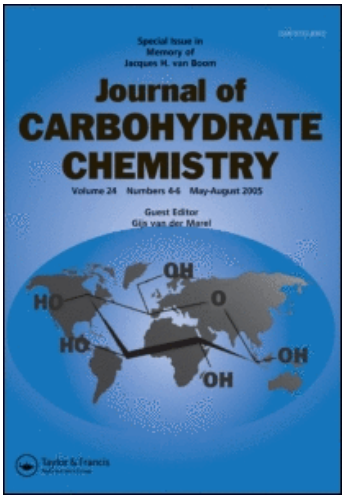

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Synthesis of the 3-*0*, 4-*0* and 6-*0* Sulfates of Methyl 2-amino-2-deoxy-α-*D*-Glucopyranoside

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SYNTHESIS OF THE 3-O, 4-O AND 6-O SULFATES OF METHYL 2-AMINO-2-DEOXY-α-D-GLUCOPYRANOSIDE

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ABSTRACT

Starting with methyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (1), the isomeric methyl 2-amino-2-deoxy-α-D-glucopyranoside 3-, 4-, and 6-sulfates have each been prepared by sulfation of suitably blocked intermediates. Tritylation and acetylation of 1 followed by detritylation gave methyl 3,4-di-O-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (3), having a free 6-hydroxyl group. Base catalyzed O-4 ⟶ O-6 acetyl migration provided the corresponding 3,6 di-O-acetyl derivative (4) posessing a free 4-hydroxyl group. Preparation of methyl 4,6-O-benzylidene-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (9) provided the intermediate bearing a free 3-hydroxyl group. O-sulfation of 3, 4 and 9 was effected with the pyridine sulfur trioxide complex in dry pyridine.

INTRODUCTION

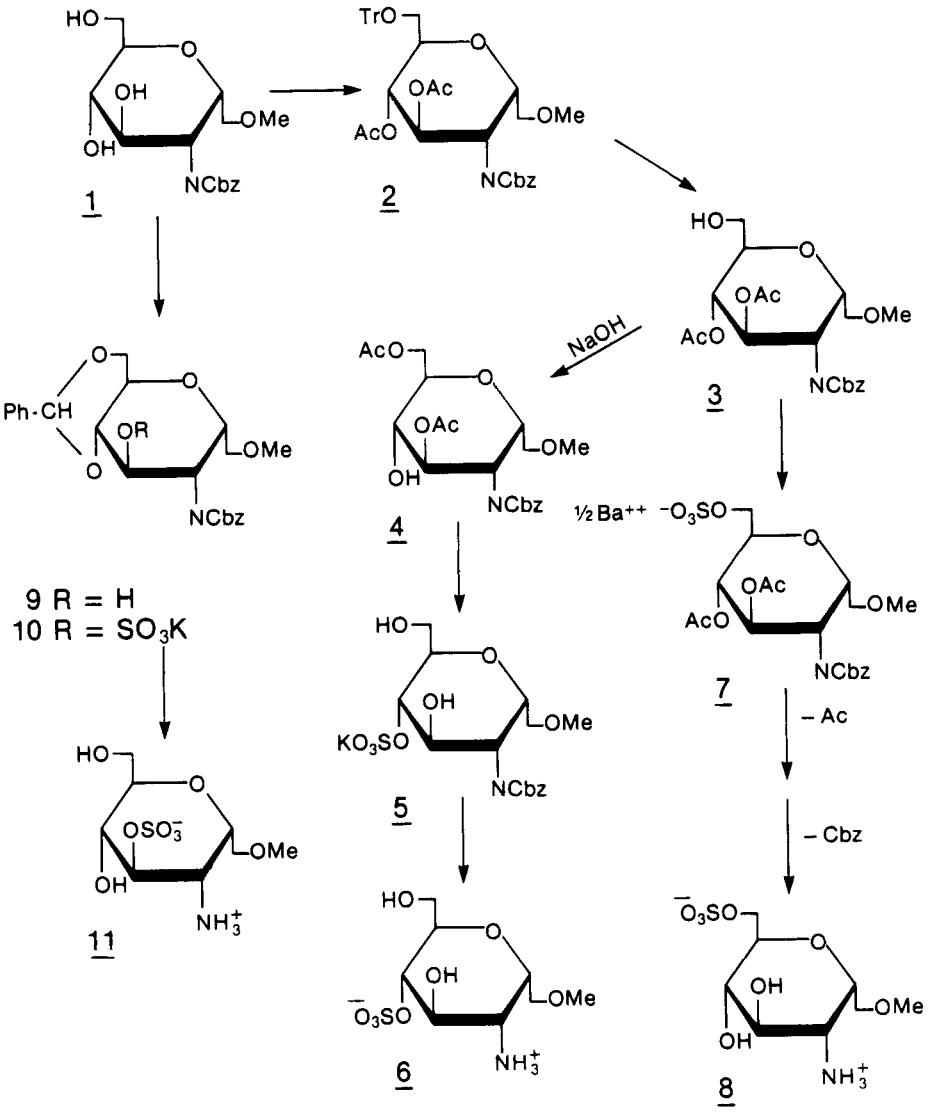
In seeking to develop a convenient and sensitive assay for a mammalian sulfamidase essential for the catabolism of heparin, O,N-disulfated derivatives of α-methylglucosaminide were prepared starting with direct sulfation of methyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (1). After removing the benzyloxycarbonyl blocking group, the nitrogen was sulfated with ^{35}S labeled pyridine sulfur trioxide complex in aqueous medium. When the resulting mixture of putative substrates was tested, no hydrolysis of the N-sulfate group was detected. Instead, the presence of a novel sulfohydrolase was revealed. By means

of the isomeric α-methylglucosaminide sulfates whose definitive synthesis is described herein, it was established that this new enzyme is a 3-O sulfohydrolase with the special requirement that the 2-amino group also be sulfated.[1] Such a 3-O,N disulfated glucosamine residue was subsequently shown, with the aid of the title compounds, to be an essential element of the antithrombin binding sequence of heparin.[2,3] Sugar sulfates are found most abundantly in glycosaminoglycans, but are also widely distributed in mucins, glycolipid sulfatides and glycoproteins. These synthetic sugar sulfates have been useful in studying cell surface binding[4] and, particularly after conversion to the corresponding tritiated anhydromannitol sulfates, in establishing the structure of the glycoprotein sugar sulfates.[5]

RESULTS AND DISCUSSION

The scheme for the sulfation of specific hydroxyl groups is shown in Fig. 1. Methyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (1) was prepared by the procedure of Fischer as described by Neuberger and Pitt-Rivers[6] except that neutralization and removal of chloride were achieved by titration with ion exchange resin AG-1 (OH^- form). The specific rotation of various preparations ($+94^o$– $+99^o$) was higher than reported therein, ($+80^o$), but close to the maximum value reported elsewhere[7] ($+100^o$). Tritylation and acetylation in the ususal manner provided essentially pure methyl 3,4-di-O-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-6-triphenylmethyl-α-D-glucopyranoside (2) after one recrystallization. Preparative TLC was employed to remove a persistant trace-contaminant revealed by TLC in solvent E. Detritylation with HBr in glacial acetic acid[8] resulted in fewer side products than when BF-methanol complex[9] was employed. The product, methyl 3,4-di-O-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (3), crystallized readily as sheaves of blunt needles by the addition of petroleum ether to a solution of the crude product in chloroform.

Conversion of 3 to 4, was achieved by base-catalyzed (0.6 mM NaOH in 95% ethanol), acetyl-group migration from O-4 to O-6.[10,11] The reaction, allowed to proceed at room temperature for 3-5 hours until a contaminant of very low Rf appeared (TLC; solvent B), was stopped by the addition of 0.2M acetic acid. The ratio of the desired 3,6- to the 3,4-di-O-acetyl derivative varied from 7:3 to 9:1. After evaporation of



SCHEME 1

the solvent, the 3,6 di-O-acetyl derivative 4, was crystallized as clusters of rods by the addition of petroleum ether to a solution of the crude product in ether. A trace of 3 as a contaminant in the crystalline product could be removed by column chromatography on Silica 60 or by preparative TLC. As expected, the product could not readily be tritylated.

Methyl 2-(benzyloxycarbonyl)amino-2-deoxy-4,6-O-benzylidene-α-D-glucopyranoside (9), bearing a free 3-hydroxyl group, was prepared by the addition of 1 to the zinc chloride-benzaldehyde complex as suggested by Hall.[12] The product was obtained as soft, hair-like crystals from boiling methanol.

Sulfation of hydroxyl groups in positions 3,4 or 6 was carried out in anhydrous pyridine employing a 20-30% excess of a pyridine:SO_3 complex as the sulfating agent. Sulfation of 3 at 50-60 °C gave the barium salt of methyl 3,4-di-O-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside 6-sulfate (7) which readily crystallized from methanol as fine needles in 50-60% yield. The barium salt was deacetylated with 0.1 molar equivalents of sodium methoxide in methanol. The benzyloxycarbonyl group was then removed by hydrogenation in glacial acetic acid in the presence of 10% palladium on charcoal. Removal of acetic acid by distillation and of cations by passage through a column of sulfonic acid resin gave crystalline amphoteric methyl 2-amino-2-deoxy-α-D-glucopyranoside 6-sulfate (8) as sheaves of needles from a concentrated aqueous solution by the addition of methanol.

Sulfation of the 3,6-di-O-acetyl derivative 4 was carried out in similar fashion, except that barium was exchanged for potassium prior to catalytic deacetylation with sodium methoxide in methanol. The potassium salt of methyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside 4-sulfate (5) crystallized spontaneously from the methanolic solution. The crystals appear to be needles but are actually very thin rectangular plates. The benzyloxycarbonyl blocking group was removed by catalytic hydrogenation providing methyl 2-amino-2-deoxy-α-D-glucopyranoside 4-sulfate (12) which crystallized as rosettes of blunt rods from a concentrated aqueous solution upon addition of methanol.

Sulfation of the benzylidene derivative (9) followed by exchange of barium for potassium as described above gave the potassium salt of methyl-2-(benzyloxycarbonyl)amino-2-deoxy-4,6-O-benzylidene-α-D-glucopyranoside 3-sulfate (10) which crystallized readily from 95% alcohol. The subsequent removal of the benzylidene group was best effected by autohydrolysis: Compound 10, dissolved in 50% methanol, was passed through a column of sulfonic acid resin and the resulting solution of the free acid warmed to 60 °C. The hydrolysis, monitored by TLC in solvent C, was complete in 30 minutes. Although the product appeared to be homo-

geneous, repeated attempts to crystallize the potassium salt of methyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside 3-sulfate were unsuccessful. However, after removing the liberated benzaldehyde by ether extraction, when the benzyloxycarbonyl group was removed as described above, crystalline methyl 2-amino-2-deoxy-α-D-glucopyranoside 3-sulfate (11) was obtained as thin rectangular plates by the addition of alcohol to an aqueous concentrate.

EXPERIMENTAL

General Procedures. Melting points were determined with a hot stage apparatus and are uncorrected. Optical rotations were determined in 1 dm tubes with a Perkin-Elmer automatic polarimeter, Model 241 MC. Thin layer chromatography (TLC) was performed on silica gel 60, 0.2 mm thick on aluminum (E. Merck, Darmstadt, Germany). Preparative TLC was performed on precoated glass plates, 2 mm thick and containing a fluorescent indicator (Brinkmann Instruments, U.S.A.). Slurry-packed columns were prepared from silica gel 60, 0.05-0.2 mm (Brinkmann Instruments, U.S.A.). Detection of compounds on aluminum plates was effected by heating on a hot plate after spraying with 5% sulfuric acid in ethanol. Tritylated compounds were revealed as yellow spots before heating.

TLC was performed with the following solvents: A, ethyl acetate-benzene 3:7 B, ethyl acetate-benzene 1:1 C, 1-butanol-acetic acid-water 10:3:7 D, isobutyric acid-0.5M NH_4OH 5:3 E, ethyl acetate-benzene 1:7. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee or by Atlantic Microlab, Atlanta, Georgia.

Pyridine was distilled over barium oxide. Benzaldehyde was washed with saturated sodium carbonate, dried and distilled. Zinc chloride was freshly fused and ground. Solvents were stored over molecular sieves 3Å or 4Å (Fisher Scientific). Evaporations were conducted under reduced pressure at less than 40 °C. Ion exchange resins (AG 1x8 OH^-, AG 50W x8 H^+, K^+) were from Bio-Rad.

Methyl 3,4-Di-O-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-6-triphenylmethyl-α-D-glucopyranoside (2). To a solution of 1 (19 g) in dry pyridine (56 mL), triphenylmethyl chloride (17 g) and Drierite (2 g) were added. The mixture was heated to 100 °C and after 45 min, acetic anhydride (31.4 mL) was added in one portion. After standing overnight overnight at room temperature, the drierite and crystals of pyridinium

chloride were removed by filtration and the filtrate added dropwise to one liter of vigorously stirred ice-water containing sufficient acetic acid to neutralize all of the excess pyridine. The granular precipitate of crude 2 was resuspended in ice water and filtered. It was then dissolved in ethyl ether (400 mL), and the ether was extracted twice with saturated sodium bisulfate (20 mL) and saturated sodium bicarbonate (the final wash should be alkaline). Drying (sodium sulfate) and concentration in vacuo gave a residual oil which crystallized from 95% ethanol. After one recrystallization from absolute ethanol, the product 2 (30 g, 75%) contained a trace impurity, with an Rf 0.1 in solvent A. Preparative chromatography of 300 mg of 2 on silica coated plates (SIL G 200 uv-254, 2mm thick) removed this impurity. Compound 2 exhibited unusual melting characteristics. It melted partially at 96-98 °C, resolidified between 100 °C and 105 °C, to melt sharply at 115 °C; $[\alpha]_D$ +90.6° (c 1, chloroform).

Anal. Calcd for $C_{38}H_{39}NO_9$: C, 69.81; H, 6.02; N, 2.14. Found: C, 69.89; H, 6.02; N, 2.06.

Methyl 3,4-Di-O-acetyl-2-(benzyloxycarbony)amino-2-deoxy-α-D-glucopyranoside (3). To a stirred solution of 2 (10 g) in glacial acetic acid (27 mL) at room temperature, HBr (3.4 mL, 5N in glacial acetic acid) was added in one portion and after 90 sec the mixture was rapidly filtered into a Buchner flask containing ice (70 g) and sodium bicarbonate (20 g). After frothing had ceased, more sodium bicarbonate (10 g) was added and the solution was extracted with chloroform (300 mL). The extract was washed sequentially with saturated sodium bicarbonate and water, dried (sodium sulfate) and concentrated to a slightly turbid solution (15 mL). The dropwise addition of petroleum ether initially clarified this solution then led to renewed turbidity. The product 3 crystallized spontaneously as sheaves of blunt needles (6 g, 95%). Trace impurities, revealed by TLC in solvent A were eliminated by recrystallization from the same solvents, mp 98-99 °C, $[\alpha]_D$ +102.3° (c 3.6 chloroform).

Anal. Calcd for $C_{19}H_{25}NO_9$: C, 55.47; H, 6.13; N, 3.40. Found: C, 55.73; H, 6.13; N, 3.30.

Methyl 3,6-Di-O-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (4). Acetyl migration from O-4 to O-6 was monitored by TLC in solvent B, in which 3 and 4 migrate with Rf values, respectively, of

0.42 and 0.57. A solution of 3 (7.1 g) in 0.2 mM sodium hydroxide in 95% alcohol (250 mL) was stirred at room temperature for 5-8 h until a contaminant (Rf of approximately 0.07) appeared. The ratio of 4 to 3 varied from 7/3 to 9/1. The reaction mixture was acidified by the addition of acetic acid (2N, 0.075 mL) and the solvent evaporated to leave a colorless oil. Residual water was removed by repeated azeotropic distillation with ether. Addition of petroleum ether to the ethereal solution (10-15 mL) then caused crystallization of impure 4 (5.3 g, 75%). A persistant contaminant (3) and an uncharacterized contaminant of low Rf were removed by column chromatography: a solution of 4 (3.5 g) in chloroform was applied to a column (32 x 2.5 cm) of Silica Gel 60 (0.05-0.2 mm) and eluted with solvent B. Appropriate fractions were combined and the product crystallized from chloroform by the addition of petroleum ether to give contaminant-free 4 (3 g): mp 99-100 °C, $[\alpha]_D$ +58.7° (c 2.1 chloroform).

Anal. Calcd for $C_{19}H_{25}NO_9$: C, 55.47; H, 6.13; N, 3.40. Found: C, 55.38; H, 6.23; N, 3.30.

Methyl 4,6-O-Benzylidene-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (9) To dry benzaldehyde (27 mL), freshly fused and ground zinc chloride (8 g) was added and the mixture shaken for fifteen min. To this gelatinous gray paste, 1 (9 g) was added and the mixture shaken for 56 h on a mechanical shaker. The mixture was transfered to a separatory funnel with the aid of petroleum ether and ice-water (1:1) and shaken vigorously. The precipitate obtained was washed sequentially four times with petroleum ether and ice-water. Crystallization from boiling methanol gave 9 (8 g, 70%). Recrystallization of a portion gave the analytical sample. The powder sublimes at approximately 185-190 °C to form clusters of needles which melt sharply at 214-215 °C; $[\alpha]_D$ +47° (c 0.97, chloroform).

Calcd for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.60; H, 6.12; N, 3.41.

Barium Methyl 3,4-Di-O-acetyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside 6-sulfate (7). To a solution of 3 (1.1 g) in dry pyridine (6 mL), Drierite (250 mg) and pyridine:SO_3 complex (600 mg) were added and the mixture stirred for one h at 50 °C and then at room temperature. After two h, TLC in solvent A, in which the sulfated product does not migrate, indicated that sulfation was complete. The reac-

tion mixture was diluted with water (20 mL) and after removing the Drierite, made alkaline (phenolphthalein) with saturated barium hydroxide. The resulting suspension was repeatedly concentrated under reduced pressure, adding water and barium hydroxide solution as required to maintain alkalinity, until the pyridine had been removed. Barium sulfate was removed by centrifugation and the precipitate extracted with water. The supernatant fluid and combined washings were concentrated to dryness and the residue taken up in methanol (3-4 mL). Addition of two volumes of ether produced fine needles of 7 (900 mg, 60%), $[\alpha]_D$ +94.6° (c 1.2, chloroform).

Anal. Calcd for $C_{38}H_{48}N_2O_{24}S_2Ba$: C, 40.81; H, 4.33; N, 2.51; S, 5.73. Found: C, 40.89; H, 4.05; N, 2.37; S, 5.77.

Methyl 2-Amino-2-deoxy-α-D-glucopyranoside 6-sulfate (8). To a solution of 7 (1.8 g) in methanol (10 mL), sodium methoxide (17 mg) was added and the mixture was left overnight at room temperature. The copious precipitate of deacetylated barium salt was converted to the potassium salt by passage of its aqueous solution through a sulfonic acid ion-exchange resin (2 mL) in acid form followed by titration with potassium hydroxide to pH 7.5. The solution obtained was lyophilized, the crude deacetylated potassium salt taken up in glacial acetic acid (45 mL) and the benzyloxycarbonyl group removed by hydrogenation in the hood for two h in an open beaker in the presence of palladium on charcoal (10%, 200 mg). The filtered reaction mixture was concentrated under diminished pressure to remove the acetic acid and an aqueous solution of the product passed through a sulfonic acid resin (3.5 mL) to remove all cations. The slightly yellow effluent was decolorized with charcoal and concentrated to approximately 0.5 mL. The addition of two volumes of ethanol provided rectangular crystals of 8 (400 mg, 66%). Recrystallization gave the analytical sample of 8: mp 192-194 °C, $[\alpha]_D$ +129.6° (c 1.4 water).

Anal. Calcd for $C_7H_{15}NO_8S$: C, 30.77; H, 5.53; N, 5.13; S, 11.73. Found: C, 30.87; H, 5.74; N, 5.14; S, 11.41.

Potassium Methyl 2-(Benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside 4-sulfate (5). To a solution of 4 (2.5 g) in dry pyridine (10 mL), pyridine:SO_3 complex (1.2 g) was added and the mixture stirred for 70 min at 50-60 °C. The mixture was worked up to remove inorganic sulfate and pyridine as described for the preparation of 7. After repla-

cing barium with potassium by passage through sulfonic acid resin (18 mL) and titration with KOH (1 N) to pH 7.5, the solution was taken to dryness under reduced pressure. Residual water was removed by repeated evaporations of added methanol. The potassium salt was taken up in methanol (40 mL) and deacetylated by the addition of sodium methylate (32 mg). The product crystallized spontaneously and after chilling provided 5 (2.34 g, 86%). Recrystallization of a portion from ethanol (95%) gave the analytical sample, mp 196-197 °C, $[\alpha]_D$ +83.2° (c .97, water).

Anal. Calcd for $C_{15}H_{20}KNO_{10}S \cdot H_2O$: C, 38.72; H, 4.77; N, 3.01; S, 6.88. Found C, 38.76; H, 4.77; N, 3.03; S, 6.85.

Methyl 2-Amino-2-deoxy-α-D-glucopyranoside 4-sulfate (6). A solution of 5 (495 mg) in glacial acetic acid (4 mL) was hydrogenated in the presence of palladium on charcoal (10%, 50 mg) for 90 min as described above. The mixture was diluted with water, filtered, and the acetic acid removed by distillation at diminished pressure. Potassium was removed by passage through a sulfonic acid resin (2 mL). The addition of methanol to an aqueous concentrate (0.3 mL) gave crystalline 6 (250 mg, 82%). Two recrystallizations from water-methanol gave the analytical sample: mp 208-210 °C, $[\alpha]_D$ +148° (c 0.66%, water).

Anal. Calcd for $C_7H_{15}NO_8 \cdot \frac{1}{2}H_2O$: C, 29.78; H, 5.71; N, 4.96 S, 11.36. Found: C, 29.85; H, 5.75; N, 4.93; S, 11.35.

Potassium Methyl 4,6-O-Benzylidene-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside 3-sulfate (10). To a solution of 9 (3.8 g) in dry pyridine (24 mL), Drierite (300 mg) and pyridine:SO_3 complex (1 g) were added and the mixture stirred at 50 °C for 90 min and then at room temperature overnight. Inorganic sulfate and pyridine were removed as described for 7. The barium sulfate was extracted with methanol (75%) and the extracted barium salt converted to the potassium salt by passage through a column (1.4 x 13 cm) of sulfonic acid resin in potassium form. Chilling overnight provided thin long needles of 10 (3.1 g, 65%). Recrystallization from ethanol (95%) provided the analytical sample, mp 192-193 °C, $[\alpha]_D$ 0° (c 2, water).

Anal. Calcd for $C_{22}H_{24}NO_{10}SK$: C, 49.52; H, 4.54; N, 2.62; S, 6.00; K, 7.33. Found: C, 49.46; H, 4.69; N, 2.74; S, 6.10; K, 7.33.

Methyl 2-Amino-2-deoxy-α-D-glucopyranoside 3-sulfate (11). A solution of 10 (1.1 g) in 50% methanol (5 mL) was passed through a column

(1x4 cm) of sulfonic acid resin and warmed to 60 °C. Hydrolysis of the benzylidene group was monitored by TLC in solvent C, in which 10 exhibits an Rf of 0.75 and the product an Rf of 0.55, and was complete in less than thirty minutes. Benzaldehyde was extracted with ether and the aqueous layer concentrated under diminished pressure to 40 mL. To one half of this material (20 mL), palladium-on-charcoal (10%, 50 mg) was added and the benzyloxycarbonyl group removed by hydrogenation for 60 min as described above for compound 8. The catalyst was removed by filtration and the filtrate concentrated to 1 mL. The addition of ethanol yielded crystalline 11 (211 mg, 75%). Two recrystallizations from water -alcohol provided the analytical sample of 11: mp 208-210 °C, $[\alpha]_D$ +124.6° (c 1.87, water).

Anal. Calcd for $C_7H_{15}O_8S$: C, 30.77; H, 5.53; N, 5.13; S, 11.73 Found: C, 30.89; H, 5.57; N, 5.09; S, 11.64.

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