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A CONVENIENT SYNTHESIS OF 4-AMINO-4-DEOXY-L-ARABINOSE AND ITS REDUCTION PRODUCT, 1,4-DIDEOXY-1,4-IMINO-L-ARABINITOL*†

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ABSTRACT

Methyl β-D-xylopyranoside in a mixture of *N,N*-dimethylformamide and 2-methoxypropene containing a little hydrogen chloride gave preponderantly the 2,3-*O*-isopropylidene derivative, which was readily converted into its 4-trifluoromethanesulfonate. The facile displacement of the triflate group gave a 4-azido-4-deoxy-α-L-arabinopyranoside derivative, and this, on mild acid treatment, was hydrolyzed to the 2,3-diol, or under more vigorous conditions to 4-azido-4-deoxy-L-arabinose. Methyl 2,3-di-*O*-acetyl-4-azido-4-deoxy-α-L-arabinopyranoside, from the diol, appears (^{1}H-n.m.r. data) to exist as an equilibrating mixture of the $^4C_1$ and $^1C_4$ conformers in chloroform solution. The reduction of the azido sugar by hydrogen over Pd/C in 6M HCl yielded 4-amino-4-deoxy-L-arabinopyranose as its hydrochloride; in 0.1M HCl, further reactions occurred to give 1,4-dideoxy-1,4-imino-L-arabinitol as the final product. The aminodeoxypentose from lipid A precursor II_A, isolated from a *Salmonella* mutant by Raetz *et al.* in 1985, was shown to be identical with the synthetic aminoarabinose by t.l.c., ^{1}H-n.m.r. spectroscopy, and g.l.c. of the acetylated reduction products.

INTRODUCTION

4-Amino-4-deoxy-L-arabinose (L-Ara4N) is of interest because of its occurrence as a component of bacterial lipopolysaccharides[1,2], and the recent finding that its reduction product, 1,4-dideoxy-1,4-imino-L-arabinitol, is a fairly strong α-D-glucosidase inhibitor[3]. Compounds of this latter class have potential biomedical applications, *inter alia* in the treatment of AIDS[4]. Thus, ready access to L-Ara4N by synthesis is desirable.

Early work by Dick and Jones on the synthetic chemistry of the 4-amino-4-

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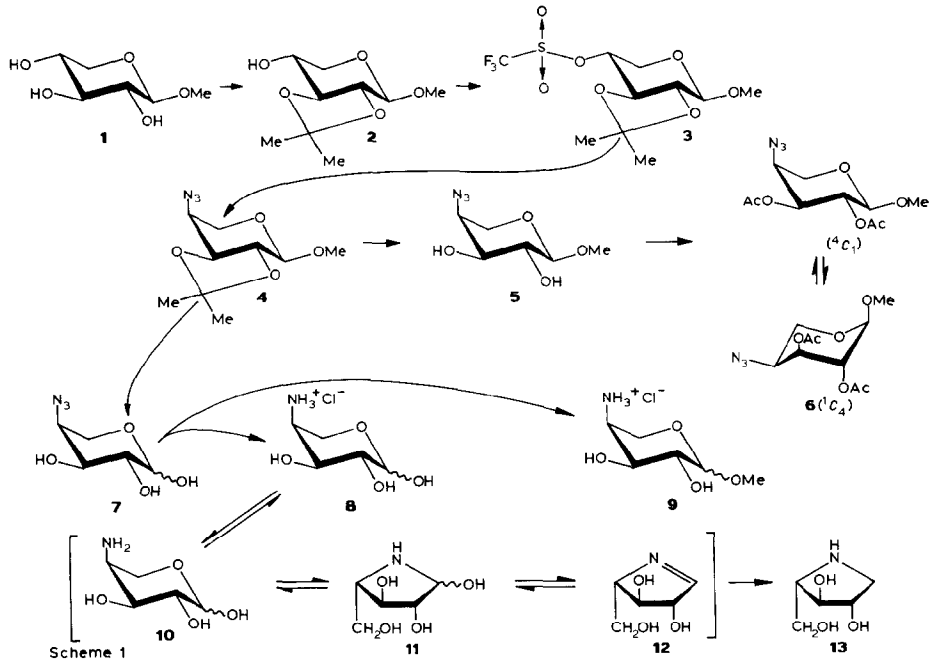
deoxy sugars yielded possible precursors[5,6], and derivatives[7], of L-Ara4N. Volk *et al.*[1] then used one of the precursors to make a chromatographic reference sample, without giving preparative details. More recently, L-Ara4N was generated, but not isolated, during the preparation of the iminoarabinitol[8]. Partial synthetic sequences have also been reported (*e.g.*, ref. 9), but these have not proceeded past the methyl glycoside stage. Thus, although samples of the sugar have remained elusive, the requirements for a successful synthesis were delineated by the studies so far undertaken.

It appeared that the most convenient route would involve the use of azide ion to displace a leaving group, with inversion, from the 4 position of a 2,3-*O*-protected D-xylopyranoside. In view of the finding that methyl 4-amino-4-deoxy-β-L-arabinopyranoside gives only decomposition products on attempted acid hydrolysis[9], the reduction of the azido group in the intermediate azidodeoxy-L-arabinoside would be deferred[10]. Removal of the protecting groups from O-2 and O-3, and cleavage of the glycosidic function, should yield 4-azido-4-deoxy-L-arabinose, and this on reduction should furnish the target sugar. We now describe a convenient synthesis of 4-amino-4-deoxy-L-arabinose and the derived 1,4-dideoxy-1,4-imino-L-arabinitol based on this plan.

RESULTS AND DISCUSSION

The individual steps in the synthesis are shown in Scheme 1. We elected to start with commercially available methyl β-D-xylopyranoside (**1**) and investigate acetonation as a possible way of protecting O-2 and O-3. Xyloside **1** was resistant to 2,2-dimethoxypropane under a variety of conditions, and to 2-methoxypropene–*N,N*-dimethylformamide with added *p*-toluenesulfonic acid[11]. However, when the catalyst in the latter system was changed to hydrogen chloride, **1** was converted into a mixture of isopropylidene acetals, separable by chromatography on silica gel. The major isomer was obtained in 72% yield, and esterified by treatment with trifluoromethanesulfonic (triflic) anhydride. A complex multiplet at δ 5.04 in the proton n.m.r. spectrum of the triflate, shifted downfield from δ 4.03 in the spectrum of the acetal, was identified by sequential decoupling as the signal for H-4. This located the triflate group at C-4, permitting the assignment of the 2,3-*O*-isopropylidene structure **2** to the acetal and structure **3** to its sulfonate.

Treatment of the crystalline but labile triflate **3** with sodium azide, under conditions milder than those originally described by Maradufu and Perlin[12], gave a modest yield (45% based on **2**) of a displacement product (**4**) that could be *O*-deacetonated to methyl 4-azido-4-deoxy-α-L-arabinopyranoside (**5**), having m.p. and $[\alpha]_D$ in satisfactory agreement with the values quoted by Dick and Jones[6]. The ^{1}H-n.m.r. spectral data for **5** in deuterium oxide (Table I) were as expected for an L-arabinopyranose derivative in the $^4C_1$ conformation. However, n.m.r. examination (Table I) of the diacetate (**6**) of **5** in chloroform at ambient temperature gave anomalous values, particularly for $J_{1,2}$, $J_{2,3}$, and $J_{4,5e}$. At −41°, **6** furnished a diffe-



rent set of values, more closely corresponding to those for **5**. It is inferred that **6** in chloroform solution is a rapidly interconverting mixture of the two chair conformers, in which the equilibrium shifts toward the $^4C_1$ form as the temperature is lowered.

In the further processing of **4**, the deprotection of O-2 and O-3 and the cleavage of the glycosidic function were accomplished simultaneously by hydrolysis with ~0.4M sulfuric acid in aqueous acetic acid. This gave the azido sugar **7**, which crystallized after purification by chromatography. The hydrogenation of **7** was then undertaken, using palladium–charcoal as the catalyst, and acidic media, which we expected would stabilize the "aminopyranose" form of the product. The outcome of this procedure varied according to the conditions used: (*a*) in methanolic 0.1M hydrogen chloride, the amino sugar **8** was formed, but it was accompanied by substantial portions of its methyl α- and β-glycopyranosides (**9**) (t.l.c. and n.m.r. analysis, data not shown); (*b*) in 0.1M hydrochloric acid, "over-reduction" occurred, giving 1,4-dideoxy-1,4-imino-L-arabinitol (**13**) as the final product; and (*c*) in 6M hydrochloric acid, the desired L-Ara4N was obtained as its hydrochloride (**8**).

The ^{1}H- and ^{13}C-n.m.r. spectra of **7** and **8** were readily interpreted as arising from mixtures of (substituted) α- and β-L-arabinopyranose anomers, all in the $^4C_1$ conformation (α:β ratio, ~2:1 for **8**). The δ and J values for the individual protons of each anomer, extracted from the ^{1}H spectra by sequential decoupling, are recorded in Table I, and the spectrum of **8** is shown in Fig. 1. A close relationship is

TABLE I

^{1}H-N.M.R. DATA

Compound	*Chemical shifts (δ) and coupling constants (Hz)*										
	H-1	($J_{1,2}$)	*H-2*	($J_{2,3}$)	*H-3*	($J_{3,4}$)	*H-4*	($J_{4,5e}$, $J_{4,5a}$)	*H-5e*[a]	($J_{5e,5a}$)	*H-5a*[a]	*OCH*$_3$	*Other*
1[b]	4.26	(7.8)	3.19	(9.0)	3.37	(9.0)	3.57	(5.2, ~9)	3.91	(11.3)	3.26	3.49	*CCH*$_3$
2[c]	4.53	(7.0)	3.33	(9.0)	3.53	(9.0)	4.03	(5.0, 7.5)	4.08	(11.0)	3.24	3.55	1.49, 1.48
3[c]	4.68	(7.0)	3.46	(10.0)	3.86	(9.0)	5.04	(5.0, 5.3)	4.24	(13.0)	3.67	3.51	1.46, 1.45
4[c]	4.47	(7.6)	3.82	(9.4)	3.68	(3.1)	4.13	(2.0, 1.6)	3.99	(12.7)	3.59	3.55	1.50, 1.47
5[b]	4.22	(7.7)	3.45	(9.5)	3.84	(3.7)	3.95	(1.9, 0.2)	3.98	(12.3)	3.68	3.49	*COCH*$_3$
6 at ~25°[c]	4.36	(5.0)	5.15	(8.0)	5.07	(3.0)	3.93	(5.0, 2.5)	4.04	(12.0)	3.62	3.45	2.12, 2.10
6 at −41°[c]	4.34	(7.0)	5.23	(9.0)	5.19	(3.0)	4.05	(3.0, 2.5)	4.09	(13.5)	3.66	3.51	2.18, 2.14
7α[b]	4.48	(7.8)	3.43	(9.6)	3.84		3.9	(~4.5, 2)	4.0	(13.2)	3.7		
7β[b]	5.18	(3.6)	3.74	(9.6)	3.97		3.9	(~4.5, 2)	4.0	(13.2)	3.7		
8α[b]	4.57	(7.7)	3.41	(9.1)	3.94	(4.6)	3.6	(1.4, 1.2)	4.02	(13.8)	3.80		
8β[b]	5.21	(3.3)	3.69	(9.1)	4.10	(4.6)	3.6	(2.3, 3.0)	4.16	(13.3)	3.71		

[a]Assignments based on the assumption that in each case the CH_2 resonance at lower field is due to the equatorial H, according to the normal pattern. [b]Measured in D_2O, using the *H*DO signal (δ = 4.80, temperature not recorded) for chemical shift calibration. [c]Measured in $CDCl_3$ containing Me_4Si.

seen (Table I) between the values assigned the α anomers and those found for the methyl azidoglycoside **5**. The reference spectrum of Ara4N facilitated the identification of the naturally occurring sugar in the present work (see below), and it should be equally useful on future occasions.

The characterization of the non-sugar reduction product as the iminoalditol **13** depended on comparison of its n.m.r. parameters with those recorded for compounds isolated from *Arachniodes standishii*[13] and *Anglyocalyx boutiqueanus*[14]. These substances were shown to be the hydrochloride and free-base forms, respectively, of a 1,4-dideoxy-1,4-iminoarabinitol (evidently the D isomer[3,8]) when the ^1H and ^{13}C spectra of authentic samples synthesized by three independent routes, two for the L compound[3,8] and one for the D enantiomer[3], were found to correspond to those of the natural materials. The chemical shifts shown by our product, when adjusted for differences in the reference standards adopted, are identical within experimental error to those reported by Furukawa *et al.*[13] for the hydrochloride from *A. standishii* (erroneously assigned the *xylo* configuration in the original paper).

In interpreting the spectra of **13**, it is important to note that, although the connectivity relationships are easily established by the usual decoupling and correlation experiments, these techniques do not distinguish between the C-1 and C-5 ends of the molecular skeleton. However, the correctness of the earlier signal

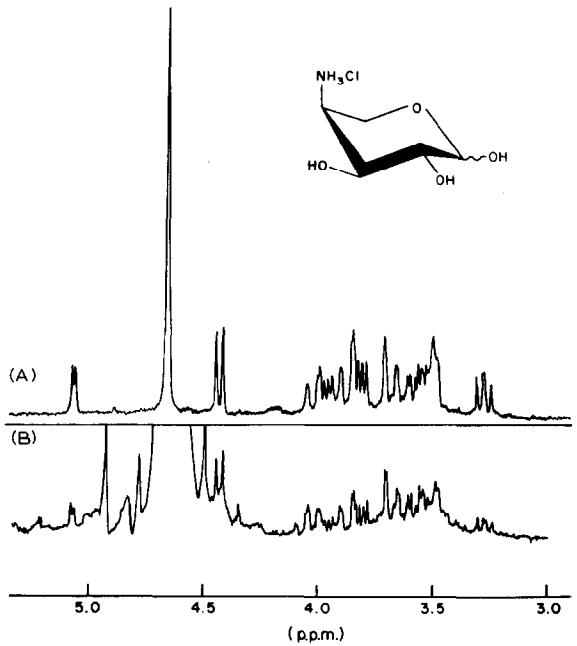


Fig. 1. 270-MHz ^1H-n.m.r. spectra (D_2O) of synthetic 4-amino-4-deoxy-L-arabinose·HCl (A) and the aminopentose from lipid A precursors II_A-II_B (B, see text).

assignments, and therefore of ours, is established by comparing the data for the hydrochloride[13] with those for the free base[14]. The only signals significantly shifted downfield on protonation of the nitrogen are those ascribed to C-1 and C-4 (sugar numbering) and their attached H atoms.

The hydrogenation of **7** to **13** has a direct precedent in the iminoarabinitol synthesis of Jones *et al.*[8], where this same transformation was accomplished in aqueous acetic acid. Its facile occurrence at pH 1 (90% complete in 1 h) nevertheless seems remarkable, as it involves a series of steps beginning with the free-base form (**10**) of the sugar. Tautomerization to the pyrrolidino sugar **11**, dehydration of this to the pyrroline **12**, and saturation of the C=N bond are unexceptional, but the concentrations of **11** and **12**, and of course **10**, must be very small at this low pH. Even without added acid, the protonated aminopyranose (**8**) form appears to be stable in water (see Fig. 1, and further discussion below). Only the "pull" of a rapid, irreversible step (**12**→**13**) could account for the funneling of the reactant into the five-membered-ring pathway.

Characterization of the aminodeoxypentose from lipid A precursors II_A-II_B. — In an earlier study, Raetz *et al.*[15] isolated a group of lipid A precursors from a 3-deoxy-D-*manno*-octulosonate-deficient mutant of *S. typhimurium*. According to mass-spectral and other data, two pairs of these compounds, designated I_A-I_B and II_A-II_B, contained an aminodeoxypentose moiety, but confirmatory evidence was not secured at the time. Therefore, the amino sugar fraction obtained by mild acid hydrolysis of II_A-II_B was now compared with our synthetic preparation (**8**).

T.l.c. of the aminopentose and standard amino compounds was accomplished using 1-butanol–acetic acid–water systems (see Experimental). The 2:1:2 mixture (solvent *A*) was particularly satisfactory, and the 5:2:1 mixture[1] (solvent *B*) gave adequate separations on repeated development of the plates. As shown in Table II, the hydrolysis product of II_A-II_B and synthetic Ara4N had identical mobilities. The ninhydrin colors of the two samples were also identical.

The ^{1}H-n.m.r. spectrum of the synthetic sample, taken in deuterium oxide at 270 MHz, is shown in Fig. 1A, and that of the natural product in Fig. 1B. In the

TABLE II

THIN-LAYER CHROMATOGRAPHIC DATA

Compound	R_F	
	Solvent A	*Solvent* B[a]
2-Amino-2-deoxy-D-glucose · HCl	0.40	0.28
4-Amino-4-deoxy-L-arabinose · HCl	0.34	0.26
Aminodeoxypentose, isolated[b]	0.34	0.26
Methyl glycosides **9**		0.34
Phosphoethanolamine	0.24	

[a]Plate developed three times to increase R_F values. [b]From hydrolysate of lipid A precursors II_A-II_B (see text).

latter spectrum, the HDO peak is much more intense, and it has side bands. Also, a few extraneous peaks are visible in the range δ 4–5, which is not surprising in view of the crude character of the sample. But with due allowance for these factors, the spectra match closely.

Finally, the natural and the synthetic amino sugars were reduced with sodium borohydride, the reduction products were acetylated, and the acetates subjected to g.l.c. on SE-30. Under the conditions employed (see Experimental), each sample gave a single sharp peak at ~18 min. Exactly the same results were obtained with a mixture of the two products, and with a sample of acetylated 1,4-dideoxy-1,4-imino-L-arabinitol (**13**). Thus, the reduction product appeared to be **13**, and this was confirmed by examining the ^{1}H-n.m.r. spectrum of a sample converted into the hydrochloride rather than the peracetate.

In the original work on the characterization of Ara4N as a component of *Salmonella* lipopolysaccharide[1], borohydride treatment of the sugar gave two compounds, presumably 4-amino-4-deoxy-L-arabinitol and the cyclic iminoalditol **13**. We cannot offer a firm explanation of the difference between this result and our obtention of the cyclic product only. However, we note that our sample solutions were evidently more alkaline at the time of addition of the borohydride than the solutions employed by the earlier workers. The higher pH would promote the formation[16] of **11** and **12**, the precursors of **13**.

Taken together, the data solidly confirm the presence, in lipid A precursors II_A-II_B, of a 4-amino-4-deoxyarabinose moiety. Assignment to a configurational series was not accomplished, but we presume the compound is the L enantiomer, so far found whenever Ara4N has been identified as a component[2,17] of lipid A.

EXPERIMENTAL

Melting points were determined in sealed tubes on a Thomas–Hoover capillary melting-point apparatus, and are uncorrected. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter. ^{1}H-N.m.r. spectra were recorded on a Bruker WH-270 instrument, at the working temperature of the probe, and ^{13}C spectra on an IBM NR-80 spectrometer. Peak assignments in the ^{1}H spectra were verified by sequential decoupling. Chemical shifts are reported in p.p.m. downfield from Me_4Si; the solvent and internal standard used are listed with the data for each compound. Elemental analyses were performed by the Galbraith Laboratories, Knoxville, TN.

Thin-layer chromatograms were done on plates coated with 0.25 mm of Silica Gel 60 F_{254} (Merck), and spots were visualized by examination under u.v. light (254 nm), by charring with H_2SO_4 (5% in EtOH), or by spraying with ninhydrin (1% in Me_2CO) and heating. Column chromatography was done on Silica Gel 60, 0.063–0.200 mm particle size (Merck). Flash chromatography procedures were essentially those of Still *et al.*[18]. Solvent systems for chromatography were: 1-butanol–acetic acid–water (*A*, 2:1:2; *B*, 5:1:2); chloroform–ethyl acetate (*C*, 19:1; *D*, 50:1);

chloroform–methanol (*E*, 9:1; *F*, 4:1; *G*, 19:1); chloroform–pyridine–formic acid–water (*H*, 40:60:15:5).

Gas–liquid chromatography was done on a Packard model 428 instrument equipped with a flame-ionization detector and a glass column (1 m × 2 mm) of 10% SE-30 on 80/100 WHP. The carrier gas was nitrogen. The temperature was held for 5 min at 150°, then increased to 240° at 4°/min; the injection port and detector were kept at 240°.

*Methyl 4-azido-4-deoxy-α-*L*-arabinopyranoside* (**5**). — To a solution of methyl β-D-xylopyranoside (1.00 g, 6.1 mmol) in dry *N,N*-dimethylformamide (2.0 mL) under dry nitrogen was added 30 μL of 4M HCl in dry methanol. Under stirring at 60°, 2-methoxypropene (2.0 mL, 21 mmol) was added, and the reaction was allowed to proceed for 2 h at 60° and then overnight at room temperature. The mixture was neutralized with triethylamine (75 μL) and the solvent removed under high vacuum at 30°. The residue (1.37 g) was dissolved in chloroform (50 mL), and the solution was washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated to dryness. The resulting syrup was chromatographed on a column (26 × 250 mm) of SiO_2 (56 g, elution with solvent *C*). Fractions containing the first major component (R_F 0.34, solvent *C*) were combined and evaporated to dryness, to yield 0.89 g (72%) of methyl 2,3-*O*-isopropylidene-β-D-xylopyranoside (**2**), homogeneous by t.l.c. The compound crystallized on standing, m.p. 138–139°, $[\alpha]_D^{20}$ −17.3° (*c* 1.2, chloroform); ^{13}C-n.m.r. ($CDCl_3$): δ ($CHCl_3$ = 77.0) 110.9 (CCH_3), 102.6 (C-1), 79.7, 76.7, 69.4, 66.9, 56.1 (OCH_3), 26.8, and 26.5 (2 × CCH_3).

Anal. Calc. for $C_9H_{16}O_5$ (204.22): C, 52.93; H, 7.90. Found, for an amorphous sample: C, 51.98; H, 8.02. Presumably the low carbon value reflects retention of chromatographic solvent ($CHCl_3$) in the sample.

A solution of **2** (2.93 g, 14 mmol, dried *in vacuo* over P_2O_5) in dry dichloromethane (140 mL) and dry pyridine (4.6 mL, 55 mmol) was cooled to −50° under dry nitrogen, and trifluoromethanesulfonic anhydride (2.9 mL, 17 mmol) was added. The mixture was stirred for 45 min at −25°, then poured into a 1-L separatory funnel containing 100 mL of crushed ice and 200 mL of saturated aqueous sodium hydrogencarbonate. The solution was quickly extracted and the aqueous layer back-extracted with chloroform (2 × 30 mL). The combined organic layers were dried (Na_2SO_4) and evaporated, and the residue was freed of pyridine by additions and evaporations of dry toluene (3 × 20 mL). This provided 4.61 g of crystalline, yellowish-white methyl 2,3-*O*-isopropylidene-4-*O*-trifluoromethylsulfonyl-β-D-xylopyranoside (**3**), which was found to deteriorate quickly at room temperature.

The crude triflate (4.58 g, 13.6 mmol) was therefore immediately dissolved in dry *N,N*-dimethylformamide (46 mL), and dry sodium azide (1.12 g, 17 mmol) was added. After stirring for 2 h at room temperature, the reaction was complete (t.l.c., solvent *C*). The mixture was diluted with anhydrous ether (100 mL) and filtered, the precipitate was washed with dry chloroform, and the combined filtrates were evaporated. The residue was applied to a column (220 g of SiO_2, elution with

solvent *D*) and the fractions containing the first major component were combined to give pure methyl 4-azido-4-deoxy-2,3-*O*-isopropylidene-α-L-arabinopyranoside (**4**; 1.49 g, 45% from **2**) as a clear syrup after evaporation, $[\alpha]_D$ −30.8° (*c* 2.0, chloroform); ^{13}C-n.m.r. ($CDCl_3$ + 5% CD_3OD): δ ($CHCl_3$ = 77.0) 110.8 (CCH_3), 103.4 (C-1), 77.4 (C-4), 73.3 (C-2/C-3), 65.2 (C-5), 57.6 (C-2/C-3), 55.8 (OCH_3), 26.1, and 25.1 (2 × CCH_3).

A sample of **4** (196 mg, 0.86 mmol) in distilled methanol (10 mL) with added water (500 μL) was stirred at room temperature with prewashed IR-120 (H^+) ion-exchange resin (~0.5 g). After 2 h, when all the starting material (R_F in solvent *E*, 0.89) had been converted into a more slowly migrating product (R_F 0.36), the resin was filtered off and washed with methanol. Concentration of the combined filtrates left 162 mg (100%) of **5**, which was chromatographed on a column (10 g of SiO_2, elution with solvent *G*). Evaporation of the eluate gave a friable solid, m.p. 94–95°, $[\alpha]_D^{22}$ −26.1° (*c* 0.5, chloroform); lit.[6] m.p. 95–96.5°, $[\alpha]_D$ −23°; ^{13}C-n.m.r. (D_2O): δ [$(CH_3)_2CO$ = 29.8] 105.4 (C-1), 73.8, 72.2, 65.2, 62.9, and 58.5 (OCH_3).

Anal. Calc. for $C_6H_{11}N_3O_4$ (189.17): C, 38.10; H, 5.86; N, 22.21. Found: C, 38.14; H, 5.91; N, 22.06.

Anal. Calc. for $C_6H_{11}N_3O_4$ (189.17): C, 38.10; H, 5.86; N, 22.21. Found: C, 38.14; H, 5.91; N, 22.06.

Acetylation of **5**. — Crude **5**, prepared from 17 mg of **4** by *p*-toluenesulfonic acid-catalyzed methanolysis, was dissolved in dry dichloromethane (5.0 mL) containing dry pyridine (25 μL), acetic anhydride (28 μL), and 4-dimethylaminopyridine (5 mg). After 12 h at room temperature, when t.l.c. indicated a single product having R_F 0.37 in solvent *C*, the reaction mixture was shaken with cold M HCl (50 mL). The organic phase was washed with saturated aqueous sodium hydrogencarbonate (50 mL) and water (50 mL), with back-extraction of the aqueous layers ($CHCl_3$, 5 mL). The combined organic layers were dried (Na_2SO_4) and evaporated. The residue, after further drying *in vacuo*, consisted of 16 mg (79%) of methyl 2,3-di-*O*-acetyl-4-azido-4-deoxy-α-L-arabinopyranoside (**6**), colorless needles, m.p. 66–67°, $[\alpha]_D^{22}$ −13.9° (*c* 0.7, $CHCl_3$); ^{13}C-n.m.r. ($CDCl_3$): δ ($CHCl_3$ = 77.0) 170.2, 169.3 (2 × *C*=O), 100.9 (C-1), 71.3, 68.7, 61.5, 57.2, 56.2, 29.7, and 20.5.

*4-Azido-4-deoxy-*L*-arabinopyranose* (**7**). — To a solution of **4** (705 mg, 3.1 mmol) in glacial acetic acid (18 mL) was added 2M sulfuric acid (5.0 mL) dropwise with stirring. This solution was held for 3 h at 95°, then cooled to room temperature, and neutralized to pH 4 with solid sodium hydrogencarbonate. The resulting solution was evaporated and the residue was treated with toluene (20 mL), which was evaporated, then taken up in water and lyophilized. The mixture of salts and organic product was then applied to a column (70 g of SiO_2, elution with solvent *E*) and the fractions containing first the deacetonated glycoside **5** (65 mg) and then **7** (310 mg, 65% based on starting material not recovered as **5**) were collected. The azido sugar (**7**) was crystallized from a methanol solution diluted with ethyl acetate by vapor-diffusion, m.p. 166–168°, $[\alpha]_D^{20}$ +82.5° (*c* 0.9, methanol); f.t.-i.r. (neat):

ν_{max} 3365 (OH stretch, br.), 2108 (N=N=N), 1340, 1269, 1080 (C–O stretch), and 992 cm^{-1}; ^{13}C-n.m.r. (D_2O): δ (external 1,4-dioxane = 66.5) 96.6 (C-1β), 92.31 (C-1α), 72.28, 71.75, 68.42, 63.65, 61.75, 61.40, and 59.78.

Anal. Calc. for $C_5H_9N_3O_4$ (175.14): C, 34.29; H, 5.18. Found: C, 34.25; H, 5.15.

*4-Amino-4-deoxy-*L*-arabinopyranose hydrochloride* (**8**). — To a solution of **7** (14 mg) in 6M aqueous HCl (3.0 mL) was added 10% Pd/C (13 mg), and the mixture was stirred under hydrogen at ambient temperature and pressure. After 2 h, the catalyst was filtered off and the filtrate evaporated under reduced pressure to give 15 mg (100%) of **8** as a yellowish glass, $[\alpha]_D^{20}$ +49.6° (*c* 0.8, D_2O); ^{13}C-n.m.r. (D_2O): δ (external 1,4-dioxane = 66.5) 103.1 (C-1β), 98.5 (C-1α), 77.7, 75.3, 74.6, 72.0, 68.2, 64.3, 57.9, 57.4. The sample showed single spots on t.l.c. in solvents *A* and *B*.

*1,4-Dideoxy-1,4-imino-*L*-arabinitol hydrochloride* (**13**). — A mixture of **7** (11.3 mg) in 0.1M aqueous HCl (5 mL) and 10% Pd/C (8.6 mg) was stirred under hydrogen at ambient temperature and pressure until all the starting material had been converted into product (6 h, t.l.c. in solvent *F*). The suspension was then filtered through a Celite pad and the filtrate lyophilized to give 10.9 mg (100%) of **13** as a clear, pale-yellow syrup, $[\alpha]_D^{22}$ −27.8°, $[\alpha]_{578}^{22}$ −29.3°, $[\alpha]_{546}^{22}$ −33.1°, $[\alpha]_{436}^{22}$ −54.9° (*c* 1, water); lit.[3] $[\alpha]_D^{20}$ −34.6°, lit.[8] $[\alpha]_{578}^{20}$ −22° (water); ^1H-n.m.r. (D_2O): δ (*H*DO = 4.80 at 15°) 3.34 (1 H, $J_{1,2}$ 2.2, $J_{1,1'}$ 12.6 Hz, H-1), 3.55 (1 H, $J_{1',2}$ 4.8 Hz, H-1′) 3.60 (1 H, $J_{4,5'}$ 4.7, $J_{4,5}$ 8.1 Hz, H-4), 3.81 (1 H, $J_{5,5'}$ 12.2 Hz, H-5), 3.94 (1 H, H-5′), 4.08 (1 H, $J_{3,4}$ 3.5 Hz, H-3), and 4.32 (1 H, $J_{2,3}$ 3.1 Hz, H-2); ^{13}C-n.m.r. (D_2O): δ 75.28 (C-3), 73.83 (C-2), 66.15 (C-4), 58.43 (C-5), and 49.58 (C-1).

Hydrolytic cleavage of precursors II_A-II_B. — A mixture of II_A and its less polar congener II_B (ref. 15; 9 mg) was dispersed in 1.8 mL of water by brief sonication in a bath type apparatus. After removal of 50 μL for use as a t.l.c. standard, the dispersion was made first 0.1M and later 0.2M in HCl, and incubated, with occasional sonication to prevent aggregation and precipitation of the lipid. As shown by t.l.c. in solvent *H*, the reaction was very slow at 37°, but, after 2 h at 80°, the starting compounds were fully converted into faster migrating products, presumably the parent lipid A precursors[15] IV_A and IV_B. The water-soluble product(s) was separated from the lipid by centrifugation and washing (H_2O) in the centrifuge tube, and the combined supernatant solutions were lyophilized to give the crude aminopentose hydrochloride.

Preparation of alditol acetates[19]. — Aqueous solutions containing ~1 mg of each amino sugar sample were placed in separate 1-mL screw-capped vials and concentrated under high vacuum. The residues were taken up in 20 μL of 1:1 glycerol–absolute ethanol, these solutions were concentrated under nitrogen jets for 20 min at 50°, and 50 μL of M ammonium hydroxide and 4 drops of freshly prepared 0.53M sodium borohydride were added. The vials were incubated overnight at 22°, then each sample was acidified with a drop of glacial acetic acid, and

methyl borate was removed by the addition and evaporation of methanol (5 × 100 μL). The residues were briefly dried at 1 mmHg, 100 μL of acetic anhydride was added, and the tubes were sealed with Teflon tape and incubated for 3 h at 123°. The cooled samples were mixed with water (400 μL) and applied to C-18 SEP-PACK cartridges (Millipore, Waters Assoc.) preconditioned with 4 mL of water and 3 mL of acetonitrile. The sample-bearing cartridges were rinsed with water (4 mL) and then eluted with acetonitrile (3 mL). The eluates were evaporated under nitrogen jets, and the residues were dried *in vacuo* (5 h) and then dissolved in chloroform (20 μL). Aliquots of 0.5 μL were injected into the g.l.c. column.

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