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STEREOSELECTIVE SYNTHESIS OF 5-*O*-CARBAMOYLPOLYOXAMIC ACID (2-AMINO-5-*O*-CARBAMOYL-2-DEOXY-L-XYLONIC ACID)*

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ABSTRACT

One of the constituents of polyoxin J, 2-amino-5-*O*-carbamoyl-2-deoxy-L-xylonic acid (**3**), has been synthesized stereoselectively from L-sorbopyranose. The amino acid function of **3** was formed in the final stage of the synthesis by reduction of the corresponding α-azido carboxylic acid.

INTRODUCTION

During work on the structural elucidation of the components of a novel antifungal antibiotic, polyoxin complex, Isono and co-workers[1] obtained 2-amino-2-deoxy-L-xylonic acid (**2**) as a common constituent of hydrolysates of polyoxins A, B, D, F, H, J, and L, and designated this compound as polyoxamic acid. Since another common constituent was 5-*O*-carbamoylpolyoxamic acid (**3**), **2** was considered to be an artefact. We have reported[2] the preparation of **3** as a part of our project[3] on the total synthesis of polyoxin J (**1**), and we now present full details.

5-*O*-Carbamoylpolyoxamic acid (**3**) has diverse functional groups and numerous asymmetric carbon atoms, and its synthesis presents several problems, including generation of the L-*xylo* configuration which is difficult as far as 2-amino sugars are concerned[4,5]. Moreover, **2**, which has been prepared already *via* 2-acetamido-2-deoxy-L-xylose[4], is a polyhydroxy amino acid that may undergo undesirable side-reactions such as racemization, lactonization, and elimination, so that it cannot be used as a synthetic intermediate for **3**. There is also the necessity for adequate protection of diverse functional groups during the synthesis. Preferential protection of one of the two terminal hydroxyl groups of acyclic polyols, which would be key intermediates to **3**, presents a particular problem.

A synthesis was therefore designed which used 1,2-*O*-isopropylidene-α-L-sorbopyranose[6] (**4**) as starting material, and involved an azide displacement reaction and generation of the amino acid function in the final stage.

*Dedicated to the memory of Dr. Hewitt G. Fletcher, Jr.

galactose dehydrogenase (see above), using lactose and melibiose as controls. Incubations with β-D-glucuronidase from *Helix pomatia* (EC 3.2.1.31; Boehringer, No. 15472; free of α-D-glucuronidase activity[42,58]) were carried out in a pH 4.5 acetate buffer[42,58,77], demonstrating the liberation of glucuronic acid by paper electrophoresis (see above).

The paper-electrophoretic analysis of partially hydrolysed, tritium-labelled oligosaccharide *H5.1* was done as follows. At 4°, 20 µl of 0.01M NaOH, containing 500 µCi of $NaBH_4/NaBT_4$ (specific activity: 480 µCi/µmole) were added to solutions of oligosaccharides *H3*, *H5.1*, and *H5.3* (~1 mg of each, in 0.01M NaOH), and the mixtures were kept at 4° overnight. After decomposition of excess reducing agent with dilute acetic acid, boric acid was removed by evaporation with methanol, and a portion of the reduced *H5.1* was partially hydrolysed (0.5M H_2SO_4, 20 min, 100°). All radioactive products were subjected to paper electrophoresis in pyridine–glacial acetic acid–water (see above), and the electropherogram strips were analysed with a Packard radiogram scanner.

P.m.r. spectroscopy. — The p.m.r. spectra of native *Klebsiella* type 11 polysaccharide, and of the polymer obtained after Smith degradation (see above) in absolute D_2O (2–3%) were run at 90 to 95°, using a Varian HR 220 instrument at 220 MHz, with the sodium salt of 3-trimethylsilylpropanesulfonic acid as an internal standard.

Serological cross-reactions. — The standard procedures described by Kauffmann[38] were used for the immunization of rabbits, as well as for bacterial slide and tube agglutinations.

RESULTS

Isolation, serological identification, homogeneity, and physical properties of Klebsiella *serotype 11 capsular polysaccharide.* — As expected[42,43] for a material carrying the serological *Klebsiella* K11 determinants, type-11 polysaccharide, as isolated from *Klebsiella* 390 (03:K11) by the phenol–water–Cetavlon technique[43,44], yielded a precipitation reaction in Ouchterlony agar gel double-diffusion[45] against a *Klebsiella* 390 OK serum—one precipitation line with alkali-treated, two (hardly separated) with native material—but none against an *E. coli* 09 serum (although the *Klebsiella* 03 and *E. coli* 09 antigens are serologically identical[29]). In the analytical ultracentrifuge, native type-11 polysaccharide (0.4% in PBS) showed a double peak with $s^c_{25,\,solv} = 2.8 \times 10^{-13}$ and 3.0×10^{-13} sec. After mild alkali-treatment, however, homogeneous sedimentation ($s^c_{25,\,solv} = 2.9 \times 10^{-13}$ sec) was observed. For the alkali-treated product, a sedimentation coefficient ($s^0_{20,w}$) of 4.55×10^{-13} sec, a partial specific volume (V_p) of 0.6129 ml/g, a molecular weight ($\overline{M}_w$) of 298,000, $[\alpha]_D^{25}$ +106° (*c* 1.0, water), and a limiting viscosity number ([η], at 37° in PBS) of 290 ml/g (against 620 ml/g for the native material) were determined.

Constituents. — Qualitative analysis of type-11 polysaccharide showed the presence of D-glucuronic acid, D-glucose, D-galactose, acetate, pyruvate, and trace

amounts of mannose, in agreement with the results reported by Nimmich[3]. The quantitative composition of the material is given in Table I; it approaches molar ratios of Gal:Glc:GlcUA:pyruvate:acetate of 2:1:1:1:0.5, if the trace amounts of mannose are considered to be due to a small contamination with *Klebsiella* 03 cell-wall lipopolysaccharide, which is known to contain large proportions of this sugar[20,78]. The analysis of type-11 polysaccharide after esterification with diazomethane and subsequent reduction with sodium borohydride is also included in Table I; 92% of the glucuronic acid, but only 23% of the pyruvic acid carboxyl-groups were reduced by this procedure (possibly due to more successful competition of hydrolysis during reduction of the latter).

Periodate oxidation. — Both the native and the alkali-treated polymer consumed 0.8 mole of periodate per mole of glucose. Periodate-oxidized material was quantitatively analysed for loss of original constituents before and after reduction with sodium borohydride and "Smith hydrolysis"[64] (Table I). The results show that approximately one mole of galactose per mole of glucose was destroyed by periodate. The material obtained after "Smith hydrolysis", which was essentially non-dialysable (72% yield from the polyalcohol), had also lost most of the pyruvate. By g.l.c. of the alditol acetates obtained from oxidized and then reduced type-11 polysaccharide, threose could be identified as one of the products formed by periodate treatment.

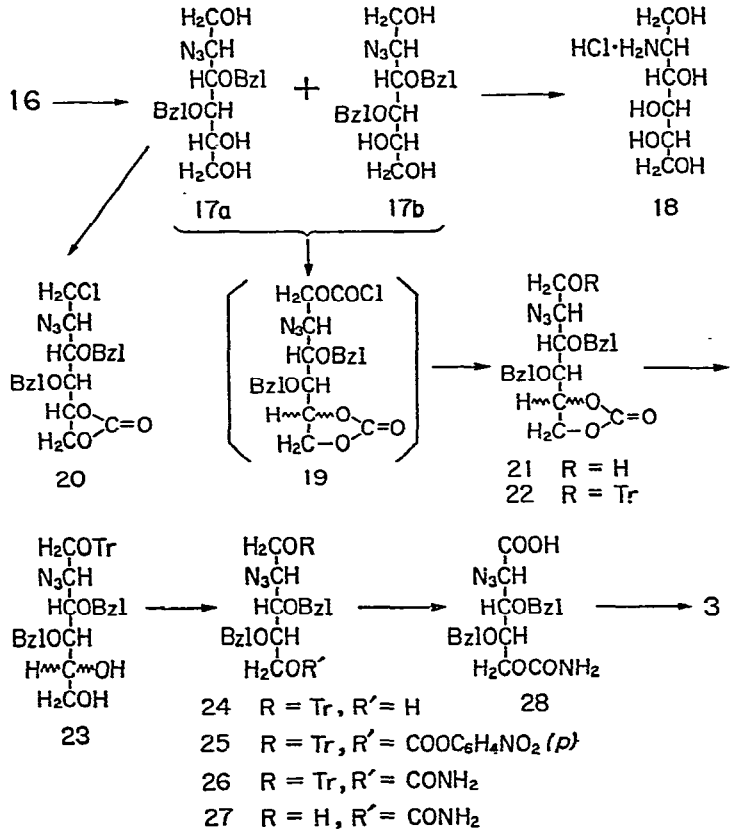
TABLE I

QUANTITATIVE COMPOSITION (PERCENT ANHYDRO RESIDUES AND THEIR MOLAR RATIOS BASED ON GLUCOSE) OF *Klebsiella* SEROTYPE 11 CAPSULAR POLYSACCHARIDE AND ITS DERIVATIVES

	I[a]	*II*	*III*	*IV*	*V*	*VI*
D-Glucose	17.4 ±0.5(1.00)	17.9(1.00)	34.2(2.00)	17.3(1.00)	17.2(1.00)	24.4(1.00)
D-Galactose	31.8 ±1.2(1.83)	32.4(1.81)	33.0(1.93)	19.4(1.12)	18.1(1.05)	24.9(1.02)
D-Glucuronic acid	20.6 ±0.8(1.09)	20.5(1.06)	1.7(0.09)	20.0(1.07)	20.7(1.11)	26.8(1.02)
Pyruvate[b]	6.9 ±0.3(0.93)	6.8(0.89)	5.3(0.72)	6.7(0.90)	6.9(0.94)	1.3(0.13)
Acetyl[b]	2.1 ±0.2(0.45)	0	0	n.d.	0	0
Total[c]	96.5					

[a]I, Native; II, alkali-treated; III, alkali-treated, esterified with diazomethane, and reduced with sodium borohydride; IV, native, oxidized with periodate; V, alkali-treated, and oxidized with periodate; VI, alkali-treated, oxidized with periodate, reduced with sodium borohydride, subjected to "Smith hydrolysis", and dialysed. [b]Calculated as CH_3–CO–COO^- and CH_3COOH minus H_2O. [c]Including 2.5% mannose, 10.0% water (loss of weight after 24 h at 50° *in vacuo* over phosphorus pentaoxide), 0.2% nucleic acid (estimated from the absorption at 260 nm with yeast RNA as a standard), and 5.0% sodium (calculated on the basis of the glucuronic and pyruvic acid values).

Methylation analysis. — Alkali-treated type-11 polysaccharide, esterified with diazomethane and then reduced with sodium borodeuteride in deuterium oxide, as well as partially autohydrolysed polymer (selective removal of 45% of the pyruvate substituents) were permethylated, and the methylated sugars obtained upon hydrolysis were analysed by g.l.c.–m.s. (Table II). The results showed that the glycan contained



2-deoxy-D-iditol and 2-azido-2-deoxy-L-glucitol derivatives. For protection of the vicinal diol grouping, **17ab** was treated with excess carbonyl chloride in chloroform–pyridine at −10°. The resulting 5,6-*O*-carbonyl-1-*O*-chloroformyl derivative **19** was not isolated but treated with water to hydrolyze the chloroformate group at C-1 to give a mixture (**21**) of 2-azido-3,4-di-*O*-benzyl-5,6-*O*-carbonyl-2-deoxy-L-iditol and the corresponding D-glucitol derivative. In a preliminary experiment, **17a** was treated with carbonyl chloride in pyridine at room temperature, but the product was 2-azido-3,4-di-*O*-benzyl-5,6-*O*-carbonyl-1-chloro-1,2-dideoxy-D-iditol (**20**), not the iditol component of **19**. The isomeric mixture **21** was converted into the 1-*O*-trityl derivative **22**, the 5,6-carbonate group of which was hydrolyzed with base to give a mixture (**23**) of 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-*O*-trityl-D-iditol and the corresponding L-glucitol derivative. The mixture **23** was cleaved between C-5 and C-6 on treatment with sodium periodate, and the product was immediately reduced at 0° with sodium borohydride to afford 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-*O*-trityl-L-xylitol (**24**). The crystalline 5-*p*-nitrophenoxycarbonate (**25**) of **24** was converted into the 5-carbamate **26** by methanolic ammonia. Treatment of **26** with aqueous trifluoroacetic acid gave 2-azido-3,4-di-*O*-benzyl-5-*O*-carbamoyl-2-deoxy-L-xylitol (**27**).

Oxidation of **27** with chromium trioxide in acetone and 3.5M sulphuric acid gave 2-azido-3,4-di-*O*-benzyl-5-*O*-carbamoyl-2-deoxy-L-xylonic acid (**28**) in moderate yield. Because of the different solubility characteristics of **28** and **3**, the catalytic reduction of **28** over palladium-on-carbon was carried out in two stages; first in 67% aqueous acetic acid, and then in aqueous media. The resulting crystalline **3** had physical constants (m.p., $[\alpha]_D$) which agreed with those of an authentic specimen derived from natural polyoxins, and the chromatographic behaviour and i.r. spectra of the two compounds were identical.

EXPERIMENTAL

General methods. — M.p.s. are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter, using a 1-dm tube. I.r. spectra were recorded with a Shimadzu IR-27G spectrometer, and n.m.r. spectra (100 MHz) with a Varian HA-100D spectrometer for solutions in $CDCl_3$ (internal Me_4Si). Kieselgel 60 (E. Merck, Darmstadt) was used for column chromatography. Organic solutions were dried over Na_2SO_4.

1,2-O-Isopropylidene-3,4,5-tri-O-mesyl-α-L-sorbopyranose (**5**). — Mesyl chloride (25 ml) was added to a solution of **4** (10.7 g) in dry pyridine (200 ml) at 0°. The mixture was kept for 2 days at 5°, then poured into ice–water, and extracted with chloroform (1000 ml). The extract was washed successively with aqueous sulphuric acid (3%) and water, dried, and concentrated *in vacuo*. The resulting syrup was crystallised from methanol (~150 ml) to give **5** (19.8 g, 90%), m.p. 125–127°, $[\alpha]_D^{24}$ −41° (*c* 0.9, chloroform); ν_{max}^{film} 1350 and 1170 cm^{-1} (SO_2). N.m.r. data: δ 4.69 (d, 1 H, $J_{3,4}$ 9 Hz, H-3), 4.73 (o, 1 H, $J_{4,5}$ 9, $J_{5,6a}$ 10.5, $J_{5,6e}$ 6 Hz, H-5), 5.09 (t, 1 H, $J_{3,4}$ 9, $J_{4,5}$ 9 Hz, H-4).

Anal. Calc. for $C_{12}H_{22}O_{12}S_3$: C, 31.72; H, 4.85; S, 21.15. Found: C, 31.79; H, 4.48; S, 21.10.

5-Azido-5-deoxy-1,2-O-isopropylidene-3,4-di-O-mesyl-β-D-fructopyranose (**6**). — A mixture of **5** (900 mg) and sodium azide (170 mg, 1.3 equiv.) in hexamethylphosphoric triamide (15 ml) was heated overnight at 110°, with stirring, then cooled, diluted with water (100 ml), and extracted with ethyl ether (200 ml). The extract was washed with water, dried, and concentrated *in vacuo* to afford syrupy **6** (800 mg, ~100%) which showed a single spot on t.l.c. Elution from silica gel with 5:1 benzene–ethyl ether gave material having $[\alpha]_D^{23}$ −107° (*c* 3.5, chloroform); ν_{max}^{film} 2100 (N_3), 1350, and 1170 cm^{-1} (SO_2). N.m.r. data: δ 3.99–4.37 (m, 4 H, $J_{5,6e}$ 1.8 Hz, H-1,1′,5,6e), 4.97 (d, 1 H, $J_{3,4}$ 10 Hz, H-3), 5.16 (q, 1 H, $J_{3,4}$ 10, $J_{4,5}$ 3.5 Hz, H-4).

Anal. Calc. for $C_{11}H_{19}N_3O_9S_2$: C, 32.91; H, 4.77; N, 10.47; S, 15.98. Found: C, 33.01; H, 4.82; N, 10.42; S, 15.88.

3,4-Anhydro-5-azido-5-deoxy-1,2-O-isopropylidene-β-D-psicopyranose (**7**). — A solution of sodium methoxide [from sodium (1.8 g) and methanol (40 ml)] was added to a solution of **6** (8 g) in methanol (50 ml) at 0°. The mixture was kept for 3 days at room temperature, then poured into ice–water (300 ml), and extracted with ethyl ether

(500 ml). The extract was washed with water, dried, and concentrated *in vacuo* to give a colourless syrup (4.5 g) which was eluted from silica gel with 8:1 benzene–ethyl ether to afford syrupy **7** (2.2 g, 49%), unchanged **6** (0.3 g), and a trace of **13**. Compound **7**, which could also be isolated from the mixture by distillation (90°/2 torr) in better yield and crystallized, had m.p. 46–50°, $[\alpha]_D^{22}$ +5° (*c* 1.4, chloroform), ν_{max}^{film} 2100 cm^{-1} (N_3). N.m.r. data: δ 3.17 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-3),

Anal. Calc. for $C_9H_{13}N_3O_4$: C, 47.57; H, 5.77; N, 18.49. Found: C, 47.66; H, 5.79; N, 18.16.

5-Azido-5-deoxy-1,2-O-isopropylidene-β-D-sorbopyranose (**11**) *and its acetate* (**12**). — A suspension of **7** (2.1 g) in aqueous potassium hydroxide (5%, 400 ml) was heated under reflux with stirring for 7 h and then cooled. The mixture was neutralized with excess of solid carbon dioxide and concentrated *in vacuo*, and the residue was extracted with acetone (200 ml). The extract was concentrated *in vacuo*, and the resulting brown syrup (1.7 g) was eluted from silica gel with 5:1 benzene–acetone to give **11** (1.3 g, 59%), m.p. 100–102°, $[\alpha]_D^{22}$ −72° (*c* 1.75, chloroform); ν_{max}^{KBr} 3580, 3400 (OH), and 2100 cm^{-1} (N_3). N.m.r. data: δ 3.13 (s, 2 H, 2 HO), 3.40–3.82 (m, 4 H), 4.00–4.40 (m, 3 H).

Anal. Calc. for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.14. Found: C, 44.31; H, 6.06; N, 17.15.

The yield of **11** from **6** increased to 41% if the mixture of **7**, unchanged **6**, and **13** was treated with aqueous potassium hydroxide containing a little *p*-dioxane and then chromatographed as described above. Therefore, the preparation of **11** on a large scale was performed without any isolation of **7**.

Treatment of **11** with acetic anhydride and pyridine, in the usual way, gave the diacetate **12**, m.p. 89–92° (from isopropyl ether), $[\alpha]_D^{25}$ −34° (*c* 0.6, chloroform); ν_{max}^{KBr} 2100 (N_3) and 1760–1750 cm^{-1} (C=O). N.m.r. data: δ 3.69 (sex, 1 H, $J_{4,5}$ 8, $J_{5,6a}$ 8, $J_{5,6e}$ 4 Hz, H-5), 4.93 (t, 1 H, $J_{3,4}$ 8, $J_{4,5}$ 8 Hz, H-4), 5.14 (d, 1 H, $J_{3,4}$ 8 Hz, H-3).

Anal. Calc. for $C_{13}H_{19}N_3O_7$: C, 47.42; H, 5.82; N, 12.76. Found: C, 47.62; H, 5.95; N, 12.75.

5-Azido-5-deoxy-1,2-O-isopropylidene-4-O-methyl-β-D-sorbopyranose (**13**) *and its acetate* (**14**). — A solution of **7** (1.1 g) and potassium hydroxide (0.56 g) in methanol (10 ml) was heated under reflux for 24 h, then neutralized with carbon dioxide, and poured into water. The mixture was extracted with dichloromethane, and the extract was washed with water, dried, and concentrated *in vacuo* to afford **13** (1.24 g, 96%), m.p. 88–89° (from cyclohexane), $[\alpha]_D^{25}$ −9° (*c* 0.8, chloroform); ν_{max}^{KBr} 3440 (OH) and 2100 cm^{-1} (N_3). N.m.r. data: δ 3.69 (bd, 1 H, sharpened on addition of water, $J_{3,4}$ 7 Hz, H-3).

Anal. Calc. for $C_{10}H_{17}N_3O_5$: C, 46.33; H, 6.61; N, 16.21. Found: C, 46.62; H, 6.64; N, 16.19.

Treatment of **13** (600 mg) with acetic anhydride and pyridine, in the usual way, gave a syrupy 2-acetate (**14**, ~100%), $[\alpha]_D^{26}$ −60° (*c* 1.9, chloroform); ν_{max}^{film} 2100 (N_3) and 1765–1735 cm^{-1} (C=O). N.m.r. data: δ 5.07 (d, 1 H, $J_{3,4}$ 6.5 Hz, H-3).

Anal. Calc. for $C_{12}H_{19}N_3O_6$: C, 47.84; H, 6.36; N, 13.95. Found: C, 47.92; H, 6.30; N, 13.96.

*5-Azido-3,4-di-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-β-*D*-sorbopyranose* (**15**).— A suspension of powdered potassium hydroxide (5 g) and **11** (2.5 g) in benzyl chloride (18 ml) was heated for 8 h at 90° with vigorous stirring, then cooled, diluted with water, and extracted with ethyl ether (150 ml). The extract was washed with water, dried, and concentrated *in vacuo* to a yellow syrup (4.4 g) which was eluted from silica gel with 15:1 benzene–ethyl ether to yield **15** (3 g, 70%), m.p. 57–62°, $[\alpha]_D^{19}$ +80° (*c* 1.85, chloroform); ν_{max}^{KBr} 2100 (N_3) and 1500 cm^{-1} (phenyl).

Anal. Calc. for $C_{23}H_{27}N_3O_5$: C, 64.93; H, 6.40; N, 9.88. Found: C, 65.12; H, 6.39; N, 9.80.

*5-Azido-3,4-di-*O*-benzyl-5-deoxy-*D*-sorbopyranose* (**16**). — Aqueous sulphuric acid (2%, 550 ml) was added to a solution of **15** (9 g) in propan-2-ol (650 ml). The mixture was heated for 6 h at 80°, then neutralized with barium carbonate, filtered, and concentrated *in vacuo*. The residue was eluted from silica gel (750 g) with 2:1 cyclohexane–ethyl acetate to afford **16** (6.5 g, 80%), m.p. 72–75° (from cyclohexane containing a little ethyl acetate), $[\alpha]_D^{22}$ +46° (*c* 0.5, chloroform, no mutarotation); ν_{max}^{KBr} 3450 (OH), 2100 (N_3), and 1500 cm^{-1} (phenyl).

Anal. Calc. for $C_{20}H_{23}N_3O_5$: C, 62.33; H, 6.02; N, 10.90. Found: C, 62.31; H, 5.81; N, 10.94.

*2-Azido-3,4-di-*O*-benzyl-2-deoxy-*D*-iditol* (**17a**) *and 2-azido-3,4-di-*O*-benzyl-2-deoxy-*L*-glucitol* (**17b**). — Sodium borohydride (1.2 g) was added in portions at 0° to a solution of **16** (1 g) in diglyme (50 ml), and the mixture was kept for 1.5 h at 0° with stirring. Aqueous acetic acid (10%, 27 ml) was then added dropwise and the resulting mixture was concentrated *in vacuo* to dryness. Methanol was thrice distilled from the residue which was then extracted with ethyl acetate. The extract was dried, and concentrated *in vacuo*, and the residue was eluted from silica gel with 94:6 chloroform–methanol to afford **17a** (550 mg, 55%) and **17b** (180 mg, 18%). Compound **17a** had m.p. 104–106° (from ethyl acetate–hexane), $[\alpha]_D^{18}$ +13° (*c* 1, ethanol); ν_{max}^{KBr} 3450, 3350 (OH), 2100 (N_3), and 1500 cm^{-1} (benzene).

Anal. Calc. for $C_{20}H_{25}N_3O_5$: C, 62.00; H, 6.50; N, 10.85. Found: C, 61.77; H, 6.37; N, 10.86.

Compound **17b** had m.p. 71–73° (from isopropyl ether), $[\alpha]_D^{19}$ −7° (*c* 1.75, ethanol); ν_{max}^{KBr} 3510, 3420 (OH), 2100 (N_3), and 1500 cm^{-1} (phenyl).

Anal. Found: C, 61.97; H, 6.27; N, 10.83.

*2-Amino-2-deoxy-*L*-glucitol hydrochloride* (**18**). Conc. hydrochloric acid (0.2 ml) was added to a solution of **17b** (0.7 g) in aqueous methanol (50%, 40 ml), and the mixture was shaken with hydrogen in the presence of palladium-on-carbon (10%). The catalyst was removed and the filtrate was concentrated *in vacuo*. The residue was recrystallized from a little aqueous ethanol to give **18** (0.2 g, 51%), m.p. 159–161°, $[\alpha]_{365}^{19}$ +7° (*c* 0.7, water); ν_{max}^{KBr} 3410, 3320–3280, 3150, 3050 (OH, NH_3^+), 1630, 1615, and 1530 cm^{-1} (NH_3^+). The i.r. spectrum and X-ray diffraction pattern were identical with those of the authentic D enantiomer[9], m.p. 159–161°, $[\alpha]_{365}^{19}$ −6.8° (*c* 0.76, water).

Anal. Calc. for $C_6H_{16}ClNO_5$: C, 33.11; H, 7.41; N, 6.44. Found: C, 33.29; H, 7.43; N, 6.44.

*2-Azido-3,4-di-*O*-benzyl-5,6-*O*-carbonyl-2-deoxy-*D*-iditol/*L*-glucitol* (**21**). — A solution of carbonyl chloride (17 g) in toluene (120 ml) was added to a mixture of chloroform (170 ml) and pyridine (43 g), and cooled in an ice–salt bath. To this solution was added a solution of **17ab** (6.9 g) in chloroform (170 ml). The resulting mixture was stirred for 1 h 40 min at −10°, and then water (200 ml) was added dropwise with vigorous stirring. The chloroform layer was separated, washed successively with aqueous sulphuric acid (3%) and water, dried, and concentrated *in vacuo* to afford crude **21** (7.3 g, 99%). Elution from silica gel with 5:2 cyclohexane–ethyl acetate gave material having ν_{max}^{film} 3500–3450 (OH), 2100 (N_3), 1800–1780 (C=O), and 1500 cm^{-1} (phenyl).

Anal. Calc. for $C_{21}H_{23}N_3O_6$: C, 61.01; H, 5.61; N, 10.16. Found: C, 60.56; H, 5.38; N, 10.11.

*2-Azido-3,4-di-*O*-benzyl-5,6-*O*-carbonyl-1-chloro-1,2-dideoxy-*D*-iditol* (**20**). — A solution of **17a** (400 mg) in dry pyridine (10 ml) was added dropwise at 0° to a large excess of carbonyl chloride in a mixture of toluene (20 ml) and pyridine (15 ml). The mixture was stirred for 3 h at room temperature, then diluted carefully with water (100 ml), and extracted with ethyl ether. The extract was washed successively with aqueous sulphuric acid (3%) and water, dried, and concentrated *in vacuo* to afford a red syrup (400 mg) which was eluted from silica gel with 5:1 benzene–ethyl ether to give syrupy **20** (290 mg, 66%), $[\alpha]_D^{24}$ −20.5° (*c* 1.9, chloroform); ν_{max}^{film} 2100 (N_3), 1810–1790 (C=O), and 1500 cm^{-1} (phenyl), but no absorption higher than 3200 cm^{-1}.

Anal. Calc. for $C_{21}H_{22}ClN_3O_5$: C, 58.40; H, 5.13; Cl, 8.21; N, 9.73. Found: C, 58.39; H, 5.11; Cl, 8.06; N, 9.74.

*2-Azido-3,4-di-*O*-benzyl-5,6-*O*-carbonyl-2-deoxy-1-*O*-trityl-*D*-iditol/*L*-glucitol* (**22**). — Trityl chloride (8.8 g) was added to a solution of **21** (6.6 g) in dry pyridine (120 ml). The mixture was kept for 6 days at room temperature, then diluted with water, and extracted with ethyl ether. The extract was washed with water, dried, and concentrated *in vacuo* (below 1 torr). The resulting syrup was eluted from silica gel with 4:1 hexane–ethyl acetate to give **22** as a glass (7.8 g, 75%); ν_{max}^{KBr} 2100 (N_3), 1800–1790 (C=O), and 1490 cm^{-1} (phenyl).

Anal. Calc. for $C_{40}H_{37}N_3O_6$: C, 73.27; H, 5.69; N, 6.41. Found: C, 73.17; H, 5.39; N, 6.33.

In another experiment, one of the diastereoisomers crystallised from the mixture and had m.p. 140–143°, $[\alpha]_D^{21}$ −2° (*c* 1.4, chloroform).

*2-Azido-3,4-di-*O*-benzyl-2-deoxy-1-*O*-trityl-*D*-iditol/*L*-glucitol* (**23**). — Methanolic sodium methoxide (1%, 70 ml) was added to a solution of **22** (7.6 g) in a mixture of *p*-dioxane (70 ml) and methanol (70 ml). The mixture was kept overnight at room temperature, then neutralized with excess of solid carbon dioxide, and concentrated *in vacuo*. The residue was extracted with chloroform (200 ml), and the extract was concentrated *in vacuo* to give a pale yellow syrup (7.3 g, ~100%). A part of this syrup

was eluted from silica gel with 6:1 benzene–ethyl ether to give material having ν_{max}^{film} 3450–3400 (OH), 2100 (N_3), and 1495 cm^{-1} (phenyl).

Anal. Calc. for $C_{39}H_{39}N_3O_5$: C, 74.38; H, 6.24; N, 6.67. Found: C, 74.67; H, 6.30; N, 6.73.

*2-Azido-3,4-di-*O*-benzyl-2-deoxy-1-*O*-trityl-*L*-xylitol* (**24**). — A solution of sodium periodate (5.3 g) in water (100 ml) was added at 0° to a solution of crude **23** (7.2 g) in ethanol (400 ml). The mixture was stirred for 2 h at 0°, then diluted with water, and extracted with ethyl acetate (1000 ml). The extract was washed with water, dried, and concentrated *in vacuo* below 40°. A solution of the resulting colourless syrup (6.3 g) in methanol (250 ml) was cooled in an ice-bath. Sodium borohydride (1.6 g) was added in several portions, the mixture was stirred for 1 h at 0°, acetone (50 ml) was then added, and the mixture was kept for several hours at room temperature before concentration *in vacuo*. The residue was extracted with chloroform, and the extract was washed with water, dried, and concentrated *in vacuo* to afford yellow, syrupy **24** (6.7 g, 97% based on **23**) which was eluted from silica gel with 4:1 cyclohexane–ethyl acetate to give material having $[\alpha]_D^{20}$ +15° (*c* 1.2, chloroform); ν_{max}^{film} 3470 (OH), 2100 (N_3), and 1495 cm^{-1} (phenyl).

Anal. Calc. for $C_{38}H_{37}N_3O_4$: C, 76.10; H, 6.22; N, 7.01. Found: C, 76.04; H, 6.00; N, 7.07.

*2-Azido-3,4-di-*O*-benzyl-2-deoxy-5-*O*-*p*-nitrophenoxycarbonyl-1-*O*-trityl-*L*-xylitol* (**25**). — A mixture of **24** (2.7 g) and *p*-nitrophenoxycarbonyl chloride (1.1 g) in pyridine (40 ml) was stirred overnight at room temperature. More *p*-nitrophenoxycarbonyl chloride (0.6 g) was added, and the resulting mixture was stirred for 2 days and then diluted with benzene (600 ml). The solution was washed successively with aqueous sulphuric acid (1.5%), 2M sodium carbonate, and water, dried, and concentrated *in vacuo*. The residue was eluted from silica gel with benzene to give **25** (2.1 g, 61%), m.p. 106–108° (from cyclohexane), $[\alpha]_D^{23}$ +8° (*c* 1.8, chloroform); ν_{max}^{film} 2100 (N_3), 1770–1760 (C=O), 1595, 1495 (phenyl), 1525, and 1350 cm^{-1} (NO_2).

Anal. Calc. for $C_{45}H_{40}N_4O_8$: C, 70.69; H, 5.27; N, 7.33. Found: C, 70.85; H, 5.25; N, 7.36.

*2-Azido-3,4-di-*O*-benzyl-5-*O*-carbamoyl-2-deoxy-1-*O*-trityl-*L*-xylitol* (**26**). — Methanolic ammonia (1.8%, 2.8 ml) was added at 0° to a solution of **25** (1.1 g) in dichloromethane (14 ml). The mixture was kept overnight at room temperature, then diluted with dichloromethane (200 ml), and washed successively with 2M sodium carbonate and water, dried, and concentrated *in vacuo*. The residual syrup was eluted from silica gel with 10:1 benzene–ethyl ether to give **26** (0.63 g, 68%), m.p. 45–50°, $[\alpha]_D^{26}$ +17° (*c* 0.9, chloroform); ν_{max}^{film} 3510, 3400–3350 (NH_2), 2100 (N_3), 1730–1720 (C=O), 1600 (NH_2, benzene), and 1495 cm^{-1} (phenyl).

Anal. Calc. for $C_{39}H_{38}N_4O_5$: C, 72.88; H, 5.96; N, 8.72. Found: C, 72.70; H, 5.89; N, 8.79.

*2-Azido-3,4-di-*O*-benzyl-5-*O*-carbamoyl-2-deoxy-*L*-xylitol* (**27**). — A solution of **26** (630 mg) in aqueous trifluoroacetic acid (90%, 5.5 ml) was kept for 15 min at room temperature, then diluted with water (200 ml), and extracted with chloroform

(200 ml). The extract was washed successively with saturated, aqueous sodium hydrogen carbonate and water, dried, and concentrated *in vacuo*. The residue was eluted from silica gel with 94:6 chloroform–methanol to give **27** as a colourless syrup (370 mg, 94%), $[\alpha]_D^{25}$ +12° (*c* 0.9, chloroform); ν_{max}^{film} 3500–3350 (OH, NH_2), 2100 (N_3), 1720–1710 (C=O), 1600 (NH_2, phenyl), and 1495 cm^{-1} (phenyl).

Anal. Calc. for $C_{20}H_{24}N_4O_5$: C, 59.99; H, 6.04; N, 13.99. Found: C, 59.86; H, 6.05; N, 13.89.

*2-Azido-3,4-di-O-benzyl-5-O-carbamoyl-2-deoxy-*L*-xylonic acid* (**28**). — A solution of chromic trioxide (104 mg) in 3.5M sulphuric acid (0.3 ml) was added dropwise to a solution of **27** (155 mg) in acetone (4 ml). The mixture was stirred for 50 min at room temperature, then poured into water (*ca.* 50 ml) saturated with sodium sulphate, and extracted with ethyl acetate (2 × 100 ml). The extract was washed with water saturated with sodium sulphate, dried, and concentrated *in vacuo* to afford a pale brown syrup which was eluted from silica gel with 40:7:1 benzene–acetone–acetic acid to yield **28** (101 mg, 63%), $[\alpha]_D^{25}$ +15° (*c* 1, chloroform); ν_{max}^{film} 3500, 3370, 3200, (NH_2), 2880–2550 (COOH), 2100 (N_3), 1730–1690 (both C=O), 1590 (NH_2, phenyl), and 1500 cm^{-1} (phenyl).

Anal. Calc. for $C_{20}H_{22}N_4O_6$: C, 57.97; H, 5.35; N, 13.52. Found: C, 57.45; H, 5.38; N, 13.25.

*2-Amino-5-O-carbamoyl-2-deoxy-*L*-xylonic acid* (**3**). — A solution of **28** (500 mg) in 2:1 acetic acid–water (50 ml) was shaken in hydrogen in the presence of palladium-on-carbon (10%) for 3 h. The filtered mixture was concentrated *in vacuo* to afford an amorphous product which showed two spots on t.l.c. The product was dissolved in water and hydrogenated again over palladium-on-carbon (10%). The product was recrystallized from water–ethanol to yield **3** (130 mg, 52%), m.p. 208° (dec.), $[\alpha]_D^{22}$ +4° (*c* 1.1, water), $[\alpha]_{365}^{22}$ +23° (*c* 1.1, water); ν_{max}^{KBr} 3430, 3350, 3300, 3220 (OH, NH_2), 2840, 2720, 2620 (COOH), 1705 (C=O of carbamoyl group), 1655, 1625 (amino acid), and 1580 cm^{-1} (NH_2 of carbamoyl group); the authentic specimen of natural origin[1] had m.p. 214–215° (dec.), $[\alpha]_{365}^{22}$ +22°.

Synthetic **3** and the natural product showed identical behaviour in t.l.c. on cellulose (Merck), using 36:36:7:21 pyridine–ethyl acetate–acetic acid–water or 4:1:2 1-butanol–acetic acid–water.

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