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Synthesis and evaluation of 1,5,6-trideoxy-6,6-difluoro-1,5-imino-D-glucitol (1,6-dideoxy-6,6-difluoronojirimycin) as a glucosidase inhibitor

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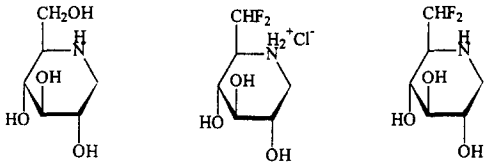
Abstract

1,5,6-Trideoxy-6,6-difluoro-1,5-imino-D-glucitol hydrochloride (**2**) and 1,5,6-trideoxy-6,6-difluoro-1,5-imino-D-glucitol (**3**) have been synthesized from L-sorbose. The formation of the C-5 difluoromethyl group was accomplished by difluorination of a carbonyl group by DAST. The results of in vitro enzymic evaluation of **3** indicate that it is a competitive inhibitor of yeast α-glucosidase ($K_i = 7.5$ mM) and a noncompetitive inhibitor of almond β-glucosidase ($K_i = 8.7$ mM). © 1997 Elsevier Science Ltd.

Keywords: 1,5,6-Trideoxy-6,6-difluoro-1,5-imino-D-glucitol hydrochloride; 1,5,6-Trideoxy-6,6-difluoro-1,5-imino-D-glucitol; Glucosidase inhibitor

1. Introduction

A very active area of research has been the synthesis and evaluation of novel inhibitors of glycosidases [1]. 1-Deoxynojirimycin (**1**) has been shown to be an effective inhibitor of glycosidases [2] and to exhibit anti-HIV activity [3]. *N*-Butyl-1-deoxynojirimycin has entered clinical trials [4]. 1-Deoxynojirimycin is an example of a polyhydroxylated alkaloid or an iminosugar, sometimes termed an 'azasugar'. The salient structural feature of the class of molecules is the substitution of the ring oxygen of the sugar by nitrogen. Many analogues of **1** have been synthesized in order not only to identify more potent inhibitors but also to probe the mechanism of cleavage of the glycosidic bond by the glycosidases [1].



1 2 3

The substitution of fluorine for atoms or functional groups in natural products has resulted in altered biological activities or physiochemical properties of the molecules. The substitution of the C-5 hydroxymethyl group of **1** by a difluoromethyl group to give 1,5,6-trideoxy-6,6-difluoro-1,5-imino-D-glucitol (**3**,

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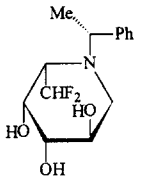
1,6-dideoxy-6,6-difluoronojirimycin) may have interesting effects on the inhibition of glycosidases. The difluoromethyl group acts as a hydrogen-bond donor [5], a structural feature which may allow for favorable interactions of the molecules with enzymes. Furthermore, this substitution alters the size and electronegativity of the functional group at the C-5 position of **1**. The synthesis and evaluation of three azasugars, namely 1,5,6-trideoxy-6,6-difluoro-1,5-imino-*N*-(α-methylbenzyl)-L-galactitol (**4**), 1,5,6-trideoxy-6,6-difluoro-1,5-imino-*N*-(α-methylbenzyl)-D-galactitol (**5**), and 1,5,6-trideoxy-6,6-difluoro-1,5-imino-*N*-(α-methylbenzyl)-L-altritol (**6**), each containing the difluoromethyl group at the C-5 position, have been reported recently [6]. The compounds were tested for antiviral activity, specifically anti-HIV transmission in cell culture; however, none was observed. In this article are described the synthesis **2** and **3** and the in vitro glycosidase evaluation of **3**.



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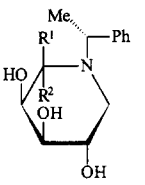
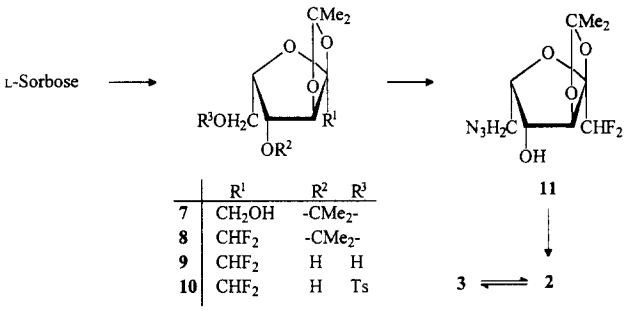


5 $R^1 = CHF_2$, $R^2 = H$
6 $R^1 = H$, $R^2 = CHF_2$

2. Results and discussion

An adaptation of the method for the synthesis of **1** by Paulsen et al. [7] and the method for the synthesis of 1,5,6-trideoxy-6-fluoro-1,5-imino-D-glucitol by Szarek et al. [8] was employed for the synthesis of **2** and **3** (see Scheme 1). Treatment of L-sorbose with acetone in the presence of iodine (see ref. [9]) gave 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose (**7**) in a good yield. Swern oxidation (oxalyl chloride) of the primary C-1 hydroxyl group of **7** resulted in the formation of an aldehyde. The difluoromethyl group in 1-deoxy-1,1-difluoro-2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose (**8**) was formed by treatment of the crude aldehyde with 4 equivalents of (diethylamino)-sulfur trifluoride (DAST) (see ref. [10]) at room temperature; a low yield (36%) was obtained in this transformation. The presence of two distinct signals (δ 134.55 and 128.46) in the ^{19}F NMR spectrum, and of an apparent triplet at δ 5.87 attributable to H-1 in the ^{1}H NMR spectrum of **8**, confirmed the presence of the difluoromethyl group.



	R^1	R^2 R^3
7	CH_2OH	$-CMe_2-$
8	CHF_2	$-CMe_2-$
9	CHF_2	H H
10	CHF_2	H Ts

Scheme 1.

The selective removal of the 4,6-*O*-isopropylidene group of **8** was achieved with 60% aqueous acetic acid to give 1-deoxy-1,1-difluoro-2,3-*O*-isopropylidene-α-L-sorbofuranose (**9**). Chemoselective tosylation of the primary hydroxyl group in **9** with *p*-toluenesulfonyl chloride in dry pyridine and a catalytic amount of 4-(dimethylamino)pyridine provided 1-deoxy-1,1-difluoro-2,3-*O*-isopropylidene-6-*O*-*p*-tolylsulfonyl-α-L-sorbofuranose (**10**). Tosylation of the secondary hydroxyl group was not observed. The introduction of nitrogen into the molecule was achieved by treatment of **10** with sodium azide in *N,N*-dimethylformamide to give 6-azido-1,6-dideoxy-1,1-difluoro-2,3-*O*-isopropylidene-α-L-sorbofuranose (**11**).

Catalytic hydrogenation of **11** over 10% palladium-on-charcoal in methanol gave the intermediate amine as a syrup. The removal of the 2,3-*O*-isopropylidene group was achieved with 2 N HCl in aqueous tetrahydrofuran at reflux temperature, and was accompanied by ring rearrangement to form a cyclic-imine intermediate that was reduced with an excess of sodium cyanoborohydride in methanol to afford crude **3**. Crude **3** was purified partially by flash chromatography, and the hydrochloride salt **2** precipitated from a solution of the product in methanol and concentrated HCl; a pure sample of **3** was obtained from the salt.

The results of an in vitro evaluation of **3** with commercially available, yeast α-glucosidase and almond β-glucosidase are given in Table 1. α-Glucosidase was inhibited weakly in the presence of **3** ($K_i = 7.5$ mM) in a competitive manner. In the case of the β-glucosidase, weak noncompetitive inhibition was observed with **3** ($K_i = 8.7$ mM).

The mechanism of inhibition of glycosidases by **1** is believed to involve the protonation of the nitrogen to form a cation which then pairs with an anion within the active site of the glucosidases [1]; the proton may be abstracted from the active site of the

Table 1
Inhibition of glucosidases by 1,6-dideoxy-6,6-difluoronojirimycin (**3**)

Enzyme	Substrate or inhibitor	K_m [a] (mM)	K_i [a] (mM)	Type of inhibition
α-Glucosidase (yeast)	*p*-Nitrophenyl α-D-glucopyranoside	0.20		
	3		7.5	Competitive
β-Glucosidase (almonds)	*p*-Nitrophenyl β-D-glucopyranoside	4.3		
	3		8.7	Noncompetitive

[a] Value is an average of trials.

enzyme. The acquisition of the proton results in a mimicking of the positive charge of the oxocarbenium ion-like transition state for glycoside hydrolysis [11]. In the case of **3**, since the difluoromethyl group is electron-withdrawing, it may destabilize the positive charge that is formed upon protonation, thereby possibly accounting for the weak inhibition by **3** of yeast α-glucosidase.

3. Experimental

General methods.—Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter for solutions in a 1-dm cell at room temperature. ^1H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker AM 400 spectrometer at 400.1, 100.6, and 376.5 MHz, respectively. $CDCl_3$ was used as the solvent, unless stated otherwise. The signals owing to residual protons in the deuterated solvents were used as internal standards. Chemical shifts (δ) are reported in ppm downfield from Me_4Si for ^1H and ^{13}C NMR spectra and downfield from $CFCl_3$ for ^{19}F NMR spectra. Infrared (IR) spectra were recorded on a Bomem MB-series FTIR spectrophotometer. Thin-layer chromatography (TLC) was performed using glass plates precoated with EM Science Silica Gel 60 F_{254}. Flash chromatography was performed using EM Science Silica Gel 60 (230–400 mesh) or EM Science Aluminum Oxide 60, Basic Activity 1 (70–230 mesh).

Preparation of 2,3:4,6-Di-O-isopropylidene-α-L-sorbofuranose (**7**).—L-Sorbose (20 g, 111 mmol) was suspended in dry Me_2CO (2 L) under an atmosphere of argon. Iodine (6 g, 23.7 mmol) was added, and the mixture was stirred at room temperature until the sorbose had dissolved. A 20% aq solution of $Na_2S_2O_3$ was added until a clear, colorless solution was obtained. The excess of Me_2CO was removed under vacuum. The remaining aq phase was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed with H_2O (25 mL) and dried ($MgSO_4$). The solution was concentrated under vacuum to give a syrup, which crystallized from diisopropyl ether to give **7** (22.1 g, 76%): R_f 0.39 (1:2 hexane–EtOAc); mp 78–79 °C, lit. 77–78 °C [12]; $[\alpha]_D$ −15.8° (*c* 1.51, Me_2CO), lit. −18.1° [12]; IR (KBr): ν 3510 cm^{-1} (OH); ^1H NMR: δ 4.48 (s, 1 H, H-1), 4.33 (d, 1 H, $J_{3,4}$ 1.6 Hz, H-4), 4.11–4.10 (m, 1 H, H-5), 4.06 (overlapping dd, 2 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 3.6 Hz, H-6a, H-6b), 3.88 (dd, 1 H, $J_{1a,1b}$ 11.8, $J_{1a,OH}$ 6.9 Hz, H-1a), 3.79 (dd, 1 H, $J_{1b,OH}$ 5.8 Hz, H-1b), 2.14 (overlapping dd, 1 H, OH), 1.51, 1.44, and 1.38 (3 s, 12 H, 4 Me); ^{13}C NMR: δ 114.26 (CMe_2), 111.93 (C-2), 97.52(CMe_2), 84.93 (C-3), 73.26 (C-5), 72.22 (C-4), 63.63 (C-1), 60.25 (C-6), 28.90, 27.31, 26.46, and 18.55 (4 Me). Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.36; H, 7.75. Found: C, 55.37; H, 7.85.

1-Deoxy-1,1-difluoro-2,3:4,6-di-O-isopropylidene-α-L-sorbofuranose (**8**).—To a solution of dry oxalyl chloride (1.05 mL, 12 mmol) in dry CH_2Cl_2 (50 mL) at −78 °C was added dropwise dry Me_2SO (1.7 mL, 24 mmol) in dry CH_2Cl_2 (20 mL), and the solution was stirred for 10 min. A solution of **7** (2.65 g, 10.2 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise, and the solution was stirred for 2.5 h at −78 °C. The temperature of the solution was increased to −50 °C, dry Et_3N (7 mL) was added, and the solution was stirred for 20 min. The temperature of the solution was increased to rt and H_2O (25 mL) was added. The aq layer was extracted with CH_2Cl_2 (3 × 20 mL), and the organic extracts were washed with satd aq NaCl (20 mL), neutralized with 1% aq HCl, washed sequentially with H_2O (20 mL), 5% aq $NaHCO_3$ (20 mL), H_2O (20 mL), and satd aq NaCl (20 mL), and dried ($MgSO_4$). The solvent was removed under vacuum to give a syrup (2.12 g, 80%). To a solution of the syrup in dry CH_2Cl_2 (25 mL) at 0 °C was added dropwise Et_2NSF_3 (4 equiv, 5.7 mL, 43.1 mmol) under an atmosphere of argon, and the solution was stirred for 2 days at rt. The solution was cooled to 0 °C and added slowly to ice-cold 5% aq $NaHCO_3$ (200 mL); the mixture was stirred vigorously until the liberation of HF ceased. The organic layer was washed

with H_2O (15 mL), dried ($MgSO_4$), and concentrated under vacuum to give a syrup. Purification by flash chromatography on silica gel (1:8 hexane–EtOAc) gave **8** as a clear syrup (0.87 g, 36%): R_f 0.49 (3:1 hexane–EtOAc); $[\alpha]_D$ −0.71° (*c* 0.99, $CHCl_3$); IR (KBr): ν 2990, 2940, and 2880 (CH), 1220 (Me of acetal), and 1090 cm^{-1} (CF_2); ^1H NMR: δ 5.87 (app t, 1 H, $J_{1,F}$ 55.1 Hz, H-1), 4.56 (s, 1 H, H-3), 4.34 (br s, 1 H, H-4), 4.19–4.18 (m, 1 H, $J_{5,6}$ 1.8 Hz, H-5), 4.07 (app dt, 2 H, H-6a, H-6b), 1.54, 1.43, 1.41, and 1.37 (4 s, 12 H, 4 Me); ^{13}C NMR: δ 114.14 (CMe_2), 111.55 (app t, $J_{1,F}$ 247.0 Hz, C-1), 111.14 (dd, $J_{2,F}$ 28.7, $J_{2,F}$ 22.6 Hz, C-2), 97.51 (CMe_2), 84.97 (C-3), 73.51 (C-5), 72.71 (C-4), 59.87 (C-6), 28.74, 27.40, 26.08, and 18.72 (4 Me); ^{19}F NMR: δ 134.55 (dd, 1 F, $J_{F,F}$ 287.8, $J_{1,F}$ 55.5 Hz), 128.46 (dd, 1 F, $J_{1,F}$ 54.6 Hz). Anal. Calcd for $C_{12}H_{18}F_2O_5$: C, 51.41; H, 6.48. Found: C, 51.50; H, 6.51.

*1-Deoxy-1,1-difluoro-2,3-*O*-isopropylidene-α-*L*-sorbofuranose* (**9**).—A suspension of **8** (8.56 g, 30.5 mmol) in 60% aq AcOH (50 mL) was stirred at a temperature of 60 °C. After 2 h, the syrup had dissolved, and TLC indicated that the deprotection was complete. The solution was evaporated under vacuum to a syrup. The syrup was purified by flash chromatography on silica gel (1:2 hexane–EtOAc) to give **9** as a white solid (6.64 g, 91%): R_f 0.24 (1:1 hexane–EtOAc); mp 85–86 °C; $[\alpha]_D$ +12.9° (*c* 1.4, $CHCl_3$); IR (KBr): ν 3450 (OH), 2990 and 2950 (CH), 1220 (Me of acetal), and 1100 cm^{-1} (CF_2); ^1H NMR: δ 5.89 (app t, 1 H, $J_{1,F}$ 55.2 Hz, H-1), 4.64 (s, 1 H, H-3), 4.38–4.36 (m, 2 H, H-4, H-5), 4.14 (ddd, 1 H, $J_{6a,6b}$ 12.6, $J_{6a,OH}$ 4.9, $J_{5,6a}$ 3.5 Hz, H-6a), 4.05 (ddd, 1 H, $J_{6b,OH}$ 8.1, $J_{5,6b}$ 2.8 Hz, H-6b), 3.61–3.59 (m, 1 H, OH-6), 2.15 (dd, 1 H, $J_{4,OH}$ 8.1, $J_{5,OH}$ 4.9 Hz, OH-4), 1.54 and 1.40 (2 s, 6 H, 2 Me); ^{13}C NMR: δ 114.20 (CMe_2), 111.48 (dd, $J_{1,F}$ 247, $J_{1,F}$ 245 Hz, C-1), 110.81 (dd, $J_{2,F}$ 28.7, $J_{2,F}$ 26.2 Hz, C-2), 85.67 (C-3), 80.80 (C-5), 76.53 (C-4), 60.97 (C-6), 27.34 and 25.99 (Me); ^{19}F NMR: δ 131.18 (dd, 1 F, $J_{F,F}$ 287.8, $J_{1,F}$ 55.4 Hz), 129.85 (dd, 1 F, $J_{1,F}$ 54.9 Hz). Anal. Calcd for $C_9H_{14}F_2O_5$: C, 44.98; H, 5.88; F, 15.83. Found: C, 45.07; H, 5.79; F, 15.67.

*1-Deoxy-1,1-difluoro-2,3-*O*-isopropylidene-6-*O*-p-tolylsulfonyl-α-*L*-sorbofuranose* (**10**).—Dry pyridine (2 equiv, 0.3 mL, 3.76 mmol) was added to a solution of **9** (1 equiv, 0.45 g, 1.88 mmol) in dry CH_2Cl_2 (2 mL). The temperature of the solution was decreased to −60 °C and TsCl (1.2 equiv, 0.43 g, 2.26 mmol) and DMAP (0.1 equiv, 23 mg, 0.188 mmol) were added. The solution was stirred at rt for 24 h and then poured into ice-water (25 mL) to give an oil. The aq layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer and extracts were dried ($MgSO_4$), the solvent was removed under vacuum, and the oily product was purified by flash chromatography on silica gel (5:1 hexane–EtOAc) to give a white solid that was recrystallized from *i*-Pr_2O to give pure **10** (0.67 g, 90%): R_f 0.38 (2:1 hexane–EtOAc); mp 140 °C; $[\alpha]_D$ +10.5° (*c* 1.7, $CHCl_3$); IR (KBr): ν 3520 (OH), 2990 and 2920 (CH), 1350 (SO_2), 1220 (Me of acetal), and 1090 cm^{-1} (CF_2); ^1H NMR: δ 7.81 and 7.36 (2 d, 4 H, $J_{a,b}$ 8.3 Hz, 4 aromatic), 5.77 (app t, 1 H, $J_{1,F}$ 55.3 Hz, H-1), 4.60 (s, 1 H, H-3), 4.49 (app dt, 1 H, $J_{5,6}$ 6.3, $J_{4,5}$ 2.8 Hz, H-5), 4.36 (dd, 1 H, $J_{6a,6b}$ 10.7, $J_{5,6a}$ 6.8 Hz, H-6a), 4.31 (dd, 1 H, $J_{4,OH}$ 7.4 Hz, H-4), 4.16 (dd, 1 H, $J_{5,6b}$ 5.8 Hz, H-6b), 2.46 (s, 3 H, aromatic Me), 2.14 (dd, 1 H, $J_{5,OH}$ 3.3 Hz, OH), 1.51 and 1.37 (2 s, 6 H, 2 Me); ^{13}C NMR: δ 145.33 (ipso), 132.26, 129.99, and 128.04 (aromatic), 114.69 (CMe_2), 111.18 (dd, $J_{1,F}$ 248, $J_{1,F}$ 245 Hz, C-1), 110.95 (dd, $J_{2,F}$ 29.2, $J_{2,F}$ 26.2 Hz, C-2), 84.82 (C-3), 79.53 (C-5), 74.09 (C-4), 66.07 (C-6), 27.28 and 25.93 (Me), 21.66 (aromatic Me); ^{19}F NMR: δ 131.88 (dd, 1 F, $J_{F,F}$ 288.0, $J_{1,F}$ 55.7 Hz), 129.71 (dd, 1F, $J_{1,F}$ 54.8 Hz). Anal. Calcd for $C_{16}H_{20}F_2SO_7$: C, 48.72; H, 5.11; F, 9.64; S, 8.11. Found: C, 48.55; H, 5.11; F, 9.82; S, 8.05.

*6-Azido-1,6-dideoxy-1,1-difluoro-2,3-*O*-isopropylidene-α-*L*-sorbofuranose* (**11**).—Sodium azide (1.3 equiv, 0.13 g, 1.98 mmol) was added to a solution of **10** (0.60 g, 1.52 mmol) in dry DMF (20 mL). The solution was stirred and heated at 100 °C for 12 h and then cooled to 0 °C; cold H_2O (20 mL) was added then. The aq layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layer and extracts were dried ($MgSO_4$), and the solvent was removed under vacuum. The crude syrup was purified by flash chromatography (2:1 hexane–EtOAc) to give **11** as a white solid (0.38 g, 98%) that was recrystallized from *i*-Pr_2O: R_f 0.49 (2:1 hexane–EtOAc); mp 88–89 °C; $[\alpha]_D$ +23.9° (*c* 1.2, $CHCl_3$); IR (KBr): ν 3450 (OH), 3000 (CH), 2110 (N_3), 1220 (Me of acetal), and 1090 cm^{-1} (CF_2); ^1H NMR: δ 5.86 (app t, 1 H, $J_{1,F}$ 55.4 Hz, H-1), 4.64 (s, 1 H, H-3), 4.44 (app dt, 1 H, $J_{5,6}$ 6.0, $J_{4,5}$ 3.0 Hz, H-5), 4.27 (dd, 1 H, $J_{4,OH}$ 7.8 Hz, H-4), 3.65 (dd, 1 H, $J_{6a,6b}$ 12.9 Hz, H-6a), 3.57 (dd, 1 H, H-6b), 2.15 (d, 1 H, OH), 1.55 and 1.39 (2 s, 6 H, 2 Me); ^{13}C NMR: δ 114.61 (CMe_2), 111.26 (app t, $J_{1,F}$ 246 Hz, C-1), 110.87 (app t, $J_{2,F}$ 28.9 Hz, C-2), 84.98 (C-3), 80.46 (C-5), 75.06 (C-4), 49.10 (C-6), 27.33 and

25.97 (2 Me); ^{19}F NMR: δ 130.75 (dd, 1 F, $J_{F,F}$ 287.0, $J_{1,F}$ 54.8 Hz), 129.97 (dd, 1 F, $J_{1,F}$ 55.4 Hz). Anal. Calcd for $C_9H_{13}F_2N_3O_4$: C, 40.74; H, 4.94; F, 14.33; N, 15.85. Found: C, 40.76; H, 4.82; F, 14.14; N, 15.90.

1,5,6-Trideoxy-6,6-difluoro-1,5-imino-D-*glucitol hydrochloride* (**2**).—To a solution of **11** (1.32 g, 4.70 mmol) in MeOH (30 mL) was added 10% Pd/C (75 mg), and the mixture was subjected to a hydrogen pressure (50 psig) at rt for 1 h. The mixture was filtered through Celite 521 (Aldrich), and the filtrate was concentrated under vacuum to give a clear syrup (1.19 g); R_f 0.48 (100:25:1 $CHCl_3$–MeOH–NH_4OH). The syrup was dissolved in a solution of THF (27 mL), H_2O (3 mL) and concd HCl (6 mL). The solution was heated at reflux temperature for 8 h, and the solvent was removed under vacuum to give a brown syrup. The syrup was dissolved in MeOH (30 mL) and $NaCNBH_3$ (1.5 g, 23.9 mmol) was added. The mixture was stirred at room temperature for 16 h. Concd HCl was added dropwise until the liberation of HCN ceased. The solvent was removed under vacuum to give a white solid that was extracted with MeOH (15 mL); the organic extract was concentrated under vacuum to a syrup. Partial purification of the product was achieved by flash chromatography on aluminum oxide (3:1 MeOH–H_2O), followed by flash chromatography on silica gel (100:25:1 $CHCl_3$–MeOH–NH_4OH). The hydrochloride salt **2** (0.46 g, 44%) precipitated from a solution of the product in MeOH (2 mL) and concd HCl (5 drops): R_f (silica gel) 0.04 (100:25:1 $CHCl_3$–MeOH–NH_4OH); R_f (aluminum oxide) 0.07 (5:1 MeOH–H_2O); mp 216–218 °C; $[\alpha]_D$ +33.2° (*c* 1.5, MeOH); IR (KBr): ν 3380 (OH), 2920 and 2780 (CH), 2480 (NH), and 1090 cm^{-1} (CF_2); ^{1}H NMR (CD_3OD): δ 6.42 (app t, 1 H, $J_{6,F}$ 53.5 Hz, H-6), 3.74–3.65 (m, 2 H, H-2, H-5), 3.58 (app t, 1 H, $J_{4,5}$ 10.0, $J_{3,4}$ 10.0, $J_{2,3}$ 10.0 Hz, H-4), 3.43 (app t, 1 H, H-3), 3.41 (dd, 1 H, $J_{1eq,2}$ 4.7, $J_{1eq,1ax}$ 11.9 Hz, H-1eq), 3.00 (app t, 1 H, $J_{1ax,2}$ 11.9 Hz, H-1ax); ^{13}C NMR (CD_3OD): δ 114.16 (app t, $J_{6,F}$ 242.6 Hz, C-6), 77.84 (C-3), 69.05 (d, $J_{4,F}$ 4.8 Hz, C-4), 60.46 (app t, $J_{5,F}$ 19.0 Hz, C-5), 47.77 (C-1); ^{19}F NMR (CD_3OD): δ 133.64 (ddd, 1 F, $J_{F,F}$ 286.9, $J_{6,F}$ 52.1, $J_{5,F}$ 3.3 Hz), 129.92–128.93 (m, 1 F). Anal. Calcd for $C_6H_{12}ClF_2NO_3$: C, 32.87; H, 5.52; Cl, 15.96; F, 17.35; N, 6.39. Found: C, 32.76; H, 5.54; Cl, 15.94; F, 17.04; N, 6.19.

1,5,6-Trideoxy-6,6-difluoro-1,5-imino-D-*glucitol* (**3**).—A solution of **2** (199 mg, 0.906 mmol) in H_2O (1 mL) was applied on to a column of Amberlite IR-120 (H^+ form) ion-exchange resin (40 mL); elution with 5% (v/v) NH_4OH in H_2O (300 mL) gave **3** as a white solid (141 mg, 85%) that was recrystallized from MeOH: mp 155–156 °C; $[\alpha]_D$ +31.3° (*c* 0.55, MeOH); IR (KBr): ν 3350 (OH), 2900 and 2850 (CH), 2480 (NH), and 1100 cm^{-1} (CF_2); ^{1}H NMR (CD_3OD): δ 6.03 (d app t, 1 H, $J_{6,F}$ 55.6, $J_{5,6}$ 1.2 Hz, H-6), 3.40–3.34 (m, 1 H, H-2), 3.27 (app t, 1 H, $J_{4,5}$ 9.5, $J_{3,4}$ 9.5 Hz, H-4), 3.17 (app t, 1 H, $J_{2,3}$ 8.9 Hz, H-3), 3.07 (dd, 1 H, $J_{1eq,2}$ 5.1, $J_{1eq,1ax}$ 12.3 Hz, H-1eq), 2.74 (dddd, 1 H, $J_{5,F}$ 21.6, $J_{5,F}$ 6.4 Hz, H-5), 2.38 (app t, 1 H, $J_{1ax,2}$ 11.7 Hz, H-1ax); ^{13}C NMR (CD_3OD): δ 116.36 (app t, $J_{6,F}$ 241.6 Hz, C-6), 80.32 (C-3), 72.24 (C-4), 72.17 (C-2), 62.87 (app t, $J_{5,F}$ 18.6 Hz, C-5), 50.77 (C-1); ^{19}F NMR (CD_3OD): δ 134.96 (ddd, 1 F, $J_{F,F}$ 283.8, $J_{6,F}$ 54.7, $J_{5,F}$ 6.0 Hz), 129.94 (ddd, 1 F, $J_{6,F}$ 55.3, $J_{5,F}$ 22.1 Hz). Anal. Calcd for $C_6H_{11}F_2NO_3$: C, 39.35; H, 6.05; F, 20.74; N, 7.65. Found: C, 39.46; H, 6.15; F, 20.45; N, 7.64.

Enzymic assays.—The assays were conducted at 25 °C using yeast α-glucosidase (EC 3.2.1.20; Sigma G889) and *p*-nitrophenyl α-D-glucopyranoside as substrate, in 50 mM sodium phosphate buffer at pH 6.7, and almond β-glucosidase (EC 3.2.1.21; Sigma G4511) and *p*-nitrophenyl β-D-glucopyranoside as substrate, in 100 mM MES buffer containing 0.1% (w/v) bovine serum albumin at pH 5.0. Five concentrations of substrate in the range 0.1–0.4 mM *p*-nitrophenyl α-D-glucopyranoside or 1.0–4.0 mM *p*-nitrophenyl β-D-glucopyranoside were used for each assay. The rate of formation of *p*-nitrophenol (ε_{400} = 6623 M^{-1} cm^{-1} at pH 6.7 and ε_{400} = 353 M^{-1} cm^{-1} at pH 5.0) was determined using a Beckman DU 7400 spectrophotometer at 400 nm. The rate was calculated 1–3 min after addition of the substrate to the enzyme or to a mixture of enzyme and inhibitor. The Michaelis constants (K_m) were calculated using the Enzyme Mechanism program (Marguardt algorithm) provided with the spectrophotometer software. Four or five concentrations of inhibitor in the range 1–8.5 mM were utilized to determine the type of inhibition and the inhibition constant (K_i). The enzyme and inhibitor were incubated together for 5 min prior to the addition of substrate. The K_i's were calculated as the negative of the intercept on the [I] axis of the K_m versus [I] or $1/\nu_{max}$ versus [I] plot for competitive or noncompetitive inhibition, respectively.

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References

[1] G. Legler, *Adv. Carbohydr. Chem. Biochem.*, 48 (1990) 319–384; G. Mooser, *The Enzymes*, 20 (1992) 187–233.
[2] A.B. Hughes and A.J. Rudge, *Nat. Prod. Rep.*, 11 (1994) 135–162.
[3] G.W.J. Fleet, A. Karpas, R.A. Dwek, L.E. Fellows, A.S. Tyms, S. Petursson, S.K. Namgoong, N.G. Ramsden, P.W. Smith, J.C. Son, F. Wilson, D.R. Witty, G.S. Jacob, and T. Rademacher, *FEBS Lett.*, 237 (1988) 128–132.
[4] J.E. Baldwin, *Chem. Br.*, 25 (1989) 583.
[5] J.A. Erickson and J.I. McLoughlin, *J. Org. Chem.*, 60 (1995) 1626–1631.
[6] T. Kitazume, K. Murata, A. Okabe, Y. Takahashi, and T. Yamazaki, *Tetrahedron: Asymmetry*, 5 (1994) 1029–1040; T. Kitatsume, Jpn. Kokai Tokkyo Koho JP 06 128 229 [94 128 229] (1994); *Chem. Abstr.*, 122 (1995) 106391f.
[7] H. Paulsen, I. Sangster, and K. Heyns, *Chem. Ber.*, 100 (1967) 802–815.
[8] J. Di, B. Rajanikanth, and W.A. Szarek, *J. Chem. Soc., Perkin Trans. 1*, (1992) 2151–2152.
[9] K.P.R. Kartha, *Tetrahedron Lett.*, (1986) 3415–3416.
[10] R.A. Sharma, I. Kavai, Y.L. Fu, and M. Bobek, *Tetrahedron Lett.*, (1977) 3433–3436.
[11] M.L. Sinnott, *Chem. Rev.*, 90 (1990) 1171–1202.
[12] T. Reichstein and A. Grüssner, *Helv. Chim. Acta*, 17 (1934) 311–328.