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SYNTHESIS OF 5-AMINO-5-DEOXY-D-GALACTOPYRANOSE AND 1,5-DIDEOXY-1,5-IMINO-D-GALACTITOL, AND THEIR INHIBITION OF α- AND β-D-GALACTOSIDASES*

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ABSTRACT

A 12-step route is presented starting from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose for the preparation of the title compounds and their L-*altro* analogues. Their synthesis is based on the reduction with Raney nickel of a protected 5-hydroxyimino derivative of L-*arabino*-hexofuranos-5-ulose, with the following improvements for the preparation of a D-galactofuranose derivative: oxidation at C-3 with pyridinium dichromate–acetic anhydride, stereospecific reduction of a 3-*O*-acetyl-hex-3-enofuranose intermediate to the D-*gulo* derivative, and inversion at C-3 of its 3-tosylate with tetrabutylammonium acetate in chlorobenzene. α-D-Galactosidase from coffee beans and from *Escherichia coli* and β-D-galactosidase from *E. coli* and *Aspergillus wentii* were inhibited with K_i values that ranged from 0.0007 to 8.2 μM. Formation of the enzyme–inhibitor complexes with the D-galactose analogue was on the time-scale of minutes, whereas the D-galactitol analogue showed a slow approach to the inhibition only with α-D-galactosidase from coffee beans and β-D-galactosidase from *A. wentii*. *N*-Alkylation of the D-galactitol analogue was detrimental to the inhibition except for β-D-galactosidase from *E. coli* and β-D-glucosidase from almonds, but, even with these enzymes, the observed affinity enhancements were 10^2 to 10^3-times smaller than those of *N*-alkylated D-galactosylamine and D-glucosylamine.

INTRODUCTION

Replacement by the NH-group of the ring oxygen in D-glucose and D-mannose and in their 1,5-anhydro derivatives enhances up to 10^4-fold their inhibition of the respective α- and β-glycohydrolases[1,2]. In many instances, the enzyme–inhibitor complex is formed slowly with each type of inhibitor. From the strong inhibition, it was inferred that a carboxyl group at the catalytic site is in close proximity to the

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anomeric carbon of the bound substrate. This situation and a restricted access of water to the reaction centre appears to be a general feature of these enzymes.

Apart from providing theoretical insights into the mechanism of glucosidases and mannosidases, the basic analogues of the two hexopyranoses have proved to be versatile tools for studies of the biological function of individual enzymes[3]. *N*-Alkylation of the 1,5-dideoxy-1,5-imino-D-glycitols affords effective affinity ligands for enzyme purification[4,5] and improves the discrimination between enzymes having the same glycon specificity, *e.g.*, α-D-glucosidase I and II involved in the biosynthesis of asparagine-linked glycans of glycoproteins[4].

In order to explore further the potential of 5-amino-5-deoxyhexopyranoses in studies of glycohydrolases, we have synthesised 5-amino-5-deoxy-D-galacto-pyranose (**1**) and 1,5-dideoxy-1,5-imino-D-galactitol (**2**) and determined their inhibitory action on several α- and β-D-glucosidases. The reaction sequence was based on that used for the corresponding derivatives of D-glucose[6] and D-mannose[2]. A synthesis of **2** starting from 1,6-anhydro-α-D-galactofuranose has been described[7], but the inhibition of galactosidases apparently has not been investigated in detail.

By analogy with the *gluco* analogue nojirimycin, the names *manno*-nojirimycin, *galacto*-nojirimycin (**1**), *galacto*-1-deoxynojirimycin (**2**), *etc.*, are used for convenience.

RESULTS AND DISCUSSION

Synthesis. — Adaptation of the routes[2,6] for the synthesis of 5-amino-5-deoxyhexofuranoses for the preparation of the D-*galacto* isomer required protected D-galactofuranose derivatives. As these are not easily accessible, 1,2:5,6-di-*O*-isopropylidene-D-glucose (**3**) was used and transformed into the D-*galacto* derivative *via* the D-*gulo* epimer **6**. Important modifications of the procedure described[8] for the preparation of **6** were the oxidation of C-3 with pyridinium dichromate–acetic anhydride[9] and the reduction of the enol acetate **5** with Pd/H_2 instead of $NaBH_4$, which was carried out at −15 to −10° to ensure stereoselectivity. The subsequent inversion at C-3 was accomplished by reacting the tosylate **7** with tetrabutylammonium acetate in chlorobenzene, conditions much milder than those with sodium benzoate in *N*,*N*-dimethylformamide[10]. Further steps paralleled those described for the synthesis of nojirimycin[6], except that the oxidation at C-5 was carried out with pyridinium dichromate–acetic anhydride. As the acetyl group of **11** was still present in the oxime **12**, the latter was deacetylated with sodium methoxide in order to avoid an *O*→*N*-acetyl migration in the reduction step **12**→**13**.

Reduction of the oxime **12** with Raney nickel gave a 1.7:1 mixture of the D-*galacto* and L-*altro* derivatives **13a** and **13b** which was resolved by chromatography. The structural assignment of the two epimers followed from the enzymic oxidation of **1a** with D-galactose dehydrogenase and NAD^+, and from their inhibitory action on galactosidases (see below). The reaction with D-galactose

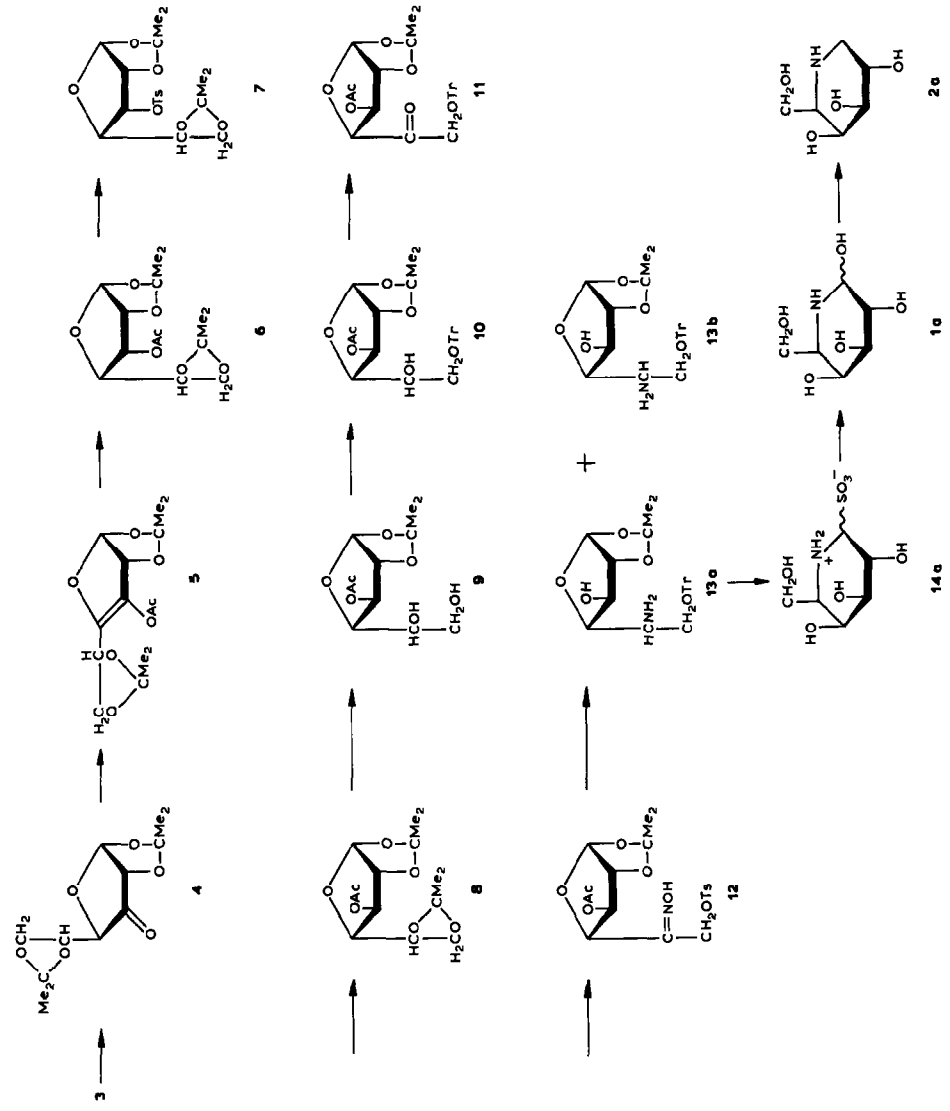

TABLE I

INHIBITION OF α- AND β-D-GALACTOSIDASES AND β-D-GLUCOSIDASES BY 5-AMINO-5-DEOXY ANALOGUES AND OTHER BASIC DERIVATIVES OF D-GALACTOSE

Enzyme	K_i *Values for competitive inhibition (μM)*				
	D-galacto-*Nojirimycin*	*1-Deoxy-*D-galacto-*nojirimycin*	*1-Deoxy-*N-*heptyl-*D-galacto-*nojirimycin*	D-*Galactosyl-amine*	D-*Galactose*
α-D-Galactosidase (coffee beans)	~0.0007[a]	0.0016[a]	3.2	7.5	180
α-D-Galactosidase (*E. coli*)	0.17[a]	0.24	1.6	86	50,000
β-D-Galactosidase (*E. coli*)	0.045[a]	12.5	0.26	7	21,000
β-D-Galactosidase (*Asp. wentii*)	0.011[a]	0.16[a]	—	13.4	1,300
β-D-Glucosidase (*Asp. wentii*)	400 (0.07[a])	— (0.28[a])	—	790 (0.17)	— (2,800)
β-D-Glucosidase (almonds)	22.4 (0.9[a])	540 (69)	47	2,000 (310)	(60,000)

[a]Slow approach to steady-state inhibition; values in brackets refer to the D-*gluco* analogue.

dehydrogenase also served to quantify the **1a** released from its hydrogensulfite adduct **14a**. Oxidation of **1a** at C-6 with D-galactose oxidase could not be observed. This enzyme apparently requires the pyranose ring oxygen of the substrate for its action.

The *N*-heptyl derivative of **2a** was prepared by reductive alkylation with heptanal as described for *N*-alkyl derivatives of 1-deoxynojirimycin[5].

Potentiometric titration gave dissociation constants corresponding to pK_a 5.1 for **1a** and 7.1 for **2a**. The value for **1a** is similar to those found for the D-*gluco*[6] and D-*manno* analogues[2], whereas the pK_a of **2a** is more like that of the D-*manno*[2] (7.2) than that of the D-*gluco* analogue[11] (6.33). Both the *manno* and *galacto* derivatives have an axial hydroxyl group in a 1,3-position with respect to the cationic nitrogen.

Inhibition studies. — Results of the inhibition of α- and β-D-galactosidases from various organisms by **1a** and **2a** are summarised in Table I. The data demonstrate that both α- and β-specific enzymes have a high affinity for these basic analogues of D-galactose. Comparison of the K_i values for **1a** and **2a** with those for D-galactose shows, in many instances, an even greater contribution of the basic nitrogen to binding than reported for analogous systems in the D-*gluco* and D-*manno* series[2]. As with the other enzymes, this strong inhibition is interpreted by the electrostatic interaction of an active-site carboxylate group with the protonated form of the inhibitor. The presence of an essential carboxylate group adjacent to C-1 of the bound galactose substrate had been demonstrated for α-D-galactosidase from coffee beans and β-D-galactosidase from *E. coli* by their covalent inhibition

by epoxides structurally related to D-galactose[12–14]. Another resemblance of the inhibition of galatosidases with other glycohydrolases is the slow onset of the inhibition in many instances. In order to minimise errors due to depletion of substrate or formation of product during the initial phase (1–5 min), measurements were made by preincubating the enzyme and inhibitor and starting the reaction by the addition of substrate.

An accurate evaluation of the inhibition of α-D-galactosidase from coffee beans by **1a** was complicated by an apparent positive co-operativity of inhibitor binding. The apparent K_i decreased from 0.9nM at [I] 0.06μM to 0.3nM with [I] 0.049μM. Dixon plots ($1/v$ *vs.* [I]) were strongly curved upwards at each substrate concentration. No anomalies were observed with **2a** as inhibitor.

The *N*-heptyl derivative of **2a** was studied because a large enhancement of the inhibition by *N*-alkyl substituents had been found with D-galactosylamine and D-glucosylamine when studied with β-D-galactosidase from *E. coli*[15] and β-D-glucosidase from almonds[16]. Also, β-D-glucosidase from calf-spleen lysosymes was inhibited up to 1000-fold better by long-chain *N*-alkyl derivatives of 1-deoxynojirimycin than by the parent compound[5]. It is seen from the data of Table I that *N*-alkylation of **2a** is detrimental to binding except for β-D-galactosidase from *E. coli* and β-D-glucosidase from almonds. But even here binding is enhanced only 50- and 11-fold, respectively, whereas *N*-heptyl-β-D-galactosylamine and *N*-benzyl-β-D-glucosylamine inhibited the respective glycosidases 10^4- and 10^3-fold, respectively, better than the parent compounds[15,16]. As the basicity of **2a** is not altered by *N*-alkylation (see above), any changes in K_i must be ascribed to steric effects. The results obtained with *N*-heptyl-**2a** indicate that enzyme–substrate interactions include the pyranose ring oxygen; substitution at this position sterically hinders binding.

Measurements with the two β-D-glucosidases were made in order to assess their glycon specificity with respect to the configuration at C-4. The enzyme from *A. wentii* exemplifies the extremely large discriminating ability[17] [K_i(galactosylamine)/K_i(glucosylamine) = 4,600] whereas, with the almond enzyme, this property is hardly developed at all: galactosylamine and glucosylamine are bound with equal affinity[18] or with K_i 6.5-fold lower for the glucosyl derivative (this study). The earlier findings with respect to the C-4 glycon specificity are fully confirmed by the data on the 5-amino-5-deoxypyranose inhibitors (Table I): K_i(Gal)/K_i(Glc) is 5,700 for the *Aspergillus* enzyme, and 7.8 (1-deoxynojirimycin) and 25 (nojirimycin) for almond β-D-glucosidase.

Data for glycosylamines are included in Table I in order to discuss the explanation by Fleet[19] of the strong inhibition of glycosidases by 1-deoxynojirimycin and its analogues in terms of the close structural relationship between good inhibitors and the transition state or labile intermediates. According to Fleet, the enzymic cleavage of a glycosidic bond could be initiated by the protonation of the pyranose ring oxygen. The resulting oxonium ion intermediate would be more similar to the protonated 1-deoxynojirimycins than the glycosyl oxocarbonium ion

normally assumed to be on the pathway of glycoside hydrolysis. The precursor to the glycosyl cation would be a substrate species protonated on, or strongly hydrogen-bonded to, the glycosyl oxygen by an acidic group of the catalytic site[20,21]. If the latter pathway predominated, glycosylamines should be better inhibitors than 1-deoxynojirimycins. On first sight, the results given in Table I seem to support Fleet's proposal for most enzymes, but a fair comparison can only be made in those instances where strong binding occurs rapidly. In the others, an initial rate of formation of product was observed that was identical, within the limits of error, with the rate in the absence of inhibitor. This then leaves α-D-galactosidase from *E. coli* as the only example among the six enzymes studied which is not in conflict with the proposal made by Fleet. Further studies are clearly required.

Inhibition by the L-*altro* derivative **1b** was studied with α-D-galactosidase from coffee beans and from *E. coli,* and K_i values of 18.4μM and >2mM, respectively, were found, *i.e.,* more than four orders of magnitude larger than for **1a**. In contrast with the slow onset of the inhibition by **1a**, the enzyme inhibitor equilibrium with the enzyme from coffee beans was reached within the temporal resolution of the assay procedure (<3 s). This finding demonstrates that the inhibition by **1b** is not due to a contamination of the L-*altro* compound by traces of the D-*galacto* isomer. It probably represents a non-specific interaction of the enzyme with basic polyhydroxy compounds as described[22] for β-D-galactosidase from *E. coli.*

EXPERIMENTAL

General procedures. — T.l.c. was performed on silica gel 60 (Merck), using toluene–methanol (9:1) and detection by charring with sulfuric acid. Melting points were determined on a Kofler hot-stage and are uncorrected. Optical rotations were measured on a Perkin–Elmer 141 polarimeter at 578 nm in 1-dm tubes. ^{1}H-N.m.r. spectra (90 MHz) were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Varian EM-390 spectrometer. Hydroxyl proton resonances were detected by deuterium exchange.

*1,2:5,6-Di-*O*-isopropylidene-α-*D*-ribo-hexofuranos-3-ulose* (**4**). — A solution of **3** (26 g), pyridinium dichromate (22.5 g), and acetic anhydride (31 mL) in dichloromethane (300 mL) was heated under reflux for 1 h, and then concentrated to about half its volume. The chromium salts were precipitated by the addition of 2 vol. of ethyl acetate and removed, and the dark filtrate was passed through a column (4 × 20 cm) of silica gel equilibrated and eluted with ethyl acetate. The eluate was concentrated, and toluene was evaporated from the residue to remove traces of pyridine and acetic acid. The resulting, colourless, viscous oil (24.4 g, 94%) was homogeneous by t.l.c.

A small sample, crystallised from toluene–light petroleum, gave **4**, m.p. 107–109°, $[\alpha]_{578}^{20}$ +39° (chloroform); lit.[8] m.p. 108–112°, $[\alpha]_D^{25}$ +40°.

*3-*O*-Acetyl-1,2:5,6-di-*O*-isopropylidene-α-*D*-gulofuranose* (**6**). — To a solu-

tion of **5**[8] (32.6 g) in methanol (750 mL) was added 20% $Pd(OH)_2$ on charcoal (~1 g, Aldrich), and the mixture was cooled to −25° and rapidly stirred under hydrogen. The temperature was then slowly raised to −15 to −10°. Monitoring by t.l.c. showed the reduction to be complete after ~7 h. The catalyst was removed and the filtrate concentrated to a viscous oil (30.5 g, 91%) which was deacetylated by methanolysis without further purification. A small sample was crystallised from light petroleum to give **6**[8] as long needles, m.p. 74–76°, $[\alpha]_{578}^{22}$ +70° (chloroform).

3-O-Acetyl-1,2:5,6-di-O-isopropylidene-α-D-galactofuranose (**8**). — A solution of 1,2:5,6-di-*O*-isopropylidene-3-*O*-tosyl-α-D-gulofuranose[10] (**7**, 29.8 g) and tetrabutylammonium acetate (20 g) in chlorobenzene (200 mL, dried over P_2O_5) was heated for 5 h under reflux and then concentrated, and the residue was partioned between water and ether. Unreacted **7** (4.6 g) was recovered from the ether phase by crystallisation from toluene–light petroleum (2:1). Addition of 4 vol. of light petroleum to the mother liquor gave **8** (14.4 g, 78% based on reacted **7**), m.p. 52–53°, $[\alpha]_{578}^{20}$ −8° (chloroform). ^{1}H-N.m.r. data: δ 1.30, 1.34, 1.41, and 1.56 (4 s, 12 H, 2 CMe_2), 2.12 (s, 3 H, Ac), 3.79–4.40 (m, 2 H, H-6,6), 4.02 (dd, 1 H, $J_{3,4}$ 2.7, $J_{4,5}$ 6.0 Hz, H-4), 4.39 (m, 1 H, H-5), 4.60 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-2), 4.97 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-3), 5.98 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1).

Anal. Calc. for $C_{14}H_{22}O_7$: C, 55.63; H, 7.28. Found: C, 55.38; H, 7.50.

3-O-Acetyl-1,2-O-isopropylidene-6-O-trityl-α-D-galactofuranose (**10**). — A solution of **8** (11.1 g) in aqueous 50% acetic acid (500 mL) was left at room temperature for 5 h, then concentrated at 25°, neutralised with saturated aqueous $NaHCO_3$, and extracted four times with chloroform. The combined extracts were dried ($MgSO_4$) and concentrated to give **9** (8.8 g) as a colourless, viscous oil which was used for the next step without further purification. ^{1}H-N.m.r. data: δ 1.38 and 1.60 (2 s, 6 H, CMe_2), 2.13 (s, 3 H, Ac), 2.50–2.95 (bm, 2 H, 2 OH), 3.76–4.27 (several m, 4 H, H-4,5,6,6), 4.74 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-2), 5.18 (s, 1 H, H-3), 6.06 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1).

A solution of **9** (7.9 g) and trityl chloride (18.1 g) in dry pyridine (180 mL) was heated at 70° for 7 h, then cooled, poured into ice–water (400 mL), and extracted with chloroform 5 × 100 mL). The combined extracts were washed with ice-cold dilute sulfuric acid until the aqueous phase had pH ~3 and then with saturated aqueous $NaHCO_3$, dried, and concentrated. The residue was crystallised from toluene–light petroleum to give **10** (14.8 g, 98%), m.p. 111–113°, $[\alpha]_{578}^{22}$ −7° (chloroform). ^{1}H-N.m.r. data: δ 1.33 and 1.55 (2 s, 6 H, CMe_2), 2.07 (s, 3 H, Ac), 2.67 (d, 1 H, $J_{5,OH}$ 5.7 Hz, OH), 3.30 (d, 2 H, $J_{5,6}$ 6.0 Hz, H-6,6), 4.0 (m, 1 H, H-5), 4.25 (dd, 1 H, $J_{3,4}$ 3.0 Hz, $J_{4,5}$ 6.0 Hz, H-4), 4.61 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-2), 5.93 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 7.25–7.56 (m, 15 H, CPh_3).

Anal. Calc. for $C_{30}H_{32}O_7$: C, 71.43; H, 6.35. Found: C, 71.68; H, 6.51.

3-O-Acetyl-1,2-O-isopropylidene-6-O-trityl-β-L-arabino-hexofuranos-5-ulose (**11**). — Compound **10** (14.0 g) was oxidised with pyridinium dichromate–acetic anhydride, as described for **4**, to give **11** (13.3 g, 95%), m.p. 158–160°, $[\alpha]_{578}^{23}$ −21° (chloroform). ^{1}H-N.m.r. data: δ 1.0 (bs, 6 H, CMe_2), 2.02 (s, 3 H, Ac), 4.16 and

4.17 (2 s, 2 H, H-6,6), 4.50 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-2), 5.60 (s, 1 H, H-3), 5.93 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 7.22–7.58 (m, 15 H, CPh_3).

Anal. Calc. for $C_{30}H_{30}O_7$: C, 71.72; H, 6.02. Found: C, 72.11; H, 6.10.

*5-Amino-5-deoxy-1,2-O-isopropylidene-6-O-trityl-α-*D*-galactofuranose* (**13a**) *and -β-*L*-altrofuranose* (**13b**). — Potassium hydrogencarbonate (11.8 g) and hydroxylammonium chloride (7.8 g) were heated under reflux in methanol (300 mL) for 10 min, **11** (12.5 g) was added, and heating continued for 30 min. The mixture was then filtered and concentrated to dryness, and the residue was extracted several times with chloroform. The combined extracts were washed with water and then concentrated to yield **12** (12.4 g, 96%) as a glassy foam. ^{1}H-N.m.r. data: δ 1.18 and 1.27 (2 s, 6 H, CMe_2), 2.00 (s, 3 H, Ac), 4.08 and 4.28 (2 d, 2 H, $J_{6,6}$ 13.5 Hz, H-6), 4.53 (d, 1 H, $J_{2,1}$ 4.5 Hz, H-2), 5.10 (s, 1 H, H-4), 5.60 (s, 1 H, H-3), 6.00 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 7.22–7.52 (m, 15 H, CPh_3), 8.5 (bs, 1 H, N-OH).

Compound **12** (3 g) was deacetylated with methanolic 0.01M sodium methoxide (50 mL). The solution was neutralised with CO_2 and concentrated, and the colourless oil was reduced with Raney-nickel, as described for nojirimycin[6], to give a mixture (2.3 g, 70%) of **13a** and **13b** as a pale-yellow viscous oil.

Elution of the mixture from a column (42 × 2.2 cm) of Silica C18 (particle size, 20 μm; Amicon) with methanol–water (3:1) containing 1.2% of ammonia at 5 mL/min (10-mL fractions and u.v. detection at 254 nm) gave **13a** in fractions 49–58 and **13b** in fractions 60–70. Base-line separation of the two isomers was possible with up to 150 mg of **13ab** per run. The yield from 600 mg of **13ab** was 305 mg of **13a** and 178 mg of **13b**, both obtained as a glassy mass; **13a** could be crystallised (toluene–light petroleum).

The D-*galacto* isomer **13a** had m.p. 135°, $[\alpha]_{578}^{23}$ −11.5° (chloroform). ^{1}H-N.m.r. data: δ 1.27 and 1.45 (2 s, 6 H, CMe_2), 1.94 (bm, 2 H, NH_2), 3.04–3.34 (m, 3 H, H-5,6), 3.78 (dd, 1 H, $J_{3,4}$ 3.9, $J_{4,5}$ 5.1 Hz, H-4), 4.08 (d, 1 H, $J_{3,4}$ 3.9 Hz, H-3), 4.46 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-2), 5.82 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 7.24–7.53 (m, 15 H, CPh_3).

Anal. Calc. for $C_{28}H_{31}NO_5$: C, 72.9; H, 6.72; N, 3.04. Found: C, 72.6; H, 6.43; N, 3.18.

The L-*altro* isomer **13b** had $[\alpha]_{578}^{20}$ −29° (chloroform). ^{1}H-N.m.r. data: δ 1.30 and 1.45 (2 s, 6 H, CMe_2), 3.65 (s, 3 H, NH_2 and OH), 3.03–3.8 (m, 4 H, H-4,5,6,6), 4.25 (d, 1 H, $J_{3,4}$ 4.5 Hz, H-3), 4.55 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-2), 5.80 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1), 7.1–7.5 (m, 15 H, CPh_3).

*5-Amino-5-deoxy-*D*-galactopyranose hydrogensulfite adduct* (**14a**). — To a solution of **13a** (700 mg) in methanol (5 mL) saturated with SO_2 at 0° was added water saturated with SO_2 (1 mL). The mixture was stored at 40° in a sealed vessel for 40 h, and then filtered to give **14a** (380 mg, 84%), m.p. 130–135° (dec.). The material was recrystallised by adding methanol saturated with SO_2 (4 vol.) to a solution of the free base **1a** (see below).

Anal. Calc. for $C_6H_{13}NO_7S$: C, 29.62; H, 5.39; N, 5.76; S, 13.18. Found: C, 27.74; H, 5.64; N, 5.83; S, 12.60.

*5-Amino-5-deoxy-*D*-galactopyranose* (**1a**). — To a suspension of **14a** (100 mg) in water was added 0.3M $Ba(OH)_2$ (1.27 mL), and the mixture was stirred at room temperature for 1 h while the pH was kept at ≥8. The mixture was then centrifuged and the precipitate was washed with water (2 mL). The pH of the combined supernatant solution and washings was adjusted to pH 7 and the solution was stored at 4° to give **1a** [0.37 mmol, 97%; determined with D-galactose dehydrogenase (see below)], which was stable for up to 5 days.

*5-Amino-5-deoxy-*L*-altropyranose hydrogensulfite adduct* (**14b**) *and 5-amino-5-deoxy-*L*-altropyranose* (**1b**). — Compound **13b** (150 mg) was treated with SO_2–methanol, as described for **14a**, to give **14b** (59 mg, 68%), m.p. 165–170 (dec.).

Anal. Calc. for $C_6H_{13}NO_7S$: C, 29.62; H, 5.39; N, 5.76; S, 13.18. Found: C, 29.73; H, 5.40; N, 5.82; S, 12.90.

Compound **14b** was converted into **1b** as described for **1a**. The concentration of **1b** was calculated from the amount of **14b** taken, assuming the same yield as for **1a**.

*1,5-Dideoxy-1,5-imino-*D*-galactitol* (**2a**). — A solution of **1a** (0.54 mmol) in water (4 mL) was hydrogenated for 2.5 h at 10 atm. after the addition of acetic acid (80 μL) and PtO_2 (20 mg), then filtered, and passed over Dowex 50 (H^+) resin (4 mL). The resin was washed with water, and **2a** (98%; potentiometric titration) was eluted with aqueous 10% ammonia (2 vol). Compound **2a** (80 mg, 74%), obtained as the hydrochloride by dissolving in water (3 mL), titrating to pH 1.5 with M HCl, concentrating to dryness, and crystallisation of the residue from methanol, had m.p. 250–255° (dec.), $[\alpha]_{578}^{22}$ +41° (water); lit.[7] m.p. 260° (dec.), $[\alpha]_D^{20}$ +46°.

*1,5-Dideoxy-*N*-heptyl-1,5-imino-*D*-galactitol.* — Compound **2a** was reductively alkylated with heptanal as described for *N*-hexyl- and *N*-decyl-1-deoxynojirimycin[5]. The product, after crystallisation from ethanol–water, had m.p. 128°, $[\alpha]_{578}^{22}$ −26° (methanol).

Anal. Calc. for $C_{13}H_{27}NO_4$: C, 59.74; H, 10.41; N, 5.36. Found: C, 59.81; H, 10.28; N, 5.52.

Determination of **1a** *with* D*-galactose dehydrogenase.* — The solution of **1a** (10 μL, previously adjusted to ~5 mM) was added to 100mM Tris/HCl buffer (pH 8.6, 3 mL) containing 0.5mM NAD^+ and D-galactose dehydrogenase from *Pseudomonas fluorescens* (0.1 unit, Boehringer Mannheim). A_{340} was recorded until a constant value was reached (~5 h), and the concentration of **1a** was calculated using $\Delta\varepsilon_{340} = 6{,}220\ M^{-1}.cm^{-1}$.

Enzymes and activity measurements. — Hydrolytic activities were assayed at 25° fluorimetrically with the respective 4-methylumbelliferyl glycosides[16] or by continuous recording of A_{410} with the respective 4-nitrophenyl glycosides. Conversion factors for the calculation of hydrolysis rates by the latter procedure were determined by adding known amounts of 4-nitrophenol to the substrate solution. Other conditions are given for each enzyme. When the steady-state hydrolysis rate in the presence of inhibitor was approached slowly, the rate was calculated from the slope of asymptotes drawn to the product curves.

α-D-Galactosidase from coffee beans (Boehringer Mannheim) was assayed with 4-nitrophenyl α-D-galactopyranoside in 100mM phosphate (pH 6.0); K_m 0.19mM. Accurate measurements in the presence of **1a** were not possible when the reaction was started by adding the enzyme to a mixture of substrate and inhibitor. The enzyme inhibitor equilibrium was approached so slowly that increasing product inhibition prevented approach to a constant rate. Therefore, the reaction was started by the addition of concentrated substrate to a mixture of enzyme and **1a** preincubated for 15 min.

α-D-Galactosidase from *Escherichia coli* (Boehringer Mannheim) was assayed as above in 50mM phosphate buffer (pH 7.2); K_m 0.06mM. Identical steady-state rates in the presence of **1a** were observed with either mode of assay.

β-D-Galactosidase from *E. coli* (Boehringer Mannheim) was assayed with 4-nitrophenyl β-D-galactopyranoside in 50mM phosphate buffer (pH 7.0) containing 145mM NaCl and mM $MgCl_2$; K_m 0.04mM.

β-D-Galactosidase from *A. wentii* was obtained as a spray-dried culture filtrate (Röhm) that was fractionated by ammonium sulfate precipitation and by chromatography on CM- and DEAE-Sephadex[23]. Assay with 4-methylumbelliferyl β-D-galactopyranoside in 50mM acetate buffer (pH 4.0); K_m 0.73mM.

β-D-Glucosidase from almonds (Sigma, type I) was assayed with 4-nitrophenyl β-D-glucoside in 50mM acetate buffer (pH 5.0); K_m 1.25mM. As judged from the relative rates with 20mM 4-nitrophenyl and mM 4-methylumbelliferyl β-D-glucopyranosides[16] (v_{PNPG}/v_{MUG} 2.8), this sample consisted of the B isoenzyme, whereas the almond β-D-glucosidase isolated from a Sigma, type II preparation[2] was the A isoenzyme.

β-D-Glucosidase A_3 from *A. wentii* was isolated from a spray-dried culture filtrate[24] and was assayed with 4-methylumbelliferyl β-D-glucopyranoside in 50mM acetate buffer (pH 5.0); K_m 0.24mM. The K_i for 1-deoxynojirimycin in Table I differs from the value reported previously[2] because of the different pH of the assay (4.0).

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