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Iminoalditol-amino acid hybrids: synthesis and evaluation as glycosidase inhibitors

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Abstract—Cyclization by double reductive amination of D-*xylo*-hexos-5-ulose with the terminal amino group of α-*N*-Boc-lysine methyl ester gave a 4:1-mixture of (1′*R*)-*N*-methoxycarbonyl-(1-*N*-Boc-amino)pentyl-1-deoxynojirimycin and the corresponding L-*ido* epimer whereas D-*lyxo*-hexos-5-ulose furnished the desired N-alkylated 1-deoxymannojirimycin derivative without any observable epimer formation at C-5. By subsequent modification of the lysine moiety, additional chain-extended derivatives as well as fluorescent compounds were obtained. All fluorescent iminoalditol-amino acid hybrids prepared in this study exhibited glycosidase inhibitory activities better than or comparable to the parent compounds'.

Keywords: Iminoalditol; Amino acid conjugate; Glycosidase inhibitor; Glucosidase; Mannosidase

1. Introduction

Iminoalditols are well-known, (usually) competitive, glycosidase inhibitors.[1] Representatives of this class of compounds, for example, glucosidase inhibitor **1** and mannosidase inhibitor **2** 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 (Miglitol by Bayer) and other metabolic disorders including Gaucher disease (Zavesca by Actelion) (Fig. 1). It was demonstrated that immobilized N-alkylated imino- alditols can be employed as affinity ligands in glycosidase isolation and purification protocols.[3]

We have been interested in derivatives of such compounds featuring 'added value' properties suitable for analytical purposes. 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 activity of the parent compound by two orders of magnitude.[4]

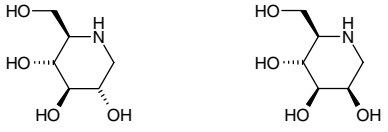

Figure 1.

In this context, we have reported syntheses and glycosidase inhibitory activities of various N-alkylated iminoalditols featuring fluorescent tags such as dansyl moieties attached to simple N-substituents.[5] Based on the encouraging inhibitory activities of these compounds, we envisaged more convenient properties with compounds providing a suitably positioned amine for tagging as well as an additional 'handle' for chain-extensions and with a view to the preparation of glycosidase-recognizing arrays by immobilization on surfaces.

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2. Results and discussion

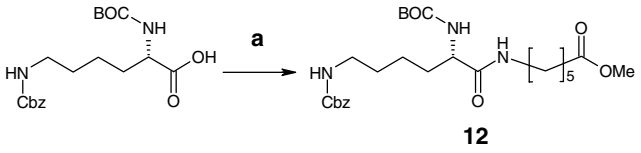
Partially protected L-lysine derivatives featuring a free terminal amino group were found to be useful reaction partners in ring-closing reactions with 5-ulohexoses by reductive amination[6] to provide lysine-iminoalditol hybrids (Scheme 1) ready for a wide range of useful follow-up modifications.

Again, the presence of a dansyl residue attached to the α-nitrogen in the L-lysine component was found to improve inhibitory potencies whereas the second functional 'handle', the terminal carboxylic moiety, can clearly be employed for the attachment, via linkers, to suitable surfaces.

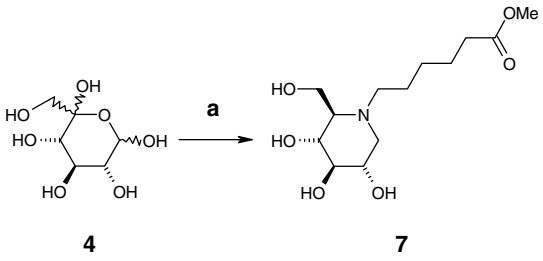
(5*R*)-Spiro-oxirane **3**,[7] readily available from methyl α-D-glucopyranoside via Garegg reaction, per-O-acetylation, AgF-mediated[8] 5,6-ene formation (78%), and *m*-CPBA oxidation (80%), gave by conventional Zemplén deprotection and acidic hydrolysis known[9] D-*xylo*-hexos-5-ulose (**4**). The latter was reacted with α-*N*-Boc-ε-*N*-benzyloxycarbonyl-L-lysine methyl ester (**5**) at ambient pressure under an atmosphere of hydrogen in the presence of $Pd(OH)_2$ (20%) on carbon. Hydrogenolytic deprotection at the terminal amine followed by double reductive amination was expected to proceed with high diastereoselectivity at the newly formed chiral centre at C-5 of desired inhibitor **6**. Contrary to previous results with methyl 6-aminohexanoate, which exclusively provided iminoglucitol **7** under the same conditions, with the amino acid, a 4:1 mixture of N-alkylated 1,5-dideoxy-1,5-imino-D-glucitol **6** and -L-iditol **8** was obtained. Separation was found to be difficult on a preparative scale. Deprotection of the secondary amine in compound **6** followed by reaction with dansyl chloride provided fluorescent iminoglucitol **9**. Chain-extended derivatives **10** and **11**, were readily available from **4** by reaction with **12**, which was prepared from commercially available α-*N*-Boc-ε-*N*-Cbz-L-lysine by standard coupling with methyl 6-aminohexanoate. Removal of the Boc group followed by N-dansylation gave compounds **13** and **14** (Schemes 2–6).

Compound **15**, featuring two fluorescent moieties of significantly different emission spectra in the same molecule was prepared by saponification of methyl ester **13** to the free acid followed by conventional coupling to the NBD fluorophor in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (Scheme 7).

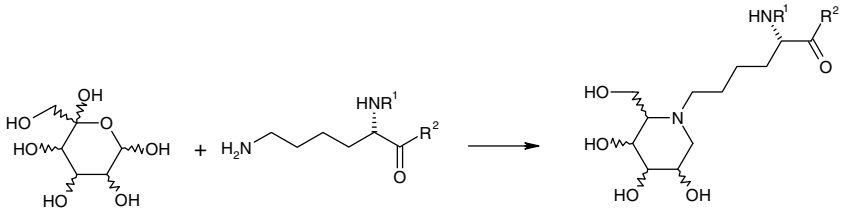
In the D-*manno* series, (5*R*)-oxirane **16**[7] (prepared in 44% overall yield from methyl α-D-mannopyranoside)



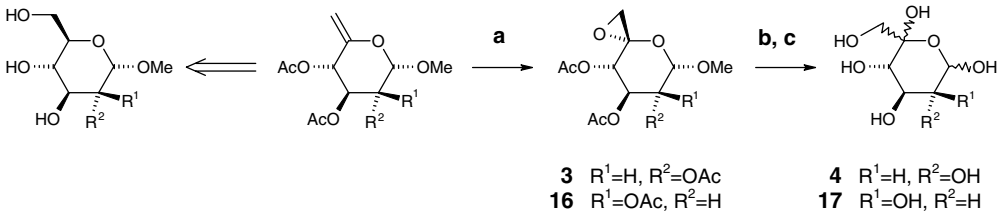
Scheme 3. Reagents and conditions: (a) methyl 6-aminohexanoate hydrochloride, NEt_3, DMF, TBTU.



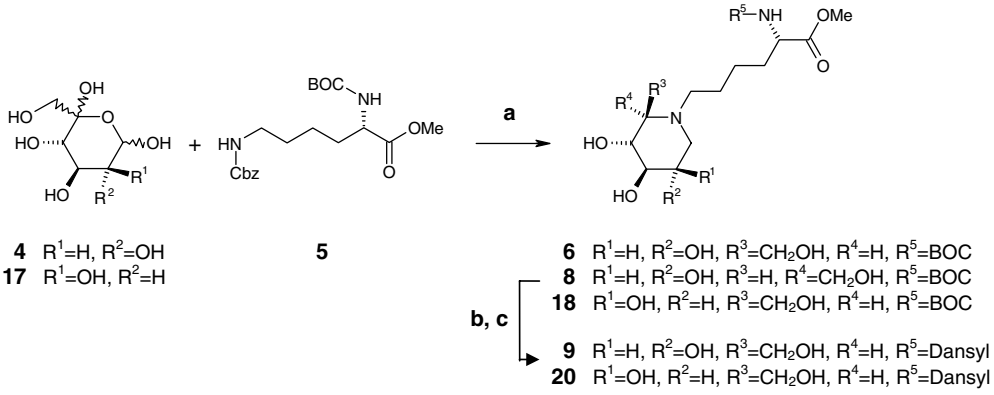
Scheme 4. Reagents and conditions: (a) methyl 6-aminohexanoate hydrochloride, aq MeOH, NEt_3, $Pd(OH)_2/C$ (20%), H_2.



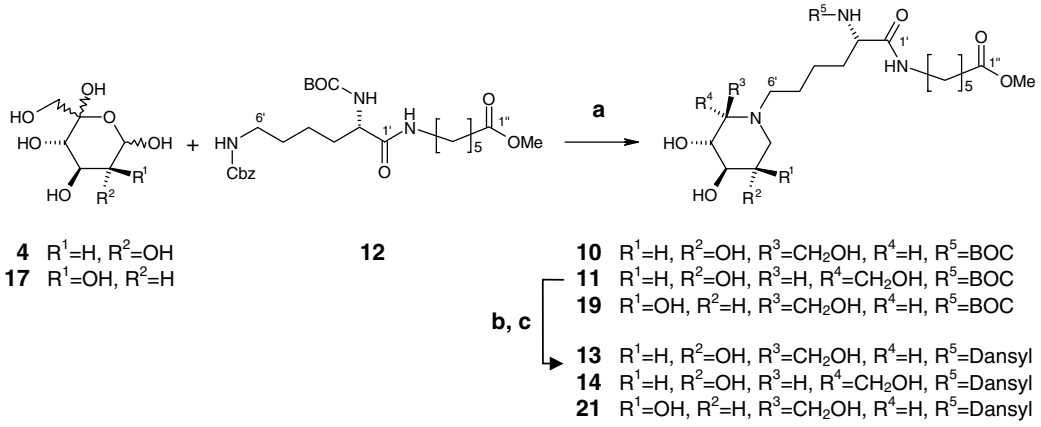
Scheme 1.



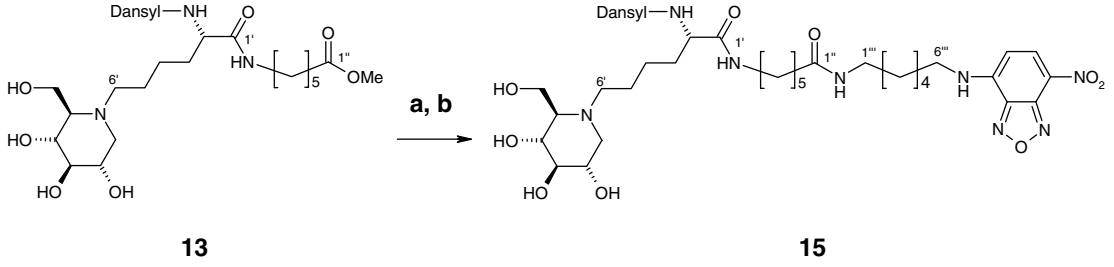
Scheme 2. Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, aq $NaHCO_3$; (b) NaOMe, MeOH, −30°C; (c) H_2O, Amberlite IR-120.



Scheme 5. Reagents and conditions: (a) MeOH, $Pd(OH)_2/C$ (20%), H_2; (b) MeOH, AcCl; (c) NEt_3, DMF, dansyl chloride.



Scheme 6. Reagents and conditions: (a) MeOH, $Pd(OH)_2/C$ (20%), H_2; (b) MeOH, AcCl; (c) NEt_3, DMF, dansyl chloride.



Scheme 7. Reagents and conditions: (a) NaOH, H_2O/dioxane (1:1 v/v); (b) DMF, NEt_3, 6-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]hexan-1-aminium trifluoroacetate, TBTU.

was efficiently hydrolyzed to give known[9] D-*lyxo*-hexos-5-ulose **17**. The latter furnished exclusively the D-*manno* configured iminoalditols **18** and **19** when reacted with L-lysine derivatives **5** and **12**, respectively. Fluorescent 1-deoxymanojirimycin derivatives **20** and **21** were prepared following the same protocols as for **9** and **13**, respectively.

Activities of compounds **9** and **13**–**15** in the D-*gluco*/L-*ido* series as well as of compounds **20** and **21** in the D-*manno* series and the respective parent compounds' **1** and **2** are shown in Table 1. In the D-*gluco* series, the new compounds prepared all exhibit better activities than the parent compound against the enzyme probed. Interestingly, despite the 'wrong' configuration at C-5, the K_i value of 9.3 μM for L-*ido* configured iminoalditol **14** is essentially the same as for unsubstituted 1-deoxynojirimycin (**1**). Similar cases of such insensitivity to this particular configurational change at C-5 have previously been observed with some 5-fluoro glycosyl fluorides as diagnostic substrates for glycosidases.[10] As previously observed for related structures,[5] the products in the *manno* series, **20** and **21**, turned out slightly less active than parent compound **2**. Nonetheless, the modifications under consideration here should be helpful tools en route to applications of iminosugars in biochemical investigations.

Table 1. K_i values of compounds, β-glucosidase, *Agrobacterium* sp.

Compd	K_i (μM)
1	12
2	18[a]
9	2.6
13	6.2
14	9.3
15	4
20	60[a]
21	79[a]

[a] α-Mannosidase, jack beans.

In conclusion, we could demonstrate that cyclization of hex-5-ulososes with the terminal amine of lysine is a suitable method to provide iminoalditol-lysine hybrids. Dansylation of the secondary amine in the lysine sub-unit provided efficient inhibitors for the glycosidases probed in this study. Based on previous work, it is expected that immobilization via the carboxylate will not significantly alter these activities thus providing new and selective tools for the construction of micro-arrays with glycosidase-scavenging and glycosidase-profiling properties.

3. Experimental

3.1. General methods

Melting points were recorded on a Tottoli apparatus and are uncorrected. 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 500.619 MHz (^{1}H), and at 125.894 MHz (^{13}C). $CDCl_3$ was employed for protected compounds and MeOH-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 Da) 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 aluminium 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-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 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 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.[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 this data ($1/V$ vs [I]) intersects the experimental line at an inhibitor concentration equal to $-K_i$.

3.3. Methyl (5*R*)-2,3,4-tri-*O*-acetyl-5,6-anhydro-5-*C*-hydroxy-α-D-*xylo*-hexopyranoside (3)

Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-*xylo*-hex-5-enopyranoside[8] (2.00 g, 6.62 mmol) was dissolved in CH_2Cl_2 (100 mL). Satd aq $NaHCO_3$ (0.6 M, 40 mL) and 3-chloroperbenzoic acid (70%, 2.00 g, 8.11 mmol, 1.2 equiv) were added and the mixture was stirred at ambient temperature for 20 h. The organic layer was washed with satd aq $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated to about 10 mL under reduced pressure at ambient temperature. Quick filtration over a short plug of silica gel (cyclohexane/ethyl acetate, 2:1 v/v) yielded product **4** as one single diastereomer (1.69 g, 5.31 mmol, 80%) as colourless syrup: $[\alpha]_D^{20}$ +91.0 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.76 (dd, 1H, $J_{2,3} = 9.8$ Hz, $J_{3,4} = 9.8$ Hz, H-3), 5.54 (d, 1H, H-4), 5.03 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1), 5.01 (dd, 1H, H-2), 3.44 (s, 3H, OCH_3), 2.85 (d, 1H, $J_{6a,6b} = 4.2$ Hz, H-6a), 2.54 (d, 1H, H-6b); ^{13}C NMR: δ 98.5 (C-1), 79.1 (C-5), 71.0, 68.3, 66.8 (C-2, C-3, C-4), 56.8 (OCH_3), 45.8 (C-6); Anal. Calcd for $C_{13}H_{18}O_9$: C, 49.06; H, 5.70. Found: C, 49.00; H, 5.76.

3.4. D-*xylo*-Hexos-5-ulose (4)

To a 2% methanolic solution of methyl (5*R*)-2,3,4-tri-*O*-acetyl-5,6-anhydro-5-*C*-hydroxy-α-D-*xylo*-hexopyranoside (**3**) (400 mg, 1.26 mmol), 1.0 M NaOMe solution (500 μL) was added dropwise at −30 °C. After 3 h reaction time at −30 °C, water (30 mL) was added and the resulting reaction mixture was concentrated to about 20 mL under reduced pressure. After addition of acetonitrile (5 mL), ion exchange resin Amberlite IR 120 [H^+] was added to the reaction mixture, which was then stirred at 45 °C for 20 h. Filtration and removal of the solvents under reduced pressure gave compound **4** (220 mg) as a slightly yellow foam. As previously observed, NMR revealed a complex mixture of several tautomeric forms.[9] The compound was used for the next step without further purification.

3.5. *N*-[5-(Methoxycarbonyl)pentyl]-1-deoxynojirimycin (7)

To a 1.5% solution of D-*xylo*-hexos-5-ulose (**4**) (510 mg, 2.60 mmol), methyl 6-aminohexanoate hydrochloride (550 mg, 3.03 mmol, 1.2 equiv) and Et_3N (200 μL, 145 mg, 1.4 mmol) in MeOH/H_2O (2:1 v/v), $Pd(OH)_2$/C (20%, 50 mg) was added and the reaction mixture was stirred under an atmosphere of hydrogen at ambient pressure for 72 h. After filtration and removal of the solvent under reduced pressure, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 300:100:4 v/v/v), yielding product **7** (437 mg, 1.50 mmol, 58%) as colourless syrup. The formation of the corresponding L-*ido*-configured product was not observed. $[\alpha]_D^{20}$ −7.0 (*c* 0.7, MeOH); ^{1}H NMR (MeOH-d_4): δ 3.96 (dd, 1H, $J_{5,6a} = 2.0$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 3.89 (dd, 1H, $J_{5,6b} = 2.9$ Hz, H-6b), 3.66 (s, 3H, OCH_3), 3.58 (ddd, 1H, $J_{1eq,2} = 4.9$ Hz, $J_{1ax,2} = 10.3$ Hz, $J_{2,3} = 8.8$ Hz, H-2), 3.47 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.25 (dd, 1H, H-3), 3.21 (dd, 1H, $J_{1eq,1ax} = 11.7$ Hz, H-1eq), 3.07 (m, 1H, H-6′a), 2.88 (m, 1H, H-6′b), 2.57 (m, 2H, H-1ax, H-5), 2.36 (t, 2H, H-2′a, H-2′b), 1.70–1.60 (m, 4H, H-3′a, H-3′b, H-5′a, H-5′b), 1.38 (m, 2H, H-4′a, H-4′b); ^{13}C NMR: δ 174.6 (C-1′), 78.2 (C-3), 69.3, 68.1 (C-2, C-4), 66.2 (C-5), 56.1, 55.1, 52.5 (C-1, C-6, C-6′), 50.9 (OCH_3), 33.4 (C-2′), 26.4 (C-5′), 24.5 (C-4′), 23.3 (C-3′); Anal. Calcd for $C_3H_{25}NO_6$: C, 53.59; H, 8.65. Found: C, 53.52; H, 8.70.

3.6. Methyl N^2-(*tert*-butoxycarbonyl)-N^6-(1,5-dideoxy-D-glucitol-1,5-diyl)-L-lysinate (6) and methyl N^2-(*tert*-butoxycarbonyl)-N^6-(1,5-dideoxy-L-iditol-1,5-diyl)-L-lysinate (8)

To a mixture of D-*xylo*-hexos-5-ulose (**4**) (220 mg, 1.12 mmol) and **5** (590 mg, 1.50 mmol, 1.3 equiv) in MeOH (40 mL), $Pd(OH)_2$/C (20%) (20 mg) was added and the heterogeneous reaction mixture was stirred under an atmosphere of hydrogen at ambient pressure for 44 h. After filtration and removal of the solvent under reduced pressure, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 500:100:6 v/v/v), yielding pure product **6** (185 mg, 0.46 mmol, 41%) as colourless syrup: $[\alpha]_D^{20}$ −18.2 (*c* 3.4, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.09 (m, 1H, H-2′), 3.85 (m, 2H, $J_{6a,6b} = 11.7$ Hz, H-6a, H-6b), 3.71 (s, 3H, OCH_3), 3.47 (ddd, 1H, $J_{1eq,2} = 4.9$ Hz, $J_{1ax,2} = 10.7$ Hz, $J_{2,3} = 8.8$ Hz, H-2), 3.35 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.14 (dd, 1H, H-3), 2.99 (dd, 1H, $J_{1eq,1ax} = 11.2$ Hz, H-1eq), 2.79 (m, 1H, H-6′a), 2.58 (m, 1H, H-6′b), 2.17 (dd, 1H, H-1ax), 2.12 (m, 1H, H-5), 1.82–1.30 (m, 6H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b); ^{13}C NMR: δ 173.9 (C-1′), 79.3 (C-3), 70.8 (C-4), 69.5 (C-2), 66.2 (C-5), 58.2, 56.4, 53.8, 52.2 (C-1, C-6, C-2′, C-6′), 51.5 (OCH_3), 31.3 (C-3′), 23.7, 23.6 (C-4′, C-5′); Anal. Calcd for $C_{18}H_{34}N_2O_8$: C, 53.19; H, 8.43. Found: C, 53.22; H, 8.50.

Side product **8** (46 mg, 0.11 mmol, 10%) was isolated as a colourless syrup: $[\alpha]_D^{20}$ −12.8 (*c* 1.0, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.09 (m, 1H, H-2′), 3.85 (m, 2H, H-6a, H-6b), 3.71 (s, 3H, OCH_3), 3.57 (nr, 1H, H-2), 3.44 (nr, 1H), 3.31 (nr, 1H), 3.10 (nr, 1H, H-1eq), 2.90–2.64 (nr, 4H, H-1ax, H-5, H-6′a, H-6′b), 1.82–1.30 (m, 6H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b); ^{13}C NMR: δ 173.9 (C-1′), 74.8 (C-3), 71.6 (C-4), 69.8 (C-2), 63.2 (C-5), 57.0, 54.0, 53.9, 51.9 (C-1, C-6, C-2′, C-6′), 51.5 (OCH_3), 31.3 (C-3′), 26.2, 23.4 (C-4′, C-5′); Anal. Calcd for $C_{18}H_{34}N_2O_8$: C, 53.19; H, 8.43. Found: C, 53.12; H, 8.55.

3.7. Methyl N^2-(dansyl)-N^6-(1,5-dideoxy-D-glucitol-1,5-diyl)-L-lysinate (9)

Acetyl chloride (0.5 mL, 0.55 g, 7.0 mmol) was slowly added to dry MeOH (15 mL) at 0 °C. After 10 min, compound **6** (37 mg, 0.091 mmol) was added to this mixture, which was then stirred for 20 h at ambient temperature when TLC indicated complete conversion of the starting material. The solvents were removed under reduced pressure and the residue was dissolved in dry DMF (15 mL). Et_3N (60 μL, 44 mg, 0.43 mmol, 5 equiv) and dansyl chloride (28 mg, 0.10 mmol, 1.1 equiv) were added, and the reaction mixture was stirred in a brown flask at ambient temperature for 4 h. Removal of the solvent under reduced pressure and purification of the residue by preparative TLC ($CHCl_3$/MeOH/concd NH_4OH, 300:100:4 v/v/v, extraction with MeOH) gave pure product **9** (32 mg, 0.059 mmol, 65%) as a bright green syrup: $[\alpha]_D^{20}$ +1.4 (*c* 0.3, MeOH); ^{1}H NMR (MeOH-d_4): δ 3.67 (m, 2H, H-6a, H-6b), 3.64 (m, 1H, H-2′), 3.33 (ddd, 1H, $J_{1eq,2} = 4.9$ Hz, $J_{1ax,2} = 10.2$ Hz,

$J_{2,3} = 8.8$ Hz, H-2), 3.22 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.10 (s, 3H, OCH_3), 3.01 (dd, 1H, H-3), 2.73 (dd, 1H, $J_{1eq,1ax} = 11.2$ Hz, H-1eq), 2.40 (m, 1H, H-6′a), 2.26 (m, 1H, H-6′b), 1.96 (dd, 1H, H-1ax), 1.93 (ddd, 1H, $J_{5,6a} = 2.0$ Hz, $J_{5,6b} = 2.9$ Hz, H-5), 1.52–1.43 (m, 2H, H-3′a, H-3′b), 1.24–0.80 (m, 4H, H-4′a, H-4′b, H-5′a, H-5′b); ^{13}C NMR: δ 172.5 (C-1′), 79.4, (C-3), 70.9 (C-4), 69.6 (C-2), 66.0 (C-5), 58.3, 56.5, 55.9, 52.0 (C-1, C-6, C-2′, C-6′), 51.1 (OCH_3), 31.9 (C-3′), 23.0, 22.9 (C-4′, C-5′); ESI-MS Calcd for $[C_{25}H_{37}N_3O_8S]$: m/z 539.65. Found: $[M+H]^+$ 540.3, $[M+Na]^+$ 562.3.

3.8. Methyl 6-{[N^6-(benzyloxycarbonyl)-N^2-(*tert*-butoxycarbonyl)-L-lysyl]amino}hexanoate (12)

To a solution of commercially available (Fluka) N^6-(benzyloxycarbonyl)-N^2-(*tert*-butoxycarbonyl)-L-lysine (3.00 g, 7.89 mmol) and Et_3N (3.0 mL, 2.2 g, 22 mmol, 2.7 equiv) in dry DMF (30 mL), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (2.66 g, 8.28 mmol, 1.05 equiv) and methyl 6-aminohexanoate hydrochloride (1.57 g, 8.64 mmol, 1.1 equiv) were added simultaneously and the reaction mixture was stirred at ambient temperature for 20 min. After removal of the solvent under reduced pressure, the residue was dissolved in CH_2Cl_2, consecutively washed with 6% aqueous HCl and satd aq $NaHCO_3$, dried (Na_2SO_4) and filtered. Removal of the solvent under reduced pressure and chromatography (cyclohexane/ethyl acetate, 2:1 v/v) gave **12** (3.99 g, 7.86 mmol, 100%) as a colourless oil: $[\alpha]_D^{20}$ −9.6 (*c* 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.01 (m, 1H, H-2), 3.64 (s, 3H, OCH_3), 3.20 (m, 4H, H-6a, H-6b, H-6′a, H-6′b), 2.29 (t, 2H, H-2′a, H-2′b), 1.80 (m, 1H, H-3a), 1.66–1.28 (m, 11H, H-3b, H-4a, H-4b, H-5a, H-5b, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b); ^{13}C NMR: δ 174.3 (C-1′), 172.3 (C-1), 54.6 (C-2), 51.7 (OCH_3), 40.6 (C-6), 39.4 (C-6′), 34.0 (C-2′), 32.2 (C-3), 29.7 (C-5), 29.3 (C-5′), 26.5 (C-4′), 24.6 (C-3′), 22.8 (C-4); Anal. Calcd for $C_{26}H_{41}N_3O_7$: C, 61.52; H, 8.14. Found: C, 61.42; H, 8.21.

3.9. Methyl 6-{[N^2-(*tert*-butoxycarbonyl)-N^6-(1,5-dideoxy-D-glucitol-1,5-diyl)-L-lysyl]amino}hexanoate (10) and methyl 6-{[N^2-(*tert*-butoxycarbonyl)-N^6-(1,5-dideoxy-L-iditol-1,5-diyl)-L-lysyl]amino}hexanoate (11)

To a solution of D-*xylo*-hexos-5-ulose (**4**) (1.30 g, 6.63 mmol, 1.03 equiv) and **12** (3.28 g, 6.46 mmol) in MeOH/H_2O (40 mL, 3:1 v/v), $Pd(OH)_2$/C (20%) (120 mg) was added and the heterogeneous reaction mixture was stirred under hydrogen at ambient pressure for 96 h. After filtration and removal of the solvent under reduced pressure, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 500:100:6 v/v/v), yielding pure compound **10** (0.30 g, 0.58 mmol, 9%) and an inseparable mixture of **10** and **11** (1.32 g, 2.54 mmol, 39%, **10**/**11** = 7:2 by integration over ^{1}H NMR spectra), as colourless syrups.

Compound **10**: $[\alpha]_D^{20}$ −10.8 (*c* 1.8, MeOH); ^{1}H NMR (MeOH-d_4): δ 3.96 (m, 1H, H-2′), 3.87 (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), 3.65 (s, 3H, OCH_3), 3.47 (ddd, 1H, $J_{1eq,2} = 4.9$ Hz, $J_{1ax,2} = 10.7$ Hz, $J_{2,3} = 8.8$ Hz, H-2), 3.36 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.25–3.12 (nr, 3H, H-3, H-6″a, H-6″b), 3.00 (dd, 1H, $J_{1eq,1ax} = 11.2$ Hz, H-1eq), 2.81 (m, 1H, H-6′a), 2.59 (m, 1H, H-6′b), 2.33 (t, 2H, H-2″a, H-2″b), 2.18 (dd, 1H, H-1ax), 2.13 (ddd, 1H, H-5), 1.80–1.30 (m, 12H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b, H-3″a, H-3″b, H-4″a, H-4″b, H-5″a, H-5″b); ^{13}C NMR: δ 174.6, 174.0 (C-1′, C-1″), 79.4 (C-3), 70.8 (C-4), 69.5 (C-2), 66.3 (C-5), 58.2, 56.5, 55.0, 52.3 (C-1, C-6, C-2′, C-6′), 50.9 (OCH_3), 38.9 (C-6″), 33.5 (C-2″), 32.1 (C-3′), 28.9 (C-5″), 26.2, 24.5, 24.5, 23.7 (C-4′, C-5′, C-3″, C-4″); Anal. Calcd for $C_{27}H_{28}O_5$: C, 74.98; H, 6.53. Found: C, 74.92; H, 6.60.

Compound **11**: ^{1}H NMR (MeOH-d_4): δ 3.96 (m, 1H, H-2′), 3.81 (nr, 2H, H-6a, H-6b), 3.53 (nr, 1H, H-2), 3.02 (nr, 1H, H-1eq), 2.73 (m, 1H, H-6′a), 2.65 (m, 1H, H-6′b); ^{13}C NMR: δ 174.7, 174.0 (C-1′, C-1″), 74.7, 71.6, 70.1, 63.2 (C-2, C-3, C-4, C-5), 57.2, 54.5, 54.1, 51.6 (C-1, C-6, C-2′, C-6′), 50.8 (OCH_3), 38.9 (C-6″), 33.5 (C-2″), 32.1 (C-3′), 28.9 (C-5″), 26.2, 24.5, 24.5, 23.7 (C-4′, C-5′, C-3″, C-4″).

3.10. Methyl 6-{[N^2-(dansyl)-N^6-(1,5-dideoxy-D-glucitol-1,5-diyl)-L-lysyl]amino}hexanoate (13)

Acetyl chloride (0.5 mL, 0.55 g, 7.0 mmol) was slowly added to dry MeOH (25 mL) at 0 °C. After 10 min, **10** (260 mg, 0.50 mmol) was added to the reaction mixture, which was then stirred for at ambient temperature 40 h. The solvents were removed under reduced pressure and the residue was dissolved in dry MeOH (10 mL). Et_3N (200 μL, 145 mg, 1.4 mmol, 3 equiv) and dansyl chloride (150 mg, 0.56 mmol, 1.1 equiv) were added, and the resulting reaction mixture was stirred in a brown flask at ambient temperature for 4 h. Removal of the solvent under reduced pressure and chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 700:100:8 v/v/v) gave pure product **13** (173 mg, 0.265 mmol, 53%) as bright green syrup: $[\alpha]_D^{20}$ +0.8 (*c* 0.7, MeOH); ^{1}H NMR (MeOH-d_4): δ 3.77 (m, 2H, H-6a, H-6b), 3.65 (s, 3H, OCH_3), 3.55 (m, 1H, H-2′), 3.43 (ddd, 1H, $J_{1eq,2} = 4.9$ Hz, $J_{1ax,2} = 10.3$ Hz, $J_{2,3} = 8.8$ Hz, H-2), 3.31 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.09 (dd, 1H, H-3), 2.82 (dd, 1H, $J_{1eq,1ax} = 11.2$ Hz, H-1eq), 2.82–2.72 (m, 2H, H-6″a, H-6″b), 2.46 (m, 1H, H-6′a), 2.33 (m, 1H, H-6′b), 2.27 (t, 2H, H-2″a, H-2″b), 2.05 (dd, 1H, H-1ax), 2.01 (ddd, 1H,

$J_{5,6a} = 2.9$ Hz, $J_{5,6b} = 2.4$ Hz, H-5), 1.60–0.90 (m, 12H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b, H-3″a, H-3″b, H-4″a, H-4″b, H-5″a, H-5″b); ^{13}C NMR: δ 174.6, 173.6 (C-1′, C-1″), 79.4 (C-3), 70.8 (C-4), 69.6 (C-2), 65.9 (C-5), 58.2, 57.5, 56.5, 52.1 (C-1, C-6, C-2′, C-6′), 50.9 (OCH_3), 38.7 (C-6″), 33.5 (C-2″), 33.1 (C-3′), 28.5 (C-5″), 26.1, 24.4, 23.3, 23.0 (C-4′, C-5′, C-3″, C-4″). ESIMS Calcd for $[C_{31}H_{48}N_4O_9S]$: m/z 652.81. Found: $[M+H]^+$ 653.7, $[M+Na]^+$ 675.7.

3.11. Methyl 6-{[N^2-(dansyl)-N^6-(1,5-dideoxy-L-iditol-1,5-diyl)-L-lysyl]amino}hexanoate (14)

Acetyl chloride (0.5 mL, 0.55 g, 7.0 mmol) was slowly added to dry MeOH (10 mL) at 0 °C. After 10 min, a mixture of **10** and **11** (70 mg, 0.13 mmol, **10**/**11** = 7:2) was added and the mixture was stirred at ambient temperature for 16 h. The solvents were removed under reduced pressure and the residue was dissolved in dry MeOH (20 mL). Et_3N (100 μL, 73 mg, 0.72 mmol, 5 equiv) and dansyl chloride (50 mg, 0.19 mmol, 1.4 equiv) were added, and the resulting mixture was stirred in a brown flask at ambient temperature for 4 h. Removal of the solvent under reduced pressure and purification of the residue by preparative TLC ($CHCl_3$/MeOH/concd NH_4OH, 500:100:6 v/v/v, extraction with MeOH) gave pure **14** (10 mg, 0.015 mmol, 11%), as a green syrup: $[\alpha]_D^{20}$ −5.1 (*c* 0.4, MeOH); ^{1}H NMR (MeOH-d_4): δ 3.78 (dd, 1H, $J_{5,6a} = 4.9$ Hz, $J_{6a,6b} = 11.2$ Hz, H-6a), 3.73 (dd, 1H, $J_{5,6b} = 6.8$ Hz, H-6b), 3.66 (s, 3H, OCH_3), 3.63 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 5.4$ Hz, H-4), 3.55 (m, 1H, H-2′), 3.46 (ddd, 1H, $J_{1eq,2} = 4.9$ Hz, $J_{1ax,2} = 9.3$ Hz, $J_{2,3} = 8.3$ Hz, H-2), 3.35 (dd, 1H, H-3), 2.88 (m, 1H, H-5), 2.84–2.72 (m, 2H, H-6″a, H-6″b), 2.64 (dd, 1H, $J_{1eq,1ax} = 12.2$ Hz, H-1eq), 2.48 (dd, 1H, H-1ax), 2.41 (m, 1H, H-6′a), 2.30 (m, 1H, H-6′b), 2.27 (t, 2H, H-2″a, H-2″b), 1.60–0.90 (m, 12H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b, H-3″a, H-3″b, H-4″a, H-4″b, H-5″a, H-5″b); ^{13}C NMR: δ 174.7, 173.8 (C-1′, C-1″), 74.7 (C-3), 71.5 (C-4), 70.0 (C-2), 62.8 (C-5), 57.6, 56.4, 54.0, 51.6 (C-1, C-6, C-2′, C-6′), 50.8 (OCH_3), 38.7 (C-6″), 33.5 (C-2″), 33.2 (C-3′), 28.5 (C-5″), 26.7, 26.1, 24.4, 23.1 (C-4′, C-5′, C-3″, C-4″); ESIMS Calcd for $[C_{31}H_{48}N_4O_9S]$: m/z 652.81. Found: $[M+H]^+$ 653.3, $[M+Na]^+$ 675.3.

3.12. N^2-(Dansyl)-N^6-(1,5-dideoxy-D-glucitol-1,5-diyl)-N^l-[6-({6-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-hexyl}amino)-6-oxohexyl]-L-lysinamide (15)

To a solution of compound **13** (131 mg, 0.201 mmol) in 1,4-dioxane/H_2O (10 mL, 1:1 v/v), 0.5 M aqueous NaOH (1.0 mL) was added at 0 °C and the resulting mixture was stirred at ambient temperature for 16 h. After neutralization with ion exchange resin Amberlite IR-120 [H^+] and filtration, the solvents were removed under reduced pressure. The residue was dissolved in dry DMF (12 mL), Et_3N (110 μL, 80 mg, 0.79 mmol, 4 equiv), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (65 mg, 0.20 mmol, 1 equiv) and 6-[(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino]hexan-1-aminium trifluoroacetate (80 mg, 0.20 mmol, 1 equiv) were added and the mixture was stirred at room temperature for 40 min. Removal of the solvent under reduced pressure and chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 700:100:16 v/v/v) yielded product **15** (75 mg, 0.083 mmol, 42%) as a red syrup: ^{1}H NMR (MeOH-d_4): δ 3.76 (dd, 1H, $J_{5,6a} = 2.9$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 3.72 (dd, 1H, $J_{5,6b} = 2.9$ Hz, H-6b), 3.56 (m, 1H, H-2′), 3.42 (m, 3H, H-2, H-6‴a, H-6‴b), 3.30 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5}$ 9.3 Hz, H-4), 3.16 (t, 2H, H-1‴a, H-1‴b), 3.10 (dd, 1H, $J_{2,3} = 9.3$ Hz, H-3), 2.84 (m, 2H, H-6″a, H-6″b), 2.79 (d, 1H, $J_{1eq,1ax} = 11.2$ Hz, $J_{1eq,2} = 4.9$ Hz, H-1eq), 2.40 (m, 1H, H-6′a), 2.26 (m, 1H, H-6′b), 2.13 (t, 2H, H-2″a, H-2″b), 2.03 (dd, 1H, $J_{1ax,2} = 10.7$ Hz, H-1ax), 2.00 (ddd, 1H, H-5), 1.80–0.80 (m, 20H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b, H-3″a, H-3″b, H-4″a, H-4″b, H-5″a, H-5″b, H-2‴a, H-2‴b, H-3‴a, H-3‴b, H-4‴a, H-4‴b, H-5‴a, H-5‴b); ^{13}C NMR: δ 174.8, 172.6 (C-1′, C-1″), 79.4 (C-3), 70.8 (C-4), 69.6 (C-2), 65.9 (C-5), 58.3, 57.0, 56.5, 52.0 (C-1, C-6, C-2′, C-6′), 44.7 (C-6‴), 39.1 (C-1‴), 38.9 (C-6″), 35.8, 32.5, 31.3, 29.1, 28.5, 26.5, 26.5, 26.2, 25.4, 23.1, 23.0 (C-3′, C-4′, C-5′, C-2″, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-5‴); MALDI-MS Calcd for $[C_{42}H_{61}N_9O_{11}S]$: m/z 900.07. Found: $[M]^+$ 900.3.

3.13. Methyl (5*R*)-2,3,4-tri-*O*-acetyl-5,6-anhydro-5-*C*-hydroxy-α-D-*lyxo*-hexopyranoside (16)

Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-α-D-*lyxo*-hex-5-enopyranoside[8] (2.40 g, 7.94 mmol) was dissolved in CH_2Cl_2 (60 mL). Satd aq $NaHCO_3$ (0.6 M, 30 mL) and 3-chloroperbenzoic acid (55%, 2.76 g, 8.80 mmol, 1.1 equiv) were added and the reaction mixture was stirred at ambient temperature for 3 h. The organic phase was washed with satd aq $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated to about 10 mL under reduced pressure at ambient temperature. Quick filtration over a short plug of silica gel (cyclohexane/ethyl acetate, 2:1 v/v) yielded **16** as the only diastereomer (1.92 g, 6.03 mmol, 76%) as colourless syrup: $[\alpha]_D^{20}$ +22.3 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.80 (d, 1H, $J_{3,4} = 10.7$ Hz, H-4), 5.58 (dd, 1H, $J_{2,3} = 3.4$ Hz, H-3), 5.40 (dd, 1H, $J_{1,2} = 2.0$ Hz, H-2), 4.77 (d, 1H, H-1), 3.41 (s, 3H, OCH_3), 2.85 (d, 1H, $J_{6a,6b} = 4.4$ Hz, H-6a), 2.56 (d, 1H, H-6b); ^{13}C NMR: δ 100.3 (C-1), 79.9 (C-5), 70.1, 67.3, 64.3 (C-2, C-3, C-4), 56.4 (OCH_3), 46.4 (C-6); Anal. Calcd for $C_3H_{18}O_9$: C, 49.06; H, 5.70. Found: C, 48.93; H, 5.78.

3.14. D-*lyxo*-Hexos-5-ulose (17)

To a 1.5% methanolic solution of methyl (5*R*)-2,3,4-tri-*O*-acetyl-5,6-anhydro-5-*C*-hydroxy-α-D-*lyxo*-hexopyranoside (**16**) (610 mg, 1.92 mmol), 1.0 M NaOMe solution (500 μL) was added dropwise at −30 °C. After 7 h reaction time at −30 °C, water (30 mL) was added and the resulting reaction mixture was concentrated to about 20 mL under reduced pressure. After addition of acetonitrile (8 mL), ion exchange resin Amberlite IR 120 [H^+] was added to the reaction mixture, which was then stirred at 45 °C for 16 h. Filtration and removal of the solvents under reduced pressure gave **17** (330 mg) as a slightly yellow syrup. As previously reported, NMR showed a complex mixture of several tautomeric forms including hydrates.[9] This was used for the next step without further purification.

3.15. Methyl N^2-(*tert*-butoxycarbonyl)-N^6-(1,5-dideoxy-D-mannitol-1,5-diyl)-L-lysinate (18)

To a solution of D-*lyxo*-hexos-5-ulose (**17**) (200 mg, 1.02 mmol) and L-lysine derivative **5** (507 mg, 1.29 mmol, 1.3 equiv) in MeOH (40 mL), $Pd(OH)_2$/C (20%) (20 mg) was added and the heterogeneous mixture was stirred under hydrogen at ambient pressure for 18 h. After filtration and removal of the solvent under reduced pressure, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 500:100:6 v/v/v), yielding pure compound **18** (210 mg, 0.52 mmol, 51%) as colourless syrup. $[\alpha]_D^{20}$ −38.9 (*c* 1.4, MeOH); ^{1}H NMR (MeOH-d_4): δ 4.09 (m, 1H, H-2′), 3.91 (dd, 1H, $J_{5,6a} = 2.9$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 3.87 (dd, 1H, $J_{5,6b} = 2.4$ Hz, H-6b), 3.85 (ddd, 1H, $J_{1eq,2} = 3.9$ Hz, $J_{1ax,2} = 2.0$ Hz, $J_{2,3} = 2.9$ Hz, H-2), 3.71 (s, 3H, OCH_3), 3.68 (dd, 1H, $J_{3,4} = 8.8$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.33 (dd, 1H, H-3), 3.01 (dd, 1H, $J_{1eq,1ax} = 12.2$ Hz, H-1eq), 2.81 (m, 1H, H-6′a), 2.61 (m, 1H, H-6′b), 2.52 (dd, 1H, H-1ax), 2.18 (ddd, 1H, H-5), 1.82–1.30 (m, 6H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b); ^{13}C NMR: δ 173.9 (C-1′), 75.2, 68.3, 68.2 (C-2, C-3, C-4), 66.0 (C-5), 57.8, 55.1, 53.8, 52.3 (C-1, C-6, C-2′, C-6′), 51.4 (OCH_3), 31.2 (C-3′), 23.7, 23.5 (C-4′, C-5′); Anal. Calcd for $C_{18}H_{34}N_2O_8$: C, 53.19; H, 8.43. Found: C, 74.92; H, 6.60. The formation of the corresponding L-*gulo*-configured product was not observed.

3.16. Methyl 6-{[N^2-(*tert*-butoxycarbonyl)-N^6-(1,5-dideoxy-D-mannitol-1,5-diyl)-L-lysyl]amino}hexanoate (19)

To a solution of D-*lyxo*-hexos-5-ulose (**17**) (1.17 g, 5.96 mmol) and **12** (3.05 g, 6.01 mmol, 1.0 equiv) in MeOH/H_2O (40 mL, 3:1 v/v), $Pd(OH)_2$/C (20%) (120 mg) was added and the heterogeneous mixture was stirred under hydrogen at ambient pressure for 120 h. After filtration and removal of the solvent under reduced pressure, the residue was purified by chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 400:100:5 v/v/v), yielding compound **19** (1.73 g, 3.33 mmol, 56%) as colourless syrup: $[\alpha]_D^{20}$ −20.3 (*c* 1.9, MeOH); ^{1}H NMR (MeOH-d_4): δ 3.96 (m, 1H, H-2′), 3.92 (dd, 1H, $J_{5,6a} = 2.4$ Hz, $J_{6a,6b} = 12.2$ Hz, H-6a), 3.89 (dd, 1H, $J_{5,6b} = 2.9$ Hz, H-6b), 3.87 (ddd, 1H, $J_{1eq,2} = 3.9$ Hz, $J_{1ax,2} = 2.0$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 3.71 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.65 (s, 3H, OCH_3), 3.36 (dd, 1H, H-3), 3.25–3.12 (m, 2H, H-6″a, H-6″b), 3.06 (dd, 1H, $J_{1eq,1ax} = 12.2$ Hz, H-1eq), 2.85 (m, 1H, H-6′a), 2.70 (m, 1H, H-6′b), 2.62 (dd, 1H, H-1ax), 2.32 (t, 2H, H-2″a, H-2″b), 2.28 (ddd, 1H, H-5), 1.77–1.25 (m, 12H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b, H-3″a, H-3″b, H-4″a, H-4″b, H-5″a, H-5″b); ^{13}C NMR: δ 174.7, 174.0 (C-1′, C-1″), 75.0, 68.0, 68.0 (C-2, C-3, C-4), 66.0 (C-5), 57.5, 55.1, 55.0, 52.4 (C-1, C-6, C-2′, C-6′), 50.9 (OCH_3), 38.9 (C-6″), 33.5 (C-2″), 32.1 (C-3′), 28.9 (C-5″), 26.2, 24.5, 24.5, 23.5 (C-4′, C-5′, C-3″, C-4″); Anal. Calcd for $C_{24}H_{45}N_3O_9$: C, 55.47; H, 8.73. Found: C, 55.42; H, 8.80. The formation of the corresponding L-*gulo*-configured product was not observed.

3.17. Methyl N^2-(dansyl)-N^6-(1,5-dideoxy-D-mannitol-1,5-diyl)-L-lysinate (20)

Acetyl chloride (0.5 mL, 0.55 g, 7.0 mmol) was slowly added to dry MeOH (15 mL) at 0 °C. After 10 min, **18** (69 mg, 0.17 mmol) was added and the mixture was stirred at ambient temperature for 18 h. The solvents were removed under reduced pressure and the residue was dissolved in dry DMF (15 mL). Et_3N (150 μL, 110 mg, 1.1 mmol, 6 equiv) and dansyl chloride (51 mg, 0.19 mmol, 1.1 equiv) were added, and the mixture was stirred in a brown flask at ambient temperature for 5 h. Removal of the solvent under reduced pressure and chromatography on silica gel ($CHCl_3$/MeOH/concd NH_4OH, 500:100:6 v/v/v) gave product **20** (68 mg, 0.13 mmol, 74%) as a bright green syrup: $[\alpha]_D^{20}$ −13.6 (*c* 0.8, MeOH); ^{1}H NMR (MeOH-d_4): δ 3.84 (dd, 1H, $J_{5,6a} = 2.4$ Hz, $J_{6a,6b} = 11.8$ Hz, H-6a), 3.81 (ddd, 1H, $J_{1eq,2} = 3.9$ Hz, $J_{1ax,2} = 2.0$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 3.76 (dd, 1H, $J_{5,6b} = 2.9$ Hz, H-6b), 3.73 (m, 1H, H-2′), 3.64 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.28 (dd, 1H, H-3), 3.21 (s, 3H, OCH_3), 2.81 (dd, 1H, $J_{1eq,1ax} = 12.2$ Hz, H-1eq), 2.52–2.42 (m, 2H, H-6′a, H-6′b), 2.40 (dd, 1H, H-1ax), 2.10 (ddd, 1H, H-5), 1.60–1.54 (m, 2H, H-3′a, H-3′b), 1.30–1.02 (m, 4H, H-4′a, H-4′b, H-5′a, H-5′b); ^{13}C NMR: δ 172.5 (C-1′), 75.2, 68.2, 68.2 (C-2, C-3, C-4), 66.0 (C-5), 57.7, 55.8, 55.1, 52.1 (C-1, C-6, C-2′, C-6′), 51.1 (OCH_3), 31.9 (C-3′), 22.9, 22.7 (C-4′, C-5′); ESIMS Calcd for [$C_{25}H_{37}N_3O_8S$]: *m*/*z* 539.65. Found: $[M+H]^+$ 540.6, $[M+Na]^+$ 562.6.

3.18. Methyl 6-{[N^2-(dansyl)-N^6-(1,5-dideoxy-D-mannitol-1,5-diyl)-L-lysyl]amino}hexanoate (21)

Acetyl chloride (0.5 mL, 0.55 g, 7.0 mmol) was slowly added to dry MeOH (15 mL) at 0 °C. After 10 min, **19** (35 mg, 0.067 mmol) was added and the mixture was stirred ambient temperature for 6 h. The solvents were removed under reduced pressure and the residue was dissolved in dry DMF (15 mL). Et_3N (100 μL, 73 mg, 0.72 mmol, 10 equiv) and dansyl chloride (23 mg, 0.085 mmol, 1.3 equiv) were added, and the resulting reaction mixture was stirred in a brown flask at ambient temperature for 4 h. Removal of the solvent under reduced pressure and preparative TLC ($CHCl_3$/MeOH/concd NH_4OH, 400:100:5 v/v/v, extraction with MeOH) gave product **21** (24 mg, 0.037 mmol, 55%) as a green syrup: $[\alpha]_D^{20}$ −9.4 (*c* 0.9, MeOH); ^1H NMR (MeOH-d_4): δ 3.84 (dd, 1H, $J_{5,6a} = 2.4$ Hz, $J_{6a,6b} = 11.7$ Hz, H-6a), 3.80 (ddd, 1H, $J_{1eq,2} = 3.9$ Hz, $J_{1ax,2} = 2.0$ Hz, $J_{2,3} = 3.4$ Hz, H-2), 3.76 (dd, 1H, $J_{5,6b} = 2.9$ Hz, H-6b), 3.65 (s, 3H, OCH_3), 3.64 (dd, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 9.3$ Hz, H-4), 3.54 (m, 1H, H-2′), 3.27 (dd, 1H, H-3), 2.80 (dd, 1H, $J_{1eq,1ax} = 12.2$ Hz, H-1eq), 2.79 (m, 1H, H-6′a), 2.70 (m, 1H, H-6′b), 2.40 (m, 2H, H-6″a, H-6″b), 2.34 (dd, 1H, H-1ax), 2.26 (t, 2H, H-2″a, H-2″b), 2.02 (ddd, 1H, H-5), 1.60–0.95 (m, 12H, H-3′a, H-3′b, H-4′a, H-4′b, H-5′a, H-5′b, H-3″a, H-3″b, H-4″a, H-4″b, H-5″a, H-5″b); ^{13}C NMR: δ 174.6, 174.3 (C-1′, C-1″), 75.4, 68.4, 68.3 (C-2, C-3, C-4), 65.4 (C-5), 57.8, 57.6, 55.2, 52.2 (C-1, C-6, C-2′, C-6′), 50.9 (OCH_3), 38.6 (C-6″), 33.5 (C-2″), 33.5 (C-3′), 28.4 (C-5″), 26.1, 24.4, 23.4, 22.9 (C-4′, C-5′, C-3″, C-4″); ESIMS: Calcd for [$C_{31}H_{48}N_4O_9S$]: *m*/*z* 652.81. Found: $[M+H]^+$ 653.3, $[M+Na]^+$ 675.3.

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