1-Deoxynojirimycins with dansyl capped N-substituents as probes for Morbus Gaucher affected cell lines

Richard F. G. Fröhlich[a], **Richard H. Furneaux**[a], **Don J. Mahuran**[b], **Brigitte A. Rigat**[b], **Arnold E. Stütz**[c,*], **Michael B. Tropak**[b], **Jacqueline Wicki**[d], **Stephen G. Withers**[d], and **Tanja M. Wrodnigg**[c]

[a]Carbohydrate Chemistry Team, Industrial Research Limited, PO Box 31-310, 5040 Lower Hutt, New Zealand [b]Department of Laboratory Medicine and Pathobiology, Sick Kids Hospital, 555 University Avenue, University of Toronto, Ontario, Canada M5G 1X8 [c]Glycogroup, Institut für Organische Chemie, Technische Universität Graz, Stremayrgasse 16, A-8010 Graz, Austria [d]Chemistry Department, University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1

Abstract

Cyclization by double reductive amination of D-*xylo*-hexos-5-ulose with methyl 6-aminohexanoate gave (methoxycarbonyl)pentyl-1-deoxynojirimycin. Reaction of the terminal carboxylic acid with *N*-dansyl- 1,6-diaminohexane provided the corresponding chain-extended fluorescent derivative. By reaction with bis(6-dansylaminohexyl)amine, the corresponding branched di-*N*-dansyl compound was obtained. Both compounds are strong inhibitors of D-glucosidases and could also be shown to distinctly improve, at sub-inhibitory concentrations, the activity of β-glucocerebrosidase in a Gaucher fibroblast (N370S) cell-line through chaperoning of the enzyme to the lysosome.

Keywords

1. Introduction

Iminoalditols and structurally related alkaloids such as compounds **1**–**3** are one of the most prominent families of low molecular weight reversible glycosidase inhibitors.[1] By virtue of their powerful interactions with many glycosidases, they exert activity against retroviruses, tumor growth and metastasis, symptoms of diabetes type 2 as well as quite a few others.[1a,1b,1e]

Various N-alkylated derivatives including the *N*-butyl (**1a**), *N*-nonyl (**1b**), and *N*-hydroxyethyl (**1c**) derivatives of compound **1** (Fig. 1) have become pharmaceutical substances in the treatment of diabetes type 2 symptoms and other metabolic disorders

*Corresponding author. Tel.: +0043 316 873 8244; fax: +0043 316 873 8740. stuetz@tugraz.at (A.E. Stütz).

including Gaucher's disease.[2] Earlier, it had been demonstrated that immobilized N-alkylated iminoalditols, for example, **1e**, can be employed as affinity ligands in glycosidase isolation and purification protocols.[3]

Recently, iminosugars have been introduced as therapeutic principles for various forms of lysosomal disorders.[4–6] This group of over 40 hereditary metabolic diseases arises from mutations in specific genes that lead to deficiencies in enzymes involved in the lysosomal degradation of glycolipids and glycans.

For Gaucher's disease, which is characterized by deficient activity of acid β-glucosidase (glucocerebrosidase), *N*-modified derivatives of 1-deoxynojirimycin, were investigated and employed in substrate reduction therapy (the *N*-butyl derivative **1a** is marketed as Zavesca).[6] Subsequently, it was suggested that sub-inhibitory concentrations of active site specific molecules (pharmacological chaperones) could be exploited for chaperone-mediated therapy (CMT).[4] Based upon this concept, these small molecules bind to and stabilize mutant proteins such as lysosomal β-glucosidase, β-galactosidase, or β-*N*-acetylhexosaminidase, once they have reached their functional folded conformations enabling exit from the endoplasmatic reticulum and subsequent transport to the lysosome. Mutant proteins that cannot obtain or retain their functional conformation are recognized as misfolded by the quality control machinery in the endoplasmatic reticulum and are eventually targeted for degradation.

N-Nonyl-1-DNJ (**1b**) has become one of the current benchmark molecules in this context. Recently, unusual structures such as bicyclic nojirimycin derivatives[7] and a lipophilic derivative of isofagomine[8] have also been found to exert excellent activities in this context. Other laboratories such as Wong's[9] as well as Aerts and Overkleeft and their co-workers[10] have observed that large, lipophilic *N*-alkyl substituents, in particular adamantyl-substituted spacer arms, provide excellent interaction between the inhibitor and the lysosomal β-glucosidase. These leading references, in line with our interest in fluorescently tagged glycosidase inhibitors for diagnostic purposes,[11,12] have recently led us to initiate the preparation of a range of *N*-modified 1,5-iminoglucitols from D-*xylo*-hexos-5-ulose (**4**), amongst them compounds **10** and **11**.

2. Results and discussion

Both compounds were synthesized from known[13] *N*-carboxy-pentyl-1-deoxynojirimycin (**1e**), which, in turn, had been accessed by reaction of D-*xylo*-hexos-5-ulose[11] (**4**) with methyl 6-aminohexanoate under reductive amination conditions (H$_2$, Pd(OH)$_2$–C, in MeOH at ambient pressure) to give **1d**, followed by saponification of the methyl ester with NaOH in water–dioxane (Scheme 1).

Reaction of intermediate **1e** with *N*-dansyl-1,6-diaminohexane **8** (prepared in four simple steps from 6-aminohexanol via *N*-dansyl derivative **5**, the corresponding mesylate **6** and azide **7**, Scheme 2) under standard peptide coupling conditions (TBTU, triethylamine, DMF) gave iminoalditol **10** as a yellow-green fluorescent syrup in 43% yield after extensive purification on silica gel.

For the synthesis of compound **11**, *N*-dansyl-1,6-diaminohexane **8** was reacted with *N*-dansyl-*O*-mesyl-6-aminohexanol (**6**) in *N*,*N*-dimethylformamide in the presence of sodium carbonate (Scheme 3).

The resulting bis(6-dansylaminohexyl)amine **9** reacted smoothly with intermediate **1e** (TBTU, triethylamine, DMF) to give final product **11** in 84% yield after chromatography (Scheme 4).

In a general screen against three selected glucosidases, compound **10** was found to be a powerful inhibitor of β-glucosidases from *Agrobacterium* sp. ($K_i = 0.99$ μM; compound **1**: $K_i = 12$ μM) as well as from almonds ($K_i = 0.73$ μM) but devoid of activity against yeast α-glucosidase. Compound **11** was more active than inhibitor **10** against the *Agrobacterium* sp. enzyme ($K_i = 0.22$ μM) but was much less potent with the almond glucosidase (K_i ca. 80 μM) and, akin to **1** and **10**, did not inhibit the α-selective glucosidase screened (**1e** was not an inhibitor of these enzymes). Both, **10** and **11**, significantly inhibited lysosomal β-glucosidase (Cerezyme). Compound **10** had a K_i value of 3.8 μM, whereas compound **11** was double as active with $K_i = 1.7$ μM. Thus, both inhibitors were tested as potential pharmacological chaperones.

Their influence on residual glucocerebrosidase in fibroblasts homozygous for the common N370S mutation found in type Gaucher patients was compared with Ambroxol (ABX) (Fig. 2). Although ABX has been used to treat humans for airway mucus hyper-secretion diseases such as chronic bronchitis, it was recently found to have a secondary side activity as a glucocerebrosidase inhibitor ($K_i = 23 \pm 3$ μM at pH 5.6) and pharmacological chaperone.[14] Relative to DMSO treated patient cells, ABX is effective at enhancing mutant lysosomal glucocerebrosidase activity more than 50% at 10–30 μM concentrations. Compound **10** was found somewhat less effective than ABX with best effects at a 20-μM concentration causing a 25% relative increase of enzymatic activity (Fig. 3). Compound **11** was found to be more effective across the range of concentrations probed and showed a statistically significant maximal activity increase of 60% at 30 μM. This effect is specific for glucocerebrosidase, as the relative activity of another lysosomal enzyme, β-*N*-acetylhexosaminidase, did not significantly differ from DMSO treated patient cells at this concentration or other concentrations of **11**.

Based on the encouraging, albeit preliminary, results of this exploratory study, we are now engaged in several structural reiterative modifications of the *N*-substituent in compound **11** looking for an optimal balance between undesired inhibition of lysosomal and other glucosidases and beneficial sub-inhibitory chaperoning effects of the particular enzyme under consideration here.

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 at 300.13 MHz (^{1}H) and at 75.4 MHz (^{13}C). CDCl$_3$ was employed for protected compounds and methanol-d^4 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. MALDI mass spectra were recorded on a MALDI Micro MX (Waters) time-of-flight instrument used in reflectron mode with 2.3 meffective flight path. Analytical TLC was performed on precoated aluminum plates 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. For column chromatography Silica Gel 60 (230–400 mesh, E. Merck 9385) was used.

3.2. Kinetic studies

Almond β-glucosidase and *Saccharomyces cerevisiae* (yeast) α-glucosidase were obtained from Sigma. *Agrobacterium* sp. β-glucosidase was produced and purified as described previously.[13,15] Purified human recombinant lysosomal acid β-glucocerebrosidase was purchased from Genzyme (Cambridge, MA, USA). All buffer chemicals and substrates were obtained from Sigma (USA).

Kinetic studies using non-lysosomal enzymes were performed at 37 °C in 50 mM sodium phosphate buffer (pH 7.0 or pH 6.5). Approximate values of K_i were determined using a fixed concentration of substrate, either 4-nitrophenyl β-D-glucopyranoside or 4-nitrophenyl α-D-glucopyranoside at a concentration equal to 1–2 times the K_m value, and inhibitor concentrations ranging from 0.2 to 5 times the K_i value ultimately determined. Initial rates were determined by continuous measurement of the release of nitrophenolate using a Unicam UV4 spectrophotometer equipped with a temperature controlled circulating water bath. Reactions were initiated by the addition of enzyme, and the release of nitrophenolate was monitored at 400 nm. The concentration of enzyme added and the length of time that the reaction was monitored in each case were selected such that less than 10% of the total substrate was converted to product. 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 $-K_i$.

Kinetic studies using β-glucocerebrosidase (7.5 μg/mL) were performed in 100 mM citrate phosphate buffer pH 5.5 containing 0.2% taurodeoxycholate at 37 °C for 30 min. Experiments were carried out using 4-nitrophenyl β-D-glucopyranoside substrate at concentrations ranging from approximately 0.2 to 5 times K_m in the presence of inhibitors at final concentrations ranging from 0.5 to 3 times the K_i value that was ultimately ascertained. K_i values were determined by fitting the data to the competitive model described by $K_{mObserved} = K_m * (1 + [I]/K_i)$ and the Michaelis–Menten equation using non-linear regression function within Prism 5.0 (Graphpad) software (where $K_{mObserved}$ is the K_m observed in the presence of inhibitor).

3.3. Treatment of Gaucher patient cells and intracellular lysosomal enzyme activity

Primary skin fibroblasts derived from a patient with Gaucher disease homozygous for the N370S mutation (c.1226A>G/c.1226A>G) were provided by the Hospital for Sick Children Tissue Culture Facility. Fibroblast cells were grown in complete minimal essential medium from Wisent Inc. (Canada) in the presence of 5% antibiotics (penicillin and streptomycin, Invitrogen), supplemented with 10% fetal bovine serum (FBS, Wisent Inc., Canada), and incubated at 37 °C in a humidified atmosphere with 5% CO_2. To evaluate the intracellular enzyme activity enhancement of the compounds, cells were grown to 70–80% confluence in 96-well tissue culture plates. Threefold serial dilutions of the compounds in DMSO were prepared and added to growth media at a 100-fold dilution. Patient cells were grown in the presence of the compounds for five days at 37 °C as described above. Prior to enzyme activity analysis, media were removed, cells were washed with two changes of phosphate buffered saline and lysed in the presence of 200 mM citrate phosphate buffer pH 5.5 containing 0.4% Triton X-100 and 0.2% taurodeoxycholate at 4 °C for 1 h. Aliquots of the lysate were added to an equal volume of 10 mM 4-methylumbelliferyl β-D-glucopyranoside or 3.2 mM 4-methylumbelliferyl β-N-acetyl-D-glucosaminide and were incubated at 37 °C for 2 h or 15 min to measure GCC or Hex activity, respectively. Reactions were terminated by the addition of four volumes of 0.1 M 2-amino-2-methyl-1-propanol (pH 10.5). Fluorescence was quantified using a Molecular Devices M2 Spectrofluorometer plate reader using excitation and emission wavelengths of 365 and 450 nm, respectively. Activity values for each dose of ABX, **10** or **11** were normalized to activities found in lysates from vehicle (0.1% DMSO) treated patient cells.

3.4. *N*-Dansyl-6-aminohexanol (5)

To a 2% solution of 6-aminohexanol (309 mg, 2.64 mmol) in dichloromethane containing triethylamine (2 mL), dansyl chloride (690 mg, 2.56 mmol) was added at ambient temperature and the mixture was stirred for 16 h in the dark. Solvents were removed under reduced pressure and the remaining residue was dissolved in dichloromethane (2.5 mL) and purified on silica gel employing cyclohexane–EtOAc 1:3 v/v as the eluant to give compound **5** (633 mg, 68.5%) as a yellow oil. ^{13}C NMR (125 MHz, CDCl$_3$): δ = 152.0, 135.1, 130.4, 130.1, 129.8, 129.6, 128.5, 123.4, 119.2, 115.3, 62.6, 45.6 (2C), 43.2, 32.4, 29.5, 26.2, 25.2. MS calcd for [C$_{18}$H$_{26}$N$_2$O$_3$S]: *m/z* 350.48352; found 351.490 [M+H]$^+$, 373.470 [M+Na]$^+$.

3.5. *N*-Dansyl-*O*-mesyl-6-aminohexanol (6)

To a 3% solution of compound **5** (570 mg, 1.63 mmol) in dichloromethane containing triethylamine (0.7 mL), methanesulfonyl chloride (0.15 mL, 2.0 mmol) was added at 0 °C and the mixture was stirred for 1 h. The reaction mixture was diluted with dichloromethane and the solution was washed with water, then dried (Na$_2$SO$_4$), filtered and the solvent was removed under reduced pressure to give 640 mg (92%) of the product which was sufficiently pure for the next step. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.53 (d, 1H, *J* = 8.5 Hz), 8.29 (d, 1H, *J* = 8.5 Hz), 8.25–8.22 (m, 1H), 759–7.49 (m, 2H), 7.18 (d, 1H, *J* = 7.5 Hz), 4.91 (br s, 1H), 4.10 (m, 2H), 2.97 (s, 3H), 2.90–2.85 (m, 8H), 1.60–1.52 (m, 2H), 1.42–1.33 (m, 2H), 1.30–1.14 (m, 4H). ^{13}C NMR (125 MHz, CDCl$_3$): δ = 152.3, 135.0, 134.9, 130.7, 130.1,

129.8, 128.7, 123.5, 119.1, 115.5, 70.1, 45.7 (2C), 43.2, 37.6, 29.5, 29.0, 25.9, 25.0. MS calcd for $[C_{19}H_{28}N_2O_5S_2]$: *m/z* 428.57341; found 428.580 $[M+H]^+$.

3.6. *N*-Dansyl-1-azido-6-aminohexane (7)

To a 4% solution of mesylate **6** (257 mg, 0.60 mmol) in *N,N*-dimethylformamide, sodium azide (119 mg, 3 equiv) was added and the mixture was stirred at 90 °C for 2 h whenTLC indicated completed conversion of the starting material. The reaction mixture was concentrated under reduced pressure and the residue was partitioned between water and dichloromethane. The aqueous layer was extracted with dichloromethane and the combined organic layers were dried (Na_2SO_4). After filtration and removal of the solvent under reduced pressure the residue was purified by chromatography to give 205 mg (90%) of product **7**. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.53 (d, 1H, *J* = 8.5 Hz), 8.32 (d, 1H, *J* = 8.5 Hz), 8.24 (d, 1H, *J* = 7.3 Hz), 7.58–7.49 (m, 2H), 7.18 (d, 1H, *J* = 7.5 Hz), 5.01 (m, 1H), 3.10 (m, 2H), 2.93–2.84 (m, 8H), 1.42–1.30 (m, 4H), 1.18–1.07 (m, 4H); ^{13}C NMR (125 MHz, CDCl^3): δ = 152.2, 135.0, 130.6, 130.1, 129.8, 129.75, 128.6, 123.4, 119.0, 115.4, 51.4, 45.7 (2C), 43.4, 29.5, 28.7, 26.2, 26.1. MS calcd for $[C_{18}H_{25}N_5O_2S]$: *m/z* 375.49625; found 376.500 $[M+H]^+$.

3.7. *N*-Dansyl-1,6-diaminohexane (8)

To a 1% solution of azide **7** (387 mg, 1.03 mmol) in MeOH, Pd/C (10%, 86 mg) was added and the mixture was stirred under an atmosphere of hydrogen at ambient pressure for 1 h. After filtration, the solvent was removed under reduced pressure and the remaining residue was purified by chromatography on silica gel employing a mixture of CHCl$_3$–MeOH–concd NH$_4$OH (30:10:1 v/v/v) as the solvent system to give pure product **8** (280 mg, 78%) as a fluorescent syrup. ^{1}H NMR (500 MHz, CD$_3$OD): δ = 8.53 (m, 1H), 8.36 (m, 1H), 8.17 (m, 1H), 7.59–7.51 (m, 2H), 7.23 (m, 1H), 2.87–2.79 (m, 8H), 2.52–2.45 (m, 2H), 1.34–1.20 (m, 4H), 1.15– 1.00 (m, 4H); ^{13}C NMR (125 MHz, CD$_3$OD): δ = 153.1, 137.2, 131.1, 131.0, 130.9, 130.1, 129.0, 124.3, 120.6, 116.4, 45.8 (2C), 43.7, 42.1, 32.9, 30.4, 27.2. MS calcd for $[C_{18}H_{27}N_3O_2S]$: *m/z* 349.49879; found 350.510 $[M+H]^+$.

3.8. Bis(6-dansylaminohexyl)amine (9)

To a solution of mesylate **6** (28 mg, 70 μmol) and amine **8** (23 mg, 66 μmol) in *N,N*-dimethylformamide (1 mL) Na$_2$CO$_3$ (42 mg, 0.40 mmol) was added and the mixture was stirred at 55 °C for 48 h. The solvent was removed under reduced pressure and the remaining material was purified by chromatography on silica gel employing CHCl$_3$–MeOH–concd NH$_4$OH 70:10:1 v/v/v as the solvent system to give product **9** (23 mg, 51%) as a slightly yellow syrup. ^{1}H NMR (500 MHz, CDCl$_3$): δ = 8.55–8.50 (m, 2H), 8.31– 8.26 (m, 2H), 8.25–8.20 (m, 2H), 7.57–7.48 (m, 4H), 7.19–7.15 (m, 2H), 2.90–2.84 (m, 16H), 2.46 (t, 4H, *J* = 7.3 Hz), 1.40–1.28 (m, 8H), 1.20–1.08 (m, 8H); ^{13}C NMR (125 MHz, CDCl$_3$): δ = 152.2, 135.0, 130.6, 130.1, 130.0 (2C), 128.6, 123.5, 119.0, 115.4, 49.9, 45.7 (2C), 43.4, 29.8, 29.6, 26.8, 26.4. MS calcd for $[C_{36}H_{51}N_5O_4S_2]$: *m/z* 681.99697; found 683.010 $[M +H]^+$.

3.9. *N*-Methoxycarbonylpentyl-1,5-dideoxy-1,5-imino-D-glucitol (1d)

To a 5% aqueous solution of D-*xylo*-hexos-5-ulose[11] (**4**, 250 mg, 1.27 mmol), triethylamine (0.25 mL, 1.78 mmol), methanol (10 mL), methyl 6-aminocaproate hydrochloride (279 mg, 1.53 mmol, 1.2 equiv), and Pd(OH)$_2$ (20%, 100 mg) were added and the mixture was stirred under an atmosphere of hydrogen for 72 h. The catalyst was removed by filtration and washed with MeOH. Filtrate and washings were combined and concentrated under reduced pressure. For easier removal of excess aminocaproate, MeOH (10 mL) and triethylamine (0.25 mL, 1.78 mmol) followed by di-*tert*-butyldicarbonate (336 mg, 1.53 mmol) were added and the mixture was stirred at ambient temp for 30 min. The reaction mixture was concentrated under reduced pressure and the remaining residue was purified by chromatography on silica gel (CHCl$_3$–MeOH–concd NH$_4$OH, 500:100:6 v/v/v) to give known[11,16] compound **1d** (59 mg, 16% as a colorless syrup). $[\alpha]_D^{20} - 12.7$ (*c* 2.0, MeOH); ^{13}C NMR (125 MHz, MeOH-*d*^{4}): δ = 175.9 (C-6′), 80.5, 72.0, 70.7, 67.3, 59.4, 57.7 (C-1, C-2, C-3, C-4, C-5, C-6), 53.5 (OMe), 52.0 (C-1′), 34.7, 28.0, 25.6, 24.8 (C-2′, C-3′, C-4′, C-5′). MS: calcd for [C$_{13}$H$_{25}$NO$_6$]: *m/z* 291.34730; ESIMS found: [M+H]$^+$ 292.510, [M+Na]$^+$ 314.250.

3.10. *N*-(Dansylamino)hexylaminocarbonylpentyl-1,5-dideoxy-1,5-imino-D-glucitol (10)

To an aqueous solution (3 mL) of *N*-methoxycarbonylpentyl-1-deoxynojirimycin (**1d**, 132 mg, 0.45 mmol), 0.5 M aqueous NaOH (1.0 mL, 0.5 mmol) was added and the mixture was kept at ambient temperature for 90 min. The mixture was brought to pH 5 by the addition of aqueous HCl (5%) and the solvent was removed under reduced pressure. Mono-*N*-dansyl-1,6-diaminohexane (**6**, 200 mg, 0.57 mmol) in dichloromethane (5 mL) was added to this crude **1e** and the solvent was again removed under reduced pressure. The residue was dissolved in DMF (4.5 mL), triethylamine (125 μL, 0.9 mmol) and TBTU (96%, 167 mg, 0.5 mmol) were added. After completed conversion (TLC, ca. 10 min), the mixture was concentrated under reduced pressure. Dichloromethane (8 mL), triethylamine (60 mL, 0.43 μmol), and di-*t*-butyldicarbonate (35 mg, 0.16 mmol) were added to the residue to derivatize excess reagent and the mixture was stirred for 15 min. After evaporation to dryness, the residue was purified by chromatography on silica gel (CHCl$_3$–MeOH–concd NH$_4$OH, 25:10:1 v/v/v) to give compound **10** (120 mg, 43%) as a pale yellow syrup. $[\alpha]_D^{20} - 5.7$ (*c* 1.2, MeOH); ^{1}H NMR (500 MHz, MeOH-*d*^{4}): δ = 8.53 (d, 1H, *J* = 8.6 Hz), 8.35 (d, 1H, *J* = 8.6 Hz), 8.18 (d, 1H, 7.1 Hz), 7.60–7.52 (m, 2H), 7.25 (m, 1H), 3.89–3.83 (m, 2H), 3.55–3.46 (m, 1H), 3.38 (dd, 1H, *J* = 9.1 Hz, *J* = 9.6 Hz), 3.07–2.97 (m, 3H), 2.95–2.77 (m, 10H), 2.63 (m, 1H), 2.26 (dd, 1H, *J* = 10.9 Hz, *J* = 11.2 Hz), 2.18–2.11 (m, 2H), 1.66–1.45 (m, 4H), 1.34–1.21 (m, 6H), 1.15–1.00 (m, 4H); ^{13}C NMR (125 MHz, MeOH-*d*^{4}): δ = 175.9 (C-6′), 153.1, 137.2, 131.1, 131.0, 130.9, 130.1, 129.0, 124.3, 120.6, 116.4 (dansyl), 80.2, 71.6, 70.4, 67.3, 58.9, 57.3 (C-1, C-2, C-3, C-4, C-5, C-6), 53.6, 45.8, 43.7, 40.1 (1′, 1″, 6″, NMe$_2$), 36.9, 30.4, 30.1, 28.0, 27.2, 27.0, 26.8, 24.7. MS: calcd for [C$_{30}$H$_{48}$N$_4$O$_7$S]: *m/z* 608.80366; ESIMS found: [M+H]$^+$ 609.8506, [M+Na]$^+$ 631.7701.

3.11. *N*-Bis(dansylaminohexyl)aminocarbonylpentyl-1,5-dideoxy-1,5-imino-D-glucitol (11)

To an aqueous solution (1.5 mL) of *N*-methoxycarbonylpentyl-1-deoxynojirimycin (**1d**, 32 mg, 0.11 mmol), 0.5 M aqueous NaOH (0.30 mL, 0.15 mmol) was added and the mixture

was kept at ambient temperature for 60 min. The mixture was brought to pH 5 by the addition of aqueous HCl (5%) and the solvent was removed under reduced pressure. Bis(6-dansylamino)hexylamine (**9**, 100 mg, 0.15 mmol) in dichloromethane (3 mL) was added to the residue and the solvent was again removed under reduced pressure. The residue was dissolved in DMF (1.5 mL), triethylamine (30 μL, 0.22 mmol) and TBTU (96%, 39 mg, 0.12 mmol) were added. After completed conversion (TLC, ca. 10 min), the mixture was concentrated under reduced pressure and the residue was purified by chromatography (CHCl$_3$–MeOH–concd NH$_4$OH, 50:10:1 v/v/v) providing compound **11** (87 mg, 84%) as a fluorescent yellow syrup. $[\alpha]_D^{20} - 3.9$ (c 1.5, MeOH); ^{1}H NMR (500 MHz, MeOH-d^4): δ = 8.53–8.46 (m, 2H), 8.36 (d, 2H, J = 8.6 Hz), 8.20–8.14 (m, 2H), 7.58– 7.49 (m, 4H), 7.24–7.17 (m, 2H), 3.89–3.78 (m, 2H), 3.69–3.53 (m, 2H), 3.37 (dd, 1H, J = 9.3 Hz, J = 9.9 Hz), 3.15 (dd, 1H), 3.11– 2.93 (m, 5H), 2.86–2.75 (m, 17H), 2.63–2.51 (m, 1H), 2.27–2.09 (m, 4H), 1.63–1.42 (m, 4H), 1.34–1.19 (m, 11H), 1.14–1.03 (m, 4H), 1.03–0.93 (m, 4H); ^{13}C NMR (125 MHz, MeOH-d^4): δ = 174.8 (C-6′), 153.1 (2C), 137.0 (2C), 131.1 (2C), 131.0 (2C), 130.9 (2C), 130.1 (2C), 129.1 (2C), 124.3 (2C), 120.6 (2C), 116.4 (2C), 80.5, 73.5, 71.9, 70.7, 67.3, 62.2, 59.3, 57.6, 53.6, 46.8, 45.9, 43.7, 43.6, 33.8, 30.4, 30.3, 29.7, 28.3, 28.2, 27.2, 27.1, 27.0 (2C), 26.5, 25.0. MS: calcd for [C$_{48}$H$_{72}$N$_6$O$_9$S$_2$]: m/z 941.27184; ESIMS found: [M+H]$^+$ 942.2888, [M+Na]$^+$ 964.2890.
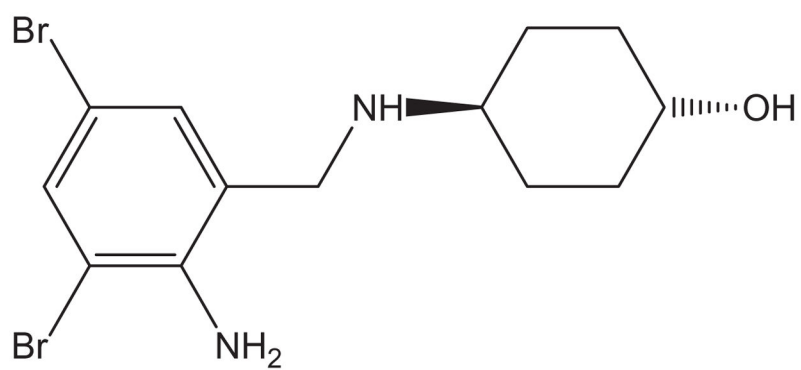
Acknowledgments

References

1. Iminosugars—From Synthesis to Therapeutic Applications; Compain P, Martin OR. Wiley. Chichester2007Martin OR, Compain P. Iminosugars: Recent Insights into their Bioactivity and Potential as Therapeutic Agents. Current Topics in Medicinal Chemistry. Betham Books2003:471–591.Wrodnigg TM. Schmid W, Stütz AE. From Lianas to Glycobiology Tools: Twenty-five Years of 2,5-dideoxy-2,5-imino-D-mannitol. Timely Research Perspectives in Carbohydrate Chemistry. SpringerVienna, New York2002:43–76.Heightman TD, Vasella AT. Angew Chem, Int Ed. 1999; 38:750–770.Stütz AE. Iminosugars as Glycosidase Inhibitors. Wiley-VCHWeinheim1999

2. Compain, P., Martin, OR. Iminosugars—From Synthesis to Therapeutic Applications. Compain, P., Martin, OR., editors. Wiley; Chichester: 2007. p. 1-6.

3. De Raadt, A., Ekhart, C., Legler, G., Stütz, AE. Iminosugars as Glycosidase Inhibitors. Stütz, AE., editor. Wiley-VCH; Weinheim: 1999. p. 207-215.

4. Wennekes T, van den Berg RJBHN, Boot RG, van der Marel GA, Overkleeft HS, Aerts JMFG. Angew Chem, Int Ed. 2009; 48:8848–8869.

5. Fan, J-Q. Iminosugars—From Synthesis to Therapeutic Applications. Compain, P., Martin, OR., editors. Wiley; Chichester: 2007. p. 225-247.

6. Butters, T. Iminosugars—From Synthesis to Therapeutic Applications. Compain, P., Martin, OR., editors. Wiley; Chichester: 2007. p. 249-268.

7. Brumshtein B, Aguilar-Moncayo M, Garcia-Moreno MI, Ortiz Mellet C, Garcia Fernandez JM, Silman I, Shaaltiel Y, Aviezer D, Sussman JL, Futerman AH. ChemBioChem. 2009; 10:1480–1485. [PubMed: 19437524]

8. Zhu XX, Sheth KA, Li S, Chang HH, Fan JQ. Angew Chem, Int Ed. 2005; 44:7450–7453.

9. Yu Z, Sawkar AR, Whalen L, Wong CH, Kelly JW. J Med Chem. 2007; 50:94–100. [PubMed: 17201413]

10. Wennekes T, van den Berg RJBHN, Donker W, van der Marel GA, Strijland A, Aerts MFG, Overkleeft HS. J Org Chem. 2007; 72:1088–1097. [PubMed: 17243712]

11. Steiner AJ, Stütz AE, Tarling CA, Withers SG, Wrodnigg TM. Carbohydr Res. 2007; 342:1850–1858. [PubMed: 17442281]

12. Steiner AJ, Stütz AE, Wrodnigg TM, Tarling CA, Withers SG, Hermetter A, Schmidinger H. Bioorg Med Chem Lett. 2008; 18:1922–1925. [PubMed: 18281216]

13. Hettkamp H, Legler G, Bause E. Eur J Biochem. 1984; 142:85–89. [PubMed: 6235111]

14. Maegawa GHB, Tropak MB, Buttner JD, Rigat BA, Fuller M, Pandit D, Tang L, Kornhaber GJ, Hamuro Y, Clarke JTR, Mahuran DJ. J Biol Chem. 2009; 284:23502–23516. [PubMed: 19578116]

15. Prade H, Mackenzie LF, Withers SG. Carbohydr Res. 1998; 305:371–381.Kempton JB, Withers SG. Biochemistry. 1992; 31:9961–9969. [PubMed: 1390780]

16. Nishimura K, Mori K, Yoshikuni Y, Fujita T. Nippon Noyaku Gakkaishi. 1990; 15:237–239.

1	R = H	**2**	**3**
1a	R = $(CH_2)_3CH_3$		
1b	R = $(CH_2)_8CH_3$		
1c	R = $(CH_2)_2OH$		
1d	R = $(CH_2)_5CO_2Me$		
1e	R = $(CH_2)_5CO_2H$		
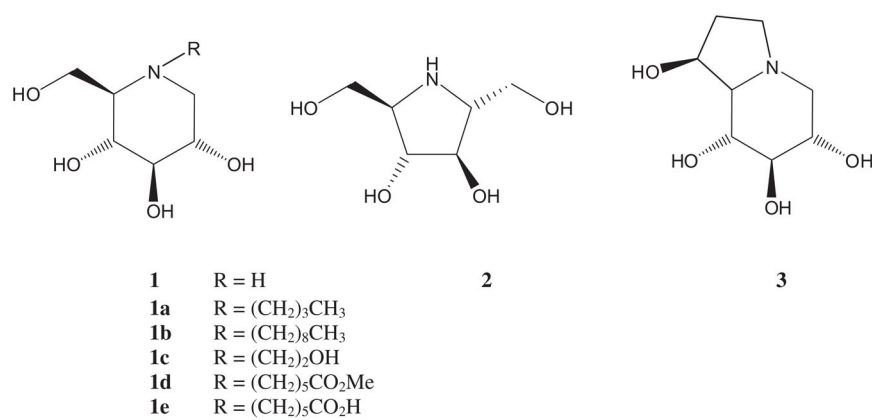
Figure 1.
Typical glucosidase inhibitors.
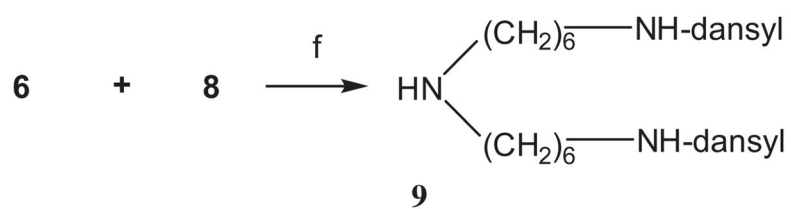
Figure 2.
Structure of Ambroxol (ABX).
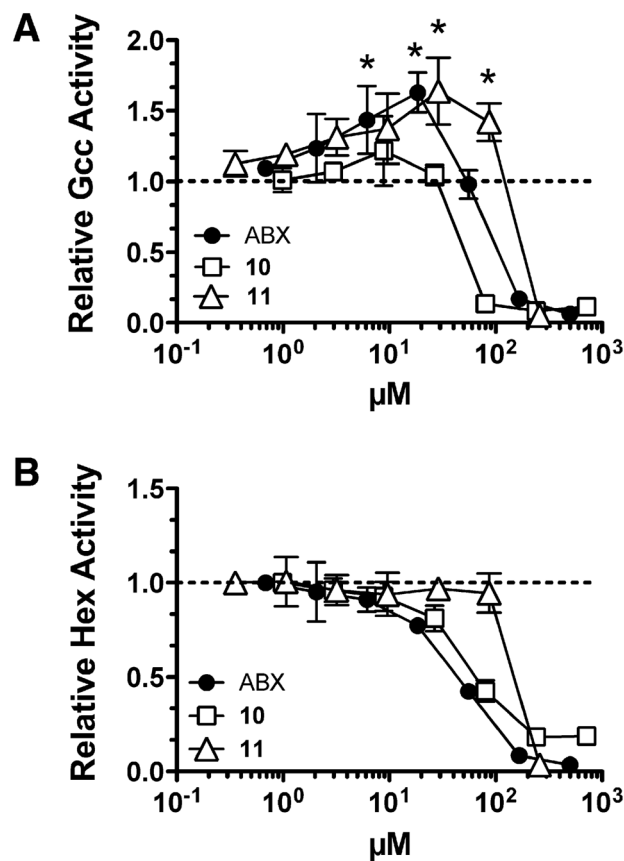
Figure 3.
Relative lysosomal β-glucosidase and β-*N*-acetylhexosaminidase activities in Gaucher
fibroblast (N370S/N370S) cell-line treated with ABX, **10** and **11**. Lysosomal enzyme
activities of β-glucosidase (A) and β-*N*-acetylhexosaminidase (B) for each dose of ABX, **10**
or **11** were normalized to activities found in lysates from vehicle (0.1% DMSO) treated
patient cells. All treatments were performed in triplicate. Treatment groups showing a
statistically significant increase relative to DMSO treated cells are marked with an asterisk
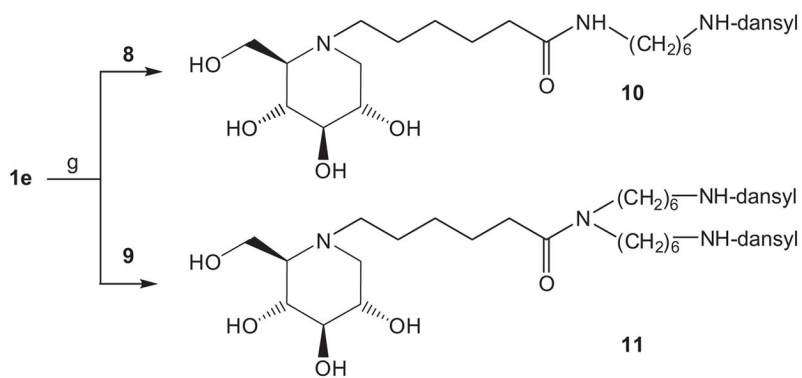($p < 0.05$). Dashed line demarcates relative activity levels greater or less than 1.
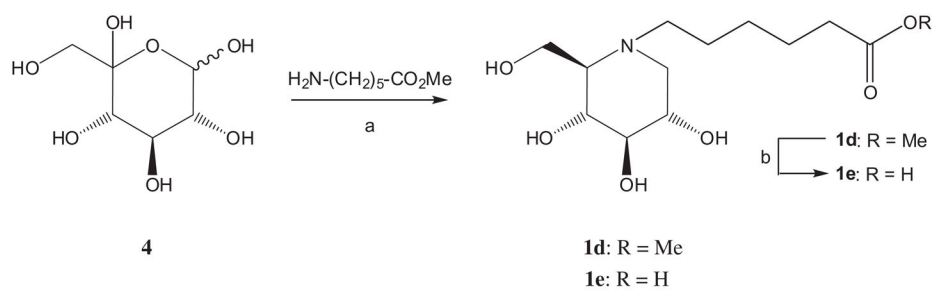
Scheme 1.
Reagents and conditions: (a) H_2, Pd–C, MeOH, 69%; (b) NaOH, dioxane–water, 95%.
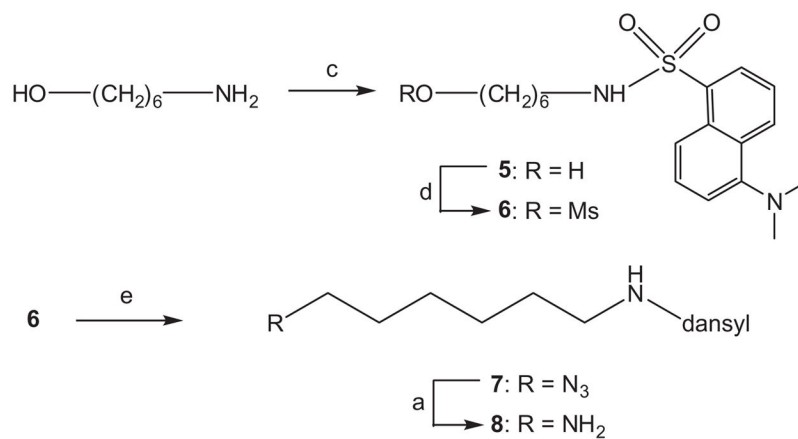
Scheme 2.
Reagents and conditions: (c) dansylCl, NEt$_3$, CH$_2$Cl$_2$; 68%; (d) MsCl, NEt$_3$, CH$_2$Cl$_2$; 92%; (e) NaN$_3$, DMF, 90%.

$$6 \quad + \quad 8 \xrightarrow{f} HN \Big\langle \begin{array}{l} (CH_2)_{\overline{6}}\!-\!NH\text{-dansyl} \\ (CH_2)_{\overline{6}}\!-\!NH\text{-dansyl} \end{array}$$

9

Scheme 3.
Reagents and condition: (f) Na_2CO_3, DMF, 51%.

Scheme 4.
Reagents and conditions: (g) DBTU, NEt$_3$, DMF (**10**: 43%; **11**: 84%).