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α- and β-Homogalactonojirimycins (α- and β-Homogalactostatins): Synthesis and Further Biological Evaluation

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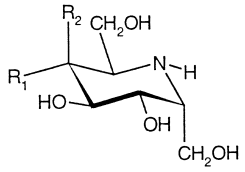
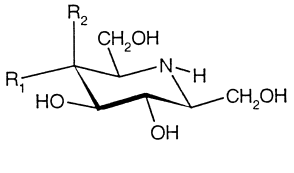
Abstract—The homoiminosugars α- and β-homogalactonojirimycins were prepared from a common intermediate, tetra-*O*-benzyl-D-*galacto*-heptenitol **6**, by way of highly stereoselective reaction sequences involving, as the key steps, an internal amidomercuration (α-epimer) and a double reductive amination (β-epimer). α-Homogalactonojirimycin retains a large part of the potent activity of the parent galactonojirimycin and 1-deoxygalactonojirimycin as an inhibitor of α-galactosidases. However, by contrast with the parent iminosugars, it does not inhibit β-galactosidases, with the exception of the Jack beans enzyme. β-Homogalactonojirimycin is a weak α-galactosidase inhibitor and is completely devoid of activity towards β-galactosidases. Thus, a marked selectivity toward one family of enzymes has been achieved by the addition of an α-CH_2OH group in the structure of the parent iminosugars.

Introduction

As a result of their remarkable biological activities, primarily as glycosidase inhibitors, iminosugars have been the object of an intense research effort during the past decade.[1] Potential therapeutic applications, for example for the treatment of lysosomal storage diseases (Fabry[2] and Gaucher[3] diseases) or of viral infections,[4] are now starting to emerge. These useful properties have prompted the search for more efficient and/or more selective compounds and iminosugar derivatives of great structural diversity have been prepared[5,6] or isolated from natural sources.[1b] One modification of the archetypal 1-deoxynojirimycin structure that has received particular attention is the addition of a CH_2OH group at the pseudoanomeric carbon such as, for example, in α- and β-homonojirimycin **1**[7] and **2**.[8,9]



1 $R_1 = OH, R_2 = H$
3 $R_1 = H, R_2 = OH$



2 $R_1 = OH, R_2 = H$
4 $R_1 = H, R_2 = OH$

Such compounds are truly homoiminosugars since they result formally from the insertion of a CH_2 group into the anomeric C–O bond of the labile 5-amino-5-deoxyhexoses, and constitute also the simplest examples of iminosugar C-glycosides. Both **1** and **2** are natural products which, interestingly, have been synthesised[7–9] before their discovery in Nature.[10,11] While **1** is a potent inhibitor of several α-glucosidases,[10,12] **2** is a very weak β-glucosidase inhibitor.[9,12] Homoiminosugars in the D-*gluco*, D-*manno*, L-*fuco*, and D-*allo*[13] series as well as *N*-alkylated derivatives have been the object of extensive studies.[12,13]

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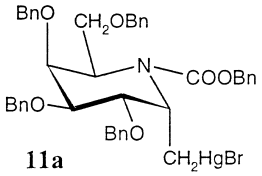
We have reported in a preliminary communication[14] the first synthesis of the *galacto* epimer of **1**, namely α-homogalactonojirimycin **3** (α-homogalactostatin). We have also recently[15] completed the synthesis of the β-epimer **4**, β-homogalactonojirimycin (β-homogalactostatin), by way of an internal double reductive amination, process that we had developed for the synthesis of β-homonojirimycin **2**.[9] We wish to report in this article the full details of our syntheses of **3** and **4** as well as to provide new data on the activities of **3** and **4** as glycosidase inhibitors. The synthesis of **3** and **4** by a different approach, as well as their activity toward selected galactosidases, have been reported recently by Fleet and co-workers.[16]

Results and Discussion

Synthesis

Our synthesis of **3** was achieved in 10 steps from a D-galactose derivative (**5**) by way of a chain–extension–amination–cyclisation sequence (Scheme 1). This approach was designed on the basis of the expectation that the cyclisation of a D-*galacto*-configured aminoheptenitol such as **11** by internal amidomercuration would proceed with a very high degree of stereoselectivity[7a] and provide the desired pseudo-α-epimer (i.e., an iminoheptitol with the D-*glycero*-L-*galacto* configuration).

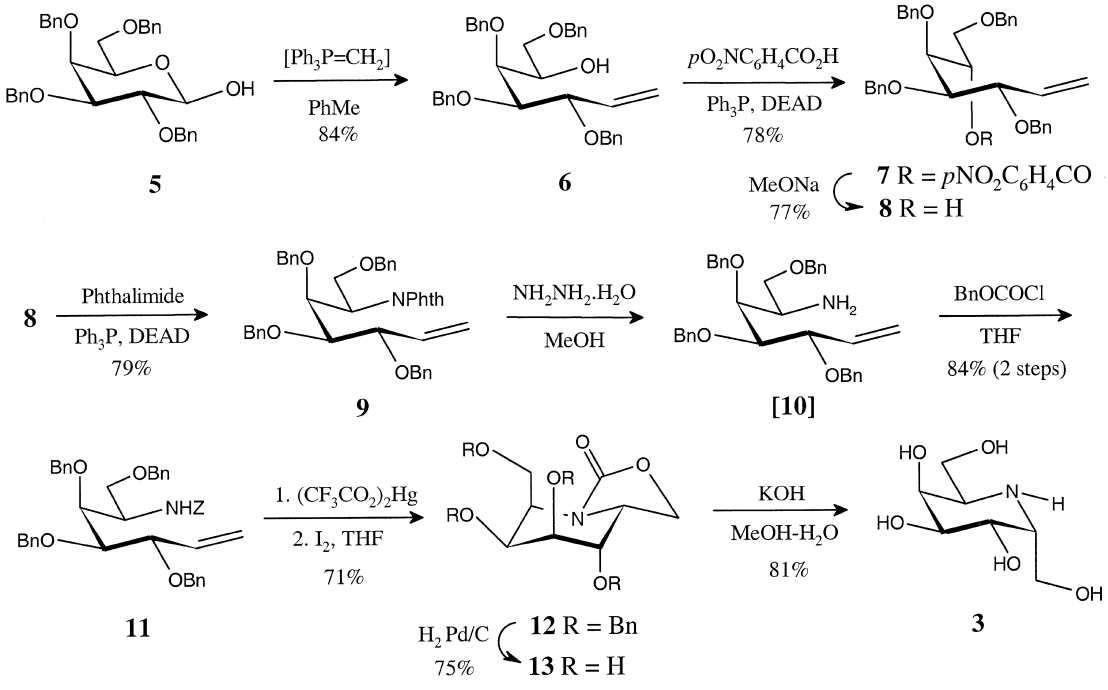
Wittig methylenation of tetra-*O*-benzyl-D-galactopyranose **5** by Sinaÿ's procedure[17] as previously described[18] gave the corresponding heptenitol **6** in high yield. The substitution of the 6-OH group in **6** by an amino group using an oxidation–reductive amination sequence or the reduction of an oxime gave preponderantly the undesired L-*altro*-epimer.[19] However, the D-*galacto* amino heptenitol derivative **9** could be obtained from **6** by a double inversion process under Mitsunobu's conditions, by way of the L-*altro* heptenitol **8**. It is noteworthy that the two displacement reactions proceeded without complications: similar reactions of the D-*gluco* epimer of **6** are indeed accompanied by rearrangements and lead to useless mixtures of several products.[20]



11a

Exchange of the protecting group of the amine function provided the benzyloxycarbonyl derivative **11** which was submitted to internal amidomercuration using mercury(II) trifluoroacetate.[21] This reaction led to a labile organomercurial which could be isolated as bromomercury derivative **11a** in 76% yield after ligand exchange with KBr and flash chromatography. This product was then treated with iodine to achieve iododemercuration; remarkably, the reaction gave directly the cyclic carbamate **12** in 89% yield. Isolation of the organomercurial intermediate was not necessary: treatment of **11** with the mercury(II) salt followed by in situ treatment of the resulting intermediate with iodine gave **12** in 71% isolated yield. This very useful reaction provided thus directly the oxygen-functionalized methylene group as a result of the participation of the vicinal benzyl carbamate. In addition, the cyclisation was highly stereoselective and no pseudo-β-epimer could be detected.

The NMR parameters of cyclic carbamate **12** indicated that the six-membered ring adopts a conformation different from the usual $^4C_1$(D) form of D-galactopyranose



5 **6** **7** **8** **9** **[10]** **11** **12** **13** **3**

Scheme 1.

derivatives. The $^3J_{H,H}$ coupling constants provide evidence that the conformation is near an inverted $^1C_4$ chair conformation, a consequence of the strain imposed by the fused five-membered ring.

Deprotection of **12** in two steps afforded α-homogalactonojirimycin **3** in 15% overall yield from **5**. The ring $^3J_{H,H}$ coupling constants and in particular the magnitude of $J_{4,5}$ (9.8 Hz) indicated that **3** adopts in solution a chair conformation similar to that of the parent D-galactopyranosides.

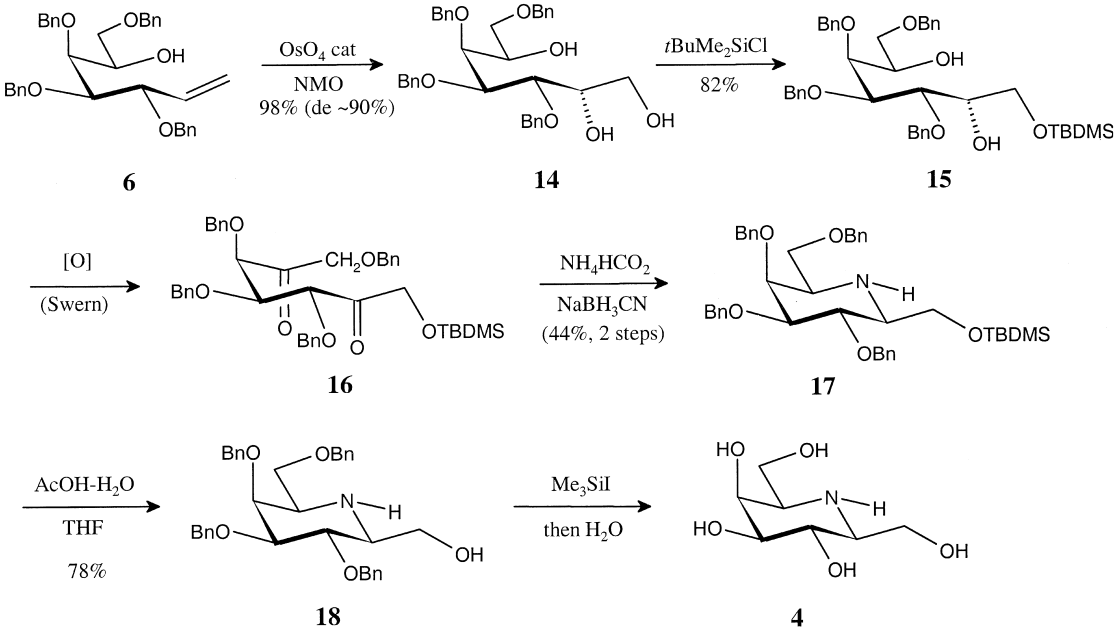
The epimeric β-homogalactonojirimycin **4** was reached in six steps from the same heptenitol **6** (Scheme 2). The synthetic plan consisted in forming the piperidine ring of **4** by way of a double reductive amination process;[22] according to our previous studies in the nojirimycin (D-*gluco*) series,[9] we had predicted that the double reductive amination of an L-*arabino*-2,6-heptodiulose derivative such as **16** would afford predominantly if not exclusively a homoiminosugar having the L-*glycero*-L-*galacto* (pseudo-β-D-*galacto*) configuration. The diulose **16** was obtained in three steps from **6**: dihydroxylation of the double bond with catalytic OsO_4 gave the L-*glycero*-L-*galacto* heptitol derivative **14** in high yield and with a high degree of stereoselectivity (6*S* configuration, d.e. ≅90%); a sample of diastereomerically pure **14** could be obtained by crystallization from MeOH and its configuration was unambiguously established by further chemical transformation (see below). Selective protection of the primary alcohol function of **14** as a *t*-butyldimethylsilyl ether gave the diol **15** which was oxidized to the corresponding diketone under Swern conditions. The crude diketone was submitted to reductive amination conditions (ammonium formate and $NaBH_3CN$): this reaction gave a single piperidine derivative, **17**, in 44% combined yield from **15**. As shown clearly by its NMR parameters, compound **17** has a pseudo-β-D-*galacto* configuration and adopts a chair conformation with four equatorial substituents and a single axial substituent. As expected, both reduction steps were highly stereoselective; their selectivity was not affected by the presence of the incipient axial substituent in the intermediate cyclic iminium ions derived from **16** and ammonia.[23]

Cleavage of the *t*-butyldimethylsilyl ether of **17** gave **18**, a partially protected β-homogalactonojirimycin derivative particularly useful for the synthesis of further glycomimetics containing an aza-galactose unit. Final debenzylation of **18** using iodotrimethylsilane or by catalytic hydrogenation afforded 'β-homogalactonojirimycin' **4**, thus completing a seven step synthesis from **5** (∼20% overall yield).

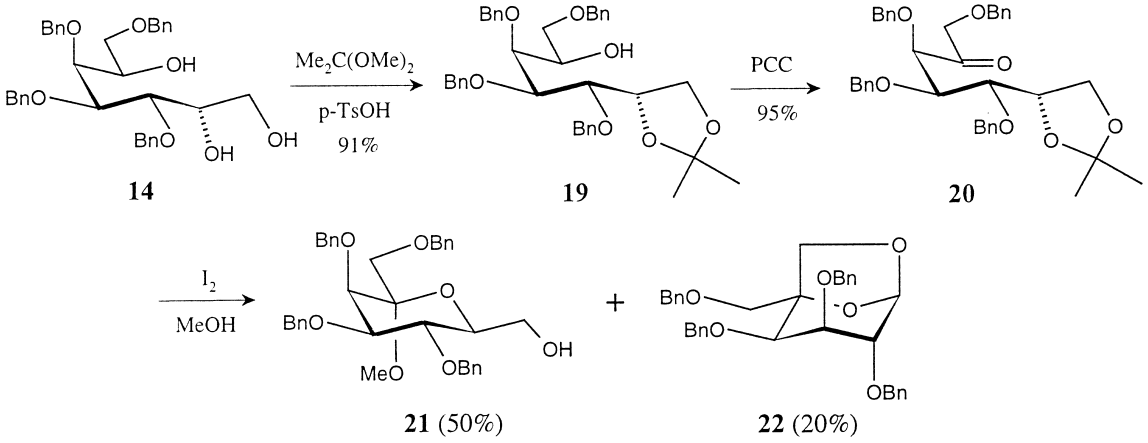
The configuration of **14** was determined as follows (Scheme 3). Protection of the vicinal diol function of **14** by formation of an isopropylidene acetal afforded compound **19**. The isolated alcohol function of **19** was then oxidized to give a fully protected 2-heptulose derivative (**20**). The L-*manno* configuration of this heptulose was established on the basis of the NMR parameters of the pyranoside **21** formed upon cleavage of the isopropylidene group from **20** using iodine in MeOH. This reaction gave also a small amount of the 2,7-anhydro-L-*manno*-heptulose derivative **22**. The major stereoisomer (**14**) resulting from the dihydroxylation of **6** is thus the one predicted from Kishi's empirical rule.[24]

Enzymatic assays

Compounds **3** and **4** have been submitted to a screening of their activity as inhibitors toward a wide range of glycosidases. The most significant results, namely their action on galactosidases, are gathered in Table 1 and compared with the reported activity of the parent

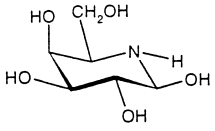
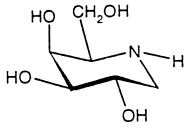
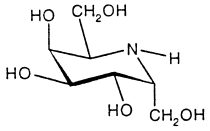
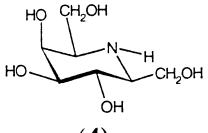


Scheme 2.



Scheme 3.

Table 1. Inhibition of α- and β-galactosidases by galactonojirimycin, 1-deoxygalactonojirimycin and their homo analogues **3** and **4**. IC_{50} and K_i values are in μM

Iminosugars					 (3)		 (4)	
Enzymes:	IC_{50}	K_i	IC_{50}	K_i	IC_{50}	K_i	IC_{50}	K_i
α-Gal, Coffee beans	—	0.0007[a]	0.003[b]	0.0016[a]	0.022	0.015	2.4	0.107
α-Gal, Rat epididymis	—	—	—	—	4.5	nd	nd	
α-Gal, Human α-Gal.A	—	—	0.004[c]	—	0.21	0.17	nd	
α-Gal, *E. Coli*	—	0.17[a]	—	0.24[a]	68	1.4	17% inh. at 1 mM	
β-Gal, *E.Coli*	0.17[d]	0.045[a]	—	12.5[a]	NI		NI	
β-Gal, Bovine liver	616[d]	—	—	NI	NI		NI	
β-Gal, *A. niger*	—		—		NI		NI	
β-Gal, Jack beans	0.494[d]	—	74% inh. at 1 mM[e]		4.7	0.86	NI	

[a]Ref 25.
[b]Ref 30.
[c]Ref 26.
[d]Ref 28.
[e]Ref 27. nd = not determined. NI = no inhibition.

galactonojirimycin (5-amino-5-deoxy-D-galactose)[25,28] and 1-deoxygalactonojirimycin.[26,27] In addition, α-homogalactonojirimycin **3** is an inhibitor of chicken liver α-*N*-acetylgalactosaminidase ($IC_{50} = 10\,\mu M$, $K_i = 1.7\,\mu M$), and the β-epimer **4** is a weaker inhibitor of the same enzyme ($IC_{50} = 220\,\mu M$, $K_i = 78\,\mu M$). It was found that **3** and **4** did not inhibit the following enzymes:

Bovine epididymis α-L-fucosidase	*Caldocellum sacch.* β-glucosidase
Yeast and rice α-glucosidases	Jack beans and almonds α-mannosidases
Bakers' yeast isomaltase	Snail β-mannosidase
A. niger amyloglucosidase	*A. niger* β-xylosidase
Rhizopus amyloglucosidase	Jack beans β-*N*-acetylglucosaminidase
Almonds β-glucosidase	Bovine epididymis A & B β-*N*-acetylglucosaminidase

The cyclic carbamate derivative of **3**, compound **13**, was also evaluated as an inhibitor of all the enzymes indicated above, with the exception of the rat epididymis galactosidase and of the human α-galactosidase A. As expected from its altered shape and of the non-basic character of its nitrogen atom, no activity was found for this compound (1 mM concentration), save a very weak effect on the coffee beans α-galactosidase (58% inhibition at 1 mM concentration).

The homogalactonojirimycins **3** and **4** are extremely specific for enzymes that hydrolyse galactosides: by contrast with galactonojirimycin[25] and its 1-deoxy derivative,[29] which retain some activity toward certain glucosidases, **3** and **4** are not inhibitors of any of the enzymes that act on non-*galacto* substrates.

Furthermore, α-homoiminosugar **3** remains a potent inhibitor of α-galactosidases, with K_i values for competitive inhibition one order of magnitude larger than those for 1-deoxygalactonojirimycin. Interestingly, compound **3** is a good inhibitor of human lysosomal galactosidase A (α-Gal A): this enzyme is responsible for one of the

steps involved in the degradation of glycosphingolipids in the lysosome (hydrolysis of the terminal galactose unit from a ceramide trihexoside) and a deficiency in this enzyme is the cause of Fabry's disease.[2] We have shown recently that low concentrations of α-Gal A inhibitors could be used to restore the mutant enzyme's activity in Fabry's patients and that compound **3** was one of the most active agents.[30] These studies provide the basis of a method of treatment of this lysosomal storage disease ('chemical chaperone therapy'). By contrast with both galactonojirimycin and its 1-deoxy derivative, α-homogalactonojirimycin is completely devoid of activity toward β-galactosidases, with the exception of the Jack beans enzyme. It is particularly significant to note that a high degree of selectivity was achieved between two galactosidases of the same microorganism: while the parent iminosugar and iminohexitol inhibit both the α- and the β-galactosidases of *E. coli*, the presence of the α-CH_2OH group in the homo analogue **3** abolished the activity toward the β-glycosidase, a result that emphasizes the usefulness of such a structural change.

β-Homogalactonojirimycin **4** is a generally weaker inhibitor than **3**. Compound **4** still retains some activity towards the coffee beans α-galactosidase. However, as had been noted by Fleet and co-workers,[16b] compound **4** is devoid of inhibitory activity towards all the β-galactosidases investigated. Thus, if an increased selectivity towards α-galactosidases is observed for the α-homoiminosugar **3**, a similar trend does not exist for the β-epimer: the presence of the β-CH_2OH group is detrimental to the biological activity of this compound. This behaviour parallels that of α- and β-homonojirimycins **1** and **2** towards α- and β-glucosidases.

The detrimental effect of the β-CH_2OH group may be justified by steric effects. However, as it is now well documented for β-glucosidases,[31] the lack of a heteroatom at the pseudo-anomeric center may be an important factor as well: the fact that galactonojirimycin (i.e. 5-amino-5-deoxy-D-galactose) is generally a potent inhibitor of mammalian β-galactosidases,[28] much more potent than its 1-deoxy derivative, may indeed be explained by the fact that the amino-sugar can exist with a β-hydroxy group and thus interact favorably with a lateral proton donor.[31]

Conclusion

The synthesis of α- and β-homogalactonojirimycins **3** and **4** has been achieved in nine and six steps respectively from a common precursor, D-*galacto*-heptenitol derivative **6**. Both compounds were obtained by way of highly stereoselective processes which provided control of the pseudoanomeric configuration, namely an internal amido-mercuration for the α-epimer, and a double reductive amination of a 2,6-heptodiulose for the β-epimer.

Compound **3** is a potent α-galactosidase inhibitor; it is much more selective for α-galactoside-hydrolysing enzymes than the parent galactonojirimycin and 1-deoxygalactonojirimycin. The β-epimer **4** is a weaker inhibitor devoid of all activity toward β-galactosidases. These results demonstrate that it is possible to modulate the selectivity of iminosugars in the 1-deoxynojirimycin series by the addition of a hydroxymethyl substituent in the α-orientation.

Experimental

Optical rotations were measured with an automatic polarimeter for solutions in a 0.1 dm cell at 22 ± 3 °C. ^1H and ^{13}C NMR spectra were recorded at 360 and 90 MHz, or at 400 and 100 MHz, respectively, on a Bruker AM-360 instrument or on a JEOL JNM-GX 400 spectrometer, using chloroform-*d* as the solvent unless otherwise indicated and TMS as the internal reference ($\delta_{TMS} = 0.00$ ppm). TSP (sodium 3-trimethylsilylpropionate) was used as the reference for spectra recorded in D_2O ($\delta_{TSP} = 0.00$ ppm). For ^{13}C NMR spectra in $CDCl_3$, the signal of the solvent ($\delta = 77.00$) was used as the reference. Chemical shifts (in ppm) and coupling constants (in Hz) were obtained from first-order analysis of the spectra. A minus sign (−) is used to indicate negative ^{13}C NMR-signals in the DEPT mode ($\theta_y = 135°$).

Analytical TLC was performed on glass plates precoated with silica gel 60 F-254 as the adsorbent (layer thickness: 0.25 mm). The developed plates were air-dried, exposed to UV light for inspection, sprayed with a solution of ammonium phosphomolybdate, and heated to 120–140 °C. Flash chromatography was performed using silica gel 60 (230–400 mesh). The following solvent systems were used: A, hexane/ethyl acetate 1:1; B, 1:3; C, 1:4; D, 1:5; E, 1:6; F, 1:9.

Separations by HPLC were achieved using a preparative HPLC system equipped with a gradient programmer, a variable wavelength UV detector, and a 21.2×250 mm preparative column of silica gel 60 (10 μm).

Solvents were evaporated under reduced pressure and below 40 °C. Catalytic hydrogenations and hydrogenolysis under pressure (P > 1 atm) were performed in a stainless steel benchtop pressure reactor equipped with a magnetic stirring drive and a pressure and temperature controller (maximum pressure: 2000 psig).

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-D-*galacto*-hept-1-enitol (6). To a suspension of methyltriphenylphosphonium bromide (7.10 g, 19.9 mmol) in dry toluene (120 mL) was added dropwise a 1.6 M solution of butyllithium in hexane (12 mL, 19.2 mmol) at 0 °C under N_2 and the reaction mixture was stirred for 2 h at room temperature. A suspension of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranose **14** (3.58 g, 6.62 mmol) in dry toluene (30 mL) was added in one portion and the reaction mixture was stirred for 48 h at the same temperature. The reaction was quenched with acetone (20 mL), the mixture was diluted with ether and extracted with water (100 mL). The aqueous phase was extracted with ether (2×100 mL), the organic layers

were combined, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (solvent C) to afford **6** (3.0 g, 84%) as a syrup: $[\alpha]_D^{18}$ −10.8 (*c* 1.2, $CHCl_3$); ^1H NMR: δ 3.04 (d, 1H, $J_{6,OH}$ = 5.2 Hz, HO-C6), 3.49 (dd, 1H, $J_{7A,6}$ = 6.5, $J_{7A,7B}$ = 9.4 Hz, H-7A), 3.54 (dd, 1H, $J_{7B,6}$ = 6.2 Hz, H-7B), 3.8 (dd, 1H, $J_{5,4}$ = 5.9, $J_{5,6}$ = 1.5 Hz, H-5), 3.83 (dd, 1H, $J_{4,3}$ = 4.0 Hz, H-4), 4.09 (dd, 1H, $J_{3,2}$ = 7.9, $J_{3,1A}$ = 0.4, $J_{3,1B}$ = 0.8 Hz, H-3), 4.13 (m, 1H, H-6), 4.34–4.77 (m, 8H, 4 OCH_AH_BPh), 5.32 (ddd, 1H, $J_{1A,1B}$ = 1.6, $J_{1A,2}$ = 10.3 Hz, H-1A), 5.35 (ddd, 1H, $J_{1B,2}$ = 17.4 Hz, H-1B), 5.89 (ddd, 1H, H-2), 7.16–7.38 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ 69.57 (C-6), 70.21, 71.11, 73.00 (2C) and 75.06 [all (−), C-7 and 4 OCH_2Ph], 76.61, 80.68 and 82.06 (C-3,4,5), 118.94 [(−), C-1], 127.4–128.2 (ArCH), 135.66 (C-2), 137.98, 138.08, 138.13 and 138.18 (4 ArC). Anal. calcd for $C_{35}H_{38}O_5$: C, 78.04; H, 7.11. Found: C, 77.72; H, 7.11.

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-6-*O*-(4-nitrobenzoyl)-L-*altro*-hept-1-enitol (7). To a cold (0 °C), stirred solution of **6** (130 mg, 0.24 mmol), triphenylphosphine (184 mg, 0.72 mmol) and 4-nitrobenzoic acid (160 mg, 0.96 mmol) in dry THF (20 mL) was added dropwise DEAD (0.15 mL, 0.95 mmol) under N_2. After having been stirred overnight, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash chromatography (solvent F) to afford **7** (130 mg, 78%) as a syrup. ^1H NMR: δ 3.74 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 5.3 Hz, H-4), 3.80 (m, 1H, $J_{7A,6}$ = 3.5, $J_{7A,7B}$ = 11 Hz, H-7A), 3.90 (m, 1H, $J_{7B,6}$ = 6.5 Hz, H-7B), 4.07 (dd, 1H, $J_{5,6}$ = 3.5 Hz, H-5), 4.12 (dd, 1H, $J_{3,2}$ = 7.6 Hz, H-3), 4.22–4.81 (m, 8H, 4 OCH_AH_BPh), 5.32 (dd, 1H, $J_{1A,1B}$ = 1.5, $J_{1A,2}$ = 10.4 Hz, H-1A), 5.38 (dd, 1H, $J_{2,1B}$ = 17.6 Hz, H-1B), 5.80 (dt, 1H, H-6), 5.92 (ddd, 1H, H-2), 7.18–7.53 (m, 20H, 4 C_6H_5), 7.86–8.24 (m, 4H, C_6H_4); ^{13}C NMR: δ 68.71, 70.54, 72.91, 73.17 and 74.49 [all (−), C-7 and 4 OCH_2Ph], 74.68, 78.77, 80.74 and 81.69 (C-3,4,5 and 6), 119.14 [(−), C-1)], 123.44, 127.47–128.33 and 130.70 (ArCH), 135.58 (C-2), 135.79, 137.97, 138.07 and 138.26 (4 ArC), 163.80 (C=O).

3,4,5,7-Tetra-*O*-benzyl-1,2-dideoxy-L-*altro*-hept-1-enitol (8). To a solution of *p*-nitrobenzoate **7** (110 mg, 0.16 mmol) in MeOH (3 mL) was added a solution of MeONa (5 mg) in MeOH (0.8 mL). The reaction mixture was stirred for 5 h, and then neutralised with Amberlite® IR-120 (H^+) ion exchange resin. The resin was removed by filtration, washed with MeOH and water, and the filtrate was concentrated. The residue was purified by flash chromatography (solvent D) to give **8** (70 mg, 77%) as a syrup: $[\alpha]_D^{18}$ −10.8 (*c* 1.2, $CHCl_3$); ^1H NMR: δ 2.82 (d, 1H, $J_{HO,6}$ = 4.0 Hz, HO-C6), 3.61 (dd, 1H, $J_{7A,6}$ = 6.3, $J_{7A,7B}$ = 9.9 Hz, H-7A), 3.67 (dd, 1H, $J_{7B,6}$ = 3.0 Hz, H-7B), 3.78 (dd, 1H, $J_{5,4}$ = 4.0, $J_{5,6}$ = 6.2 Hz, H-5), 3.8 (dd, 1H, $J_{4,3}$ = 5.4 Hz, H-4), 4.14 (m, 1H, H-6), 4.15 (br dd, 1H, $J_{3,2}$ = 7.8, $J_{3,1A}$ = 0.6, $J_{3,1B}$ = 0.9 Hz, H-3), 4.37–4.81 (s and 3 AB, 8H, 4 OCH_AH_BPh), 5.31 (ddd, 1H, $J_{1A,1B}$ = 1.7, $J_{1A,2}$ = 10.3 Hz, H-1A), 5.34 (ddd, 1H, $J_{1B,2}$ = 17.3 Hz, H-1B), 5.9 (ddd, 1H, H-2), 7.16–7.35 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ 70.52 [(−) C-7], 70.90 (C-6), 71.35, 72.74, 73.22 and 74.60 [all (−), 4 OCH_2Ph], 79.43, 81.46, 82.51 (C-3, 4, 5), 118.77 [(−), C-1], 127.39–128.24 (ArCH), 135.83 (C-2), 138.14, 138.28, 138.30 and 138.42 (4ArC). Anal. calcd for $C_{35}H_{38}O_5$: C, 78.04; H, 7.11. Found: C, 78.08; H, 7.15.

3,4,5,7-Tetra-*O*-benzyl-1,2,6-trideoxy-6-phthalimido-D-*galacto*-hept-1-enitol (9). To a solution of heptenitol **8** (730 mg, 1.36 mmol), triphenylphosphine (1.07 g, 4.08 mmol) and phthalimide (400 mg, 4.71 mmol) in dry THF (80 mL) was added dropwise DEAD (0.64 mL, 4.06 mmol) at room temperature under N_2. After having been stirred overnight, the reaction mixture was concentrated and the residue was triturated with ether (5 mL). The precipitate was removed by filtration and the filtrate concentrated under reduced pressure. The residue was purified by flash chromatography (solvent D) to afford **9** (720 mg, 79%) as a syrup: $[\alpha]_D^{18}$ +5.8 (*c* 1.55, $CHCl_3$); ^1H NMR: δ 3.74 (dd, 1H, $J_{4,3}$ = 6.6, $J_{4,5}$ = 2.8 Hz, H-4), 3.77 (dd, 1H, $J_{7A,6}$ = 5.1, $J_{7A,7B}$ = 10.0 Hz, H-7A), 4.03 (br t, 1H, $J_{7B,6}$ = 9.4 Hz, H-7B), 4.06 (br dd, 1H, $J_{3,2}$ = 7.6, $J_{3,1A}$ = 1.0, $J_{3,1B}$ = 0.5 Hz, H-3), 4.21 (dd, 1H, $J_{5,6}$ = 8.3 Hz, H-5), 4.26–4.84 (m, 8H, 4 OCH_AH_BPh), 4.92 (ddd, 1H, H-6), 5.37 (ddd, 1H, $J_{1A,1B}$ = 1.7, $J_{1A,2}$ = 17.4 Hz, H-1A), 5.38 (ddd, 1H, $J_{1B,2}$ = 10.3 Hz, H-1B), 5.92 (ddd, 1H, H-2), 6.97–7.72 (m, 24H, 4 C_6H_5, PhthN); ^{13}C NMR: δ 52.25 (C-6), 67.07, 70.71, 72.37, 73.15 and 74.64 [all (−), C-7, 4 OCH_2Ph], 77.12, 81.45, 83.05 (C-3, 4, 5), 119.41 [(−), C-1)], 122.91, 127.2–128.2 and 133.46 (ArCH), 135.48 (C-2), 132.08 and 138.02–138.5 (5ArC), 168.64 (C=O). Anal. calcd for $C_{43}H_{41}NO_6$: C, 77.34; H, 6.19. N, 2.10. Found: C, 77.26; H, 6.20; N, 2.08.

3,4,5,7-Tetra-*O*-benzyl-6-[(benzyloxycarbonyl)amino]-1,2,6-trideoxy-D-*galacto*-hept-1-enitol (11). Compound **9** (830 mg, 1.24 mmol) was heated in MeOH (80 mL) in the presence of hydrazine hydrate (3 mL) at a $T \leq 70$ °C for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was immediately dissolved in THF (100 mL) containing anhydrous K_2CO_3 (15 g) and the slurry was stirred under N_2 at 0 °C for 5 min. A solution of benzyl chloroformate (0.8 mL) in THF (5 mL) was added at 0 °C and the mixture was stirred for 1 h at the same temperature. Water (30 mL) was then added and the mixture was extracted with ether (2×50 mL). The organic phases were combined, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (solvent E) to afford compound **11** (700 mg, 84%) as a syrup; $[\alpha]_D^{23}$ +1.7 (*c* 1.15, $CHCl_3$); ^1H NMR: δ 3.45 (br t, 1H, $J_{7A,6}$ = 8.4, $J_{7A,7B}$ = 9.3 Hz, H-7A), 3.55 (dd, 1H, $J_{7B,6}$ = 5.8 Hz, H-7B), 3.59 (dd, 1H, $J_{4,3}$ = 3.7, $J_{4,5}$ = 7.5 Hz, H-4), 4.02 (dd, 1H, $J_{5,6}$ = 1.1 Hz, H-5), 4.11 (dd, 1H, $J_{3,2}$ = 7.8 Hz, H-3), 4.33 (dddd, 1H, $J_{6,NH}$ = 8.3 Hz, H-6), 4.30–4.65 (m, 8H, 4 OCH_AH_BPh), 5.04 (d, 1H, $J_{A,B}$ = 12.1 Hz, Cbz-H_A), 5.07 (d, 1H, Cbz-H_B), 5.31 (br d, 1H, $J_{1A,1B}$ = 0, $J_{1A,2}$ = 10.3 Hz, H-1A), 5.31 (d, 1H, NH), 5.37 (br d, 1H, $J_{1B,2}$ = 17.4 Hz, H-1B), 5.96 (ddd, 1H, H-2), 7.07–7.37 (m, 25H, 5 C_6H_5); ^{13}C NMR: δ 50.30 (C-6), 66.56, 69.59, 70.19, 72.79, 73.47 and 74.96 [all (−), C-7, 5 OCH_2Ph], 75.89, 80.50, 81.42 (C-3, 4, 5), 118.68 [(−), C-1)], 127.40–128.36 (ArCH), 136.07 (C-2), 138.03 (2C) and 138.34 (2C) (ArC), 156.02 (C=O). Anal. calcd for $C_{43}H_{45}NO_6$: C, 76.87; H, 6.75. N, 2.08. Found: C, 76.52; H, 6.76; N, 2.08.

1,3,4,5-Tetra-*O*-benzyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*galacto*-heptitol *O*(7), *N*-cyclic carbamate (12). A solution of compound **11** (200 mg, 0.31 mmol) and mercuric trifluoroacetate (250 mg, 0.62 mmol) in dry THF (15 mL) was stirred for 48 h at room temperature in the dark and under N_2. Solid $NaHCO_3$ (~200 mg) was then added and the suspension stirred for 2 h. To the cold (0 °C) reaction mixture was added dropwise a solution of I_2 (500 mg) in THF (30 mL) until persistence of the brown color. The mixture was stirred for 45 min (0–25 °C). An aqueous solution of $Na_2S_2O_3$ [10% (w/v), 10 mL] was then added. The mixture was extracted with ether (2×40 mL). The organic phases were combined, dried (Na_2SO_4), filtered over Celite and concentrated. The residue was purified by flash chromatography (solvent C then A) to give iminoheptitol **12** (127 mg, 71%) as a syrup: $[\alpha]_D^{19}$ +32.4 (*c* 3.3, $CHCl_3$); ^{1}H NMR: δ 3.32 (dd, 1H, $J_{5,6}$ = 2.4, $J_{4,5}$ = 3.9 Hz, H-5), 3.82 (br dd, 1H, $J_{3,4}$ = 2.7 Hz, H-4), 3.87 (dd, 1H, $J_{1A,2}$ = 3.0, $J_{1A,1B}$ = 11.3 Hz, H-1A), 3.94 (dd, 1H, $J_{2,3}$ = 6.8 Hz, H-3), 3.95 (ddd, 1H, $J_{6,7A}$ = 4.0, $J_{6,7B}$ = 8.0 Hz, H-6), 4.01 (dd, 1H, $J_{1B,2}$ = 9.9 Hz, H-1B), 4.08 (dd, 1H, $J_{7A,7B}$ = 8.6 Hz, H-7A), 4.15 (br t, 1H, H-7B), 4.30–4.72 (m, 8H, 4 OCH_2Ph), 4.56 (br ddd, 1H, H-2), 7.10–7.38 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ 49.61 and 51.63 (C-2,6), 62.82 and 65.59 (C-1,7), 71.67, 72.33, 72.38 and 73.84 [all (−), 4 OCH_2Ph], 73.35, 74.00, 75.38 (C-3, 4,5), 127.50–128.60 (ArCH), 137.13, 137.86, 138.04 and 138.48 (4ArC), 157.69 (C=O). Anal. calcd for $C_{36}H_{37}NO_6$: C, 74.59; H, 6.43; N, 2.42. Found: C, 74.67; H, 6.44; N, 2.37.

2,6-Dideoxy-2,6-imino-D-*glycero*-L-*galacto*-heptitol *O*(7), *N*-cyclic carbamate (13). To a solution of compound **12** (100 mg, 0.17 mmol) in a mixture of AcOH (10 mL) and EtOH (40 mL), Pd/C (10% Pd, 100 mg) was added and the reaction mixture was stirred overnight under H_2 (90 psi, 50 °C) in a Parr hydrogenation apparatus. The catalyst was removed by filtration and the solution concentrated under reduced pressure to give compound **13** (28 mg, 75%) as a solid: mp 210 °C (dec.); $[\alpha]_D^{18}$ −36.1 (*c* 0.36, H_2O); IR ν_{max} (film) 1728 (C=O). ^{1}H NMR (D_2O): δ 3.78 (dd, 1H, $J_{1A,1B}$ = 12.9, $J_{1A,2}$ = 1.3 Hz, H-1A), 3.80 (dd, 1H, $J_{5,6}$ = 2.0, $J_{4,5}$ = 5.8 Hz, H-5), 3.91 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 5.8 Hz, H-4), 3.95 (dd, 1H, $J_{1B,2}$ = 8.4 Hz, H-1B), 3.99 (ddd, 1H, $J_{2,3}$ = 2.9 Hz, H-2), 4.15 (dd, 1H, H-3), 4.24 (ddd, 1H, $J_{6,7A}$ = 4.2, $J_{6,7B}$ = 9.0 Hz, H-6), 4.31 (dd, 1H, H-7A), 4.41 (t, 1H, $J_{7A,7B}$ = 9.0 Hz, H-1B); ^{13}C NMR (D_2O; internal reference CH_3OH δ 49.60): δ 50.47 and 56.28 (C-2,6), 58.43 and 64.88 [(−), C-1,7], 65.03, 69.79, 71.34 (C-3, 4, 5), 160.65 (C=O). Anal. calcd for $C_8H_{13}NO_6$: C, 43.84; H, 5.98; N, 6.39. Found: C, 43.60; H, 6.05; N, 6.17.

2,6-Dideoxy-2,6-imino-D-*glycero*-L-*galacto*-heptitol (α-homogalactonojirimycin) (3). To a solution of **13** (14 mg, 0.064 mmol) in MeOH (1 mL) was added 50% aqueous KOH (5 mL) and the mixture was stirred overnight at room temperature. Thereafter the solution was heated to 60 °C for 2 h. The pH of the cooled reaction mixture was then reduced to ~9 using MeOH-washed IR-120(H^+) ion-exchange resin. The resin was removed by filtration and the volume of the filtrate was reduced to about 1 mL. The solution was loaded onto an ion-exchange column (IR-120 (H^+) resin). The resin was washed with water and the product was then eluted with 10% aqueous NH_3. The eluted fractions were concentrated, thus affording iminoheptitol **3** (10 mg, 81%). A very pure sample was obtained by chromatography on Dowex 1X2 (OH^-) ion exchange resin (elution with H_2O), followed by chromatography on CG-50 (NH_4^+) ion exchange resin (elution with water) (yield of purification: ~60%). $[\alpha]_D$ +86.3 (*c* 0.15, H_2O) [lit.[16b] $[\alpha]_D$ +72.0 (*c* 0.54, H_2O)]; ^{1}H NMR (D_2O, 400 MHz): δ 3.05 (m, 1H, H-2), 3.30 (m, 1H, H-6), 3.67 (dd, 1H, $J_{1A,2}$ = 7.5, $J_{1A,1B}$ = 11.0 Hz, H-1A), 3.70 (dd, 1H, $J_{3,4}$ = 3.0, $J_{4,5}$ = 9.8 Hz, H-4), 3.72 (dd, 1H, $J_{1B,2}$ = 5.8 Hz, H-1B), 3.78–3.85 (m, 2H, H-7A,7B), 4.00 (dd, 1H, $J_{2,3}$ = 2.6 Hz, H-3), 4.04 (dd, 1H, $J_{5,6}$ = 5.9 Hz, H-5); ^{13}C NMR (D_2O, 100 MHz): good agreement with lit. data.[16b] HR-FABMS (matrix: glycerol), calcd for $C_7H_{16}NO_5$ ($[M+H]^+$): *m*/*z* 194.1029; found: 194.1028.

1,3,4,5-Tetra-*O*-benzyl-L-*glycero*-L-*galacto*-heptitol (14). To a solution of heptenitol **6** (1.88 g, 4.49 mmol) and *N*-methylmorpholine *N*-oxide (900 mg, 7.68 mmol) in a mixture of acetone (36 mL) and water (9 mL) was added a 2.5% (w/v) solution of OsO_4 in *t*BuOH (1.8 mL) and the reaction mixture was stirred for 14 h at room temperature. A 5% (w/v) solution of $NaHSO_3$ in water (20 mL) was then added, the resulting mixture was passed through a layer of Florisil and the Florisil layer was washed with EtOAc (100 mL). The eluted phase was extracted with N H_2SO_4 (50 mL), water (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated to afford a homogeneous mixture of L-*glycero*-L-*galacto* and D-*glycero*-L-*galacto* epimers [~20:1(NMR), 1.96 g, 98%]. The major epimer **14** was obtained as a solid by crystallization from MeOH: mp 101–102 °C; $[\alpha]_D$ −16 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 3.49 (dd, 1H, $J_{1A,1B}$ = 9.4, $J_{1A,2}$ = 6.5 Hz, H-1A), 3.55 (dd, 1H, $J_{1B,2}$ = 5.9 Hz, H-1B), 3.61 (dd, 1H, $J_{7A,6}$ = 5.1, $J_{7A,7B}$ = 11.5 Hz, H-7A), 3.68 (dd, 1H, $J_{7B,6}$ = 5.1 Hz, H-7B), 3.74 (dd, 1H, $J_{5,4}$ = 4.0, $J_{5,6}$ = 7.2 Hz, H-5), 3.89 (ddd, 1H, H-6), 3.92 (dd, 1H, $J_{3,2}$ = 2.1, $J_{3,4}$ = 5.3 Hz, H-3), 3.94 (dd, 1H, H-4), 4.13 (ddd, 1H, H-2), 4.41–4.73 (m, 8H, 4 OCH_AH_BPh), 7.20–7.32 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ 63.62 [(−) C-7], 69.76 (C-2), 71.07 [(−) C-1], 71.66 (C-6), 73.30, 73.40, 74.09 and 74.13 [all (−), 4 OCH_2Ph], 78.15 (C-3), 78.51 (C-5), 79.61 (C-4), 127.7–128.4 (ArCH), 137.73, 137.78, 137.85 and 137.90 (4 ArC). Anal. calcd for $C_{35}H_{40}O_7$: C, 73.40; H, 7.04. Found C, 73.23; H, 7.08.

1,3,4,5-Tetra-*O*-benzyl-7-*O*-(*t*-butyldimethylsilyl)-L-*glycero*-L-*galacto*-heptitol (15). To a solution of the mixture of epimeric triols obtained in the preceding reaction (1.54 g, 2.7 mmol) in anhydrous CH_2Cl_2 (10 mL) was added successively DMAP (80 mg), Et_3N (0.56 mL) and *t*-butyldimethylsilyl chloride (450 mg, 3.0 mmol). The mixture was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and the residue purified by flash chromatography (solvent D) to afford a mixture of silylated epimers [1.54 g, 82%; ratio ~20:1 (NMR)]. Data for the major isomer (**15**): ^{1}H NMR: δ 0.04 and 0.05 [2s, 6H, $Si(CH_3)_2$], 0.89 [s, 9H, $SiC(CH_3)_3$], 3.52 (dd, 1 H, $J_{1A,1B}$ = 9.4, $J_{1A,2}$ = 6.5 Hz,

H-1A), 3.58 (dd, 1H, $J_{1B,2}=6.3$ Hz, H-1B), 3.73 (dd, 1H, $J_{7A,6}=5.2$, $J_{7A,7B}=10.2$ Hz, H-7A), 3.79 (dd, 1H, $J_{5,4}=3.5$, $J_{5,6}=7.7$ Hz, H-5), 3.8 (dd, 1H, $J_{7B,6}=3.6$ Hz, H-7B), 3.92 (dd, 1H, $J_{3,2}=3.5$, $J_{3,4}=6.4$ Hz, H-3), 3.93 (ddd, 1H, H-6), 4.10 (dd, 1H, H-4), 4.20 (ddd, 1H, H-2), 4.46–4.78 (m, 8H, 4 OCH_AH_BPh), 7.21–7.36 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ −5.41 [2C, $Si(CH_3)_2$], 18.20 [$SiC(CH_3)_3$], 25.85 [$SiC(CH_3)_3$], 64.03 [(−) C-7], 69.67 (C-2), 71.19 (C-6), 71.39 [(−) C-1], 72.84, 73.21, 73.42 and 74.59 [all (−), 4 OCH_2Ph], 77.65 (C-3), 78.13 (C-5), 79.52 (C-4), 127.5–128.1 (ArCH), 138.08 (2C), 138.25 and 138.44 (4 ArC). Anal. (mixture of epimers). Calcd for $C_{41}H_{54}O_7Si$: C, 71.69; H, 7.92. Found: C, 71.82; H, 8.01.

3,4,5,7-Tetra-*O*-benzyl-1-*O*-(*t*-butyldimethylsilyl)-L-*arabino*-2,6-heptodiulose (16). To a solution of oxalyl chloride (0.89 mL, 10.2 mmol) in anhydrous CH_2Cl_2 (25 mL) was added dropwise a solution of DMSO (0.79 mL, 11.1 mmol) in CH_2Cl_2 (5 mL) at −78 °C over a period of 3 min; the mixture was further stirred for 10 min at the same temperature. A solution of diol **15** (1.3 g, 1.89 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise while the temperature of the mixture was maintained below −60 °C. The mixture was stirred for 1 h at −78 °C. Et_3N (7.1 mL) was then added dropwise, the mixture was stirred for 15 min at −78 °C and then allowed to warm-up to room temperature. The solids were removed by filtration through a membrane (Magna-R, Nylon, 0.22 micron), the filtrate was concentrated, co-evaporated once with toluene (15 mL), and dried under high vacuum to give crude **16** (1.5 g). This product was used in the next step without purification. A sample of crude **16** was rapidly processed [extraction with cold N HCl and brine] for analysis by NMR. ^{13}C NMR: δ −5.89, −5.95 [$Si(CH_3)_2$], 18.20 [$SiC(CH_3)_3$], 25.35 [$SiC(CH_3)_3$], 68.13 [(−) C-1], 72.31, 72.77, 73.60, 73.81 and 73.96 [all (−), C-7 and 4 OCH_2Ph], 79.97, 80.59 and 81.41 (C-3, 4, 5), 127.5–128.1 (ArCH), 136.33, 136.52, 136.74 and 136.99 (4 ArC), 206.48 and 207.27 (C-2, 6).

1,3,4,5-Tetra-*O*-benzyl-7-*O*-(*t*-butyldimethylsilyl)-2,6-dideoxy-2,6-imino-L-*glycero*-L-*galacto*-heptitol (17). To a stirred solution of crude **16** (1.5 g) in MeOH (25 mL) was added HCO_2NH_4 (227 mg, 3.6 mmol), powdered molecular sieves (3Å, 200 mg), and then, $NaBH_3CN$ (380 mg, 6.1 mmol) in one portion. The reaction mixture was stirred for 1 h at room temperature. The solids were removed by filtration through Celite, the Celite bed was washed with MeOH (100 mL), the filtrate and washings were combined and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL), the solution washed with water (30 mL), saturated aqueous $NaHCO_3$ (30 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (solvent C), thus affording pure **17** (577 mg, 44%): syrup, $[\alpha]_D$ −29.1 (*c* 1.03, $CHCl_3$); ^1H NMR: δ 0.05 [s, 6H, $Si(CH_3)_2$], 0.86 [s, 9H, $SiC(CH_3)_3$], 2.60 (ddd, 1H, $J_{6,5}=9.6$, $J_{6,7A}=2.2$, $J_{6,7B}=3.5$ Hz, H-6), 2.88 (ddd, 1H, $J_{2,1A}=8.4$, $J_{2,1B}=5.8$, $J_{2,3}=0$ Hz, H-2), 3.39 (dd, 1H, $J_{1A,1B}=8.6$ Hz, H-1A), 3.52 (dd, 1H, H-1B), 3.58 (dd, 1H, $J_{4,3}=2.4$, $J_{4,5}=9.5$ Hz, H-4), 3.76 (dd, 1H, $J_{7A,7B}=9.7$ Hz, H-7A), 3.90 (dd, 1H, H-7B), 3.99 (dd, 1H, H-5), 4.05 (dd, 1H, H-3), 4.42–5.05 (m, 8H, 4 OCH_AH_BPh), 7.23–7.40 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ −5.65, −5.71 [$Si(CH_3)_2$], 14.16 [$SiC(CH_3)_3$], 25.27 [$SiC(CH_3)_3$], 57.01 (C-2), 60.52 (C-6), 61.95 [(−) C-7], 69.85 [(−) C-1], 71.90, 73.08, 73.78 and 75.05 [all (−), 4 OCH_2Ph], 74.01 (C-3), 76.56 (C-5), 85.92 (C-4), 126.9–128.1 (ArCH), 137.90, 138.45, 138.70 and 138.96 (4 ArC). Anal. calcd for $C_{41}H_{53}NO_5Si$: C, 73.72; H, 8.00; N, 2.10. Found: C, 73.61; H, 8.03; N, 2.15.

1,3,4,5-Tetra-*O*-benzyl-2,6-dideoxy-2,6-imino-L-*glycero*-L-*galacto*-heptitol (18). A solution of imino heptitol **17** (242 mg, 0.36 mmol) in a mixture of THF (2 mL), water (2 mL), and CH_3COOH (6 mL) was stirred overnight at 55 °C. The solution was then co-evaporated under reduced pressure with MeOH (2×50 mL) and then toluene (2×50 mL). The residue was purified by flash chromatography (solvent A for elution of impurities, then 100% EtOAc) to give pure **18** (157 mg, 78%): solid; mp 81–82 °C; $[\alpha]_D$ −11.8 (*c* 1.1, $CHCl_3$); ^1H NMR: δ 2.2 (br s, 1H, HO-C7), 2.61 (ddd, 1H, $J=9.1$, 5.4, 3.3 Hz, H-6), 2.83 (ddd, 1H, $J_{2,1A}=8.8$, $J_{2,1B}=6.2$, $J_{2,3}\sim0.0$ Hz, H-2), 3.35 (dd, 1H, $J_{1A,1B}=8.8$ Hz, H-1A), 3.47 (dd, 1H, H-1B), 3.52–3.57 (m, 2H, H-4, 5), 3.73–3.78 (m, 2H, H-7A,7B), 4.0 (br s, 1H, H-3), 4.36–4.96 (m, 8H, 4 OCH_AH_BPh), 7.17–7.37 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ 57.32 (C-2), 60.85 (C-6), 62.36 [(−) C-7], 69.82 [(−) C-1], 72.11, 73.27, 74.23 and 75.04 [all (−), 4 OCH_2Ph], 74.11 (C-3), 77.12 (C-5), 85.84 (C-4), 127.4–128.3 (ArCH), 137.80, 138.41 (2C), and 138.76 (ArC). Anal. calcd for $C_{35}H_{39}NO_5$: C, 75.92; H, 7.10; N, 2.53. Found: C, 75.84; H, 7.05; N, 2.48.

2,6-Dideoxy-2,6-imino-L-*glycero*-L-*galacto*-heptitol (β-Homogalactonojirimycin 4). To a solution of imino heptitol **18** (127.6 mg, 0.23 mmol) in CH_2Cl_2 (4 mL) was added, dropwise at 0 °C, iodotrimethylsilane (0.25 mL). The reaction mixture was allowed to warm up to room temperature and stirred for 12 h. Water (25 mL) and CH_2Cl_2 (25 mL) were added, the organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (25 mL). The volume of the aqueous phase was then reduced to 2 mL by evaporation under reduced pressure. The solution was loaded onto a column of Dowex 1x2-200 (OH^-) ion-exchange resin. The product was eluted with water (40 mL) and the fractions containing **4** were combined and lyophilized to afford **4** as a glass (34.1 mg, 68%); ^1H NMR (CD_3OD): good agreement with lit. data.[16b] ^{13}C NMR (CD_3OD): δ 60.69 (C-2), 62.78 (C-6), 63.21 and 63.74 [both (−), C-1, 7], 70.86 (C-3), 71.10 (C-5), 77.69 (C-4). HR-FABMS (matrix: 3-nitrobenzyl alcohol), calcd for $C_7H_{16}NO_5$ ($[M+H]^+$): *m*/*z* 194.1029; found: 194.1022. Note: the debenzylation can also be performed by catalytic hydrogenation [$Pd(OH)_2$ on carbon, EtOH containing HCl, 1 atm H_2, 12 h at room temperature].

1,3,4,5-Tetra-*O*-benzyl-6,7-*O*-isopropylidene-L-*glycero*-L-*galacto*-heptitol (19). To a suspension of **14** (192 mg, 0.335 mmol) in 2,2-dimethoxypropane (5 mL) was added *p*-TsOH (10 mg, 0.06 mmol). The mixture became homogeneous after 2 min and TLC analysis indicated that the reaction was complete. The solution was diluted

with CH_2Cl_2 (10 mL), washed with saturated aqueous $NaHCO_3$ (10 mL), dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (solvent D) to give **19** (187.2 mg, 91%) as a syrup; $[\alpha]_D$ −19.8 (*c* 1.1, $CHCl_3$); ^1H NMR: δ 1.29 and 1.38 [2s, 2×3H, $C(CH_3)_2$], 2.92 (d, 1H, *J* = 5.8 Hz, HO-C2), 3.45 (dd, 1H, *J* = 6.5, 9.3 Hz) and 3.56 (dd, 1H, *J* = 6.4, 9.3 Hz) (H-1A, 1B), 3.89 (m, 3H, H-3, 4, 5), 3.95 (d, 2H, *J* = 6.7 Hz, 2H-7), 4.15 (tt, *J*∼5.8 Hz, H-2), 4.26 (tt, *J* = 6.6, 5.4, 6.4 Hz, H-6), 4.42–4.80 (m, 8H, 4 OCH_AH_BPh), 7.20–7.40 (m, 20 H, 4 C_6H_5); ^{13}C NMR: δ 24.96 and 26.46 [$C(CH_3)_2$)], 66.39 [(−) C-7], 69.54 (C-2), 71.28 [(−) C-1], 73.22, 73.46, 73.53 and 74.64 [all (−), 4 OCH_2Ph], 76.37, 71.17, 79.12 and 80.21 (C-3, 4, 5, 6), 108.52 [$C(CH_3)_2$)], 127.6–128.3 (ArCH), 137.99 (2C), 138.09 and 138.35 (4 ArC). Anal. calcd for $C_{38}H_{44}O_7$: C, 74.49; H, 7.24. Found: C, 74.47; H, 7.28.

1,3,4,5-Tetra-*O*-benzyl-6,7-*O*-isopropylidene-L-*manno*-2-heptulose (20). To a solution of heptitol **19** (116.7 mg, 0.19 mmol) in CH_2Cl_2 (3 mL) containing molecular sieves (3 Å, 200 mg), PCC (150 mg, 0.69 mmol) was added in one portion. After having been stirred for 4 h at room temperature, the reaction mixture was poured into a column containing ether and flash grade silica gel (ϕ = 1 cm, 10 cm silica gel, 20 cm Et_2O). The column was rapidly eluted and the silicagel bed washed with Et_2O (100 mL). The resulting clear solution was concentrated, thus giving homogeneous **19** (110 mg, 95%): syrup; IR ν_{max} (film) 1732.1 cm^{-1} (C=O); ^1H NMR: δ 1.29 and 1.38 [2s, 2×3H, $C(CH_3)_2$], 3.87 (dd, 1H, *J* = 6.7, 8.1 Hz, H-7A), 3.88 and 3.99 (2t, 2H, *J*∼5.6 Hz, H-4, 5), 3.93 (dd, 1H, *J* = 6.5, 8.1 Hz, H-7B), 4.19 (d, 1H, $J_{1A,1B}$ = 17.9 Hz, H-1A), 4.24 (dt, 1H, *J* = 6.5, 5.6, 5.6 Hz, H-6), 4.29 (d, 1H, $J_{3,4}$ = 5.6 Hz, H-3), 4.3 (d, 1H, H-1B), 4.36–4.64 (m, 8H, 4 OCH_AH_BPh), 7.21–7.32 (m, 20H, 4 C_6H_5); ^{13}C NMR: δ 24.98, 26.43 [$C(CH_3)_2$)], 66.34 [(−) C-7], 72,47, 73.12, 74.37, 74.46 and 74.61 [all (−) C-1, 4 OCH_2Ph], 75.94, 79.08, 80.89 and 81.13 (C-3-6), 108.58 [$C(CH_3)_2$], 127.6–128.4 (ArCH), 136.89, 137.34, 137.61 and 138.04 (4 ArC), 207.22 (C-2).

Methyl 1,3,4,5-tetra-O-benzyl-L-*manno*-2-heptulopyranoside (21) and 2,7-Anhydro-1,3,4,5-tetra-O-benzyl-L-*manno*-2-heptulose (22). Compound **20** (110 mg, 0.18 mmol) was dissolved in a 1% (w/v) solution of I_2 in MeOH (5 mL) and the reaction mixture was stirred overnight at room temperature. The solution was then concentrated, the residue dissolved in CH_2Cl_2 (20 mL), the solution washed with 5% (w/v) aqueous $Na_2S_2O_3$, dried (Na_2SO_4) and concentrated, to afford a crude mixture of **21** and **22** (97.4 mg). Flash chromatography (solvent B) of the mixture afforded pure **21** (50.5 mg, 50%) as well as a sample of **22** (20 mg, 20%).

(**21**): ^1H NMR: δ 3.17 (s, 3H, OCH_3), 3.44 (d, 1 H, $J_{1A,1B}$ = 10.1 Hz, H-1A), 3.55 (ddd, 1 H, $J_{6,5}$ = 9.7, $J_{6,7A}$ = 4.6, $J_{6,7B}$ = 3.0 Hz, H-6), 3.63 (d, 1H, H-1B), 3.70 (dd, 1 H, $J_{7A,7B}$ = 11.7 Hz, H-7A), 3.78 (dd, 1H, H-7B), 3.95 (dd, 1H, $J_{5,4}$ = 9.7 Hz, H-5), 4.08 (d, $J_{3,4}$ = 2.8 Hz, H-3), 4.12 (dd, H-4), 4.40–4.94 (m, 8H, 4 OCH_AH_BPh), 7.23–7.36 (m, 20 H, 4 C_6H_5); ^{13}C NMR: δ 47.84 (OCH_3), 62.37 [(−) C-7], 65.28 [(−) C-1], 72.12, 73.45, 74.92 and 75.13 [all (−), 4 OCH_2Ph], 72.94, 74.72, 75.23, 81.29 (C-3-6), 101.12 (C-2), 127.4–128.4 (ArCH), 137.0, 138.4, 138.6 and 138.8 (4 ArC). Anal. calcd for $C_{36}H_{40}O_7$: C, 73.95; H, 6.89. Found: C, 73.85; H, 6.93.

(**22**): ^1H NMR (CD_3OD): δ 3.44 (d, 1H, $J_{1A,1B}$ = 10.7 Hz, H-1A), 3.65 (dd, 1H, $J_{5,6}$ = 1.5, $J_{5,4}$ = 1.6 Hz, H-5), 3.72 (dd, 1H, $J_{7A,6}$ = 5.9, $J_{7A,7B}$ = 7.1 Hz, H-7A), 3.82 (ddd, 1H, $J_{4,3}$ = 5.3, $J_{4,6}$ = 1.4 Hz, H-4), 3.84 (d, 1H, H-3), 3.92 (d, 1H, H-1B), 4.16 (dd, 1H, $J_{7B,6}$ = 0.9 Hz, H-7B), 4.61–4.65 (m, 2H, H-6 and 0.5 OCH_AH_BPh), 4.34–4.58 (m, 7H, 3.5 OCH_AH_BPh), 7.21–7.42 (m, 20H, 4 C_6H_5); ^{13}C NMR (CD_3OD): δ 66.04 [(−) C-7], 70.91 [(−) C-1], 72.48, 72.94, 72.16 and 74.85 [all (−), 4 OCH_2Ph], 75.26 (C-3), 76.24 (C-4), 77.17 (C-6), 78.09 (C-5), 108.52 (C-2), 127.9–129.8 (ArCH), 139.38, 139.48, 139.53 and 139.54 (4 ArC).

Enzymatic assays

Human α-Gal A was expressed from Sf-9 insect cells infected with a recombinant baculovirus encoding normal α-Gal A gene and purified to homogeneity by Concanavalin A-Sepharose and Mono-Q (Pharmacia LKB Biotechnology).[33] Other glycosidases were commercially available (Sigma). Assays were performed as previously described.[32,33] The following typical conditions were used: enzyme (0.01–0.03 U/mL) in a buffer at the appropriate pH (see below), 5 mM substrate (*p*-nitrophenyl glycopyranoside), inhibitor, for a final volume of 0.1 mL. Enzyme and inhibitor were preincubated for 5 min at room temperature in the buffer, and the reaction started by addition of the substrate. After 20 min at 37 °C, the reaction was stopped by the addition of 0.2M sodium borate buffer (pH 9.8, 0.25 mL). Concentration of the *p*-nitrophenolate ion released was measured at 410 nm.

For each enzyme, the following buffers and pH were used [buffer *A* = 0.1M Na phosphate/citrate; *B* = 0.1M Na citrate; *C* = 0.1M K phosphate; *D* = 0.1M Na citrate/0.1M $Zn(OAc)_2$]: bovine epididymis α-L-fucosidase (*B*, pH 6.5); *E. Coli* α-galactosidase (*C*, pH 7); β-galactosidases from *E. Coli* (*C*, pH 7.3), bovine liver (*A*, pH 7.3), *A. niger* (*B*, pH 4), Jack beans (*B*, 3.5); α-glucosidases from yeast (*A*, pH 6.8), rice (*B*, pH 4), Baker's yeast (isomaltase)(*A*, pH 6.8); amyloglucosidases from *A. niger* (*B*, pH 5), *Rhizopus* mold (*B*, pH 5); β-glucosidases from almonds (*B*, pH 4.5), *caldocellum saccharolyticum* (*B*, pH 5); α-mannosidases from jack beans (*D*, pH 5), almonds (*B*, pH 4.5); β-mannosidase from *helix pomatia* (*B*, pH 4); β-xylosidase from *A. niger* (*B*, pH 5); α-N-acetylgalactosaminidases from chicken liver (*B*, pH 4); β-*N*-acetylglucosaminidase from jack beans (*B*, pH 5), form bovine epididymis A and B (*B*, pH 4); human lysosomal α-galactosidase A (*B*, pH 4.5).[33]

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