A Practical Method for the Synthesis of Sialyl α-Glycosides

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Reprinted from Volume 118, Number 35, Pages 8187–8191
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Received January 18, 1996

Abstract: Addition of 2,4-dimethylbenzenesulfonyl chloride to sialic acid glycal gives crystalline 2-chloro-3-thiosialic acid 3 in 85% yield. Reaction of 3 with sodium thiomethoxide in acetonitrile at 0 °C affords the sialic acid donor α-2-(methylthio)-3-thiosialic acid 4 in quantitative yield. Sialylation of glycosyl acceptors 9 and 10 with 4 in the presence of phenylsulfonyl triflate (PST) as promoter in CH3CN at −40 °C gives α-sialosides in good yield and excellent stereoselectivity. No β-sialosides are formed in either case. Removal of the auxiliary 3-(2,4-dimethylphenyl)-thio group is achieved in high yield using Ph3SnH and AIBN in refluxing toluene. Protected GM3 trisaccharide and 6-sial-2-yllactose were obtained on a gram scale.

Introduction

Stereoselective α-sialylation is one of the unsolved problems in carbohydrate chemistry. In this paper we describe a new 2-(methylthio)-3-thiosialic acid donor that is conveniently prepared in two steps from sialyl glycal. We show that when activated with phenylsulfonyl triflate (PST), this donor gives α-sialosides in excellent yield and stereospecificity.

Practical and stereocontrolled chemical syntheses of α-sialosides are important, because sialic acid (Neu5Ac) frequently terminates the oligosaccharide chains of 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 that mediate intercellular recognition. Sialic acids are usually found in the terminal positions of oligosaccharides. The most abundant of these linkages include α2-3 to galactose, α2-6 to galactose or N-acetylgalactosamine, and α2-8 to Neu5Ac. Sialosides have been the subject of extensive research due to their potential as therapeutics, and recent reviews describe synthetic approaches to their synthesis.

Sialic acid possesses a C-3 deoxy structure and exists solely as a 2α (equatorial) glycoside which is less favored in a stereoelectronic sense than a corresponding 2β (axial) glycoside. The C-2 carbon atom of sialic acid, to which sugar residues must be attached in glycosylation reactions, is quaternary and carries an electron-withdrawing carboxylate group; the formation of the carbocation is disfavored sterically and electronically. Classical methods in glycoside synthesis are, therefore, not successful. Donors based on the methyl ester of 2-halosialic acid give poor yield and stereoselectivity. Better stereoselectivity can also be increased with thioglycosides of sialic acid, when these compounds are activated with equimolar amounts of thiophilic reagents in nitro...
solvents at low temperature.\(^7\) A substantial amount of the sialic acid-derived donor undergoes elimination to sialyl glycal during glycosylation; this side reaction leads to modest yields. In practice, an excess of these donors (they are expensive and relatively difficult to prepare) is used in order to obtain reasonable yields.

Sialyl donors that have an auxiliary Br, OH, SPh, or SePh group have recently been introduced,\(^8\) in order to overcome the problem of poor stereoselectivity and glycal formation. These donors give exclusively α-sialosides and do not undergo undesired elimination to sialyl glycal. They, however, have not found wide use in carbohydrate chemistry due to the fact that multistep syntheses are required for their preparation.

Enzymatic synthesis, on the other hand, can be used for multigram synthesis of sialosides. It is, however, generally limited to natural products and their analogs. In most cases sialosides that can be obtained using enzymes are susceptible to hydrolytic cleavage \textit{in vivo} by sialidases. Chemical synthesis offers an advantage that analogs of sialosides can be prepared that are stable to sialidases.

**Results and Discussion**

We reacted sialyl glycal 1 with 2,4-dimethylbenzenesulfonyl chloride (10 equiv) in CH\(_2\)Cl\(_2\) for two weeks at 0 \(^\circ\)C under N\(_2\) and obtained a crystalline chloride, 3, in 85\% yield.\(^5\) The chloride 5 was formed as the only byproduct (4\% yield) and remained in the mother liquor during crystallization (Scheme 1). The ratio of 3 to 5 did not change when the reaction was carried out at ambient temperature. The reaction of chloride 3 with sodium thiomethoxide (1.5 equiv) in acetonitrile at 0 \(^\circ\)C afforded methyl thioglycoside 4 in quantitative yield. Compound 4 was indefinitely stable at 4 \(^\circ\)C.

We prepared acceptors used for sialylation according to

\begin{align*}
\end{align*}
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donor 4. No β-sialosides were detected in either case. Acetylation of 11, and subsequent reductive removal of the auxiliary (2,4-dimethylphenyl)thio group using Ph$_2$SnH (10 equiv)/AIBN (1.2 equiv) in refluxing toluene, afforded 13—protected GM$_3$ trisaccharide—in 91% yield after two steps. The same procedure for 14 afforded protected 6-sial-2-ylactose 16—an element of many gangliosides—in 94% total yield. We applied this method for gram scale syntheses of 13 and 16 without any loss in yield and stereoelectivity.

We identified the site of sialylation in the products 11 and 14 on the basis of observation of a downfield shift of the C-4' proton of their acetylation products. We determined the stereochemistry of the glycosidic linkage in compound 3 to be β and in compounds 4, 11, and 14 to be α by the measurement of the long-range $^{13}$C-$^1$H coupling constants ($J^{13}$C-$^1$H) of the sialic acid residue using the method described by Hori.$^{12}$

Conclusions

Addition of 2,4-dimethylbenzenesulfonyl chloride to sialyl glycal and subsequent substitution of chlorine with sodium thiomethoxide is an effective and economic method for the synthesis of complex sialyl oligosaccharides. We are currently studying the applications of this method for the synthesis of complex sialyl oligosaccharides.

Experimental Section

General Methods. Anhydrous reagents and solvents were prepared according to literature procedures.$^{11}$ Sialic acid was obtained from extraction of edible Chinese swiftlet's nest.$^{14}$ Sialic acid glycal was obtained form Aldrich. Benzenesulfenyl chloride was synthesized according to literature procedures.$^{13}$ Sialic acid was obtained from laboratory Chemicals; Pergamon Press: New York, 1980.


mocher linker was concentrated in vacuo. The residue was distilled in vacuo to give 2 (4.71 g, 75%). bp 58 °C, 0.5 mmHg (lit.$^{16}$ bp 87 °C, 0.5 mmHg).

Methyl 5-Actamidolacto-4,7,8,9-tetra-O-acetyl-2-chloro-3-[2,4-dimethylphenylthio]-2,3,5-trideoxy-o-erythro-2-glucopyranosyl-
nonosanate (4). To a stirred suspension of sialyl glycal (3.76 g, 7.95 mmol) in CH$_2$Cl$_2$ (10 mL) cooled to −10 °C was added dropwise 2,4-dimethylbenzenesulfonyl chloride (5.43 g, 31.5 mmol), and the mixture was the left under N$_2$ in the dark at 0 °C for 14 days. The reaction mixture was loaded on a short silica gel column and eluted with 10% ethyl acetate in CH$_2$Cl$_2$ to give a mixture of chlorides 3 and 5 in a 95:1 ratio as determined by $^1$H NMR. Compound 3 (4.38 g, 85% yield) was obtained in pure form by crystallization of the mixture from CH$_2$Cl$_2$.


(17) It is important to weigh sodium thiomethoxide in a glovebox and to use dry CH$_2$Cl$_2$.
were prepared according to a modified published procedure. Reaction of TMSE-protected lactose 6 with acetone dimethyl acetal and subsequent benzylolation afforded a mixture of acetones 7 and 8, not just 7 as reported. This mixture was separated by column chromatography on silica gel eluting with 10% EtOAc in toluene. Treatment of 7 with 80% aqueous acetic acid gave known acceptor 9 in 70% overall yield. The same reaction of acetone 8 afforded acceptor 10 in 11% overall yield. Compound 10 was crystallized from EtOAc/pentane: Rf = 0.31 (33% EtOAc in hexanes); mp 112–114°C. [α]D = +23.8° (c 1.33, CHCl3). 1H NMR (500 MHz, CD3OD) δ 0.14 (s, 9 H, Si(CH3)3), 0.96–1.07 (m, 2 H, CH2TMS), 2.20–2.38 (br s, 1 H, H-1, H-2), 2.42 (d, J = 9.16 Hz, 1 H, H-14), 3.03 (m, 1 H, H-5S), 3.15 (dd, J = 3.27, 9.29 Hz, 1 H, H-13), 3.41 (br d, J = 2.51, 9.84 Hz, 1 H, H-5T), 3.55–3.66 (m, 4 H, OCH2CH2TMS, H-2a, H-2b, H-5a, H-5b), 3.71 (t, J = 9.00 Hz, 1 H, H-13), 3.74–3.84 (m, 3 H, H-3, H-4a, H-4b), 4.56 (dd, J = 4.06, 10.94 Hz, 1 H, H-6b), 4.58–4.64 (m, 1 H, OCH2CH2Si(CH3)3), 4.71 (s, 1 H, H-14), 4.52 (d, J = 12.13 Hz, 1 H), 4.58 (d, J = 7.83 Hz, 1 H, H-14), 4.78 (d, J = 11.36 Hz, 1 H, H-6b), 4.86 (d, J = 11.79 Hz, 1 H, 4.89 (d, J = 11.46 Hz, 1 H, 4.94 (d, J = 10.82 Hz, 1 H, 5.08 (d, J = 11.51 Hz, 1 H), 5.26 (d, J = 10.82 Hz, 1 H), 7.05–7.32 (m, 19 H, aromatic), 7.39 (d, J = 7.40 Hz, 2 H, aromatic), 7.44 (d, J = 7.60 Hz, 2 H, aromatic), 7.62 (d, J = 7.62 Hz, 2 H, aromatic); HRMS (FAB) calculated for C40H52O12SiNa (M + Na) 591.4116, found 591.4110.

2-(Trimethylsilyl)ethyl 2,3,6-Tri-O-benzyl-2-O-(3,4,6-tri-O-acetyl-2,3,6-tri-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,6-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-(2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-{2,3-di-O-benzyl-4-O-
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1.60, 10.94 Hz 1 H, H-6b), 4.00 (dd, J : 4.24, 10.94 Hz, 1 H, H-6a), 4.06—4.12 (m, 2 H, CH2Ph, H-5′), 4.06—4.12 (m, 3 H, CH2Ph, H-5′, H-1, H-4), 4.60 (bd, J = 11.70 Hz, 2 H, CH2Ph, H-9b), 4.77 (d, J = 12.03 Hz, 1 H, 4.83 (d, J = 11.67 Hz, 2 H, CH2Ph, H-9b), 4.87 (d, J = 12.57 Hz, 1 H, 4.90 (bd, J = 4.53, 11.86 Hz, 1 H, H-4′), 4.94—4.98 (m, 2 H, H-3′), 5.06 (bd, J = 12.00 Hz, 1 H, two CH2Ph), 5.17 (d, J = 7.60 Hz, 1 H, H-1′), 5.33 (d, J = 10.88 Hz, 1 H, H-1′), 5.43 (dd, J = 2.64, 6.88 Hz, 1 H, H-7′), 5.48 (d, J = 3.28 Hz, 1 H, H-4′), 5.56 (ddd, J = 2.63, 5.20, 8.25 Hz, 1 H, H-8′), 7.00—7.65 (m, 25 H, aromatic).

13C NMR (100 MHz, CDCl3) δ -1.45, 18.43, 20.37, 20.69, 20.75, 21.21, 23.13, 37.52, 49.10, 53.02, 62.02, 66.99, 67.24, 67.53, 68.45, 68.60, 68.90, 69.48, 71.31, 72.13, 72.75, 73.09, 73.80, 74.82, 74.92, 74.95, 76.62, 76.69, 77.00, 77.32, 79.42, 81.98, 82.82, 97.26, 102.04, 102.92, 126.95, 127.04, 127.15, 127.27, 127.38, 127.43, 127.58, 127.70, 127.90, 127.99, 128.06, 128.14, 128.18, 138.13, 138.65, 138.72, 139.26, 139.36, 167.82, 169.84, 169.91, 170.27, 170.49, 170.75; MS (FAB) calecd for C43H57NO21SiNa (M + Na) 1430, found 1430.

2-[Trimethylsilyl]ethy 2,3,6-tri-O-benzyl-4-O-[4-O-acetyl-2,3-di-O-benzyl-4-O-[4-O-[ methyl [S-acetamido 4 J $,9-tetra-O-acetyl-3$ dideoxy-D1 ry th rc -s-t- - gluc o -2-nonulopyranosidlonatel -/-o-galactopyranosyl ) -B-n-glucopyranoside (16) was prepared in 98% yield from 15 (730 mg, 0.47 mmol) utilizing the procedure for the preparation of 13: Rr : 0.38 (33% CHTCN in toluene); [al23n : -1.9o (c 2.62, CHCl3); 1H NMR (500 MHz, C6D6) 6 -0.27 (s, 9H, Si(CH3)3), 0.99 (m, 2H, CH2Si(CH3)2), 1.57 (s,3H), 1.58 (s,3H), 1.81 (s,3H), 1.84 (s,3H), 1.95 (s,3H), 2.05 (s, 3H), 2.12 (t, J = 12.73 Hz, 1 H, H-3ax′). 2.80 (dd, J = 4.65, 12.78 Hz, 1 H, H-3eq′), 3.42 (dd, J = 3.49, 9.70 Hz, 1 H, H-3′), 3.44 (s, 3 H, CO2CH2), 3.51 (ddd, J = 4.85, 9.52 Hz, 1 H, H-5′), 3.60 (dd, J = 7.76, 8.90 Hz, 1 H, H-2′), 3.63 (m, 1 H, OCH2CH2TMS), 3.70 (m, 2 H, H-5′, H-6′), 3.78 (t, J = 8.96 Hz, 1 H, H-2′), 3.80 (dd, J = 7.86, 9.50 Hz, 1 H, H-3′), 3.91 (dd, J = 1.46, 10.85 Hz, 1 H, H-6a), 3.99 (dd, J = 4.94, 10.93 Hz, 1 H, H-6b), 4.10 (dd, J = 2.05, 10.23 Hz, 1 H, H-6′), 4.12 (m, 1 H, OCH3CH2TMS), 4.18 (m, 2 H, H-6b′, NH), 4.29 (dd, J = 6.56, 12.35 Hz, 1 H, H-9a′), 4.34 (t, J = 9.52 Hz, 1 H, H-4′), 4.35 (d, J = 11.15 Hz, 1 H), 4.40—4.47 (m, 3 H, CH2CH2Ph, H-5′, H-1), 4.56 (d, J = 12.27 Hz, 1 H, 4.69 (dd, J = 2.63, 12.31 Hz, 1 H, H-9′), 4.79 (d, J = 11.10 Hz, 1 H), 4.82 (d, J = 12.43 Hz, 1 H), 4.83 (d, J = 11.69 Hz, 1 H), 4.84 (d, J = 7.80 Hz, 1 H, H-1′), 4.85 (d, J = 10.33 Hz, 1 H), 4.86 (dt, J = 4.65, 11.91 Hz, 1 H, H-4′), 5.02 (d, J = 11.18 Hz, 1 H), 5.06 (d, J = 11.62 Hz, 1 H), 5.34 (d, J = 11.15 Hz, 1 H), 5.47 (dd, J = 2.25, 7.31 Hz, 1 H, H-7′), 5.74 (d, J = 3.43 Hz, 1 H, H-4′), 5.78 (dd, J = 2.65, 6.87 Hz, 1 H, H-8′), 7.06—7.44 (m, 23 H, aromatic). 7.68 (d, J = 7.36 Hz, 2 H, aromatic); 13C NMR (100 MHz, CDCl3) δ −1.43, 18.49, 20.69, 20.80, 20.82, 20.87, 20.99, 23.19, 37.75, 49.41, 52.87, 62.28, 62.69, 66.19, 67.30, 67.35, 68.49, 68.66, 68.90, 71.28, 71.98, 72.74, 73.05, 74.73, 75.18, 76.68, 77.00, 77.21, 77.32, 79.37, 80.09, 81.92, 82.83, 98.91, 102.12, 103.07, 126.15, 126.47, 127.64, 127.67, 127.93, 128.03, 128.10, 128.20, 128.27, 128.28, 137.86, 138.45, 138.59, 138.74, 139.26, 167.81, 169.62, 170.00, 170.07, 170.19, 170.56, 170.95; MS (FAB) calecd for C43H57NO21SiNa (M + Na) 1430, found 1430.

Acknowledgment. This work was supported by NIH Grant GM30367. The NMR facilities at Harvard were supported by NIH Grants S-10-RR04870-01 and CHE-8814019. The Harvard University Mass Spectrometry Facility was supported by grants from NSF (CHE-9020043) and NIH (SIO-RR067116).

Supporting Information Available: 1H NMR and 13C NMR spectra for compounds 3, 4, 11, 13, 14, and 16; and 1H NMR for compound 10 (13 pages). See any current masthead page for ordering and Internet access instructions.

JA960148B