Synthesis of an Analog of the Aminoglycoside Antibiotics[1]

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Synthetic routes to 1D-4-*O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-3-*O*-methyl-*chiro*-inositol (**6**) are described. The key intermediate, 1D-1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-β-D-glucopyranosyl)-*chiro*-inositol (**4**), was obtained by deacetylation, tosylation, and reacetylation of the known 1D-1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*chiro*-inositol (**1**, 57%), or directly by coupling 2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-α-D-glucopyranosyl bromide (**2**) with 1D-1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-*chiro*-inositol (**3**, 52%). The ammonolysis and deacetonation of **4** gave **6** (73%). Alternatively **4** with sodium azide gave the crystalline 1D-1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)-*chiro*-inositol (**5**), which was converted into **6** (70% from **4**) by deacetylation, reduction, and deacetonation. The aminoglucoside **6** did not show antibiotic activity or cause code "misreading" in *in vitro* protein synthesis.

The synthesis of analogs of the aminoglycoside (pseudo-oligosaccharide) antibiotics, which include the streptomycins, neomycins, paromomycins, and kanamycins, is of substantial interest in view of the clinical usefulness of some members of the group and the finding that these antibiotics cause the "misreading" of messenger RNA during protein synthesis.[2] A few of the desired structures, consisting of sugars linked glycosidically to cyclitols, usually with amino groups in both moieties, have been obtained by direct coupling of the suitably blocked and activated components.[3] However, the effectiveness of this approach is restricted by the limited availability of suitably blocked aminocyclitols and the low reactivity of these toward acylglycosyl halides. An alternate route, the coupling of an ordinary sugar to an ordinary cyclitol followed by introduction of the amino group(s) into the resulting glycoside, is illustrated in this paper.

Scheme I summarizes the synthesis, which proceeded *via* the tosylated glucoside **4** to 1D-4-*O*-(6-amino-6-deoxy-β-D-glucopyranosyl)-3-*O*-methyl-*chiro*-inositol[4] (**6**). The key intermediate **4** was obtained in two ways. In one preparation the previously known 1D-1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*chiro*-inositol (**1**),[5] made by coupling 1D-1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-*chiro*-inositol (diisopropylidene-D-pinitol, **3**) and tetra-*O*-acetyl-α-D-glucopyranosyl bromide, was deacetylated, tosylated, and reacetylated to give crystalline **4** in 32% over-all yield from diisopropylidenepinitol. The second method was to couple diisopropylidene-D-pinitol with 2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-α-D-glucopyranosyl bromide (**2**) by the Koenigs–Knorr procedure. In this reaction the yield, based on diisopropylidene-D-pinitol, was 52%, unusually high for a coupling involving a secondary ring hydroxyl on a blocked sugar.

For the conversion of **4** into the aminoglucoside **6**, two methods were again used. Treatment of **4** with methanolic ammonia at 110° followed by deacetonation and chromatography of the crude product gave **6** as a colorless glass in 73% yield. Alternatively, **4** was converted into the crystalline azidoglucoside **5**. The azido compound was then deacetylated, reduced, and deacetonated, and the crude product was chromatographed. The yield of pure aminoglucoside (**6**), based on intermediate **4**, was 70%. Samples of **6** prepared by the two methods had identical chemical and spectroscopic properties, which fully substantiated the assigned stereoformula (see Experimental Section).

The good yield in the coupling step, which is one of the attractive features of the present synthesis, is probably due to the favorable conformational and electronic properties of the specific cyclitol derivative used. Suitably blocked halides of the several aminodeoxyglucoses (perhaps not the 2 isomer) could no doubt be coupled directly with diisopropylidenepinitol in good yield. However, deblocking the coupling products would be more difficult than the simple deblocking–reduction of our azidoglucoside (**5**). Also, the over-all syntheses would require several more steps than the procedure of coupling first, then aminating. This procedure should be capable of considerable extension, hopefully to the preparation of glycosides with both the cyclitol portion and the sugar aminated.

The aminoglucoside **6** showed no antibiotic activity against several bacteria and fungi, and a yeast, when it was included in the culture media at 1000 ppm.[6] It caused no misreading at 200 μg/ml in an *in vitro* protein-synthesizing system sensitive to natural aminoglycoside antibiotics.[7]

Experimental Section

Nuclear magnetic resonance spectra were taken on a Varian A-60 spectrometer. Tetramethylsilane and sodium dimethylsilapentanesulfonate (DSS) were used as internal standards. Infrared spectra were taken on a Beckman IR-5 instrument. Melting points were determined in Pyrex capillaries immersed in a heated oil bath equipped with a calibrated thermometer.

1D-1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-β-D-glucopyranosyl)-*chiro*-inositol (4).

(1) Presented at the 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965.

(2) (a) J. Davies, W. Gilbert, and L. Gorini, *Proc. Natl. Acad. Sci. U. S.*, **51**, 883 (1964); (b) J. Davies, L. Gorini, and B. D. Davis, *Mol. Pharmacol.*, **1**, 93 (1965).

(3) (a) S. Umezawa and Y. Ito, *Bull. Chem. Soc. Jap.*, **34**, 1540 (1961); (b) S. Koto, Y. Ito, and S. Umezawa, *ibid.*, **38**, 1447 (1965); (c) S. Umezawa and S. Koto, *ibid.*, **39**, 2014 (1966); (d) S. Umezawa, S. Koto, K. Tatsuta, and T. Tsumura, *J. Antibiot.* (Tokyo), **21**, 162 (1968); (e) M. Nakajima, A. Hasegawa, N. Kurihara, H. Shibata, T. Ueno, and D. Nishimura, *Tetrahedron Lett.*, 623 (1968).

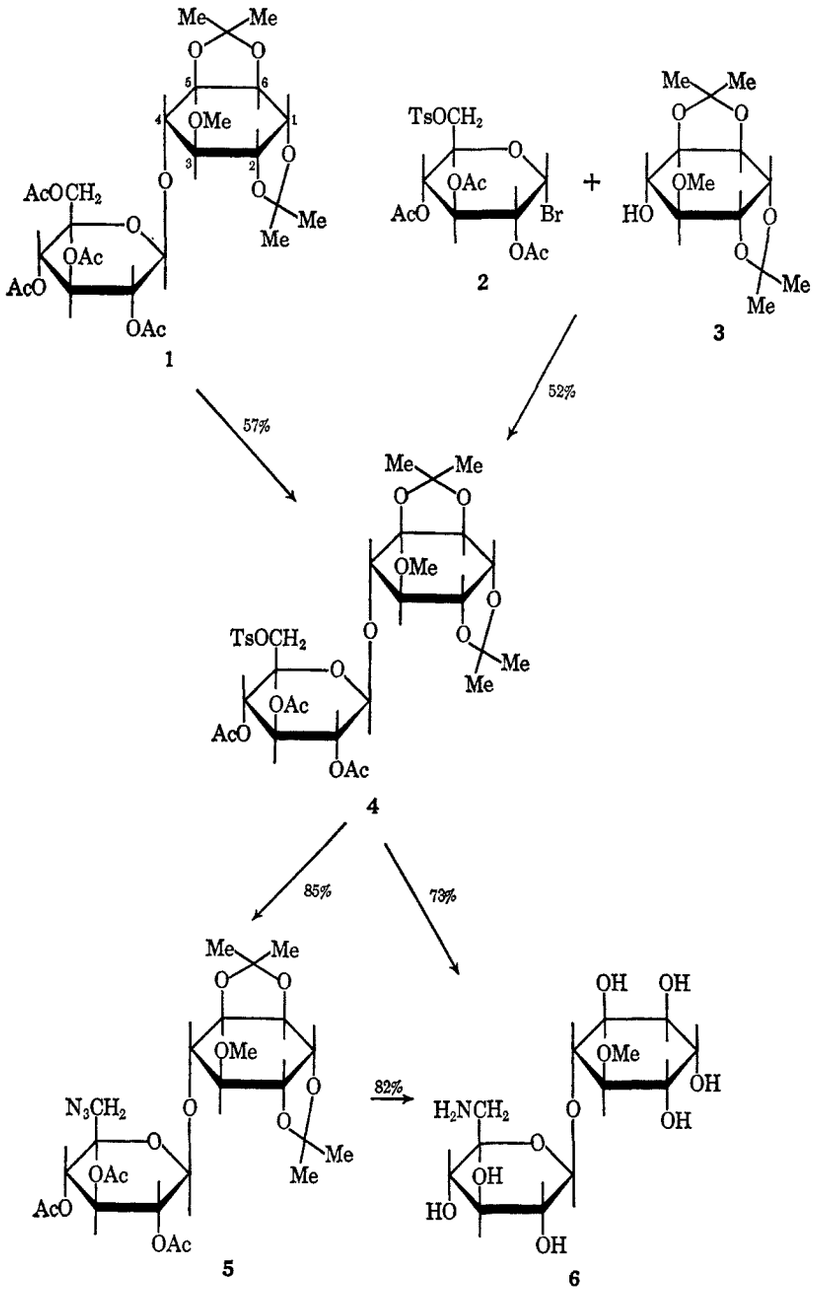
(4) The compounds described here are named as substituted cyclitols. For the cyclitol moieties, the recently adopted IUPAC/IUB Tentative Rules for Cyclitol Nomenclature (soon to be published) are used. Under these rules the parent inositol of the series, formerly known as *d*-inositol, (+)-inositol, or D-inositol, is now designated D- (or 1D-) *chiro*-inositol. The naturally occurring D-pinitol, which has been called 5-*O*-methyl-D-inositol in most recent American literature, is 1D-3-*O*-methyl-*chiro*-inositol.

(5) K. A. Caldwell, S. P. Raman, and L. Anderson, *Nature*, **199**, 373 (1963); the compound is there named 1,2:3,4-di-*O*-isopropylidene-5-*O*-methyl-6-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-D-inositol.

(6) The authors are grateful to Mr. Donald M. Murphy of the Wisconsin Alumni Research Foundation for carrying out these tests.

(7) This test was by Professor Julian Davies of this department.

SCHEME I



A. From 1D-1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*chiro*-inositol (1).—The deacetylation,[8] then tosylation, and reacetylation[9] of **1** (1.0 g) were accomplished without isolation of intermediates. The crude **4** obtained by crystallization from absolute ethanol melted at 139–142°: yield 0.67 g (57%). Recrystallization from ethanol–water gave colorless needles: mp 145.0–145.5°; $[\alpha]^{25}D$ +6° (*c* 6, $CHCl_3$); nmr ($CDCl_3$), τ 2.19 (d, 2, *J* = 8 Hz, tosyl ring H), 2.63 (d, 2, *J* = 8 Hz, tosyl ring H), 6.49 (s, 3, OCH_3), 7.54 (s, 3, tosyl CH_3), 7.96 (s, 3, acetyl CH_3), 8.01 (s, 6, acetyl CH_3), 8.50 (s, 6, isopropylidene CH_3), 8.66 ppm (s, 6, isopropylidene CH_3); ir (KBr), 2950 (C–H), 1750 cm^{-1} (C=O).

B. From 1D-1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-*chiro*-inositol (3) and 2,3,4-Tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-α-D-glucopyranosyl Bromide (2).—Silver oxide[10] (2.5 g), powdered Drierite (2.5 g), and **3**[11] (1.0 g, 3.6 mmol) were stirred (magnetic bar) with 8 ml of anhydrous, alcohol-free chloroform in a 125-ml erlenmeyer which was wrapped with black tape and protected from moisture. After 30 min 0.1 g of iodine was added, and then, dropwise over a 3-hr period, a solution of 4.0 g (7.2 mmol) of the 6-tosyltriacetylglucopyranosyl bromide **2**[12] in 10 ml of anhydrous, alcohol-free chloroform. Stirring was continued overnight. Water (*ca.* 0.5 ml) was then added to destroy any excess glycosyl halide and the mixture was filtered and concentrated.

The resulting syrup was chromatographed on a column of 60–200 mesh silica gel (200 g, 57 × 2.5 cm) with acetone–benzene (7:93, v/v as developer). Peaks were detected by the sulfuric acid char method.[13] The product, identified by thin layer chromatography (silica gel G, acetone–benzene, 30:70, v/v), emerged in the lead peak. After concentration and crystallization from ethanol the yield was 1.37 g (52%), mp 144.5–145.5°. Recrystallization from methanol–water gave pure **4**, mp 146–147°, identical

(8) A. Thompson, M. L. Wolfrom, and E. Pacsu in "Methods in Carbohydrate Chemistry," Vol. II, R. L. Whistler and M. L. Wolfrom, Ed., Academic Press Inc., New York, N. Y., 1963, p 216.

(9) E. Hardegger and R. M. Montavon, *Helv. Chim. Acta*, **29**, 1199 (1946).

(10) Prepared as described by E. L. Hirst and E. Percival [*Methods Carbohyd. Chem.*, **2**, 146 (1963)]; sodium hydroxide was used in place of barium hydroxide.

(11) Prepared from D-pinitol by the method of S. J. Angyal and R. M. Hoskinson, *J. Chem. Soc.*, 2985 (1962), with dimethoxypropane instead of diethoxypropane as the acetonating agent.

(12) B. Helferich and S. Grünler, *J. Prakt. Chem.*, [2] **148**, 107 (1937). These authors give mp 89–90° and $[\alpha]^{20}D$ +165°; our preparations agreed in rotation but were lower melting (65–69°).

(13) P. Ways, *J. Lipid Res.*, **4**, 101 (1963).

in all respects (mixture melting point, ir, specific rotation) with the product of method A.

Anal. Calcd for $C_{32}H_{44}O_{16}S$ (716.74): C, 53.61; H, 6.19. Found: C, 53.93; H, 6.26.

1D-1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)-*chiro*-inositol (5).—Sodium azide (2.5 g, 38 mmol) was stirred with **4** (4.0 g, 5.6 mmol) in 50 ml of dimethylformamide for 1 hr at 100–105°. The mixture was then concentrated under reduced pressure, the residue was dissolved in hot methanol–water, and crystallization was induced by adding more water, dropwise, to the hot solution. The pure **5** thus obtained weighed 2.8 g (85%), melted at 140–140.5°, and had $[\alpha]^{24}D$ −13.5° (*c* 7, DMF), and nmr ($CDCl_3$), τ 6.46 (s, 3, OCH_3), 7.96 (s, 3, acetyl CH_3), 7.98 (s, 6, acetyl CH_3), 8.48 (s, 6, isopropylidene CH_3), 8.66 ppm (s, 6, isopropylidene CH_3); ir (KBr), 2950 (C–H), 2090 (N_3), 1740 cm^{-1} (C=O).

Anal. Calcd for $C_{25}H_{37}O_{13}N_3$ (587.37): C, 51.10; H, 6.35. Found: C, 51.03; H, 6.27.

1D-4-*O*-(6-Amino-6-deoxy-β-D-glucopyranosyl)-3-*O*-methyl-*chiro*-inositol (6). A. From 1D-1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-β-D-glucopyranosyl)-*chiro*-inositol (4) by Ammonolysis.—A solution of **4** (4.0 g, 5.6 mmol) in 60 ml of methanol saturated with ammonia at 0° was sealed in a glass-lined steel bomb and heated at 110° for 12 hr. The pale yellow reaction mixture was decolorized with Darco G-60 and concentrated to a syrup under reduced pressure.

For deacetonation the syrup was dissolved in 10 ml of acetic acid–water (1:1, v/v) and heated 8 hr in an oil bath at 100–105°. The resulting brown solution was chromatographed on a column of Bio-Rad AG 1-X2 anion-exchange resin (OH^- form, 200–400 mesh, 65 × 2.5 cm), by development with distilled water at 0.5 ml/min. Peaks were again detected by the sulfuric acid char method.[13] The components were identified on thin layer plates of cellulose (without binder). These were developed with pyridine–ethyl acetate–acetic acid–water (5:5:1:3 by volume), and spots were visualized with silver nitrate–alkali.[14]

The first product to be eluted was pinitol, 0.16 g; the eluate volume was 430–470 ml.[15] The following peak, eluate volume 700–2000 ml, was basic and contained the desired pinitol 6-aminoglucoside **6**. The product (1.45 g, 73%) was obtained as a colorless glass by removal of the solvent: $[\alpha]^{18}D$ +27° (*c* 1, H_2O); nmr (D_2O), τ 6.40 (s, 3, OCH_3), 5.24 ppm (d, 1, J = 8 Hz, anomeric H); ir (KBr), 3320 cm^{-1} (O—H), no band for ester C=O.

Anal. Calcd for $C_{13}H_{25}O_{10}N$ (355.34): C, 43.93; H, 7.09; N, 3.94. Found: C, 43.55; H, 7.15; N, 3.89.

The 8-Hz spacing of the doublet for the anomeric proton in the nmr spectrum indicates a β configuration for the glucoside bond, as previously deduced for compound **1** on other grounds.[5] Compound **6** did not reduce Fehling's solution, but treating it with 8 *N* hydrochloric acid for 15 min at 100°, followed by removal of the chloride ion with Dowex-1 (OH^-) resin, gave a hydrolysate with reducing properties. When the hydrolysate was chromatographed on cellulose thin layer plates, it gave reduced silver spots at R_f 0.24 (w), 0.34 (w), and 0.56 (s). Control spots showed R_f 0.24 for the unhydrolyzed glycoside and R_f 0.56 for authentic pinitol. Approximately 1 molar equiv of nitrogen was evolved when **6** reacted with nitrous acid in a Van Slyke apparatus.

B. From 1D-1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-4-*O*-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-β-D-glucopyranosyl)-*chiro*-inositol (5).—The azidoglucoside **5** (4.0 g, 6.8 mmol) was deacetylated,[8] and the deacetylated product was concentrated to a syrup. This was dissolved in *ca.* 100 ml of ethanol and added to a suspension of palladium-on-carbon catalyst (0.7 g, 5% Pd) in *ca.* 50 ml of ethanol. The mixture was stirred magnetically for 3 hr under hydrogen at 1 atm of pressure with frequent exchange of the gas for fresh hydrogen. The catalyst was then filtered off and the filtrate was concentrated to a glassy solid. The infrared spectrum of the solid showed no absorption in the ester carbonyl or azide regions.

The solid was deacetonated and chromatographed as described under A. The product was in eluate volume of 1050–2050 ml from the resin column. Concentration of this gave 2.0 g of colorless glass, thus a yield of 82% based on **5**, or 70% over-all from compound **4**. This product was identical in all respects with **6** prepared by method A.

Registry No.—**4**, 16802-83-8; **5**, 16802-81-6; **6**, 16802-82-7.

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(14) L. Hough and J. K. N. Jones, *Methods Carbohyd. Chem.*, **1**, 28 (1962).

(15) The appearance of free pinitol in the eluate was probably due to the hydrolysis of pinitol 3,6-anhydroglucoside, an expected side product of the ammonolysis reaction. The 3,6-anhydroglucoside would be very acid labile, whereas the glucoside bond of **4** is stable to the deacetonation conditions.

Selective Reactions of Sulfonic Esters of Carbohydrates on Alumina[1]

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Selective hydrolysis of primary sulfonic ester groups of carbohydrates occurs on basic or neutral alumina. In the presence of aliphatic alcohols, hydrolysis is accompanied by selective alcoholysis of a sulfonic ester group at a primary position.

Hydrolysis of carboxylic[2,3] and sulfonic[4] esters of steroids on basic alumina has long been known, and more recently selective hydrolysis of a primary acetate group in the presence of a secondary acetate group has been demonstrated[5] with several steroidal esters.

In carbohydrate chemistry, acetylation is frequently employed to protect hydroxyl functions but migration[6] of acetate groups can be a source of difficulty during a definitive synthetic procedure. Few examples have been reported[7,8] of migration of sulfonic ester groups of carbohydrates, and the usefulness of these esters in synthetic work is well documented.[9] Our studies on the hydrolysis of sulfonic esters of carbohydrates stemmed from our observations on deacetylation of sugar acetates on basic alumina. The sulfonic ester derivatives examined in detail in this study are readily

(1) This work was supported in part by the Army Research Office, Durham, N. C.

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(3) R. H. Levin, A. V. McIntosh, Jr., G. B. Spero, D. E. Rayman, and E. M. Meinzer, *J. Amer. Chem. Soc.*, **70**, 511 (1948).

(4) R. J. W. Cremlyn and C. W. Shoppee, *J. Chem. Soc.*, 3515 (1954).

(5) W. F. Johns and D. M. Jerina, *J. Org. Chem.*, **28**, 2922 (1963).

(6) W. A. Bonner, *J. Amer. Chem. Soc.*, **80**, 3697 (1958).

(7) A. C. Cope and T. Y. Shen, *ibid.*, **78**, 5912 (1956).

(8) J. S. Brimacombe and L. C. N. Tucker, *Chem. Commun.*, 903 (1966).

(9) R. S. Tipson, *Advan. Carbohyd. Chem.*, **8**, 107 (1953).