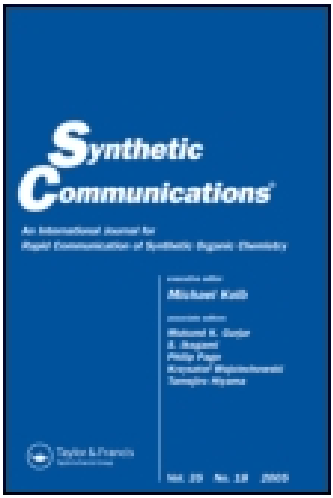

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Selective N-Sulfation of Glucosamine Derivatives using Phenyl Chlorosulfate in Non-Aqueous Solvent

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SELECTIVE *N*-SULFATION OF GLUCOSAMINE DERIVATIVES USING PHENYL CHLOROSULFATE IN NON-AQUEOUS SOLVENT.

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Abstract. The selective *N*-sulfation of 2-amino-2-deoxy-D-glucopyranose derivatives having unprotected hydroxyl groups with phenyl chlorosulfate and triethylamine in anhydrous organic solvent followed by addition of aqueous sodium bicarbonate affords high yields of the 2-deoxy-2-sulfoamino-D-glucopyranose products.

Introduction

Sulfate containing oligosaccharides and glycoconjugates play an important role in biological recognition processes.[1,2] Heparin and heparan sulfate, members of a class of sulfated polysaccharides called glycosaminoglycans (GAGs), are composed of repeating uronic acid and glucosamine residues.[2] The synthesis of heparin and heparan sulfate is complicated, in part, by the need for regiospecific introduction of *O*- and *N*-sulfate into glucosamine residues. The known methods for sulfation of amines include pyridine·SO_3, trimethylamine·SO_3, dioxane·SO_3 and chlorosulfonic acid.[3] In heparin and heparan sulfate oligosaccharides synthesis, selective *N*-sulfation is performed in the final synthetic step using trimethylamine·SO_3 or pyridine·SO_3 in basic water.[4] Only aqueous systems have been used for such selective *N*-sulfation.

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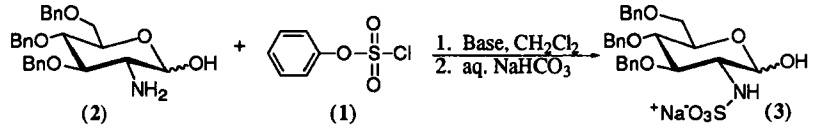
Results and Discussion

In the course of our investigation of new methods for GAG oligosaccharide synthesis, we observed the reaction of phenyl chlorosulfate (chlorosulfuric acid phenyl ester, **1**) and collidine with 2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (**2**) in anhydrous dichloromethane afforded *N*-sulfated product **3** (Figure 1).

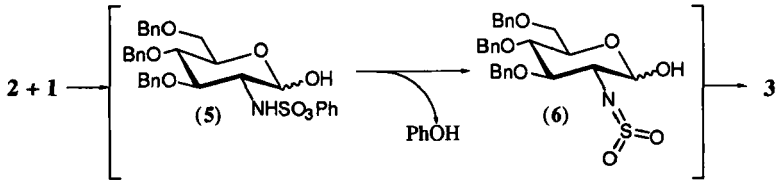
Figure 1.



A review of the literature showed phenyl chlorosulfate (**1**) was first reported as a carbohydrate sulfating reagent for primary hydroxyl groups.[5a,b] Sulfation of the primary hydroxyl groups on monosaccharides and oligosaccharides with phenyl chlorosulfate in the presence of pyridine afforded low yields of the primary sulfates even over extended reaction times. Increasing reaction temperature and the amount of added base gave moderate yields of primary sulfates but also resulted in the sulfation of some secondary hydroxyl groups.[5a-c] Recent reports have shown that a strong base, such as NaH, must be used to generate the alkoxide anion in order to obtain reasonable yields of the phenyl sulfate in the reaction of phenyl chlorosulfate with hydroxyl groups.[6,7] The use of bases such as pyridine or collidine did not promote secondary hydroxyl substitution by phenyl chlorosulfate and there was little evidence for substitution of primary hydroxyl groups under such mild conditions. When 2-napthaleneethanol was treated under the same conditions used for the formation of **3**, (Figure 1) no reaction was observed over 12 h. Since this information suggested that hydroxyl participation was not involved in the *N*-sulfate

formation, we anticipated phenyl chlorosulfate might be useful as a novel *N*-sulfation method.

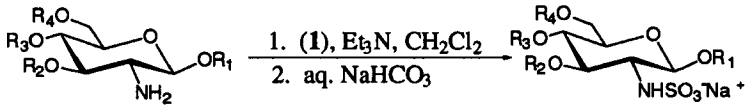
Figure 2.

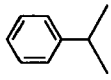


Aryl sulfamidate formation has been reported by DuBois and Stephenson[8] from the reaction of a primary amine with cyclic catechol sulfate. The alkaline hydrolysis of these aryl sulfamidates yields the sulfamic acid (*N*-sulfate) salts. This report is consistent with earlier studies by Williams and Douglas[9] suggesting an *E*1cB mechanism for the alkaline hydrolysis of aryl methylamino sulfamates. The phenyl chlorosulfate reaction described in Figure 1 probably procedes through a similar *E*1cB mechanism in which an initial sulfamidate intermediate (**5**) converts to the sulfonylamine (**6**) under basic conditions (Figure 2). While refluxing potassium hydroxide was used in the hydrolysis of 2-hydroxyphenyl *N*-alkylsulfamidates to give the *N*-alkyl sulfates,[8] the phenyl sulfamidate intermediates prepared in the current study afford the sulfonylamines (**6**) and ultimately *N*-sulfates (**3**) under very mild conditions.

The scope of this reaction was examined by treating four 2-amino-2-deoxy-D-glucopyranose derivatives with phenyl chlorosulfate (see Table). The relatively high yields obtained suggest that this new method should be applicable to a large number of amino sugars. The synthetic utility of sulfated carbohydrates **3**, **11** and **12** for the synthesis GAG oligosaccharides is currently under investigation.

Table. *N*-sulfation with phenyl chlorosulfate (1)



Compound	R_1	R_2	R_3	R_4	(1) eq	Base[a] eq	Reaction Time, h	Product	Yield, %
2	H	Bn	Bn	Bn	1.2	2.6[b]	48	**3**	< 60[b]
					6	20	5	**3**	70
					2	2.2	6[c]	**3**	81
					2	3.8	10	**3**	91
7	TBDPS	Bn	Bn	Bn	2	2.2	12	**10**	96
8	CH_3	H			2	4	6	**11**	95
9	CH_3	Bn	H	Bn	2	2.2	8	**12**	91

[a]All reactions used triethylamine as base except where noted. [b]Collidine was used as the base and the product was isolated from the incomplete reaction for characterization. [c]Phenyl chlorosulfate was initially stirred with the amine for 1.5 h before addition of base.

Experimental

General methods. Phenyl chlorosulfate (**1**) was prepared as previously reported.[6] Methyl 3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside and methyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside were purchased from Toronto Research Chemicals. All other reagents and solvents were of reagent grade and were dried using standard procedures.

All reactions were monitored by thin layer chromatography on aluminum sheets, silica gel 60 F_{254} (Merck); detection under short wavelength UV light (254 nm) or by dipping the plates into staining solution (1.0 g cerric ammonium sulfate

and 24.0 g ammonium molybdate in 31 mL sulfuric acid, 470 mL water) then heating. Flash chromatography was performed using 230-400 mesh silica gel 60 (Aldrich). Optical rotations were measured with a Perkin Elmer 141 polarimeter at 22°C. ^{1}H NMR spectra were recorded at 25°C on a Varian Unity 500 MHz spectrometer and chemical shifts are given in ppm from tetramethylsilane as internal standard. The NMR solvent, 10% CD_3OD in $CDCl_3$, was used when micelle formation resulted in signal broadening in a single solvent system. Mass spectra were obtained using a VG ZAB-HF instrument (VG Analytical, Inc.) in the fast atom bombardment (FAB) ionization mode using a Xenon beam. Triethanolamine or thioglycerol was used as the matrix.

2-Amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (2). 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose[10] (4.5 g, 9.6 mmol) was dissolved in pyridine-water, 3:1 (100 mL). H_2S gas was bubbled through the stirring solution for 8 min. The flask was sealed with a rubber septum and stirred at room temperature. After 48 h the dark brown reaction mixture was repeatedly evaporated with toluene azeotrope until a crude yellow mass remained. Flash chromatography (ethyl acetate/hexanes v/v 1:1 → 2:1 → chloroform/methanol v/v 40:1) afforded **2** (2.0 g). Additional impure fractions were combined and evaporated and re-chromatographed (chloroform/methanol v/v 36:1) to give combined yield **2** (3.1 g, 72%) as a white solid.[11] $[\alpha]_D$ = +75° (c = 1, $CHCl_3$); HRMS: Calcd for $C_{27}H_{31}N_1O_5$ $[m+Li^+]^+$ 456.2362; Found 456.2368 (FABMS required LiI in matrix for stability). Anal. Calcd for $C_{27}H_{31}N_1O_5$ (449.5) C 72.14 H 6.95 N 3.12; Found C 71.84 H 7.22 N 3.01.

***tert*-Butyldiphenylsilyl 2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (7).** *tert*-Butyldiphenylsilyl 2-azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (0.48 g, 0.67 mmol) and 1,1'-azobis(cyclohexane

carbonitrile) (cat) were combined in 3.8 mL anhydrous toluene under an argon atmosphere and heated to 95°C. After 4 h the solution was cooled to room temperature and evaporated. Purification by flash chromatography (ethyl acetate/petroleum ether v/v 1:10 → 1:6) afforded **7** (0.32 g, 70%) as a colorless oil. $[\alpha]_D$ = -8° (c = 1, $CHCl_3$); – ^{1}H NMR ($CDCl_3$) δ 1.10 (s, 9H, $C(CH_3)_3$), 2.99 (dd, 1H, $J_{1,2}$ 8 Hz, H-2), 3.08 (m, 1H, H-5), 3.32 (dd, 1H, $J_{3,4}$ 9 Hz, H-3), 3.40 (dd, 1H, $J_{5,6a}$ 2 Hz, $J_{6a,6b}$ 11 Hz, H-6a), 3.60 (dd, 1H, $J_{5,6b}$ 4 Hz, H-6b), 3.70 (dd, 1H, $J_{4,5}$ 10 Hz, H-4), 4.36 (d, 1H, $J_{1,2}$ 8 Hz, H-1), 4.30-4.92 (6H, 3 × CH_2Ph), 7.19-7.73 (25H, 5 C_6H_5); HRMS: Calcd for $C_{43}H_{49}N_1O_5Si_1$ $[m+H^+]^+$ 688.3458; Found 688.3456. Anal. Calcd for $C_{43}H_{49}N_1O_5Si_1$ (688.0) C 75.07 H 7.18 N 2.04; Found C 74.79 H 7.19 N 2.23.

Methyl 2-amino-4,6-*O*-benzylidene-2-deoxy-β-D-gluco pyranoside (8). Methyl 4,6-*O*-benzylidene-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (100 mg, 0.24 mmol) in methanol (4 mL) containing hydrazine (0.5 mL) and water (0.2 mL) was heated at 65°C for 1 h. The solution was allowed to cool to room temperature and evaporated. The residue was taken up in CH_2Cl_2 (25 mL) and transfered to a separatory funnel containing 25 mL cold water. The organic layer was separated and the aq. layer washed with CH_2Cl_2 (2 × 20 mL). Combined organics were dried over Na_2SO_4, evaporated, and purification by flash chromatography $CHCl_3/CH_3OH$ v/v 10:1 afforded **8** (63 mg, 94%) as an amorphous white solid. $[\alpha]_D$ = -72° (c = 1, Me_2SO) Lit[12] $[\alpha]_D^{25}$ = -73.9 (c = 1.1, Me_2SO). ^{1}H NMR was consistant with that previously reported.[12]

Methyl 2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (9). Removal of the *N*-phthalimido group from methyl 3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside was performed following the procedure for **8**. Purification by flash chromatography $CHCl_3/CH_3OH$ v/v 40:1 afforded

chromatographically pure **9** in 60% yield as a colorless oil. $[\alpha]_D$ = -16° (c = 1, $CHCl_3$); – ^1H NMR ($CDCl_3$) δ 2.81 (dd, 1H, $J_{2,3}$ 9 Hz, H-2), 3.34 (dd, 1H, $J_{3,4}$ 9 Hz, H-3), 3.49 (m, 1H, H-5), 3.51 (s, 3H, CH_3), 3.71-3.82 (m, 3H, H-4,6a,6b), 4.12 (d, 1H, $J_{1,2}$ 8 Hz, H-1), 4.55-4.98 (4H, 2 × CH_2Ph), 7.25-7.39 (10H, 2 C_6H_5); HRMS: Calcd for $C_{21}H_{27}N_1O_5$ $[m+H^+]^+$ 374.1968 Found 374.1963.

General Procedure: To a solution of the appropriate amine (30-100 mg, 1 eq) in anhydrous dichloromethane (0.08 - 0.1 M) phenyl chlorosulfate and base (see table for ratios) was added with stirring under dry argon at 0°C. After 5 min the ice bath was removed and stirring continued at room temperature until TLC showed disappearance of starting material . The reaction mixture was cooled to 0°C and 1 to 2 vol of saturated aq. $NaHCO_3$ was added. The ice bath was removed after 5 min and the mixture stirred vigorously for an additional 30 min at room temperature.

Purification: Method A. Compounds **3** and **10**. The reaction mixture was transferred to a separatory funnel and brought to final volume (10 mL) of equal portions dichloromethane and saturated aq. $NaHCO_3$. The organic layer was separated and the aqueous layer washed with dichloromethane (2 × 5 mL). The combined organic layers were dried over anhydr. Na_2SO_4, filtered and evaporated. Products were purified by flash chromatography $CHCl_3$-CH_3OH (4:1 for compound **3** and 6:1 for compound **7**). **Method B.** Compounds **11** and **12**. The reaction mixture was transferred to a separatory funnel and brought to final volume (10 mL) of equal portions of dichloromethane and saturated aq. $NaHCO_3$. The aqueous layer was separated and the organic layer washed with water (2 × 5 mL). The combined aqueous layers were evaporated with repeated toluene azeotrope (bath temperature kept below 35°C). The residue was dried overnight under high vacuum, scraped and crushed, and repeatedly extracted with

dichloromethane (compound **11**) or methanol (compound **12**) until extraction of organic soluble material was complete. The combined organics were filtered, evaporated and purified by flash chromatography, $CHCl_3$-CH_3OH (4:1).

After evaporation, compounds **3**, **11**, and **12** were taken up in water and lyophilized to give amorphous white solids. Compound **10** evaporated to a colorless glass.

2-Deoxy-3,4,6-tri-*O*-benzyl-2-sulfoamino-α-D-glucopyranose sodium salt (**3**). – ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 3.43 (dd, 1H, $J_{2,3}$ 10 Hz, H-2), 3.56 (dd, 1H, H-4), 3.64 (dd, 1H, $J_{6a,5}$ 2 Hz, $J_{6a,6b}$ 11 Hz, H-6a), 3.71 (dd, 1H, $J_{6b,5}$ 4 Hz, H-6b), 3.75 (dd, 1H, $J_{3,4}$ 10 Hz, H-3), 4.02 (m, 1H, H-5), 4.45-5.00 (6H, 3 × CH_2Ph), 5.50 (d, 1H, $J_{1,2}$ 4 Hz, H-1), 7.10-7.40 (15H, 3 C_6H_5); $[\alpha]_D$ = +34° (c = 1, CH_3OH); FABMS: m/z 528 $[M\text{-}Na^+]^-$. Anal. Calcd for $C_{27}H_{30}N_1O_8S_1Na_1 \cdot 1H_2O$ (569.6) C 56.93 H 5.66 N 2.46 S 5.81; Found C 56.76 H 5.69 N 2.39 S 6.14.

***tert*-butyldiphenylsilyl 2-deoxy-3,4,6-tri-*O*-benzyl-2-sulfo amino-β-D-glucopyranoside sodium salt** (**10**). – ^{1}H NMR (10% CD_3OD in $CDCl_3$) δ 1.10 (s, 9H, *t*-bu), 3.17 (m, 1H, H-5), 3.43-3.47 (m, 2H, H-2,6a), 3.52 (dd, 1H, $J_{6a,6b}$ 11 Hz, $J_{6b,5}$ 4 Hz, H-6b), 3.67-3.74 (m, 2H, H-3,4), 4.67 (d, 1H, $J_{1,2}$ 7 Hz, H-1), 4.31-5.06 (6H, 3 × CH_2Ph), 7.10-7.80 (25H, 5 C_6H_5); $[\alpha]_D$ = -5° (c = 2, $CHCl_3$); FABMS: m/z 767 $[M\text{-}Na^+]^-$. Anal. Calcd for $C_{43}H_{48}N_1O_8S_1Si_1Na_1$ (790.0) C 65.32 H 6.12 N 1.77 S 4.06; Found C 64.95 H 6.29 N 1.76 S 3.80.

Methyl 4,6-*O*-benzylidene-2-deoxy-2-sulfoamino-β-D-gluco pyranoside sodium salt (**11**). – ^{1}H NMR (CD_3OD) δ 3.13 (dd, 1H, $J_{2,3}$ 8 Hz, H-2), 3.45 (m, 1H, H-5), 3.50 (s, 3H, CH_3), 3.54 (dd, 1H, H-4), 3.77 (dd, 1H, $J_{5,6a}$ 10 Hz, $J_{6a,6b}$ 10 Hz, H-6a), 3.94 (dd, 1H, $J_{3,4}$ 9 Hz, H-3), 4.28 (dd, 1H, $J_{5,6b}$

5 Hz, H-6b), 4.46 (d, 1H, $J_{1,2}$ 8 Hz, H-1), 5.58 (s, 1H, benzylidene CH), 7.30-7.50 (5H, C_6H_5); $[\alpha]_D$ = -44° (c = 0.7, CH_3OH); HRFABMS: Calcd for $C_{14}H_{18}N_1S_1O_8[m\text{-}Na^+]^-$ 360.0753 Found 360.0753

Methyl 3,6-di-*O*-benzyl-2-deoxy-2-sulfoamino-β-D-gluco pyranoside sodium salt (12). – ^1H NMR (10% CD_3OD in $CDCl_3$) δ 3.11 (dd, 1H, $J_{2,3}$ 10 Hz, H-2), 3.47 (m, 1H, H-5), 3.53 (s, 3H, CH_3), 3.58 (dd, 1H, H-4), 3.69-3.73 (m, 2H, H-3,6a), 3.81 (dd, 1H, $J_{6a,6b}$ 11 Hz, $J_{6b,5}$ 3 Hz, H-6b), 4.5 (d, 1H, $J_{1,2}$ 8 Hz, H-1), 4.59 (s, 2H, CH_2Ph), 4.91 (dd, 2H, CH_2Ph), 7.2-7.5 (10H, 2 C_6H_5); $[\alpha]_D$ = -3° (c = 1, CH_3OH); FABMS: m/z 452 $[M\text{-}Na^+]^-$. Anal. Calcd for $C_{21}H_{26}N_1S_1O_8Na_1 \cdot 1H_2O$ (493.5) C 51.11 H 5.72 N 2.84 S 6.51; Found C 51.27 H 5.53 N 2.80 S 6.90.

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