JOC The Journal of Organic Chemistry

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Chemoenzymatic Synthesis of Sialosides Containing 7-*N*- or 7,9-Di-*N*-acetyl Sialic Acid as Stable *O*-Acetyl Analogues for Probing Sialic Acid-Binding Proteins

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Cite This: https://doi.org/10.1021/acs.joc.1c01091 Read Online

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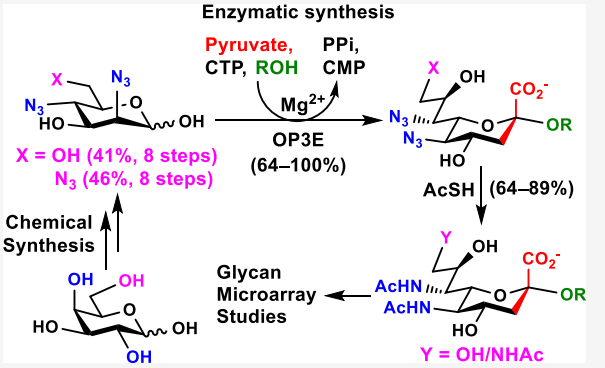
ABSTRACT: A novel chemoenzymatic synthon strategy has been developed to construct a comprehensive library of α2−3- and α2−6-linked sialosides containing 7-*N*- or 7,9-di-*N*-acetyl sialic acid, the stable analogue of naturally occurring 7-*O*-acetyl- or 7,9-di-*O*-acetyl-sialic acid. Diazido and triazido-mannose derivatives that were readily synthesized chemically from inexpensive galactose were shown to be effective chemoenzymatic synthons. Together with bacterial sialoside biosynthetic enzymes with remarkable substrate promiscuity, they were successfully used in one-pot multienzyme (OPME) sialylation systems for highly efficient synthesis of sialosides containing multiple azido groups. Conversion of the azido groups to *N*-acetyl groups generated the desired sialosides. The hydrophobic and UV-detectable benzyloxycarbonyl (Cbz) group introduced in the synthetic acceptors of sialyltransferases was used as a removable protecting group for the propylamine aglycon of the target sialosides. The resulting *N*-acetyl sialosides were novel stable probes for sialic acid-binding proteins such as plant lectin MAL II, which bond strongly to sialyl T antigens with or without an *N*-acetyl at C7 or at both C7 and C9 in the sialic acid.



INTRODUCTION

Sialic acids (Sias) are nine-carbon acidic monosaccharides that are called nonulosonic acids. They are commonly found as the terminal residues of glycan moieties on cell surface glycoproteins and glycolipids of all vertebrates[1,2] and as components of capsular polysaccharides and lipopolysaccharides of a number of pathogenic bacteria.[3,4] Sia-terminated glycans and glycoconjugates are involved in various biological and pathogenic processes, which are influenced by the natural diversities of Sia structures, sialyl linkages, and internal glycans.[5] More than 50 structurally distinct forms of Sias have been found in nature.[1,2,5] *N*-Acetylneuraminic acid (Neu5Ac, **1**; Figure 1) is the most common form of natural Sias. Modifications at one or more hydroxyl groups of Sias at C4, C5, C7, C8, and/or C9 are most commonly by the *O*-acetyl group and less commonly by the *O*-methyl, *O*-sulfate, or *O*-lactyl group.[6,7] *O*-Acetylated sialoglycans have been identified as potential cancer markers for acute lymphoblastic leukemia and have been shown to be involved in the regulation of ganglioside-mediated apoptosis.[8,9] Sialosides containing 7-*O*-acetyl Neu5Ac (Neu5,7Ac$_2$, **2**) have been found on human immune cells such as human lymphocytes,[10] and those containing 7,9-di-*O*-acetyl Neu5Ac (Neu5,7,9Ac$_3$, **3**) are preferentially recognized by bovine coronavirus.[11−15]

Even though *O*-acetylated sialoglycans were discovered as early as 1936,[16,17] the progress of elucidating their functions has been delayed due to the instability of the *O*-acetyl moiety.[18,19] The *O*-acetyl group at C7-OH of Sia is notoriously challenging to study. It was shown to spontaneously migrate to the C9-OH group even under physiological pH.[20] A sialate *O*-acetyltransferase (EC 2.3.1.45) was believed to catalyze the transfer of the *O*-acetyl group to C7-OH of the Neu5Ac in sialosides, which is then migrated to C9-OH to form the sialosides containing 9-*O*-acetyl Neu5Ac (Neu5,9Ac$_2$) as the most abundant *O*-acetylated Sia.[20,21] The *O*-acetyl group at C7 of Neu5,7,9Ac$_3$ (**3**) was also shown to migrate to C8-OH.[20]

To overcome the *O*-acetyl instability problem, we showed previously that sialosides containing 9-*N*-acetyl Neu5Ac (Neu5Ac9NAc) are suitable mimics of Neu5,9Ac$_2$-sialosides.[22−24] To explore the functions and applications of more challenging sialoside targets containing Neu5,7Ac$_2$ (**2**) or Neu5,7,9Ac$_3$ (**3**), we report here the design and synthesis of their structurally stable mimics where the labile *O*-acetyl groups are replaced by *N*-acetyl groups. A novel chemoenzymatic synthon strategy was developed based on highly

Received: May 10, 2021

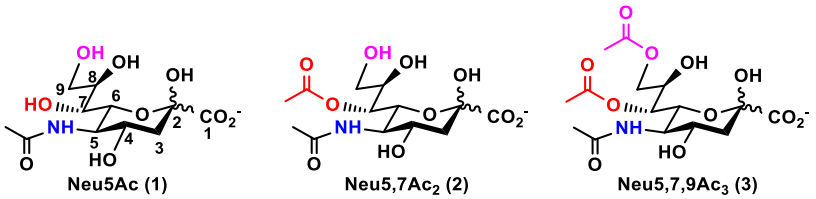


Figure 1. Structures of *N*-acetylneuraminic acid (Neu5Ac, **1**), the most abundant natural sialic acid form, and its naturally occurring *O*-acetylated forms 7-*O*-acetyl-*N*-acetylneuraminic acid (Neu5,7Ac$_2$, **2**) and 7,9-di-*O*-acetyl-*N*-acetylneuraminic acid (Neu5,7,9Ac$_3$, **3**).

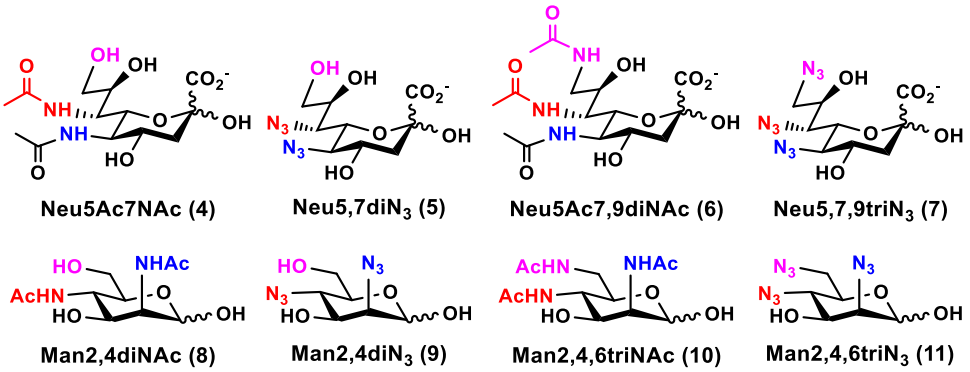
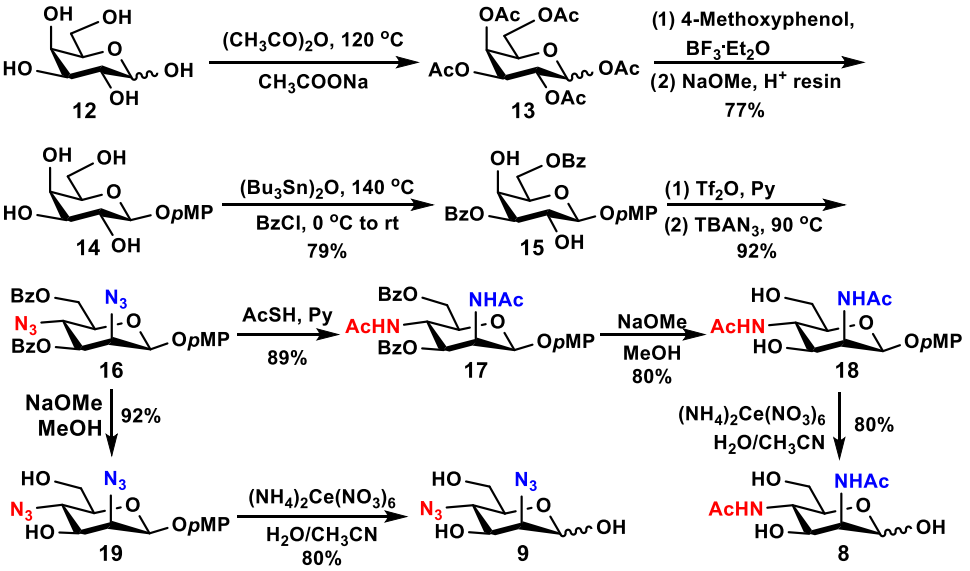


Figure 2. Structures of *N*-acetyl analogue Neu5Ac7NAc (**4**) and azido derivative Neu5,7diN$_3$ (**5**) of Neu5,7Ac$_2$ (**2**); *N*-acetyl analogue Neu5Ac7,9diNAc (**6**) and azido derivative Neu5,7,9triN$_3$ (**7**) of Neu5,7,9Ac$_3$ (**3**); as well as their corresponding hexose precursors Man2,4diNAc (**8**), Man2,4diN$_3$ (**9**), Man2,4,6triNAc (**10**), and Man2,4,6triN$_3$ (**11**).

Scheme 1. Chemical Synthesis of Man2,4diNAc (8) and Man2,4diN$_3$ (9) from Galactose (12). Ac, acetyl; *p*MP, *para*-methoxyphenyl; and Bz = benzoyl



efficient one-pot three-enzyme (OP3E) sialylation systems containing sialoside biosynthetic enzymes with remarkable substrate promiscuities. The produced sialosides containing an *N*-acetyl sialic acid analogue were shown to be stable probes for glycan microarray-based studies for Sia-binding proteins including human Siglec 7 (hSiglec 7)[25] and human Siglec 9 (hSiglec 9),[26,27] as well as plant lectins *Sambucus nigra* lectin (SNA) and *Maackia amurensis* lectin II (MAL II).

RESULTS AND DISCUSSION

Synthesis Design. In nature, *N*-acetylmannosamine (ManNAc) is the six-carbon sugar precursor for Neu5Ac biosynthesis. For vertebrate cells, Neu5Ac is activated inside the nucleus to form CMP-Neu5Ac, which is transported to the Golgi apparatus and used by sialyltransferases to form α-linked sialoglycoconjugates.[28] Our design for synthesizing α2−3- and α2−6-linked sialosides containing modified Sia is by chemical introduction of the modifications to its six-carbon precursor and using substrate promiscuous sialoside biosynthetic enzymes to convert modified precursors to Sia derivatives, which are then activated and transferred to suitable acceptors to form the target sialosides.[23] Sialoside biosynthetic enzymes including a sialic acid aldolase, a CMP-sialic acid synthetase, and a sialyltransferase can be used in one-pot for efficient sialylation.[29,30] As shown in Figure 2, 7-*N*-acetyl Neu5Ac (Neu5Ac7NAc, **4**) was designed as the stable mimic of

https://doi.org/10.1021/acs.joc.1c01091
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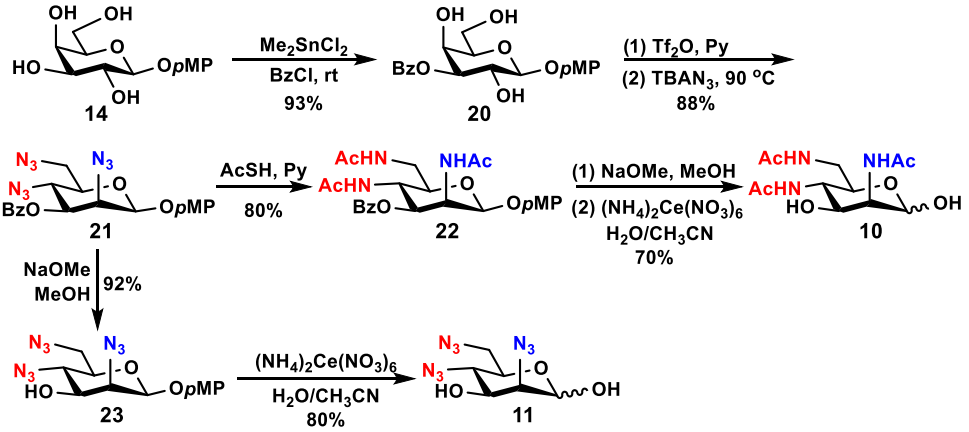
Scheme 2. Synthesis of Man2,4,6triNAc (10) and Man2,4,6triN$_3$ (11) from Galactoside 14



Neu5,7Ac$_2$ (**2**). It can be derived from the corresponding azido analogue Neu5,7diN$_3$ (**5**). Similarly, 7,9-di-*N*-acetyl Neu5Ac (Neu5Ac7,9diNAc, **6**) was proposed as the more stable mimic of Neu5,7,9Ac$_3$ (**3**) and the corresponding azido analogue is Neu5,7,9triN$_3$ (**7**). The corresponding six-carbon precursors for these sialic acid derivatives (**4**–**7**) of a sialic acid aldolase-catalyzed reaction are 2,4-diacetamindo-2,4-dideoxy-D-mannose (Man2,4diNAc, **8**), 2,4-diazido-2,4-dideoxy-D-mannose (Man2,4diN$_3$, **9**), 2,4,6-triacetamindo-2,4,6-trideoxy-D-mannose (Man2,4,6triNAc, **10**), and 2,4,6-triazido-2,4,6-trideoxy-D-mannose (Man2,4,6triN$_3$, **11**). These compounds (**8**–**11**) were chemically synthesized.

Chemical Synthesis of Mannose Derivatives. Numerous chemical synthetic methods have been developed to access amino sugars, rare sugars, and sugar derivatives by selective protection and epimerization.[31−34] However, some of them suffer from long multistep synthetic routes along with low to moderate yields.[35] We envisioned that Man2,4diNAc (**8**) and Man2,4diN$_3$ (**9**) could be synthesized from commercially available inexpensive D-galactose (**12**) by introducing two azido groups simultaneously with the inversion of the stereochemistry at both C2 and C4. As shown in Scheme 1, galactoside 14 was formed in an overall 77% yield over three steps by per-*O*-acetylation of D-galactose (**12**) followed by nucleophilic replacement of the anomeric acetate with *para*-methoxyphenyl (*p*MP) under acidic conditions using BF$_3$·Et$_2$O and de-*O*-acetylation using sodium methoxide. Regioselective benzoylation of both C3- and C6-hydroxyl groups of **14** using bistributyltin oxide (Bu$_3$Sn)$_2$O catalyst[36,37] formed a partially protected D-galactosyl-2,4-diol intermediate (**15**) in a 79% yield. Treatment of 15 with trifluoromethanesulfonic anhydride (Tf$_2$O) and pyridine followed by simultaneous displacement (S$_N$2 reaction) of both triflate groups at C2 and C4 with azido groups using tetrabutylammonium azide (TBAN$_3$)[38] in dry toluene[30,39] at 90 °C afforded diazido-D-mannose derivative 16 in a 92% yield. The azido moieties of **16** were converted to acetamido moieties by reacting with thioacetic acid in pyridine under argon[40] to produce benzoyl-protected glycoside **17** in an 89% yield. Debenzoylation under Zemplén reaction conditions in the presence of sodium methoxide and methanol followed by ceric ammonium nitrate (CAN)-catalyzed removal of the *p*MP group[41] produced the desired Man2,4diNAc (**8**). On the other hand, removal of the benzoyl groups and the anomeric *p*MP group from **16** formed the desired Man2,4diN$_3$ (**9**). Altogether, Man2,4diNAc (**8**) and Man2,4diN$_3$ (**9**) were synthesized from D-galactose (**12**) in nine and eight steps with overall yields of 32 and 41%, respectively.

Man2,4,6triNAc (**10**) and Man2,4,6triN$_3$ (**11**) were synthesized from galactoside **14** (Scheme 2) using a similar strategy except that only the 3-OH group of **14** was regioselectively protected with a benzoyl group using dimethyltin chloride (Me$_2$SnCl$_2$) as a catalyst[38,42] to produce partially protected triol **20** in a 93% yield. Simultaneous triflation of all three hydroxyl groups in **20** with Tf$_2$O in pyridine followed by concurrent displacement of all three triflates with azido groups via an S$_N$2-mechanism worked very well when TBAN$_3$ in dry toluene[30,39] was used at 90 °C for 2 h in the second step. The formation of D-mannose derivative **21** was achieved in an overall 88% yield. Conversion of all three azido groups in **21** to acetamido moieties using thioacetic acid and pyridine[40] produced **22** in an 80% yield. Debenzoylation of **22** under Zemplén reaction conditions followed by CAN-catalyzed removal of the *p*MP group[41] produced Man2,4,6-triNAc (**10**) in a 70% yield. On the other hand, stepwise removal of the benzoyl protecting group (92% yield) and the *p*MP group (80% yield) from **21** produced Man2,4,6triN$_3$ (**11**). Altogether, Man2,4,6triNAc (**10**) and Man2,4,6triN$_3$ (**11**) were formed from **14** in six and five steps with overall yields of 46 and 60%, respectively.

Enzymatic Synthesis of Sialic Acid Derivatives. Mannose derivatives Man2,4diNAc (**8**), Man2,4diN$_3$ (**9**), Man2,4,6triNAc (**10**), and Man2,4,6triN$_3$ (**11**) were tested as potential substrates for *Pasteurella multocida* sialic acid aldolase (PmAldolase).[43] Among these four compounds, Man2,4diNAc (**8**) and its diazido derivative Man2,4diN$_3$ (**9**), as well as the triazido derivative Man2,4,6triN$_3$ (**11**), were suitable substrates for PmAldolase. The corresponding Sia derivatives Neu5Ac7NAc (**4**), Neu5,7diN$_3$ (**5**), and Neu5,7,9triN$_3$ (**7**) were synthesized in the presence of 5 equiv of sodium pyruvate with 70−72% yields (Scheme 3). In contrast, Man2,4,6triNAc (**10**) was not a suitable substrate for PmAldolase, and the corresponding Neu5Ac7,9diNAc (**6**) could not be obtained directly from **10** by PmAldolase-catalyzed reaction.

One-Pot Multienzyme (OPME) Synthesis of Sialosides Containing an Azido-Modified Sialic Acid. The Neu5Ac7NAc (**4**), Neu5,7diN$_3$ (**5**), and Neu5,7,9triN$_3$ (**7**) obtained from PmAldolase-catalyzed reaction were tested as potential substrates for *Neisseria meningitidis* CMP-sialic acid synthetase (NmCSS).[44] While both Neu5,7diN$_3$ (**5**) and Neu5,7,9triN$_3$ (**7**) were suitable substrates for NmCSS, Neu5Ac7NAc (**4**) was not.

https://doi.org/10.1021/acs.joc.1c01091
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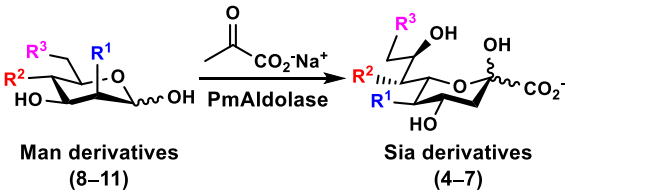
Scheme 3. PmAldolase-Catalyzed Preparative-Scale Synthesis of Sialic Acid Derivatives Neu5Ac7NAc (4), Neu5,7diN$_3$ (5), and Neu5,7,9triN$_3$ (7) from the Corresponding Mannose Derivatives Man2,4diNAc (8), Man2,4diN$_3$ (9), and Man2,4,6triN$_3$ (11), Respectively[a]



4 (72%) or 8, $R^1 = R^2 = NHAc$, $R^3 = OH$
5 (72%) or 9, $R^1 = R^2 = N_3$, $R^3 = OH$
6 (0%) or 10, $R^1 = R^2 = R^3 = NHAc$
7 (70%) or 11, $R^1 = R^2 = R^3 = N_3$

[a]Man2,4,6triNAc (**10**) was not a suitable substrate for PmAldolase-catalyzed synthesis of Neu5Ac7,9diNAc (**6**).

Man2,4diN$_3$ (**9**) and Man2,4,6triN$_3$ (**11**) were also tested as chemoenzymatic synthons[30] in one-pot three-enzyme (OP3E) sialyation systems[29] (Table 1) for enzymatic synthesis of α2−3- and α2−6-linked sialosides containing Neu5,7diN$_3$ or Neu5,7,9triN$_3$. It was remarkable that all four enzymes including PmAldolase,[43] NmCSS,[44] *P. multocida* α2−3-sialyltransferase 1 (PmST1),[45] and *Photobacterium damselae* α2−6-sialyltransferase (Pd2,6ST)[46] were able to tolerate the diazido- and triazido-derivatives of the corresponding substrates.

LacβProNHCbz (type VI glycan, **24**),[47] LacNAcβ-ProNHCbz (type II glycan, **25**), Galβ3GalNAcβProNHCbz (type IV glycan, **26**), Galβ3GalNAcαProNHCbz (type III or core 1 glycan, **27**), Galβ3GlcNAcβProNHCbz (type I glycan, **28**), Galβ3GlcNAcαProNHCbz (**29**), and GalNAcαP-roNHCbz (Tn antigen, **30**) were synthesized and used as sialyltransferase acceptors for the construction of a comprehensive library of sialosides (Table 1). The hydrophobic and UV-detectable benzyloxycarbonyl (Cbz) group in **24**−**30** was used as a removable protecting group for the propylamine aglycon of the target sialosides.

In the OP3E sialylation systems (Table 1), Man2,4diN$_3$ (**9**) or Man2,4,6triN$_3$ (**11**) was coupled with pyruvate by a PmAldolase-catalyzed reaction to form Neu5,7diN$_3$ (**5**) or Neu5,7,9triN$_3$ (**7**). The resulting azido-Sia derivative was converted by an NmCSS-catalyzed reaction in the presence of cytidine 5′-triphosphate (CTP) and magnesium cation (Mg^{2+}) to form the corresponding CMP-activated sugar nucleotide CMP-Neu5,7diN$_3$ or CMP-Neu5,7,9triN$_3$, which was used by sialyltransferases such as *P. multocida* α2−3-sialyltransferase 1 (PmST1)[45] and *P. damselae* α2−6-sialyltransferase (Pd2,6ST),[46] respectively, to produce α2−3- and α2−6-linked sialosides (**31**−**56**) from sialyltransferase acceptors **24**−**30**.

Using PmST1[45] as the sialyltransferase, α2−3-linked Neu5,7diN$_3$-containing (**31**, **35**, **39**, **43**, **47**, and **51**) and Neu5,7,9triN$_3$-containing glycosides (**32**, **36**, **40**, **44**, **48**, and **52**) were obtained in good to excellent (64−99%) yields with galactosides **24**−**30** as the sialyltransferase acceptors. Similarly, using Pd2,6ST[46] as the sialyltransferase, α2−6-linked Neu5,7-diN$_3$-containing (**33**, **37**, **41**, **45**, **49**, **53**, and **55**) and Neu5,7,9triN$_3$-containing glycosides (**34**, **38**, **42**, **46**, **50**, **54**, and **56**) were obtained in good to excellent (65−100%) yields.

Chemical Conversion of the Azido Groups in Sialosides to *N*-Acetyl Groups and Removal of the Cbz-Protecting Group. The azido-containing sialosides (**31**−**56**) obtained were readily converted to their *N*-acetyl analogues (**57**−**82**) in 64−89% yields using thioacetic acid and saturated sodium bicarbonate aqueous solution (Table 1), a condition optimized previously.[30] The Cbz group in the aglycone of the sialosides **57**−**82** was removed by hydrogenation using Pd/C catalyst in water−methanol solution for a few hours at room temperature to form sialosides containing a propylamine aglycone in quantitative yields for downstream conjugation reactions. Compared to the previous chemoenzymatic synthon strategy[30] of using sialyltransferase acceptors with a propyl chloride aglycone, the Cbz-protected propylamine aglycone has several advantages. It facilitates reaction monitoring and product purification processes due to its hydrophobic UV-detectability. It also allows selective conversion of the azido group in the enzymatic products (**31**−**56**) to the desired *N*-acetyl group and permits highly efficient conversion (in quantitative yields) of the aglycon in the sialosides (**57**−**82**) to a primary propylamine for glycan microarray printing.

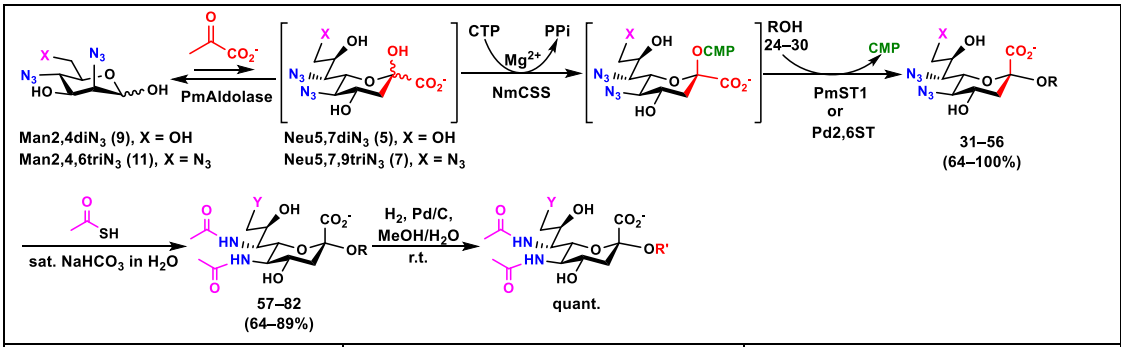
Sialosides Containing Neu5Ac7NAc or Neu5Ac7,9diNAc Can Bind to Sia-Binding Proteins as Shown by Sialoglycan Microarray Studies. The propylamine aglycon in the sialosides obtained made it easy to print these glycans on an array format for protein-binding studies in a high-throughput format. Glycan microarray experiments were carried out using α2−3- and α2−6-linked sialosides containing Neu5Ac7NAc or Neu5Ac7,9diNAc synthesized here, as well as related sialosides containing Neu5Ac, Neu5,9Ac$_2$, or Neu5-Ac9NAc synthesized previously.[22,23,45,46,48] The corresponding internal glycans (without the Sia component) were used as negative controls. As shown in Figure 3 and Table S1, the binding of hSiglec 7, hSiglec 9, and SNA to the sialosides containing Neu5Ac (gray bars) or Neu5Ac9NAc (orange bars) was blocked by substituting the C7-OH of sialic acid by a C7-NAc group (Neu5Ac7NAc red bars and Neu5Ac7,9diNAc blue bars, respectively) except for the weak binding retained for Neu5Ac7NAcα3Galβ3GalNAcβ-glycan (red bars for Js in Figure 3). Interestingly, MAL II bond to sialyl T antigens Siaα3Galβ3GalNAcα-glycans (L in Figure 3) strongly[49] regardless of the Sia forms in the sialosides tested including those containing Neu5Ac (gray bar), Neu5,9Ac$_2$ (green bar), Neu5Ac9NAc (orange bar), Neu5Ac7NAc (red bar), and Neu5Ac7,9diNAc (blue bar). On the other hand, the binding of MAL II to Neu5Acα3Galβ3GalNAcβ-glycan (gray bar in J in Figure 3), a sialoglycan differing from the sialyl T antigen only on the glycosyl linkage remotely from the Sia, was not influenced significantly by Neu5Ac 9-*O*-acetylation (green bar) or 9-NAc substitution (orange bar) but was blocked completely by Neu5Ac7NAc (red bar) or 7,9-di-NAc substitution (blue bar).

CONCLUSIONS

In conclusion, a highly efficient chemoenzymatic synthon strategy has been successfully developed to construct a comprehensive library of sialosides containing *N*-acetyl analogues of 7-*O*- or 7,9,-di-*O*-acetylated Neu5Ac. Man2,4diN$_3$ (**9**) and Man2,4,6triN$_3$ (**11**) are effective chemoenzymatic synthons that are readily accessible via chemical synthesis from inexpensive galactose. We have shown that bacterial sialoside biosynthetic enzymes including PmAldolase, NmCSS, PmST1, and Pd2,6ST have remarkable substrate promiscuity. The

https://doi.org/10.1021/acs.joc.1c01091
J. Org. Chem. XXXX, XXX, XXX−XXX

Table 1. One-Pot Three-Enzyme (OP3E) Synthesis of Sialosides Containing Neu5,7diN$_3$ or Neu5,7,9triN$_3$ (31−56) from Man2,4diN$_3$ (9) or Man2,4,6triN$_3$ (11) and Chemical Conversion of the Azido Groups to *N*-Acetyl Moieties to Form Sialosides Containing Desired Neu5Ac7NAc or Neu5Ac7,9diNAc (57−82)[c]



Acceptor (ROH)	31–56 (64–100%)		57–82 (64–89%)	
	α2–3-sialoside[a] Neu5,7diN$_3$/ Neu5,7,9triN$_3$α3OR	α2–6-sialoside[b] Neu5,7diN$_3$/ Neu5,7,9triN$_3$α6OR	α2–3-sialoside Neu5Ac7NAc/Neu5 Ac7,9diNAcα3OR	α2–6-sialoside Neu5Ac7NAc/Neu 5Ac7,9diNAcα6OR
Type VI glycan LacβProNHCbz (**24**)	X=OH, **31** (90%) X=N$_3$, **32** (92%)	X=OH, **33** (quant.) X=N$_3$, **34** (quant.)	Y=OH, **57** (68%) Y=NHAc, **58** (80%)	Y=OH, **59** (78%) Y=NHAc, **60** (80%)
Type II glycan LacNAcβProNHCbz (**25**)	X=OH, **35** (70%) X=N$_3$, **36** (66%)	X=OH, **37** (72%) X=N$_3$, **38** (65%)	Y=OH, **61** (67%) Y=NHAc, **62** (67%)	Y=OH, **63** (77%) Y=NHAc, **64** (85%)
Type IV glycan Galβ3GalNAcβProNHCbz (**26**)	X=OH, **39** (99%) X=N$_3$, **40** (67%)	X=OH, **41** (Quant.) X=N$_3$, **42** (69%)	Y=OH, **65** (64%) Y=NHAc, **66** (65%)	Y=OH, **67** (67%) Y=NHAc, **68** (70%)
Type III or Core 1 glycan Galβ3GalNAcαProNHCbz (**27**)	X=OH, **43** (95%) X=N$_3$, **44** (65%)	X=OH, **45** (68%) X=N$_3$, **46** (79%)	Y=OH, **69** (77%) Y=NHAc, **70** (65%)	Y=OH, **71** (77%) Y=NHAc, **72** (72%)
Type I glycan Galβ3GlcNAcβProNHCbz (**28**)	X=OH, **47** (87%) X=N$_3$, **48** (64%)	X=OH, **49** (quant.) X=N$_3$, **50** (91%)	Y=OH, **73** (73%) Y=NHAc, **74** (66%)	Y=OH, **75** (75%) Y=NHAc, **76** (70%)
Galβ3GlcNAcαProNHCbz (**29**)	X=OH, **51** (93%) X=N$_3$, **52** (87%)	X=OH, **53** (quant.) X=N$_3$, **54** (96%)	Y=OH, **77** (89%) Y=NHAc, **78** (69%)	Y=OH, **79** (72%) Y=NHAc, **80** (68%)
Tn antigen GalNAcαProNHCbz (**30**)		X=OH, **55** (65%) X=N$_3$, **56** (65%)		Y=OH, **81** (84%) Y=NHAc, **82** (85%)

[a]PmST1 was used as the α2−3-sialyltransferase. [b]Pd2,6ST was used as the α2−6-sialyltransferase. [c]The Cbz group in the resulting sialosides was removed from the NAc-sialosides in quantitative yields to produce sialosides containing a propylamine aglycone for glycan microarray studies. R′ differs from R by replacing the propyl NHCbz aglycon in R with the propylamine (ProNH$_2$) in R′.

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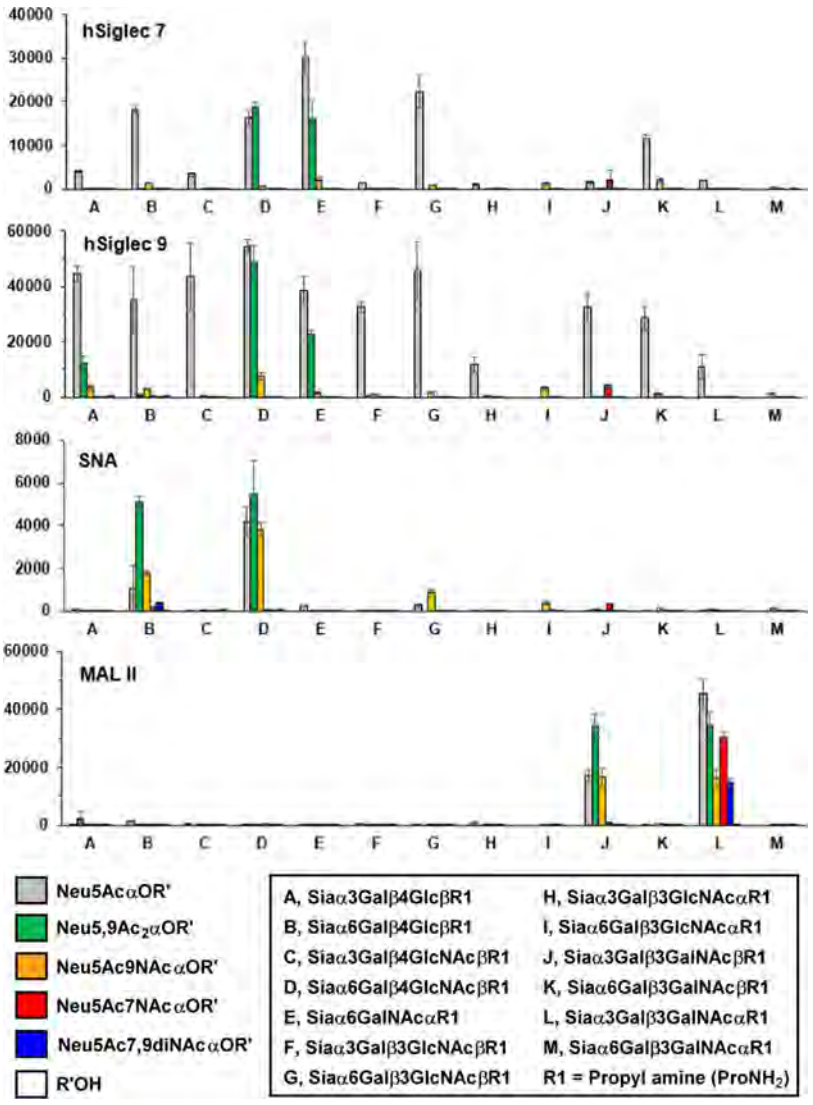

Figure 3. Glycan microarray study results for sialoside binding by hSiglec 7, hSiglec 9, SNA, and MAL II (numeric data are shown in Table S1). Asialoglycans (R′OH) corresponding to the internal glycans in the sialosides are negative controls shown in white bars. R1 = $ProNH_2$.

OPME systems are highly efficient in using Man2,4diN_3 and Man2,4,6triN_3 as effective chemoenzymatic synthons to produce sialosides containing Neu5Ac7N_3 or Neu5Ac7,9diN_3, which are readily converted to the target NAc-containing sialosides by a facile chemical conversion process. The hydrophobic and UV-detectable Cbz group introduced in the synthetic acceptors of sialyltransferases is a convenient removable protecting group for the propylamine aglycon of the target sialosides. The application of the obtained sialosides in glycan microarray studies has demonstrated that these compounds could be valuable stable probes for Sia-binding proteins. The chemoenzymatic synthon strategy has the potential to be used for the synthesis of other glycans containing *N*-acetyl groups.

■ EXPERIMENTAL SECTION

Materials and General Methods. Chemicals were purchased and used without further purification. Nuclear magnetic resonance (NMR) spectra were recorded in the NMR facility of the University of California, Davis, on 600 and 800 MHz Bruker Avance III-NMR spectrometers and a 400 MHz Bruker Avance III HD Nanobay spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale. High-resolution electrospray ionization (ESI) mass spectra were obtained using a Thermo Electron LTQ-Orbitrap Hybrid mass spectrometer or a Thermo Scientific Q Exactive HF Orbitrap Mass Spectrometer at the mass spectrometry facility in the University of California, Davis, or using an LTQ-Orbitrap Elite mass spectrometer at the Georgia State University. Column chromatography was performed using columns manually packed with silica gel 60 Å (230−400 mesh, Sorbent Technologies) or a CombiFlash Rf 200i system with either Redi*Sep* Rf silica columns or an ODS-SM (C18) column (51 g, 50 μm, 120 Å, Yamazen). Thin-layer chromatography (TLC) was performed on silica gel plates (Sorbent Technologies) using anisaldehyde sugar stain or 5% sulfuric acid in ethanol stain for detection. Gel filtration chromatography was performed with a column (100 cm × 2.5 cm) packed with Bio-Gel P-2 Fine resins (Bio-Rad). *P. multocida* sialic acid aldolase (PmAldolase),[43] *N. meningitidis* CMP-sialic acid synthetase (NmCSS),[44] *P. multocida* α2−3-sialyltransferase 1 (PmST1),[45] and *P. damselae* α2−6-sialyltransferase (Pd2,6ST)[46] were expressed and purified as described previously.

Chemical Synthesis of 2,4-Diacetamido-2,4-dideoxy-D-mannose (Man2,4diNAc, 8) and 2,4-Diazido-2,4-dideoxy-D-mannose (Man2,4diN_3, 9). *p-Methoxyphenyl-β-D-galactopyranoside (**14**).* D-Galactopyranose (**12**, 7.00 g, 38.8 mmol) and sodium acetate (3.7 g, 0.045 mole) were dissolved in acetic anhydride (50 mL). The reaction mixture was heated at 120 °C for 1 h and neutralized using

https://doi.org/10.1021/acs.joc.1c01091
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sodium bicarbonate. The compound was extracted by washing with dichloromethane. The dichloromethane solution was washed with brine. The organic layer was dried on sodium sulfate, and the solvent was removed under a reduced pressure to produce 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (**13**), which was used in the next step without further purification. Compound **13** (21.67 g, 0.055 mmol) and *para*-methoxyphenol (10.33 g, 0.083 mmol) were added to a round-bottom flask (500 mL) and dissolved in dichloromethane (50 mL). The reaction mixture was cooled in an ice−water bath. Boron trifluoride diethyl etherate (11.86 g, 0.0832 mmol) was added slowly into the reaction mixture over a period of 5 min.[30] The reaction was stirred for 6 h and quenched by adding MeOH (50 mL). The solvent was removed under a reduced pressure, and the residue was subjected to silica gel column chromatography (hexane:ethyl acetate = 3:1 by volume) to obtain *p*-methoxyphenyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranoside in an 81% yield. The column-purified intermediate was dissolved in dry MeOH (100 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. After the reaction was completed, the reaction mixture was neutralized by adding H^+-resin. The product was purified by silica gel column chromatography (ethyl acetate:methanol = 20:1 by volume) to produce *p*-methoxyphenyl-*β*-D-galactopyranoside (**14**) in a 95% yield. Compound **14** (8.56 g) was obtained as a white amorphous solid in an overall 77% yield from D-galactose in three steps. ^{1}H NMR (400 MHz, CD_3OD) δ 7.10−7.05 (m, 2H), 6.87−6.82 (m, 2H), 4.74 (d, J = 7.7 Hz, 1H), 3.91 (dd, J = 3.4, 1.1 Hz, 1H), 3.78 (s, 3H), 3.65 (ddd, J = 6.7, 5.3, 1.1 Hz, 1H), 3.57 (dd, J = 9.7, 3.4 Hz, 1H), 3.37 (s, 1H), 3.33 (p, J = 1.7 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 156.3, 152.3, 119.3, 115.4, 108.7, 84.6, 83.5, 78.2, 72.2, 64.4, 56.0. HRMS (ESI-Orbitrap) m/z: $[M + Cl]^-$ calcd for $C_{13}H_{18}ClO_7$ 321.0747; found 321.0760.

*p-Methoxyphenyl-3,6-dibenzoyl-β-D-galactopyranoside (**15**).* Compound **14** (0.60 g, 2.10 mmol) and bis(tri-*n*-butyltin) oxide (1.78 g, 3.15 mmol) were dissolved in toluene (70 mL) in a round-bottom flask (500 mL). The reaction mixture was heated to 140 °C in an oil bath for 15 min. The reaction mixture was cooled down to 0 °C in an ice−water bath, and benzoyl chloride (0.88 g, 6.30 mmol) was added. The reaction was stirred for 6 h at 0 °C and quenched by adding methanol (50 mL). The solvent was removed under a reduced pressure, and the residue was purified by silica gel column chromatography (toluene:ethyl acetate = 7:1 by volume) to produce **15** as a white amorphous solid (0.82 g, 79% yield).[36−38] ^{1}H NMR (400 MHz, CD_3OD) δ 8.22−8.09 (m, 2H), 8.08−7.99 (m, 2H), 7.64 (dt, J = 9.7, 7.5 Hz, 2H), 7.50 (td, J = 7.6, 5.3 Hz, 4H), 7.09−6.97 (m, 2H), 6.73−6.62 (m, 2H), 5.15 (dd, J = 10.1, 3.4 Hz, 1H), 4.94 (d, J = 7.8 Hz, 1H), 4.68 (dd, J = 11.4, 8.5 Hz, 1H), 4.59 (bs, 2H), 4.50 (dd, J = 11.5, 4.0 Hz, 1H), 4.30 (d, J = 3.4 Hz, 1H), 4.20 (td, J = 7.3, 2.6 Hz, 2H), 3.69 (s, 3H). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 167.74, 167.70, 156.6, 153.0, 134.43, 134.36, 131.4, 131.3, 130.9, 130.7, 130.7, 129.6, 129.5, 119.3, 115.3, 103.7, 77.6, 74.1, 69.9, 68.0, 65.1, 56.0.

*p-Methoxyphenyl-2,4-diazido-3,6-dibenzoyl-2,4-dideoxy-β-D-mannopyranoside (**16**).* *p*-Methoxyphenyl-3,6-dibenzoyl-*β*-D-galactopyranoside (**15**, 0.40 g, 0.81 mmol) was dissolved in dichloromethane (25 mL) in a round-bottom flask (100 mL) at 0 °C. Pyridine (0.65 mL, 8.09 mmol) was added followed by the slow addition of trifluoromethanesulfonic anhydride (0.68 mL, 4.04 mmol) at 0 °C and the reaction was stirred for 30 min before it was quenched by adding sodium bicarbonate (10 mL). The organic layer was washed with hydrochloric acid (1 N) and brine. The organic layer was combined and concentrated under reduced pressure to produce a crude product, which was used for the next step without any purification. To a solution of 2,4-bistriflate in toluene (20 mL), tetrabutylammonium azide (0.76 g, 2.67 mmol) was added at room temperature. The reaction was stirred at 70 °C for 1 h and then at 90 °C for another 1 h.[39] The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (toluene) to produce *p*-methoxyphenyl-2,4-diazido-3,6-dibenzoyl-2,4-dideoxy-*β*-D-mannopyranoside (**16**) as a white amorphous solid (0.40 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (ddd, J = 21.4, 8.4, 1.4 Hz, 4H), 7.71−7.60 (m, 2H), 7.52 (dt, J = 11.6, 7.8 Hz, 4H), 7.03 (d, J = 9.0 Hz, 2H), 6.75 (d, J = 9.1 Hz, 2H), 5.36−5.27 (m, 1H), 5.24 (d, J = 1.2 Hz, 1H), 4.81 (dd, J = 11.9, 2.4 Hz, 1H), 4.61 (dd, J = 11.9, 6.6 Hz, 1H), 4.57 (dd, J = 3.7, 1.1 Hz, 1H), 4.13 (t, J = 10.1 Hz, 1H), 3.76 (s, 4H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 166.1, 165.6, 155.8, 150.4, 134.1, 133.4, 130.1, 129.9, 129.7, 129.1, 128.8, 128.5, 118.5, 114.6, 98.9, 74.1, 73.0, 63.7, 61.6, 57.4, 55.6. HRMS (ESI-Orbitrap) m/z: $[M + Na]^+$ calcd for $C_{27}H_{24}N_6NaO_7$ 567.1599; found 567.1581.

*p-Methoxyphenyl-2,4-diacetamido-3,6-dibenzoyl-2,4-dideoxy-β-D-mannopyranoside (**17**).* *p*-Methoxyphenyl-2,4-diazido-3,6-dibenzoyl-2,4-dideoxy-*β*-D-mannopyranoside (**16**, 0.22 g, 0.41 mmol) was dissolved in pyridine (4.0 mL) in a round-bottom flask (50 mL), and thioacetic acid (1.0 mL) was added. The reaction mixture was stirred for 24 h under a reduced pressure at room temperature before the reaction was quenched by adding methanol (10 mL). The solvent was removed under a reduced pressure,[40] and the residue was purified by silica gel column chromatography (toluene:ethyl acetate = 2:1 by volume) to produce *p*-methoxyphenyl-2,4-diacetamido-3,6-dibenzoyl-2,4-dideoxy-*β*-D-mannopyranoside (**17**) as a white amorphous solid (0.21 g, 89% yield). ^{1}H NMR (800 MHz, $CDCl_3$) δ 8.00 (dd, J = 8.2, 1.5 Hz, 2H), 7.95 (dd, J = 8.1, 1.5 Hz, 2H), 7.62−7.56 (m, 1H), 7.55−7.49 (m, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.36 (t, J = 7.8 Hz, 2H), 6.92−6.87 (m, 2H), 6.89−6.85 (m, 1H), 6.62−6.52 (m, 3H), 5.43 (dd, J = 10.7, 4.0 Hz, 1H), 5.19 (d, J = 1.8 Hz, 1H), 5.10 (ddd, J = 9.3, 4.0, 1.6 Hz, 1H), 4.66 (dd, J = 11.9, 2.6 Hz, 1H), 4.55 (dd, J = 11.9, 8.6 Hz, 1H), 4.51−4.44 (m, 1H), 4.01−3.95 (m, 1H), 3.65 (s, 3H), 2.06 (s, 3H), 1.89 (s, 3H). ^{13}C{^{1}H} NMR (200 MHz, $CDCl_3$) δ 171.3, 171.1, 166.8, 166.3, 155.3, 150.6, 133.5, 133.3, 129.9, 129.8, 129.8, 129.3, 128.6, 128.5, 118.2, 114.4, 98.0, 74.4, 72.0, 64.4, 55.5, 50.6, 47.6, 23.5, 23.2. HRMS (ESI-Orbitrap) m/z: $[M + H]^+$ calcd for $C_{31}H_{33}N_2O_9$ 577.2181; found 577.2167.

*p-Methoxyphenyl-2,4-diacetamido-2,4-dideoxy-β-D-mannopyranoside (**18**).* *p*-Methoxyphenyl-2,4-diacetamido-3,6-dibenzoyl-2,4-dideoxy-*β*-D-mannopyranoside (**17**, 0.51 g, 0.88 mmol) was dissolved in dry MeOH (10 mL) in a round-bottom flask (50 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. After the reaction was completed, the reaction mixture was neutralized using H^+-resin. The solvent was removed under a reduced pressure, and the residue was purified by silica gel column chromatography (ethyl acetate:methanol = 9:1 by volume) to produce *p*-methoxyphenyl-2,4-diacetamido-2,4-dideoxy-*β*-D-mannopyranoside (**18**) as a white amorphous solid (0.26 g, 80% yield). ^{1}H NMR (800 MHz, CD_3OD) δ 7.01−6.94 (m, 2H), 6.86−6.76 (m, 2H), 5.12 (d, J = 1.6 Hz, 1H), 4.70 (dd, J = 3.8, 1.6 Hz, 1H), 3.93−3.86 (m, 2H), 3.74 (s, 3H), 3.72−3.66 (m, 2H), 3.40 (ddd, J = 9.7, 4.0, 2.4 Hz, 1H), 2.12 (s, 3H), 2.02 (s, 3H). ^{13}C{^{1}H} NMR (200 MHz, MeOD) δ 175.0, 174.6, 156.7, 152.4, 119.1, 115.5, 99.6, 77.5, 71.7, 62.4, 56.0, 54.5, 49.7, 22.77, 22.75. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{17}H_{23}N_2O_7$ 367.1511; found 367.1507.

*2,4-Diacetamido-2,4-dideoxy-D-mannose (Man2,4diNAc, **8**).* *p*-Methoxyphenyl-2,4-diacetamido-2,4-dideoxy-*β*-D-mannopyranoside (**18**, 0.125 g, 0.34 mmol) was added to a round-bottom flask (50 mL) and dissolved in acetonitrile (6 mL). Ammonium cerium nitrate (0.6 g) was dissolved in water (1.5 mL) and was added slowly while stirring. The reaction was carried out at room temperature for 4.5 h before the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (ethyl acetate:methanol:water = 9:1:0.5 by volume) to produce Man2,4diNAc (**8**) as a white amorphous solid (0.071 g, 80% yield). ^{1}H NMR (800 MHz, D_2O) δ 5.14 (bs, 0.6H), 4.97 (bs, 0.4H), 4.45 (d, J = 4.8 Hz, 0.4H), 4.30 (d, J = 4.8 Hz, 0.6H), 4.10 (dd, J = 10.8, 4.0 Hz, 0.6H), 3.76−3.99 (m, 2H), 3.52−3.74 (m, 2H), 3.39−3.47 (m, 0.4H), 1.97−2.31 (m, 6H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ = 175.7, 174.8, 174.7, 174.6, 92.9, 92.8, 75.3, 70.8, 69.9, 66.6, 60.6, 60.5, 53.4, 52.4, 48.1, 47.8, 21.9, 21.8. HRMS (ESI-Orbitrap) m/z: $[M + Na]^+$ calcd for $C_{10}H_{18}N_2O_6Na$ 285.1057; found 285.1058.

*p-Methoxyphenyl-2,4-diazido-2,4-dideoxy-β-D-mannopyranoside (**19**).* *p*-Methoxyphenyl-2,4-diazido-3,6-dibenzoyl-2,4-dideoxy-*β*-D-mannopyranoside (**16**) (0.50 g, 0.92 mmol) was dissolved in dry MeOH (5 mL) in a round-bottom flask (50 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. After the reaction was

https://doi.org/10.1021/acs.joc.1c01091
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completed, the reaction mixture was neutralized using H^+-resin.[41] The product was purified by silica gel column chromatography (toluene:ethyl acetate = 7:1 by volume) to produce *p*-methoxyphenyl-2,4-diazido-2,4-dideoxy-β-D-mannopyranoside (**19**) as a white amorphous solid (0.28 g, 92% yield). ^{1}H NMR (800 MHz, $CDCl_3$) δ 6.95 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 9.0 Hz, 2H), 5.13−5.09 (m, 1H), 4.21−4.15 (m, 1H), 3.93 (ddd, *J* = 12.4, 5.9, 2.4 Hz, 1H), 3.79 (d, *J* = 3.6 Hz, 1H), 3.77 (s, 3H), 3.64 (t, *J* = 9.9 Hz, 1H), 3.24 (ddd, *J* = 10.1, 4.7, 2.4 Hz, 1H), 2.61 (d, *J* = 9.2 Hz, 1H). ^{13}C{^{1}H} NMR (200 MHz, $CDCl_3$) δ 155.6, 150.1, 117.6, 114.7, 98.8, 75.1, 72.4, 63.9, 62.0, 59.4, 55.7. HRMS (ESI-Orbitrap) *m*/*z*: [M + Cl]$^-$ calcd for $C_{13}H_{16}ClN_6O_5$ 371.0876; found 371.0864.

*2,4-Diazido-2,4-dideoxy-D-mannose (Man2,4diN$_3$, **9**).* *p*-Methoxyphenyl-2,4-diazido-2,4-dideoxy-β-D-mannopyranoside (**19**, 0.28 g 0.84 mmol) was added to a round-bottom flask (50 mL) and dissolved in acetonitrile (6 mL). Ammonium cerium nitrate (1.4 g) dissolved in water (1.5 mL) was added slowly while stirring. The reaction was carried out at room temperature[41] for 2.5 h before the reaction mixture was concentrated. The residue was purified by silica gel column chromatography (toluene:ethyl acetate = 2:1 by volume) to produce Man2,4diN$_3$ (9) as a white amorphous solid (0.15 g, 80% yield). ^{1}H NMR (800 MHz, D_2O) δ 5.29 (s, 1H), 4.99 (s, 1H), 4.21 (dd, *J* = 9.9, 3.8 Hz, 1H), 4.07 (d, *J* = 3.8 Hz, 1H), 4.03−3.97 (m, 2H), 3.87 (dd, *J* = 12.5, 2.2 Hz, 1H), 3.83 (dd, *J* = 12.4, 2.3 Hz, 1H), 3.78 (dd, *J* = 12.4, 4.8 Hz, 1H), 3.74 (dd, *J* = 12.7, 5.2 Hz, 2H), 3.66 (t, *J* = 10.1 Hz, 1H), 3.54 (t, *J* = 10.1 Hz, 1H), 3.32 (ddd, *J* = 10.3, 5.5, 2.2 Hz, 1H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 93.0, 92.2, 74.7, 72.1, 70.7, 69.5, 65.6, 63.9, 61.0, 60.9, 59.1, 58.7. HRMS (ESI-Orbitrap) *m*/*z*: [M + Cl]$^-$ calcd for $C_6H_{10}ClN_6O_4$ 265.0458; found 265.0439.

Chemical Synthesis of 2,4,6-Triacetamido-2,4,6-trideoxy-D-mannose (Man2,4,6triNAc, 10) and 2,4,6-Triazido-2,4,6-trideoxy-D-mannose (Man2,4,6triN$_3$, 11). *p-Methoxyphenyl-3-benzoyl-β-D-galactopyranoside (**20**).* Compound **14** (6.14 g, 21.4 mmol) was dissolved in THF (100 mL) in a round-bottom flask (500 mL). Diisopropylethylamine (DIPEA) (5.5 g, 42.9 mmol) and a catalytic amount of dimethyltin dichloride (200 mg) were added to the reaction mixture.[38] The reaction mixture was cooled down to 0 °C in an ice−water bath, and benzoyl chloride (3.32 g, 23.6 mmol) was added dropwisely. The reaction mixture was stirred for 2 h at 0 °C and quenched by adding methanol (100 mL). The solvent was removed under a reduced pressure, and the residue was purified by silica gel column chromatography (toluene:ethyl acetate = 3:1 by volume) to produce **20** as a white amorphous solid (7.77 g, 93% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.20−8.13 (m, 2H), 7.69−7.60 (m, 1H), 7.51 (dd, *J* = 8.4, 7.1 Hz, 2H), 7.15−7.06 (m, 2H), 6.91−6.80 (m, 2H), 5.10 (dd, *J* = 10.1, 3.3 Hz, 1H), 4.93 (d, *J* = 7.7 Hz, 1H), 4.25 (d, *J* = 3.3 Hz, 1H), 4.19 (dd, *J* = 10.2, 7.7 Hz, 1H), 3.85−3.79 (m, 3H), 3.77 (s, 3H). ^{13}C{^{1}H} NMR (200 MHz, CD_3OD) δ 167.7, 156.6, 153.1, 134.3, 130.8, 129.4, 119.3, 115.4, 104.0, 77.8, 76.6, 69.9, 67.9, 62.1, 56.0. HRMS (ESI-Orbitrap) *m*/*z*: [M + Cl]$^-$ calcd for $C_{20}H_{22}ClO_8$ 425.1009; found 425.1027.

*p-Methoxyphenyl-2,4,6-triazido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**21**).* *p*-Methoxyphenyl-3-benzoyl-β-D-galactopyranoside (**20**, 0.15 g, 0.38 mmol) was dissolved in dichloromethane (10 mL) at 0 °C in a round-bottom flask (50 mL). Pyridine (0.31 mL, 3.84 mmol) was added followed by the slow addition of trifluoromethanesulfonic anhydride (0.32 mL 1.92 mmol) at 0 °C. After stirring at the same temperature for 30 min, the reaction was quenched by adding sodium bicarbonate (10 mL). The organic layer was washed with hydrochloric acid (1 N) and brine. The organic layer was combined and concentrated under reduced pressure to produce a crude product, which was used for the next step without any purification. To a solution of 2,4,6-tristriflate in toluene (20 mL), tetrabutylammonium azide (0.6 g, 2.1 mmol) was added at room temperature to a round-bottom flask (50 mL). The reaction was stirred at 70 °C for 1 h and then at 90 °C for another 1 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (toluene) to produce *p*-methoxyphenyl-2,4,6-triazido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**21**) as a colorless oil (0.15 g, 88% yield). ^{1}H NMR (400 MHz, CD_3Cl) δ 8.15 (d, *J* = 8 Hz, 2H), 7.67 (t, *J* = 7.6 Hz, 1H), 7.54 (t, *J* = 8 Hz, 2H), 7.05 (d, *J* = 8.8 Hz, 2H), 6.91−6.82 (m, 2H), 5.22, (s, 1H), 4.52 (d, *J* = 3.6 Hz, 1H), 4.08 (t, *J* = 10 Hz, 1H), 3.80 (s, 3H), 3.69−3.56 (m, 2H), 3.51−3.43 (m, 1H). ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ = 165.5, 150.2, 155.9, 134.0, 130.0, 128.7, 128.4, 118.7, 114.6, 98.8, 74.1, 73.8, 61.5, 57.4, 55.6, 51.5. HRMS (ESI-Orbitrap) *m*/*z*: [M + Cl]$^-$ calcd for $C_{20}H_{19}ClN_9O_5$ 500.1203; found 500.1190.

*p-Methoxyphenyl-2,4,6-triacetamido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**22**).* *p*-Methoxyphenyl-2,4,6-triazido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**21**, 0.18 g, 0.38 mmol) was dissolved in pyridine (4.0 mL) in a round-bottom flask (50 mL), and thioacetic acid (1.0 mL) was added dropwisely. The reaction mixture was stirred for 24 h under reduced pressure at room temperature. The reaction was quenched by adding methanol (10 mL), and the solvent was removed under a reduced pressure. The compound was purified by silica gel column chromatography (ethyl acetate:methanol = 20:1 by volume) to produce *p*-methoxyphenyl-2,4,6-tri-acetamido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**22**) as a white amorphous solid (0.16 g, 80% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.98−7.92 (m, 2H), 7.59 (t, *J* = 7.5 Hz, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.01−6.93 (m, 2H), 6.89−6.80 (m, 2H), 5.28 (d, *J* = 1.6 Hz, 1H), 5.25 (dd, *J* = 11.0, 4.2 Hz, 1H), 5.00 (dd, *J* = 4.2, 1.5 Hz, 1H), 4.34 (t, *J* = 10.7 Hz, 1H), 3.75 (s, 3H), 3.74−3.68 (m, 1H), 3.61−3.50 (m, 1H), 3.44 (dd, *J* = 14.2, 2.7 Hz, 1H), 2.06 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H). ^{13}C{^{1}H} NMR (200 MHz, CD_3OD) δ 174.1, 173.7, 173.6, 167.1, 156.9, 152.1, 134.4, 131.1, 130.7, 129.5, 119.3, 115.5, 99.1, 75.0, 73.8, 56.1, 51.9, 48.4, 41.5, 22.7, 22.6, 22.5. HRMS (ESI-Orbitrap) *m*/*z*: [M + Na]$^+$ calcd for $C_{26}H_{31}N_3O_8Na$ 536.2003; found 536.1995.

*2,4,6-Triacetamido-2,4,6-trideoxy-D-mannose (Man2,4,6triNAc, **10**).* *p*-Methoxyphenyl-2,4,6-tri-acetamido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**22**, 0.1 g, 0.19 mmol) was dissolved in dry MeOH (10 mL) in a round-bottom flask (50 mL), and 0.3 mL of sodium methoxide solution (5.4 M) was added. After the reaction was completed, the reaction mixture was neutralized using H^+-resin. The crude product without further purification was added to a round-bottom flask (50 mL) and dissolved in acetonitrile (4 mL). Ammonium cerium nitrate (0.3 g) dissolved in water (1 mL) was added slowly while stirring. The reaction was run for 2.5 h at room temperature. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (ethyl acetate:methanol:water = 5:1:0.1 by volume) to produce 2,4,6-triacetamido-2,4,6-trideoxy-D-mannopyranose (**10**) as a white amorphous solid (0.04 g, 70% yield). ^{1}H NMR (800 MHz, D_2O) δ 5.16 (bs, 1H), 4.97 (bs, 1H), 4.48 (dt, *J* = 2.8, 1.4 Hz, 1H), 4.31 (dd, *J* = 4.6, 1.5 Hz, 1H), 4.09 (ddd, *J* = 10.6, 4.6, 1.3 Hz, 1H), 4.01−3.95 (m, 1H), 3.92 (t, *J* = 10.5 Hz, 1H), 3.88 (ddt, *J* = 10.7, 4.2, 0.9 Hz, 1H), 3.83 (t, *J* = 10.5 Hz, 1H), 3.56−3.52 (m, 1H), 3.45−3.38 (m, 3H), 3.36−3.33 (m, 1H), 2.13 (s, 3H), 2.08 (s, 3H), 2.02 (s, 6H), 2.00 (s, 6H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 175.8, 174.9, 174.5, 174.2, 93.0, 73.4, 70.0, 69.0, 66.7, 53.5, 52.6, 49.3, 48.9, 40.0, 39.9, 22.0, 21.9, 21.7. HRMS (ESI-Orbitrap) *m*/*z*: [M + Na]$^+$ calcd for $C_{12}H_{21}N_3NaO_6$ 326.1323; found 326.1308.

*p-Methoxyphenyl-2,4,6-triazido-2,4,6-trideoxy-β-D-mannopyranoside (**23**).* *p*-Methoxyphenyl-2,4,6-triazido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**21**, 0.50 g, 1.1 mmol) was dissolved in dry MeOH (10 mL) in a round-bottom flask (50 mL), and 0.2 mL of sodium methoxide solution (5.4 M) was added. The reaction was carried out at room temperature for 6 h. After the reaction was completed, the reaction mixture was neutralized using H^+-resin. The product was purified by silica gel column chromatography (toluene:ethyl acetate = 35:1 by volume) to produce compound **23** as a white amorphous solid (0.36 g, 92% yield). ^{1}H NMR (400 MHz, CD_3Cl) δ 6.99 (d, *J* = 8.8 Hz, 2H), 6.83 (d, *J* = 8.8 Hz, 2H), 5.06 (s, 1H), 4.19 (bd, *J* = 3.6 Hz, 1H), 3.75 (s, 1H), 3.75 (s, 3H), 3.60−3.45 (m, 3H), 3.31−3.22 (m, 1H), 2.59 (d, *J* = 9.2 Hz, 1H). ^{13}C{^{1}H} NMR (100 MHz, CD_3Cl) δ 155.9, 150.1, 118.5, 114.7, 99.4, 74.2, 72.5, 63.9, 60.5, 55.7, 51.7. HRMS (ESI-Orbitrap) *m*/*z*: [M + Cl]$^-$ calcd for $C_{13}H_{15}ClN_9O_4$ 396.0941; found 396.0930.

https://doi.org/10.1021/acs.joc.1c01091
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*2,4,6-Triazido-2,4,6-trideoxy-D-mannose (Man2,4,6triN$_3$, **11**).* *p*-Methoxyphenyl-2,4,6-triazido-2,4,6-trideoxy-β-D-mannopyranoside (**23**, 0.72 g, 1.99 mmol) was added to a round-bottom flask (50 mL) and dissolved in acetonitrile (12 mL). Ammonium cerium nitrate (1.1 g) dissolved in water (3 mL) was added slowly while stirring. The reaction was carried out at room temperature for 2.5 h. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (toluene:ethyl acetate = 15:1 by volume) to produce compound **11** as a white amorphous solid (0.41 g, 80% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 5.14−4.86 (m, 2H), 4.20−4.11 (m, 1H), 3.95−3.73 (m, 4H), 3.64−3.36 (m, 6H), 3.35−3.25 (m, 1H). ^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 93.7, 92.4, 73.6, 72.4, 69.8, 69.7, 66.2, 64.7, 60.4, 60.0, 51.7, 51.6. HRMS (ESI-Orbitrap) *m*/*z*: [M + Na]$^+$ calcd for $C_6H_9N_9NaO_3$ 278.0721; found 278.0707.

Enzymatic Synthesis of Sia Derivatives 7-Acetamido-7-deoxy-*N*-acetylneuraminic Acid (Neu5Ac7NAc, 4), 5,7-Diazido-5,7-dideoxy-neuraminic Acid (Neu5,7diN$_3$, 5), and 5,7,9-Triazido-5,7,9-trideoxy-neuraminic Acid (Neu5,7,9triN$_3$, 7). *7-Acetamido-7-deoxy-N-acetylneuraminic Acid (Neu5Ac7NAc, **4**).* The 2,4-diacetamido-2,4-dideoxy-D-mannose (Man2,4diNAc, **8**, 0.27 g, 1.03 mmol) and sodium pyruvate (0.57 g, 5.15 mmol) were dissolved in water in a 50 mL centrifuge tube. After the addition of an appropriate amount of PmAldolase, water was added to bring the final concentration of mannose derivative to 10 mM. The reaction was carried out by incubating the solution at 30 °C with agitation at 120 rpm in an incubator for 72 h. The product formation was observed by TLC developed with ethyl acetate:methanol:water = 7:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by the addition of methanol (15 mL), and the mixture was then centrifuged. The supernatant was concentrated and passed through a Bio-Gel P-2 gel filtration (water was used as an eluent). Then, the product was further purified by silica gel chromatography (ethyl acetate:methanol:water = 7:1:0.5) to produce 5,7-di-*N*-acetyl-neuraminic acid (**4**) as a white amorphous solid (0.26 g, 72% yield). ^{1}H NMR (400 MHz, D_2O) δ 4.23 (dd, *J* = 10.5, 2.1 Hz, 1H), 4.00−3.90 (m, 2H), 3.81−3.68 (m, 2H), 3.63 (dd, *J* = 12.1, 2.7 Hz, 1H), 3.46 (dd, *J* = 12.1, 7.0 Hz, 1H), 2.22 (dd, *J* = 13.0, 4.9 Hz, 1H), 2.00 (s, 3H), 1.99 (s, 3H), 1.83 (d, *J* = 13.0 Hz, 1H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 176.6, 174.1, 173.9, 96.5, 70.2, 69.4, 67.5, 62.5, 59.3, 54.7, 49.3, 39.6, 22.1, 21.6. HRMS (ESI-Orbitrap) *m*/*z*: [M − H]$^-$ calcd for $C_{13}H_{21}N_2O_9$ 349.1253; found 349.1275.

*5,7-Diazido-5,7-dideoxy-neuraminic Acid (Neu5,7diN$_3$, **5**).* The 2,4-diazido-2,4-dideoxymannose (Man2,4diN$_3$, **9**, 0.05 g, 0.22 mmol) and sodium pyruvate (0.12 g, 1.1 mmol) were dissolved in water in a 50 mL centrifuge tube. After the addition of an appropriate amount of PmAldolase, water was added to bring the final concentration of mannose derivative to 10 mM. The reaction was carried out by incubating the solution at 30 °C with agitation at 120 rpm in an incubator for 48 h. The product formation was observed by TLC developed with ethyl acetate:methanol:water = 7:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by the addition of methanol (15 mL), and the mixture was then centrifuged. The supernatant was concentrated and passed through a Bio-Gel P-2 gel filtration (water was used as an eluent). Then, the product was further purified by silica gel chromatography (ethyl acetate:methanol:water = 7:1:0.5) to produce 5,7-diazido-5,7-dideoxy-neuraminic acid (**5**) as a white amorphous solid (0.05 g, 72% yield). ^{1}H NMR (600 MHz, D_2O) δ 4.11 (ddd, *J* = 11.6, 9.5, 5.0 Hz, 1H), 4.05 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.98−3.91 (m, 1H), 3.82−3.74 (m, 2H), 3.70−3.65 (m, 1H), 3.60−3.56 (m, 1H), 2.21 (dd, *J* = 13.1, 5.0 Hz, 1H), 1.97 (d, *J* = 13.1 Hz, 1H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 175.7, 96.4, 72.1, 69.5, 68.5, 63.1, 62.5, 61.4, 39.1. HRMS (ESI-Orbitrap) *m*/*z*: [M − H]$^-$ calcd for $C_9H_{13}N_6O_7$ 317.0851; found 317.0830.

*5,7,9-Triazido-5,7,9-trideoxy-neuraminic Acid (Neu5,7,9triN$_3$, **7**).* Man2,4,6triN$_3$ (**11**, 0.04 g, 0.16 mmol) and sodium pyruvate (0.086 g, 0.78 mmol) were dissolved in water in a 50 mL centrifuge tube. After the addition of an appropriate amount of PmAldolase, water was added to bring the final concentration of mannose derivative to 10 mM. The reaction was carried out by incubating the solution at 30 °C with agitation at 120 rpm in an incubator for 48 h. The product formation was observed by TLC developed with ethyl acetate:methanol:water = 7:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by the addition of methanol (15 mL), and the mixture was then centrifuged. The supernatant was concentrated and passed through a Bio-Gel P-2 gel filtration (water was used as an eluent). Then, the product was further purified by silica gel chromatography (ethyl acetate:methanol:water = 7:1:0.5) to produce **7** as a white amorphous solid (0.037 g, 70% yield). ^{1}H NMR (600 MHz, D_2O) δ 4.14−4.06 (m, 2H), 4.04 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.79 (dd, *J* = 8.9, 1.8 Hz, 1H), 3.72 (dd, *J* = 13.4, 2.8 Hz, 1H), 3.60−3.53 (m, 2H), 2.20 (dd, *J* = 13.1, 5.0 Hz, 1H), 1.96 (dd, *J* = 13.2, 11.7 Hz, 1H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 175.6, 96.4, 69.5, 68.5, 68.4, 64.2, 62.1, 53.9, 39.1. HRMS (ESI-Orbitrap) *m*/*z*: [M − H]$^-$ calcd for $C_9H_{12}N_9O_6$ 342.0916; found 342.0943.

Chemical Synthesis of NHCbz-Tagged Sialyltransferase Acceptors (24−30). Lacβ*Pro*NHCbz (**24**) was synthesized as described previously.[47] Compounds **25**−**30** were synthesized similarly. Briefly, in a round-bottom flask, a propylazide-tagged acceptor[23] (0.085 mmol) was dissolved in water and a catalytic amount of palladium (10%) Pd/C was added. The mixture was stirred under a hydrogen atmosphere for overnight. After the completion of reaction, palladium was removed by filtration. The solvent was removed *in vacuo*. The obtained propylamine-tagged acceptor was used directly for the next reaction without any further filtration. The compound (0.085 mmol) was dissolved in 5−10 mL of water in a round-bottom flask, and sodium carbonate (45 mg, 0.43 mmol) was added. Benzyl chloroformate (CbzCl, 30 mg, 0.17 mmol) in acetonitrile was added to the mixture in the flask immersed in an ice−water bath. After the completion of reaction, the solvent was removed. The mixture was purified using a C18 column (gradient solvent of CH_3CN in H_2O was used for elute) to produce the Cbz-tagged acceptors (**25**−**30**).

*LacNAcβ*Pro*NHCbz (**25**).* 39 mg, 80% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.43 (q, *J* = 6.2, 5.7 Hz, 5H), 5.11 (s, 2H), 4.47 (dd, *J* = 7.7, 1.9 Hz, 2H), 4.05−3.46 (m, 14H), 3.17 (d, *J* = 7.1 Hz, 2H), 2.00 (d, *J* = 11.0 Hz, 3H), 1.74 (t, *J* = 6.4 Hz, 2H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.5, 158.3, 136.6, 128.8, 128.3, 127.6, 102.9, 101.0, 78.5, 75.3, 74.7, 72.5, 72.4, 71.0, 68.5, 67.6, 66.8, 61.0, 60.1, 55.1, 37.2, 28.8, 22.1. HRMS (ESI-Orbitrap) *m*/*z*: [M + FA − H]$^-$ calcd for $C_{26}H_{39}N_2O_{15}$ 619.2356; found 619.2350.

*Galβ3GalNAcβ*Pro*NHCbz (**26**).* 38 mg, 78% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.48−7.38 (m, 5H), 5.12−5.08 (m, 2H), 4.45−4.40 (m, 2H), 4.16 (d, *J* = 3.2, 1H), 3.98 (dd, *J* = 10.9, 8.5 Hz, 1H), 3.90 (d, *J* = 3.7 Hz, 2H), 3.82 (dd, *J* = 10.9, 3.3 Hz, 1H), 3.81−3.70 (m, 4 H), 3.68−3.63 (m, 2H), 3.62−3.58 (m, 2H), 3.52 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.21−3.10 (m, 2H), 1.99 (s, 3H), 1.79−1.69 (m, 2H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 174.7, 158.3, 136.6, 128.7, 128.3, 127.6, 104.8, 101.3, 79.9, 75.0, 74.7, 72.4, 70.5, 68.5, 68.0, 67.5, 66.73, 61.0, 60.9, 51.2, 37.2, 28.7, 22.2. HRMS (ESI-Orbitrap) *m*/*z*: [M + FA − H]$^-$ calcd for $C_{26}H_{39}N_2O_{15}$ 619.2356; found 619.2356.

*Galβ3GalNAcα*Pro*NHCbz (**27**).* 39 mg, 81% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.52−7.34 (m, 5H), 5.12 (d, *J* = 2.8 Hz, 2H), 4.82 (d, *J* = 3.8 Hz, 1H), 4.43 (d, *J* = 7.6 Hz, 1H), 4.32 (dd, *J* = 11.0, 3.7 Hz, 1H), 4.22 (d, *J* = 3.1 Hz, 1H), 4.06−3.92 (m, 2H), 3.90 (d, *J* = 3.3 Hz, 1H), 3.81−3.66 (m, 5H), 3.65−3.57 (m, 2H), 3.55−3.40 (m, 2H), 3.25 (t, *J* = 6.6 Hz, 2H), 2.01 (s, 3H), 1.80 (dd, *J* = 9.8, 4.2 Hz, 2H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.5, 158.4, 136.6, 128.8, 128.3, 127.5, 104.7, 97.1, 77.4, 74.9, 72.5, 70.6, 70.6, 68.8, 68.5, 66.7, 65.0, 61.2, 60.9, 48.6, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) *m*/*z*: [M + FA − H]$^-$ calcd for $C_{26}H_{39}N_2O_{15}$ 619.2356; found 619.2351.

*Galβ3GlcNAcβ*Pro*NHCbz (**28**).* 39 mg, 80% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.49−7.24 (m, 5H), 5.03 (s, 2H), 4.41 (d, *J* = 8.2 Hz, 1H), 4.34 (d, *J* = 7.7 Hz, 1H), 3.83 (tt, *J* = 5.8, 2.9 Hz, 3H), 3.77−3.60 (m, 6H), 3.59−3.48 (m, 2H), 3.48−3.41 (m, 2H), 3.37 (ddd, *J* = 9.9, 5.6, 2.1 Hz, 1H), 3.09 (hept, *J* = 6.9 Hz, 2H), 1.92 (s, 3H), 1.66 (p, *J* = 6.6 Hz, 2H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.6, 158.3, 136.6, 128.8, 128.3,

https://doi.org/10.1021/acs.joc.1c01091
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127.6, 103.5, 100.9, 82.4, 75.3, 75.3, 72.5, 70.7, 68.7, 68.5, 67.6, 66.8, 61.0, 60.7, 54.5, 37.2, 28.8, 22.2. HRMS (ESI-Orbitrap) m/z: $[M + FA - H]^-$ calcd for $C_{26}H_{39}N_2O_{15}$ 619.2356; found 619.2353.

Galβ3GlcNAcαProNHCbz (**29**). 38 mg, 78% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.43 (q, J = 6.9 Hz, 5H), 5.12 (s, 2H), 4.41 (d, J = 7.7 Hz, 1H), 4.08 (dd, J = 10.6, 3.6 Hz, 1H), 3.98−3.86 (m, 2H), 3.84−3.39 (m, 12H), 3.34−3.14 (m, J = 7.0 Hz, 2H), 2.01 (s, 3H), 1.88−1.68 (m, 2H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 174.4, 158.4, 136.6, 128.8, 128.3, 127.5, 103.5, 97.0, 80.6, 75.2, 72.5, 71.5, 70.6, 68.6, 68.5, 66.7, 64.9, 60.9, 60.5, 52.4, 37.4, 28.5, 21.9. HRMS (ESI-Orbitrap) m/z: $[M + FA - H]^-$ calcd for $C_{26}H_{39}N_2O_{15}$ 619.2356; found 619.2349.

GalNAcαProNHCbz (**30**). 42 mg, 78% yield, white amorphous solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.45−7.23 (m, 5H), 5.17−5.02 (m, 2H), 4.77 (d, J = 3.7 Hz, 1H), 4.27 (dd, J = 10.9, 3.6 Hz, 1H), 3.89 (d, J = 3.2 Hz, 1H), 3.84−3.66 (m, 5H), 3.42 (dt, J = 10.7, 6.0 Hz, 1H), 3.32−3.17 (m, 2H), 2.01 (s, 3H), 1.79 (p, J = 6.5 Hz, 2H). $^{13}C\{^1H\}$ NMR (100 MHz, CD_3OD) δ 174.2, 159.0, 138.5, 129.5, 129.0, 128.8, 98.8, 72.5, 70.4, 70.0, 67.4, 65.9, 62.9, 51.6, 38.8, 30.7, 22.7. HRMS (ESI-Orbitrap) m/z: $[M + FA - H]^-$ calcd for $C_{20}H_{29}N_2O_{10}$ 457.1828; found 457.1822.

General Procedures for One-Pot Three-Enzyme (OP3E) Preparative-Scale Synthesis of Neu5,7diN$_3$α2−3/6-Linked Sialosides (31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53). Man2,4diN$_3$ (**9**, 50−70 mg, 0.22−0.30 mmol), sodium pyruvate (0.75−1.5 mmol), CTP (0.15−0.30 mmol), and an acceptor selected from Lacβ ProNHCbz (**24**),[47] LacNAcβProNHCbz (**25**), Galβ1−3GalNAcβProNHCbz (**26**), Galβ1−3GalNAcαProNHCbz (**27**), Galβ1−3GlcNAcβProNHCbz (**28**), Galβ1−3GlcNAcαProNHCbz (**29**), and GalNAcαProNHCbz (**30**) (0.10−0.20 mmol), were dissolved in water in a 50 mL centrifuge tube containing Tris−HCl buffer (100 mM, pH 8.5) and $MgCl_2$ (20 mM). After adding sialic acid aldolase (0.5−3 mg), NmCSS (0.5 mg), and a sialyltransferase PmST1 (1−3 mg) or Pd2,6ST (1−4 mg), water was added to bring the final concentration of Man2,4diN$_3$ (**9**) to 10 mM. The reaction mixture was incubated at 30 °C for 24−36 h. The reaction progress was monitored using TLC (ethyl acetate:methanol:water = 6:1:1 by volume) and mass spectrometry. The reaction mixture was diluted with the same volume of ethanol and incubated at 4 °C for 30 min. The mixture was then centrifuged and concentrated, which was purified using a C18 column (CH_3CN in H_2O gradient was used as running solvents) to produce the sialoside product.

Neu5,7diN$_3$α2−3LacβProNHCbz (**31**). 107 mg, 90% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.49−7.36 (m, 5H), 5.09 (s, 2H), 4.50 (d, J = 7.9 Hz, 1H), 4.41 (d, J = 8.0 Hz, 1H), 4.06 (ddd, J = 8.8, 5.6, 2.6 Hz, 1H), 4.03 (dd, J = 9.9, 3.2 Hz, 1H), 3.96−3.89 (m, 4H), 3.81−3.73 (m, 4H), 3.71−3.52 (m, 10H), 3.28 (t, J = 8.4 Hz, 1H), 3.26−3.17 (m, 2H), 2.72 (dd, J = 12.7, 4.7 Hz, 1H), 1.90 (t, J = 12.3 Hz, 1H), 1.79 (p, J = 6.6 Hz, 2H). $^{13}C\{^1H\}$ NMR (200 MHz, D_2O) δ 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 102.6, 102.1, 100.8, 78.4, 75.7, 75.1, 74.7, 74.3, 72.8, 72.4, 70.4, 69.5, 69.3, 67.9, 67.7, 66.8, 62.5, 60.9, 60.3, 60.1, 38.8, 37.3, 28.8. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{32}H_{46}N_7O_{19}$ 832.2854; found 832.2883.

Neu5,7diN$_3$α2−6LacβProNHCbz (**33**). 140 mg, 100% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.46−7.37 (m, 5H), 5.09 (s, 2H), 4.40 (dd, J = 17.6, 7.9 Hz, 2H), 4.05 (ddd, J = 8.7, 5.5, 2.5 Hz, 1H), 3.96−3.90 (m, 5H), 3.80−3.72 (m, 5H), 3.70−3.62 (m, 4H), 3.61−3.50 (m, 5H), 3.30 (t, J = 8.7 Hz, 1H), 3.26−3.18 (m, 2H), 2.71 (dd, J = 12.6, 4.7 Hz, 1H), 1.85−1.77 (m, 3H). $^{13}C\{^1H\}$ NMR (200 MHz, D_2O) δ 173.1, 158.4, 136.6, 128.8, 128.3, 127.7, 103.3, 102.0, 100.6, 80.0, 74.6, 74.5, 73.5, 72.6, 72.4, 72.1, 70.8, 70.3, 69.4, 68.3, 67.7, 66.8, 63.4, 62.5, 60.6, 60.3, 59.3, 39.8, 37.3, 28.8. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{32}H_{46}N_7O_{19}$ 832.2854; found 832.2879.

Neu5,7diN$_3$α2−3LacNAcβProNHCbz (**35**). 26.5 mg, 70% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.46−7.40 (m, 5H), 5.11 (s, 2H), 4.54 (d, J = 7.9 Hz, 1H), 4.46 (d, J = 8.3 Hz, 1H), 4.11−4.02 (m, 2H), 4.00−3.88 (m, 4H), 3.84 (dd, J = 12.4, 5.1 Hz, 1H), 3.80−3.75 (m, 3H), 3.73−3.64 (m, 7H), 3.62−3.51 (m, 4H), 3.24−3.13 (m, 2H), 2.74 (dd, J = 12.6, 4.7 Hz, 1H), 2.02 (s, 3H), 1.91 (t, J = 12.3 Hz, 1H), 1.74 (p, J = 6.5 Hz, 2H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 173.4, 158.3, 136.6, 128.8, 128.3, 127.6, 102.6, 101.1, 100.7, 78.4, 75.7, 75.1, 74.7, 72.4, 70.4, 69.5, 69.3, 67.9, 67.6, 66.7, 62.5, 61.0, 60.4, 60.1, 59.4, 55.0, 38.8, 37.2, 28.8, 22.1. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3144.

Neu5,7diN$_3$α2−6LacNAcβProNHCbz (**37**). 27 mg, 72% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.51−7.39 (m, 5H), 5.20−5.09 (m, 2H), 4.53 (d, J = 8.1 Hz, 1H), 4.43 (d, J = 8.0 Hz, 1H), 4.10 (ddd, J = 9.5, 5.5, 2.6 Hz, 1H), 4.01−3.89 (m, 5H), 3.84−3.51 (m, 15H), 3.44 (t, J = 9.9 Hz, 1H), 3.25−3.12 (m, 1H), 2.69 (dd, J = 12.6, 4.8 Hz, 1H), 2.04 (s, 3H), 1.86−1.72 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.2, 173.1, 158.3, 136.6, 128.8, 128.4, 127.6, 103.5, 100.8, 100.4, 80.8, 74.5, 73.6, 72.5, 72.2, 70.8, 70.2, 69.2, 68.4, 67.6, 66.8, 63.7, 63.4, 62.5, 60.4, 60.3, 59.3, 55.0, 39.9, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3146.

Neu5,7diN$_3$α2−3Galβ1−3GalNAcβProNHCbz (**39**). 24 mg, 99% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.48−7.38 (m, 5H), 5.14−5.07 (m, 2H), 4.49 (d, J = 7.8 Hz, 1H), 4.44 (d, J = 8.5 Hz, 1H), 4.16 (d, J = 3.2 Hz, 1H), 4.09−4.04 (m, 1H), 4.02−3.96 (m, 2H), 3.94−3.88 (m, 3H), 3.83−3.70 (m, 6H), 3.70−3.60 (m, 6H), 3.58 (t, J = 9.7 Hz, 1H), 3.52 (dd, J = 9.8, 7.8 Hz, 1H), 3.23−3.12 (m, 2H), 2.73 (d, J = 12.7, 4.7 Hz, 1H), 1.99 (s, 3H), 1.88 (t, J = 12.3 Hz, 1H), 1.80−1.69 (m, 2H). $^{13}C\{^1H\}$ NMR (200 MHz, D_2O) δ 174.7, 173.4, 158.3, 136.6, 128.8, 128.3, 127.6, 104.6, 101.3, 100.4, 80.3, 75.8, 74.72, 74.7, 72.2, 70.4, 69.5, 68.9, 67.8, 67.6, 67.5, 66.8, 63.2, 62.4, 60.9, 60.3, 51.1, 39.1, 37.2, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3142.

Neu5,7diN$_3$α2−6Galβ1−3GalNAcβProNHCbz (**41**). 25 mg, 100% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.51−7.39 (m, 5H), 5.19−5.09 (m, 2H), 4.42 (dd, J = 8.2, 6.1 Hz, 2H), 4.19 (d, J = 3.3 Hz, 1H), 4.07−4.02 (m, 1H), 4.00−3.87 (m, 5H), 3.82−3.69 (m, 8H), 3.67−3.60 (m, 4H), 3.56−3.49 (m, 2H), 3.27−3.10 (m, 2H), 2.73 (dd, J = 12.6, 4.8 Hz, 1H), 2.00 (s, 3H), 1.80−1.71 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.7, 173.0, 158.3, 136.6, 128.8, 128.3, 127.6, 104.9, 101.3, 100.8, 80.0, 74.9, 73.1, 72.5, 72.1, 70.54, 70.48, 69.3, 68.6, 67.9, 67.6, 66.8, 63.6, 63.4, 62.5, 60.9, 60.7, 51.1, 40.0, 37.2, 28.8, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3128.

Neu5,7diN$_3$α2−3Galβ1−3GalNAcαProNHCbz (**43**). 47.6 mg, 95% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.50−7.39 (m, 5H), 5.14 (s, 2H), 4.85 (d, J = 3.8 Hz, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.30 (dd, J = 11.1, 3.7 Hz, 1H), 4.22 (s, 1H), 4.10−4.03 (m, 1H), 4.02−3.89 (m, 5H), 3.84−3.62 (m, 9H), 3.62−3.43 (m, 4H), 3.32−3.21 (m, 2H), 2.73 (ddd, J = 13.3, 8.6, 4.7 Hz, 1H), 2.01 (s, 3H), 1.93−1.78 (m, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 174.5, 173.4, 158.4, 135.7, 128.8, 128.3, 127.5, 104.5, 100.4, 97.1, 77.9, 75.8, 74.7, 72.3, 70.5, 70.4, 69.5, 69.0, 68.5, 67.7, 66.7, 65.1, 62.4, 61.2, 60.9, 60.3, 59.3, 48.5, 39.1, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3149.

Neu5,7diN$_3$α2−6Galβ1−3GalNAcαProNHCbz (**45**). 34.1 mg, 68% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.55−7.40 (m, 5H), 5.14 (s, 2H), 4.83 (d, J = 3.7 Hz, 1H), 4.41 (d, J = 7.8 Hz, 1H), 4.31 (dd, J = 10.9, 3.7 Hz, 1H), 4.18 (s, 1H), 4.09−3.97 (m, 2H), 3.95−3.85 (m, 4H), 3.81−3.72 (m, 6H), 3.70−3.57 (m, 4H), 3.55−3.45 (m, 3H), 3.34−3.22 (m, 2H), 2.74 (dd, J = 12.6, 4.5 Hz, 1H), 2.01 (d, J = 4.2 Hz, 3H), 1.89−1.76 (m, 2H), 1.73 (t, J = 12.3 Hz, 1H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 173.0, 158.4, 136.6, 128.8, 128.3, 127.6, 104.5, 100.7, 97.1, 77.2, 73.2, 72.3, 72.1, 70.7, 70.5, 70.4, 69.3, 68.8, 68.4, 66.8, 65.0, 63.5, 62.5, 61.5, 60.6, 59.4, 48.6, 40.0, 37.6, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3150.

Neu5,7diN$_3$α2−3Galβ1−3GlcNAcβProNHCbz (**47**). 66.1 mg, 87% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.49−7.39 (m, 5H), 5.12 (s, 2H), 4.50 (dd, J = 14.0, 8.2 Hz, 2H), 4.08−4.00 (m, 2H), 3.96−3.89 (m, 4H), 3.79−3.69 (m, 7H), 3.70−3.65 (m, 3H), 3.64−3.56 (m, 2H), 3.53 (dd, J = 9.5, 7.7 Hz, 2H), 3.49−3.43 (m,

https://doi.org/10.1021/acs.joc.1c01091
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1H), 3.24−3.13 (m, 2H), 2.76 (dd, J = 12.7, 4.8 Hz, 1H), 2.02 (s, 3H), 1.89 (t, J = 12.3 Hz, 1H), 1.76 (p, J = 6.6 Hz, 2H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 173.4, 158.3, 136.6, 128.8, 128.3, 127.6, 103.5, 100.8, 100.3, 82.9, 75.8, 75.3, 75.1, 72.2, 70.4, 69.5, 69.0, 68.8, 67.7, 67.5, 66.8, 63.2, 62.4, 60.9, 60.7, 60.4, 59.3, 54.3, 39.2, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3150.

Neu5,7diN$_3$α2−6Galβ1−3GlcNAcβProNHCbz (**49**). 60.7 mg, 100% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.51−7.39 (m, 5H), 5.12 (s, 2H), 4.51 (d, J = 8.5 Hz, 1H), 4.37 (d, J = 7.8 Hz, 1H), 4.07 (ddd, J = 9.4, 5.5, 2.6 Hz, 1H), 4.00−3.89 (m, 5H), 3.82−3.76 (m, 5H), 3.74−3.66 (m, 3H), 3.65−3.48 (m, 7H), 3.24−3.12 (m, 2H), 2.72 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.83−1.71 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.6, 173.1, 158.3, 136.6, 128.8, 128.4, 127.6, 104.0, 100.9, 100.5, 84.4, 75.3, 73.5, 72.5, 72.1, 70.6, 70.4, 69.3, 69.1, 68.4, 67.6, 66.8, 63.6, 62.5, 61.4, 61.0, 60.6, 59.3, 54.2, 39.9, 37.2, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3150.

Neu5,7diN$_3$α2−3Galβ1−3GlcNAcαProNHCbz (**51**). 46.6 mg, 93% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.48−7.38 (m, 5H), 5.13 (s, 2H), 4.53−4.45 (m, 1H), 4.06 (ddt, J = 9.2, 5.6, 3.1 Hz, 2H), 4.01 (dd, J = 9.8, 3.1 Hz, 1H), 3.95−3.87 (m, 3H), 3.81−3.65 (m, 11H), 3.64−3.47 (m, 5H), 3.33−3.20 (m, 2H), 2.79−2.69 (m, 1H), 2.01 (s, 3H), 1.89 (t, J = 12.3 Hz, 1H), 1.85−1.74 (m, 2H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.4, 173.2, 158.4, 136.7, 128.8, 128.3, 127.5, 118.9, 103.4, 100.5, 96.9, 81.1, 75.9, 75.0, 72.3, 71.5, 70.4, 69.45, 69.37, 69.1, 68.7, 67.5, 66.7, 63.2, 62.4, 60.9, 60.5, 60.4, 59.3, 52.3, 39.1, 37.4, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3142.

Neu5,7diN$_3$α2−6Galβ1−3GlcNAcαProNHCbz (**53**). 50 mg, 100% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.52−7.34 (m, 5H), 5.14 (s, 2H), 4.34 (d, J = 7.8 Hz, 1H), 4.12−4.02 (m, 2H), 3.97−3.88 (m, 4H), 3.87−3.70 (m, 9H), 3.68 (dd, J = 9.4, 2.3 Hz, 1H), 3.62−3.54 (m, 3H), 3.53−3.45 (m, 3H), 3.35−3.17 (m, 2H), 2.72 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.86−1.74 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.4, 173.1, 158.4, 136.6, 128.8, 128.3, 127.5, 103.8, 100.6, 96.8, 82.3, 73.5, 72.5, 72.1, 71.4, 70.6, 70.3, 69.3, 68.9, 68.4, 66.7, 65.0, 63.6, 62.5, 61.4, 60.6, 59.3, 52.2, 39.9, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{49}N_8O_{19}$ 873.3119; found 873.3133.

Neu5,7diN$_3$α2−6GalNAcαProNHCbz (**55**). 112 mg, 65% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.46−7.38 (m, 5H), 5.17−5.06 (m, 2H), 4.12 (dd, J = 11.1, 3.6 Hz, 1H), 4.02 (ddd, J = 8.6, 5.4, 2.6 Hz, 1H), 3.99−3.87 (m, 3H), 3.87−3.80 (m, 2H), 3.79−3.58 (m, 7H), 3.50 (t, J = 9.8 Hz, 1H), 3.47−3.37 (m, 1H), 3.30−3.17 (m, 2H), 2.74 (dd, J = 12.7, 4.8 Hz, 1H), 2.02 (s, 3H), 1.82−1.71 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 172.8, 158.3, 136.6, 128.8, 128.3, 127.6, 101.0, 97.1, 72.1, 70.4, 69.4, 69.3, 68.2, 67.6, 66.7, 65.4, 63.5, 62.5, 60.6, 59.3, 49.9, 40.0, 37.5, 28.5, 21.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{28}H_{39}N_8O_{14}$ 711.2591; found 711.2605.

General Procedures for One-Pot Three-Enzyme (OP3E) Preparative-Scale Synthesis of Neu5,7,9triN$_3$α2−3/6-Linked Sialosides (32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56). An acceptor selected from Lacβ ProNHCbz **24**, LacNAcβProNHCbz **25**, Galβ1−3GalNAcβProNHCbz **26**, Galβ1−3GalNAcαProNHCbz **27**, Galβ1−3GlcNAcβProNHCbz **28**, Galβ1−3GlcNAcαProNHCbz **29**, GalNAcαProNHCbz **30** (0.10−0.20 mmol), Man2,4,6triN$_3$ (**11**, 50−70 mg, 0.2−0.30 mmol), sodium pyruvate (1.0−1.5 mmol), and CTP (0.3−0.45 mmol) was dissolved in water in a 50 mL centrifuge tube containing Tris−HCl buffer (100 mM, pH 8.5) and $MgCl_2$ (20 mM). After adding sialic acid aldolase (0.5−3 mg), NmCSS (0.5 mg), and a sialyltransferase PmST1 (1−3 mg) or Pd2,6ST (1−4 mg), water was added to bring the final concentration of Man2,4,6triN$_3$ (**11**) to 10 mM. The reaction mixture was incubated at 30 °C for 24−36 h. The reaction progress was monitored by TLC (ethyl acetate:methanol:-water = 6:1:1 by volume) and mass spectrometry. The reaction mixture was diluted with the same volume of ethanol and incubated at 4 °C for 30 min. The mixture was then centrifuged and concentrated, which was purified by automated flash chromatography using a C18 column (CH_3CN in H_2O gradient was used as running solvents) to produce sialosides.

Neu5,7,9triN$_3$α2−3LacβProNHCbz (**32**). 127 mg, 92% yield, white amorphous solid. ^1H NMR (800 MHz, D_2O) δ 7.46−7.38 (m, 5H), 5.10 (s, 2H), 4.49 (d, J = 7.8 Hz, 1H), 4.42 (d, J = 8.0 Hz, 1H), 4.19 (ddd, J = 9.0, 6.2, 2.7 Hz, 1H), 4.02 (dd, J = 9.8, 3.2 Hz, 1H), 3.98−3.88 (m, 3H), 3.83−3.46 (m, 15H), 3.31−3.15 (m, 3H), 2.73 (dd, J = 12.7, 4.7 Hz, 1H), 1.89 (t, J = 12.3 Hz, 1H), 1.80 (p, J = 6.6 Hz, 2H). $^{13}C\{^1H\}$ NMR (200 MHz, D_2O) δ 173.2, 158.8, 136.6, 128.8, 128.3, 127.6, 102.6, 102.6, 102.1, 78.4, 75.8, 75.1, 74.7, 74.3, 72.8, 72.2, 69.5, 69.3, 69.3, 67.8, 67.7, 66.8, 63.1, 61.3, 60.9, 60.1, 53.1, 39.0, 37.3, 28.8. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{32}H_{45}N_{10}O_{18}$ 857.2919; found 857.2948.

Neu5,7,9triN$_3$α2−6LacβProNHCbz (**34**). 132 mg, 100% yield, white amorphous solid. ^1H NMR (800 MHz, D_2O) δ 7.41 (dd, J = 25.3, 7.7 Hz, 5H), 5.08 (s, 2H), 4.40 (t, J = 8.9 Hz, 2H), 4.17 (ddd, J = 8.7, 5.8, 2.6 Hz, 1H), 3.96−3.90 (m, 4H), 3.80−3.71 (m, 6H), 3.71−3.63 (m, 4H), 3.61−3.51 (m, 5H), 3.31 (t, J = 8.7 Hz, 1H), 3.23 (dt, J = 12.5, 6.7 Hz, 2H), 2.71 (dd, J = 12.6, 4.8 Hz, 1H), 1.85−1.74 (m, 3H). $^{13}C\{^1H\}$ NMR (200 MHz, D_2O) δ 173.1, 158.3, 136.5, 128.8, 128.3, 127.7, 103.3, 102.0, 100.6, 80.0, 74.6, 74.5, 73.5, 72.6, 72.5, 72.0, 70.8, 69.5, 69.1, 68.3, 67.7, 66.8, 63.4, 63.3, 61.6, 60.4, 53.2, 39.9, 37.4, 28.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{32}H_{45}N_{10}O_{18}$ 857.2919; found 857.2939.

Neu5,7,9triN$_3$α2−3LacNAcβProNHCbz (**36**). 12.9 mg, 66% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.52−7.37 (m, 5H), 5.11 (s, 2H), 4.53 (d, J = 7.8 Hz, 1H), 4.47 (d, J = 8.3 Hz, 1H), 4.21 (ddd, J = 9.1, 6.3, 2.7 Hz, 1H), 4.04 (dd, J = 9.9, 3.2 Hz, 1H), 3.99 (dd, J = 12.4, 2.4 Hz, 1H), 3.95−3.87 (m, 2H), 3.84 (dd, J = 12.4, 5.1 Hz, 1H), 3.81−3.75 (m, 3H), 3.73−3.65 (m, 7H), 3.65−3.52 (m, 5H), 3.23−3.08 (m, 2H), 2.74 (dd, J = 12.6, 4.7 Hz, 1H), 2.02 (s, 3H), 1.90 (t, J = 12.3 Hz, 1H), 1.75 (t, J = 6.5 Hz, 2H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 173.3, 158.3, 136.6, 128.8, 128.4, 127.6, 102.6, 101.1, 100.7, 78.5, 75.8, 75.1, 74.7, 72.4, 72.2, 71.0, 69.5, 69.4, 69.3, 67.8, 66.8, 61.3, 60.9, 60.1, 59.3, 55.0, 53.2, 39.0, 37.3, 28.8, 22.1. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3200.

Neu5,7,9triN$_3$α2−6LacNAcβProNHCbz (**38**). 25.4 mg, 65% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.50−7.39 (m, 5H), 5.17−5.08 (m, 2H), 4.52 (d, J = 8.2 Hz, 1H), 4.42 (d, J = 7.9 Hz, 1H), 4.23 (ddd, J = 8.8, 5.9, 2.7 Hz, 1H), 4.01−3.88 (m, 3H), 3.86−3.76 (m, 4H), 3.73−3.51 (m, 12H), 3.44 (t, J = 12 Hz, 1H), 3.25−3.12 (m, 2H), 2.69 (dd, J = 12.6, 4.7 Hz, 1H), 2.06 (s, 3H), 1.84−1.71 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.2, 173.0, 158.3, 136.6, 128.8, 128.4, 127.6, 103.5, 102.9, 100.8, 80.8, 74.5, 73.6, 72.51, 72.47, 72.1, 70.8, 69.2, 68.9, 68.3, 67.6, 66.8, 63.6, 61.2, 60.4, 59.3, 55.0, 53.2, 39.9, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3202.

Neu5,7,9triN$_3$α2−3Galβ1−3GalNAcβProNHCbz (**40**). 7.3 mg, 67% yield, white amorphous solid. ^1H NMR (800 MHz, D_2O) δ 7.47−7.38 (m, 5H), 5.15−5.09 (m, 2H), 4.48 (d, J = 7.8 Hz, 1H), 4.44 (d, J = 8.6 Hz, 1H), 4.19−4.15 (m, 2H), 4.01−3.96 (m, 2H), 3.94−3.90 (m, 1H), 3.89 (d, J = 3.2 Hz, 1H), 3.82−3.73 (m, 5H), 3.72−3.55 (m, 9H), 3.52 (dd, J = 9.8, 7.8 Hz, 1H), 3.23−3.11 (m, 2H), 2.73 (dd, J = 12.7, 4.7 Hz, 1H), 2.00 (s, 3H), 1.87 (t, J = 12.3 Hz, 1H), 1.77−1.72 (m, 2H). $^{13}C\{^1H\}$ NMR (200 MHz, D_2O) δ 174.7, 173.3, 158.3, 136.6, 128.8, 128.3, 127.6, 104.5, 101.3, 100.3, 80.3, 75.8, 74.7, 72.1, 69.5, 69.3, 68.9, 67.8, 67.5, 66.7, 61.2, 60.92, 60.9, 59.3, 53.1, 51.1, 39.2, 37.2, 29.6, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3206.

Neu5,7,9triN$_3$α2−6Galβ1−3GalNAcβProNHCbz (**42**). 17.2 mg, 69% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.55−7.36 (m, 5H), 5.25−5.05 (m, 2H), 4.43 (ddd, J = 15.5, 11.6, 8.0 Hz, 2H), 4.24−4.12 (m, 2H), 3.93−3.69 (m, 10H), 3.66−3.50 (m, 9H), 3.43−3.29 (m, 1H), 2.90 (s, 1H), 2.77−2.70 (m, 1H), 2.01 (s, 3H), 1.84−1.71 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 173.02, 172.97, 171.0, 158.2, 136.5, 128.8, 128.4, 127.6, 104.9, 104.4, 100.8, 75.0, 72.5, 72.31, 72.28, 72.1, 70.5, 70.4, 69.3, 69.2, 69.1, 68.6, 63.4, 63.2, 61.3, 60.9, 53.2, 51.2, 45.7, 40.0, 28.8, 22.2. HRMS (ESI-

https://doi.org/10.1021/acs.joc.1c01091
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Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3212.

Neu5,7,9triN$_3$α2−3Galβ1−3GalNAcαProNHCbz (**44**). 29.2 mg, 65% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.44 (q, J = 7.3 Hz, 5H), 5.14 (s, 2H), 4.85 (d, J = 3.7 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.29 (dd, J = 11.1, 3.7 Hz, 1H), 4.24−4.13 (m, 2H), 4.04−3.86 (m, 4H), 3.81−3.46 (m, 14H), 3.35−3.19 (m, 2H), 2.74 (dd, J = 12.7, 4.7 Hz, 1H), 2.01 (s, 3H), 1.92−1.76 (m, 3H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.5, 173.4, 158.4, 136.5, 128.8, 128.3, 127.4, 104.5, 100.3, 97.1, 77.9, 77.3, 75.9, 74.7, 72.1, 70.6, 69.5, 69.2, 69.0, 68.5, 67.5, 66.7, 65.1, 63.1, 61.2, 60.9, 53.1, 48.6, 39.3, 37.4, 28.4, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3201.

Neu5,7,9triN$_3$α2−6Galβ1−3GalNAcαProNHCbz (**46**). 40.7 mg, 79% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.52−7.31 (m, 5H), 5.13 (s, 2H), 4.82−4.81 (m, 1H), 4.39 (d, J = 7.8 Hz, 1H), 4.29 (dd, J = 11.1, 3.7 Hz, 1H), 4.20−4.07 (m, 2H), 3.98 (dd, J = 11.0, 3.1 Hz, 1H), 3.94−3.82 (m, 3H), 3.79 (dd, J = 10.3, 2.3 Hz, 1H), 3.78−3.65 (m, 6H), 3.63 (t, J = 6.2 Hz, 1H), 3.60−3.52 (m, 3H), 3.52−3.43 (m, 3H), 3.26 (dq, J = 13.5, 6.9 Hz, 2H), 2.71 (dd, J = 12.6, 4.8 Hz, 1H), 2.00 (s, 3H), 1.80 (qt, J = 13.0, 7.6 Hz, 2H), 1.71 (t, J = 12.3 Hz, 1H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 174.5, 173.0, 158.4, 136.6, 128.8, 128.3, 127.6, 104.5, 100.7, 97.1, 77.2, 73.1, 72.3, 72.0, 70.7, 70.5, 69.3, 69.2, 68.8, 68.3, 66.7, 65.0, 63.4, 61.5, 61.5, 59.3, 53.1, 48.6, 40.0, 37.6, 28.4, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3216.

Neu5,7,9triN$_3$α2−3Galβ1−3GlcNAcβProNHCbz (**48**). 50 mg, 64% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.71−7.20 (m, 5H), 5.29−5.04 (m, 2H), 4.49 (d, J = 8.5 Hz, 1H), 4.46 (d, J = 7.8 Hz, 1H), 4.15 (ddd, J = 9.0, 6.4, 2.8 Hz, 1H), 4.00 (dd, J = 9.8, 3.2 Hz, 1H), 3.96−3.87 (m, 3H), 3.84−3.77 (m, 1H), 3.75−3.55 (m, 12H), 3.54−3.48 (m, 2H), 3.48−3.40 (m, 1H), 3.24−3.08 (m, 2H), 2.74 (dd, J = 12.6, 4.7 Hz, 1H), 2.01 (s, 3H), 1.86 (t, J = 12.3 Hz, 1H), 1.74 (p, J = 6.5 Hz, 2H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 174.5, 173.3, 158.3, 136.6, 128.8, 128.3, 127.6, 103.4, 100.8, 100.3, 82.9, 75.9, 75.3, 75.0, 72.1, 69.5, 69.3, 69.0, 68.8, 67.6, 67.4, 66.7, 63.1, 61.3, 60.9, 60.7, 54.3, 53.1, 39.3, 37.3, 28.7, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3201.

Neu5,7,9triN$_3$α2−6Galβ1−3GlcNAcβProNHCbz (**50**). 57.1 mg, 91% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.50−7.41 (m, 5H), 5.16−5.08 (m, 2H), 4.50 (d, J = 8.5 Hz, 1H), 4.37 (d, J = 7.8 Hz, 1H), 4.20 (ddd, J = 9.0, 6.0, 2.7 Hz, 1H), 4.01−3.88 (m, 4H), 3.84−3.74 (m, 6H), 3.71−3.66 (m, 2H), 3.65−3.56 (m, 4H), 3.55−3.45 (m, 4H), 3.26−3.05 (m, 2H), 2.71 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.82−1.70 (m, 3H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.6, 173.0, 158.3, 136.6, 128.8, 128.4, 127.6, 103.9, 100.9, 100.6, 84.4, 75.3, 73.5, 72.5, 72.0, 70.6, 69.3, 69.1, 69.1, 68.4, 67.6, 66.8, 63.5, 63.5, 61.5, 61.0, 59.3, 54.2, 53.2, 39.9, 37.2, 28.8, 22.2. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3213.

Neu5,7,9triN$_3$α2−3Galβ1−3GlcNAcαProNHCbz (**52**). 44.8 mg, 87% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.53−7.37 (m, 5H), 5.19−5.08 (m, 2H), 4.52−4.39 (m, 1H), 4.21−4.13 (m, 1H), 4.12−4.02 (m, 1H), 3.99 (dd, J = 9.8, 3.2 Hz, 1H), 3.96−3.85 (m, 2H), 3.87−3.81 (m, 1H), 3.81−3.71 (m, 5H), 3.73−3.63 (m, 4H), 3.64−3.43 (m, 7H), 3.35−3.16 (m, 2H), 2.78−2.69 (m, 1H), 2.02 (s, 3H), 1.88 (t, J = 12.3 Hz, 1H), 1.86−1.73 (m, 2H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.4, 173.3, 158.3, 136.7, 128.8, 128.3, 127.5, 103.5, 100.3, 96.9, 81.3, 75.9, 75.0, 72.5, 72.1, 71.5, 70.7, 69.5, 69.3, 69.1, 68.8, 67.4, 66.7, 63.1, 61.3, 60.9, 60.5, 53.1, 52.3, 39.3, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3209.

Neu5,7,9triN$_3$α2−6Galβ1−3GlcNAcαProNHCbz (**54**). 49.5 mg, 96% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.51−7.38 (m, 5H), 5.14 (s, 2H), 4.34 (d, J = 7.9 Hz, 1H), 4.23−4.16 (m, 1H), 4.06 (dd, J = 10.6, 3.6 Hz, 1H), 3.95−3.87 (m, 3H), 3.87−3.78 (m, 3H), 3.78−3.66 (m, 7H), 3.64−3.53 (m, 4H), 3.53−3.43 (m, 3H), 3.39−3.16 (m, 2H), 2.71 (dd, J = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.90−1.70 (m, 3H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.4, 173.0, 158.4, 136.6, 128.8, 128.3, 127.5, 103.8, 100.6, 96.8, 82.2, 73.4, 72.5, 72.0, 71.4, 70.6, 69.3, 69.1, 68.9, 68.3, 66.7, 65.0, 63.5, 63.3, 61.5, 60.7, 59.3, 53.2, 52.2, 40.0, 37.5, 28.5, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{34}H_{48}N_{11}O_{18}$ 898.3184; found 898.3209.

Neu5,7,9triN$_3$α2−6GalNAcαProNHCbz (**56**). 108 mg, 65% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.72−7.09 (m, 5H), 5.32−5.02 (m, 2H), 4.22−4.05 (m, 2H), 3.95 (s, 2H), 3.89−3.46 (m, 11H), 3.43 (dt, J = 11.3, 6.1 Hz, 1H), 3.29−3.06 (m, 2H), 2.79−2.63 (m, 1H), 2.00 (s, 3H), 1.82−1.65 (m, 3H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 174.5, 158.2, 136.6, 128.7, 128.3, 127.6, 101.3, 97.1, 72.0, 69.4, 69.2, 68.1, 67.6, 66.7, 65.4, 63.4, 63.3, 63.2, 61.5, 53.2, 53.1, 49.9, 40.0, 37.5, 28.5, 21.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{28}H_{38}N_{11}O_{13}$ 736.2656; found 736.2672.

General Procedures for Converting Azido-Containing Glycosides (31−56) to *N*-Acetyl-Containing Glycosides (57−82). To a sodium bicarbonate saturated solution in water in a round-bottom flask (100 mL), an azido-containing glycoside (30−50 mg) was added followed by the dropwise addition of 12−24 equiv of thioacetic acid under argon at room temperature and stirred at 70 °C for 20 h. After the completion of the reaction, the solvent was removed under vacuum. The mixture was passed through a Bio-Gel P-2 gel filtration (water was used as an eluent). The product-containing fractions were concentrated and further purified by silica gel chromatography using a mixed solvent (ethyl acetate:methanol:water = 10:1:0.1 by volume) as an eluent, followed by C18 purification (CH_3CN in H_2O gradient was used as running solvents) to obtain the pure product.

Neu5Ac7NAcα2−3LacβProNHCbz (**57**). 38 mg, 68% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.55−7.37 (m, 5H), 5.10 (s, 2H), 4.51 (d, J = 7.9 Hz, 1H), 4.43 (d, J = 8.0 Hz, 1H), 4.13 (ddd, J = 10.0, 3.1, 1.0 Hz, 1H), 4.00−3.87 (m, 5H), 3.82−3.67 (m, 7H), 3.66−3.61 (m, 3H), 3.61−3.54 (m, 3H), 3.53−3.49 (m, 1H), 3.29 (t, J = 8.4 Hz, 1H), 3.27−3.18 (m, 2H), 2.79 (dd, J = 12.5, 4.5 Hz, 1H), 1.98 (s, 3H), 1.93 (s, 3H), 1.80 (p, J = 6.6 Hz, 2H), 1.76 (t, J = 12.2 Hz, 1H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 174.0, 173.8, 173.8, 158.4, 136.6, 128.8, 128.3, 127.6, 102.6, 102.1, 99.6, 78.3, 75.4, 75.1, 74.7, 74.3, 72.8, 71.8, 71.6, 69.4, 68.6, 67.7, 66.9, 66.8, 62.4, 61.0, 60.1, 51.8, 49.2, 40.1, 37.3, 28.8, 22.1, 21.8. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{36}H_{54}N_3O_{21}$ 864.3255; found 864.3282.

Neu5Ac7,9diNAcα2−3LacβProNHCbz (**58**). 42 mg, 80% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.47−7.41 (m, 5H), 5.12 (s, 2H), 4.51 (d, J = 7.8 Hz, 1H), 4.45 (d, J = 8.1 Hz, 1H), 4.12 (dd, J = 9.9, 3.2 Hz, 1H), 3.99−3.95 (m, 4H), 3.87−3.53 (m, 13H), 3.48 (dd, J = 14.1, 2.4 Hz, 1H), 3.34−3.23 (m, 3H), 3.09 (dd, J = 14.1, 7.5 Hz, 1H), 2.80 (dd, J = 12.4, 4.6 Hz, 1H), 2.01 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.85−1.74 (m, 3H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.2, 174.0, 173.83, 173.79, 158.4, 136.6, 128.8, 128.3, 127.6, 102.6, 102.1, 99.6, 78.3, 75.5, 75.1, 74.7, 74.3, 72.8, 71.8, 69.7, 69.4, 68.7, 67.7, 67.0, 66.8, 61.0, 60.1, 51.9, 50.6, 41.8, 40.1, 37.3, 28.8, 22.1, 21.90, 21.88. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3554.

Neu5Ac7NAcα2−6LacβProNHCbz (**59**). 42 mg, 78% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.51−7.34 (m, 5H), 5.10 (s, 2H), 4.43 (dd, J = 19.3, 8.0 Hz, 2H), 4.04−3.87 (m, 7H), 3.84−3.72 (m, 3H), 3.71−3.52 (m, 9H), 3.49 (dd, J = 12.2, 6.7 Hz, 1H), 3.34 (t, J = 8.6 Hz, 1H), 3.27−3.20 (m, 2H), 2.73 (dd, J = 12.4, 4.6 Hz, 1H), 2.00 (s, 3H), 1.93 (s, 3H), 1.81 (p, J = 6.7 Hz, 2H), 1.75 (t, J = 12.2 Hz, 1H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 173.93, 173.86, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 103.2, 101.9, 100.4, 79.5, 74.6, 73.6, 72.8, 72.4, 71.6, 71.5, 70.7, 68.7, 68.4, 67.7, 66.8, 63.4, 62.4, 60.2, 51.9, 49.2, 40.1, 37.3, 28.8, 22.1, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{36}H_{54}N_3O_{21}$ 864.3255; found 864.3280.

Neu5Ac7,9diNAcα2−6LacβProNHCbz (**60**). 36 mg, 80% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.49−7.37 (m, 5H), 5.12 (s, 2H), 4.45 (dd, J = 15.6, 8.0 Hz, 2H), 4.04−3.90 (m, 8H), 3.86−3.54 (m, 12H), 3.45 (dd, J = 14.1, 2.5 Hz, 1H), 3.36 (t, J = 8.6 Hz, 1H), 3.09 (dd, J = 14.1, 7.6 Hz, 1H), 2.75 (dd, J = 12.4, 4.6

https://doi.org/10.1021/acs.joc.1c01091
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Hz, 1H), 2.02 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.83 (p, J = 6.5 Hz, 2H), 1.76 (t, J = 12.2 Hz, 1H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.2, 174.0, 173.8, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 103.2, 102.0, 100.4, 79.5, 74.64, 74.62, 73.6, 72.9, 72.4, 71.6, 70.7, 69.5, 68.7, 68.5, 67.7, 66.8, 63.4, 60.2, 51.9, 50.6, 41.8, 40.1, 37.3, 28.9, 22.14, 22.09, 21.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3555.

Neu5Ac7NAcα2−3LacNAcβProNHCbz (**61**). 10 mg, 67% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.44 (dq, J = 14.1, 7.3 Hz, 5H), 5.12 (s, 2H), 4.59−4.53 (m, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.19−4.11 (m, 1H), 4.03−3.96 (m, 2H), 3.91 (ddd, J = 9.5, 6.7, 2.3 Hz, 2H), 3.87−3.80 (m, 2H), 3.78−3.64 (m, 9H), 3.63−3.50 (m, 5H), 3.26−3.11 (m, 2H), 2.80 (dd, J = 12.4, 4.6 Hz, 1H), 2.03−1.93 (m, 9H), 1.80−1.73 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 174.0, 173.9, 173.8, 158.4, 136.6, 128.8, 128.3, 127.6, 102.5, 101.1, 99.5, 78.4, 75.4, 75.1, 74.7, 72.4, 71.9, 71.6, 69.4, 68.6, 67.6, 67.0, 66.8, 62.4, 61.0, 60.1, 55.0, 51.9, 49.2, 40.1, 37.2, 28.7, 22.1, 21.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3546.

Neu5Ac7,9diNAcα2−3LacNAcβProNHCbz (**62**). 8.5 mg, 67% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.56−7.34 (m, 5H), 5.14 (d, J = 20.6 Hz, 2H), 4.54 (dd, J = 7.9, 2.3 Hz, 1H), 4.50−4.45 (m, 1H), 4.12 (dd, J = 9.9, 3.1 Hz, 1H), 4.03−3.88 (m, 5H), 3.87−3.80 (m, 2H), 3.80−3.65 (m, 7H), 3.65−3.54 (m, 4H), 3.49 (dd, J = 14.0, 2.3 Hz, 1H), 3.24−3.13 (m, 2H), 3.09 (dd, J = 14.1, 7.5 Hz, 1H), 2.80 (dd, J = 12.4, 4.6 Hz, 1H), 2.05−2.02 (m, 6H), 2.01 (s, 3H), 1.96 (s, 3H), 1.87−1.72 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.2, 174.0, 173.8, 158.4, 136.8, 128.8, 128.3, 127.6, 102.5, 101.1, 100.0, 78.3, 75.5, 75.1, 74.7, 72.4, 71.8, 69.7, 69.4, 68.6, 67.6, 67.0, 66.8, 61.0, 60.1, 55.0, 51.9, 50.6, 41.8, 40.1, 37.2, 28.6, 22.12, 22.10, 21.90, 21.88. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3804.

Neu5Ac7NAcα2−6LacNAcβProNHCbz (**63**). 11.3 mg, 77% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.68−7.29 (m, 5H), 5.14 (d, J = 12.8 Hz, 2H), 4.60−4.32 (m, 2H), 4.18−3.39 (m, 21H), 3.36−2.84 (m, 2H), 2.72 (dd, J = 12.5, 4.6 Hz, 1H), 2.05 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.87−1.56 (m, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 174.5, 173.8, 173.7, 173.4, 158.6, 138.3, 128.8, 128.3, 127.6, 103.3, 100.9, 100.3, 80.0, 74.6, 73.6, 72.4, 71.7, 71.5, 70.7, 68.7, 68.3, 67.6, 66.8, 63.2, 62.5, 60.3, 55.1, 51.9, 49.2, 40.1, 36.9, 28.6, 22.2, 22.1, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3529.

Neu5Ac7,9diNAcα2−6LacNAcβProNHCbz (**64**). 19.3 mg, 85% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.44 (td, J = 7.4, 4.1 Hz, 5H), 5.14 (d, J = 12.9 Hz, 2H), 4.56−4.43 (m, 2H), 4.24−3.31 (m, 20H), 3.27−2.87 (m, 3H), 2.71 (dd, J = 12.6, 4.7 Hz, 1H), 2.19−1.91 (m, 12H), 1.77 (h, J = 11.7 Hz, 3H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 175.2, 174.2, 173.8, 173.7, 173.4, 166.2, 136.1, 128.8, 128.3, 127.6, 103.3, 101.0, 100.4, 79.9, 74.6, 73.5, 72.4, 71.9, 71.6, 70.7, 69.5, 68.7, 68.4, 67.6, 66.8, 63.3, 55.1, 51.9, 50.6, 41.8, 40.1, 36.0, 27.6, 22.1, 22.0, 21.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3805.

Neu5Ac7NAcα2−3Galβ1−3GalNAcβProNHCbz (**65**). 5.7 mg, 64% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.59−7.34 (m, 5H), 5.15 (d, J = 22.3 Hz, 2H), 4.50 (d, J = 7.8 Hz, 1H), 4.43 (dd, J = 15.4, 8.6 Hz, 1H), 4.16 (d, J = 3.5 Hz, 1H), 4.10 (dd, J = 9.9, 3.2 Hz, 1H), 4.05−3.96 (m, 2H), 3.95−3.86 (m, 3H), 3.85−3.71 (m, 6H), 3.69−3.55 (m, 6H), 3.51 (dd, J = 12.0, 6.8 Hz, 1H), 3.37−3.17 (m, 1H), 2.93 (d, J = 32.4 Hz, 2H), 2.81 (dd, J = 12.3, 4.6 Hz, 1H), 2.01−1.94 (m, 9H), 1.87−1.69 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.1, 173.6, 173.52, 173.45, 158.0, 136.3, 128.4, 127.9, 127.1, 104.1, 99.0, 96.7, 76.9, 75.2, 74.4, 71.4, 71.3, 70.2, 68.6, 68.3, 68.2, 66.5, 66.3, 64.6, 62.0, 60.9, 60.6, 51.4, 48.9, 48.3, 39.9, 37.1, 28.1, 21.72, 21.66, 21.5. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3546.

Neu5Ac7,9diNAcα2−3Galβ1−3GalNAcβProNHCbz (**66**). 4.4 mg, 65% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.44 (dtt, J = 18.5, 6.5, 3.0 Hz, 5H), 5.15 (d, J = 22.8 Hz, 2H), 4.49 (d, J = 8.0 Hz, 1H), 4.43 (dd, J = 14.7, 8.6 Hz, 1H), 4.17 (d, J = 3.1 Hz, 1H), 4.09−3.91 (m, 6H), 3.84−3.72 (m, 7H), 3.67−3.57 (m, 5H), 3.51−3.45 (m, 1H), 3.36−3.13 (m, 2H), 3.09−2.98 (m, 1H), 2.80 (dd, J = 12.4, 4.5 Hz, 1H), 2.02−1.99 (m, 6H), 1.95 (s, 6H), 1.84−1.80 (m, 1H), 1.78−1.72 (m, 2H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.1, 173.80, 173.76, 173.61, 173.58, 158.1, 136.4, 128.5, 128.1, 127.3, 103.2, 100.6, 99.2, 82.0, 75.4, 75.2, 74.8, 71.5, 69.5, 68.8, 68.42, 68.36, 67.5, 66.6, 66.5, 60.8, 60.5, 54.3, 51.6, 50.2, 41.4, 40.0, 37.1, 28.5, 21.91, 21.88, 21.7, 21.6. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3803.

Neu5Ac7NAcα2−6Galβ1−3GalNAcβProNHCbz (**67**). 10.4 mg, 67% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.61−7.33 (m, 5H), 5.15 (d, J = 20.8 Hz, 2H), 4.50−4.36 (m, 2H), 4.20 (dd, J = 19.0, 3.2 Hz, 1H), 4.02−3.83 (m, 8H), 3.78−3.59 (m, 9H), 3.56−3.48 (m, 2H), 3.43−3.10 (m, 1H), 3.00−2.87 (m, 2H), 2.84−2.70 (m, 1H), 2.01 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.89−1.63 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.0, 173.8, 173.7, 173.3, 157.9, 136.7, 128.8, 128.4, 127.6, 104.9, 104.4, 100.4, 75.0, 74.7, 73.2, 72.5, 72.4, 71.70, 71.67, 71.5, 71.4, 70.6, 70.4, 68.6, 68.5, 67.4, 66.8, 62.5, 61.2, 61.0, 52.0, 51.2, 49.23, 49.16, 45.7, 40.3, 22.2, 22.1, 21.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3530.

Neu5Ac7,9diNAcα2−6Galβ1−3GalNAcβProNHCbz (**68**). 10.4 mg, 70% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.55−7.34 (m, 5H), 5.23−5.09 (m, 2H), 4.43 (ddd, J = 14.7, 9.2, 5.6 Hz, 2H), 4.23−4.11 (m, 1H), 4.01−3.89 (m, 6H), 3.84−3.53 (m, 11H), 3.47−3.33 (m, 2H), 3.29−3.02 (m, 2H), 2.98−2.88 (m, 1H), 2.80−2.70 (m, 1H), 2.04−1.98 (m, 9H), 1.96 (s, 3H), 1.84−1.62 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.2, 173.99, 173.97, 173.7, 173.3, 158.3, 136.6, 128.8, 128.3, 127.6, 104.9, 100.5, 100.4, 75.0, 73.21, 73.16, 72.5, 71.61, 71.58, 70.6, 70.4, 69.6, 69.4, 68.6, 67.7, 67.4, 66.8, 61.0, 52.0, 51.2, 50.6, 45.7, 41.9, 40.3, 34.3, 28.9, 22.2, 22.1, 21.90, 21.87. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3805.

Neu5Ac7NAcα2−3Galβ1−3GalNAcαProNHCbz (**69**). 20 mg, 77% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.50−7.35 (m, 5H), 5.17−5.09 (m, 2H), 4.84 (d, J = 3.8 Hz, 1H), 4.56−4.48 (m, 1H), 4.37−4.30 (m, 1H), 4.25−4.19 (m, 1H), 4.11−4.06 (m, 1H), 4.00−3.92 (m, 3H), 3.88 (ddd, J = 9.5, 6.8, 2.4 Hz, 1H), 3.82 (dd, J = 10.5, 3.1 Hz, 1H), 3.80−3.67 (m, 7H), 3.63−3.54 (m, 4H), 3.53−3.46 (m, 2H), 3.32−3.20 (m, 2H), 2.81 (dd, J = 12.4, 4.6 Hz, 1H), 1.99 (s, 6H), 1.95 (s, 3H), 1.85−1.79 (m, 2H), 1.76 (t, J = 12.1 Hz, 1H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.5, 174.0, 173.9, 173.8, 158.4, 136.7, 128.8, 128.3, 127.5, 104.5, 99.4, 97.1, 77.2, 75.6, 74.8, 71.73, 71.70, 70.6, 69.0, 68.7, 68.6, 66.9, 66.7, 65.0, 62.3, 61.2, 60.9, 51.8, 49.2, 48.7, 40.2, 37.5, 28.4, 22.1, 22.0, 21.8. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3536.

Neu5Ac7,9diNAcα2−3Galβ1−3GalNAcαProNHCbz (**70**). 10.2 mg, 65% yield, white amorphous solid. ^1H NMR (600 MHz, D_2O) δ 7.53−7.31 (m, 5H), 5.17−5.09 (m, 2H), 4.85 (d, J = 3.8 Hz, 1H), 4.52−4.42 (m, 1H), 4.32 (dd, J = 11.1, 3.8 Hz, 1H), 4.26−4.20 (m, 1H), 4.10−4.00 (m, 2H), 3.99−3.89 (m, 4H), 3.83 (dd, J = 10.4, 3.1 Hz, 1H), 3.78−3.68 (m, 6H), 3.63−3.53 (m, 3H), 3.52−3.44 (m, 2H), 3.34−3.21 (m, 2H), 3.04 (dd, J = 14.0, 7.9 Hz, 1H), 2.80 (dd, J = 12.4, 4.6 Hz, 1H), 2.02−1.94 (m, 12H), 1.87−1.71 (m, 3H). $^{13}C\{^1H\}$ NMR (150 MHz, D_2O) δ 174.4, 174.04, 173.99, 173.8, 158.4, 136.7, 128.8, 128.3, 127.4, 104.4, 99.5, 97.0, 77.4, 75.6, 74.7, 71.7, 70.6, 69.7, 69.1, 68.6, 66.8, 66.7, 65.0, 61.2, 60.9, 51.8, 50.5, 48.6, 41.7, 40.2, 37.5, 28.4, 22.1, 22.0, 21.9. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3804.

Neu5Ac7NAcα2−6Galβ1−3GalNAcαProNHCbz (**71**). 13.5 mg, 77% yield, white amorphous solid. ^1H NMR (400 MHz, D_2O) δ 7.61−7.29 (m, 5H), 5.28−5.04 (m, 2H), 4.84 (d, J = 3.7 Hz, 1H), 4.43 (d, J = 7.8 Hz, 1H), 4.32 (dd, J = 11.0, 3.8 Hz, 1H), 4.24 (s, 1H), 4.11−3.40 (m, 19H), 3.38−3.19 (m, 2H), 2.78 (dd, J = 12.5, 4.6 Hz, 1H), 2.07−1.91 (m, 9H), 1.89−1.75 (m, 2H), 1.65 (t, J = 12.1 Hz, 1H). $^{13}C\{^1H\}$ NMR (100 MHz, D_2O) δ 174.3, 173.9, 173.8, 173.3, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.4, 97.0, 77.7, 73.2, 72.4, 71.7, 71.4, 70.4, 68.6, 68.5, 68.3, 66.7, 65.1, 63.7, 62.4, 61.6, 52.0, 49.1, 48.7, 40.4, 37.6, 28.5, 22.1, 22.0. HRMS (ESI-Orbitrap) m/z: $[M - H]^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3533.

https://doi.org/10.1021/acs.joc.1c01091
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*Neu5Ac7,9diNAcα2−6Galβ1−3GalNAcαProNHCbz (**72**).* 14.6 mg, 72% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.35 (q, J = 7.5 Hz, 5H), 5.05 (s, 2H), 4.76 (d, J = 3.7 Hz, 1H), 4.34 (d, J = 7.7 Hz, 1H), 4.23 (dd, J = 11.1, 3.8 Hz, 1H), 4.15 (s, 1H), 3.98−3.29 (m, 18H), 3.26−3.11 (m, 2H), 3.00 (dd, J = 14.1, 7.3 Hz, 1H), 2.69 (dd, J = 12.4, 4.6 Hz, 1H), 1.98−1.81 (m, 12H), 1.79−1.65 (m, 2H), 1.56 (t, J = 12.1 Hz, 1H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.5, 174.2, 173.9, 173.7, 173.3, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.4, 97.2, 77.7, 73.2, 72.4, 71.6, 70.4, 69.4, 68.6, 68.5, 68.3, 66.7, 64.7, 63.7, 61.6, 52.0, 50.5, 48.7, 41.8, 40.4, 22.1, 22.00, 21.96, 21.9. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3793.

*Neu5Ac7NAcα2−3Galβ1−3GlcNAcβProNHCbz (**73**).* 24.2 mg, 73% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.43 (h, J = 7.3 Hz, 5H), 5.12 (s, 2H), 4.53−4.45 (m, 2H), 4.15−4.07 (m, 1H), 3.98−3.44 (m, 20H), 3.25−3.11 (m, 2H), 2.81 (dd, J = 12.3, 4.5 Hz, 1H), 2.04−1.92 (m, 9H), 1.80−1.72 (m, 3H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.5, 174.0, 173.9, 173.8, 158.3, 136.6, 128.8, 128.3, 127.5, 103.6, 100.8, 99.3, 82.4, 75.6, 75.4, 75.1, 71.7, 71.7, 68.9, 68.62, 68.57, 67.7, 66.8, 66.7, 62.3, 61.1, 60.7, 54.5, 51.7, 49.2, 40.2, 37.3, 28.7, 22.2, 22.1, 21.8. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3534.

*Neu5Ac7,9diNAcα2−3Galβ1−3GlcNAcβProNHCbz (**74**).* 11.6 mg, 66% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.55−7.35 (m, 5H), 5.12 (s, 2H), 4.48 (dd, J = 11.2, 8.1 Hz, 2H), 4.26−3.34 (m, 20H), 3.28−3.10 (m, 2H), 3.02 (dd, J = 14.2, 7.7 Hz, 1H), 2.80 (dd, J = 12.5, 4.6 Hz, 1H), 2.07−1.88 (m, 12H), 1.76 (d, J = 10.2 Hz, 3H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.3, 174.03, 173.99, 173.84, 173.81, 158.3, 136.6, 128.8, 128.3, 127.5, 103.4, 100.8, 99.5, 82.2, 75.6, 75.4, 75.1, 71.7, 69.7, 69.0, 68.7, 68.6, 67.7, 66.8, 66.7, 61.0, 60.7, 54.5, 51.8, 50.5, 41.7, 40.2, 37.3, 28.7, 22.14, 22.11, 21.88, 21.86. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3807.

*Neu5Ac7NAcα2−6Galβ1−3GlcNAcβProNHCbz (**75**).* 25.4 mg, 75% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.84−7.09 (m, 5H), 5.13 (d, J = 10.4 Hz, 2H), 4.52 (d, J = 8.5 Hz, 1H), 4.39 (d, J = 7.8 Hz, 1H), 4.22−3.35 (m, 21H), 3.28−3.09 (m, 2H), 2.74 (dd, J = 12.4, 4.7 Hz, 1H), 2.10−1.88 (m, 9H), 1.76 (tq, J = 6.7, 3.1 Hz, 3H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.7, 173.9, 173.7, 173.5, 158.3, 136.6, 128.8, 128.4, 127.6, 104.0, 100.9, 100.1, 84.4, 75.6, 73.5, 72.5, 71.6, 71.5, 70.5, 69.0, 68.6, 68.3, 67.6, 66.8, 63.2, 62.4, 60.9, 54.2, 51.9, 49.2, 40.1, 37.2, 28.7, 22.15, 22.13, 22.10. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3531.

*Neu5Ac7,9diNAcα2−6Galβ1−3GlcNAcβProNHCbz (**76**).* 23.7 mg, 70% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.50−7.39 (m, 5H), 5.13 (d, J = 10.8 Hz, 2H), 4.51 (d, J = 8.5 Hz, 1H), 4.39 (d, J = 7.7 Hz, 1H), 4.23−3.34 (m, 20H), 3.29−2.99 (m, 3H), 2.73 (dd, J = 12.4, 4.6 Hz, 1H), 2.17−1.91 (m, 12H), 1.86−1.64 (m, 3H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.6, 174.2, 173.9, 173.6, 173.5, 158.3, 136.6, 128.8, 128.4, 127.6, 104.0, 100.9, 100.1, 84.3, 75.6, 73.5, 72.5, 71.6, 70.5, 69.5, 69.0, 68.7, 68.3, 67.6, 66.8, 63.1, 60.9, 54.2, 51.9, 50.6, 41.8, 40.1, 37.2, 28.7, 22.1, 21.9. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3799.

*Neu5Ac7NAcα2−3Galβ1−3GlcNAcaProNHCbz (**77**).* 18.4 mg, 89% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.48−7.38 (m, 5H), 5.19−5.09 (m, 2H), 4.48 (d, J = 7.9 Hz, 1H), 4.13−4.06 (m, 2H), 3.98 (dd, J = 9.8, 3.0 Hz, 1H), 3.95−3.68 (m, 12H), 3.66−3.43 (m, 7H), 3.34−3.17 (m, 2H), 2.81 (dd, J = 12.4, 4.6 Hz, 1H), 2.02−1.91 (m, 9H), 1.86−1.70 (m, 3H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.3, 174.0, 173.9, 173.8, 158.4, 136.7, 128.8, 128.3, 127.5, 103.4, 99.3, 97.0, 80.2, 75.6, 75.0, 71.75, 71.68, 71.6, 69.0, 68.57, 68.55, 66.8, 66.7, 64.9, 62.3, 61.0, 60.5, 52.5, 51.7, 49.2, 40.2, 37.4, 28.4, 22.1, 22.0, 21.8. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{38}H_{57}N_4O_{21}^-$ 905.3521; found 905.3540.

*Neu5Ac7,9diNAcα2−3Galβ1−3GlcNAcαProNHCbz (**78**).* 14.5 mg, 69% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.47−7.37 (m, 5H), 5.17−5.09 (m, 2H), 4.52−4.40 (m, 1H), 4.08 (td, J = 9.8, 3.4 Hz, 2H), 4.00−3.87 (m, 4H), 3.87−3.68 (m, 8H), 3.62−3.45 (m, 6H), 3.40 (s, 1H), 3.27 (ddt, J = 26.8, 13.3, 6.6 Hz, 2H), 3.03 (dd, J = 14.2, 7.9 Hz, 1H), 2.80 (dd, J = 12.4, 4.6 Hz, 1H), 2.07−1.91 (m, 12H), 1.86−1.78 (m, 2H), 1.75 (t, J = 12.1 Hz, 1H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.2, 174.0, 173.84, 173.80, 158.4, 136.7, 128.8, 128.3, 127.4, 103.3, 99.5, 96.9, 80.4, 78.5, 75.6, 74.9, 71.7, 71.6, 69.7, 69.1, 68.62, 68.59, 66.8, 66.7, 64.9, 61.0, 60.5, 55.3, 52.4, 51.8, 50.5, 41.7, 40.3, 37.4, 28.4, 22.1, 21.94, 21.88, 21.86. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3808.

*Neu5Ac7NAcα2−6Galβ1−3GlcNAcαProNHCbz (**79**).* 19.3 mg, 72% yield, white amorphous solid. ^{1}H NMR (400 MHz, D_2O) δ 7.50−7.36 (m, 5H), 5.13 (s, 2H), 4.36 (d, J = 7.8 Hz, 1H), 4.20−3.40 (m, 22H), 3.34−3.17 (m, 2H), 2.74 (dd, J = 12.4, 4.7 Hz, 1H), 2.00 (d, J = 6.3 Hz, 6H), 1.98−1.91 (m, 3H), 1.86−1.71 (m, 3H). ^{13}C{^{1}H} NMR (100 MHz, D_2O) δ 174.5, 173.9, 173.7, 173.5, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.1, 96.8, 82.5, 73.4, 72.5, 71.7, 71.6, 71.5, 70.5, 68.8, 68.7, 68.3, 66.7, 65.0, 63.1, 62.4, 60.6, 52.1, 51.9, 49.2, 40.1, 37.5, 28.5, 22.13, 22.09, 21.9. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{38}H_{57}N_4O_{21}$ 905.3521; found 905.3531.

*Neu5Ac7,9diNAcα2−6Galβ1−3GlcNAcαProNHCbz (**80**).* 13.8 mg, 68% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.51−7.37 (m, 5H), 5.14 (t, J = 9.1 Hz, 2H), 4.38 (dd, J = 16.6, 7.9 Hz, 1H), 4.10 (dd, J = 10.5, 3.6 Hz, 1H), 4.05−3.82 (m, 8H), 3.81−3.70 (m, 4H), 3.68−3.38 (m, 8H), 3.37−3.19 (m, 2H), 3.08 (dd, J = 14.0, 7.3 Hz, 1H), 2.73 (dd, J = 12.4, 4.7 Hz, 1H), 2.07−1.99 (m, 9H), 1.96 (s, 3H), 1.90−1.70 (m, 3H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.5, 174.2, 173.9, 173.6, 173.5, 158.4, 136.6, 128.8, 128.3, 127.5, 103.9, 100.1, 96.9, 82.4, 73.4, 72.5, 71.7, 71.5, 70.5, 69.5, 68.8, 68.7, 68.3, 66.7, 65.0, 63.0, 60.6, 52.1, 51.9, 50.6, 41.8, 40.1, 37.5, 28.5, 22.2, 22.1, 22.0, 21.9. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{40}H_{60}N_5O_{21}$ 946.3786; found 946.3794.

*Neu5Ac7NAcα2−6GalNAcαProNHCbz (**81**).* 32 mg, 84% yield, white amorphous solid. ^{1}H NMR (800 MHz, D_2O) δ 7.47−7.36 (m, 5H), 5.19−5.07 (m, 2H), 4.82 (d, J = 3.7 Hz, 1H), 4.12 (dd, J = 11.1, 3.7 Hz, 1H), 4.03−3.93 (m, 3H), 3.91−3.84 (m, 4H), 3.75 (dt, J = 11.5, 5.8 Hz, 1H), 3.71−3.66 (m, 1H), 3.64 (dd, J = 12.1, 2.3 Hz, 1H), 3.60−3.51 (m, 2H), 3.50−3.44 (m, 2H), 3.31−3.19 (m, 2H), 2.75 (dd, J = 12.5, 4.6 Hz, 1H), 2.00 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.81 (p, J = 6.3 Hz, 2H), 1.65 (t, J = 12.2 Hz, 1H). ^{13}C{^{1}H} NMR (200 MHz, D_2O) δ 174.5, 174.0, 173.7, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 100.2, 97.0, 71.6, 71.5, 69.5, 68.6, 68.5, 67.6, 66.7, 65.4, 64.3, 62.4, 52.0, 49.9, 49.2, 40.4, 37.6, 28.4, 22.1, 21.9, 21.8. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{32}H_{47}N_4O_{16}$ 743.2993; found 743.3022.

*Neu5Ac7,9diNAcα2−6GalNAcαProNHCbz (**82**).* 33 mg, 85% yield, white amorphous solid. ^{1}H NMR (600 MHz, D_2O) δ 7.48−7.41 (m, 5H), 5.14 (q, J = 12.7 Hz, 2H), 4.83 (d, J = 3.9 Hz, 1H), 4.17−4.10 (m, 1H), 3.98−3.86 (m, 6H), 3.80−3.66 (m, 3H), 3.62−3.53 (m, 2H), 3.52−3.43 (m, 2H), 3.33−3.21 (m, 2H), 3.08 (dd, J = 14.1, 7.3 Hz, 1H), 2.76 (dd, J = 12.5, 4.6 Hz, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.83 (p, J = 6.3 Hz, 2H), 1.67 (t, J = 12.2 Hz, 1H). ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 174.5, 174.2, 174.0, 173.7, 173.3, 158.4, 136.6, 128.8, 128.3, 127.6, 100.3, 97.0, 71.4, 69.7, 69.5, 68.63, 68.56, 67.6, 66.7, 65.5, 64.3, 52.0, 50.7, 49.9, 41.9, 40.4, 37.6, 28.4, 22.1, 21.91, 21.87, 21.8. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{34}H_{50}N_5O_{16}$ 784.3258; found 784.3285.

General Procedures for Synthesis of Neu5Ac7NAc- and Neu5Ac7,9diNAc-Terminated Propylamino-Glycosides by Reduction. To a stirred solution of a glycoside (selected from **57** to **82**, 1.0−6.0 mg) in a water−methanol solution (2 mL, 1:1 by volume), a catalytic amount of 10% palladium on charcoal was added to a round-bottom flask (50 mL). The mixture was stirred under a hydrogen environment for 2−5 h. The solution was passed through a filter to remove the catalyst. The solvent was removed under vacuum and lyophilized and used directly for microarray studies.

Neu5Ac7NAcα2−3LacβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) m/z: [M − H]$^-$ calcd for $C_{28}H_{48}N_3O_{19}$ 730.2887; found 730.2896.

https://doi.org/10.1021/acs.joc.1c01091
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Neu5Ac7,9diNAcα2−3LacβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ (M-H) 771.3153; found 771.3170.

Neu5Ac7NAcα2−6LacβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{28}H_{48}N_3O_{19}$ 730.2887; found 730.2898.

Neu5Ac7,9diNAcα2−6LacβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3172.

Neu5Ac7NAcα2−3LacNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3157.

Neu5Ac7,9diNAcα2−3LacNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3427.

Neu5Ac7NAcα2−6LacNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3151.

Neu5Ac7,9diNAcα2−6LacNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3416.

Neu5Ac7NAcα2−3Galβ1−3GalNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3172.

Neu5Ac7,9diNAcα2−3Galβ1−3GalNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3427.

Neu5Ac7NAcα2−6Galβ1−3GalNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3154.

Neu5Ac7,9diNAcα2−6Galβ1−3GalNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3421.

Neu5Ac7NAcα2−3Galβ1−3GalNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3150.

Neu5Ac7,9diNAcα2−3Galβ1−3GalNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3424.

Neu5Ac7NAcα2−6Galβ1−3GalNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3147.

Neu5Ac7,9diNAcα2−6Galβ1−3GalNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3422.

Neu5Ac7NAcα2−3Galβ1−3GlcNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3158.

Neu5Ac7,9diNAcα2−3Galβ1−3GlcNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3421.

Neu5Ac7NAcα2−6Galβ1−3GlcNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3159.

Neu5Ac7,9diNAcα2−6Galβ1−3GlcNAcβProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3421.

Neu5Ac7NAcα2−3Galβ1−3GlcNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3161.

Neu5Ac7,9diNAcα2−3Galβ1−3GlcNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3421.

Neu5Ac7NAcα2−6Galβ1−3GlcNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{30}H_{51}N_4O_{19}$ 771.3153; found 771.3155.

Neu5Ac7,9diNAcα2−6Galβ1−3GlcNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{32}H_{54}N_5O_{19}$ 812.3418; found 812.3421.

Neu5Ac7NAcα2−6GaNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{24}H_{41}N_4O_{14}$ 609.2625; found 609.2631.

Neu5Ac7,9diNAcα2−6GalNAcαProNH$_2$ white amorphous solid. HRMS (ESI-Orbitrap) *m/z*: [M − H]$^-$ calcd for $C_{26}H_{44}N_5O_{14}$ 650.2890; found 650.2901.

Sialoglycan Microarray Studies. Glycans were printed on PolyAn Glass Slide, 3D-NHS (Automate Scientific) at 100 μM in four replicates each in phosphate buffer (300 mM, pH 8.4). Printed glycan microarray slides were blocked with ethanolamine (0.05 M) in Tris−HCl (0.1 M, pH 9.0), washed, and dried. Slides were fitted in a multiwell microarray hybridization cassette (AHC4X8S, ArrayIt, Sunnyvale, CA) to divide them into eight subarrays. The subarrays were blocked with 1% ovalbumin in PBS, pH 7.4 at room temperature for 1 h with gentle shaking (all incubations were carried out in a humid chamber). The blocking solution was removed followed by incubation at room temperature for 2 h with gentle shaking with 30 μg/mL of Fc-fused human Siglec 7 (hSiglec 7) or Fc-fused human Siglec 9 (hSiglec 9), or 20 μg/mL of *Sambucus nigra* lectin (SNA) or *Maackia amurensis* lectin II (MAL II) (Vector Laboratories, Burlingame, CA) in blocking buffer. The slides were washed and incubated at room temperature for 1 h with goat antihuman IgG-Cy3 (1.5 μg/mL in PBS) (Jackson ImmunoResearch Laboratories, 109-165-088) for Siglecs or streptavidin-Cy3 (1.5 μg/mL in PBS) for lectins (Jackson ImmunoResearch, 109-160-084). The slides were subsequently washed and dried. The microarray slides were then scanned by a Genepix 4000B microarray scanner (Molecular Devices Corp., Union City, CA), and data analysis was performed using Genepix Pro 7.0 analysis software (Molecular Devices Corp., Union City, CA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c01091.

Microarray data, detailed synthetic procedures, nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (HRMS) data, and NMR spectra of the products (PDF)

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https://doi.org/10.1021/acs.joc.1c01091
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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the U.S. National Institutes of Health (NIH) under Award Number R01AI130684. The Bruker Avance-800 NMR spectrometer was purchased with a U.S. National Science Foundation (NSF) Shared Instrumentation Grant (grant no. DBI-0722538). The Thermo Scientific Q Exactive HF Orbitrap Mass Spectrometer was purchased with a U.S. NIH Shared Instrumentation Grant (grant no. S10OD025271). The authors would like to thank Dr. Lei Li and Dr. Ding Liu at Georgia State University for their help in obtaining high-resolution mass spectrometry (HRMS) data for some of the products.

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https://doi.org/10.1021/acs.joc.1c01091
J. Org. Chem. XXXX, XXX, XXX−XXX

Supporting Information

Chemoenzymatic Synthesis of Sialosides Containing 7-*N*- or 7,9-Di-*N*-acetyl Sialic Acid as Stable *O*-Acetyl Analogues for Probing Sialic Acid-Binding Proteins

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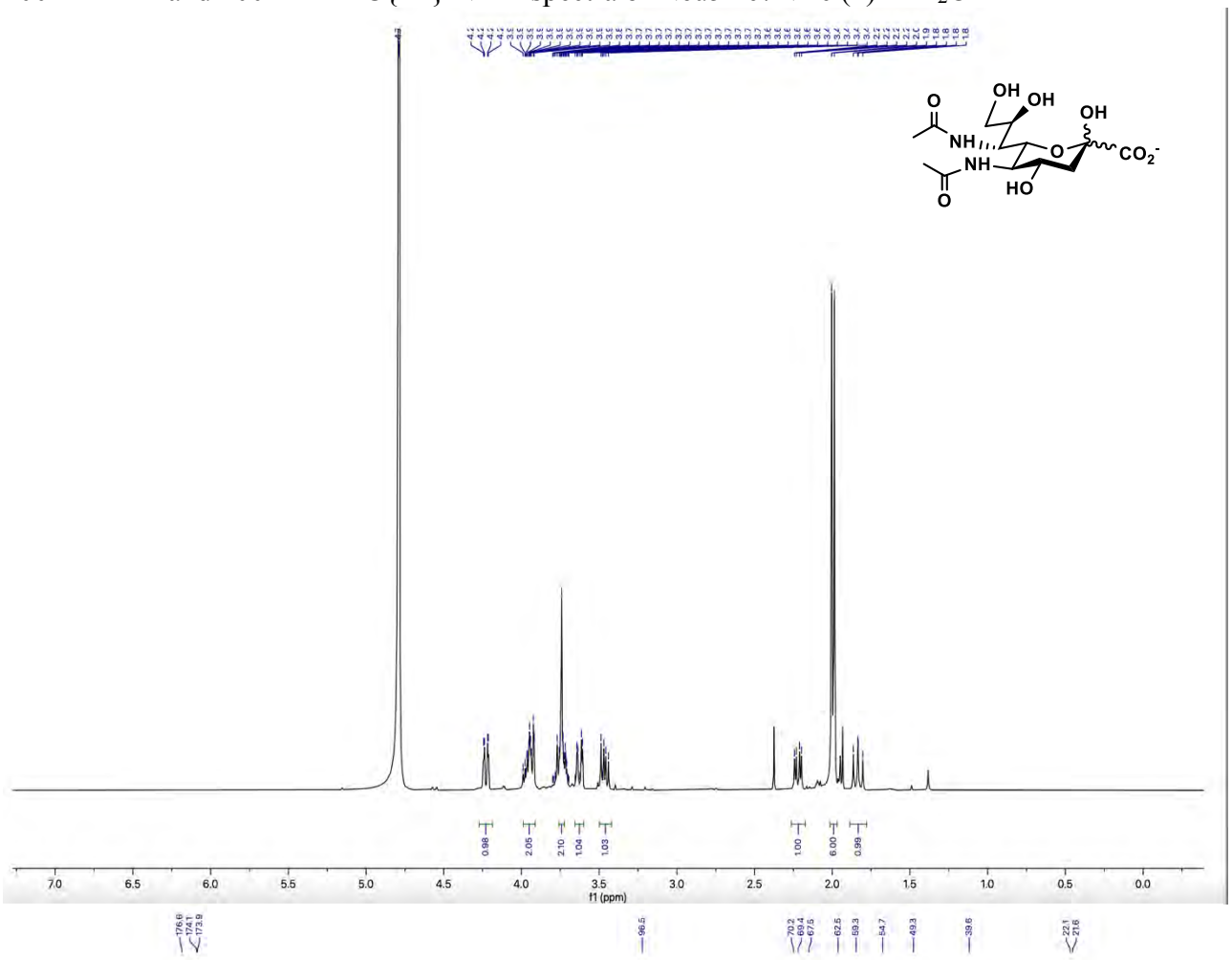
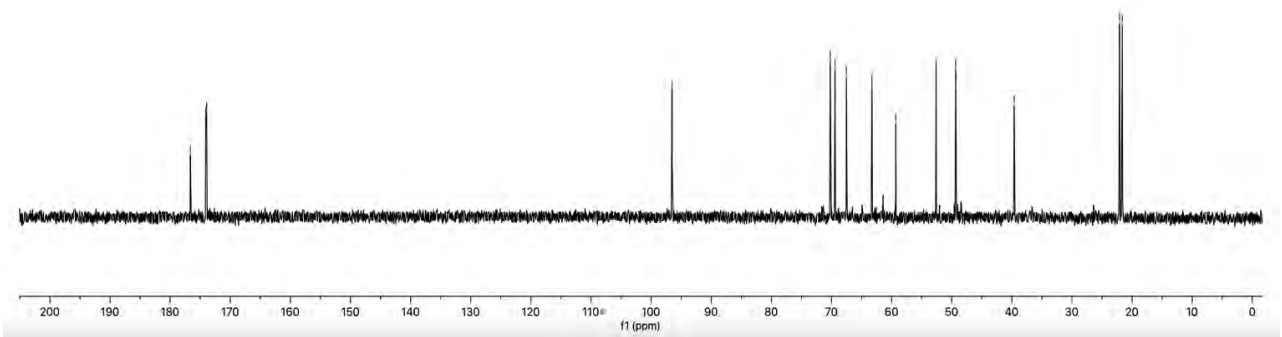
Table S1. Glycan microarray study results (See **Figure 3** for the corresponding bar figure). R1 = $ProNH_2$.

	Glycans	hSiglec 7	hSiglec 9	SNA	MAL II
		Av±SD	Av±SD	Av±SD	Av±SD
A	Neu5Acα3Galβ4GlcβR1	3873±236	44638±3098	70±22	82±16
	Neu5,9Ac_2α3Galβ4GlcβR1	128±45	12452±2402	28±11	2449±2487
	Neu5Ac9NAcα3Galβ4GlcβR1	74±5	3598±1128	13±2	44±10
	Neu5Ac7NAcα3Galβ4GlcβR1	32±13	233±31	23±4	24±3
	Neu5Ac7,9diNAcα3Galβ4GlcβR1	33±12	123±36	16±4	23±6
	Galβ4GlcβR1	86±57	455±85	20±5	61±17
B	Neu5Acα6Galβ4GlcβR1	18232±769	35090±11846	1071±1068	1456±245
	Neu5,9Ac_2α6Galβ4GlcβR1	82±21	763±332	5070±300	65±3
	Neu5Ac9NAcα6Galβ4GlcβR1	1289±104	3074±355	1773±112	52±7
	Neu5Ac7NAcα6Galβ4GlcβR1	57±14	365±205	151±37	22±2
	Neu5Ac7,9diNAcα6Galβ4GlcβR1	24±5	137±8	358±76	30±20
	Galβ4GlcβR1	86±57	455±85	20±5	61±17
C	Neu5Acα3Galβ4GlcNAcβR1	3316±442	43732±12044	24±1	339±276
	Neu5,9Ac_2α3Galβ4GlcNAcβR1				
	Neu5Ac9NAcα3Galβ4GlcNAcβR1	17±14	414±350	8±2	73±17
	Neu5Ac7NAcα3Galβ4GlcNAcβR1	40±8	248±26	20±1	21±3
	Neu5Ac7,9diNAcα3Galβ4GlcNAcβR1	20±1	101±18	15±4	20±5
	Galβ4GlcNAcβR1	111±18	316±40	96±16	81±10
D	Neu5Acα6Galβ4GlcNAcβR1	16232±1719	54607±2394	4170±682	58±11
	Neu5,9Ac_2α6Galβ4GlcNAcβR1	18675±1057	48835±6066	5452±1564	19±2
	Neu5Ac9NAcα6Galβ4GlcNAcβR1	574±63	7438±1391	3794±309	43±6
	Neu5Ac7NAcα6Galβ4GlcNAcβR1	32±10	324±98	97±23	403±232
	Neu5Ac7,9diNAcα6Galβ4GlcNAcβR1	24±9	75±80	56±11	20±3
	Galβ4GlcNAcβR1	111±18	316±40	96±16	81±10
E	Neu5Acα6GalNAcαR1	30069±3743	38633±4875	236±18	164±47
	Neu5,9Ac_2α6GalNAcαR1	15931±4038	22398±2008	56±6	48±9
	Neu5Ac9NAcα6GalNAcαR1	2180±301	1506±471	28±4	22±3
	Neu5Ac7NAcα6GalNAcαR1	37±10	291±106	12±3	12±4
	Neu5Ac7,9diNAcα6GalNAcαR1	27±12	87±18	15±5	16±17
	GalNAcαR1	47±9	179±100	32±4	42±4
F	Neu5Acα3Galβ3GlcNAcβR1	1325±212	32721±1974	40±11	662±86
	Neu5,9Ac_2α3Galβ3GlcNAcβR1	33±8	483±140	65±4	208±52
	Neu5Ac9NAcα3Galβ3GlcNAcβR1	80±11	1008±97	24±9	18±4
	Neu5Ac7NAcα3Galβ3GlcNAcβR1	36±14	189±34	23±1	23±3
	Neu5Ac7,9diNAcα3Galβ3GlcNAcβR1	19±5	138±81	13±1	15±2
	Galβ3GlcNAcβR1	53±14	232±24	29±9	114±17
G	Neu5Acα6Galβ3GlcNAcβR1	22346±3694	45726±10343	249±90	27±9
	Neu5,9Ac_2α6Galβ3GlcNAcβR1				
	Neu5Ac9NAcα6Galβ3GlcNAcβR1	774±72	1542±238	897±72	36±8
	Neu5Ac7NAcα6Galβ3GlcNAcβR1	35±10	230±156	30±12	16±7
	Neu5Ac7,9diNAcα6Galβ3GlcNAcβR1	17±12	60±18	25±2	19±1
	Galβ3GlcNAcβR1	53±14	232±24	29±9	114±17

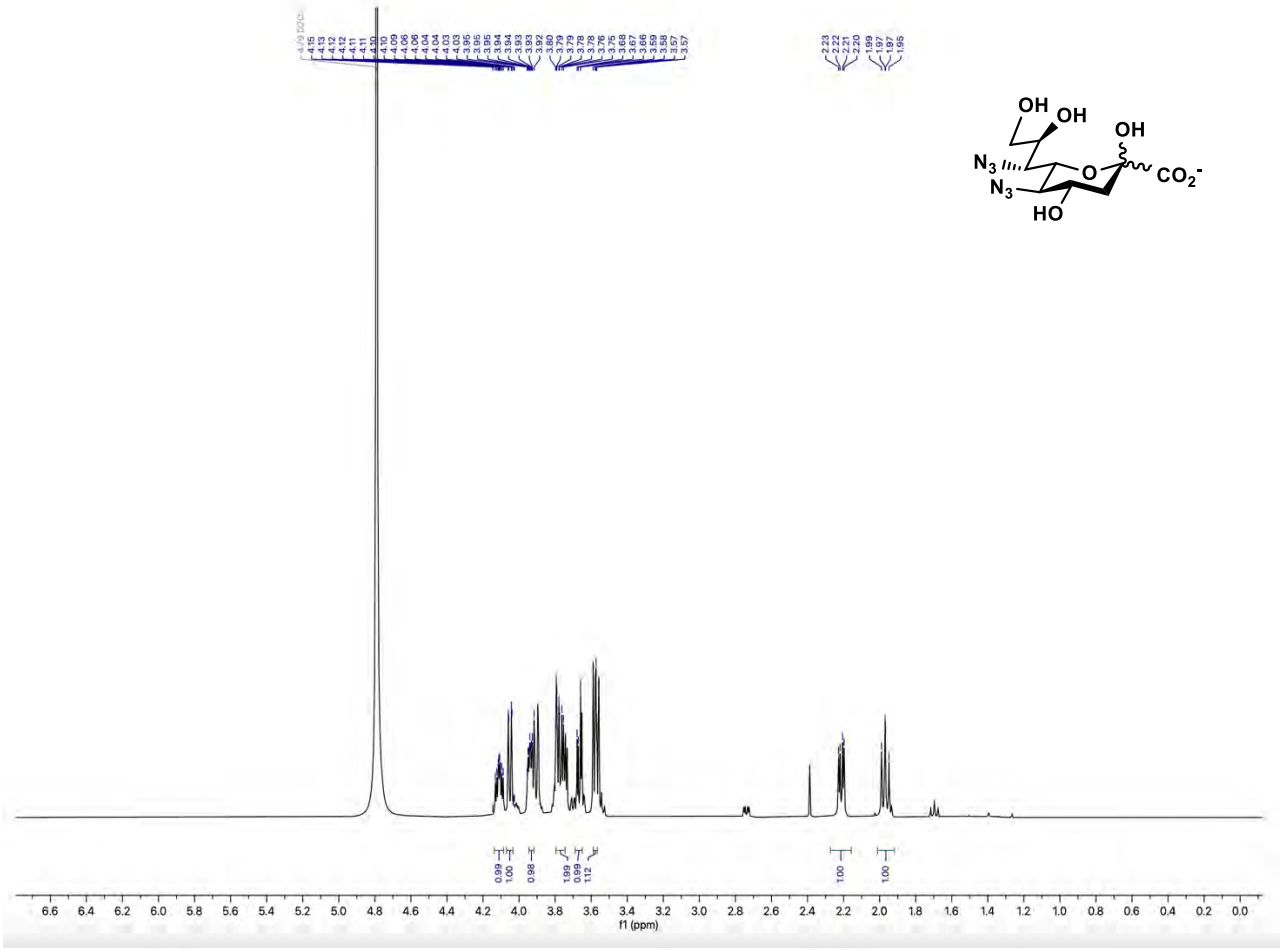
	Glycans	hSiglec 7	hSiglec 9	SNA	MAL II
		Av±SD	Av±SD	Av±SD	Av±SD
H	Neu5Acα3Galβ3GlcNAcαR1	858±249	11809±2780	34±4	915±256
	Neu5,9Ac$_2$α3Galβ3GlcNAcαR1				
	Neu5Ac9NAcα3Galβ3GlcNAcαR1	92±21	702±135	22±5	29±2
	Neu5Ac7NAcα3Galβ3GlcNAcαR1	33±11	160±42	34±5	53±3
	Neu5Ac7,9diNAcα3Galβ3GlcNAcβR1	49±21	75±8	14±4	16±5
	Galβ3GlcNAcαR1				
I	Neu5Acα6Galβ3GlcNAcαR1				
	Neu5,9Ac$_2$α6Galβ3GlcNAcαR1				
	Neu5Ac9NAcα6Galβ3GlcNAcαR1	1114±295	3071±723	384±88	214±270
	Neu5Ac7NAcα6Galβ3GlcNAcαR1	28±7	229±24	66±10	23±3
	Neu5Ac7,9diNAcα6Galβ3GlcNAcαR1	33±5	133±40	39±6	16±3
	Galβ3GlcNAcαR1				
J	Neu5Acα3Galβ3GalNAcβR1	1580±329	32410±5066	27±10	17200±1879
	Neu5,9Ac$_2$α3Galβ3GalNAcβR1	58±15	271±119	99±11	34276±4340
	Neu5Ac9NAcα3Galβ3GalNAcβR1	19±7	244±116	14±2	16576±3253
	Neu5Ac7NAcα3Galβ3GalNAcβR1	2158±2198	4010±416	375±24	778±499
	Neu5Ac7,9diNAcα3Galβ3GalNAcβR1	52±35	259±97	37±4	47±5
	Galβ3GalNAcβR1	55±9	212±42	25±4	57±8
K	Neu5Acα6Galβ3GalNAcβR1	11743±694	28518±4248	11±8	11±7
	Neu5,9Ac$_2$α6Galβ3GalNAcβR1				
	Neu5Ac9NAcα6Galβ3GalNAcβR1	1924±431	1229±394	135±18	695±125
	Neu5Ac7NAcα6Galβ3GalNAcβR1	35±4	124±29	27±6	32±4
	Neu5Ac7,9diNAcα6Galβ3GalNAcβR1	28±5	136±111	16±2	15±3
	Galβ3GalNAcβR1	55±9	212±42	25±4	57±8
L	Neu5Acα3Galβ3GalNAcαR1	1868±177	10988±4120	37±8	45417±4854
	Neu5,9Ac$_2$α3Galβ3GalNAcαR1	121±120	154±89	87±25	34313±4699
	Neu5Ac9NAcα3Galβ3GalNAcαR1	16±5	84±32	7±3	16570±2600
	Neu5Ac7NAcα3Galβ3GalNAcαR1	36±11	145±62	16±2	30441±1702
	Neu5Ac7,9diNAcα3Galβ3GalNAcαR1	37±5	202±102	34±3	14694±1436
	Galβ3GalNAcαR1	66±17	338±29	13±4	46±4
M	Neu5Acα6Galβ3GalNAcαR1				
	Neu5,9Ac$_2$α6Galβ3GalNAcαR1				
	Neu5Ac9NAcα6Galβ3GalNAcαR1	258±40	1414±167	148±2	26±14
	Neu5Ac7NAcα6Galβ3GalNAcαR1	22±3	115±12	24±7	21±2
	Neu5Ac7,9diNAcα6Galβ3GalNAcαR1	26±6	83±46	11±3	9±1
	Galβ3GalNAcαR1	66±17	338±29	13±4	46±4

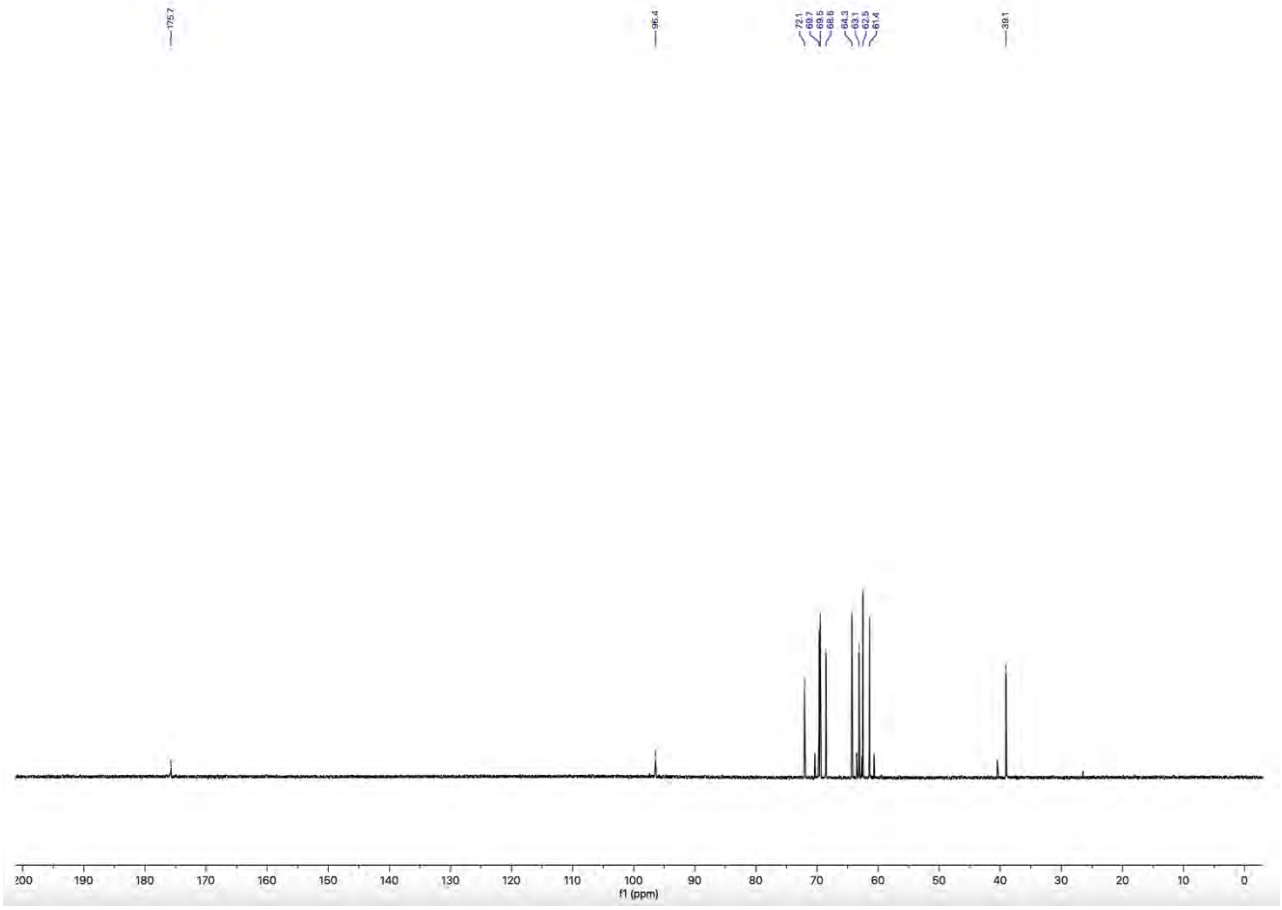
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAc (**4**) in D_2O



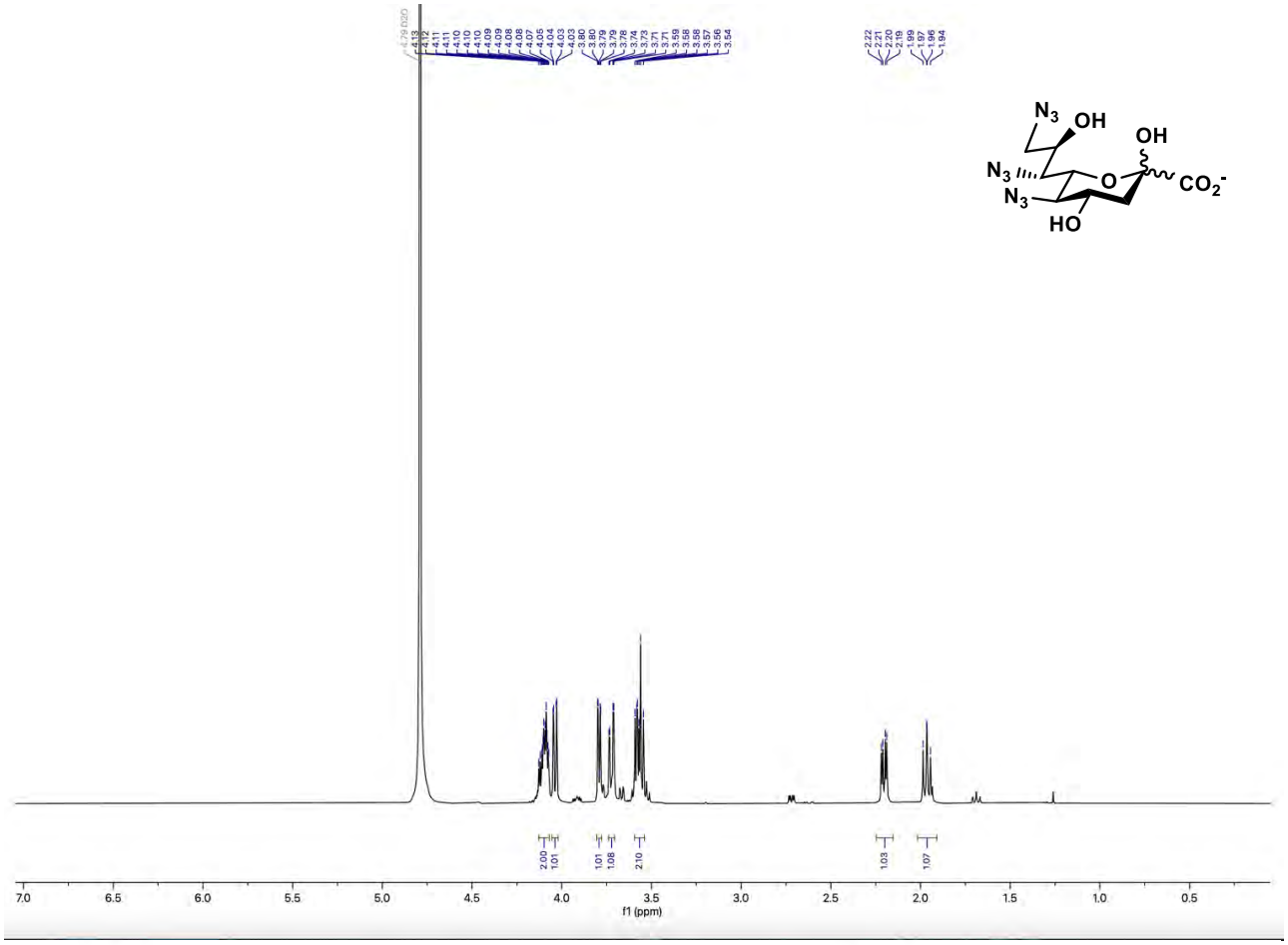


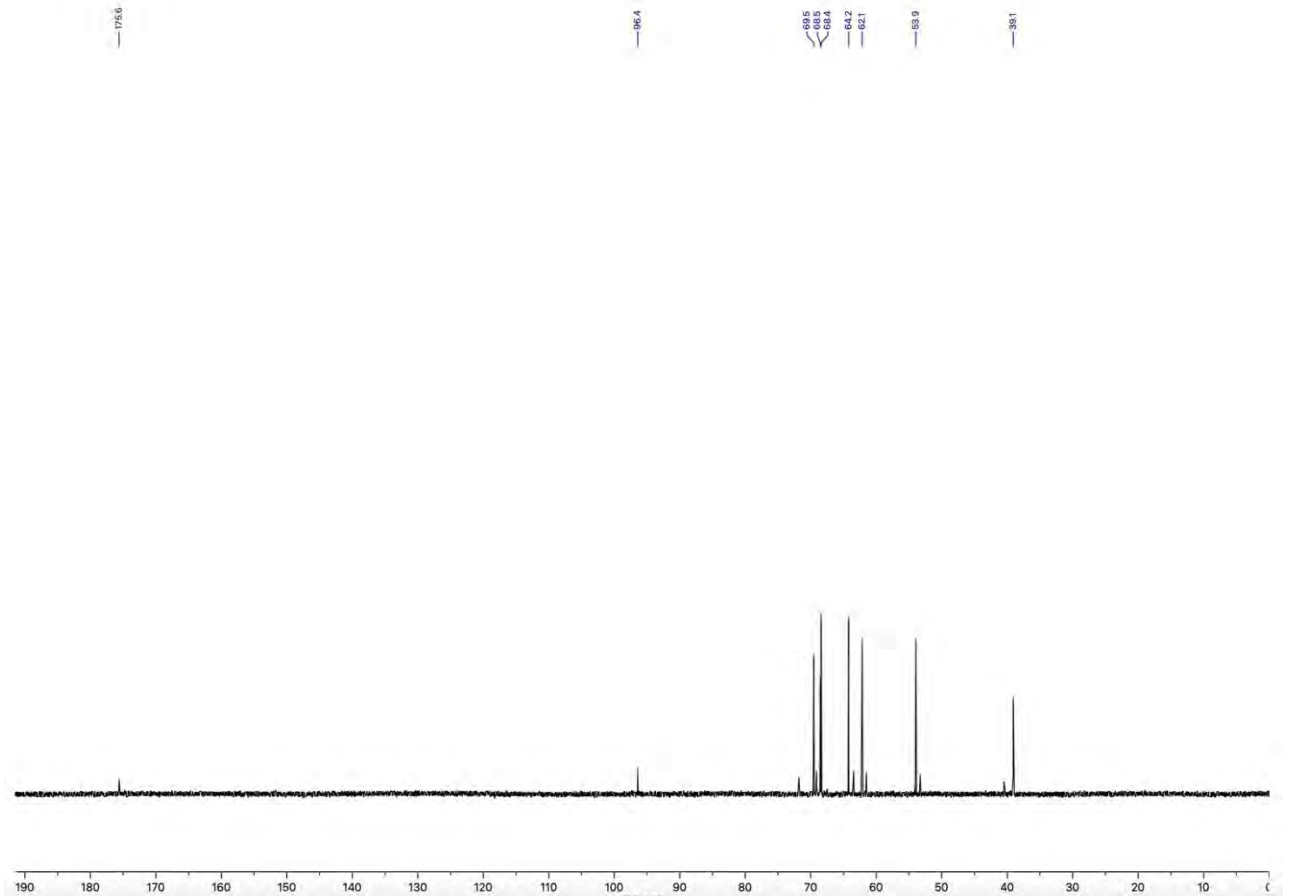
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$ (**5**) in D_2O



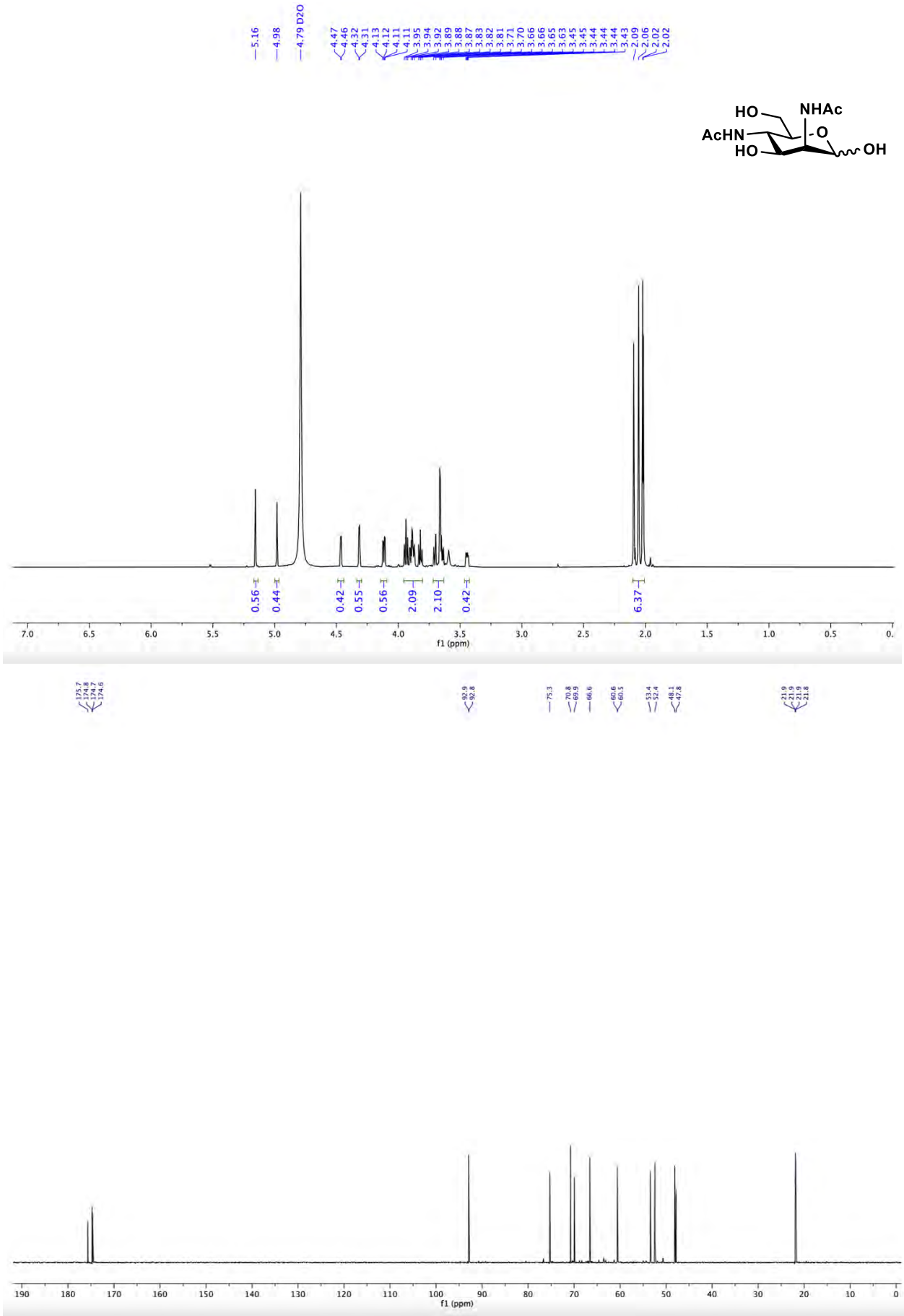


600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3 (**7**) in D_2O

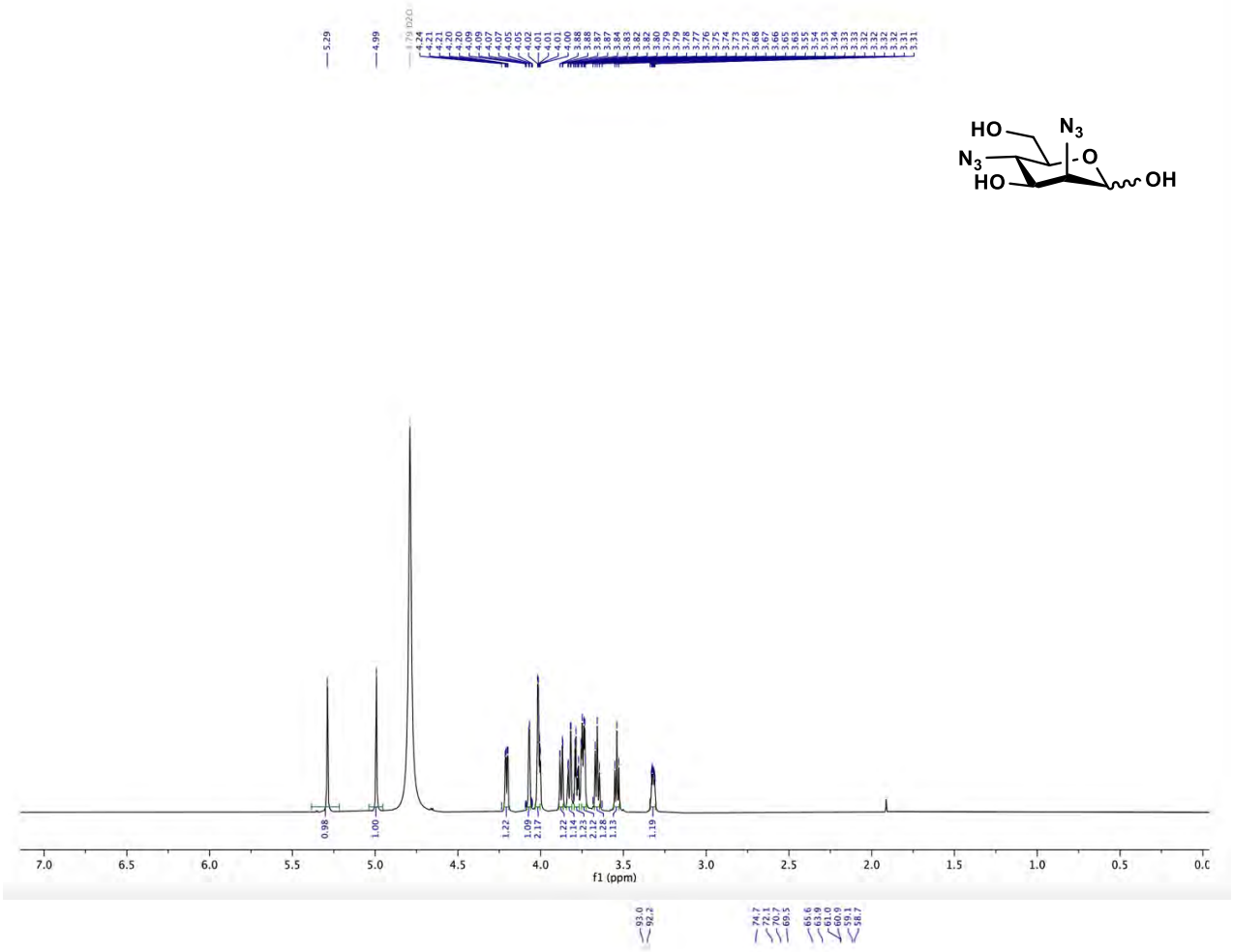


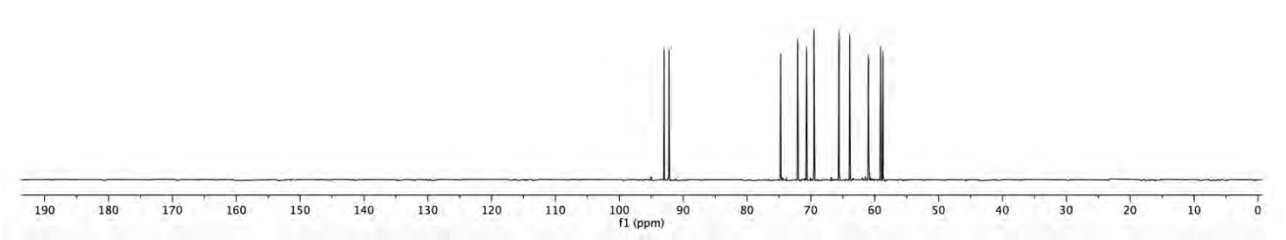


800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of 2,4-diacetamido-2,4-dideoxy-D-mannopyranose (Man2,4diNAc, **8**) in D_2O

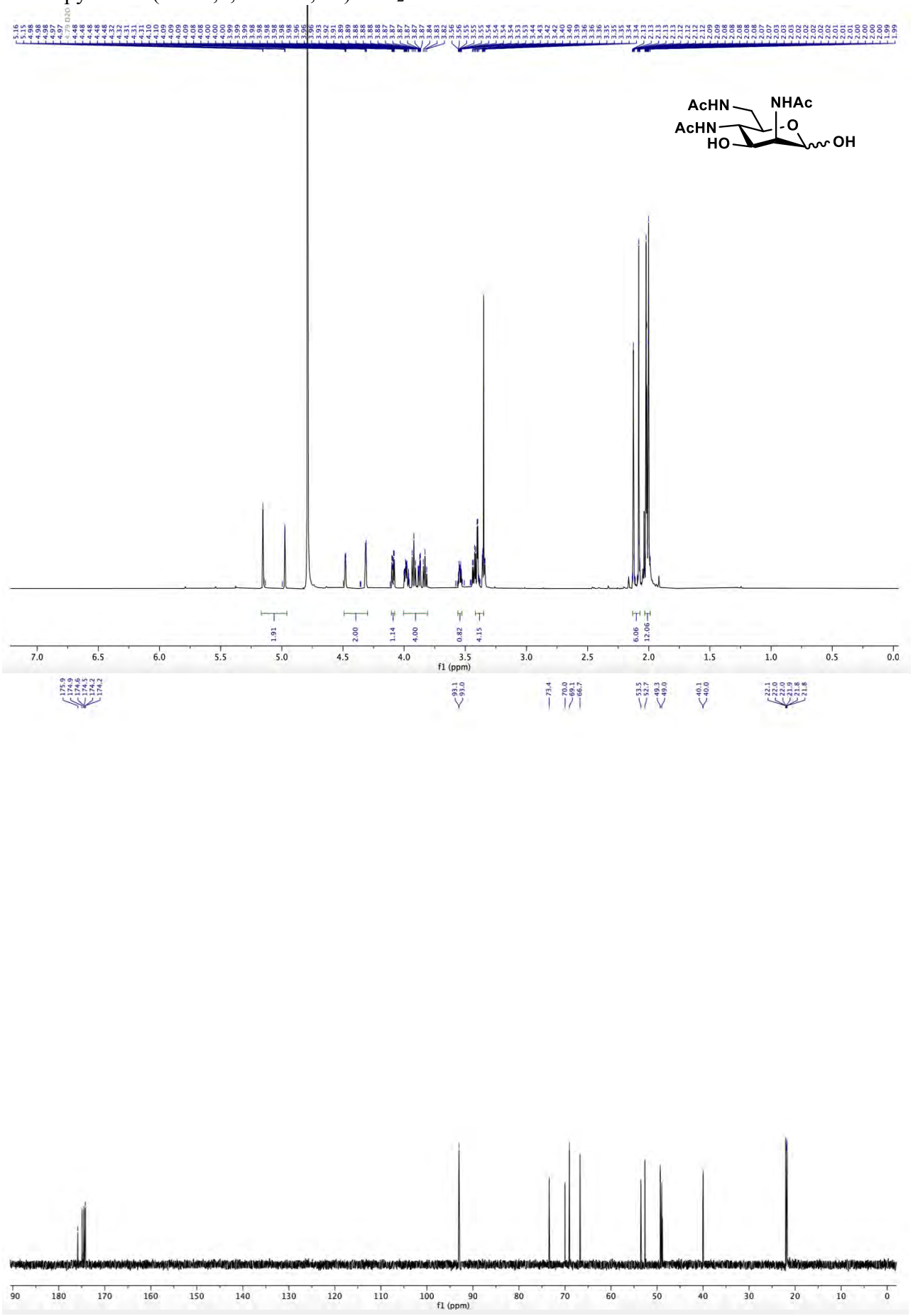


800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of 2,4-diazido-2,4-dideoxy-D-mannopyranose (Man2,4diN_3, **9**) in D_2O

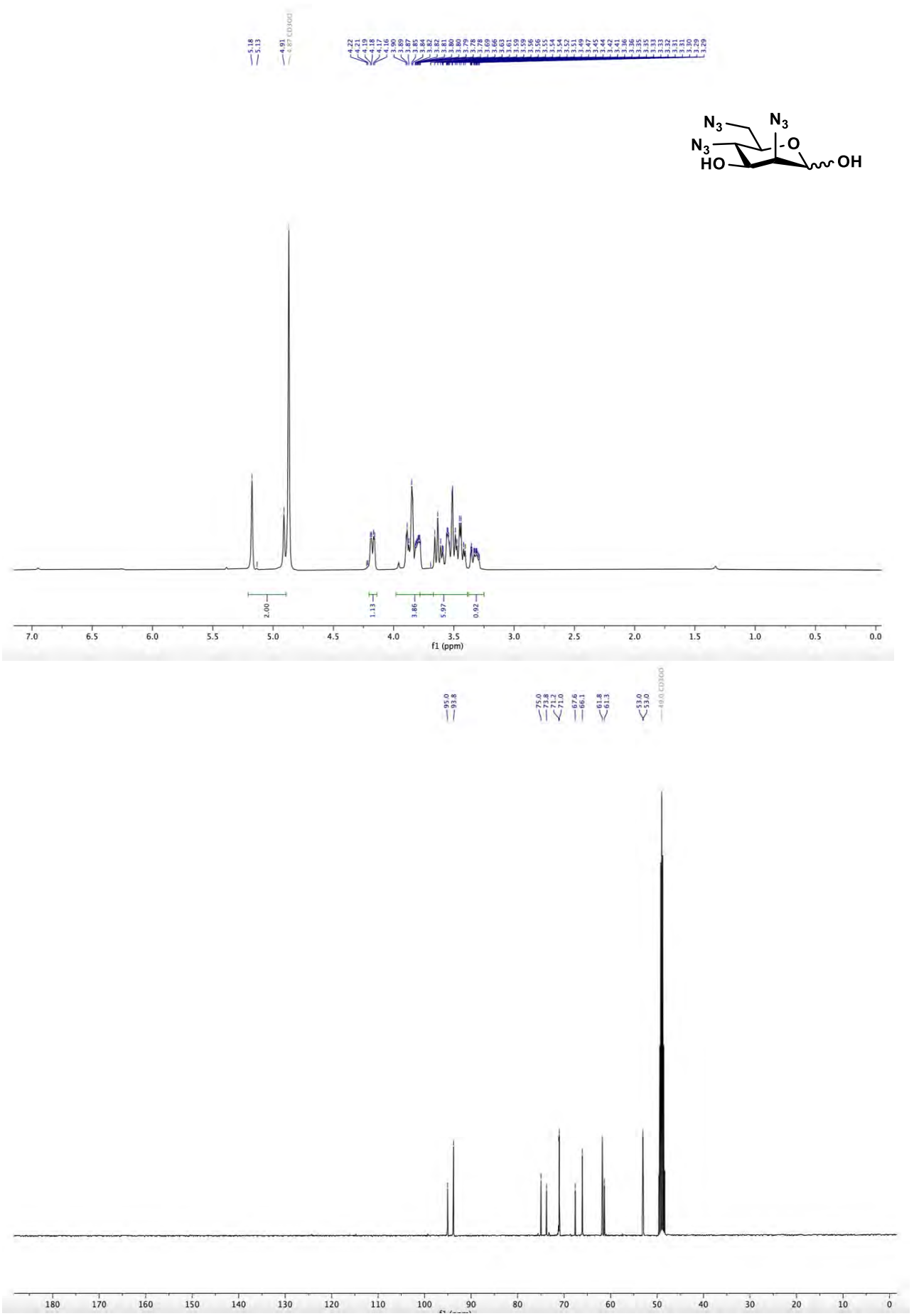




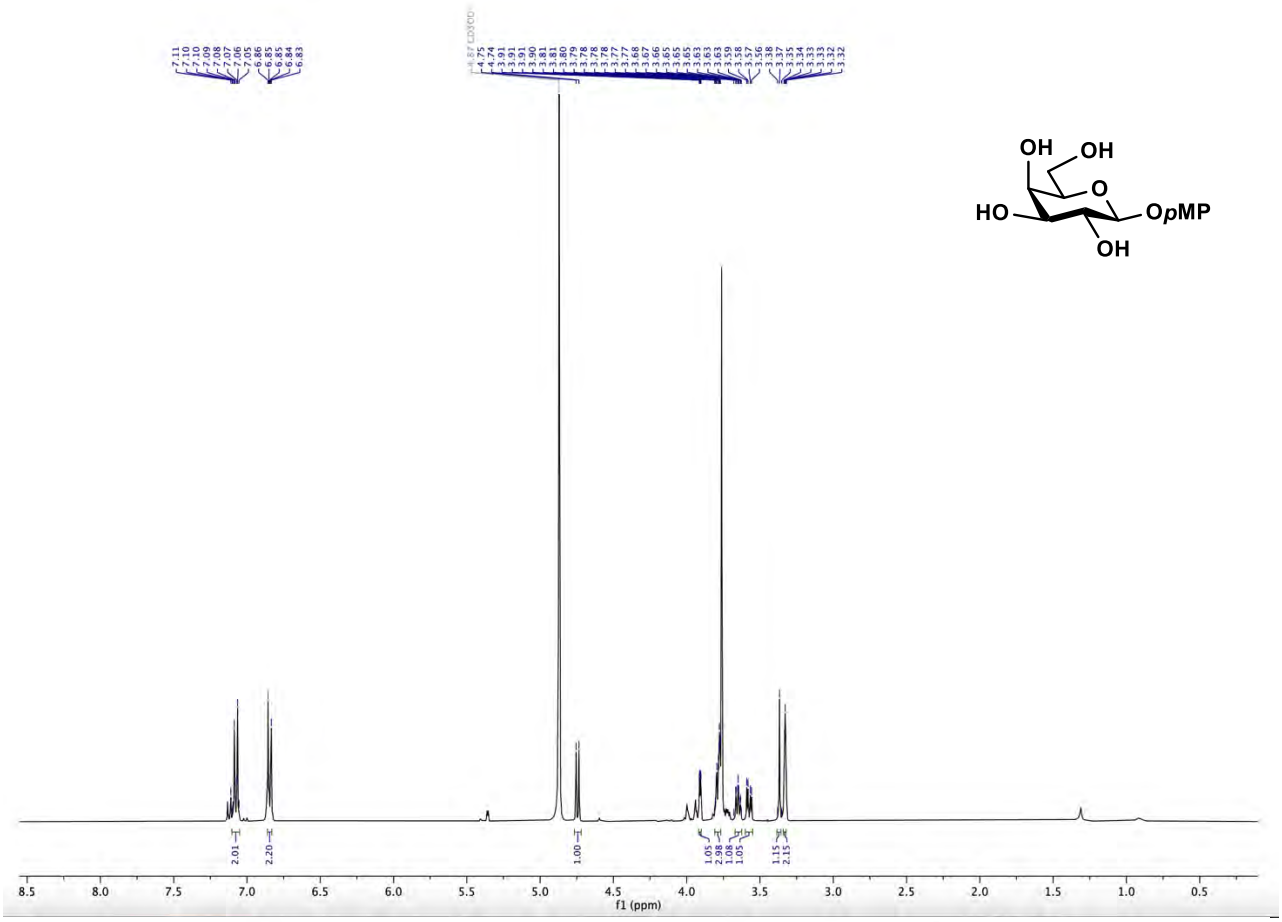
400 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of 2,4,6-triacetamido-2,4,6-trideoxy-D-mannopyranose (Man2,4,6triNAc, **10**) in D_2O

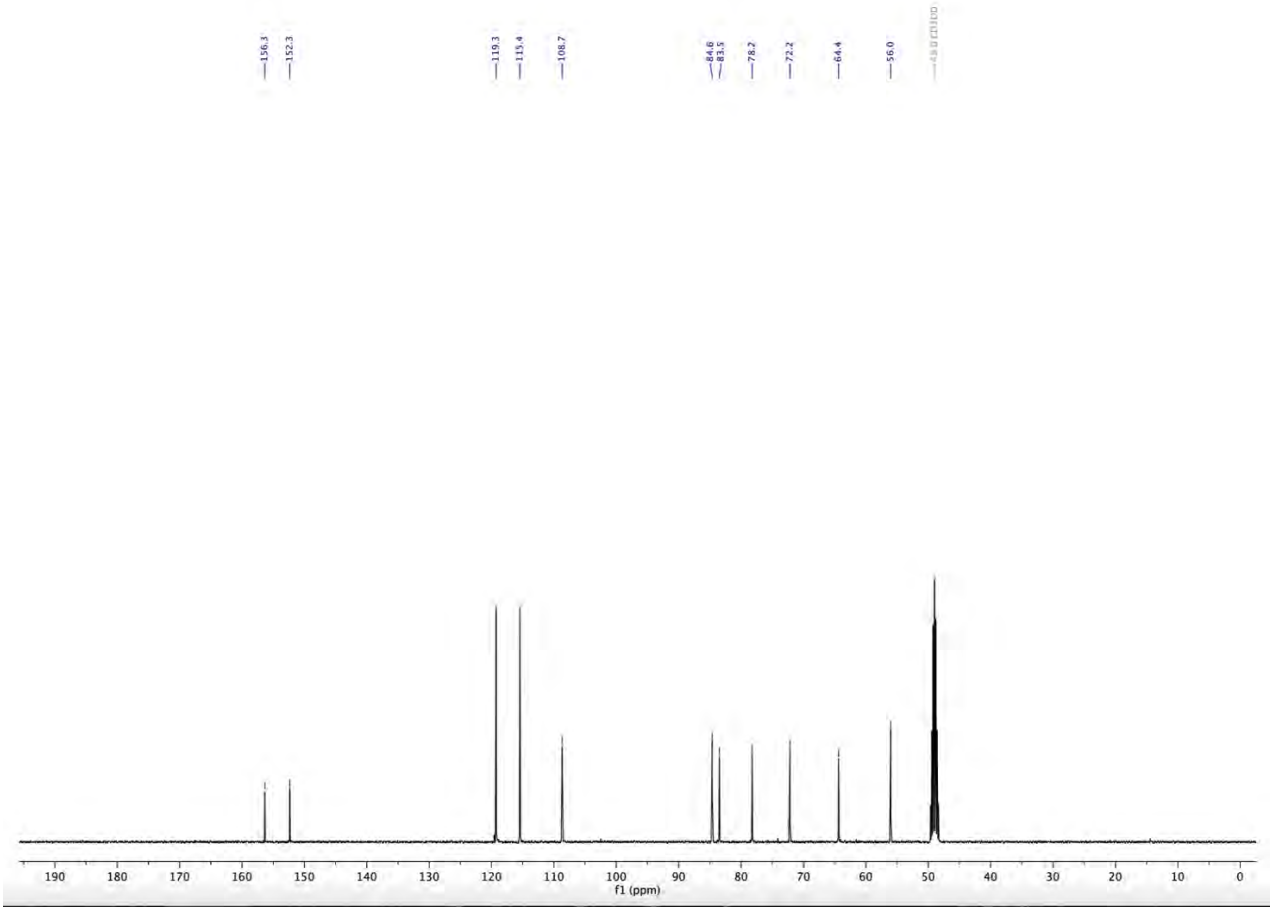


400 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of 2,4,6-triazido-2,4,6-trideoxy-D-mannopyranose (Man2,4,6triN_3, **11**) in CD_3OD

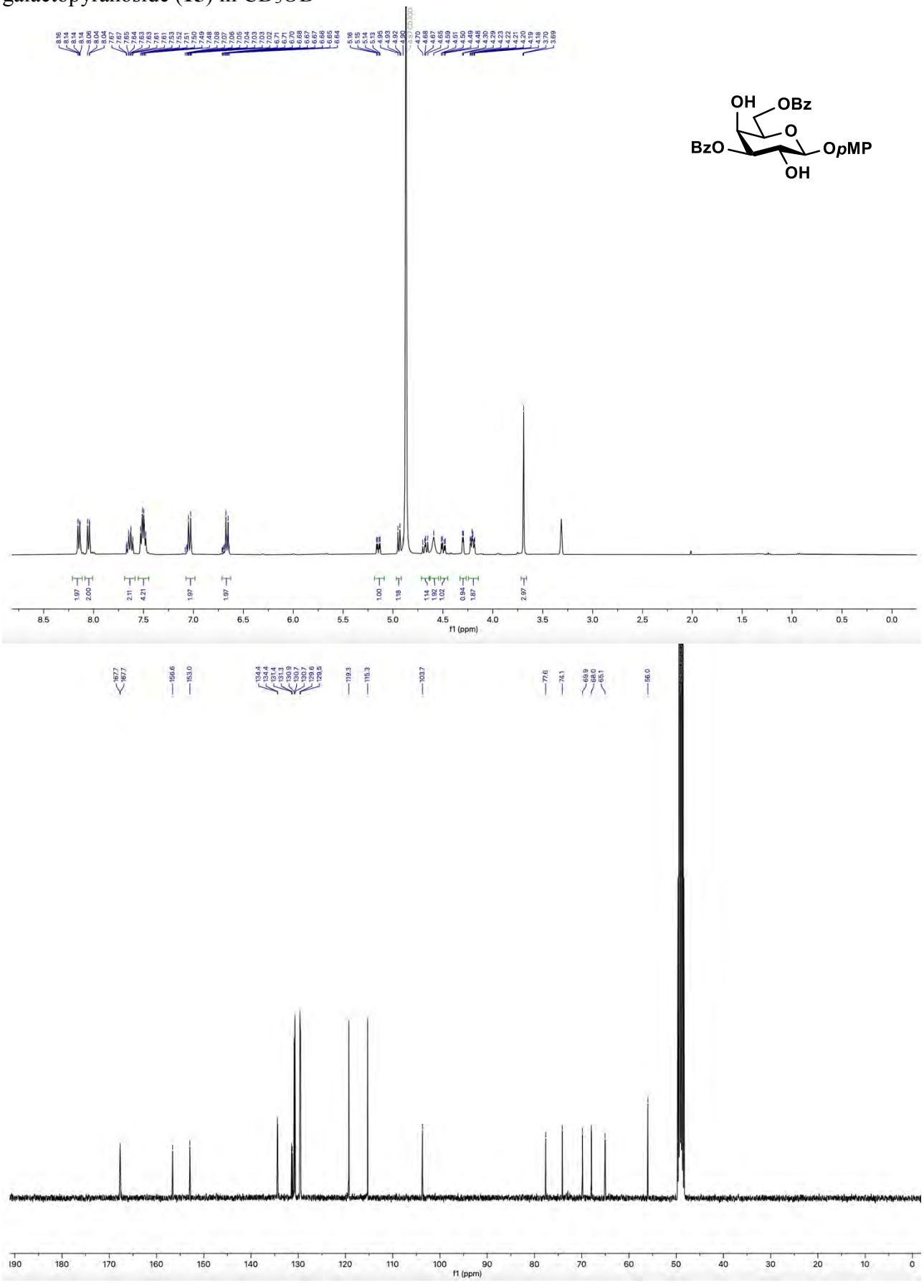


400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-β-D-galactopyranoside (**14**) in CD_3OD

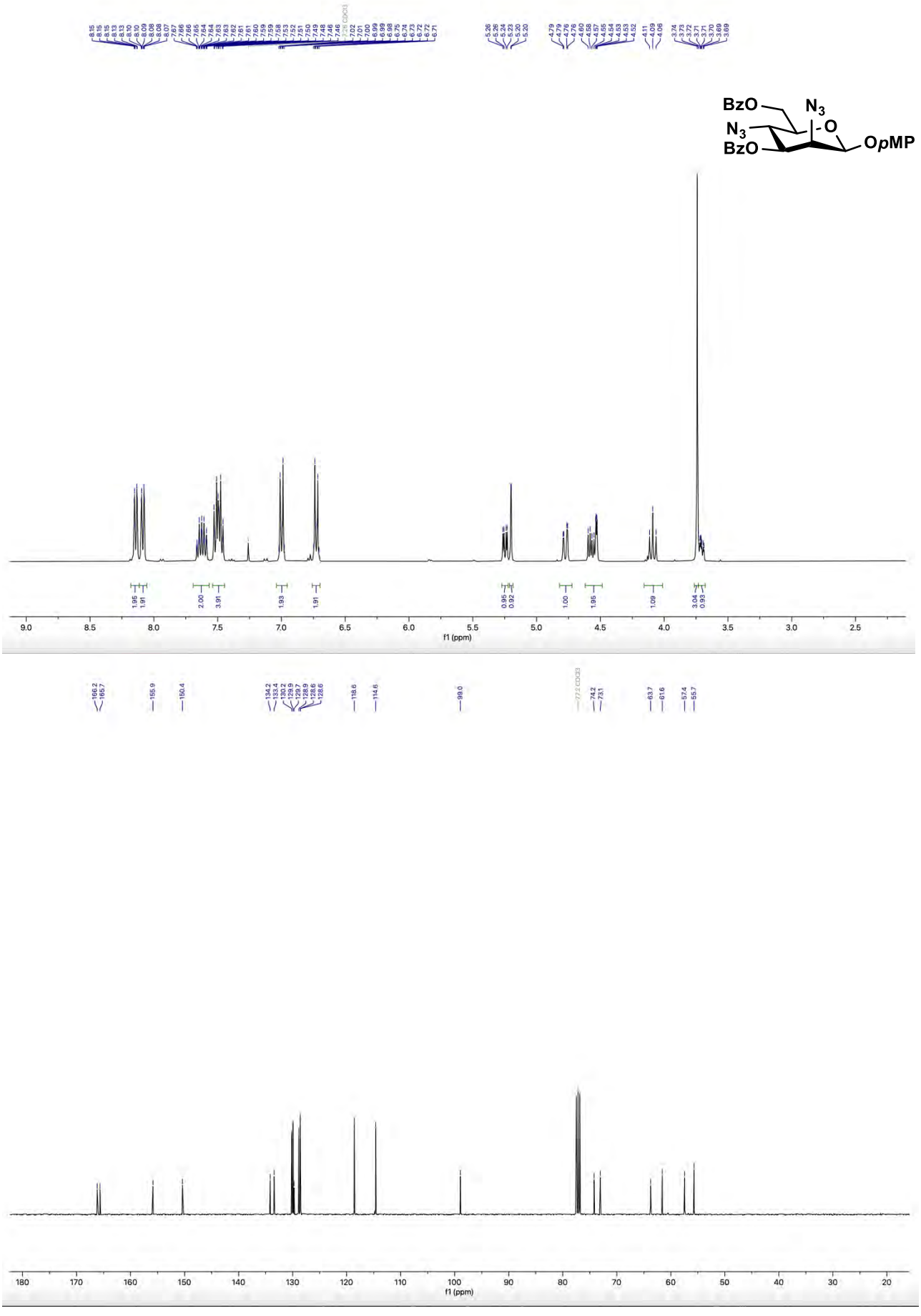




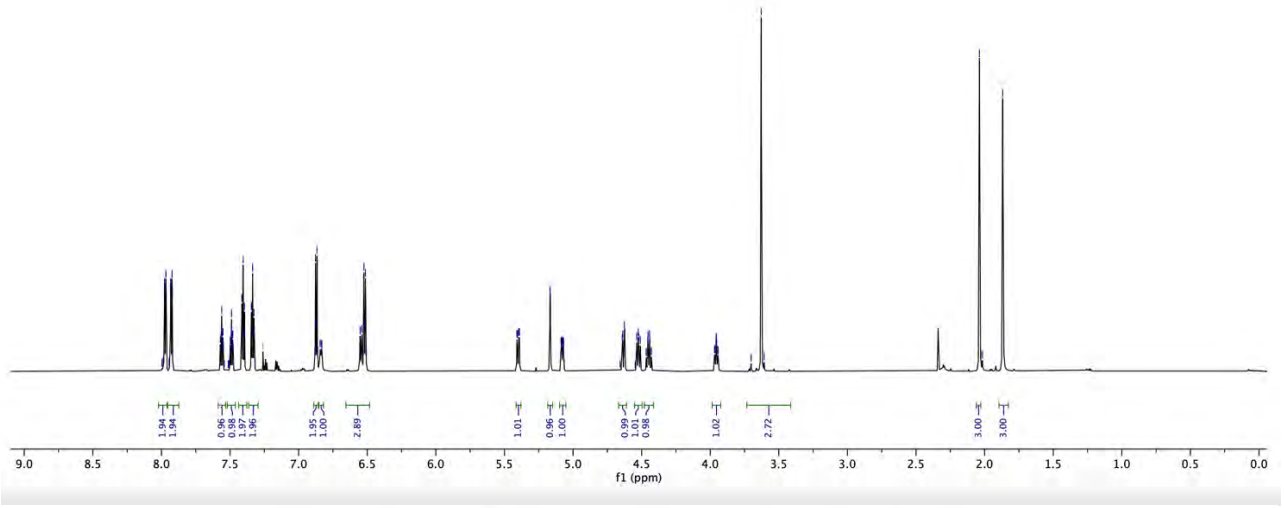
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-3,6-dibenzoyl-β-D-galactopyranoside (**15**) in CD_3OD

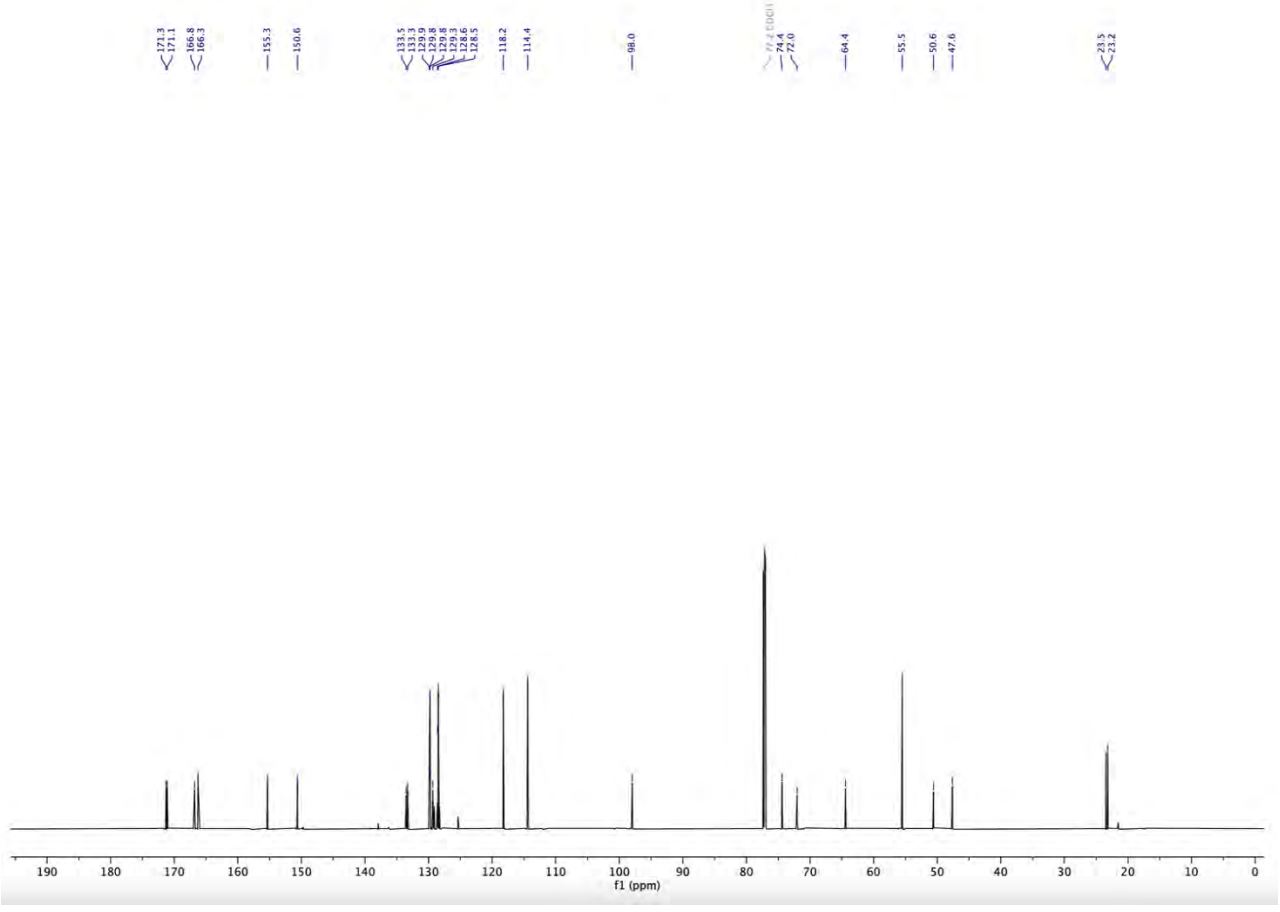


400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-2,4-di-azido-3,6-dibenzoyl-2,4-dideoxy-β-D-mannopyranoside (**16**) in $CDCl_3$

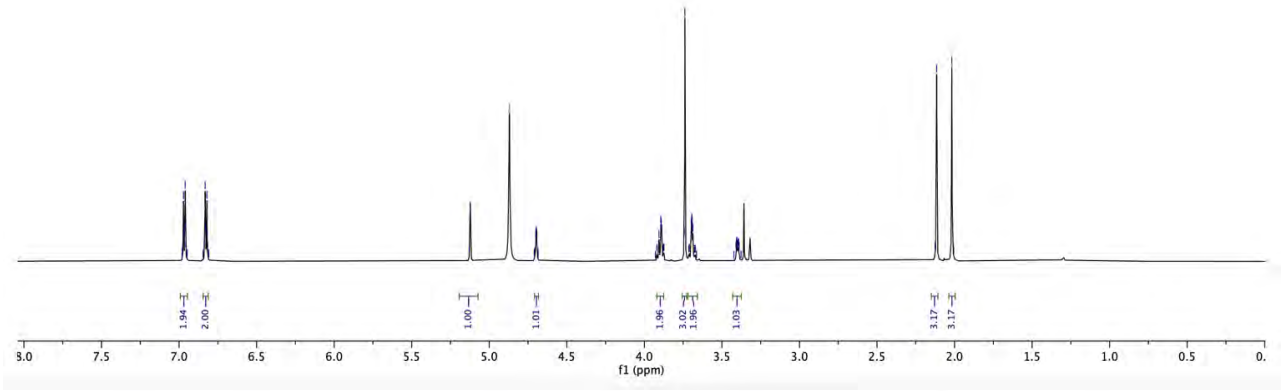


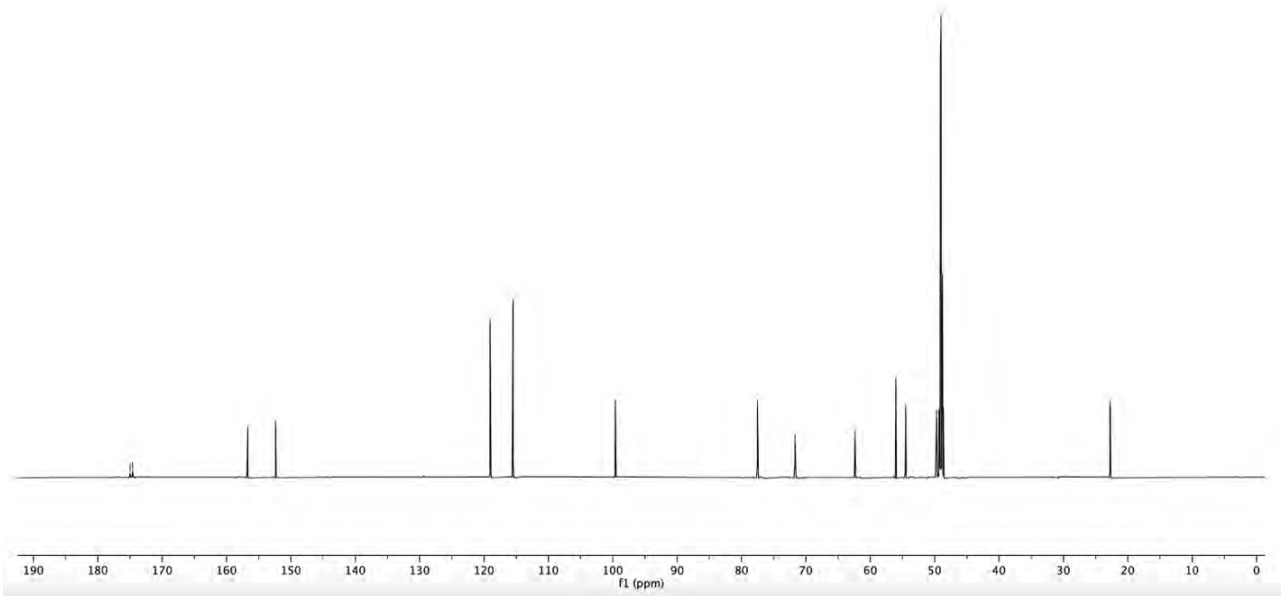
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-2,4-di-acetamido-3,6-dibenzoyl-2,4-dideoxy-β-D-mannopyranoside (**17**) in $CDCl_3$



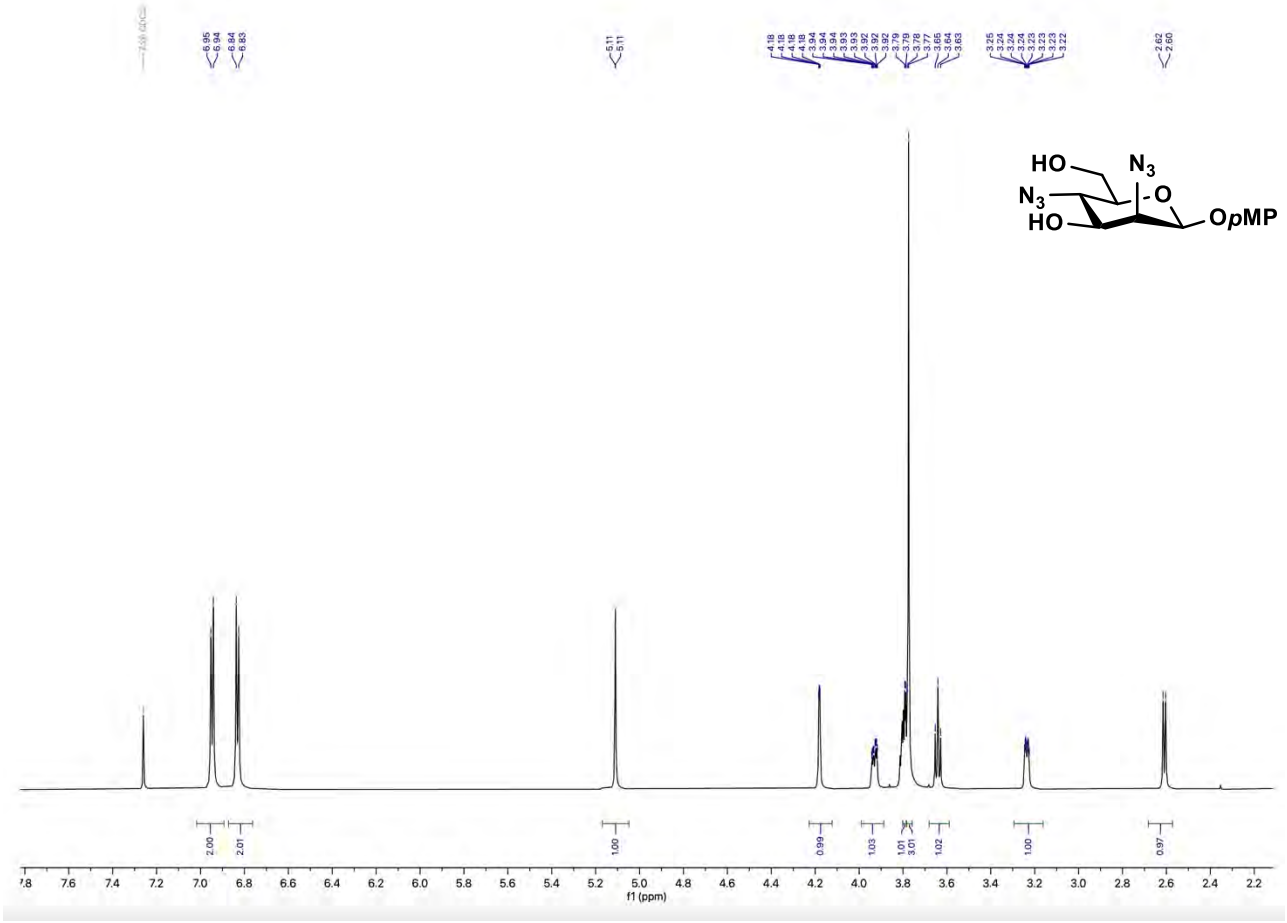


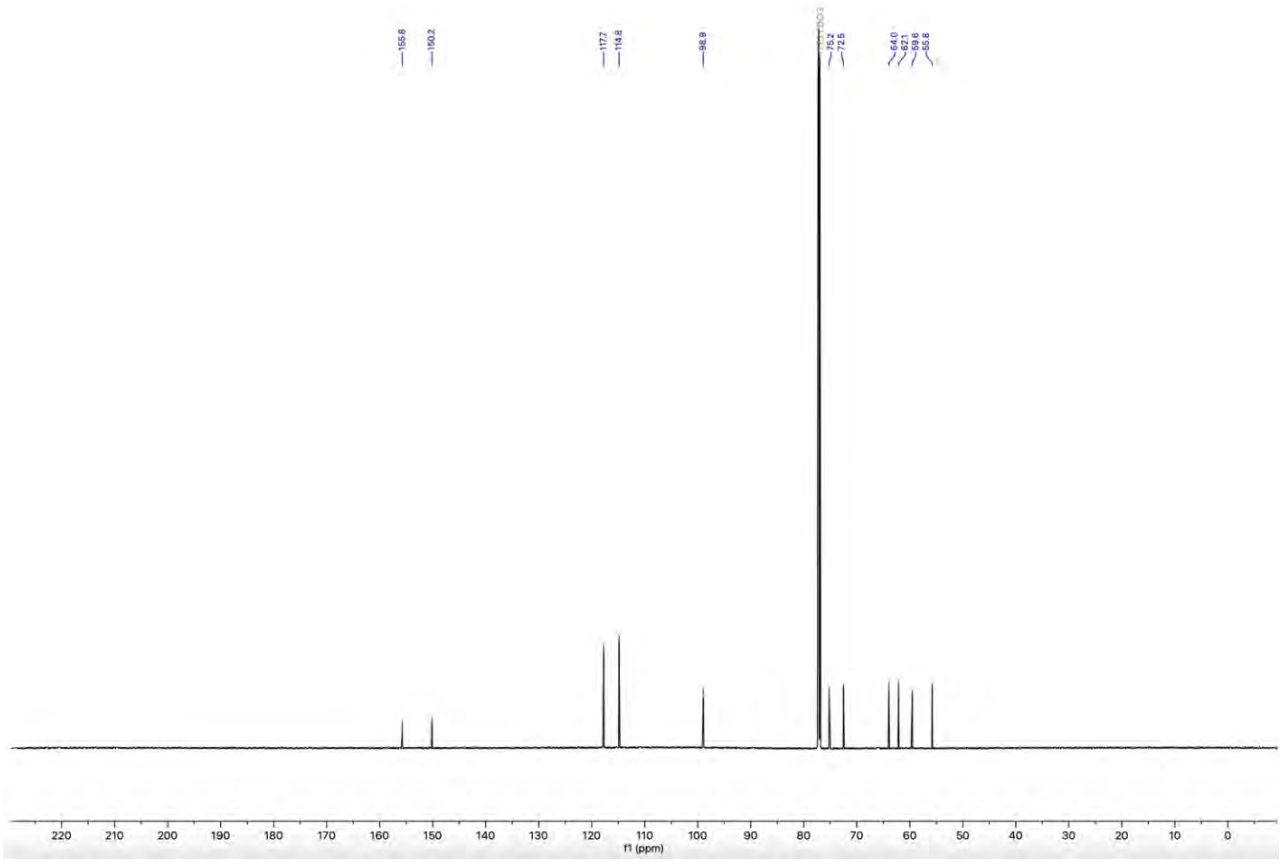
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-2,4-diacetamido-2,4-dideoxy-β-D-mannopyranoside (**18**) in CD_3OD



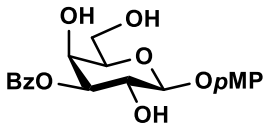


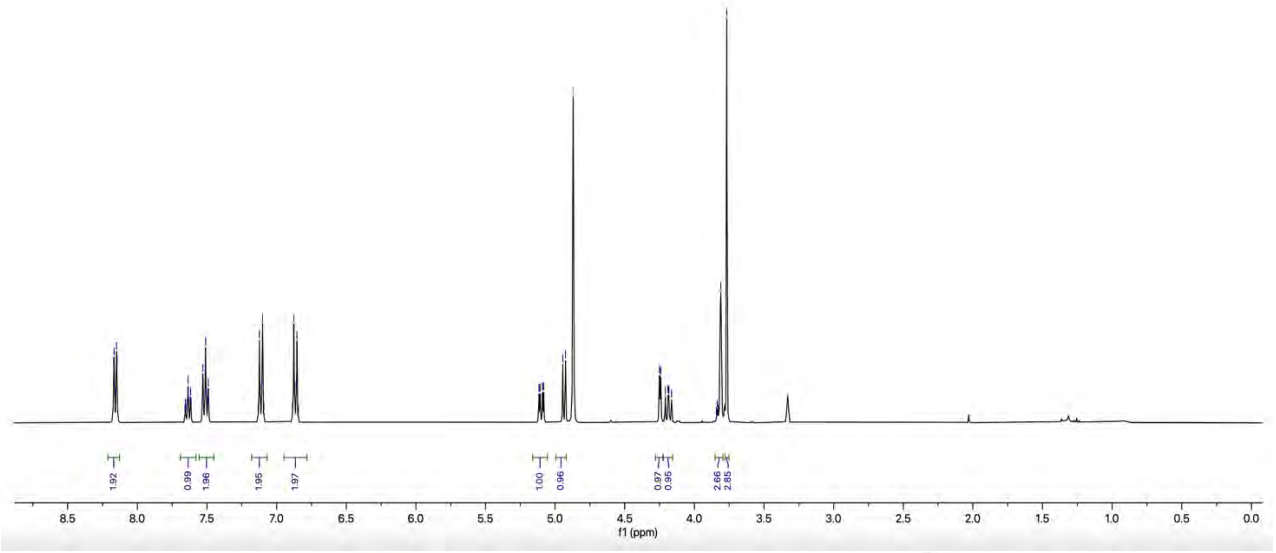
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-2,4-di-azido-2,4-dideoxy-β-D-mannopyranoside (**19**) in $CDCl_3$

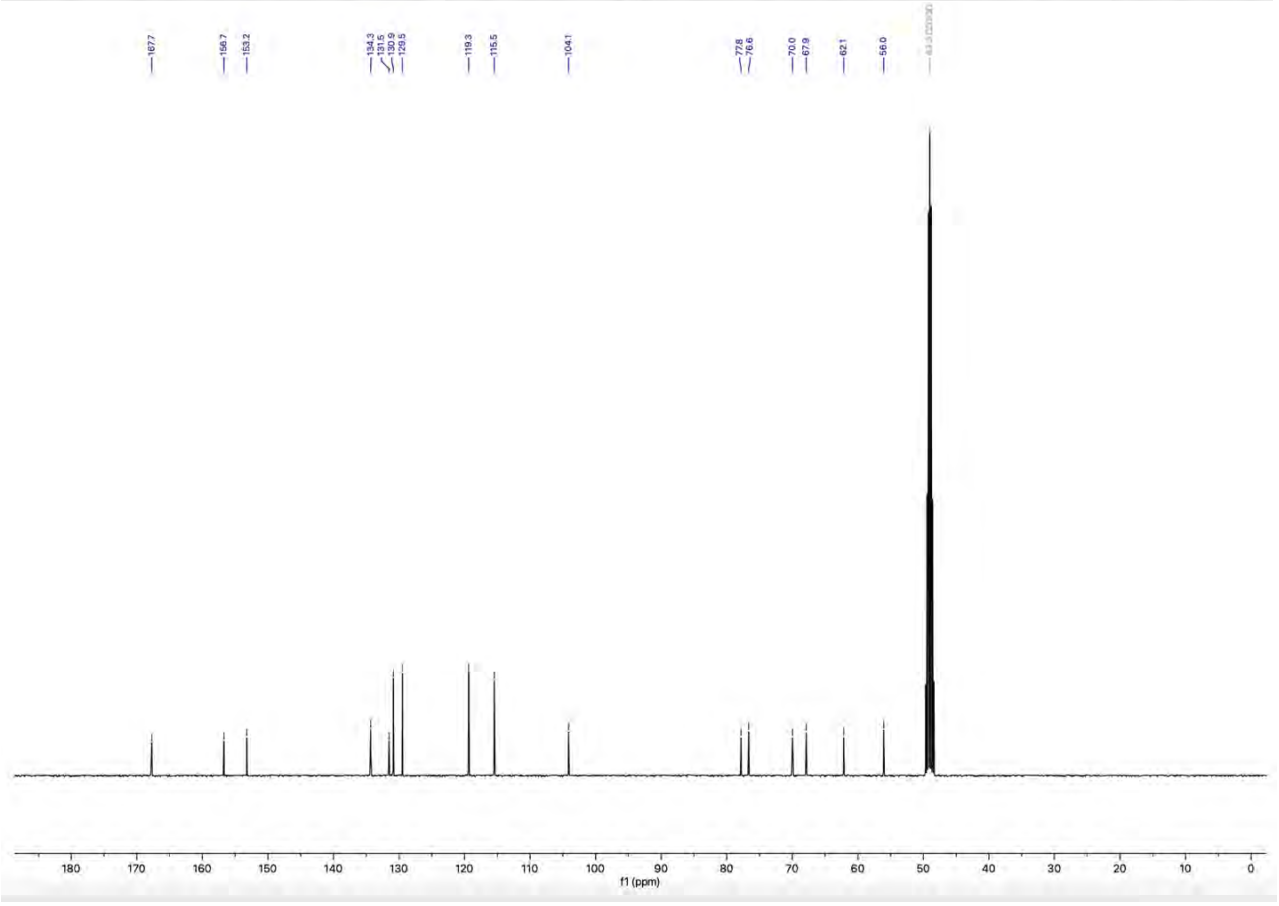




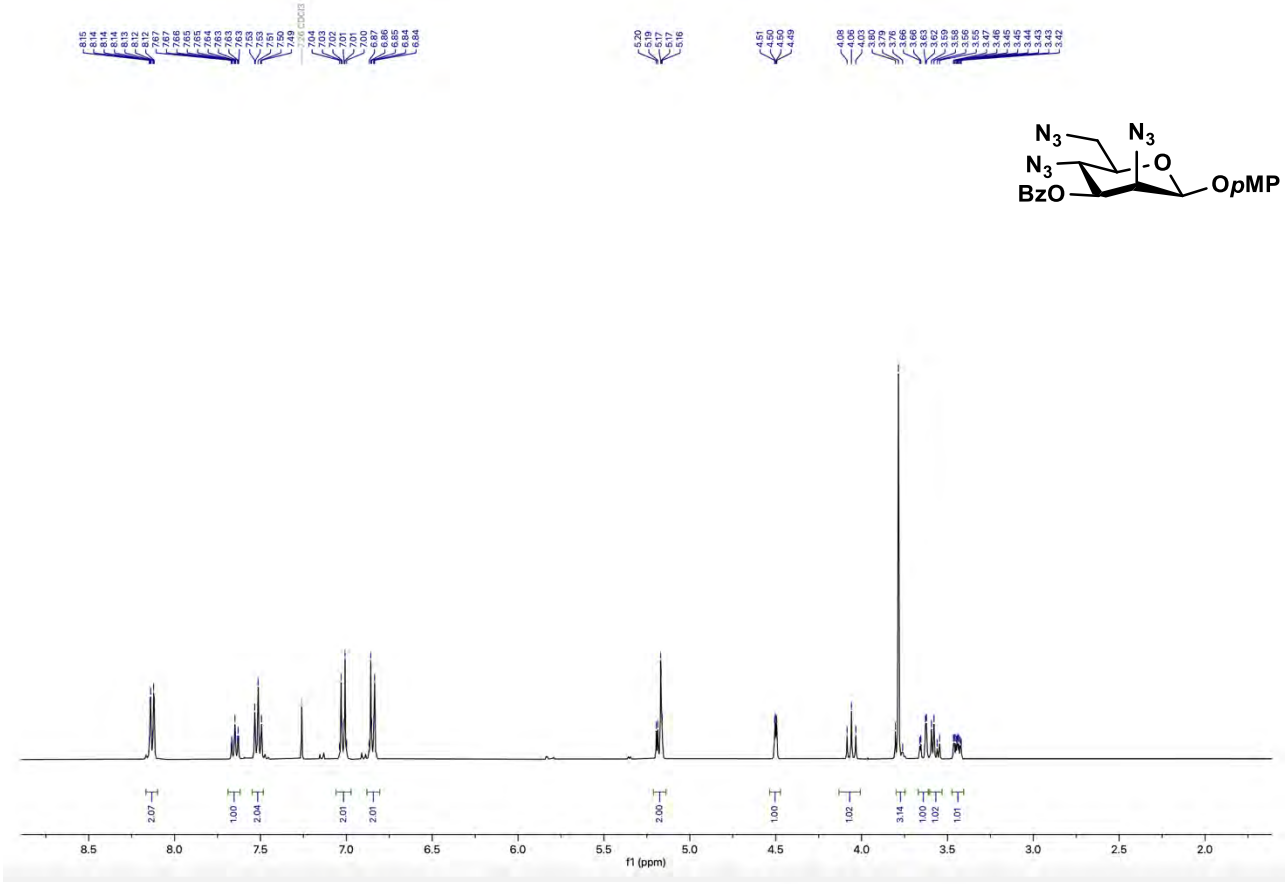
400 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-3-benzoyl-β-D-galactopyranoside (**20**) in CD_3OD

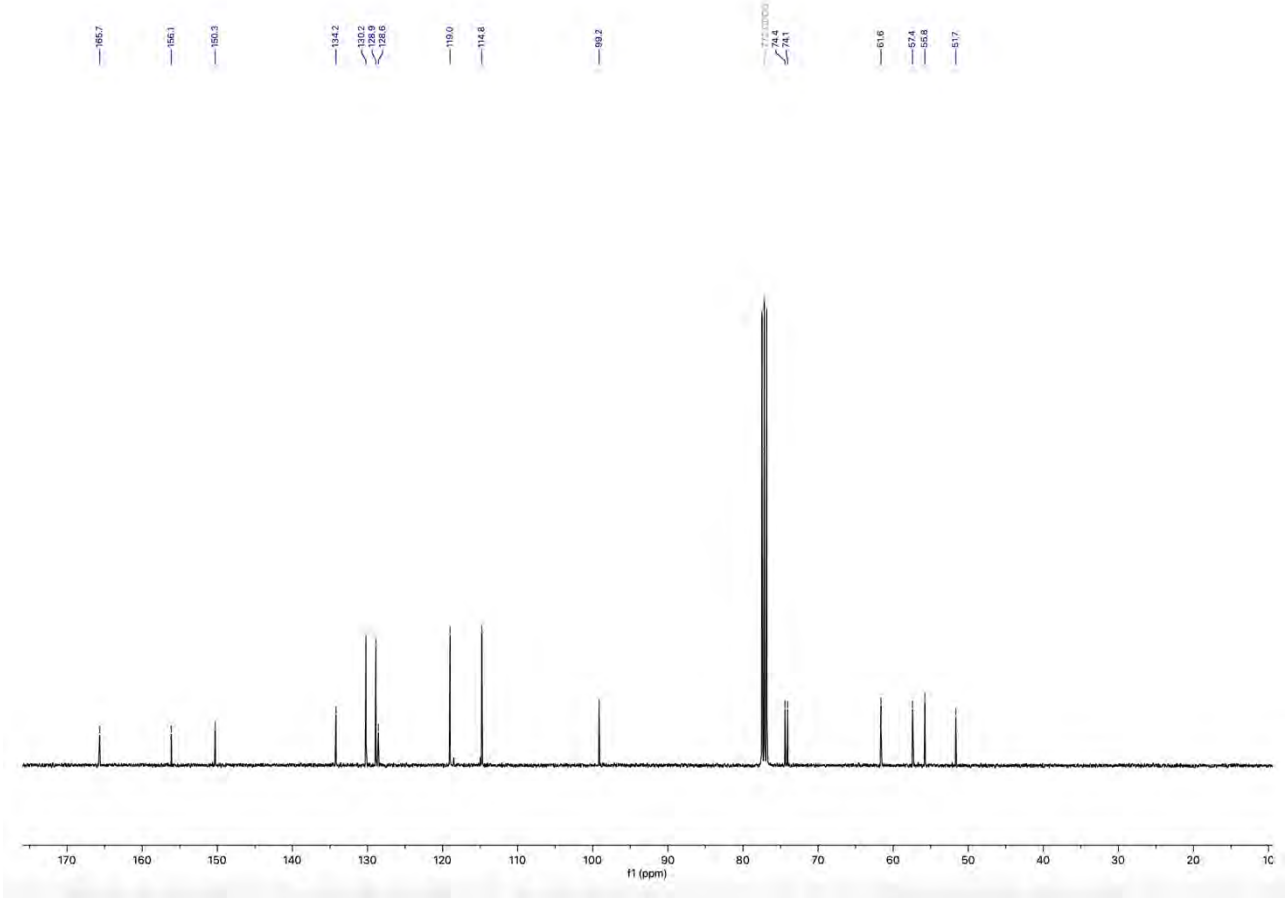




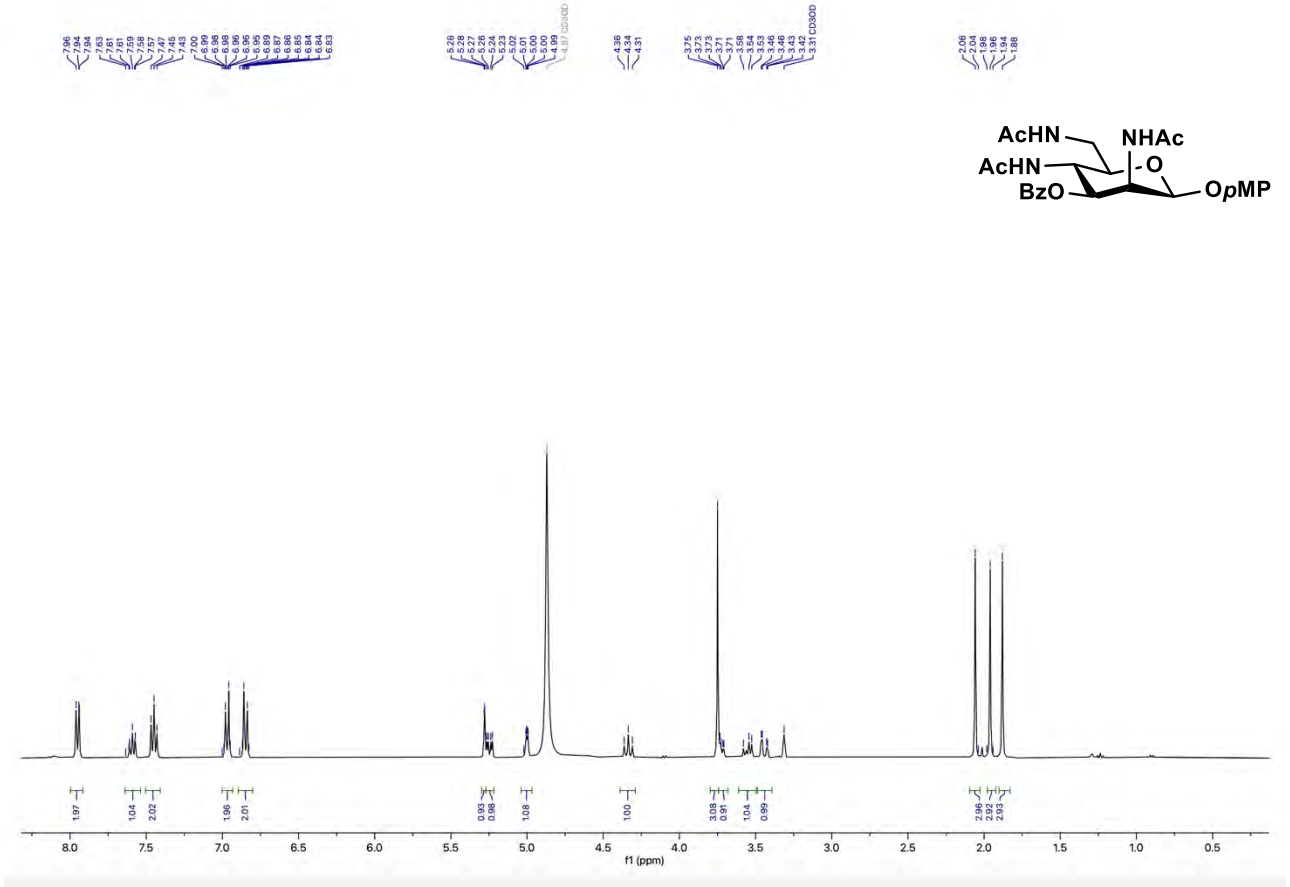


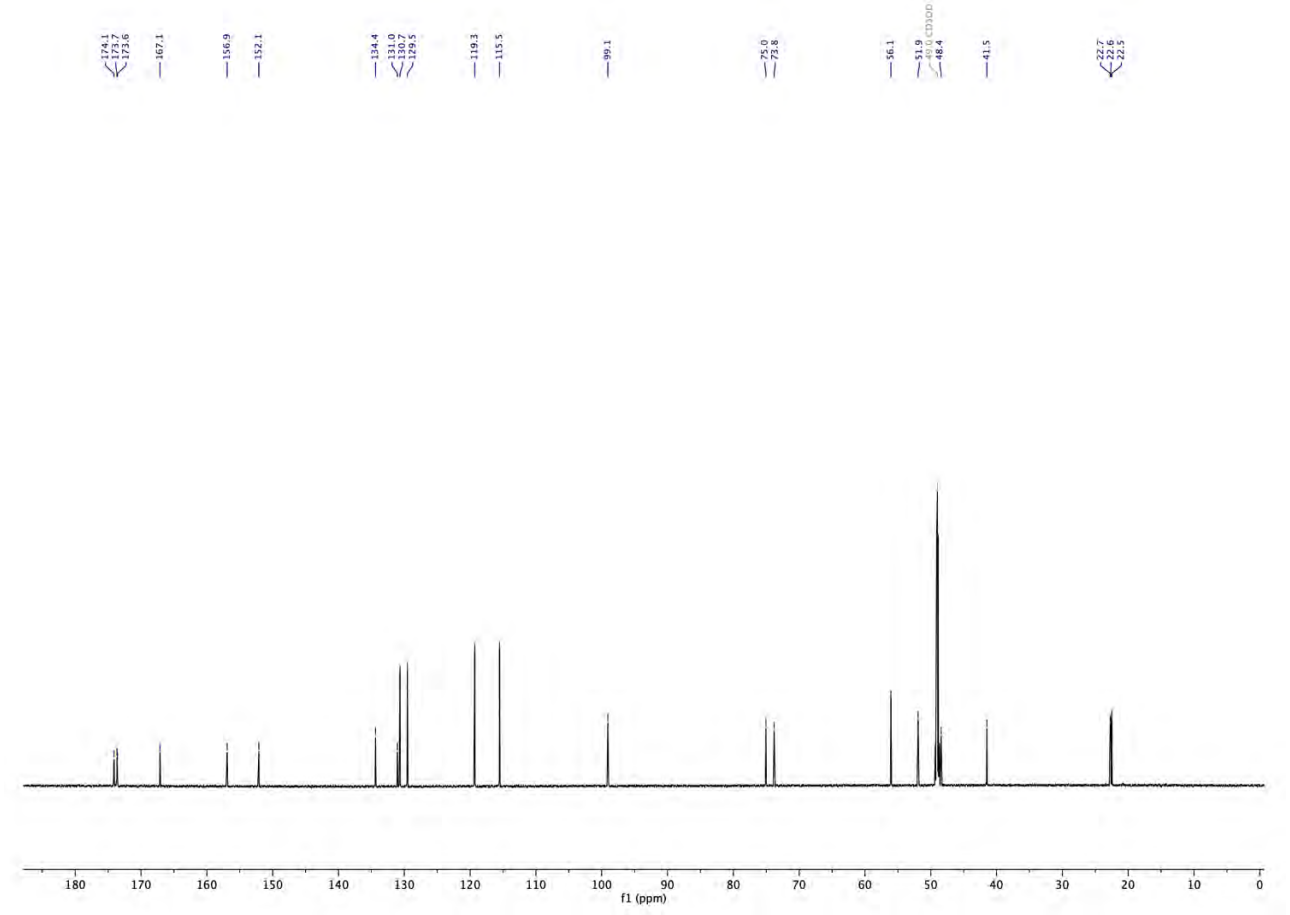
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-2,4,6-triazido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**21**) in $CDCl_3$



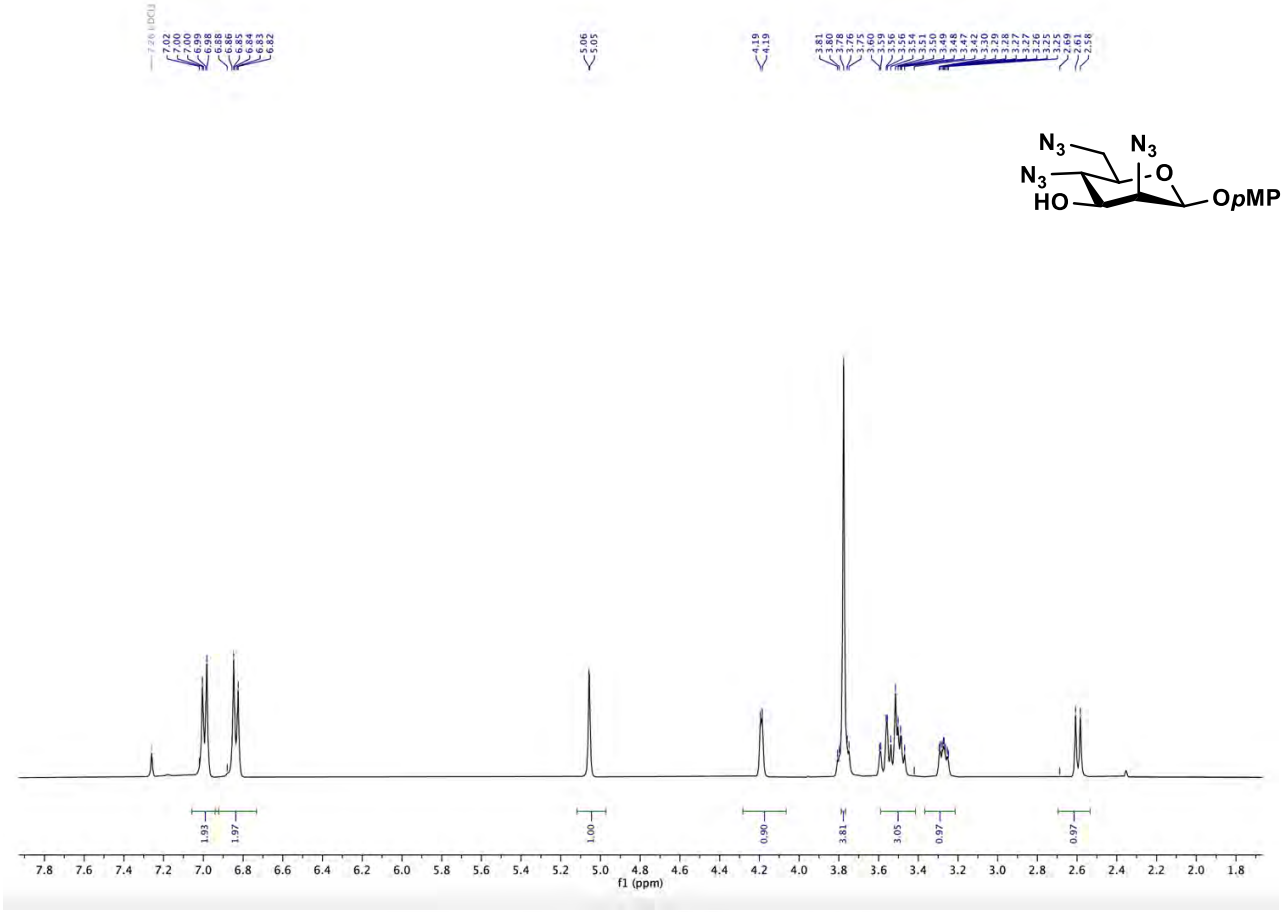


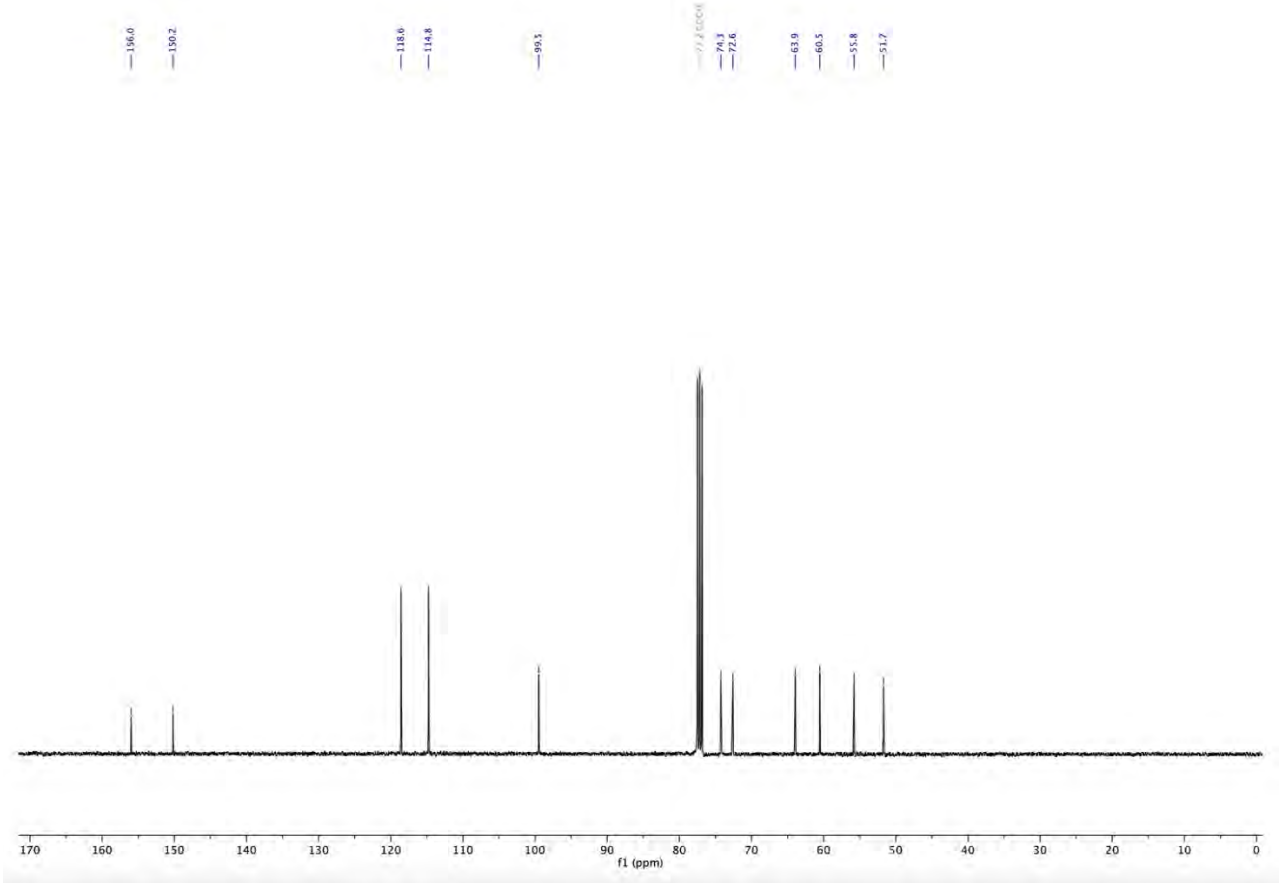
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxy phenyl-2,4,6-triacetamido-3-benzoyl-2,4,6-trideoxy-β-D-mannopyranoside (**22**) in CD_3OD



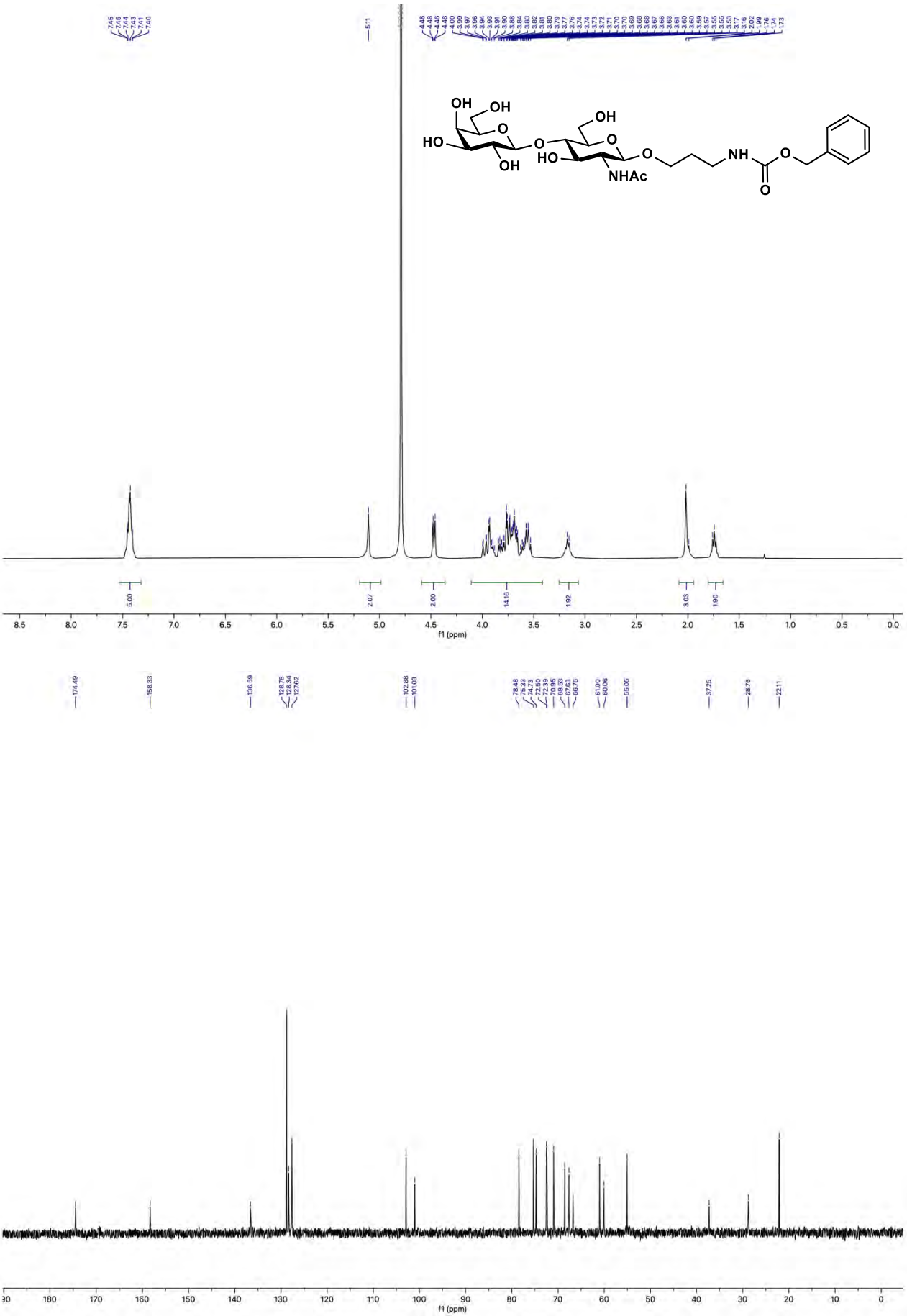


400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of *p*-methoxyphenyl-2,4,6-triazido-2,4,6-trideoxy-β-D-mannopyranoside (**23**) in $CDCl_3$

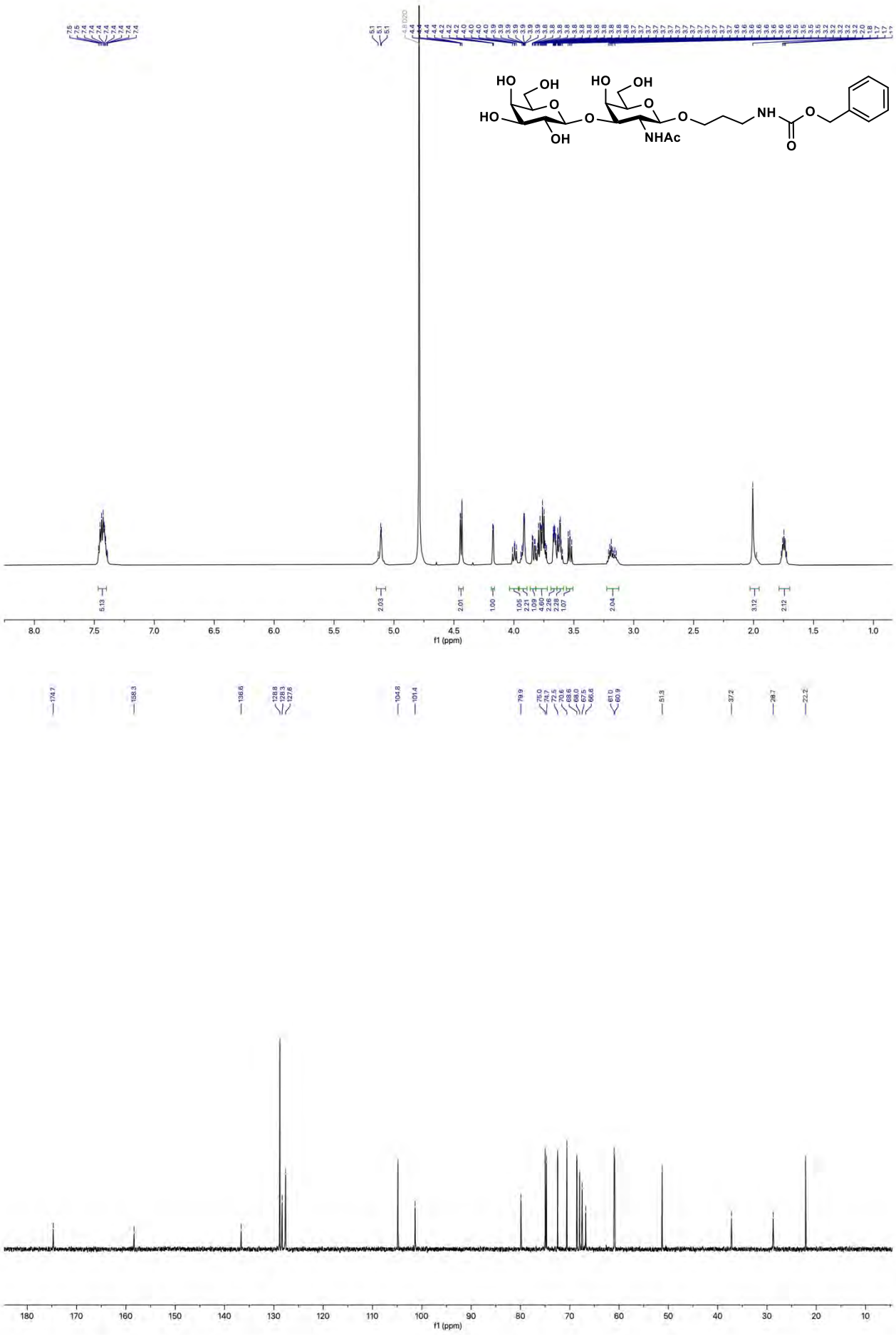




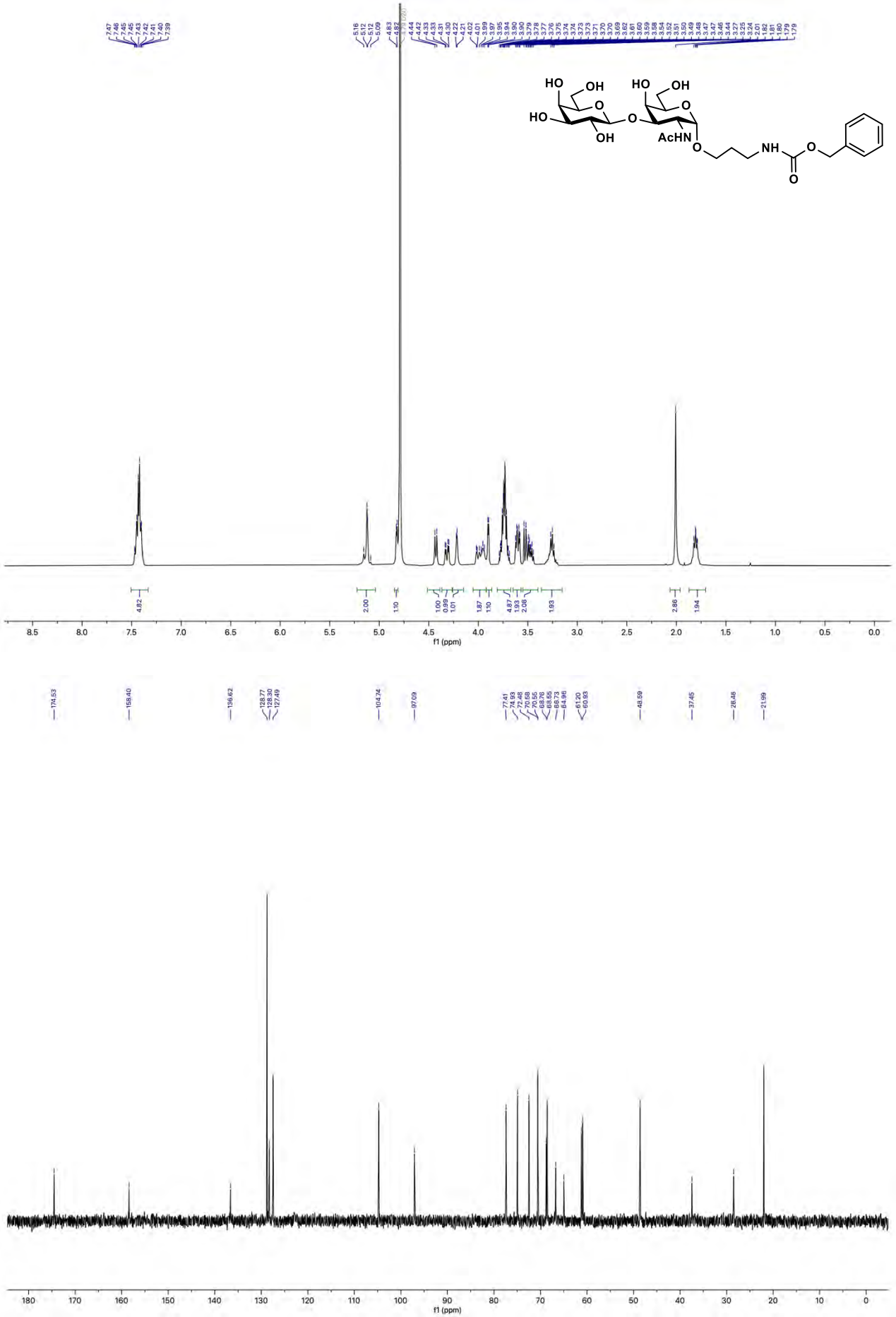
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of LacNAcβProNHCbz (**25**) in D_2O



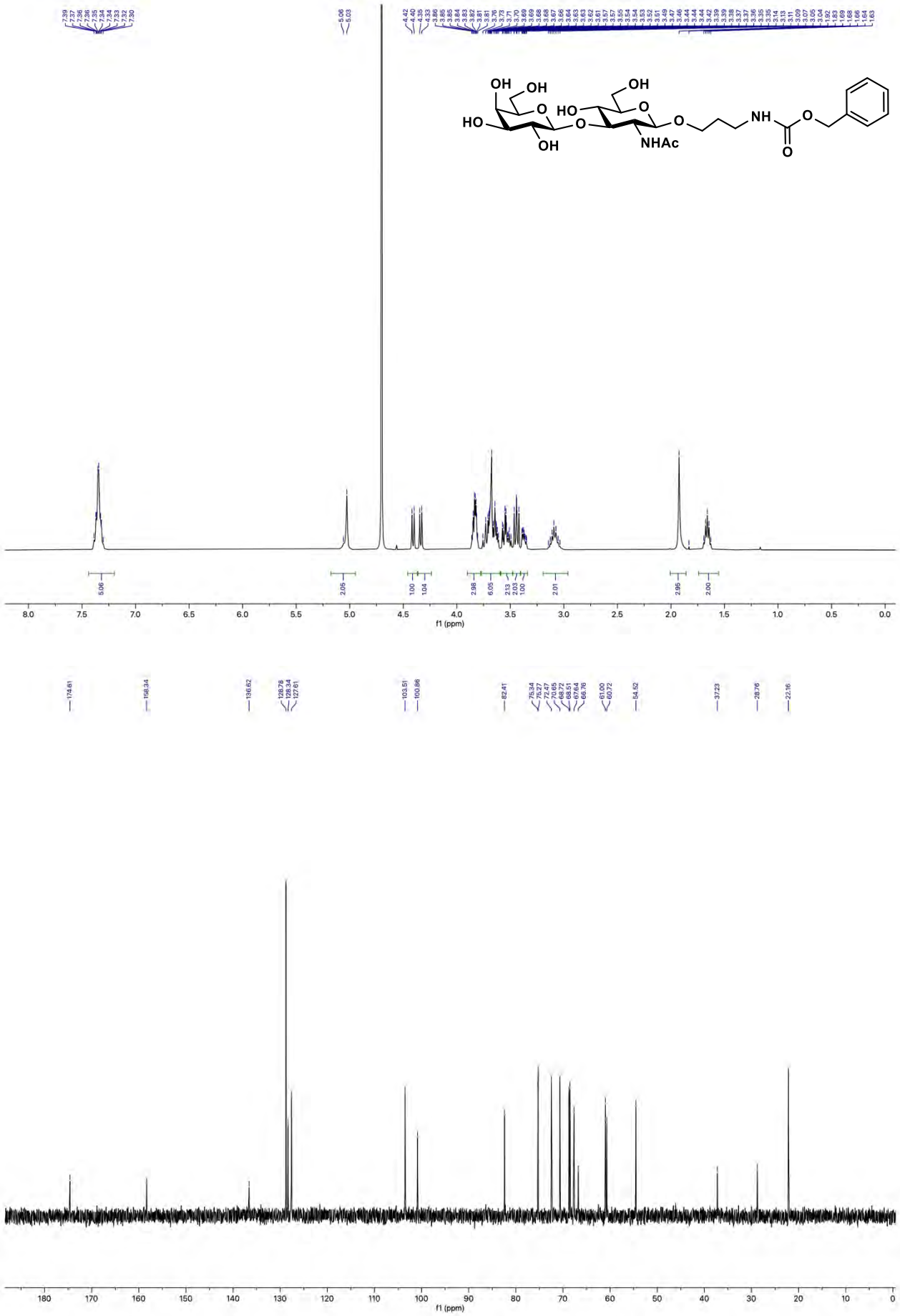
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Galβ1–3GalNAcβProNHCbz (**26**) in D_2O



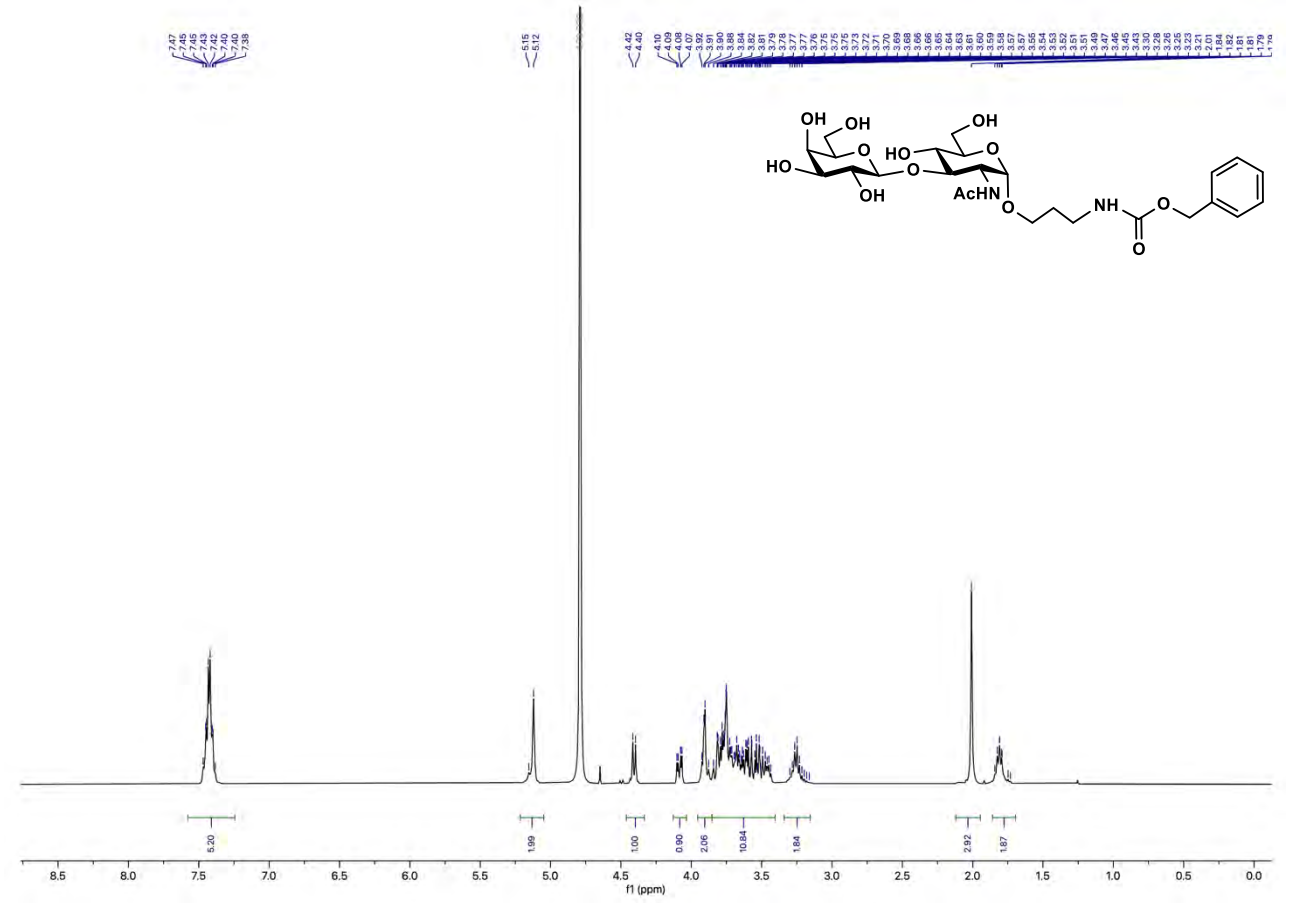
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Galβ1–3GalNAcαProNHCbz (**27**) in D_2O

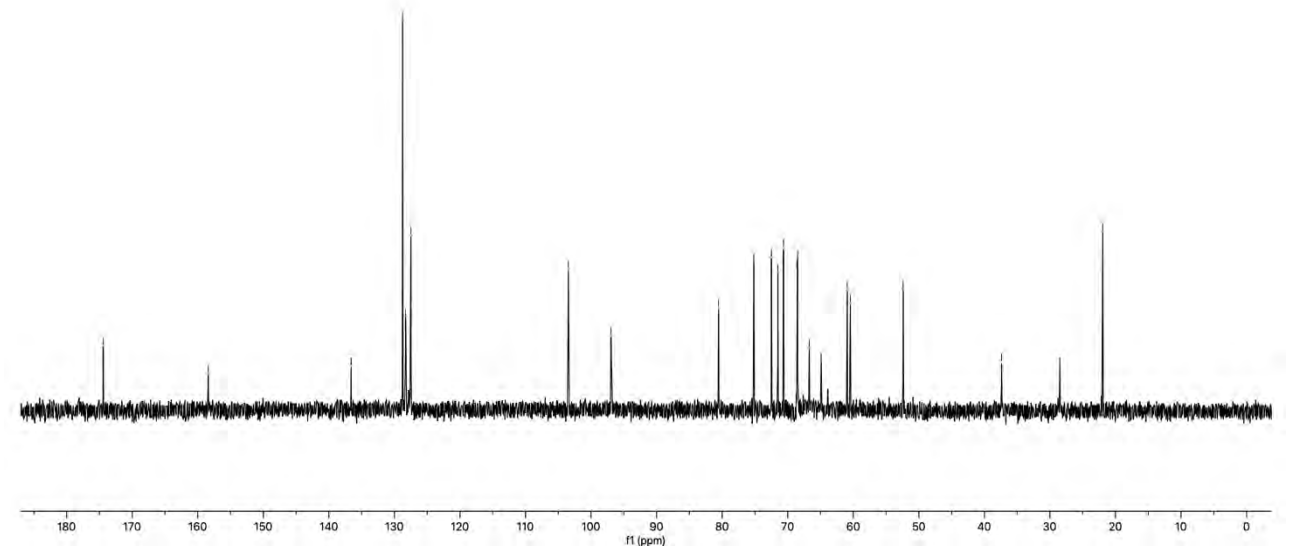


400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Galβ1−3GlcNAcβProNHCbz (**28**) in D_2O

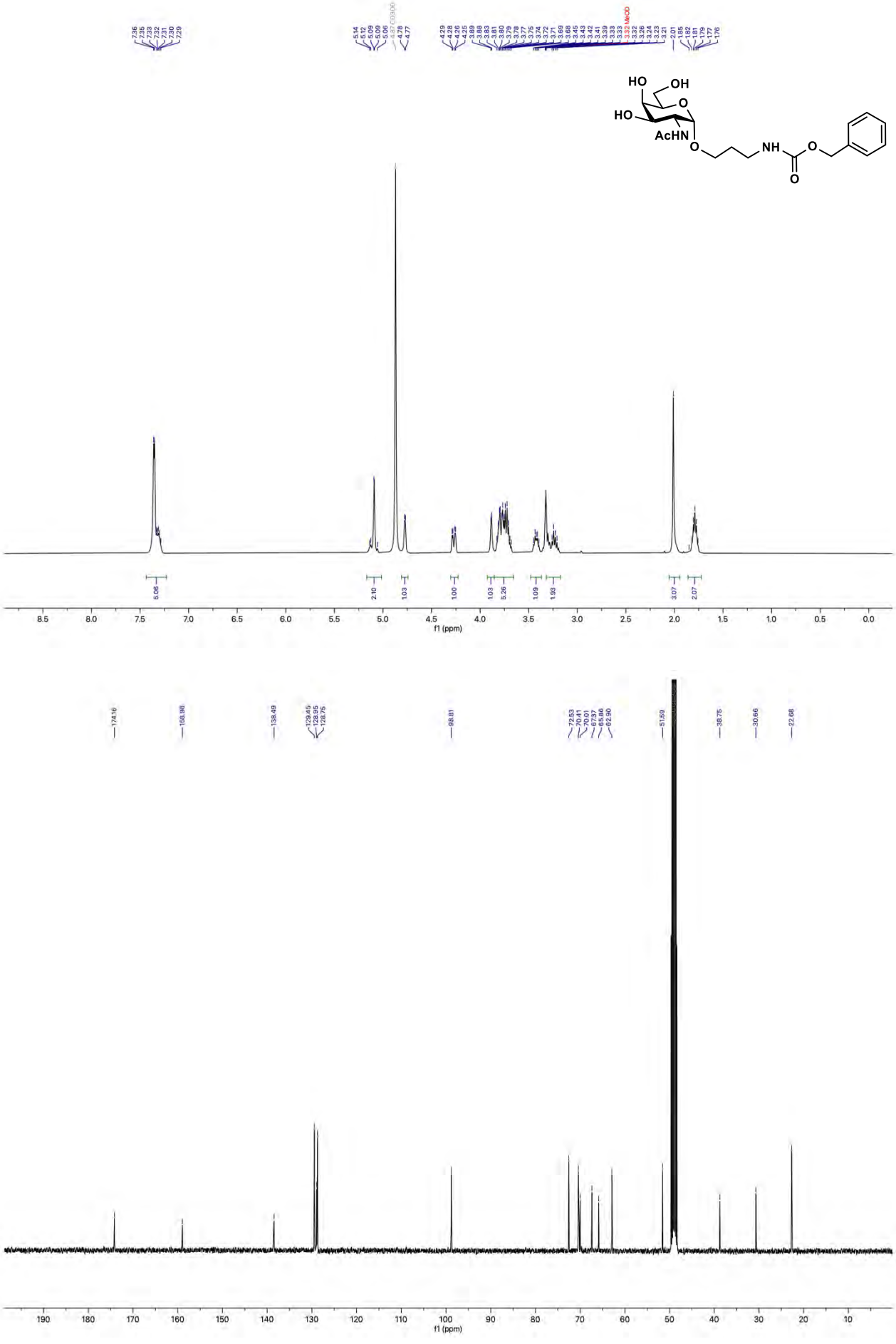


400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Galβ1–3GlcNAcαProNHCbz (**29**) in D_2O

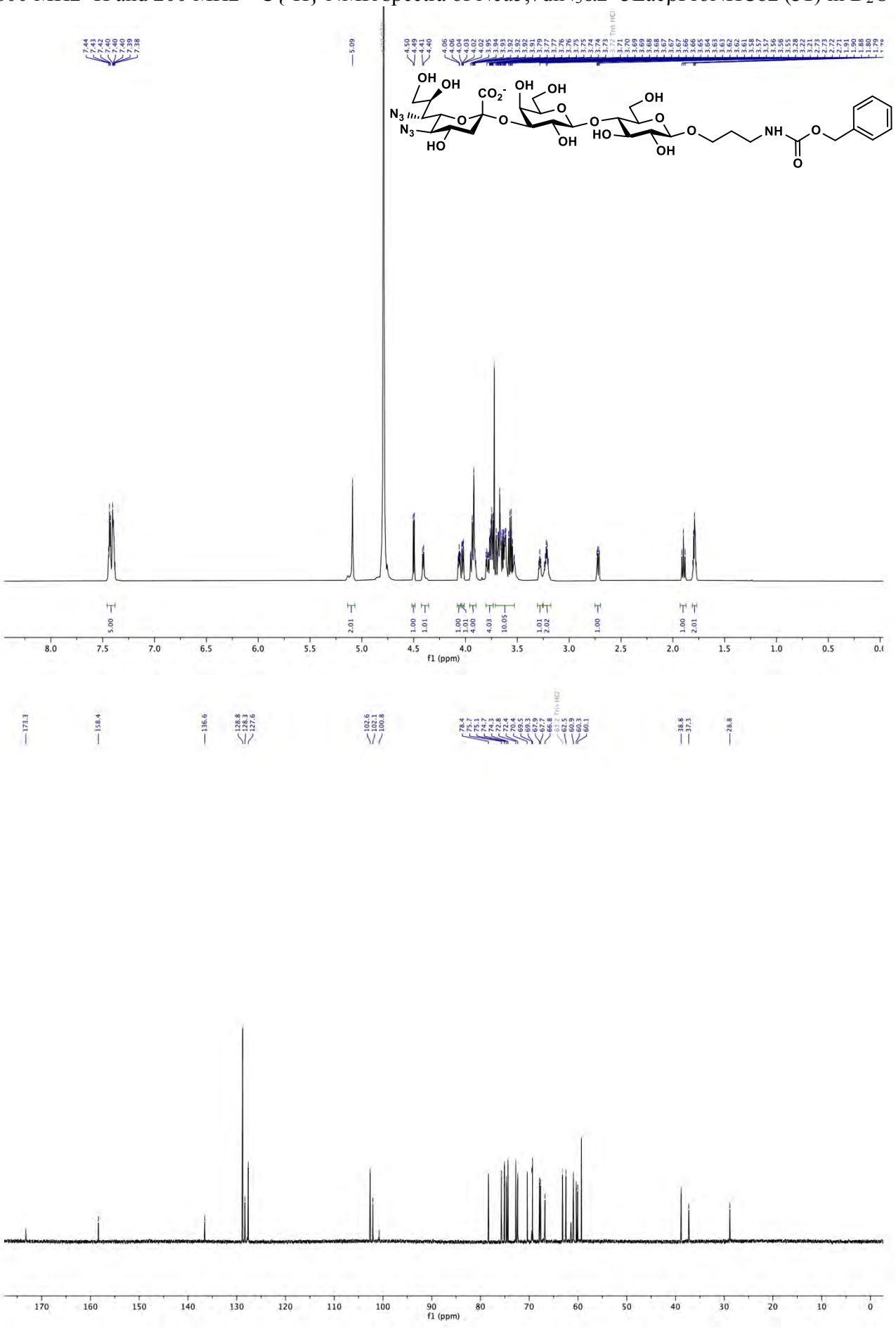




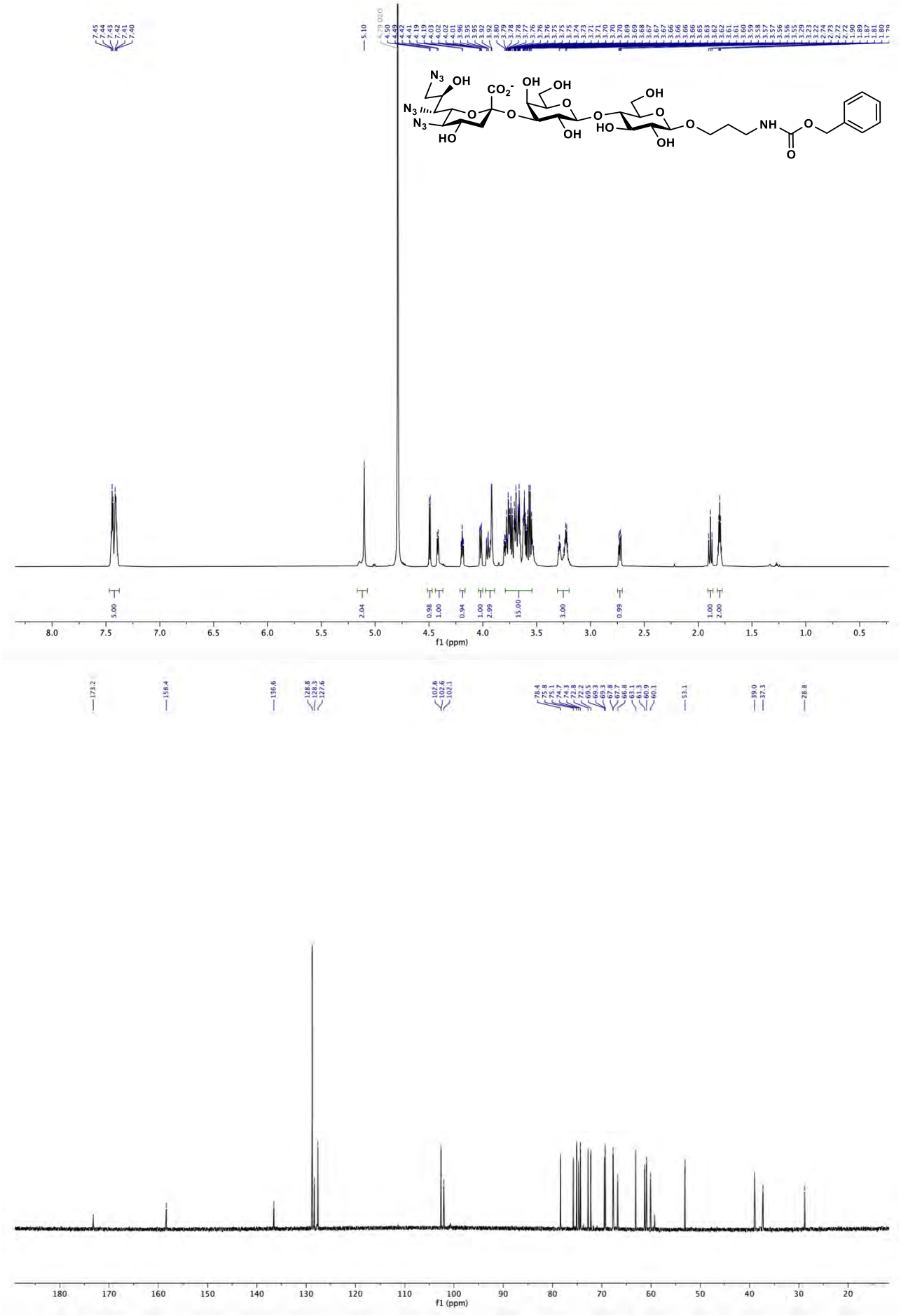
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of GalNAcαProNHCbz (**30**) in CD_3OD



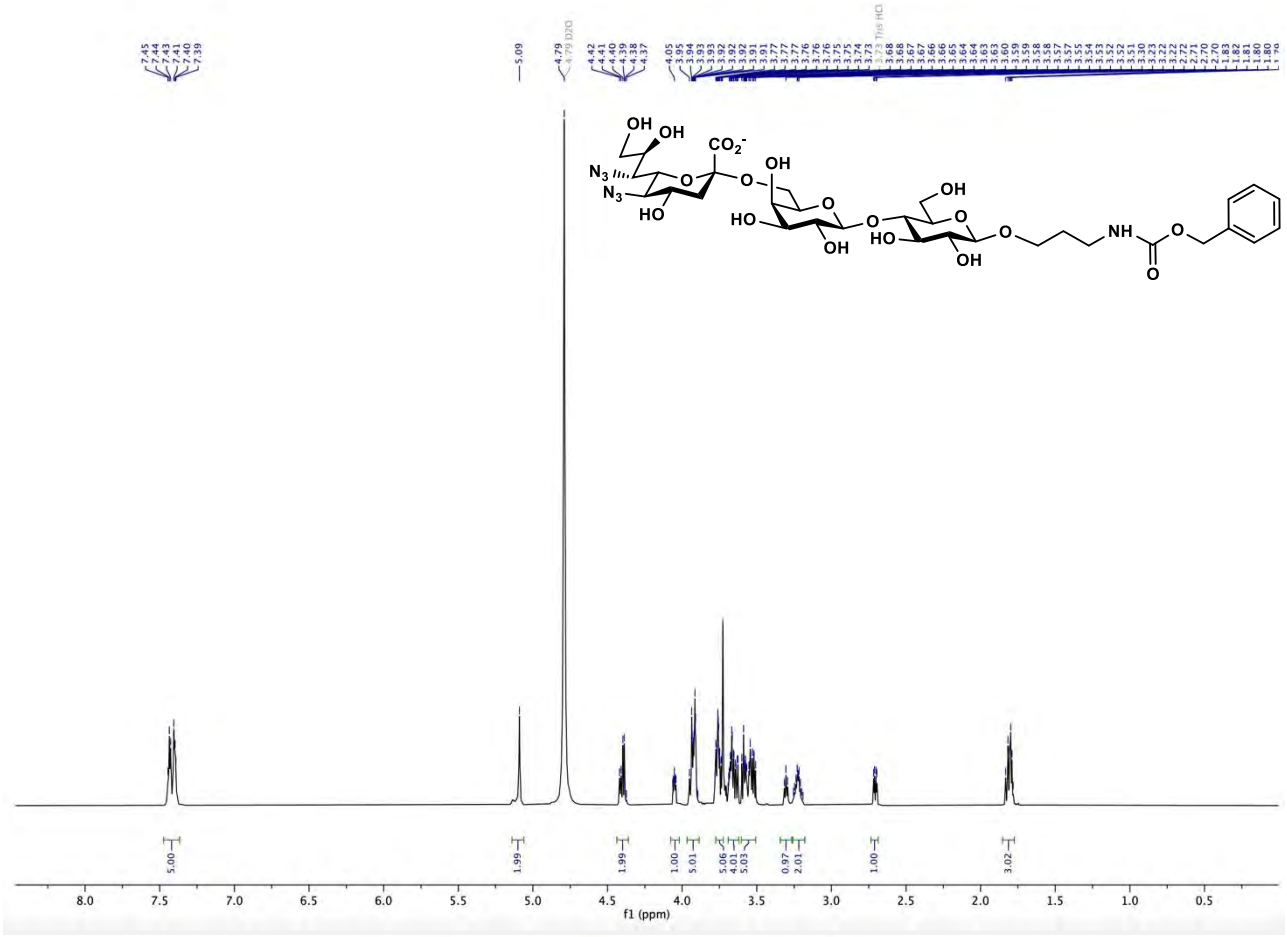
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN_3α2–3LacβProNHCbz (**31**) in D_2O

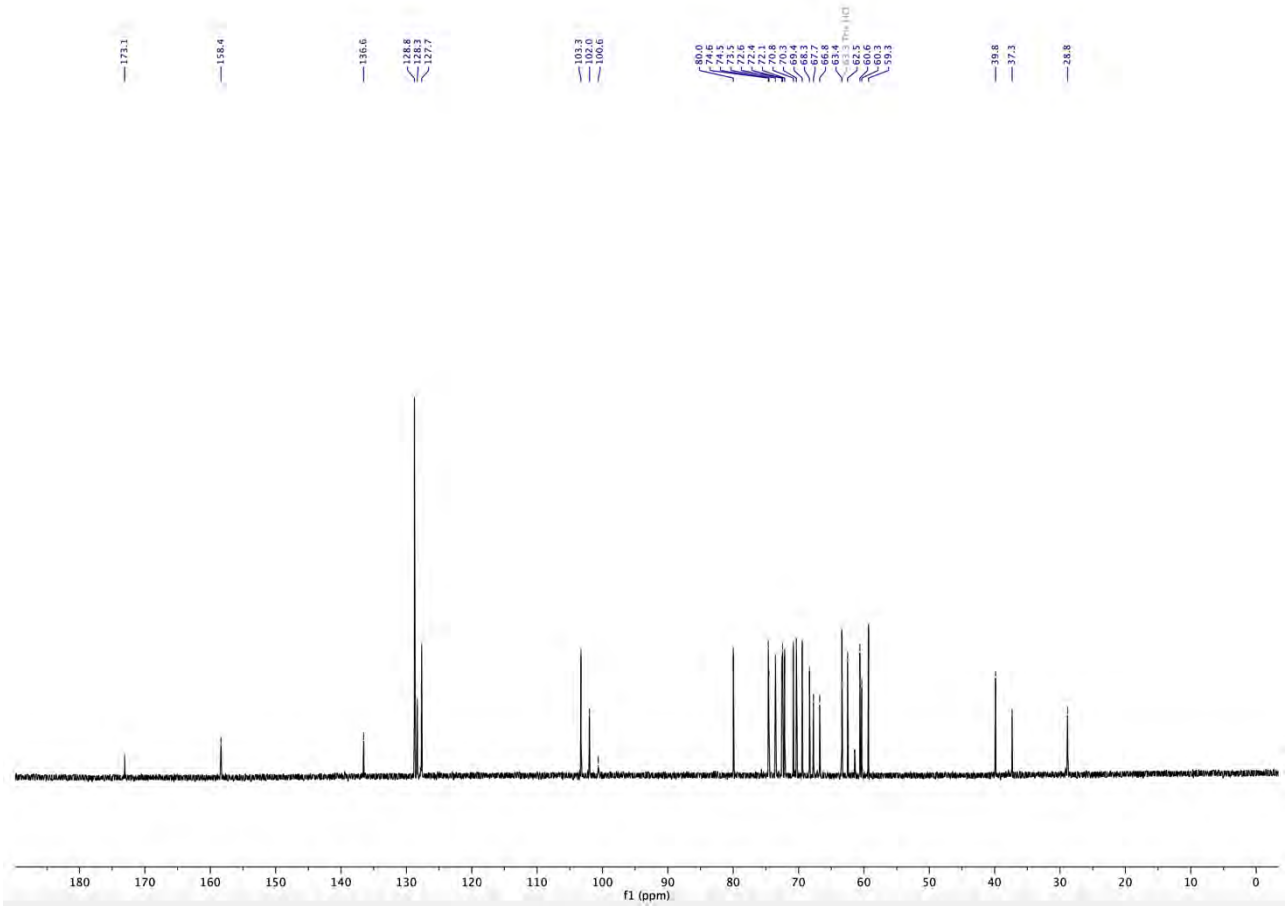


800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN$_3$α2–3LacβProNHCbz (**32**) in D_2O

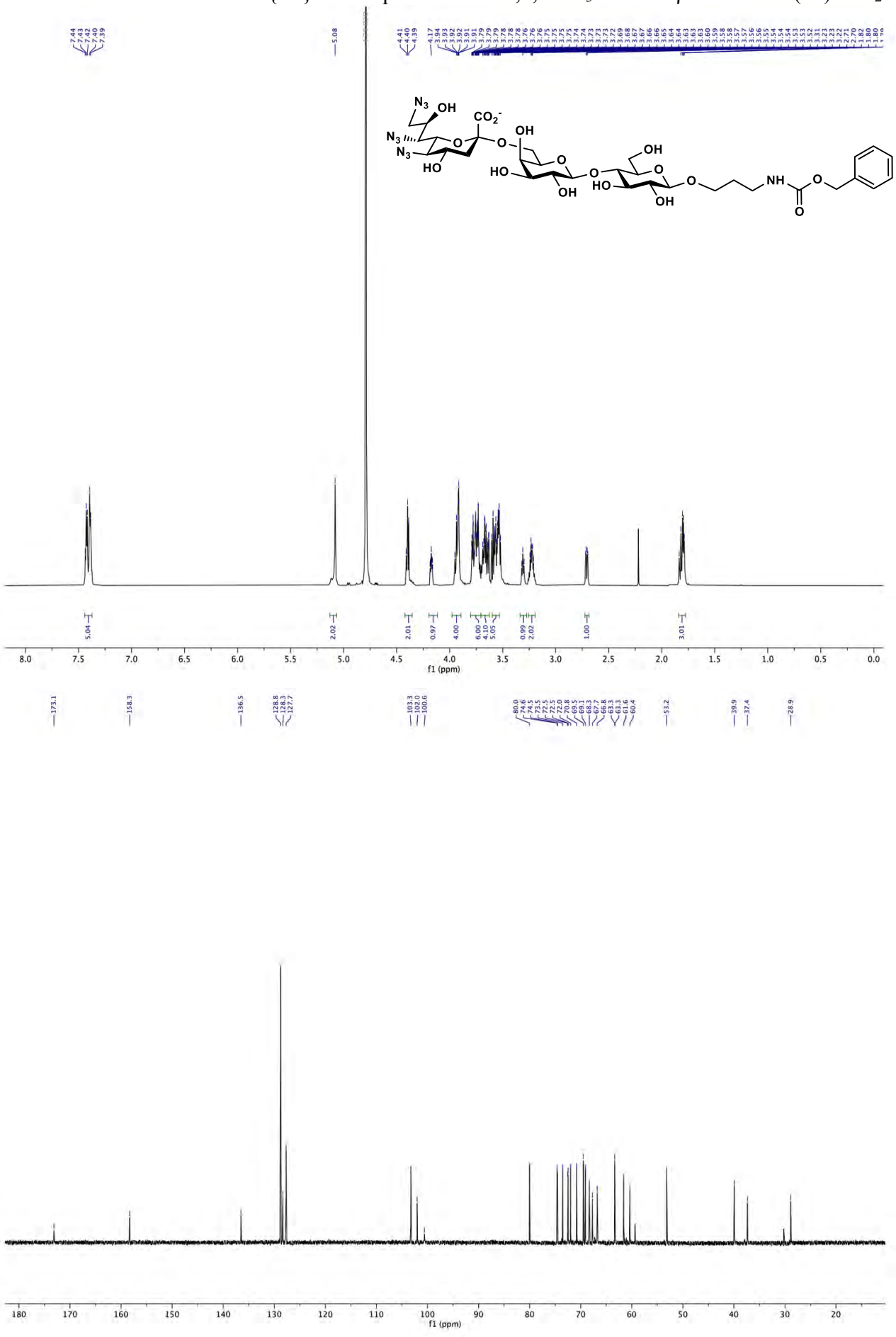


800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN_3α2–6LacβProNHCbz (**33**) in D_2O

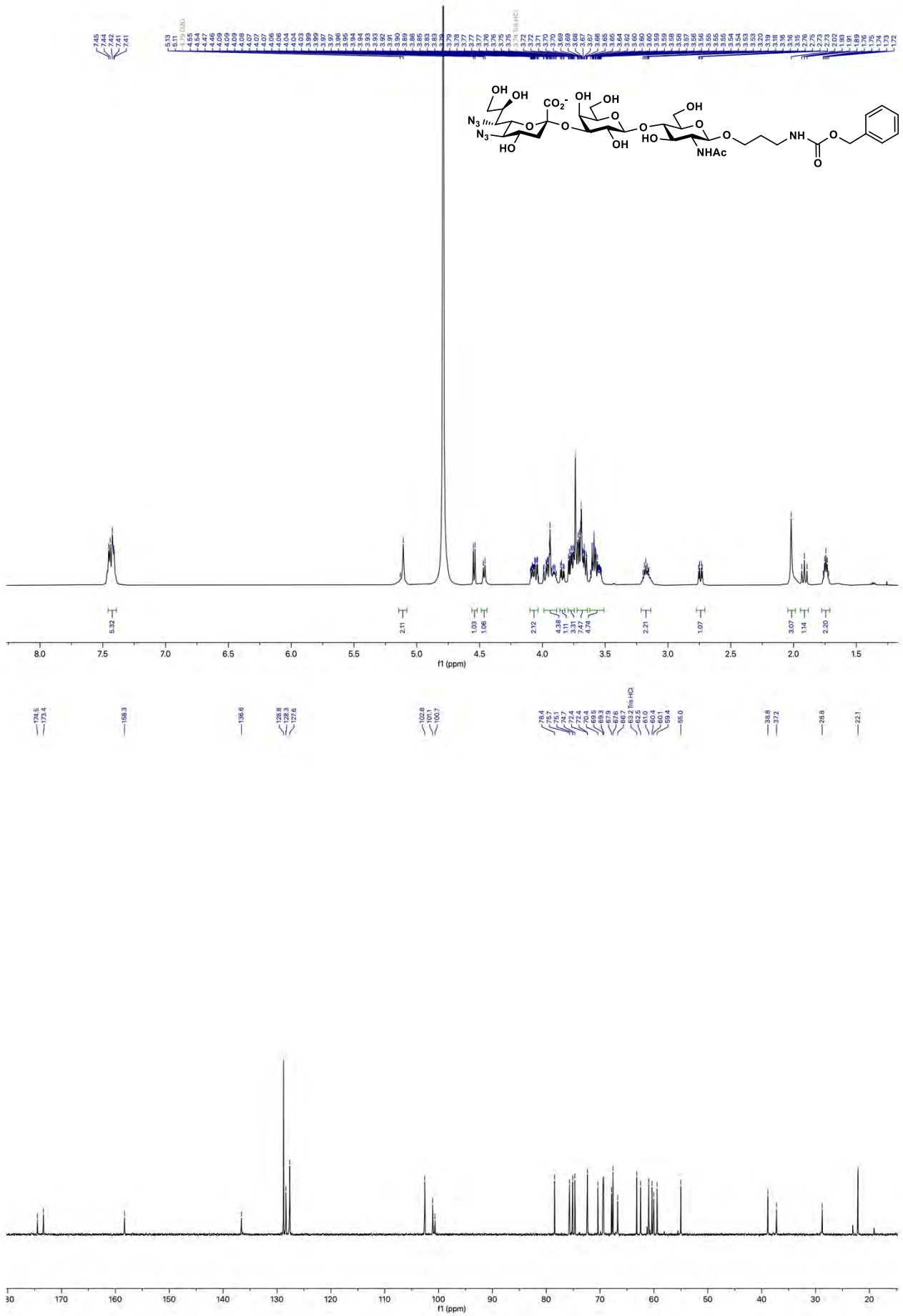




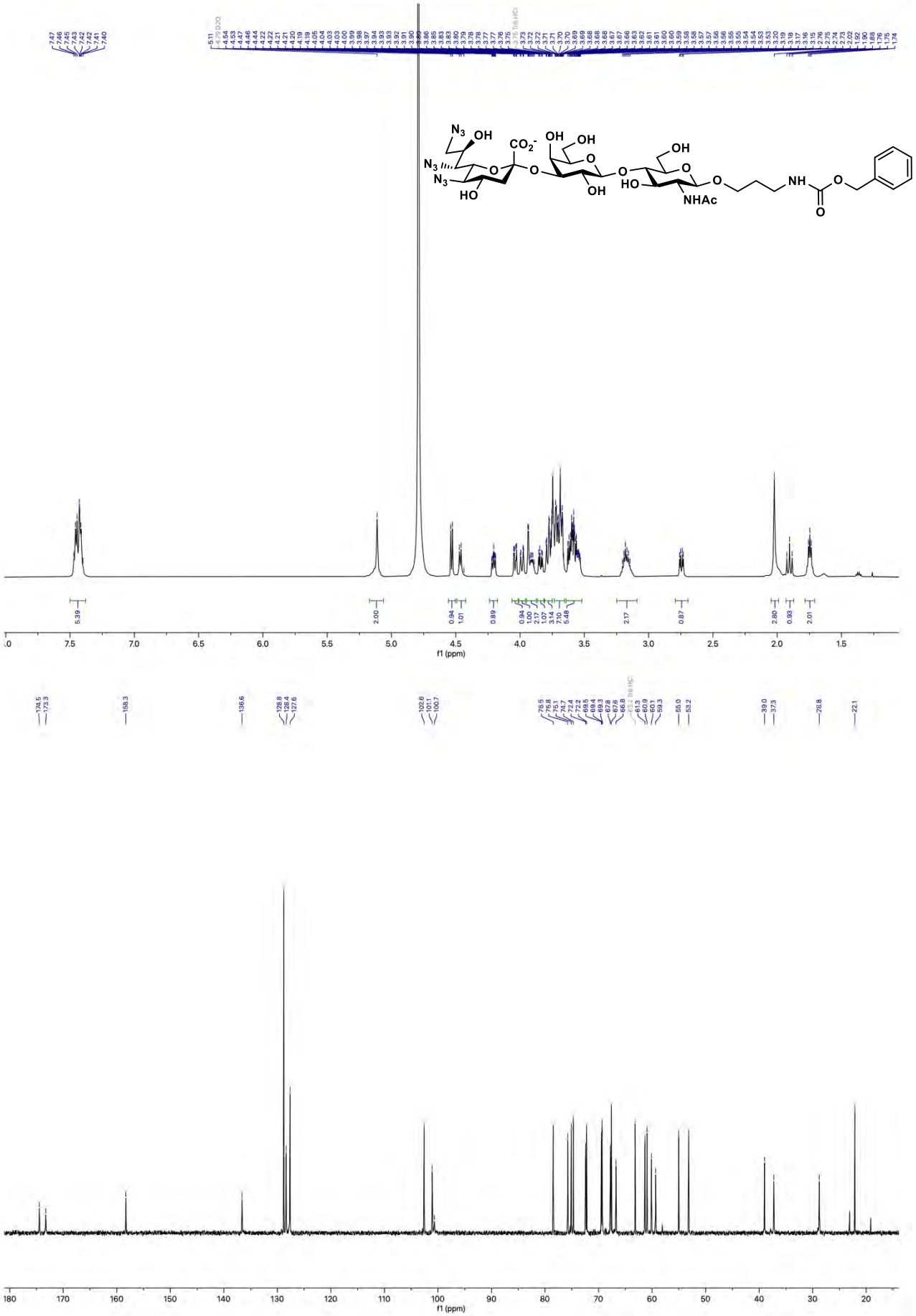
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN$_3$α2–6LacβProNHCbz (**34**) in D_2O



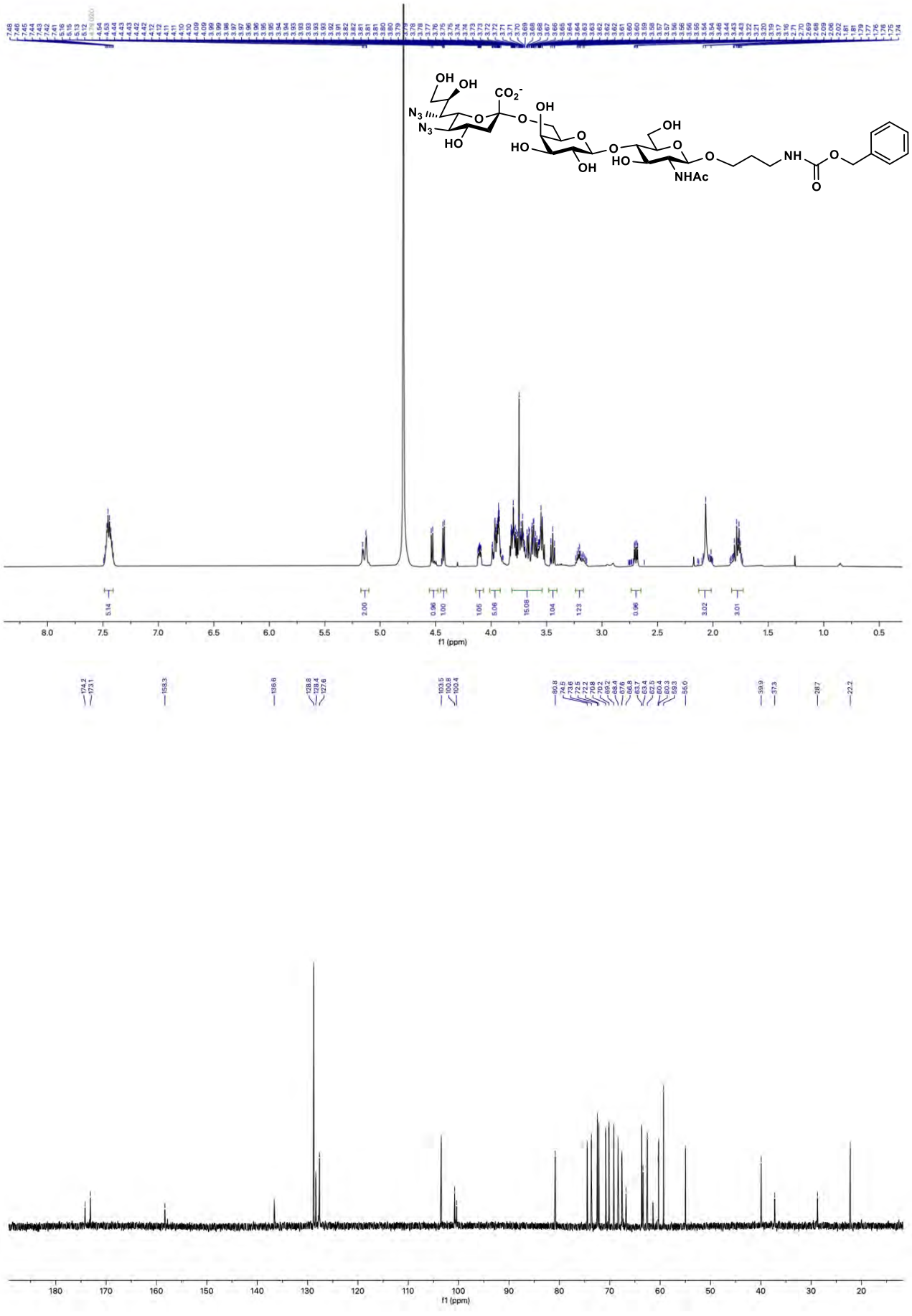
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$α2–3LacNAcβProNHCbz (**35**) in D_2O



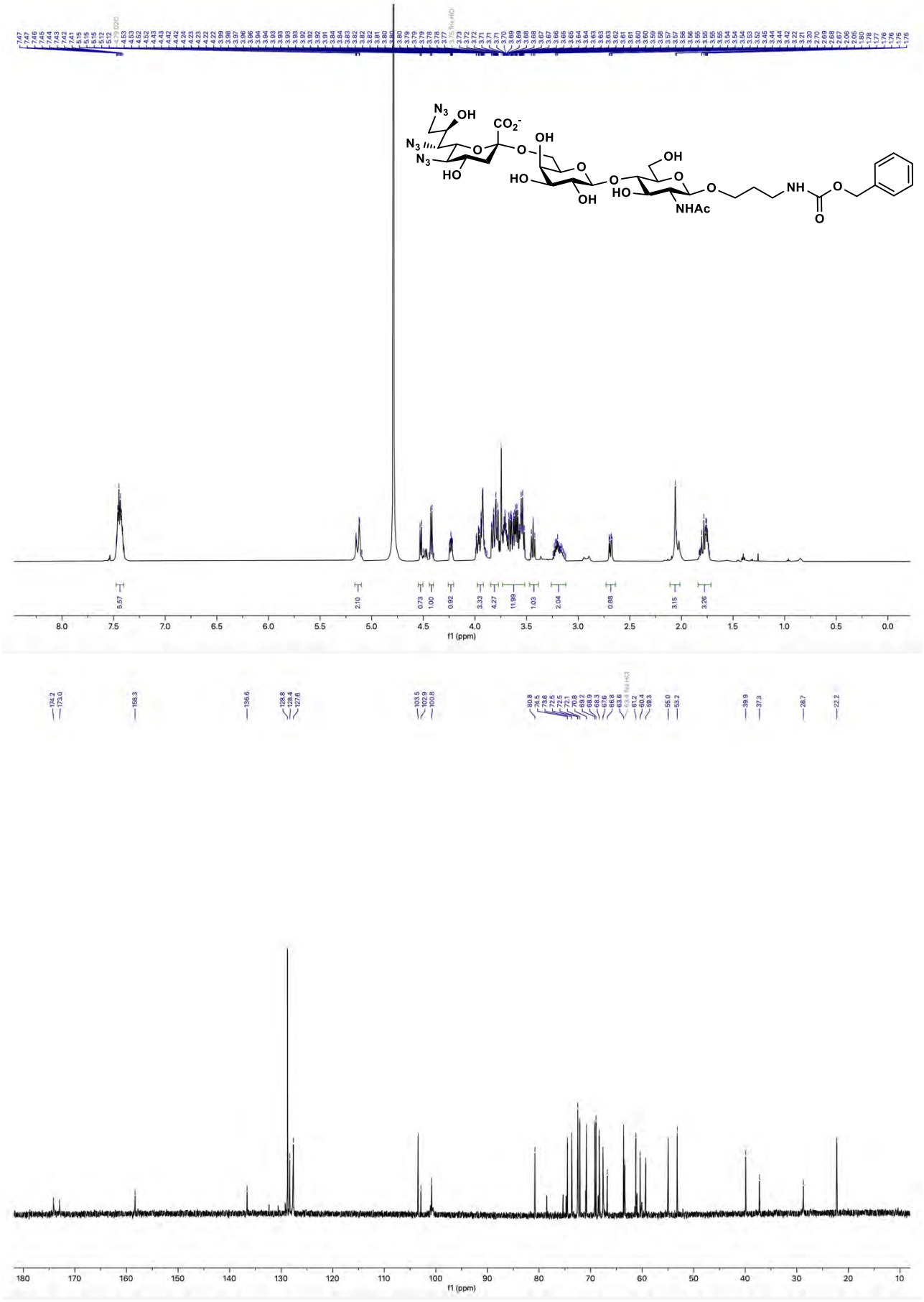
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN$_3$α2–3LacNAcβProNHCbz (**36**) in D_2O



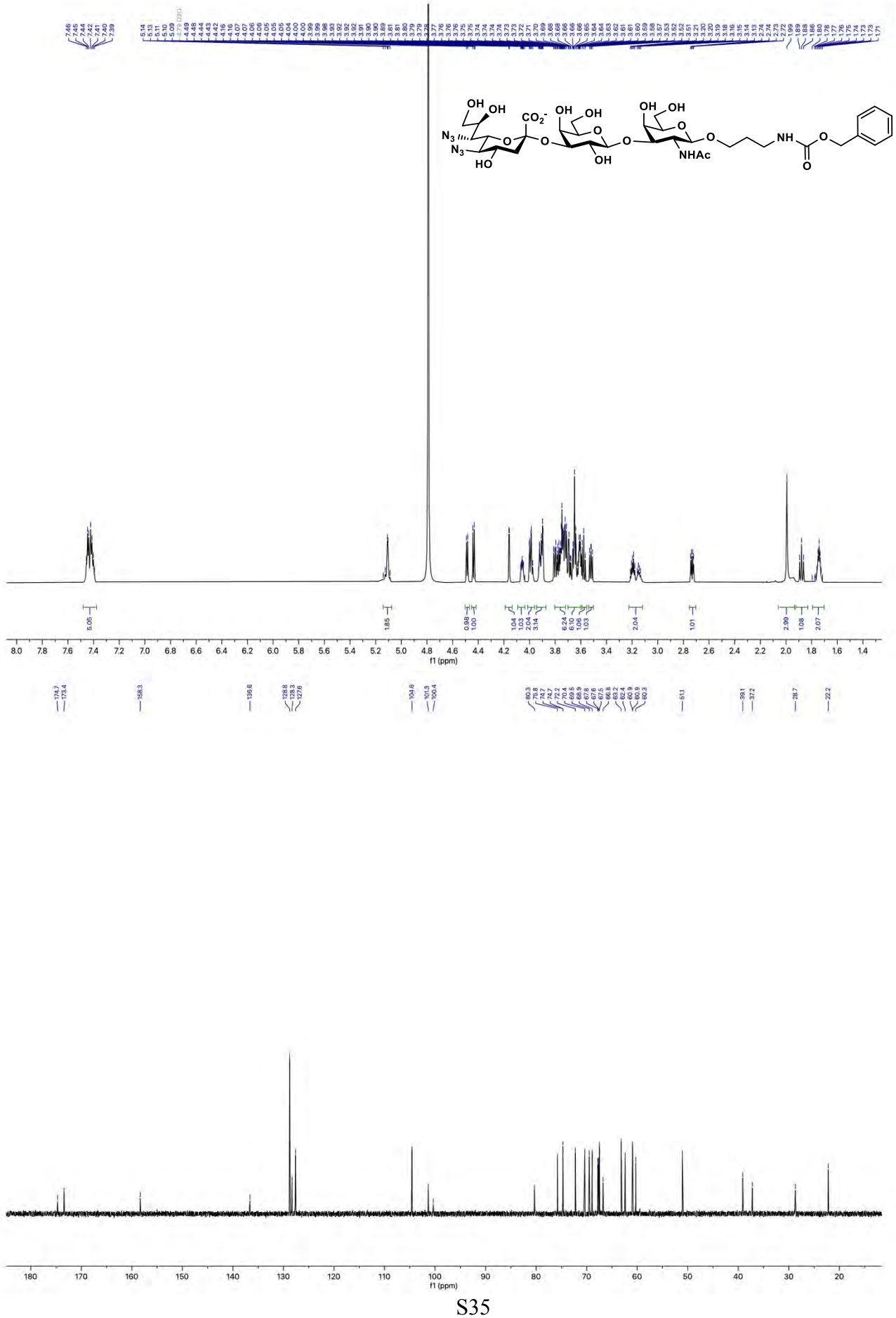
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$α2–6LacNAcβProNHCbz (**37**) in D_2O



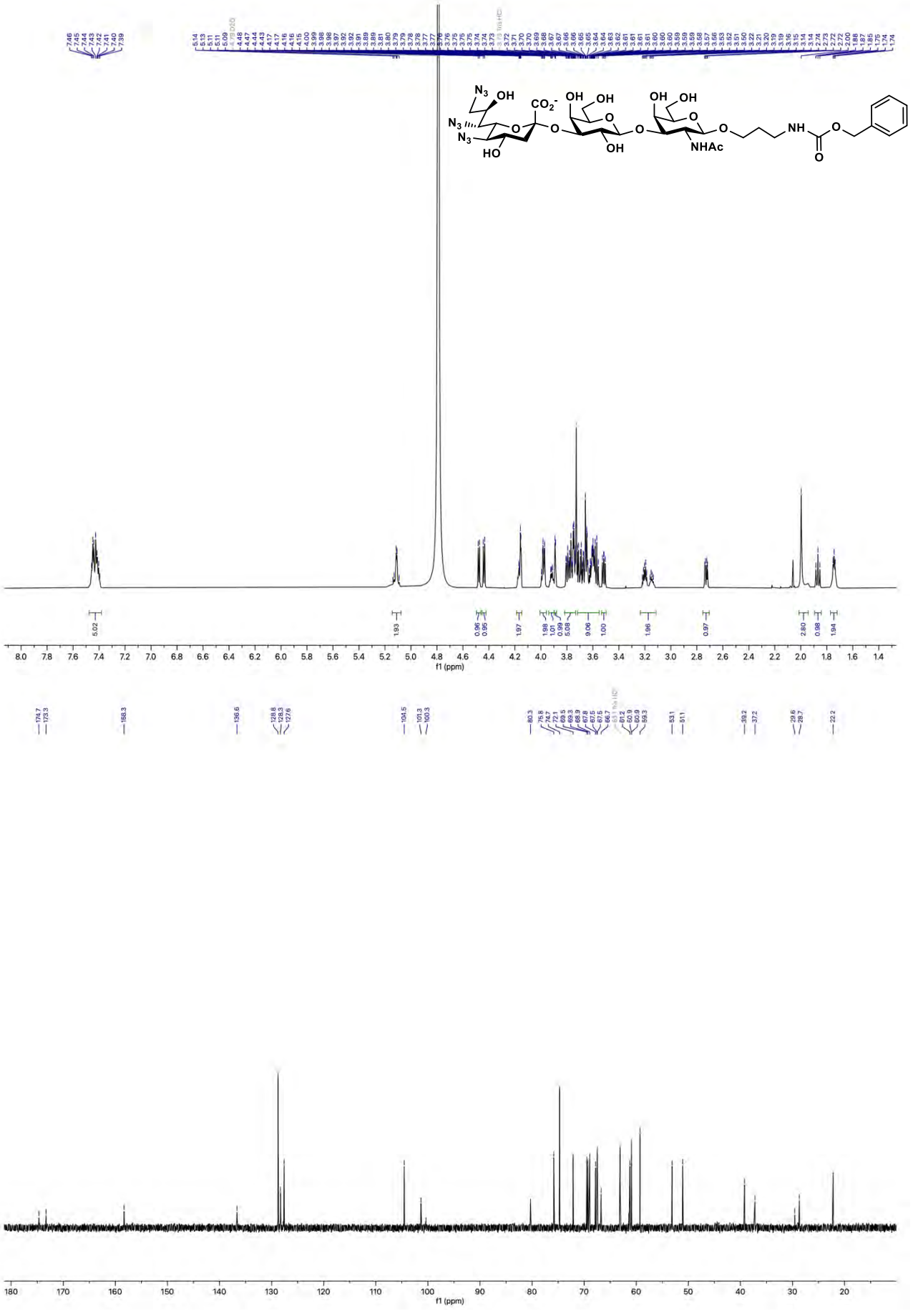
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN$_3$α2–6LacNAcβProNHCbz (**38**) in D_2O



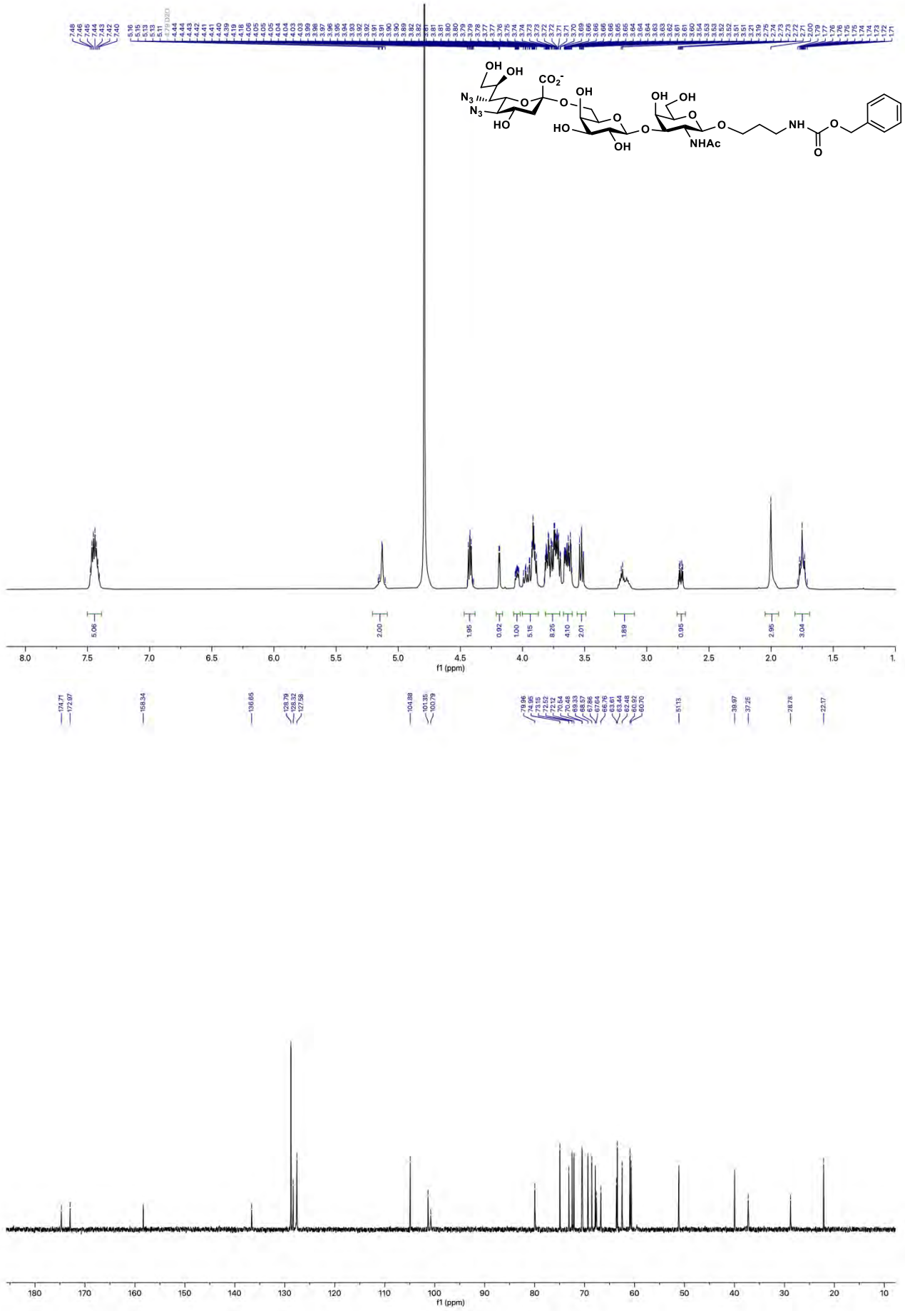
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$α2–3Galβ1–3GalNAcβProNHCbz (**39**) in D_2O



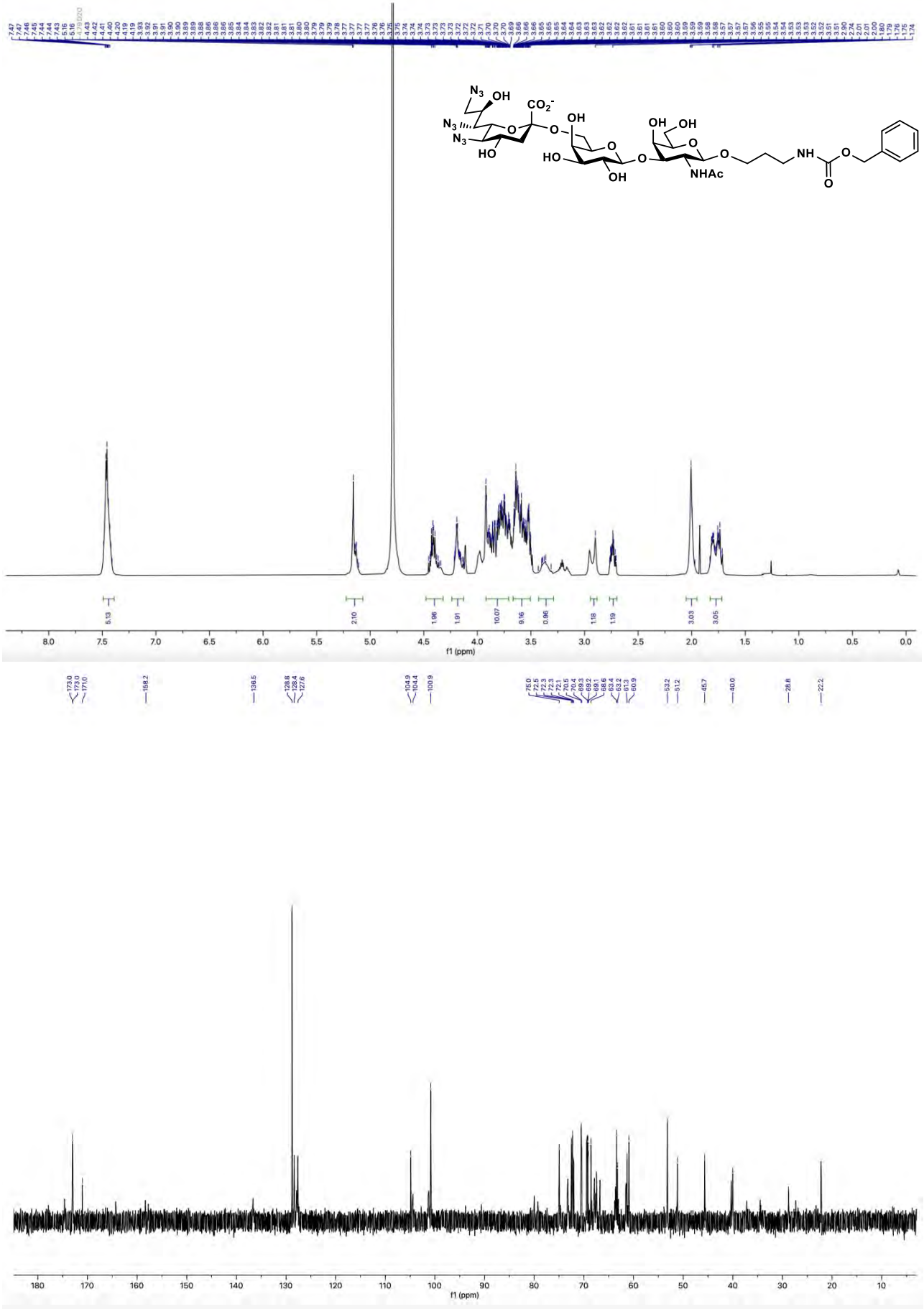
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN$_3$α2–3Galβ1–3GalNAcβProNHCbz (**40**) in D_2O



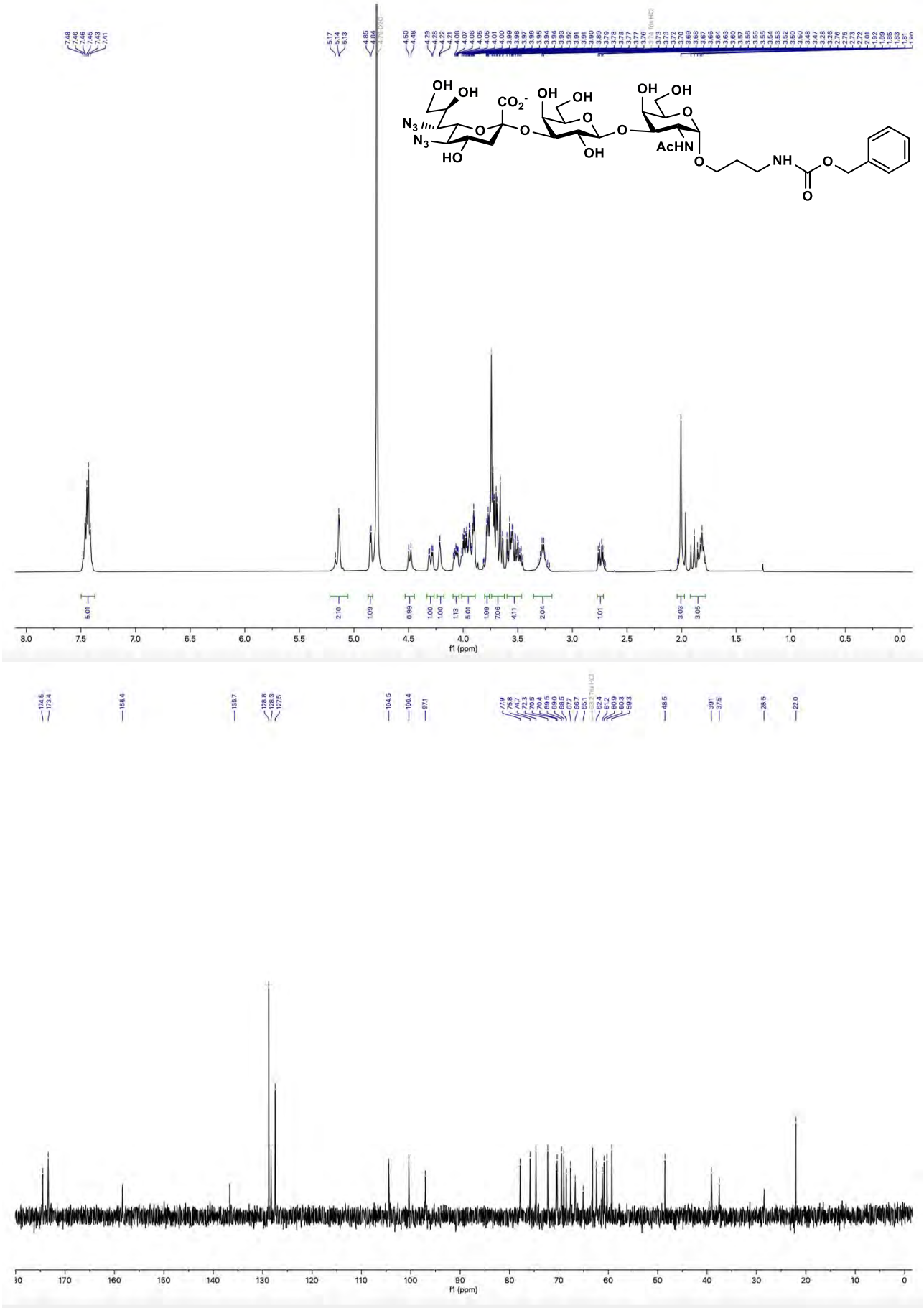
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$α2–6Galβ1–3GalNAcβProNHCbz (**41**) in D_2O



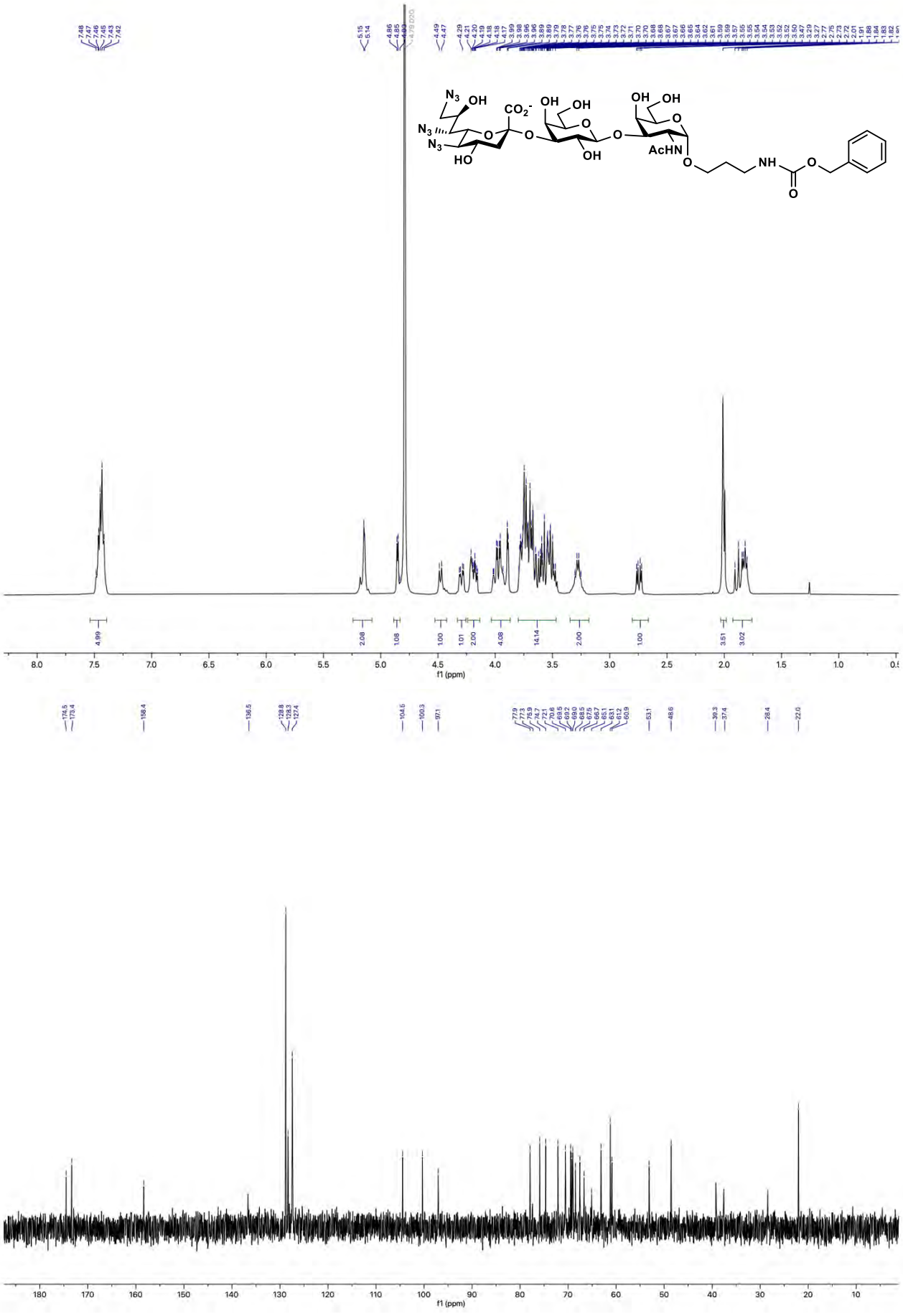
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3α2–6Galβ1–3GalNAcβProNHCbz (**42**) in D_2O



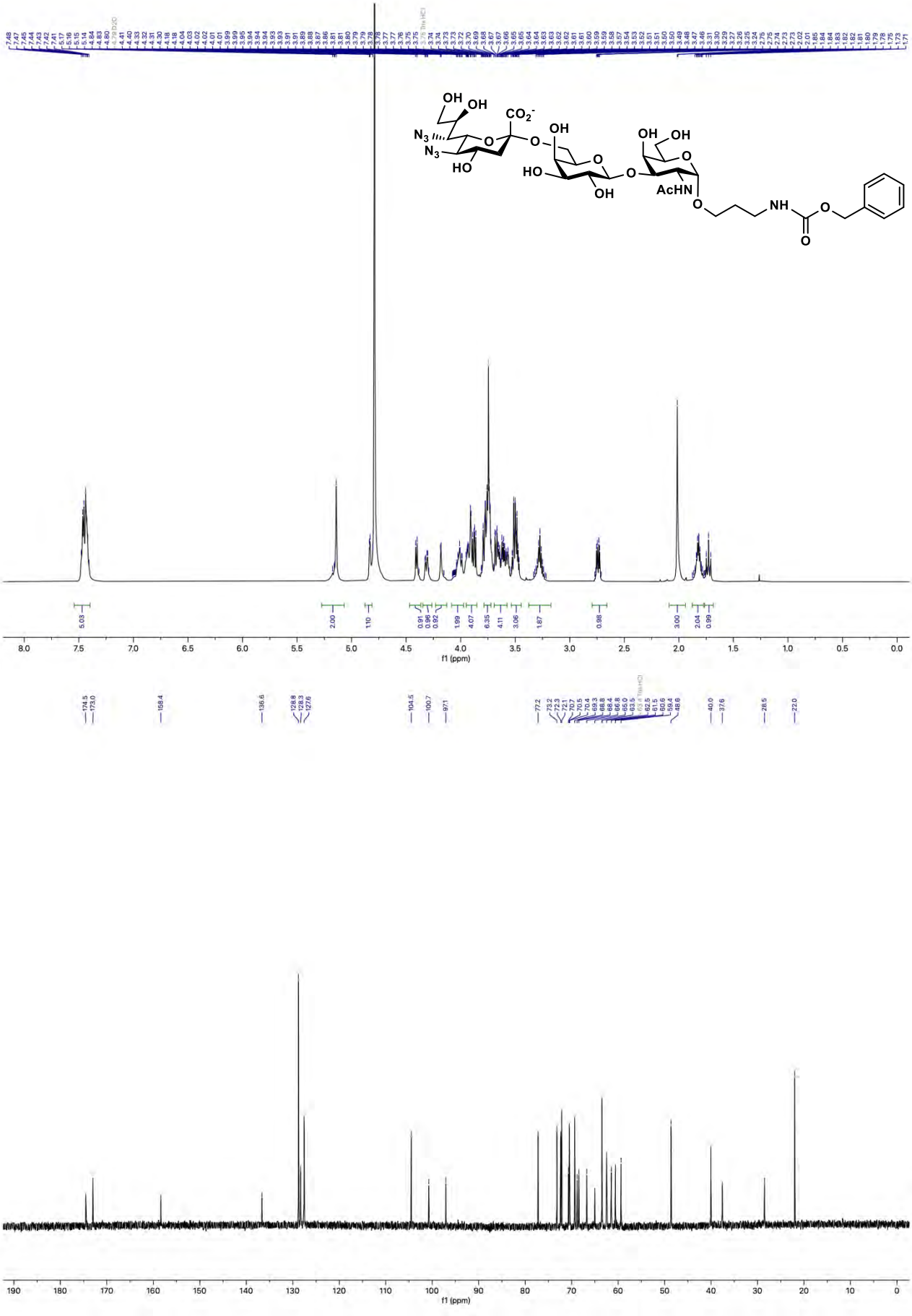
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$α2–3Galβ1–3GalNAcαProNHCbz (**43**) in D_2O



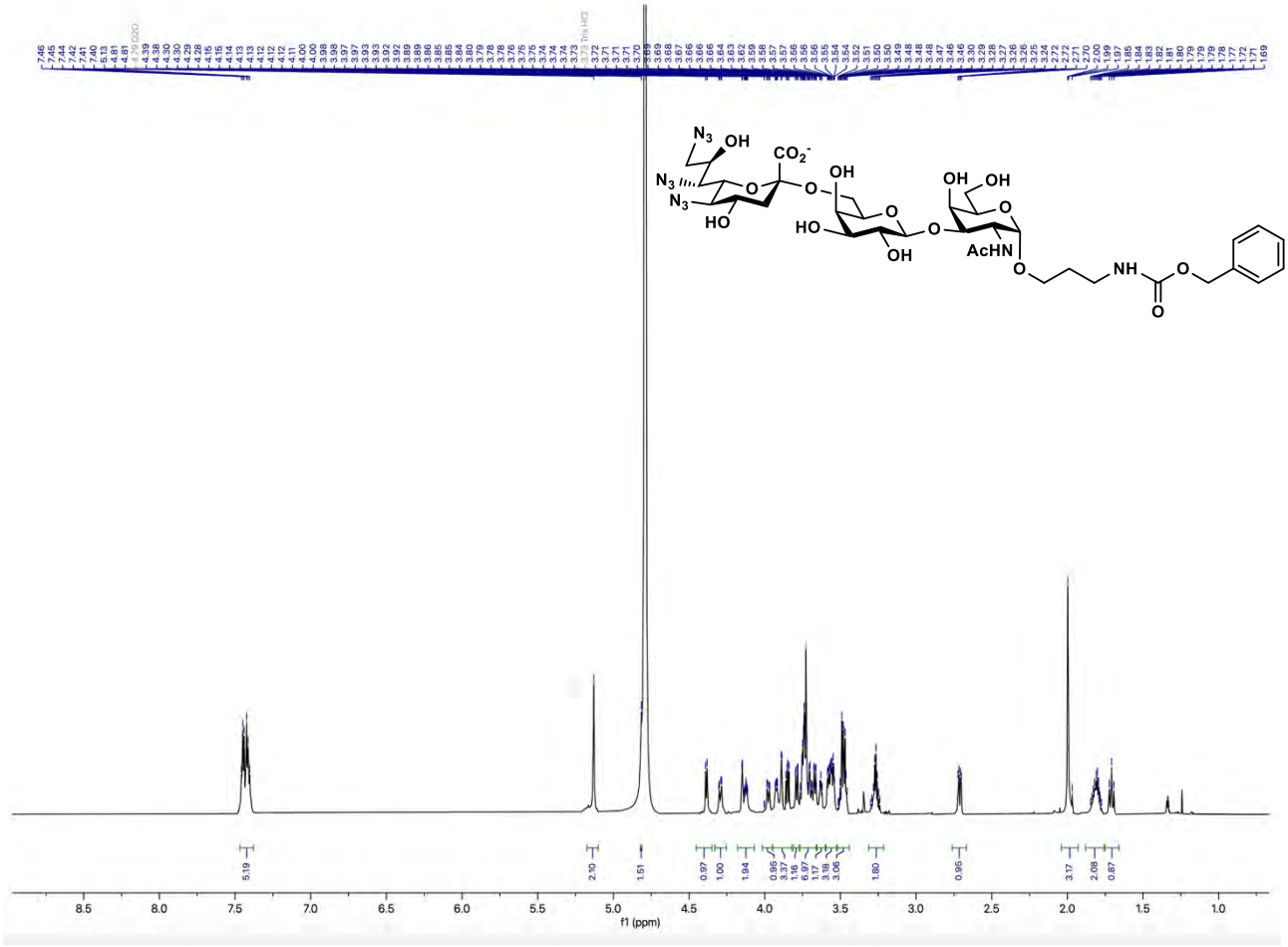
400 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3α2–3Galβ1–3GalNAcαProNHCbz (**44**) in D_2O

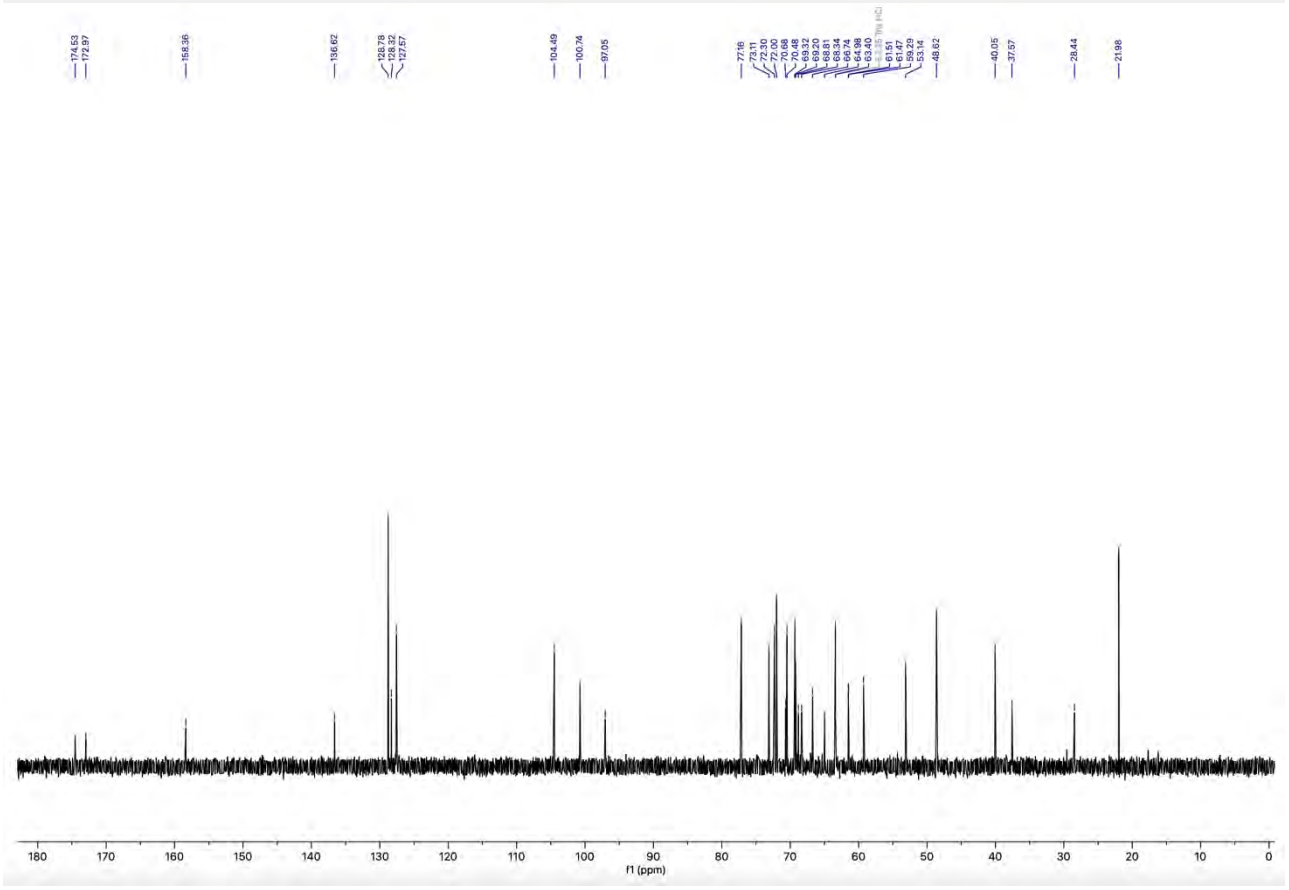


600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$α2–6Galβ1–3GalNAcαProNHCbz (**45**) in D_2O

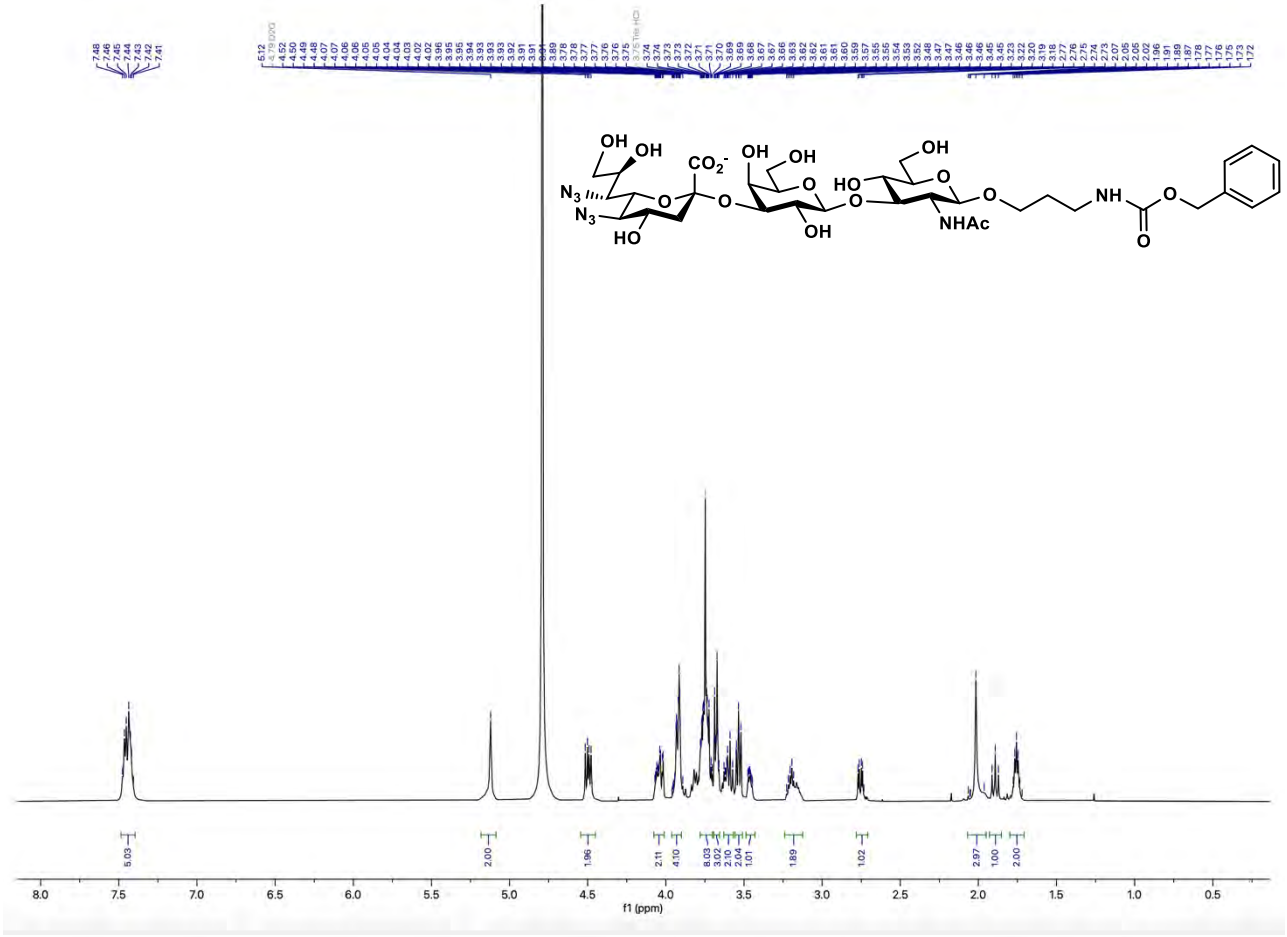


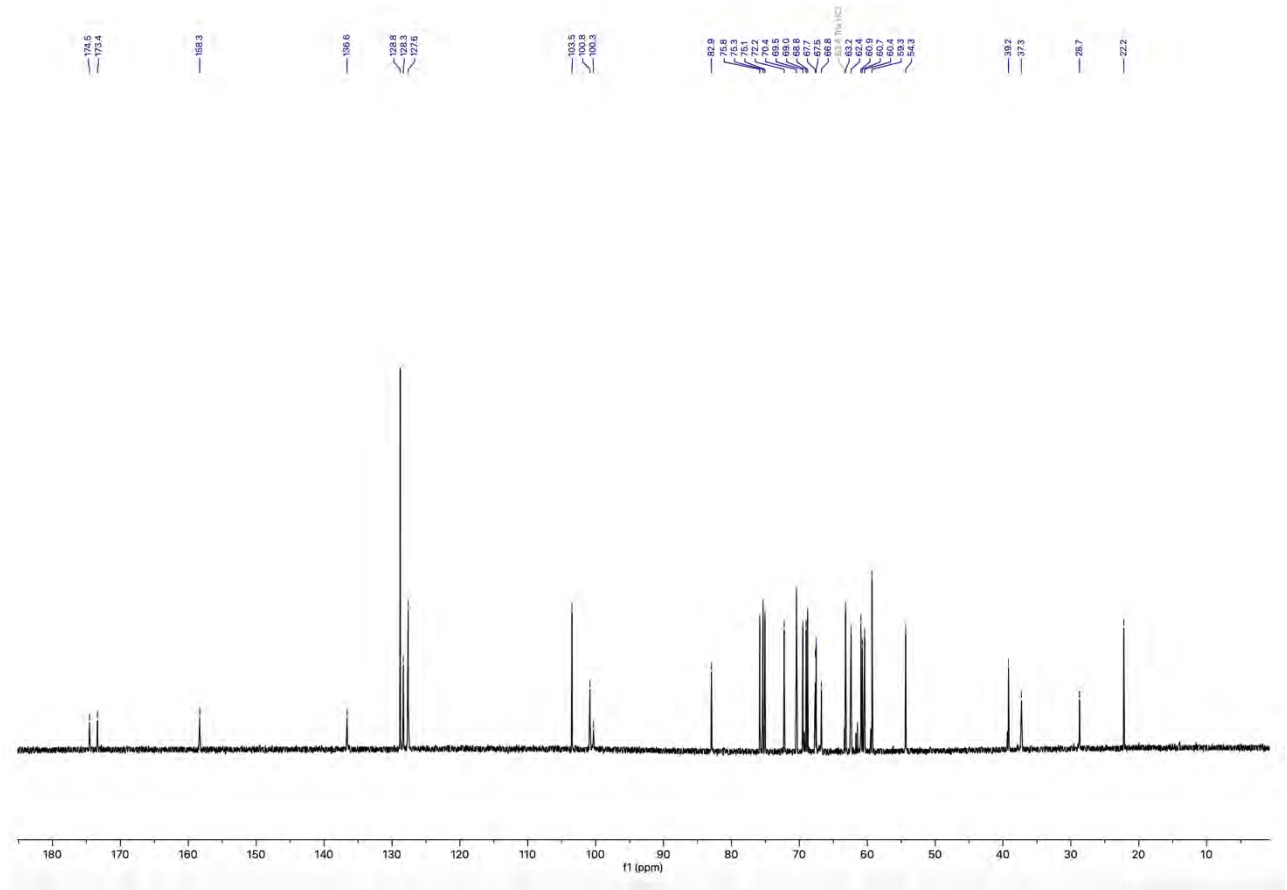
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3α2–6Galβ1–3GalNAcαProNHCbz (**46**) in D_2O



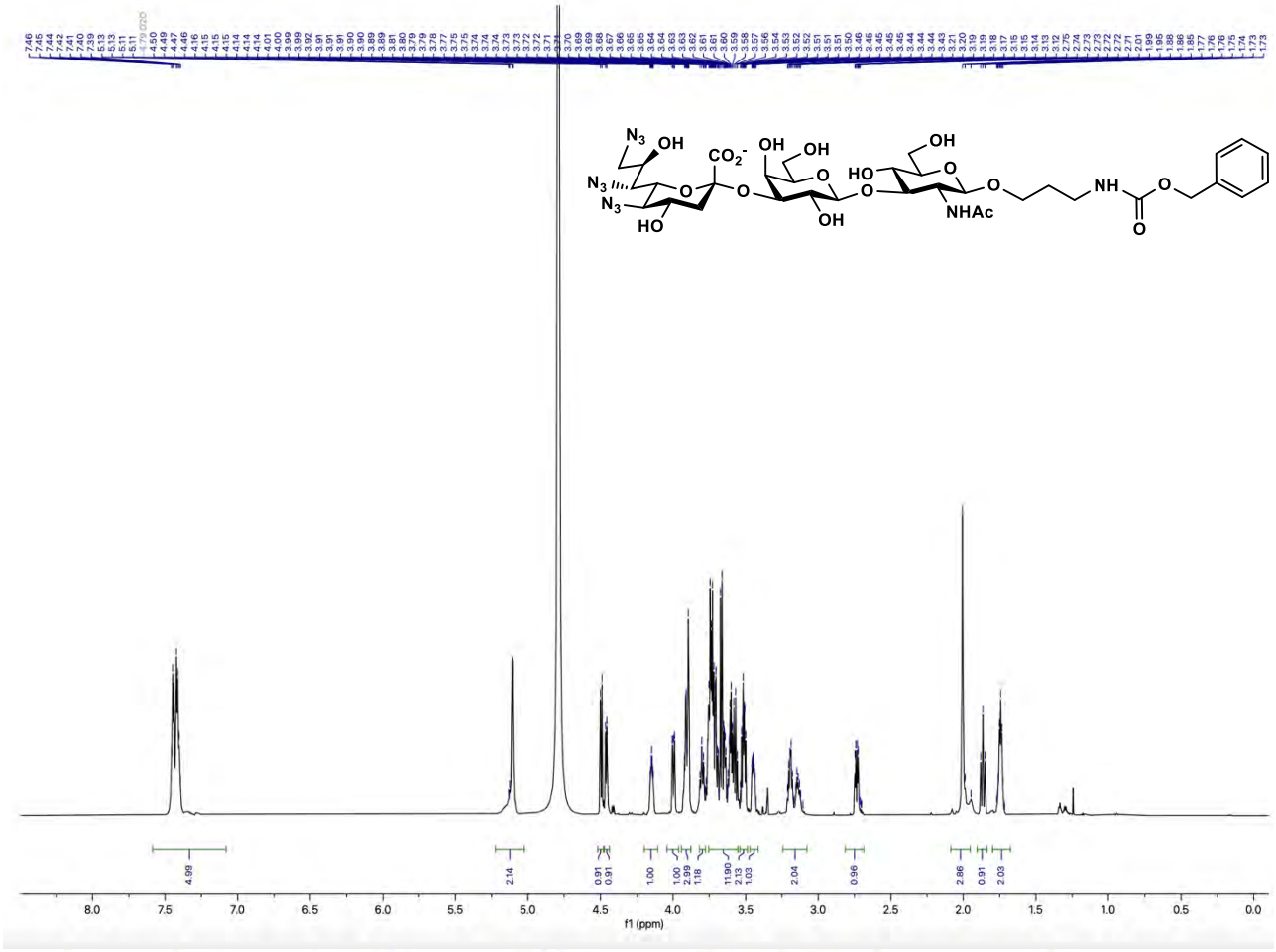


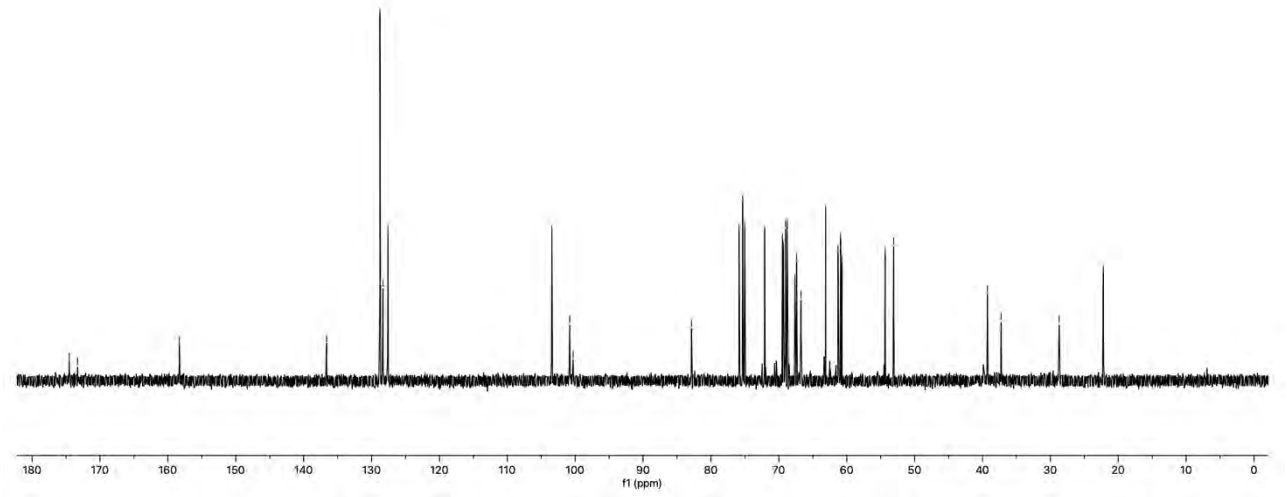
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN$_3$α2–3Galβ1–3GlcNAcβProNHCbz (**47**) in D_2O



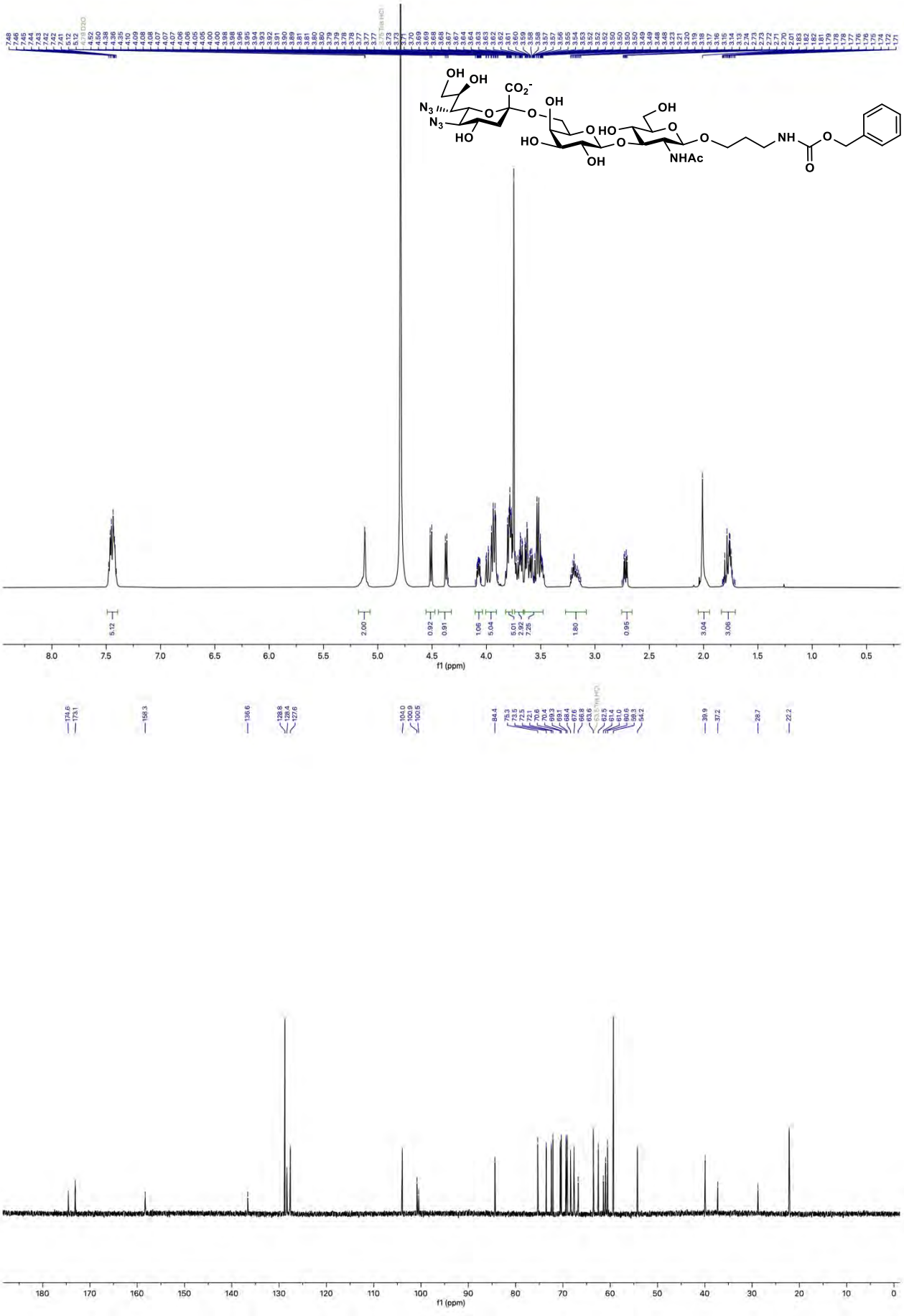


800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3α2–3Galβ1–3GlcNAcβProNHCbz (**48**) in D_2O

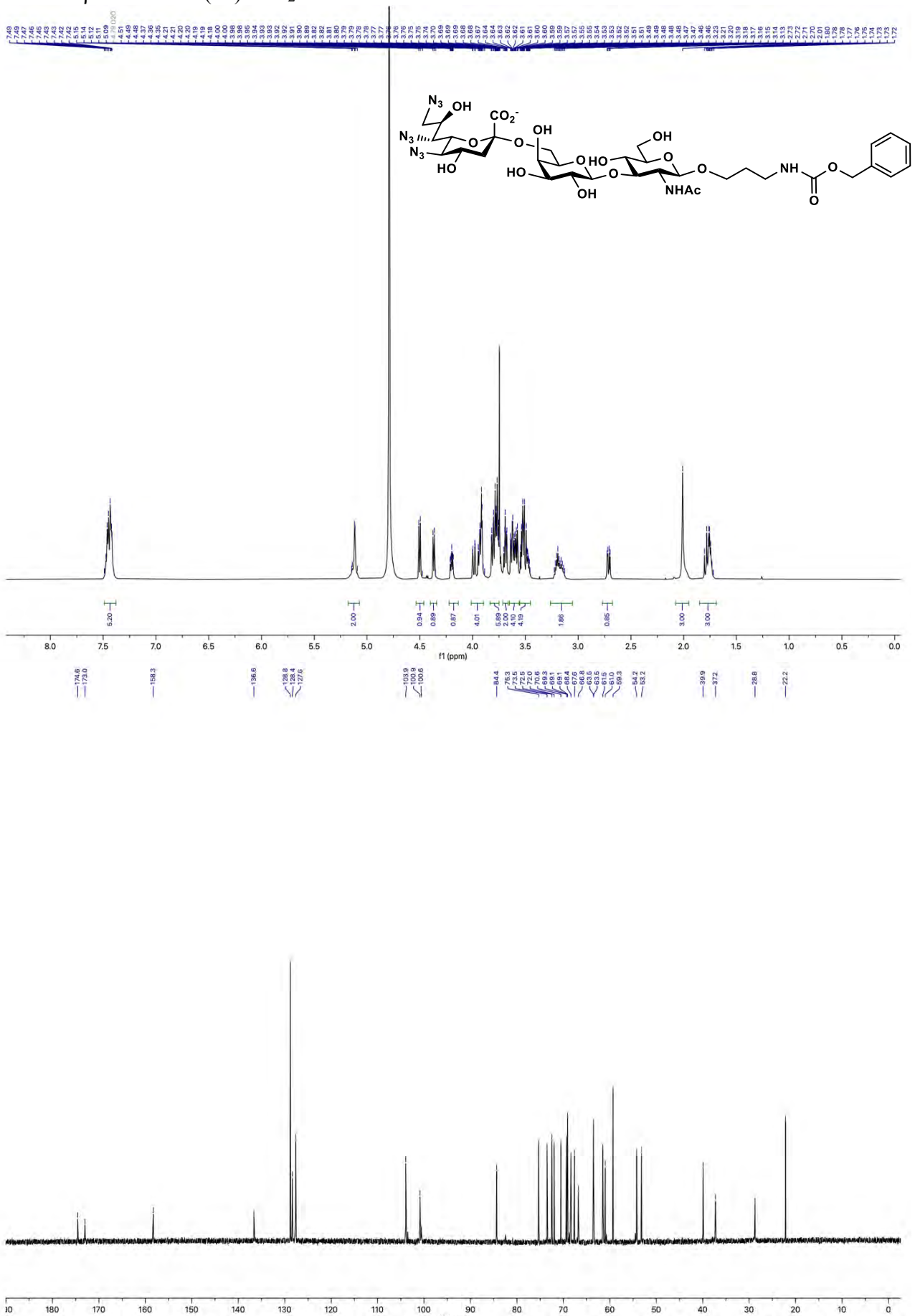




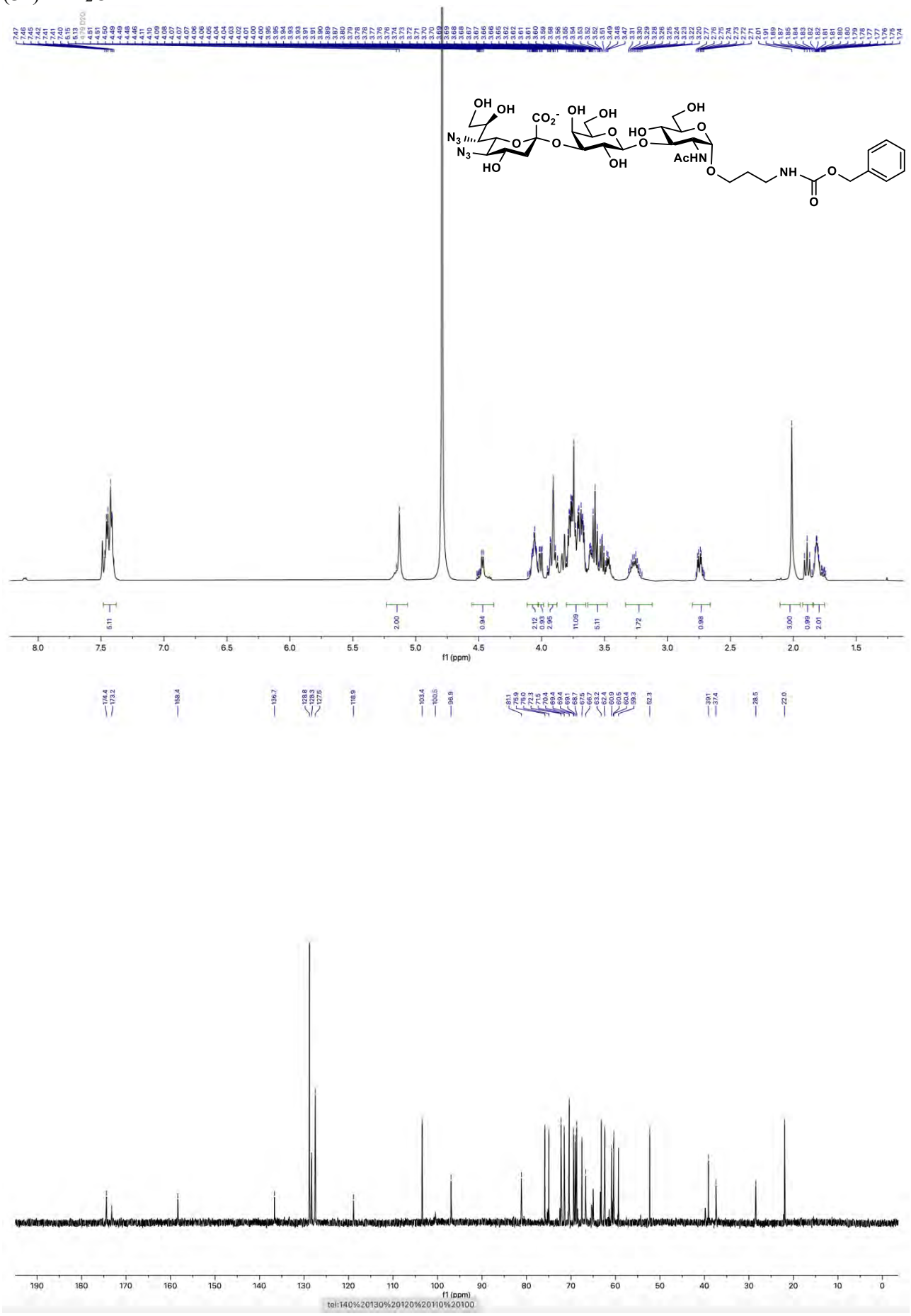
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN_3α2–6Galβ1–3GlcNAcβProNHCbz (**49**) in D_2O



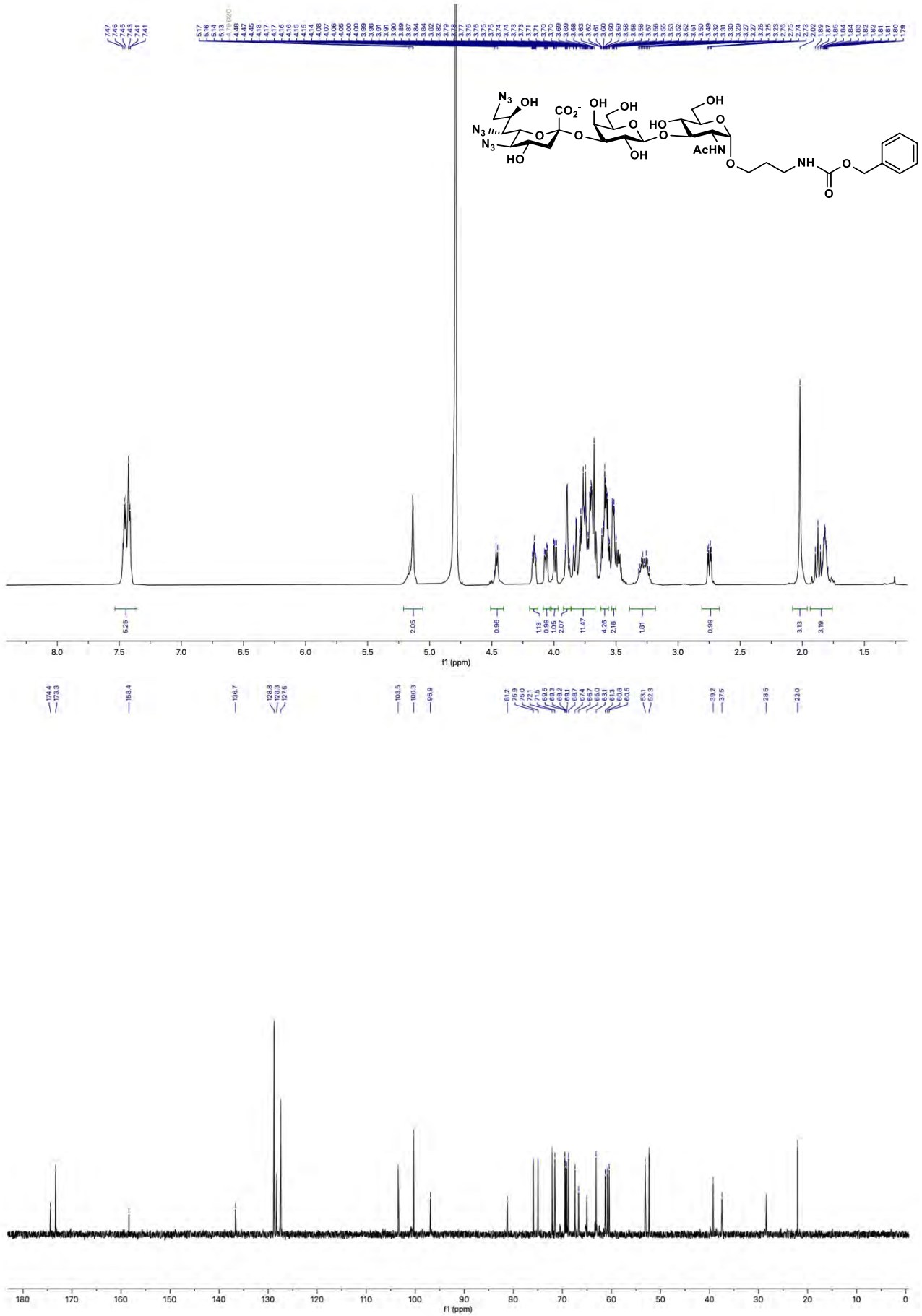
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3α2–6Galβ1–3GlcNAcβProNHCbz (**50**) in D_2O



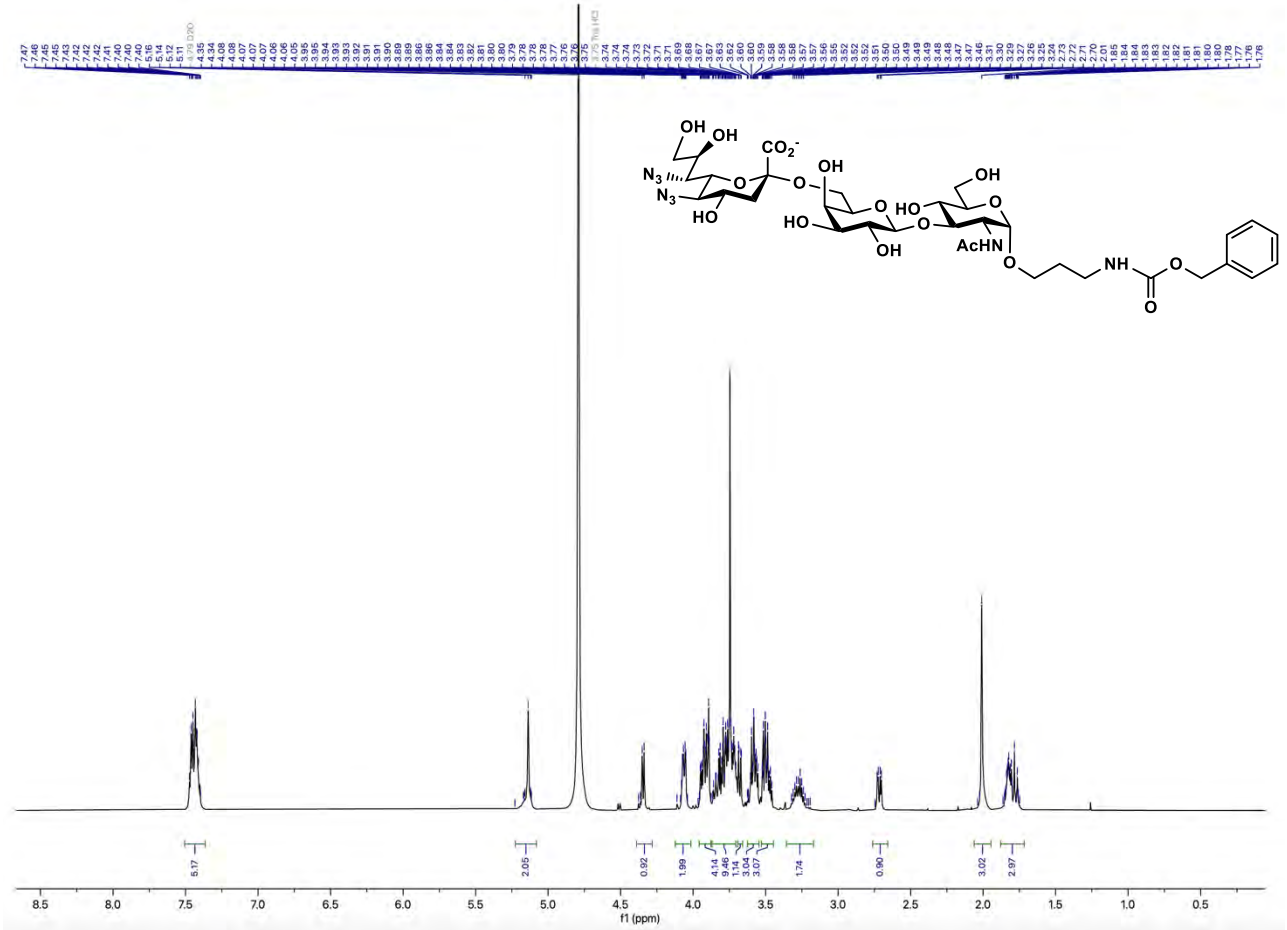
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN_3α2–3Galβ1–3GlcNAcαProNHCbz (**51**) in D_2O

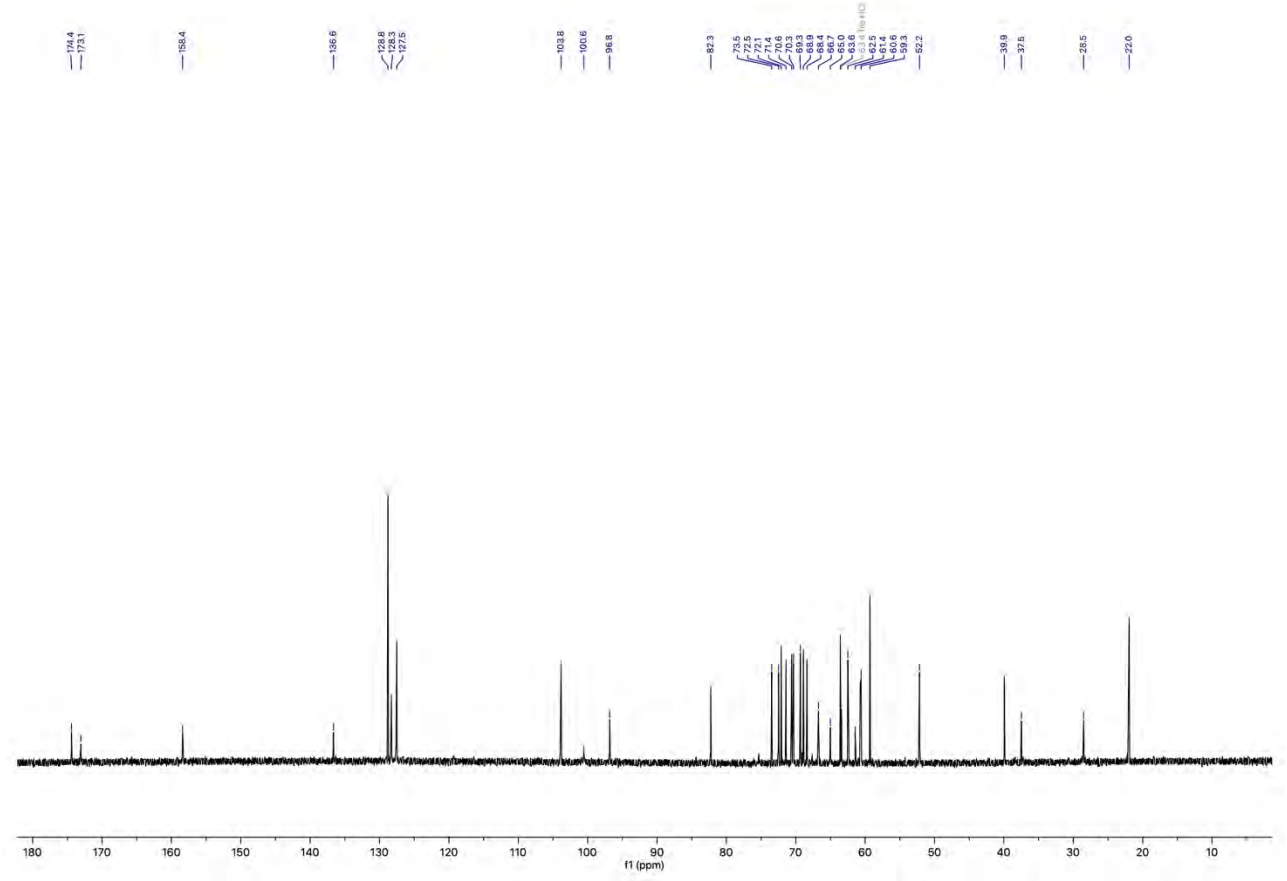


600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3α2–3Galβ1–3GlcNAcαProNHCbz (**52**) in D_2O

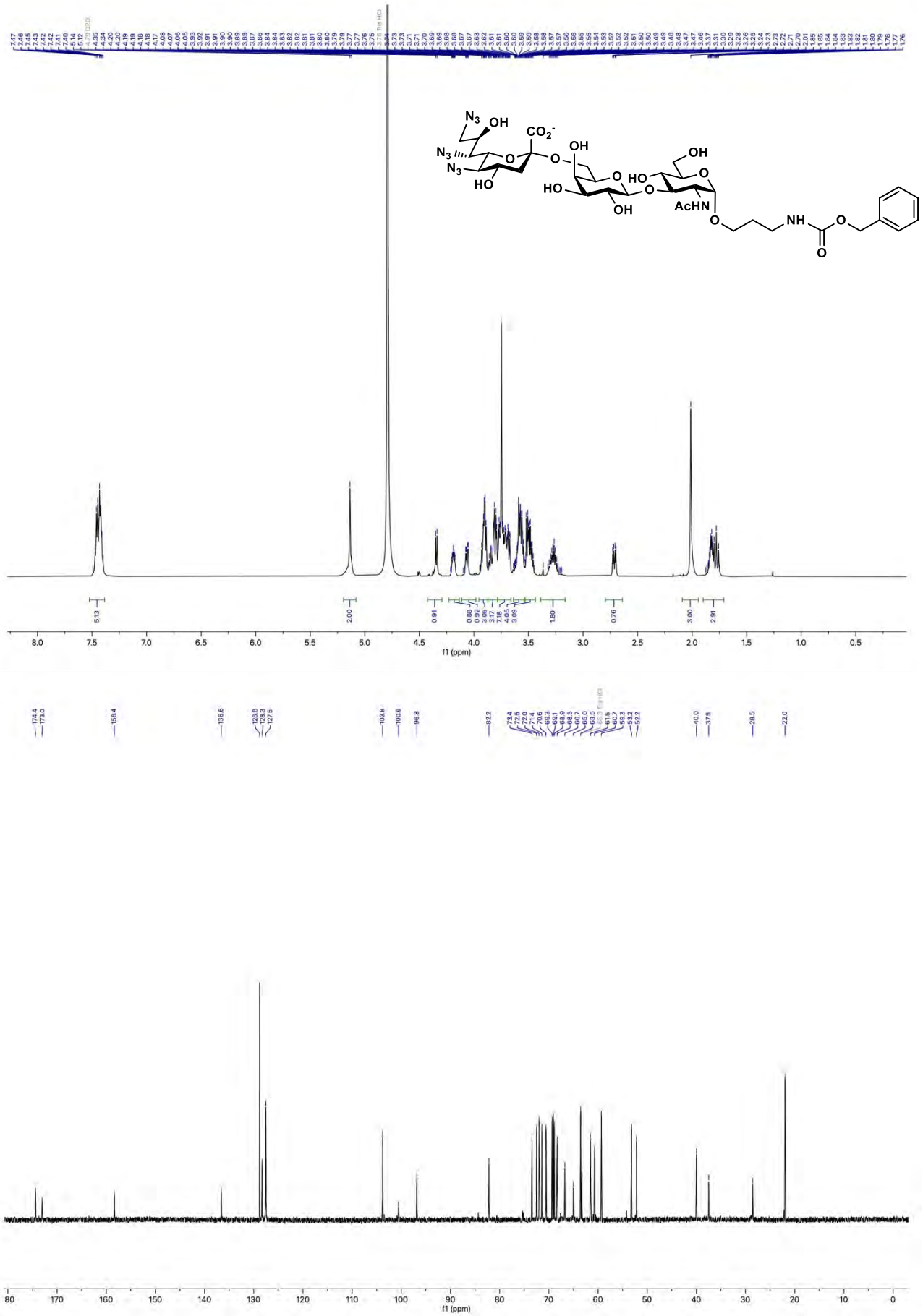


600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN_3α2–6Galβ1–3GlcNAcαProNHCbz (**53**) in D_2O

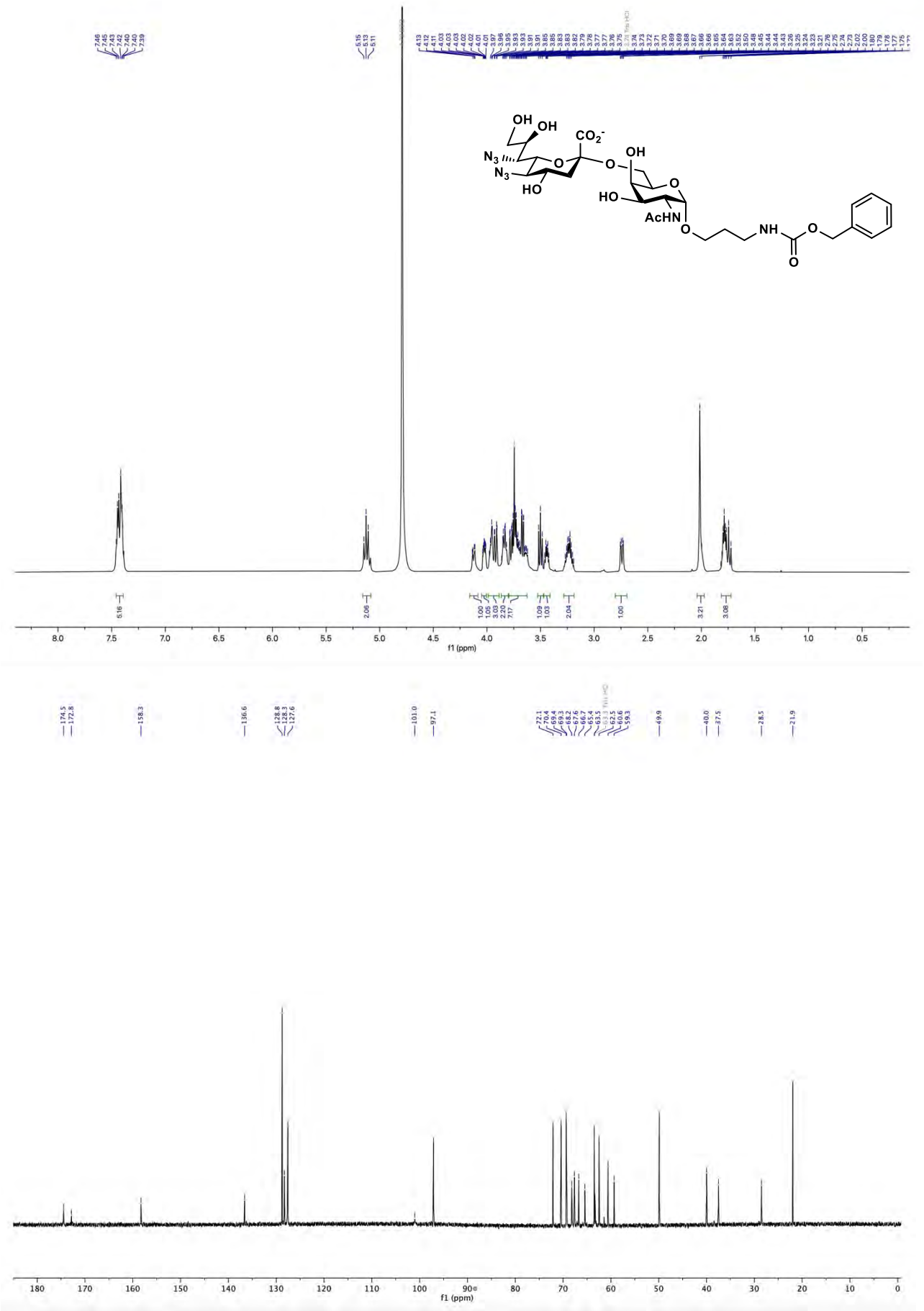




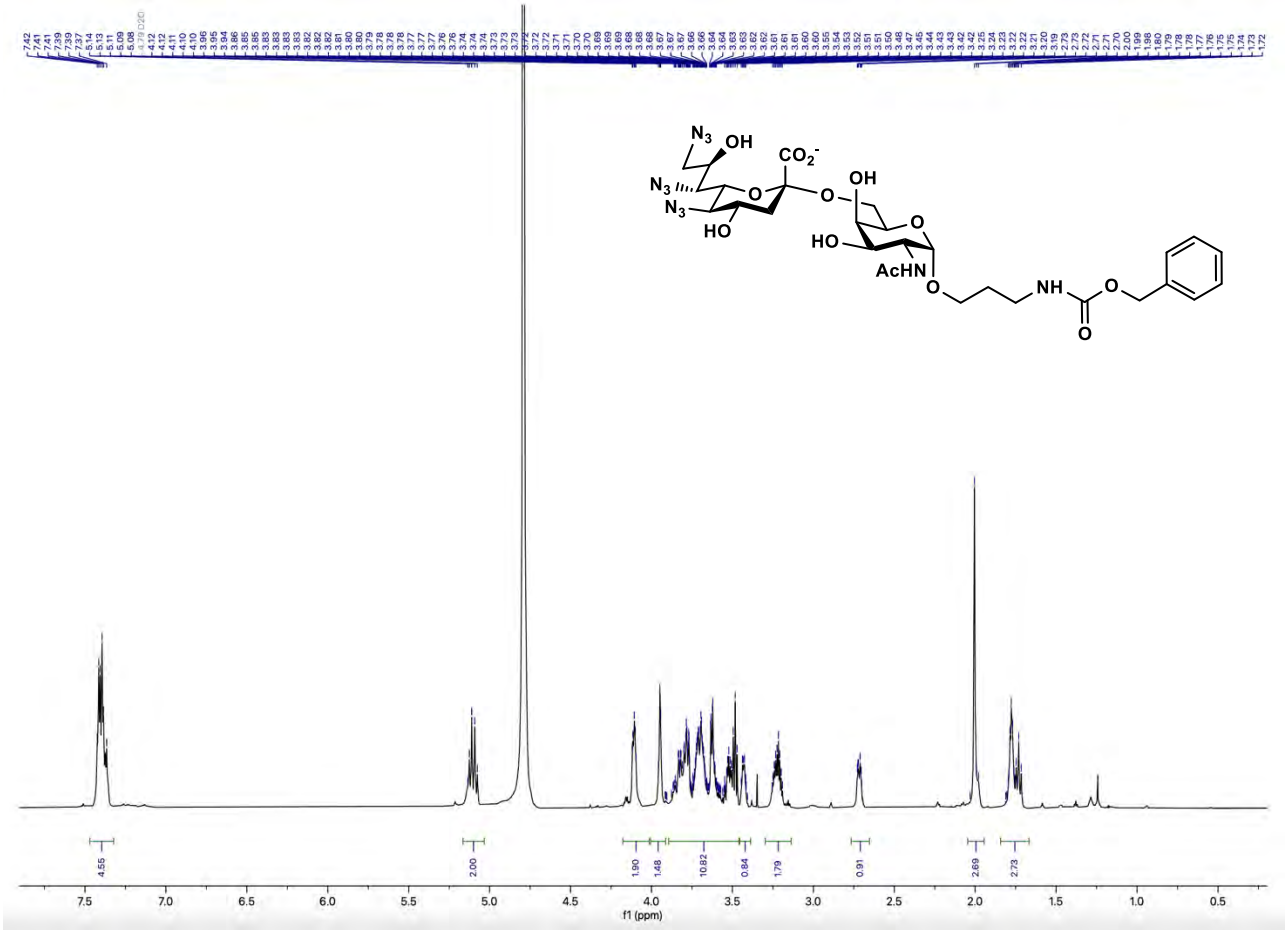
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN_3α2–6Galβ1–3GlcNAcαProNHCbz (**54**) in D_2O

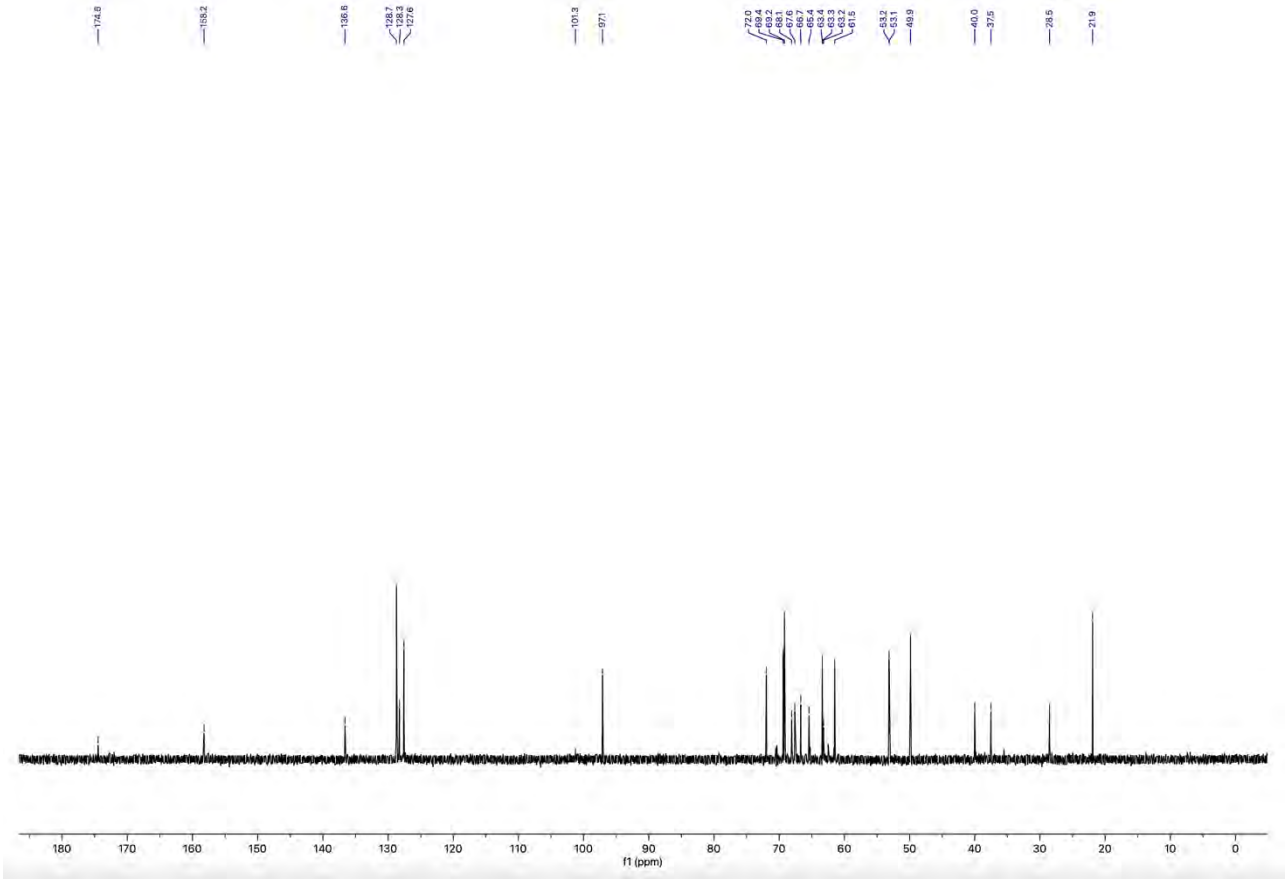


600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7diN_3α2–6GalNAcαProNHCbz (**55**) in D_2O

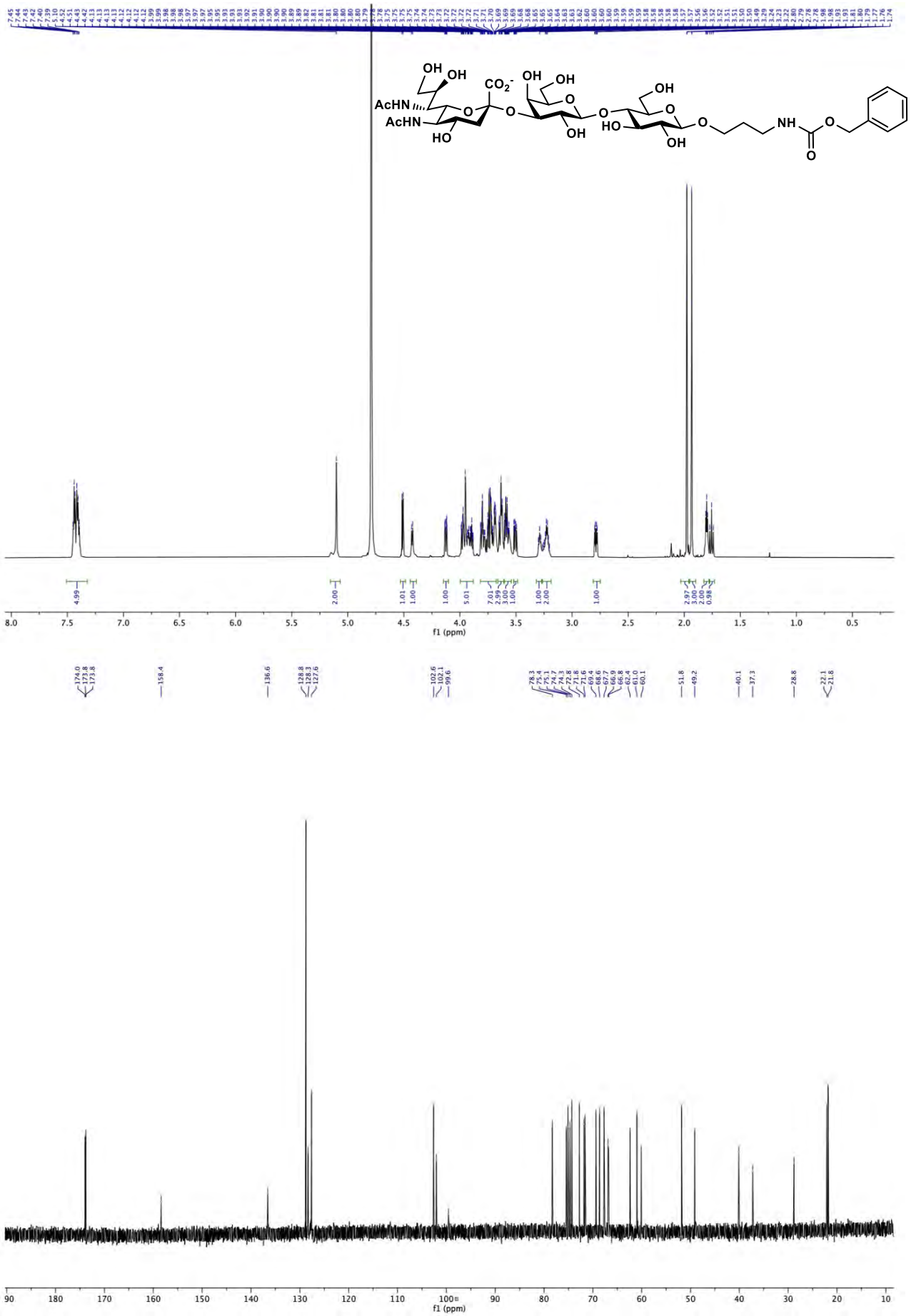


800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5,7,9triN$_3$α2–6GalNAcαProNHCbz (**56**) in D_2O

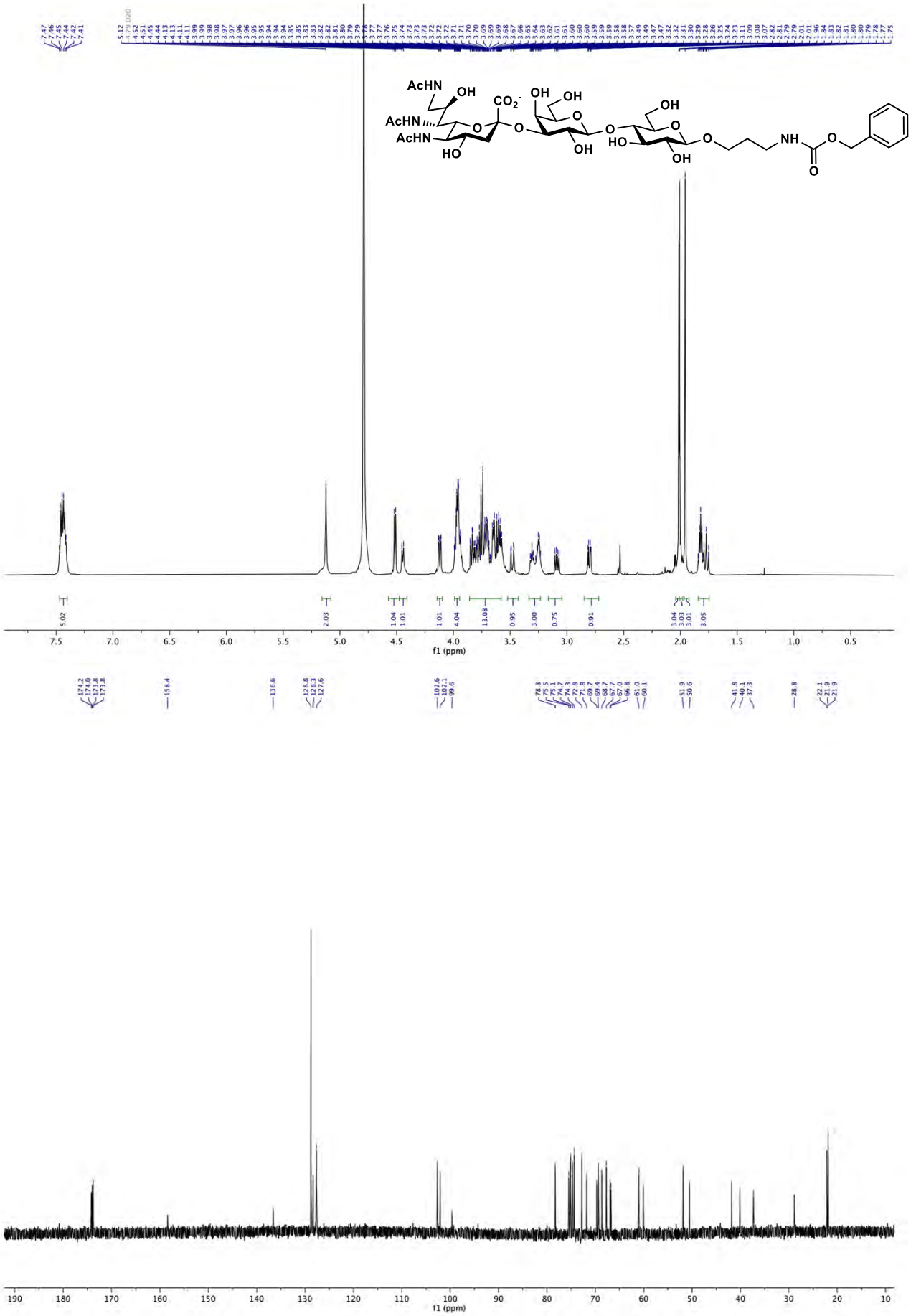




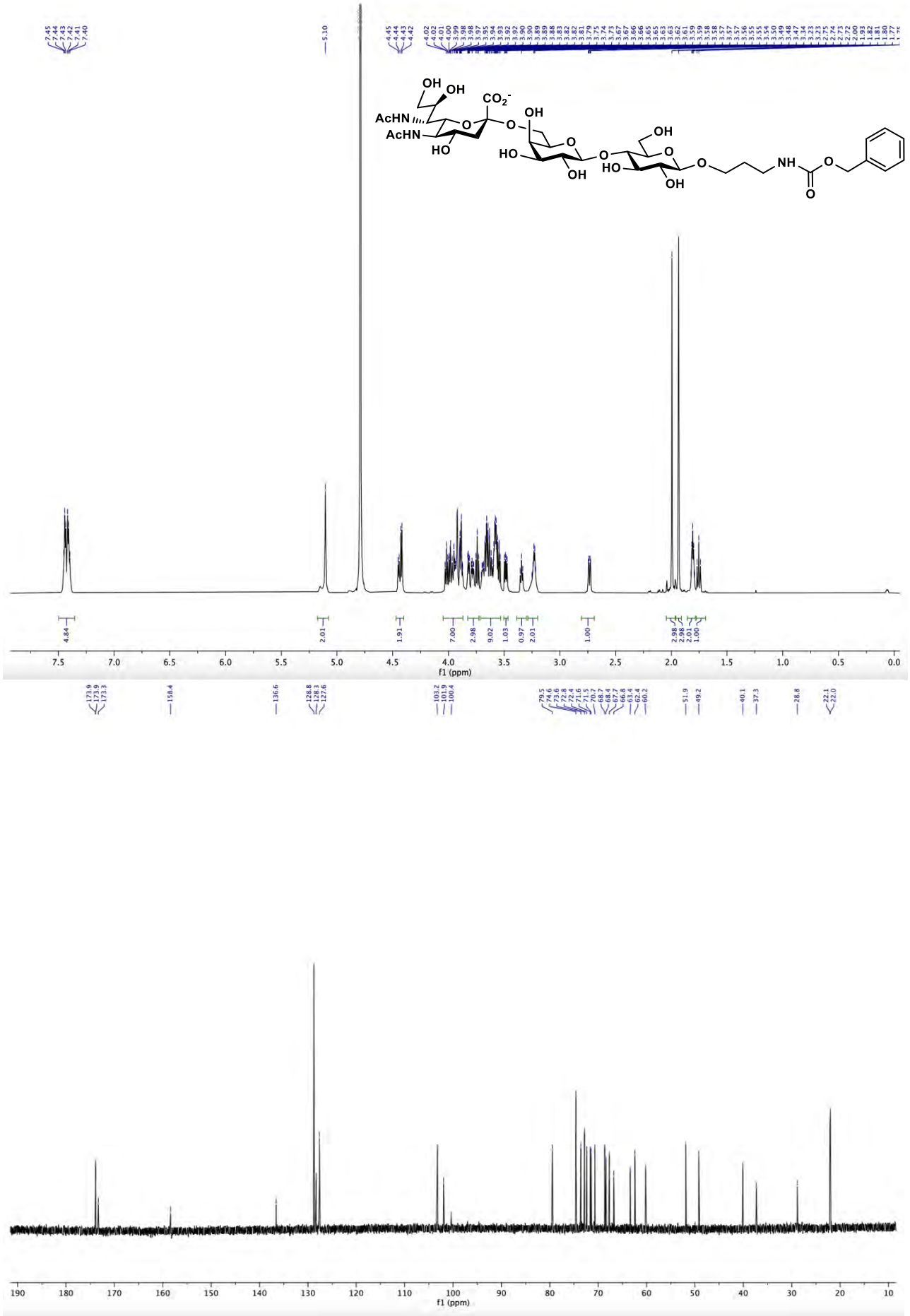
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–3LacβProNHCbz (**57**) in D_2O



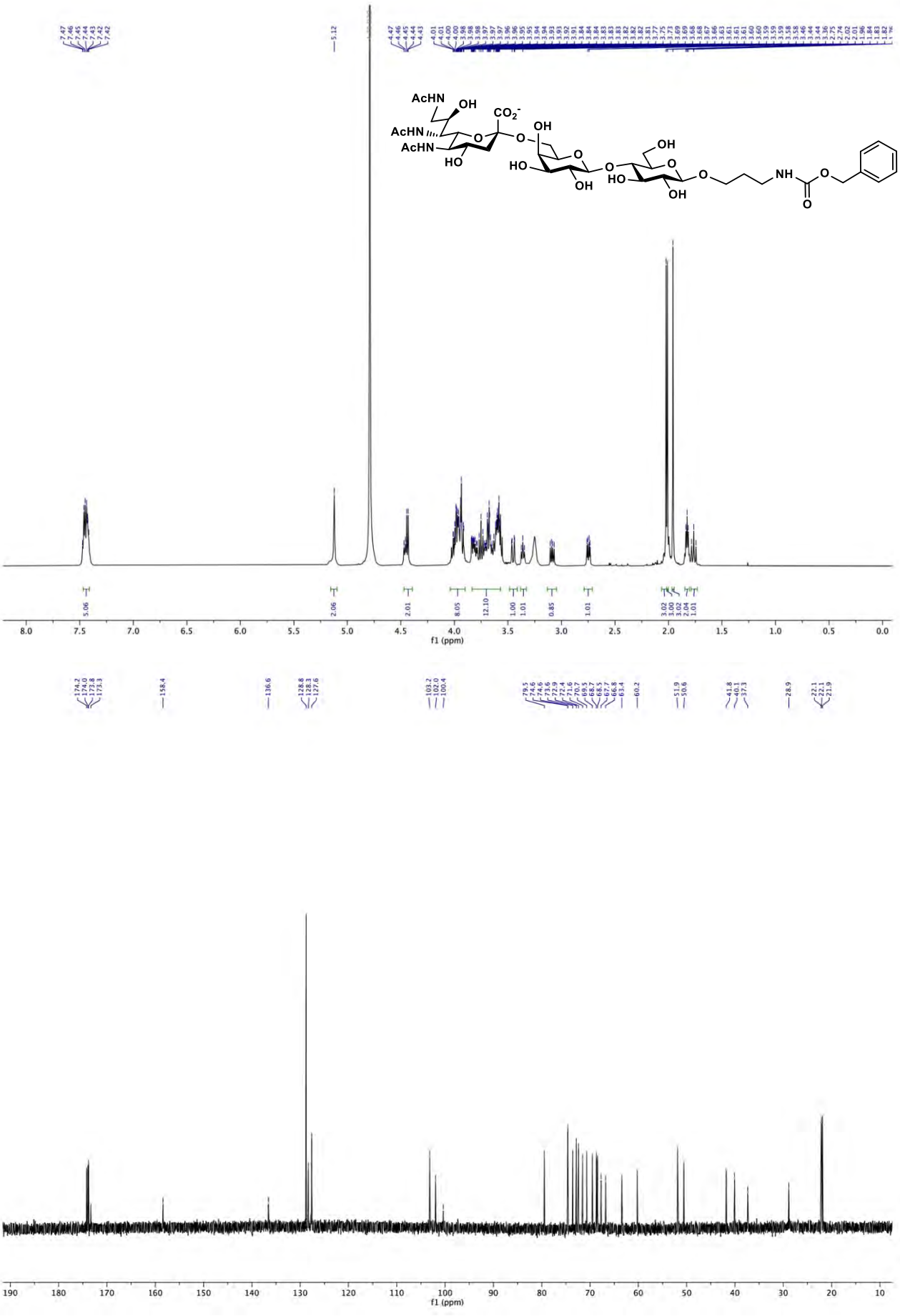
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2−3LacβProNHCbz (**58**) in D_2O



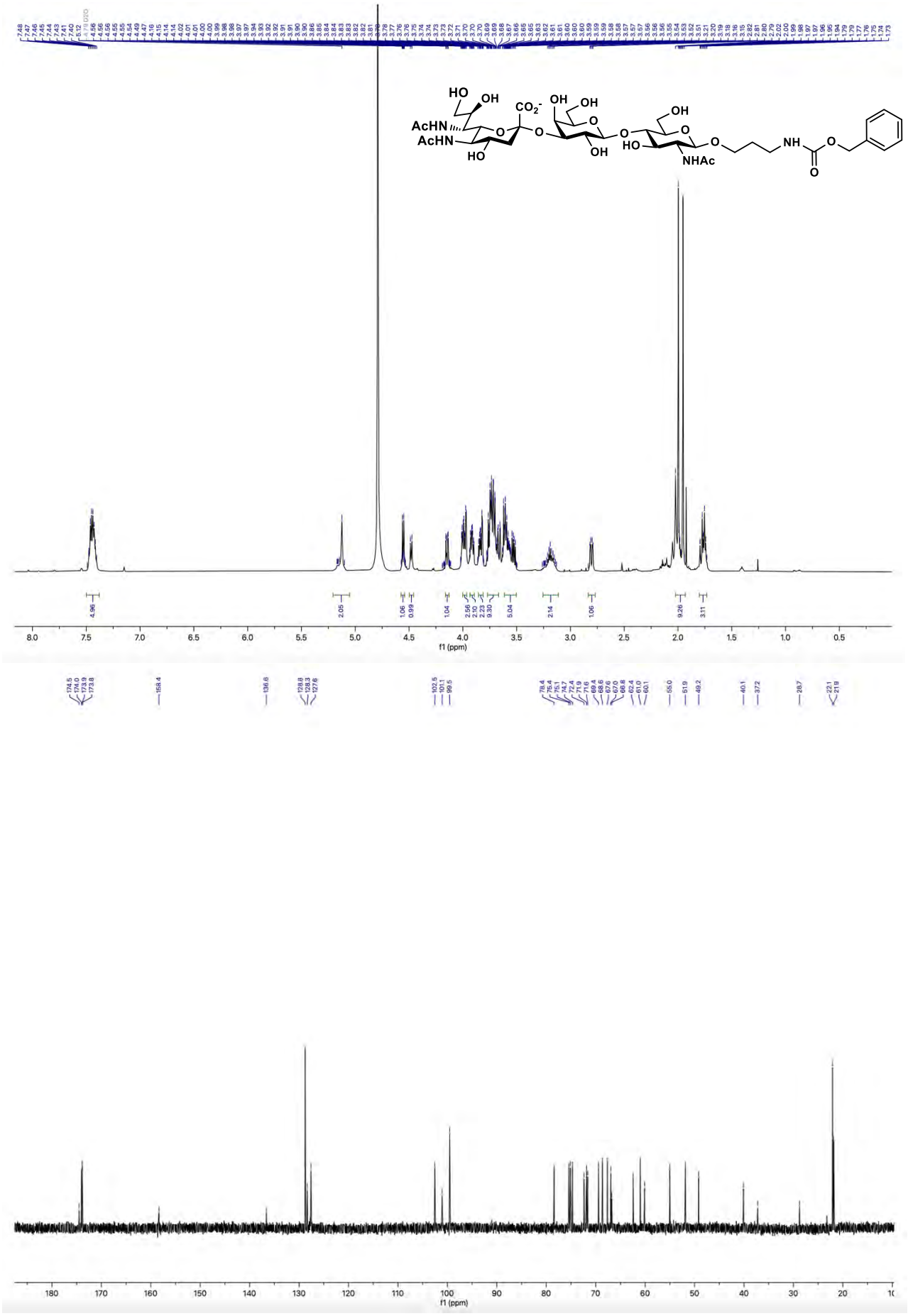
800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–6LacβProNHCbz (**59**) in D_2O



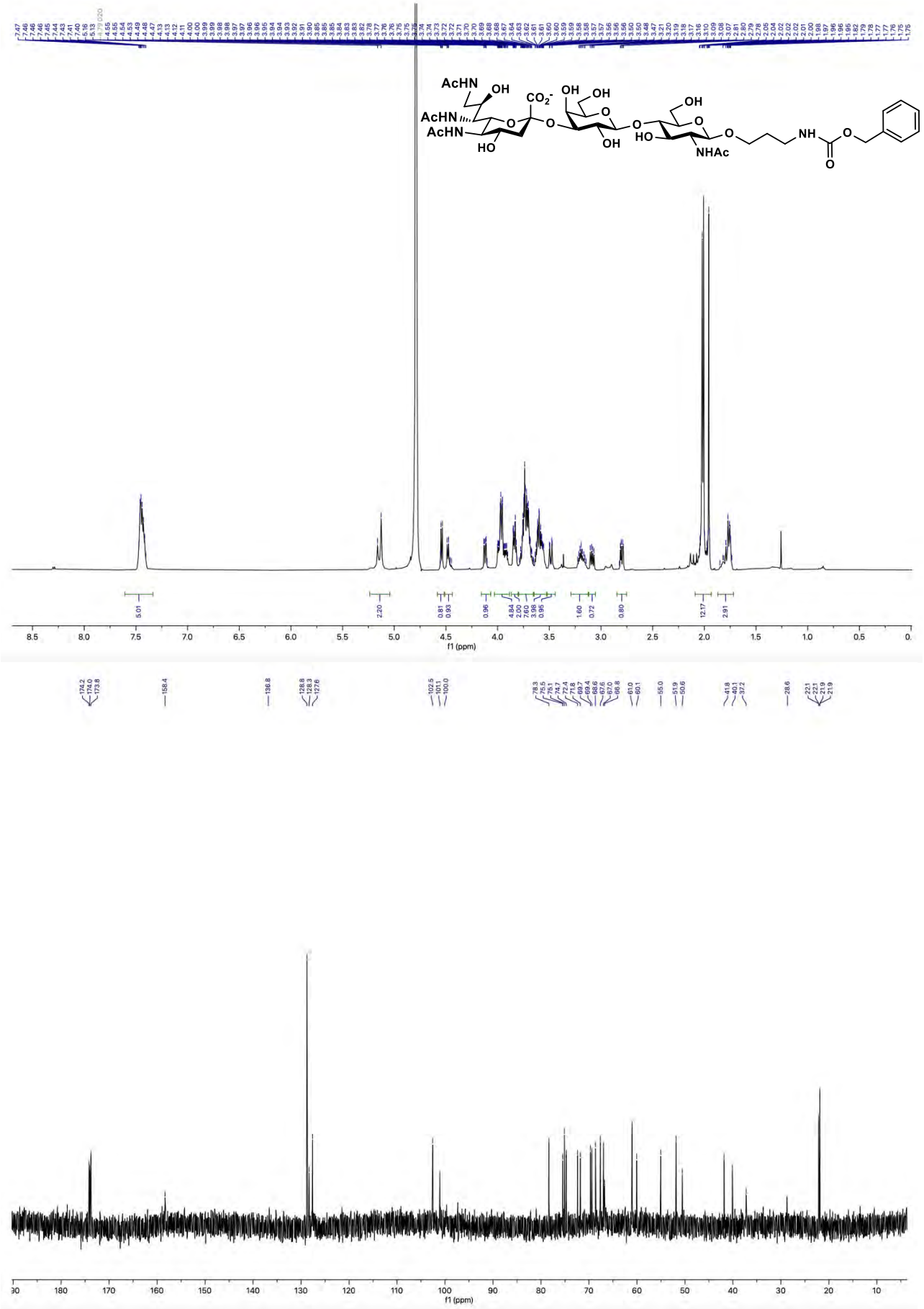
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2−6LacβProNHCbz (**60**) in D_2O



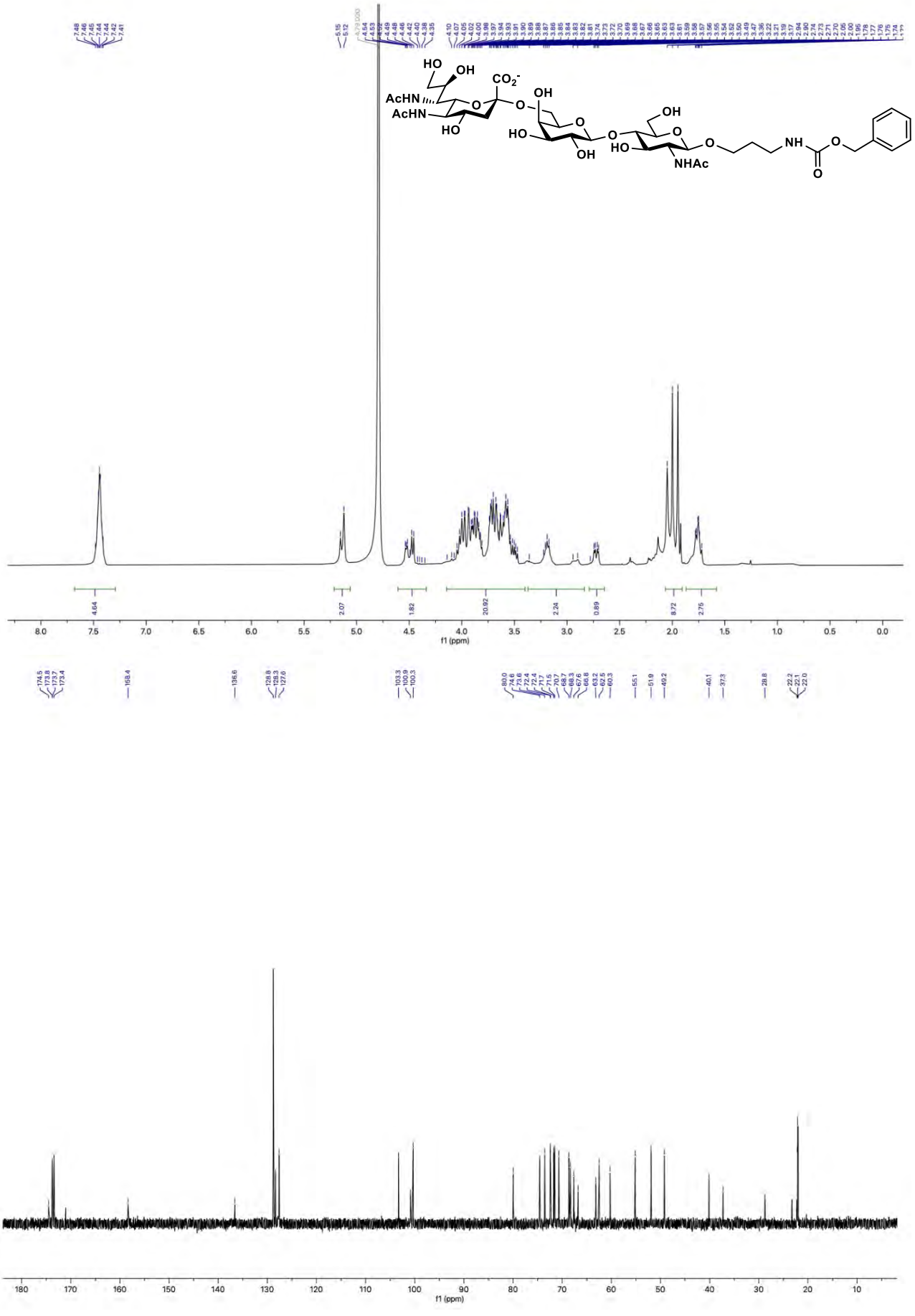
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–3LacNAcβProNHCbz (**61**) in D_2O



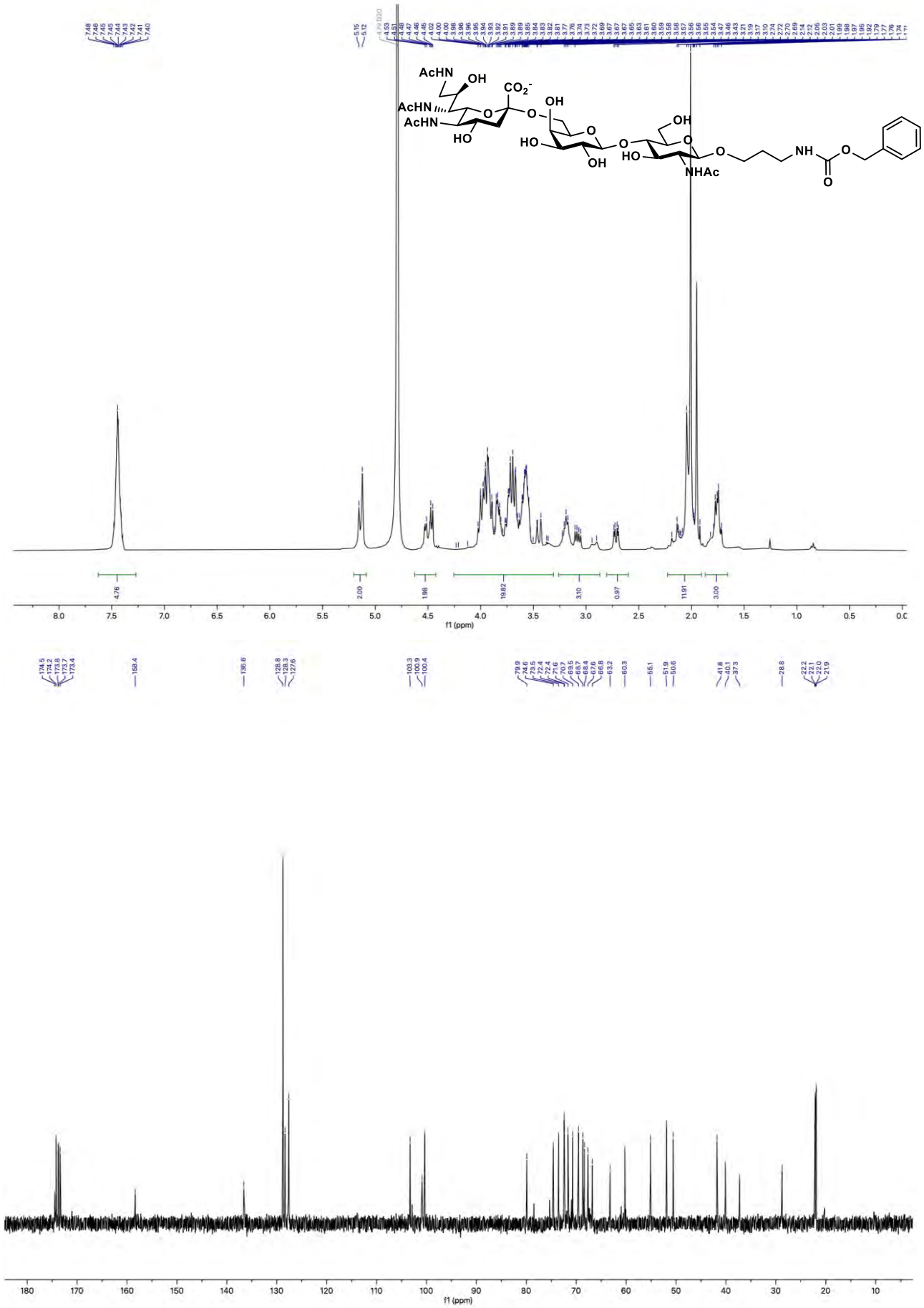
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–3LacNAcβProNHCbz (**62**) in D_2O



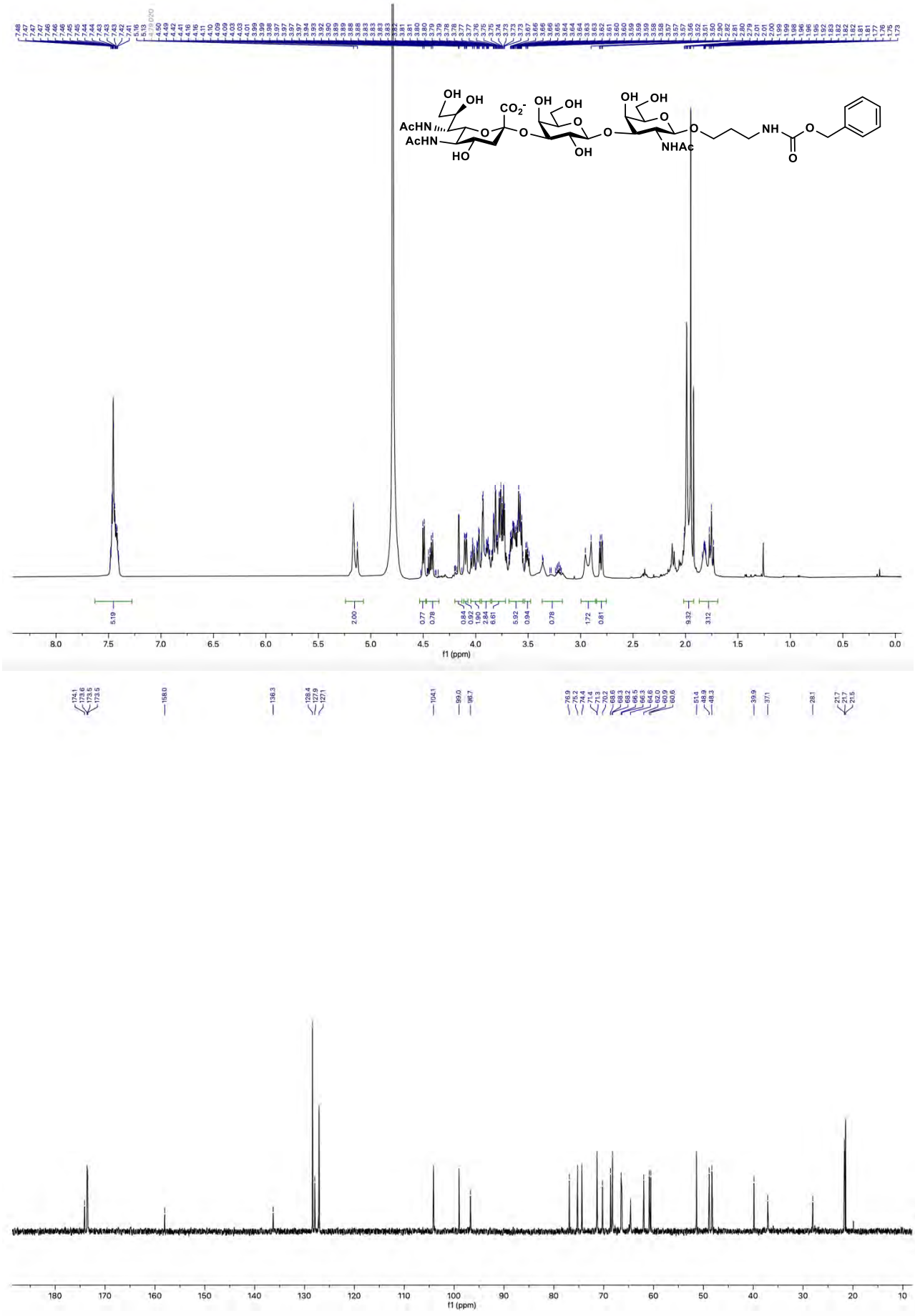
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–6LacNAcβProNHCbz (**63**) in D_2O



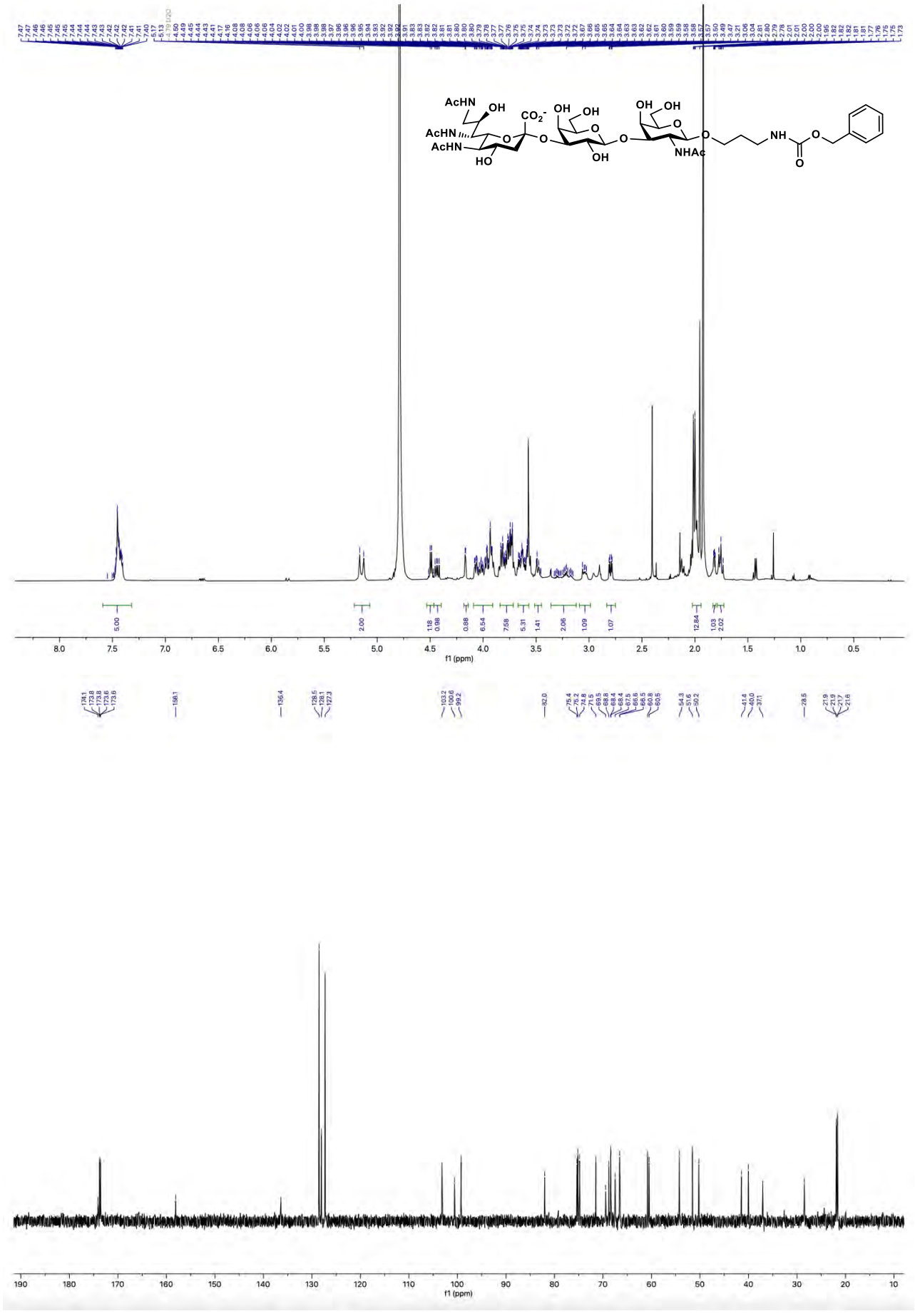
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–6LacNAcβProNHCbz (**64**) in D_2O



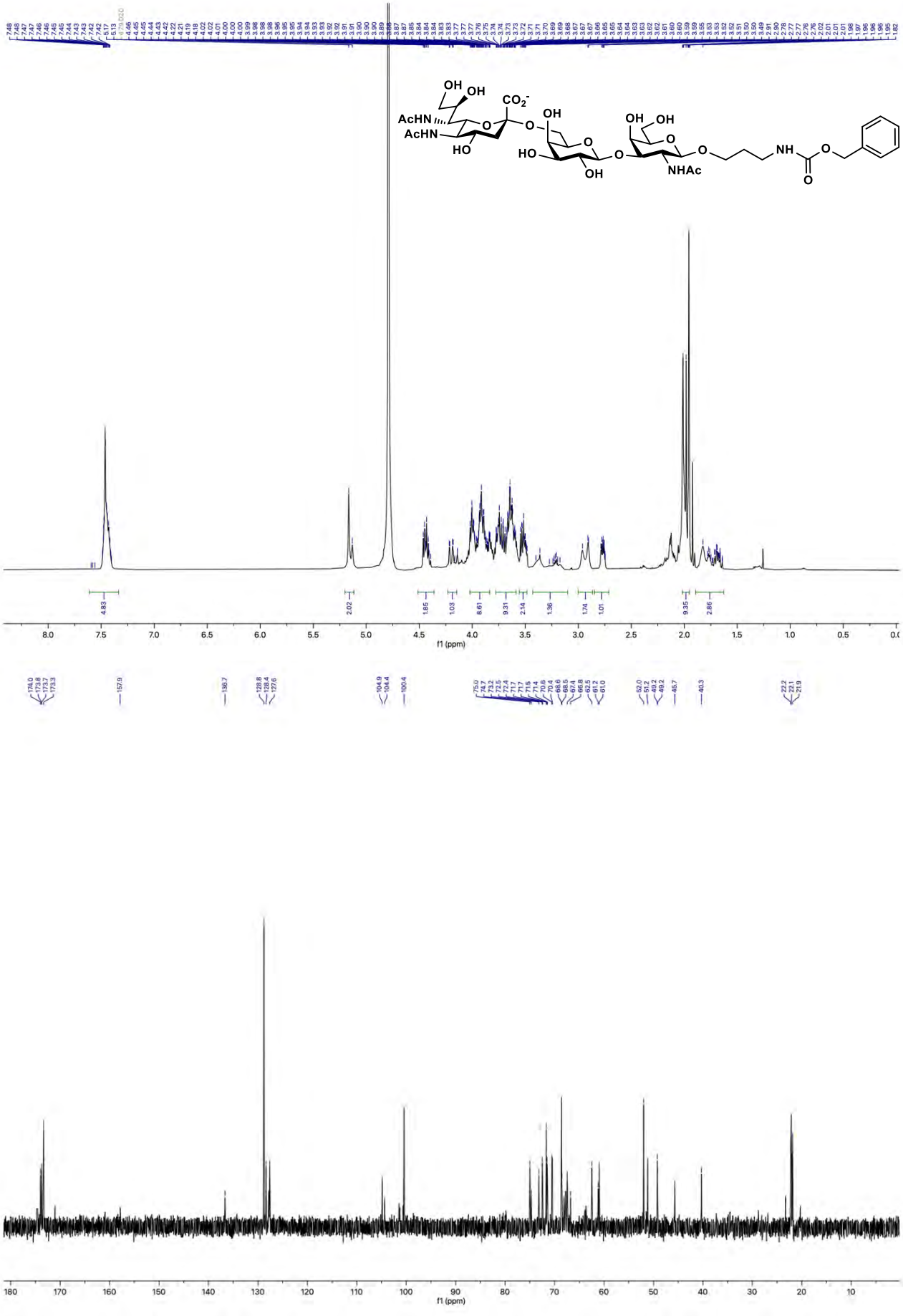
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–3Galβ1–3GalNAcβProNHCbz (**65**) in D_2O



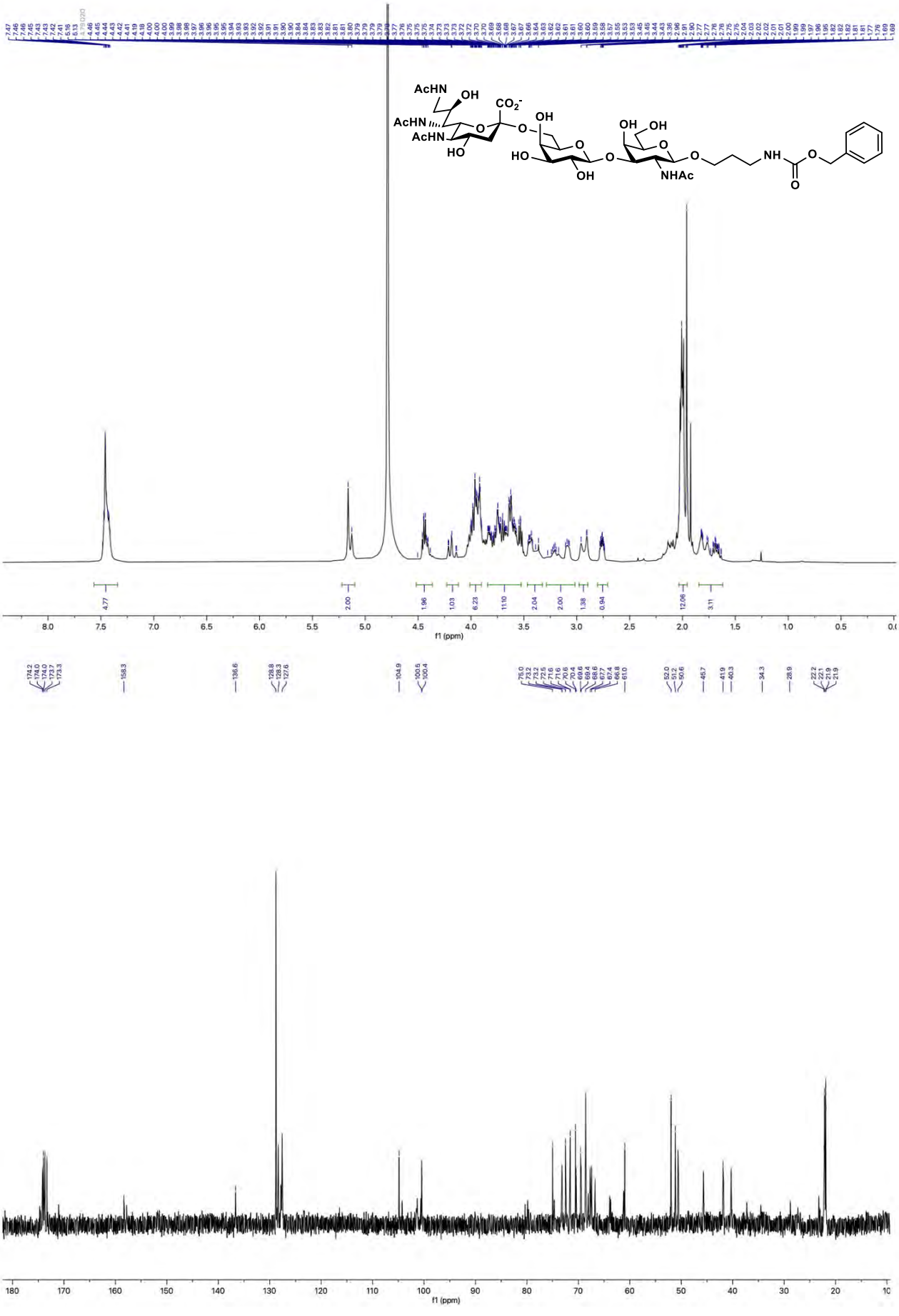
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–3Galβ1–3GalNAcβProNHCbz (**66**) in D_2O



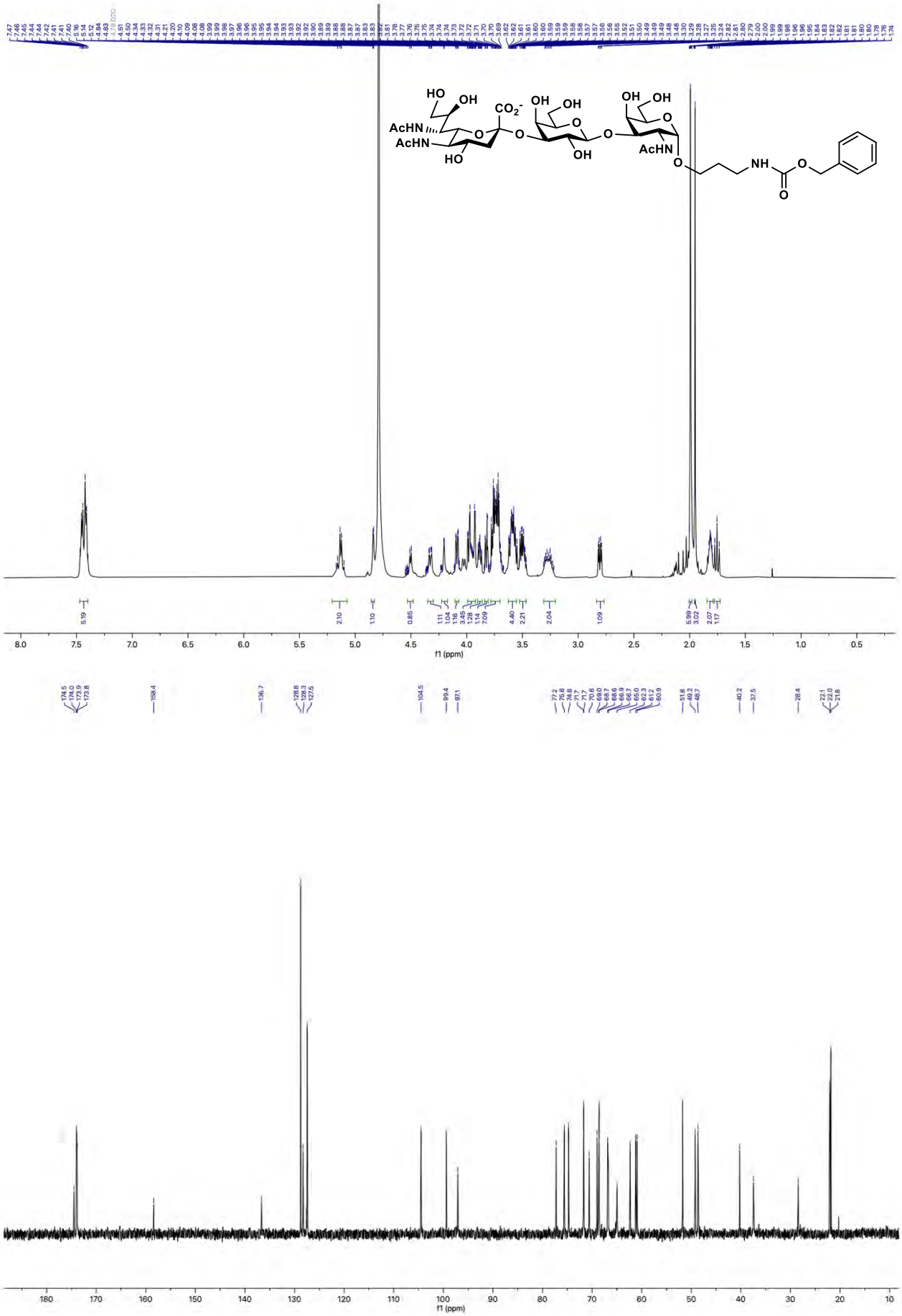
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–6Galβ1–3GalNAcβProNHCbz (**67**) in D_2O



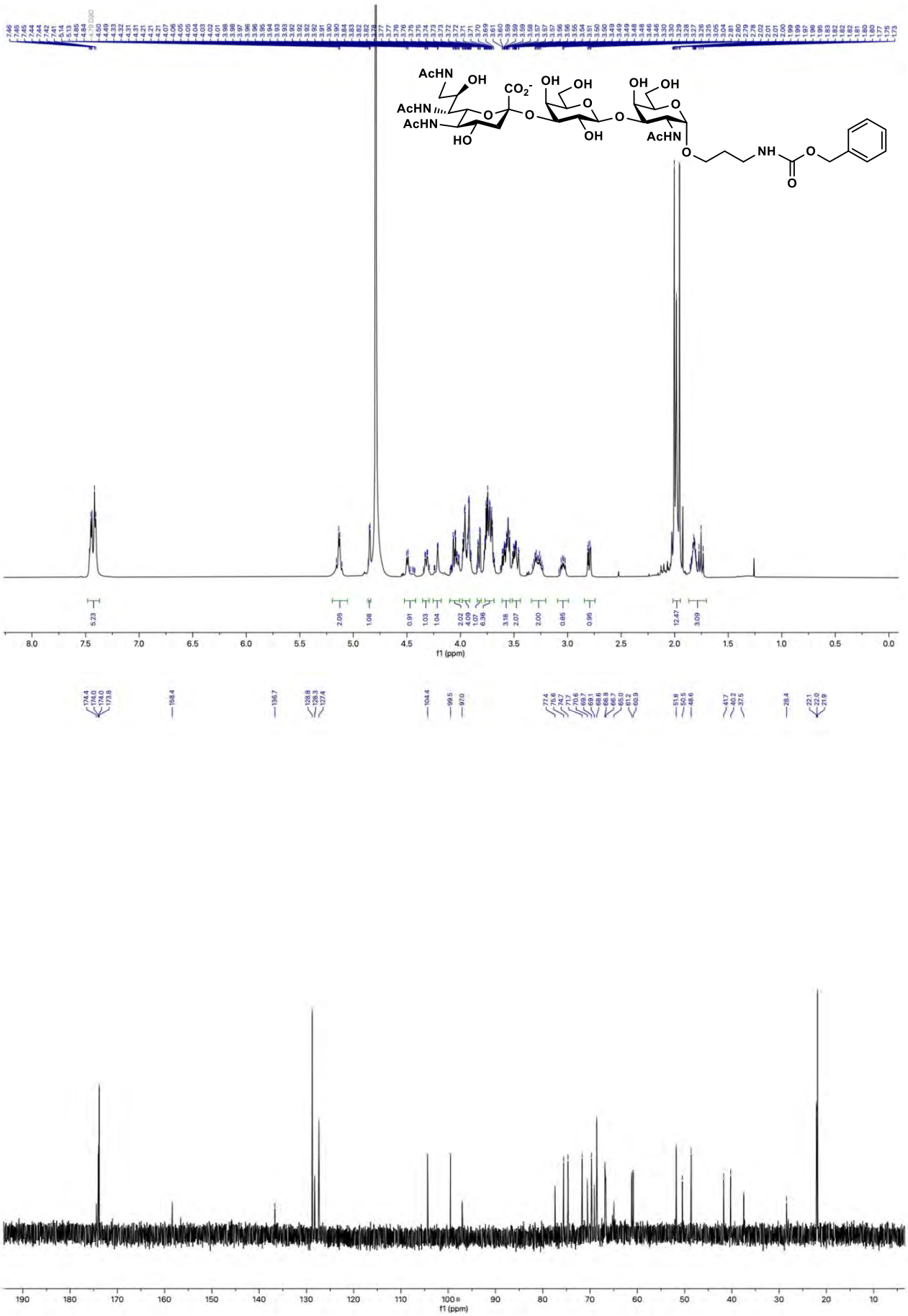
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–6Galβ1–3GalNAcβProNHCbz (**68**) in D_2O



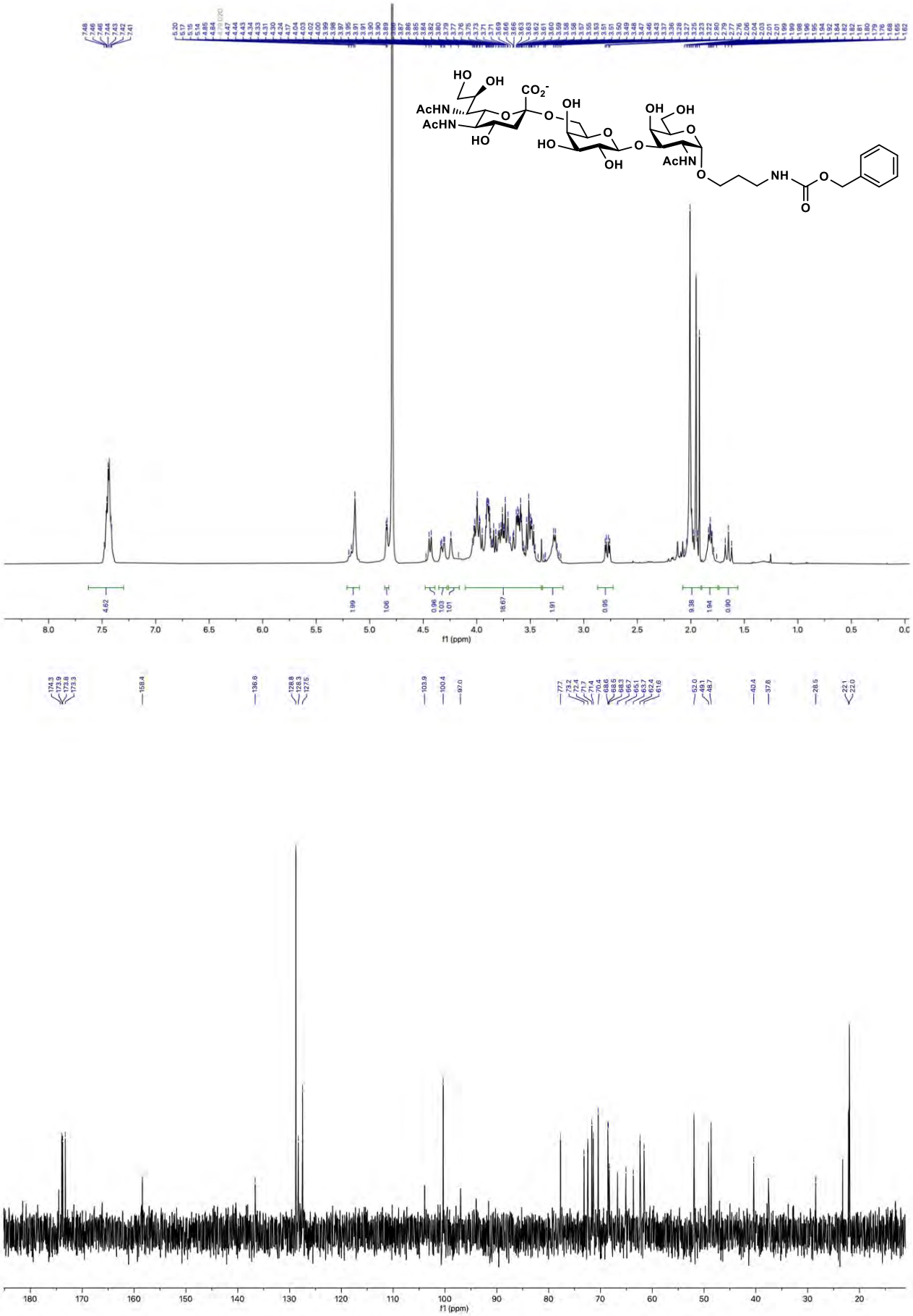
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–3Galβ1–3GalNAcαProNHCbz (**69**) in D_2O



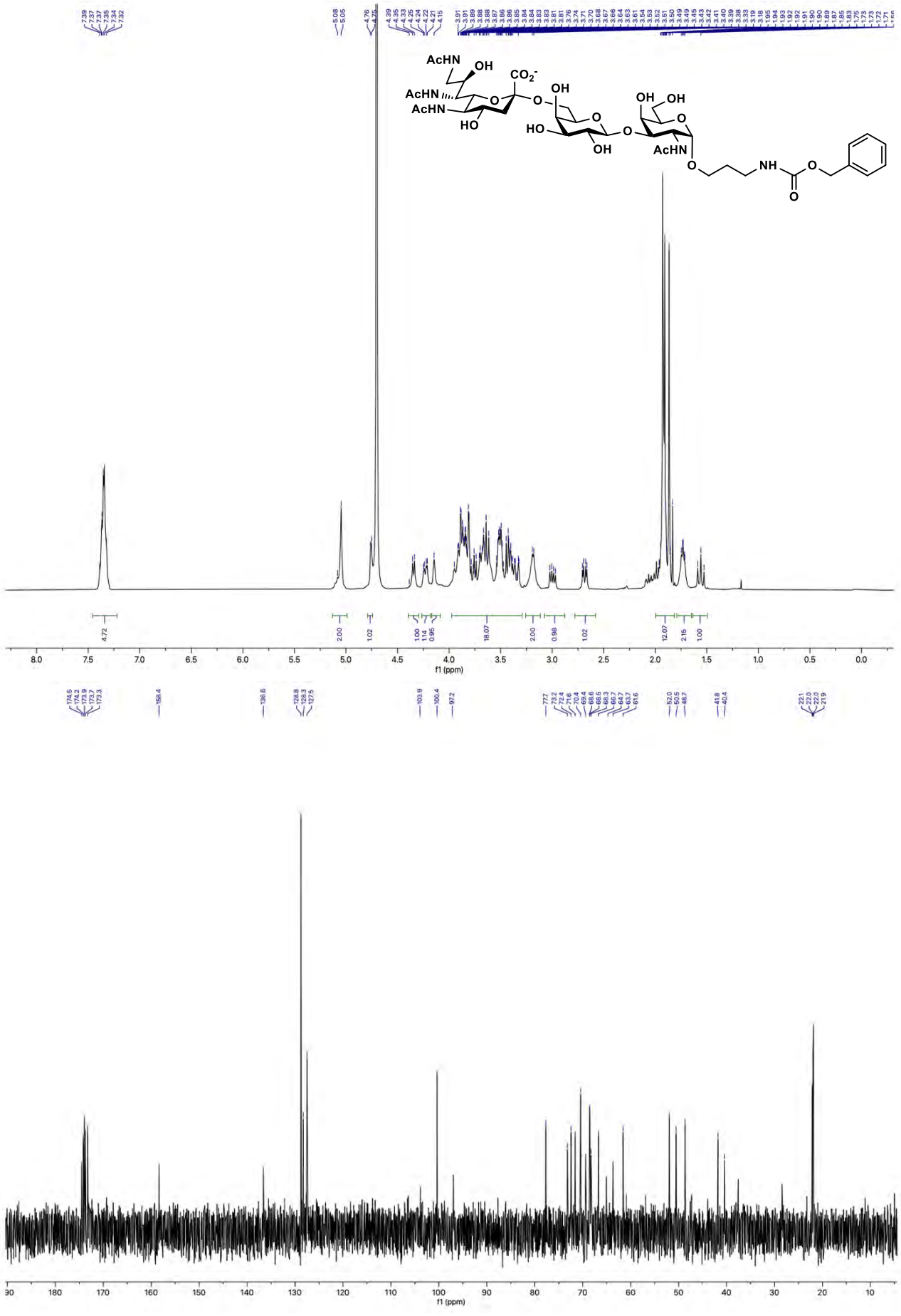
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–3Galβ1–3GalNAcαProNHCbz (**70**) in D_2O



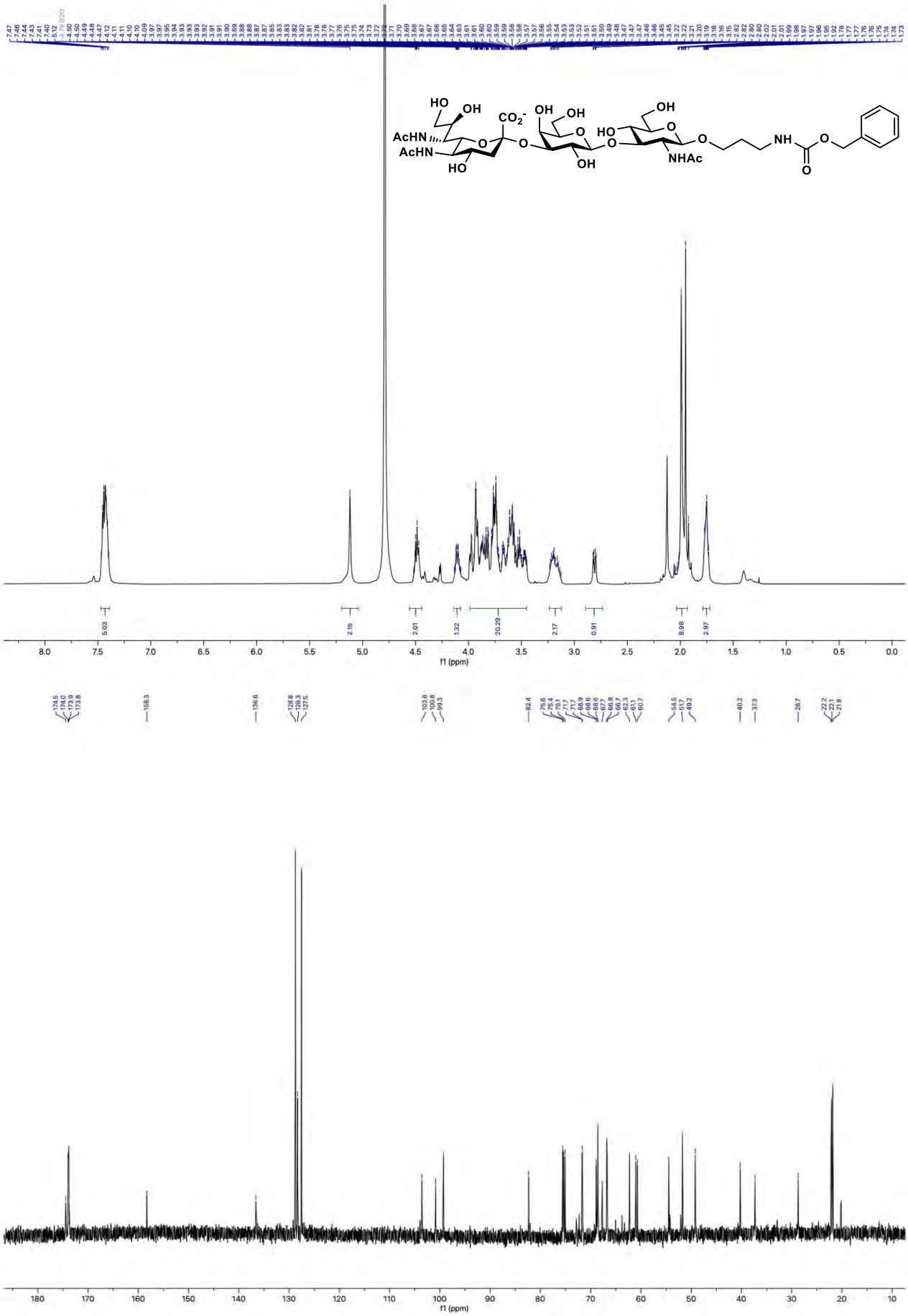
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–6Galβ1–3GalNAcαProNHCbz (**71**) in D_2O



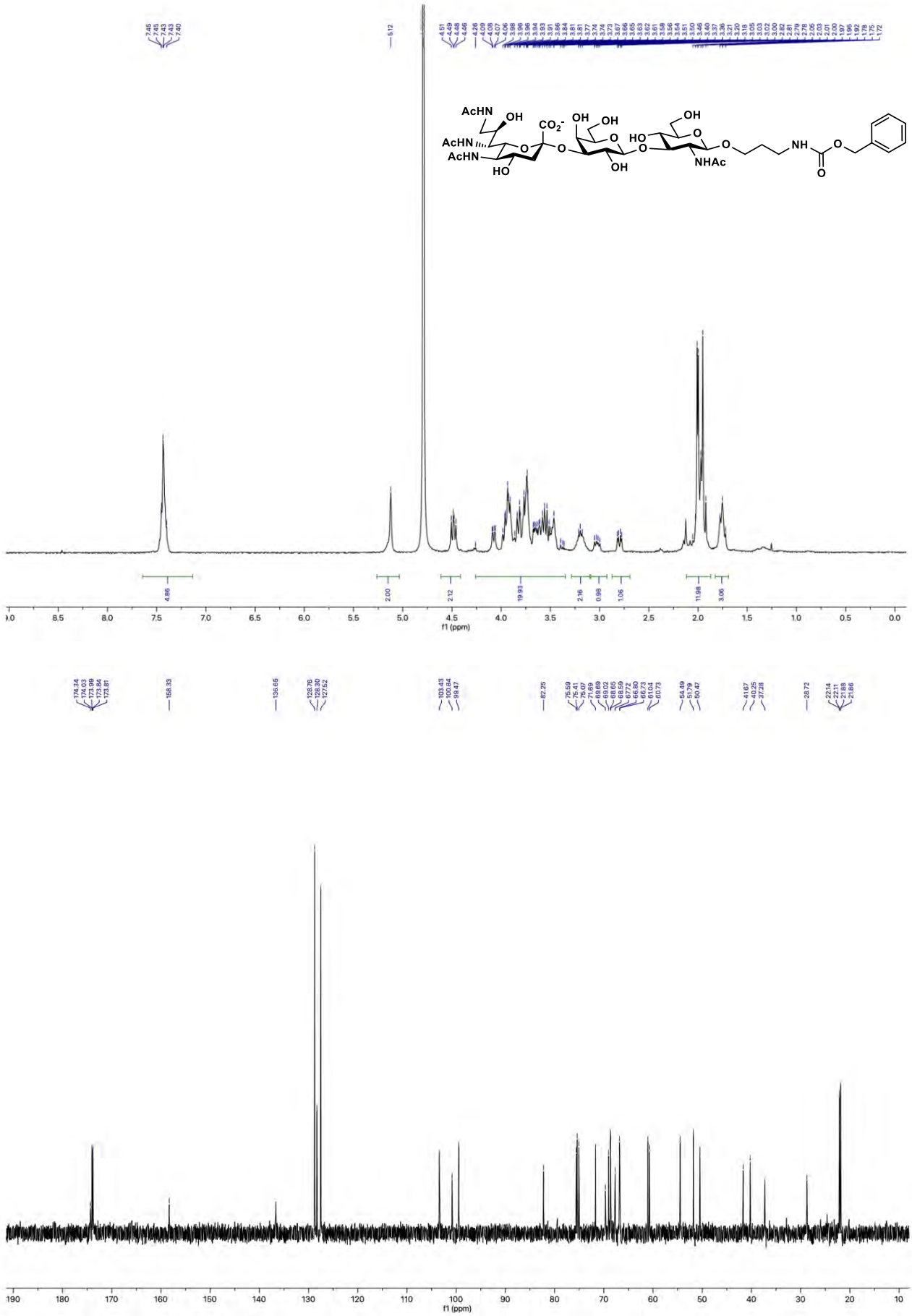
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–6Galβ1–3GalNAcαProNHCbz (**72**) in D_2O



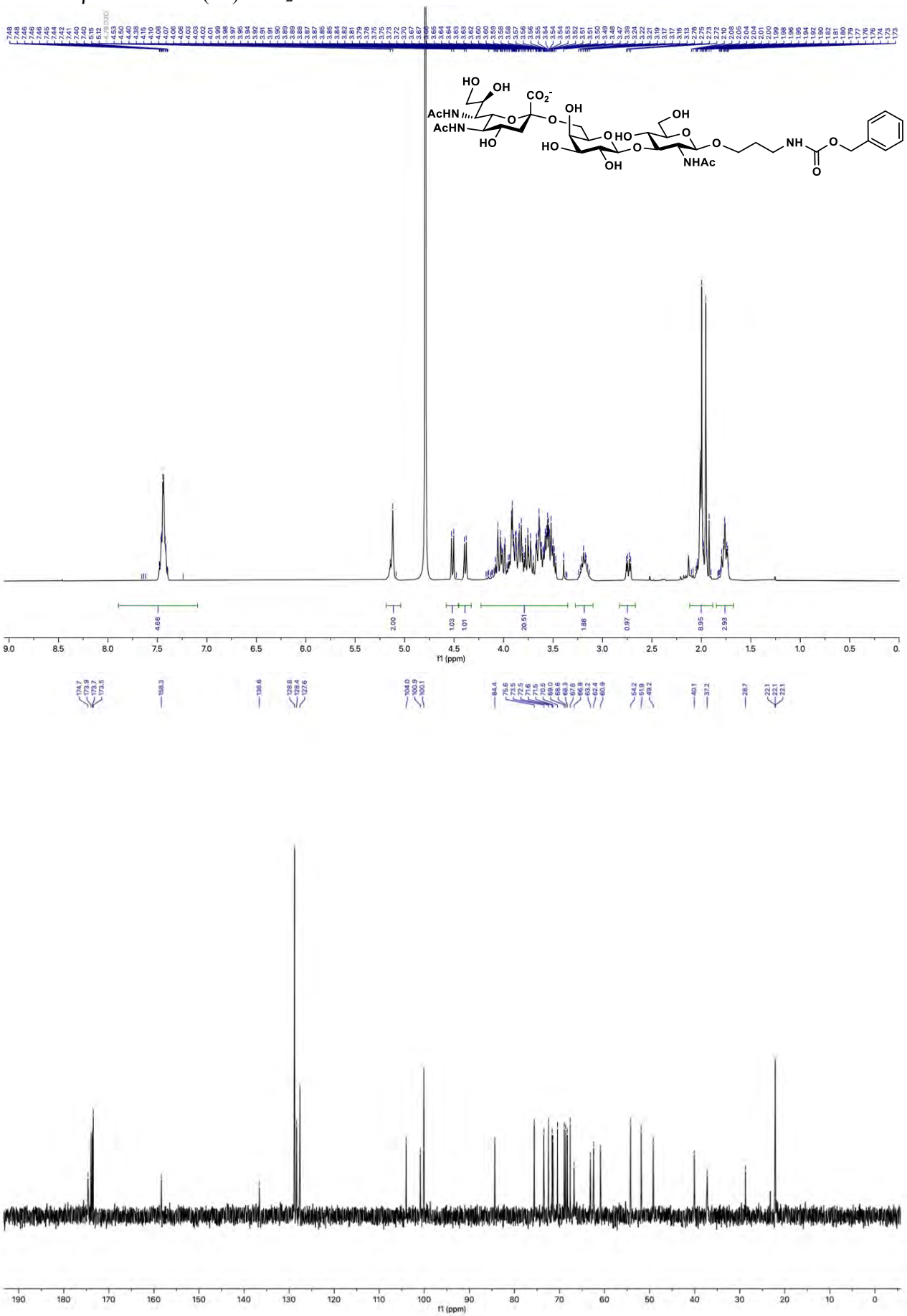
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–3Galβ1–3GlcNAcβProNHCbz (**73**) in D_2O



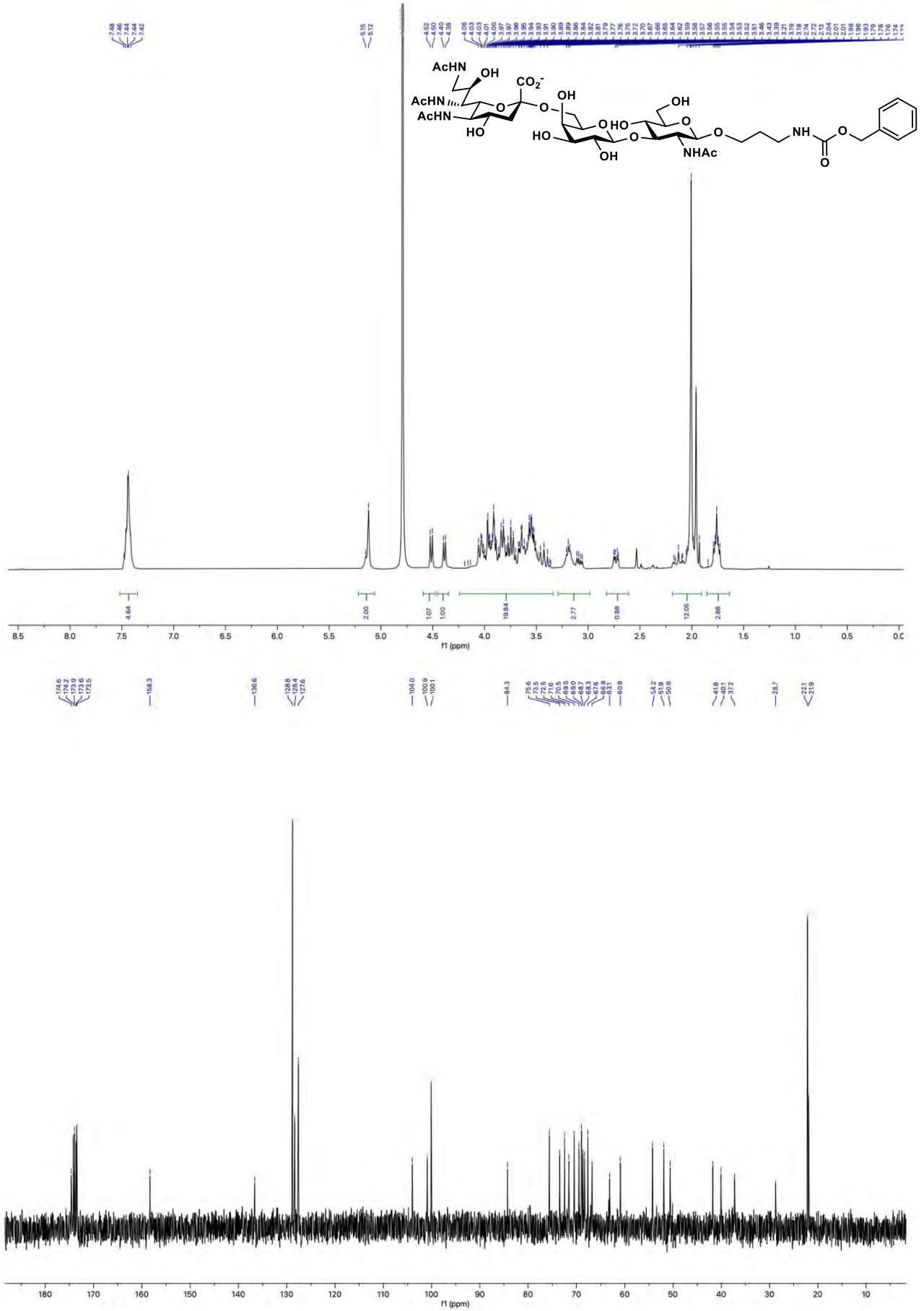
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–3Galβ1–3GlcNAcβProNHCbz (**74**) in D_2O



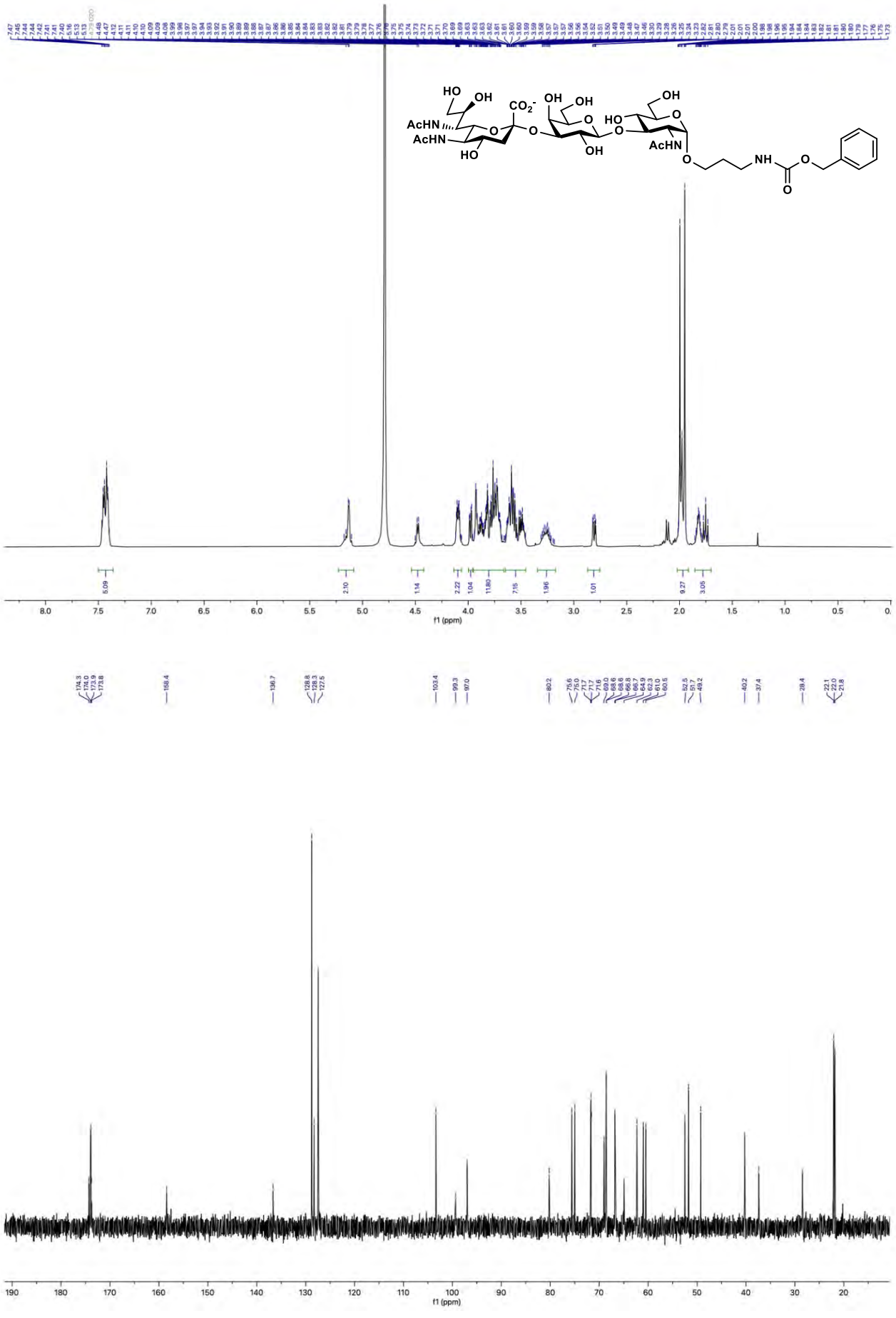
400 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–6Galβ1–3GlcNAcβProNHCbz (**75**) in D_2O



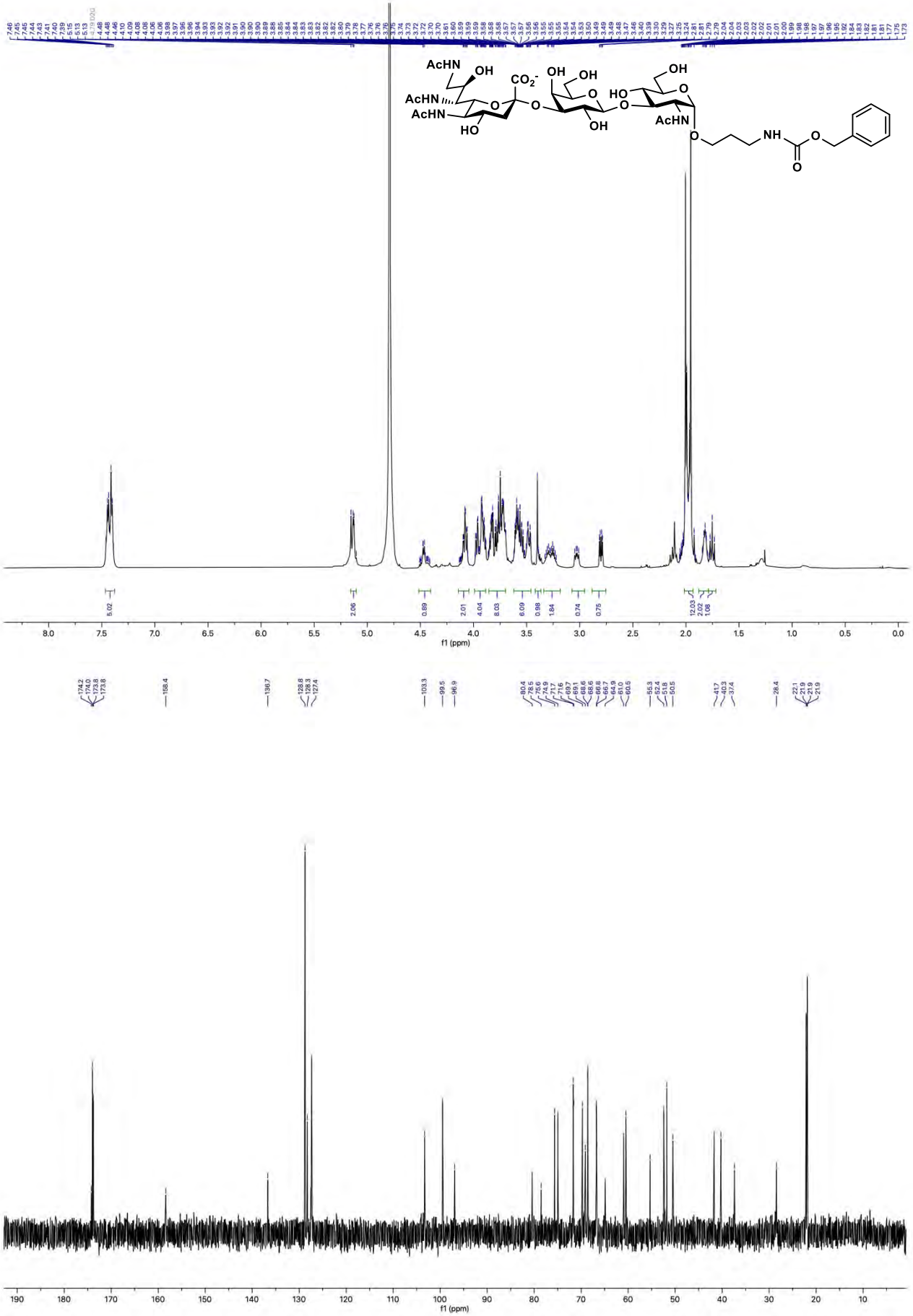
400 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–6Galβ1–3GlcNAcβProNHCbz (**76**) in D_2O



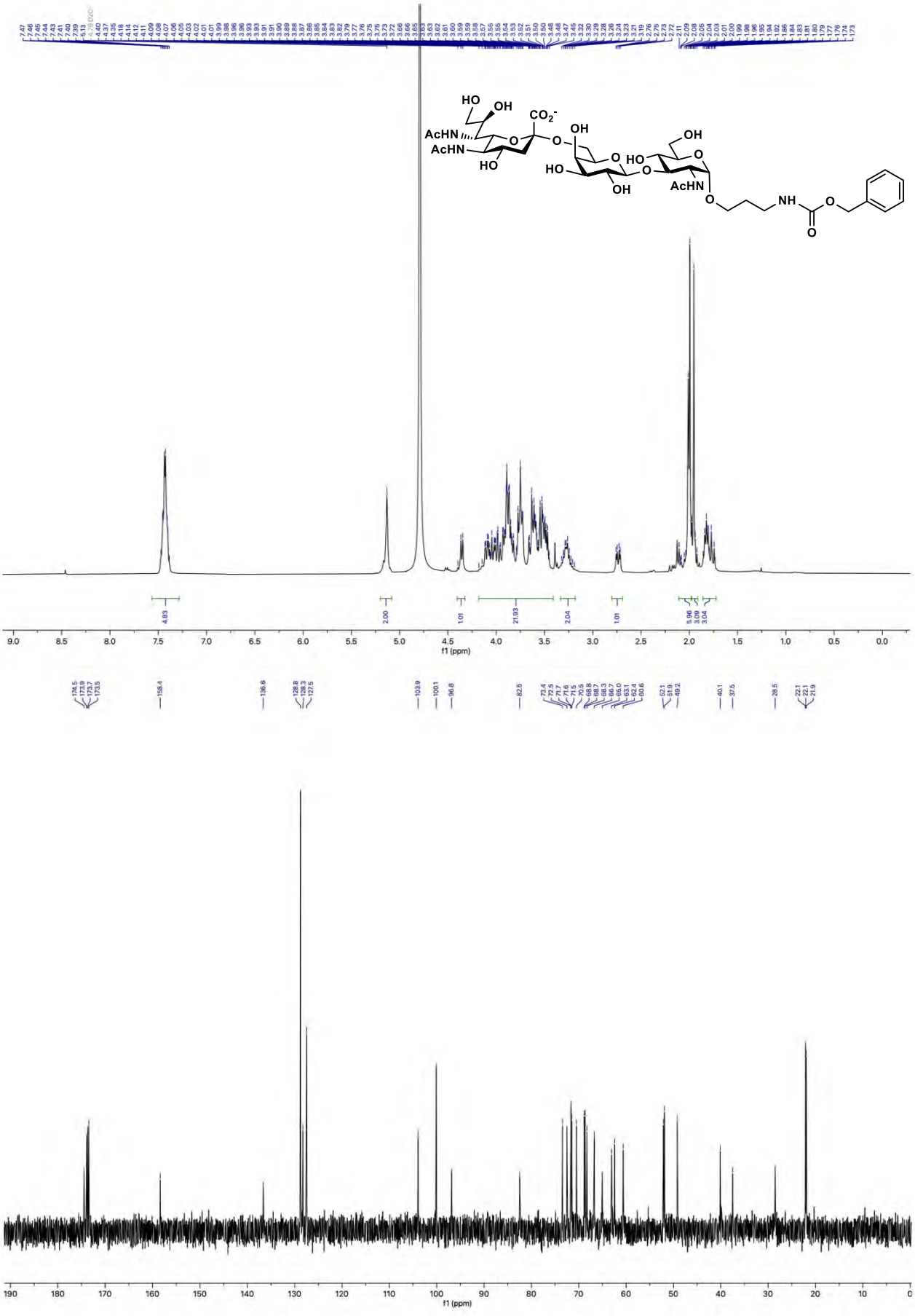
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–3Galβ1–3GlcNAcαProNHCbz (**77**) in D_2O



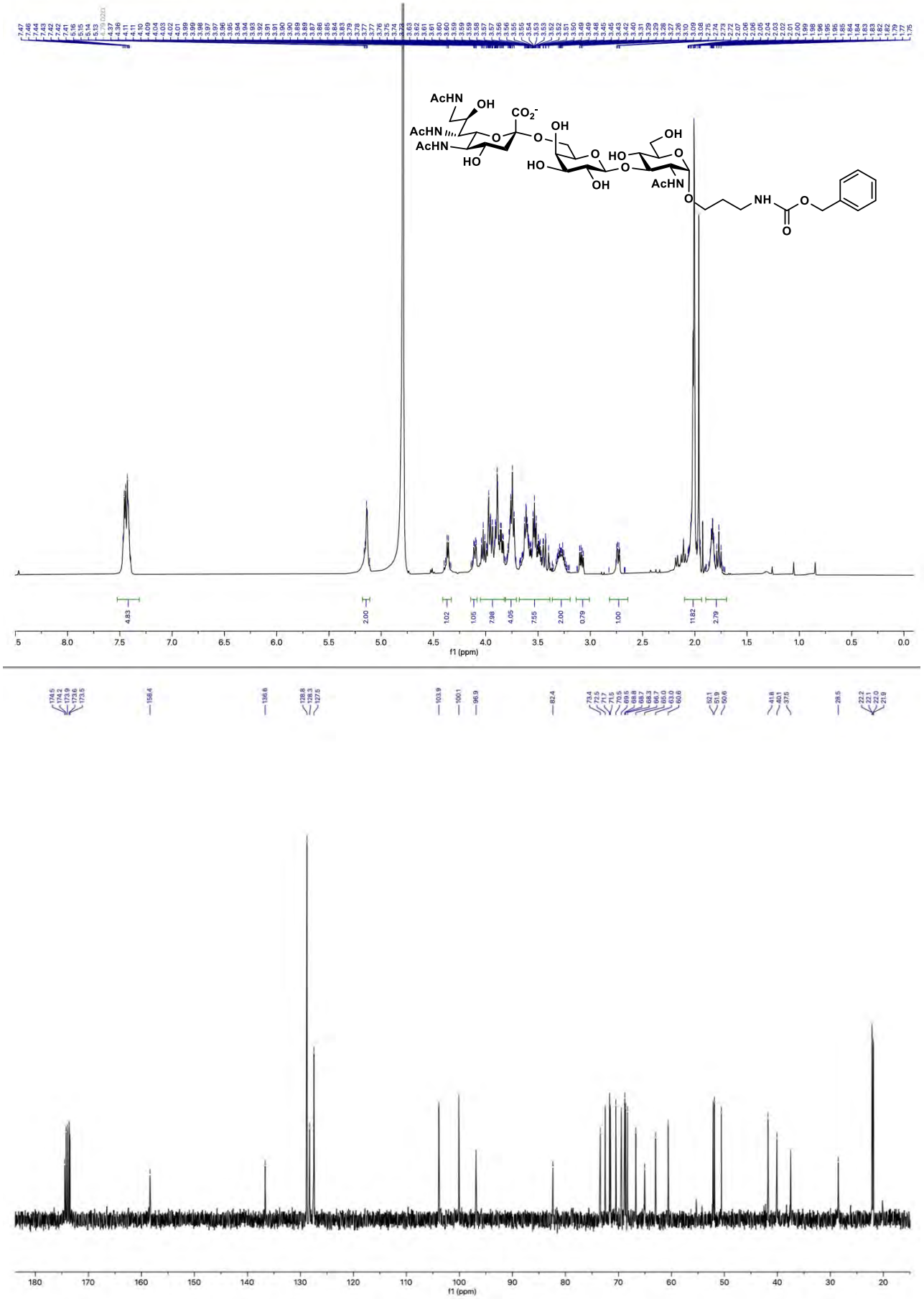
400 MHz ^{1}H and 100 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–3Galβ1–3GlcNAcαProNHCbz (**78**) in D_2O



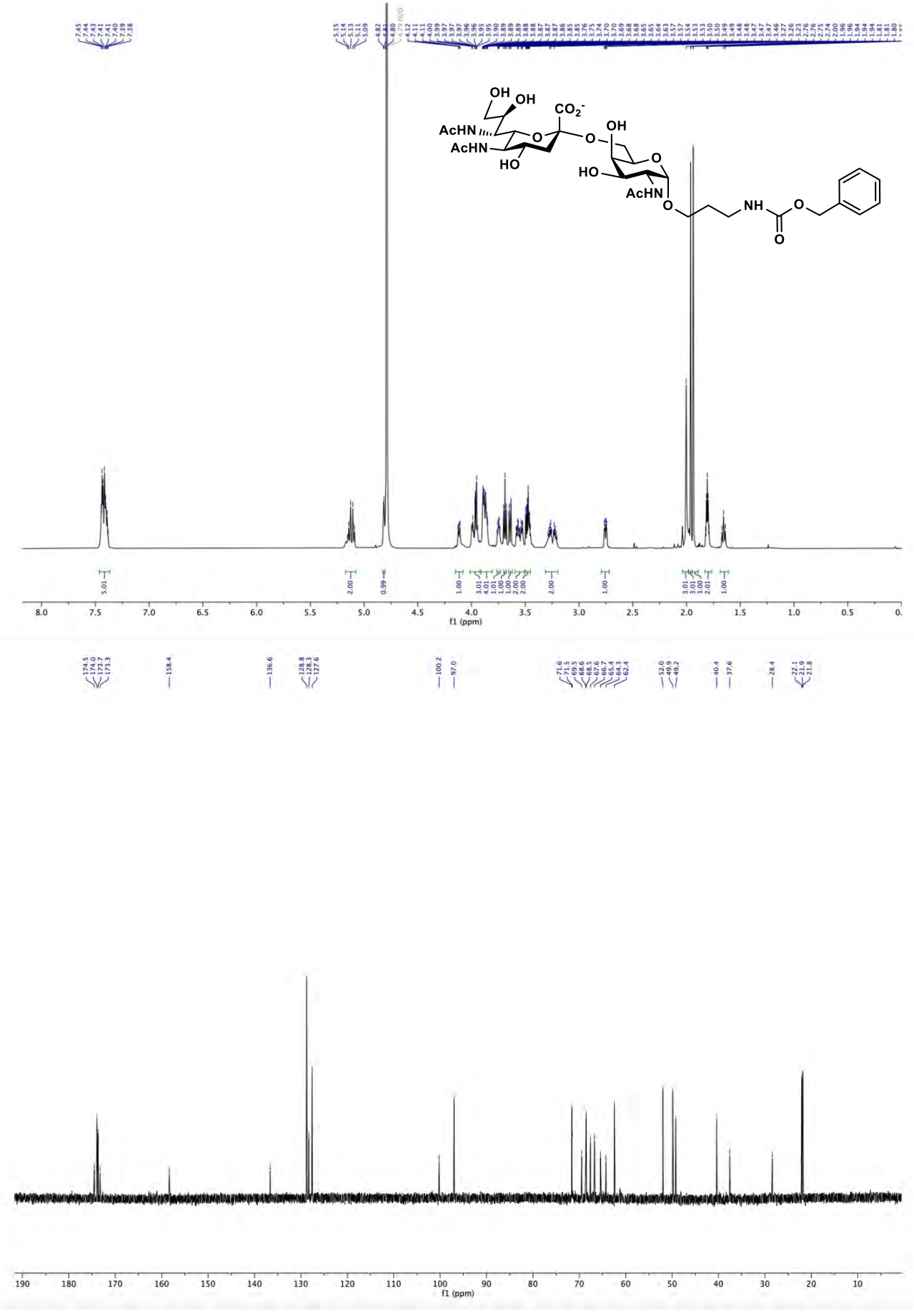
600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2–6Galβ1–3GlcNAcαProNHCbz (**79**) in D_2O



600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2–6Galβ1–3GlcNAcαProNHCbz (**80**) in D_2O



800 MHz ^{1}H and 200 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7NAcα2−6GalNAcαProNHCbz (**81**) in D_2O



600 MHz ^{1}H and 150 MHz $^{13}C\{^{1}H\}$ NMR spectra of Neu5Ac7,9diNAcα2−6GalNAcαProNHCbz (**82**) in D_2O