8

Synthesis and Biological Evaluation of Exo-N-Cyclic Deoxynojirimycin and Derivatives

8.1 Introduction

The naturally occurring iminosugar 1-deoxynojirimycin 1 and derivatives have attracted considerable attention due to their therapeutic potential and their ability to inhibit glycan-processing enzymes.1-5 An important synthetic analogue comprises N-butyl-1-deoxynojirimycin 2, which is used as a drug for the lysosomal storage disorder Gaucher disease under the trade name Zavesca.6,7 Gaucher disease is an inherited lysosomal storage disorder that is characterized by excessive storage of the endogenous substrate glucosylceramide in tissue macrophages.8,9 Glucosylceramide is a member of the glycosphingolipid family and is the crucial precursor of complex glycosphingolipids, which are involved in many (patho)physiological processes.
Excessive glucosylceramide accumulation is caused by the impaired catalytic activity of glucocerebrosidase (GBA), a lysosomal retaining β-glucosidase that catalyzes the degradation of glucosylceramide into glucose and ceramide. Treatment with N-butyl-1-deoxynojirimycin 2, also referred to as substrate reduction therapy (SRT), inhibits glucosylceramide synthase (GCS), an enzyme that is responsible for the biosynthesis of glucosylceramide. As a result, the biosynthesis of glucosylceramide is slowed down to such an extent that it is in balance with the reduced catabolic capacity of GBA.10-12

Another intriguing 1-deoxynojirimycin derivative is the lipophilic adamantan-1-yl-methoxy-pentyl 1-deoxynojirimycin 3 (AMP-DNM, MZ21) due to its dual inhibitory ability.13 AMP-DNM 3 targets not only GCS, but also inhibits GBA and GBA2 (non-lysosomal β-glucosidase) in the nanomolar range.14 GBA2 is an alternative pathway in man to process glucosylceramide and recently, this enzyme has been linked to a neurological disorder (Cerebellar Ataxia).15-17

Gravier-Pelletier and coworkers reported the synthesis of exo-N-cyclic deoxynojirimycin 4 and some derivatives as potential β-glucosidase inhibitors starting from a D-mannitol-derived bis-epoxide.18 A follow-up study confirmed the inhibitory activity of 4 on almond β-glucosidase, although the compound turned out to be less potent than the parent compound 1.19 In the same vein, it was envisioned that 4 could serve as a viable inhibitor for GCS, GBA and GBA2.

**Figure 1.** Structures of parent-and target compounds: 1-deoxynojirimycin 1, Zavesca 2, AMP-DNM 3 and exo-N-cyclic-1-deoxynojirimycin 4 and derivatives 5-8.

This Chapter presents a practical synthesis route towards exo-N-cyclic deoxynojirimycin 4 and the mono-and di-alkylated derivatives 5-8 of Zavesca 2 and AMP-DNM 3. These compounds were screened for their inhibitory potency against GCS, GBA and GBA2. In addition, the compounds were tested for inhibitory activity...
Exo-N-cyclic-deoxynojirimycin

against the cytosolic β-glucosidase GBA3 that is responsible for the hydrolysis of xenobiotic β-glucosides.

8.2 Results and Discussion

The synthesis of exo-N-cyclic deoxynojirimycin 4 was based on the known cyclophellitol precursor 9\textsuperscript{20} (see also Chapter 3) as shown in Scheme 1. Treatment of 9 with benzyl bromide and sodium hydride in DMF afforded the fully protected cyclophellitol 10 in quantitative yield. Ensuing reductive opening (LiAlH\textsubscript{4}, THF, 0 °C) of the epoxide functionality in 10 gave alcohol 11 (76%). The free hydroxyl in 11 was transformed into the corresponding mesylate 12 (MsCl, DMAP, pyridine, 0 °C) in a good yield (92%). Nucleophilic substitution of the mesylate group in 12 with sodium azide in DMF gave 13 (66%). Finally, Pd-catalyzed hydrogenation provided exo-N-deoxynojirimycin 4 in 53% yield.


Reagents and conditions: (a) BnBr, NaH, DMF, 0 °C to rt, quant.; (b) LiAlH\textsubscript{4}, THF, 0 °C to rt, 76%; (c) MsCl, DMAP, pyridine, 0 °C to rt, 92%; (d) NaN\textsubscript{3}, DMF, 120 °C, 66%; (e) 10% Pd/C, H\textsubscript{2}, 6 M HCl, MeOH, 53%.

The mono-and di-exo-N-alkylated analogues 5-8 of N-butyl-1-deoxynojirimycin 2 and AMP-DNM 3 were constructed following the strategy as outlined in Scheme 2. The common intermediate for these compounds is amine 18, which was obtained after subjecting azide 13 to Staudinger reduction (PMe\textsubscript{3}, pyridine, water). Amine 18 was used as crude, without any further purification in the ensuing reactions. For the formation of the monoalkylated 5 and 6, crude amine 18 was protected with a Boc group (Boc\textsubscript{2}O, Et\textsubscript{3}N, DCM) giving 19 in 91% over two steps. Consecutive alkylation (propylbromide, NaH, DMF, 70 °C) and Pd-catalyzed hydrogenation under acidic conditions gave exo-N-butylated Zavesca analogue 5. A similar strategy was followed for the generation of AMP-DNM derivative 6 by alkylation of 19 with bromide 16. Bromide 16 was prepared by consecutive substitution of the tosylate 14\textsuperscript{21} with 1-
adamantanemethanol, removal of trityl group with $p$TsOH and bromination under Appel conditions (CBr₄, PPh₃, ACN, 80 °C). Ensuing removal of the Boc and benzyl protective groups by hydrogenation in the presence of 6 M HCl yielded 6 (7%).

Reductive amination of amine 18 with a given aldehyde was the general approach to synthesize di-alkylated exo-N-derivatives 7 and 8. Treatment of 18 with propanal with sodium cyanoborohydride and acetic acid was followed by BCl₃-mediated deprotection to furnish 7. Reductive amination with aldehyde 17 (Dess-Martin oxidation of alcohol 16) generated a mixture of mono-and di-alkylated protected exo-N-DNM-AMP derivatives. After isolation of 20, the benzyl ether groups were removed under Pd/C-catalyzed hydrogenolysis conditions affording 8 in a moderate yield (51%).


Reagents and conditions: (a) i. 1-adamantanemethanol, NaH, DMF, 75 °C, ii. $p$TsOH, DCM, MeOH (1/1; v/v), 49%; (b) CBr₄, PPh₃, acetonitrile, 80 °C, 59%; (c) Dess-Martin periodane, DCM, quant.; (d) PMe₃, pyridine, H₂O (9/1; v/v), quant.; (e) Boc₂O, Et₃N, DCM, 91%; (f) i. propylbromide or 16, NaH, DMF, 70 °C; ii. 10% Pd/C, 6 M HCl, MeOH, 5:16%, 6: 7%; (g) i. propanal, NaBH₃CN, 4Å molecular sieves, AcOH, THF, ii. BCl₃, -78 °C to -20 °C, 40%; (h) 17, NaBH₃CN, 4Å molecular sieves, AcOH, THF, 24%; (i) 10% Pd/C, H₂, 37% HCl, EtOH, 51%.
The inhibitory potential of exo-\(N\)-cyclic deoxynojirimycin 4 and derivatives 5-8 were assessed by testing the compounds in assays for the three enzymes involved in glucosylceramide metabolism: GCS, GBA and GBA2. The compounds were also tested on GBA3, which is involved in the hydrolysis of xenobiotic \(\beta\)-glucosides. These results are summarized together with the parent compounds 1-3 in Table 1.

### Table 1. Apparent IC\(_{50}\) values of 1-8.

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<th>Compound</th>
<th>GCS ((\mu)M)</th>
<th>GBA ((\mu)M)</th>
<th>GBA2 ((\mu)M)</th>
<th>GBA3 ((\mu)M)</th>
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<td>340</td>
<td>20</td>
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<td>50</td>
<td>600</td>
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<td>0.002</td>
<td>50</td>
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<tr>
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The apparent IC\(_{50}\) values of the newly synthesized compound 4-8 showed that they were all inactive towards GCS, GBA, GBA2 and GBA3. These results implicate that the exo-\(N\)-deoxynojirimycin core is not a suitable design scaffold for GBA3 and the enzymes that are involved in the glucosylceramide metabolism. This is rather surprising as numerous related aminocyclitols were confirmed as GBA inhibitors.\(^{22,23}\) Further explorations are needed to explain these results, which will be elaborated in Chapter 9.

### 8.3 Conclusion

This Chapter describes a straightforward synthesis route towards exo-\(N\)-deoxynojirimycin 4, mono- and dialkylated derivatives 5-8 of Zavesca 2 and AMP-DNM 3 as potential GCS, GBA, GBA2 and GBA3 inhibitors, starting from the partial benzyl protected cyclophellitol 9. Determination of their IC\(_{50}\) values and comparison with the parent compounds proved that 5-8 were inactive towards the carbohydrate processing enzymes.
Experimental section

General methods: All reagents and solvents were of a commercial grade and used as received unless stated otherwise. THF and dichloromethane were stored over flamed-dried 3Å molecular sieves. All reactions were performed under an inert atmosphere unless stated otherwise. Solvents used for flash chromatography were of pro analysis quality. Reactions were monitored by TLC analysis using aluminium sheets pre-coated with silica gel 60 with detection by UV-absorption (254 nm) and by spraying with a solution of (NH₄)₆Mo₇O₂₄·H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·H₂O (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C or by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C. Column chromatography was performed using Screening Device silica gel in the indicated solvents. ¹H NMR, ¹³C NMR, COSY and HSQC spectra were recorded on a Bruker AV-400 (400/100 MHz) and Bruker AV-600 (600/150 MHz) spectrometer in the given solvent. Chemical shifts are reported as δ-values in ppm relative to the chloroform residual solvent peak or tetramethylsilane (TMS) as internal standard or the signal for deuterated solvent. Coupling constants are given in Hz. All given ¹³C spectra are proton decoupled. High resolution mass spectra were recorded with a LTQ Orbitrap (Thermo Finnigan). HPLC-MS purifications were performed on an Agilent Technologies 1200 series automated HPLC system with a Quadrupole MS 6130, equipped with a semi-preparative Gemini C18 column (Phenomenex, 250x10, 5μm particle size).

2,3,4,8-tetra-O-benzyl-cyclophellitol 10

Sodium hydride (60% dispersion in mineral oil, 450 mg, 11.2 mmol) was added in portions to a dry and cooled (0 °C) solution of 2,3-di-O-benzyl-cyclophellitol 9 (see Chapter 3, 713 mg, 2.0 mmol) and benzyl bromide (0.54 mL, 4.4 mmol) in DMF (10 mL). The reaction mixture was stirred at ambient temperature for 18 h before being quenched with MeOH at 0 °C and poured into Et₂O. The organic layer was washed with H₂O, dried over MgSO₄, filtered and concentrated in vacuo. Purification by silica column chromatography (petroleum ether/EtOAc, 94:6→92:8) afforded 10 (1.08 g, 2.0 mmol, quant.) as a light yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 7.29-7.22 (m, 18H, H ArBn), 7.20-7.10 (m, 2H, H ArBn), 4.86-4.81 (m, 3H, CH₂Bn), 4.78 (d, J = 11.2 Hz, 1H, CH₂Bn), 4.71 (d, J = 11.2 Hz, 1H, CH₂Bn), 4.52 (dd, J = 6.8, 12.4 Hz, 2H, CH₂Bn), 4.39 (d, J = 11.2 Hz, 1H, CH₂Bn), 3.86 (d, J = 8.0 Hz, 1H, H-6), 3.75 (dd, J = 4.4, 8.8 Hz, 1H, H-8), 3.59-3.53 (m, 2H, H-8, H-3), 3.47 (d, J = 3.6 Hz, 1H, H-6), 3.26 (t, J = 10.0 Hz, 1H, H-4), 3.19 (d, J = 3.6 Hz, 1H, H-1), 2.33-2.27 (m, 1H, H-5). ¹³C NMR (100 MHz, CDCl₃): δ 138.6, 138.2, 138.1, 137.7, 138.5, 128.3, 128.3, 128.3, 128.0, 127.9, 127.9, 127.7 127.6, 127.6, 127.5, 127.5, 83.9, 79.8, 75.3, 75.3, 75.1, 73.1, 73.0, 68.6, 55.5, 53.8, 42.5. HRMS: found 559.2449 [M+Na]⁺, calculated for [C₃₅H₃₆O₅Na] 559.2455.

(15S,25S,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)cyclohexan-1-ol 11. Epoxide 10 (1.08 g, 2.0 mmol) was co-evaporated thrice with toluene before being dissolved in THF (20 mL) and cooled down to 0 °C. After the portionwise addition of lithium aluminium hydride (906 mg, 24 mmol), the solution was stirred first for 30 min at 0 °C and then for 18 h at ambient temperature. The mixture was cooled down to 0 °C and carefully quenched with EtOAc and 1 M HCl. The layers were separated and the
organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/Et₂O, 60:40+56:44) furnished alcohol 11 (822 mg, 1.52 mmol, 76%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ 7.29-7.18 (m, 20H, H ArBn), 5.00 (d, J = 10.8 Hz, 1H, CH₂Bn), 4.94 (d, J = 10.8 Hz, 1H, CH₂Bn), 4.86 (d, J = 11.2 Hz, 1H, CH₂Bn), 4.68 (dd, J = 11.2, 14.4 Hz, 2H, CH₂Bn), 4.52 (d, J = 10.8 Hz, 1H, CH₂Bn), 4.48 (d, J = 12.0 Hz, 1H, CH₂Bn), 4.41 (d, J = 11.6 Hz, 1H, CH₂Bn), 4.26 (d, J = 2.0 Hz, 1H, H-1), 4.05-3.98 (m, 3H, H-3, H-5, H-7), 3.71 (dd, J = 2.4, 9.2 Hz, 1H, H-7), 3.54 (t, J = 9.2 Hz, 1H, H-4), 2.31 (dt, J = 4.0, 13.2 Hz, 1H, H-2), 1.68 (dd, J = 2.0, 11.2 Hz, 1H, H-6), 1.42 (dt, J = 1.6, 13.2 Hz, 1H, H-2). ¹³C NMR (100 MHz, CDCl₃): δ 139.0, 138.7, 138.6, 137.2, 128.5, 128.3, 128.3, 128.3, 128.0, 127.9, 127.7, 127.7, 127.6, 127.4, 127.3, 88.5, 78.1, 76.7, 75.5, 75.5, 73.6, 72.7, 70.0, 69.7, 46.2, 36.4. HRMS: found 539.2793 [M+H]⁺, calculated for [C₃₅H₄₀O₅] 539.2792.

(1S, 2R, 3R, 4R, 5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)cyclohexylmethanesulfonate 12. Mesyl chloride (1 mL, 14.8 mmol) was added dropwise to a cooled (0 °C) solution of alcohol 11 (654 mg, 1.21 mmol) and a catalytic amount of DMAP in pyridine (12 mL). The reaction was stirred at ambient temperature until TLC analysis showed full consumption of the starting material (18 h). The reaction was cooled to 0 °C and carefully quenched with 1 M HCl. Subsequently, the mixture was extracted with EtOAc, dried over MgSO₄, filtered and concentrated in vacuo. Purification by silica column chromatography (petroleum ether/EtOAc, 88:12→80:20) afforded mesylate 12 (691 mg, 1.12 mmol, 92%) as an oil. ¹H NMR (400 MHz, CDCl₃): δ 7.33-7.18 (m, 20H, H ArBn), 5.16 (br s, 1H, H-1), 4.99 (d, J = 11.2 Hz, 1H, CH₂Bn), 4.87 (d, J = 11.2 Hz, 1H, CH₂Bn), 4.82 (d, J = 10.8 Hz, 1H, CH₂Bn), 4.71-4.64 (m, 2H, CH₂Bn), 4.38 (ddd, J = 4.4, 9.2, 11.6 Hz, 1H, H-3), 3.75 (dd, J = 4.4, 9.2 Hz, 1H, H-7), 3.58 (t, J = 9.2 Hz, 1H, H-4), 3.54-3.47 (m, 1H, H-5), 3.37 (t, J = 9.6 Hz, 1H, H-7), 2.82 (s, 3H, CH₃mesyl), 2.64 (dt, J = 4.0, 14.4 Hz, 1H, H-2), 2.13-2.04 (m, 1H, H-6), 1.59-1.52 (m, 1H, H-2). ¹³C NMR (100 MHz, CDCl₃): δ 138.7, 138.4, 138.2, 138.1, 128.4, 128.3, 128.3, 128.3, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.6, 87.5, 77.9, 77.3, 76.7, 75.7, 75.5, 73.3, 72.8, 66.7, 46.1, 37.8, 35.0. HRMS: found 639.2384 [M+Na]⁺, calculated for [C₃₆H₄₀O₇SNa] 639.2387.

(1S, 2R, 3R, 4R, 5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)cyclohexyl azide 13. Mesylate 12 (691 mg, 1.12 mmol) was coevaporated thrice with anhydrous toluene before being dissolved in DMF (10 mL). After the addition of sodium azide (728 mg, 11.2 mmol), the solution was stirred at 120 °C for 18 h. The mixture was cooled down to ambient temperature and subsequently diluted with H₂O, extracted with EtO, dried over MgSO₄, filtered and concentrated in vacuo. Purification by silica column chromatography (petroleum ether/EtOAc, 96:4→95:5) afforded azide 13 (419 mg, 0.74 mmol, 66%) as an oil. ¹H NMR (400 MHz, CDCl₃): δ 7.34-7.23 (m, 18H, H ArBn), 7.20-7.16 (m, 2H, H ArBn), 4.96 (d, J = 10.8 Hz, 1H, CH₂Bn), 4.89 (d, J = 10.8 Hz, 1H, CH₂Bn), 4.83 (d, J = 10.8 Hz, 1H, CH₂Bn), 4.66 (d, J = 7.2, 9.6 Hz, 2H, CH₂Bn), 4.50 (d, J = 5.6 Hz, 1H, CH₂Bn), 4.47 (d, J = 7.2 Hz, 1H, CH₂Bn), 4.39 (d, J = 11.6 Hz, 1H, CH₂Bn), 3.79 (dd, J = 1.6, 9.2 Hz, 1H, H-7), 3.65 (dd, J = 2.0, 9.2 Hz, 1H, H-7), 3.61-3.44 (m, 4H, H-3, H-4, H-5), 2.41 (dt, J = 4.4, 12.4 Hz, 1H, H-2), 1.59-1.46 (m, 2H, H-2, H-6). ¹³C NMR (100 MHz, CDCl₃): δ 138.7, 138.4, 138.2, 138.1, 128.4, 128.3, 128.3, 127.9, 127.8, 127.7, 127.7, 127.7,
127.6, 127.6, 127.5, 87.3, 77.8, 76.7, 76.5, 75.6, 73.1, 72.5, 64.9, 55.3, 48.2, 35.1. HRMS: found 586.2670 [M+Na]+; calculated for [C35H37N3O4Na] 586.2676.

Exo-N-deoxynojirimycin 4

A catalytic amount of 10% Pd/C was added to a solution of azide 13 (28 mg, 0.5 mmol) in MeOH (0.9 mL) and 6 M HCl (0.1 mL). After stirring at ambient temperature for 18 h under H2 atmosphere, the mixture was filtered over celite and concentrated under reduced pressure. Purification by silica column chromatography (EtOAc/MeOH/NH4OH, 48:40:12) provided the title compound 4 (4.7 mg, 27 μmol, 53%) as an oil.

1H NMR (400 MHz, D2O): δ 3.95 (dd, J = 3.2, 12.0 Hz, 1H, H-7), 3.77 (dd, J = 6.8, 12.0 Hz, 1H, H-7), 3.54-3.47 (m, 1H, H-3), 3.34-3.21 (m, 3H, H-1, H-4, H-5), 2.24 (dt, J = 4.0, 12.0 Hz, 1H, H-2), 1.73-1.70 (m, 1H, H-6), 1.58 (q, J = 12.4 Hz, 1H, H-2). 13C NMR (100 MHz, D2O): δ 78.0, 69.9, 68.4, 59.7, 47.7, 46.4, 35.9. HRMS: found 178.1074 [M+H]+, calculated for [C7H16NO4] 178.1074.

3, 4, 5, 7-tetra-O-benzyl-exo-N-deoxynojirimycin 18

A solution of azide 13 (113 mg, 0.2 mmol) and trimethylphosphine (1 M in THF, 1.2 mL, 1.2 mmol) in a mixture of pyridine (1.8 mL) and water (0.2 mL) was stirred at ambient temperature until TLC-analysis showed full conversion (6 h). The mixture was then concentrated under reduced pressure providing crude amine 18, which was used without further purifications. 1H NMR (400 MHz, CDCl3): δ 7.48-6.94 (m, 20 H, HArBn), 4.98 (d, J = 10.8 Hz, 1H, CH2Bn), 4.90 (d, J = 10.8 Hz, 1H, CH2Bn), 4.84 (d, J = 10.8 Hz, 1H, CH2Bn), 4.70 (d, J = 12.0 Hz, 1H, CH2Bn), 4.66 (d, J = 12.0 Hz, 1H, CH2Bn), 4.49 (d, J = 10.8 Hz, 1H, CH2Bn), 4.46 (d, J = 12.0 Hz, 1H, CH2Bn), 4.41 (d, J = 12.0 Hz, 1H, CH2Bn), 3.74 (dd, J = 2.8, 9.4 Hz, 1H, H-7), 3.68 (dd, J = 2.8, 9.4 Hz, 1H, H-7), 3.49-3.55 (m, 3H, H-1, H-4, H-5), 2.21-2.26 (m, 1H, H-2), 1.41-1.25 (m, 2H, H-6, H-2). 13C NMR (100 MHz, CDCl3): δ 148.4, 139.1, 138.8, 138.5, 128.5, 128.5, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 88.1, 79.0, 78.8, 75.7, 76.5, 73.2, 72.4, 65.8, 51.1, 45.8, 39.6.

3, 4, 5, 7-tetra-O-benzyl-exo-N-(tertbutoxycarbonyl)-deoxynojirimycin 19

Crude amine 18 (107 mg, 0.2 mmol) was coevaporated thrice with anhydrous toluene and was taken up in DCM (2 mL). After the addition of Boc2O (66 mg, 0.3 mmol) and triethylamine (0.08 mL, 0.6 mmol), the solution was stirred at ambient temperature for 18 h before being concentrated in vacuo. Purification by silica column chromatography (petroleum ether/EtOAc, 92:8→88:12) provided 19 (105 mg, 0.18 mmol, 91%) as a colorless oil. 1H NMR (400 MHz, CDCl3): δ 7.33-7.18 (m, 20H, HArBn), 4.96 (d, J = 10.8 Hz, 1H, CH2Bn), 4.89 (d, J = 10.8 Hz, 1H, CH2Bn), 4.81 (d, J = 10.8 Hz, 1H, CH2Bn), 4.72-4.61 (m, 3H, CH2Bn, NH), 4.52 (d, J = 10.8 Hz, 1H, CH2Bn), 4.46 (d, J = 11.6 Hz, 1H, CH2Bn), 4.39 (d, J = 11.6 Hz, 1H, CH2Bn), 3.66-3.64 (m, 2H, H-1, H-7), 3.57-3.52 (m, 4H, H-3, H-4, H-5, H-7), 2.52 (d, J = 11.2 Hz, H-2), 1.62-1.50 (m, 1H, H-6), 1.42 (s, 9H, tBuBoc), 1.35-1.29 (m, 1H, H-2). 13C NMR (100 MHz, CDCl3): δ 155.2, 138.4, 138.3, 128.3, 128.0, 127.8, 127.6, 127.5, 127.4, 87.4, 79.2, 78.5, 78.4, 75.5, 73.3, 72.3, 66.5, 48.1, 46.3, 36.8, 28.4. HRMS: found 660.3295 [M+H]+, calculated for [C40H48NO6] 660.3296.
Exo-N-(propyl)-deoxynojirimycin 5

Boc-amine 19 (57 mg, 0.09 mmol) was coevaporated thrice with anhydrous toluene and then the compound was taken up in DMF (0.5 mL). Propylbromide (24.5 μL, 0.27 mmol) and sodium hydride (60% dispersion in mineral oil, 8 mg, 0.2 mmol) were added to the solution and stirred at 65 °C for 18 h. Additional bromopropane (82 μL, 0.9 mmol) and sodium hydride (60% dispersion in mineral oil, 16 mg, 0.4 mmol) were added. The reaction mixture was stirred again at 70 °C for 18 h before being quenched with methanol, diluted with water, extracted with Et₂O, dried over MgSO₄, filtered and concentrated under reduced pressure. The resulting mono-alkyl was dissolved and the reaction was stirred under H₂ atmosphere at ambient temperature for 18 h before being filtered over a small pad of celite and concentrated in vacuo.

Purification by silica column chromatography (dichloromethane/MeOH/NH₄OH, 81:18:1) afforded 5 as a white solid (3.1 mg, 14 μmol, 16% over two steps). ¹H NMR (400 MHz, MeOD): δ 4.14 (dd, J = 3.6, 10.8 Hz, 1H, H-7), 3.72 (dd, J = 8.4, 10.8 Hz, 1H, H-7), 3.39 (ddd, J = 4.4, 8.8, 11.6 Hz, 1H, H-3), 3.19 (t, J = 8.8 Hz, 1H, H-4), 3.08 (t, J = 10.0 Hz, 1H, H-5), 3.03-2.94 (m, 2H, H-1, CH₂N), 2.74 (dt, J = 7.6, 11.6 Hz, 1H, CH₂N), 2.30 (dt, J = 4.4, 12.4 Hz, 1H, H-2), 1.75-1.68 (m, 1H, H-3), 1.67-1.59 (m, 2H, CH₂propyl), 1.44 (q, J = 5.6 Hz, 1H, H-2), 1.00 (t, J = 7.6 Hz, 3H, CH₃propyl). ¹³C NMR (100 MHz, MeOD): δ 80.3, 72.6, 70.6, 64.6, 57.8, 47.9, 36.5, 22.4, 11.5. HRMS: found 220.1542 [M+H]⁺, calculated for [C₁₀H₂₂NO₄] 220.1543.

4-(Adamantan-1-yl-methoxy)-1-butanol 15

A dry solution of adamantanemethanol (2.45 g, 14.7 mmol) in DMF (35 mL) was charged with sodium hydride (60% dispersion in mineral oil, 911 mg, 23 mmol) and the solution was carefully heated to 75 °C and stirred for 1 h. Next, a dry solution of 14 (6.53 g, 13.4 mmol) was added to the reaction and the mixture was stirred at 75 °C for 2 h. The reaction mixture was allowed to cool to ambient temperature and subsequently quenched with MeOH, diluted with H₂O, extracted with Et₂O, dried over MgSO₄, filtered and concentrated under reduced pressure. The crude product was redissolved in dichloromethane (30 mL) and MeOH (30 mL) and a catalytic amount of pTsOH was added. The mixture was stirred at ambient temperature until TLC analysis showed full consumption of the starting material (2 h). Then anhydrous Et₃N was added and the solution was concentrated under reduced pressure. Purification by silica column chromatography (toluene/acetone, 96:4→95:5) afforded 15 (1.70 g, 7.2 mmol, 49%) as an oil. ¹H NMR (400 MHz, CDCl₃): δ 3.64 (t, J = 5.6 Hz, 2H, CH₂OH), 3.43 (t, J = 5.6 Hz, 2H, CH₂O), 3.00 (s, 2H, OCH₂Ada), 1.96 (br s, 3H, CHAda), 1.73-1.63 (m, 10H, CH₂alkyl, CH₂Ada), 1.53 (d, J = 2.4 Hz, 6H, CH₂Ada). ¹³C NMR (100 MHz, CDCl₃): δ 82.2, 71.7, 62.6, 39.6, 37.1, 33.9, 30.4, 28.2, 26.8. HRMS: found 253.2036 [M+H]⁺, calculated for [C₃₅H₃₆O₅Na] 253.2036.

4-(Adamantan-1-yl-methoxy)-1-bromo-butanol 16

A solution of adamantane alcohol 15 (358 mg, 1.5 mmol), carbontetrabromide (995 mg, 3.0 mmol), triphenylphosphine (1.18 g, 4.5 mmol) in acetonitrile (42 mL) was refluxed for 4 h before being concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 98:2→96:4) afforded bromide
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16 (266 mg, 0.88 mmol, 59%) as an oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.46 (t, $J$ = 6.8 Hz, 2H, CH$_2$Br), 3.41 (t, $J$ = 6.4 Hz, 2H, CH$_2$O), 2.95 (s, 2H, OCH$_2$Ada), 1.99-1.92 (m, 5H, CHAda, CH$_2$Ada), 1.73-1.63 (CH$_2$Ada, CH$_2$alkyl), 1.52 (d, $J$ = 2.0 Hz, 6H, CH$_2$Ada). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 81.9, 70.4, 39.7, 37.2, 34.0, 29.8, 28.2, 28.2. HRMS: found 303.2256 [M+H]$^+$, calculated for [C$_{15}$H$_{26}$BrO] 303.2254.

4-(Adamantan-1-ylmethoxy)-1-butanal 17

A solution of adamantane alcohol 15 (477 mg, 2 mmol) and Dess-martin periodane (1.27 g, 3 mmol) in DCM (10 mL) was stirred at ambient temperature for 3 h. The mixture was then diluted with saturated aqueous NaHCO$_3$, extracted with EtOAc, dried over MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified over a small pad of silica (petroleum ether/EtOAc, 94:6→84:16), after it was directly subjected to reductive amination with 18.

Exo-N-((4-adamantane-1-ylmethoxy)-butyl)-deoxynojirimycin 6

A solution of 19 (64 mg, 0.1 mmol), 16 (33 mg, 0.11 mmol) and sodium hydride (60% dispersion in mineral oil, 8.5 mg, 0.21 mmol) was stirred at 65 °C for 18 h before additional 16 (60 mg, 0.2 mmol) in DMF (0.5 mL) and sodium hydride (60% dispersion in mineral oil, 17 mg, 0.42 mmol) were added. After 18 h at 70 °C, the reaction mixture was quenched with MeOH and further diluted with H$_2$O. The mixture was extracted with EtOAc, dried over MgSO$_4$, filtered and concentrated in vacuo. The crude product was purified over a short pad of silica (petroleum ether/EtOAc, 96:4→94:6) and directly re-dissolved in MeOH (0.8 mL) and 6 M HCl (0.2 mL). A catalytic amount of 10% Pd/C was added and the mixture was stirred under H$_2$ atmosphere, before being filtered over celite and concentrated under reduced pressure. Purification by silica column chromatography (dichloromethane/MeOH/NH$_4$OH, 95:4:1→94:5:1) afforded 6 (2.8 mg, 7.0 μmol, 7% over two steps). $^1$H NMR (400 MHz, MeOD): $\delta$ 4.10 (dd, $J$ = 3.6, 11.2 Hz, 1H, H-7), 3.65 (dd, $J$ = 8.4, 10.8 Hz, 1H, H-7), 3.36-3.28 (m, 2H, CH$_2$NH), 3.25-3.21 (m, 1H, H-1), 3.16-3.05 (m, 2H, H-3, H-4), 3.01-2.92 (m, 3H, H-5, CH$_2$alkyl), 2.89 (s, 2H, OCH$_2$Ada), 2.24 (dt, $J$ = 4.0, 8.0 Hz, 1H, H-2), 1.85 (br s, 4H, H-6, CHAda), 1.75-1.65 (m, 4H, CH$_2$Ada, CH$_2$alkyl), 1.60-1.50 (m, 6H, CH$_2$Ada, CH$_2$alkyl), 1.47 (s, 6H, CH$_2$Ada), 1.28-1.21 (m, 1H, H-2). $^{13}$C NMR (100 MHz, MeOD): $\delta$ 132.4, 129.9, 83.2, 79.8, 72.2, 71.8, 70.2, 69.1, 64.1, 57.9, 46.7, 45.9, 40.9, 38.3, 34.8, 29.7, 27.6, 25.0. HRMS: found 398.2899 [M+H]$^+$, calculated for [C$_{22}$H$_{40}$NO$_5$] 398.2900.

Exo-N-((dipropyl)-deoxynojirimycin 7

Activated 4Å molecular sieves were added to a solution of crude amine 19 (56 mg, 0.1 mmol) in anhydrous THF (0.5 mL) and AcOH (0.2 mL). After stirring for 30 min, propanal (16 μL, 0.22 mmol) and sodium cyanoborohydride (15.7 mg, 0.25 mmol) were added and the reaction mixture was stirred at ambient temperature for 18 h. The mixture was then quenched with saturated NaHCO$_3$, extracted with EtOAc, dried over MgSO$_4$, filtered and concentrated under reduced pressure. The crude product was purified over a small plug of silica (petroleum ether/EtOAc, 90:10→85:15) and then subjected to the ensuing deprotection step. The resulting product (37 mg, 0.06 mmol) was taken up in anhydrous DCM (1 mL)
and the solution was cooled to -78 °C before boron trichloride (1 M in DCM, 0.6 mL, 0.6 mmol) was added. Over a period of 3 h, the reaction mixture was allowed to come to -20 °C. Subsequently the mixture was quenched with MeOH and concentrated under reduced pressure. Purification by silica column chromatography (dichloromethane/MeOH/NH₄OH, 93:6:1→91:8:1) afforded the title compound 7 as a colorless oil (11 mg, 0.04 mmol, 40% over three steps). 

\[ \text{HRMS: found 262.2011 [M+H]+, calculated for [C₃H₆NO₄] 262.2012.} \]

**2,3,4,7-tetra-O-benzyl-exo-N-((di-4-adamantane-1-yl-methoxy)-butyl)-deoxynojirimycin 20.**

Crude amine 18 (48 mg, 0.09 mmol) was dissolved in THF (0.5 mL) and AcOH (0.2 mL). After the consecutive addition of activated 4Å molecular sieves, aldehyde 17 (105 mg, 0.44 mmol) and NaBH₃CN (15 mg, 0.24 mmol), the reaction mixture was stirred at ambient temperature for 18 h. The mixture was quenched with saturated aqueous NaHCO₃ and EtOAc was added. The two layers were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated in vacuo. Purification by silica column chromatography (petroleum ether/EtOAc/Et₃N, 95:5:1) yielded the desired title compound 20 (22 mg, 22 μmol, 24% over two steps) as a colorless oil. 

\[ \text{HRMS: found 987.6606 [M+H]+, calculated for [C₆₅H₈₈NO₆] 978.6612.} \]

**exo-N-((di-4-adamantane-1-yl-methoxy)-butyl)-deoxynojirimycin 8.**

Tertiar amine 20 (22 mg, 0.022 mmol) was dissolved in aldehyde free ethanol (1 mL) and HCl (37%) was added until pH = 1. Oxygen was depleted from the reaction mixture by sonication under an argon atmosphere. A catalytic amount of 10% Pd/C was added and after argon bubbling for 10 min, hydrogen was bubbled for 15 min. The reaction mixture was then stirred overnight under an H₂-atmosphere. After 3 days, the reaction was complete and the crude was filtered through a pad of celite. Solvent was removed in vacuo and the crude was purified by HPLC to afford the title compound 8 (7 mg, 0.011 mmol, 51%). 

\[ \text{HRMS: found 987.6606 [M+H]+, calculated for [C₆₅H₈₈NO₆] 978.6612.} \]
MHz, CDCl3): δ 4.22 (dd, J = 3.7, 10.8 Hz, 1H, H-7), 3.61 (t, J = 10.8 Hz, 1H, H-7), 3.45 (td, J = 3.6, 12.1 Hz, 1H, H-1), 3.37-3.40 (m, 4H, CH2N), 3.32-3.36 (m, 1H, H-3), 3.25-3.31 (m, 1H, H-4), 3.16-3.15 (m, 2H, CH2alkyl), 3.10 (t, J = 9.1 Hz, 1H, H-5), 2.99 (2 x s, 4H, OCH2Ada), 2.81 (td, J = 4.4, 12.2 Hz, 1H, CH2Ada), 2.02-2.14 (m, 2H, H-2, H-6), 1.88-1.94 (m, 6H, CHAda), 1.73-1.58 (m, 21 H, H-2, CH2Ada, CH2alkyl), 1.52 (s, 12H, CH2Ada, CH2alkyl). 13C NMR (150 MHz, CDCl3): δ 83.0, 79.8, 72.3, 71.5, 69.9, 65.9, 64.2, 53.7, 51.3, 43.3, 40.9, 40.9, 38.3, 35.2, 35.2, 30.5, 29.7, 29.7, 27.5, 27.4, 24.0, 23.8. HRMS: found 618.4723 [M+H]+, calculated for [C37H64NO5] 618.4728.

References

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Exo-N-cyclic-deoxyribojirimycin

