Carbohydrate Research, 167 (1987) 305–311
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthesis of (+)-1,5-dideoxy-1,5-imino-D-galactitol, a potent α-D-galactosidase inhibitor*

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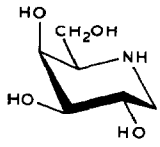
(Received October 3rd, 1986; accepted for publication in revised form, December 28th, 1986)

The glycosyl hydrolases (glycosidases) are widely distributed throughout Nature and are vital for the growth and development of most organisms[1]. Although mechanistic aspects of these enzymes remain controversial[2], current research on specific glycosidase inhibitors promises to develop exciting new therapeutic protocols for regulating the breakdown of carbohydrate foodstuffs, the processing of eukaryotic glycoproteins, and the catabolism of polysaccharides and glycoconjugates[3]. Up to now, most work in synthetic chemical[4] and biochemical[5] laboratories has focused on naturally occurring inhibitors of α- and β-D-glucosidases and α-D-mannosidases. D-Galactosidases, in particular, have been largely neglected, despite the fact that specific, genetically inherited deficiencies of these enzymes are responsible for inborn errors of glycosphingolipid metabolism[6]. In G_{M1} gangliosidosis, diminished activity of lysosomal acid β-D-galactosidase cause glycolipids and oligosaccharides having terminal β-D-galactosidic linkages to accumulate[7]. Fabry's disease, characterized by a deficiency of lysosomal α-D-galactosidase A, leads to storage of glycosphingolipids exhibiting terminal α-D-galactosyl residues in most visceral tissues[8]. Besides helping to probe questions of enzyme stereoselectivity and mechanism, the design and synthesis of sugar- and linkage-specific D-galactosidase inhibitors might be useful in developing biochemical and clinical models of these pathological conditions. Herein is described an efficient, enantiospecific, total synthesis of 1,5-dideoxy-1,5-imino-D-galactitol (**1**), first prepared by Paulsen *et al.*[9] in 1980. Compound **1**, named 1-deoxygalactonojirimycin in view of its relationship to the naturally occurring glycosidase inhibitors deoxynojirimycin[10] (**2**) and deoxymannojirimycin[11] (**3**), is a potent and selective α-D-galactosidase inhibitor that may be useful in developing a reversible, animal model of Fabry's disease.

*Presented at the XIIIth International Carbohydrate Symposium, Ithaca, August 10–15, 1986.

0008-6215/87/$ 03.50



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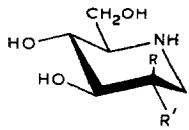
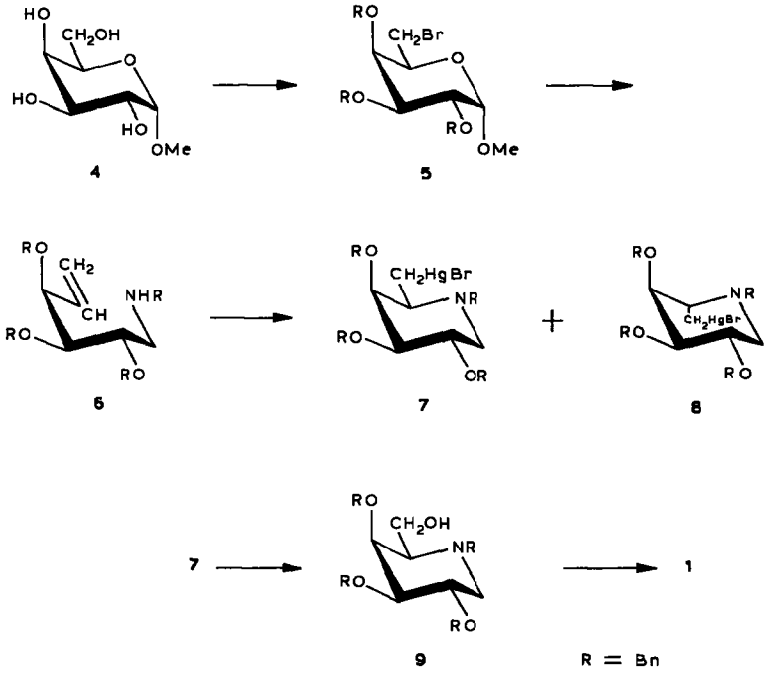


2 R = H, R′ = OH
3 R = OH, R′ = H

Recently, we reported[12] an expeditious, new synthesis of **2** and **3** from D-glucose and D-mannose, respectively. By a combination of reductive ring-opening based on a procedure developed by Bernet and Vasella[13] and subsequent intramolecular aminomercuration, each natural sugar was converted into the corresponding aza-alditol having the same relative and absolute configuration caused by substituents on the piperidine ring. Thus, commercially available methyl α-D-galactopyranoside (**4**; Sigma; dried azeotropically before use) was converted into methyl 2,3,4-tri-*O*-benzyl-6-bromo-6-deoxy-α-D-galactopyranoside (**5**) by the method of Bernet and Vasella[13], except that purifications after the tritylation and benzylation steps were omitted. Using this sequence, 30 g of **5** could be efficiently prepared in 61% overall yield from **4**. By coupling the reductive ring-opening of such bromopyranosides with an *in situ* reductive amination of the intermediate alkenic aldehydes, it proved possible to minimize the overreduction and elimination problems normally associated with such sensitive carbonyl compounds. Thus, heating bromide **5** with zinc, benzylamine, and $NaBH_3CN$ in 19:1 1-propanol–water at reflux afforded aminoalkene **6** in 90% yield. On treatment with $HgBr_2$ (conditions of thermodynamic control), **6** afforded a chromatographically inseparable mixture of **7** (45%) and **8** (45%) along with 10% of an unidentified product. Kinetically controlled cyclization with $Hg(OAc)_2$ favored the desired equatorial organomercurial (15:2:3 ratios of **6**:**7**:unknown). This crude mixture was reductively oxygenated by a modification of the Hill and Whitesides procedure[14], using $NaBH_4$–O_2–DMF to afford equatorial alcohol **9** in 54% overall yield from **6**, along with a small proportion of the axial epimer. Catalytic debenzylation of **9** afforded aza-alditol **1** (88% yield) whose physical properties and spectral constants, notably its well resolved, first-order, 300-MHz ^{1}H-n.m.r. spectrum, agreed with literature values[9].

Compound **1** proved to be an extremely potent and selective α-D-galactosidase inhibitor. Assays *in vitro* showed that **1** competitively inhibits the hydrolysis of *p*-nitrophenyl α-D-galactopyranoside (K_M 1.1mM) by green coffee-bean α-galactosidase (K_i 1.6nM; 50% inhibition of enzymic activity at 400nM). Against human-placental ceramide trihexosidase, the α-D-galactosidase implicated in Fabry's disease, the IC_{50} was determined to be 4nM. Although high concentrations (1mM of **1**) caused no inhibition of bovine β-D-galactosidase, rat-intestinal lactase was effectively inhibited at an IC_{50} of ~60mM. (Paulsen *et al.*[9] noted that **1** also inhibits pig-intestinal lactase). Jack-bean α-D-mannosidase, yeast α-D-glucosidase, almond β-D-gluco-



sidase, bovine-liver β-D-glucosiduronase, and bovine *N*-acetyl-β-D-hexosaminidase were also unaffected at mM concentration.

In preliminary animal studies, administration of **1** to female C57 mice and male beige mice cause modest to substantial elevations in total kidney-glycolipid and ceramide trihexoside levels[15].

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover Unimelt apparatus and are uncorrected. ^{1}H-n.m.r. spectra were recorded at 300 MHz with a Bruker WM-300 spectrometer. For solutions of samples in $CDCl_3$, tetramethylsilane was used as an internal standard, and for those in D_2O, the HOD peak was used as an internal reference. Chemical-ionization mass spectrometry (c.i.m.s.) was performed by using isobutane as reagent gas.

Nomenclature and numbering. — Although structures **1** and **9** are substituted piperidines, the "imino-galactitol" designation permits the conventional, carbohydrate-numbering system to be employed in n.m.r. assignments.

Methyl 2,3,4-tri-O-benzyl-6-bromo-6-deoxy-α-D-galactopyranoside (**5**). — A solution of methyl α-D-galactopyranoside (**4**, 15.0 g, 77.3 mmol) in pyridine (200 mL) was dried by distilling off 25% of the solvent. After cooling the solution to room temperature, trityl chloride (21.9 g, 77.3 mmol) was added, and the mixture

was heated for 1.5 h at 90°, cooled, diluted with saturated Na_2CO_3 (200 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The extracts were combined, dried (Na_2SO_4), and evaporated *in vacuo*, to afford the oily monotrityl derivative, which was dissolved in DMF (50 mL), and the solution added during 0.5 h to NaH (10.5 g, 440 mmol) in DMF (250 mL) maintained at 0°. After 30 min, benzyl bromide (42 mL, 350 mmol) was added and, 3 h later, the mixture was warmed to room temperature, and DMF (200 mL) was added. The mixture was stirred overnight, and then treated with sufficient acetic acid to decompose the excess of NaH. Water (800 mL) was added, and the mixture was extracted with ether (4 × 500 mL). The extracts were combined, dried ($MgSO_4$), and evaporated *in vacuo*, to afford the 2,3,4-tri-*O*-benzyl-6-*O*-trityl derivative, which was dissolved in a mixture of conc. H_2SO_4 (13 mL) and MeOH (800 mL). After stirring for 90 min at room temp., the mixture was basified with 10% NaOH, diluted with water, and extracted with CH_2Cl_2 (3 × 300 mL). The extracts were combined, dried, evaporated *in vacuo*, and chromatographed with 1:4 and then 1:1 EtOAc–hexane, to afford 31.6 g (94%) of the detritylated compound as a viscous oil; R_F 0.5 in 1: EtOAc–hexane. To a solution of this oil and triethylamine (17 mL, 0.12 mol) in CH_2Cl_2 (500 mL) at 0° was added mesyl chloride (8.0 mL, 70 mmol) and, after 2 h, the mixture was diluted with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 150 mL). The extracts were combined, dried, and evaporated *in vacuo*, to afford an oil which was heated at reflux for 2.3 h with butanone (450 mL) and LiBr (150 g). The mixture was cooled, diluted with water (500 mL), and extracted with CH_2Cl_2 (3 × 200 mL). The extracts were combined, dried, evaporated *in vacuo*, and the residue chromatographed with 1:4 EtOAc–hexane, to afford 24.8 g of **5** (61% from methyl α-D-galactopyranoside) as a viscous oil; $[\alpha]_D^{20}$ +27.1° (*c* 1.8, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.40–7.26 (15 H, benzyl), 5.00, 4.87 (ABq, OCH_2Ph, *J* 11.3 Hz), 4.82, 4.74 (ABq OCH_2Ph, *J* 12 Hz), 4.66, 4.60 (ABq, OCH_2Ph, *J* 11.5 Hz), 4.65 (d, H-1, *J* 3.6 Hz), 4.02–3.98 (m, H-2,4), 3.92 (dd, H-3, $J_{3,4}$ 2.6, $J_{2,3}$ 10.2 Hz), 3.87 (t, H-5, *J* 6.7 Hz), 3.39 (s, OCH_3), 3.29, and 3.25 (ABq, H-6,6′, J_{vic} 6.7, J_{gem} 10.1 Hz); ν_{max}^{film} 3095, 3065, 3035, 2960–2840, 1610, 1590, 1495, 1455, 1350, 1195, 1150–1040, 910, 730, and 695 cm^{-1}; c.i.m.s. *m/z* 517, 529 (3% each, M + 1), 495 (7%), and 181 (100%).

Anal. Calc. for $C_{28}H_{31}BrO_5$: C, 63.87; H, 5.89; Br, 15.02. Found: C, 63.92; H, 5.98; Br, 15.54.

(2S,3S,4S)-1-(Benzylamino)-2,3,4-tri-(benzyloxy)-5-hexene (**6**). — A solution of bromide **5** (2.97 g, 5.63 mmol) in 19:1 1-propanol–water (100 mL) was added to acid-treated zinc[13] (12 g, 320 mmol). Benzylamine (9.5 mL, 82 mmol) and $NaBH_3CN$ (0.71 g, 11 mmol) were added, and the mixture was stirred and heated at reflux for 2 h, cooled, filtered through Celite, and the solid washed with EtOH. The filtrates were combined, and evaporated *in vacuo* to a solid which was treated with ether (200 mL) and 20% HCl (20 mL). After 1 h, the mixture was basified with 15% NaOH, diluted with water (300 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The extracts were combined, dried, and evaporated *in vacuo*, and the residue chromatographed with 3:3 EtOAc–hexane, to afford 2.59 g (90%) of **6** as an oil;

$[\alpha]_D^{20}$ +8.8° (*c* 1.1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.38–7.16 (20 H, *N*-,*O*-benzyl), 5.97 (ddd, H-5, J_{vic} 8.0, J_{cis} 10.4, J_{trans} 17.4 Hz), 5.37–5.25 (m, 2 H), 4.79, (d, 1 H, *J* 11.3 Hz), 4.69–4.50 (m, 4 H), 4.28 (1 H), 4.00 (dd, 1 H, *J* 4.7, 7.9 Hz), 3.82–3.72 (2 H), 3.68 (1 H, *J* 13.4 Hz), 2.75 (dd, 1 H, *J* 1.9, 9.4 Hz), and 2.70 (dd, 1 H, *J* 3.4, 9.4 Hz); ν_{max}^{film} 3330, 3090, 3065, 3035, 2960–2840, 1610, 1590, 1495, 1455, 1205, 1140–1050, 1035, 730, and 695 cm^{-1}; c.i.m.s. *m/z* 509 (40%) and 508 (M + 1, 100%).

Anal. Calc. for $C_{34}H_{37}NO_3$: C, 80.44; H, 7.35; N, 2.76. Found: C, 80.27; H, 7.54; N, 2.71.

N-*Benzyltri*-O-*benzyl-1,5-dideoxy-1,5-imino*-D-*galactitol* (**9**). — Mercuric trifluoroacetate (2.37 g, 5.55 mmol) was added to a solution of aminoalkene **6** (2.56 g, 5.05 mmol) in THF (80 mL) at room temp. After 1 h, saturated $NaHCO_3$ (20 mL) was added, followed 10 min later by saturated KBr solution (20 mL). After stirring for 2.5 h, the mixture was diluted with water (200 mL), and extracted with CH_2Cl_2 (3 × 100 mL). The extracts were combined, dried, and evaporated *in vacuo*, to give 4.71 g of an oil which, by n.m.r. analysis, contained both **7** (75%) and **8** (10%). This mixture was dissolved in DMF (10 mL) and added during 1.5 h to a mixture of $NaBH_4$ (335 mg, 8.8 mmol) with oxygen-saturated DMF (65 mL) in a 100-mL, graduated cylinder. Oxygen was vigorously bubbled through the mixture during the addition and for 1 h after the addition was complete; then, the mixture was treated with 10% HCl (40 mL) and stirred for 30 min. The solution was diluted with water, basified to pH 12 with 15% NaOH, and extracted with ether (4 × 150 mL). The extracts were combined, dried, and evaporated *in vacuo*, and the residue was chromatographed with 1:3 EtOAc–hexane, to afford 1.43 g (54%) of **9** as an oil, along with 250 mg (10%) of recovered aminoalkene **6**.

For **9**: $[\alpha]_D^{20}$ +20.7° (*c* 2.1, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$): δ 7.36–7.21 (20 H, *N*-,*O*-benzyl), 4.80, 4.65 (ABq, OCH_2Ph, *J* 11.6 Hz), 4.73 (s, OCH_2Ph), 4.51 (s, OCH_2Ph), 4.12 (t, H-4, *J* 3.4 Hz), 3.98 (m, 1 H), 3.95 (s, NCH_2Ph), 3.82–3.66 (m, 3 H), 3.11 (dd, H-1*a*, *J* 3.3, 12.9 Hz), 2.80 (br. m, H-5), and 2.39 (bm, H-1*e*); ν_{max}^{film} 3440, 3090, 3065, 3035, 2940–2800, 1605, 1590, 1495, 1455, 1365, 1350, 1265, 1205, 1140–1020, 730, and 695 cm^{-1}; c.i.m.s. *m/z* 526 (14%), 525 (38%), and 524 (M + 1, 100%).

Anal. Calc. for $C_{34}H_{37}NO_4$: C, 77.98; H, 7.12; N, 2.67. Found: C, 77.96; H, 7.02; N, 2.61.

1,5-Dideoxy-1,5-imino-D-*galactitol hydrochloride* (**1**). — A solution of amino alcohol **9** (1.09 g, 2.08 mmol) in a mixture of EtOH (50 mL) and 4M methanolic HCl (0.6 mL) was evaporated *in vacuo*. The solid residue was dissolved in EtOH (15 mL), 10% Pd–C (290 mg) was added, and hydrogen was bubbled directly through the suspension for 28 h. The mixture was filtered through Celite, the filtrate concentrated *in vacuo*, and the concentrate stirred with elemental sulfur (100 mg) while H_2S was bubbled through the mixture, a treatment that removed colloidal Hg and traces of residual organomercurials. Compound **1**·HCl (366 mg, 88%) was obtained as a crystalline solid. The product could be recrystallized from 49:1

EtOH–H_2O by slow evaporation of the solvent under a stream of argon, to give white, irregular prisms: m.p. 240.0–241.5° (dec.), [lit.[9] m.p. 260° (dec.)], $[\alpha]_D^{20}$ +44.2° (*c* 0.45, 9:1 MeOH–water), lit.[9] $[\alpha]_D^{20}$ +46.1° (*c* 0.90, water); ^{1}H-n.m.r. (D_2O): δ 4.17 (dd, H-4), 4.09 (ddd, H-2), 3.89, 3.82 (ddd, H-6,6'), 3.65 (dd, H-3), 3.52 (dd, H-1*a*), 3.42 (ddd, H-5), and 2.88 (t, H-1*a*); $J_{1a,1e}$ 12.0, $J_{1a,2}$ 12.2, $J_{1e,2}$ 5.3, $J_{2,3}$ 9.6, $J_{3,4}$ 2.9, $J_{4,5}$ 1.3, $J_{5,6}$ 5.0, $J_{5,6'}$ 8.6, and $J_{6,6'}$ 12.2 Hz; ν_{max}^{KBr} 3430, 3380, 3340, 3300, 3020, 2980, 2500, 1605, 1455, 1420, 1395, 1080, 1020, 930, and 860 cm^{-1}; c.i.m.s. *m/z* 165 (9%), and 164 (M + 1 − HCl, 100%).

Bioassays. — Compound **1** was tested against green coffee-bean α-D-galactosidase, bovine β-D-galactosidase, jack-bean α-D-mannosidase, yeast α-D-glucosidase, almond β-D-glucosidase, bovine-liver β-D-glucosiduronase, and bovine *N*-acetyl-β-D-hexosaminidase, using the corresponding monosaccharide *p*-nitrophenyl α- or β-D-glycoside as substrate at pH 5.00 (50mM HOAc–NaOAc buffer) and a substrate concentration of 5mM. Assays were conducted in disposable, rimless culture-tubes (10 × 75 mm; Kimble Company) which had been rinsed with 1% aqueous bovine serum albumin solution, and then baked to dryness, a procedure that prevented enzyme from sticking to the test-tube walls. Enzyme–buffer–inhibitor mixtures (200 μL total volume; triplicate runs) were pre-incubated for 5 min at 37°, substrate was then added and, after incubation (15 min), each reaction was quenched with glycine buffer, pH 10.4, and the absorbance of the mixture read at 400 nm, using a glycine-buffer blank. In control runs, distilled, de-ionized water was substituted for the inhibitor.

ACKNOWLEDGMENTS

We thank Dr. James R. Rasmussen (Genzyme Corporation) for kindly testing **1** against human-placental, ceramide trihexosidase, and Dr. Peter Daniel (E. R. Shriver Center) for performing the rat-intestinal lactase assay. This work was supported by a grant from the National Institutes of Health (Grant No. GM 35712).

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