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# Study on the Synthesis and Biological Activities of N-Alkylated Deoxynojirimycin Derivatives with a Terminal Tertiary Amine

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### **Abstract**

A series of N-alkylated deoxynojirimycin (DNJ) derivatives connected to a terminal tertiary amine at the alkyl chains of various lengths were prepared. These novel synthetic compounds were assessed for preliminary glucosidase inhibition and anticancer activities in vitro. Potent and selective inhibition was observed among them. Compound 7d (IC $_{50}$  = 0.052 mM) showed improved and selective inhibitory activity against  $\beta$ -glucosidase compared to DNJ (IC $_{50}$  = 0.65 mM). In addition, analysis of the kinetics of enzyme inhibition by using Lineweaver–Burk plots indicated that 7d inhibited  $\beta$ -glucosidase in a competitive manner, suggesting that 7d was expected to bind to the active site of  $\beta$ -glucosidase. Compounds 8b and 8c were found to be moderate and selective inhibitors of  $\alpha$ -glucosidase. Nevertheless, none of compounds inhibited the growth of B16F10 melanoma cells.

Keywords: biological activities; glucosidase; 1-deoxynojirimycin; selective inhibition

#### 1. Introduction

Glucosidases are enzymes which catalyze the hydrolysis of glycosidic bonds in oligosaccharides or glycoconjugates, playing a vital role in the digestion of carbohydrates and in the processing of glycoproteins and glycolipids.1 Glucosidases are also involved in carbohydrate-mediated diseases such as diabetes,<sup>2</sup> tumor metastasis,3 viral infections,4 and lysosomal storage diseases.5 Inhibitors of α-glucosidase can significantly decrease postprandial blood glucose levels<sup>6</sup> and promote glycoprotein misfolding in the endoplasmic reticulum (ER).<sup>7</sup> In mammals, β-glucosidase enables hydrolysis of glucosylceramide into ceramide and glucose, which is in part performed by β-glucocerebrosidases (GBA1 or GCase)<sup>8</sup> and GBA2.9 Gaucher disease, the most common lysosomal storage disease, is caused by mutations in the β-glucocerebrosidase (GBA1) gene. Inhibitors of β-glucosidase could reduce the biosynthesis of glycolipids to balance the deficient activity of β-Gcase. <sup>10</sup> In tumor cells, oligosaccharides on the surface of tumor cells play an important role in expression of the malignant phenotype and the metastatic spread of tumor cells. The synthesis of these oligosaccharides in endoplasmic reticulum and Golgi is dependent on carbohydrate processing enzymes such as glycosidases. Therefore, specific glycosidase inhibitors may be candidates for cancer chemotherapy.<sup>11,12</sup>

Among the families of glycosidase inhibitors reported so far, iminosugars are particularly notable. They are carbohydrate mimetics where the endocyclic oxygen has been replaced by a nitrogen atom. 13-15 Their structures can mimic transition-state analogues of glycosidases, which interact with two carboxylic acid units to form strong ions and catalyze the cleavage of the glycoside bonds. Their most famous representative is the naturally occurring 1-deoxynojirimycin 1.<sup>2</sup> Some N-alkylated DNJ derivatives, like N-hydroxyethyl-DNJ<sup>16</sup> 2 (miglitol, an intestinal α-glucosidase inhibitor), and N-butyl-DNJ<sup>17</sup> 3 (miglustat, a glucosylceramide synthase inhibitor) have been approved for the treatment of diabetes-type 2 and Gaucher disease, respectively. Compound 6<sup>3</sup> not only inhibited α-glucosidase (Bacillus stearothermophilus), BAEC growth and migration, but also suppressed the growth of A549 cells (Figure 1). Nevertheless, despite extensive synthesis and investigations of highly bioactive iminosugars, a remaining drawback is their limited selectivity on glucosidases, and this leads to some side effects when applied therapeutically. For example, N-butyl-DNJ 3 (Figure 1) can inhibit some other

Figure 1. Known potent glycosidase inhibitors derived from 1-deoxynojirimycin.

enzymes nonrelated to lysosomal storage disease, such as sucrase, maltase, α-glucosidase I and II.<sup>18</sup> Obviously, improving the selectivity of iminosugars as glycosidase inhibitors is a challenging goal.

Modification or variation of a known iminosugar inhibitor, especially a natural product, is a feasible strategy to obtain more selective and stronger inhibitors. Generally, there are two main strategies for modification of iminosugars: introduction of different alkyl groups on the amino group and alterations of the ring hydroxyl residues. <sup>19</sup> It has been demonstrated that the potency of DNJ derivatives could be increased by introducing a hydrophobic group on the nitrogen atom of DNJ using a heteroatom linker and a carbon chain spacer. Moreover, lengthening of the alkyl chain and an increase in the size of the hydrophobic group would be also beneficial for the glucosidase inhibition. These types of modifications can be seen in the design of compounds **4**, <sup>20</sup> **5**<sup>21</sup> and **6** (Figure 1).<sup>3</sup>

Our group had done some work on the modification of DNJ, such as the synthesis of C-6 deutero DNJ, a potent α-glucosidase and the optimization of DNJ synthetic route. <sup>22,23</sup> And as a part of our ongoing program devoted to the development of new glucosidase inhibitors, we embarked on a strategy starting from DNJ as the lead compound. The key DNJ scaffold was connected to a terminal tertiary amine through introduction of alkyl chains of various length. And the introduction of a nitrogen atom may lead to a polarization different from that of oxygen atom. <sup>24,25</sup> The work reported herein describes the synthesis and biological evaluation of a small library of DNJ derivatives in which the length of the alkyl chain and the size and nature of the terminal tertiary amine substituents have been studied.

### 2. Experimental

#### 2. 1. Materials and Methods

All reagents and solvents were purchased from commercial suppliers and used without further purification. Reactions progression was monitored by Thin Layer Chromatography (TLC) using silica gel GF $_{254}$  plates (0.2 mm thickness), spots were detected under UV-light ( $\lambda$  =

254 nm). Visualization of the deprotected iminosugar was accomplished by exposure to iodine vapour. Flash column chromatography was carried out by silica gel (200–300 mesh). NMR spectra were recorded on Bruker Avance III 500 MHz spectrometer using  $CDCl_3$  or  $D_2O$  as solvents. Chemical shifts are reported in ppm. High resolution mass spectra (HRMS) were recorded by direct injection on a mass spectrometer (Thermo Scientific LTQ Orbitrap XL) equipped with an electrospray ion source in positive mode. The following abbreviations have been used to describe the signal multiplicity: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), h (hextet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets).

#### General Procedure A

Nucleophilic substitution on a nitrogen atom. The starting material (1 mM) was mixed with N-bromophthalimide (2 mM) and  $K_2CO_3$  (3 mM) in DMF (10 mL). The mixture was heated at 100 °C for 24 h. After cooling, the mixture was poured into water and extracted into ethyl acetate. The organic layer was dried over  $Na_2SO_4$  and concentrated. The residue was purified by flash column chromatography (10:1 $\rightarrow$ 3:1; PE:ethyl acetate).

#### **General Procedure B**

Hydrazinolysis. The starting material (1 mM) was mixed with 80% hydrazine hydrate (0.13 mL, 2 mM) in EtOH (10 mL). The mixture was heated under reflux for 3 h. After cooling, the solid was removed by filtration. The filtrate was concentrated and the residue was purified by flash column chromatography (20:1:0.2→20:2:0.2; ethyl acetate: MeOH:NH₄OH).

#### **General Procedure C**

**Reductive amination.** The starting material (1 mM) was mixed with formaldehyde (178.38 mg, 37% aqueous solution, 0.22 mL, 2.2 mmol) and formic acid (0.19 mL, 5.0 mmol). The mixture was heated at 105 °C for 3 h. After cooling, the mixture was poured into water and extracted into ethyl acetate. The organic layer was washed with saturated NaHCO<sub>3</sub> solution, dried and concentrated. The residue was purified by flash column chromatography (20:1:0.2 $\Rightarrow$ 20:2:0.2; ethyl acetate: MeOH:NH<sub>4</sub>OH).

#### **General Procedure D**

**Double nucleophilic substitution.** The starting material (1 mM) was mixed with alkyl dibromide (2 mM) and  $K_2CO_3$  (3 mM) in  $CH_3CN$  (10 mL). The mixture was heated at 80 °C for 12 h. After cooling, the solution was concentrated. The residue was purified by flash column chromatography (20:1:0.2 $\rightarrow$ 20:2:0.2; ethyl acetate: MeOH:NH<sub>4</sub>OH).

#### General Procedure E

Catalytic hydrogenolysis. To a solution of the benzylated intermediate (1 mmol) in EtOH was added Pd (10%)/C (100 mg) and the mixture stirred under an atmosphere of hydrogen at room temperature for 24 h. The catalyst was filtered off, the solvents removed under reduced pressure and the residue purified by flash column chromatography (8:2:0.1 $\rightarrow$ 6:4:0.1; n-propanol:H<sub>2</sub>O:NH<sub>4</sub>OH).

## 2-(2-((2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)ethyl)isoindoline-1,3-dione (10a)

Prepared according to procedure A. Compound 9 (2) g, 3.8 mmol), N-(4-bromoethyl)phthalimide (2.1 g, 7.6 mmol), K<sub>2</sub>CO<sub>3</sub> (1.6 g, 11.4 mmol), DMF (40 mL). Yield: 87% (2.4 g), colourless syrup,  $R_f = 0.45$  (3:1, PE:ethyl acetate). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.76 (dd, J = 5.4, 3.0 Hz, 2H, ArH), 7.65 (dd, J = 5.4, 3.0 Hz, 2H, ArH), 7.46– 7.00 (m, 20H, ArH), 4.95 (d, J = 10.9 Hz, 1H, Ph $CH_2$ ), 4.77 (ddd, J = 22.9, 15.6, 11.3 Hz, 4H, PhCH<sub>2</sub>), 4.46 (d, J = 12.1)Hz, 1H, Ph $CH_2$ ), 4.32 (dd, J = 26.1, 11.4 Hz, 2H, Ph $CH_2$ ), 3.94 (dt, J = 13.9, 8.0 Hz, 1H, H-8a), 3.78-3.56 (m, 4H,H-6, H-8b, H-2), 3.51-3.43 (m, 2H, H-3, H-4), 3.41 (dd, J = 11.0, 4.8 Hz, 1H, H-1a), 3.23 (dt, J = 13.7, 8.2 Hz, 1H, H-7a), 2.65 (ddd, J = 13.3, 7.2, 3.4 Hz, 1H, H-7b), 2.42 (d, J = 8.8 Hz, 1H, H-5), 2.26 (t, J = 10.7 Hz, 1H, H-1b). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 168.35, 138.97, 138.71, 138.52, 138.07, 133.81, 132.17, 128.43, 128.37, 128.33, 128.01, 127.97, 127.94, 127.84, 127.64, 127.53, 123.16, 87.20, 78.69, 78.57, 77.33, 77.08, 76.83, 75.49, 75.20, 73.10, 72.66, 66.07, 64.50, 54.25, 49.24, 34.56.

# 2-(4-((2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)butyl)isoindoline-1,3-dione (10b)

Prepared according to procedure A. Compound **9** (2 g, 3.8 mmol), N-(4-bromobutyl)phthalimide (1.9 g, 7.6 mmol),  $K_2\text{CO}_3$  (1.6 g, 11.4 mmol), DMF (40 mL). Yield: 85% (2.3 g), colourless syrup,  $R_f$ = 0.33 (3:1, PE: ethyl acetate). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.83 (dt, J = 7.4, 3.7 Hz, 2H, ArH), 7.75–7.63 (m, 2H, ArH), 7.42–7.16 (m, 18H, ArH), 7.11 (d, J = 6.4 Hz, 2H, ArH), 4.95 (d, J = 11.1 Hz, 1H, Ph $CH_2$ ), 4.86 (d, J = 10.8 Hz, 1H, Ph $CH_2$ ), 4.80 (d, J = 11.1 Hz, 1H, Ph $CH_2$ ), 4.39 (d, J = 10.8 Hz, 1H, Ph $CH_2$ ), 3.63 (dt, J = 11.0, 5.9 Hz, 4H, H-6, H-2, H-10a), 3.56 (t, J = 9.4 Hz, 2H, H-10b, H-3), 3.45 (t, J = 9.1 Hz, 1H, H-4), 3.08 (dd, J = 11.1, 4.8 Hz, 1H, H-1a), 2.72 (m, 1H, H-7a), 2.63–2.50 (m,

1H, H-7b), 2.29 (d, J = 9.5 Hz, 1H, H-5), 2.18 (t, J = 10.8 Hz, 1H, H-1b), 1.65–1.33 (m, 4H, H-8, H-9). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  168.44, 139.07, 138.57, 137.80, 133.98, 132.17, 128.53, 128.44, 128.37, 127.92, 127.70, 127.58, 127.48, 123.27, 87.35, 78.61, 78.49, 77.38, 77.12, 76.87, 75.36, 75.23, 73.43, 72.79, 65.43, 63.88, 54.40, 51.76, 37.81, 26.56, 21.43.

### 2-((2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy) methyl)piperidin-1-yl)ethan-1-amine (11a)

Prepared according to procedure B. Compound 10a (1.3 g, 1.86 mmol), 80% hydrazine hydrate (0.23 mL, 3.72 mM), EtOH (10 mL). Yield: 82% (0.87 g), colourless syrup,  $R_f = 0.43$  (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.46–7.18 (m, 18H, ArH), 7.18-7.04 (m, 2H, ArH), 4.95 (d, J = 11.0 Hz, 1H, Ph $CH_2$ ), 4.87 (d, J = 10.8 Hz, 1H, Ph $CH_2$ ), 4.81 (d, J = 11.0 Hz, 1H,  $PhCH_2$ ), 4.67 (q, J = 11.6 Hz, 2H,  $PhCH_2$ ), 4.50 (d, J = 12.0Hz, 1H, Ph $CH_2$ ), 4.42 (dd, J = 11.4, 4.6 Hz, 2H, Ph $CH_2$ ), 3.77-3.44 (m, 5H, H-6, H-2, H-3, H-4), 3.11 (dd, J = 11.4, 4.8 Hz, 1H, H-1a), 2.90–2.79 (m, 1H, H-7a), 2.79–2.64 (m, 2H, H-7b, H-8a), 2.55-2.42 (m, 1H, H-8b), 2.42-2.24 (m, 3H, H-5, N $H_2$ ), 2.20 (t, J = 10.9 Hz, 1H, H-1b). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 138.99, 138.53, 137.80, 128.52, 128.50, 128.43, 128.39, 127.98, 127.93, 127.79, 127.67, 127.57, 87.19, 78.55, 78.39, 77.46, 77.20, 76.95, 75.40, 75.26, 73.34, 72.92, 66.20, 64.83, 54.89, 54.27, 38.43.

### 4-((2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy) methyl)piperidin-1-yl)butan-1-amine (11b)

Prepared according to procedure B. Compound 10b (1.2 g, 1.66 mmol), 80% hydrazine hydrate (0.21 mL, 3.31 mM), EtOH (10 mL). Yield: 85% (0.84 g), colourless syrup,  $R_f = 0.35$  (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.39–7.18 (m, 18H, ArH), 7.18–7.06 (m, 2H, ArH), 4.95 (d, J = 11.1 Hz, 1H, Ph $CH_2$ ), 4.87 (d, J = 10.9 Hz, 1H, Ph $CH_2$ ), 4.80 (d, J = 11.1 Hz, 1H,  $PhCH_2$ ), 4.67 (q, J = 11.6 Hz, 2H,  $PhCH_2$ ), 4.53–4.37 (m, 3H, Ph $CH_2$ ), 3.66 (dt, J = 8.7, 5.5 Hz, 2H, H-6), 3.62–3.51 (m, 2H, H-3, H-2), 3.47 (t, J = 9.0 Hz, 1H, H-4), 3.08 (dd, J)= 11.2, 4.8 Hz, 1H, H-1a), 2.77-2.43 (m, 6H, H-7, H-10,  $NH_2$ ), 2.32 (d, J = 9.4 Hz, 1H, H-5), 2.21 (t, J = 10.8 Hz, 1H, H-1b), 1.37 (m, 4H, H-8, H-9). 13C NMR (126 MHz, CDCl<sub>3</sub>): δ 138.10, 137.67, 136.86, 127.48, 127.42, 126.94, 126.73, 126.63, 126.54, 86.23, 77.61, 77.54, 76.60, 76.35, 76.09, 74.35, 74.21, 72.52, 71.83, 64.59, 62.99, 53.44, 51.27, 40.79, 30.07, 20.50.

### *N*,*N*-Dimethyl-2-((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)ethan-1-amine (12a)

Prepared according to procedure C. Compound **11a** (0.3 g, 0.53 mmol), formaldehyde (37% aqueous solution, 0.12 mL, 1.17 mmol) and formic acid (0.1 mL, 2.65 mmol). Yield: 83% (0.26 g), yellow syrup,  $R_f$  = 0.83(20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.28 (m, 18H, Ar*H*), 7.12 (d, J = 6.4 Hz, 2H, Ar*H*), 4.95 (d,

J = 11.1 Hz, 1H, Ph $CH_2$ ), 4.87 (d, J = 10.8 Hz, 1H, Ph $CH_2$ ), 4.81 (d, J = 11.1 Hz, 1H, Ph $CH_2$ ), 4.67 (q, J = 11.6 Hz, 2H, Ph $CH_2$ ), 4.47 (s, 2H, Ph $CH_2$ ), 4.40 (d, J = 10.8 Hz, 1H, Ph $CH_2$ ), 3.74–3.54 (m, 4H, H-6, H-2, H-3), 3.46 (t, J = 9.1 Hz, 1H, H-4), 3.12 (dd, J = 11.2, 4.8 Hz, 1H, H-1a), 2.93–2.80 (m, 1H, H-7a), 2.80–2.66 (m, 1H, H-7b), 2.47–2.33 (m, 2H, H-5, H-8a), 2.29 (td, J = 11.2, 7.2 Hz, 2H, H-1b, H-8b), 2.16 (s, 6H, 2×CH<sub>3</sub>). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 139.05, 138.58, 137.78, 128.56, 128.48, 128.46, 128.39, 127.95, 127.93, 127.91, 127.73, 127.61, 127.52, 87.36, 78.51, 78.42, 77.39, 77.14, 76.89, 75.38, 75.24, 73.56, 72.86, 65.66, 64.07, 55.05, 54.68, 50.21, 45.91.

# *N,N*-Dimethyl-4-((2*R*,3*R*,4*R*,5*S*)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)butan-1-amine (13a)

Prepared according to procedure C. Compound 11b (0.17 g, 0.29 mmol), formaldehyde (37% aqueous solution, 64 μL, 0.64 mmol) and formic acid (55 μL, 1.45 mmol). Yield: 90% (0.16 g), yellow syrup,  $R_f = 0.75$ (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.45–7.19 (m, 18H, ArH), 7.19–7.05 (m, 2H, ArH), 4.95 (d, J = 11.1 Hz, 1H, PhCH<sub>2</sub>), 4.88 (d, J =10.9 Hz, 1H,  $PhCH_2$ ), 4.81 (d, J = 11.1 Hz, 1H,  $PhCH_2$ ), 4.67 (q, J = 11.6 Hz, 2H,  $PhCH_2$ ), 4.45 (dt, J = 10.9, 9.9 Hz, 3H, Ph $CH_2$ ), 3.72–3.61 (m, 2H, H-6), 3.57 (dt, J = 9.8, 5.6Hz, 2H, H-2, H-3), 3.45 (t, J = 9.1 Hz, 1H, H-4), 3.08 (dd, J = 11.2, 4.9 Hz, 1H, H-1a), 2.68 (m, 1H, H-7a), 2.63-2.52 $(m, 1H, H-7b), 2.37-2.09 (m, 10H, H-5, H-1a, H-10, 2 \times$ CH<sub>3</sub>), 1.54–1.20 (m, 4H, H-8, H-9). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$  138.09, 137.64, 136.89, 127.43, 126.89, 126.67, 126.56, 126.47, 86.41, 77.70, 77.63, 76.36, 76.10, 75.85, 74.36, 74.21, 72.48, 71.78, 64.61, 62.83, 58.56, 53.47, 51.23, 44.35, 24.49, 20.72.

### (2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy) methyl)-1-(2-(pyrrolidin-1-yl)ethyl)piperidine (12b)

Prepared according to procedure D. Compound 11a (0.3 g, 0.53 mmol), 1,4-dibromobutane (127 μL, 1.06 mmol), K<sub>2</sub>CO<sub>3</sub> (0.22 g, 1.59 mmol), CH<sub>3</sub>CN (5 mL). Yield: 84% (276 mg), yellow syrup,  $R_f = 0.55$  (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.45-7.21 (m, 18H, ArH), 7.18 (d, J = 6.7 Hz, 2H, ArH), 4.95 (d, J = 10.9 Hz, 1H, PhCH<sub>2</sub>), 4.90 (d, J = 10.8 Hz, 1H, $PhCH_2$ ), 4.81 (d, J = 11.0 Hz, 1H,  $PhCH_2$ ), 4.69 (d, J = 1.5Hz, 2H, Ph $CH_2$ ), 4.48 (dt, J = 18.5, 11.5 Hz, 3H, Ph $CH_2$ ), 3.74 (qd, J = 10.9, 2.4 Hz, 2H, H-6), 3.59 (m, 2H, H-2, H-3), 3.50 (t, I = 8.8 Hz, 1H, H-4), 3.27–3.10 (m, 2H, H-1a, H-8a), 2.86 (m, 7H, H-7, H-8b, H-9, H-12), 2.39 (d, J = 9.1Hz, 1H, H-5), 2.26 (t, J = 10.8 Hz, 1H, H-1b), 1.83-1.60 (m, 4H, H-10, H-11).  $^{13}$ C NMR (126 MHz, CDCl<sub>3</sub>):  $\delta$ 138.84, 138.46, 138.40, 137.76, 128.58, 128.52, 128.48, 128.45, 128.11, 128.02, 127.96, 127.93, 127.79, 127.76, 127.64, 86.59, 78.35, 78.02, 77.55, 77.30, 77.04, 75.40, 75.30, 73.40, 72.79, 66.28, 64.64, 55.05, 54.36, 51.99, 49.58, 23.15.

### (2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy) methyl)-1-(2-(piperidin-1-yl)ethyl)piperidine (12c)

Prepared according to procedure D. Compound 11a (0.3 g, 0.53 mmol), 1,4-dibromopentane (143 μL, 1.06 mmol), K<sub>2</sub>CO<sub>3</sub> (0.22 g, 1.59 mmol), CH<sub>3</sub>CN (5 mL). Yield: 80% (269 mg), yellow syrup,  $R_f = 0.64(20:2:0.2; \text{ ethyl ace-}$ tate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.42-7.19 (m, 18H, ArH), 7.14 (d, J = 6.4 Hz, 2H, ArH), 4.95 (d, J = 11.1 Hz, 1H, PhCH<sub>2</sub>), 4.88 (d, J = 10.8 Hz, 1H, $PhCH_2$ ), 4.81 (d, J = 11.1 Hz, 1H,  $PhCH_2$ ), 4.66 (q, J = 11.6Hz, 2H, Ph $CH_2$ ), 4.50 (d, J = 12.1 Hz, 1H, Ph $CH_2$ ), 4.43 (d,  $J = 11.4 \text{ Hz}, 2H, PhCH_2), 3.75-3.52 \text{ (m, 4H, H-6, H-2, H-6)}$ H-3), 3.46 (t, J = 9.1 Hz, 1H, H-4), 3.12 (dd, J = 11.2, 4.8 Hz, 1H, H-1a), 2.96-2.84 (m, 1H, H-7a), 2.84-2.70 (m, 1H, H-7b), 2.53–2.21 (m, 8H, H-8, H-9, H-13, H-5, H-1b), 1.58-1.46 (m, 4H, H-10, H-12), 1.39 (m, 2H, H-11). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 139.07, 138.59, 137.81, 128.59, 128.45, 128.40, 128.37, 127.92, 127.72, 127.61, 127.50, 87.31, 78.55, 78.42, 77.38, 77.13, 76.88, 75.36, 75.26, 73.54, 72.82, 65.52, 64.02, 55.02, 54.96, 54.19, 49.10, 25.75, 24.17.

### 4-(2-((2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)ethyl)morpholine (12d)

Prepared according to procedure D. Compound 11a (0.3 g, 0.53 mmol), 2-bromoethyl ether (137 μL, 1.06 mmol), K<sub>2</sub>CO<sub>3</sub> (0.22 g, 1.59 mmol), CH<sub>3</sub>CN (5 mL). Yield: 85% (287 mg), colourless syrup,  $R_f = 0.73$  (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.40–7.21 (m, 18H, ArH), 7.21–7.10 (m, 2H, ArH), 4.96 (d, J = 11.1 Hz, 1H, Ph $CH_2$ ), 4.89 (d, J = 10.9 Hz, 1H, Ph $CH_2$ ), 4.82 (d, J = 11.1 Hz, 1H, PhCH<sub>2</sub>), 4.66 (dd, J = 27.2, 11.6 Hz, 2H,  $PhCH_2$ ), 4.52 (d, J = 12.1 Hz, 1H,  $PhCH_2$ ), 4.45 (d, J = 10.9 Hz, 1H, PhCH<sub>2</sub>), 4.38 (d, J = 12.1 Hz, 1H, PhCH<sub>2</sub>), 3.72-3.52 (m, 8H, H-6, H-2, H-3, H-10, H-11), 3.46 (t, J =9.1 Hz, 1H, H-4), 3.10 (dd, J = 11.2, 4.8 Hz, 1H, H-1a), 2.84 (m, 1H, H-7a), 2.78-2.67 (m, 1H, H-7b), 2.49-2.20 (m, 8H, H-5, H-1b, H-8, H-9, H-12). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 139.03, 138.56, 137.74, 128.65, 128.47, 128.43, 128.39, 127.97, 127.93, 127.75, 127.67, 127.54, 87.33, 78.54, 78.42, 77.43, 77.17, 76.92, 75.40, 75.30, 73.53, 72.88, 66.93, 65.46, 63.89, 55.04, 54.03, 48.86.

### (2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy) methyl)-1-(4-(pyrrolidin-1-yl)butyl)piperidine (13b)

Prepared according to procedure D. Compound **11b** (0.3 g, 0.5 mmol), 1,4-dibromobutane (120  $\mu$ L, 1 mmol),  $K_2CO_3$  (0.21 g, 1.5 mmol),  $CH_3CN$  (5 mL). Yield: 81% (265 mg), pale yellow syrup,  $R_f = 0.49$  (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  7.39–7.19 (m, 18H, Ar*H*), 7.15 (d, J = 6.6 Hz, 2H, Ar*H*), 4.96 (d, J = 11.0 Hz, 1H, Ph*CH*<sub>2</sub>), 4.89 (d, J = 10.8 Hz, 1H, Ph*CH*<sub>2</sub>), 4.81 (d, J = 11.0 Hz, 1H, Ph*CH*<sub>2</sub>), 4.67 (q, J = 11.6 Hz, 2H, Ph*CH*<sub>2</sub>), 4.52–4.39 (m, 3H, Ph*CH*<sub>2</sub>), 3.73–3.52 (m, 4H, H-6, H-2, H-3), 3.47 (t, J = 9.0 Hz, 1H, H-4), 3.07 (dd, J = 11.2, 4.8 Hz, 1H, H-1a), 2.78–2.43 (m, 8H, H-7, H-10, H-11, H-14), 2.30 (d, J = 9.5 Hz, 1H, H-5), 2.18 (t, J = 10.8

Hz, 1H, H-1b), 1.83 (m, 4H, H-12, H-13), 1.60–1.34 (m, 4H, H-8, H-9).  $^{13}$ C NMR (126 MHz, CDCl<sub>3</sub>): δ 138.99, 138.55, 137.91, 128.45, 128.38, 127.95, 127.92, 127.89, 127.73, 127.61, 127.53, 87.35, 78.67, 78.53, 77.34, 77.08, 76.83, 75.42, 75.24, 73.47, 72.80, 65.71, 64.01, 55.98, 54.33, 53.96, 51.75, 29.36, 25.92, 23.37, 21.91.

### (2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy) methyl)-1-(4-(piperidin-1-yl)butyl)piperidine (13c)

Prepared according to procedure D. Compound 11b  $(0.3 \text{ g}, 0.5 \text{ mmol}), 1,4\text{-dibromopentane} (135 \mu\text{L}, 1 \text{ mmol}),$ K<sub>2</sub>CO<sub>3</sub> (0.21 g, 1.5 mmol), CH<sub>3</sub>CN (5 mL). Yield: 82% (274 mg), colourless syrup,  $R_f = 0.57$  (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.43–7.20 (m, 18H, ArH), 7.20–7.09 (m, 2H, ArH), 4.97 (d, J = 11.0 Hz, 1H, Ph $CH_2$ ), 4.90 (d, J = 10.8 Hz, 1H, Ph $CH_2$ ),  $4.82 \text{ (d, } J = 11.0 \text{ Hz, } 1\text{H, } PhCH_2), 4.68 \text{ (q, } J = 11.7 \text{ Hz, } 2\text{H,}$  $PhCH_2$ ), 4.45 (t, J = 5.3 Hz, 3H,  $PhCH_2$ ), 3.74–3.53 (m, 4H, H-6, H-2, H-3), 3.48 (t, J = 9.0 Hz, 1H, H-4), 3.06 (dd, J =11.2, 4.8 Hz, 1H, H-1a), 2.80-2.36 (m, 8H, H-7, H-10, H-11, H-15), 2.30 (d, J = 9.4 Hz, 1H, H-5), 2.15 (t, J = 10.8Hz, 1H, H-1b), 1.86-1.31 (m, 10H, H-8, H-9, H-12, H-13, H-14). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 138.96, 138.53, 137.99, 128.51, 128.48, 128.43, 128.37, 128.00, 127.93, 127.88, 127.78, 127.66, 127.59, 87.33, 78.68, 78.44, 77.55, 77.30, 77.04, 75.47, 75.27, 73.43, 72.77, 65.88, 64.21, 58.12, 54.24, 53.73, 51.32, 24.21, 23.17, 22.94, 22.00.

### 4-(4-((2R,3R,4R,5S)-3,4,5-Tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)butyl)morpholine (13d)

Prepared according to procedure D. Compound 11b (0.3 g, 0.5 mmol), 2-bromoethyl ether (129µL, 1 mmol), K<sub>2</sub>CO<sub>3</sub> (0.21 g, 1.5 mmol), CH<sub>3</sub>CN (5 mL). Yield: 87% (292 mg), colourless syrup,  $R_f = 0.7$  (20:2:0.2; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.44–7.18 (m, 18H, ArH), 7.18–7.04 (m, 2H, ArH), 4.95 (d,  $J = 11.1 \text{ Hz}, 1\text{H}, PhCH_2$ , 4.88 (d,  $J = 10.8 \text{ Hz}, 1\text{H}, PhCH_2$ ), 4.81 (d, J = 11.1 Hz, 1H, PhCH<sub>2</sub>), 4.67 (q, J = 11.6 Hz, 2H,  $PhCH_2$ ), 4.45 (dt, J = 13.5, 11.5 Hz, 3H,  $PhCH_2$ ), 3.63 (m, 8H, H-6, H-2, H-3, H-12, H-13), 3.46 (t, J = 9.1 Hz, 1H, H-4), 3.08 (dd, J = 11.1, 4.9 Hz, 1H, H-1a), 2.76-2.64 (m, 1H, H-7a), 2.60 (m, 1H, H-7b), 2.48-2.16 (m, 8H, H-5, H-1b, H-10, H-11, H-14), 1.51-1.28 (m, 4H, H-8, H-9). <sup>13</sup>C NMR (126 MHz, CDCl<sub>3</sub>): δ 139.04, 138.59, 137.86, 128.44, 128.39, 128.37, 127.92, 127.86, 127.70, 127.60, 127.50, 87.41, 78.64, 78.60, 77.36, 77.10, 76.85, 75.38, 75.24, 73.50, 72.83, 67.01, 65.52, 63.72, 58.77, 54.49, 53.72, 52.21, 24.45, 21.58.

### (2R,3R,4R,5S)-1-(2-(Dimethylamino)ethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol (7a)

Prepared according to procedure E. Compound **12a** (260 mg, 0.44 mmol), 10% Pd/C (100 mg), EtOH (10 mL), pH~1 with 1 M aq HCl. Yield: 91% (93 mg), pale yellow syrup,  $R_f = 0.73$  (1:1:0.5; ethyl acetate:MeOH:N-H<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ 3.84 (dd, J = 12.8,

2.1 Hz, 1H, H-6a), 3.76 (dd, J = 12.8, 2.9 Hz, 1H, H-6b), 3.47 (td, J = 10.2, 4.9 Hz, 1H, H-2), 3.29 (t, J = 9.5 Hz, 1H, H-3), 3.18 (t, J = 9.2 Hz, 1H, H-4), 2.94 (dd, J = 11.4, 4.9 Hz, 1H, H-1a), 2.89–2.77 (m, 1H, H-7a), 2.73–2.63 (m, 1H, H-7b), 2.56–2.45 (m, 2H, H-8), 2.33–2.14 (m, 8H, H-5, H-1b, 2×CH<sub>3</sub>). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O):  $\delta$  78.30, 69.95, 68.76, 65.22, 57.54, 55.92, 53.30, 48.79, 44.18. HRMS (ESI) m/z calcd for  $C_{10}H_{23}N_2O_4^+$  (M+H)<sup>+</sup> 235.16523, found 235.16492.

### (2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)-1-(2-(pyrrolidin-1-yl)ethyl)piperidine-3,4,5-triol (7b)

Prepared according to procedure E. Compound **12b** (160 mg, 0.44 mmol), 10% Pd/C (80 mg), EtOH (5 mL), pH~1 with 1 M aq HCl. Yield: 95% (64 mg), yellow syrup,  $R_f$  = 0.44 (1:1:0.5; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O):  $\delta$  3.86 (dd, J = 12.7, 1.9 Hz, 1H, H-6a), 3.79 (dd, J = 12.8, 2.8 Hz, 1H, H-6b), 3.49 (td, J = 10.3, 4.9 Hz, 1H, H-2), 3.31 (t, J = 9.5 Hz, 1H, H-3), 3.21 (t, J = 9.2 Hz, 1H, H-4), 2.98 (dd, J = 11.4, 4.9 Hz, 1H, H-1a), 2.89 (m, 1H, H-7a), 2.81–2.68 (m, 3H, H-7b, H-8), 2.62 (m, 4H, H-9, H-12), 2.30 (t, J = 11.1 Hz, 1H, H-1b), 2.24 (d, J = 9.7 Hz, 1H, H-5), 1.74 (m, 4H, H-10, H-11). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O):  $\delta$  78.31, 69.97, 68.79, 65.17, 57.55, 55.92, 53.62, 50.34, 49.49, 22.73. HRMS (ESI) m/z calcd for  $C_{12}$ H- $_{25}$ N<sub>2</sub>O<sub>4</sub>+ (M+H)+ 261.18088, found 261.18057.

### (2R,3R,4R,5S)-2-(Hydroxymethyl)-1-(2-(piperidin-1-yl)ethyl)piperidine-3,4,5-triol (7c)

Prepared according to procedure E. Compound **12c** (260 mg, 0.41 mmol), 10% Pd/C (100 mg), EtOH (10 mL), pH~1 with 1 M aq HCl. Yield: 92% (123 mg), yellow syrup,  $R_f$  = 0.52 (1:1:0.5; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ 3.82 (dd, J = 12.7, 2.2 Hz, 1H, H-6a), 3.75 (dd, J = 12.8, 2.9 Hz, 1H, H-6b), 3.45 (td, J = 10.3, 4.9 Hz, 1H, H-2), 3.26 (t, J = 9.4 Hz, 1H, H-3), 3.17 (t, J = 9.2 Hz, 1H, H-4), 2.94 (dd, J = 11.2, 5.3 Hz, 2H, H-1a, H-7a), 2.71 (m, 7H, H-7b, H-8, H-9, H-13), 2.24 (dd, J = 21.8, 10.5 Hz, 2H, H-5, H-1a), 1.65–1.48 (m, 4H, H-10, H-12), 1.43 (m, 2H, H-11). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O): δ 78.25, 69.92, 68.71, 65.08, 57.45, 55.81, 54.06, 53.38, 46.94, 24.00, 22.41. HRMS (ESI) m/z calcd for  $C_{13}H_{27}N_2O_4^+$  (M+H)<sup>+</sup> 275.19653, found 275.19635.

### (2R,3R,4R,5S)-2-(Hydroxymethyl)-1-(2-morpholino-ethyl)piperidine-3,4,5-triol (7d)

Prepared according to procedure E. Compound **12d** (270 mg, 0.42 mmol), 10% Pd/C (100 mg), EtOH (10 mL), pH~1 with 1 M aq HCl. Yield: 94% (110 mg), yellow solid,  $R_f$  = 0.68 (1:1:0.5; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ 3.81 (dd, J = 12.9, 1.8 Hz, 1H, H-6a), 3.74 (dd, J = 12.9, 2.7 Hz, 1H, H-6b), 3.65 (m, 4H, H-10, H-11), 3.45 (td, J = 10.1, 4.9 Hz, 1H, H-2), 3.26 (t, J = 9.5 Hz, 1H, H-3), 3.16 (t, J = 9.2 Hz, 1H, H-4), 2.93 (dd, J = 11.4, 4.9 Hz, 1H, H-1a), 2.88–2.80 (m, 1H, H-7a), 2.76–2.64 (m, 1H, H-7b), 2.59–2.41 (m, 6H, H-8, H-9, H-12),

2.27 (t, J = 11.1 Hz, 1H, H-1b), 2.20 (d, J = 9.7 Hz, 1H, H-5).  $^{13}$ C NMR (126 MHz, D<sub>2</sub>O):  $\delta$  78.25, 69.89, 68.70, 66.06, 65.11, 57.47, 55.87, 52.88, 47.71. HRMS (ESI) m/z calcd for  $C_{12}H_{25}N_2O_5^+$  (M+H) $^+$  277.17580, found 277.17614.

### (2R,3R,4R,5S)-1-(4-(Dimethylamino)butyl)-2-(hydroxymethyl)piperidine-3,4,5-triol (8a)

Prepared according to procedure E. Compound **13a** (200 mg, 0.32 mmol), 10% Pd/C (100 mg), EtOH (10 mL), pH~1 with 1 M aq HCl. Yield: 93% (78 mg), white solid,  $R_f$  = 0.67 (1:1:0.5; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O):  $\delta$  3.79 (d, J = 12.6 Hz, 1H, H-6a), 3.71 (d, J = 12.4 Hz, 1H, H-6b), 3.49–3.38 (m, 1H, H-2), 3.25 (t, J = 9.4 Hz, 1H, H-3), 3.14 (t, J = 9.2 Hz, 1H, H-4), 2.90 (dd, J = 11.1, 4.2 Hz, 1H, H-1a), 2.72–2.59 (m, 1H, H-7a), 2.53 (m, 1H, H-7b), 2.30 (m, 2H, H-10), 2.25–2.04 (m, 8H, H-5, H-1a, 2×CH<sub>3</sub>), 1.36 (m, 4H, H-8, H-9). <sup>13</sup>C NMR (126 MHz, D2O):  $\delta$  78.34, 70.07, 68.88, 64.96, 58.20, 57.52, 55.27, 51.81, 43.63, 24.18, 20.82. HRMS (ESI) m/z calcd for  $C_{12}H_{27}N_2O_4^+$  (M+H)<sup>+</sup> 263.19653, found 263.19635.

### (2R,3R,4R,5S)-2-(Hydroxymethyl)-1-(4-(pyrrolidin-1-yl)butyl)piperidine-3,4,5-triol (8b)

Prepared according to procedure E. Compound **13b** (220 mg, 0.34 mmol), 10% Pd/C (100 mg), EtOH (10 mL), pH~1 with 1 M aq HCl. Yield: 90% (88 mg), colorless syrup,  $R_f = 0.37$  (1:1:0.5; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O): δ 3.80 (dd, J = 12.7, 1.9 Hz, 1H, H-6a), 3.72 (dd, J = 12.8, 2.6 Hz, 1H, H-6b), 3.48–3.37 (m, 1H, H-2), 3.26 (t, J = 9.5 Hz, 1H, H-3), 3.15 (t, J = 9.2 Hz, 1H, H-4), 2.91 (dd, J = 11.4, 4.9 Hz, 1H, H-1a), 2.65 (m, 1H, H-H-7a), 2.48 (m, 7H, H-7b, H-10, H-11, H-14), 2.25–2.08 (m, 2H, H-5, H-1b), 1.66 (m, 4H, H-12, H-13), 1.39 (m, 4H, H-8, H-9). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O): δ 78.37, 70.10, 68.90, 65.00, 57.59, 55.31, 55.25, 53.19, 51.86, 25.67, 22.70, 21.09. HRMS (ESI) m/z calcd for  $C_{14}$ H- $_{29}$ N<sub>2</sub>O<sub>4</sub>+ (M+H)+ 289.21218, found 289.21194.

### (2R,3R,4R,5S)-2-(Hydroxymethyl)-1-(4-(piperidin-1-yl)butyl)piperidine-3,4,5-triol (8c)

Prepared according to procedure E. Compound **13c** (260 mg, 0.39 mmol), 10% Pd/C (100 mg), EtOH (10 mL), pH~1 with 1 M aq HCl. Yield: 95% (113 mg), yellow syrup,  $R_f$  = 0.44 (1:1:0.5; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O):  $\delta$  3.78 (d, J = 11.9 Hz, 1H, H-6a), 3.71 (dd, J = 12.7, 2.4 Hz, 1H H-6b), 3.43 (td, J = 10.0, 5.0 Hz, 1H, H-2), 3.25 (t, J = 9.5 Hz, 1H, H-3), 3.14 (t, J = 9.2 Hz, 1H, H-4), 2.89 (dd, J = 11.4, 4.9 Hz, 1H, H-1a), 2.84–2.46 (m, 8H, H-7, H-10, H-11, H-15), 2.26–2.09 (m, 2H, H-5, H-1b), 1.58 (m, 4H, H-12, H-14), 1.53–1.31 (m, 6H, H-8, H-9, H-13). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O):  $\delta$  78.33, 70.04, 68.86, 65.01, 57.58, 57.31, 55.29, 53.29, 51.54, 23.74, 22.45, 22.30, 20.75. HRMS (ESI) m/z calcd for  $C_{15}H_{31}N_2O_4^+$  (M+H)<sup>+</sup> 303.22783, found 303.22754.

### (2R,3R,4R,5S)-2-(Hydroxymethyl)-1-(4-morpholinobutyl)piperidine-3,4,5-triol (8d)

Prepared according to procedure E. Compound **13d** (270 mg, 0.34 mmol), 10% Pd/C (100 mg), EtOH (10 mL), pH~1 with 1 M aq HCl. Yield: 96% (119 mg), pale yellow solid,  $R_f$  = 0.59 (1:1:0.5; ethyl acetate:MeOH:NH<sub>4</sub>OH). <sup>1</sup>H NMR (500 MHz, D<sub>2</sub>O):  $\delta$  3.73 (m, 6H, H-6, H-12, H-13), 3.41 (td, J = 9.7, 4.6 Hz, 1H, H-2), 3.24 (t, J = 9.5 Hz, 1H, H-3), 3.12 (t, J = 9.2 Hz, 1H, H-4), 2.89 (dd, J = 11.4, 4.8 Hz, 1H, H-1a), 2.73–2.40 (m, 8H, H-7, H-10, H-11, H-14), 2.25–2.08 (m, 1H, H-5, H-1b), 1.40 (m, 4H, H-8, H-9). <sup>13</sup>C NMR (126 MHz, D<sub>2</sub>O):  $\delta$  78.25, 69.93, 68.77, 65.50, 64.97, 57.58, 57.41, 55.20, 52.21, 51.64, 22.60, 20.75. HRMS (ESI) m/z calcd for  $C_{14}H_{29}N_2O_5^+$  (M+H)<sup>+</sup> 305.20710, found 305.20676.

#### 2. 2. Glucosidase Inhibitory Assays

α-Glucosidase (yeast), β-glucosidase (sweet almonds), and α-mannosidase (jack bean) was purchased from Sigma. 1-Deoxynojirimycin, para-nitrophenyl α-D-glucopyranoside, para-nitrophenyl β-D-glucopyranoside and para-nitrophenyl α-D-mannosidase were also purchased from Sigma. Inhibitory potencies were carried out by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases on the corresponding para-nitrophenyl glycoside substrates. The α-glucosidase,<sup>26</sup> β-glucosidase assays<sup>27</sup> were performed in 50 mM phosphate buffer, pH 6.8 at 37 °C. The α-mannosidase assay<sup>28</sup> was performed in 50 mM citrate buffer, pH 5.5 at 37 °C. The test compounds were pre-incubated with the enzyme solutions and buffered in a disposable 96-well microtiter plate at 37 °C for 15 min. Next, the reactions were initiated by the addition of 20  $\mu$ L of a solution of the corresponding para-nitrophenyl glycoside substrates. After the reaction mixture was incubated at 37 °C for 15 min. Thereupon, it was quenched by adding 80 μL Na<sub>2</sub>CO<sub>3</sub> (0.2 mol/L). Enzymatic activity was quantified by measuring the absorbance at 405 nm using a BioTek µQuant Microplate Spectrophotometer. Each experiment was performed in triplicate. IC<sub>50</sub> values were determined graphically with GraphPad Prism (version 8.0).

#### 2. 3. Kinetics of Enzyme Inhibition

Inhibition constant ( $K_i$ ) measurement was performed in 50 mM phosphate buffer (pH 6.8) at 37 °C, using *para*-nitrophenyl  $\beta$ -D-glucopyranoside as the substrate. The assay was initiated by adding  $\beta$ -glucosidase ( $K_m$  = 3.5 mM) to a solution of the substrate (concentrations used: 0.875 mM, 1.75 mM, 3.5 mM, 7 mM, 10.5 mM) in the presence of inhibitors (concentrations used: 0 mM, 0.1 mM, 0.2 mM). After the reaction mixture was incubated at 37 °C for 15 min, it was quenched by adding 80  $\mu$ L Na<sub>2</sub>CO<sub>3</sub> (0.2 mol/L). The absorbance of 4-nitrophenol released from the substrate was read at 405 nm.

### 2. 4. Cell Culture and Inhibition of Proliferation B16F10 Cells<sup>29</sup>

The mouse B16F10 melanoma cell line, which is derived from C57BL/6 mice was purchased from KeyGen Biotech (Nanjing, China). The cell line was cultured in DMEM supplemented with fetal bovine serum (10%), penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37 °C in humidified 5% CO<sub>2</sub> atmosphere. Media was replenished every third day. B16F10 cells were seeded on 96well microtiter plates in DMEM supplemented with 10% FBS and incubated overnight. The compounds (1 mM, 0.05 mM) were then added to the cells and cultured for another 48 h. Each treatment was performed in six well replicates. MTT reagent (Sigma Aldrich) was added to each well incubated for 4 h at 37 °C. After the cell culture medium was removed, formazan crystals in adherent cells were dissolved in 200 µL DMSO and the absorbance of the formazan solution was measured at 570 nm.

#### 3. Results and Discussion

#### 3. 1. Chemistry

The target compounds were prepared from the key intermediate 11 through reductive amination or double nucleophilic substitution, respectively (Figure 2). The synthesis of compound 11 commenced from 2,3,4,6-tet-

ra-O-benzyl-1-deoxynojirimycin **9** which was prepared according to previously published procedures in four steps. <sup>28</sup> Treatment of O-benzyl protected DNJ **9** with N-(4-bromobutyl)phthalimide or N-(4-bromoethyl) phthalimide in the presence of  $K_2CO_3$  in DMF afforded N-phthalyl protected DNJ **10** (Scheme 1). The intermediate **10** was then converted into primary amide **11** by a hydrazinolysis reaction using  $N_2H_4$  in EtOH.

A generalized synthetic approach to the derivatives 7 and 8 was shown in Scheme 2. The reductive amination of 11 with HCHO-HCOOH gave compounds 12a and 13a. For compounds 12 and 13 which beared 5- and 6-membered rings, double nucleophilic substitution reaction was performed on primary amine 11 in basic conditions. All the intermediates 12 and 13 were obtained in good (80%) to excellent (90%) yields, independently of the chain length. Precursors 12 and 13 were then deprotected by hydrogenolysis (10% Pd/C, EtOH, 1 M HCl) to afford the target derivatives 7 and 8 in almost quantitative yield.

#### 3. 2. Biological Evaluation

The small library of DNJ derivatives were submitted to a panel of biological evaluations, which included inhibition of glycosidases, inhibition kinetics of  $\beta$ -glucosidase, as well as inhibition of B10F16 cells growth. These experiments are summarized below.

 $\textbf{Figure 2.} \ \ \textbf{The key intermediate 11} \ \ \textbf{and the general structures of the target compounds}.$ 

Scheme 1. Synthesis of the primary amide 11. Reagents and conditions: (a) N-(4-bromobutyl) phthalimide or N-(4-bromoethyl) phthalimide,  $K_2CO_3$ , DMF, 100 °C, 24 h, 87% (10a), 85% (10b); (b)  $N_2H_4$  (80%), EtOH, reflux, 3 h, 82% (11a), 85% (11b).

Scheme 2. Synthesis of N-alkylated derivatives of 1-deoxynojirimycin. Reagents and conditions: (a) HCHO, HCOOH, 105 °C, 3 h, 83% (12a), 90% (13a); (b) alkyl dibromide,  $K_2CO_3$ ,  $CH_3CN$ , 80 °C, 12 h, 84% (12b), 81% (13b), 80% (12c), 82% (13c), 85% (12d), 87% (13d); (c)  $H_2$ , 10% Pd/C, EtOH, 1 M HCl, rt, 24 h, 91% (7a), 93% (8a), 95% (7b), 90% (8b), 92% (7c), 95% (8c), 94% (7d), 96% (8d).

#### 3. 2. 1. Inhibition of Glucosidases

Glycosidase inhibitory activities of compounds 7 and 8 was evaluated against  $\alpha$ -glucosidase (yeast),  $\beta$ -glucosidase (almonds),  $\alpha$ -mannosidase (jack bean), with reference to the known standard DNJ. The results were expressed as the inhibition of glucosidase activity (IC<sub>50</sub>) and are summarized in Table 1.

Compounds **7a**, **7b** and **7c** had weak inhibitory activities against  $\alpha$ - and  $\beta$ -glucosidase at 1 mM. It was, however, interesting to note that compound **7d** bearing a morpholine ring was the only derivative in our library exhibiting higher and selective activity of  $\beta$ -glucosidase with an IC<sub>50</sub> of 0.052  $\pm$  0.004 mM compared to DNJ (IC<sub>50</sub> = 0.65  $\pm$  0.04 mM), while none of the other glycosidases

were inhibited by this compound (Table 1). This indicated that a much more favorable interaction with the  $\beta$ -glucosidase active site.

Compound **8a** also had weak inhibitory activity against  $\alpha$ - and  $\beta$ -glucosidase. Derivatives **8b** and **8c** which possessed a longer alkyl chain were found to be more selective inhibitors of  $\alpha$ -glucosidase than **7b** and **7c**, with IC<sub>50</sub> values of  $0.364 \pm 0.011$  mM and  $0.358 \pm 0.04$  mM, respectively. And they had similar potencies to  $\alpha$ -glucosidase. Compound **8d**, which beared a morpholine ring, showed decreased inhibitory activity against  $\alpha$ -glucosidase with an IC<sub>50</sub> of  $1.385 \pm 0.137$  mM compared to **8b** and **8c**. However, **8d** showed better inhibitory effect on  $\alpha$ -glucosidase than **7d** which possessed a shorter alkyl chain (Table 1). Moreover, compounds **8a**, **8b**, **8c** and **8d** have reduced

Table 1. Glycosidase inhibitory activity values  $IC_{50} \, (mM)$ 

Enzyme	7a	7 <b>b</b>	7c	7 <b>d</b>	8a	8b	8c	8d	DNJ
α-glucosidase (yeast)	10%ª	24%	29%	33%	49%	$0.364 \pm 0.011$	$0.358 \pm 0.04^{b}$	$1.385 \pm 0.137$	$0.155 \pm 0.015$
β-glucosidase (almonds)	22%	34%	33%	$0.052 \pm 0.004$	17%	40%	18%	29%	$0.648 \pm 0.036$
α-mannosidase (jack bean)	$NI^c$	NI	NI	NI	NI	NI	NI	NI	NI

<sup>&</sup>lt;sup>a</sup> The inhibition rate (%) was obtained from the 1 mM of compounds. <sup>b</sup>  $IC_{50}$  is defined as the compound concentration at which 50% activities of glucosidases. The values are mean±SD from three independent experiments. <sup>c</sup> NI indicated no inhibition at 1 mM of compounds.

inhibitory activity compared to DNJ. These results suggested that **8b** and **8c** which beared 5- and 6-membered rings would be beneficial for the interaction with  $\alpha$ -glucosidase through the hydrophobic effect more than *N*-dimethyl DNJ derivative **8a**. Ocmpounds having n=3 displayed better inhibition towards  $\alpha$ -glucosidase than compounds having n=1, with a trend correlating higher inhibition associated with increased chain length. In addition, introduction of a nitrogen atom seemed to display no or negligible inhibition against all the enzymes. Finally, none of these derivatives showed inactivation of jack bean  $\alpha$ -mannosidase.

#### 3. 2. 2. Inhibition Kinetics of β-Glucosidase

In order to explore further insight into how 7d interacted with  $\beta$ -glucosidase (almonds), the mode of inhibition and inhibition constant of 7d was determined by the Lineweaver–Burk plots (Figure 3). The double reciprocal plots of 7d showed straight lines with the same  $v_{max}$ . This indicated that 7d ( $K_i = 7 \mu M$ ) inhibited  $\beta$ -glucosidase in a competitive manner, a nearly 7-fold increase compared to DNJ<sup>26</sup> ( $K_i = 47 \mu M$ ). Hence, this competitive inhibition indicated that 7d was expected to bind to the active site of  $\beta$ -glucosidase and compete with their primary substrates. Moreover, a probable hydrogen bond acceptor was the carbonyl hydrogen atom of the catalytic acid.<sup>27</sup>

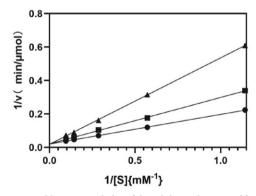
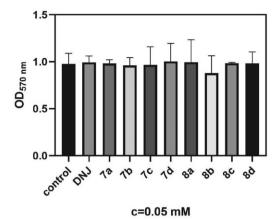
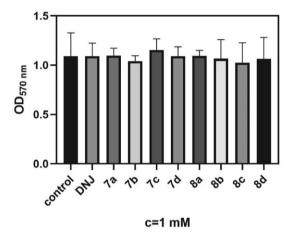


Figure 3. Double-reciprocal plot of the inhibition kinetics of β-glucosidase (almonds) by compound 7d. Substrate concentration: 0.875, 1.75, 3.5, 7, 10.5 mM, inhibitor concentration: 0 mM (control,  $\nabla$ ), 0.1 mM ( $\spadesuit$ ), 0.2 mM ( $\spadesuit$ ).

#### 3. 3. Inhibition of B16F10 Cells Growth

The inhibition of B16F10 cells growth by compounds was determined using the MTT assay and the results are summarized in Figure 4. All compounds were inactive with no significant inhibition being observed at 0.05 mM and 1 mM. This indicated that compounds by the modification of changing length of the tether, the size and nature of the terminal tertiary amine substituents had no influence on the anticancer activity.





**Figure 4.** Effect of glycosidase inhibitors on B16F10 cells growth. Each bar represents the mean ( $\pm$ SD, n = 6). P > 0.05 comparing with control

#### 4. Conclusion

In summary, a series of DNJ derivatives were designed and synthesized, and the structures of synthesized compounds were confirmed by <sup>1</sup>H NMR, <sup>13</sup>C NMR and HRMS. Moreover, the preliminary glucosidase inhibition and anticancer activities were evaluated in vitro. Compound 7d proved to be the most potent and selective β-glucosidase inhibitor in a competitive manner, and none of the other glycosidases were inhibited by this compound at micromolar level. Compounds 8b and 8c were moderate and selective α-glucosidase inhibitors. Nevertheless, all compounds could not inhibit the growth of B16F10 melanoma cells. The collective results indicated that a lengthening of the alkyl chain linking DNJ provide better selectivity towards  $\alpha$ -glucosidase. The size of the hydrophobic group at the alkyl chain, especially its nature, differs greatly for the selective inhibition aganist  $\alpha$ -and  $\beta$ -glucosidases. Compounds 7d, 8b and 8c would be a lead for designing novel compounds, and further derivatives would be prepared by altering these specific molecules. In addition, our results provides useful clues for the design of selective glucosidase inhibitors.

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#### Povzetek

Pripravili smo serijo N-alkiliranih deoksinojirimicinskih (DNJ) derivatov, povezanih s terminalno terciarno aminsko skupino na alkilni verigi različnih dolžin. Te nove sintezne spojine smo preliminarno *in vitro* analizirali za glukozidazno inhibicijo in antirakavo aktivnost. V nekaterih primerih smo opazili močno in selektivno inhibicijo. Spojina 7d (IC $_{50}$  = 0.052 mM) je, v primerjavi z DNJ (IC $_{50}$  = 0.65 mM), pokazala izboljšano in selektivno inhibitorno aktivnost proti  $\beta$ -glukozidazi. Dodatne analize kinetike encimske inhibicije s pomočjo Lineweaver–Burkovih diagramov so pokazale, da 7d inhibira  $\beta$ -glukozidazo na kompetitiven način, kar nakazuje, da se 7d verjetno veže v aktivno mesto  $\beta$ -glukozidaze. Spojini 8b and 8c sta pokazali zmerno, a vendar selektivno, inhibicijo  $\alpha$ -glukozidaze. Ne glede na to, pa nobena od spojin ni inhibirala rasti B16F10 melanomskih celic.



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