Pergamon

Tetrahedron Letters 40 (1999) 2217–2220

TETRAHEDRON LETTERS

A Novel Donor for Stereoselective α-Sialylation; Efficient Synthesis of an α(2-8)-Linked Bis-Sialic Acid Unit

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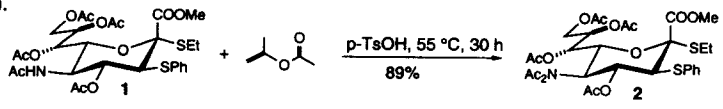
Received 4 December 1998; accepted 19 January 1999

Abstract: The novel sialyl donor methyl [ethyl 5-(*N,N*-diacetyl)-4,7,8,9-tetra-*O*-acetyl-2-thio-3-thiophenyl-2,3,5-trideoxy-D-erythro-α-L-gluco-2-nonulopyranosid]onate (**2**) was synthesized by *N*-acetylation of the corresponding 5-acetamido compound **1**. α-Sialylation of mono- and disaccharide aglycons, having one or two unprotected hydroxyl groups, furnished the corresponding di- and trisaccharides in high yield and with virtually complete regio- and stereoselectivity. As one example, a Neu5Acα(2→8)Neu5Ac derivative was thus obtained in 44% yield; the corresponding β anomer was not observed.

Glycosylation with *N*-acetyl-neuraminic acid donors (sialylation) is considered to be more difficult than other glycosylations, especially when another *N*-acetyl-neuraminic acid is used as acceptor. It seems as if steric hindrance in the acceptor enables a competing 2,3-elimination to destroy the donor, which is witnessed by the formation of a large amount of sialic acid glycal as a byproduct.[1] In order to avoid losses of donor and also to improve the α,β-ratio in sialylations, donors were introduced that carry a thiophenyl substituent in the 3-position.[2] It is believed that the thiophenyl substituent decreases the acidity of H-3 and also leads to the formation of a reactive episulfonium intermediate that can only be attacked by the acceptor molecule from the α-side. The original donors were *O*-benzylated[2] whereas two novel donors (**1** and a derivative of **1**; Scheme 1) carry *O*-acetyl protecting groups.[3,4] In a comparative study of **1** and traditional sialyl donors that lack the thiophenyl group, we found that only **1** gave sialylated products in the form of pure α-glycosides; the other donors gave α-glycosides contaminated by a small amount (4-6%) of the corresponding β-glycosides.[3] Although only donor **1** had the power to sialylate the sterically hindered acceptor **3** (Scheme 2), the yield of the desired bis-sialic acid was rather low (28%).[3] Another attempt towards improved sialyl donors includes the use of a phenoxy-thiocarbonyl group in the 3-position.[5]

It was recently reported[6] that the nitrogen atom of the acetamido residue of sialic acid donors can undergo alkylation by the strongly electrophilic reagents used for activation of the anomeric thioalkyl group. To avoid alkylation, an extra *N*-acetyl group was introduced, which reduced the nucleophilicity of the amide nitrogen, and sialylations could be performed in high yield. In a comparative study with different sialic acid acceptors, the Neu5Acα(2→8)Neu5Ac moiety was obtained in ~60% yield, but the α,β-ratio was low.[7] Following the glycosylation step, the extra *N*-acetyl group was easily removed by methanolic sodium methoxide to furnish the intact sialic acid moiety.[6,7] We now wish to report a further elaboration on this theme and introduce the donor **2**, carrying both a thiophenyl group and an extra acetyl group on the nitrogen atom (Scheme 1).



Scheme 1

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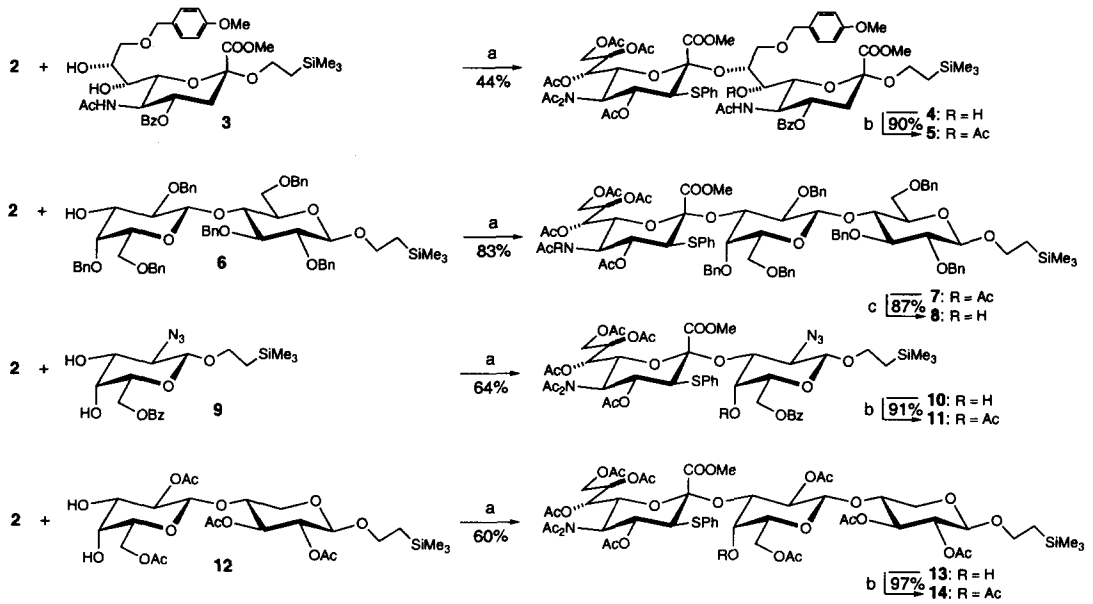
The preparation of **2** was performed according to the procedure used for *N*-acetylation of traditional sialyl donors.[6,7] The known[3] sialyl donor **1** was dissolved in isopropenyl acetate, a catalytic amount of *p*-toluenesulfonic acid was added, and the mixture was stirred at 55 °C for 30 h. Residual isopropenyl acetate was then removed, which gave pure **2** in 89% yield.

The efficiency of **2** as a sialyl donor was investigated by reaction with a series of glycosyl acceptors (**3**[3], **6**[3], **9**[3], and **12**[8]; Scheme 2), having different protecting-group patterns. After some experimentation it was found that activation of **2** with AgOTf/methylsulfenyl bromide, in the presence of the acceptor and with CH_3CN/CH_2Cl_2 as solvent, furnished the desired α-sialylated compounds in good yields after chromatographic purification. In no case was the corresponding β-glycoside observed (TLC and ^{1}H-NMR). It should be noted that the reaction conditions are not optimized.



Scheme 2. a) AgO_3SCF_3, MeSBr, MS (3 Å), MeCN, CH_2Cl_2, -45 °C, 4.5 h . b) Ac_2O, pyridine. c) MeONa, MeOH, then Ac_2O, pyridine.

Sialylation of the very demanding acceptor **3** to give the pure α-linked bis-sialic acid derivative **4** proceeded in an unprecedented yield of 44%. This should be compared with sialylation using the donor **1**, which gave the corresponding bis-sialic acid in 28% yield.[3] The results augment the suggestion that donors carrying two *N*-acetyl groups are more stable against destruction than the traditional donors.[6,7] As anticipated, acceptor **6** gave the best yield in the series, probably because it carries electron-donating *O*-benzyl protecting groups, which are known to increase the reactivity of nearby hydroxyl groups; the G_{M3}-ganglioside-derived trisaccharide **7** was obtained in 83% yield. In line with previous sialylations of galactopyranosyl-configured diols and triols,[3,8-11] the diols **9** and **12** underwent completely regio- and stereoselective sialylations, and the products **10** and **13** were obtained in 64% and 60% yields, respectively. Despite many successful examples, such pronounced regioselectivity should by no means be taken for granted; some interesting exceptions were found during our recent syntheses of the sialyl Lewis x tetrasaccharide and its analogs.[11]

The auxiliary *N*-acetyl group was easily removed[6,7] under normal de-*O*-acetylation conditions. As an example, compound **7** was treated with methanolic sodium methoxide, followed by acetic anhydride in pyridine, which gave the de-*N*-acetylated trisaccharide **8** in 87% yield. In order to simplify the determination (by ^{1}H-NMR) of the position of sialylation of the three diols **3**, **9** and **12**, the primary sialylation products **4**, **10** and **13** were *O*-acetylated to furnish the fully protected compounds **5**, **11** and **14** in 90%, 91%, and 97% yield, respectively. The α-configuration of the sialic acid moiety in the products was confirmed by measuring the long-range $J_{C1\text{-}H3ax}$ coupling constants (see Experimental).[12]

EXPERIMENTAL

Methyl [ethyl 5-(*N*,*N*-diacetyl)-4,7,8,9-tetra-*O*-acetyl-2-thio-3-thiophenyl-2,3,5-trideoxy-D-erythro-α-L-gluco-2-nonulopyranosid]onate (2). Compound **1** (1.287 g, 2.0 mmol) was treated with isopropenyl acetate (10 mL) in the presence of *p*-toluenesulfonic acid (19 mg) at 55 °C for 30 h. The reaction mixture was cooled to room temperature, Et_3N (1 drop) was added, and the solvent was removed in vacuo. The residue was chromatographed (SiO_2, toluene-MeCN 19:1→4:1) to give **2** (1.22 g, 89%). ^{1}H-NMR ($CDCl_3$): δ 7.61-7.17 (m, 5 H, Ph), 5.79 (dd, 1 H, *J* = 9.8, 10.8 Hz, H-4), 5.30 (m, 1 H, H-8), 5.12 (dd, 1 H, *J* = 1.8, 8.4 Hz, H-7), 4.83 (dd, 1 H, *J* = 1.8, 10.6 Hz, H-6), 4.33 (dd, 1 H, *J* = 10.6, 9.8 Hz, H-5), 4.27 (dd, 1 H, *J* = 2.8, 12.6 Hz, H-9), 4.14 (dd, 1 H, *J* = 4.9, 12.6 Hz, H-9'), 3.94 (s, 3 H, $COOCH_3$), 3.42 (d, 1 H, *J* = 10.8 Hz, H-3), 2.98-2.76 (m, 2 H, SCH_2CH_3), 2.36, 2.28, 2.17, 2.11, 2.03, 1.86, (s, 3 H each, Ac), 1.27 (t, 3 H, *J* = 7.5 Hz, SCH_2CH_3).

Preparation of compounds 4, 7, 10, and 13. Sialylation of compounds **3**, **6**, **9**, and **12** with the donor **2** was performed essentially as detailed for the synthesis of compound **7**:
Compounds **2** (90 mg, 0.131 mmol) and **6** (64.5 mg, 0.066 mmol) were dissolved in a mixture of MeCN (1 mL) and CH_2Cl_2 (0.7 mL), and activated, powdered molecular sieves (3Å, 200 mg) were added under N_2. The reaction mixture was stirred at room temperature for 5 min and cooled to -45 °C. A mixture of AgO_3SCF_3 (74 mg, 0.289 mmol) in MeCN (1.0 mL) was added and after 5 min, a 2 M solution of MeSBr (0.131 mL) in $ClCH_2CH_2Cl$ was added over a period of 25 min. The reaction mixture was stirred at -45 °C for 4 h and diisopropylamine (0.2 mL) was added. The reaction mixture was stirred for 45 minutes, then the temperature was raised to ~22 °C, and the mixture was filtered, washed with MeCN, and the solvent was removed. The residue was chromatographed (SiO_2, toluene-MeCN 19:1→4:1) to give **7** (88 mg, 83%).
Similar sialylation of compounds **3**, **9**, and **12** with **2** gave **4**, **10**, and **13** in 44%, 64%, and 60% yield, respectively.

Preparation of compounds 5, 11, and 14. Compounds **4**, **10**, and **13** were conventionally acetylated to give **5**, **11**, and **14** in 90%, 91%, and 97% yield, respectively.

Preparation of compound 8. Compound **7** (20 mg, 0.0124 mmol) was treated with 1 M MeONa/MeOH (0.009 mL) at ~22 °C for 4 h. The pH of the reaction mixture was adjusted to 7.0 by addition of Duolite C_{26} H^+ resin. The resin was filtered off and washed with MeOH. The combined filtrate was concentrated and the residue was treated with Ac_2O in pyridine to give the known[3] compound **8** (17 mg, 87%).

Selected ^{1}H-NMR ($CDCl_3$) data: δ Compound **4**: 6.07 (d, 1 H, *J* = 9.3 Hz, NH), 5.88 (t, 1 H, *J* = 9.8 Hz, H-4'), 5.32 (m, 1 H, H-8'), 5.19 (m, 1 H, H-4), 4.94 (m, 1 H, H-8), 3.88, 3.84, 3.79 (s, 3 H each, $COOCH_3$ and OCH_3), 2.78 (dd, 1 H, *J* = 4.9, 12.8 Hz, H-3_{eq}), 0.82 (t, 2 H, *J* = 7.8 Hz, $OCH_2CH_2SiMe_3$). Compound **5**: 6.30 (d, 1 H, *J* = 9.4 Hz, NH), 5.80 (dd, 1 H, *J* = 9.8, 10.8 Hz, H-4'), 5.69 (t, 1 H, *J* = 1.7 Hz, H-7), 5.46 (m, 1 H, H-8'), 5.23 (dt, 1 H, H-8) 3.96, 3.87, 3.78 (s, 3 H each, $COOCH_3$ and OCH_3), 2.87 (dd, 1 H, *J* = 4.7, 12.7 Hz, H-3_{eq}), 0.90-0.75 (m, 2 H, $OCH_2CH_2SiMe_3$). Compound **7**: 5.87 (dd, 1 H, *J* = 9.7, 11.0 Hz, H4"), 5.52 (m, 1 H, H-8"), 4.74 (d, 1 H, *J* = 7.4 Hz, H1'), 4.38 (d, 1 H, *J* = 8.7 Hz, H1), 3.93 (s, 3 H, $COOCH_3$). Compound **10**: 5.92 (dd, 1 H, *J* = 10.0, 10.7 Hz, H-4'), 5.42 (m, 1 H, H-8'), 3.95 (s, 3 H, $COOCH_3$), 1.04 (m, 2 H, $OCH_2CH_2SiMe_3$).

Compound **11**: 5.85 (dd, 1 H, *J* = 9.7, 10.9 Hz, H-4'), 5.51 (d, 1 H, *J* = 3.4 Hz, H-4), 5.48 (m, 1 H, H-8'), 5.19 (dd, 1 H, *J* = 2.0, 8.8 Hz, H7'), 4.93 (dd, 1 H, *J* = 3.6, 10.1 Hz, H-3), 4.74 (dd, 1 H, *J* = 2.0, 10.3 Hz, H-6'), 4.44 (d, 1 H, *J* = 8.2 Hz, H-1), 3.96 (s, 3 H, $COOCH_3$). Compound **13**: 5.78 (dd, 1 H, *J* = 9.6, 10.9 Hz, H-4"), 5.46 (m, 1 H, H8"), 5.07 (dd, 1 H, *J* = 8.0, 9.9 Hz, H2'), 4.84 (dd, 1 H, *J* = 7.3, 9.0 Hz, H-2), 4.56 (d, 1 H, *J* = 8.0 Hz, H-1'), 4.42 (d, 1 H, *J* = 7.3 Hz, H-1), 3.94 (s, 3 H, $COOCH_3$). Compound **14**: 5.73 (dd, 1 H, *J* = 10.9, 5.8 Hz, H-4"), 5.54 (m, 1 H, H-8"), 5.27 (dd, 1 H, *J* = 1.0, 3.6 Hz, H-4'), 4.98 (s, 3 H, $COOCH_3$), 3.04 (d, 1 H, *J* = 10.9 Hz, H-3"), 1.00-0.86 (m, 2 H, $OCH_2CH_2SiMe_3$).
Long-range ^{13}C-^{1}H coupling constants: compound **5**: $J_{C1'\text{-}H3'ax}$ = 6.1 Hz; compound **7**: $J_{C1''\text{-}H3''ax}$ = 6.1 Hz; compound **10**: $J_{C1'\text{-}H3'ax}$ = 6.9 Hz; compound **13**: $J_{C1''\text{-}H3''ax}$ = 6.1 Hz.

Acknowledgments: This work was supported by the Swedish Natural Science Research Council.

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