Synthesis and Biological Activity of Acyclic Analogues of Nojirimycin

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A series of acyclic compounds has been prepared which comprises compounds that mimic key structural elements of nojirimycin **1** and 1-deoxynojirimycin **2**, both of which are highly effective glucosidase inhibitors, in order to ascertain if similar biological activity can be obtained with simpler structures. All of the compounds are competitive inhibitors of yeast α-glucosidase, with varying degrees of effectiveness, but none of them, in contrast to 1-deoxynojirimycin **2**, show significant anti-HIV activity.

Nojirimycin **1** and 1-deoxynojirimycin **2** are highly effective glycosidase inhibitors,[1] and the anti-HIV activity shown by compound **2** and related compounds, such as the *N*-butyl derivative **3** and castanospermine **4**,[2] has provided the impetus for much of the recent research in this area. The anti-HIV activity of compound **3** (and possibly of castanospermine **4** also) appears to rest on its ability to inhibit key steps in glycoprotein processing,[2,3] but the involvement of glycosidases in other crucial biochemical transformations, such as the breakdown of dietary carbohydrates,[4] means that their inhibition offers new opportunities for chemotherapy. In view of the success of acyclovir **5**, which can be viewed as a truncated acyclic analogue of guanosine, as an anti-viral agent,[5] we were intrigued by the possibility that at least some of the important biological activity of compounds **1** and **2** might be retained in simpler acyclic compounds which mimic smaller structural sub-units in the parent cyclic systems. We describe here the synthesis of such compounds, their inhibitory activity towards yeast α-glucosidase, and their anti-HIV activity in infected cell cultures.

2-[(2′-Hydroxyethyl)amino]propane-1,3-diol **9**† was prepared as its hydrochloride by reaction of commercially available serinol **6** with 1-bromo-2-trityloxyethane[6] **7** to give, first the ether **8**, which was then subjected to de-*O*-tritylation through treatment with methanol containing a molar equivalent of hydrochloric acid. Alkylating agent **7** was readily made by reaction of 2-bromoethanol with trityl chloride. A similar sequence of reactions on amine **6** with (*S*)-glycidyl trityl ether[7] **10**, prepared by tritylation of (*R*)-glycidol with trityl chloride, afforded, first, (2′*S*)-2-[(2′-hydroxy-3′-trityloxypropyl)amino]-propane-1,3-diol **11**, and then (2′*S*)-2-[2′,3′-dihydroxypropyl)-amino]propane-1,3-diol **12**, as its hydrochloride.

2-Amino-2-deoxy-L-erythritol **15** has been prepared[8] by hydrogenation of the γ-lactone of (2*S*,3*R*)-2-amino-3,4-dihydroxybutanoic acid. We have synthesized compound **15**, as its hydrochloride, by ammonolysis of 2,3-anhydro-1,4-di-*O*-benzyl-L-threitol[9] **13** to give, first, 2-amino-1,4-di-*O*-benzyl-2-deoxy-L-erythritol **14**, which was then subjected to catalytic hydrogenolysis in the presence of hydrogen chloride.

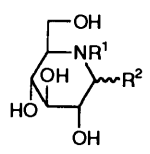
5-Amino-5-deoxy-D-glucitol **21** is the direct acyclic analogue of nojirimycin, and 2-amino-2-deoxy-L-iditol **22** is related to the recently reported[10] 1-deoxy-L-idonojirimycin, a C-5 epimer of 1-deoxynojirimycin which is a non-competitive rather than a competitive inhibitor of yeast α-glucosidase and which shows no anti-HIV activity.[11] These compounds were prepared from the known[12] 2,3,4,6-tetra-*O*-benzyl-1-*O*-trityl-D-glucitol **16**. Oxidation of compound **16** with nicotinium dichromate[13,14] gave the known[12] ketone **17**, which was converted into the corresponding oxime **18**, reduction of which afforded the epimeric mixture of amines **19** and **20**. After chromatographic separation of these epimers, they were individually deprotected in the presence of hydrochloric acid to give amino pentaols **21** and **22** as their hydrochlorides. Structural proof of this pair depended on the unequivocal synthesis of the *ido*-isomer **20** by a Mitsunobu reaction[15] on compound **16** with phthalimide, triphenylphosphine, and diethyl diazodicarboxylate (DEAD) to give compound **23**, N-protection‡ of the product **23**, and comparison of this product with the pair of compounds **19** and **20** obtained by the reduction of oxime **18**.

Three further compounds included in this study were 2-amino-2-(hydroxymethyl)propane-1,3-diol (TRIS) **25**, 2-[(2′-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol **27** and (2′*S*)-2-[(2′,3′-dihydroxypropyl)amino]-2-(hydroxymethyl)propane-1,3-diol **29**, the latter two compounds being prepared from TRIS **25** *via* compounds **26** and **28**, respectively, by similar methods to those used to prepare homologues **9** and **12** from serinol **6**. Compared with compounds **6**, **9** and **12**, respectively, these compounds possess an extra hydroxymethyl group at C-2 and a direct analogy to the natural products **1** and **2** is thereby destroyed. However, the long known[18–20] inhibitory properties of the biological buffer TRIS towards glycosidases, and the common structural elements in the two series of compounds was, we felt, sufficient reason for including these compounds in the investigation.

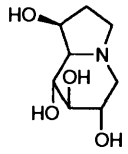
Biological Activity.—The amino alcohols **6**, **9**, **12**, **15**, **21**, **22**, **25**, **27** and **29** were tested for their inhibitory properties towards yeast α-glucosidase at pH 6.5, using standard Lineweaver–Burk analysis and 4-nitrophenyl α-D-glucopyranoside as the enzyme substrate. The solutions for kinetic studies were prepared by the sequential addition to the optical cell, over a period of less than 30 s, of solutions of buffer, inhibitor, enzyme, and substrate; measurement of the rate of liberation of 4-nitrophenol was commenced as soon as possible (within a few seconds) by UV spectroscopy. Measurement of the initial rate of liberation of the phenol in the presence of the acyclic inhibitors revealed, for all compounds except **6** and **25**, the slow onset of a tighter binding phase, the approach to steady-state inhibition taking place, typically, over a time scale of *ca.* 200 s at the highest

† The structural representations of the acyclic analogues **9**, **12**, **15**, **21**, **22**, **25**, **27** and **29** are drawn to best represent structural analogy with nojirimycin **1** and its derivatives.

‡ Using butylamine in an alcohol,[16] in this case butan-1-ol, we succeeded only in forming the mixed diamide **24** which was then N-deprotected with ethylenediamine according to the procedure of Hindsgaul and co-workers.[17]



1 R^1 = H, R^2 = OH
2 R^1 = R^2 = H
3 R^1 = Bu, R^2 = H



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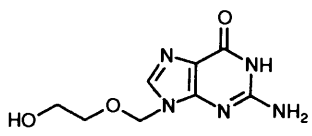

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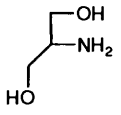

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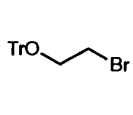

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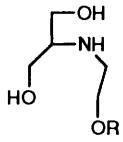

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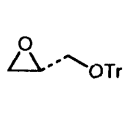
9 R = H



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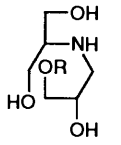
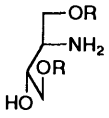


11 R = Tr
12 R = H

13



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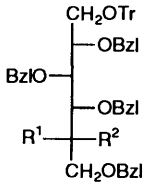
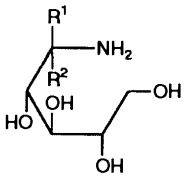
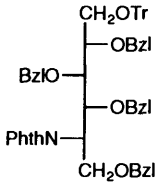
15 R = H



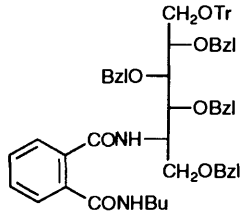




16 R^1 = H, R^2 = OH
17 R^1, R^2 = O
18 R^1, R^2 = NOH
19 R^1 = H, R^2 = NH_2
20 R^1 = NH_2, R^2 = H

21 R^1 = CH_2OH, R^2 = H
22 R^1 = H, R^2 = CH_2OH

23



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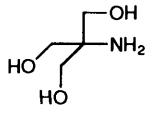

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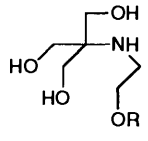
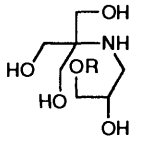


26 R = Tr
27 R = H



28 R = Tr
29 R = H

Tr = Ph_3C, Bzl = $PhCH_2$, Phth =

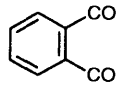


Table 1 Inhibition constants (K_i* or K_i) for the inhibition of yeast α-glucosidase by acyclic analogues of nojirimycin

Compound	K_i* (or K_i)[a]	Compound	K_i* (or K_i)[a]
6	(702)	**22**	216
9	4	**25**	(379)
12	10	**27**	3556
15	82	**29**	652
21	3.6		

[a] In μmol dm^{-3}.

inhibitor concentration used in our experiments. In these cases, therefore, measurements of reaction velocity were made over the linear portion of the plot in the final steady state,† but still at substrate conversions of less than 10% to ensure compliance with requirements for the Lineweaver–Burk analysis, to yield overall inhibition constants, K_i* (see below). Values of the inhibition constants are recorded in Table 1.

The slow onset of enzyme inhibition has been noted previously with several types of enzymes,[21] including glycosidases,[1] *N*-glycohydrolases,[22] and proteases.[23] This phenomenon has been rationalised[21] in terms of the slow attainment of the equilibrium between an enzyme (E), an inhibitor (I), and the corresponding enzyme inhibitor complex (E·I) and a distinction has been drawn between slow-binding and slow, tight-binding on the basis of [I] ≫ [E] or [I] ≈ [E], respectively.

More detailed mechanisms to describe slow-binding and slow, tight-binding have been proposed[21] and the favoured one involves a slow step in which an initial enzyme inhibitor complex E·I is converted into another complex E·I* [eqn. (1)] in which the inhibitor is more tightly bound.

$$E + I \rightleftharpoons E{\cdot}I \overset{slow}{\rightleftharpoons} E{\cdot}I^* \qquad (1)$$

In cases of slow-binding enzyme inhibition, a K_i-value obtained by measurement of the variation with inhibitor concentration of the final steady-state velocity represents[21] an overall inhibition constant K_i*, given by eqn. (2).

$$K_i^* = [E]\,[I]/\{[E{\cdot}I] + [E{\cdot}I^*]\} \qquad (2)$$

All of the new compounds proved to be competitive inhibitors of the enzyme, as is 1-deoxynojirimycin **2**, for which we had previously measured[10] an inhibition constant, K_i, of 14.6 μmol dm^{-3} (in close agreement with other workers)[24] and in constrast to 1-deoxy-L-idonojirimycin, which we found[10] to be a non-competitive inhibitor. However, their effectiveness as inhibitors differed markedly. Perhaps not surprisingly, in the serinol series, serinol **6**, which possesses the smallest common structural unit found in compound **2**, was the least effective with a K_i-value of 702 μmol dm^{-3}. However, addition of an *N*-(2-hydroxyethyl) or an *N*-[(2*S*)-2,3-dihydroxypropyl] substituent increased dramatically the efficiency of binding, compounds **9** and **12** having K_i*-values of 4 and 10 μmol dm^{-3}, respectively, a similar order as that of compound **2**. 2-Amino-2-deoxy-L-

† That this linear portion of the graph indeed represented the final steady-state velocity was substantiated through separate experiments on triol **15**, which showed that pre-incubation of the enzyme with the inhibitor in buffer solution for 10 min afforded similar reaction velocities to those obtained under non-incubation conditions with corresponding inhibitor concentrations. Incubation of the enzyme with high concentrations of triol **15**, which led to virtual cessation of enzyme activity, did not cause irreversible inhibition since activity was restored on dialysis.

erythritol **15**, which mimics C-3 to C-6 and the ring nitrogen of 1-deoxynojirimycin **2**, is also surprisingly effective as an inhibitor with a K_i*-value of 82 μmol dm^{-3}. It is interesting that 5-amino-5-deoxy-D-glucitol **21**, an acyclic analogue of nojirimycin **1**, is a remarkably good inhibitor of the enzyme with a K_i*-value of 3.6 μmol dm^{-3}, whereas the *ido*-isomer **22**, although acting as a competitive inhibitor, is much less effective with a K_i*-value of 216 μmol dm^{-3}.

Although it is reasonable to expect that for the acyclic compounds entropy factors might militate against effective occupation of the enzyme active site when compared with the parent heterocycle, it is clear that when certain structural and stereochemical features are present they are able to bring about similar levels of inhibition to the cyclic compounds. It would seem possible, therefore, that the acyclic compounds are indeed occupying the same enzyme site as do the nojirimycins **1** and **2**. The observation of slow binding with all of the acyclic compounds except **6** and **25** could be accommodated possibly by a model in which the inhibitor must adopt a specific conformation, with analogous topography to that of the cyclic inhibitors, before binding is completed.† The classical behaviour shown by compounds **6** and **25** might reflect the restricted conformational freedom they possess compared with the compounds derived from them. The similar K_i*-values of compounds **9** and **12** lend support to a similarity between the enzyme-bound conformation of the acyclic analogues and the related structural unit in 1-deoxynojirimycin **2**. Thus, studies by Wong and co-workers,[24,25] which clarified important features in the structure of 1-deoxynojirimycin and related compounds for effective binding to glycosidases, have indicated the lesser importance of 3-HO compared with other factors.

The apparently greater effectiveness of 2-amino-2-(hydroxymethyl)propane-1,3-diol **25** (K_i 379 μmol dm^{-3}) over serinol **6** (K_i 702 μmol dm^{-3}) as an inhibitor could be rationalised in terms of the greater statistical likelihood that the required stereo-arrangement for binding, which presumably involves 4-HO, 6-HO, and N in compound **2**, is obtained in the case of TRIS **25** compared with serinol **6**, since it contains three rather than two hydroxymethyl groups. However, the poor binding ability of compounds **27** and **29** (K_i* 3556 μmol dm^{-3} and 652 μmol dm^{-3}, respectively) compared with TRIS **25**, **9** and **12**, makes such an argument difficult to sustain, unless the presence of the third hydroxymethyl group adversely affects, as regards binding ability, the conformation adopted by the 2-hydroxyethyl and 2,3-dihydroxypropyl substituents on the nitrogen atom.

Anti-HIV tests were carried out in HIV-1 IIIB-infected cell (C8166) cultures to determine EC_{50}- and TC_{50}-values, these parameters representing, respectively, the concentration of compound that reduces the antigenic glycoprotein gp 120 by 50% in infected cells and the concentration of compound which reduces normal cell growth by 50%. Results are conveniently expressed as TI (= TC_{50}/EC_{50})-values, and highly effective drugs have a high TI-value. Typically, 3′-azido-3′-deoxythymidine (AZT) gives a TI-value of > 50 000 in such tests and our sample of 1-deoxynojirimycin gave a TI-value > 50. Unfortunately, none of the acyclic compounds prepared in this study showed significant activity with TI-values ranging from 1 to 2.5.

† However, slow-binding has also been observed between cyclic compounds, such as nojirimycin and 1-deoxynojirimycin, and certain glycosidases.[1] The currently accepted explanation is that slow-binding inhibitors combine at the active site of enzymes and induce conformational changes that cause the enzymes to clamp down on the inhibitors, leading to a more stable inhibitor complex.[21] It is not clear whether a similar process is occurring with our acyclic analogues. Possible models for the molecular basis of slow inhibition in glycosidases have been discussed by Legler.[1]

Experimental

^1H NMR spectra were recorded at 60 MHz on a JEOL PMX60si spectrometer, at 270 MHz on a JEOL EX270 spectrometer, or at 400 MHz on a JEOL GX400 spectrometer in [^2H]chloroform (internal Me_4Si), unless indicated otherwise. *J*-Values are given in Hz. Rotations were measured with a Perkin-Elmer 141 polarimeter for chloroform solutions unless stated otherwise and $[\alpha]_D$ units are recorded in a 10^{-1} deg cm^2 g^{-1}. IR spectra were obtained on a Perkin-Elmer 357 spectrometer and UV spectra were recorded on Pye Unicam PU-800 spectrometer. TLC and column chromatography were performed on silica gel (Machery-Nagel, SIL G-25UV$_{254}$) and Silica Gel 60 (Merck, 70–230 mesh), respectively. Preparative centrifugal chromatography was carried out on silica gel plates (Merck 7749) with a Chromatotron™ Model 7924T. Paper chromatography was performed on Whatman chromatography paper No. 1 and components were detected by spraying with either an ammoniacal solution of silver nitrate[26] or a 0.1% w/v solution of ninhydrin in butan-1-ol.[26] Organic solutions were dried over anhydrous sodium sulfate. Yeast α-glucosidase (type VI) from brewer's yeast was obtained from Sigma Chemical Co.

1-Bromo-2-trityloxyethane **7**.—The title compound, prepared[6] by treatment of 2-bromoethanol with trityl chloride in pyridine at 20 °C, had m.p. 127–128 °C (lit.,[6] 126–127 °C); ν_{max}(Nujol)/cm^{-1} no absorption near 3500 (OH); δ_H(400 MHz) 3.378–3.467 (4 H, complex, CH_2CH_2) and 7.199–7.476 (15 H, complex, 3 × Ph).

(S)-*Glycidyl Trityl Ether* **10**.—Treatment of a solution of (*R*)-glycidol with trityl chloride in pyridine in the usual manner and column chromatography [methylene dichloride–hexane (2:1 v/v)] of the crude product gave (*S*)-glycidyl trityl ether **10**, m.p. 97–98 °C (lit.,[7] 99–100 °C) (Found: C, 83.65; H, 6.3. Calc. for $C_{22}H_{20}O_2$: C, 83.5; H, 6.4%); $[\alpha]_D$ −10.0 (*c* 1.0); δ_H(60 MHz) 2.56–2.88 (2 H, complex, epoxide CH_2), 3.00–3.56 (3 H, complex, epoxide CH and CH_2OTr) and 7.20–7.60 (15 H, complex, 3 × Ph). The ^1H NMR spectrum was in agreement with that reported.[7]

2-[(2′-*Trityloxyethyl*)*amino*]*propane*-1,3-*diol* **8**.—A solution of 1-bromo-2-trityloxyethane **7** (4.24 g, 12 mmol) and serinol **6** (2.50 g, 27 mmol) was heated under reflux in methanol (60 cm^3) for 14 days, after which time TLC [ethyl acetate–methanol (9:1 v/v)] revealed the complete disappearance of bromide **7** (R_f 0.84) and the formation of a less mobile component. Concentration of the solution gave a solid, which was extracted with cyclohexane (100 cm^3) and then with water (100 cm^3). The residue was recrystallised from ethyl acetate–hexane to afford *compound* **8** (1.36 g, 36%), m.p. 96–97 °C (Found: C, 76.2; H, 7.2; N, 3.6. $C_{24}H_{27}NO_3$ requires C, 76.4; H, 7.2; N, 3.7%); ν_{max}(Nujol)/cm^{-1} 3410 (OH); δ_H(60 MHz) 2.60 (3 H, br s, NH and 2 × OH), 2.68–2.96 (3 H, complex, $NHCH_2$ and $HNCH$), 3.16–3.32 (2 H, m, CH_2OTr), 3.48–3.60 (4 H, complex, 2 × CH_2OH) and 7.16–7.56 (15 H, complex, 3 × Ph).

2-[(2′-*Hydroxyethyl*)*amino*]*propane*-1,3-*diol Hydrochloride* **9**·HCl.—A solution of 2-[(2′-trityloxyethyl)amino]propane-1,3-diol **8** (0.30 g, 0.79 mmol) in methanol (10 cm^3) to which acetyl chloride (0.06 cm^3, 0.84 mmol) had previously been added was stored at room temperature for 7 days, after which time TLC [ethyl acetate–hexane (2:8 v/v)] revealed the presence of trityl methyl ether (R_f 0.67). The residue obtained on concentration of the solution was partitioned between water (8 cm^3) and hexane (8 cm^3), and the aqueous layer was separated, and then extracted with hexane (3 × 8 cm^3). Concentration of the aqueous layer gave, as a chromatographically homogeneous [paper chromatography; pyridine–

amyl alcohol–water (7:7:6 v/v); R_f 0.21], hygroscopic solid the hydrochloride **9**·HCl (104 mg, 77%), *m/z* (CI MS) 136.0974 (MH^+, $C_5H_{14}NO_3$); δ_H(60 MHz; CD_3OD) 3.00–3.64 (3 H, complex, N^+CH_2 and CH) and 3.80–3.96 (6 H, complex, 3 × CH_2OH).

(2′S)-2-[2′-*Hydroxy*-3′-*trityloxypropyl*)*amino*]*propane*-1,3-*diol* **11**.—A solution of (*S*)-glycidyl trityl ether **10** (1.06 g, 3.3 mmol) and serinol **6** (0.30 g, 3.3 mmol) in methanol (30 cm^3) was boiled under reflux for 6 days, after which time analysis by TLC [ethyl acetate–methanol (9:1 v/v)] revealed the appearance of a new component (R_f 0.21) and the complete disappearance of the oxirane **10** (R_f 0.74). The solvent was removed and the residue was extracted sequentially with cyclohexane (100 cm^3) and water (100 cm^3), and the remaining solid was recrystallised from ethyl acetate–hexane to afford *compound* **11** (0.62 g, 46%), m.p. 133–136 °C (Found: C, 73.6; H, 7.0; N, 3.2. $C_{25}H_{29}NO_4$ requires C, 73.7; H, 7.2; N, 3.4%); $[\alpha]_D$ −11.9 (*c* 1.0, MeOH); δ_H(60 MHz; CD_3OD) 2.56–2.96 (3 H, complex, NCH and NCH_2), 3.00–4.08 (7 H, complex, C(OH)*H*, CH_2OTr and 2 × CH_2OH) and 7.16–7.56 (15 H, complex, 3 × Ph).

(2′S)-2-[(2′,3′-*Dihydroxypropyl*)*amino*]*propane*-1,3-*diol Hydrochloride* **12**·HCl.—Acetyl chloride (0.04 cm^3, 0.59 mmol) was added to methanol (10 cm^3) and compound **11** (0.24 g, 0.59 mmol) was dissolved in the resulting mixture. After storage at room temp. for 7 days, TLC [ethyl acetate–hexane (2:8 v/v)] revealed the formation of trityl methyl ether (R_f 0.84). The solvent was removed, the residue was partitioned between water (8 cm^3) and hexane (8 cm^3), and the aqueous phase was separated, and extracted with hexane (2 × 8 cm^3). Concentration of the aqueous phase afforded, as a hygroscopic, though chromatographically homogeneous [paper chromatography; pyridine–amyl alcohol–water (7:7:6 v/v); R_f 0.10] amorphous solid, the *hydrochloride* **12**·HCl (0.111 g, 93%); *m/z* (CI MS) 166.1070 (MH^+, $C_6H_{16}NO_4$) (Found: C, 35.2; H, 7.8; N, 6.6. $C_6H_{16}ClNO_4$ requires C, 35.7; H, 7.8; N, 7.0%); $[\alpha]_D$ −23.1 (*c* 1.1, MeOH); δ_H(400 MHz; CD_3OD), 3.172 (1 H, dd, $J_{1'a,2'}$ 8.9 and $J_{1'a,1'b}$ 12.5, $NH_2{}^+CH'_aH'_b$), 3.384 (1 H, dd, $J_{1'b,2'}$ 3.7, $H_2{}^+NCH'_aH'_b$), 3.395–3.431 [1 H, m, $CH(CH_2OH)_2$], 3.607 [1 H, dd, $J_{2',3'a}$ 5.3 and $J_{3'a,3'b}$ 11.3, CH(OH)C$H'_aH'_b$OH], 3.660 [1 H, dd, $J_{2',3'b}$ 4.6, CH(OH)C$H'_aH'_b$OH], 3.755–3.804 (2 H, complex, 2 × C*H*H′OH), 3.849–3.891 (2 H, complex, 2 × CH*H*′OH) and 3.975–4.030 (1 H, m, C*H*OH).

2-*Amino*-1,4-*di*-O-*benzyl*-2-*deoxy*-L-*erythritol* **14**.—A solution of 2,3-anhydro-1,4-di-*O*-benzyl-L-threitol[9] **13** (0.50 g, 1.76 mmol) in methanol (20 cm^3) previously saturated with ammonia was heated in a glass pressure vessel for 2 days at 50 °C. TLC [ethyl acetate–hexane (2:8 v/v)] indicated considerable depletion of starting material (R_f 0.34) and the presence of a new component (R_f 0.0). The solution was resaturated with ammonia and heating was continued under pressure for a further 3 days at 50 °C, when TLC showed reaction was complete. Concentration of the solution gave an oil which crystallised on storage. Recrystallisation from ethyl acetate–hexane afforded *compound* **14** (0.29 g, 55%), m.p. 63–64 °C (Found: C, 71.4; H, 7.9; N, 4.5. $C_{18}H_{23}NO_3$ requires C, 71.7; H, 7.7; N, 4.6%); $[\alpha]_D$ +3.2 (*c* 1.2); ν_{max}(Nujol)/cm^{-1} 3345; δ_H(60 MHz) 2.04 (3 H, br s, OH and NH_2), 3.08–3.32 (1 H, m, C*H*NH_2), 3.48–3.84 (5 H, complex, 2 × CH_2OBzl and C*H*OH), 4.48–4.56 (4 H, complex, 2 × CH_2Ph) and 7.32 (10 H, br s, 2 × Ph).

2-*Amino*-2-*deoxy*-L-*erythritol Hydrochloride* **15**·HCl.—A solution of compound **14** (0.46 g, 1.54 mmol) in methanol (25 cm^3) containing conc. hydrochloric acid (0.2 cm^3, 2 mmol) was stirred under H_2 in the presence of 10% palladium on charcoal (0.1 g) at room temperature until debenzylation was complete as judged by cessation of H_2 uptake. The catalyst was removed by filtration through a Kieselguhr pad, and concentration of the filtrate afforded, as a hygroscopic solid, the amine hydrochloride **15**·HCl (0.18 g, 96%), *m/z* (CI MS) 122.0817 (MH^+, $C_4H_{12}NO_3$); $[\alpha]_D$ +18.7 (*c* 1.9, MeOH); δ_H(400 MHz; CD_3OD) 3.350–3.391 (1 H, m, 2-H), 3.602 (1 H, dd, $J_{1,2}$ 6.1 and $J_{1,1'}$ 11.3, 1-H), 3.668 (1 H, dd, $J_{1',2}$ 5.2, 1-H′), 3.753 (1 H, dd, $J_{3,4}$ 7.9 and $J_{4,4'}$ 11.6, 4-H) and 3.838–3.889 (2 H, complex, 3- and 4-H′).

1,3,4,5-*Tetra*-O-*benzyl*-6-O-*trityl*-L-*sorbose* **17**.—To a solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-trityl-D-glucitol[12] **16** (0.29 g, 0.4 mmol) in toluene (30 cm^3)–pyridine (0.5 cm^3) was added nicotinium dichromate[13,14] (0.37 g, 0.8 mmol) and the vigorously stirred solution was heated at 90 °C for 4 h. TLC [ethyl acetate–hexane (2:8 v/v)] indicated the disappearance of starting material **16** (R_f 0.26) and the appearance of a new component (R_f 0.31). After filtration through a Kieselguhr pad, the solution was concentrated to give an oily solid, which was taken up in toluene (20 cm^3), and the solution was refiltered through Kieselguhr and concentrated to give a pale yellow oil. Column chromatography [ethyl acetate–hexane (2:8 v/v)] gave, as a gum, the title compound **17** (0.26 g, 83%), *m/z* (FAB MS) 783 (MH^+); $[\alpha]_D$ −5.1 (*c* 1.6) (lit.,[12] $[\alpha]_D$ −4.2); ν_{max}(film)/cm^{-1} 1730 (C=O), no absorption near 3300; δ_H(400 MHz) 3.076 (1 H, dd, $J_{1,2}$ 5.1 and $J_{1,1'}$ 10.3, C*H*H′OTr), 3.398 (1 H, dd, $J_{1',2}$ 3.5, CH*H*′OTr), 3.800–3.869 (1 H, m, CH), 3.877 (1 H, d, $J_{A,B}$ 11.3, CH_AH_BPh), 3.915 (1 H, d, $J_{6,6'}$ 12.0, C*H*HOBzl), 3.953 (1 H, d, CH*H*OBzl), 4.066 (1 H, d, *J* 6.9, 3-H), 4.095–4.147 (1 H, m, CH), 4.194 (1 H, d, $J_{A,B}$ 11.3, CH_AH_BPh), 4.238 (1 H, d, $J_{A,B}$ 12.0, CH_AH_BPh), 4.277 (1 H, d, $J_{A,B}$ 12.0, CH_AH_BPh), 4.379 (1 H, d, $J_{A,B}$ 11.3, CH_AH_BPh), 4.491 (1 H, d, $J_{A,B}$ 11.4, CH_AH_BPh) 4.555 (1 H, d, $J_{A,B}$ 11.4, CH_AH_BPh), 4.564 (1 H, d, $J_{A,B}$ 11.3, CH_AH_BPh) and 6.952–7.403 (35 H, complex, 7 × Ph).

1,3,4,5-*Tetra*-O-*benzyl*-6-O-*trityl*-L-*sorbose Oxime* **18**.—A solution of the ketone **17** (0.16 g, 0.2 mmol) in pyridine (5 cm^3)–ethanol (5 cm^3) was added dropwise to a stirred solution of hydroxylamine hydrochloride (50 mg, 0.7 mmol) in pyridine (5 cm^3) and the mixture was stirred at room temperature for 3 h. TLC [ethyl acetate–hexane (2:8 v/v)] indicated complete loss of ketone **17** (R_f 0.37) and formation of a new component (R_f 0.24). The solution was poured into ice-cold water (50 cm^3), was extracted with diethyl ether (3 × 50 cm^3), and the combined extracts were dried and concentrated. The residue was extracted with toluene (3 × 30 cm^3) and the combined extracts were evaporated to leave, as a viscous syrup, the *oxime* **18** (80 mg, 50%), *m/z* (FAB MS) 798 (MH^+, $C_{53}H_{51}NO_6$) (Found: C, 79.8; H, 6.4; N, 1.5. $C_{53}H_{51}NO_6$ requires C, 79.8; H, 6.4; N, 1.8%); $[\alpha]_D$ −3.9 (*c* 1.0); ν_{max}(film)/cm^{-1} 3300 (OH), 1670 (C=N) and 630 (CPh_3); δ_H(270 MHz) 2.928 (1 H, dd, $J_{1,2}$ 4.2 and $J_{1,1'}$ 10.3, C*H*H′OTr), 3.213–3.280 (1 H, m, CH*H*′OTr), 3.475 (1 H, dd, $J_{5,6}$ 2.5 and $J_{6,6'}$ 10.6, C*H*H′OBzl), 3.540 (1 H, d, CH*H*′OBzl), 3.728–3.842 (2 H, complex, 2 × CH), 3.941–4.790 (9 H, complex, CH and 4 × CH_2Ph) and 6.845–7.436 (35 H, complex, 7 × Ph).

5-*Amino*-2,3,4,6-*tetra*-O-*benzyl*-5-*deoxy*-1-O-*trityl*-D-*glucitol* **19** *and* 2-*Amino*-1,3,4,5-*tetra*-O-*benzyl*-2-*deoxy*-6-O-*trityl*-L-*iditol* **20** *by Reduction of Oxime* **18**.—A solution of oxime **18** (0.79 g, 0.99 mmol) in tetrahydrofuran (THF) (10 cm^3) was added over a period of 10 min to a stirred solution of lithium aluminium hydride (0.22 g, 5.98 mmol) in THF (5 cm^3) under N_2. The resultant mixture was then stirred at 60 °C for 90 min. TLC [ethyl acetate–hexane (2:8 v/v)] revealed complete disappearance of starting material (R_f 0.56) and formation of two close-running components (R_f 0.30 and 0.25). Excess of

lithium aluminium hydride was destroyed by sequential addition of ethyl acetate (0.1 cm^3), water (0.1 cm^3), 15% aq. sodium hydroxide (0.1 cm^3), and water (0.3 cm^3), and inorganic solids were removed by filtration. Concentration of the filtrate gave an oil, which was partitioned between water (30 cm^3) and methylene dichloride (30 cm^3). The organic phase was dried and concentrated to leave an oil, which was subjected to preparative centrifugal chromatography. Elution with ethyl acetate–hexane (7:13 v/v) gave a chromatographically homogeneous viscous oil,* identified by an alternative synthesis as 2-amino-1,3,4,5-tetra-*O*-benzyl-2-deoxy-6-*O*-trityl-L-iditol **20** (0.43 g, 56%), $[\alpha]_D$ +15.5 (*c* 4.3); ν_{max}(film)/cm^{-1} 3400, 3020 and 630, no absorption near 1670 (C=N); δ_H(400 MHz) 1.832 (2 H, br s, NH_2), 2.984 (1 H, m, *H*CNH$_2$), 3.284–3.351 (2 H, complex, 2 × CH), 3.423 (1 H, dd, $J_{6,6'}$ 9.9 and $J_{5,6}$ 4.7, C*H*H'OTr), 3.498 (1 H, dd, $J_{6,5}$ 6.0, CH*H'*OTr), 3.753 (1 H, dd, $J_{1,1'}$ 7.3, $J_{1,2}$ 3.1, C*H*H'OBzl), 3.805–3.840 (1 H, m, CH), 4.001 (1 H, dd, $J_{1',2}$ 3.5, CH*H'*OBzl), 4.384 (2 H, br s, CH_2Ph), 4.460, 4.565, 4.662, 4.704 and 4.785 [each 1 H (except 4.565, 2 H), 5 × d, (3 × AB systems), J_{AB} 11.6, 11.4 and 11.3, 3 × CH_2Ph] and 7.142–7.474 (35 H, complex, 7 × Ph).

Further elution gave, as a chromatographically homogeneous oil, 5-amino-2,3,4,6-tetra-*O*-benzyl-5-deoxy-1-*O*-trityl-D-glucitol **19** (0.25 g, 33%) $[\alpha]_D$ +9.4 (*c* 2.5); ν_{max}(film)/cm^{-1} 3380, 3020 and 630, no absorption near 1670 cm^{-1} (C=N); δ_H(400 MHz) 1.960 (2 H, br s, NH_2), 2.989–3.030 (1 H, m, *H*CNH$_2$), 3.240 (1 H, dd, $J_{1,1'}$ 10.1 and $J_{1,2}$ 5.2, C*H*H'OTr), 3.373 (1 H, dd, $J_{1',2}$ 4.4, CH*H'*OTr), 3.468–3.511 (2 H, complex, C*H*H'OBzl and CH), 3.545 (1 H, dd, $J_{5,6}$ 3.8 and $J_{6,6'}$ 9.5, CH*H'*OBzl), 3.807–3.843 (1 H, m, CH), 3.989–4.012 (1 H, m, CH), 4.300 (1 H, d, $J_{A,B}$ 11.6, CH_AH_BPh), 4.374 (1 H, d, $J_{A,B}$ 11.6, CH$_A$*H*$_B$Ph), 4.424 (2 H, br s, CH_2Ph), 4.530 (1 H, d, $J_{A,B}$ 11.6, CH_AH_BPh), 4.618 (2 H, s, CH_2Ph), 4.662 (1 H, d, $J_{A,B}$ 11.6, CH$_A$*H*$_B$Ph) and 7.021–7.443 (35 H, complex, 7 × Ph).

*5-Amino-5-deoxy-*D*-glucitol Hydrochloride* **21**·HCl.—A solution of compound **19** (197 mg, 0.25 mmol) in methanol (25 cm^3) containing hydrochloric acid (0.025 cm^3, 10 mol dm^{-3}) was stirred under H_2 in the presence of 10% palladium on charcoal (100 mg) until uptake of gas ceased (2 weeks). The filtered solution was concentrated and the residue was partitioned between water (8 cm^3) and hexane (8 cm^3). The aqueous phase was washed with hexane (2 × 8 cm^3) and concentrated to afford, as a chromatographically homogeneous [paper chromatography; pyridine–amyl alcohol–water (7:7:6 v/v); R_f 0.13] hygroscopic solid, the *amine hydrochloride* **21**·HCl (54 mg, 100%), *m/z* (CI MS) 182.1028 (MH^+, $C_6H_{16}O_5$); $[\alpha]_D$ +13.1 (*c* 0.4, MeOH); δ_H(60 MHz; CD_3OD) 3.28–3.44 (1 H, m, C*H*N$^+$H$_3$) and 3.48–4.00 (7 H, complex, 2 × CH_2OH and 3 × CH).

*2-Amino-2-deoxy-*L*-iditol Hydrochloride* **22**·HCl.—A solution of compound **20** (0.43 g, 0.55 mmol) in methanol (35 cm^3) containing hydrochloric acid (0.055 cm^3, 10 mol dm^{-3}) was vigorously stirred with 10% palladium on charcoal (200 mg) under H_2 until the uptake of gas ceased (8 days). The filtered solution was concentrated and the residue was partitioned between water (8 cm^3) and hexane (8 cm^3). The aqueous phase was extracted with hexane (2 × 8 cm^3) and then concentrated to afford, as a chromatographically homogeneous [paper chromatography; pyridine–amyl alcohol–water (7:7:6 v/v); R_f 0.17] hygroscopic solid, the *amine hydrochloride* **22**·HCl (114 mg, 95%), *m/z* (CI MS) 182.1028 (MH^+, $C_6H_{16}NO_5$); $[\alpha]_D$ +8.2 (*c* 0.5, MeOH); δ_H(400 MHz; CD_3OD) 3.306–3.347 (1 H, m, C*H*N$^+$H$_3$) and 3.489–3.771 (7 H, complex, 2 × CH_2OH and 3 × CH).

*Identification of 2-amino-*1,3,4,5*-tetra-*O*-benzyl-2-deoxy-*6-O*-trityl-*L*-iditol* **20** *by Preparation from Amide* **24**.—A solution of the amide **24** (see below) (0.29 g, 0.29 mmol) in toluene (1.25 cm^3), methanol (2.5 cm^3) and 1,2-diaminoethane (1.94 cm^3, 29 mmol) was boiled under reflux for 7 h under N_2 after which time analysis by TLC [ethyl acetate–hexane (9:11 v/v)] indicated complete consumption of starting material (R_f 0.51) and the formation of another component (R_f 0.35). The residue obtained on removal of the solvent was subjected to preparative centrifugal chromatography [ethyl acetate–hexane (9:11 v/v)] to afford, as an amorphous solid, 2-amino-1,3,4,5-tetra-*O*-benzyl-2-deoxy-6-*O*-trityl-L-iditol **20** (0.15 g, 67%). The ^{1}H NMR spectra of the samples of compound **20** prepared from substrates **18** and **24** were indistinguishable.

*2-Amino-*1,3,4,5*-tetra-*O*-benzyl-2-deoxy-*N*-phthaloyl-6-O-trityl-*L*-iditol* **23**.—Triphenylphosphine (0.33 g, 1.27 mmol) and phthalimide (0.19 g, 1.27 mmol) were added to a solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-trityl-D-glucitol[12] **16** (0.50 g, 0.64 mmol) in THF (10 cm^3). DEAD (0.22 cm^3, 1.40 mmol) was then added to the stirred solution. After 1 h, TLC [ethyl acetate–hexane (3:7 v/v)] revealed complete consumption of substrate **16** (R_f 0.43) and formation of one major component (R_f 0.58). The solvent was removed and the residue was subjected to column chromatography [ethyl acetate–hexane (3:17 v/v)] to give, as a solid foam, the *title compound* **23** (0.16 g, 27%) (Found: C, 79.7; H, 6.0; N, 1.7. $C_{61}H_{55}NO_7$ requires C, 80.15; H, 6.1; N, 1.5%); $[\alpha]_D$ −3.0 (*c* 2.9); ν_{max}(film)/cm^{-1} 1710 (NCO), no absorption near 3300 (OH); δ_H(400 MHz) 3.213 (1 H, dd, $J_{6,6'}$ 10.1 and $J_{5,6}$ 4.7, C*H*H'OTr), 3.379 (1 H, dd, $J_{5,6'}$ 5.2, CH*H'*OTr), 3.669–3.705 (2 H, m, 2 × CH), 3.936–3.957 (1 H, m, CH), 4.004–4.079 (2 H, complex, CH_2OBzl), 4.101 (1 H, d, $J_{A,B}$ 12.1, CH_AH_BPh), 4.137–4.167 (1 H, m, CH), 4.193 (1 H, d, $J_{A,B}$ 11.9, CH_AH_BPh), 4.239 (1 H, d, $J_{A,B}$ 12.1, CH$_A$*H*$_B$Ph), 4.321 (1 H, d, $J_{A,B}$ 11.9, CH$_A$*H*$_B$Ph), 4.575 (1 H, d, $J_{A,B}$ 11.4, CH_AH_BPh), 4.618 (2 H, br s, CH_2Ph), 4.637 (1 H, d, $J_{A,B}$ 11.4, CH$_A$*H*$_B$Ph) and 6.904–7.888 (39 H, complex, 7 × Ph and C_6H_4).

*1,3,4,5-Tetra-*O*-benzyl-2-[2'-(butylcarbamoyl)benzamido]-2-deoxy-6-*O*-trityl-*L*-iditol* **24**.—A solution of compound **23** (0.39 g, 0.43 mmol) in toluene (2.5 cm^3)–methanol (5 cm^3) containing butylamine (5 cm^3, 50.59 mmol) was heated under reflux for 30 min under N_2. TLC [ethyl acetate–hexane (3:7 v/v)] revealed complete disappearance of starting material (R_f 0.47) and formation of a slower running component (R_f 0.24). The residue obtained on concentration of the solution was subjected to preparative centrifugal chromatography [ethyl acetate–hexane (7:13 v/v)] to afford, as an amorphous solid, the *product* **24** (0.29 g, 68%) (Found: C, 79.15; H, 6.4; N, 2.8. $C_{65}H_{66}N_2O_7$ requires C, 79.1; H, 6.7; N, 2.8%); $[\alpha]_D$ +28.6 (*c* 2.9); ν_{max}(film)/cm^{-1} 1650 (NCO); δ_H(400 MHz) 0.945 (3 H, t, *J* 7.3, Me), 1.339–1.396 (2 H, complex, CH_2Me), 1.487–1.541 (2 H, complex, CH_2CH_2Me), 3.235–3.287 (1 H, m, C*H*H'OTr), 3.324–4.425 (2 H, complex, NC*H*H' and CH*H'*OTr), 3.458–3.590 (3 H, complex, NCH*H'* and 2 × CH), 3.993–4.015 (1 H, m, C*H*H'OBzl), 4.073–4.123 (1 H, m, CH), 4.310–4.350 (1 H, m, CH*H'*OBzl), 4.384–4.469 (2 H, complex, CH_2Ph), 4.558–4.677 (5 H, complex, NCH, 2 × C*H*H'Ph and CH_2Ph), 4.726–4.819 (2 H, complex, 2 × CH*H'*Ph) and 6.771–7.638 (39 H, complex, 7 × Ph and C_6H_4).

2-[(2'-Trityloxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol **26**.—A mixture of 1-bromo-2-trityloxyethane **7** (3.50 g,

* Compounds **19** and **20**, to our surprise, showed limited stability on storage and, although they afforded the expected elemental analyses for H and N, acceptable analyses for C could not be obtained (error 1.2 and 0.9%, respectively).

10 mmol) and TRIS **25** (2.30 g, 19 mmol) in methanol (65 cm^3) was stirred and heated under reflux for 21 days. TLC [methanol–ethyl acetate (9:1 v/v)] indicated the appearance of a new component (R_f 0.37). The solution was concentrated and the solid residue was stirred thoroughly with a mixture of water (75 cm^3) and cyclohexane (75 cm^3) for 20 min. The remaining solid residue was then stirred with cyclohexane (100 cm^3) for 1 h and then with water (100 cm^3) for 1 h. The remaining solid was collected by filtration, dried over phosphorus pentaoxide, and recrystallised from ethyl acetate–hexane to afford *product* **26** (1.55 g, 55%), m.p. 149–150 °C (Found: C, 73.5; H, 7.2; N, 3.4. $C_{25}H_{29}NO_4$ requires C, 73.7; H, 7.2; N, 3.4%); ν_{max}(Nujol)/cm^{-1} 3500 and 3341; δ_H(60 MHz) 2.12 (4 H, br s, NH and 3 × OH), 2.70 (2 H, t, *J* 5.0, $NHCH_2$), 3.20 (2 H, t, CH_2OTr), 3.48 (6 H, s, 3 × CH_2O) and 7.12–7.48 (15 H, complex, 3 × Ph).

2-[(2′-*Hydroxyethyl*)*amino*]-2-(*hydroxymethyl*)*propane*-1,3-*diol Hydrochloride* **27**·HCl.—Compound **26** (0.92 g, 2.25 mmol) was stirred in methanol (10 cm^3) containing acetyl chloride (0.16 cm^3, 2.25 mmol) to give a clear solution, which was stored for 2 days. Methyl trityl ether which had crystallised out of solution was collected and the filtrate was concentrated to give a syrup, which was triturated with cyclohexane (2 × 80 cm^3). The cyclohexane was removed by decantation and the remaining syrup was dissolved in water (10 cm^3) and filtered through a Kieselguhr pad. The filtrate was concentrated and the residue was dried over phosphorus pentaoxide to leave, as a chromatographically homogeneous [paper chromatography; pyridine–amyl alcohol–water (7:7:6 v/v); R_f 0.13] hygroscopic solid, the *title compound* **27**·HCl (0.42 g, 95%) (Found: C, 35.3; H, 8.1; N, 7.0; Cl, 17.9. $C_6H_{16}ClNO_4$ requires C, 35.7; H, 8.0; N, 6.5; Cl, 17.9%); ν_{max}(film)/cm^{-1} 3340; δ_H(60 MHz; CD_3OD) 3.28–3.44 (2 H, complex, $H_2N^+CH_2$) and 3.76–4.04 (8 H, complex, 4 × CH_2O).

(2′S)-2-[(2′-*Hydroxy*-3′-*trityloxypropyl*)*amino*]-2-(*hydroxymethyl*)*propane*-1,3-*diol* **28**.—A mixture of (*S*)-glycidyl trityl ether **10** (0.90 g, 2.8 mmol) and TRIS **25** (0.35 g, 2.8 mmol) in methanol (30 cm^3) was stirred and heated under reflux for 4 days. TLC [ethyl acetate–methanol (9:1 v/v)] indicated the gradual disappearance of substrate **10** (R_f 0.84) and the formation of a new component (R_f 0.21). The solution was concentrated to dryness and the solid residue was stirred thoroughly for 30 min with cyclohexane (100 cm^3), which was then decanted and discarded. The remaining solids were washed with water (100 cm^3) and the undissolved residue was dried over phosphorus pentaoxide and recrystallised from ethyl acetate–hexane to afford the *title compound* **28** (0.71 g, 58%), m.p. 81–83 °C (Found: C, 71.5; H, 7.2; N, 3.3. $C_{26}H_{31}NO_5$ requires C, 71.4; H, 7.1; N, 3.2%); $[\alpha]_D$ −10.9 (*c* 1.0, MeOH); ν_{max}(Nujol)/cm^{-1} 3311; δ_H(60 MHz; CD_3OD) 2.64–2.80 (2 H, complex, $NHCH_2$), 3.08–3.32 (2 H, complex, CH_2OTr), 3.36–3.72 (7 H, complex, CHOH and 3 × CH_2OH) and 7.12–7.68 (15 H, complex, 3 × Ph).

(2′S)-2-[(2′,3′-*Dihydroxypropyl*)*amino*]-2-(*hydroxymethyl*)-*propane*-1,3-*diol Hydrochloride* **29**·HCl.—Trityl ether **28** (0.19 g, 0.43 mmol) was stirred in methanol (10 cm^3) containing acetyl chloride (0.03 cm^3, 0.43 mmol) to afford a solution. After 6 days the crystals of methyl trityl ether which had formed were collected and the filtrate was concentrated to leave a solid, which was partitioned between hexane (8 cm^3) and water (8 cm^3). The separated aqueous phase was then washed with hexane (2 × 8 cm^3) and was concentrated to give a residue, which was dried over P_2O_5 to give, as a chromatographically homogeneous [paper chromatography; pyridine–amyl alcohol–water (7:7:6 v/v); R_f 0.18] oil, the amine hydrochloride **29**·HCl (84 mg, 81%), *m/z* (CI MS) 196.1190 (MH^+, $C_7H_{18}NO_5$); $[\alpha]_D$ −13.9 (*c* 0.8, MeOH); ν_{max}(film)/cm^{-1} 3340 and 2500 (N^+H); δ_H(400 MHz; CD_3OD) 3.220 (1 H, dd, $J_{1'a,2'}$ 8.9 and $J_{1'a,1'b}$ 12.2, N^+H_2CHH'), 3.380 (1 H, dd, $J_{1'b,2'}$ 3.4, H_2N^+CHH'), 3.628 (1 H, dd, $J_{2',3'a}$ 5.5 and $J_{3'a,3'b}$ 11.3, $CHH'OH$), 3.641 (1 H, dd, $J_{2',3'b}$ 4.6, $CHH'OH$), 3.760 (6 H, s, 3 × CH_2OH) and 3.935–4.005 [1 H, m, C(OH)*H*].

Enzyme Assays.—The buffer, enzyme (yeast α-D-glucosidase, type VI from brewer's yeast) and substrate were purchased from Sigma and used as received. Piperazine-*N*,*N*′-bis(ethanesulfonic acid)–sodium acetate buffer (PIPES–NaOAc) (0.01 mol dm^{-3} PIPES, 0.02 mol dm^{-3} NaOAc, and 1 × 10^{-4} mol dm^{-3} ethylenediaminetetraacetic acid, in Fisons' analytical reagent water, adjusted to pH 6.5 by dropwise addition of 5 mol dm^{-3} hydrochloric acid) was prepared according to the literature procedure.[27] The stock enzyme solution was prepared by dissolution of solid protein (0.5 mg) in PIPES–NaOAc buffer solution (1 cm^3) and stored at 4 °C. This enzyme solution was diluted as necessary for the enzyme assay. Assays were carried out at 30 °C with 4-nitrophenyl α-D-glucopyranoside as substrate (K_m = 1.11 × 10^{-4} mol dm^{-3} at pH 6.5) and with substrate concentrations in the range 8 × 10^{-5} to 4 × 10^{-3} mol dm^{-3}. Liberation of 4-nitrophenol was measured by monitoring the absorption of the phenoxide anion at 400 nm and experiments were conducted so that less than 10% of the substrate was consumed within 5 min. The following example illustrates the detailed procedure.

Into a 3 cm^3 quartz optical cell (1 cm pathlength) were placed PIPES–NaOAc buffer solution (1.30 cm^3), inhibitor solution (0.60 cm^3), and yeast α-D-glucosidase solution (0.10 cm^3). The solutions were well mixed, and the 4-nitrophenyl α-D-glucopyranoside solution (1.00 cm^3) was injected into the cell to start the reaction. The reaction was monitored at 400 nm on a Pye Unicam PU 8000 spectrophotometer for 5 min, and the initial hydrolysis rate was calculated. The same procedure was repeated for the three other substrate concentrations. After all the initial rates were accumulated, the corresponding Lineweaver–Burk plot at that inhibitor concentration was constructed. In the cases of those compounds which exhibited slow-binding, the rate of evolution of 4-nitrophenol was monitored and Lineweaver–Burk graphs were constructed from the data obtained when the rate of evolution of 4-nitrophenol had become constant. The slopes of Lineweaver–Burk reciprocal plots of 1/*v* against 1/[S] in the presence of increasing amounts of inhibitor were plotted against the corresponding inhibitor concentrations [I], to which they are linearly related, and inhibition constants (K_i) were calculated from the intercept of this graph on the [I] axis.[28]

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