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Preparation of sugar derived β,β′-dihydroxy α,α-disubstituted α-amino acids

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ABSTRACT

A novel strategy for the preparation of β,β′-dihydroxy α,α-disubstituted α-amino acids bearing a sugar moiety has been developed. The procedure is based on two Henry reactions: the first Henry reaction involves a sugar aldehyde and nitroethanol to furnish a sugar derived α-hydroxymethyl α-nitroalkanol while the second Henry reaction is between this nitro sugar and formaldehyde. This sequence provided the expected epimers of sugar derived α,α-dihydroxymethyl α -nitroalkanols, from which the corresponding β,β′-dibenzyloxy α-*N*-benzyloxycarbonylamino esters were easily obtained. All rights reserved.

1. Introduction

Despite the fact that proteins play a crucial role in biology, their use as therapeutics suffers from their poor metabolic stability and transport properties,[1] together with their limited structural diversity, which is related to the limited number of secondary structures and backbones resulting from the small number of proteinogenic amino acids.[2] Moreover, the conformational flexibility of short peptides constituted by natural α-amino acids allows them to interact with more than one receptor and side effects are usually observed when these systems are used as drugs. Accordingly, intensive work carried out in this field in recent times includes the synthesis of non-natural amino acids. This is a first step in the development of peptidomimetics with high levels of diversity and rigidity, and such compounds may act as new drugs that overcome the pharmacological limitations of proteins.[3] In this context, enantiomerically pure α,α-disubstituted α-amino acids are of increasing interest for the agrochemical and pharmaceutical industries.[4] The structural feature common to these amino acids is the presence of an additional substituent compared with their α-H counterparts. Peptides that incorporate one or more such amino acids are less prone to epimerization, display enhanced metabolic stability and show different folding properties.[5]

Specifically, β,β′-dihydroxy α-amino acid subunits are present in biologically active natural compounds, such as sphingofungin E10[6] and mycestericin G,[7] which display high levels of immunosuppressant activity[8] (Fig. 1). Structure–activity studies have strongly suggested the crucial role played by the polar β,β′-dihydroxy α-amino acid head groups.[9] Accordingly, several efficient synthetic routes have been developed to provide access to these amino acids for the subsequent preparation of novel peptidomimetics of potential pharmacological interest and for the discovery of new immunosuppressive treatments.[10]

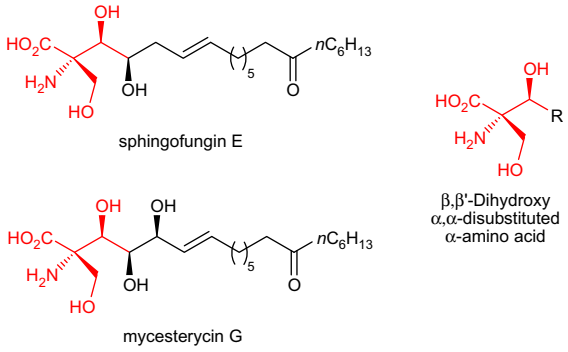

Figure 1. Natural products containing a β,β′-dihydroxy-α-amino acid unit.

Nitro sugars are valuable compounds that are powerful synthetic intermediates of growing chemical interest. This is due to the combination of the synthetic potential of sugars for the creation of chemical diversity and the chemical versatility of nitro compounds for the construction of carbon-carbon bonds prior to the transformation of the nitro group into a variety of other chemical functionalities.[11]

Our continued interest in nitro sugars led us to embark on a study of the chemistry of 6-nitroheptofuranosides and the corresponding α-nitro acids.[12] As a new contribution to this programme, we herein report the synthesis of **5a** and **5b**, the first two reported examples of sugar amino acids that incorporate a β,β′-dihydroxy α-amino acid subunit.

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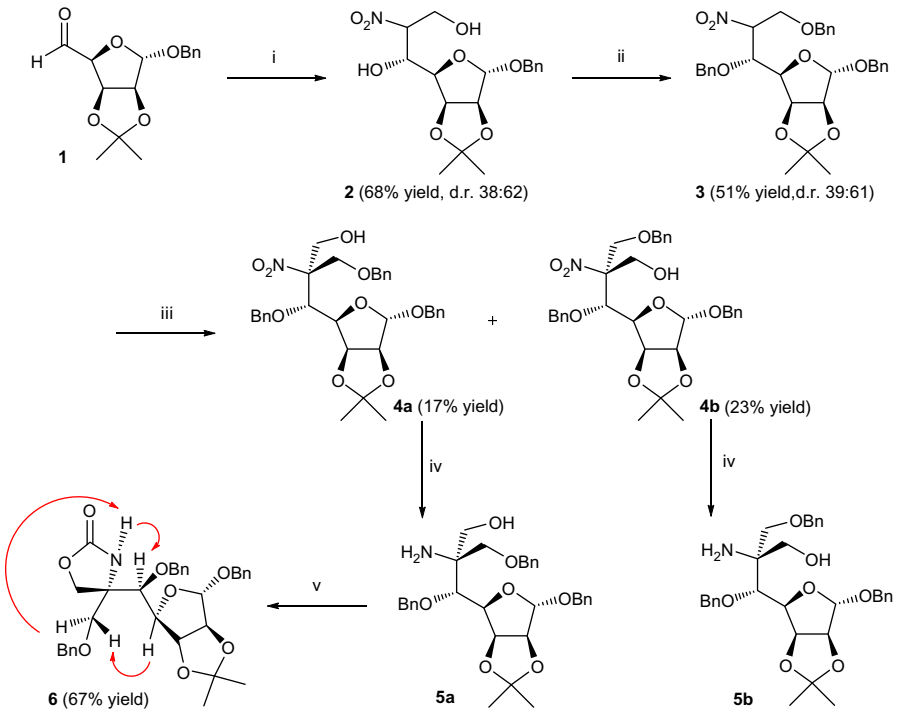
2. Results and discussion

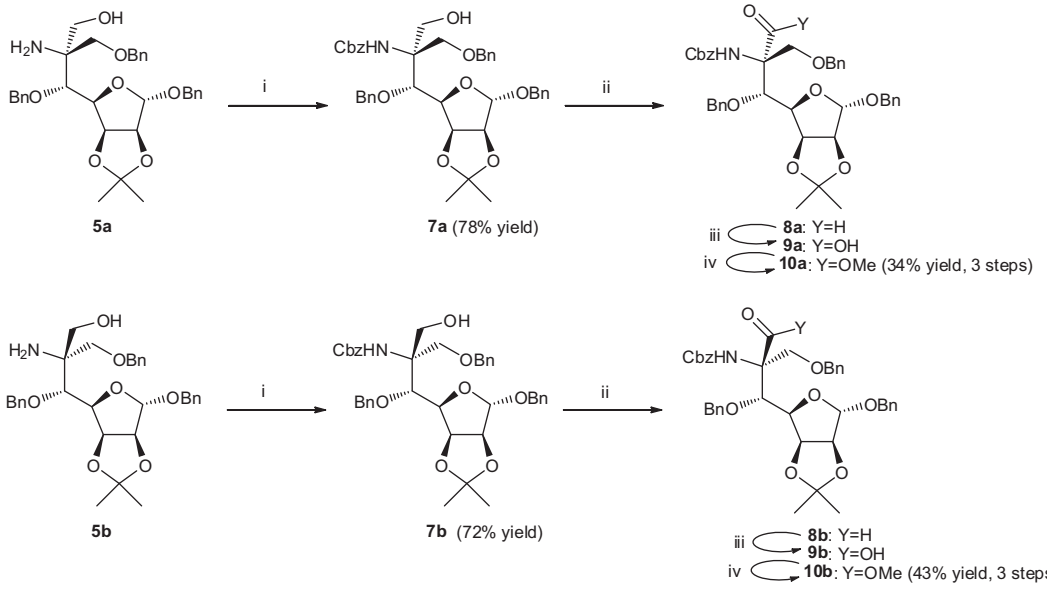
A Henry reaction between D-mannose-derived aldehyde **1** and nitroethanol,[12a] using sodium methoxide as a base, provided an epimeric mixture of nitro sugars **2** (dr 38:62) (Scheme 1). Protection of the free hydroxy groups with benzyl 2,2,2-trichloroacetimidate and catalytic trifluoromethanesulfonic acid afforded a 39:61 epimeric mixture **3**, which upon treatment with paraformaldehyde and tetrabutylammonium fluoride resulted in a 43:57 mixture of epimers **4a** and **4b**, which were easily isolated by column chromatography. Reduction of the nitro group in epimers **4a** and **4b** using zinc and hydrochloric acid afforded amino sugar epimers **5a** and **5b**, respectively.

The absolute configuration at the C-6 stereocentre of amino sugars **5a** and **5b** was established by NMR experiments carried out with compound **6**, which was easily obtained by treatment of **5a** with triphosgene. The nOe effect observed between H_4 and H_7 is only consistent with an (*S*)-configuration at the C-6 stereocentre.

According to our plan (Scheme 2), protection of the amino group of **5a** as the *N*-Cbz derivative by reaction with benzyl



Scheme 1. Reagents and conditions: (i) $NO_2CH_2CH_2OH$, NaOMe, MeOH, rt, 4 h; (ii) BTA, TfOH, DCM, C_6H_{12}, rt, 12 h; (iii) $(HCHO)_n$, TBAF, THF, rt, 12 h; (iv) Zn, 1 M HCl, *i*PrOH, rt, 2 h; (v) triphosgene, Et_3N, rt, 1 h.



Scheme 2. Reagents and conditions: (i) ClCbz, aq $NaHCO_3$, EtOAc, rt, 2 h; (ii) Dess–Martin, CH_2Cl_2, rt, 1 h; (iii) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, MeOH, H_2O, rt, 6 h; (iv) $TMSCH_2N_2$, MeOH, Et_2O, rt, 15 min.

chloroformate, followed by oxidation of the resulting **7a** with Dess–Martin periodinane, provided α-amino aldehyde **8a**, which was directly oxidized to its carboxylic acid **9a** by treatment with sodium chlorite, 2-methyl-2-butene and dihydrogen phosphate. Finally, **9a** was directly converted into α-amino acid ester **10a** by reaction with trimethylsilyldiazomethane. Amino sugar **5b** was similarly converted into α-amino acid ester **10b**, *via* compounds **7b**, **8b** and **9b**.

3. Conclusion

In conclusion, a strategy for the preparation of novel β,β′-dihydroxy sugar α-amino acids has been developed. Key steps involve a nitroaldol-mediated hydroxymethylation of a 6-nitroheptofuranoside, followed by the generation of an amino acid moiety by reduction of the nitro to an amino group and subsequent oxidation of a hydroxymethyl group to a carboxyl group.

Work is currently in progress that aimed at the incorporation of these α,α-disubstituted sugar α-amino acids into peptides in order to study their structural properties and applications. Subsequent work in this field will involve the extension of these studies to hexoses other than D-mannose in order to access a library of these novel branched-chain sugar amino acids, which would be useful for the preparation of conformationally restricted peptidomimetics and novel immunosuppressants.

4. Experimental

4.1. General

Nuclear magnetic resonance spectra were recorded on a Varian DPX-200 or a Bruker 300 apparatus. Mass spectra were obtained on a Hewlett Packard 5988A mass spectrometer. Thin layer chromatography (TLC) was performed using Merck GF-254 type 60 silica gel and ethyl acetate/hexane mixtures as eluants; TLC spots were visualized with Hanessian mixture. Column chromatography was carried out using Merck type 9385 silica gel.

4.2. 1-*O*-Benzyl-6-deoxy-2,3-di-*O*-isopropyliden-6-nitro-D,L-*glycero*-α-D-*manno*-heptofuranose 2

To a solution of 1-*O*-benzyl-2,3-*O*-isopropyliden-α-D-*lyxo*-pentodialdo-1,4-furanose **1** (1.22 g, 4.27 mmol) in dry methanol (30 mL) was added nitroethanol (0.9 mL, 12.91 mmol) and sodium methoxide (0.66 g, 12.32 mmol). 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 a mixture of 1-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropyliden-6-nitro-D and L-*glycero*-α-D-*manno*-heptofuranose **2a** and **2b** (1.48 g, 64%). **2a**: ^{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), 7.26–7.32 (m, 5H, Ar–H); ^{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). **2b**: ^{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), 7.26–7.32 (m, 5H, Ar–H); ^{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 ($-\mathbf{C}(-CH_3)_2$), 128.0, 128.1, 128.3, 128.6 (5 × –CH–), 136.8 (–C–); LRMS (ESI+, *m*/*z*, %): 392.13 $(M+Na)^+$; 370.15 $(M+H)^+$; HRMS: Calcd for $(M+H)^+$: 370.1496. Found, 370.1499.

4.3. 1,5,7-Tri-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropyliden-6-nitro-D,L-*glycero*-α-D-*manno*-heptofuranoses 3

To a solution of the mixture of nitrosugars **2** (1.65 g, 4.46 mmol) in dichloromethane (16 mL) and cyclohexane (32 mL) was added benzyl 2,2,2-trichloroacetimidate (1.5 mL, 8.04 mmol) and trifluoromethanesulfonic acid (0.01 mL) and the resulting solution was stirred at rt for 16 h. The reaction mixture was filtered and the filtrate was washed with saturated aqueous sodium hydrogen carbonate and water, dried (anhydrous sodium sulfate), filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:8 → 1:6) to give 1,5,7-tri-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropyliden-6-nitro-D,L-*glycero*-α-D-*manno*-heptofuranoses **3** (1.25 g, 51%) as a yellow oil. ^{1}H NMR (250 MHz, $CDCl_3$): 1.36, 1.37, 1.54, 1.59 (4 × s, 12H, 4 × $-CH_3$), 3.87–3.89 (m, 2H), 4.03–4.15 (m, 2H), 4.31–4.40 (m, 2H), 4.53–4.85 (m, 16H), 5.00–5.12 (m, 2H), 5.35, 5.37 (2 × s, 2H, 2 × H-1); 7.28–7.44 (m, 30H, 10 × Ar–H). ^{13}C NMR (62.8 MHz, $CDCl_3$): 24.9, 25.0, 26.0, 26.2 (4 × $-CH_3$); 66.3, 66.5, 73.5, 73.6, 74.5, 74.7 (8 × $-CH_2-$), 76.5, 77.4, 78.8, 79.0, 79.8, 79.6, 86.6, 86.8, 88.3, 88.1 (10 × –CH–), 101.4, 101.6 (2 × –CH–), 112.5, 112.7 (2 × –C–), 127.7, 127.9, 120.0, 128.1, 128.2, 128.3, 129.0 (30 × –CH–), 136.2, 136.4, 136.8, 137.0, 137.3, 137.6, (6 × –C–).

4.4. 1,5,7-Tri-*O*-benzyl-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-6-nitro-D-*glycero*-α-D-*manno*-heptofuranose 4a and 1,5,7-tri-*O*-benzyl-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-6-nitro-L-*glycero*-α-D-*manno*-heptofuranose 4b

A 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (3.2 mL, 3.2 mmol, 2.5 eq) was added to a suspension of paraformaldehyde (0.76 g, 25.2 mmol, 20 eq) and 1,5,7-tri-*O*-benzyl-6-deoxy-2,3-di-*O*-isopropyliden-6-nitro-D,L-*glycero*-α-D-*manno*-heptofuranose **3** (0.69 g, 1.26 mmol) in anhydrous tetrahydrofuran (8 mL) and the reaction mixture was stirred under a nitrogen atmosphere at room temperature for 24 h. Dichloromethane (40 mL) was added and the organic layer was washed with saturated aqueous ammonium chloride (3 × 20 mL), dried with anhydrous sodium sulfate, filtered and evaporated in vacuo. The crude product was purified by flash column chromatography (ethyl acetate/hexane 1:9) to give heptofuranoses **4a** (0.13 g, 0.22 mmol, 17%) and **4b** (0.17 g, 0.29 mmol, 23%). **4a**: $[\alpha]_D^{25} = +20.2$ (*c* 1.4, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): 1.33, 1.51 (2 × s, 6H, 2 × $-CH_3$), 4.10–4.53 (m, 12H), 4.63–4.78 (m, 2H), 5.03 (s, 1H, H-1), 7.31–7.36 (m, 15H, Ar–H); ^{13}C NMR (25.1 MHz, $CDCl_3$): 25.3, 26.35 ($-C(-CH_3)_2$), 62.9, 68.9, 69.3, 74.1, 75.6 (5 × $-CH_2-$), 77.8, 78.3, 80.4, 84.5 (4 × –CH–), 95.2 (C-6), 105.9 (C-1), 113.0 ($-C(-CH_3)_2$), 127.9, 128.1, 128.2, 128.3, 128.6, 128.7, 128.3 (15 × ArC–H), 137.00, 137.24, 137.51 (3 × ArC). **4b**: $[\alpha]_D^{25} = +36.7$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): 1.36, 1.54 (2 × s, 6H, 2 × $-CH_3$), 4.04–4.39 (m, 6H), 4.51–4.72 (m, 6H), 4.78–4.84 (m, 2H), 5.06 (s, 1H, H-1), 7.27–7.35 (m, 15H, Ar–H). ^{13}C NMR (25.1 MHz, $CDCl_3$): 25.3, 26.5 ($-C(-CH_3)_2$), 62.9, 68.9, 69.3, 74.1, 75.6 (5 × $-CH_2-$), 77.8, 78.3, 80.4, 84.5 (4 × –CH–), 95.2 (C-6), 105.9 (C-1), 113.0 ($-C(-CH_3)_2$), 127.9, 128.1, 128.2, 128.3, 128.6, 128.7, 128.3 (15 × ArC–H), 137.01, 137.36, 137.79 (3 × ArC). LRMS (ESI+, *m*/*z*, %): 602.24 $(M+Na)^+$; HRMS: Calcd for $(M+Na)^+$: 602.2369. Found, 602.2361.

4.5. 6-Amino-1,5,7-tri-*O*-benzyl-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-D-*glycero*-α-D-*manno*-heptofuranose 5a

To a stirred solution of nitro sugar **4a** (0.071 g, 0.123 mmol) in isopropanol (2.4 mL) and 1 M aqueous hydrochloric acid (1.3 mL)

was added zinc dust in four portions (0.161 g, 2.463 mmol) and the resulting mixture was stirred at rt. After 2 h, the reaction mixture was basified by the addition of saturated aqueous sodium bicarbonate and filtered through a Celite pad, washing with ethyl acetate. The layers were separated and the aqueous layer was further extracted with dichloromethane. The combined organic layers were dried (magnesium sulfate), filtered and evaporated under reduced pressure to afford 6-amino-1,5,7-tri-*O*-benzyl-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-D-*glycero*-α-D-*manno*-heptofuranose (**5a**) (0.068 g), which was used without further purification.

4.6. (4*R*,5*R*)-4-(Benzyloxymethyl)-4-[-5-(1,5-di-*O*-benzyl-2,3-di-*O*-isopropyliden-α-D-lyxofuran-5-C-yl)]oxazolidin-2-one 6

To a solution of **5a** (0.15 g, 0.27 mmol) in dichloromethane (2 mL) and DIEA (0.60 mmol, 0.10 mL) was added triphosgene (0.08 g, 0.27 mmol) and the mixture was stirred at rt for 1 h. The reaction mixture was diluted with dichloromethane and quenched by the addition of water. The organic layer was separated, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:3) to afford oxazolidinone **6** (0.10 g, 67%) as a clear oil. $[\alpha]_D^{23} = +2.2$ (*c* 1.0, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): 1.33, 1.49 (2 × s, 6H, 2 × –CH_3), 3.56 (d, 1H, *J* 9.4 Hz, H-7′), 3.66 (d, 1H, *J* 9.4 Hz, H-7), 3.95 (dd, 1H, *J* 9.4 Hz, *J* 3.2 Hz, H-4), 4.10 (d, 1H, *J* 9.4 Hz, H-5), 4.25–4.49 (m, 5H), 4.61–4.68 (m, 3H), 4.74–4.76 (m, 1H, H-3), 4.83 (d, 1H, *J* 10.9 Hz, –CHPh), 4.99 (s, 1H, H-1), 5.20 (bs, 1H, –NH–), 7.21–7.38 (m, 15H, H-Ph); ^{13}C NMR (62.8 MHz, $CDCl_3$): 26.1, 26.3 (–C(–CH_3)$_2$), 38.9 (–C–), 61.9, 69.8, 70.9, 71.0, 71.2, 73.3 (5 × –CH_2–), 75.8, 79.6, 80.1, 81.2 (4 × –CH–), 105.8 (C-1), 112.8 (–*C*(–CH_3)$_2$), 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4 (15 × –CHPh), 136.5, 138.0, 141.2 (3 × –CPh), 159.1 (–C=O).

4.7. 1,5,7-Tri-*O*-benzyl-6-benzyloxycarbonylamino-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-D-*glycero*-α-D-*manno*-heptofuranose 7a

Amine **5a** was dissolved in ethyl acetate (1.7 mL) and saturated aqueous sodium bicarbonate (0.7 mL) was added. The biphasic mixture was cooled to 0 °C and benzyloxycarbonyl chloride (0.020 mL, 0.028 g, 0.164 mmol) was added dropwise and the resulting mixture was stirred at rt for 2 h. The layers were separated and the aqueous layer was further extracted with ethyl acetate. The combined organic layers were washed with brine, dried (magnesium sulfate), filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:3) to give methyl 1,5,7-tri-*O*-benzyl-6-benzyloxycarbonylamino-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-D-*glycero*-α-D-*manno*-heptofuranoate **7a** (0.066 g, 78%). $[\alpha]_D^{23} = +48.8$ (*c* 1.35, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): 1.34, 1.49 (2 × s, 6H, 2 × –CH_3), 3.46–3.49 (m, 1H), 3.95–3.99 (m, 3H), 4.29–4.55 (m, 7H), 4.74–5.23 (m, 5H), 5.98 (s, 1H, H-1), 7.27–7.35 (m, 20H, H–Ph); ^{13}C NMR (62.8 MHz, $CDCl_3$): 25.4, 26.5 (–C(–CH_3)$_2$), 62.9 (C-6), 63.9, 66.9, 68.8, 73.8, 75.1 (6 × –CH_2–), 79.2, 81.3, 84.4 (4 × –CH–), 105.9 (C-1), 112.6 (–*C*(–CH_3)$_2$), 127.7, 127.8, 127.9, 128.0, 128.2 (20 × –CHPh), 136.7, 137.3, 138.0, 139.1 (4 × –CPh), 157.1 (–C=O); LRMS (ESI+, *m*/*z*, %): 706.32 (M+Na)$^+$, 684.30 (M+H)$^+$; HRMS: Calcd for (M+H)$^+$: 684.3167. Found, 684.3178.

4.8. Methyl 1,5,7-tri-*O*-benzyl-6-benzyloxycarbonylamino-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-D-*glycero*-α-D-*manno*-heptofuranoate 10a

To a solution of amino sugar **7a** (0.071 g, 0.104 mmol) in dichloromethane (2 mL) was added Dess–Martin periodinane (0.132 g, 0.313 mmol) and the resulting mixture was stirred at rt. After 1 h, dichloromethane (5 mL) and a solution of thiosulfate in saturated aqueous sodium bicarbonate (5 mL) were added and the biphasic mixture was stirred for 15 min. The layers were separated and the aqueous layer was further extracted with dichloromethane. The combined organic layers were dried (magnesium sulfate), filtered and evaporated under reduced pressure. The residue was dissolved in methanol/water 10:1 (12 mL) and 2-methyl-2-butene (0.021 mL, 1.390 mmol), sodium chlorite (0.024 g, 0.258 mmol) and sodium dihydrogen phosphate (0.033 g, 0.238 mmol) were added and the mixture was stirred at rt. After 2 h, more sodium chlorite (0.024 g, 0.258 mmol) and sodium dihydrogen phosphate (0.033 g, 0.238 mmol) were added and stirring was continued. After 4 h the reaction mixture was diluted with water, acidified with aqueous hydrochloric acid 10% and extracted with ethyl acetate. The combined organic layers were dried (magnesium sulfate), filtered and evaporated under reduced pressure. The residue was dissolved in a 7:3 mixture of diethyl ether/methanol (1.3 mL) and trimethylsilyldiazomethane was added (0.07 mL, 0.125 mmol). After 15 min the reaction mixture was evaporated in vacuo and the residue was purified by flash column chromatography (ethyl acetate/hexane 1:5) to yield methyl heptofuranoate **10a** (25.4 mg, 34%). $[\alpha]_D^{24} = +26.3$ (*c* 0.9, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): 1.33, 1.50 (2 × s, 6H, 2 × –CH_3), 3.73 (s, 3H, –OCH_3), 4.14 (d, 1H, *J* = 10.0 Hz), 4.31–4.57 (m, 10H), 4.79–4.83 (m, 2H), 4.96 (s, 1H), 5.08–5.21 (m, 2H), 6.73 (s, 1H, –NH–), 7.21–7.31 (m, 20H, H–Ph); ^{13}C NMR (62.8 MHz, $CDCl_3$): 25.2, 26.5 (–C(–CH_3)$_2$), 52.7 (–OCH_3), 66.2 (–C–); 66.8, 67.3, 68.3, 73.7, 75.3 (5 × –CH_2–), 78.6, 79.0, 81.0, 84.3 (4 × –CH–), 105.2 (C-1), 112.6 (–**C**(–CH_3)$_2$), 127.7, 127.8, 127.9, 128.0, 128.2, 128.4 (20 × –CHPh), 136.8, 136.9, 138.4, 138.5 (4 × –CPh), 155.2, 171.5 (2 × – C=O); LRMS (ESI+, *m*/*z*, %): 734.29 (M+Na)$^+$; 712.31 (M+H)$^+$, HRMS: Calcd LRMS (ESI+, *m*/*z*, %): 734.29 (M+Na)$^+$, 712.31 (M+H)$^+$; HRMS: Calcd for (M+H)$^+$: 712.3122. Found, 712.3129.

4.9. 6-Amino-1,5,7-tri-*O*-benzyl-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-L-*glycero*-α-D-*manno*-heptofuranose 5b

To a stirred solution of nitro sugar **4b** (0.054 g, 0.092 mmol) in isopropanol (1.8 mL) and 1 M aqueous hydrochloric acid (1 mL) was added zinc dust in four portions (0.121 g, 1.848 mmol) and the resulting mixture was stirred at rt. After 2 h, the reaction mixture was basified by the addition of saturated aqueous sodium bicarbonate and filtered through a Celite pad, washing with ethyl acetate. The layers were separated and the aqueous layer was further extracted with dichloromethane. The combined organic layers were dried (magnesium sulfate), filtered and evaporated under reduced pressure to afford 1,5,7-tri-*O*-benzyl-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-L-*glycero*-α-D-*manno*-heptofuranose **5b** (0.051 g), which was used without further purification.

4.10. 1,5,7-Tri-*O*-benzyl-6-benzyloxycarbonylamino-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-L-*glycero*-α-D-*manno*-heptofuranose 7b

Amine **5b** was dissolved in ethyl acetate (1.2 mL) and saturated aqueous sodium bicarbonate (0.5 mL) was added. The biphasic mixture was cooled to 0 °C and benzyloxycarbonyl chloride (0.017 mL, 0.021 g, 0.123 mmol) was added dropwise and the resulting mixture was stirred at rt for 2 h. The layers were separated and the aqueous layer was further extracted with ethyl acetate. The combined organic layers were washed with brine, dried (magnesium sulfate), filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexane 1:3) to give 1,5,7-tri-*O*-benzyl-6-

benzyloxycarbonylamino-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-L-*glycero*-α-D-*manno*-heptofuranose **7b** (0.045 g, 72%). ^{1}H NMR (300 MHz, $CDCl_3$): 1.33, 1.51 (2 × s, 6H, 2 × $-CH_3$), 3.76–4.39 (m, 6H), 4.52–4.69 (m, 5H), 4.77–4.85 (m, 2H), 5.02–5.13 (m, 3H), 5.87 (s, 1H, H-1), 7.25–7.42 (m, 20H, H-Ph); ^{13}C NMR (62.8 MHz, $CDCl_3$): 25.3, 26.5 ($-C(-CH_3)_2$), 62.5 (C-6); 64.3, 66.8, 69.2, 73.6, 75.2 (6 × $-CH_2-$), 79.2, 81.3, 84.4 (4 × –CH–), 105.9 (C-1), 112.6 ($-C(-CH_3)_2$), 127.7, 127.8, 127.9, 128.0, 128.2 (20 × –CHPh), 136.7, 137.3, 138.0, 139.1 (4 × –CPh), 157.1 (–C=O); LRMS (ESI+, *m*/*z*, %): 706.32 $(M+Na)^+$, 684.30 $(M+H)^+$; HRMS: Calcd for $(M+H)^+$: 684.3167. Found, 684.3178.

4.11. Methyl 1,5,7-tri-*O*-benzyl-6-benzyloxycarbonylamino-6-deoxy-6-*C*-hydroxymethyl-2,3-di-*O*-isopropyliden-L-*glycero*-α-D-*manno*-heptofuranoate 10b

To a solution of amino sugar **7b** (0.042 g, 0.061 mmol) in dichloromethane (1 mL) was added Dess–Martin periodinane (0.077 g, 0.183 mmol) and the resulting mixture was stirred at rt. After 1 h, dichloromethane (5 mL) and a solution of thiosulfate in saturated aqueous sodium bicarbonate (5 mL) were added and the biphasic mixture was stirred for 15 min. The layers were separated and the aqueous layer was further extracted with dichloromethane. The combined organic layers were dried (magnesium sulfate), filtered and evaporated under reduced pressure. The residue was dissolved in methanol/water 10:1 (6 mL) and 2-methyl-2-butene (0.012 mL, 0.812 mmol), sodium chlorite (0.014 g, 0.151 mmol) and sodium dihydrogen phosphate (0.019 g, 0.139 mmol) were added and the mixture was stirred at rt. After 2 h, more sodium chlorite (0.014 g, 0.151 mmol) and sodium dihydrogen phosphate (0.019 g, 0.139 mmol) were added and stirring was continued. After 4 h the reaction mixture was diluted with water, acidified with 10% aqueous hydrochloric acid and extracted with ethyl acetate. The combined organic layers were dried (magnesium sulfate), filtered and evaporated under reduced pressure. The residue was dissolved in a 7:3 mixture of diethyl ether/methanol (1 mL) and trimethylsilyldiazomethane (0.04 mL, 0.038 mmol) was added. After 15 min the reaction mixture was evaporated in vacuo and the residue was purified by flash column chromatography (ethyl acetate/hexane 1:5) to yield methyl heptofuranoate **10b** (18.5 mg, 43%). $[\alpha]_D^{24} = +26.3$ (*c* 0.9, chloroform); ^{1}H NMR (300 MHz, $CDCl_3$): 1.31, 1.51 (2 × s, 6H, 2 × $-CH_3$), 3.71 (s, 3H, $-OCH_3$), 4.28 (s, 2H, H-7, H-7′), 4.41–4.44 (m, 3H), 4.53–4.73 (m, 5H), 4.78–4.87 (m, 2H), 5.06 (s, 2H, $-CH_2Ph$), 5.08 (s, 1H, H-1), 6.79 (s, 1H, –NH–), 7.27–7.34 (m, 20H, H–Ph); ^{13}C NMR (62.8 MHz, $CDCl_3$): 26.3, 26.5 ($-C(-CH_3)_2$), 59.5 ($-OCH_3$), 66.2 (–C–), 66.8, 66.9, 68.7, 73.4, 75.3 (5 × $-CH_2-$), 76.0, 79.0, 81.2, 84.2 (4 × –CH–), 105.7 (C-1), 112.6 ($-C(-CH_3)_2$), 127.7, 127.8, 127.9, 128.0, 128.2, 128.4 (20 × –CHPh), 137.0, 138.5, 138.6 (4 × –CPh), 155.4, 170.8 (2 × –C=O); LRMS (ESI+, *m*/*z*, %): 734.29 $(M+Na)^+$, 712.31 $(M+H)^+$; HRMS: Calcd for $(M+H)^+$: 712.3122. Found, 712.3128.

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