

# Supporting Information for

## Synthesis of Lipophilic 1-Deoxygalactonojirimycin

### Derivatives as D-Galactosidase Inhibitors

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## Full experimental details and characterisation data

### General

Melting points were determined on a Tottoli apparatus and are uncorrected. Mass spectra were recorded on an Agilent Systems 6120 quadrupole LC-MS with APCI in the positive mode. Optical rotations were measured on a Perkin Elmer 341 with a path length of 10 cm. NMR spectra were recorded at 300.13 MHz ( $^1\text{H}$ ), and at 75.4 MHz ( $^{13}\text{C}$ ).  $\text{CDCl}_3$  was employed for protected compounds and  $\text{D}_2\text{O}$  as well as  $\text{MeOH-}d_4$  for free sugars. Chemical shifts are given as delta values employing residual, not deuterated, solvent as the internal standard. The signals of the protecting groups were found in the expected regions and are not listed explicitly. Structures of key intermediates were unambiguously assigned by 1D-TOCSY and HSQC experiments. TLC was performed on precoated aluminum sheets (E. Merck 5554). Compounds were detected by staining with concd  $\text{H}_2\text{SO}_4$  containing 5% vanillin. TLC of iminoalditols was performed employing a mixture of 10% ammonium molybdate (w/v) in 10% aqueous sulfuric acid containing 0.8% cerium sulfate (w/v). For column chromatography Silica Gel 60 (E. Merck) was used. The free inhibitors were chromatographed in  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}/\text{concd aq ammonia}$  mixtures as indicated.

### Kinetic Studies:

The inhibitory activities of new lipophilic 1-deoxygalactonojirimycin derivatives **16** – **20** as well as **22** were tested with three glycosidases. *Agrobacterium* sp.  $\beta$ -

glycosidase was purified and assayed as described [1,2]. Kinetic studies were performed at 37 °C in pH 7.0 sodium phosphate buffer (50 mM) containing 0.1 % bovine serum albumin, using  $7.2 \times 10^{-5}$  mg/mL enzyme. Approximate values of  $K_i$  were determined using a fixed concentration of substrate, 4-nitrophenyl  $\beta$ -D-glucopyranoside (0.11 mM =  $1.5 \times K_m$ ) and inhibitor concentrations ranging from 0.2 times to 5 times the  $K_i$  value ultimately determined. A horizontal line drawn through  $1/V_{max}$  in a Dixon plot of this data ( $1/V$  versus  $[I]$ ) intersects the experimental line at an inhibitor concentration equal to  $-K_i$ . Full  $K_i$  determinations where required, were performed using the same range of inhibitor concentrations while also varying substrate (4-nitrophenyl glucoside) concentrations from approximately 0.015 mM to 0.6 mM. Data were analysed by direct fit to the Michaelis Menten equation describing reaction in the presence of inhibitors using the program GraFit [3].

*E. coli*  $\beta$ -galactosidase was purchased from Sigma Chemicals. Kinetic studies were performed at 37°C in 100 mM sodium phosphate buffer, pH 7.0 containing 0.1% serum albumin. Rates of reaction were determined as a function of both varying substrate and inhibitor concentration over the concentration ranges indicated below. Data obtained were analysed using a nonlinear regression analysis program GraFit, *p*-nitrogalactopyranoside ( $K_m = 0.03$  mM), 0.045 – 0.3 mM) [4].

Green coffee bean  $\alpha$ -galactosidase was purified and assayed as described [5]. Kinetic studies were performed at 37°C in 50 mM sodium phosphate buffer, pH 6.5 containing 0.1% serum albumin. Reactions were initiated by the addition of enzyme (final concentration = 0.3  $\mu$ g/mL), bringing the total volume to 200 $\mu$ L. Reaction rates were measured by monitoring the release of *p*-nitrophenolate ( $\lambda = 400$  nm,  $\epsilon = 7.28 \times 10^{-3}$  M<sup>-1</sup> cm<sup>-1</sup>) from PNP-Gal by means of a UNICAM 8700 UV-Vis spectrophotometer equipped with a circulating water bath. Inhibition studies were performed by varying

concentrations of both PNP-Gal and inhibitor.  $K_i$  values were calculated by direct fit of the initial rates to the equation below using Grafit [3, 6].

$$v = \frac{V_{\max}[S]}{K_m(1+[I]/K_i) + [S]}$$

### **3,4-O-Isopropylidene-1,5-dideoxy-1,5-imino-D-galactitol (12):**

A solution of *m*-CPBA (13.8 g, 61.9 mmol, 77%) in CH<sub>2</sub>Cl<sub>2</sub> (150 mL) was dried over Na<sub>2</sub>SO<sub>4</sub> and added to a solution of 6-deoxy- $\alpha$ -L-arabino-hex-5-enopyranose (**10**) (10 g, 41.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL), and the mixture stirred at room temperature for 4 h. After complete conversion of the starting material (TLC), the reaction was cooled to -18 °C to furnish a white precipitate which was removed by filtration. The filtrate was washed with saturated NaHCO<sub>3</sub> solution twice and then with water until the pH of the aqueous phase was neutral. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to dryness to give crude compound **11** (14.3 g) as a brownish foam. The crude product was dissolved in MeOH (150 mL), cooled to -30 °C and NaOMe (1 M in MeOH) was added dropwise to adjust a pH of ~9-10. After complete conversion the reaction was neutralised by addition of ion exchange resin (Amberlite IR-120 H<sup>+</sup>, washed with MeOH). The resin was filtered off, the solution concentrated and purified by silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 16/1, v/v) to give compound **11** (9.8 g, 67 %) which was used for the next step immediately.

Compound **11** (1.6 g, 6.77 mmol) and benzyl amine (875 mg, 8.11 mmol) were dissolved in MeOH (250 ml), Pd/C (200 mg, ~ 0.19 mmol Pd) was added and the reaction mixture was stirred under a hydrogen atmosphere (1 atm) at room temperature for three days. After completion of the reaction (TLC), the catalyst was filtered off and the solution concentrated. Silica gel chromatography

(CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 3:1, 1%, v/v) gave compound **12** (1.04 g, 5.11 mmol, 76 %) as a colourless syrup.  $[\alpha]_D^{20} = +46.7$  (c 1.0 in MeOH).  $\delta_H$  (300 MHz, MeOD): 4.14 (1 H, dd,  $J_{4,5} = 2.7$  Hz,  $J_{3,4} = 5.3$  Hz, H-4), 3.75 (1 H, dd,  $J_{2,3} = 7.3$  Hz, H-3), 3.62 (1 H, dd,  $J_{5,6a} = 6.0$  Hz,  $J_{6a,6} = 10.9$  Hz, H-6a), 3.60-3.50 (1 H, m, H-2), 3.54 (1 H, dd,  $J_{5,6b} = 7.3$  Hz, H-6b), 2.92 (1 H, dd,  $J_{1eq,2} = 5.1$  Hz,  $J_{1eq,1ax} = 12.5$  Hz, H-1eq), 2.89 (ddd, 1H, H-5), 2.25 (1 H, dd,  $J_{1ax,2} = 10.9$  Hz, H-1ax), 1.39 (3 H, s, CCH<sub>3</sub>), 1.23 (3 H, s, CCH<sub>3</sub>).  $\delta_C$  (300 MHz, MeOD): 110.5, (C(CH<sub>3</sub>)<sub>2</sub>) 81.5 (C-3), 75.2 (C-4), 71.7 (C-2), 63.5 (C-6), 58.9 (C-5), 49.9 (C-1), 28.5, 26.7 (2 C, C(CH<sub>3</sub>)<sub>2</sub>).  $m/z$ : 204.24 (M+H)<sup>+</sup>, C<sub>9</sub>H<sub>17</sub>NO<sub>4</sub> requires M + H<sup>+</sup> 204.278.

***N*-[6-(*tert*-butoxycarbonylamino)hexyl]-3,4-O-isopropylidene-1,5-dideoxy-1,5-imino-D-galactitol (**14**):**

3,4-O-Isopropylidene-1,5-dideoxy-1,5-imino-D-galactitol **12** (220 mg, 1.08 mmol) was dissolved in DMF (15 mL), *N*-*tert*-butyloxycarbonyl-6-aminohexyl-4-toluenesulfonate (**13**) [7] (802 mg, 2.16 mmol) and Na<sub>2</sub>CO<sub>3</sub> (424 mg, 4 mmol) were added and the suspension was heated to 60 °C for 30 hours. After complete conversion of the starting material, as indicated by TLC, the solids were removed by filtration, the filtrate concentrated under reduced pressure and the resulting slurry subjected to silica gel chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 7:1:1 %, v/v) to give compound **14** (278 mg, 0.77 mmol, 71 %) as a colourless syrup.  $[\alpha]_D^{20} = + 5.3$  (c = 0.9 in MeOH).  $\delta_H$  (300 MHz, MeOD): 4.25 (1 H, dd,  $J_{3,4} = 4.5$  Hz,  $J_{4,5} = 3.6$  Hz, H-4), 3.90 (1 H, dd,  $J_{6a,5} = 5.2$  Hz,  $J_{6a,6b} = 11.9$  Hz, H-6a), 3.85 – 3.79 (3 H, m, H-2, H-3, H-6b), 3.07 – 2.98 (2 H, m, H-6'), 2.93 (1 H, dd,  $J_{1eq,2} = 3.8$  Hz,  $J_{1eq,1ax} = 11.8$  Hz, H-1eq), 2.76 – 2.64 (1 H, m, H-1'), 2.64 – 2.56 (1 H, m, H-5), 2.56 – 2.43 (1 H, m, H-1'), 2.05 (1 H, dd,  $J_{1ax,2} = 10.4$

Hz, H-1ax), 1.46 (3 H, s, C(CH<sub>3</sub>)<sub>2</sub>), 1.44 – 1.34 (8 H, m, H-2', H-3', H-4', H-5'), 1.30 (3 H, s, C(CH<sub>3</sub>)<sub>2</sub>).  $\delta_c$  (300 MHz, MeOD): 156.1 (C=O), 109.8, (C(CH<sub>3</sub>)<sub>2</sub>), 80.6 (C-3), 77.3 (C-4), 70.4 (C-2), 61.5 (C-5), 60.6 (C-6), 54.2 (C-6'), 53.4 (C-1), 52.5 (C-1'), 28.4 (2 C, C(CH<sub>3</sub>)<sub>2</sub>), 28.3, 26.9, 26.5, 26.3 (4 C, C-2', C-3', C-4', C-5'). *m/z*: 403.38 (M+H)<sup>+</sup>, C<sub>20</sub>H<sub>38</sub>N<sub>2</sub>O<sub>6</sub> requires M + H<sup>+</sup> 403.600.

### ***N*-(Aminoethyl)-1-deoxy-D-galactonojirimycin (15) [8]:**

Compound **14** (3.24 mg, 0.78 mmol) was dissolved in a mixture of MeOH and H<sub>2</sub>O (30 ml, 1/1 v/v) and 1 ml of concentrated hydrochloric acid was added to adjust the pH to ~1. If necessary, additional HCl was added. The reaction was stirred at 50 °C for 14 hours. After TLC analysis indicated complete conversion of the starting material, the reaction mixture was evaporated to dryness, the residue taken up in MeOH and evaporated again three times to remove excess of HCl. Silica gel chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 1:1:1/4 v/v) gave compound **15** (139.2 mg, 0.53 mmol, 68 %) as a pale-brown, highly hygroscopic foam after lyophilisation from H<sub>2</sub>O.  $[\alpha]_D^{20} = + 6.5$  (c 1.5 in MeOH). For NMR data see [7].

### ***N*-[6-(4-Isopropylbenzoylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (16):**

To a solution of 4-isopropylbenzoic acid (93.9 mg, 0.6 mmol) in DMF (5 mL) containing Et<sub>3</sub>N (115.6 mg, 1.14 mmol), TBTU (201.9 mg, 0.63 mmol) was added and the reaction mixture stirred for ten minutes. Consecutively, amine **15** (150 mg, 0.6 mmol) was added and the solution stirred at room temperature for one hour. After TLC showed complete conversion of the amine, the solvent was removed

under reduced pressure and the semi-solid residue subjected to silica gel chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 3/1/1% v/v) to yield compound **16** (62.6 mg, 0.15 mmol, 51 %) as a syrup. After lyophilisation from H<sub>2</sub>O, (47.1 mg, 0.12 mmol, 20.2 %) a hygroscopic white foam was obtained.  $[\alpha]_D^{20} = +5.9$  (c 2.4 in MeOH).  $\delta_H$  (300 MHz, MeOD/CDCl<sub>3</sub> 2/1 v/v): 7.76 (2 H, d,  $J = 8$  Hz, Ph), 7.23 (1 H, d,  $J = 8$  Hz, Ph), 3.99 (1 H, dd,  $J_{3,4} = 2.3$  Hz,  $J_{4,5} = 1.8$  Hz, H-4), 3.87 – 3.80 (3 H, m,  $J_{2,3} = 9.3$  Hz,  $J_{2,1eq} = 4.2$  Hz, H-2 H-6a, H-6b), 3.33 – 3.26 (3 H, m, H-3, H-6'), 3.12 (1 H, dd, H-1eq), 3.05 – 2.81 (4 H, m, H-5, H-1', H-1', CH(CH<sub>3</sub>)<sub>2</sub>), 2.55 (1 H, dd,  $J_{1eq,1ax} = 11.2$  Hz, H-1ax), 1.66 – 1.48 (4 H, m, H-2', H-5'), 1.42 – 1.25 (4 H, m, H-3', H-4'), 1.18 (3 H, s, CH(CH<sub>3</sub>)<sub>2</sub>), 1.15 (3 H, s, CH(CH<sub>3</sub>)<sub>2</sub>).  $\delta_C$  (75 MHz, MeOD/CDCl<sub>3</sub> 2/1 v/v): 168.4 (NCO), 152.1, 131.1, 129.1, 126.6, 125.7 (6 C, Ph), 72.6 (C-3), 69.8 (C-4), 64.4 (C-2), 64.2 (C-5), 59.5 (C-6), 53.2 (C-1), 52.9 (C-1'), 38.8 (C-6'), 33.4 (CH(CH<sub>3</sub>)<sub>2</sub>), 28.3, 25.5, 25.4, 21.4 (4 C, C-2', C-3' C-4', C-5'), 22.6 (2 C, CH(CH<sub>3</sub>)<sub>2</sub>).  $m/z$ : 409.42 (M+H)<sup>+</sup>, C<sub>22</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub> requires M + H<sup>+</sup> 409.603.

#### ***N*-[6-Nicotinoylaminohexyl]-1,5-dideoxy-1,5-imino-D-galactitol (**17**):**

To a solution of pyridine-3-carboxylic acid (59.1 mg, 0.48 mmol) in DMF (15 mL) containing Et<sub>3</sub>N (132.4 mg, 1.31 mmol), TBTU (154.1 mg, 0.48 mmol) was added and the solution was stirred at room temperature for ten minutes. Amine **15** (114.5 mg, 0.44 mmol) was added and the mixture stirred for 90 minutes until complete conversion of the starting material was observed by TLC. The solvent was removed under reduced pressure and the residue purified by silica gel chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 3/1/1% v/v) to yield compound **17** (34.3 mg, 0.1 mmol, 21.2 %). After lyophilisation, compound **17** was obtained as a hygroscopic, white foam.  $[\alpha]_D^{20}$

= -10.1 (c 1.0 in MeOH).  $\delta_{\text{H}}$  (300 MHz, MeOD): 8.99 (1 H, dd,  $J = 0.7$  Hz,  $J = 2.2$  Hz, Py), 8.71 (1 H, dd,  $J = 1.62$  Hz,  $J = 4.9$  Hz, Py), 8.26 (1 H, dt,  $J = 1.9$  Hz,  $J = 4.9$  Hz, Py), 7.57 (1 H, dd,  $J = 8.0$  Hz,  $J = 4.9$  Hz, Py), 4.01 (1 H, dd,  $J_{4,5} = 1.8$  Hz,  $J_{3,4} = 3.3$  Hz, H-4), 3.88 – 3.79 (3 H, m, H-2, H-6a, H-6b), 3.42 (2 H, t, H-6'), 3.24 (1 H, dd,  $J_{2,3} = 9.25$  Hz, H-3), 3.01 (1 H, dd,  $J_{1\text{eq},1\text{ax}} = 11.2$  Hz,  $J_{1\text{eq},2} = 4.8$  Hz, H-1eq), 2.85 – 2.68 (1 H, m, H-1'), 2.58 – 2.47 (1 H, m, H-1'), 2.39 (1 H, ddd,  $J_{5,6\text{a}} = 2.4$  Hz,  $J_{5,6\text{b}} = 5.2$  Hz, H-5), 2.13 (1 H, dd,  $J_{1\text{ax},2} = 10.7$  Hz, H-1ax), 1.73 – 1.61 (2 H, m, H-5'), 161 – 147 (2 H, m, H-2'), 147 – 130 (4 H, m, H-3', H-4').  $\delta_{\text{C}}$  (75 MHz, MeOD): 167.8 (NCO), 152.6, 149.3, 137.2, 132.5, 125.4 (5 C, Py), 77.6 (C-3) 72.6 (C-4), 69.3 (C-2), 65.5 (C-5), 62.7 (C-6), 58.4 (C-1), 54.3 (C-1'), 41.3 (C-6'), 30.6 (C-5'), 28.6, 28.2 (2 C, C-3', C-4'), 25.3 (C-2').  $m/z$ : 368.32 (M+H)<sup>+</sup>, C<sub>18</sub>H<sub>29</sub>N<sub>3</sub>O<sub>5</sub> requires M + H<sup>+</sup> 368.507.

***N*-[6-(4-Dimethylaminobenzoylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol  
(18):**

A solution of amine **15** (57.6 mg, 0.22 mmol) in DMF (4 mL) and Et<sub>3</sub>N (44.7 mg, 0.44 mmol) was cooled to 0 °C and 4-dimethylaminobenzoic acid chloride hydrochloride (53.4 mg, 0.2 mmol) in 1 ml of dry DMF was added dropwise with a syringe. The reaction mixture was allowed to reach room temperature and stirring was continued for two hours. After complete consumption of the acid chloride, as indicated by TLC, 1 mL of MeOH was added and the reaction mixture stirred for 20 minutes. The solvents were removed under reduced pressure and the residue subjected to silica gel chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 3/1/1% v/v). Subsequent lyophilisation from H<sub>2</sub>O gave compound **18** (20.8 mg, 0.5 mmol, 23.1 %) as an off-white, highly hygroscopic material.  $[\alpha]_{\text{D}}^{20} = +5.6$  (c 1.6 in MeOH).  $\delta_{\text{H}}$  (300 MHz, MeOD/CDCl<sub>3</sub> 2/1

v/v): 7.62 (2 H, d,  $J = 8.2$  Hz, Ph), 6.79 (2 H, d,  $J = 8.2$  Hz, Ph), 4.01 – 3.95 (1 H, m, H-4), 3.78 (1 H, dd,  $J_{2,3} = 10.6$  Hz,  $J_{2,1\text{eq}} = 4.5$  Hz, H-2), 3.75 – 3.66 (2 H, m, H-6a, H-6b), 3.32 – 3.25 (3 H, m, H-3, H-6'), 2.93 - 2.86 (7 H, m, H-1eq,  $\text{N}(\text{CH}_3)_2$ ), 2.62 – 2.49 (1 H, m, H-1'), 2.46 – 2.31 (2 H, m, H-5, H-1'), 2-10 (1 H, dd,  $J_{1\text{eq},1\text{ax}} = 11.0$  Hz, H-1ax), 1.57 – 1.45 (2 H, m, H-5'), 1.44 – 1.34 (2 H, m, H-2'), 1.33 – 1.14 (4 H, m, H-3', H-4').  $\delta_{\text{C}}$  (75 MHz, MeOD/ $\text{CDCl}_3$  2/1 v/v): 171.3 (NCO), 154.7, 129.8, 122.3, 113.6 (6 C, Ph), 76.4 (C-3), 71.4 (C-4), 68.3 (C-2), 63.9 (C-5), 61.7 (C-6), 57.2 (C-1), 53.6 (C-1'), 41.0 (2 C,  $\text{N}(\text{CH}_3)_2$ ), 40.9 (C-6), 29.9 (C-5'), 27.8, 27.3 (C-3', C-4'), 23.9 (C-2').  $m/z$ : 410.37 (M+H)<sup>+</sup>,  $\text{C}_{21}\text{H}_{35}\text{N}_3\text{O}_5$  requires M + H<sup>+</sup> 410.589

#### **1,5-Dideoxy-1,5-imino-D-galactitol (4):**

3,4-O-Isopropylidene-1,5-dideoxy-1,5-imino-D-galactitol (**12**) (0.62 g, 3.05 mmol) was dissolved in a mixture of H<sub>2</sub>O and MeOH (12 mL, 10/2 v/v), concentrated HCl (2 ml) was added (pH~1) and the mixture stirred at room temperature for 20 hours. The reaction mixture was concentrated and co-evaporated with MeOH. The resulting material was purified by silica gel chromatography ( $\text{CHCl}_3/\text{MeOH}/\text{NH}_3$  3:1, 1%, v/v) to give compound **4** (196.5 mg, 1.2 mmol, 53 %) as a colourless syrup.

#### **N-[(4-Pyrenebutanoylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (19):**

To a solution of 1-pyrenebutyric acid (123.4 mg, 0.42 mmol) in DMF (15 mL) containing Et<sub>3</sub>N (61.9 mg, 0.61 mmol) and HBTU (144 mg, 0.44 mmol), N-(6-aminohexyl)-1,5-dideoxy-1,5-imino-D-galactitol (**15**) (111.9 mg, 0.42 mmol) was added in one portion and the mixture stirred at room temperature for 45 minutes.

When TLC indicated complete conversion of the starting material MeOH (10 mL) was added and stirring continued for 10 minutes. The solvents were removed under reduced pressure and the remaining material subjected to column chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 9/1/1 % - 6/1/1 %). The resulting product was treated with basic ion exchange resin (Merck III, washed with water) to give compound **19** (134.0 mg, 0.3 mmol, 59 %) as white crystals.  $[\alpha]_D^{20} = +3.6$  (c 1.1 in MeOH/CHCl<sub>3</sub> 1/1 v/v). m.p. = 189 – 191 °C.  $\delta_H$  (500 MHz, D<sub>2</sub>O): 7.19 – 6.60 (9 H, m, pyrenyl), 3.96 – 3.88 (1 H, m, H-4), 3.86 – 3.79 (1 H, m, H-2), 3.59 – 3.47 (2 H, m, H-6a, H-6b), 3.25 – 3.18 (1 H, m, H-3), 3.14 – 3.06 (1 H, m, H-1eq), 2.80 – 2.66; 2.63 – 2.45; 2.42 – 2.26 (8 H, m, H-1ax, H-5, H1', H6', H-10'), 1.78 – 1.62 (2 H, m, H-8'), 1.46 – 1.32; 1.15 – 0.97; 0.83 – 0.5 (10 H, m, H-2', H-3', H-4', H-5', H-9').  $\delta_C$  (75 MHz, D<sub>2</sub>O): 174.5 (NCO), 135.5, 130.6, 130.2, 129.0, 127.8 126.7, 125.8, 125.2, 124.0, 122.7 (pyrenyl), 72.7 (C-3), 70.0 (C-4), 64.5, 64.1 (2 C, C-2, C-5), 59.3 (C-6), 53.4, 53.1(C-1, C-1'), 39.2 (C-6'), 35.6 (C-2'), 32.1 (C-4'), 28.5, 27.3, 25.7, 25.5, 21.7 (5 C, C-3', C-5', C-8', C-9', C-10'). *m/z*: 533.55 (M+H)<sup>+</sup>, C<sub>32</sub>H<sub>40</sub>N<sub>2</sub>O<sub>5</sub> requires M + H<sup>+</sup> 533.762

***N*-[6-(*tert*-butyloxycarbonylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (**20**):**

To a solution of iminosugar **15** (80 mg, 0.30 mmol) in DMF (15 ml) containing Et<sub>3</sub>N (121 mg, 1.20 mmol), a solution of BOC<sub>2</sub>O (80 mg, 0.37 mmol) was added and the mixture stirred at room temperature for 30 minutes. After TLC indicated complete consumption of amine **15**, MeOH (1 ml) was added and the reaction mixture concentrated under reduced pressure to yield compound **20** (94.9 mg, 0.3 mmol, 86 %) after silica gel chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 3:1:1 %, v/v).  $[\alpha]_D^{20} = -14.3$ , (c 1.1 in MeOH).  $\delta_H$  (500 MHz, D<sub>2</sub>O): 3.89 – 3.85 (1 H, m, H-4), 3.73 – 3.64 (3 H, m, H-

2, H-6a, H-6b), 3.09 (1 H, dd,  $J_{2,3} = 9.1$  Hz,  $J_{3,4} = 1.8$  Hz, H-3), 2.90 (2 H, t, H-6'), 2.86 (1 H, dd,  $J_{1eq,1ax} = 11.1$  Hz,  $J_{1eq,2} = 4.6$  Hz, H-1eq), 2.64 – 2.55 (1 H, m, H-1'), 2.43 – 2.34 (1 H, m, H-1'), 2.28 – 2.23 (1 H, m, H-5), 2.00 (1 H, dd,  $J_{1ax,2} = 10.7$  Hz), 1.45 – 1.27 (13 H, m, H-5', H-2', C(CH<sub>3</sub>)<sub>3</sub>), 1.26 – 1.12 (4 H, m, H-3', H-4').  $\delta_C$  (75 MHz, MeOD): 157.4 (NCO), 78.6 (C(CH<sub>3</sub>)<sub>3</sub>), 76.1 (C-3), 71.0 (C-4), 67.8 (C-2), 64.0 (C-5), 61.2 (C-6), 56.3 (C-1), 52.8 (C-1'), 40.1 (C-6'), 29.8 (C-5'), 27.6 (3 C, C(CH<sub>3</sub>)<sub>3</sub>), 27.1; 26.6 (C-3', C-4'), 23.8 (C-2').  $m/z$ : 363.2 (M + H)<sup>+</sup>, 366.43. C<sub>17</sub>H<sub>37</sub>N<sub>2</sub>O<sub>6</sub> requires M + H<sup>+</sup> 366.567

***N*-[6-(benzyloxycarbonylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (**22**):**

To a solution of iminosugar **4** (370.5 mg, 2.27 mmol) and *N*-Cbz-6-aminohexyl-4-toluenesulfonate **21** [9] (3.05 g, 7.52 mmol) in DMF (16 mL), Na<sub>2</sub>CO<sub>3</sub> (1.2 g, 11.32 mmol) was added and the reaction mixture stirred at 50 °C for 18 hours. After TLC showed complete conversion of the starting material, the solids were removed by filtration and the filtrate concentrated under reduced pressure. Silica gel column chromatography (CHCl<sub>3</sub>/MeOH/NH<sub>3</sub> 3:1:1 %, v/v) followed by treatment with basic ion exchange resin (Merck III, washed with water) gave compound **22** (688.9 mg, 1.7 mmol, 77 %) as a colourless syrup.  $[\alpha]_D^{20} = -14.0$  (c 1.2 in MeOH).  $\delta_H$  (500 MHz, MeOD): 7.39 – 7.23 (5 H, m, Ph), 5.25 – 5.12 (2 H, m, NHCOOCH<sub>2</sub>Ph), 4.02-3.95 (1 H, m, H-4), 3.85 – 3.74 (3 H, m, H-2, H-6<sub>a</sub>, H-6<sub>b</sub>), 3.25 – 3.17 (1 H, dd,  $J_{2,3} = 8.4$  Hz, H-3), 3.10 (t, 2H, H-6'), 3.02 (1 H, bs, NHCOOPh), 3.00 – 2.93 (1 H, m, H-1<sub>eq</sub>), 2.74 – 2.64 (1 H, m, H-1'), 2.54 – 2.44 (1 H, m, H-1'), 2.39 – 2.32 (1 H, m, H-5), 2.10 (1 H, dd,  $J_{1eq,1ax} = 1ax,2 = 10.5$  Hz, H-1<sub>ax</sub>), 1.58 – 1.43 (4 H, m, H-5', H-2'), 1.42 – 1.21 (4 H, m, H-3', H-4').  $\delta_C$  (125 MHz, MeOD): 157.8, (NCO), 137.2, 128.3, 127.8, 127.6 (6 C,

Ph), 76.1(C-3), 71.0 (C-4), 67.9 (C-2), 66.1 (OCH<sub>2</sub>Ph), 64.0 (C-5), 61.2 (C-6), 56.9 (C-1), 52.8 (C-1'), 40.5 (C-6'), 29.7 (C-5'), 27.1, 26.5 (2 C, C-3', C-4'), 23.8 (C-2').  
*m/z*: 397.29 (M+H)<sup>+</sup>, C<sub>20</sub>H<sub>32</sub>N<sub>2</sub>O<sub>6</sub> requires M + H<sup>+</sup> 397.548

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