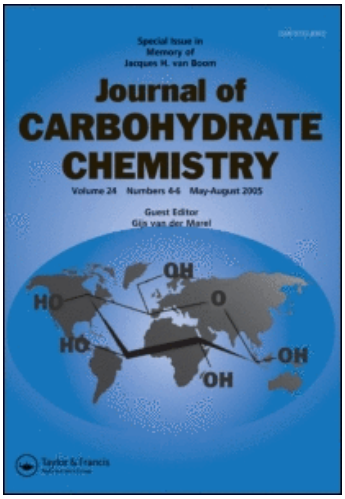

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NEW *N*-ACYLATING REAGENTS DERIVED FROM 3-DEOXY-L-*GLYCERO*-TETRONIC ACID[1]

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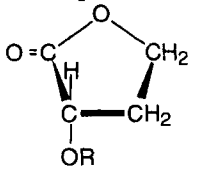
ABSTRACT

Commercially available *N*-Boc-4-*O*-benzyl-L-homoserine was treated with trifluoroacetic acid and the corresponding, *N*-deprotected derivative was submitted to deamination to give 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid (**5**). In another approach to 3-deoxy-L-*glycero*-tetronic acid protected at position 4, the carboxylic groups in L-malic acid were reduced, and the resulting triol was benzylidenated. Oxidation of the 2,4-*O*-benzylidene derivative formed with CrO_3-pyridine complex in the presence of *t*-butyl alcohol gave *t*-butyl 2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronate (**13**). The latter was saponified with aqueous sodium hydroxide to give, after Na^+ exchange for H^+, 2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronic acid (**15**). Opening of the acetal ring in **13**, followed by hydrolytic cleavage of the *t*-butyl ester function gave material indistinguishable from **5** obtained in the original way. When tested for their efficiency of *N*-acylation of derivatives of D-perosamine, both acids **5** and **15** gave the corresponding tetronamido derivatives in high yields.

INTRODUCTION

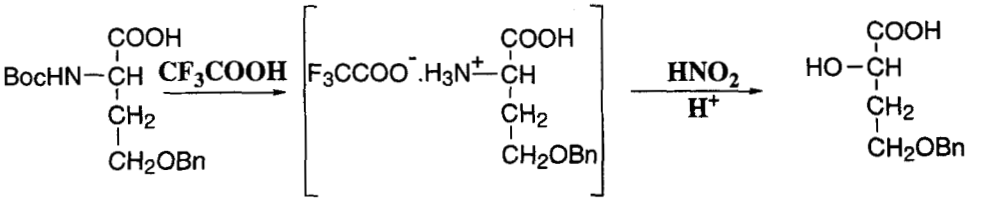
The O-specific polysaccharide (O-SP) of the two main strains of *Vibrio cholerae* O:1, Ogawa and Inaba, consists of a relatively short chain[3] of (1→2)-linked moieties of 4-amino-4,6-dideoxy-α-D-mannopyranose (D-perosamine), whose amino groups are acylated with 3-deoxy-L-*glycero*-tetronic acid. The two O-antigens differ only in that in the Ogawa O-polysaccharide the nonreducing D-perosaminyl group in the O-SP is methylated at O-2.[4,5] Our studies of the interactions of *Vibrio cholerae* O:1 antigens with homologous antibodies require a large number of synthetic ligands. Both structural constituents of the monomeric repeating unit of the O-SP of *V. cholerae*, D-perosamine and 3-deoxy-L-*glycero*-tetronic acid, are rare chemical species. While several syntheses of the methyl α-glycoside of D-perosamine have been reported[6-11] the *N*-acylating reagents used thus far to introduce the 3-deoxy-L-*glycero*-tetronyl group were lactones, namely the lactone derived from either 3-deoxy-L-*glycero*-tetronic acid itself (**1**) or its 2-*O*-acetyl- (**2**) and 2-*O*-benzyl- (**3**) derivatives. Compounds **1-3** were used successfully to *N*-acylate the methyl α-glycoside of D-perosamine and other mono-[9,11-14] and disaccharides[10] of the same class. We have recently found,[15] however, that when oligosaccharides composed of (1→2)-linked D-perosamine were treated with **1** the efficiency of *N*-acylation decreased dramatically with increasing size of the oligosaccharide. In fact, no desired product could be isolated from the attempted *N*-acylation of a hexamer of D-perosamine with **1**. This difficulty could be overcome[15] using 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid (**5**) as an acylating reagent, in conjunction with a carbodiimide type promoter. Here, we report the hitherto unpublished details of the synthesis of **5**, and the synthesis of another new, 4-*O*-protected *N*-3-deoxy-L-*glycero*-tetronylating reagent, namely 2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronic acid (**15**).



	R
1	**H**
2	**Ac**
3	**Bn**

RESULTS AND DISCUSSION

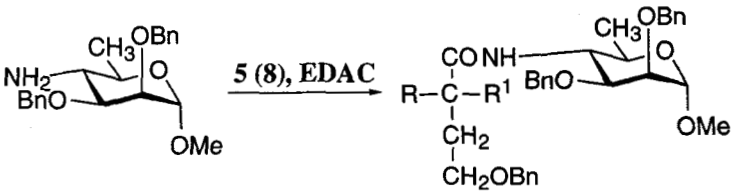
Carboxylic acids, when used in conjunction with a suitable promoter, are useful reagents for *N*-acylation. Thus, when the *N*-acylation of a hexamer composed of D-perosamine with lactone (**1**) failed,[15] the parent acid, expected to be a more efficient reagent, was our next choice for *N*-3-deoxy-L-*glycero*-tetronoylation. It is known,



4 **5**

however, that 3-deoxy-L-*glycero*-tetronic acid lactonizes spontaneously,[9] unless position 4 is suitably protected. We have now prepared 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid (**5**) from the commercially available *N*-Boc-4-*O*-benzyl-L-homoserine (**4**) by deamination. Due to the presence of a 4-*O*-benzyl group in **4** and **5**, formation of the corresponding 1,4-lactone during chemical manipulations does not occur.

The suitability of **5** for *N*-acylation of derivatives of perosamine was shown when its reaction with methyl 4-amino-2,3-di-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside

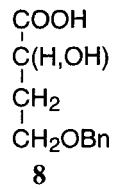


6

	R	R¹
7	**OH**	**H**
7a	**(H,OH)**	

(**6**)[14] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDAC) gave the corresponding tetronamido derivative **7** in high yield.

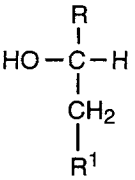
The high optical purity of acid **5** was verified by comparison of the NMR spectra of **7** and **7a**. The latter compound was prepared from **6** in the same way as **7** but using 4-*O*-benzyl-3-deoxy-D,L-*glycero*-tetronic acid (**8**) as the tetronylation reagent. The latter was synthesized starting with commercially available D,L-homoserine, which was converted to *N*-Boc-4-*O*-benzyl-D,L-homoserine as described for the pure L-enentiomer.[16] In contrast to the spectra of **7** which showed clear features of a single substance, the spectra of **7a** contained independent signals for certain nuclei belonging to each of the diastereoisomers present (see Experimental). The presence of two isomers in the product **7a** could also be seen by TLC.



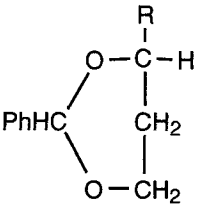
8

Since *N*-Boc-4-*O*-benzyl-L-homoserine (**4**) is a rather expensive commodity, we searched for an alternative starting material for the synthesis of a 4-*O*-protected-3-deoxy-L-*glycero*-tetronic acid. We turned our attention to the work of Altman et al.[17] who synthesized a number of substances in which the structural fragment having the 3-deoxy-L-*threo* configuration was constructed from L-malic acid (**9**). As the first step of our synthesis, we have converted the inexpensive, commercially available compound **9** to (*S*)-1,2,4-butanetriol (**10**). The latter was previously prepared[18] from the same acid by reduction with $LiAlH_4$. The low yield reported (~21%) was probably caused by the experimental difficulties during isolation of the desired, water soluble product form mixtures containing large amount of salts. We carried out the reduction with borane-dimethyl sulfide complex (BMS)[19] thereby avoiding difficulties commonly encountered with the use of $LiAlH_4$. The reduction of L-malic in this way was a smooth reaction, yielding virtually pure (NMR) **10** in theoretical yield. The specific optical rotation of a sample purified by chromatography was identical with that of a sample of a commercial product, which is in close agreement with the value in the literature;[18] unfortunately, no criteria of purity were reported for the material obtained.[18] Acetalation of **10** with

benzaldehyde dimethylacetal catalyzed with tetrafluoroboric acid[20] gave the benzylidene derivative **12** in high yield. That the HO-1 in the latter was unsubstituted followed from the NMR spectral data (see Experimental).

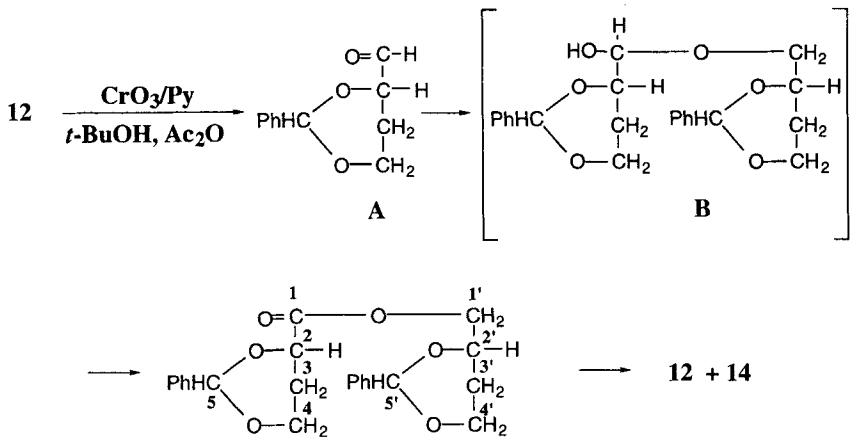


	R	R^1
9	COOH	COOH
10	CH_2OH	CH_2OH
11	COO^tBu	CH_2OBn



	R
12	CH_2OH
13	COO^tBu
14	COOMe
15	COOH

Next, the oxidation of alcohol **12** to the corresponding carboxylic acid had to be explored. In view of the presence of the acid-labile *O*-benzylidene group, we performed the oxidation with chromium trioxide-pyridine complex. According to the recent variation of the method, the use of an aprotic solvent in the presence of *t*-BuOH and acetic anhydride[21,22] facilitates the oxidation, and the product is readily isolated in the form of the corresponding *t*-butyl ester. When we performed the oxidation of **12** as recommended,[21,23] *i.e.* with the addition of *t*-BuOH at a later stage of the reaction (not described in the Experimental), the crystalline *t*-butyl ester **13** was obtained in 54% yield, following the resolution of the crude product by chromatography. In addition, a crystalline byproduct was isolated in a yield of 21%, whose spectral data (see Experimental) strongly supported structure **16** (Scheme 1). Final proof of the structure was obtained when treatment of the substance with a catalytical amount of sodium methoxide in MeOH yielded equimolar amounts of products of transesterification, the alcohol **12** and the methyl ester **14**, the latter also obtained crystalline. A plausible explanation for the formation of the ester **16** during the oxidation of **12** is as follows: Concomitantly with the oxidation of the alcohol **12** to the corresponding aldehyde (**A**), the still unoxidized alcohol reacts with **A** to form the hemiacetal **B** (Scheme 1), as suggested for the case when *t*-BuOH is the hemiacetal-forming alcohol.[21] This decreases the amount of the aldehyde available for the reaction with *t*-BuOH, as well as of the alcohol **12**

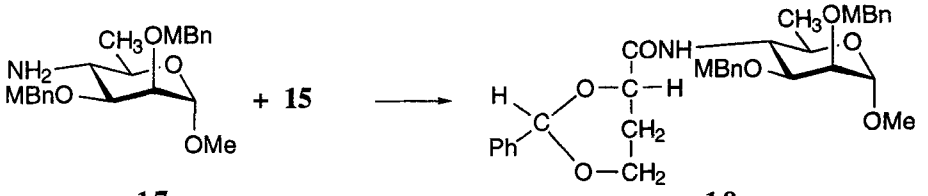


16

Scheme 1

available for the desired oxidation. Consequently, the expected *t*-butyl ester **13** is formed in a decreased yield. To verify this hypothesis and explore a possibility to increase the yield of the desired **13**, the oxidation just described was performed with very slow addition of the alcohol **12** to the stirred mixture of all other reagents, *i.e.* CrO_3-pyridine complex, acetic anhydride, and *t*-BuOH (see Experimental). In this way, *t*-BuOH was made available for the reaction with the aldehyde **A** as soon as the latter was formed. The crystalline *t*-butyl ester **13**, formed at the expense of **16**, was now isolated in 81% yield, following resolution of the crude product by chromatography. Previously, isolation of esters other than *t*-butyl esters from analogous conversions of hexose derivatives to the corresponding derivatives of hexuronic acids have not been reported. In those conversions, perhaps due to steric hindrance, the extent of the formation of esters analogous to **16** was preparatively unimportant, and the target *t*-butyl esters have often[21,23,24] been isolated in high yields. The formation of a considerable amount of **16** during the oxidation of **12** described above suggests, however, that when alcohols of small molecular mass are oxidized using Corey's method[21] the order of addition of reagents may play a critical role, and govern yields of products formed.

The ester **13** was readily saponified with aqueous sodium hydroxide, to give the corresponding acid **15** which showed itself to be a powerful *N*-acylating reagent. The reaction of the amine **17**, prepared by treatment with H_2S of methyl 4-azido-4,6-dideoxy-2,3-di-*O*-4-methoxybenzyl-α-D-mannopyranoside,[12] with **15** (used in only slight excess over the amount of the amine) gave pure (NMR) product **18** in virtually theoretical yield.



17 **18**

The opening of the benzylidene acetal ring in **13** using borane-trimethylamine complex and aluminium chloride,[25] followed by acid hydrolysis of the resulting ester **11**, gave material indistinguishable from the acid **5** prepared in the independent way described above.

EXPERIMENTAL

General methods. Unless stated otherwise, optical rotations were measured at ambient temperature for solutions in chloroform with a Perkin Elmer automatic polarimeter, Model 241MC. Thin-layer chromatography (TLC) was performed with *A*, 15:1 CH_2Cl_2-MeOH; *B*, 4:1 $CHCl_3$–MeOH; *C*, 4:1 hexane–EtOAc; *D*, 10:1 hexane–EtOAc; *E*, 10:1 $CHCl_3$–acetone; *F*, 6:1 $CHCl_3$–MeOH; and *G*, EtOAc (neat). Detection was effected by charring with 5% H_2SO_4 in EtOH and with UV light or, when required, with iodine vapors. Preparative chromatography was performed by gradient elution from columns of Silica Gel 60 (particle size 0.04–0.063 mm) using, at the onset of development, a solvent mixture slightly less polar than that used for TLC. NMR spectra were obtained at 300 MHz for ^1H and 75 MHz for ^{13}C. The measurements were done at ambient temperature, using a Varian XL 300 or a Varian Gemini spectrometer. The solvents used are given as required.

Chemical shifts are reported in ppm downfield of the signal of of Me_4Si; ^1H shifts determined in D_2O were measured relative to the signal of HOD (δ 4.78). The ^{13}C shifts were measured relative to the signal of $CDCl_3$ (δ 77.0), benzene (δ 128.0) or MeOH (δ 49.0). Assignments of NMR signals were made by first-order analysis of the spectra, and by comparison with spectra of related substances. When feasible, the assignments were supported by homonuclear decoupling experiments or homonuclear and heteronuclear 2-dimensional correlation spectroscopy, using commercial software supplied with the spectrometers. Chemical ionization mass spectra (CIMS) were measured using ammonia as the reactive gas. Before final drying, the solutions of analytical samples were filtered through Anotop 10 Plus 0.1 μm syringe filters (Whatman, Inc). We have found these devices superior to other, same porosity filtration devices for removal of coloidal material sometimes present. L-Malic acid, borane-methylsulfide complex (BMS, 2 M solution in THF) and a commercial sample of (*S*)-1,2,4-butanetriol were purchased from Aldrich Chemical Co. The solution of tetrafluoroboric acid in ether (54%) was a product of Fluka Chemical Co. D,L-Homoserine and 4-*O*-benzyl-*N*-Boc-L-homoserine were purchased from Bachem Califormia, and used as supplied. Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at or below 40°/2kPa.

4-*O*-Benzyl-3-deoxy-L-*glycero*-tetronic acid (5). a. A solution of *N*-Boc-4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid (**4**, 10 g) in concd trifluoroacetic acid (TFAA, 20 mL) was kept at room temperature for 30 min, when TLC (solvent *D*) showed that the reaction was complete. After concentration, the residue was dissolved in 3 M acetic acid (200 mL), and a solution of $NaNO_2$ (6.6 g) in water (20 mL) was added, over a period of 30 min, dropwise and with stirring at 0 °C. Stirring was continued overnight and TLC (solvent *A*) then showed that the starting material was no longer present. After concentration, the solution of the residue in dichloromethane was washed with water, dried, and concentrated. The residue was chromatographed to give **5** (4.78 g, 72%), mp 70-72 °C (from EtOAc–hexane), $[\alpha]_D$ -12° (*c* 0.9) ; ^1H NMR ($CDCl_3$) δ 7.30 (m, 5 H,

Ph), 4.53 (s, 2 H, C*H*$_2$Ph), 4.38 (dd, 1 H, $J_{2,3a}$ 4.3, $J_{2,3b}$ 6.9 H, H-2), 3.72, m, 2 H, H-4a,b), and 2.27–1.98 (2 m, 1 H each, H-3a,b); ^{13}C NMR ($CDCl_3$) δ 176.96 (C-1), 73.47 (*C*H$_2$Ph), 69.59 (C-2), 67.43 (C-4), and 33.13 (C-3); CIMS: *m/z* 228 ([M + 18]$^+$).

Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71; Found: C, 62.76; H, 6.70.

b. A solution of **11** (45 mg) in 20% TFAA in CH_2Cl_2 (1 mL) was kept at room temperature for 1 h. TLC (solvent *A* and *C*) showed that all starting material was consumed. After concentration with coevaporation of water, to remove TFAA, the NMR characteristics for the material obtained agreed with those described above. After crystallization, **5** thus obtained showed mp 71–72 °C and $[\alpha]_D$ -12° (*c* 0.5).

Methyl 2,3-di-*O*-benzyl-4-(4-*O*-benzyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (7) and methyl 2,3-di-*O*-benzyl-4-(4-*O*-benzyl-3-deoxy-D,L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (7a). A solution of the amine **6**[14] (30 mg, 0.08 mmol), **5** (35 mg, 0.16 mmol), EDAC (31 mg, 0.16 mmol) and *N*-hydroxybenzotriazole (HOBT, 22 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 1 h, when TLC (solvent *A*) showed that the reaction was essentially complete. After concentration, chromatography gave **7** (41 mg, 88 %), mp 90-91 °C (from ether-hexane), $[\alpha]_D$ -10° (*c* 1.2). ^{1}H NMR ($CDCl_3$) δ 6.67 (d, 1 H, $J_{4,NH}$ 9.6 Hz, NH), 4.74, 4.68 (2 d, partially overlapped, ^2J 12.5 Hz, C*H*$_2$Ph), 4.70 (bs, overlapped, H-1), 4.54, 4.38 (2 d, 1 H each, ^2J 12.1 Hz, C*H*$_2$Ph), 4.42 (s, 2 H, C*H*$_2$Ph), 4.24 (dd, 1 H, $J_{2',3'a}$ 3.2, $J_{2',3'b}$ 8.1 Hz, H-2'), 4.21–4.10 (m, 1 H, H-4), 3.79–3.63 (m, 5 H, H-2,3,5,4'a,b), 3.30 (s, 3 H, OCH_3), 2.22–2.14, 1.98–1.87 (2 m, 1 H each, H-3'a,b), and 1.24 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 99.35 (C-1), 76.49 (C-3), 73.46 (*C*H$_2$Ph), 73.08 (C-2'), 73.01 (C-2), 72.71, 71.12 (2 *C*H$_2$Ph), 69.74 (C-4'), 67.86 (C-5), 54.77 (OCH_3), 52.47 (C-4), 33.35 (C-3'), and 18.08 (C-6); CIMS: *m/z* 567 ([M + 18]$^+$).

Anal. Calcd for $C_{32}H_{39}NO_7$: C, 69.93; H, 7.15; N, 2.55. Found: C, 69.64; H, 7.06; N, 2.53.

When the reaction just described was performed with 4-*O*-benzyl-3-deoxy-D,L-*glycero*-tetronic acid, prepared from D,L-homoserine[16] the product **7a** contained two substances present in approximately equal amounts, as showed by TLC (solvent *A*). Definite, characteristic, structurally significant signals in the ^{1}H NMR spectrum, revealing the presence of two diastereoisomers as well, were at δ 6.69, 6.65 (2 d, partially overlapped, $NH_{D,L}$), 1.24, and 1.21 (2 d, H-$6_{D,L}$); ^{13}C NMR ($CDCl_3$) δ 99.34 (2 C, C-$1_{D,L}$), 76.45 (2 C, C-$3_{D,L}$), 73.56 (CH_2Ph_D), 73.47 (CH_2Ph_L), 73.07 (2 C, C-$2'_{D,L}$), 72.90 (2 C, C-$2_{D,L}$), 72.74 (2 C, $CH_2Ph_{D,L}$), 71.28 (CH_2Ph_D), 71.07 (CH_2Ph_L), 69.80 (C-$4'_L$), 69.50 (C-$4'_D$), 67.86 (C-5_L), 67.77 (C-5_D), 54.78 (2 C, $OCH_{3D,L}$), 52.50 (C-4_L), 52.38 (C-4_D), 33.30 (2 C, C-$3'_{D,L}$), and 18.08 (2 C, C-$6_{D,L}$).

(*S*)-1,2,4-Butanetriol (10). A solution of L-malic acid (**9**, 20 g, 150 mmol) in THF (100 mL) was added dropwise during ~30 min, under a stream of argon, to a solution of BMS in THF (2 M, 220 mL, 440 mmol) contained in a round bottomed flask equipped with a reflux condenser. After 1 h, when TLC (solvent *C*) showed that the reaction was complete, the mixture was successively concentrated with coevaporation of MeOH (three times) and CCl_4, to give virtually pure (TLC, NMR) triol **10**, (15.8 g, ~100 %) which was used for the next step without further purification. A portion, when eluted from a small column of silica gel, showed $[\alpha]_D$ -26° (*c* 5.3, EtOH). The $[\alpha]_D$ of a commercial sample of (**10**) was the same; lit.,[18] yield of a similar reduction using $LiAlH_4$, 21%, $[\alpha]_D$ -22° (*c* 1 and 10); ^{1}H NMR (D_2O) δ 3.85–3.77 (m, 1 H, H-2), 3.70 (m, 2 H, H-4a,b), 3.59 (dd, 1 H, $J_{1a,2}$ 3.8, $J_{1a,1b}$ 11.8 Hz, H-1a), 3.47 (dd, $J_{1b,2}$ 6.8 Hz, H-1b), 1.79–1.55 (m, 2 H, H-3a,b); ^{13}C NMR ($CDCl_3$) δ 71.75 (C-2), 68.34 (C-1), 61.16 (C-4), 37.52 (C-3).

When the order of addition of synthons during the reduction of L-malic acid was reversed the product **10** contained (NMR) ~10% of 3-deoxy-L-*glycero*-tetronolactone[11] formed[9] by spontaneous lactonization of the intermediate 3-deoxy-L-*glycero*-tetronic acid.

2,4-*O*-Benzylidene-(*S*)-1,2,4-butanetriol (12). Ethereal tetrafluoroboric acid (1.5 mL, ~0.01 M), was added with stirring to a solution of the triol **10** (11 g, 0.1 M)

in DMF (220 mL) and the mixture was stirred at room temperature. During the early stage of the reaction a large number of products was formed. After 4 h, TLC (solvent *D*) showed that products formed at the early stage of the reaction, showing fast chromatographic mobility, were converted to one major and one minor product. The mixture was made neutral by addition of solid $NaHCO_3$ and concentrated. The residue was triturated with CH_2Cl_2 and the mixture was filtered through a celite pad. The filtrate was concentrated, and the residue was chromatographed (solvent *E*) to give the title acetal **12** (16.1 g, 80%), $[\alpha]_D$ +106° (*c* 0.6); ^1H NMR ($CDCl_3$) δ 7.50–7.32 (m, 5 H, aromatic), 5.47 (s 1 H, C*H*Ph), 4.23 (m, 1 H, H-4a), 3.89 (m, 2 H, H-2,4b), 3.55 (m, 2 H, H-1a,b), 2.63 (bt, 1 H, OH), 1.81 (m, 1 H, H-3a), 1.33 (m, 1 H, H-3b); ^{13}C NMR ($CDCl_3$) δ 128.76, 128.07, 126.01 (aromatic), 101.17 (*C*HPh), 77.44 (C-2), 66.43 (C-4), 65.16 (C-1), 26.67 (C-3), CIMS: *m/z* 212 ($[M + 18]^+$), 195 ($[M + 1]^+$).

Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.93; H, 7.30.

***t*-Butyl 2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronate (13) and 2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronyl 2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronate (16).** *t*-BuOH (50 mL, 516 mmol) followed by acetic anhydride (19.5 mL, 206.2 mmol) was added to a solution of CrO_3 (10.3 g, 103 mmol) and pyridine (16.7 mL, 206.2 mmol) in 4:1 CH_2Cl_2-DMF (260 mL) which had been stirred for 30 min. A solution of the benzylidene derivative (**12**, 5 g, 25.8 mmol) in 4:1 CH_2Cl_2-DMF (100 mL) was added dropwise over a period of 1.5 h, and stirring was continued at room temp for 16 h. TLC (solvent *C*) then showed that all starting material was consumed and that, essentially, one major and one minor product were formed. The slower moving product (minor) showed mobility only slightly faster than **12**. After cooling (5-10 °C), solid $NaHCO_3$ (35 g) and ethanol (24 mL) was added and, after 30 min, the mixture was concentrated with coevaporation of toluene. The residue was suspended in EtOAc, the resulting mixture was filtered, the solids were washed with EtOAc, and the filtrate was concentrated. Chromatography of the residue gave first the title compound **13** (5.5 g,

81%), mp 55-56 °C (from hexane or ethanol); $[\alpha]_D$ -11° (c 1); ^{1}H NMR ($CDCl_3$) δ 7.45–7.24 (m, 5 H, aromatic protons), 5.45 (s, 1 H, C*H*Ph), 4.30 (dd, 1 H, $J_{2,3a}$ 2.8, $J_{2,3b}$ 11.8 Hz, H-2), 4.24 (ddd, 1 H, $J_{4a,3a}$ 1.4, $J_{4a,3b}$ 5.1, $J_{4a,4b}$ ~11.8 Hz, H-4a), 3.92 (dt, 1 H, $J_{4b,3a}$ 2.6, $J_{4b,3b}$ ~11.8 Hz, H-4b), 2.09–1.95 (m, 1 H, H-3a), 1.80–1.73 (m, 1 H, H-3b), 1.42 (s, 9 H, 3 CH_3); ^{13}C NMR ($CDCl_3$) δ 166.97 (C-1), 137.89 [*C*(CH_3)$_3$], 101.06 (*C*Ph), 75.63 (C-2), 66.74 (C-4), 28.23 (C-3), 27.98 [CH_3)$_3$]; CIMS: *m/z* 265 ([M + 1]$^+$), 282 ([M + 18]$^+$).

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.16; H, 7.63. Found: C, 68.19; H, 7.67.

The material obtained on continued elution with a more polar solvent mixture solidified on concentration. Crystallization from $CHCl_3$–hexane gave pure tetronyl tetronate **16** (480 mg, 9.8%), mp 100-101 °C (from ethanol), $[\alpha]_D$ +18° (*c* 0.6); ^{1}H NMR ($CDCl_3$) δ 5.55, 5.52 (2 s, 2 H, H-5,5'), 4.56 (dd, 1 H, $J_{2,3a}$ 2.7, $J_{2,3\ b}$ 11.8 Hz, H-2), 4.36–4.26 (m, 4 H, H-1'a,1'b, 4a,4'a), 4.22–4.14 (m, 1 H, H-2'), 4.06–3.93 (m, 2 H, 4b,4'b), 2.23–2.18 (m, 1 H, H-3a), 1.97–1.83 (m, 2 H, H-3b,3'a), 1.58–1.50 (H-3'b); ^{13}C NMR ($CDCl_3$) δ 169.67 (C-1), 101.26, 101.13 (C-5,5'), 75.34 (C-2), 74.55 (C-2'), 66.93 (C-1'), 66.62, 66.48 (C-4,4'), 28.14 (C-3), 27.36 (C-3')'; CIMS: *m/z* 402 ([M + 18]$^+$), 384 ([M]$^+$).

Anal. Calcd for $C_{22}H_{24}O_6$: C, 68.74; H, 6.29. Found: C, 69.30; H, 6.41.

When the above reaction was carried out as described,[21,23] compounds **13** and **16** were obtained in 54 and 21%, respectively.

Methyl 2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronate (14). The solution of tetronyl tetronate (**16**) (386 mg, 1 mmol) in methanol (20 mL) containing a few drops of sodium methoxide was kept at 0 °C for 30 min, when TLC (solvent *C*) showed that all starting material was consumed. Two products were formed, as shown by UV detection, the faster moving of which charred poorly with the sulfuric acid reagent. The mixture was treated, at 0 °C, with Amberlite IR 120 (H$^+$) resin, filtered, and concentrated. Chromatography gave first the methyl ester **14** (105 mg, 0.47 mmol, 47%), mp 51-52°

(from EtOAc–hexane), $[\alpha]_D$ -21° (*c* 0.5); ^{1}H NMR ($CDCl_3$) δ 5.53 (s, 1 H, C*H*Ph), 4.52 (dd, 1 H, $J_{2,3a}$ 2.82, $J_{2,3b}$ 11.9 Hz, H-2), 4.33 (ddd, 1 H, $J_{3a,4a}$ 1.2, $J_{3b,4a}$ 5.0, $J_{4a,4b}$ 11.7 Hz, H-4a), 4.00 (dt, 1 H, $J_{3a,4b}$ 2.6, $J_{3b,4b}$~12 Hz, H-4b), 3.71 (s, 3 H, OCH_3), 2.21–2.09, 1.91–1.84 (2 m, 2 H, H-3a,b); ^{13}C NMR ($CDCl_3$) δ δ 101.37 (*C*HPh), 75.51 (C-2), 66.67 (C-4), 52.25 (OCH_3), 28.13 (C-3); CIMS: *m/z* 240 ([M + 18]$^+$), 223 ([M + 1]$^+$).

Anal. Calcd for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.77; H, 6.42.

Eluted next was pure alcohol **12** (93 mg, 0.48 mmol, 48%), as shown by CIMS and NMR spectra.

2,4-*O*-Benzylidene-3-deoxy-L-*glycero*-tetronic acid (15). Aqueous 1 M NaOH (90 mL, 90 mmol) was added to a stirred solution of **13** (8.7 g, 33 mmol) in 1,2-dimethoxyethane, and the solution was kept at room temperature for 5 h. TLC (solvent *F*) then showed that all starting material was consumed. At 5-10 °C, the mixture was neutralized with Amberlite IR 120, H^+-resin, filtered, the resin was washed with 1,2-dimethoxyethane, and the filtrate was concentrated. Crystallization from CH_2Cl_2–ether gave **15** (6 g, 88%). Recrystallization a portion from methanol gave material melting at 120–121 °C, $[\alpha]_D$ -31° (*c* 0.9); ^{1}H NMR ($CDCl_3$) δ 7.51–7.27 (m, 5 H, aromatic protons), 6.45 (bs, 1 H, COOH), 5.59 (s, 1 H, C*H*Ph), 4.55 (dd, 1 H, $J_{2,3a}$ 2.1, $J_{2,3b}$ 11.6 Hz, H-2), 4.37 (ddd, 1 H, $J_{3a,4a}$ 1.7, $J_{3b,4a}$ 4.8 Hz, $J_{4a,4b}$ 11.6 Hz, H-4a), 4.05 (dt, 1 H, $J_{3a,4b}$ 2.9, $J_{3b,4b}$ ~11.8 Hz, H-4b), 2.19–1.97 (2 m, 2 H, H-3a,b); ^{13}C NMR ($CDCl_3$) δ 171.92 (CO), 137.12, 129.41, 128.41, 126.17 (aromatic carbons), 101.36 (*C*HPh), 74.61 (C-2), 66.78 (C-4), 27.83 (C-3); CIMS: *m/z* 209 ([M + 1]$^+$), 226 ([M + 18]$^+$).

Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.40; H, 5.90.

Methyl 4,6-dideoxy-2,3-di-*O*-(4-methoxybenzyl)-4-(2,4-*O*-benzylidene-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (18). Hydrogen sulfide was passed for 1 h through a solution of methyl 4-azido-4,6-dideoxy-

2,3-di-*O*-4-methoxybenzyl-α-D-mannopyranoside[12] (3.6 g) in 7:3 pyridine–Et_3N (200 mL). The solution was kept, in a loosely stoppered round bottomed flask, at room temperature overnight, when TLC (solvent *G*) showed that the reaction was complete and that a single product was formed. After concentration, the residue was chromatographed to give pure amine **17** (2.7 g, 75%) which was used immediately for the further conversion. ^{1}H NMR ($CDCl_3$) δ 4.69 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.64, 4.58 (2 d, 1 H each, ^2J 12.1 Hz, CH_2Ph), 4.45, 4.26 (2 d, 1 H each, ^2J 11.3 Hz, CH_2Ph), 3.78, 3.77 (2 s, 6 H, 2 CH_3OPh), 3.72 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2) 3.52–3.42 (m, 2 H, H-3,5), 3.30 (s, 3 H, OCH_3), 2.98 (t, 1 H, *J* 9.8 Hz, H-4), 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 99.14 (C-1), 79.29 (C-3), 72.08 (C-2), 71.99, 70.64 (2 *C*H_2Ph), 69.54 (C-5), 55.04 (2 C, 2 *C*H_3OPh), 54.40 (OCH_3), 53.47 (C-4), 17.99 (C-6); CIMS: *m/z* 418 ([M + 1]$^+$).

A solution of the foregoing amine **17** (1 g, 2.4 mmol), the acid **15** (220 mg, 2.64 mmol), and EDAC (0.7 g, 3.6 mmol) in CH_2Cl_2 (10 mL) was kept at room temperature for 1 h. TLC (solvent *G*) then showed that all starting material was consumed and that one product was formed. After concentration, the residue was triturated with toluene, the solid was removed, and the material in the filtrate was chromatographed to give **18** (1.6 g, 91%), $[\alpha]_D$ -5° (*c* 1.4); ^{1}H NMR ($CDCl_3$) δ 6.27 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH), 5.53 (s, 1 H, C*H*Ph), 4.64, 4.61 (2 d overlapping the signal of H-1, 3 H, ^2J 12.2 Hz, CH_2Ph), 4.45 (d, 1 H, ^2J 11.5 Hz, C*H*Ph), 4.36–4.29 (m, 3 H, H-2',4a',C*H*Ph), 4.13–4.03 (m, 1 H, H-4), 3.99 (dt, 1 H, $J_{3'a,4'b}$ 2.6 Hz, $J_{3'b,4'b} \sim J_{4'a,4'b}$ 12.0 Hz, H-4'b), 3.77–3.73 (m, 9 H, incl 2 s at 3.77 and 3.73 for 2 CH_3OPh, H-2,3,5), 2.08–2.01, 1.98–1.82 (2 m, 2 H, H-3'a,b), 1.23 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 101.07 (*C*HPh), 99.39 (C-1), 76.51 (C-2'), 75.97 (C-3), 72.56 (C-2), 72.25, 71.03 (*C*H_2Ph), 67.43 (C-5), 67.22 (C-4'), 55.11 (2 C, 2 *C*H_3OPh), 54.72 (OCH_3), 52.65 (C-4), 28.53 (C-3'), 18.04 (C-6); CIMS: *m/z* 608 ([M + 1]$^+$), 625 ([M + 18]$^+$).

Anal. Calcd for $C_{34}H_{42}NO_9$: C, 67.09; H, 6.96; N, 2.30. Found: C, 67.19; H, 6.91; N, 2.26.

***t*-Butyl 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronate (11).** Borane-TMA complex (664 mg, 9.1 mmol) was added to a suspension of **13** (0.4 g, 1.5 mmol) and powdered molecular sieves 4 Å (100 mg) in anhydrous THF (10 mL), and the mixture was stirred at room temperature for 30 min. After addition, at 0 °C, of anhydrous aluminum chloride (1.2 g, 9.1 mmol), the mixture was stirred at 0 °C for 2 h. TLC (solvent *C*) showed that all starting material was consumed and that one major product was formed. The mixture was poured into cold, aqueous $NaHCO_3$, the mixture was extracted with CH_2Cl_2, the organic phase was dried, and concentrated. The material in the residue was chromatographed, to give the major product **11** (300 mg, 75%), mp 41-43 °C (from pentane), $[\alpha]_D$ -6° (*c* 0.8); ^1H NMR ($CDCl_3$) δ 4.53, 4.48 (2 d, 1 H each, ^2J 12.0 Hz, 2 CH_2Ph), 4.20 (m, 1 H, changes to dd on deuteration, $J_{2,3a}$ 4.0, $J_{2,3b}$ 7.4 Hz, H-2), 3.64 (m, 2 H, H-4a,b), 3.05 (d, $J_{2,OH}$ 5.5 Hz, disappears on deuteration, OH), 2.17–2.05, 1.95–1.83 (2 m, 1 H each, H-3a,b), 1.46 [s, 9 H, $C(CH_3)_3$]; ^{13}C NMR ($CDCl_3$) δ 73.25 (CH_2Ph), 68.40 (C-2), 66.40 (C-4), 34.21 (C-3), 27.99 [$C(CH_3)_3$]; CIMS: *m/z* 284 ([M + 18]$^+$), 267 ([M + 1]$^+$).

Anal. Calcd for $C_{15}H_{22}O_4$; C, 67.64; H, 8.33. Found: C, 67.56; H, 8.35.

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