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1-Deoxygalactonojirimycin-lysine hybrids as potent D-galactosidase inhibitors

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ABSTRACT

Cyclization by double reductive amination of L-*arabino*-hexos-5-ulose with suitably protected D- as well as L-lysine derivatives provided 1-deoxygalactonojirimycin lysine hybrids without any observable epimer formation at C-5. Modifications on the lysine moiety by acylation gave access to lipophilic derivatives which exhibited excellent D-galactosidase inhibitory activities.

1. Introduction

Iminoalditols are well-known, (usually) competitive, glycosidase inhibitors.[1] Representatives of this class of compounds, for example, glucosidase inhibitor **1**, mannosidase inhibitor **2** as well as galactosidase inhibitor **3** (Fig. 1) have found important roles as biological probes such as in the investigation of glycoprotein trimming glycosidases.[2]

Various *N*-alkylated derivatives including the *N*-butyl (**1a**), *N*-nonyl (**1b**) and *N*-hydroxyethyl (**1c**) derivatives of compound **1** and the *N*-nonyl analogue (**3a**) of inhibitor **3** (Fig. 1) have become pharmaceutical agents in the treatment of diabetes type II symptoms and other metabolic disorders including Gaucher's disease.[3] Earlier, it had been demonstrated that immobilized *N*-alkylated iminoalditols can be employed as affinity ligands in glycosidase isolation and purification protocols.[4]

Following up on our observation that some fluorescently labeled derivatives of the D-glucosidase inhibitor 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) are powerful inhibitors exceeding the parent compound's activity by two orders of magnitude[5] 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.[6] Based on their encouraging inhibitory activities and with a view to the preparation of glycosidase-recognizing arrays by immobilization on surfaces we envisaged more convenient properties with compounds providing a suitably positioned amine for tagging as well as an additional 'handle' for chain-extensions.[7]

Relying on recent literature that lipophilic derivatives of various iminoalditols such as *N*-butyl- (**1a**) and *N*-nonyl-1-deoxynojirimycin (**1b**), as well as other examples such as adamantyl substituted compounds[8] have shown promise as molecular chaperones[9] for the experimental treatment of various lysosomal storage disease related cell lines, we have now extended our range of compounds to meet structural requirements for these interesting applications.

2. Results and discussion

Initially pyranoid ulososide **4**, prepared by Barili's method[10] was employed as the sugar component but isolated yields of this compound ranging around 30% did not match our expectations. Searching for a better yielding approach, acid treatment of compound **4** in benzyl alcohol gave 5-ulofuranoside **5** (Scheme 1) in essentially quantitative yield.

This turned out to be a very efficient reaction partner for the amines employed in the catalytic hydrogenation/reductive amination step (Scheme 2).

As amine components for the double reductive amination step, partially protected D- as well as L-lysine derivatives featuring one free amino group proved to be useful reaction partners in reactions with 5-ulohexoses[11] to provide lysine-iminoalditol hybrids[12]

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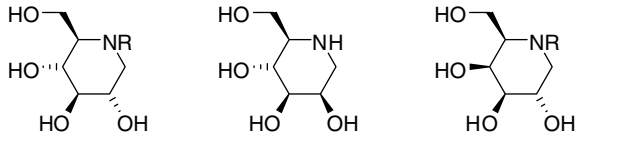
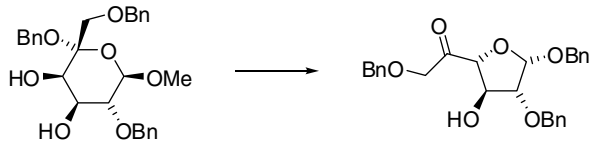


1 R = H
1a R = n-Bu
1b R = n-Non
1c R = $(CH_2)_2OH$

2

3 R = H
3a R = n-Non

Figure 1. Structures of compounds **1**–**3**.



4 **5**

Scheme 1. Rearrangement of compounds **4**–**5**.

(Scheme 2) ready for a wide range of interesting follow-up modifications.[13]

Despite the complexity of this hydrogenation/reductive amination step 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 cyclization with formation of a chiral center at C-5, isolated yields were fair and the stereoselectivities for the desired D-*galacto* configuration were excellent in all cases probed due to the favorable influence of the C-4 substituent.[13]

Thus, compound **5**, with 6-*N*-benzyloxycarbonyl-2-*N*-BOC methyl lysinate **A** as well as with the chain extended amide **B** gave iminogalactitol derivatives **6** (70%) and **7** (73%), respectively. In both cases, epimer formation at C-5 could not be detected by NMR. Likewise, reaction with tripeptide **C** gave exclusively D-*galacto* derivative **8**, albeit in 67% isolated yield.

Compounds **6**, **7**, as well as **8** were smoothly converted into the corresponding *N*-dansyl derivatives 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 **6a**, **7a**, and **8a**, respectively (Scheme 2).

For comparison in the glycosidase inhibition assays, D-lysine derivative **9**, was also prepared (89%) and converted into *N*-dansyl derivative **9a** (69%).

Reaction of compound **5** with the α-amine of suitably protected lysine **E** gave (Scheme 3) an inseparable mixture of the iminosugar substituted lysine methyl ester **10** (42%) and the lactone **11** (20%). Conventional BOC removal and N-dansylation gave the corresponding mixture of fluorescent derivatives **10a** and **11a** which for analytical purposes was converted into stable amide **12** by treatment with aqueous ammonia.

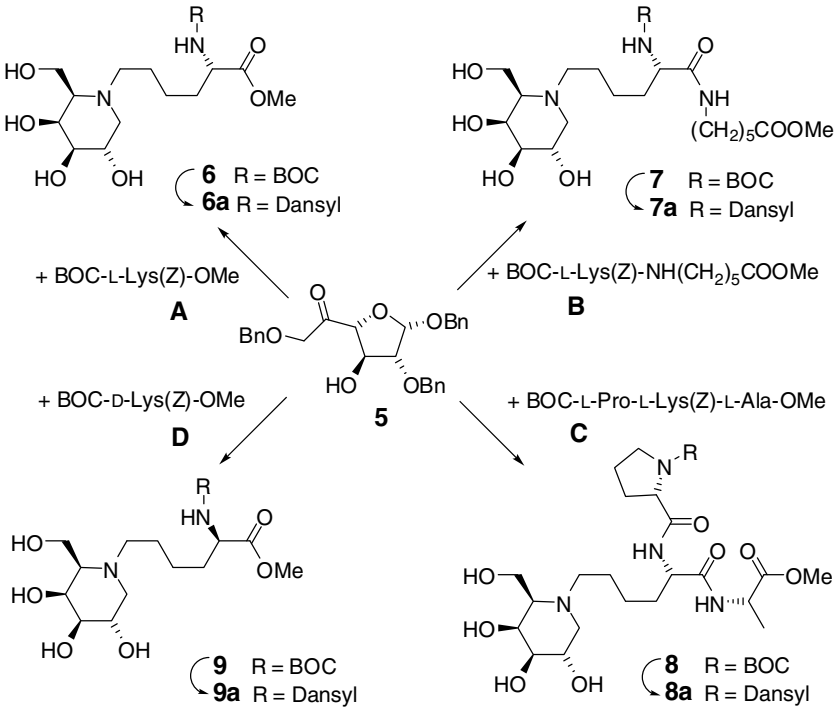
Inhibitory activities of the new compounds against a standard β-galactosidase that also has β-glucosidase activity (*Agrobacterium* sp. β-glycosidase) are summarized in Table 1. As previously[6] observed, the presence of a dansyl residue was found beneficial for inhibitory potencies when compared to the activity of unsubstituted parent compound **3**. Conveniently, the second functional 'handle', the terminal carboxylic moiety of the amino acid, allows for attachment, via linkers, to suitable surfaces.

In preliminary experiments, compound **7a** which also shows inhibitory activity against human lysosomal β-galactosidase (IC_{50}, 7.8 μM) has exhibited significant improvements of the β-galactosidase activity in lysosomal β-galactosidase deficient cell lines. Detailed results will be published in due course.

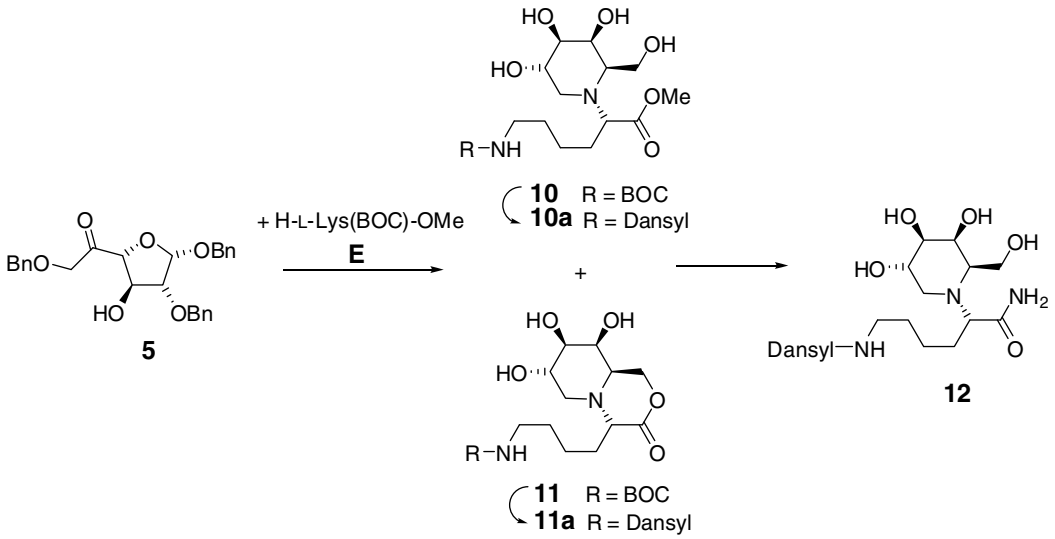
3. Experimental

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



Scheme 2. Synthesis of iminoalditol-lysine hybrids **6**–**9**.



Scheme 3. Synthesis of iminoalditol-lysine hybrid **12**.

Table 1
Inhibitory activities of compounds with *Agrobacterium* sp. β-glycosidase.

Compound	K_i [μm]	Compound	K_i [μm]	Compound	K_i [μm]
3	100	**7a**	10	**9a**	21
8	1900	**8a**	140	**12**	170
6a	3.1				

at 500.619 MHz (^{1}H), and at 125.894 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 m effective 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. Preparative TLC was performed on precoated glass plates 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

Agrobacterium sp. β-galactosidase/-glucosidase was purified and assayed as described.[14] 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 this 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 mM to 0.6 mM. Data were analyzed by direct fit to the Michaelis–Menten equation describing reaction in the presence of inhibitors using the program GraFit.

The inhibitory activity of compound **7a** with human lysosomal β-galactosidase was evaluated using 4-nitrophenyl β-D-galactopyranoside (5 mM) as the substrate and a lysosomal enzyme enriched fraction derived from human placenta[15] as the enzyme source. Reactions were performed at 37 °C in pH 4.5 citrate phosphate buffer (100 mM).

3.3. General intramolecular reductive amination procedure

To a 0.03 m solution of **5** in MeOH/H_2O (15:1, v/v), $Pd(OH)_2$/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 1 h. One equivalent of the respective peptide component was added and the reaction was continued for 24 h. After filtration and removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 500:100:6, v/v/v), yielding the target compound as a colorless syrup. The formation of L-*altro*-configured products was not observed in any of the reported cases.

3.4. General procedure for BOC removal and N-dansylation

A 0.01 m solution of the respective BOC-protected iminosugar-amino 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 dissolved in dry DMF, to give 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, $CHCl_3$/MeOH/concd NH_4OH, 300:100:4, v/v/v, extraction with MeOH) gave the desired target compound.

3.5. Benzyl 2,6-di-*O*-benzyl-β-L-*arabino*-hexofuranoside-5-ulose (5)

A mixture of methyl (5*R*)-2,6-di-*O*-benzyl-5-*C*-benzyloxy-α-L-*arabino*-hexopyranoside (**4**) (2.86 g, 5.95 mmol), benzyl alcohol

(10 mL, 96 mmol) and *p*-toluenesulfonic acid monohydrate (1.0 g, 5.3 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 24 h. CH_2Cl_2 was added and the organic phase was washed with satd aqueous $NaHCO_3$, dried over Na_2SO_4 and filtered. Removal of the solvents under reduced pressure and purification of the residue by column chromatography on silica gel (cyclohexane/ethyl acetate, 4:1, v/v) gave pure **5** (2.63 g, 5.86 mmol, 99%) as colorless crystals; mp 92–93 °C; $[\alpha]_D^{20}$ +87.2 (c = 1.3, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.99 (d, $J_{1,2}$ = 4.4 Hz, 1H; H-1), 4.56 (dd, $J_{2,3}$ = 7.3 Hz, $J_{3,4}$ = 6.3 Hz, 1H; H-3), 4.43 (d, $J_{6a,6b}$ = 18.1 Hz, 1H; H-6a), 4.39 (d, 1H; H-4), 4.35 (d, 1H; H-6b), 3.91 (dd, 1H; H-2). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 206.2 (C-5), 100.0 (C-1), 86.0, 83.1 (C-2, C-3), 75.8 (C-4), 69.9 (C-6). Anal. Calcd for $C_{27}H_{28}O_6$: C, 72.30; H, 6.29. Found: C, 72.23; H, 6.35. MS: Calcd for $[C_{27}H_{28}O_6]$: m/z 448.521; ESIMS found: $[M+H]^+$ 449.57, $[M+Na]^+$ 471.52.

3.6. Methyl N^2-*tert*-butyloxycarbonyl-N^6-(1,5-dideoxy-1,5-imino-D-galactitol-1,5-diyl)-L-lysinate (6)

Following general procedure 3.3., compound **5** (135 mg, 0.301 mmol) was reacted with BOC-L-Lys(Z)-OMe (**A**, 1.3 equiv) to give iminoalditol **6** (86 mg, 70%): $[\alpha]_D^{20}$ −16.2 (c = 0.9, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 4.09 (m, 1H), 3.98 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.80 (m, 3H; H-2, H-6a, H-6b), 3.22 (dd, $J_{2,3}$ = 9.3 Hz, 1H; H-3), 2.98 (dd, $J_{1eq,1ax}$ = 11.2 Hz, $J_{1eq,2}$ = 4.9 Hz, 1H; H-1eq), 2.73 (m, 1H), 2.50 (m, 1H), 2.40 (m, 1H; H-5), 2.14 (dd, $J_{1ax,2}$ = 10.7 Hz, 1H, H-1ax), 1.77 (m, 1H), 1.66 (m, 1H), 1.56–1.30 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.9 (C=O), 76.0 (C-3), 71.1 (C-4), 67.8 (C-2), 64.1 (C-5), 61.3 (C-6), 57.1 (C-1), 53.8, 52.5, 31.3, 23.6, 23.5. MS: Calcd for $[C_{18}H_{34}N_2O_8]$: m/z 406.480; ESIMS found: $[M+H]^+$ 407.51, $[M+Na]^+$ 429.50.

3.7. Methyl 6-[N^2-(*tert*-butyloxycarbonyl)-N^6-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinyl] aminohexanoate (7)

Following Section 3.3, compound **5** (175 mg, 0.39 mmol) was reacted with BOC-L-Lys(Z)-NH-$(CH_2)_5CO_2Me$ (**B**, 1.2 equiv) to give iminoalditol **7** (140 mg, 69%): $[\alpha]_D^{20}$ −15.9 (c = 1.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 3.97 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.95 (m, 1H), 3.80 (m, 3H; H-2, H-6a, H-6b), 3.21 (dd, $J_{2,3}$ = 9.3 Hz, 1H; H-3), 3.25–3.12 (m, 2H), 2.98 (dd, $J_{1eq,1ax}$ = 11.2 Hz, $J_{1eq,2}$ = 4.9 Hz, 1H; H-1eq), 2.72 (m, 1H), 2.50 (m, 1H), 2.38 (m, 1H; H-5), 2.32 (t, 2H), 2.11 (dd, $J_{1ax,2}$ = 10.7 Hz, 1H, H-1ax), 1.75–1.26 (m, 12H); ^{13}C NMR (125 MHz, CD_3OD): δ = 174.6, 174.0 (2 C=O), 76.1 (C-3), 71.1 (C-4), 67.8 (C-2), 64.2 (C-5), 61.3 (C-6), 56.8 (C-1), 55.0, 52.6, 38.9, 33.5, 32.2, 28.9, 26.2, 24.5, 23.7, 23.6. MS: Calcd for $[C_{24}H_{45}N_3O_9]$: m/z 519.641; ESIMS found: $[M+H]^+$ 520.66, $[M+Na]^+$ 542.70.

3.8. Methyl *N*-(*tert*-butyloxycarbonyl)-L-prolinyl-N^6-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinyl-L-alaninate (8)

Following Section 3.3, compound **5** (139 mg, 0.310 mmol) was reacted with BOC-L-Pro-L-Lys(Z)-L-Ala-OMe (**C**, 1.2 equiv) to give iminoalditol **8** (87 mg, 48.8%): $[\alpha]_D^{20}$ −55.7 (c = 0.6, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 4.42–4.20 (m, 3H), 3.98 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.81 (m, 3H; H-2, H-6a, H-6b), 3.50 (m, 1H), 3.40 (m, 1H), 3.22 (dd, $J_{2,3}$ = 9.3 Hz, 1H; H-3), 2.99 (dd, $J_{1eq,1ax}$ = 11.2 Hz, $J_{1eq,2}$ = 4.9 Hz, 1H; H-1eq), 2.75 (m, 1H), 2.55 (m, 1H), 2.42 (m, 1H; H-5), 2.16 (dd, $J_{1ax,2}$ = 10.3 Hz, 1H, H-1ax), 2.28–1.46 (m, 10H), 1.39 (d, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ = 174.3, 173.3, 172.8 (3 C=O), 76.0 (C-3), 71.0 (C-4), 67.7 (C-2), 64.2 (C-5), 61.2 (C-6), 60.2, 56.7 (C-1), 53.2, 52.6, 49.1, 46.8, 32.2, 31.3, 23.7, 23.4, 23.4, 16.1. MS: Calcd for $[C_{26}H_{46}N_4O_{10}]$: m/z 574.677; ESIMS found: $[M+H]^+$ 575.70, $[M+Na]^+$ 597.69.

3.9. Methyl N^2-*tert*-butyloxycarbonyl-N^6-(1,5-dideoxy-1,5-imino-D-galactitol-1,5-diyl)-D-lysinate (9)

Following Section 3.3, compound **5** (210 mg, 0.468 mmol) was reacted with BOC-D-Lys(Z)-OMe (**D**, 1.25 equiv) to give iminoalditol **9** (145 mg, 76%): $[\alpha]_D^{20}$ −5.8 (c = 2.4, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 4.09 (m, 1H), 3.98 (dd, $J_{3,4}$ = 2.9 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.80 (m, 3H; H-2, H-6a, H-6b), 3.23 (dd, $J_{2,3}$ = 9.3 Hz, 1H; H-3), 2.99 (dd, $J_{1eq,1ax}$ = 11.2 Hz, $J_{1eq,2}$ = 4.9 Hz, 1H; H-1eq), 2.72 (m, 1H), 2.51 (m, 1H), 2.40 (m, 1H; H-5), 2.14 (dd, $J_{1ax,2}$ = 10.3 Hz, 1H, H-1ax), 1.78 (m, 1H), 1.65 (m, 1H), 1.56–1.28 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.9 (C=O), 76.0 (C-3), 71.0 (C-4), 67.7 (C-2), 64.1 (C-5), 61.2 (C-6), 56.7 (C-1), 53.8, 52.6, 31.4, 23.7, 23.5. MS: Calcd for $[C_{18}H_{34}N_2O_8]$: m/z 406.480; ESIMS found: $[M+H]^+$ 407.50, $[M+Na]^+$ 429.58.

3.10. Methyl N^6-*tert*-butyloxycarbonyl-N^2-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinate (10) and lactone 11

Following Section 3.3, compound **5** was reacted with H-L-Lys (BOC)-OMe (**E**) to give an inseparable mixture of iminoalditol **10** and the corresponding lactone **11** in a ratio of 2:1. Compound **10**: ^{1}H NMR (500 MHz, CD_3OD): δ = 3.95 (m, 1H; H-4), 3.90–3.78 (m, 3H; H-2, H-6a, H-6b), 3.70 (m, 1H), 3.20 (m, 1H; H-3), 3.04 (m, 3H), 2.68 (m, $J_{4,5}$ = 1.5 Hz, 1H; H-5), 2.21 (dd, $J_{1eq,1ax}$ = 11.2 Hz, $J_{1ax,2}$ = 10.7 Hz, 1H; H-1ax), 1.85–1.32 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.5 (C=O), 75.3 (C-3), 71.7 (C-4), 68.5 (C-2), 65.3 (C-5), 62.0, 61.5 (C-1, C-6), 52.9, 40.1, 29.7, 29.5, 22.7. MS: Calcd for $[C_{18}H_{34}N_2O_8]$: m/z 406.480; ESIMS found: $[M+H]^+$ 407.55, $[M+Na]^+$ 429.60. Lactone **11**: ^{1}H NMR (500 MHz, CD_3OD): Characteristic signals: δ = 4.60 (dd, $J_{5,6a}$ = 4.0 Hz, $J_{6a,6b}$ = 11.7 Hz, 1H; H-6a), 4.53 (dd, $J_{5,6b}$ = 4.9 Hz, 1H; H-6b); ^{13}C NMR (125 MHz, CD_3OD): δ = 172.8 (C=O), 76.5 (C-3), 72.0 (C-4), 69.2, 69.1, 63.0, 59.7 (C-1, C-2, C-5, C-6), 53.2, 39.9, 29.7, 29.5, 23.4.

3.11. Methyl N^2-dansyl-N^6-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinate (6a)

Following Section 3.4, compound **6** (47 mg, 0.12 mmol) gave fluorescent iminoalditol-lysine hybrid **6a** (24 mg, 38%): $[\alpha]_D^{20}$ −3.8 (c = 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 3.95 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.76 (ddd, $J_{1eq,2}$ = 4.9 Hz, $J_{1ax,2}$ = 10.3 Hz, $J_{2,3}$ = 9.3 Hz, 1H; H-2), 3.73 (m, 1H), 3.70 (m, 2H; H-6a, H-6b), 3.18 (dd, 1H; H-3), 2.82 (dd, $J_{1eq,1ax}$ = 11.2 Hz, 1H; H-1eq), 2.40 (m, 1H), 2.30 (m, 1H), 2.27 (m, 1H; H-5), 1.99 (dd, 1H; H-1ax), 1.58–1.52 (m, 2H), 1.24–0.96 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD): δ = 172.9 (C=O), 76.1 (C-3), 71.0 (C-4), 67.8 (C-2), 63.7 (C-5), 61.1 (C-6), 56.8 (C-1), 56.1, 52.2, 32.2, 23.1, 22.7. MS: Calcd for $[C_{25}H_{37}N_3O_8S]$: m/z 539.65; ESIMS found: $[M+H]^+$ 540.0, $[M+Na]^+$ 562.2.

3.12. Methyl 6-[N^2-dansyl-N^6-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinyl]amino hexanoate (7a)

Following Section 3.4, compound **7** (97 mg, 0.19 mmol) gave fluorescent iminoalditol-lysine hybrid **7a** (48 mg, 39%): $[\alpha]_D^{20}$ −6.4 (c = 0.9, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 3.95 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.76 (ddd, $J_{1eq,2}$ = 4.9 Hz, $J_{1ax,2}$ = 10.7 Hz, $J_{2,3}$ = 9.3 Hz, 1H; H-2), 3.70 (m, 2H; H-6a, H-6b), 3.56 (m, 1H), 3.17 (dd, 1H; H-3), 2.82 (dd, $J_{1eq,1ax}$ = 11.2 Hz, 1H; H-1eq), 2.81 (m, 1H), 2.77 (m, 1H), 2.37 (m, 1H), 2.28 (m, 1H; H-5), 2.27 (m, 2H), 2.23 (m, 1H), 2.00 (dd, 1H, H-1ax), 1.58–0.88 (m, 12H), ^{13}C NMR (125 MHz, CD_3OD): δ = 174.6, 173.4 (2 C=O),

76.1 (C-3), 70.9 (C-4), 67.8 (C-2), 63.8 (C-5), 61.0 (C-6), 57.3 (C-1), 56.9, 52.3, 38.7, 33.5, 32.9, 28.5, 26.1, 24.4, 23.3, 22.9. MS: Calcd for $[C_{31}H_{48}N_4O_9S]$: m/z 652.81; ESIMS found: $[M+H]^+$ 653.79, $[M+Na]^+$ 675.75.

3.13. Methyl (*N*-dansyl)-L-prolinyl-N^6-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinyl-L-alaninate (8a)

Following Section 3.4, compound **8** (53 mg, 0.092 mmol) gave fluorescent iminoalditol-lysine hybrid **8a** (20 mg, 31%): $[\alpha]_D^{20}$ −54.9 (c = 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ = 4.44–4.28 (m, 3H), 3.97 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.80 (m, 3H; H-2, H-6a, H-6b), 3.58 (m, 1H), 3.40 (m, 1H), 3.21 (dd, $J_{2,3}$ = 9.3 Hz, 1H; H-3), 2.97 (dd, $J_{1eq,1ax}$ = 11.2 Hz, $J_{1eq,2}$ = 4.9 Hz, 1H; H-1eq), 2.70 (m, 1H), 2.50 (m, 1H), 2.36 (m, 1H; H-5), 2.10 (dd, $J_{1ax,2}$ = 10.7 Hz, 1H; H-1ax), 2.00–1.30 (m, 10H), 1.41 (d, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.3, 173.3, 172.6 (3 C=O), 76.1 (C-3), 71.1 (C-4), 67.9 (C-2), 64.1 (C-5), 61.7 (C-6), 61.2, 56.9 (C-1), 53.1, 52.5, 49.4, 48.3, 31.6, 31.2, 24.6, 23.6, 23.5, 16.2. MS: Calcd for $[C_{33}H_{49}N_5O_{10}S]$: m/z 707.850; ESIMS found: $[M+H]^+$ 708.90, $[M+Na]^+$ 729.95.

3.14. Methyl N^2-dansyl-N^6-(1,5-dideoxy-D-galactitol-1,5-diyl)-D-lysinate (9a)

Following Section 3.4, compound **9** (95 mg, 0.24 mmol) gave fluorescent iminoalditol-lysine hybrid **9a** (49 mg, 39%): $[\alpha]_D^{20}$ −27.9 (c = 1.8, MeOH); ^{1}H NMR (500 MHz, CD3OD): δ = 3.96 (dd, $J_{3,4}$ = 2.9 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.77 (ddd, $J_{1eq,2}$ = 4.4 Hz, $J_{1ax,2}$ = 10.7 Hz, $J_{2,3}$ = 9.3 Hz, 1H; H-2), 3.73 (m, 1H), 3.70 (m, 2H; H-6a, H-6b), 3.20 (dd, 1H; H-3), 2.85 (dd, $J_{1eq,1ax}$ = 10.7 Hz, 1H; H-1eq), 2.48 (m, 1H), 2.30 (m, 1H; H-5), 2.22 (m, 1H), 2.01 (dd, 1H; H-1ax), 1.62–1.50 (m, 2H), 1.30–0.98 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD): δ = 172.5 (C=O), 76.0 (C-3), 70.9 (C-4), 67.7 (C-2), 64.0 (C-5), 61.0 (C-6), 56.7 (C-1), 55.8, 52.4, 32.0, 23.0, 23.0. MS: Calcd for $[C_{25}H_{37}N_3O_8S]$: m/z 539.65; ESIMS found: $[M+H]^+$ 541.00, $[M+Na]^+$ 562.9.

3.15. Methyl N^6-dansyl-N^2-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinate (10a) and lactone 11a

Following Section 3.4, a mixture of compounds **10** and **11** gave a mixture of fluorescent iminoalditol-lysine hybrid **10a** and the corresponding lactone **11a** in a ratio of 7:3. Compound **10a**: ^{1}H NMR (500 MHz, CD_3OD): δ = 3.93 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.90–3.70 (m, 3H; H-2, H-6a, H-6b), 3.62 (m, 1H), 3.17 (dd, $J_{2,3}$ = 9.8 Hz, 1H; H-3), 2.96 (dd, $J_{1eq,1ax}$ = 11.2 Hz, $J_{1eq,2}$ = 4.9 Hz, 1H; H-1eq), 2.84 (t, 2H), 2.65 (m, 1H; H-5), 2.14 (dd, $J_{1ax,2}$ = 10.7 Hz, 1H; H-1ax), 1.64–1.00 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ = 173.3 (C=O), 76.4 (C-3), 71.9 (C-4), 68.5 (C-2), 65.3 (C-5), 62.9, 61.4 (C-1, C-6), 52.8, 42.5, 29.2, 29.0, 23.0.

MS: Calcd for $[C_{25}H_{37}N_3O_8S]$: m/z 539.65; ESIMS found: $[M+H]^+$ 540.0, $[M+Na]^+$ 562.2.

Lactone **11a**: ^{1}H NMR (500 MHz, CD_3OD): δ = 4.54 (dd, $J_{5,6a}$ = 4.0 Hz, $J_{6a,6b}$ = 11.7 Hz, 1H; H-6a), 4.38 (dd, $J_{5,6b}$ = 4.9 Hz, 1H; H-6b); ^{13}C NMR (125 MHz, CD_3OD): δ = 172.7 (C=O), 75.2 (C-3), 71.5 (C-4), 69.0, 68.9, 65.1, 59.4 (C-1, C-2, C-5, C-6), 53.0, 42.7, 29.6, 29.1, 23.8.

3.16. N^6-Dansyl-N^2-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysineamide (12)

A mixture of **10a** and **11a** (10 mg, 0.019 mmol, **10a**/**11a** = 7:3) in aqueous ammonia (1 mL, 25%) was stirred for 2 h at 20 °C. Removal of the solvent under reduced pressure yielded pure **12** (9.5 mg, 0.018 mmol, 96%) as green syrup; $[\alpha]_D^{20}$ −13.8 (c = 0.5, MeOH); ^{1} NMR (500 MHz, CD_3OD): δ = 3.87 (dd, $J_{5,6a}$ = 5.9 Hz, $J_{6a,6b}$ = 11.7 Hz, 1H; H-6a), 3.85 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H; H-4), 3.72 (dd, $J_{5,6b}$ = 3.4 Hz, 1H; H-6b), 3.62 (ddd, $J_{1eq,2}$ = 4.9 Hz, $J_{1ax,2}$ = 10.2 Hz, $J_{2,3}$ = 8.8 Hz, 1H; H-2), 3.23 (m, 1H), 3.19 (dd, 1H; H-3), 3.01 (dd, $J_{1eq,1ax}$ = 11.7 Hz, 1H; H-1eq), 2.84 (t, 2H), 2.74 (m, 1H; H-5), 2.05 (dd, 1H; H-1ax), 1.64–1.18 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ = 177.2 (C=O), 76.2 (C-3), 72.4 (C-4), 68.8 (C-2), 64.9 (C-5), 63.3, 62.6 (C-1, C-6), 51.3, 42.7, 29.6, 29.5, 23.7. MS: Calcd for $[C_{24}H_{36}N_4O_7S]$: m/z 524.641; ESIMS found: $[M+H]^+$ 525,70, $[M+Na]^+$ 547.69.

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