2-Acetamino-1,2-dideoxynojirimycin—lysine hybrids as hexosaminidase inhibitors

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Abstract

Cyclisation by double reductive amination of 2-acetamino-2-deoxy-D-xylo-hexos-5-ulose with *N*-2 protected L-lysine derivatives provided 2-acetamino-1,2-dideoxynojirimycin derivatives without any observable epimer formation at C-5. Modifications on the lysine moiety gave access to lipophilic derivatives that exhibited improved hexosaminidase inhibitory activities.

1. Introduction

N-Acetylhexosaminidases have attracted considerable interest, due to associations with diseases such as osteoarthritis,¹ allergy² as well as Alzheimer's disease.³ Catalytically handicapped and misfolded mutant forms of *N*-acetylhexosaminidases are responsible for hereditary lysosomal disorders.⁴ Inhibitors of this class of glycosidases have been employed for affinity purification⁵ and the characterisation⁶ of enzymes, as insecticidal as well as anti-fungal⁷ agents and have recently also been suggested as pharmacological chaperone therapeutics for Tay-Sachs' as well as Sandhoff's diseases.⁸ In particular, iminoalditols have frequently been employed as (usually) competitive glycosidase inhibitors.⁹ Representatives of this class of compound, for example glucosidase inhibitor **1** (Fig. 1) as well as mannosidase inhibitor **2** have found important roles as biological probes, such as in the investigation of glycoprotein trimming glycosidases.¹⁰ More recently, hexosaminidase inhibitor **3** was examined as a pharmacological chaperone for lysosomal β -*N*-acetylhexosaminidase A/S (Hex A/S) in fibroblasts derived from patients with Sandhoff and Tay-Sachs diseases.^{8,11}

Following up on our observation that some fluorescently labelled derivatives of the D-glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) are powerful inhibitors

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Dedicated to Professor George W. J. Fleet on the occasion of his 65th birthday

exceeding the parent compound's activity by two orders of magnitude,¹² we have reported the syntheses and glycosidase inhibitory activities of various pyranoid *N*-alkylated iminoalditols featuring fluorescent tags such as dansyl moieties attached to simple *N*-substituents.¹³ Based on their encouraging inhibitory activities, we envisaged more convenient properties with compounds providing a suitably positioned amine for tagging as well as an additional 'handle' for chain extension and with a view to variation of the *N*-substituent's chemical as well as biochemical properties.¹⁴

2. Results and discussion

*N*²-*tert*-Butyloxycarbonyl-*N*⁶-benzyloxycarbonyl-protected L-lysine methyl ester **A** as well as the chain-extended derivative, methyl 6-(*N*⁶-benzyloxycarbonyl-*N*²-*tert*-butyloxycarbonyl-L-lysiny) aminohexanoate **B** proved to be useful reaction partners in ring-closing reactions with 5-ulohexoses by double reductive amination¹⁵ to provide lysine-iminoalditol hybrids¹⁶ ready for a wide range of interesting follow-up modifications.¹⁷

Intermediate ulososide **4** (Scheme 1), which was prepared by 3-chloroperbenzoic acid oxidation of methyl 3,4-di-*O*-acetyl-2-acetamino-2,6-dideoxy-β-D-*xylo*-hex-5-enopyranoside (available by standard methodology^{18,19}) in the presence of benzyl alcohol following a slightly modified route introduced by Barili²⁰ et al. and Murphy²¹ et al. subsequent Zemplen *O*-deacetylation, was employed as the sugar component.

Compound **4** turned out to be a very efficient reaction partner for the amines employed in the catalytic hydrogenation/reductive amination step. Despite the complexity of this conversion which includes deprotection of both the sugar unit as well as the terminal lysine amino group followed by reductive amination at C-1 and subsequent intramolecular cyclisation with formation of a stereogenic centre at C-5, isolated yields were fair and the stereoselectivities for the desired D-*gluco* configuration were excellent in all cases investigated.

Thus, compound **4**, with 6-*N*-benzyloxycarbonyl-2-*N*-BOC methyl lysinate **A** as well as with the chain-extended amide **B**, gave iminoalditol derivatives **5** (58%) and **6** (72%), respectively (Scheme 2). Epimer formation at C-5 could not be detected by NMR.

Compounds **5** and **6** were smoothly converted by standard BOC removal employing methanolic HCl and subsequent reaction of the free amino group with dansyl chloride to give, after preparative TLC, fluorescent inhibitors **5a** (45%) and **6a** (74%), respectively.

D-Hexosaminidase inhibitory activities of new compounds are summarised in Table 1.

As previously^{13,17,22} observed, the presence of a lipophilic residue improved binding compared to the unsubstituted parent compound **3**. However, in contrast to these previous results, in this series of compounds the presence of the dansyl group did not result in tighter binding relative to the *N*-BOC-substituted precursor. These compounds, with their appended fluorescent moiety and reasonably tight binding, offer potential for use in monitoring chaperoning processes.

For example, compound **6a** enhanced the activity of Hex A/S in adult Tay-Sachs as well as infantile Sandhoff cell lines more than twofold relative to vehicle (DMSO) treated cells (Fig. 2). Although Hex A/S activity was previously shown to be enhanced 2.8 fold by compound **3** (100 mM) in adult Tay-Sachs cells,¹¹ compound **6a** was equally effective at increasing HexA/S activity but at a 700-fold lower concentration (140 μ M). Like other pharmacological chaperones for Tay-Sachs or Gaucher disease, the effective intracellular enhancing concentration of compound **6a** (15–140 μ M) is greater than its K_i (4.2 μ M, human Hex A). This difference is commonly attributed to bioavailability and/or intracellular metabolism of the compound.²³ These results suggest that the structural framework of compound **6a** could be exploited for the design of additional efficacious pharmacological chaperones for GM2 gangliosidoses with low toxicity.

3. Experimental

3.1. General methods

Optical rotations were measured on a Perkin Elmer 341 polarimeter at the wavelength of 589 nm and a path length of 10 cm at 20 °C. NMR spectra were recorded on a Varian INOVA 500 operating at 500.6 MHz (^1H), and at 125.9 MHz (^{13}C). CDCl_3 was employed for protected compounds and methanol- d^4 employed for unprotected inhibitors. Chemical shifts are listed in delta employing residual, non-deuterated solvent as the internal standard. The signals of the protecting groups were found in the expected regions and are not listed explicitly. Electrospray mass spectra were recorded on an HP 1100 series MSD, Hewlett Packard. Samples were dissolved in acetonitrile or acetonitrile/water mixtures. The scan mode for positive ions (mass range 100–1000 D) was employed varying the fragmentation voltage from 50 to 250 V with best molecular peaks observed at 150 V. Analytical TLC was performed on precoated aluminium plates of silica gel 60 F254 (E. Merck 5554), detected with UV light (254 nm), 10% vanillin/sulfuric acid as well as ceric ammonium molybdate (100 g ammonium molybdate/8 g ceric sulfate in 1 L 10% H_2SO_4) and heated on a hotplate. Preparative TLC was performed on precoated glass plates of silica gel 60 F254, 0.5 mm (E. Merck 5744). For column chromatography silica gel 60 (230–400 mesh, E. Merck 9385) was used.

3.2. Kinetic studies

Inhibition of β -*N*-acetylhexosaminidase from *Streptomyces plicatus* was determined as previously described.²⁴ Lysosomal β -*N*-acetylhexosaminidase A (Hex A) was purified from human placenta as reported.²⁵ Inhibitory activity (IC_{50}) of **6a** was determined using 1 mM 4-nitrophenyl β -*N*-acetyl-glucosaminide as outlined.^{8a} K_i was estimated according to the equation $K_i = \text{IC}_{50}/(1+S/K_m)$, where S is the substrate concentration.

3.2.1. Intracellular β -*N*-acetylhexosaminidase activity—Adult Tay-Sachs (homozygous for alpha subunit G269S mutation) and infantile Sandhoff (homozygous for the 16-kb *HEXB* deletion mutation) fibroblasts¹¹ were treated with an escalating dose of **6a** (dissolved in DMSO) for 5 days. Intracellular β -*N*-acetylhexosaminidase A/S activity was measured using the fluorogenic substrate 4-methylumbelliferyl *N*-acetylglucosamine-6-sulfate as previously described.¹¹

3.3. General procedure for intramolecular reductive amination

To a 0.03 M solution of **4** in MeOH/H₂O (15:1, v/v), one equivalent of the peptide component as well as Pd(OH)₂/C (20%, 0.1 equiv) was added, and the heterogeneous reaction mixture was stirred under an atmosphere of hydrogen at ambient pressure and room temperature for 24 h. After filtration and evaporation of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel (CHCl₃/MeOH/concd NH₃, 500:100:6, v/v/v), yielding the target compound as a colourless syrup. The formation of L-*ido*-configured products was not observed in any of the reported cases.

3.4. General procedure for *N*-BOC-removal and *N*-dansylation

A 0.01 M solution of the respective BOC-protected iminosugaramino acid hybrid in MeOH/AcCl (30:1, v/v, prepared at 0 °C 10 min before use) was stirred for 20 h at ambient temperature. The solvents were removed under reduced pressure, and the residue was taken up in dry DMF, yielding a 0.01 M solution. Triethylamine (5 equiv) and dansyl chloride (1.1 equiv) were added, and the resulting reaction mixture was stirred in a brown flask for 4 h at ambient temperature. Removal of the solvent under reduced pressure and purification of the residue by preparative TLC (silica gel, CHCl₃/MeOH/concd NH₃, 300:100:4, v/v/v, extraction with MeOH) gave the desired target compound as a green, fluorescent syrup.

3.5. Methyl (5*R/S*)-2-acetamino-5-*C*-benzyloxy-2-deoxy-β-*D*-xylo-hexopyranoside **4**

To a 1% solution of methyl 3,4-di-*O*-acetyl-2-acetamino-2,6-dideoxy-β-*D*-xylo-hex-5-enopyranoside (1.00 g, 3.32 mmol) in a mixture of dichloromethane/benzyl alcohol (1:1, v/v), 3-chloroperbenzoic acid (70%, 900 mg, 3.65 mmol) was added, and the mixture was stirred at ambient temperature for 2 h. The mixture was partitioned between dichloromethane (120 mL) and aqueous sodium bicarbonate (5%), the organic layer was dried (Na₂SO₄), the solvent removed under reduced pressure, and the resulting residue was chromatographed (cyclohexane/ethyl acetate 1:4, v/v) on silica gel providing a diastereomeric mixture (5*R*:5*S* ca. 2:9) of ulososides (81%). Conventional *O*-deacetylation employing sodium methoxide (1 mmol) in methanol (25 mL) gave compound **4** (98%) as a mixture of diastereomers at C-5 from which the major 5*S*-epimer was purified by

chromatography (CHCl₃/MeOH 9:1, v/v). $[\alpha]_D^{20} = -19.8$ (*c* 1.1, MeOH); ¹H NMR: (500 MHz, CD₃OD) δ 7.50–7.20 (m, 5H, benzyl); 4.76 (d, 1H, *J* = 10.7 Hz, benzyl); 4.71 (d, 1H, *J*_{1,2} = 7.3 Hz, H-1); 4.69 (d, 1H, benzyl); 4.03 (dd, 1H, *J*_{2,3} = 9.8 Hz, H-2); 3.88 (d, 1H, *J*_{6a,6b} = 12.2 Hz, H-6a); 3.85 (d, 1H, *J*_{3,4} = 7.8 Hz, H-4); 3.84 (d, 1H, H-6b); 3.71 (dd, 1H, H-3); 3.44 (s, 3H, OMe); 1.98 (s, 3H, *N*-Ac). ¹³C NMR: (125 MHz, CD₃OD) δ 72.6 (NHAc), 138.8, 128.3, 128.0 (2C), 127.9 (2C), benzyl; 101.7 (C-1); 100.6 (C-5); 74.9, 72.5 (C-3, C-4); 63.4, 61.1 (C-6, benzyl); 55.6 (OMe); 55.3 (C-2); 21.8 (*N*-Ac).

MS: Calcd for [C₁₆H₂₃NO₇]: *m/z* 341.364; ESIMS found: [M+H]⁺ 342.37, [M+Na]⁺ 364.35.

3.6. Methyl-*N*⁶-(2-acetamino-1,2,5-trideoxy-D-glucitol-1,5-diyl)-*N*²-*tert*-butyloxycarbonyl-L-lysinate **5**

Following general procedure 3.3, ulososide **4** (diastereomeric mixture, 68 mg, 0.20 mmol) was reacted with lysine derivative **A** (90 mg, 1.1 equiv) to give compound **5** (52 mg, 58%):

$[\alpha]_{\text{D}}^{20} = +1.2$ (*c* 1.1, MeOH); ¹H NMR: (500 MHz, CD₃OD) δ 4.09 (m, 1H, H-2'); 3.86 (m, 2H, *J*_{6a,6b} = 12.2 Hz, H-6a, H-6b); 3.82 (ddd, 1H, *J*_{1eq,2} = 3.9 Hz, *J*_{1ax,2} = 10.7 Hz, *J*_{2,3} = 8.8 Hz, H-2); 3.71 (s, 3H, OCH₃); 3.40 (dd, 1H, *J*_{3,4} = 9.3 Hz, *J*_{4,5} = 9.3 Hz, H-4); 3.22 (dd, 1H, H-3); 3.01 (dd, 1H, *J*_{1eq,1ax} = 11.2 Hz, H-1eq); 2.80 (m, 1H, H-6'a); 2.57 (m, 1H, H-6'b); 2.15 (m, 2H, H-1ax, H-5); 1.96 (s, 3H, NHAc); 1.77 (m, 1H, H-3'a); 1.66 (m, 1H, H-3'b); 1.56–1.30 (m, 4H, 2 × H-4', 2 × H-5'); 1.43 (s, 9H, Boc). ¹³C NMR: (125 MHz, CD₃OD) δ 173.9, 172.4 (C-1', NHAc); 157.0 (Boc); 79.4 (Boc); 76.5 (C-3); 71.5 (C-4); 66.1 (C-5); 58.5 (C-6); 54.4, 53.8, 52.0 (C-1, C-2', C-6'); 51.4 (OCH₃); 50.7 (C-2); 31.3 (C-3'); 27.6, 27.6 (Boc); 23.7, 23.6 (C-4', C-5'), 21.6 (NHAc).

MS: Calcd for [C₂₀H₃₇N₃O₈]: *m/z* 447.533; ESIMS found: [M+H]⁺ 448.54, [M+Na]⁺ 470.52.

3.7. Methyl 6-[*N*⁶-(2-acetamino-1,2,5-trideoxy-D-glucitol-1,5-diyl)-*N*²-*tert*-butyloxycarbonyl-L-lysiny]amino hexanoate (**6**)

Following general procedure 3.3, ulososide **4** (120 mg, 0.352 mmol) was reacted with lysine

derivative **B** (200 mg, 1.1 equiv) to give compound **6** (142 mg, 72%): $[\alpha]_{\text{D}}^{20} = 3.0$ (*c* 1.8, MeOH); ¹H NMR: (500 MHz, CD₃OD) δ = 3.96 (m, 1H, H-2'); 3.85 (m, 2H, *J*_{6a,6b} = 12.2 Hz, H-6a, H-6b); 3.82 (ddd, 1H, *J*_{1eq,2} = 3.9 Hz, *J*_{1ax,2} = 10.7 Hz, *J*_{2,3} = 8.8 Hz, H-2); 3.65 (s, 3H, OCH₃); 3.40 (dd, 1H, *J*_{3,4} = 9.3 Hz, *J*_{4,5} = 9.3 Hz, H-4); 3.21 (dd, 1H, H-3); 3.24–3.12 (m, 2H, 2 × H-6''); 3.01 (dd, 1H, *J*_{1eq,1ax} = 11.2 Hz, H-1eq); 2.80 (m, 1H, H-6'a); 2.55 (m, 1H, H-6'b); 2.32 (t, 2H, 2 × H-2''); 2.12 (m, 2H, H-1ax, H-5); 1.95 (s, 3H, NHAc); 1.75–1.25 (m, 12H, 2 × H-3'', 2 × H-4'', 2 × H-5'', 2 × H-3', 2 × H-4', 2 × H-5'); 1.44 (s, 9H, Boc). ¹³C NMR: (125 MHz, CD₃OD) δ = 174.7, 174.0, 172.4 (C-1'', C-1', NHAc); 156.6 (Boc); 79.4 (Boc); 76.5 (C-3); 71.5 (C-4); 66.2 (C-5); 58.5 (C-6); 55.0, 54.5, 52.1 (C-1, C-2', C-6'); 50.9 (OCH₃); 50.7 (C-2); 38.9 (C-6''); 33.5 (C-2''); 32.1 (C-3''); 28.9 (C-5''); 27.6, 27.6, 27.6 (Boc); 26.2, 24.5, 23.9, 23.7, (C-4', C-5', C-3'', C-4''); 21.6 (NHAc). MS: Calcd for [C₂₆H₄₈N₄O₉]: *m/z* 560.694; ESIMS found: [M+H]⁺ 561.70, [M+Na]⁺ 583.69.

3.8. Methyl-*N*⁶-(2-acetamino-1,2,5-trideoxy-D-glucitol-1,5-diyl)-*N*²-dansyl-L-lysinate **5a**

Following general procedure 3.4, compound **5** (45 mg, 0.1 mmol) gave fluorescent inhibitor

5a (26 mg, 45%): $[\alpha]_{\text{D}}^{20} = +16.6$ (*c* 1.3, MeOH); ¹H NMR: (500 MHz, CD₃OD) δ 8.55 (d, 1H, dansyl); 8.38 (d, 1H, dansyl); 8.18 (d, 1H, dansyl); 7.59 (t, 1H, dansyl); 7.54 (t, 1H, dansyl); 7.27 (d, 1H, dansyl); 3.76 (m, 3H, *J*_{6a,6b} = 12.2 Hz, H-2, H-6a, H-6b); 3.72 (m, 1H, H-2'); 3.35 (dd, 1H, *J*_{3,4} = 9.3 Hz, *J*_{4,5} = 9.7 Hz, H-4); 3.21 (s, 3H, OCH₃); 3.17 (dd, 1H, *J*_{2,3} = 8.8 Hz, H-3); 2.87 (s, 6H, dansyl); 2.83 (dd, 1H, *J*_{1eq,1ax} = 11.7 Hz, *J*_{1eq,2} = 4.8 Hz, H-1eq); 2.45 (m, 1H, H-6'a); 2.32 (m, 1H, H-6'b); 2.04 (ddd, 1H, *J*_{5,6a} = 2.0 Hz, *J*_{5,6b} = 2.4 Hz, H-5); 2.00 (dd, 1H, *J*_{1ax,2} = 11.2, H-1ax); 1.98 (s, 3H, NHAc); 1.60–1.50 (m, 2H, 2 × H-3'); 1.22–0.80 (m, 4H, 2 × H-4', 2 × H-5'). ¹³C NMR: (125 MHz, CD₃OD) δ 172.5,

172.4 (C-1', NHAc); 152.0, 135.8, 130.3, 129.9, 129.9, 129.3, 127.9, 123.1, 119.7, 115.2 (dansyl); 76.6, (C-3); 71.6 (C-4); 65.9 (C-5); 58.6 (C-6); 55.8, 54.5, 51.8 (C-1, C-2', C-6'); 51.1 (OCH₃); 50.7 (C-2); 44.7, 44.7 (dansyl); 31.9 (C-3'); 23.0, 23.0 (C-4', C-5'); 21.6 (NHAc). MS: Calcd for [C₂₇H₄₀N₄O₈S]: *m/z* 580.706; ESIMS found: [M+H]⁺ 581.71, [M+Na]⁺ 603.69.

3.9. Methyl 6-[N⁶-(2-acetamino-1,2,5-trideoxy-D-glucitol-1,5-diyl)-N²-dansyl-L-lysiny]amino hexanoate 6a

Following general procedure 3.4, compound **6** (50 mg, 0.089 mmol) gave fluorescent inhibitor **6a** (46 mg, 74%): $[\alpha]_D^{20} = +1.9$ (*c* 0.9, MeOH); ¹H NMR: (500 MHz, CD₃OD) δ 8.56 (d, 1H, dansyl); 8.36 (d, 1H, dansyl); 8.21 (d, 1H, dansyl); 7.60 (t, 1H, dansyl); 7.56 (t, 1H, dansyl); 7.27 (d, 1H, dansyl); 3.76 (m, 3H, H-2, H-6a, H-6b); 3.65 (s, 3H, OCH₃); 3.57 (m, 1H, H-2'); 3.34 (dd, 1H, *J*_{3,4} = 9.3 Hz, *J*_{4,5} = 9.3 Hz, H-4); 3.17 (dd, 1H, *J*_{2,3} = 8.8 Hz, H-3); 2.88 (s, 6H, dansyl); 2.83 (dd, 1H, *J*_{1eq,1ax} = 11.2 Hz, *J*_{1eq,2} = 4.4 Hz, H-1eq); 2.82 (m, 2H, 2 × H-6''); 2.41 (m, 1H, H-6'a); 2.30 (m, 1H, H-6'b); 2.27 (t, 2H, 2 × H-2''); 2.05 (ddd, 1H, *J*_{5,6a} = 2.9 Hz, *J*_{5,6b} = 2.4 Hz, H-5); 1.99 (dd, 1H, *J*_{1ax,2} = 11.7 Hz, H-1ax); 1.97 (s, 3H, NHAc); 1.56–0.84 (m, 12H, 2 × H-3', 2 × H-4', 2 × H-5', 2 × H-3'', 2 × H-4'', 2 × H-5''); ¹³C NMR: (125 MHz, CD₃OD) δ 174.7, 172.6, 172.3 (C-1C, C-1', NHAc); 152.1, 135.5, 130.4, 130.0, 129.8, 129.5, 128.2, 123.1, 119.4, 115.3 (dansyl); 76.5 (C-3); 71.5 (C-4); 65.9 (C-5); 58.5 (C-6); 56.9, 54.5, 51.8 (C-1, C-2', C-6'); 50.9 (OCH₃); 50.7 (C-2); 44.7, 44.7 (dansyl); 38.8 (C-6''); 33.5 (C-2''); 32.5 (C-3'); 28.5 (C-5''); 26.1, 24.4, 23.2, 23.1 (C-4', C-5', C-3'', C-4''); 21.6 (NHAc).

MS: Calcd for [C₃₃H₅₁N₅O₉S]: *m/z* 693.867; ESIMS found: [M+H]⁺ 694.90, [M+Na]⁺ 716.90.

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Figure 1.

Figure 2. Enhancement of β -*N*-acetylhexosaminidase activity of adult Tay-Sachs (squares) and infantile Sandhoff (circles) fibroblast patient cell lines. Relative increase in intracellular β -*N*-Acetylhexosaminidase activity in compound **6a** versus vehicle (DMSO) treated cells (y-axis).

Scheme 1.

Scheme 2.

Table 1

K_i values for inhibition of the β -*N*-acetylhexosaminidase from *Streptomyces plicatus* by 2-acetamino-1,2-dideoxynojirimycin-lysine hybrids

Compound	K_i (μ M)
3	80
5	5.0
5a	4.3
6	6.3
6a	4.6