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Synthesis of N-substituted iminosugar derivatives and their immunosuppressive activities

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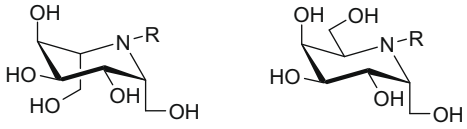
ABSTRACT

Several N-alkyl and hydroxyethyl-substituted iminosugar derivatives, including L-*altro* and D-*galacto* epimers, were synthesized and the effects of these synthetic iminosugars on proliferation of mouse splenocytes were evaluated in the MTT assay. The experimental data demonstrated that the N-alkylated D-*galacto* iminosugars showed better inhibitory activities than L-*altro* analogues, which might hold potential as immunosuppressive agents.

1. Introduction

The currently clinically used small-molecule immunosuppressive agents, such as cyclosporin A (CyA), sirolimus (Rapamycin), tacrolimus (FK506), and mycophenolate mofetil (MMF), have serious side effects including nephrotoxicity, neurotoxicity, infection, new onset post-transplant diabetes mellitus, hyperlipidemia, and hypertension. Furthermore, so far there is no antidote for the toxicity of these organ transplantation drugs. To improve transplant survival and reduce the toxicity of current agents, there is a great need to find highly efficacious immunosuppressants with low toxicity.[1–4]

Iminosugars, also known as the 'sugar-shaped alkaloids', have been found to be potent inhibitors of many carbohydrate-processing enzymes (i.e., glycosidases and glycosyltransferases).[5–8] Because these enzymes are frequently involved in essential biological processes,[9,10] iminosugars have great potential as therapeutic agents in many diseases such as cancer,[11] diabetes,[12] viral infections,[13] and lysosomal storage disorders.[14,15] However, the inhibition effects of iminosugars on immune system responses and their application as immunosuppressants have been less explored. To date only castanospermine, a naturally occurring indolizidine alkaloid, has been found to exhibit immunosuppressive activity.[16] Our preliminary results disclosed that some synthetic iminosugars (e.g., **1** and **2**, Fig. 1) displayed immunosuppressive activity.[17,18] Encouraged by these experiments, to further understand the structure–activity relationships (SARs) of this type of compounds and to find iminosugars with better activities, we report herein the synthesis of several N-alkylated iminosugar derivatives and the evaluation of their immunosuppressive activities by the MTT assay.



1: R=H
3: R=$(CH_2)_3CH_3$
4: R=$(CH_2)_5CH_3$
5 :R=$(CH_2)_8CH_3$
9: R=$(CH_2)_2OH$

2: R=H
6: R=$(CH_2)_3CH_3$
7: R=$(CH_2)_5CH_3$
8: R=$(CH_2)_8CH_3$
10: R=$(CH_2)_2OH$

Figure 1. Structures of iminosugars **1** and **2**, and their derivatives **3**–**10**.

2. Results and discussion

2.1. Design

Nojirimycin (NJ) and 1-deoxynojirimycin (DNJ), isolated from microorganism cultures and/or plants, are competitive inhibitors of *N*-glycan-processing enzymes (Fig. 2).[19,20] N-Alkylated

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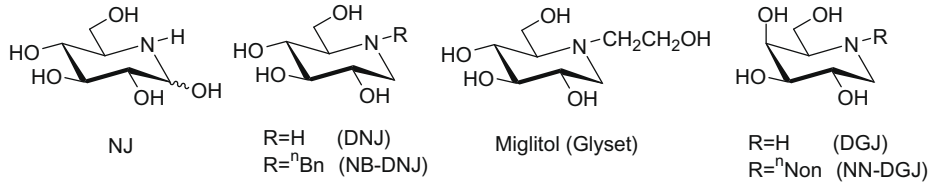
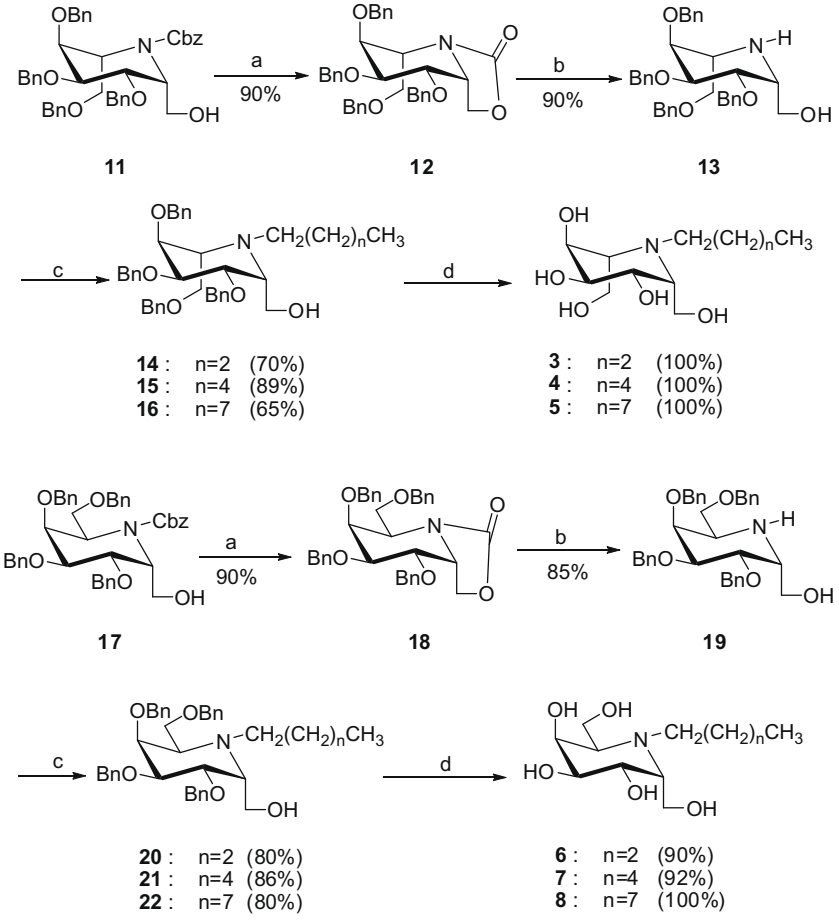


Figure 2. Some emblematic iminosugars.

deoxynojirimycin and deoxygalactonojirimycin (DGJ) span a wide range of biological activities. For instance, *N*-butyl-1-deoxynojirimycin (NB-DNJ) has been approved as medicine for type I Gaucher disease, the most common lysosomal storage disorder. In addition, NB-DNJ was evaluated in Phase II clinical trials as an anti-HIV agent.[21,22] *N*-Hydroxyethyl-DNJ (miglitol) served as an oral drug for the treatment of type II diabetes taken either alone or in combination with other anti-diabetic medicines.[23–25] In addition, the galactose analogue *N*-nonyl-1-deoxygalactonojirimycin (NN-DGJ) was in clinical trials as an anti-HBV and an anti-HCV agent.[26,27] Based on our previous report,[17] to investigate whether the N-alkylated side chain influences the immunosuppressive activity or not, we designed some N-alkylated iminosugar derivatives **3**–**10** with side chains consisting of four-, six- or nine-carbons, or a hydroxyethyl group (Fig. 1).

2.2. Chemistry

The preparation of iminosugars **3**–**8** is shown in Scheme 1. The key materials, cyclized isomeric alcohols **11** and **17**, were obtained from 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose according to a procedure described previously.[17] The alcohol **11** was treated with sodium methoxide to provide **12** smoothly, which was further treated with 50% aqueous potassium hydroxide to cleave the protecting group on the amino functionality, affording **13**.[28] The N-alkylated side chains were successfully introduced to the iminosugar moiety by reductive amination[29,30] leading to compounds **14**, **15**, and **16** in 70%, 89%, and 65% yield, respectively, based on the recovered starting materials. Debenzylation of **14**–**16** under a hydrogen atmosphere over Pd–C provided iminosugars **3**–**5** in quantitative yield. In a similar way, iminosugar derivatives **6**–**8** were prepared from the corresponding D-*galacto* alcohol **17**. Interestingly, when the above-mentioned reductive amination conditions were applied to the preparation of compounds **20**, **21**, and **22** from **19**, the reaction gave a low yield (less than 40%). However, when using ethanol instead of methanol as the solvent, satisfactory yields (80–86%) were obtained. Finally, it is noteworthy that it was not easy to obtain pure samples of iminosugars **6** and **7**. The crude product must be successively subjected to silica gel column chromatography (CH_2Cl_2–MeOH as the eluent), C-18 reverse-phase



Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, rt; (b) 50% KOH, MeOH, 80 °C; (c) $CH_3(CH_2)_nCHO$, MeOH or EtOH, HOAc then $NaBH_3CN$, 80 °C; (d) Pd/C, H_2, H_2O/THF/HOAc = 4:2:1.

column chromatography (H_2O as eluent), and ion-exchange resin column chromatography.

Starting from compound **13** and its isomer **19**, the miglitol derivatives **9** and **10** were obtained (Scheme 2). Initially, compound **13** was treated with $BrCH_2CH_2OH$ and K_2CO_3 in DMF in the hope of getting **24**, but no product was obtained. Although many other bases and solvent systems such as THF/K_2CO_3, THF/NaOH/tetra-*n*-butylammonium iodide (TBAI), and THF/Et_3N/TBAI were evaluated, all these efforts failed to give the desired product. Eventually, treatment of **13** with 40% glyoxal aqueous solution afforded the lactone **23** in 74% isolated yield.[31] The carbonyl group of **23** was subsequently reduced by $LiAlH_4$, providing tetra-*O*-benzyl-protected iminosugar derivative **24**. It was noteworthy that D-*galacto* iminosugar intermediates **25** and **26** were less stable than their corresponding L-*altro* isomers. Final deprotection of **24** by catalytic hydrogenolysis yielded the target compound **9**, which was purified by silica gel column chromatography, C-18 reverse-phase column chromatography, and ion-exchange resin. Iminosugar **10** was prepared in the same manner as described for the preparation of **9**.

2.3. Biology

With all the synthetic compounds in hand, the effect of the N-substituted iminosugars **3**–**10** and the N-unsubstituted iminosugars **1**–**2**[17] on concanavalin A (Con A)-induced proliferation of splenocytes in mice were assessed by the MTT assay.[32] The splenocytes were induced by 2.5 μg/mL of concanavalin A with 30 μM concentration of the iminosugars at 37 °C, 5% CO_2 for 48 h, using the Con A-treated splenocytes as the experimental control. Compared with the control, the levels of cell proliferation were reduced 25.4%, 16.5%, 21.6%, 17.9%, 26.6%, 35.8%, 38.3%, 27.7%, 18.4%, and 18.2% when including 30 μM of compounds **1**–**10**, respectively.

As shown in Figure 3, iminosugars **6** and **7** displayed the strongest inhibition effects. The N-alkylation of D-*galacto* iminosugar **2** was important for the improvement of the inhibitory activity and the immunosuppressive effects were increased by 1.7–2.3-fold (compared compounds **6**–**8** to **2**). The six-carbon side chain showed the best inhibitory effect among the three N-substituted iminosugars. However, in the case of the L-*altro* iminosugar epimers, N-alkylation did not improve the activity (compared compounds **3**–**5** to **1**). Comparing compounds **6**–**8** with **10**, it seemed that iminosugars with N-substituted non-polar side chains showed better inhibitory activity than that with polar chains. This suggests that the hydrophobic character may be important to the immunosuppressive activity. By comparison of the inhibition of the D-epimer **7** (38.3%) with L-epimer **4** (17.9%), it was clear that the configuration of the hydroxymethyl group was important to improve the inhibitory activity. However, the mechanisms of these differences are not understood.

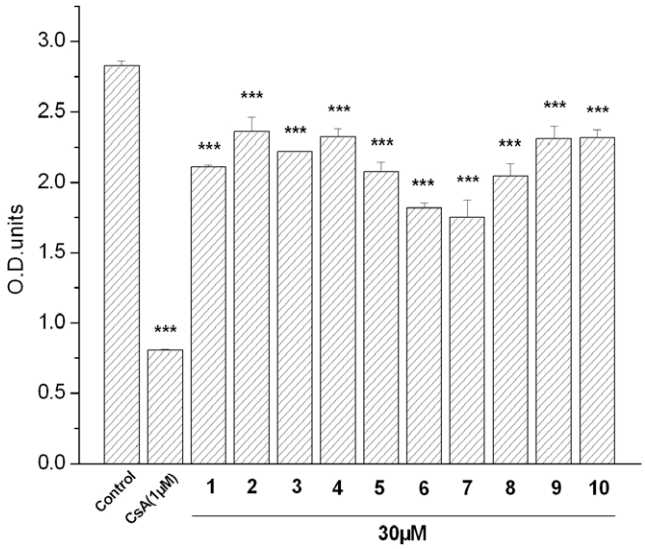


Figure 3. Effects of compounds **1**–**10** on concanavalin A-induced mouse splenocytes proliferation were assessed by the MTT assay. Values are mean ± SEM. ***p <0.001 versus control.

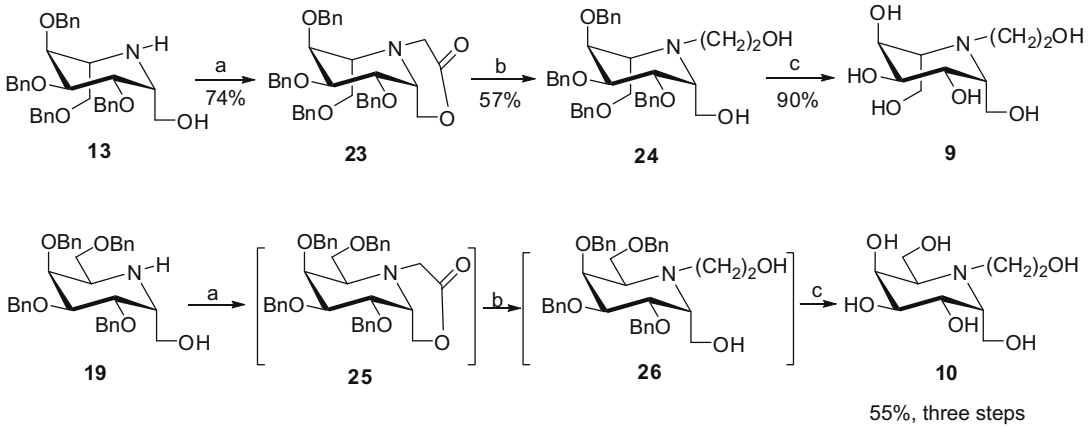
3. Conclusions

N-Alkylated iminosugars **3**–**8** and miglitol derivatives **9** and **10** were designed and synthesized, and their immunosuppressive activities were evaluated. It was clear that N-alkylation of the D-*galacto* iminosugar gave rise to better inhibitory activities than that of the L-*altro* iminosugar epimer, and the D-*galacto* iminosugar derivative with N-substituted six-carbon side chain showed the strongest inhibitory activity. With this information, our future work will focus on the preparation and evaluation of more N-substituted D-*galacto* iminosugar derivatives.

4. Experimental

4.1. General methods

All chemicals were purchased and used without further purification. Tetrahydrofuran (THF) was distilled over sodium/



Scheme 2. Reagents and conditions: (a) glyoxal, MeOH/HOAc (v/v) = 200:1, $NaBH_4$, 80 °C; (b) $LiAlH_4$, THF, rt; (c) Pd/C, H_2, H_2O/THF/HOAc = 4:2:1.

benzophenone and methylene chloride (CH_2Cl_2) over calcium hydride. The reactions were monitored with analytical TLC on Silica Gel 60-F_{254}-precoated aluminum plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate. Column chromatography was performed on silica gel (35–75 μm). NMR spectra were recorded on a Varian VXR-300M or Varian INOVA-500M spectrometer. Mass spectra were recorded using a PE SCLEX QSTAR spectrometer. Elemental analysis data were recorded on PE-2400C elemental analyzer. Concanavalin A was purchased from Sigma. Other reagents were from commercial sources.

4.2. 1,3,4,5-Tetra-*O*-benzyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*-heptitol *O* (7), *N*-cyclic carbamate (12)

To a solution of **11**[17] (210 mg, 0.31 mmol) in MeOH (15 mL) was added sodium methoxide (30% solution in MeOH) (0.5 mL, 0.009 mmol). The reaction mixture was stirred for 1 h at room temperature. MeOH was removed under reduced pressure, and the residue was extracted with EtOAc (3 × 20 mL). The organic phases were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc 4:1) to provide **12** (159 mg, 90%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.42 (d, 1H, J = 3.0 Hz), 3.80–3.81 (m, 1H), 3.87–3.91 (m, 1H), 3.98 (dd, 1H, J = 2.5, 9.5 Hz), 4.11–4.18 (m, 3H), 4.27 (dd, 1H, J = 3.5, 10.0 Hz), 4.33–4.37 (m, 2H), 4.41 (d, 1H, J = 12.0 Hz,), 4.43 (d, 1H, J = 12.5 Hz), 4.47 (d, 1H, J = 12.0 Hz), 4.51 (d, 1H, J = 11.5 Hz), 4.66 (s, 2H), 4.74 (d, 1H, J = 12.0 Hz) (8 × –CH_2Ph), 7.19–7.43 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 54.53, 55.05, 62.06, 64.65, 72.14, 72.37, 73.00, 73.07, 73.15, 73.51 (4 × –CH_2Ph), 74.58, 127.37, 127.75, 128.03, 128.09, 128.16, 128.36, 128.47, 137.30, 137.67, 137.92, 138.42 (Ph), 156.14 (CO). HRMS (ESI, positive) Calcd for $C_{36}H_{38}NO_6$: 580.2694 $[M+H]^+$. Found: 580.2705.

4.3. 3,4,5,7-Tetra-*O*-benzyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*-heptitol (13)

To a solution of **12** (220 mg, 0.38 mmol) in MeOH (15 mL) was added 50% aqueous KOH (15 mL). The reaction mixture was stirred for 20 h at 80 °C. MeOH was removed under reduced pressure, and the residue was extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc 1:1) to provide **13** (190 mg, 90%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.03 (br s, 2H, –OH and –NH), 3.15–3.21 (m, 2H, H-2, H-6), 3.50 (dd, 1H, J = 1.5, 3.5 Hz, H-5), 3.53 (dd, 1H, J = 5.5, 11.0 Hz, H-7), 3.61–3.66 (m, 2H, H-7, H-1), 3.77 (dd, 1H, J = 3.0, 10.0 Hz, H-3), 3.78–3.82 (m, 2H, H-1, H-4), 4.30 (d, 1H, J = 12.0 Hz), 4.37 (d, 1H, J = 12.5 Hz), 4.39 (br s, 2H), 4.48 (d, 1H, J = 12.0 Hz), 4.51 (d, 1H, J = 12.0 Hz), 4.56 (d, 1H, J = 12.0 Hz), 4.70 (d, 1H, J = 12.0 Hz), 7.16–7.35 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 54.17 (C-2), 54.79 (C-6), 63.59 (C-7), 69.91 (C-1), 71.79 (CH_2Ph), 71.90 (CH_2Ph), 72.26 (C-4), 72.93 (CH_2Ph), 73.26 (CH_2Ph), 74.93 (C-3), 76.75 (C-5), 127.50, 127.69, 127.81, 127.93, 127.98, 128.12, 128.30, 128.35, 137.90, 138.37, 138.42, 138.53 (Ph). HRMS (ESI, positive) Calcd for $C_{35}H_{40}NO_5$: 554.2901 $[M+H]^+$. Found: 554.2900.

4.4. 3,4,5,7-Tetra-*O*-benzyl-*N*-butyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*-heptitol(14)

To a solution of **13** (140 mg, 0.25 mmol) in MeOH and acetic acid (v/v 200:1, 10 mL) was added $CH_3(CH_2)_2CHO$ (160 μL, 1.75 mmol). After stirring for 1 h at 60 °C, a portion of $NaBH_3CN$ (80 mg, 1.25 mmol) was added. The reaction mixture was stirred overnight at 80 °C and the reaction was quenched with 1 N HCl aqueous solution (0.2 mL). The mixture was extracted with EtOAc (3 × 30 mL) and washed with aqueous $NaHCO_3$ (10 mL) and aqueous NaCl (10 mL). The organic phases were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc 4:1) to provide **14** (59 mg, 38%) as a yellow oil. The material **13** (63 mg) was recovered and the conversion was 70%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.85 (t, 3H, J = 7.5 Hz, –CH_3), 1.15–1.45 (m, 4H, –CH_2–), 2.81–2.93 (m, 2H, –NCH_2–), 3.10 (q, 1H, J = 5.0, 11.0 Hz, H-2), 3.20 (q, 1H, J = 5.5, 10.5 Hz, H-6), 3.41–3.46 (m, 2H, H-1, H-7), 3.53 (dd, 1H, J = 4.5, 10.0 Hz, H-7), 3.65 (dd, 1H, J = 2.5, 7.0 Hz, H-4), 3.75 (dd, 1H, J = 6.5, 11.5 Hz, 1H), 3.88 (dd, 1H, J = 2.5, 5.5 Hz, H-5), 3.98 (dd, 1H, J = 4.5, 7.0 Hz, H-3), 4.41 (d, 1H, J = 12.0 Hz), 4.47–4.53 (m, 5H), 4.56 (d, 1H, J = 12.5 Hz), 4.61 (d, 1H, J = 12.0 Hz), 7.22–7.32 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.95 (–CH_3), 20.21 (–CH_2–), 28.05 (–CH_2–), 53.08 (–NCH_2–), 59.05 (C-2 and C-6), 61.26 (C-1), 68.94 (C-7), 71.92, 72.33, 72.91, 73.11 (4 × –CH_2Ph), 74.09 (C-4), 75.30 (C-5), 77.00 (C-3), 127.46, 127.58, 127.63, 127.79, 127.86, 127.91, 128.03, 128.22, 128.25, 128.39, 137.98, 138.07, 138.48, 138.58. MS-ESI: 610 $[M+H]^+$. Anal. Calcd for $C_{39}H_{47}NO_5$: C, 76.82; H, 7.77; N, 2.30. Found: C, 77.06; H, 7.56; N, 2.36.

4.5. 3,4,5,7-Tetra-*O*-benzyl-*N*-hexyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*–heptitol (15)

Compound **15** was prepared from **13** (500 mg, 0.9 mmol) as described in the preparation of **14**, providing **15** (340 mg, 59%) as a yellow oil. The material **13** (170 mg) was recovered and the conversion was 89%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 7.5 Hz, –CH_3), 1.14–1.42 (m, 8H, –CH_2–), 2.80–2.92 (m, 3H, –OH, –NCH_2–), 3.10 (q, 1H, J = 6.0, 10.5 Hz, H-2), 3.20 (q, 1H, J = 5.0, 10.5 Hz, H-6), 3.43 (dd, 1H, J = 5.5, 9.5 Hz, H-7), 3.40–3.47 (m, 1H, H-1), 3.54 (dd, 1H, J = 5.0, 10.0 Hz, H-7), 3.64 (dd, 1H, J = 2.5, 6.5 Hz, H-4), 3.74 (dd, 1H, J = 6.5, 11.0 Hz, 1H), 3.87 (dd, 1H, J = 3.0, 6.0 Hz, H-5), 3.97 (dd, 1H, J = 4.5, 7.0 Hz, H-3), 4.41 (d, 1H, J = 12.0 Hz), 4.47 (d, 1H, J = 11.5 Hz), 4.49–4.52 (m, 4H), 4.55 (d, 1H, J = 12.0 Hz), 4.59 (d, 1H, J = 11.5 Hz), 7.17–7.32 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.00 (–CH_3), 22.62, 25.77, 26.75, 31.68 (4 × –CH_2–), 53.27 (–NCH_2–), 58.99 (C-6), 59.05 (C-2), 61.32 (C-1), 68.93 (C-7), 71.96, 72.38, 72.92, 73.15 (4 × –CH_2Ph), 74.05 (C-4), 75.31 (C-5), 77.00 (C-3), 127.50, 127.62, 127.67, 127.84, 127.91, 127.94, 128.07, 128.26, 128.42, 138.00, 138.07, 138.49, 138.60 (Ph). HRMS (ESI, positive) Calcd for $C_{41}H_{52}NO_5$: 638.3840 $[M+H]^+$. Found: 638.3843.

4.6. 3,4,5,7-Tetra-*O*-benzyl-*N*-nonyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*–heptitol (16)

Compound **16** was prepared from **13** (390 mg, 0.71 mmol) as described in the preparation of **14**, providing **16** (190 mg, 40%) as a yellow oil. The material **13** (150 mg) was recovered and the conversion was 65%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, 3H, J = 7.2 Hz, –CH_3), 1.23–1.27 (m, 14H, –CH_2–), 2.82–2.89 (m, 2H, –NCH_2–), 3.10 (q, 1H, J = 5.1 Hz, H-2), 3.19 (q, 1H, J = 5.4 Hz, H-6), 3.40–3.47 (m, 2H, H-7, H-1), 3.54 (dd, 1H, J = 4.8, 9.9 Hz, H-7), 3.65 (dd, 1H, J = 2.7, 6.9 Hz, H-4), 3.74 (dd, 1H, J = 6.6, 11.4 Hz, H-1), 3.87 (dd, 1H, J = 2.7, 6.0 Hz, H-5), 3.96 (dd, 1H, J = 4.5, 6.9 Hz, H-3), 4.42 (d, 1H, J = 12.0 Hz), 4.44–4.55 (m, 6H), 4.60 (d, 1H, J = 11.4 Hz), 7.23–7.30 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.09 (–CH_3), 22.64, 25.70, 27.11, 29.24, 29.52, 29.59, 31.85 (7 × –CH_2–), 53.14 (–NCH_2–), 58.91 (C-6), 58.98 (C-2), 61.35 (C-1), 68.85 (C-7), 71.94, 72.38, 72.89, 73.14 (4 × –CH_2Ph), 73.98 (C-4), 75.29 (C-5), 77.09 (C-3), 127.51, 127.68, 127.92, 128.09, 128.26, 128.43, 138.00, 138.05, 138.48, 138.59 (Ph). MS-ESI: 680 $[M+H]^+$. Anal Calcd for $C_{44}H_{57}NO_5$: C, 77.72; H, 8.45; N, 2.06. Found: C, 77.88; H, 8.67; N, 1.97.

4.7. *N*-Butyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*–heptitol (3)

A mixture of **14** (43 mg, 0.07 mmol) and 10% Pd–C (10.0 mg) in acetic acid (1.0 mL), H_2O (4.0 mL), and THF (2.0 mL) was stirred for 48 h under a H_2 atmosphere. The solid was removed by filtration through Celite and the filtrate was concentrated. The residue was passed through a C-18 reverse-phase column chromatography (H_2O as eluent) and ion-exchange resin (Dowex 1 × 8, OH^- form) to give **3** (17.5 mg, 100%) as white solids after lyophilization. $[\alpha]_D^{26}$ −21.3 (*c* 0.6, H_2O). ^1H NMR (500 MHz, D_2O): δ 0.89 (t, 3H, *J* = 7.5 Hz, $-CH_3$), 1.20–1.28 (m, 2H, $-CH_2-$), 1.40–1.46 (m, 2H, $-CH_2-$), 2.64–2.70 (m, 1H, $-NCH_2-$), 2.74 (td, 1H, *J* = 2.5, 9.0 Hz, H-2), 2.78–2.85 (m, 1H, $-NCH_2-$), 2.95–2.98 (m, 1H, H-6), 3.75 (dd, 1H, *J* = 7.0, 12.5 Hz, H-7), 3.80–3.85 (m, 3H, 2H-1, H-7), 3.87–3.90 (m, 1H, H-3), 3.92 (dd, 1H, *J* = 3.5, 6.5 Hz, H-4), 3.98 (dd, 1H, *J* = 2.5, 4.5 Hz, H-5). ^{13}C NMR (125 MHz, D_2O): δ 13.61 ($-CH_3$), 19.96 ($-CH_2-$), 24.02 ($-CH_2-$), 48.82 ($-NCH_2-$), 55.75 (C-6), 59.59 (C-1), 60.14 (C-2), 61.03 (C-7), 63.86 (C-3), 69.57 (C-5), 70.02 (C-4). HRMS (ESI, positive) Calcd for $C_{11}H_{23}NO_5Na$: 272.1468 $[M+Na]^+$. Found: 272.1472.

4.8. *N*-Hexyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*–heptitol (4)

Compound **4** was prepared from **15** (63 mg, 0.1 mmol) as described in the preparation of **3**, providing **4** (27 mg, 100%) as white solids. $[\alpha]_D^{26}$ −22.6 (*c* 0.8, H_2O). ^1H NMR (500 MHz, D_2O): δ 0.86 (t, 3H, *J* = 7.0 Hz, $-CH_3$), 1.22–1.30 (m, 6H, $-CH_2-$), 1.43–1.48 (m, 2H, $-CH_2-$), 2.64–2.70 (m, 1H, $-NCH_2-$), 2.74 (td, 1H, *J* = 3.0, 8.5 Hz, H-2), 2.79–2.85 (m, 1H, $-NCH_2-$), 2.96–2.99 (m, 1H, H-6), 3.75 (dd, 1H, *J* = 7.0, 11.5 Hz, H-7), 3.80–3.88 (m, 3H, 2H-1, H-7), 3.89 (dd, 1H, *J* = 3.0, 8.5 Hz, H-3), 3.91–3.94 (m, 1H, H-4), 3.98 (dd, 1H, *J* = 2.0, 4.0 Hz, H-5). ^{13}C NMR (125 MHz, D_2O): δ 14.04 ($-CH_2-$), 21.68, 22.72, 26.86, 31.64 (4 × $-CH_2-$), 48.21 ($-NCH_2-$), 57.59 (C-6), 58.86 (C-1), 60.16 (C-2), 61.16 (C-7), 66.51 (C-3), 70.66 (C-5), 70.94 (C-4). HRMS (ESI, positive) Calcd for $C_{13}H_{27}NO_5Na$: 300.1781 $[M+Na]^+$. Found: 300.1786.

4.9. *N*-Nonyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*–heptitol (5)

Compound **5** was prepared from **16** (77 mg, 0.11 mmol) as described in the preparation of **3**, providing **5** (36 mg, 100%) as white solids. $[\alpha]_D^{26}$ −22.8 (*c* 0.8, H_2O). ^1H NMR (500 MHz, D_2O): δ 0.86 (t, 3H, *J* = 7.0 Hz, $-CH_3$), 1.27–1.37 (m, 12H, $-CH_2-$), 1.71–1.80 (m, 2H, $-CH_2-$), 3.30–3.47 (m, 3H, 2 × $-NCH_2-$, H-2), 3.72–3.74 (m, 1H, H-6), 3.97 (dd, 1H, *J* = 4.5, 13.0 Hz, H-7), 4.02–4.09 (m, 4H, H-7, H-3, 2 × H-1), 4.19–4.23 (m, 2H, H-4, H-5). ^{13}C NMR (125 MHz, D_2O): δ 14.12 ($-CH_3$), 20.69, 22.75, 26.14, 28.84, 28.99, 29.11, 31.82 (7 × $-CH_2-$), 49.24 ($-NCH_2-$), 55.09 (C-6), 59.94 (C-1), 60.08 (C-2), 61.13 (C-7), 63.38 (C-3), 69.28 (C-5), 69.95 (C-4). HRMS (ESI, positive) Calcd for $C_{16}H_{34}NO_5$: 320.2431 $[M+H]^+$. Found: 320.2430.

4.10. 1,3,4,5-Tetra-*O*-benzyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol *O* (7), *N*-cyclic carbamate (18)

Compound **18** was prepared from **17** (210 mg, 0.71 mmol) as described in the preparation of **12**, providing **18** (159 mg, 90%) as a yellow oil. The data were in good agreement with those reported by Martin et al.[28] ^1H NMR (500 MHz, $CDCl_3$): δ 3.34 (dd, 1H, *J* = 2.5, 4.0 Hz), 3.82–3.83 (m, 1H), 3.88 (dd, 1H, *J* = 3.0, 10.5 Hz), 3.94 (dd, 2H, *J* = 2.5, 6.5 Hz), 4.02 (t, 1H, *J* = 10.5 Hz), 4.09 (dd, 1H, *J* = 4.0, 8.5 Hz), 4.13 (ABq, 1H, *J* = 7.5 Hz, H-1), 4.32 (d, 1H, *J* = 12.0 Hz), 4.40 (d, 1H, *J* = 13.0 Hz), 4.43–4.46 (m, 3H), 4.55–4.57 (m, 1H), 4.59 (d, 1H, *J* = 12.0 Hz), 4.62 (d, 1H, *J* = 12.0 Hz), 4.70 (d, 1H, *J* = 12.0 Hz), 7.12–7.35 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 49.42, 51.51, 62.69, 65.41, 71.42, 72.15, 72.19, 73.11, 73.62, 75.09, 127.38, 127.52, 127.70, 127.81, 127.98, 128.13, 128.28, 128.30, 128.44, 137.00, 137.67, 137.85, 138.29, 157.60 (CO). MS-ESI: 580 $(M+H^+)$. Anal Calcd for $C_{36}H_{37}NO_6$: C, 74.59; H, 6.43; N, 2.42. Found: C, 74.39; H, 6.58; N, 2.26.

4.11. 3,4,5,7-Tetra-*O*-benzyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (19)

Compound **19** was prepared from **18** (220 mg, 0.38 mmol) as described in the preparation of **13**, providing **19** (178 mg, 85%) as a yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 2.07 (br s, 2H, –OH, –NH–), 3.06 (br s, 1H, H-6), 3.27–3.31 (m, 1H, H-2), 3.45 (t, *J* = 8.5 Hz, 1H, H-7), 3.57–3.61 (m, 2H, H-7, H-1), 3.64 (dd, 1H, *J* = 2.5, 9.0 Hz, H-3), 3.75 (dd, 1H, *J* = 5.5, 11.0 Hz, H-1), 3.99–4.01 (m, 2H, H-5, H-4), 4.47 (s, 2H), 4.54 (d, 1H, *J* = 11.5 Hz), 4.58 (d, 1H, *J* = 12.0 Hz), 4.66 (d, 1H, *J* = 12.5 Hz), 4.68 (d, 1H, *J* = 12.5 Hz), 4.73 (d, 1H, *J* = 11.5 Hz), 4.84 (d, 1H, *J* = 11.0 Hz) (8 × $-CH_2Ph$), 7.24–7.33 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 52.25 (C-6), 53.85 (C-2), 58.84 (C-1), 68.91 (C-7), 72.83, 73.12, 73.33, 73.78 (4 × $-CH_2Ph$), 75.20 (C-5), 77.61 (C-4), 79.36 (C-3), 127.40, 127.46, 127.61, 127.73, 127.84, 127.86, 127.95, 128.27, 128.31, 128.38, 128.42, 138.06, 138.32, 138.71, 138.74. MS-ESI: 554 $[M+H]^+$. Anal Calcd for $C_{35}H_{39}NO_5$: C, 75.92; H, 7.10; N, 2.53. Found: C, 75.67; H, 7.08; N, 2.45.

4.12. 3,4,5,7-Tetra-*O*-benzyl-*N*-butyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (20)

Compound **20** was prepared from **19** (100 mg, 0.18 mmol) as described in the preparation of **14** with the exception that ethanol was used in the reaction mixture instead of MeOH, providing **20** (88 mg, 80%) as a yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 0.85 (t, 3H, *J* = 7.0 Hz, $-CH_3$), 1.14–1.29 (m, 4H, $-CH_2-$), 2.56–2.67 (m, 2H, $-NCH_2-$), 2.82 (br s, 1H, –OH), 2.93 (br s, 1H, H-6), 3.22–3.26 (m, 1H, H-2), 3.52–3.56 (m, 2H, H-1, H-7), 3.59 (dd, 1H, *J* = 2.5, 9.5 Hz, H-4), 3.66 (t, 1H, *J* = 8.0 Hz, H-7), 3.72 (dd, 1H, *J* = 5.5, 10.5 Hz, H-1), 4.07 (br s, 1H, H-5), 4.18 (br s, 1H, H-3), 4.35 (d, 1H, *J* = 12.0 Hz), 4.41 (d, 1H, *J* = 11.5 Hz), 4.46 (d, 1H, *J* = 12.0 Hz), 4.62 (d, 1H, *J* = 11.5 Hz), 4.71–4.76 (m, 3H), 4.96 (d, 1H, *J* = 11.5 Hz) (8 × $-CH_2Ph$), 7.22–7.36 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.97 ($-CH_3$), 20.19 ($-CH_2-$), 32.55 ($-CH_2-$), 49.96 ($-NCH_2-$), 54.97 (C-6), 56.27 (C-1), 59.43 (C-2), 68.64 (C-7), 72.78, 73.26, 73.38 (3 × $-CH_2Ph$), 74.34 (C-3), 74.58 ($-CH_2Ph$), 76.74 (C-5), 80.74 (C-4), 127.23, 127.30, 127.34, 127.40, 127.59, 127.75, 128.16, 128.30, 128.38, 137.96, 138.49, 138.79, 139.28. MS-ESI: 610 $[M+H]^+$. Anal. Calcd for $C_{39}H_{47}NO_5$: C, 76.82; H, 7.77; N, 2.30. Found: C, 76.64; H, 7.91; N, 2.20.

4.13. 3,4,5,7-Tetra-*O*-benzyl-*N*-hexyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (21)

Compound **21** was prepared from **19** (31 mg, 0.06 mmol) as described in the preparation of **20**, providing **21** (31 mg, 86%) as a yellow oil. ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 7.5 Hz, $-CH_3$), 1.19–1.28 (m, 8H, $-CH_2-$), 2.55–2.66 (m, 2H, $-CH_2-$), 2.93 (br s, 1H, H-6), 3.21–3.26 (m, 1H, H-2), 3.50–3.56 (m, 2H, H-1, H-7), 3.59 (dd, 1H, *J* = 3.0, 9.5 Hz, H-4), 3.65 (t, 1H, *J* = 8.5 Hz, H-7), 3.71 (dd, 1H, *J* = 5.5, 11.0 Hz, H-1), 4.06–4.07 (m, 1H, H-5), 4.17 (br s, 1H, H-3), 4.36 (d, 1H, *J* = 11.5 Hz), 4.41 (d, 1H, *J* = 12.0 Hz), 4.46 (d, 1H, *J* = 11.5 Hz), 4.62 (d, 1H, *J* = 11.5 Hz), 4.71–4.76 (m, 3H), 4.96 (d, 1H, *J* = 12.0 Hz) (8 × $-CH_2Ph$), 7.22–7.36 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.05 ($-CH_3$), 22.59, 26.70, 30.32, 31.70 (4 × $-CH_2-$), 50.24 ($-NCH_2-$), 54.98 (C-6), 56.28 (C-1), 59.47 (C-2), 68.63 (C-7), 72.77, 73.25, 73.36 (3 × $-CH_2Ph$), 74.40 (C-3), 74.57 ($-CH_2Ph$), 76.75 (C-5), 80.75 (C-4), 127.22, 127.28, 127.33, 127.38, 127.58, 127.73, 128.14, 128.28, 128.37, 137.95, 138.49,

138.78, 139.28. MS-ESI: 638 $[M+H]^+$. Anal. Calcd for $C_{41}H_{51}NO_5$: C, 77.20; H, 8.06; N, 2.20. Found: C, 76.97; H, 8.06; N, 2.10.

4.14. 3,4,5,7-Tetra-*O*-benzyl-*N*-nonyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (22)

Compound **22** was prepared from **19** (80 mg, 0.14 mmol) as described in the preparation of **20**, providing **22** (78 mg, 80%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.89 (t, 3H, J = 7.0 Hz, $-CH_3$), 1.13–1.31 (m, 14H, $-CH_2-$), 2.55–2.66 (m, 2H, $-NCH_2-$), 2.93 (br s, 1H, H-6), 3.21–3.26 (m, 1H, H-2), 3.52–3.56 (m, 2H, H-1, H-7), 3.59 (dd, 1H, J = 3.0, 10.0 Hz, H-4), 3.65 (t, 1H, J = 8.5 Hz, H-7), 3.71 (dd, 1H, J = 5.5, 11.0 Hz, H-1), 4.06 (br s, 1H, H-5), 4.18 (br s, 1H, H-3), 4.36 (d, 1H, J = 12.0 Hz), 4.41 (d, 1H, J = 11.5 Hz), 4.46 (d, 1H, J = 12.0 Hz), 4.62 (d, 1H, J = 11.5 Hz), 4.72–4.76 (m, 3H), 4.96 (d, 1H, J = 11.5 Hz) (8 × $-CH_2$Ph), 7.23–7.30 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.09 ($-CH_3$), 22.65, 27.07, 29.28, 29.54, 29.57, 30.38, 31.85 (7 × $-CH_2-$), 50.25 ($-NCH_2-$), 54.97 (C-6), 56.31 (C-1), 59.49 (C-2), 68.65 (C-7), 72.79, 73.25, 73.37 (3 × $-CH_2$Ph), 74.45 (C-3), 74.58 ($-CH_2$Ph), 76.75 (C-5), 80.77 (C-4), 127.23, 127.30, 127.34, 127.40, 127.59, 127.75, 128.15, 128.30, 128.38, 137.96, 138.49, 138.79, 139.29. HRMS (ESI, positive) Calcd for $C_{44}H_{58}NO_5$: 680.4310 $[M+H]^+$. Found: 680.4302.

4.15. *N*-Butyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (6)

Compound **6** was prepared from **20** (73 mg, 0.12 mmol) as described in the preparation of **3**, providing **6** (26.8 mg, 90%) as a white solid. $[\alpha]_D^{26}$ +26.8 (c 0.8, H_2O). ^{1}H NMR (300 MHz, D_2O): δ 0.73 (t, 3H, J = 7.2 Hz, $-CH_3$), 1.06–1.18 (m, 2H, $-CH_2-$), 1.22–1.28 (m, 2H, $-CH_2-$), 2.46–2.64 (m, 2H, $-NCH_2-$), 2.73–2.77 (m, 1H, H-2), 3.09–3.18 (m, 1H, H-6), 3.49 (dd, 1H, J = 3.6, 9.9 Hz, H-4), 3.62–3.74 (m, 4H, H-7, H-1), 3.86 (br s, 1H, H-5), 3.93 (dd, 1H, J = 5.7, 9.9 Hz, H-3). ^{13}C NMR (75 MHz, D_2O): δ 15.92 ($-CH_3$), 22.58, 33.28 (2 × $-CH_2-$), 51.51 ($-NCH_2-$), 58.32 (C-6), 60.40 (C-2), 62.71 (C-7), 63.24 (C-1), 69.60 (C-3), 72.75 (C-5), 73.73 (C-4). HRMS (ESI, positive) Calcd for $C_{11}H_{23}NO_5Na$: 272.1468 $[M+Na]^+$. Found: 272.1466.

4.16. *N*-Hexyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (7)

Compound **7** was prepared from **21** (30 mg, 0.05 mmol) as described in the preparation of **3**, providing **7** (12 mg, 92%) as white solids. $[\alpha]_D^{26}$ +30.5 (c 0.8, H_2O). ^{1}H NMR (300 MHz, D_2O): δ 0.70 (t, 1H, J = 6.3 Hz, $-CH_3$), 1.13–1.14 (m, 6H, $-CH_2-$), 1.39 (br s, 2H, $-CH_2-$), 2.76–2.78 (m, 2H, $-NCH_2-$), 3.02 (br s, 1H, H-2), 3.31–3.33 (m, 1H, H-6), 3.56 (dd, 1H, J = 3.0, 9.6 Hz, H-4), 3.65–3.74 (m, 3H, H-7, H-1), 3.78 (dd, 1H, J = 6.6, 11.7 Hz, H-1), 3.94 (br s, 1H, H-5), 3.98 (dd, 1H, J = 5.7, 9.9 Hz, H-3). ^{13}C NMR (75 MHz, D_2O): δ 15.92 ($-CH_3$), 24.54, 28.62, 30.14, 33.49 (4 × $-CH_2-$), 52.20 ($-NCH_2-$), 58.05 (C-6), 61.18 (C-2), 62.01 (C-7), 63.56 (C-1), 69.25 (C-3), 72.25 (C-5), 73.26 (C-4). HRMS (ESI, positive) Calcd for $C_{13}H_{28}NO_5$: 278.1962 $[M+H]^+$. Found: 278.1962.

4.17. *N*-Nonyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (8)

Compound **8** was prepared from **22** (43 mg, 0.06 mmol) as described in the preparation of **3**, providing **8** (20 mg, 100%) as a white solid. $[\alpha]_D^{26}$ −10.2 (c 0.4, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 0.86 (t, 3H, J = 6.0 Hz, $-CH_3$), 1.23–1.32 (m, 12H, $-CH_2-$), 1.60 (br s, 2H, $-CH_2-$), 3.07 (d, 2H, J = 7.5 Hz, $-NCH_2-$), 3.33–3.34 (m, 1H, H-2), 3.56–3.57 (m, 1H, H-6), 3.78 (d, 1H, J = 7.0 Hz, H-4), 3.84–3.94 (m, 4H, H-1, H-7), 4.16–4.20 (m, 2H, H-3, H-5). ^{13}C NMR (125 MHz, D_2O): δ 14.38 ($-CH_3$), 23.09, 27.16, 28.02, 29.62, 29.71, 29.89, 32.29 (7 × $-CH_2-$), 51.01 ($-NCH_2-$), 55.92 (C-6), 59.28 (C-2), 60.18 (C-7), 66.79 (C-1), 68.86 (C-3), 67.00 (C-5), 70.96 (C-4). HRMS (ESI, positive) Calcd for $C_{16}H_{34}NO_5$: 320.2431 $[M+H]^+$. Found: 320.2427.

4.18. 1,3,4,5-Tetra-*O*-benzyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*-heptitol- lactone (23)

To a solution of **13** (278 mg, 0.50 mmol) in MeOH and acetic acid (v/v 200:1, 10 mL) was added 40% glyoxal aqueous solution (86 μL, 1.88 mmol). After stirring for 1 h at 60 °C, a portion of $NaBH_4$ (38 mg, 1.0 mmol) was added. The reaction mixture was stirred overnight at 80 °C and the reaction was quenched by the addition of 1 N HCl aqueous solution (0.2 mL). The mixture was extracted with EtOAc (3 × 50 mL) and washed with aqueous $NaHCO_3$ (10 mL) and aqueous NaCl (10 mL). The organic phases were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc 4:1) to provide **23** (221 mg, 74%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.69 (td, 1H, J = 2.5, 10.5 Hz), 2.91 (td, 1H, J = 2.5, 10.5 Hz), 3.07 (d, 1H, J = 18.0 Hz), 3.36 (dd, 1H, J = 2.5, 4.0 Hz), 3.63–3.71 (m, 3H), 3.84 (dd, 1H, J = 2.5, 10.0 Hz), 3.93 (dd, 1H, J = 3.0, 11.0 Hz), 4.05 (d, 1H, J = 17.5 Hz), 4.19 (d, 1H, J = 12.5 Hz), 4.31 (d, 1H, J = 11.5 Hz), 4.33 (d, 1H, J = 12.5 Hz), 4.36 (d, 1H, J = 11.5 Hz), 4.44 (d, 1H, J = 12.5 Hz), 4.48 (d, 1H, J = 10.5 Hz), 4.49 (d, 1H, J = 12.5 Hz), 4.57 (d, 1H, J = 12.0 Hz), 4.67 (d, 1H, J = 12.5 Hz), 7.10–7.33 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 53.56, 53.65, 61.72, 65.98, 70.04, 70.82, 72.18, 72.40, 73.09, 73.23, 73.42, 73.63, 127.79, 127.84, 127.87, 128.20, 128.32, 128.40, 128.43, 128.46, 128.49, 128.57, 137.11, 137.83, 137.89, 138.16, 167.41 (CO). MS-ESI: 594 $[M+H]^+$. Anal. Calcd for $C_{37}H_{39}NO_6$: C, 74.85; H, 6.62; N, 2.36. Found: C, 74.62; H, 6.55; N, 2.21.

4.19. 3,4,5,7-Tetra-*O*-benzyl-*N*-hydroxyethyl-2,6-dideoxy-2,6-imino-D-*glycero*- L-*altro*–heptitol (24)

To a solution of **23** (133 mg, 0.22 mmol) in dry THF (8 mL) was added $LiAlH_4$ (70 mg, 1.76 mmol) at −15 °C under argon and the mixture was stirred for 5 h at room temperature. The reaction mixture was slowly added EtOAc (3 mL) and 1 N NaOH (1 mL) at 0 °C and was extracted with EtOAc (3 × 50 mL) and washed with aqueous $NaHCO_3$ (10 mL) and aqueous NaCl (10 mL). The organic phases were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether–EtOAc 2:1) to provide **24** (76 mg, 57%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.96 (td, 1H, J = 5.5, 14.5 Hz, $-NCH_2-$), 3.09 (td, 1H, J = 4.0, 15.0 Hz, $-NCH_2-$), 3.26 (q, 1H, J = 6.5 Hz, H-6), 3.33–3.36 (m, 1H, H-2), 3.43 (dd, 1H, J = 5.0, 10.0 Hz, H-7), 3.47–3.56 (m, 4H, 2H-1, 2 × CH_2OH), 3.68 (dd, 1H, J = 2.5, 7.5 Hz, H-4), 3.72 (dd, 1H, J = 6.5, 11.5 Hz, H-7), 3.79 (dd, 1H, J = 3.0, 5.0 Hz, H-3), 4.03 (dd, 1H, J = 5.0, 7.5 Hz, H-5), 4.44 (d, 1H, J = 12.0 Hz), 4.48–4.60 (m, 6H), 4.62 (d, 1H, J = 11.5 Hz) (8 × $-CH_2$Ph), 7.24–7.39 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 55.77 ($-NCH_2-$), 60.46 ($-CH_2OH$), 60.98 (C-2), 61.07 (C-6), 61.96 (C-7), 69.83 (C-1), 72.26, 72.63, 73.22, 73.35 (4 × $-CH_2$Ph), 74.56 (C-4), 74.92 (C-3), 77.37 (C-5), 127.60, 127.64, 127.69, 127.77, 127.82, 127.88, 127.94, 128.33, 128.34, 128.45, 137.43, 138.06, 138.11, 138.43. HRMS (ESI, positive) Calcd for $C_{37}H_{44}NO_6$: 598.3163 $[M+H]^+$. Found: 598.3170.

4.20. *N*-Hydroxyethyl-2,6-dideoxy-2,6-imino-D-*glycero*-L-*altro*-heptitol (9)

Compound **9** was prepared from **24** (43 mg, 0.07 mmol) as described in the preparation of **3**, providing **9** (15.3 mg, 90%) as a

white solid. $[\alpha]_D^{26}$ −9.0 (*c* 0.8, H_2O). ^1H NMR (500 MHz, D_2O) δ: 2.84–2.87 (m, 1H, H-2), 2.98 (t, 2H, *J* = 6.5 Hz, $-NCH_2-$), 3.06 (dt, 1H, *J* = 3.0, 6.5 Hz, H-6), 3.66 (t, 2H, *J* = 6.0 Hz, 2 × CH_2OH), 3.74–3.83 (m, 4H, 2H-7 and 2H-1), 3.90 (dd, 1H, *J* = 3.5, 6.0 Hz, H-3), 3.93 (dd, 1H, *J* = 3.0, 7.5 Hz, H-4), 4.01 (dd, 1H, *J* = 3.0, 5.5 Hz, H-5). ^{13}C NMR (75 MHz, D_2O) δ: 53.54 ($-NCH_2-$), 61.38 ($-CH_2OH$), 61.76 (C-6), 62.07 (C-1), 63.20 (C-2), 64.58 (C-7), 68.85 (C-3), 72.16 (C-5), 72.37 (C-4). HRMS (ESI, positive) Calcd for $C_9H_{19}NO_6Na$: 260.1105 $[M+Na]^+$. Found: 260.1102.

4.21. *N*-Hydroxyethyl-2,6-dideoxy-2,6-imino-D-*glycero*-D-*galacto*-heptitol (10)

Compound **10** was prepared from **19** (90 mg, 0.16 mmol) as described in the preparation of **23**, providing yellow crude product (64 mg) after column chromatography. The product was treated under the conditions as described in the preparation of **24**, yielding crude product (53 mg). Finally, catalytic hydrogenolysis of the crude product using the method in the preparation of **9** provided **10** (21 mg, 55%, three steps) as white solids. $[\alpha]_D^{26}$ +33.8 (*c* 0.8, H_2O). ^1H NMR (300 MHz, D_2O): δ 2.54–2.62 (m, 1H, $-NCH_2-$), 2.73–2.89 (m, 2H, H-2, $-NCH_2-$), 2.99–3.05 (m, 1H, H-6), 3.32–3.44 (m, 3H, H-4, $-CH_2OH$), 3.49–3.55 (m, 3H, H-7, H-1), 3.64 (dd, 1H, *J* = 7.8, 10.8 Hz, H-1), 3.77 (br s, 1H, H-5), 3.84 (dd, 1H, *J* = 5.4, 9.3 Hz, H-3). ^{13}C NMR (75 MHz, D_2O): δ 53.15 ($-NCH_2-$), 58.82 (C-6), 59.74 (C-2), 62.17 (C-7), 62.77 (CH_2OH), 64.05 (C-1), 69.56 (C-3), 73.10 (C-5), 74.12 (C-4). HRMS (ESI, positive) Calcd for $C_9H_{19}NO_6Na$: 260.1105 $[M+Na]^+$. Found: 260.1101.

4.22. Cell proliferation assay

Male BALB/c mouse splenocytes (4×10^5 cells per well), retreated with Con A alone (2.5 μg/mL concentration) or along with 30 μM concentration of synthetic iminosugar compounds, were incubated at 37 °C for 48 h under 5% CO_2 in a RPMI-1640 medium containing 10% fetal bovine serum (FBS). The proliferation of the mouse splenocytes was assayed using the MTT reduction method. MTT (5 mg/mL, 20 μL) dissolved in phosphate buffer solution (PBS) was added to each well and the plates were incubated for 4 h at 37 °C. The 96-well microplates were centrifuged at 1500*g* for 6 min and the media were aspirated. The resultant crystals were dissolved in 150 μL of DMSO for 10 min. Optical density was measured using a Microplate Reader at 570 nm. All data were presented as mean ± SEM. One-way ANOVA followed by Bonferroni was used to test the difference between groups. Statistical significance was indicated by $p < 0.05$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.01.021.

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