Carbohydrate Research, 59 (1977) 319–331

AMINOGLYCOSIDES OF D-PINITOL. THE 3-AMINO-3-DEOXY-α-D-GLUCOPYRANOSIDE, THE 3-ACETAMIDINO ANALOG, AND THE 3,6-DIDEOXY-3,6-EPIMINO-α-D-GLUCOPYRANOSIDE

JIUNN-YANN TANG AND LAURENS ANDERSON*

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison, WI 53706 (U. S. A.)

(Received January 31st, 1977; accepted for publication March 11th, 1977)

ABSTRACT

3-Azido-2,4,6-tri-*O*-benzyl-3-deoxy-α-D-glucopyranosyl chloride (**7**), prepared conventionally from the azido precursor **2**, was coupled with "diisopropylidene-D-pinitol" (**8**) to give the α-D-glucoside **9** in good yield, together with some β anomer. Removal of the *O*-benzyl groups from **9** and reduction of the azido group to $-NH_2$ were accomplished simultaneously. Further deprotection yielded **11**, a 3-amino-3-deoxy-α-D-glucoside of D-pinitol (**1a**). Compound **11** was converted into the (impure) 3-acetamidino hydrochloride **12**. The synthesis of 3,6-epimino-D-glucosides was accomplished by ring closure of the 3-*N*-tosyl-6-*O*-tosyl intermediates **17** and **13**. The products, after deprotection, were methyl 3,6-dideoxy-3,6-epimino-β-D-glucopyranoside (**20**) and the novel 3,6-epimino analog **15** of the pinitol D-glucoside **11**.

INTRODUCTION

Some years ago, in the pursuit of simple analogs of the aminocyclitol (aminoglycoside) antibiotics, we found 1D-3-*O*-methyl-*chiro*-inositol (D-pinitol, **1a**) attractive as a cyclitol moiety because it is readily converted into a derivative (**8**) having a single, readily glycosylated, hydroxyl group. The discovery that kasugamycin[1] is an aminoglycoside of the parent D-*chiro*-inositol lent further interest to the synthesis of conjugates of **1a** with amino sugars. In the course of this work, we prepared 6-amino-6-deoxy-β-D-glucopyranosyl-D-pinitol[2] and its α anomer[3]. Finally, as described in this paper, we made the 3-amino-3-deoxy-α-D-glucoside **11** and two of its further transformation products (**12** and **15**). In **12** the sugar moiety is 3-acetamidino-3-deoxy-α-D-glucose, and in **15** the novel 3,6-dideoxy-3,6-epimino-D-glucose. The simple 3,6-epimino-D-glucoside **20** was synthesized as a model for **15**.

*Please address correspondence to this author.

RESULTS AND DISCUSSION

For the synthesis of the parent aminoglycoside (**11**) of the present series, our approach was to prepare the benzyl-protected azidodeoxy-D-glucopyranosyl chloride **7**, and couple this with di-*O*-isopropylidene-D-pinitol (**8**). It was envisaged that the coupling product (**9**) could then be converted into the free aminoglycoside in two steps. Hydrogenolysis would reduce the azido group to $-NH_2$ and achieve *O*-debenzylation, and acidic hydrolysis would remove the isopropylidene acetal groups. While our work was under way, this general approach to aminoglycoside synthesis was described by others[4–6,*] but in only one of these syntheses[5] was simultaneous $N_3 \rightarrow NH_2$ conversion and *O*-debenzylation achieved, and in that case it gave poor results. In the other instances, a four-step sequence was used[4,6]: reduction of $-N_3$ to $-NH_2$, protection of $-NH_2$, *O*-debenzylation, and deprotection of $-NH_2$.

The starting material for the glucosyl chloride **7** was methyl 2-*O*-acetyl-3-azido-4,6-*O*-benzylidene-3-deoxy-β-D-glucopyranoside (**2**), derived from methyl 4,6-*O*-benzylidene-3-chloro-3-deoxy-β-D-allopyranoside by treatment with sodium azide and then acetic anhydride[8]. These reactions yield a mixture of **2** and an unsaturated product, methyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-β-D-*erythro*-hex-3-enopyranoside, which may be separated by fractional crystallization. The details of the separation were not given in the original description[8] of the preparation, but procedures that worked well in our hands are recorded in the Experimental section. The conversion of **2** into the azidotri-*O*-benzyl-D-glucoside **5**, via the 2-acetate **3**, was accomplished by standard methods.

For the hydrolysis of **5**, a mixture of acetic acid, water, and sulfuric acid was used, but because of the instability of the resulting free sugar **6** to hot acid, the reaction could not be completed in a single stage. Chromatographic separation of the partial hydrolyzate and recycling of the unreacted glucoside were required to obtain a satisfactory yield in this step. Similar difficulties in hydrolyzing benzylated methyl azidoglycosides have been noted by others[5,9]. Consequently, the alternative method of acetolysis, used by Stevens and coworkers[4,10] for the cleavage of these compounds, is to be recommended. The treatment of **6** with thionyl chloride and zinc chloride[11] readily gave the α-glucosyl chloride **7**.

The coupling of **7** with di-*O*-isopropylidene-D-pinitol (**8**) was accomplished by the procedure of Hasegawa *et al.*[12], with benzene–1,4-dioxane as the solvent and silver perchlorate–silver carbonate as the catalyst. The preponderant product was the α-glucoside **9**, obtained in 68% yield by chromatography of the mixture on silica gel. The β anomer of **9** was also isolated in 12% yield.

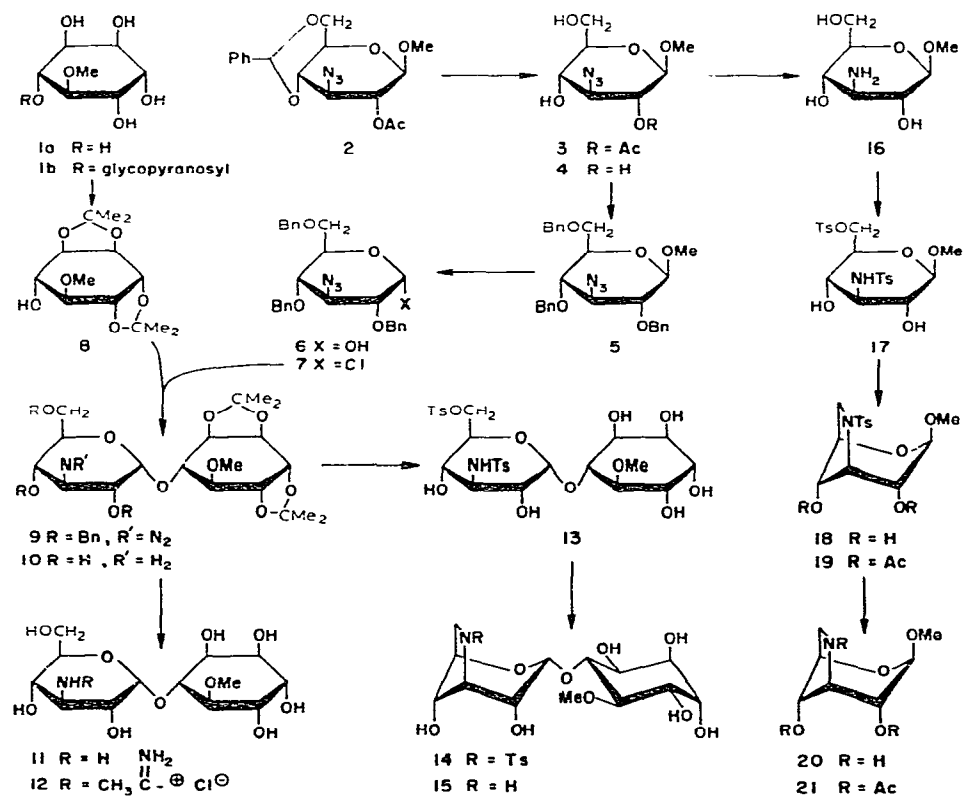
Our initial attempts to remove the benzyl groups from **9** and simultaneously convert its azido group into an amino group, by hydrogenolysis over palladium on charcoal, gave mixtures of products, and low yields of the partially deprotected aminoglycoside **10**. Parallel observations have been recorded by Y. Nishimura *et al.*[13],

*Paulsen and collaborators (ref. 7) have recently reported on the preparation of *O*-benzylated 2-azido-2-deoxy-β-glycosyl chlorides and their use in the synthesis of α-linked oligosaccharides.

who used palladium black as the catalyst. The single-step hydrogenolysis of **9** to **10** did, however, proceed satisfactorily with a catalyst prepared by depositing palladium hydroxide[14] on charcoal. Thus, we were able to realize the theoretical advantages of using a protected azidoglycosyl halide as an aminoglycoside precursor.

Mild acidic cleavage of the isopropylidene groups from **10** readily gave the primary target compound, 3-amino-3-deoxy-α-D-glucopyranosyl-D-pinitol (**11**). The acetamidino analog **12** was generated by the reaction of **11** with ethyl acetimidate hydrochloride. It was not obtained pure, but was characterized by p.m.r. and i.r. spectroscopy.

3,6-Epiminoglycosides, although not extensively studied, have been synthesized from derivatives of 3-amino-3-deoxyglycosides carrying suitable leaving-groups in the 6-position[15,16]. In the present case, it was envisaged that the 3,6-epimino analog (**15**) of **11** could be obtained by tosylation of O-6 of the sugar moiety of the partially deprotected precursor **10**, followed by internal displacement of the sulfonate group. If N-3 were left unprotected, it would be tosylated at the same time as O-6, but this was not seen as a disadvantage. The *p*-toluenesulfonamido group is readily converted by base into a nucleophilic anion, and the *N*-tosyl group could readily be removed



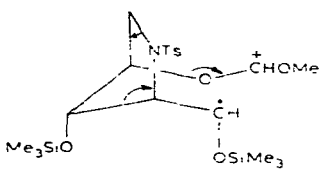
Bn = $PhCH_2$—

from the cyclized product. The projected series of reactions was conducted first on a simple model-compound, methyl 3-amino-3-deoxy-β-D-glucopyranoside (**16**) (**16**→ **17**→ **18**→ **20**), and then on **10** (**10**→ **13**→ **14**→ **15**). In the case of the pinitol amino-glucoside (**10**), the isopropylidene protecting groups were removed prior to the internal displacement, to avoid complications that might result were the 3,6-epimino-glucoside especially acid-labile.

Characterization of the model compounds **18**–**21** and the pinitol glycosides **14** and **15** as derivatives of 3,6-dideoxy-3,6-epimino-D-glucopyranose rests on both chemical and spectroscopic evidence. Elemental analysis showed that a tosyloxy group was lost during the conversion **17**→ **18**, and also in the conversion **13**→ **14**. If the loss were due to elimination to form 5,6-unsaturated products, AB quartets (or possibly 2-proton singlets), representing H-6 and 6′ should have appeared in the vinyl region of the p.m.r. spectra of **14** and **18**, but no such signals were evident. Also in accord with the proposed structures is the formation of a diacetate (**19**) from **18**.

Evidence that compounds **18**, **19**, **20**, and **21** have the $^1C_4$ conformation, as opposed to the $^4C_1$ conformation characteristic of the parent β-D-glucoside **17** and its precursors, was found in the p.m.r. spectra. The value of $J_{1,2}$ is near zero for all of the epimino compounds (the signals for H-1 are singlets), and for compound **19** the values of $J_{2,3}$ and $J_{3,4}$ are 4 and 3 Hz, respectively. These values indicate gauche relationships for H-2–H-3 and H-3–H-4. For $J_{1,2}$, one might expect a value in the order of 2 Hz, but the observed lack of coupling between H-1 and H-2 may be rationalized as being due to severe steric crowding involving the 3,6-epimino ring and C-1 methoxyl group. This factor could force the methoxyl group outward, and cause the H-1–H-2 projection-angle to approach 90°. Finally, in the spectrum of the tri-acetate **21**, two lines are observed for the methyl protons of the *N*-acetyl group, indicative of hindered rotation about the C(O)–N bond. We have previously observed hindrance of this kind in a 1,4-epiminoinositol derivative[17]. Compound **21** is closely related to methyl 2,4-di-*O*-acetyl-3,6-dideoxy-3,6-*N*-(methoxycarbonylepimino)-α-D-glucopyranoside, described by Ikeda *et al.*[16], and the p.m.r. spectra of the two compounds display the anticipated similarities.

The mass spectra of the trimethylsilyl (Me_3Si) derivatives of **18** and **20** were remarkably simple in the low-mass region (*m/e* <230). For Me_3Si-**18**, the principal lines were at *m/e* 73 (Me_3Si^+), 91 ($C_7H_7^+$, *p*-tolyl), 129, 217 ($Me_3SiOCH{=}CH{-}CHOSiMe_3^+$), and 228. In the spectrum of Me_3Si-**20** the line at *m/e* 91 was absent, but there was a prominent line at *m/e* 102 (·$CHOSiMe_3^+$?). The lines at *m/e* 73 and 217 are commonly found in the spectra of trimethylsilylated sugars[18], but the line at *m/e* 129 may be characteristic of 3,6-bridged derivatives. It is tentatively attributed to $CH_2{=}CH{-}CHOSiMe_3^+$, from the following fragment:



A corresponding line at *m/e* 71, attributed to CH_2=CH–$CHOMe^+$, appears in the mass spectrum of methyl 3,6-anhydro-2,4-di-*O*-methyl-α-D-glucopyranoside[19]. Also characteristic of the spectra of Me_3Si-**18** and Me_3Si-**20** is the absence of *m/e* 191 ($Me_3SiOCHOSiMe_3^+$), 147 ($Me_3SiOSiMe_2^+$), and 133 ($Me_3SiOCHOMe^+$), which are prominent in the spectrum of methyl tetrakis-*O*-(trimethylsilyl)-α-D-glucopyranoside[18] The spectrum of the Me_3Si-derivative of the pinitol glucoside **15** did contain lines at *m/e* 133, 147, and 191, but these are believed to have arisen from the fragmentation of the pinitol moiety. The presence of the characteristic line at *m/e* 129, and the general simplicity of the spectrum, is consistent with a 3,6-epiminoglucosidic structure for **15**.

So far as we are aware, structure **15** is novel to the aminoglycoside field. Compounds **11**, **12**, and **15**, when tested at 1 mg/ml against a standard spectrum of microorganisms (bacteria, yeasts, molds), failed to show any convincing antibiotic activity.

EXPERIMENTAL

General methods. — Melting points, which are uncorrected, were determined in sealed, Pyrex capillaries in a Hershberg-type (heated oil-bath) apparatus. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter. Infrared spectra were recorded with a Beckman IR-5 instrument calibrated on the 1605-cm^{-1} line of polystyrene. P.m.r. spectra were taken on a Varian T-60 spectrometer. Chemical shifts are referenced to internal tetramethylsilane or, for samples run in deuterium oxide, to instrument zero set with a separate tube of tetramethylsilane in chloroform. Mass spectra were obtained with an AEI MS-902 instrument, by using a direct introduction probe. T.l.c. was performed on glass plates coated with Silica Gel G (E. Merck). Spots were detected by charring with sulfuric acid or, for amino sugar derivatives, by spraying with ninhydrin. All compounds reported as pure were recrystallized to constant melting point, at which stage they gave single spots on t.l.c. Elemental analyses were performed by the Galbraith Laboratories.

Methyl 2-O-acetyl-3-azido-4,6-O-benzylidene-3-deoxy-β-D-glucopyranoside (**2**). — A suspension of methyl 4,6-*O*-benzylidene-3-chloro-3-deoxy-β-D-allopyranoside[20] (29.4 g, 98 mmol) and sodium azide (13.5 g, 208 mmol) in 130 ml of dry *N*,*N*-dimethylformamide was stirred vigorously for 4 h at 130°. T.l.c. in 54:20:46:5 chloroform–ether–Skellysolve B–methanol showed complete conversion into two products, the faster-moving one being the azide **2** and the slightly slower-moving one the unsaturated byproduct. The mixture was filtered and the undissolved material was washed with chloroform several times. Evaporation of the combined filtrate and washings under diminished pressure gave a brownish syrup, which was dissolved in 40 ml of 1:1 acetic anhydride–pyridine and heated for 30 min at 100°. The acetylation mixture was then evaporated to dryness and several portions of toluene were evaporated from the residue. Crystallization of the dark-brown product from methanol and recrystallization from chloroform–methanol–Skellysolve B gave 13.7 g

of **2** as needles. The mother liquor was evaporated and the residue was then extracted with 20:1 Skellysolve B–methanol. After evaporation of the solvent, crystallization of the residue afforded 2.1 g of additional product. The total yield was 46%, m.p. 119–121°, $[\alpha]_D^{25}$ −80°, $[\alpha]_{436}^{25}$ −166° (*c* 2.87, chloroform) (lit.[8] m.p. 107–109°, $[\alpha]_D$ −81°); p.m.r. ($CDCl_3$): δ 7.65–7.20 (m, 5, Ph-H), 5.58 (s, 1, PhC*H*), 4.90 (dd, 1, $J_{1,2}$ 8 and $J_{2,3}$ 10 Hz, H-2), 4.42 (d, 1, *J* 8 Hz, H-1), 4.40 (dd, 1, $J_{3,4}$ ~4 Hz, H-3), 3.7–3.4 (m, 4, H-4,5,6,6'), 3.51 (s, 3, OCH_3), and 2.15 (s, 3, CH_3CO).

In an alternative procedure, a suspension of sodium azide (17 g) in 350 ml of *N*,*N*-dimethylformamide was heated, with stirring to 130°. Methyl 4,6-*O*-benzylidene-3-chloro-3-deoxy-β-D-allopyranoside (40 g) was added. The mixture was stirred for ~5 h at 130° and then cooled to room temperature. Water (400 ml) and chloroform (250 ml) were added, the chloroform layer was separated, and the aqueous layer extracted twice with 100–200 ml of chloroform. The combined chloroform extracts were washed with water, dried, and evaporated to a thin syrup under diminished pressure. A solution of the syrup in 550 ml of 1:1 acetic anhydride–pyridine was heated for 1 h on a steam bath, cooled to room temperature, and stirred into ~1500 ml of crushed ice. Stirring was continued for 2 h, and then the precipitated product was collected on a filter and dissolved in ~600 ml of hot ethanol. The crystals of **2** (13.1 g) that formed during 2 h were collected, and the mother liquor was refrigerated. After 2 h, an additional 6.0 g of **2** was collected. Further refrigeration caused the deposition of a mixture of **2** and the unsaturated by product.

Methyl 2-O-acetyl-3-azido-3-deoxy-β-D-glucopyranoside (**3**). — A solution of **2** (2.16 g, 6.2 mmol) in 50% aqueous acetic acid (20 ml) was boiled for 30 min under reflux, cooled, and extracted with Skellysolve B (20 ml) to remove benzaldehyde. Evaporation of the aqueous solution under diminished pressure gave a brown solid. This was washed with cold water and recrystallized from chloroform–Skellysolve B with the addition of a few drops of methanol to give 1.38 g (85%) of crystalline **3**, m.p. 169.5–170.5°; $[\alpha]_D^{25}$ −42.5°, $[\alpha]_{436}^{25}$ −84.5° (*c* 1.52, methanol); ν_{max}^{KBr} 2100 cm^{-1} (N_3); p.m.r. (CD_3COCD_3): no signals below 5.0 (disappearance of the benzylidene group), δ 4.95 (bs, 1, D_2O exchangeable, O*H*), otherwise similar to the p.m.r. of **2**.

Anal. Calc. for $C_9H_{15}N_3O_6$ (261.23): C, 41.38; H, 5.79; N, 16.09. Found: C, 41.36; H, 5.64; N, 15.92.

Methyl 3-azido-3-deoxy-β-D-glucopyranoside (**4**). — To a solution of **3** (1.5 g, 5.75 mmol) in anhydrous methanol (30 ml) sodium methoxide (0.45 g, 8.3 mmol) was added in portions. The mixture was heated for 2 min on a steam bath and then deionized by treatment with Dowex-50 (H^+) resin (3 g). The resin was filtered off and the filtrate was concentrated *in vacuo* to a syrup (1.26 g). Trituration with ether gave crystalline product. Recrystallization from ether yielded 1.17 g (93%) of **4** as colorless needles, m.p. 105–105.5°, $[\alpha]_D^{25}$ −15.2°, $[\alpha]_{436}^{25}$ −25° (*c* 1.09, methanol); ν_{max}^{KBr} 2100 cm^{-1} (N_3); p.m.r. (CD_3COCD_3): δ 4.71 (bs, 2, O*H*), 4.28 (d, 1, $J_{1,2}$ 6.5 Hz, H-1), 3.94–3.00 (m, 7, H-2,3,4,5,6,6', and O*H*), and 3.47 (s, 3, OCH_3).

Anal. Calc. for $C_7H_{13}N_3O_5$ (219.20): C, 38.36; H, 5.98; N, 19.17. Found: C, 38.43; H, 6.02; N, 19.05.

*Methyl 3-azido-2,4,6-tri-*O*-benzyl-3-deoxy-β-*D*-glucopyranoside* (**5**). — To a stirred solution of methyl 2-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranoside (**3**, 6.1 g, 23.4 mmol) in 60 ml of dry 1,4-dioxane was added 26 g of powdered potassium hydroxide, and then, slowly and with stirring, 24 ml of α-chlorotoluene. The mixture was then heated and stirred for 3 h at 90°. T.l.c. in 25:1 benzene–ether showed the presence of a major component and several minor components. After cooling, the mixture was poured into water (700 ml) and extracted thrice with 200 ml of ether (chloroform may also be used). The extract was washed with water, dried with anhydrous sodium sulfate, and evaporated *in vacuo* to give 10.9 g of a dark syrup. Chromatography of the syrup on a column of silica gel (500 g), with 25:1 benzene–ether as eluant, gave a yellow syrup (6.0 g, 52% yield). Rechromatography gave material that slowly crystallized. On recrystallization from Skellysolve B, the compound was obtained as needles, m.p. 60–61.5°; $[\alpha]_D^{25}$ +3.9°, $[\alpha]_{436}^{25}$ +8.0° (*c* 1.12, chloroform); ν_{max}^{KBr} 2100 cm^{-1} (N_3); p.m.r. ($CDCl_3$): δ 7.39–7.31 (3 s, 15, Ph-H), 4.96–4.54 (2 q_{AB} and 1 s, total 6 H, PhCH_2), 4.27 (d, 1, $J_{1,2}$ 7.5 Hz, H-1), 3.57 (s, 3, OCH_3), and 3.80–3.10 (m, 6, H-2,3,4,5,6,6').

Anal. Calc. for $C_{28}H_{31}N_3O_5$ (489.57): C, 68.69; H, 6.38; N, 8.58. Found: C, 68.70; H, 6.53; N, 8.63.

*3-Azido-2,4,6-tri-*O*-benzyl-3-deoxy-α-*D*-glucopyranose* (**6**). — Aqueous sulfuric acid (2M, 28 ml) was added to an ice-cooled solution of **5** (6.98 g, 14.3 mmol) in acetic acid (105 ml). The solution was heated for 4 h at 95°, cooled, poured into water (150 ml), and then extracted thrice with chloroform (150 ml). The combined chloroform extracts were successively washed with sodium hydrogencarbonate solution and water, dried over anhydrous sodium sulfate, and evaporated *in vacuo* to a brown syrup (7.6 g). Crystallization from benzene–Skellysolve B gave 1.01 g of **6** as colorless needles. The mother liquor was then concentrated to a syrup, which was chromatographed on a column of silica gel (250 g, 3.2 cm diameter) with 19:1 chloroform–ethyl acetate as eluant. This procedure afforded 3.5 g of the starting material **5**, which was eventually recycled, and a further 1.54 g of **6**. Thus the total yield, based on the amount of **5** that had reacted, was 75%. The m.p. was 124.5–125.5°, $[\alpha]_D^{25}$ +43.4°, $[\alpha]_{436}^{25}$ +83.9° (*c* 2.37, chloroform); ν_{max}^{KBr} 2100 cm^{-1} (N_3); p.m.r. ($CDCl_3$): δ 5.20 (d, 1, $J_{1,2}$ 3.8 Hz, H-1), no OCH_3 signal, otherwise similar to the p.m.r. spectrum of **5**.

Anal. Calc. for $C_{27}H_{29}N_3O_5$ (475.55): C, 68.19; H, 6.14; N, 8.83. Found: C, 68.45; H, 6.15; N, 8.75.

*3-Azido-2,4,6-tri-*O*-benzyl-3-deoxy-α-*D*-glucopyranosyl chloride* (**7**). — A solution of **6** (1.08 g, 2.27 mmol) in benzene (35 ml), in a flask equipped with a drying tube, was treated with zinc chloride (1.10 g, 8.2 mmol) and thionyl chloride (2.6 g, 22 mmol). The mixture was stirred for 50 min at room temperature, at which time t.l.c. in 1:9 acetone–benzene showed one major product plus traces of starting material and minor products. The mixture was filtered through a 2-cm layer of silica gel on a sintered-glass funnel under mild suction and the silica gel was washed several times with dry benzene. Evaporation of the filtrate to dryness *in vacuo* and evaporation of 3 portions of toluene from the residue yielded 0.85 g (76%) of **7** as a pale-yellow syrup containing

traces of **6**. Further purification on silica gel (1.5 × 30 cm), with 19:1 chloroform–ethyl acetate as eluant, gave an analytical sample having $[\alpha]_D^{25}$ +103°, $[\alpha]_{436}^{25}$ +207° (*c* 0.8, chloroform); p.m.r. ($CDCl_3$): δ 6.00 (d, 1, $J_{1,2}$ 3.8 Hz, H-1), otherwise similar to the p.m.r. spectrum of **6**.

Anal. Calc. for $C_{27}H_{28}ClN_3O_4$ (493.99): C, 65.65; H, 5.71; Cl, 7.18; N, 8.51. Found: C, 65.52; H, 5.67; Cl, 7.23; N, 8.58.

*1D-3-O-(3-Azido-2,4,6-tri-O-benzyl-3-deoxy-α-D-glucopyranosyl)-1,2:5,6-di-O-isopropylidene-4-O-methyl-*chiro*-inositol* (**9**) *and its β anomer.* — 1D-1,2:5,6-Di-*O*-isopropylidene-3-*O*-methyl-*chiro*-inositol* ("di-*O*-isopropylidene-D-pinitol", **8**)[22] was prepared in this laboratory by acetonation of D-pinitol (**1a**) with 2,2-dimethoxypropane (procedure of Angyal and Hoskinson[23]). A mixture of **8** (0.84 g, 3.06 mmol), powdered Drierite (6 g), dry benzene (20 ml), 1,4-dioxane (13.3 ml), silver carbonate (1.4 g), and silver perchlorate (0.117 g) was stirred for 1 h at room temperature in the dark. After the addition of 3-azido-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl chloride(**7**) (2.0 g, 4.05 mmol) in dry benzene (33 ml), stirring was continued for an additional 17 h. The insoluble materials were filtered off on a pad of Celite and washed with chloroform. The combined filtrate and washings were evaporated *in vacuo* to a pale-yellow syrup that was chromatographed on a column of silica gel (220 g, 3-cm diameter) with 44:1:1 benzene–methanol–ammonia-saturated methanol as eluant. Partial separation of the α-and β-glucosidic products was achieved. After rechromatography of fractions containing both anomers, the yield of the pure, syrupy α-glucoside **9** was 1.49 g (66%); $[\alpha]_D^{25}$ +75.6°, $[\alpha]_{436}^{25}$ +148.2° (*c* 1.19, chloroform); ν_{max}^{KBr} 2100 cm^{-1} (N_3); p.m.r. ($CDCl_3$): δ 7.34–7.28 (3 s, 15, Ph-H), 5.40 (d, 1, *J* 4 Hz, H-1 of sugar), 5.00–4.40 (m, 6, PhCH_2), 4.35–3.40 (m, 12, sugar and cyclitol C*H*), 3.51 (s, 3, OCH_3), 1.53, 1.40, 1.33, and 1.26 (4 s, 3 each, CCH_3).

Anal. Calc. for $C_{40}H_{49}N_3O_{10}$ (731.84): C, 65.65; H, 6.75; N, 5.74. Found: C, 65.58; H, 6.60; N, 5.79.

The β *anomer* of **9**, also a syrup, was obtained in 12% yield: $[\alpha]_D^{25}$ −2.4°, $[\alpha]_{436}^{25}$ −4.7° (*c* 1.64, chloroform); ν_{max}^{KBr} 2100 cm^{-1} (N_3); p.m.r. ($CDCl_3$): signal for H-1 of sugar not distinguishable (overlapped with PhCH_2), otherwise similar to the p.m.r. spectrum of **9**. Found: C, 65.99; H, 6.69; N, 5.75.

Palladium hydroxide catalyst for hydrogenolysis (10% Pd). — Charcoal (2.5 g) was suspended in a solution of palladium chloride (0.42 g) in 1 liter of water. Lithium hydroxide (0.2 g in a few ml of water) was added, and the suspension was stirred overnight. The catalyst was collected on a suction filter, washed first with dilute acetic acid and then with water until the washings were neutral, and dried in a vacuum oven overnight at 60°.

*The pinitol derivatives described here are named and numbered according to the IUPAC–IUB 1973 Recommendations for Cyclitols (ref. 21). The assignment of the number 3 to the methylated position in **1a**, **1b**, and **8** and of the number 4 to this position in the subsequent products **9–15**, results from the application of the principle of "lowest numbering to the substituent first in alphabetical order". This principle is necessarily invoked to choose between two equivalent numberings based on the stereochemistry of the parent *chiro*-inositol.

1D-3-O-(3-Amino-3-deoxy-α-D-glucopyranosyl)-1,2:5,6-di-O-isopropylidene-4-O-methyl-chiro-*inositol* (**10**). — Catalyst (2.4 g) was added in portions, with vigorous stirring, to a solution of compound **9** (0.65 g, 0.89 mmol) in anhydrous methanol (20 ml), and the suspension was stirred under hydrogen at atmospheric pressure and room temperature. As judged by t.l.c. in 2:1 chloroform–methanol, reaction for 6 h sufficed to convert the starting material into ninhydrin-positive, polar products (major, R_F 0.36; minor, R_F 0.09). The catalyst was removed by filtration through a Celite pad and washed with methanol, and the filtrate was concentrated to a syrup *in vacuo*. The syrup was dissolved in acetone–benzene and the solution again evaporated. Chromatography of the resulting pale-yellow, glassy solid on silica gel (1.5 × 36 cm), with 4:1 chloroform–methanol as eluant, gave 0.29 g (75%) of the pure, amorphous product, $[\alpha]_D^{25}$ +84.0°, $[\alpha]_{436}^{25}$ +160° (*c* 0.5, methanol); p.m.r. ($CDCl_3$): δ 5.10 (d, 1, *J* 3 Hz, H-1 of sugar), 4.60–2.65 (m, 15, sugar and cyclitol C*H*, 3 O*H*), 3.57 (s, 3, OCH_3), 2.03 (d, 2, *J* 6 Hz, D_2O exchangeable, NH_2), 1.50 and 1.33 (2 s, 6 each, CCH_3).

Anal. Calc. for $C_{19}H_{33}NO_{10}$ (435.47): C, 52.41; H, 7.64; N, 3.22. Found: C, 52.59; H, 7.59; N, 3.07.

1D-3-O-(3-Amino-3-deoxy-α-D-glucopyranosyl)-4-O-methyl-chiro-*inositol* (**11**). — A solution of compound **10** (0.33 g, 0.76 mmol) in 50% aqueous acetic acid (4 ml) was heated for 1 h on a steam bath. The solution was then concentrated *in vacuo*, several portions of toluene were evaporated from the residue, and the latter was taken up in methanol. This solution was decolorized with charcoal and then evaporated to pale-yellow syrup. Chromatography on silica gel (60 g), with 2:3 chloroform–methanol as eluant, gave 0.2 g (72%) of the amorphous product. It showed only one spot on a paper chromatogram developed with 5:5:1:4 pyridine–ethyl acetate–acetic acid–water, and had $[\alpha]_D^{25}$ +118°, $[\alpha]_{436}^{25}$ +225° (*c* 0.58, methanol); p.m.r. (D_2O): δ 5.28 (d, 1, *J* 4 Hz), 4.40–3.10 (m, 12, sugar and cyclitol C*H*), and 3.63 (s, 3, OCH_3).

Anal. Calc. for $C_{13}H_{25}NO_{10}\cdot 0.5H_2O$ (364.35): C, 42.86; H, 7.19; N, 3.84. Found: C, 42.74; H, 7.22; N, 3.72.

Presumed 1D-3-O-(3-acetamidino-3-deoxy-α-D-glucopyranosyl)-4-O-methyl-chiro-*inositol hydrochloride* (**12**). — To a solution of compound **11** (80 mg, 0.22 mmol) in 5 ml of methanol, ethyl acetimidate hydrochloride[24] (177 mg, 1.43 mmol) was added in portions. After each addition, methanolic sodium methoxide was added[25] until a drop of the mixture diluted with water registered pH 7–7.5 on pH paper. The mixture was then boiled for 26 h under reflux. After cooling, the mixture was adjusted to pH 5.5 with methanolic hydrogen chloride, and evaporated to low volume *in vacuo*. Precipitated sodium chloride was removed by filtration, and the filtrate was evaporated to dryness. The residue was dissolved in water (3 ml) and applied to a Dowex-50 (H^+) column (1.5 × 12 cm). The column was first washed with water (70 ml) and then eluted with M hydrochloric acid. The fractions giving positive ninhydrin and nitroprusside tests were combined and adjusted to pH 4 with 10% aqueous sodium hydroxide. The solution was evaporated to low volume, and precipitated sodium chloride was filtered off. After further evaporation of the solvent,

the residue was dissolved in a mixture of methanol and benzene, and the solution was again evaporated *in vacuo*. Several repetitions of this operation gave 66 mg of a white amorphous solid that began to decompose at 173°, and had $[\alpha]_D^{25}$ +100°, $[\alpha]_{436}^{25}$ +196° (*c* 0.23, methanol); ν_{max}^{KBr} 1680 cm^{-1} (C=N); p.m.r. (D_2O): δ 5.50 (d, 1, *J* 3.8 Hz, H-1 of sugar), 4.50–3.30 (m, 12, sugar and cyclitol C*H*), 3.67 (s, 3, OCH_3), and 2.39 (*s*, ~3, CH_3C=N). A satisfactory analysis was not obtained, apparently because the product was contaminated with sodium chloride.

*1*D-*3*-O-(*3-Deoxy-3*-p-*toluenesulfonamido-6*-O-p-*tolylsulfonyl*-α-D-*glucopyranosyl*)-*4*-O-*methyl*-chiro-*inositol* (**13**). — An ice-cooled solution of compound **10** (0.293 g, 0.67 mmol) in pyridine (5 ml) was treated with portions of *p*-toluenesulfonyl chloride (total 0.279 g, 1.46 mmol), and allowed to warm to room temperature. After 24 h, the solution contained a major product (R_F 0.76) and several minor products. It was poured into ice and water and the mixture was extracted thrice with chloroform. The washed (water) and dried extract was concentrated *in vacuo* and a portion of toluene was added and evaporated off. The residual brown syrup was taken up in hot ethyl acetate, the solution was decolorized with charcoal and again evaporated, and the residue was taken up in benzene–Skellysolve B. Evaporation of this solution gave a yellow amorphous solid (0.38 g) that was purified first on a column (1.5 × 36 cm) of silica gel (elution with 15:1 benzene–ethanol), then by preparative t.l.c. This procedure yielded 97 mg (19%) of amorphous *1*D-*3*-O-(*3-deoxy-3*-p-*toluenesulfonamido-6*-O-p-*tolylsulfonyl*-α-D-*glucopyranosyl*)-*1,2:5,6-di*-O-*isopropylidene-4*-O-*methyl*-chiro-*inositol*, $[\alpha]_D^{25}$ +66° (*c* 0.99, methanol).

For removal of the *O*-isopropylidene groups, a solution of the compound in 50% aqueous acetic acid (3 ml) was heated during 35 min on a steam bath. Concentration of the solution, the addition of water to the residue and reconcentration, and the addition of toluene followed by evaporation gave the crude product. After purification by preparative t.l.c., there remained 78.5 mg (91%) of amorphous, solid **13**, $[\alpha]_D^{25}$ +82°, $[\alpha]_{436}^{25}$ +161° (*c* 0.80, methanol); p.m.r. (CD_3COCD_3): δ 7.98–7.23 (m, 8, Ph-H), 6.70 (bs, 1, TsN*H*), 2.45 and 2.39 (2 s, 3 each, PhCH_3), no signals for CCH_3 (isopropylidene).

Anal. Calc. for $C_{27}H_{37}NO_{14}S_2$ (663.71): C, 48.86; H, 5.62; N, 2.11; S, 9.66. Found: C, 48.71; H, 5.71; N, 2.04; S, 9.41.

*1*D-*3*-O-(*3,6-Dideoxy-3,6-epimino*-α-D-*glucopyranosyl*)-*4*-O-*methyl*-chiro-*inositol* (**15**). — A solution of the ditosylated pseudodisaccharide **13** (47 mg, 0.071 mmol) in anhydrous methanol (1.5 ml) was treated with sodium methoxide (4.3 mg, 0.08 mmol), and the mixture was heated for 20 min at 58°. The solution was then neutralized with Dowex-50 (pyridinium form) and the mixture filtered. Evaporation of the filtrate gave crude *1*D-*3*-O-(*3,6-dideoxy-3,6*-N-p-*tolylsulfonylepimino*-α-D-*glucopyranosyl*)-*4*-O-*methyl*-chiro-*inositol* (**14**) as a glass.

The *N*-detosylation of **14** and the purification of the product **15** on Dowex-50 were accomplished as described next for the respective model compounds (**18** and **20**). The yield of amorphous, solid **15** was 9.5 mg (40%), $[\alpha]_D^{25}$ +122°, $[\alpha]_{436}^{25}$ +234°

(*c* 0.3, methanol); p.m.r. (D_2O): δ 5.15 (d, 1, $J_{1,2}$ 4 Hz, H-1), 4.45–3.00 (m, 12, sugar and cyclitol C*H*), and 3.80 (s, 3, OCH_3).

Anal. Calc. for $C_{13}H_{23}NO_9$ (337.33): C, 46.29; H, 6.87; N, 4.15. Found: C, 46.09; H, 7.00; N, 3.95.

*Methyl 3-amino-3-deoxy-β-*D*-glucopyranoside* (**16**). — To a solution of methyl 3-azido-3-deoxy-β-D-glucopyranoside (**4**) (723 mg, 3.30 mmol) in anhydrous methanol, 10% palladium on charcoal (82 mg) was carefully added with stirring. The mixture was hydrogenated at room temperature under atmospheric pressure. After 3 h, t.l.c. in 2:3 chloroform–methanol showed complete conversion into a product giving a positive ninhydrin test. The mixture was warmed on a steam bath and then filtered through a Celite pad with suction, and the filter cake was repeatedly washed with hot methanol. Evaporation of the filtrate and recrystallization of the residue from methanol–ether afforded 0.592 g (93%) of **16** as colorless needles, m.p. 207–209° (dec.); $[\alpha]_D^{25}$ −38.5°, $[\alpha]_{436}^{25}$ −76° (*c* 0.74, methanol) (lit.[26] m.p. 207–208°, $[\alpha]_D^{20}$ −39.5°).

*Methyl 3-deoxy-3-*p*-toluenesulfonamido-6-*O*-p-tolylsulfonyl-β-*D*-glucopyranoside* (**17**). — *p*-Toluenesulfonyl chloride (2.48 g, 13 mmol) was added to a stirred suspension of **16** (1.17 g, 6.1 mmol) in dry pyridine. The mixture was stirred for 22 h at room temperature and then poured into ice and water, and extracted thrice with chloroform. The combined extract was washed with water and dried over anhydrous sodium sulfate. Evaporation of the filtrate gave a brown syrup consisting of **17** (R_F 0.38 in 1:1 benzene–ethyl acetate) plus two minor compounds later characterized by p.m.r. analysis as a di-*O*-tosylated product (R_F 0.54) and a tri-*O*-tosylated product (R_F 0.76). The crude syrup was taken up in hot ethyl acetate and decolorized with charcoal, and the filtrate was evaporated to dryness. Purification of the residual solid on a column of silica gel (70 g, 2-cm diameter), with 1:1 benzene–ethyl acetate as eluant, gave 1.46 g (48%) of **17** as a colorless glass, $[\alpha]_D^{25}$ +20°, $[\alpha]_{436}^{25}$ +46° (*c* 1.4, chloroform); p.m.r. ($CDCl_3$): δ 7.83 and 7.34 (q_{AB}, 8, *J* 8 Hz, Ph-H), 5.70 (d, 1, *J* 5 Hz, TsN*H*), and 2.40 (s, 6, PhCH_3).

Anal. Calc. for $C_{21}H_{27}NO_9S_2$ (501.57): C, 50.29; H, 5.43; N, 2.79; S, 12.79. Found: C, 50.12; H, 5.25; N, 2.71; S, 13.18.

*Methyl 3,6-dideoxy-3,6-*N*-p-tolylsulfonylepimino-β-*D*-glucopyranoside* (**18**). — To a solution of compound **17** (1.02 g, 2.03 mmol) in methanol (20 ml), sodium methoxide (119 mg, 2.20 mmol) in methanol (3 ml) was added. The mixture was heated for 50 min at 55°. T.l.c. in 1:1 benzene–ethyl acetate indicated conversion into a major product (R_F 0.40) plus traces of a minor product (R_F 0.28). Evaporation of the methanol and separation of the organic material from the salts by conventional chloroform extraction gave a glassy mass (0.66 g) that crystallized upon the addition of 1:1 benzene–ethyl acetate. Recrystallization yielded 0.47 g (70%) of **18** as colorless prisms, m.p. 134–136°, $[\alpha]_D^{25}$ − 149°, $[\alpha]_{436}^{25}$ −289° (*c* 0.68, chloroform); p.m.r. ($CDCl_3$): δ 7.80–7.40 (q_{AB}, 4, *J* 8 Hz, Ph-H), 4.70 (s, 1, H-1), and 2.40 (s, 3, PhCH_3).

Anal. Calc. for $C_{14}H_{19}NO_6S$ (329.37): C, 51.05; H, 5.81; N, 4.25; S, 9.73. Found: C, 51.39; H, 5.91; N, 4.18; S, 9.88.

Acetylation of a portion of compound **18** with acetic anhydride in pyridine gave *methyl 2,4-di-*O*-acetyl-3,6-dideoxy-3,6-*p*-tolylsulfonylepimino-β-*D*-glucopyranoside* (**19**), which was not purified. The p.m.r. ($CDCl_3$) spectrum of the diacetate showed δ 4.93 (d, 1, $J_{2,3}$ 4 Hz, H-2), 4.60 (s, 1, H-1), 4.57 (m, 1, H-4), 4.44 (t, 1, $J_{4,5}$ and $J_{5,6exo}$ 4 Hz, H-5), 4.12 (dd, 1, collapses to d on irradiation of H-2, $J_{2,3}$ 4 and $J_{3,4}$ 3 Hz, H-3), 3.77 (d, 1, J 11 Hz, H-6 *endo*), and 3.33 (dd, 1, J 4 and 11 Hz, H-6 *exo*).

*Methyl 3,6-dideoxy-3,6-epimino-β-*D*-glucopyranoside* (**20**). — Compound **18** (314 mg, 0.95 mmol) was dissolved in abs. tetrahydrofuran (2 ml) in a flask equipped with a cold-finger type of condenser filled with a Dry Ice–acetone slurry. Ammonia gas was led over potassium hydroxide pellets and condensed in the flask until the volume of the solution reached 6 ml. Freshly cut sodium was added in portions (80 mg in all) to the vigorously stirred (with a glass-coated magnet), refluxing solution until the mixture remained dark blue for 20 min. After evaporation of the ammonia at room temperature, 3 ml of methanol was added to decompose the excess of sodium and the solution was concentrated to dryness, and the residual syrup was taken up in 5 ml of water. The aqueous solution was neutralized to pH ~8.5 by stirring with a few pieces of Dry Ice, exhaustively extracted with chloroform to remove unreacted starting material, and treated with Dowex-50 (pyridinium form). The resulting suspension was applied to a column of Dowex-50 (pyridinium form) (1 × 16 cm), which was eluted with 40 ml of water, followed by 2% ammonium hydroxide solution. Evaporation of the ammonium hydroxide effluent (50 ml) gave a slightly yellow solid. Recrystallization from methanol gave 153 mg (92%) of **20**, m.p. 102.5° (dec.), $[\alpha]_D^{25}$ −178°, $[\alpha]_{436}^{25}$ −347° (*c* 0.93, 1:1 methanol–water); p.m.r. (D_2O): δ 4.81 (s, 1, H-1), 4.17–2.80 (m, 6, H-2,3,4,5,6,6′), and 3.43 (s, 3, OCH_3).

Anal. Calc. for $C_7H_{13}NO_4$ (175.18): C, 47.99; H, 7.48; N, 8.00. Found: C, 48.13; H, 7.63; N, 7.90.

Acetylation of a portion of the epiminoglucoside with acetic anhydride–pyridine gave *methyl 2,4-di-*O*-acetyl-3,6-acetylepimino-3,6-dideoxy-β-*D*-glucopyranoside* (**21**), which was not purified. The p.m.r. ($CDCl_3$) spectrum of **21** showed δ 3.45 (2 s, total 3 H, OCH_3) and 2.5–1.9 (4 s, total 9 H, $COCH_3$).

ACKNOWLEDGMENTS

This work was supported by the College of Agricultural and Life Sciences, University of Wisconsin–Madison, by the Graduate School (with funds from the Wisconsin Alumni Research Founadtion), and by Grant No. AM-10588 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, N.I.H. We thank Prof. H. K. Schnoes and coworkers for the mass spectra, Mr. D. E. Blake for assistance in preparing intermediates, and P. W. Wertz for conducting trials of potential hydrogenolysis catalysts. Mr. D. M. Murphy of the WARF Institute, Madison, kindly provided the assays for antibiotic activity.

REFERENCES

1 Y. SUHARA, K. MAEDA, H. UMEZAWA, AND M. OHNO, *Adv. Chem. Ser.*, 74 (1968) 15–40.
2 M. J. MOHLENKAMP AND L. ANDERSON, *J. Org. Chem.*, 33 (1968) 3163–3165.
3 D. L. STEBBINGS AND L. ANDERSON, unpublished data.
4 C. L. STEVENS, J. NĚMEC, AND G. H. RANSFORD, *J. Am. Chem. Soc.*, 94 (1972) 3280–3281.
5 H. UMEZAWA, T. TSUCHIYA, R. MUTO, AND S. UMEZAWA, *Bull. Chem. Soc. Jpn.*, 45 (1972) 2842–2847.
6 S. UMEZAWA, Y. NISHIMURA, H. HINENO, K. WATANABE, S. KOIKE, T. TSUCHIYA, AND H. UMEZAWA, *Bull. Chem. Soc. Jpn.*, 45 (1972) 2847–2851.
7 H. PAULSEN AND W. STENZEL, *Angew. Chem.*, 87 (1975) 547–548; H. PAULSEN, O. LOCKHOFF, B. SCHRÖDER, B. SUMFLETH, AND W. STENZEL, *Tetrahedron Lett.*, (1976) 2301–2304.
8 B. T. LAWTON, W. A. SZAREK, AND J. K. N. JONES, *Carbohydr. Res.*, 15 (1970) 397–402.
9 Y. TAKAGI, T. TSUCHIYA, AND S. UMEZAWA, *Bull. Chem. Soc. Jpn.*, 46 (1973) 1261–1262.
10 C. L. STEVENS, G. H. RANSFORD, J. NĚMEC, J. M. CAHOON, AND P. M. PILLAI, *J. Org. Chem.*, 39 (1974) 298–302.
11 V. D. GROB, T. G. SQUIRES, AND J. R. VERCELLOTTI, *Carbohydr. Res.*, 10 (1969) 595–597.
12 A. HASEGAWA, N. KURIHARA, D. NISHIMURA AND M. NAKAJIMA, *Agr. Biol. Chem.*, 32 (1968) 1123–1129; D. NISHIMURA, A. HASEGAWA, AND M. NAKAJIMA, *ibid.*, 36 (1972) 1767–1772.
13 Y. NISHIMURA, T. TSUCHIYA, AND S. UMEZAWA, *Bull. Chem. Soc. Jpn.*, 44 (1971) 2521–2528.
14 S. NISHIMURA, M. SHIMAHARA, AND M. SHIOTA, *J. Org. Chem.*, 31 (1966) 2394–2395.
15 W. MEYER ZU RECKENDORF, *Chem. Ber.*, 97 (1964) 1275–1285; W. MEYER ZU RECKENDORF AND N. WASSILIADOU-MICHELI, *ibid.*, 103 (1970) 37–45; W. MEYER ZU RECKENDORF, *ibid.*, 103 (1970) 995–996.
16 D. IKEDA, T. TSUCHIYA, AND S. UMEZAWA, *Bull. Chem. Soc. Jpn.*, 44 (1971) 2529–2537.
17 K. E. ESPELIE AND L. ANDERSON, *Carbohydr. Res.*, 46 (1976) 53–66.
18 D. C. DEJONGH, T. RADFORD, J. D. HRIBAR, S. HANESSIAN, M. BIEBER, G. DAWSON, AND C. C. SWEELEY, *J. Am. Chem. Soc.*, 91 (1969) 1728–1740.
19 K. HEYNS AND H. SCHARMANN, *Carbohydr. Res.*, 1 (1966) 371–392.
20 H. J. JENNINGS AND J. K. N. JONES, *Can. J. Chem.*, 43 (1965) 2372–2386.
21 IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC–IUB Commission on Biochemical Nomenclature, *Pure Appl. Chem.*, 37 (1974) 285–297.
22 S. J. ANGYAL AND C. G. MACDONALD. *J. Chem. Soc.*, (1952) 686–695.
23 S. J. ANGYAL AND R. M. HOSKINSON, *J. Chem. Soc.*, (1962) 2985–2991.
24 A. W. DOX, in H. GILMAN AND A. H. BLATT (Eds.), *Org. Syn. Coll. Vol.* 1, 2nd ed. (1944) 5–7.
25 M. J. CRON, R. E. SMITH, I. R. HOOPER, J. G. KEIL, E. A. RAGAN, R. H. SCHREIBER, G. SCHWAB, AND J. C. GODFREY, *Antimicrob. Agents Chemother.*, (1970) 219–224.
26 H. H. BAER, *Chem. Ber.*, 93 (1960) 2865–2871.