Stereoselective α-Sialylation with Sialyl Xanthate and Phenylsulfenyl Triflate as a Promotor

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Abstract

Here we report an efficient α-stereoselective synthesis of sialyl glycosides using phenylsulfenyl triflate (PST) as a new promotor for sialyl xanthates 2 and 3 (Scheme 1). Sialic acid (Neu5Ac) frequently terminates the oligosaccharide chains of the glycoproteins and glycolipids that play a central role in cell surface recognition phenomena. Cell surface sialosides serve as ligands for microbial toxins, microbial adhesins that mediate attachment to host cells, and lectins crucial in intercellular recognition.

Sialosides have been the subject of extensive research, and recent reviews describe synthetic approaches to their synthesis. Enzymatic synthesis has been used for multigram syntheses of sialosides. It is, however, generally limited to natural products, or to close structural analogs of them, by the specificities of these enzymes. Practical and stereocontrolled chemical syntheses are of particular interest, especially for the preparation of analogs of sialosides. Recent reports have established the potential in oligosaccharide synthesis of thioglycosides and of sialyl acids xanthate 3 when these compounds are activated with equimolar amounts of thiophilic reagents in nitrile solvents at low temperature. Dimethyl(methylthio)sulfonium triflate (DMTST) and methylsulfenyl triflate (MST) are the most effective activators; DMTST and MST are usually prepared from dimethyl disulfide and bromine and is unstable. Both DMTST and MST are toxic, and some of the intermediates and reagents used in their preparation also are toxic, unstable, and expensive.

In this paper we show that α-sialyl glycosides can be efficiently prepared from sialyl xanthates 2 and 3 using PST as a promotor. PST is, in fact, superior to both DMTST and MST. We demonstrate that this procedure can be used for a gram-scale synthesis of protected GM3 trisaccharide 10. We discuss a possible mechanism of the reaction.

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Table 1. Sialylation with Sialyl Xanthate 2

<table>
<thead>
<tr>
<th>acceptor</th>
<th>donor/acceptor ratio</th>
<th>promotor condition</th>
<th>product</th>
<th>yield (%)</th>
<th>α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1:1.3</td>
<td>PhSCI/AgOTf</td>
<td>9</td>
<td>63</td>
<td>94:6</td>
</tr>
<tr>
<td>5</td>
<td>1:1.3</td>
<td>PhSCI/AgOTf/DTBP</td>
<td>9</td>
<td>74</td>
<td>95:5</td>
</tr>
<tr>
<td>6</td>
<td>1:1.5</td>
<td>PhSCI/AgOTf</td>
<td>10</td>
<td>61</td>
<td>96:4</td>
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<tr>
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<td>1.5:1</td>
<td>PhSCI/AgOTf/DTBP</td>
<td>10</td>
<td>78*</td>
<td>96:4</td>
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<tr>
<td>7</td>
<td>1:1.2</td>
<td>PhSCI/AgOTf/DTBP</td>
<td>11</td>
<td>48</td>
<td>83:17</td>
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<tr>
<td>7</td>
<td>1:5.1</td>
<td>PhSCI/AgOTf</td>
<td>11</td>
<td>54</td>
<td>90:10</td>
</tr>
<tr>
<td>8</td>
<td>1:1.5</td>
<td>PhSCI/AgOTf</td>
<td>12</td>
<td>59</td>
<td>84:16</td>
</tr>
<tr>
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<td>1:1.3</td>
<td>MeSBr/AgOTf</td>
<td>10</td>
<td>31</td>
<td>86:14</td>
</tr>
</tbody>
</table>

a The concentration of 2 was ~0.05 mol/L; the ratio 2:PhSCI/AgOTf:DTBP was 1:1.05:1.1.2. b The yield was determined by 1H NMR and refers to a mixture of α- and β-anomers. c The isolated yield. d A ratio of > 99:1 was obtained in a diluted solution, with the concentration of the donor 2 ≈ 0.01 mol/L, albeit in 52% yield. e The ratio 2:PhSCI/AgOTf:DTBP was 1:1.05:1.1.2.5.

Scheme 1

Results and Discussion

Preparation of Sialyl Xanthates. When sialyl chloride 1 was allowed to react with O-ethyl S-potassium dithiocarbonate in acetone at ambient temperature, a mixture of α- and β-sialyl xanthates 2 and 3 and glycal 4 was obtained (Scheme 1) in molar ratio 16:1:3, as determined by 1H NMR. The proportion of glycal/product was increased when the reaction was carried out at 0 °C. α-Sialyl xanthate 2 was reported as the only product when the reaction was carried out in EtOH.11 We obtained the same product ratio in this solvent as in acetone. Chromatographic separation gave xanthates 2 and 3, which were contaminated with sialyl glycal 4, probably due to their partial decomposition on silica gel.

Sialylation. MST is less reactive than DMTST and, according to literature data, is more effective than DMTST for α-sialylation in terms of yield and stereoselectivity. We reasoned that PST, which is less reactive than MST, would be even better. PST was prepared in situ by reacting benzenesulfonyl chloride with silver triflate. Benzenesulfonyl chloride was synthesized by reacting phenyl thioacetic acid with SO2Cl2.12 It also can be conveniently prepared by treatment of thiophenol or diphenyl disulfide with Cl2 or SO2Cl2.13 We found that unlike unstable methanesulfonyl bromide, benzenesulfonyl chloride was stable for at least 10 months at 4 °C under nitrogen. Sialylation of acceptors 5–8 with xanthate 2 was studied in a 2:1 mixture of acetonitrile/dichloromethane under conditions that are summarized in Table 1. Sialylation of acceptors 5–7 occurred at the more reactive 3-OH and gave rise to the predominantly α-products 9–11. Racemic alcohol 8 was used as a model for NeuAc-2,6-Gal linkage that often occurs as an element in gangliosides. Compound 12 was obtained as a diastereomeric mixture.

The yield and purity of the product increased when di-tert-butylpyridine (DTBP) was used as a proton scavenger. We note that less reactive alcohols 5 and 6 gave a higher α/β ratio than more reactive allylic alcohol 7 and primary alcohol 8. This ratio is reversed relative to that which was observed with sialyl chloride 1 in the presence of silver or mercury salts. Furthermore, we observed that α-stereoselectivity increased when the

References

reaction was conducted in a dilute solution, although the yield was lower. Only α-product 10 was detected by 1H NMR with 1.5 equiv of donor 2 relative to acceptor 6 in a dilute solution. The yields of products 9, 10, and 12 were good; the yield of 11 was somewhat lower because PST can add to the double bond of the galactosyl glycal 7. About 30% of the unreacted xanthate 2 was detected in this reaction. Sialyl glycal 4 was formed as a byproduct of the reaction.

We found that sialylation of lactosyl acceptor 6 with pure β-xanthate 3 gave the same α/β ratio of the product 10 as sialylation with α-xanthate 2. For large-scale sialylations compounds 2, 3, and 4 were not separated. Using the crude reaction mixture of 2, 3, and 4 as sialyl donor, we prepared 10-protected GM3 trisaccharide on a gram scale.

The site of sialylation in the product 10 was determined by acetylation and observation of a downfield shift of the C-4' proton. The stereochemistry of the new glycosidic linkage in products 9–12 was determined to be α on the basis of the occurrence of the NeuAc H-4 at ~4.8 ppm as well as the $J_{\text{NeuAc-C-3'x}} > 7.0 \text{ Hz}$. The stereochemistry of 10 was also confirmed by the measurement of the long-range coupling constant $J_{\text{C-1,H-3'x}} = 5.10 \text{ Hz}$ of the sialic acid residue. In addition, the anomeric configuration of 2 and 3 was confirmed by the chemical shift difference between the two hydrogen atoms at position 9 ($\delta_\alpha (H(9) - H(9')) - \delta_\beta (H(9) - H(9'))$): $\Delta \delta = 0.50 \text{ ppm for } \alpha, \Delta \delta = 0.14 \text{ ppm for } \beta$. The α/β ratios were determined by integration of H(3)ac signals in 1H NMR spectra. Chemical shifts were smaller for β-glycosides (in the range 2.58–2.42 ppm for β-anomers of compounds 2, 3, 9–12) than for α-glycosides (in the range 2.67–2.51 ppm for compounds 2, 3, 9–12).

**Mechanism of the Reaction.** We propose that in the first step sialyl xanthate reacts with PST to give the oxonium cation 14 via the formation of the intermediate 13 (Scheme 2). This conjecture is supported by the following facts: (i) the α/β ratio of the final product is independent of the stereochemistry of the starting xanthate; (ii) compound 15 was isolated in 86% as a major byproduct of the reaction. In the second step oxonium cation 14 is stabilized by reaction with acetonitrile to form nitrilium cations 16 and 17. Nitrilium cations have been evoked to explain the stereoselectivities of glycosilation in acetonitrile solvents. According to our semiempirical MO calculations (PM3 molecular model), 16 (R = Me) is 1.46 kcal/mol more stable than 17. The nitrilium cation 16 is probably formed first due to kinetic and thermodynamic control. The attack of acetonitrile from the re side of 14 to form 17 is hindered by unfavorable steric interactions with protons at C-4 and C-6 of the sialic acid; both 16 and 17 are probably formed in this step. We assume that the nitrilium cations 16 and 17 are in equilibrium. In the third step acceptor ROH reacts with the cation 17 to give α-product 18. Attack on cation 16 is very hindered due to protons at C-4 and C-6 and to the CO$_2$Me group. A small amount of the β-product is formed by reaction of the acceptor ROH with oxonium cation 14. More reactive acceptors, such as alcohols 7 and primary alcohol 8, are more likely to compete with acetonitrile for 14 and give a lower α/β ratio. The dilution of the reaction mixture with the solvent increases the ratio [CH$_3$CN]/[ROH] and, consequently, increases the α/β ratio for the product of the reaction.

**Conclusion**

PST is superior to MST in terms of both yield and stereoselectivity of sialylation. High stability of benzene-sulfonyl chloride, combined with ease of its preparation and its low toxicity, makes PST an effective reagent for stereoselective α-sialylation. Important deficiencies still remain in sialylation, however. Although PST is a useful reagent, it is not a panacea in synthesis of carbohydrates. Among these residual problems are the following: (i) overall yields are only moderate due to the formation of sialyl glycal during preparation of sialyl xanthates and during sialylation; (ii) some amount of the undesired β-product is always formed, especially with reactive alcohols; (iii) the product of the reaction is contaminated with traces of byproducts, separation of which requires careful chromatography.

Preparation of Acceptors 5 - 7. Methyl(2,3-O-2,6,6,8,9-tetra-O-(phenylmethyl)-β-D-galactopyranosyl) (5) was prepared in 66% yield from methyl β-D-galactopyranoside following the procedure for synthesis of 6. Rf = 0.49 (50% EtOAc in hexanes); mp 76-77°C (lit.19 mp 76-77°C; [α]D20 = +11.07° (c 1.68, CHCl3), lit.20 mp 80-81°C; [α]D20 = +10.0° (c 0.40, CHCl3). 2-Trimethylsilyl methyl 2,6,8,9-bis-O-phenylmethyl)-β-D-galactopyranosyl-(1,4)-2,3,6-tris-O-(phenylmethyl)-β-D-glucopyranoside (6) was prepared in total 26% yield from lactose (six steps23). Compound 6 was crystallized from EtO/pentane: mp 88-89°C; [α]D20 = +15.9° (c 1.13, CHCl3), lit.25 mp 95-101°C (after trituration); [α]D20 (400 MHz, CDCl3) δ = -0.26 (d, J = 10.28 Hz, 1 H, H-3), 3.39 (d, d, J = 6.77 Hz, 1 H, H-2a), 3.50 (dd, J = 6.77 Hz, 1 H, H-2b), 3.57 (d, J = 7.85 Hz, 1 H, H-8a), 3.69 (m, 1 H, H-8b), 3.82 (m, 1 H, H-8c), 4.38 (d, J = 12.15 Hz, 1 H, H-7), 4.44 (d, J = 7.70 Hz, 1 H, H-1), 4.51 (d, J = 12.10 Hz, 1 H, H-6), 4.60 (d, J = 7.77 Hz, 1 H, H-1), 4.69 (d, J = 11.60 Hz, 1 H, H-6), 4.86 (d, J = 11.60 Hz, 1 H, H-1), 4.97 (d, J = 11.15 Hz, 1 H, H-5), 5.08 (d, J = 11.51 Hz, 1 H, H-5), 5.27 (d, J = 11.12 Hz, 1 H, H-4), 7.04-7.61 (m, 25 H, aromatic); 13C NMR (100 MHz, CDCl3) δ = -1.48, 18.37, 67.24, 68.29, 68.58, 68.68, 72.82, 73.05, 73.34, 73.44, 74.76, 75.02, 75.06, 76.61, 79.66, 81.82, 82.75, 102.48, 102.51, 103.05, 121.77, 123.39, 124.74, 124.79, 125.47, 126.77, 127.69, 127.87, 128.93, 128.15, 128.19, 128.28, 128.37, 129.97, 133.21, 135.81, 136.68, 139.10, HRMS (FAB) cated for C63H52O35SNa (M + Na)+, found 835.3676, 15-Andoxy-6-O-butyldimethylsilyl)-2-deoxy-o-beta-hex-
Methyl (N-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-a-neuraminosyl)(2,3)-O-2,6-bis-O-(phenylmethyl)-β-D-galactopyranoside (9): 1H NMR (500 MHz, CDCl₃) δ 2.51 (dd, J = 4.67, 12.78 Hz, 1 H, H-3eq’), 3.54 (s, 3 H, OCH₃), 3.75 (s, 3 H, CO₂CH₃), 3.95 (dd, J = 5.80, 12.51 Hz, 1 H, H-9a’), 4.00 (dd, J = 2.21, 10.65 Hz, 1 H, H-6’), 4.29 (dd, J = 2.50, 12.50 Hz, 1 H, H-9a’), 4.83 (dt, J = 4.43, 11.85 Hz, 1 H, H-7’), 5.29 (dd, J = 2.10, 8.04 Hz, 1 H, H-7’), β-anomer δ 2.43 (dd, J = 4.91, 12.90 Hz, 1 H, H-3eq’); MS (FAB) calc'd for C₃₁H₄₁NO₁₉SiNa (M + Na) 870, found 870.

(N-Acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-a-neuraminosyl)-(2,3)-O-1,5-anhydro-6-O-(tert-butyldimethylsilyl)-2-deoxy-β-D-xyl-1-enopyranose (11): 1H NMR (500 MHz, CDCl₃) δ 0.62 (s, 6 H, S(CF₃)₂), 0.87 (s, 9 H, Si-t-Bu), 2.62 (dd, J = 4.66, 12.91 Hz, 1 H, H-3eq’), 2.71 (dd, J = 3.38 Hz, 1 H, 4-OH), 3.84 (s, 3 H, CO₂CH₃), 6.36 (dd, J = 6.07 Hz, 1 H, H-1’), β-anomer δ 2.54 (dd, J = 4.96, 12.98 Hz, 1 H, H-3eq’); MS (FAB) calc'd for C₂₉H₃₇NO₁₉SiNa (M + Na) 756, found 756.

(1-Tetrahydropyranylmethyl) (N-acetyl-4,7,8,9-tetra-O-acetyl-1-methyl-a-neuraminosyl) (12) was obtained as a mixture of two diastereomers. α-Diastereomers: 1H NMR (500 MHz, CDCl₃) 2.59 (dd, J = 4.66, 13.14 Hz, 1 H, H-3eq’), 2.61 (dd, J = 4.68, 12.92 Hz, 1 H, H-9a’), 98.60 and 98.82. β-Diastereomers: 1H NMR (500 MHz, CDCl₃) 2.59 (dd, J = 4.66, 13.14 Hz, 1 H, H-3eq’), 2.61 (dd, J = 4.68, 12.92 Hz, 1 H, H-9a’), 98.60 and 98.82; MS (FAB) calc'd for C₃₀H₄₄NO₁₈SiNa (M + Na) 612.2268, found 612.2272.

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Supporting Information Available: 1H NMR spectra for crude compounds 9, 11, and 12 (3 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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