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Synthesis of *C*-(D-glycopyranosyl)ethylamines and *C*-(D-glycofuranosyl)methylamines as potential glycosidase inhibitors

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Abstract

The *C*-glucosyl aldehyde, 2-*C*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)ethanal was prepared from the *C*-glucopyranosyl propene precursor by ozonolysis. Reductive amination of the *C*-glucosyl aldehyde and subsequent deprotection gave 1-anilino-2-*C*-(α-D-glucopyranosyl)ethane. The E and Z isomers of the oxime derivative, 1-*C*-(α-D-arabinofuranosyl)methanal oxime were prepared by treating their aldehyde precursor with hydroxylamine. Acetylation of the oxime, followed by catalytic hydrogenation and deprotection, gave the corresponding 1-*C*-(α-D-arabinofuranosyl)methylamine. Reductive amination of ethyl 2,3-*O*-isopropylidene-α-D-*lyxo*-pentodialdo-1,4-furanoside using aniline gave ethyl 5-anilino-5-deoxy-D-*lyxo*-furanoside. Inhibition studies with these compounds on β-D-glucosidase from sweet almond, using *o*-nitrophenyl D-glucopyranoside as substrate, were carried out.

Keywords: *C*-Glucosyl aldehyde; α-*C*-Arabinosides; α-D-Mannofuranoside; Reductive amination; Glycosidase inhibitor

1. Introduction

Carbohydrate-processing enzymes and, in particular, glycosidases are involved in a wide range of important biological processes [1–3]. The possibility of modifying or blocking these processes for therapeutic or biotechnological applications has attracted the attention of many investigators. A range of configurational and constitutional isomers of inhibitors are presently known [1–6]. The topography of the relative position of the acid groups on the active site of the enzyme which participate in the glucosidic cleavage has been indicated from the difference in the inhibitory activity towards almond β-glucosidase of the (1*R*)-isomer of *C*-(β-D-glucopyranosyl)phenylmethylamine, which has an activity similar to that of 1-deoxynojirimycin, and the (1*S*)-epimer where the former is more potent inhibitor [7]. Similarly, there is a clear distinction between the diastereomeric α-anomeric analogues **1** and **2** with a relatively strong inhibition by isomer **1** [8]. Iminosugars exhibit a broad spectrum of glycosidase inhibition. However, the popularity of using glycosylamines in general is not like that of iminosugars as a consequence of their susceptibility to spontaneous hydrolysis and the instability of their anomeric configuration [9,10]. These problems have been overcome by inserting a methylene bridge between the sugar C-1 and the amino group as apparent in the series of homoglyco-

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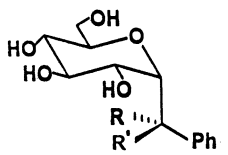
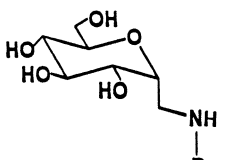
sylamines **3** and **4** [11,12]. The strength of the inhibition of *N*-substituted (glycosylmethyl)amines was found to be associated with the ability of the aglycon part of the inhibitor to allow proton donation in the active site in order to form an ion pair and the basicity as well as the hydrophobicity of the substituent [13–16]. A better inhibition was found for the *N*-(*C*-glucosylmethyl)aniline (**4**) which has a K_i value practically identical to that of 1-deoxynojirimycin. On the other hand, the *C*-(β-D-glucopyranosyl)methylamine is a weaker inhibitor of the β-glucosidase, from either calf-liver cytosol or from *Aspergillus wentii*, than the glucosylamine [1,17]. These data promoted our interest in designing glycosidase inhibitors of the *C*-glycosyl type which have an ethylene bridge between the anomeric carbon atom and the amino group of the glucosylamine, i.e., the two-carbon homologue; thus it should become clear whether the proximity of the amino group to the anomeric carbon is necessary. *N*-(*C*-Glycofuranosylmethyl)amines have also been synthesized in order to find the extent of the effect of changing the pyranosyl to a furanosyl ring on the glucosidase inhibition.



1 R = H, R' = NH_2
2 R = NH_2, R' = H



3 R = H
4 R = Ph

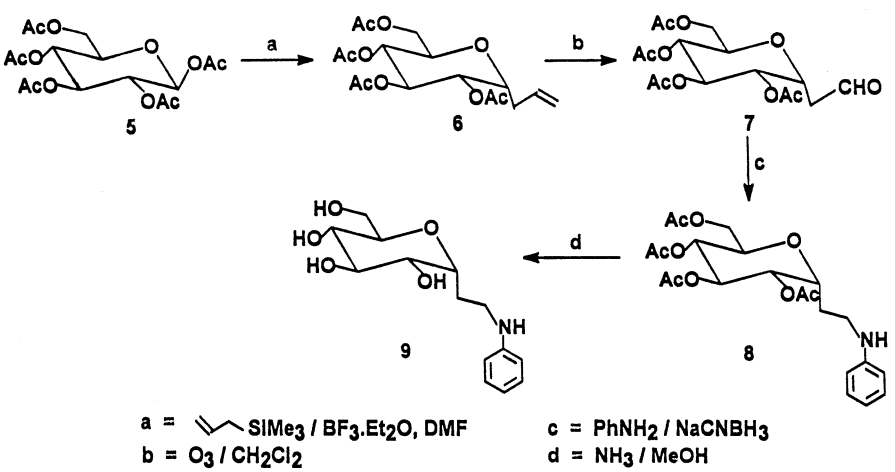
2. Results and discussion

The *C*-glucopyranosylpropene **6** was prepared from β-D-glucose pentaacetate (**5**) by reaction with allyltrimethylsilane [18,19] in the presence of boron trifluoride–diethyl ether complex as a Lewis acid. The reaction takes place stereoselectively, depending on the solvent [19] used in performing the reaction to give the α anomer **6**. The ozonolysis of **6** to give the *C*-glucosyl aldehyde **7** in 77% yield was found to be more practical than the oxidation with osmium tetraoxide, followed by cleavage with sodium metaperiodate [20]. The structure of **7** was confirmed by studying its spectral data. The aldehydic proton appeared as a triplet at δ 9.73. The α configuration of the glucosidic linkage was confirmed from the value of the coupling constant of $J_{1,2}$ (5.4 Hz), which indicated that the H-1′ and H-2′ atoms exist in a cis relation (a, e) to each other. The rest of the coupling constants of the glucopyranosyl ring indicated its existence in the $^4C_1$ conformation. Reductive amination of **7** with aniline in the presence of sodium cyanoborohydride gave the corresponding anilino derivative **8** in 61% yield, which was deprotected by using a saturated methanolic ammonia solution to give **9** in 90% yield. The structure of **8** was confirmed by studying its ^{1}H NMR spectrum, which showed four singlets at δ 2.02, 2.03, 2.04 and 2.08 corresponding to the four OAc groups. The disappearance of the aldehydic proton and the appearance of a multiplet at δ 3.24–3.31, which corresponds to the new CH_2 group, in addition to the presence of the aromatic protons at δ 6.59–7.18, indicated the successful reductive amination. The ^{13}C NMR spectrum of **8** showed signals at δ 171.69, 170.65, 169.56 (CO) and 20.64 (Me) of the four Ac groups. The four signals at δ 144.97, 129.34, 117.62 and 112.78 are attributed to the aromatic ring. The two signals of the two CH_2 groups appear at δ 40.26 and 25.23, respectively. The ^{1}H NMR spectrum of the deacetylated product **9** showed the disappearance of the four OAc groups, and the appearance of the four hydroxyl groups as a broad singlet at δ 3.09–3.11 and a multiplet at δ 3.28–3.32 (Scheme 1).

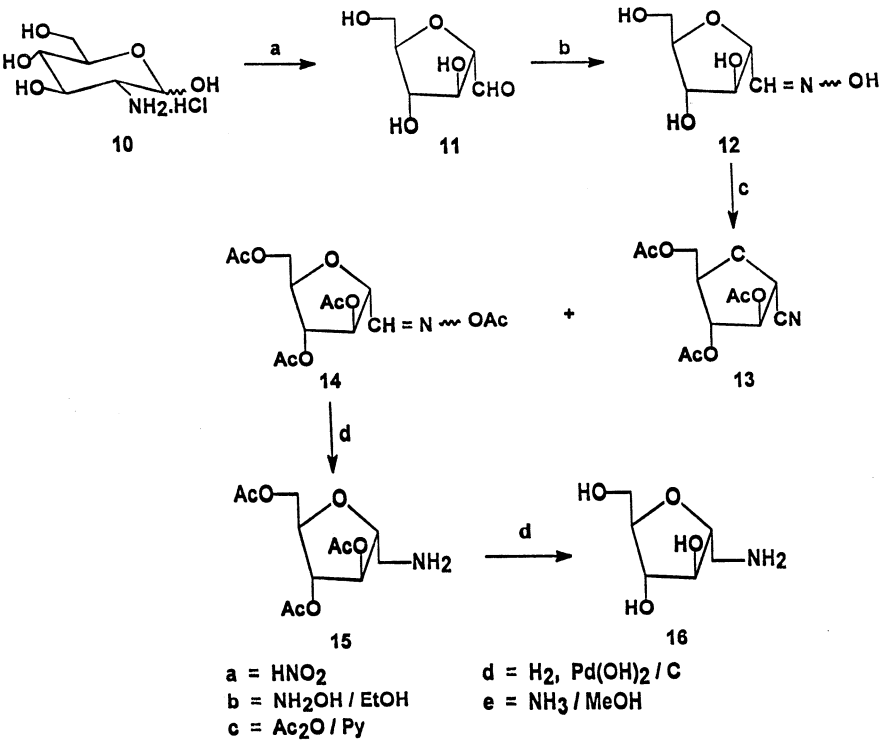
The original procedure [21] for nitrous acid deamination of 2-amino-2-deoxy-D-glucopyranose hydrochloride (**10**) to give the 2,5-anhydro-D-mannose (**11**), through attack of O-5 to C-2 with inversion of configuration at C-2, has been modified by Szarek and co-workers [22]. This procedure for the synthesis of the *C*-arabinofuranosylaldehyde **11** is a convenient route to the synthesis of *C*-nucleosides via its oxime; however, experimental details are not reported [23]. Epimerization of the α-linked *C*-glycosylaldehyde **11** has not been observed during its synthesis [21–23]; mild basic conditions (Et_3N) are required to effect that process [24]. Its use as a precursor for *C*-(α-D-arabinofuranosyl)methylamine has

been successful. Thus, treatment of **11** with hydroxylamine gave the oxime **12** in 77% yield as a mixture of the E and Z isomers. After chromatographic purification, the structure was confirmed by studying its ^{1}H NMR spectrum, which showed doublets at δ 6.67 and 7.28 corresponding to the C*H*=N group and broad singlets at δ 10.91 and 10.83 corresponding to the =N ~ O*H* group in a ratio 3:7, respectively. Acetylation of the oxime **12** was carried out using Ac_2O/pyridine to give a mixture of the nitrile **13** as the fast moving component on TLC, and the respective acetyl derivative **14** of the oxime as the slower moving component in 35% and 60% yield, respectively. The nitrile group in **13** was readily identified from its ^{13}C NMR spectrum that showed a signal at δ 114.95. Reduction of the acetylated oxime **14** was carried out using hydrogen and $Pd(OH)_2$/C to give the corresponding amino derivative **15** in 90% yield. Deprotection of **15** using a saturated methanolic ammonia solution gave **16** in 95% yield. The structure of **15** was confirmed by studying its ^{1}H NMR spectrum, which showed the disappearance of (C*H*=N) proton and the appearance of the multiplet at δ 2.89 attributed to the CH_2 group. Its ^{13}C NMR spectrum showed the disappearance of the signal at δ 154.26 corresponding to the (*C*H=N) and the appearance of the signal at δ 50.42 corresponding to the CH_2 group, thus indicating the successful reduction of the acetylated oxime. The ^{1}H NMR spectrum of the deacetylated product **16** showed the disappearance of the three OAc groups (Scheme 2).

For the synthesis of ethyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside (**18**), 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside (**17**) was alkylated [30] with ethyl iodide in the presence of silver(I) oxide to give **18** in 76% yield. Alternatively, **18** can be synthesized in 63% yield by treatment of the sodium salt of **17** with ethyl iodide. The protons in the ^{1}H NMR spectrum of **18** were assigned by ^{1}H–^{1}H homonuclear shift correlated (COSY) 2D NMR spectroscopy (Fig. 1). Partial removal of the two *O*-isopropylidene groups was carried out by treating **18** with 0.2 N HCl in ethanol to give ethyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (**19**) in 96% yield. The aldehyde **20** can be synthesized either by the oxidation of **19** by sodium metaperiodate or by treating compound **18** with periodic acid in ether solution. The latter method gave better yield (88%) than the former (71%). The structure was confirmed by the presence of a doublet at δ 9.64 in its ^{1}H NMR spectrum, which corresponds to the aldehydo group. Treatment of **20** with ethanolamine at pH 6, followed by addition of sodium cyanoborohydride, did not give the expected product **21**, but gave instead the alcohol **22**. This can be attributed to the rupture of the corresponding Schiff base before the reduction or lack of formation of it and, consequently, the reduction has taken place on the aldehyde group. On the other hand, the use of aniline instead of ethanolamine to react with **20** gave the expected product **23** in 60% yield. O-Deiso-



Scheme 1.



Scheme 2.

propylidenation of the latter product with aqueous acetic acid gave **24** in 72% yield (Scheme 3).

Inhibition studies.—The assay method [25] was based on the treatment of *o*-nitrophenyl β-D-glucopyranoside with β-D-glucosidase from sweet almond and measuring the continuous release of *o*-nitrophenol. The Michaelis–Menten constant (K_M) at pH 6.8 was determined to be 0.07 M. The kinetic parameters were determined by Lineweaver–Burk plots [26] (Figs. 2–4). The *C*-(D-glucopyranosyl)ethylamine (**9**) showed a moderate inhibition (K_i 1.5×10^{-4} M) compared with the corresponding homologue *C*-(D-glucopyranosyl)methylamine (**4**), which has a K_i of 1.1×10^{-5} M against α-glucosidase [8], indicating that the distance between the anomeric center and the NH group plays a role on the inhibition that may result in a less-than-optimum interaction with the active site of the enzyme. Although the homoglycosylamines *C*-(β-D-glucopyranosyl)phenylmethylamines are potential inhibitors of sweet almond β-glucosidases [7], the corresponding *C*-(β-D-glucopyranosyl)methylamine was found to be a weaker inhibitor of the β-glucosidases from calf-liver cytosol and from *A. wentii* [1,17] than the corresponding glucosylamine [27–29]. Moreover, changing the pyranosyl ring to a furanosyl ring such as in **16** or **24** did not improve the inhibition property, where the K_i of **16** is 1.3×10^{-4} M and that of **24** is 3.4×10^{-4} M. Further studies are needed in order to test the inhibition of other glucosylalkylamines and of other glycosidases with

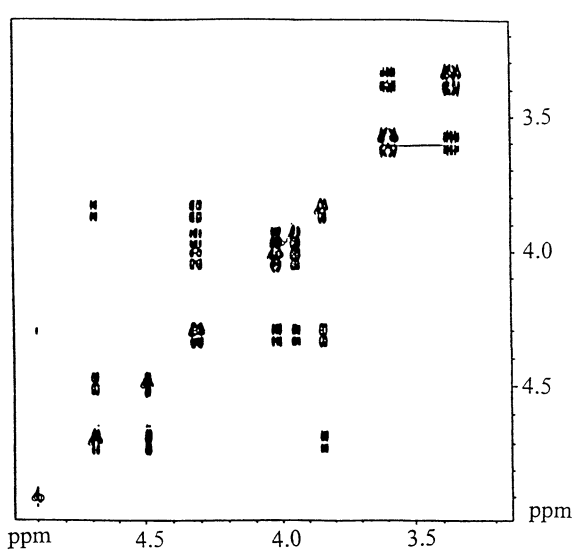
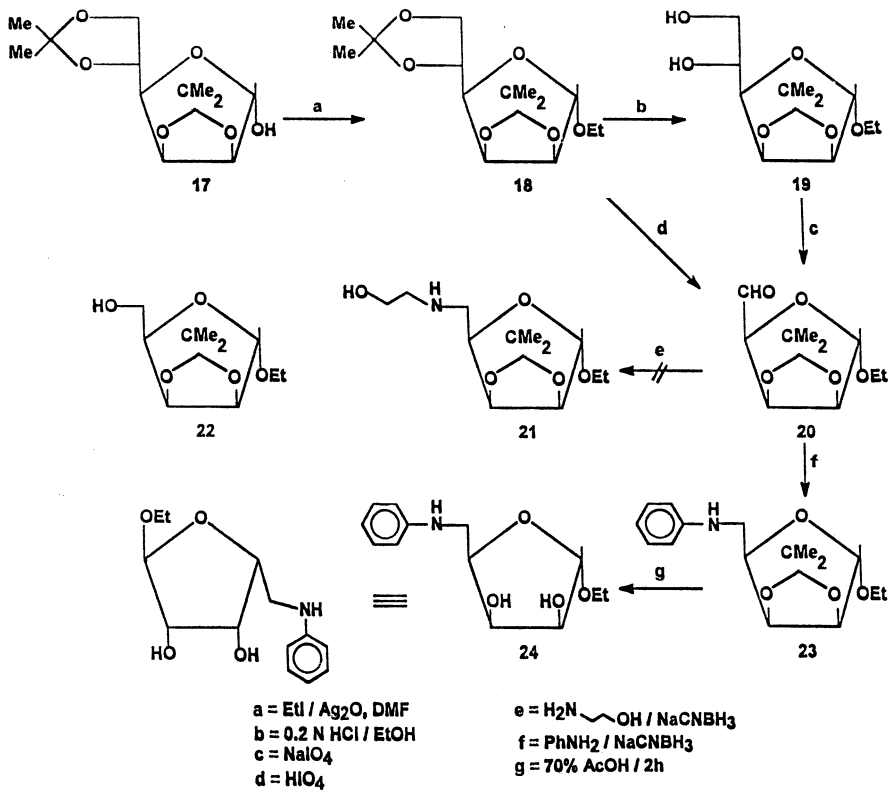


Fig. 1. $^1H-^1H$ homonuclear (COSY) 2D NMR of **18**.



Scheme 3.

these compounds in order to obtained more generalized picture about the active site of these enzymes. The K_i values of the tested compounds, which are $\sim 10^{-4}$ M, are encouraging, because even the affinity of 1-deoxynojirimycin [31] with $K_i = 1.8 \times 10^{-5}$ M against β-glucosidase from sweet almond is only one order of magnitude higher.

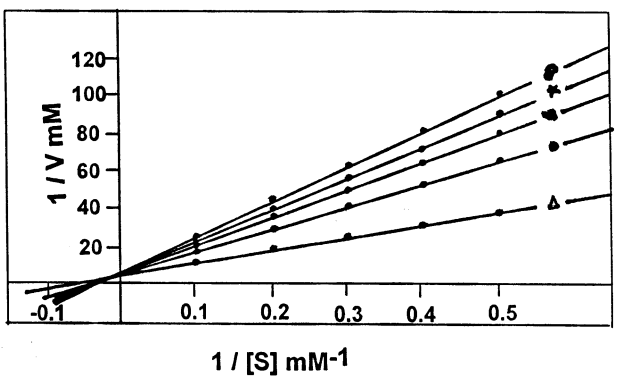


Fig. 3. Lineweaver–Burk plots in the absence (△) and presence of **16**.

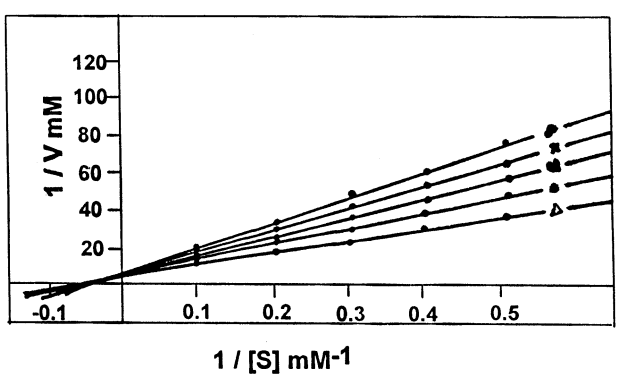


Fig. 2. Lineweaver–Burk plots in the absence (△) and presence of **9**.

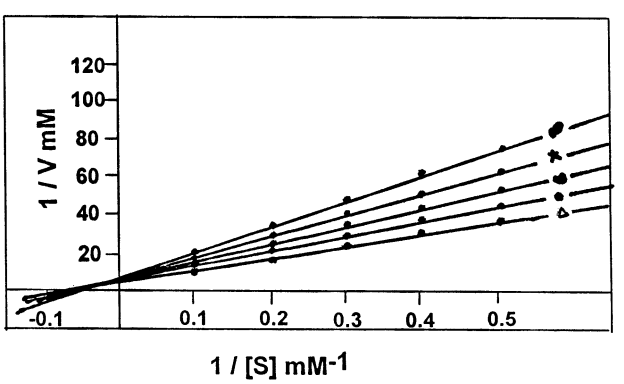


Fig. 4. Lineweaver–Burk plots in the absence (△) and presence of **24**.

3. Experimental

General methods.—Optical rotations were measured at 20 °C with a Roussel–Jouan electronic digital micropolarimeter. All reactions were monitored by TLC on Silica Gel 60 F_{254} (E. Merck) with detection by charring with sulfuric acid. Silica Gel 60 (E. Merck, 70–230 mesh) was used for flash column chromatography (FC). ^{1}H NMR spectra (250 and 600 MHz) were recorded with a Bruker spectrometer using Me_4Si as the internal standard. ^{13}C NMR spectra were recorded with a Brucker (250 and 600 MHz) instruments at (62.9 and 150.9 MHz). Mass spectra were recorded using electron-ionization (EI) on a Varian MAT 311A spectrometer and fast-atom-bombardment (FAB) on a Kratos MS 50 spectrometer. Microanalyses were performed at the Fakultät für Chemie, Universität Konstanz, Germany.

2-C-(2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl)ethanal (**7**).—A solution of **6** (0.37 g, 1.00 mmol) in absolute CH_2Cl_2 (25.0 mL) was cooled to −80 °C, and then ozone was passed in. After the blue color of ozone (∼10–15 min) appeared, argon was bubbled through the solution at −50 °C in order to remove excess ozone. The temperature was allowed to reach room temperature (rt), an excess of methylsulfide (20.0 mL) was added, and the stirring was continued for two days to cleave the ozonized product (TLC). The solvent was removed in vacuo, and the residue was purified by FC using 1:1 light petroleum–EtOAc to give **7** as a foam (0.28 g, 77.0%): Lit. [20] mp 71.5–72.5 °C; R_f 0.85 (9:1 light petroleum–EtOAc); $[\alpha]_D^{20}$ +13.1° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.04 (s, 3 H, OAc), 2.05 (s, 6 H, 2 OAc), 2.09 (s, 3 H, OAc), 2.79–2.84 (m, 2 H, H-2), 3.86–3.91 (m, 1 H, H-5′), 4.07 (dd, 1 H, $J_{5',6'a}$ 2.7, J_{gem} 12.2 Hz, H-6′a), 4.27 (dd, 1 H, $J_{5',6'b}$ 5.5, J_{gem} 12.2 Hz, H-6′b), 4.85 (q, 1 H, $J_{1,1'}$ 7.4 Hz, H-1′), 4.98 (t, 1 H, $J_{4',5'}$ 8.3 Hz, H-4′), 5.13 (dd, 1 H, $J_{1',2'}$ 5.4, $J_{2',3'}$ 8.8 Hz, H-2′), 5.26 (t, 1 H, $J_{3',4'}$ 8.3 Hz, H-3′), 9.73 (t, 1 H, $J_{1,2}$ 2.2 Hz, CHO); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 20.60 (2 OAc), 20.66 (2 OAc), 41.67 (C-2), 61.80 (C-6′), 67.37 (C-5′), 68.16 (C-4′), 69.27 (C-2′), 69.88 (C-3′), 70.27 (C-1′), 169.43 (2 OAc), 169.91 (OAc), 170.62 (OAc), 197.86 (CHO); EIMS: *m/z* (%): 331 [M-Ac, 2], 317 (3), 270 (10), 229(42). Anal. Calcd for $C_{16}H_{22}O_{10}$: C, 51.33; H, 5.92. Found: C, 50.80; H, 5.68.

1-Anilino-2-C-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)ethane (**8**).—Aniline (0.23 g, 2.47 mmol) was dissolved in MeOH (2.00 mL) under argon, and a small amount of 3Å molecular sieves was added. The pH was adjusted by addition of HCl/MeOH to pH 6. Compound **7** (0.16 g, 0.43 mmol) was dissolved in MeOH (5.00 mL) and then added to the reaction mixture by syringe. Sodium cyanoborohydride (0.17 g, 2.71 mmol) was added, whereby a white milky suspension was obtained. The reaction mixture was stirred for 20 h at rt (TLC), then filtered through Celite and washed well with MeOH. The solvent was removed in vacuo (30 °C), and the residue was purified by FC using 1:1 light petroleum–EtOAc to give **8** as a syrup (0.12 g, 61.0%); R_f 0.76 (9:1 light petroleum–EtOAc); $[\alpha]_D^{20}$ +25.2° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.67 (brs, 1 H, NH), 1.87–1.20 (m, 2 H, CH_2), 2.02 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.04 (s, 3 H, OAc), 2.08 (s, 3 H, OAc), 3.24–3.31 (m, 2 H, CH_2), 3.89–3.96 (m, 1 H, H-5′), 4.09 (dd, 1 H, $J_{5',6'a}$ 2.5, $J_{6'a,6'b}$ 12.1 Hz, H-6′a), 4.29 (dd, 1 H, $J_{5',6'b}$ 5.8, $J_{6'a,6'b}$ 12.2 Hz, H-6′b), 4.34–4.38 (m, 1 H, H-1′), 4.97 (t, 1 H, $J_{4',5'}$ 8.4 Hz, H-4′), 5.07 (dd, 1 H, $J_{1',2'}$ 5.4, $J_{2',3'}$ 9.0 Hz, H-2′), 5.28 (t, 1 H, $J_{3',4'}$ 8.5 Hz, H-3′), 6.59 (d, 2 H, *J* 9.3 Hz, Ar-H), 6.71 (t, 1 H, *J* 7.3 Hz, Ar-H), 7.18 (t, 2 H, *J* 7.3 Hz, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 20.64 (4 OAc), 25.23 (C-2), 40.26 (C-1), 62.22 (C-6′), 68.59 (C-5′), 69.47 (C-4′), 70.00 (C-2′), 70.10 (C-3′), 70.95 (C-1′), 112.78, 117.62, 129.34, 144.97 (Ar-C), 169.56 (2 OAc), 170.65 (OAc), 171.69 (OAc); EIMS: *m/z* (%): 451 (M, 38), 392 (3), 332 (2). Anal. Calcd for $C_{22}H_{29}NO_9$: C, 58.52; H, 6.47; N, 3.10. Found: C, 58.28; H, 6.14; N, 2.91.

1-Anilino-2-C-(α-D-glucopyranosyl)ethane (**9**).—Compound **8** (0.10 g, 0.22 mmol) was dissolved in MeOH (5.00 mL), and then a saturated solution of MeOH with ammonia (5.00 mL) was added at 0 °C. Stirring was continued for 1 h at rt (TLC), and the solvent was removed in vacuo (40 °C). The residue was purified by FC using 8:2 CH_2Cl_2–MeOH to give **9** (0.05 g, 90.0%) as a syrup; R_f 0.25

(8:2 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ +28.5° (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, DMSO-d_6): δ 1.77–1.81 (m, 2 H, CH_2), 2.98–3.03 (m, 2 H, CH_2), 3.09–3.11 (brs, 1 H, OH), 3.28–3.32 (m, 5 H, 3 OH, H_2O), 3.37–3.38 (m, 1 H, H-5′), 3.64–3.66 (m, 1 H, H-6′a), 3.84–3.87 (m, 1 H, H-6′b), 4.53 (brs, 1 H, H-1′), 4.83–4.84 (m, 2 H, H-2′, 4′), 4.94 (brs, 1 H, H-3′), 5.50 (brs, 1 H, NH), 6.48 (t, 1 H, *J* 7.2 Hz, Ar-H), 6.55 (d, 2 H, *J* 7.8 Hz, Ar-H), 7.04 (t, 2 H, *J* 7.8 Hz, Ar-H); ^{13}C NMR (150.9 MHz, DMSO-d_6): δ 23.82 (C-2), 40.02 (C-1), 61.62 (C-6′), 70.89 (C-5′), 71.22 (C-4′), 73.79 (C-2′, 3′), 73.82 (C-1′), 111.93, 115.31, 128.81, 141.53 (Ar-C); FABMS: *m*/*z* (%): 284 (MH, 20). Anal. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.94. Found: C, 59.02; H, 7.13; N, 4.60.

1-C-(α-D-*Arabinofuranosyl)methanal (2,5-anhydro*-D-*mannose) oxime* (**12**).—Hydroxylamine hydrochloride (2.50 g, 36 mmol) and anhydrous sodium acetate (2.84 g, 15.2 mmol) were dissolved in absolute EtOH (50.0 mL) with stirring for 1 h. The mixture was filtered, and the filtrate was added to 2,5-anhydro-D-mannose (**11**) (2.50 g, 39.0 mmol), and the stirring was continued for 1.5 h (TLC). The solvent was removed in vacuo (30 °C), and the residue was purified by FC using 5:1 EtOAc–MeOH to give a pale yellowish green syrup (2.11 g, 77.0%) as a mixture of E and Z isomers. R_f 0.45 (1:1 EtOAc–MeOH); $[\alpha]_D^{20}$ −1.5° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, DMSO-d_6): δ 1.91–4.15 (4 m, 8 H, 3 OH, furanosyl-H); major isomer: δ 7.28 (d, 0.7 H, *J* 7.8 Hz, C*H*=N), 10.83 (s, 0.7 H, =N ∼ O*H*); minor isomer: δ 6.67 (d, 0.3 H, *J* 7.8 Hz, C*H*=N), 10.91 (brs, 0.3 H, =N ∼ O*H*); ^{13}C NMR (62.9 MHz, DMSO-d_6): major isomer: δ 61.78 (C-5), 77.13 (C-3), 79.88 (C-2), 80.94 (C-4), 84.73 (C-1), 149.23 (*C*H=N); minor isomer: δ 62.04 (C-5), 77.65 (C-3), 79.08 (C-2), 80.40 (C-4), 86.84 (C-1), 151.27 (*C*H=N); EIMS: *m*/*z* (%): 177 (M, 6), 159 (55), 146 (28).

Acetylation of 1-C-(α-D-*arabinofuranosyl)methanal oxime*.—The oxime derivative **12** (0.35 g, 1.97 mmol) was dissolved in pyridine (3.00 mL) and then cooled in an ice-bath. Acetic anhydride (1.53 mL) was added dropwise with stirring at −10 °C, and the mixture was then kept at −5 °C for 24 h. It was poured onto crushed ice and then extracted (3 × 100 mL) with CH_2Cl_2. The solvent was removed in vacuo (25 °C) to leave a syrup whose TLC showed two spots. Separation was carried out by FC using 5:1 toluene–EtOAc to give **13** (0.197 g, 35%) and **14** (0.410 g, 60%).

1-C-*(2,3,5-Tri*-O-*acetyl*-α-D-*arabinofuranosyl)carbonitrile* (**13**).—An oil; R_f 0.59 (1:1 EtOAc–toluene); $[\alpha]_D^{20}$ +30.5° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 2.11 (s, 3 H, OAc), 2.15 (s, 3 H, OAc), 2.16 (s, 3 H, OAc), 4.24 (dd, 2 H, $J_{4,5'}$ 7.2 Hz, $J_{5,5'}$ 12.8 Hz, H-5′), 4.38 (m, 2 H, H-4, 5), 4.85 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 5.13 (t, 1 H, $J_{2,3}$ 2.3 Hz, H-2), 5.36 (t, 1 H, $J_{3,4}$ 1.3 Hz, H-3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 20.20 (OAc), 20.34 (2 OAc), 62.15 (C-5), 70.72 (C-3), 76.64 (C-2), 79.55 (C-4), 82.77 (C-1), 114.95 (CN), 169.02, 169.42, 170.08 (3 OAc); EIMS: *m*/*z* (%): 285 (M, 3), 243 (6), 225 (9), 45 (100). Anal. Calcd for $C_{12}H_{15}NO_7$: C, 50.52; H, 5.30; N, 4.91. Found: C, 50.22; H, 5.18; N, 4.77.

1-C-*(2,3,5-Tri*-O-*acetyl*-α-D-*arabinofuranosyl)methanal oxime acetate* (**14**).—An oil; R_f 0.48 (1:1 EtOAc–toluene); $[\alpha]_D^{20}$ +4.3° (*c* 1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$): δ 2.11 (s, 6 H, 2 OAc), 2.13 (s, 3 H, OAc), 2.18 (s, 3 H, OAc), 4.24–4.33 (m, 3 H, H-4, 5, 5′), 4.80 (dd, 1 H, $J_{1,2}$ 0.6, $J_{1,1'}$ 6.5 Hz, H-1), 5.21 (t, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 5.37 (dd, 1 H, $J_{2,3}$ 2.7, $J_{3,4}$ 3.8 Hz, H-3), 7.75 (d, 1 H, $J_{1,1'}$ 6.4 Hz, C*H*=N); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 19.00, 20.34, 20.37, 20.43 (4 OAc), 62.90 (C-5), 77.62 (C-3), 78.61 (C-2), 79.48 (C-4), 81.41 (C-1), 154.26 (*C*H=N), 167.72, 169.33, 169.46, 170.19 (4 OAc); EIMS: *m*/*z* (%): 345 (M, 35), 303 (47), 243 (42), 230 (9). Anal. Calcd for $C_{14}H_{19}NO_9$: C, 48.69; H, 5.54; N, 4.05. Found: C, 48.41; H, 5.33; N, 3.76.

1-C-*(2,3,5-Tri*-O-*acetyl*-α-D-*arabinofuranosyl)methylamine* (**15**).—Compound **14** (0.33 g, 0.96 mmol) was dissolved in 1:1 dioxane–MeOH (26.0 mL), and 8–10 drops of water were added. The stirred mixture was treated with $Pd(OH)_2/C$ (35.0%, 0.12 g) and then kept under hydrogen with continued stirring at rt for 4 h (TLC). The mixture was diluted with MeOH (100 mL), filtered through Celite, and washed well with MeOH. [**Caution:** $Pd(OH)_2/C$ mixtures with MeOH are extremely pyrophoric.] The solvent was removed under reduced pressure (35 °C) to give a red-

dish–brown gum of **15** (0.25 g, 90.0%); R_f 0.37 (1:1 EtOAc–toluene); $[\alpha]_D^{20}$ +5.4° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 2.10 (s, 9 H, 3 OAc), 2.89 (m, 2 H, CH_2), 4.18–4.24 (m, 4 H, H-1, 4, 5), 5.14 (brs, 2 H, H-2, 3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 20.62 (3 OAc), 50.42 (CH_2), 63.03 (C-5), 78.43 (C-3), 78.99 (C-2), 80.38 (C-4), 82.25 (C-1), 169.86 (2 OAc), 170.48 (OAc). Anal. Calcd for $C_{12}H_{19}NO_7$: C, 49.82; H, 6.61; N, 4.84. Found: C, 49.61; H, 6.49; N, 4.70.

1-C-*(α*-D-*Arabinofuranosyl)methylamine* (**16**). —Compound **15** (0.29 g, 1.00 mmol) was dissolved in MeOH (30.0 mL). MeOH saturated with ammonia (10.0 mL) was added at 0 °C to the reaction mixture, and the stirring was continued for 30 min at rt (TLC). The solvent was removed in vacuo (40 °C), and the residue was purified by FC using 9:1 CH_2Cl_2–MeOH to give **16** as a syrup (0.154 g, 95.0%); R_f 0.20 (7:3 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ +7.1° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.69 (m, 2 H, CH_2), 3.09–5.41 (m, furanosyl-H); ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 49.67 (CH_2), 61.50 (C-5), 76.94 (C-3), 78.99 (C-2), 79.50 (C-4), 84.95 (C-1); FABMS: *m/z:* 164 ($MH^{\bullet+}$).

Ethyl 2,3:5,6-di-O-*isopropylidene*-α-D-*mannofuranoside* (**18**)

Method A. To a solution of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (**17**) (10.4 g, 40.0 mmol) in DMF (100 mL), ethyl iodide (6.24 g, 40 mmol) and silver(I) oxide (10.0 g, 43.0 mmol) were added. The mixture was stirred for 24 h at rt (TLC). The silver salt was filtered and washed with a small amount of $CHCl_3$, and the solvent was removed under reduced pressure (30 °C). The residue was purified by FC using 5:1 light petroleum–EtOAc to give **18** as a syrup (8.80 g, 76.0%) that was identical to the product from method B.

Method B. A solution of **17** (10.4 g, 40.0 mmol) was prepared in dry DMF (100 mL). NaH (1.03 g, 43.0 mmol) was added, and the mixture was stirred for 10 min until the evolution of hydrogen was ceased. Ethyl iodide (6.24 g, 40.0 mmol) was added, and the mixture was stirred at rt for 24 h (TLC). The salt was removed by filtration, and the solvent was evaporated under reduced pressure (30 °C). The residue was purified as described in method A to give **18** (7.30 g, 63.0%); R_f 0.72 (7:3 light petroleum–EtOAc); $[\alpha]_D^{20}$ +38.3° (*c* 1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.19 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3) 1.32 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 1.46 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 3.41–3.51 (m, 1 H, OCH_2CH_3), 3.62–3.75 (m, 1 H, OCH_2CH_3), 3.93 (dd, 1 H, $J_{4,5}$ 3.5, $J_{3,4}$ 7.6 Hz, H-4), 4.01–4.14 (m, 2 H, H-6, 6′), 4.37–4.44 (m, 1 H, H-5), 4.57 (d, 1 H, $J_{2,3}$ 5.9 Hz, H-2), 4.78 (dd, 1 H, $J_{3,4}$ 3.6, $J_{2,3}$ 5.8 Hz, H-3), 4.99 (s, 1 H, H-1); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 14.89 (OCH_2CH_3), 24.47, 25.13, 25.84, 26.84 (4 CH_3), 62.76 (OCH_2CH_3), 66.89 (C-6), 73.15 (C-5), 79.52 (C-3), 80.16 (C-2), 85.11 (C-4), 106.03 (C-1), 109.12 (CMe_2), 112.46 (CMe_2); EIMS: *m/z* (%): 273 (M-Me, 48), 185 (7), 141 (22). Anal. Calcd for $C_{14}H_{24}O_6$: C, 58.31; H, 8.38. Found: C, 58.35; H, 8.13.

Ethyl 2,3-O-*isopropylidene*-α-D-*mannofuranoside* (**19**).—A solution of **18** (2.92 g, 10.1 mmol) in EtOH (40.0 mL) was treated with 0.2 N HCl (40.0 mL). The reaction mixture was stirred at rt for 6 h (TLC). An excess of basic ion-exchange resin (HCO_3^--form) was added, and the mixture was stirred for 30 min and then filtered. The solvent was removed in vacuo (40 °C), and the product was dried over $CaSO_4$ to give **19** as an oil (2.40 g, 96.0%); R_f 0.50 (7:3 light petroleum–EtOAc); $[\alpha]_D^{20}$ +28.8° (*c* 5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.18 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3) 1.33 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 3.34 (brs, 1 H, OH, D_2O exchangeable), 3.41–3.48 (m, 1 H, OCH_2CH_3), 3.63–3.74 (m, 3 H, OCH_2CH_3, H-4, 6′), 3.83–4.00 (m, 3 H, OH, H-5, 6), 4.57 (d, 1 H, $J_{2,3}$ 5.8 Hz, H-2), 4.83–4.87 (m, 1 H, H-3), 5.00 (s, 1 H, H-1); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.79 (OCH_2CH_3), 24.55, 25.80 (2 CH_3), 62.62 (OCH_2CH_3), 64.30 (C-6), 69.94 (C-5), 79.03 (C-3), 79.90 (C-2), 84.70 (C-4), 105.72 (C-1), 112.38 (CMe_2); EIMS: *m/z* (%): 233 (M-Me, 54), 217 (10), 187 (16), 171 (9). Anal. Calcd for $C_{11}H_{20}O_6$: C, 53.21; H, 8.11. Found: C, 52.98; H, 8.13.

Ethyl 2,3-O-*isopropylidene*-α-D-lyxo-*pentodialdo-1,4-furanoside* (**20**)

Method A. A solution of **19** (1.24 g, 5.00 mmol) in water (20 mL) was treated with a solution of (0.13 g, 5.25 mmol) sodium meta-

periodate in water (21.0 mL). The reaction mixture was stirred for 30 min at 10–12 °C. The solution was passed through a column of Amberlite MB-3, and the filtrate was concentrated under reduced pressure (45 °C) to give **20**, which was purified by FC using 6:1 light petroleum–EtOAc to give an oil (0.76 g, (71.0%).

Method B. Compound **18** (0.288 g, 1.00 mmol) was added at rt under nitrogen atmosphere to a well-stirred suspension of periodic acid (0.57 g, 3.00 mmol) in dry ether. Stirring was continued for 12 h at rt (TLC), and the reaction mixture was worked up by filtering and then evaporating the filtrate. Further purification was carried out as described in method A to give (0.19 g, 88.0%); R_f 0.46 (7:3 light petroleum–EtOAc); $[\alpha]_D^{20}$ + 34.2° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.19 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3) 1.28 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 3.49–3.57 (m, 1 H, OCH_2CH_3), 3.67–3.76 (m, 1 H, OCH_2CH_3), 4.40 (d, 1 H, $J_{3,4}$ 4.1 Hz, H-4), 4.62 (d, 1 H, $J_{2,3}$ 5.8 Hz, H-2), 5.09 (m, 1 H, H-3), 5.20 (s, 1 H, H-1), 9.64 (d, 1 H, $J_{5,4}$ 1.1 Hz, H-5); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.72 (OCH_2CH_3), 24.40, 25.69 (2 CH_3), 63.07 (OCH_2CH_3), 80.77 (C-4), 83.82 (C-3), 84.54 (C-2), 106.35 (C-1), 113.24 (CMe_2), 197.71 (C-5); EIMS: *m*/*z* (%): 217 (M + 1, 3), 201 (66), 187 (56). Anal. Calcd for $C_{10}H_{16}O_5$: C, 55.54; H, 7.45. Found: C, 55.39; H, 7.44.

Reductive amination of the aldehydo sugar derivatives

General procedure. Aniline and/or ethanolamine (4.17 mmol) was dissolved in MeOH (2.00 mL) under argon. A very small amount of 3Å molecular sieve was then added. The pH was adjusted by addition of HCl/MeOH to pH 6. Compound **20** (0.14 g, 0.62 mmol) was dissolved in MeOH (3.00 mL) and then added to the reaction mixture by syringe. After the addition of sodium cyanoborohydride (0.25 g, 3.79 mmol), a white milky suspension was obtained. The reaction mixture was stirred for 2 h (TLC) at rt, and then filtered through Celite. The solvent was removed in vacuo, and the residue was chromatographed by FC using 50:49:1 light petroleum–EtOAc–Et_3N to give **22** and **23** as syrups.

Ethyl 2,3-O-isopropylidene-α-D-lyxo-furanoside (**22**). Yield (0.09 g, 66.0%); R_f 0.43 (7:3 light petroleum–EtOAc); $[\alpha]_D^{20}$ + 36.5° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.18 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3) 1.31 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 2.28 (brs, 1 H, OH), 3.41–3.53 (m, 1 H, OCH_2CH_3), 3.66–3.78 (m, 1 H, OCH_2CH_3), 3.91–4.08 (m, 2 H, H-5), 4.10–4.20 (m, 1 H, H-4), 4.59 (d, 1 H, $J_{2,3}$ 5.9 Hz, H-2), 4.80 (dd, 1 H, $J_{3,4}$ 3.7, $J_{2,3}$ 5.9 Hz, H-3), 5.06 (s, 1 H, H-1); EIMS: *m*/*z* (%): 203 (MMe, 13), 187 (2), 173 (6). Anal. Calcd for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 54.75; H, 8.22.

Ethyl 5-anilino-5-deoxy-2,3-O-isopropylidene-α-D-lyxo-furanoside (**23**). Yield (0.11 g, 60%); R_f 0.53 (7:3 light petroleum–EtOAc); $[\alpha]_D^{20}$ + 33.7° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.17 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3) 1.32 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 3.34–3.50 (m, 3 H, OCH_2CH_3, H-5, 5′), 3.63–3.73 (m, 1 H, OCH_2CH_3), 4.00 (brs, 1 H, NH), 4.17–4.23 (m, 1 H, H-4), 4.59 (d, 1 H, $J_{2,3}$ 5.9 Hz, H-2), 4.73 (dd, 1 H, $J_{3,4}$ 3.7, $J_{2,3}$ 5.9 Hz, H-3), 5.03 (s, 1 H, H-1), 6.62–6.74 (m, 3 H, Ar-H), 7.17 (t, 2 H, *J* 7.4 Hz, Ar-H); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 14.95 (OCH_2CH_3), 24.82, 26.05 (2 CH_3), 42.91 (C-5), 62.81 (OCH_2CH_3), 77.83 (C-4), 79.92 (C-3), 85.26 (C-2), 105.76 (C-1), 112.56 (CMe_2), 113.12, 117.66, 129.22, 148.09 (Ar-C); EIMS: *m*/*z* (%): 293 (M, 23), 278 (2), 248 (3), 219 (4). Anal. Calcd for $C_{16}H_{23}NO_4$: C, 65.50; H, 7.90; N, 4.77. Found: C, 65.43; H, 7.81; N, 4.63.

Ethyl 5-anilino-5-deoxy-α-D-lyxo-furanoside (**24**). Compound **23** (0.08 g, 0.27 mmol) was treated with aqueous AcOH (2.00 mL, 70.0%) and then heated under reflux for 2 h (TLC). The solution was concentrated under reduced pressure (45 °C) to give a syrup that was chromatographed on a silica gel column using 5:94:1 light petroleum–EtOAc–Et_3N to give a brown gum (0.05 g, 72.0%); R_f 0.25 (6:4 EtOAc–MeOH); $[\alpha]_D^{20}$ + 39.5° (*c* 1, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.21 (t, 3 H, *J* 7.0 Hz, OCH_2CH_3), 3.39–3.56 (m, 3 H, OCH_2CH_3, H-5, 5′), 3.64–3.77 (m, 1 H, OCH_2CH_3), 4.17–4.56 (3m, 3 H, H-2, 3, 4), 5.01 (s, 1 H, H-1), 6.81 (m, 3 H, Ar-H), 7.25 (m, 2 H, Ar-H); EIMS: *m*/*z* (%): 253 ($M^{\bullet+}$, 11), 238 (5), 206 (2). Anal. Calcd for

$C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.52. Found: C, 61.51; H, 7.43; N, 5.40.

Inhibition studies.—The inhibitory activity of compounds **9**, **16** and **24** on the hydrolysis of *o*-nitrophenyl β-D-glucopyranoside (ONPG) was determined.

Materials.—Buffers (potassium dihydrogenphosphate and disodium hydrogenphosphate) were purchased from Fluka Chemical Co. and used as received. β-D-glucosidase (sweet almond) and *o*-nitrophenyl β-D-glucopyranoside were obtained from Boehringer Mannheim.

Preparation of solutions

A. The buffer solution. Potassium dihydrogenphosphate (0.07 M) (A) (9.073 g) was dissolved in distilled water, and dilution was completed to 1000 mL at 20 °C. Disodium hydrogenphosphate solution (0.07 M) (B) (11.866 g) was dissolved in distilled water, and dilution was completed to 1000 mL at 20 °C. Then 50 mL of A was mixed with 50 mL of B.

B. Enzyme solution. β-D-Glucosidase (1.0 mg) was dissolved in the buffer solution (5.0 mL) pH 6.8 and used for assay without further dilution.

C. The substrate initial solution. ONPG (82.25 mg) was dissolved in the buffer solution (6.5 mL, 42 mM), for enzyme assay.

D. The inhibitor initial solution. Compound **9**, **16** or **24** (16 mg) was dissolved in the buffer solution (50 mL, 1.799 mM). Inhibitor concentrations of 0.856, 0.571, 0.285 and 0.142 mM were used to determine the K_i value. At each inhibitor concentration, six substrate concentrations 20.00, 10.00, 5.00, 3.33, 2.50 and 2.00 mM were used.

Procedure for enzyme assays.—To a 1.0 mL disposable cuvette was added buffer solution (500 μL) and ONPG solution (500 μL). The solution was thermally equilibrated at 30 °C. The reaction was started by addition of 50 μL of β-D-glucosidase solution. Liberation of ONPG [30] was monitored using a PU 8740 UV–Vis spectrophotometer, for 4.0 min (λ = 405 nm), and the initial hydrolysis rate was calculated.

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