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Short Note

(1*R*,2*R*,3*S*,4*R*)-1-(Acetylamino)-2,4,5-tris(acetyloxy)-1-((2*S*)-4-(benzyloxy)-5-oxo-2,5-dihydrofuran-2-yl)pentan-3-yl Acetate

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Abstract: Treatment of *N*-acetylneuraminic acid with excess base in the presence of benzyl bromide gives a polyhydroxylated 1,4 lactone which after acetylation gave the title compound in 20% overall yield. The structure of the product was confirmed by single crystal X-ray diffraction analysis, as well as FT-IR, NMR spectroscopic and HRMS analysis.

Keywords: sialic acid; N-acetylneuraminic acid; 1,4 lactones; γ-lactones; polyhydroxylated compounds

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1. Introduction

N-acetylneuraminic acid (Neu5Ac) is part of a diverse family of nine carbon-containing sugars. These sugars are found to be the terminal residues of complex glycans of cell surfaces and are known to modulate biological and pathological processes such as cell signalling, immunity, influenza and COVID-19 infection, amongst others [1–5].

Due to their wide-ranging role, developing derivatives of sialic acids has been of interest. Oguara researched the formation of lactone derivatives of Neu5Ac such as 1,4 and 1,7 lactones (Figure 1) in the 1980s. The 1,4 lactones were formed when Neu5Ac was treated with excess caesium carbonate and a carboxylate alkylating agent such as methyl iodine, benzyl bromide or allyl bromide [6]. The 1,7 lactones were produced when Neu5Ac was treated with benzoyl chloride in pyridine [7]. In fact, the 1,7 lactones of Neu5Ac are known to exist naturally, they have been shown to act as a ligand for IL-4 and have been found as part of glycoproteins in human colon tissue [8]. To date, no 1,4 lactone of Neu5Ac has been detected in nature; however, 1,7 lactones are known to decay to 1,4 lactones under acidic or basic conditions, which can further hydrolyse back to the parent Neu5Ac under the same conditions. Issues surrounding the detection of these derivatives from biological samples also exist, so perhaps the true importance of 1,4 lactones is yet to be understood [9]. Herein, we report a procedure to obtain the title 1,4 lactone in a moderate yield using a non-nucleophilic base.

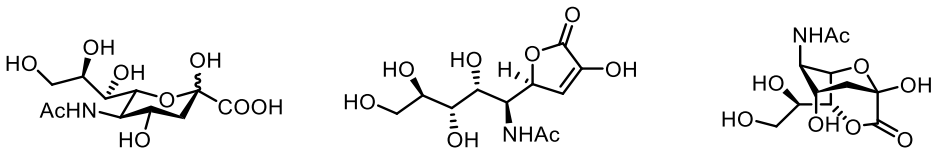


Figure 1. Neu5Ac and its lactone derivatives.

2. Results and Discussion

The precursor to the title compound **4** formed as a significant by-product during the attempted synthesis of peracetylated benzyl ester of Neu5Ac **5,** a versatile intermediate, used for further modification of Neu5Ac (Figure 2). This was carried out as described previously in the literature [10]. Thus, Neu5Ac was treated with DBU and benzyl bromide in DMF, anticipating only the isolation of the benzyl ester of Neu5Ac.

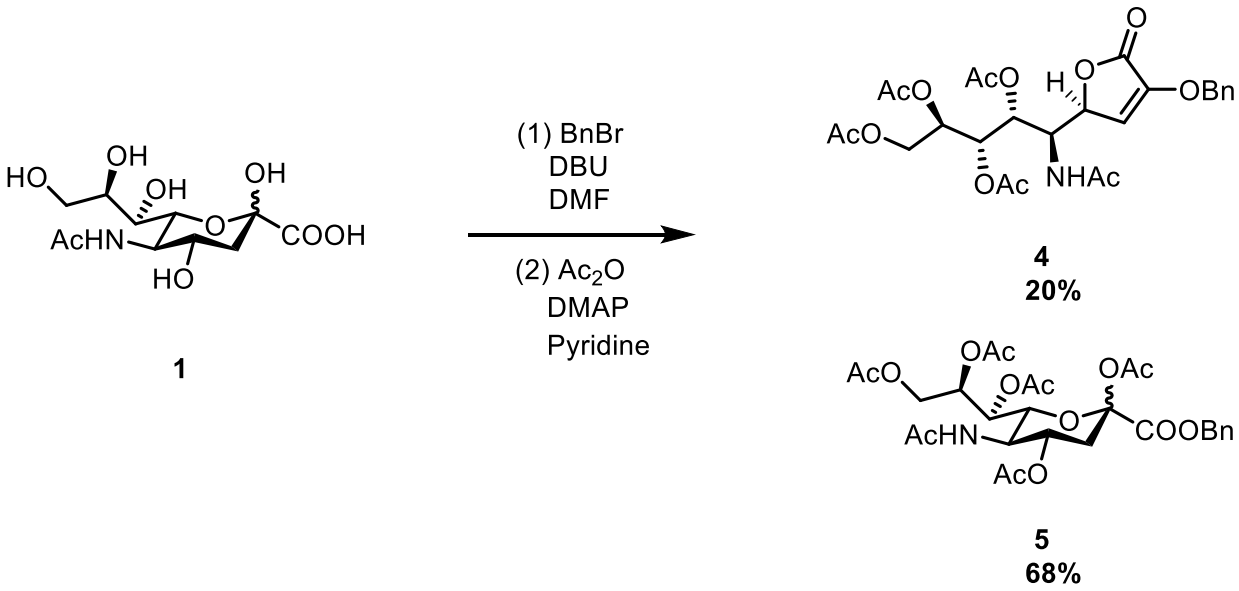


Figure 2. Preparation of a protected 1,4 lactone from Neu5Ac.

On analysis of the crude reaction mixture, a sizable secondary product was detected by TLC. Due to the polar nature of this mixture, these intermediates were peracetylated using Ac_2O, pyridine and catalytic DMAP. Chromatography then led to the isolation of **5** as a crystalline product, as well as **4**. The isolated **4** was then further purified by recrystallization from 50:50 ethyl acetate and cyclohexane. This gave crystals of high enough quality to allow the determination of its X-ray crystal structure.

3. Conclusions

The acetylated 1,4 lactone **4** was synthesised from Neu5Ac via a base which promoted intramolecular cyclisation from Neu5Ac followed by acetylation. The structure was determined by use of NMR spectroscopy IR, HRMS analysis and X-ray crystal structure.

4. Materials and Methods

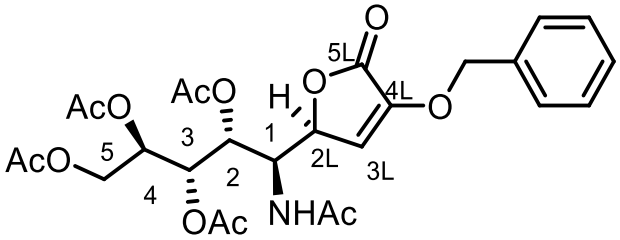
4.1. General Information

All solvents and chemicals were used as purchased unless stated otherwise; all solvents used were dried using a Puresolv purification system (Amesbury, MA, USA). All reactions were performed in oven dried apparatus with magnetic stirring under an inert atmosphere of argon or nitrogen. The reactions were followed by thin layer chromatography (TLC) carried out on aluminium foil-backed plates coated with silica gel (Merck Kieselgel 60 F_{254}, Darmstadt, Germany). The products were visualized using UV fluorescence (254 nm) or vanillin stain. Flash column chromatography was performed over Merck silica gel C60 (40–60 μm, Darmstadt, Germany) using eluent systems as described for each experiment.

All NMR spectra were recorded on an Agilent (formerly Varian) VNMRS 500 MHz spectrometer (Santa Clara, CA, USA). NMR data were processed using MNova 12.0.4 software (Mestrelab Research, S.L., Santiago de Compostela, Spain). ^{1}H and ^{13}C NMR spectra were reported as chemical shifts (δ) in parts per million (ppm) relative to the residual undeuterated solvent peak or TMS. Coupling constants (J) were reported in units

of hertz (Hz) and were rounded to the nearest 0.5. The following abbreviations were used to describe multiplets: s (singlet), d (doublet), ad (apparent doublet), t (triplet), at (apparent triplet), q (quartet), aq (apparent quartet), aqn (apparent quintet), sept (septet), m (multiplet), br (broad). IUPAC names were obtained using MNova 12.0.4 software.

*4.2. (1R,2R,3S,4R)-1-(Acetylamino)-2,4,5-tris(acetyloxy)-1-((2S)-4-(benzyloxy)-5-oxo-2,5-dihydrofuran-2-yl)pentan-3-yl Acetate (**4**)*



Neu5Ac **1** (3.0 g, 9.7 mmol) was dissolved in DMF (12.5 mL) and cooled to 0 °C, and DBU (2.34 g, 15.4 mmol) along with BnBr (2.64 g, 15.43 mmol) were added. After stirring at room temperature overnight, the solvent was removed in vacuo. The residue was dissolved in pyridine-Ac_2O (2:1, 60 mL) along with a catalytic amount of DMAP. The reaction was again stirred overnight, after which the reaction was diluted with 200 mL of DCM and washed with 100 mL of HCl 1M (x 5). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. Flash chromatography (EtOAc-cyclohexane) 50:50→100:0 gave the title compound **4** 1.06 g (20%). If required, prep HPLC was carried out. The title compound was recrystallized from 50:50 EtOAc-cyclohexane, which gave crystals suitable for X-ray crystallographic analysis.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.37–7.31 (overlapped signals, 5H, Ar-H), 5.98 (d, *J* = 2.0 Hz, 1H, alkene, H-3L), 5.76 (d, *J* = 10.0 Hz, 1H, NH), 5.50 (dd, *J* = 10.0, 2.0 Hz, 1H, H-1), 5.39 (dd, *J* = 9.0, 2.0 Hz, 1H, H-3), 5.07 (ddd, *J* = 9.0, 5.5, 3.0 Hz, 1H, H-4), 4.97 (d, *J* = 12.0 Hz, 1H, OCH_2Bn), 4.91 (d, *J* = 12.0 Hz, 1H, OCH_2Bn), 4.87 (ad, *J* = 2.0 Hz, 1H, H-2L), 4.50 (t, *J* = 10.0 Hz, 1H, H-2), 4.24 (dd, *J* = 12.5, 3.0 Hz, 1H, H-5), 4.00 (dd, *J* = 12.5, 5.5 Hz, 1H, H-5′), 2.13 (s, 3H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 1.80 (s, 3H).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 170.6 (C=O x2), 170.0(C=O), 169.7(C=O), 169.6 (C=O), 167.3 (C=O), 146.3 (C-2), 134.5 (Ar-H), 128.7 (Ar-C x2), 128.6 (Ar-C), 127.6 (Ar-C x2), 115.2 (C-3), 76.2 (C-4), 73.0 (C-10), 68.9 (C-5), 68.2 (C-8), 68.1 (H-7), 61.9 (C-9), 48.7 (C-6), 22.8 (CH_3), 20.9 (CH_3), 20.7 (CH_3 x2), 20.7 (CH_3).

HRMS: Calc. for $C_{26}H_{32}NO_{12}$ 550.1918, found $[M + H]^+$ 550.1927

IR cm^{-1}: 3349, 3109, 2938, 1780 (γ-lactone C=O), 1753, 1740, 1719, 1688, 1647, 1531, 1370, 1220, 1107, 1040, 980.

Optical Rotation: $[\alpha]^{20}_D$ = 0.9 (c 1.0, $CHCl_3$)

Crystal structure: $C_{26}H_{31}NO_{12}$ (M = 549.5290 g/mol): monoclinic space group $P2_1$, a = 10.1989(5) Å, b = 8.7455(4) Å, c = 15.8721(9) Å, α = 90°, β = 99.068(5)°, γ = 90°, V = 1398.01(12) $Å^3$, Z = 2, T = 298 K, μ = 0.104 mm^{-1}, Dcalc. = 1.31 g/cm^3, 15990 reflections were made, 8178 were unique, R = 0.0458, wR2 = 0.1185. (See Supplementary Materials). CCDC ref: 2132463 [11].

Supplementary Materials: The following are available online: 1D and 2D NMR spectra, LC-MS and crystallographic data for compound **4**.

Author Contributions: L.S.F. conceived and designed the experiments, drafted the manuscript and made revisions; L.S.F. performed the experiments; L.S.F. and P.V.M. analysed the data; C.O. solved the crystal structure. P.V.M. is principal investigator and project director and contributed to the target selection, synthesis route design as well as correcting drafts and finalising the manuscript. All authors have read and agreed to the published version of the manuscript.

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