Carbohydrate Research, 250 (1993) 185–193
Elsevier Science Publishers B.V., Amsterdam

Note

Synthesis and inhibition studies of *C*-(D-glycopyranosyl)methylamines *

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(Received March 11th, 1993; accepted May 14th, 1993)

Basic monosaccharide analogues in which either oxygen atom of the hemiacetal function is replaced by an imino (–NH–) group exhibit a high affinity for the active site of glycosidases and behave in most cases as powerful competitive inhibitors of these enzymes[1,2]. In spite of their ready availability, glycosylamines (specifically, glycopyranosylamines) have not reached the significance and popularity of "imino sugars" (5-amino-5-deoxy-hexoses and 1,5-iminohexitols), undoubtedly as a result of their susceptibility to spontaneous hydrolysis[3] and the unstability of their anomeric configuration[4]. Both of these problems would be suppressed by the insertion of a methylene group into the C-1–NH_2 bond of glycosylamines. In order to determine whether the resulting *C*-(D-glycopyranosyl)methylamines would retain the inhibitory activity of the parent glycosylamines, in spite of the increased distance between the amino group and the anomeric center, we have studied a series of such "homoglycosylamines", namely *C*-(β-D-glycopyranosyl)methylamines **1–3**, all of which are potential inhibitors of the extensively studied[5–7] sweet almond β-glucosidase, *C*-(α-D-glycopyranosyl)methylamines **4** and **5**, and the related bis-*C*-(β-D-glucopyranosyl)methylamine **14** (ref 8).

The properties of *C*-glycopyranosyl compounds bearing an amino group at the C-1′ position (i.e., at the carbon atom directly attached to the anomeric center) have been investigated only in a few isolated cases. While Legler and coworkers have shown that *C*-(β-D-glucopyranosyl)methylamine (**1**) is a weaker inhibitor of two β-glucosidases (from calf-liver cytosol[9] and from *Aspergillus wentii*[1]) than the corresponding glucosylamine (by a factor of less than 90 on K_i values), Schmidt and Dietrich have recently demonstrated[10] that the amino group could contribute effectively to the binding of the inhibitor [*C*-(β-D-glucopyranosyl)phenylmethyl-

* The recommended name for these compounds is 1-amino-2,6-anhydro-1-deoxyheptitols.

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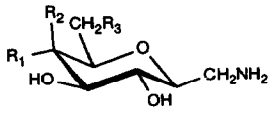
SSDI 0008-6215(93)E0186-5



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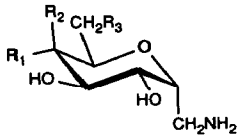

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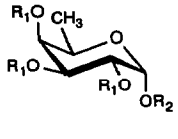
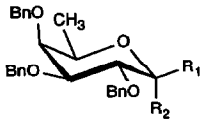
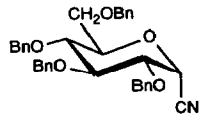


6 R_1 = H, R_2 = Me
7 R_1 = Bn, R_2 = Me
8 R_1 = Bn, R_2 = H
9 R_1 = Bn, R_2 = Ac



10 R_1 = CN, R_2 = H (β)
11 R_1 = H, R_2 = CN (α)



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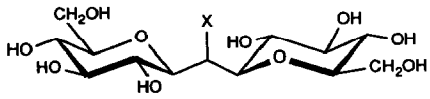


13 X = H
14 X = NH_2

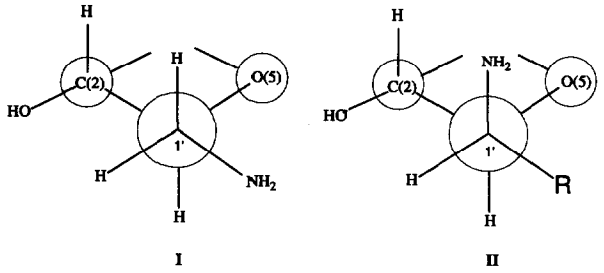
amines] provided that the basic group is properly oriented [the 1′(*R*)-isomer is a much more potent inhibitor than both the 1′(*S*)-epimer and the unsubstituted *C*-(β-D-glucopyranosyl)phenylmethane, with an activity similar to that of 1-deoxynojirimycin towards the almond β-glucosidase].

The known *C*-(β-D-glucopyranosyl)- and *C*-(β-D-galactopyranosyl)methylamines **1** (refs 11 and 12) and **2** (refs 12–15) were prepared in essentially quantitative yield by hydrogenation of the corresponding *C*-(β-D-glucopyranosyl)nitromethanes[8,16] over Adams' catalyst. The *C*-(β- and α-D-fucopyranosyl)methylamines **3** and **5** were obtained from the corresponding benzylated β- and α-D-fucopyranosyl cyanides **10** and **11** by catalytic hydrogenation under pressure (200 psi) at 75–80°C, using palladium-on-charcoal as the catalyst. The reaction of the 1-*O*-acetyl-D-fucose derivative **9** with cyanotrimethylsilane[17] afforded precursors **10** and **11** in high yield (40 and 49%, respectively, after separation by flash chromatography). Hydrogenation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl cyanide (**12**)* (ref 17) under the same conditions as **10** and **11** provided the *C*-(α-D-glucopyranosyl)methylamine **4** in 56% yield. We have recently reported[8] the preparation of *C*-disaccharides **13** and **14**.

* The recommended name is 2,6-anhydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*ido*-heptononitrile.

The activity of **1–3** as inhibitors of the sweet almond β-glucosidase was determined using *p*-nitrophenyl β-D-glucopyranoside as the substrate, and the results are reported in Fig. 1 (Lineweaver–Burk plots) and in Table I. This enzyme has a remarkably broad specificity with regard to both the aglycon and the glycosidic unit[7]. Walker and Axelrod[5] had shown that the (purified) enzyme was inhibited by the glycosylamines derived from D-glucose, D-galactose, and D-fucose (data reported in Table I), the best inhibitor being the D-fucopyranosylamine, using the *p*-nitrophenyl β-glycopyranoside derived from any of these hexoses as the substrate. These authors concluded from their studies that the hydrolytic activity of the enzyme was due to activity at a single catalytic site. It is of particular interest to compare our results with those of Walker and Axelrod (Table I). While the inhibition constant (competitive component) for the *C*-(β-D-glucopyranosyl) methylamine (**1**) is of the same order as that of D-glucopyranosylamine, the D-galacto epimer (**2**) and its 6-deoxy derivative (**3**) are much weaker inhibitors (by factors of 10 and > 200, respectively). It is difficult to rationalize the difference between the D-fucosyl derivatives; however, one notes that the ratio of the K_i values for **1** and **2** is similar to that of the K_m values of the corresponding substrates[5]. Both **1** and D-glucopyranosylamine remain relatively weak inhibitors of this enzyme [by comparison, the K_i for 1-deoxynojirimycin is 30 μM at pH 6.2 (ref 7)].

The high affinity of glycosylamines and "imino sugars" for the corresponding glycosidases is thought to be due to the formation of an ion-pair between the protonated inhibitor and an active site function (typically, carboxylate) involved in the hydrolysis of the glycosidic bond of the substrates[18]. Thus, the affinity of 1′-amino-*C*-β-D-glycopyranosyl compounds for β-glycosidases will strongly depend on the distance between the amino group and this active site function, once the substrate analogue binds to the enzyme. The activity of Schmidt's compound [1′(*R*)-epimer][10] which adopts most probably conformation **II** (R = Ph) about the



C-1–C-1′ linkage, appears to indicate that the most favorable position for the amino group in this compound is the anti orientation with respect to H-1, which is probably the closest position to the complementary function of the active site of the sweet almond enzyme. According to $J_{1,1'A}$ (7.5–8.0 Hz) and $J_{1,1'B}$ (2.5–2.6 Hz) values, *C*-(β-D-glycopyranosyl)methylamines **1–3** exhibit a conformational equilibrium about the C-1–C-1′ bond in which both conformations **I** (~ 67%*) and **II**

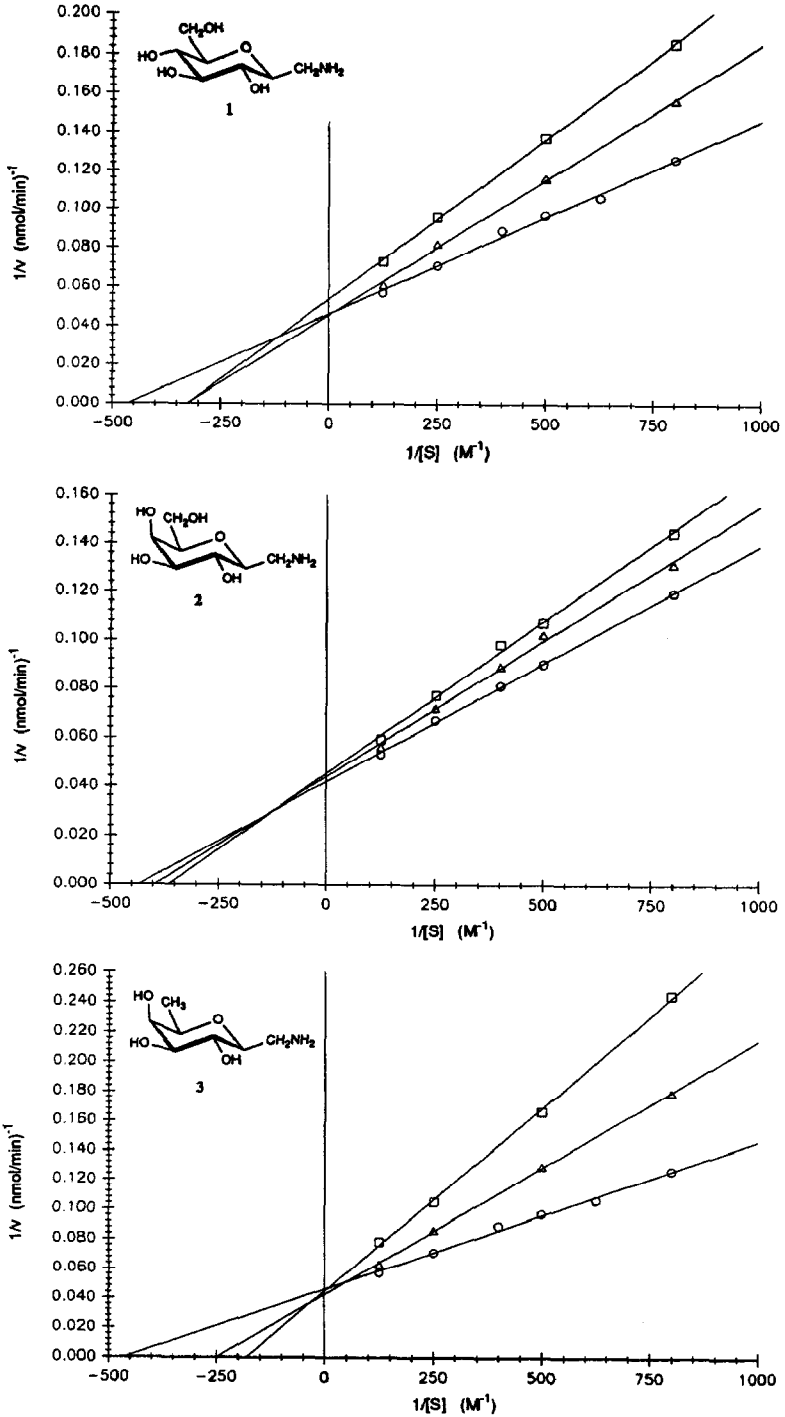


Fig. 1. Lineweaver–Burk plots for the inhibition of sweet almond β-glucosidase by compounds **1–3**: ○———○, [I] = 0; △———△, [I] = 2 mM; □———□, [I] = 4 mM.

TABLE I

K_i^c Values (mM) for **1–3** and for the corresponding D-glycopyranosylamines [a]

Compound	Inhibition type	K_i^c	D-Glycopyranosylamine	K_i^c (ref 5)
1	mixed	5.5	D-glucopyranosylamine	2.6
2	mixed	17	D-Galactopyranosylamine	1.7
3	competitive	2.8	D-Fucopyranosylamine	0.0125

[a] Enzyme: sweet almond β-glucosidase (Sigma). Substrate: *p*-nitrophenyl β-D-glucopyranoside. Procedure: see Experimental section. K_i^c = inhibition constants for competitive inhibition.

(R = H, ~33%) are present. Therefore, it is somewhat surprising to find that these compounds exhibit only a modest inhibitory activity. Since conformation **II** (R = β-D-glucopyranosyl) is the preferred one for the bis-*C*-(β-D-glucopyranosyl)methylamine **14** (ref 8), we also tested this compound, as well as "*C*-β,β-trehalose" **13**, as inhibitors of the almond enzyme under the same conditions as **1–3**. Both compounds were found, however, to be devoid of inhibitory activity. We attribute this lack of affinity of **13** and **14** for the enzyme to the hydrophilicity of their "aglycon": it is indeed well established that the sweet almond enzyme possesses a hydrophobic domain at the aglycon binding site[18] that promotes a favorable interaction with nonpolar substituents such as the phenyl group of *N*-benzyl-glycosylamines (*N*-benzyl-β-D-glucopyranosylamine inhibits the almond β-glucosidase with $K_i = 0.32$ μM, ref 6) and contributes to the activity of Schmidt's compound. Further investigations on **13** and **14** with other β-glucosidases are warranted.

In preliminary studies with yeast α-glucosidase (Sigma), the *C*-(α-D-glucopyranosyl)methylamine **4** was found to competitively inhibit the hydrolysis of *p*-nitrophenyl α-D-glucopyranoside, with a K_i in the mM range. Compound **5** did not exert any inhibitory activity on this process, in accordance with the high glycon specificity of this α-glucosidase[19].

In conclusion, the *C*-(D-glucopyranosyl)methylamines form a class of relatively weak glycosidase inhibitors, in general weaker than the corresponding glycopyranosylamines, in spite of the relative proximity of the amino group to the complementary active site function in one of the favorable conformations of the "*C*-glycosidic" linkage. That the almond β-glucosidase is inhibited to the same extent by both D-glucosylamine and **1** appears to be due to the fact that the glycosylamine is an unusually weak inhibitor for this enzyme.

EXPERIMENTAL

General methods.—See ref 20. ^1H and ^{13}C NMR spectra were recorded, respectively, at 360 and at 90 MHz on a Bruker AM 360 spectrometer. Hydrogena-

* Determined using the following limit values: in **I**, $J_{1,1'A}$ 11.0 Hz; in **II**, $J_{1,1'A} = 1.5$ Hz (from PCMODEL, Serena Software). The conformation with the C-1′–N bond anti with respect to the C-1–O-5 bond can be ignored because of the destabilizing 1,3-diaxial-like interactions between the 1′-NH_2 and 2-OH groups.

tions under pressure and at elevated temperatures were performed in a Parr benchtop minireactor (450 mL) equipped with a magnetic stirring drive and a pressure display module. Chromatographic separations were achieved by flash chromatography using Silica Gel-60 (230–400 mesh) and one of the following solvent systems (v/v): *A*, 1:8; *B*, 1:4 EtOAc–hexanes; *C*, 2:3:0.6; *D*, 2:3:0.8 $CHCl_3$–MeOH–28% aq NH_3. Elemental analyses were performed by Atlantic Microlab (Norcross, GA).

1-Amino-2,6-anhydro-1-deoxy-D-glycero-D-gulo-*heptitol[C-(β-D-glucopyranosyl) methylamine]* (**1**).—To a solution of 2,6-anhydro-1-deoxy-1-nitro-D-*glycero*-D-*gulo*-heptitol [*C*-(β-D-glucopyranosyl)nitromethane][8,16] (300 mg, 1.35 mmol) in MeOH (25 mL) was added Adams' catalyst (PtO_2, 15 mg), and the reaction flask was connected to a balloon filled with H_2. The mixture was stirred for 9 h at room temperature. The catalyst was then removed by filtration, and the solvent was evaporated under reduced pressure to give an essentially quantitative yield of **1**. Well-formed crystals of **1** (190 mg, 73%) were obtained by recrystallization of this product from MeOH; mp 171.4–171.7°C (lit.[11] mp 164–165°C); $[\alpha]_D^{20}$ −6.4° (*c* 1.4, H_2O) {lit.[11] $[\alpha]_D^{20}$ −6.7° (*c* 3.2, H_2O)}*; ^{13}C NMR (CD_3OD; ref CD_3OD $\delta = 49.0$): δ 44.06, 63.32, 71.99, 73.41, 79.68, 81.51, 81.63.

7-Amino-2,6-anhydro-7-deoxy-L-glycero-L-galacto-*heptitol[C-(β-D-galactopyranosyl)methylamine]* (**2**).—Hydrogenation of 2,6-anhydro-7-deoxy-7-nitro-L-*glycero*-L-*galacto*-heptitol [*C*-(β-D-galactopyranosyl)nitromethane][16] under the conditions described above afforded an essentially quantitative yield of **2**; mp (from MeOH) 194–195°C (lit.[12,14] mp 191–192°C); $[\alpha]_D^{20}$ +29.5° (*c* 1.0, H_2O) {lit.[14] $[\alpha]_D^{20}$ +30.0° (*c* 1.6, H_2O); lit.[12] $[\alpha]_D^{21}$ +29° (*c* 2.0 H_2O)}; ^{13}C NMR (CD_3OD): δ 43.96, 63.25, 70.57, 71.09, 76.36, 80.44, 82.14.

2,3,4-Tri-O-*benzyl-α*-D-*fucopyranose* (**8**).—Benzylation of methyl α-D-fucopyranoside[21] (prepared from D-fucose as described by Zehavi and Sharon[22] for the L-enantiomer) using benzyl bromide and NaH in DMF according to the general procedure[23] afforded methyl 2,3,4-tri-*O*-benzyl-α-D-fucopyranoside (**7**) in 76% yield {syrup; $[\alpha]_D^{20}$ +59.4° (*c* 1.9, $CHCl_3$)}. Hydrolysis of **7** under the conditions described for the L-enantiomer[24] and crystallization of the resulting product from ether–hexanes gave **8** in 56% yield; mp 94.5–95.6°C (L-enantiomer: lit.[24] mp 102–103°C; lit.[25] mp 102–104°C); $[\alpha]_D^{20}$ +24.0° (*c* 1.0, $CHCl_3$) {L-enantiomer: lit.[24] $[\alpha]_D^{25}$ −26.5° (*c* 1.0, $CHCl_3$); lit.[25] $[\alpha]_D$ −25.4° (*c*, 1.1, $CHCl_3$)}; ^{13}C NMR ($CDCl_3$): δ 16.74, 66.74, 70.81, 72.98, 73.55, 74.77, 79.09, 82.57, 91.88, 127.5–128.5, 138–139 (Ar-C's). Anal. Calcd for $C_{27}H_{30}O_5$: C, 74.63; H, 6.96. Found: C, 74.51; H, 6.94.

1-O-Acetyl-2,3,4-tri-O-*benzyl-α*-D-*fucopyranose* (**9**).—Compound **8** (650 mg, 1.5 mmol) was acetylated under standard conditions (pyridine–Ac_2O, catalytic amount of DMAP) to give, after processing of the mixture, 694 mg (97%) of homogeneous

* Our data and those of ref 11 differ markedly from those reported by Petrus[12] for **1**.

acetates ($\alpha:\beta$ ratio, 2.3:1). Pure α anomer (364 mg, 51%) was obtained by crystallization from MeOH; mp 119.6–120.6°C {L-enantiomer: lit.[26] mp 90–92°C (MeOH)}; $[\alpha]_D^{20}$ +63.5° (*c* 1.0, $CHCl_3$) {L-enantiomer: lit.[26] $[\alpha]_D^{25}$ −71.5° (c 1.1, $CHCl_3$)}; ^{13}C NMR ($CDCl_3$): δ 16.72, 21.18, 69.10, 73.22, 73.29, 74.96, 75.32, 77.21, 79.01, 90.91, 127.4–128.4, 138–139 (Ar-C's), 169.63. Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 72.94; H, 6.78.

Reaction of **9** *with cyanotrimethylsilane.*—To a solution of **9** (300 mg, 0.63 mmol) in MeCN (10 mL) were added fresh cyanotrimethylsilane (0.39 mL, 2.9 mmol) and $BF_3 \cdot OEt_2$ (0.08 mL), and the mixture was stirred for 15 min at room temperature. Water (10 mL) and a few drops of satd aq $NaHCO_3$ were then added. The organic solvent was evaporated under reduced pressure, and the residual aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were washed with water (2 × 5 mL), dried (Na_2SO_4), and concentrated. The resulting mixture of **10** and **11** was resolved by flash chromatography (solvent *A*), which afforded, in this order, pure α-cyanide **11** (139 mg, 49%) and pure β-cyanide **10** (109 mg, 40%).

*Physicochemical data for 2,6-anhydro-3,4,5-tri-*O*-benzyl-7-deoxy-*D-glycero-L-manno-*heptononitrile* (**10**).—Mp 115.5–116.2°C; $[\alpha]_D^{20}$ +14.2° (c 1.1, $CHCl_3$); R_f 0.31 (solvent *B*); ^1H NMR ($CDCl_3$): δ 1.20 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 3.47 (dq, 1 H, $J_{4,5}$ 0.9 Hz, H-5), 3.50 (dd, 1 H, $J_{2,3}$ 9.3, $J_{3,4}$ 2.8 Hz, H-3), 3.62 (dd, 1 H, H-4), 4.00 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.17 (t, 1 H, H-2), 4.67 (d, 1 H, *J* 11.5 Hz), 4.99 (d, 1 H), 4.75 (narrow AB, 2 H), 4.91 (s, 2 H) (3 OCH_AH_BPh), 7.25–7.5 (m, 15 H, 3 Ph); ^{13}C NMR ($CDCl_3$): δ 16.89, 68.01, 72.97, 75.03, 75.80, 75.96, 76.23 (2C), 83.40, 117.04 (CN), 127.6–128.6, 137.3–138.1 (Ar-C's). Anal. Calcd for $C_{28}H_{29}NO_4$: C, 75.82; H, 6.59; N, 3.16. Found: C, 75.55; H, 6.63; N, 3.15.

*Physicochemical data for 2,6-anhydro-3,4,5-tri-*O*-benzyl-7-deoxy-*D-glycero-L-gluco-*heptononitrile* (**11**).—Mp 88.5–89.5°C; $[\alpha]_D^{20}$ + 49.5° (*c*, 1.0, $CHCl_3$); R_f 0.47 (solvent *B*); ^1H NMR ($CDCl_3$): δ 1.15 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 3.66 (narrow dd, 1 H, $J_{3,4}$ 2.8, $J_{4,5} \sim 0.8$ Hz, H-4), 3.81 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-3), 3.90 (br q, 1 H, H-5), 4.10 (dd, 1 H, $J_{1,2}$6.1 Hz, H-2), 4.667 (d, 1 H, H-1), 4.63 (d, 1 H, *J* 11.4 Hz), 4.671 (d, 1 H, *J* 12 Hz), 4.76 (d, 1 H, *J* 11.8 Hz), 4.85 (d, 1 H, *J* 12 Hz), 4.87 (d, 1 H, *J* 11.8 Hz), 4.96 (d, 1 H, *J* 11.4 Hz) (3 OCH_AH_BPh), 7.25–7.5 (m, 15 H, 3Ph); ^{13}C NMR ($CDCl_3$): δ 16.61, 67.53, 72.57, 73.55, 73.58, 73.95, 75.16, 76.85, 80.66, 116.10 (CN), 127.5–128.5, 137–138 (Ar-C's). Anal. Found: C, 75.56; H, 6.58; N, 3.15.

*7-Amino-2,6-anhydro-1,7-dideoxy-*L-glycero-L-galacto-*heptitol[*C-(β-D-*fucopyranosyl)methylamine]* (**3**).—To a solution of β-cyanide **10** (50 mg, 0.113 mmol) in acetic acid (15 mL) was added 10% Pd–C (spatula tip), and the mixture was hydrogenated for 10 h at 75–80°C under an H_2-pressure of 200 psi (see General Methods). The catalyst was removed by filtration, the solvent was evaporated under reduced pressure, and the residue was submitted to flash chromatography (solvent *C*) which afforded pure **3** (15 mg, 75%) as a syrup; $[\alpha]_D^{20}$ +14° (*c* 0.9, H_2O); ^1H NMR (D_2O): δ 1.02 (d, 3 H, $J_{6,7}$ 6.5 Hz, H-7), 2.58 (dd, 1 H, $J_{1A,2}$ 7.9,

$J_{1A,1B}$ 13.6 Hz, H-1A), 2.87 (dd, 1 H, $J_{1B,2}$ 2.6 Hz, H-1B), 3.085 (ddd, 1 H, $J_{2,3}$ 9.6 Hz, H-2), 3.26 (t, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 3.41 (dd, 1 H, $J_{4,5}$ 3.4 Hz, H-4), 3.52 (q, 1 H, $J_{5,6} \sim 0$, H-6), 3.57 (d, 1 H, H-5); ^{13}C NMR (CD_3OD): δ 17.12, 43.56, 70.12, 73.65, 75.59, 76.50, 81.18. HRFABMS: Calcd for $[M+H]^+$: 178.1079. Found: 178.1077.

7-Amino-2,6-anhydro-7-deoxy-D-glycero-L-gulo-*heptitol[C-(α-D-glucopyranosyl) methylamine]* (**4**).—2,6-Anydro-3,4,5,7-tetra-*O*-benzyl-D-*glycero*-D-*ido*-heptononitrile (**12**) (prepared from 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose as described by De las Heras and co-workers[17]) (130 mg, 0.24 mmol) was hydrogenated for 10 h under the same conditions as for **10** (see preparation of **3**). The resulting product was submitted to flash chromatography (solvent *D*) which afforded pure, syrupy **4** (26 mg, 56%); $[\alpha]_D^{20}$ +59° (*c* 1.0, H_2O); ^{1}H NMR (CD_3OD; ref CD_2HOD $\delta = 3.31$): δ 2.91 (br dd, 1 H, $J_{1A,2}$ 3.9, $J_{1A,1B} \sim 13.5$ Hz, H-1A), 3.01 (br t, 1 H, $J_{1B,2}$ 10.5 Hz, H-1B), 3.21 (dd, 1 H, $J_{4,5}$ 8.6, $J_{5,6}$ 9.4 Hz, H-5), 3.44 (ddd, partially occluded, 1 H, $J_{6,7A}$ 6.6, $J_{6,7B}$ 2.3 Hz, H-6), 3.47 (t, 1 H, $J_{3,4}$ 9.3 Hz, H-4), 3.63 (dd, 1 H, $J_{7A,7B}$ 11.7 Hz, H-7A), 3.64 (dd, 1 H, $J_{2,3}$ 5.9 Hz, H-3), 3.84 (dd, 1 H, H-7B), 3.94 (ddd, 1 H, H-2); ^{13}C NMR (CD_3OD): δ 37.59, 63.25, 72.20, 72.60, 75.25, 75.30, 77.71. Anal. Calcd for $C_7H_{15}NO_5 \cdot H_2O$: C, 39.81; H, 8.11; N, 6.63. Found: C, 40.48; H, 7.99; N, 6.23.

7-Amino-2,6-anhydro-1,7-dideoxy-D-glycero-L-galacto-*heptitol[C-(α-D-fucopyranosyl)methylamine]* (**5**).—α-Cyanide **11** (150 mg, 0.338 mmol) was hydrogenated for 10 h under the same conditions as for **10** (see preparation of **3**). The resulting product was submitted to flash chromatography (solvent *C*) which afforded pure **5** (47 mg, 79%); mp 166.4–167.6°C; $[\alpha]_D^{20}$ +86° (*c* 1.0, H_2O); ^{1}H NMR (CD_3OD): δ 1.26 (d, 3 H, $J_{6,7}$ 6.5 Hz, H-7), 2.9–3.1 (br m, 2 H, H-1A, 1B), 3.63 (dd, 1 H, $J_{3,4}$ 8.4, $J_{4,5}$ 3.4 Hz, H-4), 3.71 (dd, 1 H, $J_{5,6}$ 2.2 Hz, H-5), 3.84 (dq, 1 H, H-6), 3.91–3.98 (m, 2 H, H-2,3); ^{13}C NMR (CD_3OD): δ 16.41, 38.22, 69.58, 69.78, 72.11, 72.45, 75.61. HRFABMS: Calcd for $[M+H]^+$: 178.1079. Found: 178.1086.

Inhibition studies.—The inhibitory activity of **1–3** on the hydrolysis of *p*-nitrophenyl β-D-glucopyranoside by sweet almond β-glucosidase (Sigma, β-D-glucoside glucohydrolase, EC 3.2.1.21) was determined under the following conditions: total reaction volume 1.00 mL, pH 6.0 (50 mM sodium acetate buffer), temperature 30°C, enzyme concentration 0.5 μg/mL (~ 0.015 U/mL), stock solution of inhibitor 20 mM in **1**, **2**, or **3** at pH 6.0 (prepared by dissolving **1**, **2**, or **3** in acetate buffer and adjusting the pH of the solution using aq HCl); substrate and inhibitor concentrations used are shown in Fig. 1. Initial velocities (less than 10% substrate consumed) were measured by quenching the reaction after exactly 10 min by the addition of 1.00 mL of a 16% aq solution of Na_2CO_3 and determining the *p*-nitrophenolate ion concentration from its absorption at 400 nm (reference, same solution without enzyme; ϵ_{400} 18.6×10^6 cm^2/mol). The resulting data are presented as Lineweaver–Burk plots (Fig. 1). The K_m value (2.2 mM) thus obtained is in good agreement with previously reported values (2.5 mM, refs 5 and 7). Inhibition constants for competitive inhibition (K_i^c, Table I) were calculated from

the slope in the presence of inhibitor [slope (I)] related to the slope in its absence [slope (0)] (ref 6):

$$K_i^c = \frac{[I]}{\frac{\text{slope(I)}}{\text{slope(0)}} - 1}$$

ACKNOWLEDGMENTS

Support of this research by a grant from the National Institutes of Health (DK35766) is gratefully acknowledged. Mass spectral determinations were performed by the Midwest Center for Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility (Grant CHE 8620177).

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