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# Studies on the transformation of nitrosugars into iminosugars III: synthesis of (2*R*,3*R*,4*R*,5*R*,6*R*)-2-(hydroxymethyl)azepane-3,4,5,6-tetraol and (2*R*,3*R*,4*R*,5*R*,6*S*)-2-(hydroxymethyl)azepane-3,4,5,6-tetraol

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ABSTRACT

A divergent synthesis of the two novel polyhydroxylated azepanes (2*R*,3*R*,4*R*,5*R*,6*R*)-2-(hydroxymethyl)azepane-3,4,5,6-tetraol and (2*R*,3*R*,4*R*,5*R*,6*S*)-2-(hydroxymethyl)azepane-3,4,5,6-tetraol from D-mannose is described. The method involves a Henry reaction between dimethyl-*tert*-butylsilyl 2,3-*O*-isopropylidene-α-D-*lyxo*-pentodialdo-1,4-furanoside and 2-nitroethanol followed by a reductive ring closure of the resulting epimeric nitro aldols. Glycosidase inhibition tests showed that (2*R*,3*R*,4*R*,5*R*,6*S*)-2-(hydroxymethyl)azepane-3,4,5,6-tetraol exhibits a weak but selective inhibition against α-L-fucosides.

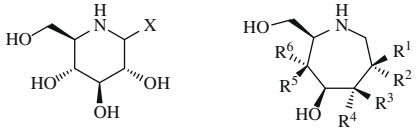


## 1. Introduction

With the breakthroughs in glycobiology, glycosidases have received considerable attention due to their use as biochemical tools and involvement in several important biological processes, such as digestion, the biosynthesis of glycoproteins, and the catabolism of glycoconjugates. Accordingly, glycosidase inhibitors have been extensively investigated over the last two decades on account of their potential as therapeutic agents.[1] Indeed, some examples have already been tested and approved for use in the treatment of various diseases such as Gaucher's disease,[2] diabetes,[3] cancer[4], and viral infections,[5] including AIDS.[6] This situation has led to a considerable effort in recent years aimed at understanding the structural types, mechanisms of action, and synthesis of glycosidase inhibitors.

The most prominent glycosidase inhibitors are imino sugars,[7] which are polyhydroxylated nitrogen-containing ring skeleton analogues of furanoses or pyranoses in which the ring oxygen is replaced by nitrogen.[8] A great deal of synthetic effort has been focused on the preparation of substituted pyrrolidine or piperidine mimics of the corresponding sugars, including nojirimycin **1a** and deoxynojirimycin **1b**, the former being an imino sugar that can be regarded as the result of replacing the ring oxygen of D-glucose by a nitrogen.[9] However, only a few higher homologues such as azepanes **2** have been reported to date even though, despite the fact that they could probably adapt better to the active site of glycosidases due to them being structurally more flexible than the corresponding pyrrolidines and piperidines.[10,11]

In 2004, Sinaÿ et al. reported for the first time the preparation and biological evaluation of a number of 1,6-dideoxy-1,6-iminoheptitols **2**, a novel family of polyhydroxylated azepanes that have an extra hydroxymethyl group compared to the previously reported analogues, so they can be considered as higher homologues of deoxynojirimycin **1b** (Fig. 1).[12] Some of these derivatives have shown potent and specific glycosidase inhibition; for example, compound **2a** is a potent and selective inhibitor of coffee bean α-galactosidase and compound **2b** strongly inhibits bovine liver β-galactosidase. Further intensive work in this field by Blériot et al. resulted in the development of alternative synthetic routes for the preparation of additional azepanes **2**[13] and related acetamido tri- and tetra-hydroxyazepanes,[14] some of which show salientable inhibition properties.




**1a**: X=OH, **1b**: X=H
**2a:** $R^1$=$R^3$=$R^5$=H, $R^2$=$R^4$=$R^6$=OH
**2b:** $R^2$=$R^4$=$R^5$=H, $R^1$=$R^3$=$R^6$=OH
**2c:** $R^2$=$R^4$=$R^5$=H, $R^2$=OBn, $R^3$=NHAc, $R^6$=OH
**2d:** $R^1$=$R^4$=$R^6$=H, $R^2$=$R^3$=OH, $R^5$=Me
**2e:** $R^2$=$R^3$=$R^5$=H, $R_2$=$R^6$=OH, $R^4$=NHAc

**Figure 1.**

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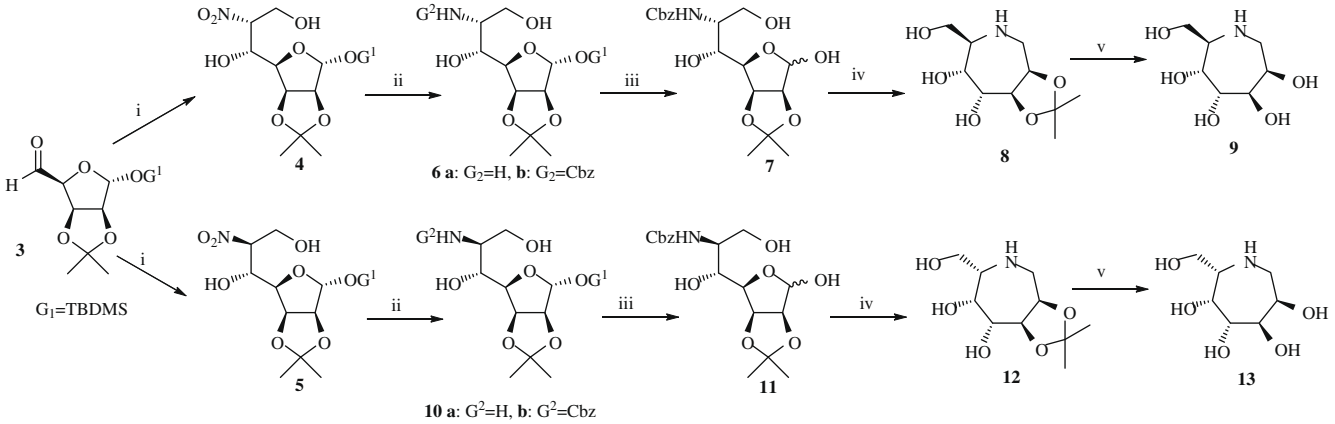



**Scheme 1.** Reagents and conditions: (i) $NO_2CH_2CH_2OH$, NaOMe, MeOH, rt, 4 h (28% of **4** and 31% of **5**); (ii) (a) $H_2$, Raney nickel, methanol, rt, 2 h; (b) ClCbz, $HNaCO_3$, EtOAc, rt, 4 h (72% of **6b** from **5** and 85% of **10b** from **5**); (iii) TBAF, THF, rt, 15 h, (100% of **8** from **6b** and 94% of **12** from **10b**); (iv) $NH_4COO$, Pd black, methanol, AcOH, rt, 12 h then 50 °C, 4 h (97% of **8** from **7** and 95% of **12** from **11**); (v) TFA/$H_2O$ 1:1, rt, 15 h (100% of **9** from **8** and 98% of **13** from **12**).

Azepane **2c** displays potent and selective β-*N*-acetylhexosaminidase inhibition;[14c] azepane **2d** is a potent β-glucosidase inhibitor[14b] while azepane **2e** has been shown to inhibit GH84 glycoside hydrolases.[13b] On the other hand, due to the unusual spatial distribution of the hydroxyl groups,[15] these azepanes not only display a different inhibition profile compared to the previously reported polyhydroxylated azepanes, but they are also more prone to form hydrogen bonds with nitrogenated bases, thus improving their ability to bind to the minor groove of DNA.[16]

Nitro sugars are powerful synthetic materials because they combine the synthetic potential of sugars and the chemical versatility of the nitro group.[17] The nitroaldol condensation (the Henry reaction) is a classical method for the construction of carbon–carbon bonds. After the carbon framework has been set up, the nitro group can be converted into a range of other functionalities, including reduction to an amino group.

As a continuation of our interest in new synthetic applications of nitrosugars,[18,19] and specifically on their utility in the synthesis of imino sugars,[18] we herein report a divergent synthesis of azepanes **9** and **13**. This approach involves the early introduction of the 2-nitroethanol subunit at position C-6 of nitrosugars **4** and **5** by means of a Henry reaction between compound **3** and formaldehyde (Scheme 1). This step is followed by the reduction of the respective nitro groups to amino groups and a heteroannulation protocol previously used in the synthesis of six-membered iminosugars.[20]

## 2. Results and discussion

The starting material used was the D-mannose-derived aldehyde **3** which has an orthogonal protecting system designed for the easy and selective deprotection of the OH at the anomeric position in an advanced stage of the synthesis. A Henry reaction of aldehyde **3** with nitroethanol and sodium methoxide as the base provided a good yield of a 1:1 epimeric mixture of nitrosugars **4** and **5**,[19g] which were isolated after column chromatography, crystallized from ethyl acetate/hexane and unambiguously characterized[21,22] by means of X-ray experiments (see Fig. 2 and Fig. 3 respectively). In this way it was established that the two compounds are the epimers predicted by the Felkin–Ahn rule. These compounds have the same configuration at their C-5 positions, while the nitro group at C-6 has an (*R*)-configuration in compound **4** (Fig. 2) and an (*S*)-configuration in compound **5** (Fig. 3).

Compounds **4** and **5** were immediately subjected to a reductive ring closure protocol.[23] Hydrogenation of nitrosugar **4** with a Raney nickel as the catalyst provided an unstable amine **6a**, which directly reacted with benzyl chloroformate to give derivative **6b**, a fully protected compound with three orthogonal protecting groups (Scheme 2). Treatment of **6b** with TBAF in THF gave hemiacetal **7**, which upon hydrogenation using acetic acid as the solvent and Pd/C as the catalyst resulted in the expected direct formation of azepane **8**, a compound that was finally converted into azepane **9** after the removal of its isopropylidene-protecting group under acidic conditions. Application of this protocol to nitrosugar **5** allowed the epimeric azepane **13** to be obtained in good yield *via* compounds **10a**, **10b**, **11**, and **12**.

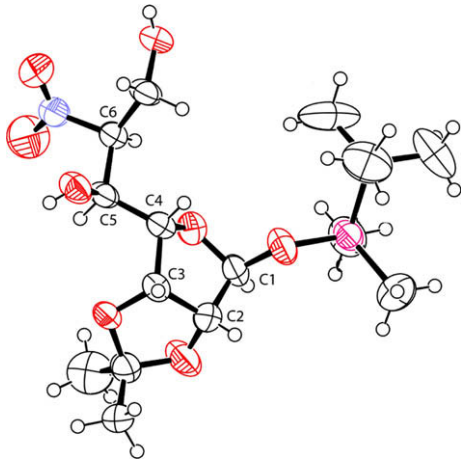



**Figure 2.** ORTEP diagram of compound **4**.

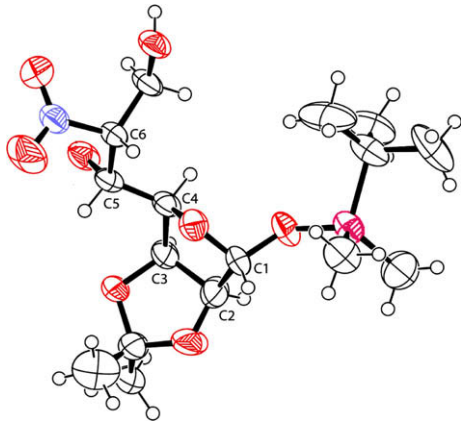
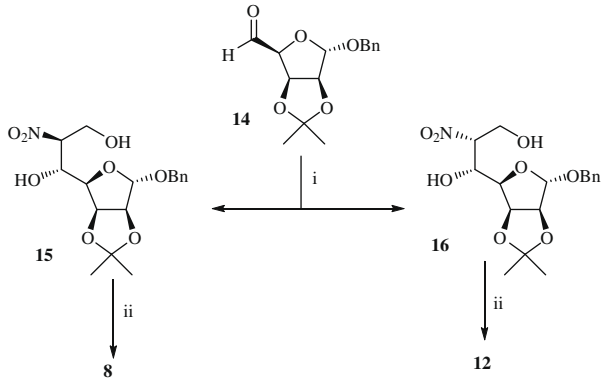



**Figure 3.** ORTEP diagram of compound **5**.



**Scheme 2.** Reagents and conditions: (i) $NO_2CH_2CH_2OH$, NaOMe, MeOH, rt, 4 h (28% of **15** and 31% of **16**); (ii) $H_2$, Raney nickel, methanol, rt, 2 h.

In order to achieve a shorter and more efficient access to our targets **9** and **13**, the route described in Scheme 2 was designed. This route started from aldehyde **14**, which is similar to compound **3** except that its anomeric hydroxyl group is protected as a benzyloxy derivative in order to satisfy the requirement of orthogonal protection with respect to hydroxy groups at positions C-2 and C-3.

According to this new route, a Henry reaction of aldehyde **14** with nitroethanol, using sodium methoxide as a base, provided a 4:1 epimeric mixture of nitrosugars **15** and **16**, from which the two epimers were isolated by column chromatography. The absolute stereochemistry of both nitroaldols was readily established when compound **15** was transformed into azepane **8** and compound **16** into azepane **12**. Hydrogenation of nitrosugar **15** using Pd/C as the catalyst directly provided the previously obtained azepane **8**, which is the precursor of **9**. Finally, hydrogenation of the epimeric nitrosugar **16** under the same conditions gave compound **12**, the precursor of azepane **13**. This modified approach formally constitutes a second synthesis of azepanes **9** and **13**, which were now obtained in 10% global yield after a five-step sequence. This route is clearly simpler and more efficient than the synthetic approach represented in Scheme 1, which led to the same two azepanes in 5% global yield in a seven-step sequence.

The new iminoheptitols **9** and **13** were assayed for their inhibitory activity toward commercially available glycosidases.[24] Compounds **9** and **13** showed no significant activity with the following glycosidases at a concentration of 143 μg/mL at the enzymes optimal pH: α-D-glucosidase from *Saccharomyces cerevisiae*, α-D-glucosidase from *Bacillus sterothermophilus*, β-D-glucosidase from Almond (*Prunus* sp.), α-D-galactosidase from green coffee bean (*Coffea* sp.), β-D-galactosidase from bovine liver, α-D-mannosidase from jack bean (*Canavalia ensiformis*), β-D-mannosidase from *Cellullomonas fimi*, and β-D-galactosidase from bovine liver. Compound **13** was also tested on bovine epididymis α-L-fucosidase and gave weak but selective inhibition with an $IC_{50}$ of 1.7 mM.

## 3. Conclusion

In conclusion, we have reported herein the preliminary results on a novel strategy for the preparation of imino sugars from nitrosugars. Specifically, this article concerns the preparation of the novel polyhydroxylated azepanes **9** and **13**. The evaluation of these compounds as glycosidase inhibitors allowed us to establish that the latter compound has a weak but selective inhibition against α-L-fucosidase.

Work is now in progress and is aimed at extending this methodology to the pool of tetroses, pentoses, and hexoses, in order to gain access to a wide range of novel five-, six-, and seven-membered imino sugars for pharmacological purposes.

## 4. Experimental

Melting points were determined using a Kofler Thermogerate apparatus and are uncorrected. Specific rotations were recorded on a JASCO DIP-370 optical polarimeter. Infrared spectra were recorded on a MIDAC Prospect-IR spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Bruker DPX-250 apparatus. Mass spectra were obtained on a Hewlett Packard 5988A mass spectrometer. Elemental analyses were obtained from the Elemental Analysis Service at the University of Santiago de Compostela. Thin layer chromatography (TLC) was performed using Merck GF-254 type 60 Silica Gel and ethyl acetate/hexane mixtures as eluents; the TLC spots were visualized with Hanessian mixture. Column chromatography was carried out using Merck type 9385 silica gel.

### 4.1. 1-*O*-*tert*-Butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-D-*glycero*-α-D-*manno*-heptofuranose 4 and 1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-L-*glycero*-α-D-*manno*-heptofuranose 5

To a solution of 1-*O*-*tert*-butyldimethylsilyl-2,3-*O*-isopropylidene-α-D-*lyxo*-pentodialdo-1,4-furanose (**3**) (0.18 g, 0.60 mmol) in dry methanol (5 mL) were added nitroethanol (0.15 mL, 1.81 mmol) and sodium methoxide (0.09 g, 1.75 mmol) and the resulting mixture was stirred at rt for 4 h. The reaction was neutralized with DOWEX 50 W resin, filtered, and the filtrate was preabsorbed onto silica, and purified by flash column chromatography (ethyl acetate/hexane 2:7) to afford 1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-D-*glycero*-α-D-*manno*-heptofuranose **4** (72 mg, 31%) and 1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-L-*glycero*-α-D-*manno*-heptofuranose **5** (66 mg, 28%). Both isomers were crystallized from ethyl acetate/hexane. Data for **5**. Mp: 129–131 °C; $[\alpha]_D^{23} = +30.6$ (*c* 1.0, chloroform); $^1$H NMR (500 MHz, $CDCl_3$): 0.09, 0.11 (2 × s, 6H, –$SiMe_2$); 0.88 (s, 9H, –Si$^t$Bu); 1.34, 1.49 (2 × s, 6H, 2 × –$CH_3$); 2.63 (t, 1H, *J* 5.4 Hz, –OH); 3.14 (d, 1H, *J* 5.4 Hz, –OH); 4.07–4.10 (m, 1H, H-4); 4.23–4.27 (m, 1H, H-7); 4.32–4.36 (m, 1H, H-7′); 4.57 (d, 1H, $J_{2,3}$ 6.1 Hz, H-3); 4.72–4.74 (m, 2H, H-5, H-6); 4.90 (dd, 1H, $J_{6,7'}$ 4.1 Hz, H-2); 5.33 (s, 1H, H-1); $^{13}$C NMR (62.8 MHz, $CDCl_3$): −5.6, −4.6 (–$SiMe_2$); 17.7 (–Si*C*($CH_3$)$_3$); 24.3, 25.6 (–*C*(–$CH_3$)$_2$); 25.3 (–Si*C*($CH_3$)$_3$); 59.8 (–$CH_2$–); 78.7, 79.5, 86.4, 88.3 (4 × –CH–); 101.3 (C-1); 112.8 (–*C*(–$CH_3$)$_2$); LRMS (*m*/*z*, %): 378 ($M^+$ −15, 0.3); 318 (0.7); 260 (2); 187 (6); 129 (27); 58 (100); IR ($\nu$, $cm^{-1}$): 3427 (–OH); 1556, 1378 (–$NO_2$); EA: Calcd for $C_{16}H_{31}NO_8Si$: C, 48.84; H, 7.94; N, 3.56. Found: C, 49.21; H, 8.04; N, 3.30. Data for **6**. Mp: 116–123 °C; $[\alpha]_D^{23} = +3.2$ (*c* 1.0, chloroform); $^1$H NMR (500 MHz, $CDCl_3$): 0.09, 0.11 (2 × s, 6H, –$SiMe_2$); 0.88 (s, 9H, –Si$^t$Bu); 1.34, 1.49 (2 × s, 6H, 2 × –$CH_3$); 2.60 (t, 1H, *J* 6.4 Hz, –OH); 3.10 (d, 1H, *J* 6.1 Hz, –OH); 4.08–4.10 (m, 1H, H-3); 4.23–4.27 (m, 1H, H-5); 4.32–4.37 (m, 1H, H-4); 4.57 (d, 1H, $J_{6,7}$ 5.8 Hz, H-7); 4.72–4.74 (m, 2H, H-2, H-7′); 4.90 (dd, 1H, $J_{6,7'}$ 4.0 Hz, H-6); 5.33 (s, 1H, H-1); $^{13}$C NMR (62.8 MHz, $Cl_3CD$): −5.7, −4.7 (2 × –$CH_3$, –$SiMe_2$); 17.6 (–Si*C*($CH_3$)$_3$); 24.4, 25.7 (–C($CH_3$)$_2$, –Si*C*($CH_3$)$_3$); 59.7 (–$CH_2$–, C-7); 69.2, 78.7, 79.4 (3 × –CH–); 86.4, 88.4 (2 × –CH–); 101.3 (–CH–, C-1); 112.7 (s, –C–); LRMS (*m*/*z*, %): 378 ($M^+$ – 15, 0.3); 318 (0.7); 260 (2); 187 (6); 129 (27); 58 (100); IR ($\nu$, $cm^{-1}$): 3427 (–OH); 1556, 1378 (–$NO_2$).

### 4.2. 6-Benzyloxycarbonylamino-1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-D-*glycero*-α-D-*manno*-heptofuranose 6b

Raney nickel (6.7 mL, 10% w/w) was added to a degassed solution of 1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-D-glycero-α-D-*manno*-heptofuranose **4** (0.41 g, 1.03 mmol) in methanol (15 mL) and the resulting mixture was

stirred under a hydrogen atmosphere at room temperature for 2 h. The suspension was then filtered through a Celite pad and the filtrate was evaporated in vacuo to afford 6-amino-1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-D-glycero-α-D-*manno*-heptofuranose **6a**, which was used for the next step without further purification. To a solution of this amine in ethyl acetate (8 mL) was added a saturated aqueous solution of sodium hydrogen carbonate (5.5 mL) and the mixture was cooled to 0 °C. Benzyl chloroformate (1.13 mmol) was added dropwise and the resulting mixture was stirred at rt for 3 h. The layers were separated and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried (sodium sulfate), filtered, and evaporated in vacuo to give a residue, which was purified by flash column chromatography (ethyl acetate/hexane 1:2) to afford 6-benzyloxycarbonylamino-1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-D-*glycero*-α-D-*manno*-heptofuranose **6b** (0.37 g, 72%) as a white foam. $[\alpha]_D^{23} = +14.5$ (*c* 0.23, chloroform); $^1$H NMR (300 MHz, $CDCl_3$): 0.00, 0.03 (2 × s, 6H, –$SiMe_2$); 0.80 (s, 9H, –Si$^t$Bu); 1.26, 1.40 (2 × s, 6H, 2 × –$CH_3$); 2.29 (br s, 1H, –OH); 2.87 (br s, 1H, –OH); 3.66–4.06 (m, 5H, H-7, H-7′, H-6, H-5, H-4); 4.46 (d, 1H, $J_{2,3}$ 5.7 Hz, H-2); 4.88–4.89 (m, 1H, H-3); 5.02–5.03 (m, 2H, –$CH_2$Ph); 5.27 (s, 1H, H-1); 5.63 (d, 1H, *J* 7.6 Hz, –NH–); 7.23–7.28 (m, 5H, H-Ph); $^{13}$C NMR (62.8 MHz, $CDCl_3$): −5.0, −4.1 (–$SiMe_2$); 18.2 (–Si*C*($CH_3$)$_3$); 25.0, 26.3 (–C(–*C*$H_3$)$_2$); 26.0 (–SiC(*C*$H_3$)$_3$); 54.5 (–CH–); 62.6, 67.3 (2 × –$CH_2$–); 72.3, 79.3, 80.8, 87.0 (4 × –CH–); 101.9 (C-1); 112.9 (–*C*(–$CH_3$)$_2$); 128.5, 129.0 (5 × –CHPh); 136.7 (–CPh); 157.2 (–C=O); LRMS (IQ+, *m*/*z*, %): 498 [(M+H)$^+$, 35]; 366 (100); IR (*ν*, cm$^{-1}$): 3432 (–OH); 1722 (–C=O); EA: Calcd for $C_{24}H_{39}NO_8Si$: C, 57.92; H, 7.90; N, 2.81. Found: C, 58.27; H, 8.06; N, 2.74.

### 4.3. 6-Benzyloxycarbonylamino-6-deoxy-2,3-di-*O*-isopropylidene-D-*glycero*-α-D-*mano*-heptofuranose 7

To a solution of 6-benzyloxycarbonylamino-1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-D-*glycero*-α-D-*manno*-heptofuranose **6b** (0.33 g, 0.67 mmol) in THF (6 mL) was added tetrabutylammonium fluoride (0.77 mL, 1 M solution in THF). The mixture was stirred at rt for 16 h, after which the solvent was evaporated, and the residue was taken up in ethyl acetate (10 mL) and washed with water (3 × 5 mL). The organic layer was dried (sodium sulfate), filtered, and evaporated in vacuo. Purification of the resulting residue by flash column chromatography (methanol/dichloromethane 10:1) yielded 6-benzyloxycarbonylamino-6-deoxy-2,3-di-*O*-isopropylidene-D-*glycero*-α-D-*manno*-heptofuranose **7** (0.26 g, quantitative) as a clear oil. $[\alpha]_D^{23} = +3.7$ (*c* 1.0, methanol); $^1$H NMR (300 MHz, MeOD): 1.23 1.37 (2 × s, 6H, 2 × –$CH_3$); 3.86–4.13 (m, 5H); 4.50 (d, 1H, $J_{2,3}$ 5.5 Hz, H-2); 4.84 (br s, 1H); 5.00 (s, 2H, –$CH_2$Ph); 5.33 (s, 1H, H-1); 6.14 (d, 1H, *J* 7.8 Hz, –NH–); 7.26–7.28 (m, 5H, H-Ph); $^{13}$C NMR (62.8 MHz, $CDCl_3$): 26.0, 26.3 (–C(–*C*$H_3$)$_2$); 54.6 (–CH–); 61.9, 67.4 (2 × –$CH_2$–); 71.3, 78.1, 80.7, 85.7 (4 × –CH–); 101.3 (C-1); 113.0 (–*C*(–$CH_3$)$_2$); 128.4, 128.9 (5 × –CHPh); 136.6 (–CPh); 157.5 (–C=O); LRMS (IQ+, *m*/*z*, %): 384 [(M+H)$^+$, 45]; 366 (100); IR (*ν*, cm$^{-1}$): 3395 (–OH); 1699 (–C=O); EA: Calcd for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65. Found: C, 55.99; H, 7.01; N, 3.44.

### 4.4. (3a*R*,6*R*,7*R*,8*R*,8a*S*)-Hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol 8

Palladium black (0.02 g, 20% w/w) and ammonium formate (0.15 g, 2.30 mmol) were added to a degassed solution of 6-benzyloxycarbonylamino-6-deoxy-2,3-di-*O*-isopropylidene-D-*glycero*-α-D-*mano*-heptofuranose **7** (0.09 g, 0.23 mmol) in methanol (7 mL) and acetic acid (two drops). The resulting mixture was stirred under a nitrogen atmosphere at rt for 12 h and then heated at 50 °C for 4 h. The suspension was filtered through a Celite pad and the filtrate was evaporated in vacuo to afford a residue, which was purified by flash column chromatography (chloroform/methanol 85:15) to obtain (2*R*,3*S*,4*R*,5*R*,6*R*)-hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol **8** (0.05 g, 97%) as a clear oil. $[\alpha]_D^{23} = +6.3$ (*c* 0.9, methanol); $^1$H NMR (300 MHz, MeOD): 1.35, 1.48 (2 × s, 6H, 2 × –$CH_3$); 2.93–3.19 (m, 2H, H-8, H-8′); 3.67–3.73 (m, 3H, H-6, –*C*$H_2$OH); 4.01 (d, 1H, *J* 5.2 Hz, H-3a); 4.18–4.29 (m, 3H, H-8a, H-4, H-5); $^{13}$C NMR (62.8 MHz, MeOD): 24.4, 26.9 (–C(–*C*$H_3$)$_2$); 46.4 (C-4); 60.9 (C-6); 62.3 (–$CH_2$OH) 70.1, 75.7, 76.5, 77.6 (C-3a, C-7, C-8, C-8a); 109.9 (–*C*(–$CH_3$)$_2$); LRMS (ESI+, *m*/*z*, %): 384 [(M+H)$^+$, 100]; HRMS (ESI+): Calcd for $C_{10}H_{20}NO_5$ [(M+H)$^+$]: 234.1341. Found: 234.1336.

### 4.5. (2*R*,3*R*,4*R*,5*R*,6*R*)-2-(Hydroxymethyl)azepane-3,4,5,6-tetraol 9

A solution of azepine **8** (30 mg, 0.13 mmol) in a 1:1 mixture of trifluoroacetic acid/water (3.3 mL) was stirred at rt for 17 h. The solvents were evaporated and the residue was coevaporated with toluene, to afford (2*R*,3*R*,4*R*,5*R*,6*R*)-2-(hydroxymethyl)azepane-3,4,5,6-tetraol **9** (25 mg, quantitative) as a clear oil. $[\alpha]_D^{23} = -3.6$ (*c* 3.2, methanol/water 1:1); $^1$H NMR (300 MHz, MeOD): 3.09 (dd, 1H, $J_{7,7'}$ 13.4 Hz, $J_{7,6}$ 3.0 Hz, H-7); 3.22–3.32 (m, 2H, H-7′, H-2); 3.70 (dd, 1H, *J* 11.7 Hz, *J* 7.0 Hz, –*C*HOH); 3.83 (dd, 1H, *J* 11.7 Hz, *J* 3.8 Hz, –*C*HOH); 3.89–3.98 (m, 2H); 4.06–4.12 (m, 2H); $^{13}$C NMR (62.8 MHz, MeOD): 45.3 (C-7); 59.9 (–$CH_2$OH); 60.1 (C-2); 66.3, 67.4, 72.4, 74.2 (C-3, C-4, C-5, C-6); LRMS (ESI+, *m*/*z*, %): 194 [(M+H)$^+$, 100]; HRMS (ESI+): Calcd for $C_7H_{16}NO_5$ [(M+H)$^+$]: 194.1028. Found: 194.1020.

### 4.6. 6-Benzyloxycarbonylamino-1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranose 10b

1-*O*-*tert*-Butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-L-*glycero*-α-D-*manno*-heptofuranose **5** (0.83 g, 2.10 mmol) was hydrogenated and protected following the same procedure used for **4**, to afford 6-benzyloxycarbonylamino-1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranose **10b** (0.89 g, 85%) as a white foam. $[\alpha]_D^{23} = +18.1$ (*c* 0.35, chloroform); $^1$H NMR (300 MHz, $CDCl_3$): −0.04, 0.02 (2 × s, 6H, –$SiMe_2$); 0.77 (s, 9H, –Si$^t$Bu); 1.23, 1.39 (2 × s, 6H, 2 × –$CH_3$); 3.19 (br s, 1H, –OH); 3.62–4.16 (m, 5H, H-7, H-7′, H-6, H-5, H-4); 4.45 (d, 1H, $J_{2,3}$ 6.0 Hz, H-2); 4.72–4.76 (m, 1H, H-3); 4.99 (s, 2H, –$CH_2$Ph); 5.22 (s, 1H, H-1); 5.58 (d, 1H, *J* 8.5 Hz, –NH–); 7.24–7.28 (m, 5H, H-Ph); $^{13}$C NMR (62.8 MHz, $CDCl_3$): −5.7, −4.7 (–$SiMe_2$); 17.6 (–Si*C*($CH_3$)$_3$); 24.5, 25.8 (–C(–*C*$H_3$)$_2$); 25.4 (–SiC(*C*$H_3$)$_3$); 52.8 (–CH–); 64.1, 66.7 (2 × –$CH_2$–); 69.8, 78.2, 79.9, 86.6 (4 × –CH–); 101.2 (C-1); 112.3 (–*C*(–$CH_3$)$_2$); 128.1, 128.4 (5 × –CHPh); 136.2 (–CPh); 156.5 (–C=O); LRMS (CI$^+$, *m*/*z*, %): 498 [(M+H)$^+$, 20]; 366 (100); IR (*ν*, cm$^{-1}$): 3437 (–OH); 1707 (–C=O); EA: Calcd for $C_{24}H_{39}NO_8Si$: C, 57.92; H, 7.90; N, 2.81. Found: C, 57.98; H, 8.24; N, 2.67.

### 4.7. 6-Benzyloxycarbonylamino-6-deoxy-2,3-di-*O*-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranose 11

6-Benzyloxycarbonylamino-1-*O*-*tert*-butyldimethylsilyl-6-deoxy-2,3-di-*O*-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranose **10b** (0.19 g, 0.40 mmol) was desilylated following the same procedure used for **7b**, yielding 6-benzyloxycarbonylamino-6-deoxy-2,3-di-*O*-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranose **11** (0.14 g, 94%) as a clear oil. $[\alpha]_D^{23} = -0.1$ (*c* 0.9, methanol); $^1$H NMR (300 MHz, MeOD): 1.24 1.36 (2 × s, 6H, 2 × –$CH_3$); 3.55–3.59 (m, 2H); 4.02–4.08 (m, 3H); 4.47 (d, 1H, $J_{2,3}$ 5.8 Hz, H-2); 5.01 (s, 2H, –$CH_2$Ph); 5.16 (s, 1H, H-1); 7.23–7.25 (m, 5H, H-Ph); $^{13}$C NMR

(62.8 MHz, MeOD): 25.5, 26.9 (–C(–$CH_3$)$_2$); 55.6 (–CH–); 63.2, 68.0 (2 × –$CH_2$–); 68.6, 80.4, 81.9, 102.7 (4 × –CH–); 102.7 (C-1); 113.8 (–$C$(–$CH_3$)$_2$); 129.4, 129.9 (5 × –CHPh); 138.7 (–CPh); 159.3 (–C=O); LRMS (IQ+, *m*/*z*, %): 384 [(M+H)$^+$, 11]; 366 (100); IR (*ν*, cm$^{-1}$): 3395 (–OH); 1699 (–C=O).

### 4.8. (3a*R*,6*S*,7*R*,8*R*,8a*S*)-Hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol 12

6-Benzyloxycarbonylamino-6-deoxy-2,3-di-*O*-isopropylidene-L-*glycero*-α-D-*manno*-heptofuranose **11** (0.10 g, 0.25 mmol) was hydrogenated following the same procedure used for **7**, to obtain (2*R*,3*S*,4*R*,5*R*,6*S*)-hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol **12** (55 mg, 95%) as a clear oil. $[\alpha]_D^{23} = -1.7$ (*c* 0.12, methanol); $^1$H NMR (300 MHz, MeOD): 1.26, 1.39 (2 × s, 6H, 2 × –$CH_3$); 2.93–3.00 (m, 1H, H-8′); 3.11–3.22 (m, 2H, H-8, H-6); 3.70–3.78 (m, 3H); 4.07 (d, 1H, *J* 4.0 Hz, H-3a); 4.18–4.29 (m, 3H); $^{13}$C NMR (62.8 MHz, MeOD): 23.9, 26.4 (–C(–$CH_3$)$_2$); 47.4 (C-4); 61.9 (C-6); 62.3 (–$CH_2$OH) 72.4, 73.7, 75.6, 80.7 (C-3a, C-7, C-8, C-8a); 109.6 (–$C$(–$CH_3$)$_2$); LRMS (ESI+, *m*/*z*, %): 384 [(M+H)$^+$, 100]; HRMS (ESI+): Calcd for $C_{10}H_{20}NO_5$ [(M+H)$^+$]: 234.1341. Found: 234.1336.

### 4.9. (2*S*,3*R*,4*R*,5*R*,6*R*)-2-(Hydroxymethyl)azepane-3,4,5,6-tetraol 13

Azepine **12** (47 mg, 0.20 mmol) was deprotected following the same procedure used for **8**, to afford (2*S*,3*R*,4*R*,5*R*,6*R*)-2-(hydroxymethyl)azepane-3,4,5,6-tetraol **13** (38 mg, 98%) as a clear oil. $[\alpha]_D^{23} = -59.0$ (*c* 0.9, in methanol/water 1:1); $^1$H NMR (300 MHz, MeOD): 3.00 (dd, 1H, $J_{7,7'}$ 13.3 Hz, $J_{7,6}$ 3.0 Hz, H-7); 3.17–3.25 (m, 2H, H-7′, H-2); 3.62 (dd, 1H, *J* 12.1 Hz, *J* 7.2 Hz, –*C*HOH); 3.74 (dd, 1H, *J* 12.1 Hz, *J* 4.0 Hz, –*C*HOH); 3.80–3.88 (m, 2H); 3.97–4.05 (m, 2H); $^{13}$C NMR (62.8 MHz, MeOD): 46.4 (C-7); 61.0 (–$CH_2$OH); 61.2 (C-2); 67.5, 68.5, 73.6, 75.4 (C-3, C-4, C-5, C-6); LRMS (ESI+, *m*/*z*, %): 194 [(M+H)$^+$, 100]; HRMS (ESI+): Calcd for $C_7H_{16}NO_5$ [(M+H)$^+$]: 194.1028. Found: 194.1023.

### 4.10. 1-*O*-Benzyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-D-*glycero*-α-D-*manno*-heptofuranose 15 and 1-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-L-*glycero*-α-D-*manno*-heptofuranose 16

To a solution of 1-*O*-benzyl-2,3-*O*-isopropylidene-α-D-*lyxo*-pentodialdo-1,4-furanose **14** (1.22 g, 4.27 mmol) in dry methanol (30 mL) were added nitroethanol (0.9 mL, 12.91 mmol) and sodium methoxide (0.66 g, 12.32 mmol) and the resulting mixture was stirred at rt for 14 h. The reaction was neutralized with DOWEX 50 W resin, filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was partitioned between water and dichloromethane and the organic layer was dried (magnesium sulfate), filtered, and evaporated in vacuo. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:2) to afford 1-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-D-*glycero*-α-D-*manno*-heptofuranose **15** (1.00 g, 43%) and 1-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-L-*glycero*-α-D-*manno*-heptofuranose **16** (0.25 g, 11%). Data for **16**: $^1$H NMR (500 MHz, $CDCl_3$): 1.33, 1.50 (2 × s, 6H, 2 × –$CH_3$); 3.98–4.22 (m, 3H); 4.48–4.72 (m, 5H); 4.86–4.88 (m, 1H, H-6); 5.12 (s, 1H, H-1); $^{13}$C NMR (62.8 MHz, $CDCl_3$): 24.6, 25.9 (–C(–$CH_3$)$_2$); 60.1, 69.6 (2 × –$CH_2$–); 79.0, 79.6, 84.7, 88.6 (5 × –CH–); 105.8 (C-1); 113.1 (–$C$(–$CH_3$)$_2$), 128.0, 128.1, 128.6 (5 × –CH–), 137.1 (–C=O). Data for **17**: $^1$H NMR (500 MHz, $CDCl_3$): 1.33, 1.48 (2 × s, 6H, 2 × –$CH_3$); 4.04–4.23 (m, 3H); 4.31–4.43 (m, 2H); 4.51–4.64 (m, 2H); 4.81–4.84 (m, 2H); 5.11 (s, 1H, H-1); $^{13}$C NMR (62.8 MHz, $CDCl_3$): 24.5, 25.9 (–C(–$CH_3$)$_2$); 61.7, 69.3 (2 × –$CH_2$–); 78.8, 79.6, 84.6, 89.7 (5 × –CH–); 105.4 (C-1); 113.0 (–$C$(–$CH_3$)$_2$), 128.0, 128.1, 128.3, 128.6 (5 × –CH–), 136.8 (–C=O).

### 4.11. (3a*R*,6*R*,7*R*,8*R*,8a*S*)-Hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol 8

Palladium black (0.02 g, 20% w/w) and ammonium formate (0.15 g, 2.30 mmol) were added to a degassed solution of 1-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-D-*glycero*-α-D-*manno*-heptofuranose **15** (0.09 g, 0.23 mmol) in methanol (7 mL) and acetic acid (two drops) and the resulting mixture was heated at 50 °C for 16 h. The suspension was filtered through a Celite pad and the filtrate was evaporated in vacuo to afford a residue, which was purified by flash column chromatography (chloroform/methanol 85:15) to obtain (2*R*,3*S*,4*R*,5*R*,6*R*)-hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol **8** (0.05 g, 97%) as a clear oil.

### 4.12. (3a*R*,6*S*,7*R*,8*R*,8a*S*)-Hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol 12

1-*O*-Benzyl-6-deoxy-2,3-di-*O*-isopropylidene-6-nitro-L-*glycero*-α-D-*manno*-heptofuranose **16** (0.10 g, 0.25 mmol) was hydrogenated following the same procedure used for **15**, to obtain (2*R*,3*S*,4*R*,5*R*,6*S*)-hexahydro-6-(hydroxymethyl)-2,2-dimethyl-3a*H*-[1,3]dioxolo[4,5-*c*]azepine-7,8-diol **12** (55 mg, 95%) as a clear oil.

### 4.13. Biological assays[21]

All enzymes and *para*-nitrophenyl substrates were purchased from Sigma, with the exception of β-mannosidase, which was obtained from Megazyme. Enzymes were assayed at 27 °C in 0.1 M citric acid/0.2 M disodium hydrogen phosphate buffers at the optimum pH for the enzyme. The incubation mixture consisted of 10 μL enzyme solution, 10 μL of 1 mg/mL aqueous solution of inhibitor, and 50 μL of the appropriate 5 mM *para*-nitrophenyl substrate made up in buffer at the optimum pH for the enzyme. The reactions were stopped by the addition of 70 μL 0.4 M glycine (pH 10.4) during the exponential phase of the reaction, which had been determined at the beginning using uninhibited assays in which water replaced inhibitor. Final absorbances were read at 405 nm using a Versamax microplate reader (Molecular Devices). Assays were carried out in triplicate, and the values given are means of the three replicates per assay.

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21. Crystallographic data for the structure of compound **4** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 759124. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax (+44)1223-336-033; e-mail deposit@ccdc.cam.ac.uk).
22. Crystallographic data for the structure of compound **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 761857. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax (+44)1223-336-033; e-mail deposit@ccdc.cam.ac.uk).
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