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Syntheses of fluorinated ligands to probe binding of antigenic determinants of *Vibrio cholerae* O:1, serotypes Inaba and Ogawa, to antibodies

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Abstract

Derivatives of methyl α-glycosides of antigenic determinants of *Vibrio cholerae* O:1, serotypes Inaba and Ogawa, specifically fluorinated at position 2′ or 4′ have been synthesized by coupling the appropriately fluorinated derivatives of 3-deoxy-L-*glycero*-tetronic acid with the methyl α-glycosides of perosamine. The compound having the fluorine atom at position 2 was obtained by electrophilic addition of fluorine to the glycal derived from the parent antigenic determinant, serotypes Inaba, using Selectfluor™ as a fluorination reagent.

1. Introduction

Reports in the literature[1–4] suggest that carbohydrate antigens interact with antibodies, at least to a significant extent, by hydrogen bonding. Information germane to understanding the details of these interactions can be obtained by studies of binding, using as probes, deoxy and deoxyfluoro analogs of components of the antigenic material. As part of our work towards a synthetic vaccine for cholera, we have studied[5] binding of monoclonal anti *V. cholerae* O:1 antibodies with a large series of ligands related to the O-antigen, some of which were specifically deoxygenated. It was the purpose of this work to synthesize specifically fluorinated derivatives of the methyl α-glycosides of the monosaccharide determinants[5,6] of the O-antigen of *V. cholerae* O:1, serotypes Inaba and Ogawa (**1** and **2**, respectively, Fig. 1).

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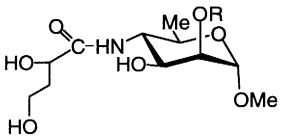


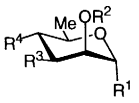
Fig. 1. The methyl α-glycosides of the monosaccharide determinants of *Vibrio cholerae* O:1, serotypes Inaba (**1**, R=H) and Ogawa (**2**, R=Me)

2. Results and discussion

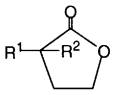
Synthesis of the target compounds fluorinated at positions 2′ involved preparation of appropriately fluorinated derivatives of 3-deoxy-L-*glycero*-tetronic acid and their coupling with the known[7–9] derivatives of perosamines **3** and **4**. Our previous study[5] showed that replacement of the 3-deoxy-L-*glycero*-tetronamido group for the D enantiomer did not result in abolition of binding. Therefore, with the intention to examine binding of the corresponding fluoro analogs, 2′-fluoro derivatives of **1** and **2** in both D- and L-*glycero* series were prepared.

Syntheses of the 2-fluoro derivatives of 3-deoxy-D- and L-*glycero*-tetronolactone have been described. Starting from enantiomerically pure D- and L-malic acid, Shiuey et al.[10] described multi-step preparations of both 2-fluorinated, enantiomerically pure enantiomers. Starting with the commercially available α-hydroxy-γ-butyrolactone, Schwartz et al.[11] prepared the racemic 3-deoxy-2-fluoro-tetronolactone. Dimethylaminosulfur trifluoride (DAST) was the fluorinating reagent in both approaches. To prepare 2-fluoro derivatives **9**–**12** we followed, essentially, the latter protocol.[11] Losses due to high volatility[10] of the fluoro lactones **6** and **8** could not be avoided by modifying the original protocol and conducting evaporation of solutions in dichloromethane at atmospheric pressure. However, we have obtained comparable yields of coupling products when we used crude vs chromatographically purified fluorinated lactones **6** or **8** for introduction of the tetronamido side-chain. The fluorinated racemic lactone was treated with each of the amines **3** and **4**, and the resulting mixtures of diastereoisomers were separated by chromatography. Compound **11** was also prepared from enantiomerically pure lactone **8**, which was obtained starting from the known[12] 2-*O*-acetyl derivative of lactone **7**. The amorphous compound **11** was characterized via the crystalline acetyl derivative **11a**.

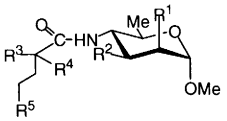
Our strategy to prepare analogs of **1** and **2** fluorinated at position 4′ involved reactions of amines **3** and **4** with 4-deoxy-4-fluoro-3-deoxy-L-*glycero*-tetronic acid **24**. Preparation of **24** required a derivative of 3-deoxy-L-*glycero*-tetronic acid fully protected, except at HO-4. We have attempted opening of the *O*-benzylidene ring in the known[13] ester **18** using a variety of reagents,[14–16] including that shown to effect conversion of benzylidene acetals into secondary benzyl ethers.[17] However, all attempts to prepare *t*-butyl 2-*O*-benzyl-3-deoxy-L-*glycero*-tetronate in such a way failed: the corresponding 4-benzyl ether[13] **19** was formed instead. We have prepared compound **19** previously by opening the 2,4-*O*-benzylidene ring in **18** with borane–trimethylamine complex and $AlCl_3$.[13] That conversion, as we have found now, can be conveniently performed with the same or better efficiency using the triethylsilane (TES)–trifluoroacetic acid (TFA) reagent.[18] Compound **19**, obtained in this way in 78% yield, was acetylated and product **20** was subjected to catalytic hydrogenolysis to give **21**. A small amount of **22**, a product of acetyl group migration, formed along with the desired compound **21** was also isolated and fully characterized. The position of the acetyl group in **22** followed from the NMR spectral data. Similar acetyl group migration has been observed previously[12] in a related derivative of 3-deoxy-L-*glycero*-tetronic acid.



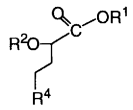
	R¹	R²	R³	R⁴
3	OMe	H	H	NH_2
4	OMe	Me	H	NH_2



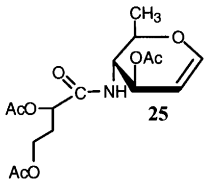
	R¹	R²
5	H,OH	
6	F,H	
7	H	OH
8	F	H



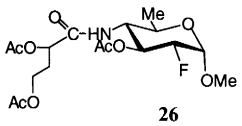
	R¹	R²	R³	R⁴	R⁵
9	OH	OH	F	H	OH
10	OH	OH	H	F	OH
11	OMe	OH	F	H	OH
11a	OMe	OAc	F	H	OAc
12	OMe	OH	H	F	OH
13	OH	OH	OH	H	F
14	OMe	OH	OH	H	F
14a	OMe	OAc	OAc	H	F
15	F	OAc	OAc	H	OAc
16	OAc	OAc	OAc	H	OAc
17	F	OH	OH	H	OH



	R¹	R²	R⁴
18	*t*-Bu	CHPh	
19	*t*-Bu	H	OBn
20	*t*-Bu	Ac	OBn
21	*t*-Bu	Ac	OH
22	*t*-Bu	H	OAc
23	*t*-Bu	Ac	F
24	OH	H	F



25



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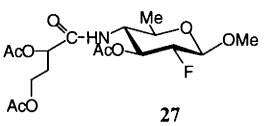

27

Fluorination of ester **21** with diethylaminosulfur trifluoride (DAST) was uneventful. That acetyl group migration did not occur under the condition of fluorination, and the presence of fluorine at the desired position 4, was evident from the ^{1}H NMR spectrum of **23** which showed a downfield shift of signals for H-4a,b, compared to the chemical shift of these protons in **21**. Also, as a result of fluorination at C-4, the signal for H-4a,b appears in the spectrum of **23** as two 1-proton multiplets, showing a typical $^3J_{F,H}$ coupling of 47.0 Hz. This is in contrast to the spectrum of **21**, where the signal for H-4a,b appears as one 2-proton multiplet. Because of high volatility of the fully protected, 4-fluorinated *t*-butyl ester **23**, considerable losses occurred when attempts were made to isolate this material using protocols involving concentrations of large volumes of solutions, such as those resulting after aqueous work-up and/or purification by column chromatography. Therefore, such operations were avoided, as in the preparation of **6** and **8**. The crude fluorinated *t*-butyl ester **23** was deprotected by treatment with aqueous TFA. Removal of both of the acetyl and *t*-butyl protecting groups was evident from the ^{1}H NMR spectrum. Compared to the spectrum of **23**, the high-field signals characteristic of the *t*-butyl group were not present in the spectrum of **24**, and the doublet of doublets for H-2 was shifted upfield, due to the absence of the electron-withdrawing acetyl group at O-2. Reaction of **24** with each of amines **3** and **4**, using [*O*-(7-azabezotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] (HATU) as a coupling reagent,[19,20] then gave **13** and **14**, respectively.

A simple method for synthesizing compound **17** fluorinated at position 2 appeared to be the recently described fluorination of glycals[21] using Selectfluor™ (1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane). When applied to tri-*O*-acetyl-L-glucal in the presence of benzyl alcohol,[21] the method was reported to afford benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-mannopyranoside as the major product. When glycal **25**, obtained conventionally[22] from 1,2,3-tri-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside,[23] was similarly treated, five compounds were formed, as shown by TLC, and they were isolated by column chromatography. The two fastest moving compounds were products of difluorination and were not further investigated. Next eluted were three substances whose NMR spectra showed general features indicative that they belonged to the desired class of compounds, namely that they contained the acetylated tetronamido side-chain, the methyl aglycon, and were fluorinated at position 2. At this point it was important to establish which of them, if any, was the desired compound **15**.

Evidence that two of the compounds isolated (**26** and **27**) had the *gluco* configuration was a large coupling observed for $J_{2,3}$ (8–10 Hz). The anomeric configuration for these two substances was readily assigned from the $J_{1,2}$ coupling constants observed in their proton spectra, namely 3.8 and 7.7 Hz, respectively. That the third compound isolated had the *manno* configuration also followed from the ^{1}H NMR spectrum (Tables 1 and 2), and the α-anomeric configuration, i.e. structure **15**, was assigned from the observed $J_{H\text{-}1,C\text{-}1}$ carbon–proton coupling constant[24] of 169.9 Hz (Table 3). Simple deacetylation (Zemplén) of **15** then afforded the desired, target compound **17**. The lower yield of the methyl α-D-*manno* compound observed in the present case, compared to the derivative of benzyl α-D-mannopyranoside reported previously,[21] can be explained by the use of different synthons in these two independently performed conversions.

Attempts to prepare the 3-deoxy-3-fluoro derivatives of **1** or **2** have failed thus far. It appears from our preliminary work that positioning of fluorine at position 3 in derivatives of perosamine will be a more formidable task than anticipated, and the chemistry involved in that synthesis will be reported at a later date.

3. Experimental

Unless stated otherwise, optical rotations were measured at 25°C 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). Detection of derivatives of perosamine was effected by charring with 5% sulfuric acid in ethanol and, when applicable, by UV light. Lactones were detected by spraying with the hydroxylamine–$FeCl_3$ reagent.[25] Compounds **21**–**24** which char poorly with 5% sulfuric acid in ethanol were detected by spraying with 5% ethanolic phosphomolybdic acid. 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. Assignments of NMR (300 MHz for ^{1}H and 75 MHz for ^{13}C) signals, obtained at 25°C, were made by first-order analysis of spectra supported by APT and/or DEPT experiments, homonuclear decoupling and/or homo- and heteronuclear 2-dimensional correlation spectroscopy, using commercial software supplied with the spectrometers (Varian Gemini or Varian Mercury). Chemical ionization mass spectra (CIMS) were measured using ammonia as the reactive gas. Palladium-on-charcoal catalyst (5%, ESCAT 103) was a product of Engelhard Industries. α-Hydroxy-γ-butyrolactone and Selectfluor™ (1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane) were purchased from Aldrich Chemical Company, and used as supplied. HATU (*O*-7-azobenzotriazol-1-yl-1,1,3,3-tetramethyluronium hexafluorophosphate) was

Table 1
^{1}H NMR (300 MHz in $CDCl_3$, unless indicated otherwise) chemical shifts (δ, relative to Me_4Si) and peak multiplicities[1] for compounds **6**, **8**–**15**, **17**, and **20**–**27**

Compound	Chemical shifts											
	H-1	H-2	H-3	H-4	H-5	H-6	H-2′	H-3′a,b	H-4′a,b	OCH_3-1	OCH_3-2	NH
6							5.31dt	2.60m	4.91m			
8[2]												
9[3]	4.62d	3.80dd	3.85dd	4.00t	3.72m	1.18d	5.08ddd	2.08m	3.72m	3.31s		
10[4]	4.59s	3.75m	3.75m	3.93t	3.75m	1.15d	5.02ddd	2.11m	3.75m	3.33s		
11	4.78dd	3.46dd	3.78m	3.97m	3.65m	1.24d	5.10dt	2.20m	3.78m	3.38s	3.50s	6.49dd
11a	4.74d	3.54dd	5.20dd	4.28bq	3.73m	1.26d	5.00ddd	2.30m	4.20dd	3.38s	3.50s	6.48dd
12	4.77d	3.45m	3.78m	3.78m	3.78m	1.21d	5.10ddd	2.10m	3.78m	3.36s	3.49s	6.67dd
13[5]	4.62s	3.78[6]	3.79dd	3.93t	3.75m	1.14d	4.17dd	2.04m	4.60m	3.30s		
14	4.79d	3.45dd	3.79m	3.85t	6.17m	1.23d	4.32dd	2.22m	4.69m	3.38s	3.50s	6.68bd
14a	4.74d	3.50[7]	5.19dd	4.25q	3.62m	1.22d	5.15dd	2.20m	4.52m	3.38s	3.51s	6.10d
15	4.84dd	4.65dt	5.20ddd	4.22q	3.71m	1.24d	5.09dd	2.15m	4.11m	3.41s		6.12d
17[8]	4.94dd	4.76dt	4.00ddd	3.00m	3.00m	1.20d	4.28dd	1.92m	3.73dd	3.42s		*
20[9]							5.04dd	2.05m	3.56dd			
21[10]							5.05dd	2.05m	3.55m			
22[11]							4.06dd	1.93m	4.18m			
23[12]							5.02dd	2.22m	4.57m			
24							4.19dd	2.05m	4.62m			
25	6.45dd	4.79dd	5.32m	4.20m	4.07m	1.34d	5.16dd	2.15m	4.14m			
26	4.91d	4.52ddd	5.35ddd	3.91q	3.71m	1.19d	5.08dd	2.16m	4.13m	3.45s		6.20d
27[13]	4.50dd	4.19ddd	5.23ddd	3.78m	3.78m	1.19d	4.97dd	2.10m	4.12m	3.49.s		7.31d

[1] b, broad; d, doublet; t, triplet; q, quartet; m, multiplet. [2] The spectrum is identical to that of **6**, except for minor variations resulting from different conditions of measurements. [3] Measured in acetone-d_6. [4] Measured in methanol-d_4. [5] Spectrum taken in acetone-d_6. [6] Overlapped signal. [7] Overlapped with OCH_3-2 signal.. [8] Measured in D_2O. [*] Not observed. [9] δ_{COCH_3} 2.07 ppm; $\delta_{C(CH_3)_3}$ 1.44 ppm; δ_{PhCH_2} 4.50, ^2J 12.1 Hz. [10] δ_{COCH_3} 2.15 ppm; $\delta_{C(CH_3)_3}$ 1.47 ppm. [11] δ_{COCH_3} 1.98 ppm; $\delta_{C(CH_3)_3}$ 1.43 ppm. [12] δ_{COCH_3} 2.14 ppm; $\delta_{C(CH_3)_3}$ 1.47 ppm. [13] Measured in acetone-d_6.

Table 2
^{1}H NMR coupling constants for compounds **6**, **8–15**, **17**, and **20–27**[1,2,3]

Compound	Coupling constants (Hz)													
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{2',3'a}$	$J_{2',3'b}$	$J_{2',F}$	$J_{4,NH}$	$J_{F,NH}$	$J_{4',F}$	$J_{1,F}$	$J_{2,F}$	$J_{3,F}$
6						7.8	7.8	52.0						
8[4]														
9	1.5	3.2	10.0	10.0	6.2	4.0	7.6	49.5						
10	*	3.1	9.9	9.9	6.2	3.4	8.6	49.4						
11	1.2	3.3	**	**	6.0	5.3	5.3	49.1	9.6	4.6				
11a	2.0	3.1	11.2	10.2	6.1	3.4	8.5	49.7	9.5	4.1				
12	1.1	**	**	**	6.0	4.7	7.6	49.0	8.7	4.5				
13	*	3.3	9.9	10.0	6.1	3.9	8.7				47.6			
14	1.5	3.3	9.9	9.9	6.2	3.5	8.4		7.9		47.0			
14a	1.9	3.0	11.3	10.2	6.6	5.3	7.2		9.4		46.8			
15	2.1	2.4	11.0	**	6.2	4.8	7.6		9.3			6.9	50.3	29.3
17	1.7	2.3	10.3	**	6.1	3.9	8.6		*			7.3	49.0	30.7
20[5]						4.4	8.8							
21						4.7	7.8							
22						4.7	8.0							
23						4.7	8.3				46.6			
24						4.4	7.7				47.0			
25	6.1	3.0	**	**	6.3	4.6	7.6		8.36					
26	3.8	9.7	10.7	9.9	6.1	4.6	8.0		9.2			*	49.6	20.2
27	7.7	8.9	10.0	**	6.0	4.5	8.9		8.7			2.6	51.6	19.1

[1] For conditions of measurements, see Table 1.
[2] Nuclei of the L-tetronic acid residue are referred to as primed.
[3] Coupling constants $J_{3'a,b}$, $J_{3'a,4'a}$, $J_{3'a,4'b}$, $J_{3'b,4'a}$, $J_{3'b,4'b}$, $J_{4'a,b}$ not determined due to overlapping signals.
* Not observed.
** Coupling constant not determined due to overlapping signals.
[4] The data are identical to those for **6**, except for minor variations resulting from different conditions of measurements.
[5] $J_{3'a,4'a,b}$ 5.7, $J_{3'b,4'a,b}$ 7.7 Hz.

Table 3
^{13}C NMR (75 MHz in $CDCl_3$, unless indicated otherwise) chemical shifts (δ) for compounds **6**, **8**–**15**, **17**, and **20**–**27**[1]

Compound	Chemical shifts											
	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	OCH_3-1	OCH_3-2
6[2]							172.08	85.19	28.85	64.67		
8[3]												
9[4]	93.01	61.80	60.59	44.84	58.63	9.28		81.00	27.45	48.42	45.74	
10[5]	102.62	71.22	69.79	54.32	68.12	18.21		89.93	36.72	58.07	54.91	
11[6]	97.58	79.07	69.46	53.74	66.90	17.77	171.34	89.70	35.03	58.10	54.93	58.89
11a[7]	98.34	77.46	71.03	50.97	67.64	17.71		77.46	31.37	59.25	54.75	59.43
12[8]	97.25	79.28	68.67	54.27	66.68	17.53	177.36	89.42	35.20	57.60	54.87	58.84
13[9]	93.04	61.91	60.97	45.01	58.88	9.38		59.70	27.34	72.38	45.72	
14[10]	97.44	79.31	66.86	54.27	69.44	17.86		69.91	34.94	81.85	54.97	58.88
14a[11]	98.53	77.76	70.88	51.48	68.24	17.82		70.52	32.07	79.73	54.88	59.58
15[12]	97.94	86.70	69.00	51.33[13]	68.17	17.66		79.0	30.50	59.77	55.16	
17[14]	98.17	98.53	67.12	53.16	67.35	16.80		69.07	66.05	57.92	55.19	
20[15]								69.87	31.28	65.33		
21[16]								70.10	33.76	58.26		
22[17]								67.63	33.31	60.51		
23[18]								69.00	32.00	79.48		
24[19]							180.60	68.75	34.85	81.45		
25	146.10	99.01	67.58	51.91	74.05	17.05		70.91	30.57	59.80		
26[20]	96.88	87.70	70.10	54.81	66.94	17.51		70.84	30.57	59.81	55.45	
27[21]	100.91	89.71	72.59	54.65	71.39	17.51		70.82	30.49	59.82	56.95	

[1] For conditions of measurements, see Table 1. [2] $J_{2',F}$ 191.2 Hz, $J_{1',F}$ 18.2 Hz, $J_{3',F}$ 18.0 Hz, $J_{4',F}$ 9.0 Hz. [3] The spectrum is identical to that of **6**, except for minor variations resulting from different conditions of measurements. [4] [1] $J_{2',F}$ 184.0 Hz, $J_{3',F}$ 19.4 Hz, $J_{4',F}$ 4.1 Hz. [5] $J_{2',F}$ 183.4 Hz, $J_{3',F}$ 20.4 Hz, $J_{4',F}$ 4.8 Hz. [6] $J_{2',F}$ 183.4 Hz, $J_{3',F}$ 20.4 Hz, $J_{4',F}$ 4.8 Hz. [7] $J_{2',F}$ 185.9 Hz,, $J_{3',F}$ 19.4 Hz, $J_{4',F}$ 3.5 Hz. [8] $J_{2',F}$ 185.6 Hz, $J_{1',F}$ 18.3 Hz, $J_{3',F}$ 19.5 Hz, $J_{4',F}$ 4.9 Hz. [9] $J_{2',F}$ 6.6 Hz, $J_{3',F}$ 19.9 Hz, $J_{4',F}$ 161.4 Hz. [10] $J_{3',F}$ 19.4 Hz, $J_{4',F}$ 163.1 Hz. [11] $J_{2',F}$ 3.9 Hz, $J_{3',F}$ 20.3 Hz, $J_{4',F}$ 165.9 Hz. [12] $J_{2,F}$ 179.5 Hz, $J_{1,F}$ 28.7 Hz, $J_{3,F}$ 17.3 Hz, $J_{C\text{-}1,H\text{-}1}$ 169.9 Hz. [13] Broad. [14] $J_{2,F}$ 175.5 Hz, $J_{1,F}$ 29.4 Hz, $J_{3,F}$ 18.3 Hz, $J_{C\text{-}1,H\text{-}1}$ 170.9 Hz. [15] $\delta_{CO(CH_3)}$ 20.60 ppm; $\delta_{C(CH_3)_3}$ 82.05 ppm; $\delta_{Ph(CH_2}$ 72.94, $\delta_{C((CH_3)_3}$ 27.91 ppm. [16] $\delta_{CO(CH_3}$ 20.60 ppm; $\delta_{C(CH_3)_3}$ 82.32 ppm; $\delta_{C((CH_3)_3}$ 27.83 ppm. [17] $\delta_{CO(CH_3}$ 20.60 ppm; $\delta_{C(CH_3)_3}$ 82.32 ppm; $\delta_{C((CH_3)_3}$ 27.83 ppm. [18] $\delta_{CO(CH_3}$ 20.64 ppm; $\delta_{C(CH_3)_3}$ 82.40 ppm; $\delta_{C((CH_3)_3}$ 28.00 ppm. [19] $J_{2',F}$ 4.3 Hz, $J_{3',F}$ 19.2 Hz, $J_{4',F}$ 158.9 Hz. [20] $J_{2,F}$ 194.8 Hz, $J_{1,F}$ 19.4 Hz, $J_{3,F}$ 18.2 Hz, $J_{4,F}$ 6.1 Hz, $J_{C\text{-}1,H\text{-}1}$ 168.2 Hz. [21] $J_{2,F}$ 188.7 Hz, $J_{1,F}$ 22.1 Hz, $J_{3,F}$ 18.9 Hz, $J_{4,F}$ 6.6 Hz, $J_{C\text{-}1,H\text{-}1}$ 158.9 Hz.

purchased from PerSeptive Biosystems. Unless stated otherwise, solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 40°C/2 kPa.

3.1. *Methyl 4-(2,3-dideoxy-2-fluoro*-D- ***10*** *and* L-glycero-*tetronamido)-4,6-dideoxy-α*-D-*mannopyranoside* ***9***

DAST (7.55 mL, 56.8 mmol) was added at −70°C, by way of a syringe, to a solution of the commercially available, racemic α-hydroxy-γ-butyrolactone (5 g) in dry CH_2Cl_2 (10 mL). The solution was stirred for 12 h at room temperature, when TLC (4:1 EtOAc:hexane, lactone-specific detection, see above) showed complete conversion of the starting material into one faster moving product. The mixture was cooled to −20°C, methanol (~1 mL) was added, and the mixture was allowed to reach ambient temperature. Aqueous $NaHCO_3$ was added portionwise until effervescence ceased, the mixture was partitioned between CH_2Cl_2 and water, and the CH_2Cl_2 phase was concentrated. The residue was chromatographed to give 2,3-dideoxy-2-fluoro-D,L-*glycero*-tetronolactone (**6**, 2.8 g, 55%). The ^{1}H NMR data agreed with those reported[10] (for ^{13}C NMR spectra, see Table 3). The spectra did not contain signals suggesting the presence of either byproducts or residual solvents. CIMS: *m/z* 122 ($[M+18]^+$).

One drop of pyridine was added to a mixture of amine **3**[7,8] (1 g, 5.65 mmol) and lactone **6** (0.86 g, 8.26 mmol) and the mixture was stirred for 6 h at 100°C, when TLC (5:1 CH_2Cl_2:CH_3OH) showed that all starting amine had been consumed and that two products were formed. The mixture was chromatographed to afford first the L diastereoisomer **9** (627 mg, 39.7%), mp 115.5–117.5 (from acetone), $[\alpha]_D$ +20.2 (*c* 1, H_2O); CIMS: *m/z* 299 ($[M+18]^+$). Anal. calcd for $C_{11}H_{20}FNO_6$: C, 46.97; H, 7.17; N, 4.98. Found: C, 46.91; H, 7.06; N, 4.93.

Eluted last was the D diastereoisomer **10** (516 mg, 32.6%), mp 133–135° (from EtOAc), $[\alpha]_D$ + 69.3 (*c* 0.9, H_2O); CIMS: *m/z* 299 ($[M+18]^+$). Anal. calcd for $C_{11}H_{20}FNO_6$: C, 46.97; H, 7.17; N, 4.98. Found: C, 46.76; H, 7.03; N, 4.92. An intermediate, mixed fraction was also obtained.

3.2. *Methyl 4-(2,3-dideoxy-2-fluoro*-D- ***12*** *and* L-glycero-*tetronamido)-4,6-dideoxy-2*-O-*methyl-α*-D-*mannopyranoside* ***11***

(a) Amine **4**[9] (1.5 g, 7.8 mmol) was treated with the fluorinated racemic lactone **6** (1 g, 9.6 mmol) as described for the preparation of **9** and **10** to give, after chromatography, first the amorphous L diastereoisomer **11** (775 mg, 39.7%), $[\alpha]_D$ +19.5 (*c* 1.3, $CHCl_3$); CIMS: *m/z* 313 ($[M+18]^+$).

Eluted last was the D diastereoisomer **12** (623 mg, 31.9%), mp 114.5–116°C (from EtOAc), $[\alpha]_D$ +71.7 (*c* 1, $CHCl_3$); CIMS: *m/z* 313 ($[M+18]^+$). Anal. calcd for $C_{12}H_{22}FNO_6$: C, 48.81; H, 7.51; N, 4.74. Found: C, 48.93; H, 7.48; N, 4.83. An intermediate, mixed fraction was also obtained.

(b) A solution of 2-*O*-acetyl-3-deoxy-D-*glycero*-tetronolactone[26] (2.8 g) in 1 M TFA (60 mL) was heated at 60°C for 3 h. TLC (1:3 hexane:EtOAc) showed disappearance of the starting material and formation of a mixture of 3-deoxy-D-*glycero*-tetronolactone **7** and the corresponding free acid. The solution was concentrated and toluene was evaporated from the residue (three times), to remove residual TFA,[9] resulting in virtually complete lactonization of the free acid, as shown by TLC and NMR spectroscopy. Chromatography gave pure 3-deoxy-D-*glycero*-tetronolactone **7** (1.63 g, 82%).

The foregoing lactone **7** (0.318 g, 3.1 mmol) was fluorinated and the mixture was processed as already described for the racemic lactone **6**, except that all concentrations of solutions in CH_2Cl_2 were performed at 45°C (bath) and atmospheric pressure. Without chromatography, the crude 2,3-dideoxy-2-fluoro-L-*glycero*-tetronolactone **8** thus obtained was treated with amine **4**[9] (475 mg, 2.5 mmol) as described in (a), to give after chromatography **11** (270 mg, 36.7%), which was identical (NMR) with the compound

just described. For characterization, compound **11** was acetylated conventionally, using acetic anhydride and pyridine, to give methyl 3-*O*-acetyl-4-(4-*O*-acetyl-2,3-dideoxy-2-fluoro-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside **11a**, mp 65–67°C (from ether–isopropyl ether), $[\alpha]_D$ +91.0 (*c* 0.8, $CHCl_3$); CIMS: *m/z* 397 ($[M+18]^+$). Anal. calcd for $C_{16}H_{26}FNO_8$: C, 50.65; H, 6.91; N, 3.69. Found: C, 50.47; H, 6.92; N, 3.72.

A sample of the fluorinated lactone **8** purified by chromatography gave NMR spectra identical to those of **6**, except for minor variations resulting from different conditions of measurements.

3.3. t-*Butyl 4*-O-*benzyl-3-deoxy*-L-*tetronate* ***19***

2,4-*O*-Benzylidene-(*S*)-1,2,4-butanetriol was prepared as described.[13] Crystallization from ethyl acetate gave material, mp 65.5–67°C, $[\alpha]_D$ +12 (*c* 1.2). Previously,[13] the specific optical rotation of $[\alpha]_D$ +106 was erroneously reported for the amorphous material. The foregoing alcohol was oxidized[13] to give the corresponding *t*-butyl ester **18**.

Compound **18** (1.07, 4.03 mmol) was added to a mixture of triethylsilane (3.25 mL, 20.25 mmol) in CH_2Cl_2 (15 mL), the mixture was cooled to −5°C, and TFA (1.6 mL, 20.25 mmol) was added dropwise and with stirring. The cooling was removed, and the stirring was continued until TLC (4:1 hexane:EtOAc) showed that the reaction was complete (∼2 h). After neutralization with aq. $NaHCO_3$, the mixture was partitioned between water and CH_2Cl_2, the organic phase was dried, concentrated and the residue was chromatographed to give **19** (0.81 g, 75.2%), which was identical with the previously described material.[13]

3.4. t-*Butyl 2*-O-*acetyl-4*-O-*benzyl-3-deoxy*-L-*tetronate* ***20***

A solution of **19** (0.81 g) in a mixture of 1:1 pyridine:Ac_2O (1 mL) was kept at room temperature overnight. TLC (6:1 hexane:EtOAc) then showed that the conversion of the starting material to a single faster moving product was complete. After concentration and elution from a small column of silica gel, the title substance **20** was obtained in virtually theoretical yield as an oil, $[\alpha]_D$ −34.4 (*c* 1.5, $CHCl_3$); CIMS: *m/z* 326 ($[M+18]^+$). Anal. calcd for $C_{17}H_{24}O_5$: C, 66.21; H, 7.84. Found: C, 66.40; H, 7.92.

3.5. t-*Butyl 2*-O- ***21*** *and 4*-O-*acetyl-3-deoxy*-L-*tetronate* ***22***

A mixture of **20** (11.66 g) and palladium-on-charcoal catalyst (2.5 g) in methanol was stirred at room temperature and atmospheric pressure overnight. TLC (3:1 hexane:EtOAc) showed that the starting material was consumed and that two products were formed. After filtration, the filtrate was concentrated and the residue was chromatographed.

Eluted first was the 4-*O*-acetyl derivative **22** resulting from acetyl group migration (0.4 g, ∼5%). The material solidified on standing, mp 32–33°C, $[\alpha]_D$ −9.9 (*c* 0.7, $CHCl_3$). Recrystallization from common solvents was unsuccessful. The compound is highly volatile and sublimed when dried at room temperature at 133 Pa. The analytical sample was kept in a desiccator over P_2O_5 and paraffin shavings overnight at atmospheric pressure and room temperature. CIMS: *m/z* 236 ($[M+18]^+$). Anal. calcd for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 55.02; H, 8.20.

Eluted next was the desired material **21** (7.1 g, 86%), in admixture with a small amount of **22**, resulting from the spontaneous conversion **21**→**22**.

3.6. Methyl 4,6-dideoxy-4-(3,4-dideoxy-4-fluoro-L-glycero-tetronamido)-α-D-mannopyranoside **13**

DAST (15.45 mL, 0.116 M) was added at –60°C to a solution of **21** (6.3 g, 0.29 M) in THF (12.6 mL) containing Et_3N (16.14 mL, 0.116 M). The cooling was removed and the mixture was stirred for 3 h. TLC (3:1 hexane:EtOAc) showed that starting material was virtually consumed and that one major product was formed. After cooling (−20°C), methanol (15 mL) was added, the mixture was stirred for 2 h, and partitioned between aq. $NaHCO_3$ and CH_2Cl_2. The organic phase was dried and concentrated at atmospheric pressure, mainly to remove methanol. A solution of the residue in CH_2Cl_2 was filtered through a layer of silica gel to remove bulk of the colored and base-line material, the eluate was concentrated at atmospheric pressure, and the crude *t*-butyl 2-*O*-acetyl-4-fluoro-3,4-dideoxy-L-tetronate (**23**, CIMS: *m/z* 238 ($[M+18]^+$), thus obtained, was used for the next step.

A solution of the foregoing material in 1 M TFA (120 mL) was heated at 80°C for 4 h. TLC (1:1 EtOAc:MeOH) showed that the reaction was essentially complete and that one major product was formed. The solution was concentrated, water was evaporated from the residue to remove TFA, and the residue was chromatographed to obtain material (2.1 g) containing ~70% (NMR) of 3,4-dideoxy-4-fluoro-L-*glycero*-tetronic acid **24**; CIMS: *m/z* 140 ($[M+18]^+$).

A mixture of amine **3** (1 g, 6.28 mmol), acid **24** (1.09 g, 6.28 mmol, based on the purity of **24** already described), diisopropylethylamine (DIEA) (2.2 mL, 12.6 mmol), and HATU (4.8 g, 12.6 mmol) in 1:1 MeOH:CH_2Cl_2 (4 mL) was stirred at room temperature for 2 h, when TLC (5:1 CH_2Cl_2:MeOH) showed that most of the amine had been converted to a faster moving product. After concentration, chromatography yielded **13** (0.98 g, 62%), mp 111.5–111.5°C (from acetone–EtOAc), $[\alpha]_D$ +34 (*c* 0.9, H_2O); CIMS: *m/z* 299 ($[M+18]^+$). Anal. calcd for $C_{11}H_{20}FNO_6$: C, 46.97; H, 7.17; N, 4.98. Found: C, 46.99; H, 7.23; N, 4.96.

3.7. Methyl 4,6-dideoxy-4-(3,4-dideoxy-4-fluoro-L-glycero-tetronamido)-2-O-methyl-α-D-mannopyranoside **14**

The amine **4** (1.4 g, 6.3 mmol) was treated with the fluorinated acid **24** (1.1 g, 6.3 mmol), as described for the preparation of **13**. After work-up, as already described, chromatography gave **14** (1.15 g, 53%), mp 121–122°C (from EtOAc), $[\alpha]_D$ +44.5 (*c* 1, $CHCl_3$); CIMS: *m/z* 313 ($[M+18]^+$). Anal. calcd for $C_{12}H_{22}FNO_6$: C, 48.81; H, 7.51; N, 4.74. Found: C, 48.99; H, 7.54; N, 4.68.

Compound **14** was further characterized as methyl 3-*O*-acetyl-4,6-dideoxy-4-(2-*O*-acetyl-3,4-dideoxy-4-fluoro-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside **14a**, which was obtained from **14** by conventional acetylation with acetic anhydride in pyridine, mp 106–107°C, $[\alpha]_D$ +62.2 (*c* 1, $CHCl_3$); CIMS: *m/z* 397 ($[M+18]^+$). Anal. calcd for $C_{16}H_{26}FNO_8$: C, 50.65; H, 6.91; N, 3.69. Found: C, 50.54; H, 6.97; N, 3.95.

3.8. 3-O-Acetyl-1,5-anhydro-2,4,6-trideoxy-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-α-D-arabino-hex-1-enitol **25**

1,2,3-Tri-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside[23] **16** (7.55 g) was treated with 40% HBr in AcOH for 1 h, when TLC (5:1 CH_2Cl_2:acetone) showed that the reaction was complete. The mixture was concentrated with co-evaporation of toluene, to remove excess reagents, to give the desired glycosyl bromide, 2,3-di-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl bromide, which was used for the next step without further purification.

Commercially available zinc dust was activated by successive washes with 10% HCl, water, acetone, and ether. To a solution of the foregoing bromide in THF (50 mL) was added air-dried activated zinc powder (12.4 g, 12 equiv.), the mixture was heated and 1-methylimidazole (1.2 mL, 1 equiv.) was added when the inner temperature reached 45°C. The heating was continued for 45 min when TLC (5:1 toluene:acetone) showed that the reaction was complete and two products had been formed. Chromatography gave the faster moving, desired glycal **25** (3.6 g, 63%), mp 86.5–87.5°C, $[\alpha]_D$ −54.8 (*c* 0.8, $CHCl_3$); CIMS: *m/z* 357 ($[M+18]^+$). Anal. calcd for $C_{16}H_{23}NO_8$: C, 53.78; H, 6.49; N, 3.92. Found: C, 53.93; H, 6.58; N, 3.92.

*3.9. Methyl 3-*O*-acetyl-2,4,6-trideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-2-fluoro-α-*D*-glucopyranoside **26**, methyl 2,4,6-trideoxy-4-(3-deoxy-*L*-glycero-tetronamido)-2-fluoro-α-*D*-mannopyranoside **15**, and methyl 3-*O*-acetyl-2,4,6-trideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-2-fluoro-β-*D*-glucopyranoside **27***

To a mixture of glycal **25** (2.94 g, 8.2 mmol) and 4 Å molecular sieves (2 g) in acetonitrile (9 mL) were added MeOH (3 mL), 2,6-di-*t*-butyl-4-methylpyridine (2.54 g, 12.4 mmol) and Selectfluor™ (4.4 g, 12.4 mmol). The mixture was stirred in an argon atmosphere overnight, when TLC (3:2 toluene:EtOAc) showed that all starting material was consumed and that, in addition to some base-line material, five products had been formed. After filtration through a pad of Celite, the filtrate was concentrated and the residue was chromatographed.

Eluted first were two compounds (~30 mg each) whose CI mass spectra showed a peak at *m/z* 413, indicating that each of these compounds contained two fluorine atoms. These were not investigated further.

Eluted next was material enriched in a compound whose NMR data confirmed that it was the α-*gluco* derivative **26**. Crystallization (twice) from ethanol gave material (120 mg, 3.6%), melting at 154–155°C, $[\alpha]_D$ +121.5 (*c* 1.1, $CHCl_3$); CIMS: *m/z* 425 ($[M+18]^+$). Anal. calcd for $C_{17}H_{26}FNO_9$: C, 50.12; H, 6.43; N, 3.44. Found: C, 49.97; H, 6.43; N, 3.47.

Eluted next was material whose NMR data confirmed that it was the α-*manno* derivative **15** (0.234 g, 7%); $[\alpha]_D$ +57.7 (*c* 0.9, $CHCl_3$); CIMS: *m/z* 425 ($[M+18]^+$).

Eluted last was material whose NMR data confirmed that it was the β-*gluco* derivative **27** (0.458 g, 14.7%), mp 96–98°C (from ether), $[\alpha]_D$ +16.2 (*c* 1.4, $CHCl_3$); CIMS: *m/z* 425 ($[M+18]^+$). Anal. calcd for $C_{17}H_{26}FNO_9$: C, 50.12; H, 6.43; N, 3.44. Found: C, 50.26; H, 6.37; N, 3.36. Intermediate, mixed fractions were also obtained.

*3.10. Methyl 2,4,6-trideoxy-4-(3-deoxy-*L*-glycero-tetronamido)-2-fluoro-α-*D*-mannopyranoside*

A solution of **15** in methanol was deacetylated (Zemplén) to give the desired 2-fluoro derivative **17** in virtually theoretical yield, mp 128–130°C (from EtOAc), $[\alpha]_D$ +40.5C (*c* 0.8, H_2O). Anal. calcd for $C_{11}H_{26}FNO_6$: C, 46.97; H, 7.17; N, 4.98. Found: C, 47.03; H, 7.26; N, 4.9.

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