Synthesis of a Phosphonate Analog of Sialic Acid (Neu5Ac) Using Indium-Mediated Allylation of Unprotected Carbohydrates in Aqueous Media

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Introduction

Sialic acid (Neu5Ac, 1), a constituent of sialoglycoproteins and gangliosides, mediates a number of intercellular and cell–virus recognition events. Analogs of sialic acid are interesting as potential inhibitors for medinally important target proteins, such as neuraminidase of the influenza virus. The syntheses of sialic acid and its analogs have been the subject of extensive research. Herein we describe a short synthesis of a phosphonate analog 3 of sialic acid (1). Vasella and others have shown that phosphonate analogs of sialic acid inhibit neuraminidase more effectively than sialic acid.

Our synthesis is based on an indium-mediated allylation of an unprotected carbohydrate in aqueous solution; this type of reaction has become a useful addition to carbohydrate chemistry. This note describes the synthesis of phosphonate analogs of sialic acid.

Results and Discussion

Stirring a suspension of indium, 4, and 5 in EtOH at rt for 24 h resulted in the complete consumption of the starting materials and afforded products as a 1:1 mixture of erythro and threo stereoisomers (results not shown). We found that when the organoindium reagent was generated in a separate step by stirring indium metal and indium in aqueous solution; this reaction gives only the desired threo product 6 in high yield. The subsequent deprotection and ozonolysis of 6 produces 3, the phosphonate analog of sialic acid, in 50% overall yield (Scheme 2). After this work was completed, Chan and Xin described a very similar synthesis, with results that are comparable to ours.

(a) In, dimethyl [1-(bromomethyl)ethenyl]phosphonate (4), EtOH, H2O, 81%, 6 days; (b) O3, MeOH, −78 °C; MeS, MeOH, −78 °C to rt, 90%

allylation of N-acetyl-β-D-mannosamine (5) with dimethyl [1-(bromomethyl)ethenyl]phosphonate (4) and indium in aqueous solution; this reaction gives only the desired threo product 6 in high yield. The subsequent deprotection and ozonolysis of 6 produces 3, the phosphonate analog of sialic acid, in 50% overall yield (Scheme 2). After this work was completed, Chan and Xin described a very similar synthesis, with results that are comparable to ours.

(a) deNinno, M. P.; Synthesis 1991, 583.
sition of 2 to the deprotected form 3, however, is difficult since 2 is not stable under either slightly acidic or basic conditions. We observed the formation of the lactone product from elimination of the phosphate group in 2. We then modified the reaction procedure to deprotect the dimethyl ester first and to permit subsequent oxidation of the olefin to ketone by ozonolysis (Scheme 2). First, trimethylsilylamine (TMS-I) was introduced to the solution of 6 in CH3Cl2 and MeOH. After 20 min at 0 °C, the reaction was stopped, and the solution was concentrated in vacuo. Crude 1H NMR spectroscopy showed that the dimethyl ester had reacted completely. The mixture was purified by gel filtration column chromatography, and the resulting material was subjected to ozonolysis. After the ozonolysis, the reaction mixture was separated by anion exchange chromatography and recrystallized in hot aqueous ethanol to produce the ammonium salt of 3 (62% from 6). Product 3 appears to be stable in both acidic and basic solutions for several days as tested by TLC (Rf = 0.35 using 6:3:1 mixture of propanol/NH3(30%)/H2O as an eluent).

Recently, Chan and Xin have reported a similar synthesis of 3 using indium chemistry.4 In their synthesis, allylation of 5 gave 6 as a 5:1 mixture of three/erthyro product, and the synthesis of 3 was achieved by the hydrolysis of the phosphate ester of acetylated 6 using TMS-Br. Our independent synthesis took a very similar course with very comparable results. We found, however, that by separately forming the indium reagents in EtOH before its addition to an aqueous solution of 5, we were able to obtain the three product diastereospecifically. Furthermore, use of TMS-I did not require 6 to be protected to hydrolyze the phosphate ester. We also discovered that the ammonium salt of 3 was readily recrystallized in aqueous ethanol. Overall, the two approaches to 3 are very similar and differ only in technical points. The results from two independent labs suggest the reproducibility of the method.

Conclusion

This paper summarizes a short synthesis of a phosphonate analog 3 of sialic acid (1, 50% yield based on consumed N-acetyl-β-D-mannosamine (5)). Compound 3 may be useful in studying a number of aspects of the glycobiology of sialic acid: for example, for the syntheses of phosphonate analogs of gangliosides or sialyl Lewis x; for the preparation of C-glycosides of the phosphate analog of sialic acid; as a basis for phosphonate-based probes to study sialic acid–receptor interactions by 31P NMR analysis; and as isosteres for sialic acid to study the influence of charge–charge interactions on the binding of derivatives of sialic acid to their receptors.

Experimental Section

Materials and Methods. Indium (powder, containing 1% Mg) was purchased from Aldrich. N-Acetyl-β-D-mannosamine monohydrate (5) was purchased from Sigma and used without further purification. Trimethylsilylamine was purchased from Fluka. Dimethyl [1-(bromomethyl)ethenyl]phosphonate (4) was prepared following literature procedures.6 MeOH and CH2Cl2 were freshly distilled before use. Reaction mixtures were monitored by thin layer chromatography on precoated silica gel plates (Merck, Kieselgel 60 F254). Flash chromatography was performed on silica gel (Merck 60, 40–63 µm). Size exclusion chromatography was performed on Biogel P2 resin. Anion exchange chromatography was performed on HiTrap Q columns from Pharmacia Biotech.

Preparation of Indium Reagent. The indium reagent was prepared freshly each time by stirring indium metal (83 mg, 0.7 mg-atm) with 4 (160 mg, 0.7 mmol) in 0.5 mL of EtOH for 10 min under Ar. Thin layer chromatography was used to monitor the reaction (eluent: CHCl3/MeOH 1:1). Formation of indium reagent resulted in the disappearance of 4 and the appearance of a new UV-active spot (Rf = 0–0.2). This suspension of indium reagent was introduced by syringe into the aqueous solution of 5.

5-[Acetamido]-1,2,3,5-tetra deoxy-2-(dimethoxyphosphoryl)-o-glycero-o-galacto-non-1-enitol (6). N-Acetyl-β-D-mannosamine monohydrate (5, 0.34 g, 1.4 mmol) was dissolved in 7 mL of water, and the solution was heated and maintained at 50 °C under Ar. The indium reagent was introduced by syringe in three portions (the preparation for each portion was described above; 12 h between each addition) to the above aqueous solution. The mixture was stirred for 6 days at 50 °C. The reaction mixture was diluted with 25 mL of EtOH and filtered through a plug of Celite. The Celite plug was rinsed with EtOH (25 mL), and the combined filtrate was concentrated in vacuo. The residue was purified by flash chromatography on silica gel (4:1 to 2:1 EtOAc/MeOH) to give 6 (252 mg, 48%) and recovered 5 (140 mg). The yield was 81% based on consumed 5. 1H NMR (400 MHz, CD3OD): δ 6.09 (d, 1 H, J = 23 Hz), 6.01 (d, 1 H, J = 49 Hz), 4.40 (t, 1 H, J = 6.8 Hz), 3.88 (s, 2 H), 3.73 (m, 8 H), 3.60 (dd, 1 H, J = 5.6 and 11.0 Hz), 3.39 (d, 2 H, J = 8.5 Hz), 2.10 MeOH, 0.03 ppm (H2O). 13C NMR (100 MHz, CD3OD): δ 174.5, 135.4 (d, J = 203 Hz, C2), 134.4 (d, J = 38 Hz, C1), 72.5, 71.4, 69.8, 67.9, 65.2, 54.9, 53.4 (d, J = 5.9 Hz), 38.4 (d, J = 11.2 Hz), 22.6. MS m/z 394 (M + Na)+. HRMS calcld for C12H24NO10P: (M + Na)+ 394.1246. Found: 394.1246.

Dimethyl Esters of the Phosphonate Analog 2 of Sialic Acid. Ozone was bubbled through a solution of vinylphosphonate 6 (74 mg, 0.20 mmol) in MeOH (15 mL) at −78 °C for 20 min. After ozonolysis, the reaction was purged with O2 for 5 min, and Me2S (0.5 mL) was added. The reaction was warmed to rt and stirred for 2 h until the TLC (1:1 EtOAc/MeOH) showed complete decomposition of the ozone. The mixture was concentrated in vacuo at rt and purified by size exclusion chromatography using a P2 gel filtration column (elution: water was used as eluent) to give 6 as a mixture of α and β-sialoside (5:1 (Rfα, 67 mg, yield 90%). 1H NMR for the β-sialoside (400 MHz, CD3OD): δ 4.01 (d, 1 H, J = 11.4 Hz), 3.9–3.7 (m, 10 H), 3.61 (dd, 1 H, J = 5.7 and 14.4 Hz), 3.46 (d, 1 H, J = 9.3 Hz), 2.23 (dd, 1 H, J = 4.9 and 12.9 Hz), 2.03 (s, 3 H), 1.77 (add, 1 H, J = 5.5, 11.2, and 12.8 Hz). 23Na NMR (100 MHz, CD3OD): δ 175.3, 99.2, 97.0, 72.0, 71.9, 71.7, 71.6, 70.2, 67.6, 65.9, 65.4, 55.1, 55.0, 54.9, 50.4, 39.7, 22.5. The chemical shifts of C(1) are 99.2 and 97.0 for the α- and β-isomer of 2, respectively. 31P NMR (200 MHz, CD3OD, using 85% phosphoric acid as an external reference) gave 19.93 ppm for the β- and 19.07 ppm for the α-isomer. MS m/z 374 (M + H)+. HRMS calcld for C13H23NaNO3P: (M + H)+ 374.1216. Found: 374.1207.

Ammonium Salt of Phosphonate Analog 3 of Sialic Acid. MeOH (50 mL) was added under Ar at 0 °C to a suspension of 74 mg (0.20 mmol) of 6 in CH2Cl2 (1.0 mL) to make 6 soluble. TMS-I (136 µL, 1.0 mmol) was introduced dropwise into this solution by syringe. After 20 min at 0 °C, MeOH (5 mL) was added, and the mixture was concentrated in vacuo. The residue was dissolved in 3 mL of H2O, decolorized with activated charcoal, filtered, lyophilized, and further purified with size exclusion chromatography using a P2 gel filtration column (deionized water as the eluent). The resulting solid material was dissolved in MeOH (15 mL), and ozone was bubbled through the solution at −78 °C for 20 min. After ozonolysis, the reaction was purged with O2 for 5 min, Me2S (0.5 mL) was added, and
the solution was allowed to warm to rt and stirred for 2 h. The mixture was concentrated in vacuo and separated by anion exchange column chromatography (HiTrap Q columns, Pharmacia Biotech) using NH₄OAc solution (0–200 mM, pH = 7) as the eluent. The mixture was lyophilized three times to remove residual NH₄OAc. The resulting solid material was recrystallized in hot aqueous ethanol to give 47 mg (62% from 6) of ammonium salt of 3. Rf = 0.35 (6:3:1 propanol/NH₄OAc(30%)/H₂O).

1H NMR (500 MHz, CD₃OD): δ 4.02 (m, 2 H), 3.76 (m, 3 H), 3.57 (dd, 1 H, J = 5.9 and 11.3 Hz), 3.42 (d, 1 H, J = 9.4 Hz), 2.31 (dd, 1 H, J = 4.8 and 13.0 Hz), 1.99 (s, 3H), 1.83 (td, 1 H, J = 12.5 and 4.8 Hz). 13C NMR (100 MHz, CD₃OD): δ 174.8, 98.0 (d, J C-P = 190 Hz), 71.5, 70.9 (d, J C-P = 10.6 Hz), 70.5, 68.2 (d, J C-P = 10.6 Hz), 65.2, 54.5, 39.8 (d, J C-P = 6.8 Hz), 22.7. 31P NMR (200 MHz, CD₃OD, using 85% phosphoric acid as an external reference): δ 14.37. MS (FAB): m/z 344 (M – H)⁻. HRMS calcd for C₁₀H₂₀NO₁₀P: (M – H)⁻ 344.0747. Found: 344.0752.

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Supporting Information Available: 1H, 13C, and 31P NMR spectra of 2, 3, and 6 (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal and can be ordered from the ACS; see any current masthead page for ordering information.

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