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Bioorganic & Medicinal Chemistry 14 (2006) 1737–1742
Bioorganic & Medicinal Chemistry

Fluorescently tagged iminoalditol glycosidase inhibitors as novel biological probes and diagnostics

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Received 1 August 2005; revised 4 October 2005; accepted 13 October 2005
Available online 7 November 2005

Abstract—1,5-Dideoxy-1,5-imino-D-glucitol, the corresponding D-*manno* and L-*ido* epimers as well as the powerful β-glucosidase inhibitor isofagomine were N-alkylated with di-, tri-, as well as tetraethylene glycol derived straight chain spacer arms by a set of simple standard procedures. The terminal functional groups of the spacer arms, primary amines, were employed to introduce fluorescent dansyl moieties. Resulting derivatives showed glycosidase inhibitory activities comparable to those of the parent compounds'.

1. Introduction

Iminosugars including iminoalditols and related bicyclic alkaloids are well known, (usually) competitive, glycosidase inhibitors.[1] Representatives of this class of compounds have found important roles as biological probes, such as in the investigation of glycoprotein trimming glycosidases[2] or as pharmaceutical substances such as in the treatment of diabetes type II symptoms.[3] Other biological activities associated with their glycosidase inhibitory properties are anti-viral, anti-cancer and anti-metastatic, anti-infective as well as insect anti-feedant and plant growth regulating effects.[4]

It was demonstrated that immobilized *N*-alkylated iminoalditols can be employed as affinity ligands in glycosidase isolation and purification protocols.[5] This derivatisation has frequently been associated with a loss of inhibitory activity of around 1–2 orders of magnitude but in this case the derivatives were found to be sufficiently active for the purpose.[5]

Recently, we have found that some fluorescently labelled derivatives of the glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol (or DMDP) are powerful inhibitors exceeding the parent compound's activity by 2 orders of magnitude.[6]

2. Results and discussion

With the aim of evaluating a more general means of tagging of iminosugars than was employed in the previously mentioned, structurally very restricted case, we resorted to the connection of fluorophores to the iminosugars via medium length spacer-arms which had been found suitable in previous immobilization studies.[5] In particular, oligoethylene glycol derived spacer arms, which minimise the non-specific adsorption of virtually all proteins over a wide range of conditions, have frequently been prepared and employed as spacer arms between ligands and their molecular tags as well as functioning as constituents of self-assembling monolayers.[7]

2.1. Syntheses

Initially deoxynojirimycin (**1a**) was alkylated with three linker arms of different lengths to yield the three azido-terminated species **1b–d**. The azides on each of these linkers were then reduced and treated with dansyl chloride to yield the three fluorescent derivatives **1e–g**. Compounds in the D-*manno* (**2a–c**), L-*ido* (**3a–c**) and isofagomine (**4a–c**) series were each converted into a

Keywords: Iminoalditol; Glycosidase inhibitor; Fluorescent tag; Diagnostic.
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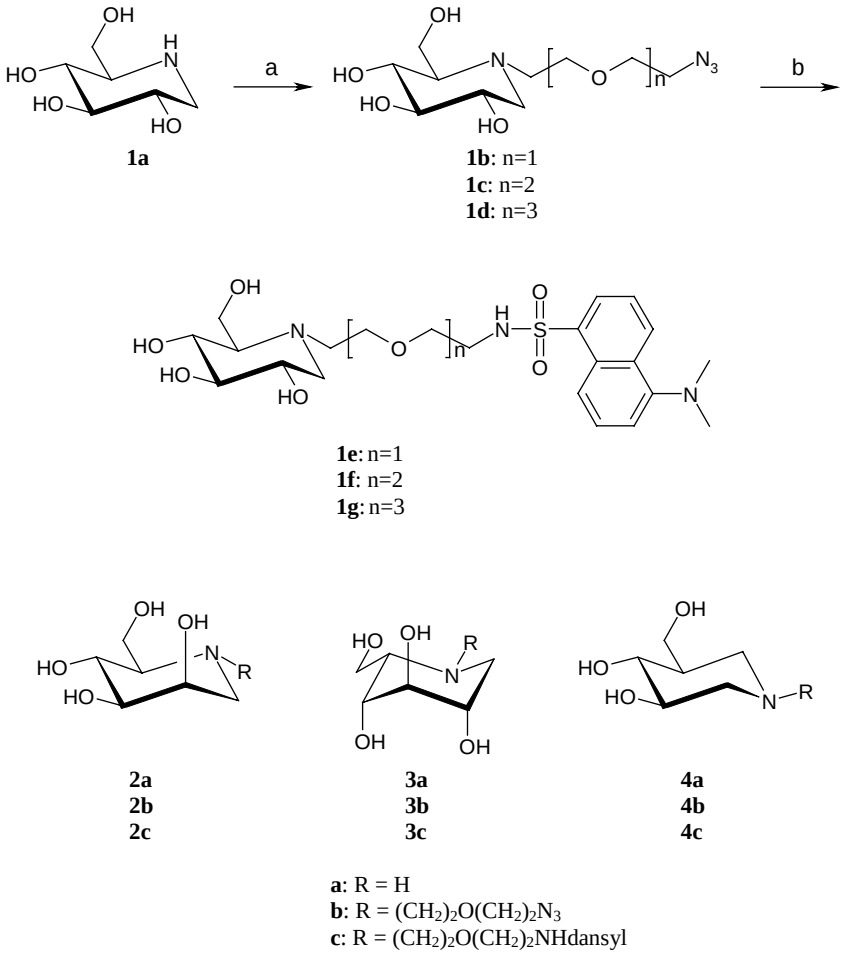
doi:10.1016/j.bmc.2005.10.021



Scheme 1. Reagents: (a) DMF, Na_2CO_3, $TosOCH_2(CH_2OCH_2)_nCH_2N_3$, b: $n = 1$, **c:** $n = 2$, **d:** $n = 3$; (b) i: Pd/C, MeOH, H_2; ii: Dansy1Cl.

single linker arm derivative with the 5-azido-3-oxapentyl linker obtaining **2b**, **3b** and **4b**, and then in each case this derivative was reduced and dansylated to yield **2c**, **3c** and **4c**. The choice of this specific linker arm length was based upon the kinetic results obtained with the deoxynojirimycin series, as described below (Scheme 1).

2.2. Inhibitory activities

Inhibition constants determined for each of the compounds synthesized are presented in Tables 1–3. The deoxynojirimycin analogues were tested as inhibitors of the *Agrobacterium* sp. β-glucosidase.[8] Addition of the azido linker arms (**1b**–**d**, Table 1) substantially reduced activities relative to deoxynojirimycin **1a**, with the inhibition worsening as the linker arm lengthened. This is presumably a consequence both of conversion of the secondary amine function to a tertiary amine, thereby possibly sterically hindering the interaction of the nitrogen with the active site carboxylic acid. This interaction has been shown to be important in structural studies with other Clan GH-A members.[9] Further, mutants of the *Agrobacterium* sp. β-glucosidase in which the acid/base side-chain carboxylate has been replaced are known to bind deoxynojirimycin significantly more weakly. Fortunately, subsequent dansylation of the reduced linker arm largely restored the inhibitory potency to that of the parent deoxynojirimycin (**1e**–**g**, Table 1). The substantially worse inhibition seen for the azido-terminated linker arms likely has its origins in the relatively high water solubility of the polyethylene glycol-based linker arm, an effect which increases with length. Partitioning of the linker arm of the inhibitor between free aqueous solution and the (relatively) hydrophobic interior of the enzyme active site becomes progressively worse as the linker arm lengthens. These differences in affinity are

Table 1. K_i values of *N*-substituted compounds against *Agrobacterium* sp. β-glucosidase

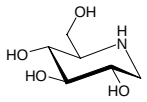
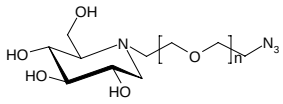
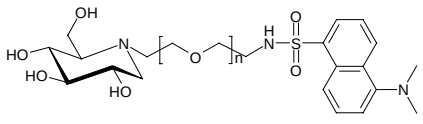
Compound		K_i (μM)
	**1a**	12
	**1b** $n = 1$	74
	1c $n = 2$	800
	1d $n = 3$	1400
	**1e** $n = 1$	12
	1f $n = 2$	39
	1g $n = 3$	34

Table 2. K_i values of *N*-substituted 1-deoxymannonojirimycin analogues against jack bean α-mannosidase

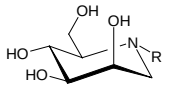
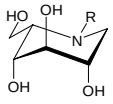
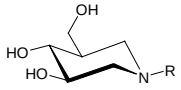
Compound		K_i (μM)
	**2a** R = H	18
	2b R = $(CH_2)_2O(CH_2)_2N_3$	155
	2c R = $(CH_2)_2O(CH_2)_2$Nhdansyl	78

Table 3. K_i values of *N*-substituted 1-deoxyidonojirimycin and isofagomine analogues against *Agrobacterium* sp. β-glucosidase

Compound		K_i (μM)
	**3a** R = H	26
	3b R = $(CH_2)_2O(CH_2)_2N_3$	720
	3c R = $(CH_2)_2O(CH_2)_2$NHdansyl	32
	**4a** R = H	0.007
	4b R = $(CH_2)_2O(CH_2)_2N_3$	31
	4c R = $(CH_2)_2O(CH_2)_2$NHdansyl	19

largely suppressed in the dansylated derivatives, possibly because the hydrophobic dansylated terminus dominates its properties. Similar effects had been observed with coumarin, dapoxyl as well as acrylodan fluorophors.[10]

Based upon these results, only the shortest linker arm was employed in the synthesis of the *manno*- and *ido*-deoxynojirimycin and iosofagomine derivatives. Once again, in each case, introduction of the linker reduced affinity, but this deleterious effect was again largely reversed upon dansylation (Tables 2 and 3). This effect of linker arm addition was largest in the isofagomine series—most likely because interactions of the active site nucleophile with the ring nitrogen seem to be particularly important, as seen in structural studies on the Clan GH-A enzyme *Cex*.[9] These classes of fluorescently tagged inhibitors are now under investigation as tools for proteomic analyses. Their uses could include activity-based staining of polyacrylamide gel separations of proteomes or in competitive displacement assays of other enzyme inhibitors for arrayed enzymes.

3. Experimental

3.1. General methods

^{1}H NMR spectra were recorded on a Varian INOVA 500 operating at 499.925 MHz. ^{13}C NMR spectra were recorded at 75.47 or 50.29 MHz. Residual non-deuterated solvent was used as internal standard for determination of chemical shifts. Signals of aromatic systems and their substituents were found in the expected regions and are not listed explicitly. 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 silica gel 60 F254 (Merck 5554), detected with UV light (254 nm), 5% vanillin/sulfuric acid as well as ceric ammonium molybdate (100 g ammonium molybdate/4 g ceric sulfate in 1 L 10% H_2SO_4) and heated on a hotplate. For column chromatography silica gel 60 (230–400 mesh, Merck 9385) was used. Optical rotation was measured on a Perkin Elmer 341 polarimeter at the wavelength of 589 nm and a path length of 10 cm at 20 °C.

3.1.1. Kinetic studies. *Agrobacterium* sp. β-glucosidase was purified and assayed as described.[11] Kinetic studies were performed at 37 °C in pH 7.0 sodium phosphate buffer (50 mM) containing 0.1% bovine serum albumin, using 7.2×10^{-5} mg/mL enzyme. Approximate values of K_i were determined using a fixed concentration of substrate, 4-nitrophenyl-β-D-glucopyranoside (0.11 mM = $1.5 \times K_m$), and inhibitor concentrations ranging from 0.2 times to 5 times the K_i value ultimately determined. A horizontal line drawn through $1/V_{max}$ in a Dixon plot of these data ($1/V$ vs [I]) intersects the experimental line at an inhibitor concentration equal to $-K_i$. Full K_i determinations, where required, were performed using the same range of inhibitor concentrations while also varying substrate (4-nitrophenyl glucoside) concentrations from approximately 0.015 to 0.6 mM. Data were analysed by direct fit to the Michaelis Menten equation describing the reaction in the presence of inhibitors using the program GraFit.[12]

α-Mannosidase from jack bean was purchased from Sigma. Kinetic studies were performed at 25 °C in pH 6.8 sodium phosphate buffer (50 mM) containing 0.1% bovine serum albumin, using 3.7×10^{-3} mg/mL enzyme. Approximate values of K_i were determined using a fixed concentration of substrate, 4-nitrophenyl-α-D-mannopyranoside (1.3 mM = $1.5 \times K_m$), and inhibitor concentrations ranging from 0.2 times to 5 times the K_i value ultimately determined. A horizontal line drawn through $1/V_{max}$ in a Dixon plot of these data ($1/V$ vs [I]) intersects the experimental line at an inhibitor concentration equal to $-K_i$.

3.2. General procedures

3.2.1. General procedure for α-azidodeoxy-ω-*O*-tosyloligoethyleneglycol formation. A 10% solution of the respective ethylene glycol di-tosylate in dry *N*,*N*-dimethylformamide (DMF) was stirred with NaN_3 (1.1 equiv) at ambient temp for 20 h. CH_2Cl_2 was added to the reaction mixture to precipitate the salts. After filtration, the solution was concentrated under reduced pressure and the remaining material was chromatographed on silica gel to furnish the respective mono-azido compound as colourless to slightly yellow oil.

3.2.2. General procedure for N-alkylation of iminoalditols. A 2% solution of the respective iminoalditol in dry DMF was stirred with the respective azidodeoxyethylenegylcol tosylate at 45 °C for 120 h. The solvent was removed under reduced pressure and the remaining material was chromatographed on silica gel ($CHCl_3$/MeOH/concd NH_4OH 4:1:1). To remove counterions, 5% aqueous

solutions of the resulting products were treated with strongly basic ion exchange resin Merck III. After filtration and removal of H_2O, compounds were passed over short plugs of silica gel to yield free bases.

3.2.3. General procedure for hydrogenation of terminal azide groups. 2% methanolic solutions of the respective terminal azido compounds were stirred at ambient temp under an atmosphere of H_2 at ambient pressure for 20 h employing Pd/C (10%) as catalyst. After removal of the catalyst by filtration, MeOH was removed under reduced pressure and the crude, air-sensitive resulting materials were immediately used in the next step.

3.2.4. General procedure for N-dansylation. To a 2% DMF solution of the respective terminal amine, dansyl chloride (1.7 equiv) and Et_3N (2 equiv) were added and the mixture was stirred at ambient temp for 3 h. Excess MeOH was added to the mixture and the solvents were removed under reduced pressure. Chromatography of the green-fluorescent residue gave the respective dansylamino-tagged iminoalditol.

3.3. α-Azidodeoxy-ω-*O*-tosyldiethyleneglycol (5-azido-3-oxapentyl-1-toluenesulfonate), *n* = 1

Following the general procedure for α-azidodeoxy-ω-*O*-tosyloligothyleneglycol formation, diethyleneglycol ditosylate (7.55 g, 18.2 mmol) gave the monoazide (2.50 g, 48%) as a yellowish oil. ^{13}C NMR ($CDCl_3$): δ 70.4, 69.4, 68.9, 50.8.

3.4. α-Azidodeoxy-ω-*O*-tosyltriethyleneglycol (8-azido-3,6-dioxaoctyl-1-toluenesulfonate), *n* = 2

Following the general procedure for α-azidodeoxy-ω-*O*-tosyloligothyleneglycol formation, triethyleneglycol ditosylate (2.00 g, 4.36 mmol) gave the monoazide (615 mg, 42.8%) as a colourless syrup. ^{13}C NMR ($CDCl_3$): δ 71.0, 70.3, 69.5, 69.0, 50.9.

3.5. α-Azidodeoxy-ω-*O*-tosyltetraethyleneglycol (11-azido-3,6,9-trioxaundecanyl-1-toluenesulfonate), *n* = 3

Following the general procedure for α-azidodeoxy-ω-*O*-tosyloligothyleneglycol formation, tetraethyleneglycol ditosylate (2.05 g, 4.08 mmol) gave the monoazide (691 mg, 45.4%) as a slightly yellow syrup. ^{13}C NMR ($CDCl_3$): δ 71.0, 70.9, 70.8, 70.3, 69.5, 68.9, 50.8.

3.6. *N*-(5-Azido-3-oxapentyl)-1-deoxynojirimycin, 1b

Following the general procedure for N-alkylation of iminoalditols, 1-deoxynojirimycin (**1a**, 100 mg, 0.613 mmol) gave **1b** (60 mg, 50%) as a colourless wax. $[\alpha]_D^{20}$ +8.9 (*c* 1.25, MeOH). ^{13}C NMR (MeOH-d_4): δ 79.2 (C-3), 70.5, 69.8, 69.4, 68.1, 66.4, 58.0, 57.3, 51.5 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′) 50.6 (C-5′). ^1H NMR: δ 3.94 (dd, 2H, $J_{5,6a} = J_{5,6b}$ 2.4 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a, H-6b), 3.68 (m, 4H, 2H-2′, 2H-4′), 3.48 (ddd, 1H, $J_{1a,2}$ 9.9 Hz, $J_{1e,2}$ 4.9 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.41–3.36 (m, 3H), 3.17 (dd, 1H, $J_{3,4}$ 9.3 Hz, H-3), 3.11 (dd, 1H, $J_{1a,1e}$ 11.2 Hz, H-1e), 3.08 (m, 1H, H-1′a), 2.80 (m, 1H, H-1′b), 2.36 (dd, 1H, H-1a), 2.26 (m, 1H, H-5). MS Calcd for $[C_{10}H_{20}N_4O_5]$: *m*/*z* 276.295. Found: *m*/*z* 276.29.

3.7. *N*-(8-Azido-3,6-dioxaoctyl)-1-deoxynojirimycin, 1c

Following the general procedure for N-alkylation of iminoalditols, 1-deoxynojirimycin (**1a**, 67 mg, 0.41 mmol) gave **1c** (48 mg, 36.5%) as a colourless wax. ^{13}C NMR (MeOH-d_4): δ 79.0 (C-3), 70.7, 70.4, 70.3, 70.0, 69.5, 68.4, 66.5, 58.2, 57.3, 51.5 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′, C-5′, C-7′) 50.6 (C-8′). ^1H NMR: δ 3.92 (dd, 1H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.83 (dd, 1H, $J_{5,6b}$ 2.4 Hz, H-6b), 3.70–3.61 (m, 8H), 3.46 (ddd, 1H, $J_{1e,2}$ 4.9 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.41–3.36 (m, 3H), 3.15 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 3.06 (dd, 1H, $J_{1a,1e}$ 11.2 Hz, H-1e), 3.04 (m, 1H, H-1′a), 2.70 (m, 1H, H-1′b), 2.29 (dd, 1H, $J_{1a,2}$ 10.8 Hz, H-1a), 2.20 (m, 1H, $J_{4,5}$ 9.7 Hz, H-5). MS: Calcd for $[C_{12}H_{24}N_4O_6]$: *m*/*z* 320.448. Found: *m*/*z* 320.44.

3.8. *N*-(11-Azido-3,6,9-trioxaundecanyl)-1-deoxynojirimycin, 1d

Following the general procedure for N-alkylation of iminoalditols, 1-deoxynojirimycin (**1a**, 50 mg, 0.31 mmol) gave **1d** (48 mg, 42.5%) as a colourless wax. ^{13}C NMR (MeOH-d_4): δ 79.2 (C-3), 70.6, 70.4, 70.3 (2 C), 70.2, 70.0, 69.4, 68.3, 66.5, 58.1, 57.2, 51.5 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′, C-5′, C-7′, C-8′, C-10′), 50.6 (C-11′). ^1H NMR: δ 3.93 (dd, 1H, $J_{5,6a}$ 2.9 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.84 (dd, 1H, $J_{5,6b}$ 2.9 Hz, H-6b), 3.70–3.61 (m, 12H), 3.47 (m, 1H), 3.41 (m, 2H), 3.16 (t, 2H), 3.07 (dd, 1H, $J_{1e,2}$ 4.9 Hz, $J_{1a,1e}$ 11.3 Hz, H-1e), 3.04 (m, 1H), 2.67 (m, 1H), 2.27 (dd, 1H, $J_{1a,2}$ 10.7 Hz, H-1a), 2.21 (ddd, $J_{4,5}$ 9.3 Hz, H-5). MS: Calcd for $[C_{10}H_{20}N_4O_5]$: *m*/*z* 364.502. Found: *m*/*z* 364.50.

3.9. *N*-(5-Dansylamino-3-oxapentyl)-1-deoxynojirimycin, 1e

Following the general procedures for azide hydrogenation and N-dansylation, **1b** (60 mg, 0.22 mmol) gave dansyl-tagged compound **1e** as a green-fluorescent glass (25 mg, 23.8%). $[\alpha]_D^{20}$ −1.4 (*c* 1.3, MeOH). ^{13}C NMR (MeOH-d_4): δ 79.2 (C-3), 70.7, 69.6, 69.4, 67.8, 66.7, 58.2, 57.1, 51.4 (C-1, C-2, C-4, C-5, C-6, C-1′,C-2′, C-4′), 42.7 (C-5′). ^1H NMR: δ 3.82 (dd, 1H, $J_{5,6a}$ 2.9 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.77 (dd, 1H, $J_{5,6b}$ 2.9 Hz, H-6b), 3.44 (ddd, $J_{1a,2}$ 10.3 Hz, $J_{1e,2}$ 4.9 Hz, $J_{2,3}$ 9.3 Hz, H-2), 3.35–3.21 (m, 4H), 3.13 (dd, 1H, H-3), 3.04 (t, 2H), 2.97 (dd, 1H, $J_{1a,1e}$ 11.2 Hz, H-1e), 2.87–2.81 (m, 1H, H-1′a), 2.44 (m, 1H, H-1′b), 2.15 (dd, 1H, H-1a), 2.10 (ddd, 1H, $J_{4,5}$ 9.3 Hz, H-5). MS: Calcd for $[C_{22}H_{33}N_3O_7S]$: *m*/*z* 483.588. Found: *m*/*z* 483.59.

3.10. *N*-(8-Dansylamino-3,6-dioxaoctyl)-1-deoxynojirimycin, 1f

Following the general procedures for azide hydrogenation and N-dansylation, **1c** (48 mg, 0.15 mmol) gave dansyl-tagged compound **1f** as a green-fluorescent glass (31 mg, 39%). ^{13}C NMR (MeOH-d_4): δ 79.3 (C-3), 70.5,

70.0, 69.8, 69.5, 69.4, 68.6, 66.6, 58.0, 57.5, 51.3 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′, C-5′, C-7′), 42.7 (C-8′). ^{1}H NMR: δ 3.91 (dd, 1H, $J_{5,6a}$ 2.9 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.85 (dd, $J_{5,6b}$ 2.4 Hz, H-6b), 3.63–3.45 (m, 3H), 3.40–3.37 (m, 3H), 3.32–3.24 (m, 4H), 3.17 (dd, 1H, $J_{2,3}$ 8.8 Hz, $J_{3,4}$ 9.3 Hz, H-3), 3.04–2.98 (m, 4H, incl H-1e and H-1′a), 2.58 (m, 1H, H-1′b), 2.26 (dd, 1H, $J_{1a,1e} = J_{1e,2}$ 10.7 Hz, H-1a), 1.98 (ddd, 1H, $J_{4,5}$ 9.3 Hz, H-5). MS: Calcd for [$C_{24}H_{37}N_3O_8S$]: *m*/*z* 527.642. Found: *m*/*z* 527.64.

3.11. *N*-(11-Dansylamino-3,6,9-trioxaundecanyl)-1-deoxynojirimycin, 1g

Following the general procedures for azide hydrogenation and N-dansylation, **1d** (51 mg, 0.14 mmol) gave dansyl-tagged compound **1g** as a green-fluorescent glass (45 mg, 56%). ^{13}C NMR (MeOH-d_4): δ 79.0 (C-3), 70.6, 70.1 (3 C), 69.9, 69.4 (2 C), 68.2, 66.5, 58.1, 58.0, 51.4 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′, C-5′, C-7′, C-8′, C-10′), 42.7 (C-11′). ^{1}H NMR: δ 3.90 (dd, 1H, $J_{5,6a}$ 2.9 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.83 (dd, $J_{5,6b}$ 2.4 Hz, H-6b), 3.62–3.51 (m, 6H), 3.47 (ddd, $J_{1a,2}$ 9.8 Hz, $J_{1e,2}$ 4.4 Hz, $J_{2,3}$ 3 Hz, H-2), 3.41–3.29 (m, 7H), 3.18 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-3), 3.08–2.98 (m, 4H, incl H-1e, H-1′a), 2.65 (m, 1H, H-1′b), 2.27 (dd, 1H, $J_{1a,1e} = J_{1a,2}$ 10.7 Hz, H-1a), 2.21 (ddd, 1H, $J_{4,5}$ 9.3 Hz, H-5). MS: Calcd for [$C_{26}H_{41}N_3O_9S$]: *m*/*z* 571.695. Found: *m*/*z* 571.68.

3.12. *N*-(5-Azido-3-oxapentyl)-1-deoxymannojirimycin, 2b

Following the general procedure for N-alkylation of iminoalditols, 1-deoxymannojirimycin (**2a**, 75 mg, 0.46 mmol) gave **2b** (62 mg, 49%) as a colourless wax. $[\alpha]_D^{20}$ −38.4 (*c* 0.7, MeOH). ^{13}C NMR (MeOH-d_4): δ 75.3 (C-3), 69.7, 68.5, 68.4, 68.3, 66.0, 58.1, 56.1, 51.6 (C-1, C-2, C-4, C-5, C-6, C-2′, C-4′, C-5′), 50.7 (C-1′). ^{1}H NMR: δ 3.94 (dd, 1H, $J_{5,6a}$ 2.9 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.89 (dd, 1H, $J_{5,6b}$ 2.4 Hz, H-6b), 3.81 (m, 1H, H-2), 3.68–3.62 (m, 5H), 3.38 (t, 2H), 3.33 (m, 1H), 3.04 (dd, 1H, $J_{1a,1e}$ 12.7 Hz, $J_{1e,2}$ 3.9 Hz, H-1e), 3.02 (m, 1H, H-1′a), 2.85 (m, 1H, H-1′b), 2.65 (dd, 1H, $J_{1a,2}$ 1.5 Hz, H-1a), 2.27 (ddd, 1H, $J_{4,5}$ 9.3 Hz, H-5). MS: Calcd for [$C_{10}H_{20}N_4O_5$]: *m*/*z* 276.295. Found: *m*/*z* 276.30.

3.13. *N*-(5-Dansylamino-3-oxapentyl)-1-deoxymannojirimycin, 2c

Following the general procedures for azide hydrogenation and N-dansylation, **2b** (44 mg, 0.16 mmol) gave dansyl-tagged compound **2c** as a green-fluorescent glass (41 mg, 68%). $[\alpha]_D^{20}$ −21 (*c* 2.0, MeOH). ^{13}C NMR (MeOH-d_4): δ 75.2 (C-3), 69.5, 68.3, 68.2, 67.7, 66.3, 57.8, 55.7, 51.4 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′), 42.8 (C-5′). ^{1}H NMR: δ 3.85 (dd, 1H, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.81 (dd, 1H, $J_{5,6b}$ 2.5 Hz, H-6b), 3.79 (m, 1H, H-2), 3.65 (dd, 1H, $J_{2,3}$ 9.3 Hz, $J_{3,4}$ 8.8 Hz, H-3), 3.36–3.26 (m, 6H), 3.04 (t, 2H), 2.92 (dd, 1H, $J_{1e,2}$ 3.9 Hz, $J_{1a,1e}$ 12.2 Hz, H-1e), 2.88–2.82 (m, 7H, aryl-NMe_2, H-1′a), 2.44 (m, 1H, H-1′b), 3.99 (dd, 1H, $J_{1a,2}$ 1.5 Hz, H-1a), 2.09 (ddd, $J_{4,5}$ 8.8 Hz, H-5). MS: Calcd for [$C_{22}H_{33}N_3O_7S$]: *m*/*z* 483.588. Found: *m*/*z* 483.58.

3.14. *N*-(5-Azido-3-oxapentyl)-1-deoxy-L-idonojirimycin, 3b

Following the general procedure for N-alkylation of iminoalditols, 1-deoxy-L-idonojirimycin, **3a** (140 mg, 0.86 mmol) gave **3b** (60 mg, 25%) as a colourless wax. $[\alpha]_D^{20}$ −13.5 (*c* 0.8, MeOH). ^{13}C NMR (MeOH-d_4): δ 74.8 (C-3), 71.3, 69.9, 69.8, 69.6, 63.9, 56.8, 54.1, 51.7 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′), 50.6 (C-5′). ^{1}H NMR: δ 3.84 (dd, 1H, $J_{5,6a}$ 4.4 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.80 (dd, 1H, $J_{5,6b}$ 7.3 Hz, H-6b), 3.71 (dd, 1H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 5.4 Hz, H-4), 3.66–3.60 (m, 4H), 3.54 (ddd, 1H, $J_{1a,2}$ 9.8 Hz, $J_{1e,2}$ 5.4 Hz, $J_{2,3}$ 8 Hz, H-2), 3.07–2.96 (m, 2H), 2.91 (m, 1H, H-5), 2.87 (dd, 1H, $J_{1a,1e}$ 12.7 Hz, H-1e), 2.67 (dd, 1H, H-1a). MS: Calcd for [$C_{10}H_{20}N_4O_5$]: *m*/*z* 276.295. Found: *m*/*z* 276.28.

3.15. *N*-(5-Dansylamino-3-oxapentyl)-1-deoxy-L-idonojirimycin, 3c

Following the general procedures for azide hydrogenation and N-dansylation, **4b** (40 mg, 0.15 mmol) gave dansyl-tagged compound **3c** as a green-fluorescent glass (5.3 mg, 10%). $[\alpha]_D^{20}$ −8 (*c* 0.3, MeOH). ^{13}C NMR (MeOH-d_4): δ 74.7 (C-3), 71.3, 69.9, 69.5, 69.1, 63.8, 56.9, 54.0, 51.8 (C-1, C-2, C-4, C-5, C-6, C-1′, C-2′, C-4′), 34.8 (C-5′). ^{1}H NMR: δ 3.82 (dd, 1H, $J_{5,6a}$ 5 Hz, $J_{6a,6b}$ 11.3 Hz, H-6a), 3.78 (dd, 1H, $J_{5,6b}$ 7.3 Hz, H-6b), 3.68 (dd, 1H, $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 5.4 Hz, H-4), 3.57 (t, 2H), 3.52 (m, 1H, H-2), 3.50–3.45 (m, 2H), 3.42 (t, 2H), 3.01 (m, 1H), 2.88–2.74 (m, 2H, H-1e, H-5), 2.62 (dd, 1H, $J_{1a,1e}$12.7 Hz, $J_{1a,2}$ 9.8 Hz, H-1a). MS: Calcd for [$C_{22}H_{33}N_3O_7S$]: *m*/*z* 483.588. Found: *m*/*z* 483.58.

3.16. *N*-(5-Azido-3-oxapentyl)-isofagomine, *N*-(5-azido-3-oxapentyl)-(3*R*,4*R*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-piperidine, 4b

Following the general procedure for N-alkylation of iminoalditols, isofagomine, **4a** (88.3 mg, 0.48 mmol) gave **4b** (101 mg, 81%) as a colourless wax. $[\alpha]_D^{20}$ +13.5 (*c* 1.5, MeOH). ^{13}C NMR (MeOH-d_4): δ 74.8 (C-4), 71.9 (C-3), 69.8, 68.6 (C-2′, C-3′), 61.5 (C-5′), 59.0 (C-2), 57.2 (C-1′), 55.7 (C-6), 50.5 (C-4′), 43.8 (C-4) ^{1}H NMR: δ 3.80 (dd, 1H, $J_{5,5'a}$ 3.9 Hz, $J_{5'a,5'b}$ 11.0 Hz, H-5′a), 3.64 (dd, 2H, H-2′, H-3′), 3.52 (m, 2H, $J_{2ax,3}$ 6.8 Hz, $J_{2eq,3}$ 3.4 Hz, $J_{3,4}$ 7.3 Hz, H-3, H-5′b), 3.37 (t, 2H, H-4′), 3.08 (m, 3H, H-2ax, H-4, H-6ax), 2.67 (m, 2H, H-1′), 1,98 (m, 2H, $J_{2ax,2eq\ =\ 6ax,6eq}$ 11.7 Hz, $J_{2eq,3}$ 2.9 Hz, $J_{6eq,5}$ 2.4 Hz, H-2eq, H-6eq), 1.76 (m, 1H, H-5). MS: Calcd for [$C_{10}H_{20}N_4O_4$]: *m*/*z* 260.295. Found: *m*/*z* 260.29.

3.17. *N*-(5-Dansylamino-3-oxapentyl)-isofagomine, *N*-(5-dansylamino-3-oxapentyl)-(3*R*,4*R*,5*R*)-3,4-dihydroxy-5-hydroxymethyl-piperidine, 4c

Following the general procedures for azide hydrogenation and N-dansylation, **4b** (70 mg, 0.27 mmol) gave dansyl-tagged compound **4c** as a green-fluorescent glass

(79 mg, 63%). $[\alpha]_D^{20}$ +7.0 (*c* 1.5, MeOH). ^{13}C NMR (MeOH-d_4): δ 74.8 (C-4), 71.7 (C-3), 69.2, 67.6 (C-2′, C-3′), 61.5 (C-5′), 58.8 (C-2), 57.1 (C-1′), 55.6 (C-6), 44.7 (2C, 2× NCH_3), 43.6 (C-5), 42.7 (C-4′). ^1H NMR: δ 3.80 (dd, 1H, $J_{5,5'a}$ 3.4 Hz, $J_{5'a,5'b}$ 11.0 Hz, H-5′a), 3.52 (m, 2H, H-3, H-5′b), 3.25 (m, 4H, H-2′, H-3′), 3.06 (m, 3H, H-2ax, H-4, H-6ax), 2.97 (m, 2H, H-4′), 2.35 (m, 2H, H-1′), 1.83 (m, 2H, $J_{2ax,2eq = 6ax,6eq}$ 11.2 Hz, $J_{2eq,3}$ 2.9 Hz, $J_{6eq,5}$ 3.4 Hz, H-1eq, H-5eq), 1.75 (m, 1H, H-5). MS: Calcd for $[C_{22}H_{33}N_3O_6S]$: *m*/*z* 467.589. Found: *m*/*z* 467.59.

Acknowledgments

Financial support by the Austrian Fonds zur Förderung der Wissenschaftlichen Forschung (FWF), Vienna (Project 15726-N03), as well as by the Protein Engineering Network of Centres of Excellence of Canada (PENCE) is gratefully acknowledged. A. E. S. thanks the Danish Technical University for a guest professorship in March 2005.

References and notes

1. For example: Martin, O. R.; Compain, P., Eds., Iminosugars: Recent Insights into their Bioactivity and Potential as Therapeutic Agents; *Curr. Top. Med. Chem.* 2003, Vol. 3, pp 471–591; From lianas to glycobiology tools: Twenty-five years of 2,5-dideoxy-2,5-imino-D-mannitol, Wrodnigg, T. M. In *Timely Research Perspectives in Carbohydrate Chemistry*; Schmid, W., Stütz, A. E., Eds.; Springer: Vienna, New York, 2002; pp 43–76; Heightman, T. D.; Vasella, A. T. *Angew. Chem., Int. Ed.* **1999**, *38*, 750; Stütz, A. E., Ed.; *Iminosugars as Glycosidase Inhibitors*; Wiley-VCH: Weinheim, 1999; Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1; Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319.
2. Spiro, R. G. In *Carbohydrates in Chemistry and Biology*. In *Part II: Biology of Saccharides*; Ernst, B., Hart, G. W., Sinay, P., Eds.; Wiley-VCH: Weinheim, 2000; Vol. 3, pp 65–79; Elbein, A. D.; Molyneux, R. J. In *Iminosugars as Glycosidase Inhibitors*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, 1999; pp 216–251.
3. Miglitol® by BAYER.
4. Simmonds, M. S. J.; Kite, G. C.; Porter, E. A. In *Iminosugars as Glycosidase Inhibitors*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, 1999; pp 8–30.
5. De Raadt, A.; Ekhart, C.; Legler, G.; Stütz, A. E. In *Iminosugars as Glycosidase Inhibitors*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, 1999; pp 207–215.
6. Hermetter, A.; Scholze, H.; Stütz, A. E.; Withers, S. G.; Wrodnigg, T. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1339.
7. Bertozzi, C. R.; Bednarski, M. *J. Org. Chem.* **1991**, *56*, 4326; Schwabacher, A. W.; Lane, J. W.; Schiesher, M. W.; Leigh, K. M.; Johnson, C. W. *J. Org. Chem.* **1998**, *63*, 1727; Mrksich, M.; Dike, L. E.; Tien, J.; Ingber, D. E.; Whitsides, G. M. *Exp. Cell Res.* **1997**, *235*, 305; Sigal, G. B.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1998**, *120*, 3464.
8. Kempton, J. B.; Withers, S. G. *Biochemistry* **1992**, *31*, 9961.
9. Notenboom, V.; Williams, S. J.; Hoos, R.; Withers, S. G.; Rose, D. R. *Biochemistry* **2000**, *39*, 11553.
10. Wrodnigg, T. M.; Withers, S. G.; Stütz, A. E. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1063.
11. (a) Prade, H.; Mackenzie, L. F.; Withers, S. G. *Carbohydr. Res.* **1998**, *305*, 371; (b) Kempton, J. B.; Withers, S. G. *Biochemistry* **1992**, *31*, 9961.
12. Leatherbarrow, R. J. GraFit Version 4.0, Erithacus Software, Staines, U.K., 1992.