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Straightforward access to 2,3- and 3,4-unsaturated derivatives of *N*-glycolyl neuraminic acid

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PII: S0040-4020(20)30912-1

DOI: https://doi.org/10.1016/j.tet.2020.131699

Reference: TET 131699

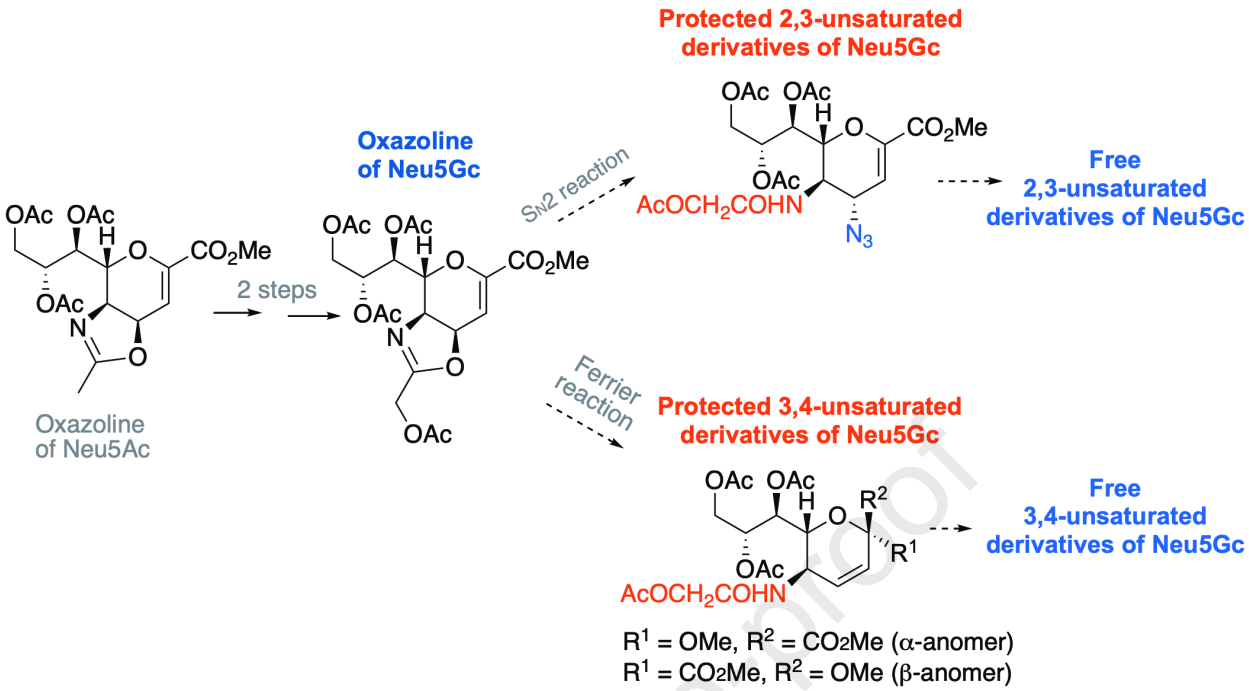
To appear in: *Tetrahedron*

Received Date: 31 August 2020

Revised Date: 14 October 2020

Accepted Date: 17 October 2020

Please cite this article as: Rota P, La Rocca P, Franco V, Allevi P, Straightforward access to 2,3- and 3,4-unsaturated derivatives of *N*-glycolyl neuraminic acid, *Tetrahedron*, https://doi.org/10.1016/j.tet.2020.131699.



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Straightforward access to 2,3- and 3,4-unsaturated derivatives of *N*-glycolyl neuraminic acid

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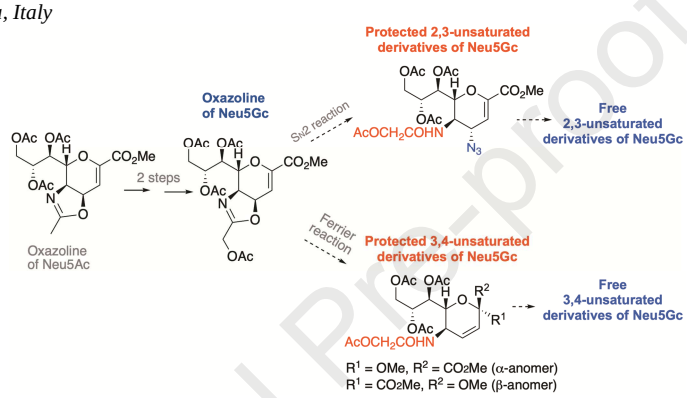

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This paper is dedicated to the memory of our mentor Professor Mario Anastasia, who passed away.

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Keyword_1 Sialidase inhibitor
Keyword_2 *N*-glycolylneuraminic acid
Keyword_3 Glycals
Keyword_4 Oxazoline of sialic acid
Keyword_5 Ferrier reaction

ABSTRACT

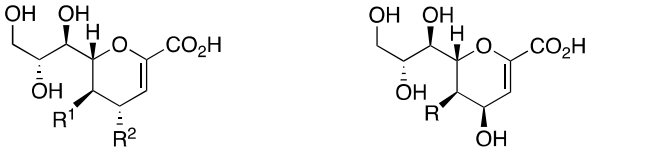
The facile syntheses of previously unreported 2,3- and 3,4-unsaturated derivatives of *N*-glycolyl neuraminic acid are herein described. The procedure involves a ring-opening of the 4,5-oxazoline of *N*-acetyl neuraminic acid, the acylation of the intermediate free amine, its conversion into the 4,5-oxazoline of *N*-glycolyl neuraminic acid and the subsequent Ferrier reaction or C4 nucleophilic substitution.

1. Introduction

Sialic acids (Sias) are a family of nine-carbons acidic monosaccharides present at the terminal portion of glycoconjugates, affecting their chemical and biological properties. Significantly, Sias are involved in several molecular recognition events associated with immune regulation, cell-cell interaction, inflammation processes, and bacterial or viral infections [1]. The most abundant mammalian-relevant members of the sialic acid family are *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). In contrast to other mammalians, Neu5Gc is not biosynthesized genetically in humans; however, the presence of exogenous Neu5Gc epitope in human cancers has generated high interest in the scientific community [1-2].

As well known, Sias removal from glycoconjugates is catalyzed by sialidases, enzymes widely distributed in vertebrates and a variety of microorganisms and viruses [1,3]. Considerable interest was devoted to studying the sialidase activity of viruses due to their crucial role in the release and spreading of newly synthesized viral progenies from the host cells. As a consequence of these researches, several 2,3-unsaturated derivatives of the Neu5Ac have been synthesized as sialidase inhibitors for therapeutic purposes [4].

This class of inhibitors includes the 5-acetamido-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enoic acid **1a** (Neu5Ac2en, DANA, derived from natural *N*-acetyl neuraminic acid, Neu5Ac) and various C5 *N*-acyl modified derivatives, as the *N*-trifluoroacetylated congener **1b** (FANA) [4] and, the less-studied, *N*-glycolyl derivative **1c** (Neu5Gc2en, derived from natural *N*-glycolyl neuraminic acid, Neu5Gc) [2b,5].



1a R^1 = NHAc, R^2 = OH; DANA
1b R^1 = $NHCOCF_3$, R^2 = OH; FANA
1c R^1 = $NHCOCH_2OH$, R^2 = OH
1d R^1 = NHAc, R^2 = N_3
1e R^1 = $NHCOCF_3$, R^2 = N_3
1f R^1 = NHAc, R^2 = $NH(C{=}NH)NH_2$; Zanamivir
1g R^1 = $NHCOCH_2OH$, R^2 = N_3

2a R = NHAc; 4-epiDANA
2b R = $NHCOCF_3$; 4-epiFANA
2c R = $NHCOCH_2OH$

Fig. 1. Chemical structures of some 2,3-unsaturated sialidase inhibitors.

Interestingly, the C4 position is also ideal for the insertion of structural modifications necessary to improve molecule-enzyme catalytic site interactions [4b,d,e,6]. In this regard, several C4 modified derivatives of DANA and FANA have been extensively developed [4b,d,e,6,7], as potent sialidase and hemagglutinin-

ne C4

such as the azido derivatives **1d** and **1e** [4c,d,7] and, the well-known, guanidino derivative **1f**, commercially available as Zanamivir [8]. On the other hand, the modified C4 analogs of Neu5Gc, such as compound **1g**, have not yet been synthesized and studied. Analogously, while the inhibitory activity, on different sialidases, of the DANA and FANA C4 epimers, **2a** and **2b**, has been investigated [4e,9], that of 4-epi Neu5Gc2en **2c** has never been described.

The limited literature on C4-modified glycals of NeuGc is not justified based on the sialidase activity inhibition values reported for the parent compound **1c** [2b,5]. Indeed, the *Ki* or IC_{50} values, found on some sialidases, are comparable to those of DANA **1a** (e.g., *Ki* 2.61 μM or IC_{50} 8.6 ± 1 μM for Neu5Gc2en **1c** versus *Ki* 3.56 μM or IC_{50} 8.6 ± 1 μM for DANA **1a** against *Vibrio cholerae*; IC_{50} 34 ± 4 μM for Neu5Gc2en **1c** versus IC_{50} 18.0 ± 1 μM for DANA **1a** against NEU2) [5b,d]. Probably, the restricted data in Neu5Gc glycals, it could be due to the need for quick synthetic protocols to obtain these derivatives.

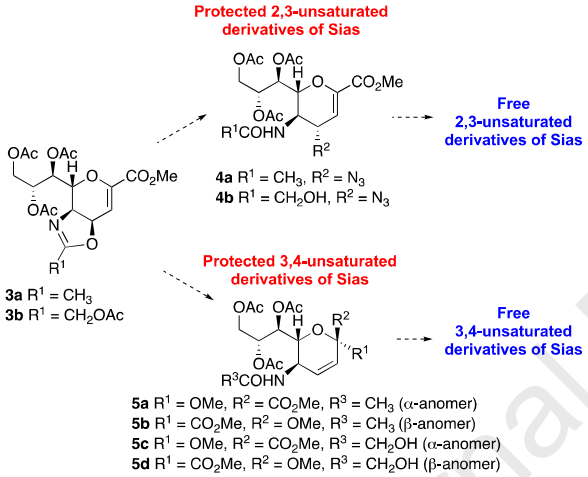


Fig. 2. Chemical structures of protected 2,3- and 3,4-unsaturated Sias derivatives **4a,b** and **5a,d**, achieved starting from 4,5-oxazoline **3a** or **3b**.

It is noteworthy that the 4,5-oxazoline of Neu5Ac **3a** [8] is a key intermediate for the synthesis of the C4-azido derivative **4a**, precursor of free glycals **1d-f** [4c,d,7,8]. Moreover, the compound **3a** is a useful starting material for the synthesis of 3,4-unsaturated derivatives of DANA (i.e., **5a** and **5b**), *via* Ferrier reaction [9], as described in the literature and recently reported by us [9d,e]. Finally, as we have recently demonstrated [8b], the ring-opening of oxazoline can be directed both to the synthesis of the 4-epi derivative of Neu5Ac **2a** and to a C5 amino intermediate, variously modifiable.

With these premises, we thought that the previously unreported protected glycolyl 4,5-oxazoline **3b** could be a good precursor for the synthesis of both: a) the protected glycal derivative **4b** of Neu5Gc to generate various 2,3-unsaturated derivatives (i.e., **1g**); b) the 3,4-unsaturated derivatives of Neu5Gc (i.e., **5c** and **5d**), *via* Ferrier reaction.

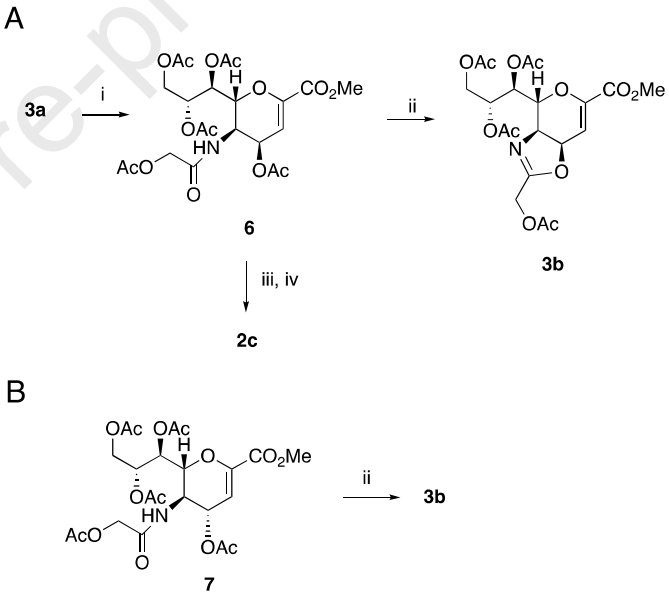
Thus, in our continuous effort to understand the Sias role in biological matrices and to provide new synthetic tools in this field [10], we report here an efficient protocol to obtain the 4,5-oxazoline of Neu5Gc **3b**, exploiting our procedure involving the generation of a C5 amino group by the 4,5-oxazoline **3a** ring-opening. We, therefore, showed the effectiveness of the 4,5-oxazoline intermediate **3b** in the synthesis of new 2,3- and 3,4-unsaturated glycolyl derivatives. Just by way of example, we compound **4b**, the precursor of several potential glycal inhibitors, including compound **1g**, and the 3,4-unsaturated derivatives **5c,d**.

2. Results and discussion

We recently developed a procedure of high synthetic value involving the conversion of the 4,5-oxazoline **3a** [8b], into the C5 amino derivative and, then, its direct transformation in a C5 amido compound, employing a suitable acylating agent and an excess of a weak basic resin, having a tertiary amine functionality (IRA-67).

We anticipated the protocol could be used to prepare compound **6**, having a C5 protected glycolic amido substituent (acetoxyacetyl amido group) and a C4β-geometry, and to convert this into the oxazoline **3b**. Moreover, a further advantage of this procedure could accrue through the direct hydrolysis of **6** into the new compound **2c**, the C4 epimer of **1c**.

To this end, the compound **6** was able to be synthesized in high yield (78%) from oxazoline **3a** by treatment with aqueous acetonitrile containing trifluoroacetic acid (TFA) and, after the disappearance of the starting material, addition of acetoxyacetyl chloride in the presence of excess of basic resin (IRA-67) (Scheme 1, path A).



Scheme 1. Synthesis of the oxazoline **3b** and the free 2,3-unsaturated compound **2c**. *Reagents and conditions:* i) TFA, CH_3CN-H_2O, 40/1 v/v, 23°C, 10 min, then acetoxyacetyl chloride, weak basic resin IRA 67, 0°C to 23°C, 40 min, 78%; ii) BF_3 Et_2O, CH_2Cl_2, 23°C to 80°C, 20 min, 63-65%; iii) NaOMe, MeOH, 23°C, 1h, 68%; iv) NaOH aq. 1 M, MeOH, 23 °C, 30 min, then strong acidic resin (Dowex 50WX8, H^+), 80%.

Then, by simple treatment of **6** with BF_3 Et_2O in dichloromethane, we obtained the unreported oxazoline **3b** (63% yield), having correct physicochemical properties (mass, ^1H and ^{13}C NMR spectra). Alternatively, 4,5-oxazoline **3b** was obtained (65% yield), using the same reaction conditions, but starting from the known compound **7** [5d] (scheme 1, path B).

Remarkably, compound **6** could be rapidly transformed into the 4-epi glycolyl derivative **2c**, in good overall yield (54%), by deacetylation with NaOMe in methanol, followed by chromatography purification, and basic hydrolysis of the corresponding methyl ester with NaOH in water solution.

Then, to demonstrate the utility of this approach for the synthesis of 2,3-unsaturated Neu5Gc glycals, we subjected the oxazoline **3b** to reaction with azidotrimethyl silane in *t*-butyl alcohol to

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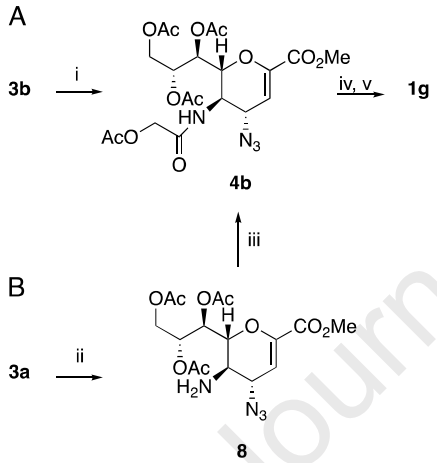
reportedpreviously [11] (scheme 2, path A). Thus, we were able to obtain the key intermediate **4b** in acceptable yield (44%).
In order to confirm the structure of the azide **4b**, and to improve its yield formation, we also attempted a second approach based on a longer synthetic protocol (scheme 2, path B), previously used by the von Itzstein group to synthesize structural analogues [4c].
Oxazoline **3a** was transformed into the C5 amino derivative **8** in five steps (39%), according to literature protocol [4c, 11]. Compound **8** was dissolved in CH_2Cl_2 and acylated with acetoxyacetyl chloride in the presence of an excess of Et_3N, to give the desired compound **4b** in good yield (75%).

As expected, both the synthetic routes led to the desired product **4b**, showing NMR and MS spectra superimposable and in agreement with the reported structure.

The two routes are, in our hands, comparable in terms of overall yields starting from oxazoline **3a** (22% first way versus 29% second way); however, the first pathway is definitely shorter in terms of synthetic passages (tree versus six steps) and reagent/solvent and time-consuming.

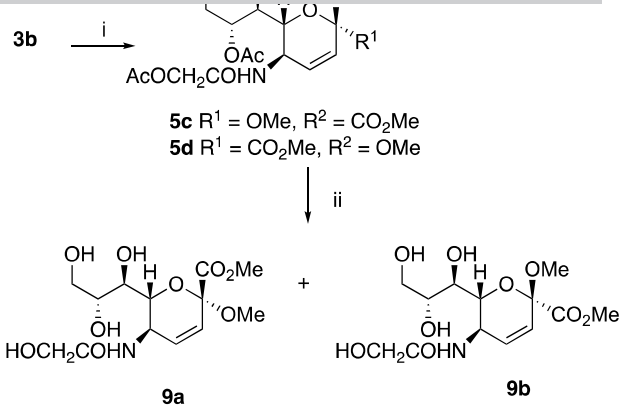
Then compound **4b** was transformed in free new glycal **1g** by Zemplén deacetylation and, subsequent, hydrolytic treatment with NaOH.



Scheme 2. Two strategies to synthesized compound **4b**. *Reagents and conditions:* i) $TMSN_3$, *t*BuOH, 80°C, overnight, 44%; ii) ref [4c,11], overall yield 39%; iii) Acetoxyacetyl chloride, Et_3N, CH_2Cl_2, 0°C to 23°C, overnight, 75%; iv) NaOMe, MeOH, 23°C, 1 h, 66%; v) NaOH aq. 1 M, MeOH, 23 °C, 30 min, then strong acidic resin (Dowex 50WX8, H^+), 76%.

At this stage, we tested the possibility also to achieve the 3,4-unsaturated derivatives, *via* our optimized Ferrier reaction conditions [9d, 12], starting from oxazoline **3b**. At this purpose we dissolved compound **3b** in anhydrous CH_3CN, using methanol as a nucleophile and Montmorillonite K-10 as catalyst (scheme 3). The reaction mixture was stirred at 80°C, until the disappearance of the starting material (1h). The reaction gave, in a short time (1h), an inseparable mixture of **5c** and **5d** in high β-anomeric selectivity (19/81, α/β), and satisfactory yields (62%). Moreover, minor traces of by-products were detectable by TLC.

The correct anomeric stereochemistry was assigned based on the new empirical rules, we recently set-up for 3,4-unsaturated derivatives of Neu5Ac [12]. In this previous work, we concluded that the ^{13}C chemical shift (δ) of the C6 signal was diagnostic for the anomers stereochemistry assignment, the α anomers showed a C6 chemical shift > 74 ppm, whereas a value < 72 ppm was indicative for the β-ones.



Scheme 3. Synthesis of 3,4-usaturated derivatives of Neu5Gc *via* Ferrier reaction. *Reagents and conditions:* i) MeOH (as nucleophile), montmorillonite K-10 40%w/w, CH_3CN, 80°C, 1h, 62% (inseparable mixture **5c** + **5d**, yield after chromatography); ii) NaOMe, MeOH, 23°C, 30 min, 80%.

In the present case, we observed that the C6 value of compound **5c** (α anomer) was of δ = 73.9 ppm, slightly lower than that reported in our rule, while that of **5d** (δ = 70.0 ppm β anomer) was in agreement with that described.

In the aforementioned work [12], we noticed that chemical shift differences were particularly significant for anomers bearing unprotected hydroxyl functions. Therefore, to further support the correct anomeric stereochemistry of **5c** and **5d** we transformed the mixture of these compounds into the corresponding free, chromatographically separable, alcohols **9a** and **9b**.
The NMR analyses of the obtained derivatives confirmed the general applicability of empirical rules. Indeed, the ^{13}C chemical shifts of C6 signal showed a value of 76.8 ppm (δ > 74 ppm) for the α derivative **9a** and a value of 71.6 ppm (δ < 72 ppm) for the β anomer **9b**.

3. Conclusions

In this study we set-up an efficient synthetic protocol to obtain the 2,3- and 3,4-unsaturated *N*-glycolyl derivatives. In particular, we demonstrated that the 4,5-oxazoline of Neu5Gc **3b** could be readily obtained from the 4,5-oxazoline of Neu5Ac **3a** and could be used as a starting material for the synthesis of glycal **4b** or the synthesis of 3,4-unsaturated Ferrier products **5c,d**. Moreover, we synthesized a key azido compound **4b**, precursor of different free glycals, by an additional independent way. Furthermore, the synthetic protocol allowed for the efficient synthesis of 4-epi Neu5Gc2en **2c** in few steps from **3a**. The biological evaluation of these molecules as sialidase inhibitors will be the subject of future studies and, it is currently ongoing in our labs.

4. Experimental

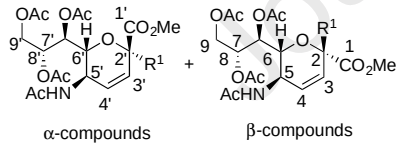
4.1 Chemistry

4.1.1 General information.

All chemicals and solvents used were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was prepared by filtering water on a Milli-Q Simplicity 185 filtration system from Millipore (Bedford, MA, USA). Solvents were dried using standard methods and distilled before use. The progress of all reactions was monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Sigma-Aldrich silica gel plates (60 F254) using UV light, anisaldehyde /H_2SO_4 /EtOH solution or 0.2% ninhydrin in ethanol and heat as the developing agent. Flash chromatography was performed with

no H_2), gel), following the general protocol of Still. Nuclear magnetic resonance spectra were recorded at 303K on a Bruker AM-500 spectrometer equipped with a 5-mm inverse-geometry broadband probe and operating at 500.13 MHz for ^1H and 125.76 MHz for ^{13}C. Chemical shifts are reported in parts per million and are referenced for ^1H spectra, to a solvent residue proton signal (δ = 7.26 and 3.31 ppm, respectively, for $CDCl_3$ and CD_3OD) and for ^{13}C spectra, to solvent carbon signal (central line at δ = 77.0 and 49.05 ppm, respectively, for $CDCl_3$ and CD_3OD). The ^1H and ^{13}C resonances were assigned by ^1H–^1H (COSY) and ^1H–^{13}C (HSQC and HMBC) correlation 2D experiments. The ^1H NMR data are tabulated in the following order: multiplicity (s=singlet, d=doublet, t=triplet, br s=broad singlet, m=multiplet, app=apparent), coupling constant(s) (J) are given in Hertz ([Hz]), number of protons and assignment of proton(s). Optical rotations were taken on a Perkin-Elmer 241 polarimeter equipped with a 1 dm tube; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} and the concentrations are given in g per 100 mL. Elemental analyses were performed on a Perkin Elmer series II, 2400 CHN analyzer and experimental data were within ± 0.4% of the theoretical values. Mass spectrometry was performed by using an ABSciex 4000Qtrap mass spectrometer equipped with an ESI ion source. The spectra were collected in a continuous flow mode by connecting the infusion pump directly to the ESI source. Solutions of the compounds were infused at a flow rate of 0.01 mL min^{-1}, the spray voltage was set at 4.5 kV in the negative ion mode with a capillary temperature of 550°C. Full-scan mass spectra were recorded by scanning an *m/z* range of 100–2000. The preparative HPLC purifications were performed on a Dionex Ultimate 3000 instrument equipped with a Dionex RS variable wavelength detector, using an Atlantis C-18-Preper T3 ODB (5 µm, 19 x 10 mm) column and starting from 100% aqueous 0.1% (v/v) formic acid to 100% CH_3CN as the eluent. The crude product was dissolved in water and the solution was filtered (polypropylene, 0.45 µm, 13 mm ø, PK/100) and injected into the HPLC, affording purified products.

For the obtained chromatographically inseparable mixtures of



5c,d the ^1H and ^{13}C NMR assignments refer to:

*4.1.2 Preparation of methyl 5-acetoxyacetamido-4,7,8,9-tetra-O-acetyl-2,6-anhydro-3,5-dideoxy-D-glycero-D-talo-non-2-enoate (**6**).*To a solution of oxazoline **3a** [8] (250 mg, 0.6 mmol) in CH_3CN-H_2O, 40:1 v/v (4 mL) TFA (0.05 mL) was added and the reaction was stirred at 23 °C until the complete disappearance of the starting material (10 minutes), by monitoring with TLC (AcOEt). At this time, a weak basic resin (IRA-67), in excess compared to the acylating agent, was added and the reaction mixture was immediately treated with acetoxyacetyl chloride (0.1 mL, 0.9 mmol) at 0 °C. The mixture was stirred at 23 °C until the complete formation of the desired compound **6** (40 min). Methanol (1 mL) was added and the reaction mixture was stirred for 15 minutes, filtered (washing with 12 mL of AcOEt) and evaporated. After purification by flash chromatography (eluting with AcOEt/hexane, 7/3 to 8/2 v/v), the compound **6** (251 mg, 78%) was obtained as a white amorphous solid: ^1H NMR (500 MHz, $CDCl_3$): δ=6.27 (d, $J_{NH,5}$=10.3 Hz, 1H; N*H*COCH$_2$O), 6.20 (d, $J_{3,4}$=5.6 Hz, 1H; H-3), 5.45 (dd, $J_{7,6}$=2.3, $J_{7,8}$=4.1 Hz, 1H; H-7), 5.31 (ddd, $J_{8,9a}$=2.5, $J_{8,7}$=4.1, $J_{8,9b}$=7.5 Hz, 1H; H-8), 5.16 (dd, $J_{4,5}$=4.3, $J_{4,3}$=5.6 Hz, 1H; H-4), 4.76 (dd, $J_{9a,8}$=2.5, $J_{9a,9b}$=12.5 Hz, 4.30 (dd, $J_{6,7}$=2.3, $J_{6,5}$=11.0 Hz, 1H; H-6), 4.18 (dd, $J_{9b,8}$=7.5, $J_{9b,9a}$=12.5 Hz, 1H; H-9b), 3.79 (s, 3H; COOC*H*$_3$), 2.16 (s, 3H; OCOC*H*$_3$), 2.10 (s, 3H; OCOC*H*$_3$), 2.08 (s, 3H; OCOC*H*$_3$), 2.07 (s, 3H; OCOC*H*$_3$), 2.04 ppm (s, 3H; OCOC*H*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=170.5 (O*C*OCH$_3$ at C-9), 170.4 (O*C*OCH$_3$ at C-8), 170.1 (O*C*OCH$_3$ at C-7), 169.3 (O*C*OCH$_3$ at C-4), 169.2 (CH$_3$*C*OOCH$_2$CO at C-5), 166.8 (OCH$_2$*C*O at C-5), 161.5 (C-1), 146.4 (C-2), 105.8 (C-3), 73.8 (C-6), 71.7 (C-8), 67.6 (C-7), 64.7 (C-4), 63.3 (O*C*H$_2$CO), 62.1 (C-9), 52.6 (COO*C*H$_3$), 44.0 (C-5), 20.9, 20.7, 20.6 ppm (5C; 5 x OCO*C*H$_3$); MS (ESI positive): *m/z* 532.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{22}H_{29}NO_{14}$: C 49.72, H 5.50, N 2.64; found: C 49.93, H 5.32, N 2.50.

*4.1.3 Preparation of 2,6-anhydro-3,5-dideoxy-5-hydroxyacetamido-D-glycero-D-talo-non-2-enoic acid (**2c**).* Compound **6** (53 mg, 0.10 mmol) was treated with a methanolic solution of NaOMe, freshly prepared by dissolving sodium metal (5 mg, 0.22 mmol) in anhydrous MeOH (2 mL). The reaction mixture was stirred at 23 °C for 1 h, and then quenched with acidic resin (Dowex 50WX8, H^+). The resin was filtered off and washed with MeOH (2 mL x 3) and the filtrate was evaporated under vacuum. The crude compound was purified by chromatography (eluting with AcOEt/MeOH, 9/1 v/v), affording the methyl ester of compound **2c** (22 mg, 68%) as a white amorphous solid: ^1H NMR (500 MHz, CD_3OD): δ=6.14 (d, $J_{3,4}$=5.6 Hz,1H; H-3), 4.24-4.14 (overlapping, 3H; H-4, H-5 and H-6), 4.11-4.01 (AB system, 2H; CH_2), 3.94 (ddd, $J_{8,9a}$=2.9, $J_{8,9b}$=5.4, $J_{8,7}$=9.3 Hz, 1H; H-8), 3.83 (dd, $J_{9a,8}$=2.9, $J_{9a,9b}$=11.4 Hz, 1H; H-9a), 3.78 (s, 3H; COOC*H*$_3$), 3.68 (dd, $J_{9b,8}$=5.4, $J_{9b,9a}$=11.4 Hz, 1H; H-9b), 3.56 ppm (dd, $J_{7,6}$<1.0, $J_{7,8}$=9.3 Hz, 1H; H-7); ^{13}C NMR (125 MHz, CD_3OD): δ=176.3 (OCH$_2$*C*O at C-5), 164.6 (C-1), 146.4 (C-2), 110.7 (C-3), 74.0 (C-6), 71.3 (C-8), 70.0 (C-7), 65.0 (C-9), 62.6 (O*C*H$_2$CO), 61.9 (C-4), 52.9 (COO*C*H$_3$), 49.0 ppm (C-5 under solvent signal). Then, the purified methyl ester of **2c** was dissolved in methanol (0.5 mL) and subjected to hydrolysis with aqueous 1M NaOH solution (0.5 mL) and kept at 23°C for 0.5 h. At this time, the reaction mixture was treated with acidic resin (Dowex 50WX8, H^+) until acidic pH, and then, the resin was filtered and washed with MeOH (2 mL x 3). Finally, the solvent was removed under reduced pressure and the residue was purified by preparative HPLC and the desired free glycal **2c** was obtained after lyophilization as a white amorphous solid (17 mg, 80%): ¹H NMR (500 MHz, CD_3OD): δ=6.18 (d, $J_{3,4}$=5.7 Hz,1H; H-3), 4.31-4.23 (overlapping, 3H; H-4, H-5 and H-6), 4.19-4.08 (AB system, 2H; CH_2), 3.96 (ddd, $J_{8,9a}$=2.7, $J_{8,9b}$=5.5, $J_{8,7}$=9.4 Hz, 1H; H-8), 3.87 (dd, $J_{9a,8}$=2.7, $J_{9a,9b}$=11.8 Hz, 1H; H-9a), 3.70 (dd, $J_{9b,8}$=5.5, $J_{9b,9a}$=11.8 Hz, 1H; H-9b), 3.62 ppm (br d, $J_{7,8}$=9.4 Hz, 1H; H-7); ^{13}C NMR (125 MHz, CD_3OD): δ=174.9 (OCH$_2$*C*O), 165.2 (C-1), 145.3 (C-2), 109.0 (C-3), 72.2 (C-6), 70.0 (C-8), 68.4 (C-7), 63.4 (C-9), 61.1 (O*C*H$_2$CO), 60.5 (C-4), 49.0 ppm (C-5 under solvent signal); MS (ESI positive): *m/z* 306.1 $[M-H]^+$; elemental analysis calcd (%) for $C_{11}H_{17}NO_9$: C 43.00, H 5.58, N 4.56; found: C 43.23, H 5.30, N 4.61.

*4.1.4 Preparation of methyl oxazolo[5,4]-fused 7,8,9-tri-O-acetyl-2,3,4,5-tetradeoxy-2,3-didehydro-4',5'-dihydro-2'-(acetoxymethyl)-D-glycero-D-talo-non-2-enoate (**3b**).* To a solution of compound **6** (200 mg, 0.38 mmol) in CH_2Cl_2 (1.5 mL), $BF_3.Et_2O$ (0.13 mL, 1.50 mmol) was added at 23 °C and the mixture was stirred at 80 °C for 20 min in a sealed tube. Then, the reaction mixture was diluted with CH_2Cl_2 (8 mL) containing Et_3N (1.04 mL, 7.5 mmol), washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and evaporated. Then, the crude was purified by a flash chromatography (eluting with AcOEt/hexane, 6/4 v/v containing the 0.3% of Et_3N).

amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.36 (d, $J_{3,4}$=4.0 Hz, 1H; H-3), 5.57 (dd, $J_{7,6}$=2.8, $J_{7,8}$=5.9 Hz, 1H; H-7), 5.41 (ddd, $J_{8,9a}$=2.6, $J_{8,7}$=5.9, $J_{8,9b}$=6.3 Hz, 1H; H-8), 4.90 (dd, $J_{4,3}$=4.0, $J_{4,5}$=8.5 Hz, 1H; H-4), 4.74-4.65 (AB system, 2H; CH_2), 4.52 (dd, $J_{9a,8}$=2.6, $J_{9a,9b}$=12.4 Hz, 1H; H-9a), 4.21 (dd, $J_{9b,8}$=6.3, $J_{9b,9a}$=12.4 Hz, 1H; H-9b), 4.03 (m, 1H; H-5), 3.79 (s, 3H; $COOCH_3$), 3.44 (dd, $J_{6,7}$=2.8, $J_{6,5}$=10.0 Hz, 1H; H-6), 2.12 (s, 3H; $OCOCH_3$), 2.11 (s, 3H; $OCOCH_3$), 2.05-2.03 ppm (overlapping, 6H; 2 x $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=170.5, 170.0, 169.8, 169.5 (4C; 3 x O*C*OCH$_3$ and CH$_3$*C*OOCH$_2$CO at C-5), 164.8 (OCH$_2$*C*O at C-5), 161.7 (C-1), 147.4 (C-2), 106.7 (C-3), 76.3 (C-6), 73.1 (C-4), 70.1 (C-8), 68.8 (C-7), 61.9 (C-9), 61.8 (C-5), 57.9 (O*C*H$_2$*C*O), 52.5 (COO*C*H$_3$), 20.8, 20.7, 20.6, 20.4 ppm (4C; 4 x OCO*C*H$_3$); MS (ESI positive): *m/z* 472.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{20}H_{25}NO_{12}$: C 50.96, H 5.35, N 2.97; found: C 50.81, H 5.03, N 3.02.

Compound **3b** was alternatively obtained starting from compound **7** [5d] (200 mg, 0.38 mmol) in CH_2Cl_2 (1.5 mL), adding $BF_3.Et_2O$ (0.13 mL, 1.50 mmol) at 23 °C. The mixture was stirred at 80 °C for 20 min in a sealed tube and, then, the reaction mixture was diluted with CH_2Cl_2 (8 mL) containing Et_3N (1.04 mL, 7.5 mmol), washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and evaporated. The crude was purified by a flash chromatography (eluting with AcOEt/hexane, 6/4 v/v containing the 0.3% of Et_3N). Compound **3b** (115 mg, 65%) was obtained as a white amorphous solid. All the chemical-physical properties were superimposable with those reported above.

*4.1.5 Preparation of methyl 5-acetoxyacetamido-7,8,9-tri-O-acetyl-2,6-anhydro-4-azido-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enoate (**4b**).* Starting from oxazoline **3b** (52 mg, 0.11 mmol) in *tert*-butyl alcohol (1.0 mL) containing azido-trimethyl silane (0.07 mL, 0.55 mmol), according to the literature procedure [11] (performed on a different oxazoline), compound **4b** (25 mg, 44%) was obtained after flash chromatography (eluting with CH_2Cl_2/acetone, 9/1 v/v), as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.48 (d, $J_{NH,5}$=8.2 Hz, 1H; N*H*COCH$_2$OCOCH$_3$), 5.98 (d, $J_{3,4}$=2.7 Hz, 1H; H-3), 5.37-5.31 (overlapping 2H; H-7 and H-8), 4.79 (dd, $J_{4,3}$=2.7, $J_{4,5}$=8.8 Hz, 1H; H-4), 4.62 (d, $J_{H,H}$=15.5 Hz, 1H; CH_2), 4.60 (dd, $J_{6,7}$=1.8, $J_{6,5}$=10.3 Hz, 1H; H-6), 4.55 (dd, $J_{9a,8}$=2.2, $J_{9a,9b}$=12.4 Hz, 1H; H-9a), 4.45 (d, $J_{H,H}$=15.5 Hz, 1H; CH_2), 4.22 (dd, $J_{9b,8}$=5.2, $J_{9b,9a}$=12.4 Hz, 1H; H-9b), 3.80 (s, 3H; $COOCH_3$), 3.61 (m, 1H; H-5), 2.19 (s, 3H; $OCOCH_3$), 2.13 (s, 3H; $OCOCH_3$), 2.07 (s, 3H; $OCOCH_3$), 2.05 ppm (s, 3H; $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ=170.6 (2C, O*C*OCH$_3$ at C8 and O*C*OCH$_3$ at C9), 169.9 (O*C*OCH$_3$ at C7), 169.5 (CH$_3$*C*OOCH$_2$CO at C-5), 168.0 (OCH$_2$*C*O at C-5), 161.4 (C-1), 145.1 (C-2), 107.2 (C-3), 74.6 (C-6), 70.1 (C-8), 67.8 (C-7), 63.0 (O*C*H$_2$CO), 61.7 (C-9), 56.5 (C-4), 52.6 (COO*C*H$_3$), 49.6 (C-5), 20.9, 20.8, 20.6 ppm (4C; 4 x OCO*C*H$_3$); MS (ESI positive): *m/z* 515.4 $[M+H]^+$; elemental analysis calcd (%) for $C_{20}H_{26}N_4O_{12}$: C 46.70, H 5.09, N 10.89; found: C 46.91, H 5.15, N 10.62.

Compound **4b** was alternatively obtained starting from the amine compound **8**. To a solution of compound **8** (41 mg, 0.10 mmol), prepared from oxazoline **3a** according to the literature procedure (five steps, overall yield 39%) [4c,11], in CH_2Cl_2 (2 mL) under argon was added Et_3N (70 µL, 0.5 mmol) and acetoxyacetyl chloride (16 µL, 0.15 mmol) at 0°C. The mixture was stirred at 23°C overnight and then the crude product was evaporated and purified by a flash chromatography (eluting with AcOEt/hexane, 2/1 v/v). Compound **4b** (38 mg, 75%) was

properties were superimposable with those above reported. As example, ^{13}C NMR (125 MHz, $CDCl_3$): δ=170.6 (2C, O*C*OCH$_3$ at C8 and O*C*OCH$_3$ at C9), 169.9 (O*C*OCH$_3$ at C7), 169.5 (CH$_3$*C*OOCH$_2$CO at C-5), 168.0 (OCH$_2$*C*O at C-5), 161.4 (C-1), 145.1 (C-2), 107.3 (C-3), 74.8 (C-6), 70.2 (C-8), 67.8 (C-7), 62.9 (O*C*H$_2$CO), 61.8 (C-9), 56.6 (C-4), 52.6 (COO*C*H$_3$), 49.4 (C-5), 20.8, 20.7, 20.6 ppm (4C; 4 x OCO*C*H$_3$).

*4.1.6 Preparation of 2,6-anhydro-4-azido-3,4,5-trideoxy-5-hydroxyacetamido-D-glycero-D-galacto-non-2-2-enoic acid (**1g**).* Compound **4b** (35 mg, 0.068 mmol) was treated with a methanolic solution of NaOMe, freshly prepared by dissolving sodium metal (5 mg, 0.22 mmol) in anhydrous MeOH (1 mL). The reaction mixture was stirred at 23 °C for 1 h, and then quenched with acidic resin (Dowex 50WX8, H^+). The resin was filtered off and washed with MeOH (1 mL x 3) and the filtrate was evaporated under vacuum. The crude compound was purified by chromatography (eluting with CH_2Cl_2/MeOH, 9/1 to 8/2 v/v), affording the methyl ester of compound **1g** (16 mg, 66%) as a white amorphous solid: ^{1}H NMR (500 MHz, CD_3OD): δ=5.95 (d, $J_{3,4}$=2.5 Hz,1H; H-3), 4.49 (dd, $J_{4,3}$=2.5, $J_{4,5}$=9.4 Hz, 1H; H-4), 4.39 (dd, $J_{6,7}$<1.0, $J_{6,5}$=10.9 Hz, 1H; H-6), 4.24 (m, 1H; H-5), 4.10-4.01 (AB system, 2H; CH_2), 3.88 (ddd, $J_{8,9a}$=2.9, $J_{8,9b}$=5.3, $J_{8,7}$=9.4 Hz, 1H; H-8), 3.82 (dd, $J_{9a,8}$=2.9, $J_{9a,9b}$=11.5 Hz, 1H; H-9a), 3.80 (s, 3H; $COOCH_3$), 3.66 (dd, $J_{9b,8}$=5.3, $J_{9b,9a}$=11.5 Hz, 1H; H-9b), 3.61 ppm (dd, $J_{7,6}$<1.0, $J_{7,8}$=9.4 Hz, 1H; H-7); ^{13}C NMR (125 MHz, CD_3OD): δ=176.5 (OCH$_2$*C*O at C-5), 163.9 (C-1), 146.9 (C-2), 108.5 (C-3), 78.0 (C-6), 71.3 (C-8), 69.7 (C-7), 64.9 (C-9), 62.7 (O*C*H$_2$CO), 59.7 (C-4), 53.0 (COO*C*H$_3$), 49.0 ppm (C-5 under solvent signal); MS (ESI positive): *m/z* 347.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{12}H_{18}N_4O_8$: C 41.62, H 5.24, N 16.18; found: C 41.85, H 5.17, N 16.02. Then, the purified methyl ester of **1g** was dissolved in methanol (0.5 mL) and subjected to hydrolysis with aqueous NaOH (0.5 mL, 1M) solution and kept at 23°C for 0.5 h. At this time, the reaction mixture was treated with acidic resin (Dowex 50WX8, H^+) until acidic pH, and then, the resin was filtered and washed with MeOH (2 mL x 3). Finally, the solvent was removed under reduced pressure and the residue was purified by preparative HPLC and the desired free glycal **1g** was obtained after lyophilization as a white amorphous solid (12 mg, 76%): ^{1}H NMR (500 MHz, CD_3OD): δ=5.87 (d, $J_{3,4}$=2.2 Hz,1H; H-3), 4.44 (dd, $J_{4,3}$=2.2, $J_{4,5}$=9.4 Hz, 1H; H-4), 4.40 (br d, $J_{6,5}$=10.9 Hz, 1H; H-6), 4.25 (m, 1H; H-5), 4.15-4.04 (AB system, 2H; CH_2), 3.91 (ddd, $J_{8,9a}$=3.0, $J_{8,9b}$=5.3, $J_{8,7}$=9.8 Hz, 1H; H-8), 3.82 (dd, $J_{9a,8}$=3.0, $J_{9a,9b}$=11.6 Hz, 1H; H-9a), 3.70-3.63 ppm (overlapping, 2H; H-7 and H-9b); ^{13}C NMR (125 MHz, CD_3OD): δ=176.4 (OCH$_2$*C*O at C-5), 166.8 (C-1), 148.3 (C-2), 106.9 (C-3), 77.2 (C-6), 71.6 (C-8), 69.2 (C-7), 64.5 (C-9), 62.5 (O*C*H$_2$CO), 59.8 (C-4), 49.0 ppm (C-5 under solvent signal); MS (ESI negative): *m/z* 331.1 $[M-H]^-$; elemental analysis calcd (%) for $C_{11}H_{16}N_4O_8$: C 39.76, H 4.85, N 16.86; found: C 39.96, H 4.68, N 16.99.

*4.1.7 Preparation of the α/β-anomeric mixture of methyl (2-methyl-5-acetoxyacetamido-7,8,9-tri-O-acetyl-3,4,5-trideoxy-D-manno-non-3-en-2-ulopyranosid)-onates (**5c,d**).* To a solution of oxazoline **3b** (100 mg, 0.21 mmol) in CH_3CN (3.5 mL) under argon, methanol, selected as nucleophile (0.086 mL, 2.10 mmol) and Montmorillonite K-10 (40 mg; 40% w/w) were added. The reaction mixture was stirred at 80°C, until the disappearance (1h) of the starting material. Subsequently, the reaction was filtered over Celite and the solvent was then evaporated. Finally, the residue was purified by silica gel chromatography (eluting with AcOEt/hexane 8/2, v/v) to provide a mixture (19:81) of the desired 3,4-unsaturated protected anomers **5c** and **5d** (66 mg, 62%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$): δ=6.33 (d,

J_N N*H*COCH$_3$), 6.10 (dd, $J_{3',5'}$=1.8, $J_{3',4'}$=10.1 Hz, 1H; H-3'), 5.97-5.91 (overlapping, 2H; H-3 and H-4), 5.81 (dd, $J_{4',5'}$=2.5, $J_{4',3'}$=10.1 Hz, 1H; H-4'), 5.43 (m, 1H; H-8'), 5.36-5.30 (overlapping, 2H; H-7 and H-8), 5.28 (dd, $J_{7',6'}$=1.9, $J_{7',8'}$=7.0 Hz, 1H; H-7'), 4.63-4.56 (overlapping, 3H; H-9a, CH_2 and CH_2'), 4.53 (m, 1H; H-5), 4.50-4.43 (overlapping, 3H; H-9a', CH_2 and CH_2'), 4.40 (m, 1H; H-5'), 4.35 (d, $J_{6',7'}$=1.9, $J_{6',5'}$=9.6 Hz, 1H; H-6'), 4.29-4.22 (overlapping, 2H; H-9b and H-9b'), 4.15 (dd, $J_{6,7}$=1.6, $J_{6,5}$=10.2 Hz, 1H; H-6), 3.81 (s, 3H; COOCH$_3$), 3.79 (s, 3H; COOCH$_3$'), 3.34 (s, 3H; OCH$_3$'), 3.30 (s, 3H; OCH$_3$), 2.20 (s, 3H; OCOCH$_3$'), 2.19 (s, 3H; OCOCH$_3$), 2.15 (s, 3H; OCOCH$_3$), 2.13 (s, 3H; OCOCH$_3$'), 2.12 (s, 3H; OCOCH$_3$'), 2.09 (s, 3H; OCOCH$_3$), 2.05 (s, 3H; OCOCH$_3$'), 2.04 ppm (s, 3H; OCOCH$_3$); ^{13}C NMR (125 MHz, CDCl$_3$): δ=170.6 (O*C*OCH$_3$'), 170.6 (O*C*OCH$_3$), 170.5 (O*C*OCH$_3$'), 170.4 (2 x C, O*C*OCH$_3$), 170.3 (O*C*OCH$_3$'),169.4 (2C; O*C*OCH$_3$' and O*C*OCH$_3$), 168.5 (C-1'), 167.4 (C-1), 167.1 (OCH$_2$*C*O' at C-5) 167.0 (OCH$_2$*C*O at C-5), 134.7, 133.0, 126.0, 125.8 (C-3', C-3, C-4' and C-4), 97.7 (C-2'), 96.5 (C-2), 73.9 (C-6'), 70.6 (C-8), 70.0 (C-6), 69.6 (C-8'), 68.3 (C-7'), 68.2 (C-7), 63.0 (O*C*H$_2$CO), 63.0 (O*C*H$_2$CO'), 62.1 (C-9), 62.1 (C-9'), 52.9 (2C; COO*C*H$_3$ and COO*C*H$_3$'), 52.1 (OCH$_3$), 51.3 (OCH$_3$), 43.4 (C-5'), 43.2 (C-5), 21.1 (OCO*C*H$_3$'), 21.0 (OCO*C*H$_3$), 20.7 (OCO*C*H$_3$'), 20.7 ppm (3C; OCO*C*H$_3$', 2 x OCO*C*H$_3$); MS (ESI positive): *m/z* 504.5 [M+H]$^+$; elemental analysis calcd (%) for $C_{21}H_{29}NO_{13}$: C 50.10, H 5.81, N 2.78; found: C 50.36, H 5.73, N 3.00.

*4.1.8 Preparation of the 2β-methyl (5-hydroxyacetamido-3,4,5-trideoxy-D-manno-non-3-en-2-ulopyranosid) methyl ester (**9b**) and the 2α- methyl (5- hydroxyacetamido-3,4,5-trideoxy-D-manno-non-3-en-2-ulopyranosid) methyl ester (**9a**):*

The peracetylated mixture of **5c** and **5d** (66 mg, 0.13 mmol) was treated with a methanolic solution of NaOMe (0.5 mL, 1M) freshly prepared in anhydrous MeOH (0.5 mL). The reaction mixture was stirred at 23°C for 30 min and then quenched with acidic resin (Dowex 50WX8, H$^+$). The resin was filtered off and washed with MeOH (2 mL) and the combined filtrates were evaporated under vacuum. The crude compound was purified by flash chromatography (AcOEt/MeOH, 95/5 to 8/2, v/v), at first affording the corresponding 2α anomeric methyl ester **9a** as a white solid (7 mg, 15%), showing: ^{1}H NMR (500 MHz, CD$_3$OD): δ=6.14 (dd, $J_{H,H}$=1.7, $J_{H,H}$=10.1 Hz, 1H; H-3 or H-4), 5.88 (dd, $J_{H,H}$=2.7, $J_{H,H}$=10.1 Hz, 1H; H-3 or H-4), 4.81 (m, 1H; H-5), 4.04-3.98 (overlapping, 3H; CH$_2$ and H-6), 3.92 (ddd, $J_{8,9a}$=2.6, $J_{8,9b}$=5.8, $J_{8,7}$=9.1 Hz, 1H; H-8), 3.86 (dd, $J_{9a,8}$=2.6, $J_{9a,9b}$=11.4 Hz, 1H; H-9a), 3.81 (s, 3H; COOCH$_3$), 3.66 (dd, $J_{9b,8}$=5.8, $J_{9b,9a}$=11.4 Hz, 1H; H-9b), 3.55 (dd, $J_{7,6}$=1.6, $J_{7,8}$=9.1 Hz, 1H; H-7), 3.34 ppm (s, 3H; OCH$_3$); ^{13}C NMR (125 MHz, CD$_3$OD): δ=176.1 (OCH$_2$*C*O), 171.8 (C-1), 137.0 (C-3 or C-4), 127.1 (C-3 or C-4), 99.3 (C-2), 76.9 (C-6), 72.5 (C-8), 69.9 (C-7), 64.7 (C-9), 62.7 (O*C*H$_2$CO), 53.7 (COOCH$_3$), 51.3 (OCH$_3$), 44.1 ppm (C-5); MS (ESI positive): *m/z* 336.3 [M+H]$^+$. Further elution afforded 2β anomeric methyl ester **9b** (28 mg, 65%), showing: ^{1}H NMR (500 MHz, CD$_3$OD): δ=5.98 (dd, $J_{H,H}$=1.7, $J_{H,H}$=10.1 Hz, 1H; H-3 or H-4), 5.91 (dd, $J_{H,H}$=2.4, $J_{H,H}$=10.1 Hz, 1H; H-3 or H-4), 4.86-4.80 (overlapping to water signal, 1H; H-5), 4.16 (dd, $J_{6,7}$=1.0, $J_{6,5}$=10.3 Hz, 1H; H-6), 4.02-3.99 (AB system, 2H; CH$_2$), 3.86-3.79 (overlapping, 5H; H-8, H-9a, COOCH$_3$), 3.67 (m, 1H; H-9b), 3.52 (dd, $J_{7,6}$=1.0, $J_{7,8}$=9.4 Hz, 1H; H-7), 3.32 ppm (s, 3H; OCH$_3$); ^{13}C NMR (125 MHz, CD$_3$OD, 23°C): δ= 175.7 (OCH$_2$*C*O), 171.1 (C-1), 135.2 (C-3 or C-4), 127.1 (C-3 or C-4), 97.8 (C-2), 71.6 (C-6), 71.4 (C-8), 69.9 (C-7), 65.2 (C-9), 62.7 (O*C*H$_2$CO), 53.6 (COO*C*H$_3$), 52.3 (OCH$_3$), 44.1 ppm (C-5); MS (ESI positive): *m/z* 336.3 [M+H]$^+$.

We gratefully acknowledge Irene Delcarro for her the technical assistance.

Appendix A. Supplementary Data

See Electronic Supplementary Material (ESI).

Supplementary data (^{1}H and ^{13}C NMR spectra of all new compounds) associated with this article can be found, in the online version, at

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Highlights

- Protocol to obtain the 2,3- and 3,4-unsaturated *N*-glycolyl derivatives.
- Transformation of the 4,5-oxazoline of Neu5Ac into 4,5-oxazoline of Neu5Gc.
- Sialidase inhibitors derived from *N*-glycolylneuraminic acid.
- 3,4-Unsaturated Ferrier products of sialic acid.
- 2,3-Unsaturated glycals of sialic acid.

Electronic Supplementary Information

Straightforward access to 2,3- and 3,4-unsaturated derivatives of *N*-glycolyl neuraminic acid

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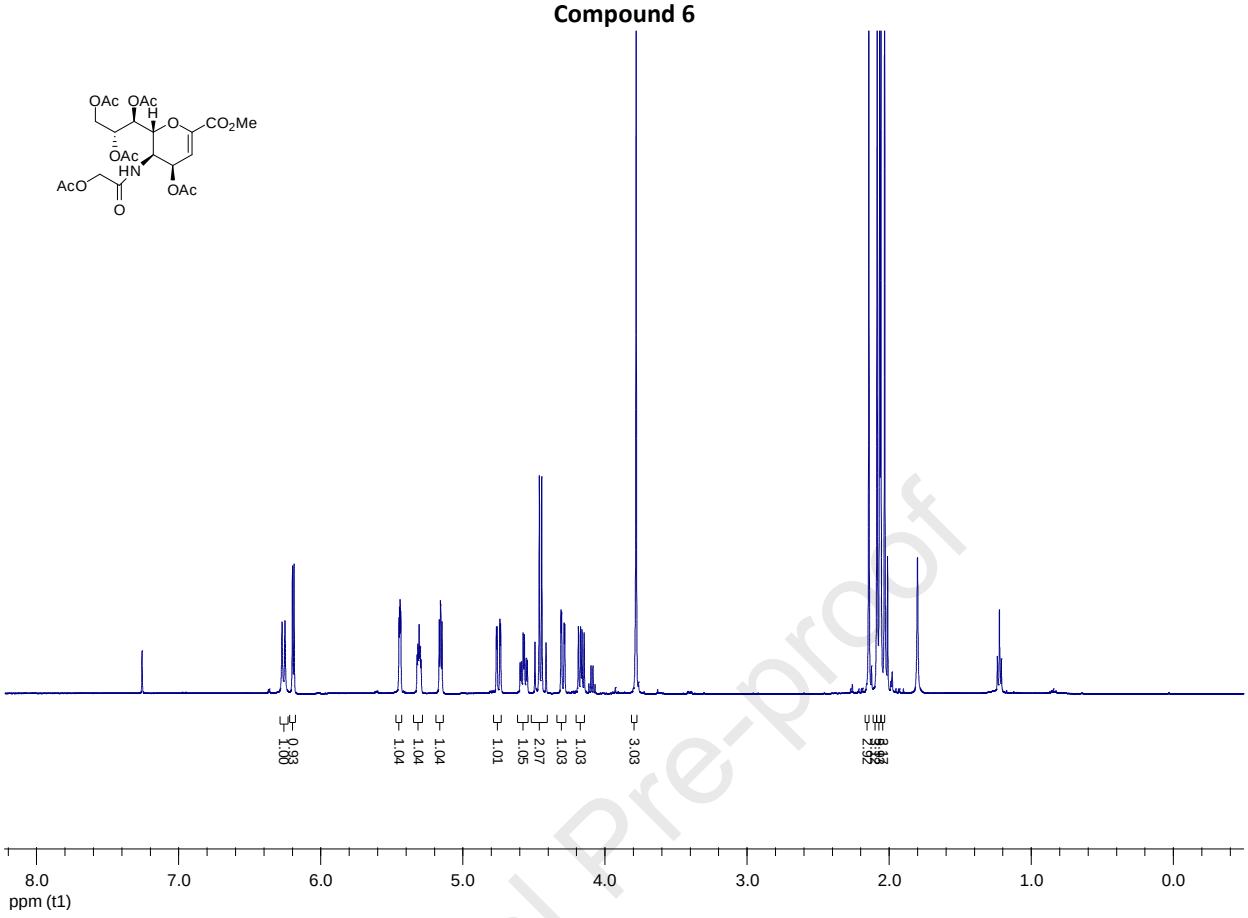
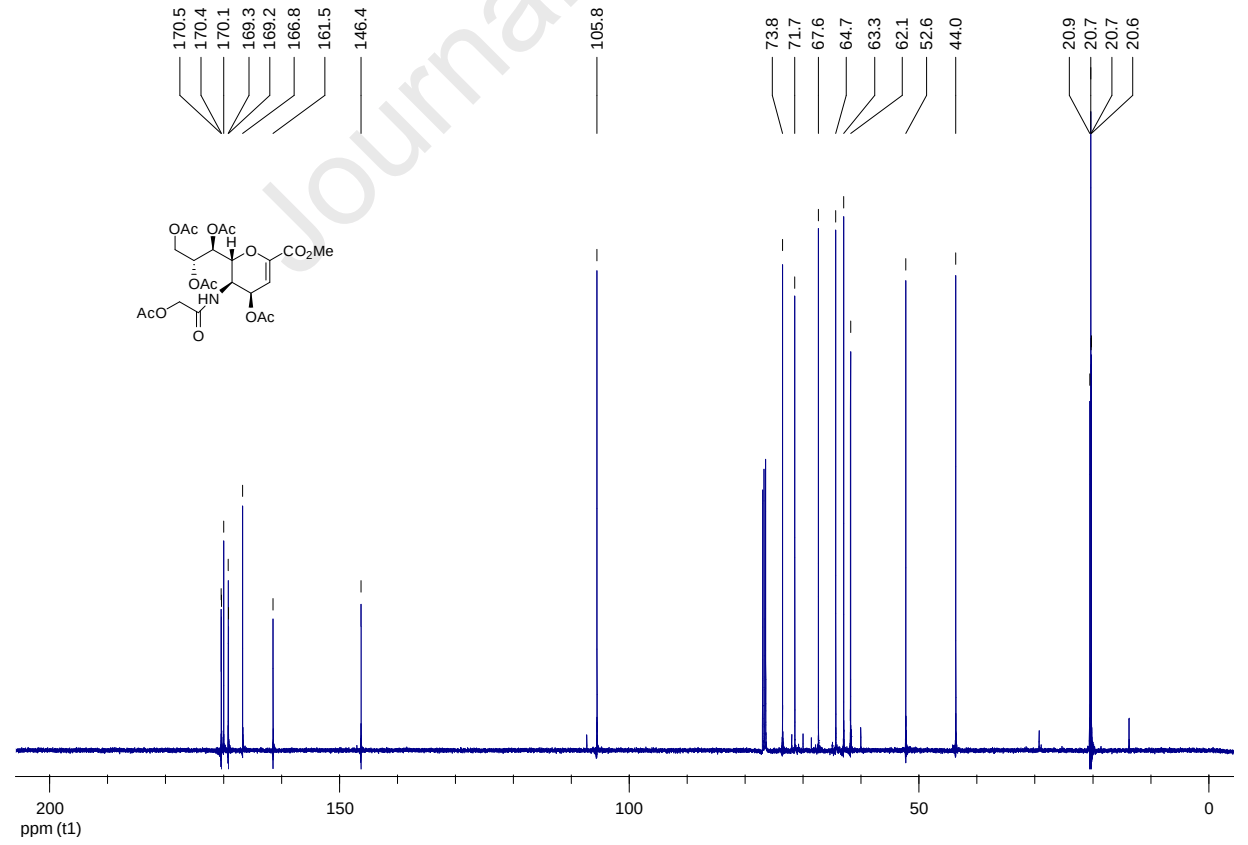
^{1}H and ^{13}C NMR of compound **2c**	S4
^{1}H and ^{13}C NMR of compound **3b**	S5
^{1}H and ^{13}C NMR of compound **4b**: **first and second pathways**	S6-S7
^{1}H and ^{13}C NMR of **methyl ester of 1g**	S8
^{1}H and ^{13}C NMR of compound **1g**	S9
^{1}H and ^{13}C NMR of mixture of compounds **5c,d**	S10
^{1}H and ^{13}C NMR of compound **9a** alpha anomer	S11
^{1}H and ^{13}C NMR of compound **9a** beta anomer	S12

Compound 6





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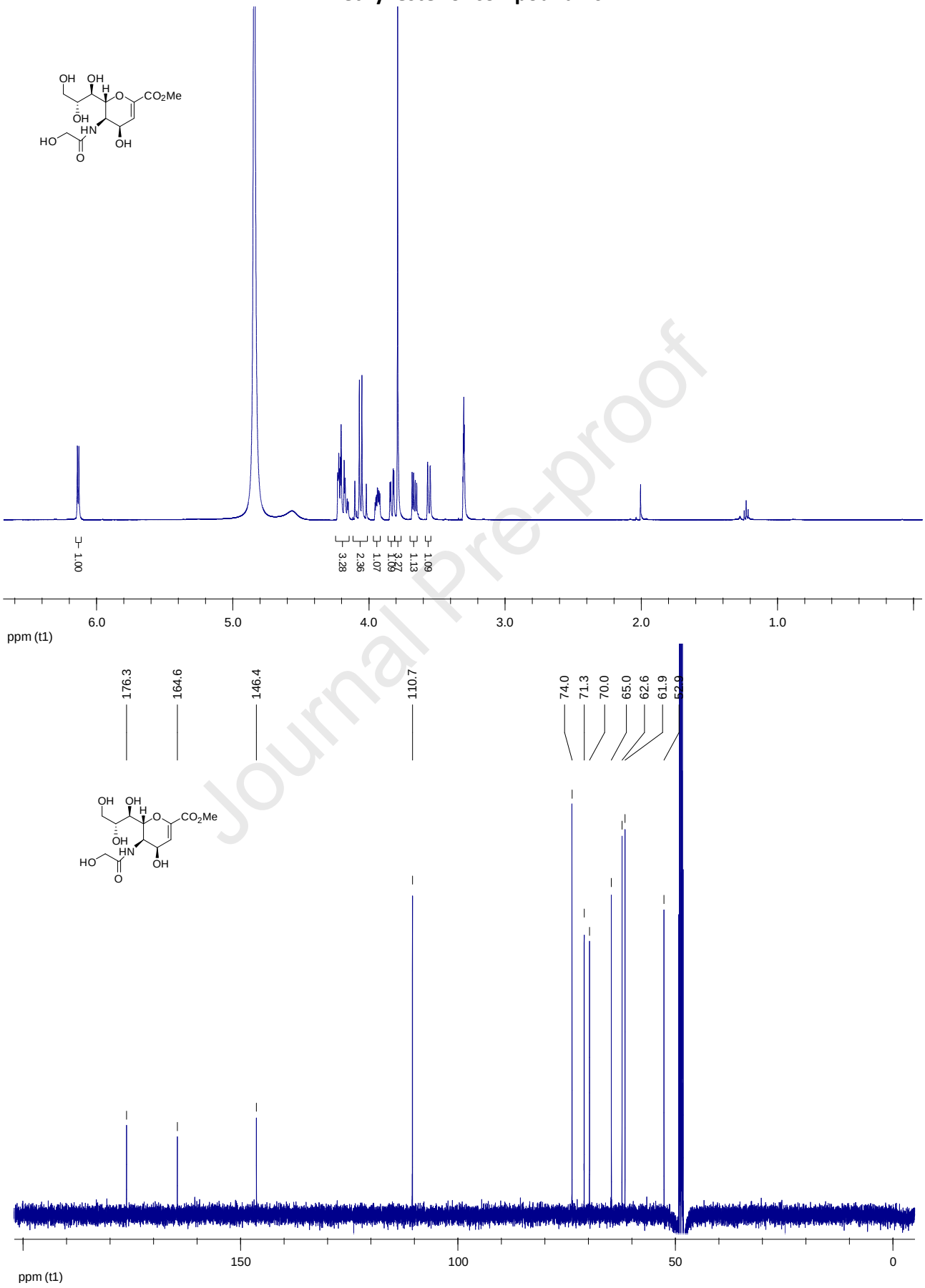

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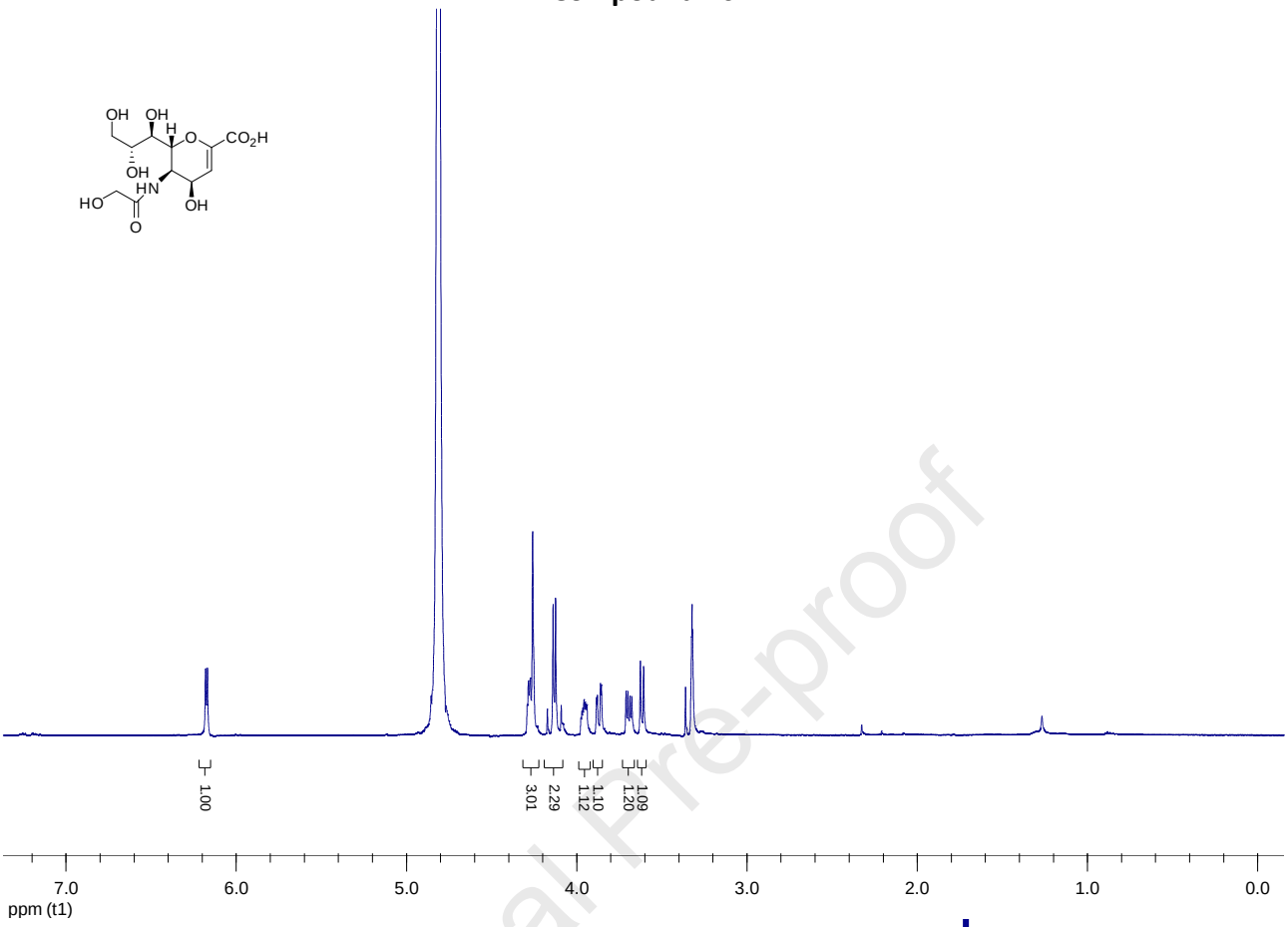

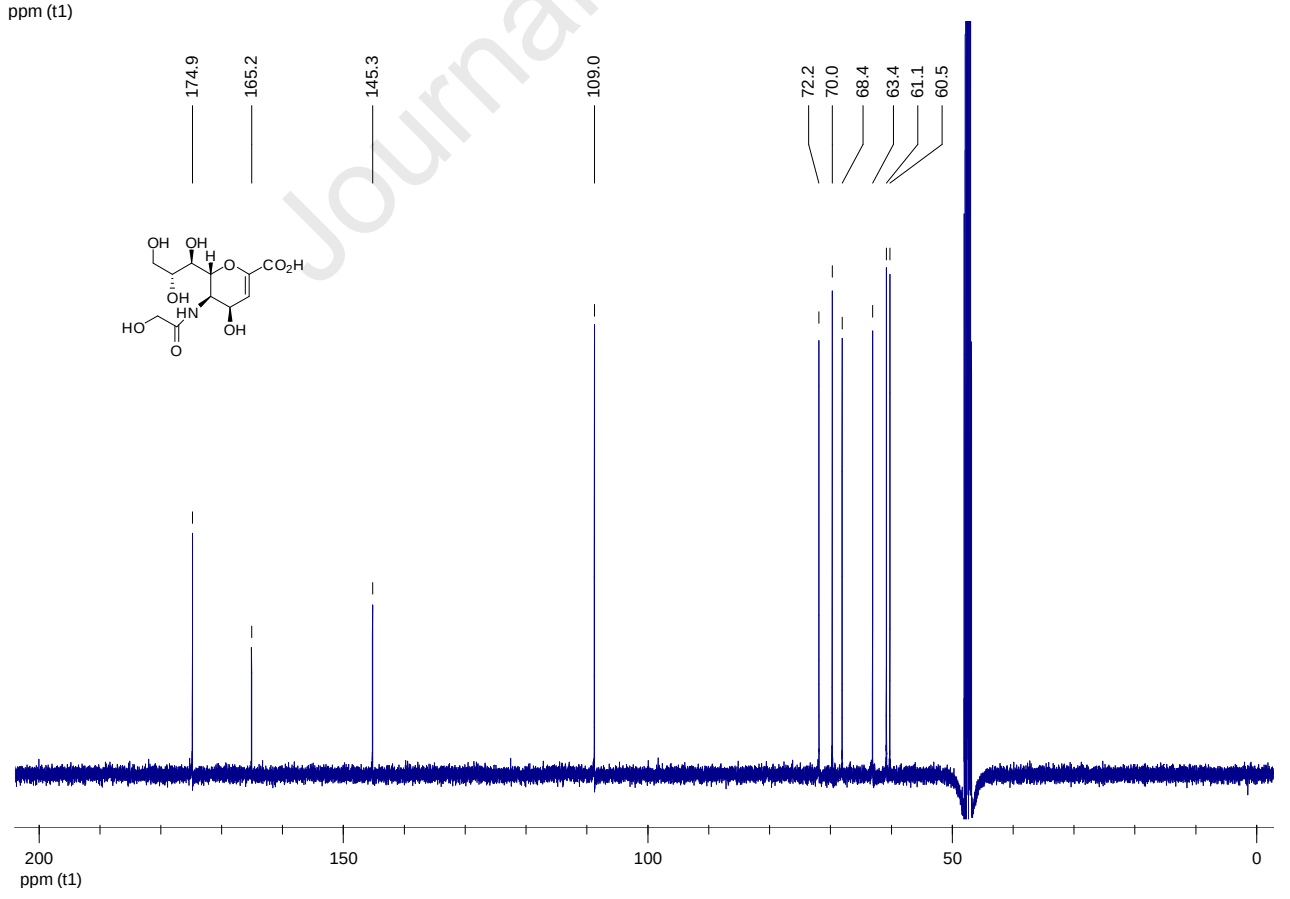

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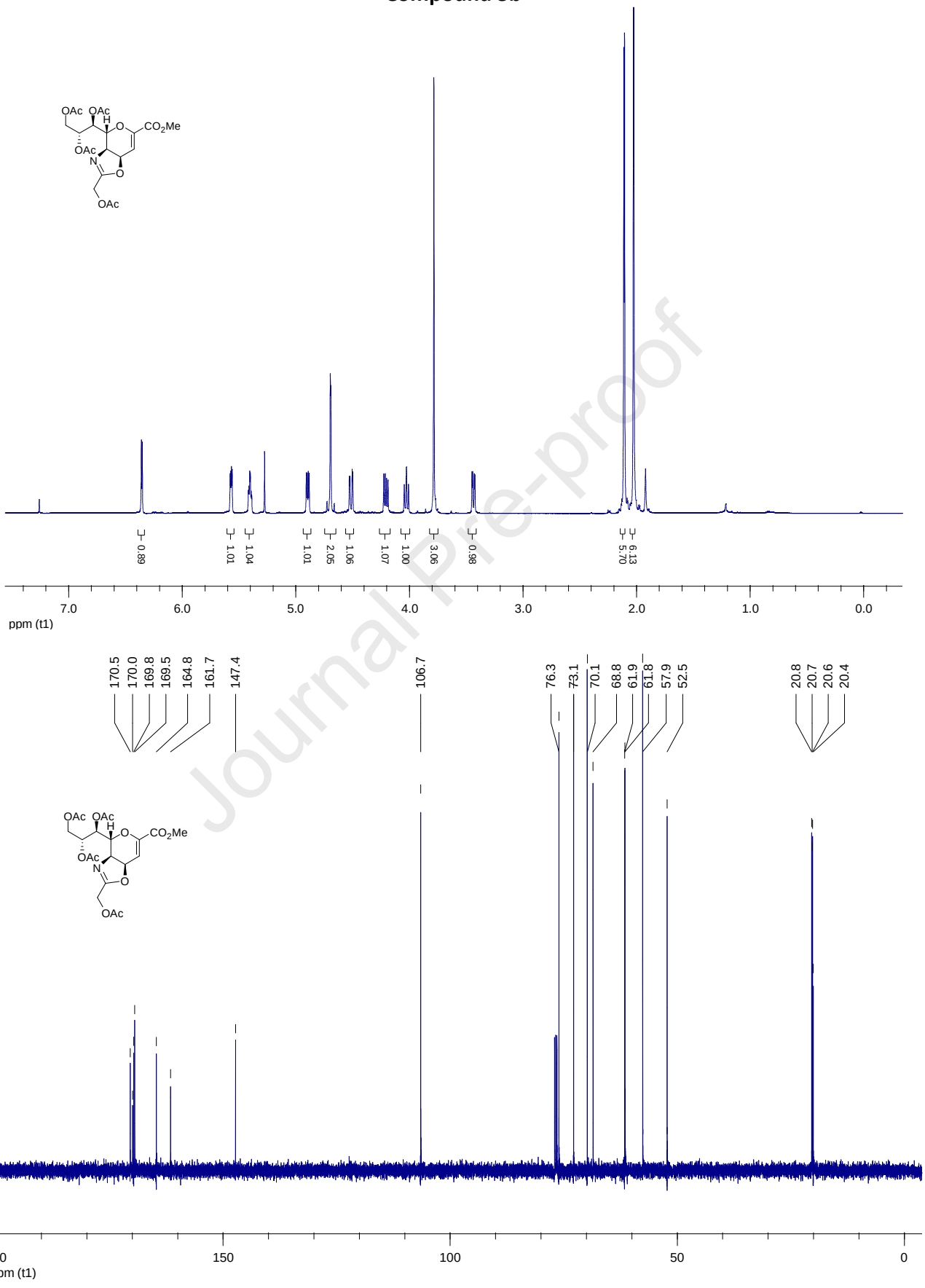

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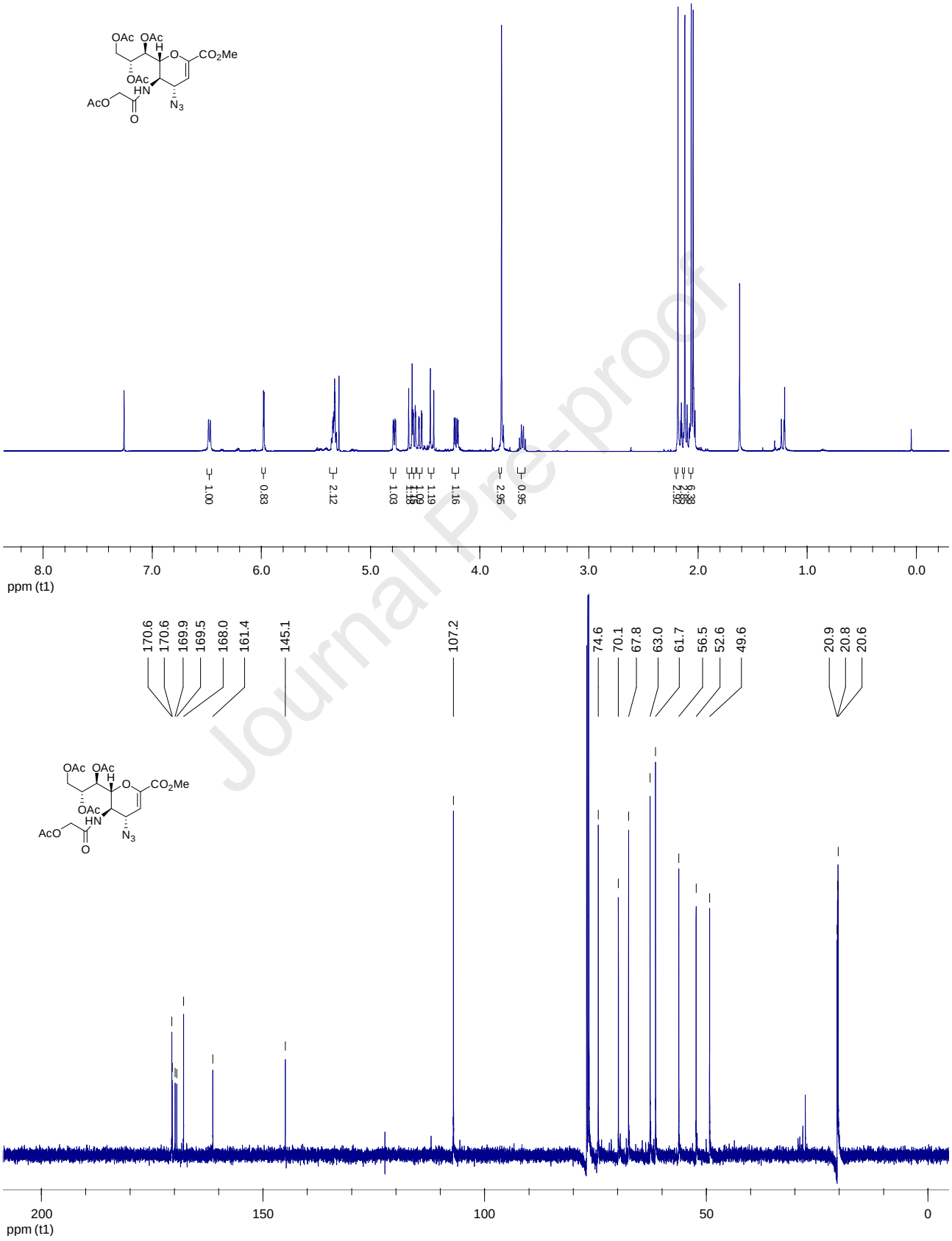

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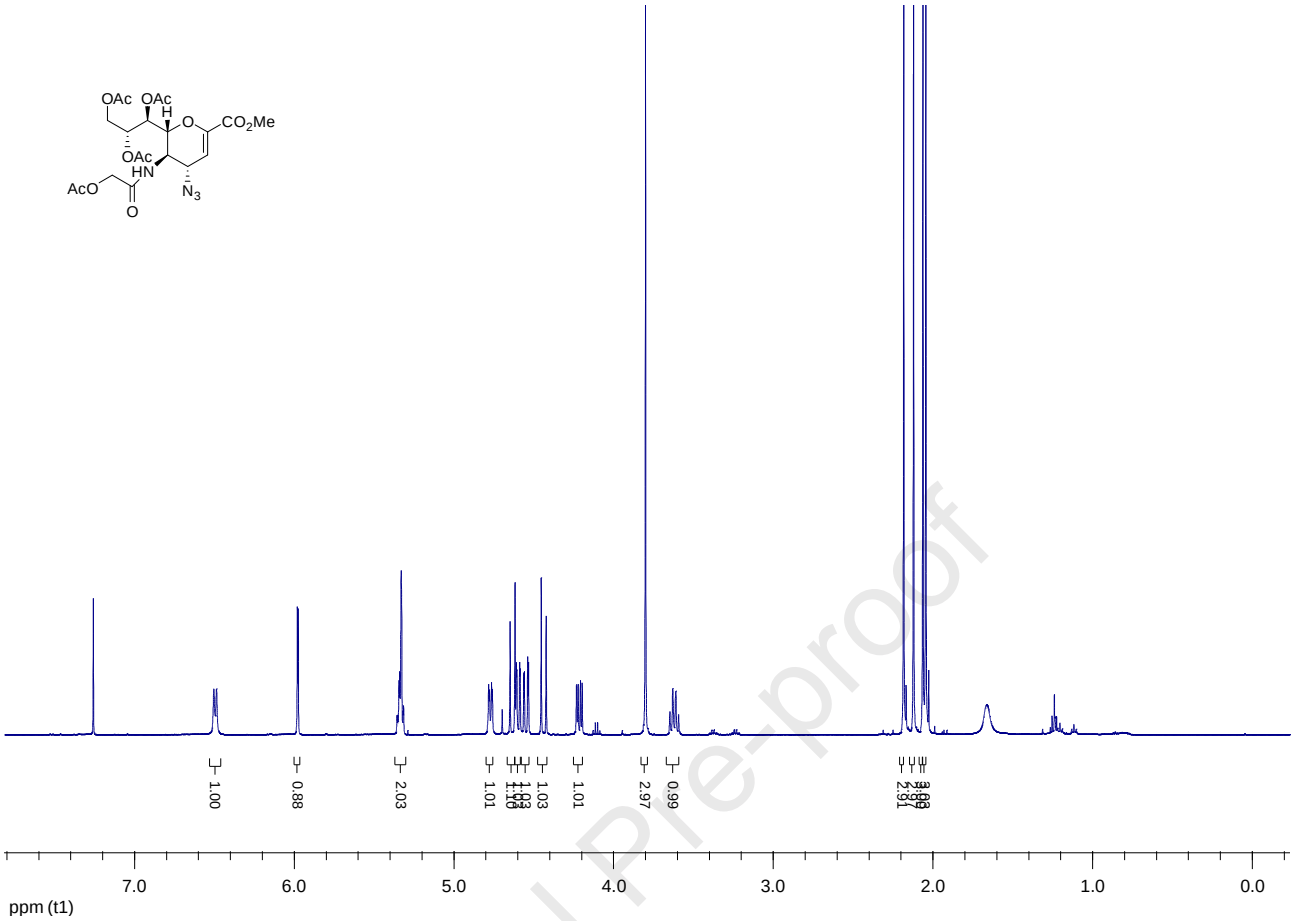
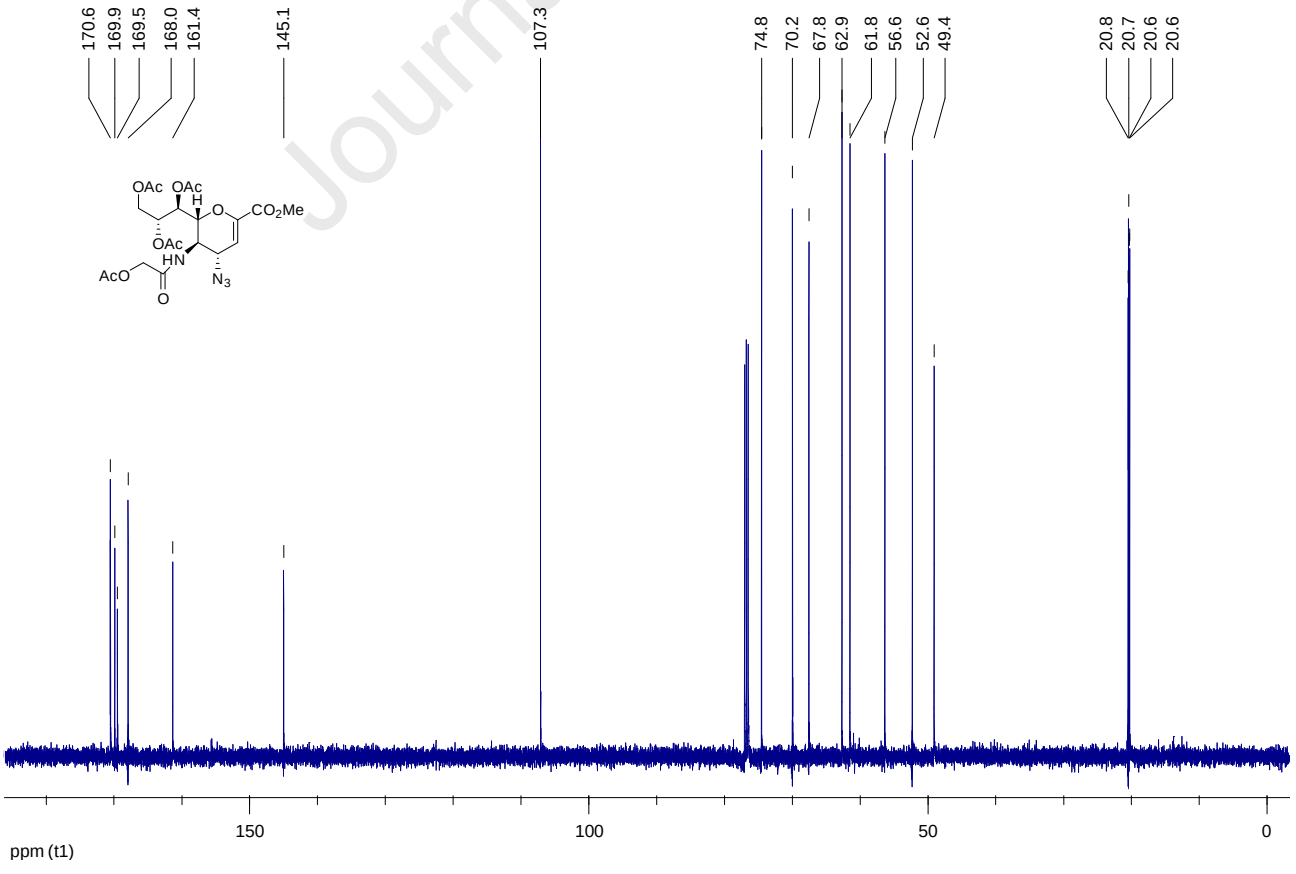
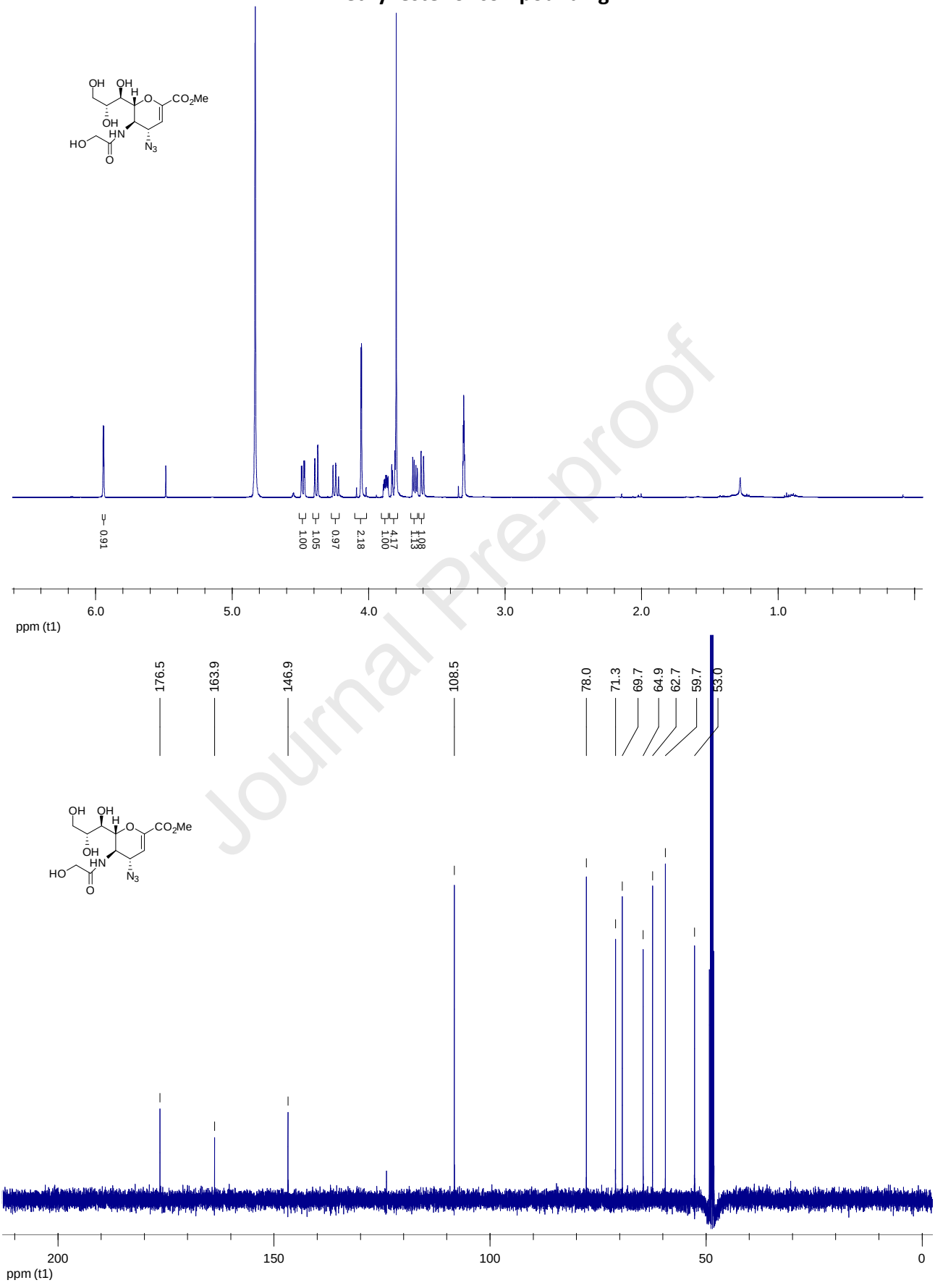
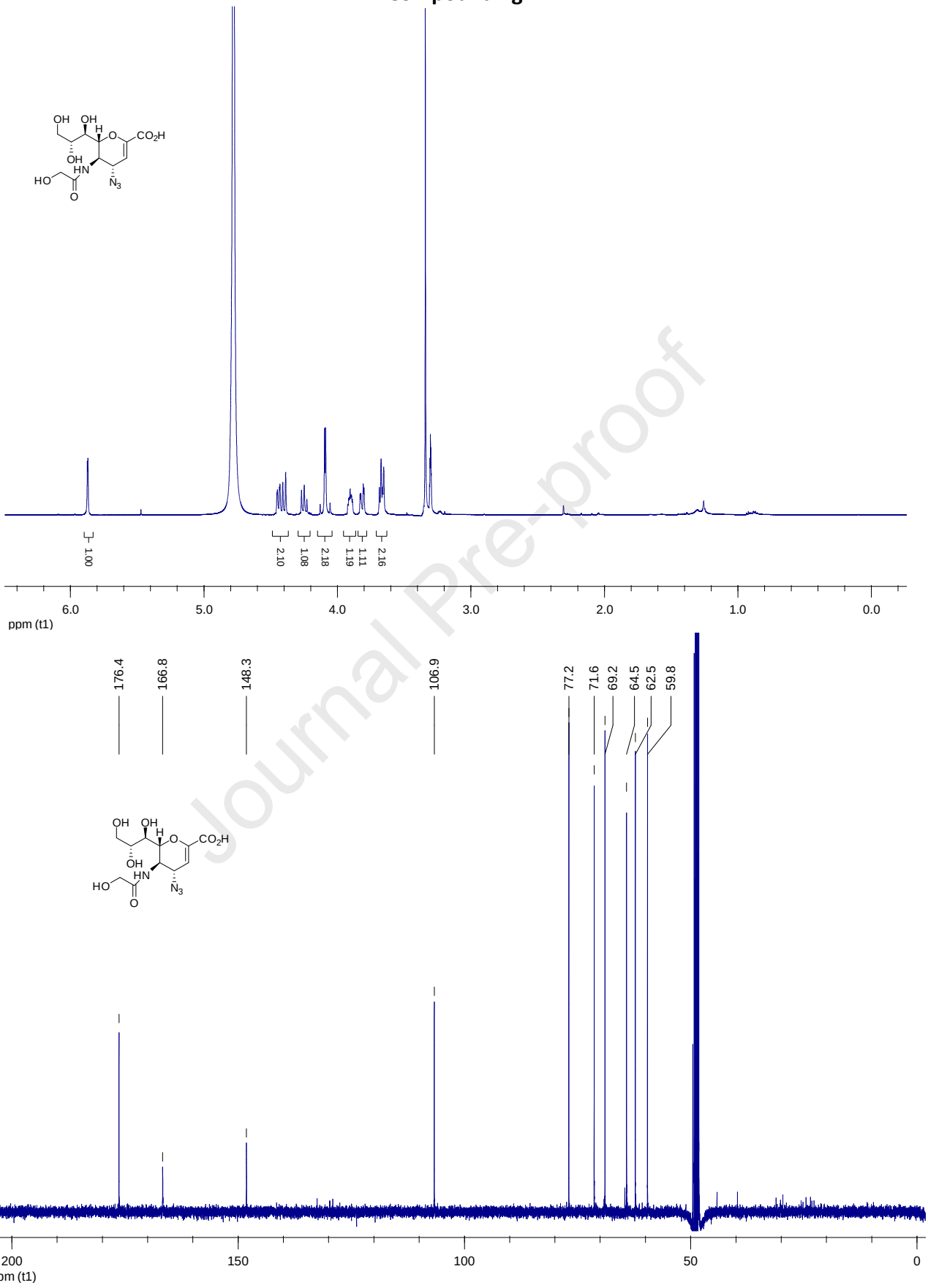




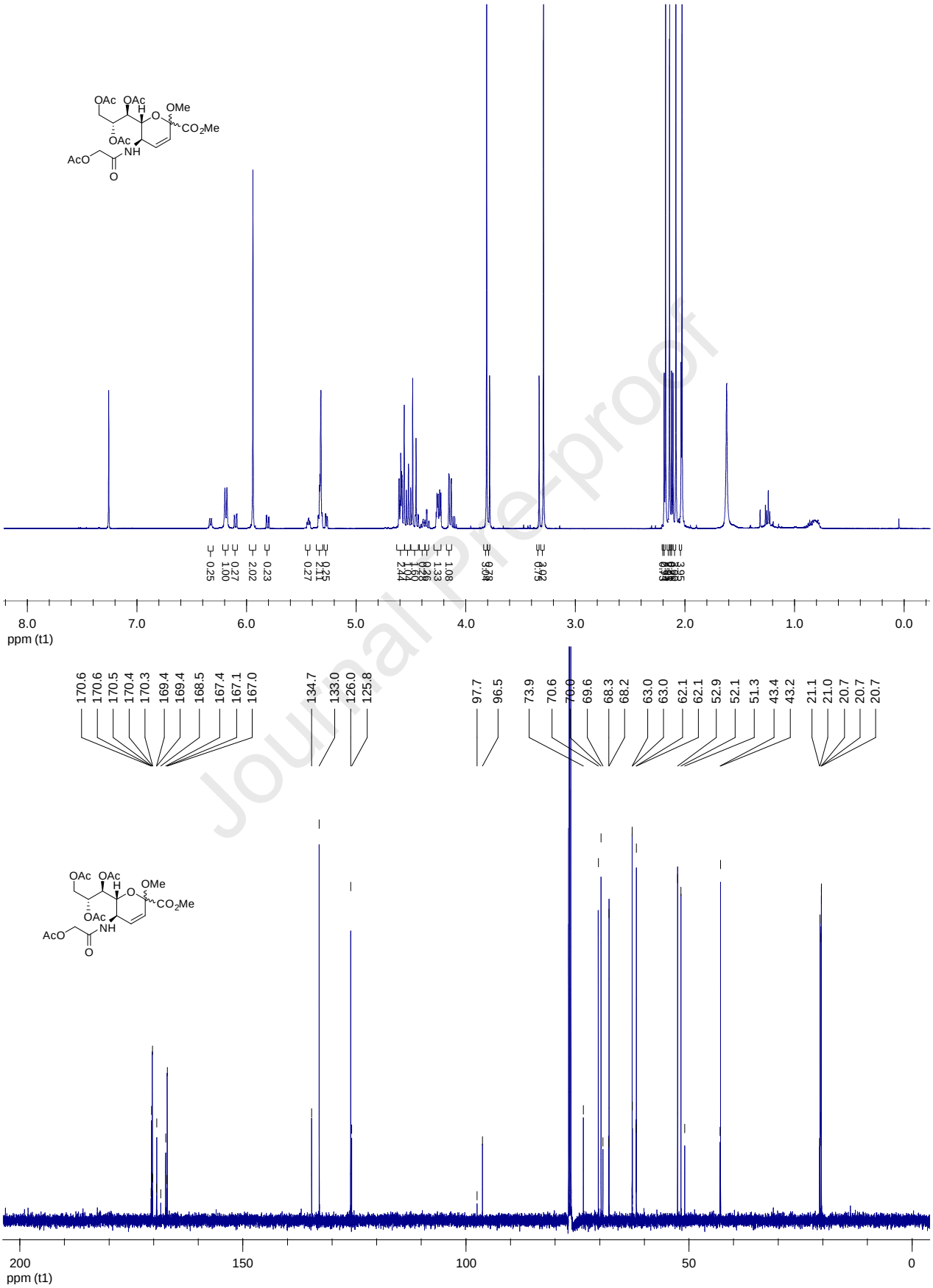
Methyl ester of compound 1g



Compound 1g



Mixture of compounds 5c,d



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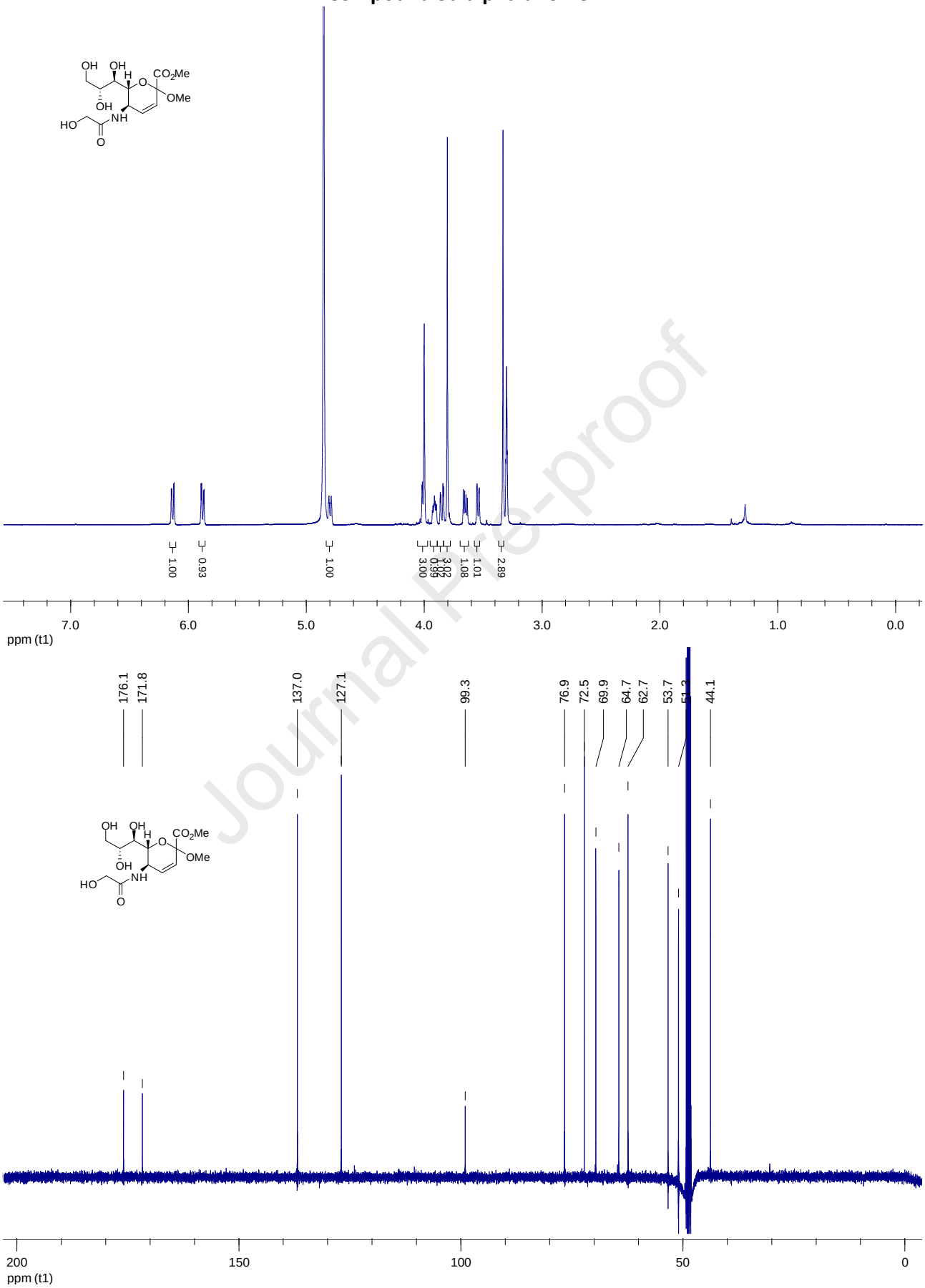
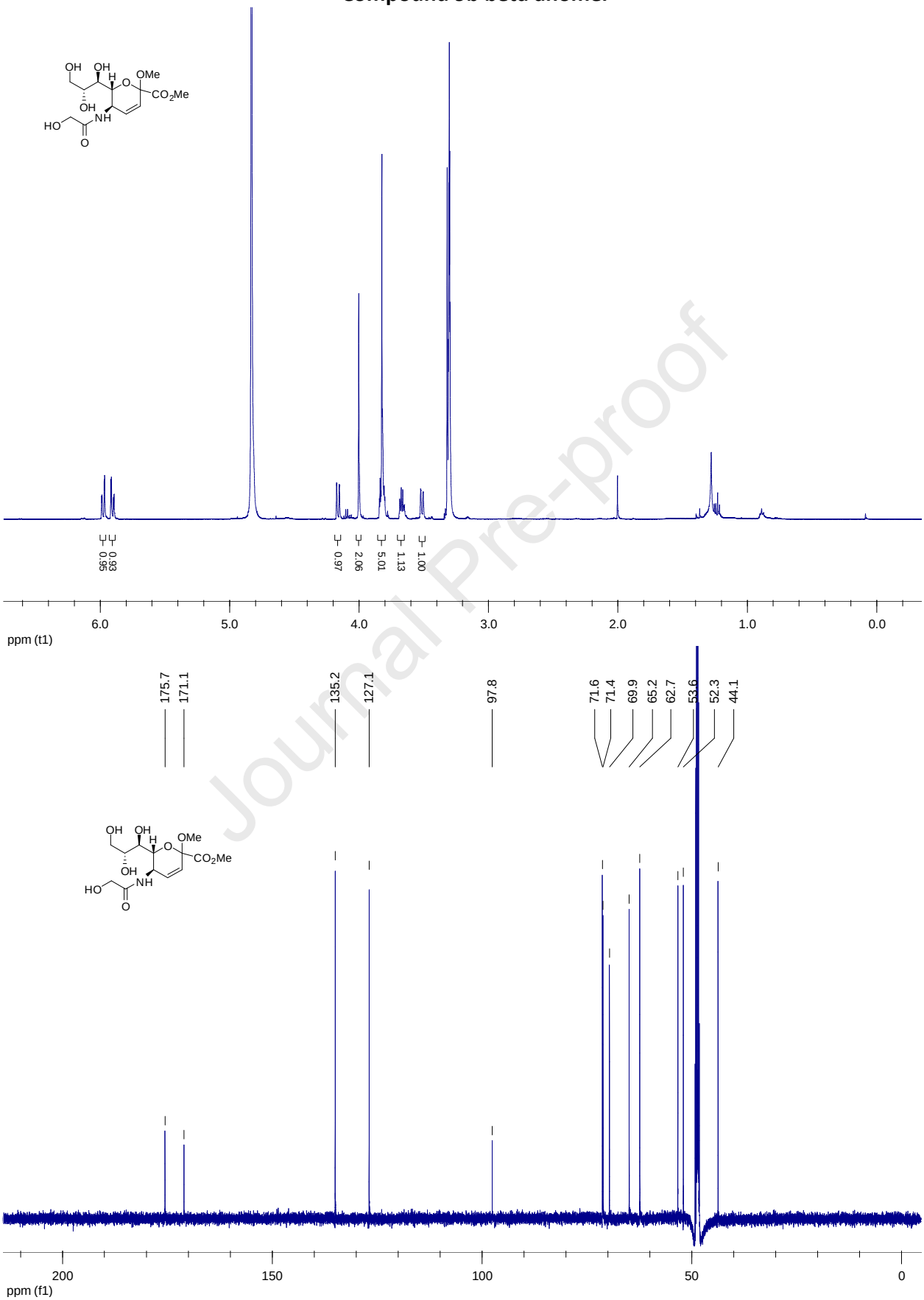


Compound 9b beta anomer



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: