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Jinming Gao, Valeri Martichonok, and George M. Whitesides
Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

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

Jinming Gao, Valeri Martichonok, and George M. Whitesides*
Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138

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Introduction
Sialic acid (Neu5Ac, 1), a constituent of sialoglycoproteins and gangliosides, mediates a number of intercellular and cell—virion recognition events. Analogs of sialic acid are interesting as potential inhibitors for medically 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). We and others have shown that phosphonate analogs of sialic acid inhibit neuraminidase more effectively than sialic acid.α,β

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

Notes

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. We found that when the organoindium reagent was generated in a separate step by stirring indium metal with 4 in EtOH for 10 min at rt and added to a solution of 5 in H2O, the yield of the threo product 6 was 48% (87% based on recovered 5) after purification by column chromatography (Scheme 1).

Ozonolysis of the vinylphosphonate 6, followed by reductive decomposition of the ozonide, produced 2 (90%). Compound 2 exists as a 1:5 mixture of α- and β-sialoside in MeOH. The major isomer of 2 was assigned as the β-sialoside by comparison of the 1H NMR spectrum of 2 with that of the β-sialoside of the methyl ester of sialic acid. The ratio of the β-sialoside over α-sialoside was determined based on the 31P NMR spectrum and the -NHCOCH3 singlets in the 1H NMR spectrum. Conver-

(a) In, dimethyl [1-(bromomethyl)ethenyl]phosphonate (4), EtOH, H2O, 81%, 6 days; (b) O3, MeOH, −78 °C; Me2S, MeOH, −78 °C to rt, 90%.

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tion 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 phosphonate 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, trimethylsilylosilane (TMS-1) was introduced to the solution of 6 in CH₂Cl₂ 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 (627° from 6). Product 3 appears to be stable in both acidic and basic solutions for several days as tested by TLC (Rₐ = 0.35 using 6:3:1 mixture of propanol/NH₄H₂O/30%/H₂O as an eluent).

Recently, Chan and Xin have reported a similar synthesis of 3 using indium chemistry. In their synthesis, allylation of 5 gave 6 as a 5:1 mixture of threo/erythro product, and the synthesis of 3 was achieved by the hydrolysis of the phosphonate 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 threo product diasteroselectively. Furthermore, use of TMS-1 did not require 6 to be protected to hydrolyze the phosphonate 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 gangiosides or sialyl Lewis x; for the preparation of C-glycosides of the phosphonate 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. Trimethylsilylosilane was purchased from Fluka. Dimethyl [1-(bromomethyl)ethenyl]phosphonate (4) was prepared following literature procedures. 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 (85 mg, 0.7 mg-at) 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: 1:10 MeOH/CH₂Cl₂). The formation of indium reagent resulted in the disappearance of 4 and the appearance of a new UV-active spot (Rₛ = 0.2). This suspension of indium reagent was introduced by syringe into the aqueous solution of 3.

5-(Acetylamino)-1,2,3,5-tetrahydroxy-2-(dimethylphosphinyl)-β-glycero-β-galacto-non-1-enitol (6). N-Acetyl-β-D-mannosamine monohydrate (5, 0.34 g, 1.4 mmol) was dissolved in 74 mL of water, and ozone was bubbled through a solution of 5. The reaction mixture was diluted with 25 mL of EtOH and warmed to rt and stirred for 2 h until the TLC (1:1 EtOAc/MeOH) showed complete decomposition of the ozonide. The mixture was concentrated in vacuo, the residue was purified by flash chromatography on silica gel (4:1 to 1:2 EtOAc/MeOH) to give 6 (232 mg, 48%) and recovered 5 (140 mg). The yield was 81% based on consumed 5. ¹H NMR (400 MHz, CD₂OD): ø 6.69 (t, J = 8.0 Hz, 1 H, J₁ = 5.4 Hz), 3.98 (s, 2 H), 2.08 (dd, 1 H, J = 4.5 and 12.9 Hz), 1.57 (dd, 1 H, J = 5.5, 11.3, and 12.8 Hz). ³¹P NMR (100 MHz, CD₂OD): ø 174.5, 135.4 (d, J_g-p : 205 Hz, C2), 134.4 (d, J_C-P : 38 Hz, C1), 72.5, 71.4, 69.8, 67.9, 65.2, 54.9, 53.4 (d, J_C-P : 38 Hz), 38.4 (d, J_C-P : 11.2 Hz), 22.6. MS m/z 394 (M + Na)⁺. HRMS calcd for C₁₁H₁₂O₆P·(M + Na)⁺ 394.1243. Found: 394.1246.

Dimethyl Ester 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 heated and maintained at 50 °C under Ar. The indium reagent was introduced by syringe into the mixture 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 filtrates were concentrated in vacuo. The residue was purified by flash chromatography on silica gel (4:1 to 1:2 EtOAc/MeOH) to give 6 (232 mg, 48%) and recovered 5 (140 mg). The yield was 81% based on consumed 5. ¹H NMR (400 MHz, CD₂OD): ø 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, 1 H, J = 8.5 Hz), 2.39 (m, 2 H, 2.03 (s, 3 H). ³¹P NMR (100 MHz, CD₂OD): ø 174.5, 135.4 (d, J_C-P : 203 Hz, C2), 134.4 (d, J_C-P : 38 Hz, C1), 72.5, 71.4, 69.8, 67.9, 65.2, 54.9, 53.4 (d, J_C-P : 38 Hz), 38.4 (d, J_C-P : 11.2 Hz), 22.6. MS m/z 394 (M + Na)⁺. HRMS calcd for C₁₁H₁₂O₆P·(M + Na)⁺ 394.1243. Found: 394.1246.

Ammonium Salt of Phosphonate Analog 3 of Sialic Acid. MeOH (50 μL) 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. The resulting clear solution was added dropwise into the 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) to give 2 as a mixture of diastereomers (15.1% of β-sialoside (5:1 β/α, 67 mg, yield 90%). ¹H NMR for the β-sialoside (400 MHz, CD₂OD): ø 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 (dd, 1 H, J = 5.5, 11.2, and 12.8 Hz). ³¹P NMR (200 MHz, CD₂OD, using 85% phosphoric acid as an external reference) gave 19.93 ppm for the β-isomer and 19.07 ppm for the α-isomer. MS m/z 394 (M + H)⁺. HRMS calcd for C₁₁H₁₂O₆P·(M + H)⁺ 374.1216. Found: 374.1207.
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, 99.0 (d, Jc-p = 190 Hz), 71.5, 70.9 (d, Jc-p = 10.6 Hz), 70.5, 68.2 (d, Jc-p = 10.6 Hz), 65.2, 54.5, 39.8 (d, Jc-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 C₁₂H₁₄NO₅P⁻ (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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