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Looking glass inhibitors: synthesis of a potent naringinase inhibitor L-DIM [1,4-dideoxy-1,4-imino-L-mannitol], the enantiomer of DIM [1,4-dideoxy-1,4-imino-D-mannitol] a potent α-D-mannosidase inhibitor

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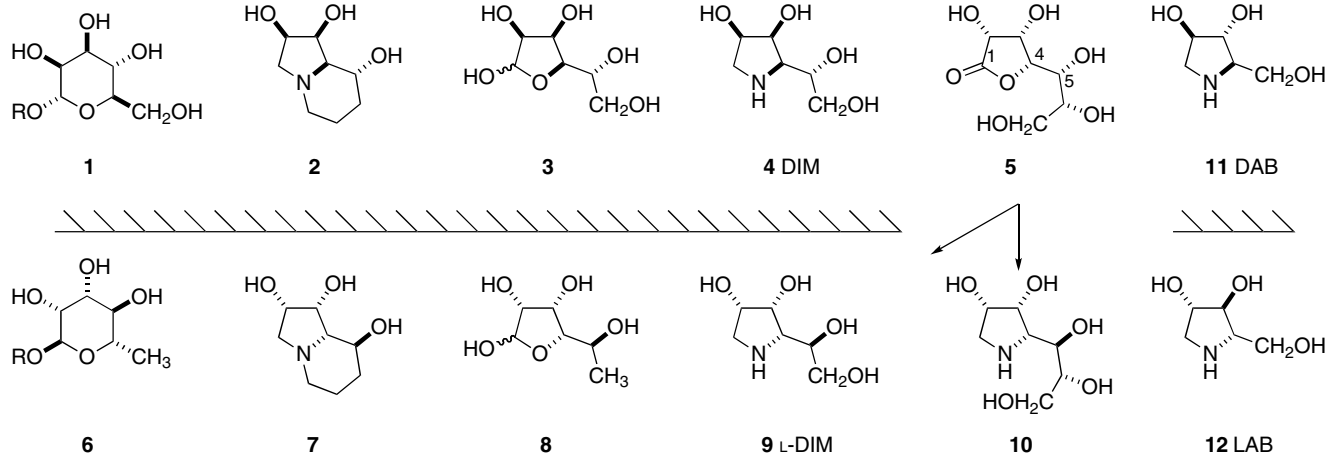
Abstract—The synthesis of L-DIM [1,4-dideoxy-1,4-imino-L-mannitol] and of 1,4-imino-D-*glycero*-L-*talo*-heptitol from D-*glycero*-D-*gulo*-heptono-1,4-lactone depends on the use of pentan-3-one to form two five ring ketals as described by Burke, rather than the formation of one five ring and one six ring ketal formed with acetone. L-DIM, the enantiomer of the potent α-D-mannosidase inhibitor DIM [1,4-dideoxy-1,4-imino-D-mannitol] is a good inhibitor of naringinase (an α-L-rhamnosidase) with a K_i of 3.63 μM. 1,4-Imino-D-*glycero*-L-*talo*-heptitol is a moderate inhibitor of naringinase.

1. Introduction

Carbohydrate mimics in which the ring oxygen of a sugar is replaced by nitrogen—because of their inhibition of glycosidases[1] and other sugar processing enzymes[2]—have considerable therapeutic potential.[3] D-Swainsonine **2**, a natural product isolated[4] from *Swainsona canescens*, is a powerful inhibitor of α-mannosidases that cleave the



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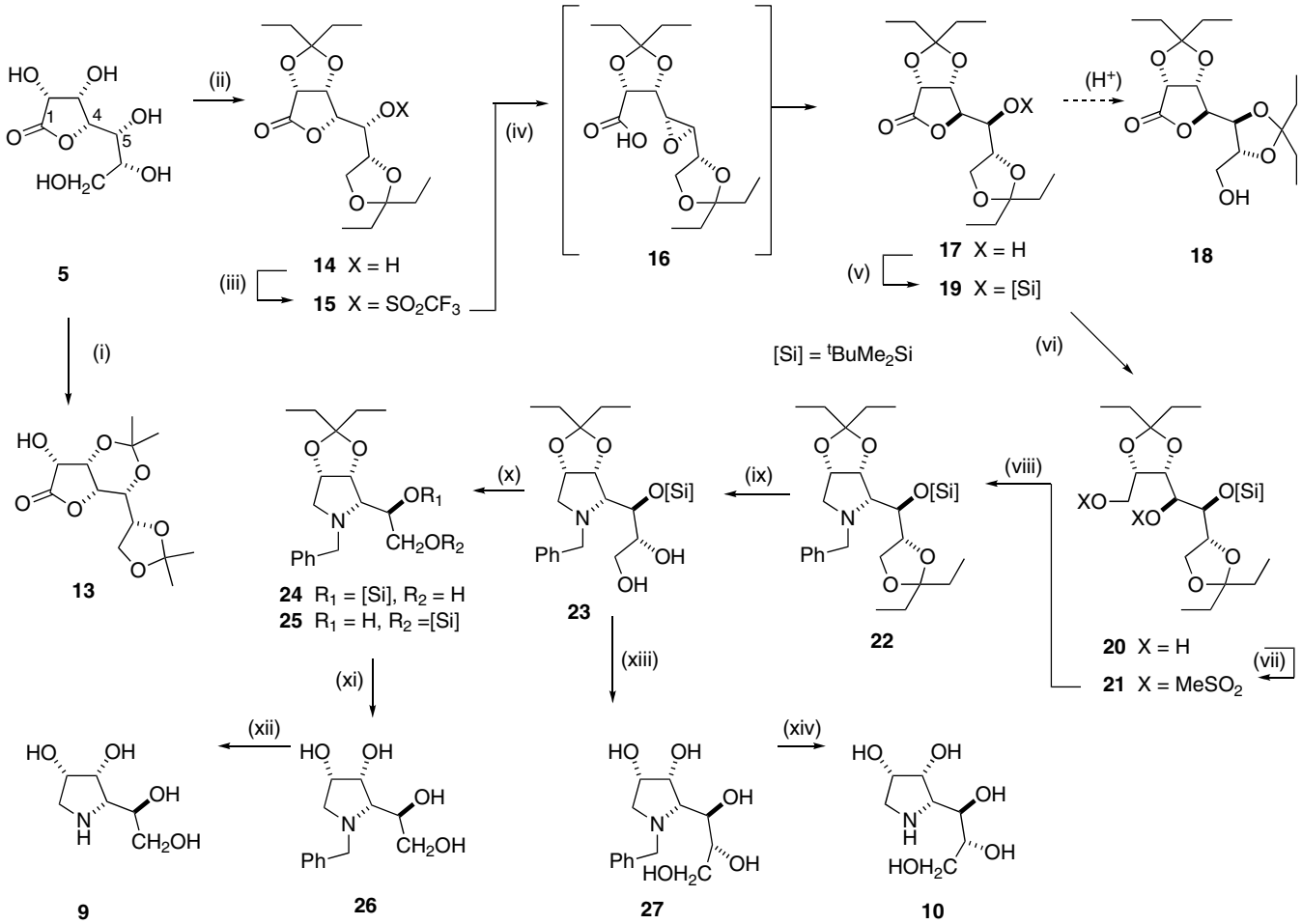
glycosidic bond of α-D-mannopyranosides **1**. In particular swainsonine **2** inhibits a mannosidase of glycoprotein processing;[5] this may be the basis for the potential of **2** in the chemotherapy of cancer[6] and the reason why more than 40 syntheses of swainsonine have appeared.[7] Swainsonine is a mimic of mannofuranose **3**; the nitrogen analogue DIM [1,4-dideoxy-1,4-imino-D-mannitol] **4**[8] is also a potent inhibitor of α-mannosidases,[9] including a mannosidase of glycoprotein processing,[10] and has been the subject of considerable synthetic interest.[11] The crystal structure of the hydrochloride of DIM **4** has been reported.[12] Mirror images of glycosidase inhibitors can also be powerful inhibitors of either the same or different enzymes;[13] for example, natural product **11** DAB and its synthetic enantiomer **12** LAB are both powerful inhibitors of α-glucosidases.[14]

α-L-Rhamnopyranosides **6** are the enantiomers of α-D-mannopyranosides **1** in which the C-6 hydroxymethyl is replaced by a methyl group. L-Swainsonine **7**, the enantiomer of the natural product **2**, is the imino sugar mimic of L-rhamnofuranose **8** and is a potent inhibitor of naringinase—an α-rhamnosidase;[15] there are a few syntheses[16] of the enantiomeric L-swainsonine **7** and none of L-DIM **9**, the mirror image of DIM **4**. Although pyrrolidine iminoheptitols have been shown to act as glycosidase inhibitors, no study of 1,4-imino-D-*glycero*-L-*talo*-heptitol **10** as an enzyme inhibitor has been reported. L-Rhamnose is a sugar, which does not occur in mammals but does occur in various pathogens; thus the mimics of L-rhamnose may have a therapeutic potential.

Herein the first synthesis of L-DIM **9**, and of iminoheptitol **10**[17] in which there is an additional CHOH in the side chain from D-*glycero*-D-*gulo*-heptono-1,4-lactone, the only cheaply available seven carbon sugar lactone, **5**. The paper also reports that L-DIM **9** is a good—and iminoheptitol **10** is a moderate—inhibitor of naringinase.

2. Synthesis of L-DIM 9 and of 1,4-imino-D-*glycero*-L-*talo*-heptitol 10

The value of the seven carbohydrate lactone **5** as a starting material has been illustrated by the syntheses of antibiotic gonifurfuranone[18] and a number of imino sugars;[19] otherwise the use of protected seven carbon sugars has been rare.[20] The synthesis of L-DIM **9** and of the related iminoheptitol **10** from the seven carbon sugar lactone **5** required



Scheme 1. Reagents and conditions: (i) Ref. 21; (ii) Ref. 22; (iii) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2; (iv) 4 M KOH, dioxane, then Amberlyst 15, 60% (two steps); (v) *tert*-$BuMe_2SiCl$, imidazole, DMF, 140 °C, 99%; (vi) $LiBH_4$, THF, 85%; (vii) $MeSO_2Cl$, DMAP, pyridine, 96%; (viii) $BnNH_2$, 120 °C, 89%; (ix) 80% AcOH (aq), 80 °C, 57% (from **19**: 47%, four steps); (x) $NaIO_4$, $MeOH/H_2O$; then $NaBH_4$ 81%; (xi) 90% CF_3CO_2H (aq), reflux, 97%; (xii) H_2, $Pd(OH)_2/C$, $dioxane/H_2O$, quant; (xiii) 90% CF_3CO_2H (aq), reflux, 68%; (xiv) H_2, $Pd(OH)_2/C$, $dioxane/H_2O$, quant.

(a) the formation of the pyrrolidine ring by the introduction of nitrogen between C-1 and C-4 with the overall retention of configuration and (b) the inversion of configuration at C-5. The required stereochemistry was efficiently achieved by an initial double inversion at both C-4 and C-5 in **5** to give the protected lactone **17**. Acetone protection of lactone **5** gave diacetonide **13** in a good yield—containing both a five and a six ring ketal—leaving the C-2 hydroxyl group free.[21] However treatment of **5** with pentan-3-one by the procedure described by Burke[22] gave the corresponding diketal derivative **14** in a 55% yield in which the C-5 hydroxyl group was unprotected, ideally set up for the required double inversion of both C-4 and C-5 (Scheme 1).

Esterification of the free hydroxyl group at C-5 in **14** with triflic anhydride in dichloromethane gave the corresponding triflate **15**, which with potassium hydroxide in dioxane afforded epoxide **16**. Epoxide **16** under acidic conditions gave the doubly inverted lactone **17**. The yield of **17** was highly dependant on the precise experimental procedure, since 6,7-diethylketal **17** was prone to acid catalysed equilibration to the more stable 5,6-diethylketal **18**; this isomerism was minimised by the use of an acidic resin (Amberlyst 15), but other acidic work-up conditions gave substantial amounts of **18**. The structure of **18** is strongly supported by the ^{13}C NMR spectrum in which the singlets of the ketal carbon are at 117.6 and 114.0, values not consistent with a six-membered ring ketal. The doubly inverted lactone **17** was thus obtained in a 60% yield from **14**; the structure of **17** was firmly established by X-ray crystal analysis.[23]

The benzylation at the C-5 position in **17** gave only a moderate yield of the corresponding benzyl ether. However, alcohol **17** could be efficiently protected as its *tert*-butyldimethylsilyl (TBDMS) ether **19** in a 99% yield by treatment with a very concentrated solution of TBDMS chloride and imidazole in anhydrous DMF at 140 °C overnight. Silyl ether **19** was reduced by lithium borohydride in tetrahydrofuran to diol **20** in a 85% yield; trace amounts of a product in which the silyl group had migrated could be removed by column chromatography. The reaction of diol **20** with methanesulfonyl chloride in the presence of *N*,*N*-dimethylamino pyridine (DMAP) gave dimesylate **21** (96% yield), which on treatment with neat benzylamine at elevated temperatures for a prolonged time gave pyrrolidine **22** in an 89% yield. Some problems were encountered during the removal of excess benzylamine but any impurities were easily removed after the selective deprotection of 6,7-diethylketal in **22** to afford diol **23** in a 57% yield [overall yield from **19** was 47% without purification of the intermediates]. Sequential treatment of **23** with periodate followed by reduction with sodium borohydride gave an inseparable mixture of **24** and **25**; migration of the TBDMS ether in **24** to the primary position in **25** was unavoidable under the basic conditions of the reduction. However, treatment of the mixture of silyl ethers **24** and **25** with aqueous trifluoroacetic acid gave **26** as a single product in a 78% yield from **23**. The benzyl group in **26** was removed by hydrogenation in the presence of Pearlman's catalyst (palladium hydroxide on carbon) to afford L-DIM **9** in a quantitative yield with identical physical and spectral data [other than the specific rotation] to those of DIM **4**.[11a] The overall yield of the synthesis of L-DIM **9** from the protected lactone **17** was 12.1%.

Iminoheptitol **10** with an extra carbon in the side chain was obtained by removal of the silyl and ketal protecting groups in **23** with aqueous trifluoroacetic acid to give benzyl derivative **27** (68% yield); subsequent hydrogenation of **27** in the presence of Pearlman's catalyst gave the seven carbon sugar mimic **10** in a 76% yield.[17]

3. Glycosidase inhibition

Inhibition was tested using 1 mg ml^{-1} aqueous solutions of L-DIM **9** and L-swainsonine **7** (the concentration of the compounds in the assays was 915.8 and 860.6 μM, respectively) and compared with D-swainsonine **2**. Assays were carried out in triplicate, and the values of percentage inhibition of the glycosidases are given in Table 1 and are means of the three replicates per assay. The substrate con-

Table 1. % Inhibition of various glycosidases by L-DIM **9**, L-**7** and D-**2** swainsonine

Assay (enzyme source)	pH	[Enzyme] working stock (U/ml)	[Enzyme] in assay (U/ml)	Reaction conditions (min at °C)	L-DIM **9**	L-Swain **7**	D-Swain **2**
α-D-Glucosidase (Yeast [*S. cerevisiae*])	6.0	1.0	0.0007	10 at 27	8.0	−6.2	3.19
α-D-Glucosidase (*Bacillus*)	6.8	0.5	0.00035	10 at 27	21.7	0.7	15.04
α-D-Glucosidase (Rice)	4.0	5.0	0.0035	20 at 27	2.0	12.1	2.08
β-D-Glucosidase (Almond)	5.0	0.25	0.000175	10 at 27	9.1	2.7	2.43
α-D-Galactosidase (Green coffee bean)	6.5	0.2	0.00014	10 at 27	2.3	−4.1	0.89
β-D-Galactosidase (Bovine liver)	7.3	0.1	0.00007	10 at 27	12.6	−2.8	7.07
α-L-Fucosidase (Bovine kidney)	5.5	0.25	0.000175	10 at 27	5.8	6.9	9.92
α-D-Mannosidase (Jack bean)	4.5	0.1	0.00007	10 at 27	17.1	11.4	99.65
β-D-Mannosidase (*Cellulomonas fimi*)	6.5	1.0	0.0007	20 at 27 (10 min for D-swain)	−7.3	−2.0	−4.61
Naringinase (*Penicillium decumbens*)	4.0	0.1	0.00007	20 at 27 (10 min for D-swain)	97.2	99.2	3.63
N-Acetyl-β-D-gluc. (Bovine kidney)	4.25	0.25	0.000175	10 at 27	17.0	13.2	4.93
N-Acetyl-β-D-gluc. (Jack bean)	7.0	0.5	0.00035	10 at 27	−0.5	2.9	23.76
N-Acetyl-β-D-hexos. (*Aspergillus oryzae*)	5.0	0.25	0.000175	10 at 27	−3.3	−0.7	14.38
Amyloglucosidase (*Aspergillus niger*)	4.5	0.5 (1 U ml^{-1} for D-swain)	0.00035	10 at 32	0.6	−5.6	0.09

centration was 5 mM in all assays.[24] The only significant inhibition of glycosidases by the L-sugar mimics **7** and **9** is of naringinase, whereas only α-D-mannosidase was inhibited by D-swainsonine **2.**

The IC_{50} and K_i values for the inhibition of naringinase for L-DIM **9** and L-swainsonine **7** are shown in Table 2. The IC_{50} values were determined using serial dilutions from the 1 mg ml^{-1} solutions used for screening glycosidase inhibition. Inhibition was tested using 0.1 U ml^{-1} enzyme solution and 5 mM substrate solution, using deionised water (dH_2O) as a control. Reactions were carried out at 28 °C. The K_i values for the inhibition were determined using a substrate dilution series (0.5–5 mM substrate in 0.5 mM steps) and 0.1 U ml^{-1} enzyme solution. Three inhibitor concentrations were tested for each compound, using the IC_{50} concentration as the highest. Control (uninhibited) reactions were carried out using dH_2O. Reactions were carried out at 28 °C. Velocity (rate of PNP release) was calculated using an absorption coefficient for PNP in 0.4 M glycine solution, pH 10.3, which was determined using the same microplate reader as used for the inhibition assays. K_i values were calculated from transformed absorbance data using Lineweaver–Burke double reciprocal plots.

The IC_{50} (μM) values of naringinase inhibition of **7** and **9** were determined separately for comparison with the homologous pyrrolidine 1,4-imino-D-*glycero*-L-*talo*-heptitol **10** and its *N*-benzyl derivative **27** (Table 3).

There was still significant inhibition of naringinase by the pyrrolidines **10** and **27** with an additional CHOH in the side chain and these may have value as rhamnose mimics. None of the rhamnose mimics have any significant inhibition of other glycosidases and are thus highly specific inhibitors.

4. Conclusion

Herein we have reported the efficient synthesis of a number of monocylic pyrrolidines, which are the mimics of L-rhamnofuranose; the compounds show an excellent to good inhibition of naringinase. Further investigation of the properties of these compounds is currently in progress.

Table 2. Inhibition of naringinase by L-DIM **9** and L-swainsonine **7**

	L-DIM **9**	L-Swainsonine **7**
IC_{50} (μM)	27.47	0.52
K_i (μM)	3.63	0.08
Mode of inhibition	Competitive	Competitive

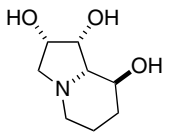
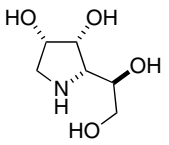
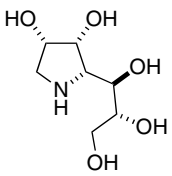
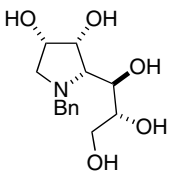
5. Experimental

All solvents were used as supplied (Analytical or HPLC grade), without further purification, except for DMF and pyridine, which were dried on molecular sieves prior to use. The reactions performed under a hydrogen atmosphere were maintained by an inflated balloon. Flash chromatography was performed using Sorbsil C60 40/60 silica. Thin layer chromatography (TLC) was carried out on aluminium backed sheets coated with 60 F^{254} silica from Merck. Plates were developed using a spray of 0.2% w/v cerium sulfate and 5% w/v ammonium molybdate in 2 M sulfuric acid with subsequent heating or 6% w/v phosphomolybdic acid in ethanol. Melting points were recorded on a Kofler hot block and are corrected. NMR spectra were recorded on a Bruker DQX 400 (400 MHz) instrument and were calibrated according to the chemical shift of the deuterated solvent. High resolution mass spectra (HRMS) were recorded on a Micromass LCT (ESI) spectrometer. Infrared spectra (IR) were recorded on a Bruker Tensor Fourier Transform IR spectrometer using thin film on NaCl plates. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. Microanalyses were carried out by the analytical service of the ICL in Oxford.

5.1. 2,3:6,7-Di-*O*-diethylidene-D-*glycero*-L-*talo*-heptono-1,4-lactone 17 and 2,3:5,6-di-*O*-diethylidene-D-*glycero*-L-*talo*-heptono-1,4-lactone 18

Triflic anhydride (0.96 ml, 5.71 mmol) was added to a solution of diethylidene ketal **14**[22] (1.31 g, 3.81 mmol) dissolved in dichloromethane (25 ml) with pyridine (0.92 ml, 11.5 mmol) under argon at −20 °C. The reaction mixture was stirred for 2 h at −20 °C, quenched with brine (20 ml) and was washed with aqueous hydrochloric acid (1 M, 20 ml). The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo in the cold. The residue of crude triflate **15** was dissolved in dioxane (25 ml) and potassium hydroxide (4 M, 3 ml, 12.0 mmol) was added. The mixture was stirred for 2 h at room temperature after which Amberlyst 15 (3 g) was added. After stirring for an additional 75 min, the reaction mixture was filtered and the resin was washed with ethyl acetate. The combined organic layers were washed with a saturated aqueous solution of sodium bicarbonate (50 ml), dried (so-

Table 3. IC_{50} (μM) of naringinase inhibition by L-DIM **9** and other rhamnose mimics

			
7	**9**	**10**	**27**
IC_{50} 0.134	IC_{50} 52.8	IC_{50} 125	IC_{50} 528

dium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:4) to afford the doubly inverted lactone **17** (781 mg, 60%) as a white, crystalline solid. $R_f = 0.70$ (ethyl acetate/cyclohexane, 1:1); mp 93–94 °C (heptane); $[\alpha]_D^{21} = -28.4$ (*c* 1.93, $CHCl_3$); IR (film) $\nu = 3443$ (OH), 1789 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 4.84$ (d, $J_{2,3} = 6.0$ Hz, 1H, H-3), 4.79 (d, $J_{2,3} = 6.0$ Hz, 1H, H-2), 4.40 (s, 1H, H-4), 4.23 (q, $J = 6.8$ Hz, 1H, H-6), 4.15–4.07 (m, 1H, H-7a), 3.75–3.67 (m, 2H, H-5, H-7b), 2.82 (br s, 1H, 4-OH), 1.72–1.56 (m, 8H, CH_2CH_3), 0.87 ppm (m, 12H, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 174.1$ (C=O), 117.5, 114.2 ($C(CH_2CH_3)_2$), 81.2 (C-4), 79.0 (C-2), 75.5 (C-6), 75.4 (C-3), 73.0 (C-5), 66.1 (C-7), 29.8, 29.5, 29.3, 29.1 ($C(CH_2CH_3)_2$), 8.3, 8.2, 7.9, 7.3 ppm ($C(CH_2CH_3)_2$); HRMS *m*/*z* (ESI +ve) calcd for $C_{17}H_{28}O_7Na$: 367.1733 $[M+Na]^+$; found: 367.1728; elemental analysis calcd (%) for $C_{17}H_{28}O_7$: C 59.29, H 8.19; found: C 59.28, H 8.26.

In the presence of acid catalysts, the 6,7-ketal **17** isomerised to the slightly more polar isomeric 5,6-ketal **18** oil, $R_f = 0.59$ (ethyl acetate/cyclohexane, 1:1); $[\alpha]_D^{22} = -62.6$ (*c* 1.60, $CHCl_3$); IR (film) $\nu = 3483$ (OH), 1791 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 4.79$ (d, $J_{2,3} = 5.6$ Hz, 1H, H-3), 4.77 (d, $J_{2,3} = 5.6$ Hz, 1H, H-2), 4.61 (br s, 1H, H-4), 4.17–4.07 (m, 2H, H-5, H-6), 3.89 (dd, $J_{6,7a} = 3.9$ Hz, $J_{7a,7b} = 12.1$ Hz, 1H, H-7a), 3.73 (dd, $J_{6,7b} = 3.2$ Hz, $J_{7a,7b} = 12.1$ Hz, 1H, H-7b), 1.72–1.48 (m, 8H, CH_2CH_3), 0.92–0.78 ppm (m, 12H, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 174.0$ (C=O), 117.6, 114.0 ($C(CH_2CH_3)_2$), 79.0 (C-2), 78.8 (C-4), 77.0, 75.9 (C-5, C-6), 75.2 (C-3), 60.9 (C-7), 29.9, 29.8, 29.6, 29.2 ($C(CH_2CH_3)_2$), 8.2, 8.2, 7.7, 7.3 ppm ($C(CH_2CH_3)_2$); HRMS *m*/*z* (ESI +ve) calcd for $C_{17}H_{28}O_7Na$: 367.1727 $[M+Na]^+$; found: 367.1725.

5.2. 5-*O*-*tert*-Butyldimethylsilyl-2,3:6,7-di-*O*-diethylidene-D-*glycero*-L-*talo*-heptono-1,4-lactone 19

Imidazole (7.29 g, 107 mmol) and TBDMS chloride (8.20 g, 54.4 mmol) were added to a suspension of lactone **17** (6.13 g, 17.8 mmol) in anhydrous DMF (6 ml) under argon. The reaction mixture was heated to 140 °C for 15 h, concentrated in vacuo and the residue was partitioned between ether and water (100 ml, 1:1). The aqueous layer was extracted with ether (50 ml) and the combined organic layers over dried sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:19) to afford silyl ether **19** (8.097 g, 99%) as a white, crystalline solid. $R_f = 0.55$ (ethyl acetate/cyclohexane, 1:3); mp 92–94 °C (ethyl acetate/cyclohexane); $[\alpha]_D^{21} = -14.6$ (*c* 2.5, $CHCl_3$); IR (film) $\nu = 1786$ (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 4.79$ (d, $J_{2,3} = 5.9$ Hz, 1H, H-2/H-3), 4.77 (d, $J_{2,3} = 5.9$ Hz, 1H, H-2/H-3), 4.44 (d, $J_{4,5} = 2.6$ Hz, 1H, H-4), 4.14 (app. dt, $J = 6.1$ Hz, $J = 8.7$ Hz, 1H, H-6), 4.07 (dd, $J_{6,7a} = 6.3$ Hz, $J_{7a,7b} = 7.6$ Hz, 1H, H-7a), 3.92 (dd, $J_{4,5} = 2.6$ Hz, $J_{5,6} = 5.9$ Hz, 1H, H-5), 3.67 (dd, $J_{7a,7b} = 7.7$ Hz, $J_{6,7b} = 8.7$ Hz, 1H, H-7b), 1.67 (q, $J_{Et} = 7.4$ Hz, 4H, $C(CH_2CH_3)_2$), 1.59 (q, $J_{Et} = 7.7$ Hz, 4H, $C(CH_2CH_3)_2$), 0.93–0.84 (m, 12H, $C(CH_2CH_3)_2$), 0.89 (s, 9H, $C(CH_3)_3$), 0.15 (s, 3H, $SiCH_3$), 0.13 ppm (s, 3H, $SiCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 173.6$ (C=O), 117.6, 113.8 ($C(CH_2CH_3)_2$), 83.2 (C-4), 78.5 (C-2/C-3), 76.6 (C-6), 75.6 (C-2/C-3), 73.0 (C-5), 66.0 (C-7), 29.8, 29.7, 29.3 (2x) ($C(CH_2CH_3)_2$), 25.7 ($C(CH_3)_3$), 18.1 ($C(CH_3)_3$), 8.2, 8.1, 8.0, 7.4 ($C(CH_2CH_3)_2$), −4.3, −4.6 ppm ($SiCH_3$); HRMS *m*/*z* (ESI +ve) calcd for $C_{23}H_{42}O_7SiNa$: 481.2592 $[M+Na]^+$; found: 481.2587.

5.3. 5-*O*-*tert*-Butyldimethylsilyl-2,3:6,7-di-*O*-diethylidene-D-*glycero*-L-*talo*-heptitol 20

Lithium borohydride (624 mg, 28.7 mmol) was added to the silylated lactone **19** (6.58 g, 14.3 mmol) in anhydrous THF (70 ml) under argon at 0 °C and the reaction mixture stirred at room temperature for 27 h. Additional lithium borohydride (232 mg, 10.7 mmol) was added at 0 °C. After a total of 50 h, the reaction mixture was quenched by the slow addition of a saturated aqueous solution of ammonium chloride (100 ml), extracted with dichloromethane (3 × 100 ml) and the combined organic layers were dried (sodium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:4) to afford diol **20** (5.604 g, 85%) as an oil. $R_f = 0.30$ (ethyl acetate/cyclohexane, 1:3); $[\alpha]_D^{21} = +24.1$ (*c* 3.1, $CHCl_3$); IR (film) $\nu = 3424$ (O–H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 4.43$–4.36 (m, 1H, H-2), 4.21–4.13 (m, 1H, H-6), 4.08–4.01 (m, 3H, H-3, H-5, H-7a), 3.85 (dd, $J_{1a,1b} = 11.8$ Hz, $J = 7.7$ Hz, 1H, H-1a), 3.77 (dd, $J_{1a,1b} = 11.8$ Hz, $J = 5.1$ Hz, 1H, H-1b), 3.60 (t, $J = 8.2$ Hz, 1H, H-7b), 3.46 (d, $J = 9.8$ Hz, 1H, H-4), 3.08 (br s, 2H, OH), 1.71–1.51 (m, 8H, $C(CH_2CH_3)_2$), 0.94–0.84 (m, 12H, $C(CH_2CH_3)_2$), 0.92 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, $SiCH_3$), 0.15 ppm (s, 3H, $SiCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 113.5$, 112.7 ($C(CH_2CH_3)_2$), 77.8 (C-6), 77.3 (C-2), 75.4, 72.3 (C-3, C-5), 69.5 (C-4), 66.3 (C-7), 60.8 (C-1), 29.7, 29.5, 29.2, 28.6 ($C(CH_2CH_3)_2$), 26.0 ($C(CH_3)_3$), 18.3 ($C(CH_3)_3$), 8.6, 8.2, 8.0, 7.8 ($C(CH_2CH_3)_2$), −4.1, −4.9 ppm ($SiCH_3$); HRMS *m*/*z* (ESI +ve) calcd for $C_{23}H_{46}O_7SiNa$: 485.2905 $[M+Na]^+$; found: 485.2904.

5.4. 5-*O*-*tert*-Butyldimethylsilyl-2,3:6,7-di-*O*-diethylidene-1,4-di-*O*-methanesulfonyl-D-*glycero*-L-*talo*-heptitol 21

Dimethylaminopyridine (66 mg, 0.54 mmol) was added to a solution of diol **20** (1.02 g, 2.20 mmol) in anhydrous pyridine (10 ml) under argon at 0 °C. Methanesulfonyl chloride (0.7 ml, 8.9 mmol) was then added and the reaction mixture stirred at room temperature for 14 h. The reaction mixture was concentrated in vacuo, the residue dissolved in dicholoromethane (20 ml) and washed sequentially with aqueous hydrochloric acid (1 M, 2 × 15 ml) and a saturated aqueous solution of sodium bicarbonate (15 ml). The organic layer was dried (sodium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:4) to afford dimesylate **21** (1.307 g, 96%) as a clear oil. $R_f = 0.30$ (ethyl acetate/cyclohexane, 1:3); $[\alpha]_D^{20} = -44.2$ (*c* 0.85, $CHCl_3$); IR (film) $\nu = 1358$ (O–SO_2), 1177 (O–SO_2) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 4.77$ (t, $J = 4.0$ Hz, 1H, H-4), 4.65–4.57 (m, 2H, H-2, H-3), 4.48 (dd, $J_{1a,1b} = 10.7$ Hz, $J = 2.5$ Hz, 1H, H-1a), 4.33 (dd,

$J_{1a,1b} = 10.7$ Hz, $J = 7.3$ Hz, 1H, H-1b), 4.30–4.24 (m, 1H, H-6), 4.12 (dd, $J = 7.7$ Hz, $J = 6.7$ Hz, 1H, H-7a), 3.97 (t, $J = 4.1$ Hz, 1H, H-5), 3.68 (t, $J = 7.9$ Hz, 1H, H-7b), 3.16 (s, 3H, SCH_3), 3.06 (s, 3H, SCH_3), 1.75–1.57 (m, 8H, C(CH_2CH$_3$)$_2$), 0.97–0.85 (m, 12H, C(CH$_2$CH_3)$_2$), 0.92 (s, 9H, C(CH_3)$_3$), 0.16 (s, 3H, SiCH_3), 0.14 ppm (s, 3H, SiCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 114.0$, 112.5 (*C*(CH$_2$CH$_3$)$_2$), 79.3 (C-4), 75.2 (C-6), 75.1, 73.7 (C-2, C-3), 71.7 (C-5), 69.7 (C-1), 66.0 (C-7), 38.7, 37.7 (S*C*H$_3$), 29.8, 29.4, 28.9, 27.9 (C(*C*H$_2$CH$_3$)$_2$), 25.8 (C(*C*H$_3$)$_3$), 18.2 (*C*(CH$_3$)$_3$), 8.6, 8.3, 8.2, 8.1 (C(CH$_2$*C*H$_3$)$_2$), −4.5, −4.9 ppm (Si*C*H$_3$); HRMS *m*/*z* (ESI +ve) calcd for $C_{25}H_{50}O_{11}S_2SiNa$: 641.2456 $[M+Na]^+$; found: 641.2454.

5.5. *N*-Benzyl 5-*O*-*tert*-butyldimethylsilyl-1,4-dideoxy-2,3:6,7-di-*O*-diethylidene-1,4-imino-D-*glycero*-L-*talo*-heptitol 22

A solution of dimesylate **21** (1.168 g, 1.89 mmol) in benzylamine (5 ml) was heated under argon to 120 °C for 40 h. The reaction mixture was concentrated in vacuo, dissolved in dichloromethane (20 ml) and washed with a saturated aqueous solution of sodium bicarbonate (15 ml). The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:24) to afford pyrrolidine **22** (0.900 g, 89%) as a clear oil. $R_f = 0.75$ (ethyl acetate/cyclohexane, 1:3); $[\alpha]_D^{20} = +20.4$ (*c* 0.85, $CHCl_3$); IR (film) $\nu = 2937$, 1463, 1132 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.39$ (d, $J = 7.3$ Hz, 2H, Ar*H*), 7.26 (t, $J = 7.1$ Hz, 2H, Ar*H*), 7.18 (t, $J = 7.2$ Hz, 1H, Ar*H*), 4.85 (d, $J_{ArCHa,ArCHb} = 13.4$ Hz, 1H, ArC*H*a), 4.69 (ddd, $J_{6,7b} = 9.0$ Hz, $J_{6,7a} = 6.6$ Hz, $J_{5,6} = 2.5$ Hz, 1H, H-6), 4.60–4.52 (m, 2H, H-2, H-3), 4.16–4.14 (m, 1H, H-5), 4.07 (dd, $J_{7a,7b} = 7.5$ Hz, $J_{6,7a} = 6.6$ Hz, 1H, H-7a), 3.79 (dd, $J_{6,7b} = 9.0$ Hz, $J_{7a,7b} = 7.5$ Hz, 1H, H-7b), 3.05 (d, $J = 11.4$ Hz, 1H, H-1a), 2.77 (d, $J_{ArCHa,ArCHb} = 13.4$ Hz, 1H, ArC*H*a), 2.43–2.41 (m, 1H, H-4), 1.91–1.70 (m, 3H, H-1b, C(CH_2CH$_3$)$_2$), 1.62–1.51 (m, 6H, C(CH_2CH$_3$)$_2$), 0.98 (t, $J_{Et} = 7.5$ Hz, 3H, C(CH$_2$CH_3)$_2$), 0.93 (s, 9H, C(CH_3)$_3$), 0.90 (t, $J_{Et} = 7.4$ Hz, 3H, C(CH$_2$CH_3)$_2$), 0.84 (t, $J_{Et} = 7.4$ Hz, 3H, C(CH$_2$CH_3)$_2$), 0.77 (t, $J_{Et} = 7.5$ Hz, 3H, C(CH$_2$CH_3)$_2$), 0.14 (s, 3H, SiCH_3), 0.10 ppm (s, 3H, SiCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 140.3$, 128.3, 127.8, 126.3 (Ar*C*), 115.8, 112.7 (*C*(CH$_2$CH$_3$)$_2$), 81.6 (C-3), 78.1 (C-2), 77.2 (C-6), 73.9 (C-4), 70.6 (C-5), 67.2 (C-7), 60.5 (C-1), 60.0 (Ar*C*H$_2$), 29.9, 29.1, 29.0, 28.0 (C(*C*H$_2$CH$_3$)$_2$), 26.0 (C(*C*H$_3$)$_3$), 18.1 (*C*(CH$_3$)$_3$), 8.4, 8.4, 8.2, 8.0 (C(CH$_2$*C*H$_3$)$_2$), −4.2, −4.5 ppm (Si*C*H$_3$); HRMS *m*/*z* (ESI +ve) calcd for $C_{30}H_{52}NO_5Si$: 534.3609 $[M+H]^+$; found: 534.3600.

5.6. *N*-Benzyl 5-*O*-*tert*-butyldimethylsilyl-1,4-dideoxy-2,3-*O*-diethylidene-1,4-imino-D-*glycero*-L-*talo*-heptitol 23

Procedure A: Diethylidene ketal **22** (664 mg, 1.21 mmol) in 80% aqueous acetic acid (30 ml) was heated to 80 °C for 16 h. The reaction mixture was concentrated in vacuo; the residue was dissolved in dichloromethane (20 ml) and the extract washed with a saturated aqueous solution of sodium bicarbonate (5 ml). The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:4) to afford **23** (320 mg, 57%) as a clear oil.

Procedure B: Lithium borohydride (798 mg, 36.6 mmol) was added to a solution of silyl ether **19** (8.097 g, 17.6 mmol) in anhydrous THF (75 ml) under argon and cooled to 0 °C and stirred at room temperature for 23 h. Additional lithium borohydride (382 mg, 17.5 mmol) was added at 0 °C and after a total of 49 h, the mixture was quenched by the slow addition of a saturated aqueous solution of ammonium chloride (100 ml). The reaction mixture was extracted with dichloromethane (3×100 ml) and the combined organic layers dried (sodium sulfate), filtered and concentrated in vacuo. The concentrate was filtered through a plug of silica gel and concentrated in vacuo. Crude diol **20** was dissolved in anhydrous pyridine (70 ml) under Ar and cooled to 0 °C, treated sequentially with dimethylaminopyridine (434 mg, 3.56 mmol) and methanesulfonyl chloride (5.5 ml, 69.7 mmol). The resulting reaction mixture was stirred at room temperature for 20 h, concentrated in vacuo, dissolved in dichloromethane (20 ml) and washed with aqueous hydrochloric acid (1 M, 2×15 ml) and a saturated aqueous solution of sodium bicarbonate (15 ml). The organic layer was dried over sodium sulfate, filtered and concentrated in vacuo. Crude dimesylate **21** was dissolved in benzylamine (50 ml) under Ar and heated to 120 °C for 24 h. The reaction mixture was concentrated in vacuo; the residue was dissolved in dicholoromethane (100 ml) and washed with a saturated aqueous solution of sodium bicarbonate (50 ml). The organic layer was dried (sodium sulfate), filtered and concentrated in vacuo. The residue was filtered through a plug of silica gel to remove excess benzylamine. Crude diethylidene ketal **22** was dissolved in 80% aqueous acetic acid (225 ml) and heated to 80 °C for 15 h. The reaction mixture was concentrated in vacuo, dissolved in dichloromethane (100 ml) and washed with a saturated aqueous solution of sodium bicarbonate (15 ml). The organic layer was dried (sodium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/cyclohexane, 1:4) to afford monoacetonide **23** (3.84 g, 47%, four steps) as a clear oil. $R_f = 0.32$ (ethyl acetate/cyclohexane, 1:3); $[\alpha]_D^{23} = +35.1$ (*c* 1.05, $CHCl_3$); IR (film) $\nu = 3447$ (O–H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.37$–7.20 (m, 5H, Ar*H*), 5.03 (br s, 1H, OH), 4.91 (d, $J_{ArCHa,ArCHb} = 13.0$ Hz, 1H, ArC*H*a), 4.65 (dd, $J = 6.3$ Hz, $J = 4.6$ Hz, 1H, H-3), 4.59–4.55 (m, 1H, H-2), 4.47–4.41 (m, 1H, H-6), 4.19 (s, 1H, H-5), 3.83 (dd, $J_{7a,7b} = 11.0$ Hz, $J_{6,7a} = 6.3$ Hz, 1H, H-7a), 3.65–3.60 (m, 1H, H-7b), 3.07 (d, $J_{1a,1b} = 11.7$ Hz, 1H, H-1a), 2.98 (s, 1H, OH), 2.84 (d, $J_{ArCHa,ArCHb} = 13.0$ Hz, 1H, ArC*H*b), 2.57 (app. d, $J = 4.0$ Hz, 1H, H-4), 1.98 (dd, $J_{1b,2} = 5.3$ Hz, $J_{1a,1b} = 11.7$ Hz, 1H, H-1b), 1.80 (app. q, $J_{Et} = 7.5$ Hz, 2H, C(CH_2CH$_3$)$_2$), 1.55 (q, $J_{Et} = 7.5$ Hz, 2H, C(CH_2CH$_3$)$_2$), 0.97 (t, $J_{Et} = 7.5$ Hz, 3H, C(CH$_2$CH_3)$_2$), 0.92 (s, 9H, C(CH_3)$_3$), 0.84 (t, $J_{Et} = 7.5$ Hz, 3H, C(CH$_2$CH_3)$_2$), 0.15 (s, 3H, SiCH_3), 0.12 ppm (s, 3H, SiCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 138.4$, 128.5, 128.4, 127.1 (Ar*C*), 116.0 (*C*(CH$_2$CH$_3$)$_2$), 80.7 (C-3), 77.3 (C-2), 74.3 (C-4), 73.6 (C-6), 71.6 (C-5), 64.4 (C-7), 61.3 (Ar*C*H$_2$), 60.1 (C-1), 28.2, 27.1 (C(*C*H$_2$CH$_3$)$_2$), 25.8

($C(CH_3)_3$), 18.0 ($C(CH_3)_3$), 8.6, 8.2 ($C(CH_2CH_3)_2$), −4.1, −5.0 ppm ($SiCH_3$); HRMS *m/z* (ESI +ve) calcd for $C_{25}H_{44}NO_5Si$: 466.2983 $[M+H]^+$; found: 466.2977.

5.7. *N*-Benzyl 1,4-dideoxy-1,4-imino-L-mannitol 26

Sodium periodate (620 mg, 2.90 mmol) was added to a solution of the protected pyrrolidine **23** (1.031 g, 2.21 mmol) in a mixture of methanol/water (4:1, 50 ml). The reaction mixture was stirred at room temperature for 1 h after which sodium borohydride (499 mg, 13.2 mmol) was added slowly. The reaction mixture was stirred for another 3 h, filtered and concentrated in vacuo. The residue was partitioned between chloroform (30 ml) and water (30 ml). The aqueous layer was extracted with chloroform (2 × 20 ml) and the combined organic layers were dried over sodium sulfate, filtered and concentrated in vacuo. The residue was filtered through a plug of silica gel to give a mixture of silyl ethers **24** and **25**, which in aqueous trifluoroacetic acid (9 trifluoroacetic acid/1 water, 25 ml) were stirred for 6.5 h at reflux temperature after which the solvents were evaporated in vacuo. The residue was purified using ion exchange resin Dowex 50WX8-200 (H^+ form) and aqueous 2 M ammonium hydroxide as an eluent to afford benzyl pyrrolidine **26** (438 mg, 78%) as an off-white solid. $R_f = 0.56$ (acetone/*n*-BuOH/H_2O, 5:4:1); mp 108–109 °C (MeOH); $[\alpha]_D^{21} = +37.7$ (*c* 1.20, H_2O); ^1H NMR (400 MHz, D_2O) $\delta = 7.43$–7.34 (m, 5H, Ar*H*), 4.34–4.29 (m, 1H, H-3), 4.13 (dt, $J_{1,2} = 6.6$ Hz, $J_{2,3} = 4.6$ Hz, 1H, H-2), 3.93 (dt, $J_{5,6b} = 6.3$ Hz, $J_{5,6a} = 3.7$ Hz, 1H, H-5), 3.89 (d, $J_{ArCHa,ArCHb} = 13.3$ Hz, 1H, ArC*H*a), 3.79 (dd, $J_{6a,6b} = 11.8$ Hz, $J_{5,6a} = 3.7$ Hz, 1H, H-6a), 3.72 (dd, $J_{6a,6b} = 11.8$ Hz, $J_{5,6b} = 6.3$ Hz, 1H, H-6b), 3.56 (d, $J_{ArCHa,ArCHb} = 13.3$ Hz, 1H, ArC*H*b), 3.01–2.97 (m, 1H, H-4), 2.83 (dd, $J_{1a,1b} = 11.4$ Hz, $J_{1a,2} = 6.6$ Hz, 1H, H-1a), 2.76 ppm (dd, $J_{1a,1b} = 11.4$ Hz, $J_{1b,2} = 6.6$ Hz, 1H, H-1b); ^{13}C NMR (100 MHz, D_2O) $\delta = 137.1$, 130.3, 129.0, 128.3 (Ar*C*), 72.8 (C-3), 71.2 (C-5), 70.0 (C-2), 66.3 (C-4), 63.7 (C-6), 59.7 ($ArCH_2$), 55.8 ppm (C-1); HRMS *m/z* (ESI +ve) calcd for $C_{13}H_{19}NO_4Na$: 276.1206 $[M+Na]^+$; found: 276.1204.

5.8. L-DIM (1,4-dideoxy-1,4-imino-L-mannitol) 9

Benzyl pyrrolidine **26** (389 mg, 1.54 mmol) in dioxane/water (4:1, 10 ml) with 20% palladium on carbon was stirred under an atmosphere of hydrogen for 19 h and then filtered through a plug of Celite. The filtrate was concentrated in vacuo to give L-DIM **9** (250 mg, quantitative) as a clear glasslike solid. $[\alpha]_D^{21} = +10.3$ (*c* 1.20, H_2O); [lit.[11a] $[\alpha]_D^{20} = -10.4$ (*c* 0.12, H_2O)]; ^1H NMR (400 MHz, D_2O) $\delta = 4.29$ (dt, $J_{1,2} = 8.3$ Hz, $J_{2,3} = 4.1$ Hz, 1H, H-2), 4.16 (app. t, $J = 3.9$ Hz, 1H, H-3), 3.81 (ddd, $J_{4,5} = 9.4$ Hz, $J_{5,6b} = 6.4$ Hz, $J_{5,6a} = 2.8$ Hz, 1H, H-5), 3.71 (dd, $J_{6a,6b} = 12.0$ Hz, $J_{5,6a} = 2.8$ Hz, 1H, H-6a), 3.51 (dd, $J_{6a,6b} = 12.0$ Hz, $J_{5,6b} = 6.4$ Hz, 1H, H-6b), 3.12 (dd, $J_{1a,1b} = 11.2$ Hz, $J_{1a,2} = 8.1$ Hz, 1H, H-1a), 3.07 (dd, $J_{4,5} = 9.4$ Hz, $J_{3,4} = 3.5$ Hz, 1H, H-4), 2.72 ppm (dd, $J_{1a,1b} = 11.2$ Hz, $J_{1b,2} = 3.5$ Hz, 1H, H-1b); ^{13}C NMR (100 MHz, D_2O) $\delta = 72.5$ (C-3), 71.8 (C-2), 70.7 (C-5), 64.0 (C-6), 61.0 (C-4), 48.8 ppm (C-1); HRMS *m/z* (ESI +ve) calcd for $C_6H_{13}NO_4Na$: 186.0737 $[M+Na]^+$; found: 186.0740.

5.9. *N*-Benzyl 1,4-imino-D-*glycero*-L-*talo*-heptitol 27

A solution of benzyl pyrrolidine **23** (192 mg, 0.41 mmol) in aqueous trifluoroacetic acid (9 trifluoroacetic acid/1 water, 25 ml) was stirred for 7.5 h at reflux temperature after which the solvents were evaporated in vacuo. The crude material was purified using ion exchange resin Dowex 50WX8-200 (H^+ form) and aqueous 2 M ammonium hydroxide as eluent to afford benzyl *N*-benzyl pyrrolidine **27** (79 mg, 68%) as a clear glass. $R_f = 0.44$ (acetone/*n*-BuOH/H_2O, 5:4:1); $[\alpha]_D^{21} = +12.7$ (*c* 2.04, MeOH); ^1H NMR (400 MHz, CD_3OD) $\delta = 7.50$–7.36 (m, 5H, Ar*H*), 4.65 (d, $J_{ArCHa,ArCHb} = 13.1$ Hz, 1H, ArC*H*a), 4.48 (dd, $J_{2,3} = 4.6$ Hz, $J_{3,4} = 6.8$ Hz, 1H, H-3), 4.22 (dd, $J_{2,3} = 4.6$ Hz, $J = 9.3$ Hz, 1H, H-2), 4.17 (dt, $J_{5,6} = 2.2$ Hz, $J_{6,7} = 5.9$ Hz, 1H, H-6), 4.11 (dd, $J_{5,6} = 2.2$ Hz, $J_{4,5} = 4.2$ Hz, 1H, H-5), 3.92 (d, $J_{ArCHa,ArCHb} = 13.1$ Hz, 1H, ArC*H*b), 3.70 (d, $J_{6,7} = 5.9$ Hz, 2H, H-7), 3.62–3.54 (m, 1H, H-4), 3.09–2.96 ppm (m, 2H, H-1); ^{13}C NMR (100 MHz, CD_3OD) $\delta = 134.8$, 131.1, 130.1, 130.0 (Ar*C*), 73.5 (C-6), 73.3 (C-3), 71.1 (C-4), 70.9 (C-2), 69.8 (C-5), 64.7 (C-7), 60.7 ($ArCH_2$), 57.1 ppm (C-1); HRMS *m/z* (ESI +ve) calcd for $C_{14}H_{22}NO_5$: 284.1492 $[M+H]^+$; found: 284.1491.

5.10. 1,4-Imino-D-*glycero*-L-*talo*-heptitol 10

Benzyl pyrrolidine **27** (79 mg, 0.27 mmol) in dioxane/water (4:1, 5 ml) was stirred under an atmosphere of hydrogen in the presence of 20% palladium on carbon for 19 h and then filtered through a plug of Celite. The filtrate was concentrated in vacuo and the residue purified using ion exchange resin Dowex 50WX8-200 (H^+ form) and aqueous 2 M ammonium hydroxide as an eluent to afford **10** (41 mg, 76%) as a clear glasslike solid, $[\alpha]_D^{23} = +9.5$ (*c* 1.70, H_2O) {lit.[17] $[\alpha]_D^{23} = +27$ (*c* 1, MeOH)}; ^1H NMR (400 MHz, D_2O) $\delta = 4.35$ (app. dt, $J = 8.2$ Hz, $J = 4.0$ Hz, 1H, H-2), 4.23–4.19 (m, 1H, H-3), 3.84 (app. d, $J = 9.5$ Hz, 1H, H-5), 3.75–3.69 (m, 1H, H-6), 3.68–3.64 (m, 2H, H-7), 3.35 (dd, $J = 9.2$ Hz, $J = 2.6$ Hz, 1H, H-4), 3.22 (dd, $J = 10.9$ Hz, $J = 8.3$ Hz, 1H, H-1a), 2.79 ppm (app. t, $J = 10.1$ Hz, 1H, H-1b); ^{13}C NMR (100 MHz, D_2O) $\delta = 72.2$ (C-2), 71.6 (C-3), 71.3 (C-6), 69.2 (C-5), 63.4 (C-7), 60.7 (C-4), 48.5 ppm (C-1); HRMS *m/z* (ESI +ve) calcd for $C_7H_{16}NO_5$: 194.1023 $[M+H]^+$; found: 194.1022.

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