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SYNTHESIS OF 5-AMINO-5-DEOXY-D-MANNOPYRANOSE AND 1,5-DIDEOXY-1,5-IMINO-D-MANNITOL, AND INHIBITION OF α- AND β-D-MANNOSIDASES

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ABSTRACT

The title compounds and the corresponding L-*gulo* derivatives were synthesised in 6 steps from benzyl 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranoside. The K_i values, determined from inhibition studies with α-D-mannosidases from jack beans, almonds, and calf liver, and β-D-mannosidase from *Aspergillus wentii*, ranged from 70 to 400μM for the mannitol derivative and from 1.2 to 20μM for 5-amino-5-deoxy-D-mannopyranose, *i.e.*, inhibition is 10^2–10^4-fold stronger than with D-mannose. Marked enhancement of inhibition with increasing pH is ascribed to the ionisation of a carboxyl group at the active site, forming an ion pair with the protonated inhibitor. The inhibition equilibrium between the jack-bean enzyme and the mannose derivative was approached slowly with k_{app} $2.0 \times 10^5 M^{-1}.min^{-1}$. The mannose-derived inhibitor was also inhibitory against β-D-glucosidases from almonds and *Asp. wentii*, with K_i values only 20–150-times larger than those for the inhibition of these enzymes by 5-amino-5-deoxy-D-glucopyranose. This moderate discrimination in binding of D-*gluco* and D-*manno* derivatives is in marked contrast to the high specificity shown by the glucosidase in catalysing the hydrolysis of mannosidases. A similar low specificity with respect to binding, combined with highly specific catalysis, was also seen with the mannosidases acting on inhibitors and substrates with the D-*gluco* configuration.

INTRODUCTION

The antibiotic nojirimycin[1] (5-amino-5-deoxy-D-glucopyranose) and its 1-deoxy derivative (1,5-dideoxy-1,5-imino-D-glucitol) inhibit α- and β-D-glucosidases up to 10^5-times better than D-glucose (Table I). An as yet unexplained peculiarity of this marked inhibition is the slow formation of the enzyme–inhibitor complex with most enzymes. Apparent, second-order rate constants for the formation of the complex range from 0.015×10^6 (calf-liver α-D-glucosidase)[2] to $6 \times 10^6 M^{-1}.min^{-1}$ (intestinal sucrase)[3], and are thus several orders of magnitude below the diffusion-controlled limit. The approach to steady-state inhibition is on the time scale of minutes with the inhibitor concentrations usually employed.

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TABLE I

INHIBITION OF α- AND β-D-GLUCOSIDASES BY 5-AMINO-5-DEOXY ANALOGUES OF D-GLUCOSE

Enzyme	K_i (μM) *for competitive inhibition*		
	Nojirimycin	*1-Deoxynojirimycin*	D-*Glucose*
α-D-*Glucosidases*			
Yeast[2]	6.3	12.6	1,900
Sucrase[3] (Rabbit intestine)	0.13[a]	0.032[a]	19,000
Calf liver[4] (Microsomes)	50	2[a]	n.d.[b]
β-D-*Glucosidases*			
Asp. wentii (A_3)[5]	0.36[a]	2.7[a]	2,800
Almonds (A)[6,7]	0.9[a]	47	60,000
Helix pomatia[8]	1.1[a]	60	46,000

[a]Slow approach to steady-state inhibition. [b]Not determined.

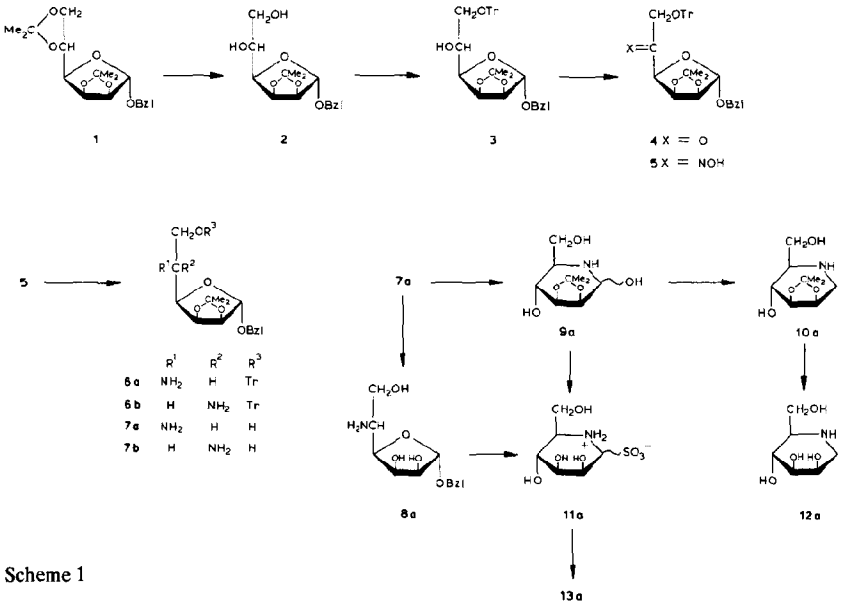
In order to test whether the marked but slow inhibition of glycoside hydrolases by 5-amino-5-deoxy derivatives of hexoses and hexitols is a general phenomenon, we have synthesised the corresponding D-mannose analogues and have studied their inhibitory properties with α- and β-D-mannosidases. 1,5-Dideoxy-1,5-imino-D-mannitol was recently synthesised in low yield from 2-amino-2-deoxy-D-mannofuranuronolactone[9]. No data have been reported on its inhibitory properties with mannosidases or other glycoside hydrolases.

RESULTS AND DISCUSSION

Synthesis. — A convenient route to 5-amino-5-deoxyhexoses starts with the 5-keto derivative of a suitably protected hexofuranose, as first described for nojirimycin[1]. Application of this principle to D-mannose results in the pathway depicted in Scheme 1.

For the synthesis of the benzyl glycoside **1**, phase-transfer catalysis with the methyltri-*n*-octylammonium ion was used in the reaction of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose with benzyl chloride, instead of sodium hydride as described by Brimacombe *et al.*[10]. The oxidation of **3** to **4** with methyl sulfoxide–acetic anhydride gave ~20% of a by-product tentatively identified as the 5-*O*-methylthiomethyl derivative of **3**; formation of methylthiomethyl ethers is a common side-reaction with this type of oxidation[11].

The crucial step in this synthesis of **12** and **13** is the deprotection of **7** and its rearrangement to the six-membered cyclic derivative. This latter step is so rapid that the intermediate 5-amino-5-deoxyfuranoid form cannot be trapped[9,12]. An open question is to what extent **9** would survive the conditions of reductive debenzylation. A report[13] that this is not the case with Pd/H_2 was confirmed: only 5% of **11a** was isolated from the reduction products of **7a** when the catalyst was prepared



Scheme 1

from palladium hydroxide on charcoal[14] and the isopropylidene group was removed by hydrolysis with sulfuric acid. The main product was **12a**. No debenzylation was observed with palladium black or $PdCl_2$ on charcoal.

A convenient route to **11a** and **11b** involved hydrolysis of **7** with aqueous toluene-*p*-sulfonic acid supersaturated with sulfur dioxide. It was shown by t.l.c. and isolation of the intermediate **8a** that the isopropylidene group is released faster than the benzyl group. The presence of a high concentration of sulfur dioxide provides an efficient protection of **13** (in the form of its hydrogensulfite adduct **11**) against acid-catalysed dehydration to 5-hydroxy-2-pyridinemethanol[1,12], even under the drastic conditions required for debenzylation (4 days at 40° in 0.2M toluene-*p*-sulfonic acid).

The configurational assignment of the compounds with suffix **a** and **b** (Scheme 1) to the D-*manno* and L-*gulo* series was made on the following basis. Compounds **6a** and **6b** gave *N*-salicylidene derivatives which had $[\alpha]_{578}$ +104° and +7.3°, respectively. As Inouye *et al.*[1] reported a difference in specific rotation of $\Delta[\alpha]_{589}$ (D − L) = + 78° for the corresponding D-*gluco* and L-*ido* derivatives, we ascribe the D-*manno* configuration to **6a**, the less-polar isomer in t.l.c. and alumina chromatography. This assignment is supported by applying Hudson's rule to the molar rotations of α-D-glucose and 1-deoxynojirimycin (DN)[1], and α-D-mannose and **12a**, respectively, which gives ΔM_{589} (α-D-Glc − DN) = +126° and ΔM_{578} (α-D-Man − **12a**) = +142°. Support is also provided by a comparison of melting points with a sample of 1,5-dideoxy-1,5-imino-D-mannitol kindly provided by Dr. E. Truscheit (Wuppertal). What cannot be explained at present is the difference

between our optical rotation values and those given by Leontein *et al.*[9] for the acetate salts of **12a** and **12b**. Calculation of M_{578} from their data gives −11° for the D-*manno* isomer and +4.4° for the L-*gulo* isomer, whereas we find, in the presence of a small molar excess of acetic acid, −31° for **12a** and +9.3° for **12b**, respectively.

The stability of **13a** in aqueous solution was monitored by its inhibition of jack-bean α-D-mannosidase, u.v. spectroscopy, and testing for α-hydroxyketones with alkaline *o*-dinitrobenzene[14]. No decomposition was observed after 2 days at 25° at pH 5.5 or 1.0. At 50°, inhibitory activity was lost with half-lives of ~4 h at pH 5.5 and 5.5 h at pH 1.0. In spite of these similar decomposition rates, different products were formed under nearly neutral and acidic conditions. A strongly positive reaction with *o*-dinitrobenzene developed at pH 5.5, whereas changes in u.v. absorbance were barely detectable. With the solution at pH 1.0, on the other hand, the *o*-dinitrobenzene test reached only ~10% of the pH 5.5 value, but a strong absorbance maximum appeared at 288 nm. When the u.v. spectrum was measured at pH 13, it showed maxima at 296 and 243 nm with relative intensities (based on the pH 1.0 value) of 0.78 and 1.58, respectively.

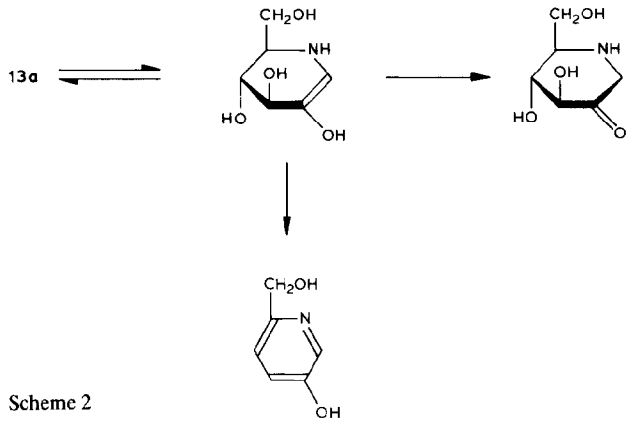
These observations can be explained qualitatively by the dehydration and isomerisation reactions (Scheme 2) first described by Paulsen[15] for 5-amino-5-deoxy-D-xylose, but further studies are required to elucidate the small influence of protonation of **13a** (pK_a 5.6) on the initial decomposition step, and to correlate the loss of inhibitory activity with the appearance of the various products. A quantitative evaluation of A_{288} with the data given by Inouye *et al.*[1] for 5-hydroxy-2-

TABLE II

INHIBITION OF α- AND β-D-MANNOSIDASES BY 5-AMINO-5-DEOXY ANALOGUES OF D-MANNOSE AND BY NOJIRIMYCIN

Enzyme	*pH*	K_i *Values (μM) for competitive inhibition*			
		5-Amino-5-deoxy-D-mannopyranose	*1,5-Dideoxy-1,5-imino-D-mannitol*	D-*Mannose*[a]	*Nojirimycin*
α-D-Mannosidase (jack beans)	4.5	6.5	400	20,000	
	5.5	1.2[b]	68		500
α-D-Mannosidase (almonds)	5.0	21	110	37,000	—
α-D-Mannosidase (calf liver)	4.5	—	380		—
	5.5	4.4	83	9,000	
β-D-Mannosidase *(Asp. wentii)*	4.5	7.7	4.600	6,000	900
β-D-Glucosidase *(Asp. wentii)*	5.0	11	—	—	0.07
β-D-Glucosidase (almonds)	5.0	20	5,300	100,000	0.9

[a] K_i calculated from total D-mannose concentration, α,β-equilibrium (68.8% α, 31.2% β). [b] Slow approach to steady-state inhibition (see Fig. 1).



Scheme 2

pyridinemethanol suggests that this compound represents only 20% of the decomposition products formed at pH 1.0.

Inhibition studies. — The data in Table II show that both 5-amino-5-deoxy-D-mannopyranose (**13a**) and 1,5-dideoxy-1,5-imino-D-mannitol (**12a**) inhibit α- and β-D-mannosidases up to 10^4-times better than D-mannose, although, on average, the inhibition is less marked than that of glucosidases by nojirimycin and its 1-deoxy derivative (Table I). Also, only one example of a slow approach to steady-state inhibition was found.

The strong inhibition of glycoside hydrolases by 5-amino-5-deoxy analogues of pyranoses is comparable to the inhibition of these enzymes by glycosylamines[16] and can be rationalised by an additional electrostatic interaction between the protonated inhibitor and one or two carboxylate groups of the active site close to C-1 of the bound pyranose[17,18]. The inhibition of jack-bean α-D-mannosidase by **13a** and **12a** is more marked at pH 5.5 than at pH 4.5, by a factor of 5.4 and 5.8, respectively. As judged by the constancy of K_m in this pH range, the enzyme undergoes no drastic conformational change with pH. In view of the different basicities of **13a** (pK_a 5.6) and **12a** (pK_a 7.2), this effect of pH on inhibition is, therefore, best explained by the ionisation of a carboxyl group with pK_a ~5.5 at the active site involved in ion-pair formation with the inhibitor.

When the L-*gulo* derivative **13b** was tested with jack-bean α-D-mannosidase, it gave the same degree of inhibition as **13a** at 600-fold higher concentrations. This is ascribed to a contamination of **13b** with ~0.17% of **13a**, which escaped detection by t.l.c., rather than to an incomplete specificity of the enzyme to discriminate between D-*manno* and L-*gulo* derivatives.

The relatively poor ability of mannosidases and glucosidases to discriminate between inhibitors epimeric at C-2 (Table II) is surprising. The enzymes tested bind the correct inhibitor only 120–400-times better than its C-2 epimer; for β-D-glucosidase from almonds, the discrimination factor is only 22. This is in marked contrast to the high catalytic specificity shown by these enzymes. An explanation could be that enzyme–substrate interactions with individual hydroxyl groups pro-

vide greatly differing contributions to binding *per se* and to catalysis, *e.g.*, by an induced fit orientation of the functional groups essential for bond breaking. With β-D-glucosidase A_3 from *Aspergillus wentii*, for example, replacement of HO-2 in D-glucosyl substrates and inhibitors by hydrogen decreased binding only 130-fold while catalysis was impaired up to 10^6-fold[19]. Similar observations were made with β-D-galactosidase from *Escherichia coli* to which 2-deoxy-D-*lyxo*-hexose (2-deoxygalactose) binds 3-times less tightly than D-galactose[20] while degalactosylation with the 2-deoxygalactosylenzyme proceeds at least 10^5-times slower than that for the galactosylenzyme[31,32].

α-D-Galactosidase from coffee beans and β-D-galactosidase from *E. coli* were not inhibited by 5mM **13a**.

These comparative studies show that the success of selectively inhibiting enzymes with different glycon specificities in the presence of each other, *e.g.*, for *in vivo* studies, will strongly depend on the enzymes involved. Use of too high concentrations of inhibitor may give erroneous results, as more than one type of enzyme may be affected.

In the presence of **13a**, the steady-state rate with jack-bean α-D-mannosidase is reached slowly with a rate constant that increases with the inhibitor concentration (Fig. 1). Thus, **13a** can be classified as a slow-binding inhibitor, as discussed by Morrison[21]. Hanozet *et al.*[3] have proposed a model where the rapid formation of

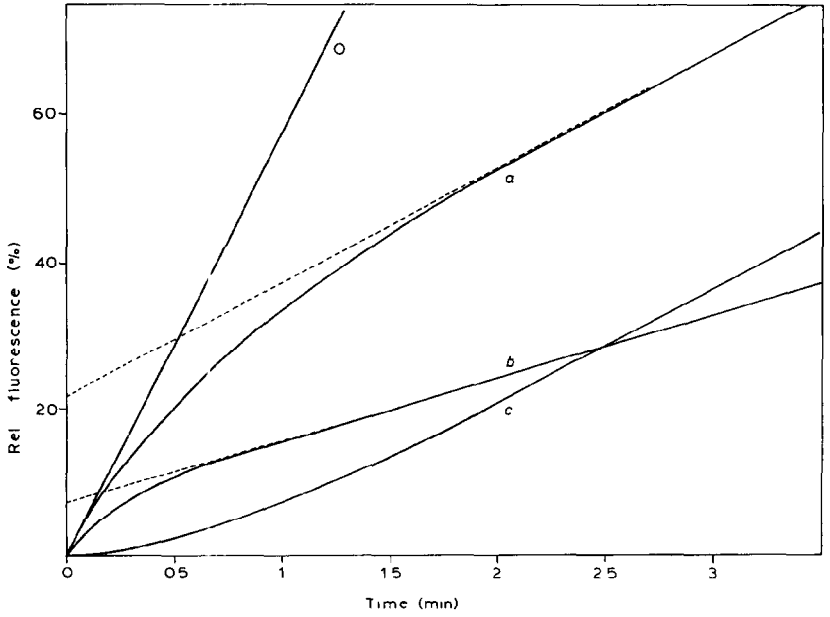


Fig. 1. Hydrolysis of mM 4-methylumbelliferyl α-D-mannopyranoside (K_m 0.42mM) by jack-bean α-D-mannosidase at pH 5 5 and 25° in the absence (o) and in the presence of 10μM (*a*) and 25μM (*b*) 5-amino-5-deoxy-D-mannopyranose. For trace *c*, the enzyme was preincubated for ≥5 min in 200μM inhibitor and then added to 18 vol. of substrate; 100% fluorescence corresponds to 5% hydrolysis.

a loose E–I complex is followed by slow, reversible isomerisation to a tight E*–I complex. The isomerisation is thought to involve a conformational change, as examples of slow inhibition are found with both nojirimycin and 1-deoxynojirimycin (Table I). Only with the former do facile chemical reactions like the dehydrations and isomerisations discussed above (Scheme 2) appear feasible.

Formally, the above model corresponds to mechanism B of ref. 21, but, if the inhibitor concentration is much smaller than the dissociation constant of the loose E–I complex, it cannot be distinguished from the simpler mechanism A of ref. 21, with only a single species of enzyme–inhibitor complex that is formed slowly. A direct application of either model to the observed data (Fig. 1) is complicated by the fact that **13a** showed mixed competitive/non-competitive inhibition, *i.e.*, the measured rate constant k_{app} will be a combination of terms for the free enzyme and the enzyme–substrate complex. The results depicted in Fig. 1 show, however, that the inhibition is reversible: the same degree of inhibition is reached starting from the free enzyme (trace *a*) and from the preformed enzyme–inhibitor complex (trace *c*). Since trace *c* was independent of preincubation of enzyme and inhibitor (10 min to 4 h), it is concluded that the jack-bean enzyme does not catalyse any irreversible transformation of **13a**.

EXPERIMENTAL

*Benzyl 2,3:5,6-di-*O*-isopropylidene-α-*D*-mannofuranoside* (**1**). — A solution of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose[22] (90 g), benzyl chloride (90 mL), and methyltri-*n*-octylammonium chloride (9 g) in benzene (700 mL) was stirred rapidly with 10M sodium hydroxide (180 mL) for 30 h at room temperature. The benzene solution was washed with water and concentrated to a syrup that crystallised from methanol–water (9:1), to yield **1** (94.8 g, 78%), m.p. 55°, $[\alpha]_{578}^{20}$ +79° (acetone); lit.[10] m.p. 54–55°, $[\alpha]_D^{20}$ +76.5° (acetone).

*Benzyl 2,3-*O*-isopropylidene-α-*D*-mannofuranoside* (**2**). — To a solution of **1** (75 g) in methanol (920 mL) were added 12M hydrochloric acid (11.5 mL) and sufficient water to cause a faint turbidity. After 7 h at room temperature, the solution was neutralised with ammonia and concentrated, and a solution of the syrupy residue in acetone (250 mL) was filtered and concentrated. Crystallisation of the residue from ethyl acetate–light petroleum gave **2** (62.5 g, 94%), m.p. 63–64°, $[\alpha]_{578}^{20}$ +95° (acetone); lit.[10] m.p. 60–61°, $[\alpha]_D^{20}$ +90° (acetone).

*Benzyl 2,3-*O*-isopropylidene-6-*O*-trityl-α-*D*-lyxo-hexofuranosid-5-ulose* (**4**). — A solution of **2** (30 g) and trityl chloride (31 g) in dry pyridine (180 mL) was stored at 25° for 18 h and then poured into ice–water (800 mL). A solution of the sticky product in toluene (300 mL) was washed with ice-cold aqueous acetic acid and then with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated, to give **3** as a viscous oil (66 g).

To a solution of crude **3** (61 g) in methyl sulfoxide (400 mL) was added acetic anhydride (60 mL). After 16 h at 25°, the solution was poured into ice–water (1 L)

and stirred with cyclohexane (500 mL). The organic phase was washed with water (3 ×) and concentrated. T.l.c. (benzene–methanol, 99:1) revealed two components. A sample (0.5 g) was purified by preparative t.l.c. The main product (R_F 0.61) crystallised from light petroleum, and was identified as **4** by ^{1}H-n.m.r. and i.r. spectroscopy. Crystallisation of the crude reaction product from light petroleum at 4°, with seeding, slowly gave **4** (40 g, 65%), m.p. 68–70°, $[\alpha]_{578}^{20}$ +11° (chloroform). ^{1}H-N.m.r. data ($CDCl_3$, 90 MHz): δ 1.23 (s, 6 H, CMe_2), 4.02 (s, 2 H, H-6), 4.53 (d, 2 H, PhCH_2), 4.58–4.73 (m, 2 H, H-2,3), 5.16 (s, 1 H, H-1), 5.15 (d, 1 H, H-4), and 7.2–7.5 (m, 20 H, 4 Ph).

Anal. Calc. for $C_{35}H_{13}O_6$: C, 76.06; H, 6.75. Found: C, 75.84; H, 6.81.

The minor compound (R_F 0.76) obtained by preparative t.l.c. was tentatively identified as the 5-*O*-methylthiomethyl derivative of **3**. ^{1}H-N.m.r. data ($CDCl_3$, 90 MHz): δ 1.25, 1.36 (2 s, 6 H, CMe_2), 2.12 (s, 3 H, S-Me), 3.33–3.48 (m, 2 H, H-6), 4.1–4.3 (m, 1 H, H-5), 4.45 (d, 2 H, PhCH_2), 4.68 (s, 2 H, O-CH_2-S), 4.3–4.9 (m, H-2,3,5), 4.96 (s. 1 H, H-1), and 7.1–7.6 (m, 20 H, 4 Ph).

*Benzyl 5-amino-5-deoxy-2,3-O-isopropylidene-6-O-trityl-α-*D*-mannofuranoside* (**6a**) *and -α-*L*-gulofuranoside* (**6b**). — The ketone **4** (33 g) was converted into the oxime and reduced with Raney nickel, to give a mixture of **6a** and **6b**, as described by Inouye *et al.*[1] for the corresponding nojirimycin precursor.

A sample (4 g) of the product mixture was subjected to column chromatography on basic alumina (500 g, Brockmann grade I, deactivated with 8 mL of water), using benzene → benzene–chloroform (1:1). The fractionation was monitored by t.l.c. (benzene–methanol, 96:4); the original mixture gave two spots [R_F 0.33 (**6a**) and 0.25 (**6b**)] when sprayed with ninhydrin. Fractions from the column gave **6a** (1.3 g), **6b** (0.63 g), and a mixture (1.4 g).

Samples (100 mg) of **6a** and **6b** were reacted[1] with salicylaldehyde; the respective salicylidene derivatives had m.p. 146–150°, $[\alpha]_{578}^{20}$ +104° (methanol), and m.p. 60–61°, $[\alpha]_{578}^{20}$ +7.3° (methanol).

Anal. Calc. for $C_{39}H_{41}NO_7$: C, 73.68; H, 6.50; N, 2.20. Found: (derivative from **6a**) C, 73.91; H, 6.38; N, 2.15; (derivative from **6b**) C, 74.05; H, 6.41; N, 2.11.

*Benzyl 5-amino-5-deoxy-2,3-O-isopropylidene-α-*D*-mannofuranoside* (**7a**) *and -α-*L*-gulofuranoside* (**7b**). — Solutions of **6a** and **6b** in 30 vol. of methanol–water (4:1) containing 0.5M HCl were left for 18 h at room temperature. Trityl-cleavage products were extracted with ether after 3-fold dilution with water, and **7a** and **7b** were then extracted into chloroform after adding excess of Na_2CO_3 to the aqueous phase. Each extract was concentrated and the residue was crystallised from ether–light petroleum, to give **7a**, and from ether, to give **7b**.

Using the crystalline samples, it was possible to fractionally crystallise **7a** and **7b** from the product mixture obtained by reduction and detritylation of the oxime of **4**. Thus, **4** (30 g) gave **7a** (1.9 g, 12%), **7b** (1.85 g, 11%), and a mixture (2.65 g, 15%).

Compound **7a** had m.p. 66°, $[\alpha]_{578}^{20}$ +86°. ^{1}H-N.m.r. data ($CDCl_3$, 90 MHz):

δ 1.32, 1.45 (2 s, 6 H, CMe_2), 1.83 (bs, disappeared on exchange with D_2O, 3 H, NH_2 and OH), 3.22 (m, 1 H, H-5), 3.50 (d, 1 H, H-6), 3.65 (d, 1 H, H-6), 3.83 (q, 1 H, H-3), 4.53 (d, 2 H, PhCH_2), 4.56-4.83 (m, 2 H, H-2,4), 5.18 (s, 1 H, H-1), and 7.33 (s, 5 H, Ph).

Anal. Calc. for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.08; H, 7.58; N, 4.45.

Compound **7b** had m.p. 112°, $[\alpha]_{578}^{20}$ +88° (methanol). ^{1}H-N.m.r. data ($CDCl_3$, 90 MHz): δ 1.29, 1.44 (2 s, 6 H, CMe_2), 1.95 (bs, disappeared on exchange with D_2O, 3 H, NH_2 and OH), 3.30 (m, 1 H, H-5), 3.60 ("t", 2 H, H-6), 3.84 (q, 1 H, H-3), 4.55 (d, 2 H, PhCH_2), 4.6–4.75 (m, 2 H, H-2,4), 5.08 (s, 1 H, H-1), and 7.33 (s, 5 H, Ph).

Anal. Found: C, 61.98; H, 7.52; N, 4.50.

*5-Amino-5-deoxy-*D*-mannopyranose hydrogensulfite adduct* (**11a**) *and benzyl 5-amino-5-deoxy-α-*D*-mannofuranoside* (**8a**). — A solution of **7a** (875 mg) and toluene-*p*-sulfonic acid (800 mg) in methanol–water (1:1, 8.5 mL previously saturated with SO_2 at 0°) was heated at 40° for 3 days in a sealed tube. The reaction was monitored by t.l.c. (chloroform–methanol–aqueous 5% ammonia, 20:23:10); R_F values: **7a**, 0.95, **8a**, 0.6; **11a** (or **13a**) 0.35. All compounds were detected with $KMnO_4$; only **7a** and **8a** gave a positive reaction with ninhydrin.

The main amount of **11a** crystallised on cooling to 0°, and an additional small amount was obtained on passing the mother liquor through Dowex 50 (H^+) resin (10 mL), concentrating the effluent, and crystallising the residue from aqueous sulfur dioxide–acetonitrile. Recrystallisation from the same solvent gave **11a** (195 mg, 28%), m.p. 163–165°, $[\alpha]_{578}^{20}$ +2° (water).

Anal. Calc. for $C_6H_{13}NO_7S$: C, 29.62; H, 5.39; N, 5.76; S, 13.18. Found: C, 29.79; H, 5.38; N, 5.82; S, 13.16.

Elution of the column of Dowex 50 with aqueous 5% ammonia, with crystallisation of the product from methanol–ether, gave **8a** (157 mg, 21%), m.p. 100–103°. ^{1}H-N.m.r. data (CD_3OD, 90 MHz): δ 3.17 (m, 1 H, H-5), 3.38–4.36 (m, 5 H, H-2,3,4,6), 4.55, 4.63 (2 s, 2 H, PhCH_2), 6.00 (d, 1 H, H-1), and 7.30 (s, 5 H, Ph).

Anal. Calc. for $C_{13}H_{19}NO_5$: C, 57.98; H, 7.11; N, 5.20. Found: C, 57.09; H, 7.20; N, 5.12.

The L-*gulo* isomer (**11b**) of **11a**, prepared from **7b** by the method described above, had m.p. 141°, $[\alpha]_{578}^{20}$ −4° (water).

Anal. Found: C, 29.53; H, 5.49; N, 5.59; S, 12.98.

*1,5-Dideoxy-1,5-imino-*D*-mannitol* (**12a**). — $Pd(OH)_2$–Charcoal[14] (2 g, moist) was added to methanol (7.5 mL) and water (2 mL), and hydrogenated for 30 min at 10 atm. A solution of **7a** (1 g) in methanol (2 mL) was added, together with acetic acid (2.5 mL), and hydrogenation was continued overnight under the same conditions. The catalyst was removed, and the filtrate was made 0.1M in HCl and left at room temperature for 2 days to cleave the isopropylidene group. The solution was diluted with water (3 vol.) and passed over Dowex 50 (H^+) resin. Elution with aqueous 5% ammonia, with crystallisation of the product from methanol–

ether, gave **12a** (395 mg, 67%), m.p. 185–187°, which was identical with that of a sample of **12a** provided by Dr. E. Truscheit (Bayer AG, Wuppertal), $[\alpha]_{578}^{20}$ −39° (water).

1,5-Dideoxy-1,5-imino-L-*gulitol* (**12b**). — Hydrogenation of **7b** under the above conditions gave **12b**, m.p. 150–151°, $[\alpha]_{578}^{20}$ −21° (water).

Anal. Calc. for $C_6H_{13}NO_4$: C, 44.17; H, 13.10; N, 8.58. Found: C, 43.98; H, 13.29; N, 8.50.

5-Amino-5-deoxy-D-*mannopyranose* (**13a**). — A solution of **11a** (122 mg, 0.5 mmol) in water (87.5 mL) was stirred with a 10-fold molar excess of Dowex 1 (HO^-) resin (4 g) for at least 1 h. The resin was washed with water, and the combined filtrate and washings were made up to the volume required.

Test for enolisable α-hydroxy ketones with o-*dinitrobenzene*[14]. — The following conditions were employed: 20 μL of 60 mM **13a** in 50mM sodium acetate adjusted to pH 5.5 (or in 50mM glycine adjusted to pH 1.0) were added, after various times of heating, to 770 μL of 0.2% of *o*-dinitrobenzene in ethanol followed by 10 μL of 2M NaOH; A_{550} was measured after 45 min at room temperature.

Enzymes. — *(a)* α-D-*Mannosidases.* The enzyme from jack beans was from Boehringer Mannheim; the almond enzyme was β-D-glucosidase, type II (from sweet almonds), from Sigma and was used without further purification (α-D-mannosidase activity, 0.5 U/mg); the liver enzyme was a crude preparation from fresh calf-liver homogenate in 3 vol. of 20mM phosphate (pH 5.0) containing 0.5% of Triton X 100. The supernatant solution from a 15,000*g* centrifugation (1 h) was used as enzyme source (α-D-mannosidase activity, 0.05 U/mL).

(b) β-D-*Mannosidase.* The spray-dried culture filtrate from *Asp. wentii* (Röhm GmbH, Darmstadt) was fractionated by ammonium sulfate precipitation and chromatography on CM-Sephadex, as described previously[23]. β-D-Mannosidase activity was eluted from DEAE-cellulose in the fractions preceding β-D-glucosidase A_3. The enzyme was isolated by saturating the appropriate fractions with ammonium sulfate.

(c) β-D-*Glucosidases.* The enzyme from *Asp. wentii* was isolated as described[23], and the almond enzyme was isoenzyme A from the Sigma type II preparation[7].

(d) D-*Galactosidases.* α-D-Galactosidase from coffee beans and β-D-galactosidase from *Escherichia coli* were from Boehringer Mannheim.

Activity determinations and evaluation of inhibition constants. — Substrates were prepared according to published procedures: 4-methylumbelliferyl α-D-galactopyranoside[24], β-D-galactopyranoside[25], β-D-glucopyranoside[26], α-D-mannopyranoside[27], and *p*-nitrophenyl α- and β-D-mannopyranoside[28].

The activities of jack-bean α-D-mannosidase, α- and β-D-galactosidases, and β-D-glucosidases were measured fluorimetrically[29] at 25° in 50mM acetate buffer (pH 4.5 and 5.5, respectively).

α-D-Mannosidase from almonds was determined with *p*-nitrophenyl α-D-mannopyranoside (0.2 to 1mM) by recording the increase in A_{350} at pH 4.5 and 25°.

Product formation was calculated on the basis of $\Delta\varepsilon_{350} = 3250\ M^{-1}.cm^{-1}$.

α-D-Mannosidase activity from calf liver was measured as follows. Supernatant solution (50 μL, see above) was added to a solution (100 μL) of *p*-nitrophenyl α-D-mannopyranoside (0.5 to 5mM in 50mM acetate, pH 4.5). Aqueous 10% Na_2CO_3 (3 mL) and aqueous 1% sodium dodecyl sulfate (100 μL) were added after 30 and 60 min, respectively, at 37°. The concentration of product was calculated from A_{410} with $\varepsilon_{410} = 18{,}500$.

β-D-Mannosidase was determined as described for α-D-mannosidase.

Dissociation constants for competitive inhibition by the various inhibitors were calculated[30] from the slopes of plots of 1/v against 1/[S] from the rates of substrate hydrolysis in the absence [slope (O)] and presence of inhibitor [slope (I)]: $K_i = [I]/\{[\text{slope(I)}/\text{slope(O)}] - 1\}$.

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REFERENCES

1 S. INOUYE, T. TSURUOKA, T. ITO, AND T. NIIDA, *Tetrahedron*, 23 (1968) 2125–2144.
2 G. LEGLER, unpublished data.
3 G. HANOZET, H. P. PIRCHER, P. VANNI, B. OESCH, AND G. SEMENZA, *J. Biol. Chem.*, 256 (1981) 3703–3711.
4 H. HETTKAMP, Thesis, University of Cologne; 1983.
5 R. RUTTER, Diplom-Thesis Biology, University of Cologne, 1980.
6 A. K. GROVER AND R. J. CUSHLEY, *Biochim. Biophys. Acta*, 482 (1977) 109–124.
7 U. PETZOLD, Diplom-Thesis Biology, University of Cologne, 1982.
8 R. DONSIMONI, Thesis, University René Descartes, Paris, 1982.
9 K. LEONTEIN, B. LINDBERG, AND J. LONNGREN, *Acta Chem. Scand., Ser. B*, 36 (1982) 515–518.
10 J. S. BRIMACOMBE, F. HUNEDY, AND L. C. N. TUCKER, *J. Chem. Soc., C*, (1968) 1381–1384.
11 K. OMURA AND D. SWERN, *Tetrahedron* 34 (1978) 1651–1660.
12 H. PAULSEN AND K. TODT, *Adv. Carbohydr. Chem.*, 23 (1968) 116–222.
13 H. PAULSEN, Y. HAYAUCHI, AND V. SINNWELL, *Chem. Ber.*, 113 (1980) 2601–2608.
14 W. R. FEARSON AND E. KAWERAU, *Biochem. J.*, 37 (1943) 326–330.
15 H. PAULSEN, *Justus Liebigs Ann. Chem.*, 683 (1965) 187–198.
16 H.-Y. L. LAI AND B. AXELROD, *Biochem. Biophys. Res. Commun.*, 54 (1973) 463–468.
17 P. LALEGERIE, G. LEGLER, AND J. YON, *Biochimie*, 64 (1982) 977–1000.
18 G. LEGLER, M. L. SINNOTT, AND S. G. WITHERS, *J. Chem. Soc., Perkin Trans. 2*, (1980) 1376–1383.
19 K.-R. ROESER AND G. LEGLER, *Biochim. Biophys. Acta*, 657 (1981) 321–333.
20 R. E. HUBER AND M. T. GAUNT, *Can. J. Biochem.*, 60 (1982) 608–612.
21 J. F. MORRISON, *Trends Biochem. Sci.*, 7 (1982) 102–105.
22 J. C. IRVINE AND A. F. SKINNER, *J. Chem. Soc.*, (1926) 1089–1097.
23 G. LEGLER, *Hoppe-Seyler's Z. Physiol. Chem.*, 348 (1967) 1359–1366.
24 N. CONSTANZAS AND J. KOCOUREK, *Collect. Czech. Chem. Commun.*, 24 (1959) 1099–1103.
25 D. H. LEABACK, *Biochem. J.*, 78 (1961) 22P–23P.
26 D. ROBINSON, *Biochem. J.*, 63 (1956) 39–44.

27 J. D. HOCKING, D JOLLY, AND R. D. BATT, *Biochem. J.*, 128 (1972) 69–78.
28 L. ROSENFELD AND Y. C. LEE, *Carbohydr. Res.*, 46 (1970) 155–158.
29 G. LEGLER, *Biochim. Biophys. Acta*, 524 (1978) 94–101.
30 K. J. LAIDLER AND P. S. BUNTING, *The Chemical Kinetics of Enzyme Action*, 2nd edn., Clarendon Press, Oxford, 1973, p. 95.
31 D. F. WENTWORTH AND R. WOLFENDEN, *Biochemistry*, 13 (1974) 4715–4720.
32 M. L. SINNOTT, S. G. WITHERS, AND O. M. VIRATELLE, *Biochem. J.*, 175 (1978) 539–546.