JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
Cite This: J. Am. Chem. Soc. 2019, 141, 4547−4552
pubs.acs.org/JACS

Redox-Controlled Site-Specific α2−6-Sialylation

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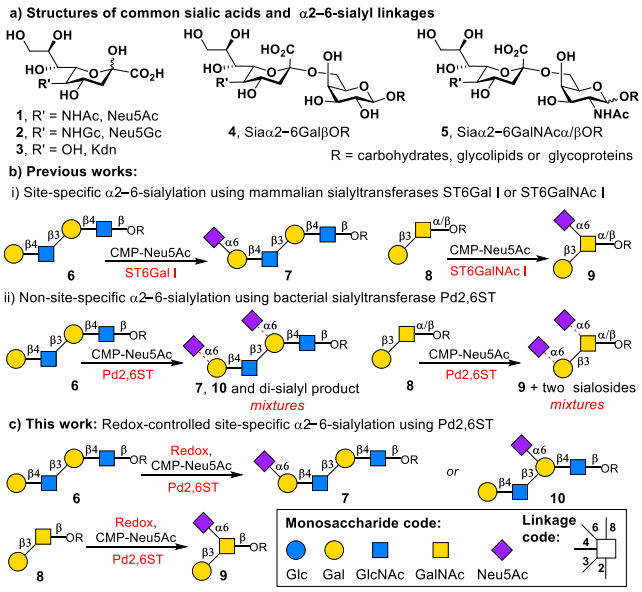
Supporting Information

ABSTRACT: The first bacterial α2−6-sialyltransferase cloned from *Photobacterium damselae* (Pd2,6ST) has been widely applied for the synthesis of various α2−6-linked sialosides. However, the extreme substrate flexibility of Pd2,6ST makes it unsuitable for site-specific α2−6-sialylation of complex substrates containing multiple galactose and/or *N*-acetylgalactosamine units. To tackle this problem, a general redox-controlled site-specific sialylation strategy using Pd2,6ST is described. This approach features site-specific enzymatic oxidation of galactose units to mask the unwanted sialylation sites and precisely controlling the site-specific α2−6-sialylation at intact galactose or *N*-acetylgalactosamine units.

Sialic acids (Sias) are the most common termini and among the most abundant monosaccharides of mammalian glycans.[1] As ubiquitous components of glycoproteins and glycolipids, three common sialic acids of *N*-acetylneuraminic acid (Neu5Ac, **1**), *N*-glycolylneuraminic acid (Neu5Gc, **2**) and 2-keto-3-deoxy-nonulosonic acid (Kdn, **3**) are found α2−6-linked to galactose (Gal) or *N*-acetylgalactosamine (GalNAc) residues (Scheme 1a). Owing to their remarkable structural diversity, sialic acid-containing glycans play important roles in many physiological and pathological processes, and most sialic acid-related biological processes require specific sialic acid forms, glycosidic linkage and defined underlying glycan chains.[2] For example, avian influenza viruses primarily bind to α2−3-linked sialic acid, whereas human influenza viruses preferentially recognize α2−6-linked sialic acid.[3] Recent studies also demonstrated that α2−6-sialylation is required for the anti-inflammatory activities of intravenous immunoglobulin (IVIG), and *in vitro* glycoengineered IVIG with uniform α2−6-sialylated *N*-glycans showed 10-fold enhancement in anti-inflammatory activities compared to unfractionated IVIG.[4] It is also well-known that the high expression levels of α2−6-sialylated glycans on a number of carcinomas are correlated with cancer progression and poor prognosis.[2b,5]

The past few decades have witnessed increasing attention on using glycosyltransferases for the synthesis of various glycans and glycoconjugates.[6] However, only very few recombinant sialyltransferases (SiaTs) from both mammals and bacteria have been widely applied for the construction of both α2−6-sialyl linkages **4** and **5** (Scheme 1a). ST6Gal I is a mammalian α2−6-SiaT which has been widely used for the α2−6-sialylation of terminal Gal residue, but it has very strict substrate specificity that can only use terminal type-2 glycan (Galβ1−4GlcNAc) as acceptor substrate (Scheme 1b, i).[7] ST6GalNAc I is another recombinant mammalian α2−6-SiaT which has been used for the α2−6-sialylation of GalNAc residue, but it can only use Tn antigen or T antigen as acceptor substrates (Scheme 1b, i).[8] The α2−6-sialylation of terminal Gal residues of type-1 glycan (Galβ1−3GlcNAc) and LacdiNAc (GalNAcβ1−4GlcNAc) have also been identified

Scheme 1. (a) Common Sialic Acid Forms and α2−6-Linkages; (b) Known and (c) Proposed Enzymatic α2−6-Sialylation Approaches



Received: January 9, 2019
Published: March 7, 2019

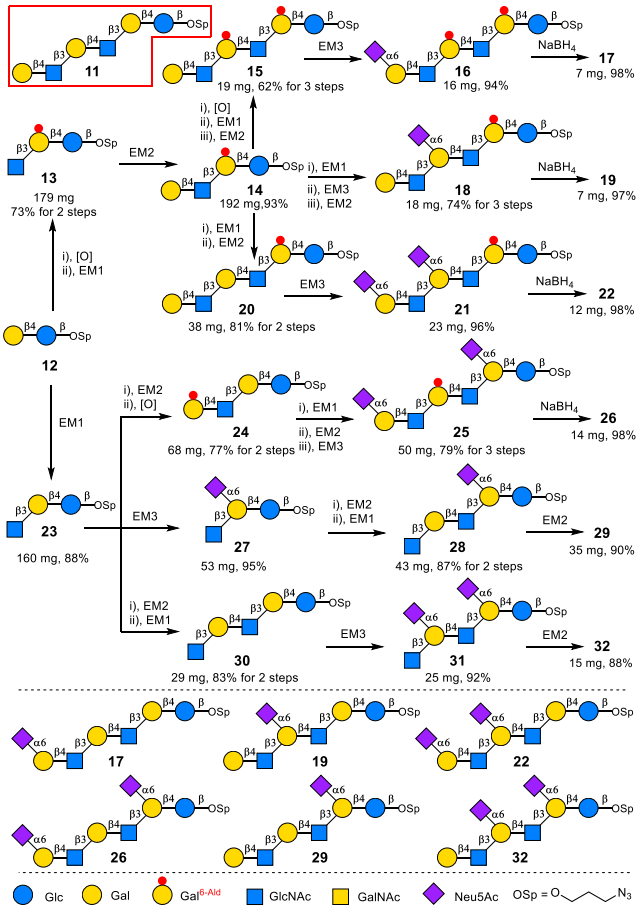
DOI: 10.1021/jacs.9b00044
J. Am. Chem. Soc. 2019, 141, 4547−4552

in a number of naturally occurring glycans;[9] however, the SiaTs responsible for the α2−6-sialylation modification are still unknown. Moreover, α-series cholinergic neuron-specific gangliosides, a subgroup of gangliosides with α2−6-sialylation on the GalNAc residue of extended type 1 glycan chain, play important roles in the development and regeneration of nervous system.[10] However, due to the substrate restriction and unavailability of siaT for modification of GalNAc residue, none of these complex sialoglycans have been enzymatically synthesized yet.[11]

In contrast to mammalian α2−6-SiaTs, the first recombinant bacterial α2−6-SiaT cloned from *Photobacterium damselae* (Pd2,6ST) can be overexpressed in a conventional *Escherichia coli* strain.[12] Owing to its remarkable activity and extreme substrate specificities, the Pd2,6ST has been extensively used for the construction of both Siaα2−6Gal and Siaα2−6GalNAc sequences for the synthesis of various *O*-glycans, *N*-glycans and human milk oligosaccharides.[7d,12a,13] Unfortunately, previous studies showed that the Pd2,6ST can recognize both internal and terminal Gal and GalNAc moieties of complex substrates, resulting a mixture of sialylated products (Scheme 1b, ii).[7d,13e−g] To overcome the limitations of both mammalian and bacterial α2−6-SiaTs, we describe herein a novel redox-controlled site-specific α2−6-sialylation approach to precisely control the reaction sites of Pd2,6ST for the synthesis of complex α2−6-linked sialosides (Scheme 1c).

To validate the feasibility of our proposed redox-controlled site-specific α2−6-sialylation strategy, lacto-*N*-neohexaoside (LNnH) **11** containing three Gal residues (Scheme 2) was investigated first as a model substrate. LNnH potentially has seven α2−6-sialylated products, including three monosialylated heptasaccharides **17**, **19** and **29**, and three disialylated octasaccharides **22**, **26** and **32**. Galactose oxidase (GOase), a commercially available copper metalloenzyme, has been widely used in biosensor for detecting Gal or terminal Gal-containing glycans by selective oxidation of the C6-hydroxyl group of free galactose or terminal Gal residue into C6-aldehyde galactose (Gal$^{6\text{-Ald}}$).[14] The resulting C6-aldehyde group has also been extensively used as a chemical handle for further labeling and derivatization purposes.[14,15] We envisioned that the Gal$^{6\text{-Ald}}$ would act as a protected Gal residue and would not be sialylated by Pd2,6ST. Therefore, the site-specifically introduction of α2−6-linked sialic acid to the intact Gal residues could be achieved. To obtain monosialylated heptasaccharide **17** with an α2−6-linked sialic acid at nonreducing end of hexasaccharide **11**, both internal Gal residues of **11** need to be protected by oxidation, while the terminal Gal was left intact for site-specific α2−6-sialylation. As shown in Scheme 2, lactoside **12** was treated with oxidation module in the presence of GOase and peroxidase to convert the terminal Gal residue into Gal$^{6\text{-Ald}}$. The resulting disaccharide was then extended by a one-pot three-enzyme β1−3-*N*-acetylglucosaminylation module (EM1)[13b,d] to give trisaccharide **13** in 73% yields for 2 steps. Trisaccharide **13** was elongated by a β1−4-linked Gal using enzyme module 2 (EM2),[13b,d] and the nascent terminal Gal was also converted into Gal$^{6\text{-Ald}}$ by oxidation module, and then extended by sequential glycosylation with EM1 and EM2 to afford the hexasaccharide intermediate **15**. Although, all C6-aldehyde groups of oxidized Gal moieties exist as hydrated germinal diols, none of them could be utilized by Pd2,6ST in EM3[13b,d] as sialylation site. The monosialylated **16** was isolated as only product in 94% yield. Two Gal$^{6\text{-Ald}}$ residues of **16** can be reduced back to the Gal moieties by simply

Scheme 2. Redox-Controlled Site Specific α2−6-Sialylation of Lacto-*N*-neohexaoside (LNnH) 11[a]



[a]Reagents and conditions: [O], one-pot two enzyme oxidation module with commercial galactose oxidase and peroxidase; EM1, one-pot three-enzyme β1−3-*N*-acetylglucosaminylation module with BlNahK, EcGlmU and HpLgtA; EM2, one-pot three-enzyme β1−4-galactosylation module with EcGalK, BLUSP and NmLgtB; EM3, one-pot two-enzyme α2−6-sialylation module with NmCSS and Pd2,6ST, see Supporting Information for details.

treating with $NaBH_4$ in aqueous solution to provide monosialylated heptasaccharide **17** in 98% yield. The same redox-controlled site-specific α2−6-sialylation strategy was also successfully applied in the synthesis of monosialylated heptasaccharide **19**, disialylated octasaccharides **22** and **26** using corresponding Gal$^{6\text{-Ald}}$-containing glycans as intermediates. The syntheses of monosialylated heptasaccharide **29** and disialylated octasaccharide **32** can be realized by simply changing the glycosylation sequence of three same enzyme modules (EM1−EM3) without the need of oxidation module (Scheme 2).

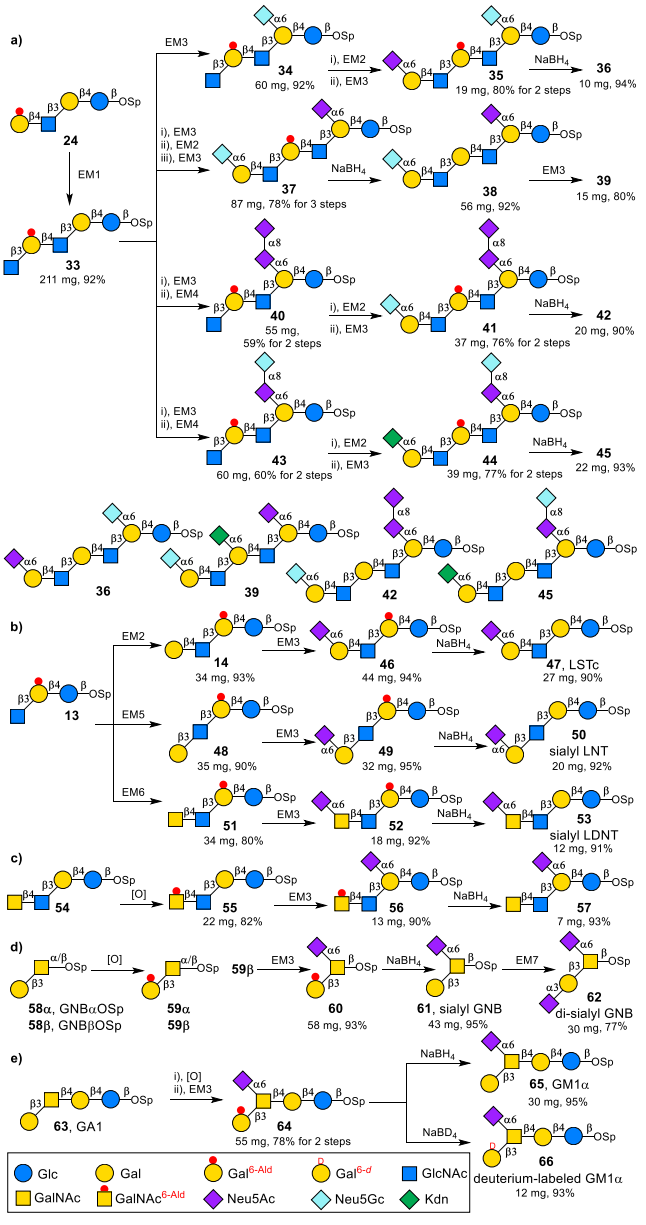
The Siaα2−6Gal sequence is not only a common terminal component of various *N*-, *O*-glycans and glycolipids, but also has been identified as internal moieties in a number of naturally occurring glycans.[9d,16] However, the SiaT from either mammalian or bacteria source that can catalyze site-specific α2−6-sialylation at the internal Gal is still unknown. The redox-controlled α2−6-sialylation strategy provides a practical approach to harness the extreme substrate flexibility offered by readily available bacterial sialyltransferase Pd2,6ST, thus

DOI: 10.1021/jacs.9b00044
J. Am. Chem. Soc. 2019, 141, 4547−4552

provides the first synthetic approach for on demand site-specific α2−6-sialylation of poly-LacNAc glycans.

This redox-controlled site-specific α2−6-sialylation strategy also provides an easy access for the synthesis of complex sialosides bearing different α2−6-linked sialic acid forms. As shown in Scheme 3a, the tetrasaccharide **24** with a terminal Gal$^{6\text{-Ald}}$ was extended using EM1, then the innermost intact Gal was modified with an α2−6-linked Neu5Gc using EM3 in the presence of Neu5Gc as the donor precursor to give hexasaccharide **34**. The **34** was treated with EM2 to form a LacNAc termini which was further modified with an α2−6-linked Neu5Ac by EM3 in the presence of Neu5Ac as the donor precursor to afford intermediate **35**. The Gal$^{6\text{-Ald}}$ moiety of **35** was reduced back to Gal by $NaBH_4$ to give disialyl octasaccharide **36** containing hybrid sialic acid forms in excellent overall yields. Taking advantage of this redox-controlled site-specific sialylation strategy, octasaccharide **38** with two different sialic acid forms, and nonasaccharide **39** with three different sialic acid forms at designated positions were also achieved from **24** (Scheme 3a). Moreover, a one-pot two-enzyme α2−8-sialylation enzyme module (EM4) comprising a recombinant α2−8-sialyltransferase from *Campylobacter jejuni* (CjCstII)[17] was applied for the modification of innermost α2−6-linked Neu5Ac to produce nonasaccharide **42** and **45** in good overall yields, respectively (Scheme 3a, see Supporting Information for details).

Scheme 3. Redox-Controlled Site Specific α2−6-Sialylation with Different Sialic Acid Forms and Substrate Scope and Application of Redox-Controlled Site-Specific α2−6-Sialylation Strategy[a]



[a]Reagents and conditions: EM4, one-pot two-enzyme α2−8-sialylation module with NmCSS and CjCstII; EM5, one-pot three-enzyme β1−3-galactosylation module with EcGalK, BLUSP and EcWbgO; EM6, one-pot three-enzyme β1−4-*N*-acetylgalactosaminylation module with BlNahK, EcGlmU and GalT1 Y289L; EM7, one-pot two-enzyme α2−3-sialylation module with NmCSS and PmST1, see Supporting Information for details.

Having established the redox-controlled site-specific α2−6-sialylation strategy for poly-LacNAc glycan receptors, the substrate scope and general applicability of the strategy was explored next. As shown in Scheme 3b, the terminal GlcNAc moiety of trisaccharide **13** was parallelly elaborated to type 2 chain by EM2 to give **14**, type 1 chain by EM5 to give **48**, and LacdiNAc by EM6 to give **51**, respectively. In addition to bacterial β1−4GalT NmLgtB in EM2, the recombinant β1−3GalT from *E. coli* (EcWbgO)[18] in EM5, and recombinant bovine GalT 1 mutant (GalT1 Y289L)[19] in EM6 all utilize trisaccharide **13** as receptor efficiently. As anticipated, the extreme substrate flexibility of Pd2,6ST in EM3 ensured the α2−6-sialylation at the terminal Gal of **14** and **48**, and terminal GalNAc of **51** furnishing pentasaccharides **46**, **49** and **52**, which were then treated with $NaBH_4$ to give the sialyl LNnT (LSTc) **47**, sialyl LNT **50** and sialyl LacdiNAc (sialyl LDNT) **53** in excellent yields, respectively (Scheme 3b). The GOase can also selectively oxidize the terminal GalNAc unit. Therefore, the selective oxidation of terminal GalNAc unit of LDNT **54** could achieve site-specific α2−6-sialylation at the internal Gal. After reduction, the monosialylated LDNT **57** was obtained in 69% overall yields for 3 steps (Scheme 3c).

The redox-controlled sialylation strategy could also be applied for the site-specific α2−6-sialylation of internal GalNAc residue for the synthesis of sialyl GNB **61**, disialyl GNB **62** and α-series ganglioside glycan GM1α **65**. It was shown that the Pd2,6ST could modify both Gal and GalNAc residues of GNB **58*α*** or **58*β***, resulting a mixture of two monosialylated and one disialylated products for each of them (Scheme 1b, ii).[13f] For site-specific α2−6-sialylation at internal GalNAc residue, the terminal Gal of **58*α*** or **58*β*** was converted into Gal$^{6\text{-Ald}}$ by oxidation enzyme module to give **59*α*** or **59*β***. Both **59*α*** or **59*β*** were treated with α2−6-sialylation module EM3, however, only **59*β*** could be utilized by Pd2,6ST to afford the sialoside **60**. These results were consistent with previous report that the α-linked GalNAc is not a good substrate for Pd2,6ST.[12a] Sialoside **60** was reduced with $NaBH_4$ to afford sialyl GNB **61** in 73% yields for 3 steps. The **61** could be further elaborated to disialyl GNB **62** by an α2−3-sialylation enzyme module (EM7)[13b,20] (Scheme 3d).

As aforementioned, owing to the substrate restriction and unavailability of siaT for modification of GalNAc residue, none of α-series cholinergic neuron-specific gangliosides has been enzymatically synthesized yet. The first enzymatic synthesis of α-series ganglioside glycan GM1α **65** using redox-controlled

DOI: 10.1021/jacs.9b00044
J. Am. Chem. Soc. 2019, 141, 4547−4552

sialylation strategy was also explored. Starting from known asialo-GM1 (GA1) **63**,[21] a similar sequence involving enzymatic oxidation, selective α2−6-sialylation of internal GalNAc, and subsequent reduction provided ganglioside GM1α **65** in 74% yields for 3 steps (Scheme 3e). Interestingly, the Pd2,6ST in EM3 can only introduce α2−6-linked Neu5Ac at GalNAc residue to give pentasaccharide **64**, while sialylation of innermost Gal residue was not observed. The redox-controlled sialylation strategy utilized for the synthesis of GM1α **65** provides a novel approach for the synthesis of other α-series gangliosides. Besides, by replacing $NaBH_4$ with $NaBD_4$ in the reduction step, the $Gal^{6\text{-}Ald}$-containing intermediates in this study, such as **64**, can be transformed into deuterium labeled products (e.g., GM1α **66**), which could be used as probe in elucidating multiple biochemical processes (Scheme 3d, see Supporting Information for details).

The binding profiles of synthesized sialosides with sialic acid-recognition proteins were also examined using printed sialoglycan slides. The plant lectin *Sambucus nigra* agglutinin (SNA) is known to be able to specifically recognize α2−6-linked sialosides.[22] SNA exhibited very strong binding exclusively to all sialosides with a terminal α2−6-sialylated LacNAc moiety, while no significant binding was observed for internal α2−6-sialylated glycans (Figure S7). Chicken polyclonal anti-Neu5Gc antibody IgY (pChGc) bound to all Neu5Gc-containg glycans with a preference toward terminal α2−6-linked Neu5Gc (Figure S8). In contrast, human anti-Neu5Gc antibody rich serum only bound tightly with terminal α2−6-linked Neu5Gc (Figure S9). The His-tagged typhoid toxin (PltB-His) recognized all Neu5Ac modified glycans (Figure S10), while human sialic acid-binding lectin Siglec-9 (hSiglec-9-Fc) only bound tightly to terminal Neu5Ac-modified glycans and Neu5Acα2−8Neu5Acα2−6-linked glycan (Figure S11). Plant lectins *Maackia amurensis* lectin I and II (MAL-I, -II) were also examined. As expected only α2−3-linked sialoside **62** exhibited strong affinity to MAL-II (Figure S12−S13).[22]

In summary, a novel substrate engineering strategy[23] was developed to harness the extreme substrate flexibility of Pd2,6ST for site-specific α2−6-sialylation of both Gal and GalNAc residues of various substrates. This strategy overcomes the limitation of availability and substrate specificities of known mammalian and bacterial α2−6-sialyltransferases, thereby providing a general and concise approach for the synthesis of complex α2−6-sialylated glycans with a single bacterial α2−6-sialyltransferase Pd2,6ST.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.9b00044.

Detailed experimental procedures and product characterization (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. Xi Chen at the University of California at Davis for sharing plasmids and providing helpful discussions, and Sandra Diaz at UCSD for technical help. This project was financially supported by National Natural Science Foundation of China (Grant Nos. 21672128, 21877072, 21807064), State Key Laboratory of Microbial Technology (M2016-06), Department of Science and Technology of Shandong Province (2016GGH4502, 2016GSF121002, ZR201709190252), Shandong University (2018JC053) and NIH grant R01GM32373.

■ REFERENCES

(1) Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C.-W.; Seeberger, P. H. Exploring the structural diversity of mammalian carbohydrates ("glycospace") by statistical databank analysis. *ACS Chem. Biol.* **2007**, *2*, 685−691.

(2) (a) Chen, X.; Varki, A. Advances in the biology and chemistry of sialic acids. *ACS Chem. Biol.* **2010**, *5*, 163−176. (b) Varki, A. Sialic acids in human health and disease. *Trends Mol. Med.* **2008**, *14*, 351−360. (c) Angata, T.; Varki, A. Chemical diversity in the sialic acids and related α-keto acids: an evolutionary perspective. *Chem. Rev.* **2002**, *102*, 439−470. (d) Schauer, R. Achievements and challenges of sialic acid research. *Glycoconjugate J.* **2000**, *17*, 485−499.

(3) Rogers, G.; Paulson, J.; Daniels, R.; Skehel, J.; Wilson, I.; Wiley, D. Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity. *Nature* **1983**, *304*, 76.

(4) Anthony, R. M.; Nimmerjahn, F.; Ashline, D. J.; Reinhold, V. N.; Paulson, J. C.; Ravetch, J. V. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. *Science* **2008**, *320*, 373−376.

(5) (a) Rodrigues, E.; Macauley, M. Hypersialylation in cancer: modulation of inflammation and therapeutic opportunities. *Cancers* **2018**, *10*, 207. (b) Büll, C.; Stoel, M. A.; den Brok, M. H.; Adema, G. J. Sialic acids sweeten a tumor's life. *Cancer Res.* **2014**, *74*, 3199−3204.

(6) For recent reviews, see: (a) Wen, L.; Edmunds, G.; Gibbons, C.; Zhang, J.; Gadi, M. R.; Zhu, H.; Fang, J.; Liu, X.; Kong, Y.; Wang, P. G. Toward automated enzymatic synthesis of oligosaccharides. *Chem. Rev.* **2018**, *118*, 8151−8187. (b) Yu, C. C.; Withers, S. G. Recent developments in enzymatic synthesis of modified sialic acid derivatives. *Adv. Synth. Catal.* **2015**, *357*, 1633−1654. (c) Schmaltz, R. M.; Hanson, S. R.; Wong, C.-H. Enzymes in the synthesis of glycoconjugates. *Chem. Rev.* **2011**, *111*, 4259−4307. (d) Palcic, M. M. Glycosyltransferases as biocatalysts. *Curr. Opin. Chem. Biol.* **2011**, *15*, 226−233.

(7) (a) Moremen, K. W.; Ramiah, A.; Stuart, M.; Steel, J.; Meng, L.; Forouhar, F.; Moniz, H. A.; Gahlay, G.; Gao, Z.; Chapla, D.; et al. Expression system for structural and functional studies of human glycosylation enzymes. *Nat. Chem. Biol.* **2018**, *14*, 156−162. (b) Prudden, A. R.; Liu, L.; Capicciotti, C. J.; Wolfert, M. A.; Wang, S.; Gao, Z.; Meng, L.; Moremen, K. W.; Boons, G.-J. Synthesis of asymmetrical multiantennary human milk oligosaccharides. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 6954−6959. (c) Wang, Z.; Chinoy, Z. S.; Ambre, S. G.; Peng, W.; McBride, R.; de Vries, R. P.; Glushka, J.; Paulson, J. C.; Boons, G.-J. A general strategy for the chemoenzymatic synthesis of asymmetrically branched N-glycans. *Science* **2013**, *341*, 379−383. (d) Nycholat, C. M.; Peng, W.; McBride, R.; Antonopoulos, A.; de Vries, R. P.; Polonskaya, Z.; Finn, M.; Dell, A.; Haslam, S. M.; Paulson, J. C. Synthesis of biologically active N-and O-linked glycans with multisialylated poly-N-acetyllactosamine extensions using *P. damsela* α2−6 sialyltransferase. *J. Am. Chem. Soc.* **2013**, *135*, 18280−18283.

DOI: 10.1021/jacs.9b00044
J. Am. Chem. Soc. 2019, 141, 4547−4552

(8) For selected examples, see: (a) Blixt, O.; Allin, K.; Pereira, L.; Datta, A.; Paulson, J. C. Efficient chemoenzymatic synthesis of O-linked sialyl oligosaccharides. *J. Am. Chem. Soc.* **2002**, *124*, 5739−5746. (b) George, S. K.; Schwientek, T.; Holm, B.; Reis, C. A.; Clausen, H.; Kihlberg, J. Chemoenzymatic synthesis of sialylated glycopeptides derived from mucins and T-cell stimulating peptides. *J. Am. Chem. Soc.* **2001**, *123*, 11117−11125.

(9) (a) Anugraham, M.; Jacob, F.; Nixdorf, S.; Everest-Dass, A. V.; Heinzelmann-Schwarz, V.; Packer, N. H. Specific glycosylation of membrane proteins in epithelial ovarian cancer cell lines: glycan structures reflect gene expression and DNA methylation status. *Mol. Cell. Proteomics* **2014**, *13*, 2213−2232. (b) Saarinen, J.; Welgus, H. G.; Flizar, C. A.; Kalkkinen, N.; Helin, J. N-Glycan structures of matrix metalloproteinase-1 derived from human fibroblasts and from HT-1080 fibrosarcoma cells. *Eur. J. Biochem.* **1999**, *259*, 829−840. (c) Suzuki, Y.; Nakao, T.; Ito, T.; Watanabe, N.; Toda, Y.; Guiyun, X.; Suzuki, T.; Kobayashi, T.; Kimura, Y.; Yamada, A.; et al. Structural determination of gangliosides that bind to influenza A, B, and C viruses by an improved binding assay: strain-specific receptor epitopes in sialo-sugar chains. *Virology* **1992**, *189*, 121−131. (d) Podolsky, D. K. Oligosaccharide structures of isolated human colonic mucin species. *J. Biol. Chem.* **1985**, *260*, 15510−15515. (e) Mizuochi, T.; Yamashita, K.; Fujikawa, K.; Kisiel, W.; Kobata, A. The carbohydrate of bovine prothrombin. Occurrence of Galβ1,3-GlcNAc grouping in asparagine-linked sugar chains. *J. Biol. Chem.* **1979**, *254*, 6419−6425.

(10) Yang, L.; Zeller, C. B.; Shaper, N. L.; Kiso, M.; Hasegawa, A.; Shapiro, R. E.; Schnaar, R. L. Gangliosides are neuronal ligands for myelin-associated glycoprotein. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 814−818.

(11) (a) Hotta, K.; Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. Synthetic studies on sialoglycoconjugates 61: synthesis of α-series ganglioside GM1α. *J. Carbohydr. Chem.* **1994**, *13*, 665−677. (b) Prabhanjan, H.; Aoyama, K.; Kiso, M.; Hasegawa, A. Synthesis of disialoganglioside GD1α and its positional isomer. *Carbohydr. Res.* **1992**, *233*, 87−99. (c) Prabhanjan, H.; Kiso, M.; Hasegawa, A. Total synthesis of a disialoganglioside GD1α. *Carbohydr. Res.* **1991**, *222*, C1−C4.

(12) (a) Yu, H.; Huang, S.; Chokhawala, H.; Sun, M.; Zheng, H.; Chen, X. Highly efficient chemoenzymatic synthesis of naturally occurring and non-natural α-2,6-linked sialosides: a *P. damsela* α-2,6-sialyltransferase with extremely flexible donor-substrate specificity. *Angew. Chem., Int. Ed.* **2006**, *45*, 3938−3944. (b) Yamamoto, T.; Nakashizuka, M.; Terada, I. Cloning and expression of a marine bacterial β-galactoside α2,6-sialyltransferase gene from *Photobacterium damsela* JT0160. *J. Biochem.* **1998**, *123*, 94−100.

(13) For selected examples, see: (a) Yu, H.; Yan, X.; Autran, C. A.; Li, Y.; Etzold, S.; Latasiewicz, J.; Robertson, B. M.; Li, J.; Bode, L.; Chen, X. Enzymatic and chemoenzymatic syntheses of disialyl glycans and their necrotizing enterocolitis preventing effects. *J. Org. Chem.* **2017**, *82*, 13152−13160. (b) Yu, H.; Chen, X. One-pot multienzyme (OPME) systems for chemoenzymatic synthesis of carbohydrates. *Org. Biomol. Chem.* **2016**, *14*, 2809−2818. (c) Shivatare, S. S.; Chang, S.-H.; Tsai, T.-I.; Tseng, S. Y.; Shivatare, V. S.; Lin, Y.-S.; Cheng, Y.-Y.; Ren, C.-T.; Lee, C.-C. D.; Pawar, S.; Tsai, C.-S.; Shih, H.-W.; Zeng, Y.-F.; Liang, C.-H.; Kwong, P. D.; Burton, D. R.; Wu, C.-Y.; Wong, C.-H. Modular synthesis of N-glycans and arrays for the hetero-ligand binding analysis of HIV antibodies. *Nat. Chem.* **2016**, *8*, 338−346. (d) Chen, C.; Zhang, Y.; Xue, M.; Liu, X. W.; Li, Y.; Chen, X.; Wang, P. G.; Wang, F.; Cao, H. Sequential one-pot multienzyme (OPME) synthesis of lacto-N-neotetraose and its sialyl and fucosyl derivatives. *Chem. Commun.* **2015**, *51*, 7689−7692. (e) Yu, H.; Lau, K.; Thon, V.; Autran, C. A.; Jantscher-Krenn, E.; Xue, M.; Li, Y.; Sugiarto, G.; Qu, J.; Mu, S.; Ding, L.; Bode, L.; Chen, X. Synthetic disialyl hexasaccharides protect neonatal rats from necrotizing enterocolitis. *Angew. Chem., Int. Ed.* **2014**, *53*, 6687−6691. (f) Meng, X.; Yao, W.; Cheng, J.; Zhang, X.; Jin, L.; Yu, H.; Chen, X.; Wang, F.; Cao, H. Regioselective chemoenzymatic synthesis of ganglioside disialyl tetrasaccharide epitopes. *J. Am. Chem. Soc.* **2014**, *136*, 5205−5208. (g) Chien, W. T.; Liang, C. F.; Yu, C. C.; Lin, C. H.; Li, S. P.; Primadona, I.; Chen, Y. J.; Mong, K. K.; Lin, C. C. Sequential one-pot enzymatic synthesis of oligo-N-acetyllactosamine and its multi-sialylated extensions. *Chem. Commun.* **2014**, *50*, 5786−5789. (h) Malekan, H.; Fung, G.; Thon, V.; Khedri, Z.; Yu, H.; Qu, J.; Li, Y.; Ding, L.; Lam, K. S.; Chen, X. One-pot multi-enzyme (OPME) chemoenzymatic synthesis of sialyl-Tn-MUC1 and sialyl-T-MUC1 glycopeptides containing natural or non-natural sialic acid. *Bioorg. Med. Chem.* **2013**, *21*, 4778−4785. (i) Teo, C. F.; Hwang, T. S.; Chen, P. H.; Hung, C. H.; Gao, H. S.; Chang, L. S.; Lin, C. H. Synthesis of sialyl TN glycopeptides-enzymatic sialylation by α2,6-sialyltransferase from *Photobacterium damsela*. *Adv. Synth. Catal.* **2005**, *347*, 967−972. (j) Kajihara, Y.; Yamamoto, T.; Nagae, H.; Nakashizuka, M.; Sakakibara, T.; Terada, I. A novel α-2,6-sialyltransferase: transfer of sialic acid to fucosyl and sialyl trisaccharides. *J. Org. Chem.* **1996**, *61*, 8632−8635.

(14) (a) Parikka, K.; Master, E.; Tenkanen, M. Oxidation with galactose oxidase: multifunctional enzymatic catalysis. *J. Mol. Catal. B: Enzym.* **2015**, *120*, 47−59. (b) Rannes, J. B.; Ioannou, A.; Willies, S. C.; Grogan, G.; Behrens, C.; Flitsch, S. L.; Turner, N. J. Glycoprotein labeling using engineered variants of galactose oxidase obtained by directed evolution. *J. Am. Chem. Soc.* **2011**, *133*, 8436−8439.

(15) (a) Laaf, D.; Bojarová, P.; Pelantova, H.; Kren, V.; Elling, L. Tailored multivalent neo-glycoproteins: synthesis, evaluation, and application of a library of galectin-3-binding glycan ligands. *Bioconjugate Chem.* **2017**, *28*, 2832−2840. (b) Kupper, C. E.; Rosencrantz, R. R.; Henßen, B.; Pelantová, H.; Thönes, S.; Drozdová, A.; Křen, V.; Elling, L. Chemo-enzymatic modification of poly-N-acetyllactosamine (LacNAc) oligomers and N,N-diacetyllactosamine (LacDiNAc) based on galactose oxidase treatment. *Beilstein J. Org. Chem.* **2012**, *8*, 712−725.

(16) (a) Naumenko, O. I.; Zheng, H.; Senchenkova, S. N.; Wang, H.; Li, Q.; Shashkov, A. S.; Wang, J.; Knirel, Y. A.; Xiong, Y. Structures and gene clusters of the O-antigens of *Escherichia albertii* O3, O4, O6, and O7. *Carbohydr. Res.* **2017**, *449*, 17−22. (b) Deutschmann, R.; Boncheff, A. G.; Daraban, L.; MacInnes, J. I.; Monteiro, M. A. Common sialylated glycan in *Actinobacillus suis*. *Glycobiology* **2010**, *20*, 1227−1232. (c) Eserstam, R.; Rajaguru, T. P.; Jansson, P. E.; Weintraub, A.; Albert, M. J. The structure of the O-chain of the lipopolysaccharide of a prototypal diarrheagenic strain of *Hafnia alvei* that has characteristics of a new species under the genus *Escherichia*. *Eur. J. Biochem.* **2002**, *269*, 3289−3295. (d) Slomiany, B. L.; Murty, V.; Slomiany, A. Isolation and characterization of oligosaccharides from rat colonic mucus glycoprotein. *J. Biol. Chem.* **1980**, *255*, 9719−9723.

(17) (a) Yu, H.; Cheng, J.; Ding, L.; Khedri, Z.; Chen, Y.; Chin, S.; Lau, K.; Tiwari, V. K.; Chen, X. Chemoenzymatic synthesis of GD3 oligosaccharides and other disialyl glycans containing natural and non-natural sialic acids. *J. Am. Chem. Soc.* **2009**, *131*, 18467−18477. (b) Gilbert, M.; Brisson, J.-R.; Karwaski, M.-F.; Michniewicz, J.; Cunningham, A.-M.; Wu, Y.; Young, N. M.; Wakarchuk, W. W. Biosynthesis of ganglioside mimics in *Campylobacter jejuni* OH4384 identification of the glycosyltransferase genes, enzymatic synthesis of model compounds, and characterization of nanomole amounts by 600-MHz ^{1}H and ^{13}C NMR analysis. *J. Biol. Chem.* **2000**, *275*, 3896−3906.

(18) Liu, X.-w.; Xia, C.; Li, L.; Guan, W.-y.; Pettit, N.; Zhang, H.-c.; Chen, M.; Wang, P. G. Characterization and synthetic application of a novel β1,3-galactosyltransferase from *Escherichia coli* O55:H7. *Bioorg. Med. Chem.* **2009**, *17*, 4910−4915.

(19) Ramakrishnan, B.; Qasba, P. K. Structure-based design of β1,4-galactosyltransferase I (β4Gal-T1) with equally efficient N-acetylgalactosaminyltransferase activity point mutation broadens β4Gal-T1 donor specificity. *J. Biol. Chem.* **2002**, *277*, 20833−20839.

(20) Yu, H.; Chokhawala, H.; Karpel, R.; Yu, H.; Wu, B.; Zhang, J.; Zhang, Y.; Jia, Q.; Chen, X. A multifunctional *Pasteurella multocida* sialyltransferase: a powerful tool for the synthesis of sialoside libraries. *J. Am. Chem. Soc.* **2005**, *127*, 17618−17619.

(21) Fair, R. J.; Hahm, H. S.; Seeberger, P. H. Combination of automated solid-phase and enzymatic oligosaccharide synthesis

DOI: 10.1021/jacs.9b00044
J. Am. Chem. Soc. 2019, 141, 4547−4552

provides access to α(2,3)-sialylated glycans. *Chem. Commun.* **2015**, *51*, 6183−6185.
(22) (a) Padler-Karavani, V.; Song, X.; Yu, H.; Hurtado-Ziola, N.; Huang, S.; Muthana, S.; Chokhawala, H. A.; Cheng, J.; Verhagen, A.; Langereis, M. A.; Kleene, R.; Schachner, M.; de Groot, R. J.; Lasanajak, Y.; Matsuda, H.; Schwab, R.; Chen, X.; Smith, D. F.; Cummings, R. D.; Varki, A. Cross-comparison of protein recognition of sialic acid diversity on two novel sialoglycan microarrays. *J. Biol. Chem.* **2012**, *287*, 22593−22608. (b) Song, X.; Yu, H.; Chen, X.; Lasanajak, Y.; Tappert, M. M.; Air, G. M.; Tiwari, V. K.; Cao, H.; Chokhawala, H. A.; Zheng, H.; Cummings, R. D.; Smith, D. F. A sialylated glycan microarray reveals novel interactions of modified sialic acids with proteins and viruses. *J. Biol. Chem.* **2011**, *286*, 31610−31622.
(23) Lairson, L. L.; Watts, A. G.; Wakarchuk, W. W.; Withers, S. G. Using substrate engineering to harness enzymatic promiscuity and expand biological catalysis. *Nat. Chem. Biol.* **2006**, *2*, 724−728.

DOI: 10.1021/jacs.9b00044
J. Am. Chem. Soc. 2019, 141, 4547−4552

Supporting Information

for

Redox-Controlled Site-Specific α2–6-Sialylation

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Table of Contents

1. General Synthesis

1.1 General Information

All chemicals were obtained from commercial suppliers and used without further purification unless noted. Thin layer chromatography (TLC) was performed on silica gel plates 60 F_{254} (Merck, Billerica MA). Plates were visualized under UV light and/or by treatment with 5% sulfuric acid in ethanol or *p*-anisaldehyde sugar stain followed by heating. Silica gel 60 (300-400 mesh, Haiyang, Qingdao, China) was used for flash silica gel column chromatography. DEAE Sepharose Fast Flow (GE, Piscataway, NJ) was used for ion exchange chromatography. Gel filtration chromatography was performed using a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad, Hercules, CA). ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on Bruker AVANCE-600 spectrometer, or Agilent VNMRS-600 spectrometer at 25 ℃. NMR spectra were calibrated using solvent signals (^{1}H: δ 7.26 for $CDCl_3$, δ 4.79 for D_2O, ^{13}C: δ 77.0 for $CDCl_3$). High resolution electrospray ionization (ESI) mass spectra were obtained at the National Glycoengineering Research Center and Drug Testing and Analysis Center in Shandong University.

The fusion enzyme, NahK/GlmU, was constructed with *Bifidobacterium longum* *N*-acetylhexosamine-1-kinase (BlNahK) and *Escherichia coli* *N*-acetylglucosamine uridyltransferase (EcGlmU)[1]. *Helicobacter pylori* β1–3-*N*-acetylglucosaminyltransferase (HpLgtA)[2], *Escherichia coli* K-12 galactokinase (EcGalK)[3], *Bifidobacterium longum* UDP-sugar pyrophosphorylase (BLUSP)[4], *Neisseria meningitides* β1–4-galactosyltransferase (NmLgtB)[5], *Escherichia coli* β1–3-galactosyltransferase (EcWbgO)[6], Bovine β1–4-galactosyltransferase (GalT1 Y289L)[7], *Neisseria meningitides* CMP-sialic acid synthetase (NmCSS)[8], *Pasteurella multocida* α2–3-sialyltransferase 1 (PmST1 M144D)[9], *Photobacterium damselae* α2–6-sialyltransferase (Pd2,6ST)[10], and *Campylobacter jejuni* α2–8-sialyltransferase (CjCstII)[11] were expressed in *E. coli* system and purified as described previously. Galactose oxidase of *Fusarium*

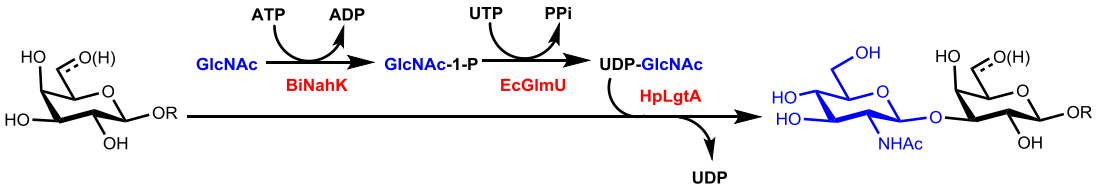
graminearum was obtained from Worthington Biochemical Corporation and peroxidase of *Horseradish* was obtained from Tokyo Chemical Industry.

1.2 General Procedures

Table of Enzyme Modules

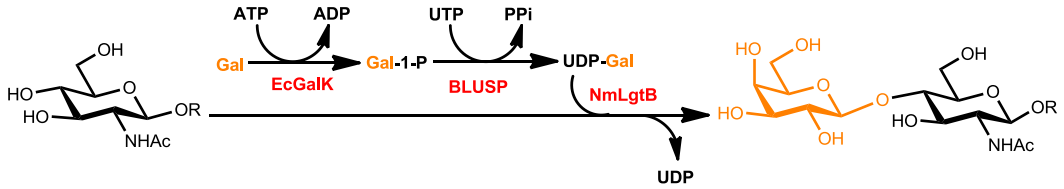
Enzyme Modules	Abbreviation	Full Name
Enzyme module 1 (EM1)	BlNahK	*Bifidobacterium longum N*-acetylhexosamine-1-kinase[1]
	EcGlmU	*Escherichia coli N*-acetylglucosamine uridyltransferase[1]
	HpLgtA	*Helicobacter pylori* β1–3-*N*-acetylglucosaminyltransferase[2]
Enzyme module 2 (EM2)	EcGalK	*Escherichia coli* K-12 galactokinase[3]
	BLUSP	*Bifidobacterium longum* UDP-sugar pyrophosphorylase[4]
	NmLgtB	*Neisseria meningitides* β1–4-galactosyltransferase[5]
Enzyme module 3 (EM3)	NmCSS	*Neisseria meningitides* CMP-sialic acid synthetase[8]
	Pd2,6ST	*Photobacterium damselae* α2–6-sialyltransferase[10]
Enzyme module 4 (EM4)	NmCSS	*Neisseria meningitides* CMP-sialic acid synthetase[8]
	CjCstII	*Campylobacter jejuni* α2–8-sialyltransferase[11]
Enzyme module 5 (EM5)	EcGalK	*Escherichia coli* K-12 galactokinase[3]
	BLUSP	*Bifidobacterium longum* UDP-sugar pyrophosphorylase[4]
	EcWbgO	*Escherichia coli* β1–3-galactosyltransferase[6]
Enzyme module 6 (EM6)	BlNahK	*Bifidobacterium longum N*-acetylhexosamine-1-kinase[1]
	EcGlmU	*Escherichia coli N*-acetylglucosamine uridyltransferase[1]
	GalT1 Y289L	Bovine β1–4-galactosyltransferase Y289L mutant[7]
Enzyme module 7 (EM7)	NmCSS	*Neisseria meningitides* CMP-sialic acid synthetase[8]
	PmST1	*Pasteurella multocida* α2–3-sialyltransferase 1 M144D mutant[9]
Oxidation module	GOase	galactose oxidase of *Fusarium graminearum* from Worthington Biochemical Corporation
	peroxidase	peroxidase of *Horseradish* was obtained from Tokyo Chemical Industry

General procedure of β1–3-*N*-acetylglucosaminylation with Enzyme Module 1:



Acceptor (GalOR or Gal$^{6\text{-Ald}}$OR, 1.0 equiv.), *N*–acetylglucosamine (GlcNAc, 1.3 equiv.), adenosine 5'-triphosphate (ATP, 1.3 equiv.) and uridine 5'-triphosphate (UTP, 1.3 equiv.) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.0) and $MgCl_2$ (20 mM). After the addition of appropriate amount of NahK/GlmU and HpLgtA, the reaction mixture was incubated at 37 ℃ with agitation at 140 rpm in an isotherm incubator. The product formation was monitored by TLC (EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v/v*) and stained with *p*-anisaldehyde sugar stain. The reaction was stopped by adding the same volume of ice-cold ethanol and incubation at 4 ℃ for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

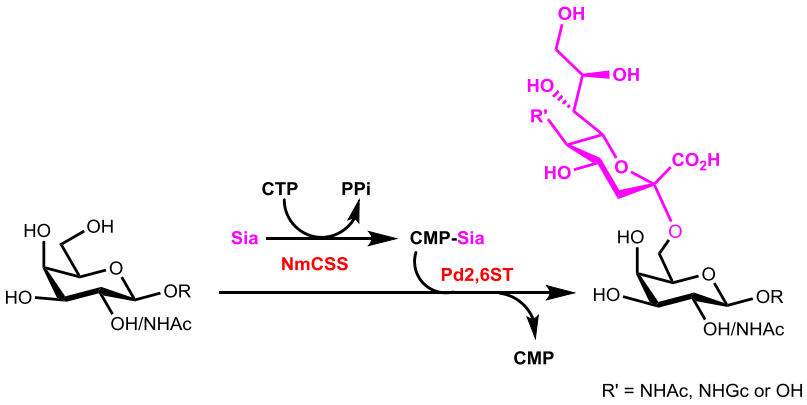
General procedure of β1–4-galactosylation with Enzyme Module 2:



Acceptor (GlcNAcOR, 1.0 equiv.), galactose (Gal, 1.3 equiv.), adenosine 5'-triphosphate (ATP, 1.3 equiv.) and uridine 5'-triphosphate (UTP, 1.3 equiv.) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.0) and $MgCl_2$ (20 mM). After the addition of appropriate amount of EcGalK, BLUSP and NmLgtB, the reaction mixture was incubated at 37 ℃ with agitation at 140 rpm in an isotherm incubator. The product formation was monitored by TLC

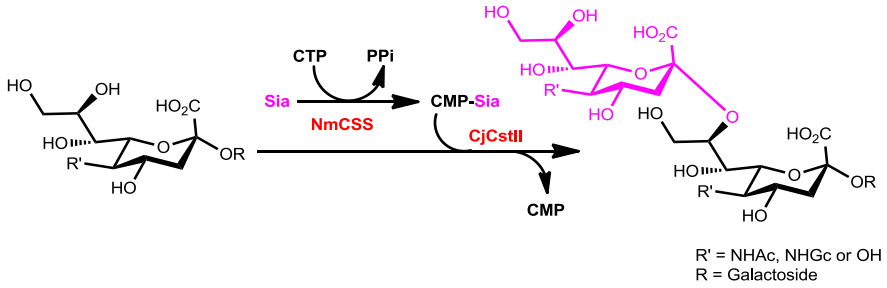
(EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v/v*) and stained with *p*-anisaldehyde sugar stain. The reaction was stopped by adding the same volume of ice-cold ethanol and incubation at 4 ℃ for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

General procedure of α2–6-sialylation with Enzyme Module 3:



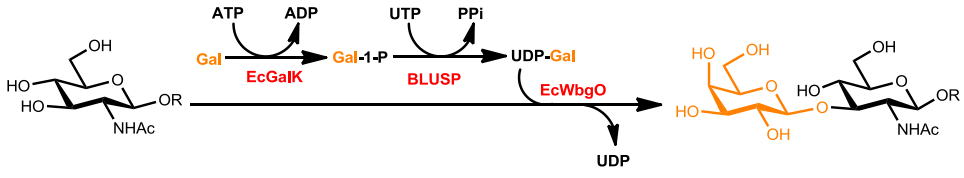
Acceptor (GalOR or GalNAcOR, 1.0 equiv.), a sialic acid precursor (Neu5Ac, Neu5Gc, or Kdn, 1.5 equiv.) and cytidine 5'-triphosphate (CTP, 1.5 equiv.) were dissolved in Tris-HCl buffer (100 mM, pH 8.5) containing $MgCl_2$ (20 mM) and appropriate amounts of NmCSS, and Pd2,6ST. The reaction mixture was incubated at 37 ℃ with agitation at 140 rpm in an isotherm incubator. The product formation was monitored by TLC (EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v/v*) and stained with *p*-anisaldehyde sugar stain. The reaction was stopped by adding the same volume of ice-cold EtOH and incubation at 4 ℃ for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

General procedure of α2–8-sialylation with Enzyme Module 4:



Acceptor (Sialosides, 1.0 equiv.), a sialic acid precursor (Neu5Ac, Neu5Gc, or Kdn, 1.5 equiv.) and cytidine 5'-triphosphate (CTP, 1.5 equiv.) were dissolved in Tris-HCl buffer (100 mM, pH 8.5) containing $MgCl_2$ (20 mM) and appropriate amounts of NmCSS, and CjCstII. The reaction mixture was incubated at 37 ℃ with agitation at 140 rpm in an isotherm incubator. The product formation was monitored by TLC (EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v/v*) and stained with *p*-anisaldehyde sugar stain. The reaction was stopped by adding the same volume of ice-cold EtOH and incubation at 4 ℃ for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

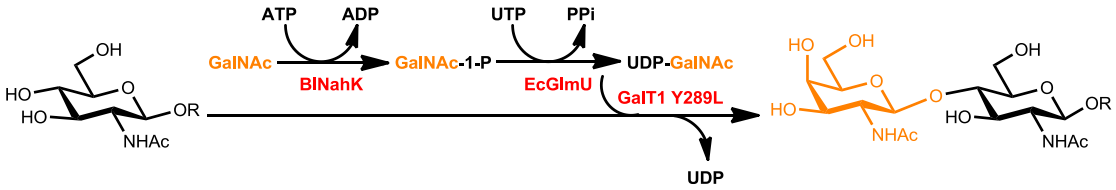
General procedure of β1–3-galactosylation with Enzyme Module 5:



Acceptor (GlcNAcOR, 1.0 equiv.), galactose (Gal, 1.3 equiv.), adenosine 5'-triphosphate (ATP, 1.3 equiv.) and uridine 5'-triphosphate (UTP, 1.3 equiv.) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.0) and $MgCl_2$ (20 mM). After the addition of appropriate amount of EcGalK, BLUSP and EcWbgO, the reaction mixture was incubated at 37 ℃ with agitation at

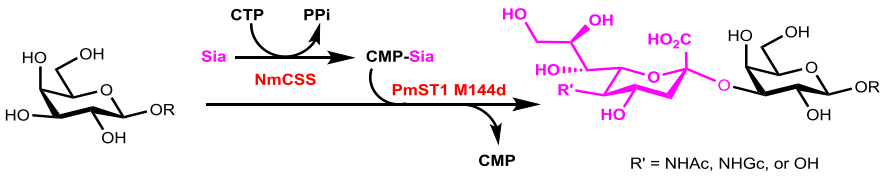
140 rpm in an isotherm incubator. The product formation was monitored by TLC (EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v/v*) and stained with *p*-anisaldehyde sugar stain. The reaction was stopped by adding the same volume of ice-cold ethanol and incubation at 4 ℃ for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

General procedure of β1–4-*N*-acetyl-galactosylation with Enzyme Module 6:



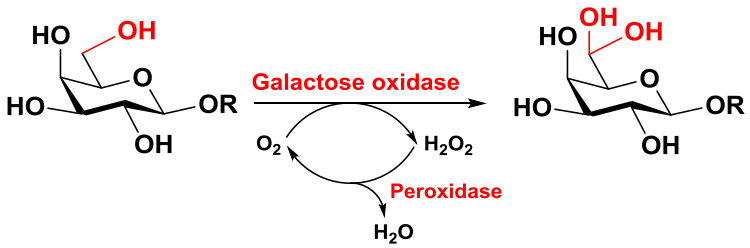
Acceptor (GlcNAcOR, 1.0 equiv.), *N*–acetylgalactosamine (GalNAc, 1.3 equiv.), adenosine 5'- triphosphate (ATP, 1.3 equiv.) and uridine 5'-triphosphate (UTP, 1.3 equiv.) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (25 mM, pH 8.0) and $MnCl_2$ (10 mM). After the addition of appropriate amount of NahK/GlmU and GalT1 Y289L, the reaction mixture was incubated at 37 ℃ with agitation at 140 rpm in an isotherm incubator. The product formation was monitored by TLC (EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v/v*) and stained with *p*-anisaldehyde sugar stain. The reaction was stopped by adding the same volume of ice-cold ethanol and incubation at 4 ℃ for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

General procedure of α2–3-sialylation with Enzyme Module 7:



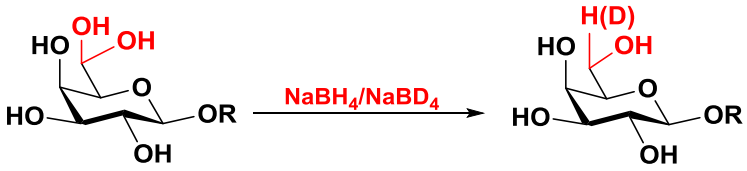
Acceptor (GalOR, 1.0 equiv.), a sialic acid precursor (Neu5Ac, Neu5Gc, or Kdn, 1.5 equiv.) and cytidine 5'-triphosphate (CTP, 1.5 equiv.) were dissolved in Tris-HCl buffer (100 mmol, pH 8.5) containing $MgCl_2$ (20 mmol) and appropriate amounts of NmCSS, and PmST1. The reaction mixture was incubated at 37 ℃ with agitation at 140 rpm in an isotherm incubator. The product formation was monitored by TLC (EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v*/*v*) and stained with *p*-anisaldehyde sugar stain. The reaction was stopped by adding the same volume of cold EtOH and incubation at 4 ℃ for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

General procedure of Oxidation Module:



The substrate (GalOR, 0.1 mmol) was dissolved in sodium phosphate buffer (50 mM, pH 6.5). Then, galactose oxidase (155 U) and peroxidase (3220 U) were added. The solution was stirred at 30 ºC under gentle mixing at 100 rpm in oxygen atmosphere. The product formation was monitored by TLC (EtOAc/MeOH/H_2O/HOAc, 4:2:1:0.2, *v*/*v*, detected by *p*-anisaldehyde sugar stain). The reaction was terminated by adding the same volume of ice-cold ethanol and incubating at 4 ºC for 30 min. The mixture was then centrifuged and the precipitates were removed. The supernatant containing the product was concentrated, purified by BioGel P-2 column (eluted with H_2O) to provide purified product.

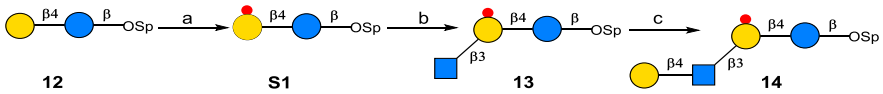
General procedure of Reduction reaction:



To a solution of acceptor (1.0 equiv.) in water (5 mL), sodium borohydride ($NaBH_4$, 1.2 equiv.) or sodium borodeuteride ($NaBD_4$, 1.2 equiv.) was added. The reaction was stirred at room temperature for 1 h. The product formation was monitored by TLC (*n*-BuOH/MeOH/H_2O/HOAc, 2:2:1:1, *v*/*v*) and stained with *p*-anisaldehyde sugar stain. The reaction was then neutralized with 1 M HCl, concentrated and purified by a BioGel P-2 column (eluted with H_2O) to provide purified product.

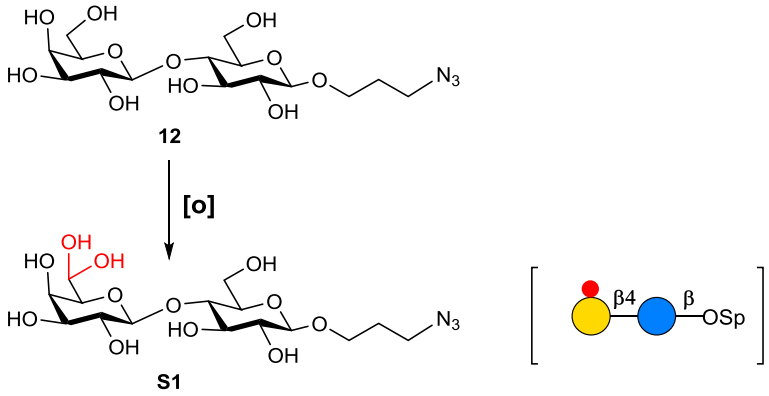
2. Experimental Procedures

2.1 Site-specific α2–6-sialylation of LNnH 11



Scheme S1. Synthesis of compounds **S1, 13** and **14** from **12**.

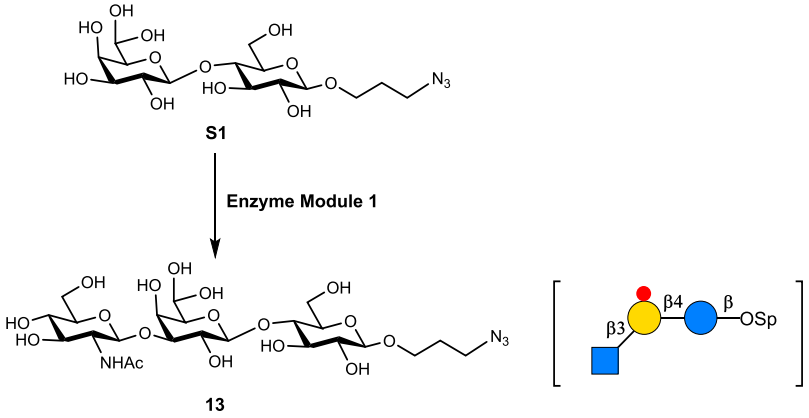
Reagents and conditions: a) Oxidation module: **12** (266 mg), galactose oxidase (970 U), peroxidase (20146 U), sodium phosphate buffer (50 mM, pH 6.5), 30 ℃, 81 %; b) Enzyme module 1: *N*–acetylglucosamine (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), NahK/GlmU, HpLgtA, Tris-HCl (100 mM, pH 8.0), 37 ℃, 90%; c) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 93%.



3-Azidopropyl 6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S 1)

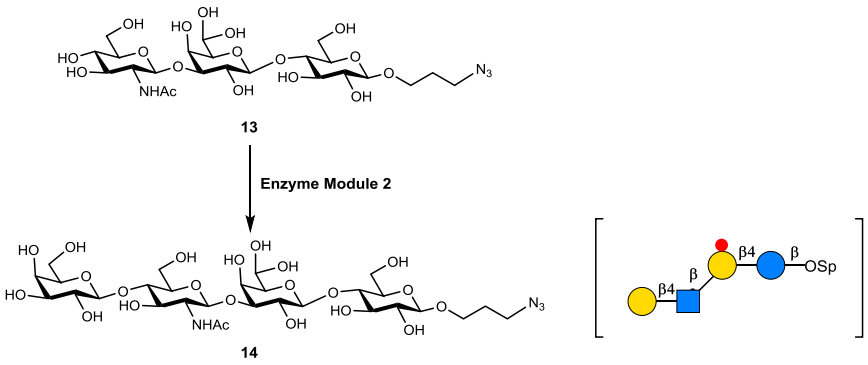
Disaccharide **S1** (214 mg, 81 %), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.10 (d, *J* = 7.3 Hz, 1H), 4.45 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.04 (d, *J* = 3.0 Hz, 1H), 3.99 – 3.90 (m, 2H), 3.79 – 3.69 (m, 3H), 3.67 – 3.48 (m, 5H), 3.42 (t, *J* = 6.6 Hz, 2H), 3.32 – 3.23 (m, 1H), 1.88 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 103.17, 102.01, 87.94, 79.39, 76.88, 74.58, 74.51, 72.71, 72.41, 70.64, 67.96, 67.32, 60.14, 47.84, 28.20; HRMS (ESI) *m/z* calcd for

$C_{15}H_{27}N_4O_{12}Na$ $[M+H_2O+Na]^+$ 464.1487, found 464.1479.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (13)

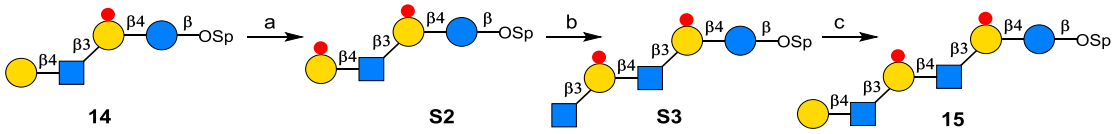
Trisaccharide **13** (271 mg, 90 %), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.11 (d, *J* = 7.3 Hz, 1H), 4.67 (d, *J* = 8.5 Hz, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.28 (d, *J* = 3.2 Hz, 1H), 4.01 – 3.92 (m, 2H), 3.91 – 3.85 (m, 1H), 3.79 – 3.70 (m, 5H), 3.66 – 3.51 (m, 5H), 3.47 – 3.40 (m, 5H), 3.29 (t, *J* = 8.6 Hz, 1H), 2.01 (s, 3H),1.90 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.85, 103.12, 102.76, 101.93, 87.76, 81.78, 79.32, 76.36, 75.53, 74.51, 74.43, 73.47, 72.62, 69.62, 69.60, 67.65, 67.26, 60.38, 60.06, 55.56, 47.76, 28.13, 22.07; HRMS (ESI) *m/z* calcd for $C_{23}H_{40}N_4O_{17}Na$ $[M+H_2O+Na]^+$ 667.2281, found 667.2279.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (14)

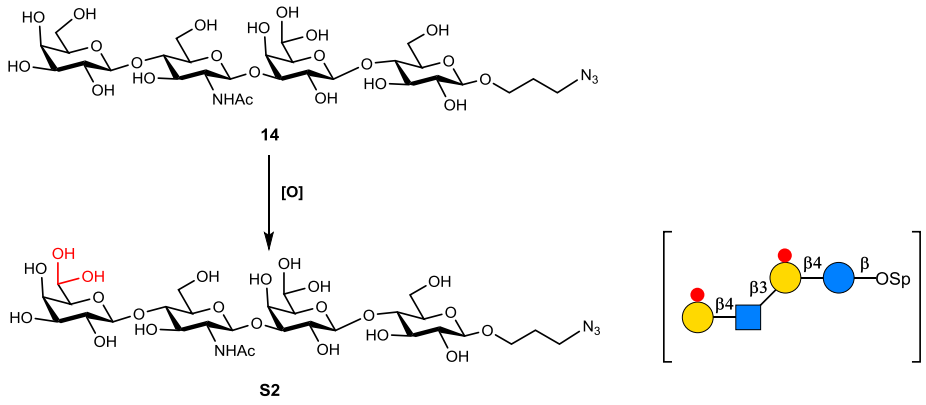
Tetrasaccharide **14** (226 mg, 93%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.15 (d, *J* = 7.2 Hz, 1H), 4.74 (d, *J* = 8.4 Hz, 1H), 4.50 (dd, *J* = 7.9, 5.4 Hz, 2H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.33 (d, *J* = 3.2 Hz, 1H), 4.04 – 3.96 (m, 3H),

3.94 (d, J = 3.4 Hz, 1H), 3.89 – 3.72 (m, 11H), 3.71 – 3.58 (m, 6H), 3.47 (t, J = 7.0 Hz, 3H), 3.33 (t, J = 8.6 Hz, 1H), 2.05 (s, 3H), 1.94 (p, J = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.88, 103.19, 102.83, 102.72, 102.01, 87.87, 81.95, 79.40, 78.14, 76.44, 75.33, 74.59, 74.50, 72.72, 72.47, 72.18, 70.95, 69.68, 68.54, 67.73, 67.35, 61.03, 60.15, 59.86, 55.18, 47.86, 28.22, 22.19; HRMS (ESI) *m/z* calcd for $C_{29}H_{50}N_4O_{22}Na$ $[M+H_2O+Na]^+$ 829.2809, found 829.2814.



Scheme S2. Synthesis of compounds **S2, S3** and **15** from **14**.

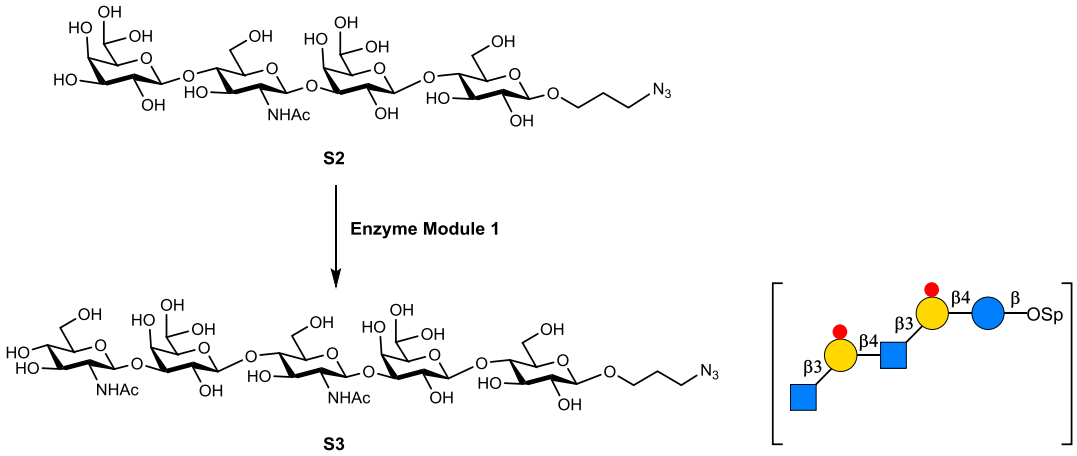
Reagents and conditions: a) Oxidation module: **14** (70 mg), galactose oxidase (138 U), peroxidase (2859 U), sodium phosphate buffer (50 mM, pH 6.5), 30 ℃, 77 %; b) Enzyme module 1: *N*–acetylglucosamine (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), NahK/GlmU, HpLgtA, Tris-HCl (100 mM, pH 8.0), 37 ℃, 89%; c) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 90%.



3-Azidopropyl 6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S2)

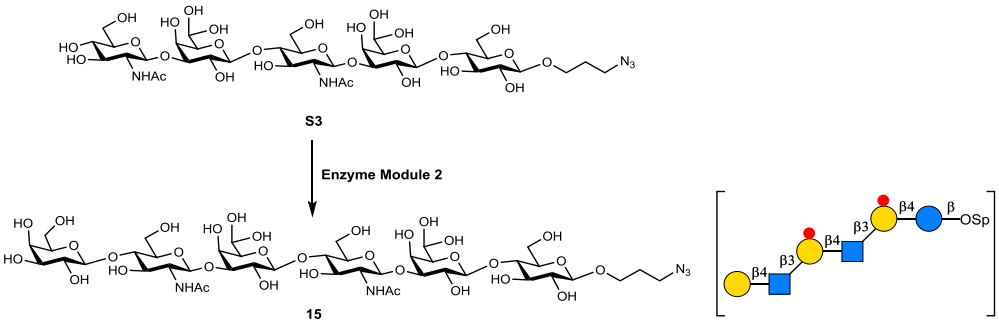
Tetrasaccharide **S2** (54 mg, 77%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.14 – 5.09 (m, 2H), 4.69 (d, J = 8.3 Hz, 1H), 4.46 (d, J = 7.9 Hz, 2H),

4.42 (d, *J* = 7.9 Hz, 1H), 4.29 (d, *J* = 2.8 Hz, 1H), 4.06 (d, *J* = 3.1 Hz, 1H), 4.00 – 3.91 (m, 4H), 3.85 – 3.51 (m, 13H), 3.44 – 3.41 (m, 4H), 3.31 – 3.27 (m, 1H), 2.01 (s, 3H), 1.89 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.80, 103.11, 103.06, 102.60, 101.93, 87.88, 87.74, 81.85, 79.33, 79.03, 76.80, 76.36, 74.50, 74.39, 74.29, 72.61, 72.29, 72.22, 70.57, 69.58, 67.88, 67.64, 67.25, 60.06, 59.85, 54.98, 47.76, 28.13, 22.09; HRMS (ESI) *m/z* calcd for $C_{29}H_{47}N_4O_{21}$ $[M+H]^+$ 787.2727, found 787.2807.



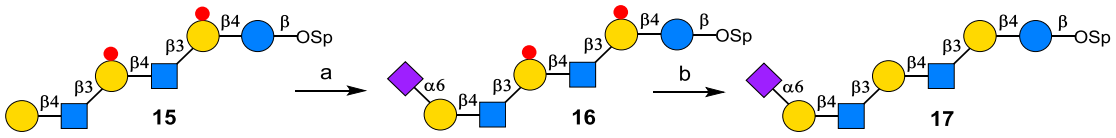
3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S3)

Pentasaccharide **S3** (33 mg, 89%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.10 (t, *J* = 7.0 Hz, 2H), 4.70 – 4.63 (m, 2H), 4.48 – 4.38 (m, 3H), 4.28 (d, *J* = 2.1 Hz, 2H), 3.99 – 3.90 (m, 3H), 3.87 (d, *J* = 12.0 Hz, 1H), 3.81 – 3.51 (m, 18H), 3.46 – 3.39 (m, 5H), 3.28 (t, *J* = 8.5 Hz, 1H), 2.00 (s, 3H), 2.00 (s, 3H), 1.88 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.83, 174.80, 103.11, 103.06, 102.76, 102.59, 101.91, 87.76, 87.75, 81.84, 81.77, 79.30, 79.04, 76.34, 75.52, 74.49, 74.41, 74.27, 73.45, 72.60, 72.20, 69.62, 69.58, 67.63, 67.24, 60.36, 60.04, 59.82, 59.29, 55.54, 54.92, 47.75, 28.12, 22.07, 22.05; HRMS (ESI) *m/z* calcd for $C_{37}H_{63}N_5O_{28}Na$ $[M+H_2O+H_2O+Na]^+$ 1048.3552, found 1048.3469.



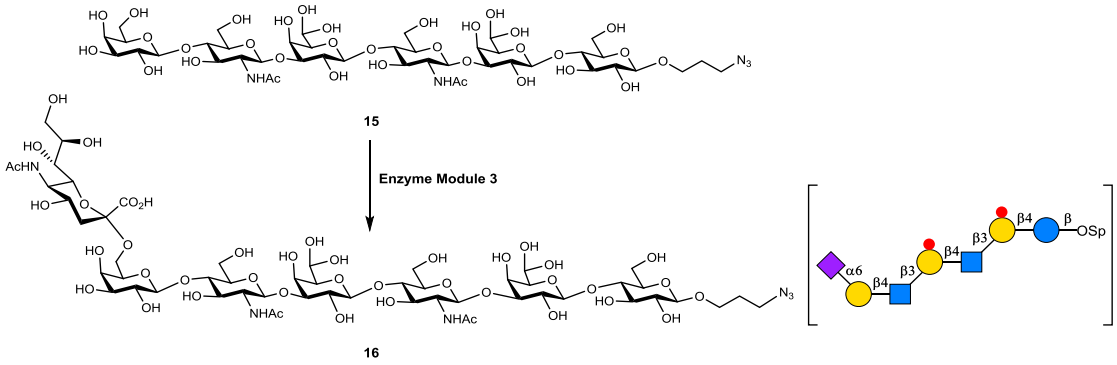
3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (15)

Hexasaccharide **15** (19 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 5.7 Hz, 2H), 4.73 – 4.69 (m, 2H), 4.51 – 4.46 (m, 3H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.37 – 4.22 (m, 2H), 4.02 – 3.94 (m, 4H), 3.93 (d, *J* = 3.1 Hz, 1H), 3.87 – 3.58 (m, 24H), 3.54 (d, *J* = 17.8 Hz, 1H), 3.50 – 3.42 (m, 3H), 3.32 (t, *J* = 8.6 Hz, 1H), 2.03 (s, 6H), 1.92 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.89, 174.88, 103.18, 102.83, 102.74, 102.68, 102.01, 87.82, 87.80, 81.93, 81.92, 79.41, 79.16, 78.14, 75.32, 74.59, 74.51, 74.37, 72.69, 72.47, 72.30, 72.18, 70.94, 69.67, 68.52, 67.65, 67.33, 61.01, 60.13, 59.92, 59.84, 55.16, 55.02, 47.84, 28.20, 22.14; HRMS (ESI) *m/z* calcd for $C_{43}H_{69}N_5O_{31}Na$ $[M+Na]^+$ 1174.3869, found 1174.3860.



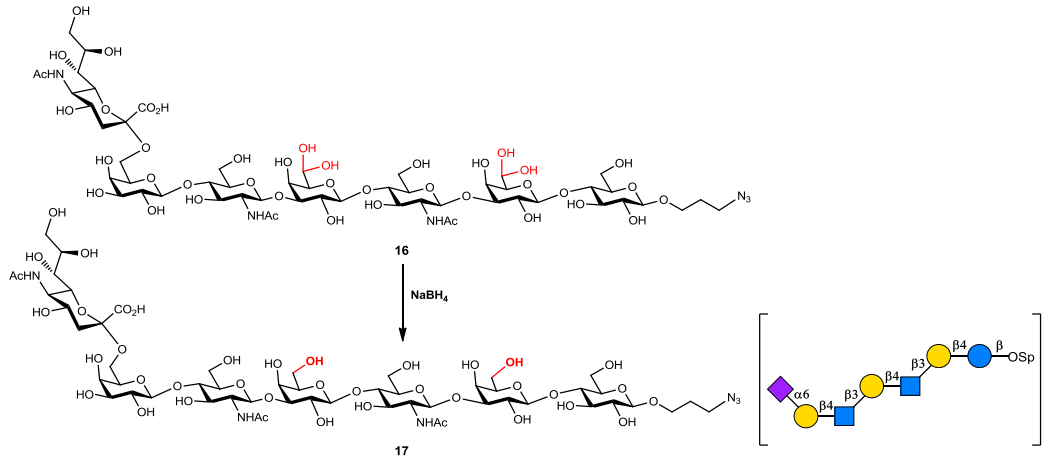
Scheme S3. Synthesis of compounds **16** and **17** from **15**.

Reagents and conditions: a) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 94%; b) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 98%.



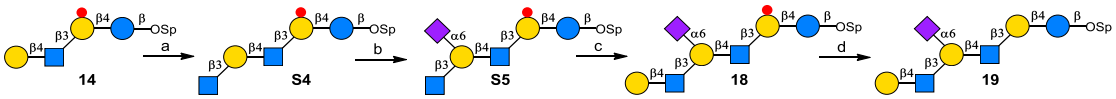
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (16)

Heptasaccharide **16** (16 mg, 94 %), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 – 5.09 (m, 2H), 4.71 (d, *J* = 7.9 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.47 – 4.41 (m, 4H), 4.29 (brs, 2H), 4.00 – 3.48 (m, 37H), 3.45 – 3.40 (m, 3H), 3.31 – 3.27 (m, 1H), 2.64 (dd, *J* = 12.5, 4.6 Hz, 1H), 2.03 (s, 3H), 2.00 (s, 6H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.70 (t, *J* = 12.3 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.89, 173.53, 103.45, 103.20, 103.17, 102.68, 102.57, 102.01, 100.11, 87.87, 87.84, 81.93, 81.88, 80.46, 79.40, 79.15, 76.43, 74.59, 74.51, 74.37, 74.23, 73.67, 72.69, 72.51, 72.39, 72.30, 72.24, 71.69, 70.70, 69.69, 69.67, 68.37, 68.34, 68.19, 67.33, 63.33, 62.62, 60.12, 60.03, 59.90, 55.00, 54.92, 51.86, 47.84, 40.05, 28.20, 22.25, 22.15, 22.01; HRMS (ESI) *m/z* calcd for $C_{54}H_{89}N_6O_{41}$ $[M+H_2O+H_2O-H]^-$ 1477.5069, found 1477.4774.



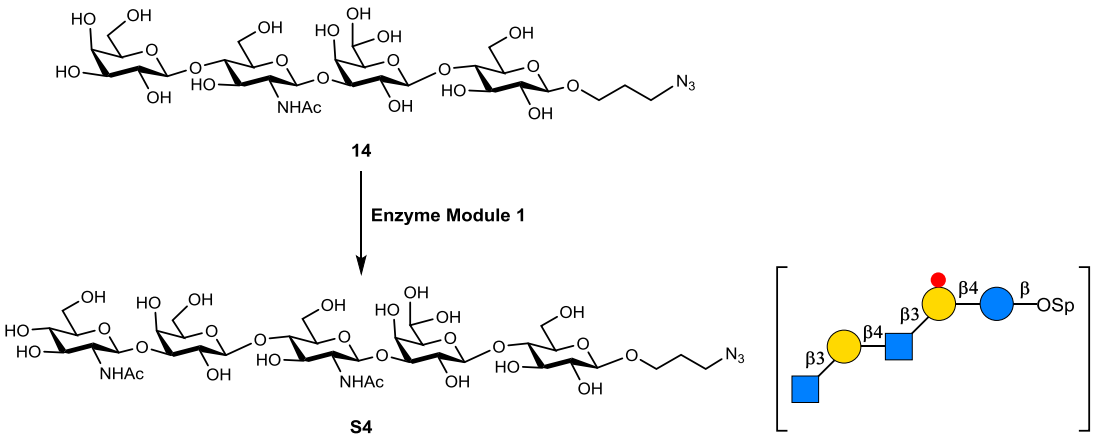
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (17)

Heptasaccharide **17** (7 mg, 98%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.73 (d, *J* = 8.0 Hz, 1H), 4.70 (d, *J* = 8.3 Hz, 1H), 4.53 – 4.38 (m, 4H), 4.16 (t, *J* = 2.7 Hz, 2H), 4.02 – 3.51 (m, 42H), 3.46 (t, *J* = 6.7 Hz, 2H), 3.35 – 3.27 (m, 1H), 2.67 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.91 (p, *J* = 6.6 Hz, 2H), 1.72 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.89, 173.53, 103.43, 102.90, 102.85, 102.73, 102.57, 102.08, 100.11, 82.02, 81.99, 80.42, 78.31, 78.11, 74.85, 74.74, 74.52, 74.33, 74.23, 73.66, 72.76, 72.50, 72.39, 72.21, 72.14, 71.68, 70.70, 69.93, 68.37, 68.34, 68.29, 68.28, 68.18, 67.34, 63.32, 62.62, 60.93, 60.10, 60.02, 59.80, 55.11, 54.91, 51.86, 47.83, 40.04, 28.20, 22.26, 22.15, 22.01; HRMS (ESI) *m/z* calcd for $C_{54}H_{89}N_6O_{39}$ $[M-H]^-$ 1445.5171, found 1445.5091.



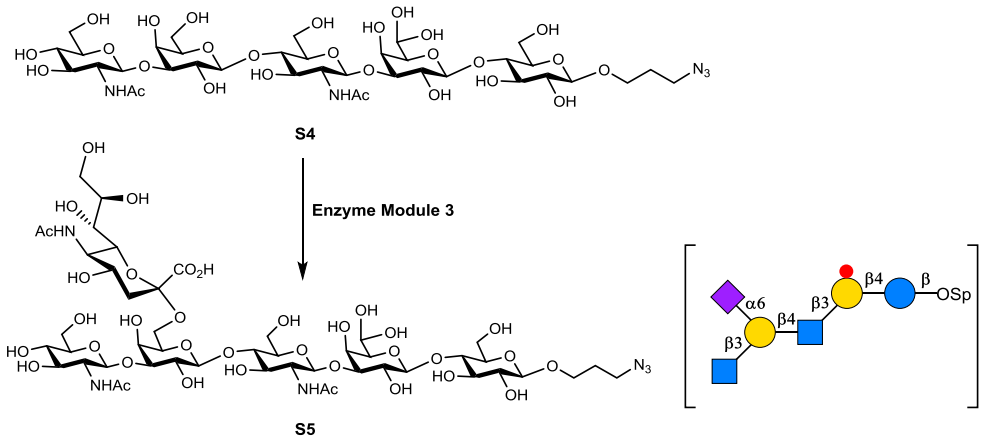
Scheme S4. Synthesis of compounds **S4, S5, 18** and **19** from **14**.

Reagents and conditions: a) Enzyme module 1: *N*–acetylglucosamine (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), NahK/GlmU, HpLgtA, Tris-HCl (100 mM, pH 8.0), 37 ℃, 89%; b) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 94%; c) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 88%; d) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 97%.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S4)

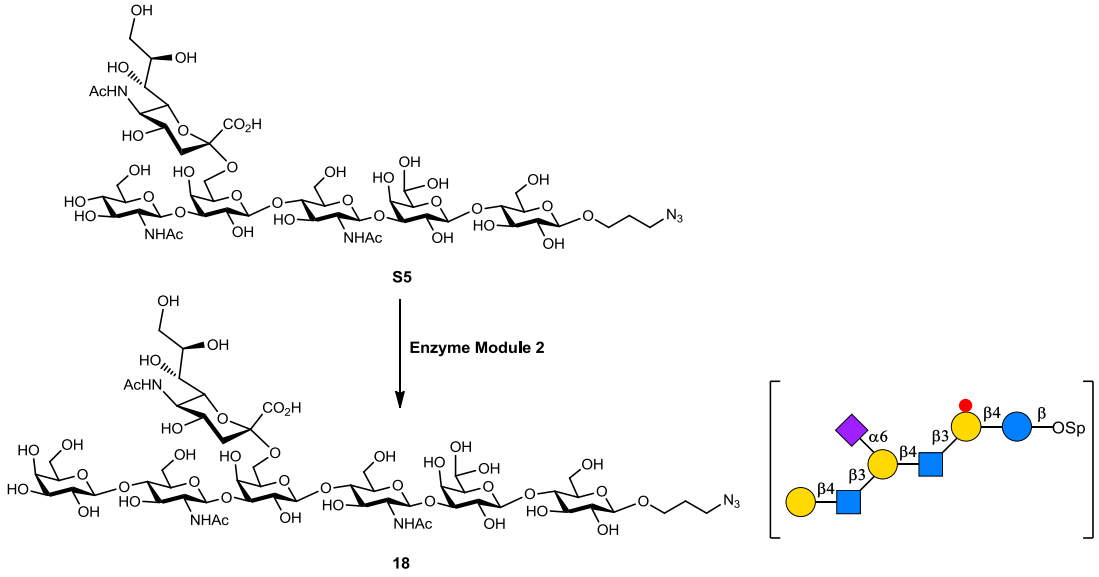
Pentasaccharide **S4** (111 mg, 89%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.14 – 5.13 (m, 1H), 4.71 (d, *J* = 8.4 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.50 – 4.43 (m, 3H), 4.31 (d, *J* = 3.1 Hz, 1H), 4.15 (d, *J* = 3.3 Hz, 1H), 4.02 – 3.93 (m, 3H), 3.89 (dd, *J* = 12.4, 2.0 Hz, 1H), 3.83 (dd, *J* = 12.5, 4.9 Hz, 1H), 3.86 – 3.70 (m, 12H), 3.67 – 3.53 (m, 7H), 3.49 – 3.42 (m, 5H), 3.31 (dd, *J* = 9.1, 8.1 Hz, 1H), 2.03 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.92, 174.85, 103.18, 102.84, 102.83, 102.71, 102.00, 87.81, 81.94, 81.91, 79.40, 78.15, 76.43, 75.62, 74.85, 74.57, 74.50, 73.52, 72.69, 72.15, 69.96, 69.65, 68.29, 67.68, 67.32, 60.93, 60.43, 60.13, 59.83, 55.62, 55.12, 47.83, 28.20, 22.13; HRMS (ESI) *m/z* calcd for $C_{37}H_{63}N_5O_{27}Na$ $[M+H_2O+Na]^+$ 1032.3603, found 1032.3620.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosy

l-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galacto pyranosyl-(1→4)-β-D-glucopyranoside (S5)

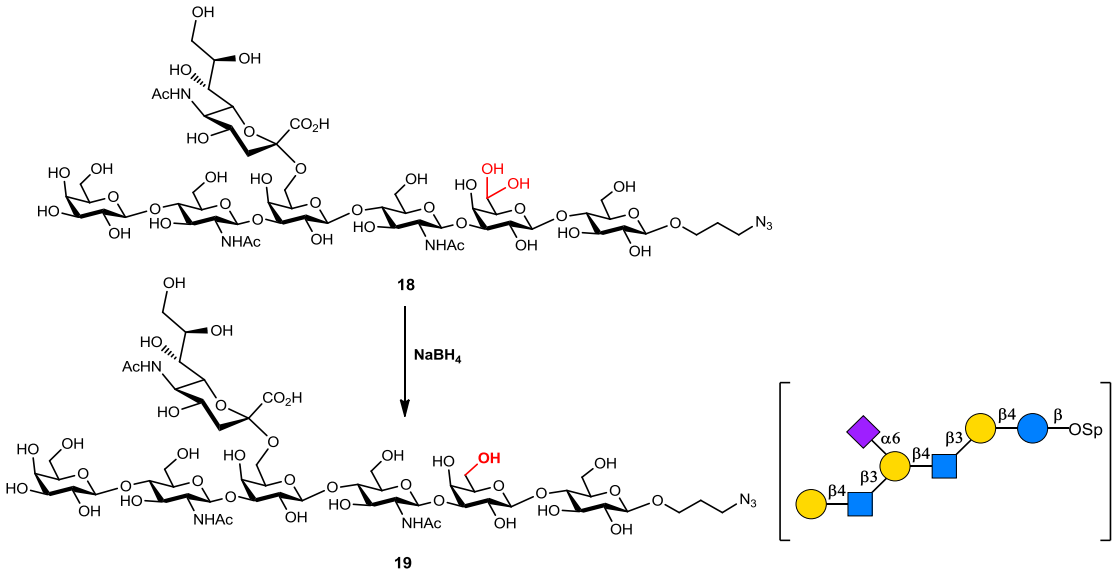
Hexasaccharide **S5** (33 mg, 94%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.14 – 5.09 (m, 1H), 4.71 (d, *J* = 8.0 Hz, 1H), 4.63 (d, *J* = 8.5 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 8.0, 1H), 4.41 (d, *J* = 8.0, 1H), 4.29 (d, *J* = 2.2 Hz, 1H), 4.14 (d, *J* = 3.3 Hz, 1H), 4.01 – 3.37 (m, 36H), 3.29 (t, *J* = 8.4 Hz, 1H), 2.64 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.69 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.80, 173.44, 103.38, 103.12, 102.81, 102.48, 101.92, 100.02, 87.76, 82.12, 81.79, 80.42, 79.33, 76.36, 75.54, 74.51, 74.43, 74.15, 73.52, 73.16, 72.61, 72.43, 72.18, 71.56, 69.58, 69.49, 68.27, 68.10, 67.93, 67.63, 67.25, 63.12, 62.53, 60.36, 60.04, 55.55, 54.80, 51.77, 47.75, 39.99, 28.12, 22.16, 22.05, 21.92; HRMS (ESI) *m/z* calcd for $C_{48}H_{79}N_6O_{35}$ $[M+H_2O-H]^-$ 1299.4592, found 1299.4600.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyra nosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 →3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (18)

Heptasaccharide **18** (18 mg, 88%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.14 – 5.11 (m, 1H), 4.72 (d, *J* = 8.0 Hz, 1H), 4.66 (d, *J* = 8.3 Hz, 1H), 4.48 – 4.43 (m, 4H), 4.29 (d, *J* = 3.4 Hz, 1H), 4.15 (d, *J* = 3.3 Hz, 1H), 4.01 – 3.41 (m,

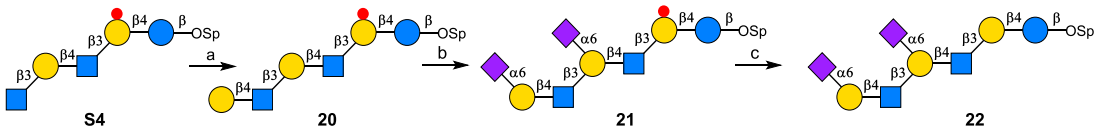
42H), 3.30 (t, *J* = 8.4 Hz, 1H), 2.65 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.04 (s, 3H), 2.01 (s, 6H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.69 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.79, 173.44, 103.37, 103.11, 102.73, 102.71, 102.47, 101.91, 100.01, 87.74, 82.18, 81.78, 80.40, 79.32, 77.96, 76.35, 75.21, 74.50, 74.42, 74.14, 73.14, 72.60, 72.42, 72.36, 72.12, 71.56, 70.84, 69.58, 69.45, 68.43, 68.26, 68.09, 67.91, 67.61, 67.23, 63.12, 62.52, 60.91, 60.01, 59.71, 55.07, 54.78, 51.76, 47.74, 39.97, 28.11, 22.15, 22.06, 21.91; HRMS (ESI) *m/z* calcd for $C_{54}H_{89}N_6O_{40}$ $[M+H_2O-H]^-$ 1461.5120, found 1461.5136.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (19)

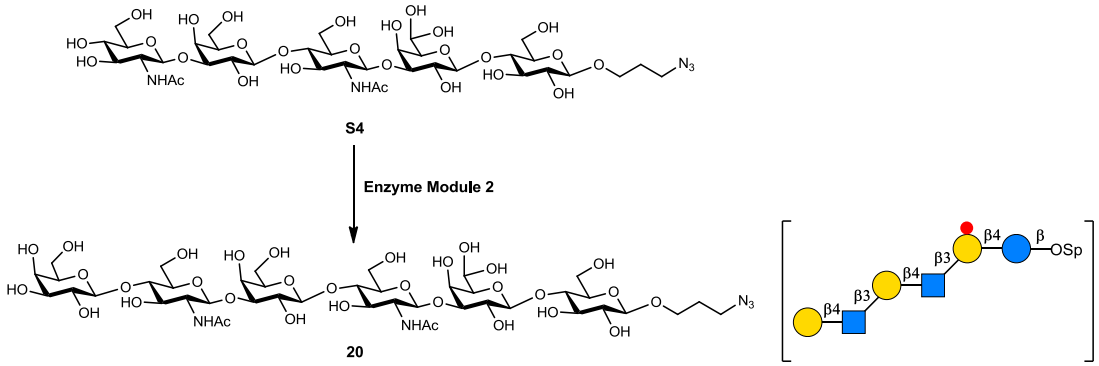
Heptasaccharide **19** (7 mg, 97%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.65 (d, *J* = 8.0 Hz, 1H), 4.61 (d, *J* = 8.4 Hz, 1H), 4.45 – 4.33 (m, 4H), 4.10 (dd, *J* = 9.8, 3.3 Hz, 2H), 4.01 – 3.42 (m, 42H), 3.39 (t, *J* = 6.7 Hz, 2H), 3.27 – 3.20 (m, 1H), 2.59 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.98 (s, 3H), 1.96 (s, 6H), 1.84 (p, *J* = 6.6 Hz, 2H), 1.70 – 1.57 (m, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.90, 174.88, 174.83, 173.51, 103.43, 102.91, 102.82, 102.78, 102.55, 102.07, 100.10, 82.26, 81.96, 80.44, 78.33, 78.08, 75.29, 74.85, 74.74, 74.51, 74.33, 74.24, 73.22, 72.75, 72.51, 72.45, 72.21, 71.65, 70.92, 69.93, 69.54, 68.52, 68.35, 68.28, 68.17, 68.00, 67.33,

62.61, 60.99, 60.93, 60.10, 60.00, 59.80, 55.16, 54.87, 51.85, 47.83, 40.06, 28.20, 22.25, 22.15, 22.00; HRMS (ESI) *m/z* calcd for $C_{54}H_{89}N_6O_{39}$ $[M-H]^-$ 1445.5171, found 1445.4611.



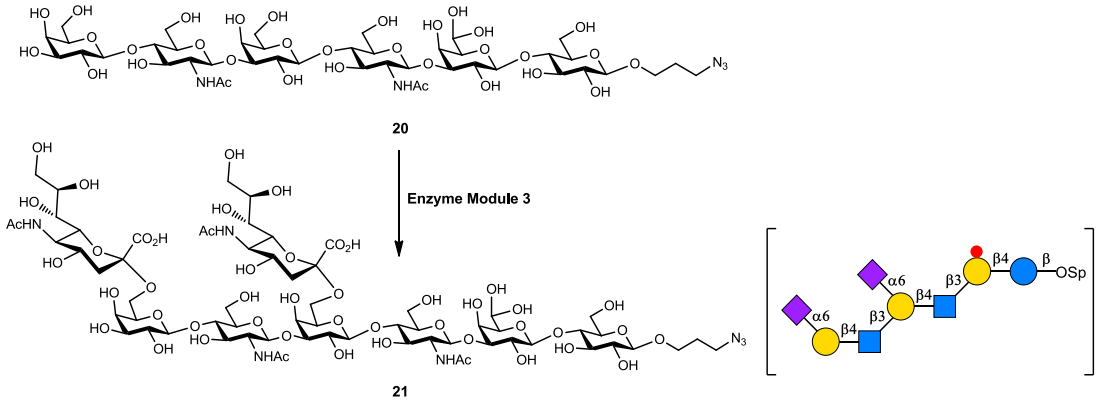
Scheme S5. Synthesis of compounds **20** to **22** from **S4**.

Reagents and conditions: a) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 91%; b) Enzyme module 3: Neu5Ac (3.0 equiv), CTP (3.0 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 96%; c) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 98%.



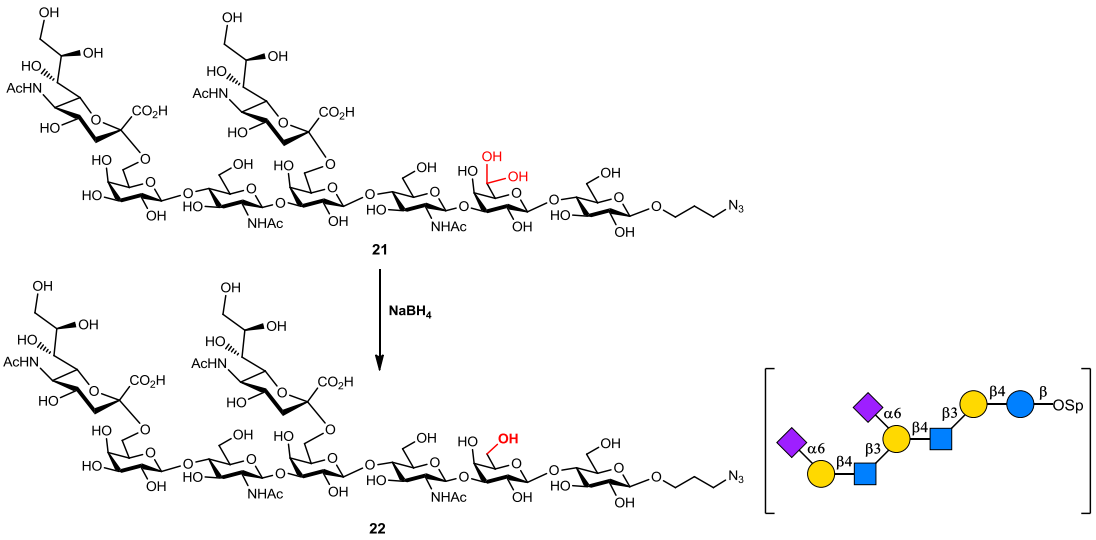
3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (20)

Hexasaccharide **20** (38 mg, 91%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.16 – 5.11 (m, 1H), 4.72 – 4.69(m, 2H), 4.50 – 4.42 (m, 4H), 4.31 (dd, *J* = 3.3, 1.9 Hz, 1H), 4.15 (d, *J* = 3.3 Hz, 1H), 4.07 – 3.92 (m, 5H), 3.87 – 3.69 (m, 18H), 3.68 – 3.51 (m, 9H), 3.45 (q, *J* = 7.1 Hz, 3H), 3.31 (dd, 1H), 2.03 (s, 6H), 1.90 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.86, 103.19, 102.85, 102.82, 102.73, 102.71, 102.00, 87.83, 87.80, 82.02, 81.91, 79.40, 78.14, 78.11, 76.43, 75.31, 74.84, 74.57, 74.51, 72.68, 72.46, 72.15, 70.93, 69.92, 69.65, 68.51, 68.28, 67.71, 67.32, 61.00, 60.92, 60.13, 59.82, 55.15, 55.12, 47.83, 28.20, 22.14; HRMS (ESI) *m/z* calcd for $C_{43}H_{72}N_5O_{31}$ $[M+H]^+$ 1154.4206, found 1154.4322.



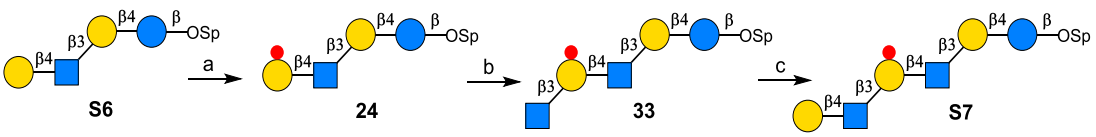
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (21)

Octasaccharide **21** (23 mg, 96%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.12 (d, *J* = 7.2 Hz, 1H), 4.72 (d, *J* = 8.1 Hz, 1H), 4.69 (d, *J* = 7.9 Hz, 1H), 4.47 (d, *J* = 8.1 Hz, 1H), 4.43 (d, *J* = 8.0 Hz, 3H), 4.31 – 4.3 (brs, 1H), 4.15 (brs, 1H), 4.02 – 3.48 (m, 46H), 3.46 – 3.41 (m, 3H), 3.30 (t, *J* = 8.6 Hz, 1H), 2.65 (dd, *J* = 12.5, 4.5 Hz, 2H), 2.06 – 1.98 (m, 12H), 1.90 (p, *J* = 6.6 Hz, 2H), 1.70 (td, *J* = 12.2, 4.7 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.79, 173.45, 103.44, 103.39, 103.13, 102.55, 102.49, 101.93, 100.03, 87.77, 82.15, 81.81, 80.44, 79.34, 76.37, 74.52, 74.44, 74.16, 73.57, 73.16, 72.62, 72.43, 72.30, 72.23, 72.19, 71.61, 71.56, 70.62, 69.60, 69.50, 68.29, 68.12, 67.93, 67.62, 67.25, 63.24, 63.15, 62.54, 60.03, 54.82, 51.78, 47.76, 39.99, 28.13, 22.19, 21.93; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{48}$ $[M+H_2O-2H]^{2-}$ 875.8001, found 875.7912.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (22)

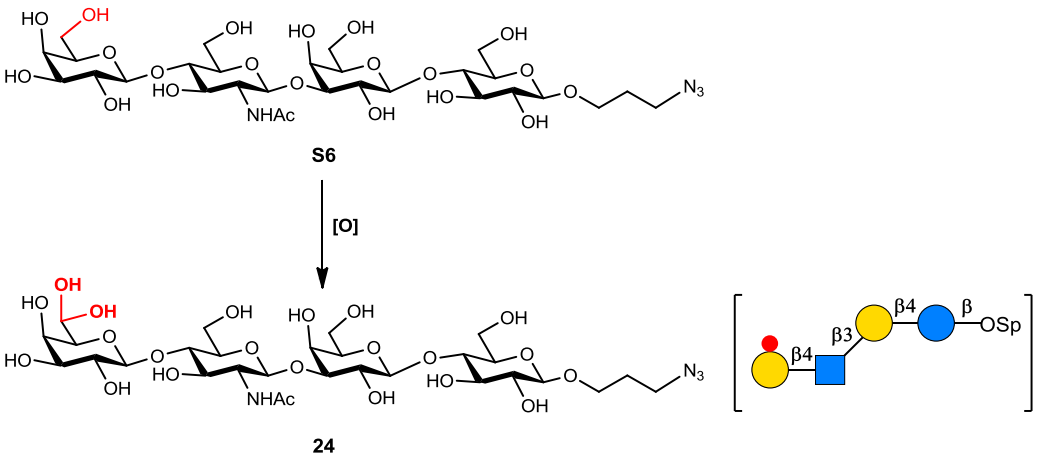
Octasaccharide **22** (12 mg, 98%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.73 – 4.69 (m, 2H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.50 – 4.43 (m, 3H), 4.16 (dd, *J* = 8.1, 3.1 Hz, 2H), 4.07 – 3.49 (m, 49H), 3.46 (t, *J* = 6.7 Hz, 2H), 3.36 – 3.26 (m, 1H), 2.70 – 2.62 (m, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 2.03 (s, 6H), 1.91 (p, *J* = 6.6 Hz, 2H), 1.72 (td, *J* = 12.2, 5.2 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.91, 174.88, 173.54, 103.51, 103.44, 102.92, 102.63, 102.57, 102.08, 100.11, 82.23, 81.98, 80.51, 80.46, 78.34, 74.86, 74.75, 74.34, 74.25, 74.23, 73.65, 73.24, 72.76, 72.51, 72.39, 72.31, 72.24, 71.69, 71.65, 70.70, 69.95, 69.58, 68.37, 68.35, 68.29, 68.20, 68.01, 67.34, 63.31, 63.23, 62.63, 60.95, 60.09, 60.01, 54.90, 54.88, 51.86, 47.84, 40.07, 40.04, 28.20, 22.26, 22.01; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{47}$ $[M-2H]^{2-}$ 867.8026, found 867.7924.



Scheme S6. Synthesis of compounds **24, 33** and **S7** from **S6**.

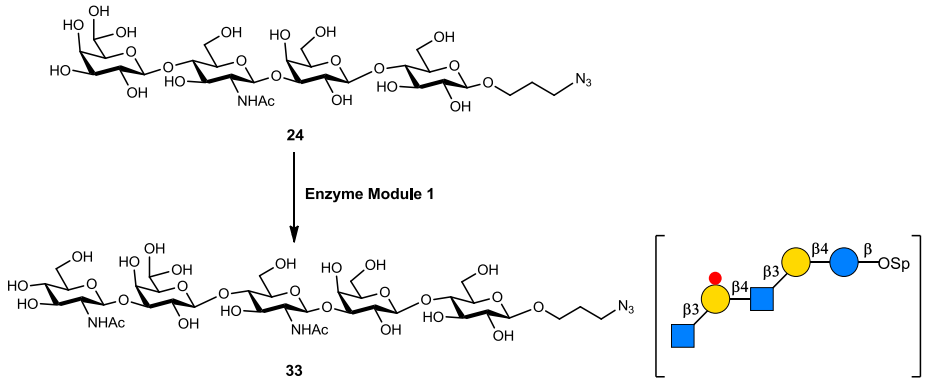
Reagents and conditions: a) Oxidation module: **S6** (354 mg), galactose oxidase (694

U), peroxidase (14423 U), sodium phosphate buffer (50 mM, pH 6.5), 30 ℃, 83%; b) Enzyme module 1: *N*–acetylglucosamine (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), NahK/GlmU, HpLgtA, Tris-HCl (100 mM, pH 8.0), 37 ℃, 92%; c) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 90%.



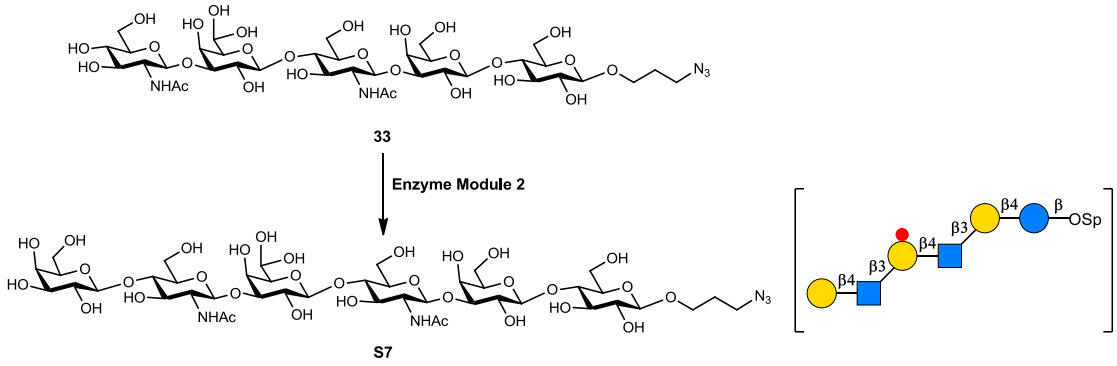
3-Azidopropyl 6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (24)

Tetrasaccharide **24** (68 mg, 83%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.11 (d, *J* = 7.2 Hz, 1H), 4.68 (d, *J* = 8.3 Hz, 1H), 4.46 (d, *J* = 7.9 Hz, 2H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.13 (d, *J* = 3.3 Hz, 1H), 4.06 (d, *J* = 3.3 Hz, 1H), 4.01 – 3.89 (m, 3H), 3.86 – 3.49 (m, 17H), 3.48 – 3.39 (m, 3H), 3.32 – 3.25 (m, 1H), 2.01 (s, 3H), 1.89 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.82, 103.07, 102.83, 102.61, 102.00, 87.89, 81.94, 79.01, 78.22, 76.81, 74.77, 74.67, 74.30, 74.25, 72.68, 72.32, 72.19, 70.58, 69.85, 68.22, 67.88, 67.26, 60.85, 59.95, 59.82, 54.98, 47.76, 28.13, 22.08; HRMS (ESI) *m/z* calcd for $C_{29}H_{50}N_4O_{22}Na$ $[M+H_2O+Na]^+$ 829.2809, found 829.2806.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (33)

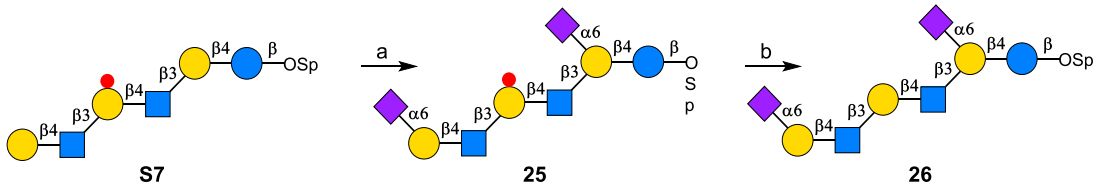
Pentasaccharide **33** (316 mg, 92%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.10 (d, *J* = 7.3 Hz, 1H), 4.66 (d, *J* = 5.6 Hz, 1H), 4.65 (d, *J* = 5.6 Hz, 1H), 4.46 (d, *J* = 8.2 Hz, 1H), 4.44 (d, *J* = 8.2 Hz, 1H), 4.40 (d, *J* = 7.9 Hz, 1H), 4.28 (d, *J* = 3.2 Hz, 1H), 4.12 (d, *J* = 3.2 Hz, 1H), 3.99 – 3.85 (m, 4H), 3.83 – 3.64 (m, 13H), 3.64 – 3.49 (m, 7H), 3.49 – 3.36 (m, 5H), 3.31 – 3.24 (m, 1H), 2.00 (s, 3H), 2.00 (s, 3H), 1.87 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.92, 174.89, 103.15, 102.91, 102.83, 102.67, 102.08, 87.86, 82.01, 81.87, 79.12, 78.31, 76.43, 75.61, 74.84, 74.74, 74.37, 74.33, 73.55, 72.76, 72.27, 69.93, 69.71, 69.68, 68.30, 67.71, 67.34, 60.92, 60.46, 60.03, 59.91, 55.63, 55.02, 47.84, 28.21, 22.16, 22.14; HRMS (ESI) *m/z* calcd for $C_{37}H_{63}N_5O_{27}Na$ $[M+H_2O+Na]^+$ 1032.3603, found 1032.3618.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S7)

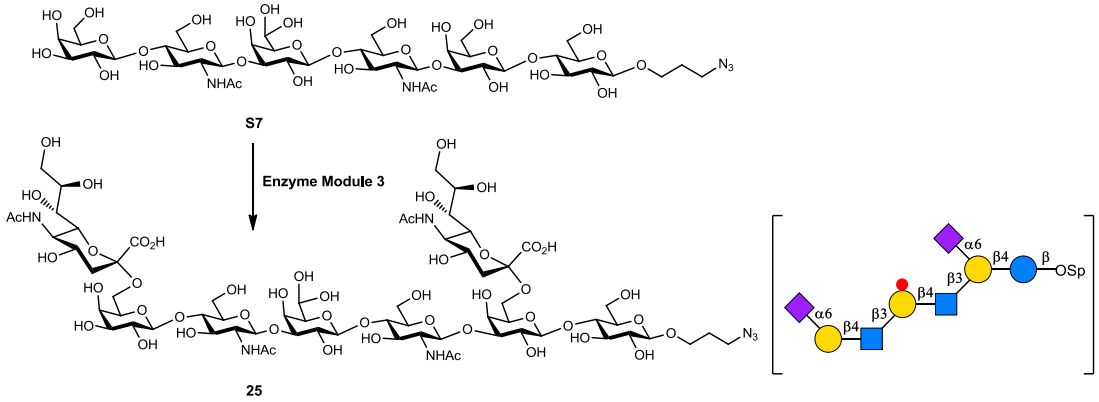
Hexasaccharide **S7** (45 mg, 90%), white solid after lyophilization. ^{1}H NMR (600

MHz, D_2O) δ 5.13 (s, 1H), 4.71 (d, *J* = 8.5 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.52 – 4.45 (m, 3H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.31 (d, *J* = 3.2 Hz, 1H), 4.15 (d, *J* = 3.3 Hz, 2H), 4.04 – 3.90 (m, 5H), 3.90 – 3.51 (m, 27H), 3.46 (t, *J* = 6.7 Hz, 2H), 3.35 – 3.26 (m, 1H), 2.03 (brs, 6H), 1.91 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.88, 103.13, 102.91, 102.82, 102.73, 102.67, 102.07, 87.82, 82.00, 81.94, 79.13, 78.30, 78.13, 75.32, 74.84, 74.74, 74.51, 74.37, 74.32, 72.75, 72.46, 72.26, 72.17, 72.14, 70.93, 69.93, 69.66, 68.51, 68.29, 67.64, 67.34, 61.00, 60.91, 60.01, 59.90, 59.83, 55.16, 55.01, 47.83, 28.20, 22.14; HRMS (ESI) *m/z* calcd for $C_{43}H_{73}N_5O_{32}Na$ $[M+H_2O+Na]^+$ 1194.4131, found 1194.4194.



Scheme S7. Synthesis of compounds **25** and **26** from **S7**.

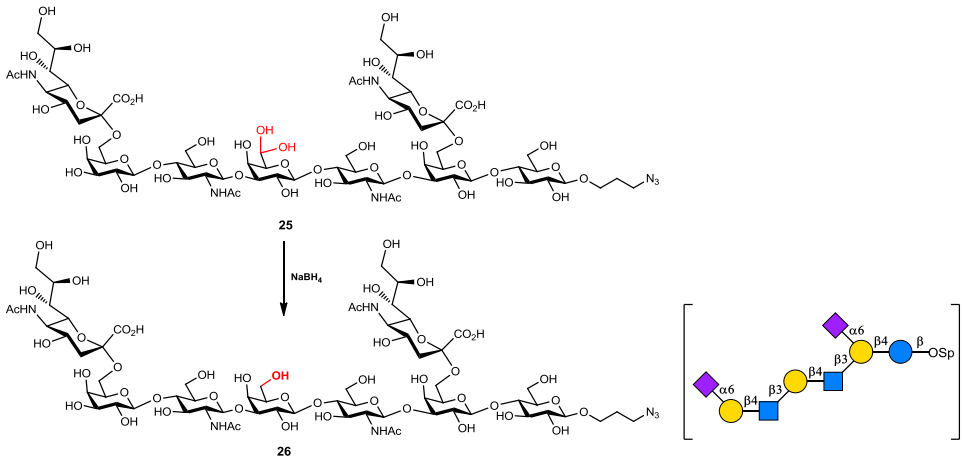
Reagents and conditions: a) Enzyme module 3: Neu5Ac (3.0 equiv), CTP (3.0 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 95%; b) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 98%.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (25)

Octasaccharide **25** (50 mg, 95%), white solid after lyophilization. ^1H NMR (600

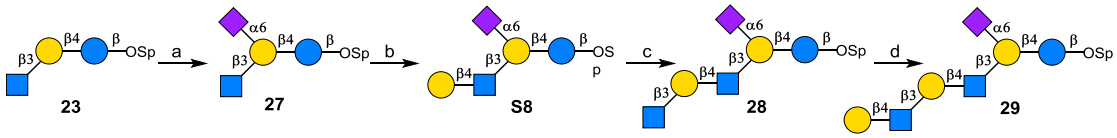
MHz, D_2O) δ 5.09 (d, *J* = 7.2 Hz, 1H), 4.70 (d, *J* = 8.0 Hz, 1H), 4.64 (d, *J* = 8.6 Hz, 1H), 4.48 – 4.40 (m, 3H), 4.38 (d, *J* = 8.0 Hz, 1H), 4.28 (d, *J* = 3.2 Hz, 1H), 4.14 (d, *J* = 3.3 Hz, 2H), 4.01 – 3.47 (m, 45H), 3.45 – 3.39 (m, 3H), 3.29 (t, *J* = 8.6 Hz, 1H), 2.67 (dd, *J* = 12.5, 4.7 Hz, 1H), 2.63 (dd, *J* = 12.4, 4.8 Hz, 1H), 2.02 (s, 3H), 1.99 (s, 9H), 1.88 (p, *J* = 6.6 Hz, 2H), 1.69 (t, *J* = 12.1 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.78, 173.41, 103.35, 103.09, 103.06, 102.64, 102.49, 101.86, 100.16, 100.01, 87.77, 82.07, 81.75, 80.32, 79.46, 79.01, 76.32, 74.52, 74.47, 74.27, 74.12, 73.56, 73.10, 72.58, 72.41, 72.28, 72.22, 72.15, 71.63, 71.59, 70.60, 69.60, 69.54, 68.26, 68.23, 68.09, 68.04, 67.62, 67.20, 63.31, 63.23, 62.52, 62.50, 60.10, 60.00, 59.79, 54.91, 54.80, 51.76, 51.66, 47.75, 39.99, 39.95, 28.11, 22.16, 22.08, 21.95, 21.93; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{47}$ $[M+H_2O-2H]^{2-}$ 875.8001, found 875.7878.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (26)

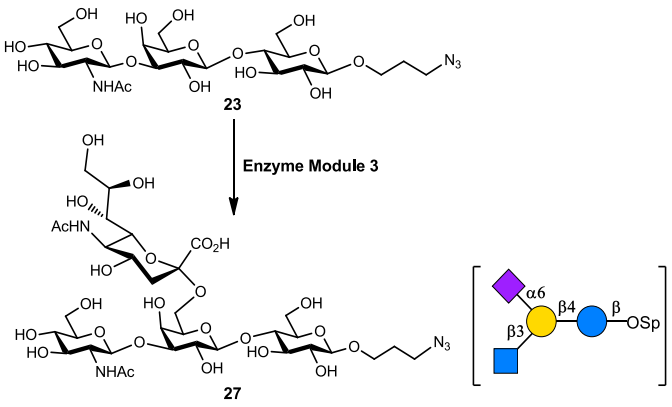
Octasaccharide **26** (14 mg, 98%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.73 (d, *J* = 7.9 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.48 – 4.44 (m, 2H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.16 (d, *J* = 3.4 Hz, 1H), 4.03 – 3.51 (m, 49H), 3.47 (t, *J* = 6.7 Hz, 2H), 3.36 – 3.31 (m, 1H), 2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.67 (dd, *J* = 12.3, 4.8 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 9H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.76 – 1.69 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ

174.88, 174.84, 173.53, 173.45, 103.43, 103.19, 102.85, 102.77, 102.57, 101.96, 100.26, 100.11, 82.16, 81.94, 80.39, 79.59, 78.11, 74.83, 74.62, 74.58, 74.53, 74.23, 73.66, 73.21, 72.67, 72.51, 72.39, 72.22, 72.20, 71.73, 71.69, 70.70, 69.97, 69.64, 68.36, 68.33, 68.18, 68.14, 67.30, 63.41, 63.32, 62.62, 62.60, 60.95, 60.21, 60.09, 59.81, 55.12, 54.90, 51.86, 51.76, 47.85, 40.09, 40.05, 28.21, 22.25, 22.15, 22.03, 22.01; HRMS (ESI) *m/z* calcd for $C_{65}H_{106}N_7O_{47}$ $[M-2H]^{2-}$ 867.8026, found 867.7948.



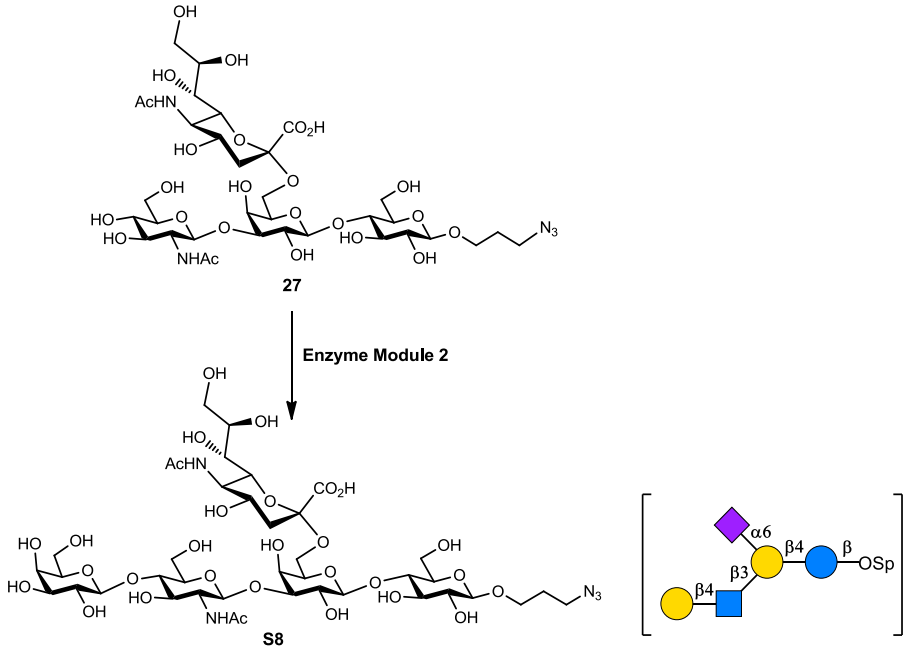
Scheme S8. Synthesis of compounds **27, S8, 28** and **29** from **23**.

Reagents and conditions: a) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 95%; b) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 94%; c) Enzyme module 1: *N*–acetylglucosamine (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), NahK/GlmU, HpLgtA, Tris-HCl (100 mM, pH 8.0), 37 ℃, 93%; d) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 90%.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3, 5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (27)

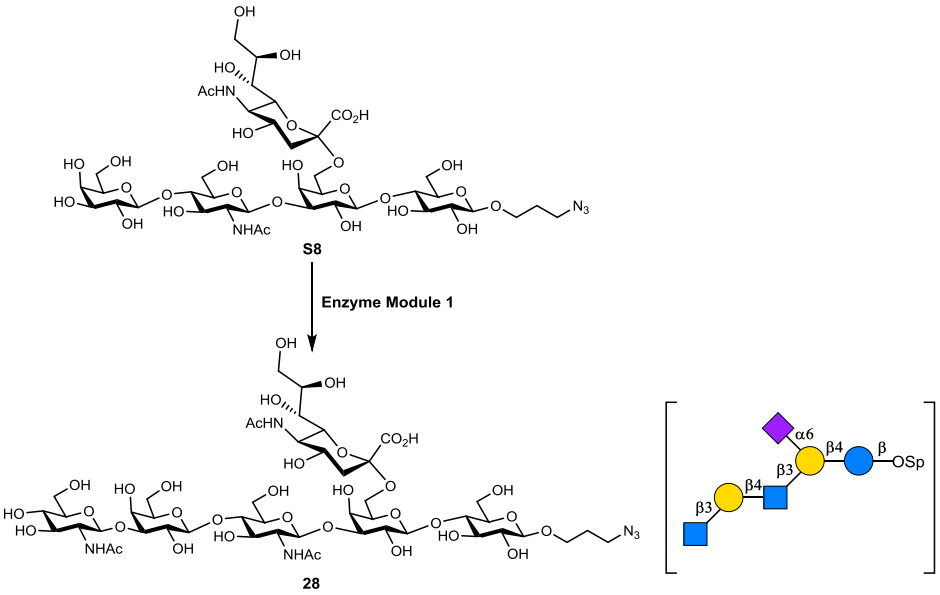
Tetrasaccharide **27** (53 mg, 95%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.66 (d, *J* = 8.5 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.17 (d, *J* = 3.4 Hz, 1H), 4.03 – 3.51 (m, 23H), 3.50 – 3.39 (m, 4H), 3.33 (t, *J* = 8.6 Hz, 1H), 2.70 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.03 (s, 3H), 2.02 (s, 3H), 1.91 (p, *J* = 6.7 Hz, 2H), 1.72 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.86, 173.42, 103.17, 102.87, 101.96, 100.25, 82.09, 79.54, 75.62, 74.61, 74.56, 73.58, 73.20, 72.69, 72.50, 71.73, 69.70, 69.67, 68.32, 68.30, 68.14, 67.29, 63.37, 62.61, 60.48, 60.21, 55.63, 51.77, 47.85, 40.09, 28.22, 22.17, 22.06; HRMS (ESI) *m/z* calcd for $C_{34}H_{56}N_5O_{24}$ $[M-H]^-$ 918.3321, found 918.3263.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S8)

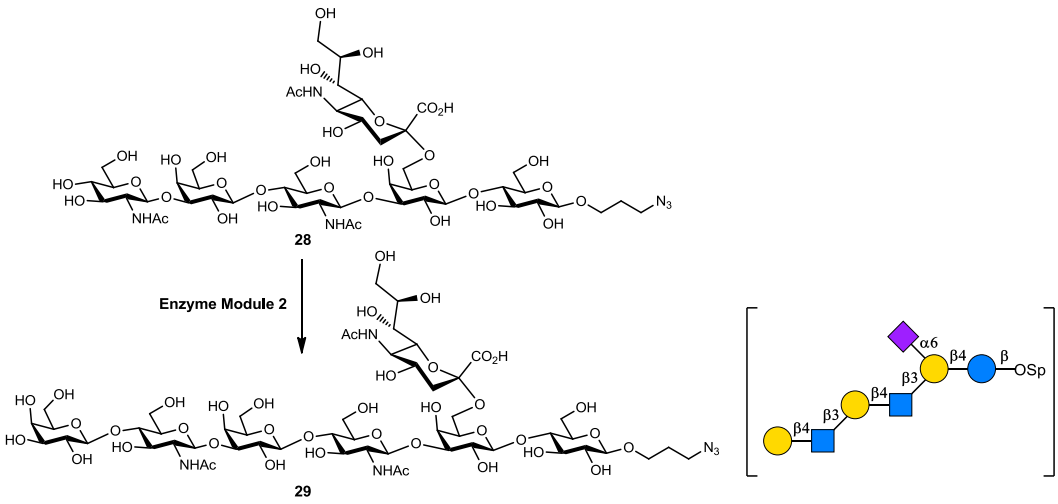
Pentasaccharide **S8** (49 mg, 94%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.68 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.03 – 3.50 (m, 31H), 3.46 (t, *J* = 6.7 Hz, 2H), 3.33 (dd, *J* = 9.2, 8.1 Hz, 1H), 2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.03 (s, 6H), 1.92 (p, *J* = 6.7 Hz, 2H), 1.73 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.88, 174.83, 173.45, 103.19, 102.82, 102.76, 101.95, 100.26, 82.19, 79.59, 78.11, 75.29, 74.61, 74.57, 74.52, 73.21, 72.67, 72.50, 72.45, 72.21, 71.72, 70.93, 69.62, 68.52, 68.32,

67.29, 63.40, 62.59, 60.99, 60.21, 59.83, 55.16, 51.75, 47.84, 40.08, 28.20, 22.14, 22.01; HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{29}$ $[M-H]^-$ 1080.3849, found 1080.3832.



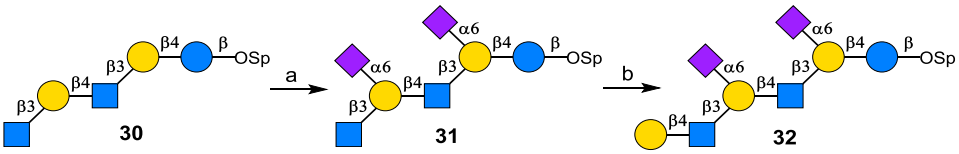
3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (28)

Hexasaccharide **28** (43 mg, 93%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.68 (d, *J* = 8.4, 1H), 4.68 (d, *J* = 8.4, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.46 (d, *J* = 7.9 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.15 (d, *J* = 3.3 Hz, 1H), 4.03 – 3.54 (m, 34H), 3.49 – 3.42 (m, 4H), 3.33 (dd, *J* = 9.0, 8.2 Hz, 1H), 2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.73 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.96, 174.90, 174.85, 173.46, 103.20, 102.87, 102.86, 102.78, 101.97, 100.27, 82.21, 81.91, 79.59, 78.13, 75.62, 74.84, 74.62, 74.58, 74.53, 73.54, 73.20, 72.68, 72.51, 72.20, 71.74, 69.99, 69.65, 69.62, 68.33, 68.13, 67.30, 63.40, 62.60, 60.93, 60.44, 60.21, 59.83, 55.62, 55.13, 51.76, 47.85, 40.09, 28.21, 22.14, 22.13, 22.02; HRMS (ESI) m/z calculated for $C_{48}H_{79}N_6O_{34}$ $[M-H]^-$ 1283.4643, found 1283.4204.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (29)

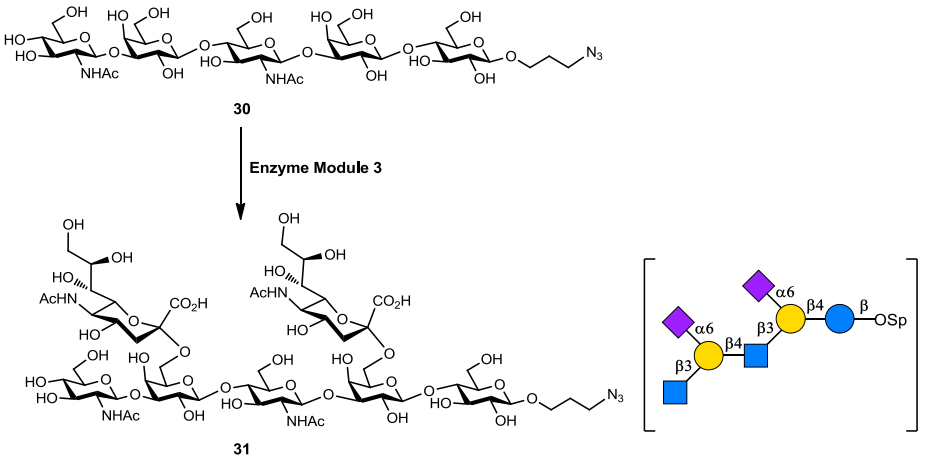
Heptasaccharide **29** (35 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.71 (d, *J* = 8.3 Hz, 1H), 4.69 (d, *J* = 8.3 Hz, 1H), 4.50 (d, *J* = 6.8 Hz, 1H), 4.48 (d, *J* = 6.7 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.16 (d, *J* = 3.3 Hz, 1H), 4.03 – 3.92 (m, 6H), 3.91 – 3.52 (m, 36H), 3.47 (t, *J* = 6.7 Hz, 2H), 3.34 (t, *J* = 8.6 Hz, 1H), 2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.93 (p, *J* = 6.6 Hz, 2H), 1.73 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.89, 174.84, 173.46, 103.19, 102.86, 102.83, 102.77, 102.74, 101.96, 100.27, 82.19, 81.99, 79.57, 78.11, 75.32, 74.84, 74.62, 74.57, 74.52, 73.20, 72.69, 72.51, 72.48, 72.20, 72.15, 71.74, 70.94, 69.96, 69.64, 68.53, 68.33, 68.14, 67.30, 63.40, 62.61, 61.01, 60.94, 60.21, 59.83, 55.15, 55.13, 51.77, 47.86, 40.09, 28.21, 22.17, 22.05; HRMS (ESI) *m/z* calcd for $C_{54}H_{89}N_6O_{39}$ $[M-H]^-$ 1445.5171, found 1445.5004.



Scheme S9. Synthesis of compounds **31** and **32** from **30**.

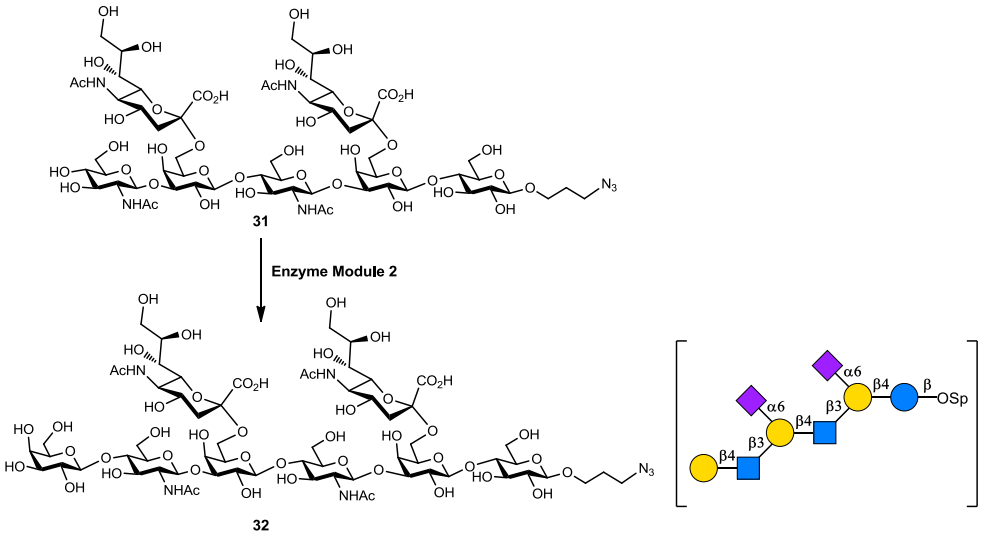
Reagents and conditions: a) Enzyme module 3: Neu5Ac (3.0 equiv), CTP (3.0 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 92%;

b) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 88%.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (31)

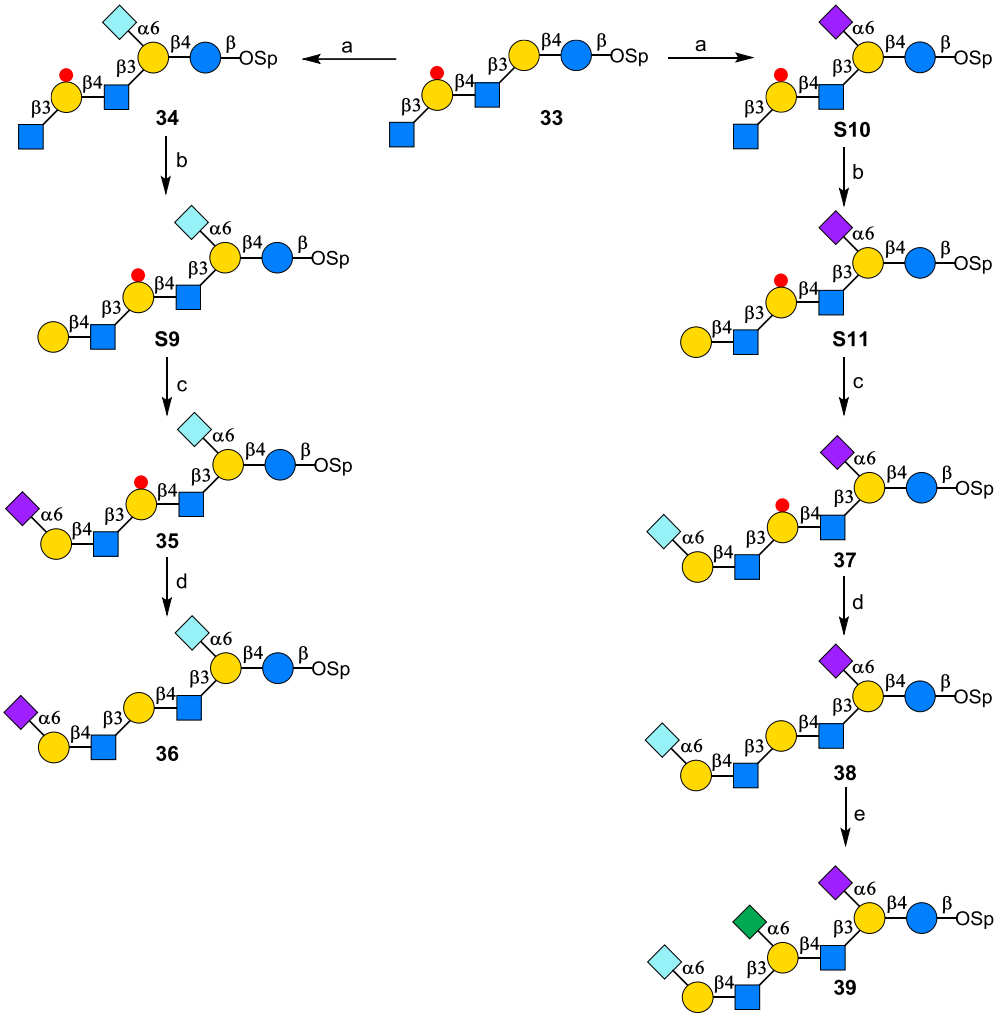
Heptasaccharide **31** (25 mg, 92%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.70 (d, *J* = 8.1 Hz, 1H), 4.66 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.44 (d, *J* = 8.2 Hz, 1H), 4.42 (d, *J* = 8.1 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.16 (d, *J* = 3.2 Hz, 1H), 4.03 – 3.93 (m, 5H), 3.91 – 3.69 (m, 21H), 3.68 – 3.52 (m, 15H), 3.49 – 3.41 (m, 4H), 3.33 (dd, *J* = 9.0, 8.3 Hz, 1H), 2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.66 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.73 (t, *J* = 11.7 Hz, 1H), 1.71 (t, *J* = 11.7 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.91, 174.88, 173.53, 173.46, 103.54, 103.21, 102.90, 102.63, 101.96, 100.27, 100.11, 82.17, 82.15, 80.58, 79.63, 75.62, 74.63, 74.59, 74.24, 73.61, 73.23, 72.68, 72.51, 72.32, 71.72, 71.64, 69.67, 69.64, 69.60, 68.35, 68.19, 68.13, 68.05, 67.30, 63.43, 63.19, 62.61, 62.60, 60.44, 60.20, 60.12, 55.62, 54.88, 51.86, 51.76, 47.85, 40.09, 28.21, 22.25, 22.13, 22.02, 22.00; HRMS (ESI) *m/z* calcd for $C_{59}H_{95}N_7O_{42}$ $[M-2H]^{2-}$ 786.7762, found 786.7707.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (32)

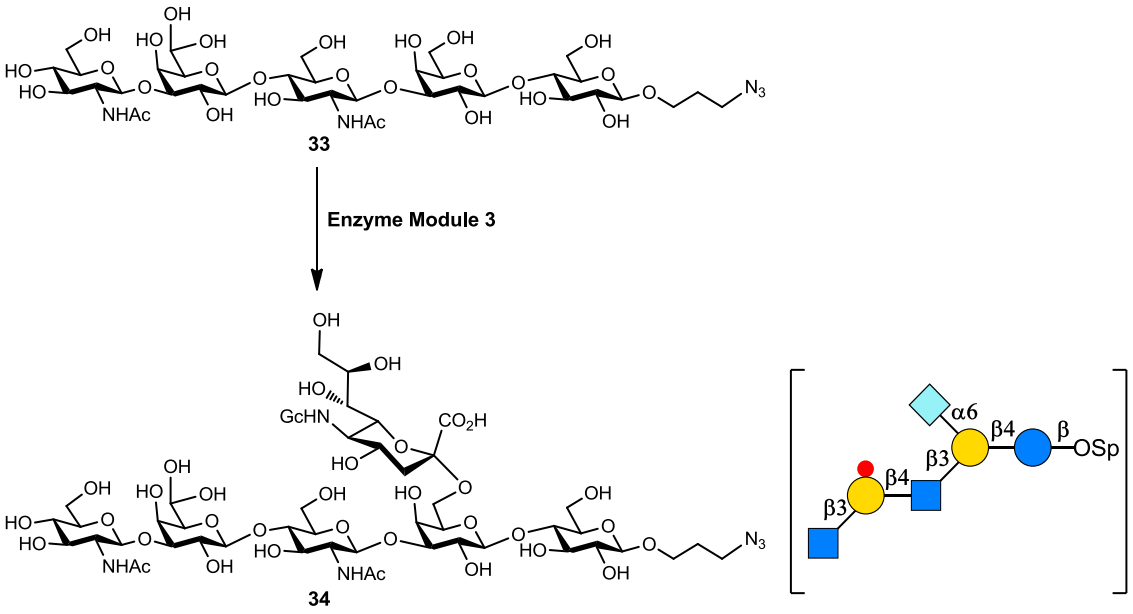
Octasaccharide **32** (15 mg, 88%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.71 (d, *J* = 8.1 Hz, 1H), 4.69 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.44 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.18 (d, *J* = 3.2 Hz, 1H), 4.17 (d, *J* = 3.3 Hz, 1H), 4.03 – 3.91 (m, 7H), 3.91 – 3.51 (m, 42H), 3.47 (t, *J* = 6.7 Hz, 2H), 3.34 (t, *J* = 8.7 Hz, 1H), 2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.66 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 9H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.74 (t, *J* = 11.8 Hz, 1H), 1.70 (t, *J* = 11.7 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.86, 173.52, 173.45, 103.53, 103.20, 102.82, 102.79, 102.61, 101.95, 100.26, 100.10, 82.20, 82.16, 80.56, 79.62, 78.07, 75.29, 74.62, 74.58, 74.51, 74.23, 73.21, 72.66, 72.50, 72.45, 72.31, 72.21, 71.71, 71.64, 70.92, 69.63, 69.56, 68.52, 68.34, 68.18, 68.12, 68.04, 67.29, 63.42, 63.19, 62.59, 60.99, 60.19, 60.10, 59.82, 55.15, 54.87, 51.85, 51.75, 47.84, 40.09, 40.07, 28.20, 22.25, 22.14, 22.02, 21.99; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{47}$ $[M-2H]^{2-}$ 867.8026, found 867.7927.

2.2 Site-specific α2–6-sialylation with different sialic acid forms



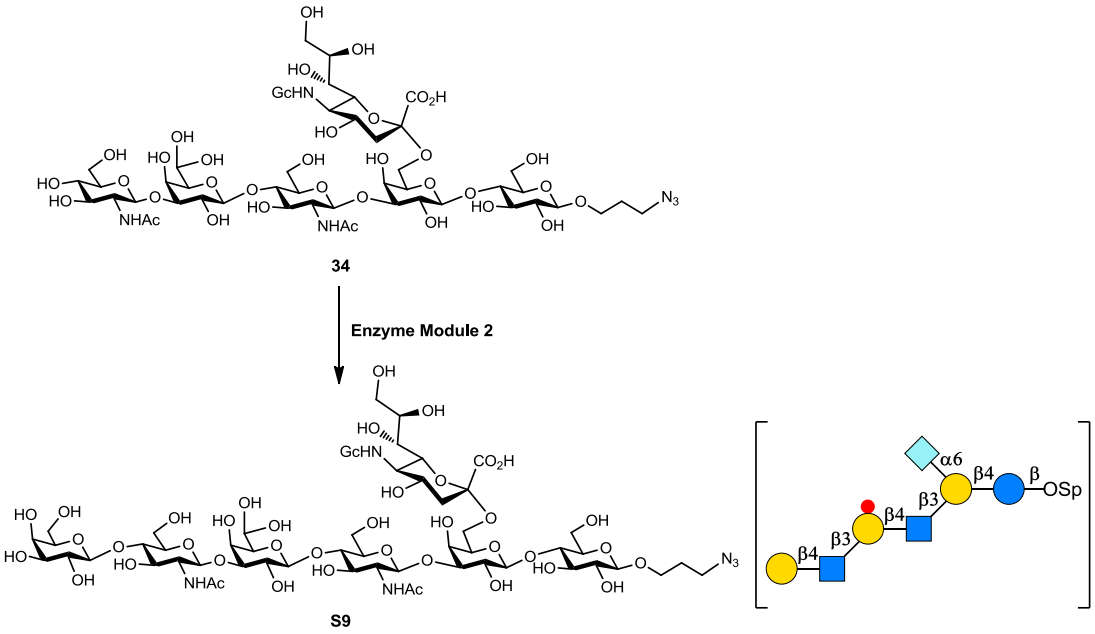
Scheme S10. Synthesis of compounds **S9** to **S11**, and **34** to **39** from **33**.

Reagents and conditions: a) Enzyme module 3: Neu5Gc (1.5 equiv) or Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 92% for **34** , 95% for **S10**; b) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 89% for **S9**, 90% for **S11**; c) Enzyme module 3: Neu5Ac (1.5 equiv) or Neu5Gc (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 90% for **35**, 91% for **37**; d) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 94% for **36**, 92% for **38**; e) Enzyme module 3: Kdn (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 80%.



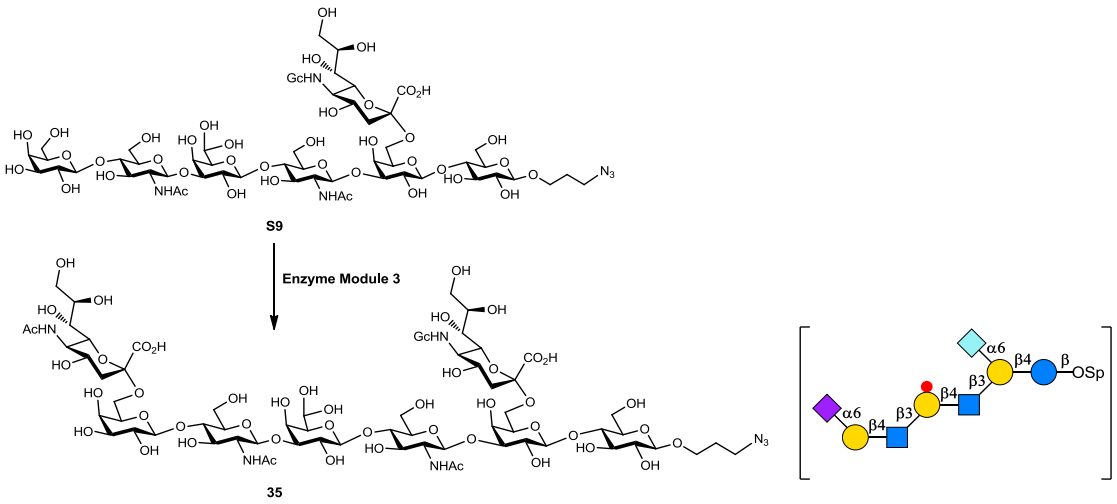
3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (34)

Hexasaccharide **34** (60 mg, 92%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.09 (d, *J* = 7.3 Hz, 1H), 4.66 (d, *J* = 8.5 Hz, 1H), 4.64 (d, *J* = 8.3 Hz, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.38 (d, *J* = 7.9 Hz, 1H), 4.28 (d, *J* = 3.2 Hz, 1H), 4.15 (d, *J* = 3.3 Hz, 1H), 4.09 (s, 2H), 4.00 – 3.50 (m, 31H), 3.47 – 3.40 (m, 5H), 3.31 (t, *J* = 8.6 Hz, 1H), 2.69 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.01 (s, 3H), 1.99 (s, 3H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.71 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D2O) δ 175.57, 174.85, 174.77, 173.39, 103.11, 103.08, 102.77, 102.65, 101.87, 100.18, 87.78, 82.11, 81.73, 79.50, 79.03, 76.34, 75.52, 74.53, 74.49, 74.28, 73.46, 73.13, 72.59, 72.24, 72.14, 71.69, 69.63, 69.59, 69.52, 68.17, 68.06, 67.97, 67.66, 67.21, 63.35, 62.46, 60.86, 60.36, 60.11, 59.82, 55.53, 54.93, 51.37, 47.75, 40.05, 28.12, 22.07, 22.04; HRMS (ESI) *m/z* calcd for $C_{48}H_{79}N_6O_{36}$ $[M+H_2O-H]^-$ 1315.4541, found 1315.4362.



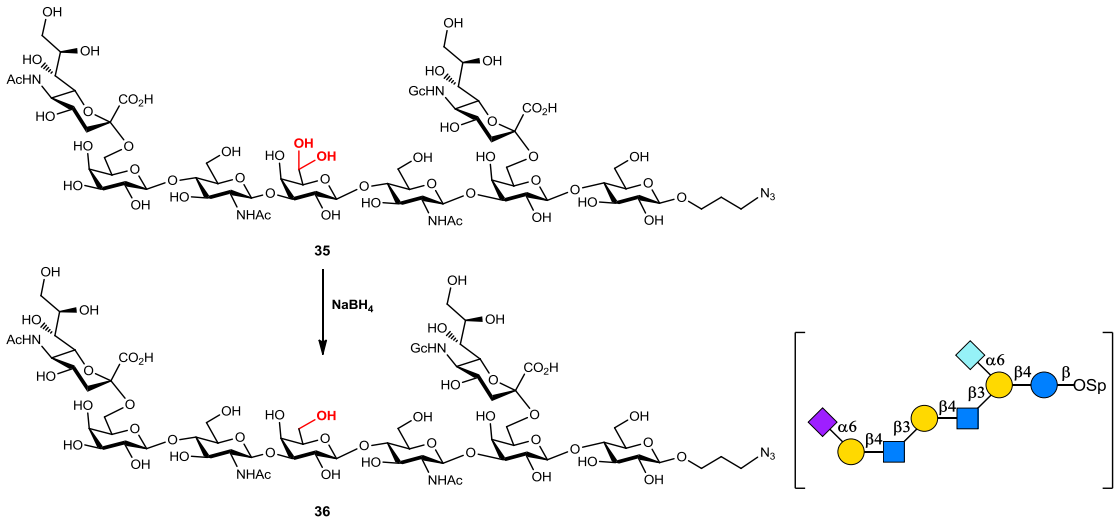
3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S9)

Heptasaccharide **S9** (48 mg, 89%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.2 Hz, 1H), 4.72 (d, *J* = 8.4 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.51 – 4.45 (m, 3H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.31 (d, *J* = 3.2 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.12 (s, 2H), 4.04 – 3.51 (m, 39H), 3.49 – 3.43 (m, 3H), 3.34 (t, *J* = 8.6 Hz, 1H), 2.72 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.74 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.65, 174.87, 174.85, 173.47, 103.19, 103.17, 102.82, 102.74, 102.72, 101.96, 100.27, 87.87, 82.18, 81.88, 79.59, 79.12, 78.12, 76.42, 75.31, 74.61, 74.58, 74.51, 74.37, 73.22, 72.68, 72.47, 72.32, 72.23, 72.17, 71.78, 70.93, 69.69, 69.62, 68.52, 68.26, 68.15, 68.05, 67.74, 67.30, 63.44, 62.56, 61.00, 60.95, 60.21, 59.91, 59.83, 55.15, 55.02, 51.46, 47.85, 40.14, 28.21, 22.16; HRMS (ESI) *m/z* calcd for $C_{54}H_{89}N_6O_{41}$ $[M+H_2O-H]^-$ 1477.5070, found 1477.4873.



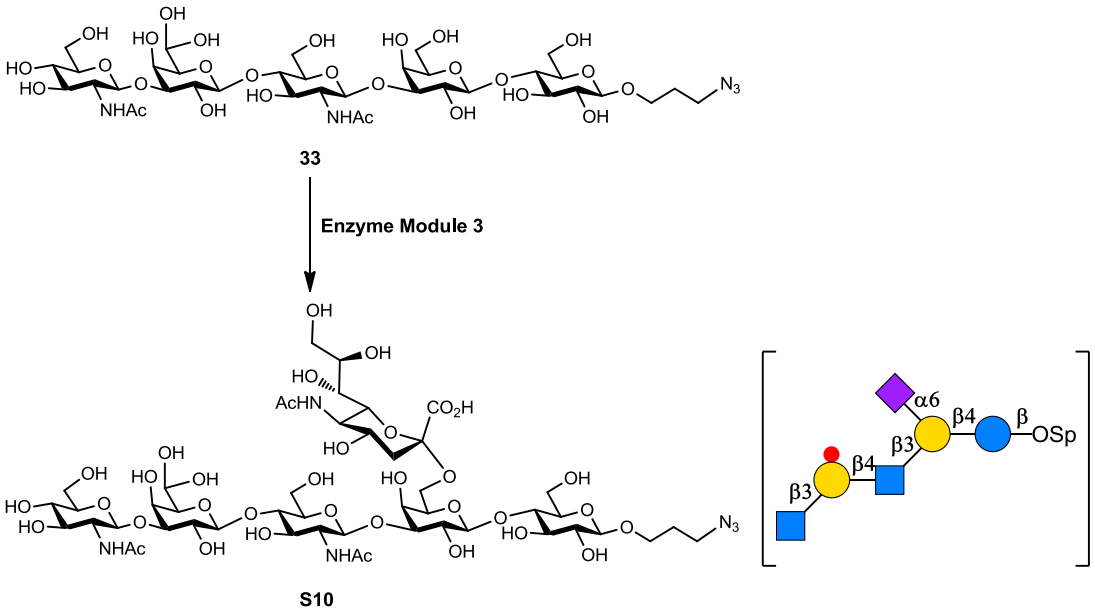
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (35)

Octasaccharide **35** (19 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.2 Hz, 1H), 4.74 (d, *J* = 8.5 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.31 (t, *J* = 2.5 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.12 (s, 2H), 4.03 – 3.51 (m, 47H), 3.48 – 3.43 (m, 3H), 3.34 (dd, 1H), 2.72 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.67 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.91 (p, *J* = 6.6 Hz, 2H), 1.74 (t, *J* = 12.7 Hz, 1H), 1.72 (t, *J* = 12.6 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.66, 174.90, 174.88, 174.85, 173.52, 173.47, 103.44, 103.19, 103.15, 102.73, 102.57, 101.95, 100.26, 100.11, 87.88, 82.15, 81.83, 80.42, 79.60, 79.11, 76.42, 74.61, 74.58, 74.38, 74.22, 73.66, 73.23, 72.68, 72.51, 72.39, 72.33, 72.24, 72.22, 71.77, 71.69, 70.70, 69.70, 69.64, 68.36, 68.34, 68.27, 68.19, 68.16, 68.06, 67.29, 63.46, 63.32, 62.62, 62.56, 60.95, 60.21, 60.11, 59.90, 55.01, 54.91, 51.86, 51.46, 47.85, 40.14, 40.05, 28.20, 22.25, 22.16, 22.01; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{49}$ $[M+H_2O-2H]^{2-}$ 883.7976, found 883.7901.



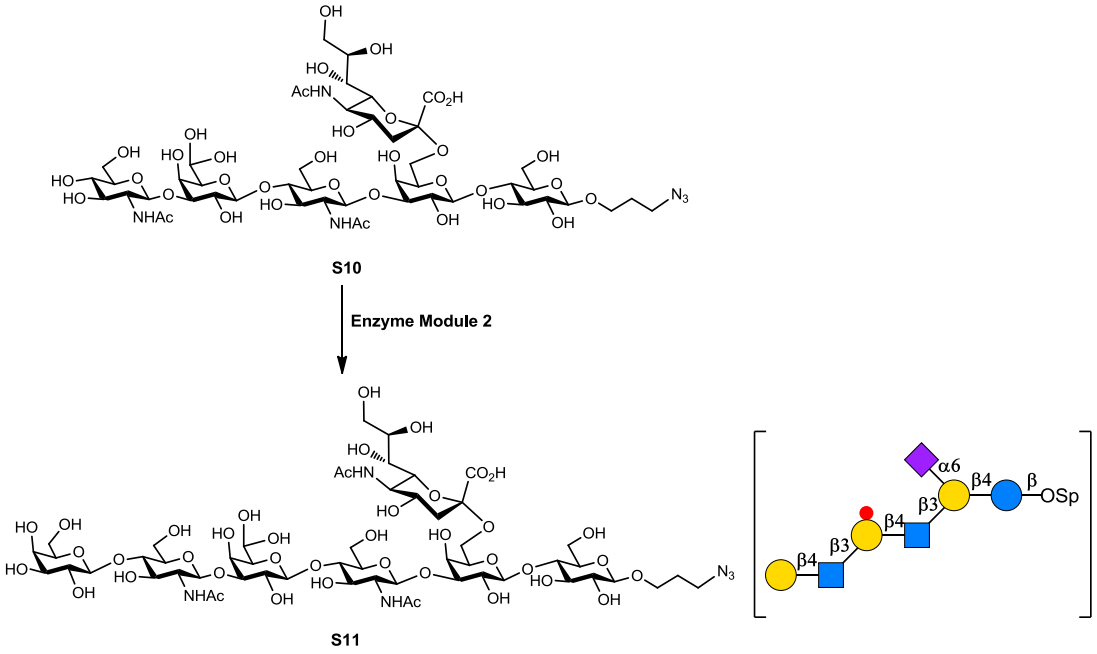
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (36)

Octasaccharide **36** (10 mg, 94%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.65 (d, *J* = 7.9 Hz, 1H), 4.61 (d, *J* = 8.3 Hz, 1H), 4.42 (d, *J* = 8.0 Hz, 1H), 4.39 (d, *J* = 6.8 Hz, 1H), 4.38 (d, *J* = 6.8 Hz, 1H), 4.34 (d, *J* = 7.9 Hz, 1H), 4.11 (d, *J* = 3.3 Hz, 1H), 4.09 (d, *J* = 3.1 Hz, 1H), 4.05 (s, 2H), 3.97 – 3.44 (m, 49H), 3.39 (t, *J* = 6.7 Hz, 2H), 3.27 (t, *J* = 8.7 Hz, 1H), 2.65 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.60 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.98 (s, 3H), 1.96 (s, 6H), 1.84 (p, *J* = 6.6 Hz, 2H), 1.68 (t, *J* = 12.6 Hz, 1H), 1.66 (t, *J* = 12.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.66, 174.91, 174.88, 174.84, 173.52, 173.47, 103.43, 103.19, 102.84, 102.77, 102.57, 101.95, 100.26, 100.10, 82.15, 81.94, 80.38, 79.59, 78.10, 74.83, 74.61, 74.58, 74.52, 74.22, 73.66, 73.23, 72.68, 72.51, 72.38, 72.22, 71.77, 71.69, 70.70, 69.96, 69.64, 69.56, 68.36, 68.26, 68.18, 68.05, 67.29, 63.45, 63.32, 62.62, 62.56, 60.95, 60.20, 60.09, 59.81, 55.12, 54.90, 51.86, 51.46, 47.84, 40.13, 40.05, 28.20, 22.25, 22.15, 22.01; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{48}$ $[M-2H]^{2-}$ 875.8001, found 875.7963.



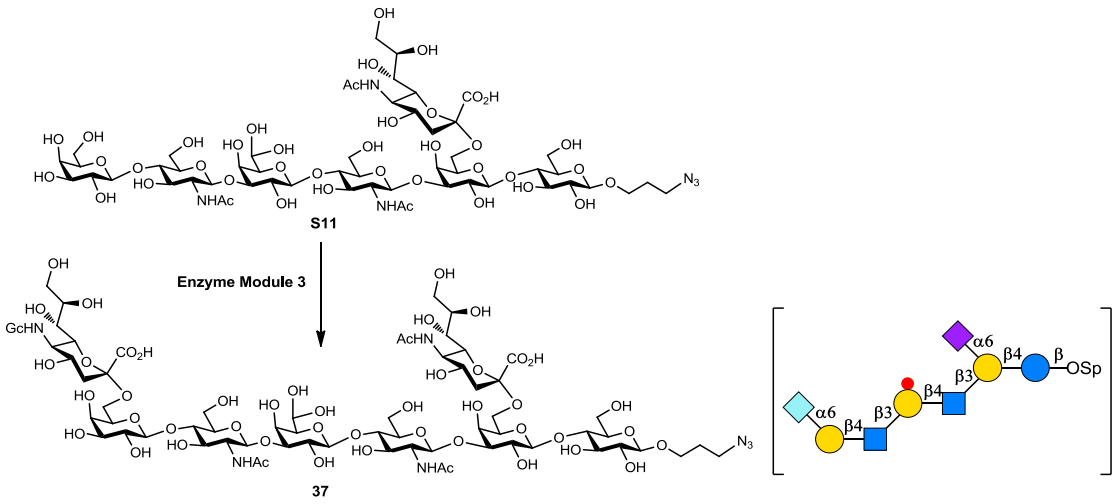
3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S10)

Hexasaccharide **S10** (260 mg, 95%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.3 Hz, 1H), 4.69 (d, *J* = 8.5 Hz, 1H), 4.68 (d, *J* = 8.3 Hz, 1H), 4.49 (d, *J* = 8.1 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.31 (d, *J* = 3.2 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.04 – 3.42 (m, 36), 3.36 – 3.30 (m, 1H), 2.71 (dd, *J* = 12.0, 4.2 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.72 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.93, 174.88, 174.85, 173.45, 103.19, 103.16, 102.84, 102.72, 101.95, 100.26, 87.87, 82.20, 81.81, 79.58, 79.13, 76.42, 75.60, 74.61, 74.57, 74.37, 73.55, 73.20, 72.67, 72.50, 72.32, 71.73, 69.72, 69.68, 69.61, 68.32, 68.13, 67.75, 67.29, 63.39, 62.60, 60.45, 60.21, 59.92, 55.62, 55.02, 51.75, 47.84, 40.09, 28.20, 22.15, 22.12, 22.02; HRMS (ESI) *m/z* calcd for $C_{48}H_{79}N_6O_{35}$ $[M+H_2O-H]^-$ 1299.4592, found 1299.4387.



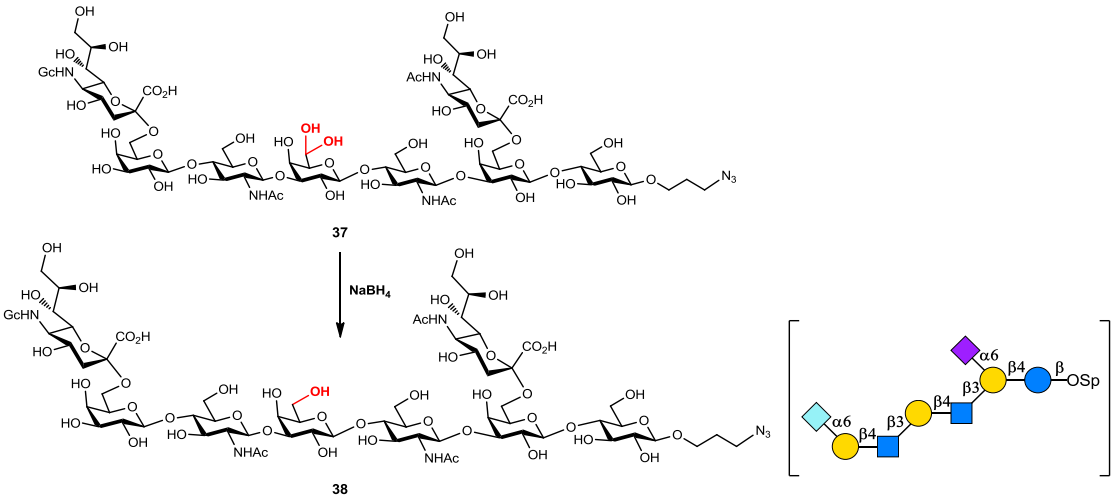
3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S11)

Heptasaccharide **S11** (99 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.11 (d, *J* = 7.3 Hz, 1H), 4.69 (d, *J* = 8.4 Hz, 1H), 4.65 (d, *J* = 8.3 Hz, 1H), 4.50 – 4.42 (m, 3H), 4.39 (d, *J* = 7.9 Hz, 1H), 4.29 (d, *J* = 3.3 Hz, 1H), 4.16 (d, *J* = 3.3 Hz, 1H), 4.01 – 3.49 (m, 39H), 3.46 – 3.42 (m, 3H), 3.31 (t, *J* = 8.6 Hz, 1H), 2.68 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.01 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.70 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.93, 174.88, 174.85, 173.45, 103.19, 103.17, 102.82, 102.74, 102.72, 101.96, 100.26, 87.87, 82.19, 81.89, 79.57, 79.12, 78.11, 76.42, 75.32, 74.61, 74.57, 74.51, 74.37, 73.20, 72.68, 72.50, 72.47, 72.32, 72.17, 71.73, 70.93, 69.68, 69.62, 68.52, 68.32, 68.13, 67.74, 67.30, 63.40, 62.60, 61.01, 60.20, 59.91, 59.83, 55.15, 55.02, 51.76, 47.85, 40.09, 28.21, 22.16, 22.03; HRMS (ESI) *m/z* calcd for $C_{54}H_{89}N_6O_{40}$ $[M+H_2O-H]^-$ 1461.5120, found 1461.4906.



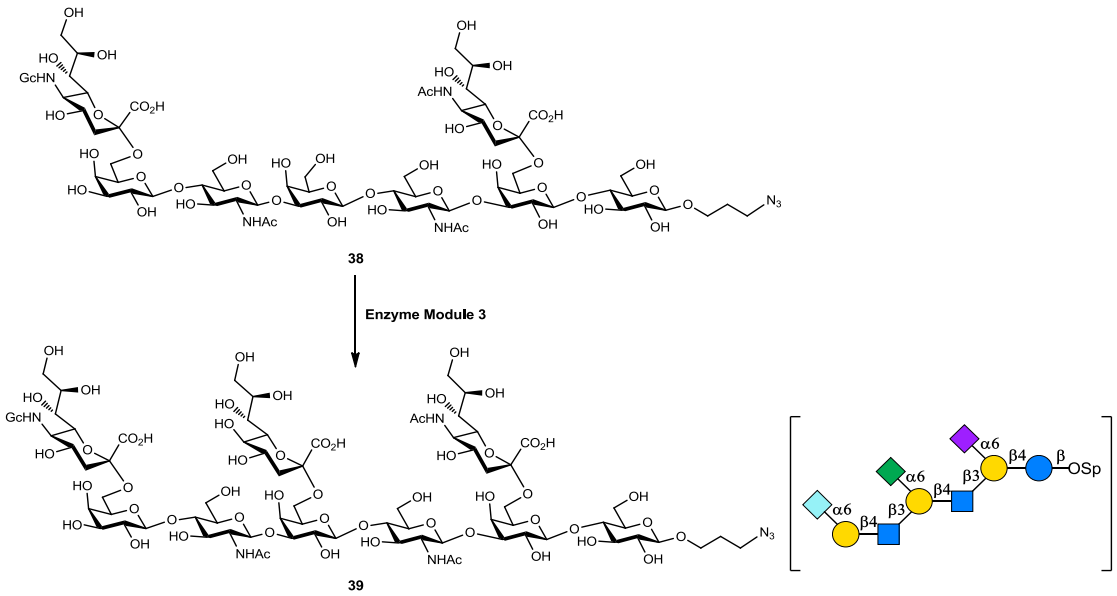
3-Azidopropyl 3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (37)

Octasaccharide **37** (87 mg, 91%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.2 Hz, 1H), 4.74 (d, *J* = 7.9 Hz, 1H), 4.68 (d, *J* = 8.3 Hz, 1H), 4.51 – 4.44 (m, 3H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.32 (d, *J* = 3.2 Hz, 1H), 4.18 (d, *J* = 3.3 Hz, 1H), 4.12 (s, 2H), 4.05 – 3.51 (m, 46H), 3.49 – 3.43 (m, 3H), 3.33 (t, *J* = 8.6 Hz, 1H), 2.71 (dd, *J* = 8.6, 3.9 Hz, 1H), 2.69 (dd, *J* = 8.5, 3.9 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.74 (t, *J* = 11.1 Hz, 1H), 1.73 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.65, 174.91, 174.88, 174.85, 173.54, 173.44, 103.42, 103.19, 103.15, 102.72, 102.58, 101.95, 100.26, 100.14, 87.88, 82.16, 81.84, 80.35, 79.58, 79.12, 76.42, 74.62, 74.57, 74.37, 74.24, 73.67, 73.21, 72.68, 72.50, 72.38, 72.33, 72.23, 71.73, 70.70, 69.70, 69.64, 68.35, 68.32, 68.30, 68.14, 67.93, 67.72, 67.30, 63.41, 63.35, 62.60, 60.96, 60.21, 60.10, 59.90, 55.01, 54.93, 51.76, 51.56, 47.85, 40.09, 28.21, 22.27, 22.17, 22.03; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{48}$ $[M+H_2O-2H]^{2-}$ 883.7976, found 883.7873.



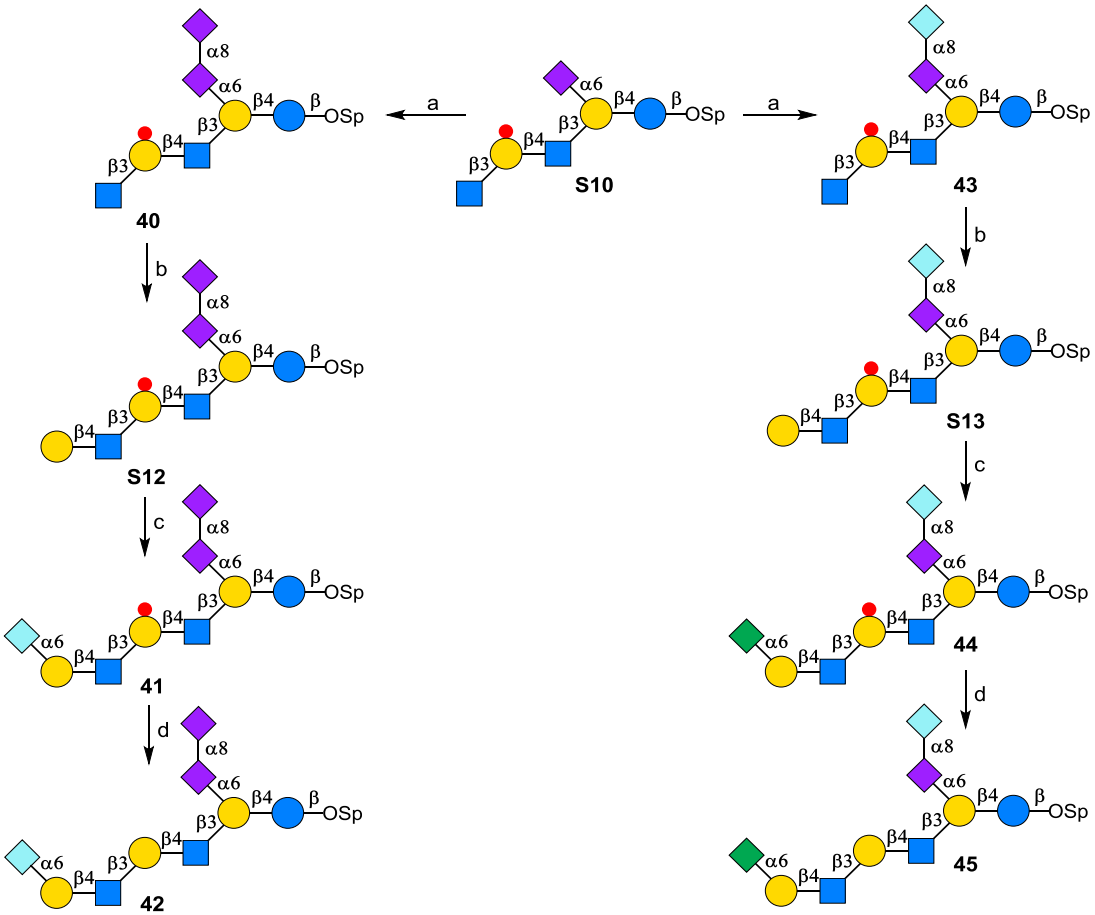
3-Azidopropyl 3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (38)

Octasaccharide **38** (56 mg, 92%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.70 (d, *J* = 7.9 Hz, 1H), 4.65 (d, *J* = 8.3 Hz, 1H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.44 (d, *J* = 7.8 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.39 (d, *J* = 7.9 Hz, 1H), 4.15 (d, *J* = 3.3 Hz, 1H), 4.13 (d, *J* = 3.2 Hz, 1H), 4.09 (s, 2H), 4.01 – 3.48 (m, 49H), 3.44 (t, *J* = 6.7 Hz, 2H), 3.31 (t, *J* = 8.6 Hz, 1H), 2.68 (dd, *J* = 8.9, 3.5 Hz, 1H), 2.66 (dd, *J* = 8.8, 3.3 Hz, 1H), 2.03 (s, 3H), 2.00 (s, 6H), 1.89 (p, *J* = 6.6 Hz, 2H), 1.71 (t, *J* = 12.3 Hz, 1H), 1.70 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.57, 174.79, 173.41, 103.33, 103.11, 102.76, 102.71, 102.51, 101.87, 100.18, 100.06, 82.08, 81.87, 80.24, 79.48, 78.00, 74.75, 74.53, 74.49, 74.44, 74.15, 73.58, 73.12, 72.59, 72.42, 72.30, 72.15, 72.12, 71.65, 70.61, 69.88, 69.55, 68.26, 68.23, 68.21, 68.05, 67.84, 67.21, 63.32, 63.26, 62.51, 60.87, 60.11, 59.99, 59.71, 55.03, 54.83, 51.67, 51.47, 47.76, 40.00, 28.13, 22.19, 22.08, 21.96; HRMS (ESI) *m/z* calcd for $C_{65}H_{105}N_7O_{48}$ $[M-2H]^{2-}$ 875.8001, found 875.7903.



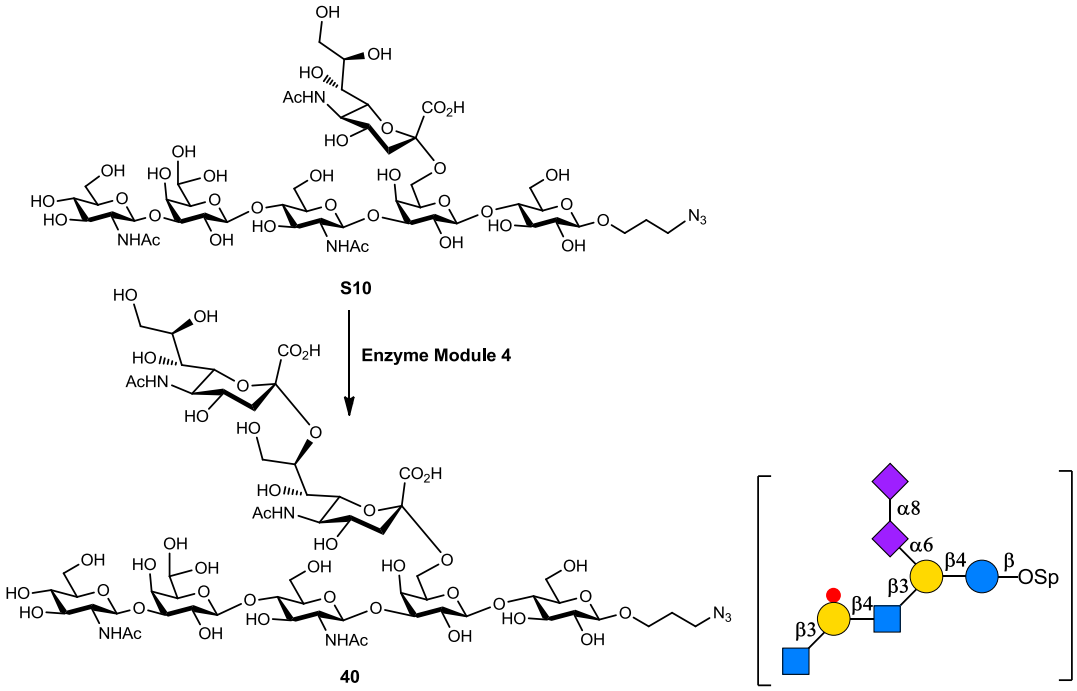
3-Azidopropyl 3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (39)

Nonasaccharide **39** (15 mg, 80%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.71 (t, *J* = 8.5 Hz, 2H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.46 (d, *J* = 7.9 Hz, 1H), 4.43 (dd, *J* = 7.9, 4.8 Hz, 2H), 4.19 (d, *J* = 3.4 Hz, 1H), 4.17 (d, *J* = 3.4 Hz, 1H), 4.13 (s, 2H), 4.03 – 3.44 (m, 58H), 3.37 – 3.31 (m, 1H), 2.70 (td, *J* = 12.1, 4.7 Hz, 2H), 2.62 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.06 (s, 6H), 2.04 (s, 3H), 1.91 (p, *J* = 7.0 Hz, 2H), 1.74 (td, *J* = 12.2, 4.9 Hz, 2H), 1.66 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.65, 174.89, 174.73, 173.66, 173.56, 173.46, 103.48, 103.34, 103.20, 102.66, 102.59, 101.96, 100.26, 100.15, 100.01, 82.16, 82.09, 80.40, 80.13, 79.63, 74.62, 74.59, 74.31, 74.24, 73.65, 73.49, 73.34, 73.24, 72.68, 72.50, 72.38, 72.28, 72.24, 72.07, 71.74, 71.73, 70.71, 70.12, 69.99, 69.65, 69.58, 68.34, 68.12, 67.93, 67.30, 63.50, 63.43, 63.33, 62.66, 62.60, 62.59, 60.96, 60.22, 60.01, 55.01, 54.91, 51.76, 51.57, 47.86, 40.09, 39.75, 28.21, 22.30, 22.27, 22.03; HRMS (ESI) *m/z* calcd for $C_{74}H_{119}N_7O_{56}$ $[M-2H]^{2-}$ 1000.8345, found 1000.8405.



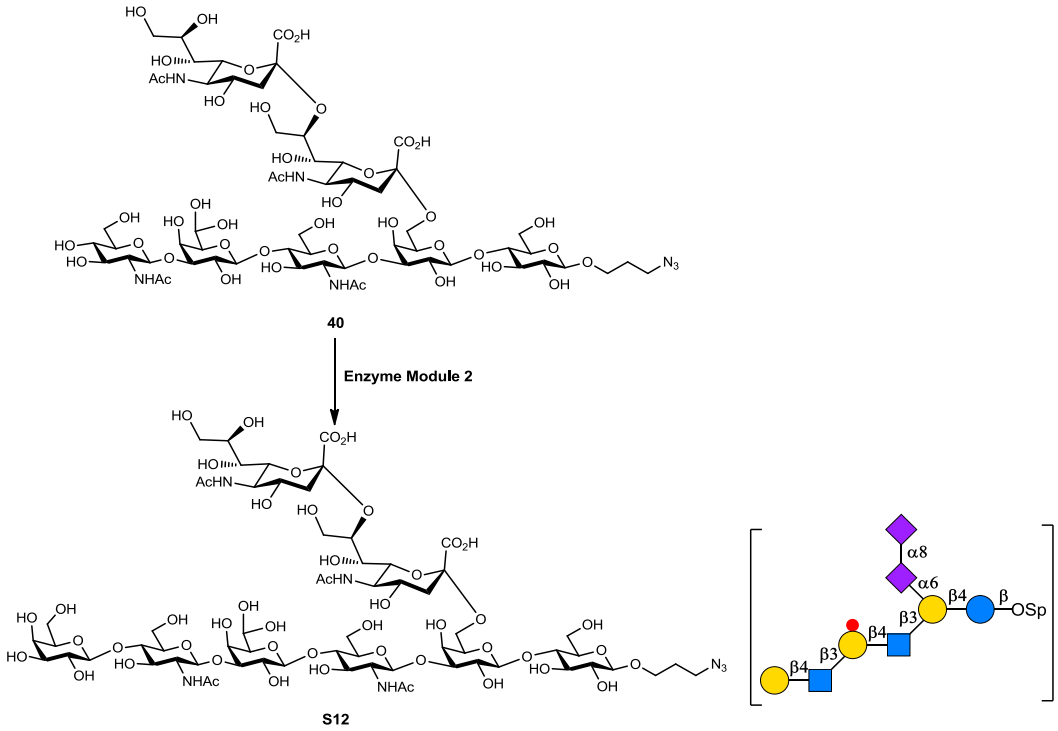
Scheme S11. Synthesis of compounds **42** to **47**, and **S12** to **S13** from **S10**.

Reagents and conditions: a) Enzyme module 4: Neu5Ac (1.5 equiv) or Neu5Gc (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, CjCstII, Tris-HCl (100 mM, pH 8.5), 37 ℃, 62% for **40**, 63% for **43**; b) Enzyme module 2: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, NmLgtB, Tris-HCl (100 mM, pH 8.0), 37 ℃, 84% for **S12**, 86% for **S13**; c) Enzyme module 3: Neu5Gc (1.5 equiv) or Kdn (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 91% for **41**, 90% for **44**; d) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 90% for **42**, 93% for **45**.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (40)

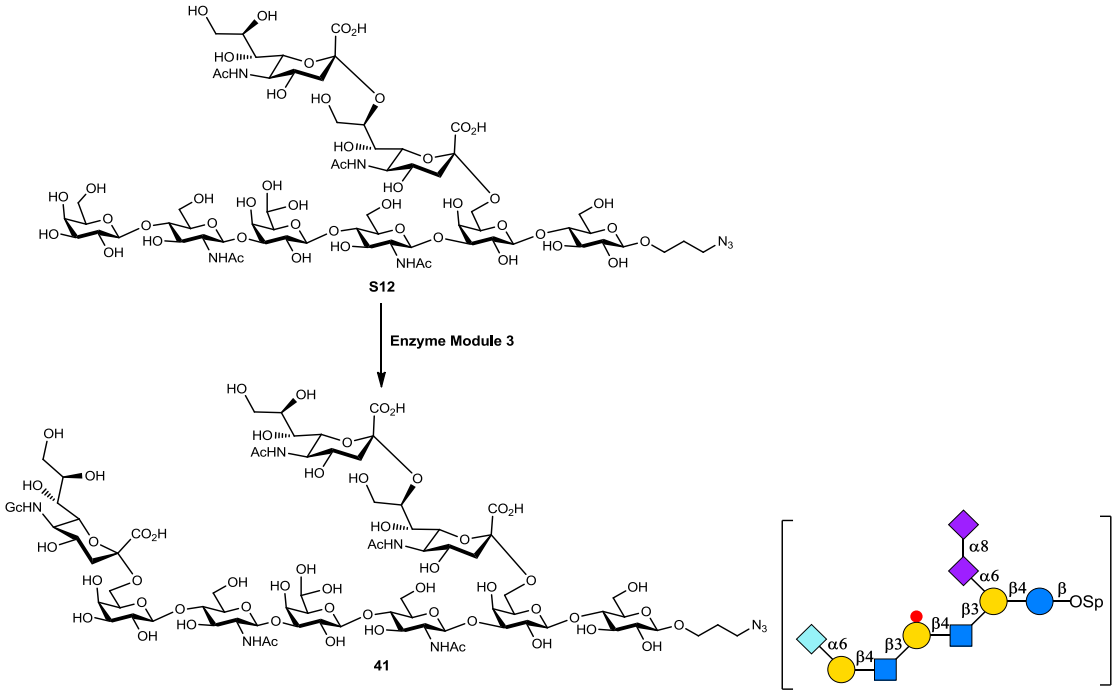
Heptasaccharide **40** (55 mg, 62 %), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.12 (d, *J* = 7.3 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.67 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.30 (d, *J* = 3.0 Hz, 1H), 4.21 – 4.17 (m, 2H), 4.11 (dd, *J* = 12.1, 3.7 Hz, 1H), 4.03 – 3.43 (m, 41H), 3.32 (t, *J* = 8.7 Hz, 1H), 2.77 (dd, *J* = 12.3, 4.5 Hz, 1H), 2.61 (dd, *J* = 12.3, 4.3 Hz, 1H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.91 (p, *J* = 6.6 Hz, 2H), 1.74 (t, *J* = 12.0 Hz, 1H), 1.67 (t, *J* = 12.3 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.96, 174.84, 173.39, 103.24, 103.18, 102.86, 102.70, 101.96, 100.96, 100.37, 87.89, 82.33, 81.78, 79.60, 79.10, 78.54, 76.42, 75.61, 74.66, 74.61, 74.39, 74.16, 73.56, 73.26, 72.68, 72.62, 72.36, 71.69, 69.74, 69.69, 69.61, 69.59, 68.48, 68.15, 68.06, 67.84, 67.78, 67.28, 63.59, 62.55, 61.58, 60.45, 60.26, 59.91, 55.62, 55.05, 52.27, 51.71, 47.85, 40.47, 40.13, 28.21, 22.27, 22.16, 22.13, 22.01; HRMS (ESI) *m/z* calcd for $C_{59}H_{93}N_7O_{42}$ $[M-2H]^{2-}$ 785.7684, found 785.7675.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S12)

Octasaccharide **S12** (42 mg, 84%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.3 Hz, 1H), 4.72 (d, *J* = 8.4 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.52 – 4.44 (m, 3H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.31 (d, *J* = 3.2 Hz, 1H), 4.21 – 4.17 (m, 2H), 4.11 (dd, *J* = 12.1, 3.7 Hz, 1H), 4.03 – 3.42 (m, 47H), 3.33 (t, *J* = 8.4, 1H), 2.77 (dd, *J* = 12.3, 4.6 Hz, 1H), 2.62 (dd, *J* = 12.3, 4.4 Hz, 1H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.92 (p, *J* = 6.7 Hz, 2H), 1.73 (t, *J* = 12.0 Hz, 1H), 1.67 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.95, 174.92, 174.91, 174.84, 173.38, 173.30, 103.24, 103.18, 102.83, 102.75, 102.70, 101.96, 100.95, 100.36, 87.89, 82.32, 81.84, 79.61, 79.09, 78.54, 78.11, 76.41, 75.31, 74.66, 74.61, 74.51, 74.39, 74.16, 73.27, 72.68, 72.62, 72.47, 72.35, 72.18, 71.69, 70.93, 69.71, 69.62, 69.59, 68.53, 68.48, 68.14, 68.06, 67.84, 67.78, 67.28, 63.60, 62.55, 61.59, 61.00, 60.26, 59.90, 59.83, 55.15, 55.05, 52.27, 51.71, 47.85, 40.47, 40.13, 28.21,

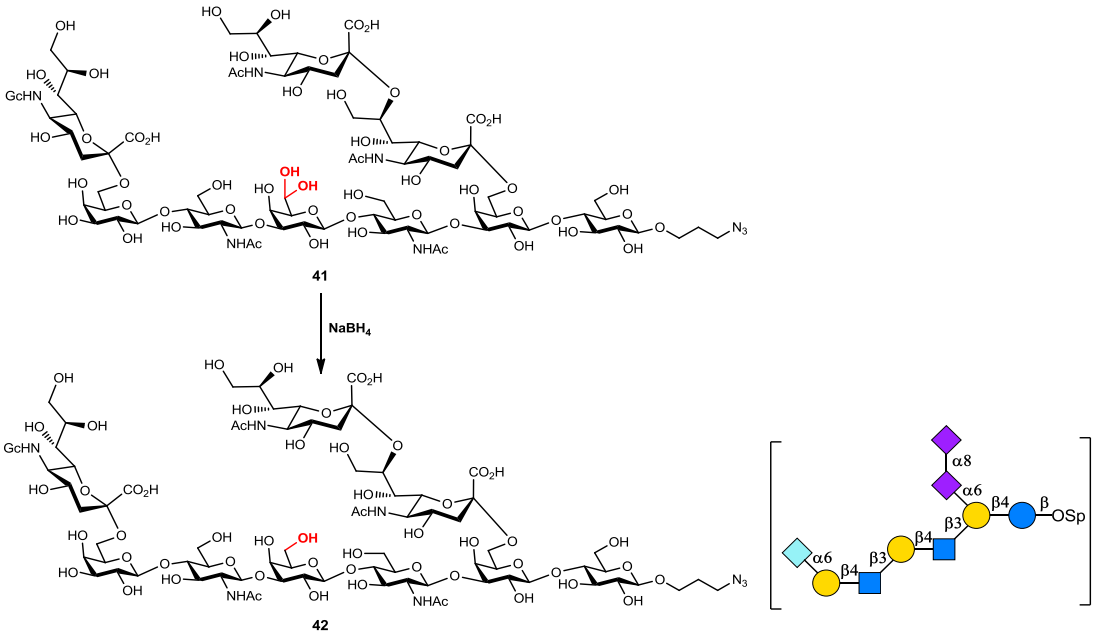
22.27, 22.16, 22.14, 22.01; HRMS (ESI) *m/z* calcd for $C_{65}H_{103}N_7O_{47}$ $[M-2H]^{2-}$ 866.7948, found 866.7922.



3-Azidopropyl 3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (41)

Nonasaccharide **41** (37 mg, 91%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.3 Hz, 1H), 4.74 (d, *J* = 8.2 Hz, 1H), 4.68 (d, *J* = 8.3 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 8.5 Hz, 1H), 4.46 (d, *J* = 8.4 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.31 (d, *J* = 3.1 Hz, 1H), 4.22 – 4.17 (m, 2H), 4.14 – 4.09 (m, 3H), 4.04 – 3.53 (m, 51H), 3.46 (t, *J* = 6.8 Hz, 3H), 3.32 (t, *J* = 8.7, 1H), 2.77 (dd, *J* = 12.3, 4.6 Hz, 1H), 2.69 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.61 (dd, *J* = 12.2, 4.3 Hz, 1H), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.92 (p, *J* = 6.7 Hz, 2H), 1.74 (t, *J* = 12.1 Hz, 2H), 1.67 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.67, 174.95, 174.92, 174.84, 173.56, 173.39, 173.30, 103.43, 103.24, 103.15, 102.71, 102.59, 101.96, 100.96, 100.37, 100.16, 87.90, 82.25, 81.81, 80.33, 79.63, 79.07, 78.54, 76.41,

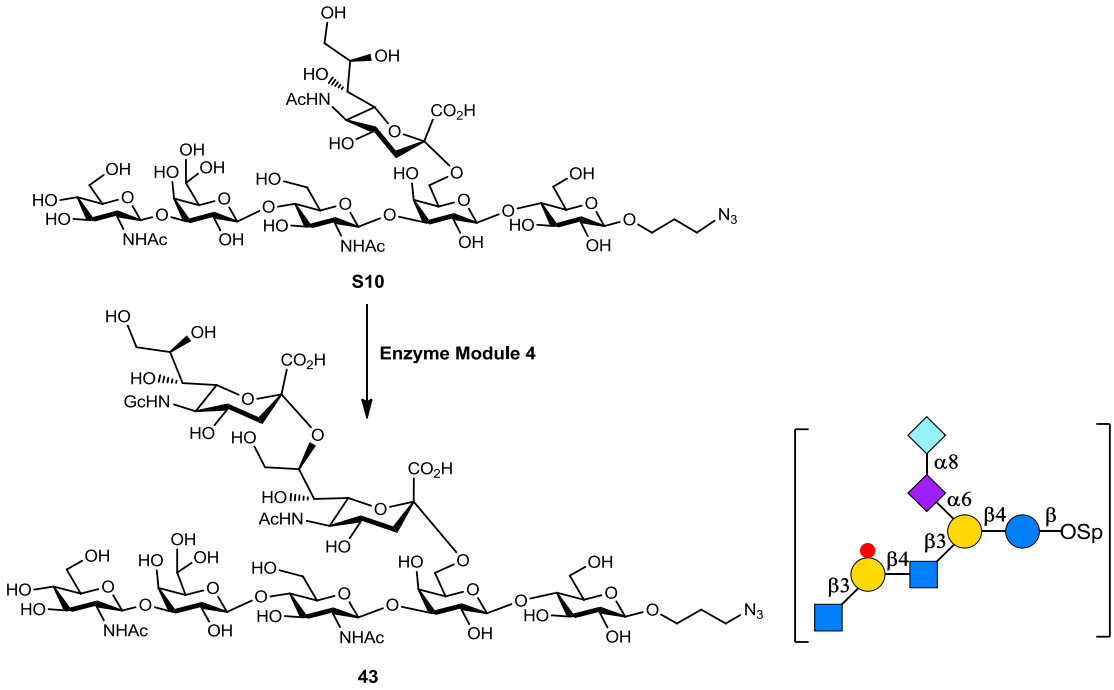
74.66, 74.62, 74.40, 74.24, 74.15, 73.67, 73.29, 72.68, 72.62, 72.38, 72.36, 72.24, 71.75, 71.70, 70.70, 69.72, 69.63, 68.49, 68.36, 68.30, 68.14, 68.08, 67.93, 67.84, 67.76, 67.28, 63.62, 63.35, 62.59, 62.56, 61.58, 60.96, 60.26, 60.09, 59.90, 59.30, 55.04, 54.93, 52.27, 51.72, 51.56, 47.85, 40.47, 40.14, 40.09, 28.21, 22.28, 22.26, 22.17, 22.02; HRMS (ESI) *m/z* calcd for $C_{76}H_{120}N_8O_{56}$ $[M-2H]^{2-}$ 1020.3400, found 1020.3338.



3-Azidopropyl 3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (42)

Nonasaccharide **42** (20 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.76 (1H), 4.74 (d, *J* = 8.1 Hz, 1H), 4.55 (d, *J* = 8.0 Hz, 1H), 4.52 (d, *J* = 7.8 Hz, 1H), 4.51 (d, *J* = 7.8 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.28 – 4.22 (m, 2H), 4.21 (d, *J* = 3.3 Hz, 1H), 4.19 – 4.14 (m, 3H), 4.08 – 3.59 (m, 54H), 3.52 (t, *J* = 6.7 Hz, 2H), 3.38 (t, *J* = 8.7 Hz, 1H), 2.83 (dd, *J* = 12.3, 4.6 Hz, 1H), 2.75 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.67 (dd, *J* = 12.3, 4.4 Hz, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.09 (d, *J* = 2.2 Hz, 3H), 2.08 (s, 3H), 1.97 (p, *J* = 6.7 Hz, 2H), 1.79 (t, *J* = 12.2 Hz, 1H), 1.78 (t, *J*

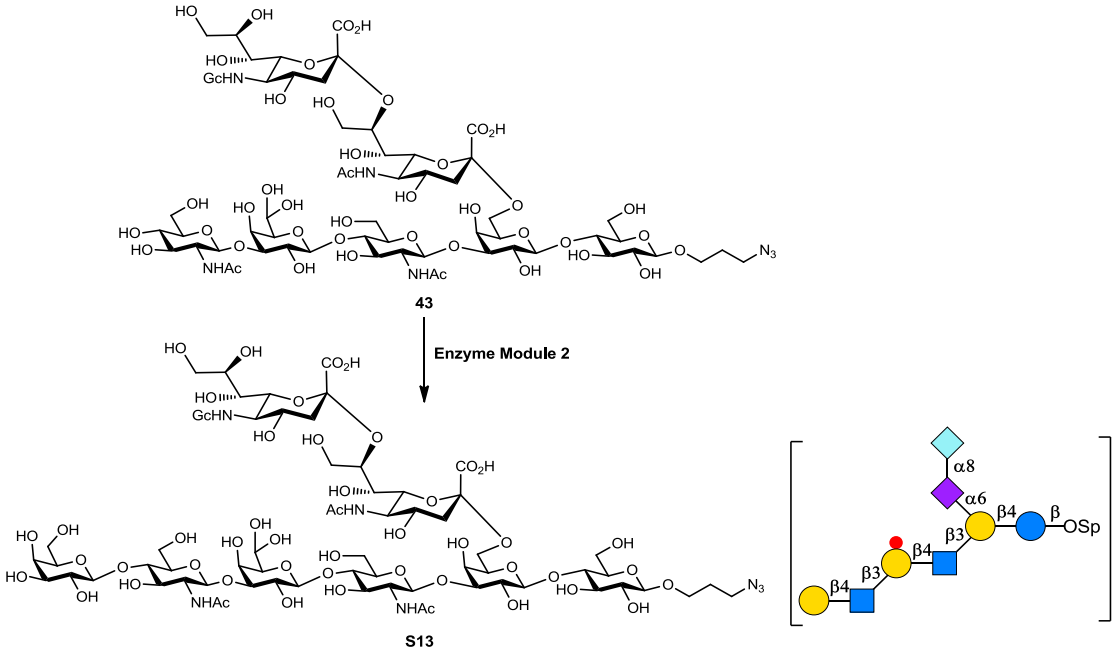
= 12.2 Hz, 1H),z 1.72 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.72, 175.03, 175.00, 174.98, 174.88, 173.59, 173.44, 173.33, 103.47, 103.29, 102.91, 102.79, 102.63, 102.03, 101.03, 100.47, 100.24, 82.31, 81.98, 80.35, 79.71, 78.56, 78.21, 74.89, 74.73, 74.69, 74.62, 74.33, 74.20, 73.74, 73.37, 72.76, 72.69, 72.47, 72.32, 71.82, 71.75, 70.79, 70.06, 69.73, 68.54, 68.44, 68.39, 68.23, 68.00, 67.91, 67.36, 63.41, 62.68, 62.65, 61.65, 61.05, 61.00, 60.36, 60.18, 59.91, 55.23, 55.02, 52.35, 51.80, 51.64, 47.94, 40.53, 40.21, 40.17, 28.28, 22.35, 22.23, 22.09; HRMS (ESI) *m/z* calcd for $C_{76}H_{122}N_8O_{56}$ $[M-2H]^{2-}$ 1021.3478, found 1021.3403.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (43)

Heptasaccharide **43** (60 mg, 63%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.3 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.68 (d, *J* = 8.4 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.31 (d, *J* = 3.0 Hz, 1H), 4.23 – 4.17 (m, 2H), 4.15 – 4.10 (m, 3H), 4.04 – 3.43 (m, 41H), 3.33 (t, *J* = 8.7 Hz, 1H), 2.79 (dd, *J* = 12.2, 4.5 Hz, 1H), 2.62 (dd, *J* = 12.2, 4.2 Hz,

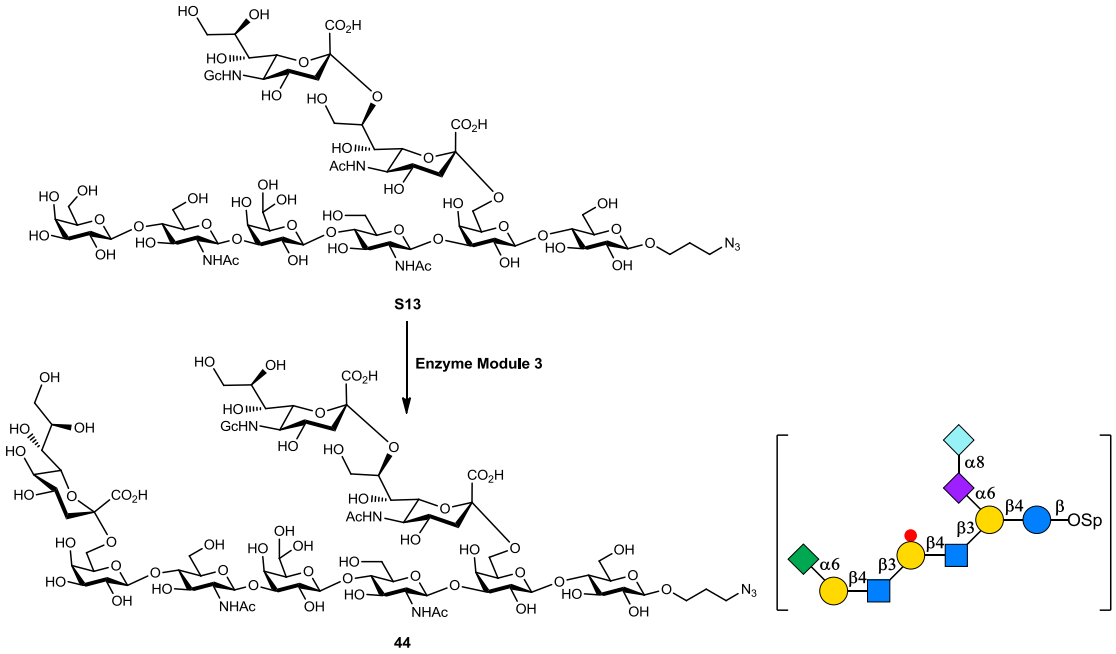
1H), 2.07 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.92 (p, J = 6.7 Hz, 2H), 1.76 (t, J = 12.0 Hz, 1H), 1.67 (t, J = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.72, 174.96, 174.94, 174.86, 173.43, 173.31, 103.24, 103.17, 102.86, 102.72, 101.97, 100.96, 100.41, 87.90, 82.28, 81.81, 79.59, 79.11, 78.53, 76.43, 75.62, 74.67, 74.61, 74.40, 74.16, 73.56, 73.27, 72.70, 72.35, 71.78, 69.75, 69.70, 69.65, 69.62, 68.22, 68.08, 67.83, 67.77, 67.30, 63.61, 62.54, 61.60, 60.97, 60.47, 60.25, 59.93, 59.58, 55.63, 55.05, 52.28, 51.43, 47.87, 40.52, 40.14, 28.22, 22.31, 22.19, 22.17; HRMS (ESI) *m/z* calcd for $C_{59}H_{93}N_7O_{43}$ $[M-2H]^{2-}$ 793.7659, found 793.7652.



3-Azidopropyl β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S13)

Octasaccharide **S13** (48 mg, 86%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, J = 7.3 Hz, 1H), 4.72 (d, J = 8.4 Hz, 1H), 4.68 (d, J = 8.4 Hz, 1H), 4.51 – 4.45 (m, 3H), 4.42 (d, J = 7.9 Hz, 1H), 4.31 (d, J = 3.2 Hz, 1H), 4.23 – 4.17 (m, 2H), 4.14 – 4.10 (m, 3H), 4.04 – 3.44 (m, 47H), 3.32 (t, J = 8.7 Hz, 1H), 2.79 (dd, J = 12.3, 4.6 Hz, 1H), 2.62 (dd, J = 12.2, 4.3 Hz, 1H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.92 (p, J = 6.7 Hz, 2H), 1.76 (t, J = 12.1 Hz, 1H), 1.67 (t, J = 12.2

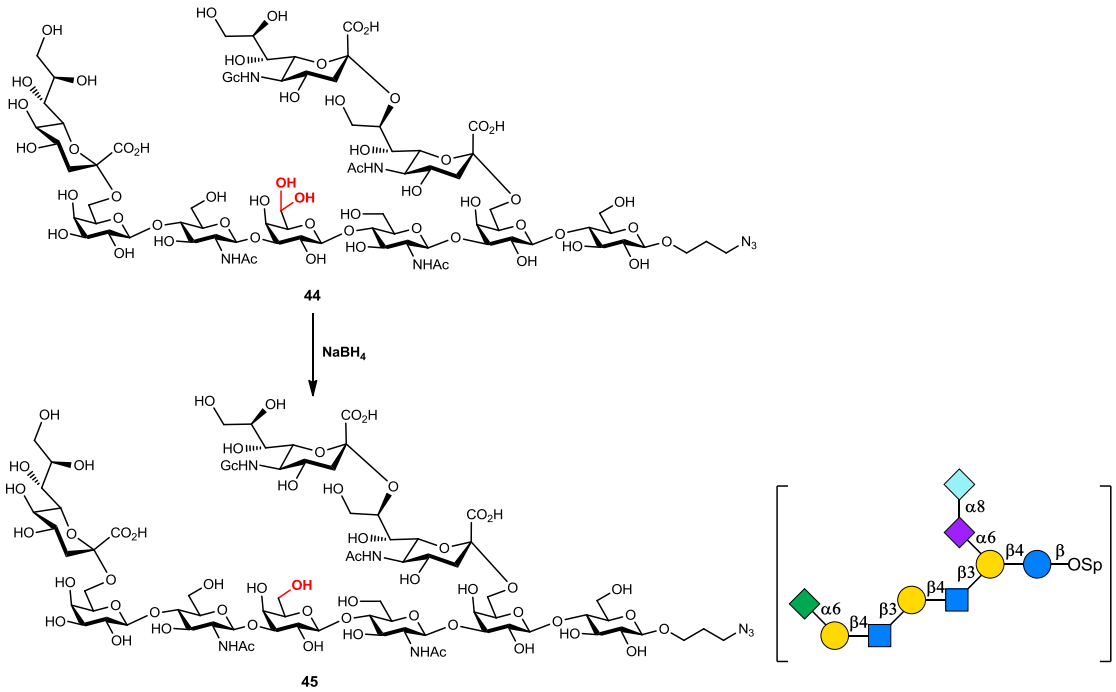
Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.71, 174.92, 174.91, 174.84, 173.42, 173.30, 103.24, 103.18, 102.83, 102.75, 102.71, 101.96, 100.96, 100.38, 87.89, 82.32, 81.84, 79.61, 79.09, 78.55, 78.11, 76.42, 75.32, 74.66, 74.62, 74.51, 74.40, 74.16, 73.27, 72.68, 72.47, 72.34, 72.18, 71.75, 70.94, 69.71, 69.62, 69.60, 68.53, 68.22, 68.07, 67.84, 67.29, 63.61, 62.52, 61.59, 61.00, 60.95, 60.26, 59.91, 59.83, 55.15, 55.05, 52.28, 51.42, 47.85, 40.53, 40.13, 28.21, 22.28, 22.17, 22.15; HRMS (ESI) *m/z* calcd for $C_{65}H_{103}N_7O_{48}$ $[M-2H]^{2-}$ 874.7923, found 874.7890.



3-Azidopropyl 3-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (44)

Nonasaccharide **44** (39 mg, 90%), white solid after lyophilization.. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, *J* = 7.3 Hz, 1H), 4.73 (d, *J* = 8.4 Hz, 1H), 4.68 (d, *J* = 8.3 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.32 (d, *J* = 3.0 Hz, 1H), 4.23 – 4.17 (m, 2H), 4.15 – 4.10 (m, 3H), 4.03 – 3.43 (m, 54H), 3.33 (t, *J* = 8.6 Hz, 1H), 2.79 (dd, *J* = 12.0, 4.1 Hz, 1H), 2.63 (dd, *J* = 12.2, 4.4 Hz, 2H), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* =

6.7 Hz, 2H), 1.76 (t, *J* = 12.0 Hz, 1H), 1.67 (t, *J* = 12.0 Hz, 1H), 1.66 (t, *J* = 12.0 Hz, 1H),; ^{13}C NMR (150 MHz, D_2O) δ 175.73, 174.88, 174.80, 103.29, 103.24, 103.16, 102.72, 102.56, 101.97, 101.00, 100.41, 100.04, 87.89, 82.26, 81.85, 79.99, 79.61, 79.11, 78.54, 76.42, 74.67, 74.62, 74.40, 74.30, 74.16, 73.78, 73.50, 73.28, 72.69, 72.34, 72.20, 72.15, 71.77, 70.67, 70.13, 69.99, 69.71, 69.63, 68.46, 68.22, 68.12, 68.09, 67.84, 67.75, 67.30, 63.62, 62.67, 62.53, 61.60, 60.96, 60.26, 60.01, 59.91, 55.05, 52.28, 51.42, 47.86, 40.52, 40.15, 39.74, 28.21, 22.44, 22.30, 22.29, 22.18, 22.03; HRMS (ESI) *m/z* calcd for $C_{74}H_{117}N_7O_{56}$ $[M-2H]^{2-}$ 999.8267, found 999.8255.

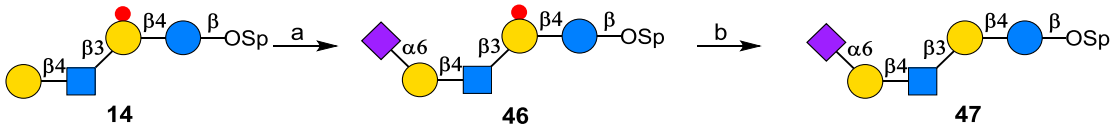


3-Azidopropyl 3-deoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-[3,5-dideoxy-5-hydoxyacetamido-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→8)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (45)

Nonasaccharide **45** (22 mg, 93%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.5 Hz, 1H), 4.69 (d, *J* = 8.3 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.9 Hz, 1H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.23 – 4.18 (m, 2H), 4.16 (d, *J* = 2.6 Hz, 1H), 4.14 – 4.10 (m, 3H), 4.04 – 3.44 (m, 56H),

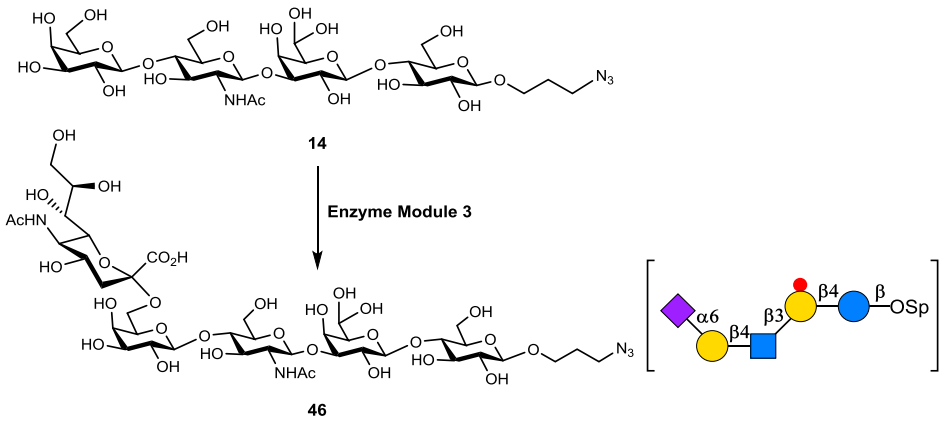
3.33 (t, *J* = 8.6 Hz, 1H), 2.79 (dd, *J* = 12.2, 4.3 Hz, 1H), 2.65 – 2.60 (m, 2H), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.7 Hz, 2H), 1.76 (t, *J* = 12.0 Hz, 1H), 1.67 (t, *J* = 12.0 Hz, 1H), 1.66 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.71, 174.92, 174.83, 174.78, 173.65, 173.41, 103.27, 103.23, 102.84, 102.76, 102.55, 101.96, 100.95, 100.39, 100.02, 82.25, 81.95, 79.95, 79.60, 78.53, 78.09, 74.83, 74.66, 74.61, 74.54, 74.29, 74.15, 73.77, 73.49, 73.27, 72.68, 72.32, 72.17, 72.14, 71.76, 70.67, 70.12, 69.98, 69.63, 68.45, 68.33, 68.21, 68.10, 68.07, 67.83, 67.29, 63.61, 62.66, 62.52, 62.45, 61.59, 60.95, 60.25, 55.15, 55.03, 52.27, 51.42, 47.85, 40.52, 40.12, 39.73, 28.21, 22.29, 22.16; HRMS (ESI) *m/z* calcd for $C_{74}H_{119}N_7O_{56}$ $[M-2H]^{2-}$ 1000.8345, found 1000.8284.

2.3 Substrate scope and application of site-specific α2–6-sialylation strategy



Scheme S12. Synthesis of compounds **46** and **47** from **14**.

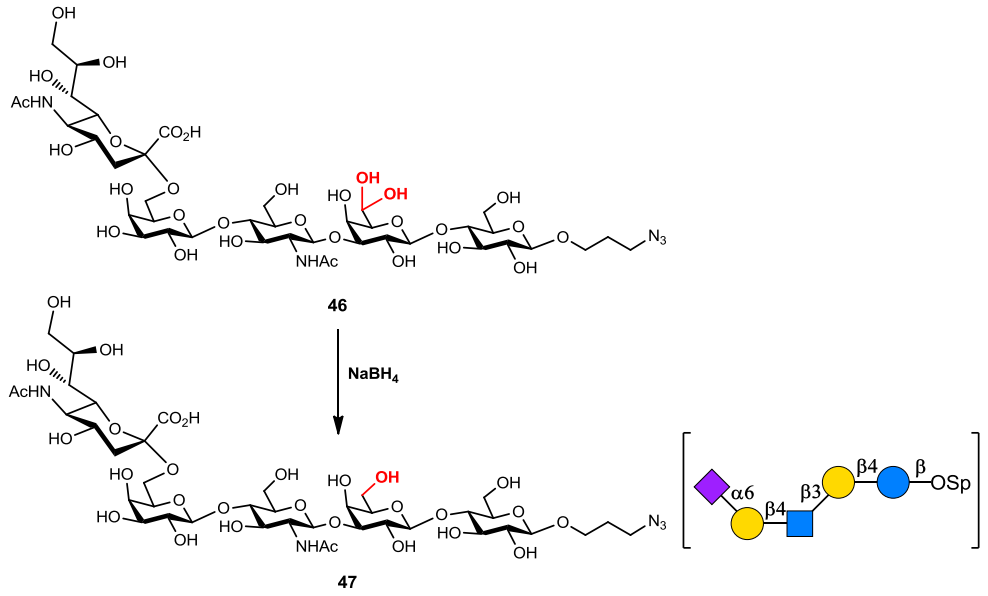
Reagents and conditions: a) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 94%; b) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 90%.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-

(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (46)

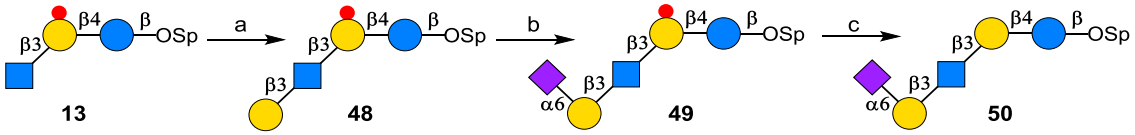
Pentasaccharide **46** (44 mg, 94%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.15 (d, *J* = 7.2 Hz, 1H), 4.75 (d, *J* = 8.0 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.45 (d, *J* = 8.4 Hz, 1H), 4.32 (dd, *J* = 3.4, 1.8 Hz, 1H), 4.05 – 3.51 (m, 28H), 3.46 (m, 3H), 3.32 (t, *J* = 8.6 Hz, 1H), 2.67 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.72 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.89, 173.52, 103.43, 103.20, 102.55, 102.00, 100.10, 87.85, 81.88, 80.43, 79.41, 76.43, 74.58, 74.51, 74.23, 73.66, 72.69, 72.51, 72.38, 72.24, 71.68, 70.70, 69.67, 68.37, 68.34, 68.18, 67.70, 67.33, 63.32, 62.62, 60.12, 54.91, 51.86, 47.84, 40.04, 28.20, 22.26, 22.01; HRMS (ESI) *m/z* calcd for $C_{40}H_{64}N_5O_{29}$ $[M-H]^-$ 1078.3692, found 1078.8687.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (47)

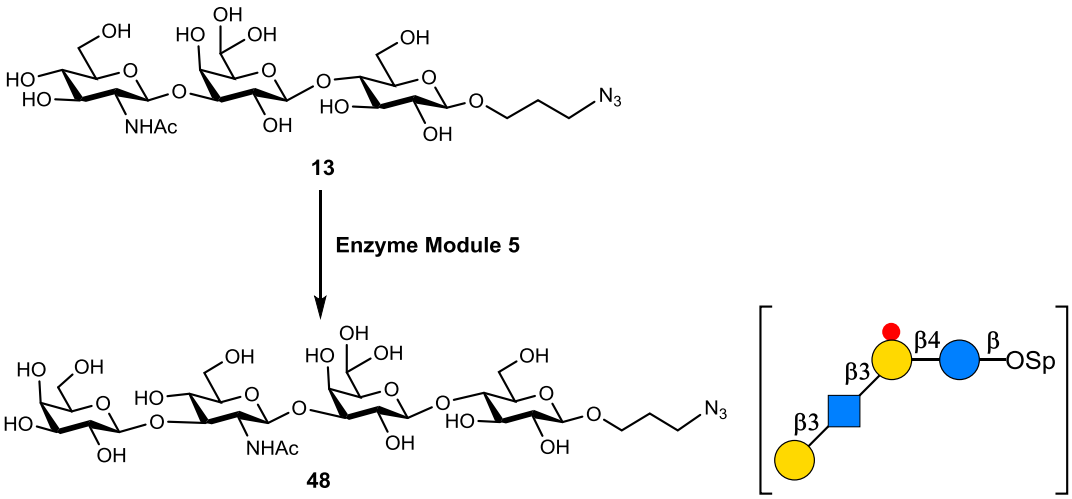
Pentasaccharide **47** (27 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.73 (d, *J* = 7.7 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 8.2 Hz, 1H), 4.43 (d, *J* = 8.4 Hz, 1H), 4.16 (d, *J* = 3.3 Hz, 1H), 4.03–3.51 (m, 31H), 3.46 (t, *J* = 6.7 Hz, 2H), 3.33–3.28 (m, 1H), 2.67 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.05 (s, 3H), 2.03 (s, 3H), 1.92 (p, *J* = 6.6 Hz, 2H), 1.72 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.89, 174.88, 173.51, 103.43, 102.91, 102.55, 102.07, 100.10, 81.97, 80.44, 78.34,

74.85, 74.74, 74.33, 74.23, 73.66, 72.75, 72.51, 72.39, 72.21, 71.68, 70.70, 69.93, 68.37, 68.33, 68.28, 68.18, 67.33, 63.32, 62.62, 60.93, 60.11, 60.01, 54.91, 51.86, 47.84, 40.05, 28.20, 22.25, 22.00; HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{29}$ $[M-H]^-$ 1080.3849, found 1080.3798.



Scheme S13. Synthesis of compounds **48** to **50** from **13**.

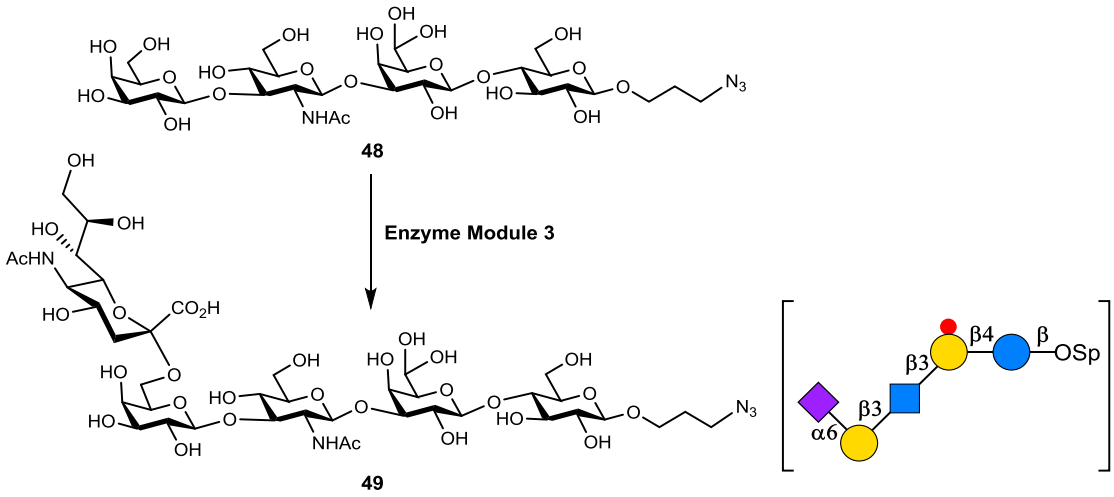
Reagents and conditions: a) Enzyme module 5: Galactose (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MgCl_2$ (20 mM), EcGalK, BLUSP, EcWbgO, Tris-HCl (100 mM, pH 8.0), 37 ℃, 90%; b) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 95%; c) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 92%.



3-Azidopropyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (48)

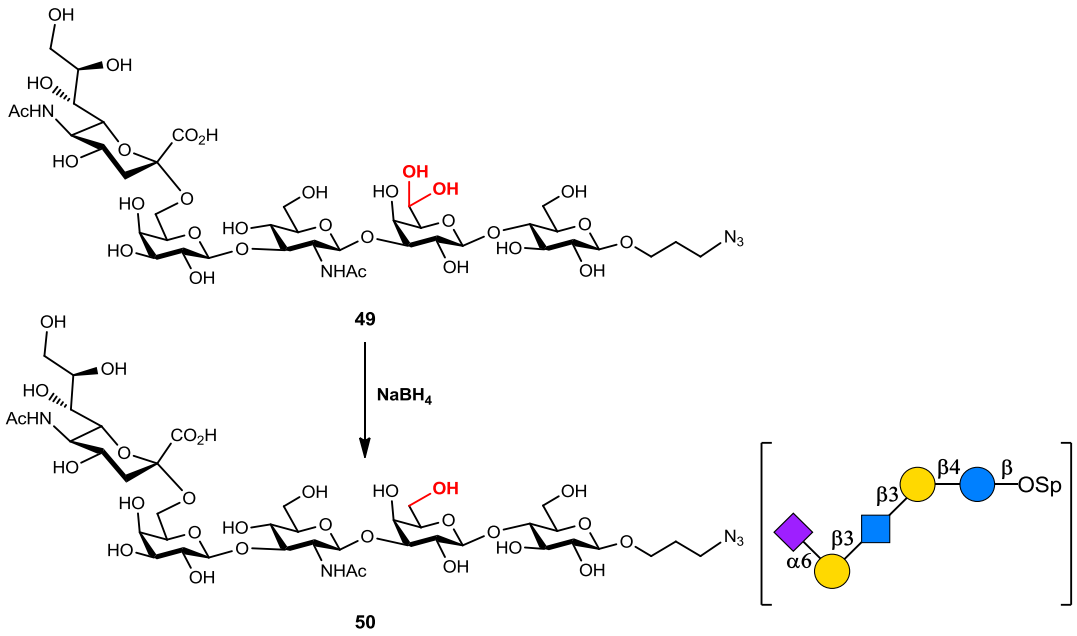
Tetrasaccharide **48** (35 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.15 (d, *J* = 7.3 Hz, 1H), 4.75 (d, *J* = 7.5 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.44 (d, *J* = 7.8 Hz, 1H), 4.31 (d, *J* = 3.0 Hz, 1H), 4.04 – 3.95 (m, 2H), 3.94 – 3.90 (m, 3H), 3.85 – 3.43 (m, 19H), 3.32 (t, *J* = 8.7 Hz, 1H), 2.03 (s, 3H), 1.91 (p, *J* = 6.7 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.92, 103.46,

103.18, 102.54, 102.00, 87.83, 82.04, 81.88, 79.41, 76.44, 75.25, 75.14, 74.58, 74.50, 72.69, 72.43, 70.64, 69.70, 68.50, 68.45, 67.70, 67.33, 61.00, 60.47, 60.14, 54.67, 47.84, 28.20, 22.20; HRMS (ESI) *m/z* calcd for $C_{29}H_{50}N_4O_{22}Na$ $[M+H_2O+Na]^+$ 829.2809, found 829.2849.



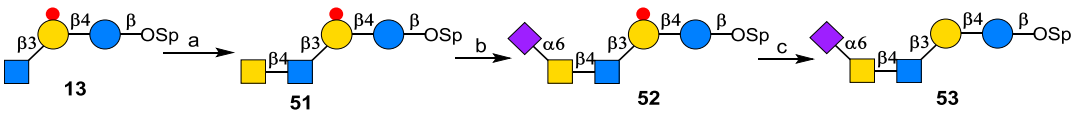
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (49)

Pentasaccharide **49** (32 mg, 95%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.14 (d, *J* = 7.3 Hz, 1H), 4.75 (d, *J* = 8.5 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.9 Hz, 1H), 4.39 (d, *J* = 7.8 Hz, 1H), 4.32 (d, *J* = 3.2 Hz, 1H), 4.04 – 3.71 (m,17H), 3.70 – 3.50 (m, 11H), 3.47 – 3.45 (m, 3H), 3.32 (dd, *J* =9.1, 8.1 Hz, 1H), 2.70 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.91 (p, *J* = 6.7 Hz, 2H), 1.70 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.93, 174.87, 173.46, 103.85, 103.17, 102.51, 102.00, 100.10, 87.84, 83.65, 81.86, 79.42, 76.46, 75.25, 74.58, 74.51, 73.56, 72.68, 72.42, 72.34, 71.79, 70.49, 69.71, 68.65, 68.42, 68.37, 68.26, 67.32, 63.49, 62.59, 60.62, 60.13, 59.33, 54.42, 51.77, 47.83, 40.11, 28.20, 22.18, 22.01; HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{30}$ $[M+H_2O-H]^-$ 1096.3798, found 1096.3822.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (50)

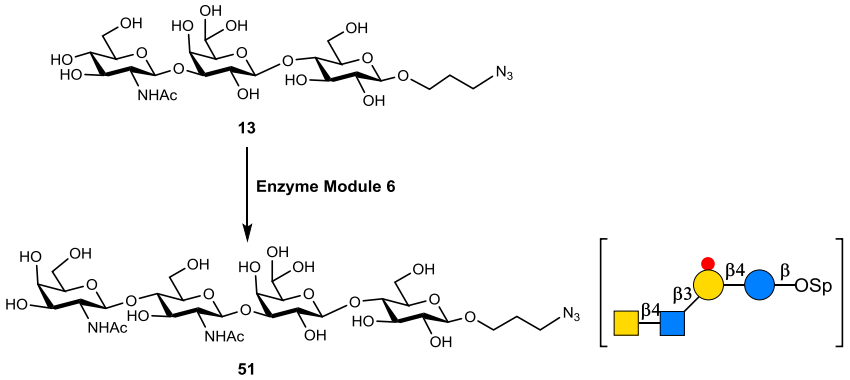
Pentasaccharide **50** (20 mg, 92%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.75 (d, *J* = 8.5 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.9 Hz, 1H), 4.40 (d, *J* = 7.8 Hz, 1H), 4.17 (d, *J* = 3.2 Hz, 1H), 4.04 – 3.70 (m, 19H), 3.70 – 3.49 (m, 12H), 3.47 (t, *J* = 6.7 Hz, 2H), 3.35 – 3.28 (m, 1H), 2.70 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.91 (p, *J* = 6.7 Hz, 2H), 1.70 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.94, 174.87, 173.47, 103.85, 102.89, 102.51, 102.07, 100.10, 83.62, 81.94, 78.34, 75.26, 74.89, 74.74, 74.33, 73.56, 72.75, 72.42, 72.34, 71.79, 70.49, 69.98, 68.62, 68.42, 68.37, 68.26, 67.33, 63.49, 62.59, 60.94, 60.61, 60.03, 54.41, 51.77, 47.83, 40.11, 28.20, 22.18, 22.02; HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{29}$ $[M-H]^-$ 1080.3849, found 1080.3867.



Scheme S14. Synthesis of compounds **51** to **53** from **13**.

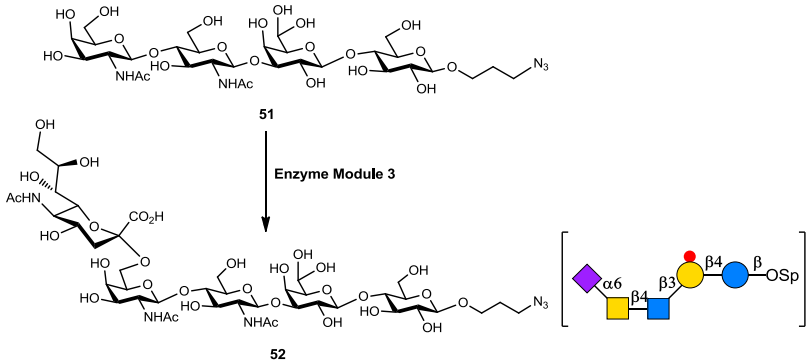
Reagents and conditions: a) Enzyme module 6: *N*–acetylgalactosamine (1.3 equiv), ATP (1.3 equiv), UTP (1.3 equiv), $MnCl_2$ (10 mM), NahK/GlmU, GalT1 Y289L,

Tris-HCl (25 mM, pH 8.0), 37 ℃, 80%; b) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 92%; c) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 91%.



3-Azidopropyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (51)

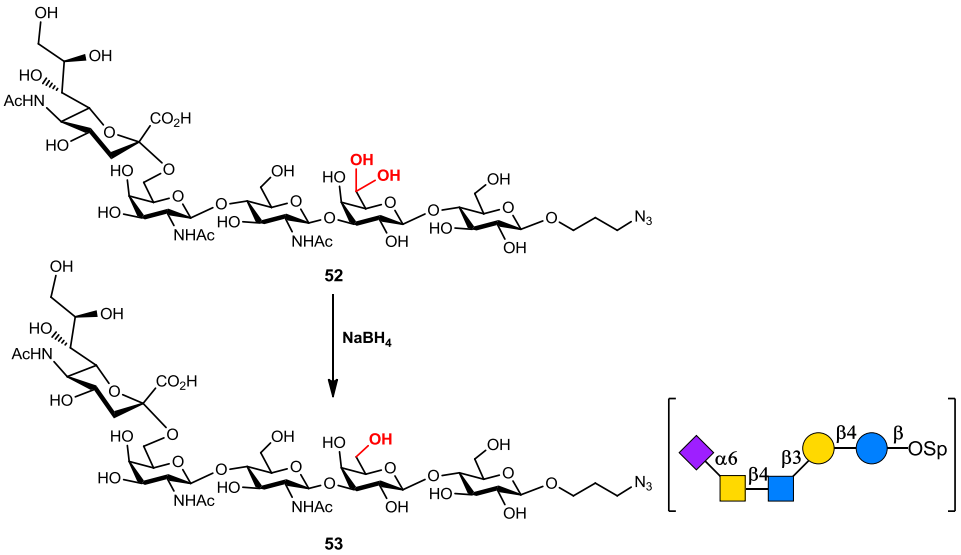
Tetrasaccharide **51** (34 mg, 80 %), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.12 (d, *J* = 7.2 Hz, 1H), 4.67 (d, *J* = 8.4 Hz, 1H), 4.51 (d, *J* = 8.4 Hz, 1H), 4.47 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 8.0 Hz, 1H), 4.28 (d, *J* = 3.0 Hz, 1H), 4.01 – 3.88 (m, 5H), 3.85 – 3.47 (m, 16H), 3.46 – 3.41 (m, 3H), 3.30 (dd, *J* = 9.1, 8.0 Hz, 1H), 2.05 (s, 3H), 2.01 (s, 3H), 1.89 (p, *J* = 6.7 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.80, 174.67, 103.12, 102.63, 101.93, 101.60, 87.77, 81.86, 79.32, 78.77, 76.35, 75.21, 74.52, 74.43, 74.20, 72.62, 72.19, 70.55, 69.59, 67.64, 67.49, 67.25, 60.85, 60.06, 59.86, 54.85, 52.44, 47.76, 28.13, 22.07; HRMS (ESI) *m/z* calcd for $C_{31}H_{52}N_5O_{21}$ $[M+H]^+$ 830.3150, found 830.3247.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-

(2→6)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-6-aldehyde-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (52)

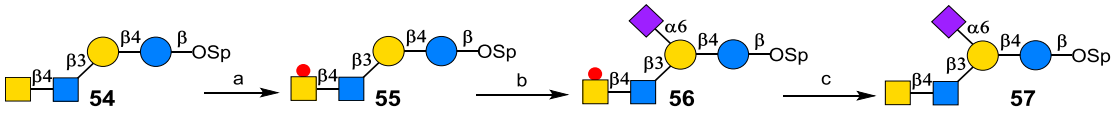
Pentasaccharide **52** (18 mg, 92%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.12 (d, *J* = 7.3 Hz, 1H), 4.70 (d, *J* = 8.4 Hz, 1H), 4.48 (d, *J* = 8.5 Hz, 1H), 4.47 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.28 (d, *J* = 3.0 Hz, 1H), 4.06 – 3.47 (m, 28H), 3.47 – 3.40 (m, 3H), 3.33 – 3.26 (m, 1H), 2.64 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H) , 2.01 (s, 3H), 1.89 (p, *J* = 6.7 Hz, 2H), 1.70 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.89, 174.68, 173.51, 103.16, 102.56, 102.21, 101.99, 100.05, 87.82, 81.86, 80.96, 79.40, 74.59, 74.50, 73.90, 73.67, 72.68, 72.51, 72.39, 71.67, 70.61, 69.67, 68.37, 68.11, 67.62, 67.32, 63.31, 62.61, 60.10, 54.65, 52.40, 51.89, 47.83, 40.06, 28.19, 22.26, 22.17, 22.00; HRMS (ESI) *m/z* calcd for $C_{42}H_{69}N_6O_{30}$ $[M+H_2O-H]^-$ 1137.4064, found 1137.4045.



3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (53)

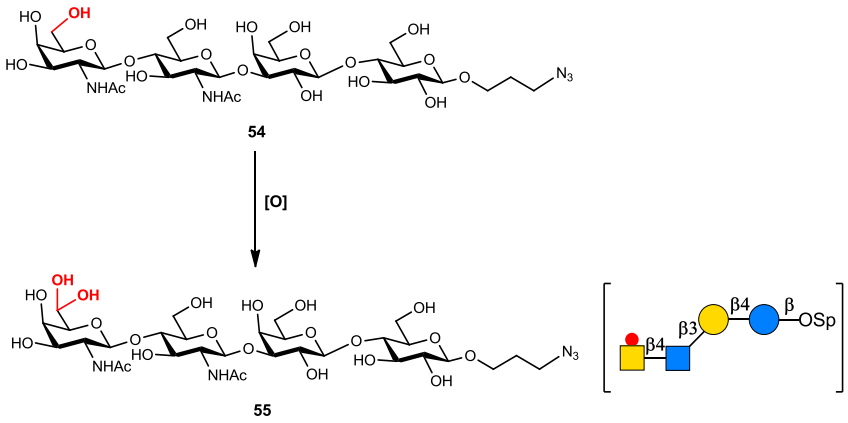
Pentasaccharide **53** (12 mg, 91%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.70 – 4.67 (m, 1H), 4.48 (d, *J* = 9.6 Hz, 1H), 4.47 (d, *J* = 8.4 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.12 (d, *J* = 3.2 Hz, 1H), 4.04 – 3.49 (m, 31H), 3.44 (t, *J* = 6.7 Hz, 2H), 3.28 (t, *J* = 8.4 Hz, 1H), 2.64 (dd, *J* = 12.5, 4.6 Hz, 1H), 2.05 (s, 3H),

2.03 (s, 3H), 2.00 (s, 3H), 1.89 (p, J = 6.7 Hz, 2H), 1.70 (t, J = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.84, 174.63, 173.46, 102.82, 102.50, 102.15, 102.00, 99.99, 81.90, 80.87, 78.25, 74.68, 74.26, 73.85, 73.61, 72.69, 72.45, 72.30, 71.60, 70.55, 69.86, 68.30, 68.05, 67.27, 63.24, 62.53, 60.81, 59.96, 54.58, 52.33, 51.82, 47.77, 40.00, 28.13, 22.20, 22.11, 21.93; HRMS (ESI) *m/z* calcd for $C_{42}H_{69}N_6O_{29}$ $[M-H]^-$ 1121.4114, found 1121.4107.



Scheme S15. Synthesis of compounds **57** from **54**.

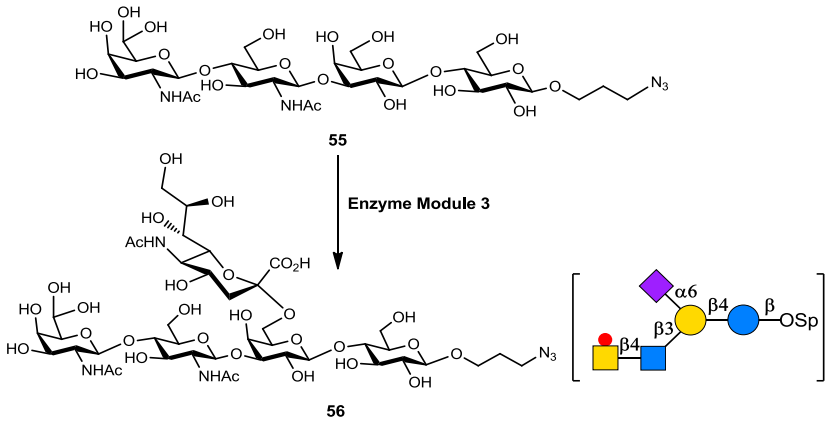
Reagents and conditions: a) Oxidation module: **54** (27 mg), galactose oxidase (50 U), peroxidase (1046 U), sodium phosphate buffer (50 mM, pH 6.5), 30 ℃, 82 %; b) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 90%; c) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 93%.



3-Azidopropyl 2-acetamido-6-aldehyde-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (55)

Tetrasaccharide **55** (22 mg, 82 %), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.19 (d, J = 7.4, 1H), 4.71 (d, J = 7.8, 1H), 4.57 (d, J = 8.4, 1H), 4.51 (d,

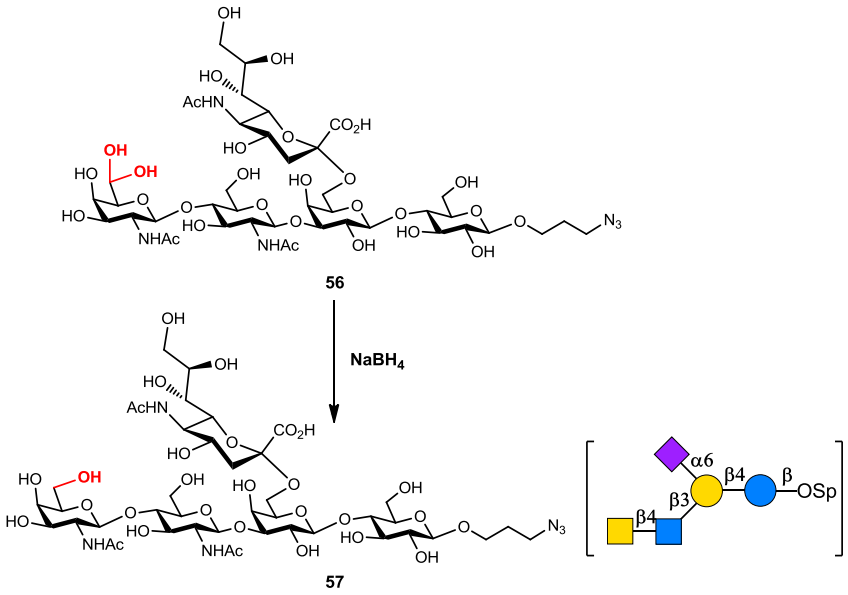
J = 8.0, 1H), 4.46 (d, *J* = 7.9, 1H), 4.16 (d, *J* = 3.3 Hz, 1H), 4.13 (d, *J* = 3.2 Hz, 1H), 4.05 – 3.52 (m, 20H), 3.51 – 3.45 (m, 3H), 3.35 – 3.30 (m, 1H), 2.09 (s, 3H), 2.05 (s, 3H), 1.93 (p, *J* = 6.6 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.93, 174.78, 102.90, 102.67, 102.08, 101.98, 87.86, 82.06, 79.79, 78.32, 76.85, 74.85, 74.75, 74.34, 74.13, 72.79, 72.41, 70.56, 69.96, 68.30, 67.37, 67.03, 60.95, 60.04, 59.99, 54.83, 52.27, 47.87, 28.23, 22.22; HRMS (ESI) *m/z* calcd for $C_{31}H_{50}N_5O_{21}$ $[M-H]^-$ 828.3004, found 828.3028.



3-Azidopropyl 2-acetamido-6-aldehyde-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (56)

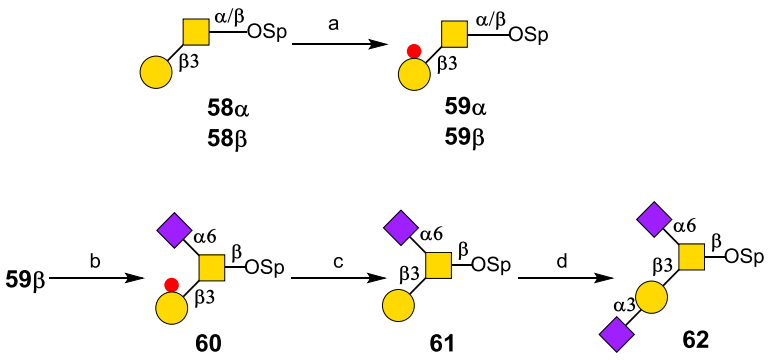
Pentasaccharide **56** (13 mg, 90%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.16 (brs, 1H), 4.66 (d, *J* = 8.1 Hz, 1H), 4.53 (d, *J* = 8.5 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.41 (d, *J* = 8.0 Hz, 1H), 4.16 (d, *J* = 3.7 Hz, 1H), 4.10 (d, *J* = 3.2 Hz, 1H), 4.04 – 3.49 (m, 28H), 3.47 (t, *J* = 6.7 Hz, 2H), 3.33 (t, *J* = 8.6 Hz, 1H), 2.71 (dd, *J* = 12.3, 4.6 Hz, 1H), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.92 (p, *J* = 6.5 Hz, 2H), 1.73 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.83, 174.81, 174.71, 173.39, 103.13, 102.67, 101.91, 100.21, 87.76, 82.11, 79.73, 79.53, 74.57, 74.53, 74.07, 73.14, 72.63, 72.45, 72.4, 71.68, 70.52, 69.59, 68.28, 68.09, 67.25, 66.92, 63.33, 62.56, 60.17, 59.93, 54.79, 52.19, 51.72, 47.81, 40.03, 28.16, 22.11, 21.98;

HRMS (ESI) *m/z* calcd for $C_{42}H_{69}N_6O_{30}$ $[M+H_2O-H]^-$ 1137.4064, found 1137.41 42.



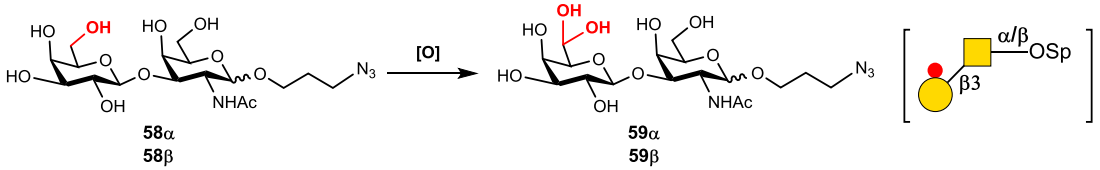
3-Azidopropyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-acetamido -2-deoxy-β-D-glucopyranosyl-(1→3)-5-acetamido-3,5-dideoxy-D-glycero-α-D-*galac to*-2-nonulopyranosyl-(2→6)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (57)

Pentasaccharide **57** (7 mg, 93%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.67 (d, *J* = 8.4 Hz, 1H), 4.53 (d, *J* = 8.5 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.17 (d, *J* = 3.3 Hz, 1H), 4. 13 – 3.49 (m, 31H), 3.47 (t, *J* = 6.7 Hz, 2H), 3.34 (dd, *J* = 9.2, 8.1 Hz, 1H), 2.71 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.08 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1. 92 (p, *J* = 6.8, 2H), 1.73 (t, *J* = 12.2, 1H); ^{13}C NMR (150 MHz, D_2O) δ 17 4.89, 174.84, 174.76, 173.44, 103.18, 102.75, 101.96, 101.63, 100.26, 82.16, 79. 58, 78.79, 74.62, 74.58, 74.30, 73.19, 72.68, 72.51, 72.29, 71.73, 70.63, 69.64, 68.33, 68.14, 67.53, 67.30, 63.38, 62.61, 60.85, 60.22, 59.93, 54.95, 52.50, 51. 77, 47.86, 40.09, 28.21, 22.15, 22.03; HRMS (ESI) *m/z* calcd for $C_{42}H_{69}N_6O_{29}$ $[M-H]^-$ 1121.4114, found 1121.4184.



Scheme S16. Synthesis of compounds **61** to **62** from **58**.

Reagents and conditions: a) Oxidation module: **58α** (37 mg), galactose oxidase (123 U), peroxidase (2556 U) or **58β** (54 mg), galactose oxidase (216 U), peroxidase (4490 U), sodium phosphate buffer (50 mM, pH 6.5), 30 ℃, 81% for **59α**, 83% for **59β**; b) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 93%; c) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 95%; d) Enzyme module 7: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, PmST1, Tris-HCl (100 mM, pH 8.5), 37 ℃, 77%.

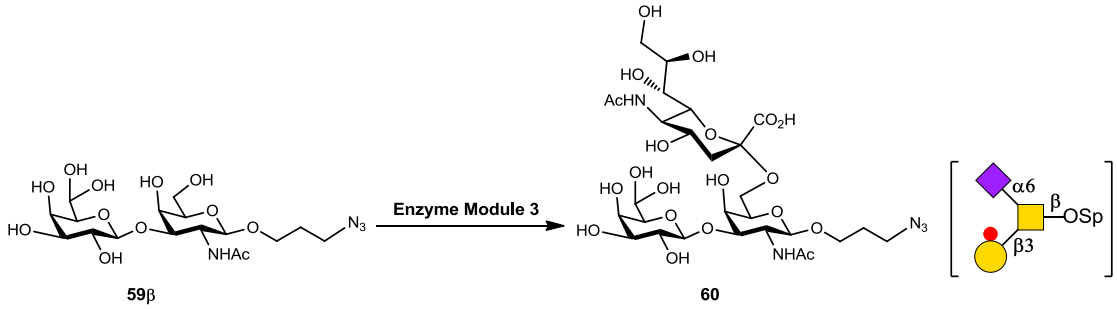


3-Azidopropyl 6-aldehyde-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α/β-D-galactopyranoside (59α/59β)

Disaccharide **59α** (30 mg, 81%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.09 (d, *J* = 7.1 Hz, 1H), 4.87 (d, *J* = 3.7 Hz, 1H), 4.44 (d, *J* = 7.8 Hz, 1H), 4.30 (dd, *J* = 11.1, 3.8 Hz, 1H), 4.24 (d, *J* = 2.9 Hz, 1H), 4.03 (d, *J* = 3.2 Hz, 1H), 4.01 (dd, *J* = 11.1, 2.9 Hz, 1H), 3.96 (dd, *J* = 7.7, 4.9 Hz, 1H), 3.81 – 3.68 (m, 3H), 3.59 (dd, *J* = 10.0, 3.3 Hz, 1H), 3.54 – 3.39 (m, 4H), 3.34 (d, *J* = 7.3 Hz, 1H), 2.00 (s, 3H), 1.88 (p, *J* = 6.5 Hz, 2 H); ^{13}C NMR (150 MHz, D_2O) δ 174.45, 104.65, 97.09, 88.03, 77.29, 76.52, 72.37, 70.47, 70.33, 68.56, 68.04, 64.77, 61.10, 48.55, 48.06, 27.88, 21.88; HR MS (ESI) *m/z* calcd for $C_{17}H_{27}N_4O_{11}$ $[M-H]^-$ 463.1682, found 463.1690.

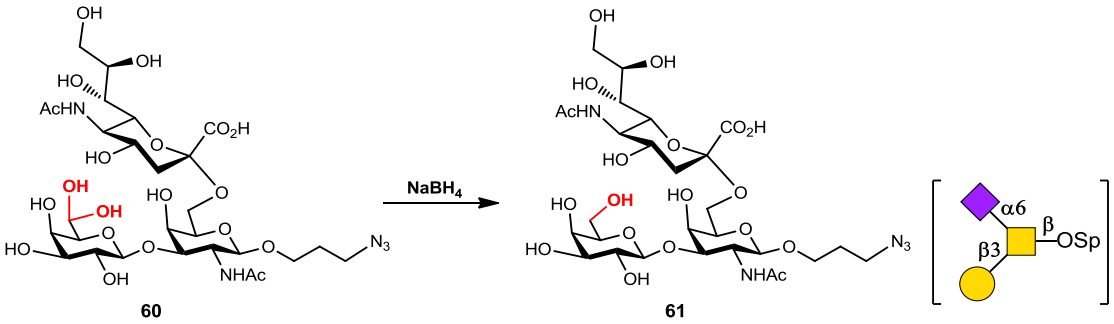
Disaccharide **59β** (54 mg, 83%), white solid after lyophilization. ^{1}H NMR (600

MHz, D_2O) δ 5.10 (d, *J* = 7.2 Hz, 1H), 4.49 (d, *J* = 8.5 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.18 (d, *J* = 3.2 Hz, 1H), 4.04 (dd, *J* = 3.4, 1.0 Hz, 1H), 4.00 – 3.94 (m, 2H), 3.85 (dd, *J* = 11.0, 3.2 Hz, 1H), 3.81 – 3.64 (m, 4H), 3.60 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.52 (dd, *J* = 9.9, 7.7 Hz, 1H), 3.39 – 3.34 (m, 3H), 2.02 (s, 3H), 1.83 (p, *J* = 6.7 Hz, 2H); ^{13}C NMR (150 MHz, D_2O) δ 174.67, 104.79, 101.31, 88.07, 80.01, 76.50, 74.62, 72.33, 70.32, 68.05, 67.81, 66.95, 60.84, 51.14, 47.71, 28.03, 22.16; HRMS (ESI) *m/z* calcd for $C_{17}H_{29}N_4O_{11}$ $[M+H]^+$ 465.1828, found 465.1864.



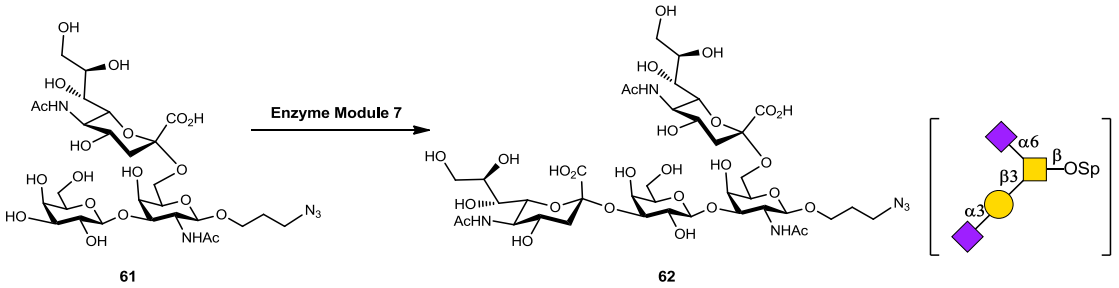
3-Azidopropyl 6-aldehyde-β-D-galactopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranoside (60)

Trisaccharide **60** (58 mg, 93%), white solid after lyophilization. ^1H NMR (600 MHz, D_2O) δ 5.12 (d, *J* = 7.2 Hz, 1H), 4.49 (d, *J* = 8.6 Hz, 1H), 4.43 (d, *J* = 7.7 Hz, 1H), 4.23 (brs, 1H), 4.05 (brs, 1H), 4.03 – 3.92 (m, 3H), 3.92 – 3.75 (m, 5H), 3.75 – 3.56 (m, 7H), 3.53 (t, *J* = 8.7 Hz, 1H), 3.42 – 3.33 (m, 3H), 2.73 (dd, *J* = 12.5, 4.7 Hz, 1H), 2.03 (s, 6H), 1.84 (p, *J* = 6.6 Hz, 2H), 1.68 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.01, 174.67, 173.38, 104.96, 101.45, 100.40, 88.09, 80.19, 76.58, 73.08, 72.60, 72.45, 71.72, 70.35, 68.19, 68.14, 67.79, 67.21, 63.39, 62.60, 51.82, 51.10, 47.76, 40.20, 28.13, 22.18, 21.99; HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{20}$ $[M+H_2O-H]^-$ 772.2742, found 772.2619.



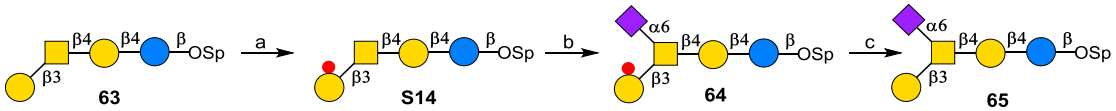
3-Azidopropyl β-D-galactopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranoside (61)

Trisaccharide **61** (43 mg, 95%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.49 (d, *J* = 8.5 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.20 (d, *J* = 3.2 Hz, 1H), 4.01 – 3.93 (m, 3H), 3.92 – 3.75 (m, 7H), 3.75 – 3.60 (m, 8H), 3.58 (dd, *J* = 9.0, 1.8 Hz, 1H), 3.52 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.38 (td, *J* = 6.6, 3.3 Hz, 2H), 2.72 (dd, *J* = 12.5, 4.7 Hz, 1H), 2.03 (s, 3H), 2.03 (s, 3H), 1.85 (p, *J* = 6.6 Hz, 2H), 1.68 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 175.01, 174.67, 173.40, 104.86, 101.45, 100.39, 79.87, 74.94, 73.10, 72.61, 72.49, 71.70, 70.52, 68.58, 68.18, 67.85, 67.22, 63.36, 62.60, 60.94, 51.82, 51.17, 47.76, 40.19, 28.13, 22.20, 21.99; HRMS (ESI) *m/z* calcd for $C_{28}H_{46}N_5O_{19}$ $[M-H]^-$ 756.2792, found 756.2689.



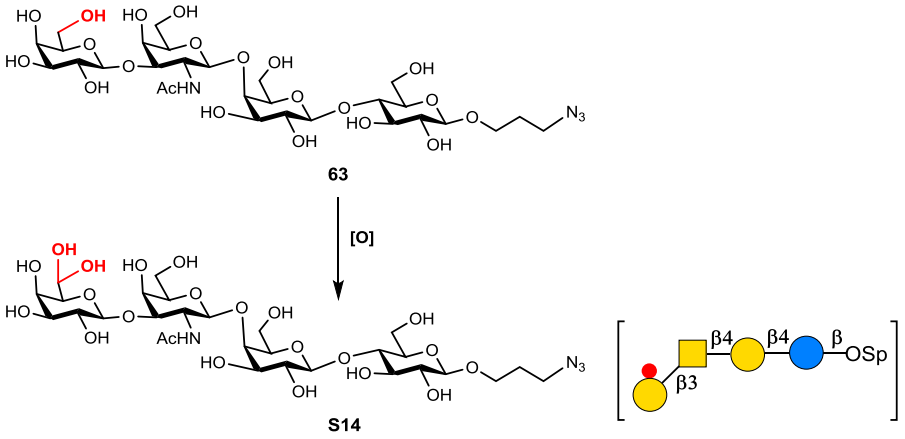
3-Azidopropyl 5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→3)-β-D-galactopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranoside (62)[12]

Tetrasaccharide **62** (30 mg, 77 %), white solid after lyophilization.^{1}H NMR (600 MHz, D_2O) δ 4.50 (d, *J* = 8.2 Hz, 1H), 4.49 (d, *J* = 8.2 Hz, 1H), 4.19 (d, *J* = 3.3 Hz, 1H), 4.07 (dd, *J* = 9.8, 3.2 Hz, 1H), 4.03 – 3.51 (m, 26H), 3.42 – 3.34 (m, 2H), 2.75 (dd, *J* = 12.4, 4.6 Hz, 2H), 2.71 (dd, *J* = 12.4, 4.6 Hz, 2H), 2.04 – 2.02 (m, 9H), 1.85 (p, *J* = 6.6 Hz, 2H), 1.79 (t, *J* = 12.1 Hz, 1H), 1.68 (t, *J* = 12.2 Hz, 1H); HRMS (ESI) *m/z* calcd for $C_{39}H_{63}N_6O_{27}$ $[M-H]^-$ 1047.3746, found 1047.3667.



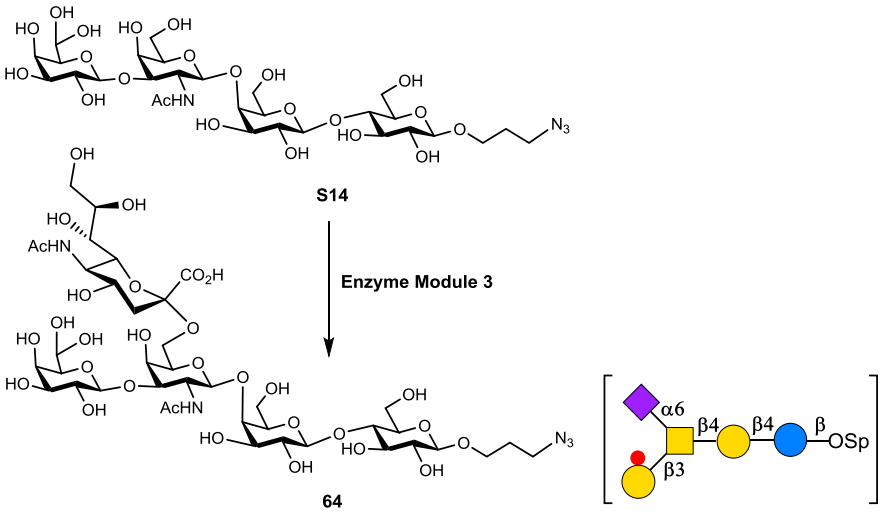
Scheme S17. Synthesis of compounds **S14**, **64** and **65** from **63**.

Reagents and conditions: a) Oxidation module: **63** (67 mg), galactose oxidase (131 U), peroxidase (2730 U), sodium phosphate buffer (50 mM, pH 6.5), 30 ℃, 85%; b) Enzyme module 3: Neu5Ac (1.5 equiv), CTP (1.5 equiv), $MgCl_2$ (20 mM), NmCSS, Pd2,6ST, Tris-HCl (100 mM, pH 8.5), 37 ℃, 92%; c) Reduction: $NaBH_4$ (1.2 equiv), room temperature, 95%.



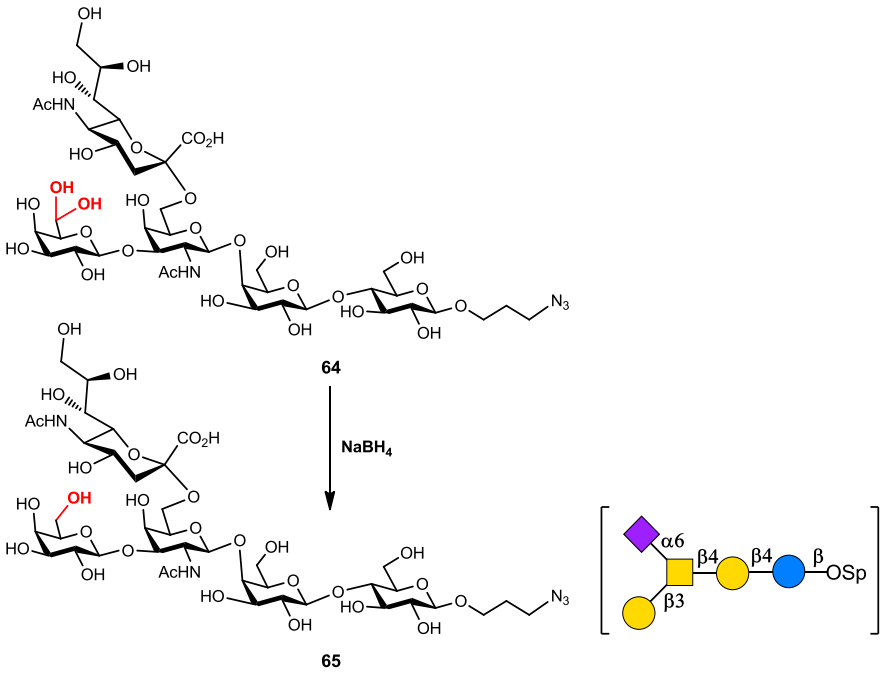
3-Azidopropyl 6-aldehyde-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (S14)

Tetrasaccharide **S14** (57 mg, 85%), white solid after lyophilization. ^{1}H NMR (400 MHz, D_2O) δ 5.16 (d, *J* = 7.2 Hz, 1H), 4.75 (d, *J* = 8.4 Hz, 1H), 4.54 (d, *J* = 8.0 Hz, 1H), 4.51 (d, *J* = 7.6 Hz, 1H), 4.49 (d, *J* = 7.6 Hz, 1H), 4.22 (d, *J* = 2.8 Hz, 1H), 4.16 (d, *J* = 2.8 Hz, 1H), 4.11 (dd, *J* = 3.3, 1.0 Hz, 1H), 4.09 – 3.40 (m, 22H), 3.35 (dd, *J* = 9.0, 8.0 Hz, 1H), 2.09 (s, 3H), 1.96 (p, *J* = 6.5 Hz, 2H); ^{13}C NMR (100 MHz, D_2O) δ 174.94, 104.86, 102.99, 102.39, 102.12, 88.22, 79.99, 78.53, 76.62, 76.12, 74.80, 74.51, 74.44, 74.34, 72.77, 72.50, 72.43, 71.10, 70.48, 68.18, 67.96, 67.41, 61.04, 60.79, 60.07, 51.49, 47.93, 28.27, 22.47; HRMS (ESI) *m/z* calcd for $C_{29}H_{47}N_4O_{21}$ $[M-H]^-$ 787.2738, found 787.2724.



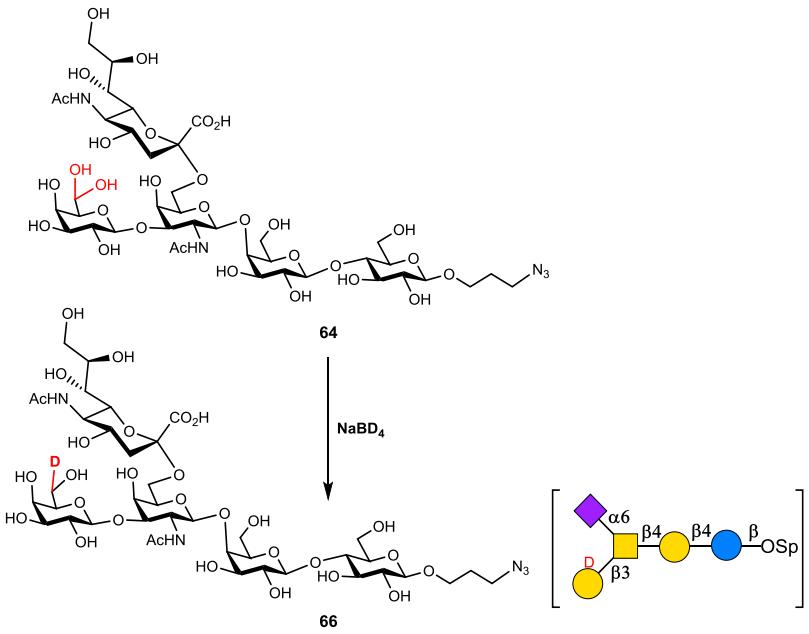
3-Azidopropyl 6-aldehyde-β-D-galactopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (64)

Pentasaccharide **64** (55 mg, 92%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 5.11 (d, *J* = 7.1 Hz, 1H), 4.64 (d, *J* = 8.5 Hz, 1H), 4.48 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.19 (d, *J* = 3.2 Hz, 1H), 4.12 (d, *J* = 3.1 Hz, 1H), 4.04 (d, *J* = 3.4 Hz, 1H), 4.02 – 3.50 (m, 25H), 3.45 (t, *J* = 6.7 Hz, 2H), 3.39 (dd, *J* = 9.9, 7.9 Hz, 1H), 3.35 (d, *J* = 7.3 Hz, 1H), 3.29 (t, *J* = 9.2 Hz, 1H), 2.70 (dd, *J* = 12.3, 4.6 Hz, 1H), 2.02 (s, 3H), 2.02 (s, 3H), 1.90 (p, *J* = 6.6 Hz, 2H), 1.64 (t, *J* = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.85, 174.80, 173.32, 104.86, 102.88, 102.40, 101.99, 100.31, 88.04, 79.90, 78.43, 76.53, 76.14, 74.71, 74.23, 74.09, 72.83, 72.64, 72.47, 72.35, 72.24, 71.76, 70.93, 70.32, 68.28, 68.22, 68.08, 67.83, 67.26, 63.65, 62.58, 60.60, 59.96, 51.73, 51.22, 47.78, 40.14, 28.15, 22.31, 21.96. HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{30}$ $[M+H_2O-H]^-$ 1096.3798, found 1096.3765.



3-Azidopropyl β-D-galactopyranosyl-(1→3)-[5-acetamido-3,5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (65)

Pentasaccharide **65** (30 mg, 95%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.63 (d, *J* = 8.5 Hz, 1H), 4.47 (d, *J* = 8.0 Hz, 1H), 4.42 (dd, *J* = 7.8 Hz, 1H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.16 (d, *J* = 3.3 Hz, 1H), 4.11 (d, *J* = 3.0 Hz, 1H), 4.02 – 3.48 (m, 29H), 3.45 (t, *J* = 6.7 Hz, 2H), 3.38 (dd, *J* = 10.0, 7.9 Hz, 1H), 3.29 (dd, *J* = 9.3, 8.0 Hz, 1H), 2.70 (dd, *J* = 12.3, 4.6 Hz, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 1.90 (p, *J* = 6.6 Hz, 1H), 1.64 (t, *J* = 12.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.83, 174.80, 173.31, 104.76, 102.87, 102.39, 101.98, 100.28, 79.58, 78.41, 76.13, 74.88, 74.70, 74.22, 74.06, 72.84, 72.63, 72.46, 72.37, 72.21, 71.73, 70.92, 70.48, 68.50, 68.26, 68.21, 67.87, 67.24, 63.62, 62.56, 60.87, 60.58, 59.94, 51.72, 51.28, 47.77, 40.12, 28.14, 22.31, 21.94; HRMS (ESI) *m/z* calcd for $C_{40}H_{66}N_5O_{29}$ $[M-H]^-$ 1080.3849, found 1080.3827.



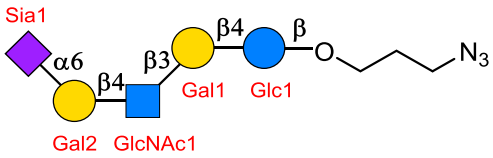
3-Azidopropyl 6-mono-deuterated-β-D-galactopyranosyl-(1→3)-[5-acetamido-3, 5-dideoxy-D-glycero-α-D-*galacto*-2-nonulopyranosyl-(2→6)]-2-acetamido-2-deoxy -β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (66)

Pentasaccharide **66** (12 mg, 93%), white solid after lyophilization. ^{1}H NMR (600 MHz, D_2O) δ 4.62 (d, *J* = 8.6 Hz, 1H), 4.47 (d, *J* = 8.0 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 2H), 4.15 (brs, 1H), 4.10 (brs, 1H), 4.02 – 3.48 (m, 28H), 3.44 (t, *J* = 6.8 Hz, 2H), 3.37 (t, *J* = 9.0 Hz, 1H), 3.28 (t, *J* = 8.7 Hz, 1H), 2.69 (dd, *J* = 11.1, 3.4 Hz, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.89 (p, *J* = 6.5 Hz, 2H), 1.63 (t, *J* = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.82, 174.79, 173.31, 104.76, 102.86, 102.39, 101.98, 100.28, 79.57, 78.40, 76.12, 74.81, 74.70, 74.21, 74.06, 72.84, 72.62, 72.46, 72.37, 72.21, 71.73, 70.91, 70.48, 68.49, 68.26, 68.21, 67.24, 63.62, 62.55, 60.58, 59.94, 51.71, 51.28, 47.76, 40.12, 28.13, 22.31, 21.94; HRMS (ESI) *m/z* calcd for $C_{40}H_{65}DN_5O_{29}$ $[M-H]^-$ 1081.3911, found 1081.3887; The mono-deuterated C6 of compand **66** was verified by comparing with the gHSQC spectrum of compand **65**.

3. NMR assignment of final products

3.1 NMR analysis method

NMR signals were assigned on the basis of ^{1}H NMR, ^{13}C NMR, COSY (Correlated Spectroscopy), HMBC (Heteronuclear Multiple Bond Correlation), HSQC (^{1}H-^{13}C Heteronuclear Single Quantum Coherence), and HSQC-TOCSY (Heteronuclear Single Quantum Coherence-Total Correaltion Spectroscopy) experiments. The compound **47** was taken for example to elucidate the NMR signal assignment method.



Scheme S18. The structrue of compound 47

The protons of middle CH_2 in the linker could be assigned at δ 1.92, and all the carbons and protons in the spin system could be found out in HSQC-TOCSY spectra (Figure S1). The triplet at δ 3.46 is the protons of CH_2 linked to N_3 group. Then all the protons and carbons in the linker could be assigned by HSQC spectra (Figure S2). The H1 of the reducing end glucose could be assigned by three-bond coupling signal in HMBC spectra (Figure S3). The C4 of Glc1 was assigned at δ 78.34 in the HSQC-TOCSY spectra (Figure S4), which is in downfield due to the glycosidic bond. Then the H1 of Gal1 could be figured out in the HMBC spectra (Figure S5). And the C3 of Gal1 was assigned at δ 81.97 in the HSQC-TOCSY spectra (Figure S4). Then the H1 of GlcNAc1 could be figured out in the HMBC spectra (Figure S5). The H1 of Gal2 could also be assigned by HSQC-TOCSY spectra (Figure S4) and HMBC spectra (Figure S5). In other way, because the H4 of Gal shows characteristic downfield shift in the ^{1}H NMR spectra and the H4 of internal Gal is more downfield than the H4 of non-reducing terminal Gal. In the HSQC-TOCSY spectra, the H4s of Gal1 and Gal2 were assigned at δ 4.16 and δ 3.93, respectively. Then, the H1s of Gal1

and Gal2 could also assigned, which was consistent with the assignment with HMBC spectra. The anomeric carbons of Glc, Gal, and GlcNAc can be assigned in the HSQC or HSQC-TOCSY spectra (Figure S6). The anomeric carbon signal of Sia1 is in highfield compared to the Glc, Gal, and GlcNAc anomeric signals as it is the only linkage with an α-configuration. The remaining protons which show intra-spin system signals were assigned by COSY, HSQC and HSQC-TOCSY analysis.

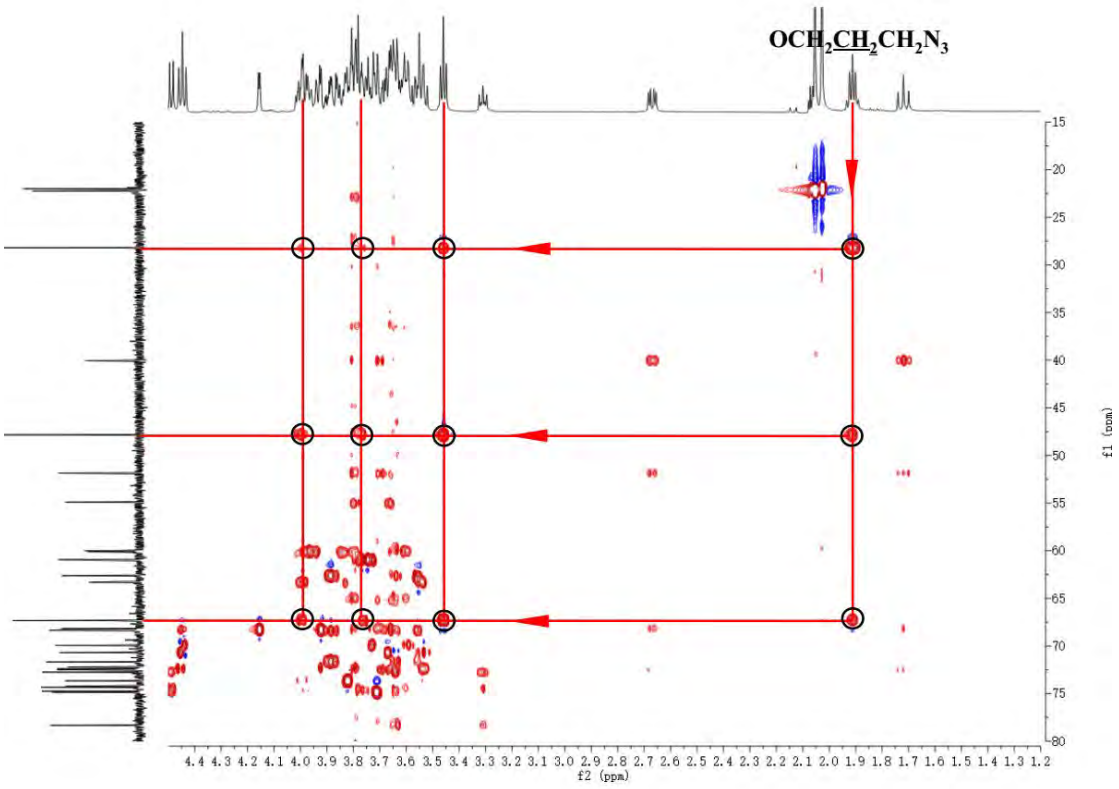


Figure S1. HSQC-TOCSY spectra of 47

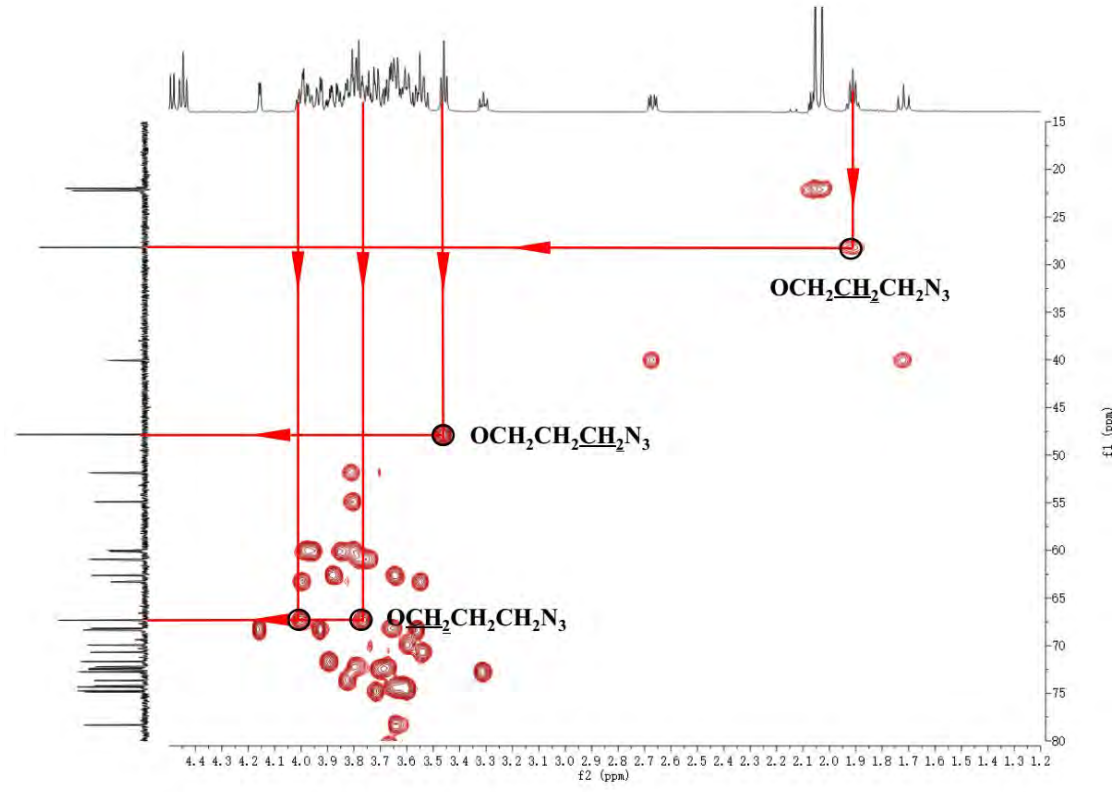


Figure S2. HSQC spectra of 47

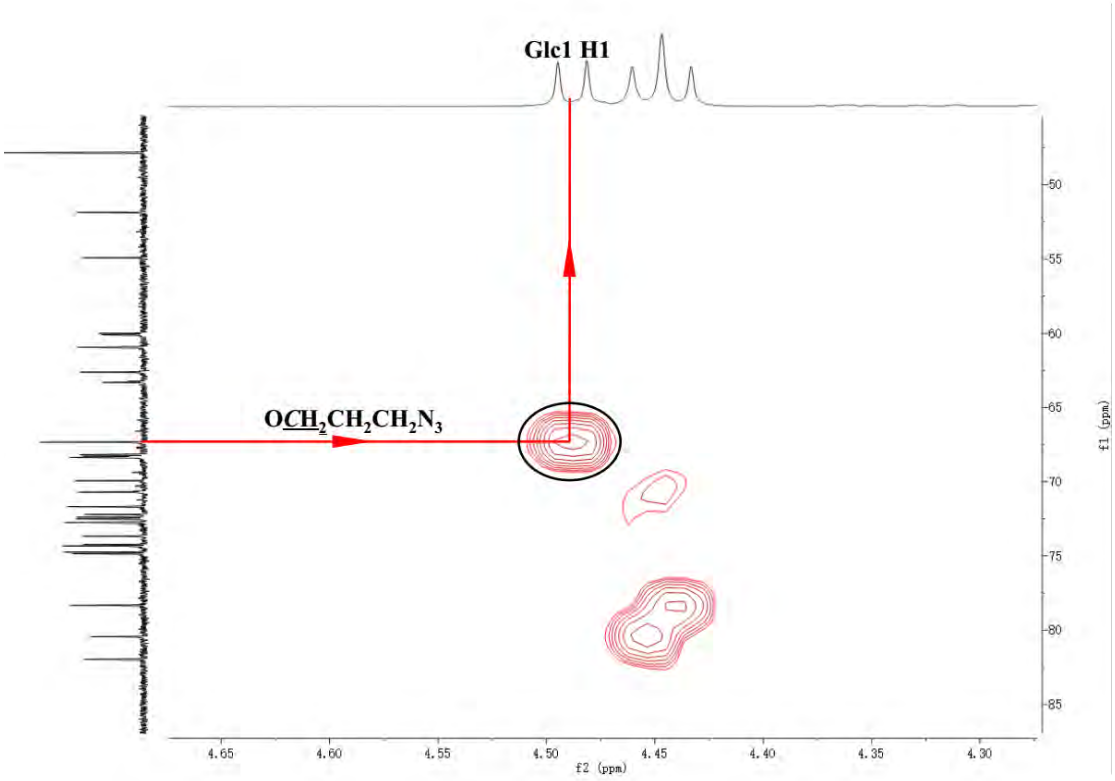


Figure S3. HMBC spectra of 47

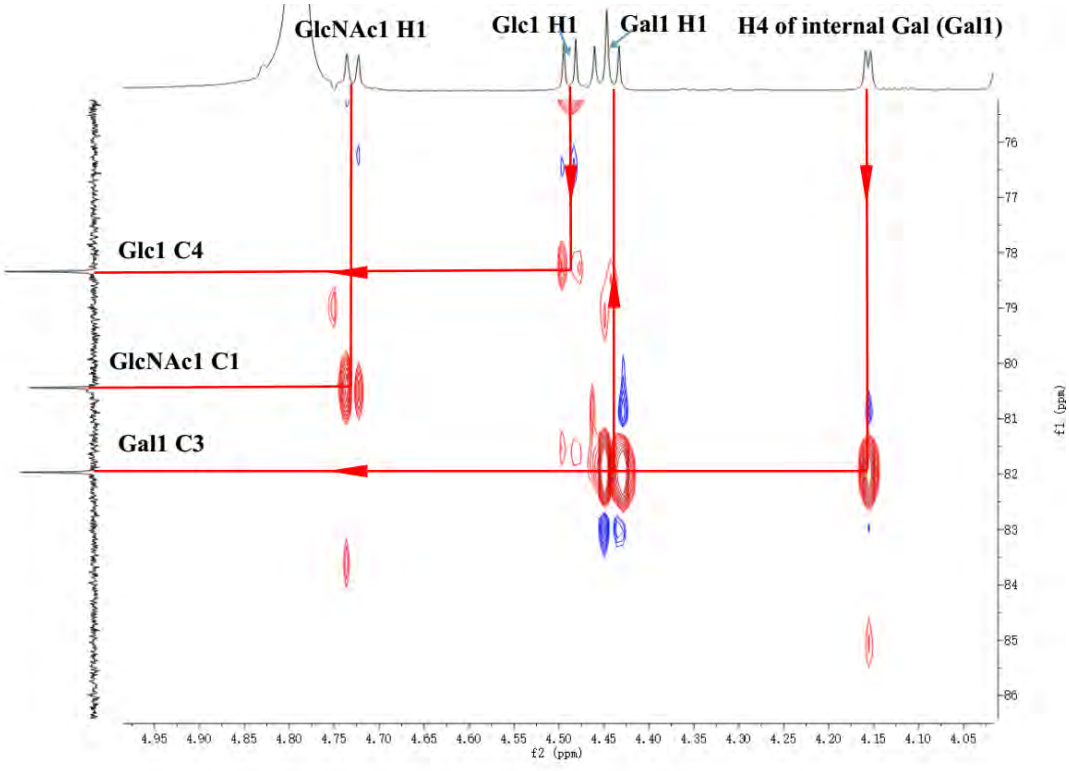


Figure S4. HSQC-TOCSY spectra of 47

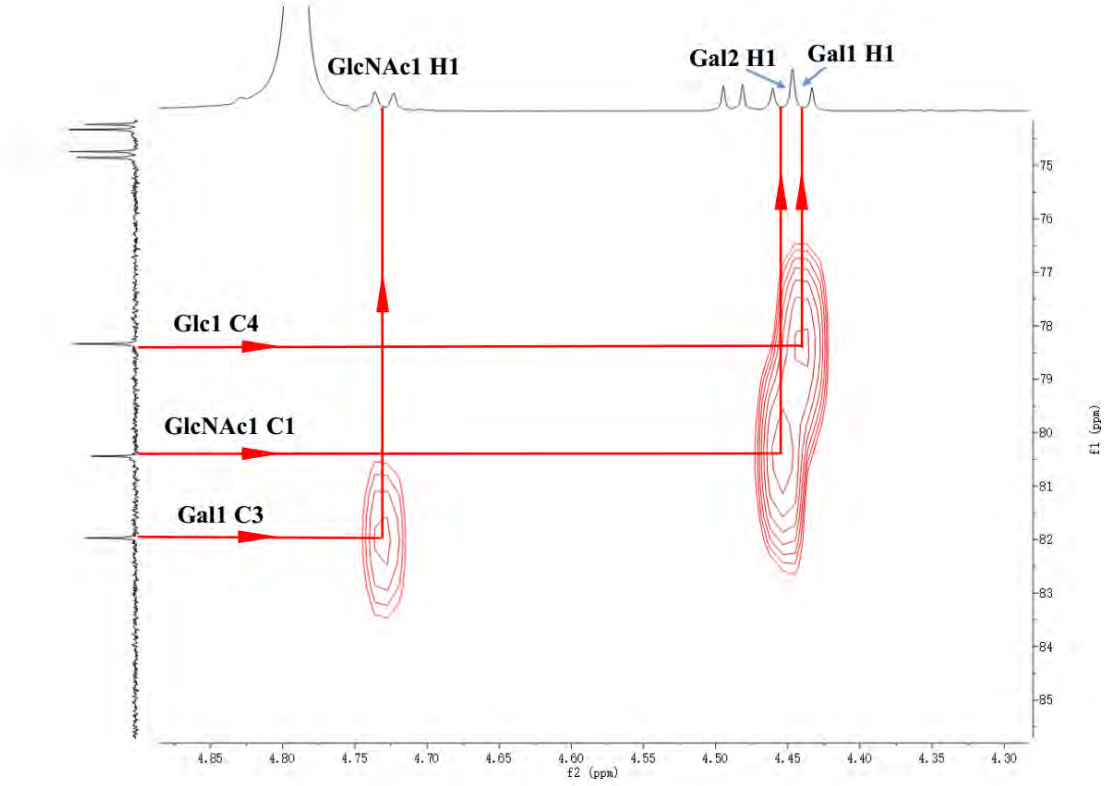


Figure S5. HMBC spectra of 47

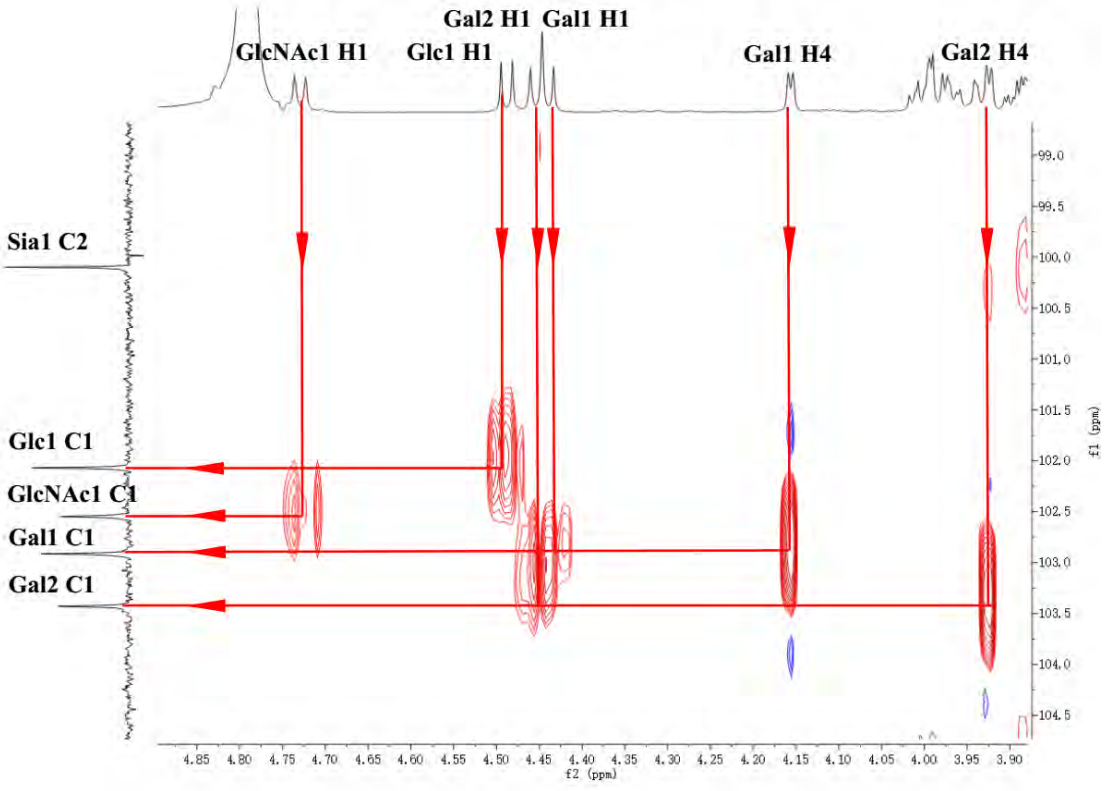
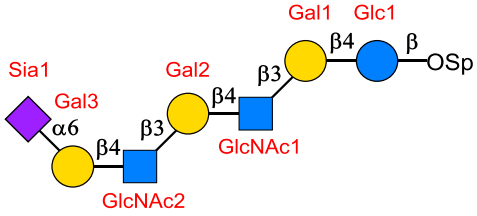


Figure S6. HSQC-TOCSY spectra of 47

3.2 NMR assignment data for final products

NMR assignment of compound 17



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.49	3.31	3.64	3.64	N/A[a]	N/A	-[b]	102.08
Gal1	4.44	3.58	3.73	4.16	N/A	N/A	-	102.90
Gal2	4.47	3.59	3.73	4.16	N/A	N/A	-	102.85
Gal3	4.45	3.56	3.67	3.93	N/A	N/A	-	103.43
GlcNAc1	4.70	3.80	3.74	3.73	N/A	N/A	2.06 or 2.03	102.73
GlcNAc2	4.73	3.80	3.74	3.66	N/A	N/A	2.06 or 2.03	102.57
Sia1[c]	-	-	2.67, 1.72	3.66	3.80	3.70	2.06 or 2.03	100.11

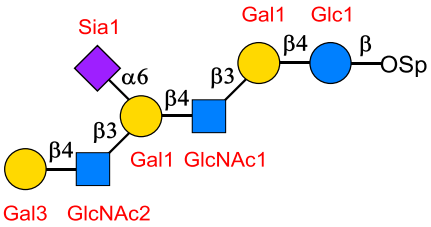
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.34	28.20	47.83
H	3.99, 3.78	1.91	3.46

NMR assignment of compound 19



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.42	3.24	3.58	3.57	N/A[a]	N/A	-[b]	102.07
Gal1	4.37	3.51	3.66	4.10	N/A	N/A	-	103.43
Gal2	4.37	3.51	3.66	4.09	N/A	N/A	-	102.91
Gal3	4.40	3.47	3.59	3.86	N/A	N/A	-	102.82
GlcNAc1	4.65	3.74	3.68	3.58	N/A	N/A	1.98 or 1.96	102.55
GlcNAc2	4.61	3.74	3.68	3.67	N/A	N/A	1.98 or 1.96	102.78
Sia1[c]	-	-	2.59, 1.65	3.58	3.72	3.62	1.98 or 1.96	100.10

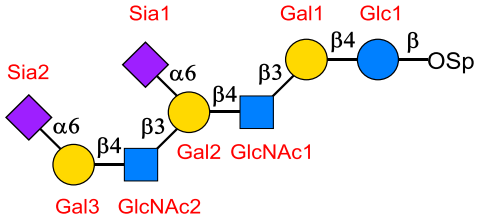
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.33	28.20	47.83
H	3.94, 3.69	1.84	3.39

NMR assignment of compound 22



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.49	3.31	3.64	3.64	N/A[a]	N/A	-[b]	102.08
Gal1	4.44	3.60	3.73	4.16	N/A	N/A	-	102.92
Gal2	4.45	3.53	3.73	4.17	N/A	N/A	-	103.44
Gal3	4.45	3.53	3.67	3.93	N/A	N/A	-	103.51
GlcNAc1	4.73	3.80	3.74	3.66	N/A	N/A	2.06-2.03	102.57
GlcNAc2	4.71	3.80	3.74	3.66	N/A	N/A	2.06-2.03	102.63
Sia1[c]	-	-	2.67, 1.72	3.65	3.79	3.69	2.06-2.03	100.11
Sia2[c]	-	-	2.67, 1.71	3.65	3.79	3.69	2.06-2.03	100.11

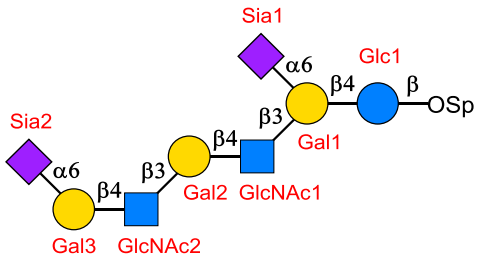
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.34	28.20	47.84
H	4.00, 3.76	1.91	3.46

NMR assignment of compound 26



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.49	3.31	3.65	3.62	N/A[a]	N/A	-[b]	101.96
Gal1	4.42	3.58	3.71	4.18	N/A	N/A	-	103.19
Gal2	4.47	3.57	3.74	4.16	N/A	N/A	-	102.85
Gal3	4.45	3.56	3.67	3.93	N/A	N/A	-	103.43
GlcNAc1	4.68	3.81	3.75	3.72	N/A	N/A	2.06 or 2.03	102.77
GlcNAc2	4.73	3.81	3.75	3.66	N/A	N/A	2.06 or 2.03	102.57
Sia1[c]	-	-	2.71, 1.73	3.66	3.78	3.68	2.06 or 2.03	100.26
Sia2[c]	-	-	2.67, 1.72	3.66	3.78	3.68	2.06 or 2.03	100.11

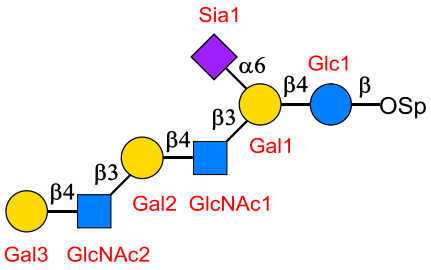
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.30	28.21	47.85
H	4.00, 3.77	1.92	3.47

NMR assignment of compound 29



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.50	3.34	3.66	3.63	N/A[a]	N/A	-[b]	101.96
Gal1	4.42	3.58	3.71	4.18	N/A	N/A	-	103.19
Gal2	4.47	3.59	3.74	4.16	N/A	N/A	-	102.86
Gal3	4.48	3.55	3.68	3.94	N/A	N/A	-	102.83
GlcNAc1	4.69	3.80	3.75	3.74	N/A	N/A	2.04 or 2.03	102.77
GlcNAc2	4.71	3.81	3.75	3.74	N/A	N/A	2.04 or 2.03	102.74
Sia1[c]	-	-	2.71, 1.73	3.66	3.78	3.68	2.04 or 2.03	100.27

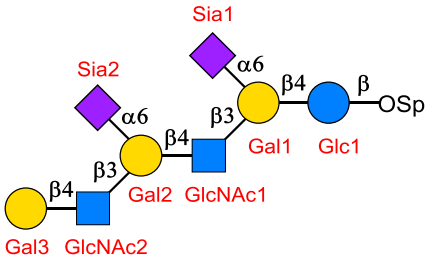
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.30	28.21	47.86
H	4.00, 3.77	1.92	3.47

NMR assignment of compound 32



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.49	3.33	3.65	3.61	N/A[a]	N/A	-[b]	101.95
Gal1	4.42	3.58	3.71	4.18	N/A	N/A	-	103.20
Gal2	4.43	3.58	3.71	4.17	N/A	N/A	-	103.53
Gal3	4.47	3.54	3.67	3.92	N/A	N/A	-	102.82
GlcNAc1	4.70	3.80	3.72	3.64	N/A	N/A	2.05 or 2.03	102.61
GlcNAc2	4.68	3.80	3.72	3.73	N/A	N/A	2.05 or 2.03	102.79
Sia1[c]	-	-	2.71, 1.73	3.65	3.77	3.67	2.05 or 2.03	100.26
Sia2[c]	-	-	2.66, 1.71	3.65	3.77	3.67	2.05 or 2.03	100.10

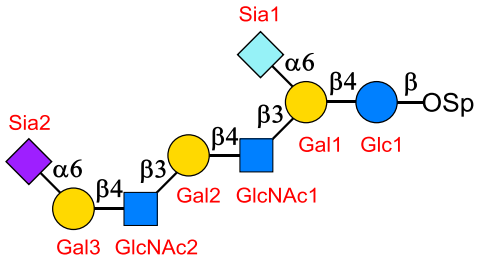
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.29	28.20	47.84
H	4.00, 3.77	1.92	3.47

NMR assignment of compound 36



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.42	3.27	3.57	3.54	N/A[a]	N/A	-[b]	101.95
Gal1	4.34	3.50	3.63	4.11	N/A	N/A	-	103.19
Gal2	4.39	3.49	3.66	4.09	N/A	N/A	-	102.84
Gal3	4.38	3.49	3.60	3.86	N/A	N/A	-	103.43
GlcNAc1	4.61	3.74	3.68	3.65	N/A	N/A	1.98 or 1.96	102.77
GlcNAc2	4.65	3.74	3.68	3.59	N/A	N/A	1.98 or 1.96	102.57
Sia1[c] (Neu5Gc)	-	-	2.65, 1.68	3.69	3.86	3.76	4.05 (NGc)	100.26
Sia2[c] (Neu5Ac)	-	-	2.60, 1.66	3.58	3.71	3.61	1.98 or 1.96	100.10

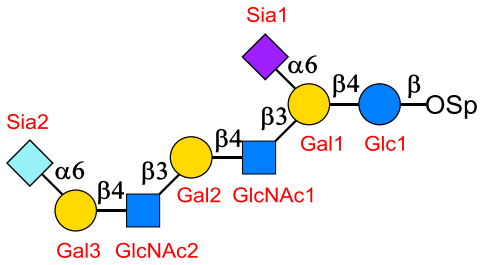
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.29	28.20	47.84
H	3.92, 3.69	1.84	3.39

NMR assignment of compound 38



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.46	3.31	3.61	3.58	N/A[a]	N/A	-[b]	101.87
Gal1	4.39	3.55	3.68	4.15	N/A	N/A	-	103.11
Gal2	4.44	3.55	3.71	4.13	N/A	N/A	-	102.76
Gal3	4.43	3.51	3.64	3.91	N/A	N/A	-	103.33
GlcNAc1	4.65	3.77	3.73	3.70	N/A	N/A	2.03 or 2.00	102.71
GlcNAc2	4.70	3.79	3.73	3.64	N/A	N/A	2.03 or 2.00	102.51
Sia1[c] (Neu5Ac)	-	-	2.68, 1.71	3.62	3.75	3.65	2.03 or 2.00	100.18
Sia2[c] (Neu5Gc)	-	-	2.66, 1.70	3.73	3.90	3.80	4.09 (NGc)	100.06

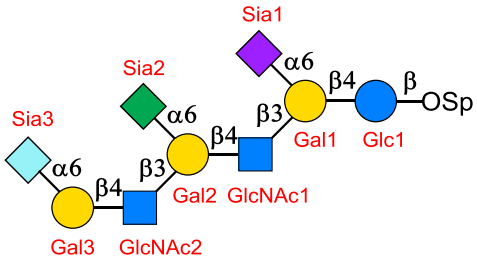
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.21	28.13	47.76
H	3.97, 3.74	1.89	3.44

NMR assignment of compound 39



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.50	3.34	3.65	3.61	N/A[a]	N/A	-[b]	101.96
Gal1	4.43	3.59	3.71	4.19	N/A	N/A	-	103.20
Gal2	4.44	3.59	3.72	4.17	N/A	N/A	-	103.34
Gal3	4.46	3.54	3.61	3.94	N/A	N/A	-	103.48
GlcNAc1	4.70	3.81	3.73	3.67	N/A	N/A		102.58
GlcNAc2	4.72	3.81	3.73	3.67	N/A	N/A		102.66
Sia1[c] (Neu5Ac)	-	-	2.71, 1.73	3.65	3.78	3.68		100.26
Sia2[c] (Kdn)	-	-	2.62, 1.66	3.56	3.48	3.67	-	100.01
Sia3[c] (Neu5Gc)	-	-	2.69, 1.74	3.76	3.93	3.83	4.13 (NGc)	100.15

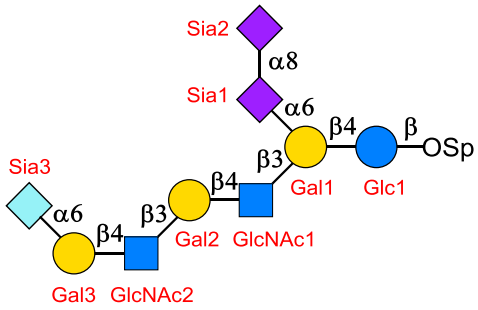
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.30	28.21	47.86
H	4.00, 3.77	1.92	3.47

NMR assignment of compound 42



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.55	3.38	3.71	3.67	N/A[a]	N/A	-[b]	102.02
Gal1	4.47	3.64	3.76	4.24	N/A	N/A	-	103.29
Gal2	4.52	3.62	3.80	4.21	N/A	N/A	-	102.91
Gal3	4.51	3.62	3.73	3.99	N/A	N/A	-	103.47
GlcNAc1	4.74	3.85	3.63	3.77	N/A	N/A	2.12-2.08	102.79
GlcNAc2	4.76	3.85	3.65	3.72	N/A	N/A	2.12-2.08	102.63
Sia1[c] (Neu5Ac)	-	-	2.83, 1.79	3.73	3.89	N/A	2.12-2.08	100.47
Sia2[c] (Neu5Ac)	-	-	2.75, 1.79	3.82	3.87	N/A	2.12-2.08	101.03
Sia3[c] (Neu5Gc)	-	-	2.67, 1.72	3.62	3.95	N/A	4.17 (NGc)	100.24

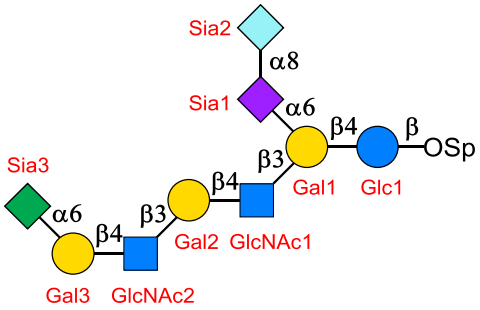
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.36	28.28	47.94
H	4.05, 3.82	1.97	3.52

NMR assignment of compound 45



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.50	3.33	3.66	3.61	N/A[a]	N/A	-[b]	101.96
Gal1	4.42	3.59	3.72	4.19	N/A	N/A	-	103.23
Gal2	4.47	3.60	3.72	4.16	N/A	N/A	-	102.84
Gal3	4.44	3.54	3.67	3.93	N/A	N/A	-	103.27
GlcNAc1	4.69	3.81	3.59	3.73	N/A	N/A	2.07-2.03	102.76
GlcNAc2	4.72	3.82	3.59	3.69	N/A	N/A	2.07-2.03	102.55
Sia1[c] (Neu5Ac)	-	-	2.79, 1.76	3.77	3.93	N/A	2.07-2.03	100.95
Sia2[c] (Neu5Gc)	-	-	2.62, 1.67	3.55	3.83	N/A	4.13 (NGc)	100.39
Sia3[c] (Kdn)	-	-	2.62, 1.67	3.55	3.48	N/A	-	100.02

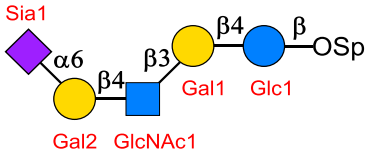
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.29	28.21	47.85
H	4.01, 3.76	1.92	3.47

NMR assignment of compound 47



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.49	3.31	3.65	3.63	N/A[a]	N/A	-[b]	102.07
Gal1	4.43	3.59	3.73	4.16	N/A	N/A	-	102.91
Gal2	4.45	3.53	3.68	3.93	N/A	N/A	-	103.43
GlcNAc1	4.73	3.81	3.56	3.67	N/A	N/A	2.05 or 2.03	102.55
Sia1[c]	-	-	2.67, 1.72	3.66	3.80	3.70	2.05 or 2.03	100.10

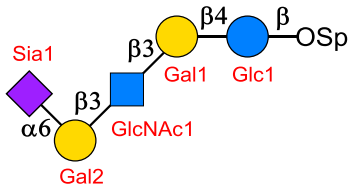
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.33	28.20	47.84
H	4.00, 3.76	1.92	3.46

NMR assignment of compound 50



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.49	3.31	3.66	3.64	N/A[a]	N/A	-[b]	102.07
Gal1	4.44	3.60	3.74	4.16	N/A	N/A	-	102.89
Gal2	4.39	3.53	3.63	3.92	N/A	N/A	-	103.85
GlcNAc1	4.74	3.89	3.78	3.56	N/A	N/A	2.03 or 2.02	102.51
Sia1[c]	-	-	2.70, 1.70	3.66	3.84	3.72	2.03 or 2.02	100.10

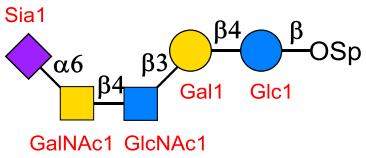
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	OCH_2CH_2CH2N3	$OCH_2CH_2CH_2N_3$	$OCH_2CH_2CH_2N_3$
C	67.33	28.20	47.83
H	4.00, 3.76	1.91	3.46

NMR assignment of compound 53



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.47	3.28	3.62	3.60	N/A[a]	N/A	-[b]	102.00
Gal1	4.41	3.57	3.71	4.12	N/A	N/A	-	102.82
GlcNAc1	4.68	3.78	3.77	3.54	N/A	N/A	2.05-2.01	102.50
GalNAc1	4.48	3.92	3.73	3.92	N/A	N/A	2.05-2.01	102.15
Sia1[c]	-	-	2.64, 1.70	3.64	3.77	N/A	2.05-2.01	99.99

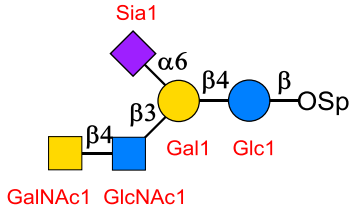
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.27	28.13	47.77
H	3.98, 3.74	1.89	3.44

NMR assignment of compound 57



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.50	3.34	3.66	3.64	N/A[a]	N/A	-[b]	101.96
Gal1	4.42	3.56	3.71	4.17	N/A	N/A	-	103.18
GlcNAc1	4.67	3.80	3.79	3.67	N/A	N/A	2.07-2.03	102.75
GalNAc1	4.53	3.94	3.74	3.95	N/A	N/A	2.07-2.03	101.63
Sia1[c]	-	-	2.71, 1.73	3.66	3.84	3.73	2.07-2.03	100.26

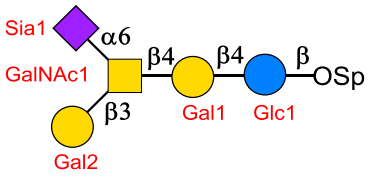
[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	$O\underline{CH_2}CH_2CH2N3$	$OCH_2\underline{CH_2}CH_2N_3$	$OCH_2CH_2\underline{CH_2}N_3$
C	67.30	28.21	47.85
H	4.00, 3.77	1.92	3.47

NMR assignment of compound 65



	H1	H2	H3	H4	H5	H6	NAc	Anomeric Carbon
Glc1	4.47	3.28	3.64	3.58	N/A[a]	N/A	-[b]	101.98
Gal1	4.42	3.38	3.75	4.11	N/A	N/A	-	102.86
Gal2	4.42	3.51	3.60	3.88	N/A	N/A	-	104.76
GalNAc1	4.63	3.99	3.85	4.16	N/A	N/A	2.02 or 2.01	102.39
Sia1[c]	-	-	2.69, 1.63	3.64	3.80	3.69	2.02 or 2.01	100.28

[a] Not assigned

[b] Not applicable

[c] The H7 to H9 of sialic acid are not assigned

Linker	OCH_2CH_2CH2N3	$OCH_2CH_2CH_2N_3$	$OCH_2CH_2CH_2N_3$
C	67.24	28.14	47.77
H	3.98, 3.75	1.90	3.45

4. Glycan Microarray

4.1 General procedure of converting 3-azidopropyl linker of glycans to ready-for-print amine-terminated linker via CuAAC

The 3-azidopropyl glycan (10 mg) and triethylene glycol 2-aminoethyl propargyl ether (H_2N-PEG_4-ALK, 1.0 equiv., Sigma) were added to a stirred solution of $CuSO_4$ (0.2 equiv.), sodium ascorbate (0.5 equiv.) and *tris*-benzyltriazolylmethyl amine (TBTA, 0.2 equiv.) in *tert*-butyl alcohol/water (2.0 mL, 2:1, *v/v*). The reaction mixture was stirred at room temperature. After 1 h, the solvent was removed under reduced pressure and the residue was purified by Bio-Gel P2 gel filtration chromatography (eluted with water) to afford the product as a white solid after lyophilization. All the resulted glycans were quantitated using DMB-HPLC[13] method before the slide printing.

4.2 General procedure for glycan microarray analysis

Each glycan with amine-terminated linker was dissolved in 300 mM sodium phosphate buffer (pH 8.4) to obtain a 100 μM glycan solution. Glycan was printed as replicates of four spots by ArrayIt SpotBot® Extreme instrument. Glycan microarrays were fabricated using PolyAn 3-D NHS-functionalized glass slides (AutoMate Scientific, CA, USA). Printed glycan microarray slides were left to dry at 20 °C for 10 hours and then the unreacted NHS esters on slides were blocked by prewarmed ethanolamine solution (50 mM in 100 mM Tris-HCl, pH 9.0), washed with warm Milli-Q water, dried, and then fitted in a multi-well microarray hybridization cassette (ArrayIt, CA, USA) to divide into subarrays. The subarrays were blocked with Ovalbumin (1% *w/v*) in PBS (pH 7.4) for 1 hour at room temperature in a humid chamber with gentle shaking. Subsequently, the blocking solution was discarded, and properly diluted primary antibodies/lectins which were diluted as described in figure legends were added to each subarray. After incubating for 2 hours at room temperature with gentle shaking, the slides were extensively washed (first with PBS with 0.1% Tween and then only PBS, pH 7.4) to remove non-specifically bound proteins. The corresponding secondary antibodies were then added and after 1 hour of

incubation followed the same washing cycle. The developed glycan microarray slides were then washed, dried and subjected to scanning by a Genepix 4000B microarray scanner (Molecular Devices Corp., Union City, CA). Data analysis was done using the Genepix Pro 7.3 analysis software (Molecular Devices Corp., Union City, CA). Plant lectins of *Sambucus nigra* agglutinin (SNA) and *Maackia amurensis* lectin I (MAL-I), MAL-II were purchased from Vector Laboratories (Burlingame, CA, USA). Chicken polyclonal anti-Neu5Gc IgY antibody was purchased from BioLeagend (San Diego, USA). Human anti-Neu5Gc antibody rich serum[14], and human sialic acid-binding lectin Siglec-9 (hSiglec-9-Fc)[14-16], and His-tagged typhoid toxin (PltB-His)[17-19] were prepared as described previously.

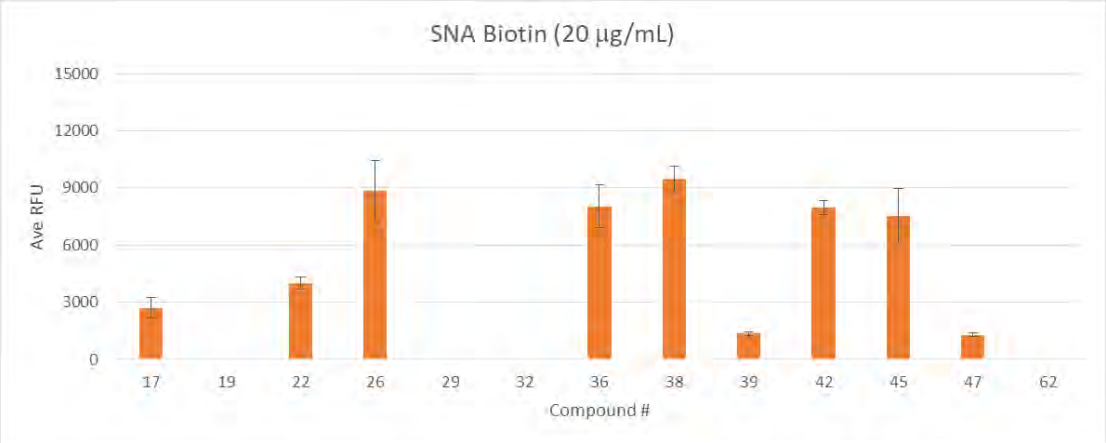


Figure S7. Binding profiles of glycans with plant lectins SNA

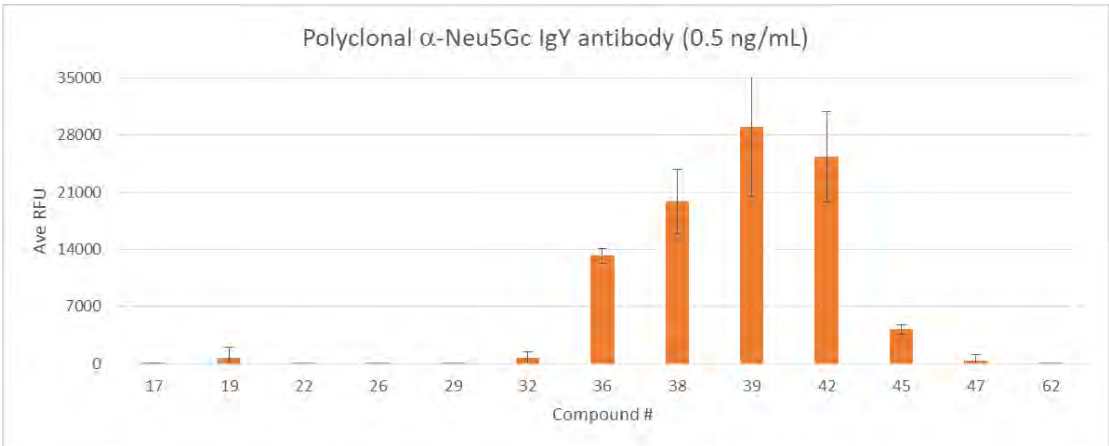


Figure S8. Binding profiles of glycans with chicken polyclonal anti-Neu5Gc IgY antibody

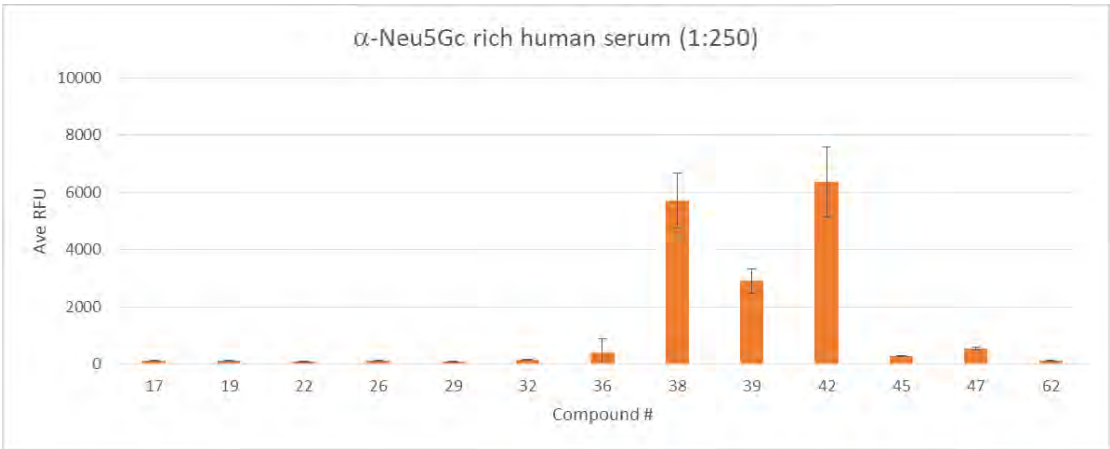


Figure S9. Binding profiles of glycans with human anti-Neu5Gc antibody rich serum

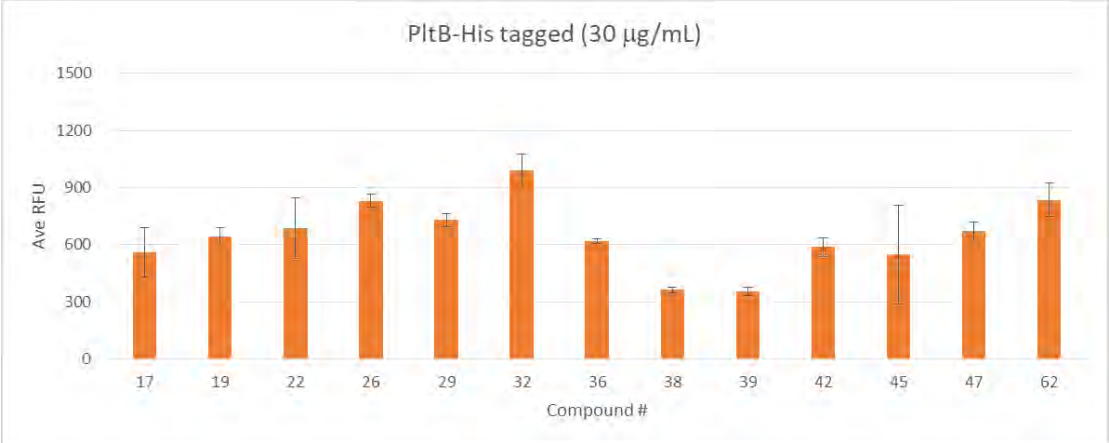


Figure S10. Binding profiles of glycans with His-tagged typhoid toxin

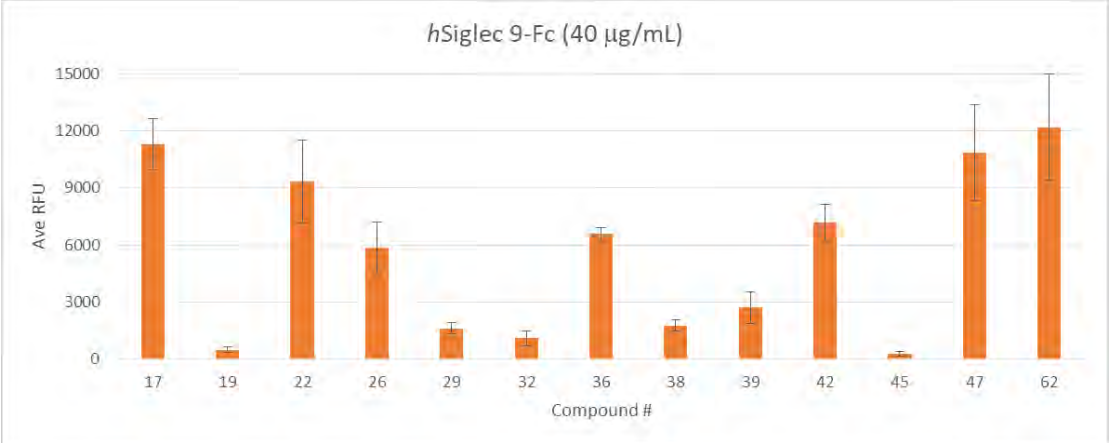


Figure S11. Binding profiles of glycans with human sialic acid-binding lectin Siglec-9

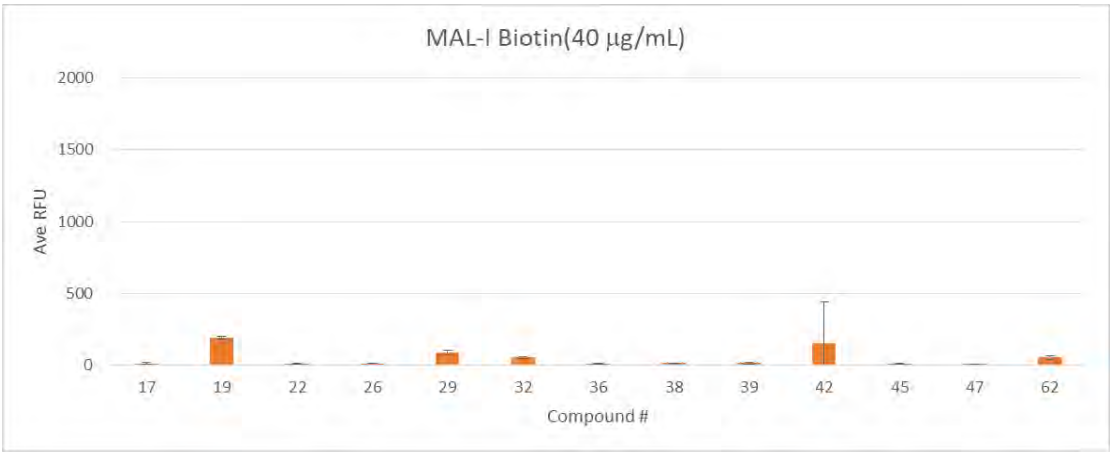


Figure S12. Binding profiles of glycans with *Maackia amurensis* lectin I

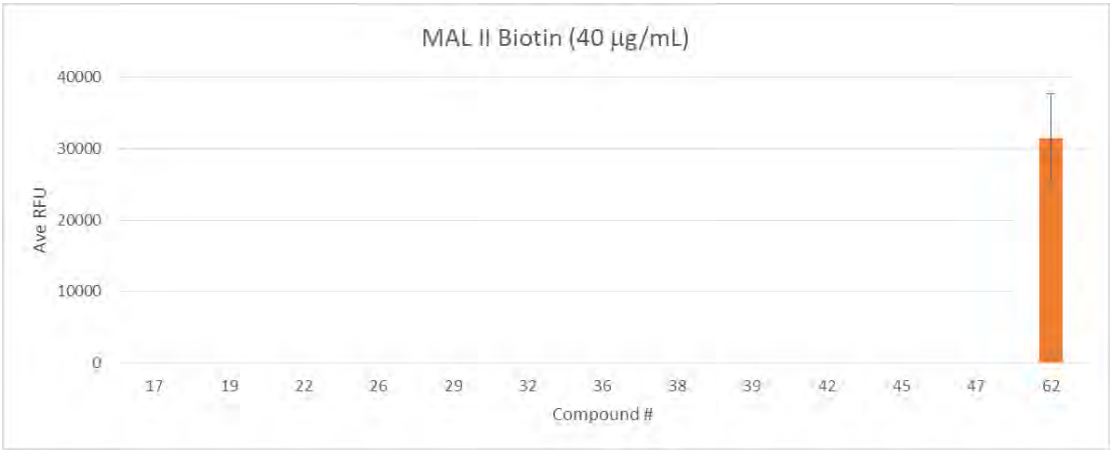


Figure S13. Binding profiles of glycans with *Maackia amurensis* lectin II

5. References

(1) Zhai, Y.; Liang, M.; Fang, J.; Wang, X.; Guan, W.; Liu, X. W.; Wang, P.; Wang, F. *Biotechnol. Lett.* **2012**, *34*, 1321.

(2) Peng, W.; Pranskevich, J.; Nycholat, C.; Gilbert, M.; Wakarchuk, W.; Paulson, J. C.; Razi, N. *Glycobiology* **2012**, *22*, 1453.

(3) Chen, X.; Fang, J.; Zhang, J.; Liu, Z.; Shao, J.; Kowal, P.; Andreana, P.; Wang, P. G. *J. Am. Chem. Soc.* **2001**, *123*, 2081.

(4) Muthana, M. M.; Qu, J.; Li, Y.; Zhang, L.; Yu, H.; Ding, L.; Malekan, H.; Chen, X. *Chem. Commun.* **2012**, *48*, 2728.

(5) Lau, K.; Thon, V.; Yu, H.; Ding, L.; Chen, Y.; Muthana, M. M.; Wong, D.; Huang, R.; Chen, X. *Chem. Commun.* **2010**, *46*, 6066.

(6) Liu, X. W.; Xia, C.; Li, L.; Guan, W. Y.; Pettit, N.; Zhang, H. C.; Chen, M.; Wang, P. G. *Bioorg. Med. Chem.* **2009**, *17*, 4910.

(7) Ramakrishnan, B.; Qasba, P. K. *J. Biol. Chem.* **2002**, *277*, 20833.

(8) Li, Y.; Yu, H.; Cao, H.; Muthana, S.; Chen, X. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 2411.

(9) Sugiarto, G.; Lau, K.; Qu, J.; Li, Y.; Lim, S.; Mu, S.; Ames, J. B.; Fisher, A. J.; Chen, X. *ACS Chem. Biol.* **2012**, *7*, 1232.

(10) Cheng, J.; Huang, S.; Yu, H.; Li, Y.; Lau, K.; Chen, X. *Glycobiology* **2010**, *20*, 260.

(11) Cheng, J.; Yu, H.; Lau, K.; Huang, S.; Chokhawala, H. A.; Li, Y.; Tiwari, V. K.; Chen, X. *Glycobiology* **2008**, *18*, 686.

(12) Meng, X.; Yao, W.; Cheng, J.; Zhang, X.; Jin, L.; Yu, H.; Chen, X.; Wang, F.; Cao, H. *J. Am. Chem. Soc.* **2014**, *136*, 5205.

(13) Hara, S.; Yamaguchi, M.; Takemori, Y.; Nakamura, M.; Ohkura, Y. *J. Chromatogr.* **1986**, *377*, 111.

(14) Padler-Karavani, V.; Song, X.; Yu, H.; Hurtado-Ziola, N.; Huang, S.; Muthana, S.; Chokhawala, H. A.; Cheng, J.; Verhagen, A.; Langereis, M. A.; Kleene, R.; Schachner, M.; de Groot, R. J.; Lasanajak, Y.; Matsuda, H.; Schwab, R.; Chen,

X.; Smith, D. F.; Cummings, R. D.; Varki, A. *J. Biol. Chem.* **2012**, *287*, 22593.

(15) Angata, T.; Varki, A. *J. Biol. Chem.* **2000**, *275*, 22127.

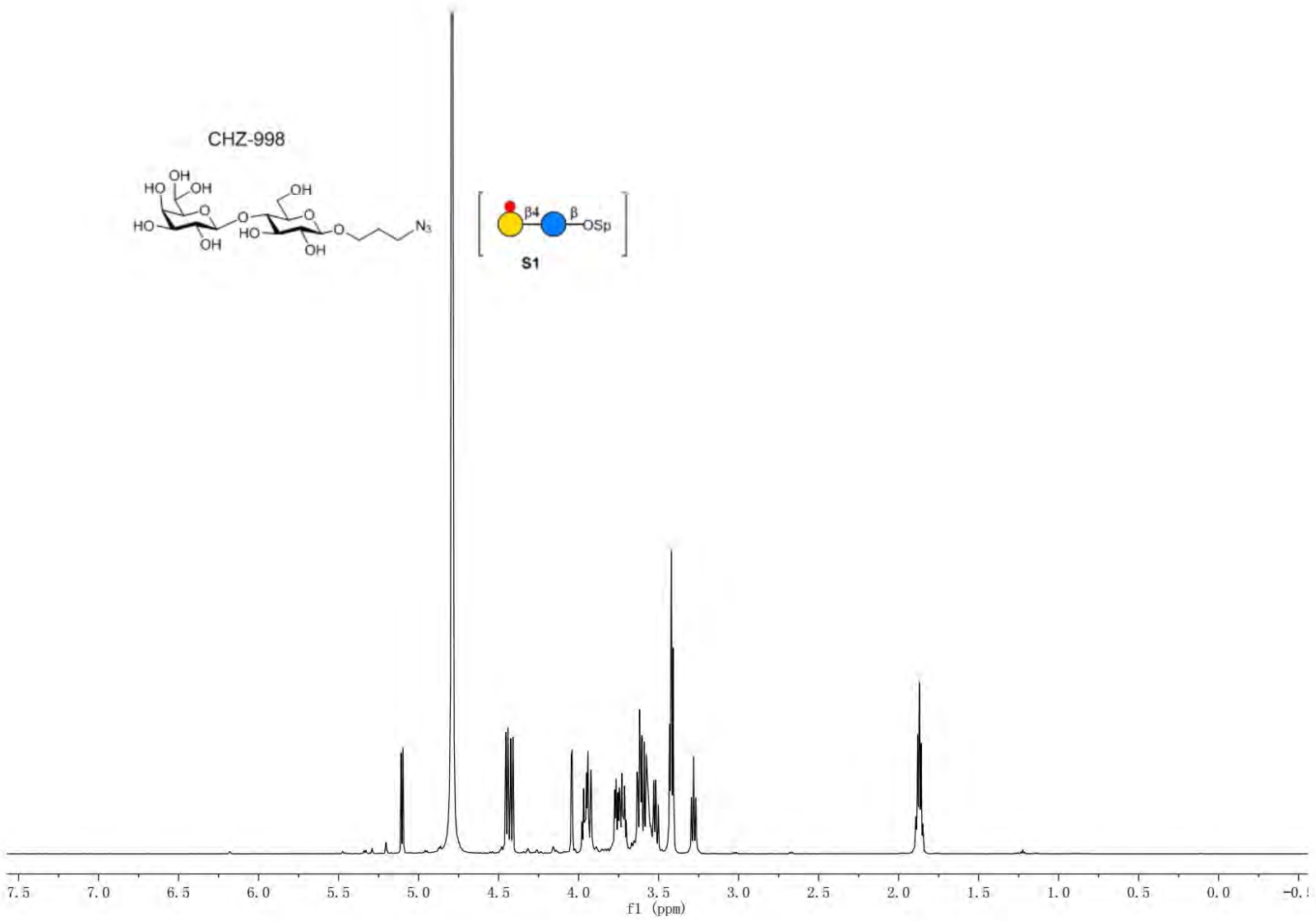
(16) Song, X.; Yu, H.; Chen, X.; Lasanajak, Y.; Tappert, M. M.; Air, G. M.; Tiwari, V. K.; Cao, H.; Chokhawala, H. A.; Zheng, H.; Cummings, R. D.; Smith, D. F. *J. Biol. Chem.* **2011**, *286*, 31610.

(17) Song, J.; Gao, X.; Galan, J. E. *Nature* **2013**, *499*, 350.

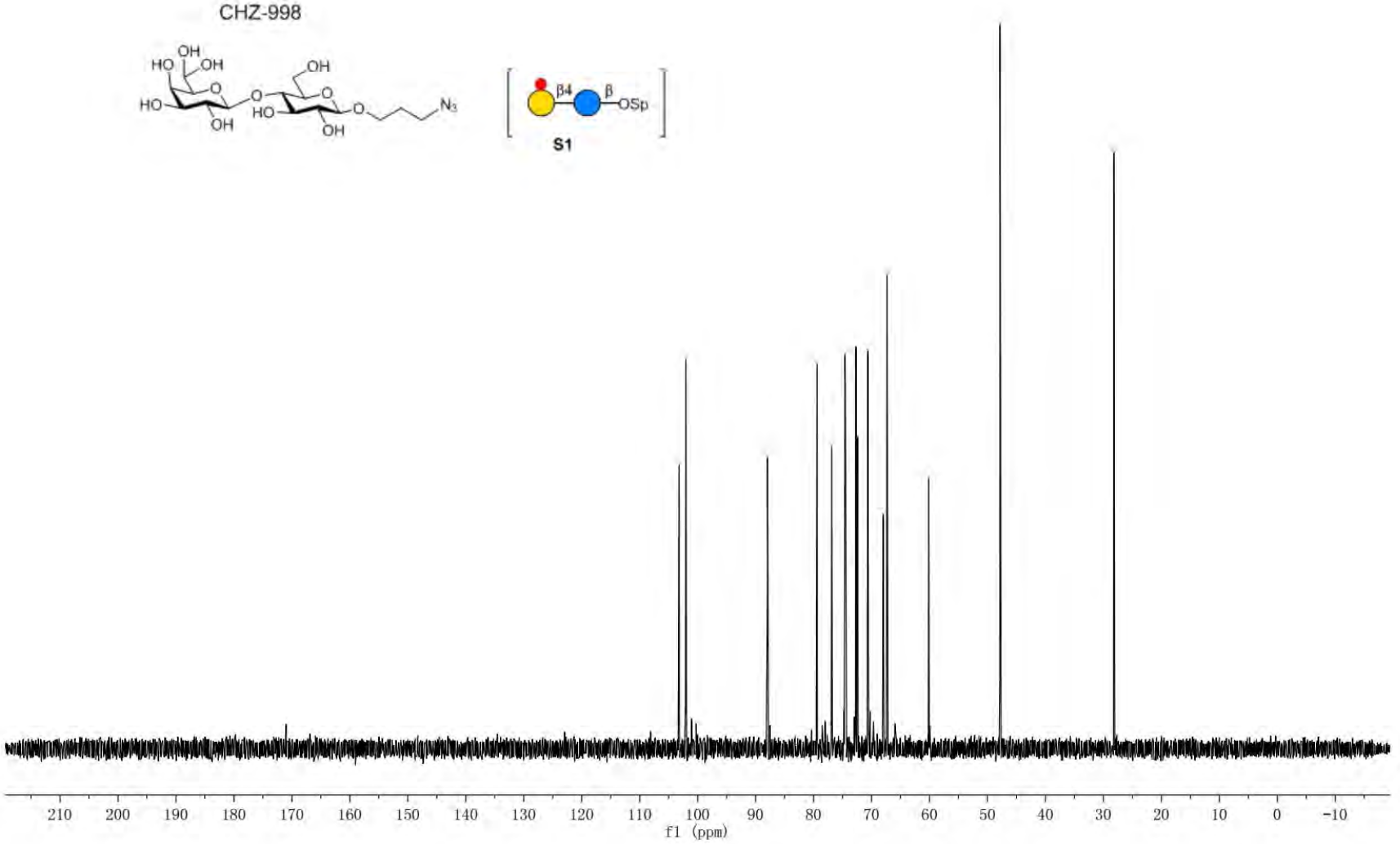
(18) Deng, L.; Song, J.; Gao, X.; Wang, J.; Yu, H.; Chen, X.; Varki, N.; Naito-Matsui, Y.; Galán, Jorge E.; Varki, A. *Cell* **2014**, *159*, 1290.

(19) Gao, X.; Deng, L.; Stack, G.; Yu, H.; Chen, X.; Naito-Matsui, Y.; Varki, A.; Galán, J. E. *Nat. Microbiol.* **2017**, *2*, 1592.

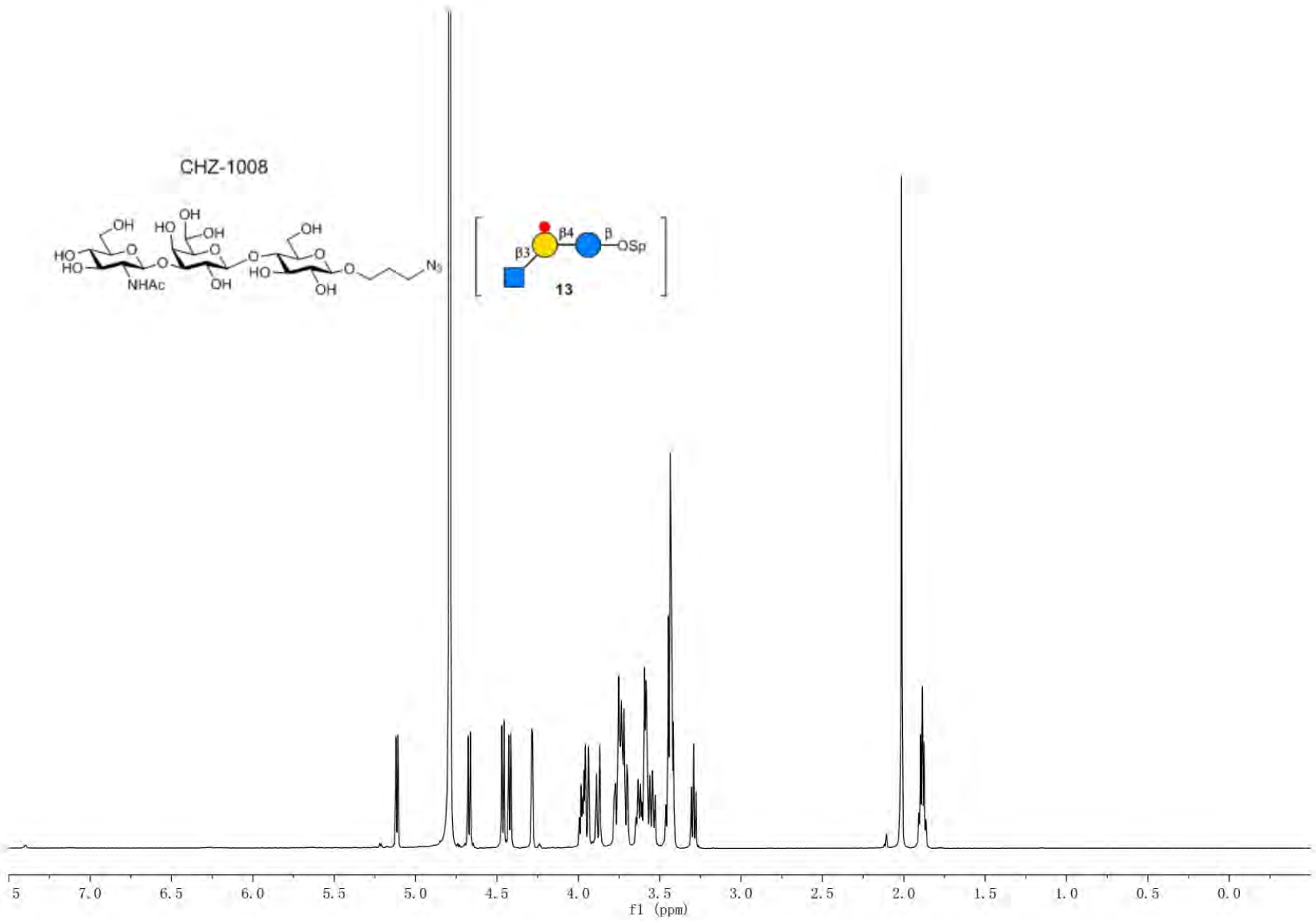
6. NMR Spectra



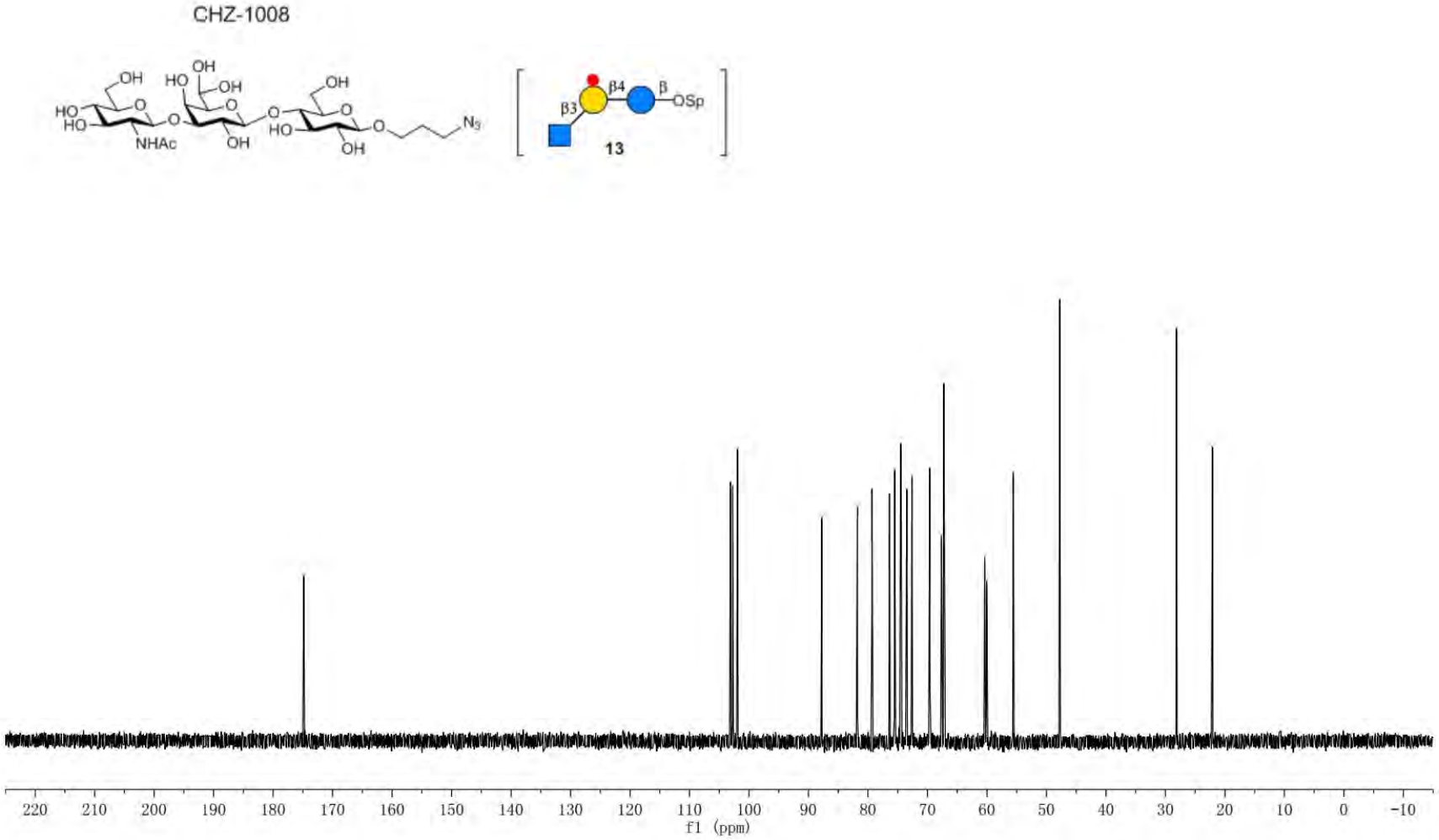
^{1}H NMR of S1


CHZ-998
HO
OH
OH
HO
O
O
OH
HO
O
O
N3
OH
OH
β4
β
OSp
S1
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

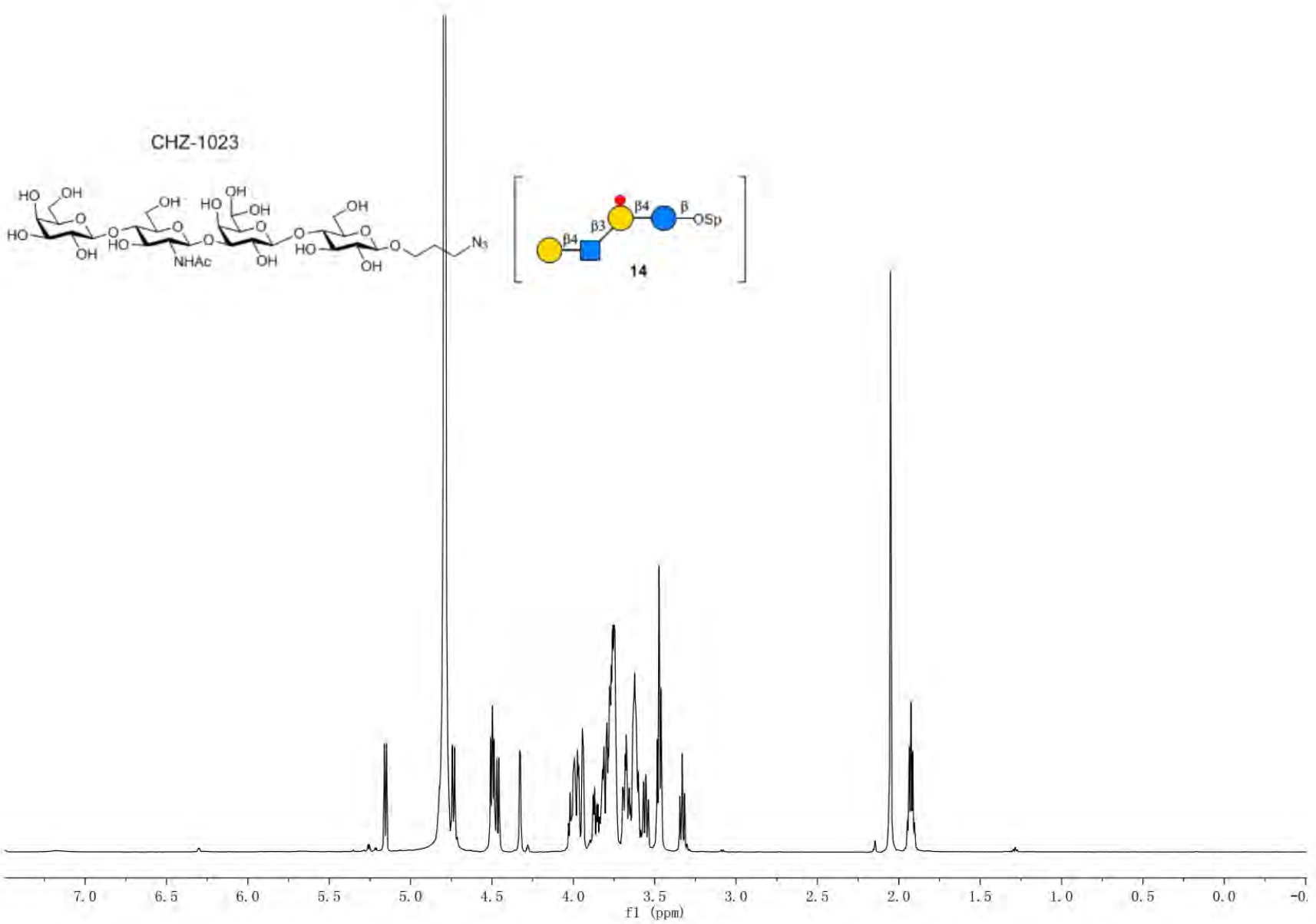
^{13}C NMR of S1



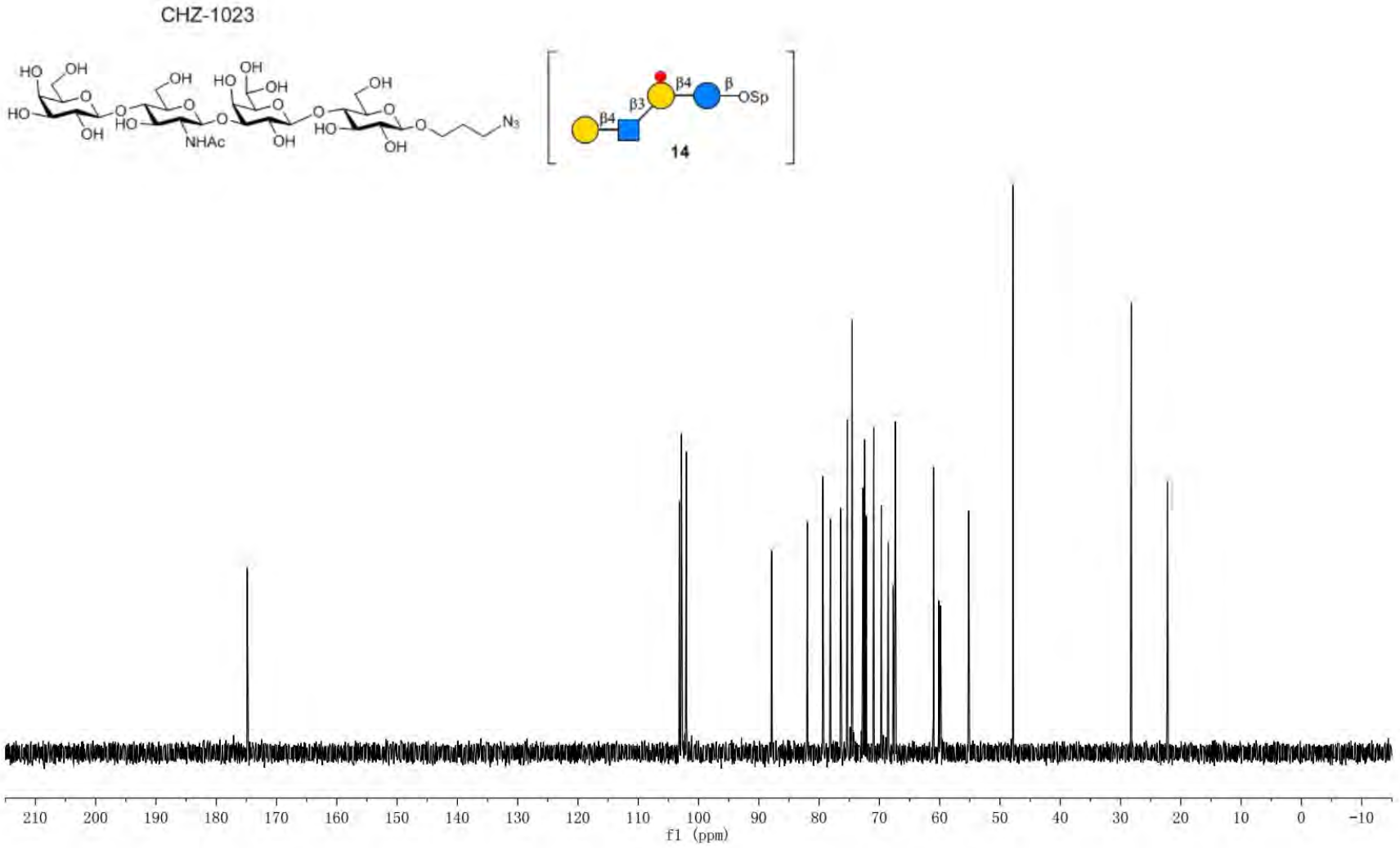
^{1}H NMR of 13



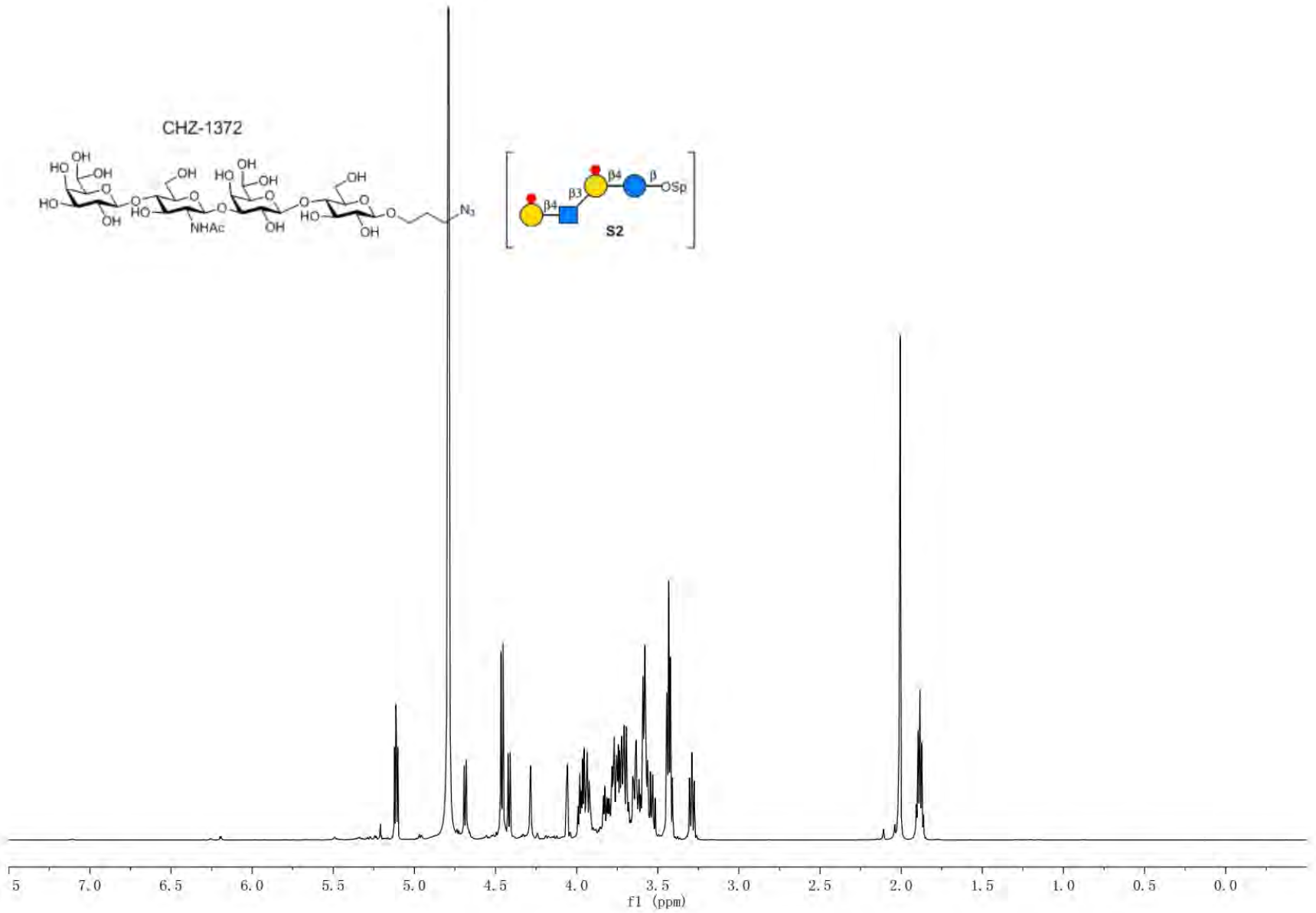
^{13}C NMR of 13



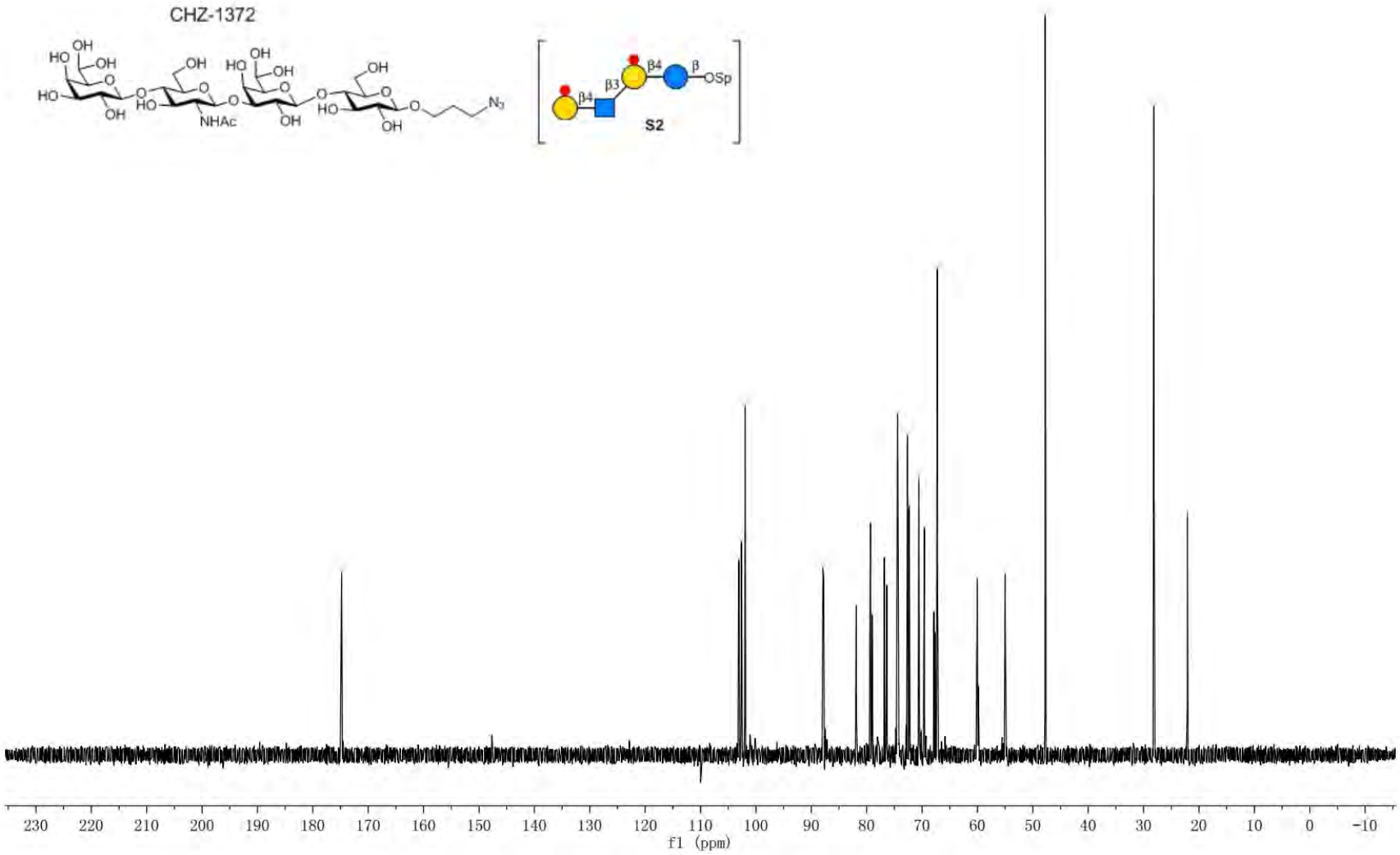
^{1}H NMR of 14



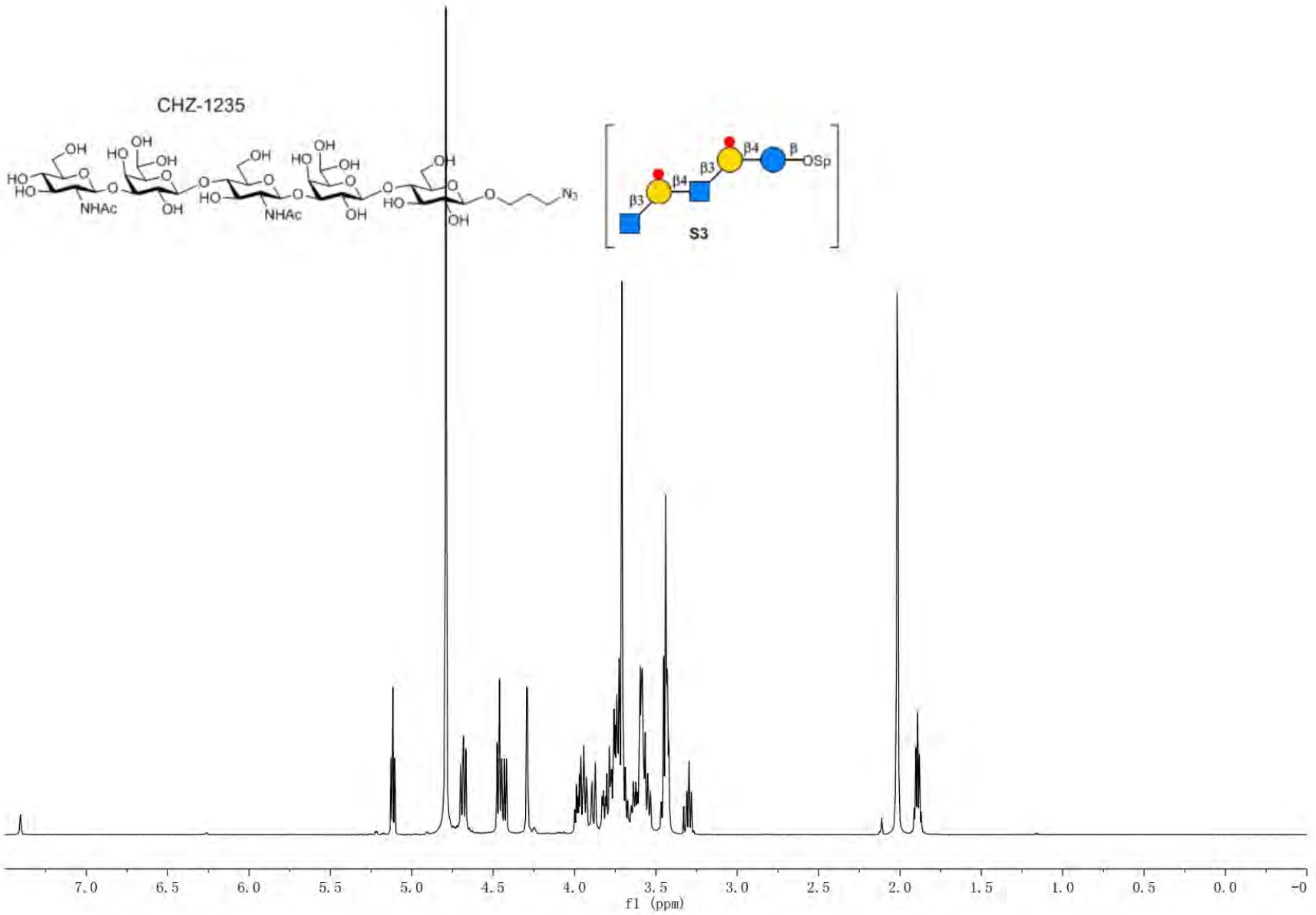
^{13}C NMR of 14



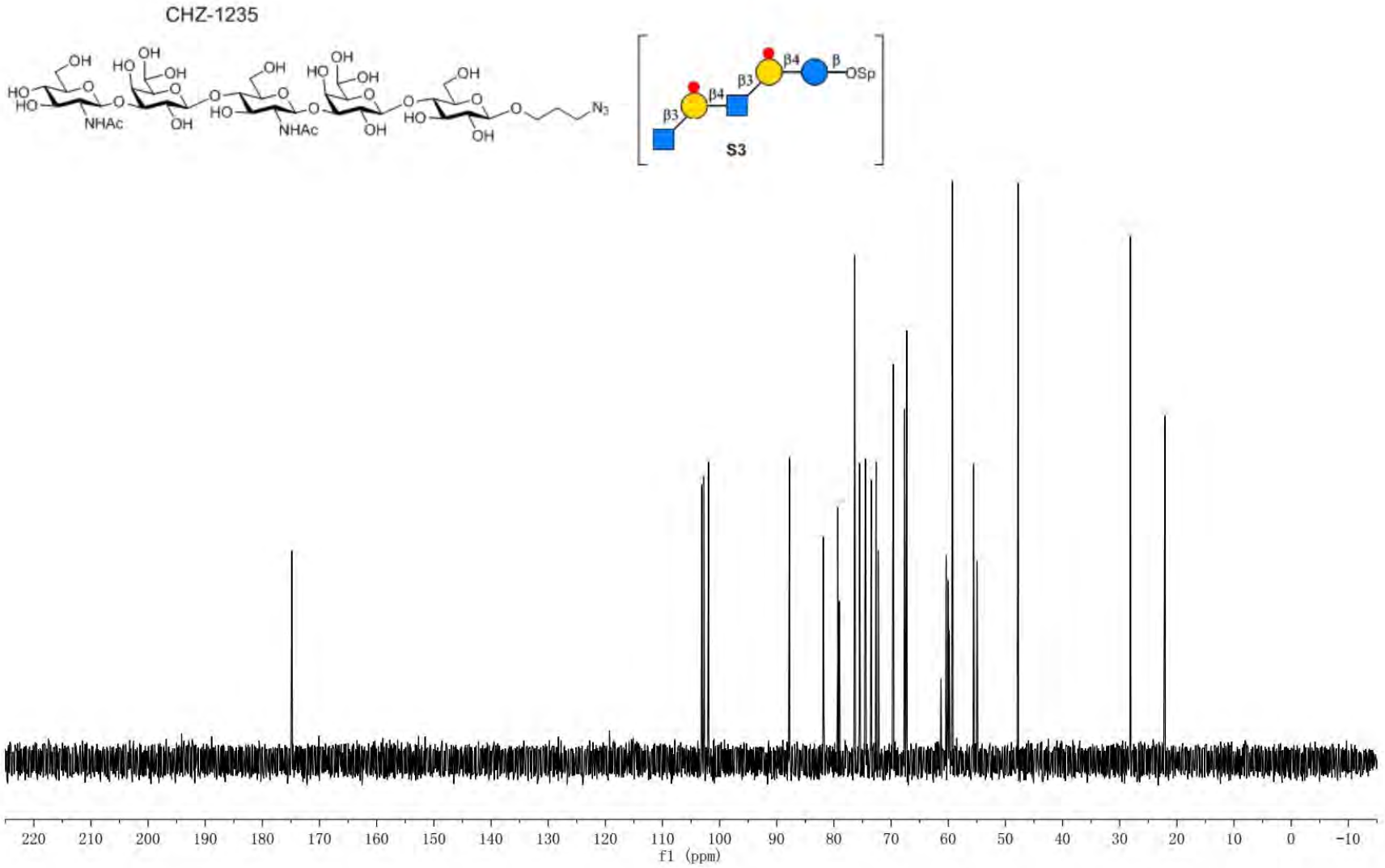
^{1}H NMR of S2


CHZ-1372
S2
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

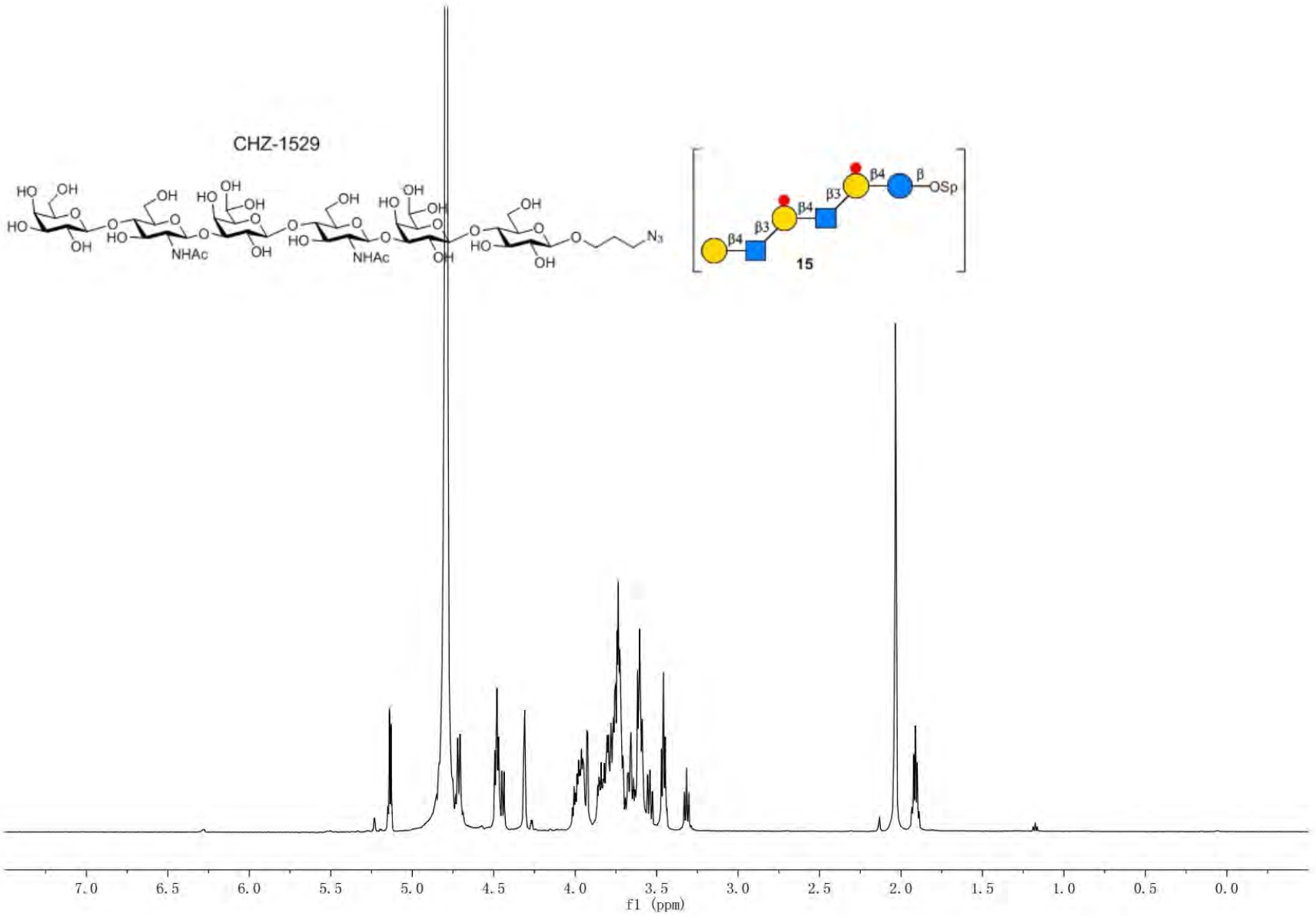
^{13}C NMR of S2


CHZ-1235
S3
β4
β3
β
OSp
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

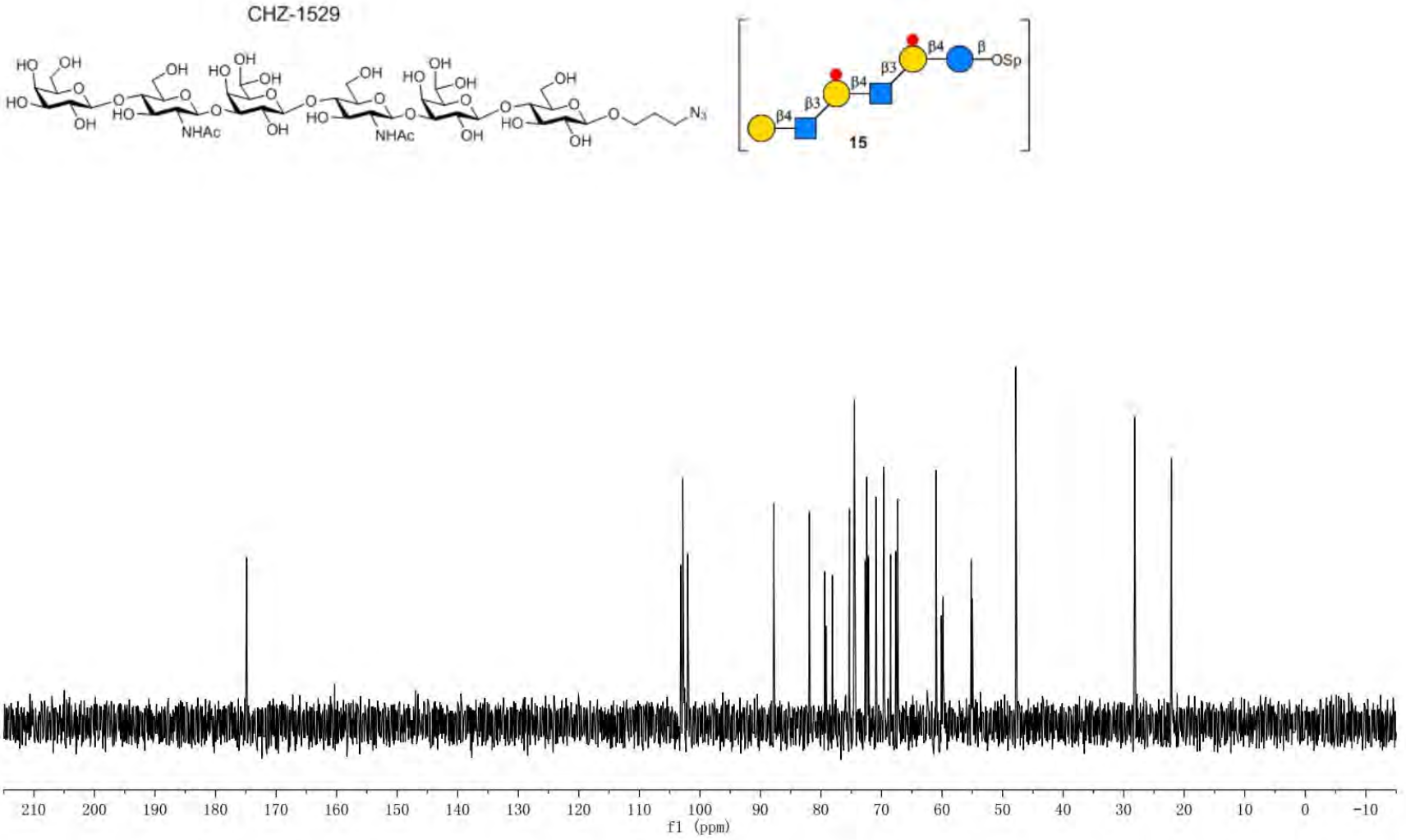
^{1}H NMR of S3


CHZ-1235
S3
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

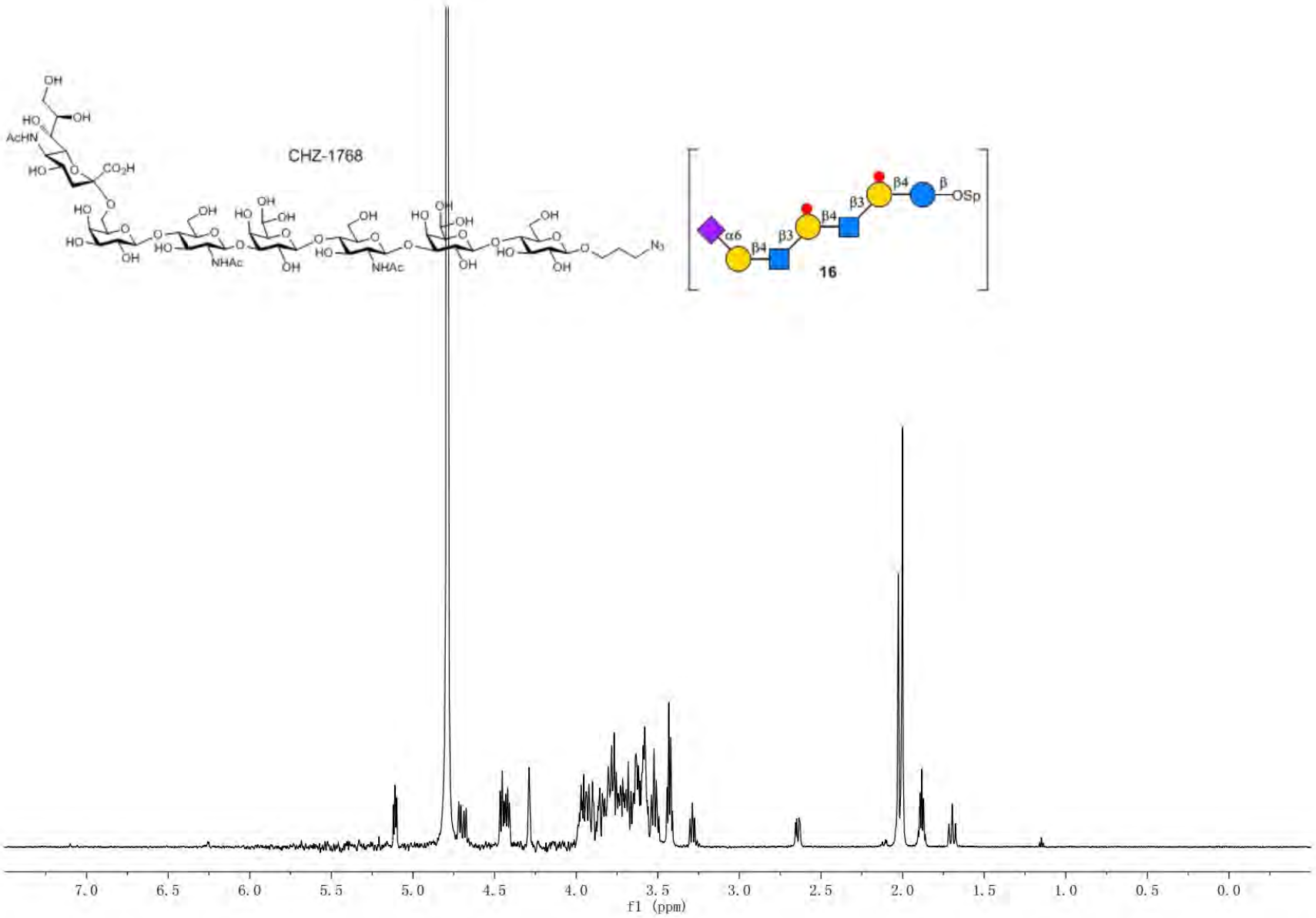
^{13}C NMR of S3


CHZ-1529
15
β4
β3
β4
β3
β4
β
OSp
NHAc
NHAc
N3
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

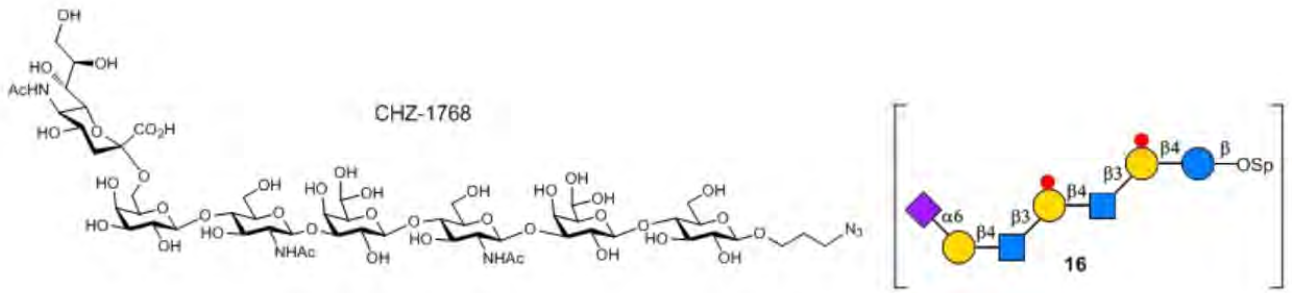
^{1}H NMR of 15

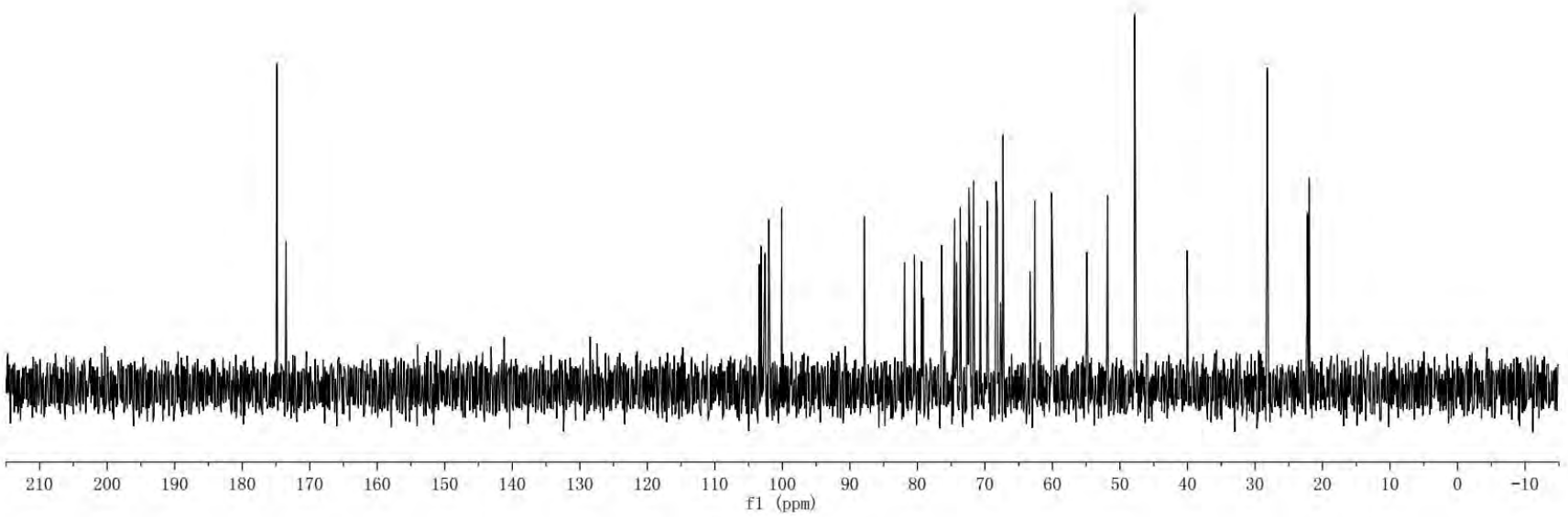

CHZ-1529
15
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{13}C NMR of 15

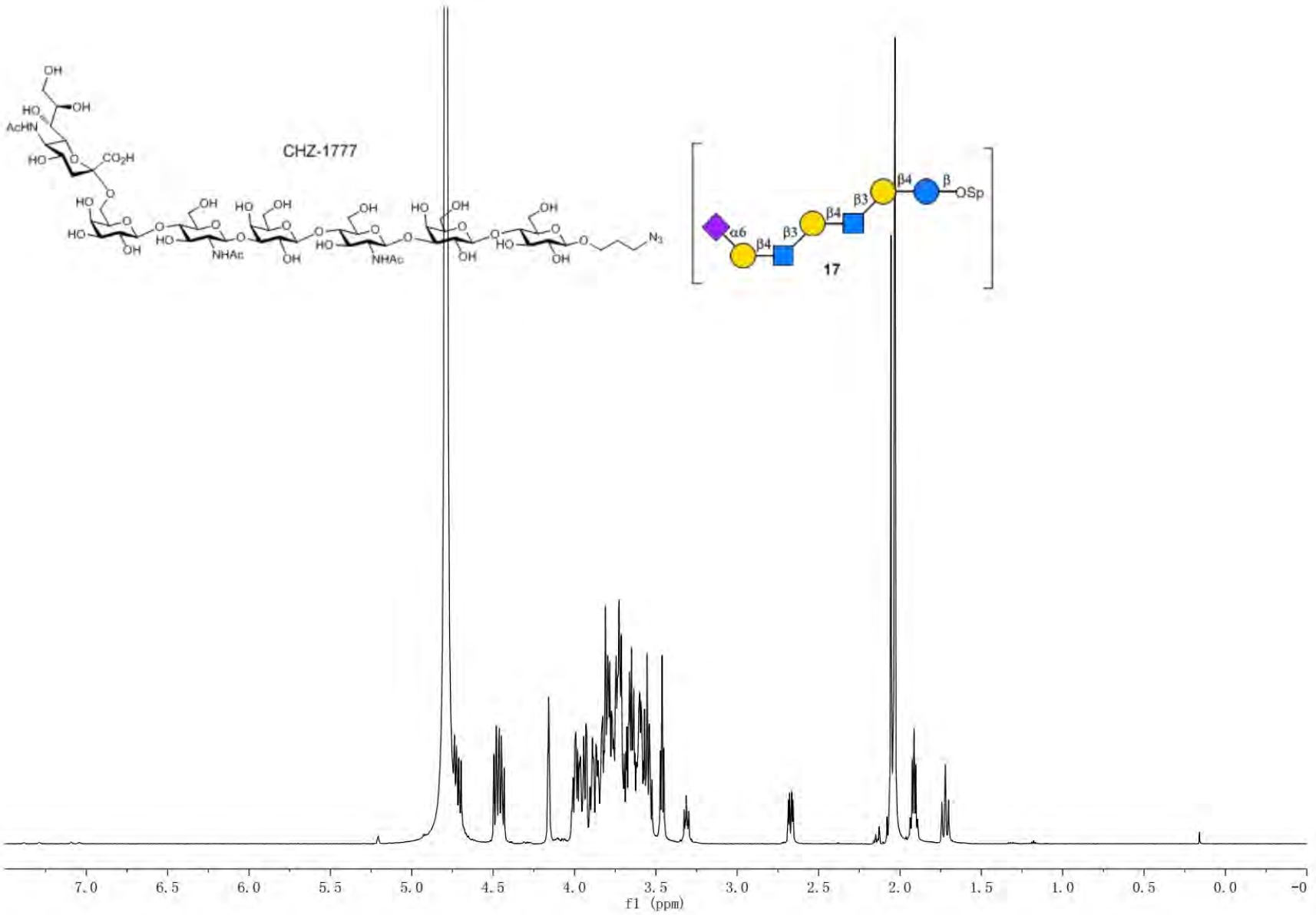


^{1}H NMR of 16

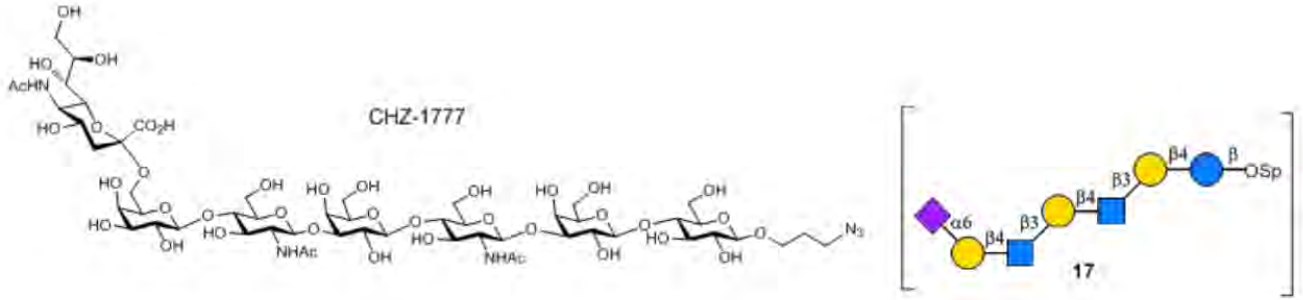

CHZ-1768
16

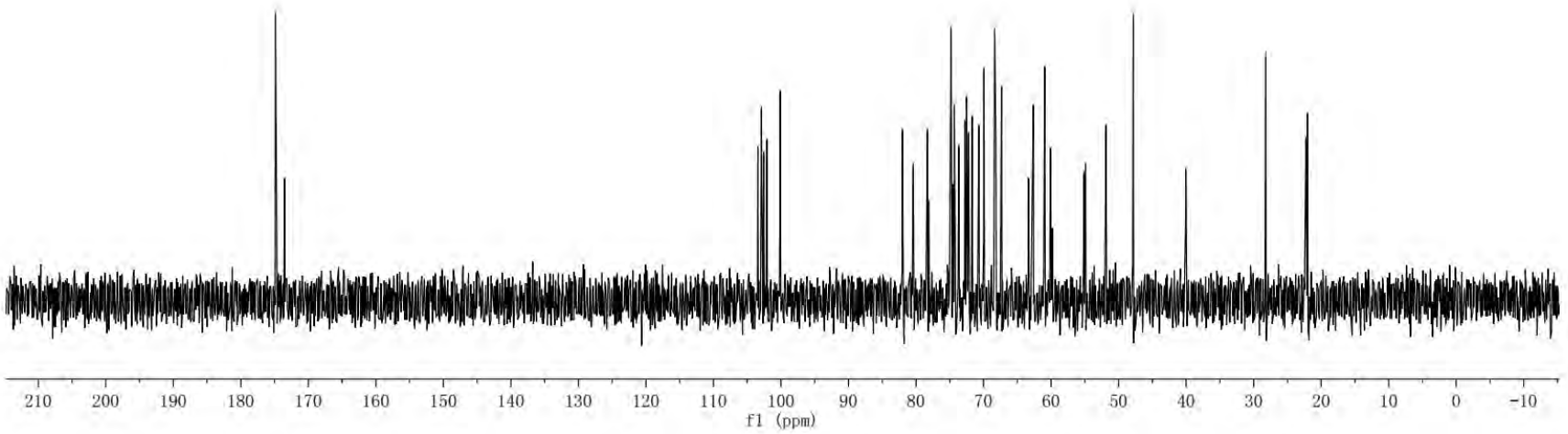

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

^{13}C NMR of 16

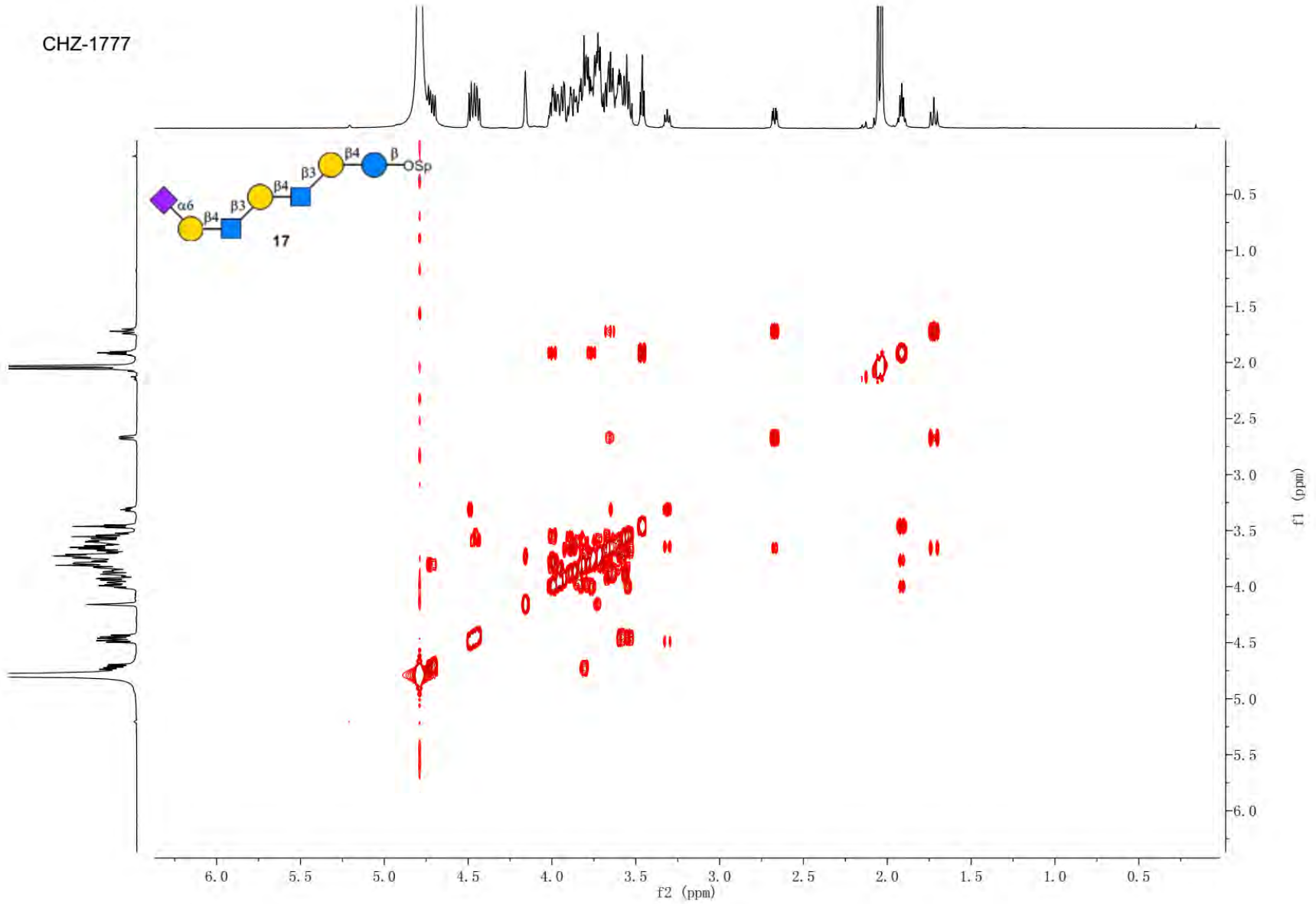


^{1}H NMR of 17

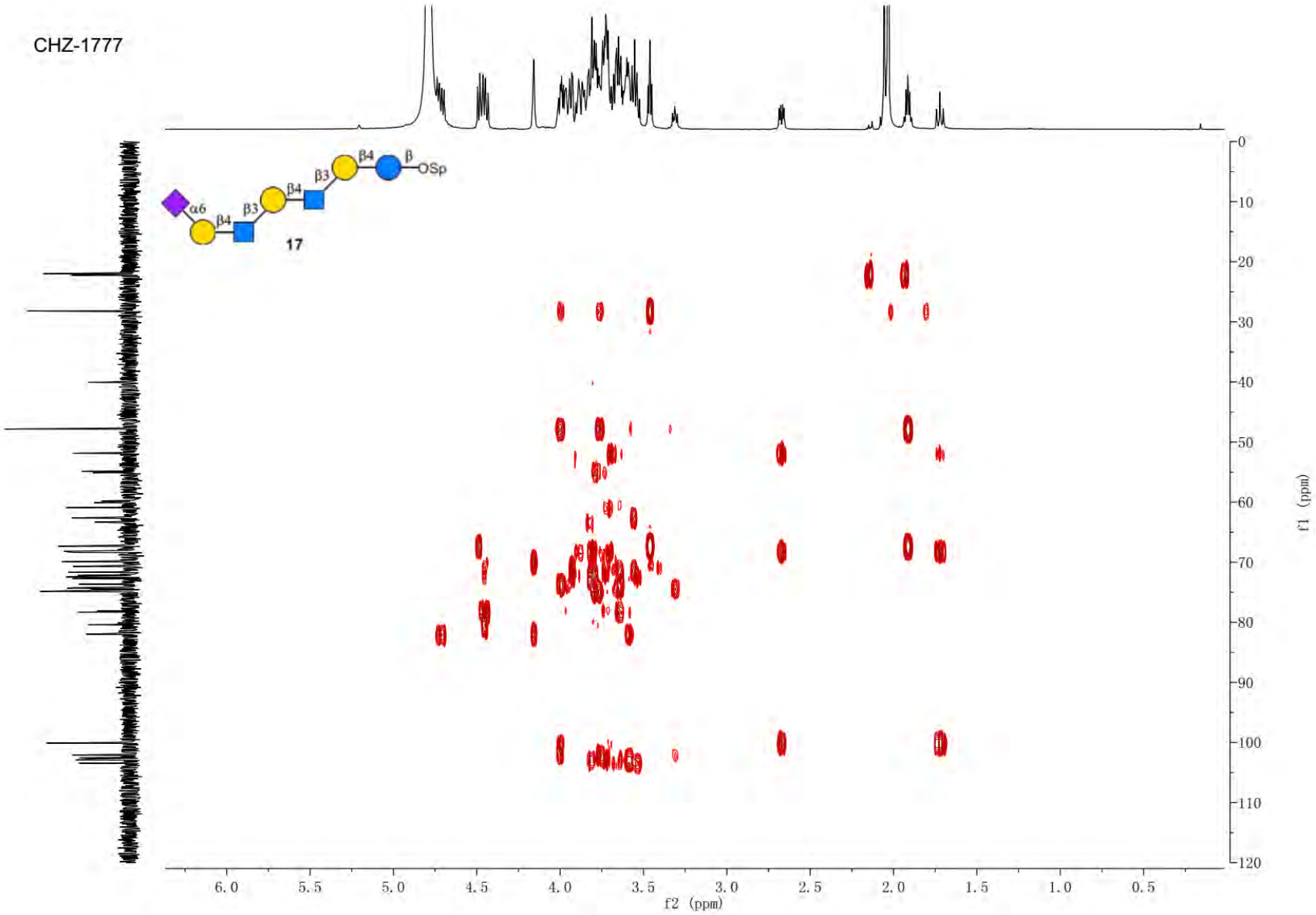

CHZ-1777
17


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

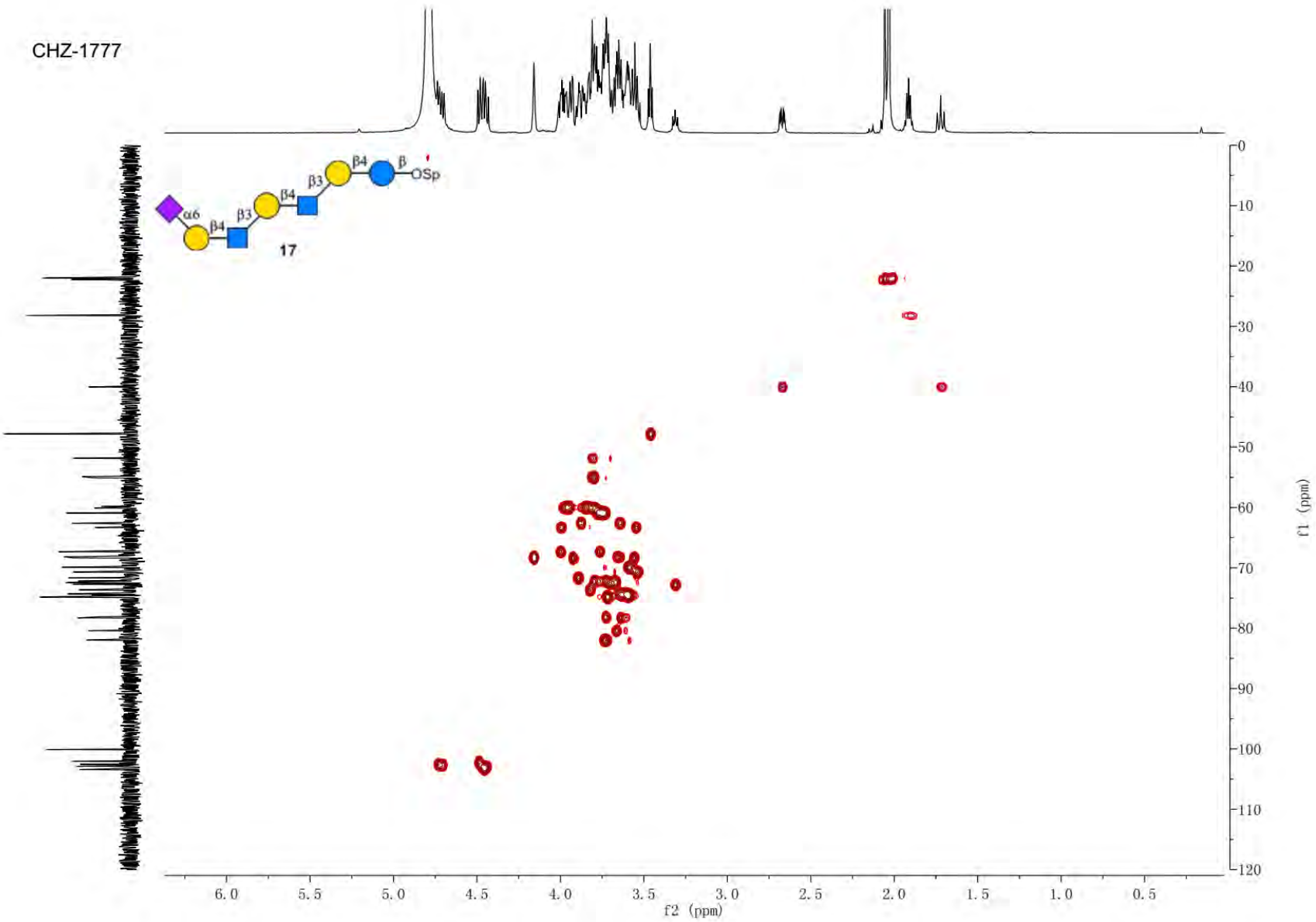
^{13}C NMR of 17



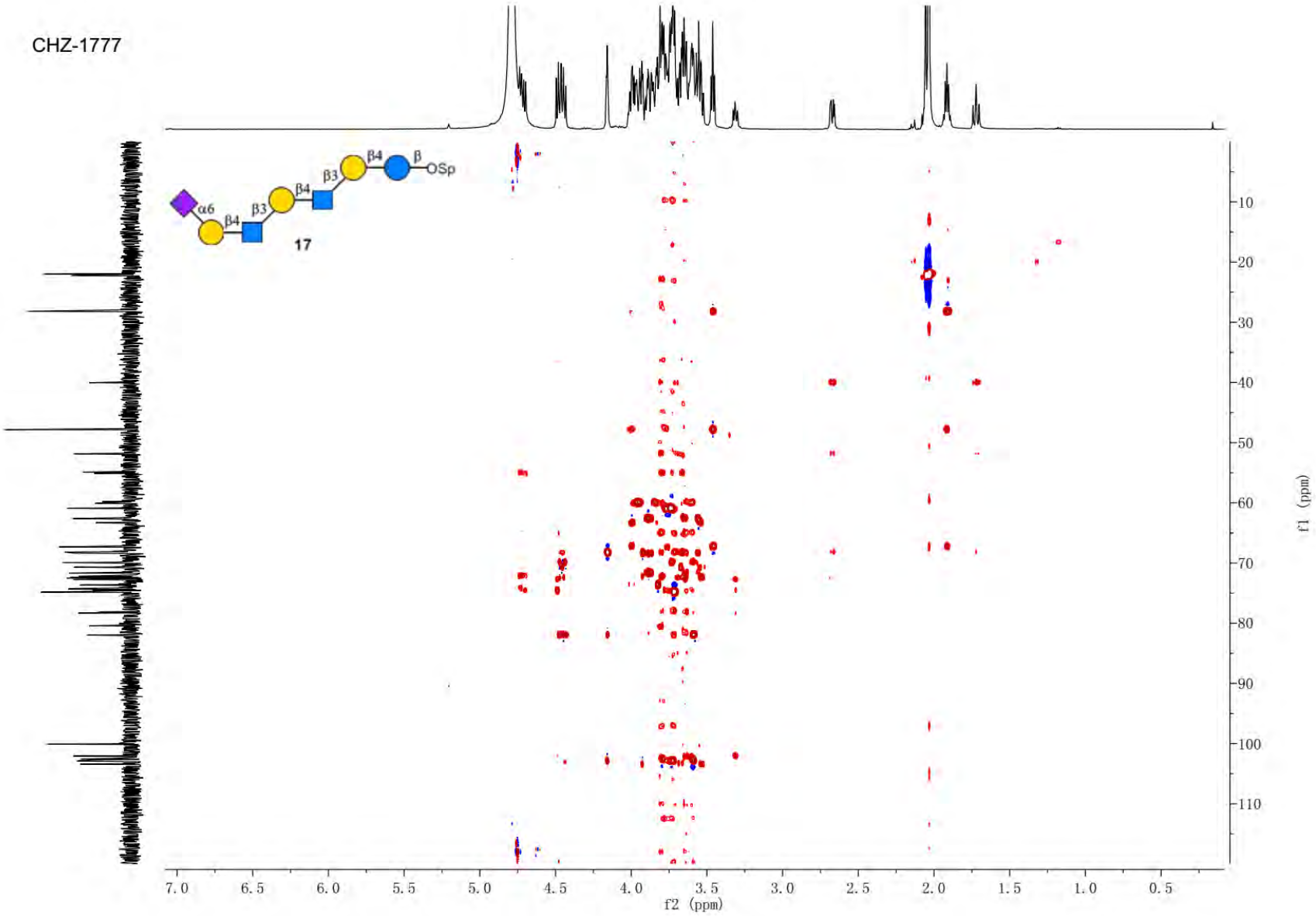
COSY spectra of 17



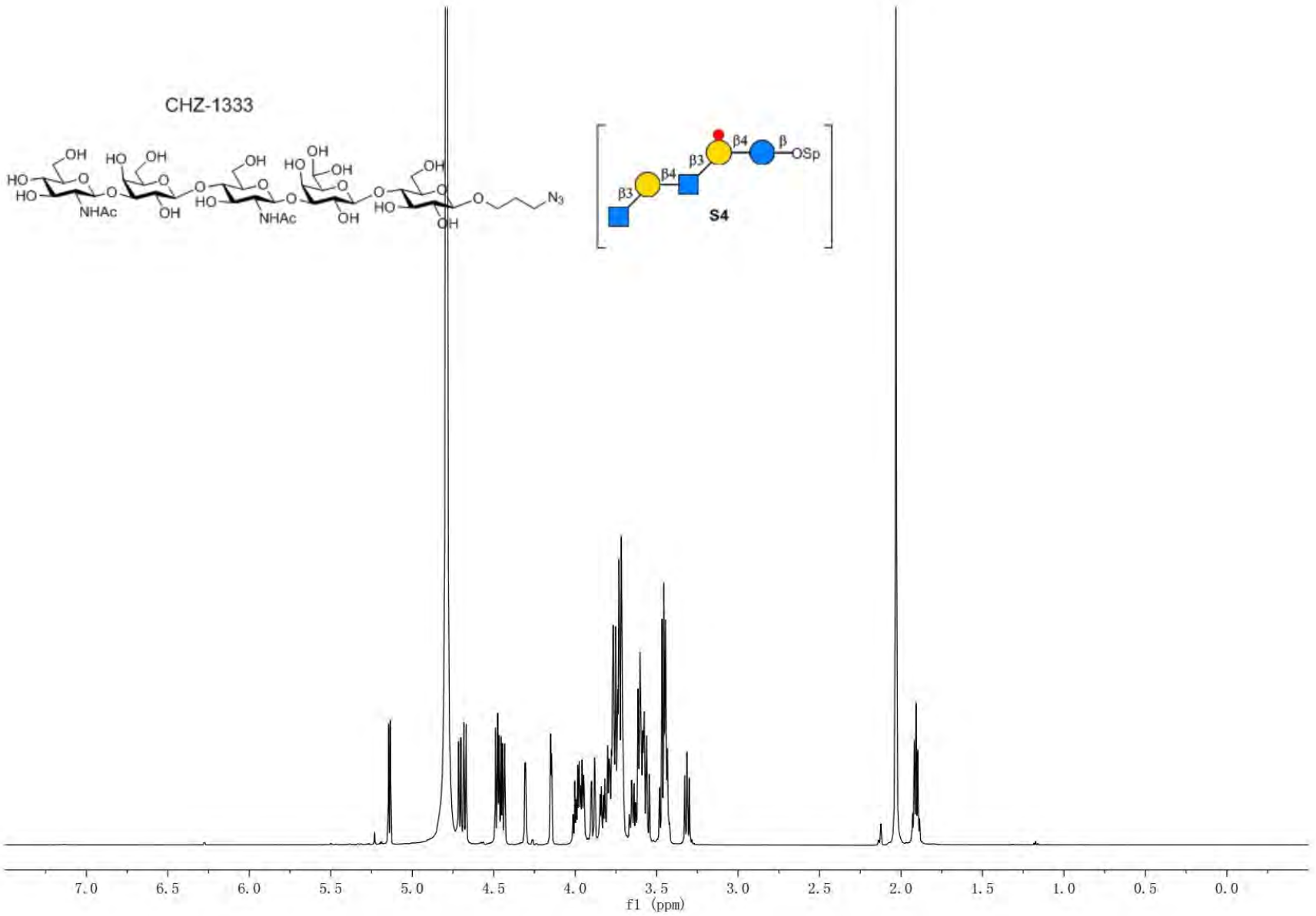
HMBC spectra of 17



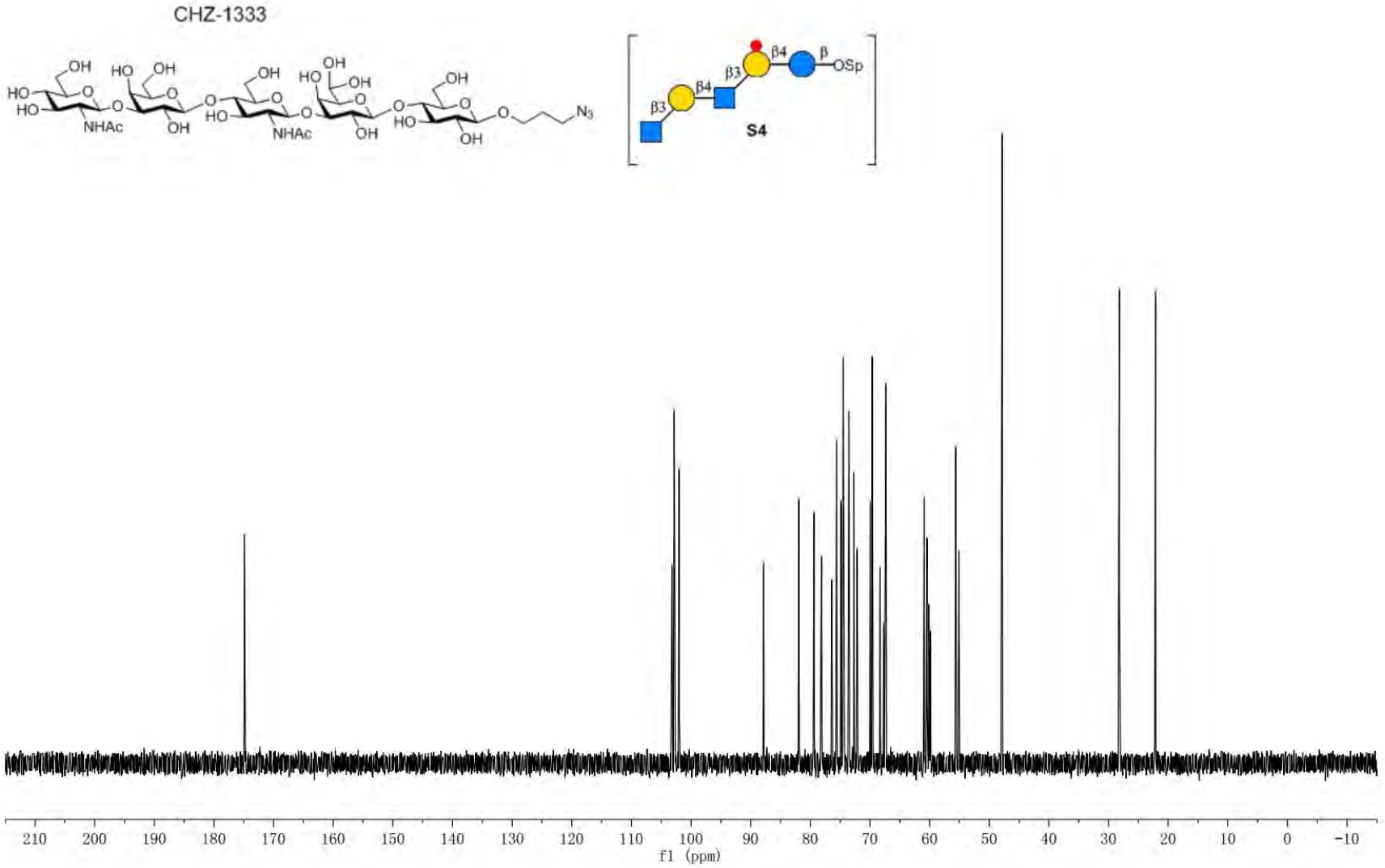
HSQC spectra of 17



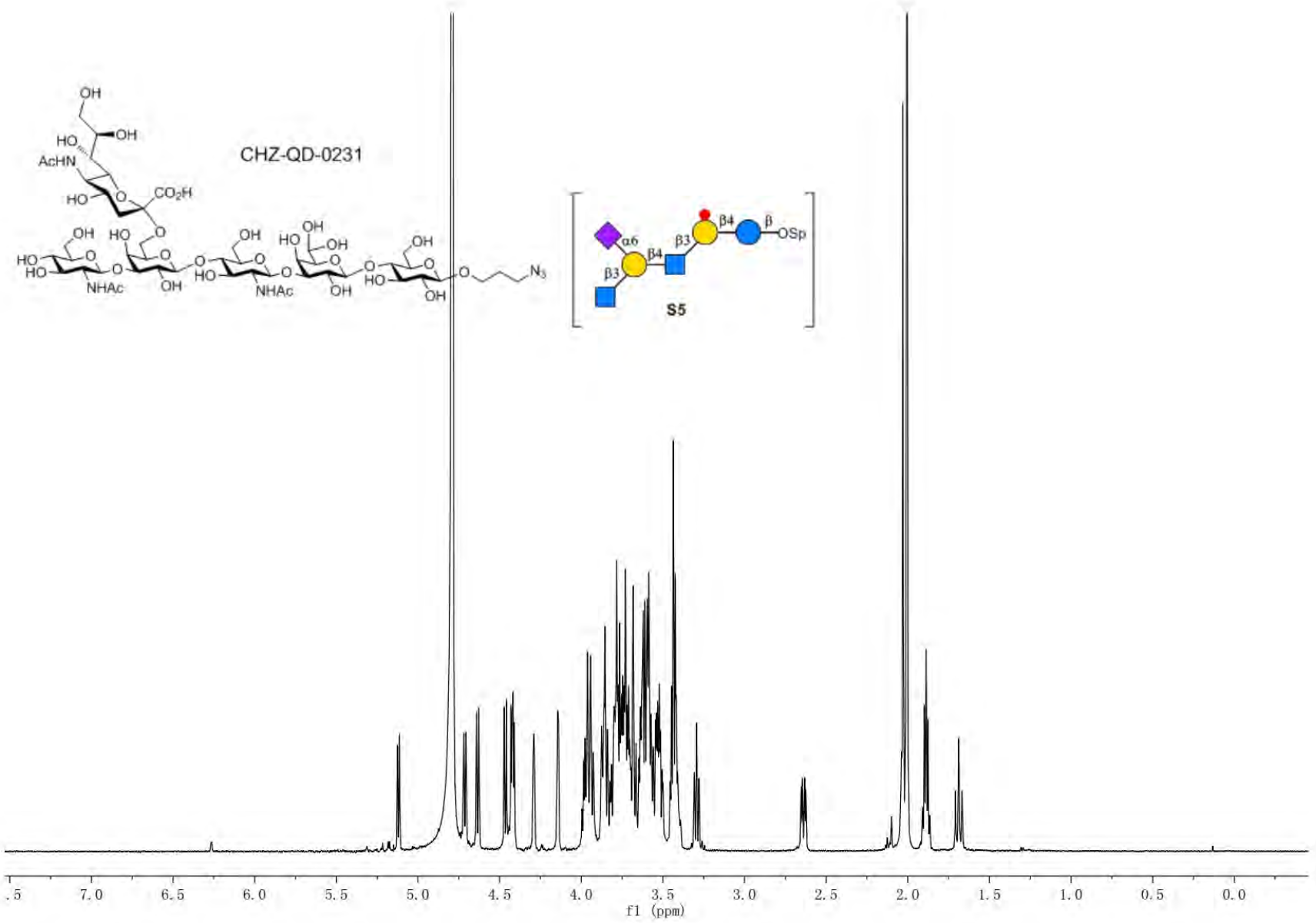
HSQC-TOCSY spectra of 17



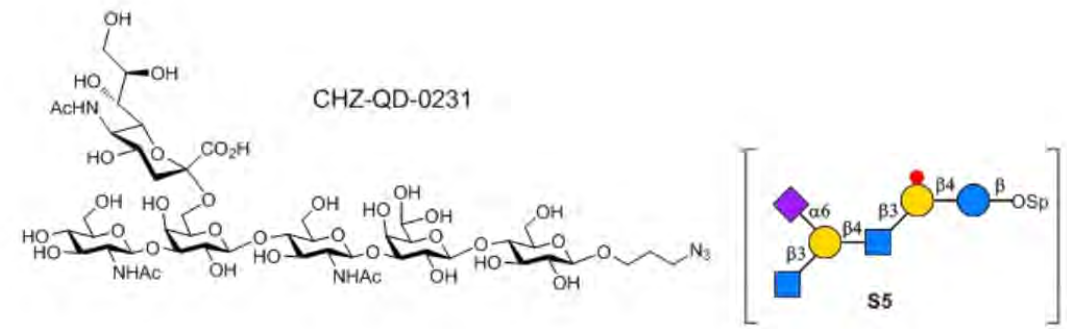
^{1}H NMR of S4

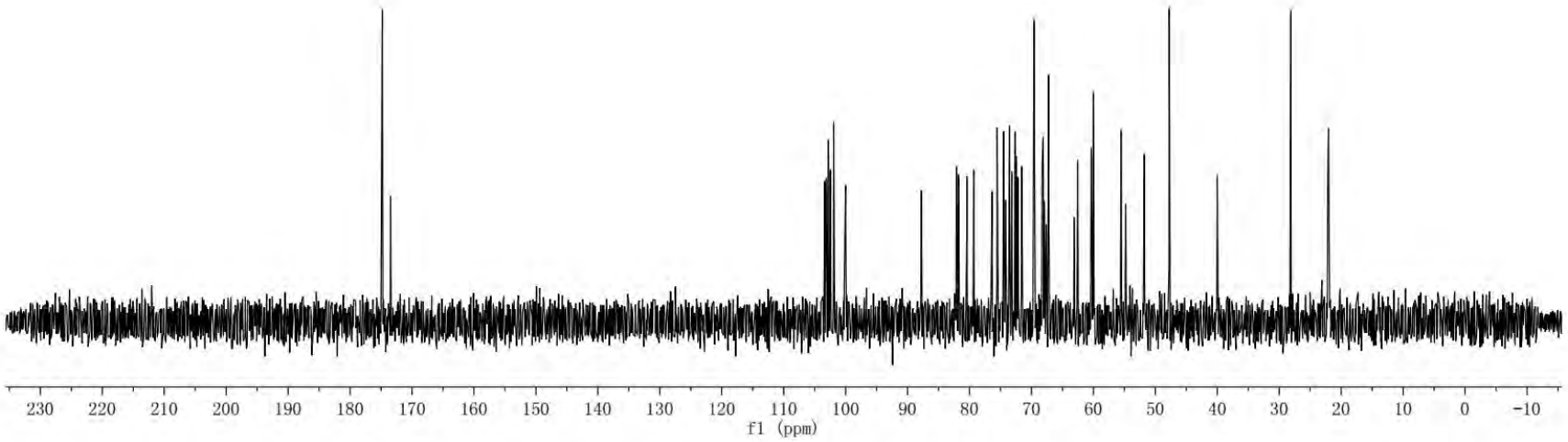

CHZ-1333
S4
β4
β3
β4
β3
β
OSp
NHAc
NHAc
N3
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

^{13}C NMR of S4

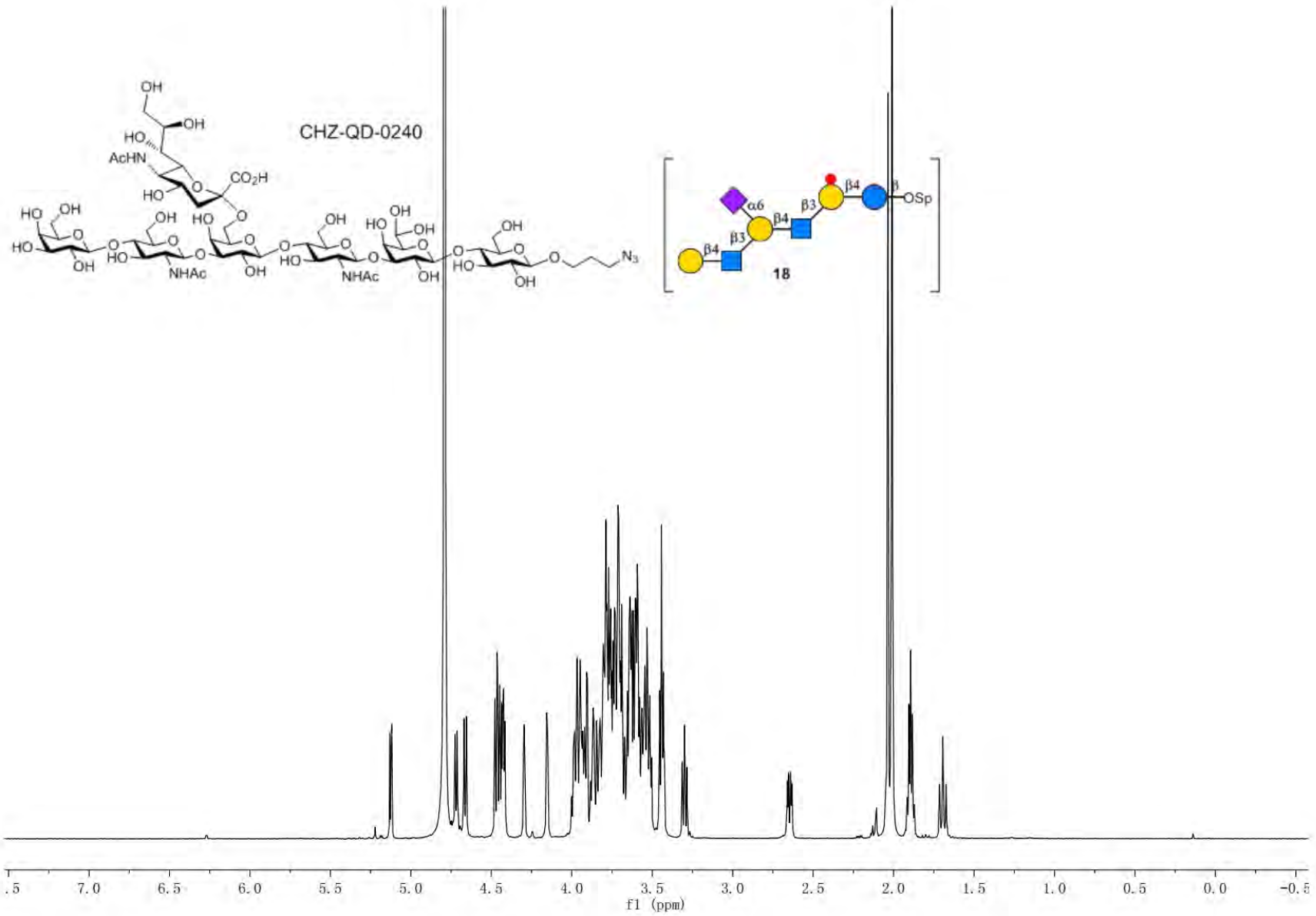


^{1}H NMR of S5

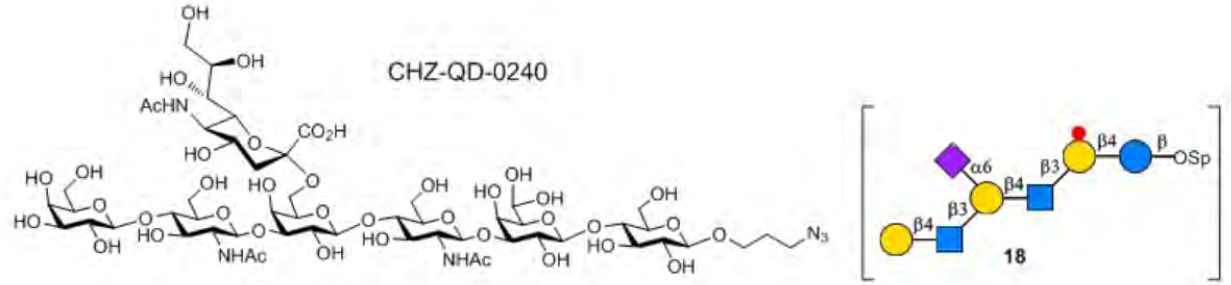

CHZ-QD-0231
S5

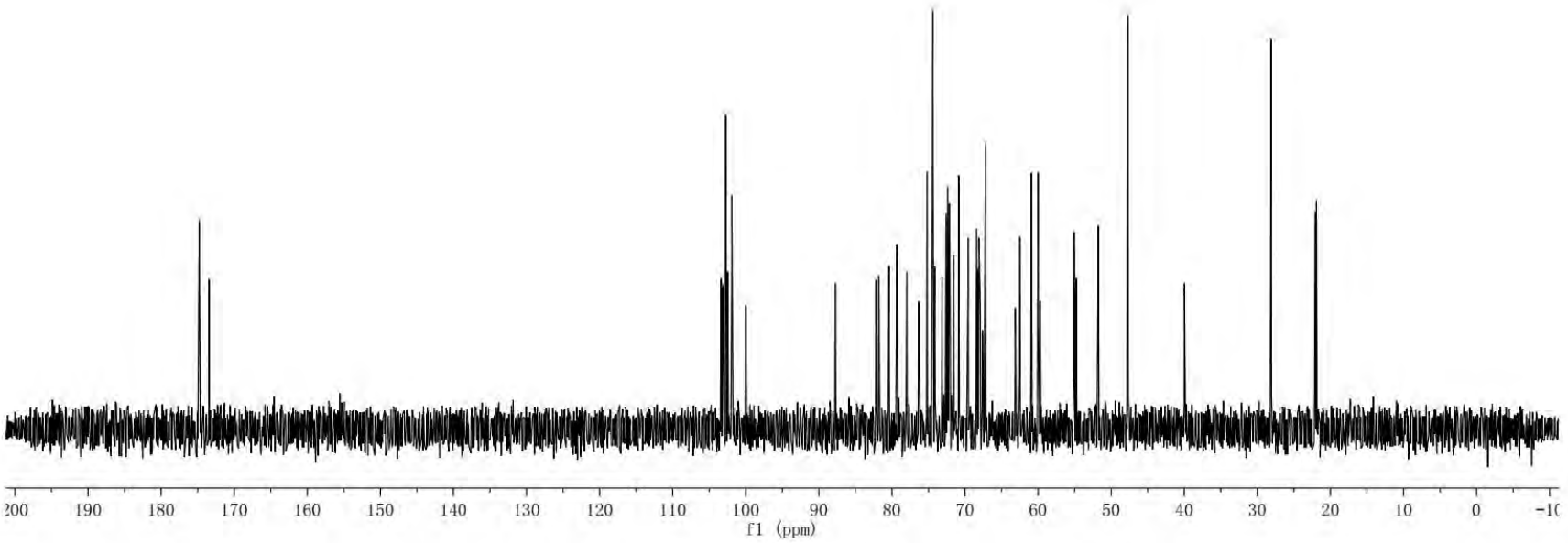

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

^{13}C NMR of S5

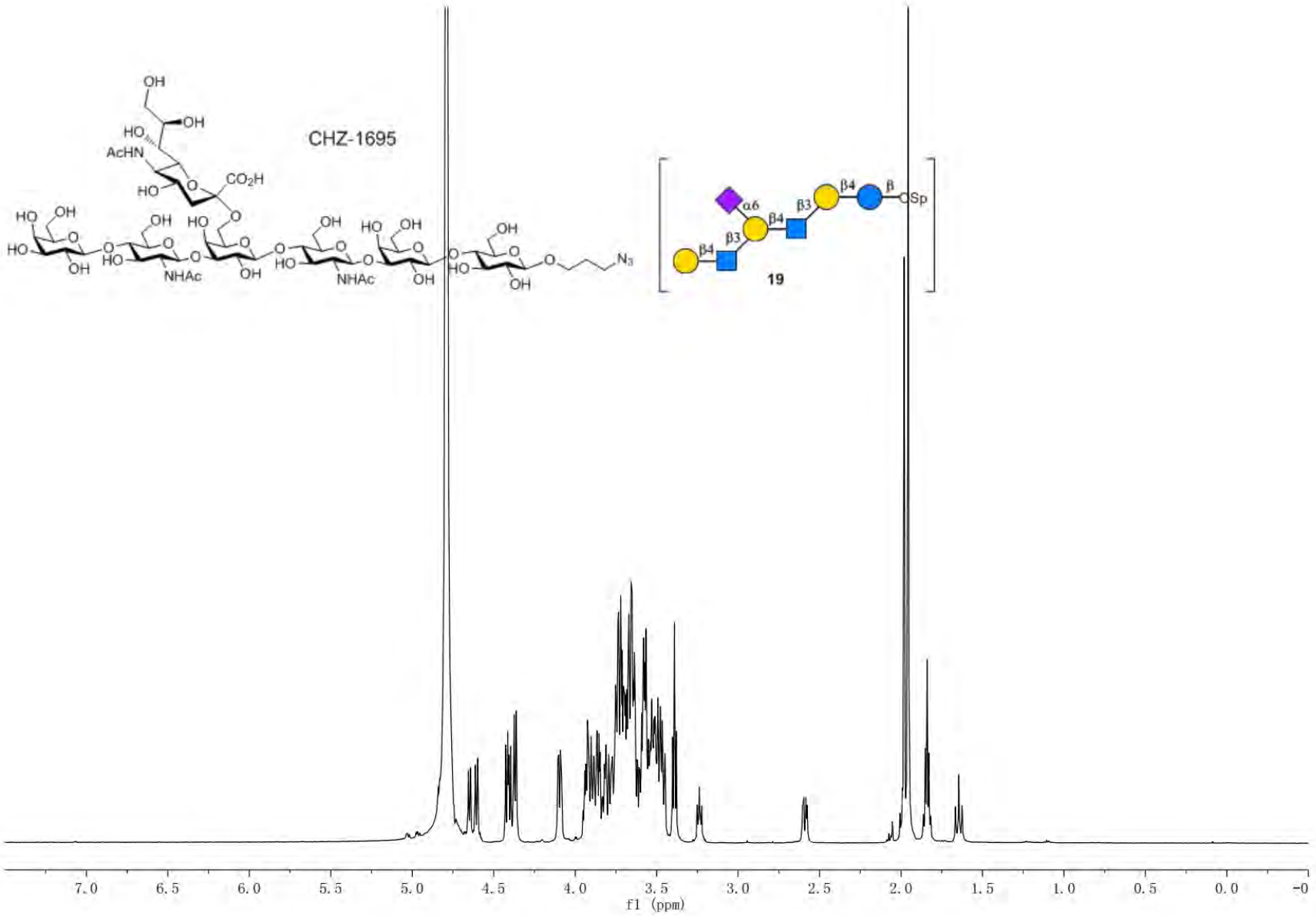


^{1}H NMR of 18

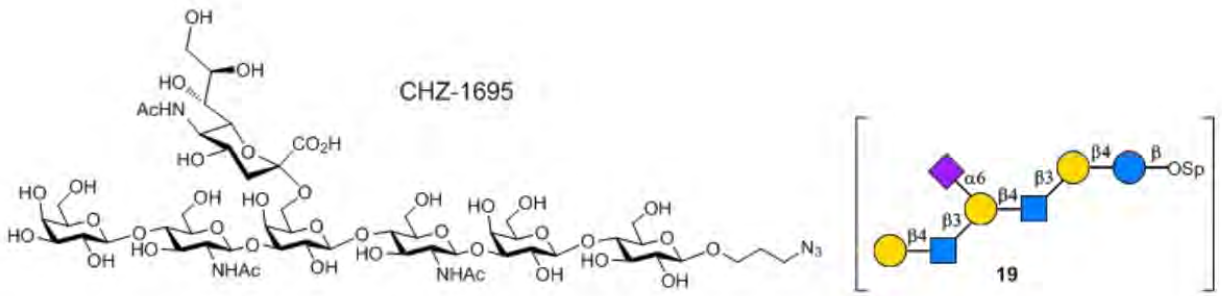

CHZ-QD-0240
18

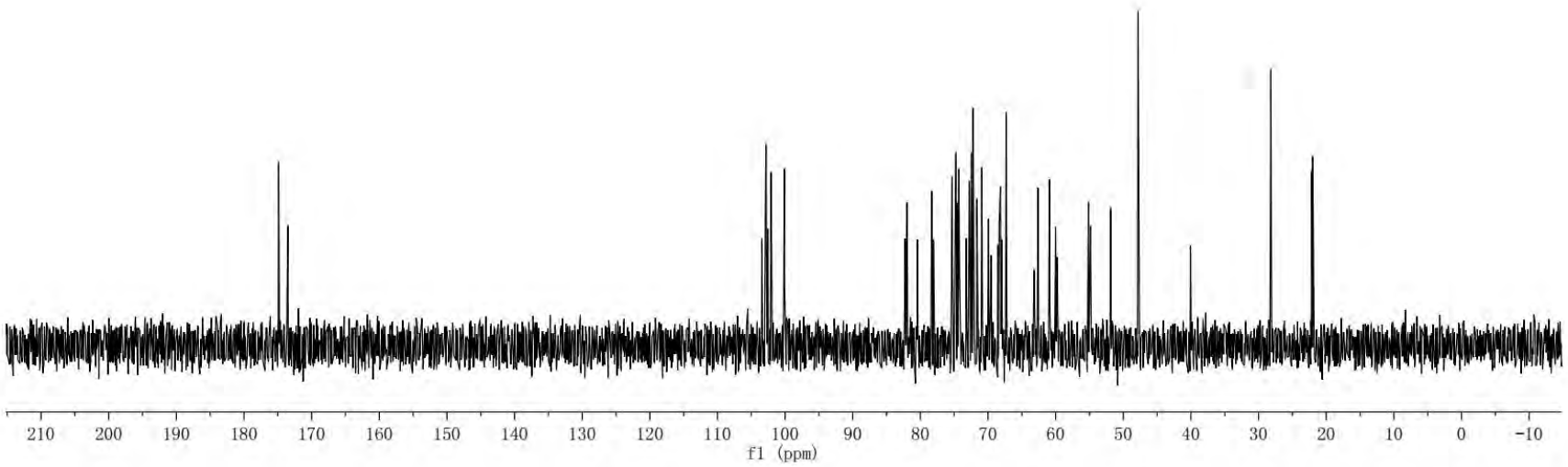

200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{13}C NMR of 18

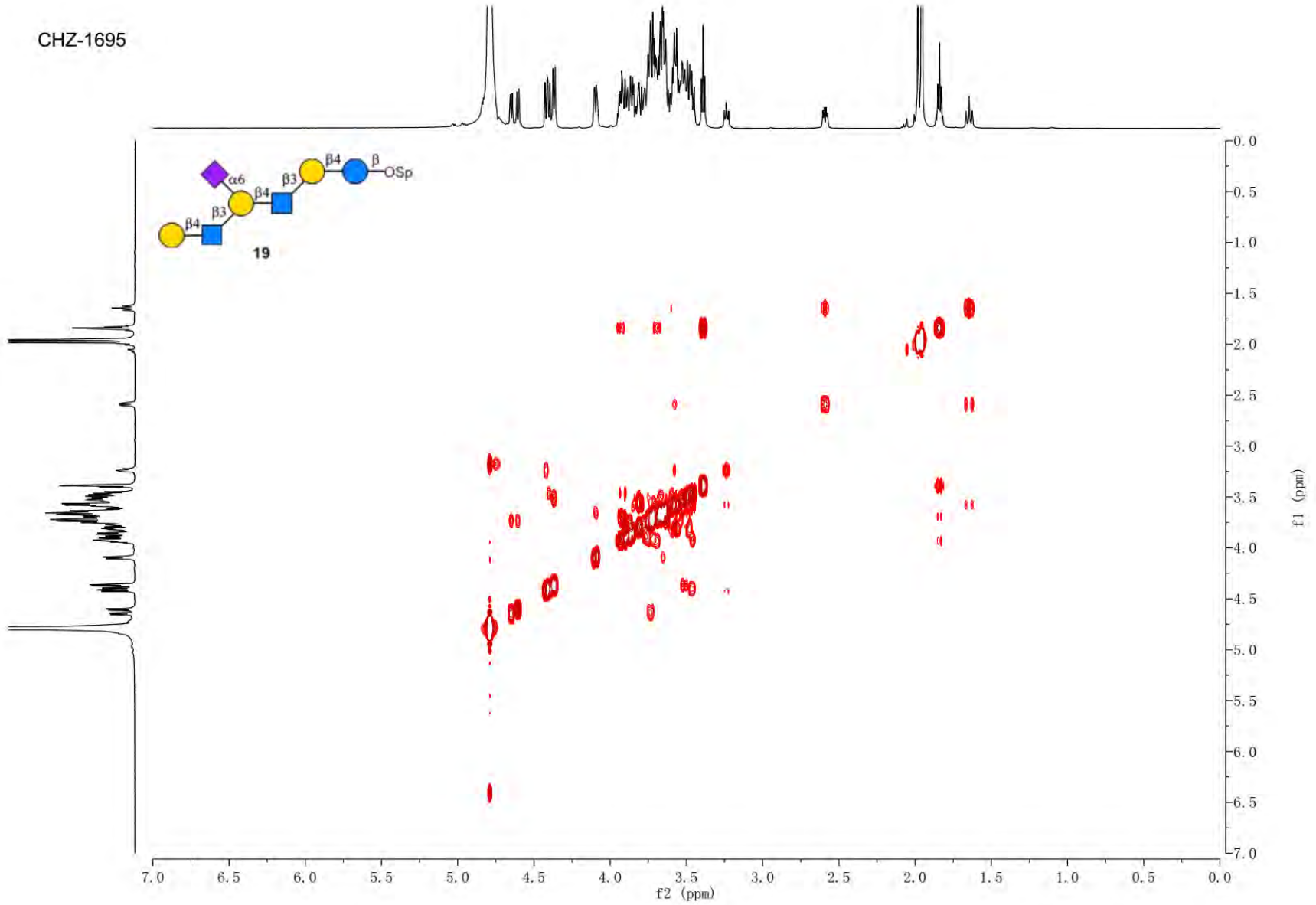


^{1}H NMR of 19

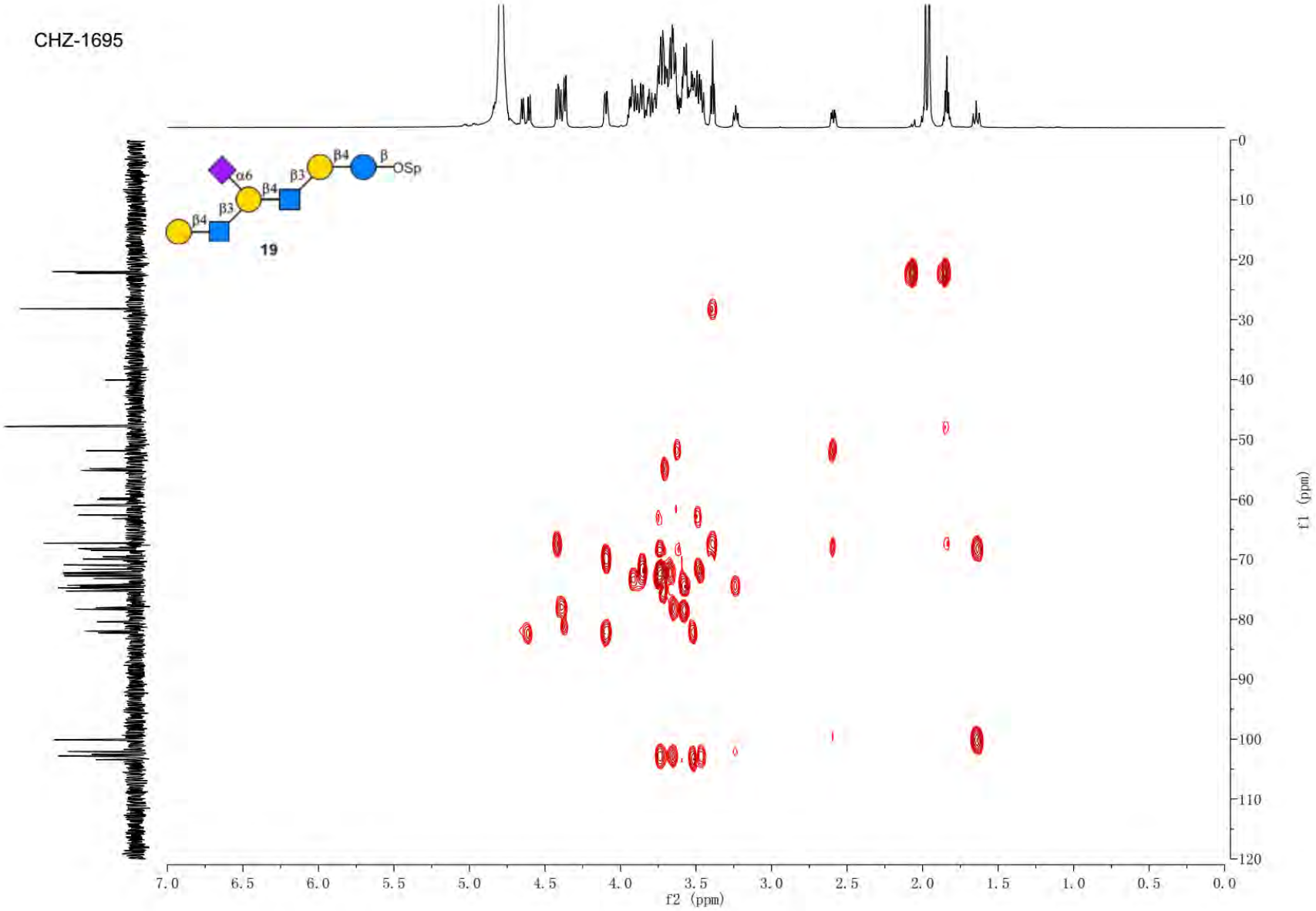

CHZ-1695
19


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

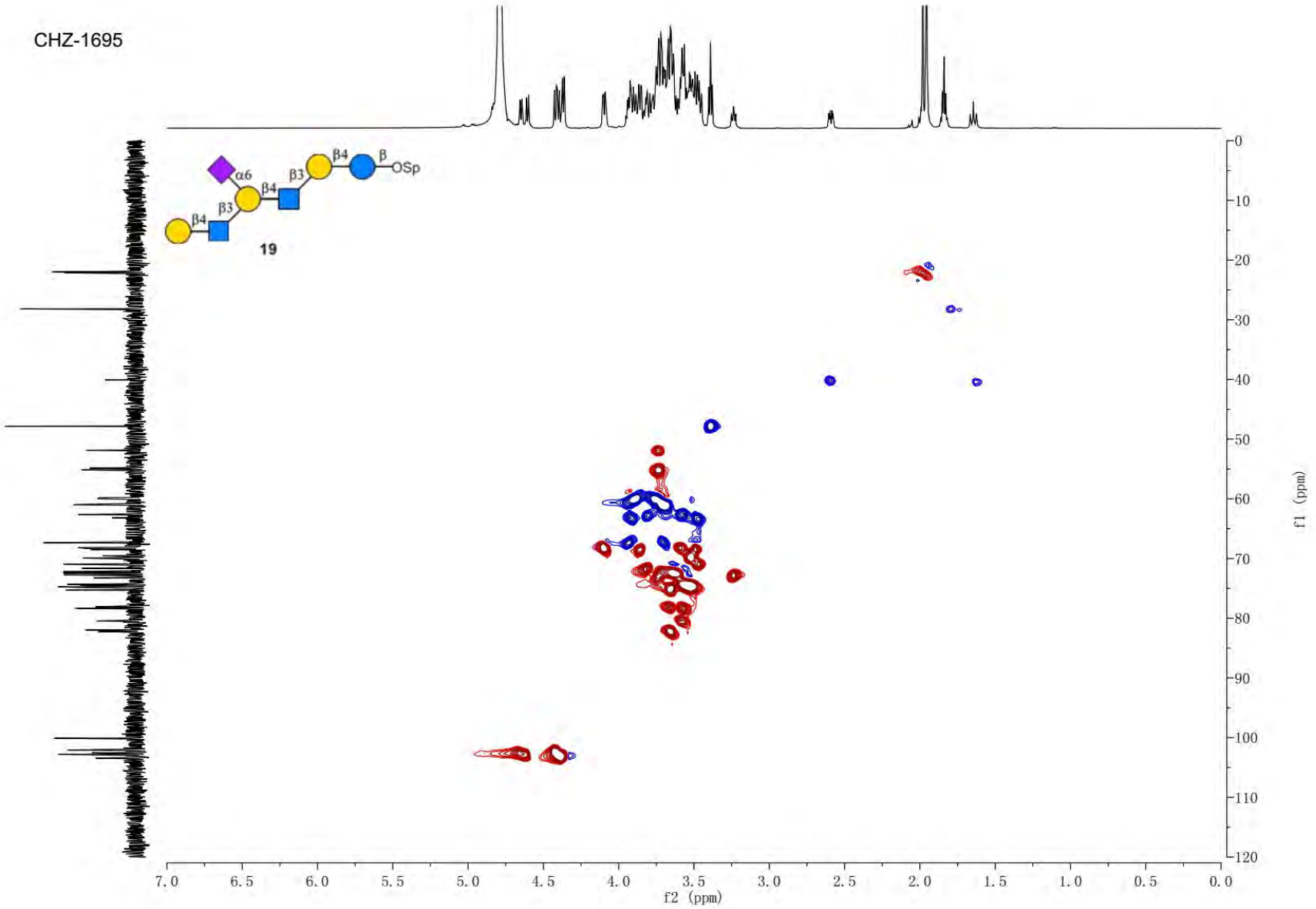
^{13}C NMR of 19



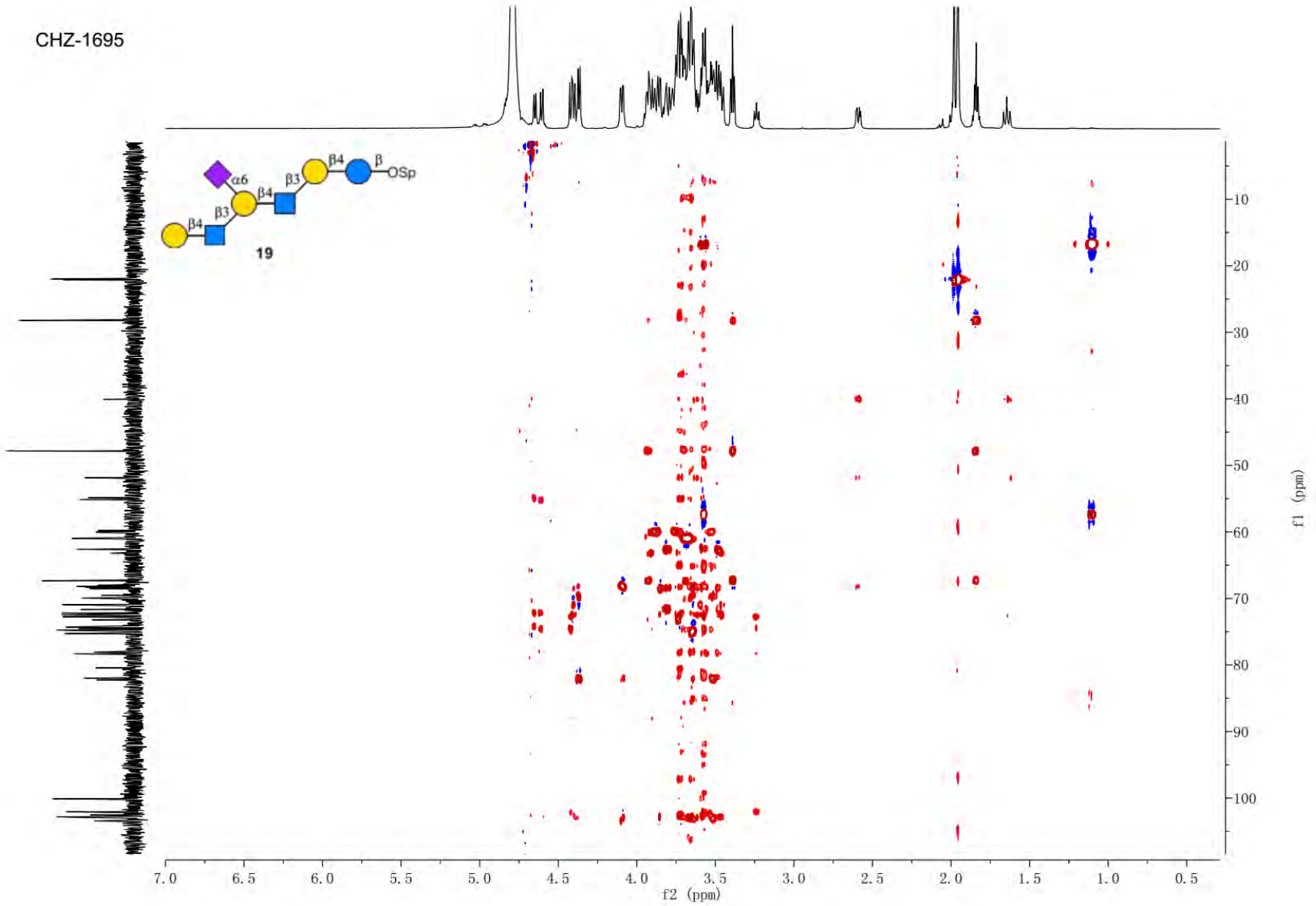
COSY spectra of 19



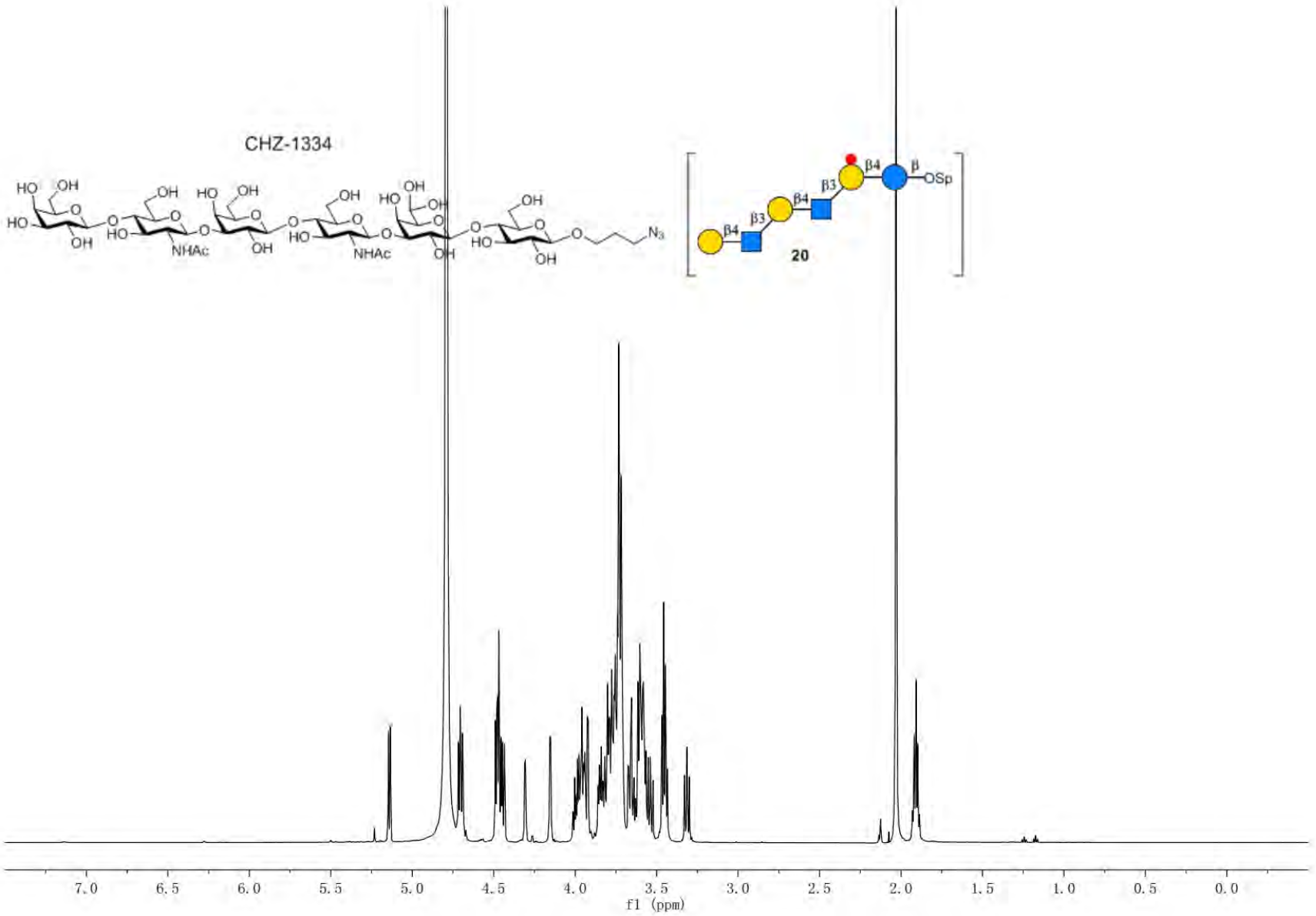
HMBC spectra of 19



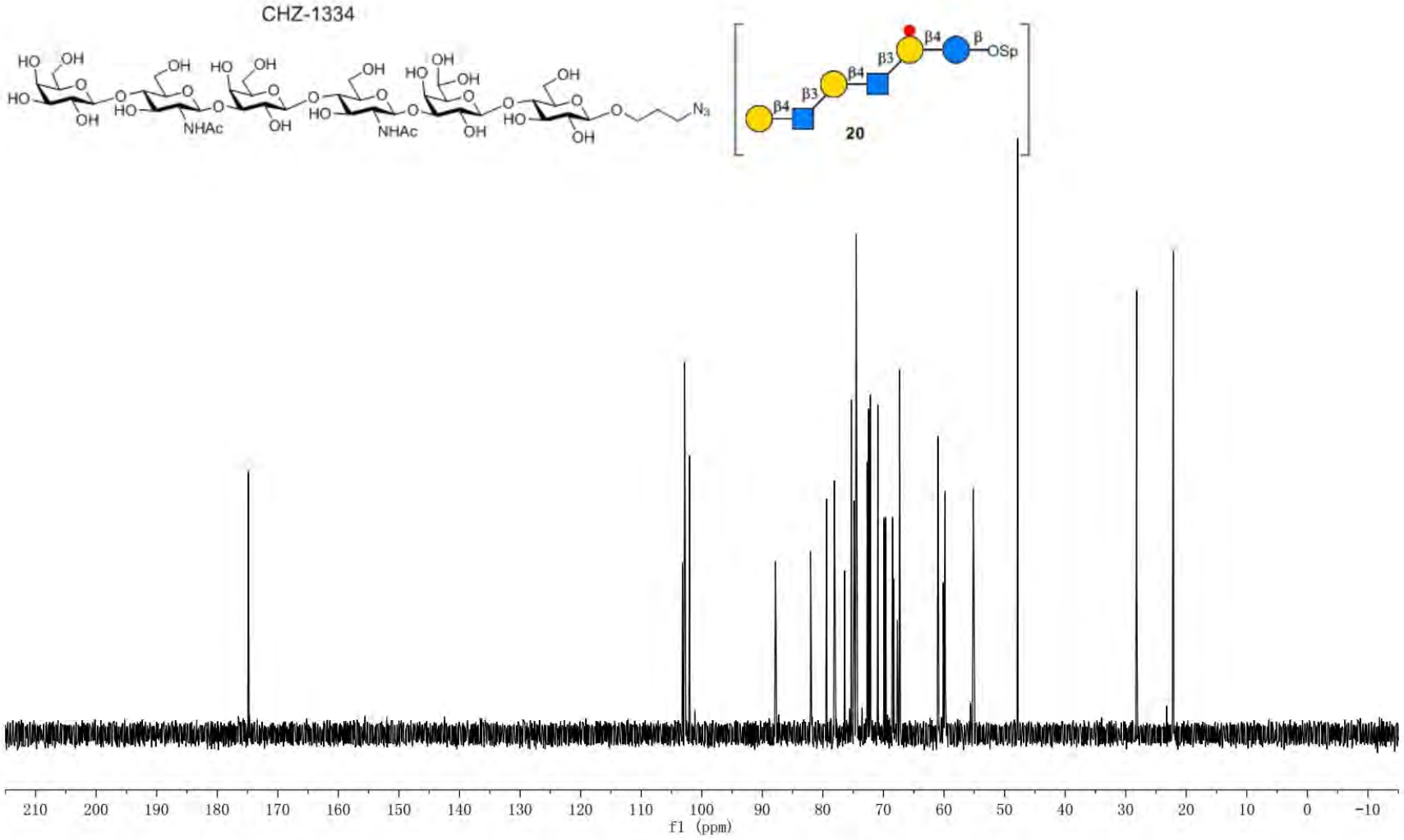
HSQC spectra of 19



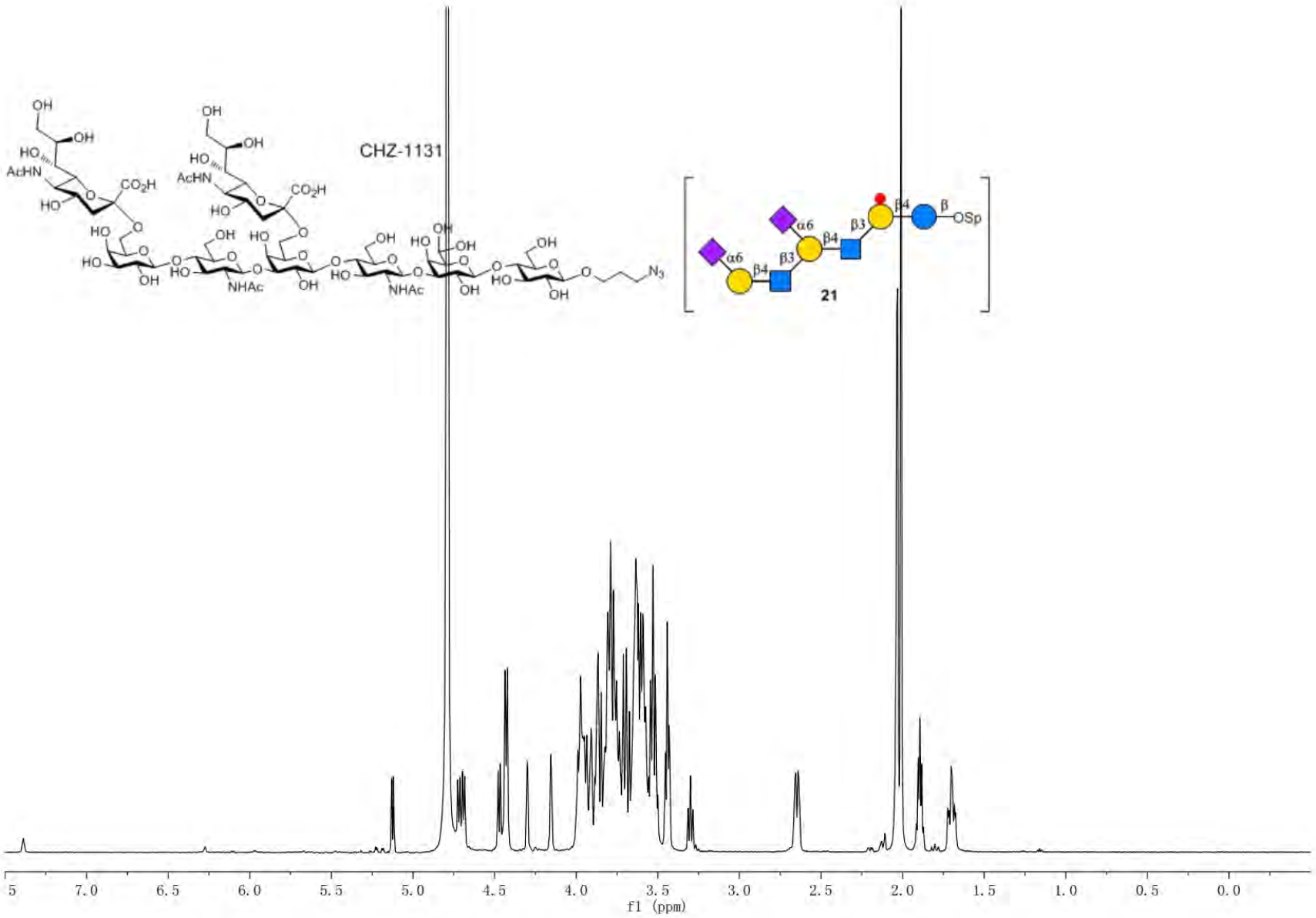
HSQC-TOCSY spectra of 19


CHZ-1334
20
β4
β3
β4
β3
β4
β
OSp
NHAc
NHAc
N3
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

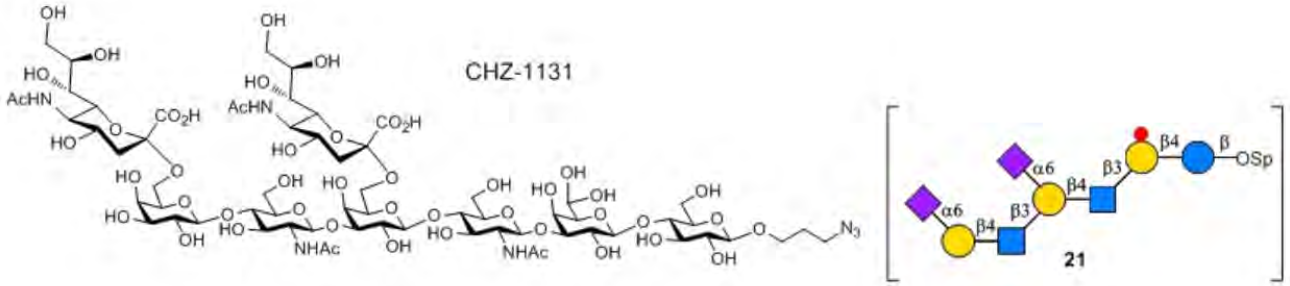
^{1}H NMR of 20

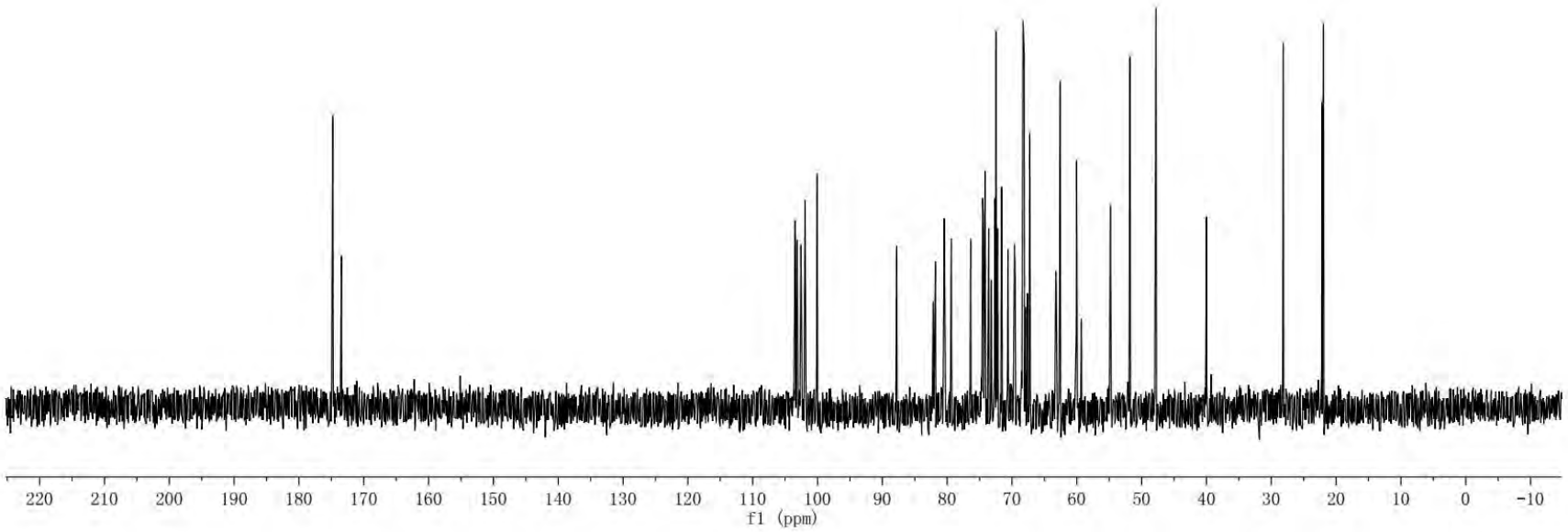

CHZ-1334
20
β4
β
OSp
β3
β4
β3
β4
NHAc
NHAc
N3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

^{13}C NMR of 20

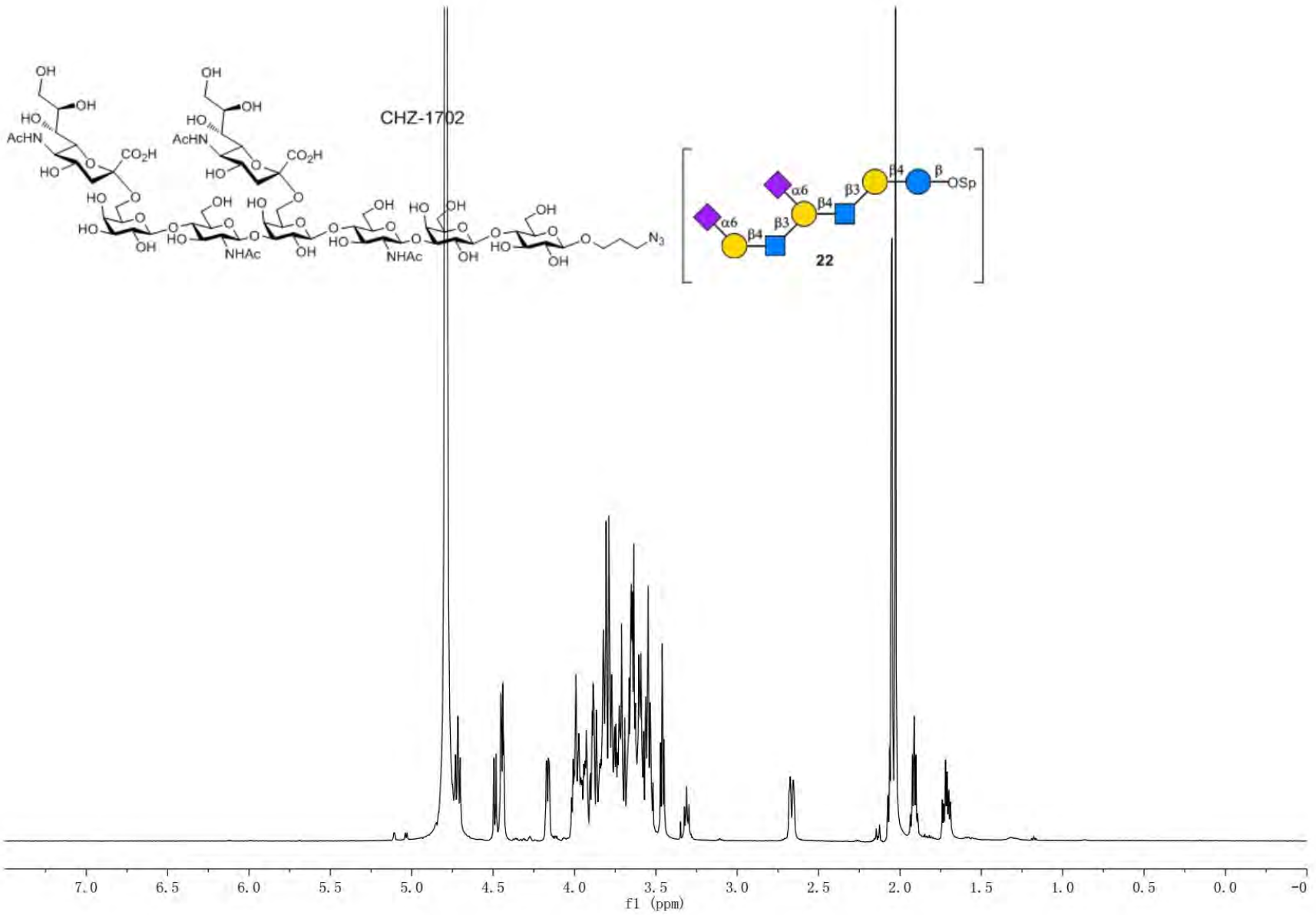


^{1}H NMR of 21

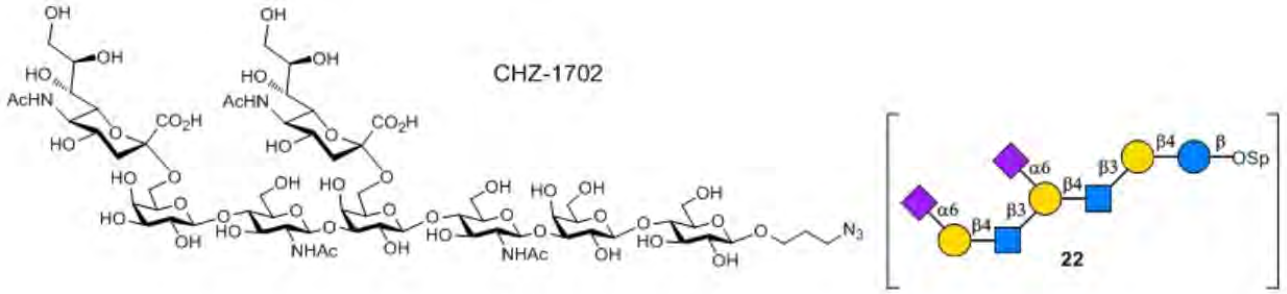

CHZ-1131
21

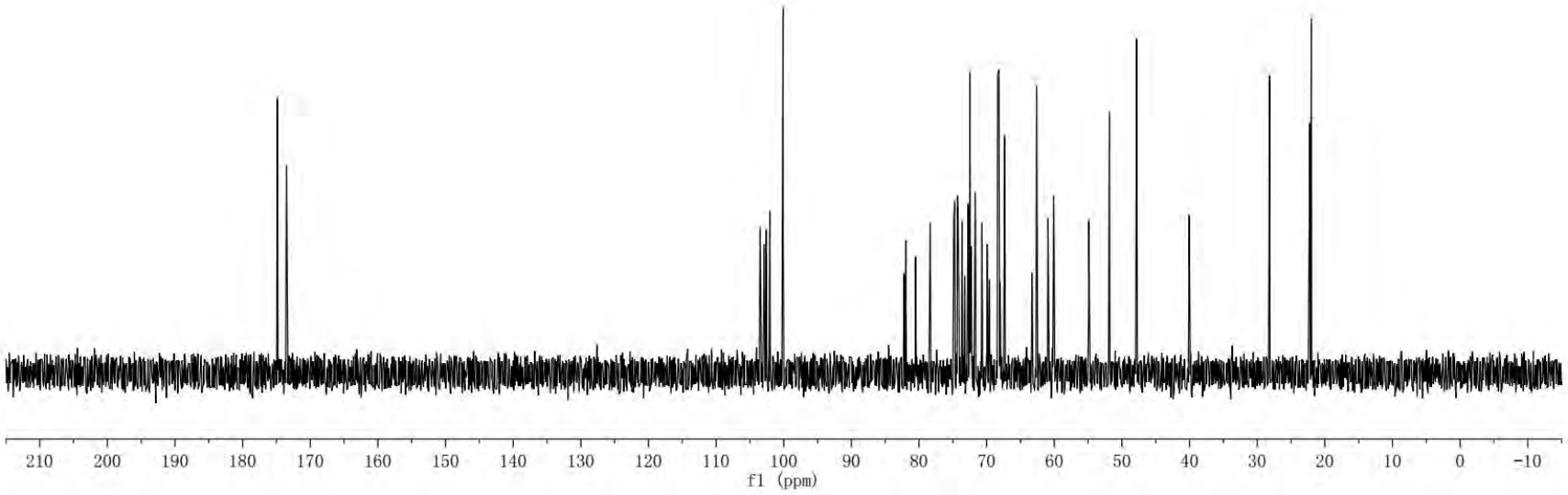

220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{13}C NMR of 21

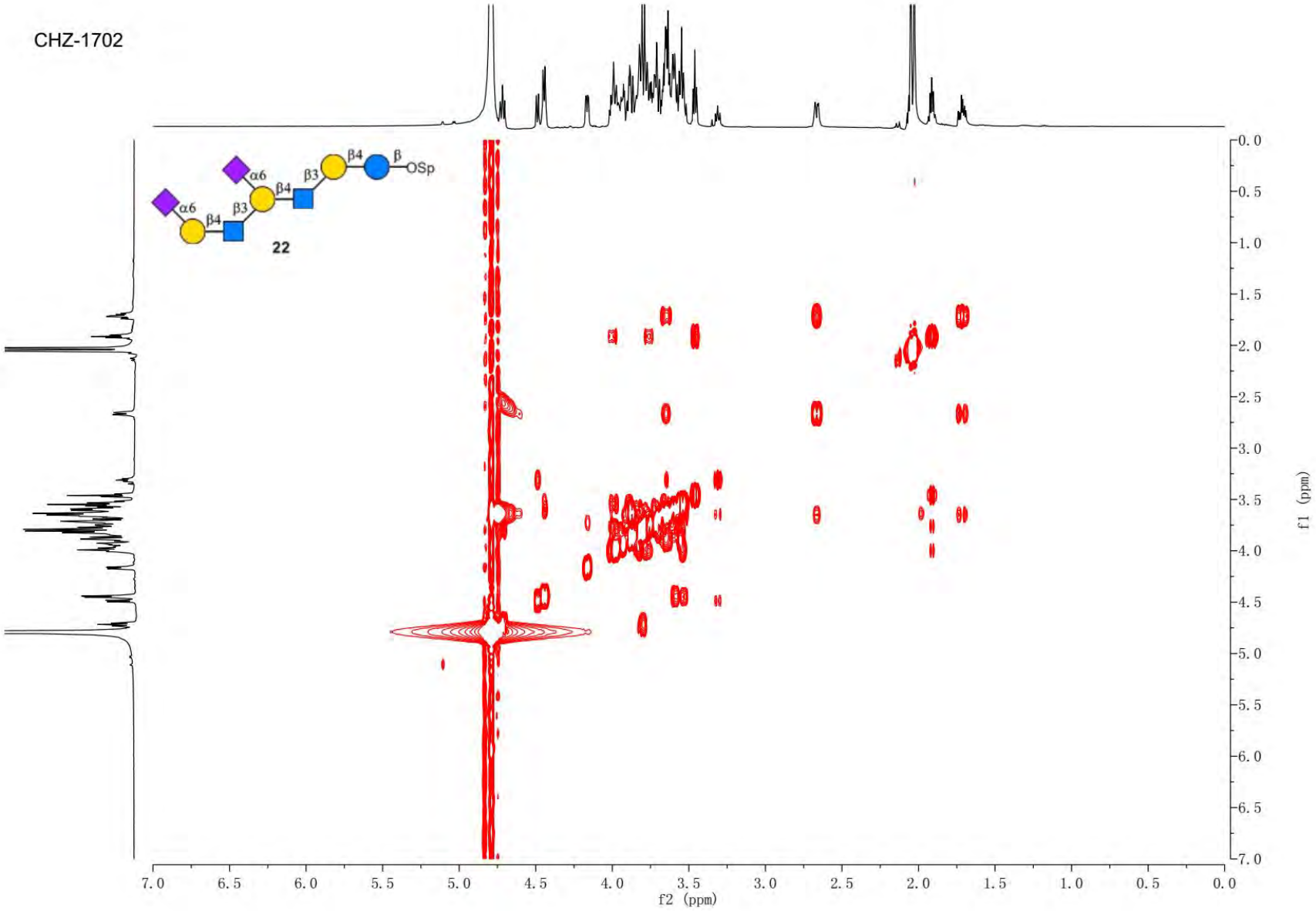


^{1}H NMR of 22

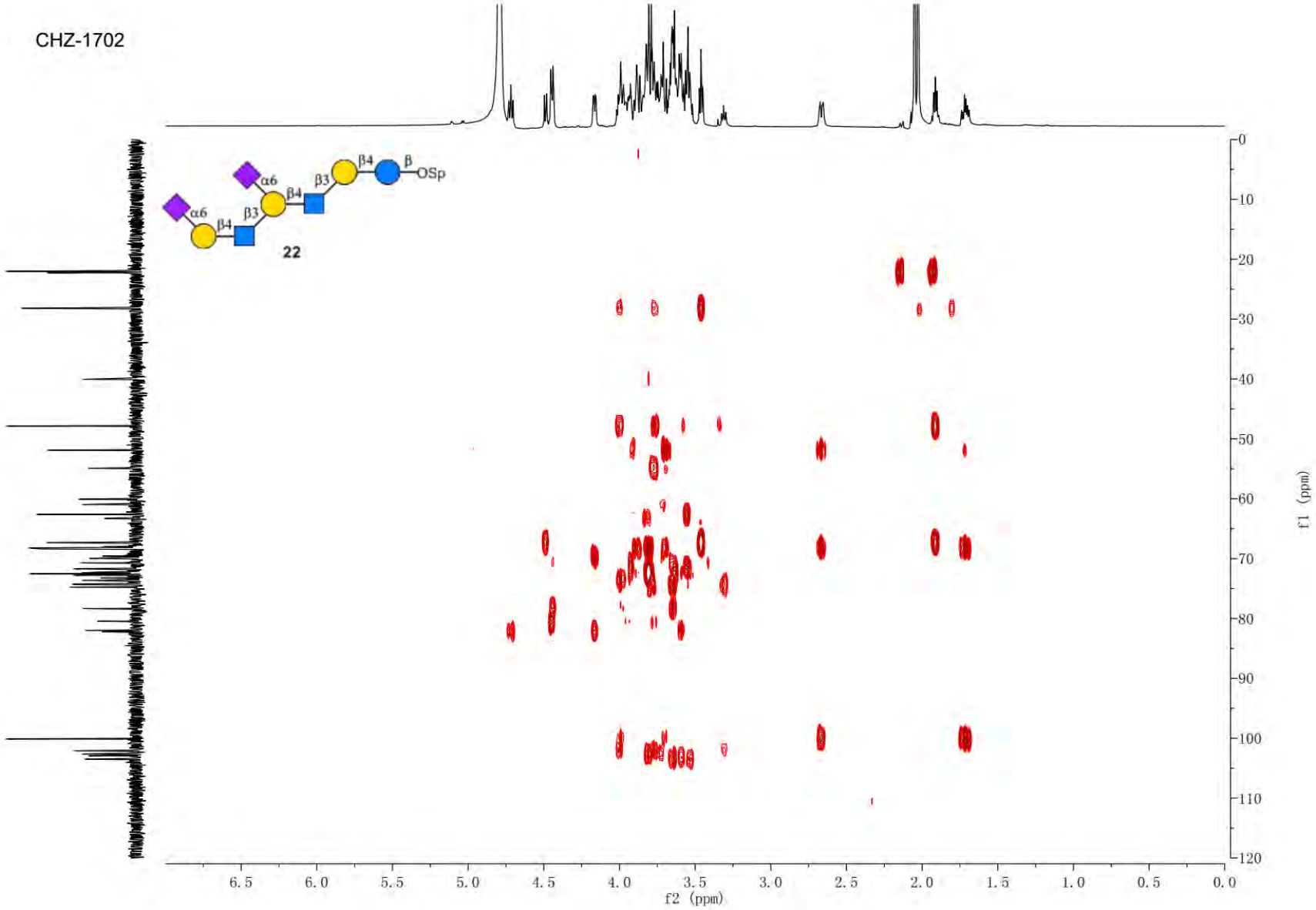

CHZ-1702
22


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

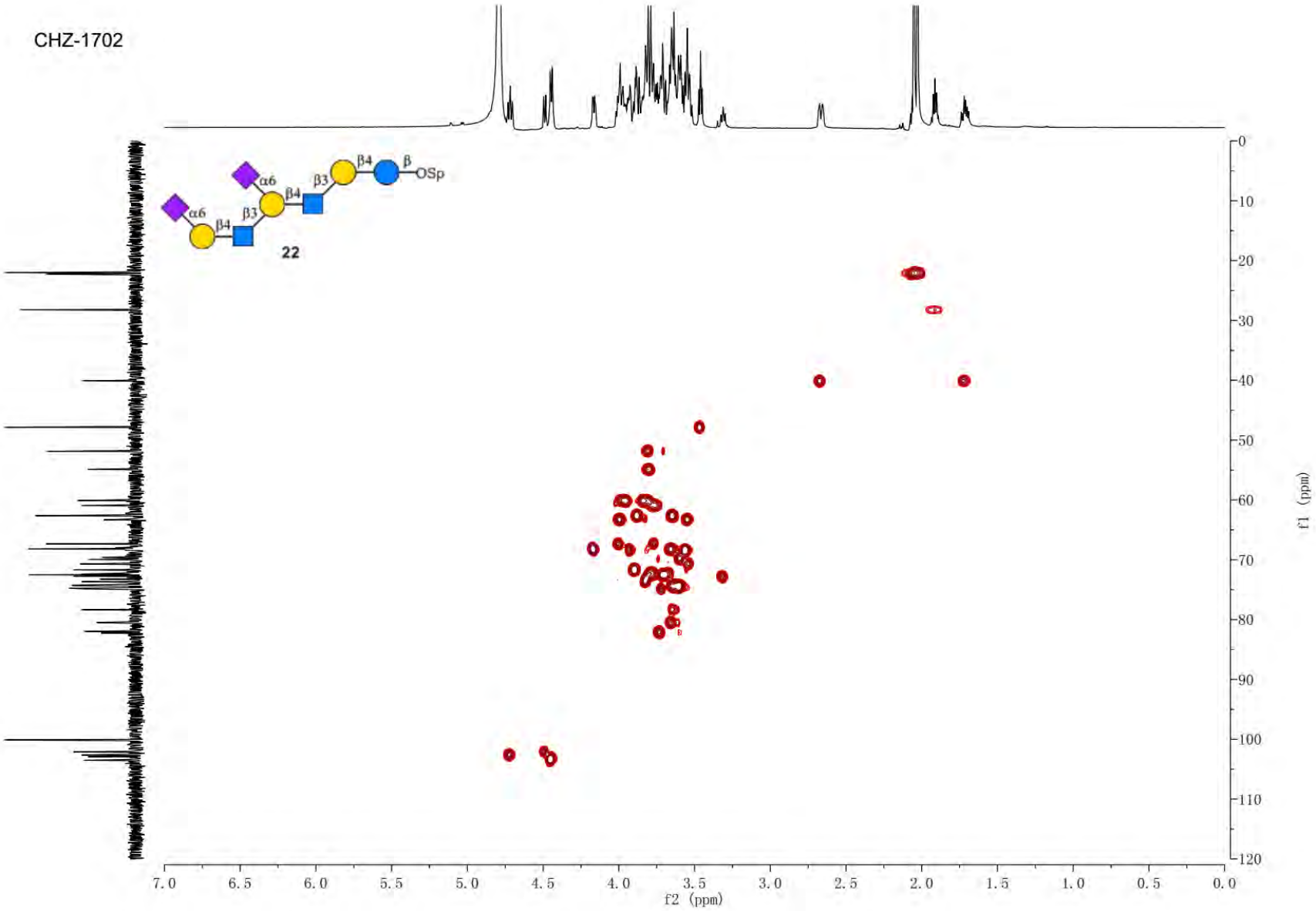
^{13}C NMR of 22



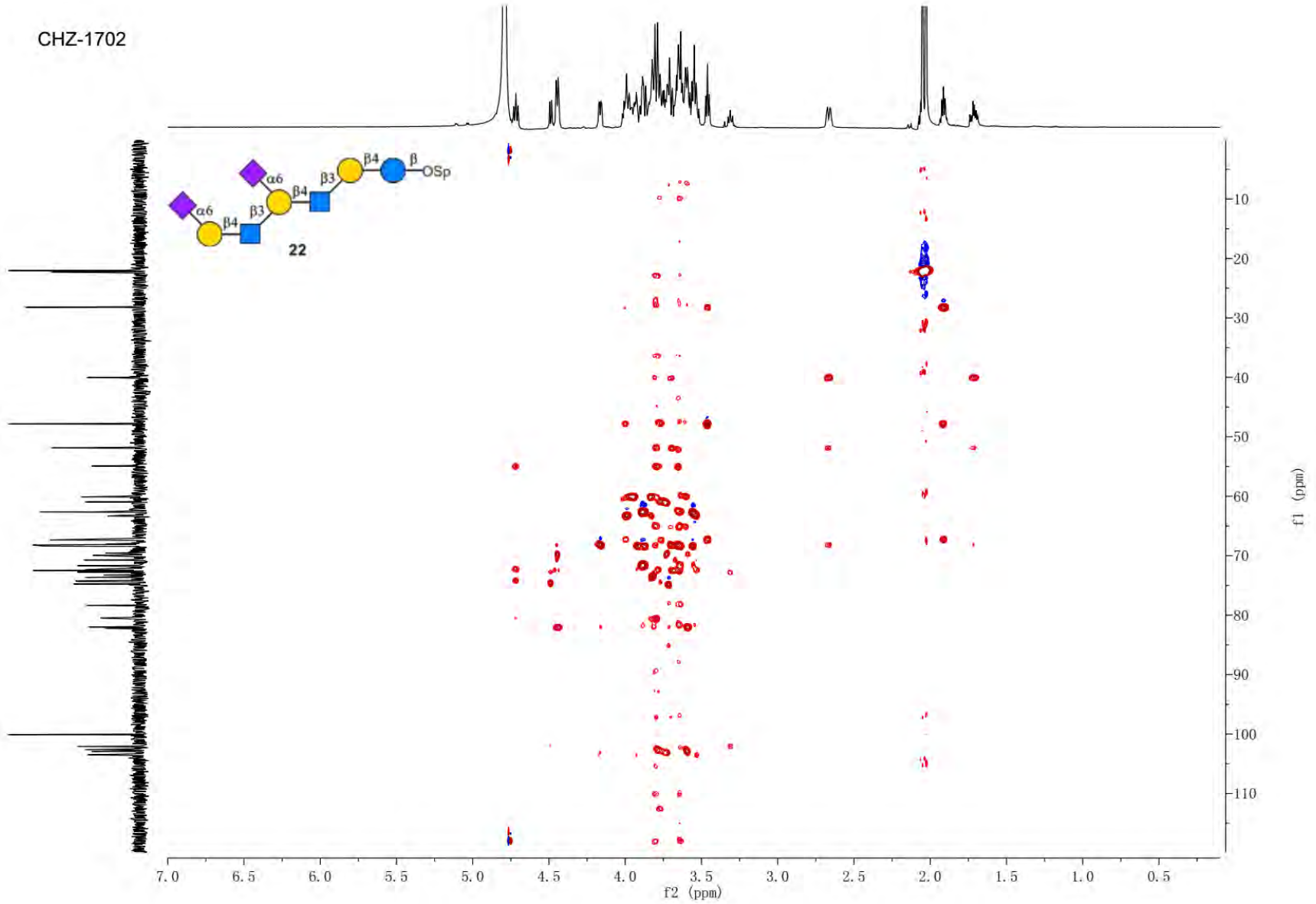
COSY spectra of 22



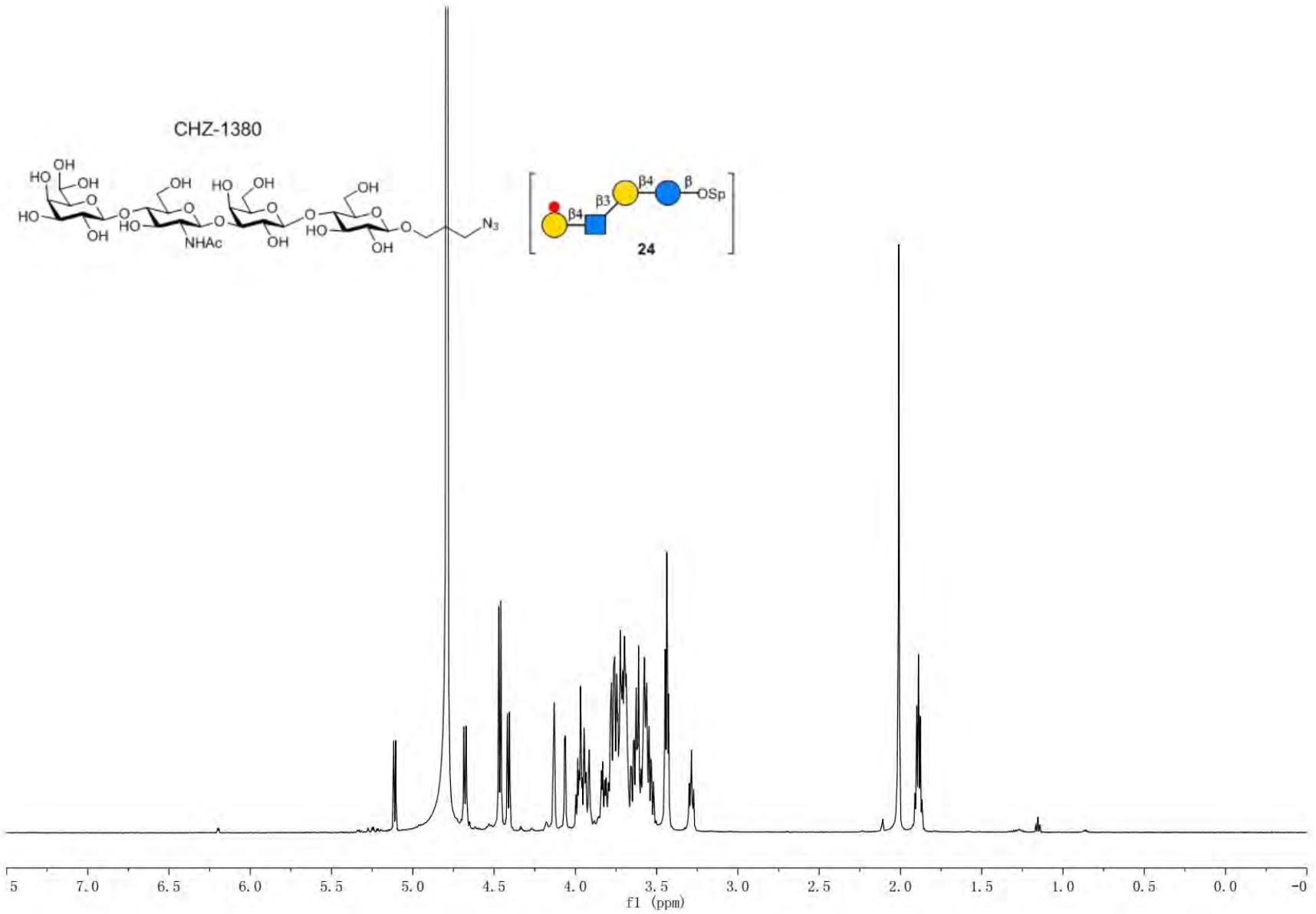
HMBC spectra of 22



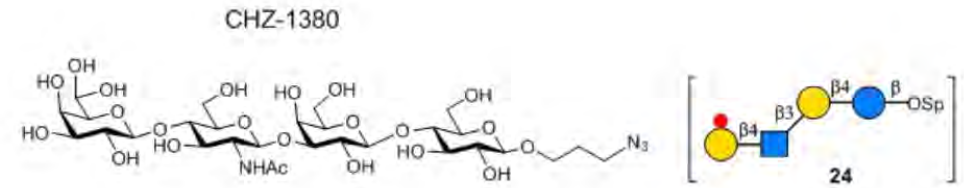
HSQC spectra of 22

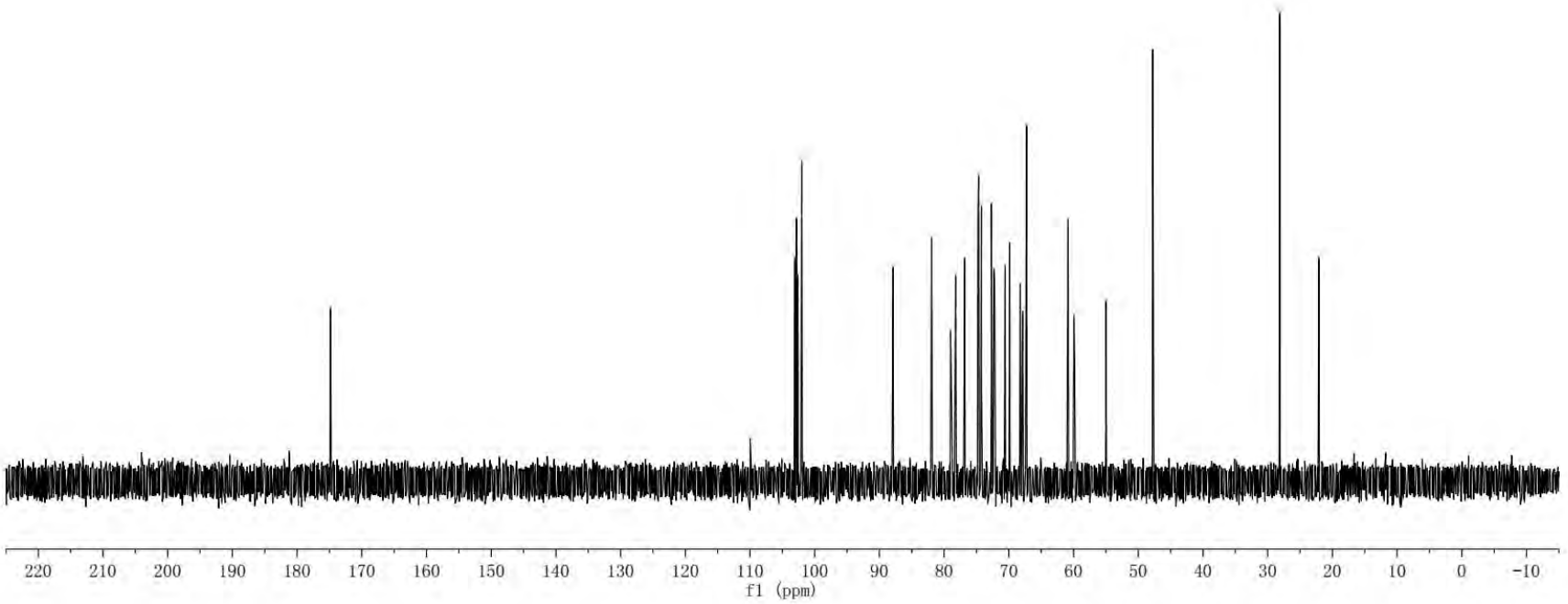


HSQC-TOCSY spectra of 22

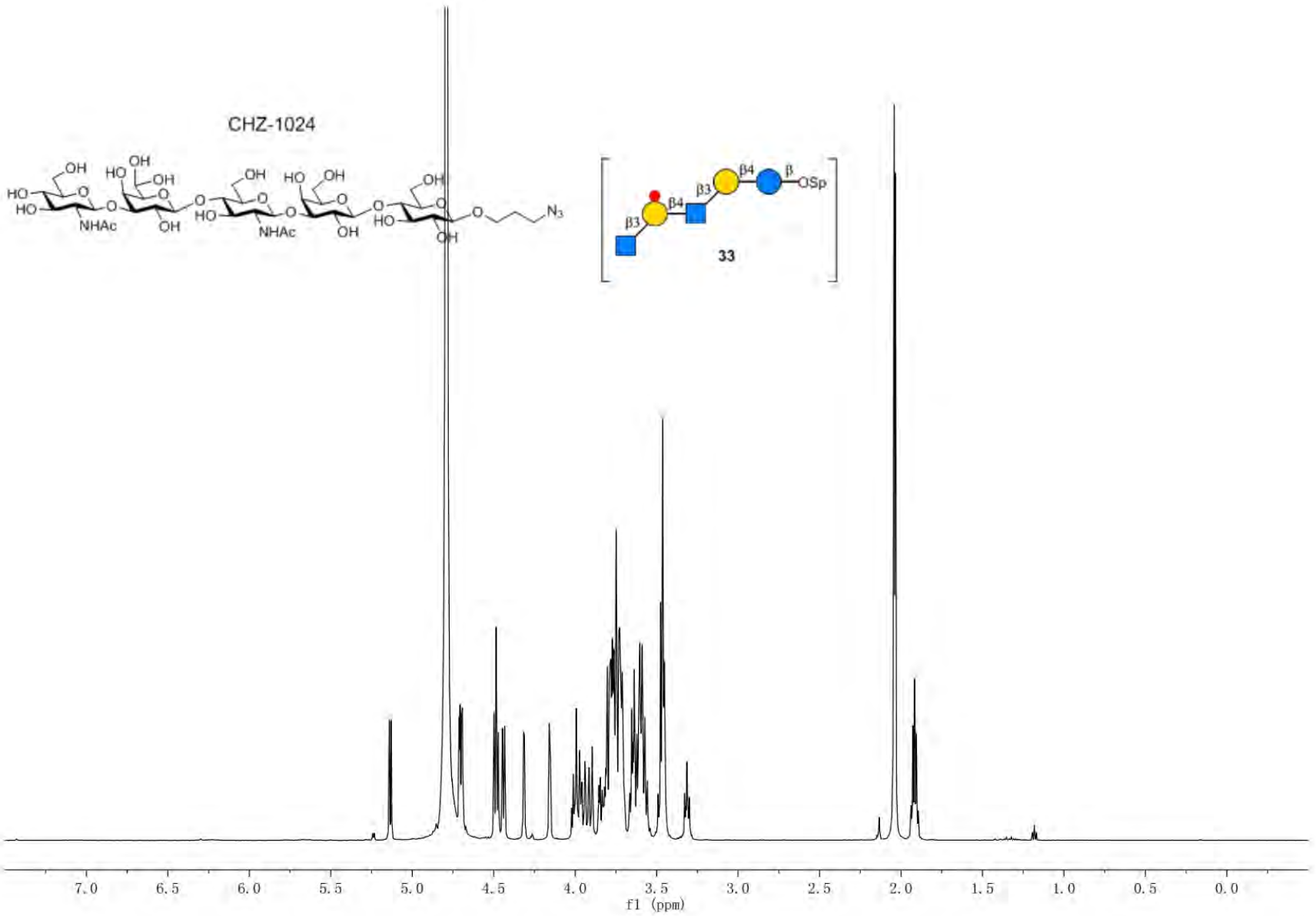


^{1}H NMR of 24

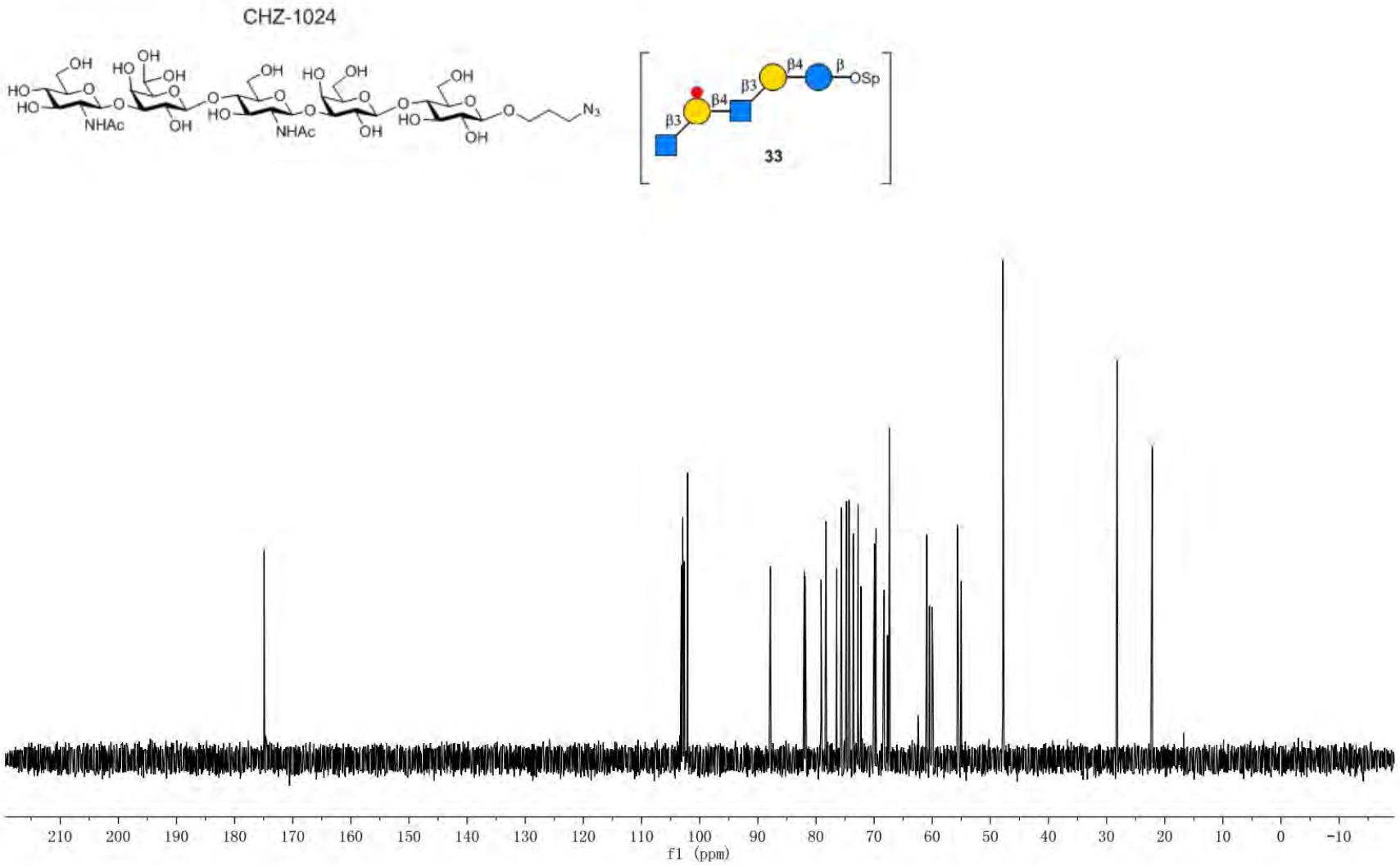

CHZ-1380
24


220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

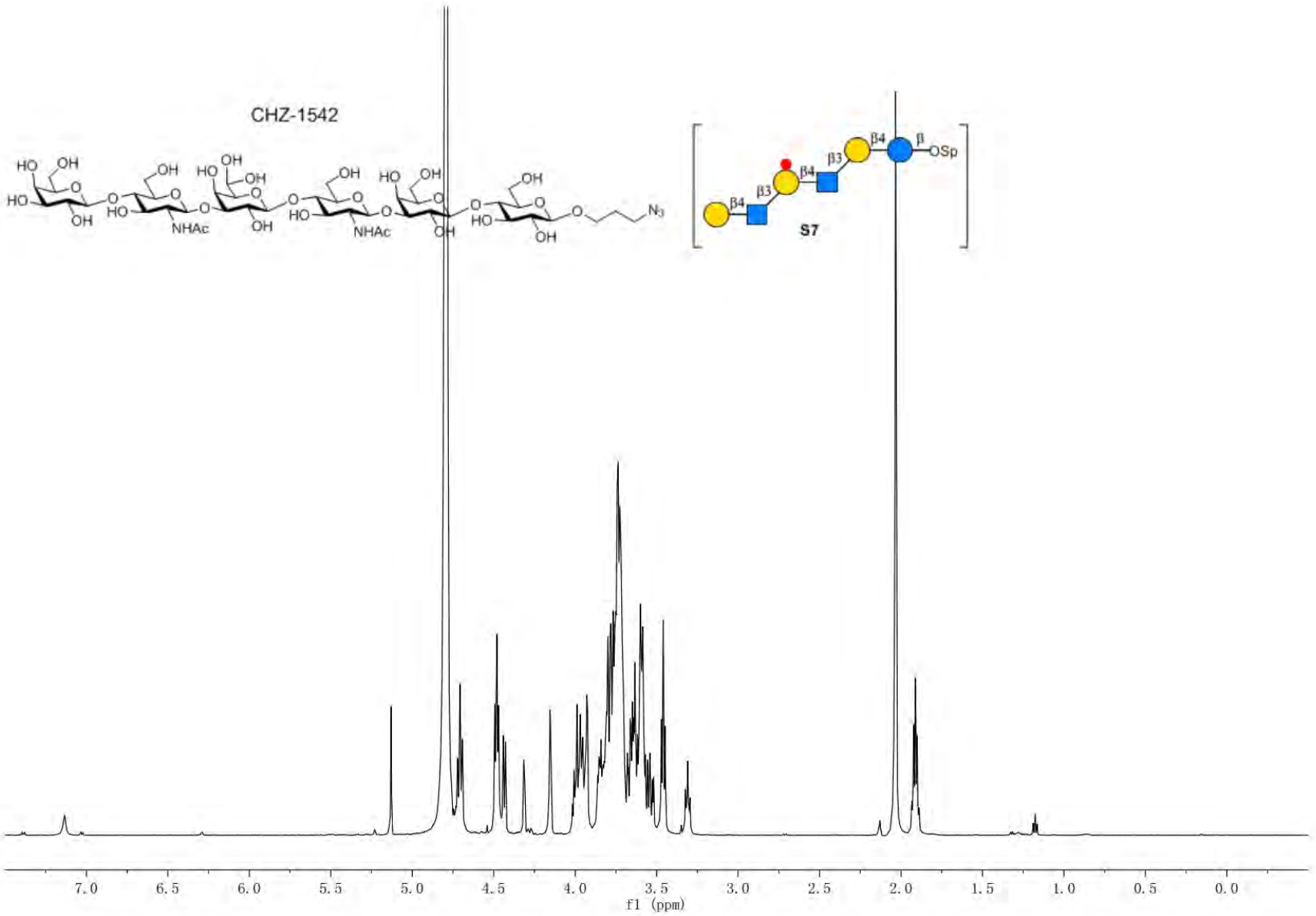
^{13}C NMR of 24



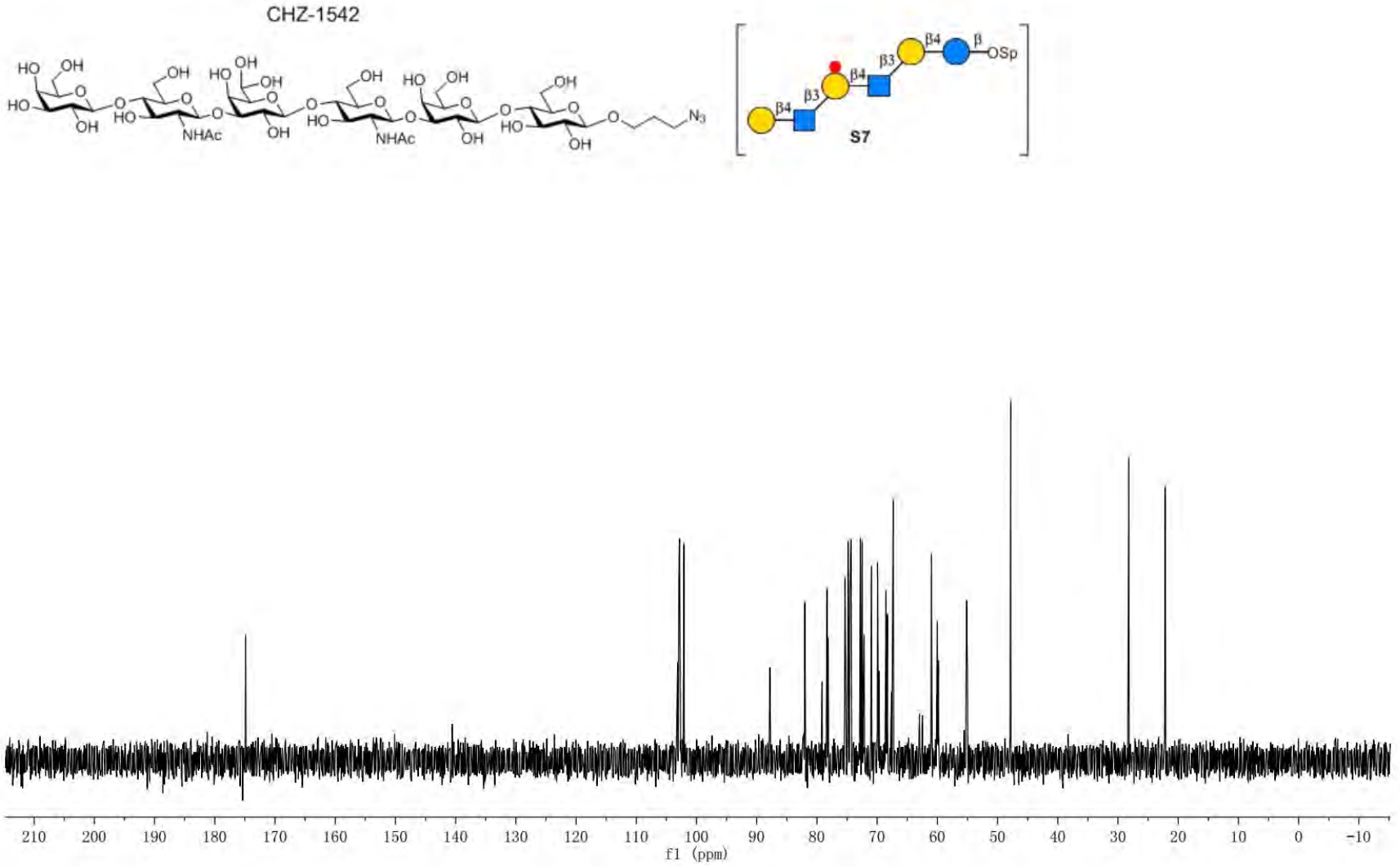
^{1}H NMR of 33



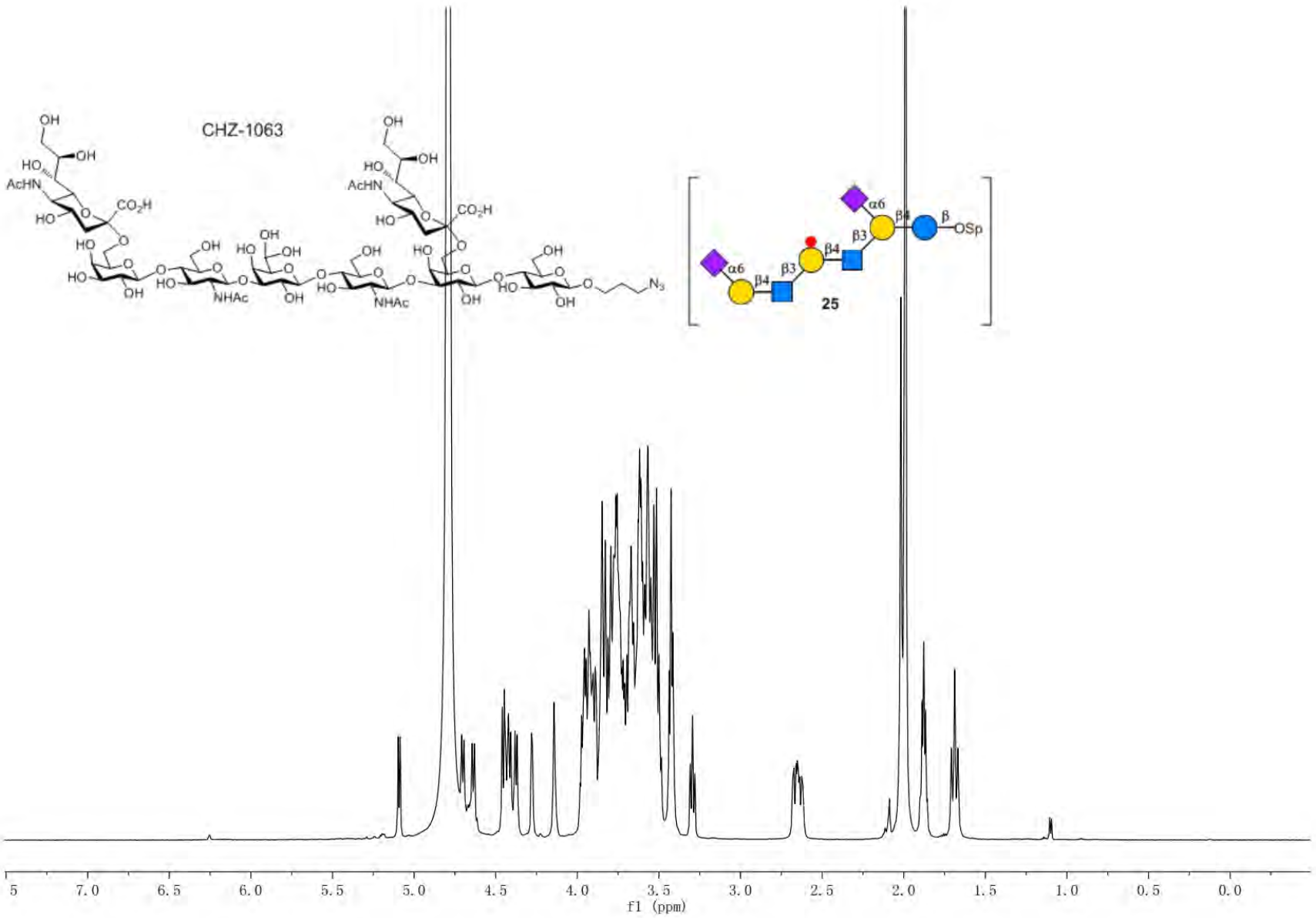
^{13}C NMR of 33



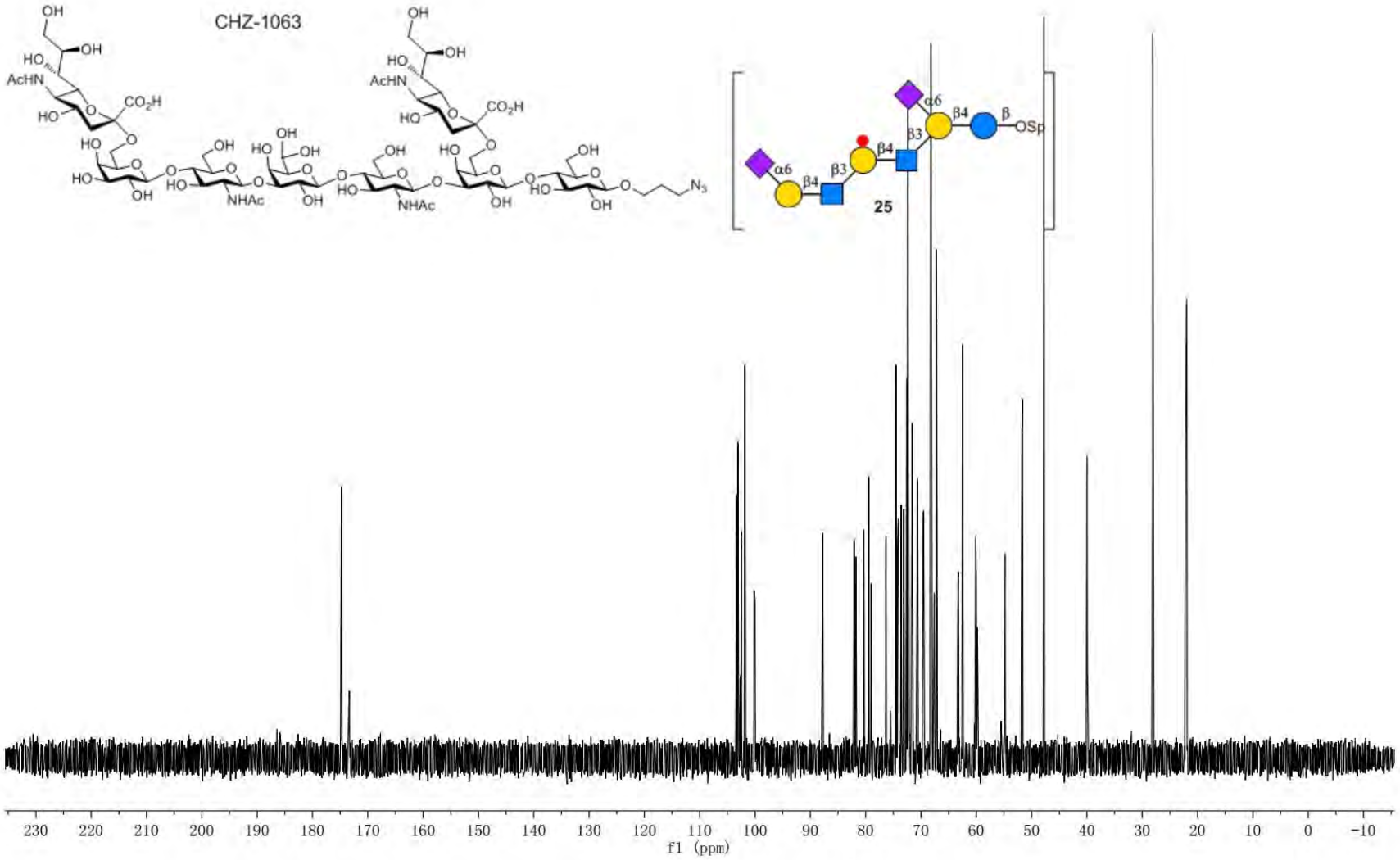
^{1}H NMR of S7



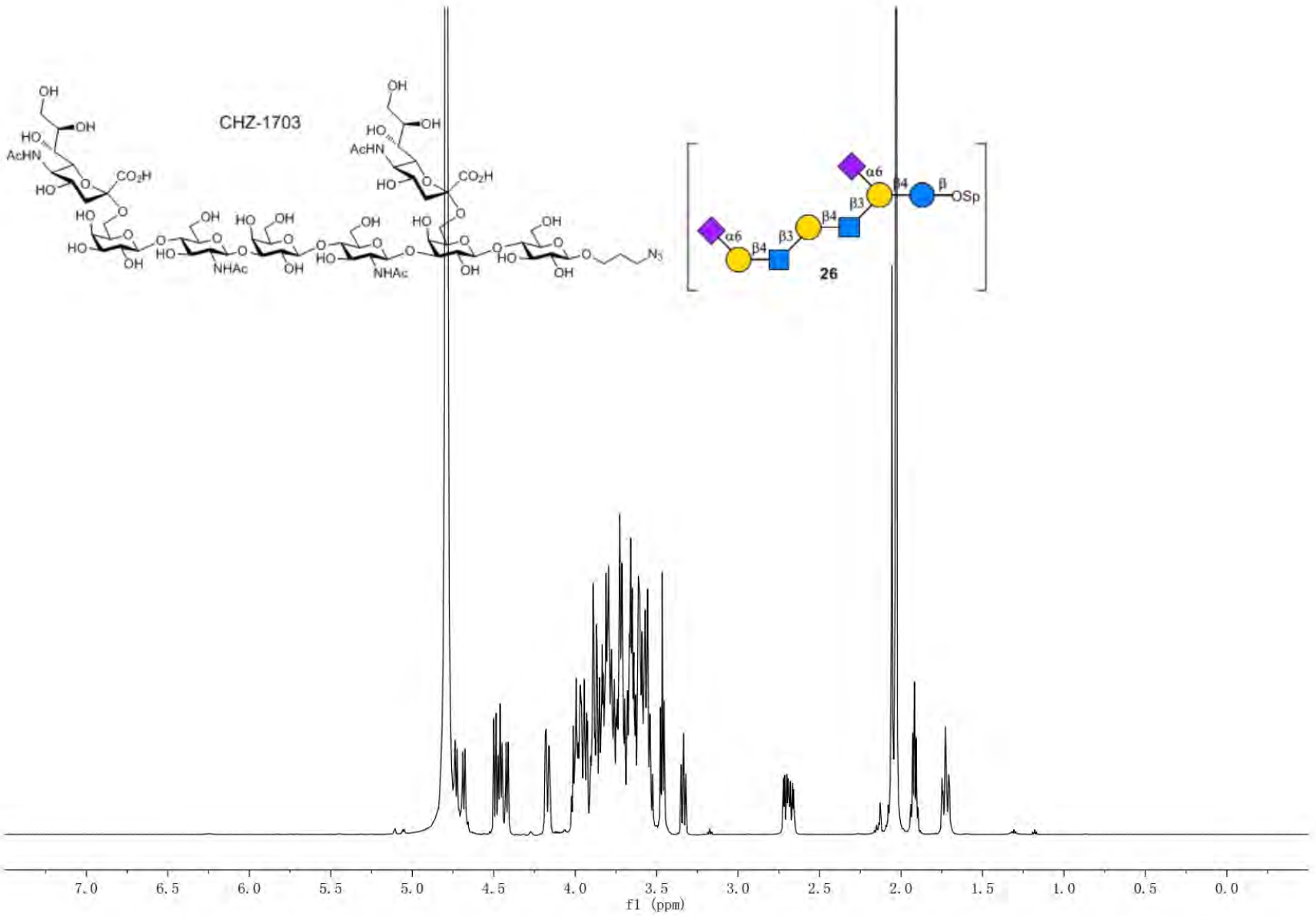
^{13}C NMR of S7



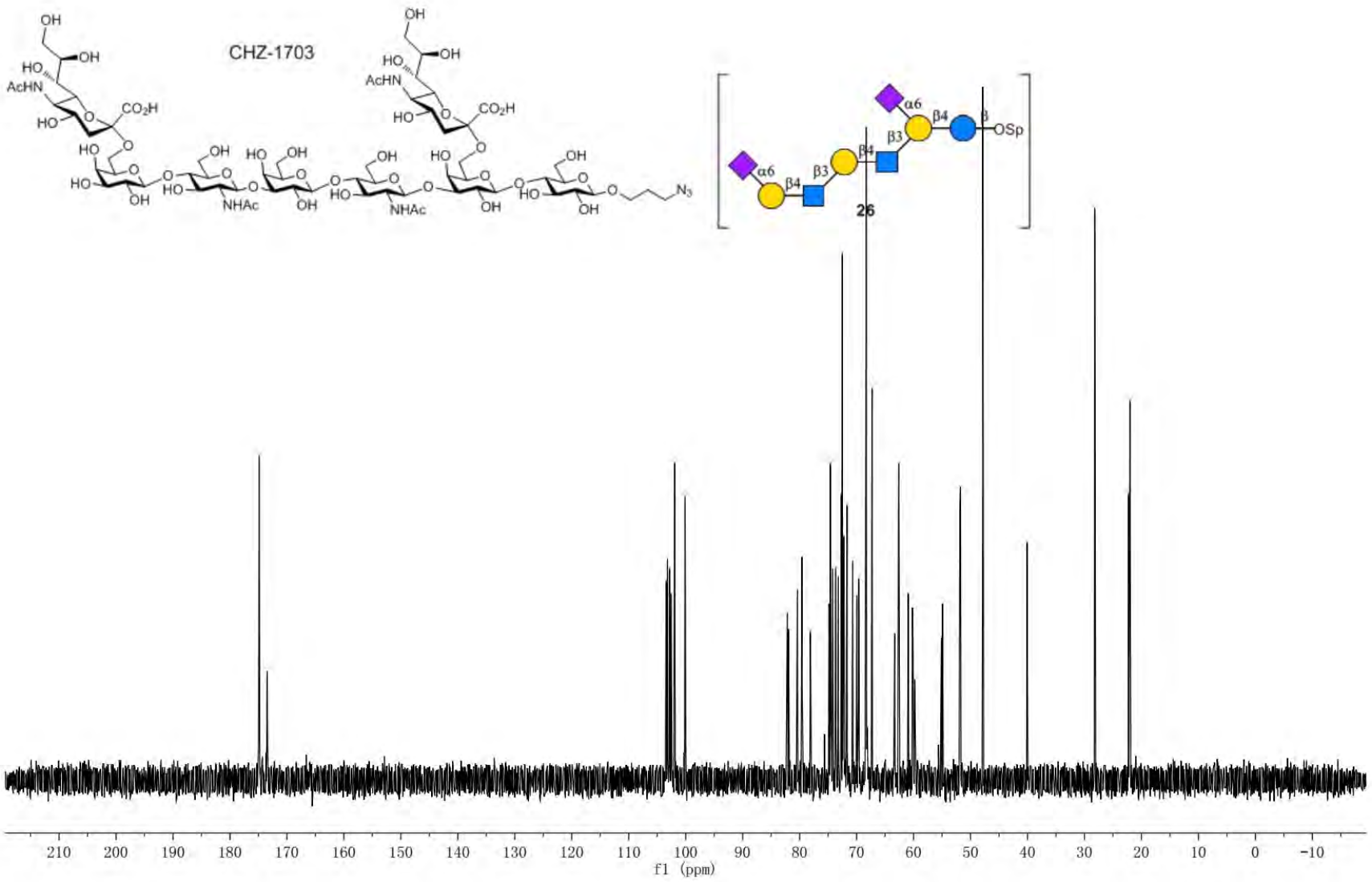
^{1}H NMR of 25



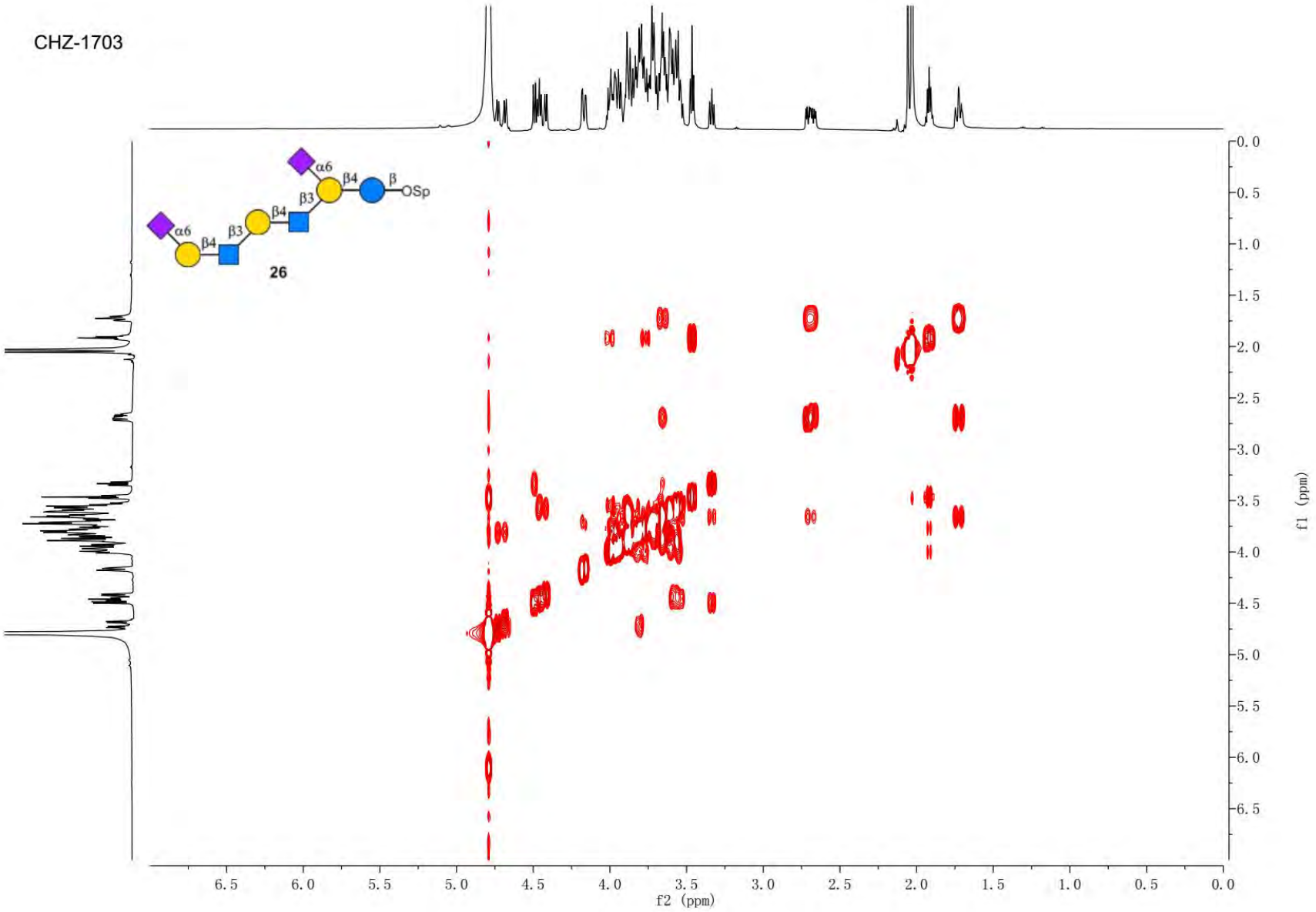
^{13}C NMR of 25



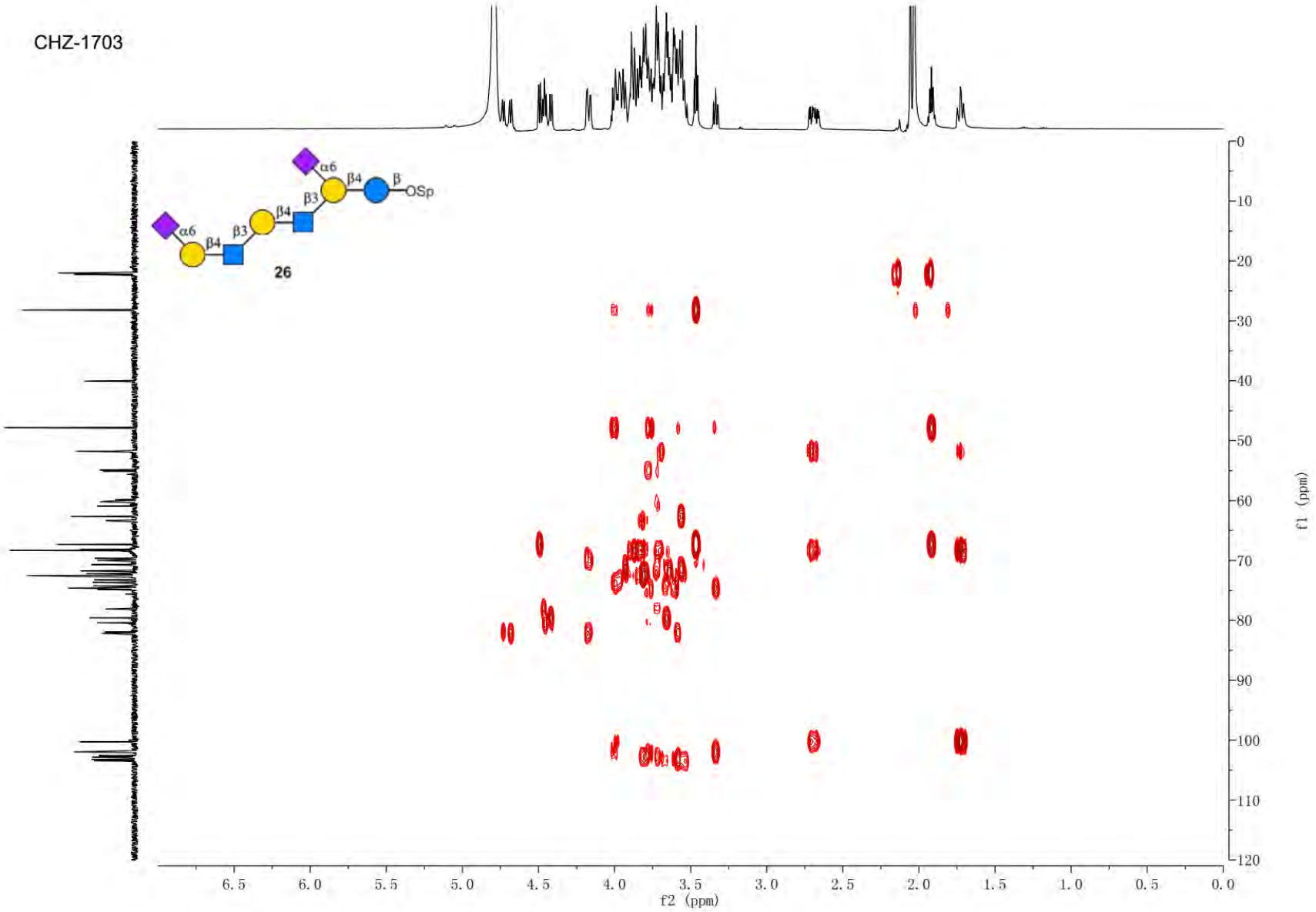
^{1}H NMR of 26



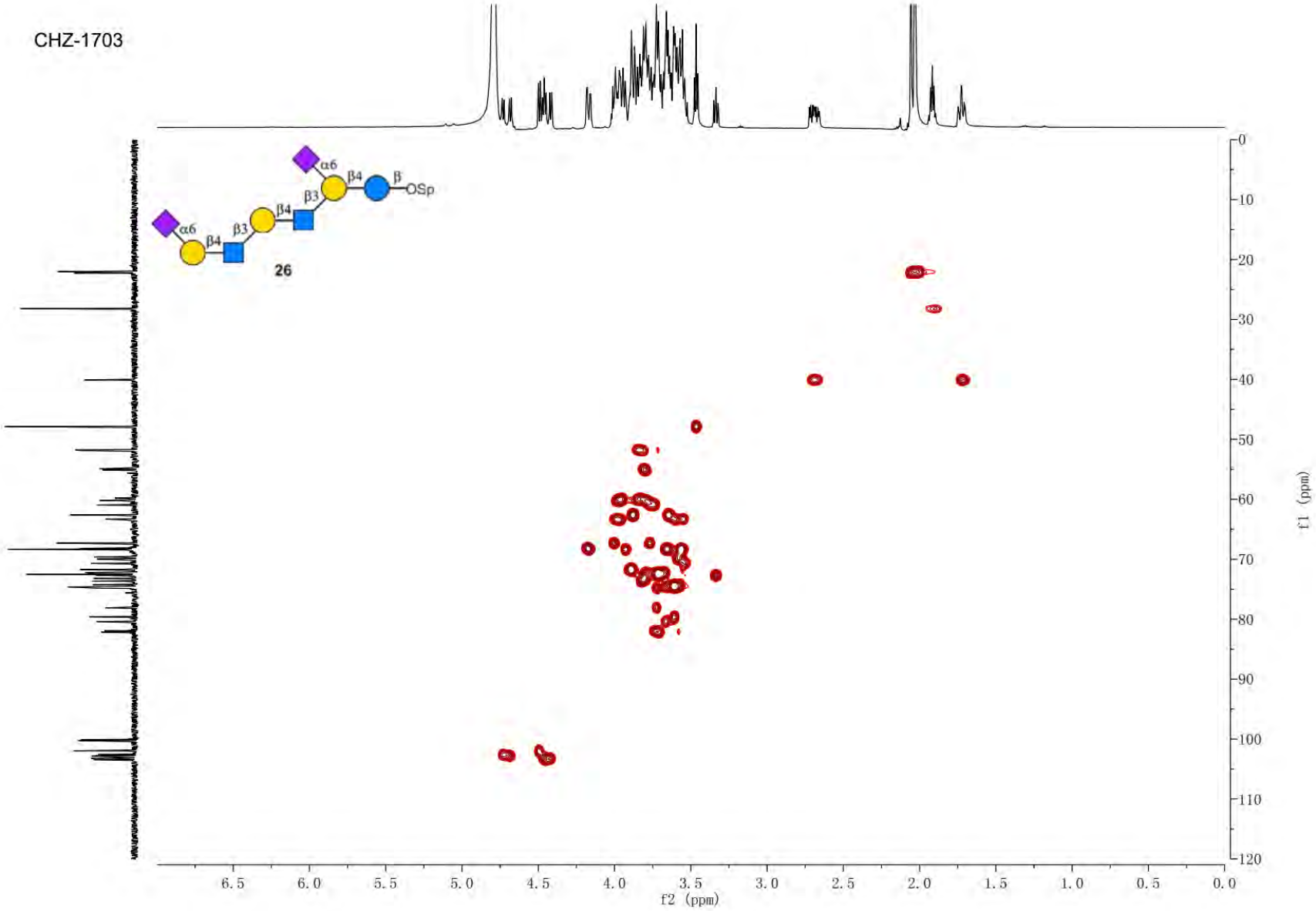
^{13}C NMR of 26



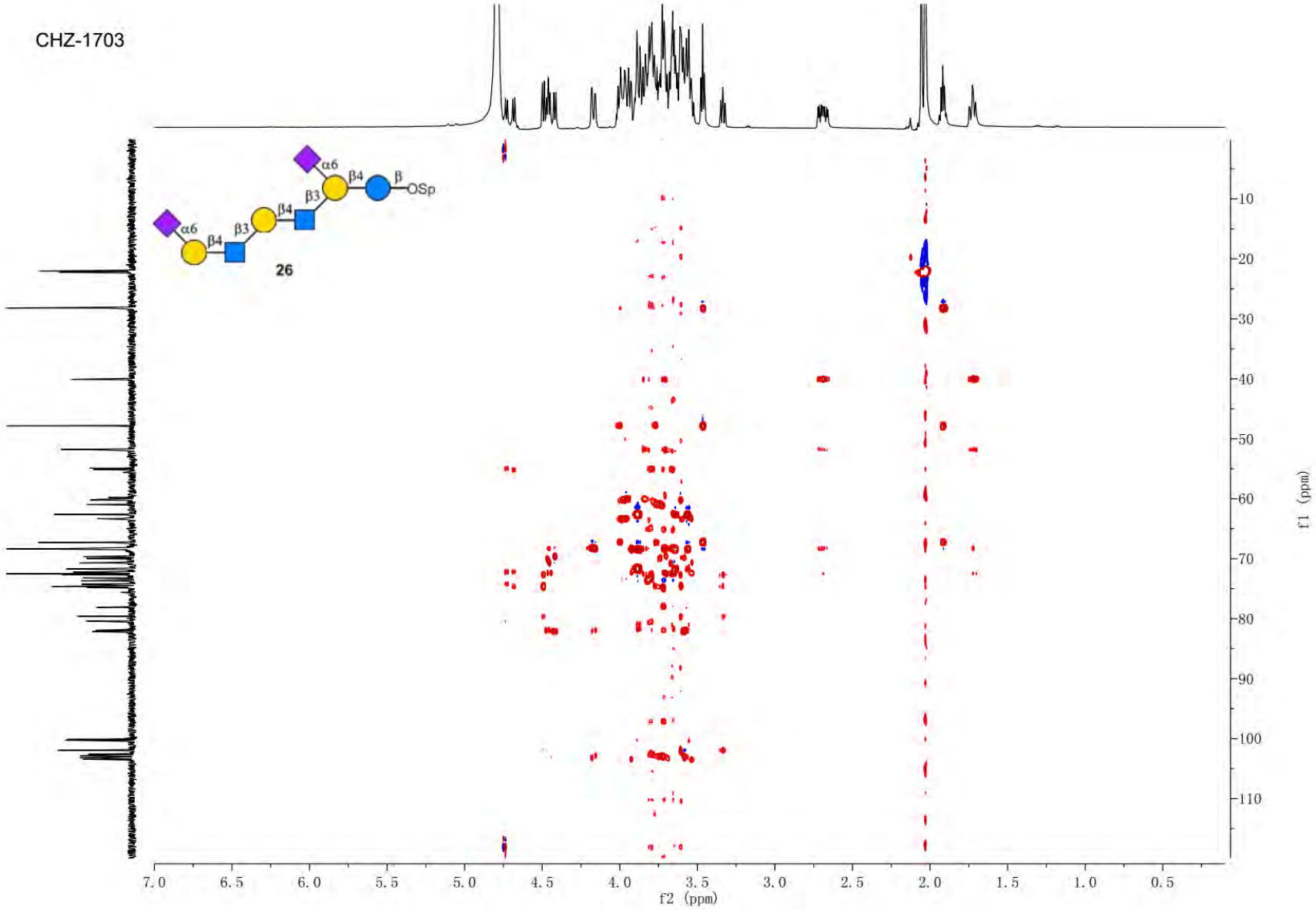
COSY spectra of 26



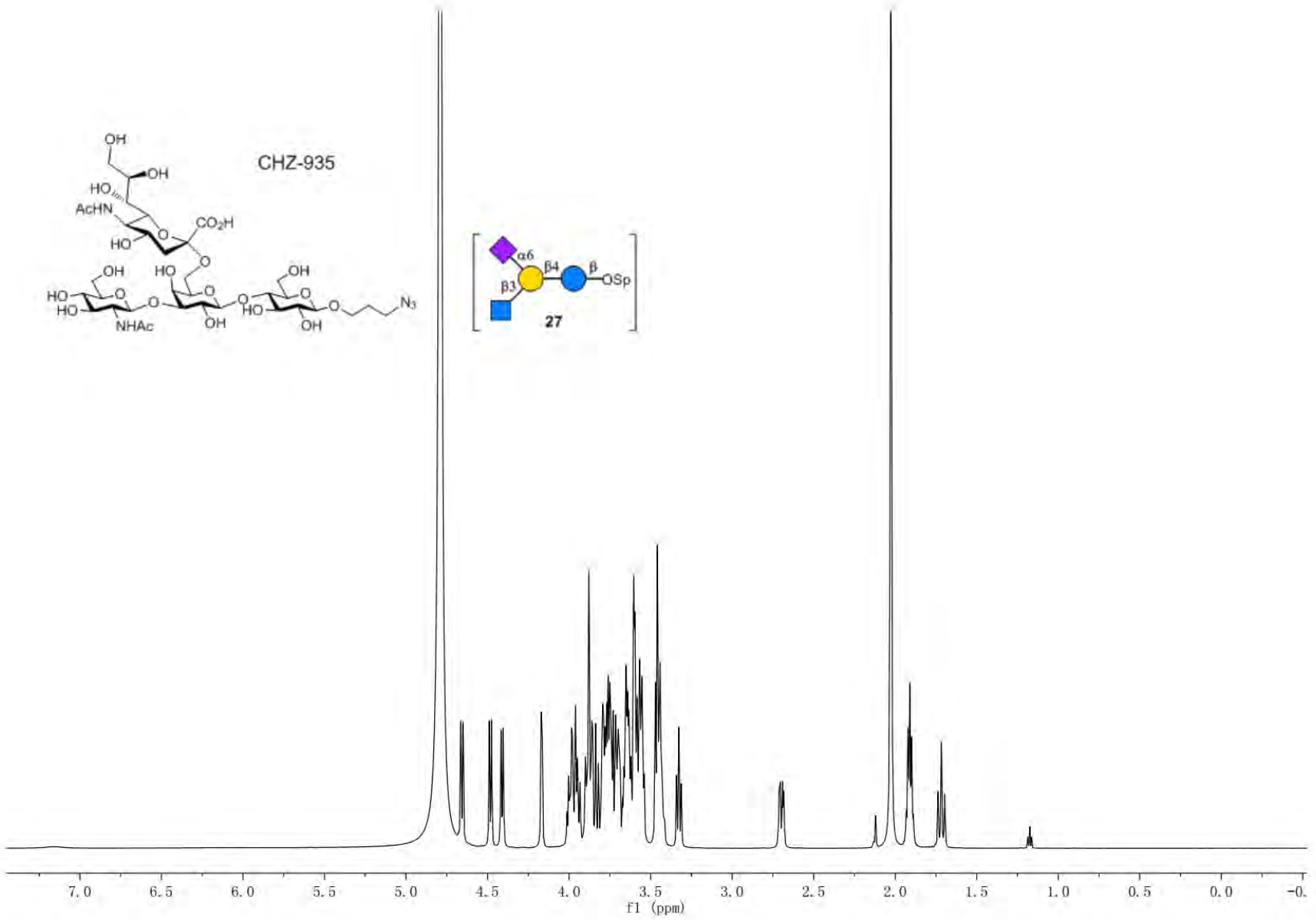
HMBC spectra of 26



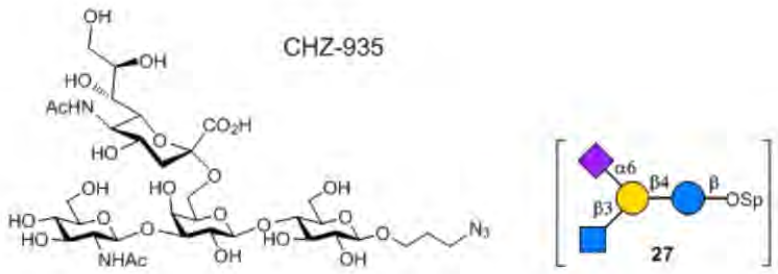
HSQC spectra of 26



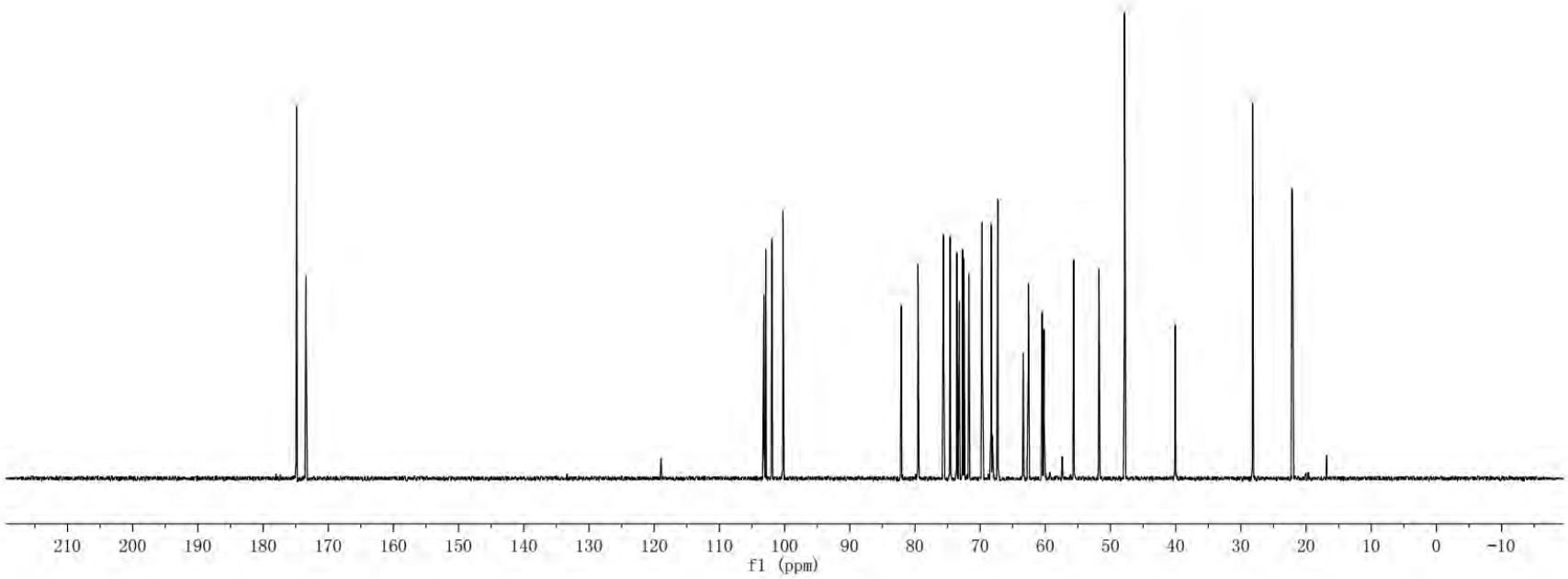
HSQC-TOCSY spectra of 26


CHZ-935
27
α6
β4
β
β3
OSp
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

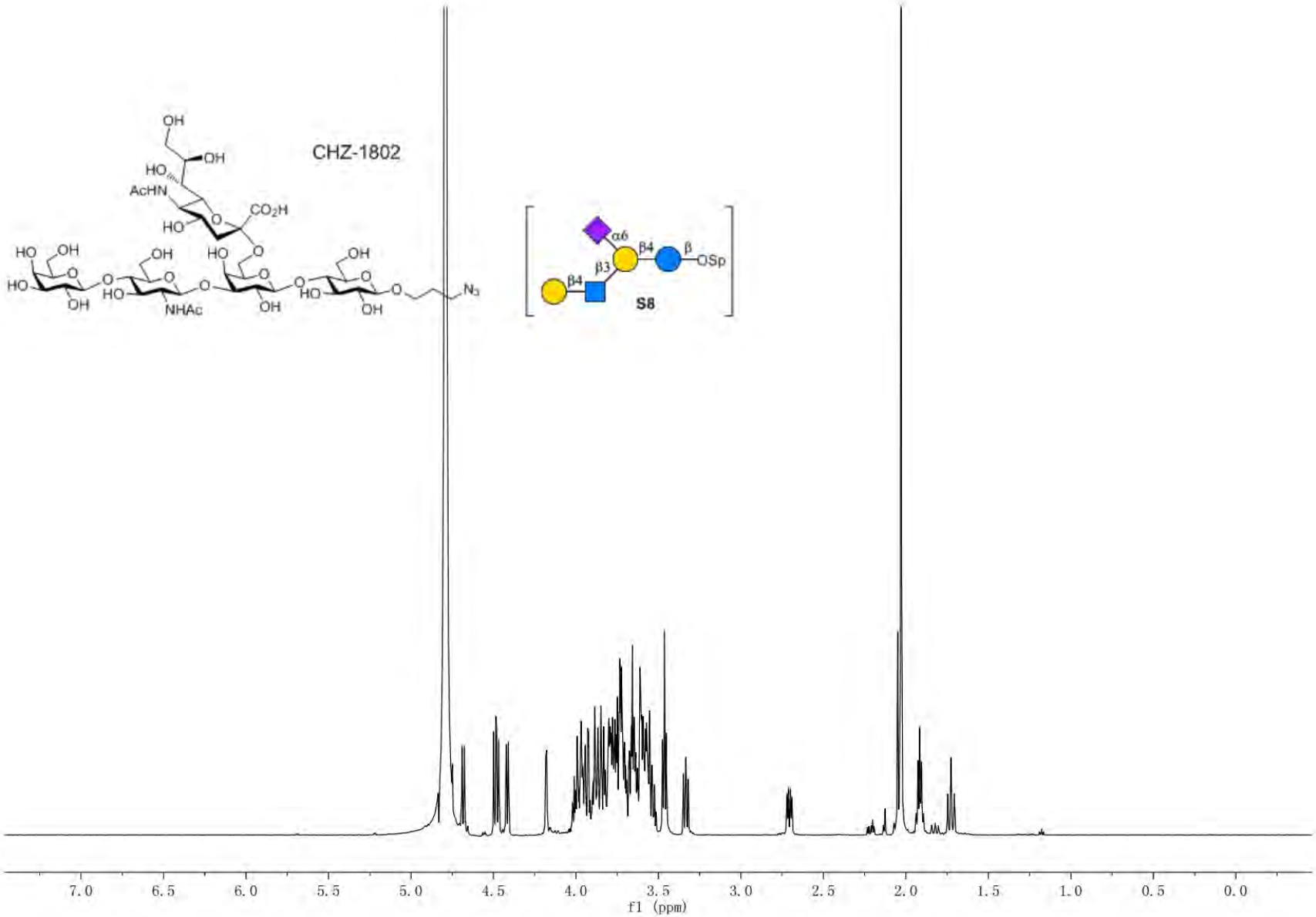
^{1}H NMR of 27


CHZ-935
OH
OH
HO
AcHN
CO2H
HO
O
OH
HO
OH
HO
O
O
O
O
HO
HO
O
O
HO
N3
NHAc
OH
OH

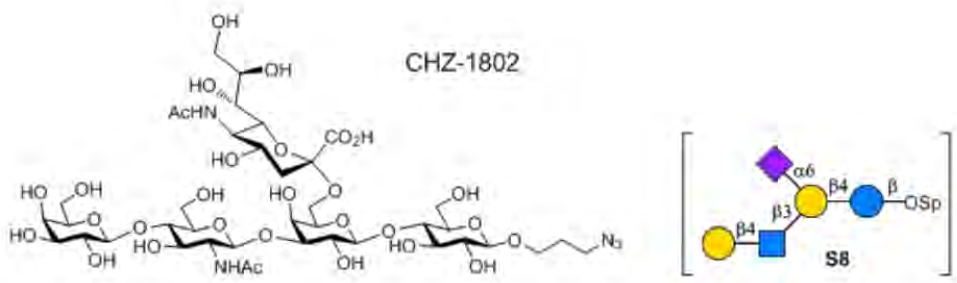
α6
β4
β
OSp
β3
27

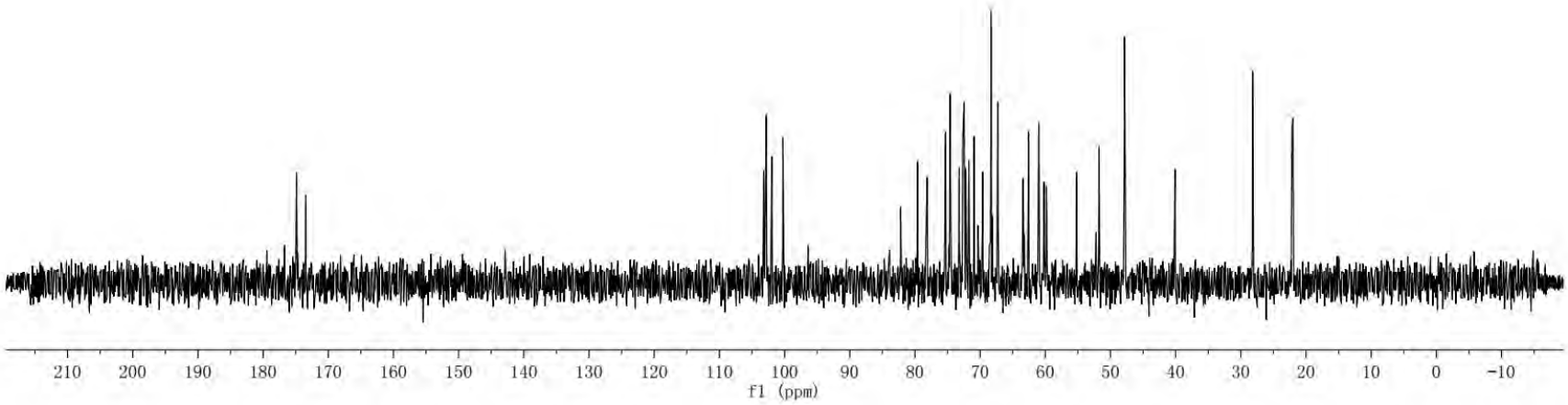

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

^{13}C NMR of 27

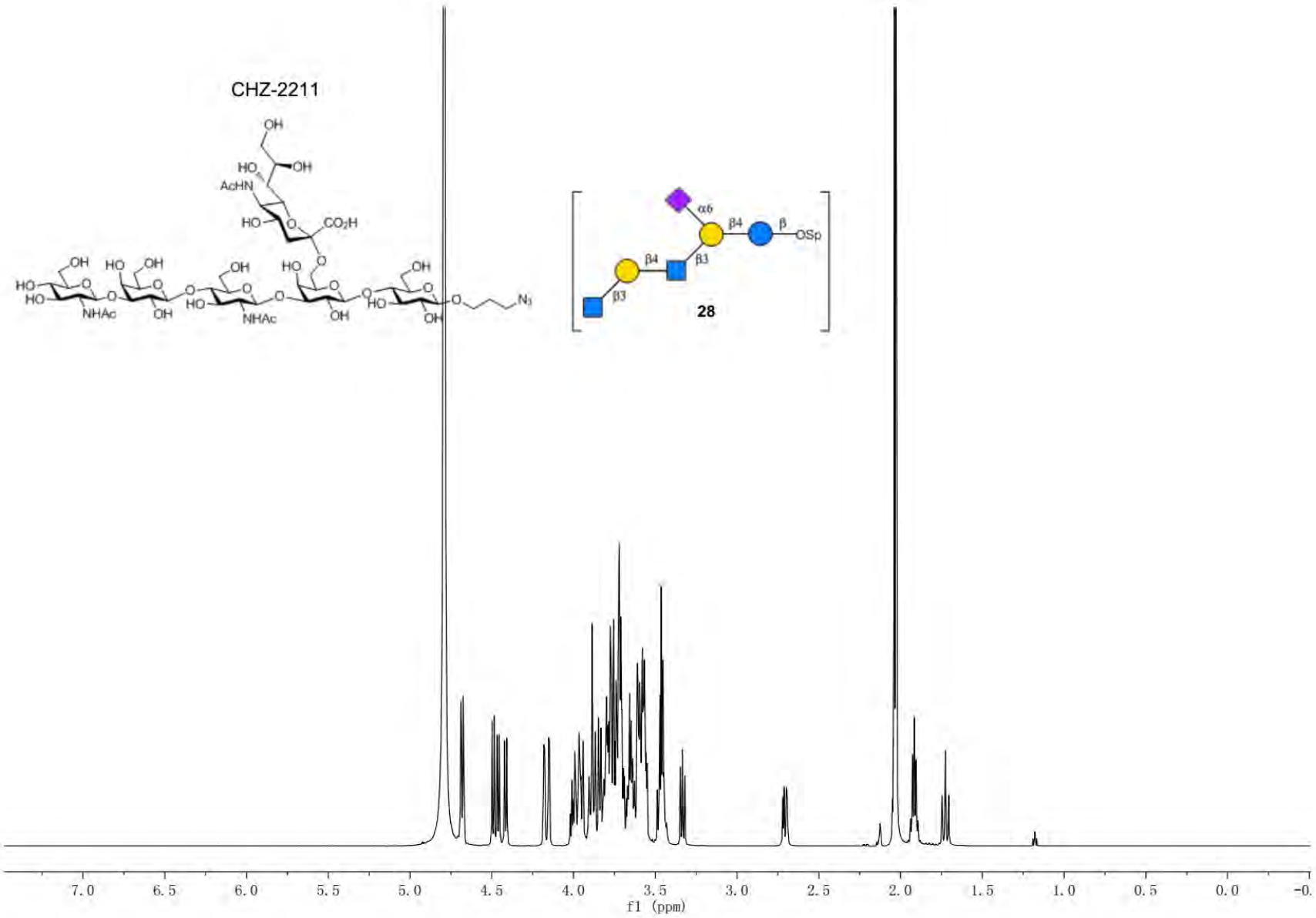


^{1}H NMR of S8

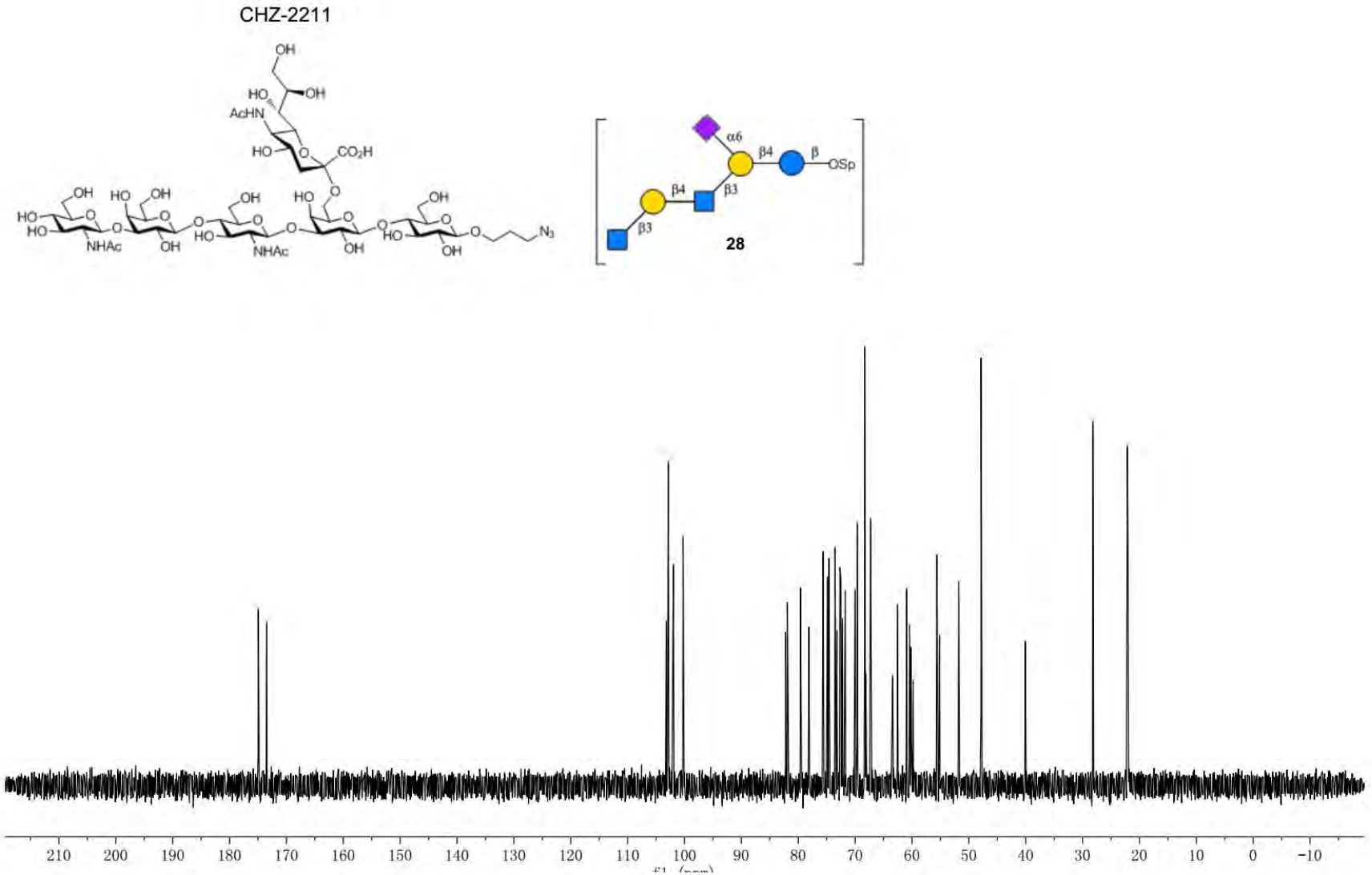

CHZ-1802
S8


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

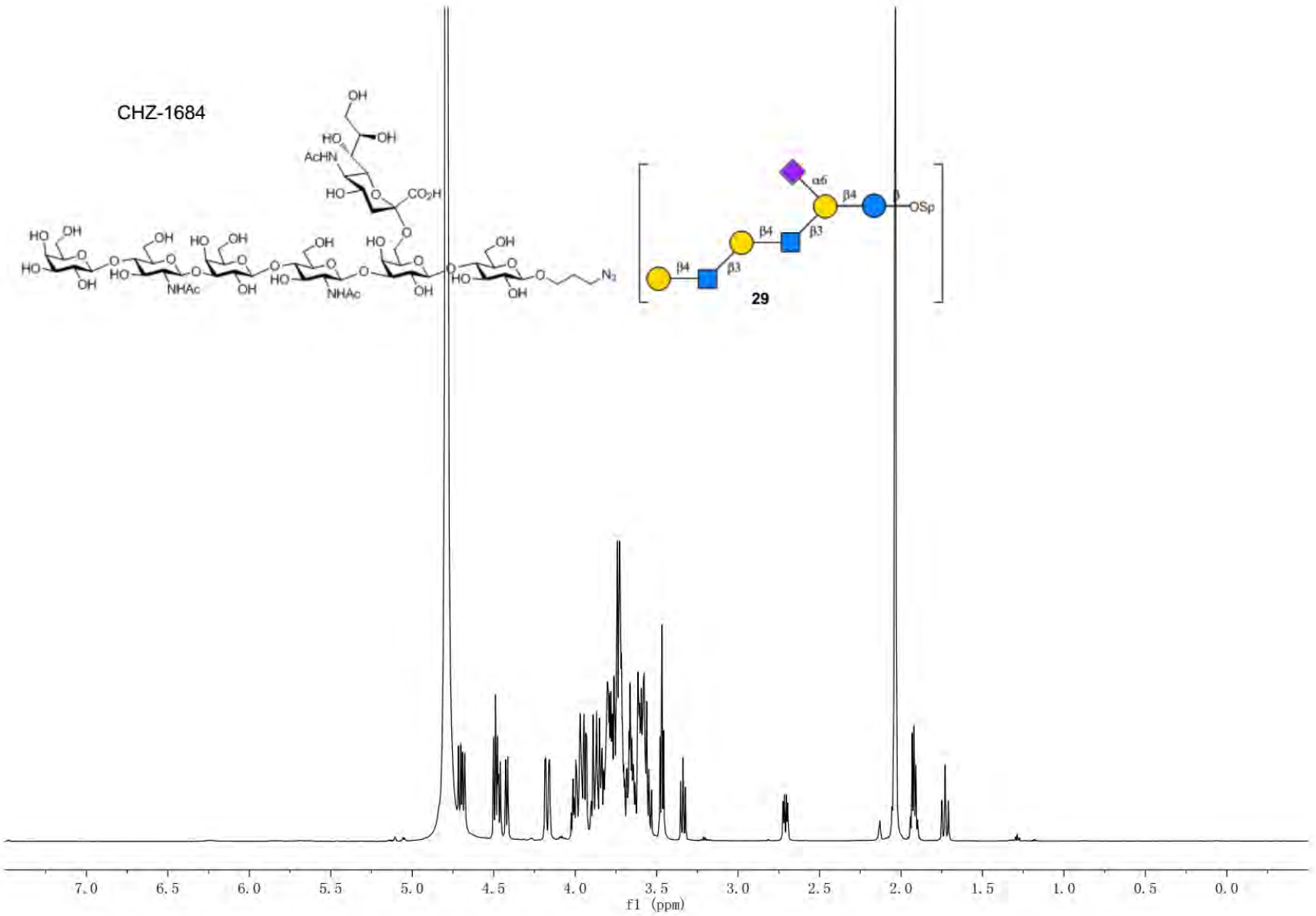
^{13}C NMR of S8



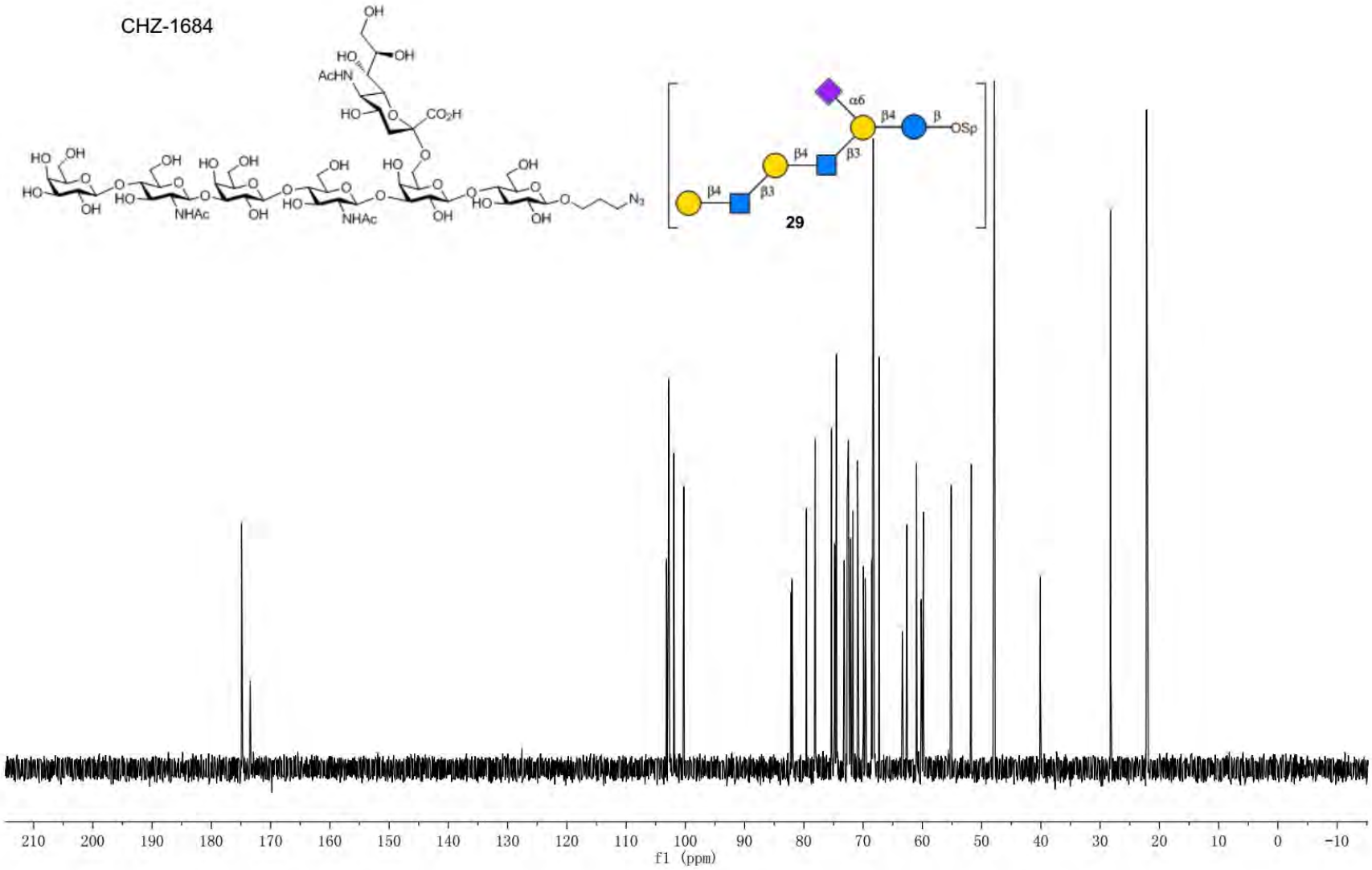
^{1}H NMR of 28


CHZ-2211
28
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10

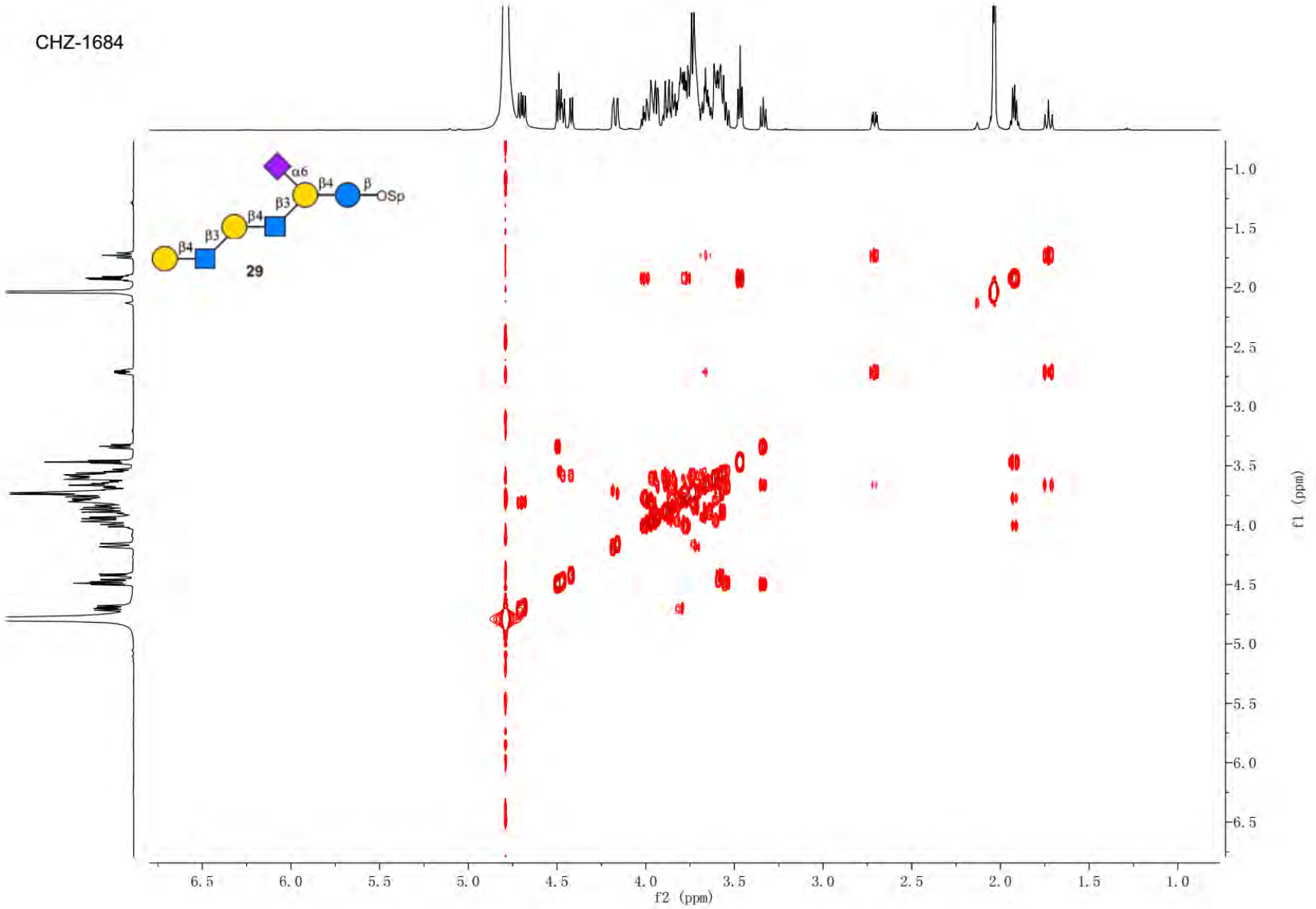
^{13}C NMR of 28



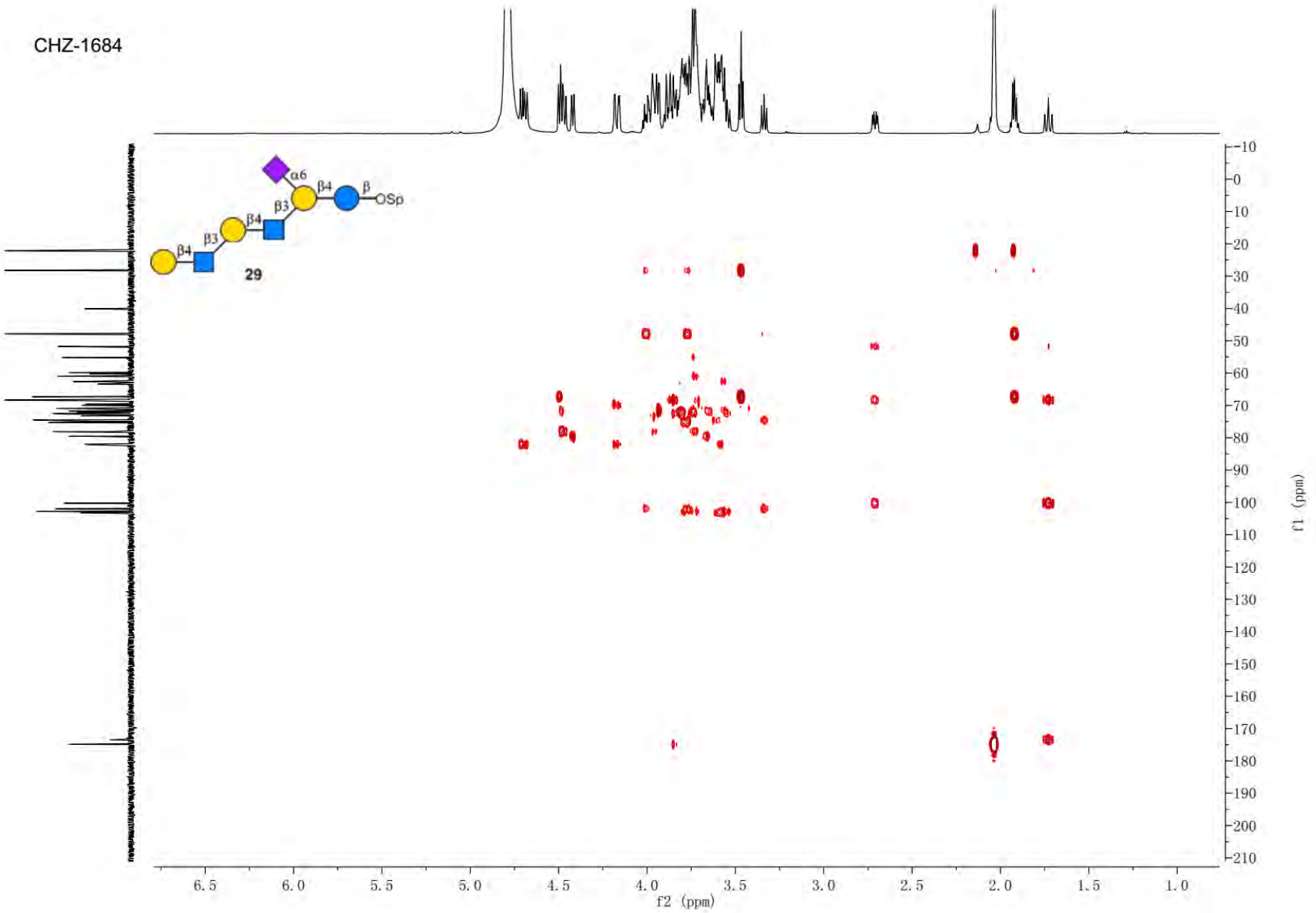
^{1}H NMR of 29



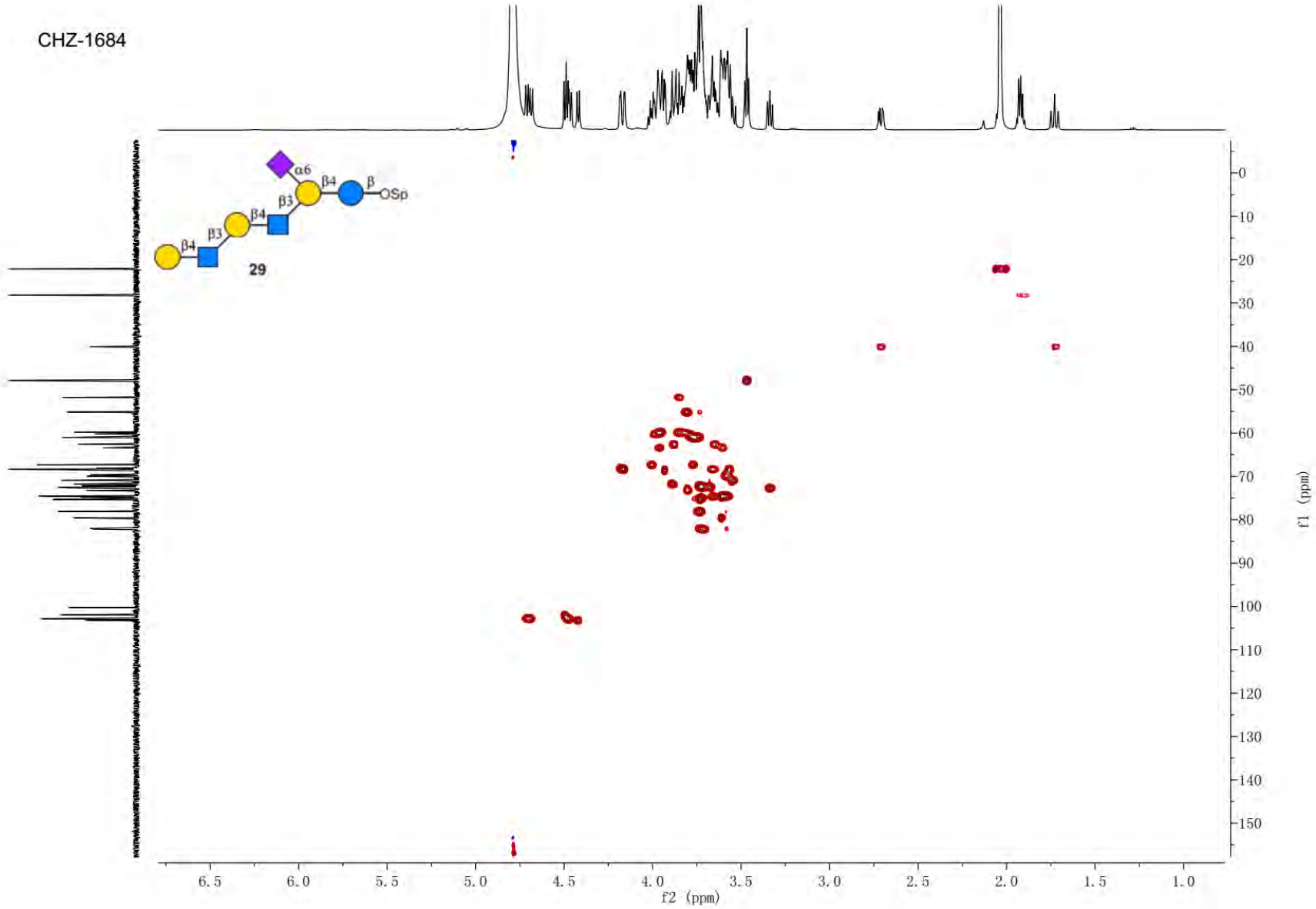
^{13}C NMR of 29



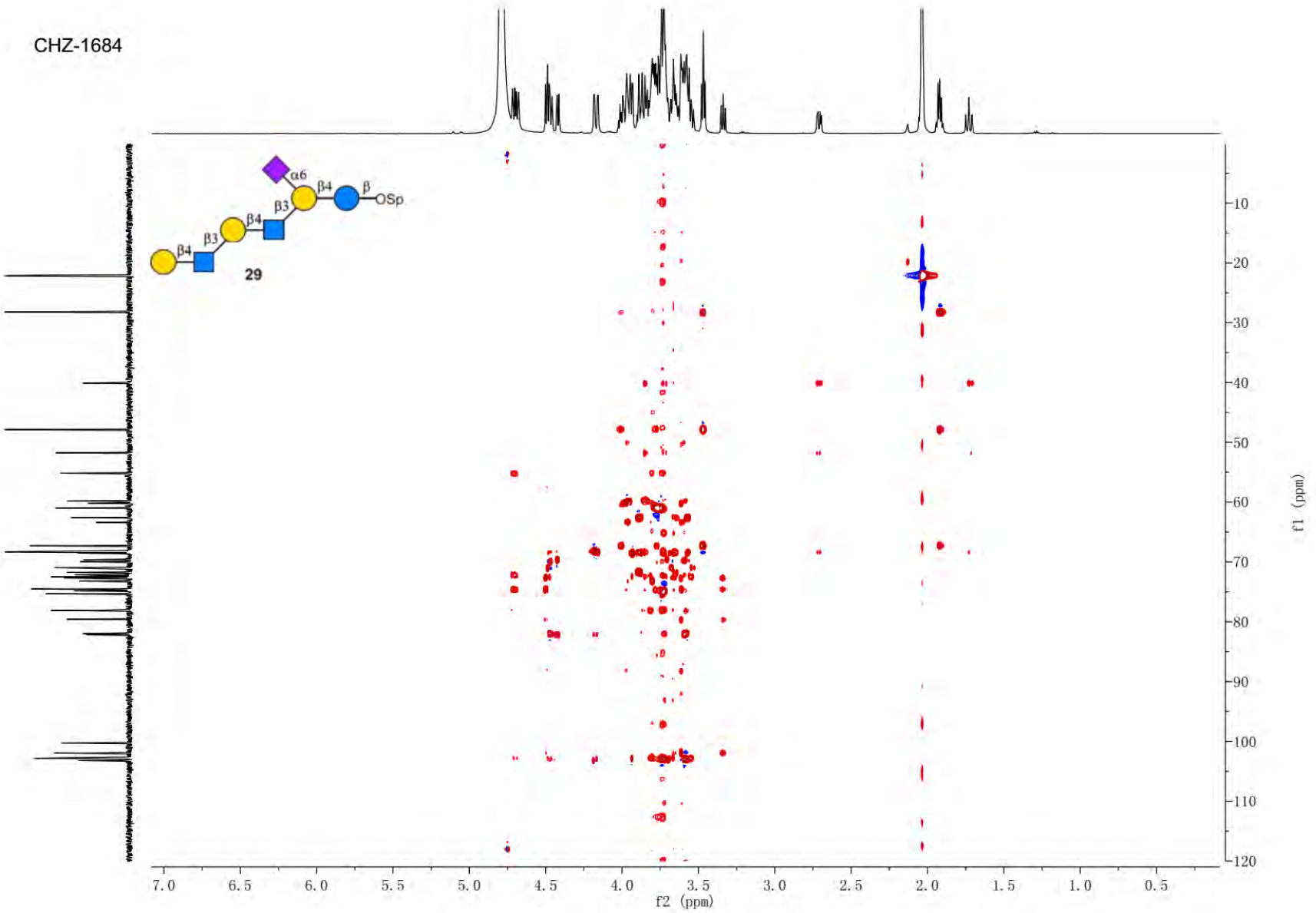
COSY spectra of 29



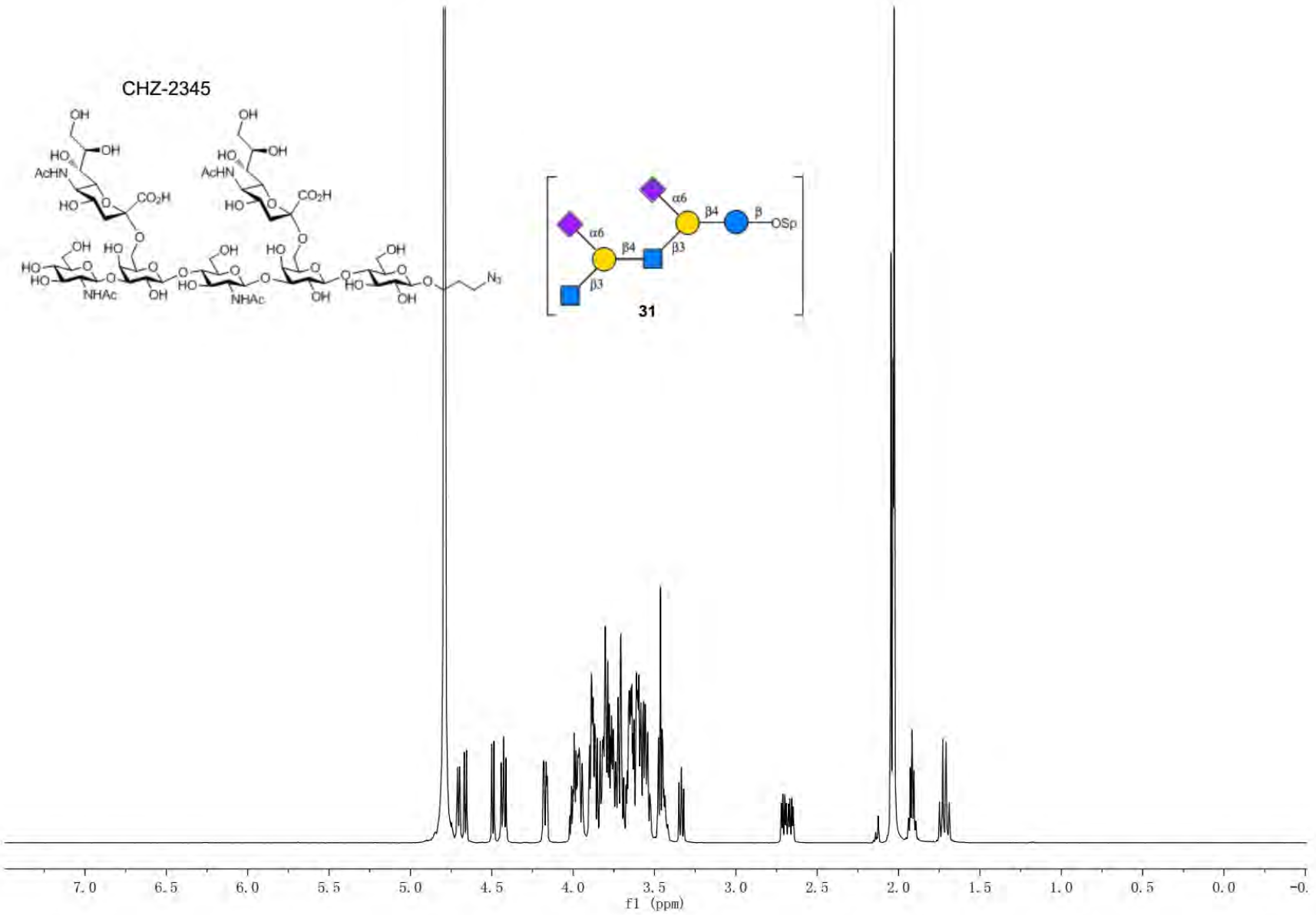
HMBC spectra of 29



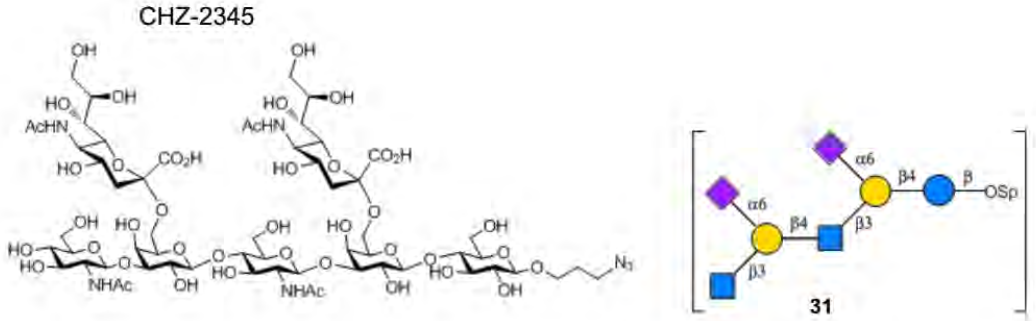
HSQC spectra of 29

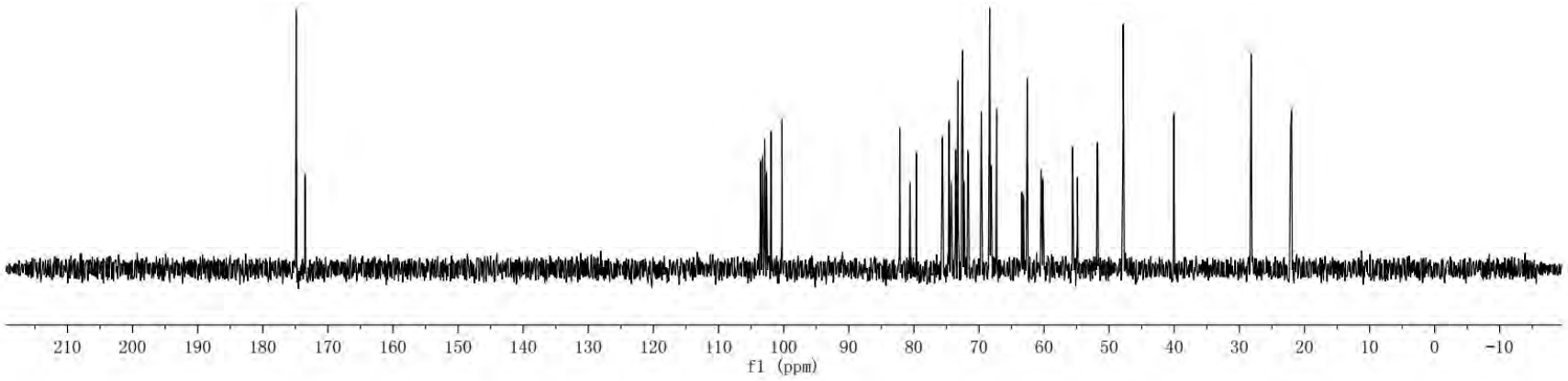


HSQC-TOCSY spectra of 29

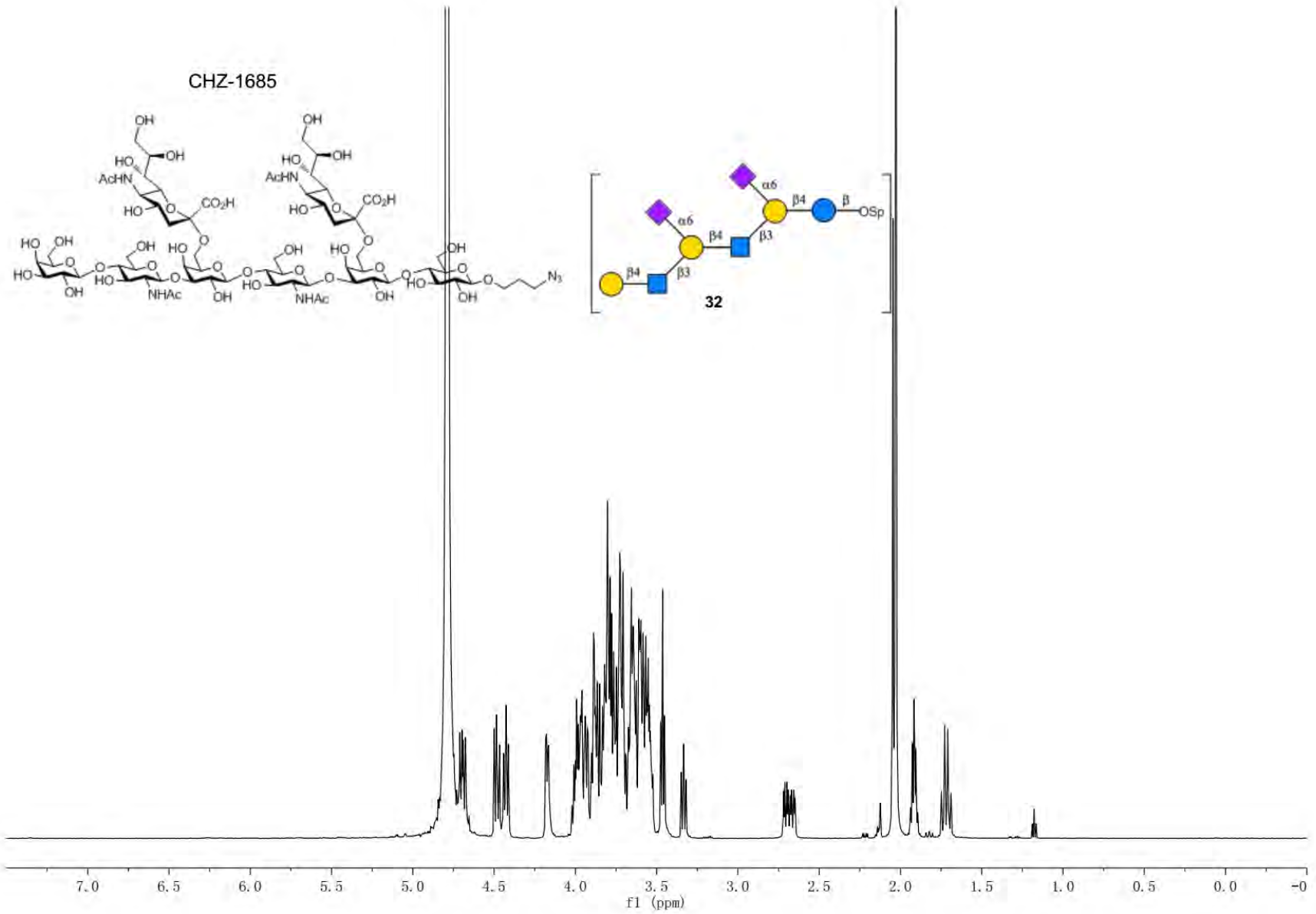


^{1}H NMR of 31


CHZ-2345
31

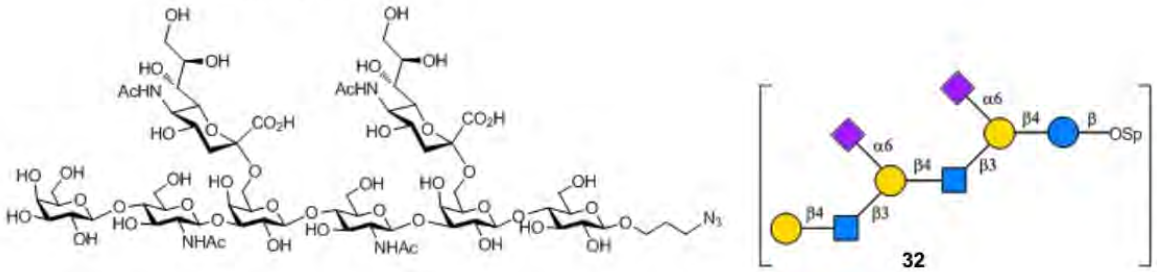

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

^{13}C NMR of 31

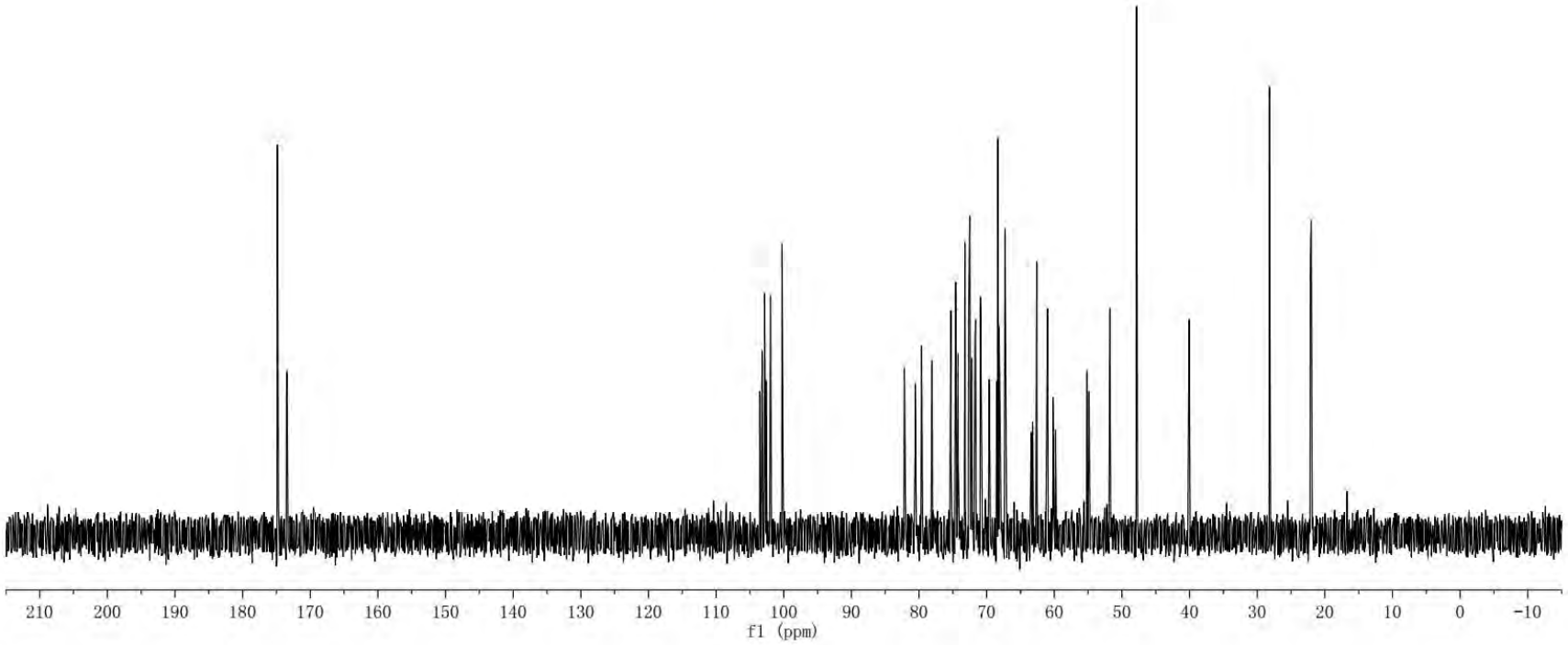


^{1}H NMR of 32

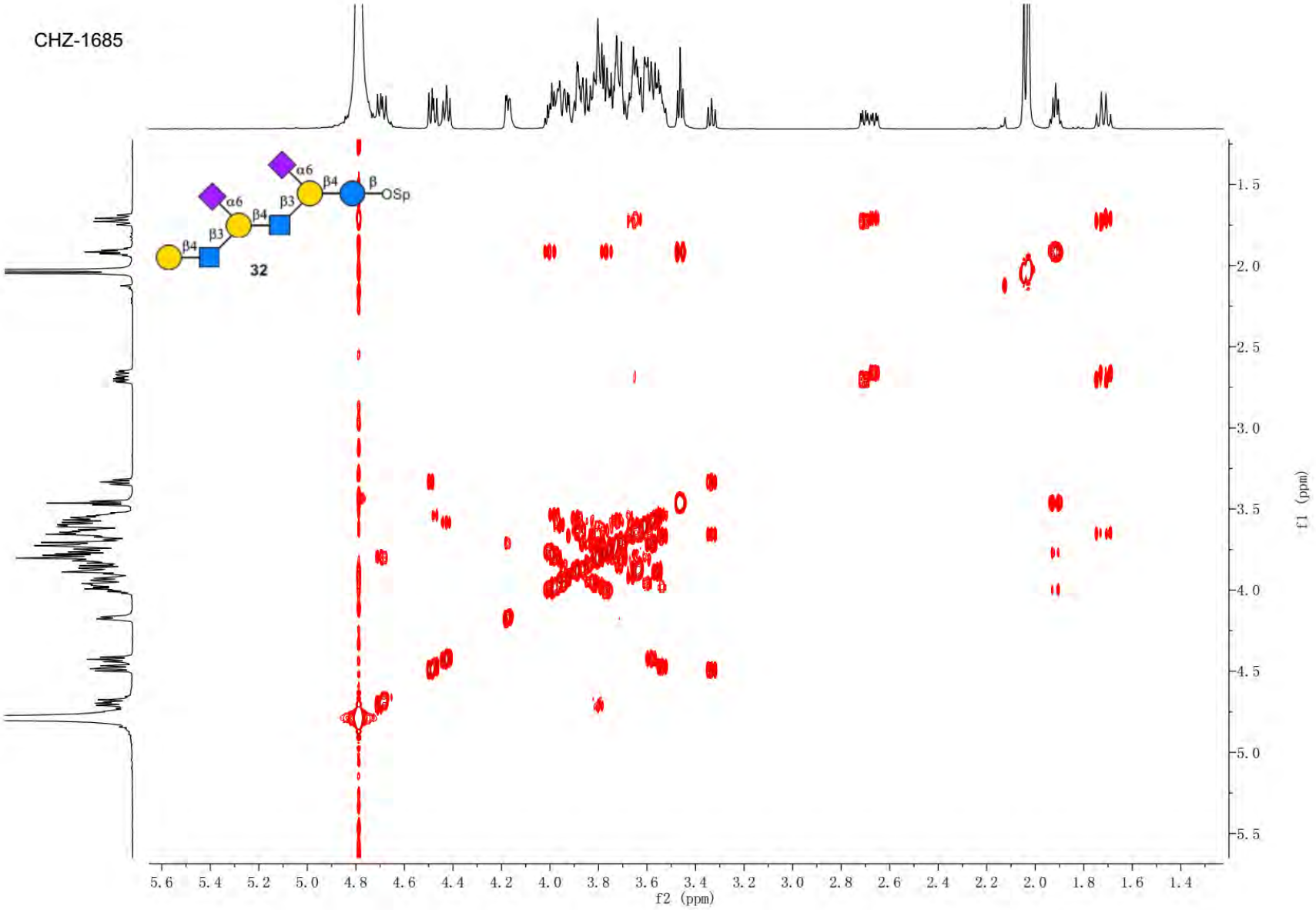
CHZ-1685



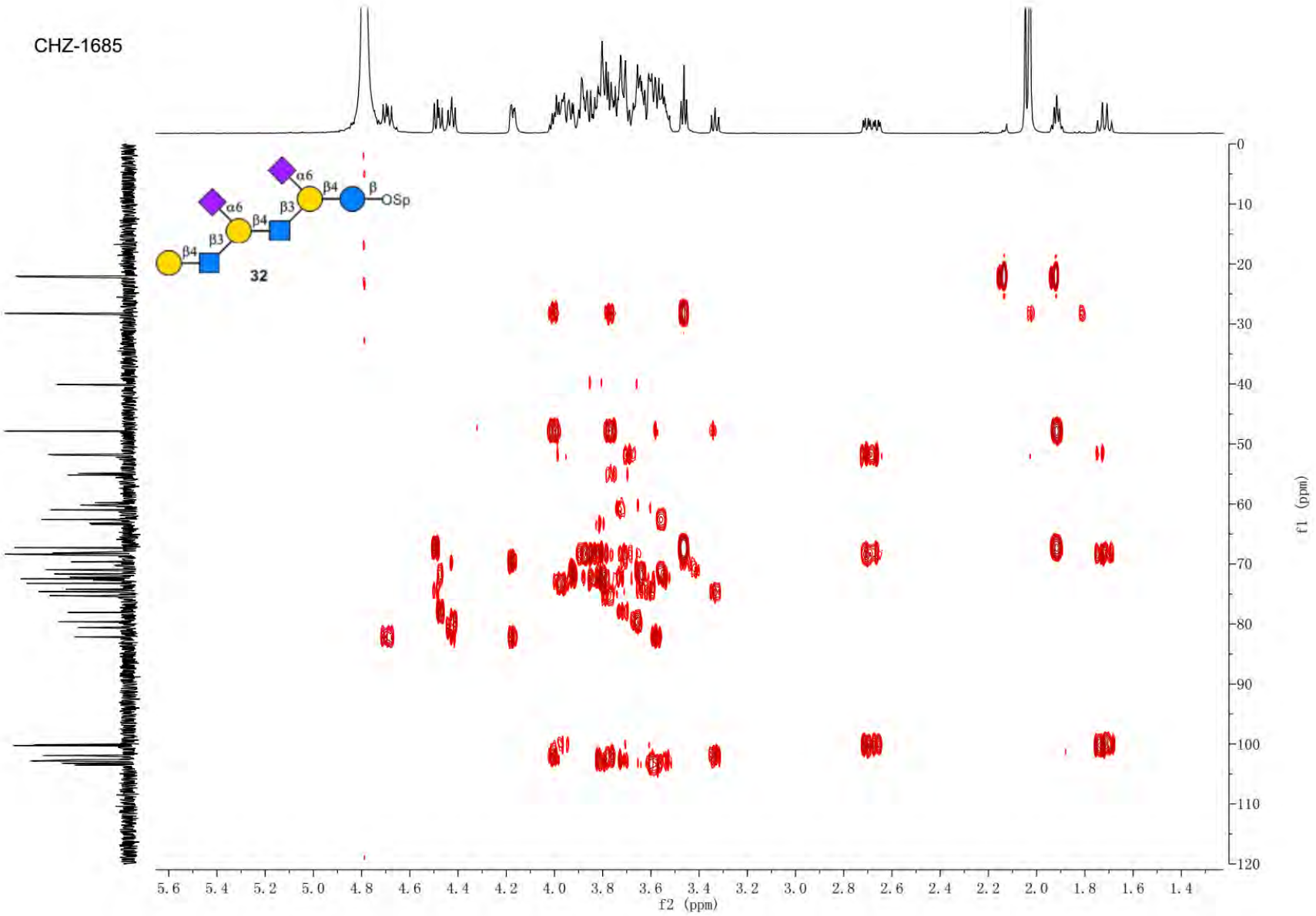
32



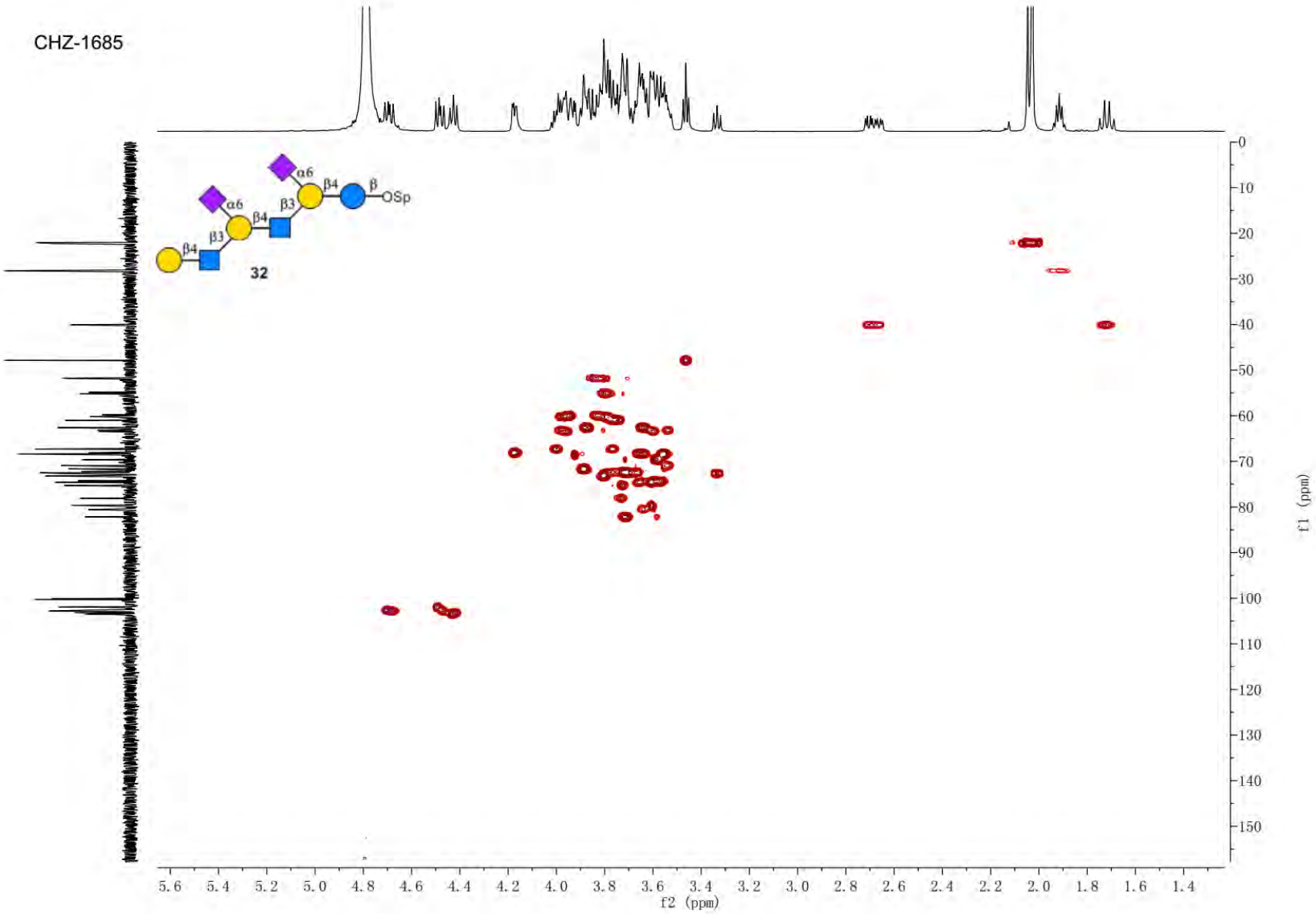
^{13}C NMR of 32



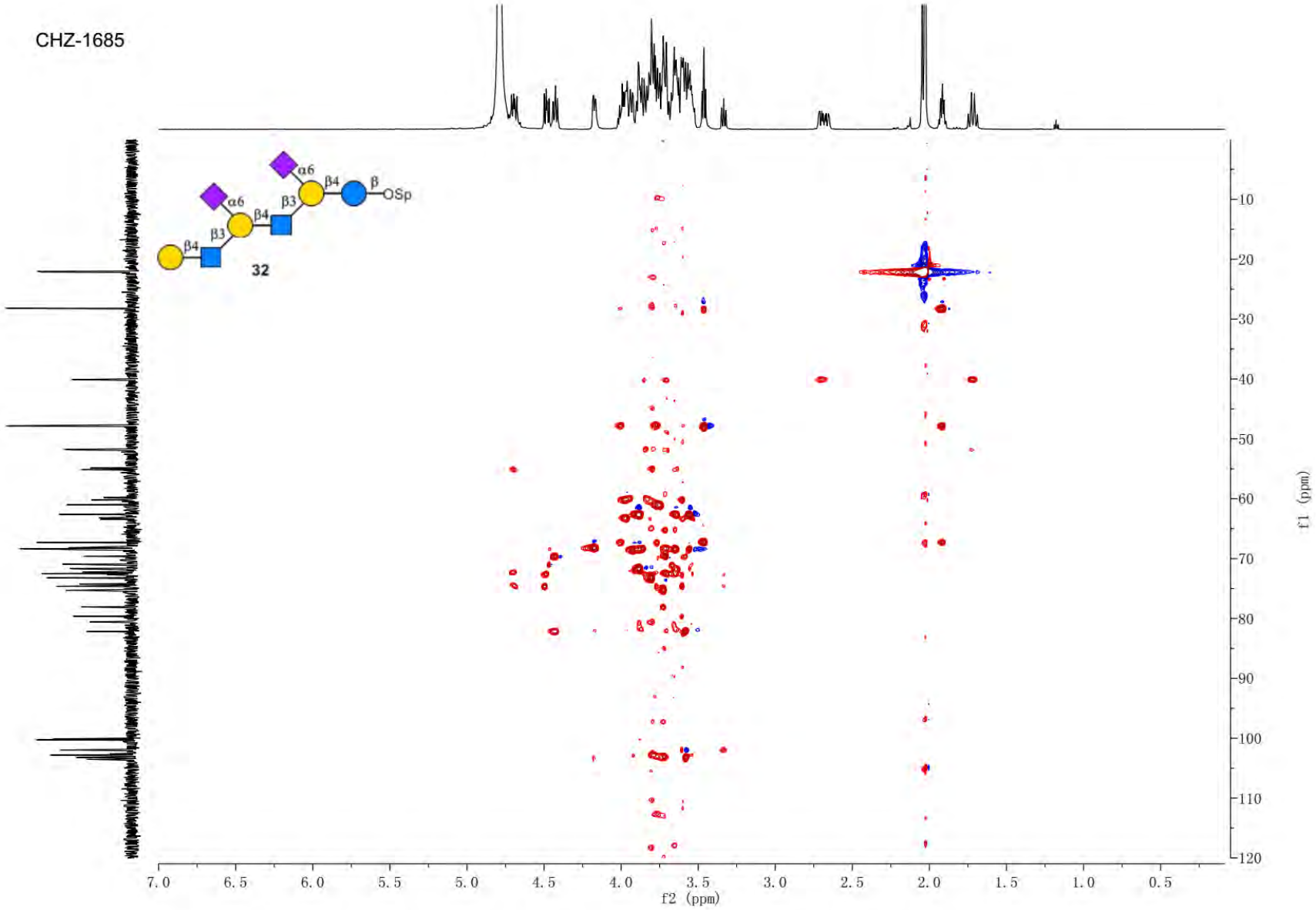
COSY spectra of 32



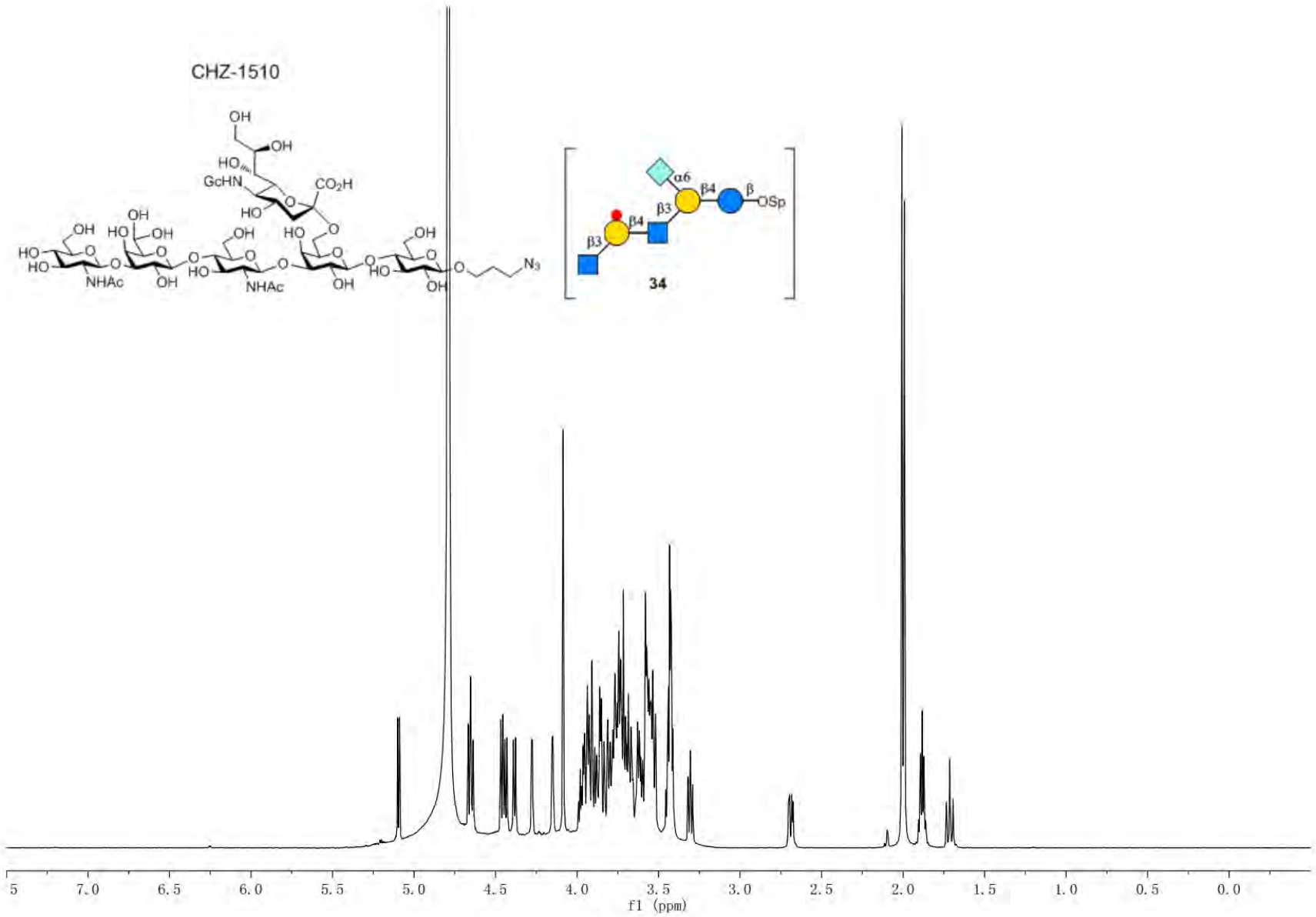
HMBC spectra of 32



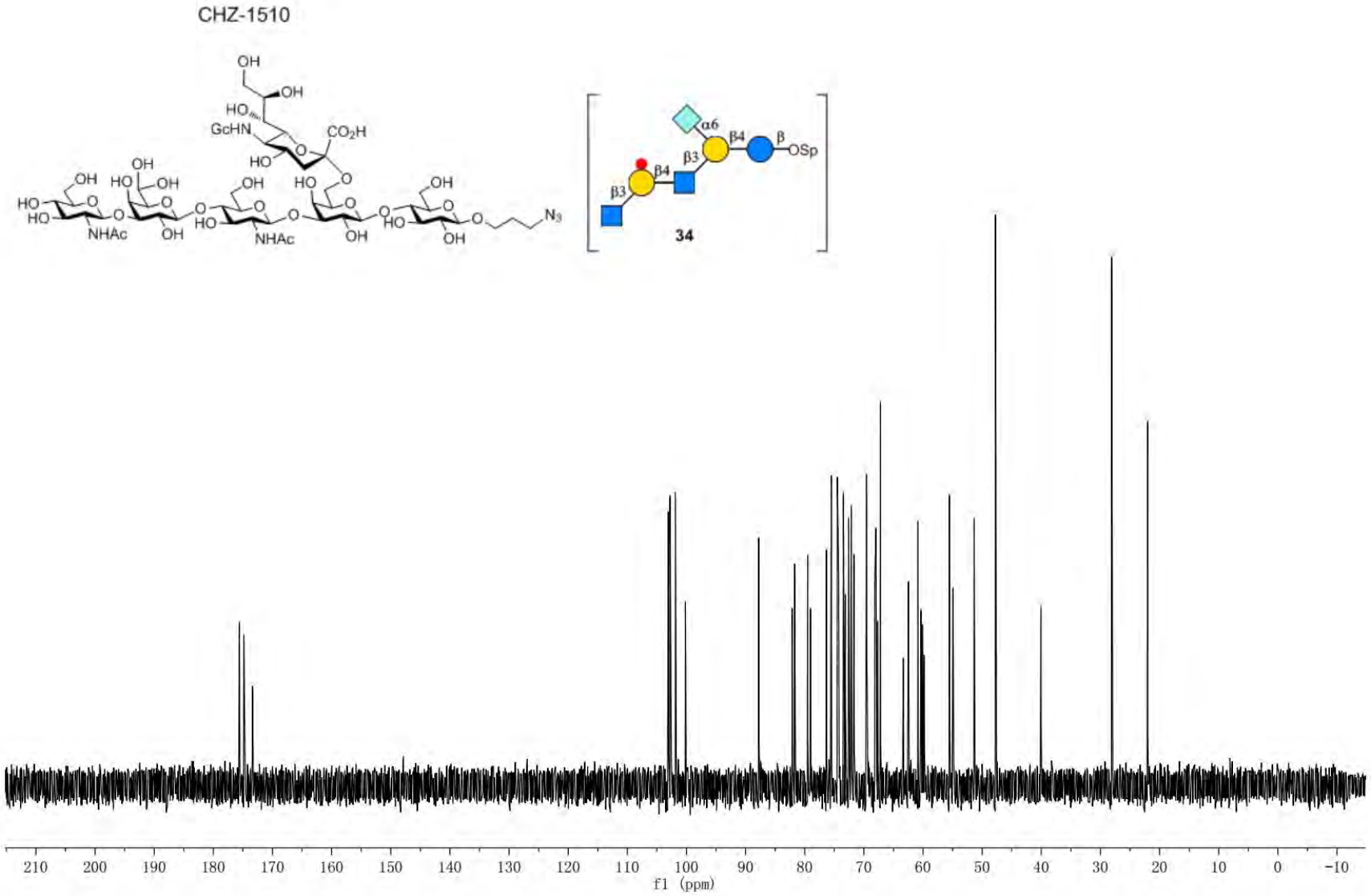
HSQC spectra of 32



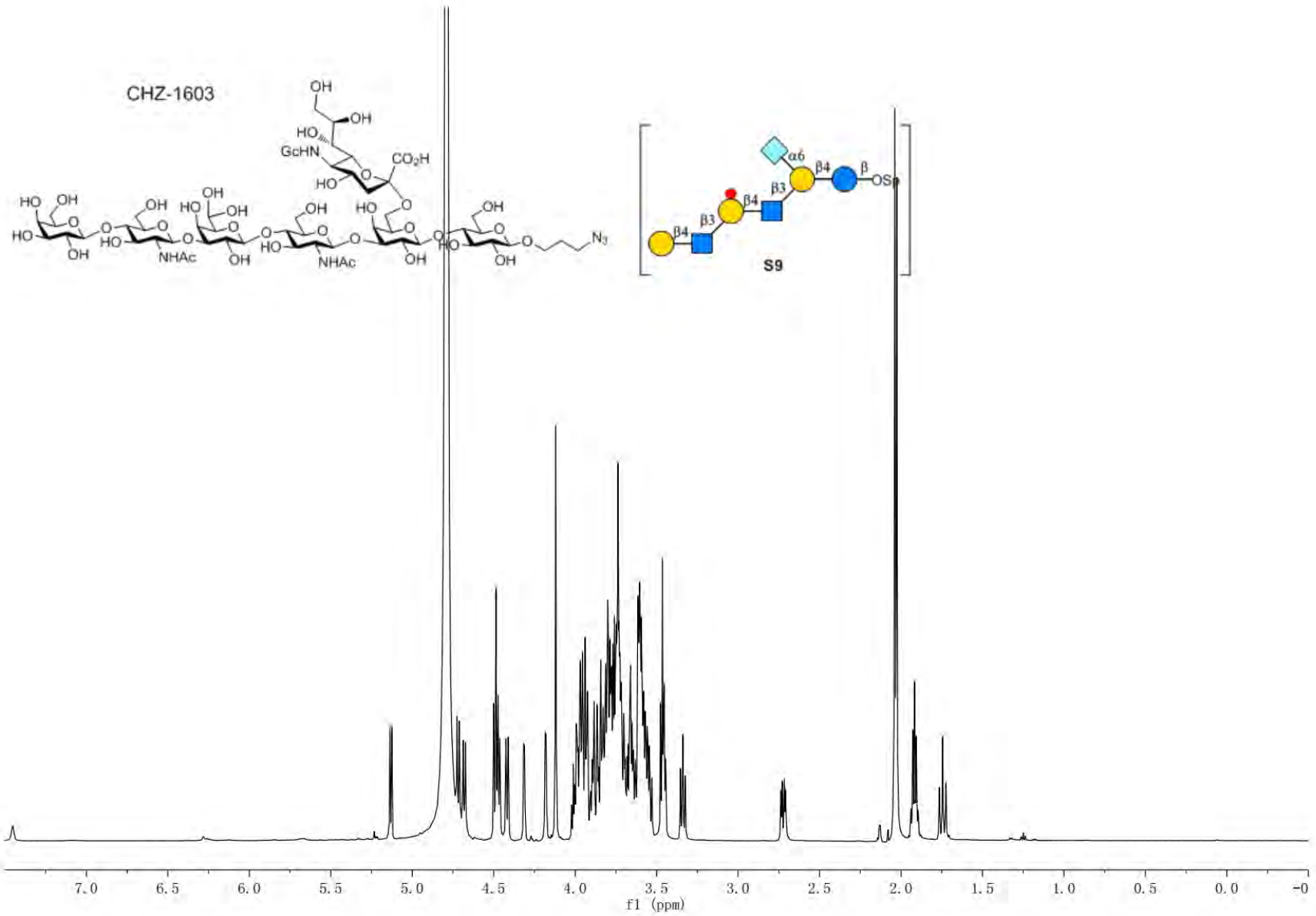
HSQC-TOCSY spectra of 32



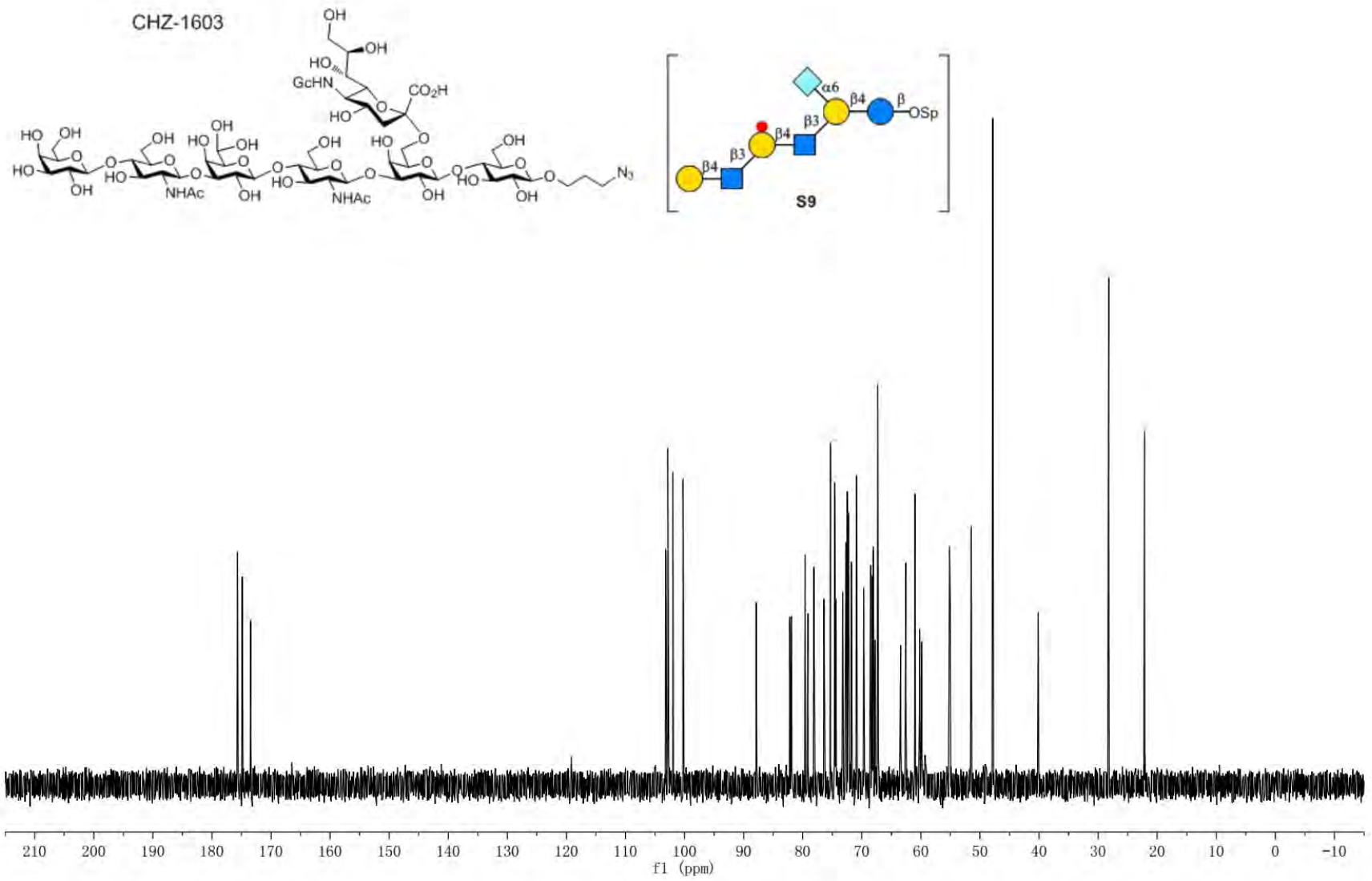
^{1}H NMR of 34



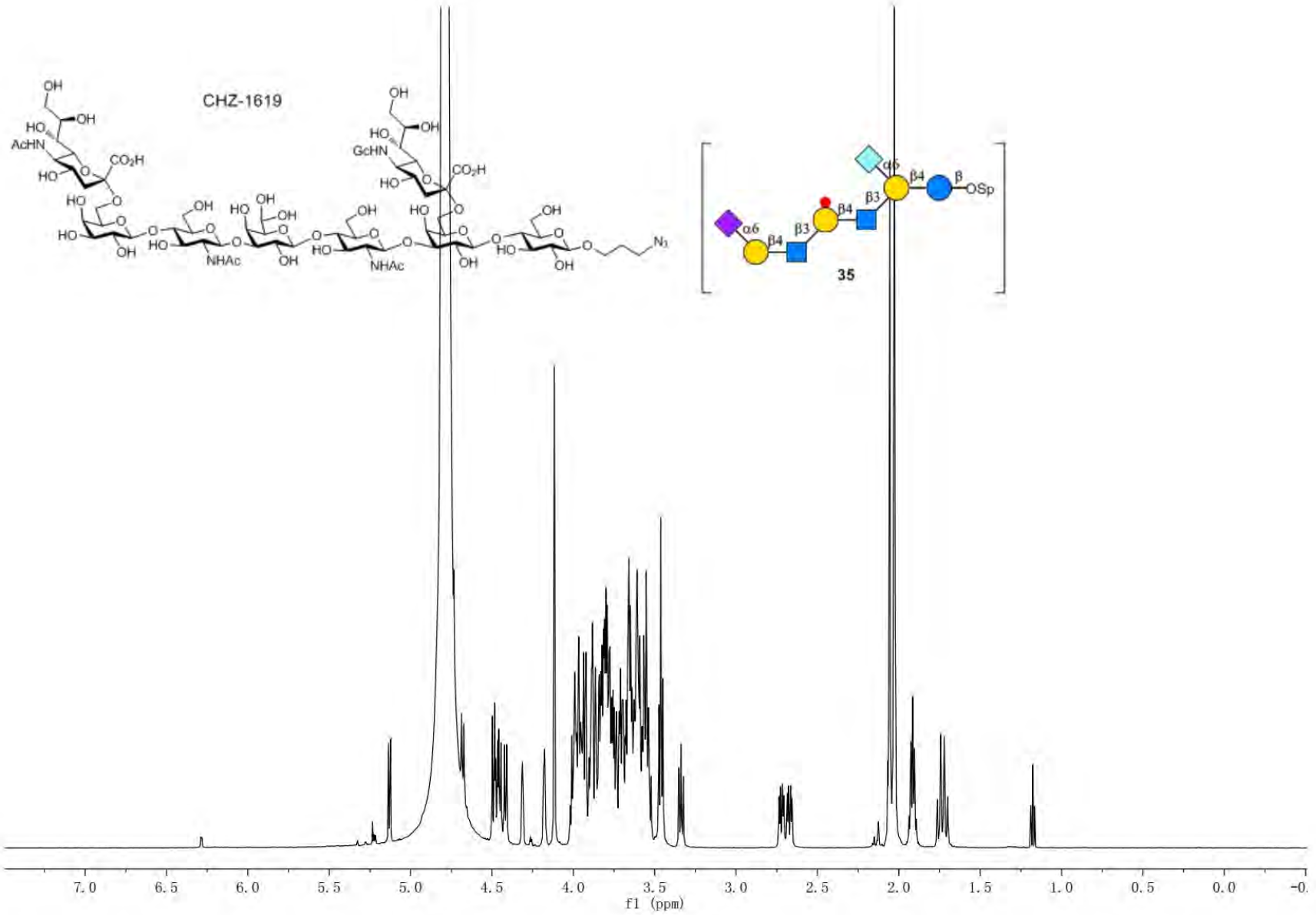
^{13}C NMR of 34



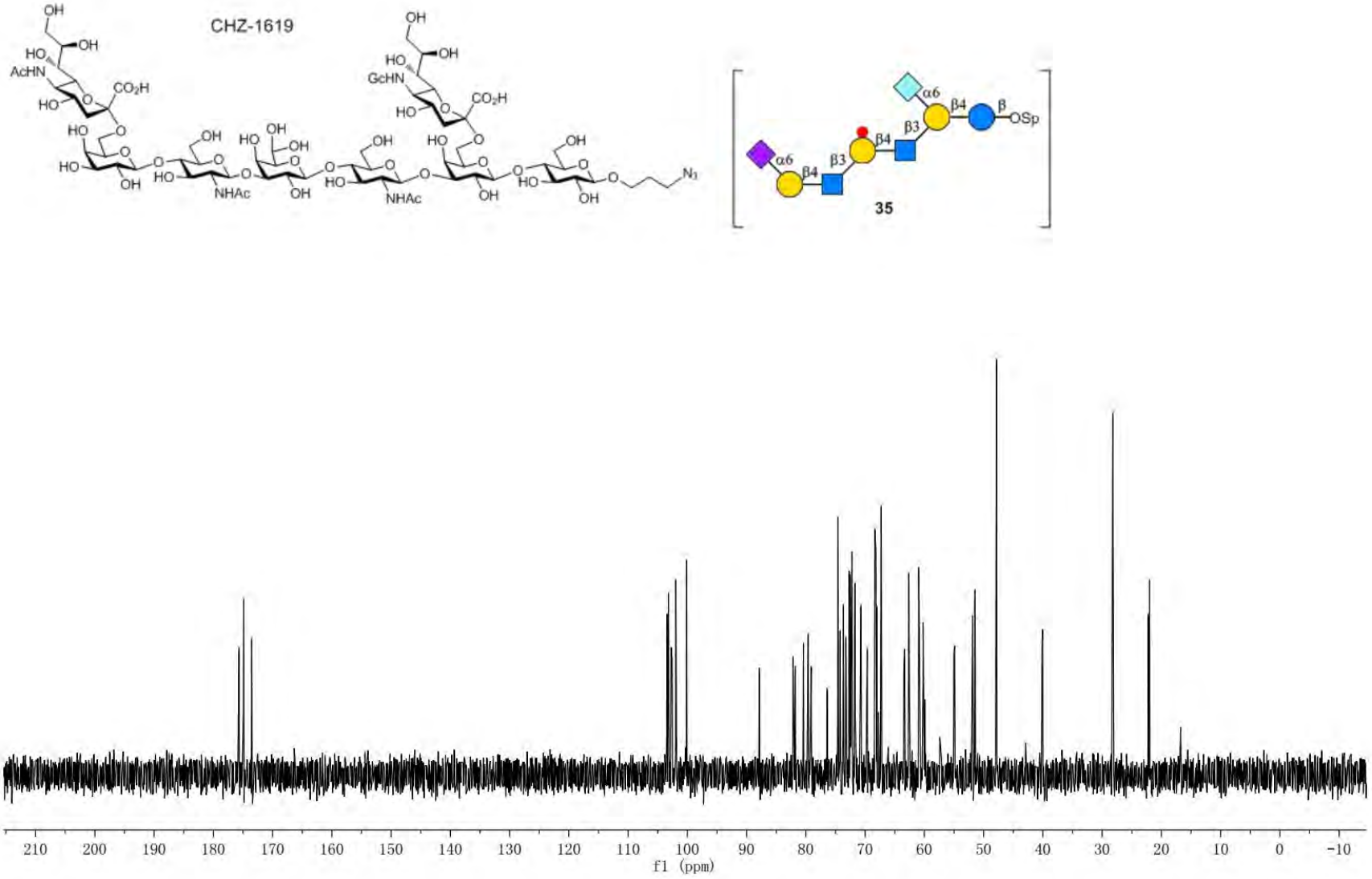
^{1}H NMR of S9



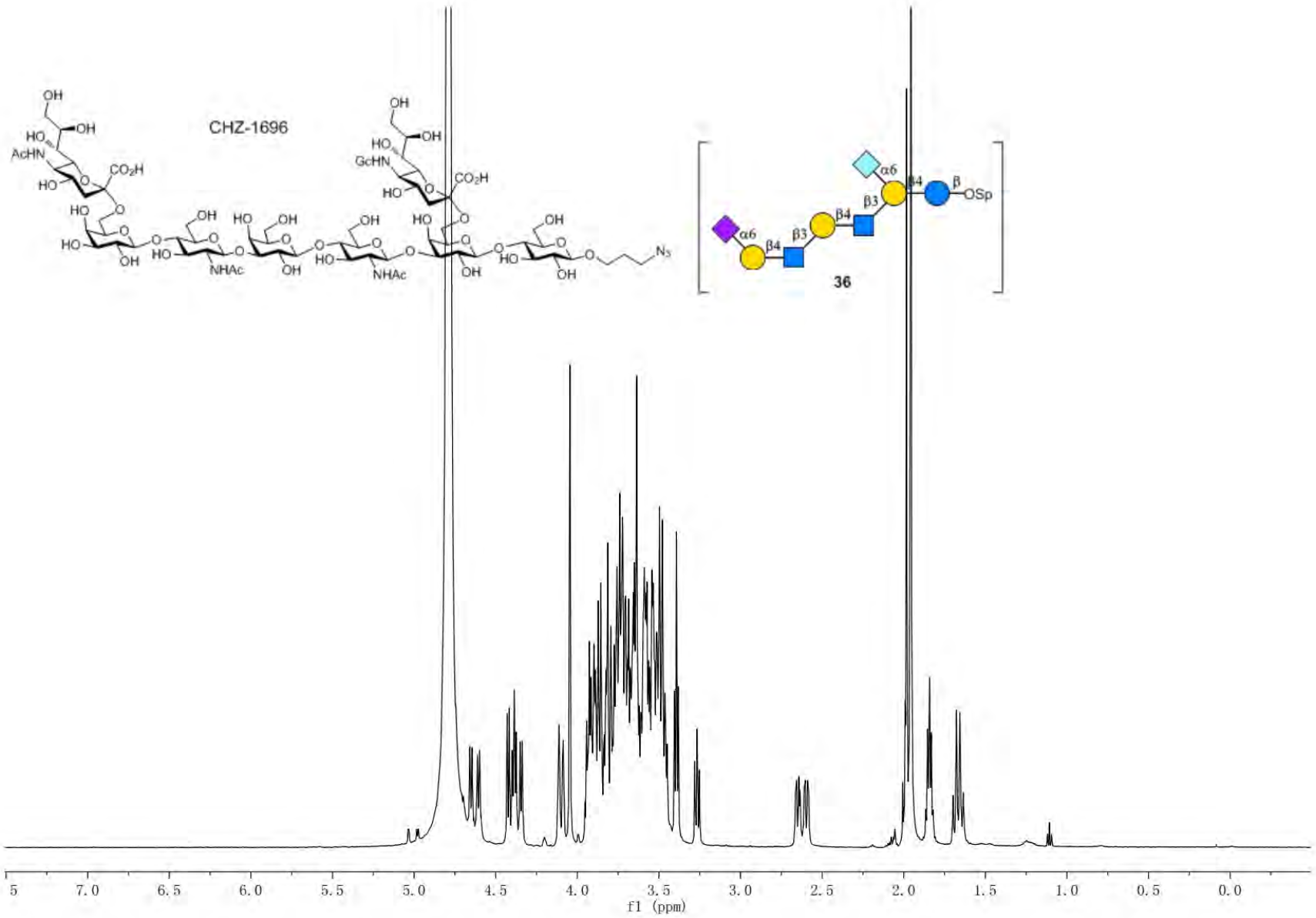
^{13}C NMR of S9



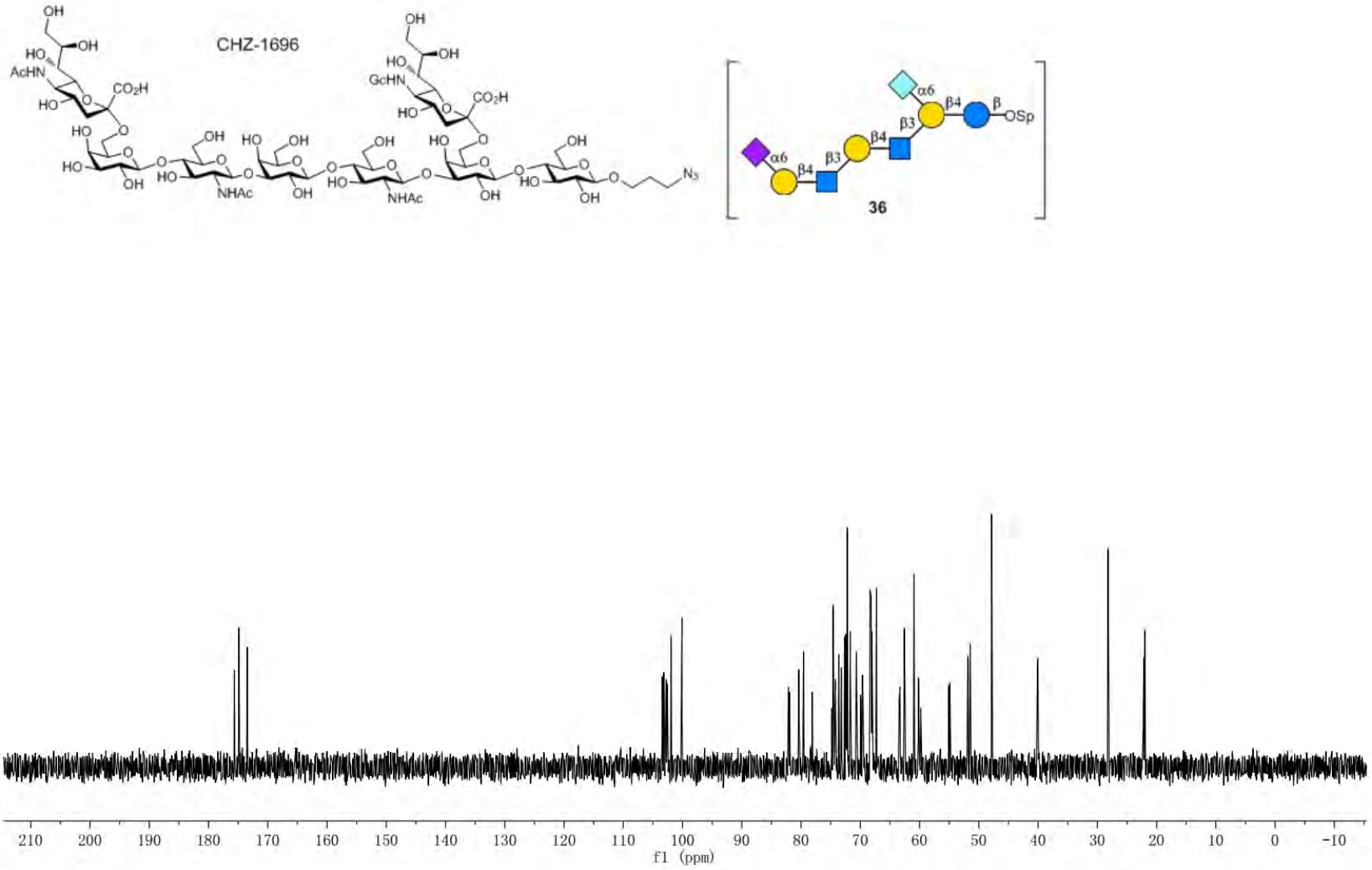
^{1}H NMR of 35



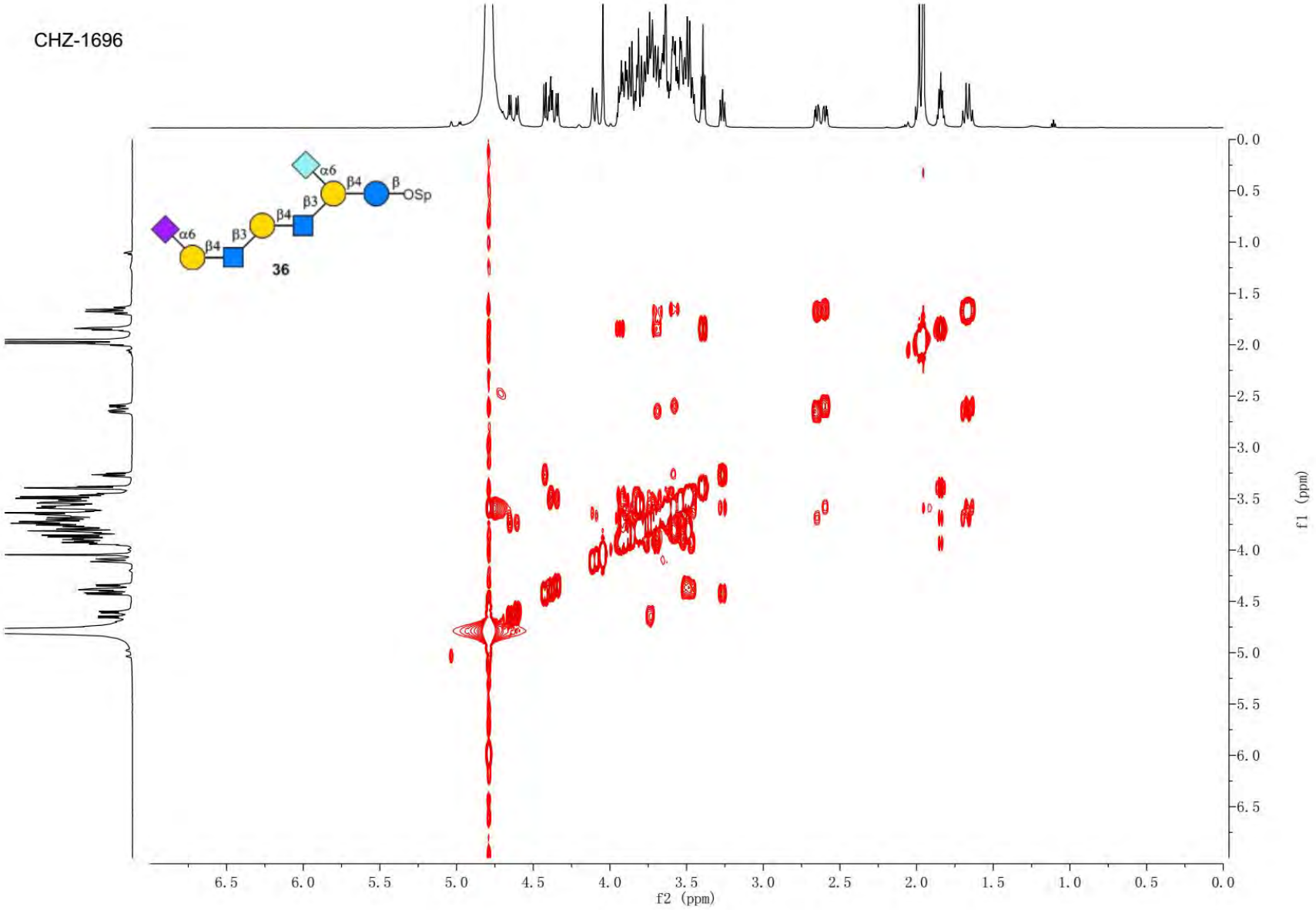
^{13}C NMR of 35



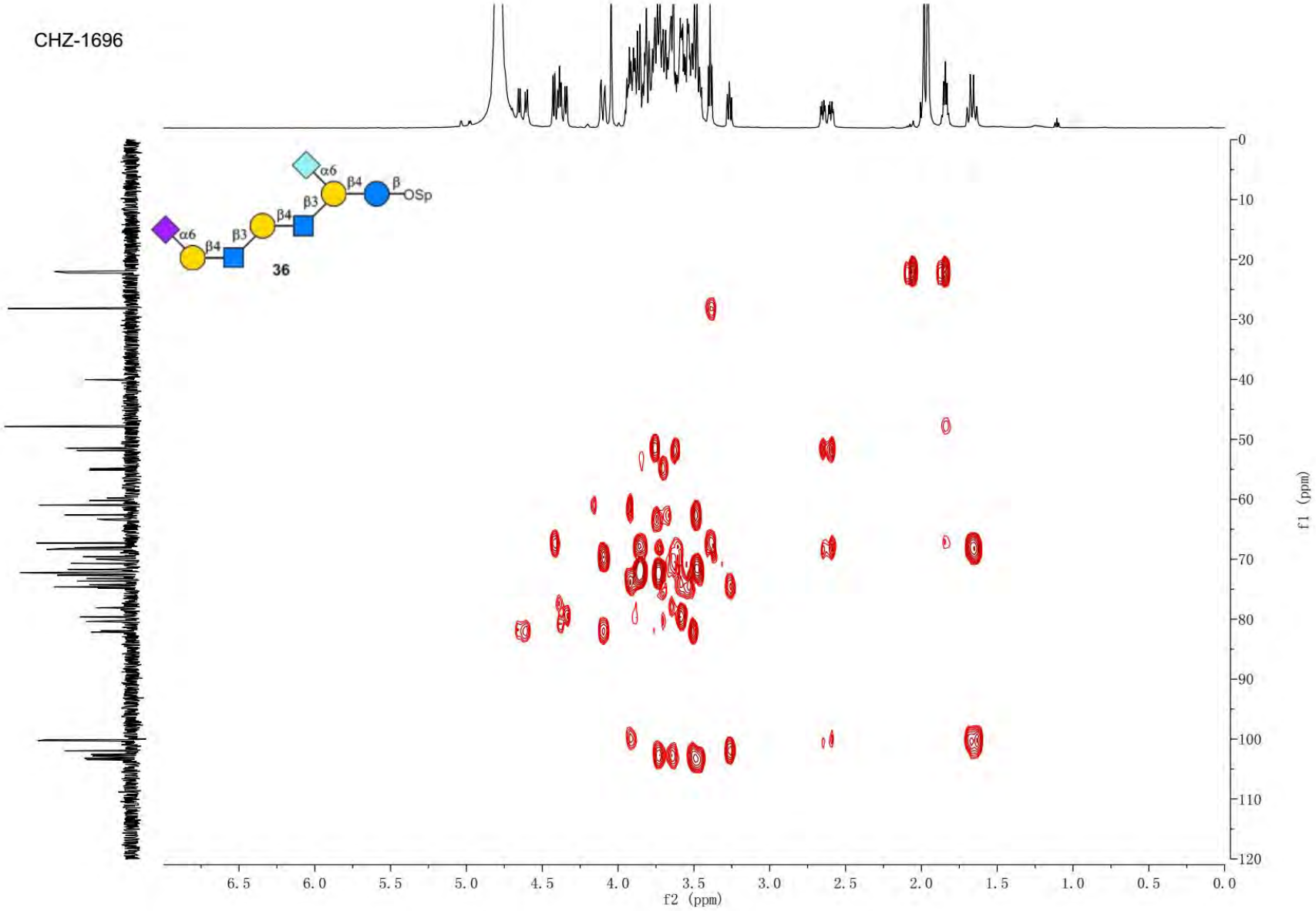
^{1}H NMR of 36


CHZ-1696
36
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

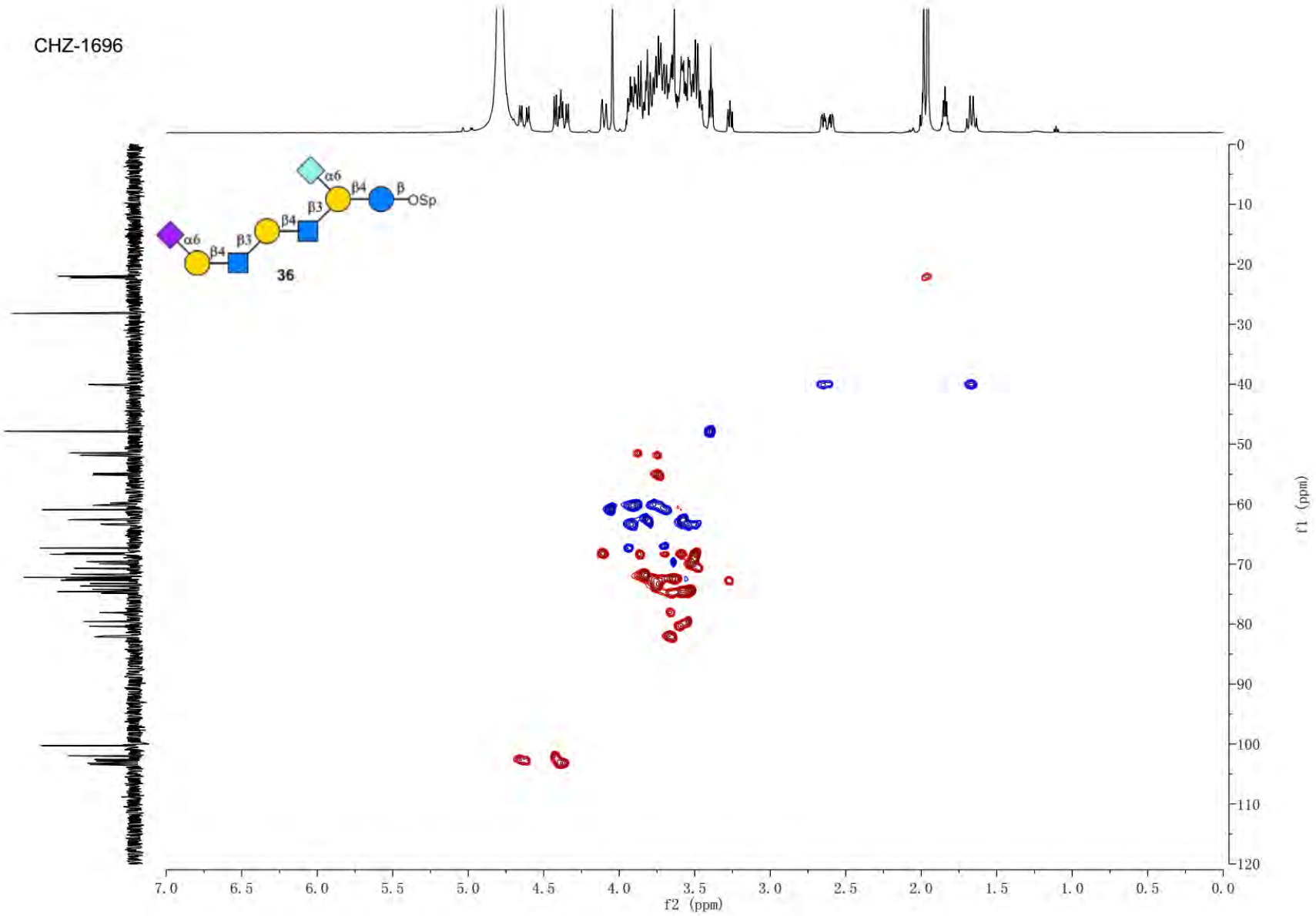
^{13}C NMR of 36



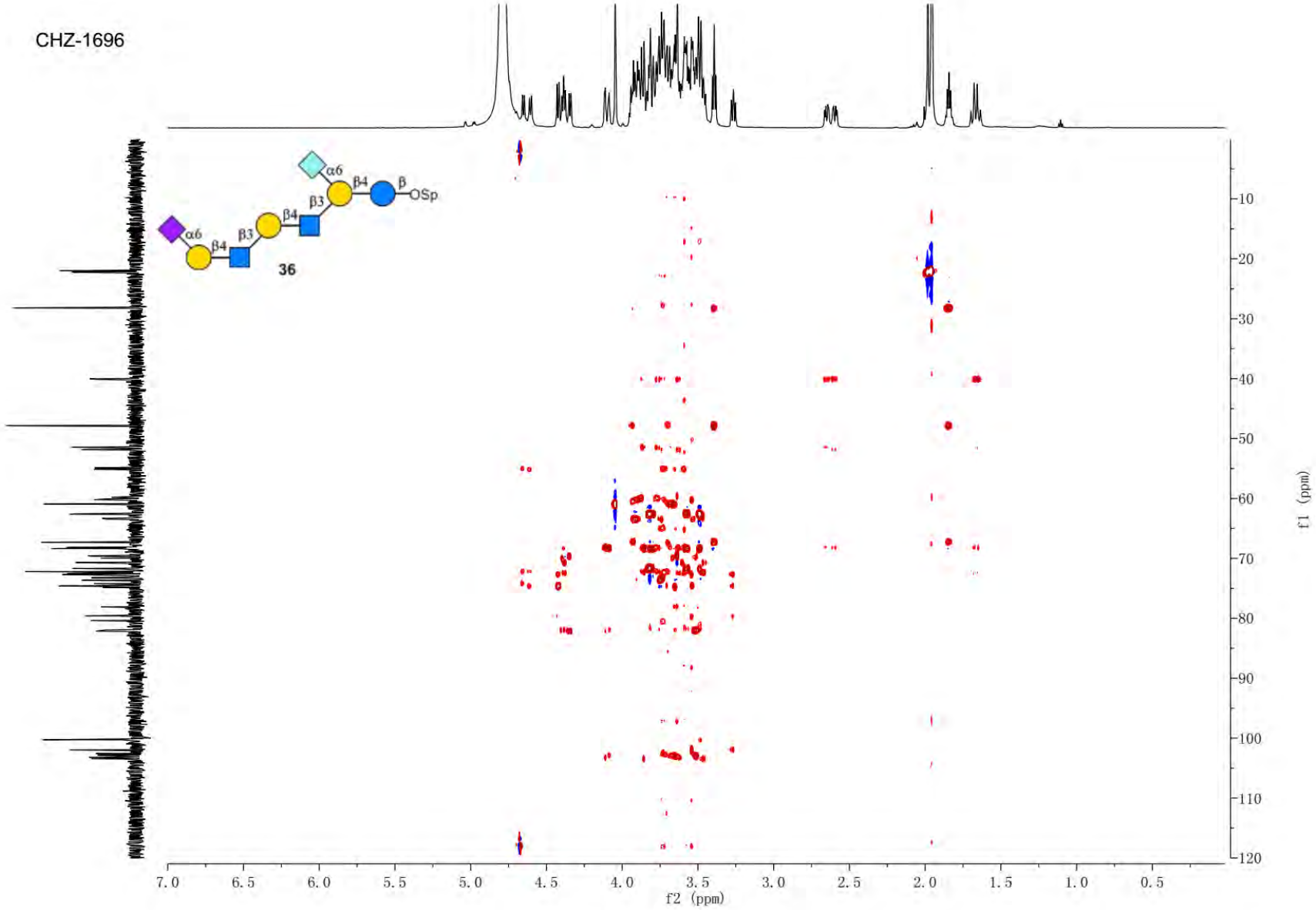
COSY spectra of 36



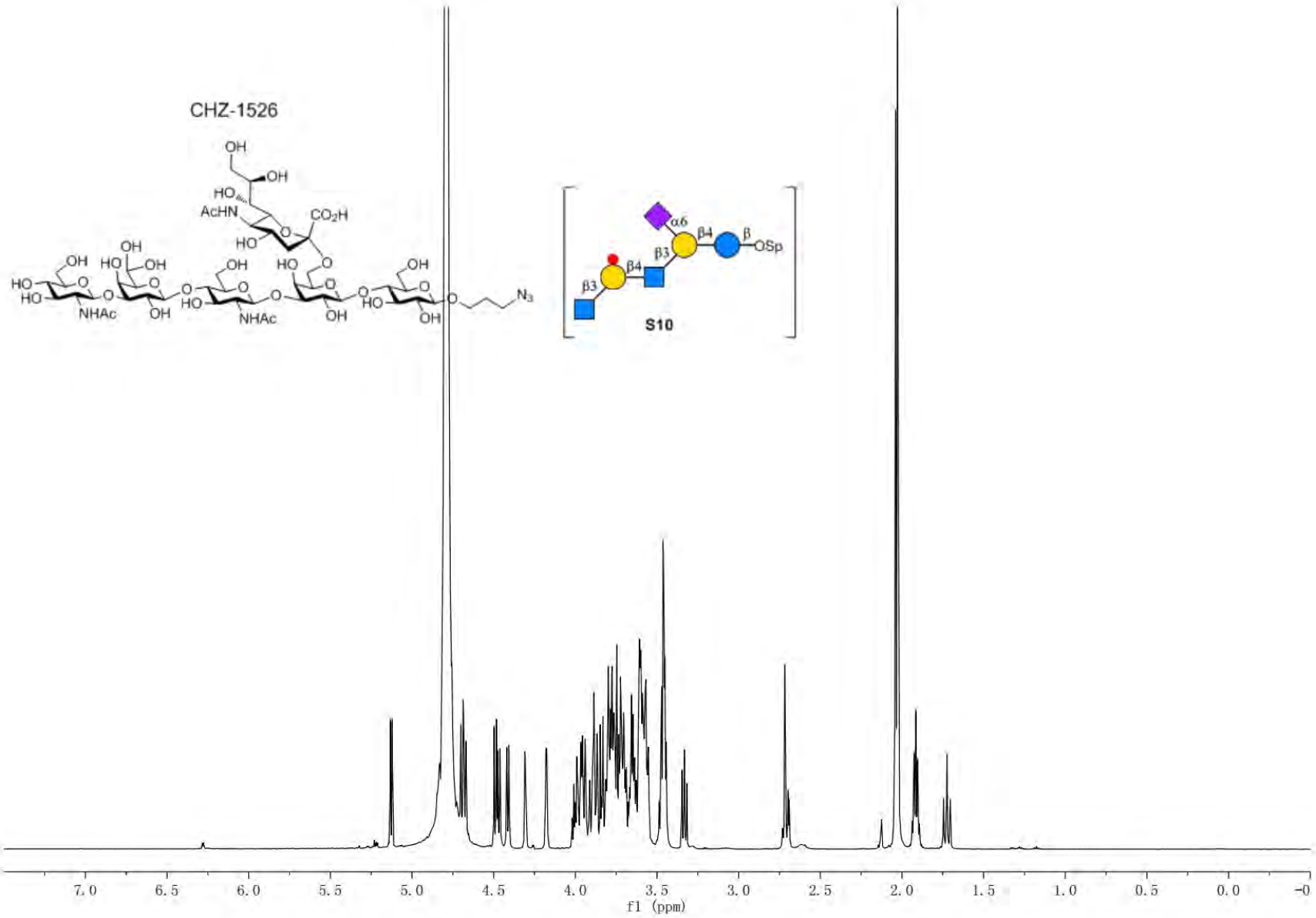
HMBC spectra of 36



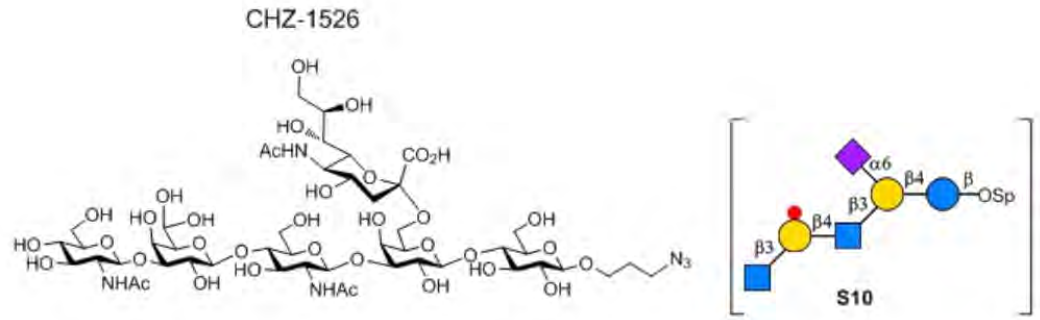
HSQC spectra of 36

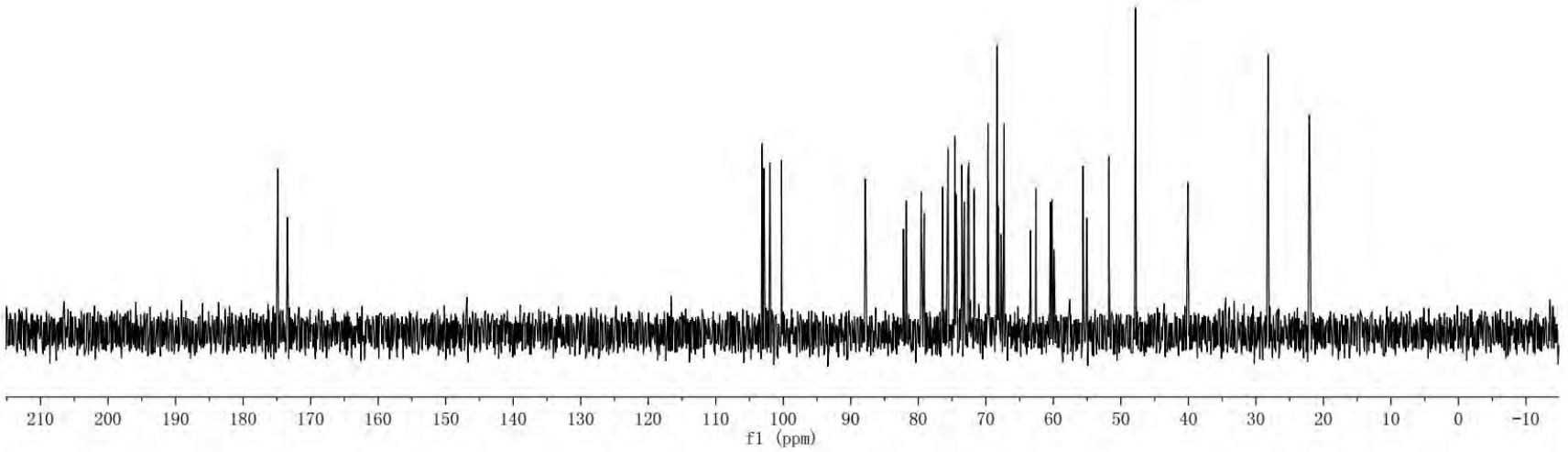


HSQC-TOCSY spectra of 36

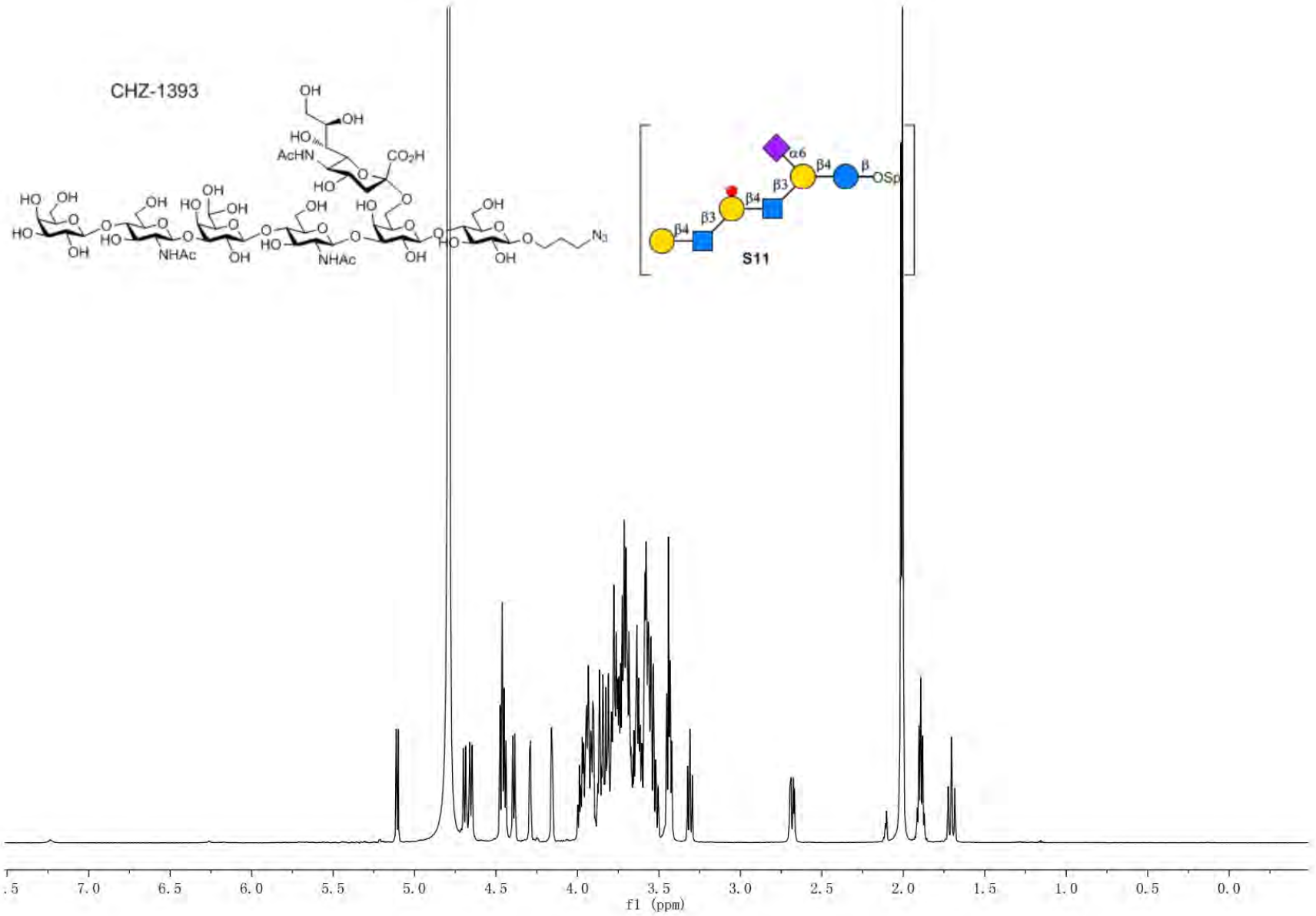


^{1}H NMR of S10

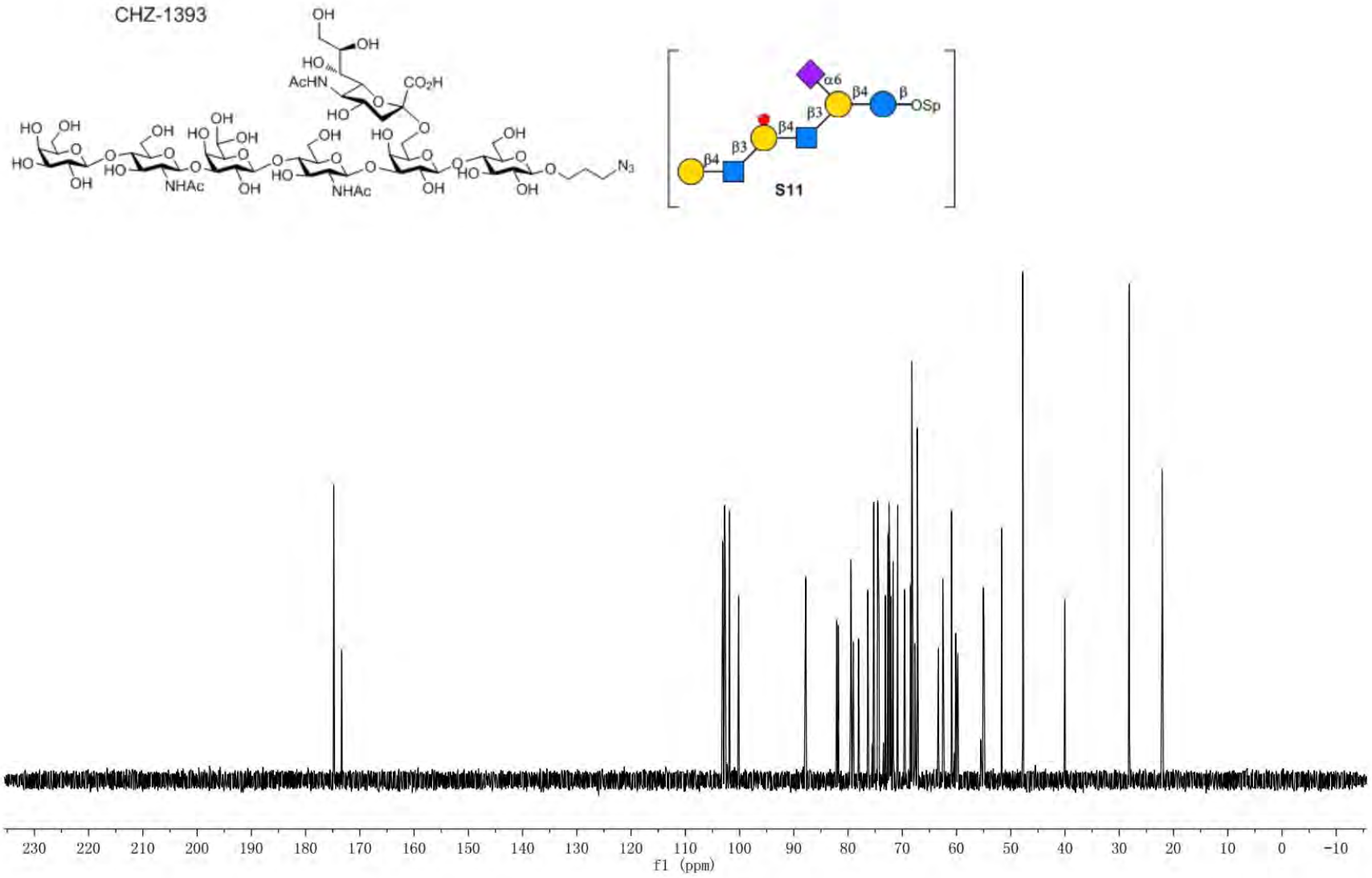




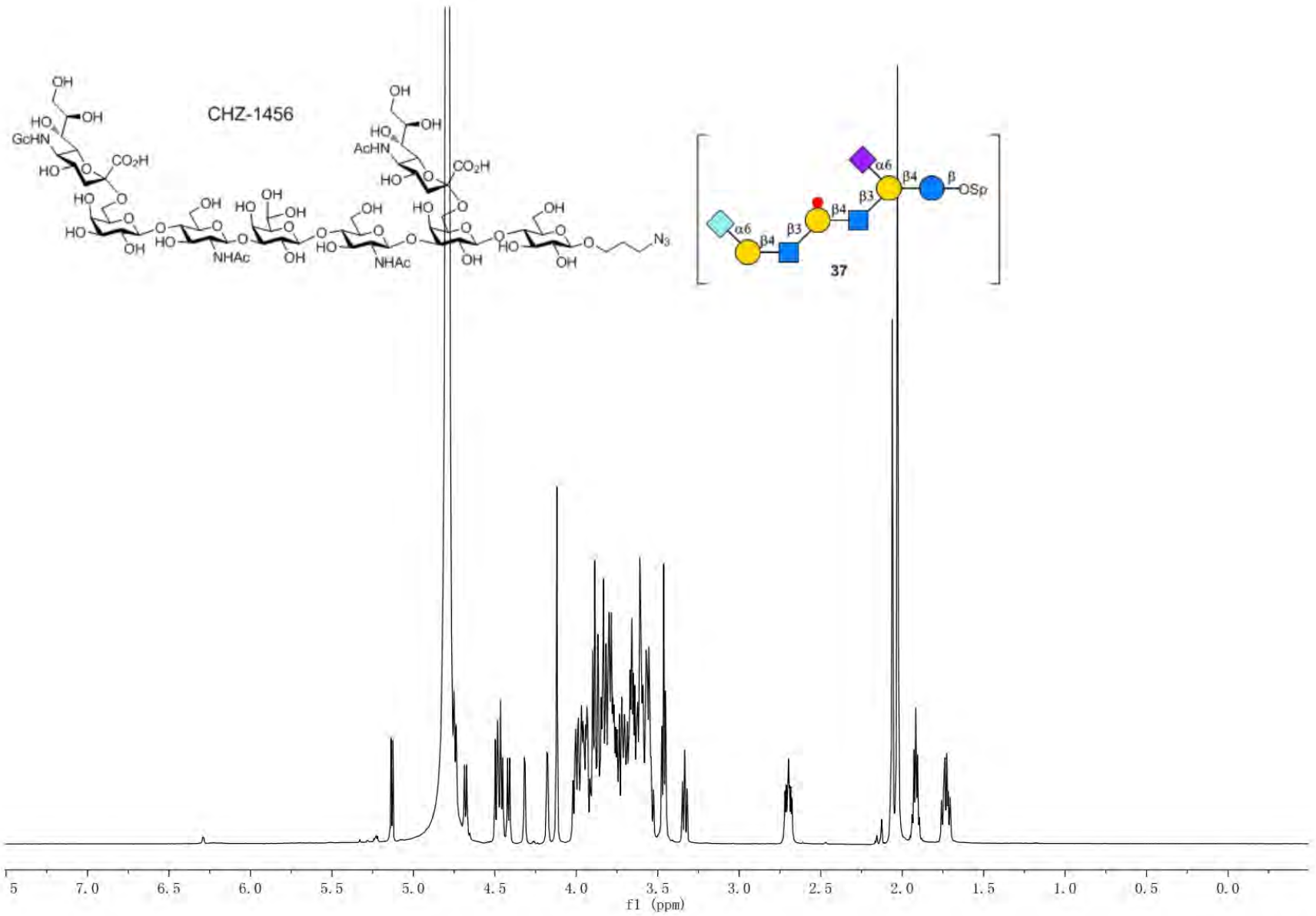
^{13}C NMR of S10



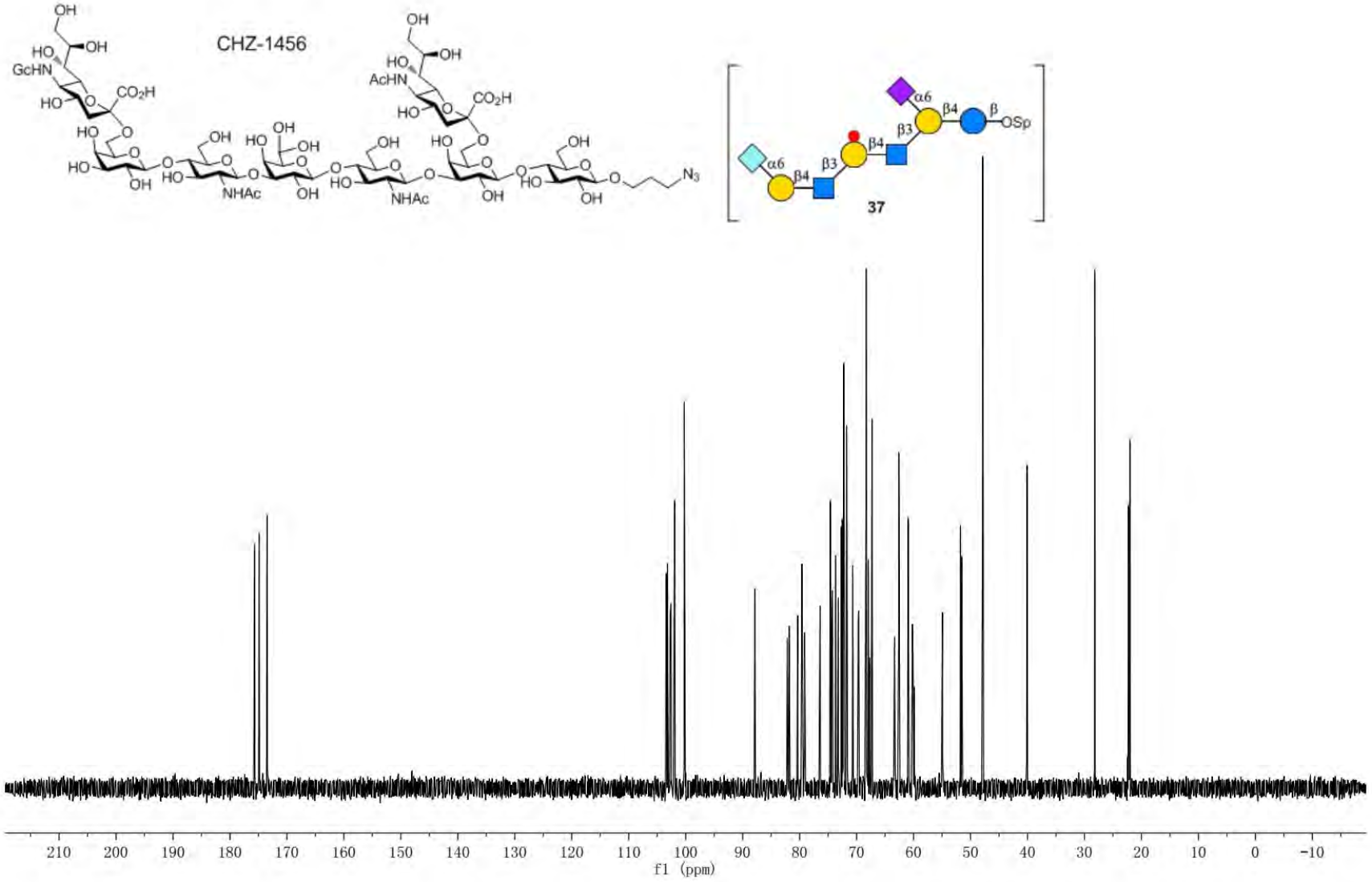
^{1}H NMR of S11



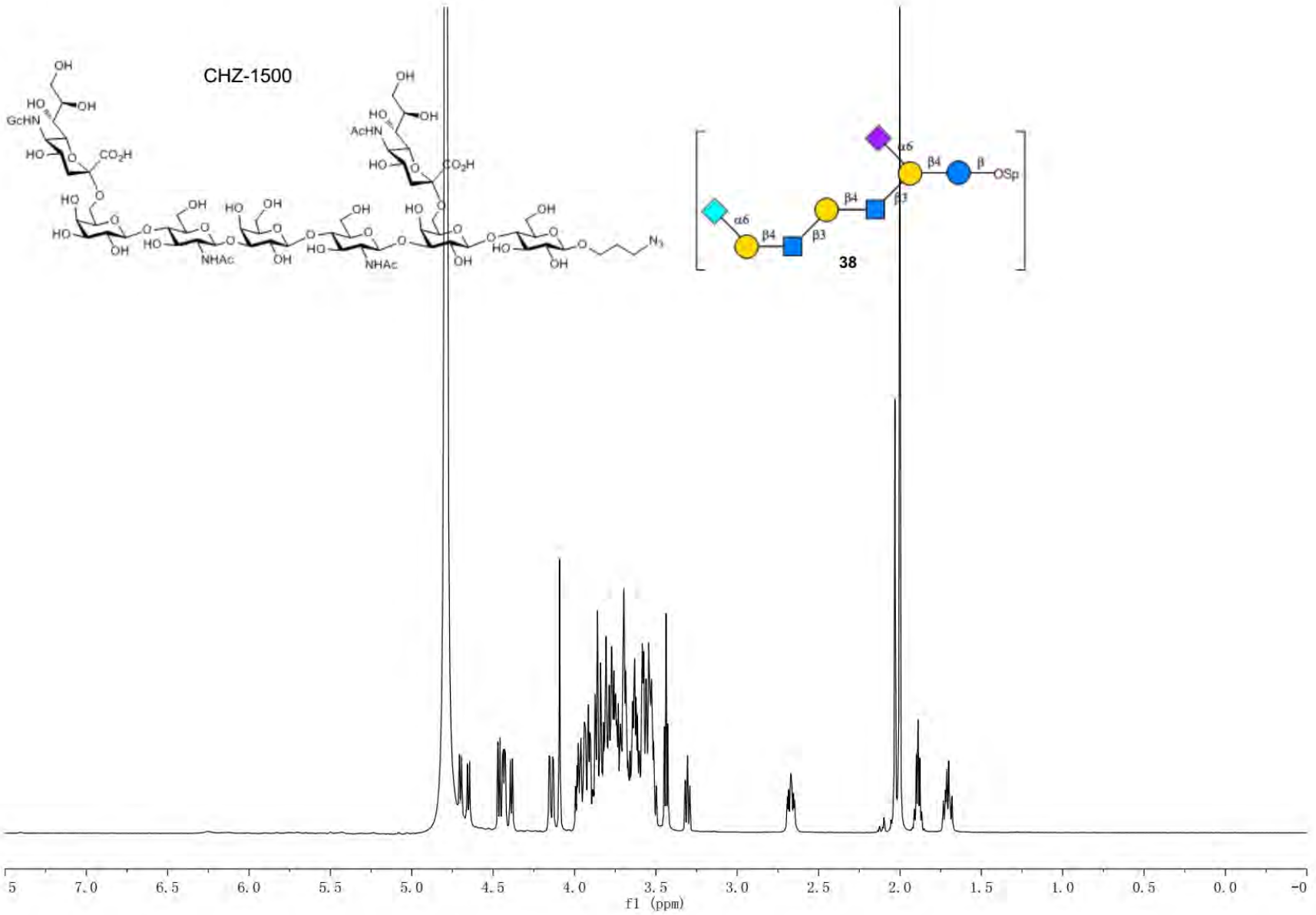
^{13}C NMR of S11



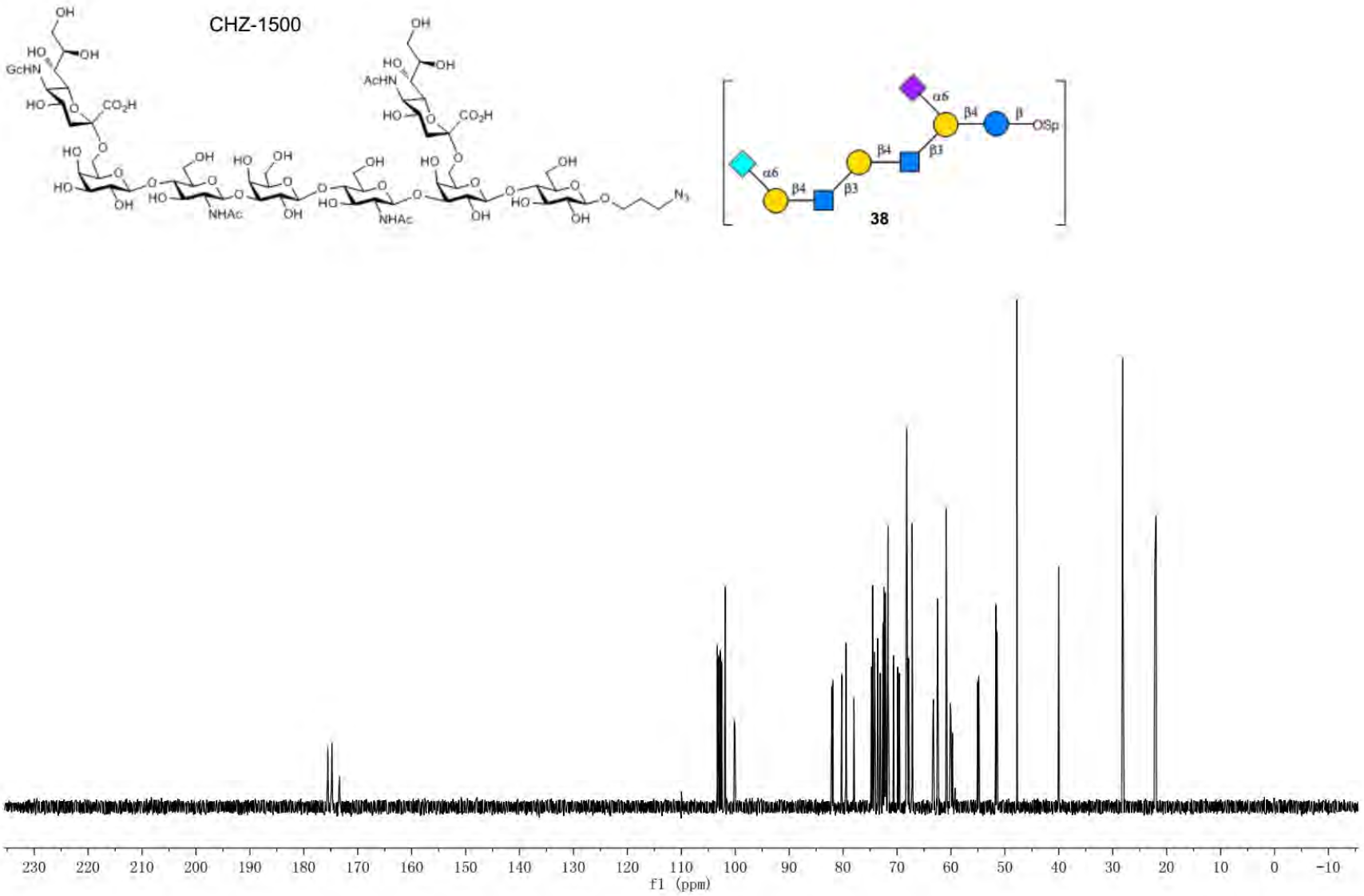
^{1}H NMR of 37



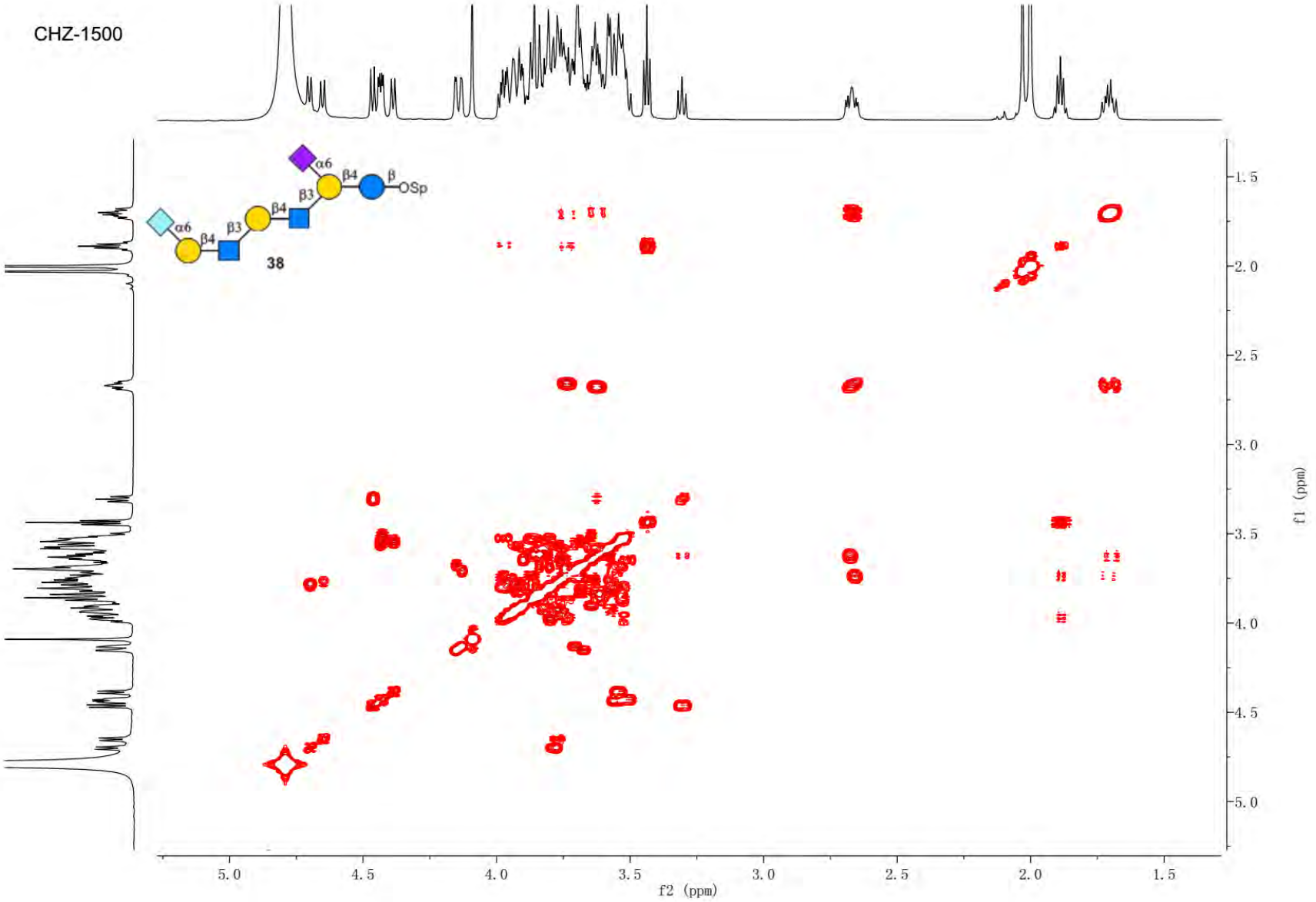
^{13}C NMR of 37



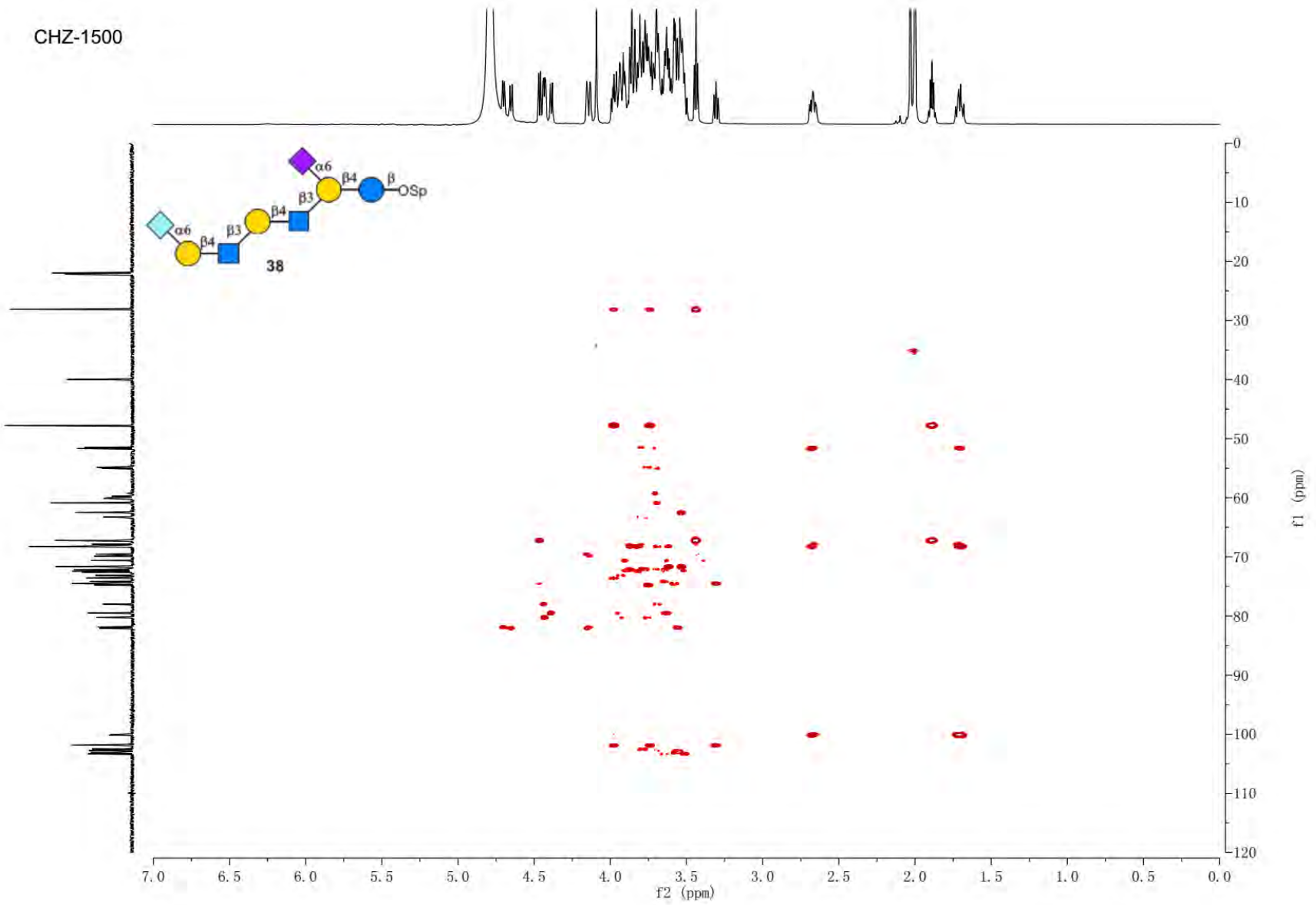
^{1}H NMR of 38



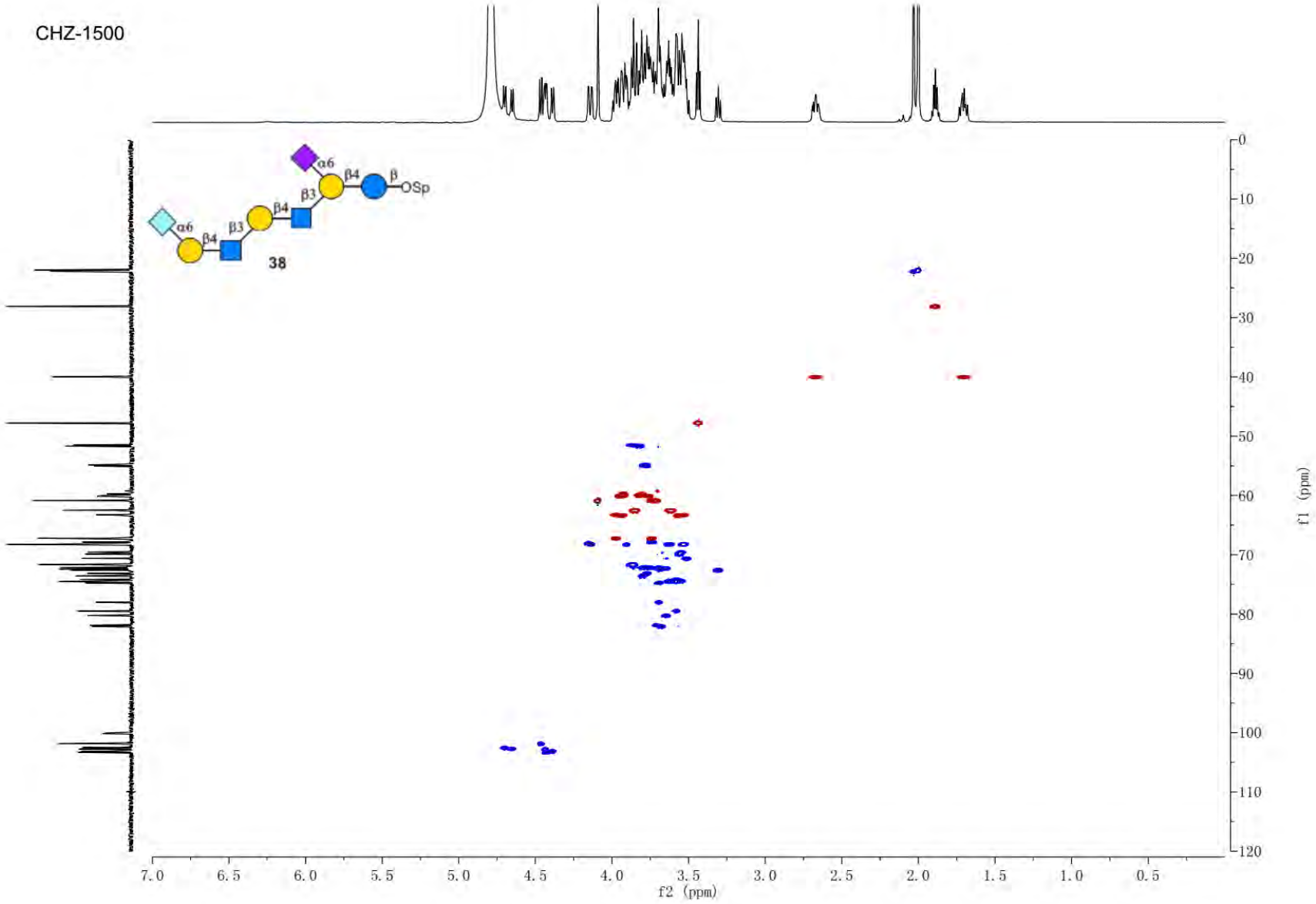
^{13}C NMR of 38



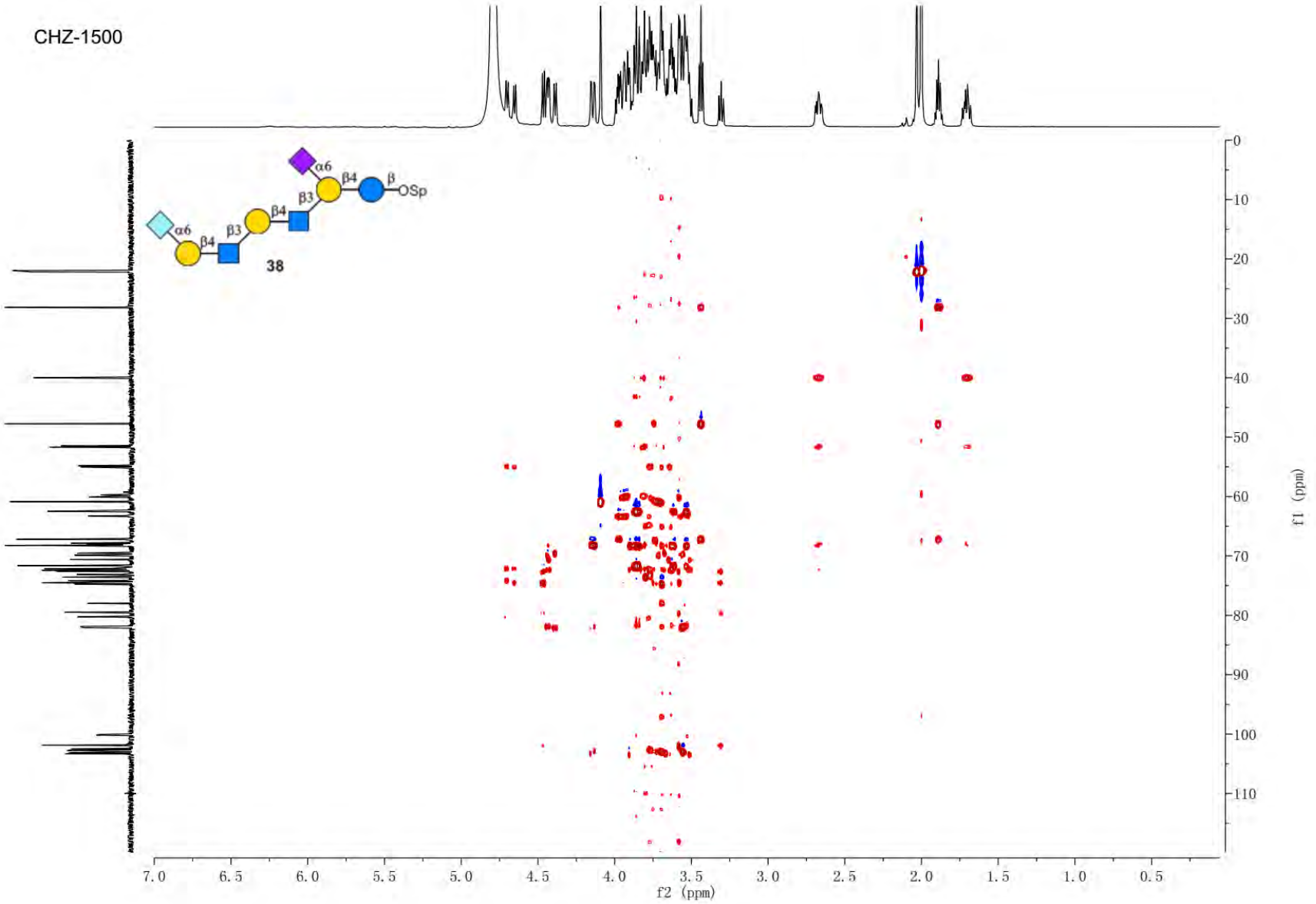
COSY spectra of 38



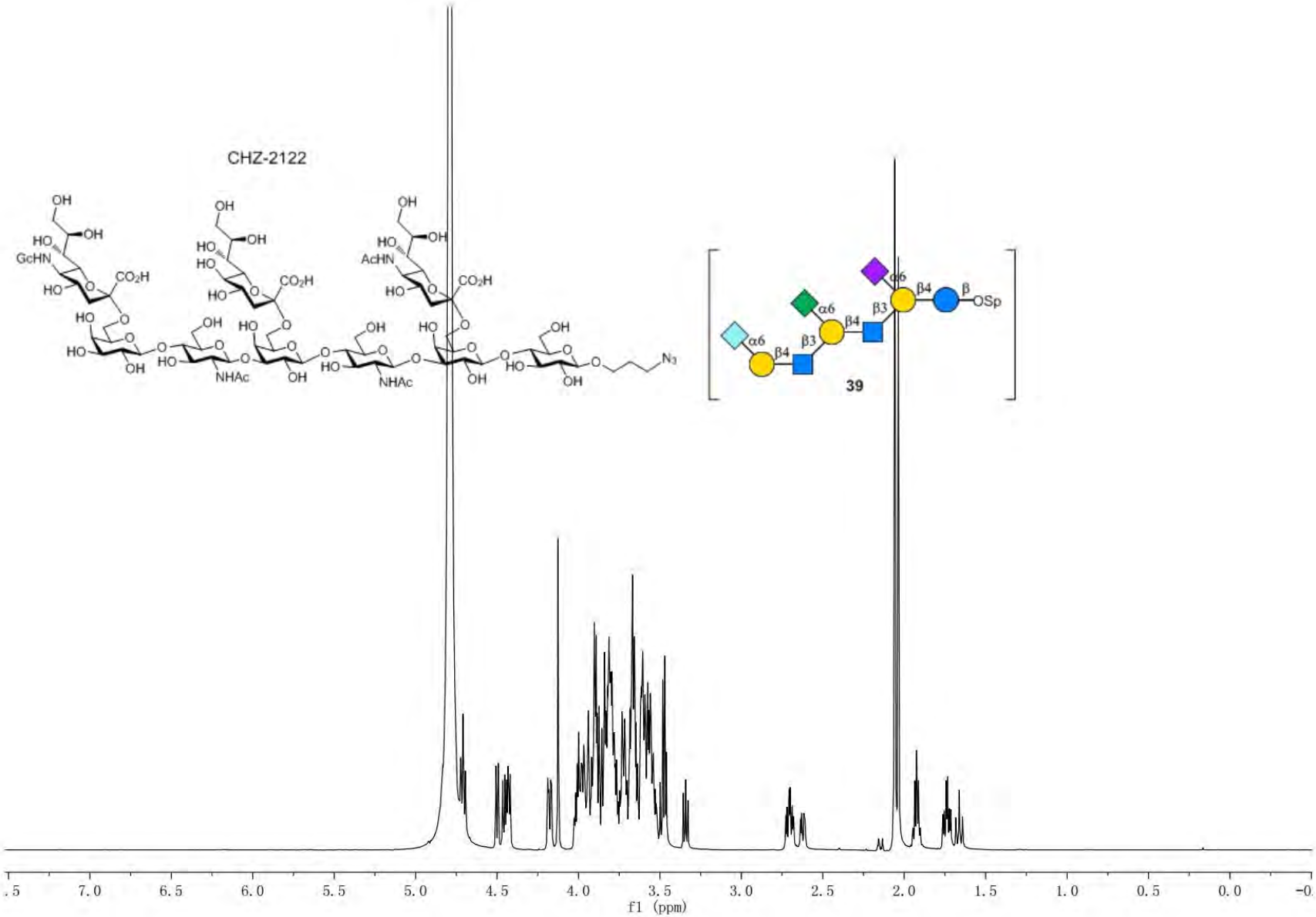
HMBC spectra of 38



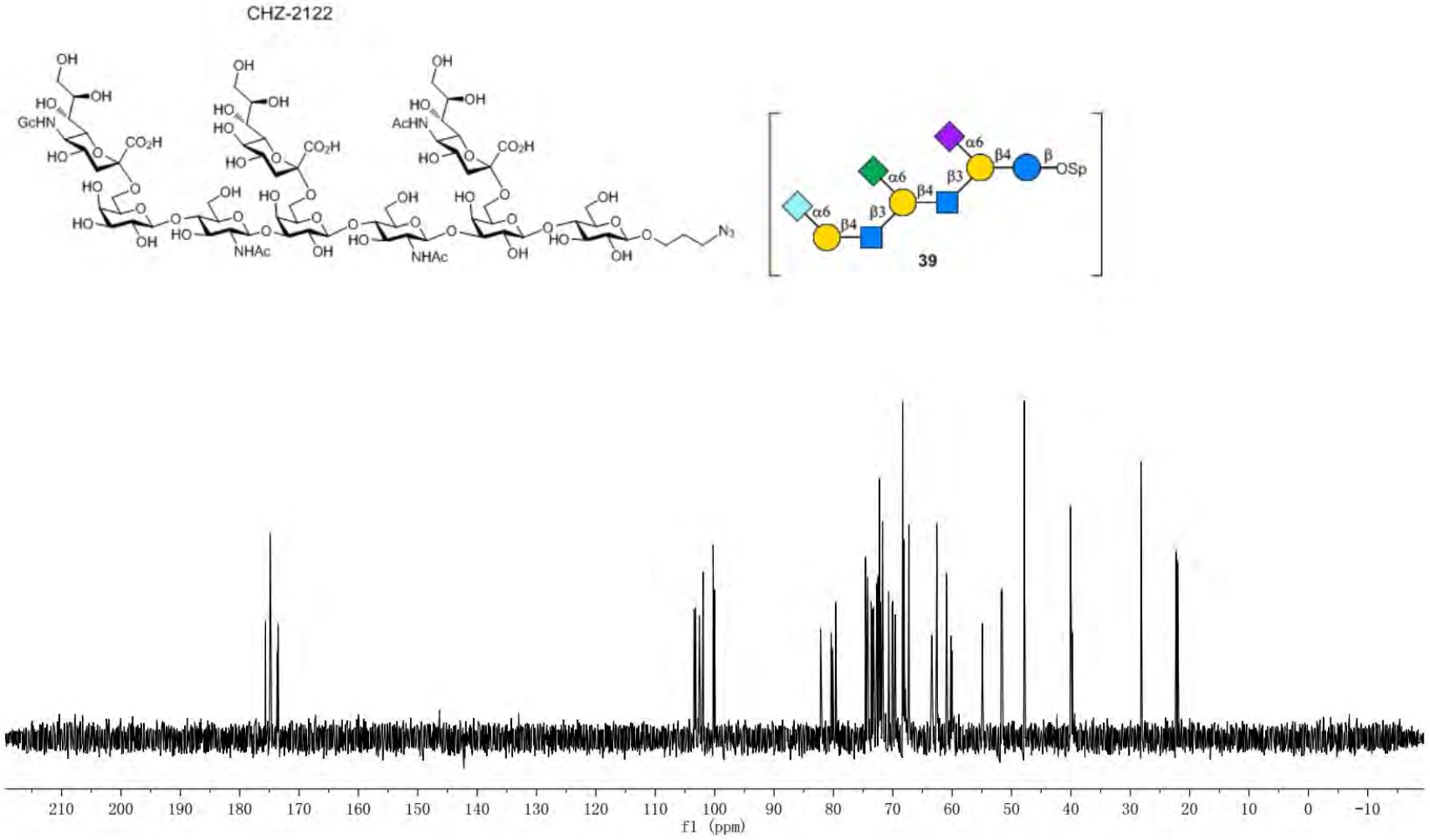
HSQC spectra of 38



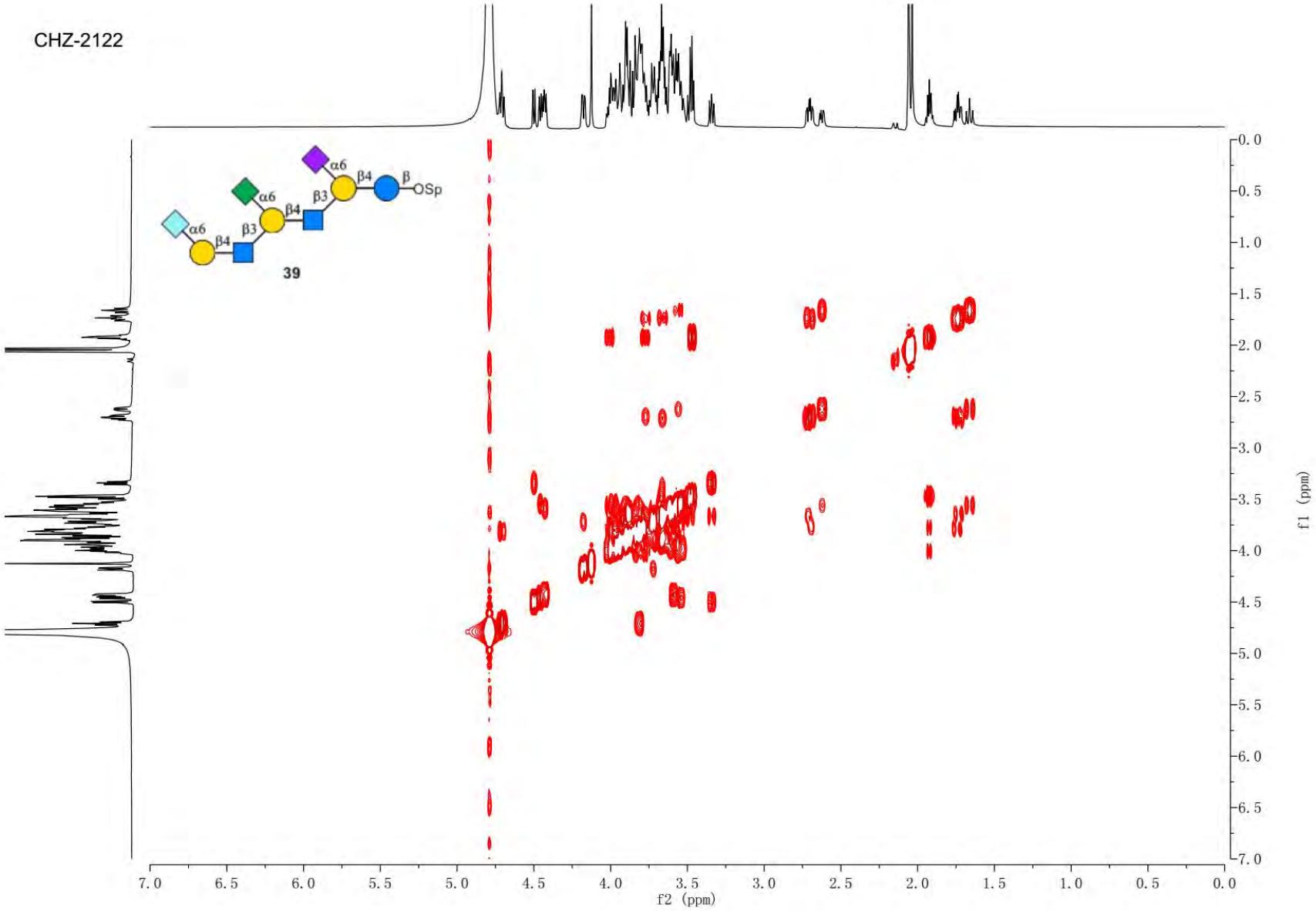
HSQC-TOCSY spectra of 38



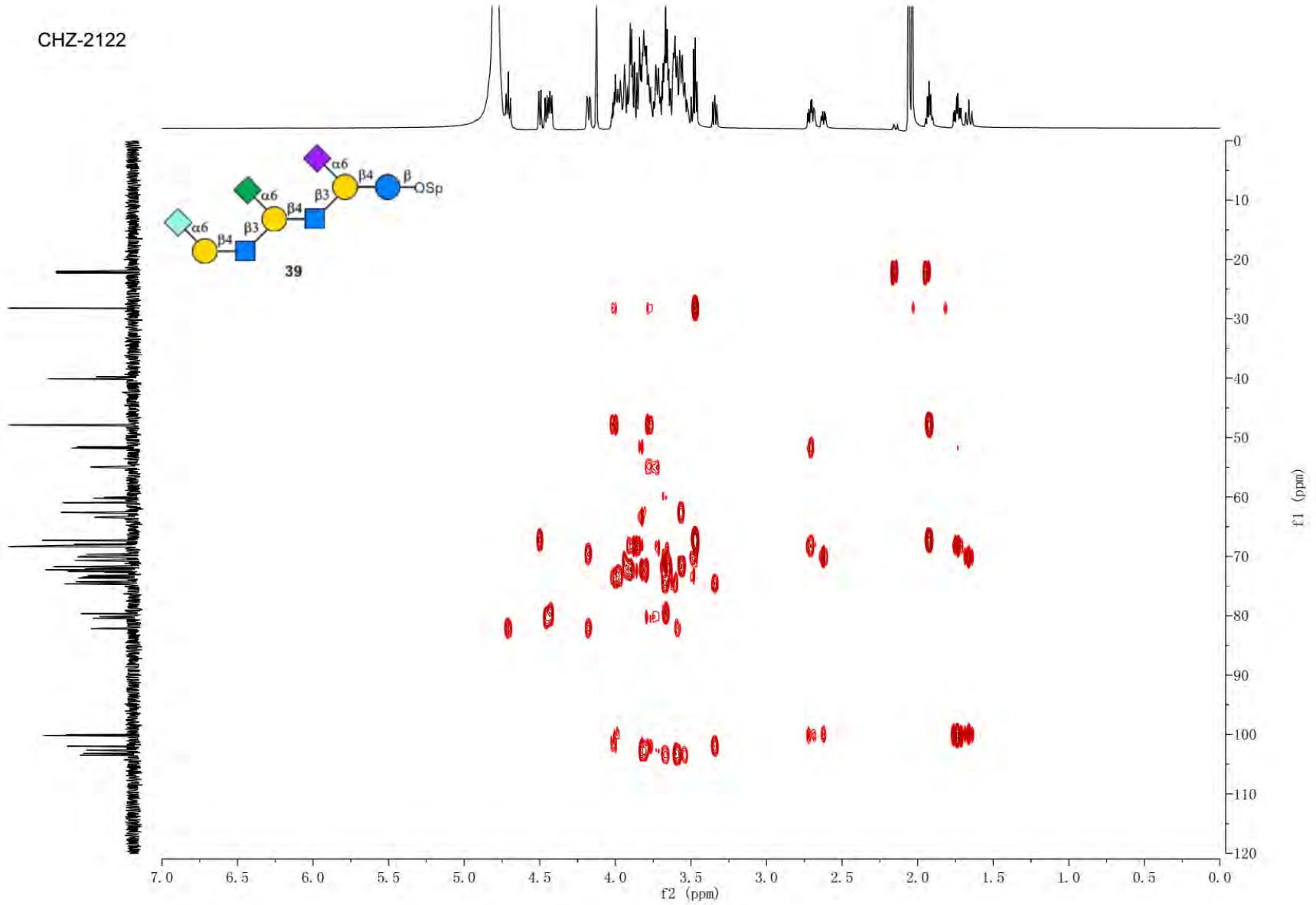
^{1}H NMR of 39


CHZ-2122
39
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

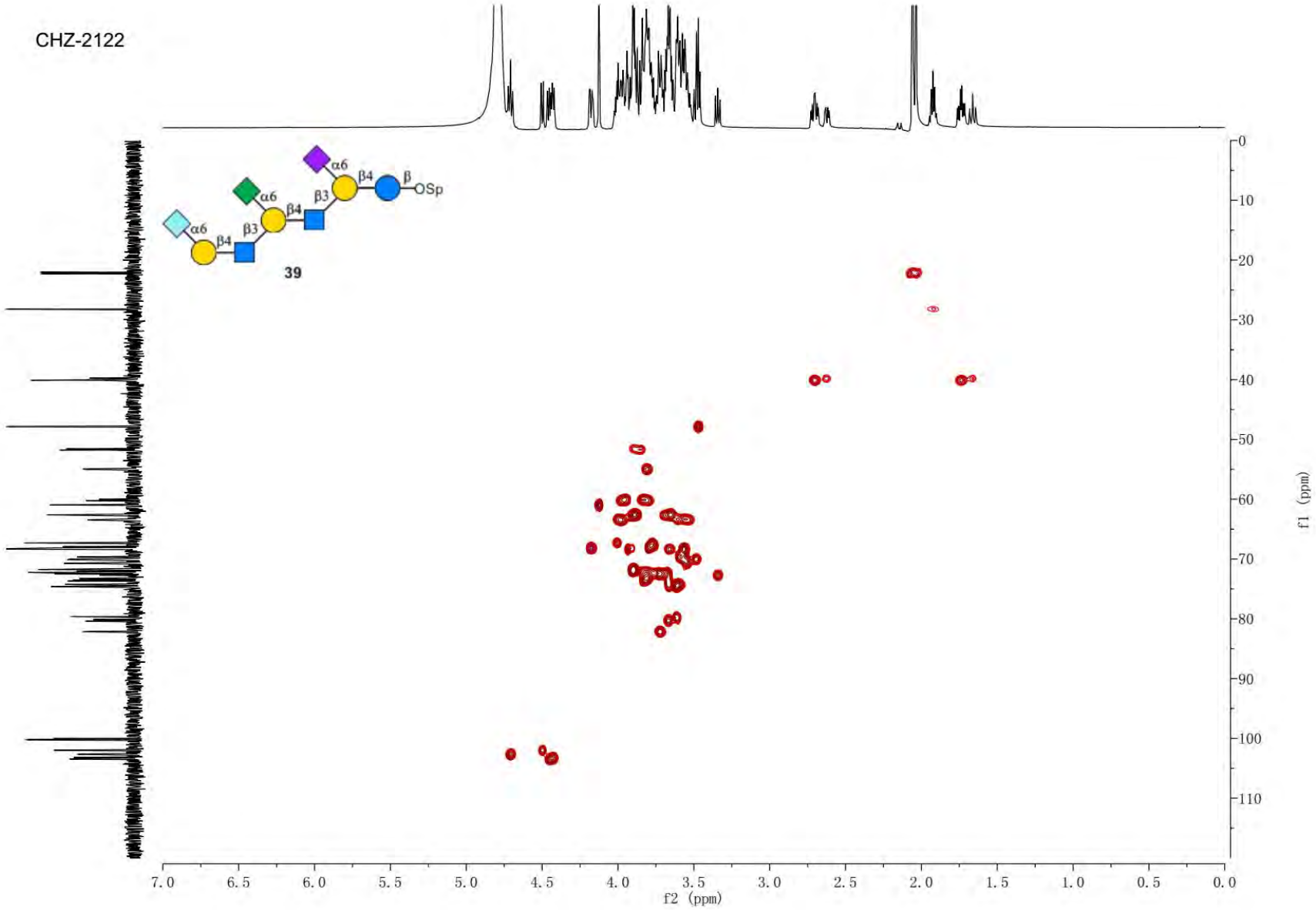
^{13}C NMR of 39



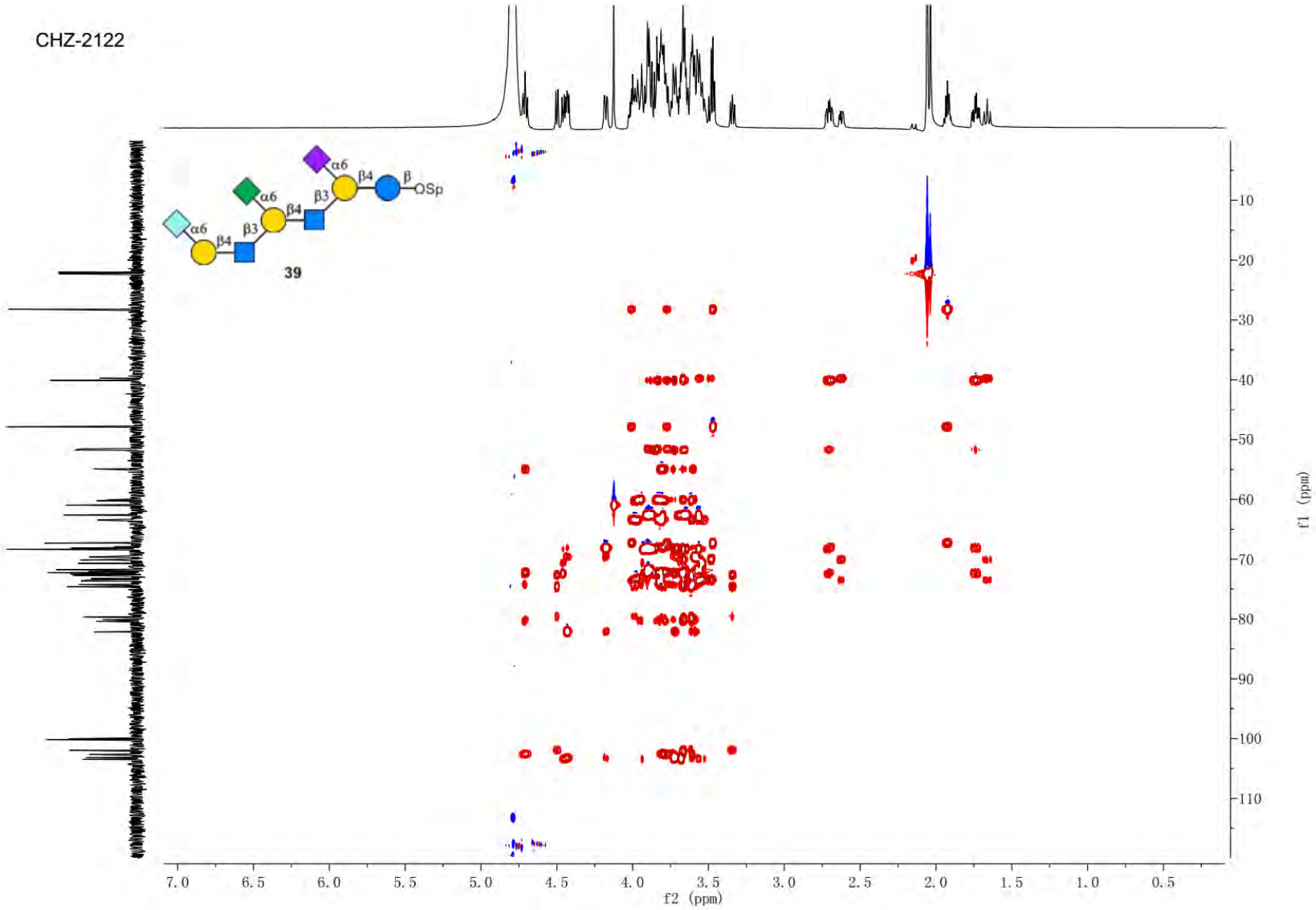
COSY spectra of 39



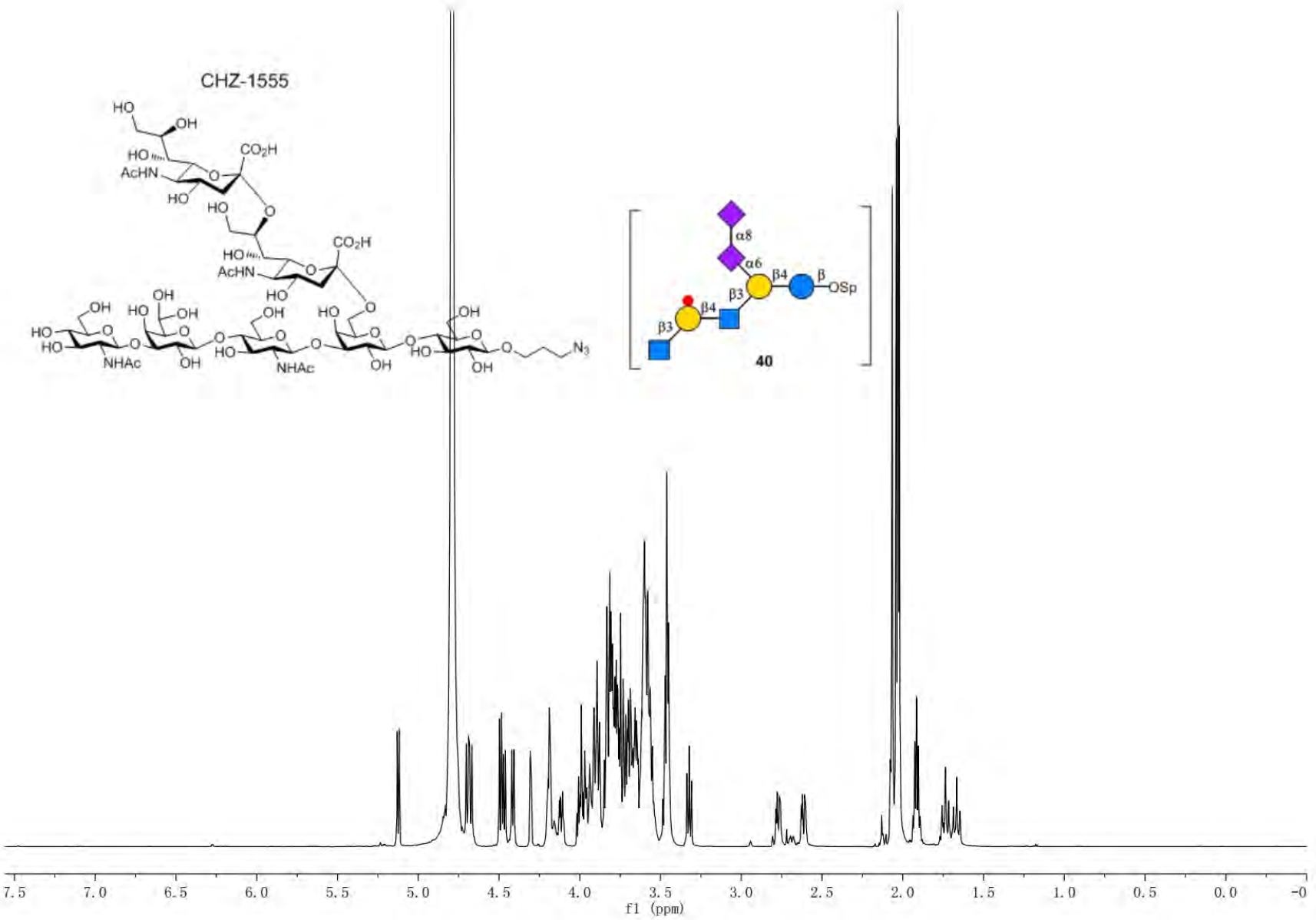
HMBC spectra of 39



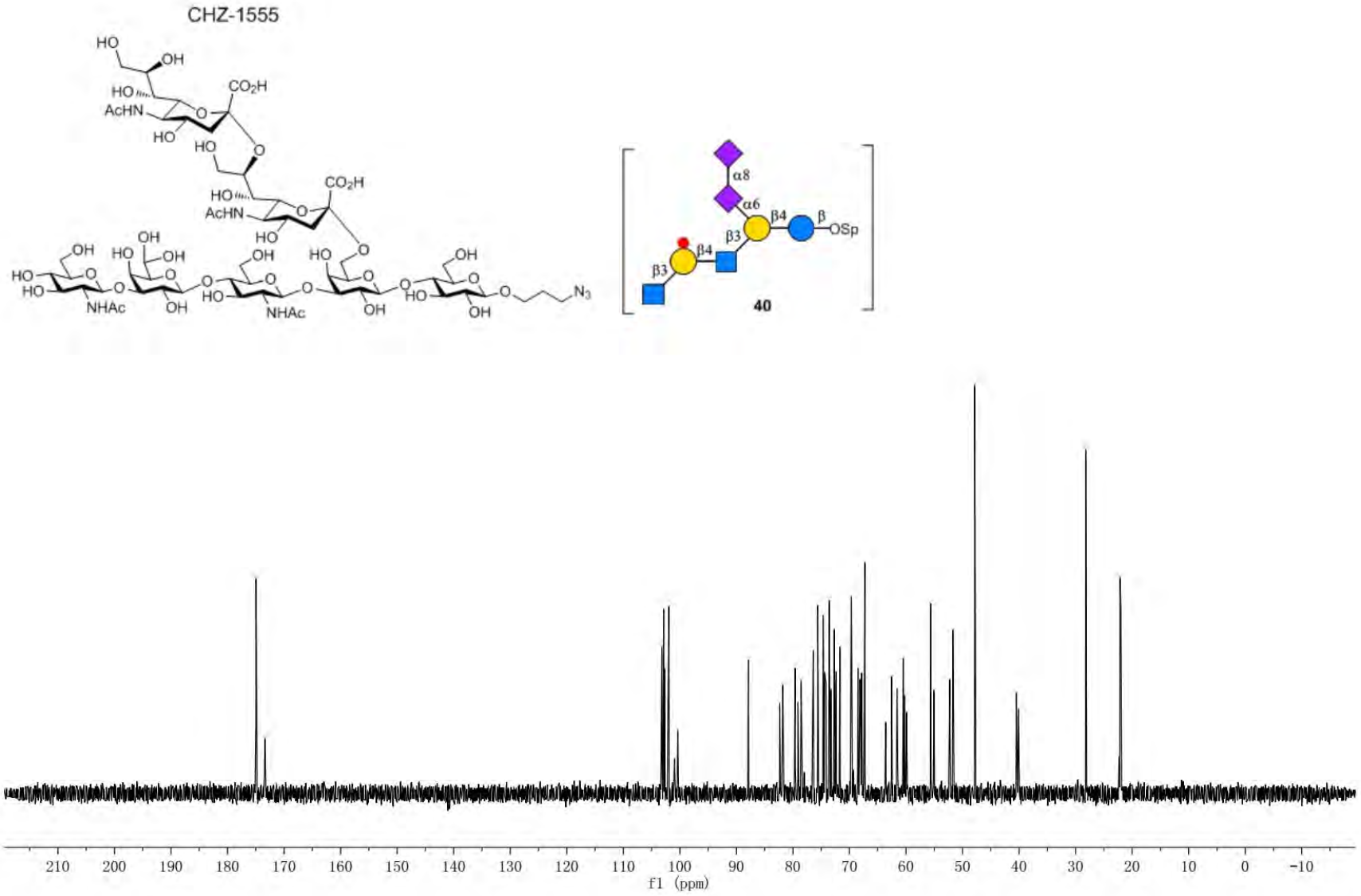
HSQC spectra of 39



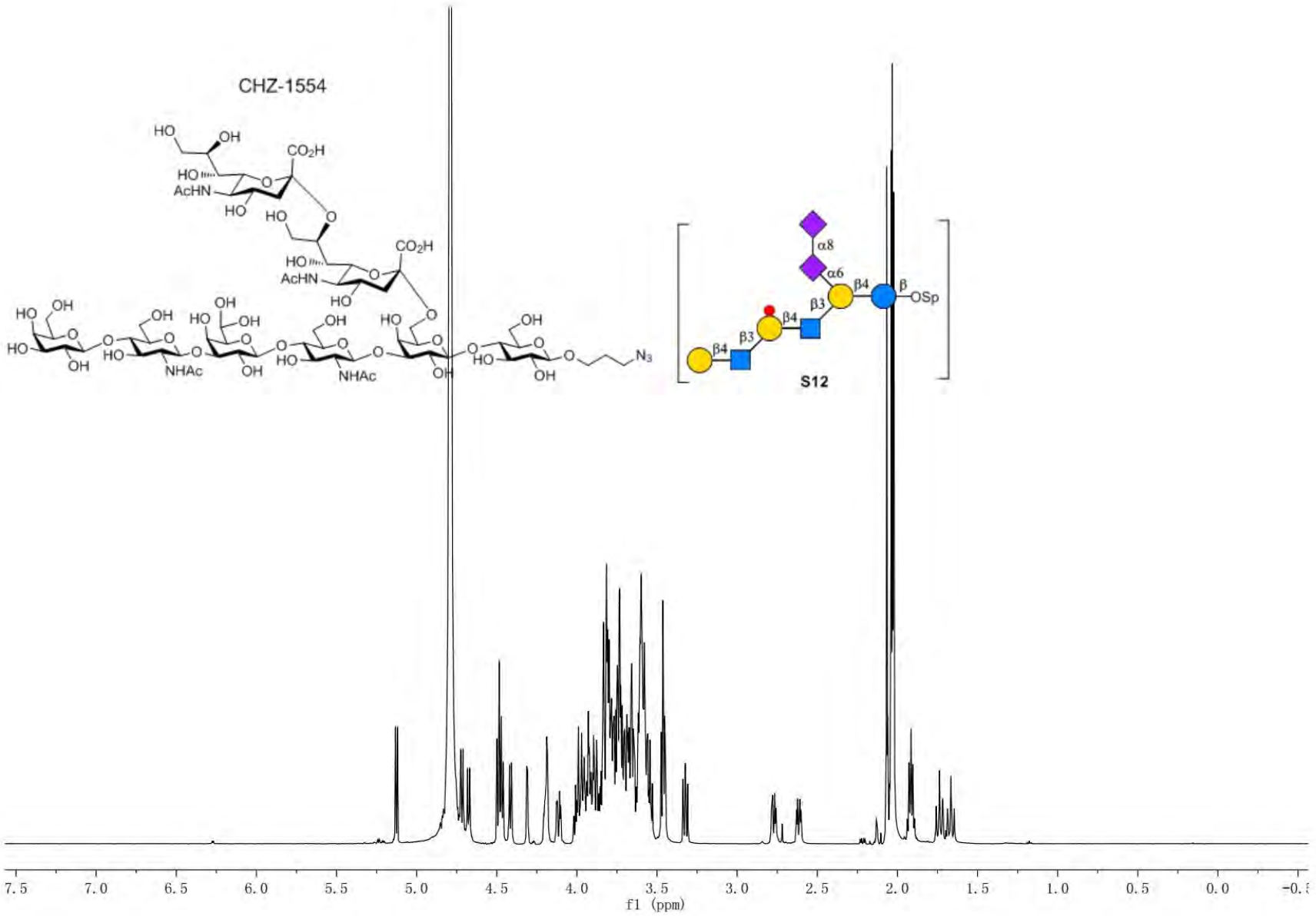
HSQC-TOCSY spectra of 39



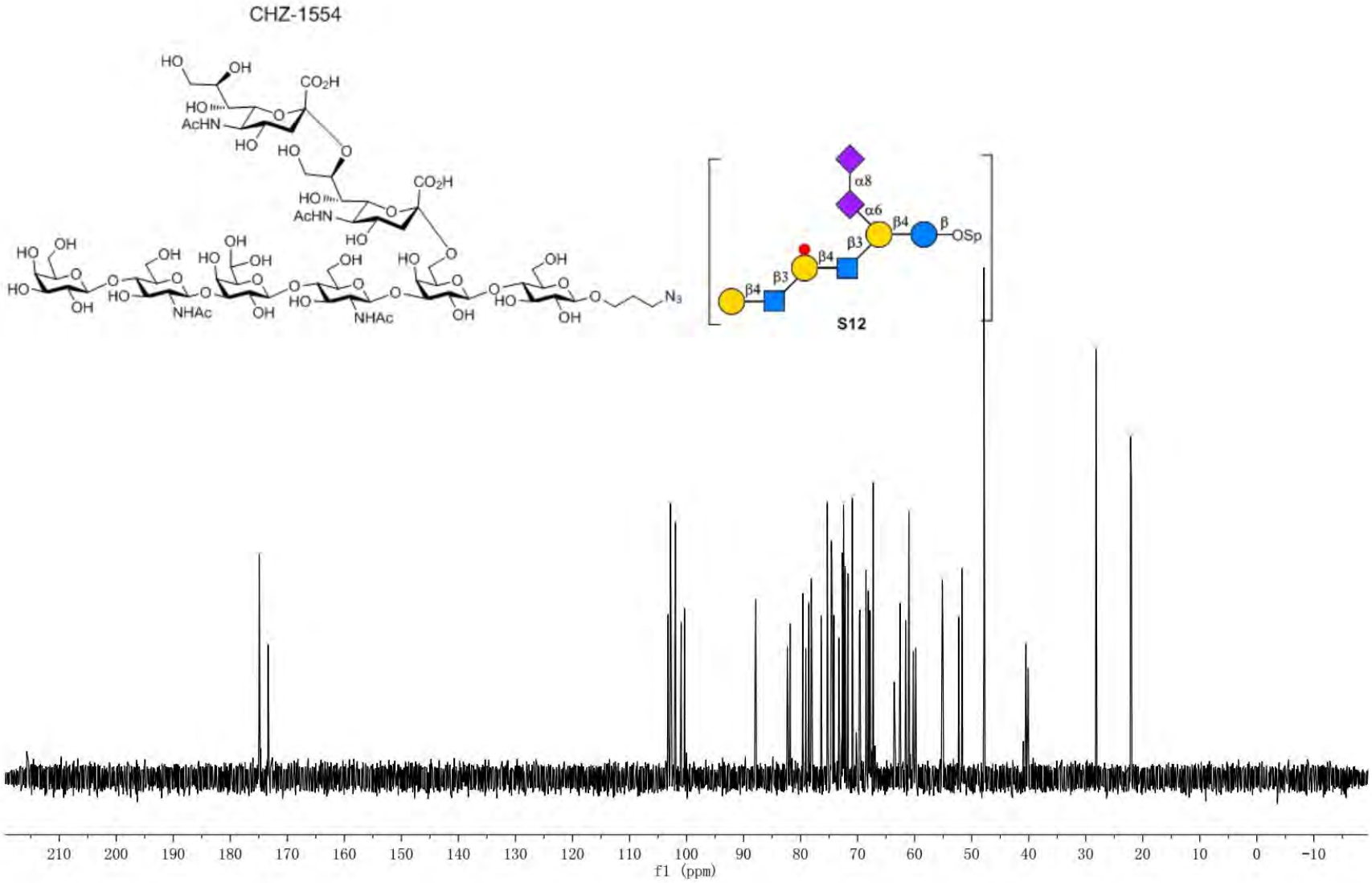
^{1}H NMR of 40



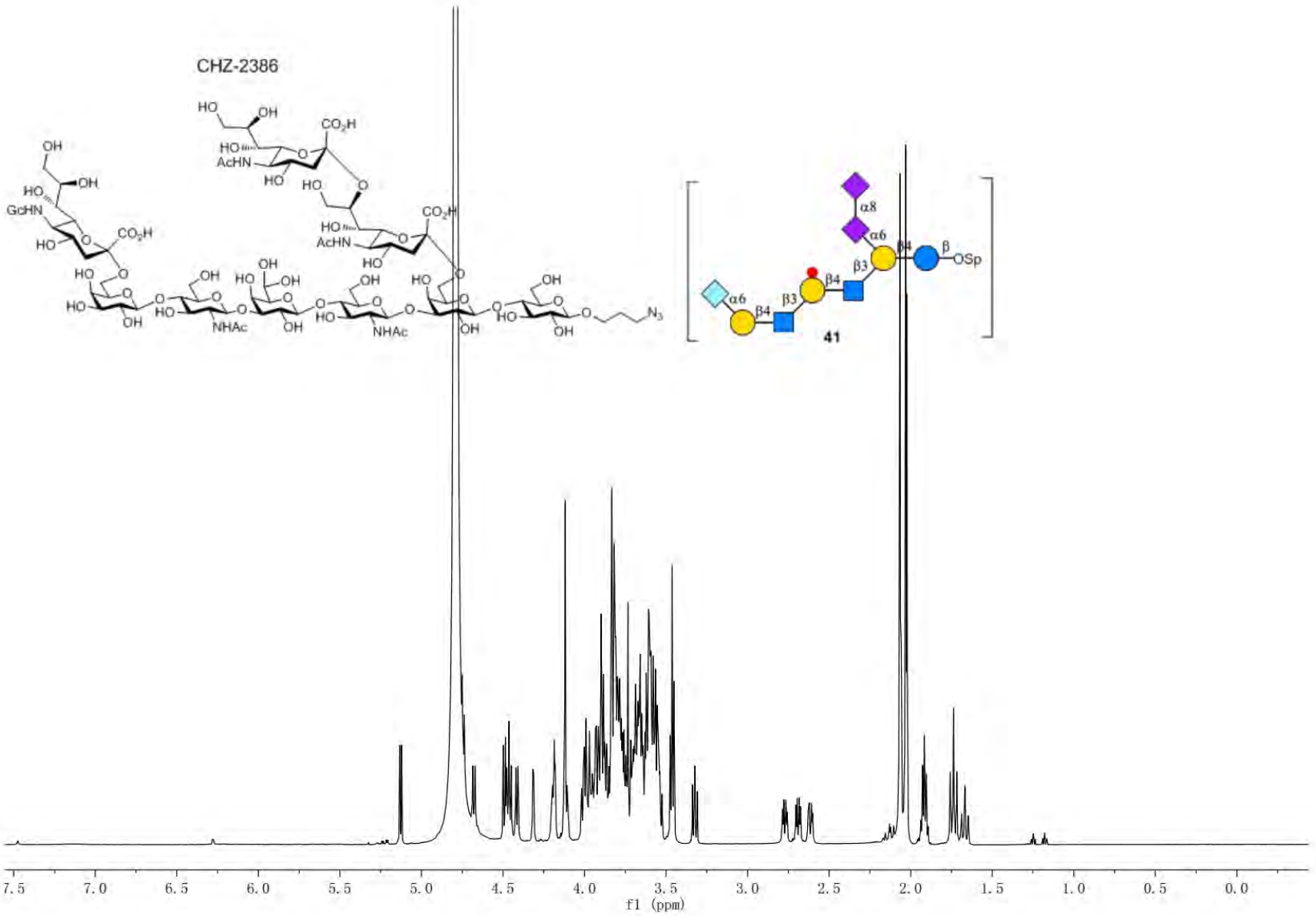
^{13}C NMR of 40



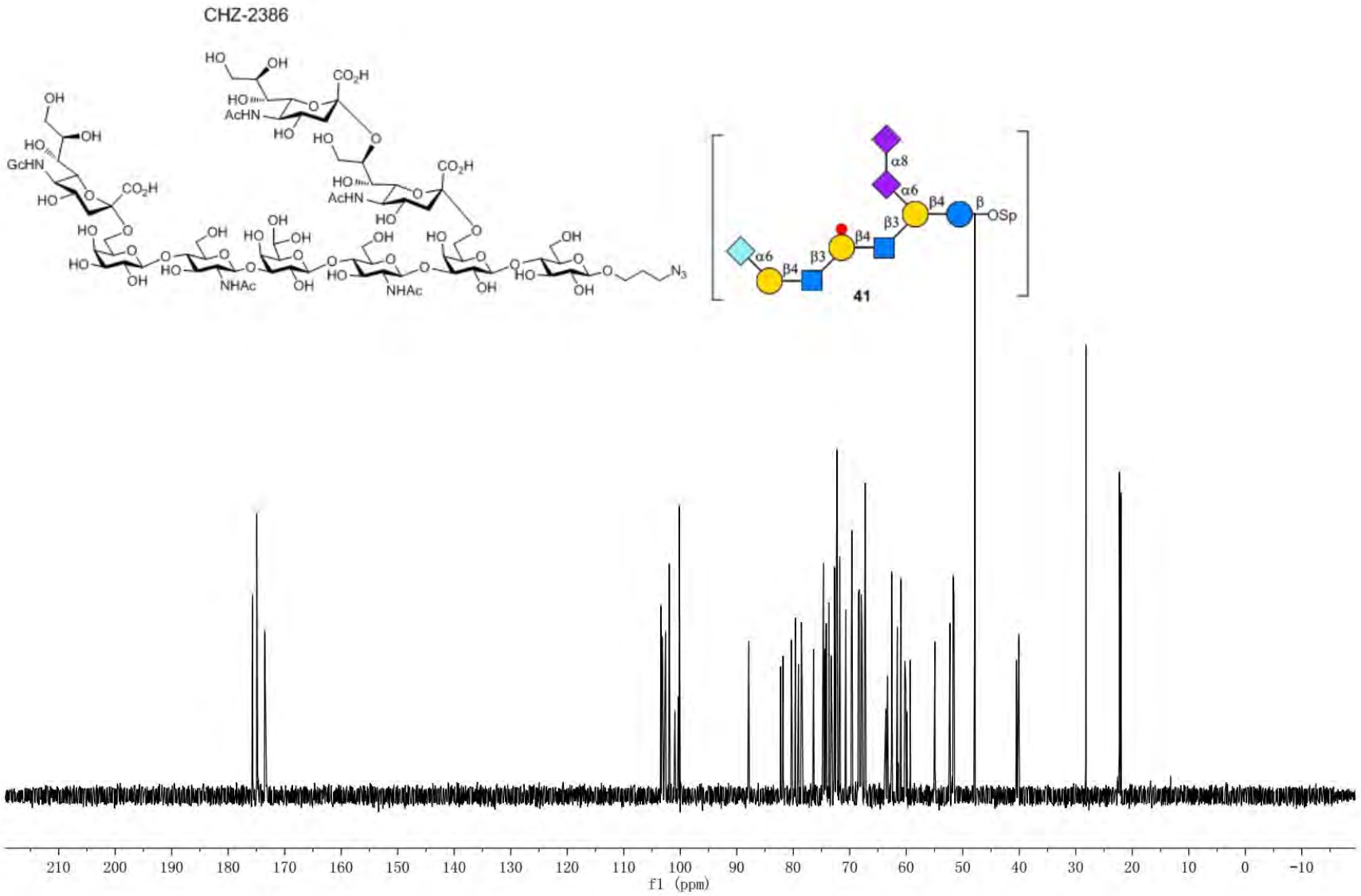
^{1}H NMR of S12



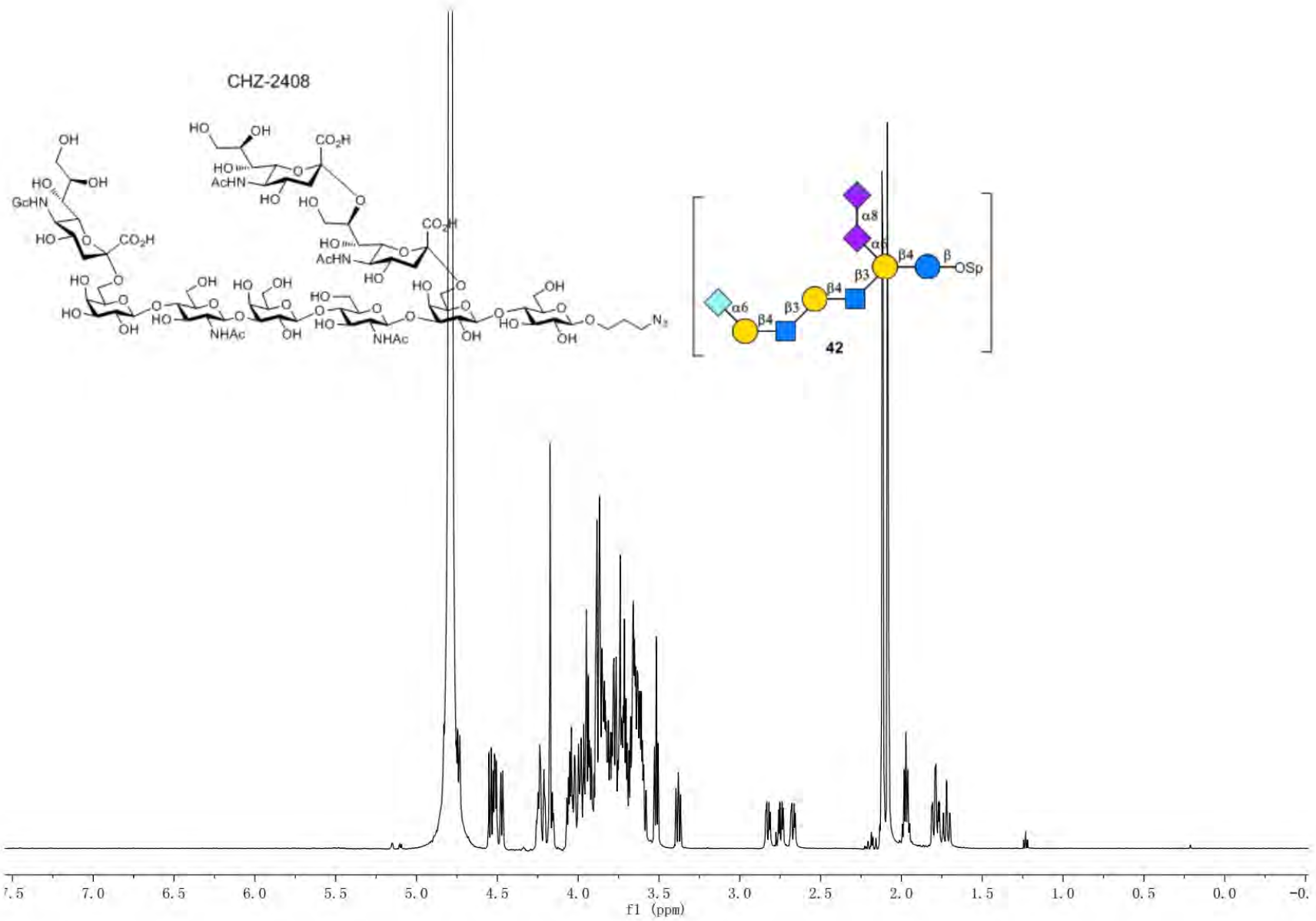
^{13}C NMR of S12



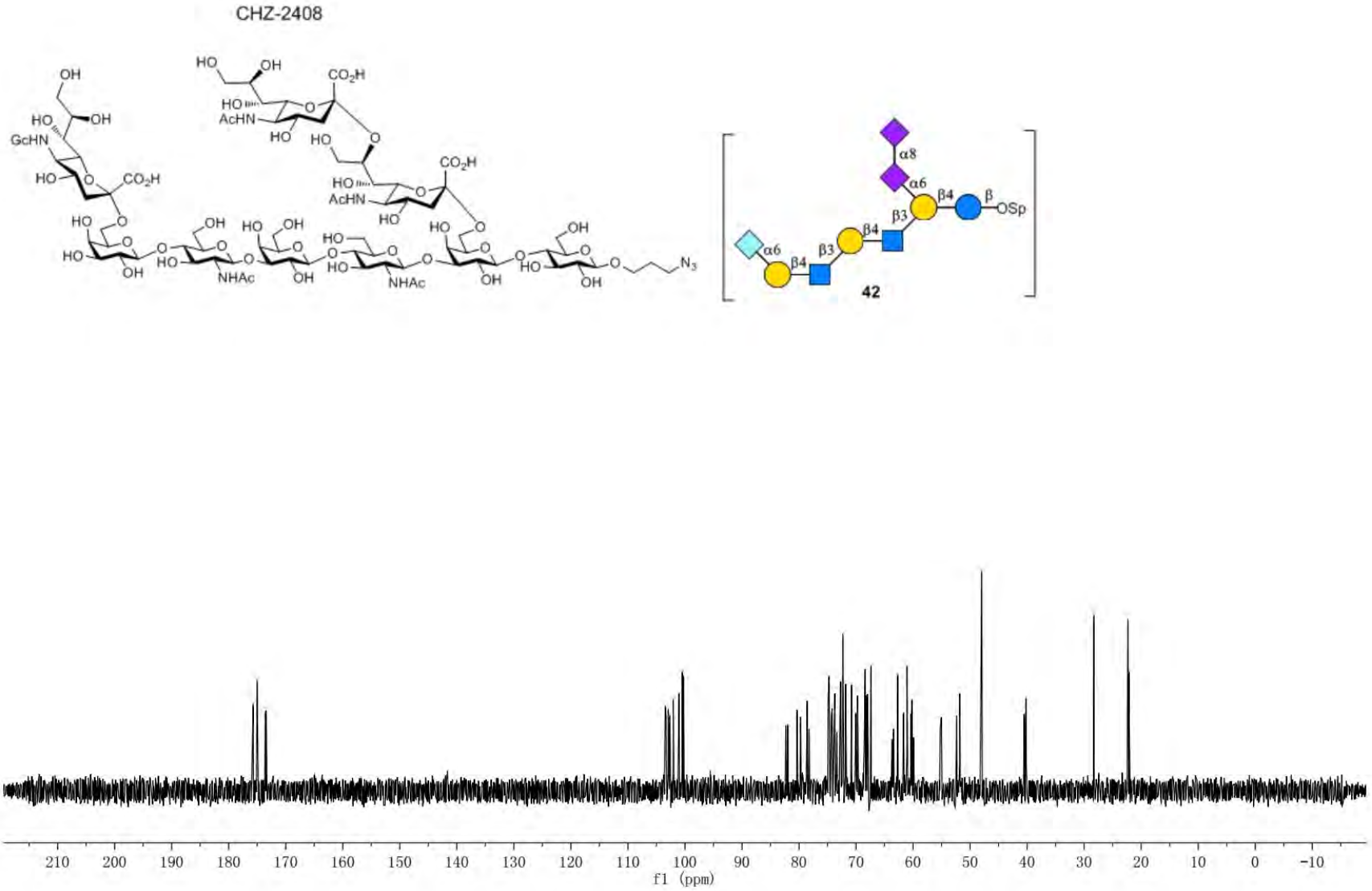
^{1}H NMR of 41



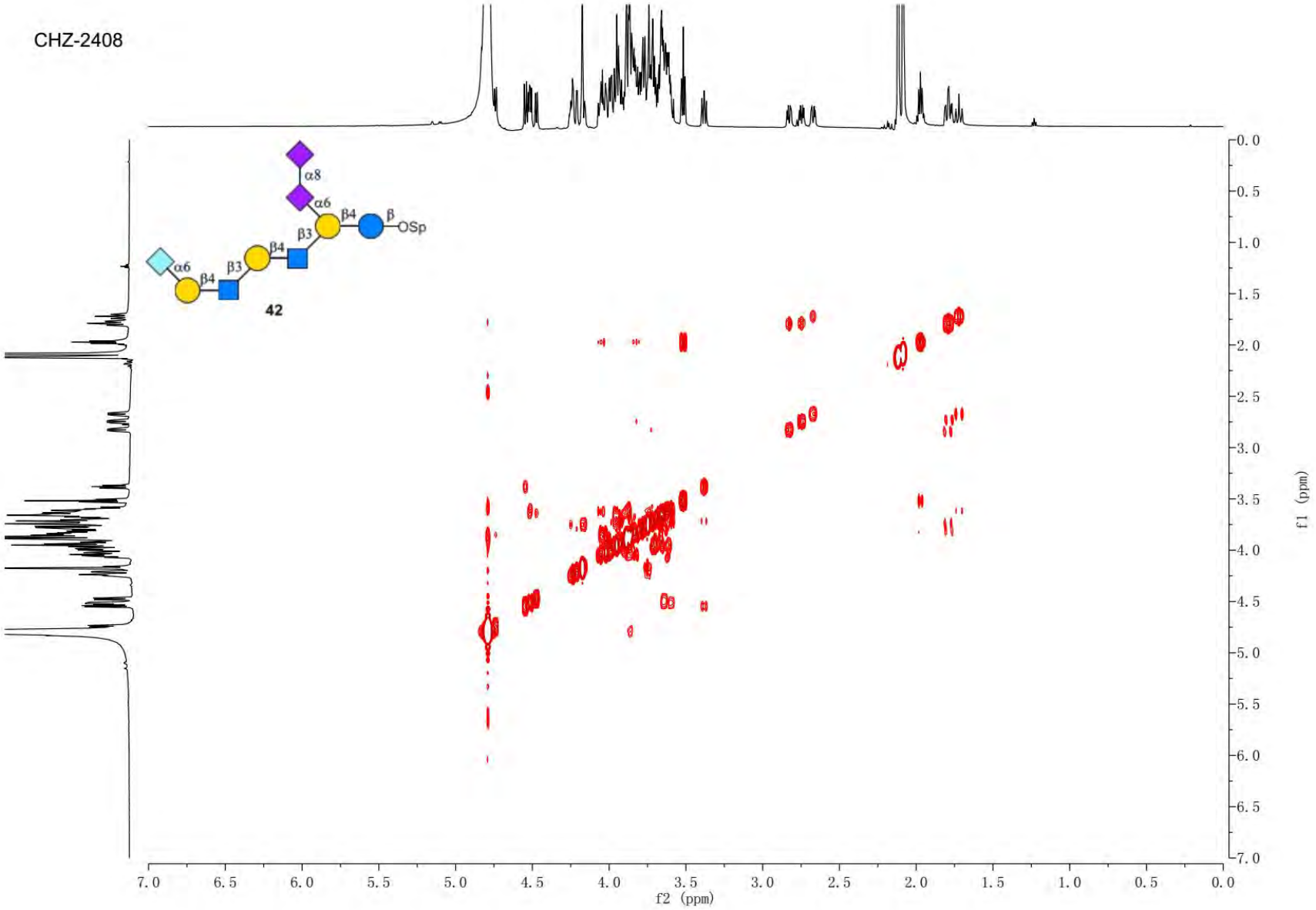
^{13}C NMR of 41



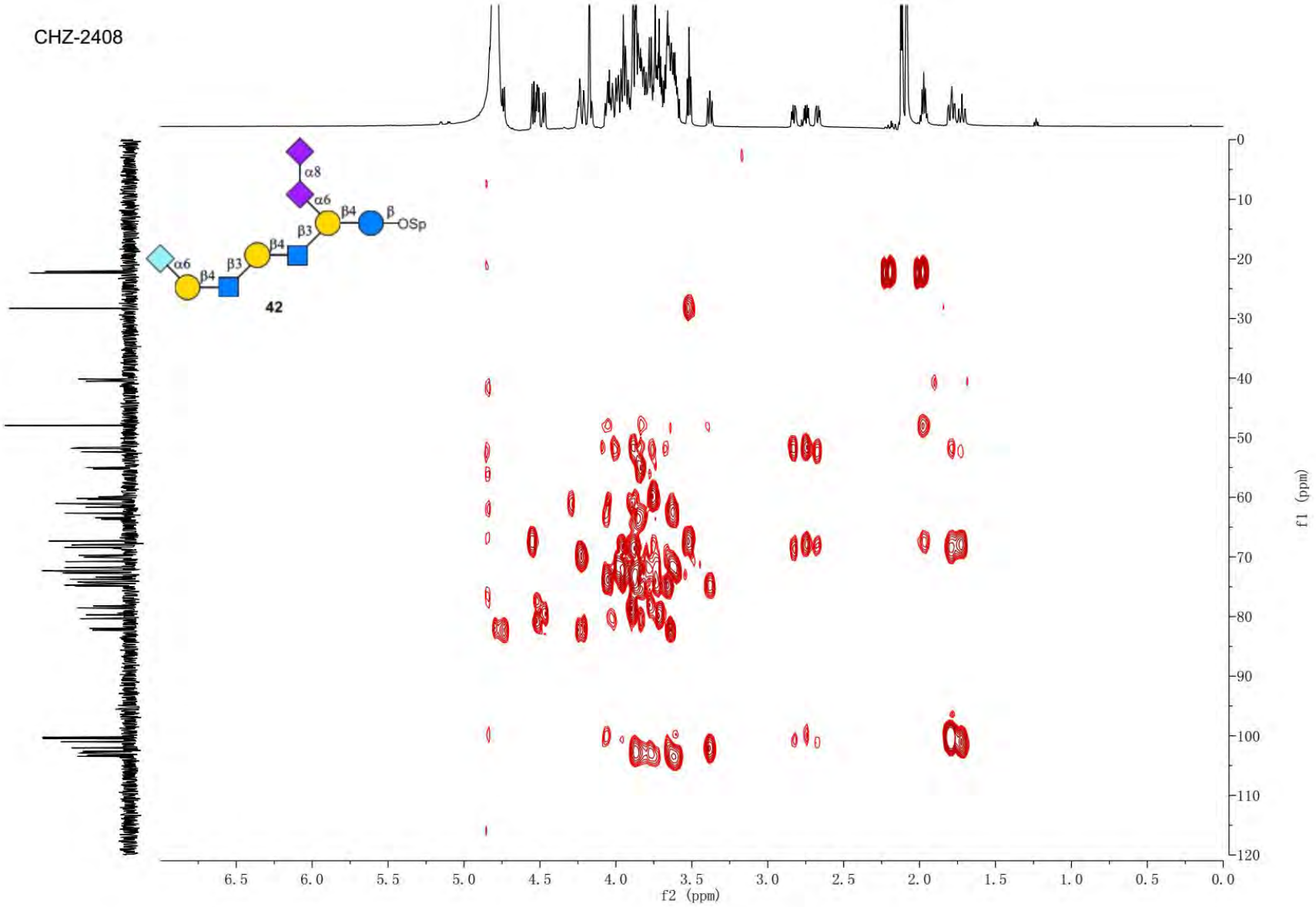
^{1}H NMR of 42



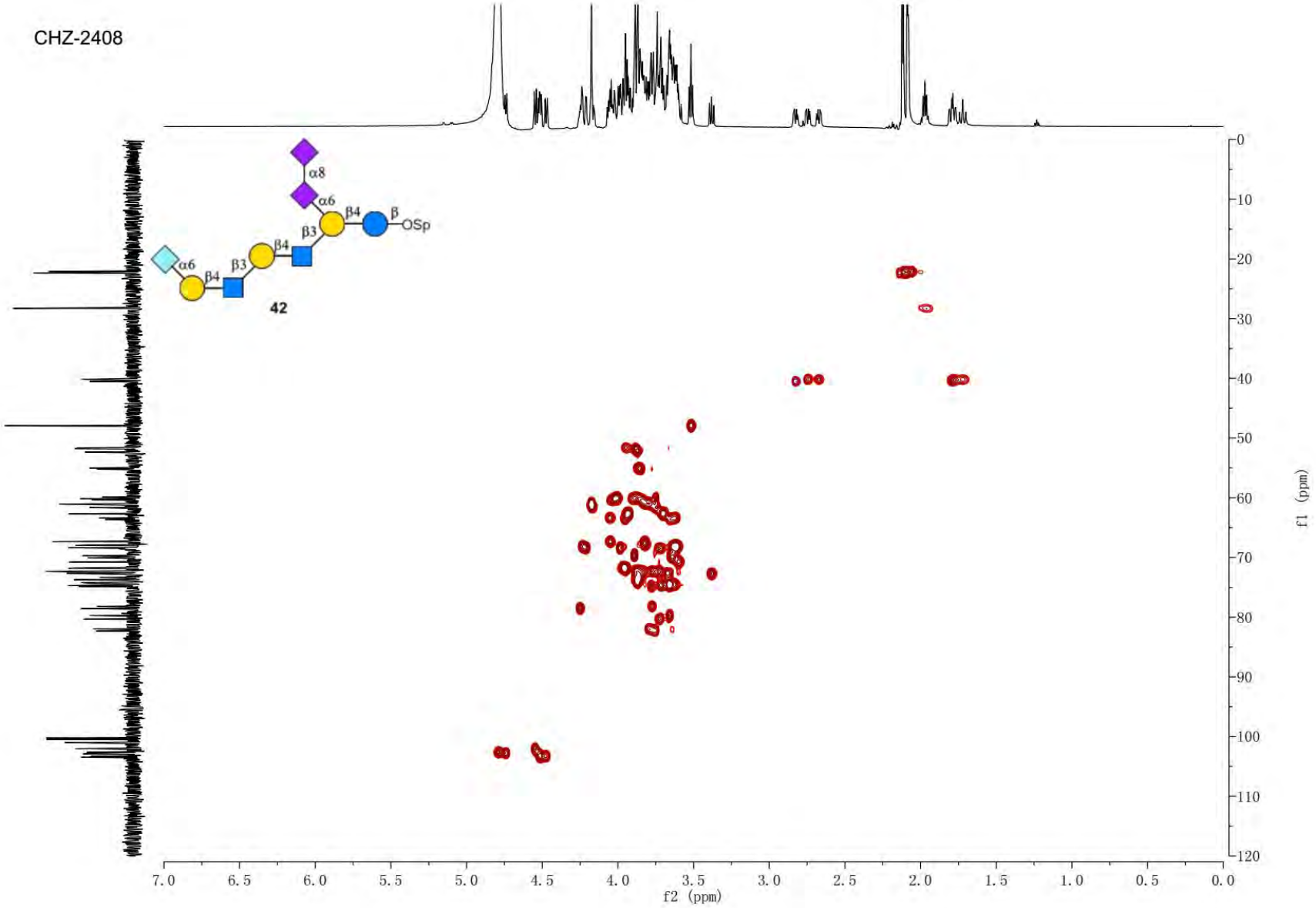
^{13}C NMR of 42



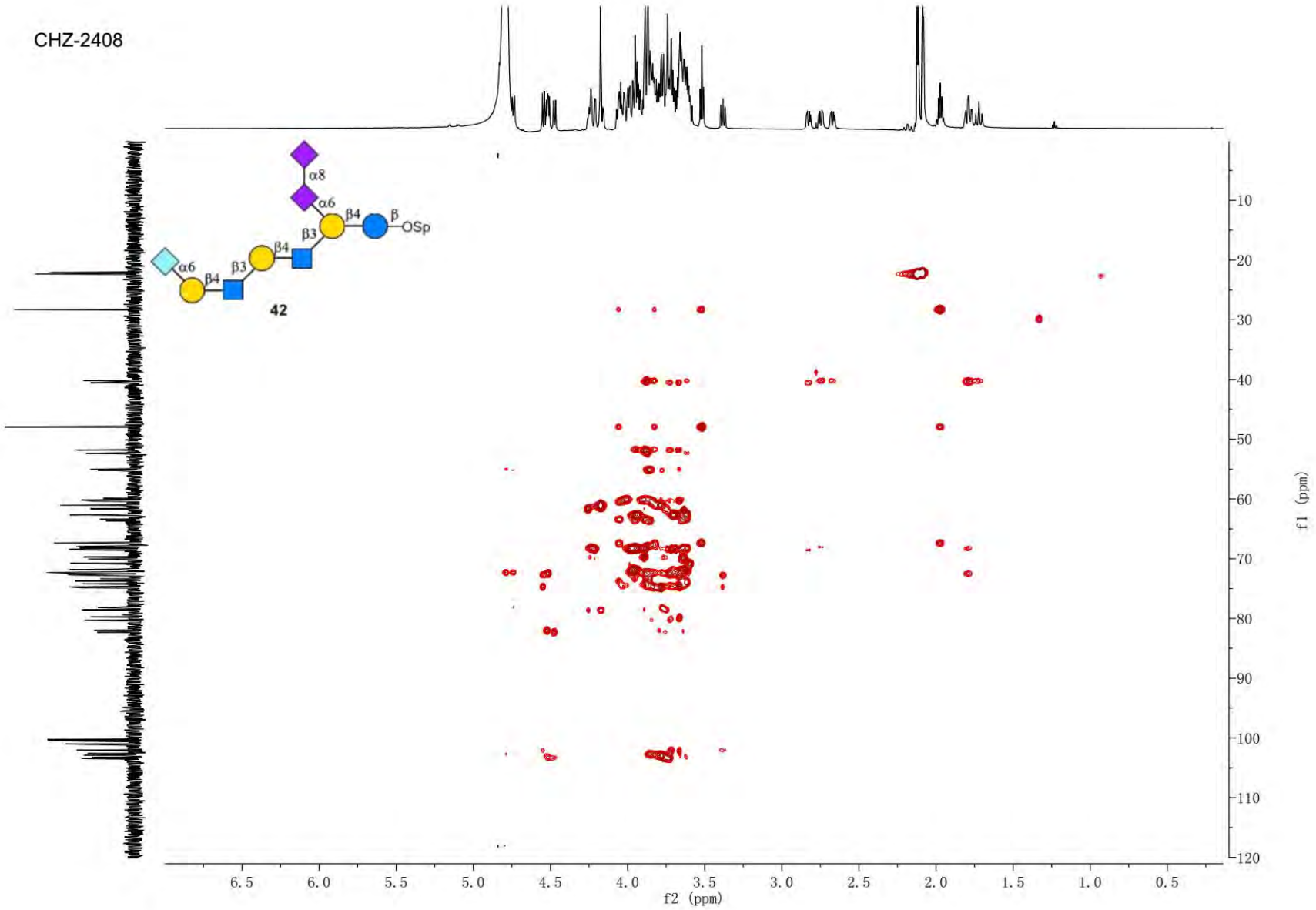
COSY spectra of 42



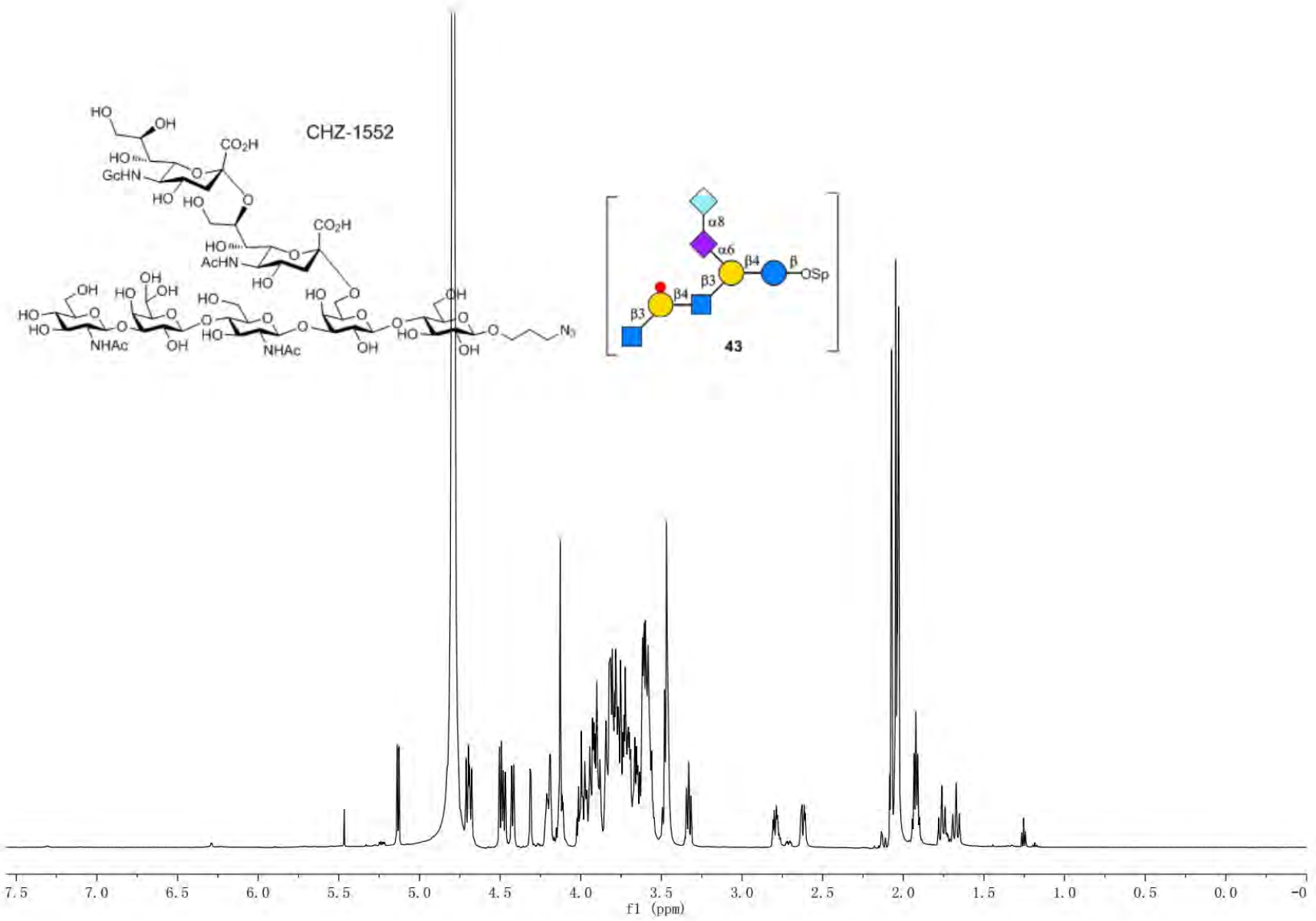
HMBC spectra of 42



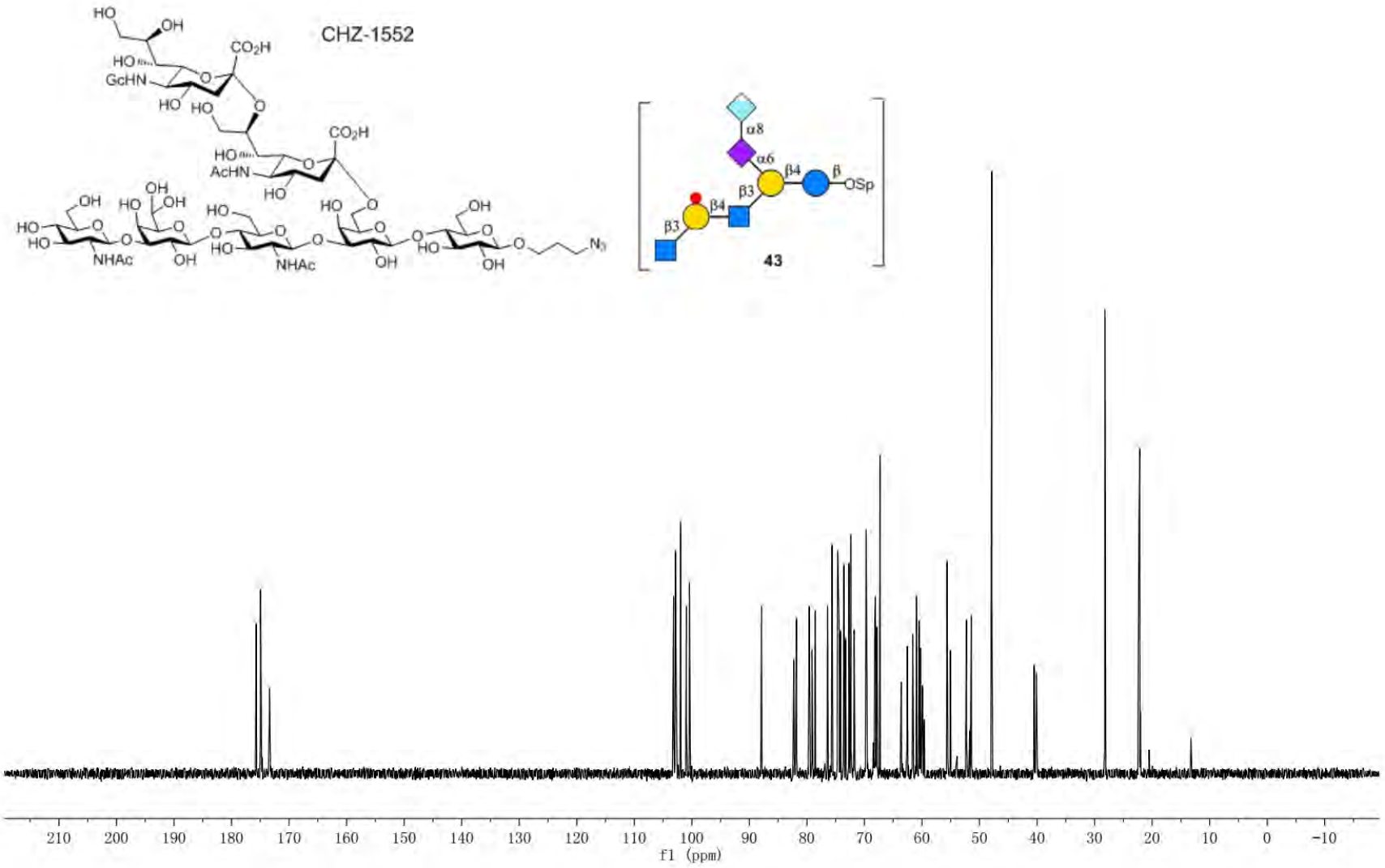
HSQC spectra of 42



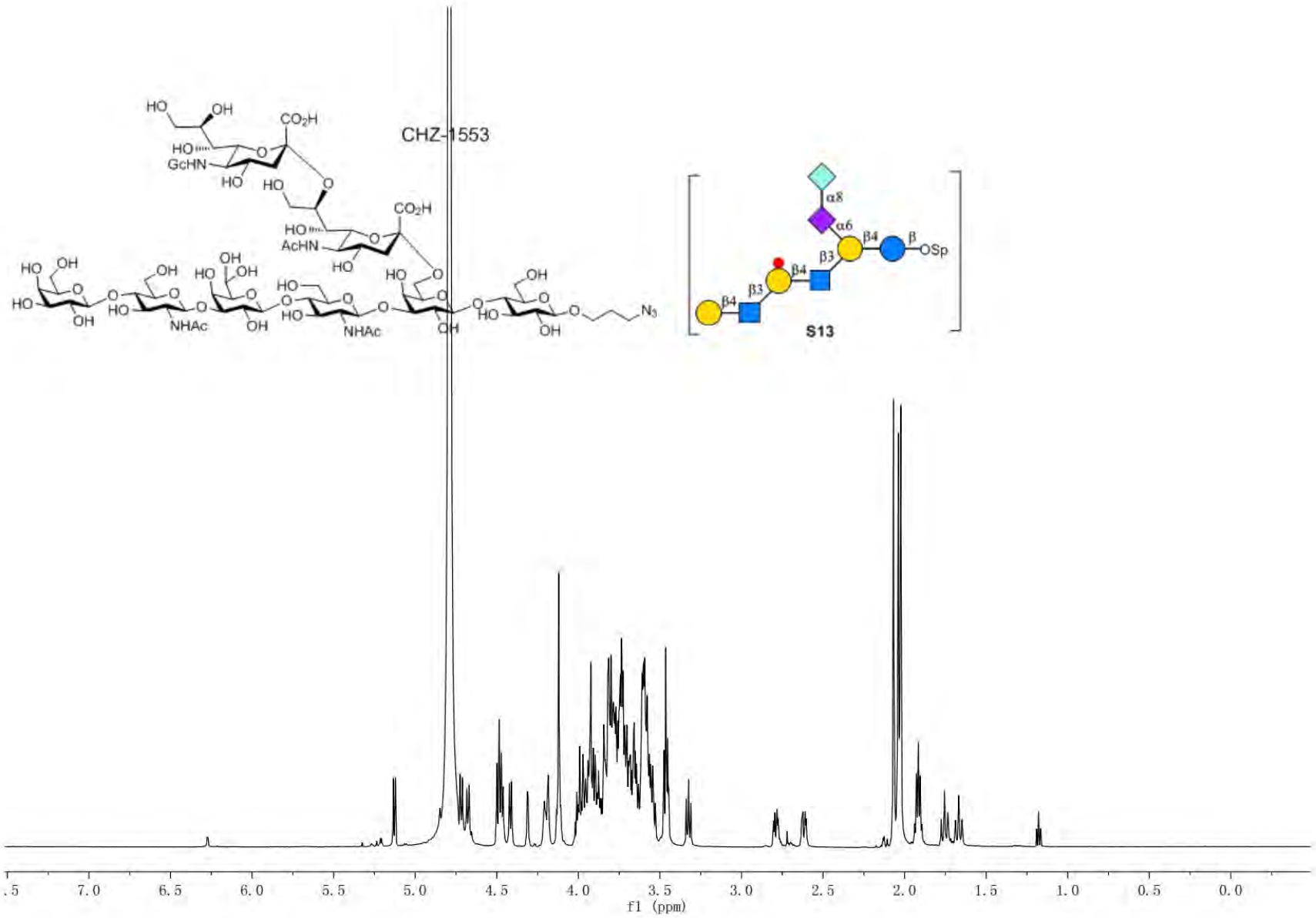
HSQC-TOCSY spectra of 42



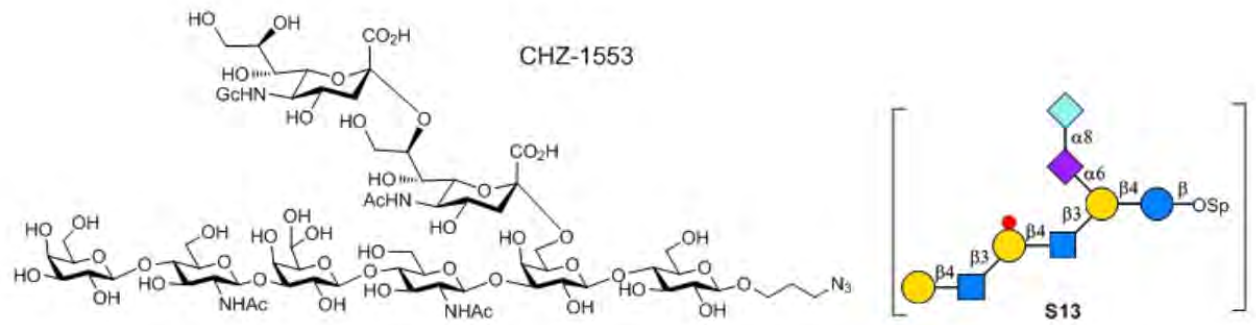
^{1}H NMR of 43

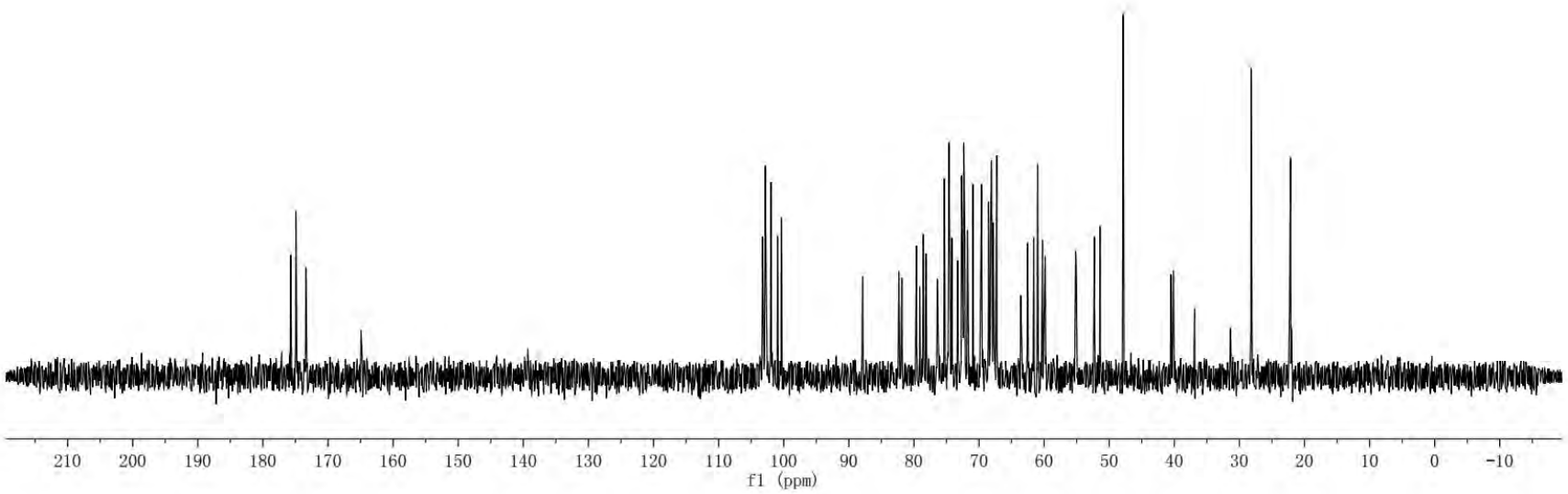


^{13}C NMR of 43

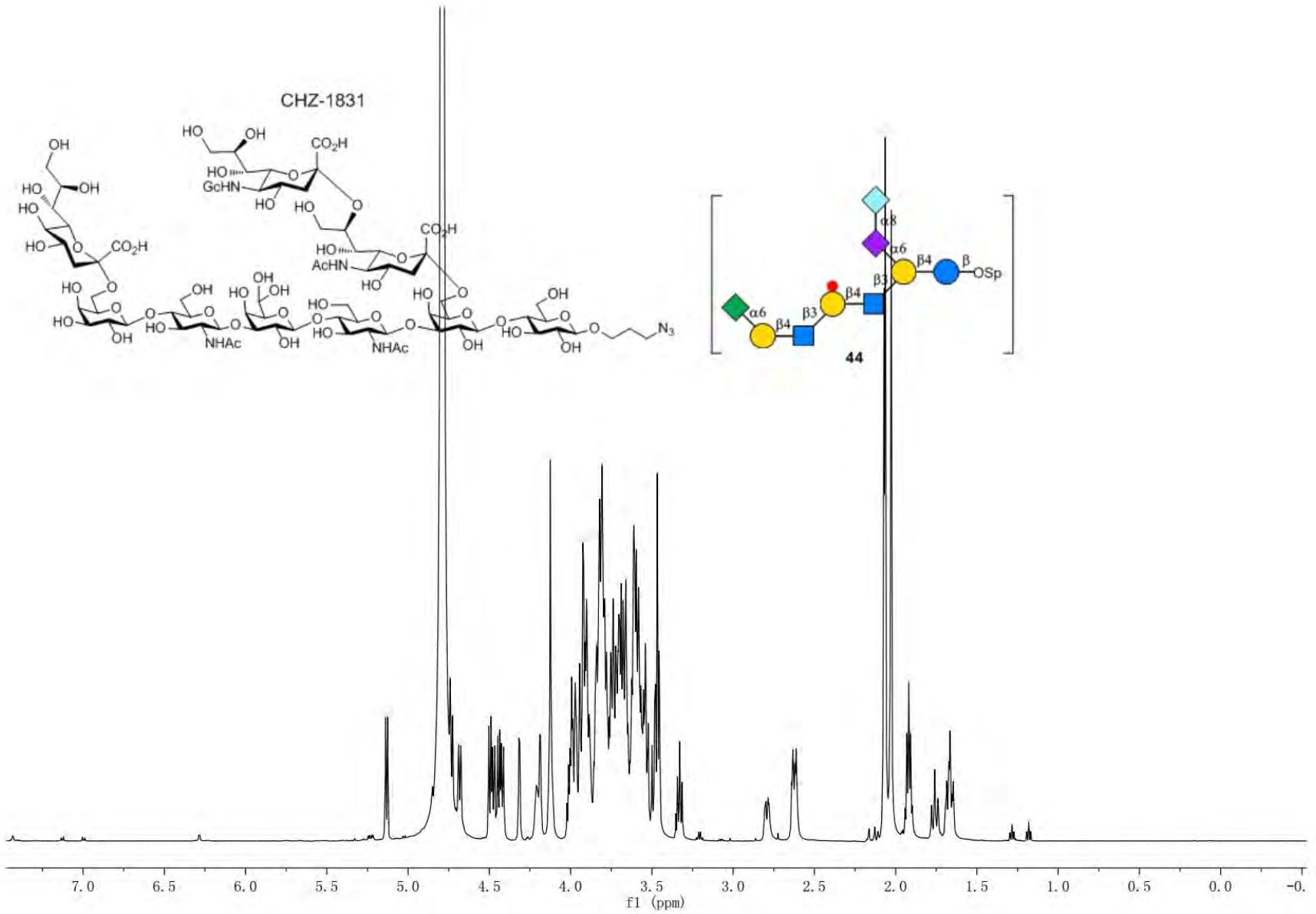


^{1}H NMR of S13

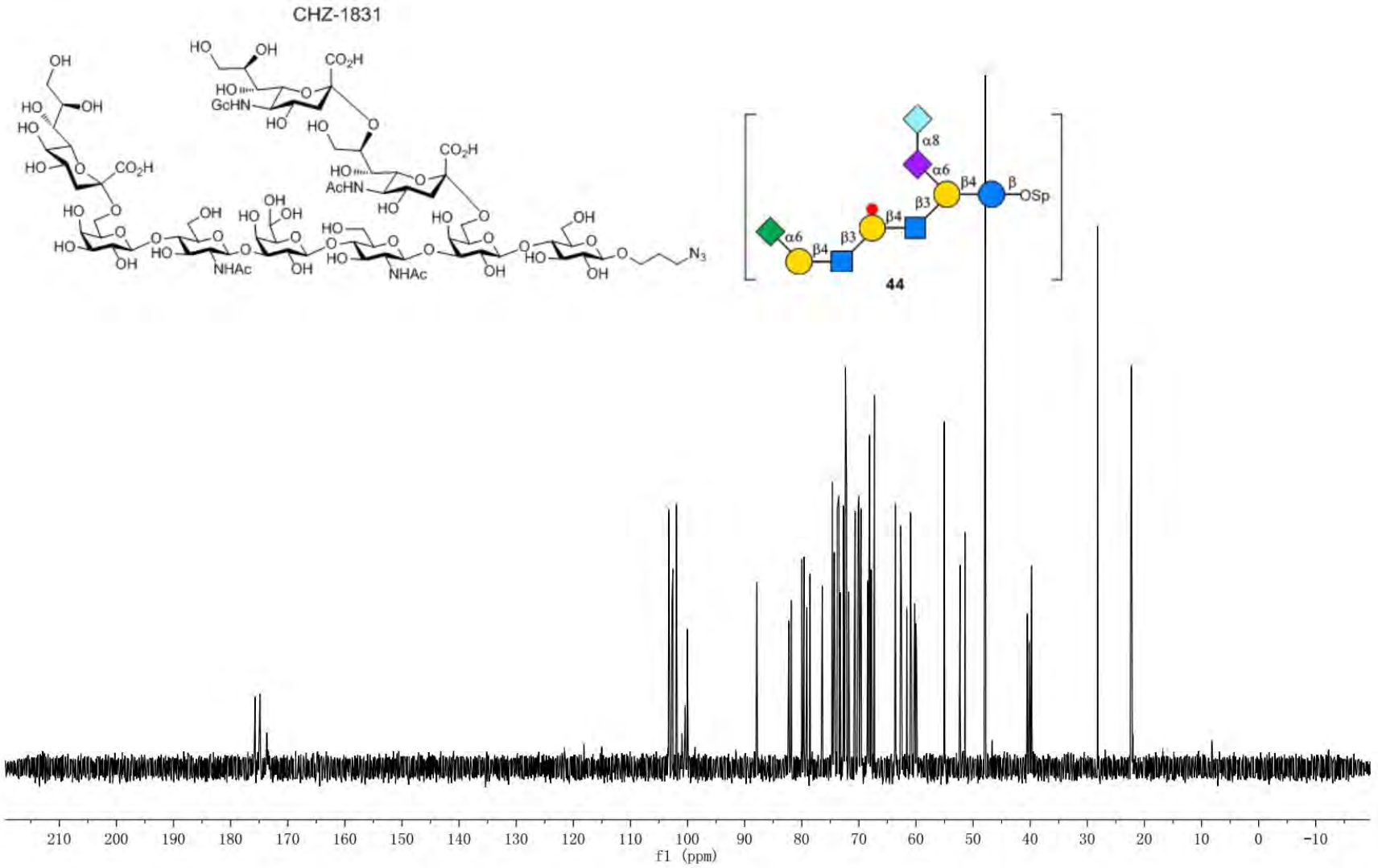




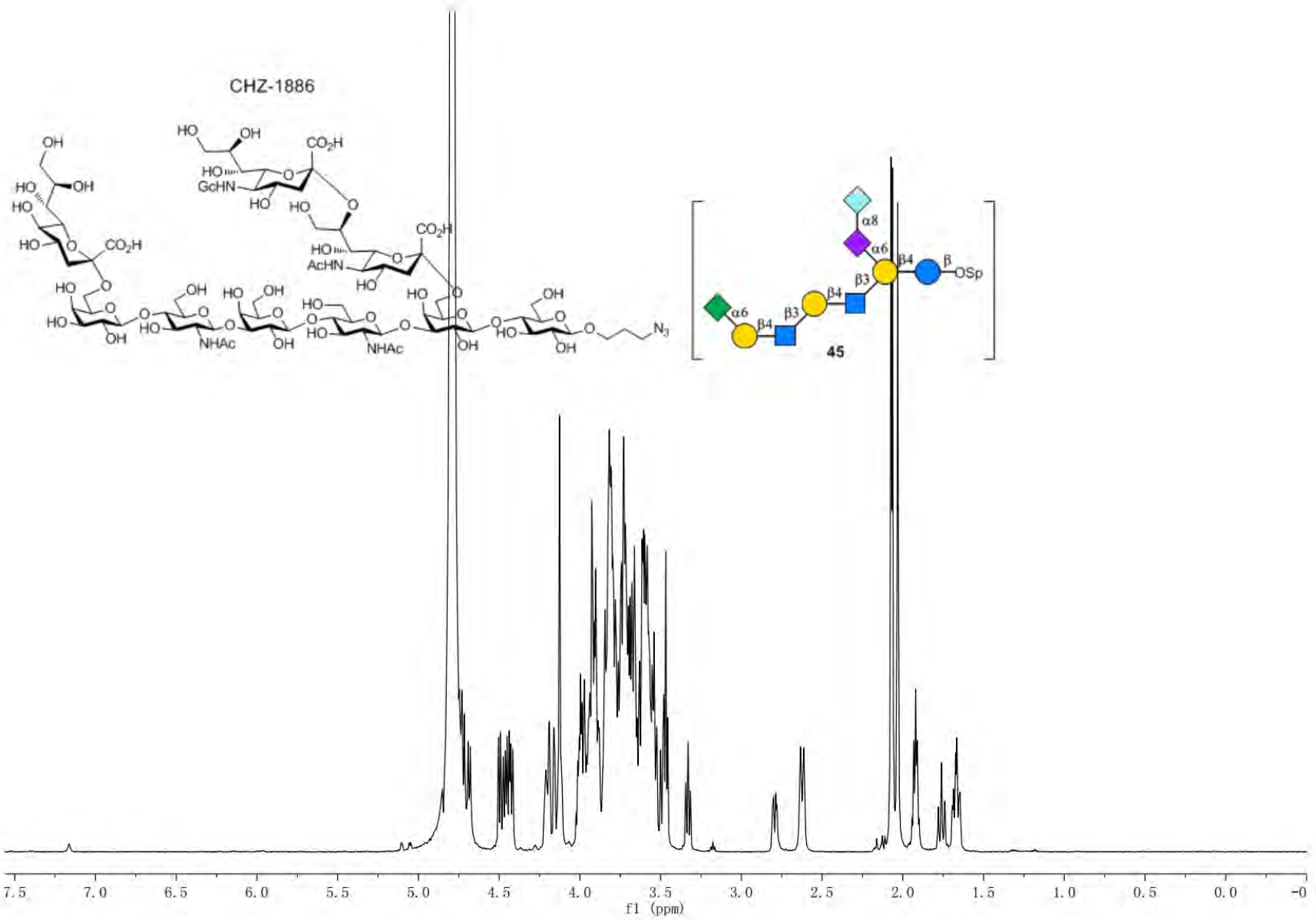
^{13}C NMR of S13



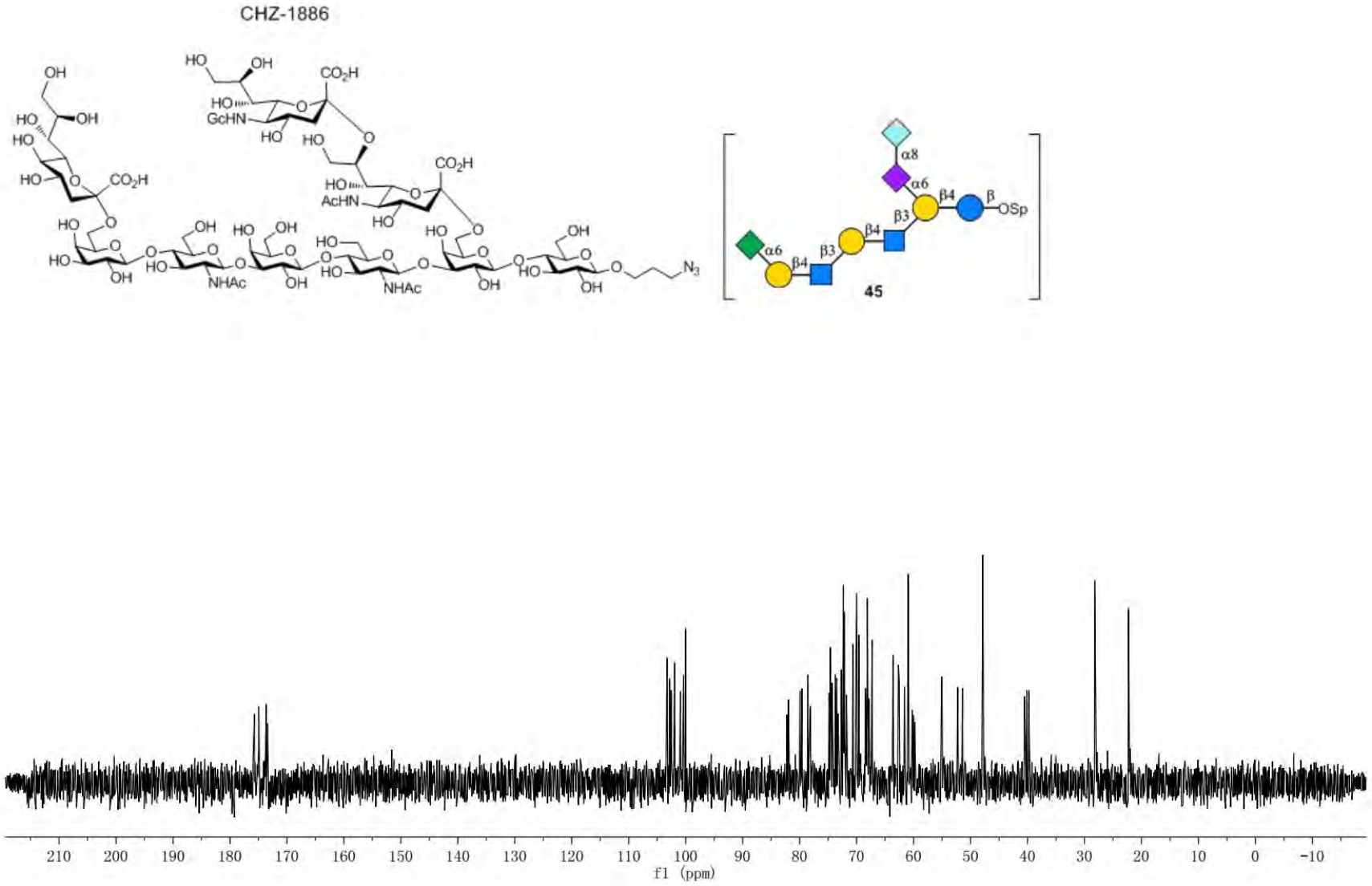
^{1}H NMR of 44



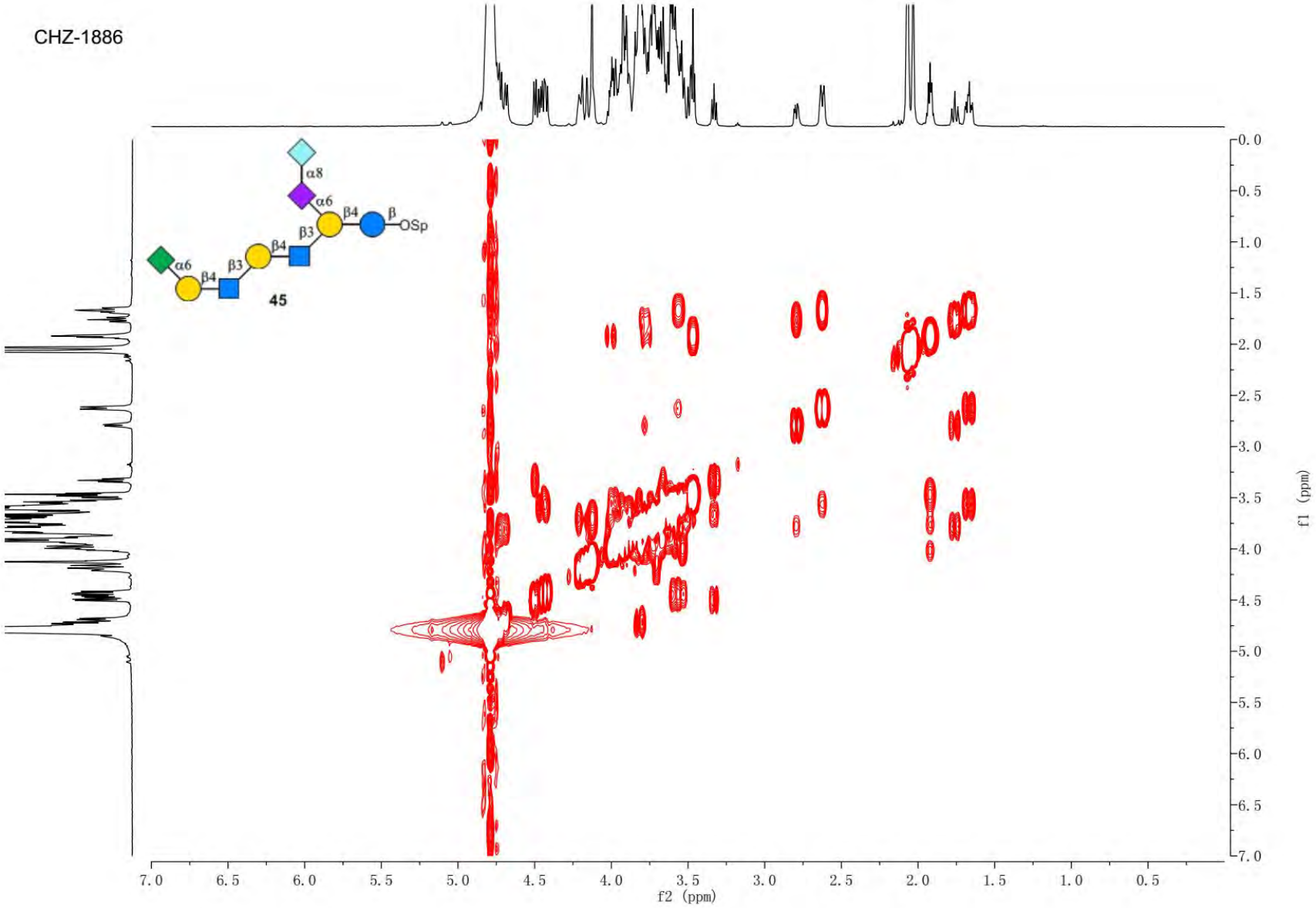
^{13}C NMR of 44



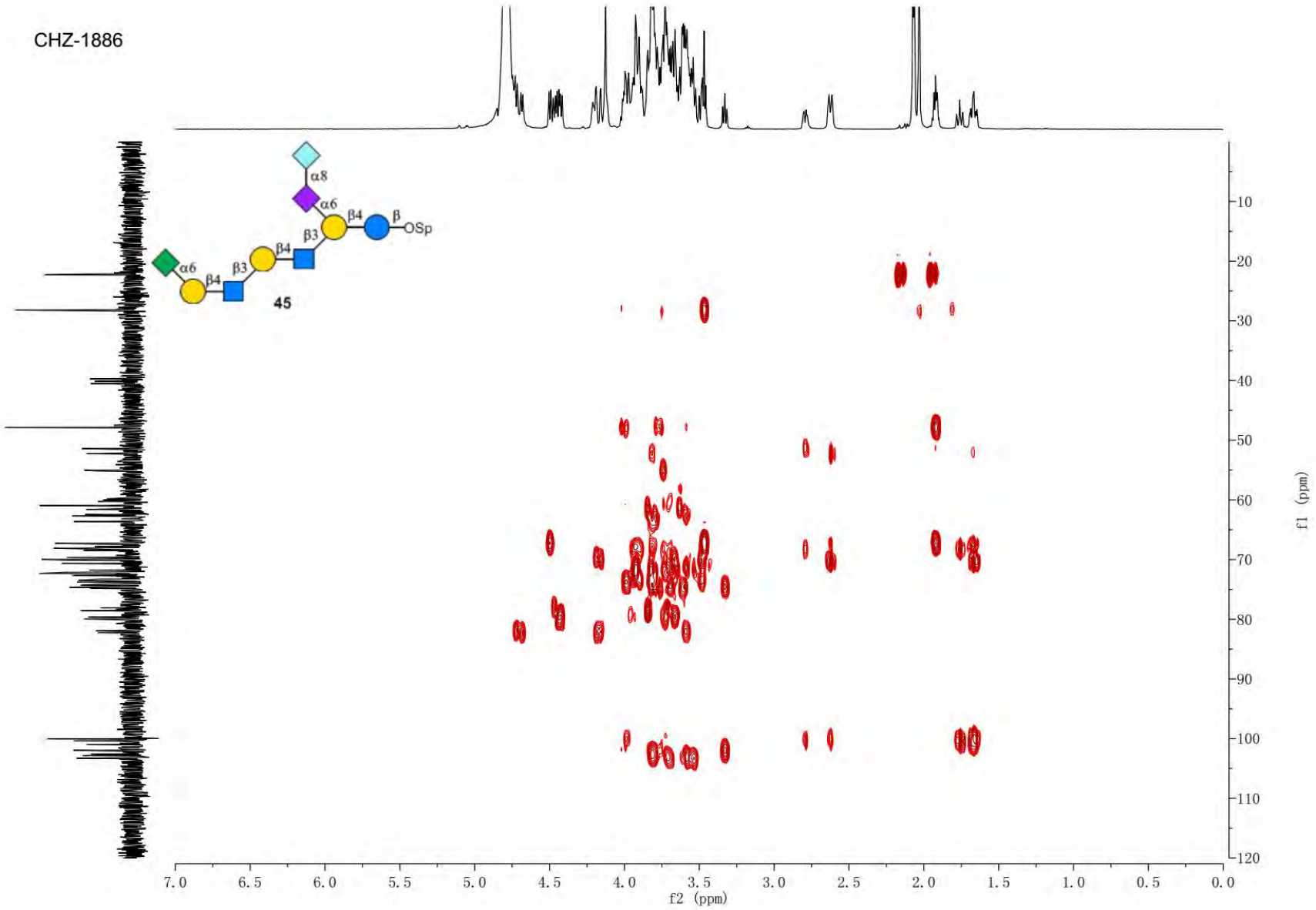
^{1}H NMR of 45



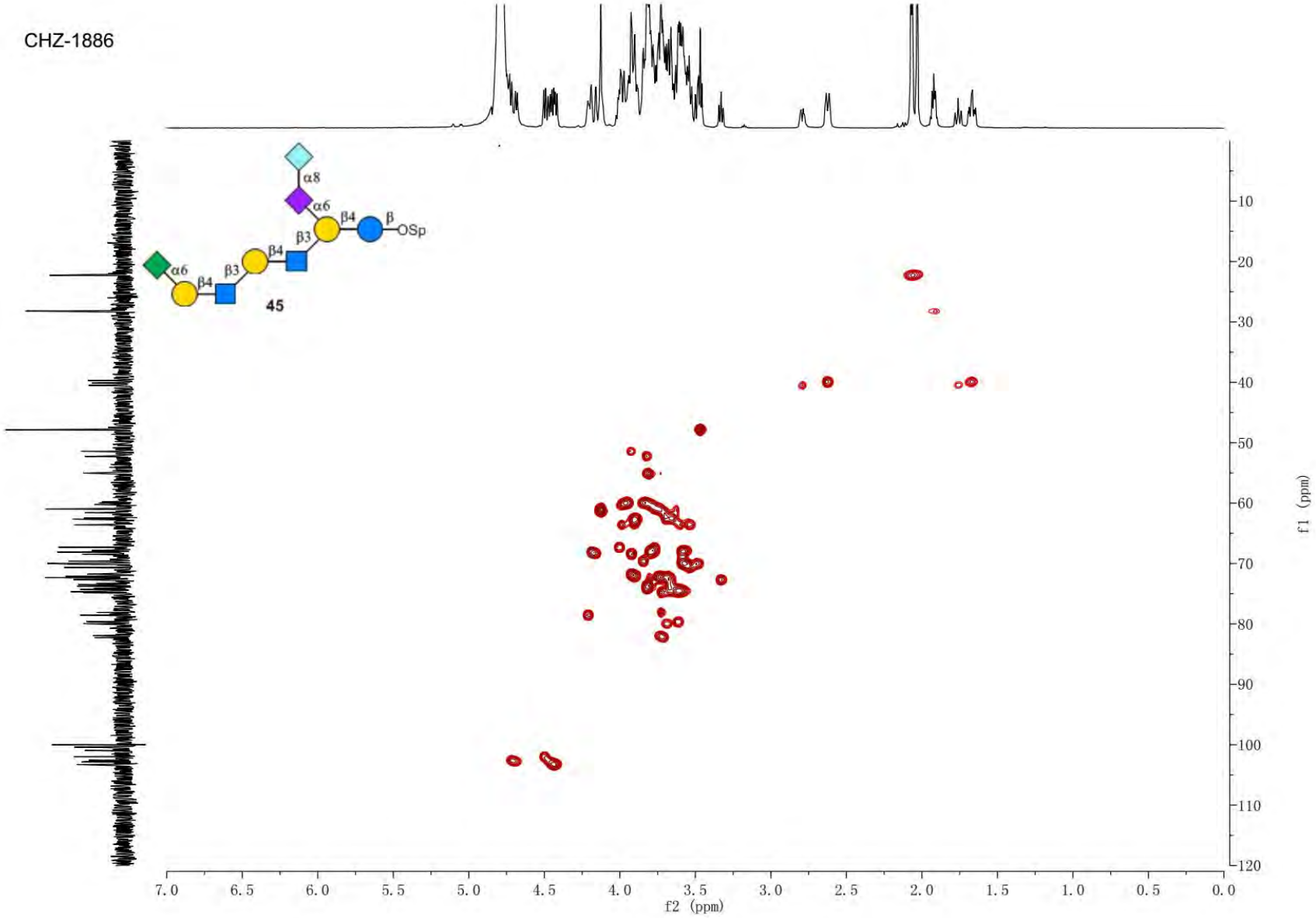
^{13}C NMR of 45



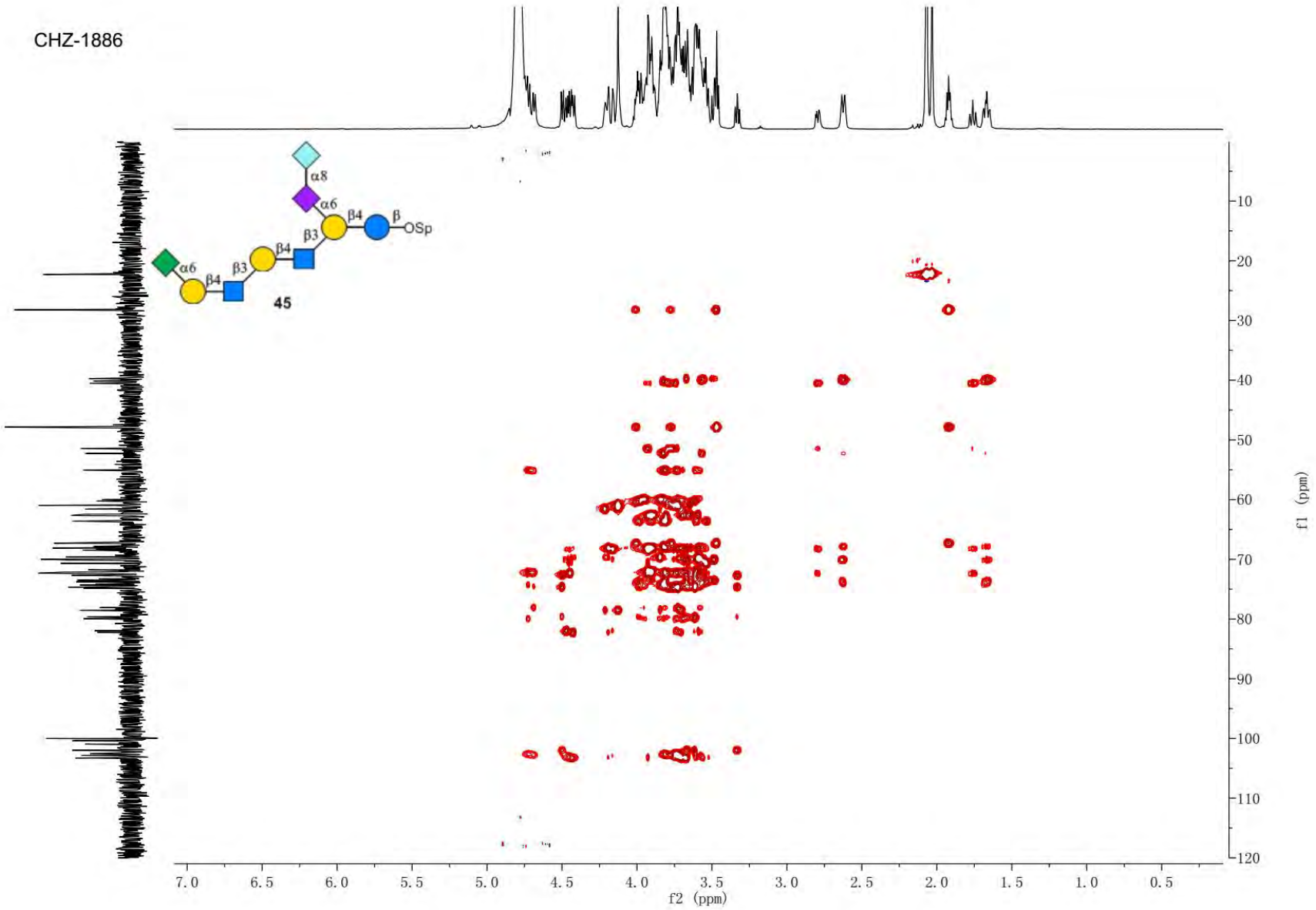
COSY spectra of 45



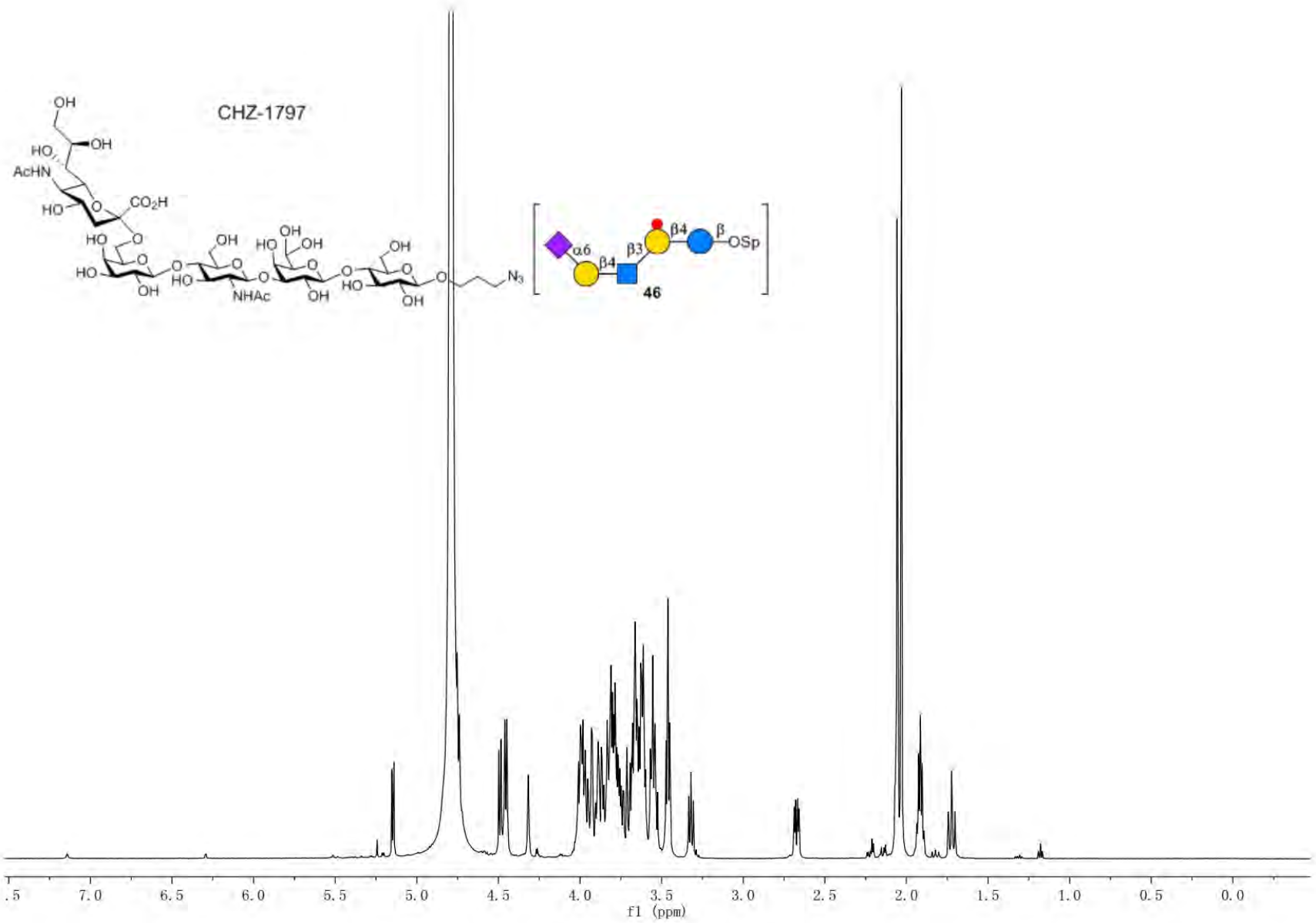
HMBC spectra of 45



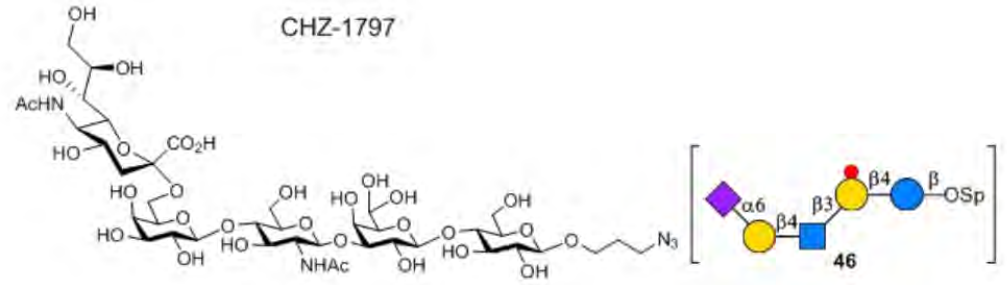
HSQC spectra of 45

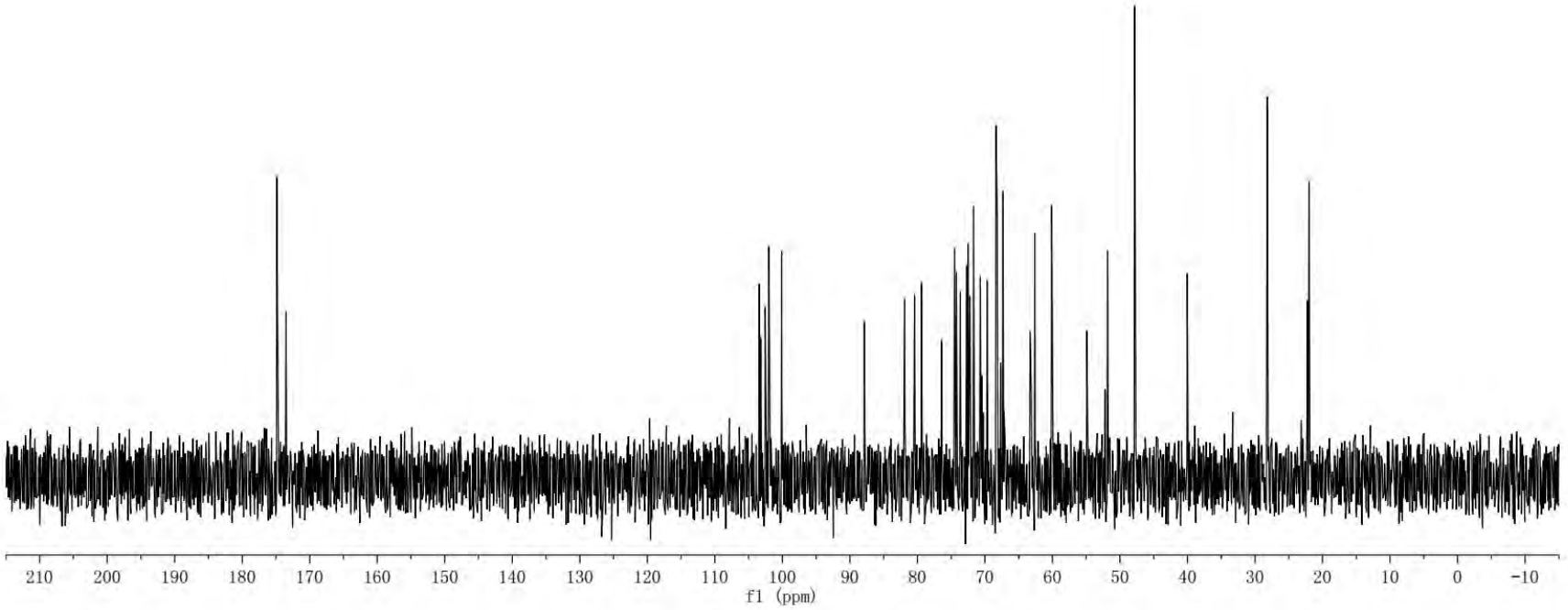


HSQC-TOCSY spectra of 45

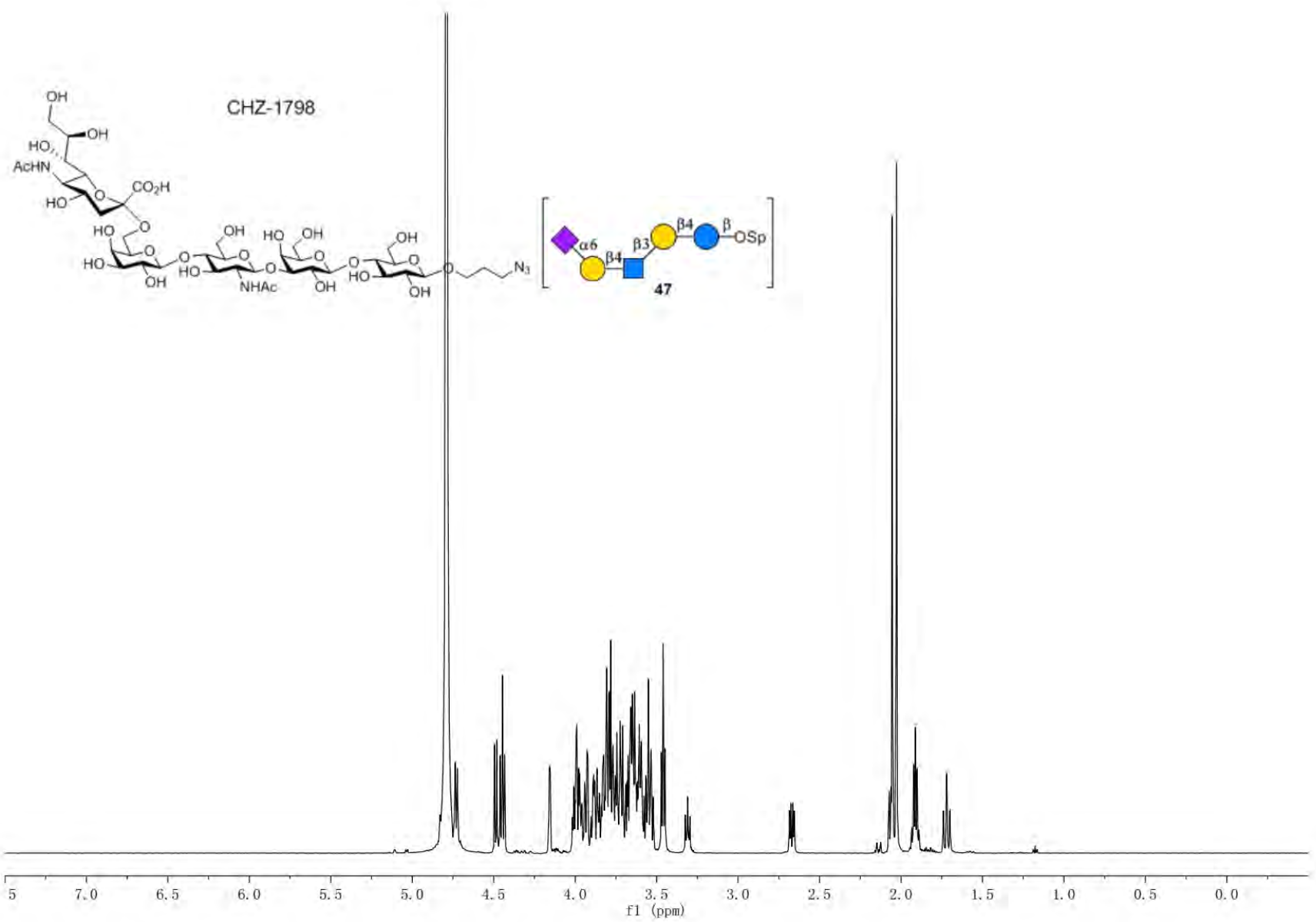


^{1}H NMR of 46

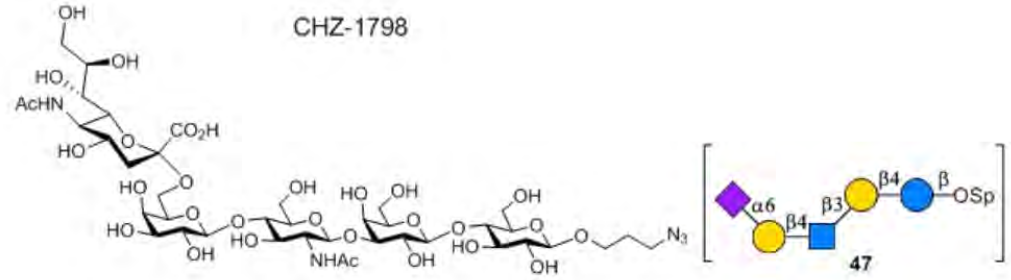

CHZ-1797
46

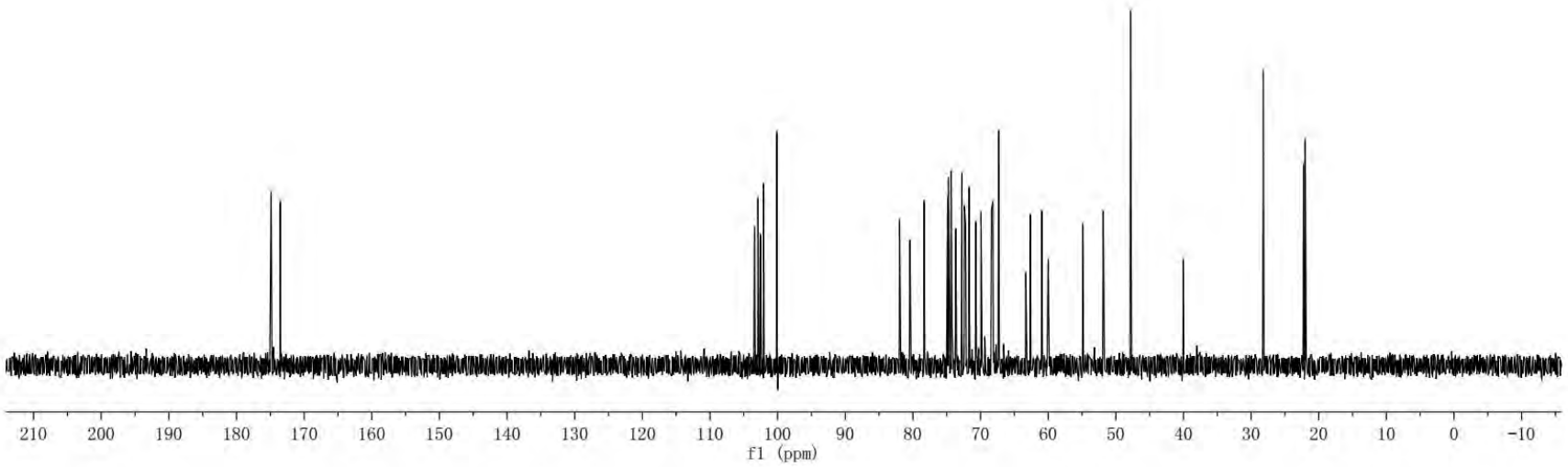

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

^{13}C NMR of 46

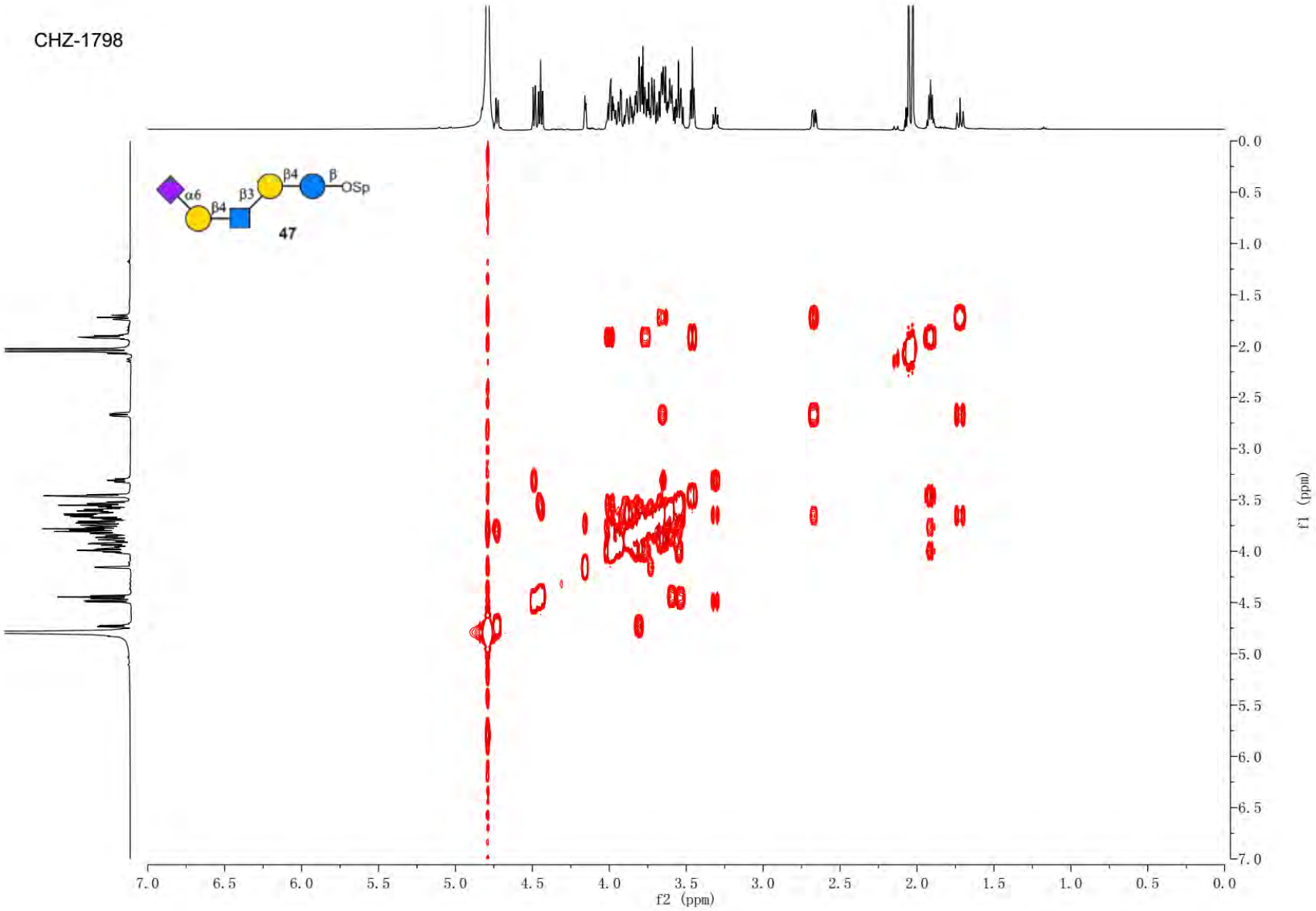


^{1}H NMR of 47

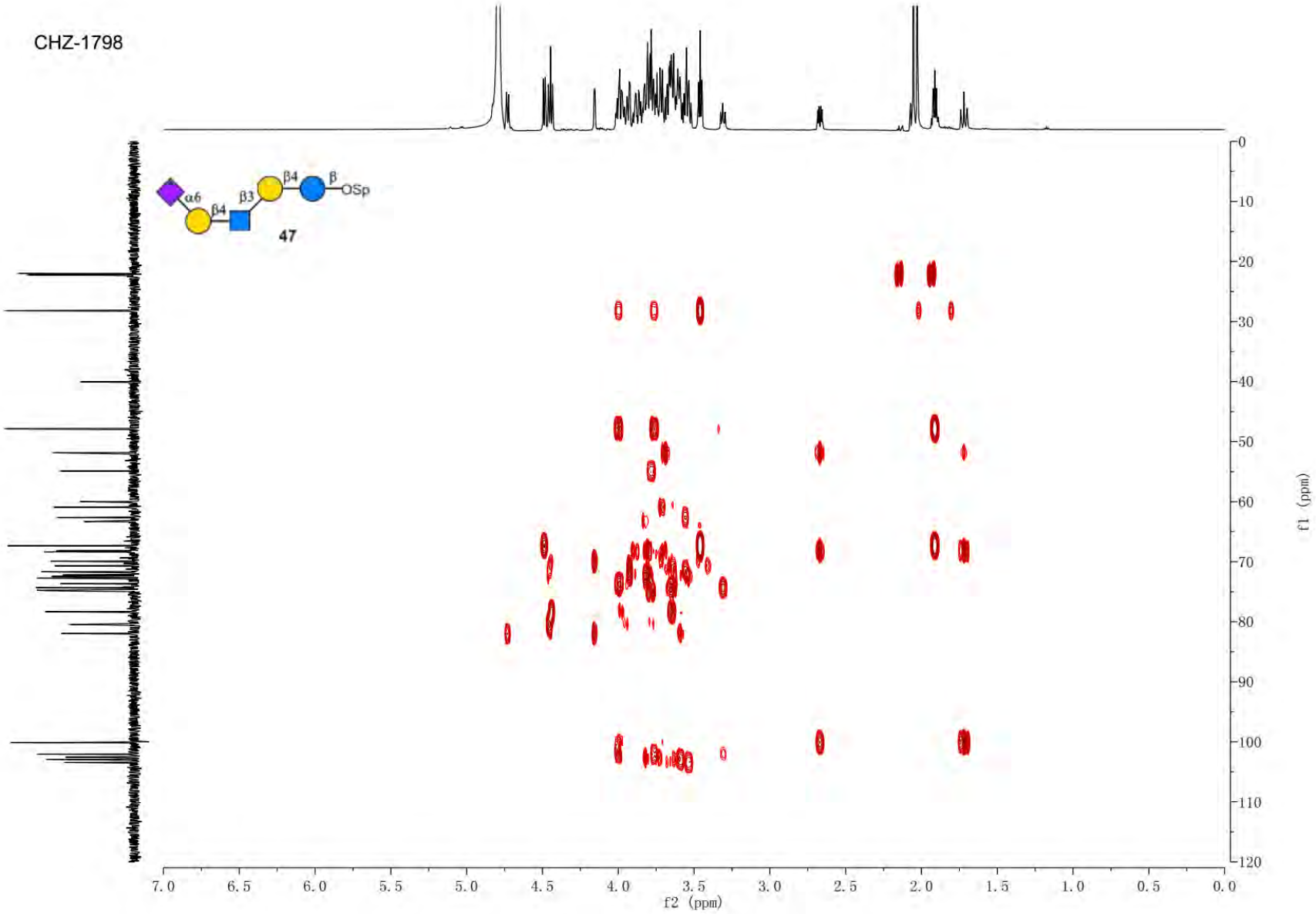

CHZ-1798
47


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

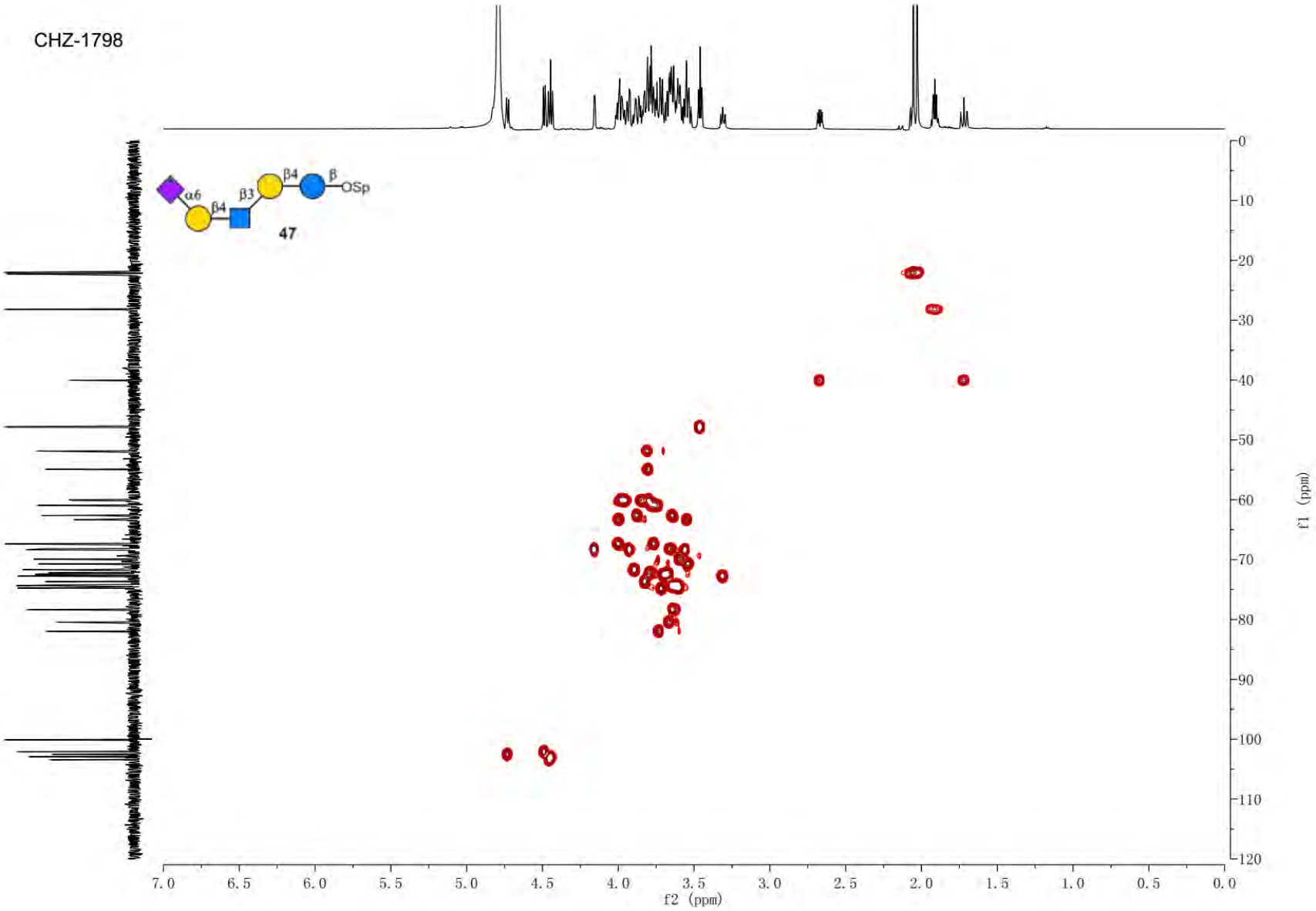
^{13}C NMR of 47



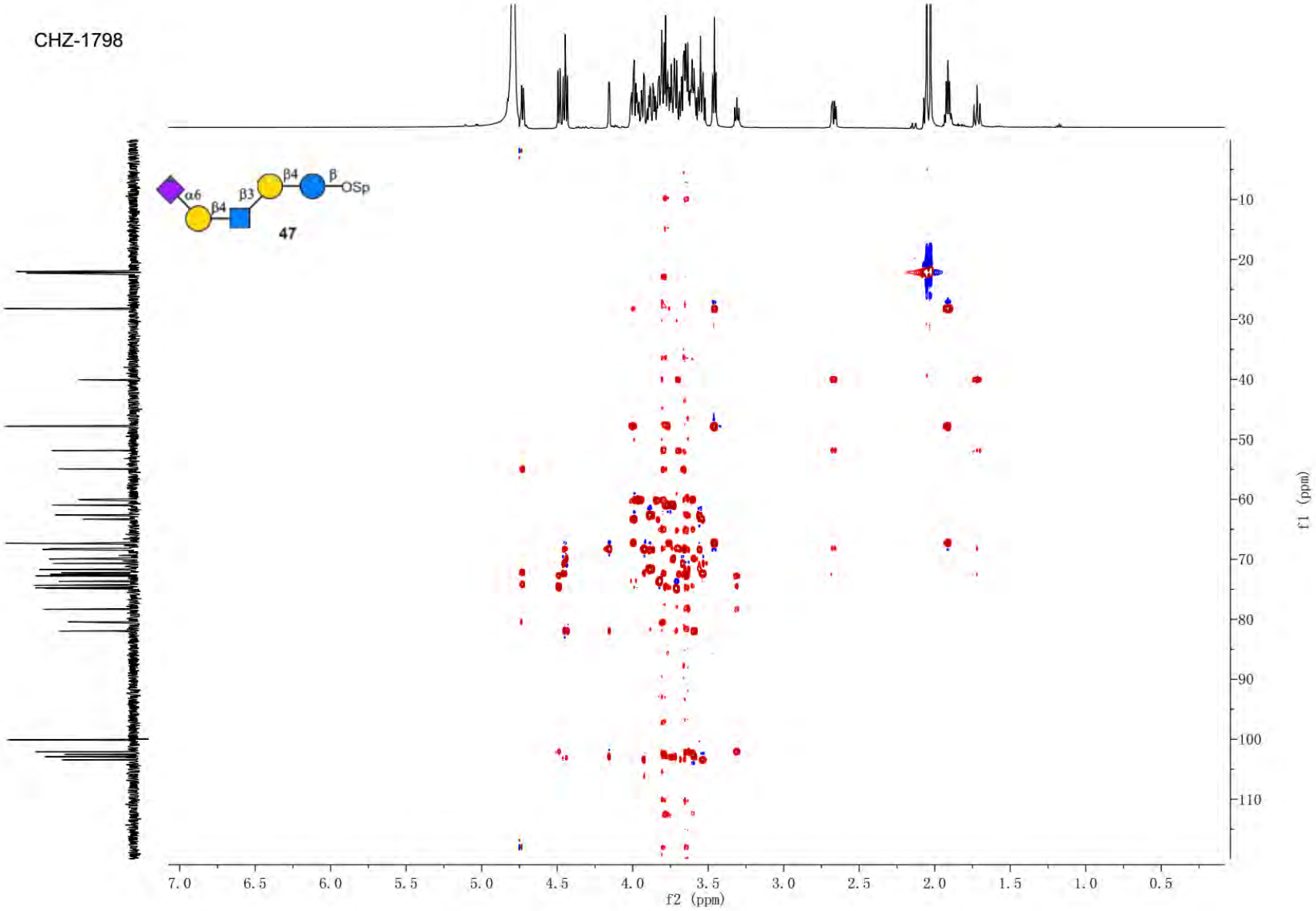
COSY spectra of 47



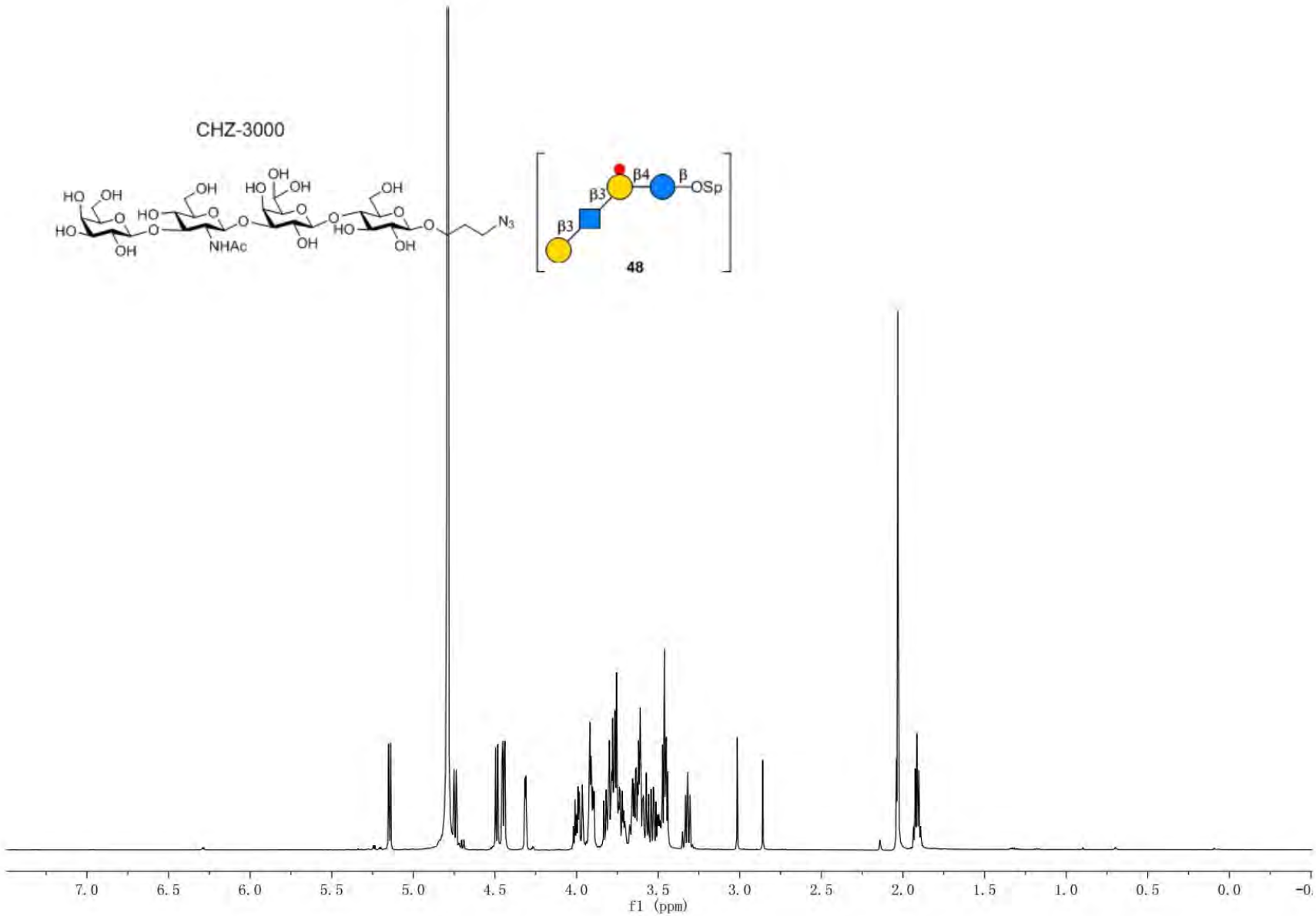
HMBC spectra of 47



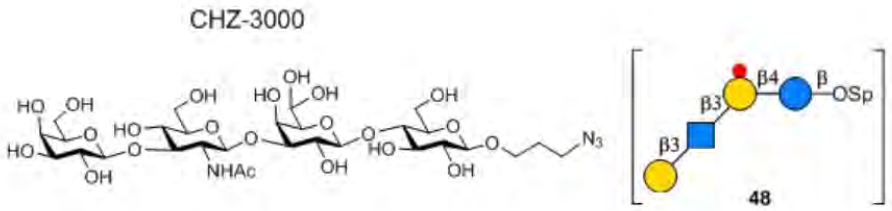
HSQC spectra of 47

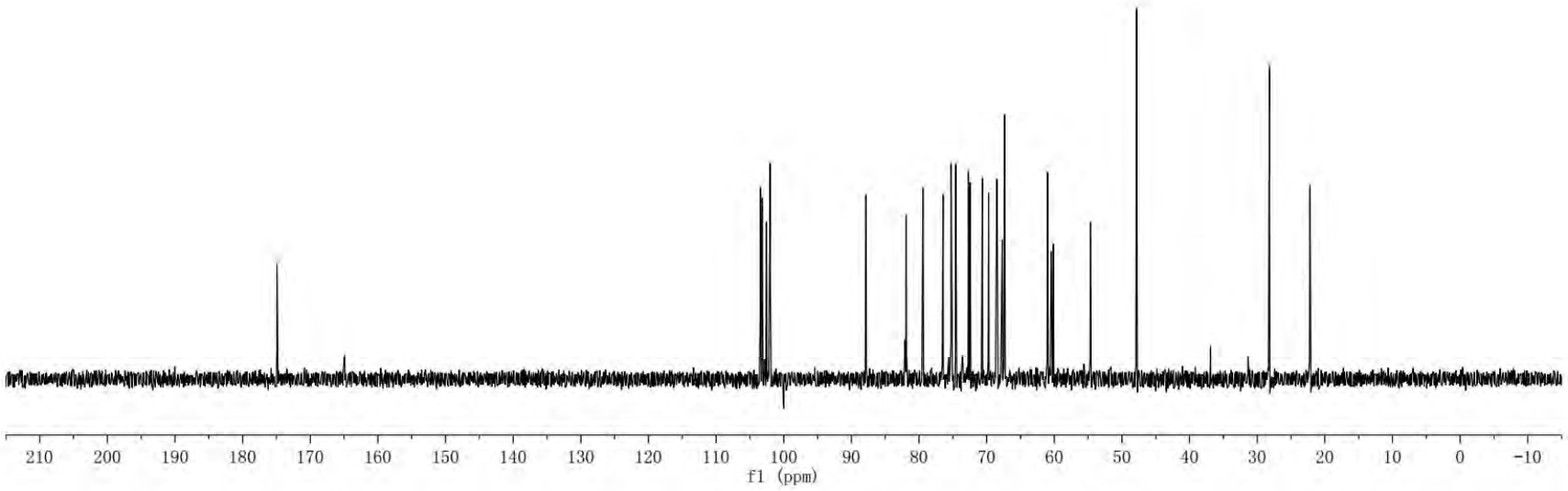


HSQC-TOCSY spectra of 47



^{1}H NMR of 48


CHZ-3000
HO
OH
NHAc
N3
β4
β
OSp
β3
β3
48


210
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160
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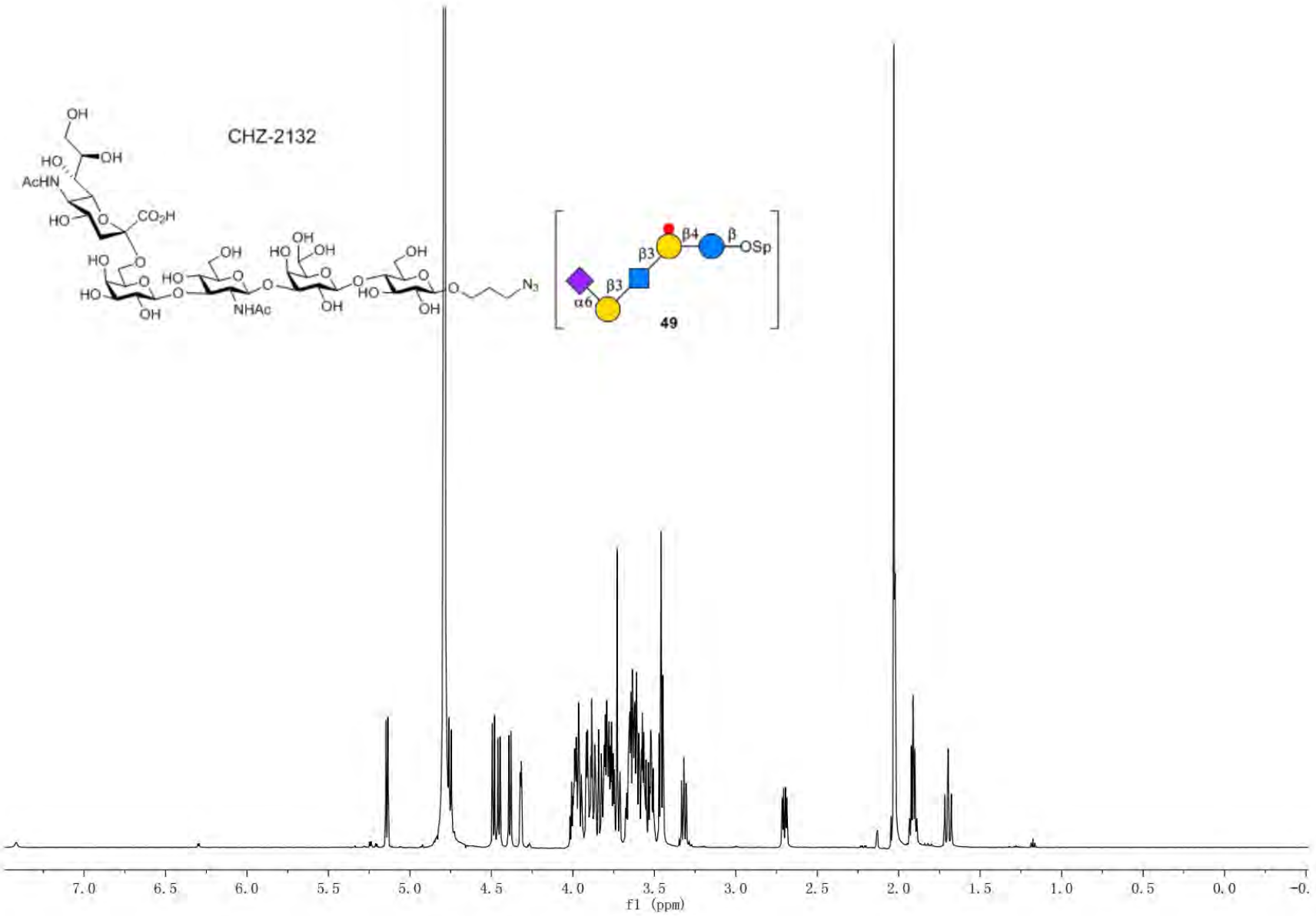
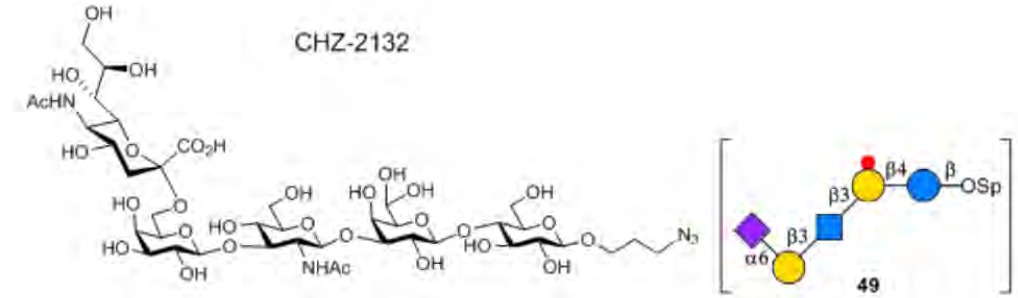
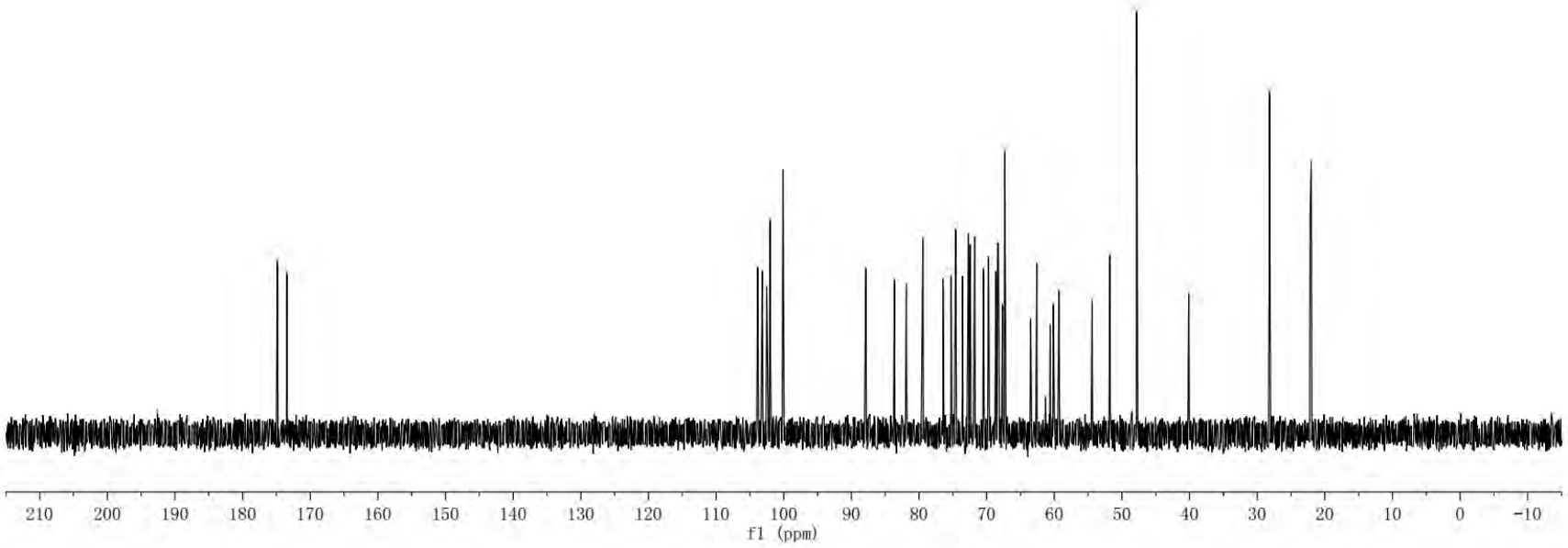
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

^{13}C NMR of 48



^{1}H NMR of 49


CHZ-2132
49


210
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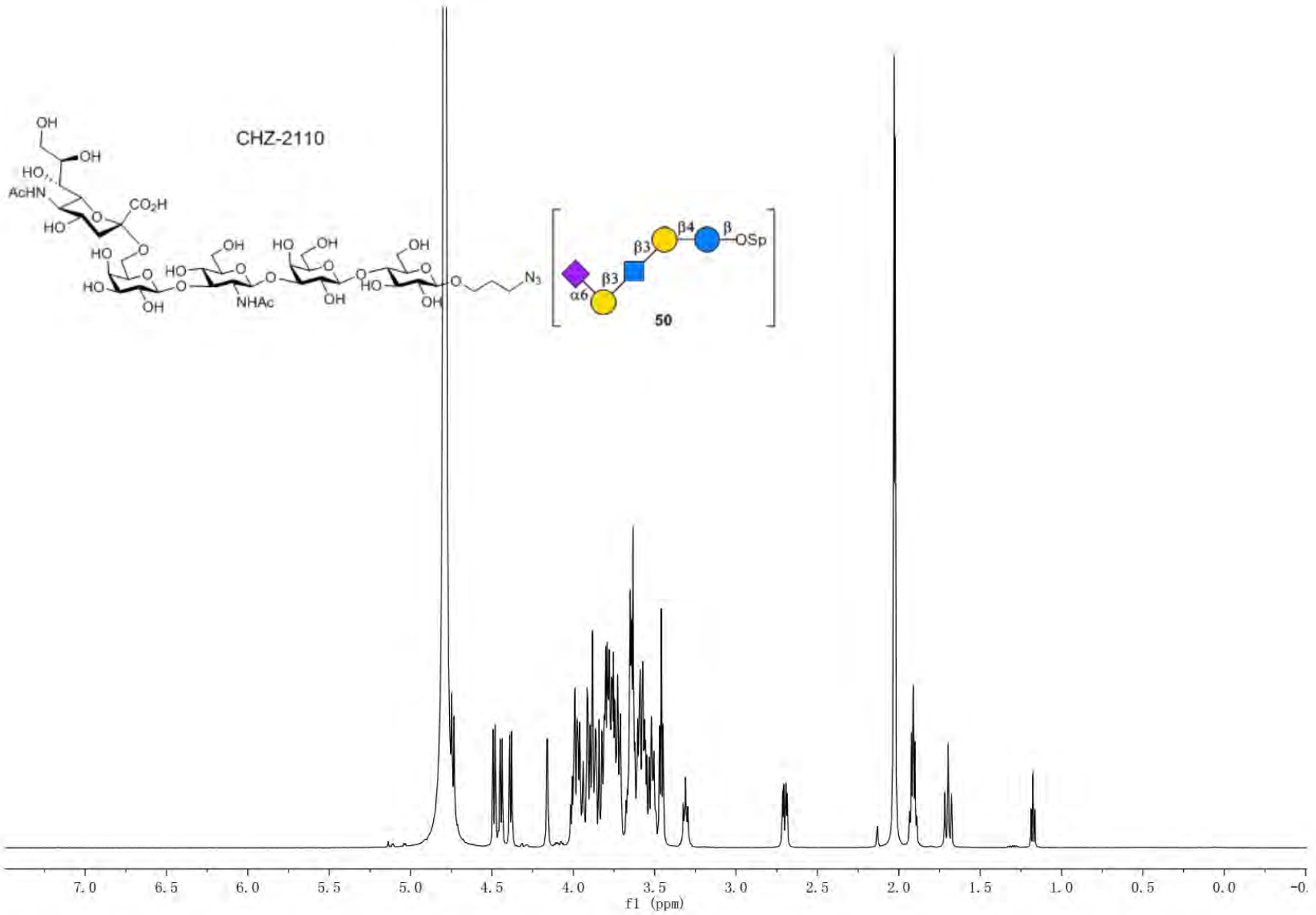
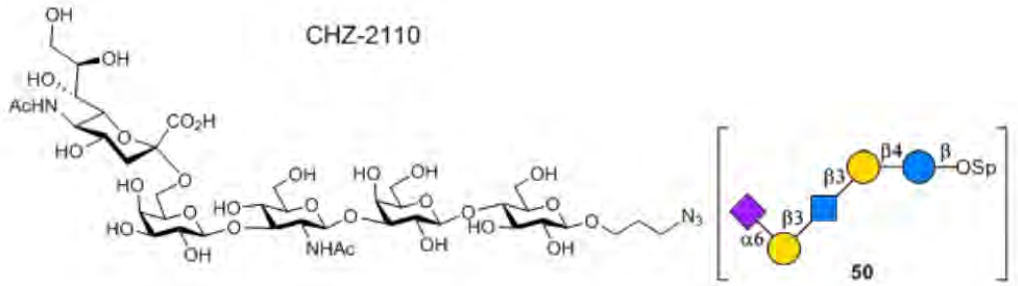
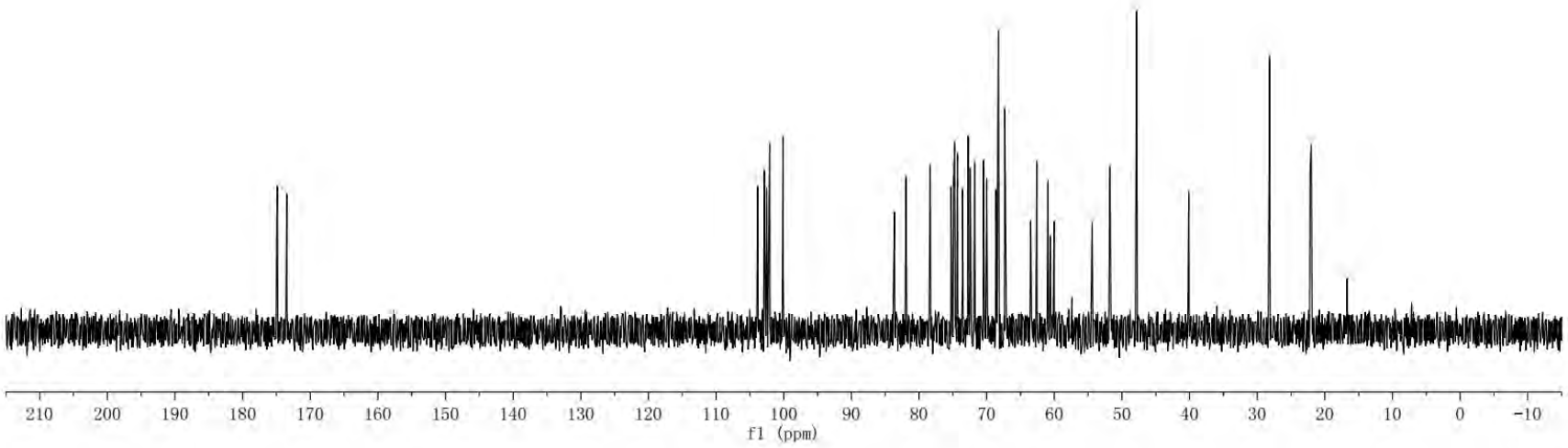
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

^{13}C NMR of 49

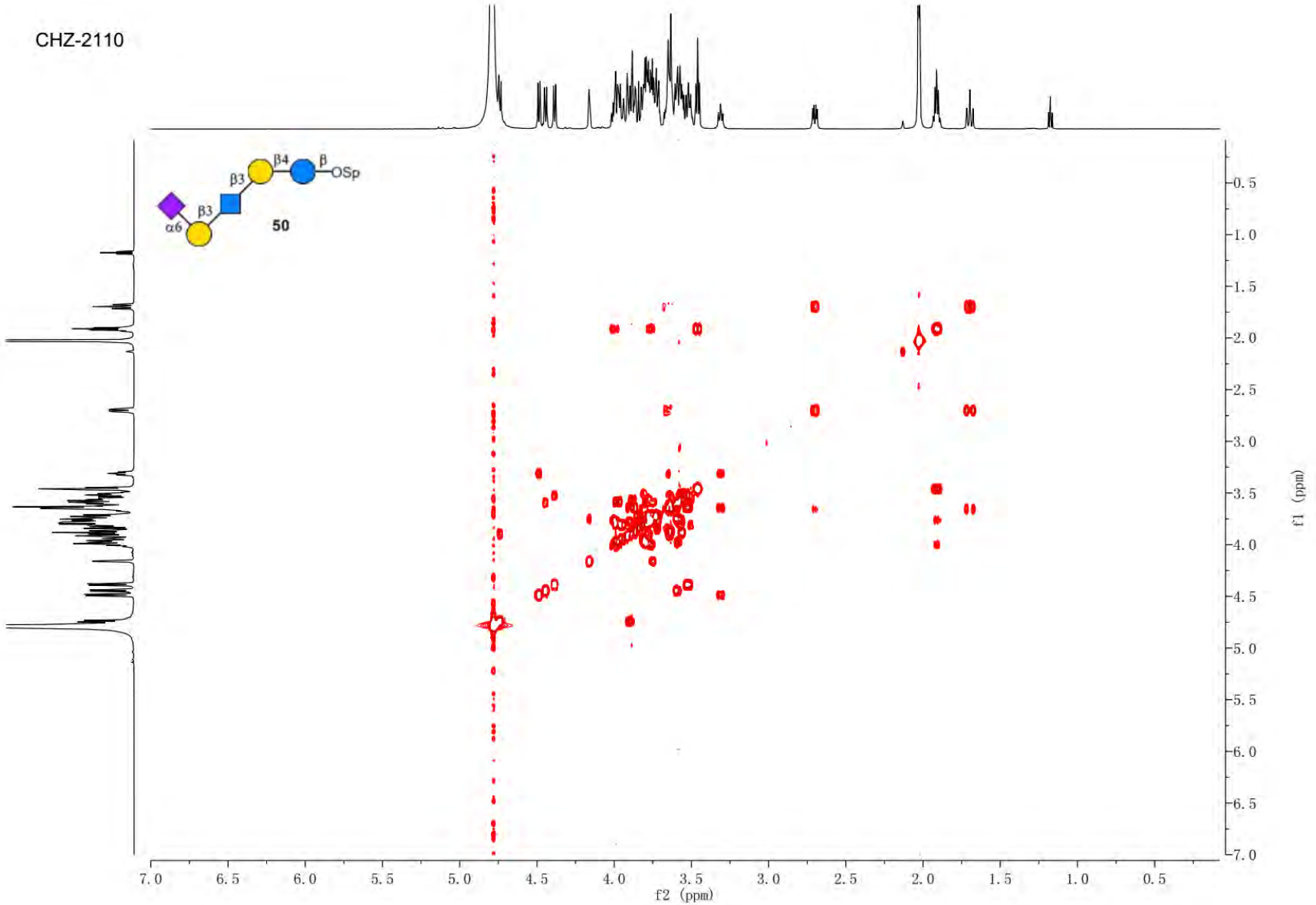


^{1}H NMR of 50

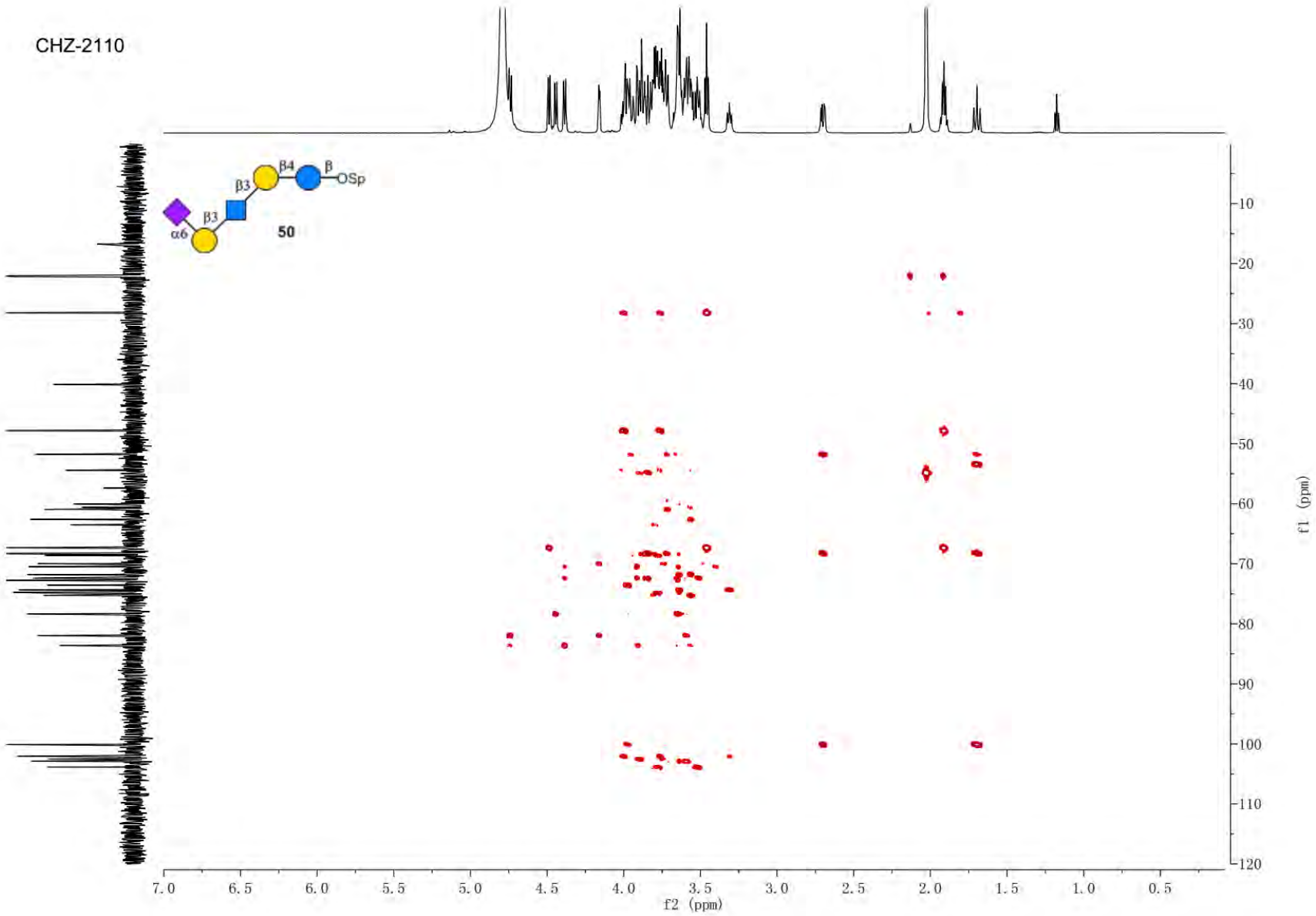

CHZ-2110
50


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

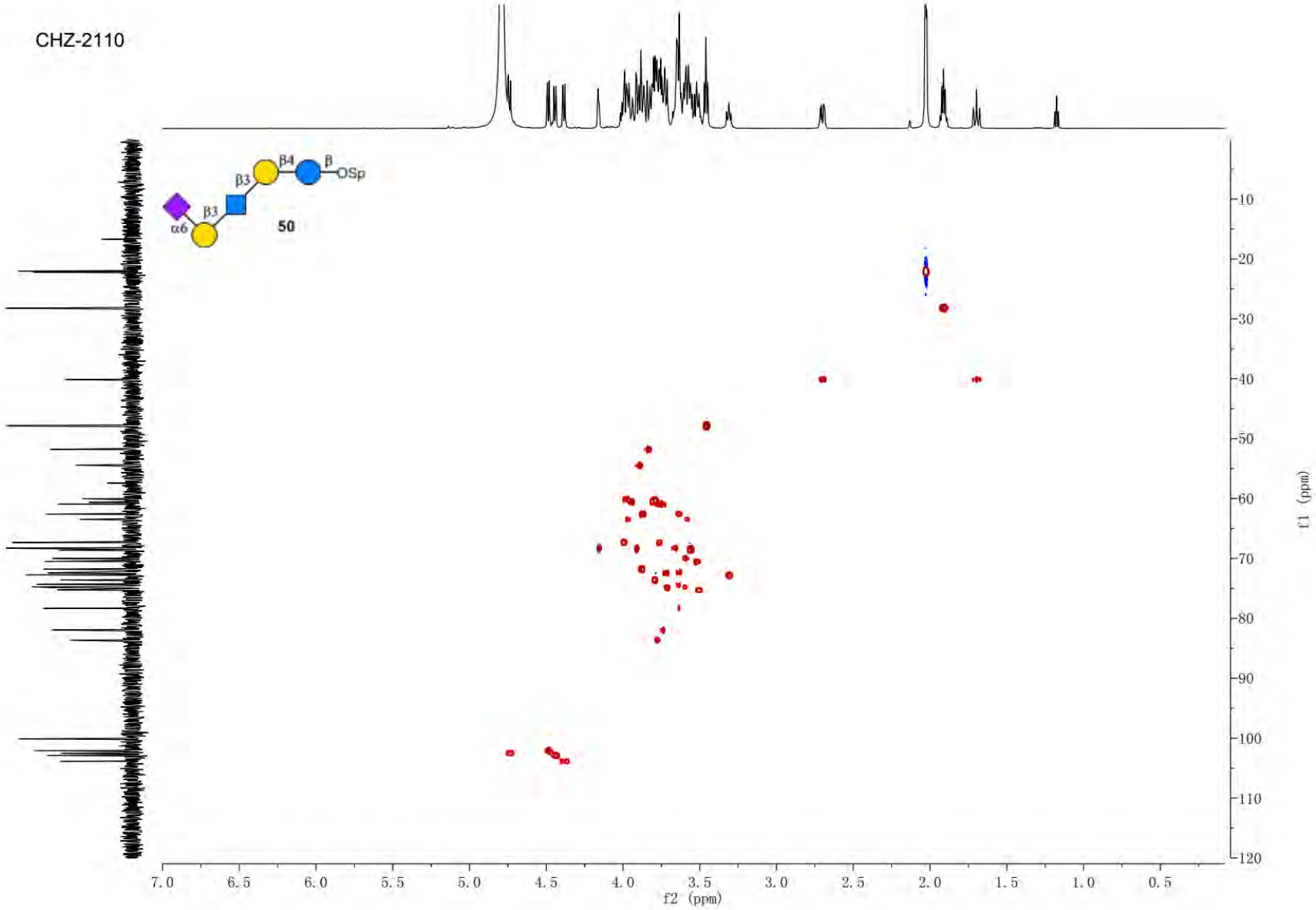
^{13}C NMR of 50



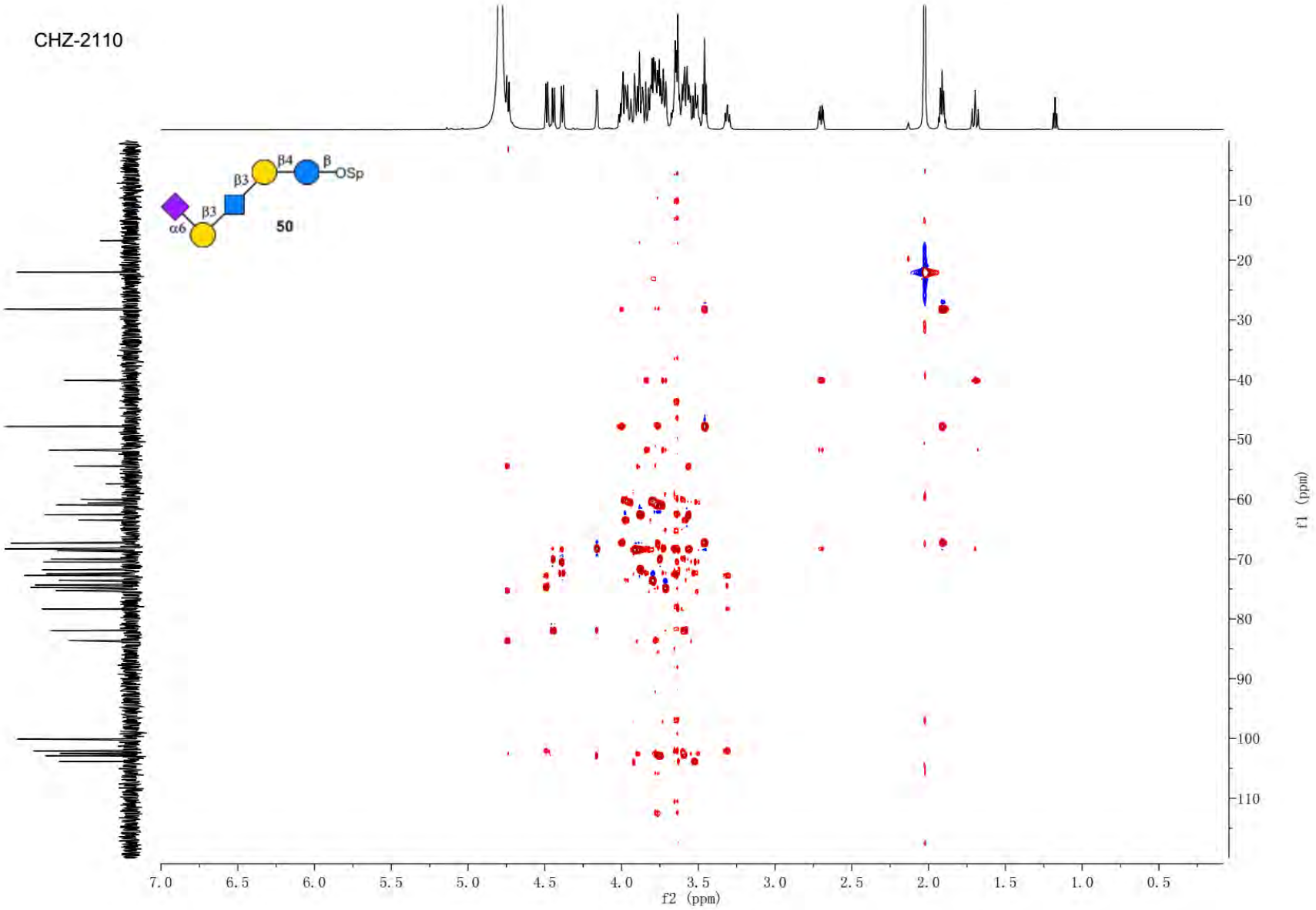
COSY spectra of 50



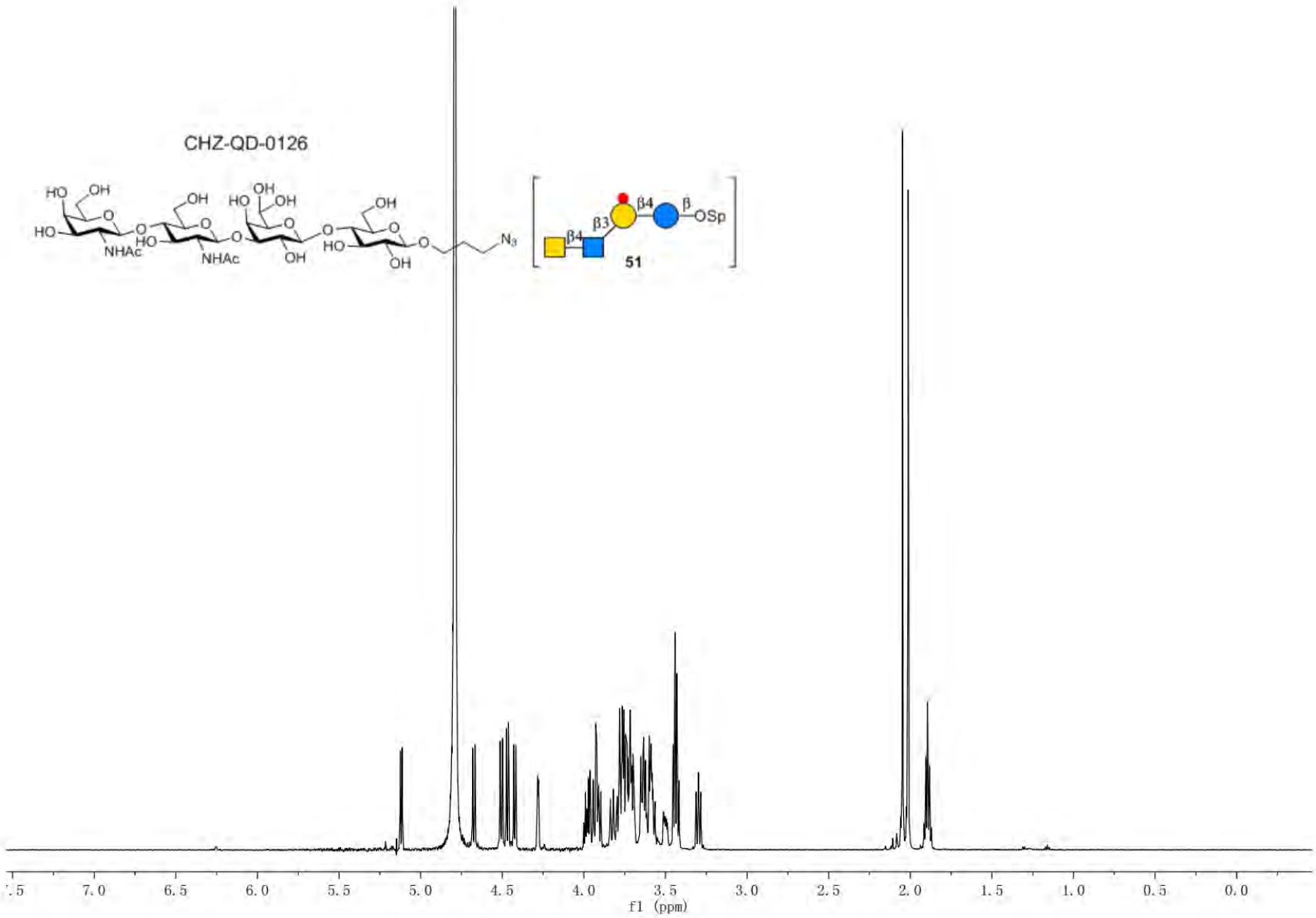
HMBC spectra of 50



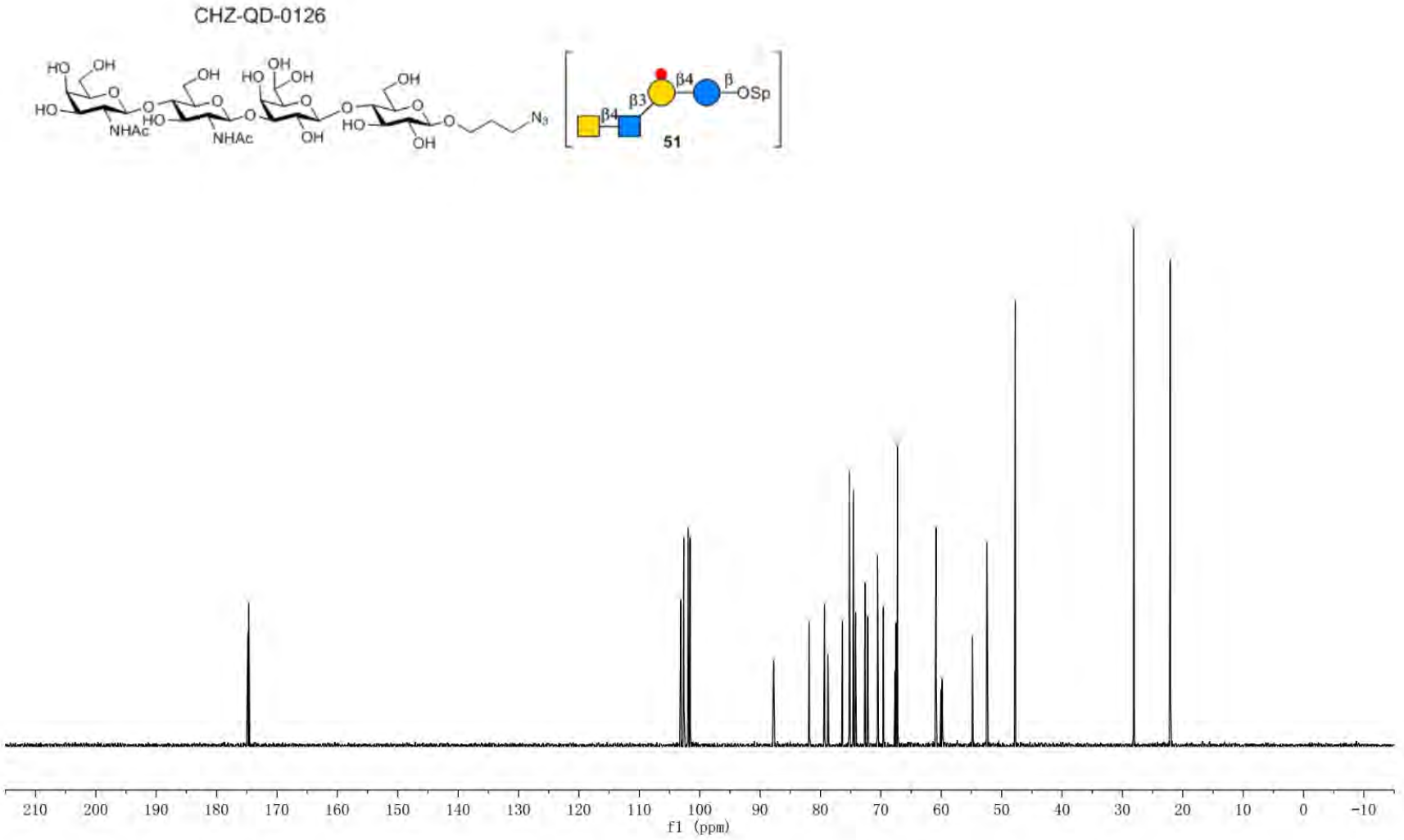
HSQC spectra of 50



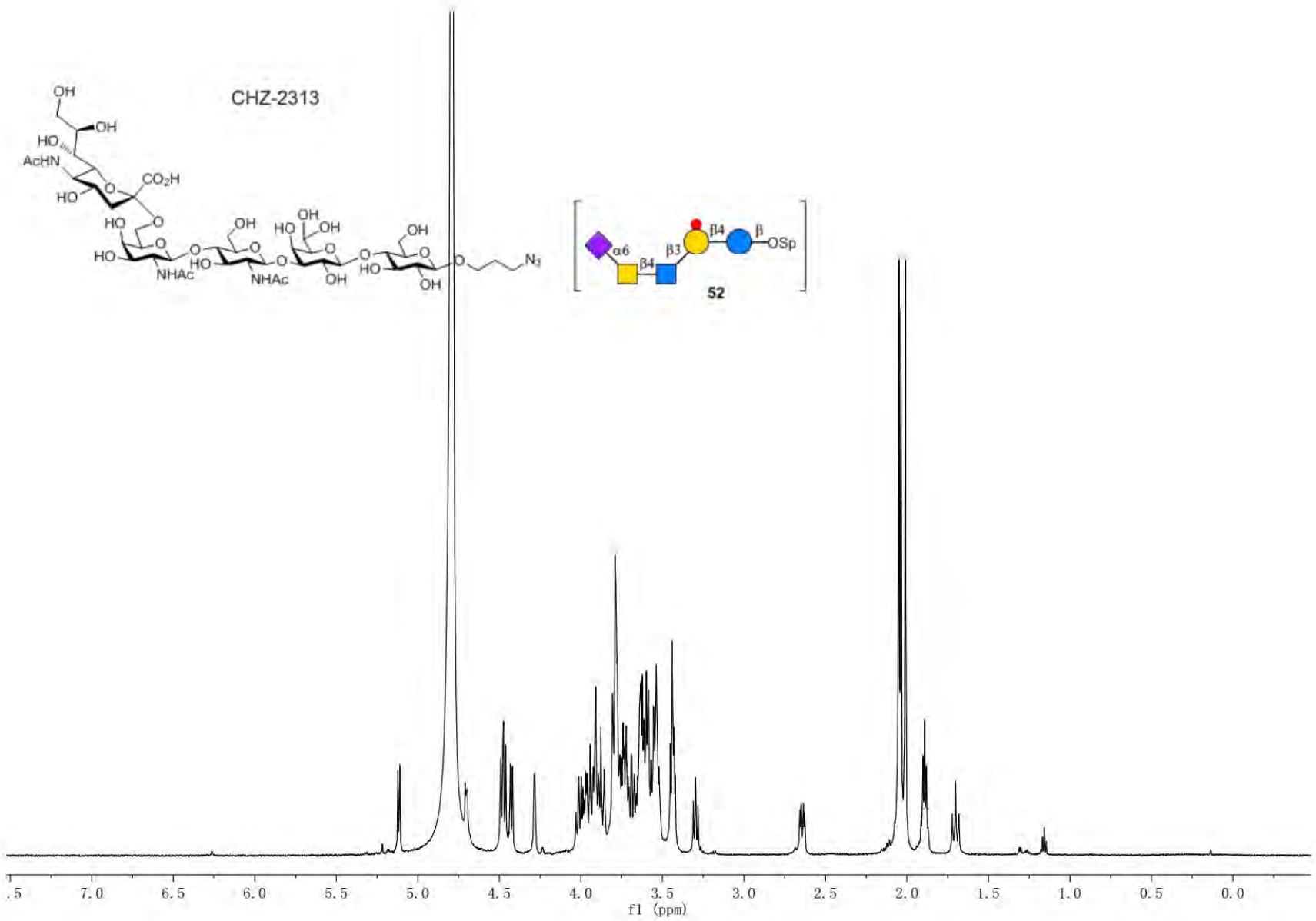
HSQC-TOCSY spectra of 50



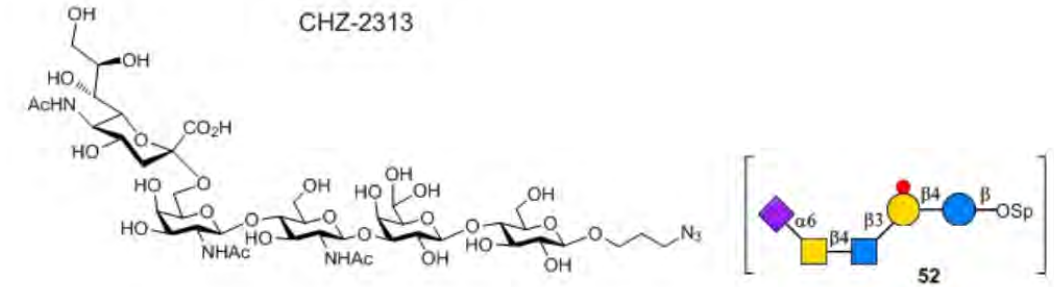
^{1}H NMR of 51

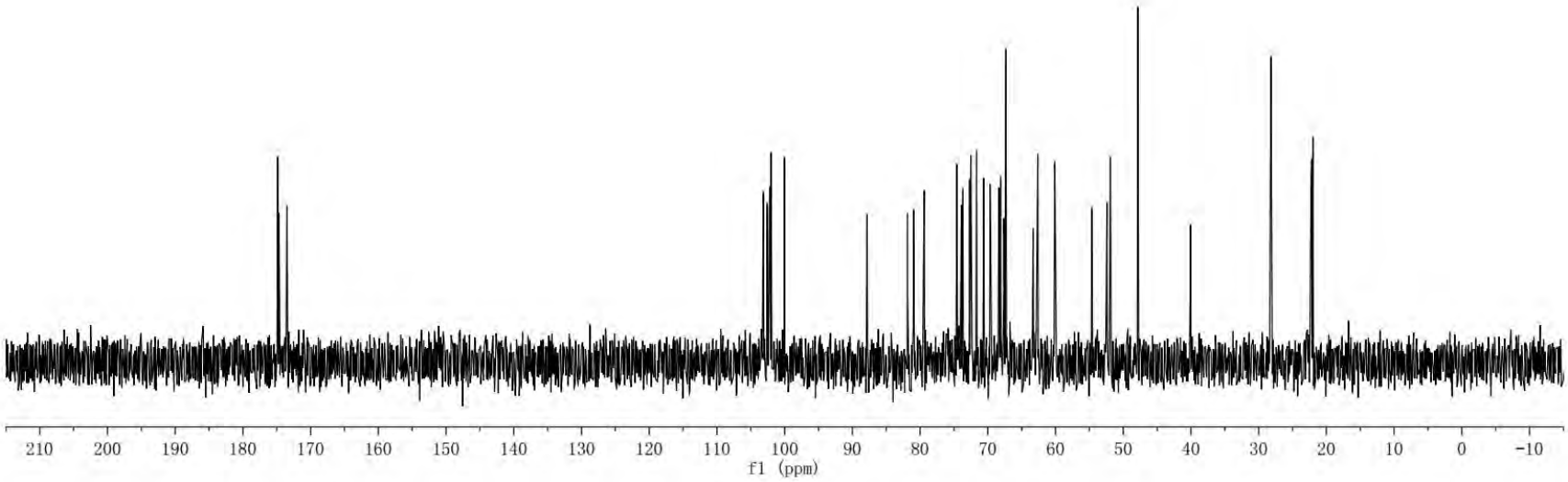


^{13}C NMR of 51

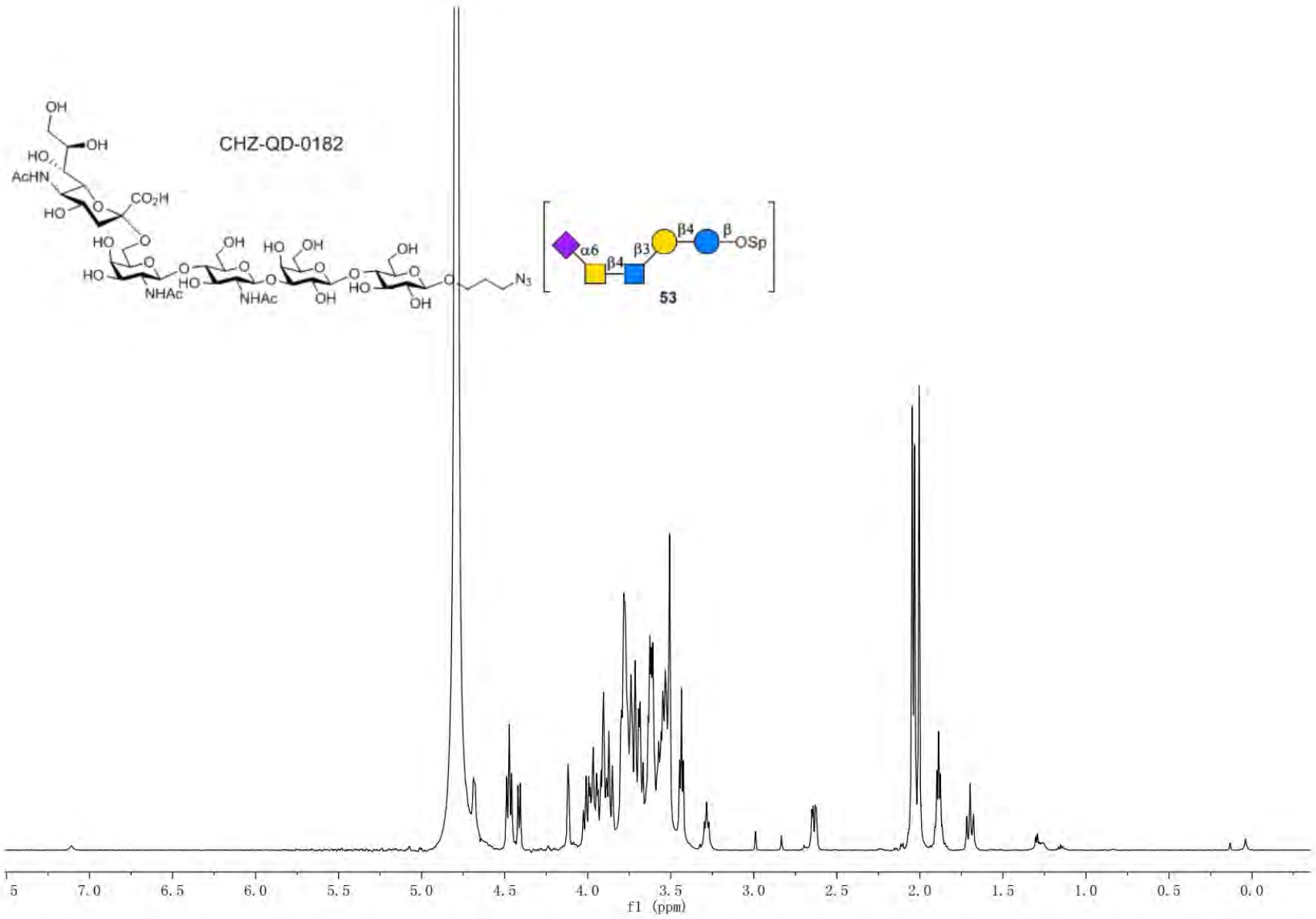


^{1}H NMR of 52

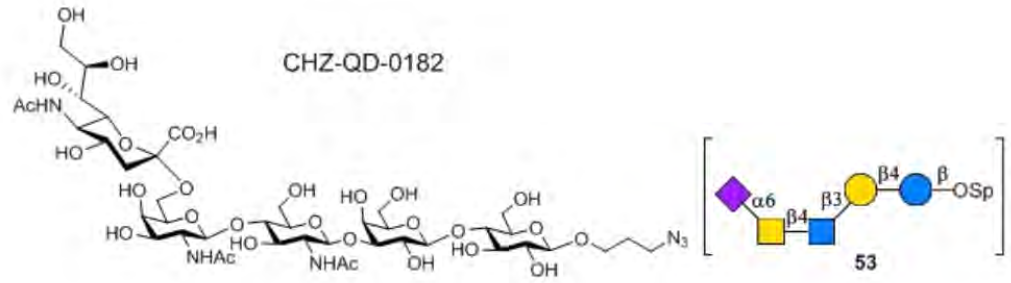

CHZ-2313
OH
OH
HO
AcHN
CO2H
HO
HO
O
HO
NHAc
OH
HO
NHAc
OH
HO
OH
OH
HO
OH
N3
α6
β4
β3
β4
β
OSp
52

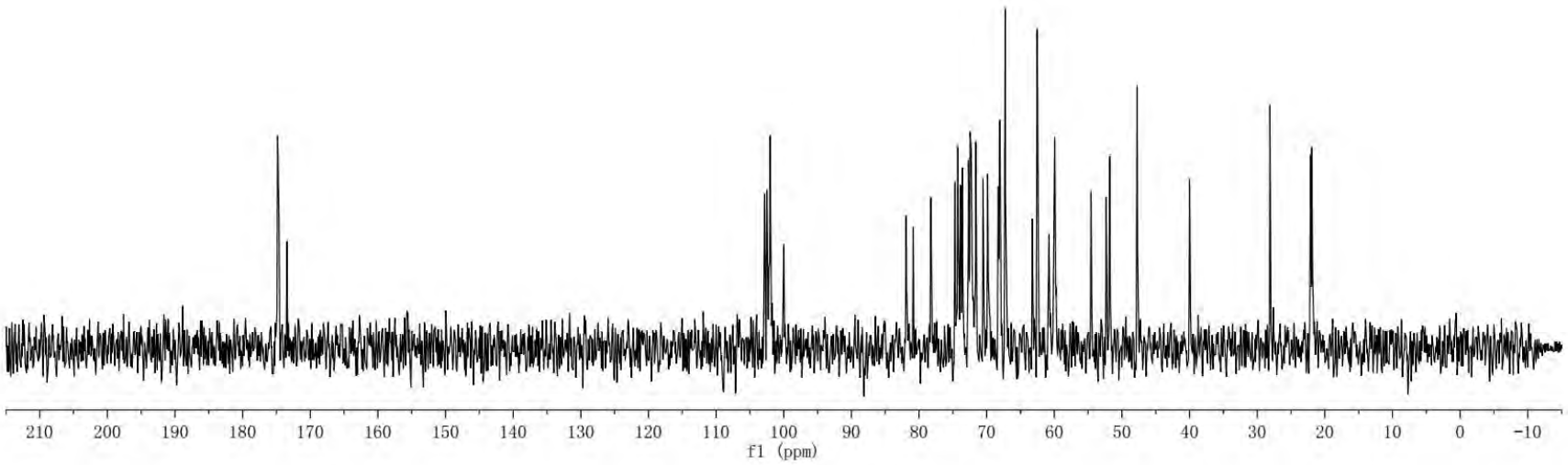

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

^{13}C NMR of 52

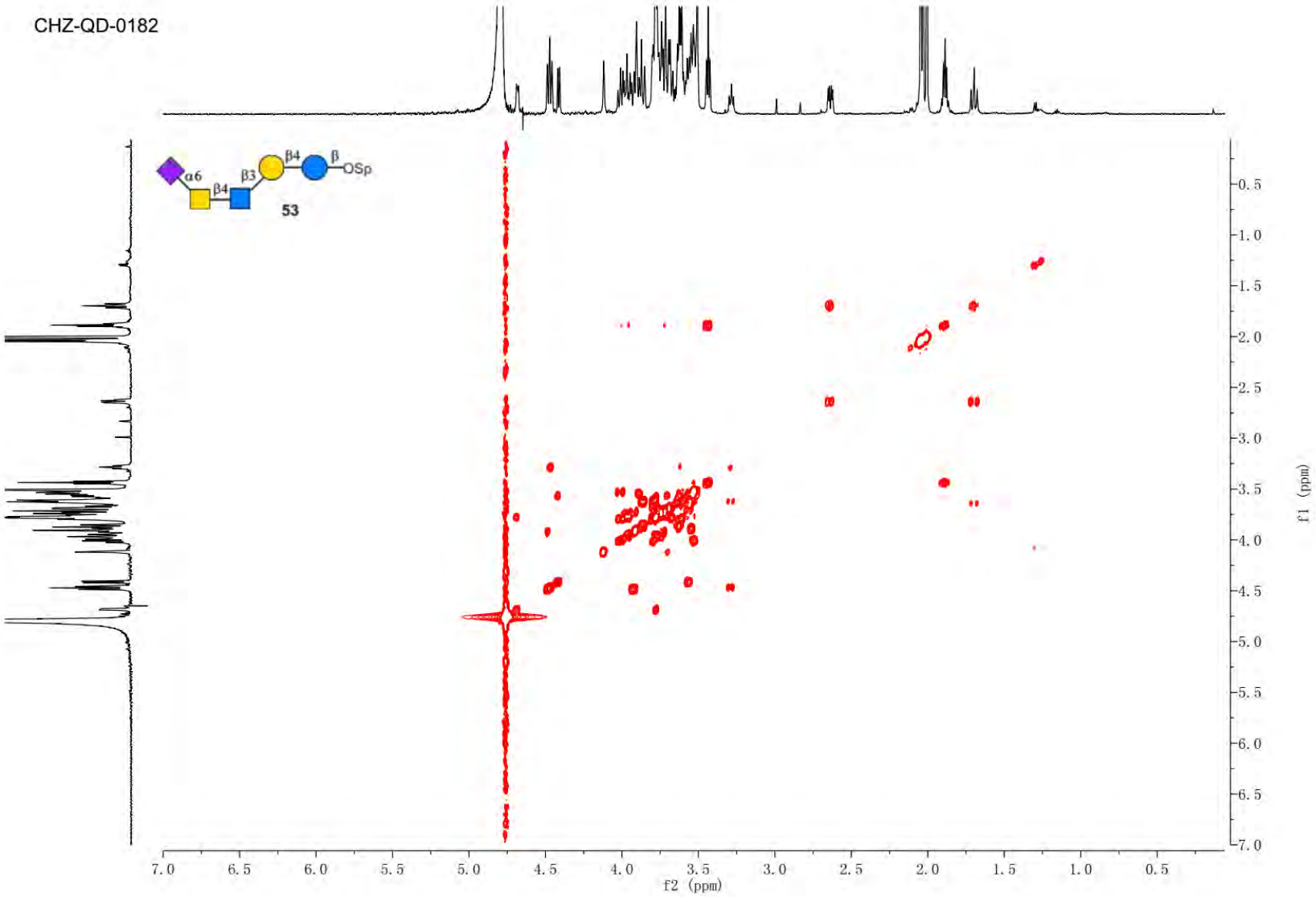


^{1}H NMR of 53

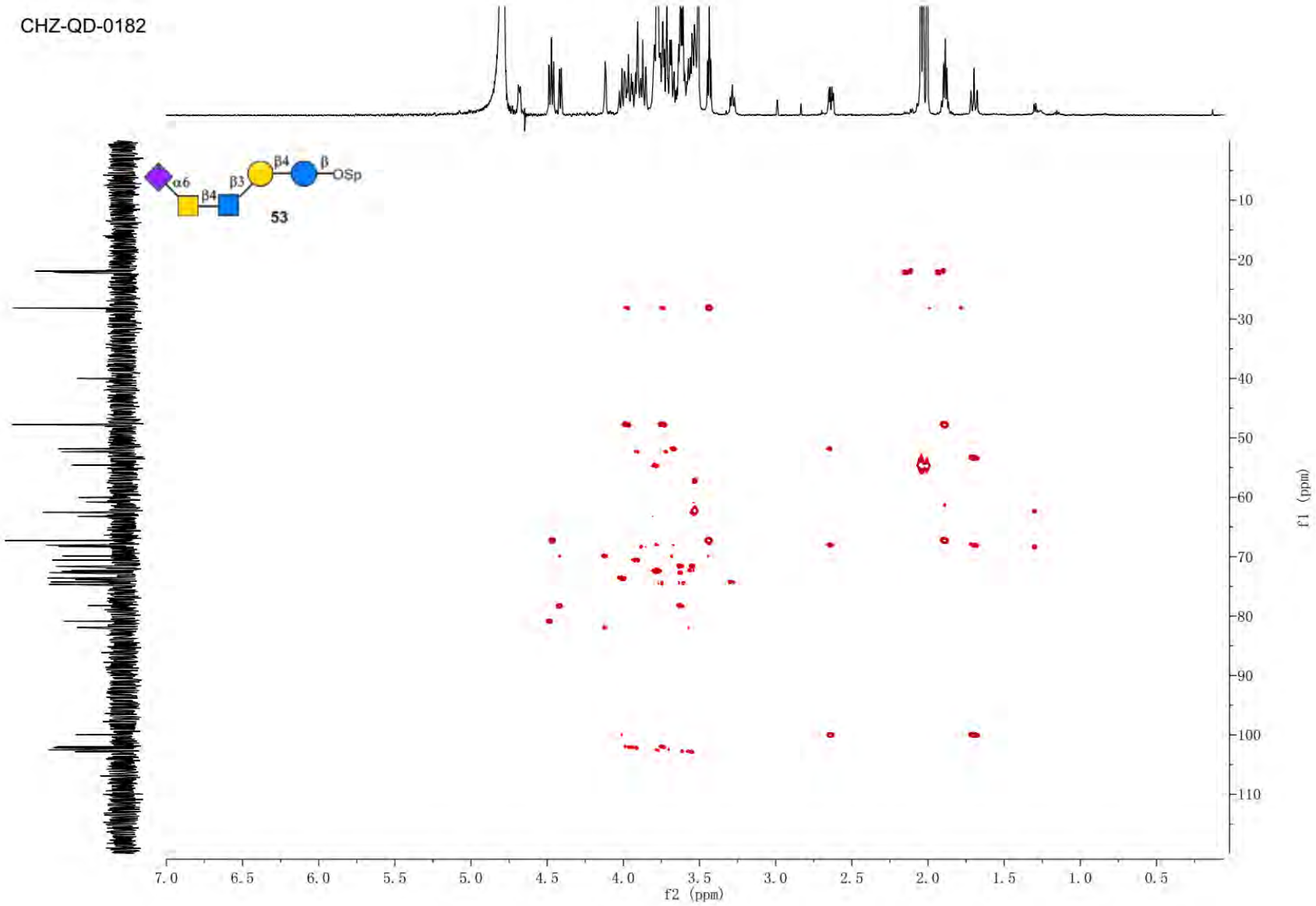

CHZ-QD-0182
OH
HO
AcHN
CO2H
HO
HO
NHAc
HO
NHAc
HO
OH
HO
OH
OH
HO
OH
N3
α6
β4
β3
β4
β
OSp
53


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

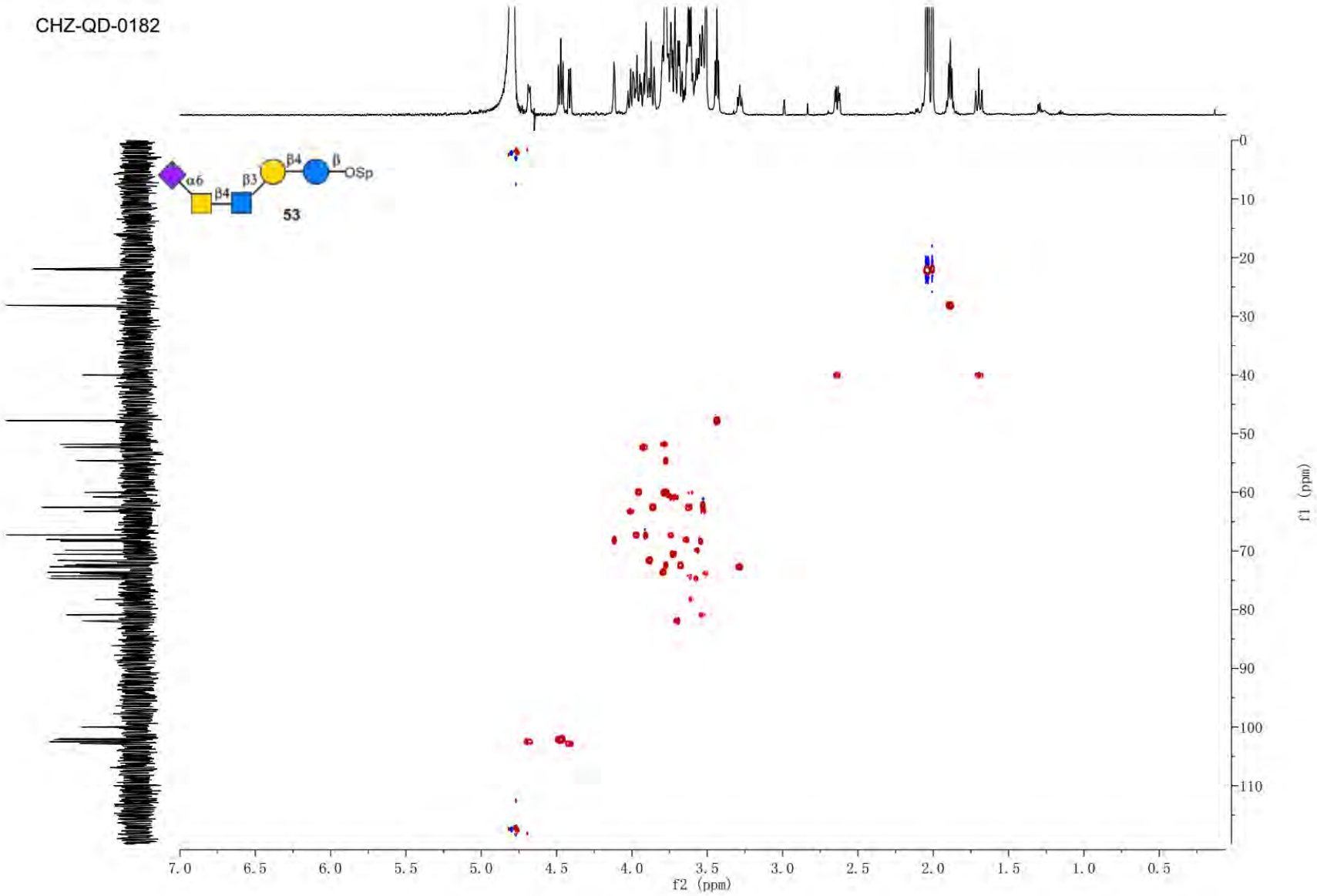
^{13}C NMR of 53



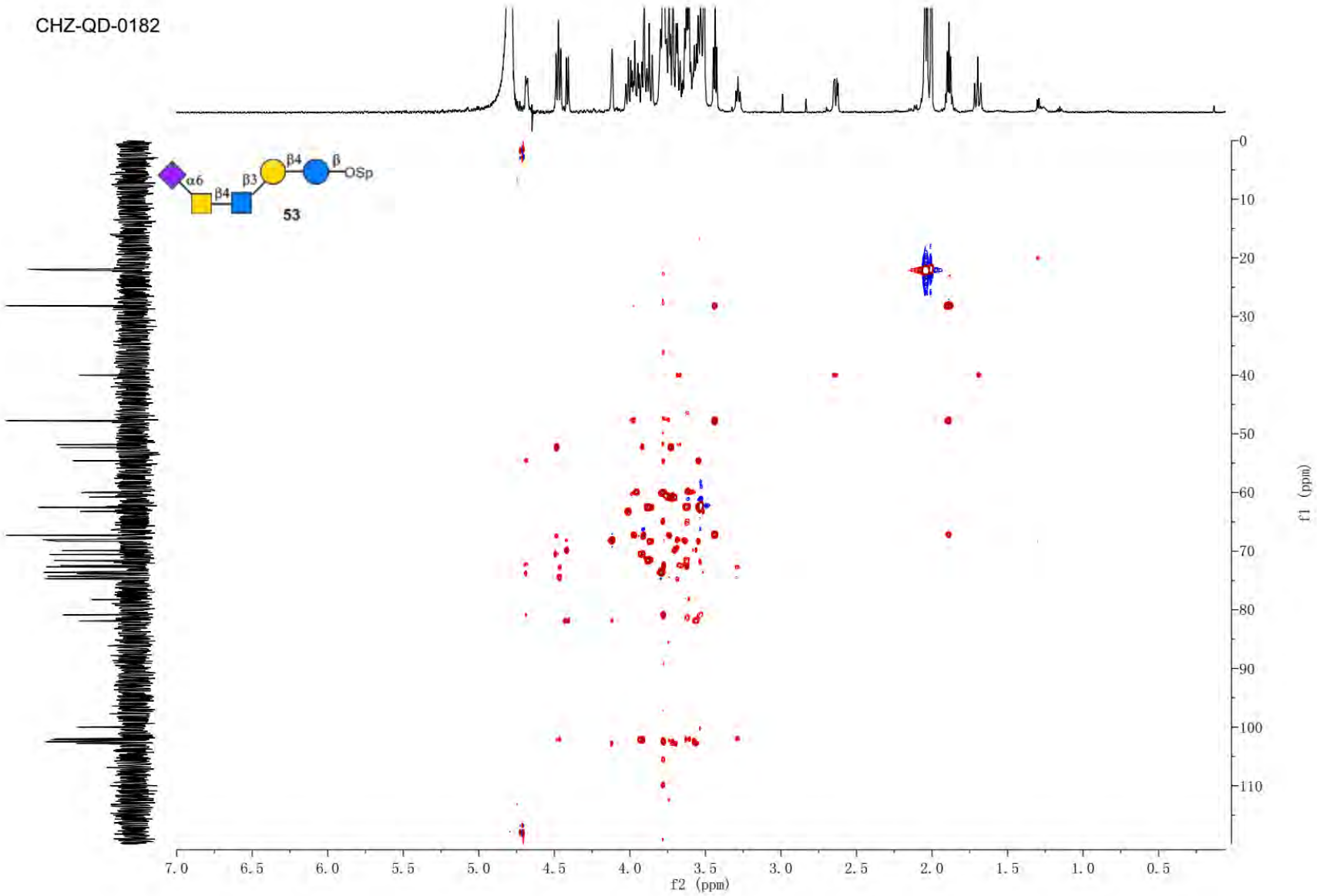
COSY spectra of 53



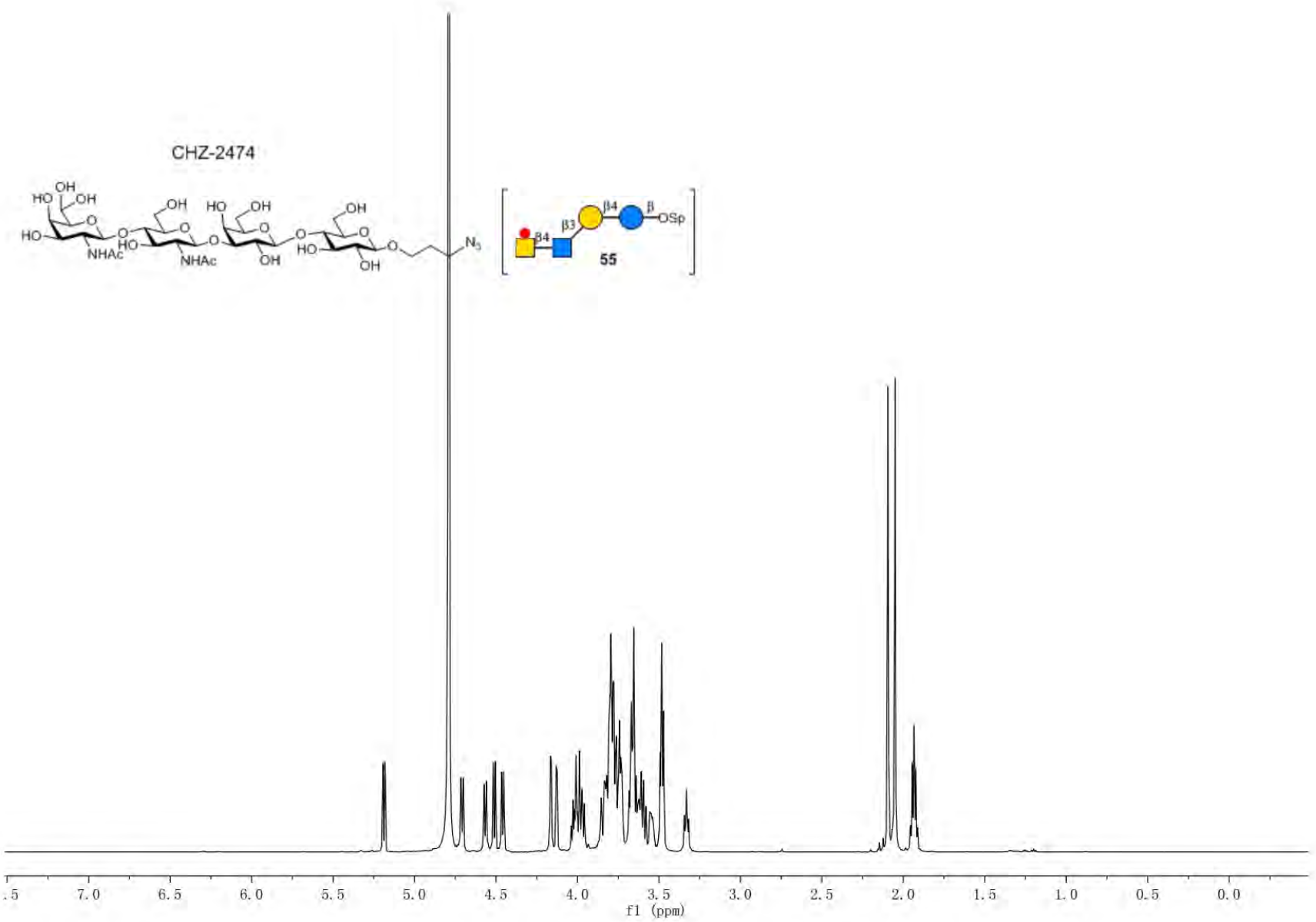
HMBC spectra of 53



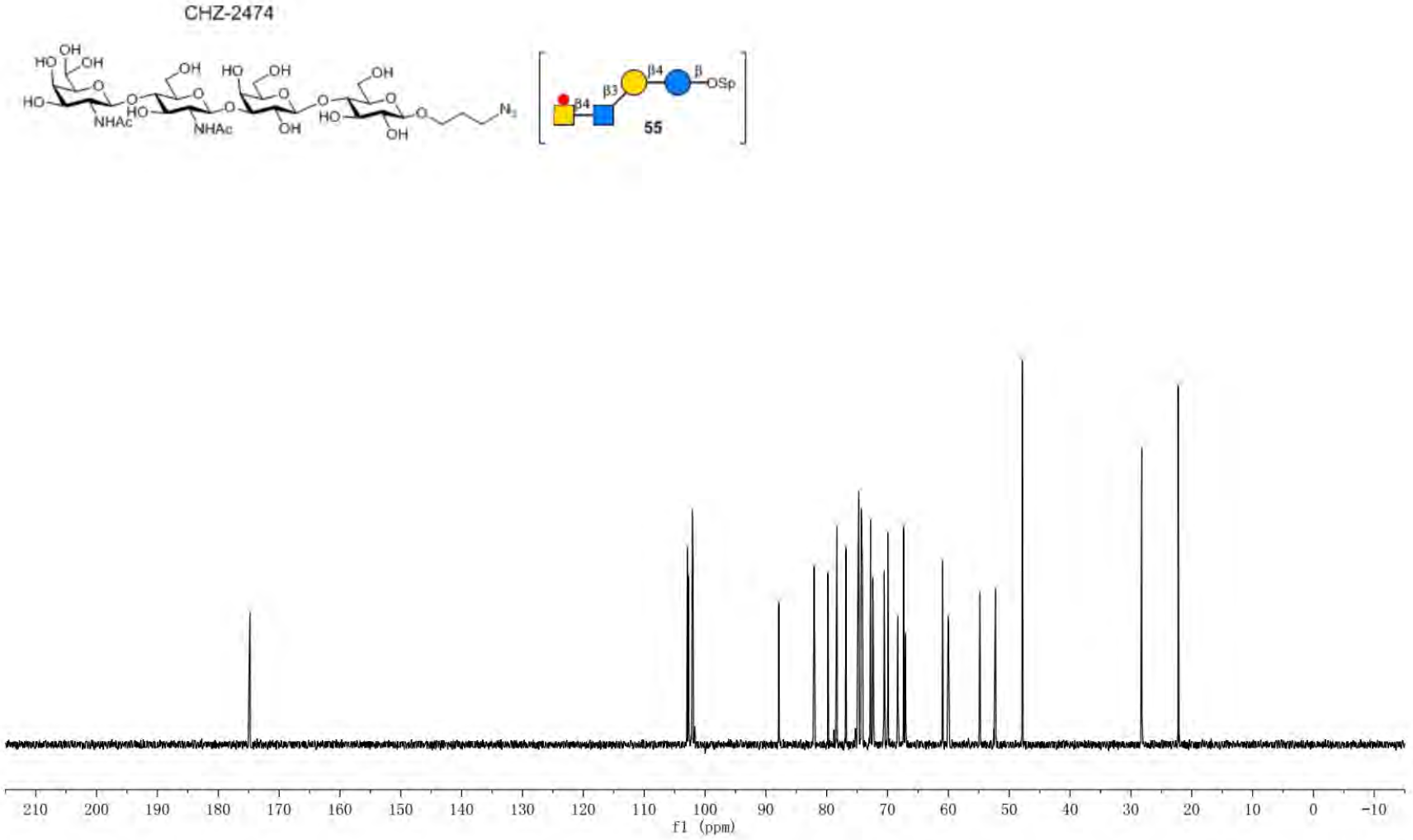
HSQC spectra of 53



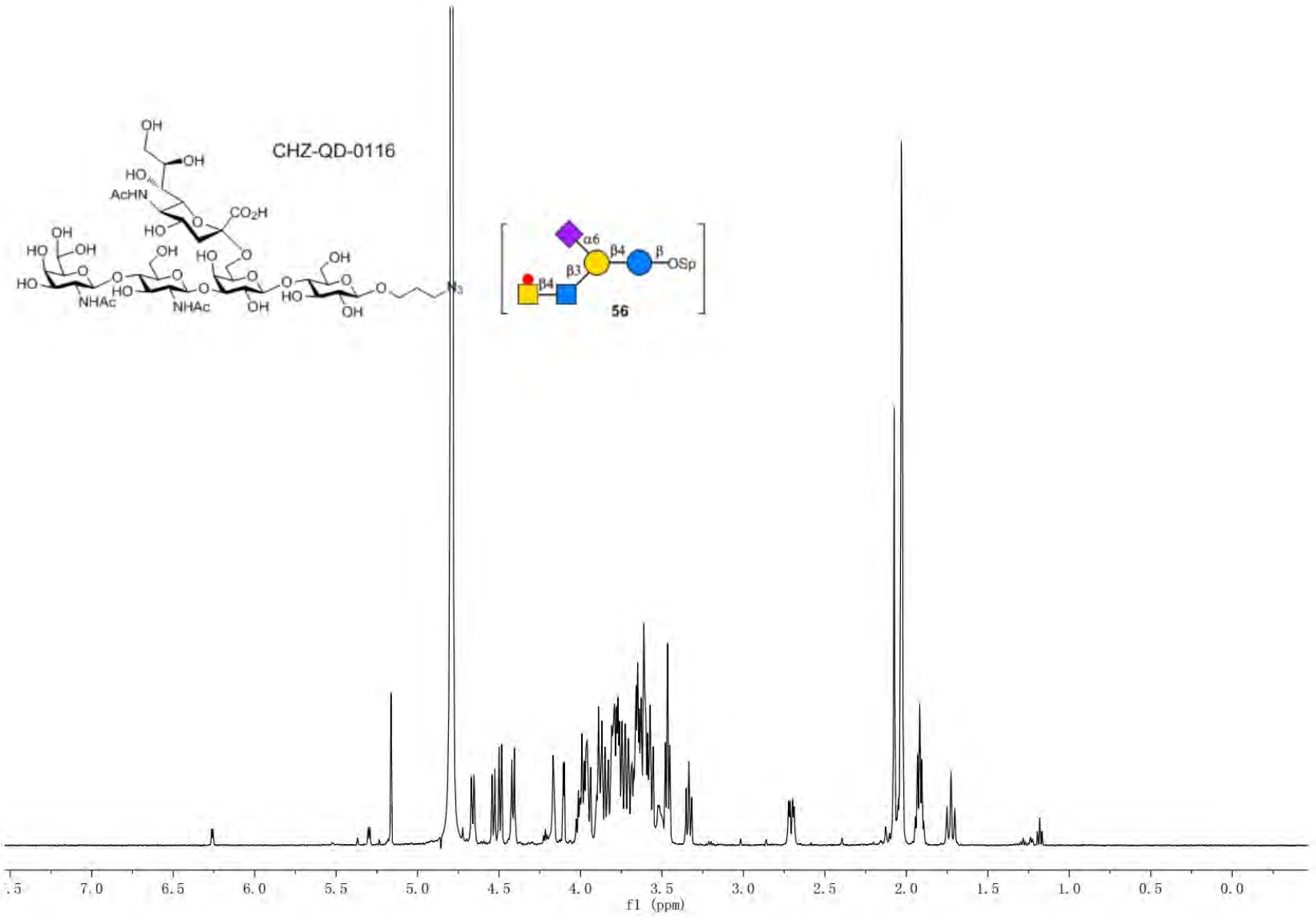
HSQC-TOCSY spectra of 53



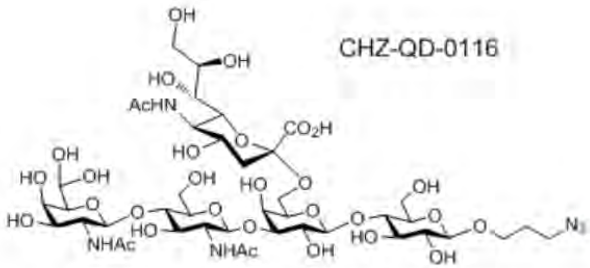
^{1}H NMR of 55

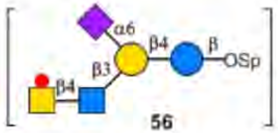


^{13}C NMR of 55

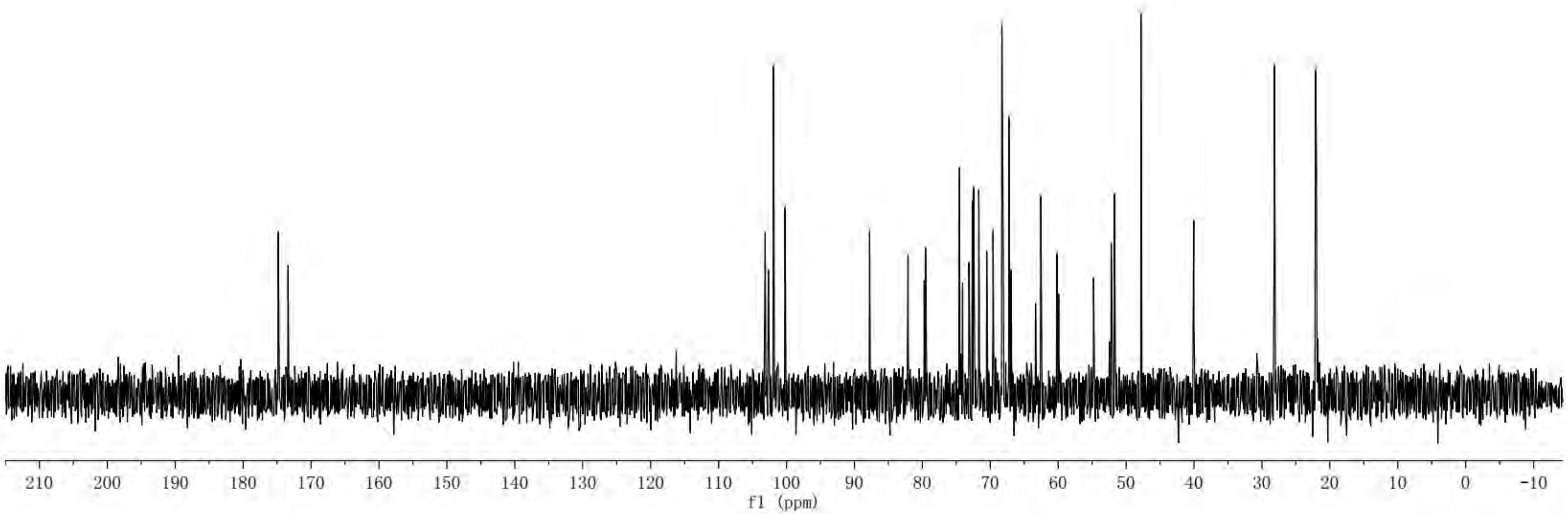


^{1}H NMR of 56

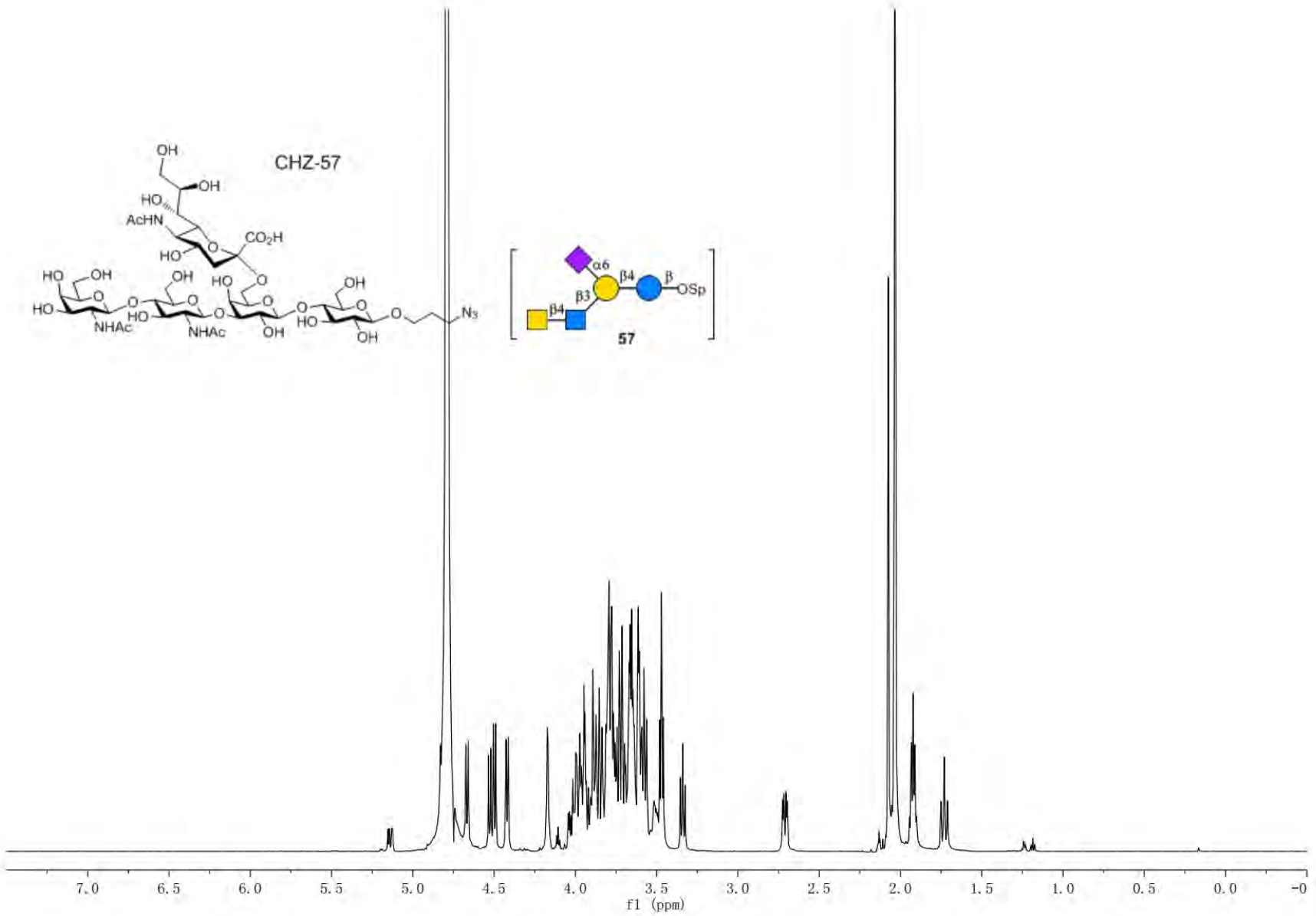




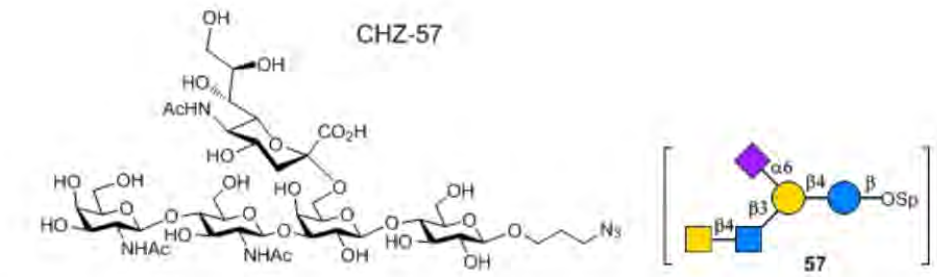
56

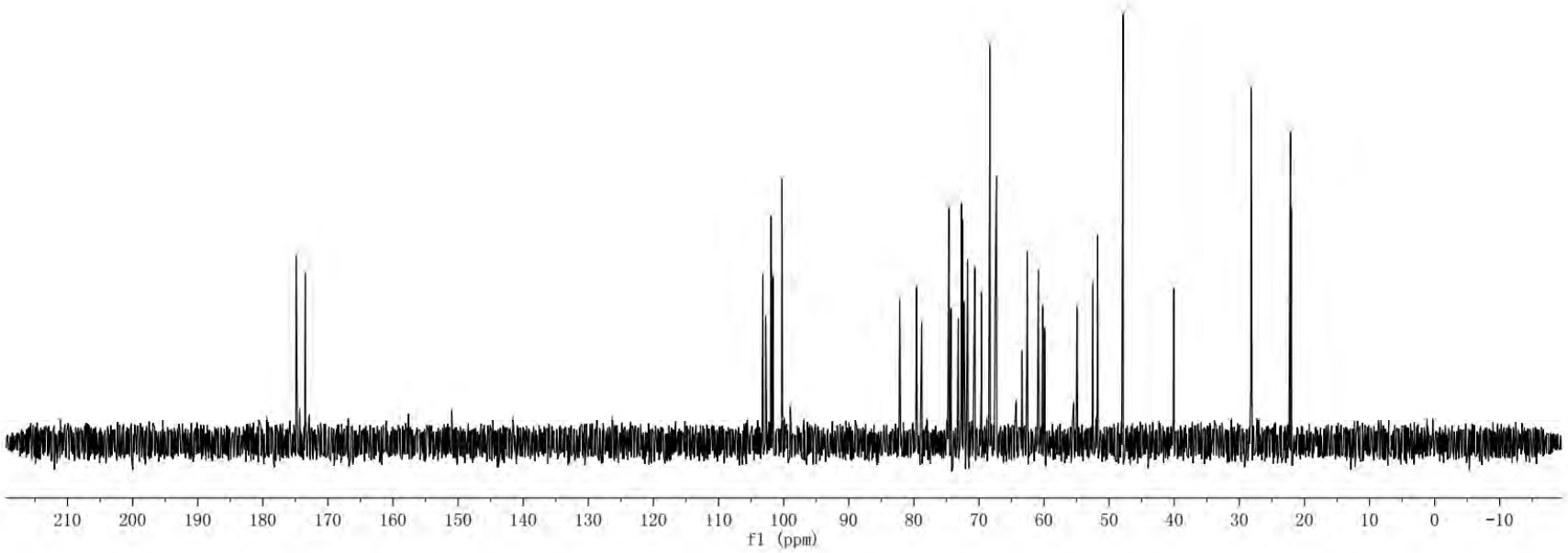


^{13}C NMR of 56

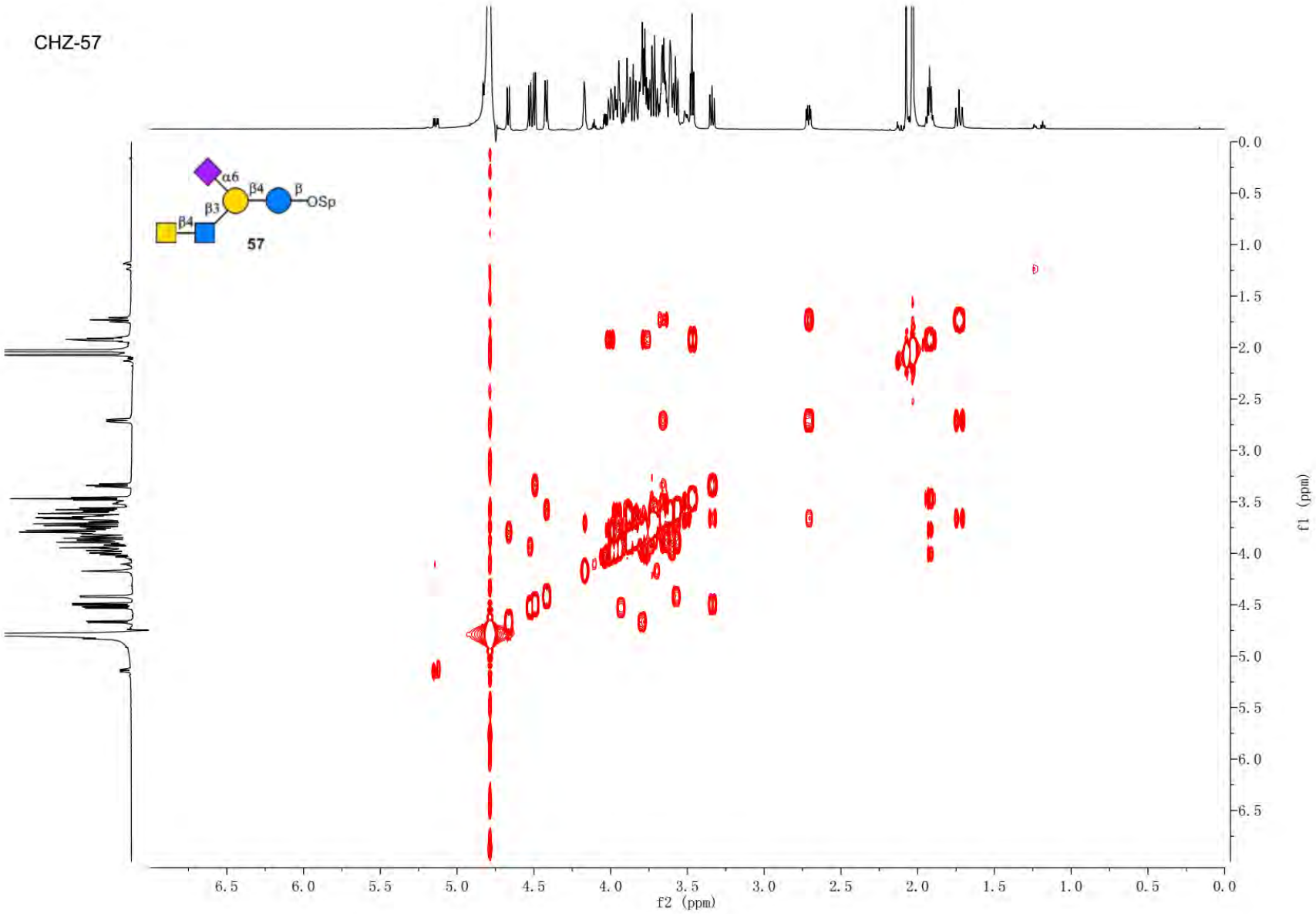


^{1}H NMR of 57

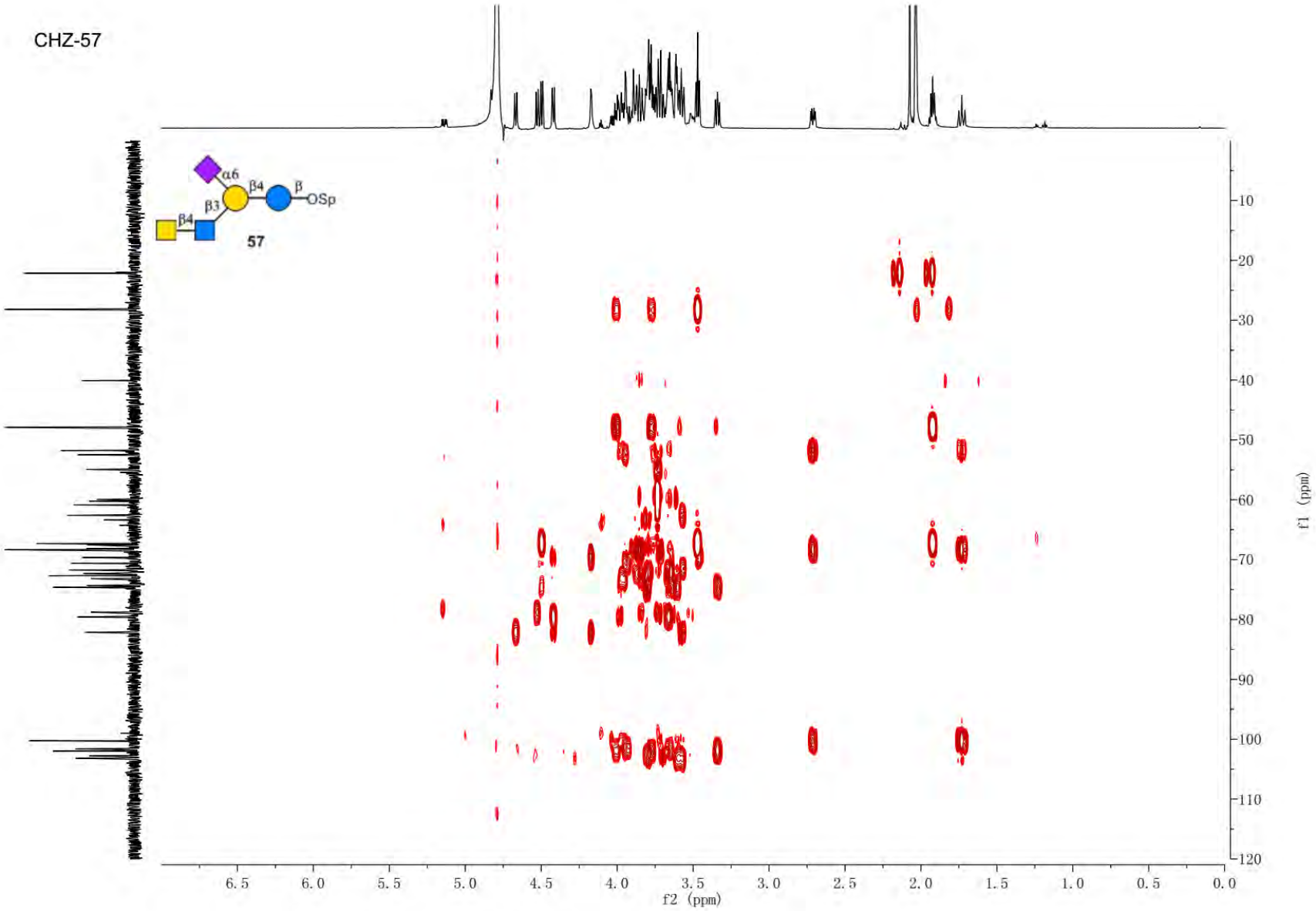

CHZ-57
OH
OH
HO
AcHN
CO2H
HO
HO
OH
O
NHAc
OH
HO
O
HO
NHAc
O
OH
HO
O
OH
O
N3
α6
β4
β
OSp
β3
β4
57


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

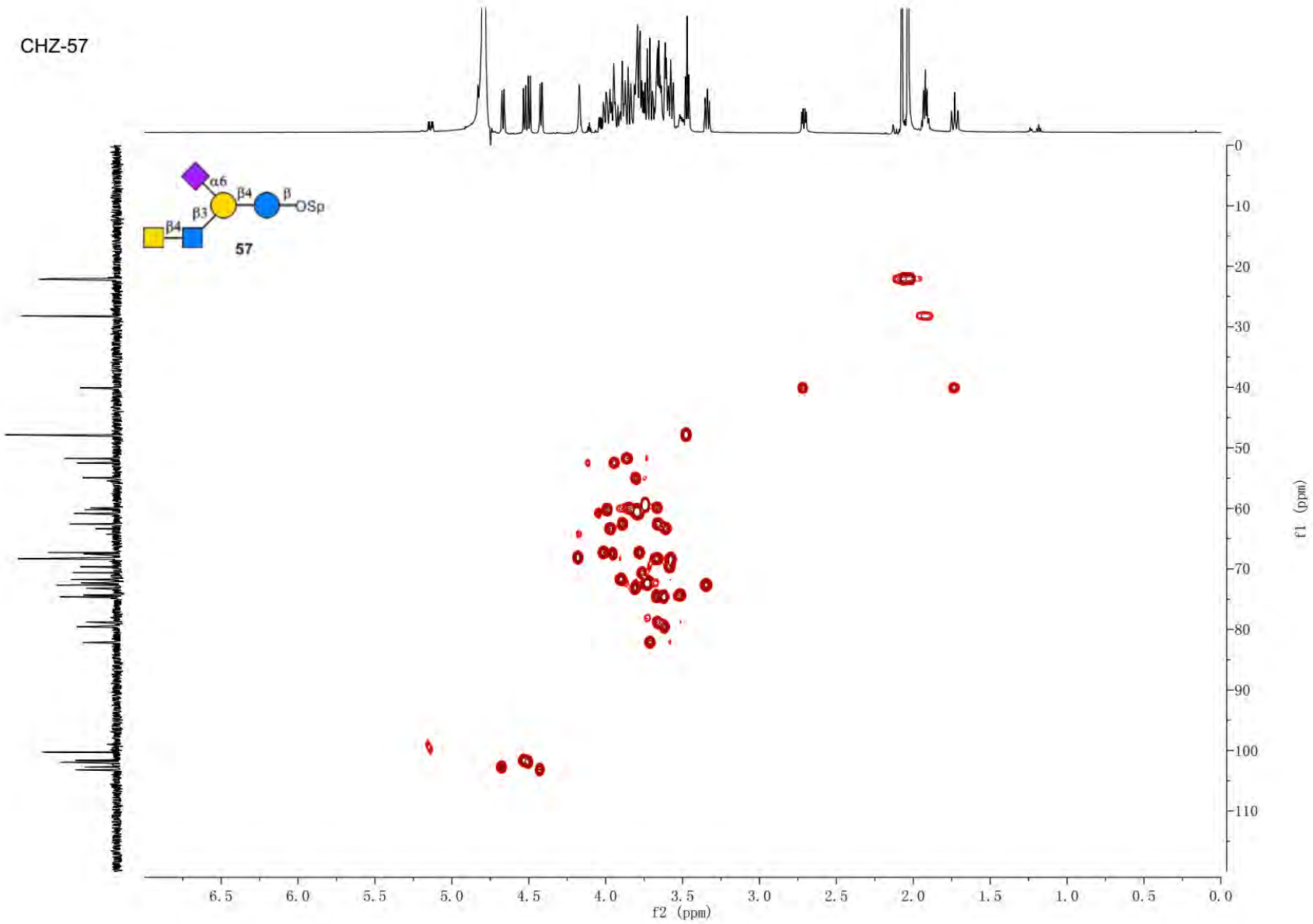
^{13}C NMR of 57



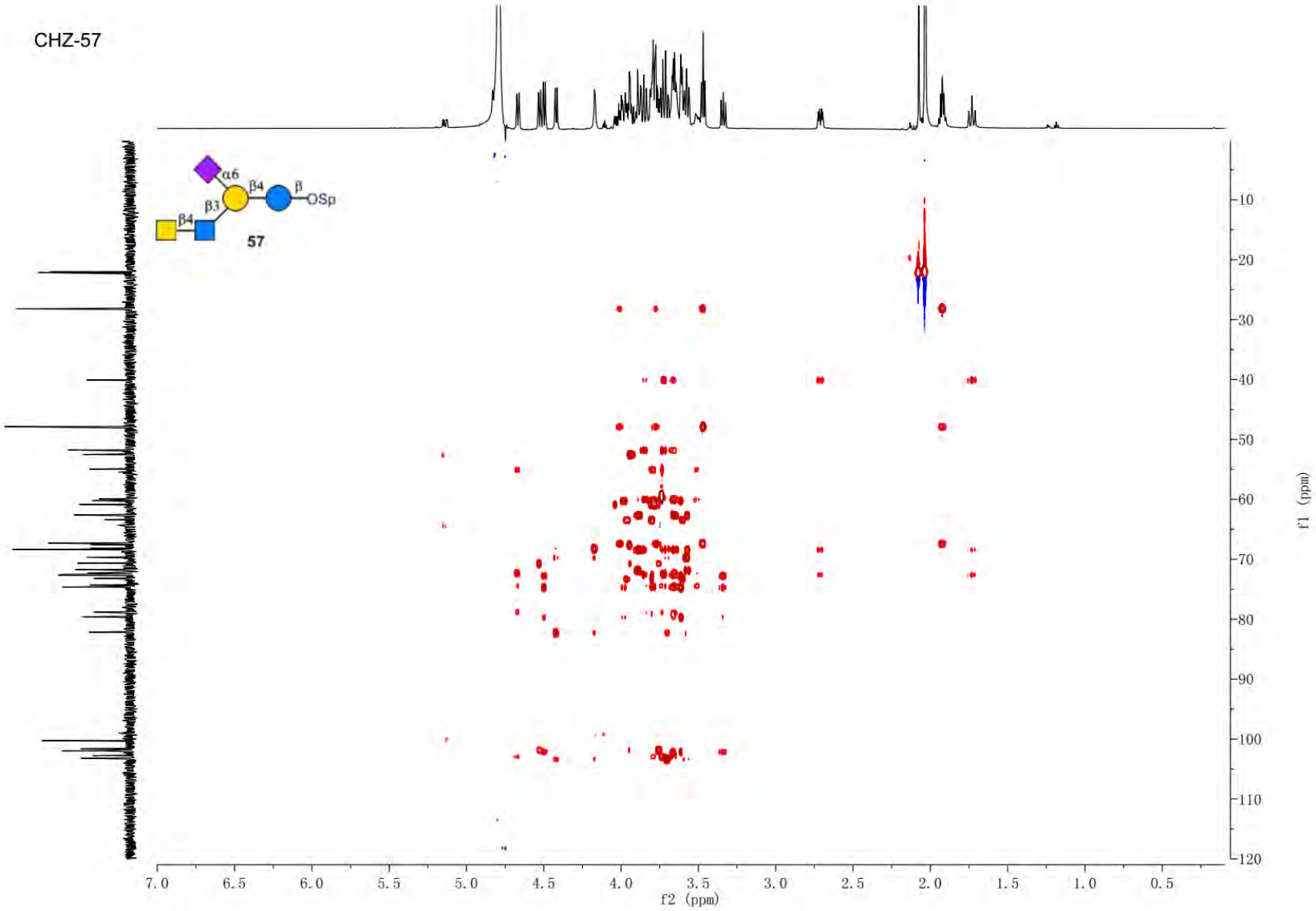
COSY spectra of 57



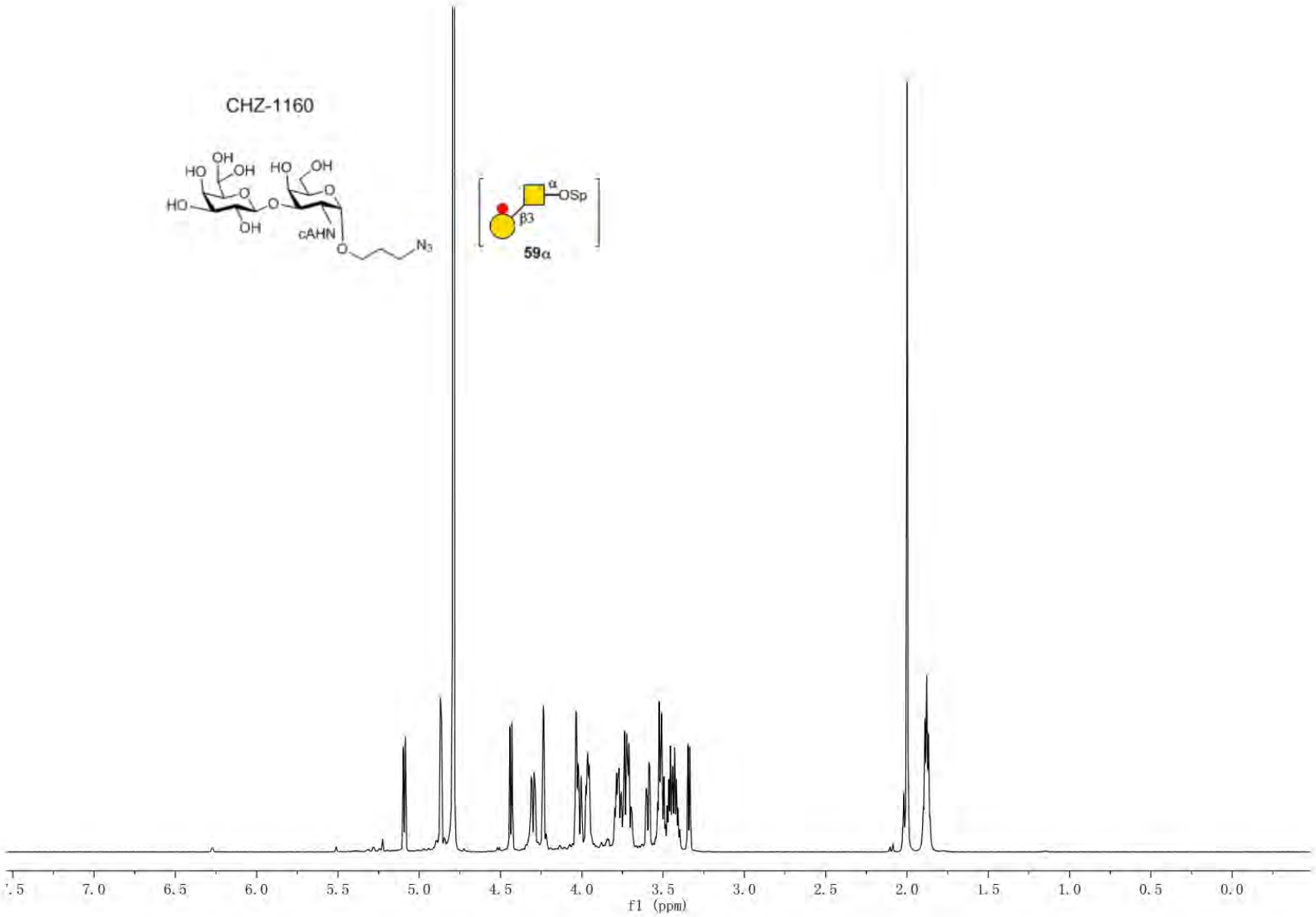
HMBC spectra of 57



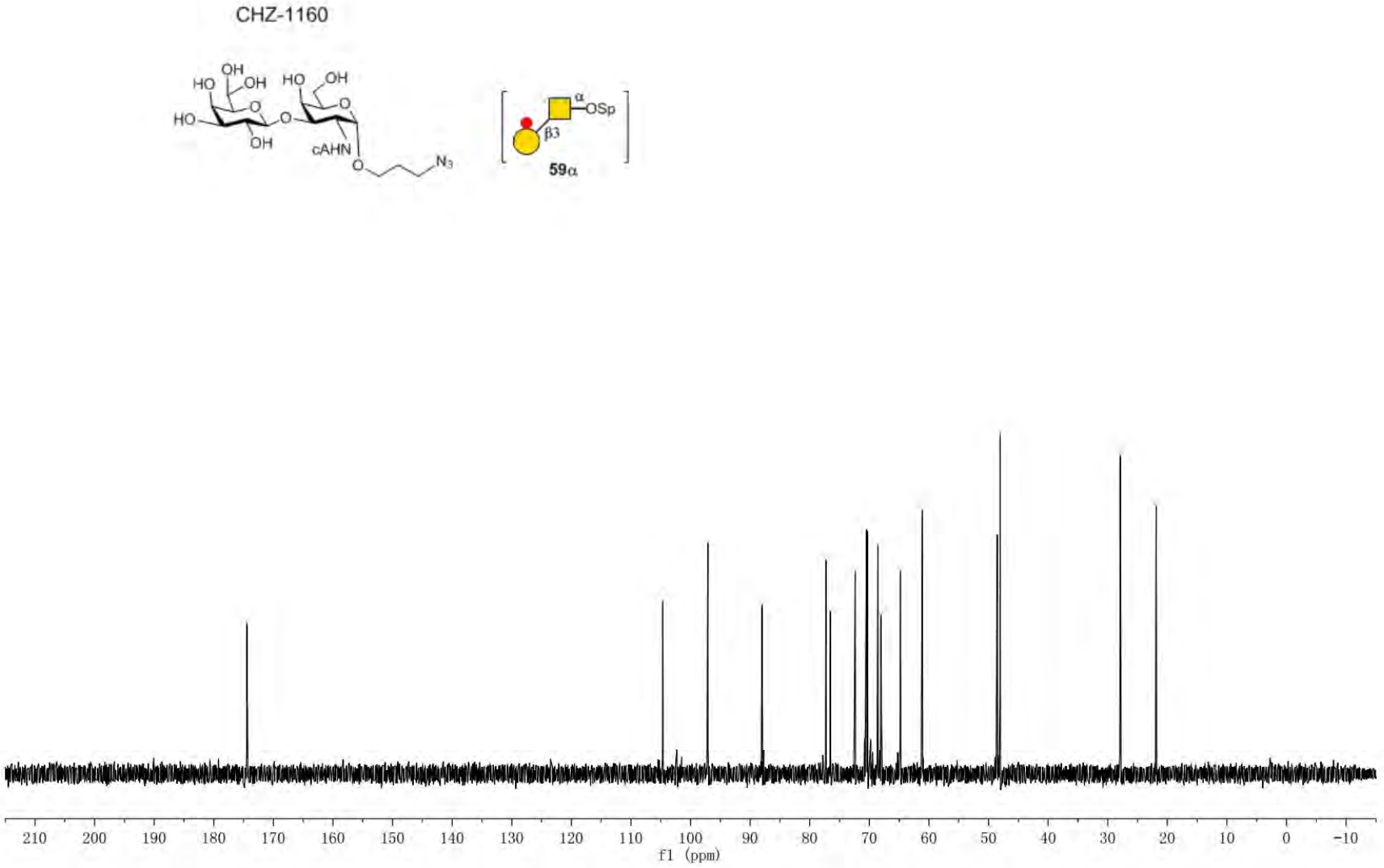
HSQC spectra of 57



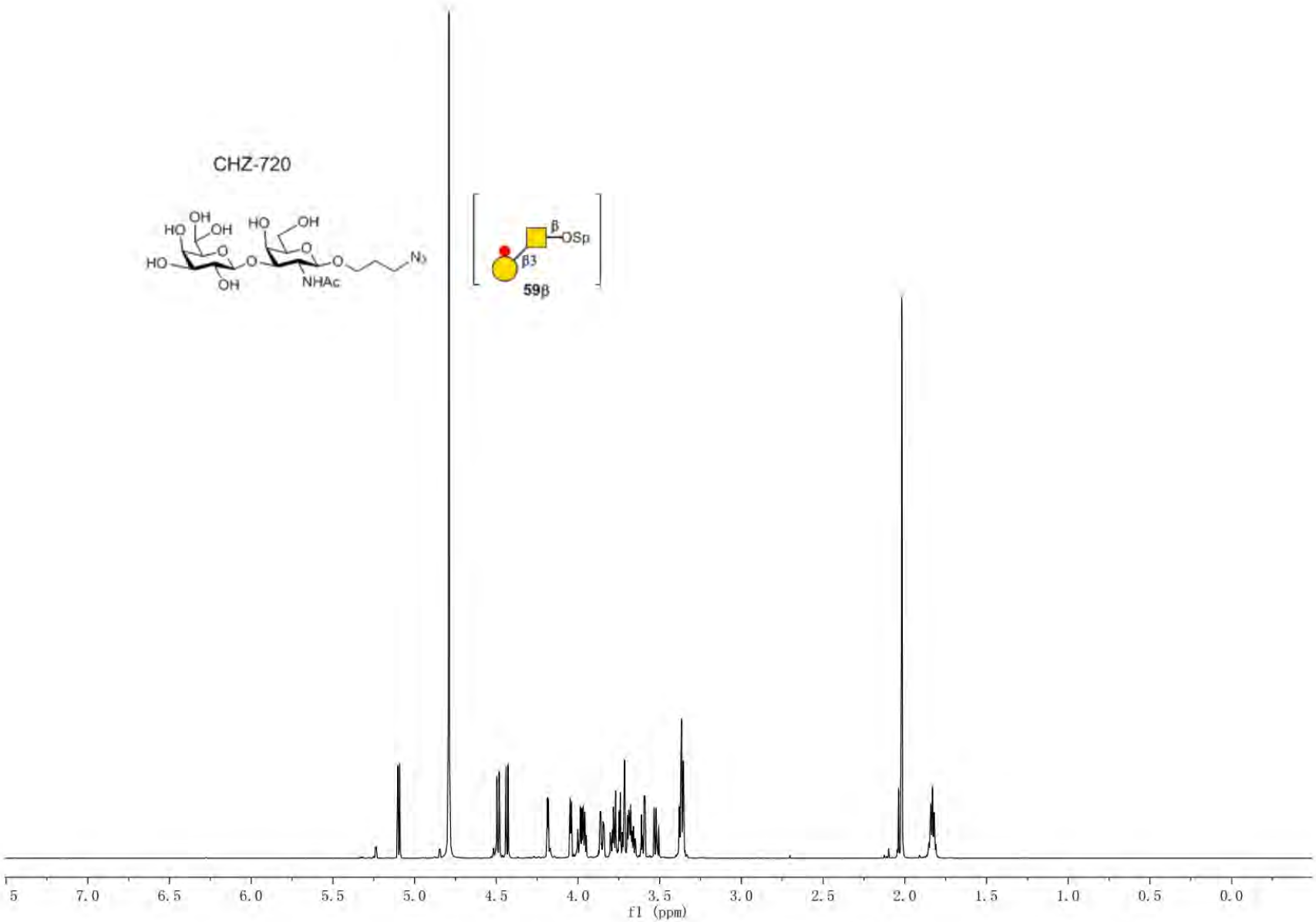
HSQC-TOCSY spectra of 57


CHZ-1160
HO
OH
OH
HO
OH
HO
OH
O
O
O
cAHN
O
N3
α
OSp
β3
59α
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

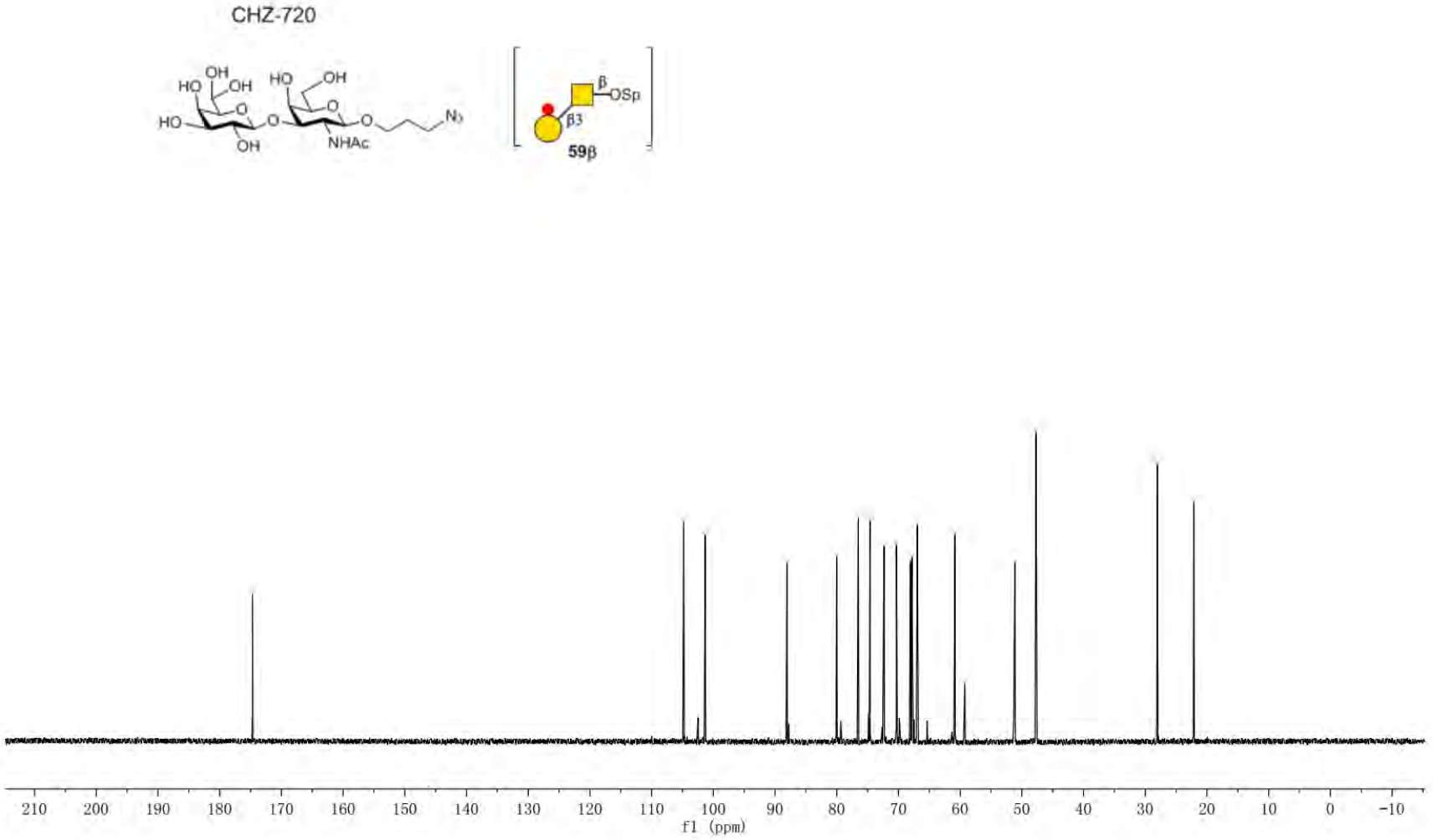
^{1}H NMR of 59α



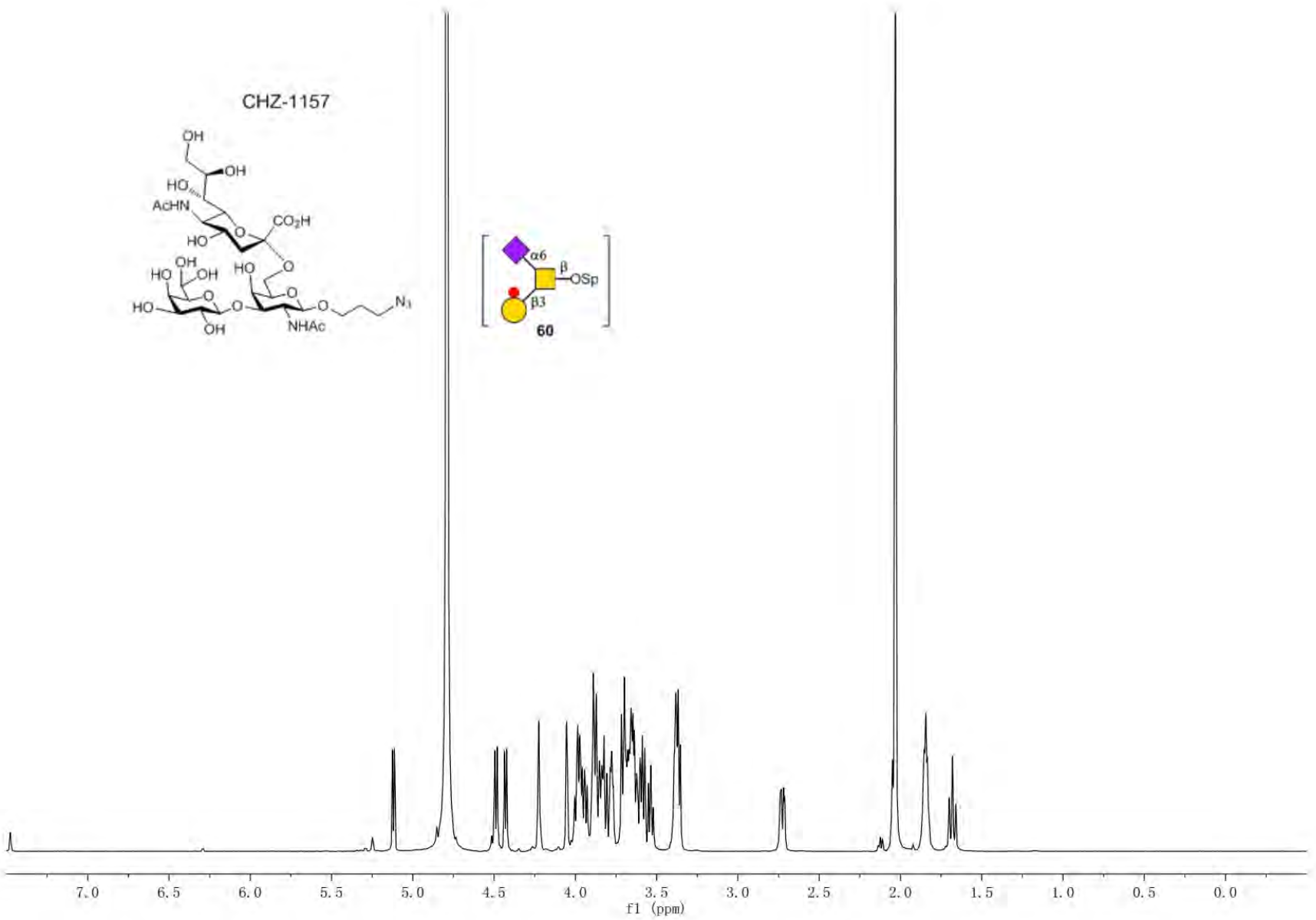
^{13}C NMR of 59α


CHZ-720
HO
OH
OH
HO
O
HO
OH
O
O
N3
OH
NHAc
β
OSp
β3
59β
5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

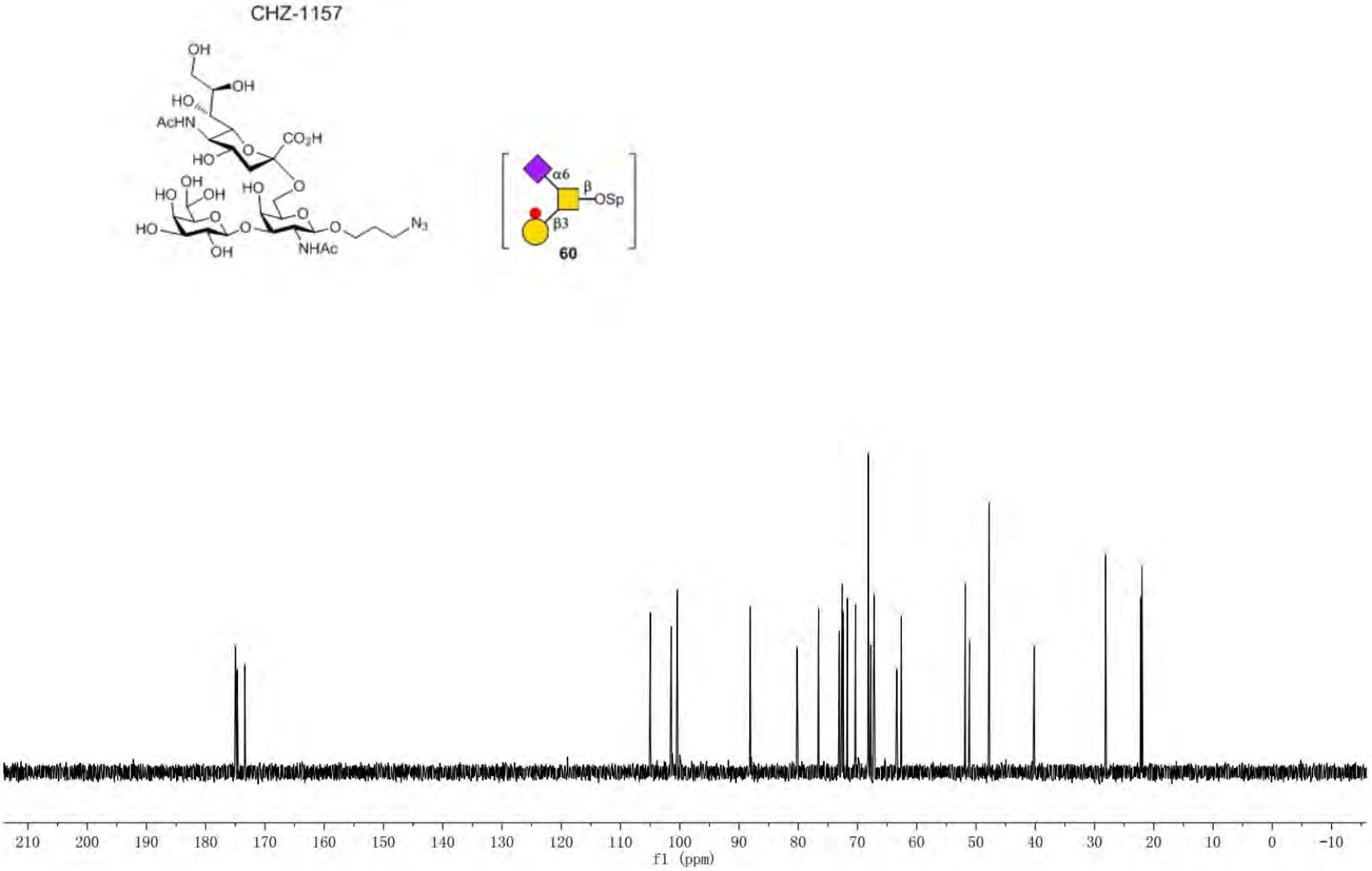
^{1}H NMR of 59β



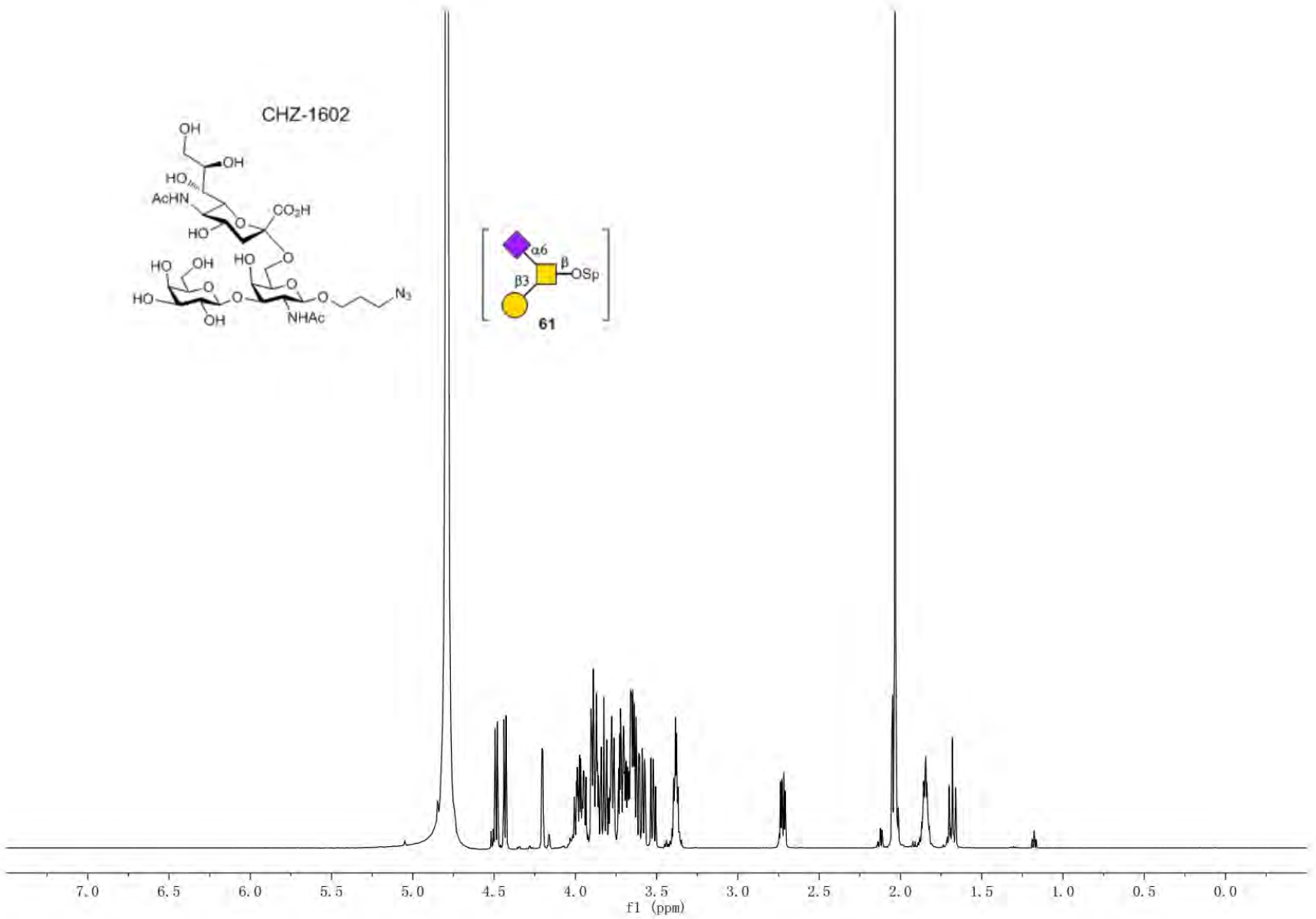
^{13}C NMR of 59β



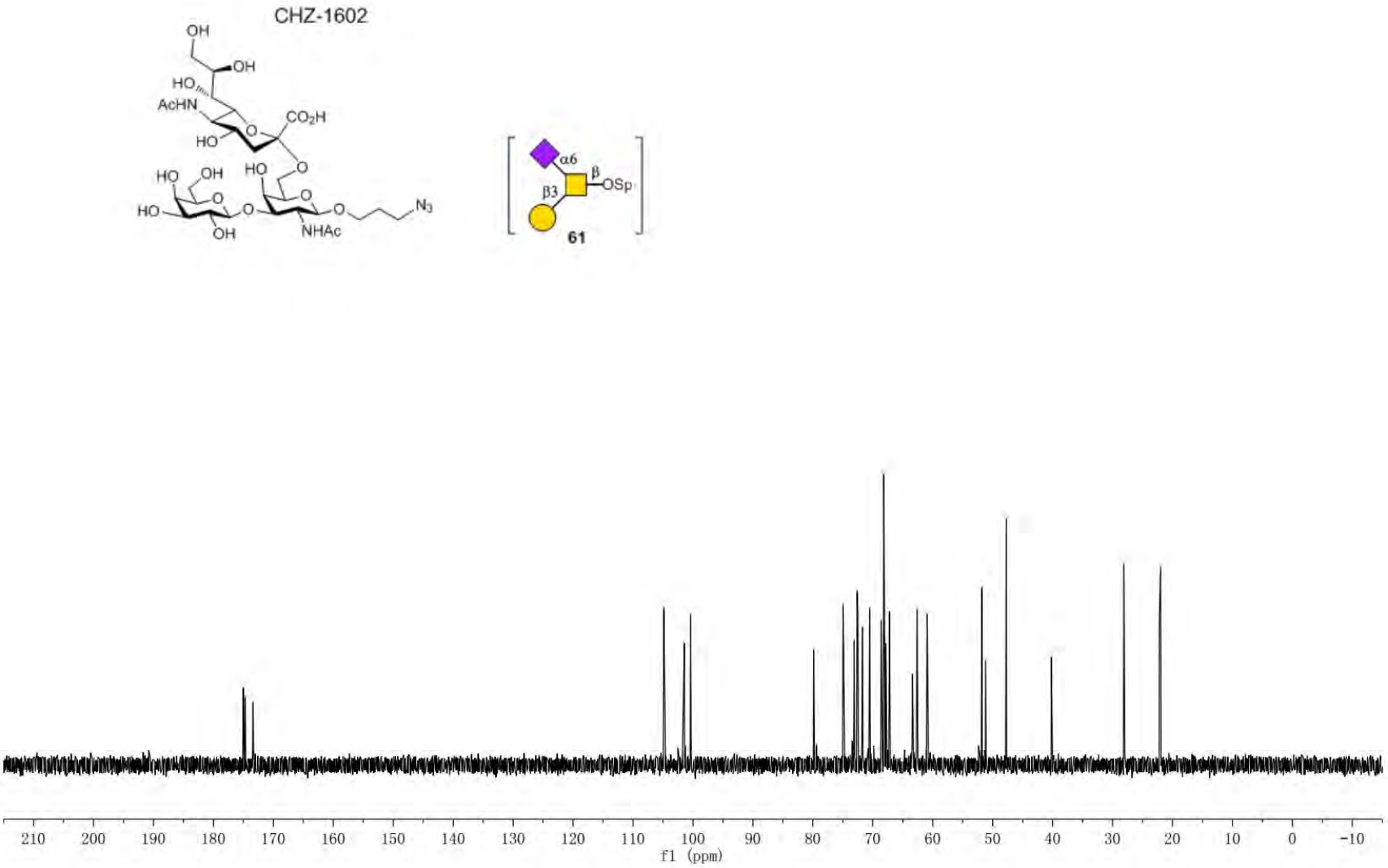
^{1}H NMR of 60


CHZ-1157
OH
OH
HO
AcHN
CO2H
HO
O
HO
OH
HO
OH
HO
O
O
HO
O
O
N3
OH
NHAc
α6
β
OSp
β3
60
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

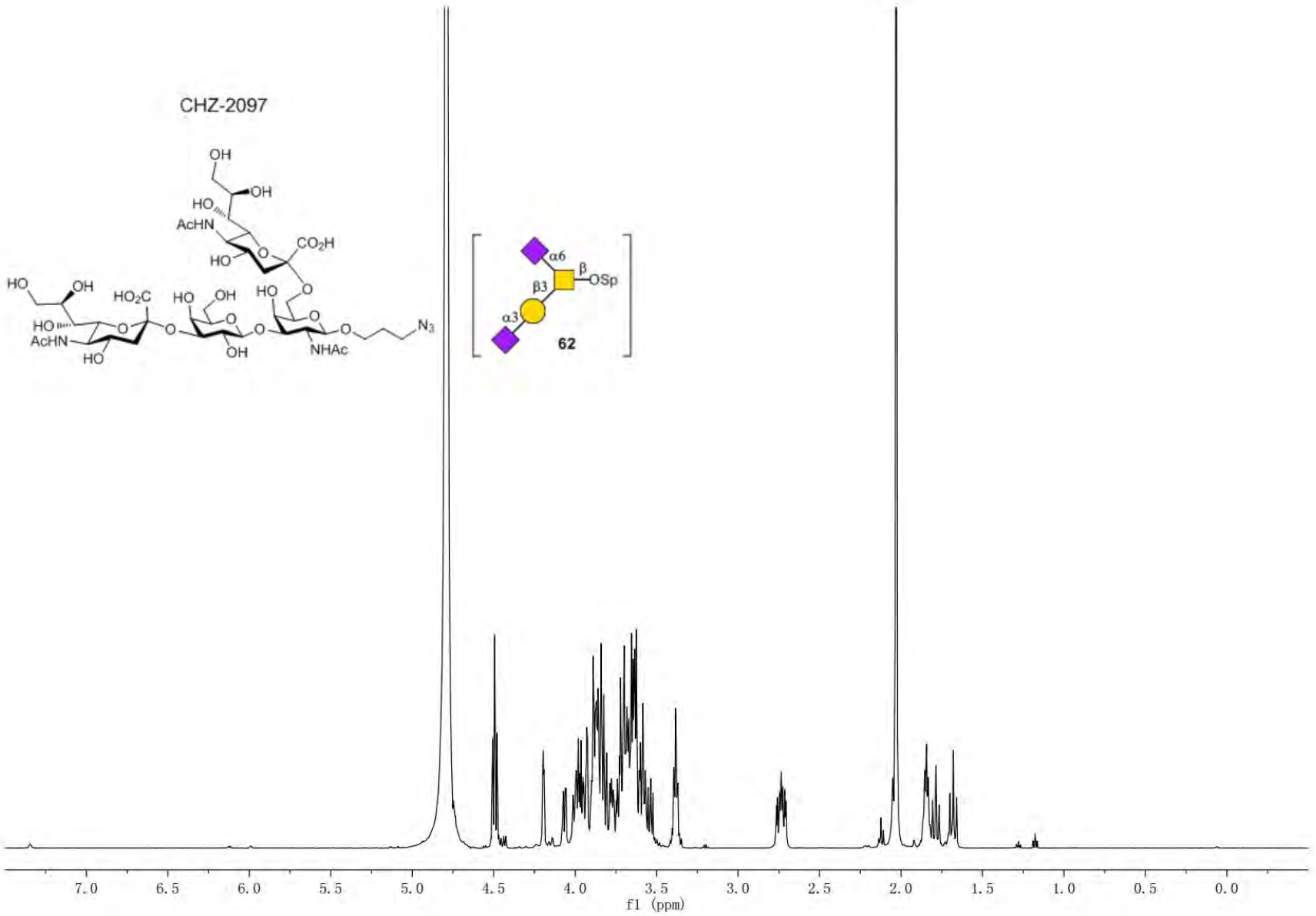
^{13}C NMR of 60



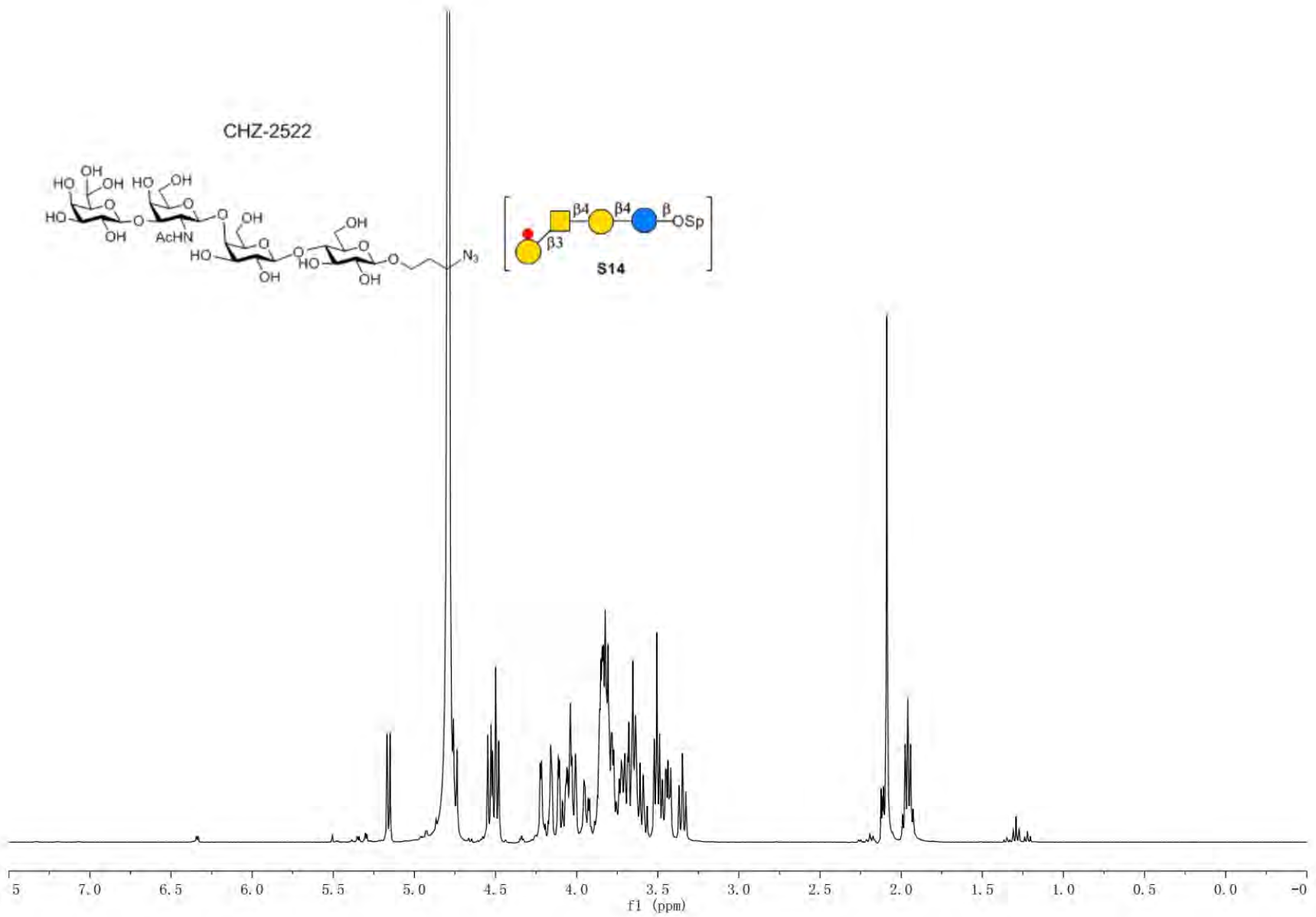
^{1}H NMR of 61


CHZ-1602
OH
OH
HO
AcHN
CO2H
HO
O
HO
OH
HO
O
HO
O
O
O
N3
OH
NHAc
α6
β
OSp
β3
61
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

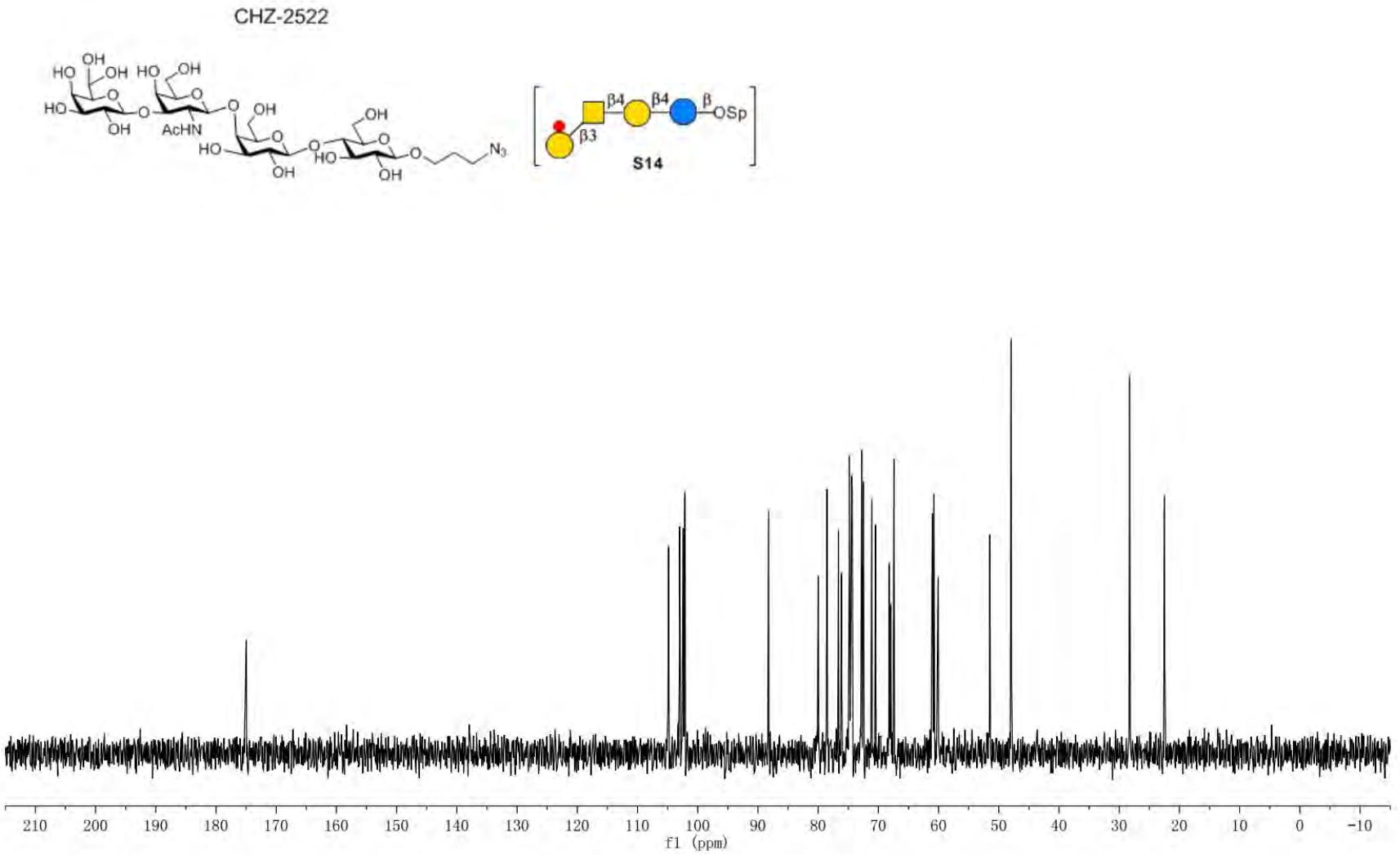
^{13}C NMR of 61



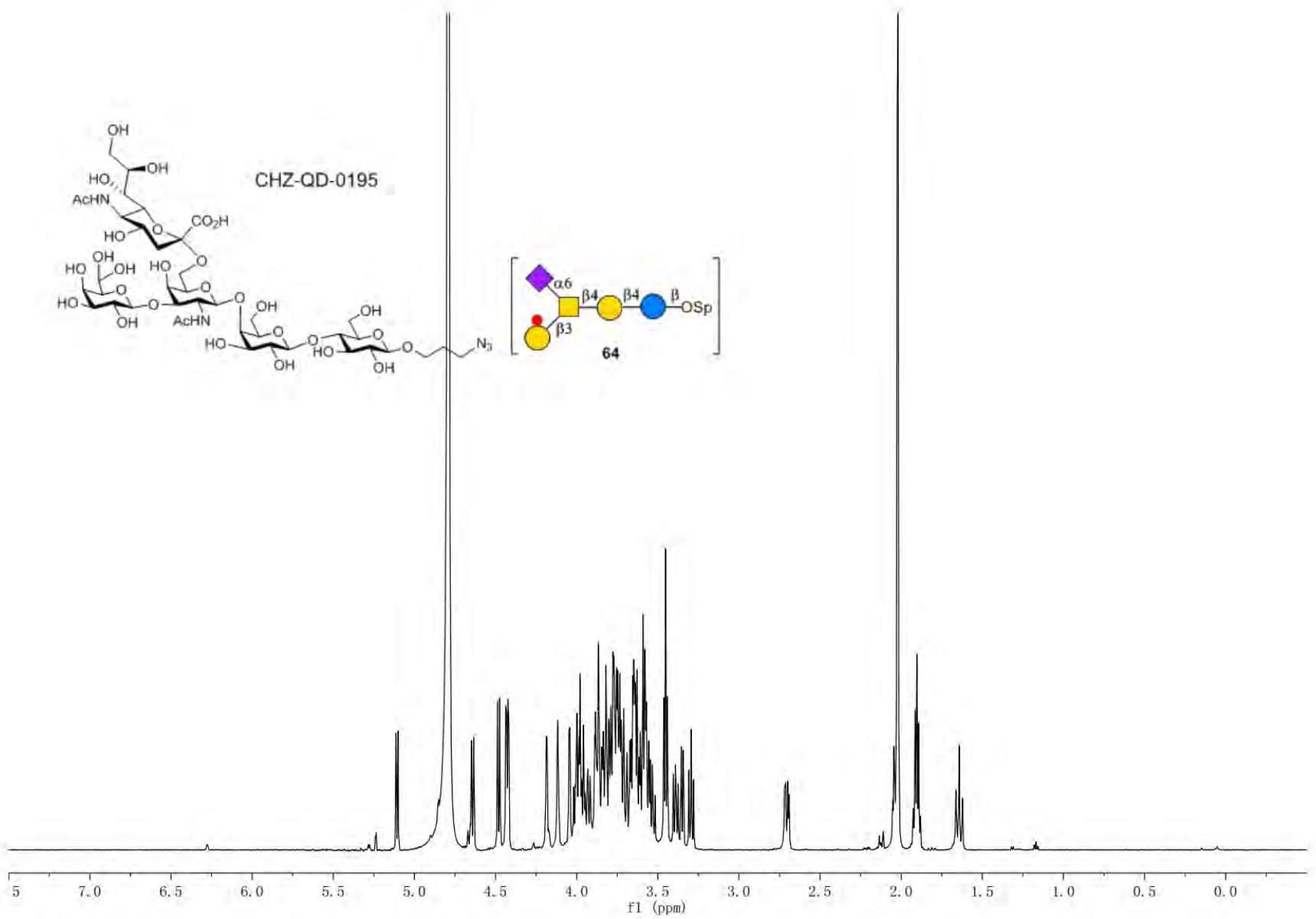
^{1}H NMR of 62


CHZ-2522
S14
β4
β4
β
OSp
β3
N3
AcHN
5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0
f1 (ppm)

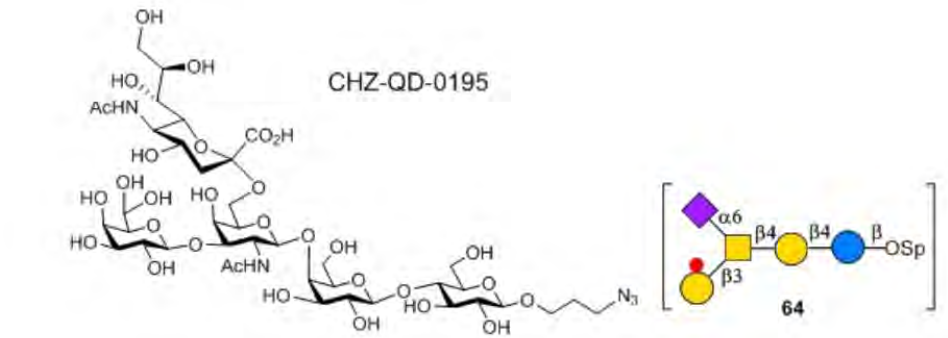
^{1}H NMR of S14

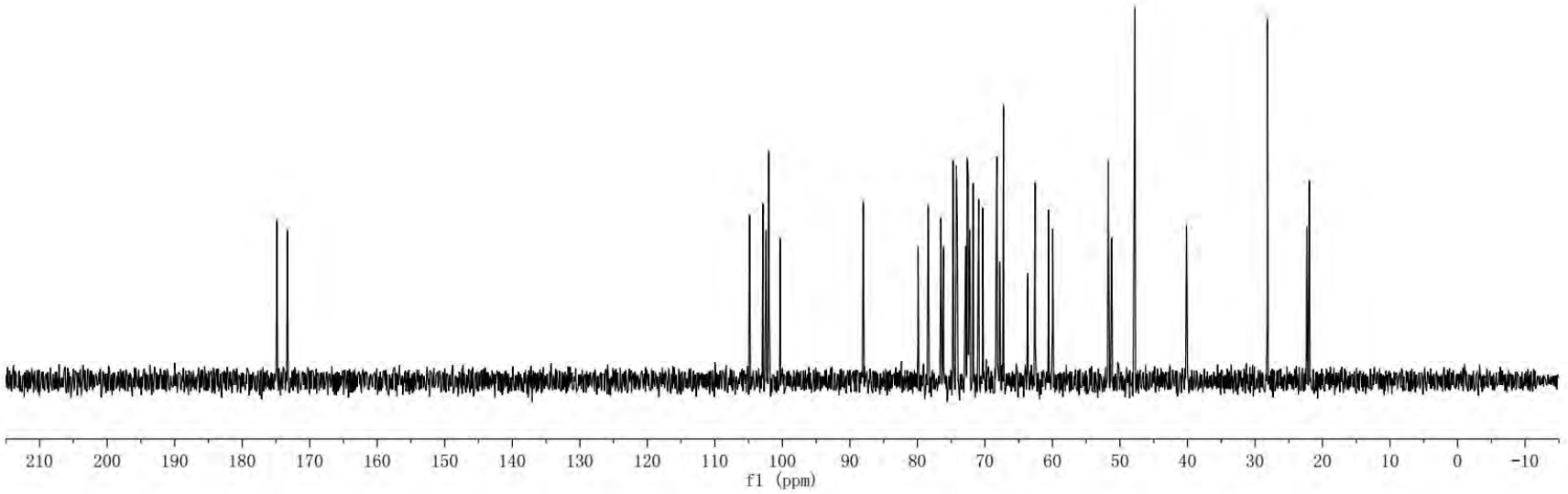


^{13}C NMR of S14

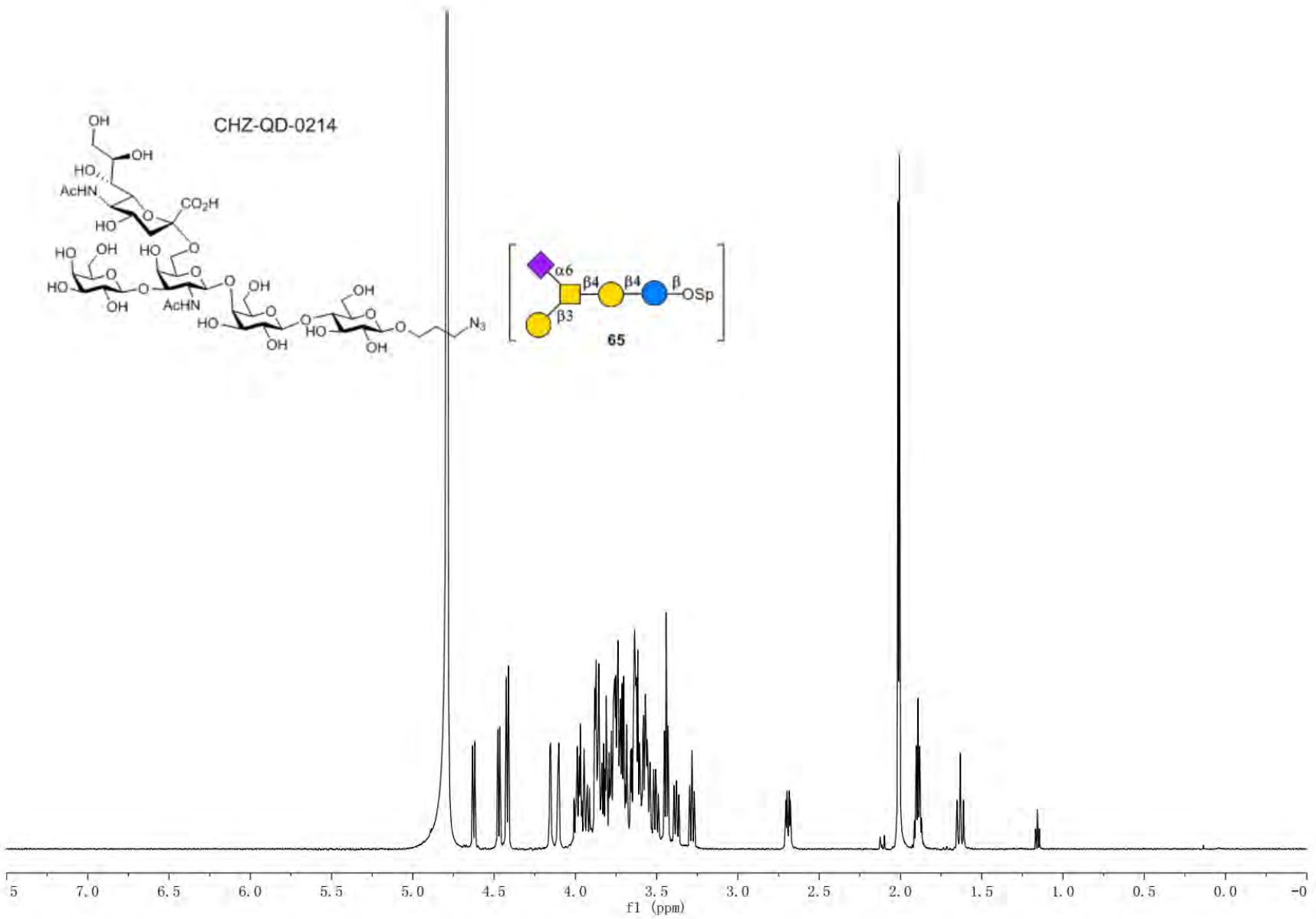


^{1}H NMR of 64

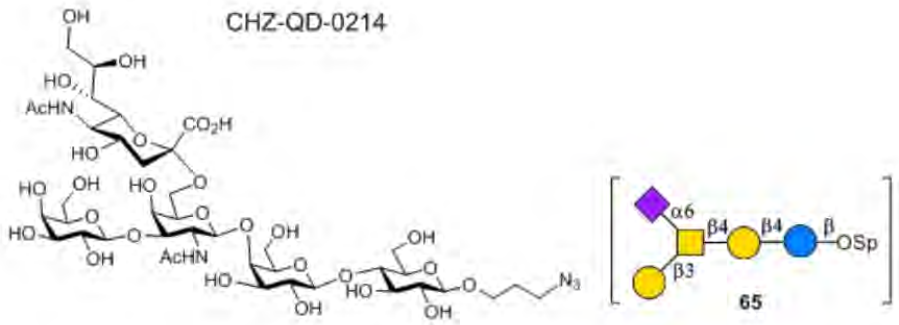

CHZ-QD-0195
64

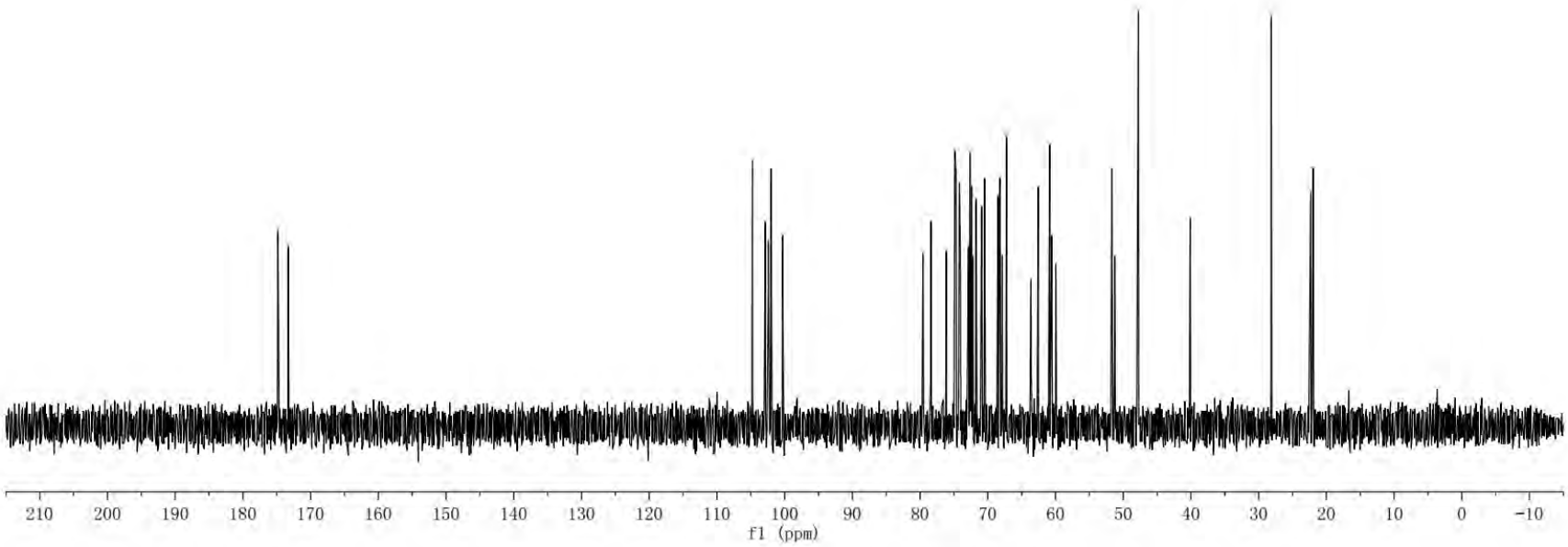

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)

^{13}C NMR of 64

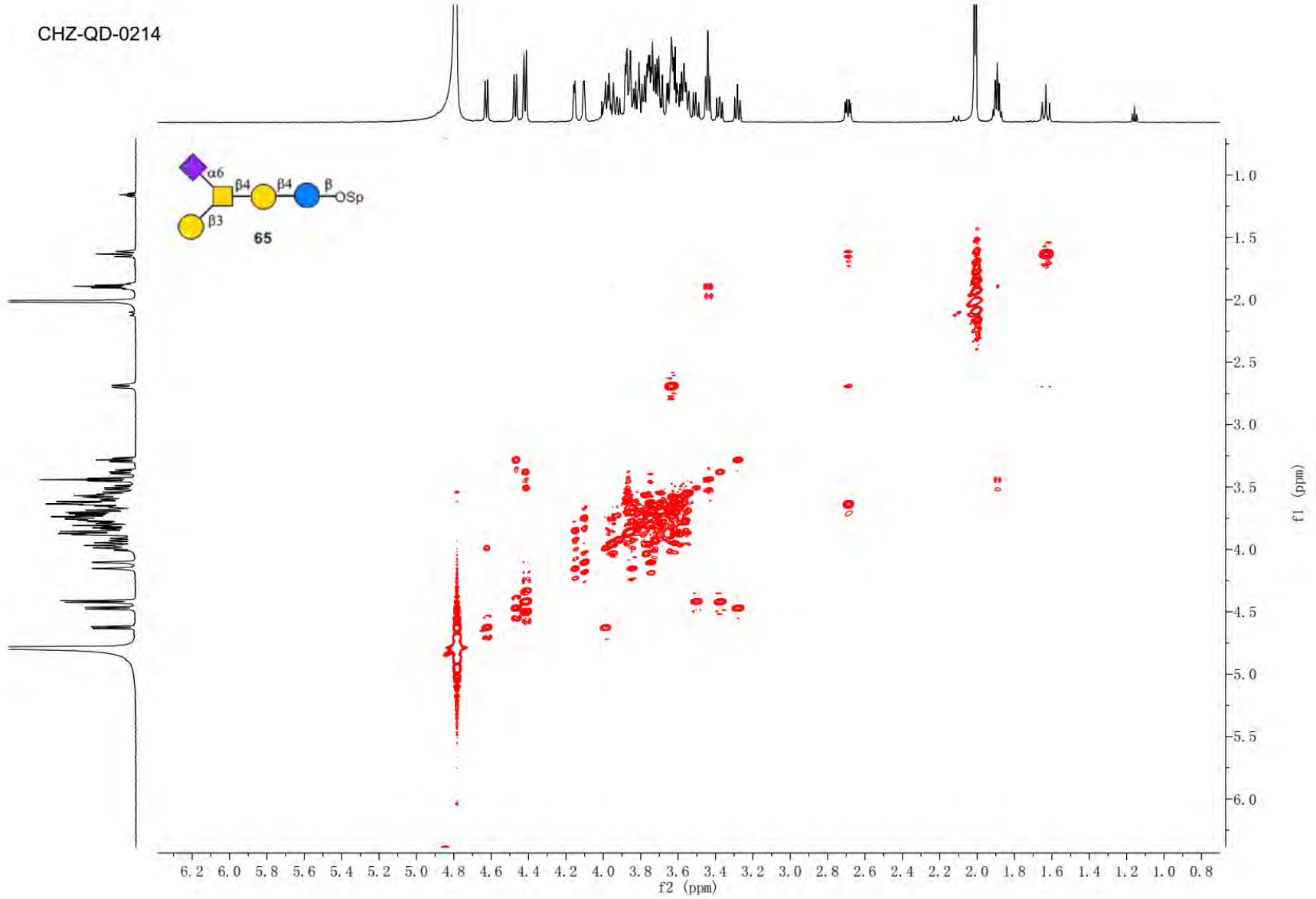


^{1}H NMR of 65

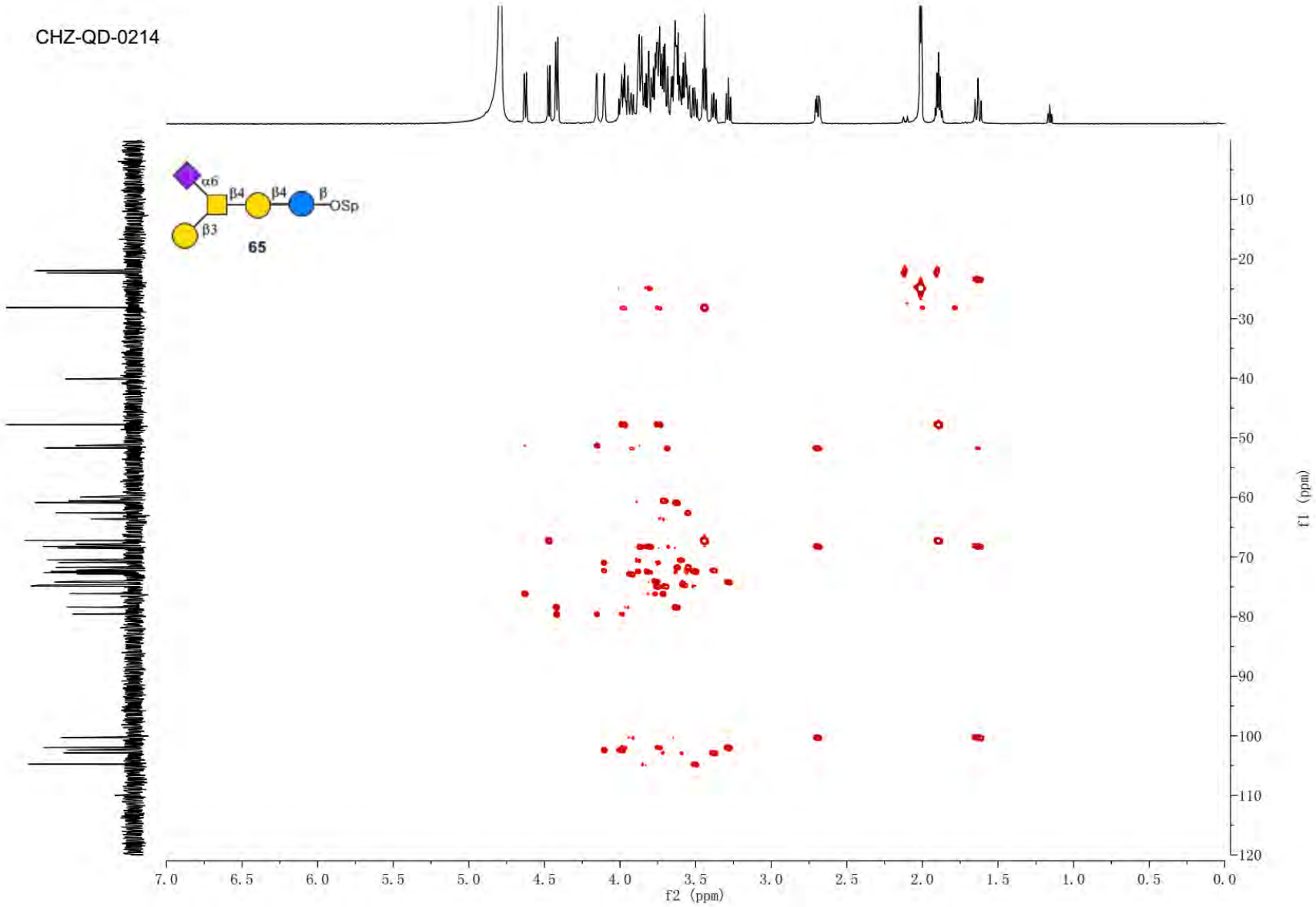

CHZ-QD-0214
OH
OH
HO
AcHN
CO2H
HO
HO
OH
HO
HO
OH
HO
AcHN
OH
HO
OH
OH
HO
OH
N3
α6
β4
β4
β
OSp
β3
65


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)

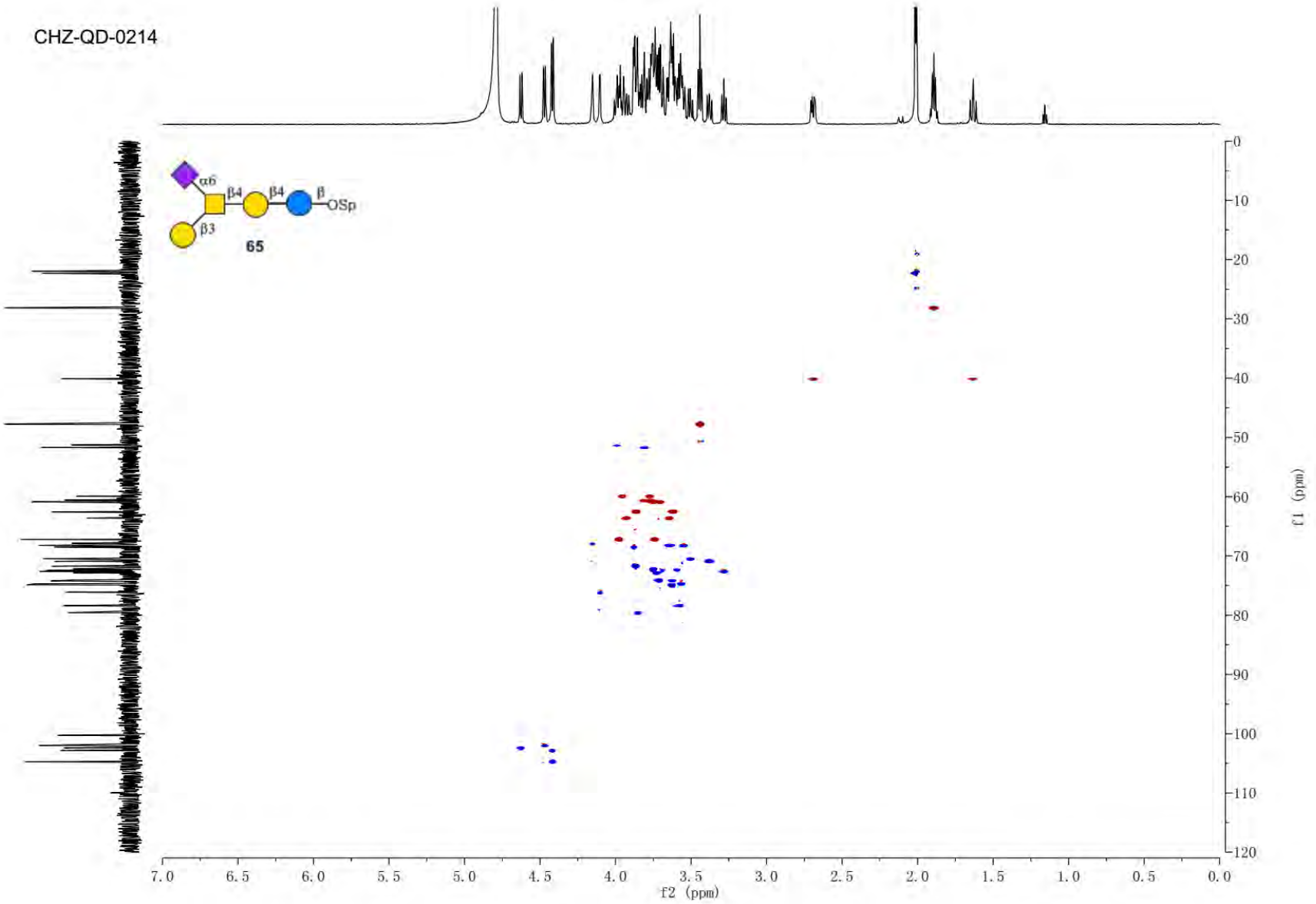
^{13}C NMR of 65



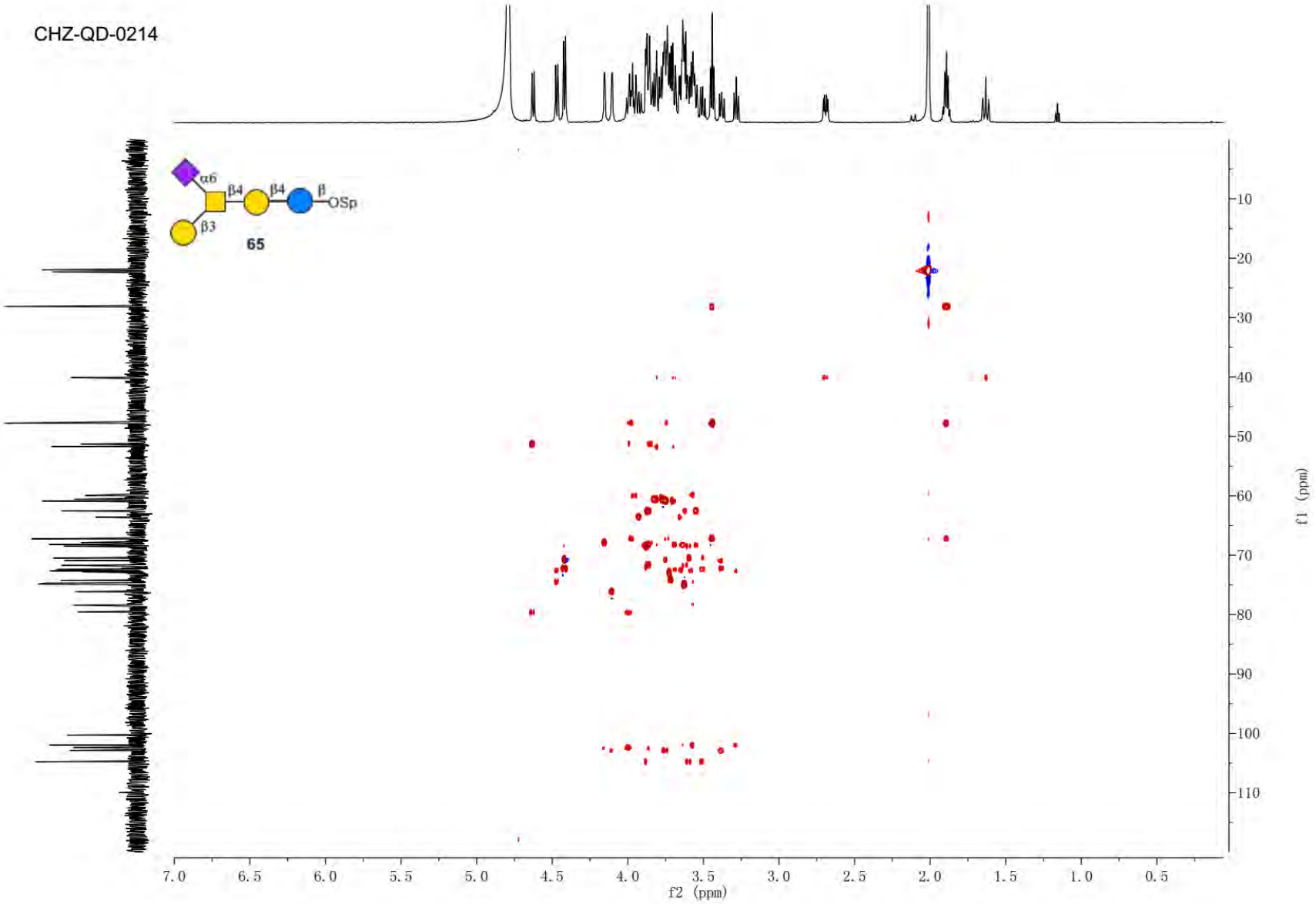
COSY spectra of 65



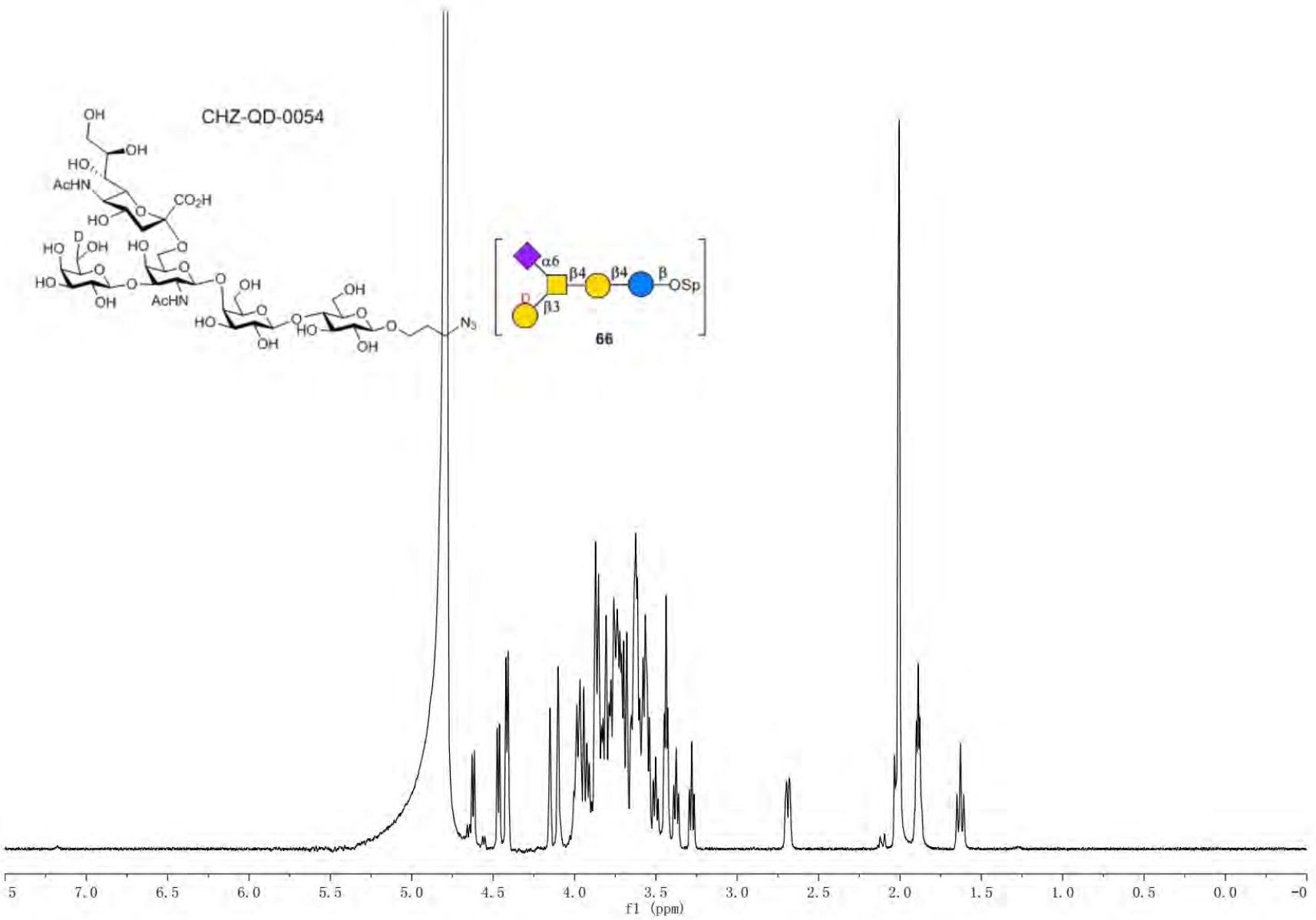
HMBC spectra of 65



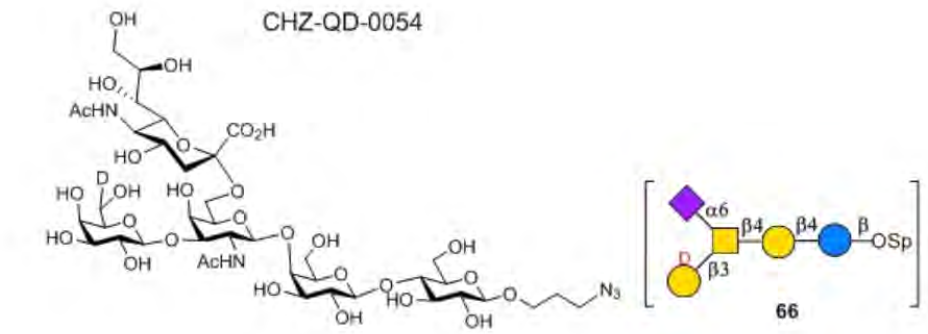
HSQC spectra of 65

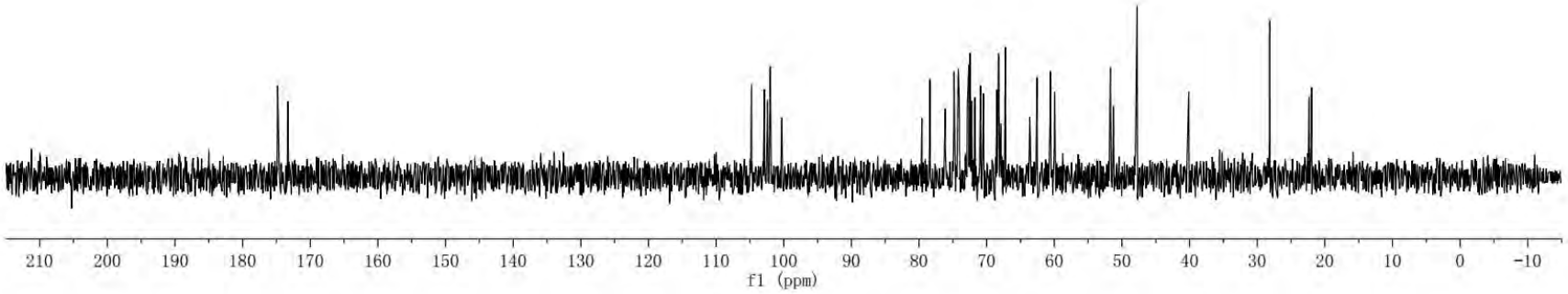


HSQC-TOCSY spectra of 65

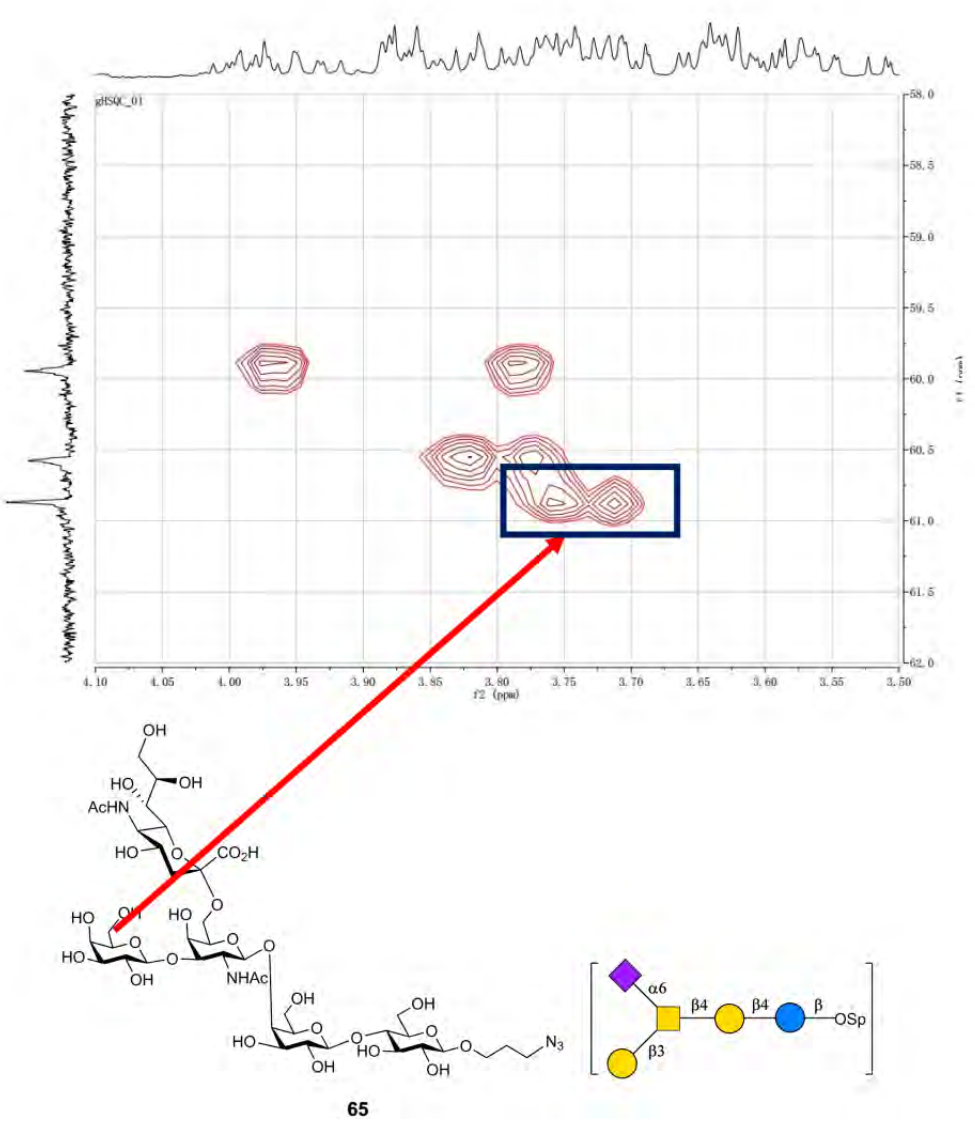


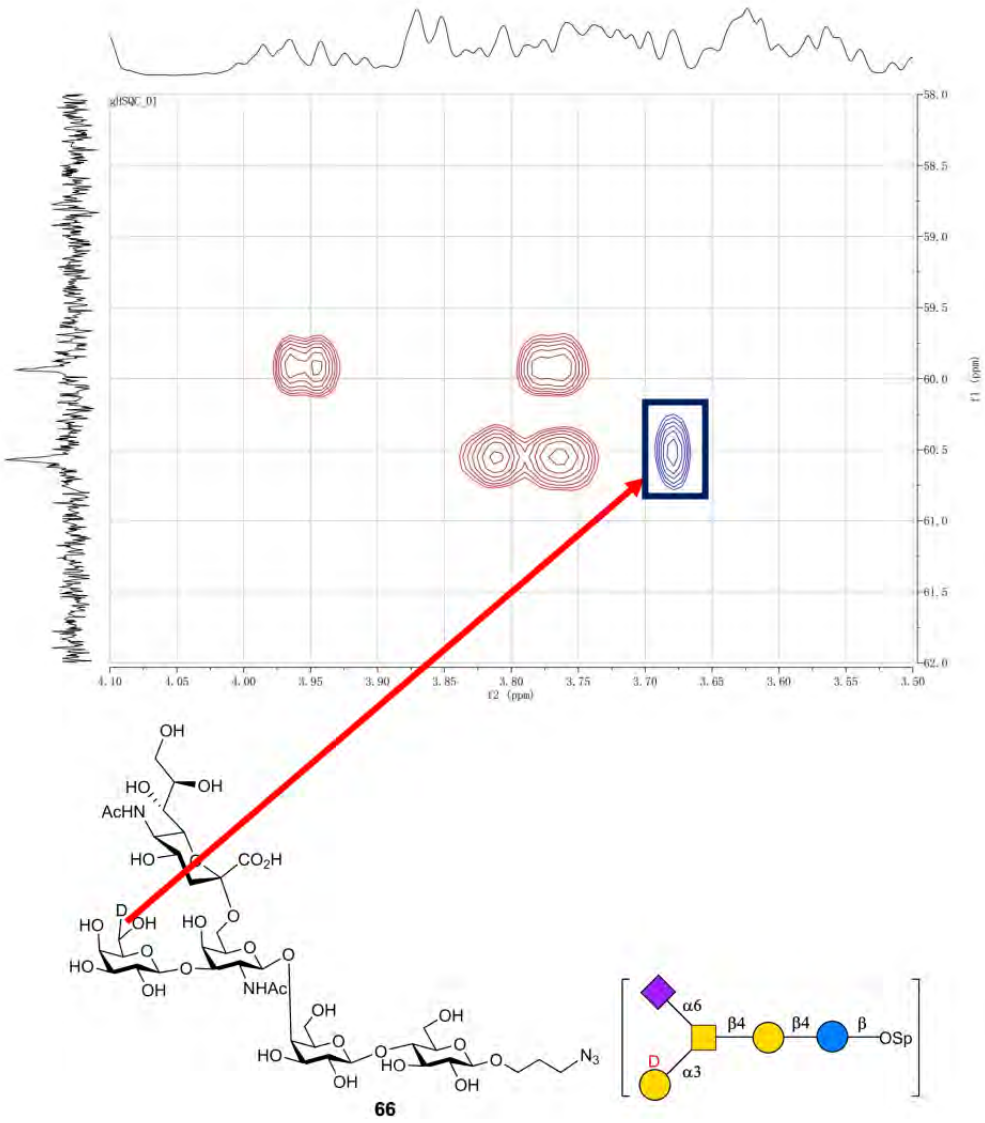
^{1}H NMR of 66


CHZ-QD-0054
66


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

^{13}C NMR of 66





HSQC spectra of 65 and 66