2BF_4/HBF_4 was also indicated. Therefore, arenecarboxaldehydes **77** are converted into benzocyclic ketones **78** in a straightforward process (Fig. 2.1.3.4).[49]



77 **78**

Figure 2.1.3.4 Iodoarylation reactions for the aldehyde to ketone conversion.

A mechanistic proposal to account for the formation of the ketones from the aldehydes is outlined (Fig. 2.1.3.5). First, the acid protonates the pyridine molecules, which are initially associated with the iodonium species. The pyridinium salt formed precipitates at low temperature and is removed by filtration. The interaction of the resulting solution with the starting aldehyde would give **79**. Such a complex could then further react to yield the ketone product in two different ways. It could undergo formal addition of IF to form intermediate **80**. Further oxidation would give **81**. In situ activation of the resulting acyl fluoride by the BF_3 present in the reaction medium would lead to an acyl cation, which could undergo cyclization to form the benzocyclic ketone product **84**. Alternatively, a direct addition of the arene to the activated carbonyl group is also presented. Subsequent loss of HI would give the ketone **84**. Both mechanistic pathways are compatible and plausible with the formation of the observed products. These structures have frequently demonstrated the superiority of IPy_2BF_4 to NIS on a source of iodonium ion.

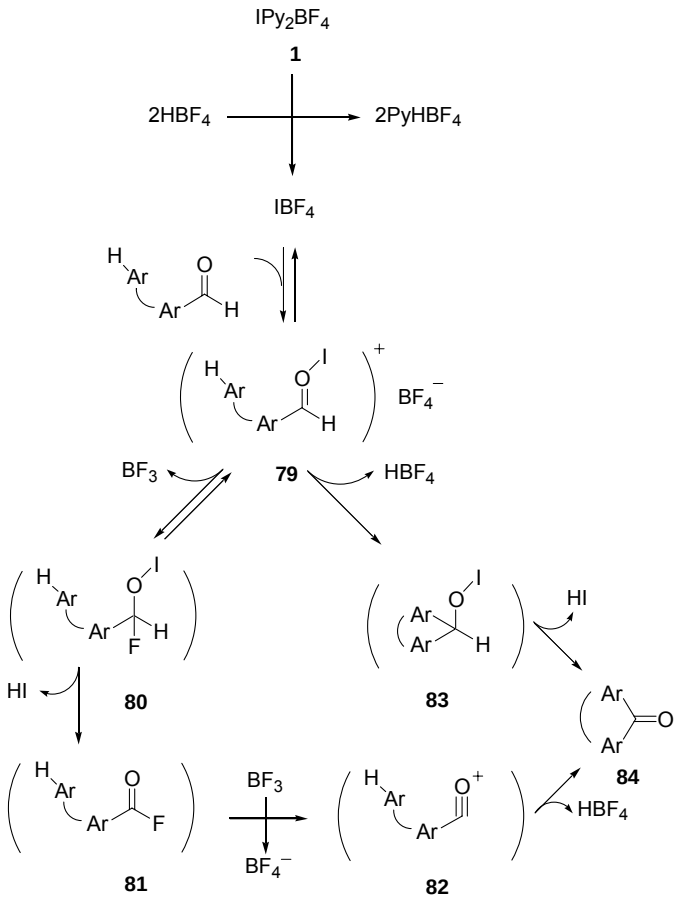


Figure 2.1.3.5 Proposed mechanistic pathways for the observed aldehyde to ketone.

2.1.4 Programmable one-pot oligosaccharide synthesis

One-pot syntheses of oligosaccharides involve several glycosyl donors selected to react in a specific order based on their reactivity and it results in a single product (Fig. 2.1.4.1).[50] The ability to control glycosyl donor reactivity through hydroxyl protecting groups is one of the underlying principles of programmable one-pot oligosaccharide synthesis. Wong and coworkers tabulated the reactivity of wide array of glycosyl donor by a competitive HPLC experiment.[51]

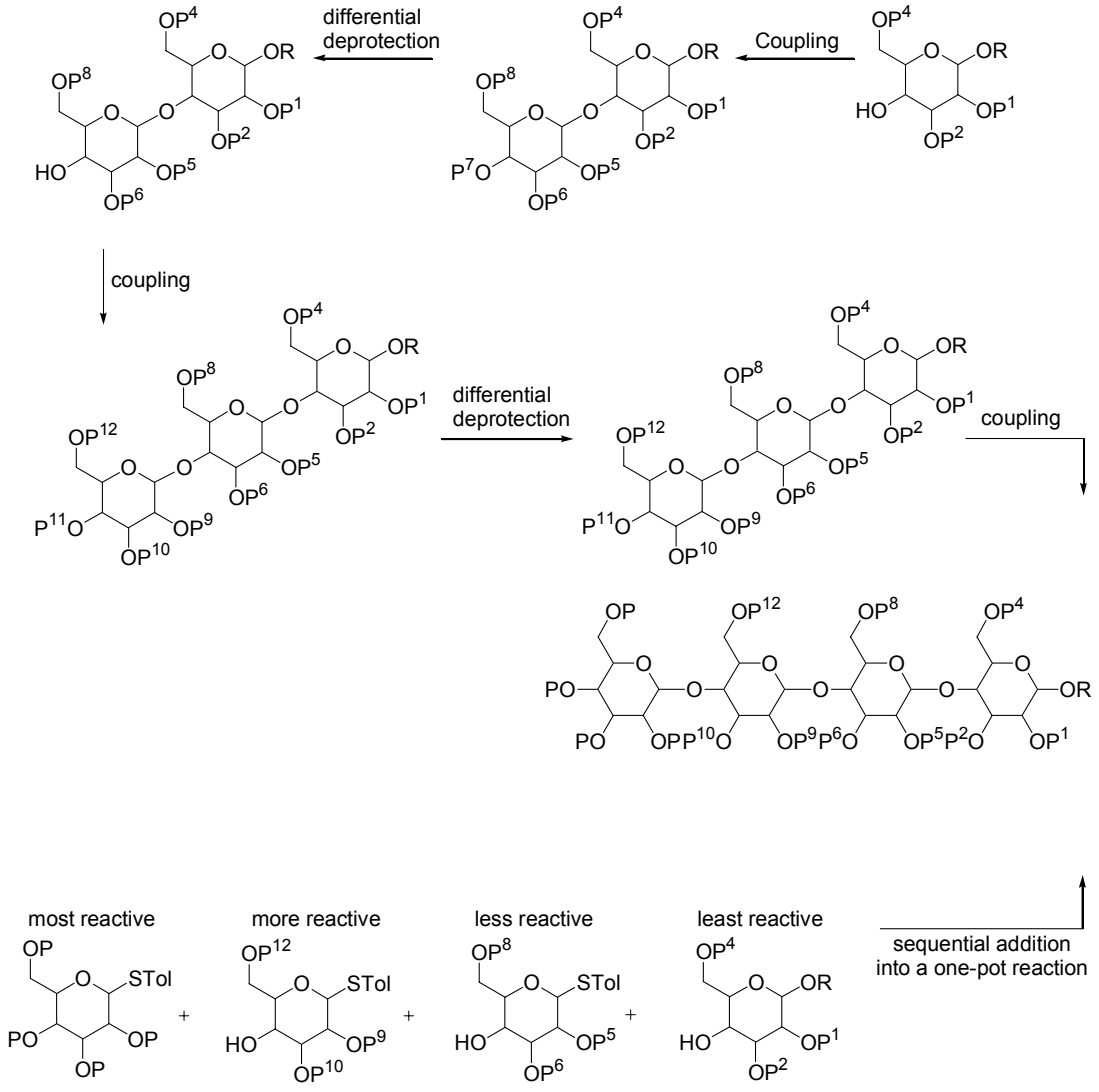


Figure 2.1.4.1 Traditional synthesis and one-pot synthesis of oligosaccharides.

Donors and donor-acceptors with various protecting group patterns provide a set of building blocks with diverse reactivities. The relative reactivity values (RRVs) can now be used with a computer program (OptiMer) to determine the optimal sequence.

Based on this strategy, oligosaccharides containing three to six monosaccharides are rapidly assembled in minutes or hours by mixing of selected building blocks in sequence. No intermediate workup or purification procedures are required.

The programmable one-pot synthesis of oligosaccharides has the potential to affect many areas of drug discovery, as it provides scientists of all fields with access to complex carbohydrate structures. For example, Globo H, a glycosyl ceramide found as an antigen in prostate and breast cancer cells. Lewis Y, expressed on tumor cell surfaces in colon-rectal adenocarcinoma, has been studied as vaccines for these cancer types. [52,53] The globo H hexasaccharide can be readily assembled by a double one-pot procedure (Fig. 2.1.4.2).[54]

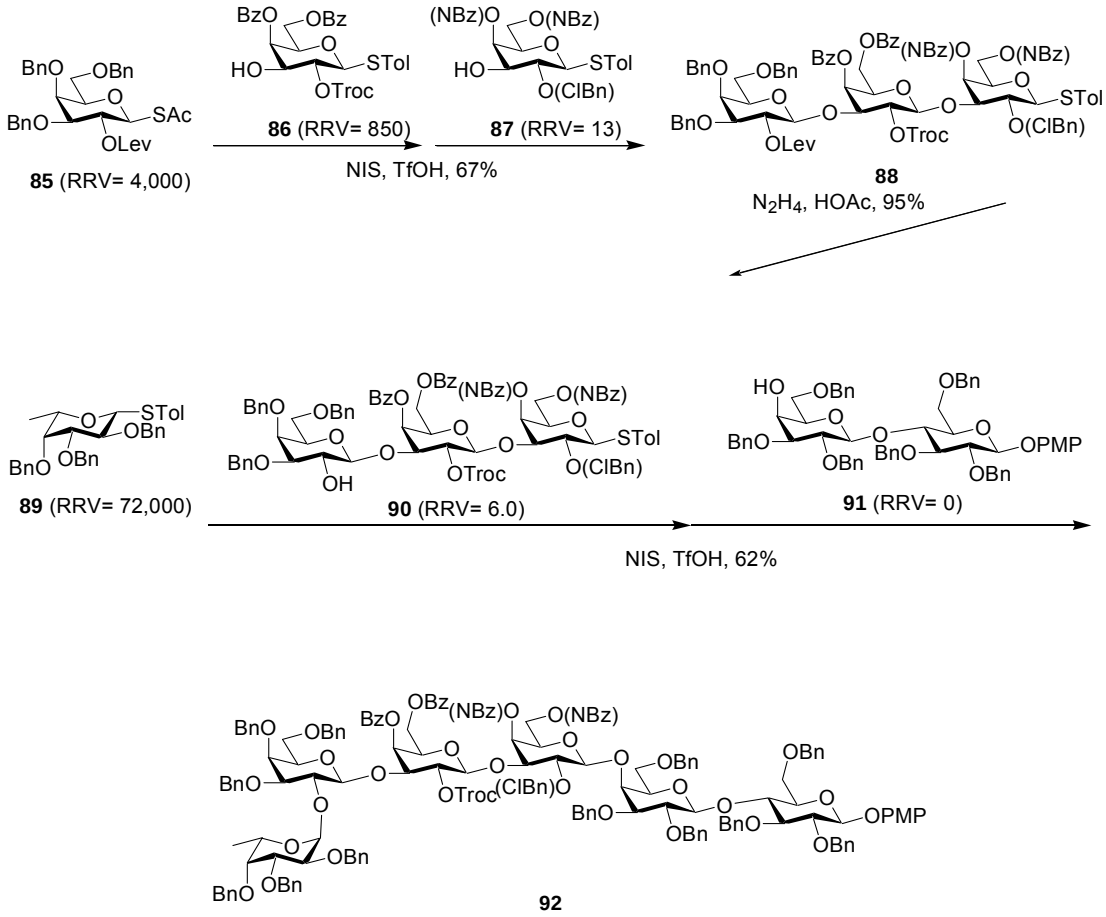


Figure 2.1.4.2 Programmable one-pot synthesis of the glycans of globo H.

Lewis Y can be obtained through one-pot coupling of two fucosyl and two lactosaminyl building blocks (Fig. 2.1.4.3).[55] These synthetic strategies could be used in the rapid synthesis of antigen analogues for identification of the optimal

structures to be used in vaccine development.

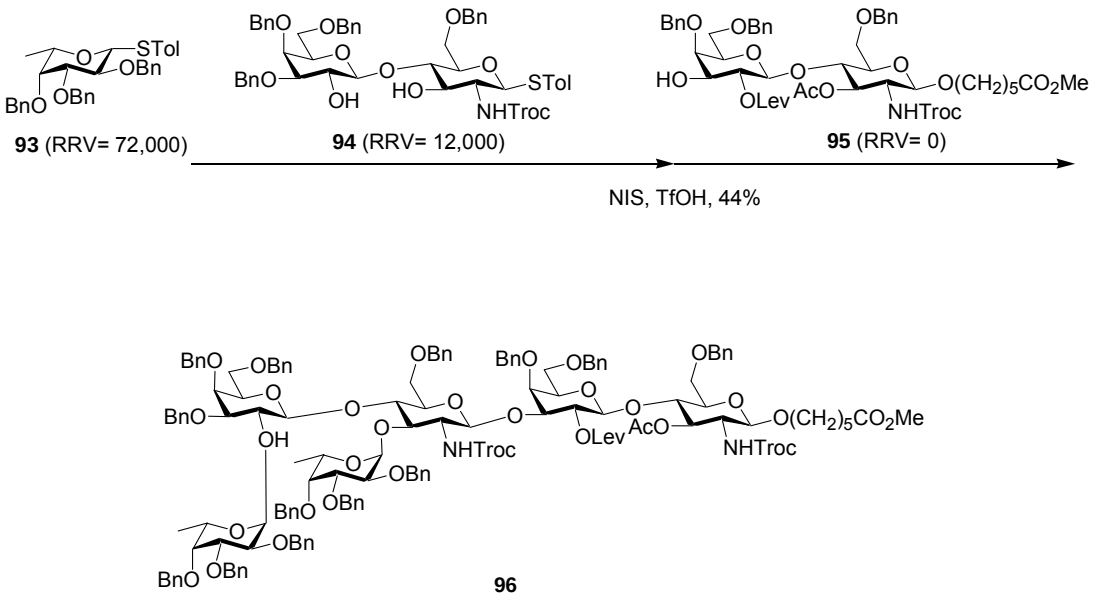
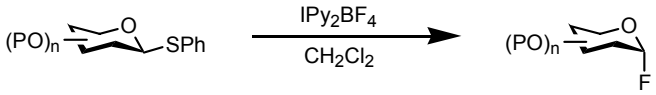


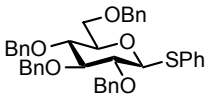
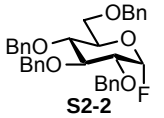
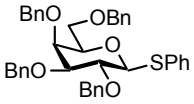
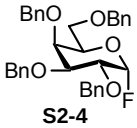
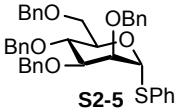
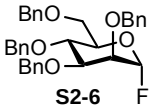
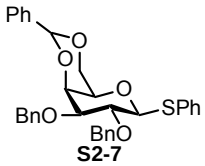
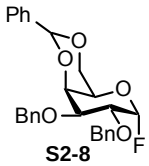
Figure 2.1.4.3 Programmable one-pot synthesis of Lewis Y.

2.2 Results and discussion

The iodonium activation of thioglycosides is well known and typically carried out with NIS/TfOH, the efficiency of IPy_2BF_4 has never been evaluated in glycosylation reactions. IPy_2BF_4 has proven to be an extremely powerful source of iodonium ion and it has become commercially available in large quantities. Thus we explored the use of IPy_2BF_4 to convert thioglycosides to glycosyl fluorides and its application as a promoter of glycosylation by using thioglycosides. We found that in the absence of acid, IPy_2BF_4 smoothly converted armed thioglycosides into glycosyl fluorides. As shown in (Table 2.2.1), perbenzylated thioglucose, thiogalatose, thiomannose, and 4,6-benzylidenegalatose afforded the glycosyl fluoride in moderate to good yield (50-86%).

Table 2.2.1 IPy_2BF_4 mediated conversion of thioglycosides into glycosyl fluorides.

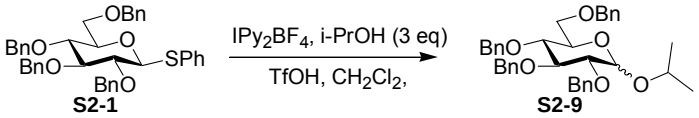


Entry	Saccharide	Product	IPy_2BF_4 (eq)	T (^oC)	Time(h)	Yield (%)
1	 **S2-1**	 **S2-2**	1.5	rt	3.0	50 α:β 80:20
2	 **S2-3**	 **S2-4**	1.5	rt	1.5	86
3	 **S2-5**	 **S2-6**	1.5	rt	3.0	75
4	 **S2-7**	 **S2-8**	1.5	rt	3.0	76

We then turned our attention to the use of IPy_2BF_4 to promote glycosylation. It is known that a protic acid or Lewis acid is necessary to coordinate to pyridine to reveal the iodonium ion. In fact, this can be monitored visually with the appearance of a deep

red color upon addition of TfOH, HBF_4, TMSOTf or BF_3Et_2O. Glycosylation was found to be effective with all these acids but further optimization of glycosylations were carried out strictly with TfOH. With the use of isopropyl alcohol as a model glycosyl acceptor, glycosylation reaction was not effective from -100°C to -60°C (Table 2.2.2, Entries 1-3) but proceeded smoothly at -35°C in the presence of 1 equip. of TfOH. A catalytic amount of acid afforded mixtures of glycosyl fluoride and glycosyl product (Table 2.2.2, Entries 4-7). It is interesting to note the predominace of the β-glycosylative product in these reactions as glycosylation of perbenzylated donors is known to proceed through either an S_N2 mechanism or through the oxocarbenium intermediate which tends to favor the α-product.

Table 2.2.2 Optimization of IPy_2BF_4 mediated glycosylation.



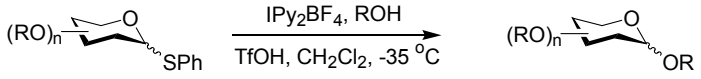
S2-1 **S2-9**

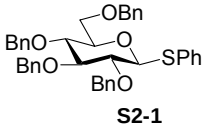
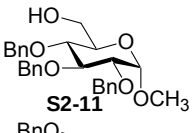
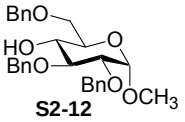
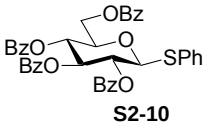
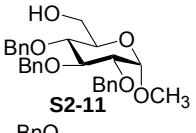
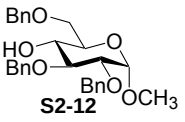
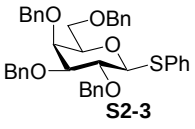
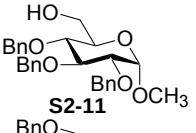
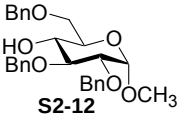
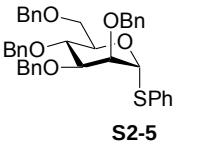
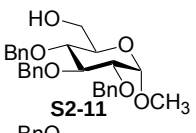
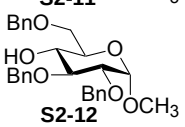
Entry	IPy_2BF_4 (eq)	TfOH (eq)	T (°C)	Time(h)	Yield (%)	α:β:F(α)
1	2.0	2.0	-100	1.0	No Reaction	
2	2.0	6.0	-100	1.0	No Reaction	
3	1.5	Cat	-60	1.0	No Reaction	
4	1.5	Cat	-35	1.0	86	40:46:14
5	1.5	Cat	-20	1.0	89	38:48:14
6	1.5	Cat	-0	1.0	90	34:52:14
7	1.5	Cat	-35 → rt	1.0	90	36:51:13
8	1.5	1.5	-35	1.0	89	42:58: 0
9	1.5	3.0	-35	1.0	90	34:66: 0

With these results in hand, we then investigated the utility of IPy_2BF_4 for the preparation of disaccharides. As shown in (Table 2.2.3), the reaction proceeds smoothly with a variety of donors to afford the product in good-to-excellent yield for both armed and disarmed donors. For perbenzylated thioglucose (Table 2.2.3, Entries 1 and 2), the reaction afforded 80% of the β product for a 1-6 glycosylation and 64% of the β-form product for a 1-4 glycosylation. The perbenzoylated thioglucose afforded exclusively the β-form product in both reactions (Table 2.2.3, Entries 3 and 4) by virtue of neighboring group participation. The armed perbenzylated thiogalatose

afforded 85% of the β product for a 1-6 glycosylation (Table 2.2.3, Entries 5); however, the selectivity was rather poor for a 1-4 glycosylation (Table 2.2.3, Entries 6). In the case of mannose, the 1-6 glycosylation reaction afforded strictly the β-form product (Table 2.2.3, Entries 7) which is impressive considering the challenge associated with 1,2-cis glycosylation.[56] The reaction for a 1-4 glycosylation afforded the α-anomer in good yield.

Table 2.2.3 IPy_2BF_4 mediated disaccharide glycosylation.



Entry	Donor	Acceptor	Time(hr)	Yield (%)	α/β
1	 **S2-1**	 **S2-11**	2.0	87	20:80 **S2-13**
2		 **S2-12**	1.0	84	36:64 **S2-14**
3	 **S2-10**	 **S2-11**	1.0	74	β **S2-15**
4		 **S2-12**	1.0	70	β **S2-16**
5	 **S2-3**	 **S2-11**	1.0	85	15:85 **S2-17**
6		 **S2-12**	1.0	80	60:40 **S2-18**
7	 **S2-5**	 **S2-11**	1.0	40	β **S2-19**
8		 **S2-12**	1.0	83	α **S2-20**

A significant advance in the field of carbohydrate synthesis is the development of one-pot sequential glycosylation methods that rely predominantly on the tuning of the donor reactivity. The spectrum of reactivity from armed to disarmed glycosyl donors can be tuned by choosing the appropriate protecting group. In this context, the ability

to form a β-glycosidic bond with a fully armed glycosyl donor is valuable. This is exemplified by the one-pot synthesis of trisaccharide from monomeric units (Fig. 2.2.4). Trisaccharide[57] was obtained in 42% yield as a 6:4 ratio mixture in favor of the β-form product.

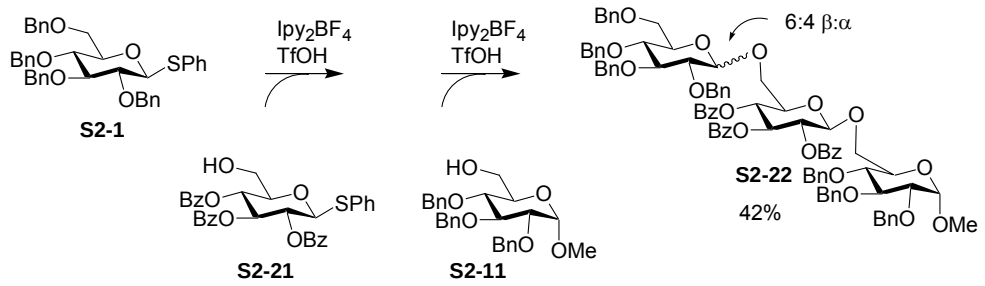


Figure 2.2.4 IPy_2BF_4 used in one-pot glycosylation reaction.

In conclusion, the activation of thioglycosides with IPy_2BF_4 was demonstrated either to yield glycosyl fluorides or to promote glycosylation, depending on the reaction conditions. Glycosylation of perbenzylated armed glycosides afforded predominantly β-form products with unhindered acceptors, which provides unique reactivity. This can be exploited in one-pot glycosylation reactions as was exemplified with the formation of a trisaccharide containing β-linkages prepared from an armed and a disarmed donor.

2.3 Experimental section

General Procedure for the Conversion of Thioglycoside to Glycosyl Fluoride: IPy_2BF_4 (55.8 mg, 0.15 mmol, 1.5 equiv.) was added to a solution of thioglycoside (0.10 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) at 23 °C, and the mixture was stirred for 3 h. The reaction mixturewas then diluted with ethyl acetate and washed sequentially with $NaHCO_3$ (satd.) and brine. The organic layer was dried with sodium sulfate, concentrated in vacuo, and purified by silica gel flash column chromatography.

General Procedure for IPy_2BF_4-Mediated Glycosylation: A solution of thioglycoside (0.15 mmol, 1.5 equiv.) and acceptor (0.1 mmol, 1.0 equiv.) in CH_2Cl_2 (5 mL) were stirred in the presence of 3 Å molecular sieves for 20 min at 23 °C room temperature and then cooled to –35 °C prior to the addition of TfOH (13.3 µL, 0.15 mmol, 1.5 equiv.). The reaction mixture was stirred at this temperature for 10 min and IPy_2BF_4 (55.8 mg, 0.15 mmol, 1.5 equiv.) was added. The solution was stirred at –35 °C for 1 h then quenched with an excess diisopropylethylamine (82.6 µL, 0.5 mmol, 5 equiv.). The reaction was diluted with ethyl acetate (20 mL) and was washed sequentially with $NaHCO_3$ (satd.) (2×15 mL) and brine (15 mL). The organic layer was dried with Na_2SO_4, concentrated in vacuo and purified by silica gel flash column chromatography.

One-Pot Sequential Glycosylation: A solution of thioglycoside **S2-1** (0.11 mmol, 1.1 equiv.) in CH_2Cl_2 (5 mL) of was stirred for 20 min at room temperature in the presence of 3 Å molecular sieves and then cooled to –35 °C followed by the addition of TfOH (0.11 mmol, 1.1 equiv.). The solution was stirred at this temperature for 10 min, and IPy_2BF_4 (0.11 mmol, 1.1 equiv.) was added. The solution was stirred at –35 °C for 20 min, and glycosyl acceptor **S2-21** was added at –35 °C. After completion of the first glycosylation reaction (1 h, monitored by TLC), glycosyl acceptor **S2-11** (0.11 mmol, 1.1 equiv.) was added followed by a second batch of TfOH (0.11 mmol, 1.1 equiv.) and Ipy2BF4 (0.11 mmol, 1.1 equiv.). The reaction was stirred for an additional 60 min at –35 °C before the addition of excess diisopropylethylamine (82.6 µL, 0.5 mmol, 5 equiv.). The reaction was diluted with ethyl acetate (20 mL) and washed sequentially with $NaHCO_3$ (satd.) (2×15 mL) and brine (15 mL). The organic layer was dried with Na_2SO_4, concentrated in vacuo, and purified by silica gel flash column chromatography to obtain trisaccharide **S2-22** in 42% yield.

2,3,4,6-tetra-*O*-benzyl-1-deoxy-1-fluoro-*α*-D-glucopyranoside[58] **(S2-2):** *Rf* = 0.40 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4-7.1 (m, 20H), 5.56 (dd, J_{HF} = 53.2, 2.7 Hz, 1H), 4.96 (d, J = 10.9 Hz, 1H), 4.86 (d, J = 10.9 Hz, 1H), 4.84 (d, J = 10.9 Hz, 1H), 4.81 (d, J = 12.0 Hz, 1H), 4.73 (d, J = 12.1 Hz, 1H), 4.60 (d, J = 12.0 Hz, 1H), 4.52 (d, J = 10.8 Hz, 1H), 4.48 (d, J = 12.1 Hz, 1H), 4.03-3.96 (m, 2H), 3.76 (dd, J = 10.8, 2.9 Hz, 1H), 3.74 (t, J = 9.5 Hz, 1H), 3.67 (dd, J = 10.9, 2.0 Hz, 1H), 3.57 (ddd, J = 25.7, 9.7, 2.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.0, 137.8, 128.6, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 105.6 (d, $J_{C\text{-}F}$ = 224.0 Hz), 81.5, 79.3 (d, $J_{C\text{-}F}$ = 24.7 Hz), 76.7, 75.9, 75.2, 73.6, 73.5, 72.7 (d, $J_{C\text{-}F}$ = 3.6 Hz), 67.8; HRMS (MALDI-TOF) *m*/*z* $C_{34}H_{35}FO_5Na$ ($M+Na^+$) 565.293.

2,3,4,6-tetra-*O*-benzyl-1-deoxy-1-fluoro-α-D-galactopyranoside (S2-4): *Rf* = 0.40 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.4-7.1 (m, 20H), 5.64 (dd, J_{HF} = 53.5, 2.1 Hz, 1H), 5.00 (d, J = 11.3 Hz, 1H), 4.90 (d, J = 11.8 Hz, 1H), 4.87 (d, J = 11.8 Hz, 1H), 4.80 (d, J = 11.8 Hz, 1H), 4.77 (d, J = 11.8 Hz, 1H), 4.62 (d, J = 11.3 Hz, 1H), 4.54 (d, J = 11.8 Hz, 1H), 4.47 (d, J = 11.8 Hz, 1H), 4.17-3.98 (m, 4H), 3.60 (d, J = 6.4 Hz, 1H); ^{13}C NMR (100 MHz, CDCl3) δ 138.4, 138.1, 137.8, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 127.9, 127.7, 127.7, 127.6, 106.3 (d, $J_{C\text{-}F}$ = 224.8 Hz), 78.5, 75.8 (d, $J_{C\text{-}F}$ = 23.2 Hz), 74.9, 74.3, 73.8, 73.5, 73.1, 71.8 (d, $J_{C\text{-}F}$ = 2.9 Hz), 68.3; HRMS (MALDI-TOF) *m*/*z* $C_{34}H_{35}FO_5Na$ ($M+Na^+$) 565.222.

2,3,4,6-tetra-*O*-benzyl-1-deoxy-1-fluoro-α-D-mannopyranoside (S2-6): *Rf* = 0.40 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.18 (m, 20H), 5.62 (dd, J_{HF} = 50.6, 1.4 Hz, 1H), 4.90 (d, J = 12.4 Hz, 1H), 4.82 (d, J = 12.4 Hz, 1H), 4.73-4.65 (m, 4H), 4.56 (d, J = 11.8 Hz, 1H), 4.10 (t, J = 9.4 Hz, 1H), 3.96-3.90 (m, 3H), 3.82-3.72 (m, 2H); ^{13}C NMR (100 MHz, CDCl3) δ 138.2, 138.2, 138.2, 137.9, 128.5, 128.4, 128.0, 127.9, 127.9, 127.8, 127.7, 106.5 (d, *J*C-F = 221.1 Hz), 79.2, 79.2, 75.2, 74.2 (d, *J*C-F = 1.5 Hz), 74.1, 73.5, 73.5 (d, $J_{C\text{-}F}$ = 34.9 Hz), 73.3, 72.6, 68.6; HRMS (MALDI-TOF) *m*/*z* C34H35FO5Na ($M+Na^+$) 565.232.

2,3-di-*O*-benzyl-4,6-O-benzylidene-1-deoxy-1-fluoro-α-D-galactopyranoside (S2-8): (76%) *Rf* = 0.47 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (m, 2H), 7.40-7.26 (m, 13H), 5.67 (dd, J_{HF} = 53.4, 2.2 Hz, 1H), 5.48 (s, 1H), 4.88 (d, J = 11.8 Hz, 1H), 4.81 (d, J = 11.8 Hz, 1H), 4.75 (d, J = 11.8 Hz, 1H), 4.71 (d, J = 11.8 Hz, 1H), 4.26–4.23 (m, 2H), 4.10–3.96 (m, 3H), 3.81 (br s, 1H); ^{13}C NMR (100 MHz, CDCl3) δ 138.3, 138.1, 137.5, 129.0, 128.4, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 126.2, 106.9 (d, $J_{C\text{-}F}$ = 224.0 Hz), 101.0, 75.4, 74.8 (d, $J_{C–F}$ = 23.3

Hz), 74.0, 72.2, 69.0, 64.8 (d, J_{C-F} = 1.8 Hz); HRMS (MALDI-TOF) *m*/*z* $C_{27}H_{26}FO_5$ (M-H) 449.379.

Isopropyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside[59] **(S2-9).** (89%, *α*:*β*=42:58) *α*-Anomer: *Rf* = 0.43 (33% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.4-7.1 (m, 20 H), 5.00 (d, *J* = 10.8Hz, 1H), 4.88 (d, *J* =3.8 Hz, 1H), 4.83 (d, *J* = 10.6 Hz, 1H), 4.82 (d, *J* = 10.8 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* =12.0 Hz, 1H), 4.62 (d, *J* = 12.3 Hz, 1H), 4.47 (d, *J* = 12.1 Hz, 2H), 4.00 (t, *J* = 9.3 Hz, 1H), 3.90 (m, 1 H), 3.85 (ddd, *J* = 10.2, 3.4, 2.0 Hz, 1H), 3.74 (dd, *J* = 10.5, 3.6 Hz, 1H), 3.64 (m, 2 H), 3.56 (dd, *J* = 9.6, 3.8 Hz, 1H), 1.13 (d, *J* = 6.3 Hz, 3H), 1.18 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, CDC_{l3}) *δ* 139.0, 138.3, 138.3, 138.0, 128.5, 128.4, 128.4, 128.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 94.8, 82.2, 79.9, 77.9, 75.7, 75.2, 73.5, 73.2, 70.0, 69.0, 68.5, 23.2, 21.2;
β-Anomer: *Rf* = 0.33 (33% ethyl acetate in hexane); ^{1}H NMR (400 MHz, CDC_{l3}) *δ* 7.4-7.1 (m, 20 H), 5.00 (d, *J* = 11.0 Hz, 1H), 4.95 (d, *J* = 10.9 Hz, 1H), 4.84 (d, *J* = 11.1 Hz, 1H), 4.81 (d, *J* = 10.8 Hz, 1H), 4.73 (d, *J* = 10.9 Hz, 1H), 4.63 (d, *J* = 12.3 Hz, 1H), 4.59 (d, *J* = 11.0 Hz, 1H), 4.57 (d, *J* = 10.8 Hz, 1H), 4.50 (d, *J* = 7.8 Hz, 1H), 4.05 (m, 1 H), 3.76 (dd, *J* = 10.8, 1.8 Hz, 1H), 3.70 (m, 1 H), 3.58 (t, *J* = 9.64 Hz, 1H), 3.48 (m, 1 H), 3.46 (dd, *J* = 9.1, 7.9 Hz, 1H), 1.34 (d, *J* = 6.2 Hz, 3H), 1.27 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CDC_{l3}) *δ* 138.7, 138.6, 138.4, 138.2, 128.4, 128.3, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 102.2, 84.9, 82.4, 78.0, 75.7, 75.0, 74.9, 74.9, 73.5, 72.4, 69.2, 23.8, 22.3; HRMS (MALDI-TOF) *m*/*z* $C_{37}H_{42}O_6Na$ (M +Na^+) 605.371.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-(1-6)-2,3,4-tri-*O*-benzyl-D-glucopyranoside[59] **(S2-13).** (87%, *α*:*β*=20:80) *α*-Anomer: *Rf* = 0.65 (33% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.4-7.1 (m, 35 H), 4.98 (d, *J* = 3.7 Hz, 1H), 4.96 (d, *J* = 11.0 Hz, 1H), 4.94 (d, *J* = 10.9 Hz, 1H), 4.91 (d, *J* = 11.0 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.81 (d, *J* = 10.9 Hz, 1H), 4.77 (d, *J* = 10.9 Hz, 1H), 4.71 (d, *J* = 11.9 Hz, 1H), 4.66 (s, 2 H), 4.64 (d, *J* = 11.3 Hz, 1H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.55 (d, *J* = 3.5 Hz, 1H), 4.45 (d, *J* = 11.0 Hz, 1H), 4.42 (d, *J* = 12.1 Hz, 1H), 3.98 (t, *J* = 9.3 Hz, 1H), 3.95 (t, *J* = 9.3 Hz, 1H), 3.79 (m, 3 H), 3.71 (dd, *J* = 11.3, 2.7 Hz, 1H), 3.65 (m, 3 H), 3.55 (dd, *J* = 10.7, 2.2 Hz, 1H), 3.54 (dd, *J* = 9.7, 3.5 Hz, 1H), 3.44 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.35 (s, 3 H); 13C NMR (100 MHz, CDCl3) *δ* 138.8, 138.5, 138.4, 138.2, 138.1, 128.4, 128.4, 128.3, 128.3, 128.0, 128.0, 127.9, 127.9, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 98.0, 97.3, 82.2, 81.7, 80.1, 80.0, 77.8, 77.6, 75.7, 75.5, 75.0, 74.9, 73.4, 72.3, 70.4, 70.2, 68.5, 66.0, 55.1;
β-Anomer: *Rf* = 0.61 (33% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) *δ*

7.4-7.1 (m, 35 H), 4.97 (d, *J* = 11.0 Hz, 1H), 4.96 (d, *J* = 10.7 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 4.79 (m, 4 H), 4.74 (d, *J* = 11.2 Hz, 1H), 4.71 (d, *J* = 11.1 Hz, 1H), 4.65 (d, *J* = 12.1 Hz, 1H), 4.60 (d, *J* = 3.4 Hz, 1H), 4.58 (d, *J* = 10.9 Hz, 1H), 4.52 (m, 3 H), 4.34 (d, *J* = 7.8 Hz, 1H), 4.18 (dd, *J* = 10.6, 2.0 Hz, 1H), 3.98 (t, *J* = 9.3 Hz, 1H), 3.82 (m, 1 H), 3.72 (dd, *J* = 11.1, 1.7 Hz, 1H), 3.67 (dd, *J* = 10.8, 4.9 Hz, 1H), 3.62 (t, *J* = 9.0 Hz, 1H), 3.56 (t, *J* = 9.4 Hz, 1H), 3.51 (m, 3 H), 3.42 (m, 1 H), 3.32 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 138.6, 138.4, 138.4, 138.3, 138.2, 138.2, 128.5, 128.4, 128.4, 128.4, 128.2, 128.0, 128.0, 127.9, 127.9, 127.9, 127.7, 127.6, 127.6, 127.5, 103.8, 98.1, 84.8, 82.1, 82.0, 79.8, 78.0, 77.9, 75.5, 75.1, 75.0, 74.9, 73.5, 73.4, 69.9, 69.9, 68.6, 55.2; HRMS (MALDI-TOF) *m*/*z* $C_{62}H_{66}O_{11}Na$ (M +Na^+) 1009.620.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-(1-4)-2,3,6-tri-*O*-benzyl-D-glucopyranoside[59] **(S2-14).** (84%, α:β=36:64) α-Anomer: *Rf* = 0.21 (20% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32-7.06 (m, 35 H), 5.70 (d, *J* = 3.6 Hz, 1H), 5.04 (d, *J* = 11.5 Hz, 1H), 4.88 (d, *J* = 10.84 Hz, 1H), 4.80 (d, *J* = 11.7 Hz, 1H), 4.78 (d, *J* = 10.6 Hz, 1H), 4.77 (d, *J* = 10.9 Hz, 1H), 4.70 (d, *J* = 12.17 Hz, 1H), 4.60 (d, *J* = 3.57 Hz, 1H), 4.59 (d, *J* = 11.9 Hz, 1H), 4.57 (d, *J* = 12.2 Hz, 1H), 4.54 (d, *J* = 11.9 Hz, 1H), 4.52 (d, *J* = 12.2 Hz, 1H), 4.49 (s, 2H), 4.41 (d, *J* = 10.86 Hz, 1H), 4.27 (d, *J* = 12.1 Hz, 1H), 4.09 (t, *J* = 8.8 Hz, 1H), 4.07 (t, *J* = 9.0 Hz, 1H), 3.90 (dd, *J* = 9.8, 8.6 Hz, 1H), 3.84 (m, 2H), 3.67 (m, 3H), 3.59 (dd, *J* = 9.2, 3.7 Hz, 1H), 3.49 (dd, *J* = 9.7, 3.6 Hz, 1H), 3.48 (dd, *J* = 10.7, 2.9 Hz, 1H), 3.38 (dd, *J* = 10.66, 1.56 Hz, 1H), 3.37 (s, 3H); ^{13}C NMR (100 MHz, CDC_{l3}) δ 138.9, 138.8, 138.5, 138.2, 138.0, 138.0, 128.5, 128.4, 128.3, 128.3, 128.3, 128.1, 128.0, 127.9, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 127.1, 126.8, 110.0, 97.8, 96.7, 82.1, 82.1, 80.2, 79.5, 77.8, 76.8, 75.0, 74.4, 73.5, 73.4, 73.3, 73.2, 72.3, 71.0, 69.5, 69.0, 68.2, 55.2;

β-Anomer: *Rf* = 0.16 (20% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.20 (m, 35 H), 5.14 (d, *J* = 11.1 Hz, 1H), 4.91 (d, *J* = 10.8 Hz, 1H), 4.85 (d, *J* = 11.02 Hz, 1H), 4.85 (d, *J* = 11.1 Hz, 1H), 4.84 (s, 1 H), 4.83 (d, *J* = 11.05 Hz, 1H), 4.80 (d, *J* = 10.6 Hz, 1H), 4.80 (d, *J* = 11.4 Hz, 1H), 4.65 (d, *J* = 12.2 Hz, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.62 (d, *J* = 3.7 Hz, 1H), 4.60 (d, *J* = 11.0 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.44 (d, *J* = 11.8 Hz, 1H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 4.01 (dd, *J* = 10.0, 9.0 Hz, 1H), 3.90 (t, *J* = 9.2 Hz, 1H), 3.89 (dd, *J* = 11.0, 3.3 Hz, 1H), 3.88 (dd, *J* = 11.0, 3.2 Hz, 1H), 3.76 (dd, *J* = 11.0, 1.9 Hz, 1H), 3.65 (dd, *J* = 9.8, 8.8 Hz, 1H), 3.64 (dd, *J* = 9.8, 2.8 Hz, 1H), 3.59 (dd, *J* = 11.2, 4.8 Hz, 1H), 3.53 (dd, *J* = 10.9, 2.0 Hz, 1H), 3.51 (t, *J* = 8.9 Hz, 1H), 3.51 (m, 1 H), 3.41 (dd, *J* = 9.0, 7.9 Hz, 1H), 3.41 (s, 3H), 3.34 (ddd, *J* = 9.8, 4.6, 1.8 Hz, 1H); ^{13}C NMR (100 MHz, CDC_{l3}) δ 139.8, 138.8, 138.8, 138.6, 138.6, 138.1, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3,

128.2, 128.2, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.5, 127.3, 102.7, 98.6, 85.1, 83.0, 80.6, 79.0, 78.3, 76.9, 76.8, 75.8, 75.6, 75.4, 75.1, 75.0, 73.8, 73.6, 73.5, 70.2, 70.1, 69.2, 68.1, 55.5; HRMS (MALDI-TOF) *m/z* $C_{62}H_{66}O_{11}Na$ ($M+Na^+$) 1009.561.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-(1-6)-2,3,4-tri-*O*-benzyl-*α*-D-glucopyranoside[59] **(S2-15).** (74%) *Rf* = 0.29 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03-7.84 (m, 8 H), 7.57-7.08 (m, 27 H), 5.89 (t, *J* = 9.6 Hz, 1H), 5.68 (t, *J* = 9.69 Hz, 1H), 5.60 (dd, *J* = 9.6, 7.8 Hz, 1H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.83 (d, *J* = 7.8 Hz, 1H), 4.74 (d, *J* = 12.1 Hz, 1H), 4.69 (d, *J* = 11.1 Hz, 1H), 4.61 (dd, *J* = 12.1, 3.6 Hz, 1H), 4.60 (d, *J* = 12.1 Hz, 1H), 4.51 (m, 3 H), 4.29 (d, *J* = 11.1 Hz, 1H), 4.15 (d, *J* = 10.3 Hz, 1H), 4.15 (m, 1 H), 3.89 (t, *J* = 9.3 Hz, 1H), 3.74 (m, 2 H), 3.43 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.83 (t, *J* = 9.3 Hz, 1H), 3.21 (s, 3 H); ^{13}C NMR (100 MHz, CDCl3) δ 166.2, 165.9, 165.2, 165.0, 138.8, 138.2, 138.1, 133.5, 133.3, 133.2, 133.1, 129.9, 129.8, 129.7, 129.6, 129.4, 129.2, 128.8, 128.7, 128.5, 128.4, 128.3, 128.3, 128.3, 128.3, 128.2, 127.9, 127.9, 127.6, 127.5, 127.5, 127.0, 124.8, 101.3, 98.0, 81.9, 79.8, 77.4, 75.6, 74.7, 73.4, 72.9, 72.2, 71.8, 69.8, 69.5, 68.3, 63.3, 55.0; HRMS (MALDI-TOF) *m/z* $C_{62}H_{58}O_{16}Na$ ($M+Na^+$) 1065.224.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl)-(1-4)-2,3,6-tri-*O*-benzyl-*α*-D-glucopyranoside[59] **(S2-16).** (70%) *Rf* = 0.31 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01-7.81 (m, 8 H), 7.57-7.20 (m, 27 H), 5.62 (t, *J* = 9.5 Hz, 1H), 5.55 (t, *J* = 9.6 Hz, 1H), 5.46 (dd, *J* = 9.6, 8.0 Hz, 1H), 5.07 (d, *J* = 11.2 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 8.1 Hz, 1H), 4.76 (d, *J* = 12.9 Hz, 1H), 4.75 (d, *J* = 12.4 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.56 (d, *J* = 3.5 Hz, 1H), 4.40 (dd, *J* = 12.1, 3.6 Hz, 1H), 4.34 (d, *J* = 12.2 Hz, 1H), 4.26 (dd, *J* = 12.1, 5.0 Hz, 1H), 3.96 (t, *J* = 9.4 Hz, 1H), 3.88 (t, *J* = 9.1 Hz, 1H), 3.73 (m, 2 H), 3.50 (m, 1 H), 3.46 (dd, *J* = 9.5, 3.7 Hz, 1H), 3.42 (dd, *J* = 10.7, 1.0 Hz, 1H), 3.28 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 165.8, 165.1, 164.9, 145.6, 139.3, 138.4, 137.9, 133.4, 133.4, 133.2, 133.0, 131.1, 129.8, 129.7, 129.7, 129.4, 129.1, 128.9, 128.9, 128.5, 128.4, 128.4, 128.3, 128.1, 127.8, 127.4, 127.2, 124.8, 100.4, 98.5, 80.0, 78.8, 77.3, 75.4, 73.6, 73.6, 73.2, 72.3, 71.8, 69.9, 69.5, 67.6, 63.2, 55.4; HRMS (MALDI-TOF) *m/z* $C_{62}H_{58}O_{16}Na$ ($M+Na^+$) 1065.208.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-(1-6)-2,3,4-tri-*O*-benzyl-*α*-D-glucopyranoside[58] **(S2-17).** (85%, *α*:*β*=15:85) *α*-Anomer: *Rf* = 0.49 (5% acetonitrile in benzene); ^{1}H NMR (400 MHz, C_6D_6) δ 7.40-7.32 (m, 10H), 7.27-7.24 (m, 4H), 7.18-7.04 (m, 21H), 5.28 (d, *J* = 3.7 Hz, 1H), 5.10 (d, *J* = 11.2 Hz, 1H), 5.05 (d, *J* = 11.5 Hz, 1H), 5.01 (d, *J* = 11.2 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.80 (d, *J* =

11.2 Hz, 1H), 4.71 (d, *J* = 12.2 Hz, 1H), 4.63 (d, *J* = 11.2 Hz, 1H), 4.60 (d, *J* = 3.4 Hz, 1H), 4.58 (d, *J* = 11.9 Hz, 1H), 4.55 (s, 2H), 4.49 (d, *J* = 12.2 Hz, 1H), 4.37 (d, *J* = 11.9 Hz, 1H), 4.34 (d, *J* = 11.9 Hz, 1H), 4.29-4.27 (m, 2H), 4.26-4.20 (m, 2H), 4.10 (dd, *J* = 10.0, 2.7 Hz, 1H), 4.01 (dd, *J* = 11.9, 3.7 Hz, 1H), 3.98 (d, *J* = 1.7 Hz, 1H), 3.94-3.87 (m, 2H), 3.86-3.80 (m, 2H), 3.73 (dd, *J* = 9.0, 5.9 Hz, 1H), 3.49 (dd, *J* = 9.5, 3.4 Hz, 1H), 3.11 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6) δ 139.8, 139.7, 130.6, 139.59, 139.5, 139.3, 138.9, 128.6, 128.5, 128.49, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.58, 127.5, 127.4, 98.4, 98.3, 82.4, 81.2, 78.6, 78.3, 77.7, 76.2, 75.5, 75.3, 75.0, 73.5, 73.0, 72.9, 72.7, 71.3, 70.1, 69.5, 66.3, 54.9;
β-Anomer: *Rf* = 0.42 (5% acetonitrile in benzene); ^{1}H NMR (400 MHz, C_6D_6) δ 7.43-7.33 (m, 4H), 7.31-7.23 (m, 9H), 7.17- 7.06 (m, 22H), 5.07 (d, *J* = 11.5 Hz, 1H), 5.04 (d, *J* = 11.5 Hz, 1H), 5.01 (d, *J* = 11.2 Hz, 1H), 4.90 (d, *J* = 11.7 Hz, 1H), 4.78 (d, *J* = 11.2 Hz, 1H), 4.77 (d, *J* = 11.2 Hz, 1H), 4.68 (d, *J* = 3.4 Hz, 1H), 4.66 (d, *J* = 10.3 Hz, 1H), 4.61 (d, *J* = 11.2 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.51 (t, *J* = 12.2 Hz, 2H), 4.46 (d, *J* = 11.9 Hz, 1H), 4.44 (d, *J* = 7.6 Hz, 1H), 4.37 (dd, *J* = 10.7, 1.7 Hz, 1H), 4.31-4.23 (m, 3H), 4.14 (dd, *J* = 9.8, 7.6 Hz, 1H), 4.04 (ddd, *J* = 10.0, 4.9, 1.5 Hz, 1H), 3.84-3.81 (m, 2H), 3.78-3.73 (m, 2H), 3.64-3.60 (m, 2H), 3.41 (t, *J* = 6.6 Hz, 1H), 3.37 (dd, *J* = 9.8, 2.9 Hz, 1H), 3.12 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6) δ 139.8, 139.7, 139.6, 139.2, 139.1, 138.8, 128.6, 128.5, 128.49, 128.46, 128.42, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 104.7, 98.2, 82.7, 82.3, 81.1, 79.9, 76.6, 75.5, 75.3, 75.2, 74.8, 74.6, 73.7, 73.5, 72.9, 72.7, 70.7, 69.0, 68.7, 54.9; HRMS (MALDI-TOF) *m*/*z* $C_{62}H_{66}O_{11}Na$ ($M + Na^+$) 1009.437.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[57] **(S2-18).** (80%, α:β=60:40) α-Anomer: *Rf* = 0.50 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.15 (m, 35H), 5.75 (d, *J* = 4.0 Hz, 1H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.86 (d, *J* = 11.3 Hz, 1H), 4.81 (d, *J* = 11.3 Hz, 1H), 4.72–4.61 (m, 4H), 4.59 (d, *J* = 12.5 Hz, 1H), 4.57 (d, *J* = 3.4 Hz, 1H), 4.51–4.56 (m, 3H), 4.42 (d, *J* = 12.2 Hz, 1H), 4.30 (d, *J* = 11.6 Hz, 1H), 4.24 (d, *J* = 11.6 Hz, 1H), 4.06 (dd, *J* = 9.5, 8.9 Hz, 1H), 3.98 (dd, *J* = 10.7, 4.0 Hz, 1H), 3.91–3.98 (m, 2H), 3.78–3.89 (m, 3H), 3.70 (dd, *J* = 10.4, 4.3 Hz, 1H), 3.63–3.68 (m, 1H), 3.54 (dd, *J* = 9.5, 3.4 Hz, 1H), 3.52–3.40 (m, 2H), 3.37 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 139.0, 138.6, 138.4, 138.3, 138.0, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.4, 127.0, 126.7, 97.7, 97.5, 82.0, 80.2, 79.2, 75.6, 74.8, 74.7, 74.3, 73.8, 73.4, 73.3, 73.1, 72.8, 72.7, 69.9, 69.5, 69.4, 68.7, 55.1;
β-Anomer: *Rf* = 0.50 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.10 (m, 35H), 5.02 (d, *J* = 10.7 Hz, 1H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.82 (d, *J* = 12.2 Hz, 1H), 4.80 (d, *J* = 11.3 Hz, 1H), 4.76 (d, *J* = 11.3 Hz, 1H), 4.72 (d, *J* = 10.7

Hz, 1H), 4.71 (d, *J* = 11.6 Hz, 1H), 4.67 (d, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 12.2 Hz, 1H), 4.56 (d, *J* = 3.7 Hz, 1H), 4.55 (d, *J* = 11.3 Hz, 1H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.36 (d, *J* = 11.9 Hz, 1H), 4.33 (d, *J* = 11.9 Hz, 1H), 4.30 (d, 1H, *J* = 7.6 Hz), 4.24 (d, 1H, *J* = 11.9 Hz), 3.94–3.86 (m, 2H), 3.85–3.79 (m, 2H), 3.74 (dd, *J* = 9.5, 7.6 Hz, 1H), 3.62–3.57 (m, 1H), 3.55–3.47 (m, 2H), 3.47 (dd, 1H, *J* = 9.8, 3.7 Hz), 3.37 (s, 3H), 3.42–3.28 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.4, 139.0, 138.9, 138.5, 138.2, 138.1, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.0, 102.8, 98.4, 82.5, 80.3, 80.1, 78.9, 76.6, 75.5, 75.2, 74.7, 73.8, 73.7, 73.4, 73.1, 73.1, 72.6, 70.0, 68.2, 68.0, 55.3; HRMS (MALDI-TOF) *m*/*z* $C_{62}H_{66}O_{11}Na$ (M +Na^+) 1009.394.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzyl-*β*-D-mannopyranosyl)-(1-6)-2,3,4-tri-*O*-benzyl-*α*-D-glucopyranoside[58] **(S2-19)**. (40%) *Rf* = 0.39 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.14 (m, 35H), 5.01 (d, *J* = 10.8 Hz, 1H), 4.93 (d, *J* = 12.0 Hz, 1H), 4.88 (d, *J* = 10.8 Hz, 1H), 4.83 (d, *J* = 3.2 Hz, 1H), 4.82 (d, *J* = 12.0 Hz, 1H), 4.81 (d, *J* = 11.0 Hz, 1H), 4.78 (d, *J* = 11.6 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.57 (s, 2H), 4.57 (br s, 1H), 4.55–4.49 (m, 1H), 4.52 (d, *J* = 11.8 Hz, 1H), 4.51 (d, *J* = 11.0 Hz, 1H), 4.47 (d, *J* = 11.8 Hz, 1H), 4.16 (dd, *J* = 10.0, 1.2 Hz, 1H), 4.11 (br s, 1H), 4.01 (dd, *J* = 9.0, 9.0 Hz, 1H), 3.88–3.66 (m, 5H), 3.50 (dd, *J* = 9.2, 3.0 Hz, 1H), 3.50–3.34 (m, 4H), 3.32 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 138.8, 138.7, 138.5, 138.3, 138.2, 138.1, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 101.5, 97.8, 82.3, 82.2, 79.9, 77.7, 77.2, 76.0, 75.7, 75.2, 75.0, 74.8, 73.7, 73.6, 73.5, 73.4, 71.6, 69.8, 69.8, 68.3, 55.1; HRMS (MALDI-TOF) *m*/*z* $C_{62}H_{66}O_{11}Na$ (M +Na^+) 1009.477.

Methyl-*O*-(2,3,4,6-tetra-*O*-benzyl-*α*-D-mannopyranosyl)-(1-4)-2,3,6-tri-*O*-benzyl-*α*-D-glucopyranoside[58] **(S2-20)**. (83%) *Rf* = 0.40 (33.3% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.11 (m, 35H), 5.29 (d, *J* = 1.2 Hz, 1H), 5.08 (d, *J* = 11.2 Hz, 1H), 3.39 (s, 3H), 4.83 (d, *J* = 10.8 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.64–4.52 (m, 7H,), 4.48 (d, *J* = 10.6 Hz, 1H), 4.42 (d, *J* = 11.4 Hz, 1H), 4.32 (d, *J* = 12.0 Hz, 1H), 4.21 (d, *J* = 11.8 Hz, 1H), 4.10 (d, *J* = 11.8 Hz, 1H), 3.97 (dd, *J* = 9.0, 9.0 Hz, 1H), 3.89–3.61 (m, 9H), 3.60–3.50 (m, 2H), 3.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 138.7, 138.6, 138.5, 138.4, 137.9, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 127.2, 127.2, 126.8, 100.6, 97.7, 81.6, 80.0, 79.8, 77.8, 76.3, 75.1, 75.0, 74.9, 73.4, 73.3, 73.2, 73.0, 72.3, 72.1, 69.8, 69.4, 55.3; HRMS (MALDI-TOF) *m*/*z* $C_{62}H_{66}O_{11}Na$ (M +Na^+) 1009.604.

Trisaccharide (S2-22)[57] in 42 % yield. α-Anomer: *Rf* = 0.50 (40% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.9 Hz, 2H), 7.87 (d, *J* = 7.9 Hz, 2H), 7.79 (d, *J* = 7.9 Hz, 2H), 7.48 (dd, *J* = 7.9, 7.9 Hz, 1H), 7.43–7.14 (m, 39H), 7.13–7.08 (m, 2H), 6.99 (d, *J* = 7.0 Hz, 2H), 5.83 (dd, *J* = 9.8, 9.5 Hz, 1H), 5.53 (dd, *J* = 9.5, 7.9 Hz, 1H), 5.48 (dd, *J* = 9.8, 9.8 Hz, 1H), 4.87 (d, *J* = 10.4 Hz, 1H), 4.86 (d, *J* = 10.4 Hz, 1H), 4.80 (d, *J* = 11.3 Hz, 1H), 4.76 (d, *J* = 7.9 Hz, 1H), 4.72 (d, *J* = 3.4 Hz, 1H), 4.71 (d, *J* = 10.4 Hz, 1H), 4.71 (d, *J* = 10.4 Hz, 1H), 4.87 (d, *J* = 12.2 Hz, 1H), 4.65 (d, *J* = 11.3 Hz, 1H), 4.59 (d, *J* = 12.2 Hz, 1H), 4.56 (d, *J* = 12.2 Hz, 1H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.45 (d, *J* = 3.7 Hz, 1H), 4.44 (d, *J* = 11.3 Hz, 1H), 4.38 (d, *J* = 11.3 Hz, 1H), 4.35 (d, *J* = 12.2 Hz, 1H), 4.20 (d, *J* = 11.3 Hz, 1H), 4.10 (d, *J* = 10.4 Hz, 1H), 4.07–4.01 (m, 1H), 3.91 (dd, *J* = 9.8, 9.2 Hz, 1H), 3.87 (dd, *J* = 11.3, 6.7 Hz, 1H), 3.83 (dd, *J* = 9.5, 9.2 Hz, 1H), 3.66–3.58 (m, 4H), 3.58–3.52 (m, 1H), 3.51 (dd, *J* = 9.8, 3.4 Hz, 1H), 3.43–3.34 (m, 2H), 3.03 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 165.8, 165.1, 164.8, 138.8, 138.4, 138.3, 138.3, 138.1, 133.3, 133.1, 133.0, 129.8, 129.7, 129.2, 128.9, 128.8, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.6, 127.6, 127.5, 127.4, 127.3, 100.8, 97.9, 97.3, 81.8, 81.7, 80.1, 79.7, 77.4, 77.1, 75.5, 75.4, 74.8, 74.5, 73.4, 73.4, 73.1, 73.0, 71.9, 70.2, 69.8, 69.4, 68.4, 67.8, 67.1, 54.9;

β-Anomer: *Rf* = 0.50 (40% ethyl acetate in hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 8.2, 1.2 Hz, 2H), 7.85 (d, *J* = 8.2 Hz, 2H), 7.79 (dd, *J* = 8.5, 1.2 Hz, 2H), 7.47-7.53 (m, 1H), 7.44–7.14 (m, 39H), 7.14–7.06 (m, 2H), 6.96–6.90 (m, 2H), 5.84 (dd, *J* = 9.8, 9.5 Hz, 1H), 5.53 (dd, *J* = 9.8, 8.2 Hz, 1H), 5.39 (dd, *J* = 9.8, 9.5 Hz, 1H), 4.94 (d, *J* = 11.0 Hz, 1H), 4.86 (d, *J* = 11.0 Hz, 1H), 4.85 (d, *J* = 11.0 Hz, 1H), 4.76 (d, *J* = 11.0 Hz, 1H), 4.76-4.68 (m, 3H), 4.66 (d, *J* = 8.2 Hz, 1H), 4.64 (d, *J* = 12.2 Hz, 1H), 4.57 (d, *J* = 12.2 Hz, 1H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.52 (d, *J* = 7.3 Hz, 1H), 4.48 (d, *J* = 3.4 Hz, 1H), 4.48 (d, *J* = 11.0 Hz, 1H), 4.42 (d, *J* = 12.2 Hz, 1H), 4.32 (d, *J* = 11.0 Hz, 1H), 4.11 (d, *J* = 11.0 Hz, 1H), 4.09-4.00 (m, 3H), 3.89 (dd, *J* = 11.9, 8.5 Hz, 1H), 3.81 (dd, *J* = 9.5, 8.9 Hz, 1H), 3.68–3.62 (m, 1H), 3.62–3.50 (m, 4H), 3.43–3.35 (m, 3H), 3.31 (dd, *J* = 9.8, 8.9 Hz, 1H), 3.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 165.4, 164.8, 138.8, 138.5, 138.2, 138.2, 138.1, 133.5, 133.2, 133.0, 129.8, 129.7, 129.1, 128.8, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 103.9, 100.8, 98.0, 84.7, 82.1, 81.8, 80.1, 79.7, 77.6, 77.2, 75.5, 74.9, 74.8, 74.6, 73.5, 73.3, 73.0, 71.9, 70.0, 69.4, 68.6, 67.9, 55.0; HRMS (MALDI-TOF) *m/z* $C_{89}H_{88}O_{19}Na$ (M +Na+) 1483.547.

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3. Novel Synthesis Methods

3.1 Glycosylation by decarboxylative condensation

3.1.1 Introduction

Chemoselective ligation reactions make possible covalent formation between two fragments which contain unprotected functional groups. The current standard reactions for joining two peptide segments together to create a longer native peptide have been the native chemical ligation (NCL)[1,2] and more recently the application of Staudinger reaction in the form of the traceless Staudinger ligation.[3-5] In the pursuit of more general chemistry for peptide synthesis, amide ligation by decarboxylative condensation between a hydroxylamine and a peptide bearing an α-keto acid moiety at C-terminus was published (Fig. 3.1.1.1).[6-8] This process proceeds in polar solvents, requires no reagents or catalysis, produces only water and carbon dioxide as byproducts, and readily tolerates unprotected functional groups. This reaction involves the decarboxylation and elimination of water from a hemiaminal. The hemiaminal is formed via a nitrone intermediate between the hydroxylamine and the a-ketoacid. This reaction should be useful for diverse applications that require the coupling of unprotected molecules.[9-10] Based on this concept, we were inspired to ask whether similar reactivity could be utilized for glycosylation reaction, especially in synthesis of β-mannoside which are notoriously difficult to occur.

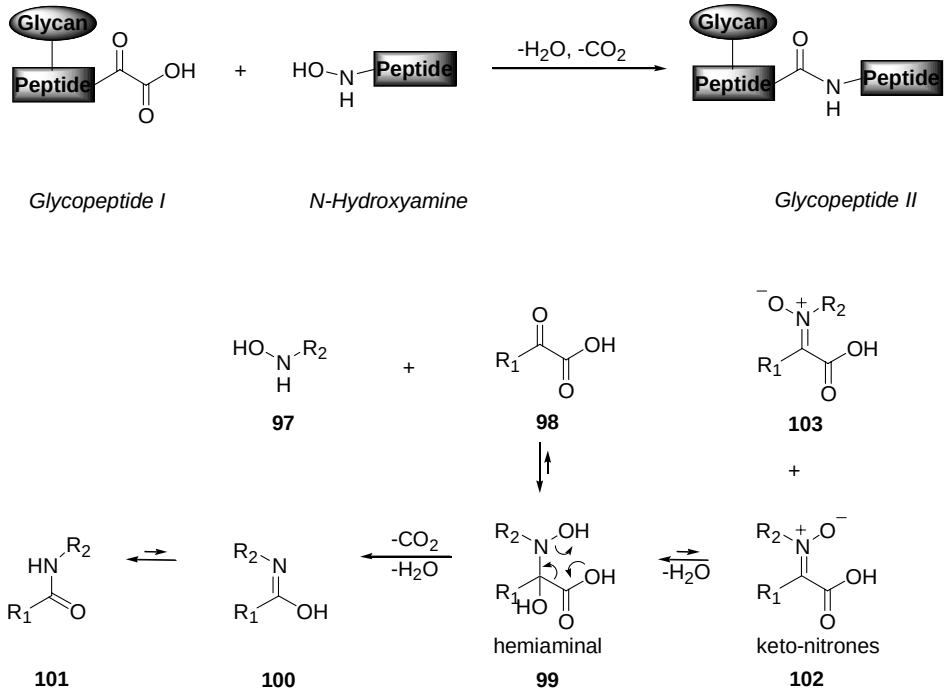


Figure 3.1.1.1 General concept for decarboxylative condensation.

3.1.2 Results and discussion

The *β*-*O*-mannopyranosidic bond, as present in the common core pentasaccharide of the *N*-linked glycoproteins, in various mannans and glycosphingolipids and in lipopolysaccharides, is the most difficult type of glycosidic linkage with which nature has challenged the synthetic chemist.[11-15] Despite these efforts, the stereoselective synthesis of *β*-mannosides is recognized as one of the most challenging problems in carbohydrate chemistry,[16,17] because both the anomeric effect and the steric repulsion between a nonparticipating group disposed axially at C-2 and an incoming alcohol uniformly favor the formation of *α*-mannosidic linkages. Several diverse and innovative strategies for *β*-mannopyranosylation have been developed,[18-25] and Crich and co-workers have made a significant breakthrough in *β*-mannoside synthesis by employing 4,6-*O*-benzylidene mannopyranosyl sulfoxides or thiomannopyranosides as glycosyl donors.[26-32] The effect of the 4,6-*O*-acetal group on the construction of *β*-mannosyl linkages was further confirmed with other mannosyl donors.[33] Direct *β*-mannosylation reaction, a variation on Kahne's sulfoxide method,[34,35] involved the low temperature activation of a 4,6-*O*-benzylidene-protected mannopyranosyl sulfoxide, bearing additional nonparticipating protecting groups at O2 and O3, with triflic anhydride at low temperature to give the corresponding *α*-mannosyl triflate. Subsequent addition of the acceptor alcohol results in an S_N2-like displacement of the triflate, with selective formation of the *β*-mannoside (Fig. 3.1.1.2). The reaction is typically conducted in the presence of a hindered base such as 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) or 2,4,6-tri-tert-butylpyrimidine (TTBP), which is our present choice owing to its ready availability and crystalline, nonhygroscopic nature.[36]

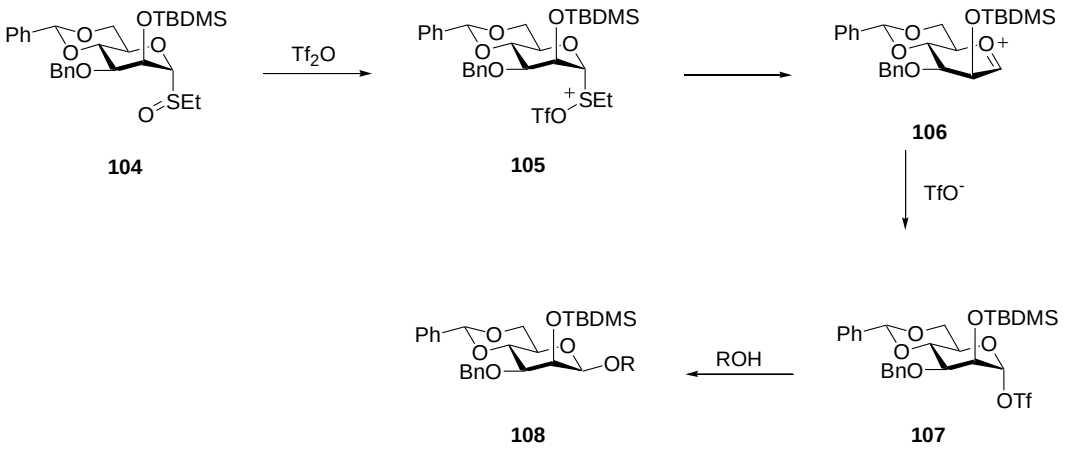


Figure 3.1.1.2 Plausible mechanism of glycosylation for *β*-mannoside.

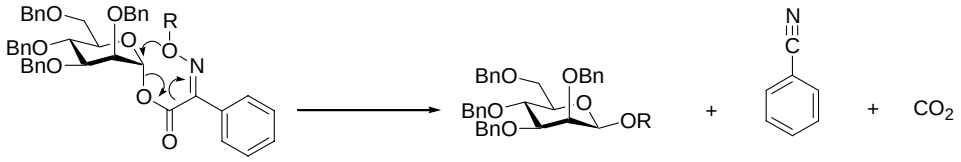


Figure 3.1.1.3 Envisioned mechanism of decarboxylative glycosylation.

We wanted to study whether decarboxylative condensation could be used for glycosylation reaction, especially in synthesis of *β*-mannoside which are notoriously difficult to occur. Benzoylformic tetrabenzylmannoside was readily prepared from the corresponding 1-hydroxy sugar[37] by simple treatment with commercially available benzoylformic acid in the presence of 1,3-diisopropylcarbodiimide (DIC) and a catalytic amount of 4-dimethylamino-pyridine (DMAP). In the presence of the acid catalyst, benzoylformic tetrabenzylmannoside was reacted with available benzylhydroxy amine to afford the mannoside derivative for the carbohydrate intramolecular decarboxylative condensation (Fig. 3.1.1.4). Since the stereochemistry of the oxime was not firmly established, we reasoned that an acid catalyst could promote the isomerization to provide the equilibrium of the desired Z-oxime.

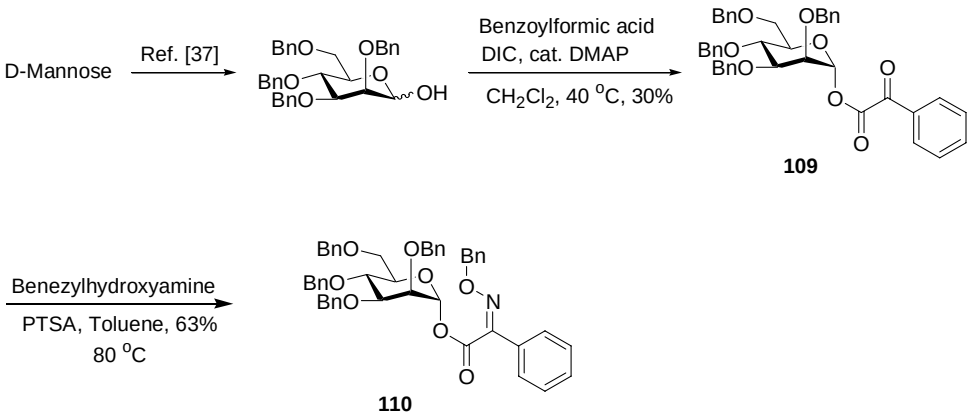


Figure 3.1.1.4 General concept for decarboxylative condensation.

We initially examined the carbohydrate intramolecular decarboxylative condensation of mannoside derivative with various conditions (Fig. 3.1.1.5). While at high temperature in the presence of an acid, only the *α*-glycosylation product was obtained, suggesting the reaction was not intramolecular. Unfortunately, we did not find any *β*-mannoside product through decarboxylative condensation in the presence of triflic acid.

Entry	Catalyst	Solvent	α/β	Yield (%)
1		DMF 80^{o}C		No reaction
2	PTSA	DMF 80^{o}C		No reaction
3		Toluene 100^{o}C		No reaction
4	PTSA	Toluene 100^{o}C	α	70
5	PTSA	CH_3CN 85^{o}C	α	66
6	PTSA	Toluene 100^{o}C/i-PrOH	α	68
7	TfOH	CH_2Cl_2 0^{o}C		unknown

Figure 3.1.1.5 Carbohydrate intramolecular decarboxylative condensation.

3.2 Organocatalysis

3.2.1 Introduction

The direct intermolecular aldol reaction between two carbonyl compounds is central to sugar metabolism. Class I aldolases catalyze this process by using an enamine mechanism.[38] Proline is an abundant chiral molecule with bifunctional group including a carboxylic acid and an amine portion. These two functional groups can both act as acid or base and can also facilitate chemical transformations in concert, similar to enzymatic catalysis. In addition, Proline is a chiral bidentate ligand that can form catalytically active metal complexes (Fig. 3.2.1.1).[39] The most important difference to other amino acids is proline`s effective aminocatalysis that facilitates iminium and enamine based transformation. The asymmetric proline-catalyzed intramolecular aldol cyclization, also termed the Hajos-Parrish-Eder-Sauer-Wiechert reaction, has been applied to several substrates since its invention over 30 years ago.[40] The Hajos-Parrish-Eder-Sauer-Wiechert reaction has not only been used in steroid synthesis but also in other natural product synthesis.[41]

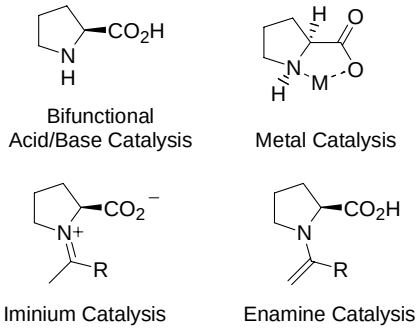


Figure 3.2.1.1 Models of action in Proline-catalysis.

List and coworkers reported that the amino acid Proline is an effective asymmetric catalyst for the direct aldol reaction between unmodified acetone and a variety of aldehydes.[42] The reaction of acetone with 4-nitrobenzaldehyde reacted with Proline in DMSO at room temperature for 4 h to obtain aldol product (Fig. 3.2.1.2).

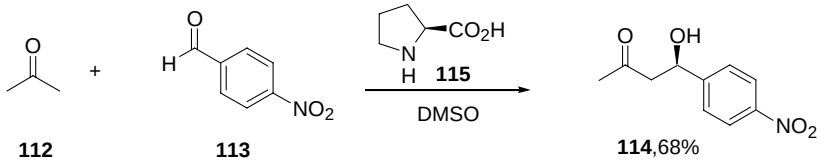


Figure 3.2.1.2 Models of action in proline-catalysis.

Proposed enamine mechanism of the Proline-catalyzed asymmetric aldol reaction was presented (Fig. 3.2.1.3). This catalyst may facilitate each individual step of the mechanism, including the nucleophilic attack of the amino group (a), the dehydration of the carbinol amine intermediate (b), the deprotonation of the iminium species (c), the carbon-carbon bond forming step (d), and both steps of the hydrolysis of the iminium-aldol intermediate (e and f).

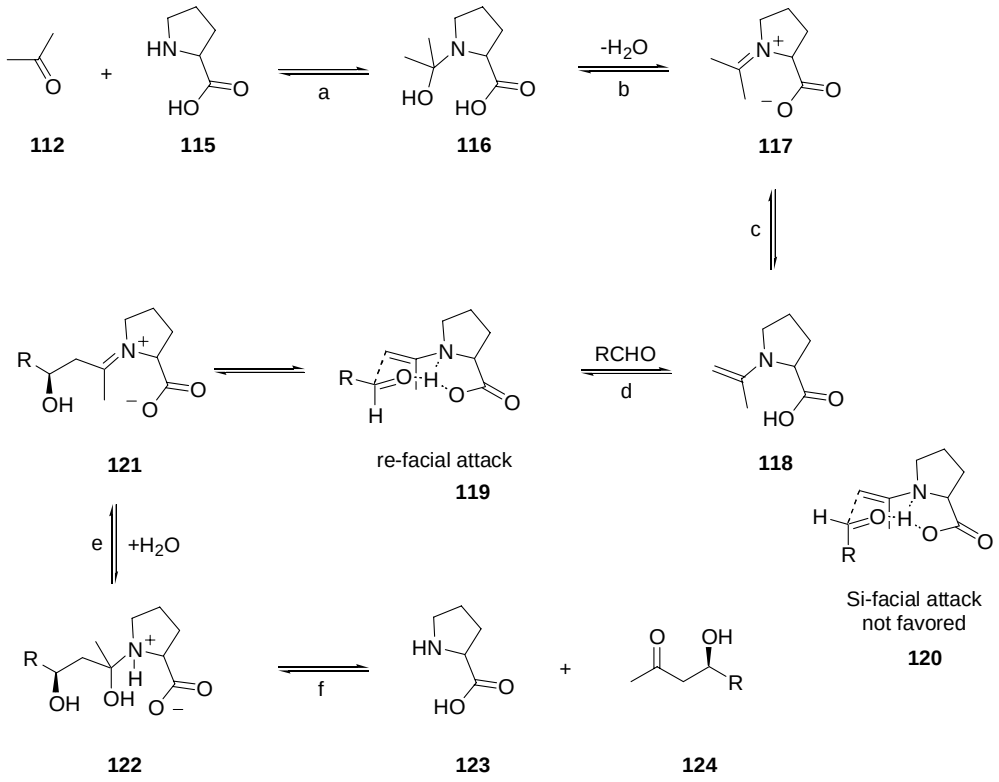


Figure 3.2.1.3 Proposed mechanism of the Proline-catalyzed asymmetric aldol reaction.

Hexose carbohydrates play important roles in biological process. However, the study of this fundamental class of biomolecular has been hindered by the paucity of chemical methods for the efficient synthesis and coupling of hexose system to form polysaccharides and other derivatives. Chemists have focused on using iterative alcohol protection-deprotection strategies, an approach that requires tedious and laborious steps.[43,44] MacMillan and coworkers developed two-step synthesis of carbohydrate by selective aldol reactions.[45] The dimerization of α-oxyaldehydes, catalyzed by L-Proline, is then followed by a tandem Mukaiyama aldol addition cyclization step catalyzed by a Lewis acid. Differentially protected glucose, allose, and mannose stereoisomers can each be selected, in high yield and stereochemical

purity, simply by changing the solvent and Lewis acid used (Fig. 3.2.1.4). Upon this concept, we investigated whether this strategy could be adopted to synthesize oligosaccharides by the condensation of glycosylated α-oxyaldehydes. Our particular interest was the apparent suitability of this technology to access C- or O- glycosides which can be laborious to access from native glycosides.

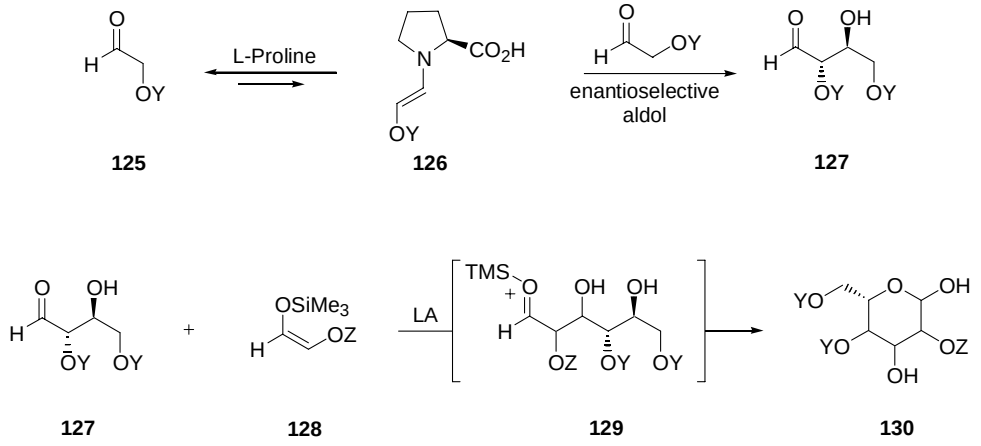


Figure 3.2.1.4 Two-step synthesis of carbohydrate.

3.2.2 Results and discussion

The synthesis of compound **136** began with D-mannose as shown in (Fig. 3.2.2.1). After the acetylation, *C*-glycosylation[46] of D-mannose pentaacetate with allyltrimethylsilane in the presence of $BF_3{\cdot}Et_2O$ and TMSOTf in CH_3CN at 0 ^oC afforded the *C*-glycoside **133** in good yield. Deacetylation of this product with NaOMe in MeOH gave tetraol **134** (anomeric mixture). After the benzylation, this mixture was separated through silica gel chromatograph purification to obtain *α*-glycoside **135.** The conversion of the alkene to the alcohol by the hydroboration-oxidation was accomplished in two steps. *C*-mannosyl aldehyde **137** was obtained through the swern oxidation. The synthesis of *O*-mannosyl aldehyde began with D-mannose pentaacetate. After the allylation, deacetylation, benzylation and ozonolysis, the *O*-mannosyl aldehyde was obtained **141** (Fig. 3.2.2.2).

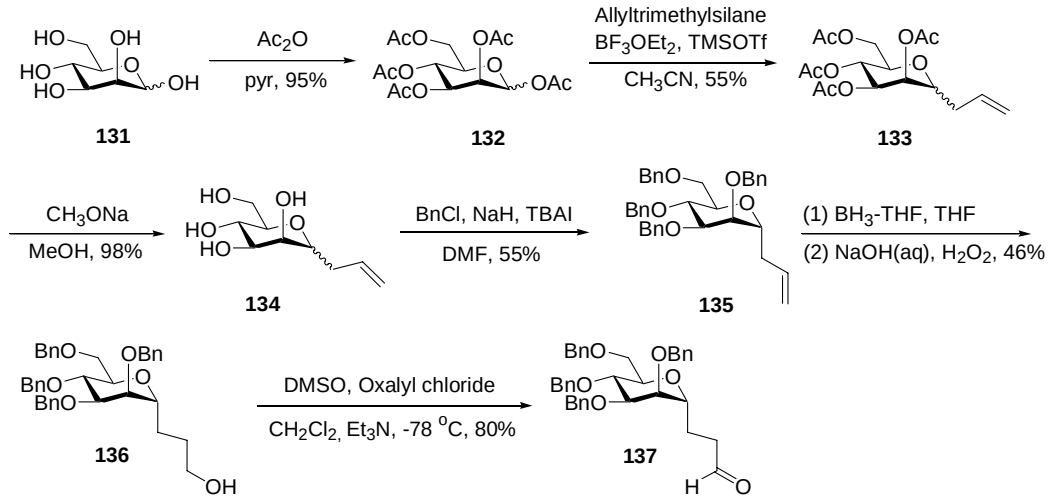


Figure 3.2.2.1 Synthesis of *C*-mannosyl aldehyde.

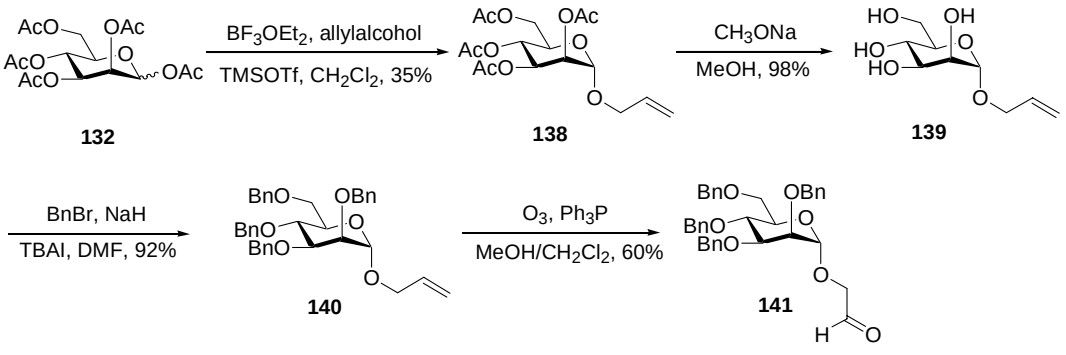
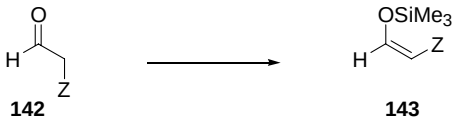


Figure 3.2.2.2 Synthesis of *O*-mannosyl aldehyde.

With the *O*- and *C*-mannosyl aldehydes in hand, we investigated the silyl enol formation (Fig. 3.2.2.3). Various silanes and bases were utilized to prepare mannosyl *α*-oxyenolsilane. We found that the crude mannosyl *α*-oxyenolsilane was easy to decompose. Hexose carbohydrate was obtained only in the presence of acetyl *α*-oxyenolsilane and the dimerization of *α*-oxyaldehydes catalyzed by Lewis acid. In order to synthesize mannosyl *α*-oxyenolsilane, we also employed the olefin cross-metathesis by using Grubb catalyst **147**.[47,48] Tetra-*O*-benzyl-1-α-*C*-allylmannoside and tetra-*O*-acetyl-1-*C*-allylmannoside were utilized to the silyl enol formation with vinyltrimethylsilane and trimethyl (vinyloxy)silane in the presence of Grubbs catalyst **147**. Various concentrations of catalyst **147** and large excess amount of vinyltrimethylsilane were explored in the olefin cross-metathesis reaction (Fig. 3.2.2.4). Homo-dimer of tetra-*O*-benzyl-1-α-*C*-allylmannoside **146** and starting material **144** were obtained. In the presence of trimethyl (vinyloxy) silane with benzyl-1-*C*-allylmannoside or acetyl-1-*C*-allylmannoside, only starting material was found in the reaction.



142 **143**

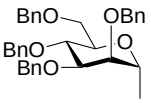
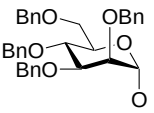
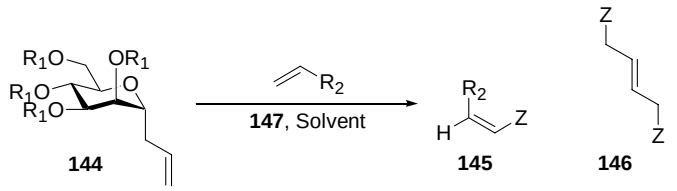
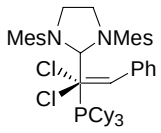
Entry	Z	Reagent	Solvent	Yield (%)
1		TMSCl/Et_3N	CH_3CN/ rt	-
2		TMSCl/Et_3N	DMF/ 100^oC	-
3		TMSCl/BuLi/DIPEA	THF/ rt	Crude
4		TBDMSCl/BuLi/DIPEA	THF/ rt	-
5		TIPSCl/BuLi/DIPEA	THF/ rt	-
6	OAc	TMSCl/Et_3N	CH_3CN/ rt	50%
7		TMSCl/BuLi/DIPEA	THF/ rt	Crude
8		TMSCl/BuLi/DIPEA	THF/ rt	Crude

Figure 3.2.2.3 Synthesis of *α*-oxyenolsilane.

After the study of the mannosyl *α*-oxyenolsilane, we focused on organocatalytic sugar aldehydrate dimerization **149**. Unfortunately, only unknown product and elimination product were obtained in the organocatalytic sugar aldehydrate dimerization (Fig. 3.2.2.5). We found that the *O*- and *C*-mannosyl aldehyde were difficult to the silyl enol formation and *α*-oxyaldehyde dimerization. We tried to pay our attention to *α*-1,2 *C*-linkage glycoside **150** (Fig. 3.2.2.6).

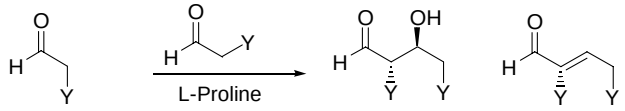


Entry	Z= **144** R_1	R_2	**147**	Time	Solvent	Product **144/145/146**
1	Bn	TMS(1.5 eq)	10 mol%	24 h	CH_2Cl_2/ 40°C	20/1/29
2	Bn	TMS(15 eq)	10 mol%	12 h	CH_2Cl_2/ 40°C	26/1/20
3	Bn	TMS(15 eq)	50 mol%	12 h	CH_2Cl_2/ 40°C	23/2/13
4	Bn	TMS(20 eq)	20 mol%	0.5 h	CH_2Cl_2/ 120°C(M)	10/2/3
5	Bn	OTMS(2 eq)	10 mol%	24 h	CH_2Cl_2/ 40°C	**144**
6	Ac	OTMS(2 eq)	10 mol%	24 h	CH_2Cl_2/ 40°C	**144**
7	Ac	OTMS(2 eq)	10 mol%	0.5 h	CH_2Cl_2/ 100°C(M)	**144**



Grubb catalyst **147**

Figure 3.2.2.4 Synthesis of *α*-oxyenolsilane through cross-metathesis.



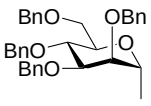
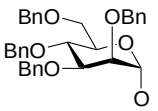
Entry	Y	Solvent	Yield (%)
1	OTIPS	DMSO/ 0°C	70%
2		DMSO/ 0°C	Crude
3		DMF/ 0°C	Crude
4		DMSO/ 0°C	Crude
5		DMF/ 0°C	Elimination
6		Dioxane/ 0°C	Elimination
7		Toluene/ 0°C	Elimination

Figure 3.2.2.5 Synthesis of *α*-oxyaldehyde dimerization.

The retrosyntheisis of *α*-1,2 *C*-linkage glycoside is shown in (Fig. 3.2.2.6). Compound **150** will be obtained through proline organocatalysis in the presence of *C*-mannosyl

aldehyde **152** and *α*-oxyaldehyde dimerization **153**, followed by cyclization. There are two other possible synthetic pathways for the synthesis of Compound **151**. One of them is sugar allyboronate **155** in the presence of aldehyde **153**.[49-51] Another one is Palladium-catalyzed coupling of sugar allyl acetates **156** with aldehyde **153** in the presence of Bis(pinacolato)diboron,[52,53] followed by ozonolysis.

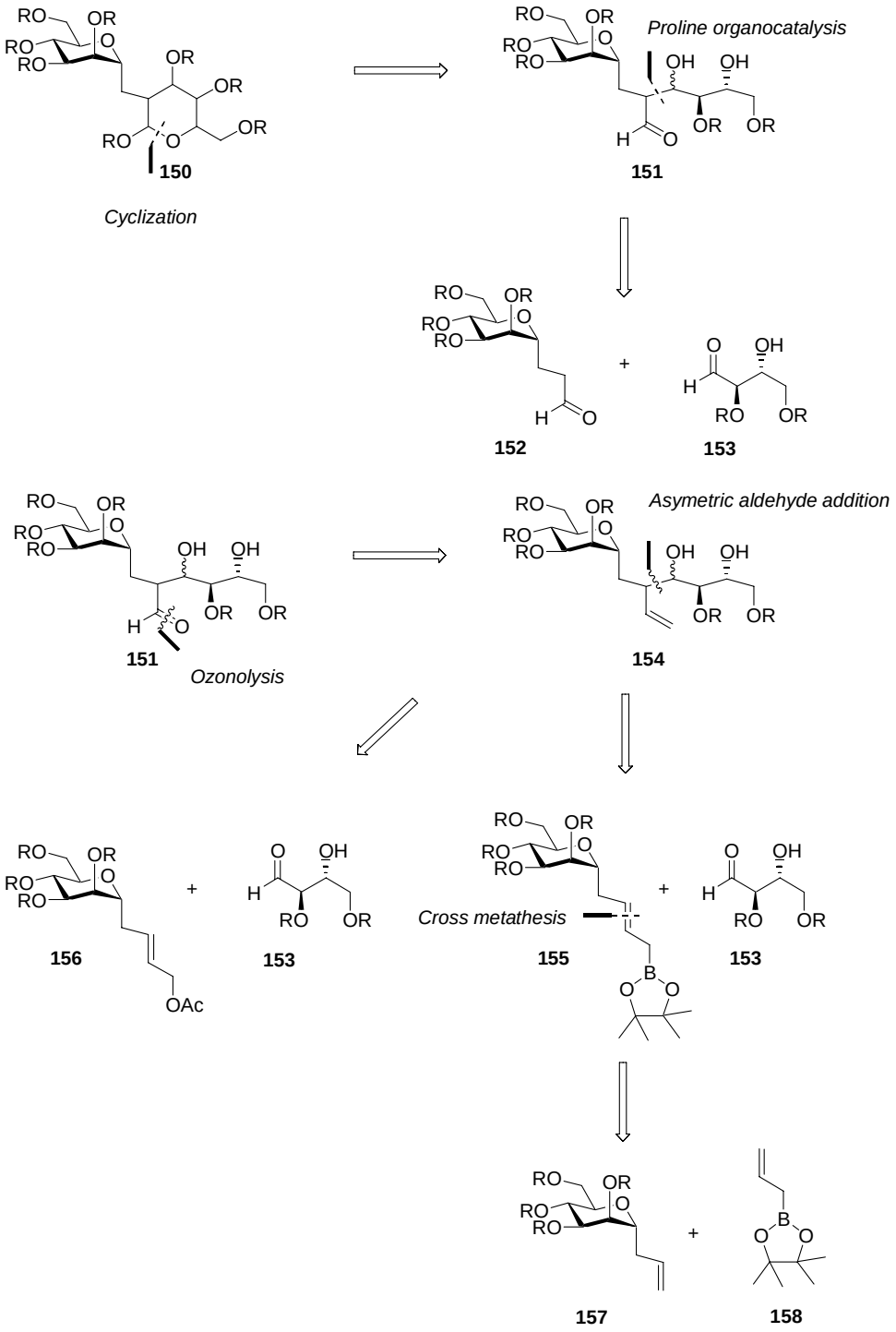
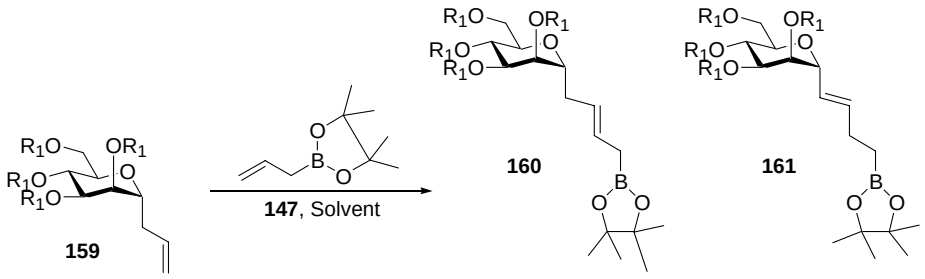


Figure 3.2.2.6 Retrosynthesis of *α*-1,2 *C*-linkage glycoside.

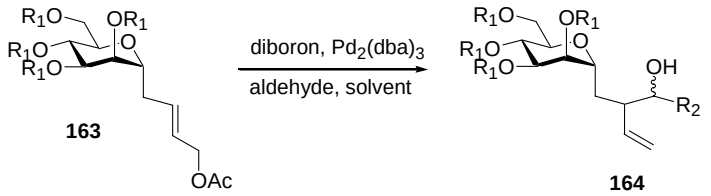
The study of synthesis of sugar allylboronate through cross-metathesis is shown in (Fig. 3.2.2.7). Only the isomerization product **161** was obtained in the cross-metathesis reaction of the different protected allylglycosides with allylboronate. Palladium-catalyzed coupling of sugar allyl acetates **163** with aldehyde in the presence of Bis(pinacolato)diboron was also investigated (Fig. 3.2.2.8). Only the nitrobenzaldehyde in the presence of sugar allyl acetates **163** with Bis(pinacolato)diboron and Palladium catalyst is effective. Proline organocatalysis in the presence of *C*-mannosyl aldehyde **152** and *α*-oxyaldehyde dimerization compound **153** was investigated. The product **151** is difficult to identify by NMR spectrum. After the silyl group deprotection and cyclization in the presence of acetic anhydride and pyridine, the estimated product *α*-1,2 *C*-linkage glycoside **150** was obtained through mass identification.

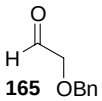
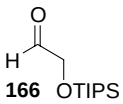
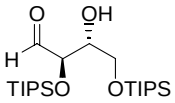
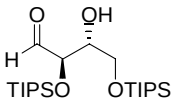
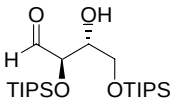
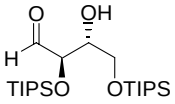


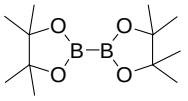
Entry	R_1	**147**	Time	Solvent	Product
1	Ac	3 mol%	24 h	CH_2Cl_2/ 40°C	**161**
2	Ac	5 mol%	0.5 h (M)	CH_2Cl_2/ 100°C	**161**
3	Ac (β-glucose)	5 mol%	24 h	CH_2Cl_2/ 40°C	**161**
4	Ac (β-glucose)	5 mol%	0.5 h (M)	CH_2Cl_2/ 100°C	**161**
5	Me	25 mol%	24 h	CH_2Cl_2/ 40°C	**161**
6	AcO	10 mol%	12 h	CH_2Cl_2/ 40°C	**162**
7	Me	10 mol% p-benzoquinone (10%)	24 h	CH_2Cl_2/ 40°C	**161**

162

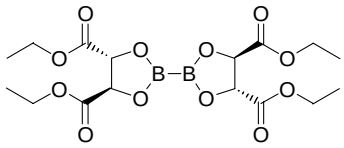
Figure 3.2.2.7 Synthesis of sugar allylboronate through cross-metathesis.



Entry	R_1	R_2	Solvent	Yield (%)
1	Bn	 **165**	DMSO/ 60°C	**163**
2	Bn	 **166**	DMSO/ 60°C	**163**
3	Bn	 **167**	DMSO/ 60°C	**163**
4	Bn	Nitrobenzaldehyde **168**	DMSO/ 60°C	55%
5	Me	 **167**	DMSO/ 60°C	**163**
6	Bn	 **167**	DMSO/ 60°C chiral diboron	**163**
7	Bn	 **167**	DMSO/Toluene/ 60°C chiral diboron	**163**
8	Bn	Nitrobenzaldehyde **168**	DMSO/Toluene/ 60°C chiral diboron	**163**



diboron



chiral diboron

Figure 3.2.2.8 Palladium-catalyzed coupling of sugar allyl acetates with aldehyde.

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4. Functional Carbohydrate Oligomers: Chemical Modification of Carbohydrate for Conjugation

4.1 Introduction

4.1.1 Receptor binding mechanisms of multivalent carbohydrate ligands

Carbohydrate binding component can possess multiple binding sites or it can be present in multiple copies at the cell membrane or distributed along a glycoprotein. Multivalent protein carbohydrate interactions mediate many important processes. Multivalent ligands with various architectures have been developed as effectors or inhititors of biological processes. To optimize the ligand activity, efforts to investigate how multivalent ligand structure influences binding are critical. Understanding the protein carbohydrate interaction binding mechanisms should result in significant advance in therapeutic agents.[1]

Various scaffolds of natural, synthetic, and semisynthetic multivalent ligands have been used to investigate receptor function.[2-4] These scaffolds are difference in size, shape, and physical characteristics. For example, protein and dendrimer are globular scaffolds and may effectively occupy multiple binding sites on an oligomeric receptor. The architecture of nature ligands is diverse. It is very useful to investigate the structure complexity of nature multiple ligands to understand how to design the synthetic multivalent ligands.

4.1.2 Multivalent carbohydrate ligands as inhibitors

Many examples of the use of multivalent ligands as potent inhibitors of biological functions are presented.[5-15] The following example illustrates the use of synthetic design strategies to develop potent inhibitors of protein carbohydrate interactions. The family of AB_5 bacterial toxins is characterized by a pentagonal arrangement of five B subunits with a single A subunit in the center.[16] The doughnut shape of the B pentamer presents a symmetrical display of five identical carbohydrate binding sites on a single face. Kitov and coworkers have designed starfish-like multivalent ligands with dramatic enhancements in inhibitor potency for the Shiga-like toxin.[7] Shiga-like toxin cause gastrointestinal diseases after entering mammalian cells through multivalent binding of the B subunit to the saccharide portion of glycolipids on the cell surface. From X-ray crystallographic data,[17] two binding sites were observed to be separated by 10 Å. Two methods were pursued to generate ligands capable of blocking binding of Shiga-like toxin. In the beginning they focused on the synthesis of the trisaccharide P^k **169**. The linker connecting these saccharides **170** was designed to span the 10 Å to place the saccharides in both binding sites (Fig. 4.1.2.1). When dimer of the trisaccharide P^k **170** was used for the Shiga-like toxin inhibition, it was only 40 fold more effective than trisaccharide P^k alone. This increase in activity was not sufficient to block Shiga-like toxin activity. The ligand design was modified in order to increase the binding activity of the multivalent ligands. The dimers were attached to a central pentavalent scaffold. The decavalent molecule **171** could occupy the two carbohydrate binding sites on each of the five B Subunits of Shiga-like toxin. Decavalent carbohydrate ligand **171** showed million-fold increase in activity as an inhibitor of Shiga-like toxin than the P^k trisaccharide alone.

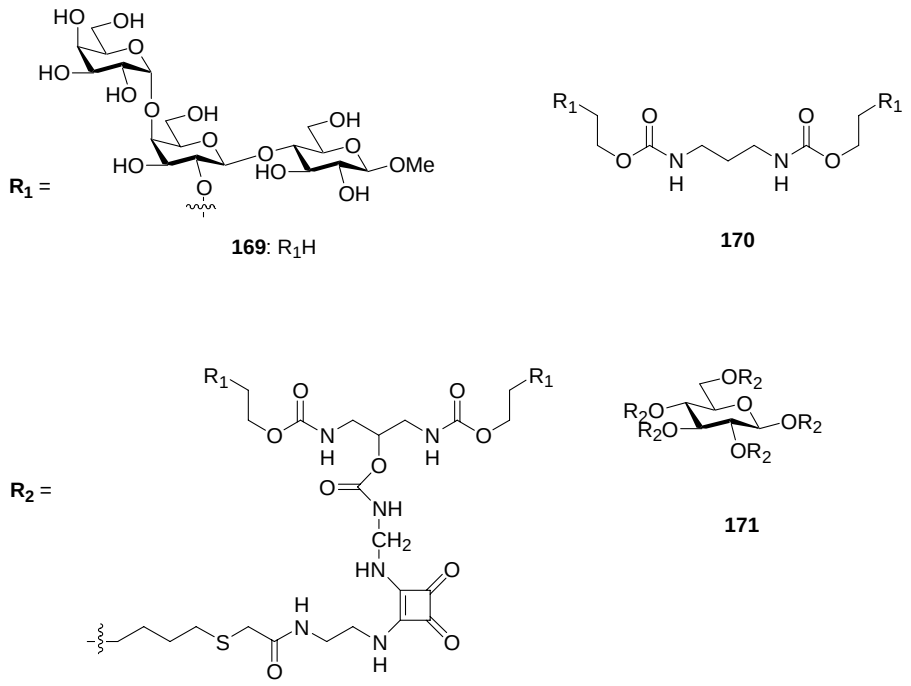


Figure 4.1.2.1 Potent shiga-like toxin inhibitor.

Cell-cell interaction mediated by protein-carbohydrate interactions are very important for many biological processes.[18,19] The galabiose (Galα1-4Gal) **172** is found as a constituent of glycolipids on the surfaces of mammalian cells of some species. Galabiose serves as an anchor for the attachment of bacteria such as the pathogen *Streptococcus suis*. *S. suis* infections cause meningitis, septicemia, and pneumonia in pigs. They are also known to cause bacterial meningitis in humans. To study the interaction of low molecular weight multivalent carbohydrates to prevent adhesion of *S. suis* to mammalian cells. Magnusson and coworkers synthesized and evaluated a series of multivalent dendrimers containing galabiose.[20] Low molecular weight monovalent, divalent, trivalent and tetravalent galabiosides were synthesized as candidate *S. suis* inhibitors (Fig. 4.1.2.2). The oligovalent ligands were evaluated based on their ability to inhibit the agglutination process over a range of concentrations. The data reveal many potent ligands among the multivalent galabiosides. Tetravalent ligands **181** completely inhibits agglutination at concentrations of 3 nM and 2 nM for the two strains of *S. suis* tested.

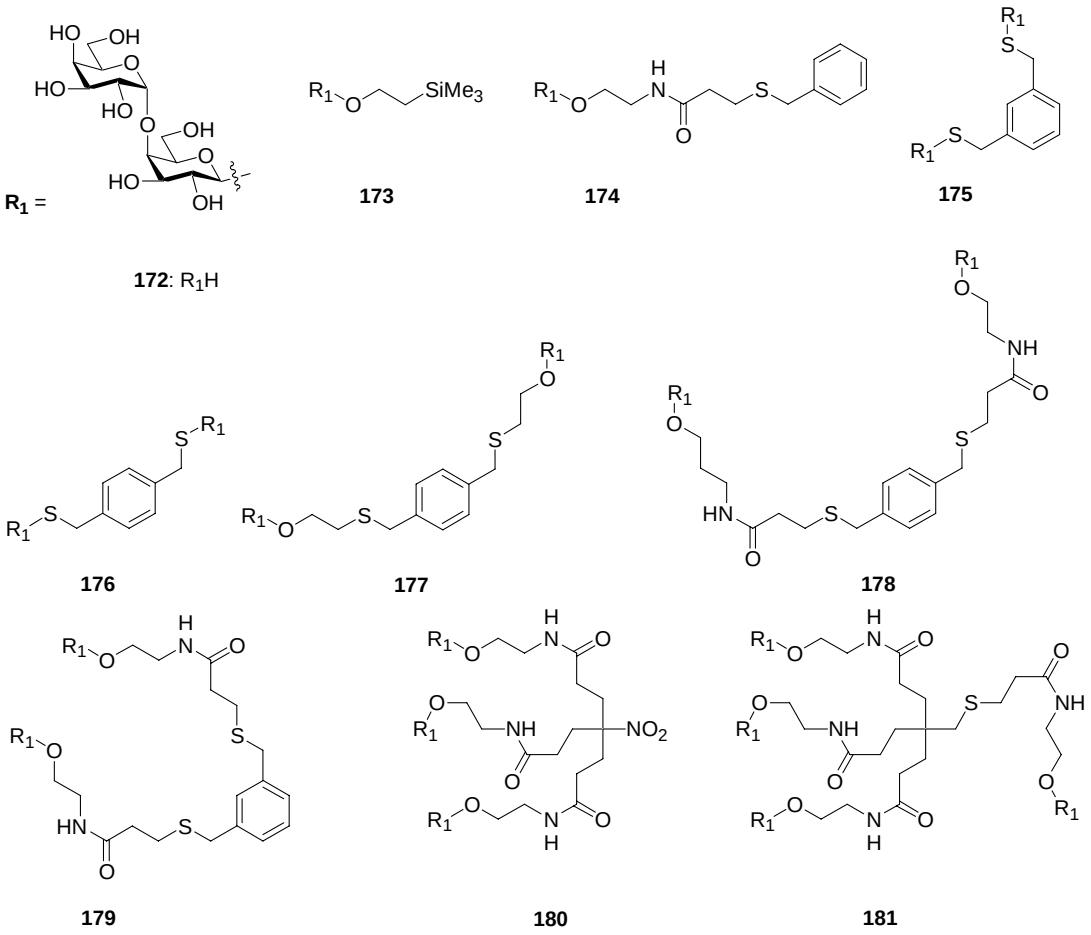


Figure 4.1.2.2 Low molecular weight monovalent, divalent, trivalent and tetravalent galabiosides.

4.1.3 Targeting the carbohydrate on HIV

AIDS is caused by the infection of the human immunodeficiency virus (HIV).[21] HIV infection is a massive global health problem with more than 33 million infected worldwide. There is widespread agreement that the most promising approach to control the ongoing HIV pandemic is through the development of an effective vaccine. [22,23] Human antibody 2G12 neutralizes a broad range of human immunodeficiency virus (HIV) isolates by binding an unusually dense cluster of carbohydrate moietes on the silent face of the gp 120 envelope glycoprotein.[24,25] A successful vaccine approach would try to elicit a immune response of 2G12-like antibodies by exposing the immune system to an effective structural mimic of the 2G12 epitope. However, HIV vaccine design has faced many difficulties, including the lack of an immunogen able to elicit broadly neutralizing antibody 2G12. Crystal structures of Fab 2G12 were shown (Fig. 4.1.3.1).[26] Monomer of Fab 2G12 in the crystal shows that the V_H clearly separates from its normal interaction with the V_L. The light and heavy chains are shown in cyan and red. The monomer does not exist in the crystal. Structure of the two domains within the dimer is assembled in the crystal. Both light chains are shown in cyan, with the heavy chains from Fab 1 and Fab 2 shown in red and purple. The two fabs are arranged side by side with their respective combining sites facing in the same direction and separated by approximately 35 Å.

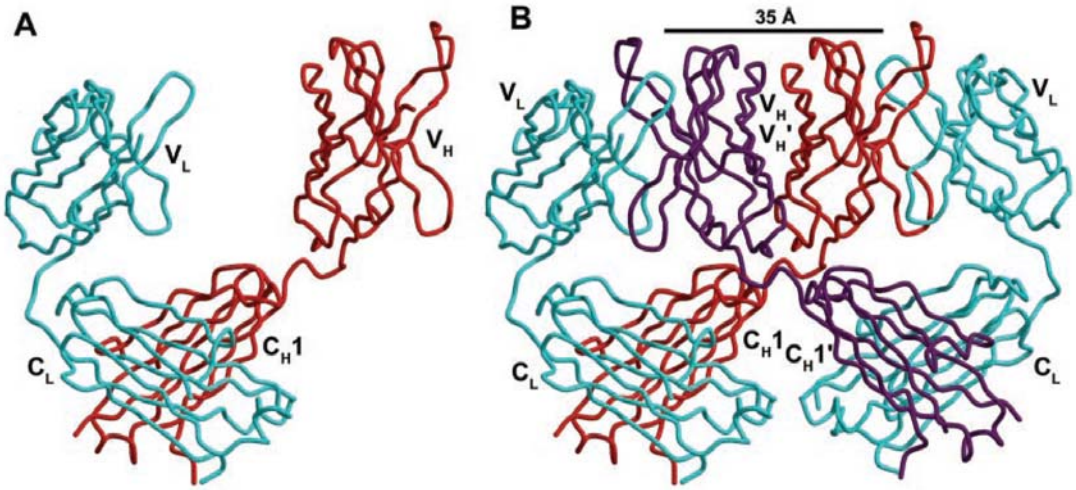
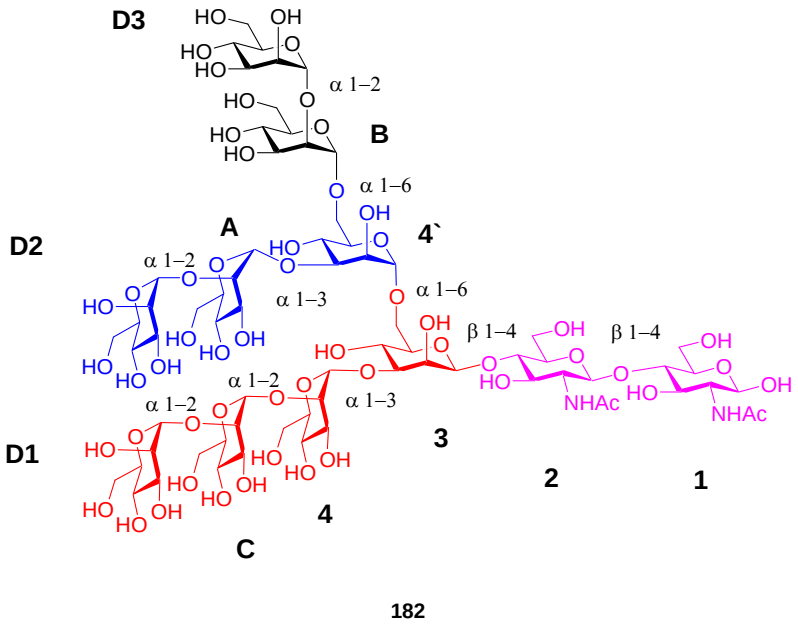


Figure 4.1.3.1 Crystal structure of Fab 2G12.

2G12 recognizes $Man_9GlcNAc_2$ **182** moieities (Fig. 4.1.3.2) covalently attached to gp 120. To explore the binding specificity, the crystal structure of Fab 2G12 with $Man_9GlcNAc_2$ are shown (Fig. 4.1.3.3).[25,27] Red sugars make contacts with Fab

2G12 at the primary binding site (conventional combining pocket), whereas blue sugars contact Fab 2G12 at the secondary binding site (the unusual $V_H/V_H`$ interface). *N*-Acetylglucosamine residues are colored in purple. In the primary combining site of the $Man_9GlcNAc_2$ complex, 2G12 contacts four sugars (3, 4, C, and D1) in the D1 arm, with the terminal Manα1-2Man disaccharide. Manα1-2Man occupies only the two conventional combining site pockets, which are separated by about 35 Å.



182

Figure 4.1.3.2 Chemical structure of $Man_9GlcNAc_2$.

This binding mode suggests that these are higher-affinity sites for the mannose linkage. The specificity of the primary combining site of 2G12 for Manα1-2Man at the tip of the D1 arm of $Man_9GlcNAc_2$ stems from a combination of several factors. First, the primary combining site forms a deep pocket that can only accommodate terminal sugar residue. Second, the highly complementary geometry of the binding sites enables formation of hydrogen bonds specific to Manα1-2Man. Last, the additional interactions with the mannose 3 and mannose 4 sugars provide specificity for the Manα1-2Man linkage at the tip of the D1 arm. The secondary binding site is formed by the $V_H/V_H`$ domain swap, which creates a surface not previously described in antibody structures. The D2 arms of the $Man_9GlcNAc_2$ residues in the crystal interact with this composite $V_H/V_H`$ surface. It can provide two additional sugar-binding sites (Fig. 4.1.3.3). The $V_H/V_H`$ interface interactions are mainly with the central mannose A of the D2 arm and also with 4` sugars. Furthermore, the carbohydrate chain runs parallel to the antibody surface in a relatively shallow binding site. Hence, it is not clear whether this secondary binding site is as restricted

for the D2 arm as compared with the highly specific D1 arm interaction in the primary binding site.

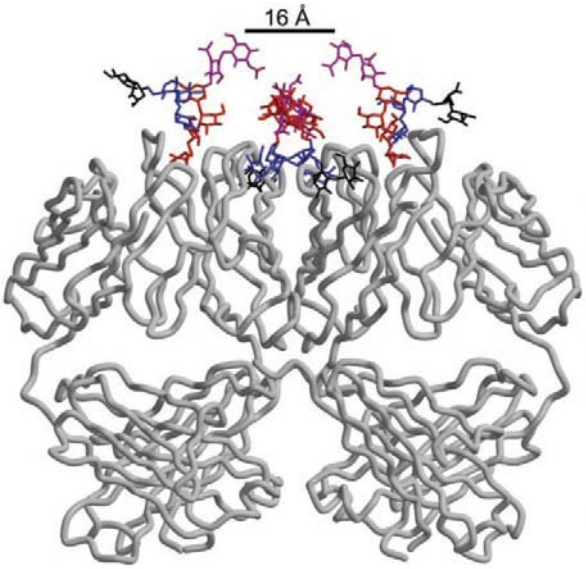


Figure 4.1.3.3 Overall structure of the Fab 2G12 dimer bound to $Man_9GlcNAc_2$.

On the basis of the 2G12 glycan recognition of gp120 model, there are three separate $Man_9GlcNAc_2$ moieties (shown in red) which potential mediate the binding of 2G12 to gp120 (two in the primary combining sites and one in the $V_H/V_H`$ interface) (Fig. 4.1.3.4).[26] The glycans at the primary combination sites originate from $Asn^{332}$ and $Asn^{392}$ in gp120, whereas the carbohydrate located at the $V_H/V_H`$ interface would arise from Asn^{339}. *N*-linked glycans occurring at Asn^{332} and Asn^{392} in gp120 have previously been implicated as critical for 2G12 binding.[25] The *N*-linked glycan occurring at Asn^{339} is not critical for 2G12 binding, although this glycan could potential interact with the $V_H/V_H`$ interface.[26] Immunogens designed to mimic the unique cluster of oligomannose sugars binding to antidody 2G12 can be tested for their ability to elicit a 2G12-like immune response. The 2G12 structure further provides a scaffold for engineering high-affinity antibodies to other molecular clusters in general, not only carbohydrates, as might be found on pathogens and tumor cells, but also other naturally occurring or synthetic clusters.

Based on these crystal structural results, several groups have launched the design of novel immunogens that will elicit 2G12-like antibody.[28-32] The synthesis of high mannose-type oligosaccharides, $Man_9GlcNAc_2$ **182** and its analogues have been extensively explored. [33-51]

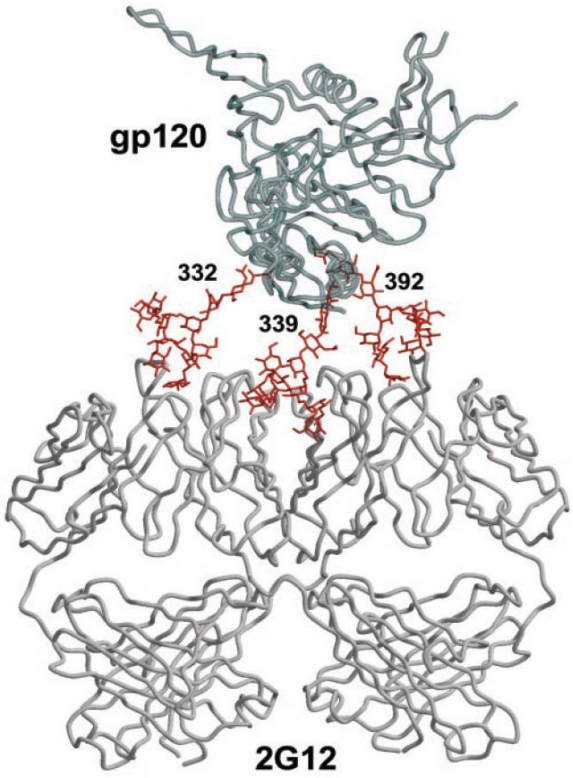


Figure 4.1.3.4 Model of 2G12 glycan recognition of gp 120.

Wong and coworkers have prepared four previously undescribed Manα1-2Man-containing oligomannose derivatives (Man_4 **183**, Man_5 **184**, Man_7 **185**, and Man_8 **186**) (Fig. 4.1.3.5) that compete with gp120 for binding to 2G12 antibody. In the combined studies reveal that 2G12 is capable of binding both the D1 and D3 arms of the $Man_9GlcNAc_2$ **182** moiety, which would provide more flexibility to make the required multivalent interactions between the 2G12 antibody and the gp120 oligomannose cluster.[28] Dendrons of $Man_9GlcNAc_2$ **182** moiety have also been shown to bind 2G12.[32]

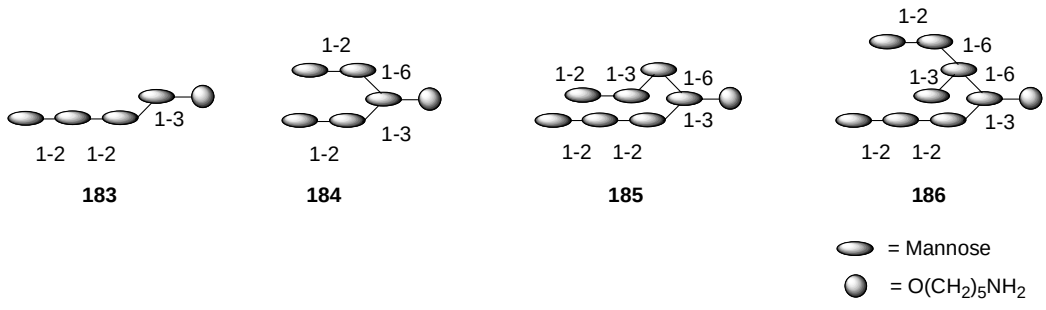


Figure 4.1.3.5 Chemical structure of oligomannose derivatives.

Danishefsky and coworkers also have been involved for some years in the synthesis and study of carbohydrate-based HIV antigen design.[29,31,39,41]

4.1.4 Glycosyl thiols and glycoconjugate

Naturally occurring glycopeptides most commonly incorporate an *O*- or *N*-glycosidic linkage between the saccharide moiety and the side chain of an appropriate amino acid residue. Replacement of the anomeric oxygen or nitrogen atom by sulfur results in the corresponding S-linked glycopeptide, which is known to be more stable chemically,[52] more resistant to the action of glycosidases, and tolerated by most biological systems. Furthermore, members of the closely related S-linked oligosaccharides have been used as enzyme inhibitors[53,54] and are suggested to be better immunogens than their natural *O*-linked analogues.[55,56] An alternate modification is the *C*-glycosyl linkage, for which several synthetic methods exist.[57] Recently, glycosyl thiols have become useful building blocks for the synthesis of certain glycoconjugates that may be considered to be analogues of glycopeptides and glycoproteins.[58–62] The common synthetic approach for S-linked glycosyl amino acids uses an anomeric thiolate nucleophile in reaction with an alanine derivative equipped with a leaving group (Fig. 4.1.4.1). The major side reaction is *β*-elimination **209**, and the subsequent Michael addition results in a diastereomeric mixture at the *α*-carbon of the amino acid product **211**. Several procedures have emerged to overcome this problem. Their use has allowed specific glycosylation of peptides to form *S*-linked glycopeptides through alkylation[63-66] or conjugate-addition strategies.[67,68]

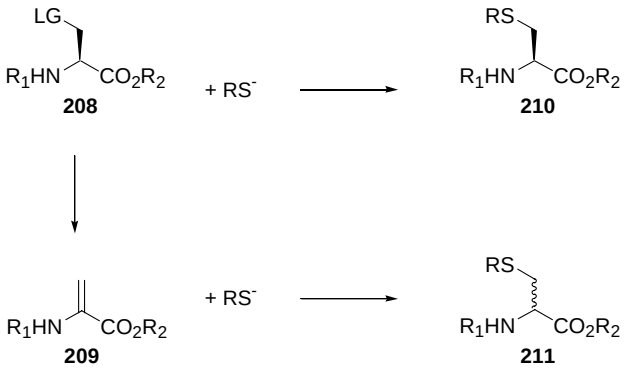


Figure 4.1.4.1 The common synthetic approach used for the synthesis of S-linked glycosyl amino acids.

The most frequently employed method for the synthesis of glycosyl thiols **215** involves the treatment of glycosyl halides **212** with a sulphur nucleophile (either thiourea[69,70] or potassium thioacetate) in acetone,[71] followed by mild hydrolysis (Fig. 4.1.4.2).[72,73] However, this procedure requires special precautions and generates

a complex mixture of products. The step for the generation of glycosyl thiols requires a special precaution, as it may give low yields due to the facile formation of disulfides during deacetylation of the sulfur atom and subsequent silica gel chromatography.

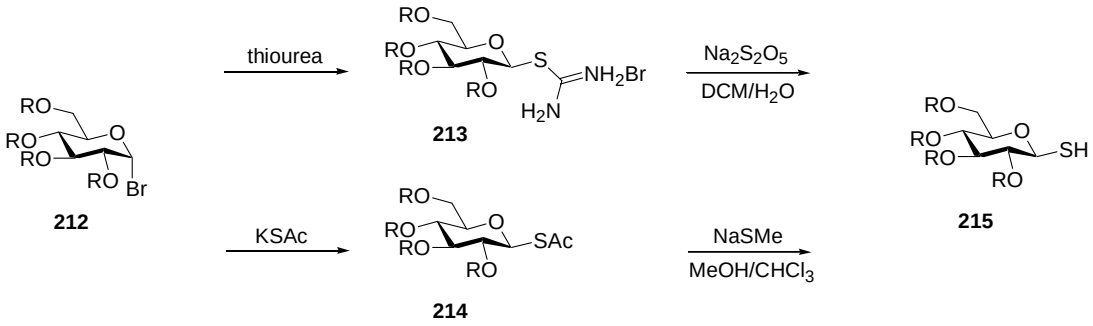


Figure 4.1.4.2 The common synthetic approaches for the synthesis of glycosyl thiols.

Davis and coworkers considered that a direct method of thiol formation in combination with protein[62] would allow a one-pot protein glycosylation method that could utilize sugars directly isolated from natural sources.[74] With the goal of finding a more efficient strategy for the direct synthesis of glycosyl thiols, Davis and coworkers speculated a reagent that operated through a concerted Lewis acid activation and displacement might allow chemo- and regioselective thionation of C1. However, to date, no such reagent or method exists. Such a C1-selective thionation might be considered mechanistically in two pathways (Fig. 4.1.4.3).[75]

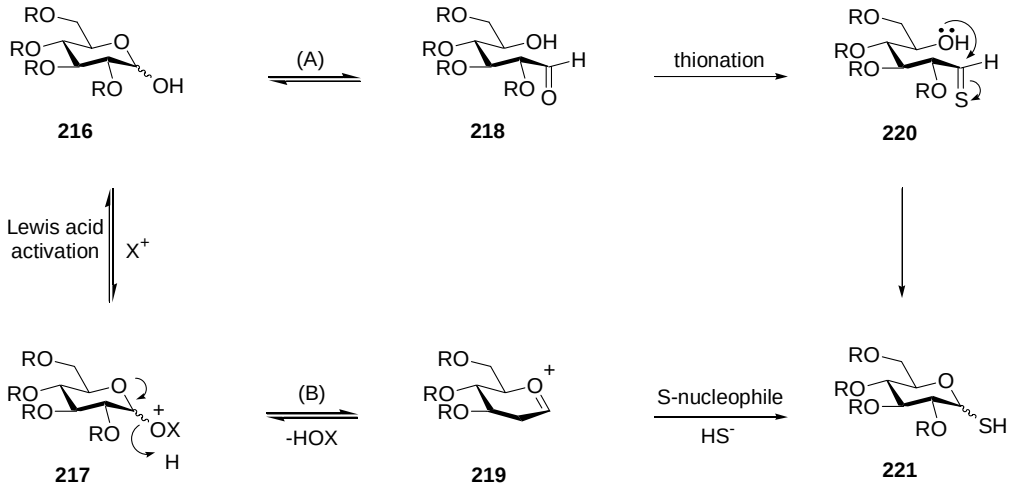


Figure 4.1.4.3 Two possible mechanisms for the direct formation of glycosyl thiols from reducing sugars through path (A) open chain or path (B) an oxacarbenium ion.

The mechanism of Lawesson's reagent[76,77] (LR) (Fig. 4.1.4.4) suggests that it might serve either pathway, acting potentially as both an oxaphilic electrophile and a sulphur source. This reagent has been extensively used for the efficient conversion of a wide variety of carbonyl functions into their corresponding thiocarbonyl functionalities.[78,79] Moreover, an earlier report had highlighted a rare single use of LR in the conversion of a benzylic alcohol into the corresponding thiol,[80,81] thereby suggesting potential utility in S_N1 or S_N1-like pathways. Therefore, owing to the enhanced reactivity of the anomeric hydroxy group in S_N1-like processes, it was thought that this procedure might proceed in an analogous fashion with sugar substrates. This approach appeared attractive for several reasons: (1) allowing direct formation of the anomeric thiosugars in one step from the corresponding anomeric alcohols; (2) application to differently protected and to unprotected sugars and (3) the possibility for direct site-selective glycosylation of proteins with sugars isolated from natural sources in just two steps and in one pot. Lawesson's reagent may be used in a direct and general manner for the preparation of glycosyl thiols from the corresponding anomeric lactols. Notably, this procedure has also been shown to be fully compatible with unprotected sugars (Fig. 4.1.4.4).

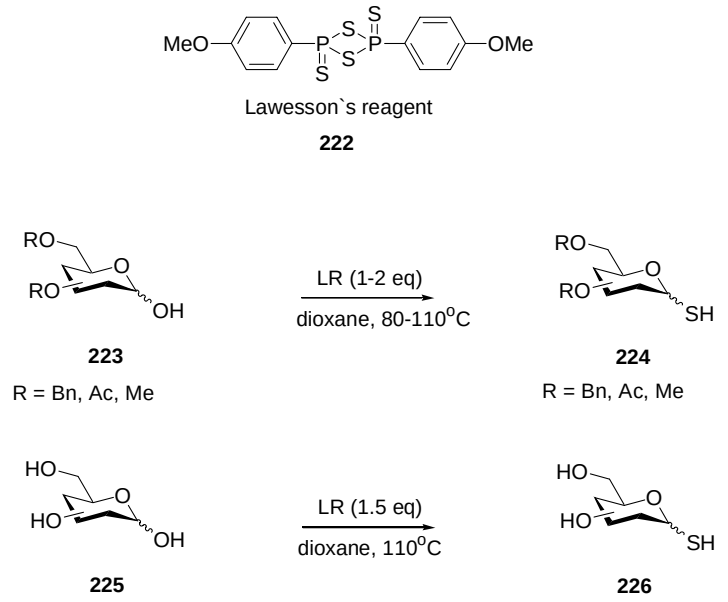


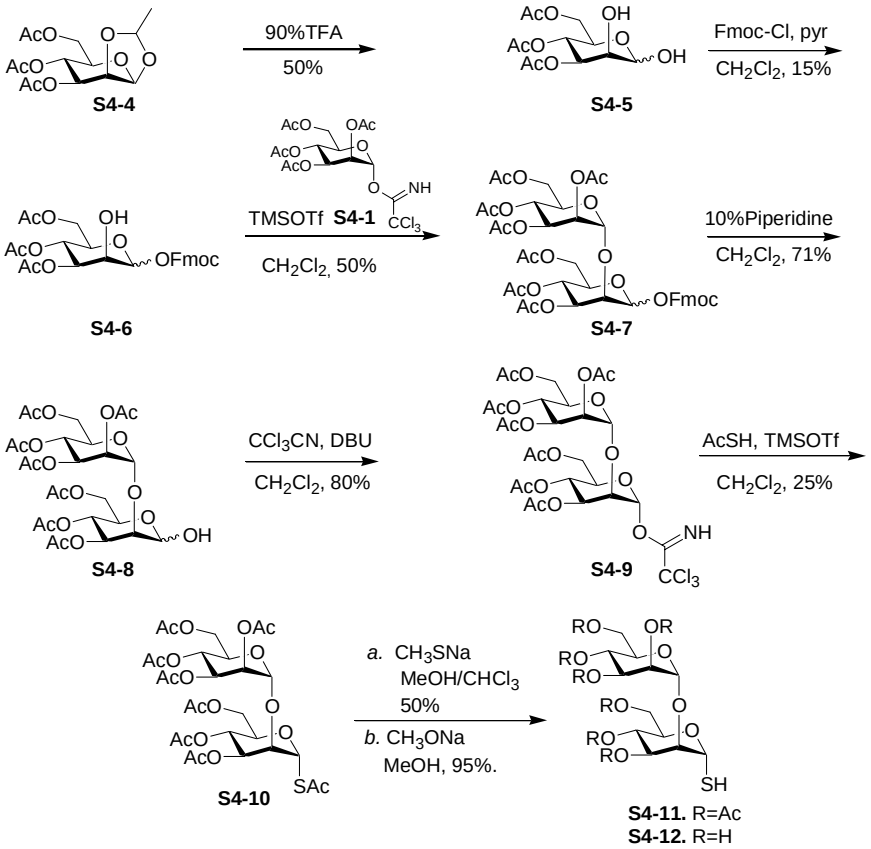
Figure 4.1.4.4 Direct formation of glycosyl thiols using Lawesson's reagent.

4.2 Results and discussion

Peptide nucleic acids[82,83] are attractive tags for programming self-assembled structures, since their chemistry is significantly more permissive than that of natural oligonucleotides. Furthermore, the higher affinity of PNAs for natural oligonucleotides allows for shorter tags, which are more specific and less sensitive to the ionic strength of the solution.[84] For example, we have shown that they could be used to encode combinatorial libraries of peptides.[85–88] In fact, only monosaccharide–DNA conjugates have been reported thus far for microarraying[89] and to study lectin interactions,[90,91] whereas larger oligosaccharides with more complex branching patterns have never been reported to a chloroacetamide with a mild base (such as Hunig's base or DBU). For the purpose at hand, a 10 mer PNA was deemed appropriate as it would provide a melting temperature (T_m) of greater than 50°C and would present adjacent ligands on the same face of the helix. Thus 10 mer PNA obtained by standard Fmoc chemistry and bearing a short polyethyleneglycol (PEG) spacer (10 Å) was coupled to chloroacetyl chloride and treated with thioacetals or carbohydrates bearing a thioalkyl group at the anomeric position. Our initial efforts relied on oligosaccharide synthesis to access the required carbohydrate. It will be discussed in the following chapter. It is clear that accessing these sugars without oligosaccharide synthesis will be important to streamline the process.

The synthesis of the D1 arm terminal Manα1-2Man dimannosyl thiols **S4-11** and **S4-12** is summarized (Scheme 4.2.1). The synthesis started with the triacetyl 1,2-ethylidene-β-D-mannoside **S4-4**,[92] which was prepared by the reaction of D-mannose through peracetylation, bromination followed by the treatment with $NaBH_4$ in acetronitrile. 1,2-ethylidene protection group was selectively removed by treatment with 90%TFA to yield compound **S4-5**. The remaining free hydroxyl groups **S4-5** was treated with Fmoc-Cl (1.2 eq) in the presence of pyridine at 0 °C to get the Fmoc protection isomers, followed by difficult and complicated silica gel column chromatography purification gave compound **S4-6**. Following a literature procedure, peracetylation of D-mannose followed by selective deprotection and activation at the anomeric position afforded mannosyl trichloroacetimidate **S4-1**.[93] TMSOTf catalyzed glycosylation between the mannosyl trichloroacetimidate **S4-1** and acceptor **S4-6** gave the disaccharide **S4-7**. Anomeric Fmoc deprotection with 10% piperidine and reaction with trichloroacetonitrile gave **S4-9** (56%, two steps). Coupling of **S4-9** with the thioacetic acid using TMSOTf yielded the requisite dimannose **S4-10** (25%). Anomeric deacetylation with sodium thiomethoxide at 0 °C gave **S4-11** (50%). Addition of sodium methoxide to the compound **S4-10** followed by global

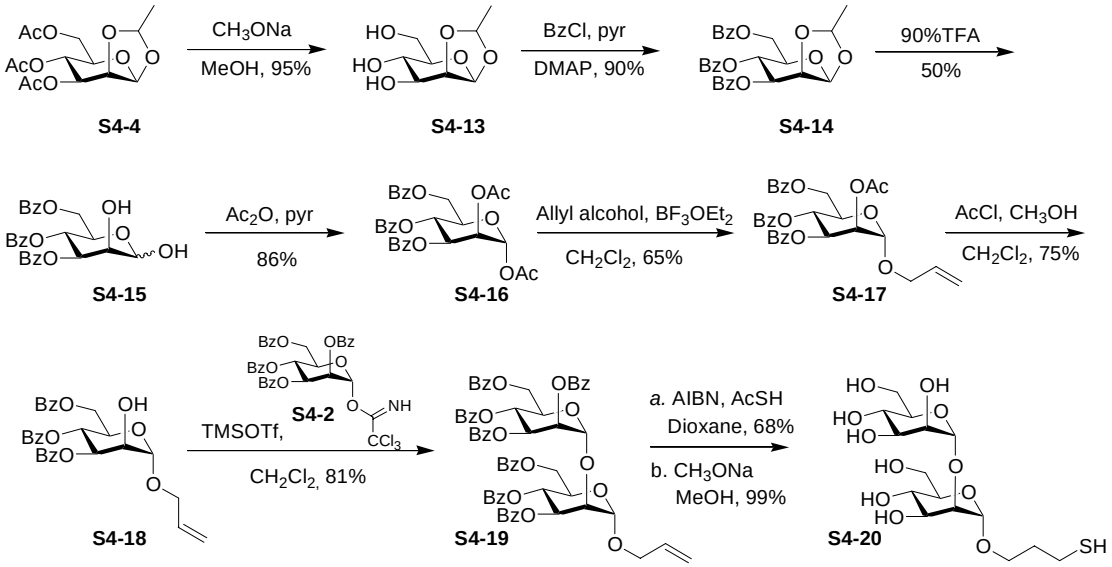
deacetylation resulted in compound **S4-12**.



Scheme 4.2.1 Synthesis of Manα1-2Man dimannosyl thiols.

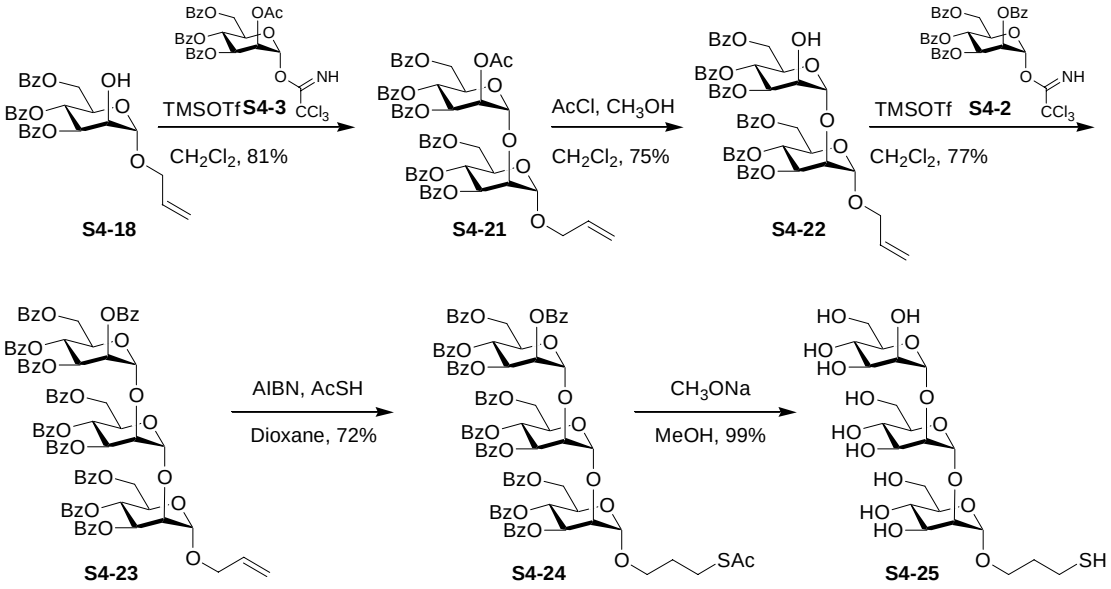
After the traditional carbohydrate synthesis in seven steps, we obtained the anomeric selectively deacetylation dimannosyl thiols **S4-11** in 0.2% yield. It will be a time-consuming and laborious synthetic strategy to prepare more complicated D1 arm terminal Manα1-2Manα1-2Man trimannosyl thiols. Therefore, synthetic routes must be developed to attach a linker moiety to the reducing end of the saccharide. The development of an appropriately functionalized linker moiety onto the saccharide is particular interest and the present work describes an efficient synthesis of dimannose and trimannose with a linker that bears a thioalkyl group at the anomeric position (Scheme 4.2.2).[94] Base-catalyzed selectively removal of the acetyl protecting groups led to the **S4-13**. The benzoylation of the free hydroxyl groups of compound **S4-13** was achieved using benzoyl chloride in the presence of DMAP and pyridine to produce **S4-14**. Acid hydrolysis of ethylidene protection group yielded **S4-15**. **S4-17** was prepared by the reaction of compound **S4-15** through peracetylation and allylation. The 2-*O*-acetyl group in **S4-17** was selectively removed by mild acidic hydrolysis in MeOH-CH_2Cl_2 co-solvent to give the intermediate 2-OH acceptor **S4-18**.

TMSOTf catalyzed glycosylation between the mannosyl trichloroacetimidate **S4-2**[95] and acceptor **S4-18** gave the disaccharide **S4-19**. The addition of thiols to the double bond in allyl glycosides by the use of radical chemistry has been shown to be useful on per-acetylated derivatives by Vliegenthart and co-workers.[96] **S4-19** was treated with thiolacetic acid in the presence of AIBN at 75 °C to give the sulfide spacer glycosides **S4-19** in good yields (68%).[94] The thioacetoxy group and benzoyl protection groups of the compound can be cleaved using sodium methoxide in MeOH to yield the corresponding thiol spacers **S4-19**.



Scheme 4.2.2 Synthesis of Manα1-2Man dimannose bearing a thioalkyl group at the anomeric position.

In order to synthesize of Manα1-2Manα1-2Man trimannose bearing a thioalkyl group at the anomeric position, the similar synthetic routes was shown (Scheme 4.2.3). TMSOTf catalyzed glycosylation between the mannosyl trichloroacetimidate **S4-3**[97] and acceptor **S4-18** gave the disaccharide **S4-21**. The 2-*O*-acetyl group in **S4-21** was selectively removed by mild acidic hydrolysis to give the intermediate 2-OH acceptor **S4-22**. Glycosylation of **S4-22** the mannosyl trichloroacetimidate **S4-2** gave the trisaccharide derivative **S4-23**. The radical elongation and global deacetylation were smoothly carried out and trimannose bearing a thioalkyl group at the anomeric position **S4-23** was provided in 70%. Synthetic procedures of thioacetals **S4-11**, **S4-12** and carbohydrates bearing a thioalkyl group at the anomeric position **S4-20**, **S4-25** were described. Further PNA-Carbohydrate conjugation and relative 2G12 antidody binding affinity study will be introduced in the chapter 5.



Scheme 4.2.3 Synthesis of Manα1-2Manα1-2Man trimannose bearing a thioalkyl group at the anomeric position.

4.3 Experimental section

2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (S4-1)[93]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.17 (s, 3H), 4.11-4.18 (m, 2H), 4.25 (dd, J = 11.8, 4.8 Hz, 1H), 5.36-5.38 (m, 2H), 5.44 (s, 1H), 6.26 (d, J = 1.6 Hz, 1H), 8.81 (s, 1H); ^{13}C NMR (100 MHz, CDCl3) δ 20.5, 20.6, 20.7, 62.0, 65.3, 67.8, 68.7, 71.1, 94.4, 159.6, 169.5, 169.6, 169.7, 170.4.

2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (S4-2)[95]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.56 (dd, J = 12.4, 4.0 Hz, 1H), 4.67-4.71 (m, 1H), 4.78 (dd, J = 12.4, 2.4 Hz, 1H), 5.99-6.06 (m, 2H), 6.29 (t, J = 10.0 Hz, 1H), 6.63 (d, J = 1.6 Hz, 1H),7.29-7.67 (m, 12H), 7.87-8.15 (m, 12H), 8.92 (s,1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 62.4, 66.1, 68.9, 69.8, 71.6, 90.6, 94.7, 128.4, 128.5, 128.5, 128.7, 128.8, 128.8, 128.9, 129.8, 129.9, 130.0, 133.1, 133.4, 133.6, 133.7, 159.9, 165.1, 165.4, 165.5, 166.0.

2-*O*-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl trichloroacetimidate (S4-3) [97]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.19 (s, 3H), 4.55 (dd, J = 12.0, 4.8 Hz, 1H), 4.63-4.67 (m, 1H), 4.71 (dd, J = 12.0, 2.8 Hz, 1H), 5.79 (t, J = 3.2 Hz, 1H), 5.92 (dd, J = 10.2, 3.2 Hz, 1H), 6.11 (t, J = 10.4 Hz, 1H), 6.49 (d, J = 2.0 Hz, 1H), 7.36-7.58 (m, 9H), 7.94-8.09 (m, 6H), 8.92 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 62.8, 66.3, 68.3, 69.7, 71.6, 90.6, 94.5, 128.4, 128.5, 128.7, 128.9, 129.7, 129.7, 129.9, 133.2, 133.5, 133.6, 159.7, 165.4, 165.5, 166.0, 169.5.

3,4,6-tri-*O*-acetyl-1,2-ethylidene-β-D-mannopyranoside (S4-4)[92]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (d, J = 4.8 Hz, 3H), 1.92 (s, 3H), 1.93 (s, 3H), 1.98 (s, 3H), 3.59-3.63 (m, 1H), 4.00 (dd, J = 12.0, 2.8 Hz, 1H), 4.08 (dd, J = 12.0, 4.8 Hz, 1H), 4.14 (t, J = 2.4 Hz, 1H), 5.09-5.22 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 21.5, 62.3, 65.9, 70.4, 71.3, 77.3, 96.4, 104.5, 169.4, 170.0, 170.4; HRMS (MALDI-TOF): m/z: calcd for $C_{14}H_{20}O_9Na$ $(M+Na)^+$: 355.1005; found 355.1006.

3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (S4-5): A solution of compound **S4-4**[92] (14.0 g, 42.2 mmol) in 90% TFA (90 mL) was stirred at room temperature for 10 h, at the end of which time TLC indicated that the reaction was complete. The reaction was diluted with toluene (200 mL) and then concentrated to dryness. The residue was purified by flash silica gel column chromatography (1:1 petroleum ether-EtOAc) to afford compound **S4-5** (6.5 g, 50%); Rf = 0.16 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s, 3H), 2.06 (br, 6H), 4.06-4.11 (m, 2H),

4.17-4.23 (m, 2H), 5.22-5.30 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 62.7, 66.4, 68.1, 69.4, 71.5, 94.0, 170.3, 170.5, 171.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{12}H_{18}O_9Na$ $(M+Na)^+$: 329.0849; found 329.0849.

1-*O*-Fluoren-9-ylmethoxycarbonyl-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (S4-6): Compound **S4-5** (5.1 g, 16.7 mmol) in CH_2Cl_2 (40 mL) was cooled to 0°C, and then Fmoc-Cl (5.4 g, 20.8 mmol) and pyridine (1.7 mL, 20.8 mmol) was added. The reaction mixture was stirred at room temperature overnight. Water was added to the reaction mixture and the aqueous layer was extracted 2 times with EtOAc. The organic layer was back extracted with saturated sodium bicarbonate solution and brine. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to gave compound **S4-6** (1.3 g, 15%); α/β =5:3; *Rf* = 0.38 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 3.65 (br, 1H), 4.11-4.51 (m, 7H), 5.32-5.36 (m, 1H), 5.50 (t, *J* = 10.0 Hz, 1H), 6.09 (d, *J* = 1.6 Hz, 1H), 7.36-7.74 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 46.6, 62.1, 65.5, 67.9, 70.5, 70.8, 71.1, 120.2, 125.1, 127.3, 128.0, 141.3, 143.0, 153.0, 169.9, 170.3, 171.0; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{27}H_{28}O_{11}Na$ $(M+Na)^+$:551.1530; found 551.1581.

1-*O*-Fluoren-9-ylmethoxycarbonyl (2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl) -(1-2)-3,4,6-tri-*O*-acetyl-α/β-D-mannopyranoside (S4-7): Compound **S4-1** (0.92 g, 1.87 mmol) and compound **S4-6** (0.79 g, 1.50 mmol) were dissolved in CH_2Cl_2 (20 mL). The solution was cooled to -30°C and TMSOTf (81 μL, 0.45 mmol) was added. The reaction mixture was stirred overnight at room temperature. The mixture was neutralized with saturated aqueous sodium bicarbonate solution and diluted with CH_2Cl_2. After washing with water and brine, the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-7** (0.64 g, 50%); α/β =5:3 for the anomeric center protected with Fmoc; *Rf* = 0.44 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97-2.18 (m, 41H), 4.04-4.28 (m, 16H), 4.42-4.54 (m, 3H), 4.97 (d, *J* = 2.0 Hz, 1H), 5.02-5.05 (m, 1H), 5.22-5.50 (m, 10H), 5.99 (d, *J* = 1.6 Hz, 0.6H), 6.17 (d, *J* = 2.0 Hz, 1H), 7.30-7.77 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.8, 46.6, 65.3, 65.9, 66.2, 66.9, 68.1, 68.3, 69.4, 69.5, 69.6, 69.7, 69.7, 69.9, 70.5, 70.8, 70.9, 74.1, 75.8, 77.4, 94.1, 94.8, 98.8, 99.3, 120.2, 124.9, 124.9, 125.0, 125.0, 127.2, 128.0, 141.3, 142.9, 152.6, 152.8, 169.2, 169.4, 169.5, 169.7, 169.8, 169.8, 170.0, 170.0, 170.3, 170.5, 170.5, 170.7; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{41}H_{46}O_{20}Na$ $(M+Na)^+$: 881.2481; found

881.2412.

(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (S4-8): The compound **S4-7** (740 mg) was dissolved in a solution of 10% piperidine in CH_2Cl_2 (10 mL). The reaction mixture was stirred for 4 h at room temperature and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (1:1 petroleum ether-EtOAc) to afford compound **S4-8** (390 mg, 71%); *Rf* = 0.22 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.07 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 4.05-4.23 (m, 7H), 4.46 (br, 1H), 4.93 (d, *J* = 1.6 Hz, 1H), 5.21-5.41 (m, 6H); 13C NMR (100 MHz, CDCl3) δ 20.6, 20.7, 20.8, 62.3, 62.4, 66.3, 66.4, 68.3, 68.4, 69.0, 69.8, 70.0, 92.7, 99.0, 169.5, 169.7, 169.8, 170.5, 170.7, 171.1; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{26}H_{36}O_{18}Na$ $(M+Na)^+$: 659.1800; found 659.1772.

(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyltrichloroacetimidate (S4-9): Compound **S4-8** (370 mg, 0.58 mmol) was dissolved in CH_2Cl_2 (8 mL), then trichloroacetonitrile (0.18 mL, 1.74 mmol) and DBU (26 μL, 0.174) were added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-9** (362 mg, 80%); *Rf* = 0.29 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 4.04-4.21 (m, 5H), 4.91 (d, *J* = 1.2 Hz, 1H), 5.17-5.41 (m, 7H), 6.34 (d, *J* = 2.0 Hz, 1H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.5, 20.5, 20.7, 61.5, 62.2, 65.1, 66.0, 68.2, 69.4, 69.5, 69.7, 71.1, 74.9, 95.4, 99.0, 159.8, 169.1, 169.3, 169.5, 169.7, 170.1, 170.4, 170.6.

1-*S*-acetyl-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (S4-10): A solution of **S4-9** (340 mg, 0.43 mmol) and thioacetic acid (63 μL, 0.87 mmol) in anhydrous CH_2Cl_2 (5 mL) was cooled to -30°C, and treated with TMSOTf (20 μL, 0.109 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized with Et_3N (0.5 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-10** (76 mg, 25%); *Rf* = 0.27 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.39 (s, 3H), 3.79-3.83 (m, 1H), 4.04-4.09 (m, 2H),

4.18-4.29 (m, 4H), 4.95 (d, J = 1.2 Hz, 1H), 5.05 (dd, J = 10.0, 2.8 Hz, 1H), 5.22-5.41 (m, 4H), 6.07 (d, J = 1.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.8, 31.3, 61.8, 62.1, 65.5, 66.0, 68.4, 69.6, 69.7, 70.8, 72.8, 78.6, 80.7, 99.2, 169.1, 169.4, 169.6, 169.9, 170.3, 170.6, 170.7, 190.7; HRMS (MALDI-TOF): m/z: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1738.

(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-1-thio-α-D-mannopyranoside (S4-11): The compound **S4-10** (75 mg, 0.108 mmol) was dissolved in CH_3Cl/MeOH(1/1) (1 mL) and cooled to 0°C. Nitrogen was bubbled through the solution for 5 min followed by the addition of NaSMe (7.6 mg, 0.108 mmol). After 20 min, the mixture was neutralized with Amberlite IRC-50. The mixture was filtered and the filtrates were concentrated. The residue was purified by flash silica gel column chromatography (1:1 petroleum ether-EtOAc) to afford compound **S4-11** (35 mg, 50%); Rf = 0.16 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.07 (br, 6H), 2.12 (br, 6H), 2.17 (br, 6H), 4.15-4.32 (m, 8H), 4.94 (s, 1H), 5.26-5.42 (m, 5H), 5.77 (d, J = 6.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 20.8, 20.8, 61.9, 62.5, 66.1, 66.4, 68.4, 69.5, 69.5, 69.6, 69.7, 69.9, 79.5, 99.2, 169.3, 169.5, 169.7, 169.9, 170.4, 170.4, 170.9; HRMS (MALDI-TOF): m/z: calcd for $C_{26}H_{36}O_{17}SNa$ $(M+Na)^+$: 675.1572; found 675.1555.

(α-D-mannopyranosyl)-(1→2)-1-thio-α-D-mannopyranoside (S4-12): Compound **S4-11** (70 mg, 0.10 mmol) was dissolved in MeOH (2 mL) and NaOMe (0.55 mg, 0.01 mmol) was added. The mixture was stirred at room temperature for 4 h. The mixture was neutralized by adding Amberlite IRC-50. After filtration, the filtrates were concentrated *in vacuo* to give compound **S4-12** which was used without further purification (34.3 mg, 95%); HRMS (MALDI-TOF): m/z: calcd for $C_{12}H_{22}O_{10}SNa$ $(M+Na)^+$: 381.0832; found 381.0806.

1,2-Ethylidene-β-D-mannopyranoside (S4-13): Compound **S4-4** (10.6 g, 31.6 mmol) was dissolved in MeOH (80 mL) and NaOMe (0.26 mg, 4.74 mmol) was added. The mixture was stirred at room temperature for 4 h. The mixture was neutralized by adding Amberlite IRC-50. After filtration, the filtrates were concentrated. The residue was purified by flash silica gel column chromatography (16:1 $CHCl_3$-MeOH) to afford compound **S4-13** (6.18 g, 95%); Rf = 0.22 (12.5% methanol in chloroform); ^{1}H NMR (400 MHz, CD_3OD) δ 1.46 (d, J = 4.8 Hz, 3H), 3.29-3.33 (m, 1H), 3.62-3.71 (m, 2H), 3.81-3.89 (m, 2H) 4.14-4.15 (m, 1H) 5.26-5.30 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 20.8, 61.6, 67.6, 71.6, 75.8, 80.2, 96.7, 103.8; HRMS (MALDI-TOF): m/z: calcd for $C_8H_{14}O_6Na$ $(M+Na)^+$: 229.0688; found 229.0677.

3,4,6-tri-O-benzoyl-1,2-ethylidene-β-D-mannopyranoside (S4-14)[97]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (d, J = 4.8 Hz, 3H), 4.10-4.14 (m, 1H), 4.50 (dd, J = 12.4, 4.8 Hz, 1H), 4.55-4.56 (m, 1H), 4.65 (dd, J = 11.6, 3.2 Hz, 1H), 5.36 (q, J = 4.8 Hz, 1H), 5.52 (d, J = 2.4 Hz, 1H), 5.66 (dd, J = 9.8, 3.6 Hz, 1H), 6.01 (t, J = 10.0 Hz, 1H), 7.36-7.58 (m, 9H), 7.94-8.07 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.6, 63.4, 66.8, 71.5, 71.9, 77.5, 96.8, 104.9, 128.3, 128.4, 129.0, 129.0, 129.7, 130.0, 133.0, 133.4, 133.5, 165.3, 166.0, 166.2; HRMS (MALDI-TOF): m/z: calcd for $C_{29}H_{26}O_9Na$ $(M+Na)^+$: 541.1475; found 541.1724.

3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-15)[97]: 1H NMR (400 MHz, CD3OD) δ 4.33 (br, 1H), 4.51-4.65 (m, 3H), 5.33 (br, 1H), 5.78 (d, J = 9.2 Hz, 1H), 6.05 (t, J = 9.2 Hz, 1H), 7.30-7.51 (m, 9H), 7.89-8.00 (m, 6H); 13C NMR (100 MHz, CD3OD) δ 63.7, 67.8, 68.2, 69.5, 72.7, 94.8, 128.1, 128.2, 129.3, 129.4, 132.9, 133.0, 133.1, 165.9, 166.0, 166.4; HRMS (MALDI-TOF): m/z: calcd for $C_{27}H_{24}O_9Na$ $(M+Na)^+$: 515.1312; found 515.1286.

1,2-Di-O-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-16)[97]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.13 (s, 3H), 2.20 (s, 1H), 4.50 (d, J = 10.8 Hz, 2H), 4.67 (d, J = 12.0 Hz, 1H), 5.56 (s, 1H), 5.86 (d, J = 10.0 Hz, 1H), 6.07 (t, J = 10.0 Hz, 1H), 6.28 (s, 1H), 7.33-7.54 (m, 9H), 7.92-8.07 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.8, 62.8, 66.5, 68.8, 69.9, 70.9, 90.6, 128.4, 128.5, 128.8, 128.9, 129.7, 129.8, 133.1, 133.5, 133.5, 165.3, 165.5, 166.0, 168.1, 169.4; HRMS (MALDI-TOF): m/z: calcd for $C_{31}H_{28}O_{11}Na$ $(M+Na)^+$: 599.1524; found 599.1480.

Allyl 2-O-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-17): Compound **S4-16** (5.2 g, 9.02 mmol) was dissolved in CH_2Cl_2 (50 mL), cooled to 0°C and allyl alcohol (1.23 mL, 18.05 mmol) was added. BF_3Et_2O (4.6 mL, 36.1 mmol) was added dropwise over the course of 5 min, and the reaction mixture was stirred at room temperature for 24 h. The mixture was neutralized with saturated aqueous sodium bicarbonate solution and diluted with CH_2Cl_2. After washing with water and brine, the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-17** (3.37 g, 65%); Rf = 0.47 (33.3% ethyl acetate in petroleum ether); 1H NMR (400 MHz, CDCl3) δ 2.16 (s, 3H), 4.15 (dd, J = 12.8, 6.0 Hz, 1H), 4.33 (dd, J = 12.8, 5.6 Hz, 1H), 4.44-4.48 (m, 1H), 4.55 (dd, J = 11.8, 5.6 Hz, 1H), 4.67 (dd, J = 12.0, 3.2 Hz, 1H), 5.05 (d, J = 1.6 Hz, 1H), 5.27 (dd, J = 10.4, 1.2 Hz, 1H), 5.37 (dd, J = 17.2, 1.2 Hz, 1H), 5.55-5.56 (m, 1H), 5.89 (dd, J = 10.0, 3.2 Hz, 1H), 5.95-6.01 (m, 2H), 7.35-7.58 (m, 9H), 7.93-8.11 (m, 6H); 13C NMR (100 MHz,

CDCl3) δ 20.8, 63.3, 67.2, 68.8, 69.0, 69.9, 70.0, 96.6, 118.4, 128.4, 128.4, 129.0, 129.2, 129.7, 129.8, 133.1, 133.1, 133.3, 133.5, 165.4, 165.6, 166.1, 169.8; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{32}H_{30}O_{10}Na$ $(M+Na)^+$: 597.1731; found 597.1687.

Allyl-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-18): Compound **S4-17** (2.9 g, 5.05 mmol) was dissolved in CH_2Cl_2 (10 mL) and MeOH (150 mL). To the reaction mixture was added acetyl chloride (1.46 mL, 20.45 mmol) at 0°C. After being stirred at room temperature for 32 h, Et_3N (2 mL) was added and the reaction mixture was concentrated. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to gave compound **S4-18** (2.02 g, 75%); *Rf* = 0.37 (33.3%
ethyl acetate in petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) δ 3.16 (br, 1H), 4.13 (dd, *J* = 12.8, 6.0 Hz, 1H), 4.32 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.39-4.45 (m, 2H), 4.53 (dd, *J* = 12.0, 6.0 Hz, 1H), 4.62 (dd, *J* = 12.0, 3.2 Hz, 1H), 5.07 (d, *J* = 1.6 Hz, 1H), 5.26 (dd, *J* = 10.6, 1.2 Hz, 1H), 5.36 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.76 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.94-6.05 (m, 2H), 7.29-7.55 (m, 9H), 7.96-8.06 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.7, 67.3, 68.6, 68.9, 69.4, 72.8, 98.9, 118.1, 128.4, 128.4, 129.1, 129.3, 129.7, 129.8, 133.1, 133.3, 133.3, 133.4, 165.7, 165.8, 166.3; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{30}H_{28}O_9Na$ $(M+Na)^+$: 555.1625; found 555.1598.

Allyl (2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-19): Compound **S4-2** (0.59 g, 0.79 mmol) and compound **S4-18** (0.38 g, 0.72 mmol) were dissolved in CH_2Cl_2 (10 mL). The solution was cooled to -30°C and treated with TMSOTf (33 μL, 0.18 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by Et_3N (1.0 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-19** (0.65 g, 81%); *Rf* = 0.33 (33.3% ethyl acetate in petroleum ether); ^1H NMR (400 MHz, $CDCl_3$) δ 4.10 (dd, *J* = 12.8, 6.0 Hz, 1H), 4.33 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.51-4.61 (m, 3H), 4.67-4.80 (m, 4H), 5.28-5.40 (m, 4H), 5.94-6.08 (m, 3H), 6.14-6.26 (m, 3H), 7.28-7.61 (m, 21H), 7.95-8.21 (m, 14H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.0, 63.7, 67.0, 67.7, 68.8, 69.1, 69.9, 70.2, 70.9, 77.2, 97.9, 99.7, 118.3, 128.4, 128.5, 128.6, 128.6, 128.9, 128.9, 129.1, 129.2, 129.8, 129.9, 130.0, 130.0, 133.1, 133.2, 133.3, 133.4, 133.5, 165.0, 165.1, 165.3, 165.7, 165.7, 166.1, 166.3, 166.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{64}H_{54}O_{18}Na$ $(M+Na)^+$: 1133.3203; found 1133.3162.

3-Thioacetoxypropyl (2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-19a)[94]: A solution of compound **S4-19** (0.55 g, 0.49 mmol), AIBN (0.41 g, 2.47 mmol) and thioacetic acid (0.53 mL, 7.42 mmol) in 1,4-dioxane (8 mL) was thoroughly degassed by bubbling N_2. The reaction mixture was stirred at 80°C for 2h. The reaction was quenched with cyclohexene (1 mL), concentrated and co-concentrated twice with toluene. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-19a** (0.40 g, 68%); *Rf* = 0.30 (33.3% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93-1.97 (m, 2H), 2.33 (s, 3H), 2.97-3.08 (m, 2H), 3.48-3.50 (m, 1H), 3.84-3.88 (m, 1H), 3.44-4.48 (m, 1H), 4.50 (s, 1H), 4.56 (dd, *J* = 12.0, 4.8 Hz, 1H), 4.63-4.79 (m, 4H),5.25 (s, 1H), 5.39 (s, 1H), 5.97-6.21 (m, 5H), 7.29-7.61 (m, 21H), 7.93-8.18 (m, 14H); 13C NMR (100 MHz, CDCl3) δ 25.8, 29.3, 30.6, 63.1, 63.8, 66.5, 67.0, 67.7, 69.1, 69.8, 70.2, 70.9, 77.4, 98.8, 99.6, 128.4, 128.5, 128.6, 128.9, 129.1, 129.2, 129.8, 129.9, 130.0, 133.1, 133.2, 133.3, 133.4, 133.5, 165.0, 165.1, 165.4, 165.6, 165.7, 166.1, 166.4, 195.6; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{66}H_{58}O_{19}SNa$ $(M+Na)^+$: 1209.3185; found 1209.3202.

3-Thiopropyl (α-D-mannopyranosyl)-(1→2)-α-D-mannopyranoside (S4-20): Compound **S4-19a** (170 mg, 0.14 mmol) was dissolved in MeOH (3 mL) and NaOMe (0.76 mg, 0.014 mmol) was added. The mixture was stirred at room temperature for 4 h. The mixture was neutralized by adding Amberlite IRC-50. After filtration, the filtrates were concentrated to give compound **S4-20** (59.4 mg, 99%); HRMS (MALDI-TOF): *m*/*z*: calcd for C15H28O11SNa (M +Na)+: 439.1251; found 439.1295.

Allyl (2-*O*-acetyl-3,4,6-tri-O-benzoyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-21): Compound **S4-3** (1.3 g, 1.92 mmol) and compound **S4-18** (0.79 g, 1.48 mmol) were dissolved in CH_2Cl_2 (20 mL). The solution was cooled to -30°C and treated with TMSOTf (81 μL, 0.45 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by Et_3N (0.5 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-21** (1.26 g, 81%); *Rf* = 0.39 (33.3% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.07 (s, 3H), 4.00 (dd, *J* = 12.8, 6.0 Hz, 1H), 4.26 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.46-4.61 (m, 2H), 4.55-4.74 (m, 5H), 5.22-5.35 (m, 4H), 5.82 (t, *J* = 2.4 Hz, 1H), 5.90-6.04 (m, 4H), 6.12 (t, *J* = 10.0 Hz, 1H), 7.32-7.56 (m, 18H), 7.99-8.18 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 63.4, 63.7, 67.2, 67.6, 68.8, 69.0, 69.6, 69.9, 77.0, 98.0, 99.6, 118.1, 128.4,

128.4, 128.5, 128.5, 128.6, 128.8, 128.9, 129.1, 129.3, 129.7, 129.9, 130.0, 130.0, 133.1, 133.2, 133.2, 133.3, 133.4, 133.5, 165.1, 165.3, 165.6, 166.1, 166.3, 169.3; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{59}H_{52}O_{18}Na$ $(M+Na)^+$: 1071.3046; found 1071.3055.

Allyl (3,4,6-tri-O-benzoyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzoyl-α-D-Mannopyranoside (S4-22): Compound **S4-21** (1.19 g, 1.14 mmol) was dissolved in CH_2Cl_2 (5 mL) and MeOH (40 mL). To the reaction mixture was added acetyl chloride (0.33 mL, 4.60 mmol) at 0°C. After being stirred at room temperature for 32 h, Et_3N (1 mL) was added and the reaction mixture was concentrated. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-22** (0.86 g, 75%); *Rf* = 0.37 (33.3% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.00 (br, 1H), 3.98 (dd, *J* = 12.8, 6.0 Hz, 1H), 4.23 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.43-4.69 (m, 8H), 5.22-5.34 (m, 4H), 5.85-6.09 (m, 5H), 7.33-7.54 (m, 18H), 7.96-8.14 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.7, 63.7, 67.1, 67.5, 68.8, 68.9, 69.4, 69.8, 71.4, 72.4, 98.0, 101.8, 118.0, 128.4, 128.4, 128.5, 128.6, 129.0, 129.0, 129.1, 129.3, 129.7, 129.8, 129.8, 129.9, 133.1, 133.2, 133.3, 133.4, 133.4, 165.3, 165.4, 165.7, 165.8, 166.2, 166.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{57}H_{50}O_{17}Na$ $(M+Na)^+$: 1029.2940; found 1029.2949.

Allyl (2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-O-benzoyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-23): Compound **S4-22** (0.69 g, 0.69 mmol) and compound **S4-2** (0.76 g, 1.03 mmol) were dissolved in CH_2Cl_2 (15 mL). The solution was cooled to -30°C and treated with TMSOTf (31 μL, 0.17 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by Et_3N (0.1 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-23** (0.84 g, 77%); *Rf* = 0.45 (33.3% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02 (dd, *J* = 12.2, 6.0 Hz, 1H), 4.20-4.29 (m, 2H), 4.38-4.69 (m, 10H), 4.98 (s, 1H), 5.18 (s, 1H), 5.27 (d, *J* = 10.4 Hz, 1H), 5.35 (d, *J* = 17.2 Hz, 1H), 5.47 (s, 1H), 5.76-5.80 (m, 2H), 5.93-6.07 (m, 6H), 7.00-7.67 (m, 30H), 7.89-8.18 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.1, 63.7, 63.9, 66.7, 67.5, 67.6, 68.8, 69.0, 69.8, 69.9, 70.1, 70.3, 71.4, 76.5, 76.9, 77.2, 77.5, 77.5, 78.1, 98.2, 99.8, 100.2, 118.1, 128.4, 128.5, 128.6, 128.6, 128.9, 129.0, 129.1, 129.2, 129.3, 129.8, 129.9, 129.9, 130.0, 130.1, 133.1, 133.2, 133.3, 133.5, 133.6, 164.8, 165.1, 165.4, 165.5, 165.7, 165.9, 166.3, 166.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{91}H_{76}O_{26}Na$ $(M+Na)_+$: 1607.4518; found 1607.4496.

3-Thioacetoxypropyl (2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-O-benzoyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside (S4-24)[94]: A solution of compound **S4-23** (0.83 g, 0.52 mmol), AIBN (0.43 g, 2.62 mmol) and thioacetic acid (0.57 mL, 7.86 mmol) in 1,4-dioxane (12 mL) was thoroughly degassed by bubbling N_2 The reaction mixture was stirred at 80°C for 2h. The reaction was quenched with cyclohexene (1 mL), concentrated and co-concentrated twice with toluene. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S4-24** (0.63 g, 72%); *Rf* = 0.43 (33.3% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94-2.00 (m, 2H), 2.34 (s, 3H), 2.98-3.11 (m, 2H), 3.46-3.51 (m, 1H), 3.84-3.89 (m, 1H), 4.28 (dd, *J* = 12.0, 4.0 Hz, 1H), 4.43-4.75 (m, 10H), 5.06 (s, 1H), 5.19 (s, 1H), 5.55 (s, 1H), 5.82 (dd, *J* = 9.6, 3.2 Hz, 1H), 5.87 (t, *J* = 2.8 Hz, 1H), 6.02-6.16 (m, 5H), 7.04-7.65 (m, 30H), 7.92-8.19 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 29.4, 30.6, 63.0, 63.8, 64.0, 66.5, 66.6, 67.5, 67.6, 69.0, 69.7, 69.9, 70.1, 70.3, 71.4, 76.4, 77.4, 77.9, 98.9, 99.7, 100.1, 128.4, 128.5, 128.6, 128.6, 128.9, 129.0, 129.1, 129.2, 129.3, 129.8, 129.9, 129.9, 130.0, 130.1, 133.0, 133.1, 133.3, 133.4, 133.4, 133.6, 164.8, 165.1, 165.4, 165.5, 165.6, 165.7, 165.9, 166.3, 166.3, 195.5; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{93}H_{80}O_{27}SNa$ $(M+Na)^+$: 1683.4534; found 1683.4531.

3-Thiopropyl (α-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-α-D-mannopyranoside (S4-25): The compound **S4-24** (0.29 g, 0.17 mmol) was dissolved in MeOH (4 mL) and NaOMe (1 mg, 0.017 mmol) was added. The mixture was stirred at room temperature for 2 h. The mixture was neutralized by adding Amberlite IRC-50. After filtration, the filtrates were concentrated to afford compound **S4-25** (99.9 mg, 99%); HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{21}H_{38}O_{16}SNa$ $(M+Na)^+$: 601.1780; found 601.1738.

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5. Synthesis of Azidodimannose building block containing carboxylic acid linker for PNA-encoded carbohydrates targeting 2G12

5.1 Introduction

5.1.1 DNA templated homo and heterodimerization of PNA encoded oligosaccharide that mimick the carbohydrate epitope of HIV

A pilot library of over 30 architectures were tested for their affinity to 2G12 by surface plasmon resonance (SPR) (Fig. 5.1.1.1).[1] The antibody was immobilized accordingly to a previously described protocol.[2] Under these conditions, no notable binding was observed for nonasaccharide **182**, as previously reported by Danishefsky and co-workers,[2,3] and in agreement with the binding mode reported by Wilson[4,5] which involves four units of mannose disaccharide from multiple units of **182**. Significant binding (μm) was observed for conjugates having the key α-1,2-mannose disaccharide units. However, the distance between the two carbohydrate units of this disaccharide was critical for the binding. Only conjugates bearing an 11 atom spacer between the two disaccharide units (structure **231** and **239**) displayed any binding (entries 12–18, Fig. 5.1.1.1). It is interesting to note that the distance between the two mannose units involved in the binding of nonasaccharide **182** is also 11 atoms (Fig. 5.1.1.2). The shorter spacer present in structures **230** and **238** (Fig. 5.1.1.3) clearly do not adequately replicate this geometry (entries 9–11), whereas longer spacers in structures **232**, **234**, **240**, and **241** (Fig. 5.1.1.3) do not provide the adequate level of cooperativity (entry 22–25, 30–32). Following the same argument, architectures based on the mannose trisaccharide fail to provide significant binding (entries 26–28; Fig. 5.1.1.1). The topology of the supramolecular architecture also has a significant impact on binding. While the PEG spacer between the carbohydrate moiety and the PNA does provide a certain level of flexibility, a clear trend emerges on comparison of entries 12 to 15 of (Fig. 5.1.1.3), with the shortest distance (entry 12) being best. Considering that a distance of about 35 Å is required for cooperative binding to 2G12, the architecture in entry 12 (the maximum distance between the branch points joining the carbohydrate units is 38.5 Å) would be most suitable, whereas the architecture of entry 15 shows a lower level of cooperative binding.

The novelty of the approach reported herein is that it exploits the programmability of hybridization to generate a library of architectures which emulate the topologies of complex carbohydrates. The gp120 epitope has stimulated tremendous efforts towards the production of vaccines.[2,6,7–9] While this example illustrates the importance of multimeric recognition with controlled topology, the generality of this concept extends far beyond pathogen recognition.[10]

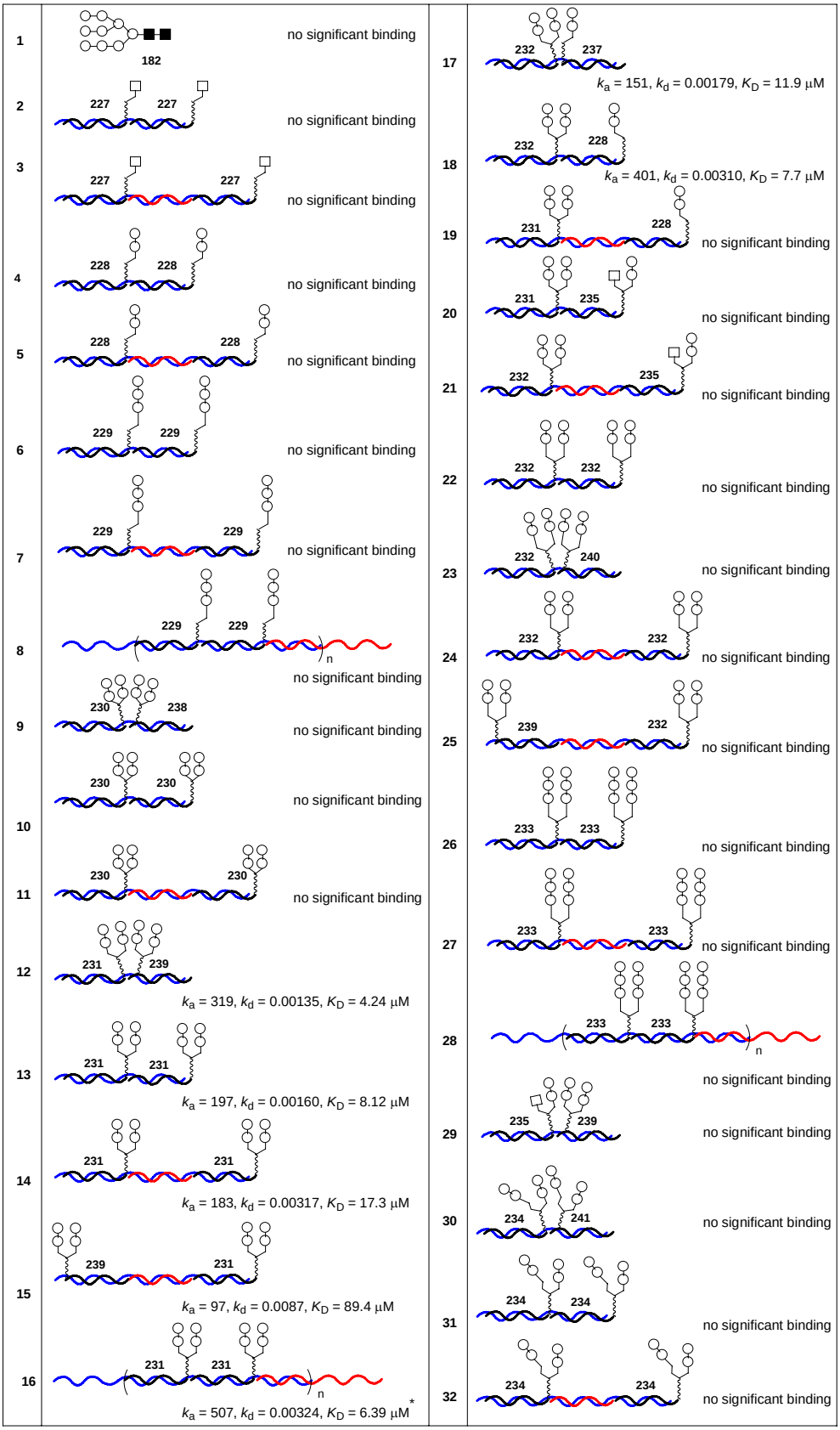
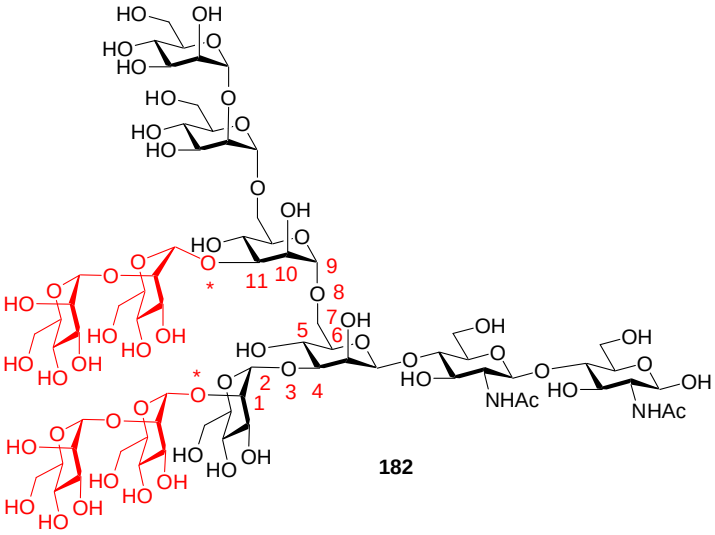
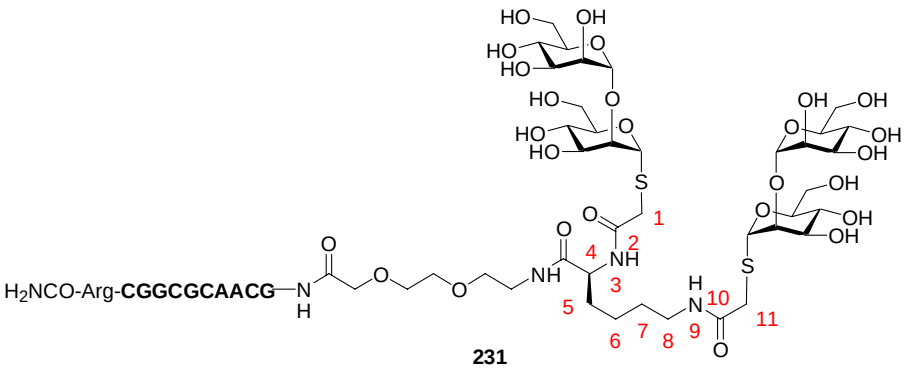


Figure 5.1.1.1 Affinity of supramolecular complexes to 2G12.





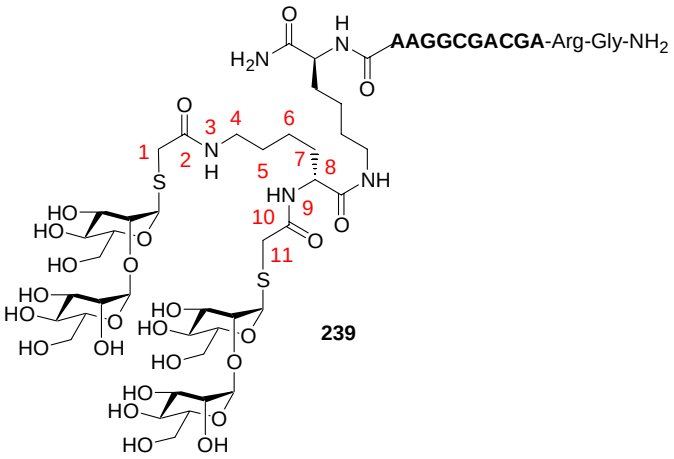


Figure 5.1.1.2 *N* terminus of PNA and *C* terminus of PNA.

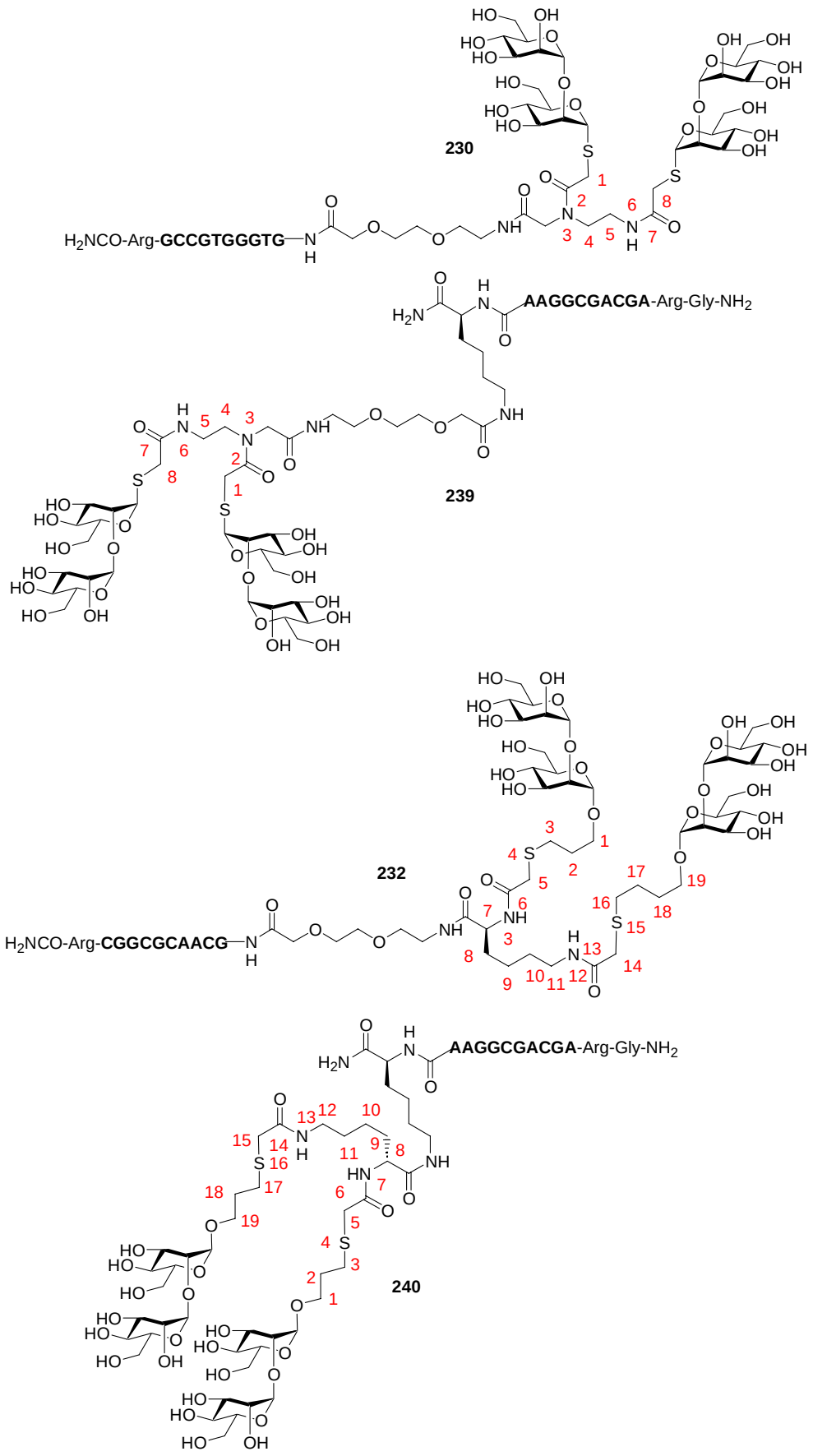


Figure 5.1.1.3 *N* terminus and *C* terminus of PNA with shorter and longer spacer.

5.1.2 PNA combinatorial library preparation

Combinatorial synthesis has opened the door for small research teams to build chemical libraries in search of selective small molecule inhibitors as probes for investigating biological phenomena. Several thousand libraries have been reported in the literature.[11-15] The two main strategies for the synthesis of libraries are parallel synthesis and split and mix combinatorial synthesis.[16,17] The synthesis of the library with five elements of diversity used in two steps yields 25 products (Fig. 5.1.2.1).[18]

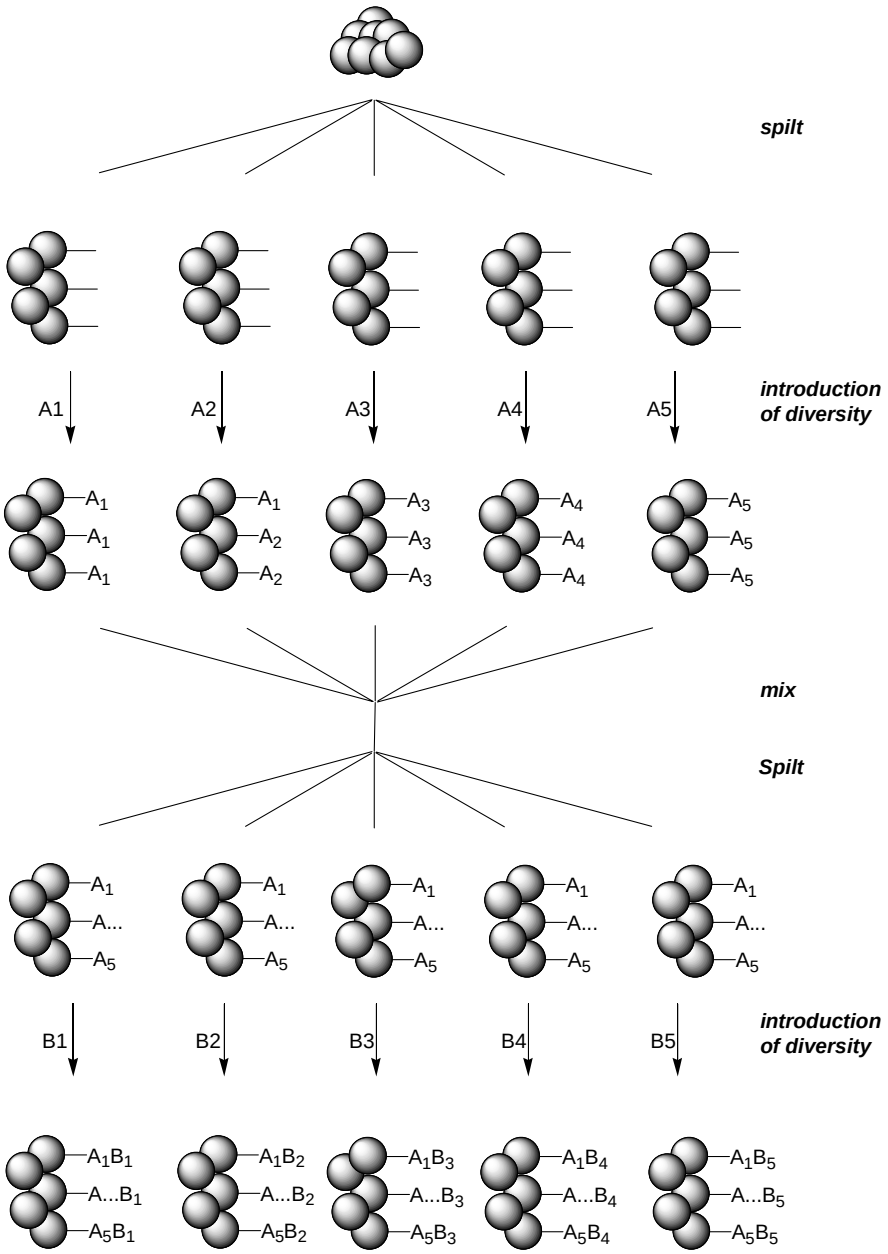


Figure 5.1.2.1 Schematic representation of split and mix synthesis

A batch of resin is split into five pools and first element of diversity (A_1-A_5) is introduced. The pools are then mixed and split into five new pools such that a member of each first give pools should be present in the second pools. Then, the second element of diversity (B_1-B_2) is added to yield a library containing all permutations of $A_{1\text{-}5}B_{1\text{-}5}$. The power of this method comes from the fact that the number formed is exponentially related to the number of steps. One of the major drawbacks of the split and mix synthesis is that the libraries are obtained as a mixture. This has stimulated the development of a number of encoding methods to track the synthetic path of the bead through the split and mixing processes.[19]

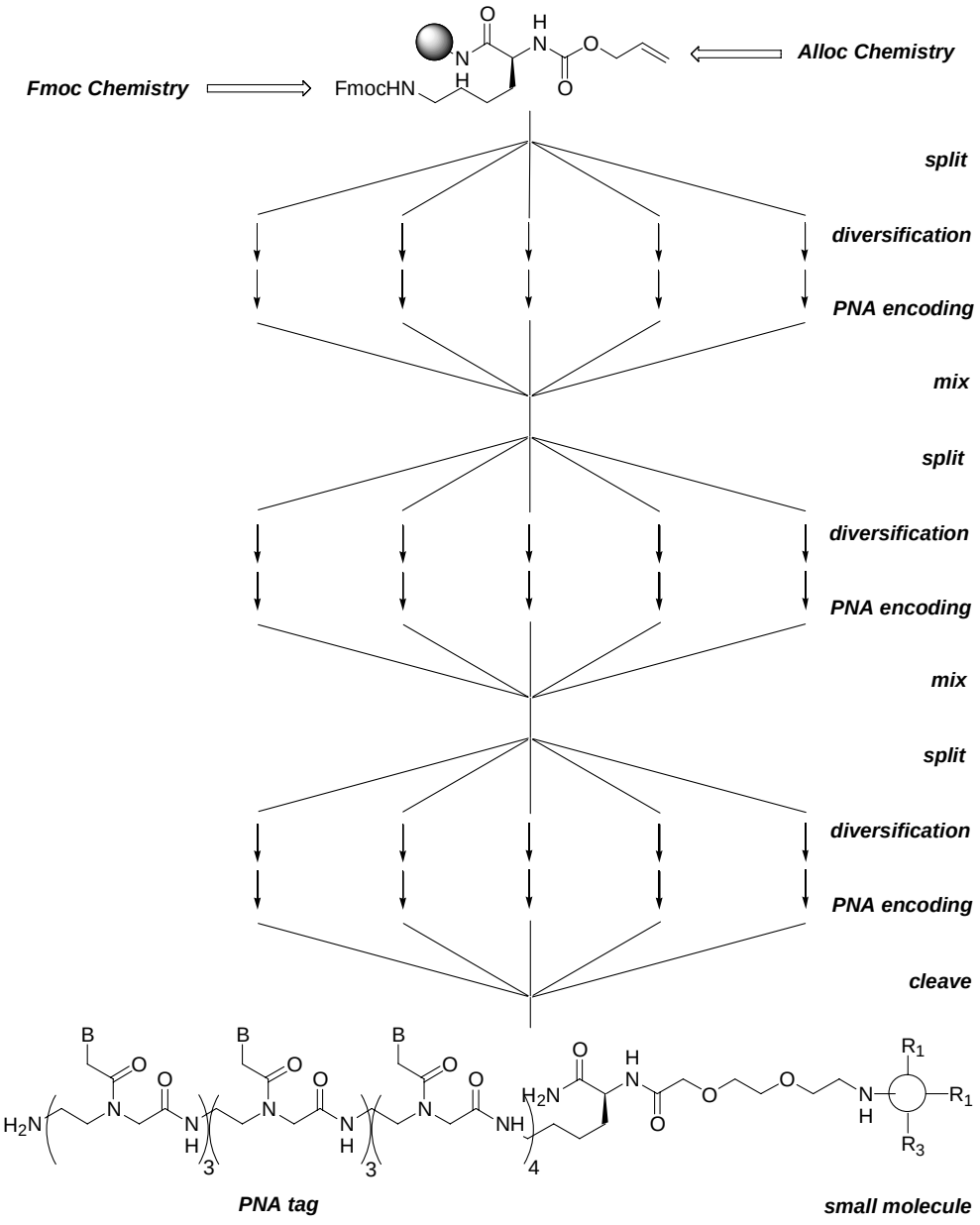


Figure 5.1.2.2 PNA-encoded split and mix combinatorial synthesis

Our group reasoned that PNA[20,21] could be used to encode library synthesis. A bifunctional linker such as a lysine with mutually orthogonal Fmoc and Alloc protecting groups can be used to elaborate the PNA tag and the small library, respectively (Fig. 5.1.2.2). The resin is split into pools in which the diverse building blocks are introduced followed by their respective PNA codons and the resin is then remixed. Repetition of this cycle affords a library wherein every molecule is tagged with its own synthetic path and its unique structure. Final cleavage of the solid support yields the library as a mixture in solution that can be converted to an organized format by hybridization to the DNA microarray (Fig. 5.1.2.3).[18]

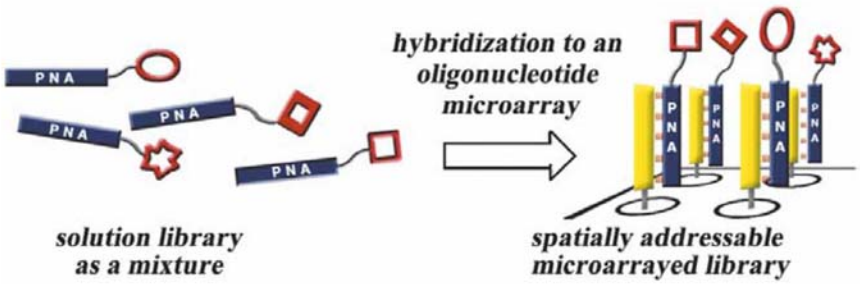


Figure 5.1.2.3 Conversion to an organized microarray.

5.2 Results and discussion

The copper-catalyzed coupling of azides with alkynes[22,23] has emerged as one of the most robust conjugation technologies.[24,25] The reliability of the Huisgen reaction have found application in the study of biological system.[26] Most commonly, an azide group is introduced into a molecule by the formation of a C-N bond, usually by the nucleophilic displacement by an azide ion.[27] In some system, this approach leads to the formation of elimination products or with incorrect stereochemical configuration. In contrast to the former approach is the diazotransfer reaction. This process utilizes trifluoromethanesulfonyl azide (TfN_3) **242** as a diazo donor in the Cu^I-catalyzed conversion of a primary amine into an azide.[28-30] The explosive nature of neat TfN_3 and its relatively poor shelf life require its preparation in solution prior to use.[31] Goddard-Borger and coworkers envisaged that imidazole-1-sulfonyl azide **243** (Fig. 5.2.1) would mimic TfN_3 in its ability to act as a diazotransfer reagent but might also be less costly to prepare, more stable, and produce more easily removed byproducts.

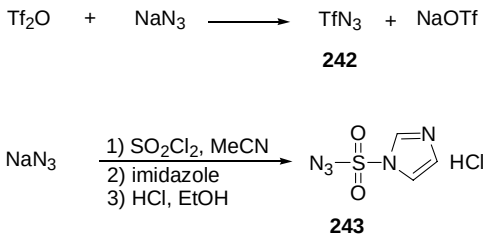


Figure 5.2.1 Diazotransfer reagents.

In our previous report, significant binding was observed for conjugates having the key α-1,2-mannose disaccharide units to the 2G12 antibody. Only conjugates bearing an 11 atom spacer between the two disaccharide units displayed binding affinity.[1] In order to further study and improve the binding affinity of synthetic dimannose with 2G12 antibody, we decided to selectively modify the mannose residue at the 6-position with azido function group followed by the highly efficient Cu^I-catalyzed 1,3-dipolar azide-alkyne cycloaddition during the the process of PNA-encoded combinatorial synthesis. The anchoring of the saccharide units to scaffolds (PNA) depends critically on the introduction of an appropriately functionalized linker moiety onto the saccharide unit.[32] Allyl alcohol can be efficiently attached to the reducing end of the saccharide in only a few steps.[33] The allyl moiety can be easily functionalized to a carboxylic acid.[34] The PNA-encoded methodology allows for combinatorial libraries synthesized in a split and mix format to be organized into microarrays by a self-sorting assembly.[18,35]

For the purpose at hand, we developed convenient synthetic path to synthesize azidodimannose building block containing carboxylic acid linker. Carbohydrate derivatives will be efficiently coupled to polymer-bound PNAs at the C terminus or the N terminus through amide bond formation. D-Glucosamine hydrochloride **S5-1** was treated with Troc-Cl under $NaHCO_3$ solution, following acetylation, to give **S5-2**. Compound **S5-2** was treated with allyl alcohol in the presence of $BF_3{\cdot}OEt_2$ and then the trichloroethoxycarbonyl group was removed with zinc and acetic acid in THF to obtain **S5-4**. Diazotransfer reagent **243**[27] was added to a stirred suspension of the amine **S5-4**. Upon completion of the reaction (TLC), deacetylation mixtures were obtained. The mixture was concentrated and then acetic anhydride was added in pyridine to get **S5-5**. Oxidative cleavage of an olefin can be achieved by various procedures leading to the carboxylic acid. Allyl azidoglucoside **S5-5** was treated with oxidant in CH_2Cl_2/MeCN/H_2O to give the corresponding carboxylic acid derivative **S5-6** in 72% (Fig. 5.2.2).

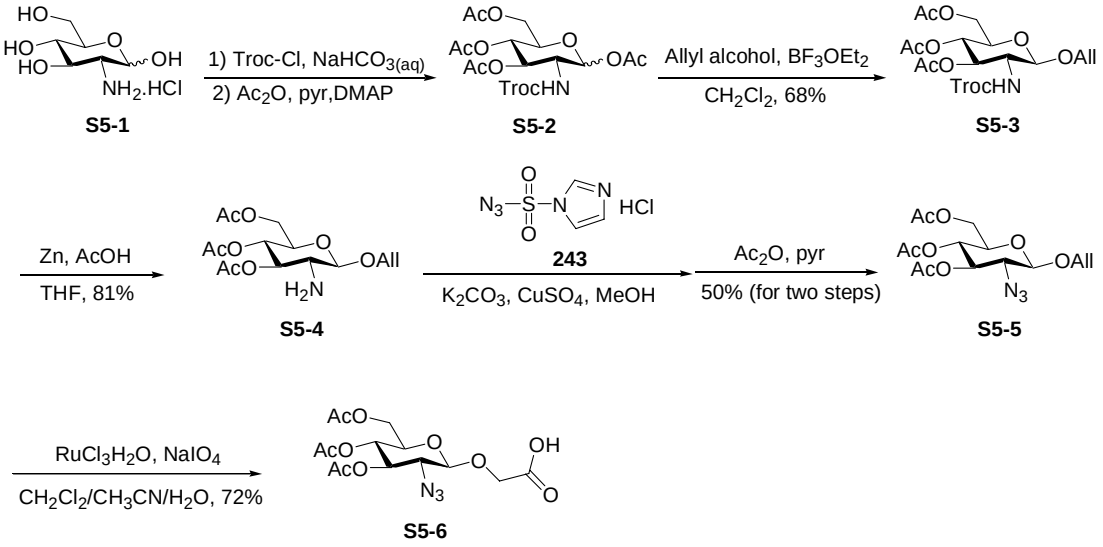


Figure 5.2.2 Synthesis of carboxyl linker containing azideglucose.

D-Mannose was treated with 4-toluenesulfonyl chloride in the presence of pyridine, followed by acetylation.[39] After the bromination,[39] Compound **S5-8** was treated with $NaBH_4$ in acetronitrile to obtain **S5-10**. Azide ion displaced the tosyl group in the 6-position to afford **S5-11**. Acetate protecting groups were removed under Zemplen conditions (NaOMe-MeOH) to produce **S5-12**, followed by the benzoylation in pyridine. Acid hydrolysis of ethylidene protection group yielded **S5-14**. **S5-16** was prepared by the reaction of compound **S5-14** through peracetylation and allylation. The 2-*O*-acetyl group in **S5-16** was selectively removed by mild acidic hydrolysis in

MeOH-CH_2Cl_2 to give the intermediate 2-OH acceptor **S5-17**[40] (Fig. 5.2.3).

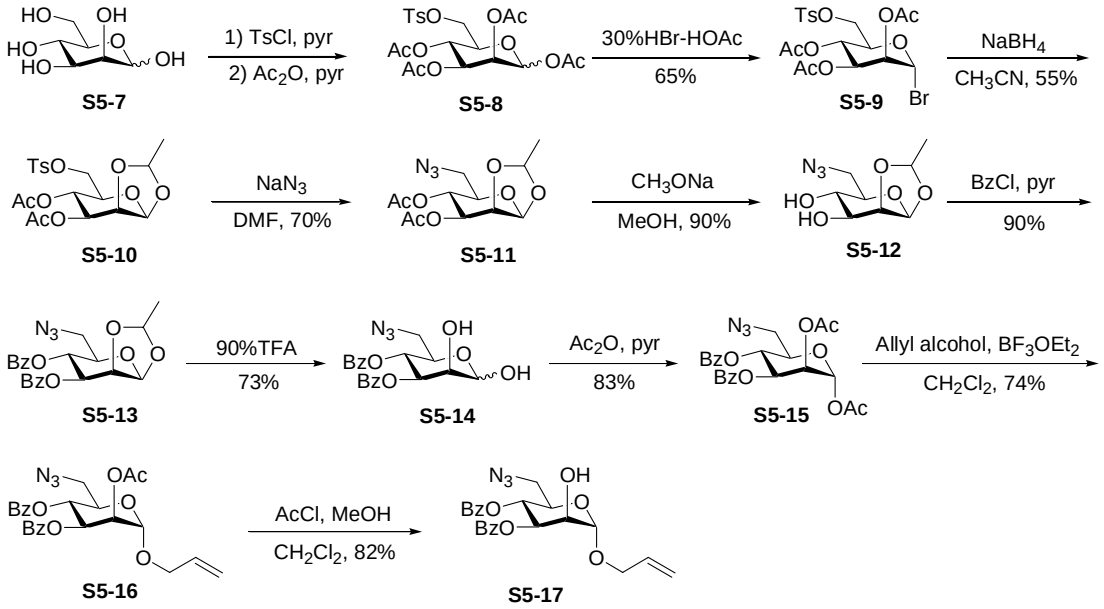


Figure 5.2.3 Synthesis of building block.

1.2-ethylidene protection group was selectively removed by treatment with 90%TFA to yield compound **S5-18**. The remaining free hydroxyl groups were treated with acetic anhydride in the presence of pyridine to get compound **S5-19**. Anomeric deacetylation with hydrazine acetate and reaction with trichloroacetonitrile afforded azidomannosyl trichloroacetimidate **S5-21** (Fig. 5.3.4).[36,37]

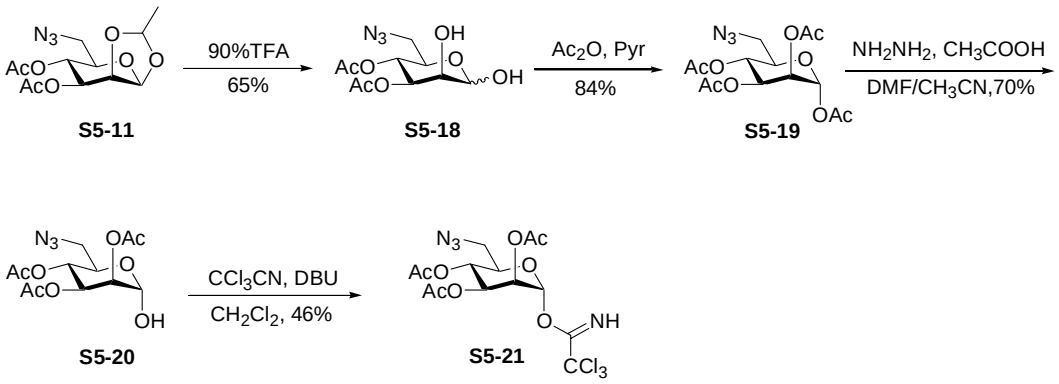


Figure 5.2.4 Synthesis of building block.

Synthesis of disaccharides employed the standard TMSOTf catalyzed glycosylation between the mannosyl donors[41] and acceptors[1]. Oxidation of the allyl dimannoside used oxidant to give azidedimannose containing carboxyl acid linker (Fig. 5.2.5).[34]

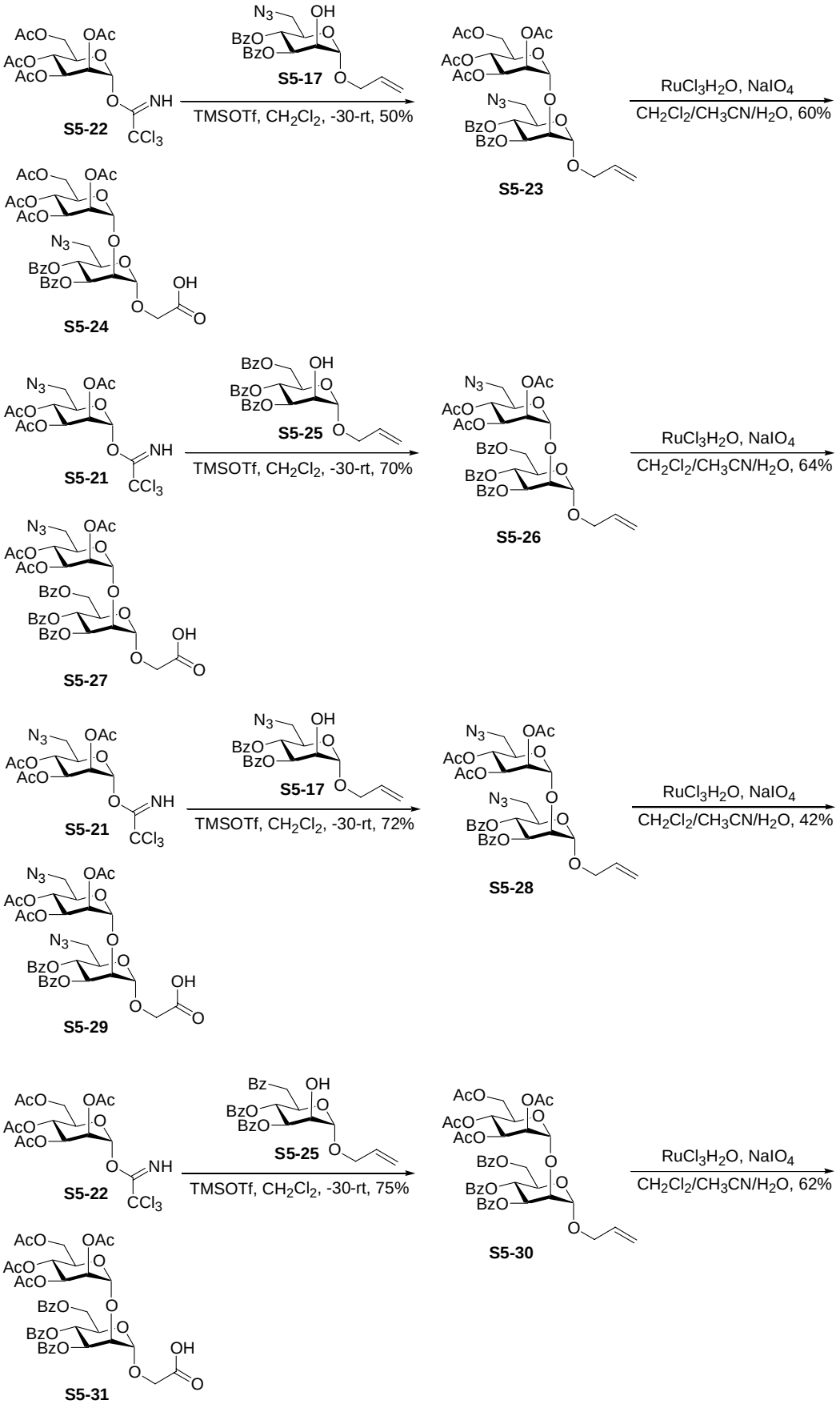


Figure 5.2.5 Synthesis of azidedimannose containing carboxyl acid linker.

5.3 Experimental section

General Procedure for glycosyl trichloroacetimidate glycosylation **(S5-23)**, **(S5-26)**, **(S5-28)**, **(S5-30)**

Glycosyl donor (0.69 mmol) and glycosyl trichloroacetimidate (1.03 mmol) were dissolved in CH_2Cl_2 (15 mL). The solution was cooled to -30°C and treated with TMSOTf (31 μL, 0.17 mmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was neutralized by Et_3N (0.1 mL) and then concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound.

1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (S5-2)[38]: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.15 (s, 3H), 3.98-4.04 (m, 2H), 4.13-4.24 (m, 2H), 4.58 (d, J = 12.0 Hz, 1H), 4.78 (d, J = 12.0 Hz, 1H), 5.14 (t, J = 10.0 Hz, 1H), 5.25 (t, J = 10.0 Hz, 1H), 5.53 (d, J = 9.2 Hz, 1H), 6.18 (d, J = 3.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 20.8, 53.1, 61.5, 67.6, 69.6, 70.3, 74.5, 90.4, 95.3, 154.1, 168.7, 169.2, 170.6, 171.1; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{17}H_{22}O_{11}NCl_3Na$ $(M+Na)^+$: 544.0156; found 544.0104.

Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-*β*-D-glucopyranose (S5-3): Compound **S5-2** (9.02 mmol) was dissolved in CH_2Cl_2 (50 mL), cooled to 0°C and allyl alcohol (18.05 mmol) was added. BF_3Et_2O (36.1 mmol) was added dropwise over the course of 5 min, and the reaction mixture was stirred at room temperature for 24 h. The mixture was neutralized with saturated aqueous sodium bicarbonate solution and diluted with CH_2Cl_2. After washing with water and brine, the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S5-3** (68%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.05 (s, 3H), 2.12 (s, 3H), 4.00-4.14 (m, 4H), 4.22 (dd, J = 12.6, 5.2 Hz, 1H), 4.28 (dd, J = 12.2, 4.8 Hz, 1H), 4.67 (d, J = 12.0 Hz, 1H), 4.82 (d, J = 12.0 Hz, 1H), 4.96 (d, J = 4.0 Hz, 1H), 5.13 (t, J = 10.0 Hz, 1H), 5.26-5.36 (m, 4H), 5.87-5.97 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 20.7, 53.9, 61.9, 67.9, 68.2, 68.9, 71.0, 74.6, 95.4, 96.3, 118.8, 132.9, 154.2, 169.4, 170.6, 170.9; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{18}H_{24}O_{10}NCl_3Na$ $(M+Na)^+$: 542.0363; found 542.0335.

Allyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-*β*-D-glucopyranose (S5-4): Compound **S5-3** (5.40 mmol) was dissolved in THF (10 mL) and Acetic acid (10 mL). Zinc (2.0 g)

was added to the solution. After the 6 hours, the reaction was complete and the mixture was filtered through celite. The solution was concentrated and the crude product purified by flash chromatography (MeOH/$CHCl_3$) to give the compound **S5-4** (81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.86 (s, 3H), 1.91 (s, 3H), 1.92 (s, 3H), 2.78 (dd, J = 10.2, 4.0 Hz, 1H), 3.84-3.92 (m, 3H), 4.03-4.14 (m, 2H), 4.75 (d, J = 3.6 Hz, 1H), 4.79 (t, J = 10.0 Hz, 1H), 4.98 (t, J = 10.0 Hz, 1H), 5.07 (dd, J = 10.4, 1.2 Hz, 1H), 5.17 (dd, J = 17.2, 1.6 Hz, 1H), 5.73-5.83 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.5, 20.7, 54.4, 62.1, 67.7, 68.6, 68.7, 74.6, 98.7, 117.7, 133.4, 169.5, 170.3, 170.6; HRMS (MOLDI-TOF): m/z: calcd for $C_{15}H_{23}O_8NNa$ $(M+Na)^+$: 368.1321; found 368.1306.

Allyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*β*-D-glucopyranose (S5-5)[27]: Imidazole-1-sulfonyl azide hydrochloride 1.HCl (1.2 mmol) was added to the amine (1.0 mmol), K_2CO_3 (1.5 mmol) and $CuSO_4$(10 μmol) in MeOH (20 mL) and the mixture stirred at room temperature for 3 hours. The mixture was concentrated and co-evaporated with toluene. Acetic anhydride (9.0 mmol) was added to the residue in pyridine (5 mL) and the mixture stirred (2 h.). The mixture was concentrated, diluted with H_2O and extracted with EtOAc. The combined organic layers were dried with $MgSO_4$, filtered and concentrated. Flash chromatography gave the compound **S5-5** (50%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.02 (s, 6H), 3.29 (dd, J = 10.6, 3.2 Hz, 1H), 3.98-4.06 (m, 3H), 4.16-4.24 (m, 2H), 4.98 (dd, J = 11.4, 8.0 Hz, 1H), 5.21 (dd, J = 10.6, 1.2 Hz, 1H), 5.30 (dd, J = 17.2, 1.6 Hz, 1H), 5.44 (t, J = 9.6 Hz, 1H), 5.83-5.93 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 60.7, 61.7, 67.6, 68.5, 68.8, 70.3, 96.7, 118.3, 132.7, 169.5, 169.7, 170.3; HRMS (MOLDI-TOF): m/z: calcd for $C_{15}H_{21}O_8N_3Na$ $(M+Na)^+$: 394.1226; found 394.1266.

Carboxymethyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-*β*-D-glucopyranose (S5-6)[34]**:** (72%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H), 2.00 (s, 3H), 2.00 (s, 3H), 3.36 (dd, J = 10.8, 3.2 Hz, 1H), 4.09-4.13 (m, 1H), 4.16-4.31 (m, 3H), 4.97 (t, J = 9.6 Hz, 1H), 5.06 (d, J = 3.6 Hz, 1H), 5.41 (dd, J = 10.4, 9.2 Hz, 1H), 9.36 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 60.6, 61.8, 64.2, 68.1, 68.3, 70.2, 97.8, 169.9, 170.2, 171.0, 172.4; HRMS (MOLDI-TOF): m/z: calcd for $C_{14}H_{19}O_{10}N_3Na$ $(M+Na)^+$: 412.0968; found 412.0984.

3,4-di-*O*-acetyl-6-*O*-tosyl-1,2-ethylidene-*β*-D-mannopyranoside (S5-10)[39,45]**:** (55%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (d, J = 4.8 Hz, 3H), 1.92 (s, 3H), 2.01 (s, 3H), 2.36 (s, 3H), 3.68-3.71 (m, 1H), 4.00-4.09 (m, 2H), 4.16 (t, J = 2.4 Hz, 1H), 5.14-5.18 (m, 3H), 5.21 (d, J = 2.4 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H), 7.70 (d, J = 8.0 Hz, 2H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 21.3, 21.5, 66.2, 68.2, 70.1, 71.2, 77.1, 96.5, 104.4, 128.0, 129.8, 132.4, 145.0, 169.5, 170.1; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{19}H_{24}O_{10}SNa$ $(M+Na)^+$: 467.0988; found 467.1010.

3,4-di-*O*-acetyl-6-azido-1,2-ethylidene-*β*-D-mannopyranoside (S5-11)[44]**:** (70%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (d, *J* = 4.8 Hz, 3H), 1.96 (s, 3H), 2.02 (s, 3H), 3.23 (dd, *J* = 13.0, 6.0 Hz, 1H), 3.33 (dd, *J* = 13.2, 3.2 Hz, 1H), 3.57-3.61 (m, 1H), 4.17 (dd, *J* = 3.6, 2.4 Hz, 1H), 5.13-5.26 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 21.4, 51.0, 66.8, 70.3, 72.6, 77.2, 96.4, 104.6, 169.4, 170.1; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{12}H_{17}O_7N_3Na$ $(M+Na)^+$: 338.0964; found 338.0946.

6-azido-1,2-ethylidene-*β*-D-mannopyranoside (S5-12): Compound **S5-11** (31.6 mmol) was dissolved in MeOH (80 mL) and NaOMe (4.74 mmol) was added. The mixture was stirred at room temperature for 4 h. The mixture was neutralized by adding Amberlite IRC-50. After filtration, the filtrates were concentrated. The residue was purified by flash silica gel column chromatography to afford compound **S5-12** (90%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (d, *J* = 4.8 Hz, 3H), 3.38-3.45 (m, 2H), 3.56-3.61 (m, 1H), 3.73 (t, *J* = 8.8 Hz, 1H), 3.80 (dd, *J* = 7.2, 4.0 Hz, 1H), 4.14 (dd, *J* = 3.8, 2.4 Hz, 1H), 5.28-5.31 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 51.0, 67.6, 71.4, 74.5, 80.4, 96.6, 104.0; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_8H_{13}O_5N_3Na$ $(M+Na)^+$: 254.0753; found 254.0757.

3,4-di-*O*-benzoyl-6-azido-1,2-ethylidene-*β*-D-mannopyranoside (S5-13)[1]**:** (90%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (d, *J* = 4.8 Hz, 3H), 3.48 (dd, *J* = 13.2, 6.0 Hz, 1H), 3.55 (dd, *J* = 13.2, 2.8 Hz, 1H), 3.90-3.94 (m, 1H), 4.53 (dd, *J* = 3.6, 2.4 Hz, 1H), 5.35 (dd, *J* = 9.8, 4.8 Hz, 1H), 5.49 (d, *J* = 2.8 Hz, 1H), 5.64 (dd, *J* = 10.0, 4.0 Hz, 1H), 5.86 (t, *J* = 9.6 Hz, 1H), 7.39-7.43 (m, 4H), 7.52-7.57 (m, 2H), 7.96-8.04 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.5, 51.3, 67.3, 71.2, 73.2, 77.4, 96.7, 104.9, 128.5, 128.5, 128.9, 128.9, 129.8, 130.0, 133.5, 133.6, 165.4, 166.0; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{22}H_{21}O_7N_3Na$ $(M+Na)^+$: 462.1277; found 462.1257.

3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S5-14)[1]**:** (73%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (dd, *J* = 13.2, 2.8 Hz, 1H), 3.48 (dd, *J* = 13.2, 6.0 Hz, 1H), 4.26 (dd, *J* = 3.0, 2.0 Hz, 1H), 4.41-4.46 (m, 1H), 5.28 (d, *J* = 2.0 Hz, 1H), 5.68 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.80 (t, *J* = 10.0 Hz, 1H), 7.33-7.53 (m, 6H), 7.91-7.98 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.4, 68.1, 69.5, 72.6, 94.6, 128.0, 128.2, 129.1, 129.2, 129.3, 129.5, 133.0, 133.2, 165.8, 165.9; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{20}H_{19}O_7N_3Na$ $(M+Na)^+$: 436.1121; found 436.1086.

1,2-di-*O*-acetyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S5-15) [1]**:** (83%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.23 (s, 3H), 3.44 (dd, J = 13.6, 5.6 Hz, 1H), 3.53 (dd, J = 13.6, 2.8 Hz, 1H), 4.26-4.31 (m, 1H), 5.51 (dd, J = 3.2, 2.0 Hz, 1H), 5.79 (dd, J = 10.0, 3.2 Hz, 1H), 5.87 (t, J = 10.0 Hz, 1H), 6.26 (d, J = 2.0 Hz, 1H), 7.34-7.54 (m, 6H), 7.89-7.99 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.8, 50.8, 66.9, 68.6, 69.3, 72.1, 90.4, 128.5, 128.5, 128.7, 128.9, 129.6, 129.8, 133.5, 133.7, 165.3, 165.5, 168.0, 169.6; HRMS (MOLDI-TOF): m/z: calcd for $C_{24}H_{23}O_9N_3Na$ $(M+Na)^+$: 520.1332; found 520.1282

Allyl 2-*O*-acetyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S5-16): Compound **S5-15** (9.02 mmol) was dissolved in CH_2Cl_2 (50 mL), cooled to 0°C and allyl alcohol (18.05 mmol) was added. BF_3Et_2O (36.1 mmol) was added dropwise over the course of 5 min, and the reaction mixture was stirred at room temperature for 24 h. The mixture was neutralized with saturated aqueous sodium bicarbonate solution and diluted with CH_2Cl_2. After washing with water and brine, the organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S5-16** (74%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 3H), 3.42 (dd, J = 13.4, 2.4 Hz, 1H), 3.52 (dd, J = 13.4, 7.2 Hz, 1H), 4.15 (dd, J = 12.6, 6.0 Hz, 1H), 4.24-4.29 (m, 1H), 4.33 (dd, J = 12.8, 5.2 Hz, 1H), 5.04 (d, J = 2.8 Hz, 1H), 5.29 (dd, J = 10.4, 1.2 Hz, 1H), 5.41 (dd, J = 17.2, 1.6 Hz, 1H), 5.52 (dd, J = 3.2, 2.0 Hz, 1H), 5.77 (t, J = 10.0 Hz, 1H), 5.84 (dd, J = 10.0, 3.2 Hz, 1H), 5.92-6.02 (m, 1H), 7.31-7.52 (m, 6H), 7.90-7.98 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 51.2, 67.7, 68.8, 69.7, 69.9, 70.4, 96.5, 118.5, 128.4, 128.5, 128.8, 129.2, 129.6 129.8, 132.9, 133.3, 133.6, 165.3, 165.5, 169.8; HRMS (MOLDI-TOF): m/z: calcd for $C_{25}H_{25}O_8N_3Na$ $(M+Na)^+$: 518.1539; found 518.1581.

Allyl 3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranoside (S5-17)[40]**:** (82%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24 (br, 1H), 3.34 (dd, J = 12.8, 2.4 Hz, 1H), 3.57 (dd, J = 13.4, 7.6 Hz, 1H), 4.16 (dd, J = 13.0, 6.4 Hz, 1H), 4.21-4.26 (m, 1H), 4.33-4.38 (m, 2H), 5.06 (d, J = 1.6 Hz, 1H), 5.30 (dd, J = 10.4, 1.6 Hz, 1H), 5.43 (dd, J = 17.2, 1.6 Hz, 1H), 5.70 (dd, J = 9.8, 2.8 Hz, 1H), 5.82 (t, J = 10.0 Hz, 1H), 5.95-6.05 (m, 1H), 7.29-7.53 (m, 6H), 7.94-7.97 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 51.4, 67.9, 68.6, 69.3, 70.4, 72.5, 98.7, 118.3, 128.4, 128.5, 128.9, 129.2, 129.8, 133.2, 133.3, 133.5, 165.7, 165.8; HRMS (MOLDI-TOF): m/z: calcd for $C_{23}H_{23}O_7N_3Na$ $(M+Na)^+$: 476.1434; found 476.1455.

3,4-di-*O*-acetyl-6-azido-*α*/*β*-D-mannopyranoside (S5-18)[1]: (65%) HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{10}H_{15}O_7N_3Na$ $(M+Na)^+$: 312.0808; found 312.0839.

1,2,3,4-tetra-*O*-acetyl-6-azido-*α*-D-mannopyranoside (S5-19)[1]: (84%) ^1H NMR (400 MHz, $CDCl_3$) δ 1.92 (s, 3H), 1.97 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 3.23 (dd, J = 13.6, 5.6 Hz, 1H), 3.32 (dd, J = 13.6, 3.2 Hz, 1H), 3.92-3.93 (m, 1H), 5.16 (t, J = 2.0 Hz, 1H), 5.24-5.25 (m, 2H), 6.00 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.5, 20.6, 20.6, 50.5, 66.3, 68.2, 68.5, 71.7, 90.2, 168.0, 169.4, 169.6, 169.8; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{14}H_{19}O_9N_3Na$ $(M+Na)^+$: 396.1019; found 396.1018.

2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranoside (S5-20)[43]: (70%) ^1H NMR (400 MHz, $CDCl_3$) δ 1.93 (s, 3H), 1.99 (s, 3H), 2.10 (s, 3H), 3.24-3.33 (m, 2H), 4.11-4.16 (m, 1H), 4.82 (d, J = 3.6 Hz, 1H), 5.15-5.20 (m, 3H), 5.33 (dd, J = 10.0, 2.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.8, 50.9, 67.2, 68.8, 69.4, 70.2, 91.7, 170.2, 170.4, 170.5; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{12}H_{17}O_8N_3Na$ $(M+Na)^+$: 354.0913; found 354.0927.

2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl trichloroacetimidate (S5-21): Compound **S5-20** (0.58 mmol) was dissolved in CH_2Cl_2 (8 mL), then trichloroacetonitrile (1.74 mmol) and DBU (0.174) were added. The reaction mixture was stirred at room temperature overnight. The solution was concentrated *in vacuo* and the residue was purified by flash silica gel column chromatography (2:1 petroleum ether-EtOAc) to afford compound **S5-21** (46%); ^1H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.01 (s, 3H), 2.14 (s, 3H), 3.27-3.36 (m, 2H), 4.09-4.12 (m, 1H), 5.29-5.36 (m, 2H), 5.40 (t, J = 2.0 Hz, 1H), 6.24 (d, J = 1.2 Hz, 1H), 8.84 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 50.6, 66.2, 67.7, 68.5, 72.4, 90.4, 94.1, 159.4, 169.5, 169.6, 169.7;

(2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (S5-23): (50%) ^1H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 3.37 (dd, J = 13.4, 2.0 Hz, 1H), 3.52 (dd, J = 13.6, 3.2 Hz, 1H), 4.08-4.35 (m, 7H), 4.94 (d, J = 1.6 Hz, 1H), 5.11 (d, J = 1.6 Hz, 1H), 5.24-5.42 (m, 4H), 5.47 (dd, J = 10.0, 3.2 Hz, 1H), 5.68 (t, J = 10.0 Hz, 1H), 5.78 (dd, J = 10.2, 3.2 Hz, 1H), 5.91-6.01 (m, 1H), 7.32-7.49 (m, 6H), 7.90-7.93 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 51.3, 61.0, 62.5, 66.2, 67.2, 67.5, 67.8, 68.4, 68.7, 68.8, 69.2, 69.3, 70.5, 72.6, 75.8, 76.7, 97.4, 99.4, 118.3, 128.4, 128.5, 128.8, 128.8, 129.8, 129.8, 133.0, 133.4, 165.3, 165.4, 169.4, 169.5, 169.8, 170.5;

HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{37}H_{41}O_{16}N_3Na$ $(M+Na)^+$: 806.2385; found 806.2312.

(2,3,4,6-Tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (S5-24)[34]**:** (60%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.07 (s, 3H), 3.42 (dd, *J* = 13.6, 2.4 Hz, 1H), 3.51 (dd, *J* = 9.8, 6.4 Hz, 1H), 4.13-4.29 (m, 4H), 4.38-4.44 (m, 3H), 5.01 (d, *J* = 1.2 Hz, 1H), 5.25 (s, 1H), 5.30 (t, *J* = 10.0 Hz, 1H), 5.44-5.50 (m, 2H), 5.72 (t, *J* = 10.0 Hz, 1H), 5.82 (dd, *J* = 10.0, 3.2 Hz, 1H), 7.32-7.49 (m, 6H), 7.91-7.94 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 51.2, 62.5, 64.1, 66.2, 67.6, 68.9, 69.2, 70.2, 71.0, 75.9, 98.5, 99.2, 128.4, 128.5, 128.7, 129.8, 129.9, 133.5, 165.3, 165.5, 169.7, 169.8, 170.0, 171.1, 172.5; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{36}H_{39}O_{18}N_3Na$ $(M+Na)^+$: 824.2127; found 824.2185.

(2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (S5-26): (70%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 3.28 (dd, *J* = 13.2, 2.4 Hz, 1H), 3.36 (dd, *J* = 13.4, 6.8 Hz, 1H), 4.12-4.17 (m, 2H), 4.29-4.34 (m, 2H), 4.39-4.43 (m, 1H), 4.51 (dd, *J* = 12.0, 5.2 Hz, 1H), 4.62 (dd, *J* = 12.2, 2.4 Hz, 1H), 4.98 (d, *J* = 1.2 Hz, 1H), 5.20-5.27 (m, 3H), 5.35 (dd, *J* = 17.2, 1.2 Hz, 1H), 5.46-5.48 (m, 2H), 5.87 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.92-6.02 (m, 2H), 7.29-7.53 (m, 9H), 7.92-8.08 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 51.1, 63.6, 67.1, 67.4, 68.7, 68.7, 68.8, 69.2, 70.7, 70.9, 77.0, 77.3, 97.4, 99.5, 118.3, 128.3, 128.4, 128.5, 128.9, 129.0, 129.7, 129.8, 129.9, 133.0, 133.1, 133.3, 133.4, 165.2, 165.5, 166.2, 169.4, 169.4, 169.8; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{42}H_{43}O_{16}N_3Na$ $(M+Na)^+$: 868.2541; found 868.2459.

(2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (S5-27)[34]**:** (64%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.04 (s, 6H), 3.31 (s, 2H), 4.12-4.14 (m, 1H), 4.39-4.62 (m, 6H), 5.03 (s, 1H), 5.23 (t, *J* = 9.6 Hz, 1H), 5.30 (s, 1H), 5.44-5.48 (m, 2H), 5.90 (d, *J* = 10.0 Hz, 1H), 5.99 (t, *J* = 10.0 Hz, 1H), 7.28-7.51 (m, 9H), 7.90-8.04 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 51.0, 63.5, 65.0, 67.0, 67.3, 68.7, 69.0, 69.2, 70.7, 76.6, 98.8, 99.2, 128.3, 128.4, 128.5, 128.8, 129.0, 129.6, 129.8, 129.9, 133.0, 133.3, 133.4, 165.2, 165.5, 166.5, 169.5, 170.0, 170.0, 174.9; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{41}H_{41}O_{18}N_3Na$ $(M+Na)^+$: 886.2283; found 886.2224.

(2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (S5-28): (72%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.01 (s,

3H), 2.03 (s, 3H), 2.03 (s, 3H), 3.30-3.41 (m, 3H), 3.51 (dd, *J* = 13.4, 6.8 Hz, 1H), 4.13-4.22 (m, 3H), 4.26 (t, *J* = 2.0 Hz, 1H), 4.33 (dd, *J* = 12.8, 5.2 Hz, 1H), 4.96 (d, *J* = 1.6 Hz, 1H), 5.19 (d, *J* = 1.6 Hz, 1H), 5.23 (t, *J* = 10.0 Hz, 1H), 5.29 (d, *J* = 10.4 Hz, 1H), 5.37-5.44 (m, 2H), 5.49 (dd, *J* = 9.8, 3.2 Hz, 1H), 5.71 (t, *J* = 10.0 Hz, 1H), 5.82 (dd, *J* = 10.0, 3.2 Hz, 1H), 5.92-6.02 (m, 1H), 7.31-7.49 (m, 6H), 7.91-7.95 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 20.7, 51.2, 51.3, 67.1, 67.9, 68.6, 68.7, 69.2, 70.5, 70.7, 76.9, 77.3, 97.2, 99.3, 118.4, 128.4, 128.5, 128.9, 129.8, 129.8, 133.0, 133.4, 165.3, 165.4, 169.4, 169.9; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{35}H_{38}O_{14}N_6Na$ $(M+Na)^+$: 789.2344; found 789.2356.

(2,3,4-tri-*O*-acetyl-6-azido-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4-di-*O*-benzoyl-6-azido-*α*-D-mannopyranose (S5-29)[34]**:** (42%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 3.37-3.46 (m, 4H), 4.22-4.43 (m, 5H), 5.02 (s, 1H), 5.26 (t, *J* = 10.0 Hz, 1H), 5.33 (s, 1H), 5.46 (s, 1H), 5.51 (dd, *J* = 9.8, 3.2 Hz, 1H), 5.76 (t, *J* = 10.0 Hz, 1H), 5.86 (dd, *J* = 10.0, 2.4 Hz, 1H), 7.33-7.49 (m, 6H), 7.93-7.96 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 20.7, 51.0, 51.1, 64.9, 67.1, 67.7, 68.7, 69.2, 70.3, 70.8, 76.7, 98.3, 99.2, 128.4, 128.5, 128.8, 128.9, 129.8, 129.9, 133.4, 165.3, 165.5, 169.6, 169.7, 170.0, 174.7; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{34}H_{36}O_{16}N_6Na$ $(M+Na)^+$: 807.2086; found 807.2097.

(2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-allyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (S5-30): (75%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.01 (s, 6H), 2.05 (s, 3H), 4.06-4.51 (m, 7H), 4.49 (dd, *J* = 12.0, 5.6 Hz, 1H), 4.61 (dd, *J* = 12.2, 2.4 Hz, 1H), 4.97 (s, 1H), 5.13 (s, 1H), 5.23-5.36 (m, 3H), 5.45-5.48 (m, 2H), 5.84 (dd, *J* = 10.2, 3.2 Hz, 1H), 5.91-6.00 (m, 2H), 7.28-7.51 (m, 9H), 7.91-8.06 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 62.5, 63.5, 66.2, 67.3, 68.7, 68.8, 68.9, 69.2, 69.3, 70.9, 76.8, 97.6, 99.4, 118.2, 128.3, 128.4, 128.5, 128.8, 129.0, 129.7, 129.8, 129.9, 133.0, 133.1, 133.3, 133.4, 165.2, 165.5, 166.2, 169.3, 169.4, 169.7, 170.4; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{44}H_{46}O_{18}Na$ $(M+Na)^+$: 885.2582; found 885.2543.

(2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosyl)-(1→2)-carboxylmethyl-3,4,6-tri-*O*-benzoyl-*α*-D-mannopyranose (S5-31)[34]**:** (62%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 4.14-4.23 (m, 3H), 4.35-4.48 (m, 5H), 4.60 (d, *J* = 9.2 Hz, 1H), 5.02, (s, 1H), 5.27-5.47 (m, 4H), 5.86 (d, *J* = 10.0 Hz, 1H), 6.00 (t, *J* = 10.0 Hz, 1H), 7.28-7.52 (m, 9H), 7.90-8.05 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 62.5, 63.4, 64.9, 66.2, 67.2, 68.9, 69.1, 69.2, 70.7, 76.3, 98.5, 99.4, 128.3, 128.5, 128.5, 128.8, 129.0, 129.6, 129.7, 129.8, 129.9, 133.0, 133.2, 133.4,

165.1, 165.5, 166.4, 169.4, 169.5, 169.8, 171.5, 14.7; HRMS (MOLDI-TOF): *m*/*z*: calcd for $C_{43}H_{44}O_{20}Na$ $(M+Na)^+$: 903.2324; found 903.2303

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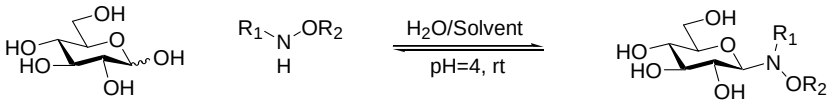
6. Glycan Fragment for the Combinatorial Self-Assembly into Microarrays

6.1 Oxime and hydrazone ligation of carbohydrates to PNA

6.1.1 Introduction

The critical role of specific oligosaccharide structures in the biological function of many glycoproteins is well appreciated. The development of elegant chemical and enzymatic approaches for the construction of glycoproteins with defined glycoforms has been highlighted.[1-4] However, the regiospecific chemical approach to glycoconjugates remains a challenging task due to the requirement for extensive protecting group manipulations and the chemical sensitivity of glycosidic linkages. Methods employing the chemoselective bond formation have been introduced for rapid access to neoglycopeptides. Chemoselective ligation methodologies are based on the introduction of reactive moieties on to fragments that makes coupling of these fragments possible in aqueous solution without protecting groups or activation procedures.[5-10] Dumy and coworkers described a general method for the stereoselective coupling of unprotected oligosaccharides with substrate containing a *N,O*-disubstituted hydroxylamine group.[8] The reaction makes use of oxime bond chemistry to stereoselectively glycosylated molecules such as peptides or lipids with anaturally or chemically accessible oligosaccharides (Fig. 6.1.1.1). Alkyl *N,O*-disubstituted hydroxylamine **224** were reacted with available carbohydrate molecules **245a-g**. The reaction proceeded under mild conditions in aqueous buffer (pH 4) or in polar organic solvents (generally a mixture of acetic acid and DMF).

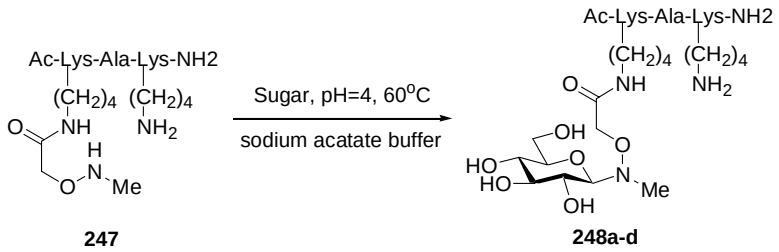


245 a **244** **246**

Product	Carbohydrate **245a-g**	R1	R2	Times (h)	Yield (%)
246a	Glucose	Et	Bn	20	90
246b	Glucose	Me	Me	20	92
246c	GluNAc	Me	Me	20	40
246d	Galatose	Et	Bn	20	80
246e	Lactose	Me	Me	60	95
246f	Mannose	Me	Me	144	60 (α)
246g	Glucose	Octyl	Me	20	30

Figure 6.1.1.1 Glycosylation of *N,O*-substituted hydroxylamines.

The N(Me)-O-peptide **247** was also exploited to the *N*-glycosylation. The desired glycopeptides **248a-d** were obtained in moderate to good yields with the *β*-form (Fig. 6.1.1.2).



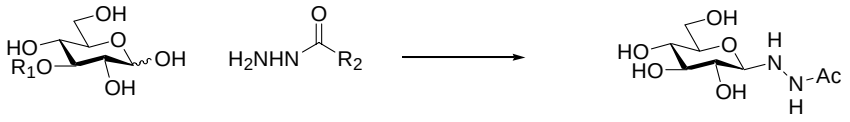
247 **248a-d**

Product	Carbohydrate	Times (h)	Yield (%)
248a	Glucose	48	62
248b	Lactose	144	57
248c	Maltotriose	144	35
248d	GalNAc	48	70

Figure 6.1.1.2 Glycosylation of aminooxy-peptide.

6.1.2 Results and discussion

As indicated above, hydrazone ligation of carbohydrate to PNA was explored in homogeneous (solution phase) and heterogeneous (solid phase) conditions. Glucose and lactose were used for the formation in different temperatures and reaction times. The better condition was shown in entry 14 (fig, 6.1.2.1).



251 **252** **253**

Entry	R1	R_2	condition	Temperature (^{o}C)	SM/Product
1	Glucose (3 eq)	Me	Sodium Phosphate buffer (pH=5.0)/ 24 h	50	5/2
2	Glucose (3 eq)	Me	Sodium Phosphate buffer (pH=5.0)/ 24 h	60	6/5
3	Glucose (10 eq)	PNA(resin)	Sodium Phosphate buffer (pH=5.0)/ 15 h	50	SM (major)
4	Glucose (10 eq)	PNA(resin)	Sodium Phosphate buffer (pH=5.0)/ 48 h	60	SM (major)
5	Glucose (10 eq)	PNA(resin)	Sodium Phosphate buffer (pH=5.0)/ 48 h	80	unknown
6	Glucose (30 eq)	PNA(resin)	Sodium Phosphate buffer (pH=5.0)/ 48 h	60	SM (major)
7	Glucose (30 eq)	PNA(resin)	Buffer/DMF=1/1 15 h	60	SM (major)
8	Lactose (10 eq)	PNA(TT)	Ammonium acetate buffer Aniline/ 2 h	50	1/1
9	Lactose (10 eq)	PNA(TT)	Ammonium acetate buffer Aniline/ 20 h	50	1/1
10	Lactose (10 eq)	PNA(TT)	Ammonium acetate buffer Aniline/ 20 h	60	1/1
11	Glucose(2 eq)	PNA(TT)	Sodium Phosphate buffer Aniline/ 20 h	60	2/3
12	Lactose (2 eq)	PNA(TT)	Sodium Phosphate buffer Aniline/ 20 h	60	1/1
13	Lactose (10 eq)	PNA(TT)	Sodium Phosphate buffer Aniline/ 20 h	80	unknown
14	Lactose (10 eq)	PNA(TC)	Sodium Phosphate buffer 20 h	60	1/4
15	Lactose (10 eq)	PNA(TC)	Sodium Phosphate buffer 20 h	80	unknown

Figure 6.1.2.1 Hydrazone formation ligation of carbohydrates to PNA.

6.2 Synthesis of thioglycan fragment for the combinatorial self-assembly into microarrays

6.2.1 Introduction

Microarray technologies have enabled a tremendous miniaturization and have instigated its implementation in a number of screening contexts beyond their original application for oligonucleotides ("DNA chips").[11,12] Microarrays containing immobilized carbohydrates ("glycan arrays") have been particularly useful to examine the binding selectivity of carbohydrate binding proteins while minimizing sample requirements.[13-16] The development of glycan arrays has enabled the high-sensitivity and high-throughput analysis of carbohydrate-protein interactions and contributed to significant advances in glycomics. However, acquiring a large collection of glycans suitably derivatized for chemoselective immobilization in a microarray format is a formidable challenge which has restrained most studies to focused glycan libraries. Remarkable efforts from the Consortium for Functional Glycomics and its collaborators have yielded arrays[17] which currently contain 465 glycans (http://www.functionalglycomics.org/). Recent advances in the area have taken place on two fronts: methodologies to attach glycans to the microarray surface[18-20] and synthesis of glycans.[21-24] Glycan arrays are not only a powerful tool for basic research, but also a promising technique for medical diagnosis, and detection of pathogens and cancers.[16] We have developed a supramolecular immobilization based on programmable hybridization of peptide nucleic acid[25] (PNA)-tagged small molecules with readily available DNA microarrays.[26-30] For our purpose, simple procedure affording the acetylated thioglycan was found to be more practical. We report the application of this strategy to combinatorially assemble two libraries of 25 thioglycan fragments into 625 discrete combinations (Fig. 6.2.1.1).

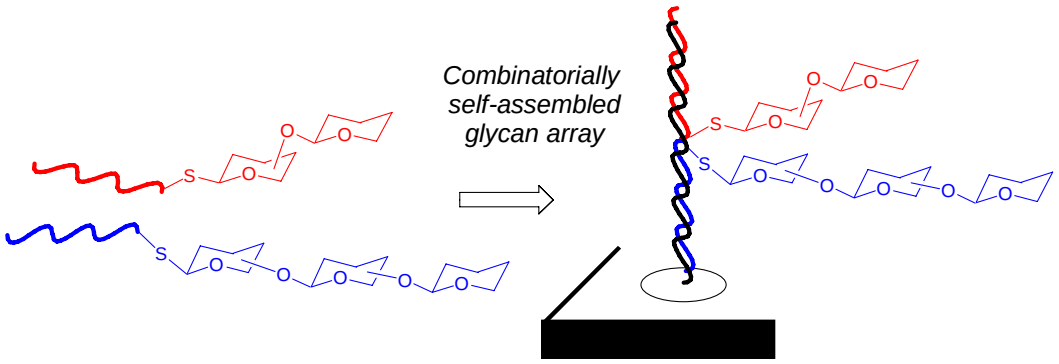


Figure 6.2.1.1 Two libraries of PNA-encoded carbohydrate fragments combinatorially self assemble into a microarray.

6.2.2 Results and discussion

We had previously shown that carbohydrates bearing a thiol at the anomeric position (glycosyl thiols) could be smoothly coupled to PNAs derivatives with a chloroacetamide using mild bases.[31] In this previous work, the glycans were prepared by classical oligosaccharide synthesis requiring laborious protecting group manipulations. Several technologies have been reported to prepare glycosyl thiols from native oligosaccharides including direct conversion of native reducing carbohydrates with Lawesson's reagent.[32] For our purpose, acetyl-protected glycans are required in some cases and a two to three steps procedure affording the acetylated thioglycan was found to be more practical. The sequence involved peracetylation of the native reducing carbohydrate, formation of a glycosyl halide (in the case of 2-aminoglucose, the first two transformation can be carried out concomitantly) and displacement with potassium thioacetate. This sequence could be carried out without intermittent purification and was practical on milligram to gram scale. As shown in (Fig. 6.2.2.1), for carbohydrates bearing a 2-amino group on the reducing saccharide, the formation of the glycosyl halide was achieved with the HCl generated in situ by the acetylation with neat AcCl[33] (2 equiv per hydroxyl residue). The reaction mixture was concentrated and treated with an excess of KSAc in DMF to afford the glycosyl thioacetate in good yield. For 2-amino glucose, the product was isolated in 84% yield as the β-anomer. For the more complex trisaccharide (Gal-α-1-3-Gal-α-1-4GlcNAc), the product was isolated in 46% yield. For other carbohydrates, the peracetylation with Ac_2O in pyridine was followed by a treatment with TMSI (1.2 equiv),[34] concentration of the reaction mixture and treatment with KSAc in DMF afforded the glycosyl thioacetate. For monosaccharides, the worst yields were obtained for mannose (56%). The reaction yielded exclusively the β-anomer except for manose and xylose where 10:1 and 5:1 mixtures in favor of the β-anomer were obtained respectively. More complex carbohydrates generally performed well with the same trend as observed for monosaccharides, carbohydrates bearing a mannose at the reducing end afforded poorer yields. The only identified side products in the reaction were the corresponding lactols due to adverse hydrolysis. DNA microarrays to sort carbohydrate-DNA conjugates have been reported,[35,36] previous efforts were restricted to monosaccharides and did not explore a broad diversity of glycan structures nor their combinatorial assembly. Importantly, the PNA-tagged glycans can be readily prepared from native glycans obtained from natural or commercial sources by conversion of the anomeric position into a thiol via a two to three step process. The simplicity of the protocol described should make glycan arrays more broadly accessible.

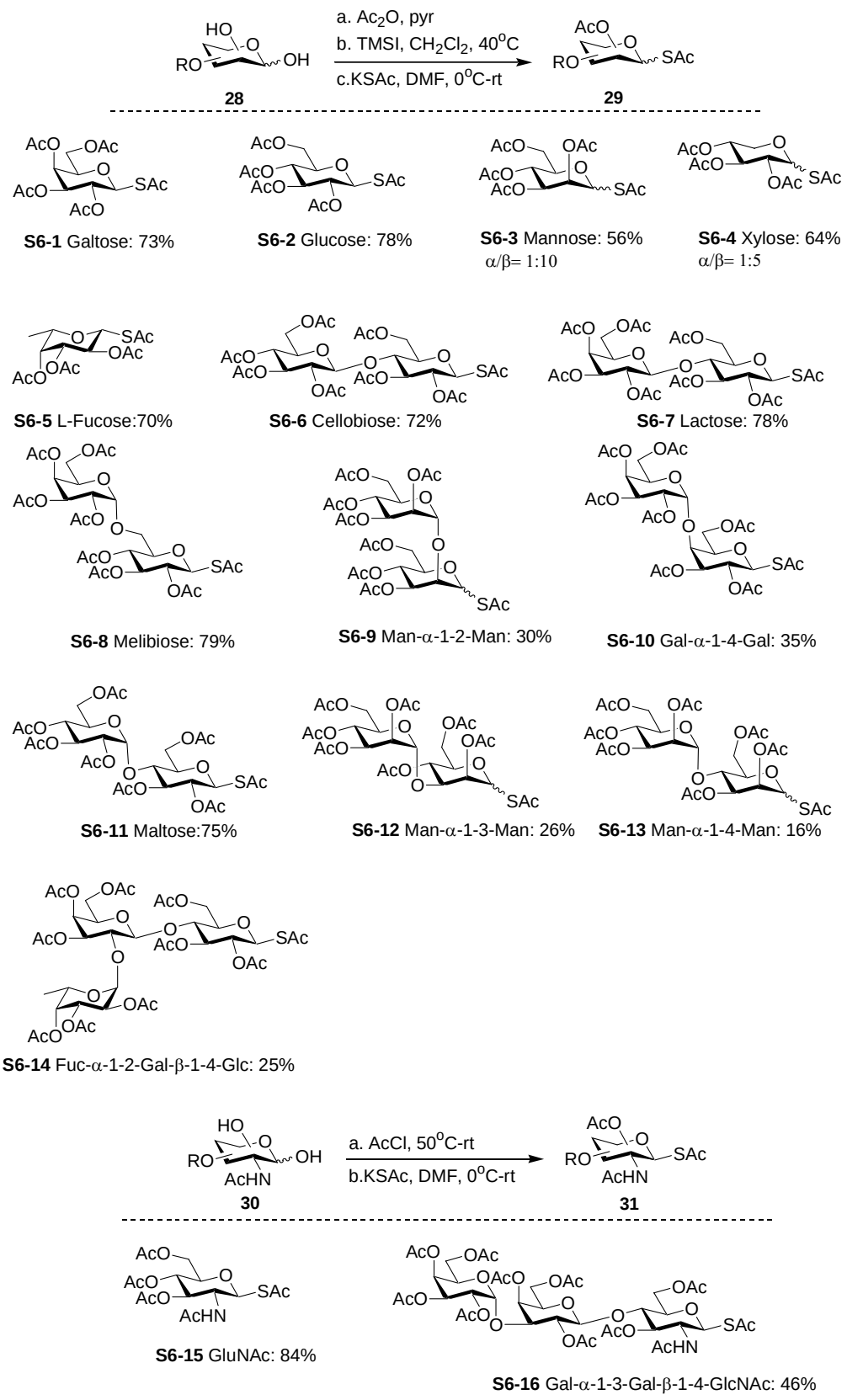


Figure 6.2.2.1 Synthesis of glycosyl thioacetate.

Our previous experience had shown that a 10mer PNA was sufficient to get stable hybridization on DNA microarrays at room temperature.[31] The sequences assigned to each member of the library were based on a previously designed codon system validated to have homogeneous melting temperatures (Tm) and designed to position both glycan fragments on adjacent nucleotides.[27] To validate the combinatorial self assembly of the two glycan fragment libraries (Fig. 6.2.2.2), concanavalin A (ConA) was selected as this carbohydrate-binding protein has been profiled with different glycan array technologies[17,20,48] and would thus offer a broad range of comparison. This lectin is well known to bind preferentially terminal α-mannose and to a lesser extent terminal α-glucose.[49,50] The microarray was incubated with two different library concentrations (4 and 16 nM) for 8 h then treated with the ConA labeled with a Cy5 at two different concentrations (25 and 100 nM) for two hours.

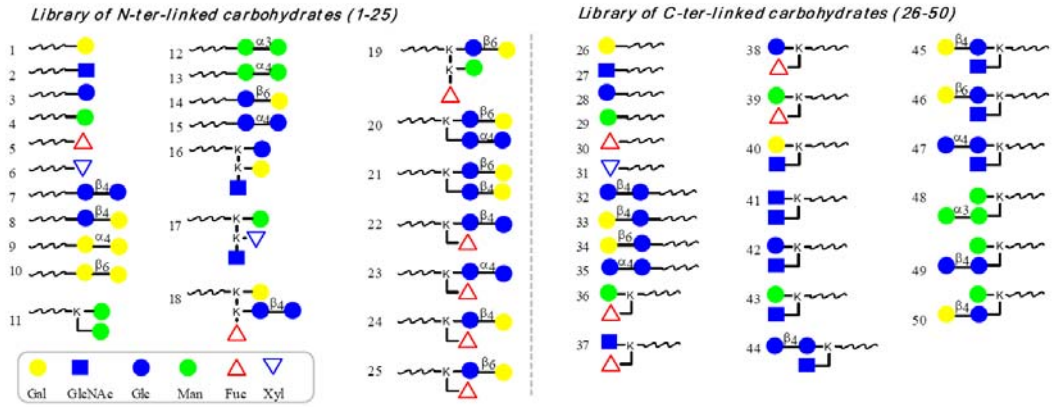


Figure 6.2.2.2 Structure of PNA encoded carbohydrate libraries.

Figure 6.2.2.3 shows a histogram of fluorescence intensity for the highest concentration (16 nM of library and 100 nM of ConA). As evident from the relative intensities, glycan fragment **12** (Manα1-3Man) is preferred amongst **1-25** followed by **20** [Glcα1,4-Glc-(Galβ1,6-Glc)-K] while glycan **48** [Manα1,3-Man-(Man)-K] is the preferred glycan fragment amongst **26-50** (the pattern is evident from the periodicity of peaks corresponding to fragment **48**). These observations are highly consistent with the known specificity of ConA. Several crystallographic structures of ConA with trisaccharides have highlighted the importance of the Manα1,3Man configuration.[51, 52] Indeed, fragment **13** (Manα1-4Man) does not show notable binding relatively to other fragments. The secondary preference for an α-linked glucose is again consistent with structural information and previous microarray data. However, the important question is whether both fragments do cooperate in their binding and the combinatorial self assembly provides more information than the sum of individual

libraries. The highest intensity spot is for the combination of **12**+**48**. Thus while glycan fragment **12** is sufficient to achieve tight binding, there is a clear trend amongst the different combinations of **12** with **26-50**. The second best combination is fragments **12**+**47** which stems from the cooperativity between a Manα1-3Man and Glcα1-4Glc. On the other hand, the combination of **12** with a fragment containing the same glycosidic units but with a β-linked glycoside (**44**) is significantly less intense. The importance of cooperativity is corroborated by its recurrence in distinct pairs (**20**+**48** and **15**+**48**). Thus while fragment **12** cooperates most efficiently with another oligomannose fragment (**48**), the second most productive cooperation is with an α1,4-linked glucose fragment (**47**). Similarly, if one considers the 25 permutions of **48** with **1**-**25**, after the aforementioned **48**+**12**, the most productive two are **48**+**20** and **48**+**15** wherein both **15** and **20** contain a terminal α-1,4-linked glucose. It is interesting to note that this pattern is identical for the arrays prepared using a lower concentration of fragments as well as a lower concentration of protein albeit with lower overall intensities.

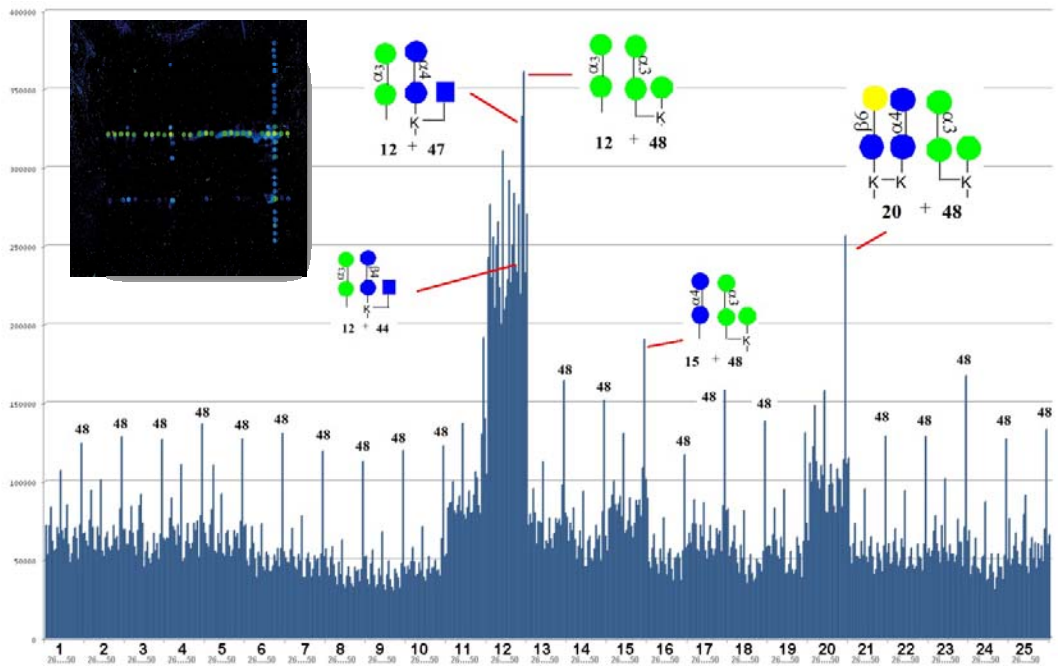


Figure 6.2.2.3 Fluorescence intensity for the highest concentration.

In summary, we've demonstrated that complex glycan arrays can be accessed by the combinatorial self assembly of PNA-encoded carbohydrate fragments. While the use of DNA microarrays to sort carbohydrate-DNA conjugates have been reported,[53, 54] previous efforts were restricted to monosaccharides and did not explore a broad diversity of glycan structures nor their combinatorial assembly. The cooperativity of the fragments in their interaction with carbohydrate-binding proteins was

demonstrated with concanavalin A. The binding profile showed the strongest interaction for a unique combination of two fragments. Importantly, the PNA-tagged glycans can be readily prepared from native oligosaccharides obtained from natural or commercial sources by conversion of the anomeric position into a thiol via a two to three step process. The simplicity of the protocols described should make glycan arrays more broadly accessible. The immobilization of the glycans by hybridization offers a reliable approach to obtain a homogeneous distribution of ligands within a microarray spot and allows the use of microarrays with higher density than accessible by contact printing which is the currently standard practice. Last but not least, combinatorial self assembly of fragments onto a DNA microarrays should be widely applicable beyond glycan fragments described herein.

6.2.3 Experimental section

General procedure for the synthesis of per-O-acetyl-*N*-acetyl-1-thioacetyl glycoside[33] The glycoside (1.0 mmol) was dissolved in AcCl (2 equiv per hydroxyl group). The solution was heated for 24 hours at 50°C. The solution was concentrated in vacuo and then residues were coevaporated three times with toluene. The product was dissolved in DMF, cooled to 0°C and reacted with KSAc (1.2 mmol). The reaction was stirred for 10 hours. Then the reaction mixture was diluted with ethylacetate and water. The organic layer washed with $NaHCO_3$, brine, and dried with Na_2SO_4. The organic layer was concentrated in vacuo and product purified by silica gel flash column chromatography.

General procedure for the synthesis of per-O-acetyl-1-thioacetyl glycoside[34]
To a solution of per-o-acetyl pyranoses (1.0 mmol) in dry CH_2Cl_2 (0.5M solution) was added TMSI (1.2 mmol) under nitrogen. The solution was refluxed under nitrogen for 1 hour and concentrated in vacuo. The resulting residue was dissolved in DMF, cooled to 0°C and treated with KSAc (1.2 mmol). The reaction was stirred for 10 hours, ethylacetate and water was added. The organic layer washed with $NaHCO_3$, brine, and dried with Na_2SO_4. After filtration and evapotated, the residue was purified by silica gel flash column chromatography.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-β-D-galatopyranosie) (S6-1): **(73%)** ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.09 (s, 3H), 2.34 (s, 3H), 4.01-4.08 (m, 3H), 5.08 (dd, J = 9.0, 3.6 Hz, 1H), 5.03-5.09 (m, 2H), 5.20-5.28 (m, 2H), 5.40 (d, J = 3.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 30.7, 61.2, 66.3, 67.2, 71.8, 74.9, 80.5, 169.4, 169.4, 170.1, 170.2, 192.0; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{16}H_{22}O_{10}SNa$ $(M+Na)^+$: 429.0832; found 429.0852.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) (S6-2): (78%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.02 (s, 3H), 2.34 (s, 3H), 3.79-3.83 (m, 1H), 4.04 (dd, J = 12.4, 2.0 Hz, 1H), 4.21 (dd, J = 12.8, 4.4 Hz, 1H), 5.03-5.09 (m, 2H), 5.21-5.25 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 30.8, 61.6, 67.8, 68.9, 73.9, 76.2, 80.1, 169.2, 169.3, 169.9, 170.5, 191.9; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{16}H_{22}O_{10}SNa$ $(M+Na)^+$: 429.0832; found 429.0822.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-β-D-mannopyranoside) (S6-3): **(**56%, α/β = 1/10) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.17 (s, 3H), 2.35 (s, 3H), 3.80-3.84 (m, 1H), 4.09 (dd, J = 12.4, 2.4 Hz, 1H), 4.25 (dd, J =

12.4, 5.2 Hz, 1H), 5.14 (dd, *J* = 10.2, 3.6 Hz, 1H), 5.24 (t, *J* = 10.0 Hz, 1H), 5.47 (t, *J* = 3.6 Hz, 1H), 5.49 (d, *J* = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 30.6, 62.3, 65.2, 70.6, 71.7, 76.8, 77.1, 77.4, 79.2, 169.6, 169.9, 170.7, 191.6; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{16}H_{22}O_{10}SNa$ $(M+Na)^+$: 429.0832; found 429.0799.

1-*S*-acetyl-1-thio-(2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside) (S6-4): (64%, α/β=1/5) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.03 (s, 6H), 2.36 (s, 3H), 3.52 (dd, *J* = 12.0, 8.4 Hz, 1H), 4.11 (dd, *J* = 12.2, 4.4 Hz, 1H), 4.87-4.92 (m, 1H), 4.98 (t, *J* = 8.0 Hz, 1H), 5.17 (t, *J* = 8.0 Hz, 1H), 5.34 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.6, 30.8, 65.5, 68.3, 69.1, 71.4, 80.2, 169.3, 169.5, 169.7, 192.1; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{13}H_{18}O_{10}SNa$ $(M+Na)^+$: 357.0617; found 357.0584.

1-*S*-acetyl-1-thio-(2,3,4-Tri-*O*-acetyl-β-L-fucopyranoside) (S6-5): (70%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16 (d, *J* = 6.4 Hz, 3H), 1.94 (s, 3H), 1.99 (s, 3H), 2.14 (s, 3H), 2.35 (s, 3H), 3.95 (dd, *J* = 13.0, 6.4 Hz, 1H), 5.08 (dd, *J* = 9.0, 3.6 Hz, 1H), 5.19-5.28 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.2, 20.5, 20.6, 20.7, 30.8, 66.4, 70.3, 72.3, 73.7, 80.2, 169.5, 169.8, 170.5, 192.3; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{14}H_{20}O_8SNa$ $(M+Na)^+$: 371.0777 found 371.0781.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-6): (72%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 1.98 (s, 3H), 1.99 (s, 3H), 1.99 (s, 3H), 2.01 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.34 (s, 3H), 3.62-3.66 (m, 1H), 3.71-3.80 (m, 2H), 4.02 (dd, *J* = 12.4, 2.0 Hz, 1H), 4.07 (dd, *J* = 12.2, 4.8 Hz, 1H), 4.33 (dd, *J* = 12.4, 4.8 Hz, 1H), 4.45 (dd, *J* = 12.4, 1.6 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.90 (t, *J* = 8.0 Hz, 1H), 5.00-5.05 (m, 2H), 5.11 (t, *J* = 8.8 Hz, 1H), 5.17-5.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 20.8, 30.8, 61.6, 61.8, 67.8, 69.2, 71.5, 71.9, 72.9, 73.4, 75.9, 80.0, 100.6, 168.9, 169.2, 169.5, 169.6, 170.1, 170.2, 170.4, 191.8; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1691.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-β-D-galatopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-7): (78%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3H), 1.97 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.10 (s, 3H), 2.32 (s, 3H), 3.71-3.80 (m, 2H), 3.85 (t, *J* = 6.8 Hz, 1H), 4.01-4.10 (m, 3H), 4.39 (d, *J* = 10.8 Hz, 1H), 4.44 (d, *J* = 10.0 Hz, 1H), 4.90 (dd, *J* = 10.6, 3.2 Hz, 1H), 4.96-5.07 (m, 2H), 5.16-5.23 (m, 2H), 5.29 (d, *J* = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$)

δ 20.4, 20.6, 20.7, 20.8, 30.8, 60.9, 62.0, 66.6, 68.9, 69.2, 70.7, 70.9, 73.6, 75.7, 77.2, 79.9, 100.8, 168.9, 169.5, 170.0, 170.1, 170.2, 170.3, 191.8; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1725.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-α-D-galatopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (S6-8): (79%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (s, 3H), 1.98 (s, 3H), 2.00 (s, 3H), 2.03 (s, 6H), 2.11 (s, 3H), 2.12 (s, 3H), 2.37 (s, 3H), 3.58 (dd, *J* = 11.6, 2.4 Hz, 1H), 3.68 (dd, *J* = 11.6, 4.8 Hz, 1H), 3.77-3.81 (m, 1H), 4.00-4.09 (m, 2H), 4.17 (t, *J* = 6.8 Hz, 1H), 5.01-5.10 (m, 3H), 5.13 (t, *J* = 2.8 Hz, 1H), 5.20-5.28 (m, 2H), 5.30 (dd, *J* = 10.8, 3.2 Hz, 1H), 5.41 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.7, 20.8, 30.8, 61.7, 65.6, 66.3, 67.5, 68.0, 68.0, 68.4, 69.0, 74.0, 77.3, 80.0, 96.0, 169.2, 169.3, 169.7, 170.0, 170.1, 170.3, 170.5, 191.9; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1737.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α/β-D-mannopyranoside (S6-9): (30%) HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1737.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-β-D-galatopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galatopyranoside (S6-10): (35%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.03 (s, 3H), 2.07 (s, 3H), 2.09 (s, 3H), 2.13 (s, 3H), 2.17 (s, 3H), 2.18 (s, 3H), 2.40 (s, 3H), 3.85-3.89 (m, 2H), 4.09-4.20 (m, 4H), 4.38 (dd, *J* = 12.0, 4.4 Hz, 1H), 4.45 (dd, *J* = 8.0 Hz, 1H), 4.99-5.05 (m, 2H), 5.18-5.28 (m, 3H), 5.38-5.40 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.7, 20.9, 30.8, 61.3, 63.5, 66.8, 68.6, 70.7, 74.2, 74.5, 76.4, 80.2, 101.9, 169.2, 169.5, 170.0, 170.2, 170.5, 170.7, 192.3; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1737.

1-*S*-acetyl-1-thio-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-11): (75%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95 (s, 3H), 1.95 (s, 3H), 1.96 (s, 3H), 1.98 (s, 3H), 2.01 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.33 (s, 3H), 3.78-3.81 (m, 1H), 3.90 (dd, *J* = 10.2, 2.0 Hz, 1H), 3.93-3.99 (m, 2H), 4.14-4.21 (m, 2H), 4.40 (dd, *J* = 12.2, 2.0 Hz, 1H), 4.81 (dd, *J* = 10.6, 4.0 Hz, 1H), 4.93 (t, *J* = 8.0 Hz, 1H), 5.00 (t, *J* = 10.0 Hz, 1H), 5.23-5.35 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.7, 20.8, 30.7, 61.4, 62.7, 67.9, 68.5, 69.2, 69.7, 70.0, 74.0, 72.6, 76.2, 76.5, 77.3, 79.7, 95.6, 169.3, 169.5, 169.7, 169.9, 170.3, 170.4, 170.5, 191.7; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1739.

1-*S*-acetyl-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-3,4,6-tri-*O*-acetyl-1-thio-α/β-D-mannopyranoside (S6-12): (26%) HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1733.

1-*S*-acetyl-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→4)-3,4,6-tri-*O*-acetyl-1-thio-α/β-D-mannopyranoside (S6-13): (16%) HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{28}H_{38}O_{18}SNa$ $(M+Na)^+$: 717.1677; found 717.1665.

1-*S*-acetyl-1-thio-[2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galatopyranosyl]-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-14): (25%) HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{38}H_{52}O_{24}SNa$ $(M+Na)^+$: 947.2468; found 947.2503.

1-*S*-acetyl-1-thio-(3,4,6-Tri-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside) (S6-15): (84%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92 (s, 3H), 2.04 (s, 3H), 2.04 (s, 3H), 2.07 (s, 3H), 2.37 (s, 3H), 3.82-3.85 (m, 1H), 4.10 (dd, J = 12.4, 2.0 Hz, 1H), 4.24 (dd, J = 12.4, 4.8 Hz, 1H), 4.36 (dd, J = 20.6, 10.0 Hz, 1H), 5.09-6.20 (m, 3H), 6.17 (d, J = 9.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.7, 23.1, 30.8, 51.9, 61.9, 68.0, 72.6, 72.7, 74.1, 76.4, 81.5, 169.3, 170.1, 170.7, 171.3, 193.5; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{16}H_{23}O_9SNNa$ $(M+Na)^+$: 428.0992; found 428.1818.

1-*S*-acetyl-1-thio-[2,4,6-Tri-*O*-acetyl-α-D-galatopyranosyl-(1→3)-2,3,6-tri-*O*-acetyl-β-D-galatopyranosyl]-(1→4)-3,6-di-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside: (S6-16): (46%) HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{40}H_{55}O_{25}SNNa$ $(M+Na)^+$: 1004.2666; found 1004.2638.

6.3 Synthesis of glycosyl azide for the combinatorial self-assembly into microarrays

6.3.1 Results and discussion

Glycosyl azides are one of the most important synthetic intermediates in sugar chemistry. The recent developments of click chemistry[37] and Staudinger ligation[38] have dramatically increased the potential of sugars possessing an azide function as precusors of glycoarrays[39-41] and glycoconjugates[42-44]. The introduction of an azide moiety onto the anomeric center requires protection and deprotection of the hydroxy groups. Shoda and coworkers reported a series of studies on the direct activation of unprotected sugars in aqueous media.[45-47] Various glycosyl azides have been synthesized directly in water by the reaction mediated by 2-chloro-1,3-dimethyllimidazolinium chloride (Fig. 6.3.1.1).[47]

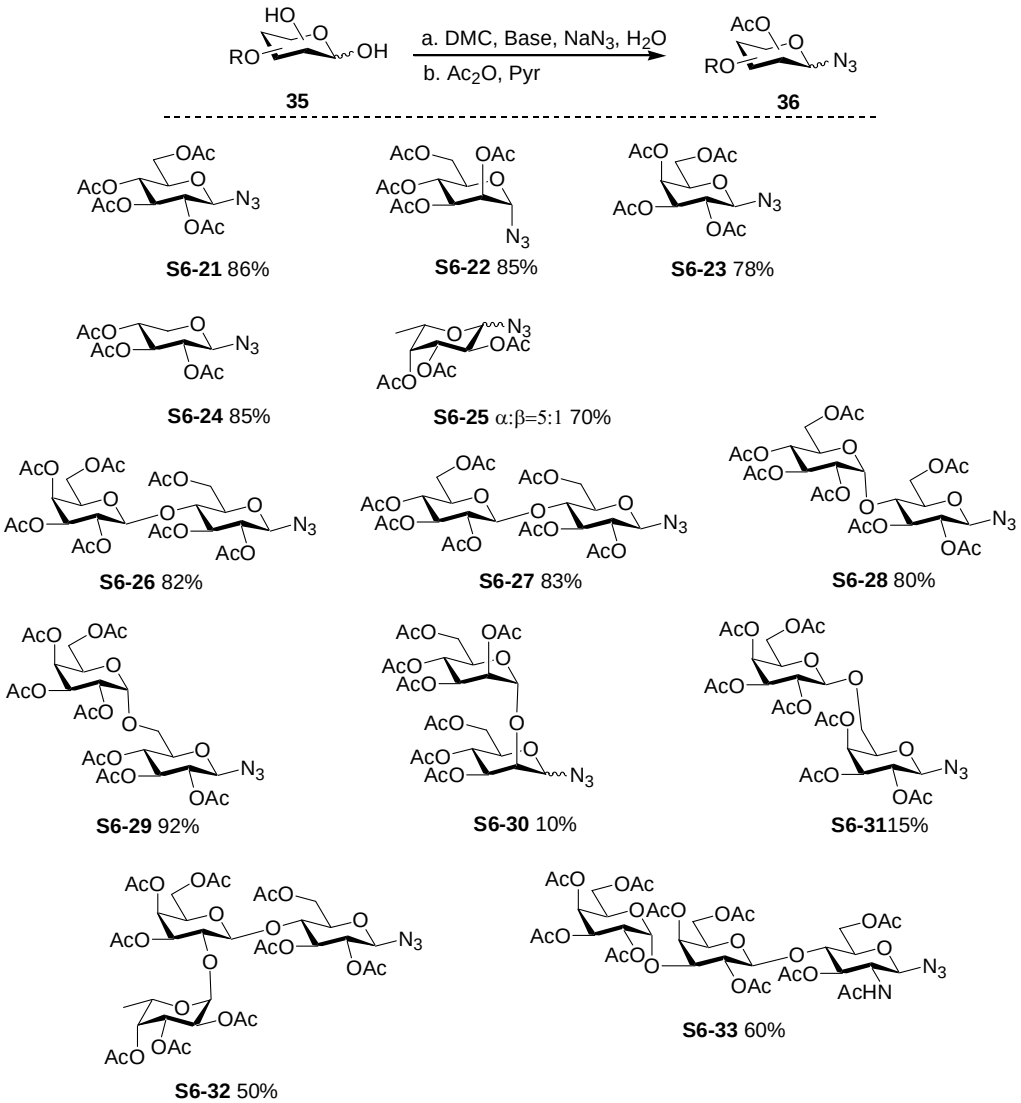


Figure 6.3.1.1 Carbohydrate libraries of azidesugar.

6.3.2 Experimental section

General procedure for the synthesis of per-O-acetyl-1-azide glycoside[47]

To a water solution of unprotected sugar, triethylamine or diisopropylethylamine (10 equiv.) and NaN_3 (10 equiv.) was added 2-chloro-1,3-dimethyllimidazolinium chloride (DMC). The resulting mixture was stirred for 2 h at 0^oC and then did the acetylation. Then the reaction mixture was diluted with ethylacetate and water. The organic layer washed with $NaHCO_3$, brine, and dried with Na_2SO_4. The organic layer was concentrated in vacuo and product purified by silica gel flash column chromatography.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside) (S6-21): (86%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 3H), 1.96 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 3.75-3.79 (m, 1H), 4.10 (dd, J = 12.4, 2.4 Hz, 1H), 4.21 (dd, J = 12.4, 4.8 Hz, 1H), 4.62 (d, J = 9.6 Hz, 1H), 4.89 (t, J = 9.6 Hz, 1H) , 5.04 (t, J = 9.6 Hz, 1H) , 5.17 (t, J = 9.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.6, 61.6, 67.8, 70.6, 72.5, 73.9, 87.7, 169.1, 169.2, 169.9, 170.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{14}H_{19}O_9N_3Na$ $(M+Na)^+$: 396.1020; found 396.0992.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside) (S6-22): (85%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3H), 1.97 (s, 3H), 2.02 (s, 3H), 2.08 (s, 3H), 4.06-4.10 (m, 2H), 4.22 (dd, J = 12.8, 5.6 Hz, 1H), 5.07 (t, J = 2.4 Hz, 1H), 5.15 (dd, J = 9.6, 3.6 Hz, 1H), 5.20 (t, J = 10.0 Hz, 1H) , 5.34 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 20.5, 20.6, 62.0, 65.5, 68.2, 69.0, 70.5, 87.3, 169.5, 169.6, 169.6, 170.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{14}H_{19}O_9N_3Na$ $(M+Na)^+$: 396.1020; found 396.1043.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-β-D-galatopyranoside) (S6-23): (78%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.15 (s, 3H), 4.02 (t, J = 2.0 Hz, 1H), 4.15 (d, J = 2.0 Hz, 1H), 4.16 (d, J = 2.8 Hz, 1H), 4.60 (d, J = 9.6 Hz, 1H), 5.03 (dd, J = 10.6, 3.2 Hz, 1H), 5.14 (dd, J = 10.0, 8.8 Hz, 1H), 5.41 (d, J = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 20.5, 61.2, 66.9, 68.0, 70.6, 72.7 88.1, 169.2, 169.8, 170.0, 170.2; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{14}H_{19}O_9N_3Na$ $(M+Na)^+$: 396.1020; found 396.1049.

1-azido-(2,3,4-Tri-*O*-acetyl-β-D-xylopyranoside) (S6-24): (85%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 3.41 (dd, J = 11.6, 9.6 Hz, 1H), 4.16 (dd, J = 11.8, 5.6 Hz, 1H), 4.61 (d, J = 8.0 Hz, 1H), 4.83 (t, J = 8.8 Hz, 1H),

4.91-4.97 (m, 1H), 5.15 (t, J = 8.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.5, 20.5, 64.2, 68.3, 70.4, 71.4, 88.2, 169.2, 169.6, 169.8; HRMS (MALDI-TOF): m/z: calcd for $C_{11}H_{15}O_7N_3Na$ $(M+Na)^+$: 324.0808; found 324.0794.

1-azido-(2,3,4-Tri-*O*-acetyl-α/β-L-fucopyranoside) (S6-25): (70%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (d, J = 6.8 Hz, 3H), 1.91 (s, 3H), 2.01 (s, 3H), 2.11 (s, 3H), 3.88 (dd, J = 12.4, 6.0 Hz, 1H), 4.55 (d, J = 8.4 Hz, 1H), 4.98 (dd, J = 10.4, 3.2 Hz, 1H), 5.05 (dd, J = 10.2, 8.8 Hz, 1H), 5.19 (dd, J = 3.4, 0.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9, 20.4, 20.5, 20.6, 68.1, 69.9, 71.0, 71.4, 88.0, 169.3, 169.9, 170.4; HRMS (MALDI-TOF): m/z: calcd for $C_{12}H_{17}O_7N_3Na$ $(M+Na)^+$: 338.0965; found 338.0963.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-β-D-galatopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-26): (82%) ^{1}H NMR (400 MHz, $CDCl_3$) δ1.95 (s, 3H), 2.03 (s, 6H), 2.05 (s, 3H), 2.06 (s, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 3.69-3.72 (m, 1H), 3.81 (t, J = 9.6 Hz, 1H), 3.88 (t, J = 6.4 Hz, 1H), 4.08-4.13 (m, 3H), 4.48-4.51 (m, 2H), 4.63 (d, J = 9.2 Hz, 1H), 4.84 (t, J = 9.2 Hz, 1H), 4.95 (dd, J = 10.4, 3.2 Hz, 1H), 5.09 (dd, J = 10.4, 8.0 Hz, 1H), 5.20 (t, J = 9.6 Hz, 1H), 5.33 (d, J = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.6, 20.7, 20.7, 60.8, 61.7, 66.6, 69.1, 70.7, 70.9, 71.0, 72.5, 74.8, 75.7, 87.6, 101.0, 169.0, 169.4, 169.5, 170.0, 170.0, 170.2; HRMS (MALDI-TOF): m/z: calcd for $C_{26}H_{35}O_{17}N_3Na$ $(M+Na)^+$: 684.1865; found 684.1870.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-27): (83%) ^{1}H NMR (400 MHz, $CDCl_3$) δ1.96 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.12 (s, 3H), 3.64-3.71 (m, 2H), 3.79 (t, J = 9.6 Hz, 1H), 4.02 (dd, J = 12.6, 2.4 Hz, 1H), 4.10 (dd, J = 12.2, 4.8 Hz, 1H), 4.35 (dd, J = 12.4, 4.4 Hz, 1H), 4.50-4.53 (m, 2H), 4.61 (d, J = 8.8 Hz, 1H), 4.84 (t, J = 9.6 Hz, 1H), 4.90 (t, J = 9.2 Hz, 1H), 5.04 (t, J = 9.6 Hz, 1H), 5.10-5.19 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.5, 20.5, 20.6, 20.7, 61.5, 61.6, 67.7, 70.9, 71.5, 72.0, 72.0, 72.8, 74.8, 76.0, 87.7, 100.8, 169.0, 169.2, 169.4, 169.6, 170.1, 170.2, 170.4; HRMS (MALDI-TOF): m/z: calcd for $C_{26}H_{35}O_{17}N_3Na$ $(M+Na)^+$: 684.1865; found 684.1865.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-28): (80%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00 (s, 3H), 2.01 (s, 3H), 2.03 (s, 3H), 2.04 (s, 3H), 2.05 (s, 3H), 2.10 (s, 3H), 2.16 (s, 3H), 3.77-3.81 (m, 1H), 3.96 (dd, J = 10.2, 2.4 Hz, 1H), 4.00-4.07 (m, 2H), 4.23 (d, J = 3.6 Hz, 1H), 4.26 (d, J = 3.6 Hz, 1H), 4.51 (dd, J = 12.2, 2.4 Hz, 1H), 4.71 (d, J = 8.4 Hz, 1H), 4.79 (t, J

= 8.8 Hz, 1H), 4.86 (dd, *J* = 10.4, 4.0 Hz, 1H), 5.05 (t, *J* = 10.0 Hz, 1H), 5.26 (t, *J* = 8.8 Hz, 1H), 5.35 (t, *J* = 10.4 Hz, 1H), 5.41 (d, *J* = 4.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.7, 20.8, 61.5, 62.5, 68.0, 68.6, 69.2, 70.0, 71.5, 72.4, 74.2, 75.1, 87.4, 95.7, 169.3, 169.4, 169.9, 170.0, 170.3, 170.4; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{26}H_{35}O_{17}N_3Na$ $(M+Na)^+$: 684.1865; found 684.1867.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-α-D-galatopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (S6-29): (92%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89 (s, 3H), 1.91 (s, 3H), 1.94 (s, 3H), 1.95 (s, 3H), 1.97 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 3.55 (d, *J* = 11.2 Hz, 1H), 3.66-3.74 (m, 2H), 3.98-4.02 (m, 2H), 4.13 (t, *J* = 6.8 Hz, 1H), 4.57 (d, *J* = 8.8 Hz, 1H), 4.82 (t, *J* = 9.6 Hz, 1H), 4.98-5.05 (m, 1H), 5.08 (d, *J* = 3.6 Hz, 1H), 5.14 (t, *J* = 9.6 Hz, 1H), 5.25 (dd, *J* = 10.8, 3.2 Hz, 1H), 5.35 (d, *J* = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.4, 20.4, 20.5, 20.5, 20.6, 61.5, 65.8, 66.3, 67.3, 67.9, 68.2, 70.5, 72.5, 74.6, 87.4, 96.1, 169.0, 169.1, 169.7, 169.9, 170.0, 170.1, 170.3; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{26}H_{35}O_{17}N_3Na$ $(M+Na)^+$: 684.1865; found 684.1867.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-acetyl-α/β-D-mannopyranoside (S6-30): (10%) HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{26}H_{35}O_{17}N_3Na$ $(M+Na)^+$: 684.1865; found 684.1914.

1-azido-(2,3,4,6-Tetra-*O*-acetyl-β-D-galatopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galatopyranoside (S6-31): (15%) HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{26}H_{35}O_{17}N_3Na$ $(M+Na)^+$: 684.1865; found 684.1878.

1-azido-[2,3,4-Tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galatopyranosyl]-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (S6-32): (50%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (d, *J* = 1.2 Hz, 3H), 2.01 (s, 6H), 2.03 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 2.19 (s, 6H), 3.77-3.80 (m, 1H), 3.84-3.91 (m, 3H), 4.09-4.20 (m, 2H), 4.32 (dd, *J* = 12.2, 6.0 Hz, 1H), 4.41-4.44 (m, 2H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.67 (d, *J* = 8.8 Hz, 1H), 5.31 (d, *J* = 3.2 Hz, 1H), 5.34 (d, *J* = 2.8 Hz, 1H), 5.41 (d, *J* = 3.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5, 20.6, 20.6, 20.8, 29.7, 60.8, 62.0, 65.0, 67.0, 67.4, 68.0, 70.5, 70.8, 71.0, 71.3, 71.8, 73.4, 75.2, 88.0, 95.5, 100.2, 169.3, 169.7, 170.0, 170.1, 170.3, 170.6, 170.7; HRMS (MALDI-TOF): *m*/*z*: calcd for $C_{36}H_{49}O_{23}N_3Na$ $(M+Na)^+$: 914.2656; found 914.2679.

1-azido-[2,4,6-Tri-*O*-acetyl-α-D-galatopyranosyl-(1→3)-2,3,6-tri-*O*-acetyl-β-D-galatopyranosyl]-(1→4)-3,6-di-acetyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranoside

(S6-33): (60%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3H), 2.02 (s, 3H), 2.09 (s, 6H), 2.10 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 2.17 (s, 3H), 2.17 (s, 3H), 2.18 (s, 3H), 3.73-3.77 (m, 1H), 3.80-3.90 (m, 3H), 4.06-4.14 (m, 4H), 4.17-4.26 (m, 3H), 4.51 (d, J = 7.6 Hz, 1H), 4.51 (dd, J = 11.8, 2.4 Hz, 1H), 4.57 (d, J = 8.4 Hz, 1H), 5.07-5.21 (m, 3H), 5.27-5.31 (m, 3H), 5.76 (d, J = 9.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.5, 20.6, 20.6, 20.6, 20.7, 20.8, 23.2, 29.7, 52.9, 61.0, 61.2, 62.0, 64.6, 66.5, 66.9, 67.1, 67.6, 69.7, 70.9, 72.3, 72.7, 74.7, 75.1, 88.5, 93.5, 101.2, 169.0, 169.7, 169.9, 170.1, 170.1, 170.2, 170.3, 170.4, 170.8; HRMS (MALDI-TOF): m/z: calcd for $C_{38}H_{52}O_{24}N_4Na$ $(M+Na)^+$: 971.2871; found 971.2896.

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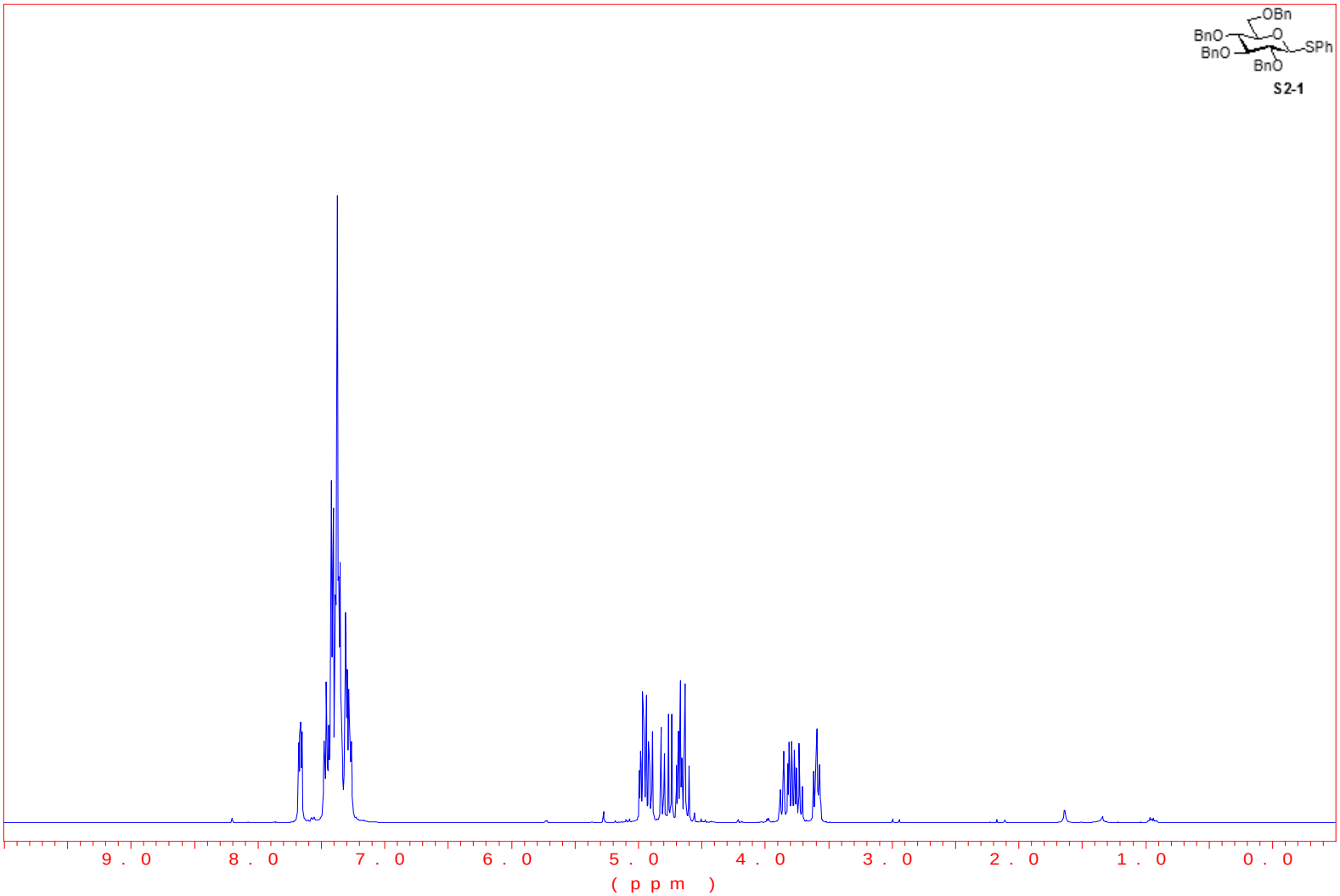
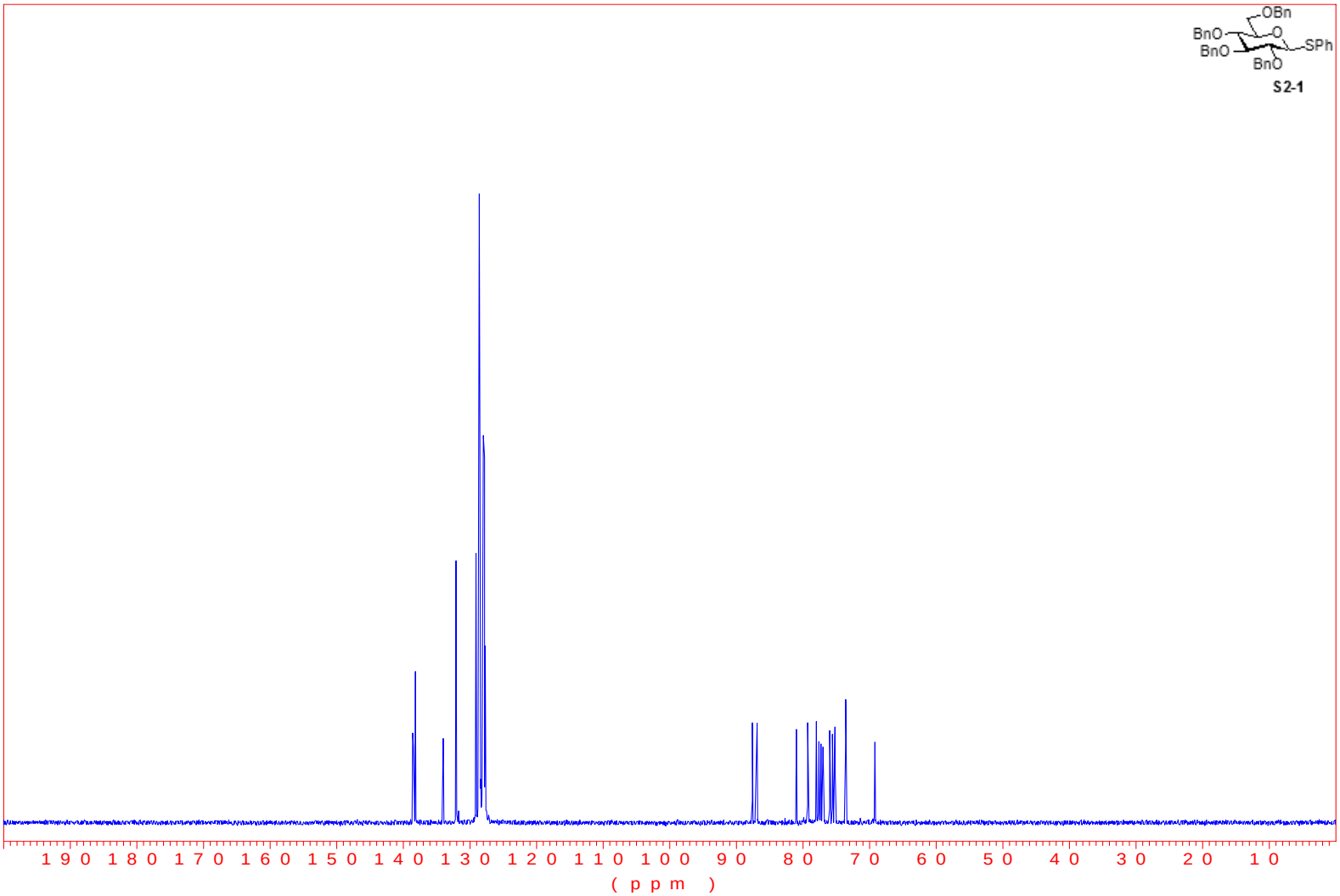
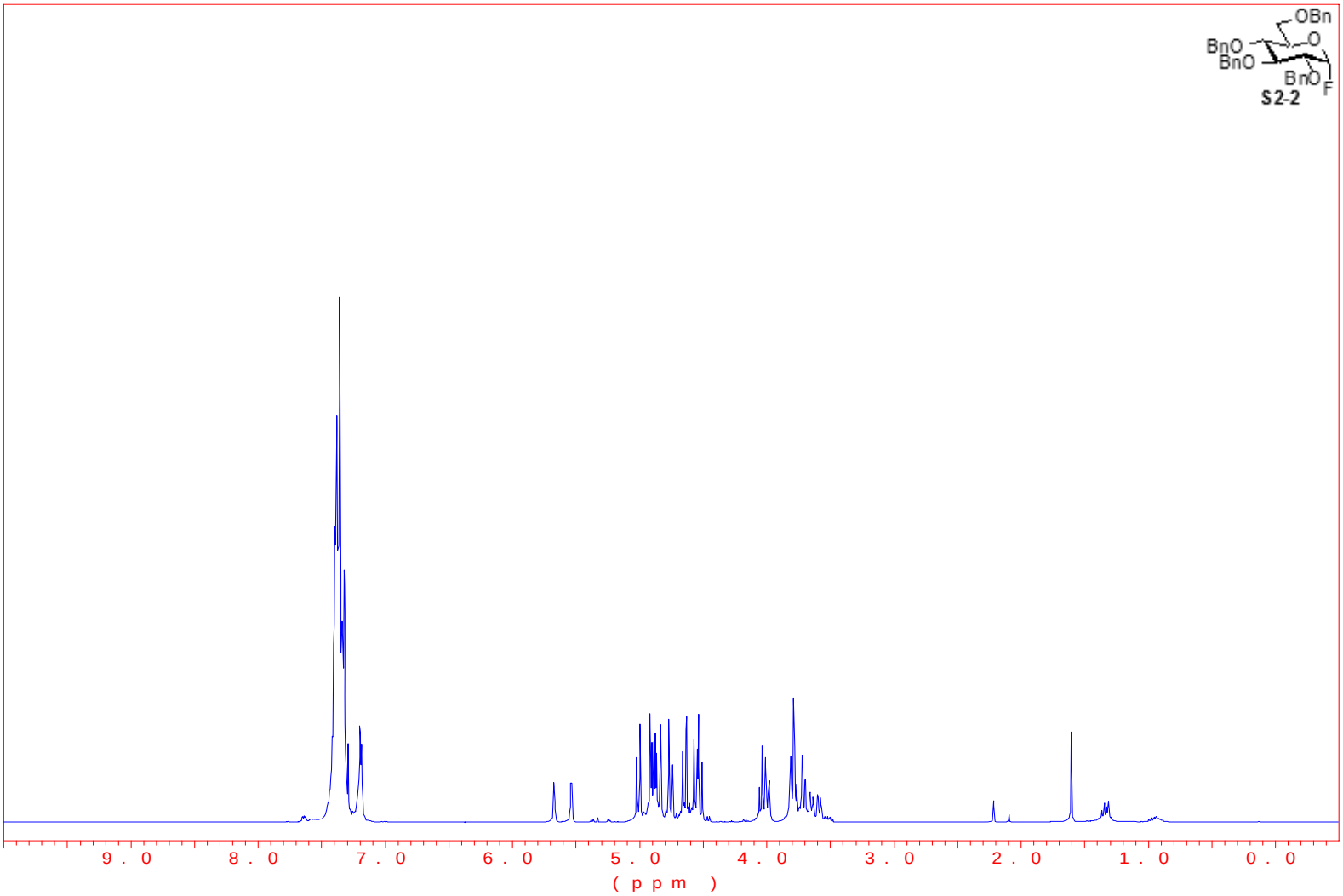
NMR Spectra


OBn
BnO
O
SPh
BnO
BnO
S2-1
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


OBn
BnO
O
SPh
BnO
BnO
S2-1
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


OBn
O
BnO
BnO
BnO
F
S2-2
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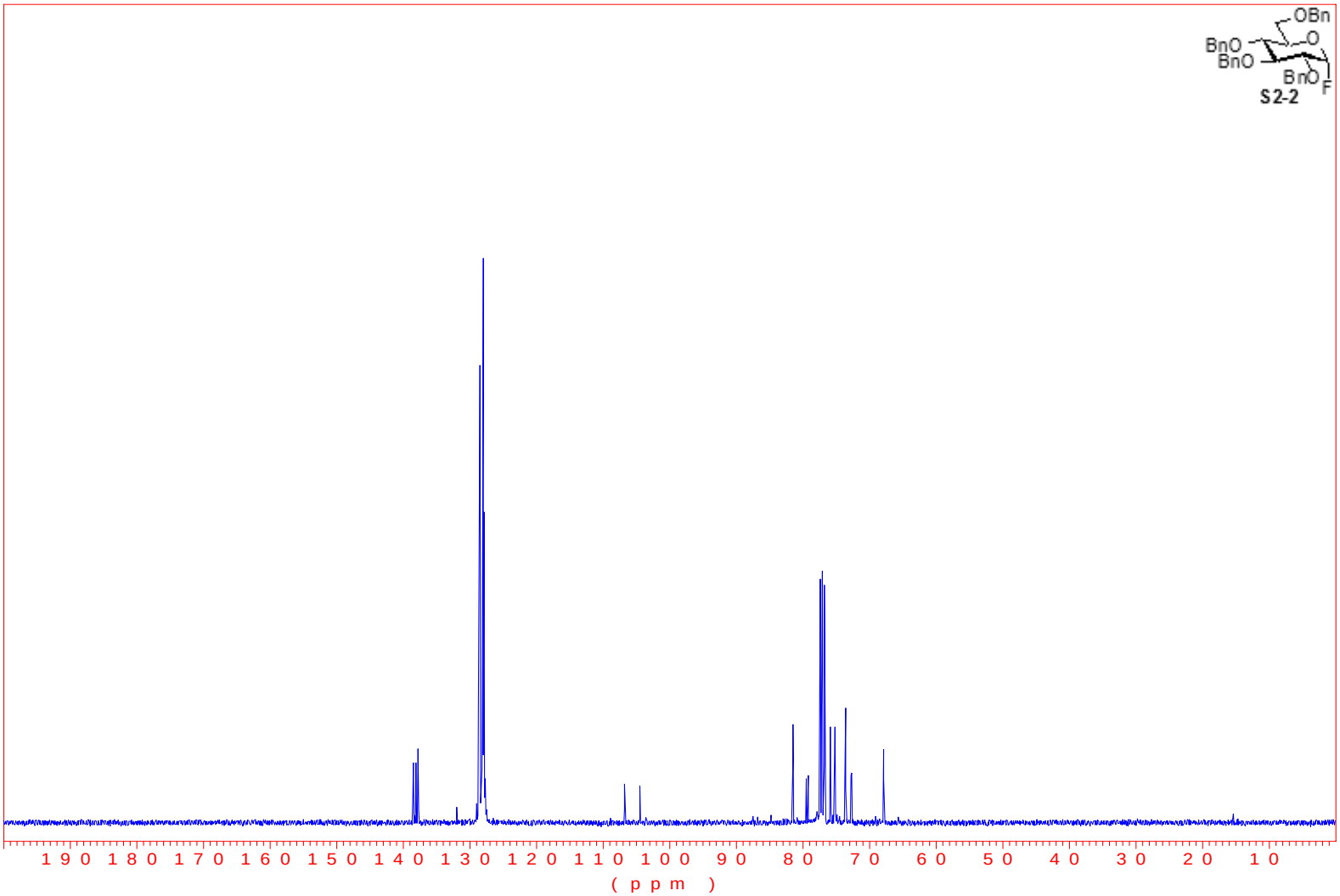
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)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O
BnO
BnO
BnO
F
S2-2
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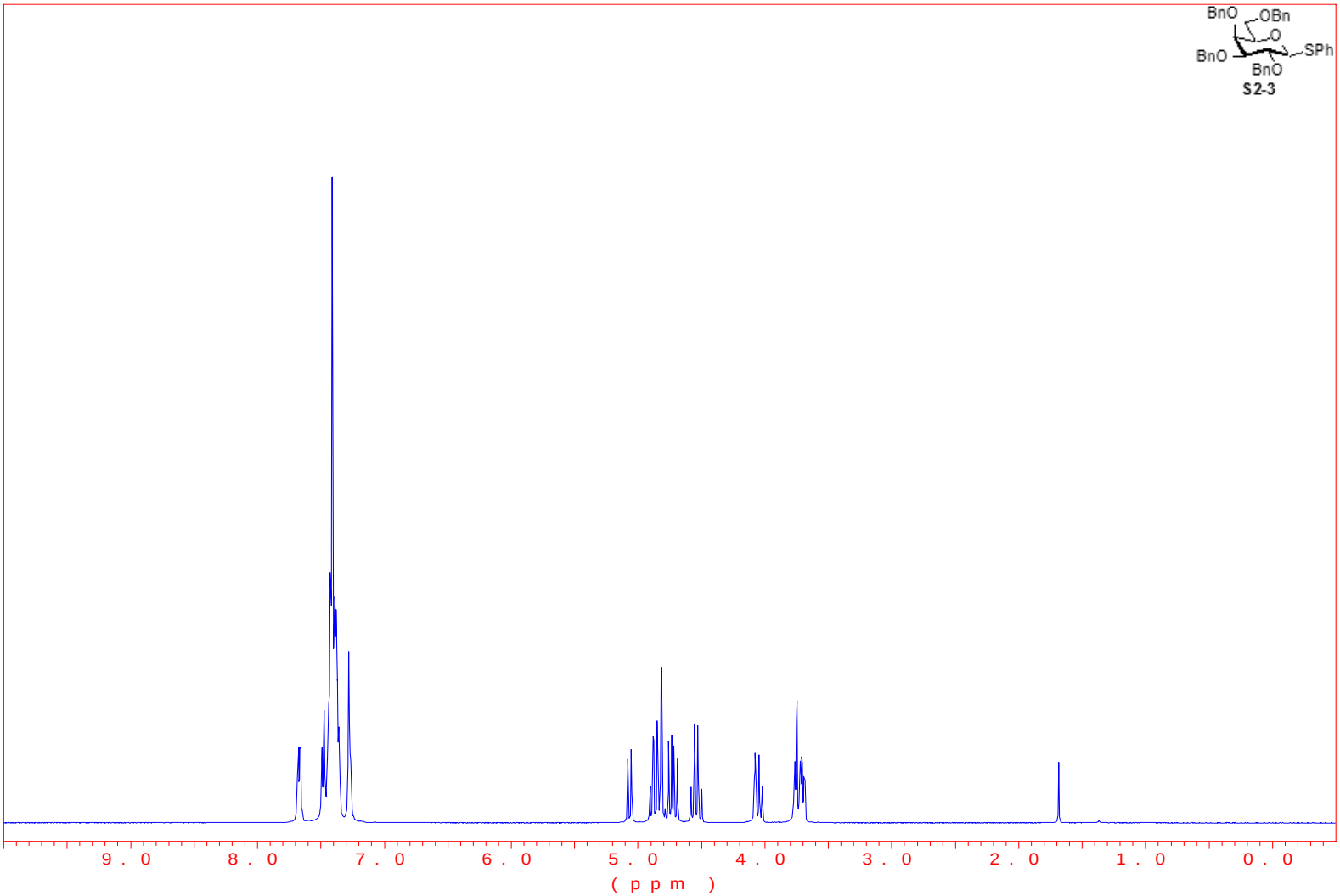
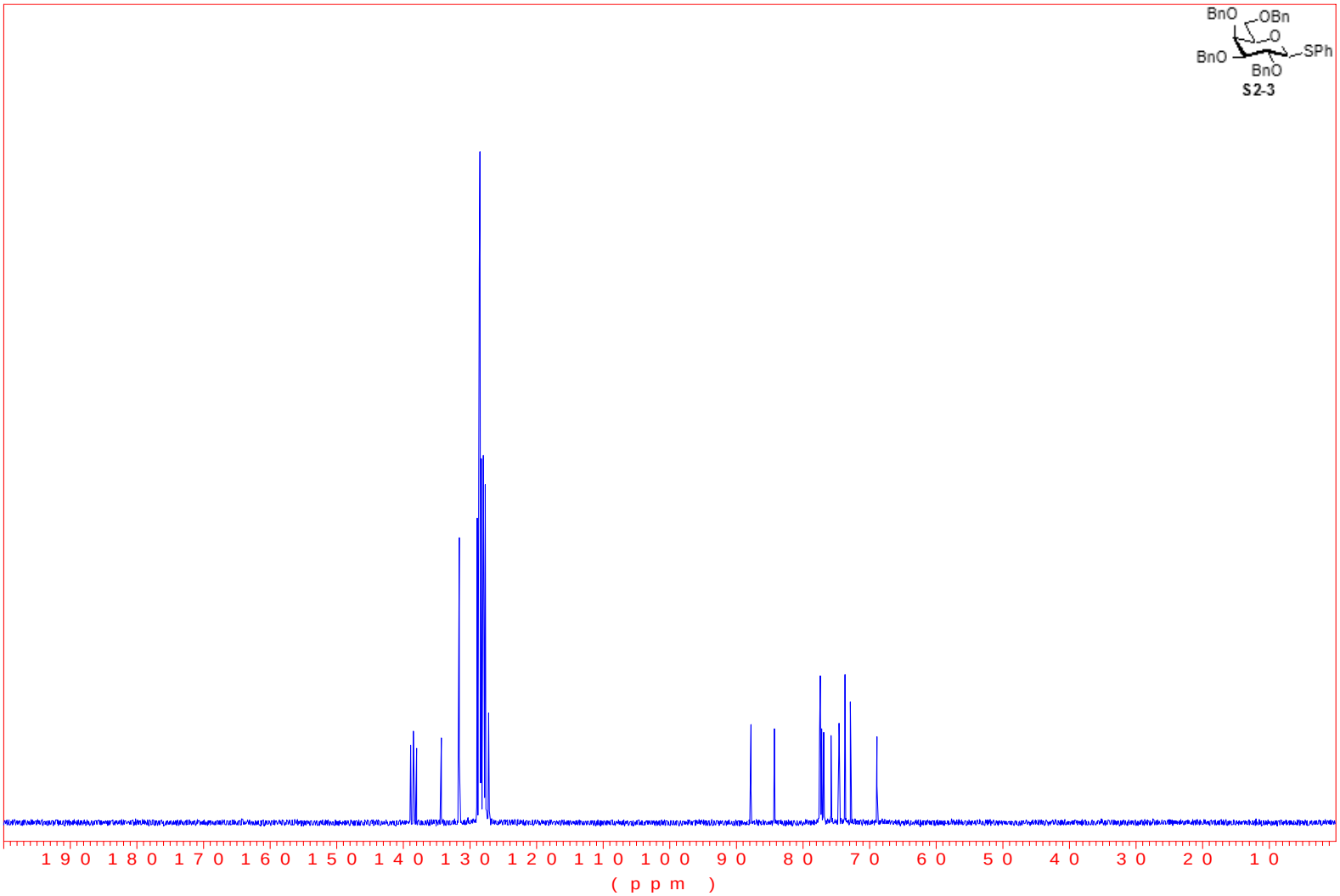
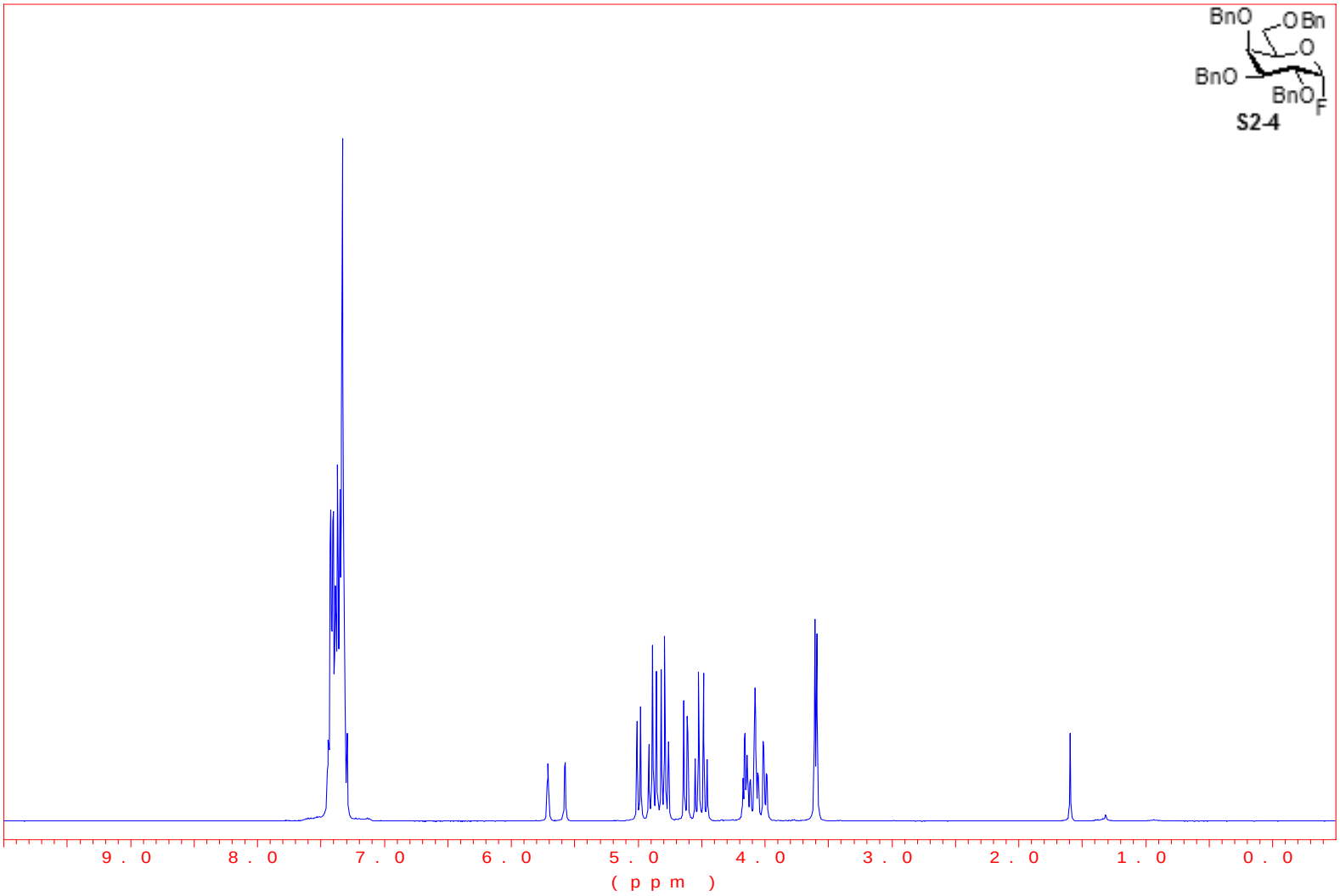
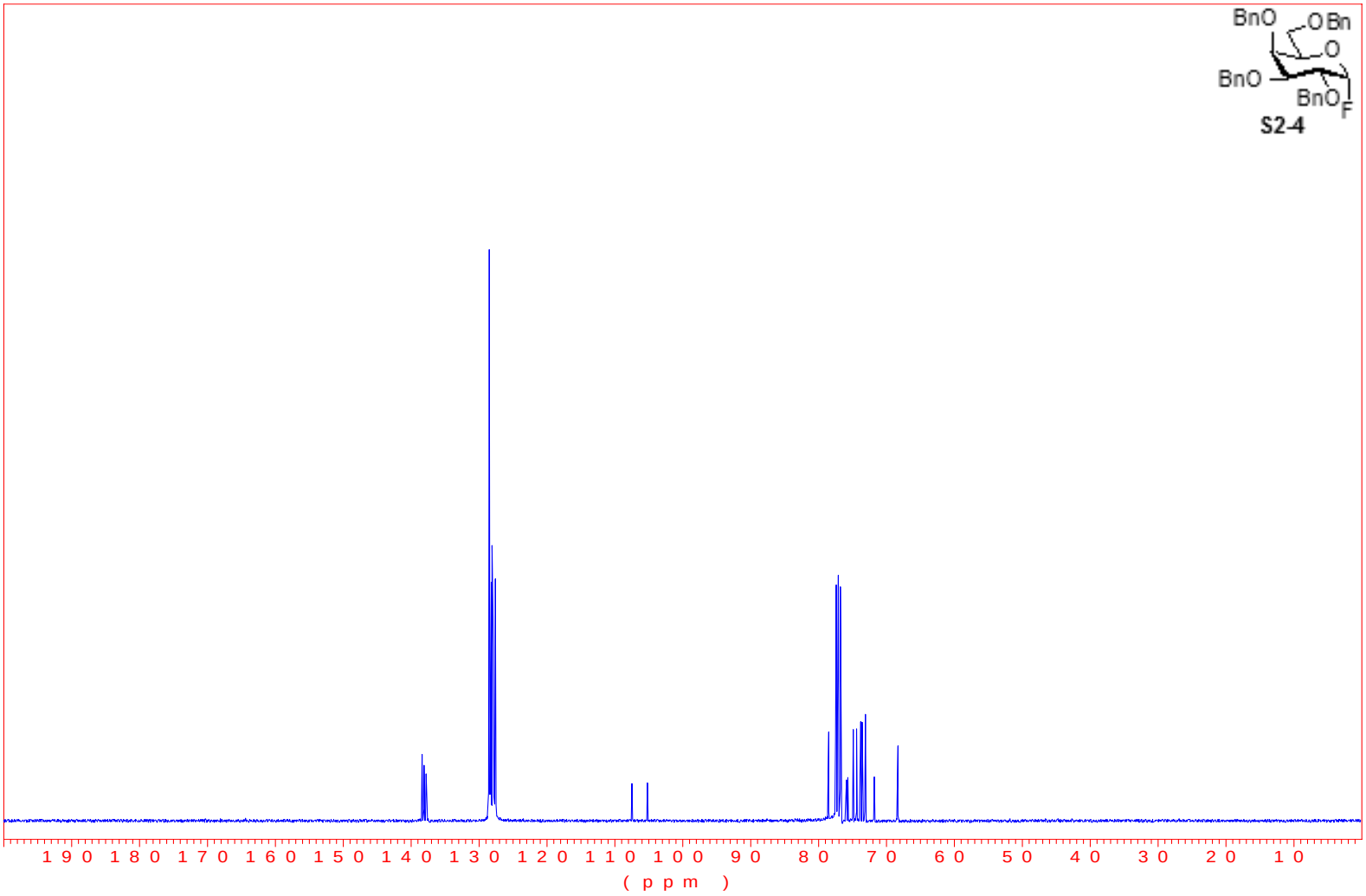
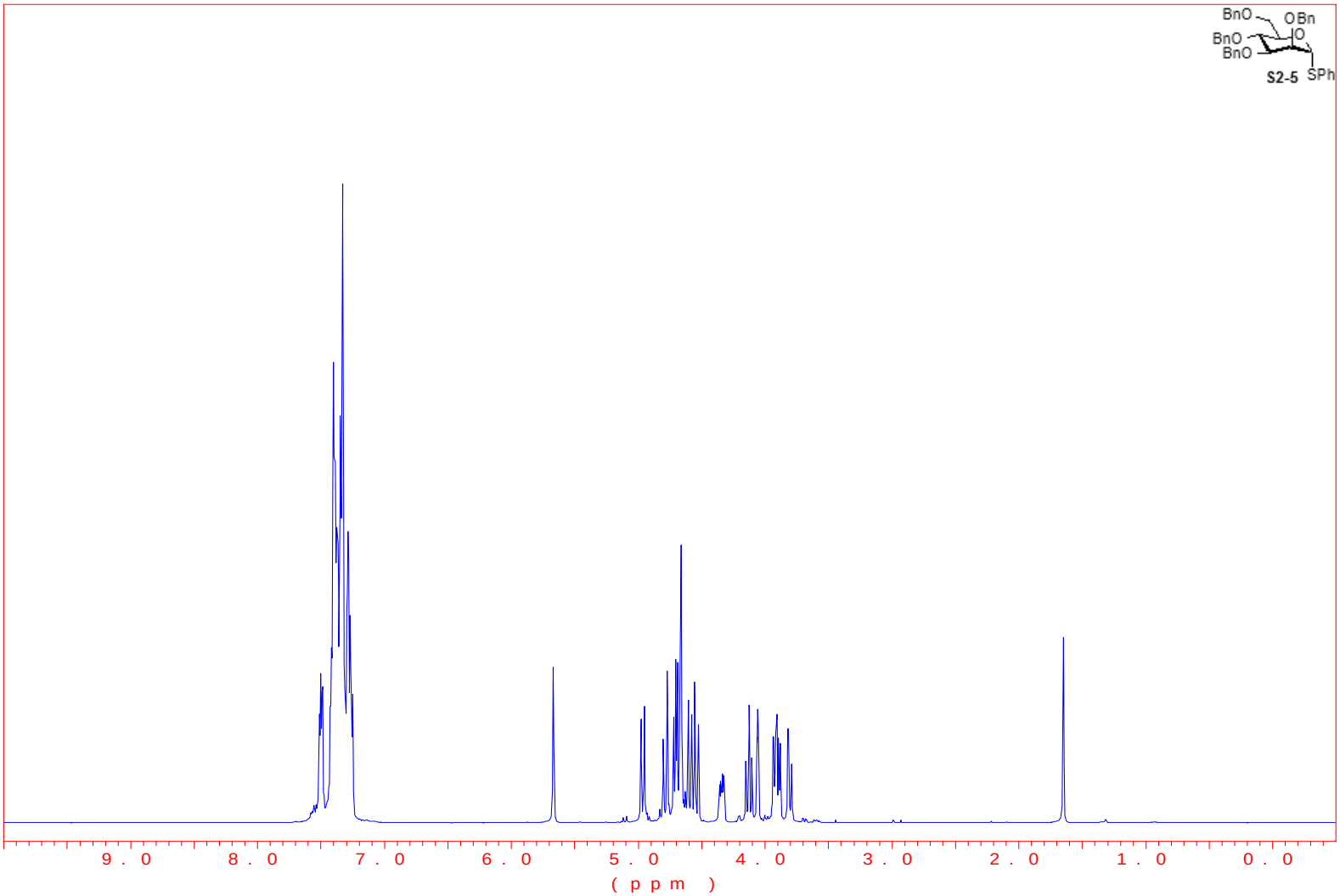
(ppm)

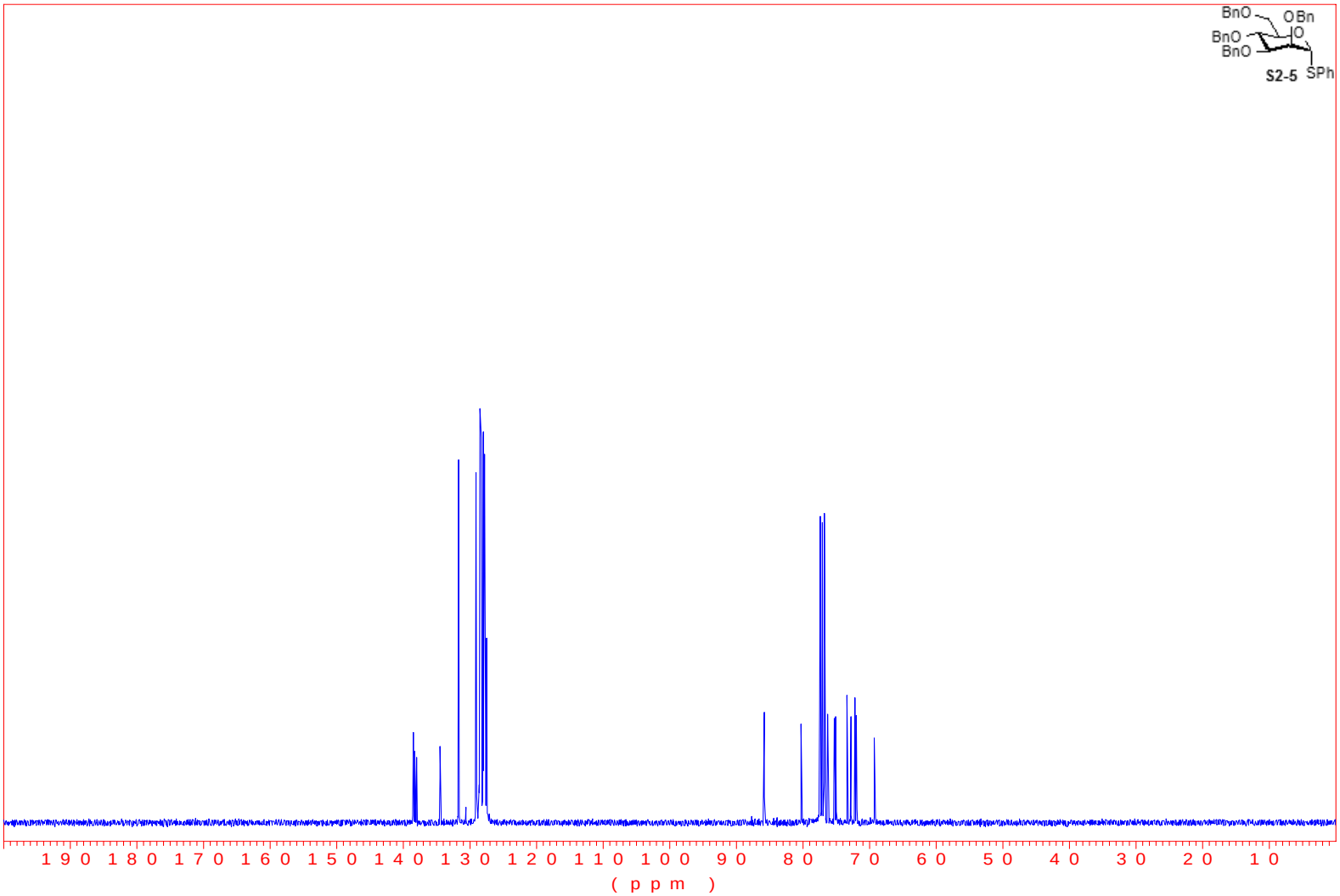

BnO
OBn
O
BnO
SPh
BnO
S2-3
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

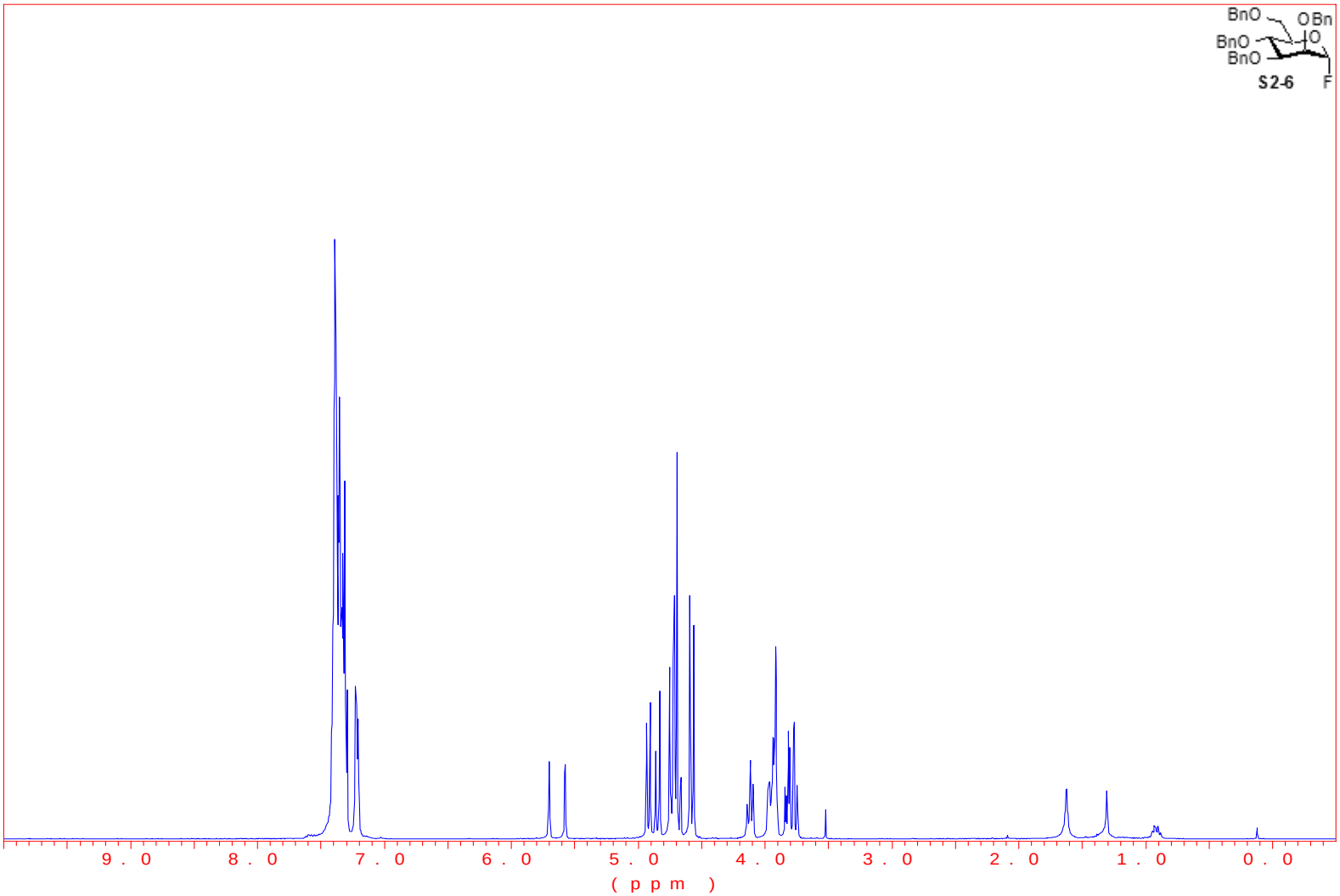

BnO
OBn
O
BnO
SPh
BnO
S2-3
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

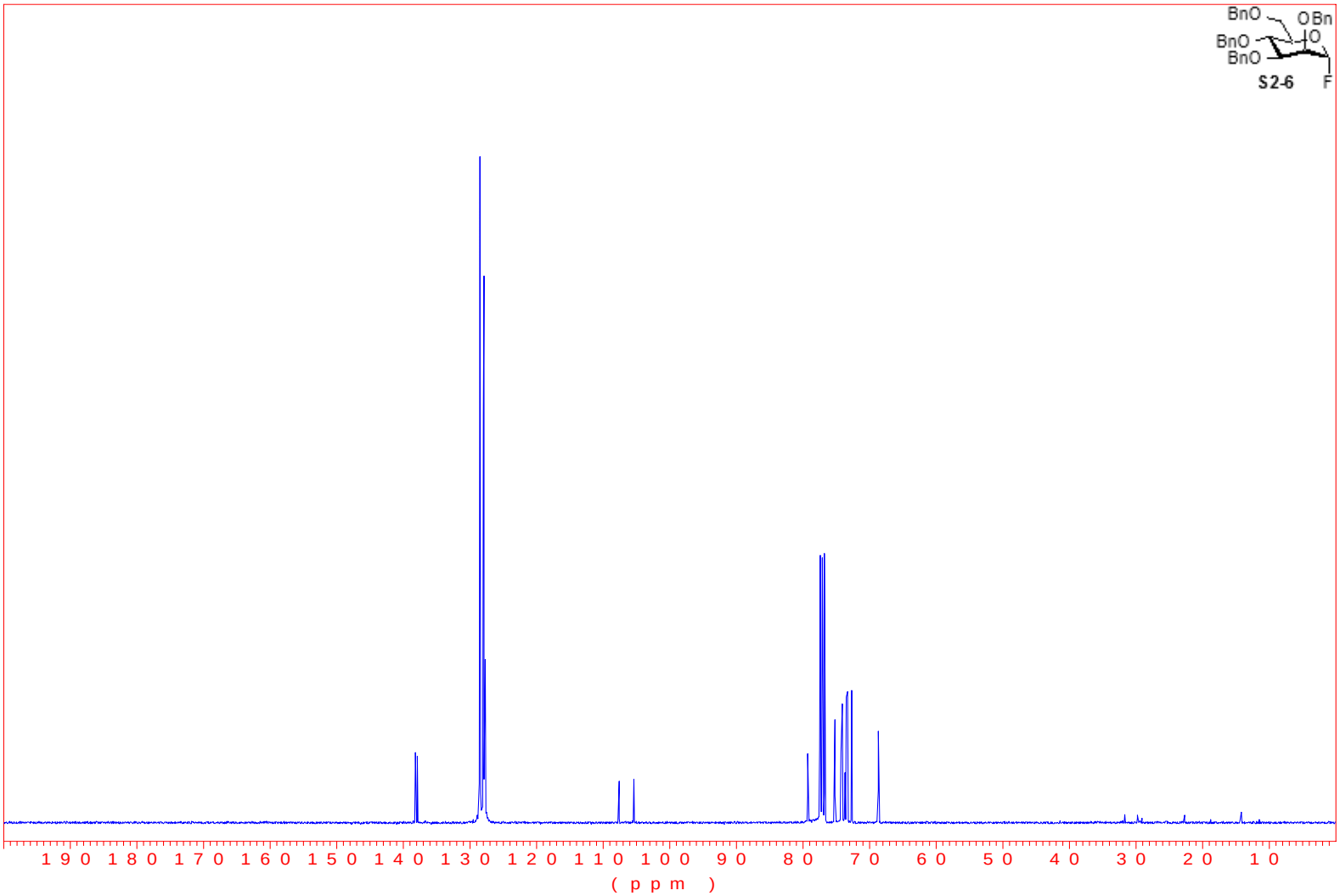

BnO
O Bn
O
BnO
BnO
F
S2-4
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

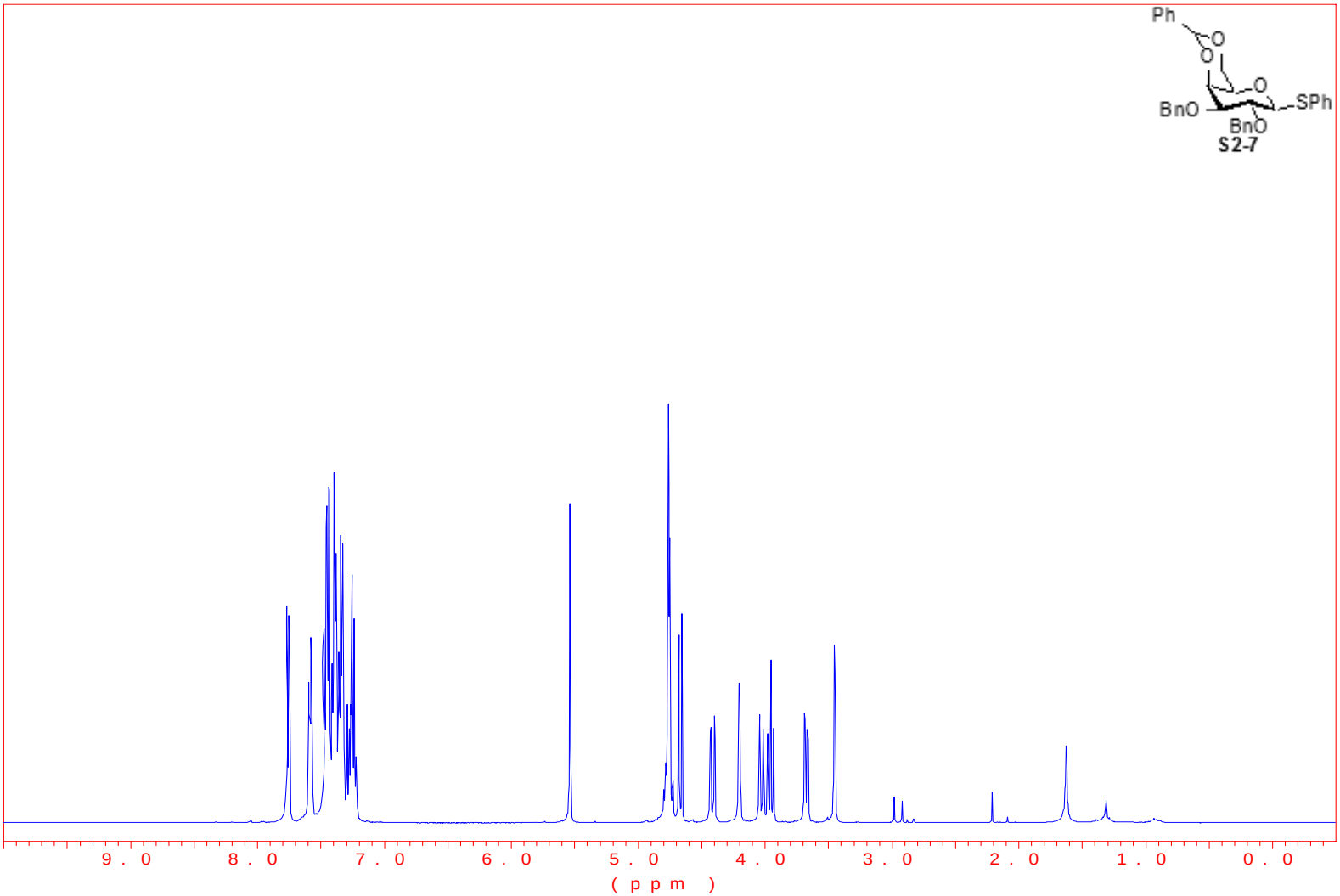

BnO
O Bn
O
BnO
BnO
F
S2-4
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

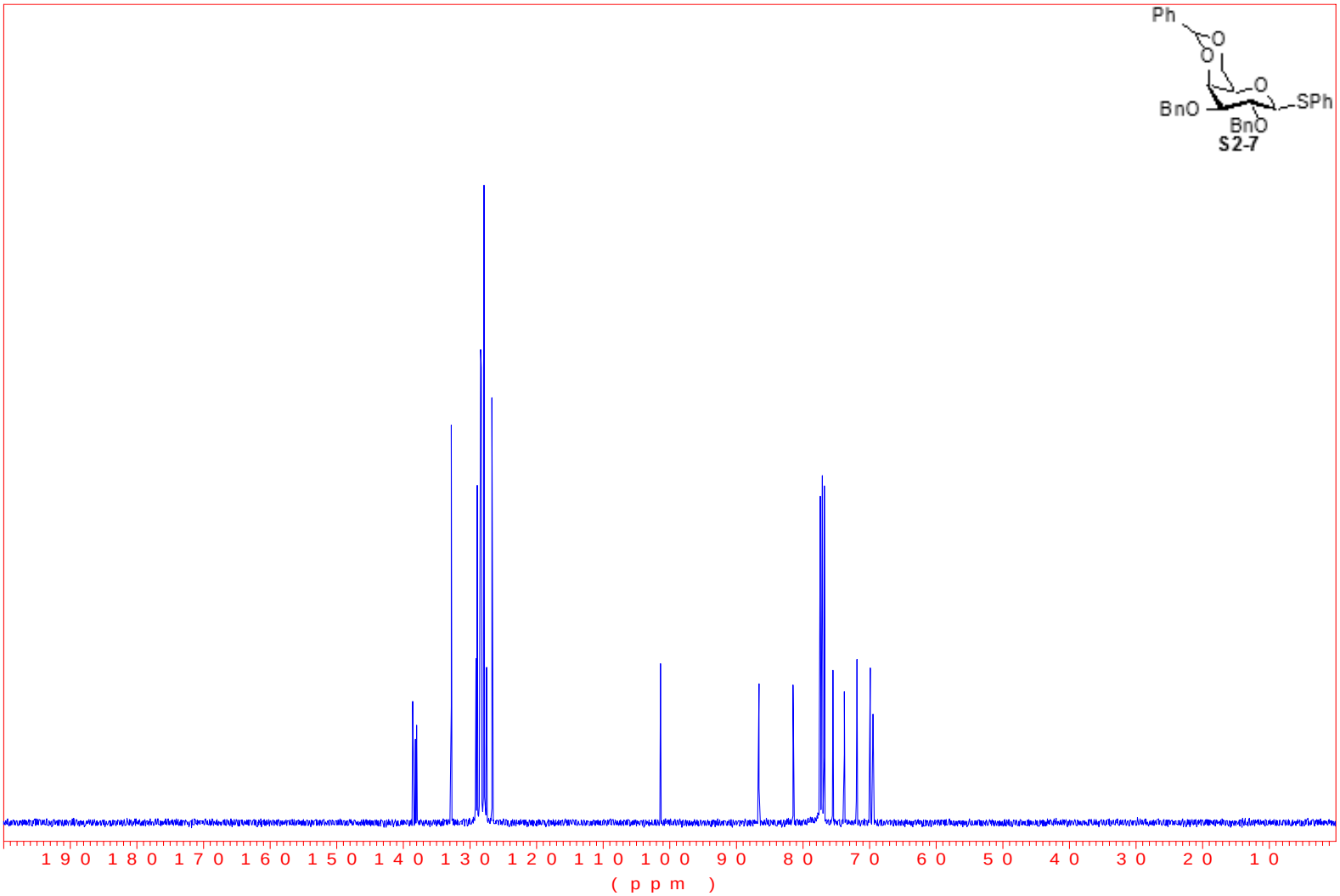

BnO
OBn
BnO
O
BnO
S2-5
SPh
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

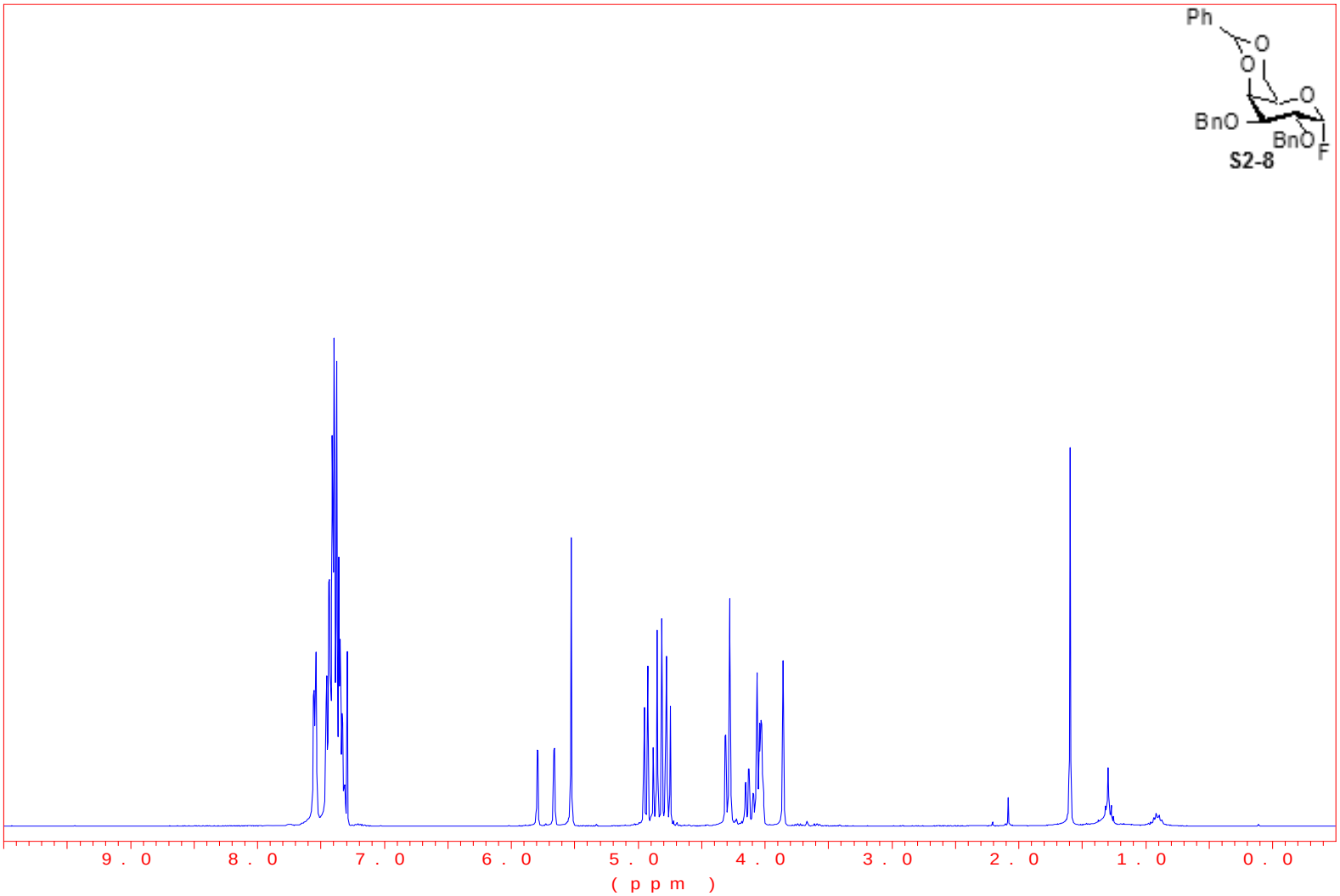

BnO
OBn
BnO
O
BnO
S2-5
SPh
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(p p m)

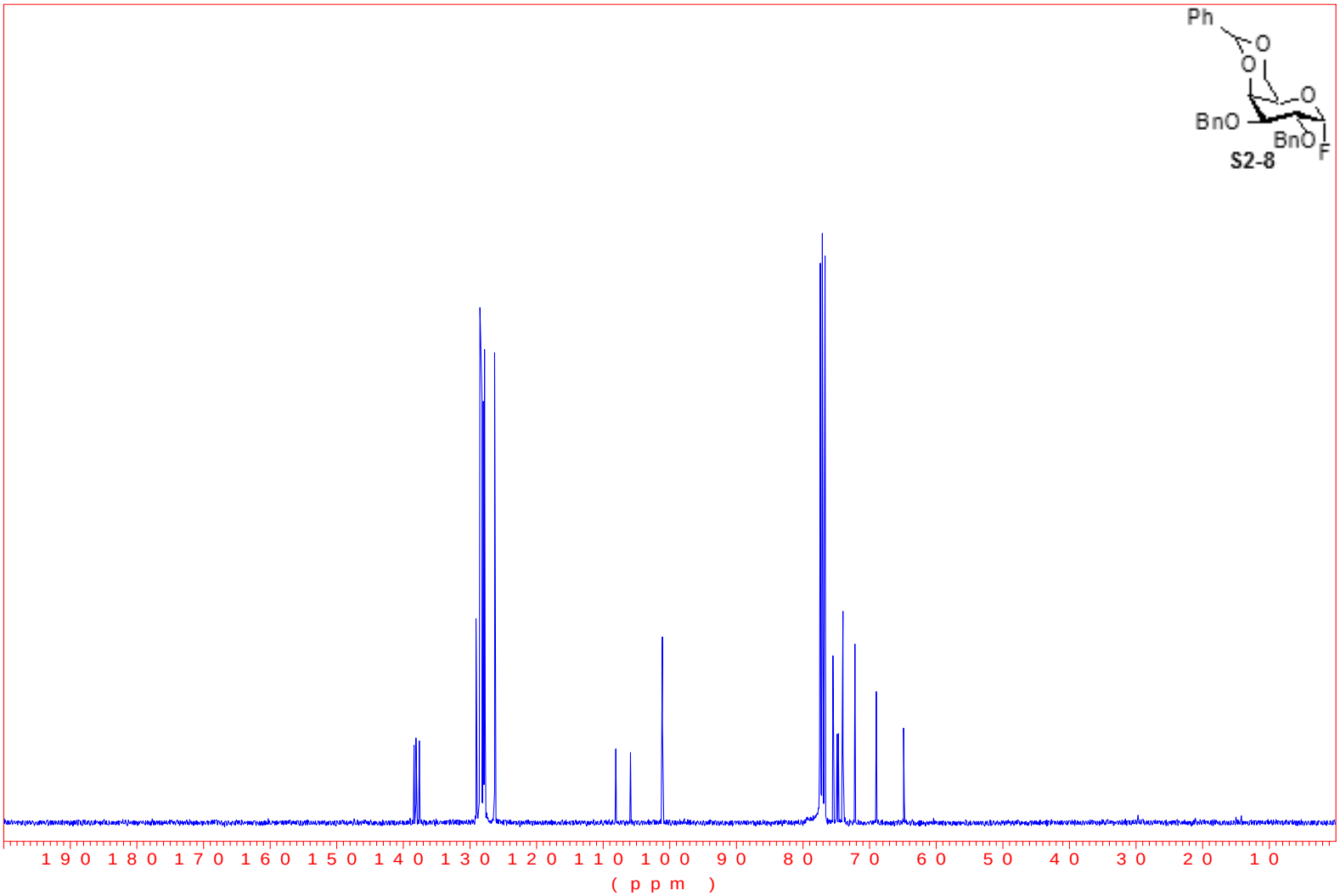

BnO
OBn
BnO
O
BnO
S2-6
F
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

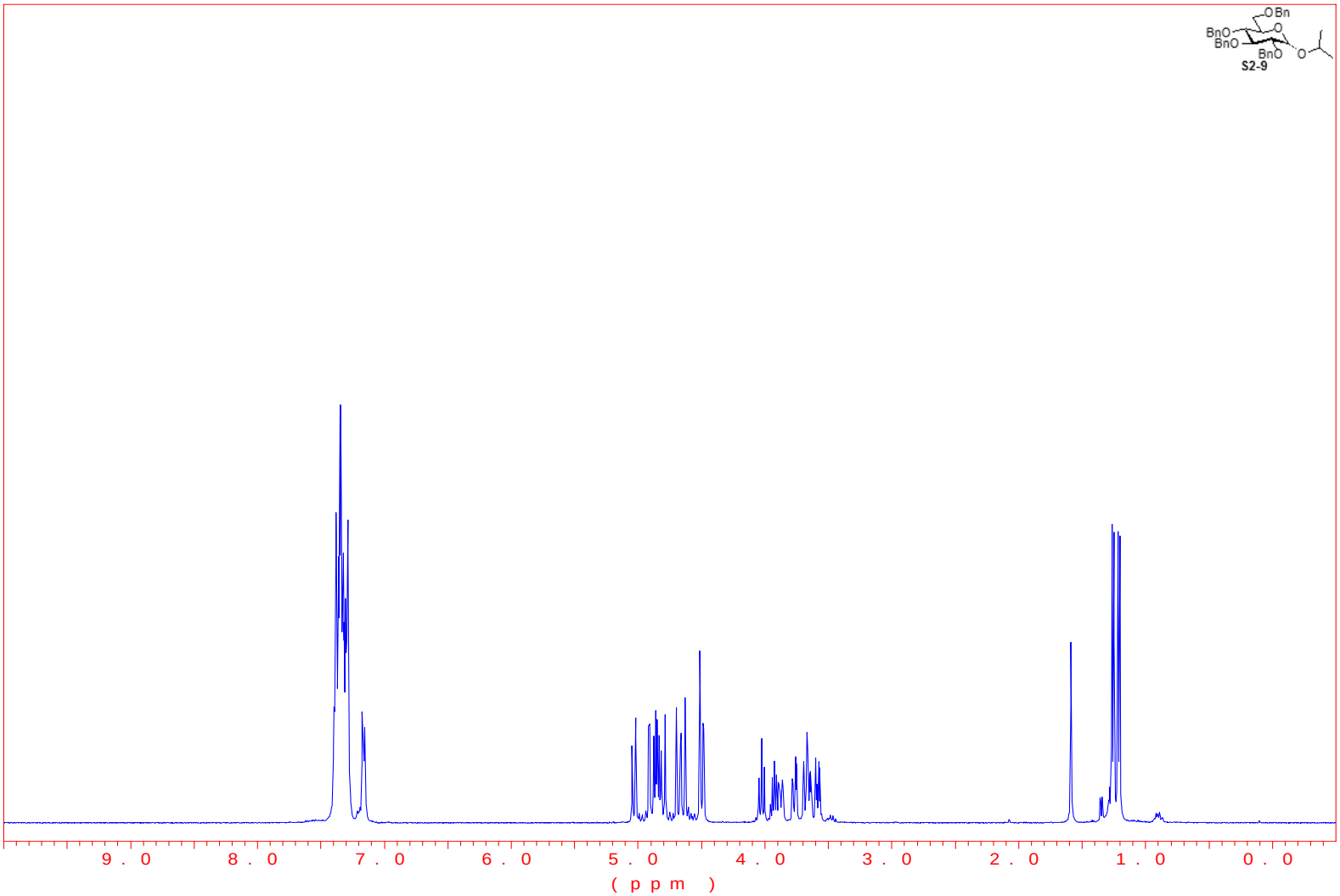

BnO
OBn
BnO
O
BnO
S2-6
F
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


Ph
O
O
O
BnO
SPh
BnO
S2-7
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


Ph
O
O
O
BnO
SPh
BnO
S2-7
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


Ph
O
O
O
BnO
BnO
F
S2-8
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


Ph
O
O
O
BnO
BnO
F
S2-8
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


OBn
BnO
BnO
BnO
O
O
S2-9
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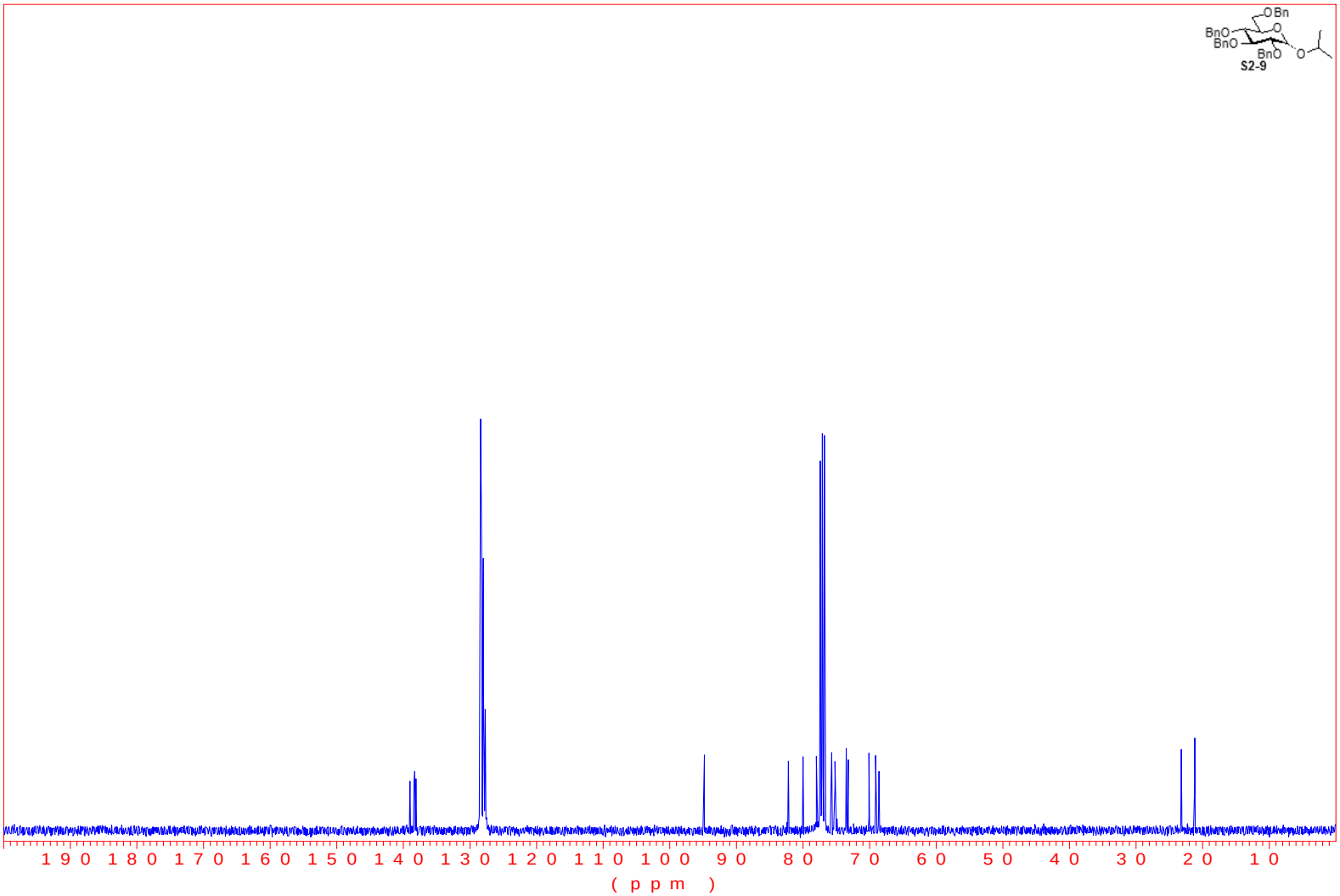
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)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BnO
BnO
O
BnO
O
S2-9
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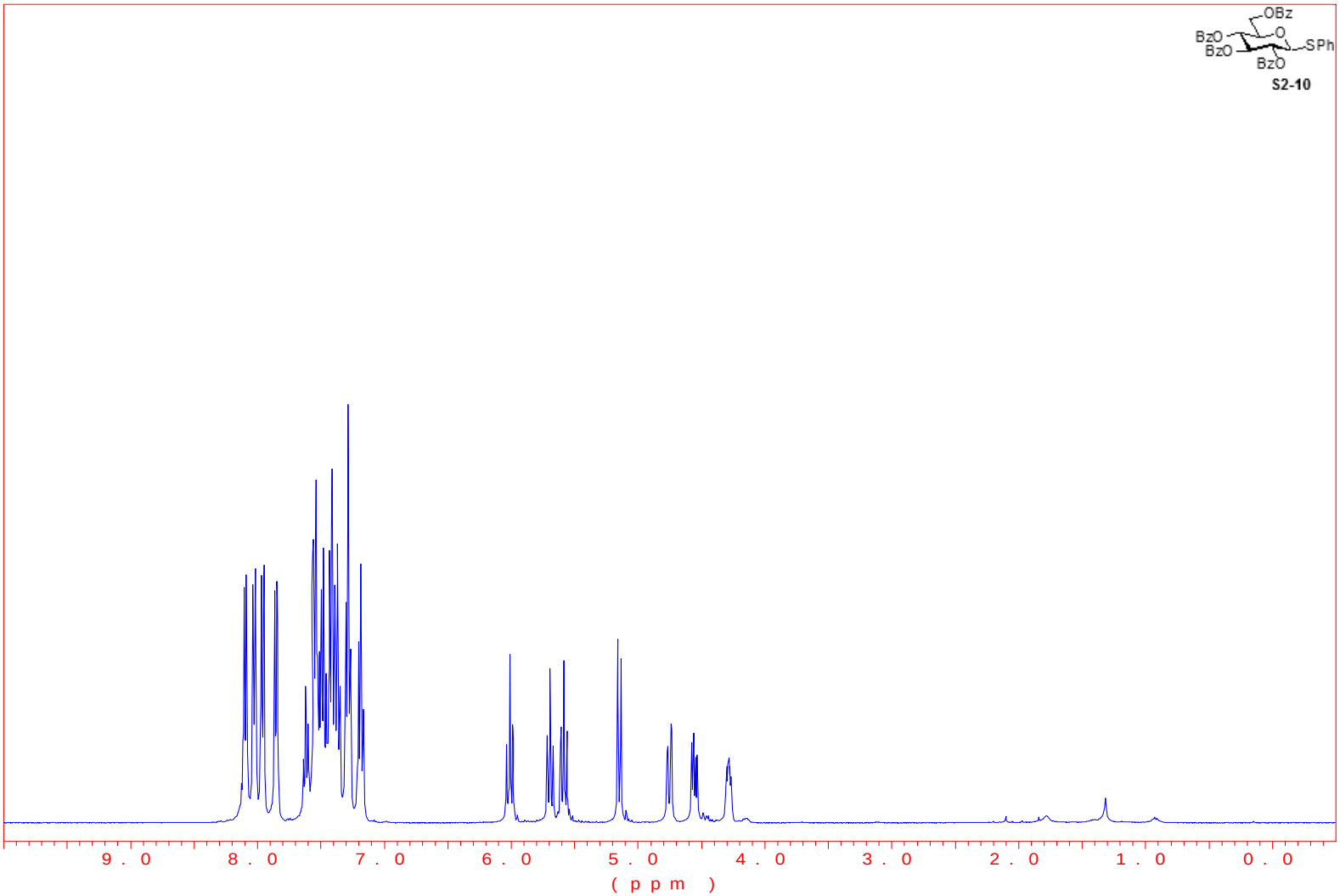
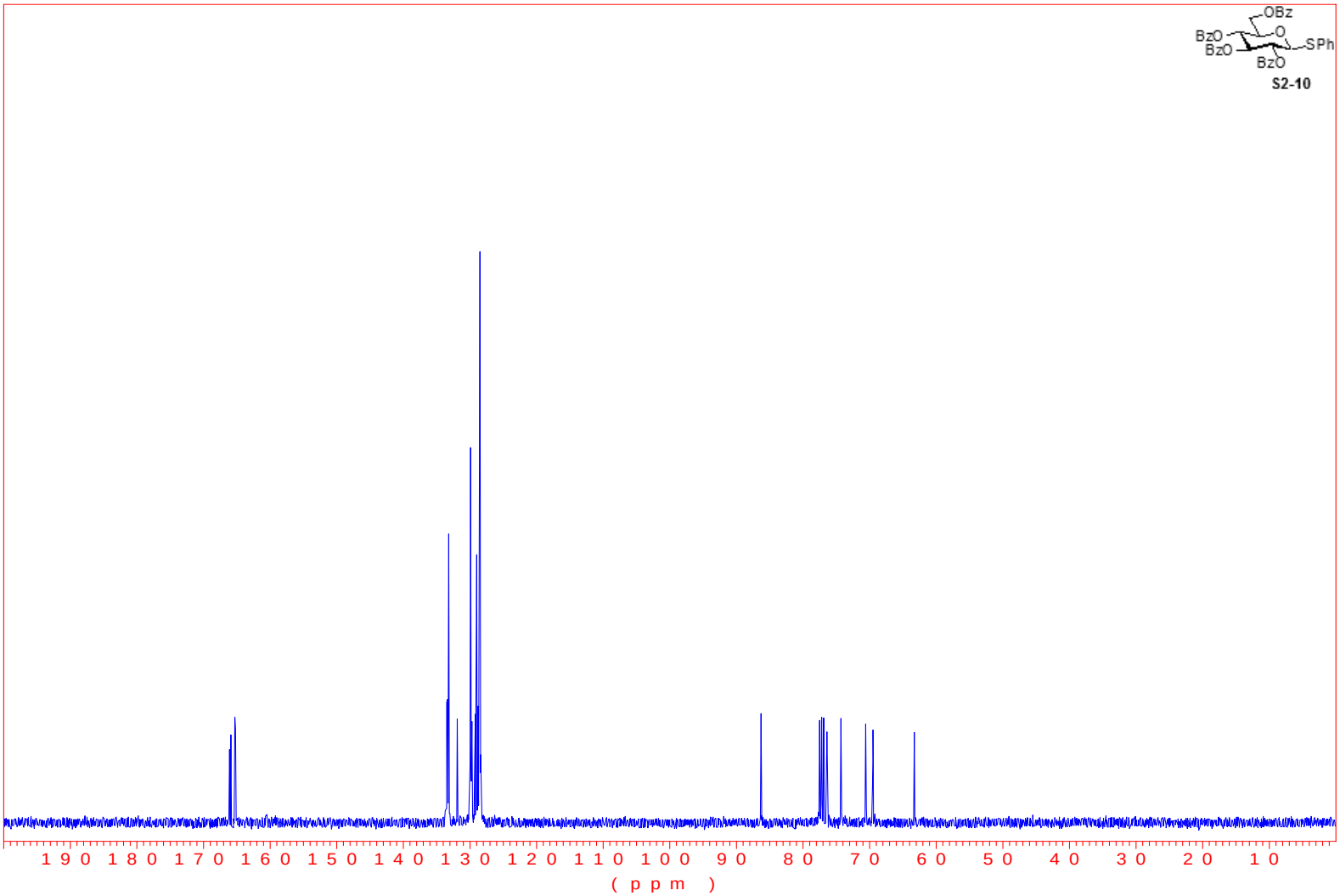
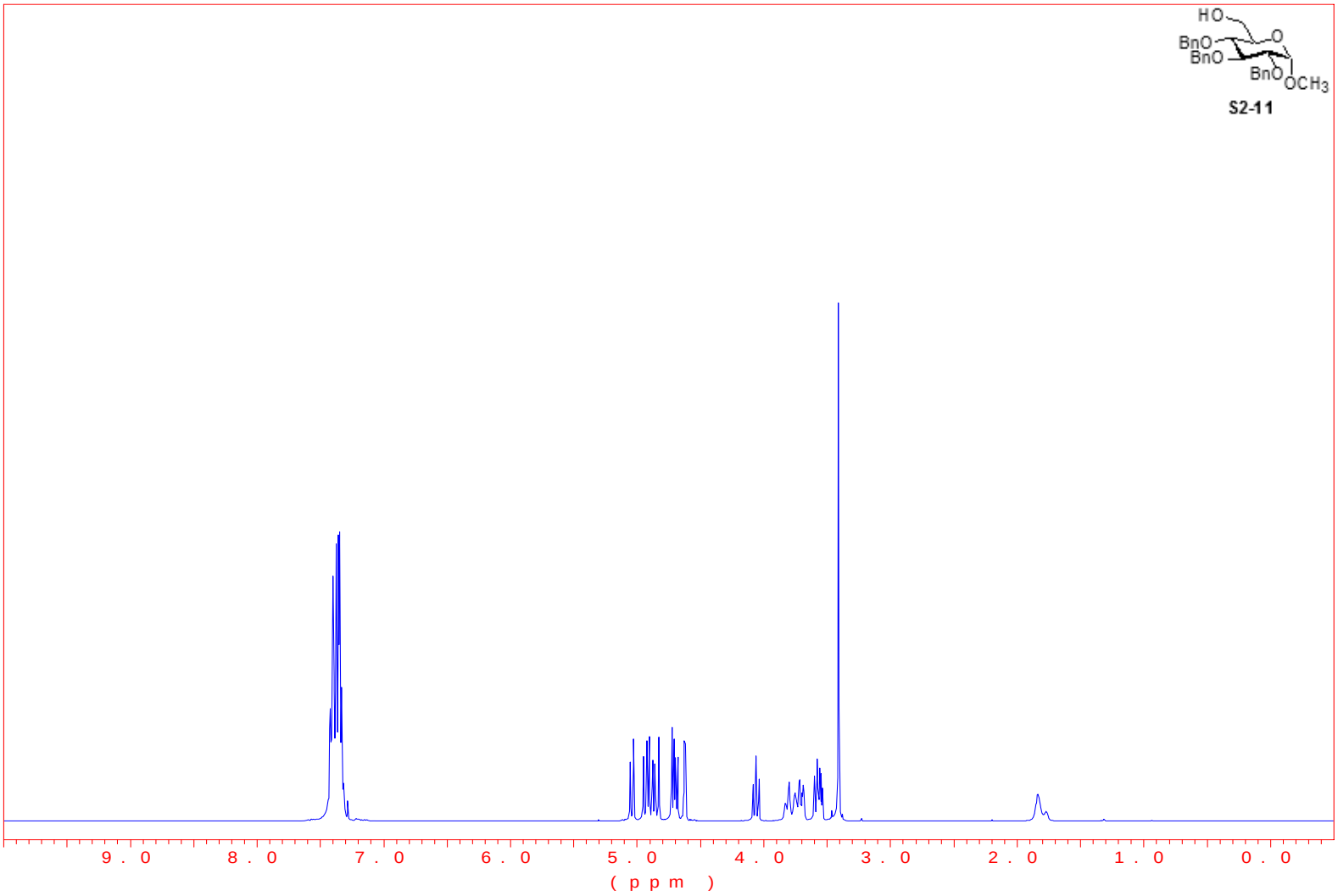
(ppm)


OBz
BzO
O
SPh
BzO
BzO
S2-10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


OBz
BzO
BzO
O
SPh
BzO
S2-10
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(p p m)


HO
BnO
BnO
O
BnO
OCH3
S2-11
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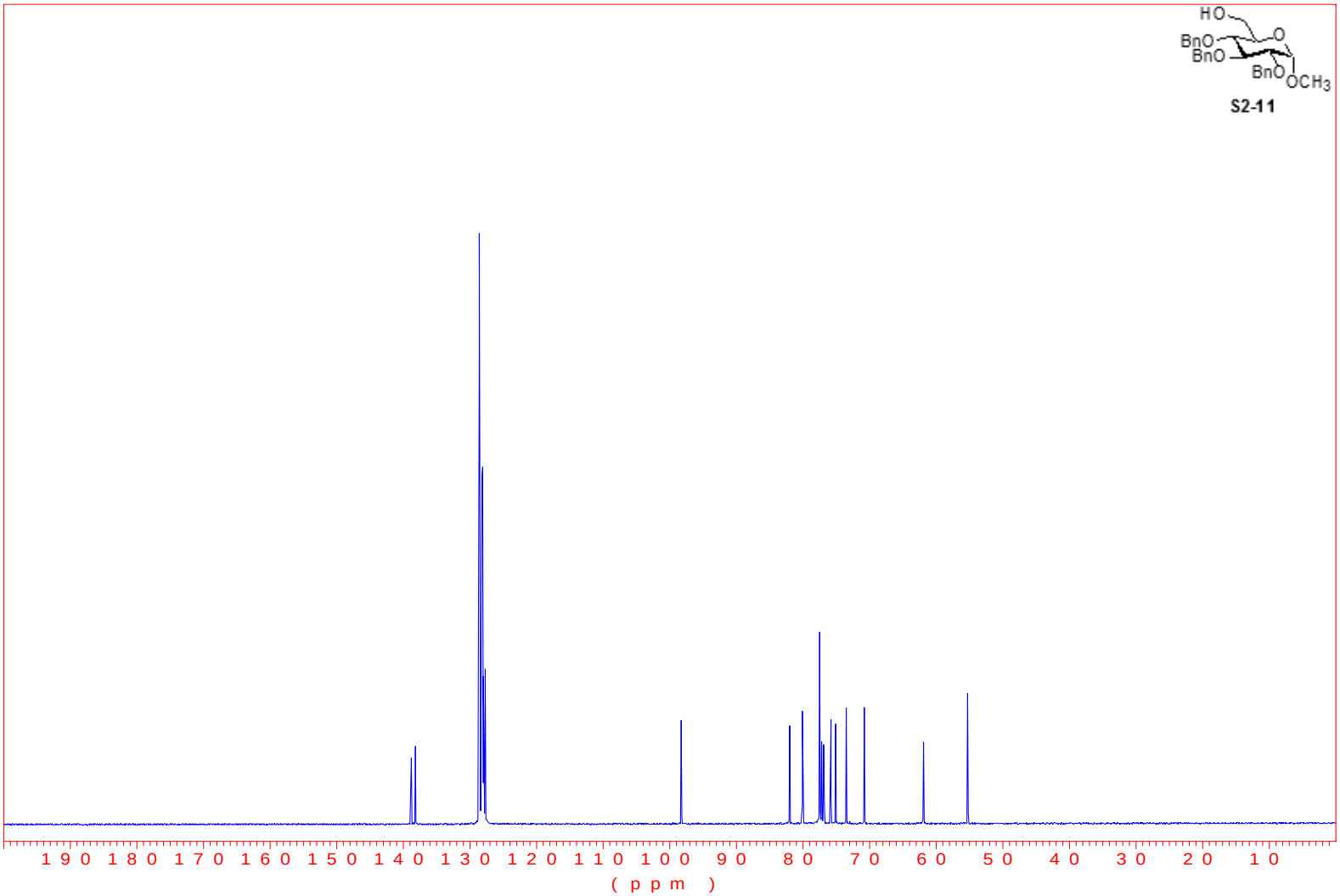
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


HO
BnO
BnO
O
BnO
OCH3
S2-11
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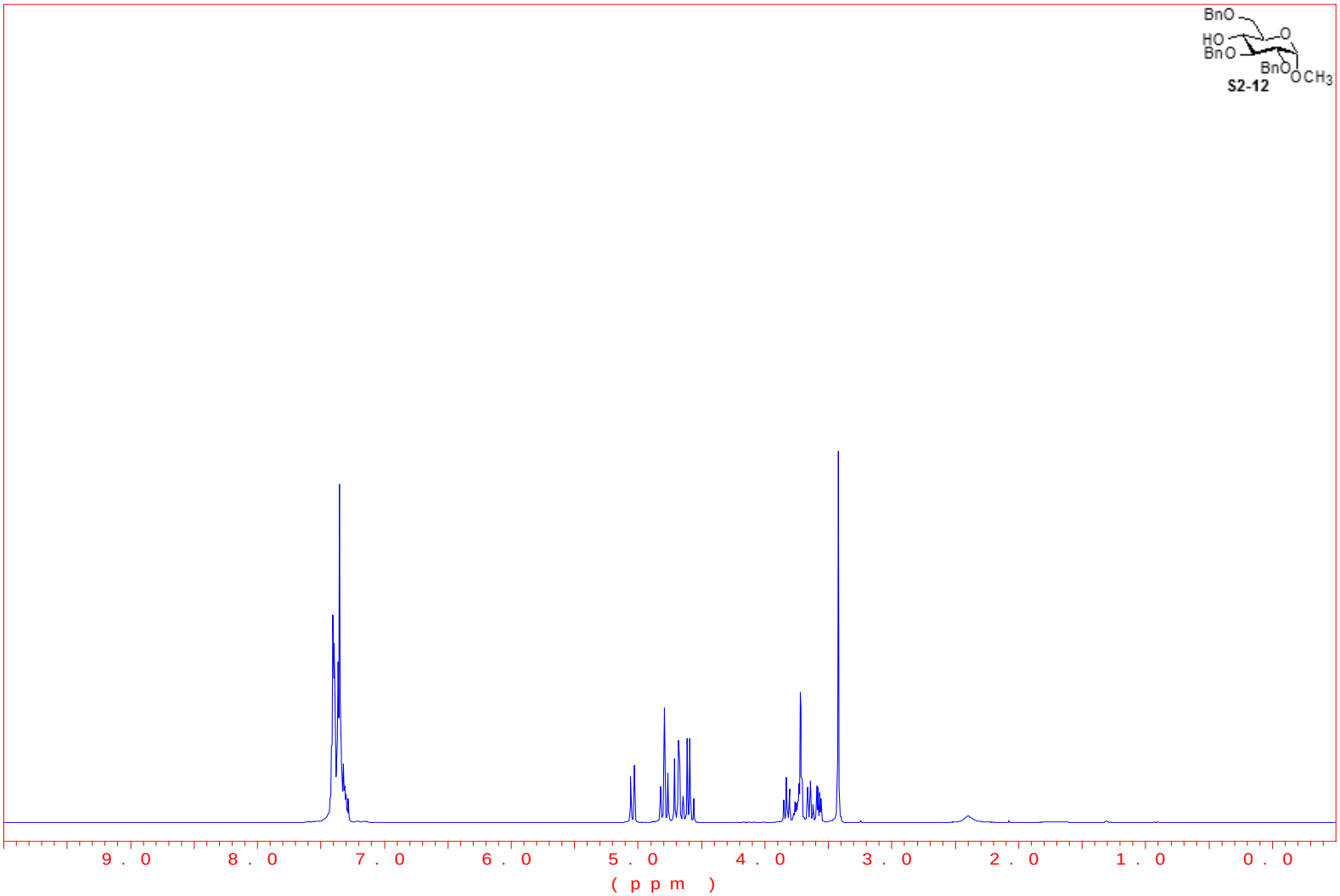
(ppm)


BnO
HO
BnO
O
BnO
OCH3
S2-12
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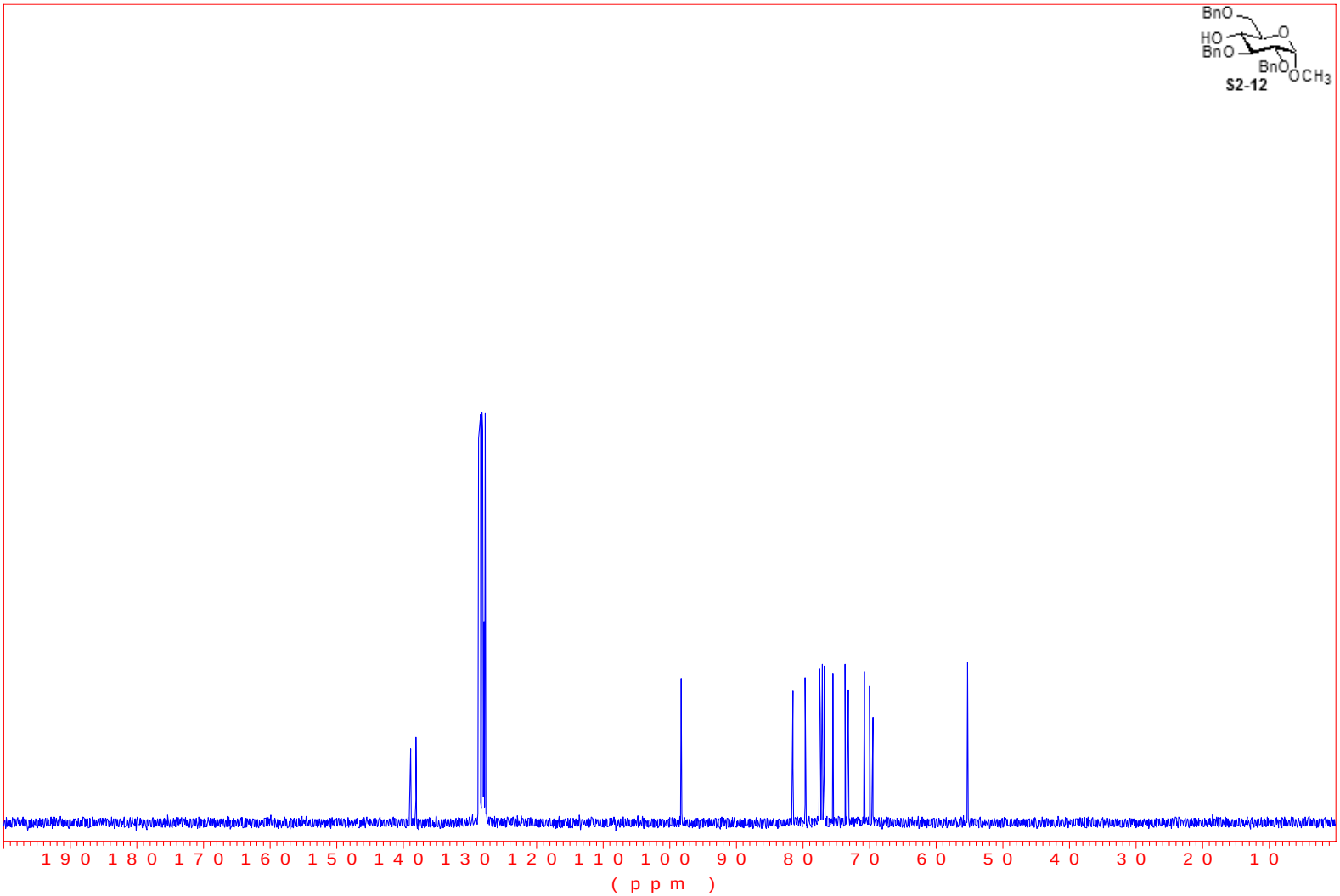
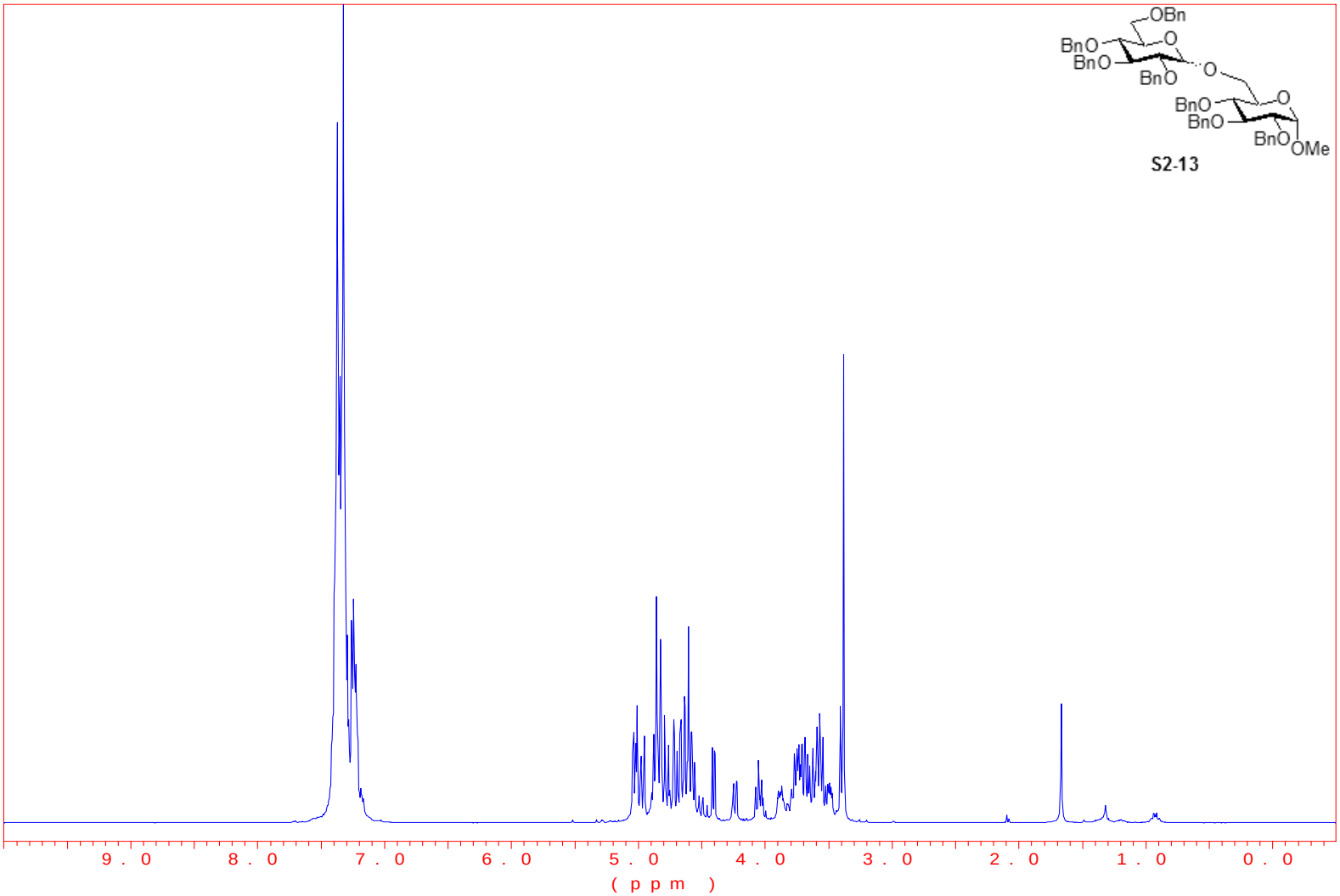
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


BnO
HO
BnO
O
BnO
OCH3
S2-12
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)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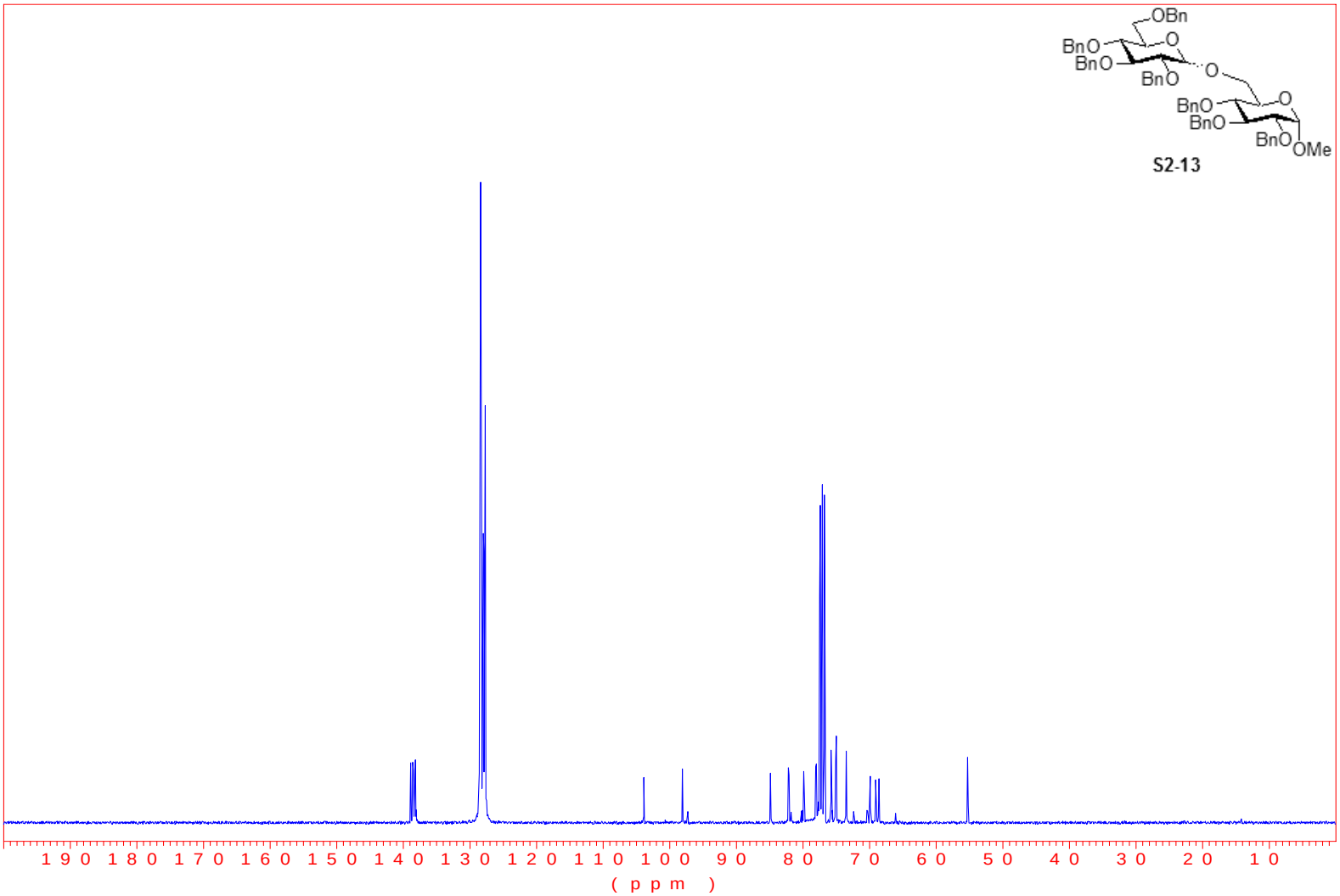
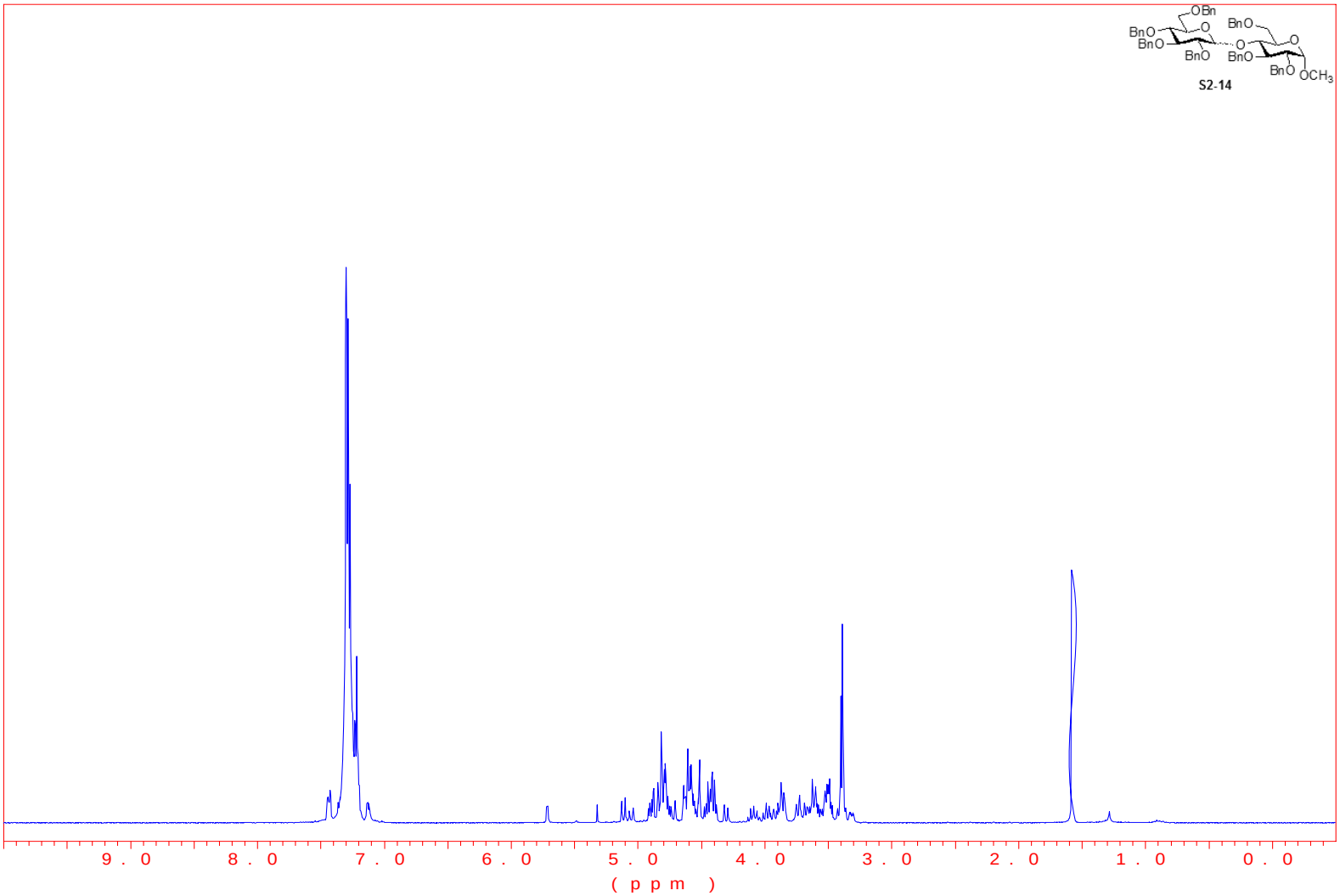
BnO
BnO
BnO
O
O
BnO
BnO
BnO
OMe
S2-13
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


OBn
BnO
BnO
BnO
O
O
BnO
BnO
BnO
OMe
S2-13
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


S2-14
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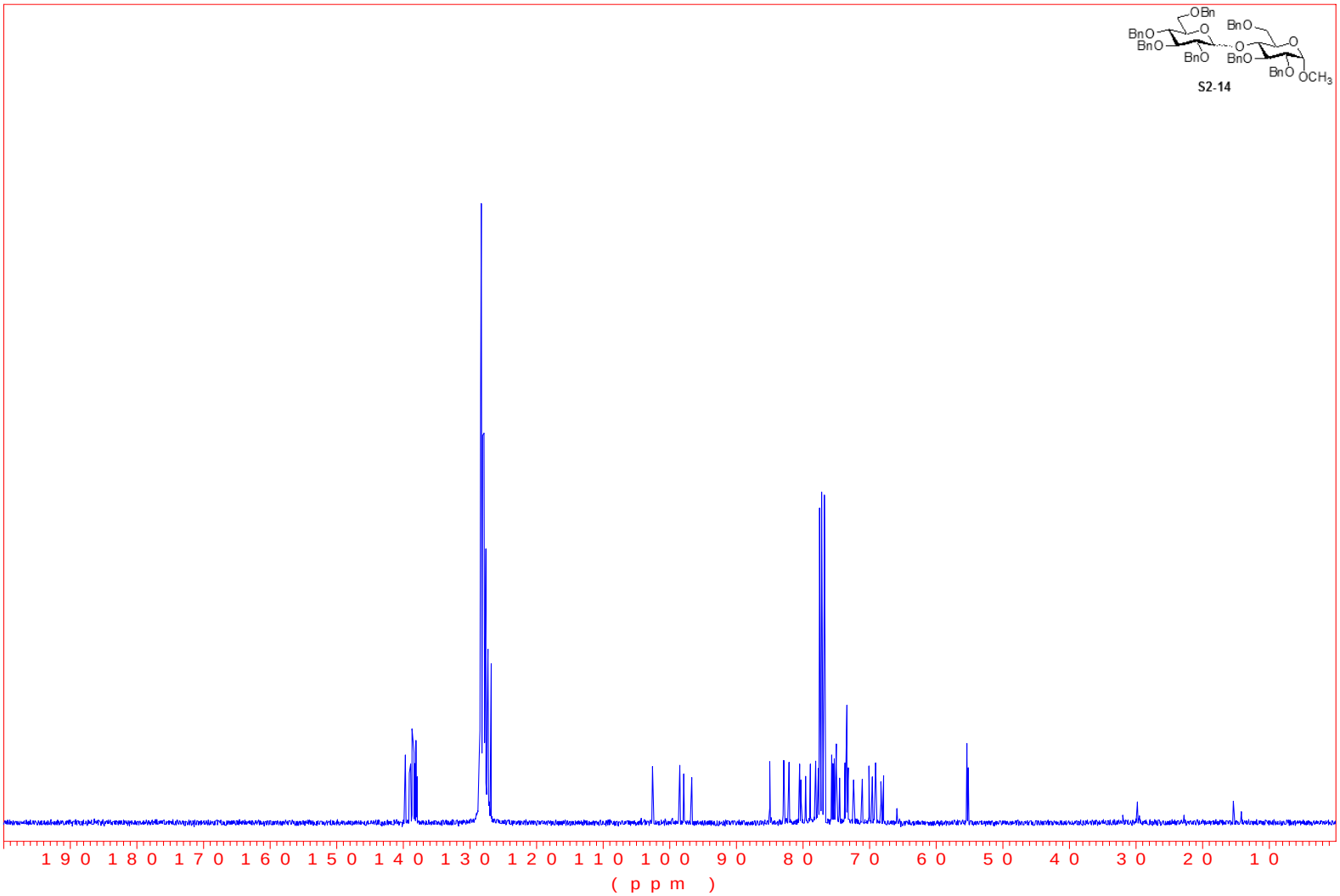
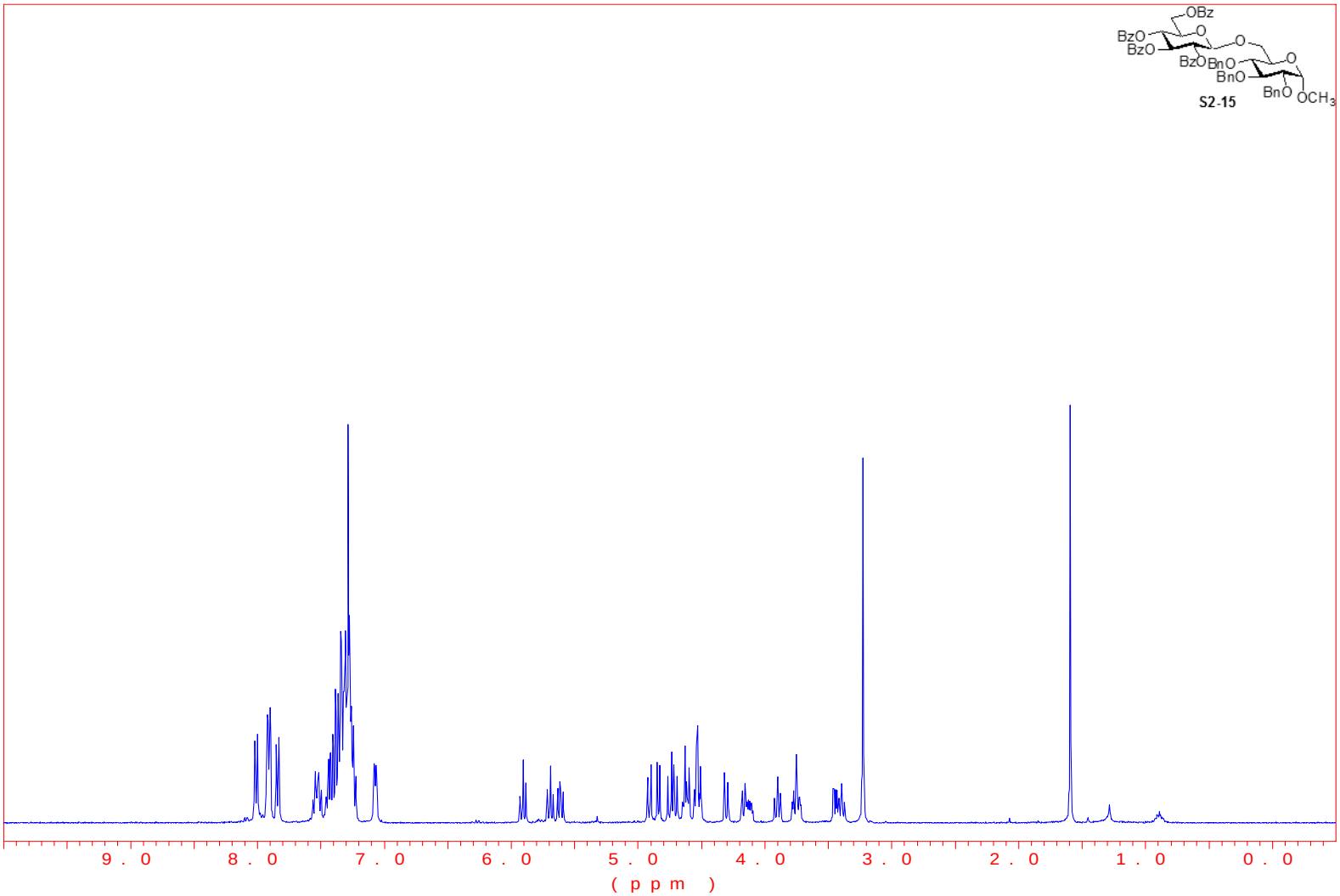
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


OBn
BnO
BnO
O
BnO
BnO
O
BnO
BnO
O
BnO
OCH3
S2-14
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


OBz
BzO
BzO
O
O
BzO
BnO
BnO
O
BnO
OCH3
S2-15
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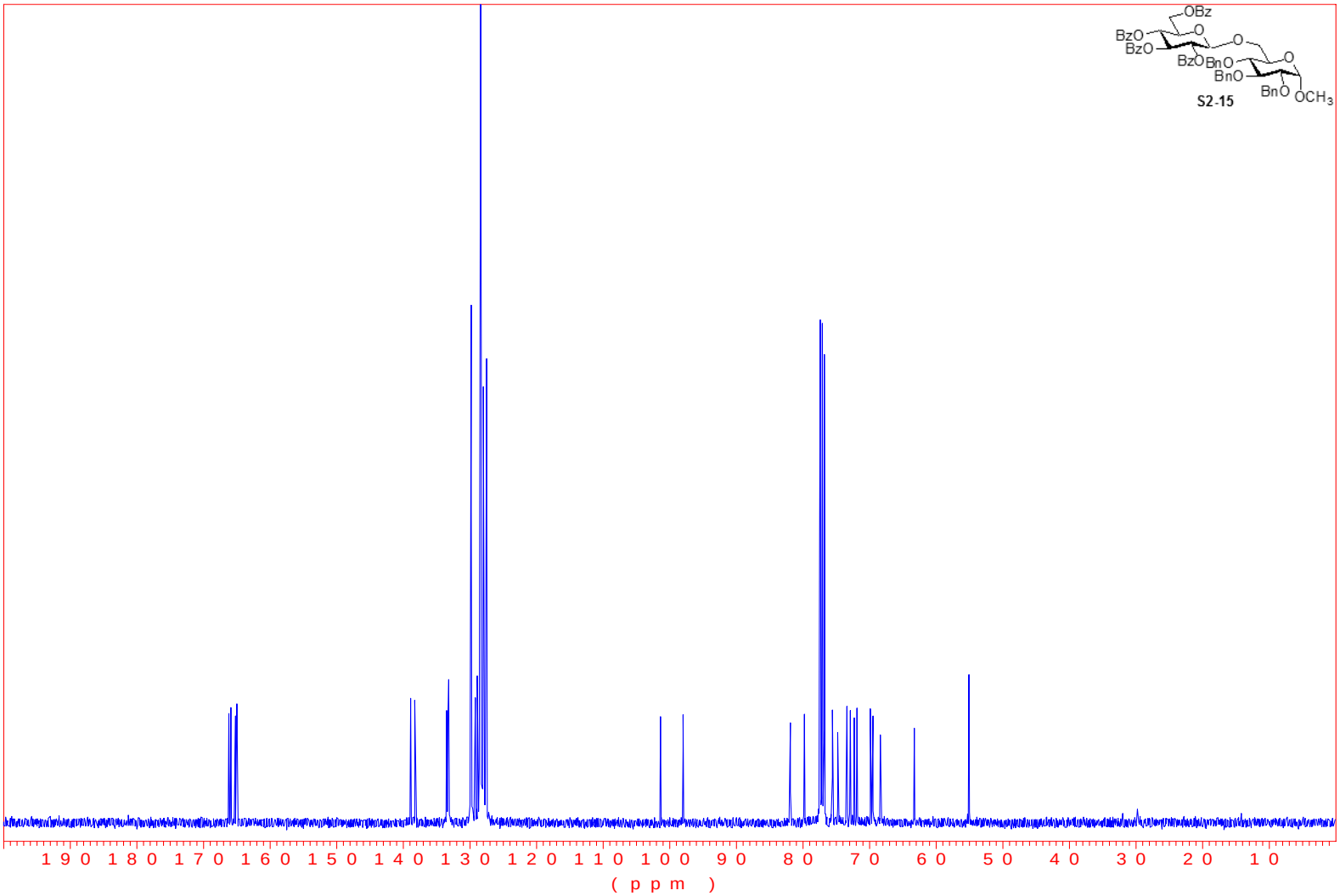
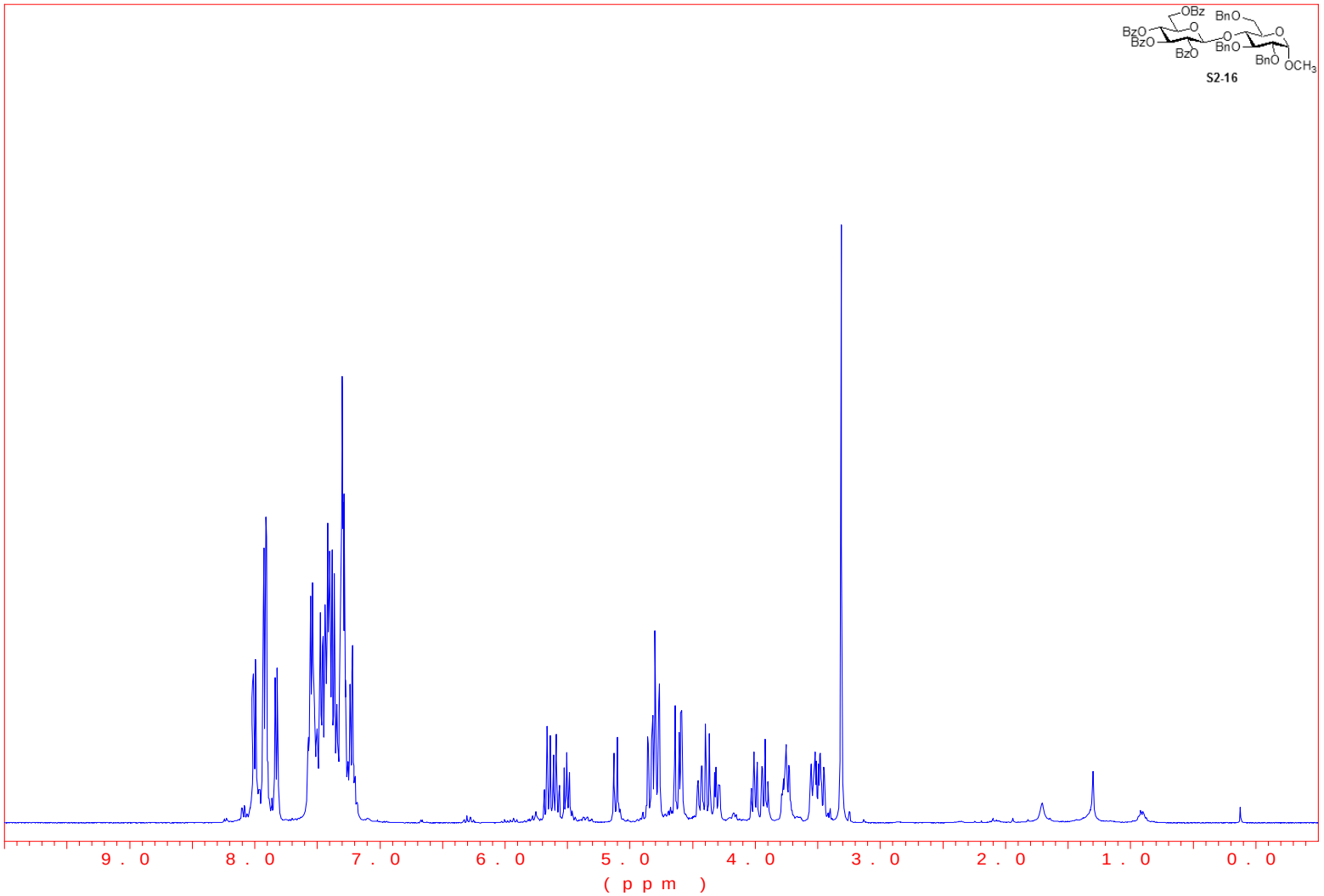
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


OBz
BzO
BzO
O
O
BzO
BnO
BnO
O
BnO
OCH3
S2-15
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


OBz
BnO
BzO
BzO
BzO
BnO
BnO
BnO
OCH3
S2-16
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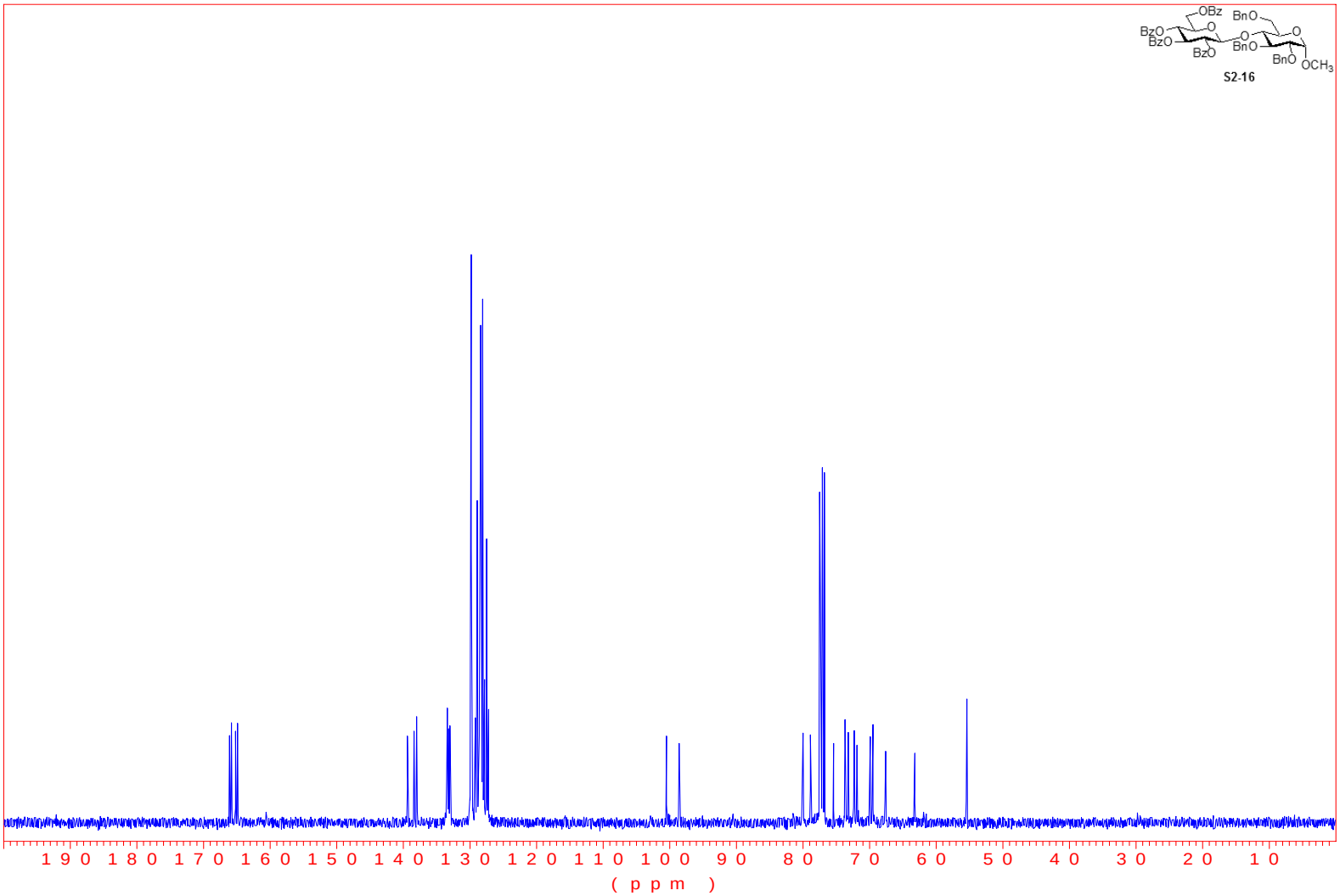
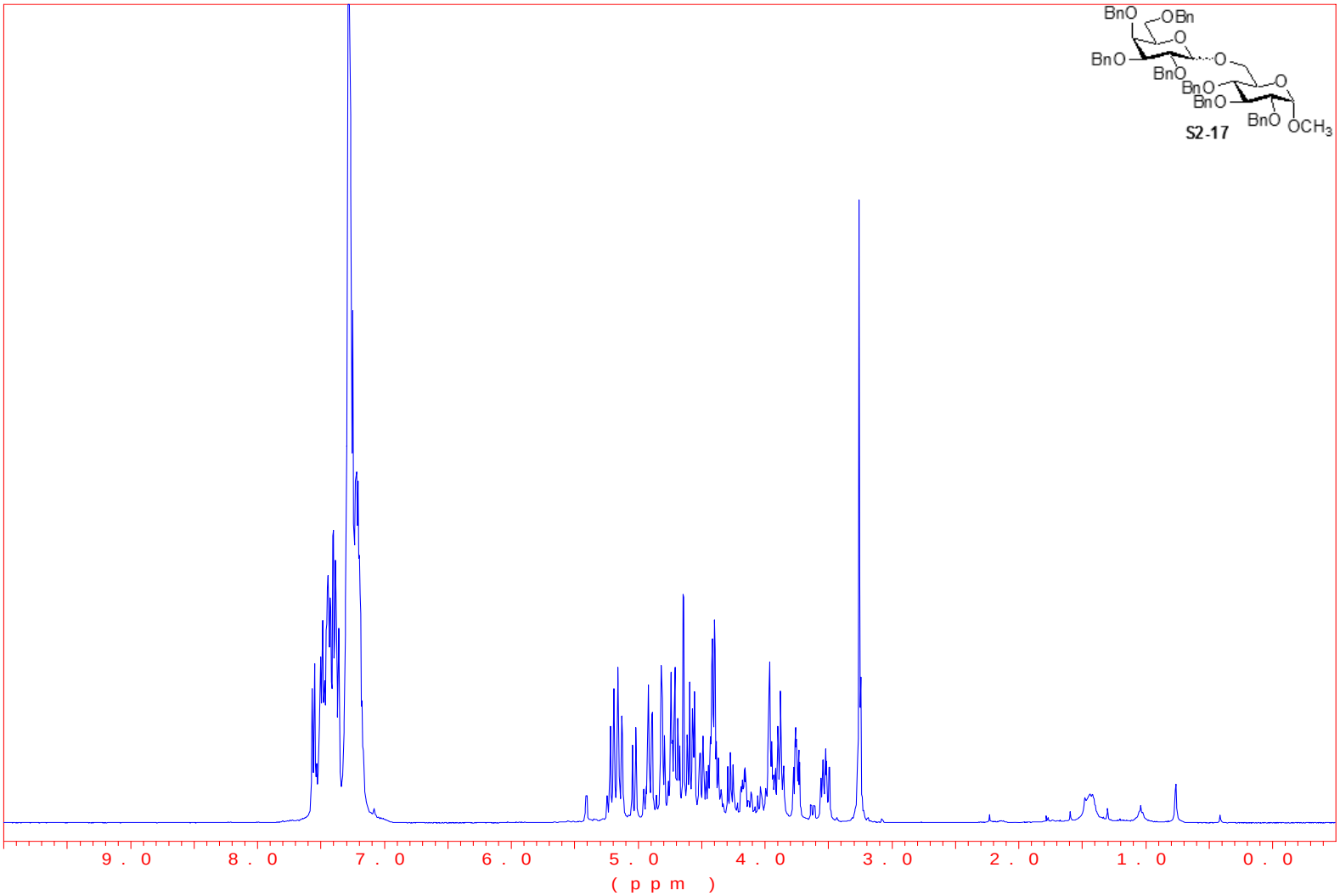
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)


OBz
BnO
BzO
O
BzO
O
BnO
BzO
BnO
BnO
OCH3
S2-16
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(p p m)


BnO
OBn
O
BnO
BnO
O
BnO
BnO
O
BnO
OCH3
S2-17
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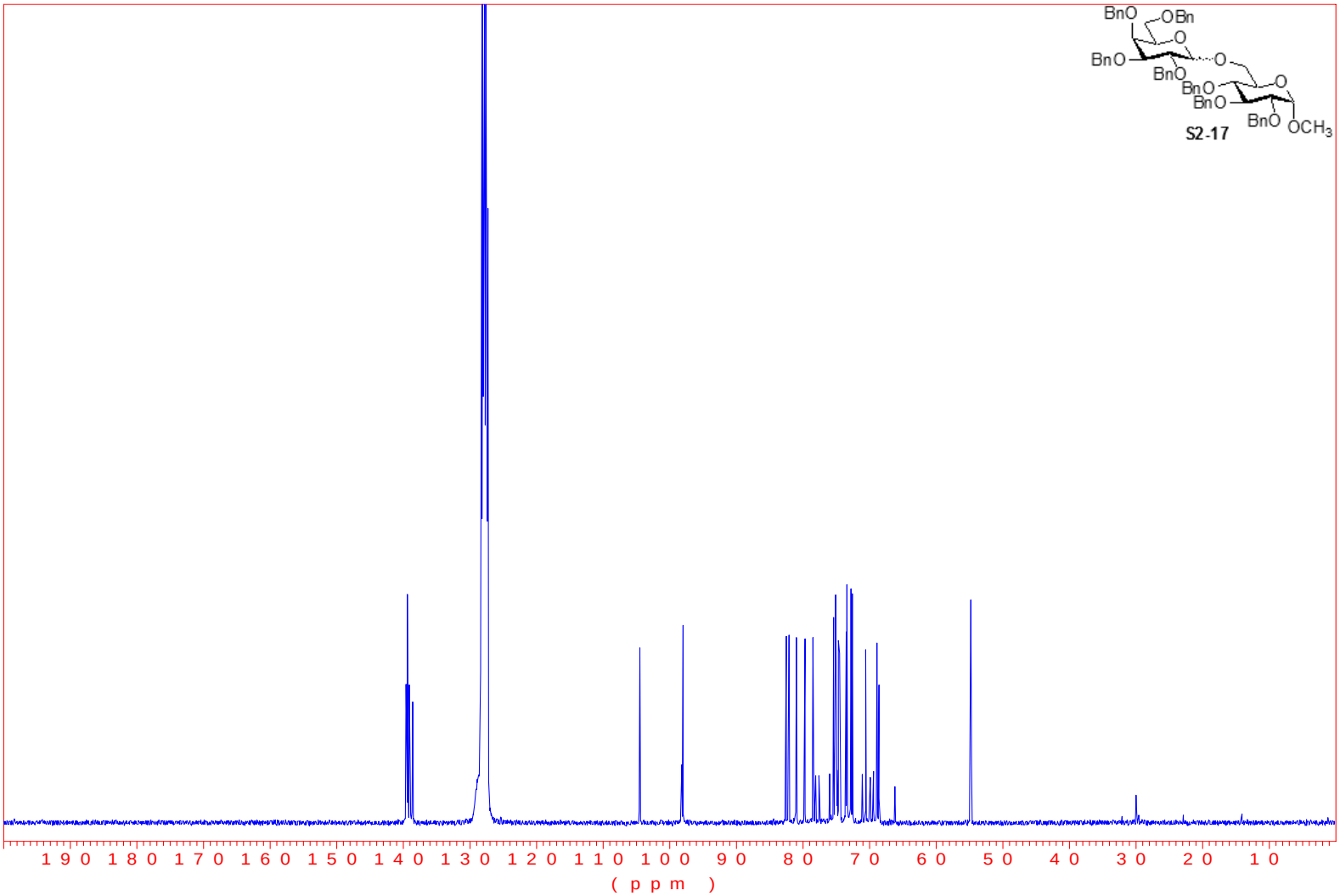
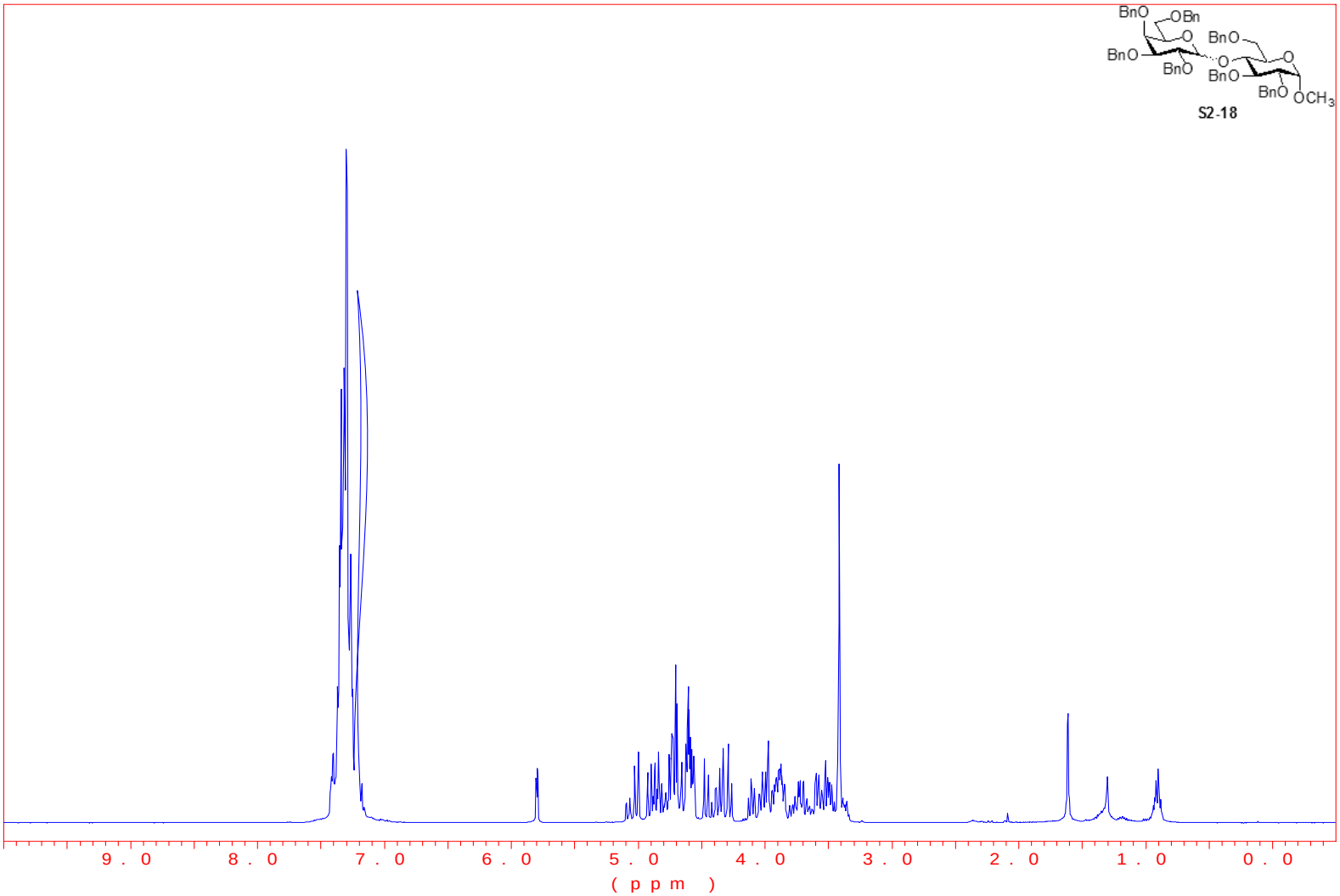
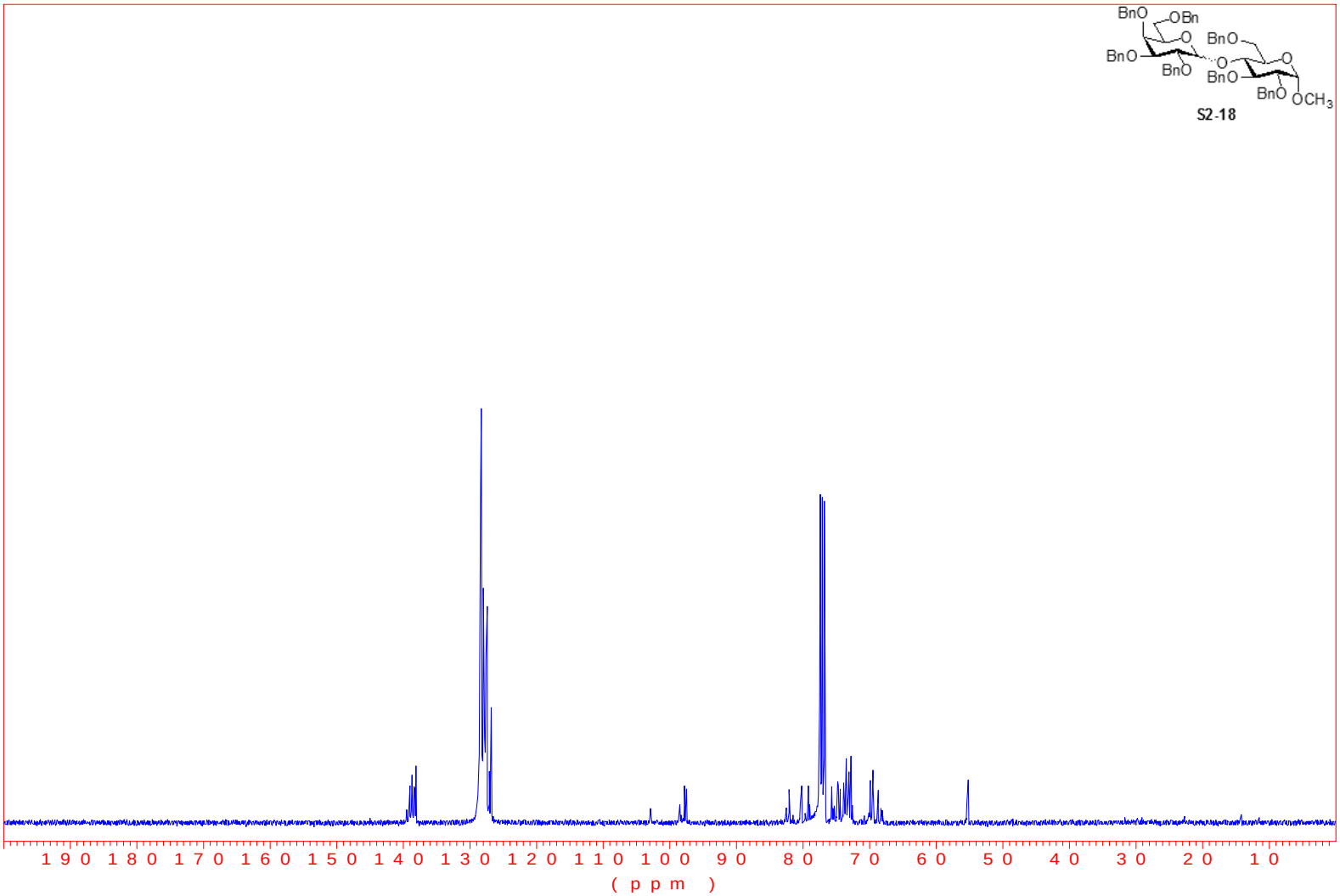
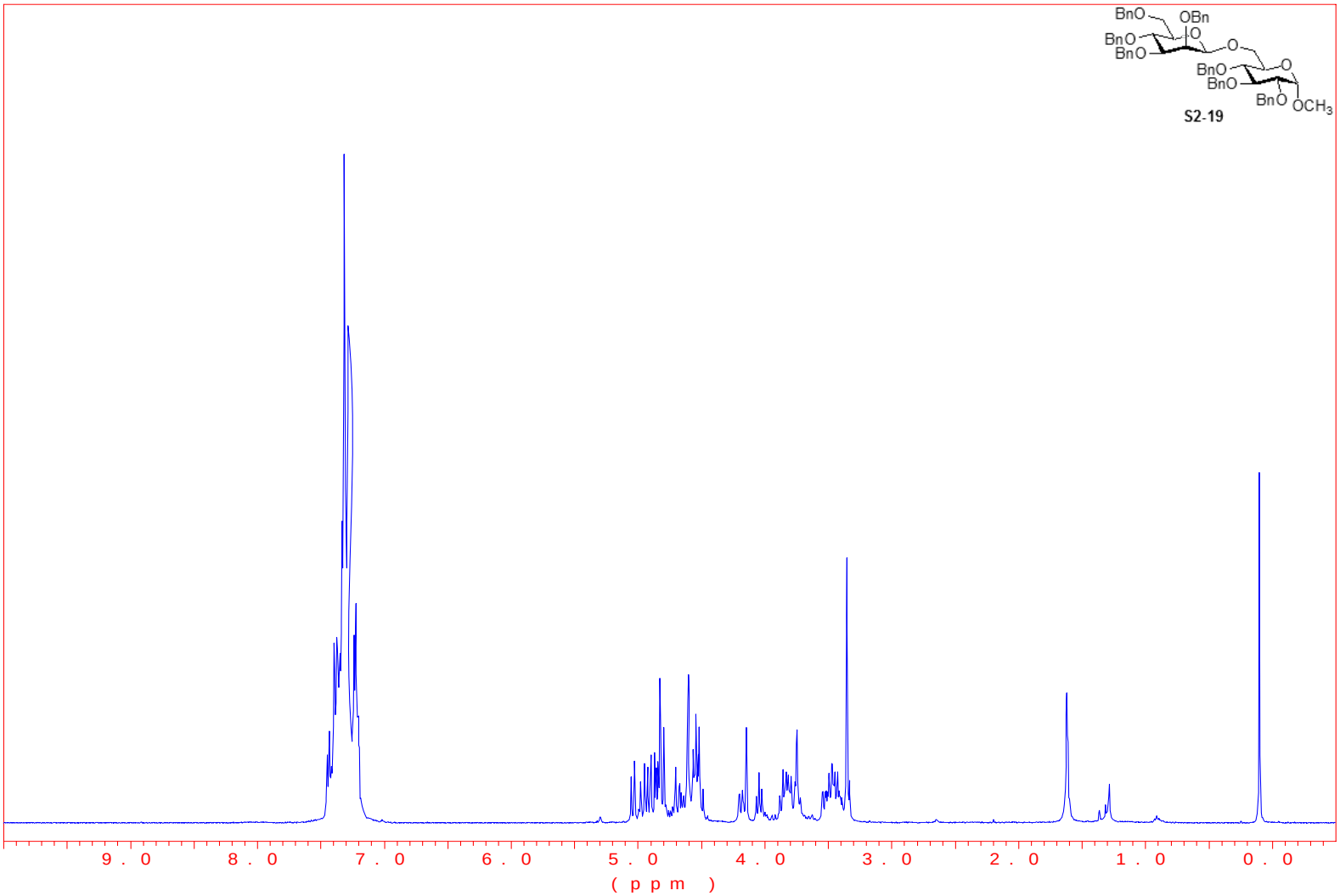
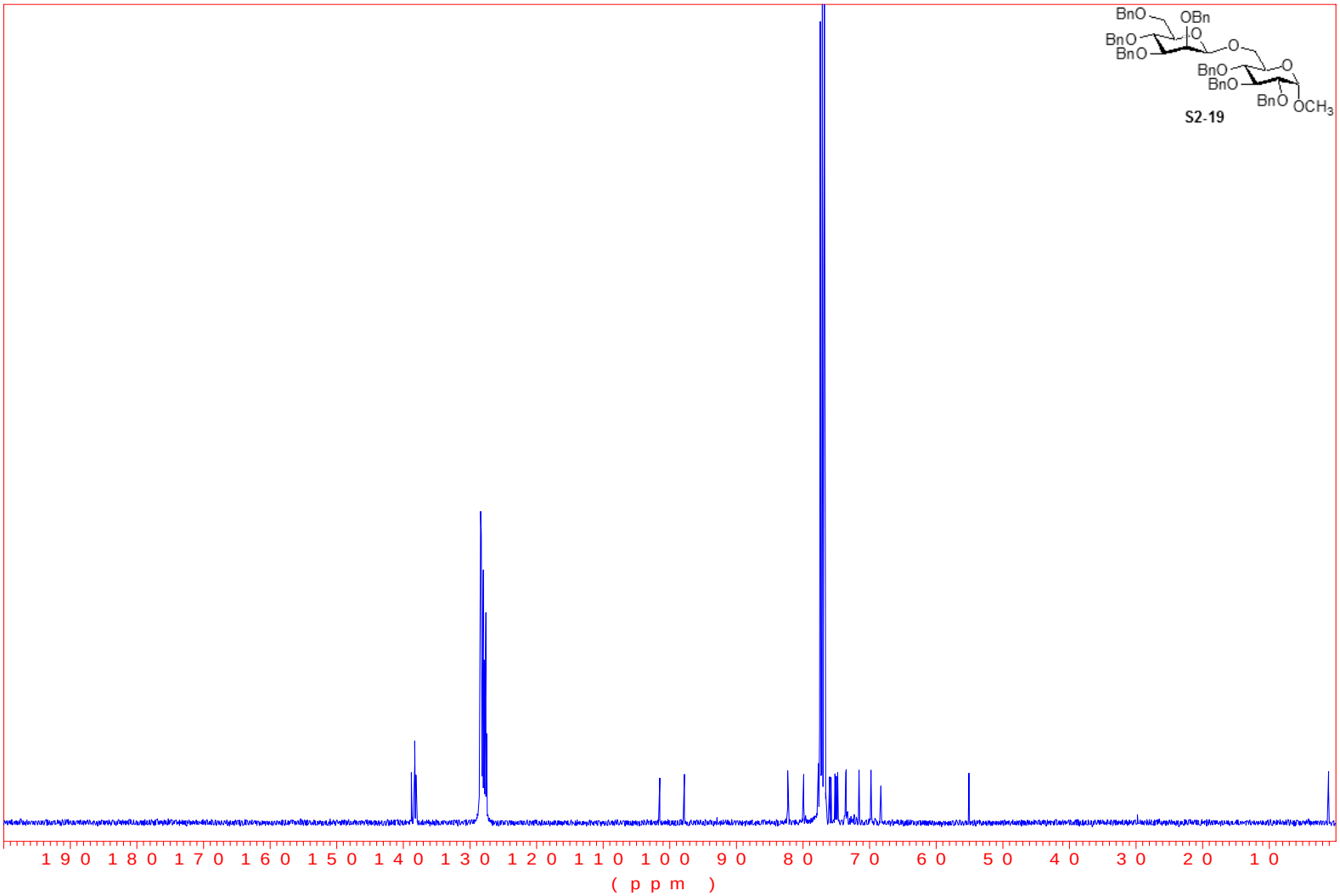
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

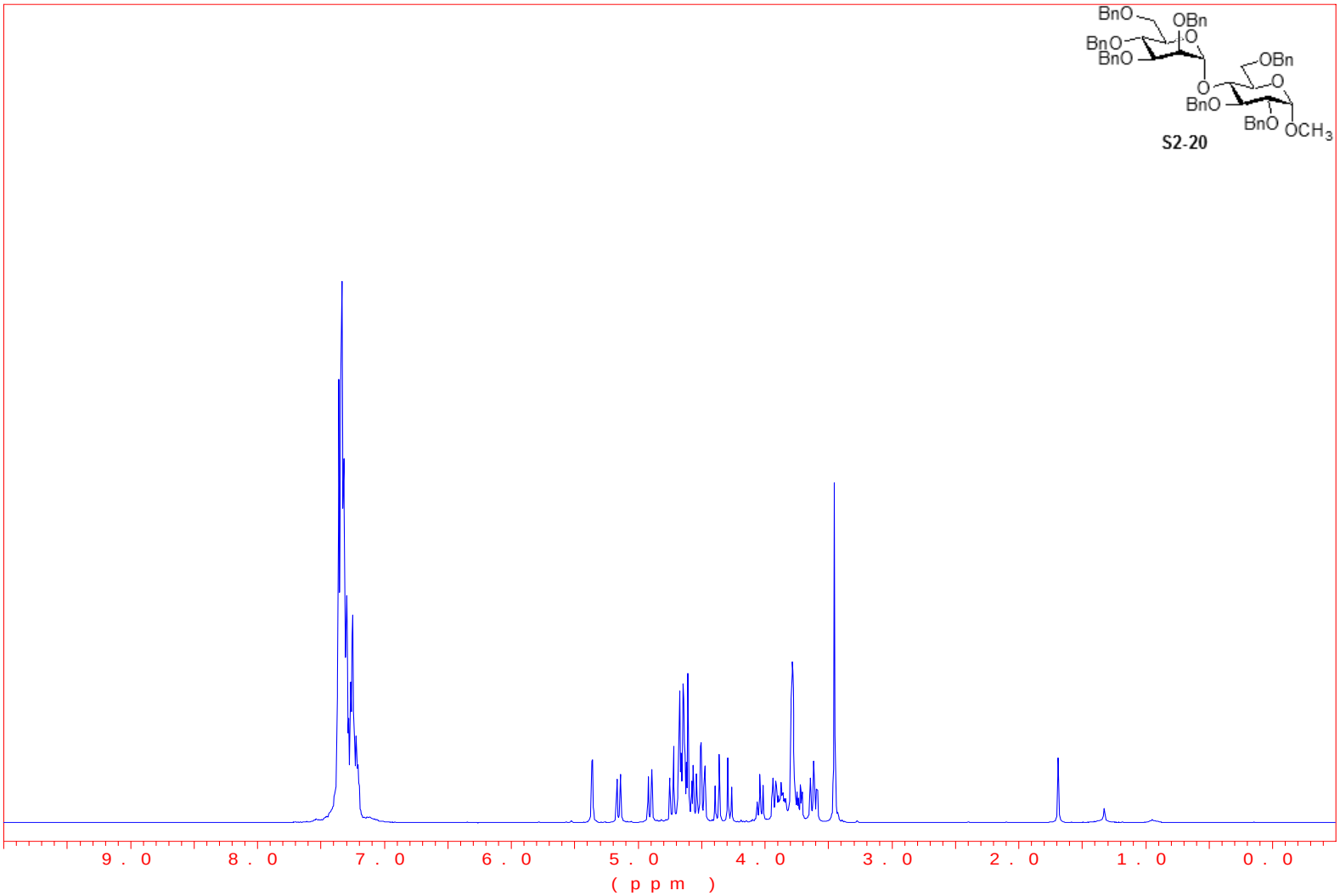

BnO
OBn
O
BnO
BnO
O
BnO
BnO
O
BnO
OCH3
S2-17
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(p p m)

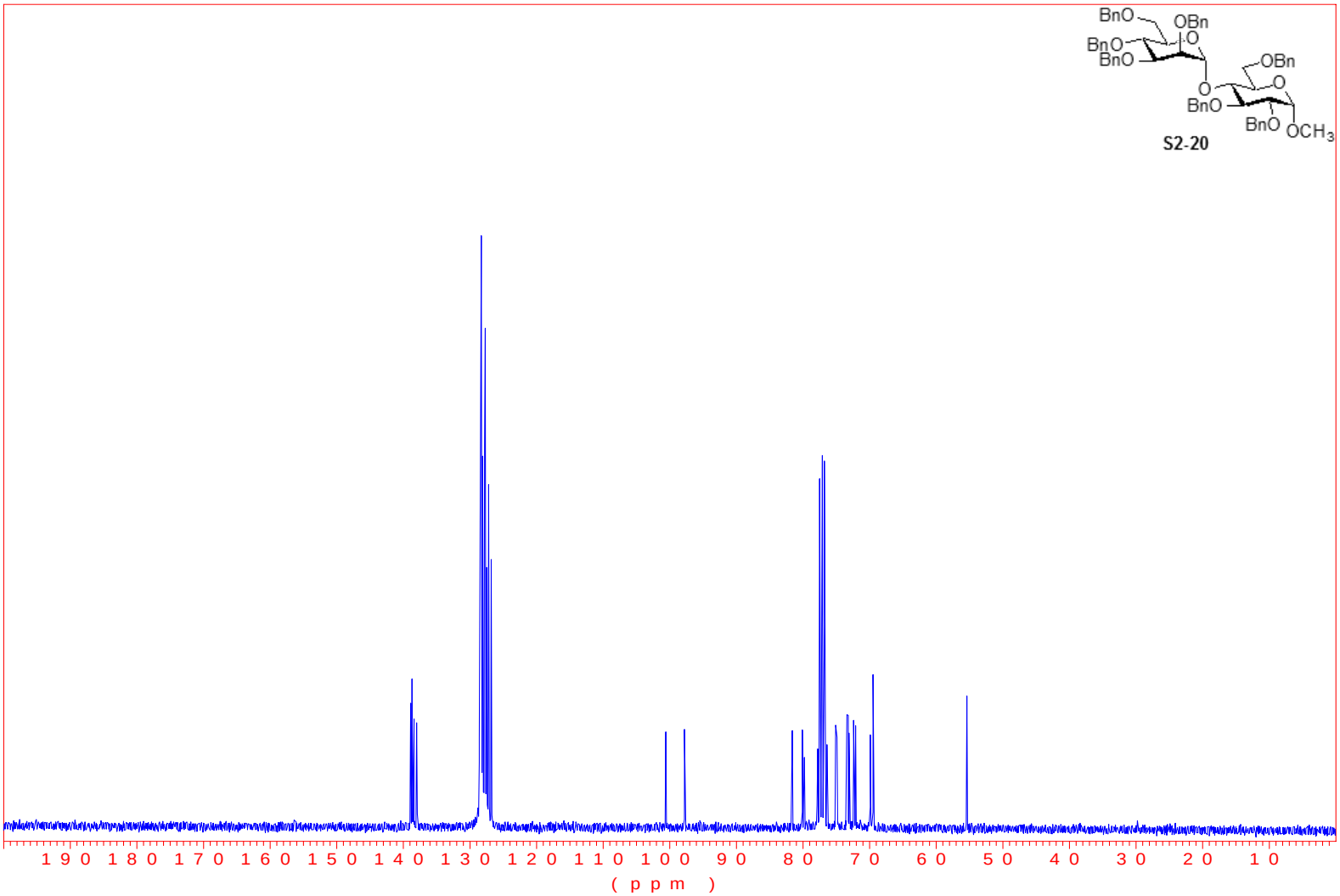

BnO
OBn
O
BnO
BnO
O
BnO
BnO
O
BnO
OCH3
S2-18
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

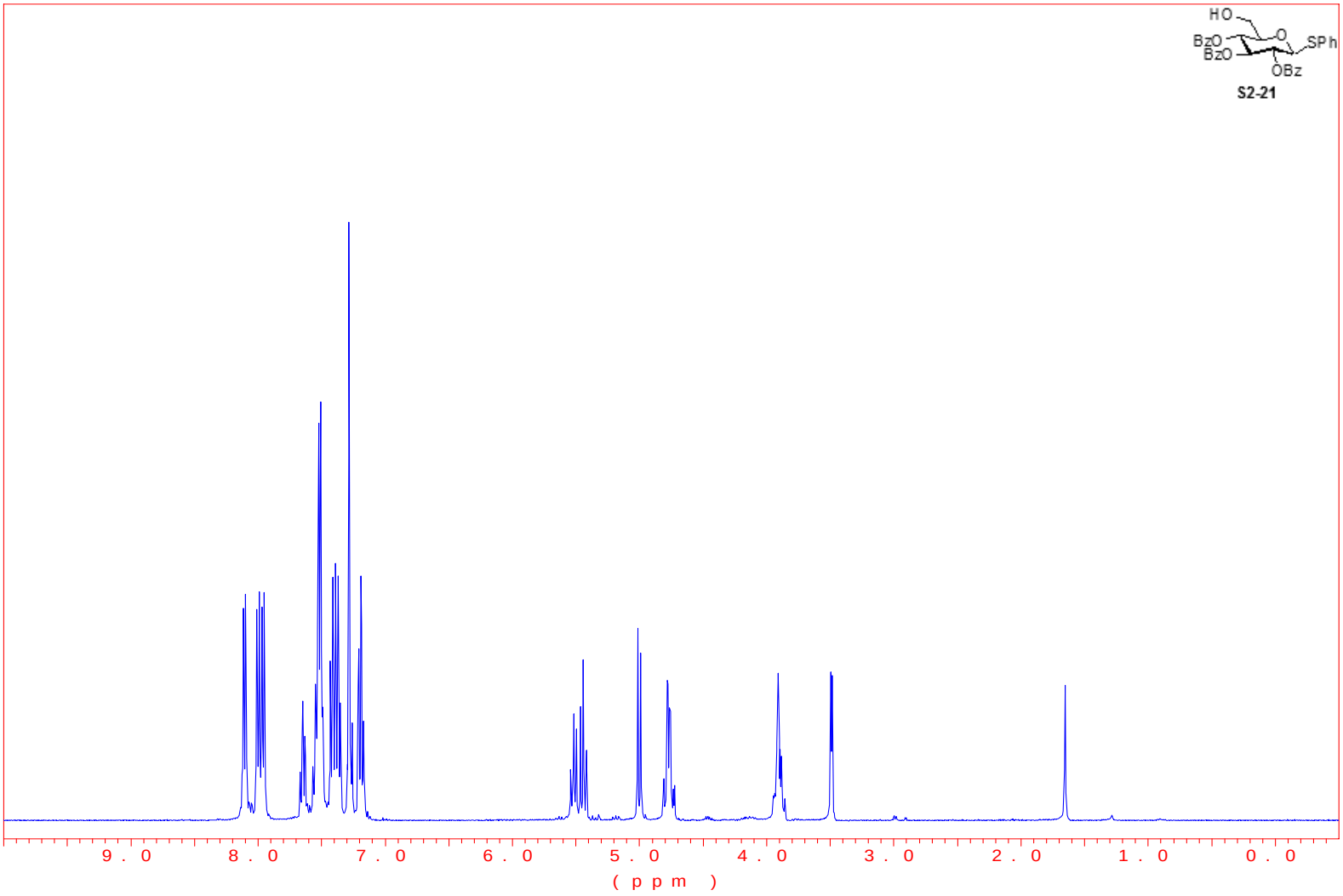

BnO
OBn
O
BnO
BnO
O
BnO
BnO
BnO
OCH3
S2-18
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

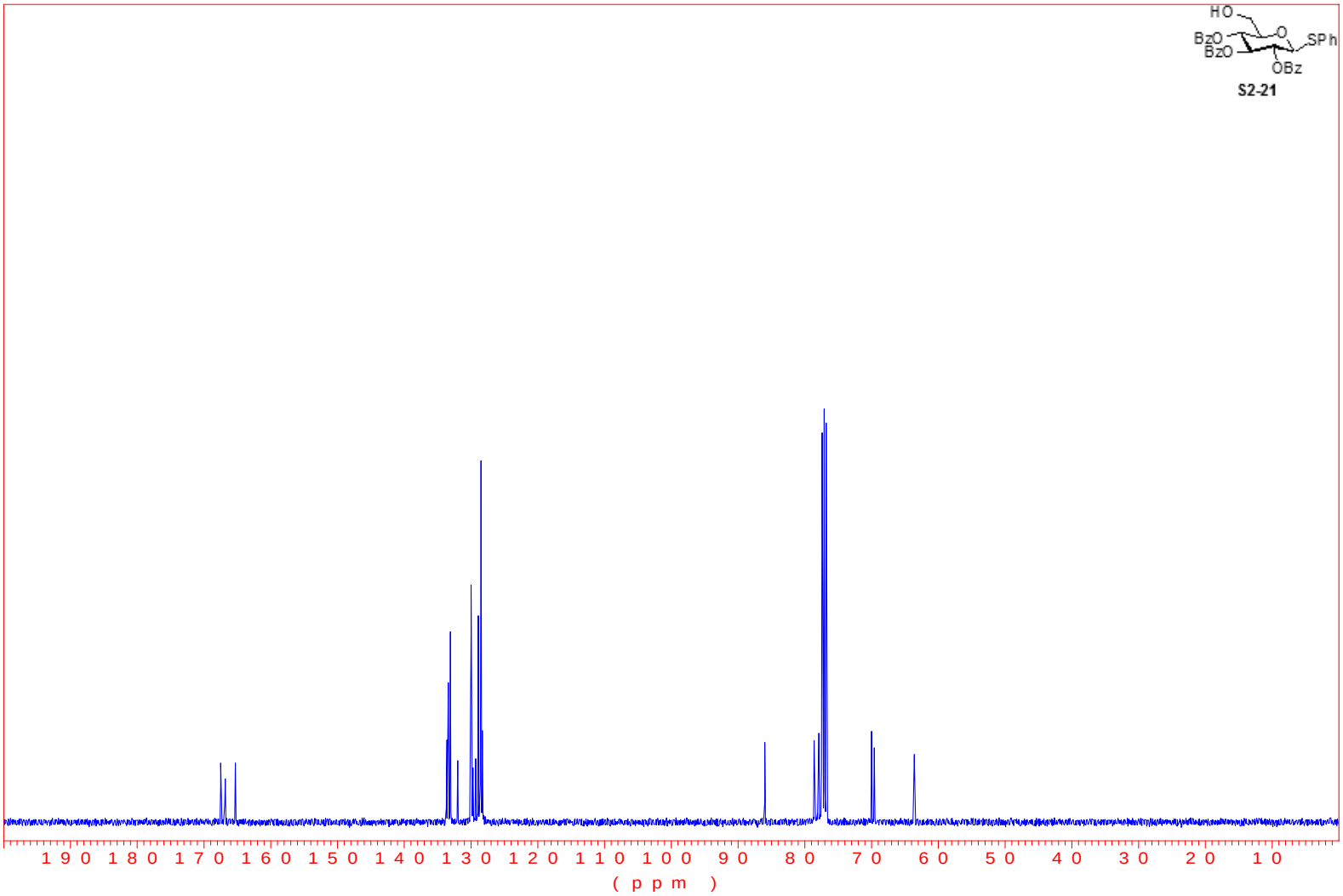

BnO
OBn
BnO
BnO
O
O
BnO
BnO
BnO
OCH3
S2-19
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

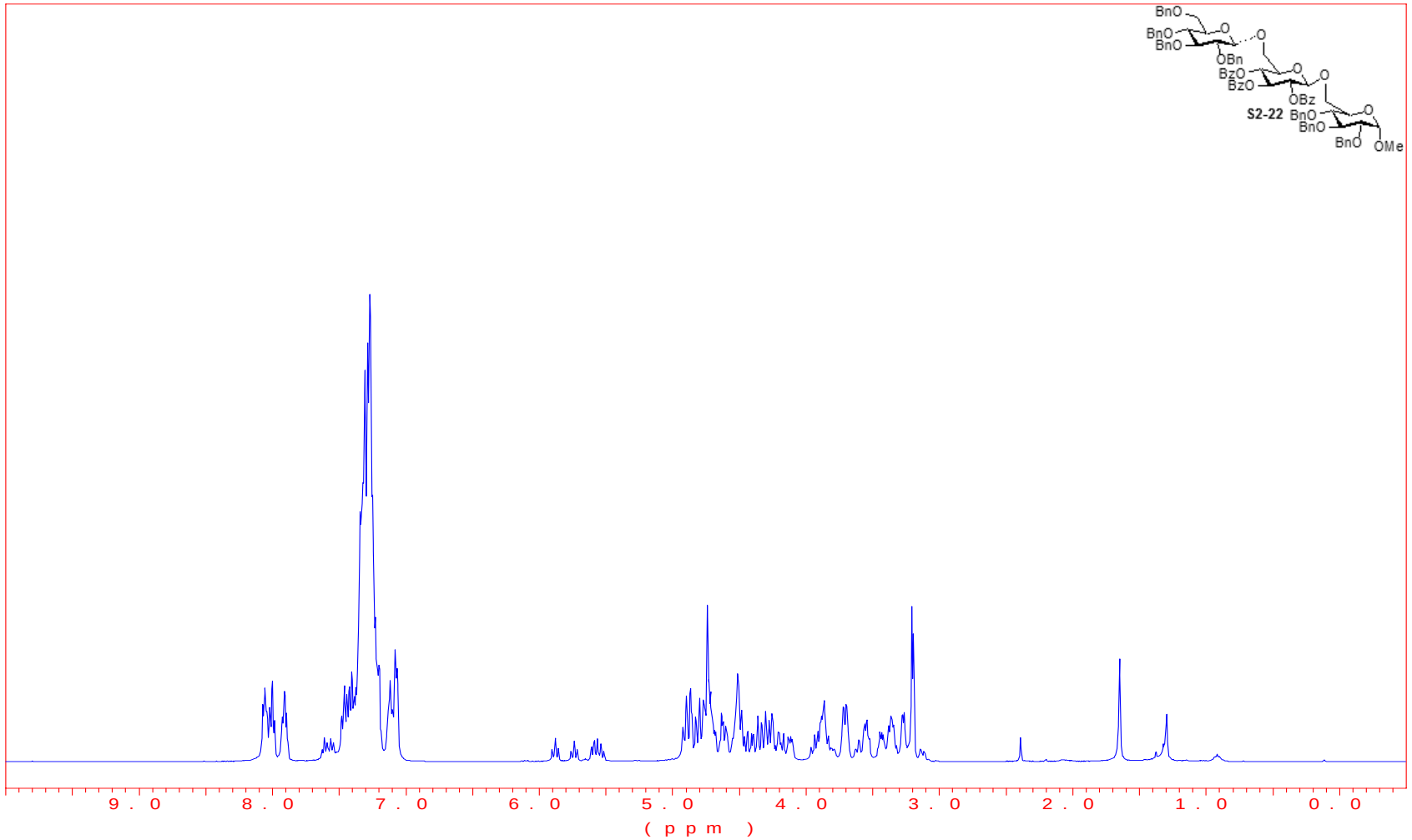

BnO
OBn
BnO
BnO
O
O
BnO
BnO
BnO
OCH3
S2-19
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)


BnO
OBn
BnO
BnO
O
OBn
O
BnO
BnO
OCH3
S2-20
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

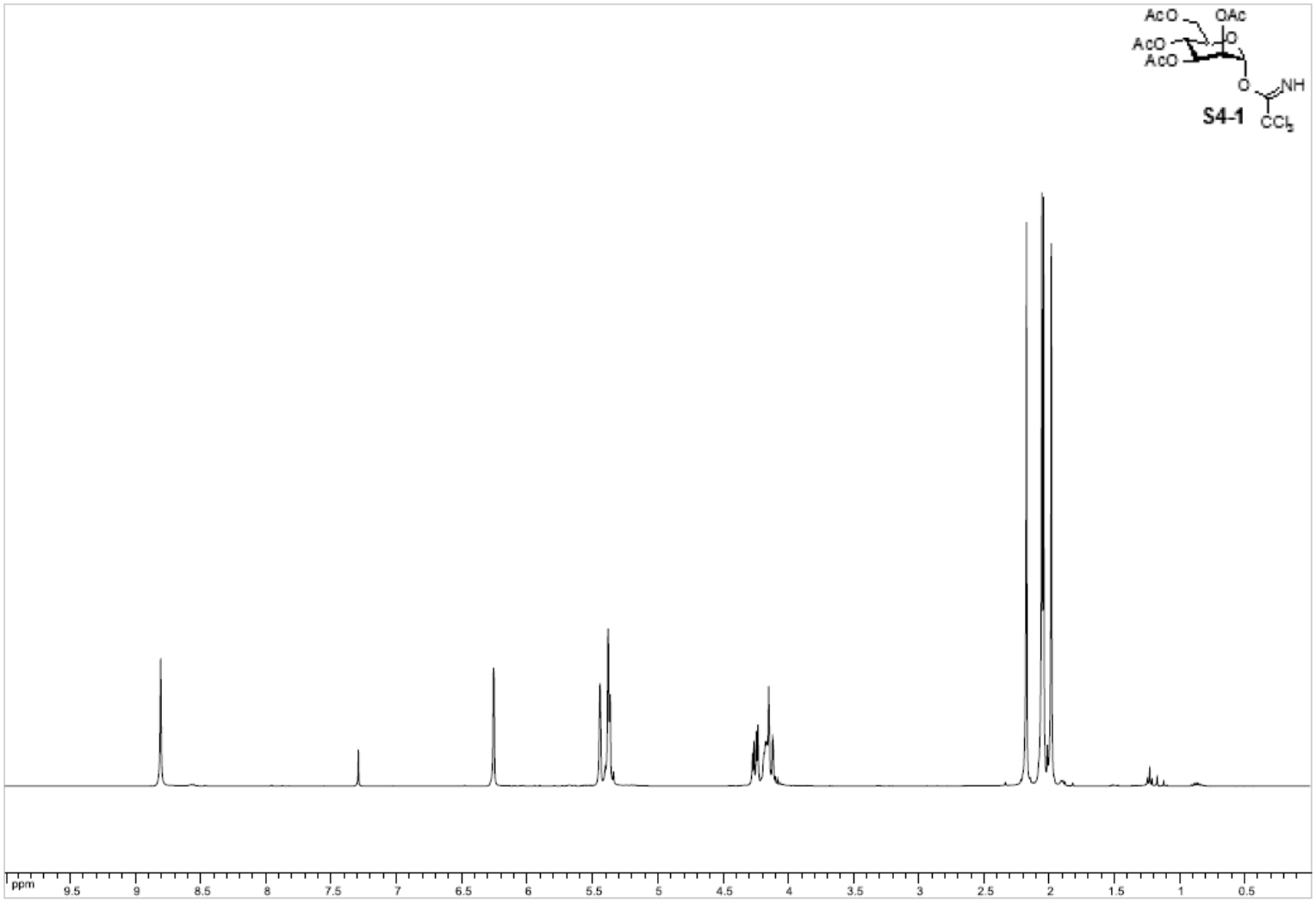

BnO
OBn
BnO
BnO
O
OBn
O
BnO
BnO
OCH3
S2-20
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

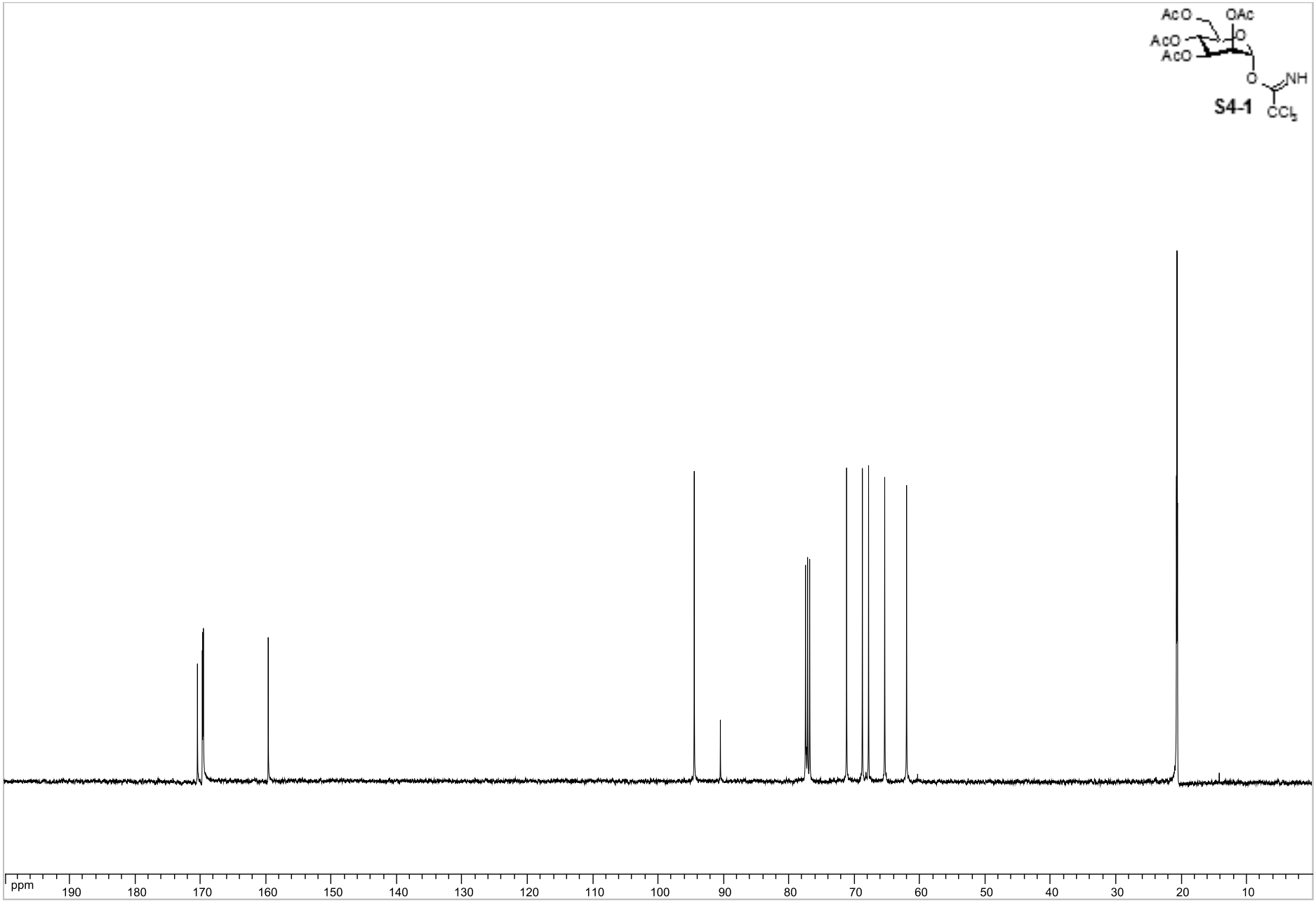

HO
BzO
BzO
O
SPh
OBz
S2-21
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

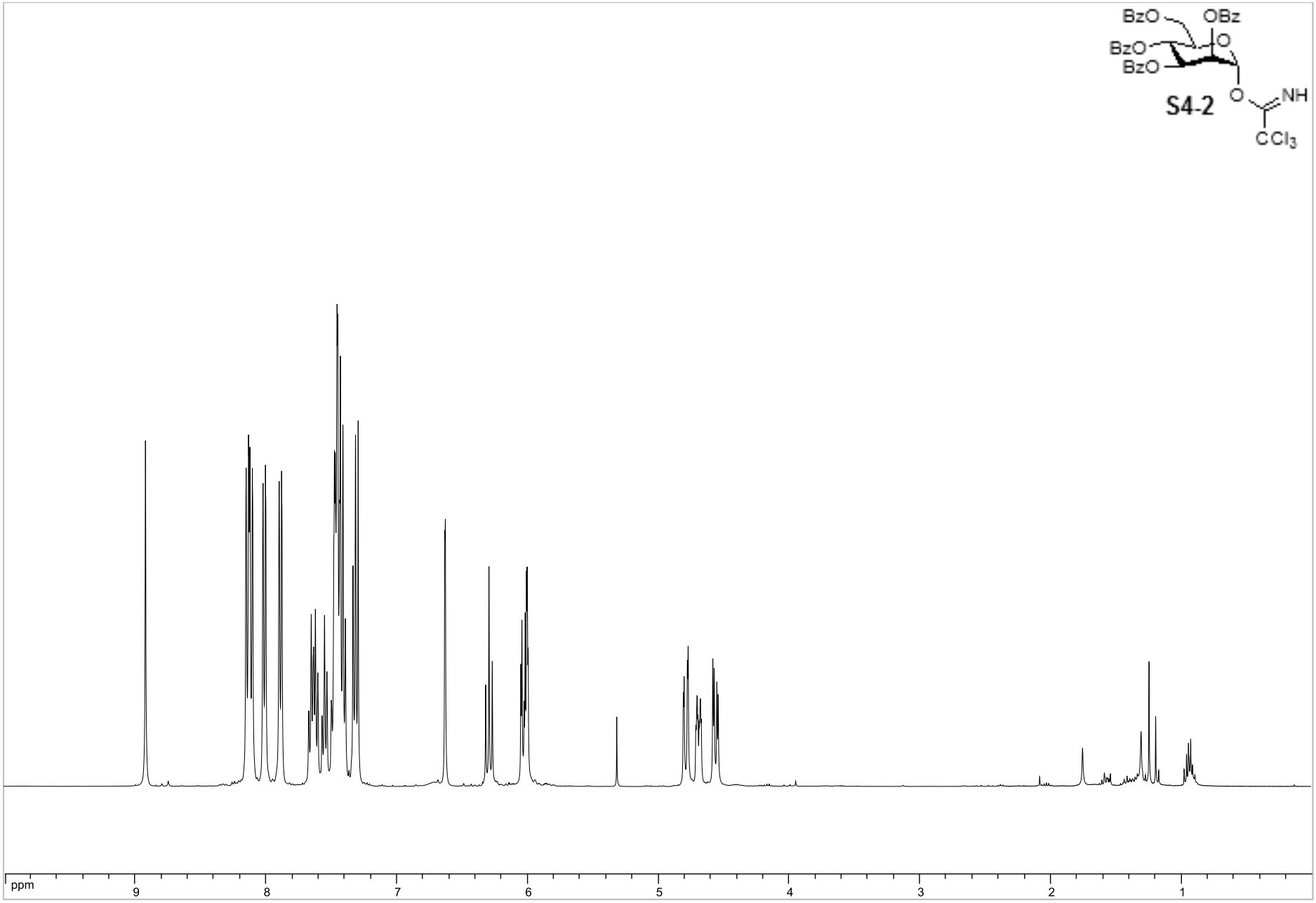

HO
BzO
BzO
O
SPh
OBz
S2-21
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(p p m)

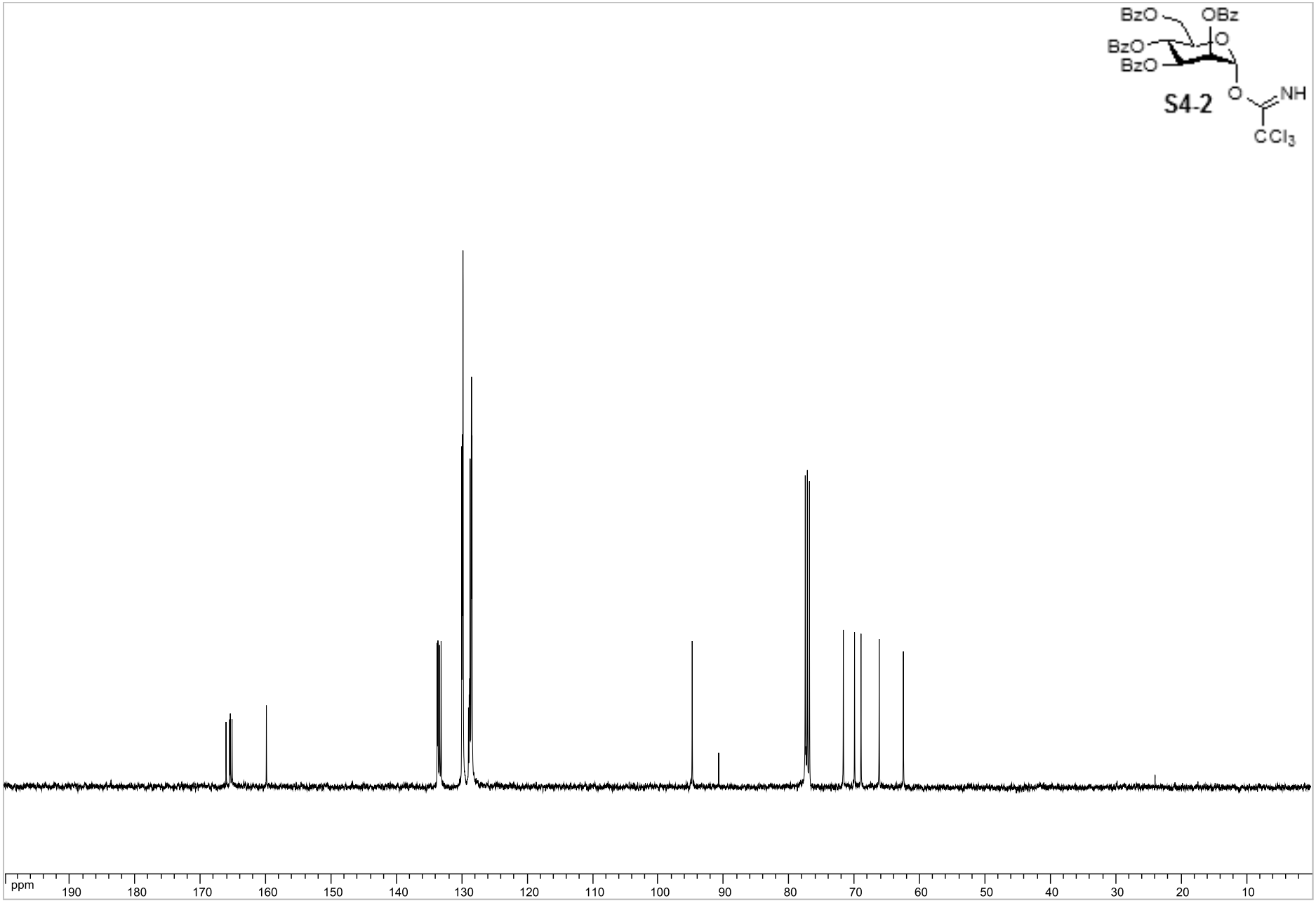

BnO
BnO
BnO
O
OBn
BzO
BzO
O
O
OBz
S2-22
BnO
BnO
O
BnO
OMe
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
(ppm)

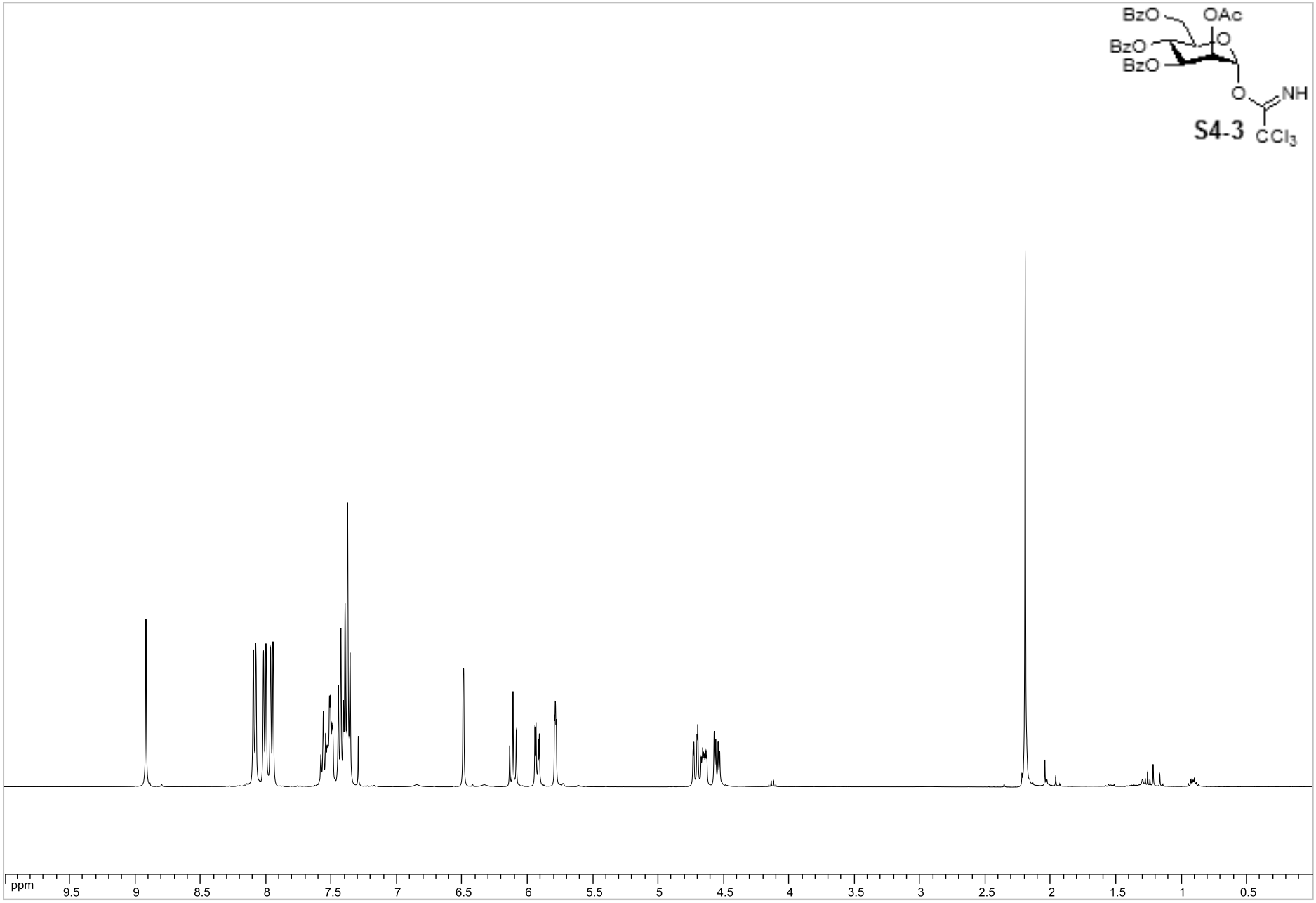

BnO
BnO
BnO
O
OBn
BzO
BzO
O
O
OBz
S2-22
BnO
BnO
O
BnO
OMe
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
(ppm)

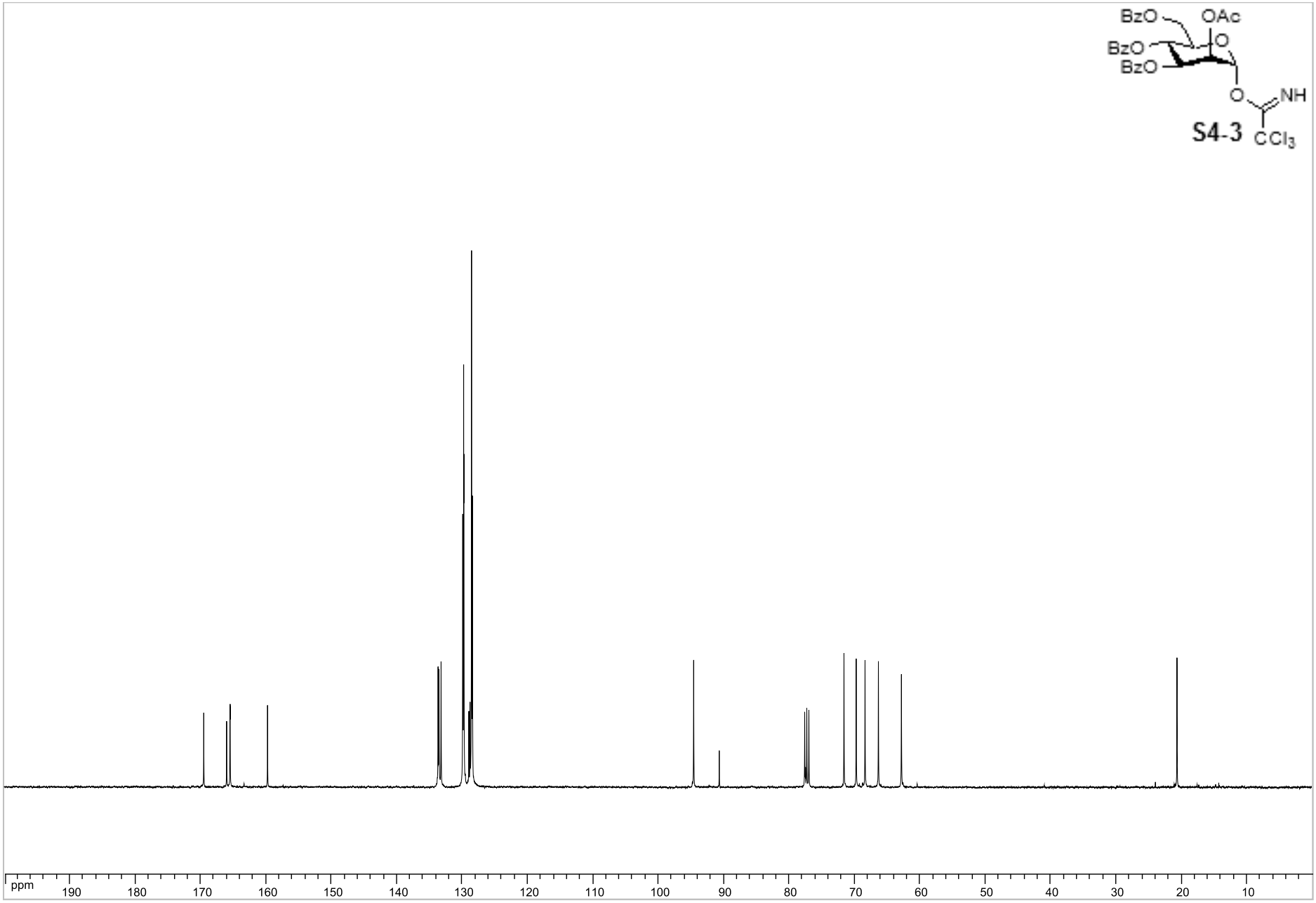

AcO
OAc
AcO
O
AcO
O
NH
S4-1
CCl3
ppm
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

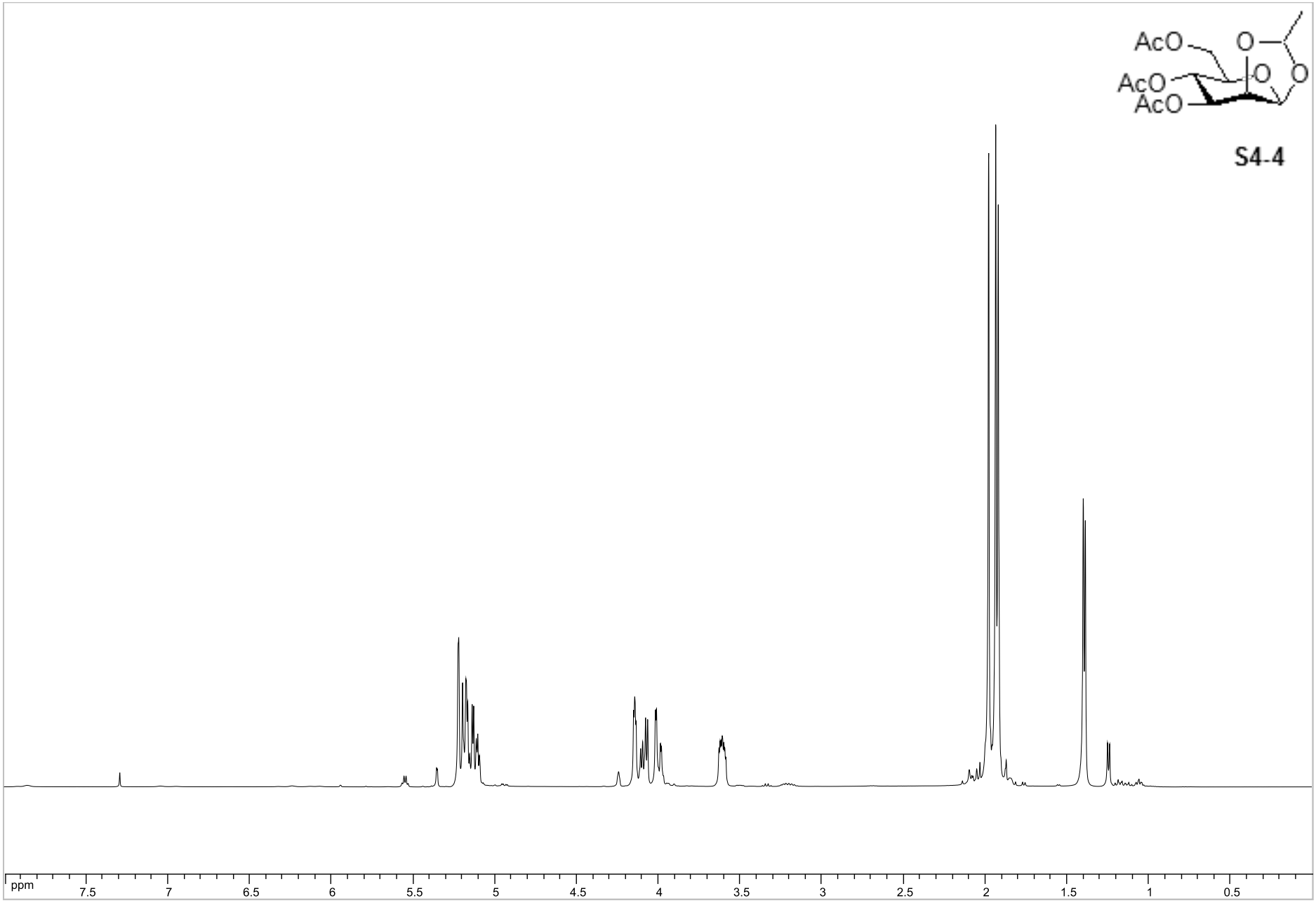

AcO
OAc
AcO
AcO
O
O
NH
CCl3
S4-1
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

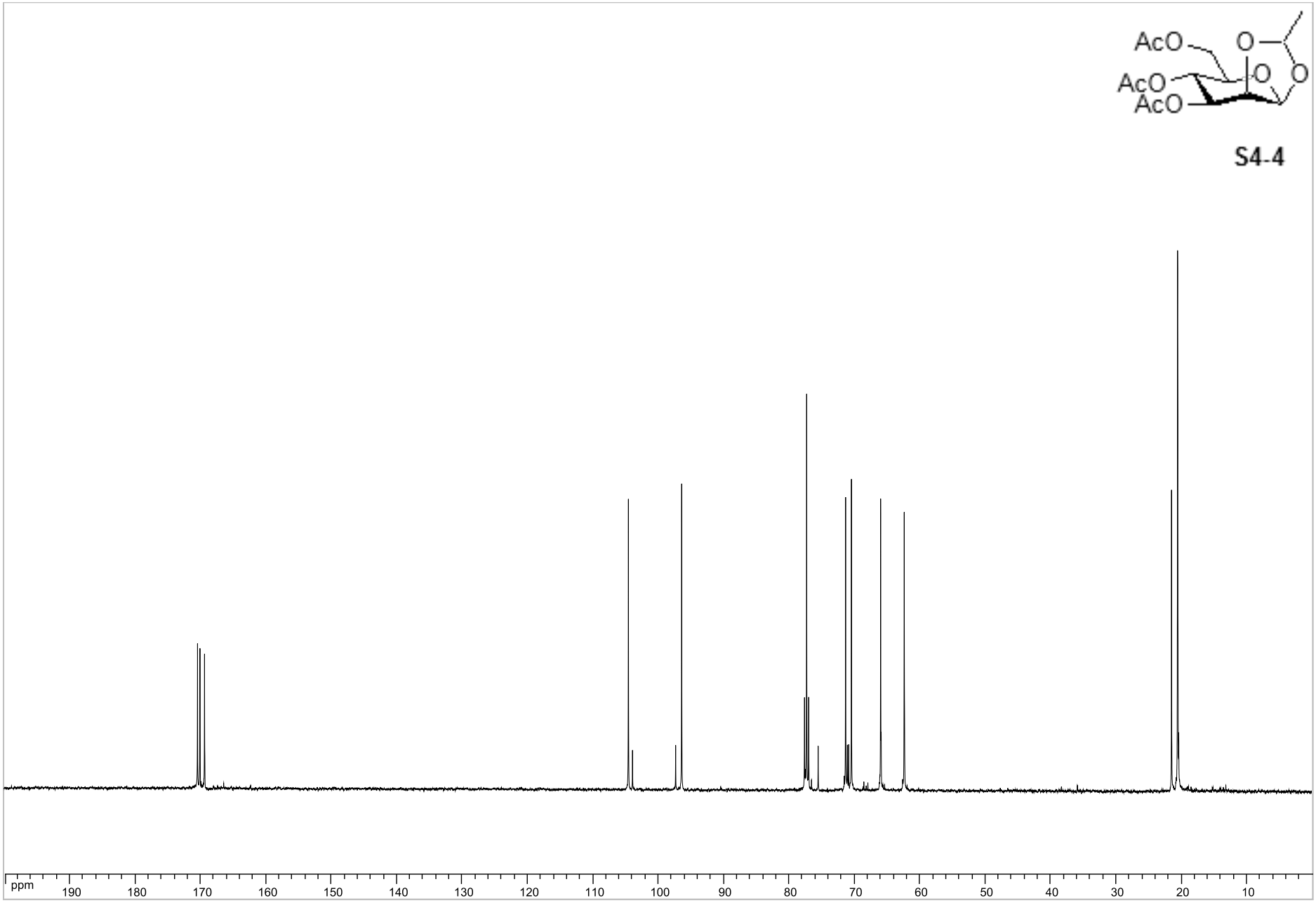

BzO
OBz
BzO
O
BzO
S4-2
O
NH
CCl3
ppm
9
8
7
6
5
4
3
2
1

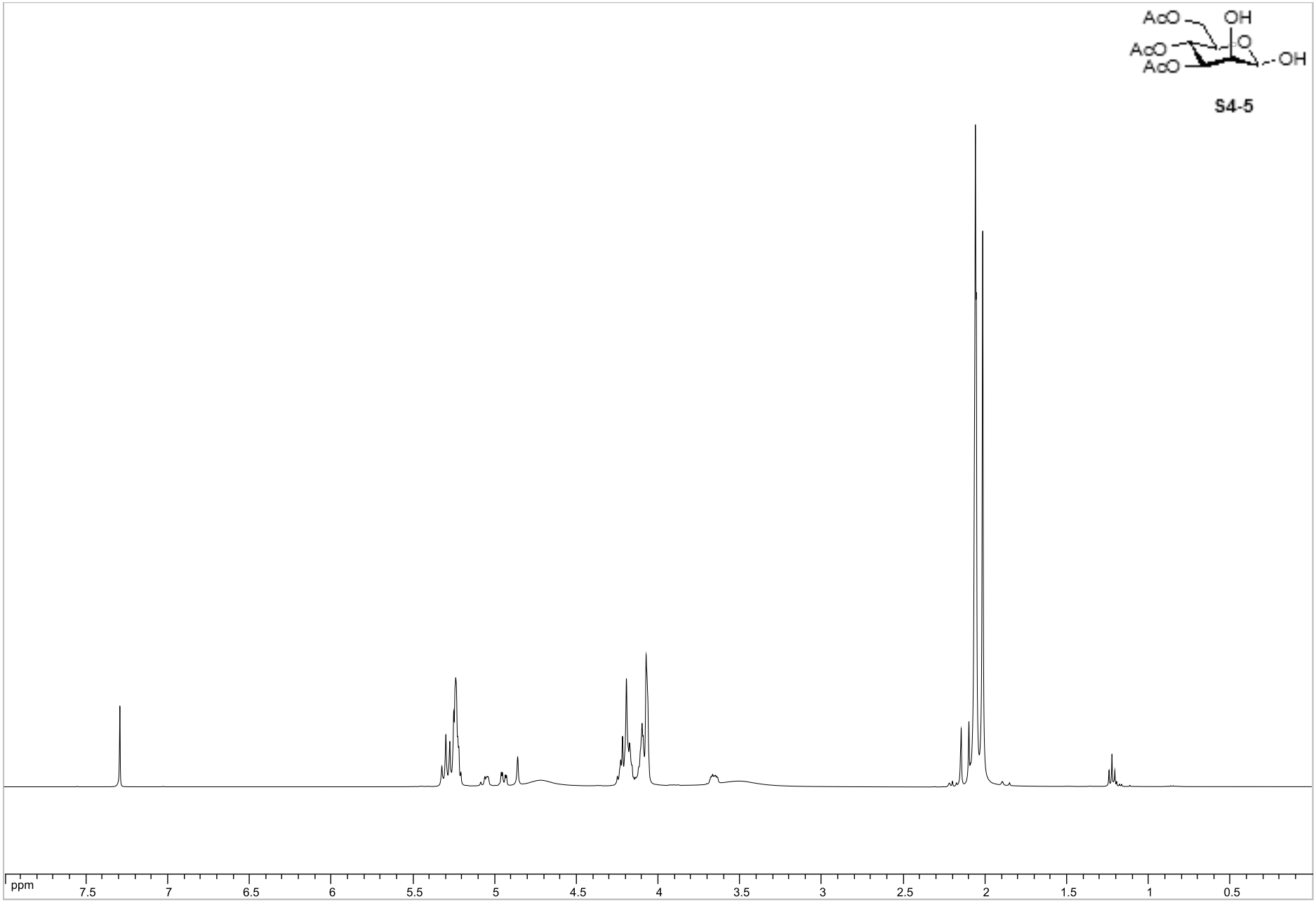

BzO
OBz
BzO
O
BzO
S4-2
O
NH
CCl3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

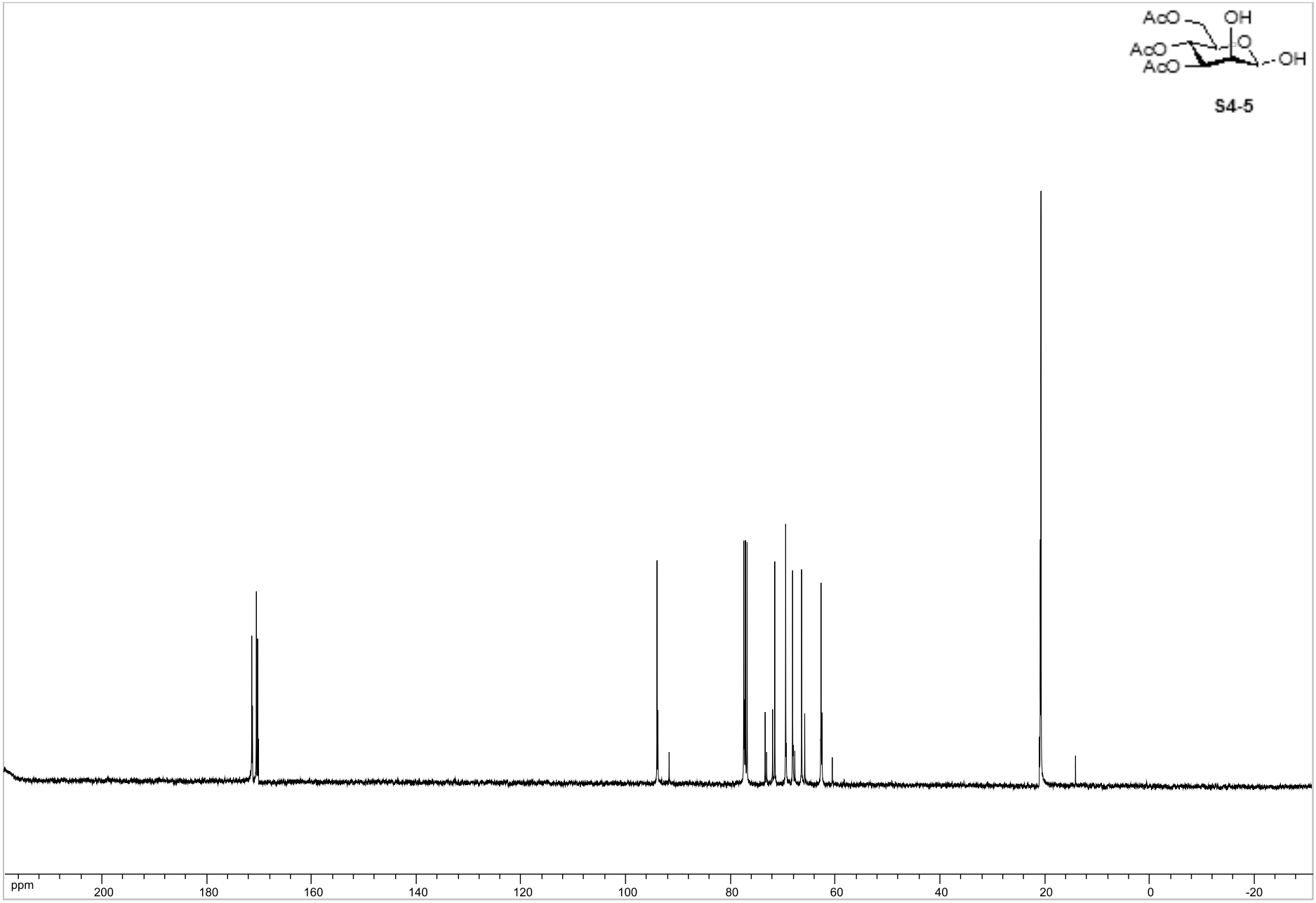

BzO
OAc
O
BzO
BzO
O
NH
S4-3
CCl3
ppm
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

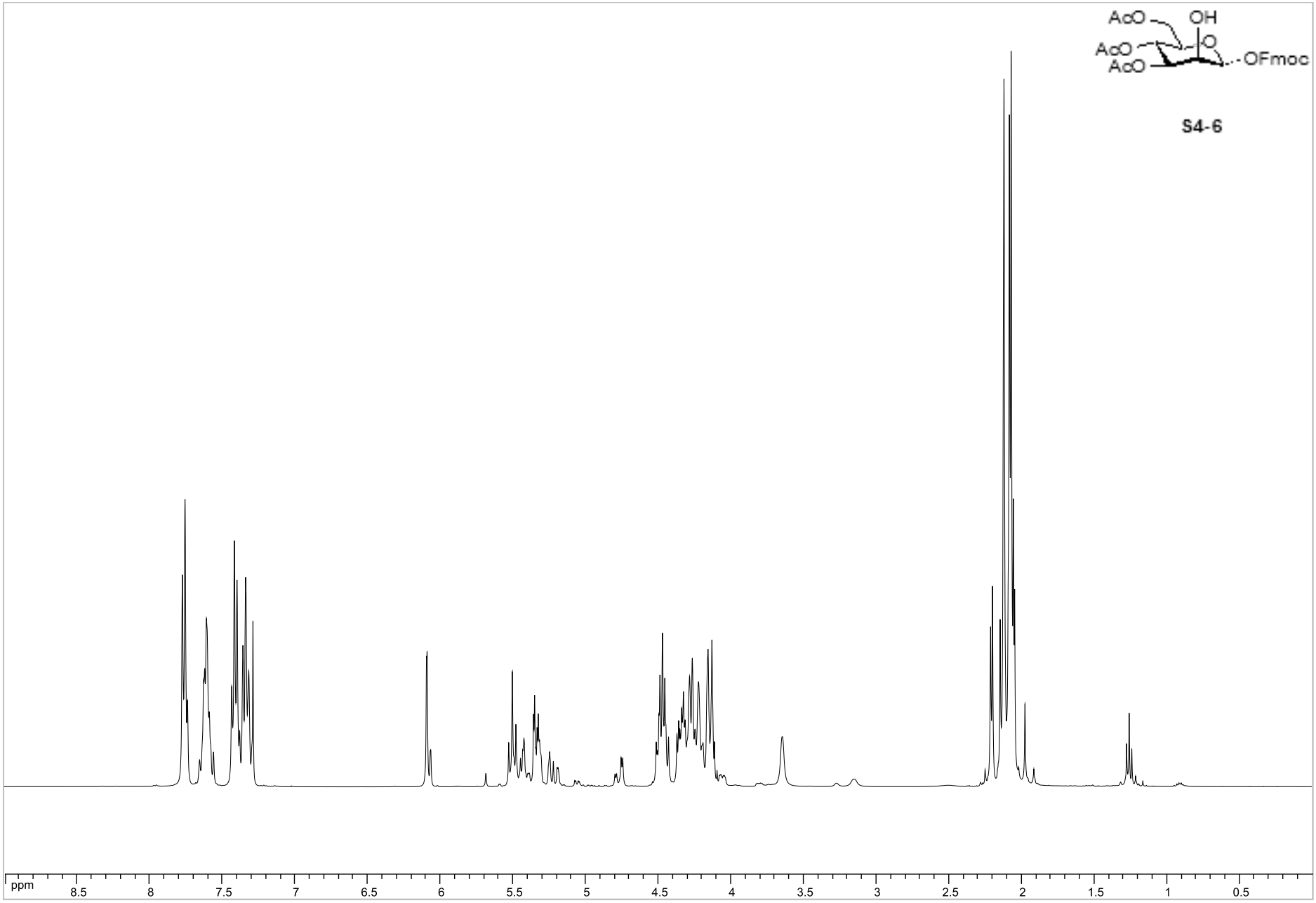

BzO
OAc
O
BzO
BzO
O
NH
S4-3
CCl3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

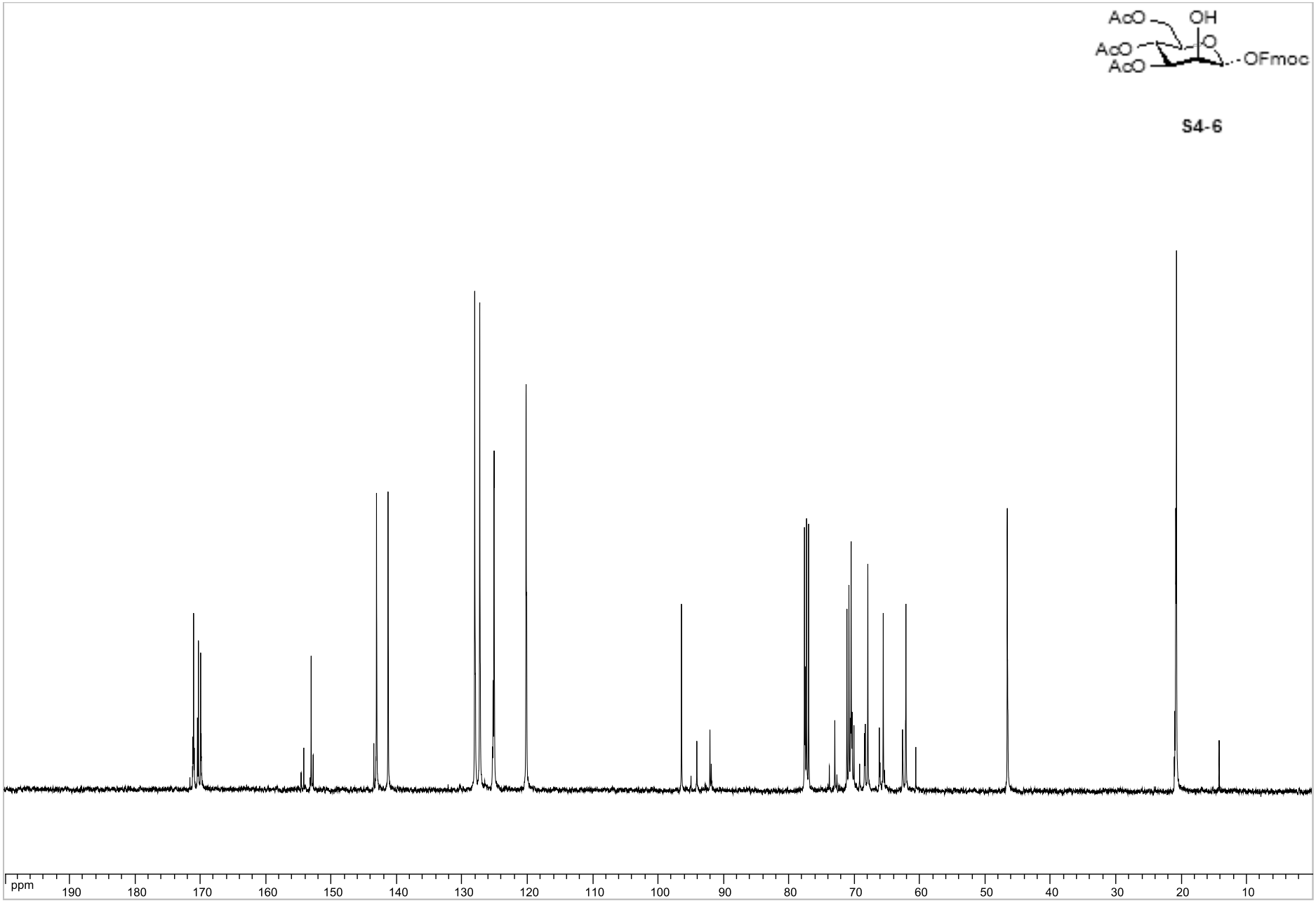

AcO
AcO
AcO
O
O
O
S4-4
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

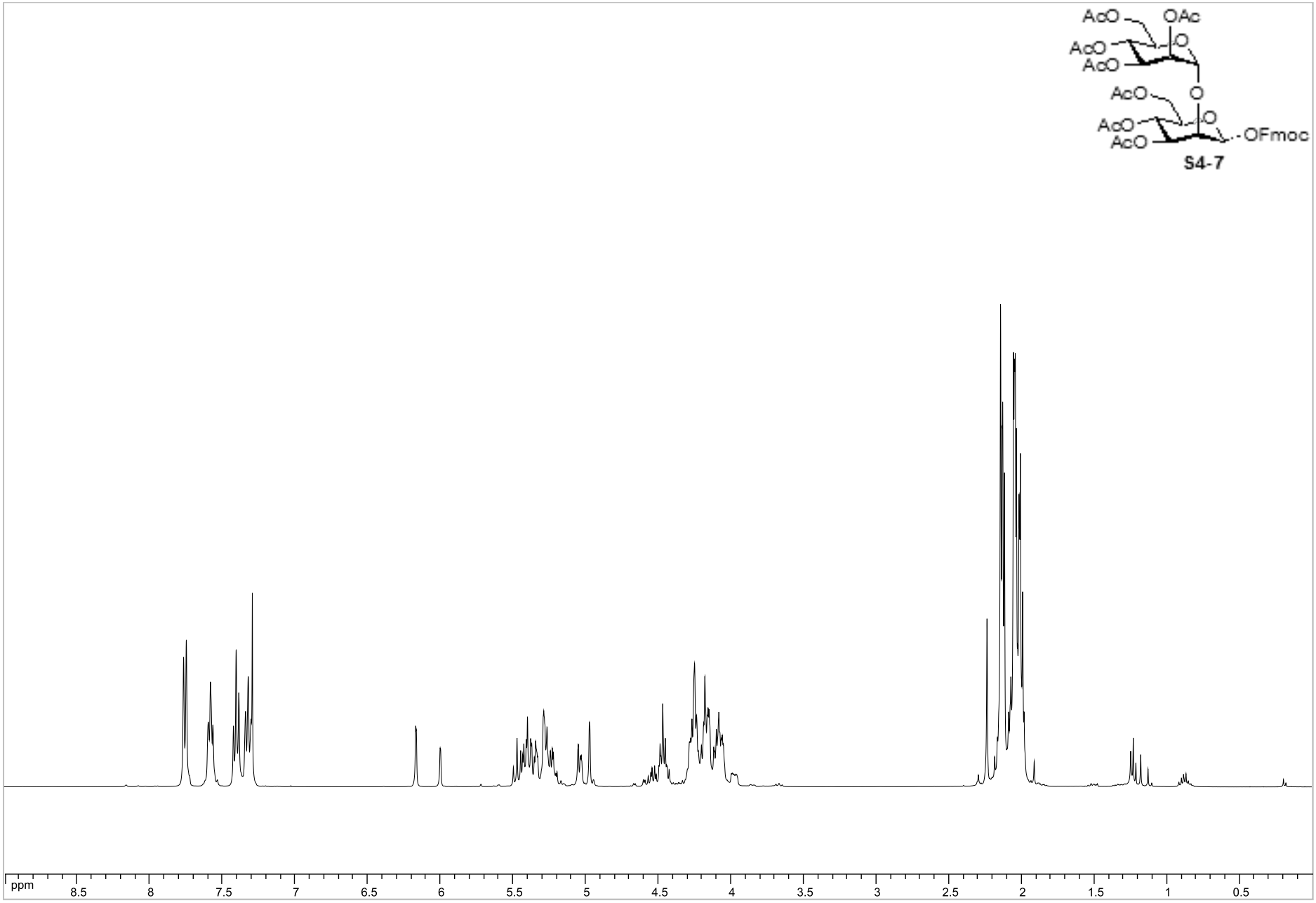

AcO
AcO
AcO
O
O
O
S4-4
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

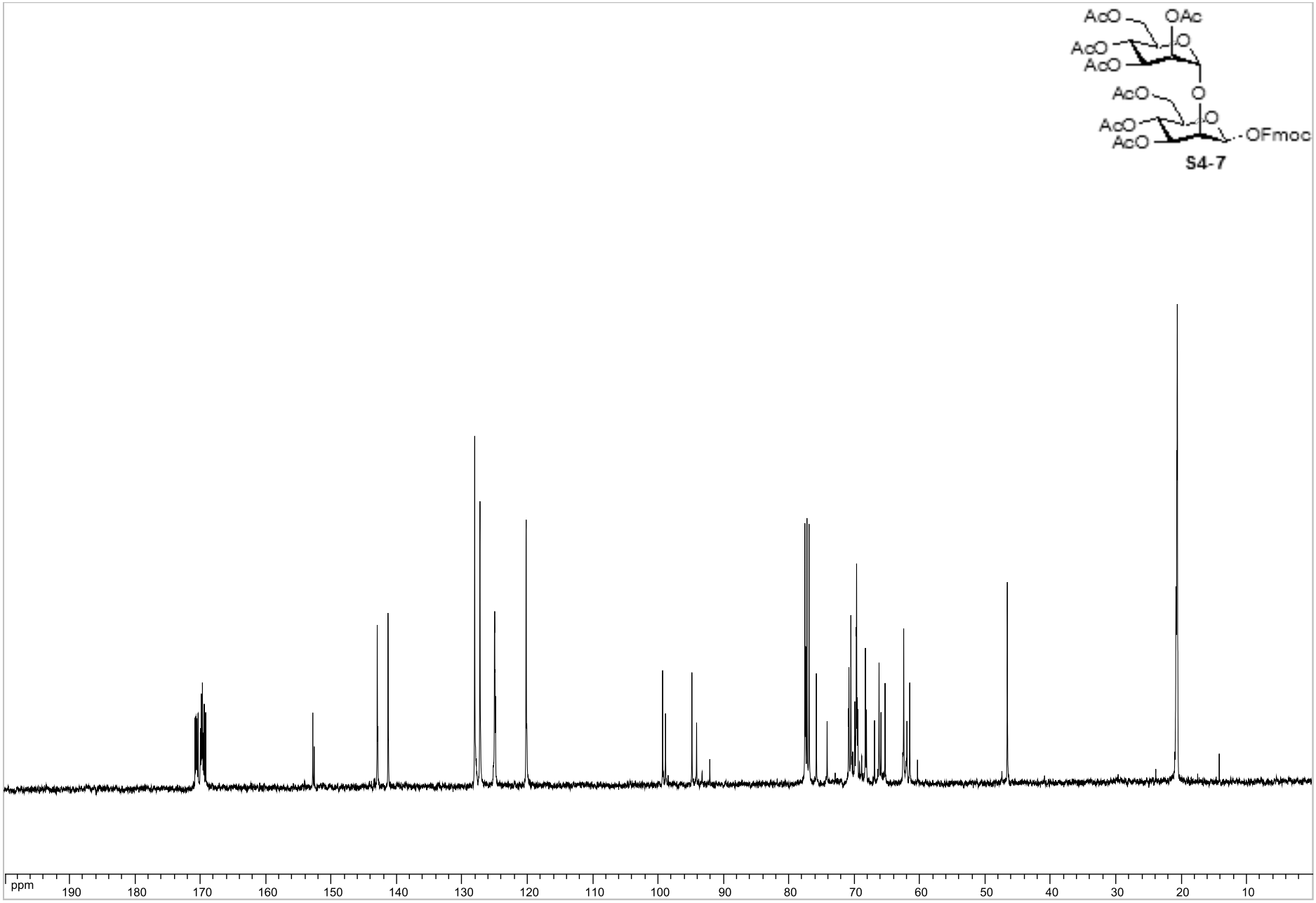

AcO
OH
AcO
O
AcO
OH
S4-5
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

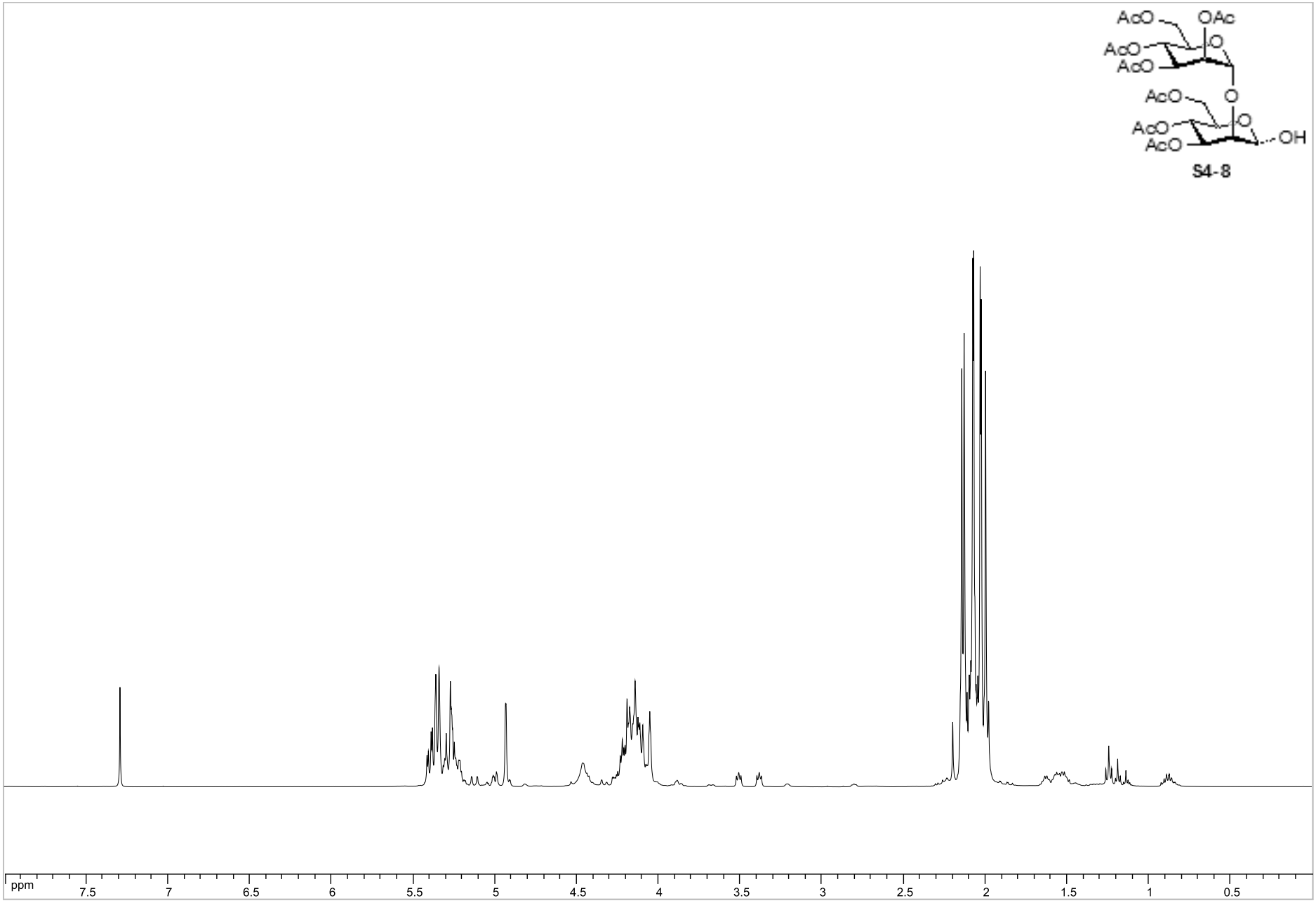

AcO
OH
AcO
O
OH
AcO
S4-5
ppm
200
180
160
140
120
100
80
60
40
20
0
-20

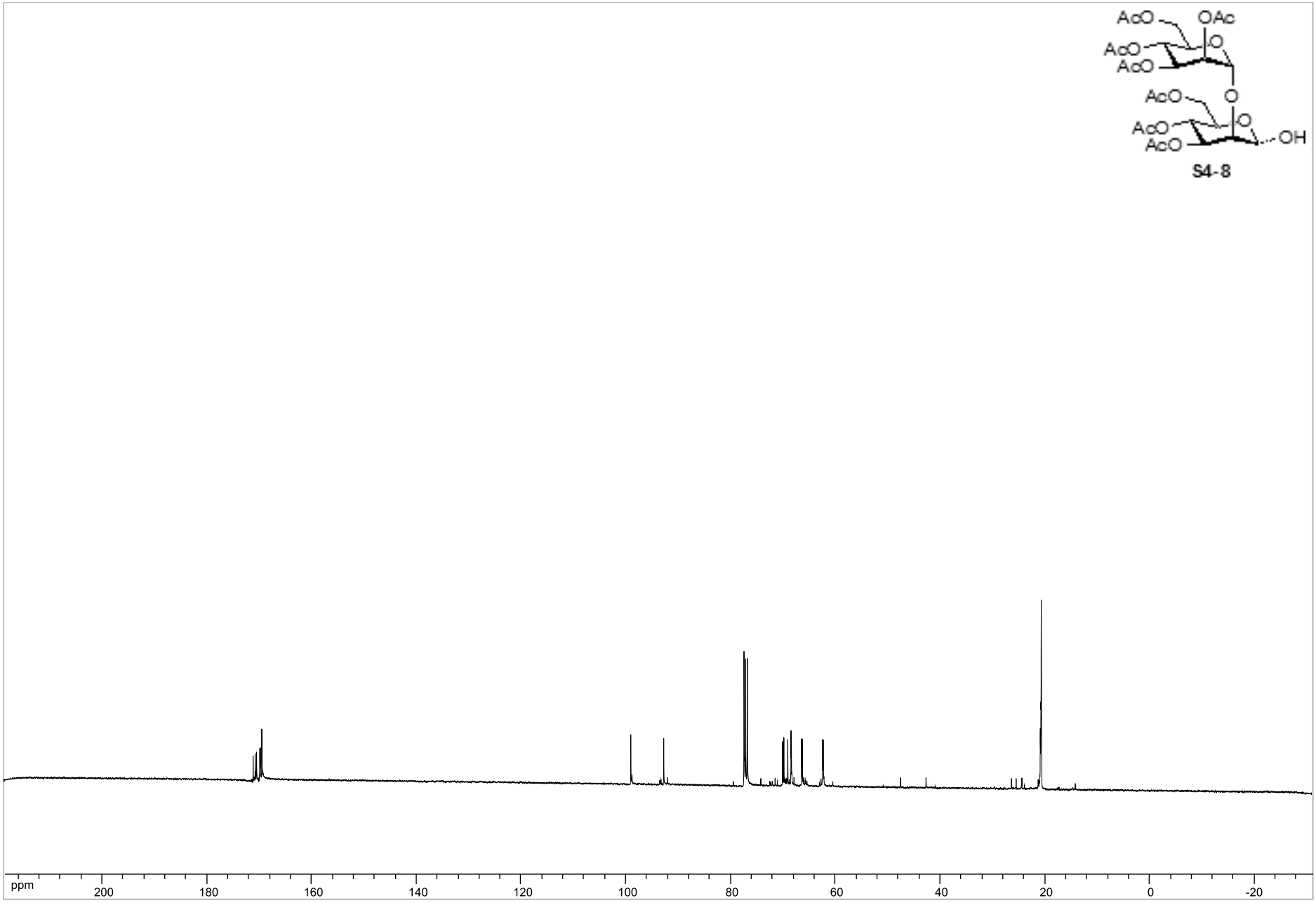

AcO
OH
AcO
O
AcO
OFmoc
S4-6
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

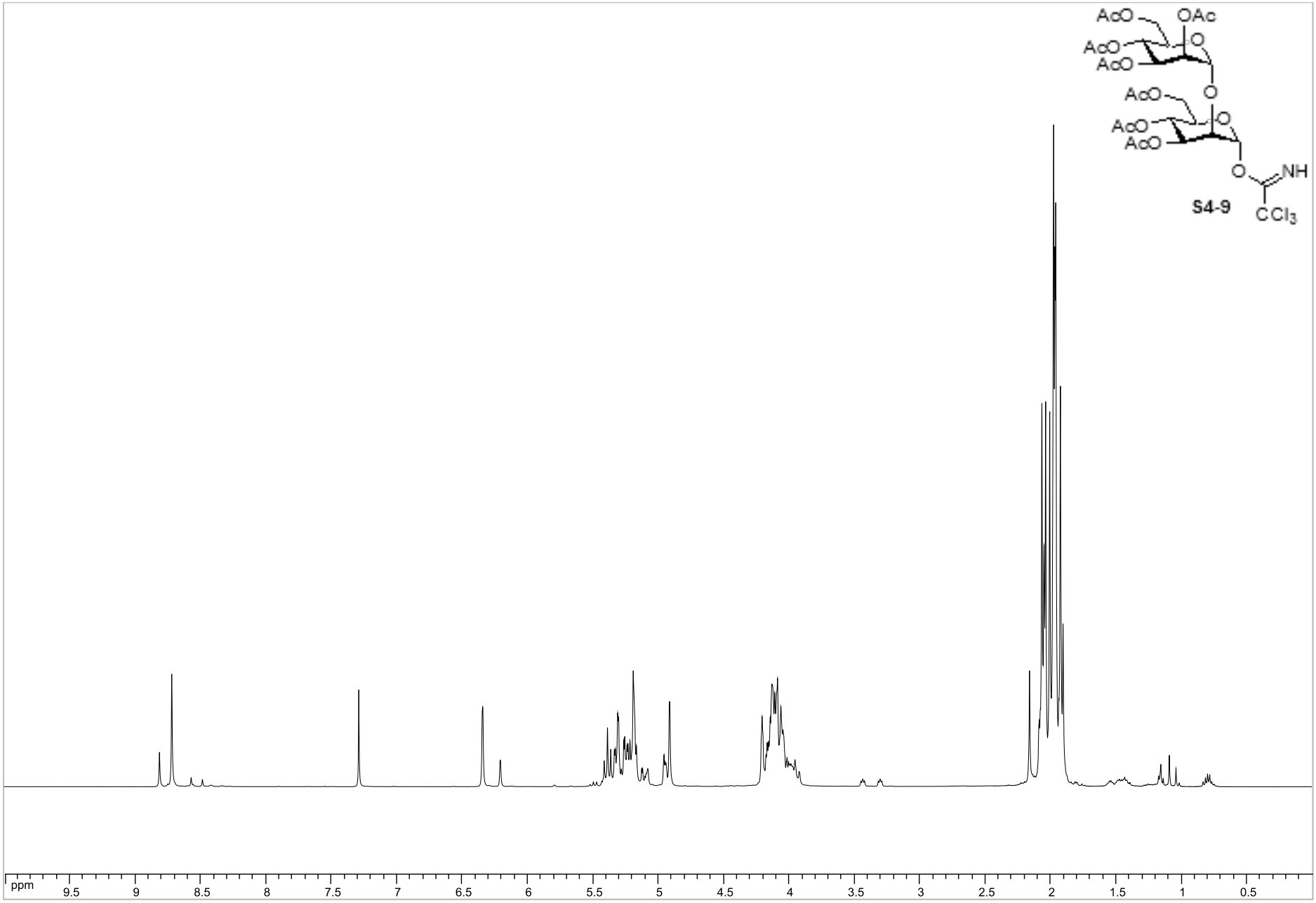

AcO
OH
AcO
O
AcO
OFmoc
S4-6
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

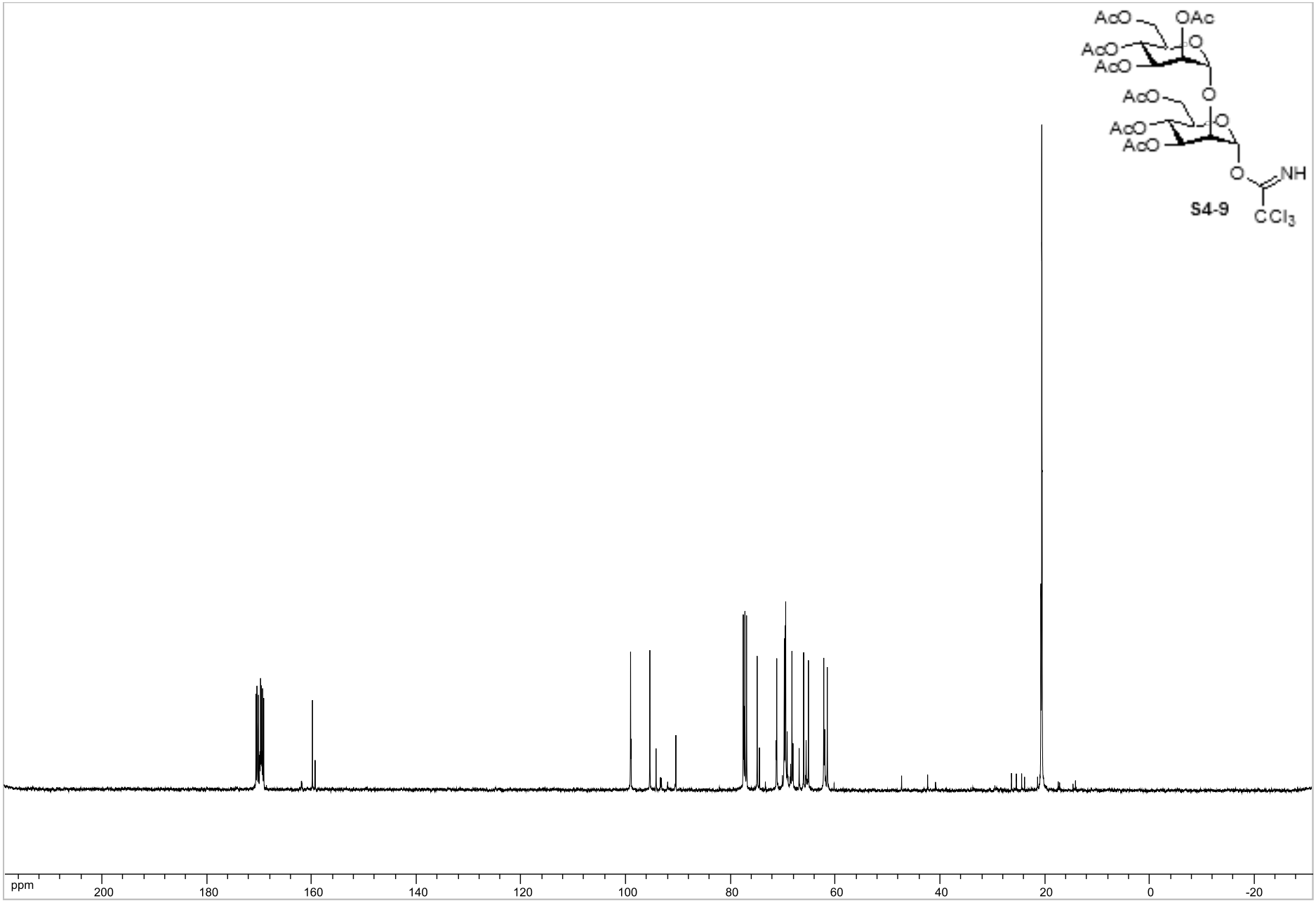

AcO
OAc
AcO
O
AcO
AcO
O
AcO
O
AcO
OFmoc
S4-7
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

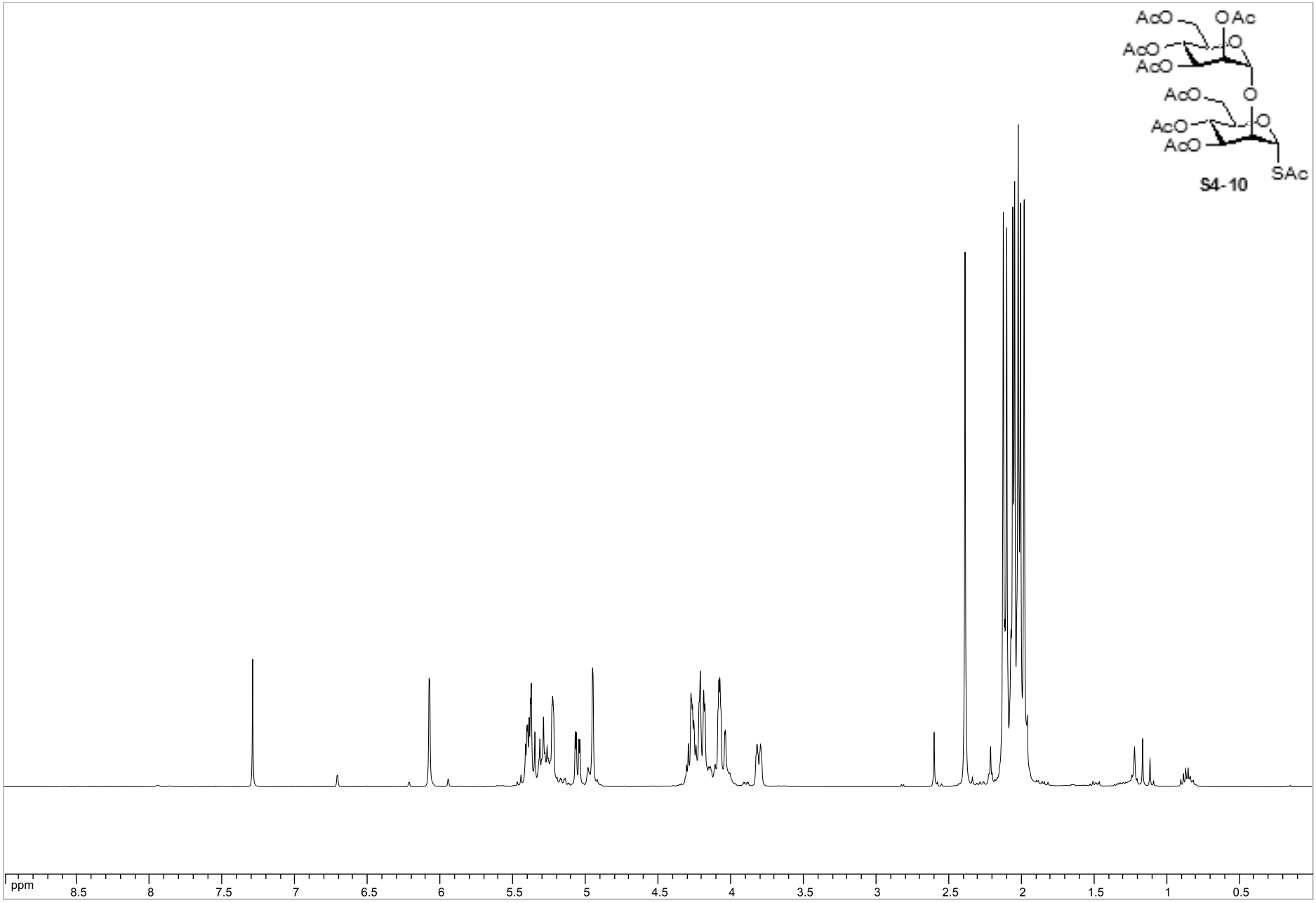

AcO
OAc
AcO
O
AcO
AcO
O
AcO
O
AcO
OFmoc
S4-7
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

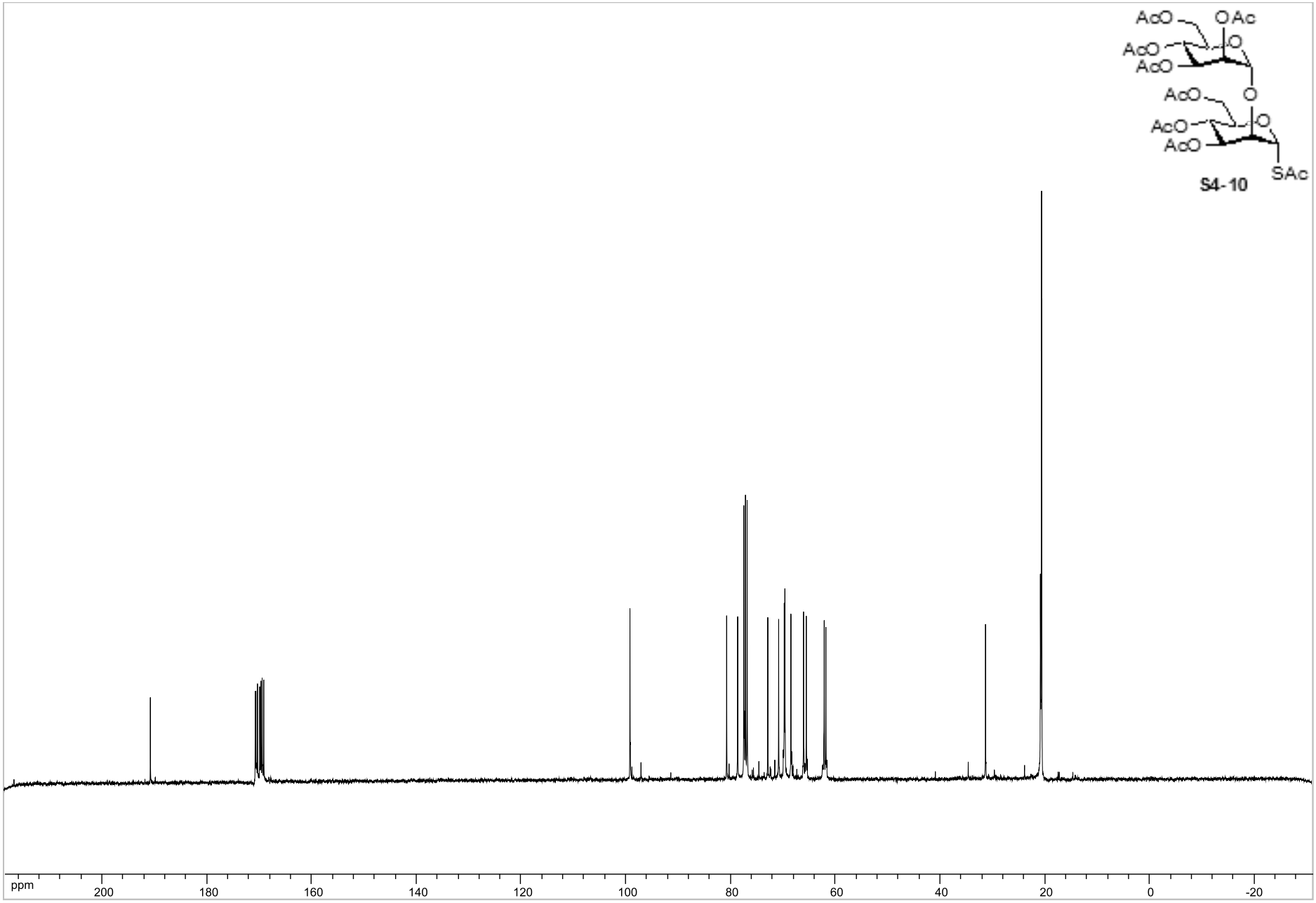

AcO
OAc
AcO
O
AcO
AcO
O
AcO
O
AcO
OH
AcO
S4-8
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

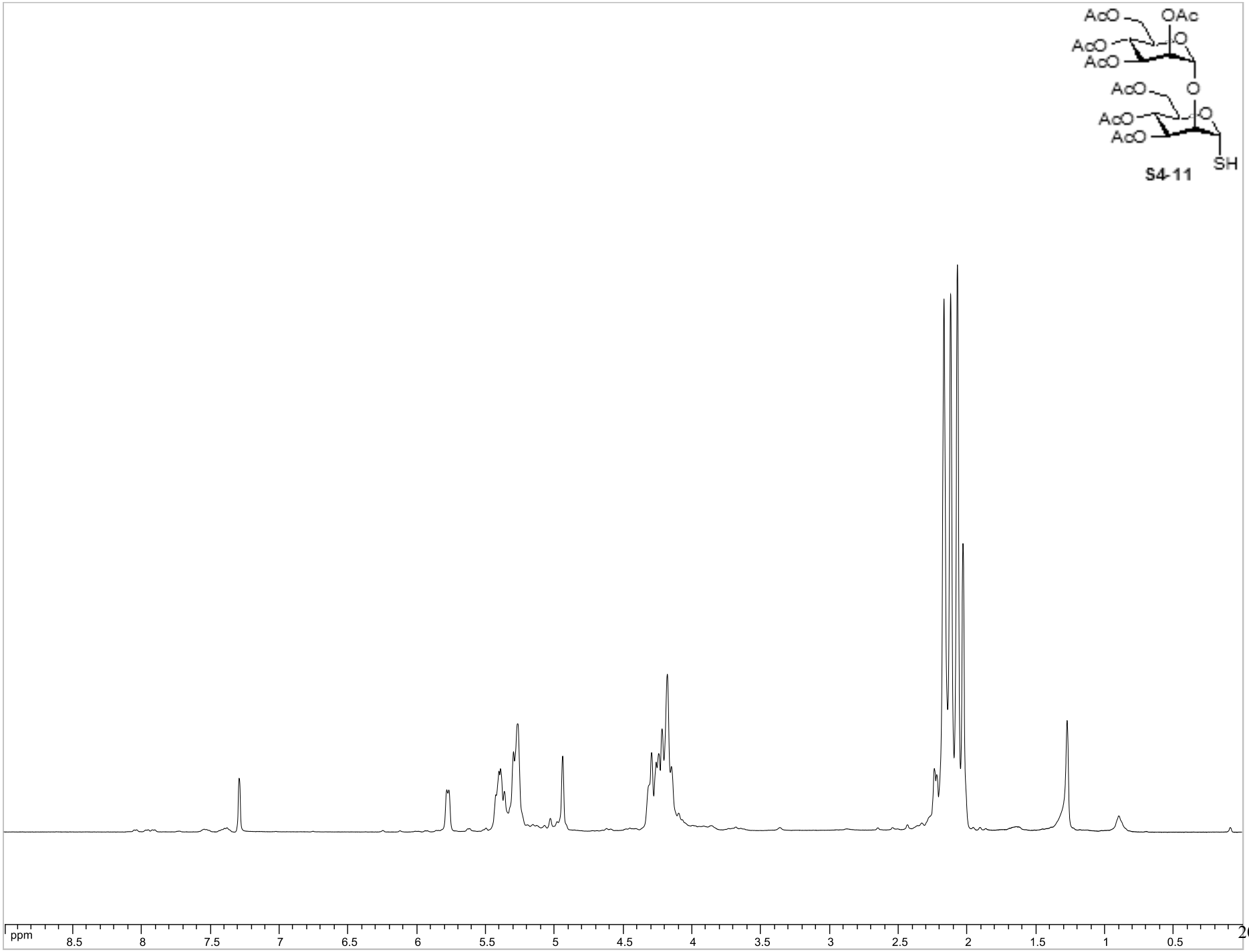

AcO
OAc
AcO
O
AcO
AcO
O
AcO
AcO
O
OH
AcO
S4-8
ppm
200
180
160
140
120
100
80
60
40
20
0
-20

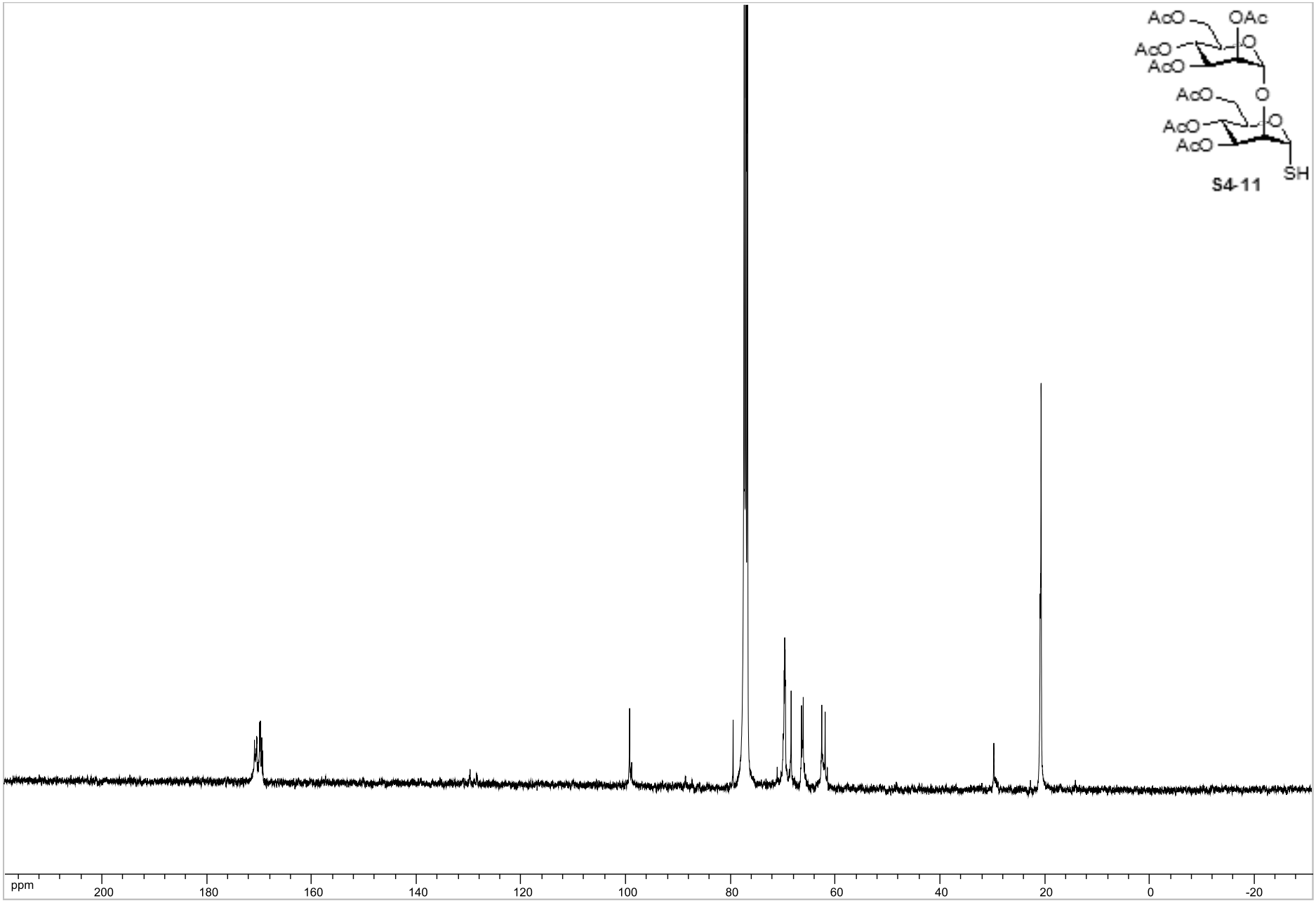

AcO
OAc
AcO
AcO
O
AcO
AcO
AcO
O
O
NH
CCl3
S4-9
ppm
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

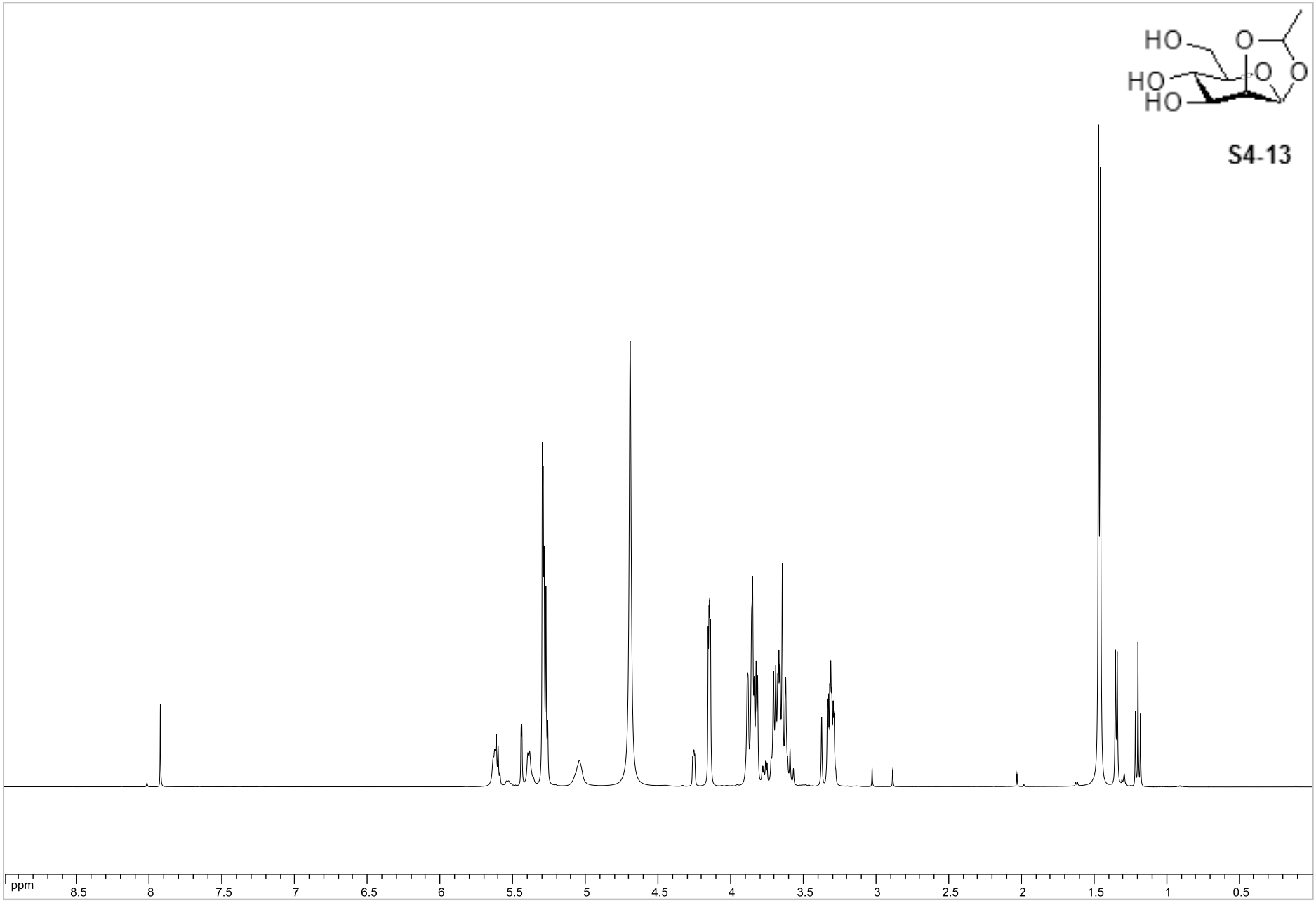

AcO
OAc
AcO
AcO
O
AcO
O
AcO
AcO
O
NH
CCl3
S4-9
ppm
200
180
160
140
120
100
80
60
40
20
0
-20

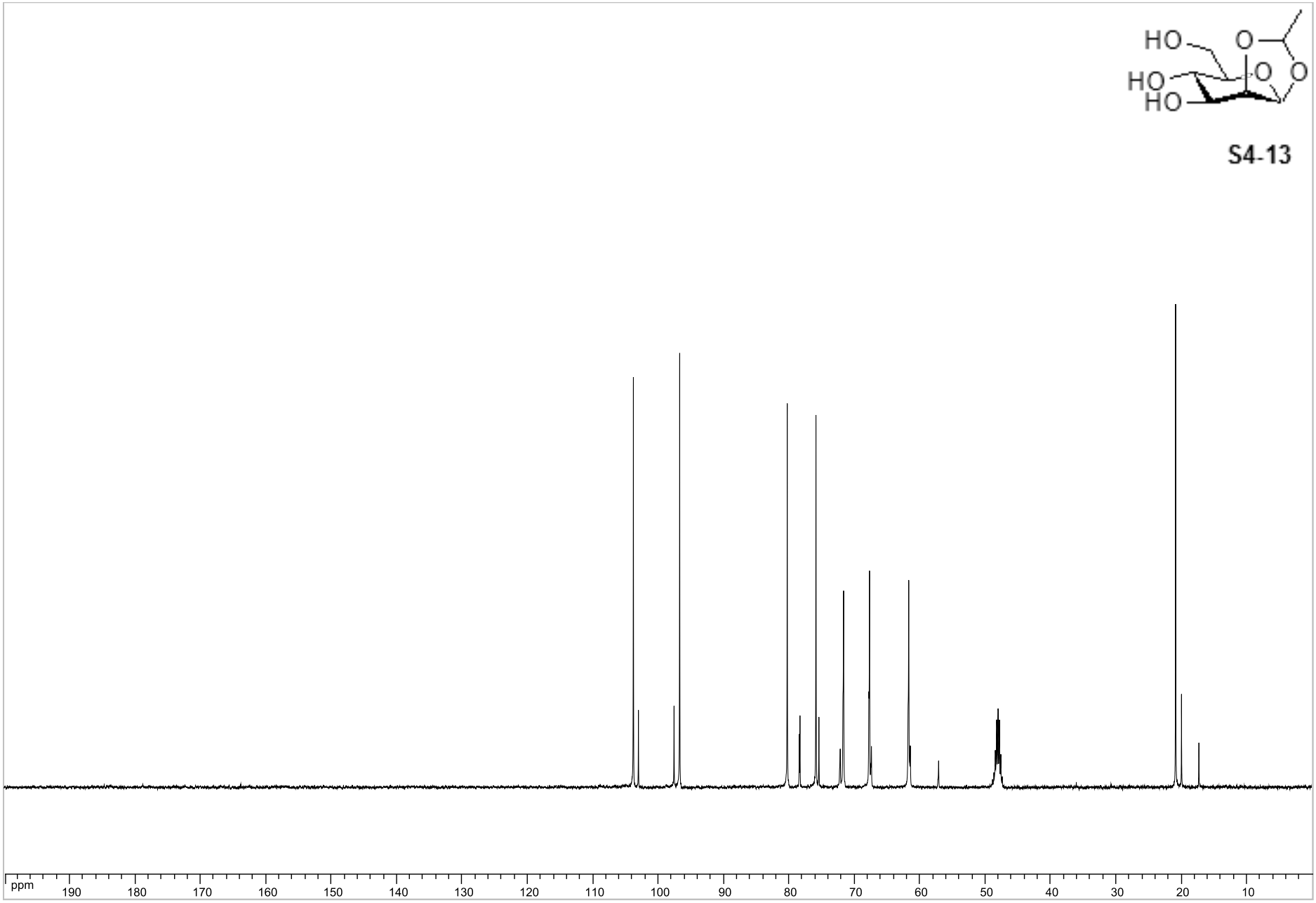

AcO
OAc
AcO
O
AcO
AcO
O
AcO
AcO
O
AcO
SAc
S4-10
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

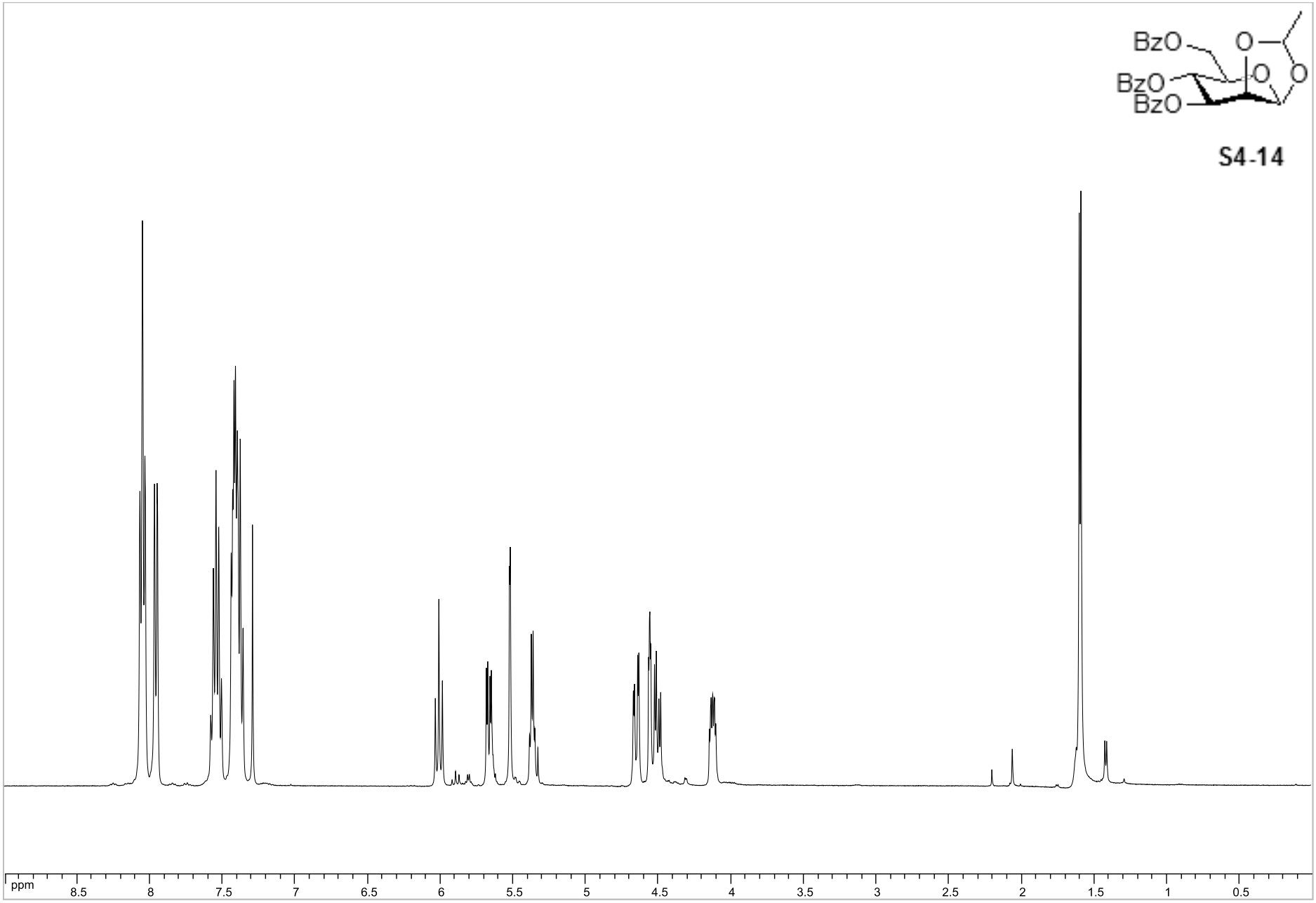

AcO
OAc
AcO
O
AcO
AcO
O
AcO
AcO
O
AcO
SAc
S4-10
ppm
200
180
160
140
120
100
80
60
40
20
0
-20

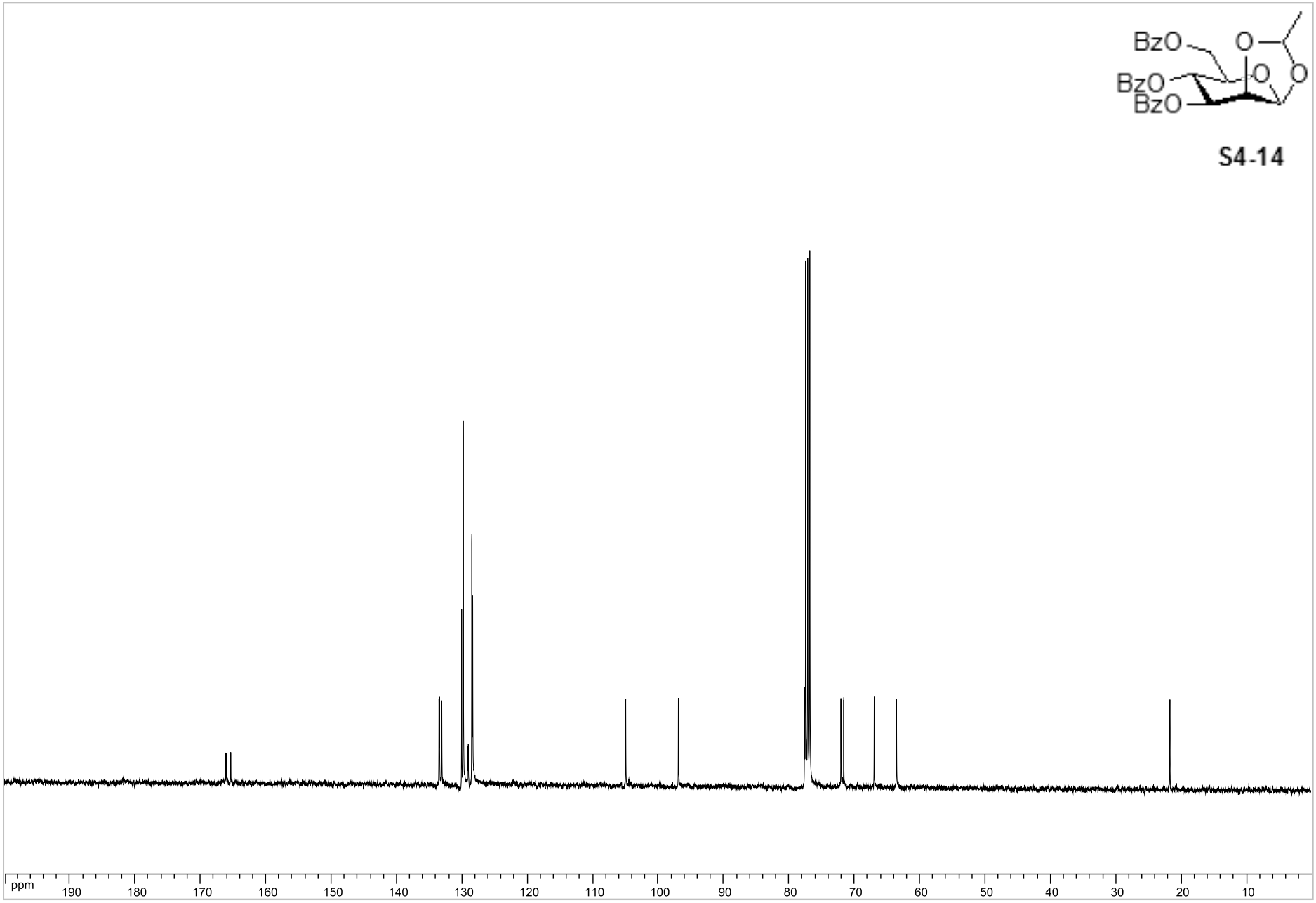

AcO
OAc
AcO
O
AcO
AcO
O
AcO
O
AcO
SH
S4-11
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

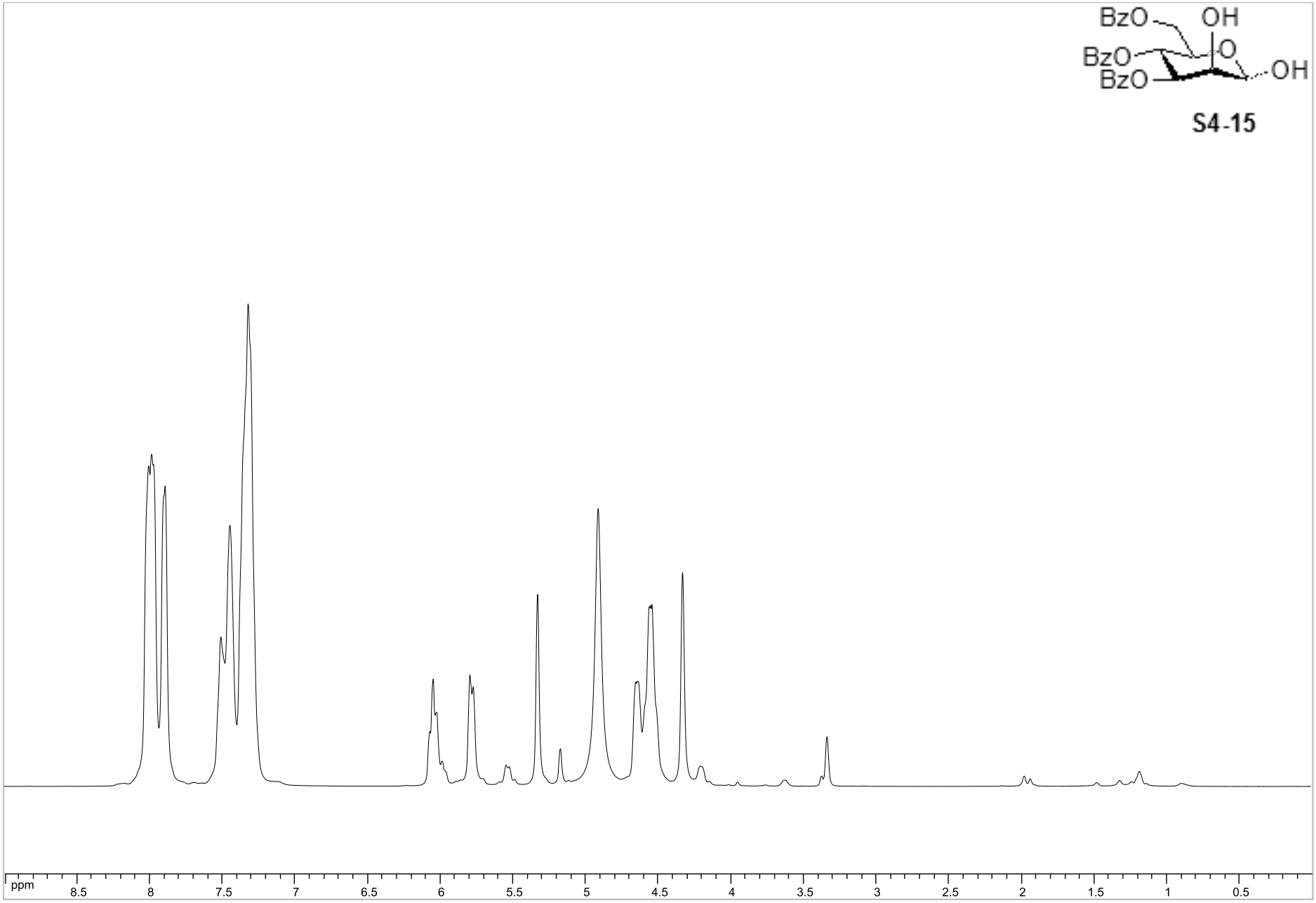

AcO
OAc
AcO
O
AcO
AcO
O
AcO
O
AcO
AcO
SH
S4-11
ppm
200
180
160
140
120
100
80
60
40
20
0
-20

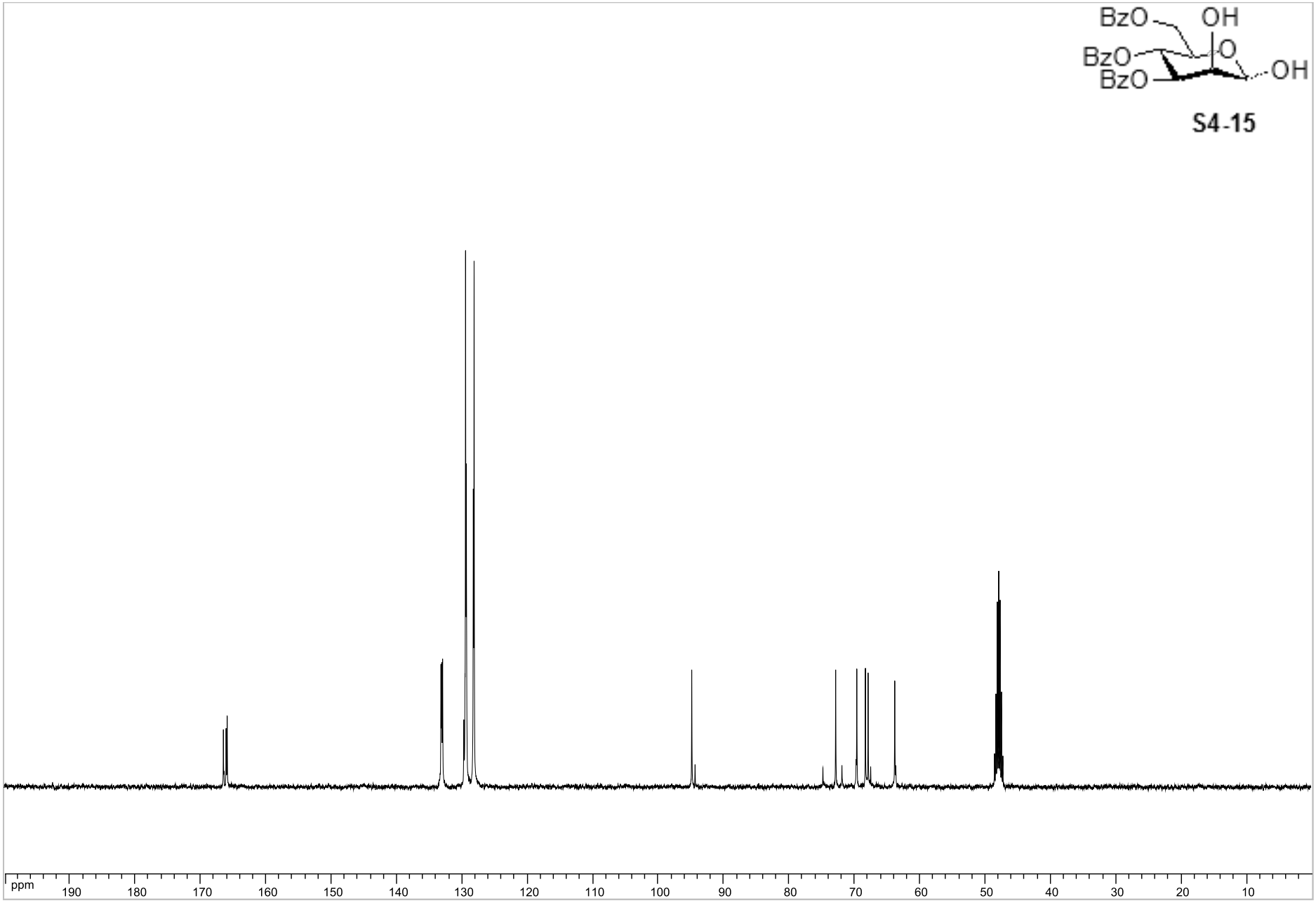

HO
O
HO
O
O
HO
S4-13
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

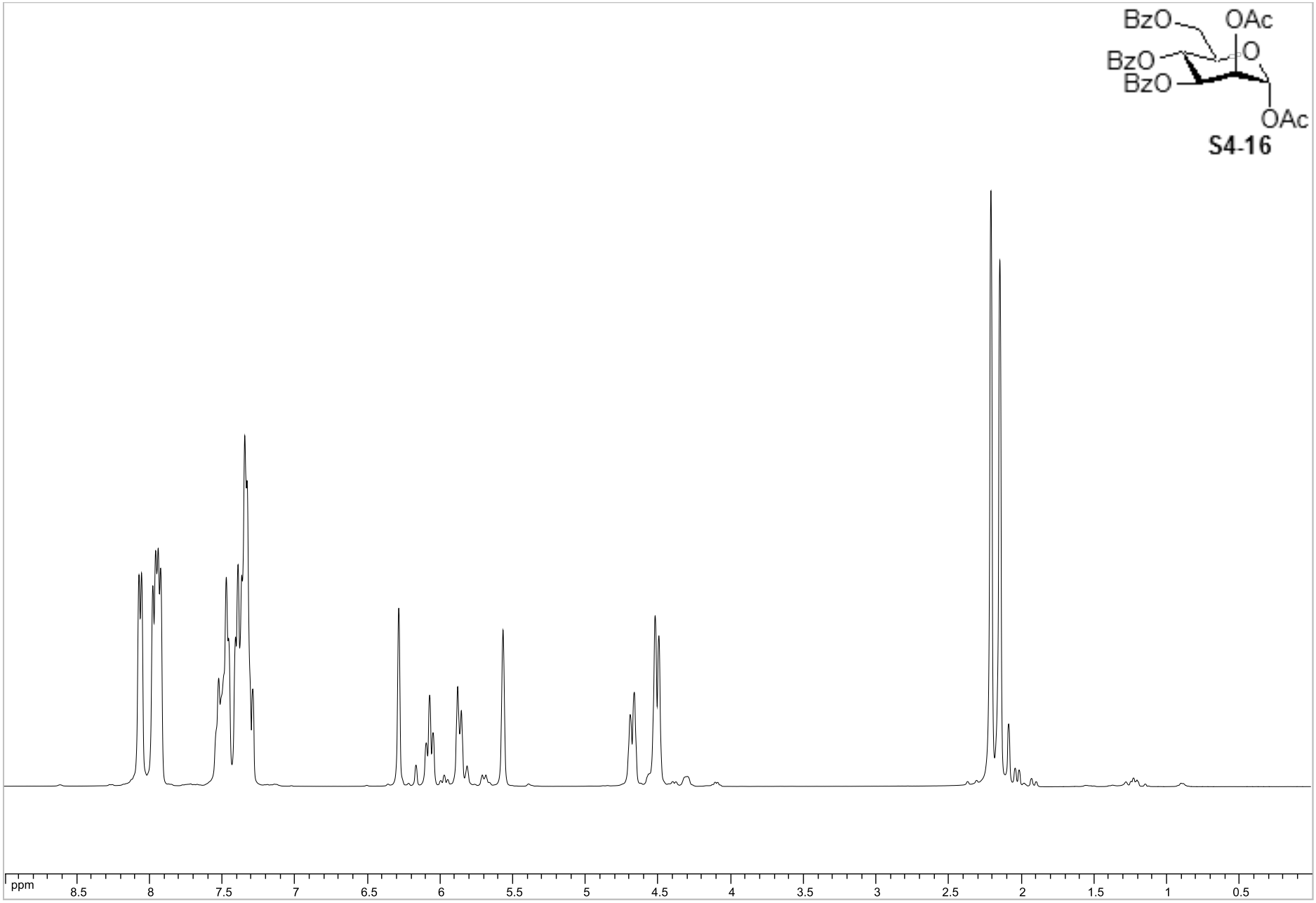

HO
O
HO
O
O
HO
S4-13
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

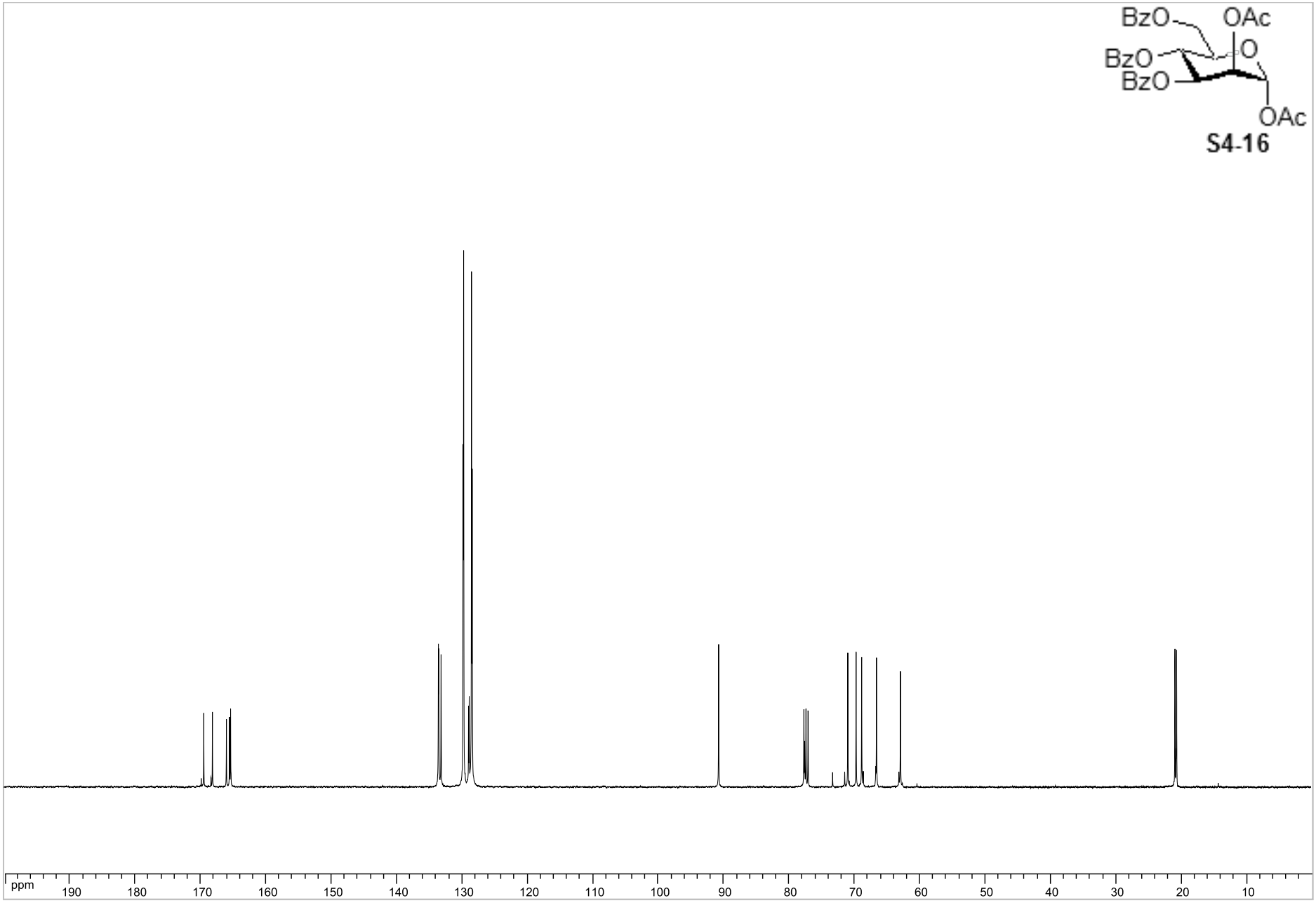

BzO
O
BzO
O
O
BzO
S4-14
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

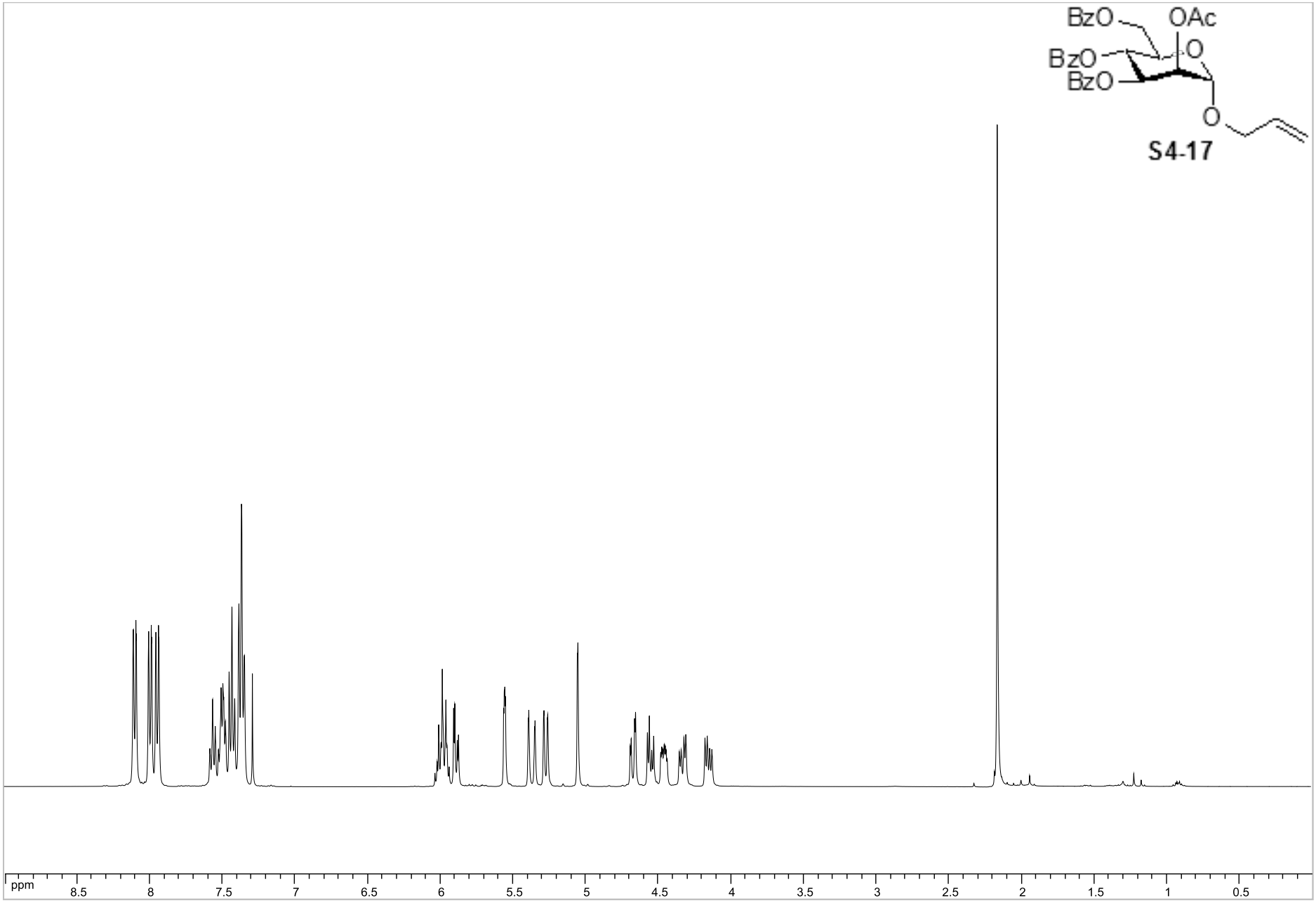

BzO
BzO
BzO
O
O
O
S4-14
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

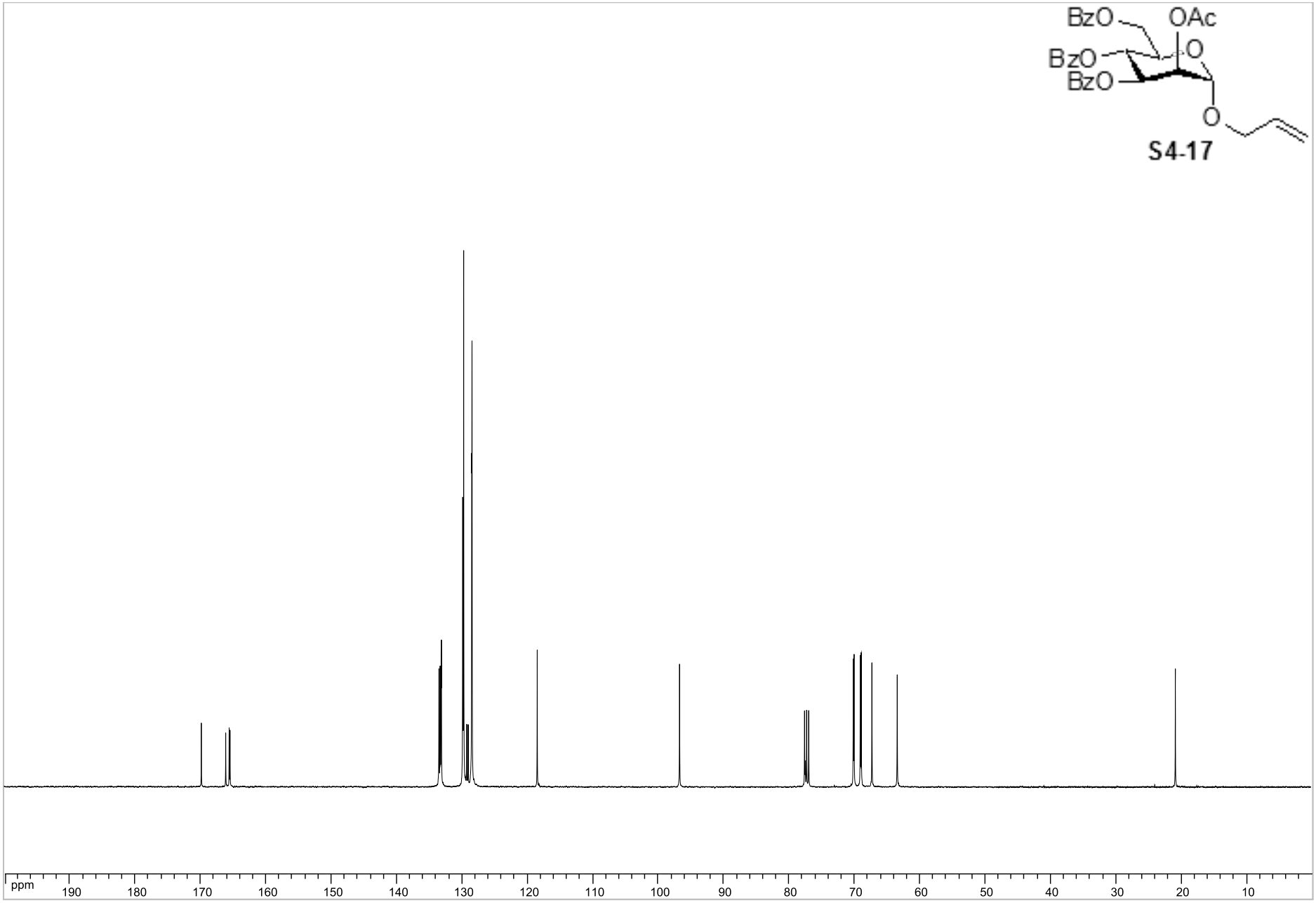

BzO
OH
BzO
O
BzO
OH
S4-15
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

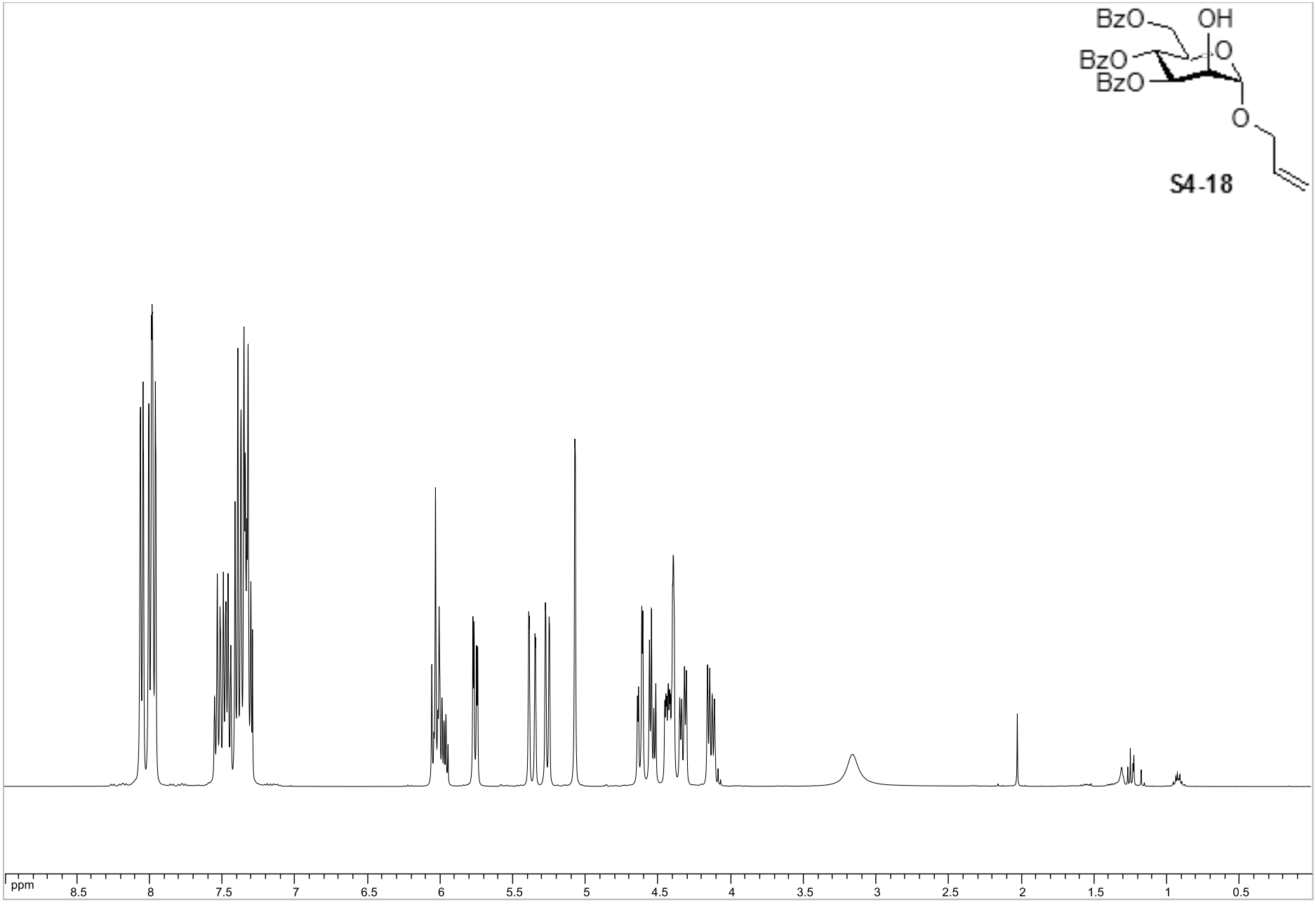

BzO
OH
BzO
O
OH
BzO
S4-15
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

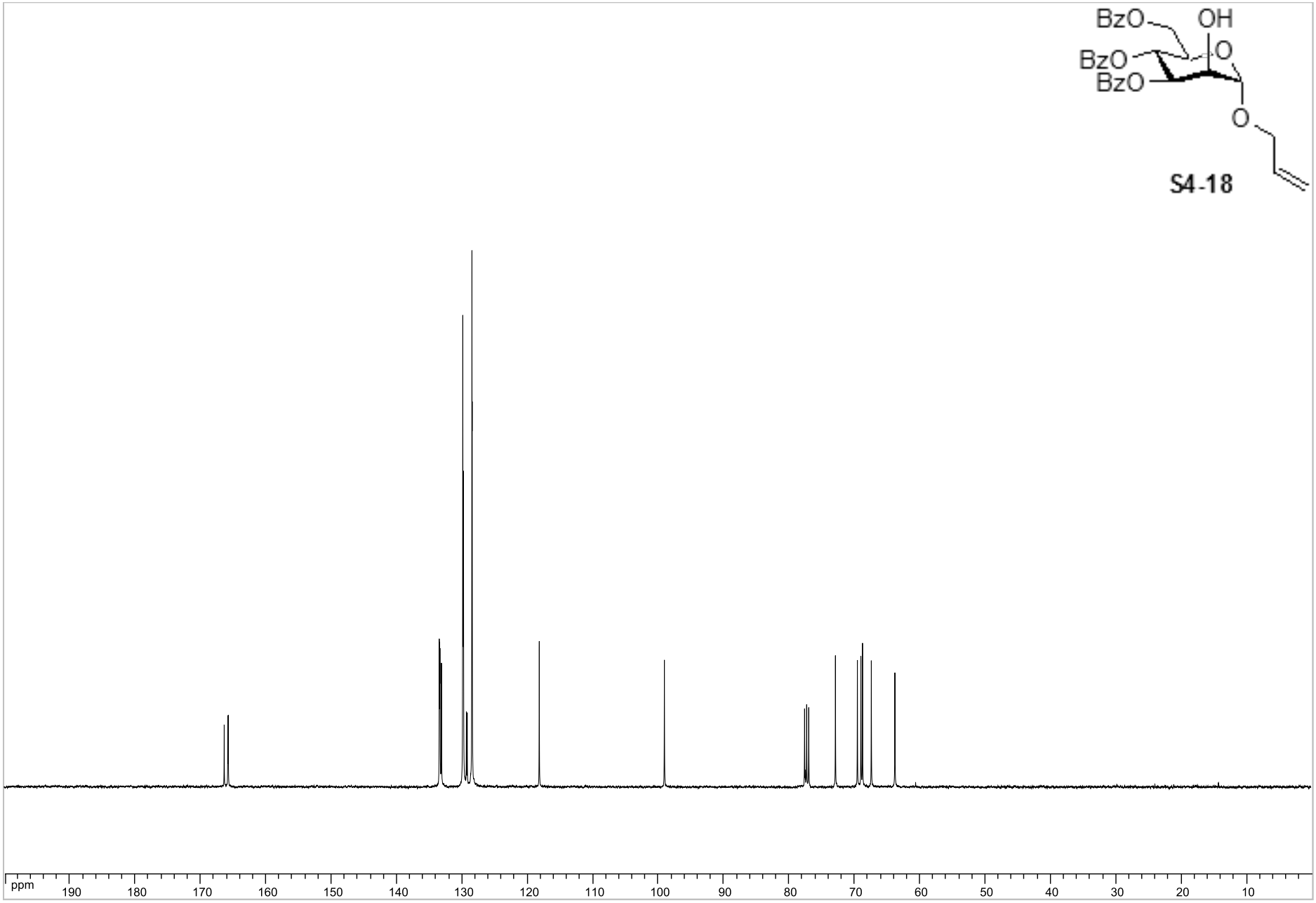

BzO
OAc
BzO
O
BzO
OAc
S4-16
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

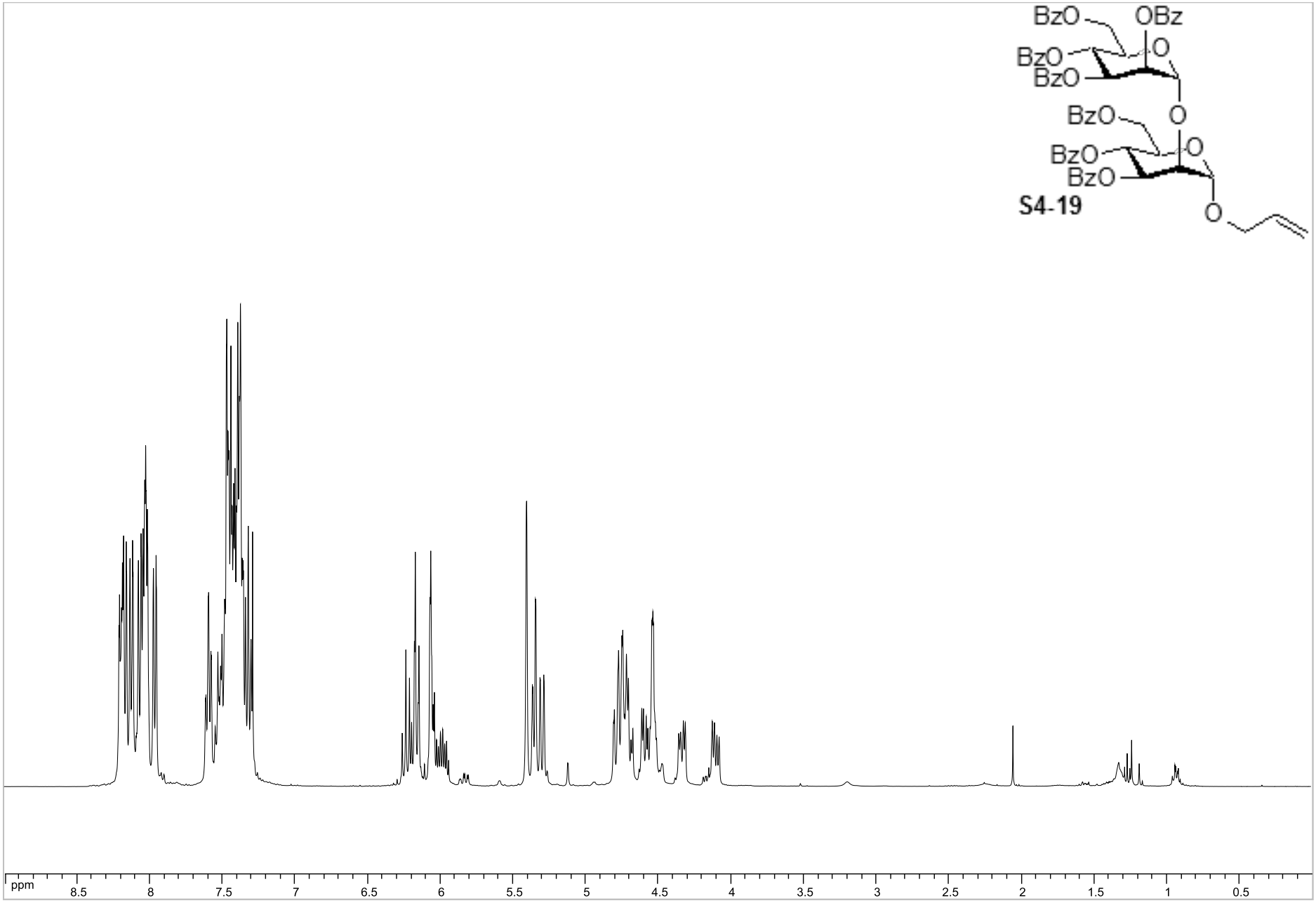

BzO
OAc
BzO
O
BzO
OAc
S4-16
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

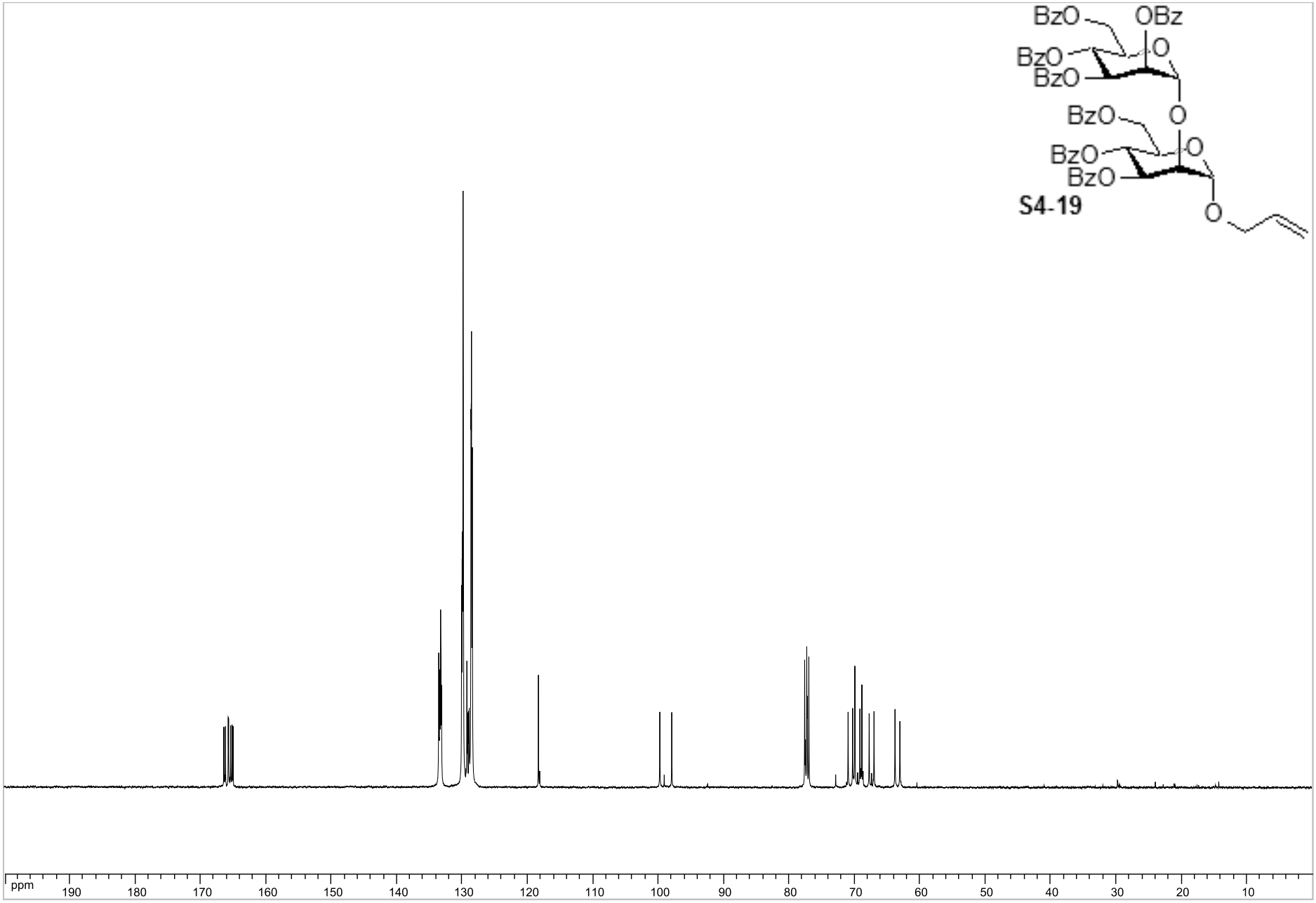

BzO
OAc
O
BzO
BzO
O
S4-17
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

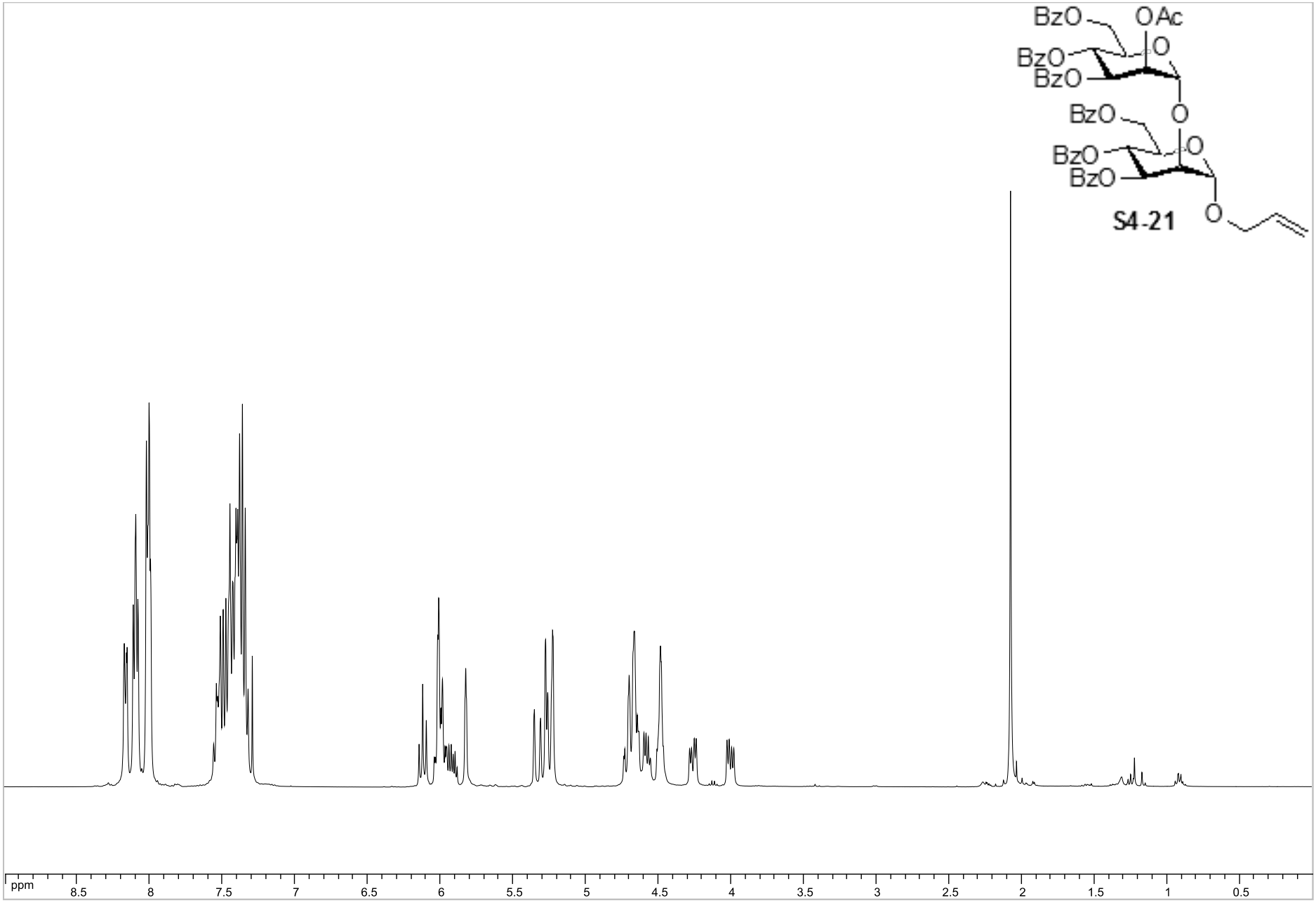

BzO
OAc
BzO
O
BzO
O
S4-17
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

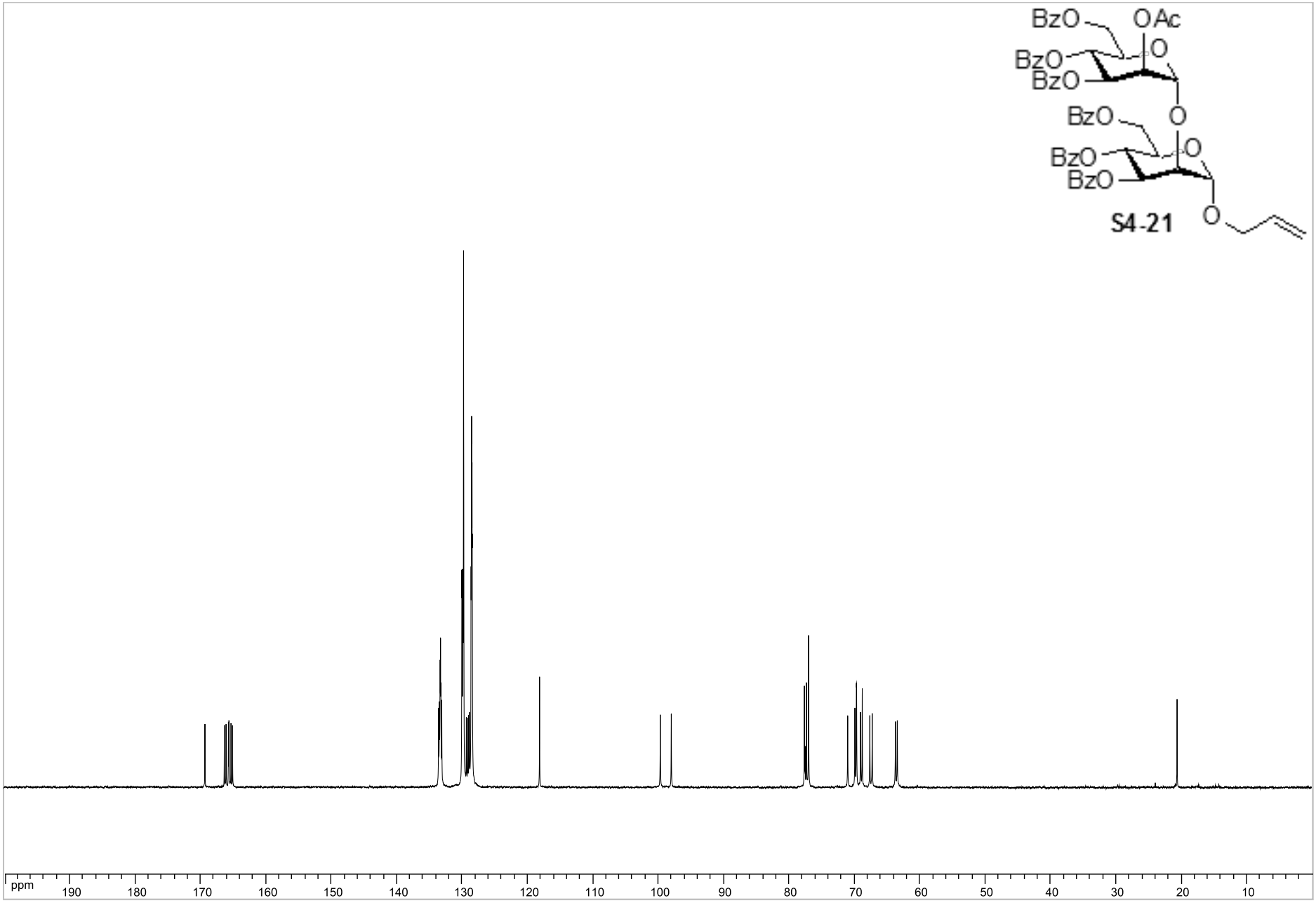

BzO
OH
BzO
O
BzO
O
S4-18
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

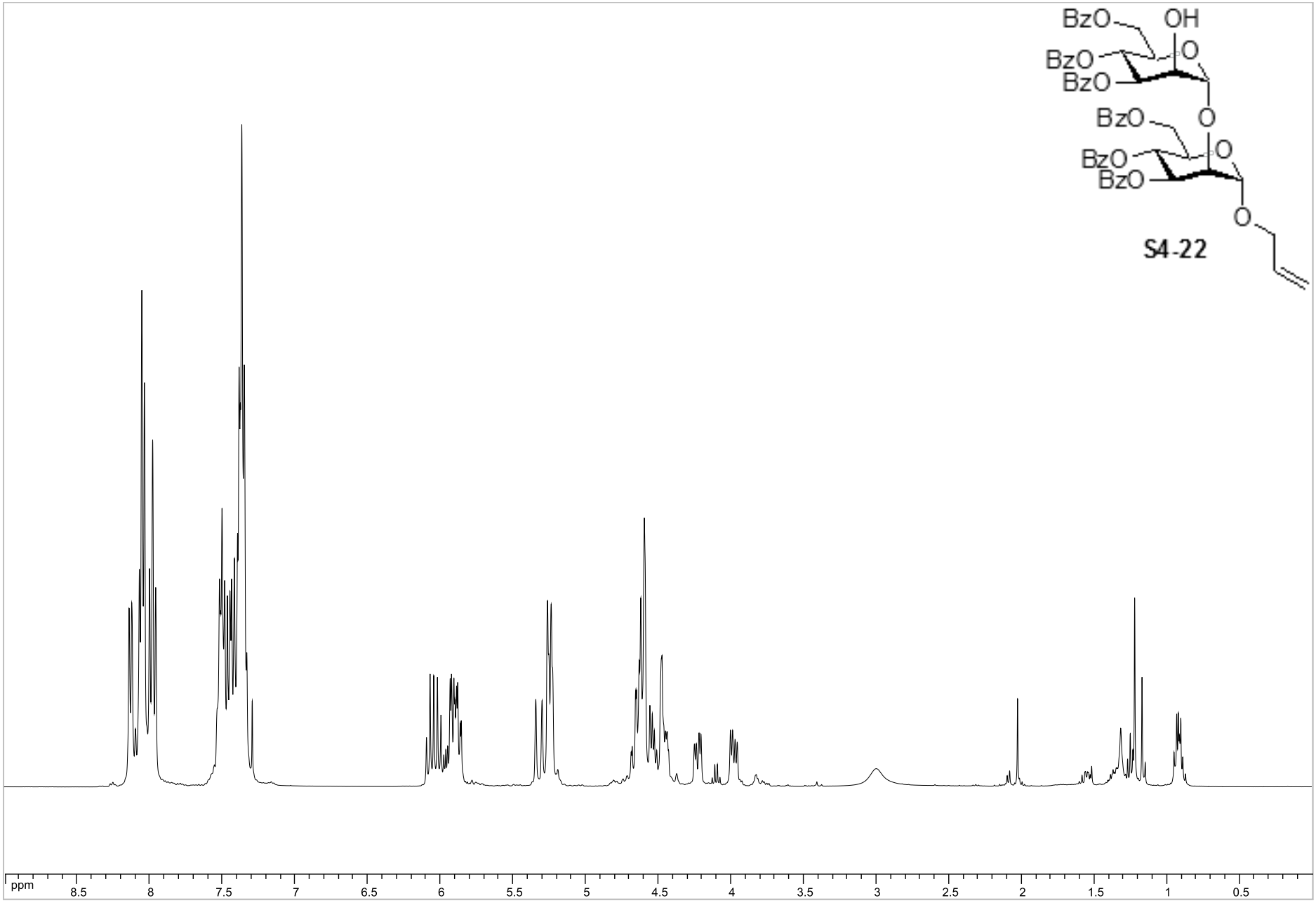

BzO
OH
BzO
O
BzO
O
S4-18
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

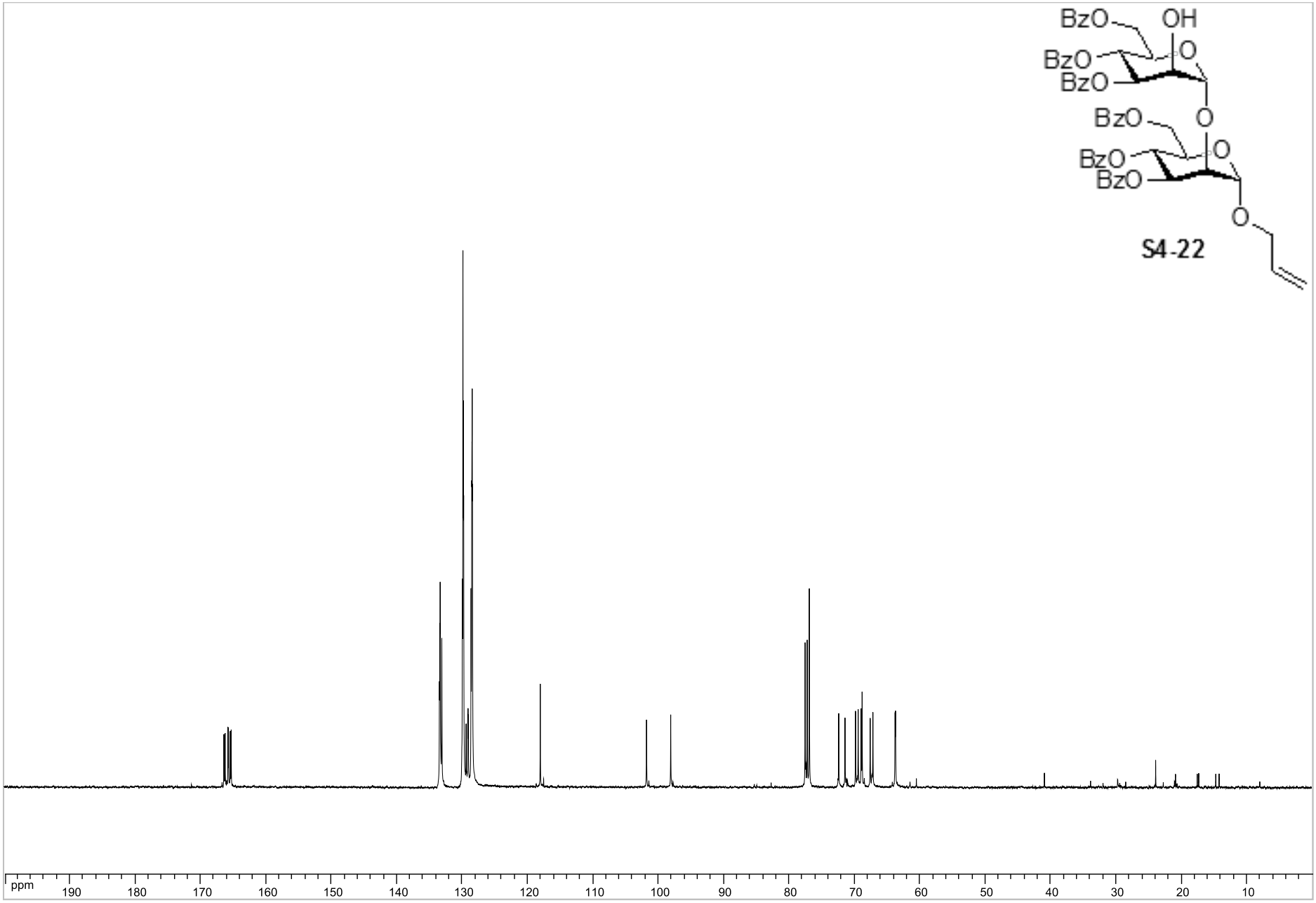

BzO
OBz
BzO
BzO
O
BzO
O
BzO
O
BzO
O
S4-19
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

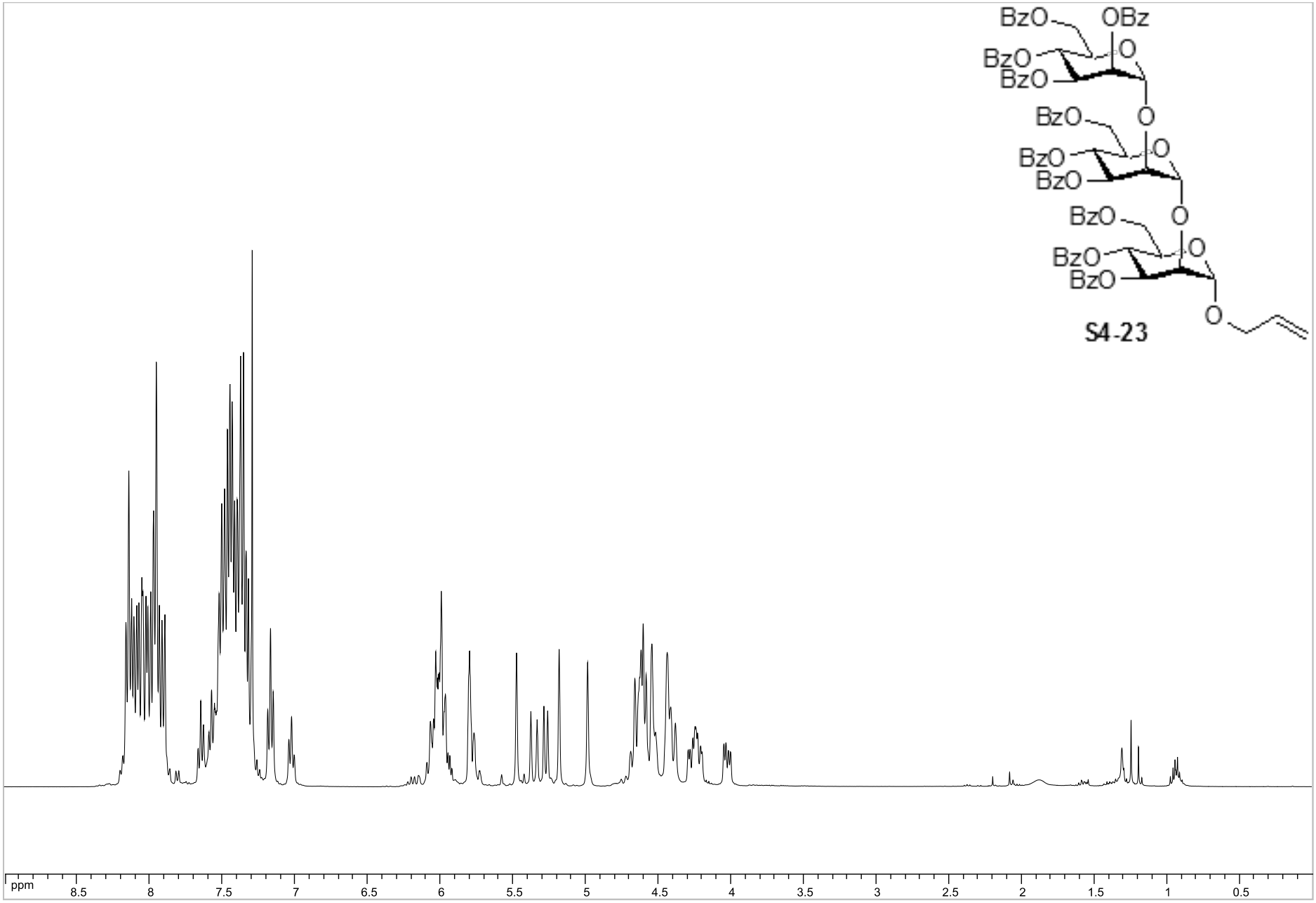

BzO
OBz
BzO
BzO
O
BzO
O
BzO
O
BzO
S4-19
O
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

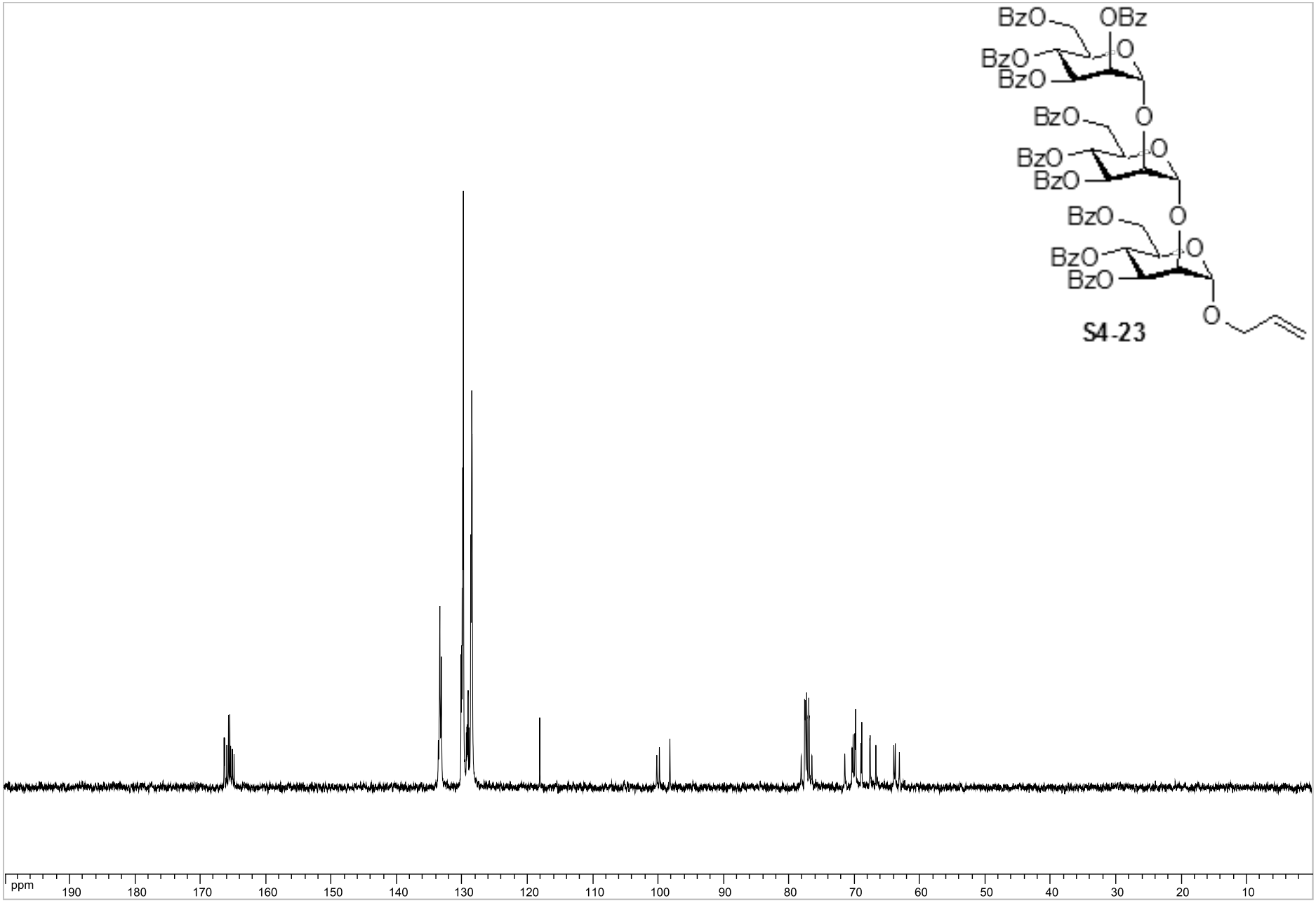

BzO
OAc
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
S4-21
O
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

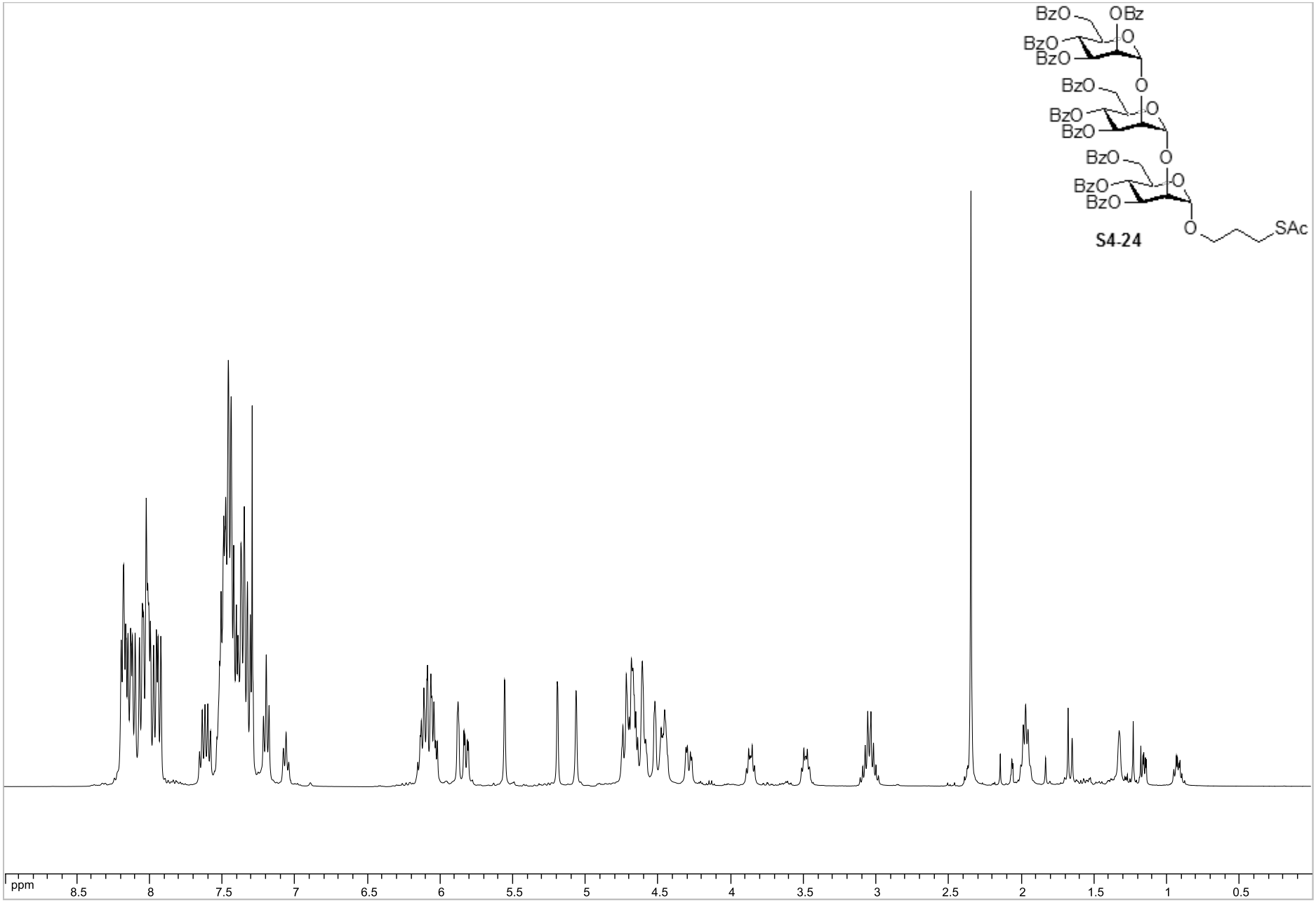

BzO
OAc
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
O
S4-21
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

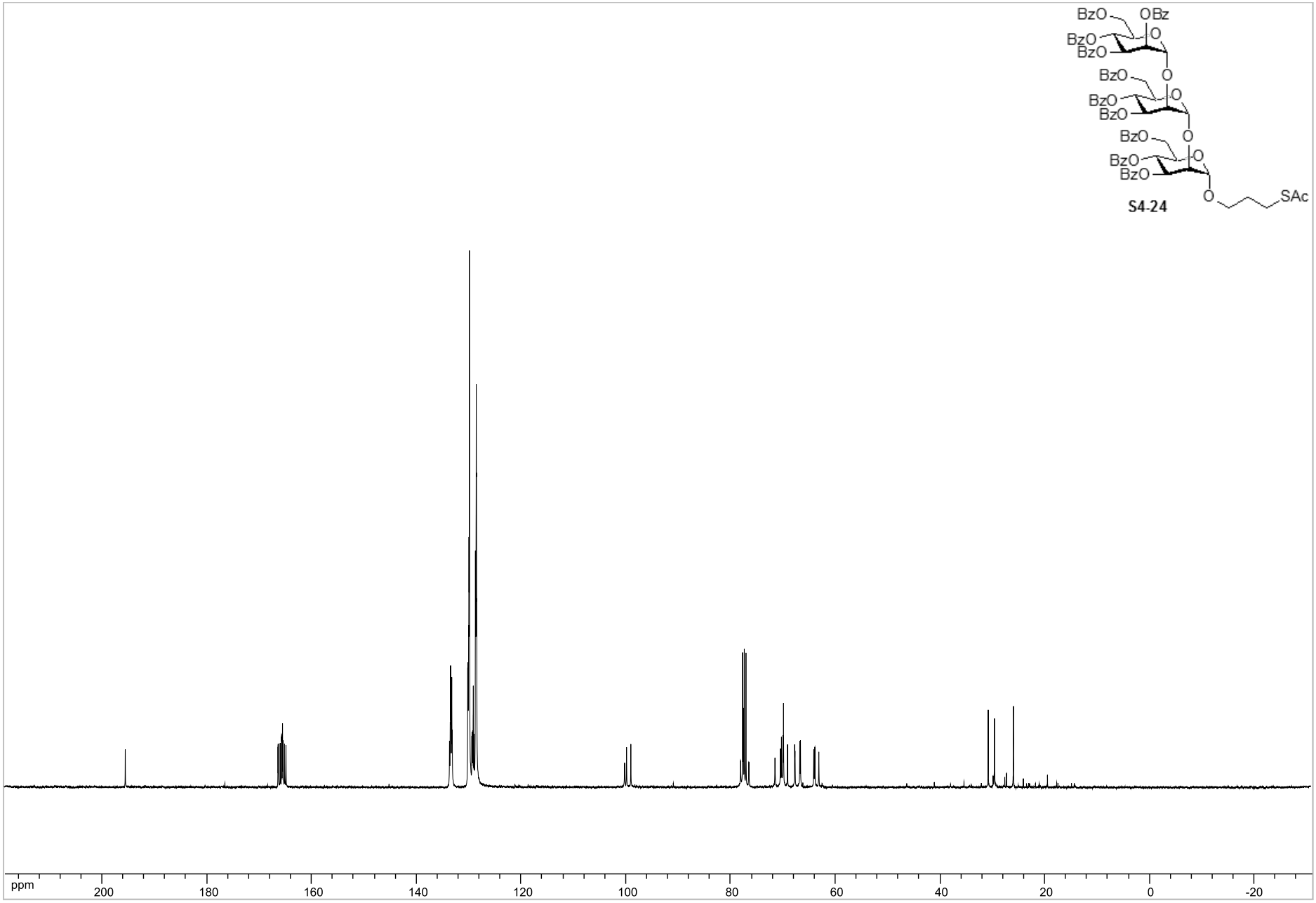

BzO
OH
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
O
S4-22
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5


BzO
OH
BzO
O
BzO
BzO
O
BzO
O
BzO
BzO
O
S4-22
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

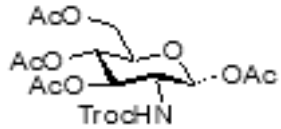

BzO
OBz
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
O
S4-23
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5


BzO
OBz
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
O
S4-23
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

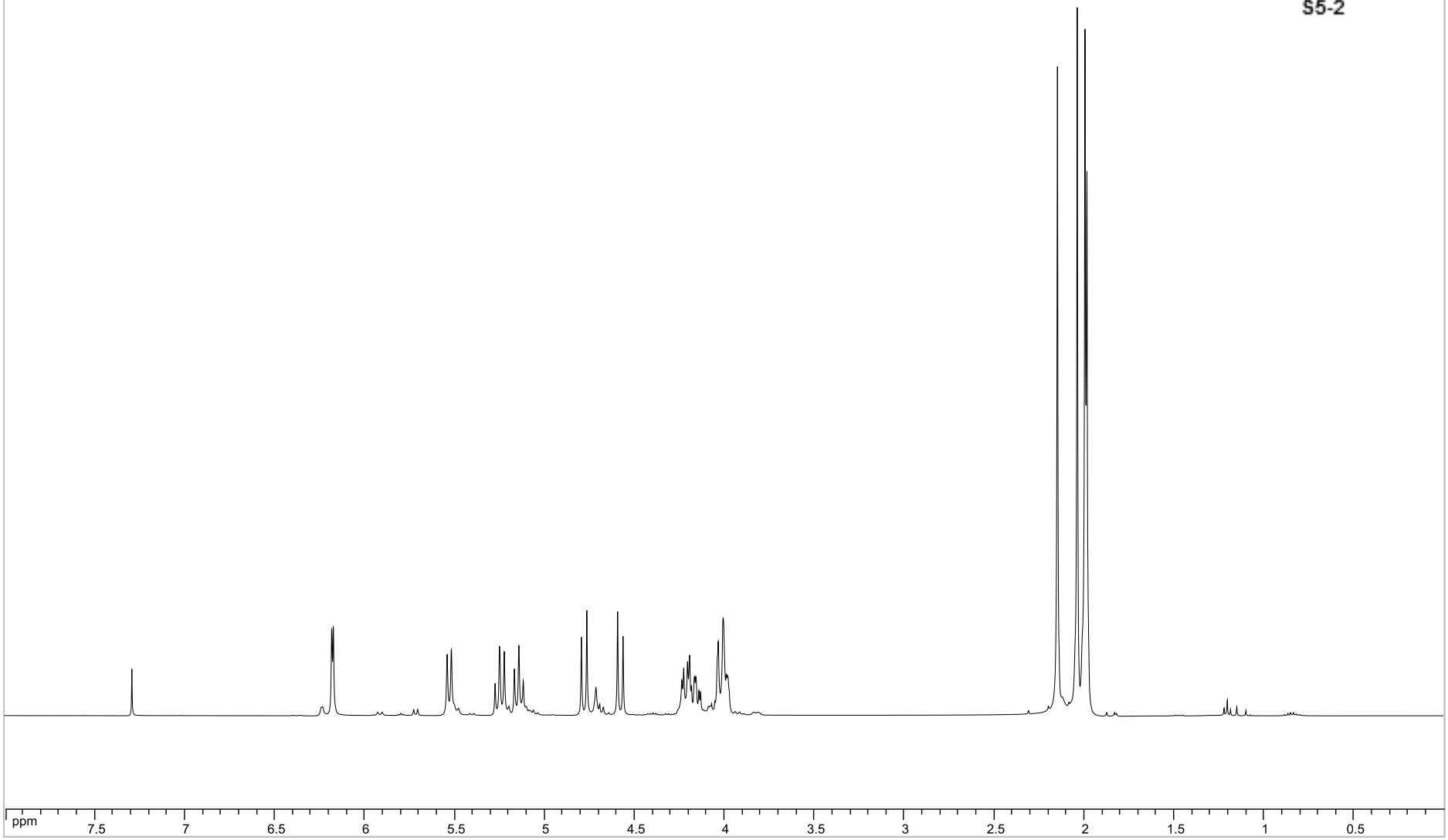

BzO
OBz
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
BzO
O
BzO
O
BzO
BzO
BzO
O
SAc
S4-24
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5


BzO
OBz
BzO
BzO
O
BzO
O
BzO
BzO
O
BzO
O
BzO
BzO
O
SAc
S4-24
ppm
200
180
160
140
120
100
80
60
40
20
0
-20

HKT3-240 1 (1D 1H) CDCl3 400MHz

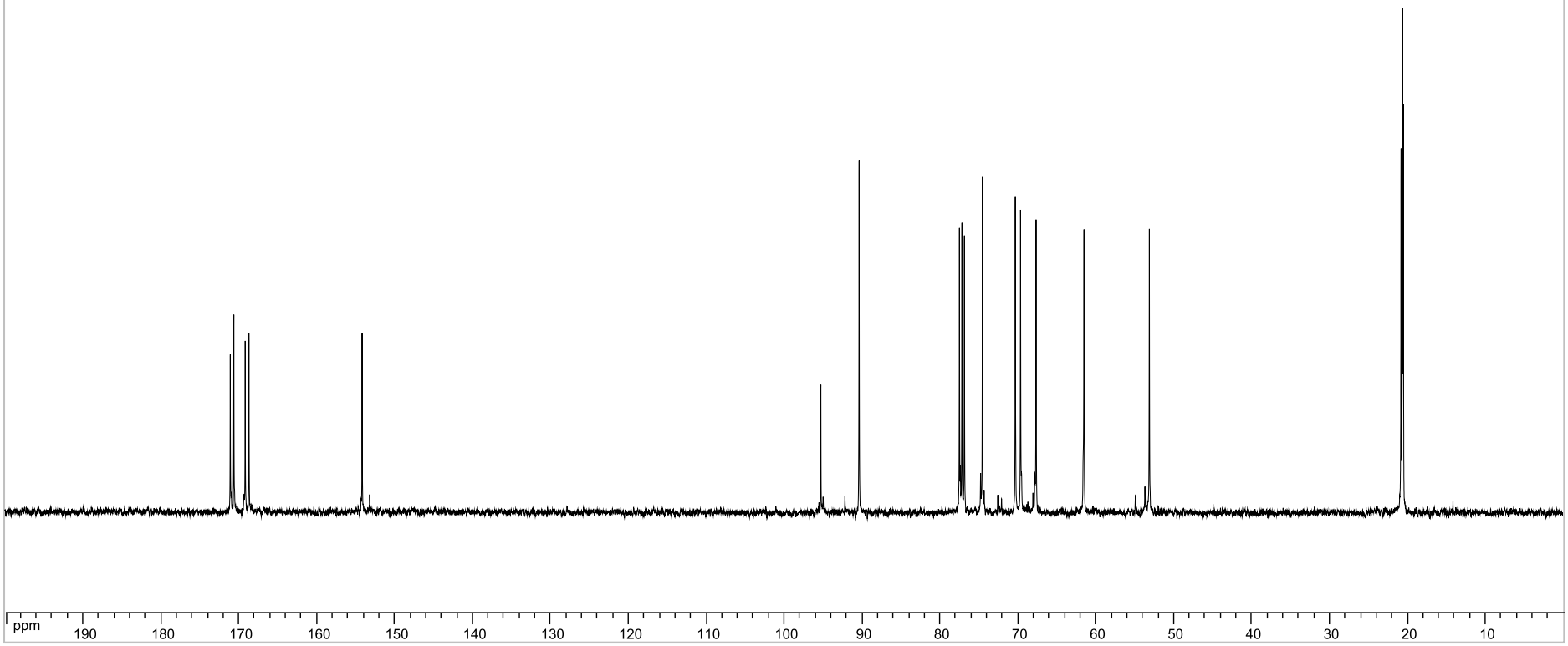


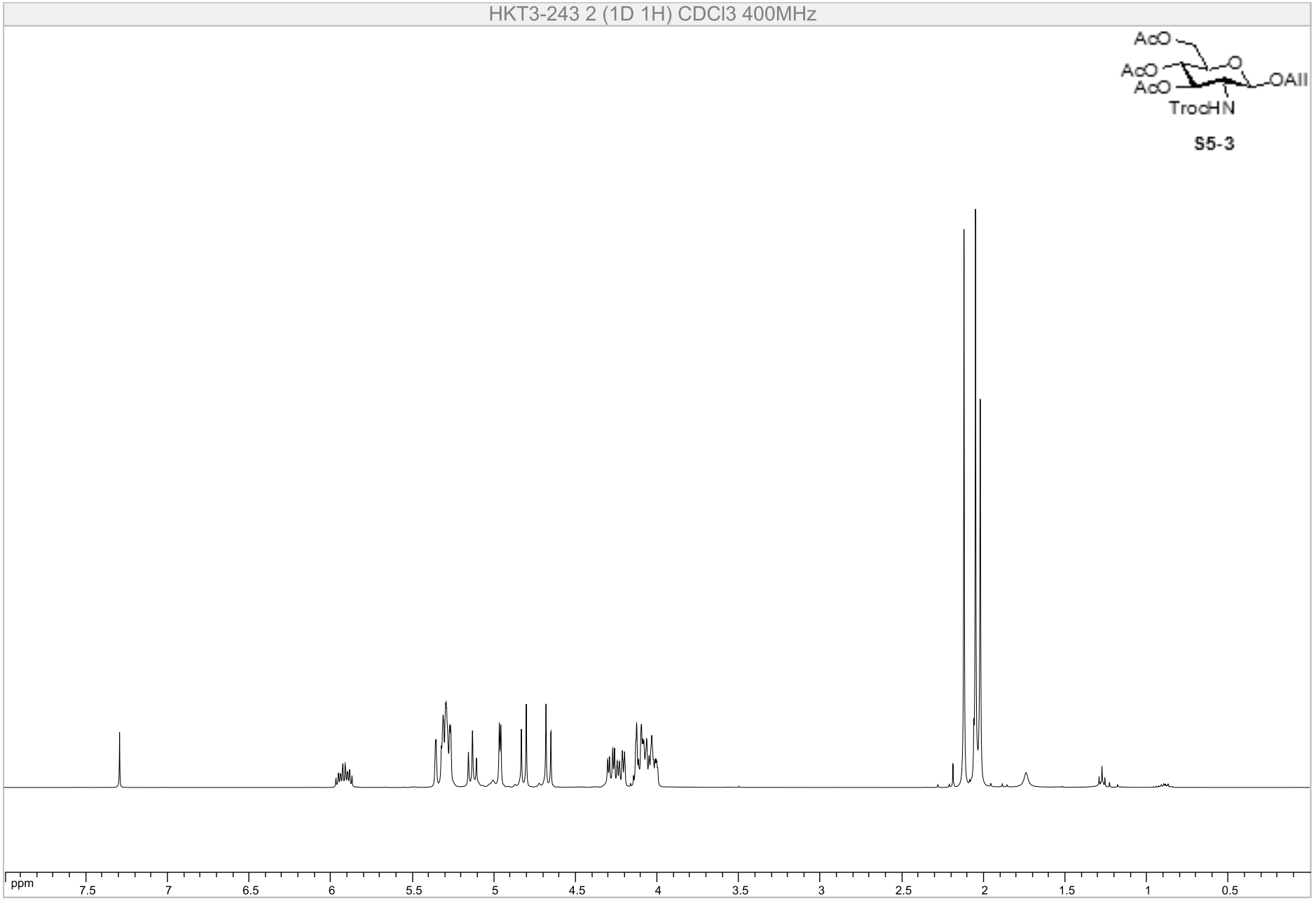
S5-2

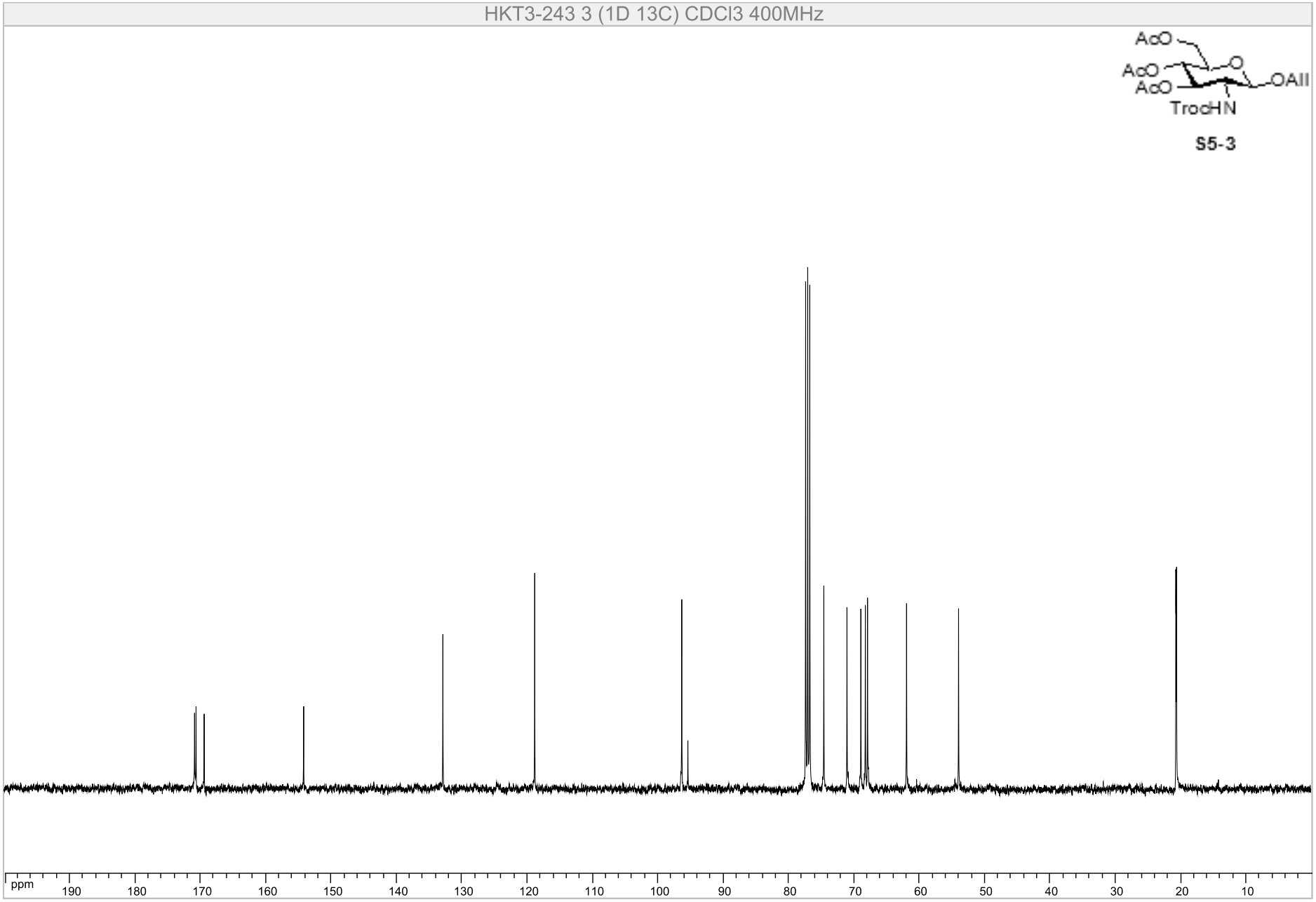


HKT3-240 2 (1D 13C) CDCl3 400MHz

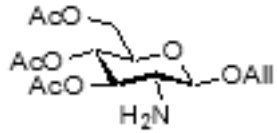
S5-2



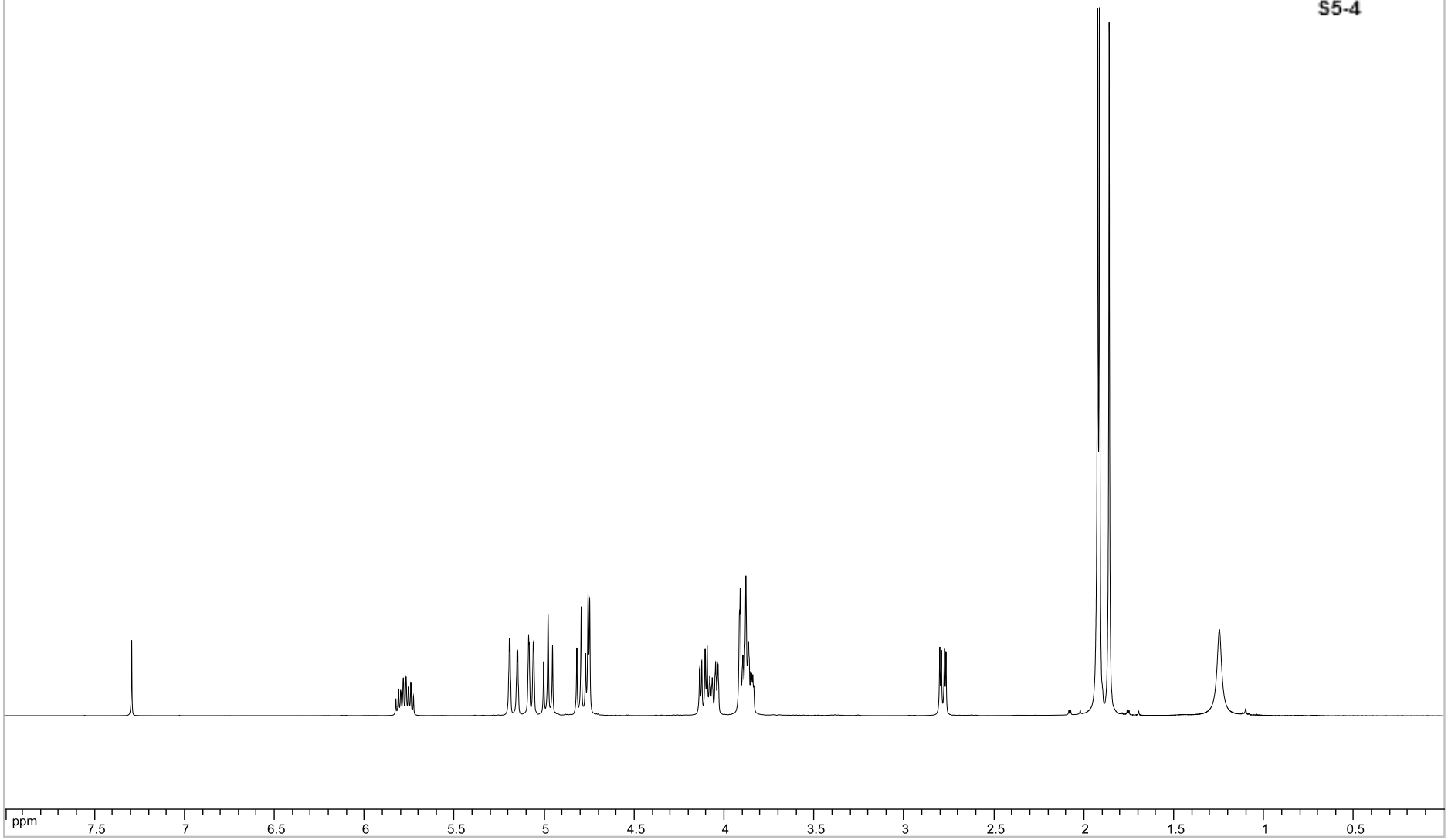

HKT3-243 2 (1D 1H) CDCl3 400MHz
AcO
AcO
AcO
O
OAll
TrocHN
S5-3
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5


HKT3-243 3 (1D 13C) CDCl3 400MHz
AcO
AcO
AcO
O
OAll
TrocHN
S5-3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

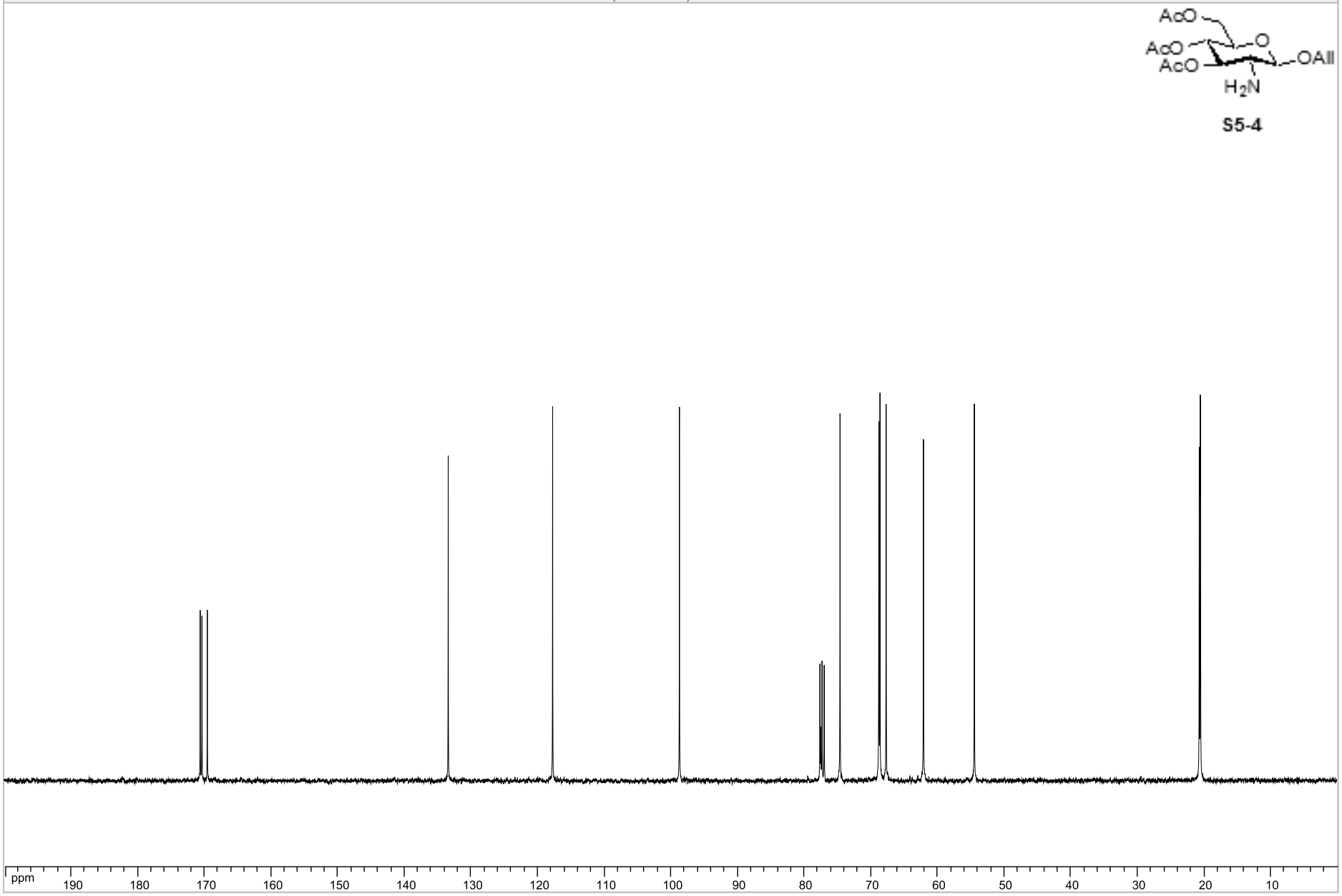
HKT3-251 2 (1D 1H) CDCl3 400MHz

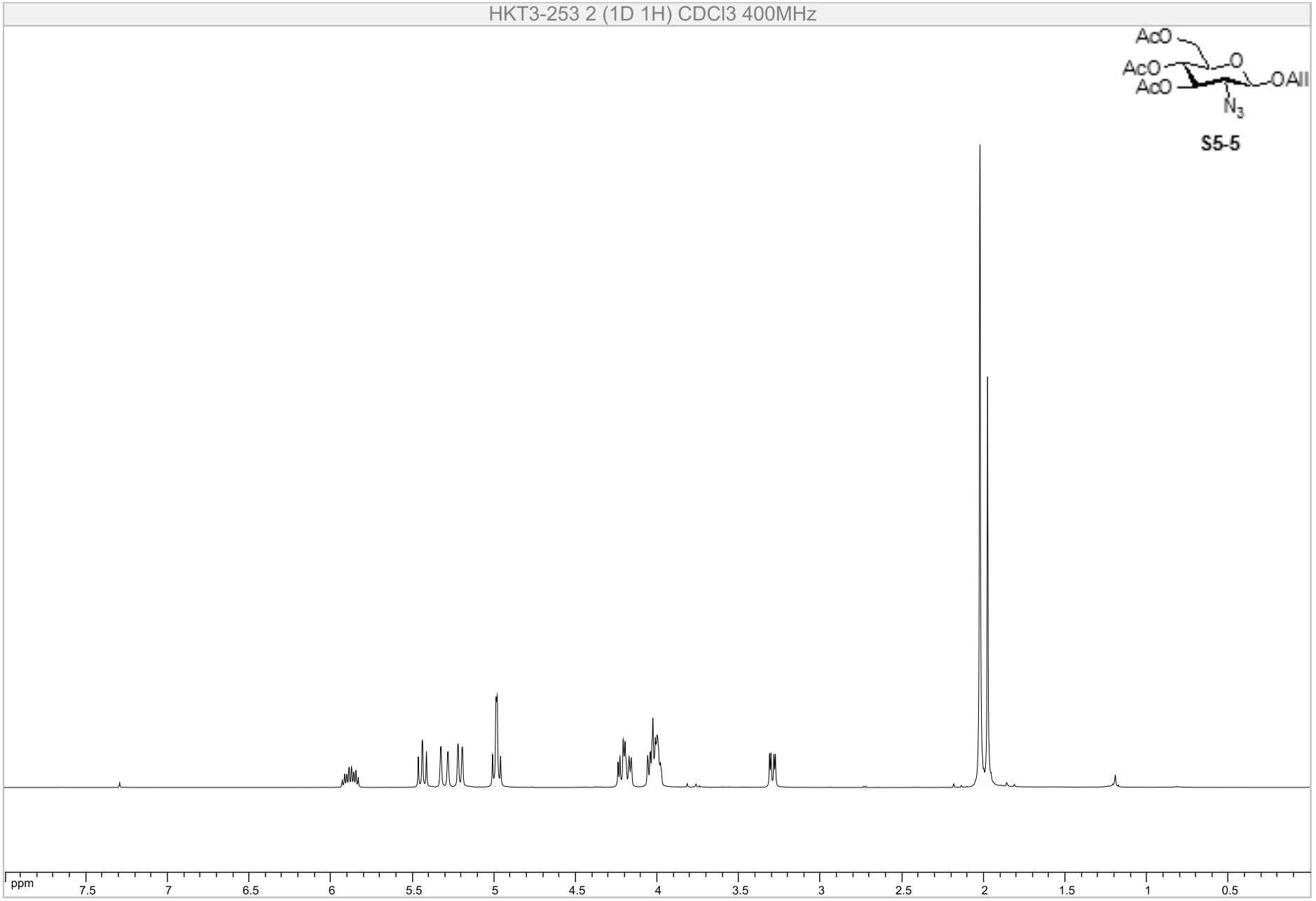


S5-4

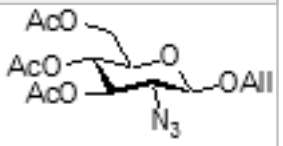


HKT3-251 3 (1D 13C) CDCl3 400MHz

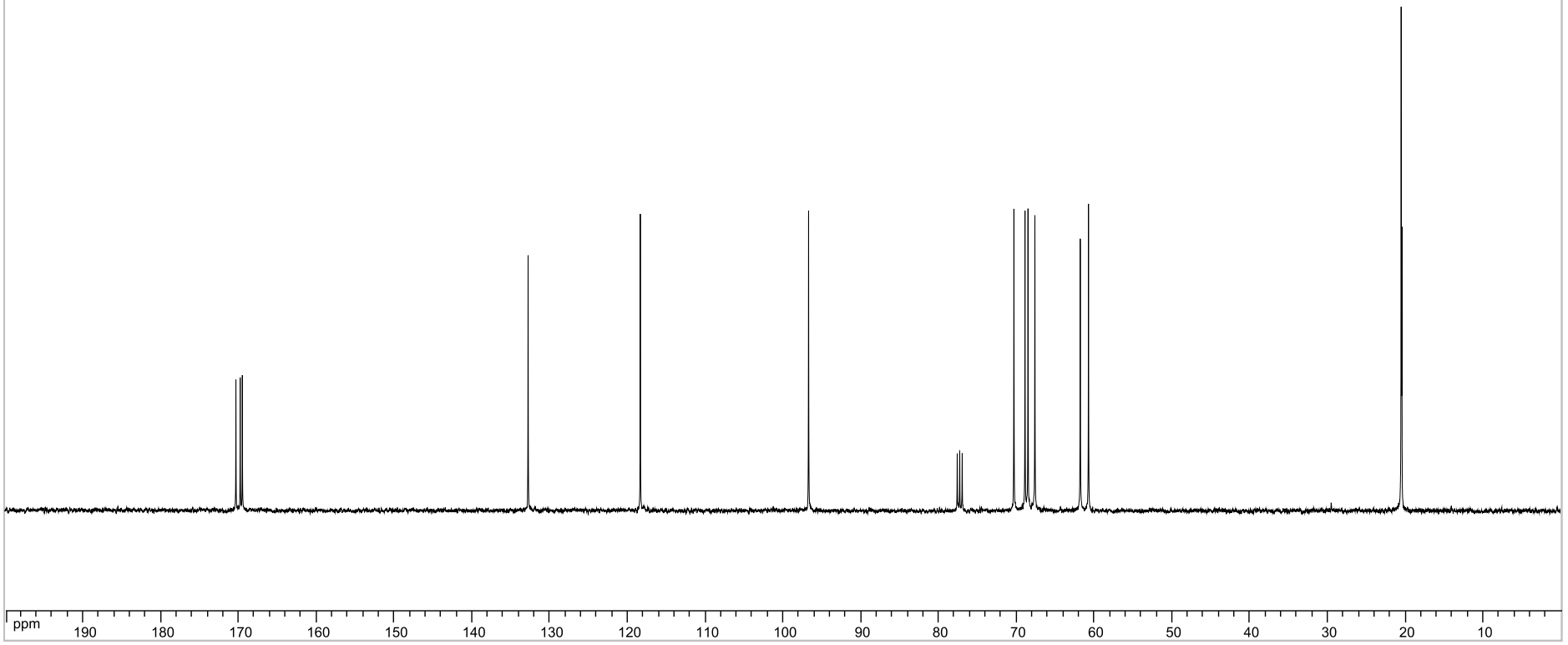



HKT3-253 2 (1D 1H) CDCl3 400MHz
AcO
AcO
AcO
O
OAll
N3
S5-5
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

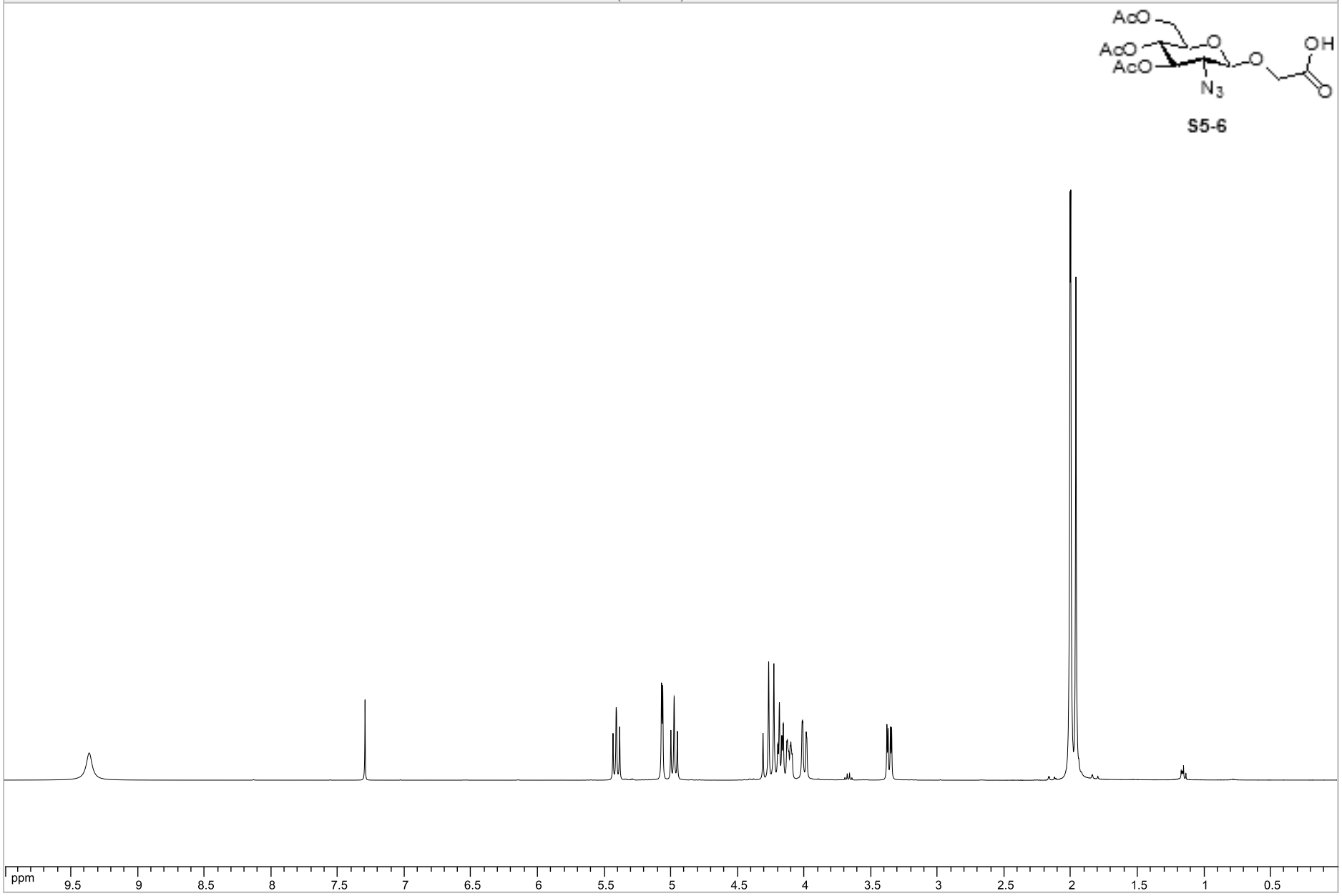
HKT3-253 3 (1D 13C) CDCl3 400MHz



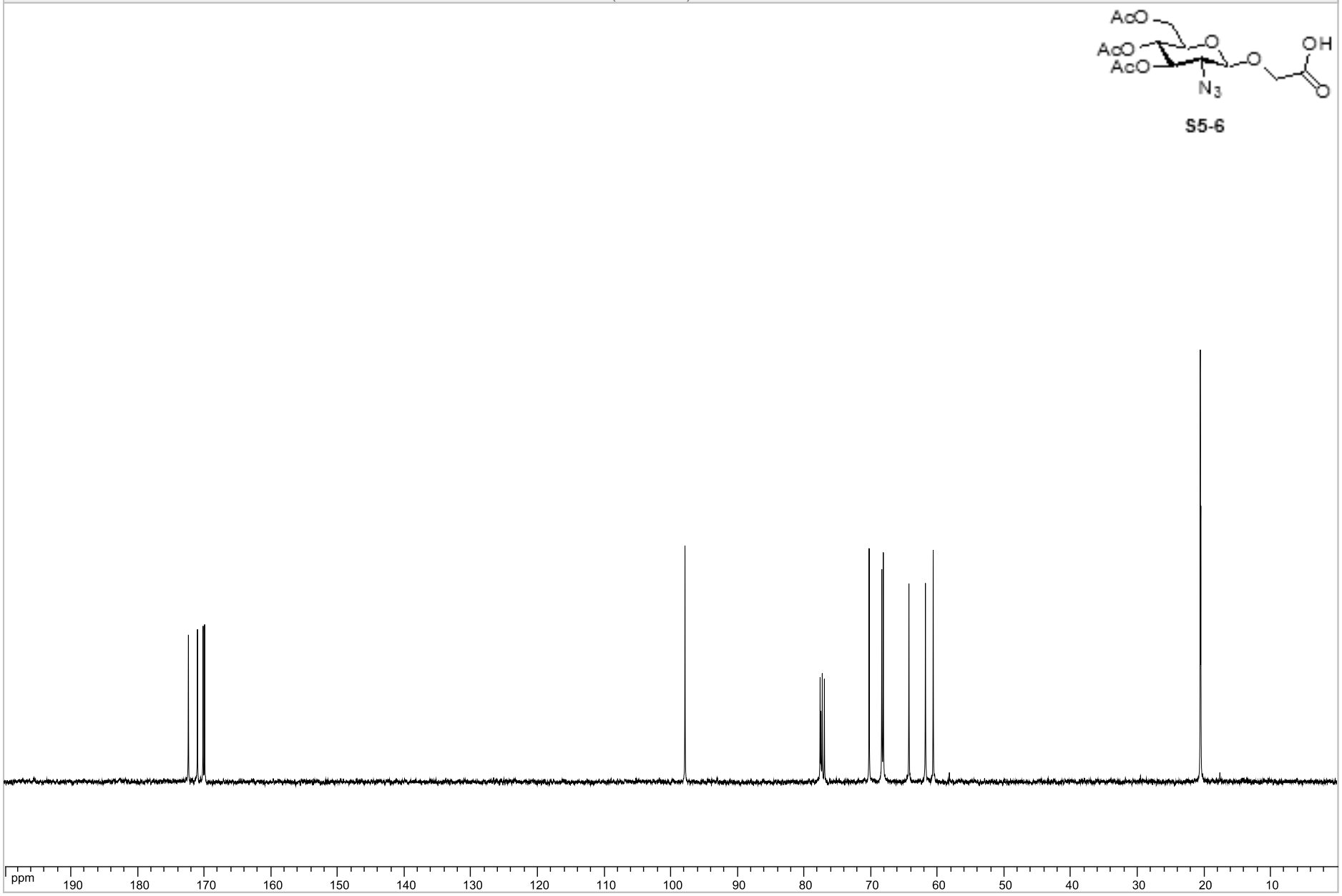
S5-5

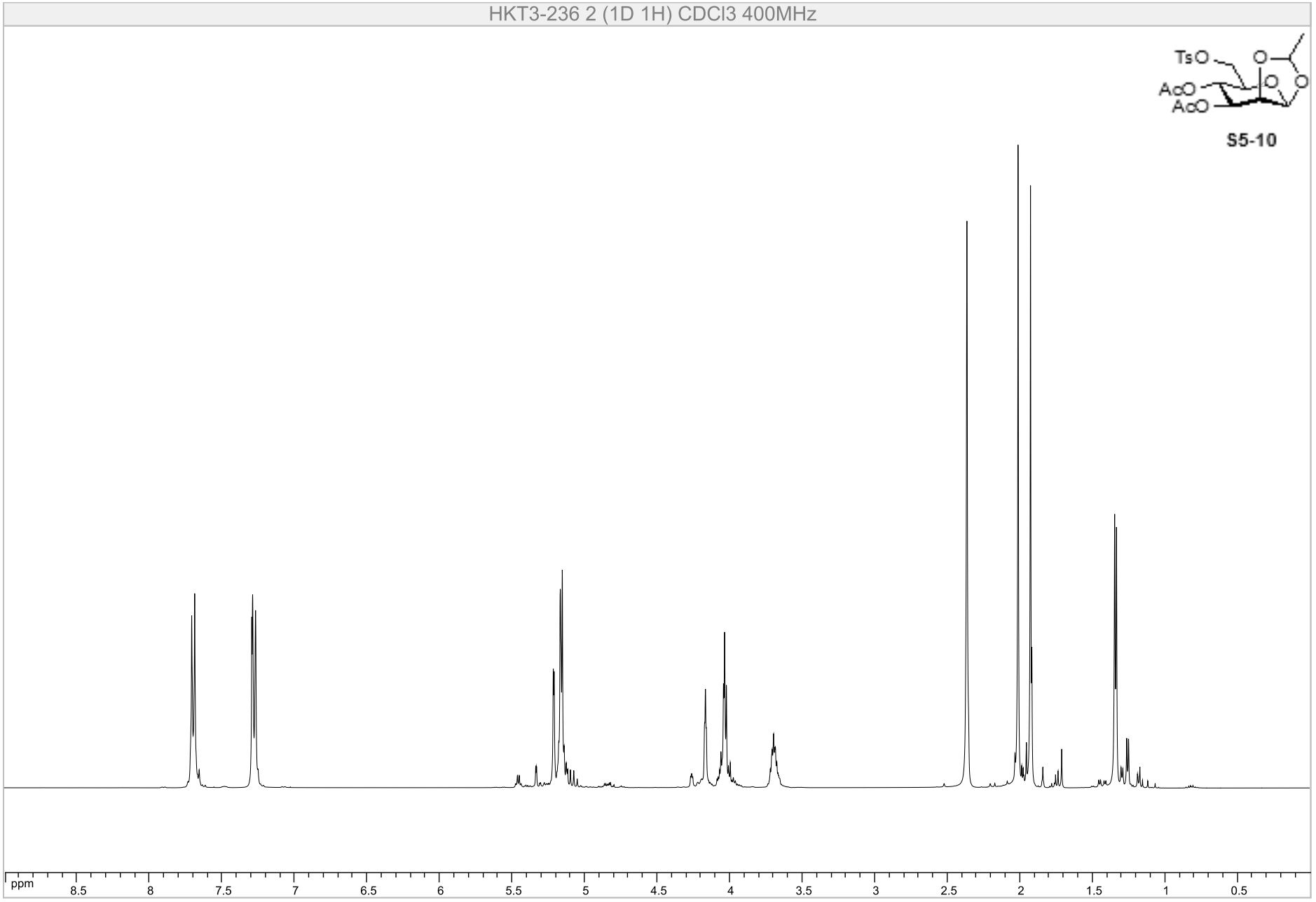


HKT3-254 1 (1D 1H) CDCl3 400MHz

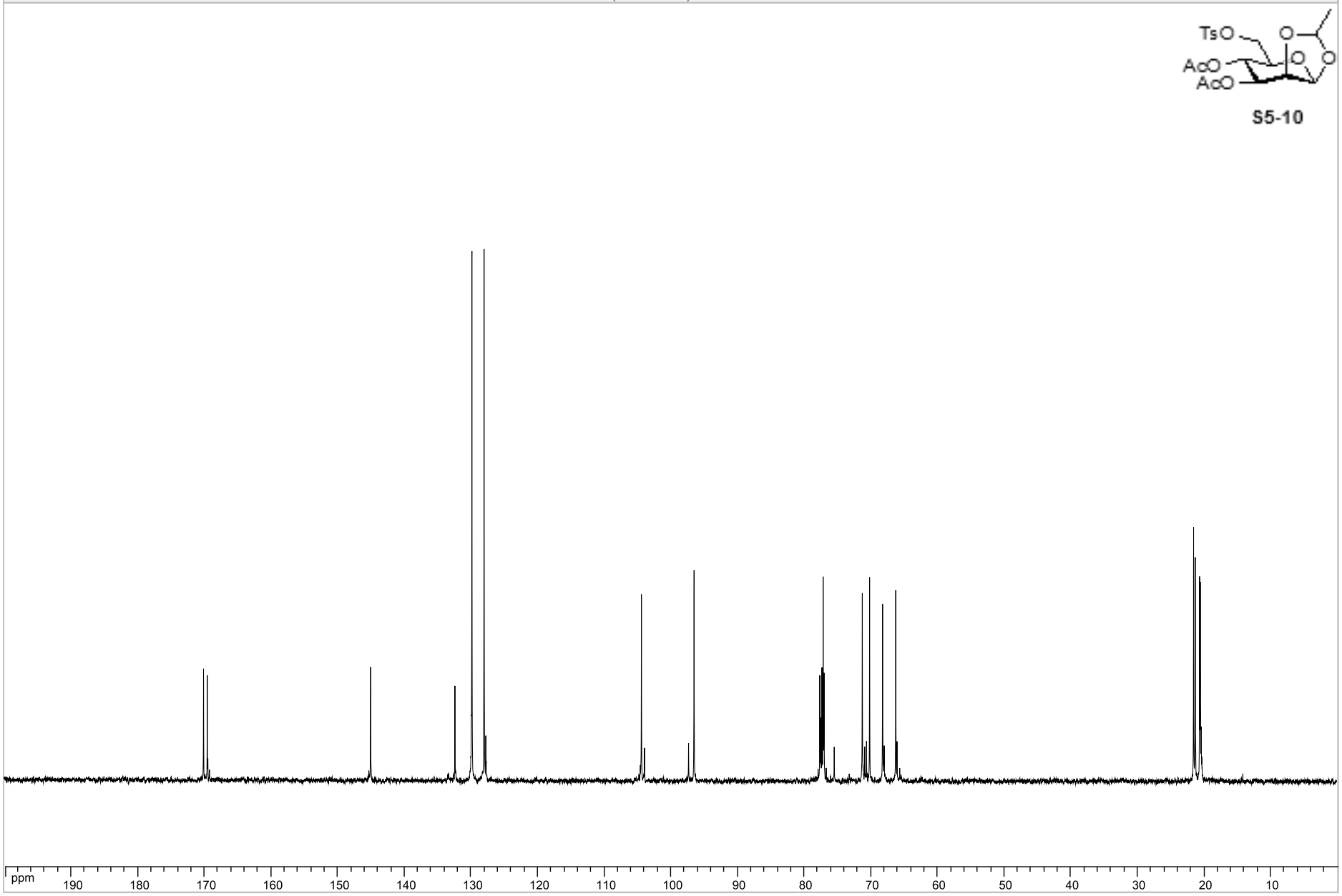


HKT3-254 2 (1D 13C) CDCl3 400MHz

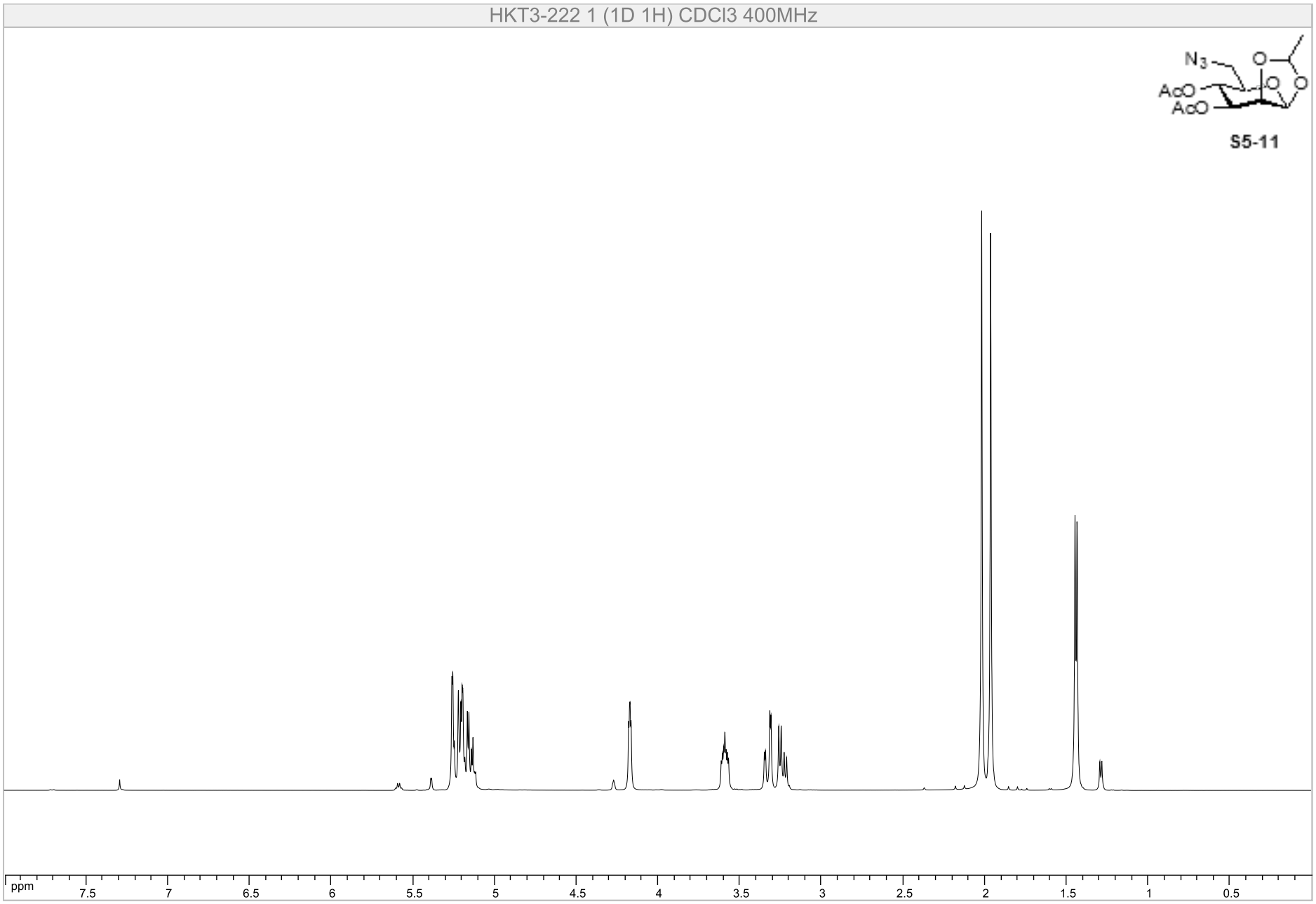



HKT3-236 2 (1D 1H) CDCl3 400MHz
TsO
AcO
AcO
S5-10
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

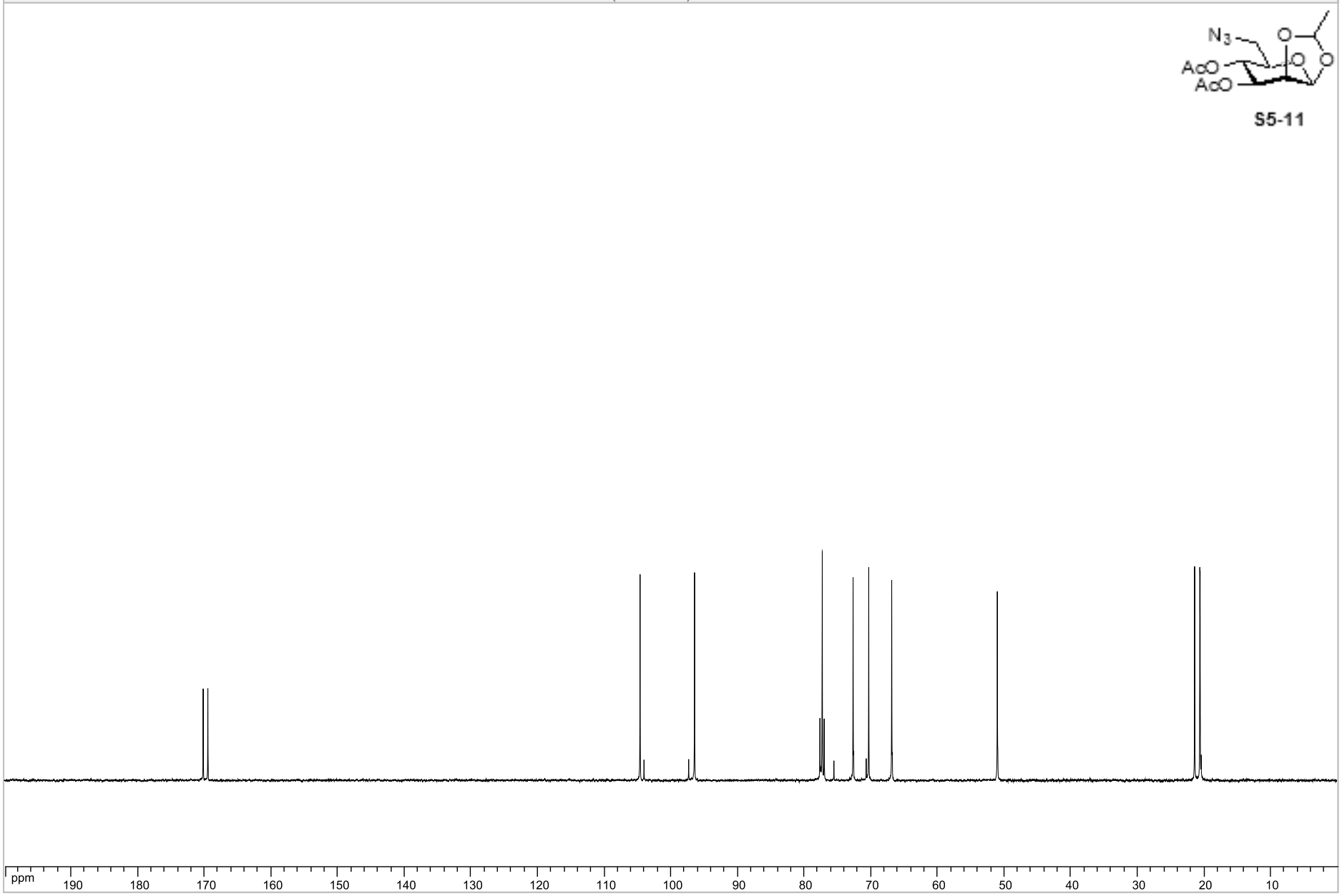
HKT3-236 3 (1D 13C) CDCl3 400MHz



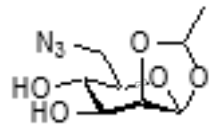
HKT3-222 1 (1D 1H) CDCl3 400MHz



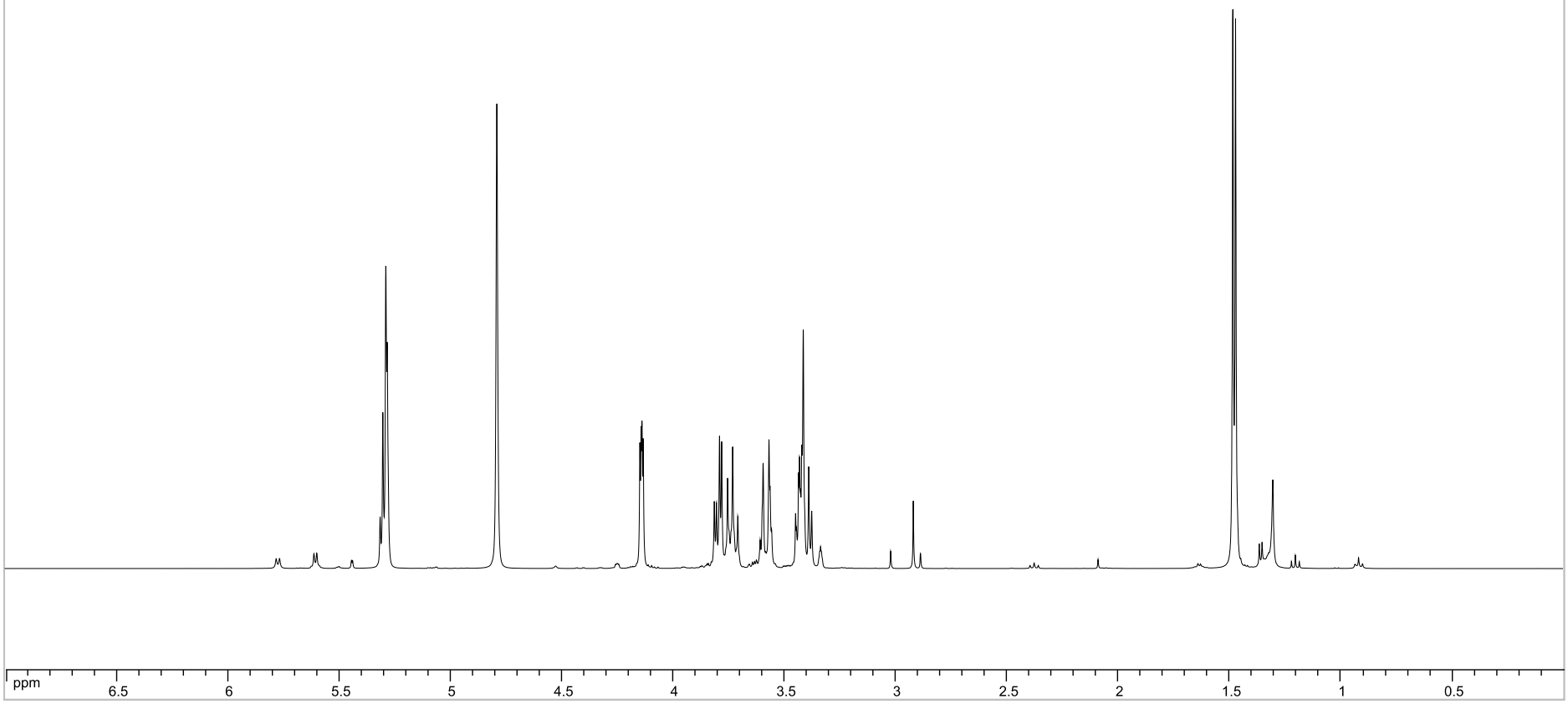
HKT3-222 2 (1D 13C) CDCl3 400MHz



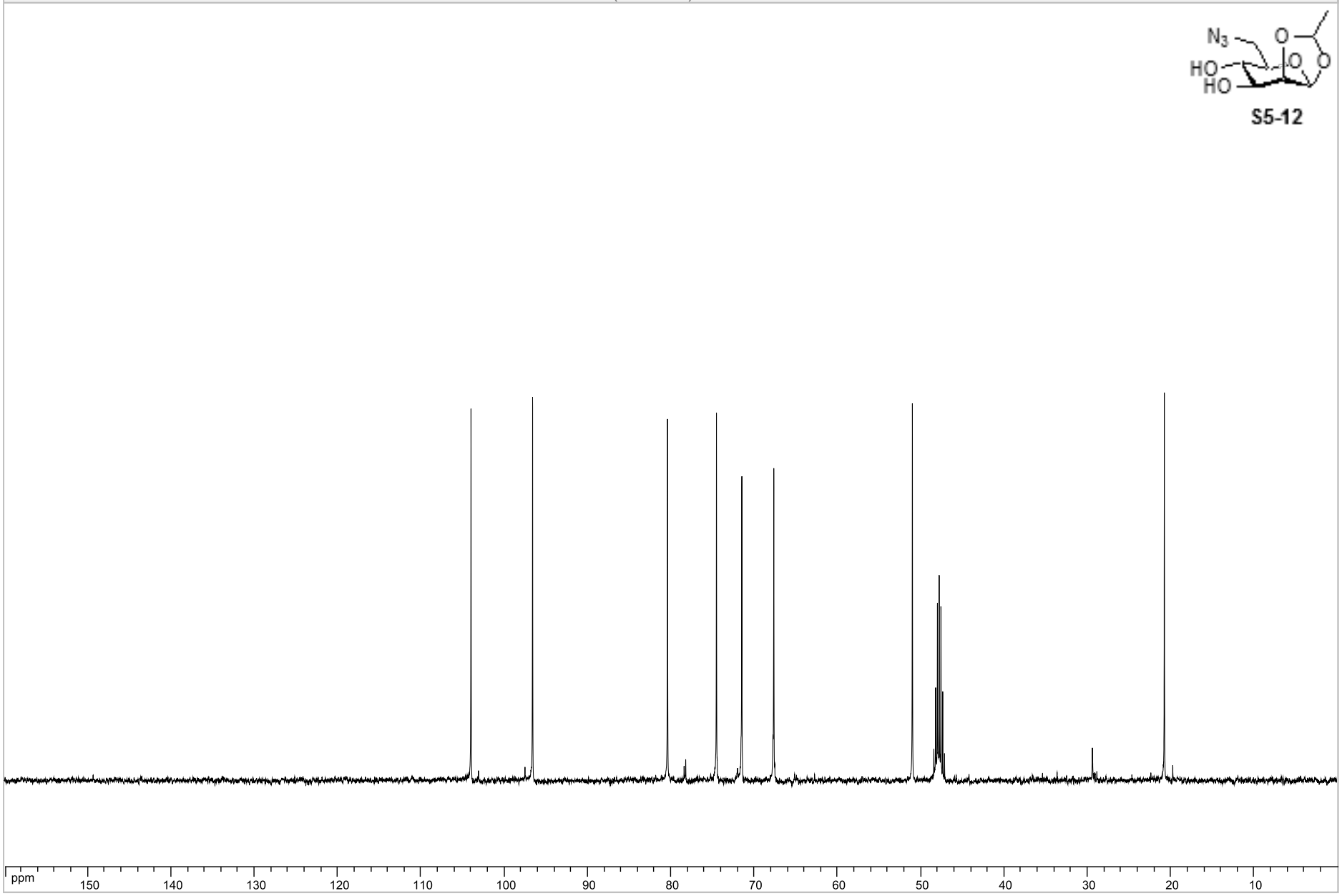
HKT3-199 1 (1D 1H) MeOD 400MHz



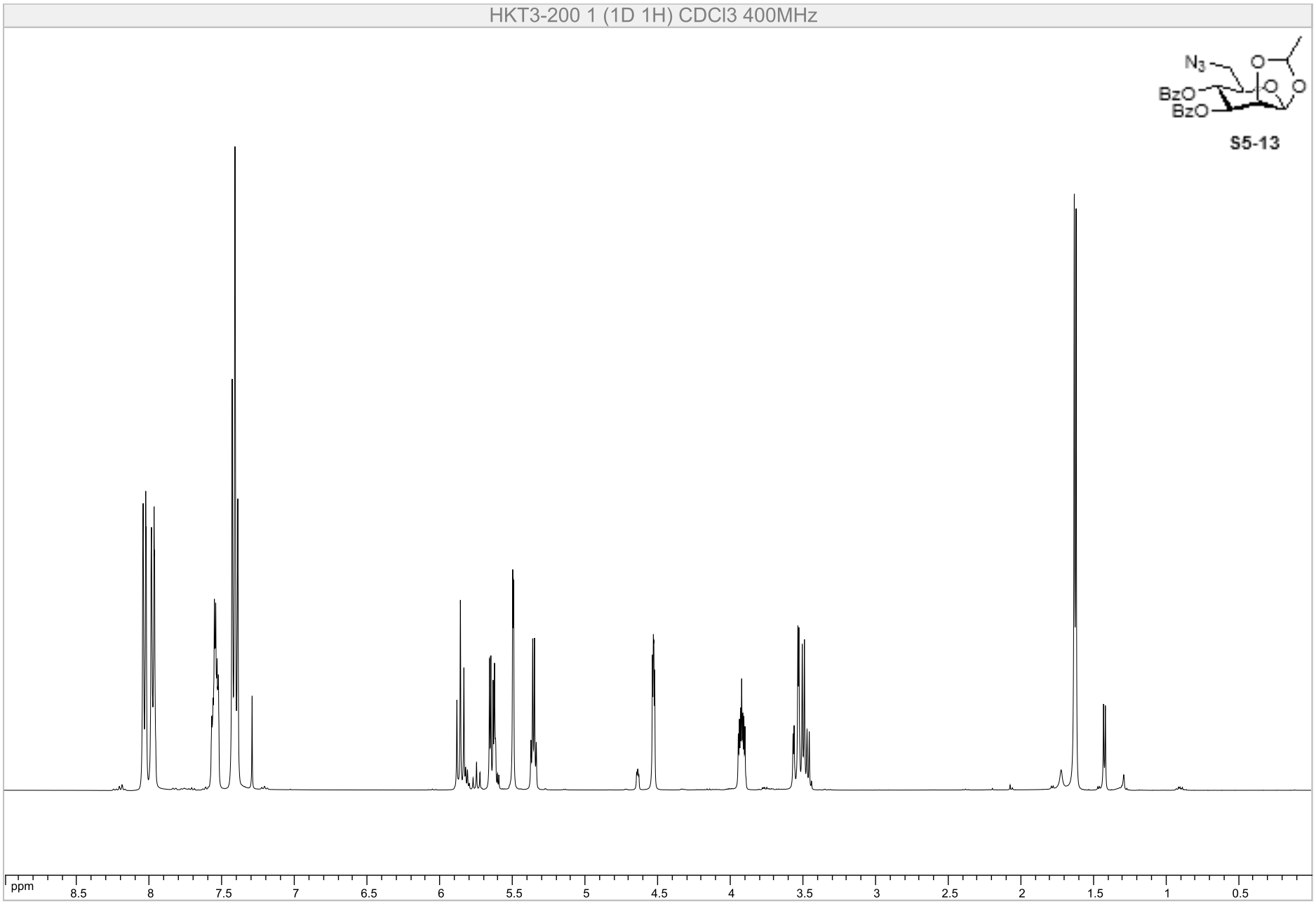
S5-12



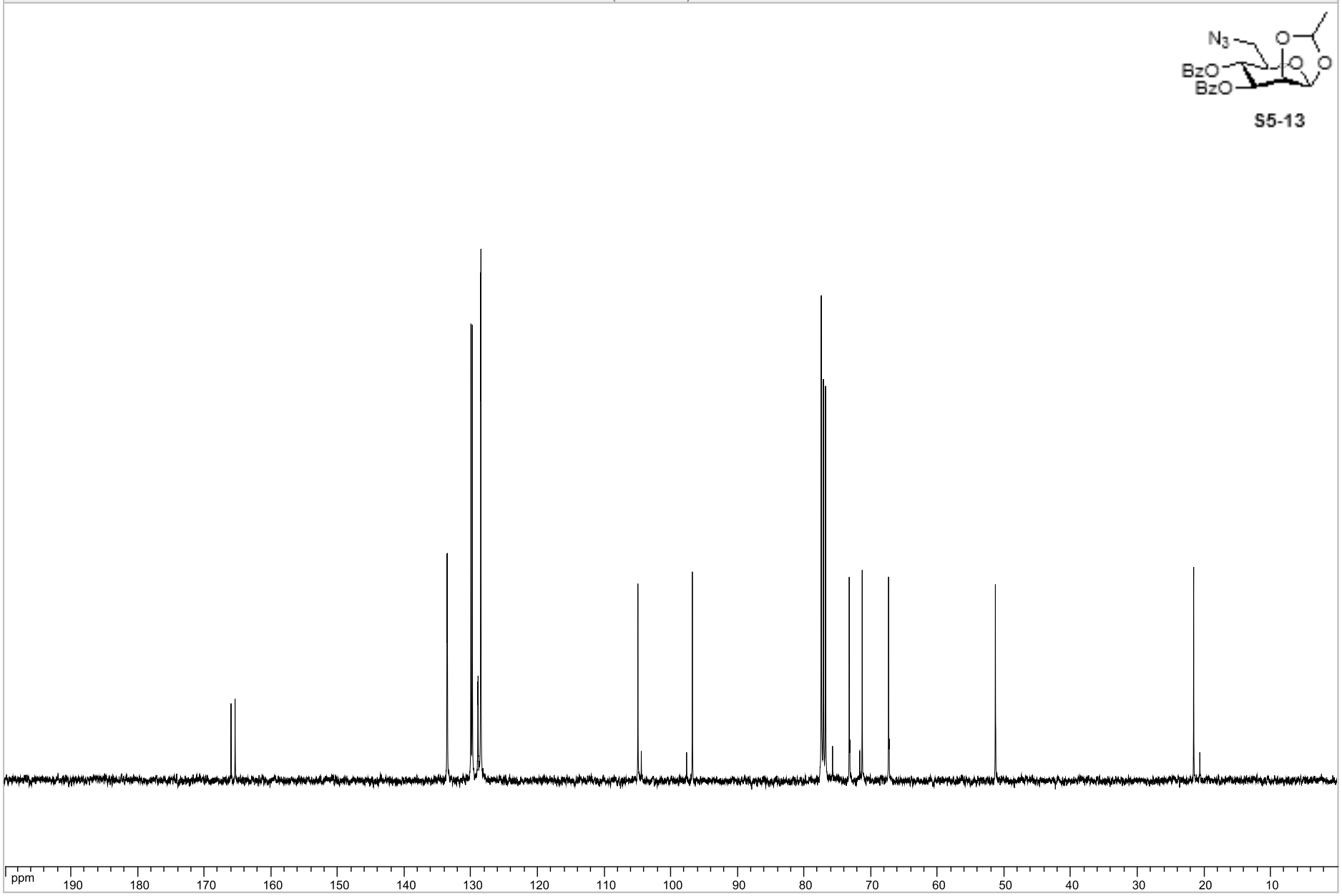
HKT3-199 2 (1D 13C) MeOD 400MHz



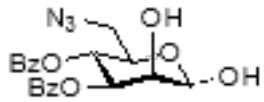
HKT3-200 1 (1D 1H) CDCl3 400MHz



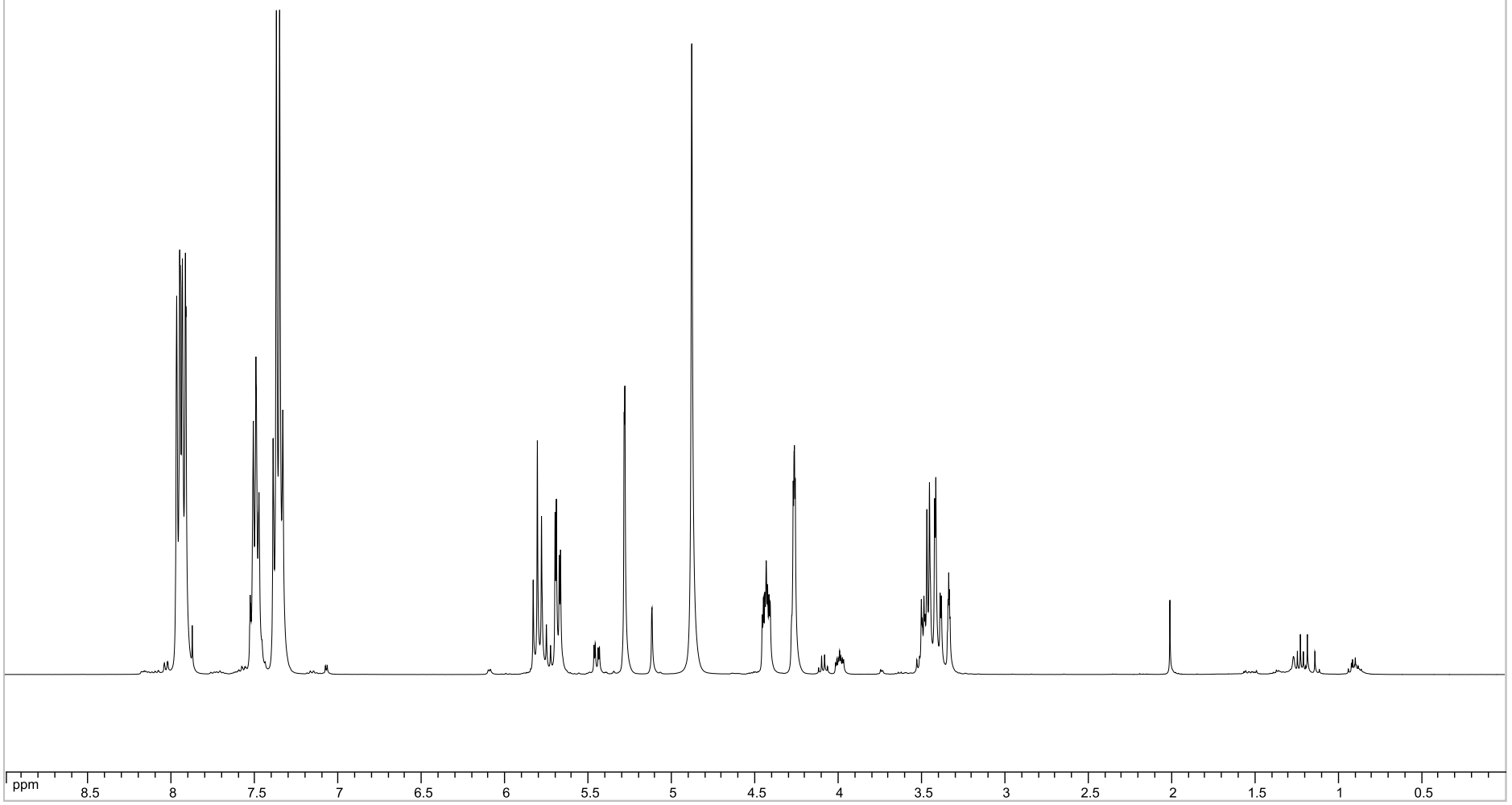
HKT3-200 2 (1D 13C) CDCl3 400MHz



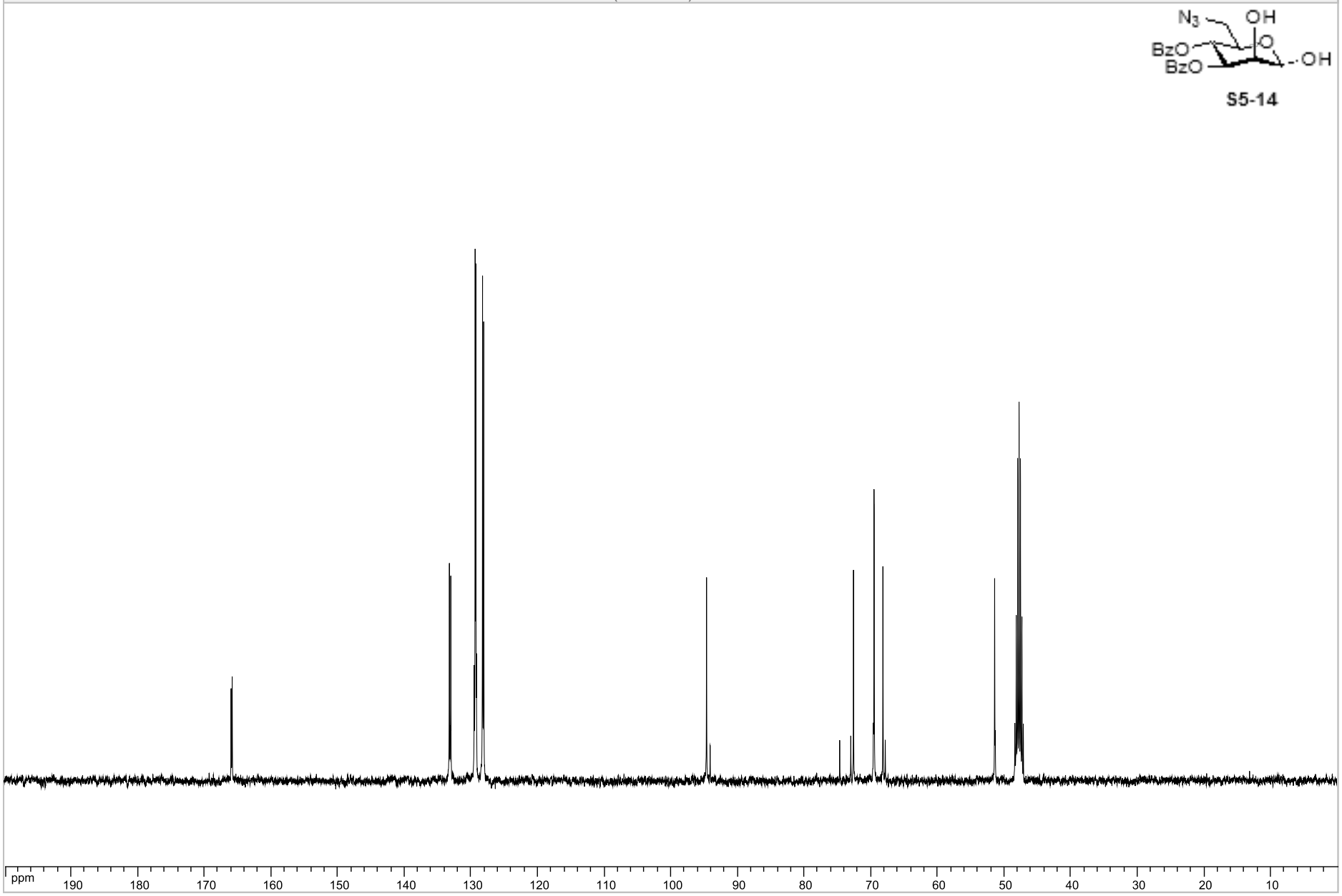
HKT3-202 1 (1D 1H) MeOD 400MHz



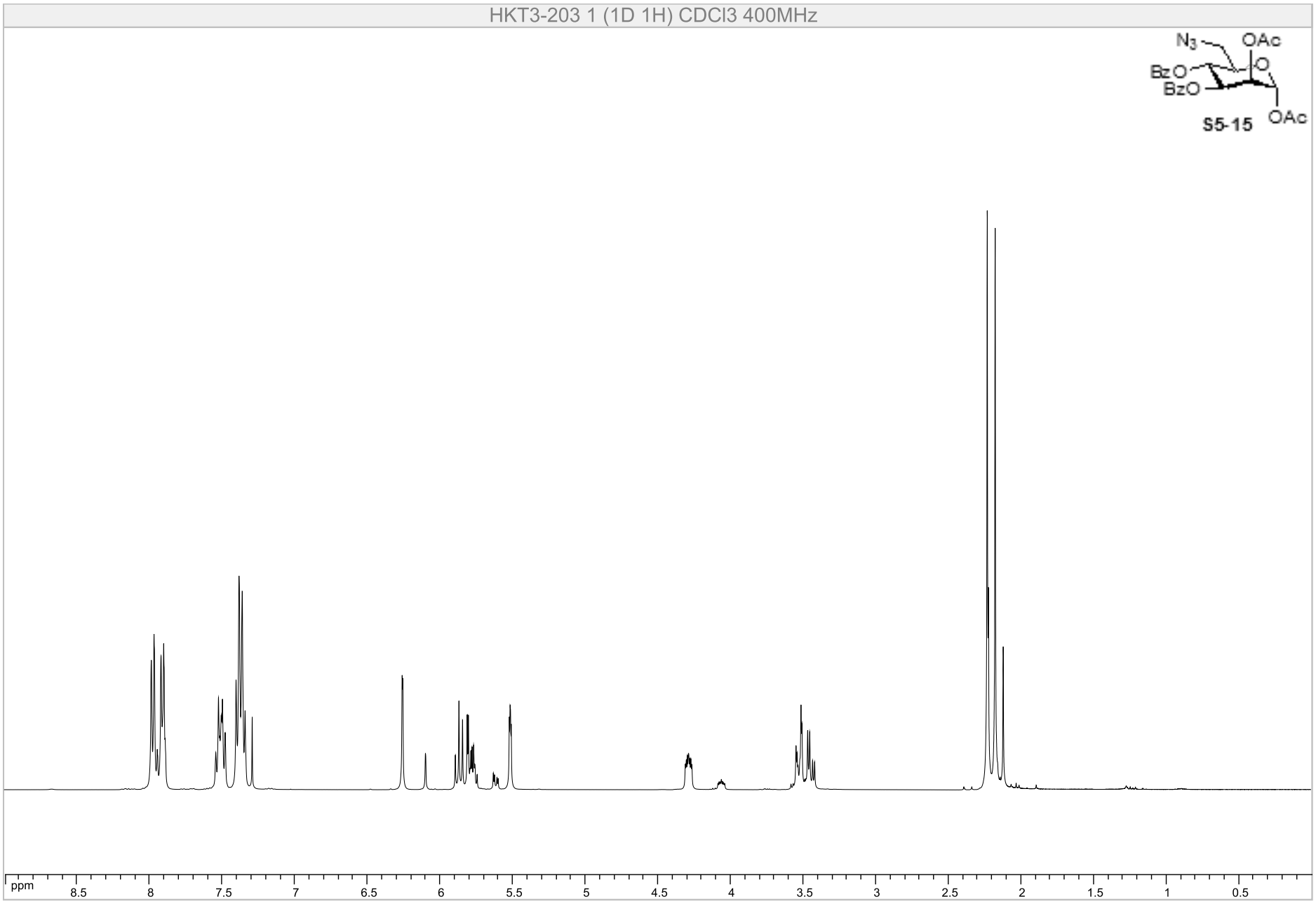
S5-14

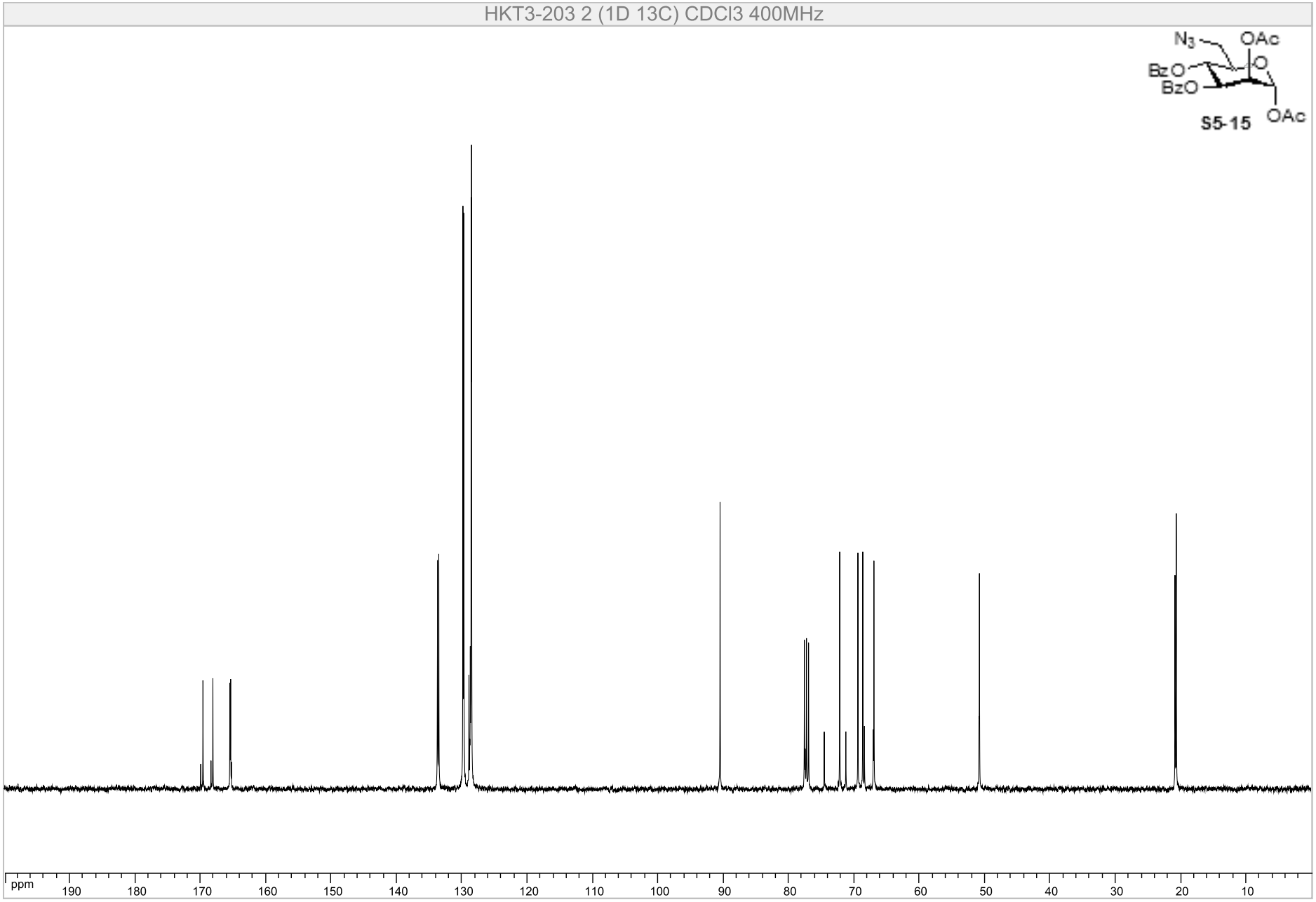


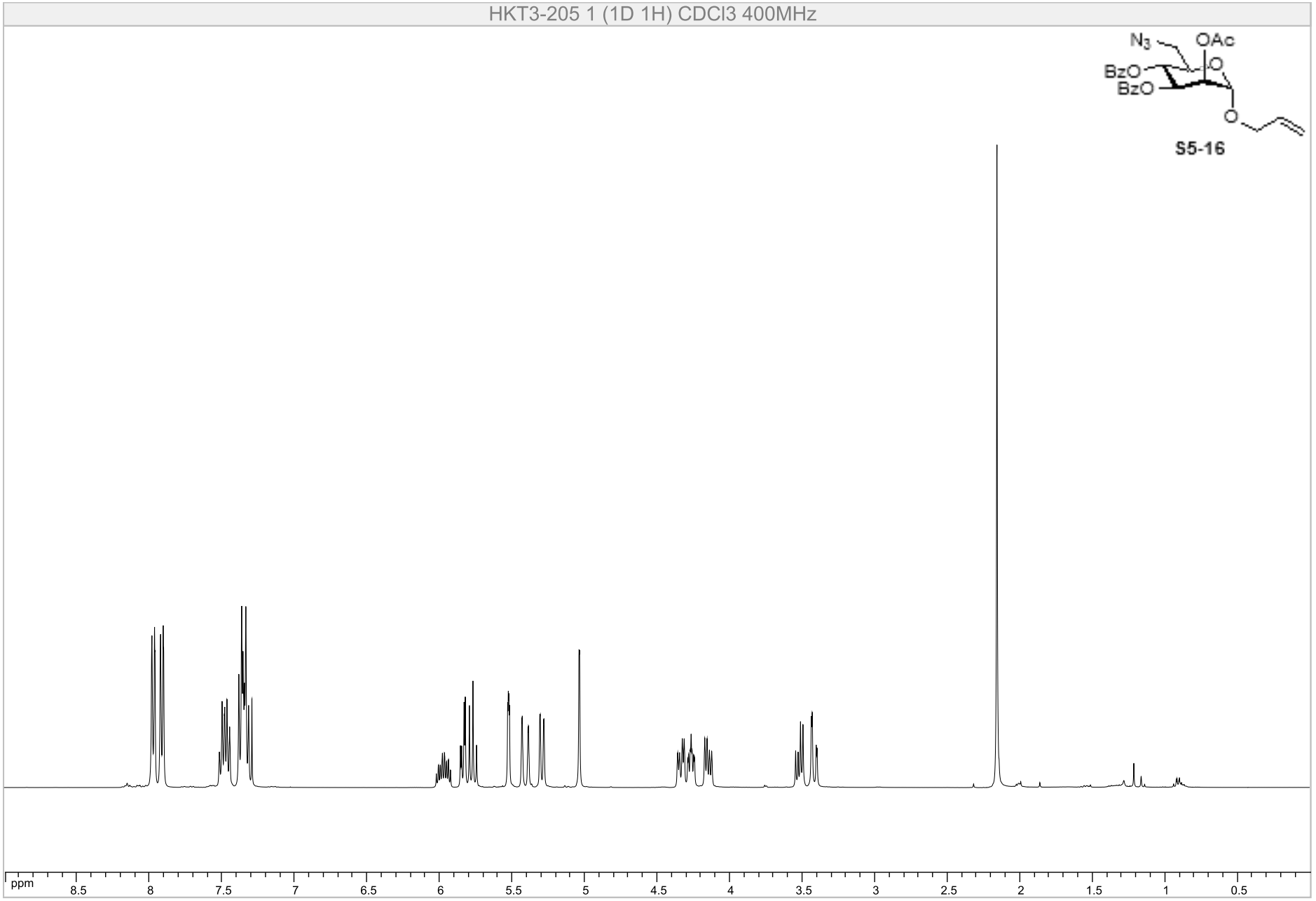
HKT3-202 2 (1D 13C) MeOD 400MHz



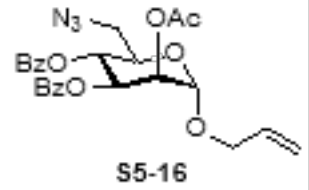
HKT3-203 1 (1D 1H) CDCl3 400MHz



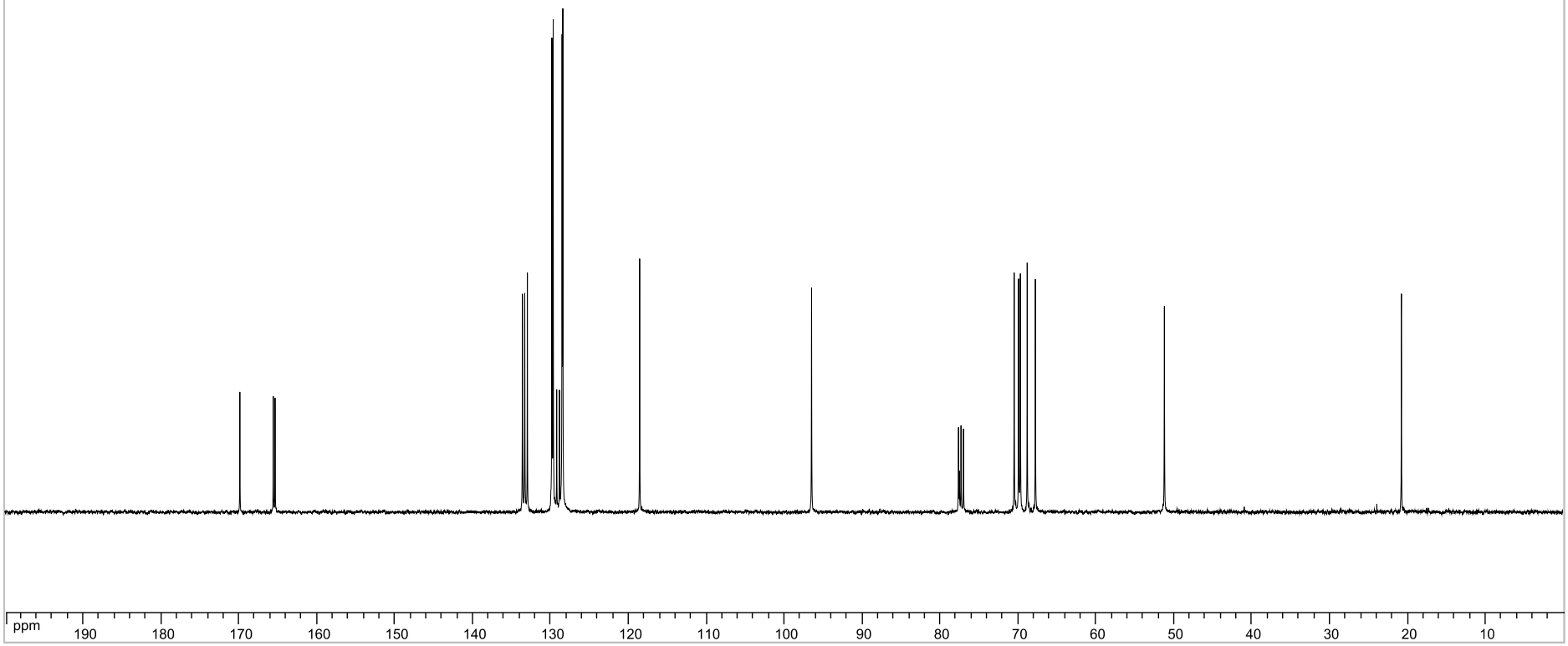

HKT3-203 2 (1D 13C) CDCl3 400MHz
N3
OAc
BzO
BzO
O
OAc
S5-15
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


HKT3-205 1 (1D 1H) CDCl3 400MHz
N3
OAc
BzO
BzO
O
O
S5-16
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

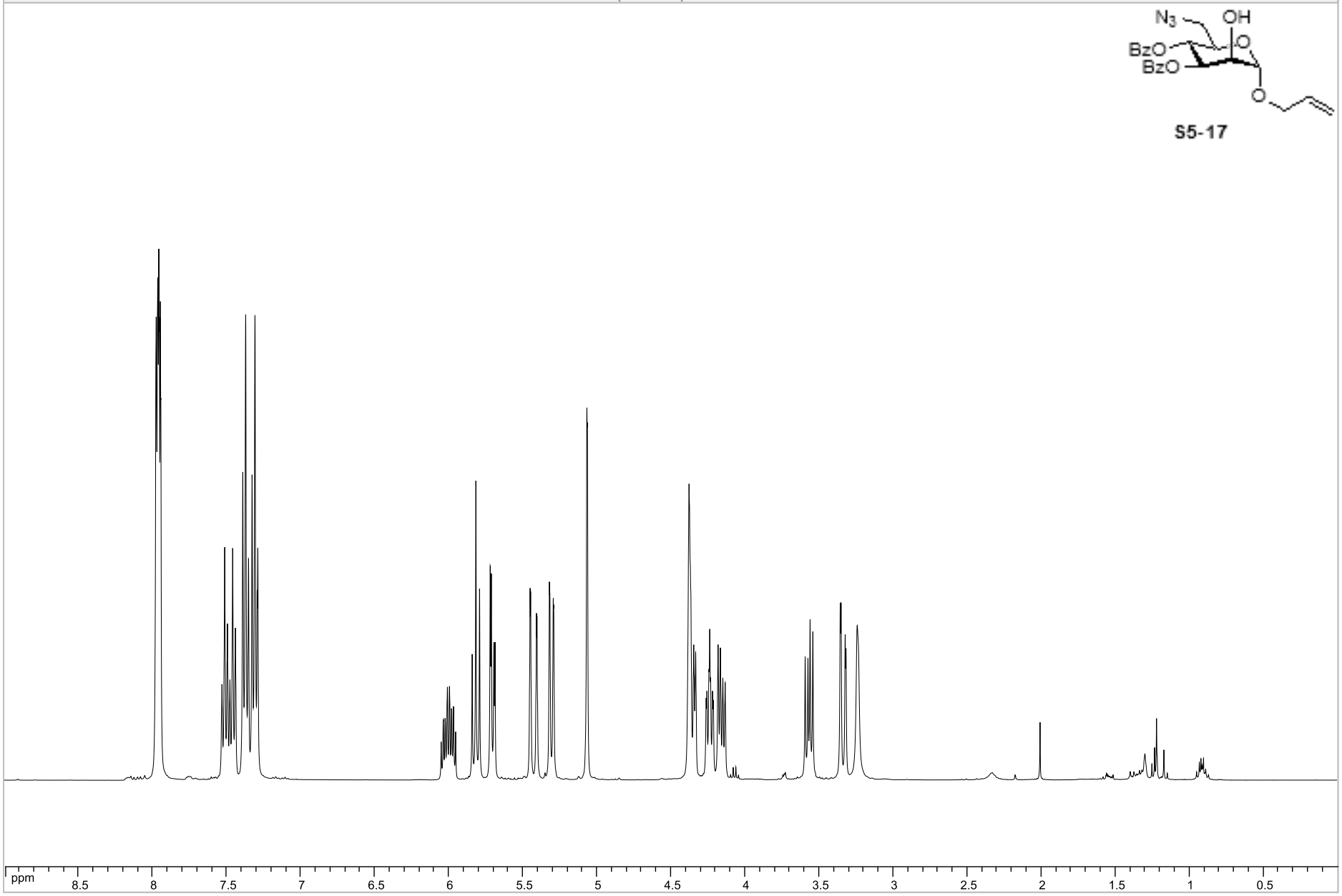
HKT3-205 2 (1D 13C) CDCl3 400MHz



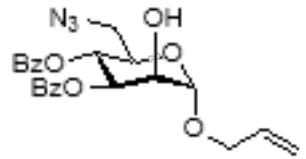
S5-16



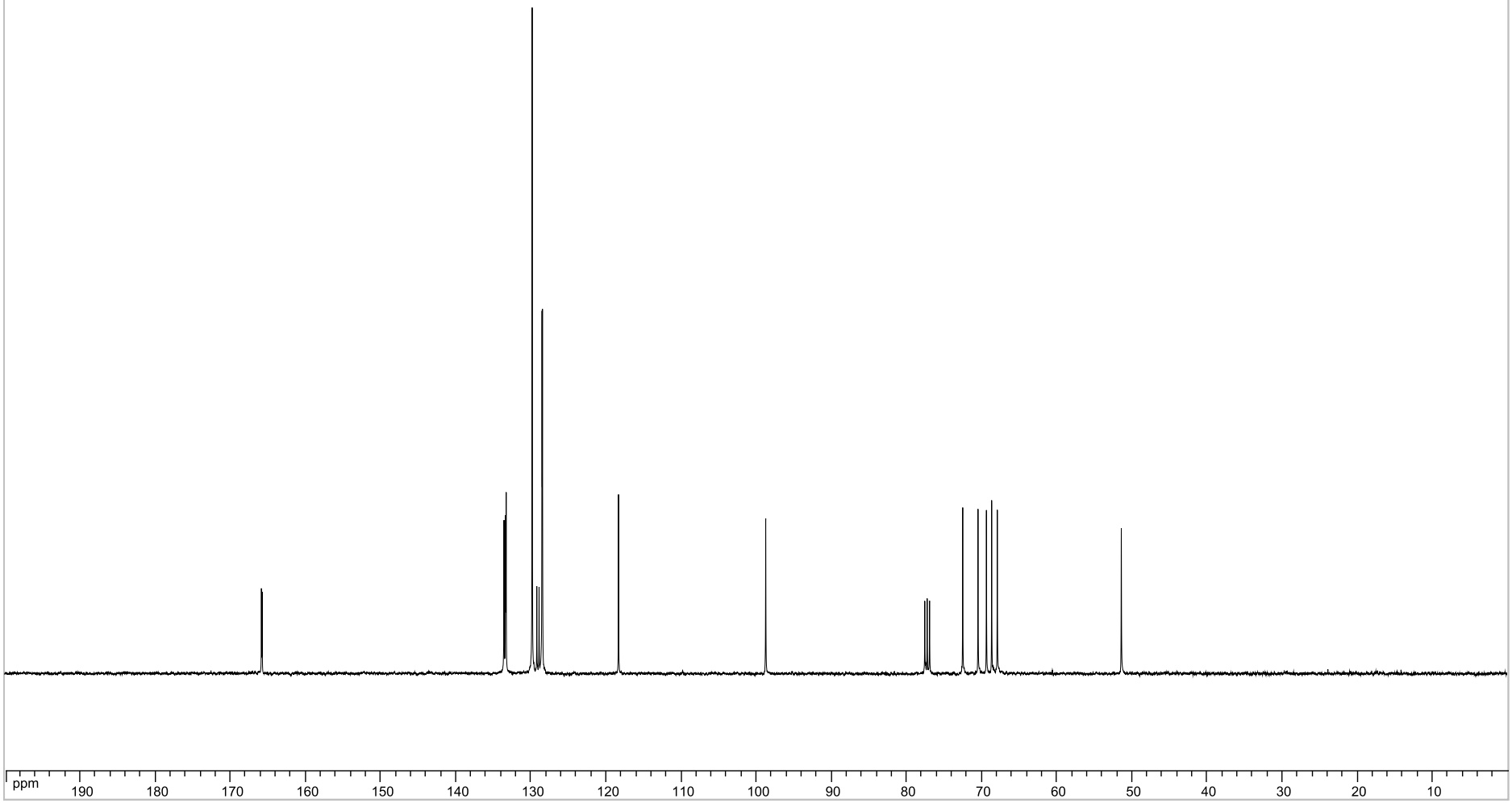
HKT3-207 1 (1D 1H) CDCl3 400MHz



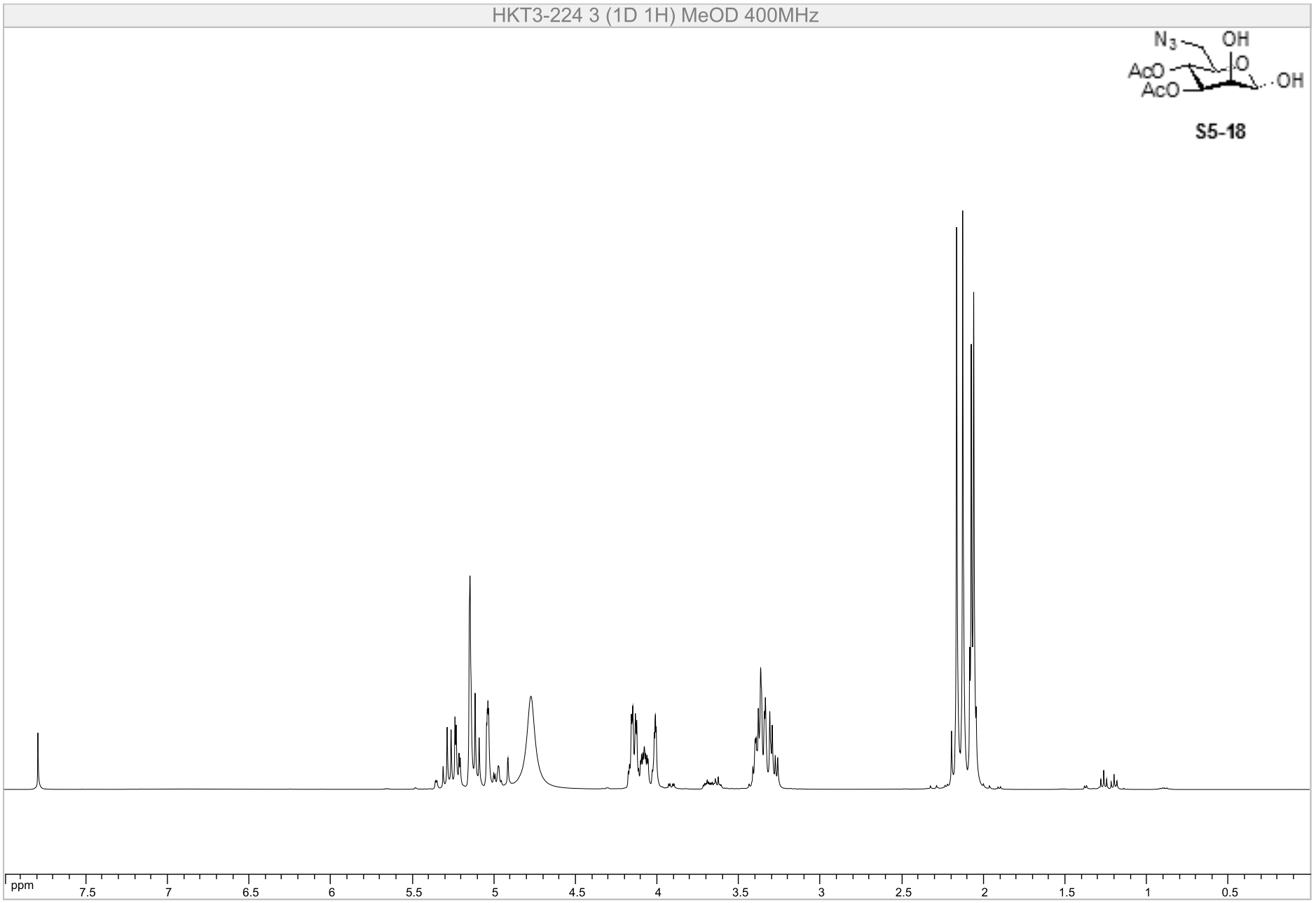
HKT3-207 2 (1D 13C) CDCl3 400MHz



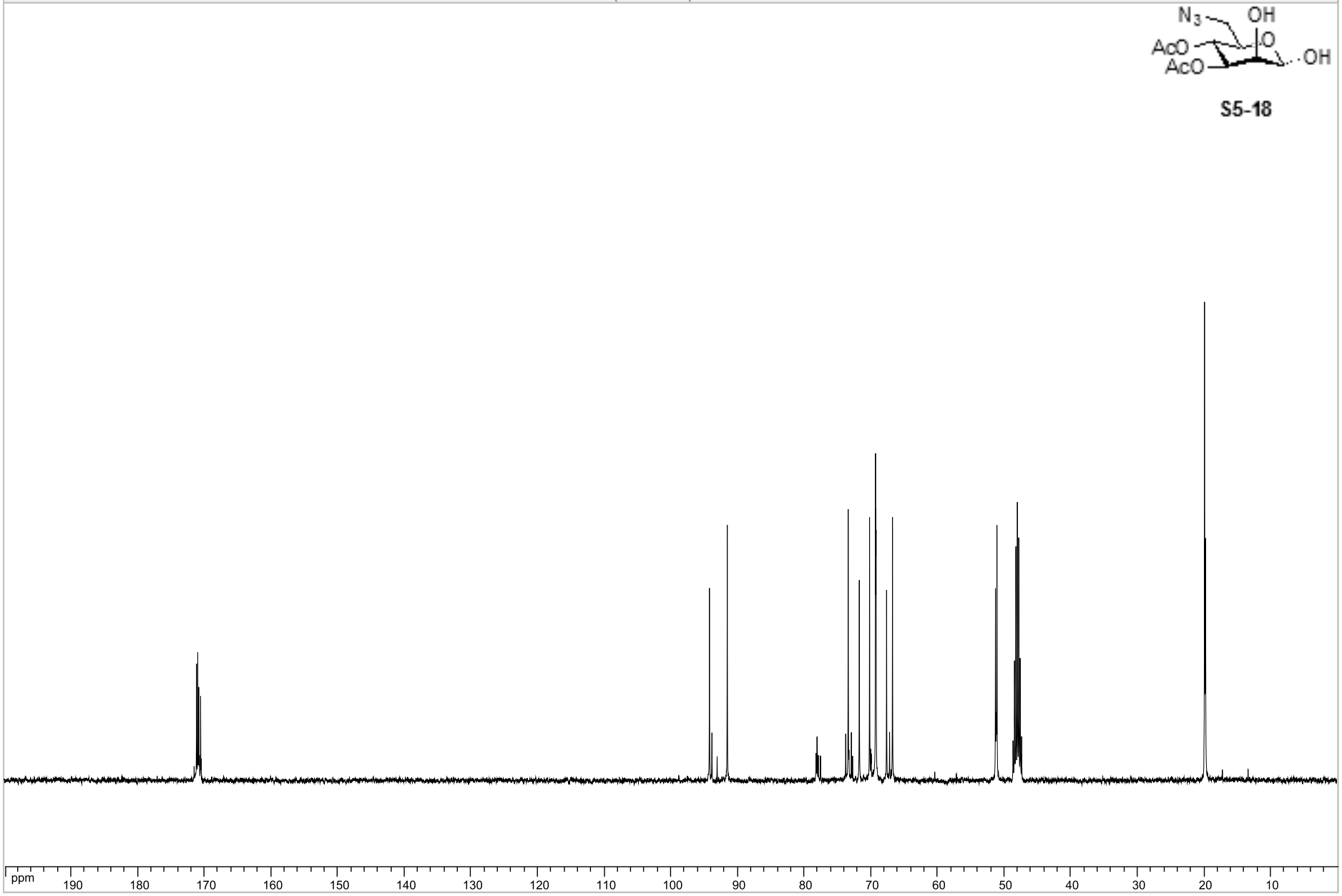
S5-17



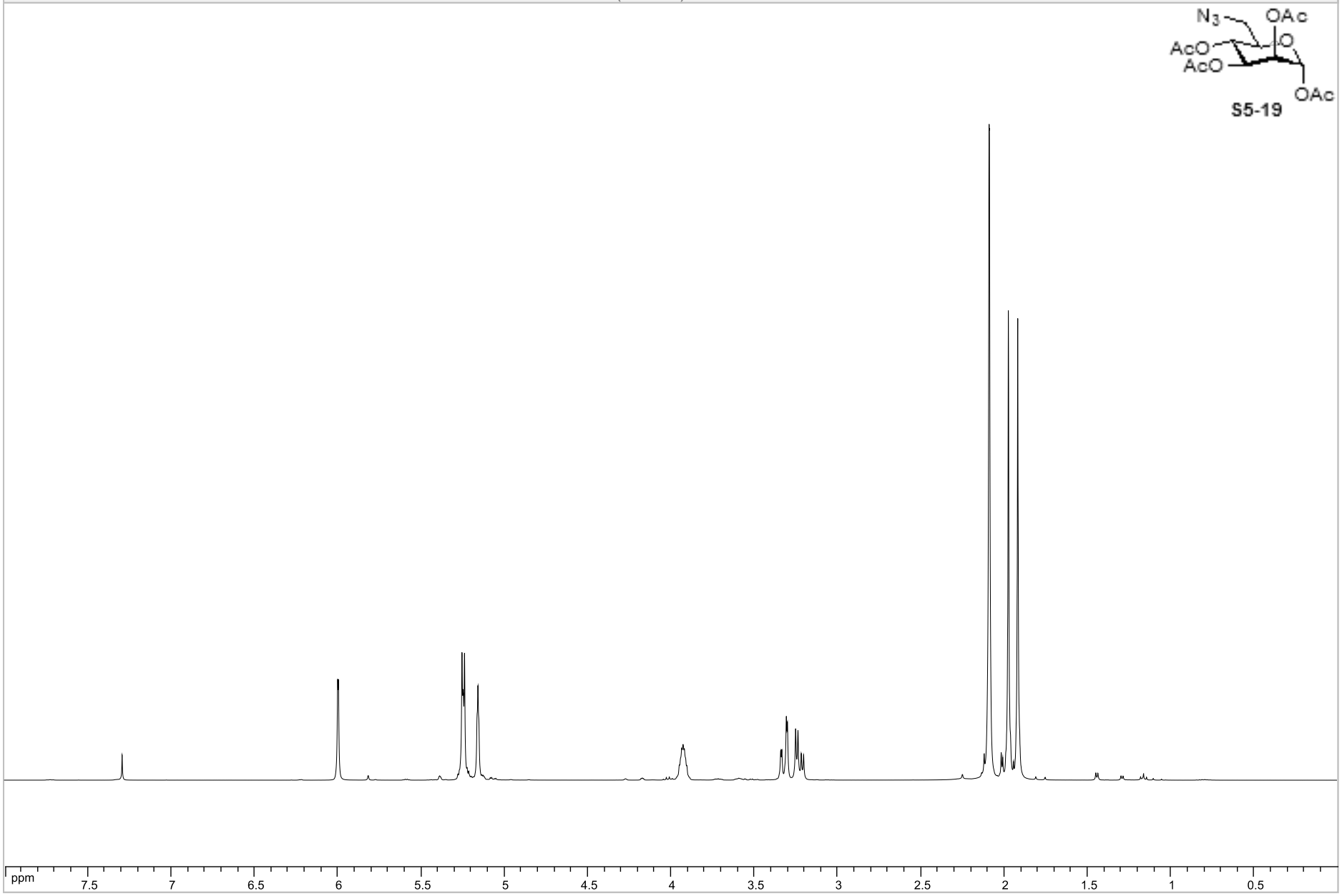
HKT3-224 3 (1D 1H) MeOD 400MHz

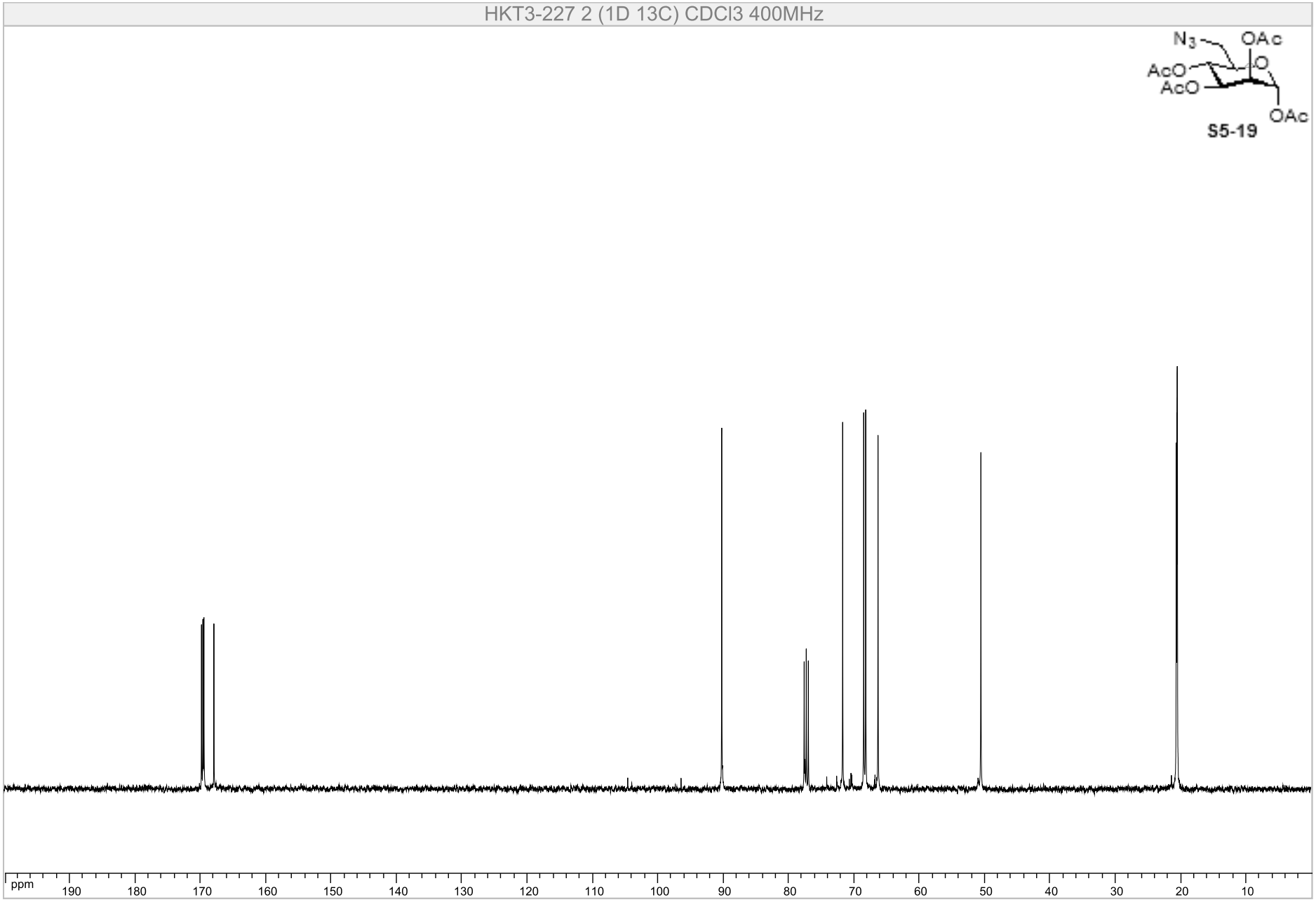


HKT3-224 4 (1D 13C) MeOD 400MHz

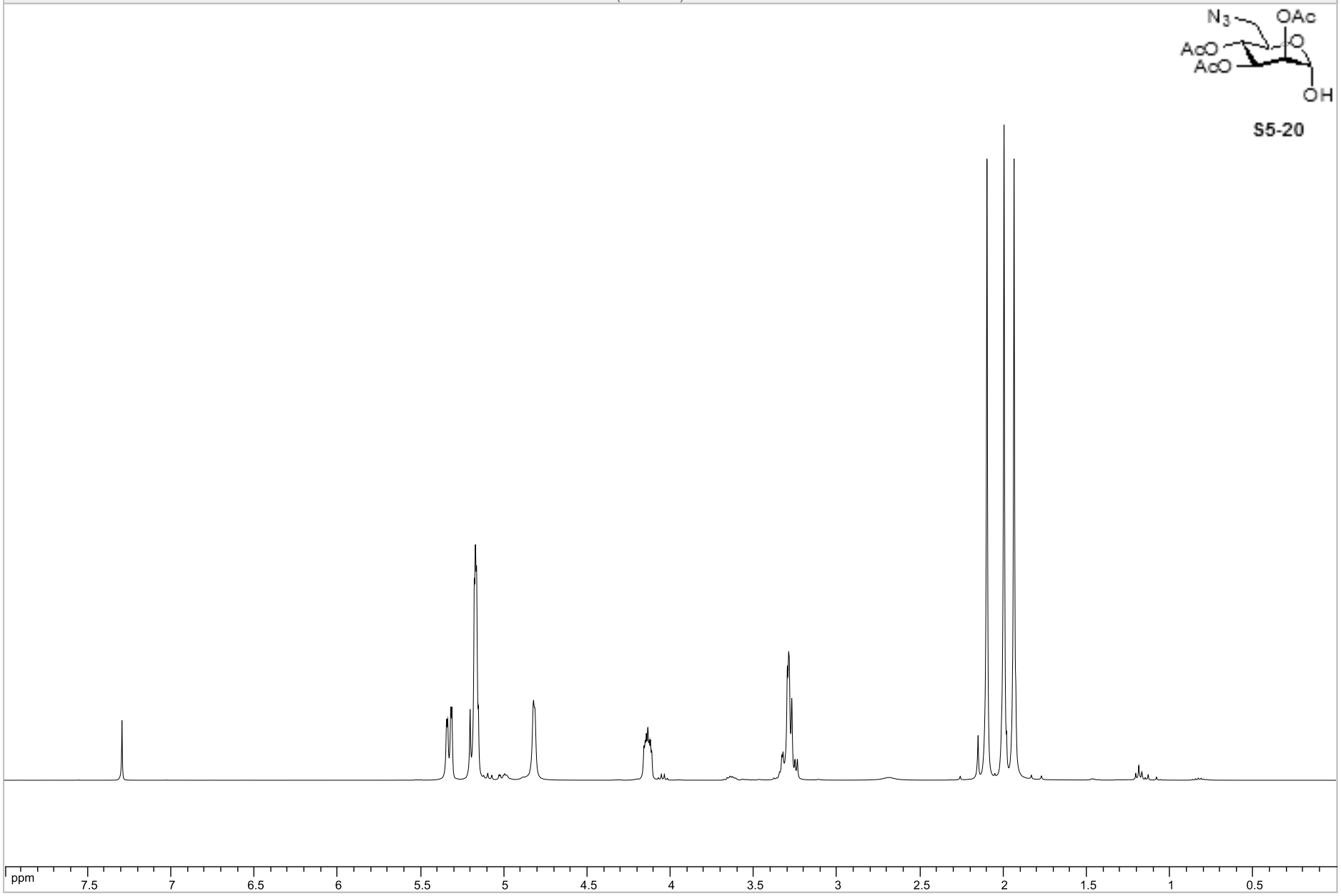


HKT3-227 1 (1D 1H) CDCl3 400MHz

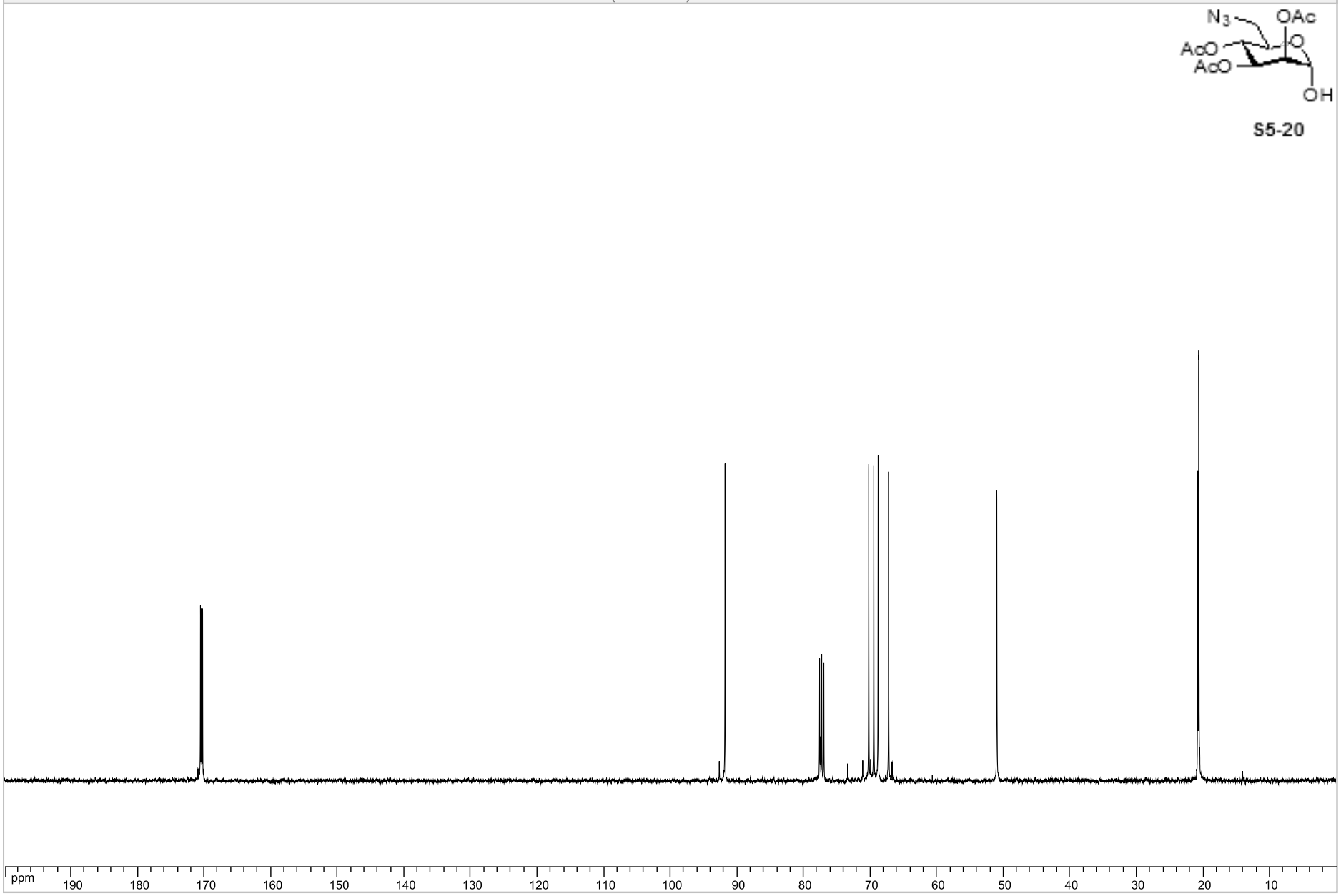


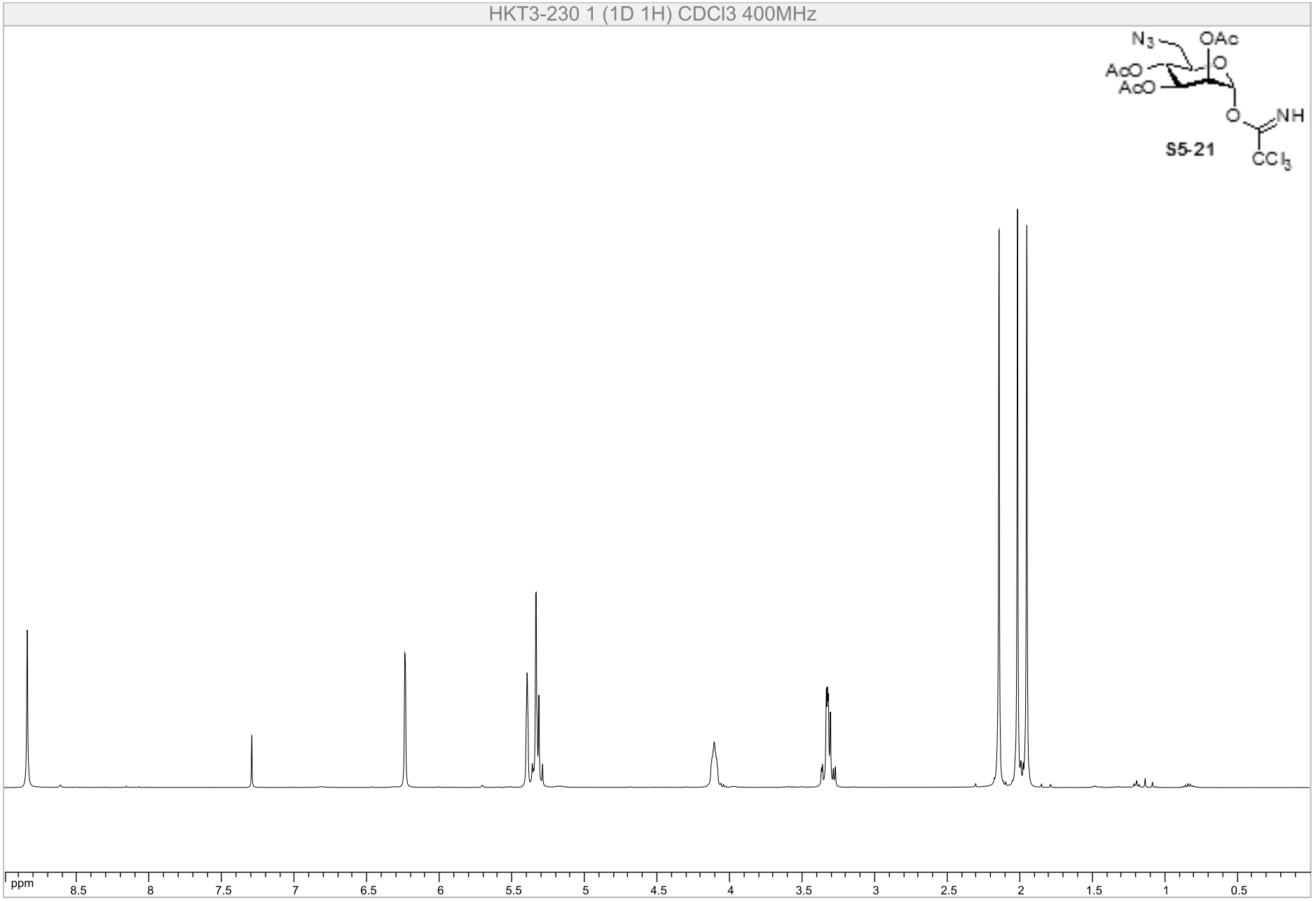

HKT3-227 2 (1D 13C) CDCl3 400MHz
N3
OAc
AcO
O
AcO
OAc
S5-19
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

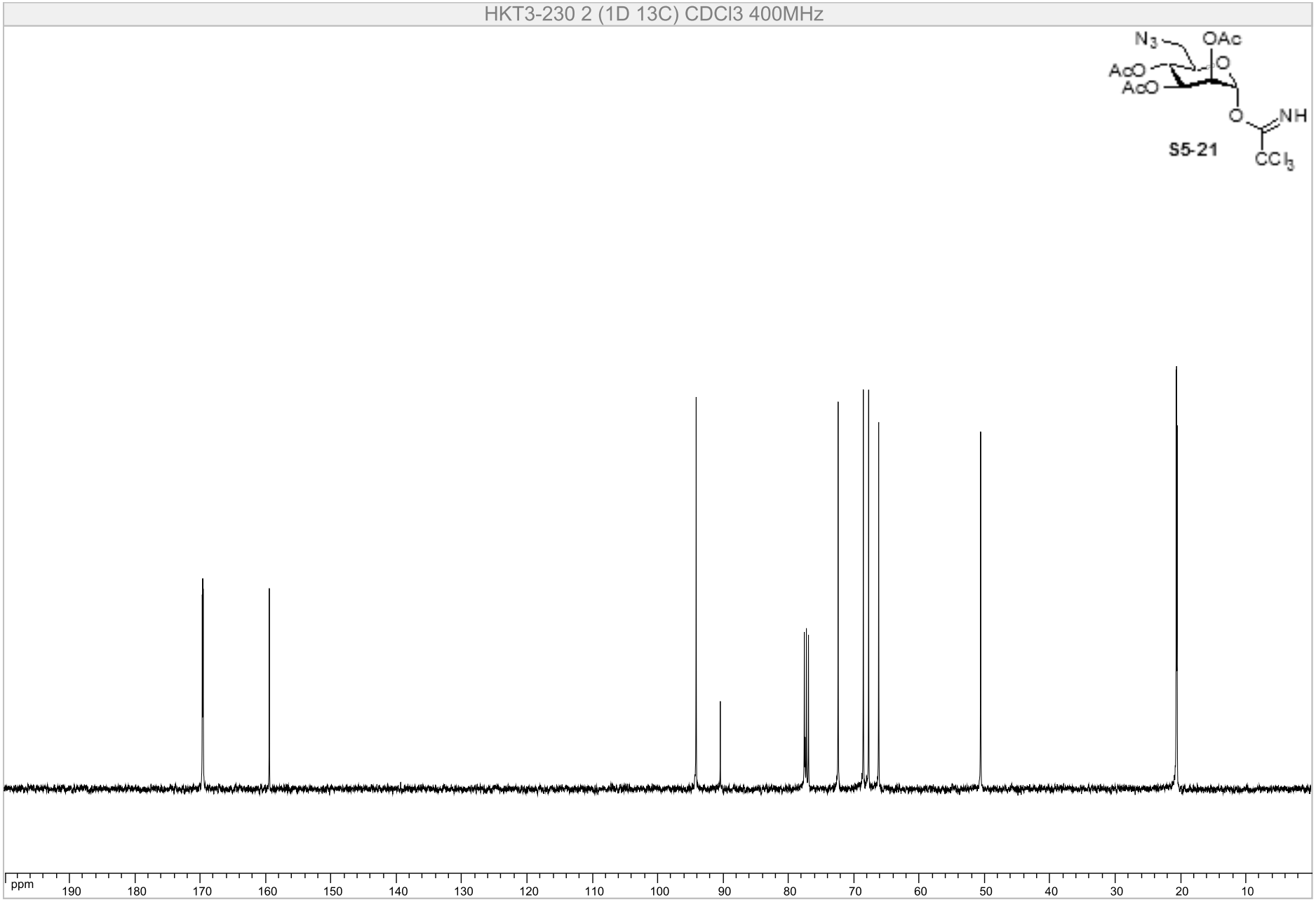
HKT3-228 2 (1D 1H) CDCl3 400MHz



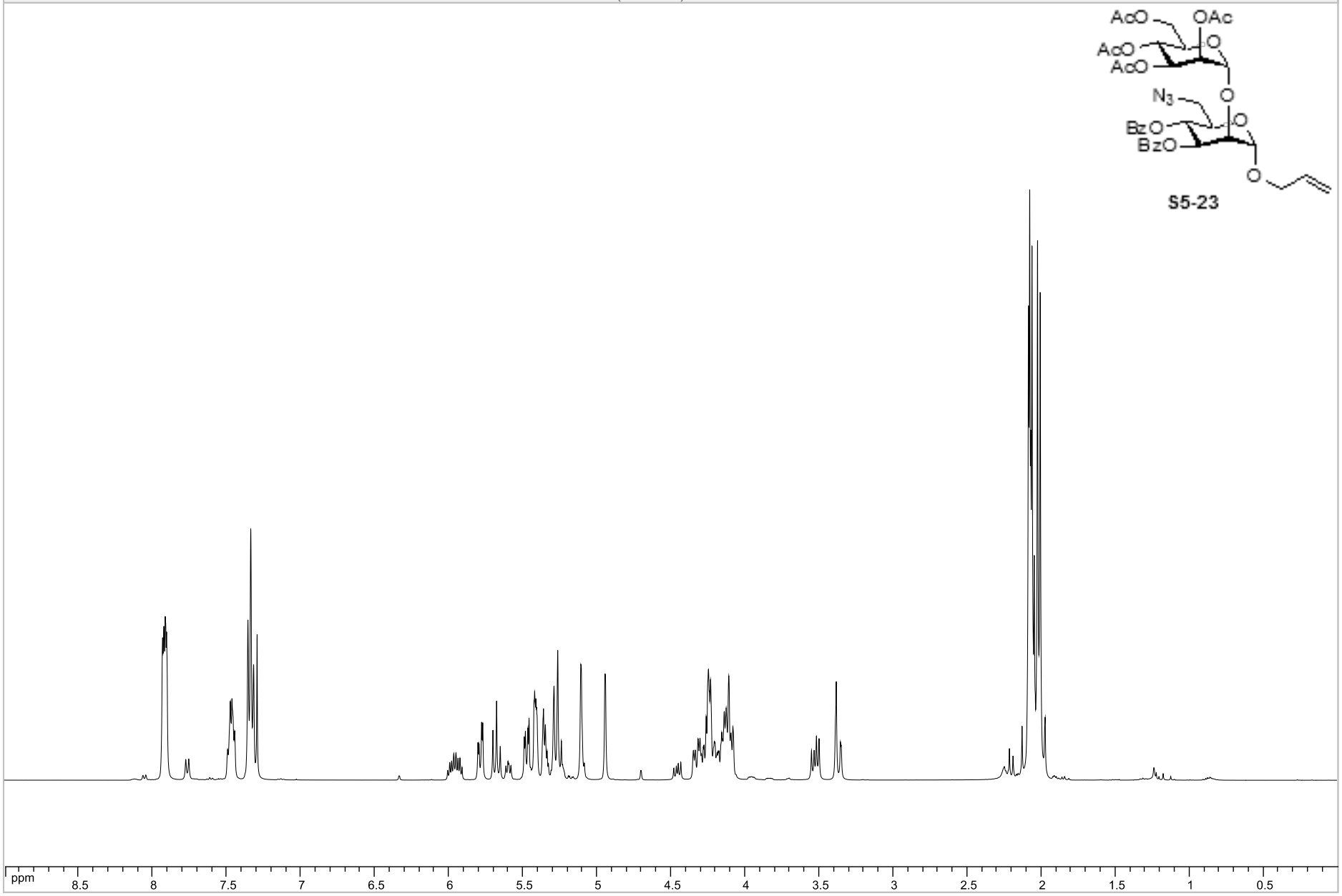
HKT3-228 3 (1D 13C) CDCl3 400MHz

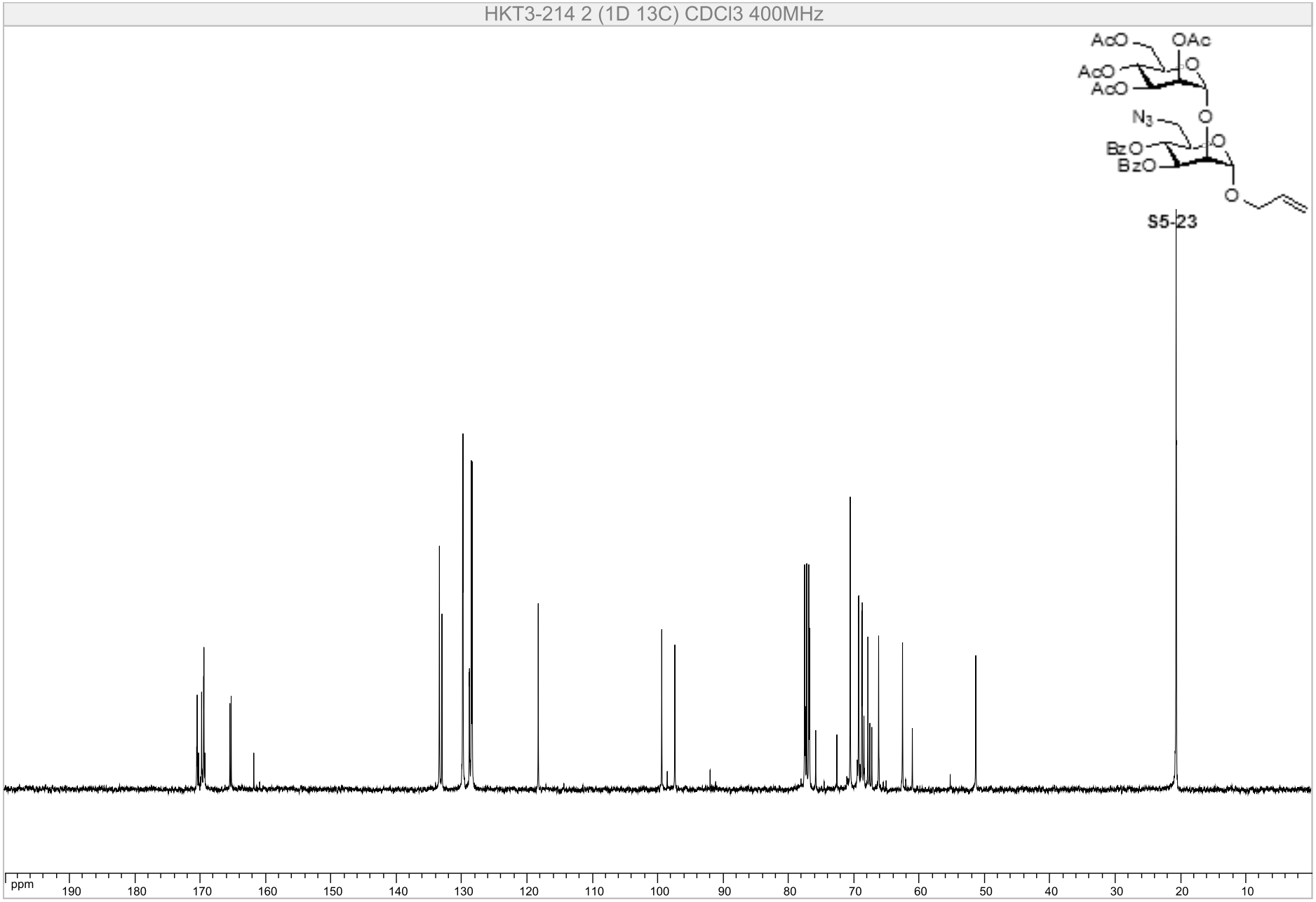



HKT3-230 1 (1D 1H) CDCl3 400MHz
N3
OAc
AcO
AcO
O
O
NH
CCl3
S5-21
ppm
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

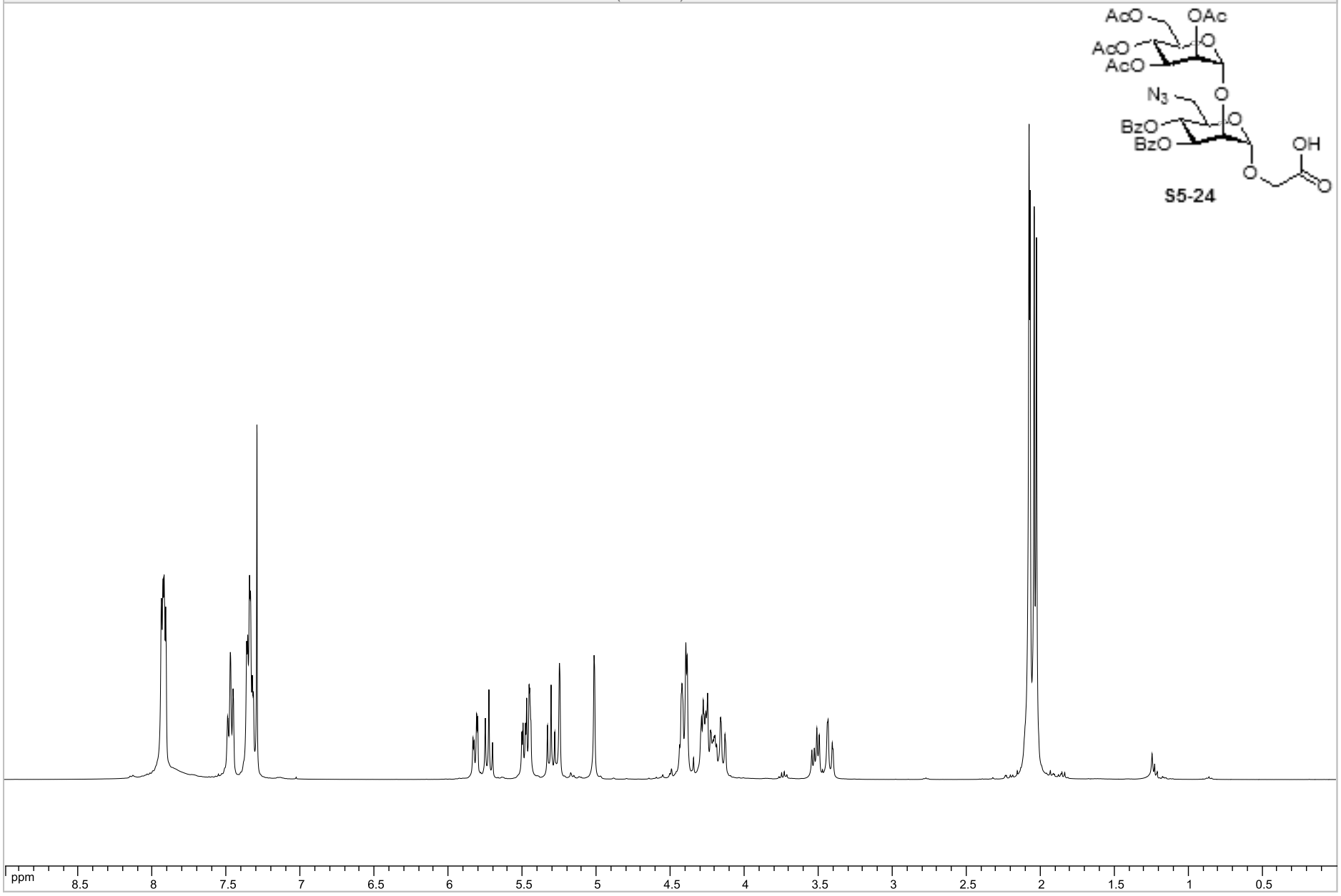

HKT3-230 2 (1D 13C) CDCl3 400MHz
N3
OAc
AcO
AcO
O
O
NH
S5-21
CCl3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

HKT3-214 1 (1D 1H) CDCl3 400MHz

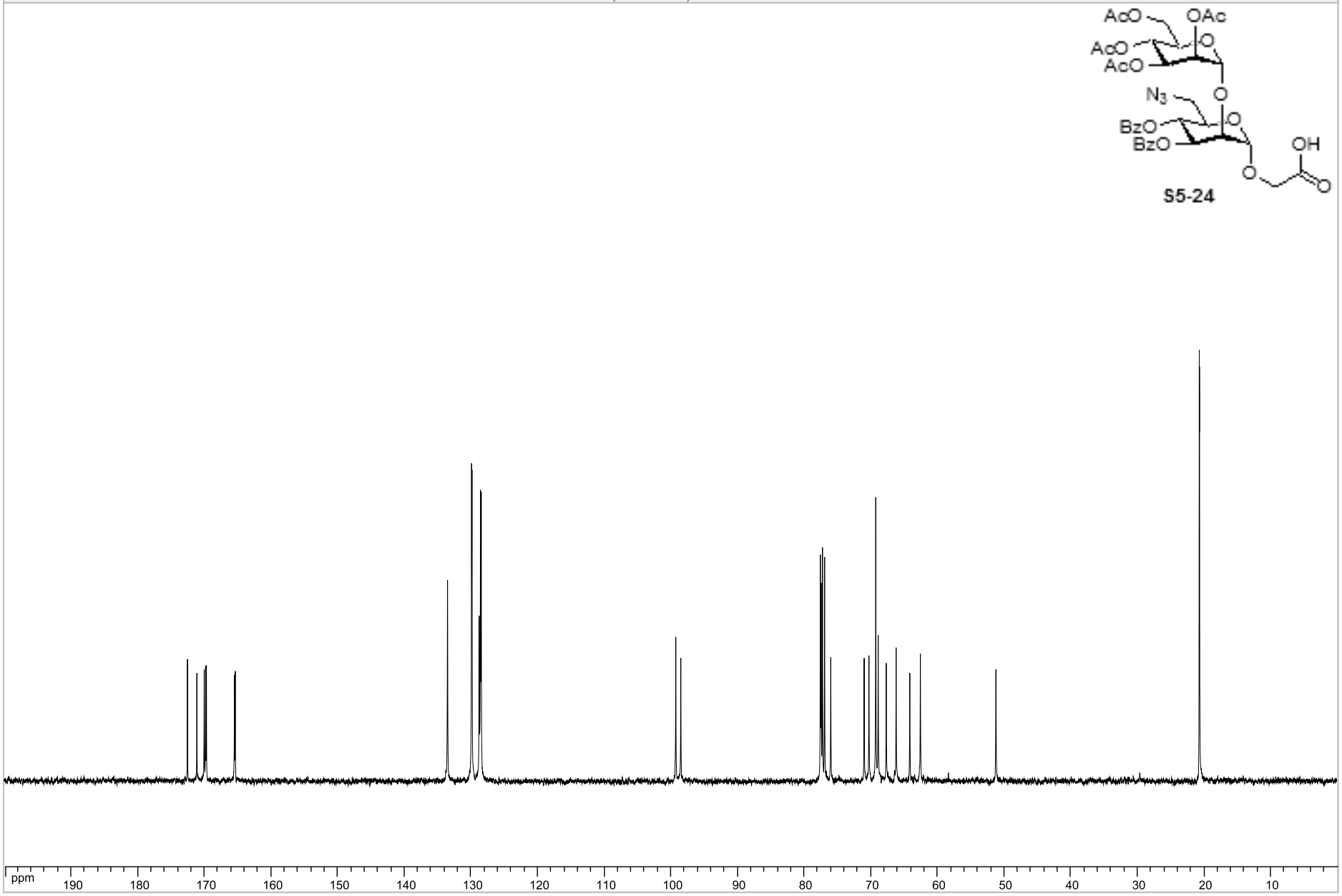



HKT3-214 2 (1D 13C) CDCl3 400MHz
AcO
OAc
AcO
AcO
O
N3
O
BzO
O
BzO
O
S5-23
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

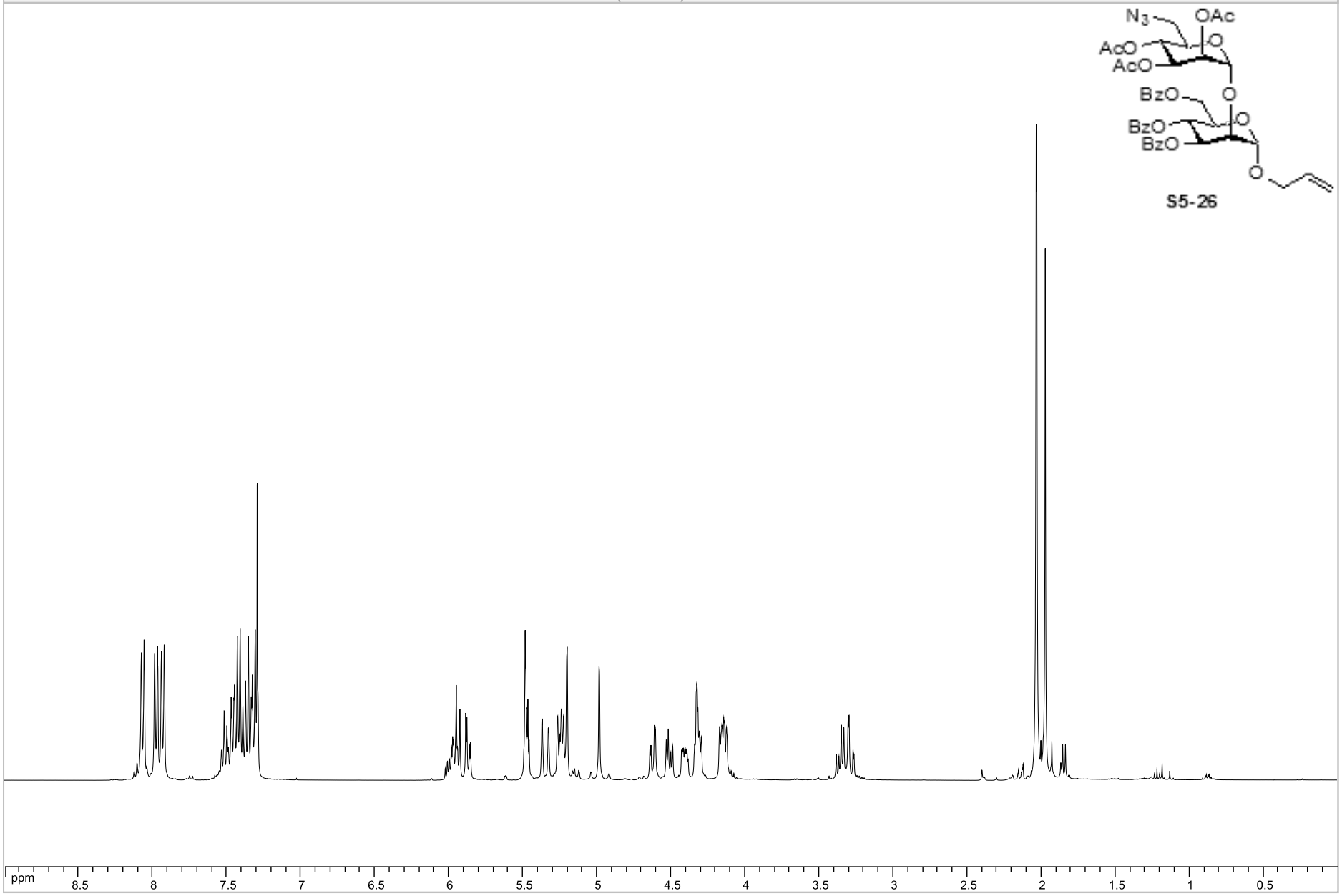
HKT3-245 1 (1D 1H) CDCl3 400MHz



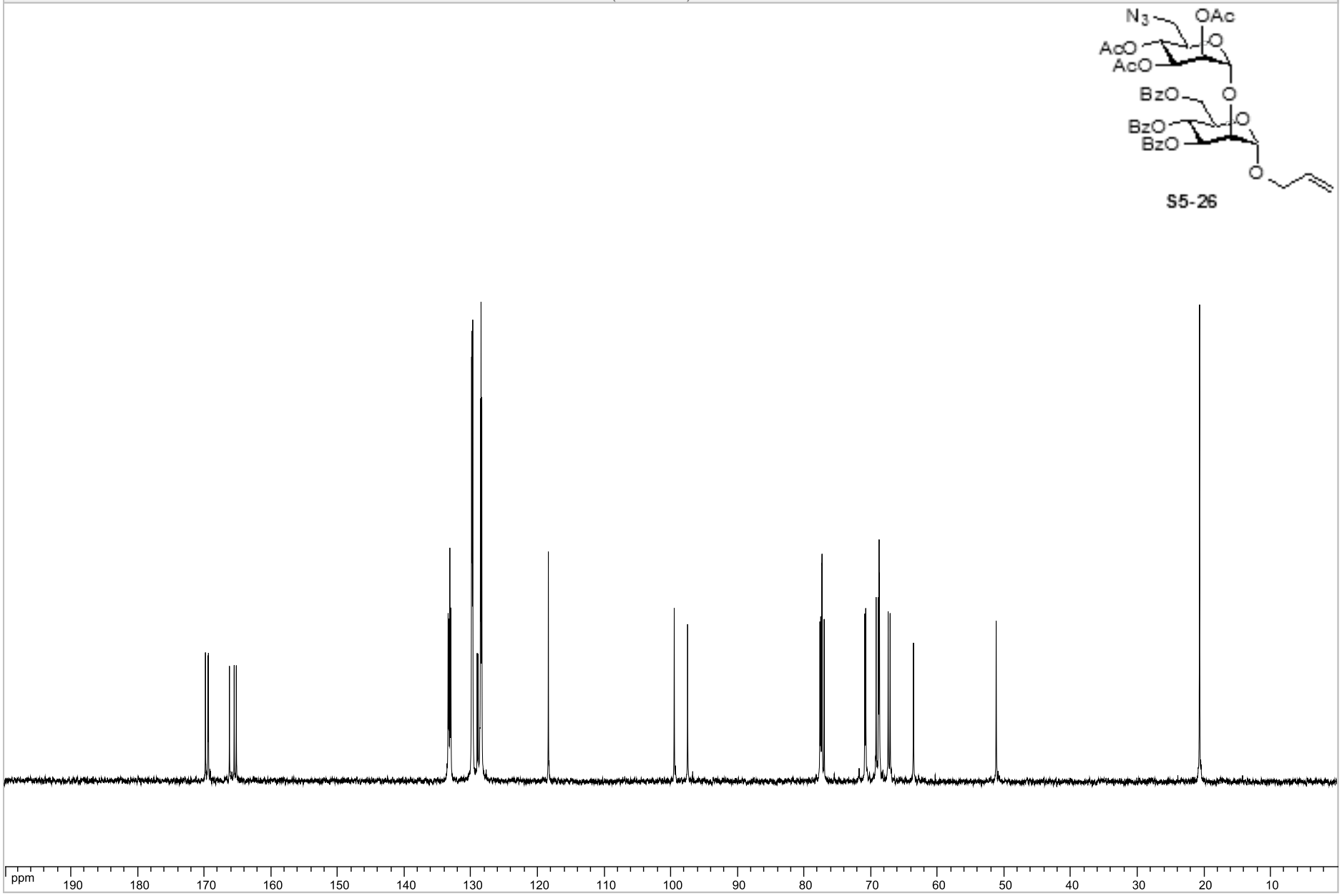
HKT3-245 4 (1D 13C) CDCl3 400MHz



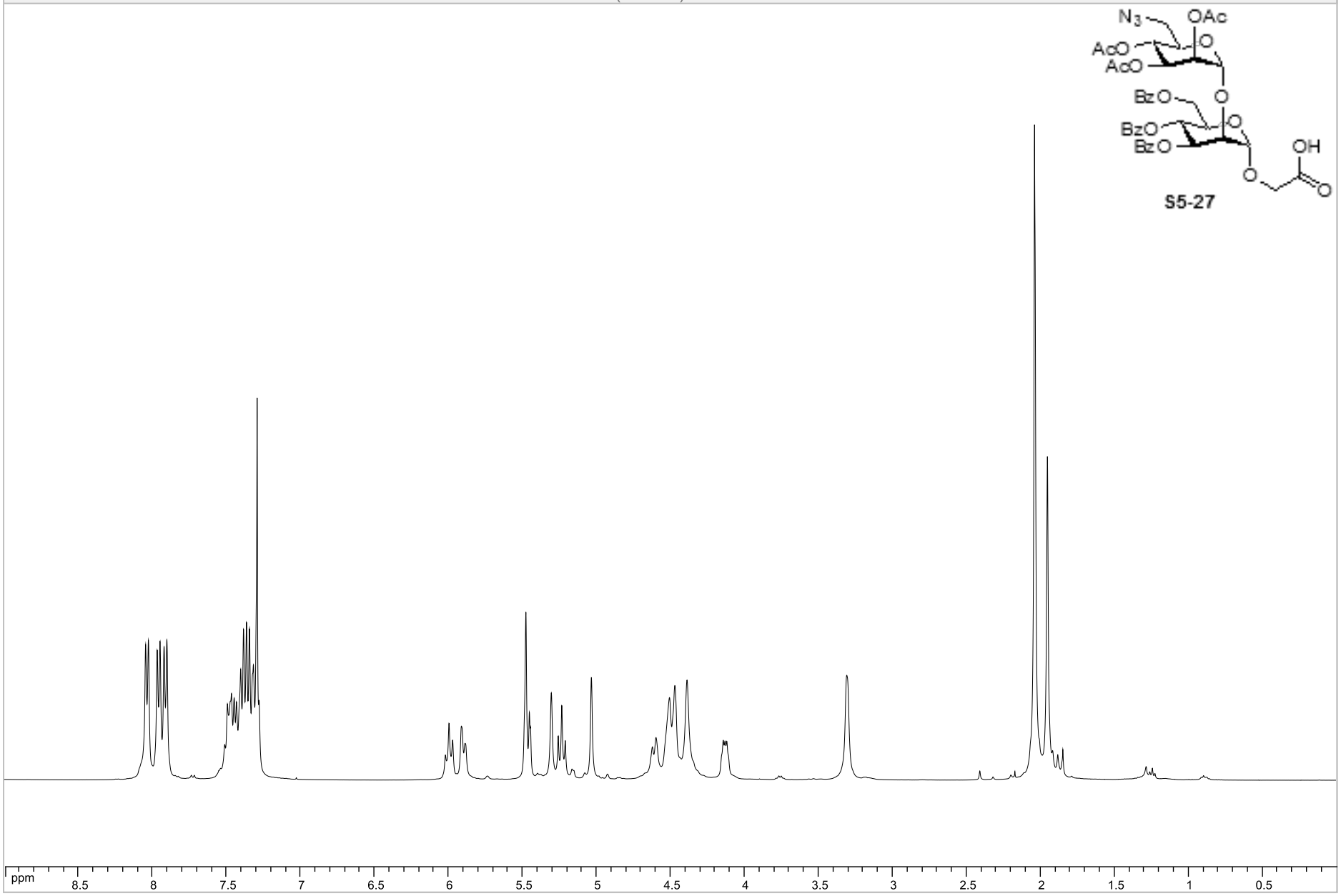
HKT3-267 2 (1D 1H) CDCl3 400MHz



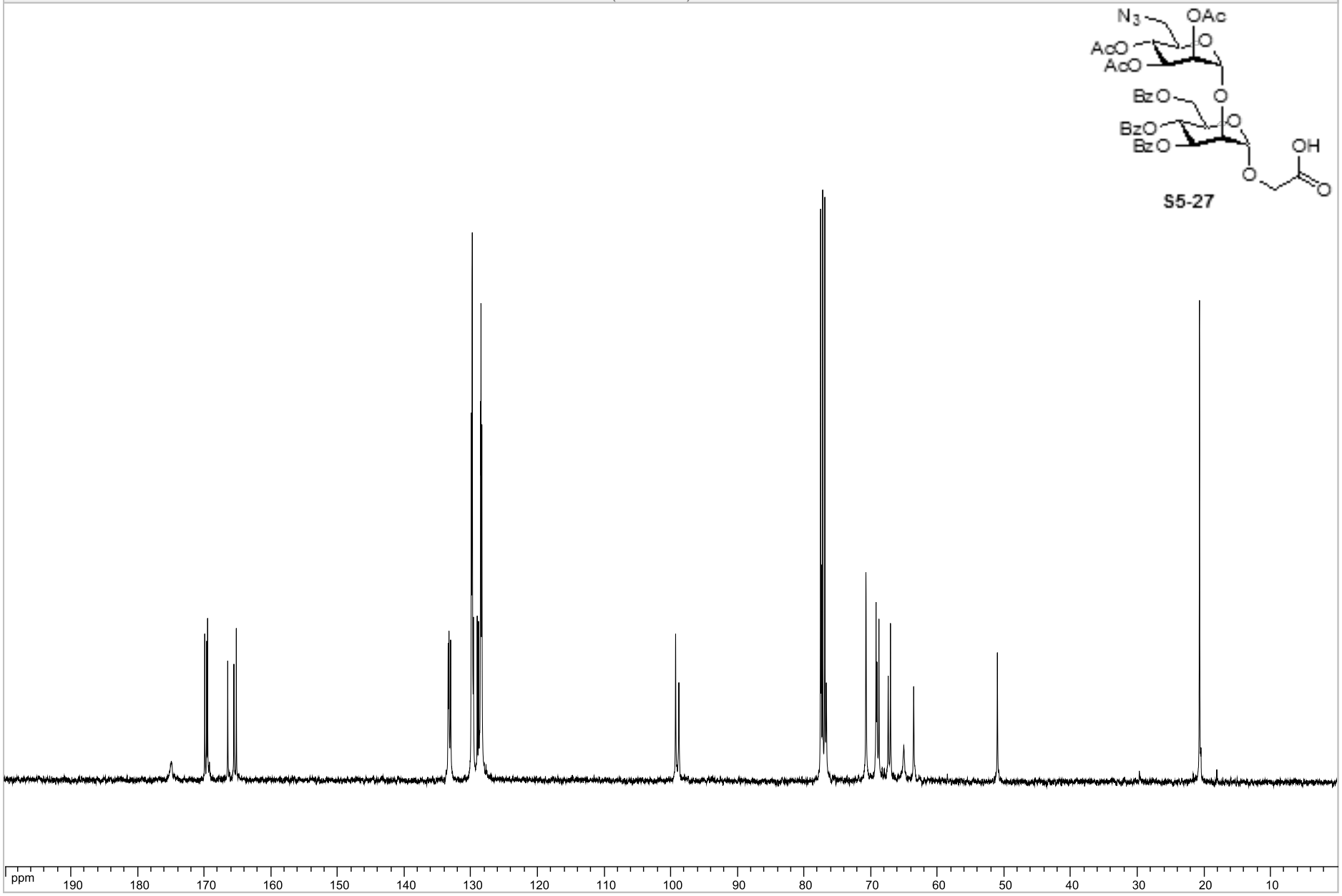
HKT3-267 3 (1D 13C) CDCl3 400MHz



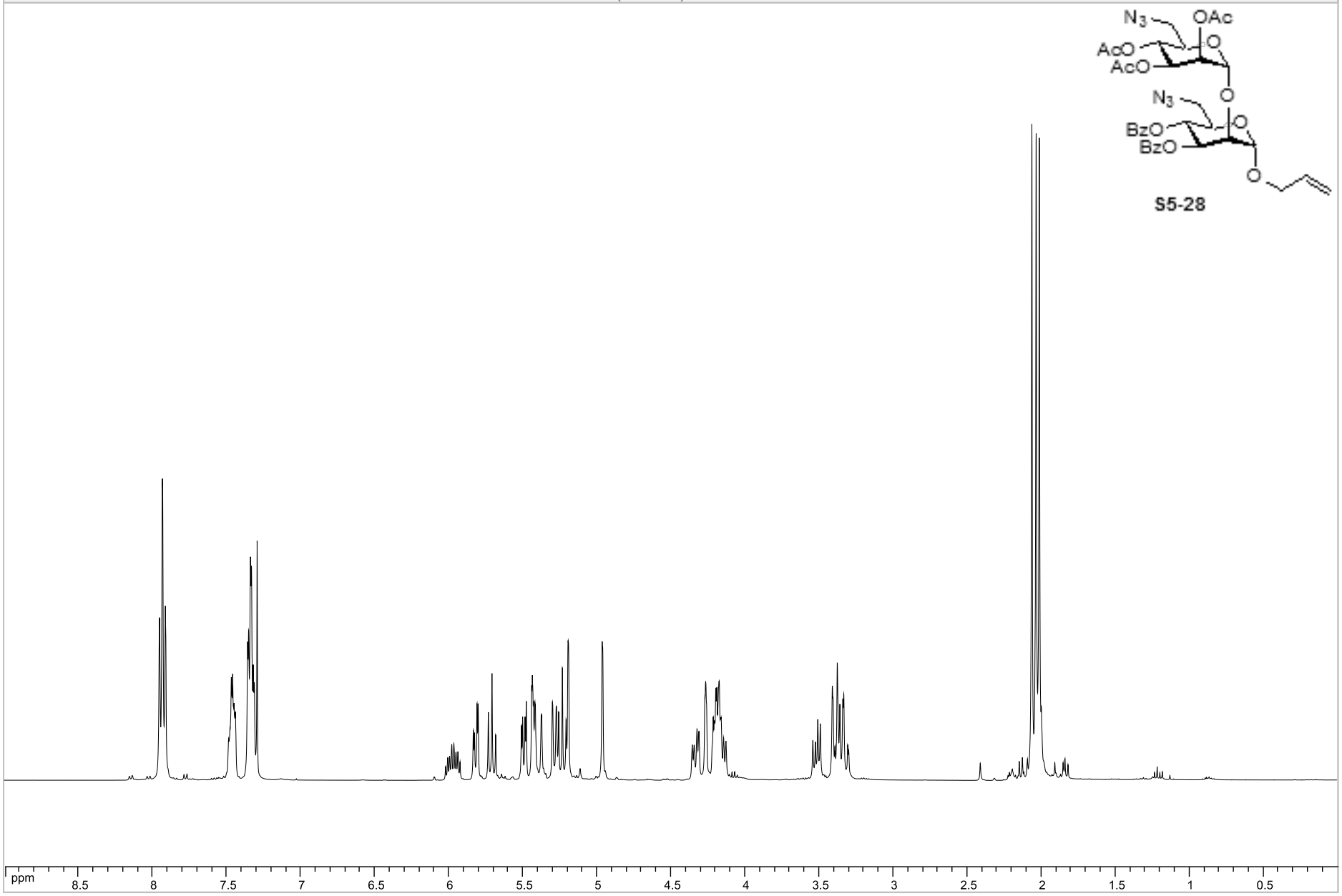
HKT3-269 2 (1D 1H) CDCl3 400MHz



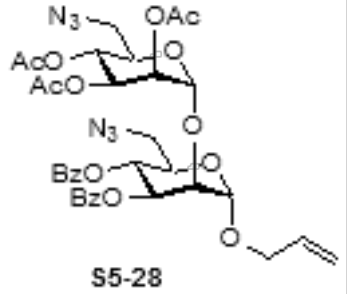
HKT3-269 3 (1D 13C) CDCl3 400MHz



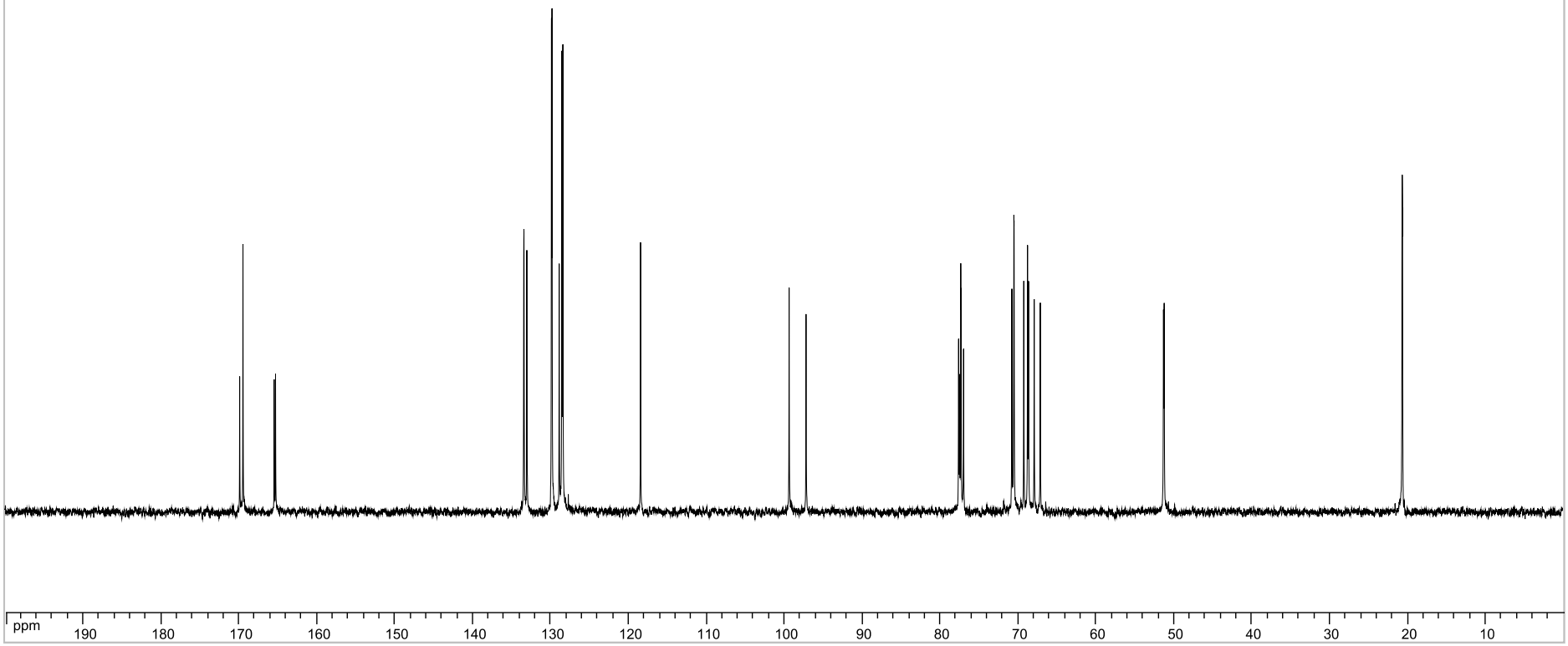
HKT3-285 1 (1D 1H) CDCl3 400MHz



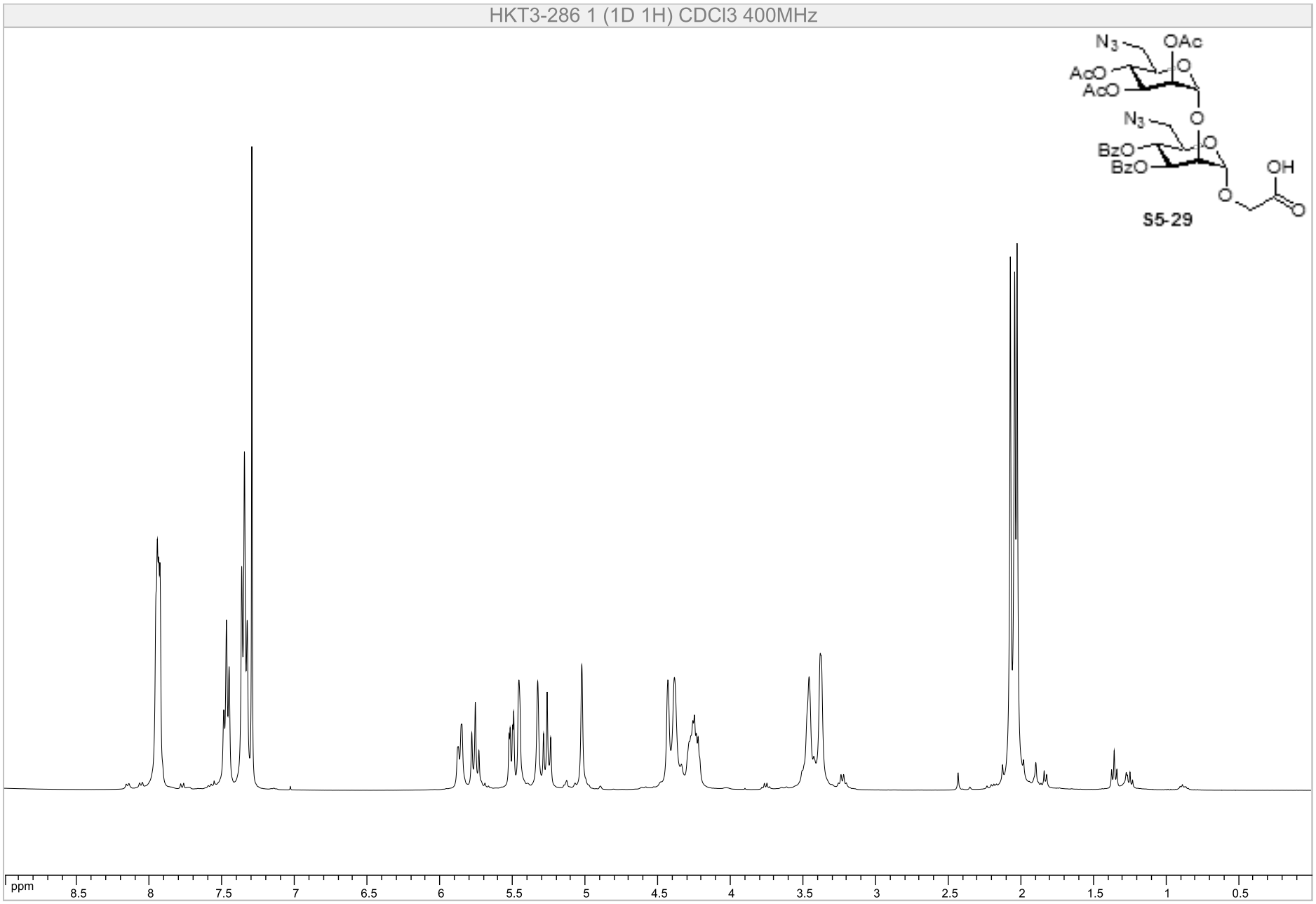
HKT3-285 2 (1D 13C) CDCl3 400MHz



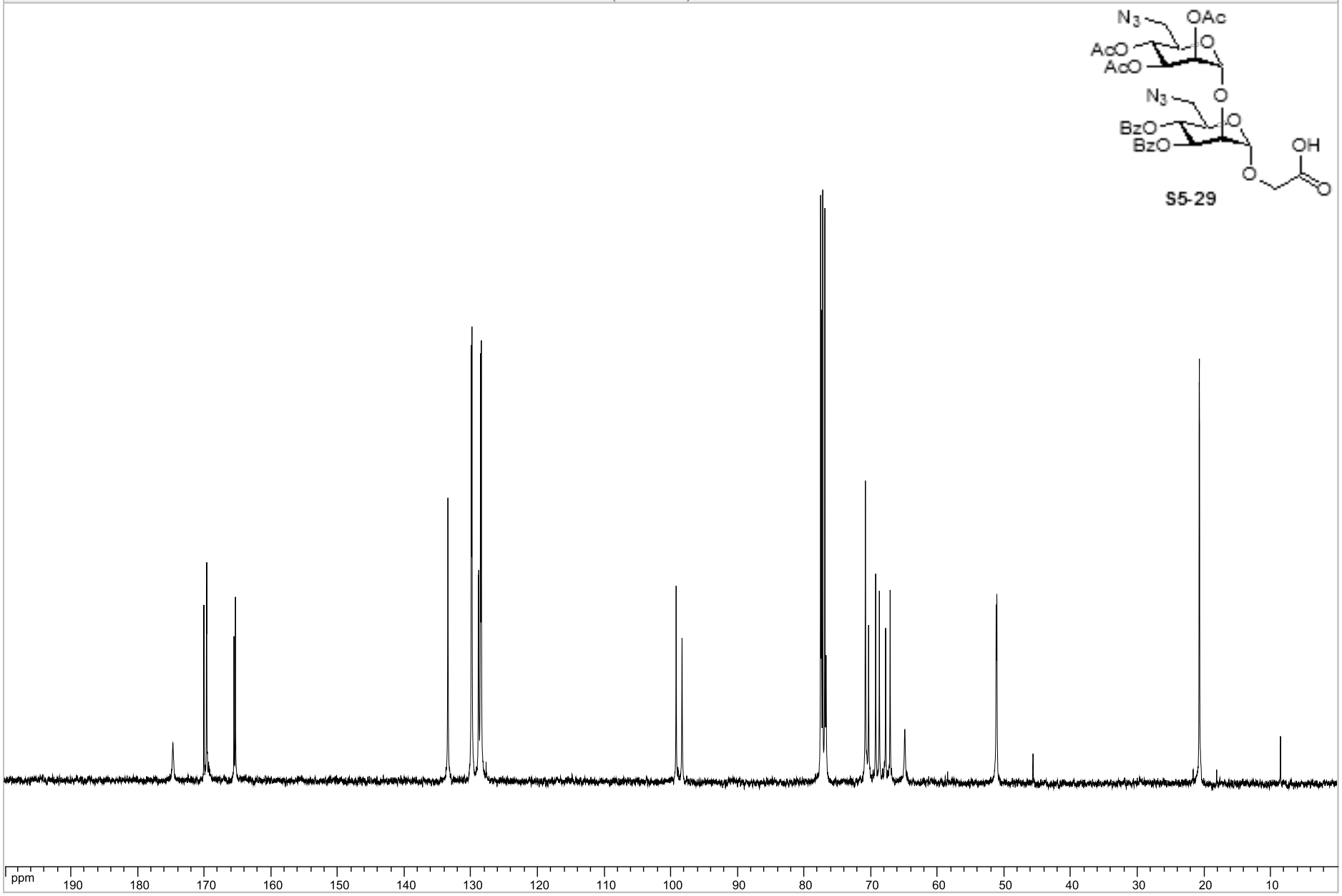
S5-28



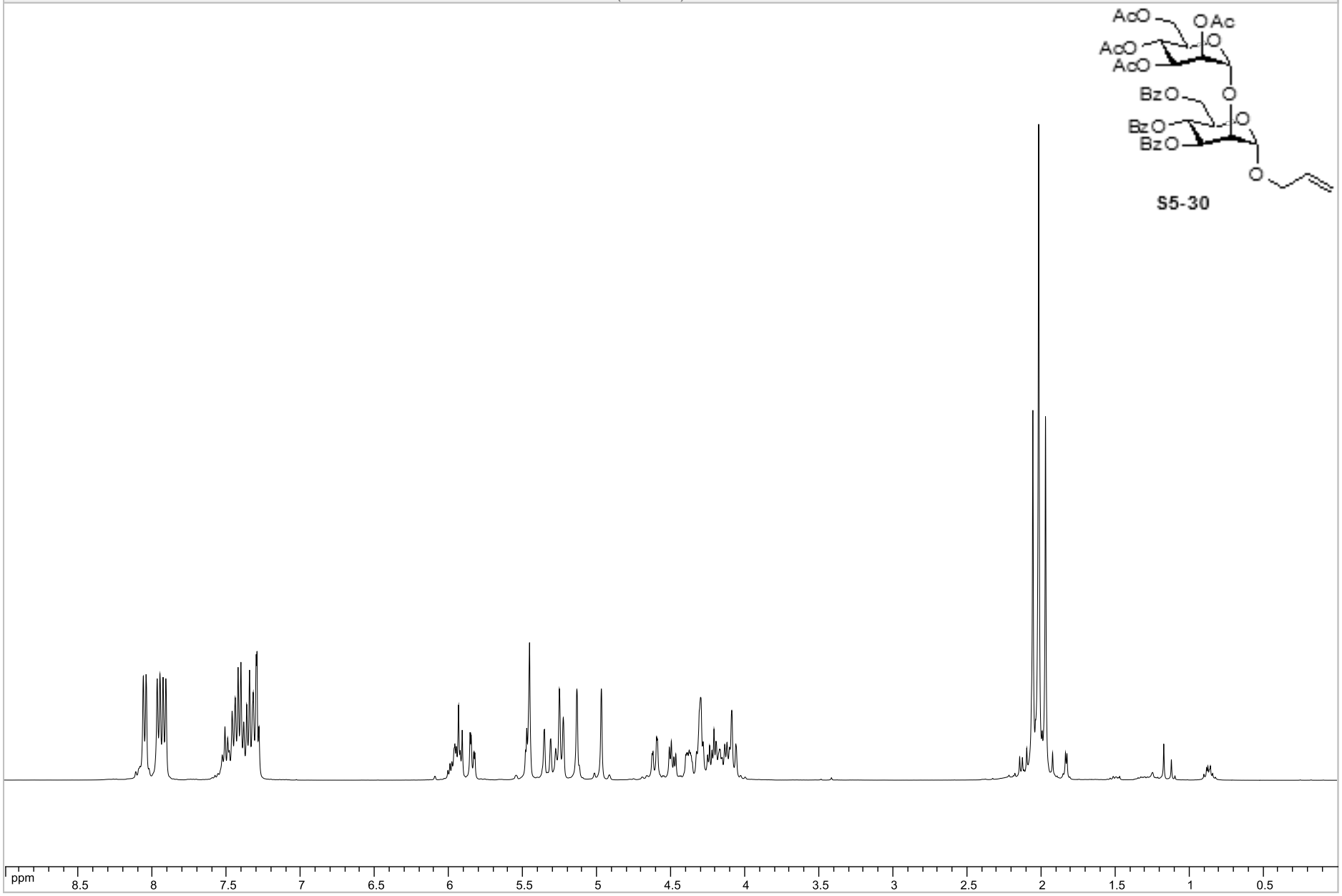
HKT3-286 1 (1D 1H) CDCl3 400MHz



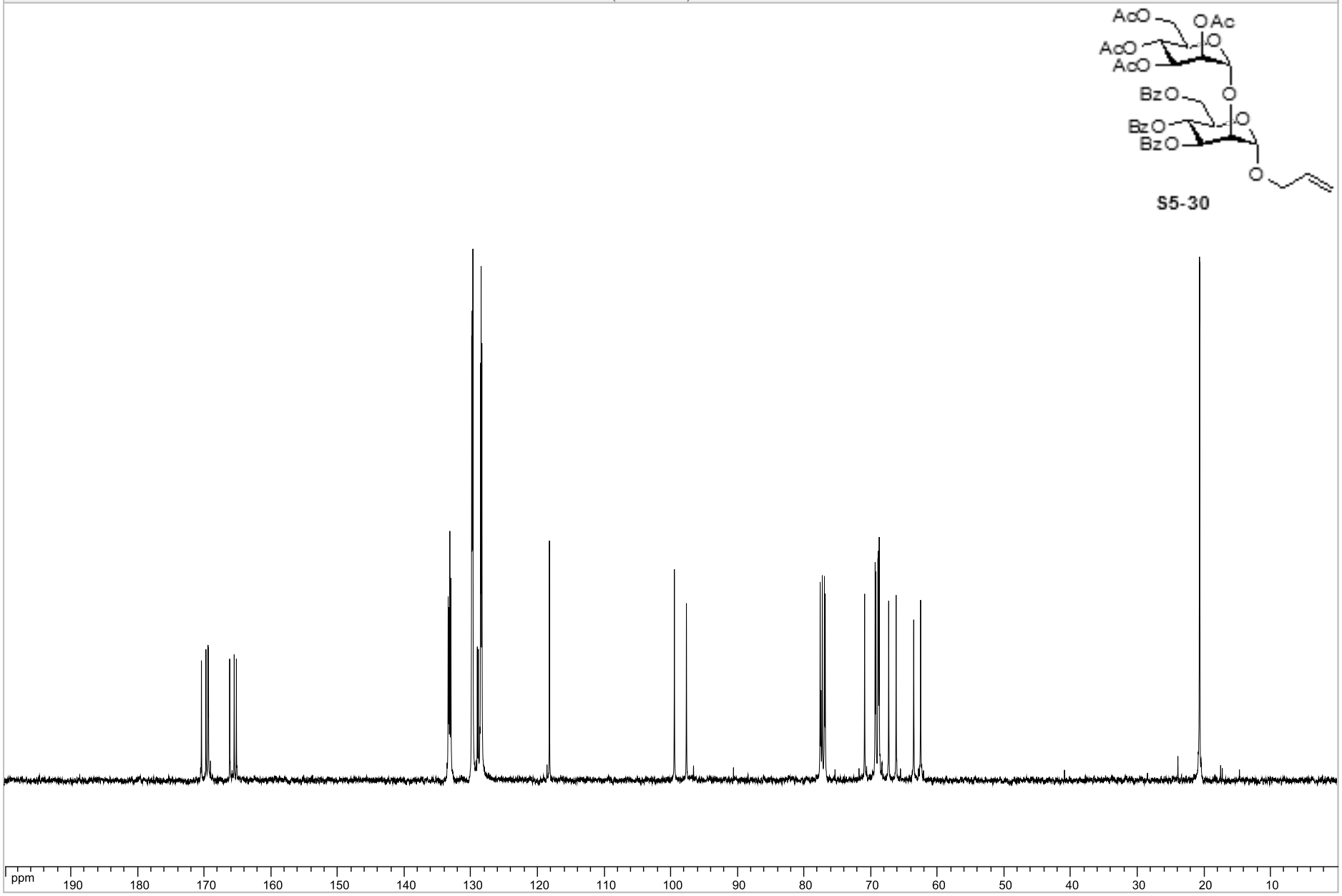
HKT3-286 2 (1D 13C) CDCl3 400MHz



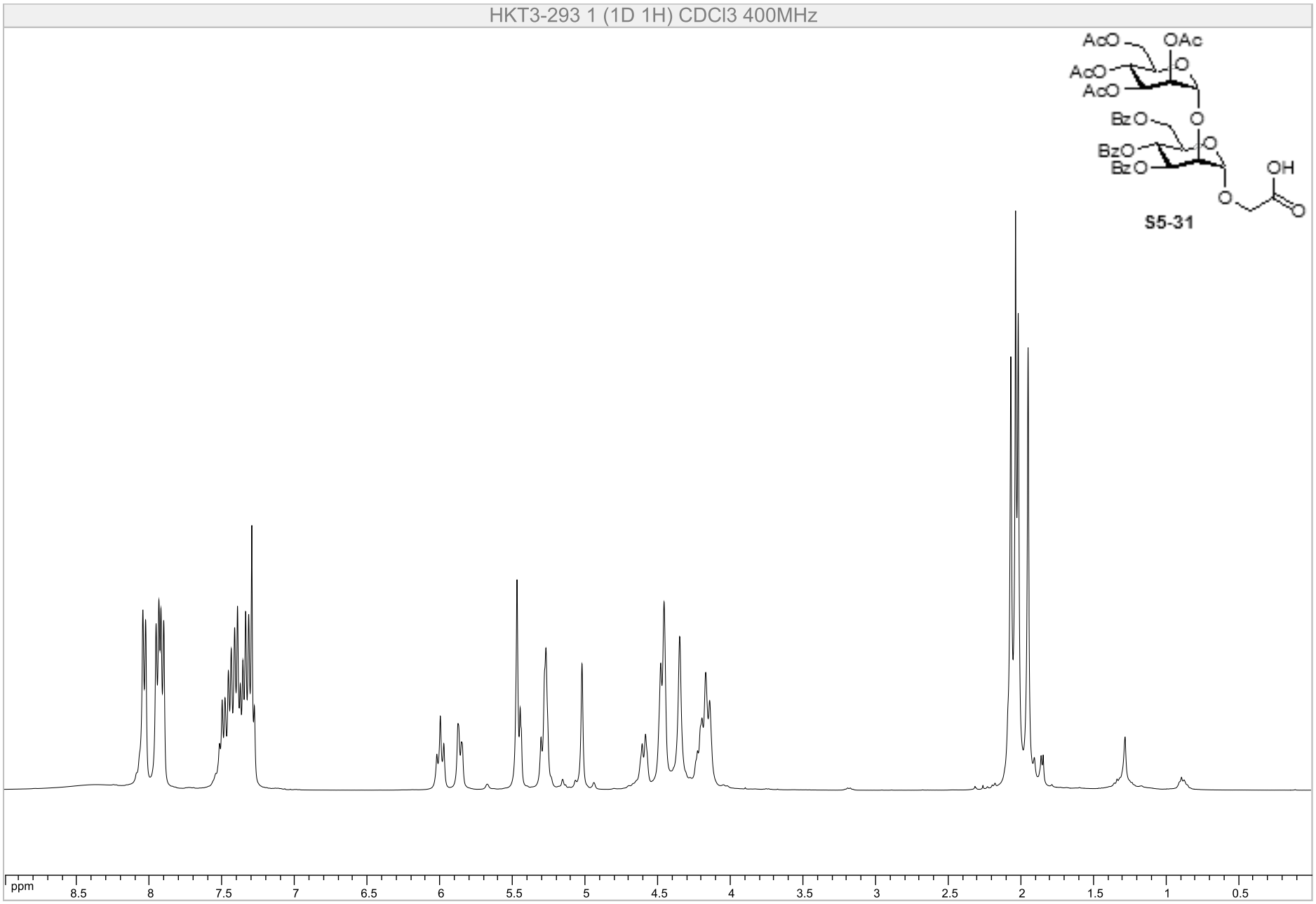
HKT3-292 1 (1D 1H) CDCl3 400MHz



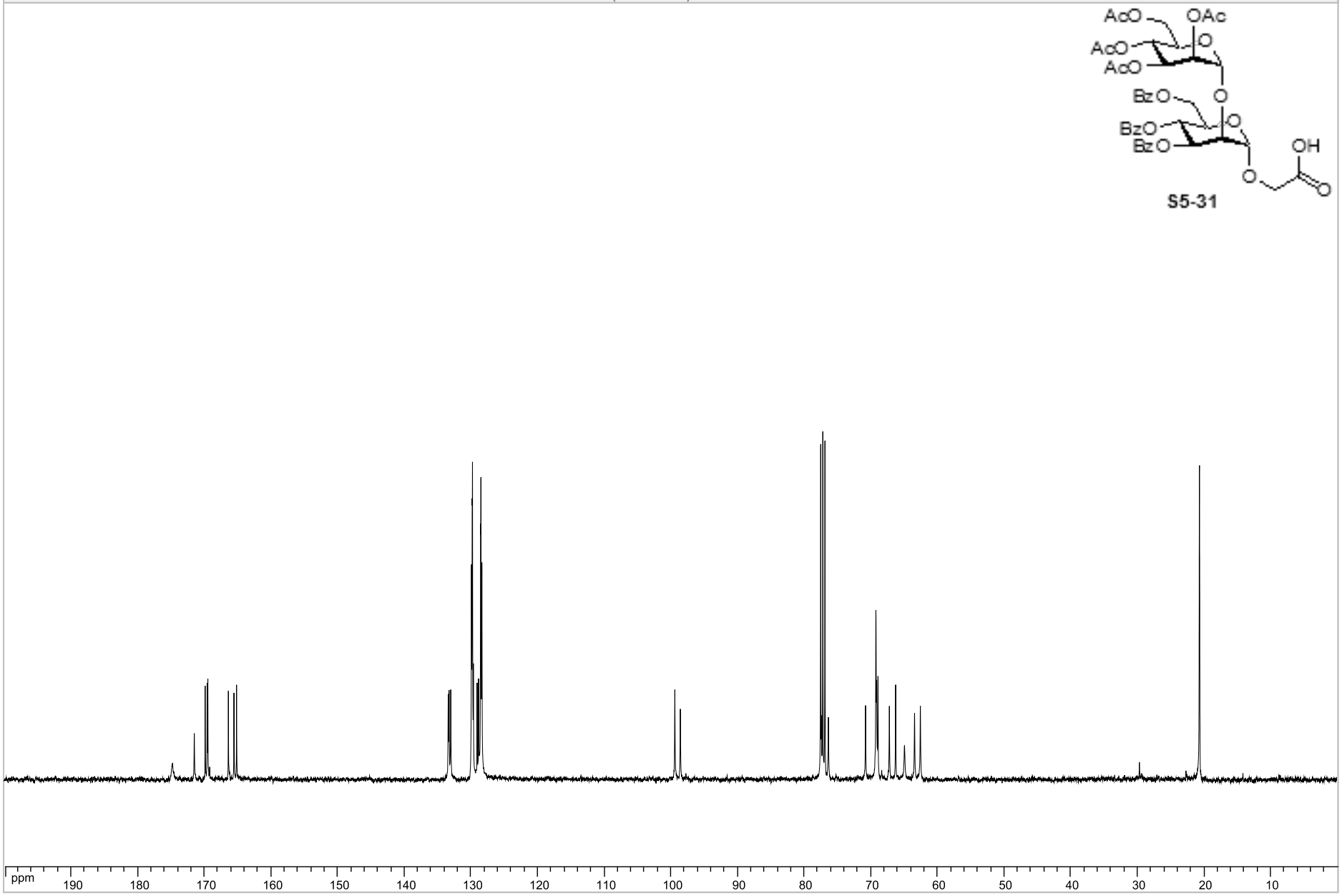
HKT3-292 2 (1D 13C) CDCl3 400MHz

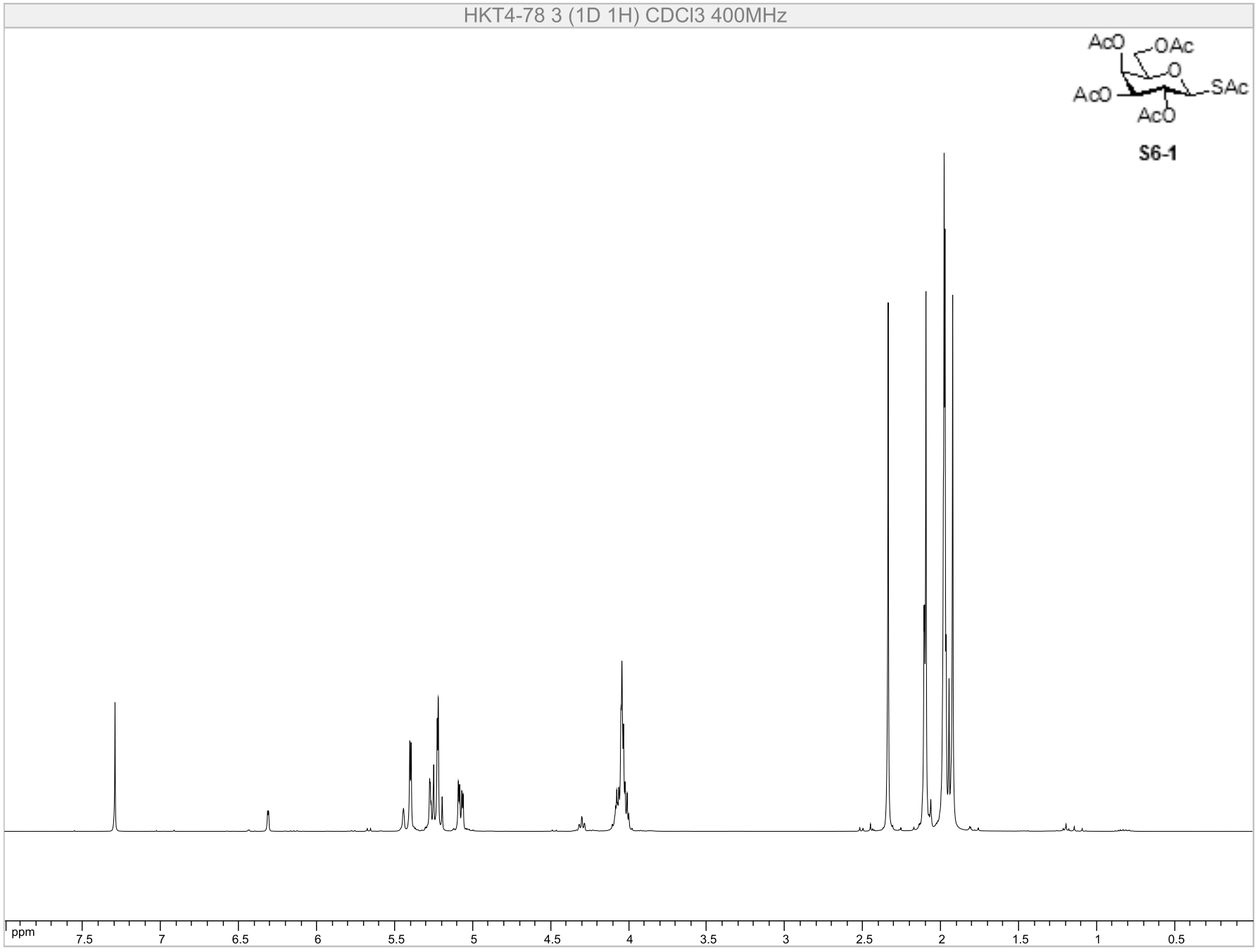


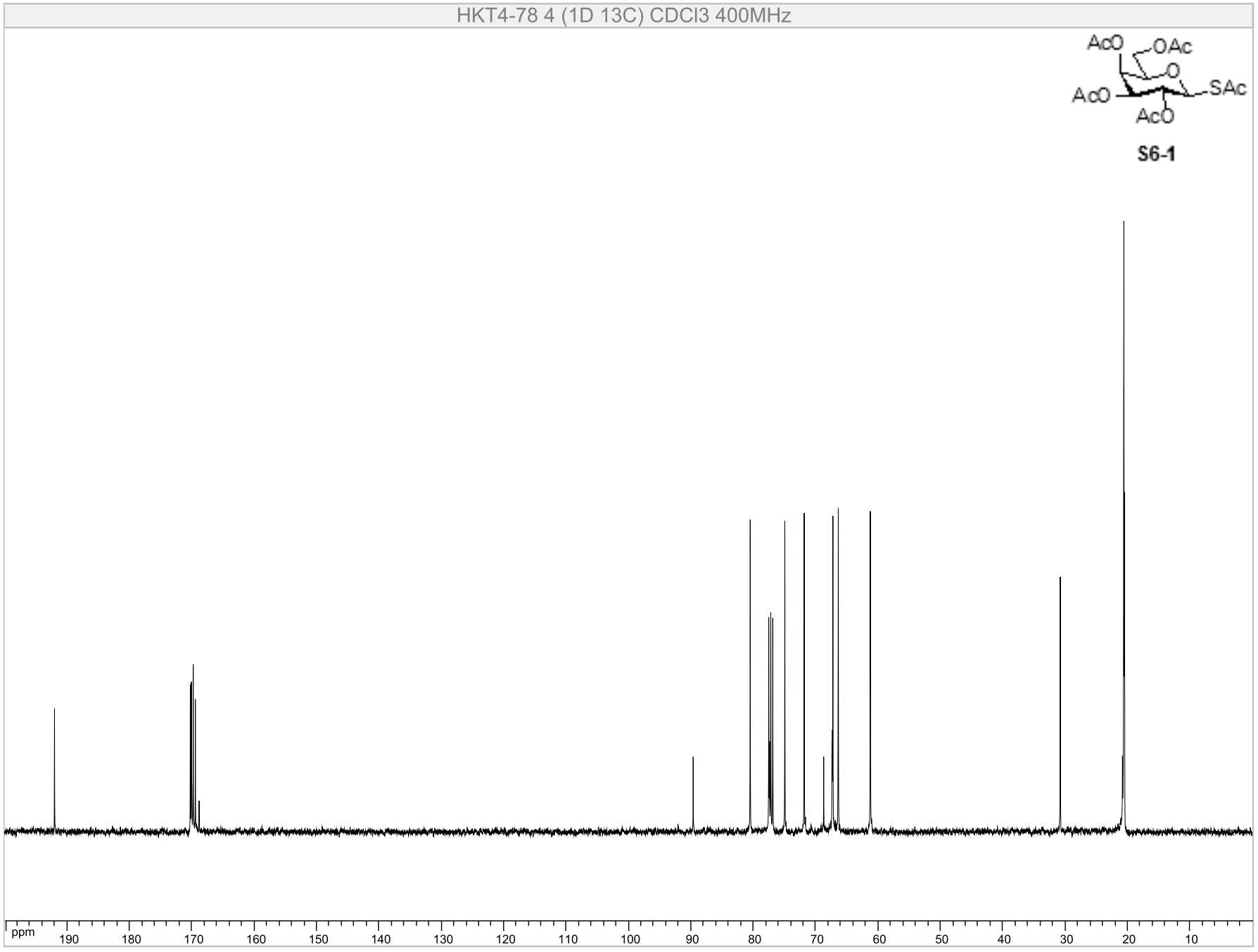
HKT3-293 1 (1D 1H) CDCl3 400MHz

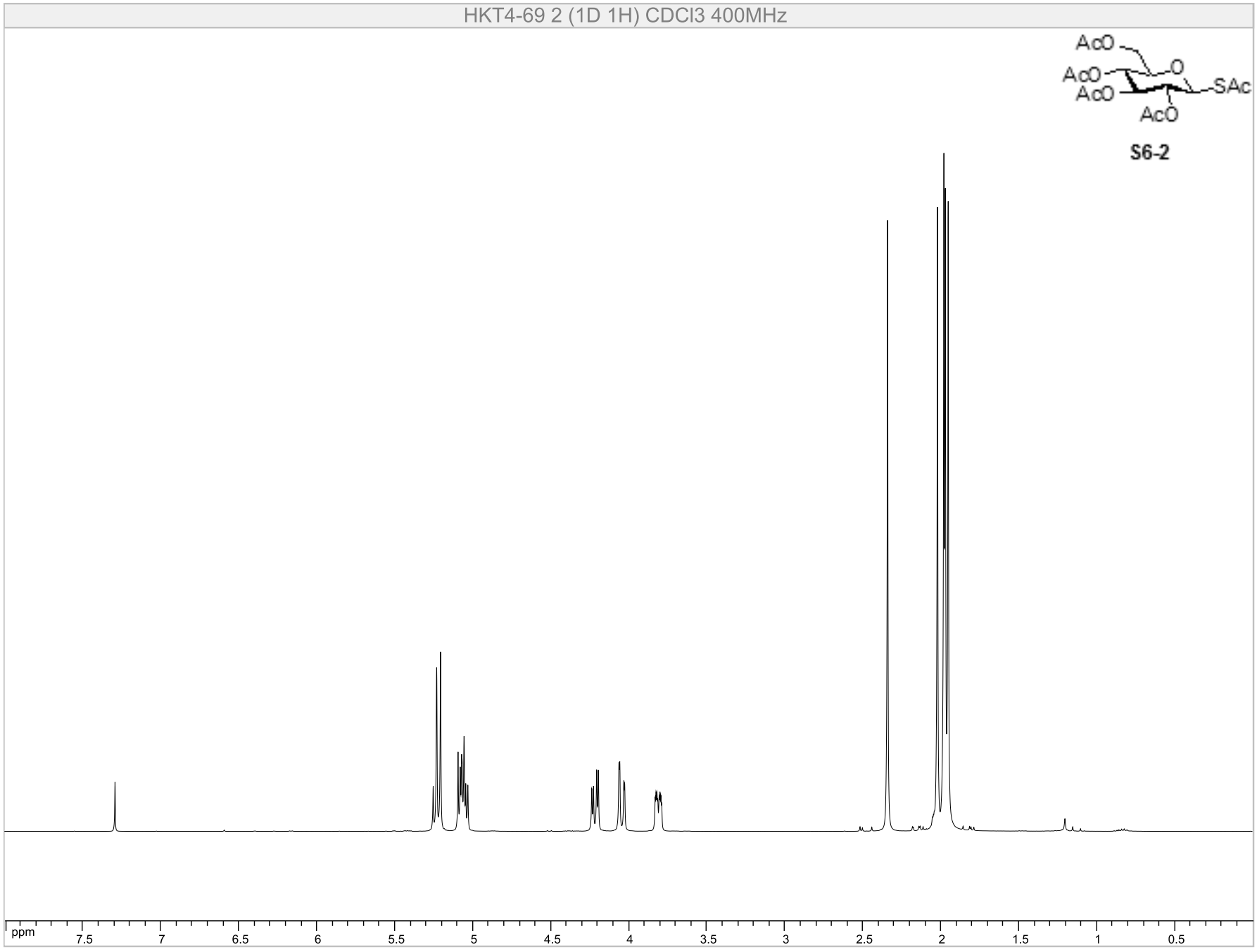


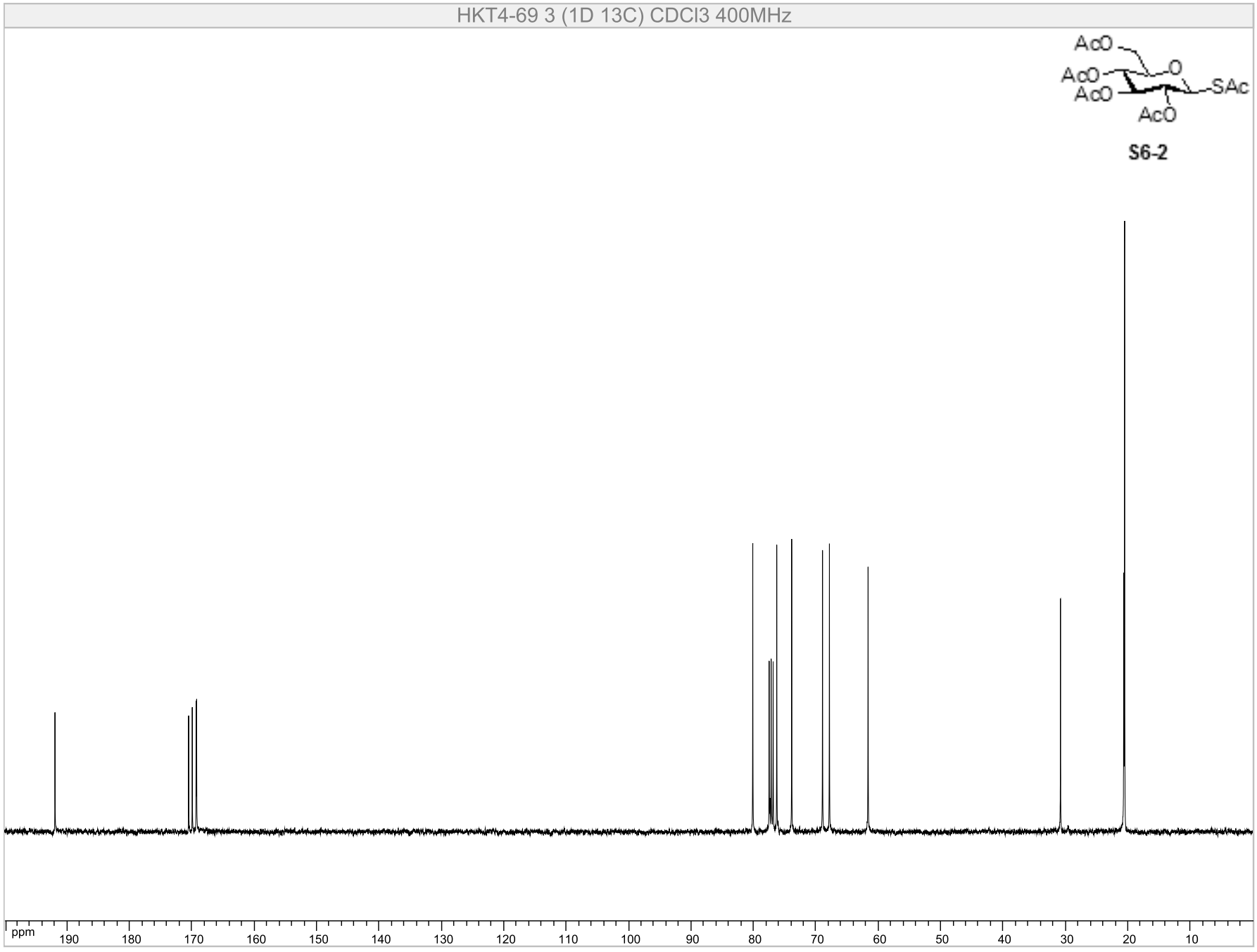
HKT3-293 2 (1D 13C) CDCl3 400MHz

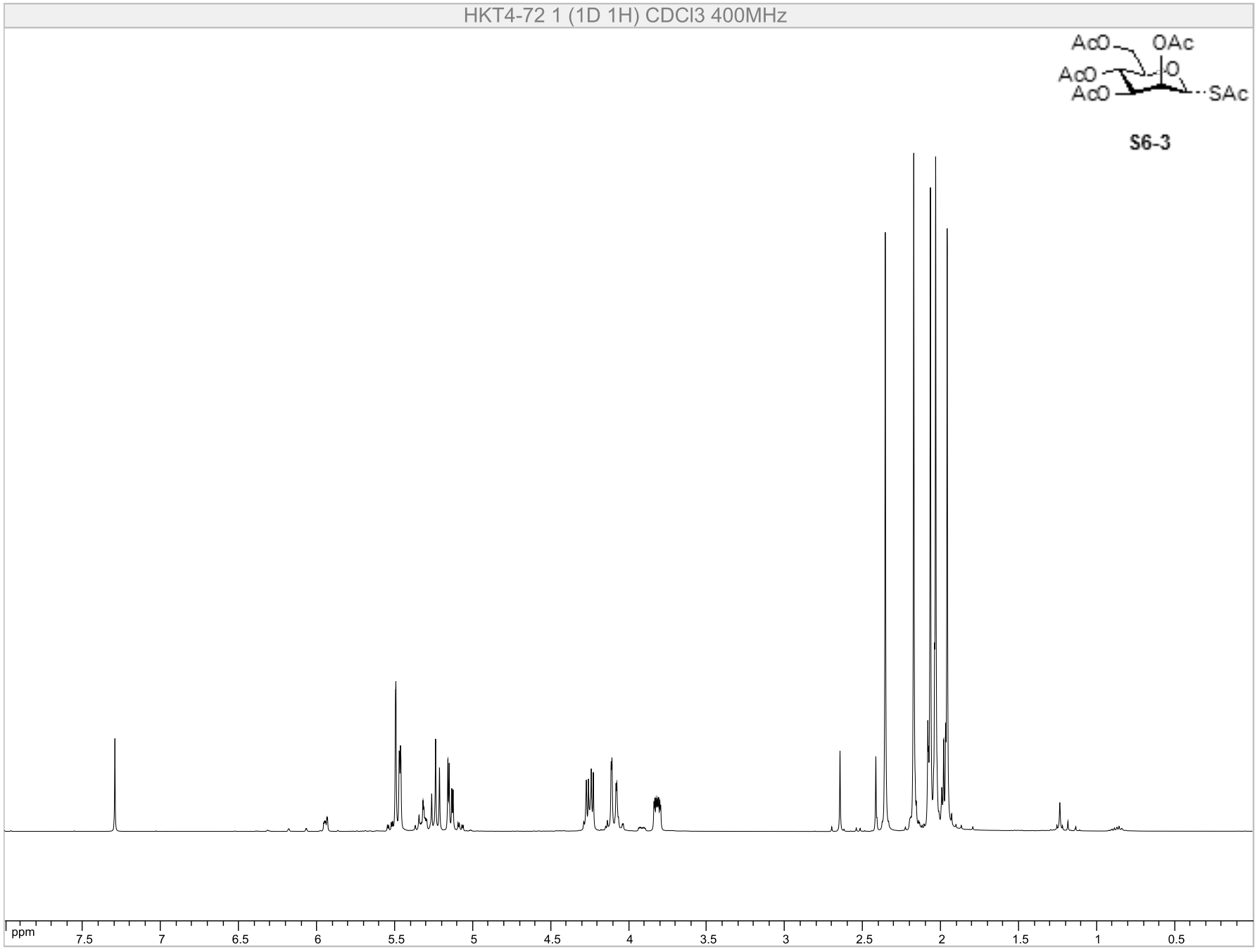


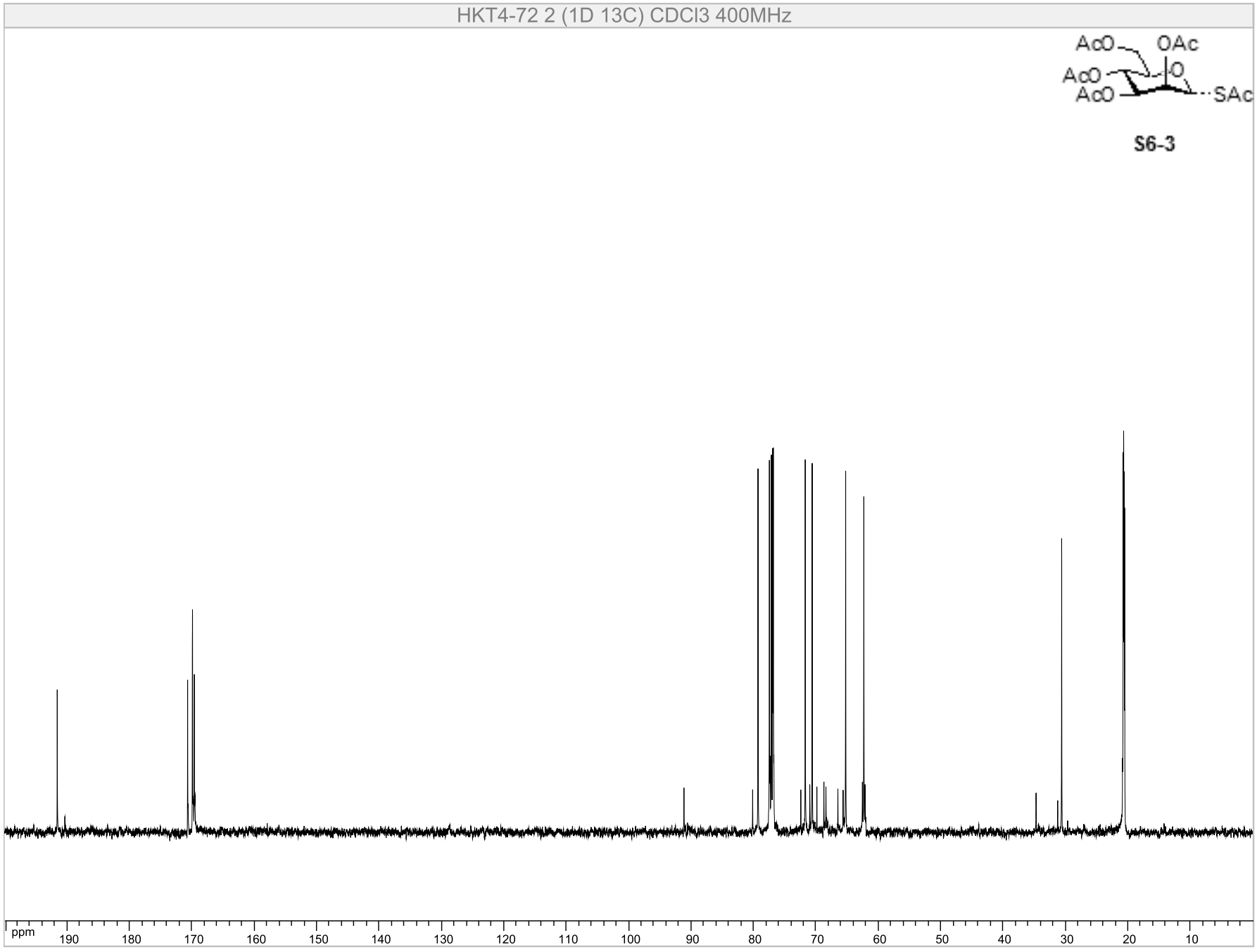

HKT4-78 3 (1D 1H) CDCl3 400MHz
AcO
OAc
O
AcO
SAc
AcO
S6-1
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

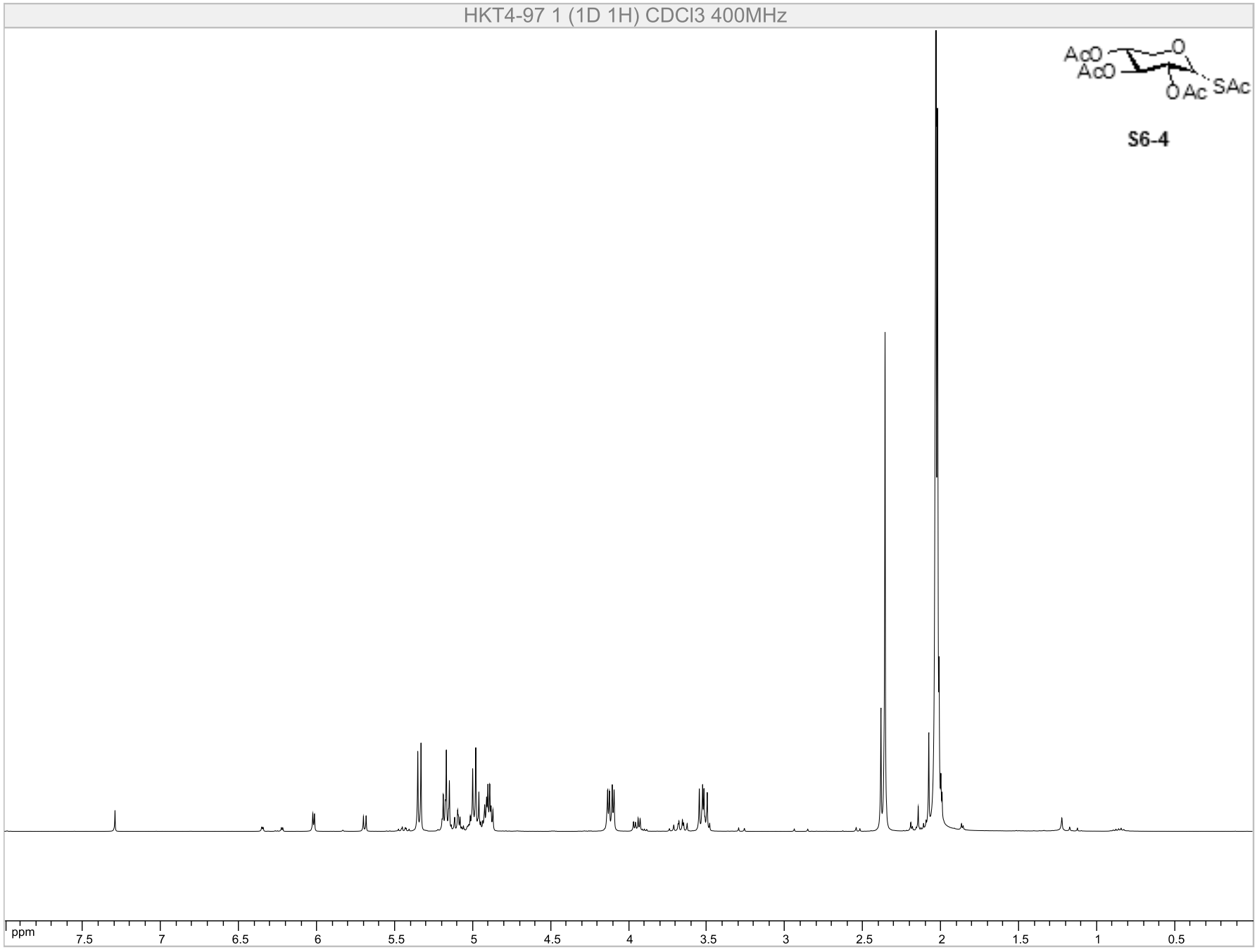

HKT4-78 4 (1D 13C) CDCl3 400MHz
AcO
OAc
O
AcO
SAc
AcO
S6-1
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


HKT4-69 2 (1D 1H) CDCl3 400MHz
AcO
AcO
AcO
AcO
O
SAc
S6-2
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

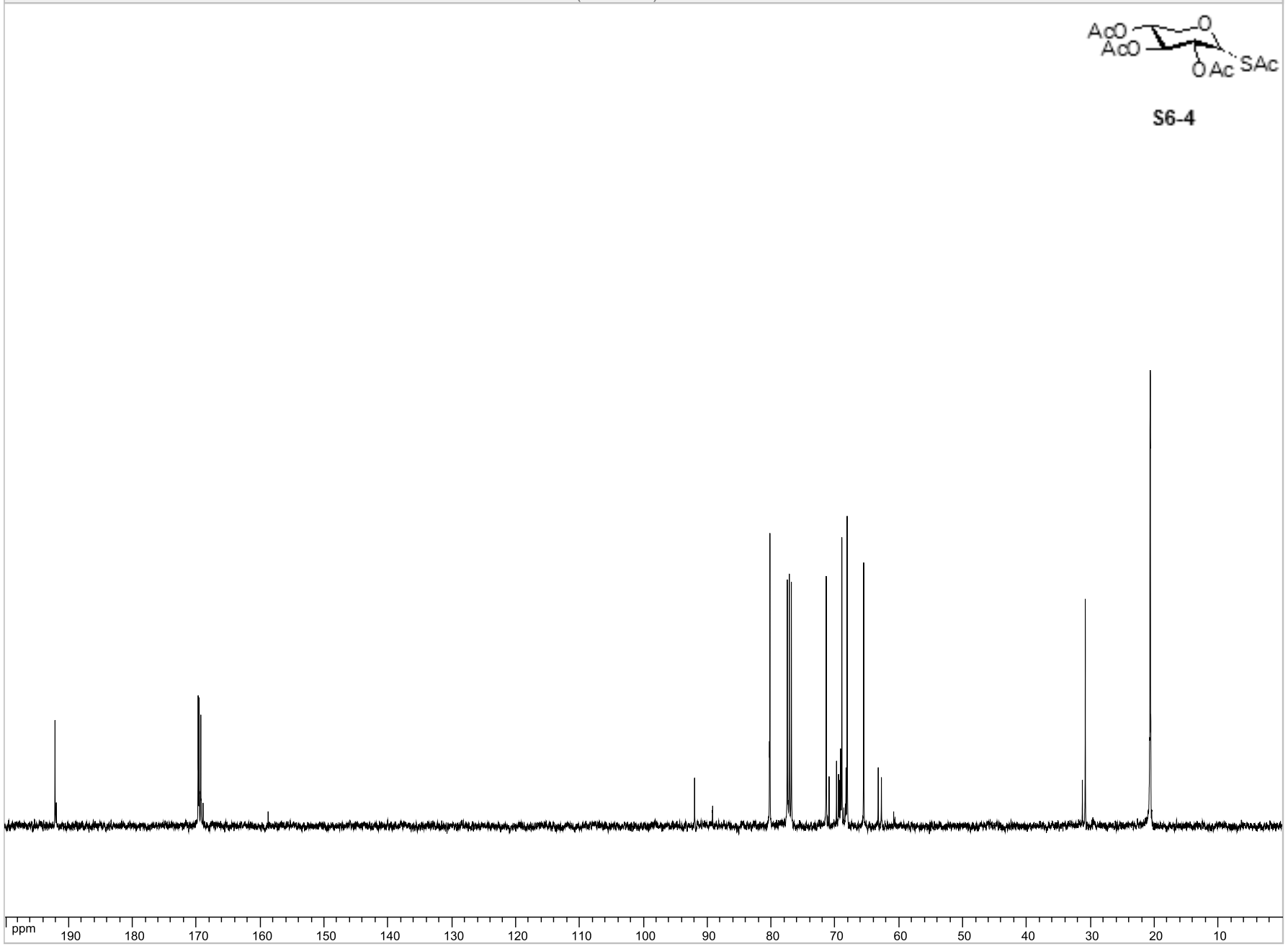

HKT4-69 3 (1D 13C) CDCl3 400MHz
AcO
AcO
AcO
O
SAc
AcO
S6-2
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


HKT4-72 1 (1D 1H) CDCl3 400MHz
AcO
OAc
AcO
O
AcO
SAc
S6-3
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

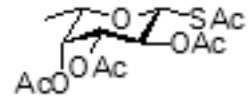

HKT4-72 2 (1D 13C) CDCl3 400MHz
AcO
OAc
AcO
O
AcO
SAc
S6-3
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10


HKT4-97 1 (1D 1H) CDCl3 400MHz
AcO
AcO
O
OAc
SAc
S6-4
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

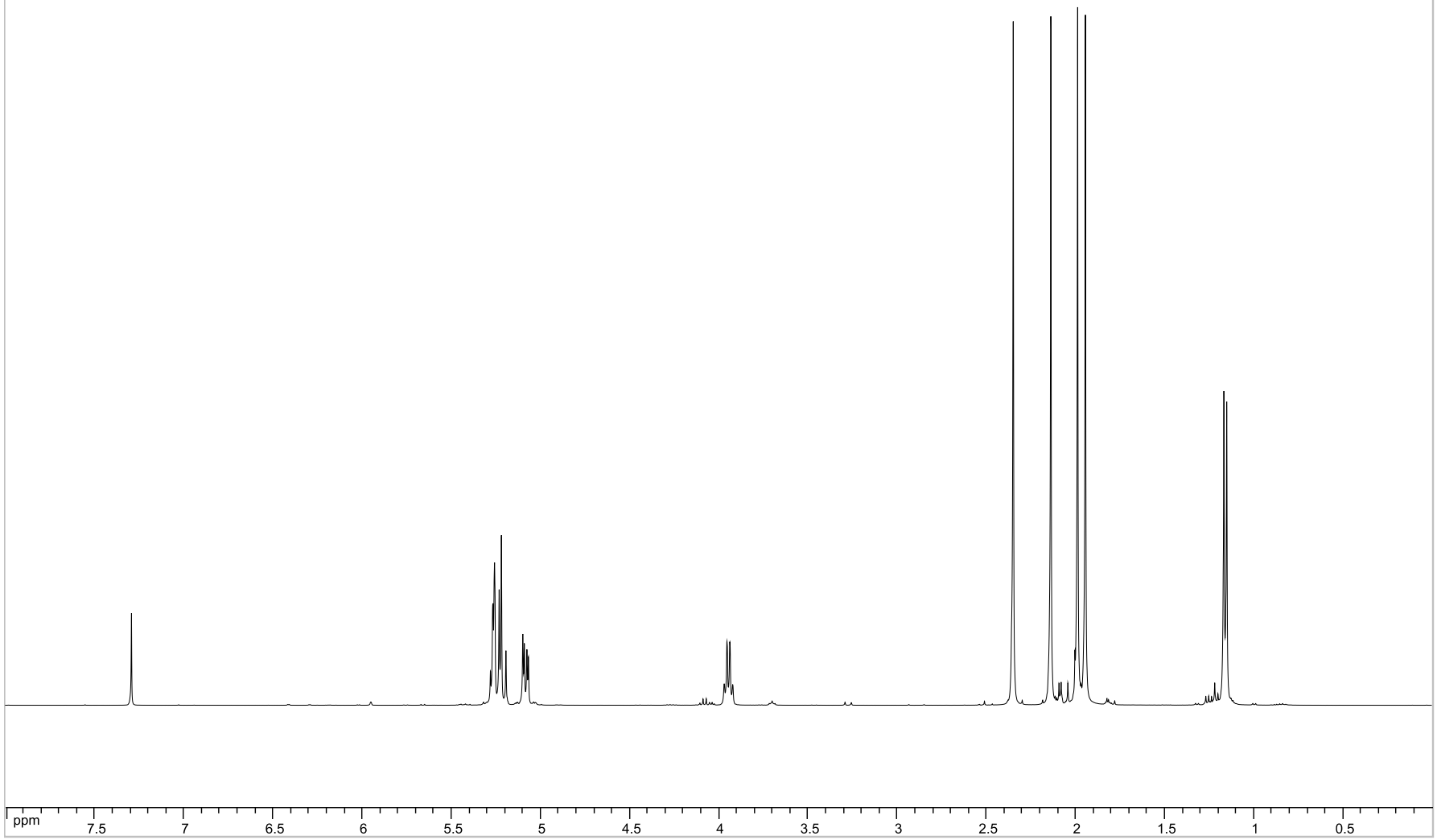
HKT4-97 2 (1D 13C) CDCl3 400MHz



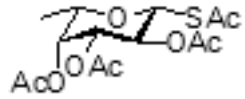
HKT4-92 2 (1D 1H) CDCl3 400MHz



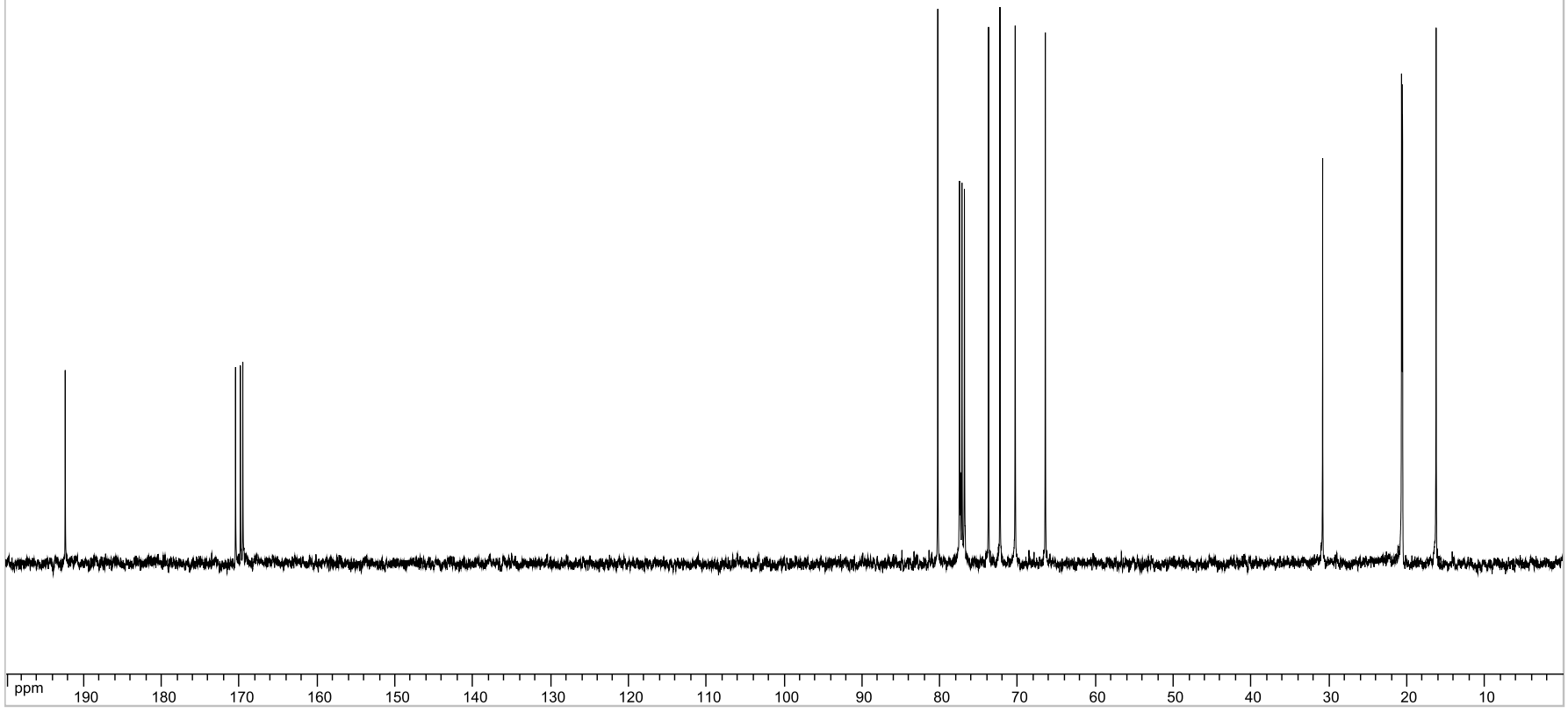
S6-5



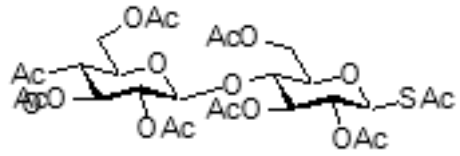
HKT4-92 3 (1D 13C) CDCl3 400MHz



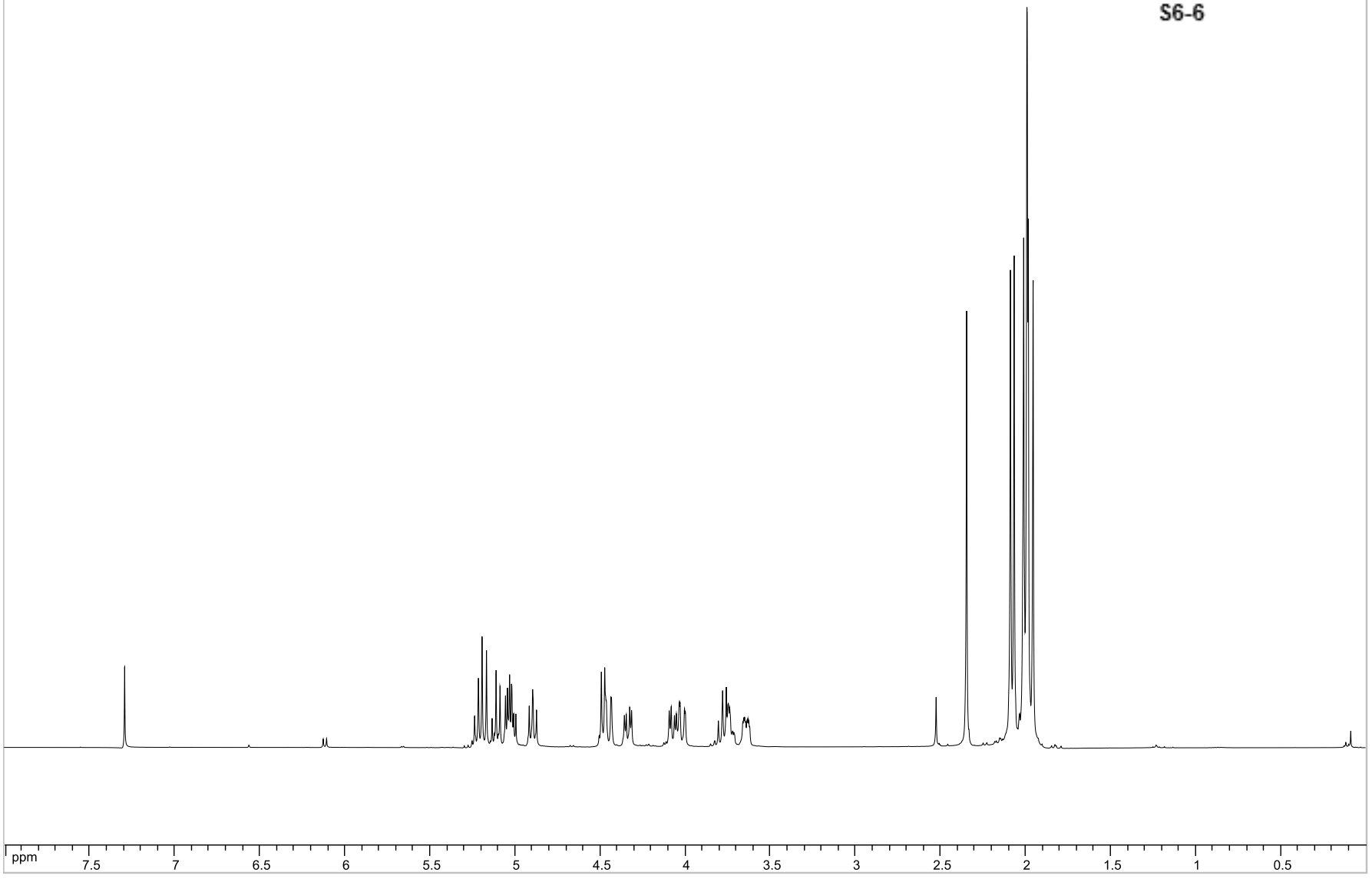
S6-5



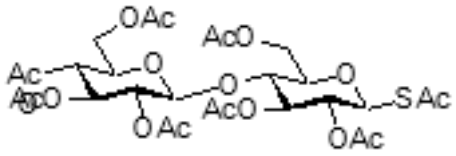
HKT4-82 1 (1D 1H) CDCl3 400MHz



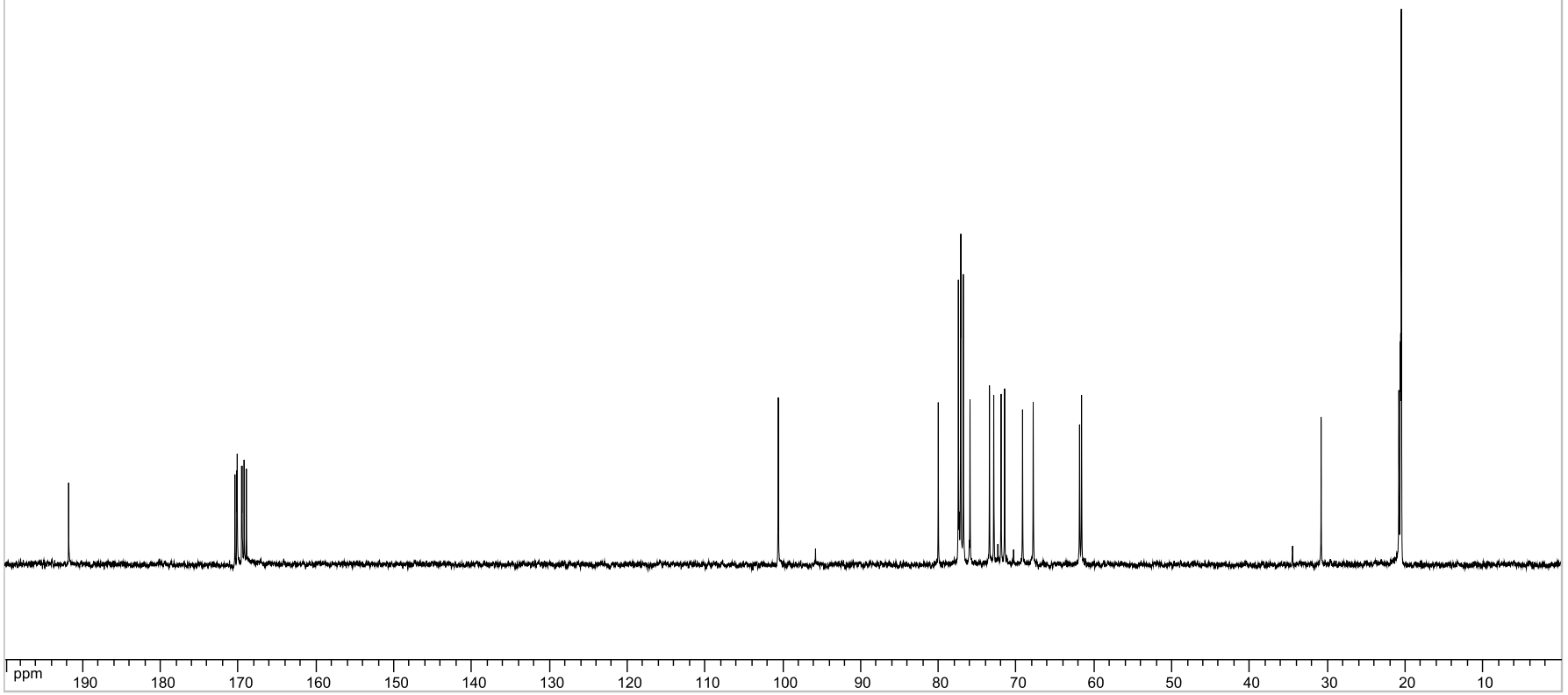
S6-6

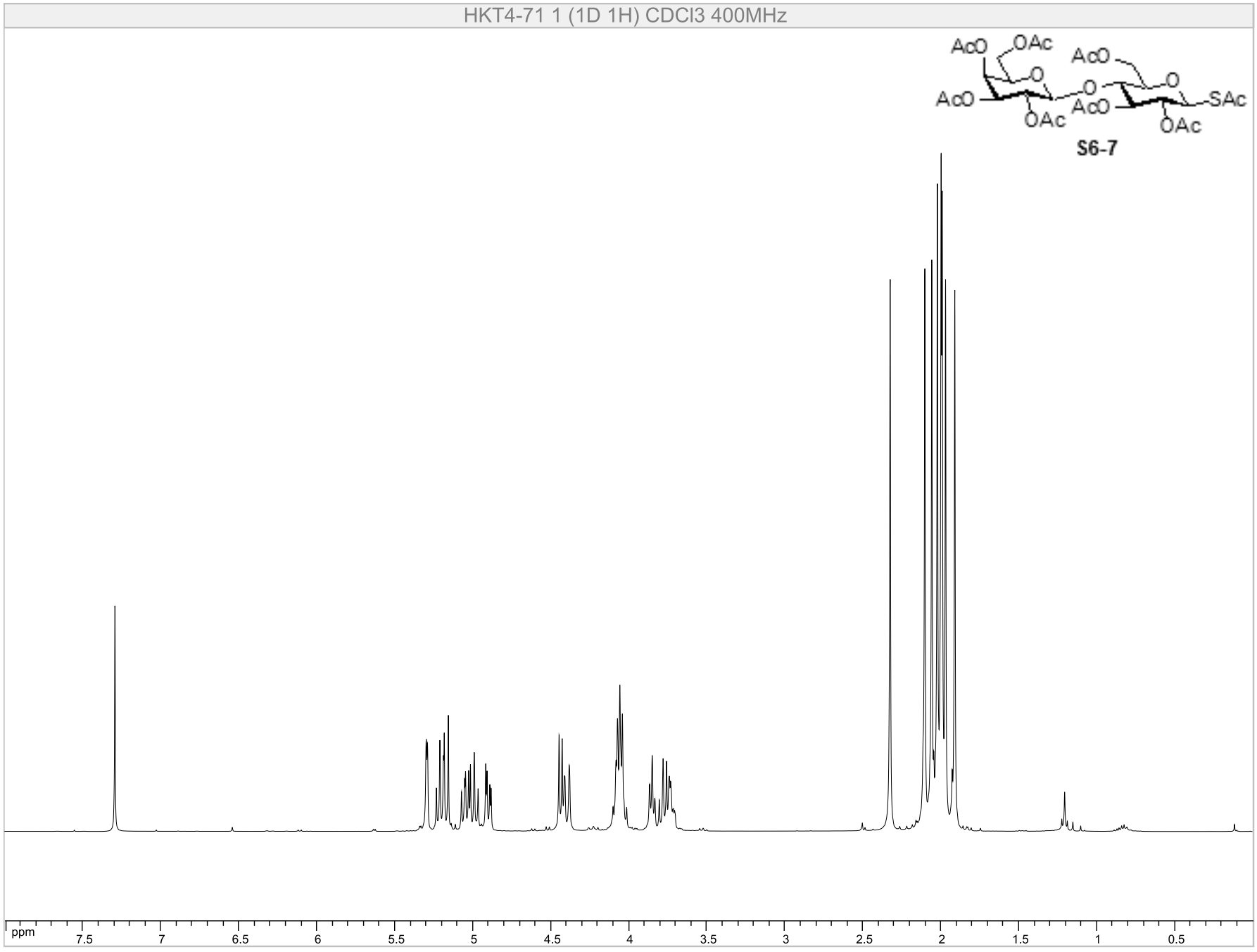


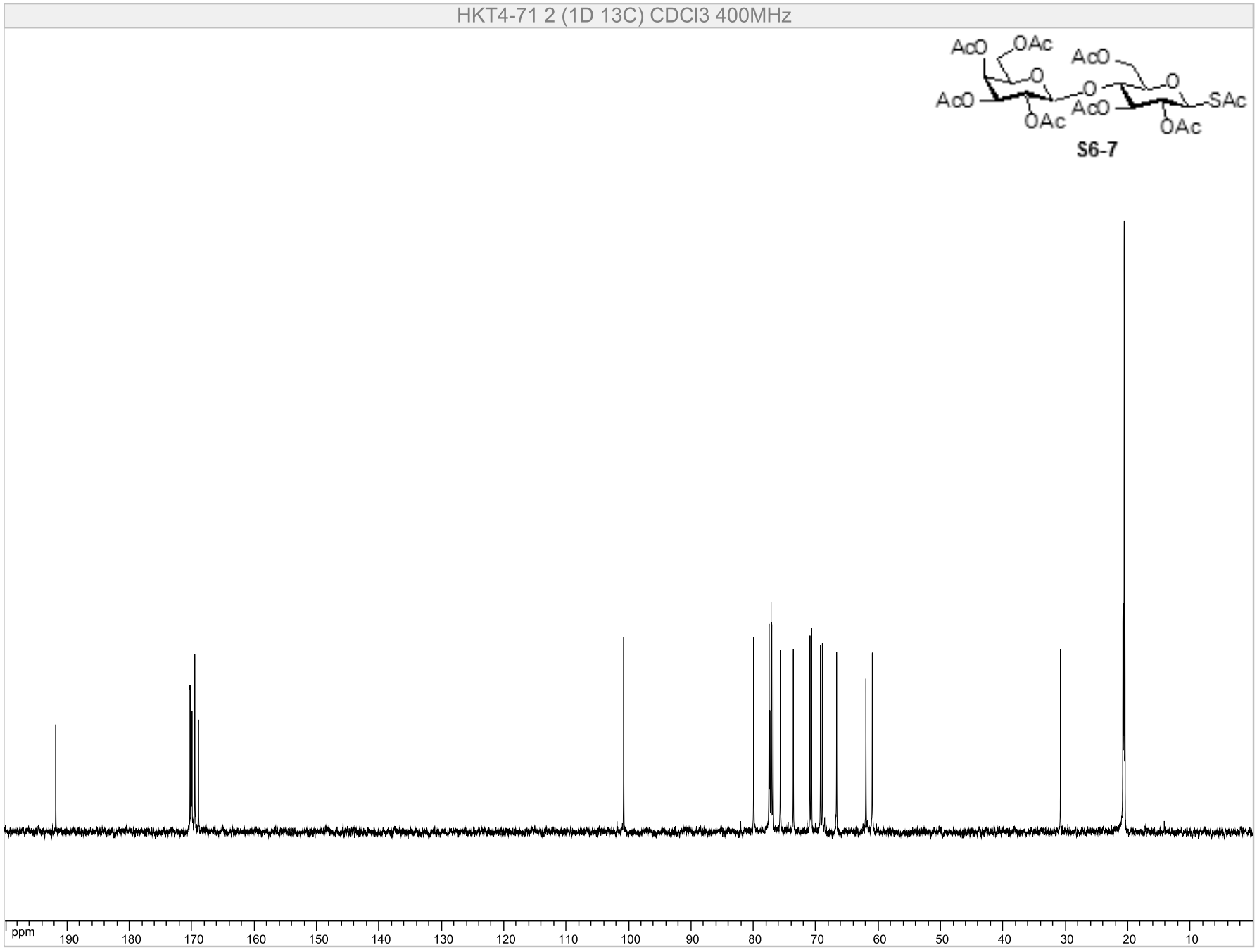
HKT4-82 2 (1D 13C) CDCl3 400MHz

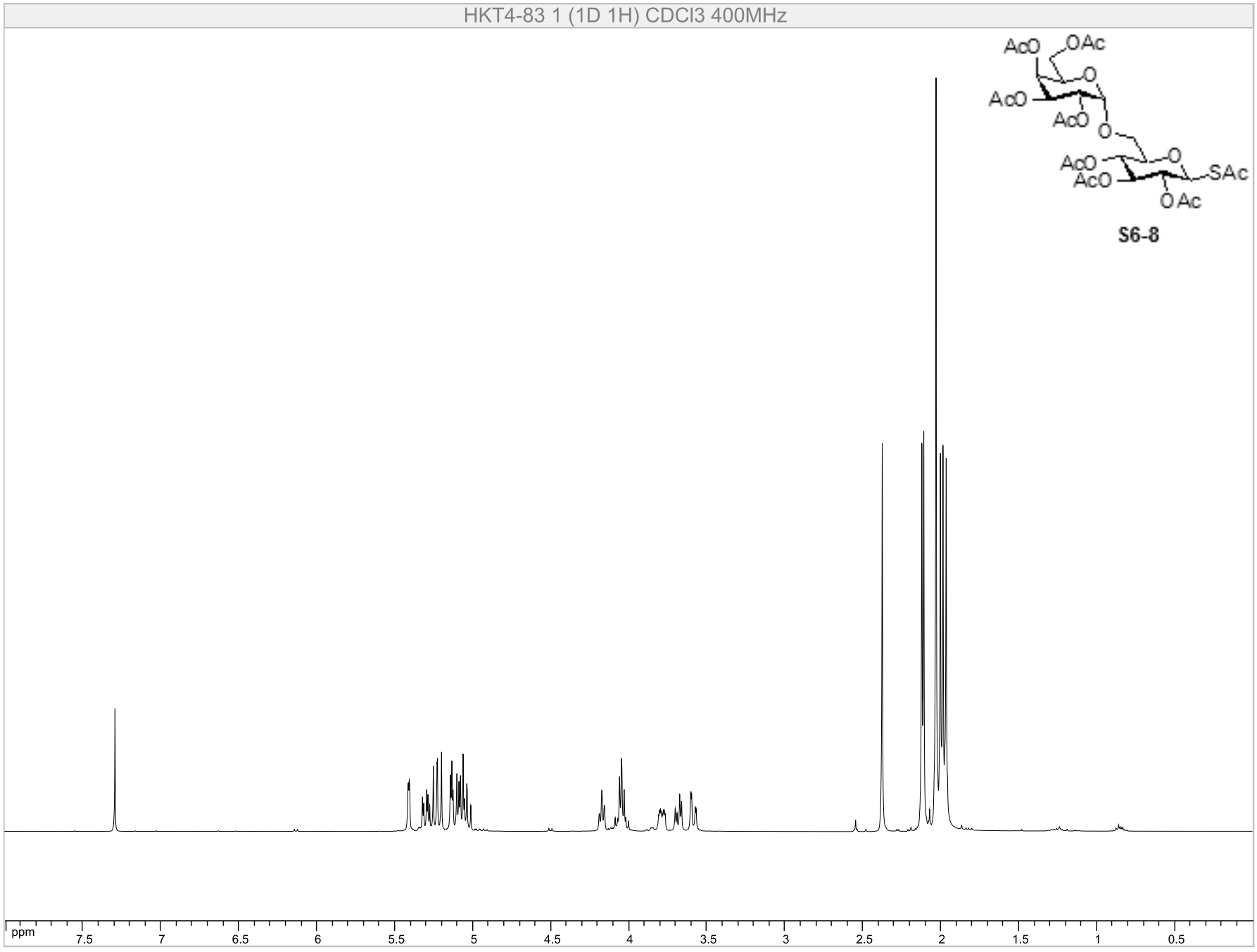


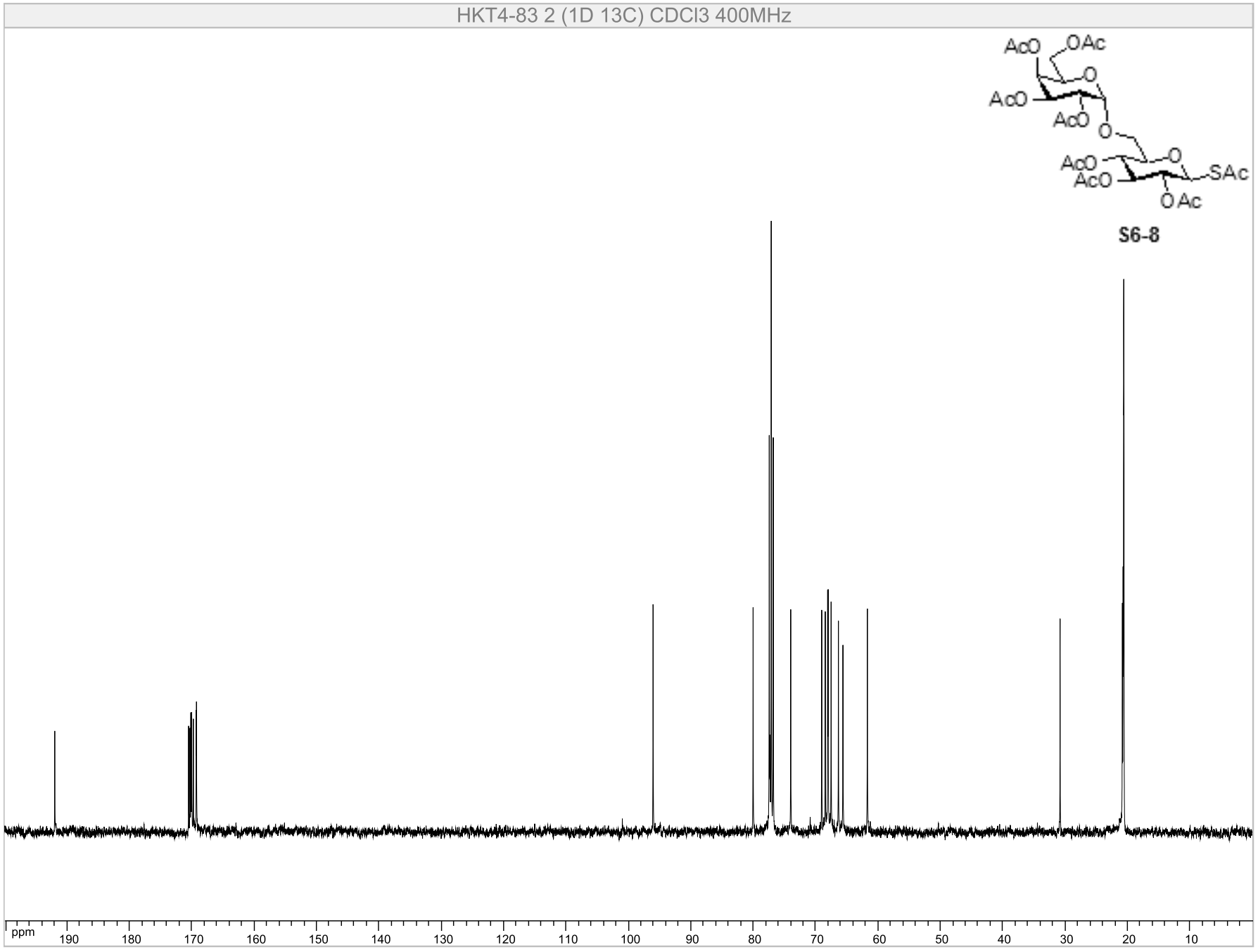
S6-6

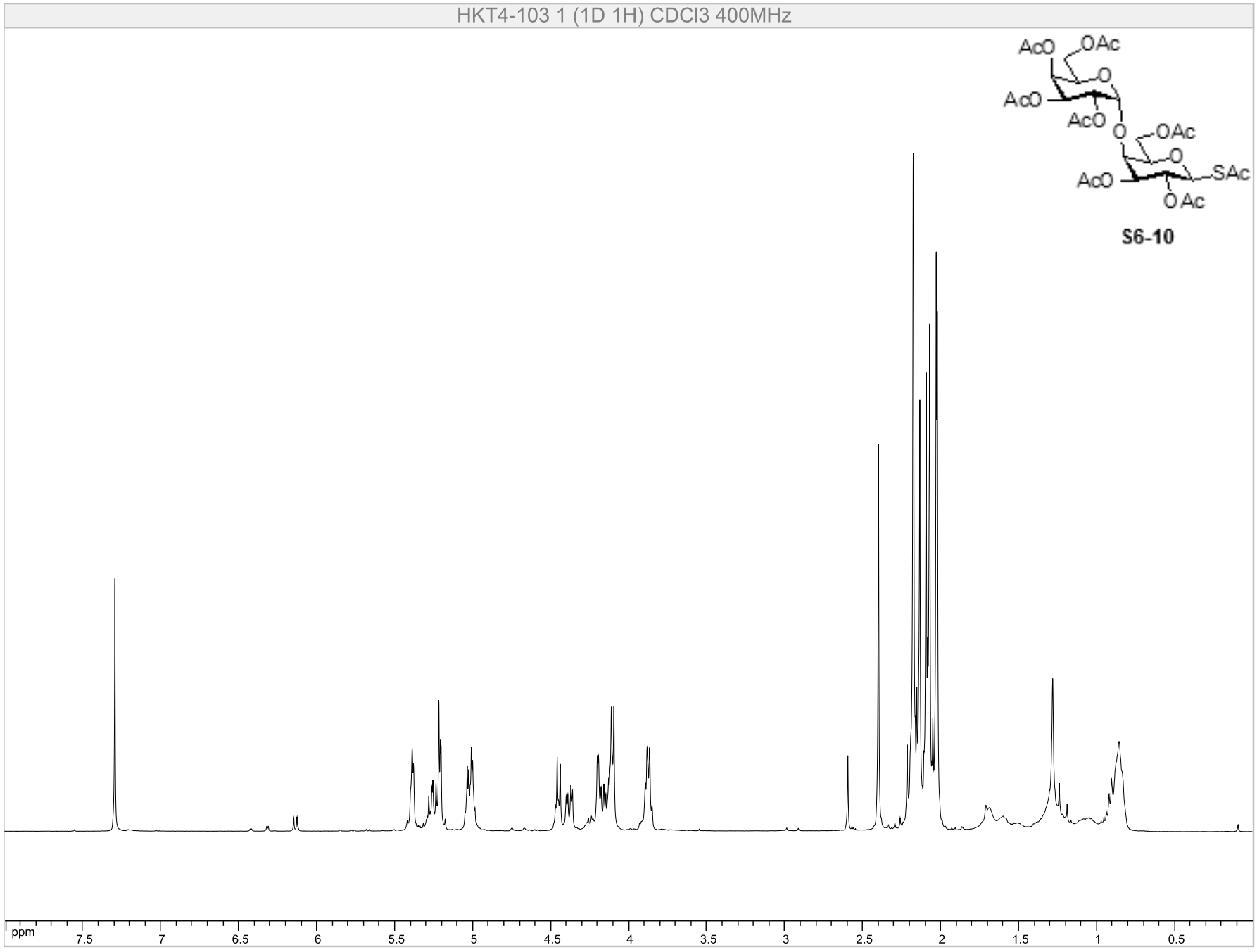


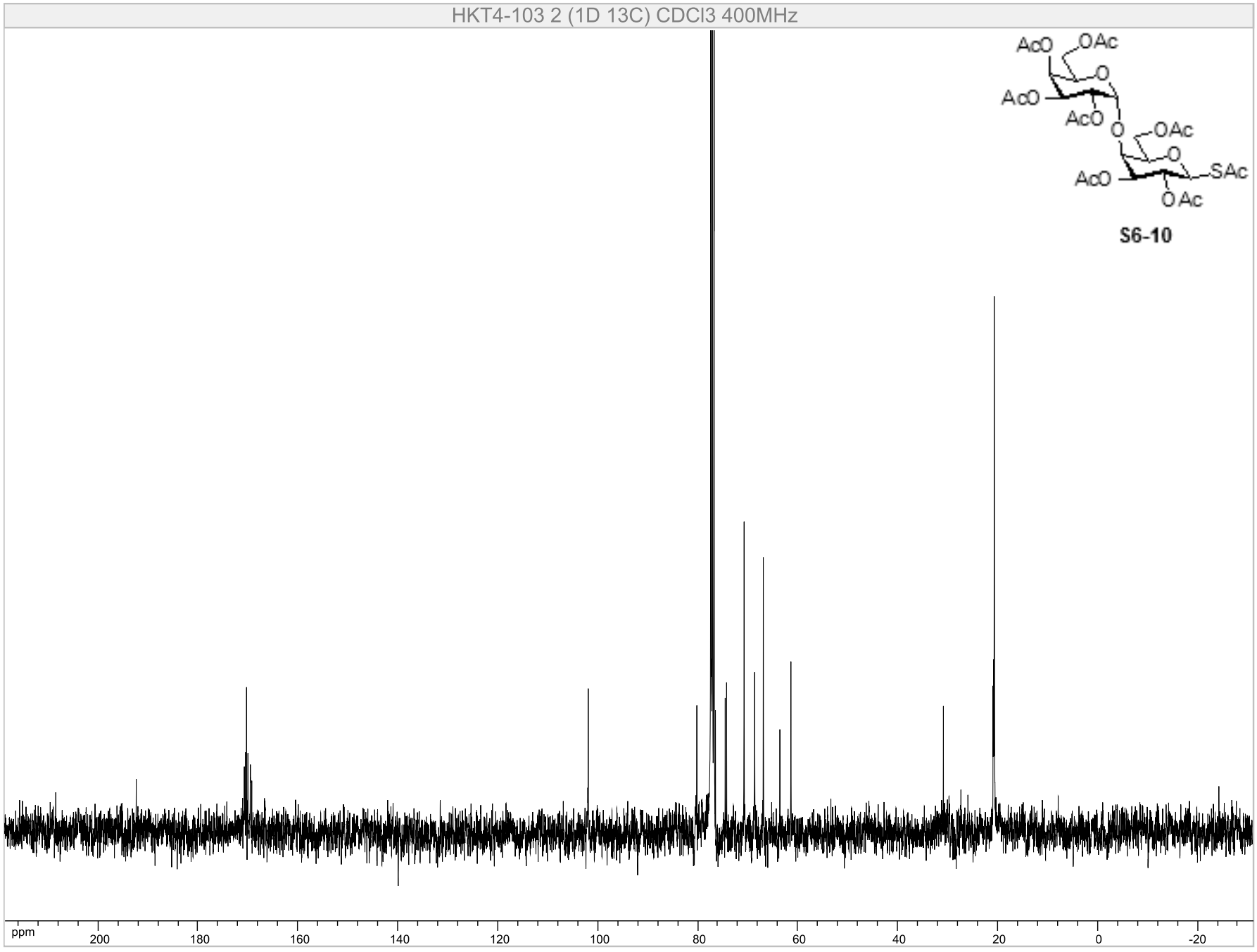

HKT4-71 1 (1D 1H) CDCl3 400MHz
AcO
OAc
AcO
O
O
AcO
AcO
O
SAc
OAc
OAc
S6-7
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

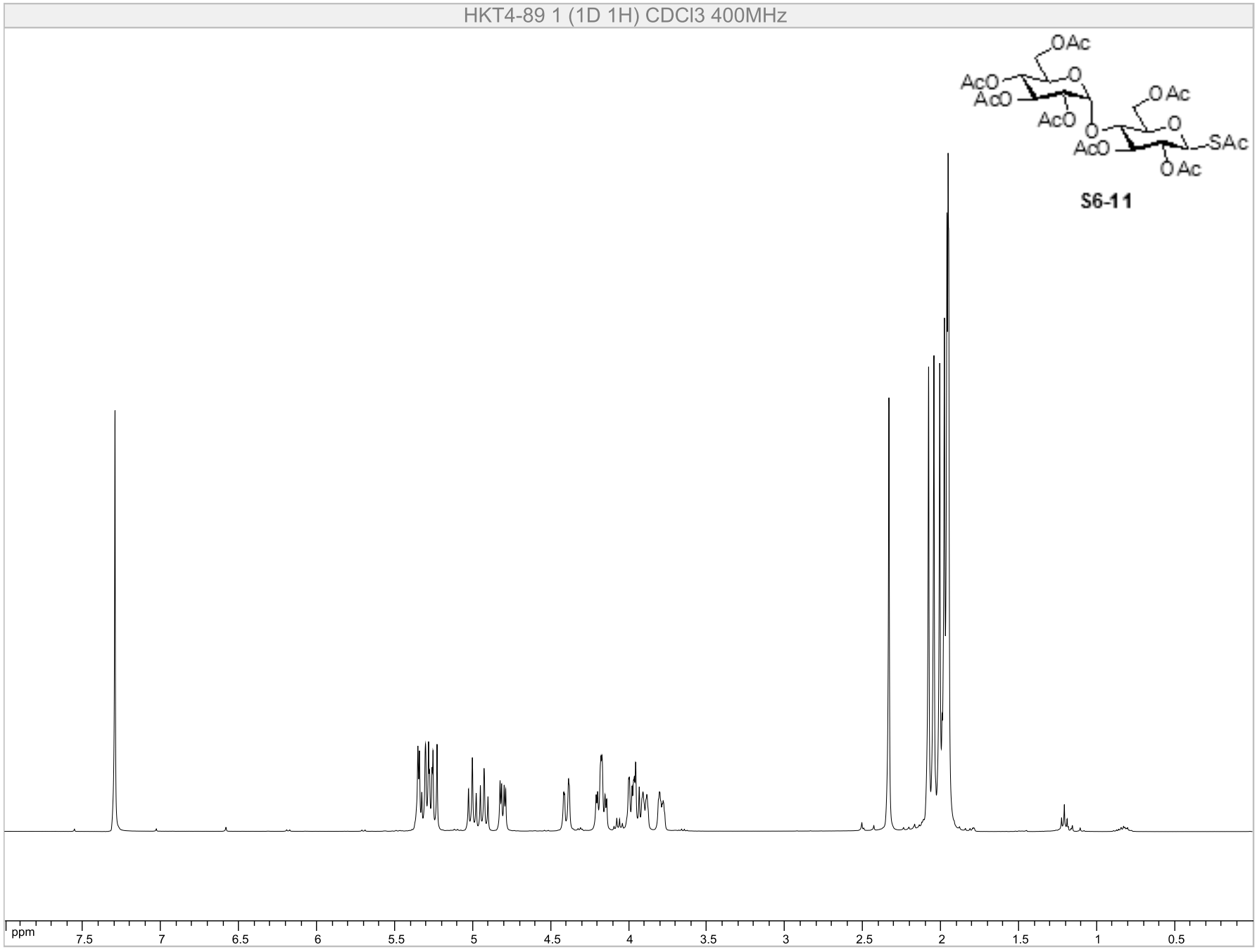

HKT4-71 2 (1D 13C) CDCl3 400MHz
AcO
OAc
AcO
O
AcO
O
AcO
O
SAc
OAc
AcO
OAc
S6-7
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

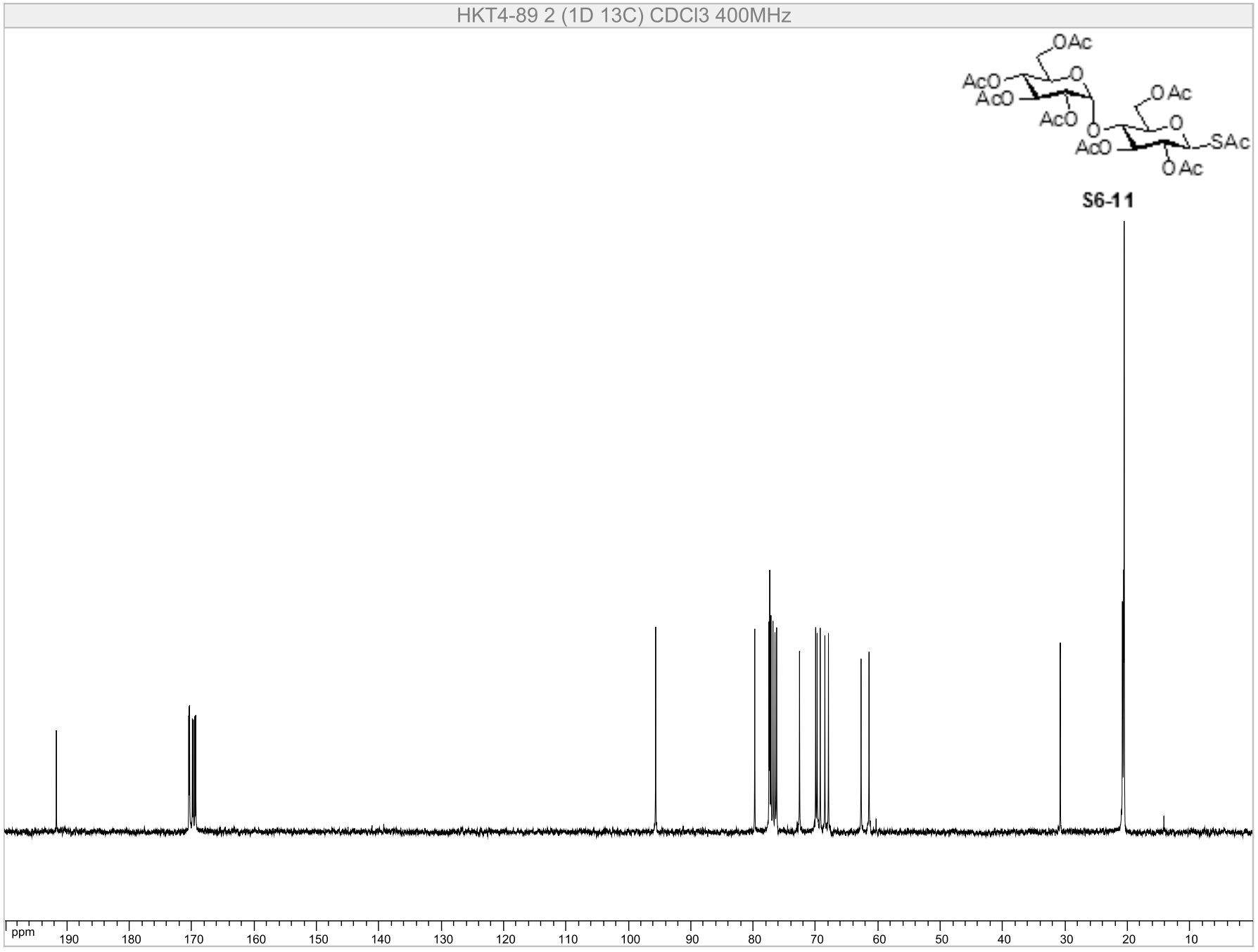

HKT4-83 1 (1D 1H) CDCl3 400MHz
AcO
OAc
O
AcO
AcO
O
AcO
O
AcO
SAc
O Ac
S6-8
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

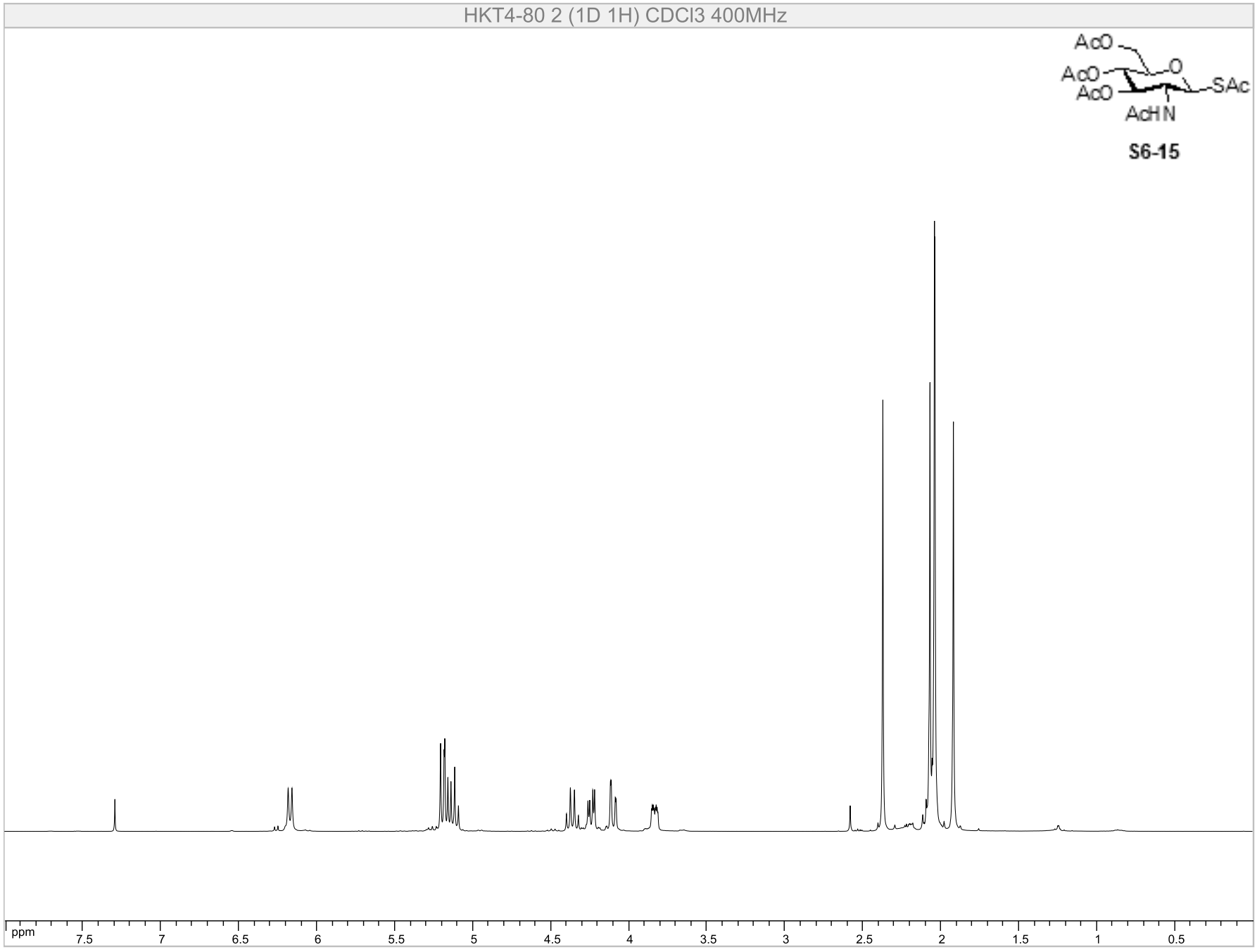

HKT4-83 2 (1D 13C) CDCl3 400MHz
AcO
OAc
O
AcO
AcO
O
AcO
O
AcO
SAc
O Ac
S6-8
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

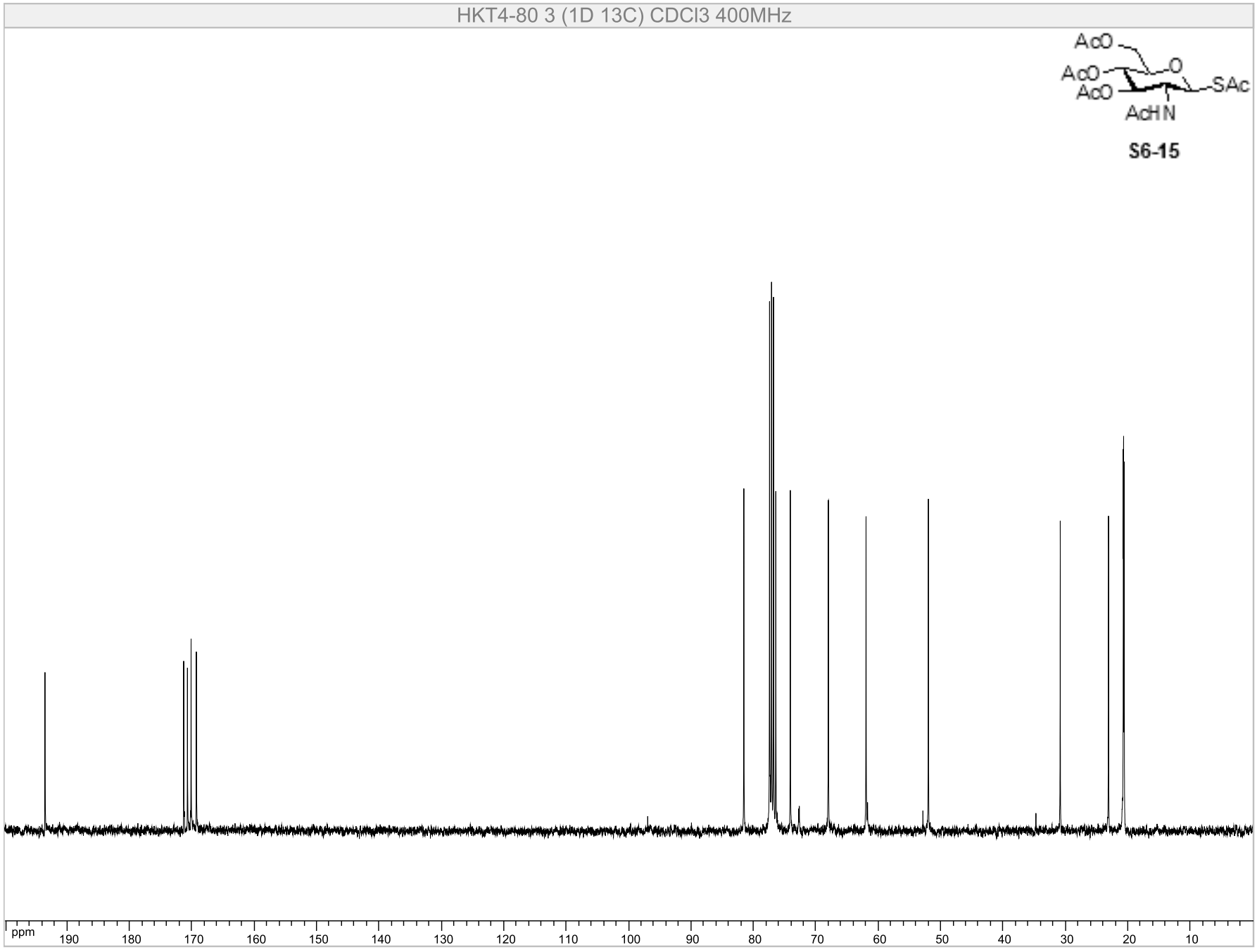

HKT4-103 1 (1D 1H) CDCl3 400MHz
AcO
OAc
O
AcO
AcO
O
OAc
O
AcO
SAc
OAc
S6-10
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

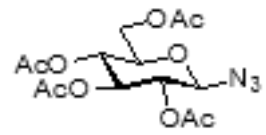

HKT4-103 2 (1D 13C) CDCl3 400MHz
AcO
OAc
O
AcO
AcO
O
OAc
O
AcO
SAc
OAc
S6-10
ppm
200
180
160
140
120
100
80
60
40
20
0
-20


HKT4-89 1 (1D 1H) CDCl3 400MHz
OAc
AcO
AcO
AcO
O
O
OAc
O
AcO
SAc
OAc
S6-11
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5

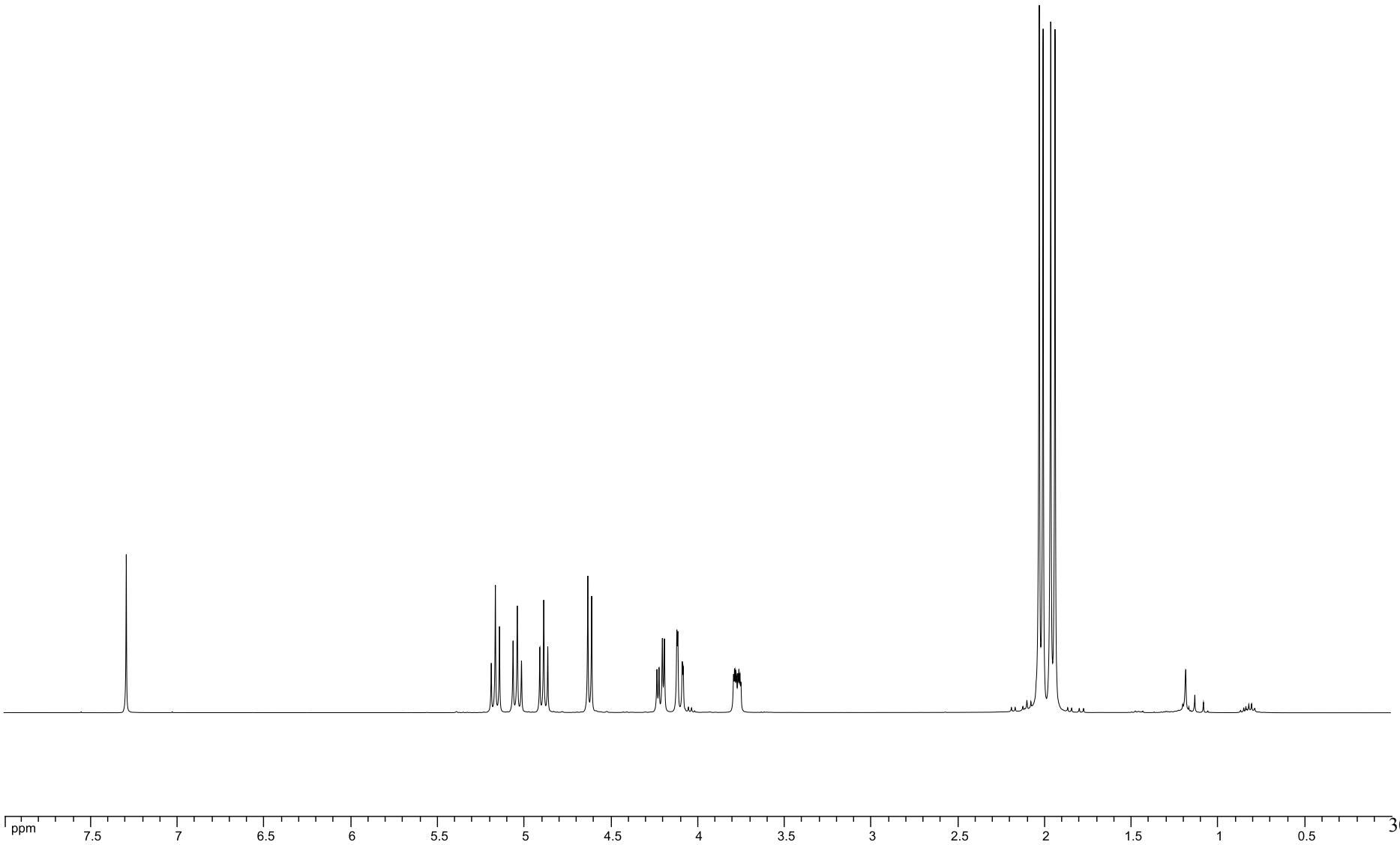

HKT4-89 2 (1D 13C) CDCl3 400MHz
OAc
AcO
AcO
O
AcO
O
AcO
O
OAc
O
SAc
OAc
S6-11
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

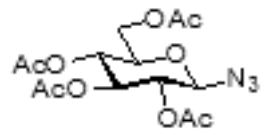

HKT4-80 2 (1D 1H) CDCl3 400MHz
AcO
AcO
AcO
O
SAc
AcHN
S6-15
ppm
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5


HKT4-80 3 (1D 13C) CDCl3 400MHz
AcO
AcO
AcO
O
SAc
AcHN
S6-15
ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

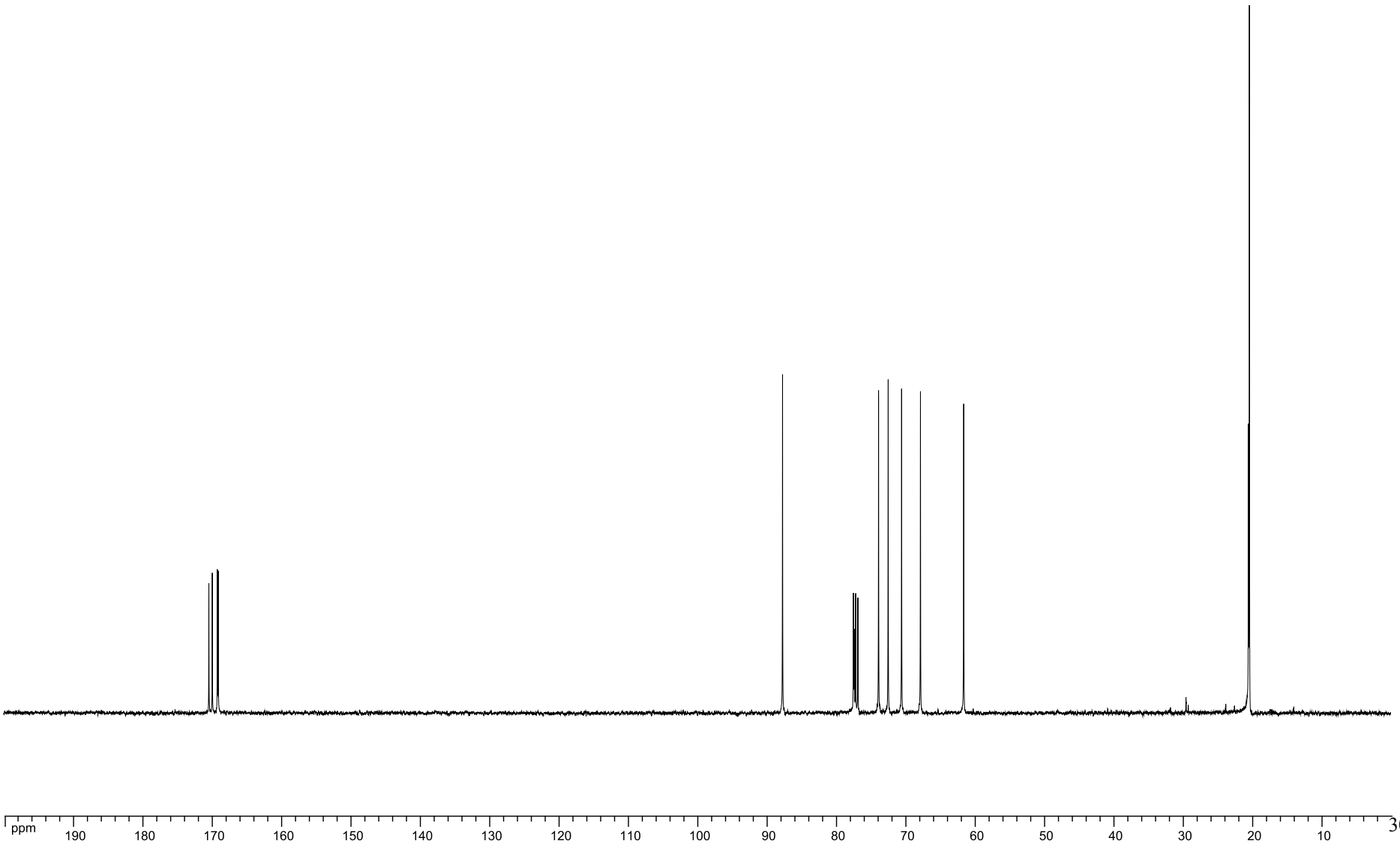


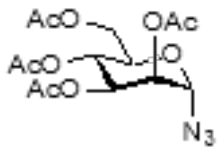
S6-21



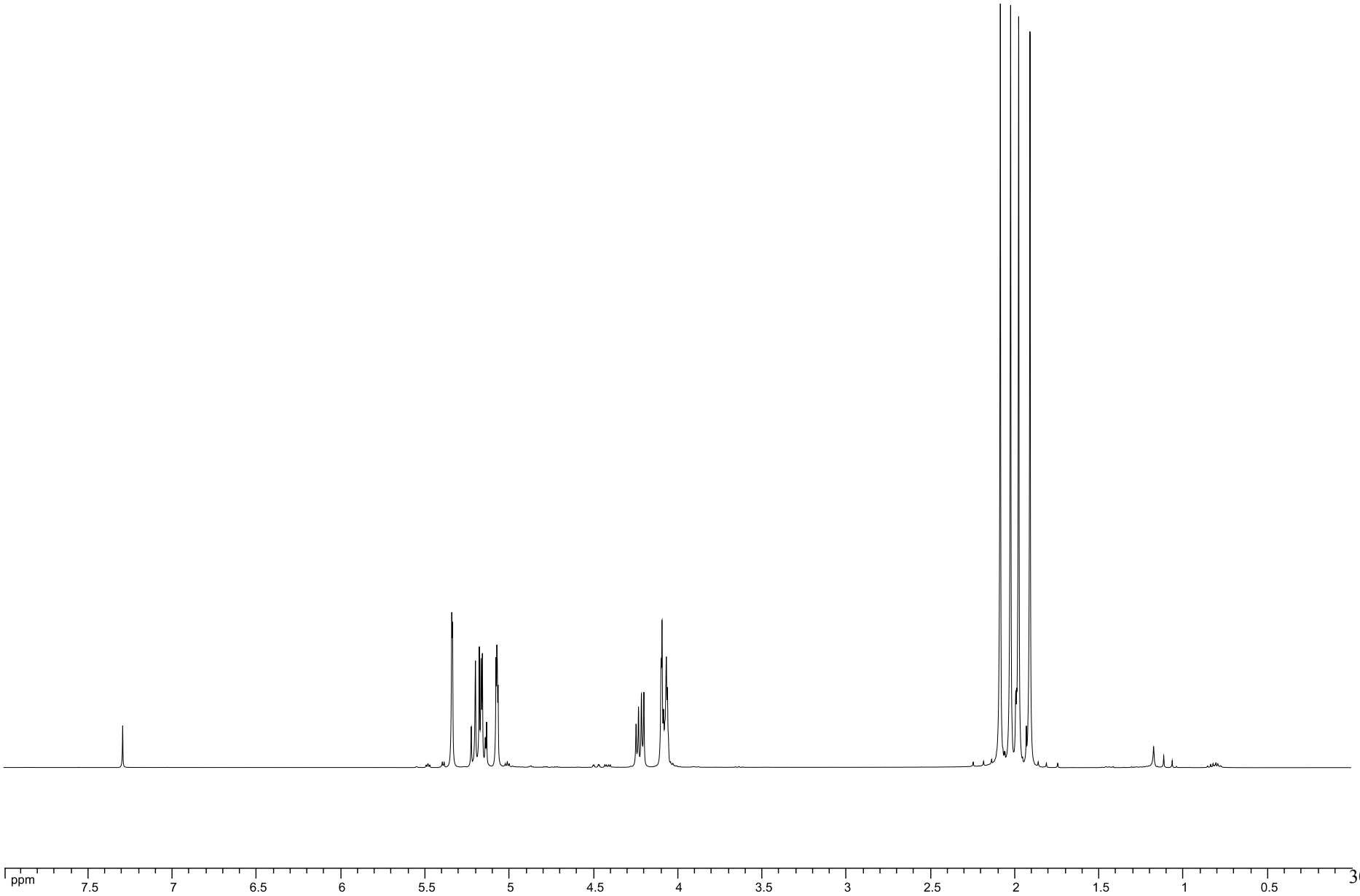

OAc
AcO
O
N3
AcO
OAc

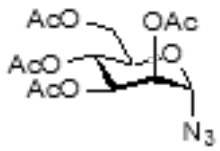
S6-21


ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

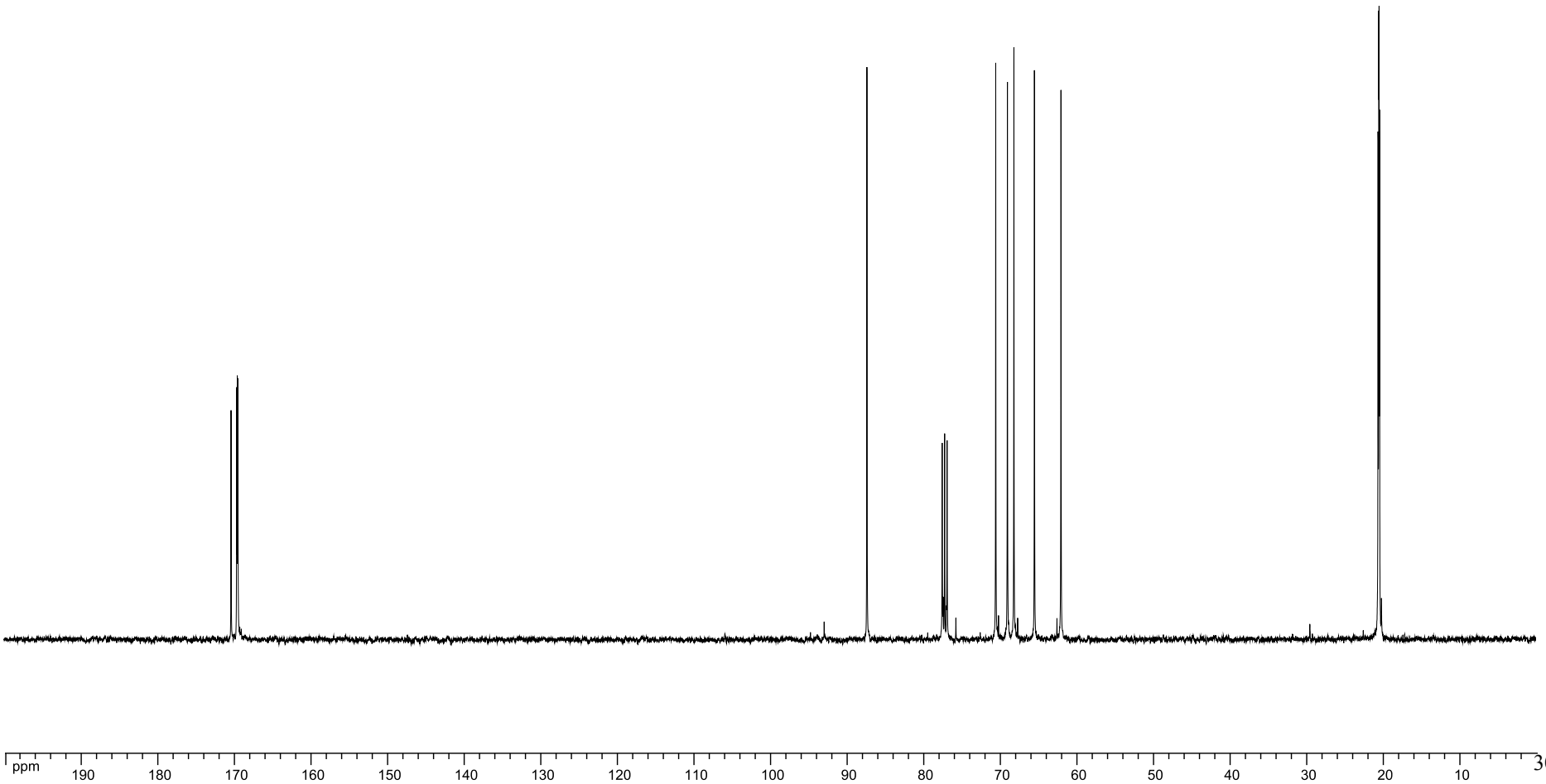


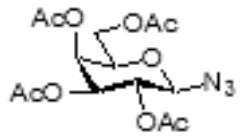
S6-22



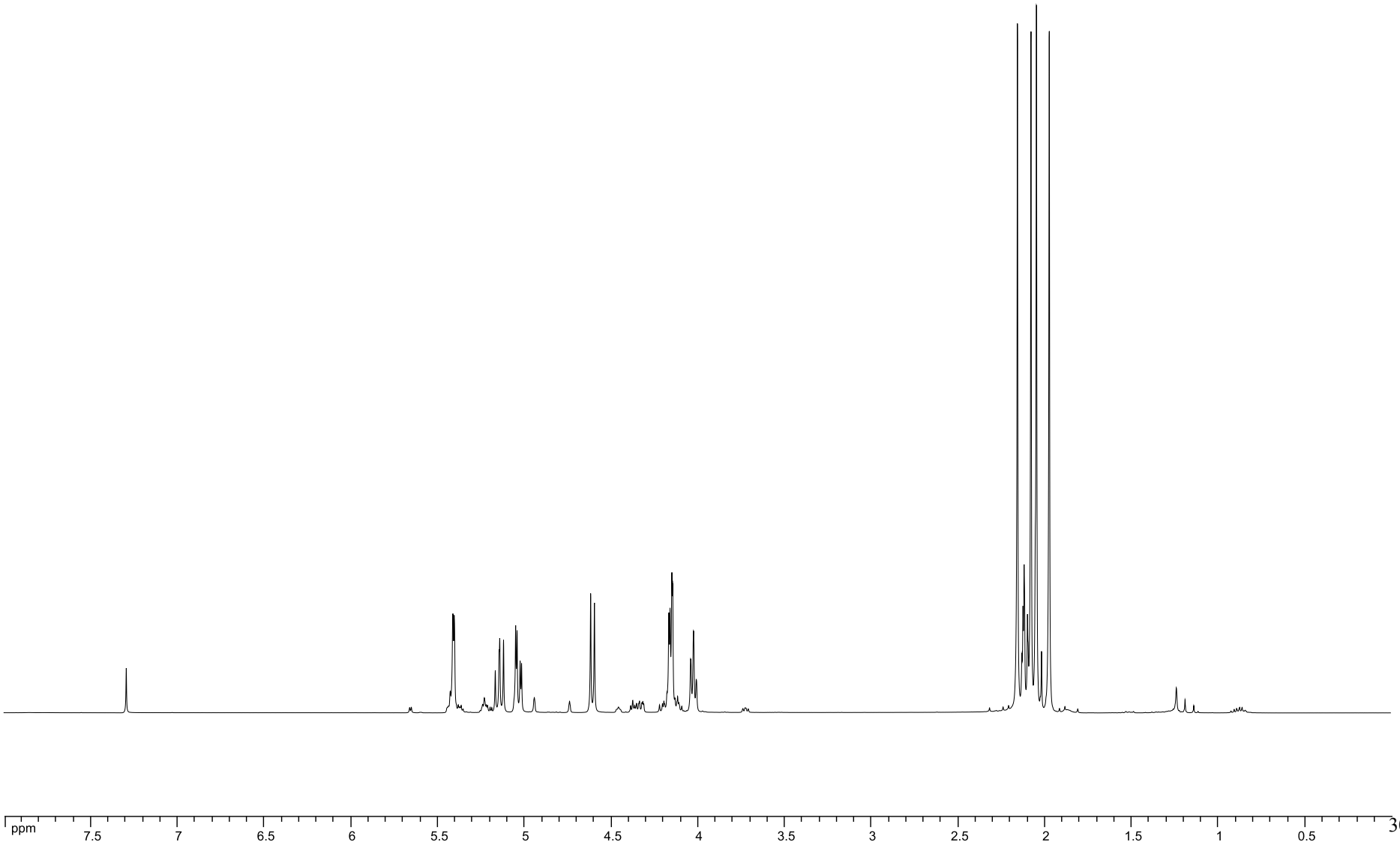

AcO
OAc
AcO
O
AcO
N3

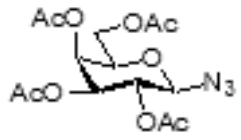
S6-22


ppm
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

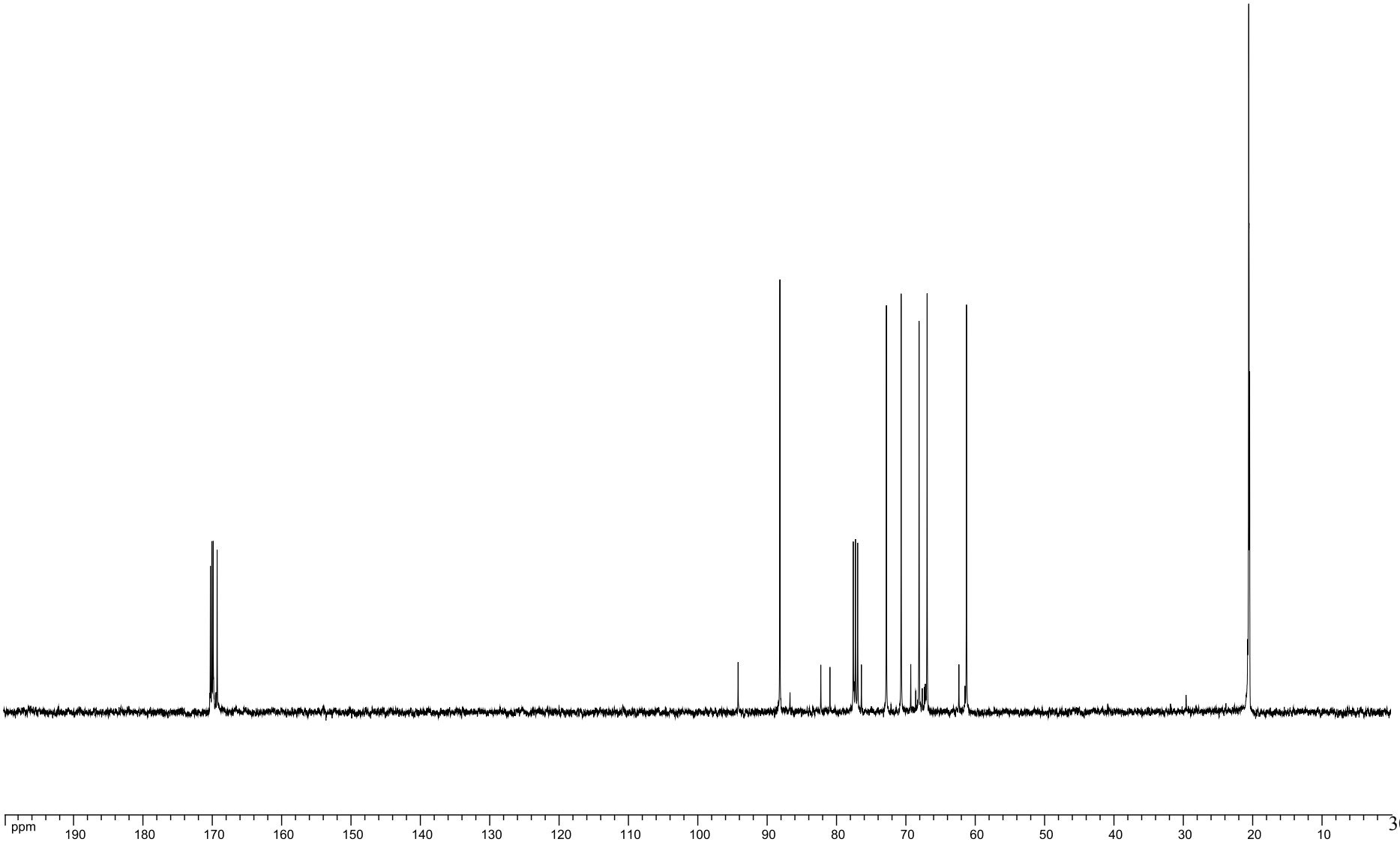


S6-23




AcO
OAc
O
AcO
N3
OAc

S6-23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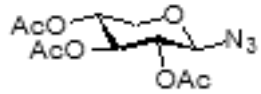

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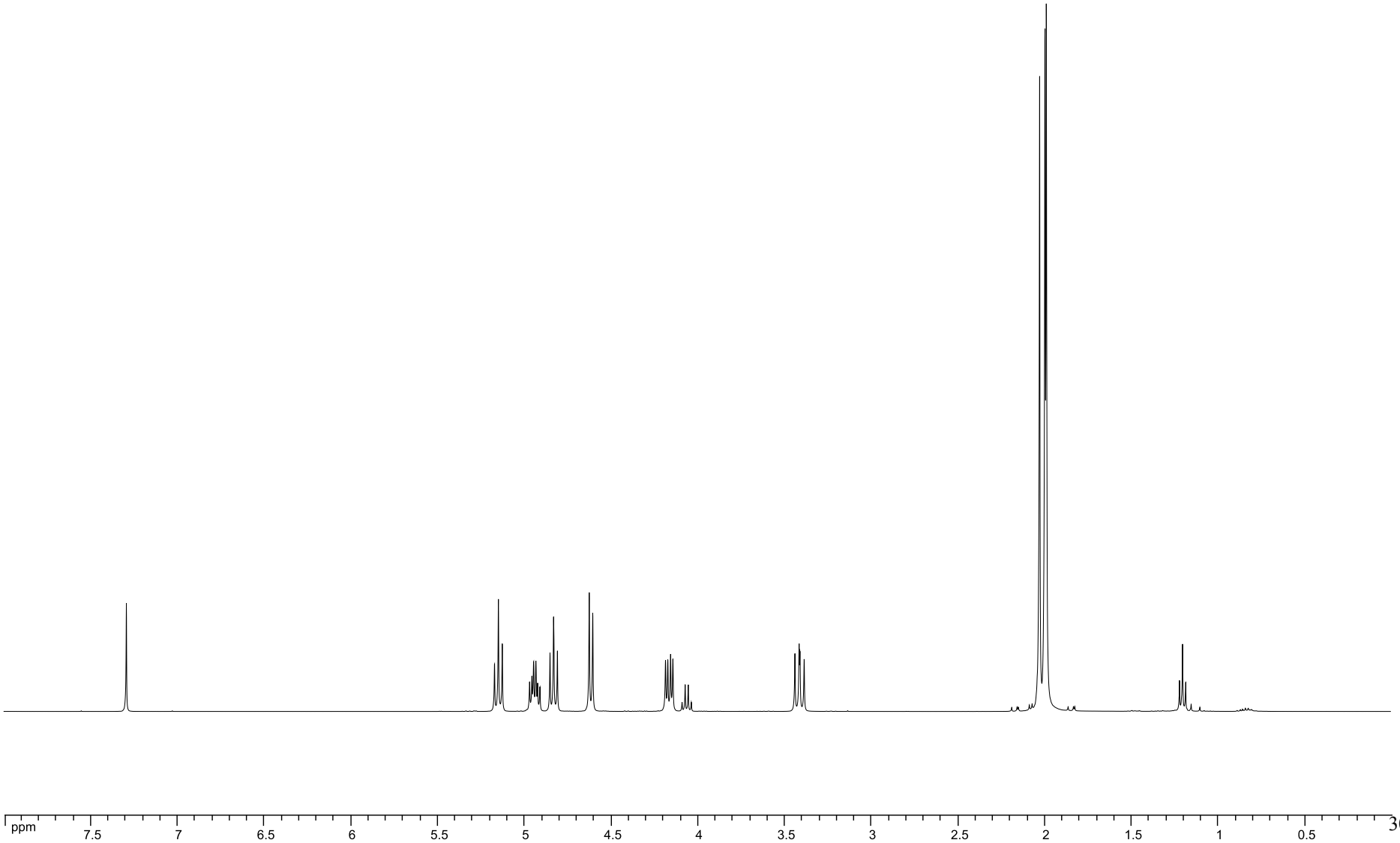

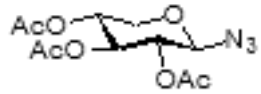
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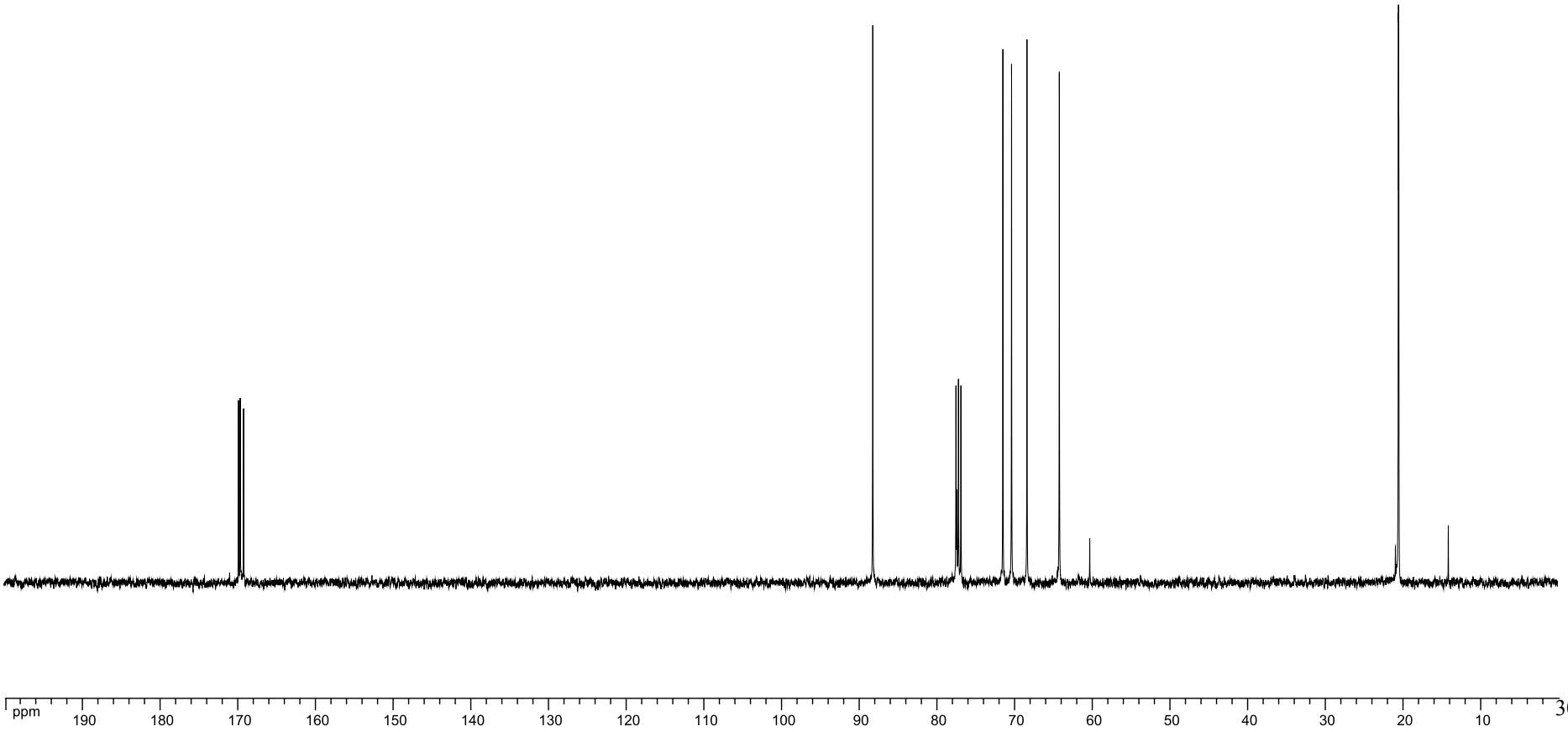
S6-24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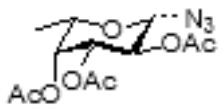

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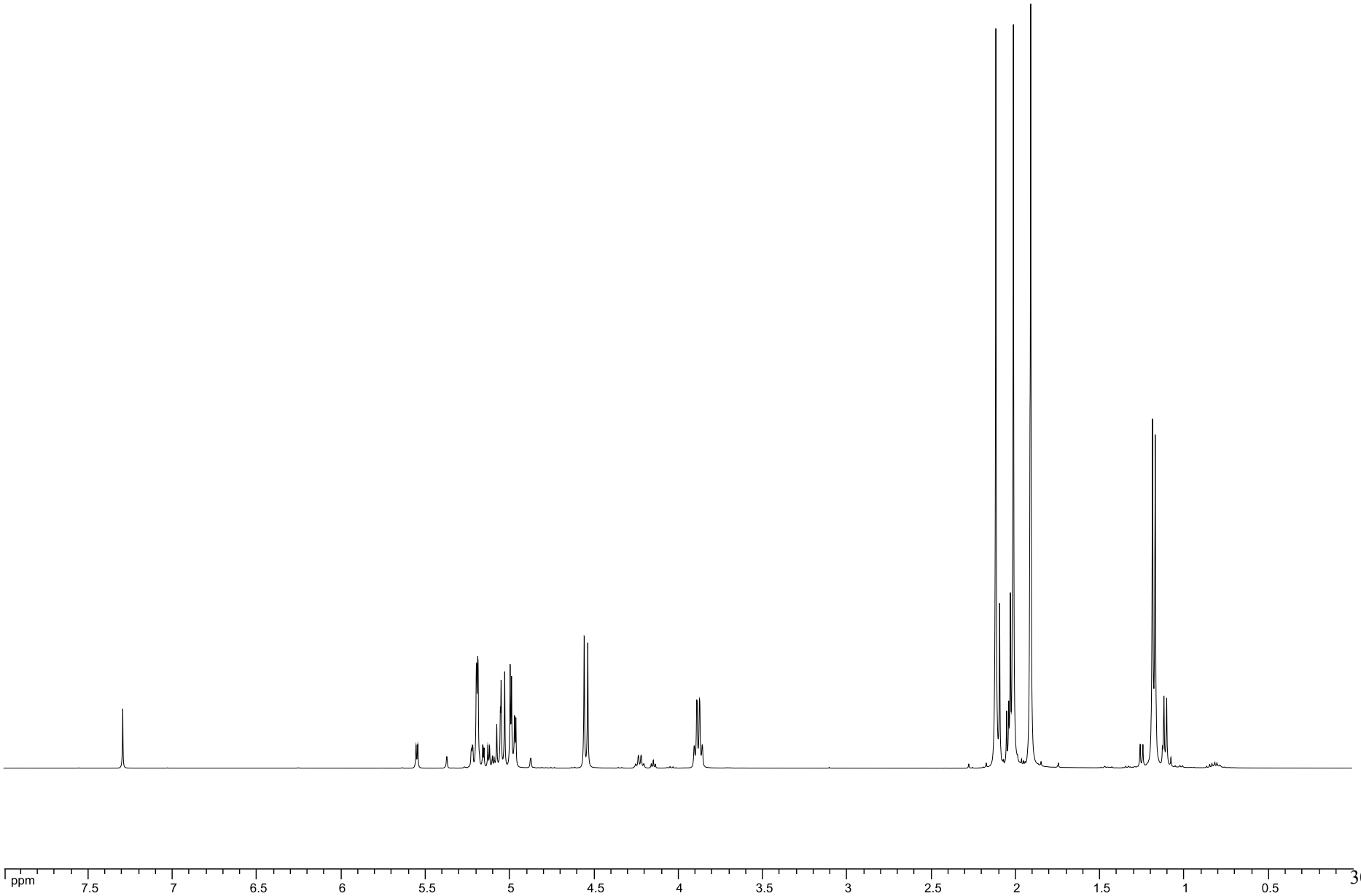

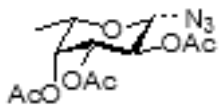
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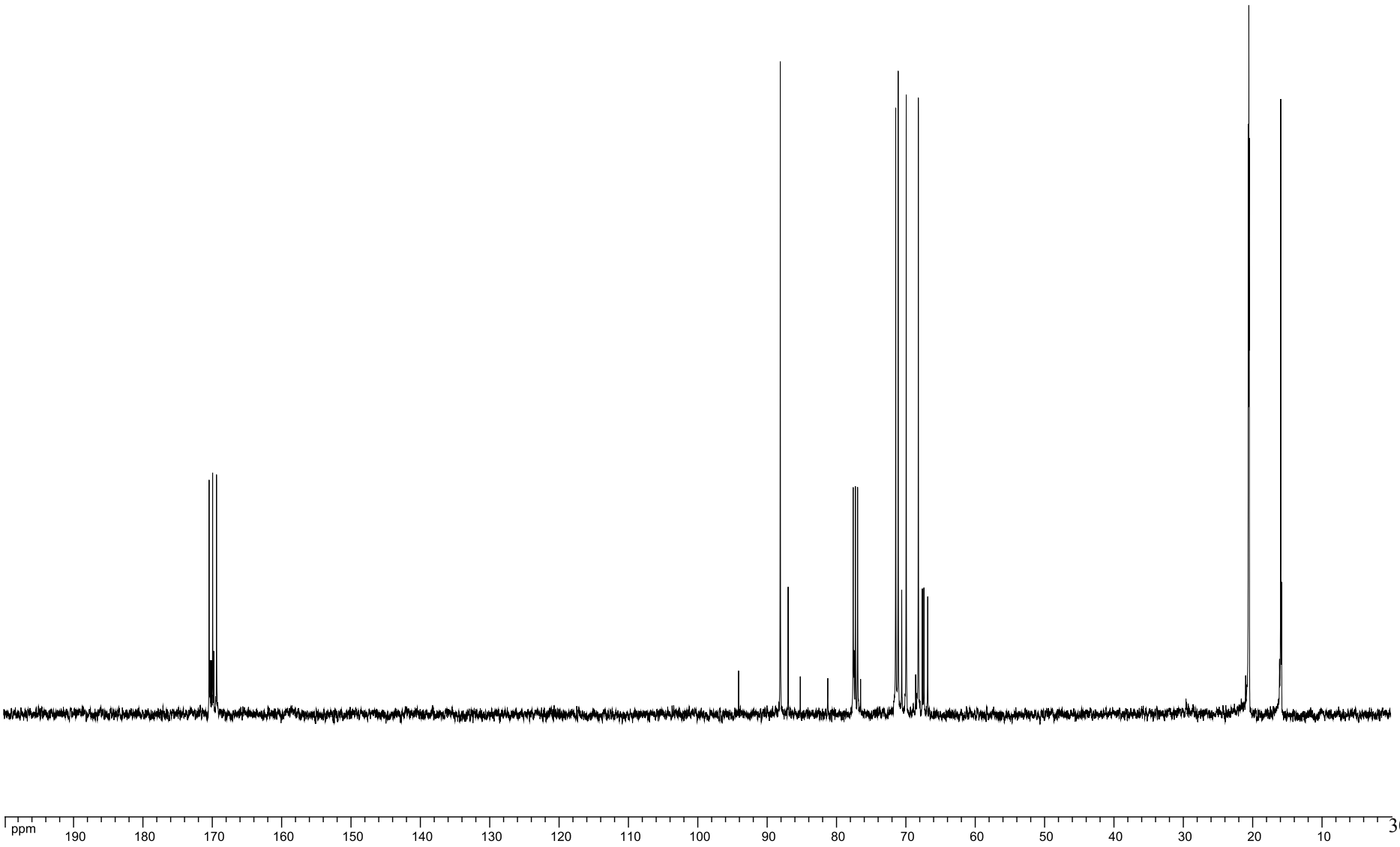
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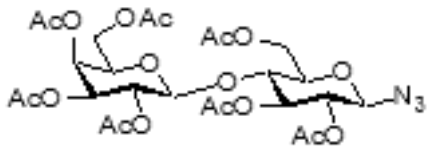
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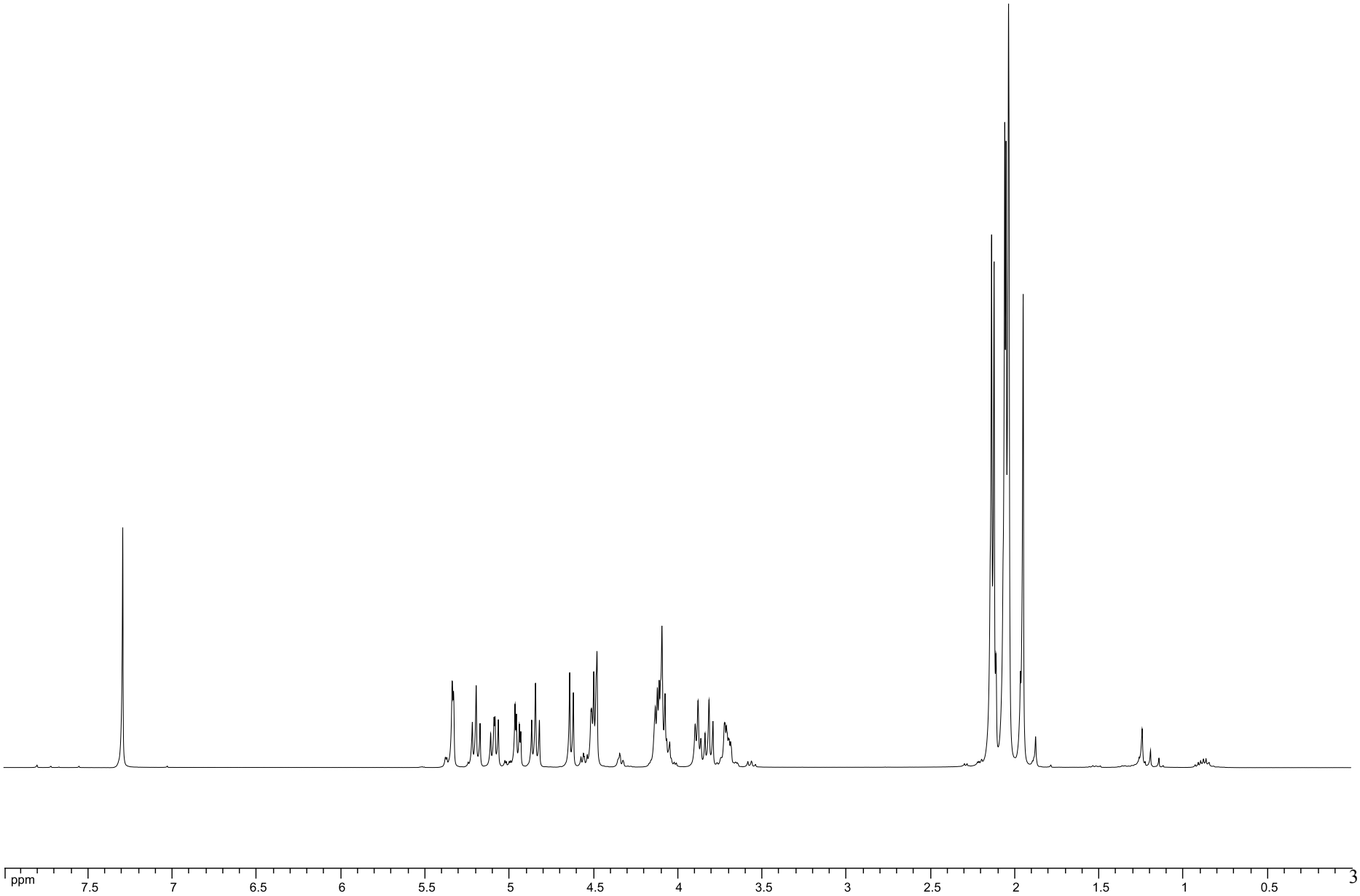
S6-26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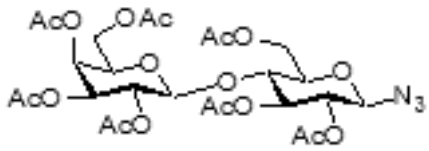
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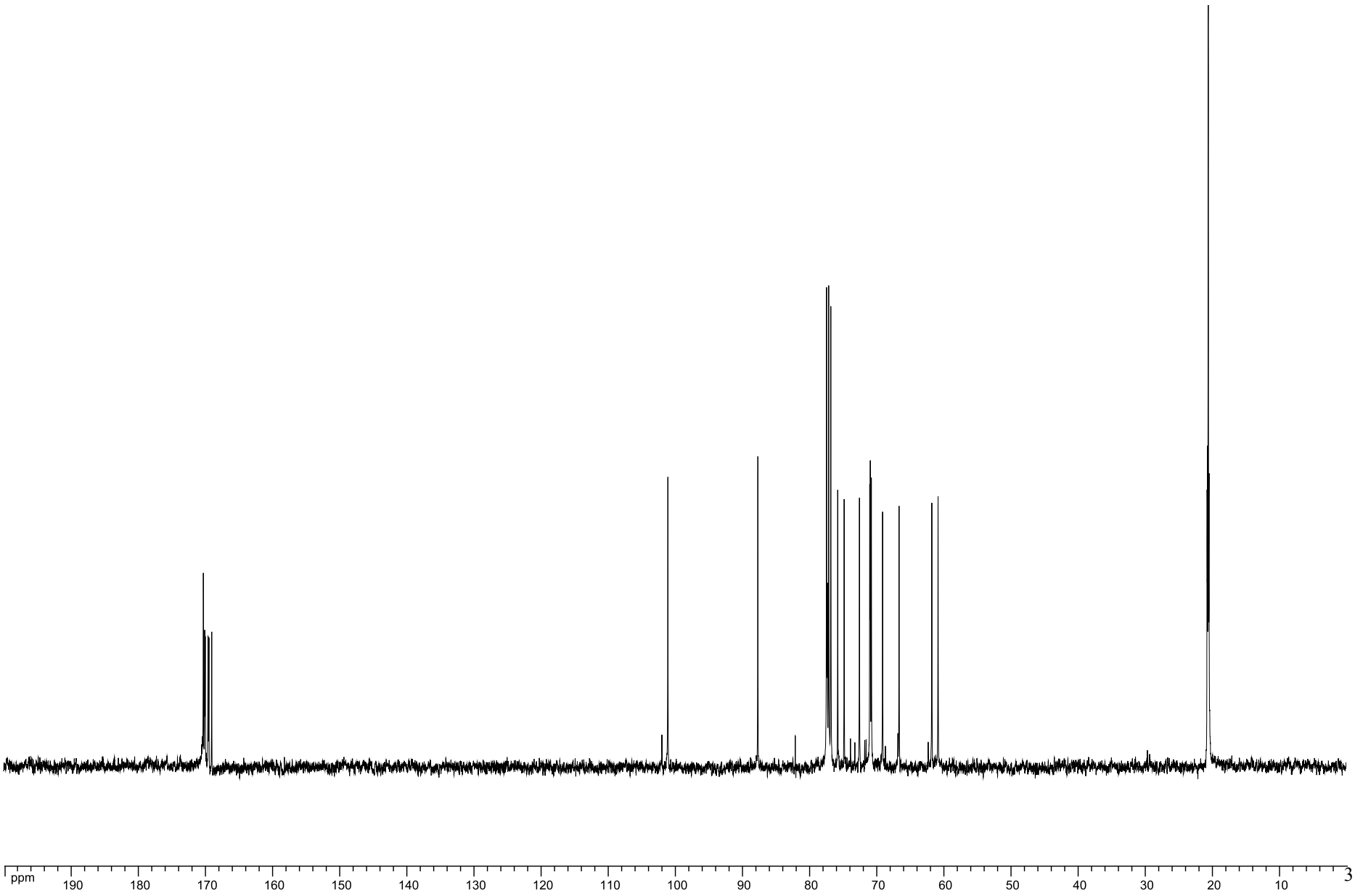
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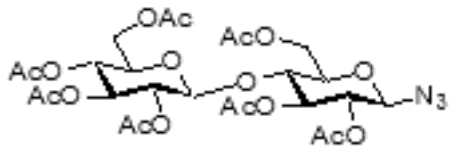
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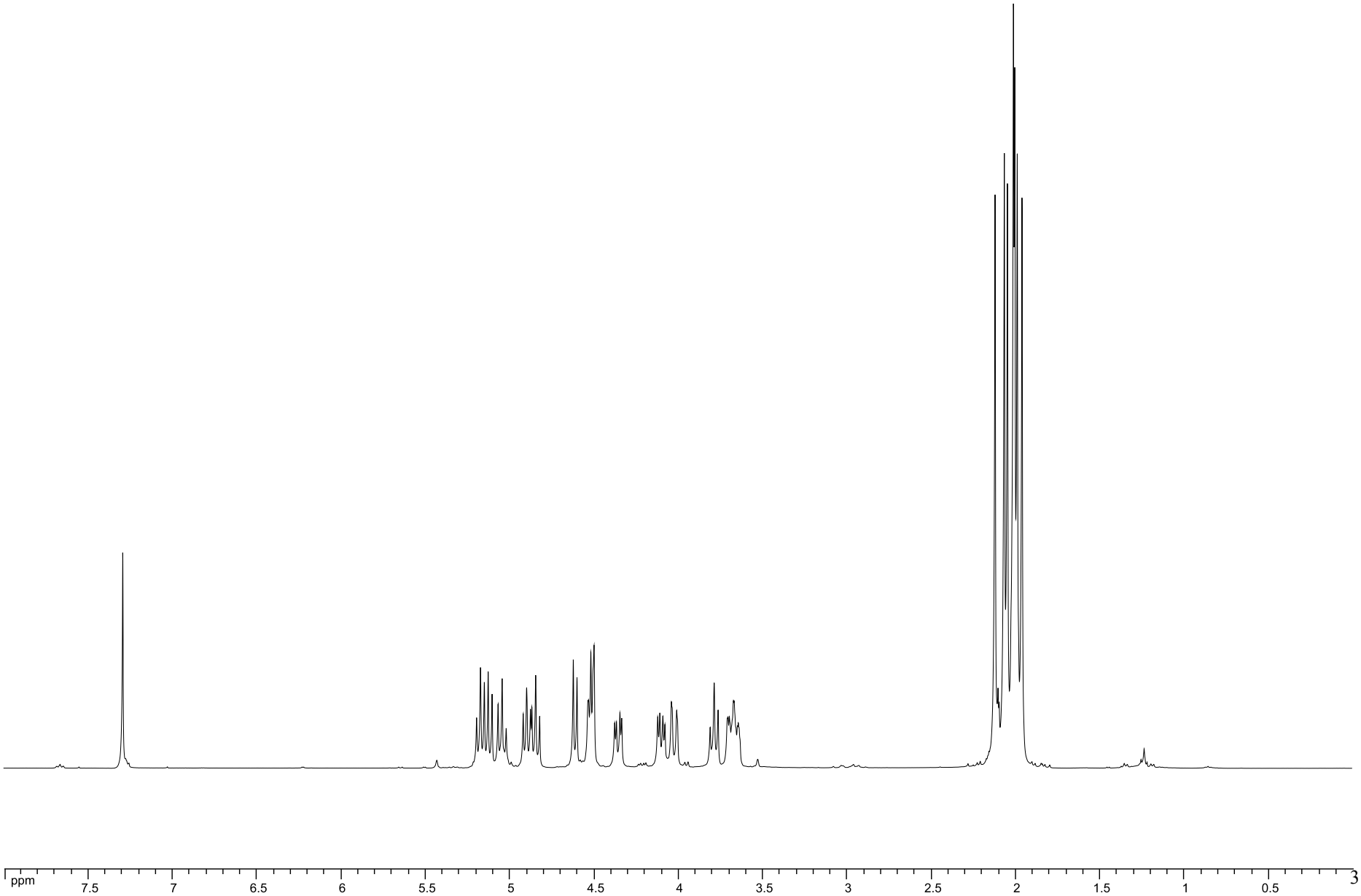
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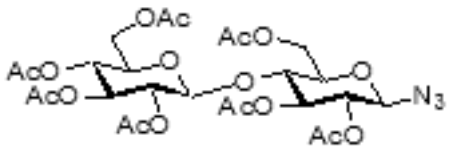
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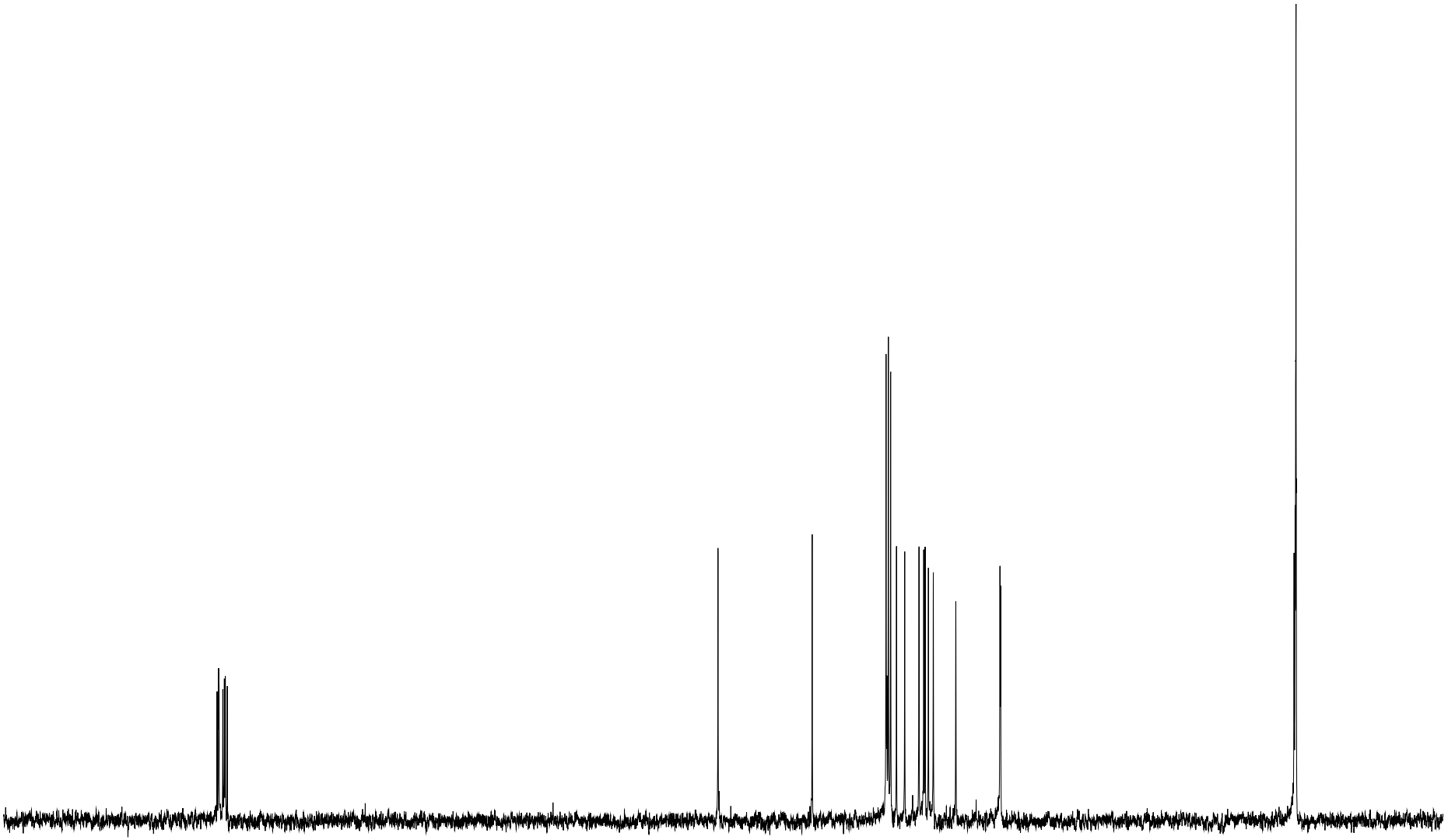

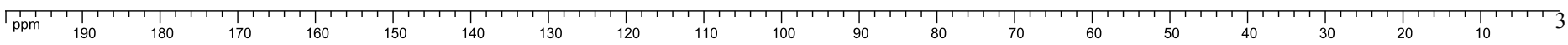
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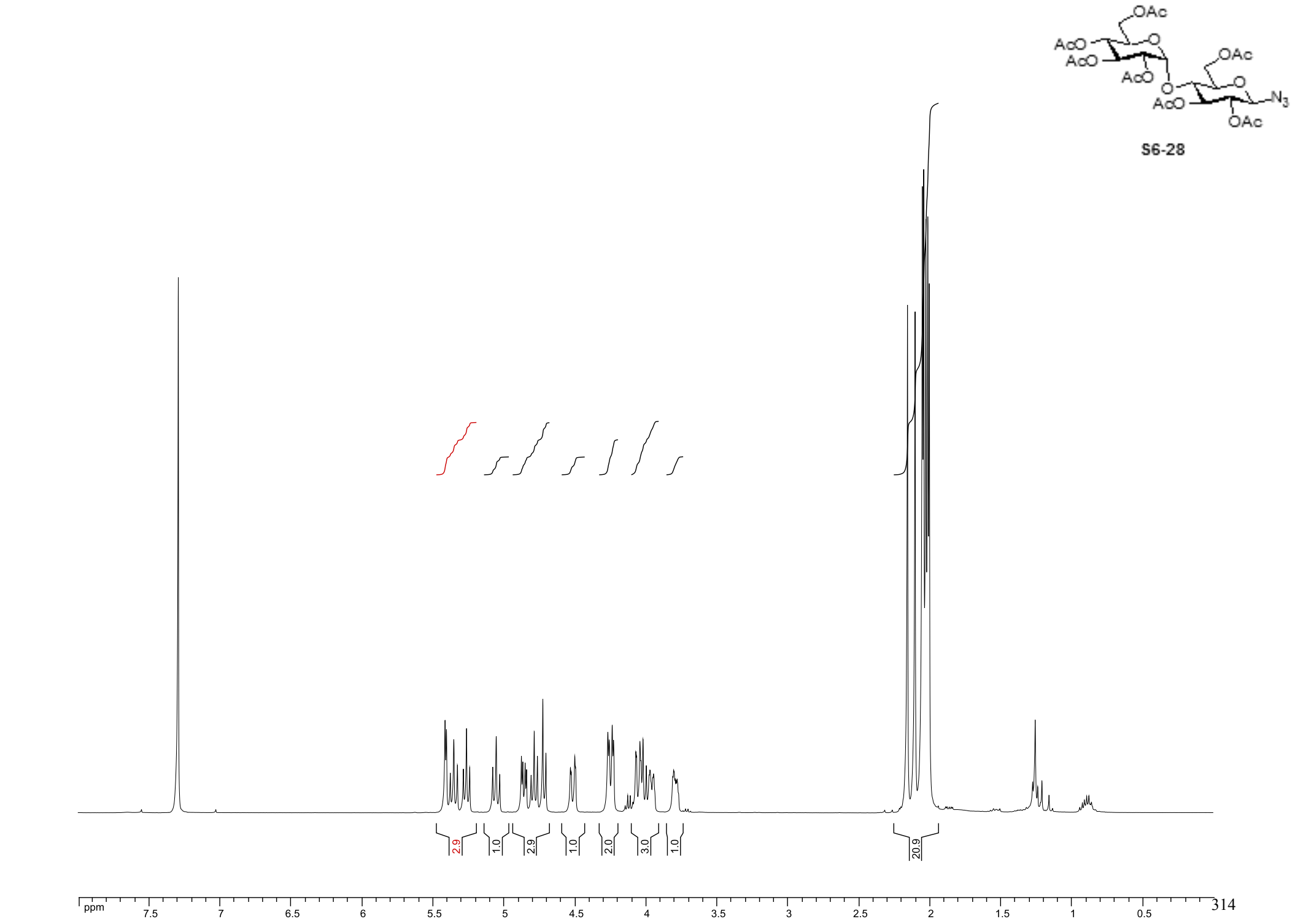
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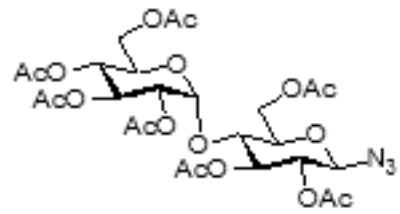
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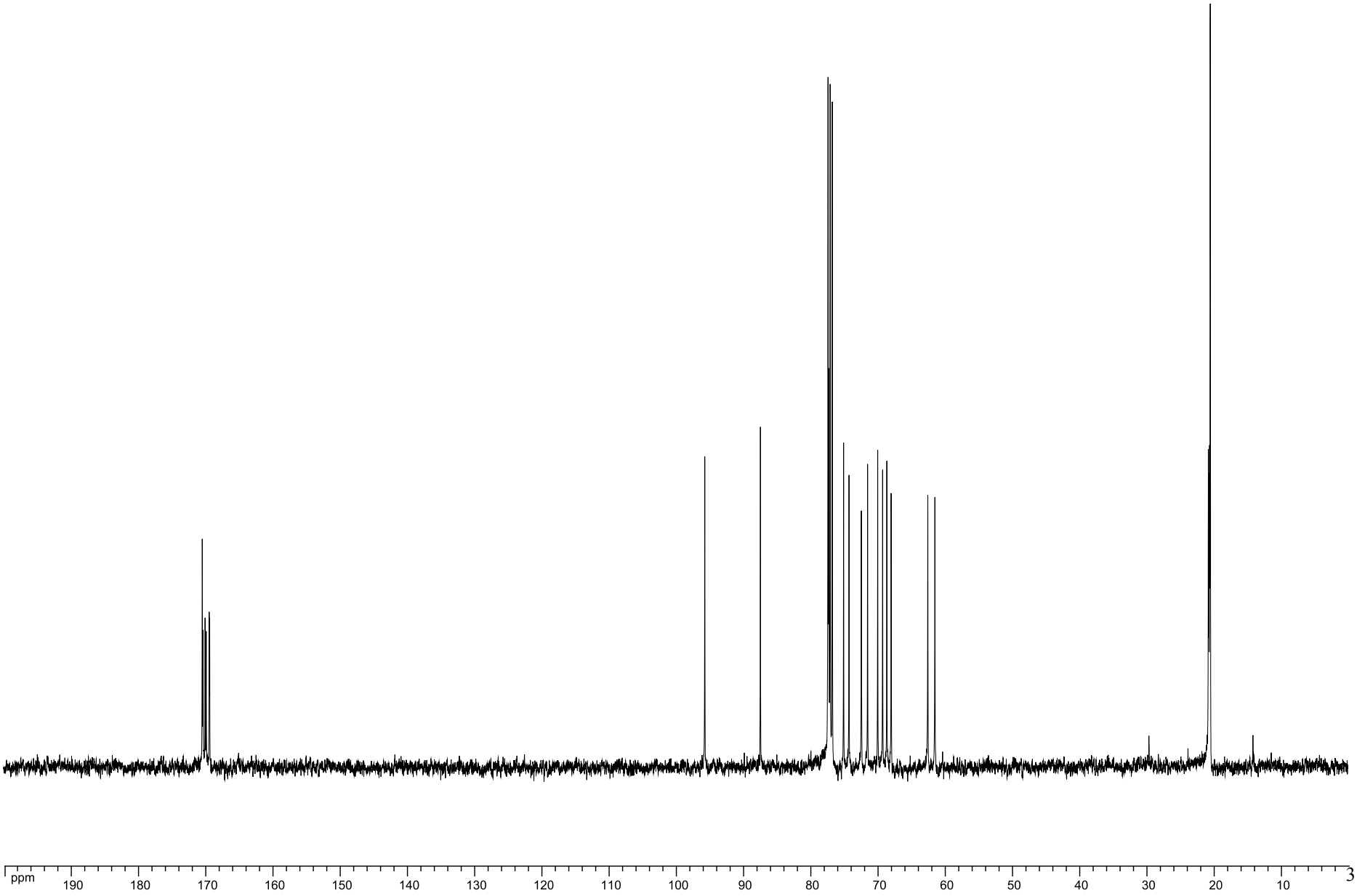
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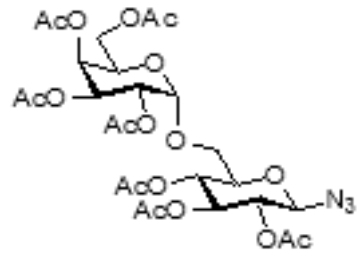
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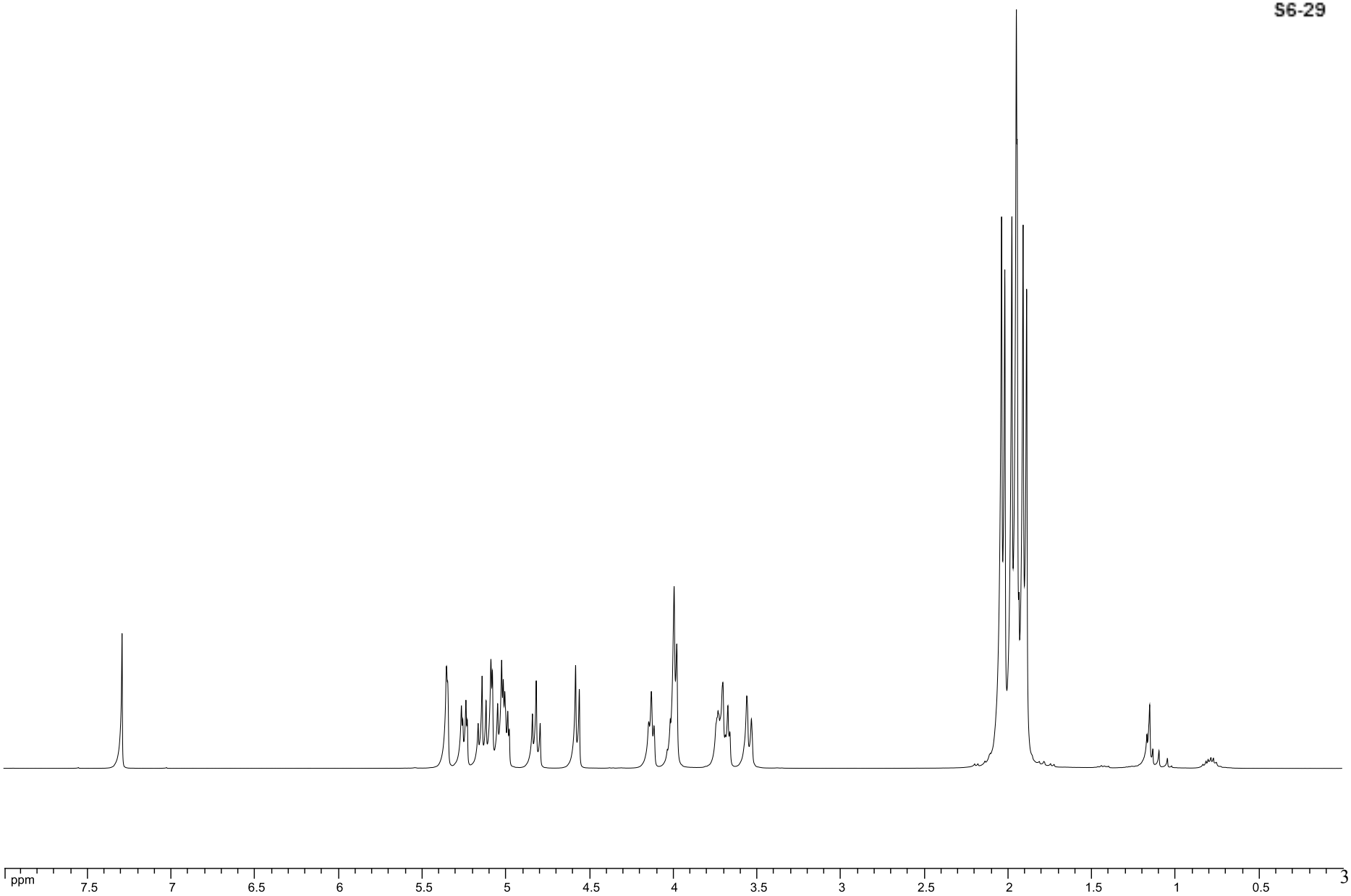
S6-29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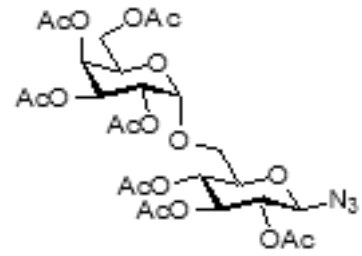
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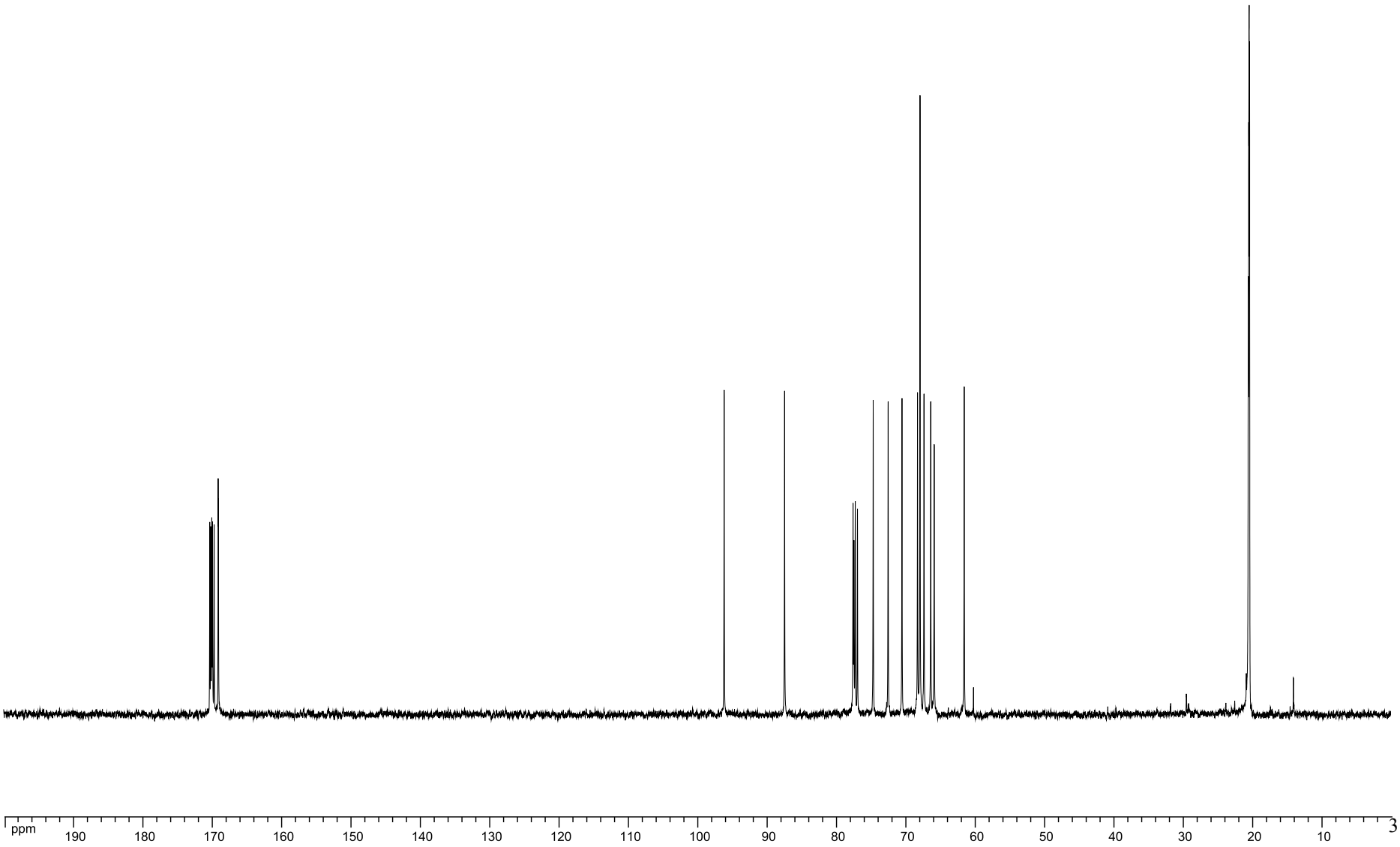
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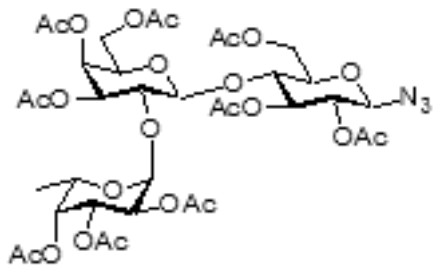
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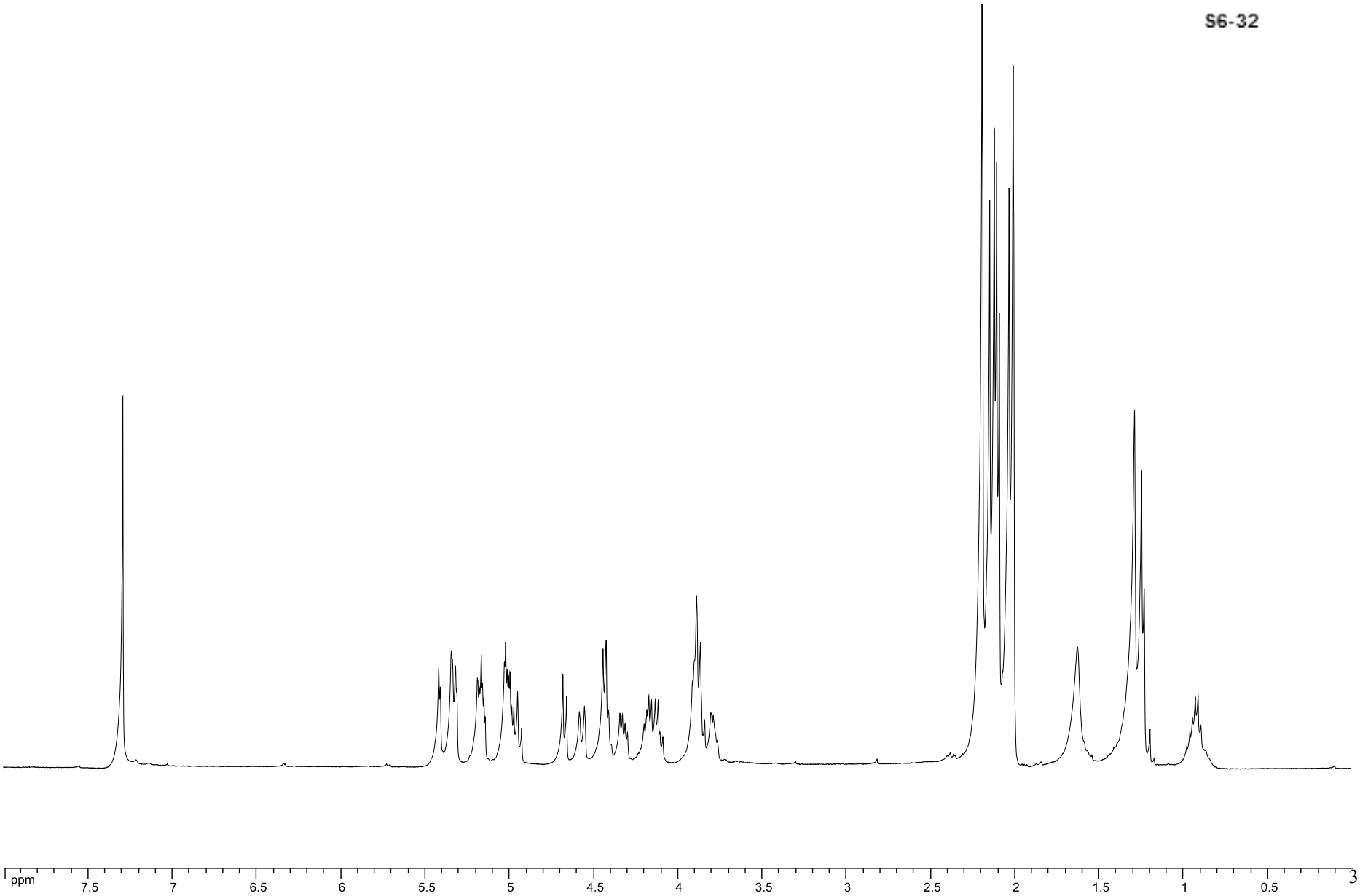
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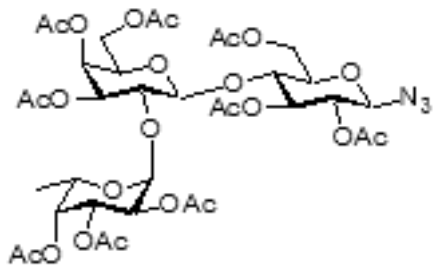

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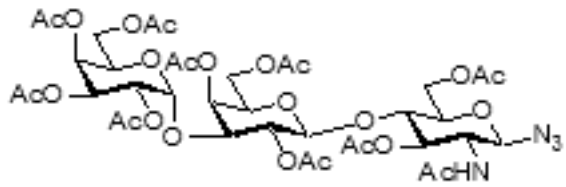
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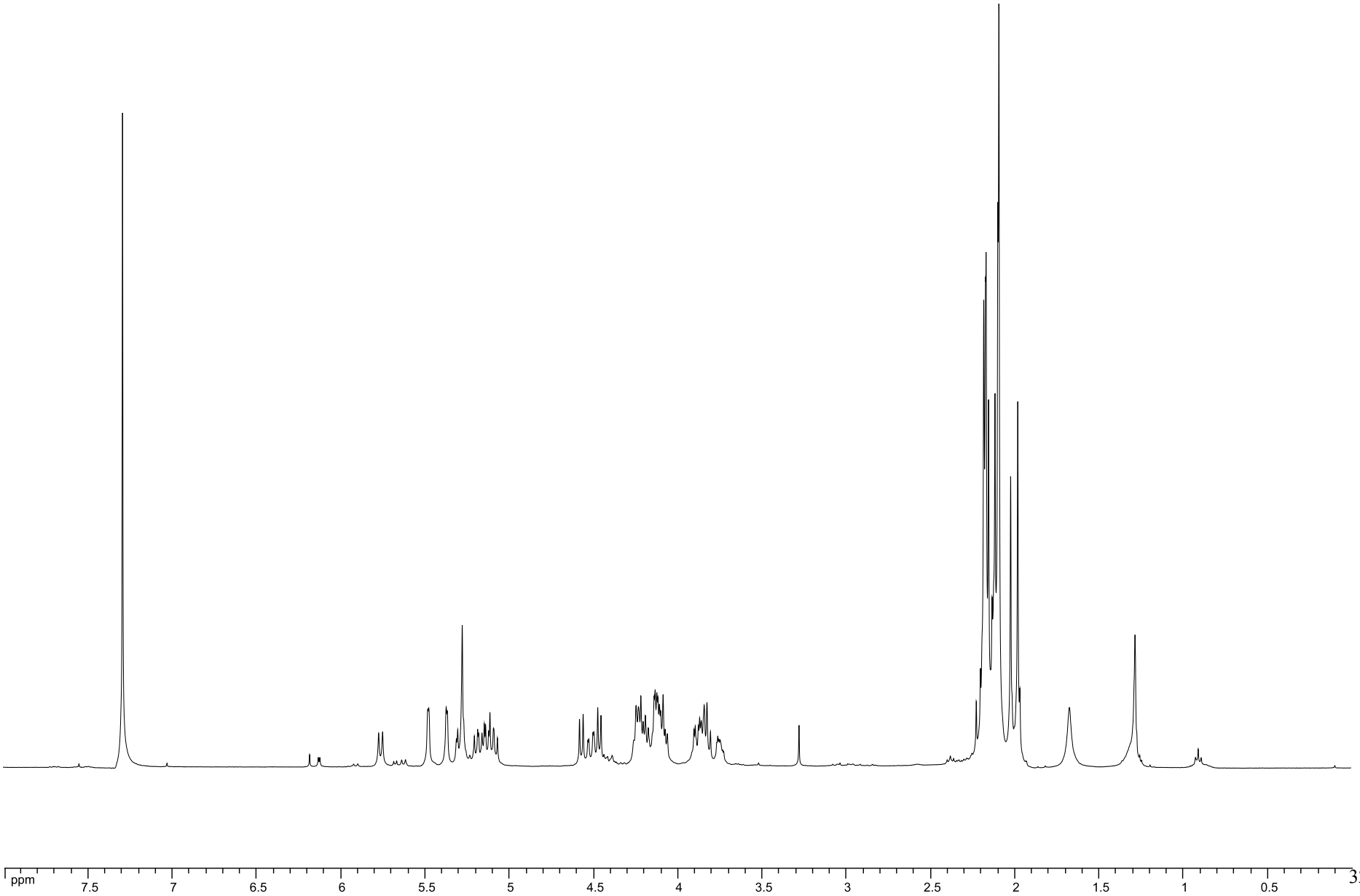
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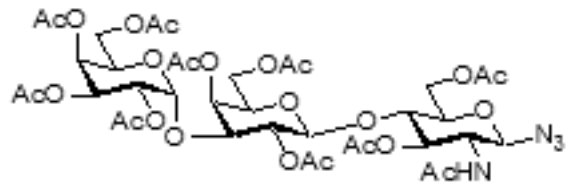
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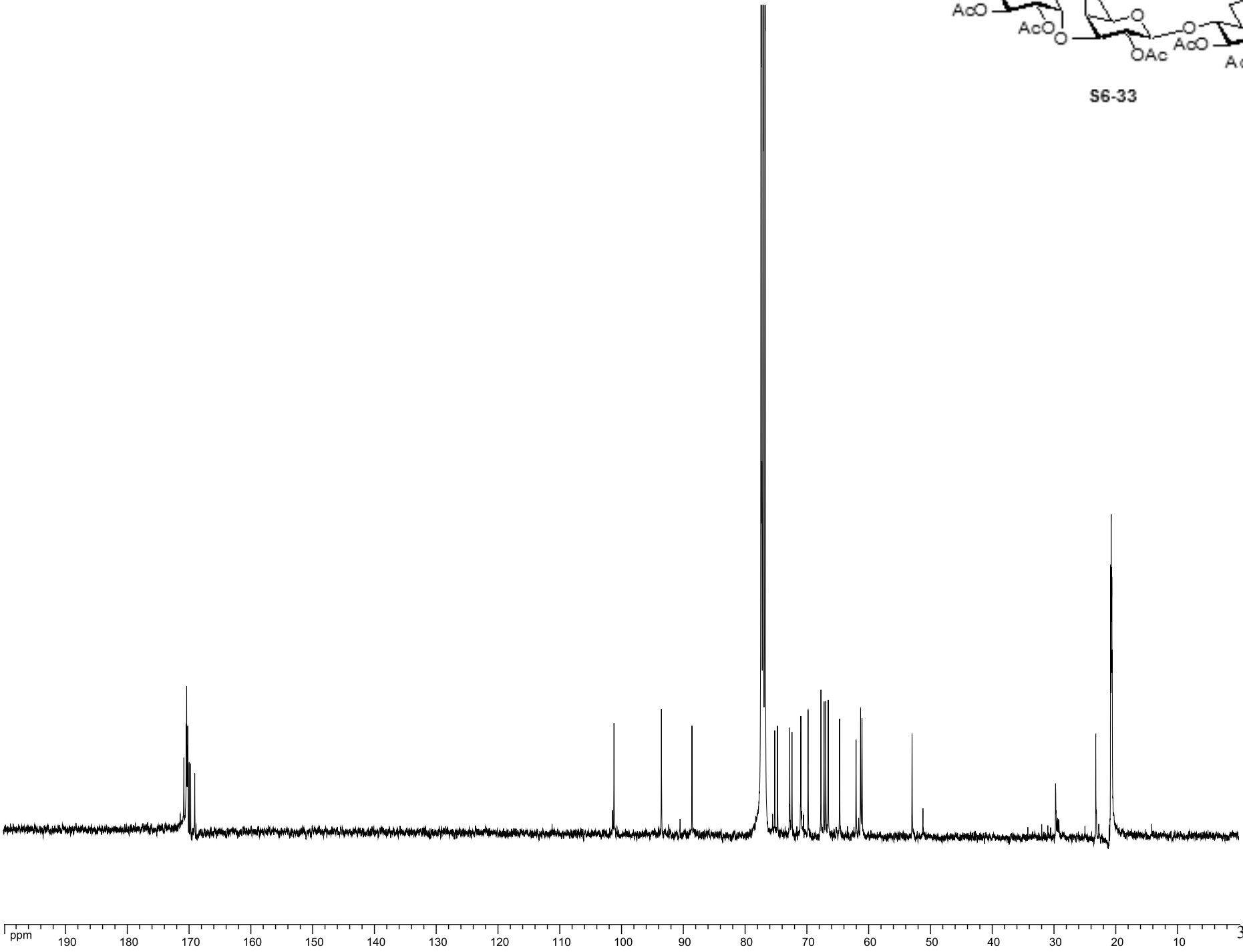
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Kuo-Ting Huang

Born in Taipei, Taiwan (1976)

CONTACT

Institut de Science et d'Ingénierie Supramoléculaires (ISIS), Université de Strasbourg
8 allée Gaspard Monge, 67000 Strasbourg, France.
Tel: 33 (0)3 68 85 51 57
Email: kt.huang@isis.u-strasbg.fr

EDUCATION

2006-2010 Université de Strasbourg, Strasbourg, France
Ph.D Institut de Science et d'Ingénierie Supramoléculaires (ISIS)
Advisor: Professor Nicolas Winssinger
Thesis: Novel Strategies for Carbohydrate Synthesis and Assemblies

1999-2001 National Dong Hwa University, Hualien, Taiwan
M.Sc Department of Chemistry
Advisor: Professor Chung-Ming Sun
Thesis: Liquid-Phase Combinatorial Synthesis of Heterocyclic Compounds

1995-1999 Chung Yuan Christian University, Chung Li, Taiwan
B.Sc Department of Chemistry

RESEARCH AND WORK EXPERIENCE

01.2005-05.2006 Academia Sinica, Taipei, Taiwan
R.A Institute of Chemistry
Advisor: Professor Chun-Cheng Lin
Research fields: Bioorganic Chemistry and Carbohydrate Chemistry

01.2002-01.2005 Academia Sinica, Taipei, Taiwan
R.A Institute of Chemistry
Advisors: Professor Chi-Huey Wong and Professor Chun-Cheng Lin
Research fields: Bioorganic Chemistry and Carbohydrate Chemistry

08.2001-09.2001 National Dong Hwa University, Hualien, Taiwan
R.A Department of Chemistry
Advisor: Professor Chung-Ming Sun

PUBLICATIONS

1. "Liquid Phase Parallel Synthesis of Ureas" K.-T. Huang, C.-M. Sun, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 271-273.
2. "Liquid Phase Combinatorial Synthesis of Amino- benzimidazoles" K.-T. Huang, C.-M. Sun, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1001-1003.
3. "*N*-Trifluoroacetyl Sialyl Phosphite Donors for the Synthesis of α(2→9) Oligosialic Acids" C.-C. Lin, K.-T. Huang, C.-C. Lin, *Org. Lett.* **2005**, *7*, 4169-4172.
4. "Tuning the conformation properties of a peptide by glycosylation and phosphorylation" F.-C. Liang, R. P.-Y. Chen, C.-C. Lin, K.-T. Huang, S.-I. Chan, *Biochem. Biophys. Res. Commun.* **2006**, *342*, 482-488.
5. "Site-Specific Protein Modification via Copper(Ⅰ)-Catalyzed 1,2,3-Triazole Formation and Its Implementation in Protein Microarray Fabrication" P.-C. Lin, S.-H. Ueng, M.-C. Tseng, J.-L. Ko, K.-T. Huang, S.-C. Yu, A. K. Adak, Y.-J. Chen, C.-C. Lin, *Angew. Chem. Int. Ed.* **2006**, *45*, 4286-4290.
6. "Multi-Enzyme One-Pot Strategy for the Synthesis of Sialyl Lewis X-Containing PSGL-1 Glycopeptide" K.-T. Huang, B.-C. Wu, C.-C. Lin, S.-H. Luo, C. Chen, C.-H. Wong, C.-C. Lin *Carbohydr. Res.* **2006**, *341*, 2151-2155.
7. "IPy2BF4-Mediated Glycosylation and Glycosyl Fluoride Formation" K.-T. Huang, N. Winssinger, *Eur. J. Org. Chem.* **2007**, 1887-1890.
8. "Tuning the conformational properties of the prion peptide" C.-C. Ho, L. Y.-L. Lee, K.-T. Huang, C.-C. Lin, M.-Y. Ku, C.-C. Yang, S.-I. Chan, R.-L. Hsu, R. P.-Y. Chen, *Proteins.* **2009**, *76*, 213-225.
9. "DAST-Mediated Regioselective Anomeric Group Migration in Saccharides" P.-C. Lin, A. K. Adak, S.-H. Ueng, L.-D. Huang, K.-T. Huang, J.-A. A. Ho, C.-C. Lin, *J. Org. Chem.* **2009**, *74*, 4041-4048.
10. "DNA-Templated Homo and Heterodimerization of PNA-encoded Oligosaccharides Mimicking HIV's Carbohydrate Epitope" K. Gorska, K.-T. Huang, O. Chaloin, N. Winssinger, *Angew. Chem. Int. Ed.* **2009**, *48*, 7695-7700. (KG and KTH contributed equally to this work)
11. "Synthesis of *N*-modified sTn analogs and evaluation of their immunogenicities by microarray-based immunoassay" S. Sahabuddin, T.-C. Chang, C.-C. Lin**,** F.-D. Jan, H.-Y. Hsiao, K.-T. Huang, J.-H. Chen, J.-C. Horng, J.-A. A. Ho, C.-C. Lin, *Tetrahedron* **2010**, in press.
12. "Combinatorial Self-Assembly of Glycan Fragments into Microarrays" K.-T. Huang, K. Gorska, S. Alvarez, S. Barluenga, N. Winssinger, *Angew. Chem. Int. Ed.* **2010**, revised

CONFERENCE PRESENTATION

09.2010 MipTec Conference, Basel, Switzerland; *Poster*.

07.2010 12th Belgian Organic Synthesis Symposium, Namur, Belgium; *Poster*

07.2010 2nd Joint Symposium Strasbourg – Osaka, Strasbourg, France; *Poster*

11.2007 6th JSPS Forum in France, Strasbourg, France; *Poster*

10.2007 5th International Congress of Young Chemists, Jurata, Poland; *Oral Presentation.*

06.2007 8th Tetrahedron Symposium, Berlin, Germany; *Poster*

10.2005 10th International Chemical Conference in Taipei, Hsinchu, Taiwan; *Poster*

FELLOWSHIP

2006-2010 French Ministry of Research and Education

2008-2010 Taiwan Ministry of Education