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Synthetic explorations towards 3-deoxy-3-fluoro derivatives of D-perosamine

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Dedicated to Professor Joachim Thiem on the occasion of his 60th birthday.

Abstract

Based on a literature precedent, preparation of methyl 4-azido-3,4,6-trideoxy-3-fluoro-α-D-mannopyranoside (**18**) was attempted via fluorination of methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-altropyranoside with diethylaminosulfur trifluoride (DAST). Contrary to expectations, the reaction took place with retention of configuration at the site of the fluorination yielding methyl 4-azido-2-*O*-benzyl-3,4,6-trideoxy-3-fluoro-α-D-altropyranoside. Treatment with DAST of methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-allopyranoside (**8**), or its 2-(*p*-methoxybenzyl) analog **9** resulted in fluorination with inversion of configuration at position 3, to give the corresponding 3-deoxy-3-fluoro glucopyranosides **10** and **11**, respectively. Accordingly, compound **18** was prepared from **11**, by de-*p*-methoxybenzylation at O-2, followed by inversion of configuration at C-2 in the resulting methyl 4-azido-3,4,6-trideoxy-3-fluoro-α-D-glucopyranoside. The 2-*O*-methyl analog of **18** (**19**) was prepared by methylation of **18**. Compounds **18** and **19** were converted, conventionally, into the 3-fluoro analogs of the terminal determinants of the O-PS of *Vibrio cholerae* O:1, serotype Inaba and Ogawa, respectively.

Keywords: 3-Deoxy-3-fluoro-D-mannose; Methyl 4-azido-3-fluoro-3,4,6-trideoxy-α-D-mannopyranoside; Fluorination with DAST; Nucleophilic displacement; *Vibrio cholerae* O:1

1. Introduction

During the past few years, we have been interested in developing conjugate vaccines for infectious diseases using synthetic fragments of lipopolysaccharides as their antigenic components. It is generally believed that antibody–antigen binding characteristics of such fragments can reveal their potential utility as components of synthetic vaccines. We have studied the interaction of carbohydrate antigens and antibodies in many antigen–antibody systems.[1–6] Using fragments of polysaccharide antigens and analogs thereof that have been specifically deoxygenated or fluorinated, we have been able to obtain a great deal of detailed information on binding at the molecular level. As part of our current work towards a synthetic vaccine for cholera, we have synthesized a number of specifically deoxygenated and fluorinated analogs of the terminal determinants of the *O*-specific polysaccharide (O-PS) of *Vibrio cholerae* O:1 (compare Ref. 7 and papers cited therein).

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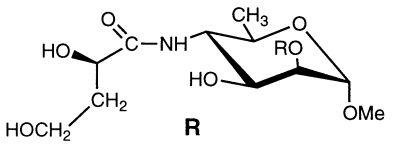
Herein we describe an approach towards substances related to 3-deoxy-3-fluoro-D-perosamine (4-amino-4,6-dideoxy-D-mannose) and their conversion into the terminal determinants of the O-PS of *V. cholerae* O:1 fluorinated at position 3.

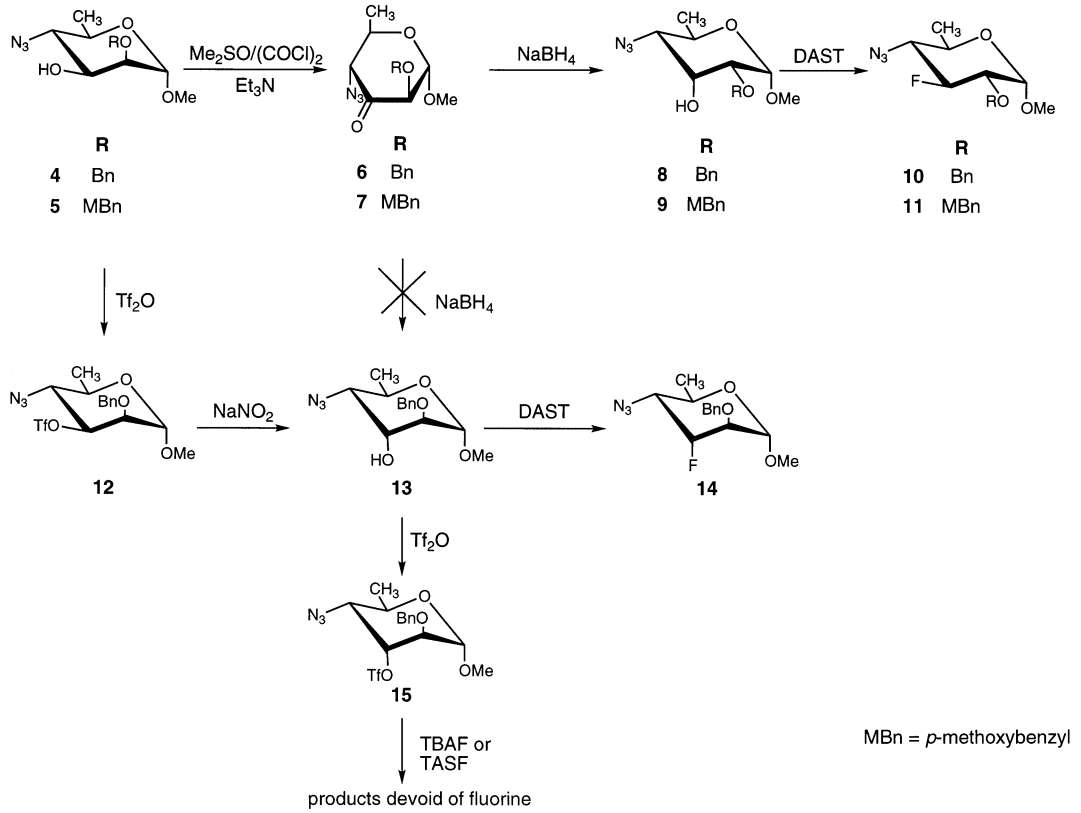
2. Results and discussion

Syntheses of 2-, 2′- and 4′-deoxyfluoro analogs of the terminal monosaccharide determinants of the O-PS of *Vibrio cholerae* O:1, serotype Inaba (**1**) and Ogawa (**2**) have been described previously.[8] The objective of this work was to prepare the 3-fluoro analogs, compounds **23** and **21**, respectively. The synthesis of the key precursor of these substances, methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (**3**), is well established.[9–11] Therefore, we focused initially on preparation of the 3-fluoro derivatives **18** and **19** from the known[12] 2-*O*-benzyl derivative of **3**, azide **4**. The synthesis was to involve first inversion of the configuration at position 3 in **4**, to arrive at the corresponding D-altro compound **13**. Subsequent S_N2 process of a suitable leaving group at C-3 mediated with fluoride ion was expected to give the desired methyl 4-azido-2-*O*-benzyl-3,4,6-trideoxy-3-fluoro-α-D-mannopyranoside. The route starting from **4** seemed feasible, as the theoretical analysis[13] of stereochemical factors suggested that the displacement at C-3 in α- and β-altropyranosides should occur readily. More importantly, we surmised, the claim of a successful, analogous displacement with a fluoride ion, effecting the altro → manno conversion reported by Sharma et al.[14] rendered an important literature precedent.



R	
1	H
2	Me

Following the foregoing strategy (Scheme 1), the inversion of configuration at C-3 in the known 2-benzyl ether (**4**) was attempted via



Scheme 1.

Table 1
^{1}H NMR chemical shifts [a] and peak multiplicities [b] in $CDCl_3$ for compounds **6**–**11**, **13**, **14**, and **16**–**27**

Compound	Chemical shifts (δ)								
	H-1	H-2	H-3	H-4	H-5	H-6	OCH_3-1	OCH_3-2	OCH_3-Ph
6 [c]	4.97d	4.11dd		3.58dd	3.87m	1.40d	3.40s		
7	4.92d	4.08dd		3.57dd	3.86m	1.40d	3.39s		3.81s
8 [d]	4.72dd	3.42t	4.39m	2.72dd	4.05m	1.34d	3.44s		
9 [e]	4.68bd [f,g]	3.39t	4.36m	2.72dd	4.04m	1.31d	3.42s		
9 [h,i]	4.50bd [l]	3.04t	4.30m	2.24dd	4.05m	1.20d	2.89s		
10 [h,j]	4.46bt	3.36dd	4.94dt	2.82ddd	3.40m	1.05d	2.92s		
11 [h,k]	4.47bt	3.46–3.38m	4.98dt	2.84ddd	3.46–3.38m	1.06d	2.94s		3.30s
13 [l]	4.72s	3.62dd	4.13m	3.24dd	4.00m	1.38d	3.40s		
14 [m]	4.66d	3.74ddd	4.85dt	3.25ddd	4.15m	1.38d	3.39s		
16	4.76bt	3.77m [n]	4.54dt	3.24dt	3,57m	1.34d	3.41s		
17 [g]	4.57bt	4.55ddd	4.57dt	2.48ddd	3.14m	0.92d	2.76s		
18	4.71dd	4.13ddd	4.67ddd	3.62dt [o]	3.53m	1.36	3.33s		
19	4.72dd	3.66–3.40m	4.71ddd	3.66–3.40m	3.66–3.40m	1.35d	3.33s	3.53s	
20	4.71bd	3.59m	4.48ddd	3.03q	3.45m	1.29d	3.32s	3.48	
21 [p]	4.76dd	3.82–3.67m	4.76ddd	4.29–4.20m	3.82–3.67m	1.25d	3.37s	3.53	
22	4.71bd	4.06m	4.57ddd	3.07q	3.53m	1.32d	3.35s		
23 [q]	4.80dd	4.22ddd	4.81ddd	4.15q	3.90	1.23d	3.39s		
24 [r]	4.59d	3.46t	5.78t	2.96dd	4.31m	1.28d	3.44s		
25 [s]	4.58s	3.66dd	5.21t	3.39dd	4.14m	1.36d	3.94s		
26 [t]	4.71dd	5.30ddd	4.82ddd	4.18m	3.72m	1.27d	3.28s		
27 [u]	4.73dd	~3.7m	4.78ddd	~4.11m	~3.7m	1.23d	3.41s	3.50s	

[a] Nuclei of the L-tetronic acid residue are referred to with primes.
[b] d, doublet; t, triplet; q, quartet; m, multiplet, b, broad.
[c] δ_{PhCH_2} 4.78, ^2J 12.4 Hz.
[d] δ_{PhCH_2} 4.68, ^2J 12.4 Hz.
[e] δ_{PhCH_2}, 4.61, ^2J 12.0 Hz; δ_{PhOCH_3} 3.80; δ_{OH} 3.5, J $J_{3,OH}$ 8.2 Hz, disappears on deuteration.
[f] Partially overlapped with the $PhCH_2$ signal.
[g] Broadening of the signal due to long-range interaction with H-3.
[h] Measured in C_6D_6.
[i] δ_{PhCH_2}, 4.31, ^2J 11.5 Hz; δ_{PhOCH_3} 3.30; δ_{OH} 3.66, ^2J 7.8 Hz, disappears on deuteration.
[j] δ_{PhCH_2} 4.52, ^2J 12.2 Hz.
[k] δ_{PhCH_2} 4.54, ^2J 11.9 Hz.
[l] δ_{PhCH_2} 4.61(s).
[m] δ_{PhCH_2} 4.64, ^2J 11.9 Hz.
[n] On deuteration, it appears as ddd, $J_{1,2}$ 4.0, $J_{2,3}$ 8.8, $J_{F,2}$ 13.1 Hz.
[o] Partially overlapped with the H-5 signal.
[p] Measured in ~3:1 $CDCl_3$–CD_3OD; $\delta_{H\text{-}2'}$ 4.27 (dd, partially overlapped, J 3.9, 8.5), $\delta_{H\text{-}3'a,b}$ 2.05, 1.84 (2 m), $\delta_{H\text{-}4'a,b}$ 3.79 (dd, partially overlapped, J 5.1, 6.3 Hz).
[q] Measured in D_2O; $\delta_{H\text{-}2'}$ 4.30 (dd, J 4.0, 8.6), $\delta_{H\text{-}3'a,b}$ 2.03, 1.84 (2 m), $\delta_{H\text{-}4'a,b}$ 3.72 (dd, J 6.2, 7.9 Hz).
[r] δ_{PhCH_2} 4.64, ^2J 12.7 Hz, δ_{COCH_3} 2.19.
[s] δ_{COCH_3} 2.10, δ_{PhCH_2} 4.68, ^2J 12.0 Hz.
[t] δ_{NH}, 6.03, $J_{4,NH}$ 9.2 Hz, $\delta_{H\text{-}2'}$ 5,24 (dd, J 5.1, 7.3), $\delta_{H\text{-}3'a,b}$ 2.2 (m), $\delta_{H\text{-}4'a,b}$ 4.15(m), δ_{COCH_3} 2.18, 2.17, 2.15.
[u] δ_{NH}, 6.08, $J_{4,NH}$ 9.7 Hz, $\delta_{H\text{-}2'}$ 5.2 (dd, J 4.9, 7.3), $\delta_{H\text{-}3'a,b}$ 2.1 (m), $\delta_{H\text{-}4'a,b}$ 4.15 (m), δ_{COCH_3} 2.15, 2.03.

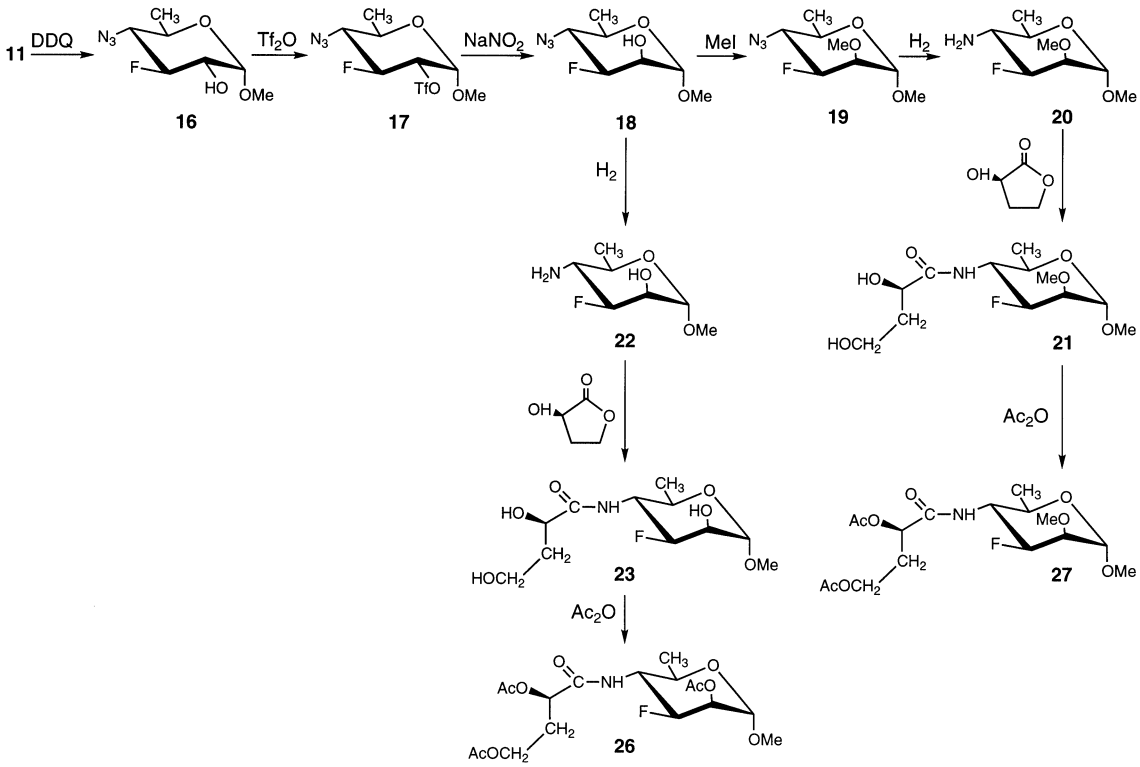
Swern[15,16] oxidation (**4**→**6**), followed by reduction with $NaBH_4$ of the ketone formed. The conformational change, as a result of the conversion **4**→**6**, evidenced from NMR spectra of **6** (Tables 1 and 2), prevented ready identification of any configurational change that may have occurred during the oxidation. [To obtain a definitive proof of the stereochemistry at C-2 in **6** (and **7**) would be beyond the scope of this work, and these structures are shown (Scheme 1), arbitrarily, as having the manno configuration at that position.] However, the ^{1}H NMR spectrum of the product of the following reduction showed

clearly that inversions at both C-3 and C-2 had occurred during the oxidation/reduction steps, yielding the allo compound **8**. The $J_{1,2}$ coupling constant in the desired product having the altro configuration would be expected to remain essentially the same as in the starting manno compound **4** (compare, $J_{1,2}$ 1.6 Hz[9]), and the large diaxial $J_{3,4}$ in **4** (compare, $J_{3,4}$ 9.6 Hz[9]) was expected to become small, typical for an axial–equatorial system. The ^{1}H NMR spectrum of **8** (Tables 1 and 2) showed the expected change of $J_{3,4}$, but it also showed $J_{1,2}$ 3.1 Hz, a value typical for α-glucopyranosides. Notwithstanding that the desired compound **13** was not obtained in this way, we proceeded with fluorination of the allo compound **8** with diethylaminosulfur trifluoride (DAST). NMR spectra of the major product isolated showed that the fluorination took place with inversion of configuration at C-3 giving the gluco compound **10**. This further confirmed the inversion of configuration at C-2 that had occurred during the oxidation/reduction steps (**4**→**6**→**8**, compare, Tables 1 and 2, showing the insignificant change of $J_{1,2}$ value as a result of the conversion). A plausible explanation for such inversion lies in the basic conditions during the foregoing transformations. Thus, we contemplated, it was important that the manno→altro conversion be carried out under nonbasic conditions, so that the undesired inversion of configuration at C-2 would not take place. This was accomplished by nucleophilic displacement of a 3-*O*-trifluoromethanesulfonyl (triflyloxy) group in **12** with nitrite ion, as described by Albert et al.,[17] which yielded directly the epi-hydroxy compound **13**. However, when the foregoing

Table 2
^{1}H NMR coupling constants [a,b] for compounds **6**–**11**, **13**, **14**, and **16**–**27**

Compound	Coupling constants (Hz)								
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{1,F}$	$J_{2,F}$	$J_{3,F}$	$J_{4,F}$
6 [c]	4.5			9.9	6.2				
7 [d]	4.2			9.8	6.1				
8	3.1	3.3	2.5	10.2	6.1				
9	3.5	~3.5	2.7	10.4	6.2				
9 [e]	3.5	~3.4	2.6	10.1	6.4				
10	~3.4	9.3	9.3	9.9	6.0	[f]	[g]	53.4	13.4
11	~3.6	9.0	9.0	9.7	6.0	[f]	[g]	53.9	13.0
13	1.2	3.6	3.5	10.3	6.1				
14	1.2	3.7	2.5	10.0	6.3	[g]	7.7	48.4	30.1
16	~3.9	9.2	9.2	9.5	6.0	~3.9	[g]	52.8	12.2
17	3.7	9.1	9.1	10.0	6.2	~3.7	18.0	55.1	19.1
18	1.8	3.4	9.5	~10	6.1	5.0	6.4	48.9	11.5
19	1.6	3.4	9.9	9.9	6.1	4.8	[g]	48.8	[g]
20	[f]	3.4	10.2	9.7	6.7	[f]	[g]	48.9	9.7
21	1.8	3.4	10.4	[g]	6.2	4.9	[g]	48.6	[g]
22	[f]	3.2	9.9	10.0	6.2	[f]	[g]	48.9	10.0
23	1.8	3.3	10.4	10.2	6.1	[g]	6.6	48.0	10.2
24	4.2	3.2	3.2	10.2	6.3				
25	1.5	3.6	3.2	9.6	6.3				
26	1.7	3.6	10.7	[g]	6.3	4.8	5.7	48.1	[g]
27	2.0	3.0	10.6	[g]	6.1	~4.5	[g]	49.0	[g]

[a] For conditions of measurements, see Table 1.
[b] Nuclei of the L-tetronic acid residue are referred to with primes.
[c] $J_{2,4}$ 1.2 Hz (long range).
[d] $J_{2,4}$ 1.2 Hz (long range).
[e] Measured in C_6D_6.
[f] Not determined due to broad signals.
[g] Not determined due to overlapping signals.



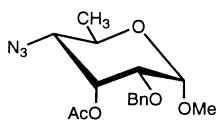
Scheme 2.

compound was treated with DAST, the fluorination occurred with retention of configuration at C-3, yielding methyl 4-azido-2-*O*-benzyl-3,4,6-trideoxy-3-fluoro-α-D-altropyranoside (**14**). This was revealed unequivocally by ^{1}H NMR spectroscopy (Tables 1 and 2). The spectra did not show the expected, structurally significant changes of either $J_{2,3}$ or $J_{3,4}$ in the product of fluorination, as compared with these values in the starting altropyranoside **13**, thereby providing evidence that the fluoro compound **14** had been formed. A retention of configuration during an analogous fluorination has been previously observed[18]. Considering the foregoing results, together with the lack of evidence Sharma et al.[14] provide for the structure of the product of their fluorination (compare, Ref. 14, compound **16** therein), it is not inconceivable that that fluorination had also occurred with retention of configuration. No products of fluorination could be isolated from treatments of triflate **15**, prepared conventionally from the altro alcohol **13**, with either tetrabutylammonium fluoride (TBAF) or tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF).[19,20]

While other routes to compounds **21** and **23** could be envisioned (Scheme 2), we concluded from the observations just described that the most straightforward route to the mannopyranoside **18** would result from taking advantage of the serendipitous inversion of configuration at C-2 (**4**→**6**→**8**). The fact that fluorination at C-3 in **8** positions the fluorine atom at that carbon atom with the desired stereochemistry (→**10**, Scheme 1) opens a relatively simple way to the manno derivative **18**. It only requires inversion of configuration at C-2 in the 3-fluoro gluco compound **10**. This idea was pursued with the *p*-methoxybenzyl derivative **11** (Scheme 1), which was obtained from the known[21] 2-ether **5** through a similar sequence of transformations as described for **10** (**5**→**7**→**9**→**11**). The use of the *p*-methoxybenzyl group as a temporary protecting group at O-2 was dictated by the presence of the azido group at C-4, as it allowed high-yielding regeneration of HO-2 under nonreductive conditions.

Different results have been reported on the efficiency of S_N2 displacements at C-2 in α-glucopyranosides. When treated with the ben-

zoate anion, a 2-*O*-mesyl derivative gave only a poor yield of the corresponding manno compound.[22] Using the better leaving, *O*-triflyloxy group, Haradahira et al.[23] reported a 30% yield in the displacement with fluoride ion. On the other hand, yields reported more recently of the displacement of the 2-*O*-triflyloxy group with fluoride and azide ions, were 60 and 90%, respectively.[18] Treatment of the corresponding 2-hydroxy-α-glucopyranoside with DAST led mainly to a rearrangement,[18] an observation that also others have made.[24–26] Thus, it seemed interesting to attempt the α-gluco → α-manno conversion (**16** → **18**) by inversion of configuration at C-2 via a nucleophilic S_N2 displacement reaction in triflate **17**. Accordingly, the *p*-methoxybenzyl group in **11** was removed by treatment with 2,3-dichloro - 5,6 - dicyano - 1,4 - benzoquinone (DDQ), and the triflate **17**, obtained from **16** conventionally, was treated[17] with $NaNO_2$ in a polar aprotic solvent. That the product of this reaction was the desired manno compound **18** (60%) was evident from the ^1H NMR spectrum showing $J_{1,2}$ 1.8 Hz (a change of $J_{1,2}$ from 3.9 Hz as a result of the conversion **16** → **18**), as expected for the newly formed diequatorial arrangement of H-1 and H-2. Also, the spectrum reflected a change of the diaxial arrangement of H-2 and H-3 in **16** to the axial–equatorial arrangement of the same protons in **18**, by showing $J_{2,3}$ 3.4 Hz (cf., $J_{2,3}$ 9.2 Hz in **16**, Table 2). Conversion of **18** into **21** and **23** required now only well established chemistry. Methylation of **18** gave the fully protected compound **19**, which was converted into amine **20** by catalytic hydrogenation. Amine **22** was similarly obtained. N-Acylation of each of amines **20** and **22** with the commercially available 3-deoxy-L-glycero-tetronolactone then gave the target compounds **21** and **23**, which were most conveniently isolated as the corresponding peracetates **27** and **26**, respectively. These were deacetylated to give the respective desired products whose NMR spectra were consistent with anticipated structures.



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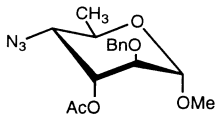

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3. Experimental

General methods.—Unless stated otherwise, optical rotations were measured at ambient temperature for solutions in $CHCl_3$ (*c* 1) with a Perkin–Elmer automatic polarimeter, model 241 MC. All reactions were monitored by thin-layer chromatography (TLC). The detection was effected by charring with 5% H_2SO_4 in EtOH and, when applicable, by UV light. Preparative chromatography was performed by gradient elution from columns of Silica Gel 60 (particle size 0.04–0.063 mm, E. Merck) using, at the onset of development, a solvent mixture slightly less polar than that used for TLC. NMR spectra (25 °C) were obtained at 300 MHz for ^1H and 75 MHz for ^{13}C with a Varian Mercury spectrometer. ^1H NMR chemical shifts reported in Table 1 are referenced to that of Me_4Si (0 ppm) and the HDO solvent peak (4.78 ppm) for measurements in organic solvents and D_2O, respectively. ^{13}C NMR chemical shifts reported in Table 3 are referenced to that of $CDCl_3$ (77.0 ppm) and the center MeOH peak (49.0 ppm). Assignments of signals were made by first-order analysis of spectra and, when feasible, they were supported by APT and/or DEPT experiments, homonuclear decoupling and/or homo- and heteronuclear two-dimensional correlation spectroscopy, using commercial software supplied with the spectrometer. Chemical ionization-mass spectra (CIMS) were measured with a Finnigan spectrometer using ammonia as the reagent gas. Palladium-on-charcoal catalyst (5%, ESCAT 103) was a product of Engelhard Industries. (*S*)-(−)-α-Hydroxy-γ-butyrolactone (3-deoxy-L-*glycero*-tetronolactone) was purchased from Aldrich Chemical Company, and used as supplied. HATU (*O*-7-azobenzotriazol - 1 - yl) - 1,1,3,3 - tetramethyluronium hexafluorophosphate) was purchased from PerSeptive Biosystem, Inc. Unless stated otherwise, solutions in organic solvents were dried with anhyd Na_2SO_4, and concentrated at 40 °C/2 kPa.

*Methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-*D*-arabino(ribo?)-hexopyranosid-3-ulose* (**6**).—A solution of oxalyl chloride (1.63 mL, 18.75 mmol) in dry CH_2Cl_2 (33 mL) was cooled to −60 °C. A solution of Me_2SO (2.66 mL, 37.5 mmol) in CH_2Cl_2 (8.5 mL) was added dropwise, while the inside temperature was kept slightly below −60 °C. When the addition was complete, the mixture was stirred at the same temperature for 30 min. A solution of **4** (4 g, 18.75 mmol) in CH_2Cl_2 (20 mL) was added at −60 °C, and the mixture was stirred for 30 min. Triethylamine (13 mL, 93 mmol) was added at −60 °C, and the mixture was allowed to warm to rt. TLC (4:1 hexane–EtOAc) then showed disappearance of the starting material and formation of a major, faster moving product. The mixture was partitioned between CH_2Cl_2 and water, the organic layer was dried and concentrated. Chromatography (10:1 hexane–EtOAc) gave pure **6** (3.78 g, 94.5%): mp 105–106 °C (from EtOAc); $[\alpha]_D$ −9.9° (*c* 0.7); CIMS: *m/z* 309 ($[M+18]^+$). Anal. Calcd for $C_{14}H_{17}N_3O_4$: C, 57.72; H,

Table 3
^{13}C NMR chemical shifts [a,b] for compounds **6–11**, **14**, and **16–27**

Compound	Chemical shifts									
	C-1	C-2	C-3	C-4	C-5	C-6	Ph*C*H_2	OCH_3-1	OCH_3-2	Ph*O*CH_3
6	100.85	79.99	198.31	69.17	68.63	18.11	72.54	55.57		
7	100.89	79.57	198.63	69.13	68.57	19.06	72.15	55.49		55.20
8	98.94	73.58	68.76	63.86	61.34	17.94	70.41	55.92		
9	99.00	73.16	68.63	63.81	61.26	17.9	70.04	55.82		55.21
9 [c]	99.77	74.69	69.23	64.13	61.81	18.32	70.16	55.14		55.80
10 [d]	97.48	76.55	93.07	65.91	64.37	16.96	71.90	54.06		
11 [b,e]	99.05	77.58	94.56	67.39	65.67	18.35	73.01	55.43		55.12
14	98.62	74.29	87.89	60.34	61.65	18.15	72.98	55.48		
16 [f]	99.20	70.95	94.28	66.12	65.63	17.97		55.56		
17 [g]	97.03	82.16	89.74	65.95	65.34	17.26	55.26			
18 [h]	100.60	68.42	91.65	63.18	66.29	17.97		55.04		
19 [i]	99.01	77.52	86.66	63.55	66.44	17.96		54.85	59.60	
20 [j]	99.02	77.44	93.59	53.36	68.86	17.37		54.47	59.27	
21 [k]	99.19	77.36	89.87	51.58	67.04	17.38		54.89	59.61	
22 [l]	93.76	68.12	93.76	53.16	68.67	17.51		54.77		
23 [m]	101.01	67.43	89.66	51.30	66.82	16.61		54.98		
24 [n]	97.81	72.32	66.68	62.15	61.45	17.88	70.74	55.82		
25 [o]	99.51	73.86	69.36	59.83	62.67	18.31	72.71	55.38		
26 [p]	98.54	68.89	86.64	52.05	66.88	17.38		55.01		
27 [q]	99.32	77.48	89.63	52.36	67.06	17.54		55.00	59.83	

[a] For conditions of measurements, see Table 1.
[b] Nuclei of the L-tetronic acid residue are referred to with primes.
[c] Measured in C_6D_6.
[d] $J_{1,F}$ 9.9, $J_{2,F}$ 15.5, $J_{3,F}$ 183.7, $J_{4,F}$ 15.5, $J_{5,F}$ 5.7 Hz.
[e] $J_{1,F}$ 11.8, $J_{2,F}$ 18.0, $J_{3,F}$ 183.5, $J_{4,F}$ 15.0, $J_{5,F}$ 6.2 Hz.
[f] $J_{1,F}$ 10.0, $J_{2,F}$ 17.3, $J_{3,F}$ 184.1, $J_{4,F}$ 16.2, $J_{5,F}$ 6.2 Hz.
[g] $J_{1,F}$ 7.8, $J_{2,F}$ 16.6, $J_{3,F}$ 189.7, $J_{4,F}$ 15.2, $J_{5,F}$ 6.6 Hz
[h] $J_{1,F}$ 7.8, $J_{2,F}$ 15.1, $J_{3,F}$ 182.3, $J_{4,F}$ 15.8, $J_{5,F}$ 6.8 Hz.
[i] $J_{1,F}$ 8.5, $J_{2,F}$ 14.5, $J_{3,F}$ 187.5, $J_{4,F}$ 15.5, $J_{5,F}$ 6.5 Hz.
[j] $J_{1,F}$ 9.7, $J_{2,F}$ 14.6, $J_{3,F}$ 182.2, $J_{4,F}$ 17.5, $J_{5,F}$ 7.8, $J_{6,F}$ 2.0, $J_{F,OMe-2}$ 2.0 Hz.
[k] $J_{1,F}$ 8.7, $J_{2,F}$ 14.6, $J_{3,F}$ 187.9, $J_{4,F}$ 17.5, $J_{5,F}$ 6.2, $J_{F,OMe-2}$ 3.1 Hz.
[l] $J_{1,F}$ 9.6, $J_{2,F}$ 17.4, $J_{3,F}$ 179.2, $J_{4,F}$ 17.1, $J_{5,F}$ 8.3, $J_{6,F}$ 2.0 Hz.
[m] $J_{1,F}$ 8.5, $J_{2,F}$ 16.6, $J_{3,F}$ 184.3, $J_{4,F}$ 17.7, $J_{5,F}$ 7.0 Hz.
[n] δ_{COCH_3} 21.05 ppm.
[o] δ_{COCH_3} 20.98 ppm.
[p] $\delta_{C\text{-}2'}$ 71.12, $\delta_{C\text{-}3'}$ 30.63, $\delta_{C\text{-}4'}$ 59.76 ppm; δ_{COCH_3} 20.88, 20.75, 20.68 ppm.
[q] $\delta_{C\text{-}2'}$ 71.26, $\delta_{C\text{-}3'}$ 30.66, $\delta_{C\text{-}4'}$ 59.83 ppm; δ_{COCH_3} (2 C) 20.79 ppm.

5.88; N 14.42. Found: C, 57.64; H, 5.97; N, 14.39.

*Methyl 4-azido-4,6-dideoxy-2-*O*-p-methoxybenzyl-α-*D*-arabino(ribo?)-hexopyranosid-3-ulose* (**7**).—Treatment of **5** (21.1 g, 65.26 mmol) as described for the preparation of **6** gave **7** (19 g 91%): mp 95–96 °C (from EtOH): $[\alpha]_D$ −31.5° (*c* 1.1); CIMS: *m/z* 339 ($[M+1]^+$). Anal. Calcd for $C_{15}H_{19}N_3O_5$: C, 56.07; H, 5.96; N, 13.08. Found: C, 55.85, H, 5.96, N, 13.06.

*Methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-*D*-allopyranoside* (**8**).—Sodium borohydride (277 mg, 7.32 mmol) was added at 0 °C to a solution of **6** (3.05 g, 10.5 mmol) in 7:2 EtOH–H_2O (90 mL), the cooling was removed, and the mixture was stirred for 1 h. TLC (10:1 toluene–acetone) then showed that no starting material remained. Acetone (2 mL) was added to decompose excess reagent, the mixture was concentrated, and the residue was partitioned between water and CH_2Cl_2. The organic phase was dried, concentrated and the residue was chromatographed to give first a small amount of **3** resulting from the non-stereospecific reduction.

Eluted later was pure, amorphous **8** (2.33 g, 75%): $[\alpha]_D$ +1.2° (*c* 0.4; CIMS: *m/z* 311 ($[M+18]^+$). Anal. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.53; N 14.33. Found: C, 57.45; H, 6.45; N, 14.36.

A small amount of an intermediate, mixed fraction was also obtained.

Compound **8** was further characterized as methyl 3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-allopyranoside (**24**), which was prepared by conventional acetylation: mp 61–62 °C (from ether–hexane): $[\alpha]_D$ −41.9° (*c* 0.7); CIMS: *m/z* 353 ($[M+18]^+$). Anal. Calcd for $C_{16}H_{21}N_3O_5$: C, 57.30; H, 6.31; N 12.53. Found: C, 57.41; H, 6.26; N, 12.53.

*Methyl 4-azido-4,6-dideoxy-2-*O*-p-methoxybenzyl-α-*D*-allopyranoside* (**9**).—Reduction of ketone **7** (10.07 g, 31.34 mmol), as described for the preparation of **8** gave, after chromatography (7:3 hexane–EtOAc), the allo compound **9** (9.80 g, 97%) as an oil: $[\alpha]_D$ −4.2° (*c* 1.1); CIMS: *m/z* 341 ($[M+1]^+$). Anal. Calcd for $C_{15}H_{21}N_3O_5$: C, 55.72; H, 6.55; N, 13.00. Found: C, 55.54, H, 6.59, N, 12.79.

*Methyl 4-azido-2-*O*-benzyl-3,4,6-trideoxy-3-fluoro-α-*D*-glucopyranoside* (**10**).—DAST (0.26 mL, 2.13 mmol) was added at −20 °C to a solution of **8** (420 mg, 1.42 mmol) in toluene (4 mL), and the mixture was stirred at rt for 30 min. TLC (4:1 hexane–EtOAc) then showed that all starting material was consumed and that one major product was formed. Methanol (1 mL) was added with stirring at −20 °C, followed by aq $NaHCO_3$ and, when effervescence ceased, the mixture was partitioned between CH_2Cl_2 and water. The organic phase was concentrated and chromatography (9:1 → 4:1 hexane–EtOAc) gave **10** (210 mg, 50%): $[\alpha]_D$ +99.6° (*c* 0.5); CIMS: *m/z* 313 ($[M+18]^+$). Anal. Calcd for $C_{14}H_{18}FN_3O_3$: C, 56.94; H, 6.14; N 14.23. Found: C, 56.77; H, 6.05; N, 14.14.

*Methyl 4-azido-3-fluoro-2-*O*-p-methoxybenzyl-3,4,6-trideoxy-α-*D*-glucopyranoside* (**11**).—Fluorination of alcohol **9** (9.8 g, 30.31 mmol) with DAST in toluene, as described for the preparation of **10** gave, after chromatography, the fluoro compound **11** (4.85 g, 49%): mp 62–63.5 °C (from ether–hexane): $[\alpha]_D$ +35° (*c* 1.1); CIMS: *m/z* 343 ($[M+1]^+$). Anal. Calcd for $C_{15}H_{20}FN_3O_4$: C, 55.38; H, 6.20; N, 12.92. Found: C, 55.23, H, 6.22, N, 12.90.

*Methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-*D*-altropyranoside* (**13**).—Trifluoromethanesulfonic anhydride (0.2 mL, 1.2 mmol) was added at −5 °C to a solution of alcohol **4**[12] (293 mg, 1 mmol) in CH_2Cl_2 (5 mL) containing pyridine (0.25 mL, 2.5 mmol), and the mixture was stirred at rt until TLC (4:1 hexane–EtOAc) showed that the reaction was complete (~1 h). The mixture was successively washed with 1 M HCl, aq $NaHCO_3$ and water, the organic phase was dried and concentrated at <35 °C, to give methyl 4-azido-2-*O*-benzyl-3-*O*-trifluoromethylsulfonyl-4,6-dideoxy-α-D-mannopyranoside (**12**, CIMS: *m/z* 425 ($[M+18]^+$)), which was sufficiently pure for the next step.

Sodium nitrite (0.150 g, 0.17 mmol) was added to a solution of the foregoing triflate in DMF (3.5 mL), and the mixture was stirred at 50 °C for 2 h, when TLC (4:1 hexane–EtOAc) showed that all starting material was consumed. The mixture was concentrated, the residue was triturated with acetone, the solids

were removed by filtration, the filtrate was concentrated, and the residue was chromatographed to give the amorphous methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-altropyranoside (**13**, 150 mg, 51%): $[\alpha]_D$ +70° (*c* 1.5); CIMS: *m*/*z* 313 ($[M+18]^+$). Anal. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.53; N 14.33. Found: C, 57.49; H, 6.63; N, 14.03.

For further characterization by NMR spectroscopy, a portion of **13** was acetylated with Ac_2O–pyridine, to give methyl 3-*O*-acetyl-4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-altropyranoside (**25**): CIMS: *m*/*z* 335 ($[M+18]^+$).

Attempted fluorinations of **13**.—(a) DAST (0.12 mL, 1.02 mmol) was added at −10 °C to a solution of the altroside **13** (100 mg, 0.34 mmol) in dry toluene (1 mL), and the mixture was stirred at 50 °C for 30 min, when TLC (4:1 hexane–EtOAc) showed that all starting material was consumed. The mixture was processed as described above for similar fluorinations and chromatographed to give methyl 4-azido-2-*O*-benzyl-3-fluoro-3,4,6-trideoxy-α-D-altropyranoside (**14**, 55 mg, 55%), as shown by NMR spectroscopy; CIMS: *m*/*z* 313 ($[M+18]^+$).

(b) Compound **13** (293 mg, 1 mmol) was converted into the corresponding 3-trifluoromethanesulfonate (**15**), as described for the preparation of **12**, and the product was divided into two equal portions.

To a solution of **15** (∼0.5 mmol) in CH_2Cl_2 (8 mL) was added, at −30 °C, TASF (0.826 g, 3 mmol), and the mixture was stirred at rt for 20 min, when TLC (4:1 hexane–EtOAc) showed that all starting material was consumed. After concentration, the residue was partitioned between water and CH_2Cl_2, and the organic phase was dried and concentrated to give material containing essentially one charring component. NMR spectra of the material showed that fluorination did not occur, and the material was not investigated further.

(c) To a solution of **15** (∼0.5 mmol) in dry THF (5 mL) was added 1 M solution of Bu_4NF in THF (1 mL), and the mixture was stirred overnight at rt, when TLC (4:1 hexane–EtOAc) showed that the reaction was almost complete. NMR spectroscopy indicated that the same product as in (b), devoid of fluorine, was formed.

Methyl 4-azido-3-fluoro-3,4,6-trideoxy-α-D-glucopyranoside (**16**).—Water (∼0.2 mL) followed by DDQ (1.736 g, 7.6 mmol) was added to a solution of the methoxybenzylated compound **11** (1.77 g, 5.46 mmol) in CH_2Cl_2 (∼50 mL). The solution, which became dark, was stirred at rt overnight, when TLC (4:1 hexane–EtOAc) showed that the reaction was complete. After dilution with CH_2Cl_2, the mixture was washed with brine, dried, and concentrated. Chromatography (9:1 → 6:1 hexane–EtOAc) gave **16** in essentially theoretical yield: mp 60–61 °C (from ether–pentane): $[\alpha]_D$ +24.5° (*c* 0.8). CIMS: *m*/*z* 223 ($[M+1]^+$). Anal. Calcd for $C_7H_{12}FN_3O_3$: C, 40.97; H, 5.89; N, 20.48. Found: C, 41.09, H, 5.76, N, 20.42.

Methyl 4-azido-3-fluoro-3,4,6-trideoxy-α-D-mannopyranoside (**18**).—Pyridine (1.1 mL, 13.6 mmol) followed by triflic anhydride (1.2 mL, 7.1 mmol) was added with stirring at 0 °C to a solution of the fluoro compound **16** (1.12 g, 5.46 mmol) in CH_2Cl_2 (∼50 mL). After 30 min, TLC (4:1 hexane–EtOAc) showed that the reaction was complete. The solution was diluted with CH_2Cl_2, washed successively with aq HCl (M), $NaHCO_3$ (saturated, twice), water, dried, and concentrated. Chromatography (95:5 hexane–EtOAc) gave triflate **17** (1.63 g, 88.5%): CIMS: *m*/*z* 355 ($[M+1]^+$), which was used immediately for the next step.

Sodium nitrite (666 mg, 9.66 mmol) was added to a solution of the foregoing compound **17** (1.63 g, 4.83 mmol) in DMF (∼20 mL), and the mixture was stirred at 85 °C for 4 h. Heating was discontinued, and after 12 h TLC (4:1 hexane–EtOAc) showed that all starting material had been consumed. The mixture was concentrated, and the residue triturated with CH_2Cl_2. After filtration and concentration of the filtrate through a Celite pad, chromatography afforded the fluorinated α-mannopyranoside **18**, 590 mg (60%) as a colorless oil: $[\alpha]_D$ +124° (*c* 1.3); CIMS: *m*/*z* 223 ($[M+1]^+$). Anal. Calcd for $C_7H_{12}FN_3O_3$: C, 40.97; H, 5.89; N, 20.48. Found: C, 40.94, H, 5.85, N, 20.39.

*Methyl 2-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-glycero-tetronamido)-3-fluoro-3,4,6-trideoxy-α-D-mannopyranoside* (**26**).—A mixture of azide **18** (263 mg, 1.28 mmol) and 5%

Pd–C (160 mg) in EtOH (~ 4 mL) was stirred under hydrogen for 3 h, when TLC (4:1 hexane–EtOAc and neat EtOAc) showed complete disappearance of the starting material and formation of one product. After filtration through a Celite pad, the filtrate was concentrated, and chromatography gave methyl 4-amino-3-fluoro-3,4,6-trideoxy-α-D-mannopyranoside (**22**, 213 mg, 93%) as a colorless oil: CIMS: m/z 180 ($[M+1]^+$), 197 ($[M+18]^+$).

A mixture of the foregoing amine (200 mg, 1.116 mmol) and 3-deoxy-L-*glycero*-tetronolactone [(*S*)-(−)-α-hydroxy-δ-butyrolactone] (228 mg, 2.23 mmol) was stirred at 95 °C for 24 h. The mixture was cooled to rt and chromatographed (twice, 1st column, 6:1 CH_2Cl_2–methanol; 2nd column, 10:1 EtOAc–MeOH) to give amide **23** (277 mg, 88%) as a yellow oil. It was dissolved in pyridine (4 mL), and the mixture was treated with Ac_2O (2 mL) overnight at rt. The mixture was concentrated, and the residue was chromatographed (1:1 hexane–EtOAc) to give **26** (293 mg, 73%): mp 101–102 °C (from EtOAc); $[\alpha]_D$ −9.3° (*c* 1.2): CIMS: m/z 408 ($[M+1]^+$), 425 ($[M+18]^+$). Anal. Calcd for $C_{17}H_{26}FNO_9$: C, 50.12; H, 6.43; N, 3.44. Found: C, 49.89, H, 6.58, N, 3.34.

Methyl 4-(3-deoxy-L-glycero-*tetronamido)-3-fluoro-3,4,6-trideoxy*-α-D-*mannopyranoside* (**23**).—Deacetylation (Zemplén), of acetate **26** gave compound **23** in virtually theoretical yield: mp 126–127.5 °C (from EtOH–EtOAc); $[\alpha]_D$ +29° (*c* 0.8, MeOH); CIMS: m/z 282 ($[M+1]^+$). Anal. Calcd for $C_{11}H_{20}FNO_6$: C, 46.97; H, 7.17; N, 4.98. Found: C, 46.87, H, 7.26, N, 4.92.

Methyl 4-azido-3-fluoro-2-O-*methyl-3,4,6-trideoxy*-α-D-*mannopyranoside* (**19**).—To a solution of the foregoing mannoside **18** (323 mg, 1.57 mmol) in DMF (~ 3 mL) was added NaH (82 mg, 2.04 mmol), followed by MeI (0.13 mL, 2.04 mmol), and the mixture was stirred at rt for 30 min. TLC (4:1 hexane–EtOAc) then showed that the methylation was complete. A few drops of MeOH were added to decompose the excess of reagents, and the mixture was concentrated. Chromatography (9:1 hexane–EtOAc) of the material in the residue gave **19** (311 mg, 90%) as a colorless syrup: $[\alpha]_D$ +115.0° (*c* 1.5); CIMS: m/z 237 ($[M+1]^+$). Anal. Calcd for $C_8H_{14}FN_3O_3$: C, 43.83; H, 6.44; N, 19.17. Found: C, 44.07, H, 6.44, N, 18.89.

Methyl 2-O-*acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-3-fluoro-2*-O-*methyl-3,4,6-trideoxy*-α-D-*mannopyranoside* (**27**).—Hydrogenation of the foregoing azide **19** (311 mg), as described for the preparation of **22**, gave methyl 4-amino-3-fluoro-2-*O*-methyl-3,4,6-trideoxy-α-D-mannopyranoside (**20**) (175 mg, 61%): CIMS: m/z 194 ($[M+1]^+$), 211 ($[M+18]^+$).

A mixture of the foregoing amine **20** (175 mg, 0.905 mmol), and 3-deoxy-L-*glycero*-tetronolactone [(*S*)-(−)-α-hydroxy-δ-butyrolactone, 185 mg, (1.811 mmol)] was stirred at 95 °C for 24 h. After cooling to rt the crude product was treated with a mixture of 3:1 pyridine–Ac_2O (4 mL), and the product isolated as described for the preparation of **26**. After conventional processing and chromatography (2:1 hexane–EtOAc), the crystalline acetate **27** (212 mg, 62%) showed mp 107–109 °C (from EtOH–Et_2O) and $[\alpha]_D$ +13.0° (*c* 0.7); CIMS: m/z 397 ($[M+1]^+$). Anal. Calcd for $C_{16}H_{26}FNO_8$: C, 50.65; H, 6.91; N, 3.69. Found: 50.10, H, 7.16, N, 3.44. We could not obtain closer analytical figures than those just shown, which fit a tetartohydrate (0.25 water) of **27**.

Methyl 4-(3-deoxy-L-glycero-*tetronamido)-3-fluoro-2*-O-*methyl-3,4,6-trideoxy*-α-D-*mannopyranoside* (**21**).—Deacetylation (Zemplén) of **27** (147 mg) gave pure **21** (96 mg, 84%): mp 173–175 °C (from acetone): $[\alpha]_D$ +27.3° (*c* 0.7); CIMS: m/z 296 ($[M+1]^+$), 313 ($[M+18]^+$). Anal. Calcd for $C_{12}H_{22}FNO_6$: C, 48.81; H, 7.51; N, 4.74. Found: C, 48.85, H, 7.69, N, 4.69.

References

1. Nashed, E. M.; Perdomo, G. R.; Padlan, E. A.; Kováč, P.; Kabat, E. A.; Glaudemans, C. P. J. *J. Biol. Chem.* **1990**, *265*, 20699–20707.
2. Glaudemans, C. P. J.; Kováč, P.; Nashed, E. M.; Padlan, E. A.; Arepalli, S. R. *ACS Symp. Ser.* **1994**, *580*, 157–183.
3. Glaudemans, C. P. J.; Kováč, P.; Nashed, E. M. *Methods Enzymol.* **1994**, *247B*, 305–322.
4. Glaudemans, C. P. J.; Kováč, P.; Rao, A. S. *Carbohydr. Res.* **1989**, *190*, 267–277.

5. Glaudemans, C. P. J.; Kováč, P.; Rasmussen, K. *Biochemistry* **1984**, *23*, 6732–6736.
6. Pavliak, V.; Pozsgay, V.; Kováč, P.; Karpas, A.; Chu, C.; Schneerson, R.; Robbins, J.; Glaudemans, C. P. J. *J. Biol. Chem.* **1993**, *268*, 25797–25802.
7. Wang, J.; Zhang, J.; Miller, C. E.; Villeneuve, S.; Ogawa, Y.; Lei, P.-s.; Lafaye, P.; Nato, F.; Karpas, A.; Bystrický, S.; Szu, S. C.; Robbins, J. B.; Kováč, P.; Fournier, J.-M.; Glaudemans, C. P. J. *J. Biol. Chem.* **1998**, *273*, 2777–2783.
8. Chang, A. H. C.; Horton, D.; Kováč, P. *Tetrahedron: Asymmetry* **2000**, *11*, 595–606.
9. Eis, M. J.; Ganem, B. *Carbohydr. Res.* **1988**, *176*, 316–323.
10. Bundle, D. R.; Gerken, M.; Peters, T. *Carbohydr. Res.* **1988**, *174*, 239–251.
11. Gotoh, M.; Kováč, P. *J. Carbohydr. Chem.* **1994**, *13*, 1193–1213.
12. Peters, T.; Bundle, D. R. *Can. J. Chem.* **1989**, *67*, 497–502.
13. Hough, L.; Richardson, A. C. In *Nucleophilic Displacement Reactions*, *Part F*; Coffey, S., Ed.; New York: Elsevier, 1967; Vol. I, pp. 403–407.
14. Sharma, M.; Bernacki, R. J.; Hillman, M. J.; Korytnyk, W. *Carbohydr. Res.* **1993**, *240*, 85–93.
15. Mancuso, A. J.; Huang, S. L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480–2482.
16. Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651–1660.
17. Albert, R.; Dax, K.; Link, R. W.; Stütz, A. E. *Carbohydr. Res.* **1983**, *118*, C5–C6.
18. Faghih, R.; Escribano, C. F.; Castillon, S.; Garcia, J.; Lukacs, G.; Olesker, A.; Thang, T. T. *J. Org. Chem.* **1986**, *51*, 4558–4564.
19. Szarek, W. A.; Hay, G. W.; Doboszewski, B. *J. Chem. Soc., Chem. Commun.* **1985**, 663–664.
20. Doboszewski, B.; Hay, G. W.; Szarek, W. A. *Can. J. Chem.* **1987**, *65*, 412–419.
21. Lei, P.-s.; Ogawa, Y.; Flippen-Anderson, J. L.; Kováč, P. *Carbohydr. Res.* **1995**, *275*, 117–129.
22. Miljkovic, M.; Gligorijevic, M.; Glisin, D. *J. Org. Chem.* **1974**, *39*, 3223–3226.
23. Haradahira, T.; Maeda, M.; Omae, H.; Yano, Y.; Kojima, M. *Chem. Pharm. Bull.* **1984**, *32*, 4758–4766.
24. Hasegawa, A.; Goto, M.; Kiso, M. *J. Carbohydr. Chem.* **1985**, *4*, 627–637.
25. Nicolaou, K. C.; Ludduwahetty, T.; Randall, J. L.; Chucholowski, A. *J. Am. Chem. Soc.* **1986**, *108*, 2466–2467.
26. Kováč, P.; Yeh, H. J. C.; Jung, G. L.; Glaudemans, C. P. J. *J. Carbohydr. Chem.* **1986**, *5*, 497–512.