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Spacer-separated sialyl LewisX cyclopeptide conjugates as potential E-selectin ligands

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Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—Completely protected sialyl LewisX azide was synthesized from a neolactosamine azide precursor carrying a 3-*O*-allyloxycarbonyl group as the temporary protecting group. After its Pd(0)-catalyzed deprotection and stereoselective α-fucosylation, the obtained LewisX azide was subjected to O-deacetylation in the galactose unit and subsequent regio- and stereoselective sialylation. Reduction of the anomeric azido group afforded the sialyl LewisX amine building block. Two molecules of this tetrasaccharide ligand were conjugated to a preformed cyclooctapeptide containing two equidistant L-asparagine units equipped with carboxy-terminated tetraethyleneglycol side chains to give, after deprotection, the target glycopeptide conjugate. Preliminary biological evaluation of the synthesized bivalent sialyl LewisX cyclopeptide conjugate showed only slightly enhanced inhibition of E-selectin binding in spite of the given flexibility of the two linked saccharide determinants.
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Keywords: Glycopeptides; Sialyl LewisX; E-Selectin ligand; Oligoethylene glycol spacer; Glycosyl azides

1. Introduction

Biological recognition of cell membrane glycoconjugates plays an important role in regulatory processes within mammalian organisms. However, carbohydrate ligands only weakly bind to their receptors in most cases. Multivalent presentation of the saccharide ligands often strongly amplifies the binding efficiency and selectivity.[1] For example, multivalency obviously is important for the binding of carbohydrate ligands like sialyl LewisX to selectins.[2] Synthetic oligosaccharide selectin ligands can function as inhibitors of the inflammatory cascade[3,4] and of tumour cell metastasis.[5] As a consequence, a number of multivalent sialyl LewisX derivatives have been synthesized by chemical and chemoenzymatic methods including dendrimers terminating with sialyl LewisX,[6] polymer-linked sialyl LewisX,[7] polylysine-bound sialyl LewisX[8] or sialyl LewisX presented on liposomes.[9] An interesting concept of multivalent presentation of sialyl LewisX is based on simultaneous enzymatic formation of seven sialyl LewisX units linked to β-cyclodextrin as a cyclic scaffold.[10] In the course of our investigations of sialyl LewisX glycopeptides it was found that a trivalent sialyl LewisX cycloheptapeptide is a selective selectin inhibitor.[11] It showed binding to E-selectin, but not to P-selectin. Influence of the peptide structure on the inhibitory effect of sialyl LewisX towards selectins was also observed for other synthetic glycopeptides.[12] Since the binding affinity of oligovalent sialyl LewisX has been found dependent on the distance between the sialyl LewisX structures,[1,13] it appeared interesting to find out the effect a spacer between the sialyl LewisX saccharide and the cyclopeptide backbone has on the binding affinity. Because information about the optimal distance between two sialyl LewisX saccharides in the natural ligands of E-selectin is not available, the chosen spacer should be flexible enough in order to ascertain an adjustable fit in the binding sites of the selectin. Furthermore, the two sialyl LewisX structures

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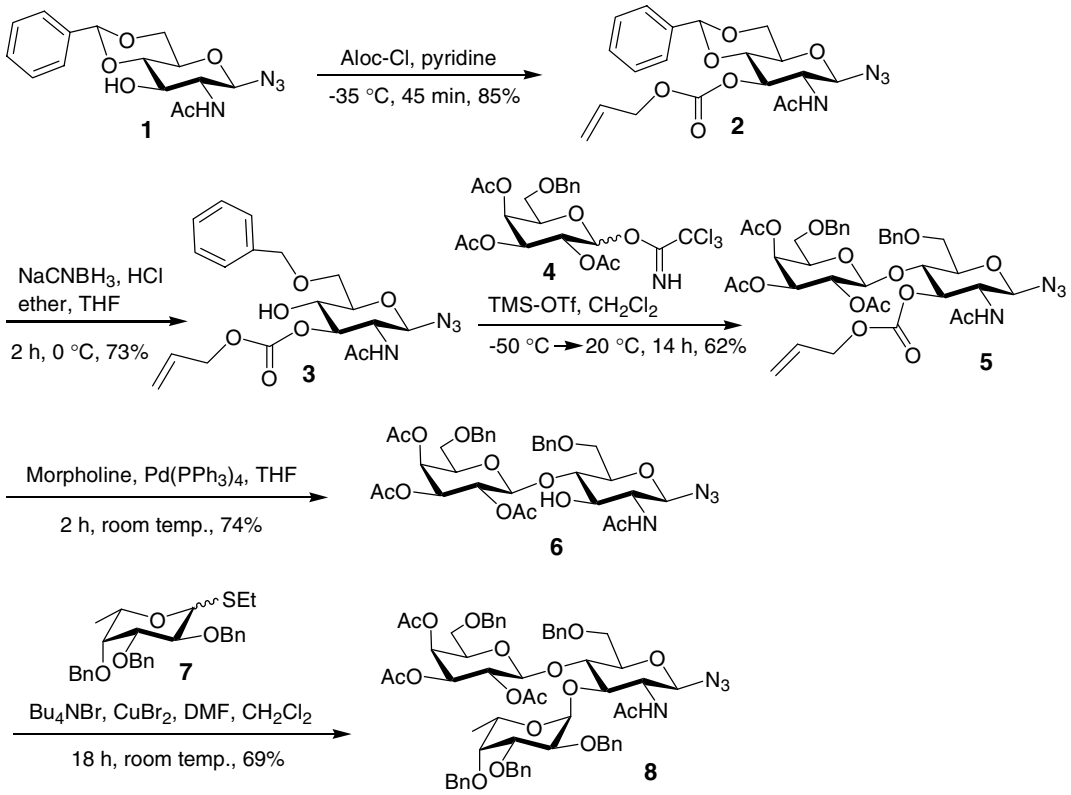


Scheme 1. Synthesis of a selectively deprotectable LewisX azide **8**.

should be equivalent. As a model compound, which meets these requirements a symmetrical cyclooctapeptide containing two equidistant spacer-separated sialyl LewisX side chains was synthesized.

2. Results and discussion

Apart from efficient enzymatic syntheses of the sialyl LewisX tetrasaccharide[14] most strategies for chemical syntheses start with fucosylation of a glucosamine derivative.[4b,11,12,15] As the stereoselective galactosylation of the fucosyl-glucosamine structure suffered from only moderate yields in many cases, a variation of the alternative fucosylation of an appropriately preformed neolactosamine structure[16] was pursued in this work. The 4,6-benzylidene glucosamine derivative **1** served as the starting material.[12a] It contained the azido group as anomeric protecting group and precursor of the glycosylamine required for later coupling reactions.[17] After introduction of the *O*-allyl carbonate[18] to give **2** and regioselective reductive opening of the benzylidene-acetal,[19] galactosylation of **3** with trichloroacetimidate **4**[20] furnished the fully protected type 2 lactosamine azide **5** (Scheme 1).

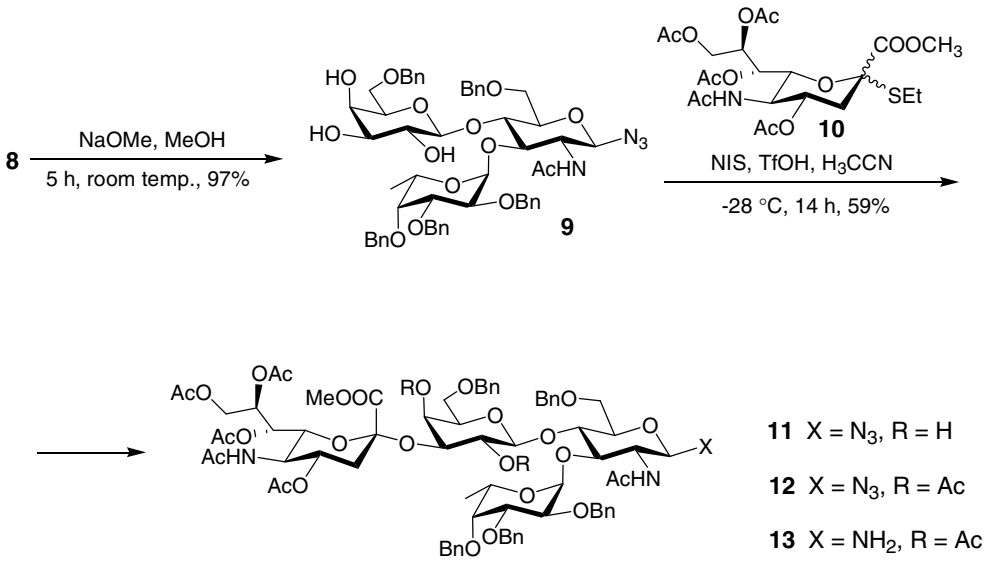
Removal of the allyloxycarbonyl (Aloc) group by Pd(0)-catalyzed allyltransfer to morpholine as a weakly basic allyl-trapping nucleophile[21] proceeded without affecting the *O*-acetyl groups. Fucosylation using donor **7**[22] gave the selectively deprotectable LewisX azide **8**.

After O-deacetylation of **8** with methanol/sodium methoxide to furnish **9**,[12a] and sialylation[†] using ethylthio sialoside **10**[23] under conditions described by Hasegawa et al.,[24] sialyl LewisX azide **11** was obtained with satisfying regio- and stereoselectivity (Scheme 2). The remaining free hydroxy groups were acetylated to afford **12**. The reduction of the azido group of **12** to furnish the glycosylamine **13** is best carried out immediately prior to further conversion. The best results were obtained by hydrogenolysis catalyzed by Raney-nickel that was washed to neutral conditions (pH 7.5).[17a,25] In this way benzyl ether groups are not affected, and anomerization of the glycosylamines is prevented.

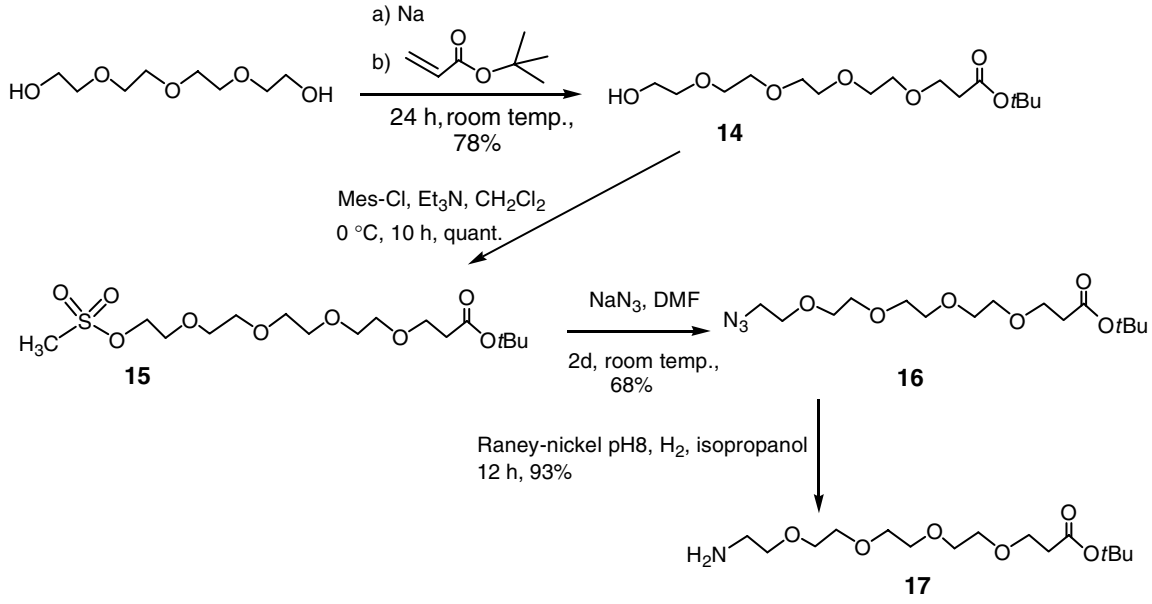
The spacer amino acid required for the construction of spacer-separated sialyl LewisX cyclopeptide conjugates was synthesized from tetraethylene glycol and its base catalyzed conjugate addition to *tert*-butyl acrylate to yield **14**.

After mesylation, **15** was subjected to nucleophilic introduction of the azido group. Finally, **16** was hydrogenolyzed to furnish spacer amino acid ester **17**[26] (Scheme 3).

[†] The sialylation of **9** was not optimized, higher, albeit varying yields of sialylation reactions with O-glycosides of LewisX acceptor molecules have been reported in the literature.



Scheme 2. Synthesis of sialyl LewisX amine **13**.



Scheme 3. Formation of spacer amino acid derived from tetraethylene glycol.

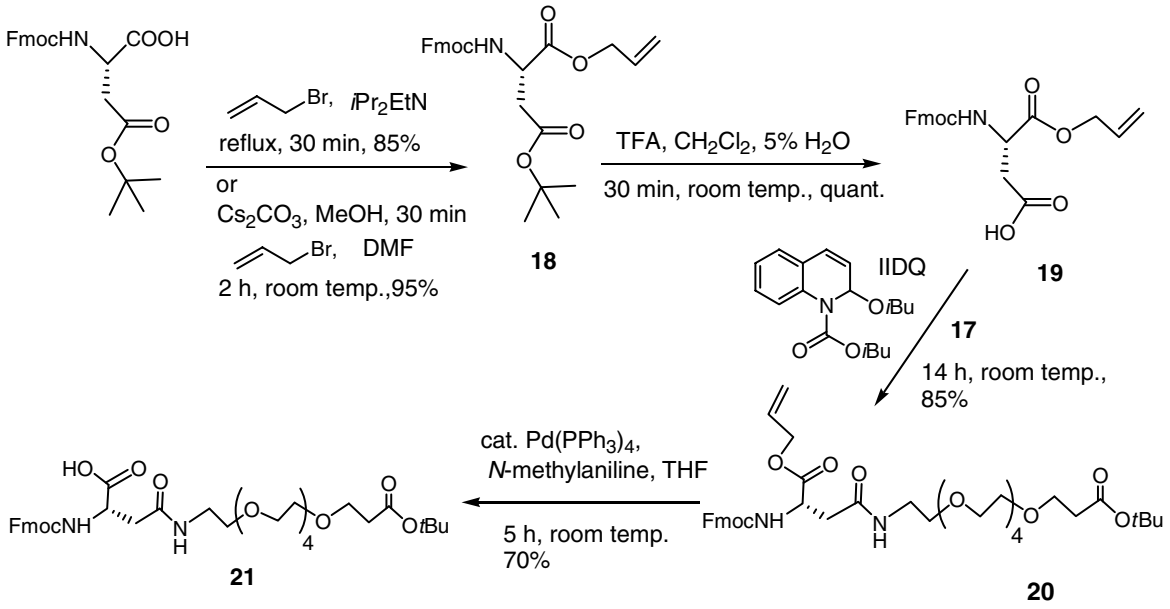
In order to explore the conjugation technique Fmoc protected β-*tert*-butyl aspartate was converted to the α-allyl ester **18**. Acidolysis of the *tert*-butyl ester gave the desired α-allyl Fmoc aspartate **19** (Scheme 4).

Condensation of **19** with the glycol spacer **17** promoted by isobutyl 2-isobutoxy-1,2-dihydroquinoline-1-carboxylate (IIDQ)[27] afforded spacer-linked asparagine **20** carrying three orthogonally stable protecting groups. Selective removal of the allyl ester protection using Pd(0)-catalysis and *N*-methylaniline as the allyl scavenger[28] furnished Fmoc asparagine building block **21**.

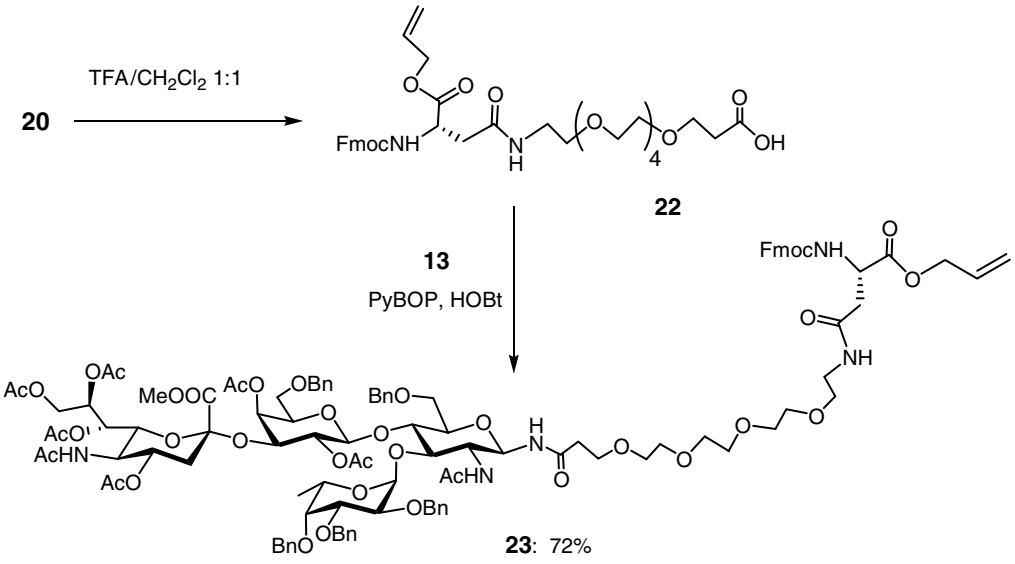
Alternative selective acidolysis of the side chain *tert*-butyl ester of **20** formed **22**, which served as a model compound for coupling reactions with sialyl LewisX amine **13**. Among the condensing reagents tested *O*-benzotriazolyl tripyrrolidinophoshonium hexafluorophosphate (PyBOP)[29] afforded the highest yield of the sialyl LewisX-spacer asparagine conjugate **23** (Scheme 5).

Due to the long reaction time partial anomerization of the glycosylamine occurred. Its condensation gave the α-anomer of **23** as a side product (25%).

The use of the benzyl ester-containing sialyl LewisX analogue of **13** and *O*-pentafluorophenyl-bis-tetrameth-



Scheme 4. Synthesis of the protected spacer-equipped asparagines **21**.



Scheme 5. Model condensation to form the spacer-separated sialyl LewisX asparagines **23**.

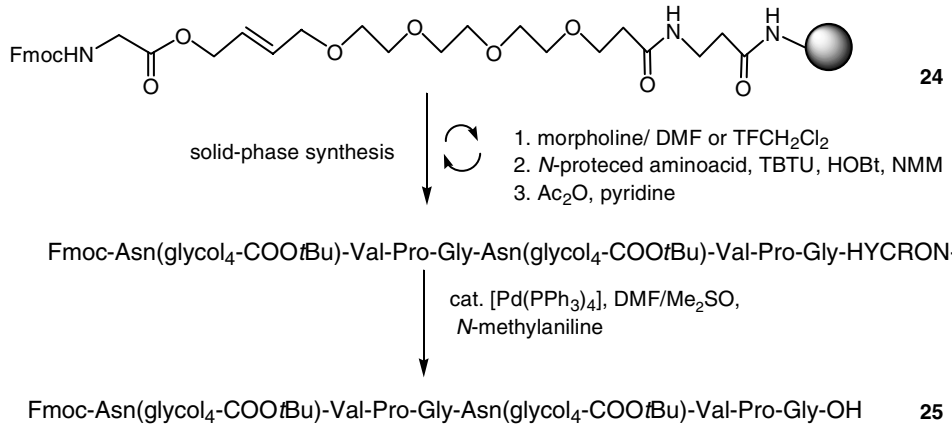
ylene-uronium-hexafluorophosphate (PfPyU)[30] or (7-aza-benzotriazol-1-yl)-tetramethyluronium-hexafluorophosphate (HATU)[31] as the condensing agents resulted in lower yields.

For the solid-phase synthesis of an octapeptide containing two spacer-equipped aspartate residues polystyrene-based resin having the allylic HYCRON anchor[26b] **24** was applied according to Scheme 6. Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium-tetrafluoroborate (TBTU)[32] in combination with 1-hydrobenzotriazol (HOBt)[33] served as the condensing reagent.

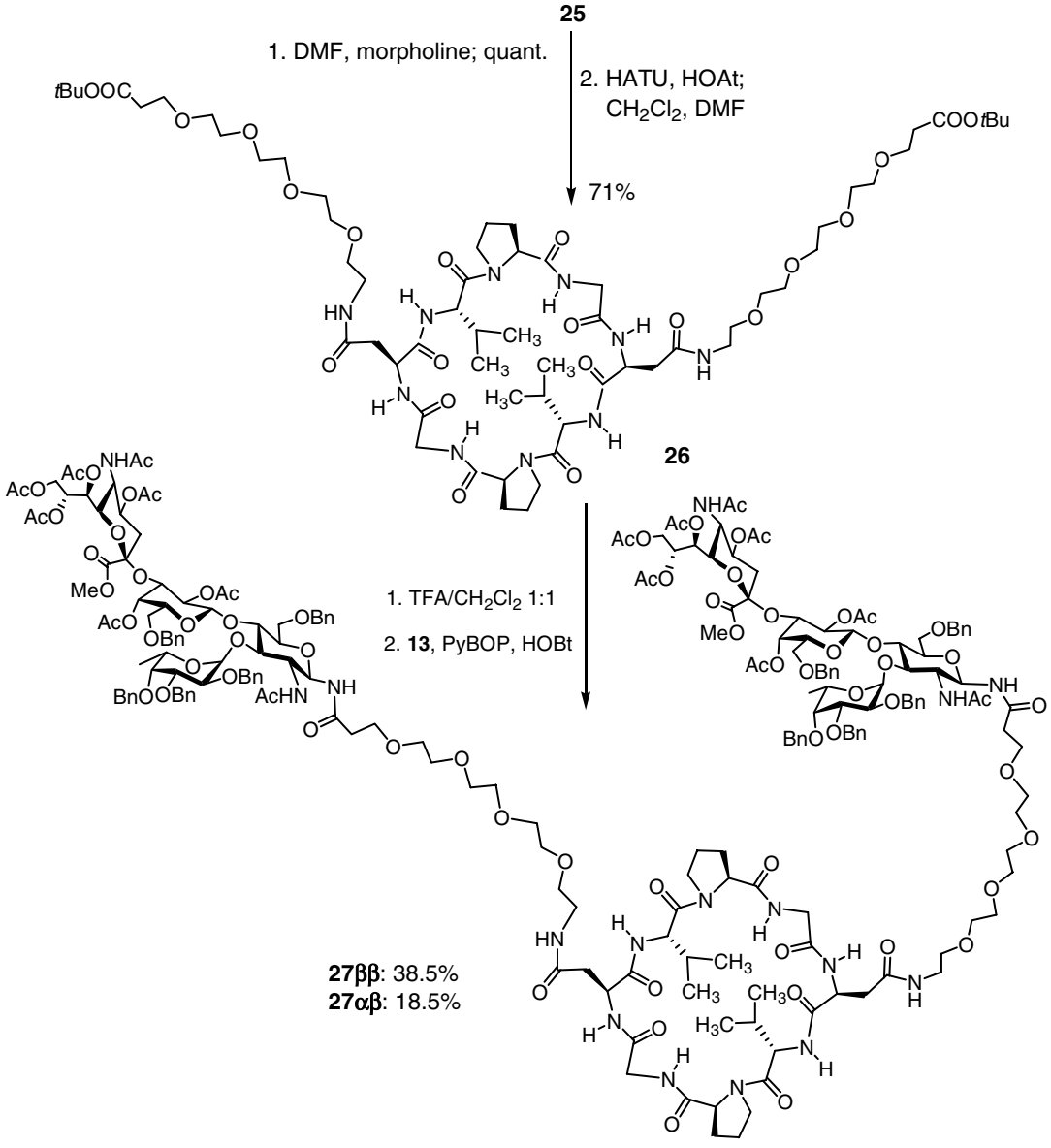
Palladium(0)-catalyzed detachment of the octapeptide **25** using the very weak base *N*-methyl aniline as the allyl scavenger[28,30b] did not affect the Fmoc group.

After purification of peptide **25** and removal of the Fmoc group cyclization was achieved using HATU and 1-hydroxy-7-aza-benzotriazole (HOAt).[31] The symmetric cyclooctapeptide **26** was subjected to acidolysis of the side chain *tert*-butyl esters. Subsequently the two spacer carboxylic groups were condensed simultaneously with sialyl LewisX amine **13** (Scheme 7).

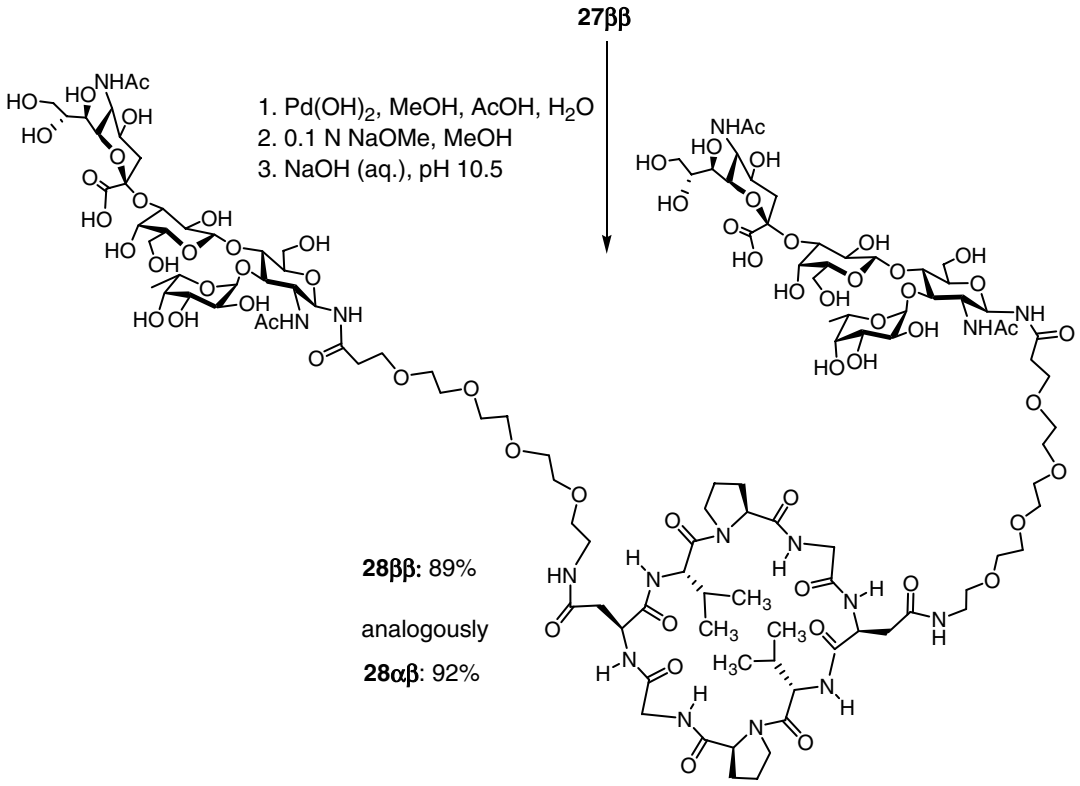
Again the long reaction time resulted in some anomerization of **13**. The cyclic sialyl LewisX glycopeptide **27ββ**



Scheme 6. Solid-phase synthesis of spacer-containing octapeptide **25**.



Scheme 7. Peptide cyclization and condensation with sialyl LewisX amine.



Scheme 8. Deprotection of the spacer-separated sialyl LewisX cyclopeptide.

containing two β-glycosyl amide bonds was isolated in a yield of 39%. The analogue having one α- and one β-glycosyl amide linkage was obtained as a by-product (18%). The NMR spectra give evidence of the structure of compounds **27**. While the spectrum of **27ββ** shows only one methyl ester signal, that of **27αβ** shows two ($\delta = 3.833$ and 3.823 ppm). The separated signals of H-1α of **27αβ** at $\delta = 5.74$ ppm has only half of the intensity compared to that of H-4″ of Gal at $\delta = 5.03$ ppm in the same range of the spectrum. Further characterization was carried out for the deblocked compounds.

Deprotection was achieved by Pd-catalyzed hydrogenation in methanol/acetic acid/water (30:3:0.2), subsequent Zemplén transesterification in methanol/sodium methoxide at pH 10 and saponification of methyl ester groups in aqueous NaOH at pH 10.5. Neutralization was performed with solid carbondioxide (Scheme 8).

The resulting crude products containing salts were purified by gel permeation chromatography (Sephadex LH15) in water giving the sialyl LewisX-spacer-cyclopeptide conjugates **28ββ** and **28αβ** in a pure form and high yield.

Preliminary evaluation of inhibitory effects showed an $IC_{50} \sim 0.8$ mM in an assay of adhesion of 32Dc3 cells (murine neutrophiles) to a E-selectin-IgG construct.[12b,26a] From this result the bivalent **28ββ** is just twice as active as sLeX itself. Obviously, the inserted spacer is too flexible. The binding of **28ββ** to E-selectin proceeds in a statistical fashion and shows no exponential enhancement. It should be noticed that in the same assay the monomeric sialyl LewisX asparagine showed an $IC_{50} \sim 2.4$ mM suggesting that in this series the amino acid decreases the binding affinity to E-selectin compared to the tetrasaccharide itself.

3. Experimental

3.1. General methods

NMR spectra were recorded on a Bruker AC-200 or a Bruker AM-400 spectrometer. The following abbreviations were used to explain multiplicities: s (singlet), d (doublet), t (triplet), m (multiplet). Indication of ^{1}H and ^{13}C NMR signals to monosaccharide units: no index GlcNAc, index ′ Fuc, index ″ Gal, index ‴ NeuNAc. Mass spectra were recorded on a ESI Navigator-1 (ThermoQuest), a Tofspec E instrument (Micromass, 2,5-dihydroxy-benzoic acid (dhb) or α-cyanocinnamic acid (cca) as the matrix) or a Finnigan MAT 95 (FD and FAB) instrument. Optical rotations were recorded using a Perkin Elmer 241 polarimeter. Elemental analyses were performed by the microanalytical laboratory of the Institut fuer Organische Chemie, Universitaet Mainz.

All reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm silica gel plates

($60F_{254}$/RP-$C18_{254}$ E. Merck, Darmstadt, Germany) using UV light, $KMnO_4$ or *p*-anisaldehyde solns and heat for visualization. Silica gel 60 (particle size 0.04–0.0063, E. Merck, Darmstadt, Germany) was used for flash chromatography, silica particle size 0.63–0.2 mm (J. T. Baker, Gross-Gerau, Germany) for atmospheric pressure chromatography. Gel permeation chromatography was carried out on Sephadex LH 15 (Pharmacia). All reactions were carried out under an argon atmosphere with dried solvents. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Analytical RP-HPLC was performed on a Phenomenex LUNA C_{18} (5 μm column, 250 * 4.6 mm), flow 1 mL/min using a Knauer HPLC equipment (MaxiStar K1000, DAD 2026 detector). Solvent: (MeCN-water + 0.1% TFA); For semi-preparative (flow 10 mL/min) and preparative HPLC (flow 20 mL/min) a Knauer HPLC equipment (two MaxiStar K500, DAD 2026 detector) was used. Columns are specified for the different compounds.

3.1.1. 2-Acetamido-3-*O*-allyloxycarbonyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide 2 (3-Aloc-4,6-BzdGlcNAc-N_3). In dry CH_2Cl_2 (200 mL) and pyridine (300 mL), 10.75 g (32.15 mmol) glycosyl azide[17b] **1** was dissolved and cooled to −35 °C. Allyl chloroformate (12.0 mL, 113.1 mmol) was added within 30 min, and the soln was stirred for 15 min. The precipitating pyridinium hydrochloride was filtered off. The soln was washed with 100 mL of satd aq $NaHCO_3$ and 100 mL of water. After drying with magnesium sulfate and evaporation of the solvents under diminished pressure, the residue was co-distilled three times with toluene. The solid residue was dissolved in boiling ethyl acetate (300 mL) and crystallized by cooling and addition of light petroleum. Concentration of the mother liquor and further addition of light petroleum afforded additional amounts of product **2**. Yield: 11.47 g (85%), colourless crystals; mp: 168 °C; $[\alpha]_D^{22}$ −82.2 (*c* 1, $CHCl_3$); $R_f = 0.28$ (1:1 light petroleum–ethyl acetate); ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.97 (s, 3H, CH_3CO–); 3.54–3.72 (m, 3H, H-2, H-5, H-6a); 4.00 (dd, 1H, J_{gem} 19.5 Hz, J_{vic} 9.3 Hz, –O–CH_{2a}–CH=CH_2); 4.30 (dd, 1H, $J_{6a,6b}$ 10.3 Hz, $J_{6a,5}$ 4.4 Hz, H-6a); 4.56–4.66 (m, 3H, H-1, H-4, –O–CH_{2b}–CH=CH_2); 5.16 (t, 1H, $J_{3,2} \approx J_{3,4}$ 9.8 Hz, H-3); 5.23 (dd, 1H, J_{cis} 11.2 Hz, J_{gem} 1.0 Hz, –CH=CH_{2cis}); 5.30 (dd, 1H, J_{trans} 17.1 Hz, J_{gem} 1.0 Hz, –CH=CH_{2trans}); 5.49 (s, 1H, =C*H*–Bzd); 5.76–5.97 (m, 1H, –C*H*=CH_2); 6.16 (d, 1H, $J_{NH,2}$ 9.3 Hz, –N*H*–C2); 7.31–7.48 (m, 5H, H-benzylidene).

^{13}C NMR (Me_2SO-d_6, 50.3 MHz): δ 22.57 (*C*H_3CO–); 52.92 (C-2); 67.25, 67.57, 67.88 (C-3, C-6, –*C*H_2–CH=CH_2); 75.38, 77.55 (C-4, C-5); 88.11 (C-1); 100.26 (=*C*H–Bzd); 117.77 (*C*H_2=CH–CH_2–); 126.29, 128.08 (C–Ar_{ortho}, C–Ar_{meta}); 128.92 (C–Ar_{para}); 137.06 (C–Ar_{ipso}); 153.96 (–O–*C*O–OAll); 169.52 (*C*H_3CO–). FDMS calcd: 418.2. Found: 419.2 (100%) $[M+H]^+$. Anal. Calcd for $C_{19}H_{22}N_4O_7$: C, 54.54; H, 5.30; N, 13.39. Found: C, 54.48; H, 5.57; N, 13.21.

3.1.2. 2-Acetamido-3-*O*-allyloxycarbonyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide 3 (3-Aloc-6-BzlGlcNAc-N_3). To a soln of benzylidene acetal **2** (11.30 g, 27.0 mmol) in dry tetrahydrofuran (130 mL), 10 g of pulverized molecular sieves (3 Å) and 17.0 g (270 mmol) of sodium cyanoborohydride were added. After cooling to 0 °C saturated (at 0 °C) HCl in diethyl ether was added dropwise within 1 h until the evolution of gas deceased (about 35 mL). Under careful monitoring by TLC, additional small amounts of $NaCNBH_3$ and HCl in ether (5 mL) were added twice. Compound **2** had then been completely consumed. After neutralization by addition of solid $NaHCO_3$, the solid materials were separated by centrifugation. The soln was diluted with CH_2Cl_2 (700 mL), and the organic layer was washed with satd aq $NaHCO_3$ and water. After drying with $MgSO_4$ and evaporation of the solvents, colourless crystals of **3** remained. The product was purified by flash chromatography in 60:1 CH_2Cl_2–MeOH. Yield: 8.32 g (73%), colourless crystals; mp: 164 °C; $[\alpha]_D^{23}$ −63.5 (*c* 1, $CHCl_3$); $R_f = 0.42$ (2:3 light petroleum–ethyl acetate).

^{1}H NMR (Me_2SO-d_6, 400 MHz): δ 1.77 (s, 3H, CH_3CO–); 3.40–3.47 (m, 1H, H-2); 3.58–3.64 (m, 2H, H-3, H-4); 3.67–3.75 (m, 2H, H-1, H-5); 4.53 (s, 2H, –CH_2Bn); 4.53 (s, 2H, –CH_2Bn); 4.55 (d, 1H, J_{gem} 16.7 Hz, J_{vic} 5.3 Hz, CH_2=CH–CH_2–O–); 4.62 (d, 1H, J_{gem} 16.7 Hz, J_{vic} 4.7 Hz, CH_2=CH–CH_2–O–); 4.66–4.74 (m, 2H, H-6a, H-6b); 5.22 (d, 1H, J_{cis} 10.6 Hz, CH_2=CH–CH_2–O–); 5.28 (d, 1H, J_{trans} 17.3 Hz, CH_2=CH–CH_2–O–); 5.72 (d, 1H, $J_{4,OH}$ 6.5 Hz, 4-OH); 5.90 (m_c, 1H, CH_2=C*H*–CH_2–O–); 7.24–7.37 (m, 5H, Ar–H); 8.06 (d, 1H, $J_{NH,2}$ 9.4 Hz, –N*H*Ac). ^{13}C NMR (Me_2SO-d_6, 50.3 MHz) DEPT: δ 22.51 (*C*H_3CO–); 52.77 (C-2); 67.55 (C-3); 67.61, 68.88 (C-6, CH_2=CH–*C*H_2–O–); 72.30 (–*C*H_2–Bn); 77.25, 79.42 (C-4, C-5); 87.43 (C-1); 117.65 (*C*H_2=CH–CH_2–O–); 127.22, 127.25, 128.06 (C–Ar); 132.03 (CH_2=*C*H–CH_2–O–); 138.29 (C_{ipso}); 154.16 (O–*C*O–O–); 169.17 (CH_3*C*O–). Anal. Calcd for $C_{19}H_{24}N_4O_7$: C, 54.28; H, 5.75; N, 13.33; Found: C, 53.82; H, 5.75; N, 13.31.

3.1.3. 2-Acetamido-3-*O*-allyloxycarbonyl-4-*O*-(6″-*O*-benzyl-2″,3″,4″-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide 5 (β-3-Aloc-4-(β-6″-Bn-Ac_3Gal)-6-BnGlcNAc-N_3). Under argon atmosphere a soln of **3** (250 mg, 0.462 mmol) and of galactosyl trichloroacetimidate[20] **4** (352 mg, 0.837 mmol) in dry CH_2Cl_2 (30 mL) was stirred with 0.5 g of pulverized molecular sieves 4 Å at room temperature for 1 h. After cooling to −50 °C, a soln of trimethylsilyl trifluoromethanesulfonate (triflate) (20 μL, 0.111 mmol) in CH_2Cl_2 (3 mL) was added via a syringe

within 1 h. The cooling was removed after 15 min and the soln stirred at room temperature for 14 h. The soln was neutralized with solid $NaHCO_3$, filtered through *Hyflo*, which was washed with CH_2Cl_2, and the combined organic layers were washed with satd aq $NaHCO_3$ and water, dried with $MgSO_4$, and the solvents were evaporated under diminished pressure. The obtained brownish-yellow solid (730 mg) was purified by flash chromatography with light petroleum/ethyl acetate. Yield: 229 mg (62%), colourless amorphous solid; $[\alpha]_D^{24}$ −50.0 (*c* 1, $CHCl_3$); $R_f = 0.32$ (2:3 light petroleum–ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz), ^{1}H, ^{1}H-COSY: δ 1.91 (s, 3H, CH_3CO–); 2 × 1.94 (s, 6H, CH_3CO–); 1.98 (s, 3H, CH_3CO–); 3.33 (t, $J_{6''b,6''a} \approx J_{6''b,5''}$ 8.8 Hz, H-6″b); 3.42 (dd, $J_{6''a,6''b}$ 9.0 Hz, $J_{6''a,5''}$ 5.3 Hz, H-6″a); 3.55 (dt, 1H, $J_{5,4}$ 9.4 Hz, $J_{5,6a}$ $J_{5,6b}$ 2.5 Hz, H-5); 3.67 (dd, 1H, $J_{5'',6''a}$ 6.1 Hz, $J_{5'',6''b}$ 8.4 Hz, H-5″); 3.68–3.71 (m, 2H, H-6a, H-6b); 3.89 (q, 1H, $J_{2,1} \approx J_{2,NH} \approx J_{2,3}$ 9.6 Hz, H-2); 3.95 (t, 1H, $J_{4,3} \approx J_{4,5}$ 9.1 Hz, H-4); 4.34 (d, 1H, J_{gem} 11.9 Hz, –CH_2Bn); 4.37 (ddt, ^4J 1.4 Hz, J_{gem} 13.1 Hz, J_{vic} 5.3 Hz, CH$_2$=CH–CH_2–O–); 4.48 (d, 1H, $J_{1'',2''}$ 7.9 Hz, H-1″); 4.50 (d, 1H, J_{gem} 11.7 Hz, –CH_2Bn); 4.51 (d, 1H, J_{gem} 12.3 Hz, –CH_2Bn); 4.55 (ddt, ^4J 1.4 Hz, J_{gem} 13.1 Hz, J_{vic} 7.4 Hz, CH$_2$=CH–CH_2–O–); 4.59 (d, 1H, $J_{1,2}$ 9.1 Hz, H-1); 4.69 (d, 1H, J_{gem} 12.0 Hz, –CH_2Bn); 4.86 (dd, 1H, $J_{3'',4''}$ 3.2 Hz, $J_{3'',2''}$ 10.3 Hz, H-3″); 4.92 (dd, 1H, $J_{3,2}$ 10.3 Hz, $J_{3,4}$ 9.1 Hz, H-3); 5.00 (dd, 1H, $J_{2'',3''}$ 10.3 Hz, $J_{2'',1''}$ 7.9 Hz, H-2″); 5.18 (dd, 1H, J_{gem} 1.2 Hz, J_{cis} 10.6 Hz, CH_2=CH–CH$_2$–O–); 5.27 (dd, 1H, J_{gem} 1.2 Hz, J_{trans} 17.0 Hz, CH_2=CH–CH$_2$–O–); 5.41 (d, 1H, $J_{4'',3''}$ 3.2 Hz, H-4″); 5.79 (m_c, 1H, CH$_2$=CH–CH$_2$–O–); 5.82 (d, 1H, $J_{NH,2}$ 8.5 Hz, –NHAc); 7.22–7.37 (m, 10H, H–Ar). ^{13}C NMR (Me_2SO-d_6, 100.6 MHz), DEPT, ^{1}H, ^{13}C-COSY: δ 20.44, 20.48, 20.63, 23.13 (*C*H$_3$CO–); 53.86 (C-2); 66.65 (C-6″); 67.20 (C-4″); 67.36 (C-6); 68.56 (CH$_2$=CH–*C*H$_2$–O); 69.32 (C-2″); 71.06 (C-3″); 73.53 (–*C*H$_2$Bn); 73.81 (–*C*H$_2$Bn); 74.81 (C-4); 71.92 (C-5″); 76.72 (C-5); 76.75 (C-3); 88.25 (C-1); 100.64 (C-1″); 118.76 (*C*H$_2$=CH–CH$_2$–O); 127.82, 127.90, 128.37, 128.47 (C–Ar); 131.17 (CH$_2$=*C*H–CH$_2$–O); 137.51, 137.68 (C_{ipso}); 154.71 (–O–*C*O–O–); 169.17, 169.76, 169.79, 170.08 (CH$_3$*C*O–). Anal. Calcd for $C_{38}H_{46}N_4O_{15}$: C, 57.14; H, 5.80; N, 7.01. Found: C, 57.19; H, 5.78; N, 6.91.

3.1.4. 2-Acetamido-4-*O*-(6″-*O*-benzyl-2″,3″,4″-tetra-*O*-acetyl-β-D-galactopyranosyl)-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide 6 (β-4-(β-6″-Bn-Ac$_3$Gal)-6-BnGlcNAc-N$_3$).

To a soln of allyloxycarbonyl protected disaccharide **5** (4.90 g, 6.13 mmol) in dry and oxygen free tetrahydrofuran (200 mL) under argon atmosphere, morpholine (3.20 mL, 36.81 mmol) was added trough a syringe. Under an argon counter stream a catalytic amount of $(PPh_3)_4Pd(0)$ (30 mg) was added, and the soln was stirred at room temperature for 2 h. The major portion (about 170 mL) of THF was distilled off, the remaining suspension was diluted with ethyl acetate (500 mL) and extracted with water. After drying with $MgSO_4$, the solvent was evaporated under diminished pressure. The remaining viscous oil (4.90 g) was purified by flash chromatography in light petroleum/ethyl acetate. Yield: 3.24 g (74%), colourless amorphous solid; $[\alpha]_D^{21}$ −17.9 (*c* 1, $CHCl_3$); $R_f = 0.24$ (1:2 light petroleum–ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz), ^{1}H, ^{1}H-COSY: δ 1.96 (s, 6H, CH_3CO–); 1.97 (s, 3H, CH_3CO–); 2.03 (s, 3H, CH_3CO–); 3.29–3.37 (m, 2H, H-6b, H-6″b); 3.47 (t, 1H, $J_{6''a,6''b} \approx J_{6''a,5''}$ 8.4 Hz, H-6″a); 3.55–3.68 (m, 4H, H-2, H-4, H-6a, H-5″); 3.79–3.83 (m, 1H, H-5); 4.05 (dd, 1H, $J_{3,4}$ 10.3 Hz, $J_{3,2}$ 8.2 Hz, H-3); 4.33 (d, J_{gem} 12.0 Hz, –CH_2Bn); 4.48 (d, 12.0 Hz, –CH_2Bn); 4.51 (d, J_{gem} 12.9 Hz, –CH_2Bn); 4.53 (d, 1H, $J_{1'',2''}$ 9.1 Hz, H-1″); 4.94 (dd, $J_{3'',4''}$ 3.2 Hz, $J_{3'',2''}$ 10.3 Hz, H-3″); 5.00 (d, 1H, $J_{1,2}$ 9.1 Hz, H-1); 5.11 (dd, $J_{2'',3''}$ 10.3 Hz, $J_{2'',1''}$ 7.9 Hz, H-2″); 5.17 (d, 1H, $J_{4'',3''}$ 2.9 Hz, H-4″); 5.96 (d, 1H, $J_{NH,2}$ 7.6 Hz, –NHAc); 7.23–7.35 (m, 10H, H–Ar). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 20.49, 20.64, 23.44 (*C*H$_3$CO–); 56.85 (C-2); 67.33, 67.65, 67.81 (C-6, C-4″, C-6″); 69.12 (C-2″); 70.89 (C-3″); 71.44, 72.36 (C-4, C-5″); 73.67 (2 × –CH_2Bn); 76.17 (C-5); 80.79 (C-3); 87.70 (C-1); 101.15 (C-1″); 127.75, 127.85, 128.02, 128.52 (H–Ar); 137.18, 137.92 (H_{ipso}); 169.19, 169.98, 170.68 (CH$_3$*C*O–). Anal. Calcd for $C_{34}H_{42}N_4O_{13}$ (714.72): C, 57.14; H, 5.92; N, 7.84. Found: C, 56.94; H, 6.03; N, 7.85.

3.1.5. 2-Acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-6-*O*-benzyl-2-deoxy-4-*O*-(2″,3″,4″-tri-*O*-acetyl-6″-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl azide 8 (β-3-(α-Bn$_3$Fuc)-4-(β-Ac$_3$-6-BnGal)-6-BnGlcNAc-N$_3$).

To a soln of lactosamine azide **6** (3.10 g, 4.34 mmol) and thiofucoside[22] **7** (2.91 g, 6.08 mmol) in dry DMF (70 mL) and dry CH_2Cl_2 (70 mL), 7 g of pulverized molecular sieve (4 Å) were added. The mixture was stirred for 1 h at room temperature. After addition of tetrabutylammonium bromide (2.38 g, 7.38 mmol) and copper(II)bromide (1.45 g, 6.51 mmol), the stirring was continued for 18 h at room temperature. After filtration through *Hyflo*, which was washed with CH_2Cl_2 (400 mL), the organic soln was extracted with brine and six times with approx. 80 mL of satd aq $NaHCO_3$. The aqueous extracts were re-extracted with 40 mL of CH_2Cl_2. The combined organic solns were dried over $MgSO_4$, and the solvent was evaporated under diminished pressure to give 9.3 g of a brown, viscous oil, which was purified by flash chromatography (4:1 light petroleum–ethyl acetate). Yield: 3.40 g (69%), colourless amorphous solid; $[\alpha]_D^{21}$ −66.3 (*c* 1, $CHCl_3$); $R_f = 0.37$ (1:1 light petroleum–ethyl acetate). ^{1}H NMR ($CDCl_3$, 400 MHz), ^{1}H, ^{1}H-COSY: δ 1.10 (d, 3H, $J_{6',5'}$ 6.5 Hz, H-6′); 1.72 (s, 3H, CH_3CO–); 1.95 (s, 3H, CH_3CO–); 1.98 (s, 3H, CH_3CO–); 3.34 (t,

$J_{5'',6''a} \approx J_{5'',6''b}$ 8.4 Hz, H-5″); 3.42 (q, 1H, $J_{2,1} \approx J_{2,3} \approx J_{2,NH}$ 7.3 Hz, H-2); 3.46–3.58 (m, 4H, H-5, H-4′, H-6″a, H-6″b); 3.76–3.82 (m, 2H, H-6a, H-6b); 3.83 (dd, 1H, $J_{3',2'}$ 10.3 Hz, $J_{3',4'}$ 2.5 Hz, H-3′); 3.93 (t, 1H, $J_{4,3} \approx J_{4,5}$ 7.3 Hz, H-4); 4.08 (dd, 1H, $J_{2',1'}$ 3.8 Hz, $J_{2',3'}$ 10.3 Hz, H-2′); 4.10 (t, 1H, $J_{3,2} \approx J_{3,4}$ 8.2 Hz, H-3); 4.26 (m, 1H, H-5′); 4.28 (d, 1H, J_{gem} 12.3 Hz, –CH_2–Bn); 4.43 (d, 1H, J_{gem} 12.0 Hz, –CH_2–Bn); 4.45 (d, 1H, J_{gem} 12.0 Hz, –CH_2–Bn); 4.53 (d, 1H, $J_{1'',2''}$ 8.2 Hz, H-1″); 4.62 (d, 1H, J_{gem} 12.0 Hz, –CH_2–Bn); 4.63 (d, 1H, J_{gem} 11.5 Hz, –CH_2–Bn); 4.65 (d, 1H, J_{gem} 12.0 Hz, –CH_2–Bn); 4.66 (d, 1H, J_{gem} 12.3 Hz, –CH_2–Bn); 4.71 (d, 1H, J_{gem} 11.7 Hz, –CH_2–Bn); 4.84 (dd, $J_{3'',2''}$ 10.6 Hz, $J_{3'',4''}$ 3.7 Hz, H-3″); 4.85 (d, 1H, J_{gem} 11.5 Hz, –CH_2–Bn); 4.94 (d, 1H, J_{gem} = 12.0 Hz, –CH_2–Bn); 4.99 (dd, 1H, $J_{2'',1''}$ 8.2 Hz, $J_{2'',3''}$ 10.6 Hz, H-2″); 5.02 (d, 1H, $J_{1',2'}$ 3.8 Hz, H-1′); 5.12 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1); 5.39 (d, 1H, $J_{4'',3''}$ 3.2 Hz, $J_{4'',5''} \ll 1$ Hz, H-4″); 5.90 (d, 1H, $J_{-NH-,2}$ 5.9 Hz, –N*H*–COCH$_3$); 7.14–7.38 (m, 25H, Ar–H). ^{13}C NMR ($CDCl_3$, 100.6 MHz), DEPT, ^{1}H, ^{13}C-COSY: δ 16.61 (C-6′); 20.42, 20.50, 20.63, 22.98 (4 × *C*H$_3$CO–); 54.78 (C-2); 66.69 (C-6″); 66.70 (C-5″); 67.32 (C-4″); 68.09 (C-6); 69.18 (C-2″); 70.77 (C-3″); 71.80 (C-5); 72.75 (–*C*H$_2$–Bn); 73.33 (–*C*H$_2$–Bn); 73.46 (C-3); 73.52 (–*C*H$_2$–Bn); 73.53 (C-5′); 73.67 (C-4); 74.46 (–*C*H$_2$–Bn); 76.71 (C-2′); 77.14 (C-4′); 79.76 (C-3′); 87.44 (C-1); 97.29 (C-1′); 99.47 (C-1″); 127.15, 127.51, 127.54, 127.58, 127.65, 127.71, 127.79, 127.95, 128.14, 128.23, 128.34, 128.40, 128.46, 128.49 (C–Ar); 170.23, 169.77, 169.68, 169.31 (4 × CH$_3$*C*O–). Anal. Calcd for $C_{61}H_{70}N_4O_{17}$: C, 64.77; H, 6.24; N, 4.95. Found: C, 64.41; H, 6.20; N, 4.76.

3.1.6. 2-Acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-6-*O*-benzyl-2-deoxy-4-*O*-(6″-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranosyl azide 9 (β-3-(α-Bn$_3$Fuc)-4-(β-6″-BnGal)-6-BnGlcNAc-N$_3$).

To a soln of LewisX derivative **8** (3.13 g,2.77 mmol) in dry MeOH (100 mL), 3.3 mL of freshly prepared 0.1 M sodium methoxide in MeOH was added. The soln was stirred for 5 h at room temperature. After neutralization with acidic ion-exchange resin (Amberlite IR-120) and filtration, the solvent was evaporated under diminished pressure. The remaining colourless amorphous solid was purified by flash chromatography with 40:1 CH_2Cl_2–MeOH. Yield: 2.67 g (97%), colourless amorphous solid; $[\alpha]_D^{22}$ −63.1 (*c* 1, CH_2Cl_2); R_f = 0.33 (18:1 CH_2Cl_2–MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz), ^{1}H, ^{1}H-COSY: δ 1.12 (d, 3H, $J_{6',5'}$ 6.3 Hz, H-6′); 1.62 (s, 3H, CH_3CO–); 3.37 (q, 1H, $J_{2,1} \approx J_{2,3} \approx J_{2,NH}$ 9.0 Hz, H-2); 3.41 (dd, 1H, $J_{3'',4''}$ 3.1 Hz, $J_{3'',2''}$ 9.4 Hz, H-3″); 3.47 (t, 1H, $J_{5'',6''a} \approx J_{5'',6''b}$ 5.9 Hz, H-5″); 3.52 (dd, 1H, $J_{2'',1''}$ 7.8 Hz, $J_{2'',3''}$ 9.0 Hz, H-2″); 3.58–3.61 (m, 2H, H-5, H-6″a); 3.70 (d, 1H, $J_{4',3'}$ 2.3 Hz, H-4′); 3.71 (dd, 1H, $J_{6''b,6''a}$ 9.8 Hz, $J_{6''b,5''}$ 6.7 Hz, H-6″b); 3.76 (dd, 1H, $J_{6a,6b}$ 11.7 Hz, $J_{6a,5}$ 1.6 Hz, H-6a); 3.89 (d, 1H, $J_{4'',3''}$ 2.7 Hz, H-4″); 3.90 (dd, 1H, $J_{3',2'}$ 10.2 Hz, $J_{3',4'}$ 2.3 Hz, H-3′); 3.95 (dd, 1H, $J_{6a,6b}$ 11.7 Hz, $J_{6b,5}$ 3.5 Hz, H-6b); 4.02–4.09 (m, 2H, H-4, H-2′); 4.10 (t, 1H, $J_{2,3} \approx J_{3,4}$ 8.6 Hz, H-3); 4.30 (q, 1H, $J_{5',6'}$ 6.3 Hz, H-5′); 4.46 (s, 2H, –CH_2Bn); 4.48 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1″); 4.51 (d, 1H, J_{gem} 12.1 Hz, –CH_2Bn); 4.55 (d, 1H, J_{gem} 11.4 Hz, –CH_2Bn); 4.60 (d, 1H, J_{gem} 9.8 Hz, –CH_2Bn); 4.63 (d, 2H, J_{gem} 11.7 Hz, –CH_2Bn); 4.72 (d, 1H, J_{gem} 11.4 Hz, –CH_2Bn); 4.81 (d, 1H, $J_{1,2}$ 9.0 Hz, H-1); 4.89 (d, 1H, J_{gem} 11.4 Hz, –CH_2Bn); 4.90 (d, 1H, J_{gem} 11.3 Hz, –CH_2Bn); 5.13 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1′); 6.16 (d, 1H, $J_{NH,2}$ 5.9 Hz, –NH–); 7.21–7.36 (m, 25H, H–Ar). ^{13}C NMR ($CDCl_3$, 100.6 MHz), DEPT, ^{1}H, ^{13}C-COSY: δ 16.85 (C-6′); 23.00 (*C*H$_3$CO–); 56.59 (C-2); 67.26 (C-5′); 68.16 (C-6); 68.25 (C-4″); 68.69 (C-6″); 72.03 (–*C*H$_2$Bn); 72.13 (C-2″); 72.89 (C-5″); 73.30 (–*C*H$_2$Bn); 73.45 (–*C*H$_2$Bn, C-3″); 74.13 (C-4); 74.47, 74.97 (–*C*H$_2$Bn); 76.07 (C-3); 76.77 (C-5); 76.94 (C-2′); 77.30 (C-4′); 79.70 (C-3′); 87.81 (C-1); 98.16 (C-1′); 100.98 (C-1″); 127.12, 127.47, 127.56, 127.64, 127.70, 127.73, 127.94, 127.99, 128.17, 128.35, 128.41, 128.67 (C–Ar); 137.68, 137.71, 138.10, 138.28, 138.50 (C$_{ipso}$); 170.91 (CH$_3$*C*O–). Anal. Calcd for $C_{55}H_{64}N_4O_{14}$: C, 65.72; H, 6.42; N, 5.57. Found: C, 65.45; H, 6.60; N, 5.41.

3.1.7. 2-Acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-6-*O*-benzyl-2-deoxy-4-*O*-(6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosyl azide 11 (β-Ac$_4$-Bn$_5$sLe^x-COOMe-N$_3$).

To a soln of LewisX azide **9** (1.023 g, 1.018 mmol) and ethyl thiosialoside[23,24] **10** (1.090 mg, 2.035 mmol) in dry acetonitrile (80 mL) under an argon atmosphere, molecular sieves (3 Å, 4 g) were added. After stirring for 40 min and subsequent cooling to −40 °C, a soln of *N*-iodosuccinimide (0.962 g, 4.276 mmol) in 20 mL of acetonitrile was added. Within 40 min, 1.30 mL of a 0.4 N soln of trifluoromethanesulfonic acid in acetonitrile was added dropwise, and the soln was stirred for 15 min at −40 °C. After keeping the soln overnight at −28 °C, the mixture was neutralized with solid $NaHCO_3$. The suspension was filtered through *Hyflo*, and the filtrate was diluted with CH_2Cl_2 (500 mL) and washed twice with satd aq $NaHCO_3$. The brownish soln became wine red. After decolourizing by washing with 80 mL of 20% aq sodium thiosulfate and water, the organic layer was dried with $MgSO_4$ and concentrated under diminished pressure to give 2.03 g of a yellow amorphous solid. Column chromatography in 6:1 light petroleum–ethyl acetate and a second chromatography in 60:1 CH_2Cl_2–MeOH gave the sialyl LewisX **11** in a pure form.

Yield: 882 mg (59%), colourless amorphous solid; $[\alpha]_D^{26}$ −45.3 (*c* 1, $CHCl_3$); R_f = 0.32 (1:1 toluene–acetone); ^{1}H NMR ($CDCl_3$, 400 MHz), ^{1}H, ^{1}H-COSY: δ 1.05

(d, 3H, $J_{6',5'}$ 6.5 Hz, H-6′); 1.63 (s, 3H, –CO–CH_3); 1.87 (s, 3H, –CO–CH_3); 1.96 (s, 3H, –CO–CH_3); 2.02 (s, 3H, –CO–CH_3); 2.03 (m, 1H, H-3‴ax); 2.07 (s, 3H, –CO–CH_3); 2.08 (s, 3H, –CO–CH_3); 2.68 (dd, 1H, $J_{3'''eq,3'''ax}$ 12.9 Hz, $J_{3'''eq,4'''}$ 4.5 Hz, H-3‴eq); 3.48–3.73 (m, 7H, H-5, H-4′, H-4″, H-5″, H-6″a, H-6″b, H-6‴); 3.74 (s, 3H, –COOCH_3); 3.76–4.19 (m, 10H, H-2, H-4, H-3, H-6a, H-6b, H-2′, H-3′, H-5′, H-3″, H-5‴, H-9a‴); 4.20–4.32 (m, 2H, –CH_2Bn, H-9‴b); 4.43 (s, 2H, –CH_2Bn); 4.52–4.77 (m, 7H, H-1, H-1″, 5 × –CH_2Bn); 4.84–4.92 (m, 3H, 2 × –CH_2Bn, H-4‴); 5.18 (d, 1H, $J_{1',2'}$ 3.3 Hz, H-1′); 5.24–5.33 (m, 2H, H-2″, H-7‴); 5.43 (m_c, 1H, H-8‴); 6.11 (d, 1H, J_{vic} 7.2 Hz, –NH–C2); 7.21–7.36 (m, 25H, H–Ar). ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 16.81 (C-6′); 20.61, 20.69, 20.77, 21.09, 23.08, 23.12 (–CO–*C*H_3); 37.49 (C-3‴); 49.61 (C-5‴); 53.05 (–COO*C*H_3); 55.98 (C-2); 62.31 (C-9‴); 67.07, 67.23, 67.67, 68.24 (C-5, C-4″, C-7‴, C-8‴); 68.29, 68.50 (C-6, C-6″); 68.63, 70.01 (C-2″, C-4‴); 72.14 (–*C*H_2Bn); 72.53, 72.70, 73.48 (C-3, C-3″, C-5″, C-6‴); 73.01, 73.28, 74.38, 75.03 (–*C*H_2–Bn); 76.13, 77.02, 77.29, 77.72 (C-4, C-2′, C-4′, C-5′); 79.93 (C-3′); 88.23 (C-1); 97.95 (C-2‴); 98.17 (C-1′); 101.14 (C-1″); 127.15, 127.34, 127.42, 127.48, 127.52, 127.65, 128.04, 128.07, 128.16, 128.23, 128.37, 128.64 (C–Ar); 138.05, 138.29, 138.42, 138.54, 138.75 (C-*ipso*); 168.16, 169.78, 169.91, 170.28, 170.42, 170.47, 170.76 (–*C*O–CH$_3$, –*C*OOMe). ESI-MS calcd for $C_{75}H_{91}N_5O_{26}$: 1477.8. Found: 1501.1 (100%), $[M+Na]^+$; 1517.1 (5%), $[M+K]^+$.

3.1.8. 2-Acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-6-*O*-benzyl-2-deoxy-4-*O*-(2″,4″-di-*O*-acetyl-6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosyl azide 12 (β-Ac$_6$-Bn$_5$sLe^{x}COOMe-N$_3$). The sLeX derivative **11** (773.5 mg, 0.523 mmol) was dissolved in pyridine (15 mL) and Ac_2O (15 mL). After addition of 50 mg of 4-dimethylaminopyridine (DMAP), the soln was stirred for 3 h at room temperature. After concentration in high vacuum the residue was co-distilled three times with 30 mL of toluene and purified by flash chromatography in 6:1 light petroleum–ethyl acetate. Yield: 725 mg (89%), colourless amorphous solid; $[\alpha]_D^{22}$ −59.9 (*c* 1, $CHCl_3$); $R_f = 0.35$ (12:1 CH_2Cl_2 – MeOH). ^{1}H NMR ($CDCl_3$, 600 MHz), ^{1}H, ^{1}H-COSY: δ 1.00 (d, 1H, $J_{6',5'}$ 6.2 Hz, H-6′); 1.65 (t, 1H, $J_{3'''a,3'''e} \approx J_{3'''a,4'''}$ 12.3 Hz, H-3‴a); 1.79 (s, 3H, CH_3CO–); 1.80 (s, 3H, CH_3CO–); 1.88 (s, 3H, CH_3CO–); 1.89 (s, 3H, CH_3-CO–); 1.94 (s, 3H, CH_3CO–); 2.01 (s, 3H, CH_3CO–); 2.04 (s, 3H, CH_3CO–); 2.14 (s, 3H, CH_3CO–); 2.50 (dd, 1H, $J_{3'''e,3a'''}$ 12.4 Hz, $J_{3'''e,4'''}$ 4.3 Hz, H-3‴e); 3.28 (t, 1H, $J_{6''a,6''b} \approx J_{6''a,5}$ 8.5 Hz, H-6″a); 3.39 (dd, 1H, $J_{6''b,6''a}$ 9.4 Hz, $J_{6''b,5''}$ 5.6 Hz, H-6″b); 3.42 (s, breit, H-4); 3.56 (dd, 1H, $J_{6''',7'''}$ 2.1 Hz, $J_{6''',5'''}$ 10.7 Hz, H-6‴); 3.66–3.73 (m, 3H, H-2, H-3′, H-5″); 3.78–3.81 (m, 6H, –COOCH_3, H-6a, H-6b, H-4′); 3.88–3.98 (m, 5H, H-3, H-5, H-5′, H-5‴, H-9‴b); 4.01 (dd, 1H, $J_{2',1'}$ 3.5 Hz, $J_{2',3'}$ 10.3 Hz, H-2′); 4.25 (d, 1H, J_{gem} 12.0 Hz, –CH_2Bn); 4.26 (t, 1H, $J_{9'''a,9'''b} \approx J_{9'''a,8'''}$ 10.8 Hz, H-9‴a); 4.32 (d, 1H, J_{gem} 12.0 Hz, –CH_2Bn); 4.36 (d, 1H, J_{gem} 11.7 Hz, –CH_2Bn); 4.50 (d, 1H, J_{gem} 12.0 Hz, –CH_2Bn); 4.52–4.59 (m, 3H, 2 × –CH_2Bn, H-3″); 4.67 (d, 2H, J_{gem} 12.0 Hz, 2 × –CH_2Bn); 4.68 (d, 1H, $J_{1'',2''}$ 7.0 H, H-1″); 4.72 (d, 1H, J_{gem} 11.7 Hz, –CH_2Bn); 4.81–4.88 (m, 3H, H-2″, H-4‴, –CH_2Bn); 4.91 (d, 1H, $J_{1,2}$ 5.6 Hz, H-1); 4.99 (d, 1H, $J_{4'',3''}$ 2.6 Hz, H-4″); 5.02 (d, 1H, $J_{NH,5'''}$ 10.3 Hz, –N*H*–C5‴); 5.06 (d, 1H, $J_{1',2'}$ 3.2 Hz, H-1′); 5.30 (dd, 1H, $J_{7''',6'''}$ 2.1 Hz, $J_{7''',8'''}$ 9.2 Hz, H-7‴); 5.53 (ddd, 1H, $J_{8''',9'''a}$ 11.8 Hz, $J_{8''',9'''b}$ 3.2 Hz, $J_{8''',7'''}$ 6.1 Hz, H-8‴); 6.14 (d, 1H, $J_{NH,2}$ 7.9 Hz, –NH–C2); 7.14–7.32 (m, 25H, H–Ar). ^{13}C NMR ($CDCl_3$, 100.6 MHz), DEPT, ^{1}H, ^{13}C-COSY: δ 16.64 (C-6′); 20.60, 20.74, 2 × 20.78; 20.92; 21.39; 23.03; 23.12 (*C*H_3CO–); 37.54 (C-3‴); 49.03 (C-5‴); 52.13 (C-2); 53.19 (–O–*C*H_3); 62.47 (C-9‴); 66.96 (C-5); 67.22 (C-7‴); 67.46 (C-6″); 67.64 (C-8‴); 67.87 (C-4″); 68.84 (C-6); 69.37 (C-4‴); 70.27 (C-2″); 71.22 (C-3″); 71.81 (C-5″); 71.97 (C-6‴); 72.78 (C-3); 72.86 (–*C*H_2Bn); 72.92 (–*C*H_2Bn); 73.09 (C-5′); 2 × 73.27 (2 × –*C*H_2Bn); 74.53 (–*C*H_2Bn); 75.60 (C-4′); 76.50 (C-2′); 77.31 (C-4); 79.35 (C-3′); 87.63 (C-1); 96.59 (C-1′); 96.88 (C-2‴); 99.05 (C-1″); 127.21, 127.24, 127.43, 127.53, 127.59, 127.64, 127.70, 127.79, 128.20, 128.23, 128.32, 128.38, 128.43 (C–Ar); 137.69, 138.28, 138.57, 138.62, 138.75 (C_{ipso}); 167.83, 169.68, 169.89, 170.30, 170.33, 170.42, 2 × 170.51, 170.82 (8 × –*C*OCH$_3$, C-1‴). MALDI-MS (dhb) calcd for $C_{79}H_{95}N_5O_{28}$: 1562.6. Found 1585.7 $[M+Na]^+$; 1601.5 $[M+K]^+$. Anal. Calcd for $C_{79}H_{95}N_5O_{28}\cdot H_2O$: C, 60.00; H, 6.19; N, 4.43. Found: C, 59.84, H, 6.32; N, 4.43.

3.1.9. 2-Acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-6-*O*-benzyl-2-deoxy-4-*O*-(6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosylamine 13 (β-Ac$_6$-Bn$_5$sLe^x-COOMe-NH$_2$). To a soln of sLe^x azide **12** (444.0 mg, 0.284 mmol) in isopropanol (12 mL) and 2 mL of water a catalytic amount of neutrally washed Raney nickel was added. After exchange of the air for argon and then for hydrogen, hydrogenation was carried out under vigorous stirring for 1–2 h at atmospheric pressure. After filtration through *Hyflo* and washing of the catalyst with isopropanol, evaporation of the solvents under diminished pressure at 35 °C and drying in high vacuum quantitatively gave unstable sLe^x amine **13** ($R_f = 0.74$, 8:1 CH_2Cl_2–MeOH), which was immediately subjected to further conversion.

3.1.10. *tert*-Butyl 15-hydroxy-4,7,10,13-tetraoxapentadecanoate 14 (Glycol$_4$-(CH$_2$)$_2$COO*t*-Bu). To dry tetraethylene glycol (172.6 mL, 1.00 mol) in 400 mL of dry

tetrahydrofuran sodium (0.2 g, 8.7 mmol) was added. After 2 h, the sodium had been dissolved and *tert*-butyl acrylate (43.5 mL, 0.3 mol) was added. The soln was stirred under exclusion of moisture for 24 h. After neutralization with 8 mL of 1 M HCl, the solvent was evaporated under diminished pressure. The residue was dissolved in 400 mL of brine and extracted three times with 250 mL of ethyl acetate. The combined organic layers were washed with water (100 mL) and dried with $MgSO_4$. The solvent was evaporated under diminished pressure, and the remaining **14** was dried in high vacuum. Yield: 43.15 g (78%), clear, pale yellow liquid, $n_D^{20} = 1.448$; $R_f = 0.52$ (4:1 EtOAc–EtOH). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.41 (s, 9H, –C(CH_3)$_3$); 2.47 (t, 2H, J_{vic} 6.6 Hz, –CH_2–COO*t*-Bu); 2.76 (s, 1H, –OH); 3.53–3.75 (m, 18H, –CH_2–O–). ^{13}C NMR ($CDCl_3$, 50.3 MHz), BB, δ [ppm]: 28.03 (–C(*C*H$_3$)$_3$); 36.20 (–*C*H$_2$–COO*t*-Bu); 61.59 (–*C*H$_2$OH); 66.81 (–*C*H$_2$CH$_2$COO*t*-Bu); 70.49, 70.43, 70.29 (–*C*H$_2$–O–); 72.49 (HO-CH$_2$–*C*H$_2$–O–); 80.39 (–*C*(CH$_3$)$_3$); 170.83 (–*C*OO*t*-Bu). FDMS calcd for $C_{15}H_{30}O_7$: 322.4. Found 345.7 (23%) $[M+Na]^+$, 646.1 (9%) $[2M+H]^+$ 668.1 (9%) $[2M+Na]^+$.

3.1.11. *tert*-Butyl 15-methylsulfonyloxy-4,7,10,13-tetraoxapentadecanoate 15 (MesO-Glycol$_4$-(CH$_2$)$_2$COO*t*-Bu). To a soln of **14** (40.0 g, 124 mmol) in 300 mL of dry CH_2Cl_2 and 42.4 mL (300 mmol) of Et_3N at 0 °C, 20.3 mL (260 mmol) of methanesulfonyl chloride were added dropwise within 15 min. After stirring for 15 h at 0 °C, filtration through *Hyflo* and washing with water and brine, the CH_2Cl_2 soln was dried with $MgSO_4$ and evaporated to dryness under diminished pressure. Yield: 49.69 g (124 mmol, quant.); dark brown oil; $R_f = 0.66$ (ethyl acetate). The product was used for further conversion without purification.

3.1.12. *tert*-Butyl 15-azido-4,7,10,13-tetraoxapentadecanoate 16 (N$_3$-Glycol$_4$-(CH$_2$)$_2$COO*t*-Bu). Sodium azide (16.3 g, 250 mmol) was added to soln of mesylate **15** (49.69 g, 124 mmol) in DMF (300 mL). The mixture was stirred under exclusion of moisture for 2 d at room temperature. After centrifugation and extraction of the precipitate with DMF (twice 50 mL), the combined organic layers were evaporated in high vacuum. The residue was dissolved in diethyl ether (400 mL) and washed with 200 mL of satd aq $NaHCO_3$. The aq soln was re-extracted three times with 80 mL of ether. The combined ether solns were dried with $MgSO_4$ and evaporated under diminished pressure to give 40.02 g of a brown oil, which was purified by flash chromatography in light petroleum/ethyl acetate. Yield: 28.47 g (66%), pale yellow oil; $n_D^{23} = 1.449$; $R_f = 0.39$ (1:2 light petroleum–ethyl acetate). FT-IR: 2104 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.41 (s, 9H, –C(CH_3)$_3$); 2.47 (t, 2H, J_{vic} 6.7 Hz, –CH_2–COO*t*-Bu); 3.36 (t, 2H, J_{vic} 5.08 Hz, –CH_2N$_3$); 3.56–3.64 (m, 14H, –O–CH_2–); 3.67 (t, 2H, J_{vic} 6.7 Hz, –CH_2–CH$_2$–COO*t*-Bu). ^{13}C NMR ($CDCl_3$, 50.3 MHz), δ 28.03 (–C(*C*H$_3$)$_3$); 36.23 (–*C*H$_2$–COO*t*-Bu); 50.62 (–*C*H$_2$N$_3$); 66.84 (–*C*H$_2$CH$_2$COO*t*-Bu); 69.97, 70.32, 70.45, 70.62 (–*C*H$_2$–O–); 80.36 (–*C*(CH$_3$)$_3$); 170.79 (–*C*OO*t*-Bu). FDMS calcd for $C_{15}H_{29}N_3O_6$: 347.4, gef.: 322.8 (100%) $[M-N_2+2H]^+$, 348.8 (36%) $[M+H]^+$, 668.2 (64%) $[2M-N_2+H]^+$. Anal. Calcd for $C_{15}H_{29}N_3O_6 \cdot 1/2H_2O$: C, 50.55; H, 8.48, N, 11.79. Found: C, 50.44, H; 8.37; N, 11.93.

3.1.13. *tert*-Butyl 15-amino-4,7,10,13-tetraoxapentadecanoate 17 (H$_2$N-Glycol$_4$-(CH$_2$)$_2$COO*t*-Bu). To a soln of azide **16** (10.0 g, 28.78 mmol) in 120 mL of isopropanol freshly prepared Raney nickel (7 g, pH 9) was given. Air was exchanged for argon and then for hydrogen. After hydrogenation for 12 h, the catalyst was filtered off through *Hyflo* and washed with 50 mL of isopropanol. The solvent was evaporated and the remaining product **17** filtered through a syringe filter in order to remove traces of Raney nickel. Yield: 8.57 g (93%), colourless liquid; $R_f = 0.05$ (ethyl acetate); ^{1}H NMR ($CDCl_3$, 200 MHz): 1.40 (s, 9H, –C(CH_3)$_3$); 1.56 (s, br, 2H, –NH_2); 2.46 (t, 2H, J_{vic} 6.6 Hz, –CH_2–COO*t*-Bu); 2.81 (t, 2H, J_{vic} 5.4 Hz, H$_2$N–CH_2–); 3.46 (t, J_{vic} 5.4 Hz, –CH_2–CH$_2$–NH$_2$); 3.54–3.62 (m, 12H, –O–CH_2–); 3.66 (t, 2H, J_{vic} 6.6 Hz, –CH_2–CH$_2$–COO*t*-Bu). ^{13}C NMR (Me_2SO-d_6, 50.3 MHz): δ 28.10 (–C(*C*H$_3$)$_3$); 36.27 (–*C*H$_2$–COO*t*-Bu); 41.84 (–*C*H$_2$–NH$_2$); 66.89 (–*C*H$_2$–CH$_2$–COO*t*-Bu); 70.31, 70.37, 70.51, 70.58, 70.61 (–*C*H$_2$–O–); 73.52 (–CH$_2$–CH$_2$–NH$_2$); 80.49 (–*C*(CH$_3$)$_3$); 170.90 (–*C*OO*t*-Bu). FDMS: calcd for $C_{15}H_{31}N_1O_6$: 321.4. Found: 322.1 (2%) $[M+H]^+$, 643.6 (100%) $[2M+H]^+$. Anal. Calcd for $C_{15}H_{31}N_1O_6$ (321.41): C, 56.05; H, 9.72; N, 4.36. Found: C, 56.10; H, 9.62; N, 4.37.

3.1.14. α-Allyl β-*tert*-butyl *N*-fluorenylmethoxycarbonyl-L-aspartate 18 (Fmoc-Asp(O*t*-Bu)-OAll)

3.1.14.1. Method (a). Fmoc-Asp(O*t*-Bu)-OH (3.0 g, 7.51 mmol) and diisopropylethylamine (2.57 mL, 15.02 mmol) in allylbromide (15 mL) were heated under reflux for 30 min. The resulting clear slightly brownish soln, after cooling, was poured into ethyl acetate (200 mL). Precipitating diisopropylethylammonium hydrobromide was filtered off, and the filtrate was rapidly washed with 0.1 N HCl, $NaHCO_3/Na_2CO_3$ buffer (pH 9.5) and brine. After drying over $MgSO_4$, the solvent was evaporated under diminished pressure and resulting brown oil was lyophilized from benzene. Yield: 3.39 g (quant.), beige-coloured amorphous solid.

3.1.14.2. Method (b). To Fmoc-Asp(O*t*-Bu)-OH (2.50 g, 6.08 mmol) in 120 mL of dry MeOH, dry Cs_2CO_3 (0.99 g, 3.04 mmol) was added. The soln was stirred for 30 min at room temperature, the MeOH was evaporated under diminished pressure, and the salt dried

in high vacuum. The thus obtained caesium salt was dissolved in dry dimethylformamide (90 mL). At 0 °C a soln of allylbromide (1.10 mL, 13.00 mmol) in dry dimethylformamide (10 mL) was added within 30 min. After stirring for 2 h at room temperature, the precipitating CsBr was filtered off and DMF evaporated in high vacuum. The residue was dissolved in 300 mL of CH_2Cl_2 and washed twice with 50 mL of water. Drying with $MgSO_4$ afforded a yellow viscous oil, which was lyophilized from benzene. Yield: 2.75 g (quant.), colourless amorphous solid; $R_f = 0.78$ (2:1 light petroleum–ethyl acetate).

3.1.15. α-Allyl *N*-fluorenylmethoxycarbonyl-L-aspartate 19 (Fmoc-Asp-OAll). A soln of Fmoc-Asp(*t*-Bu)-OAll **18** (2.74 g, 6.07 mmol) in a mixture of TFA (25 mL), CH_2Cl_2 (25 mL) and water (1.8 mL) was stirred for 40 min at room temperature. CH_2Cl_2 and TFA were evaporated under diminished pressure, and the residue was co-distilled three times with toluene (30 mL). Crude **19** was purified by flash chromatography in 20:10:1 light petroleum–ethyl acetate–acetic acid. Yield: 2.29 g (95%), colourless crystals; mp: 93 °C, $R_f = 0.66$ (100:10:1 CH_2Cl_2–MeOH–acetic acid), $[\alpha]_D^{23}$ 22.6 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): 2.94 (dd, 1H, J_{gem} 17.6 Hz, $J_{\beta,\alpha}$ 4.4 Hz, (Asp-β-H)$_a$); 3.11 (dd, 1H, J_{gem} 17.6 Hz, $J_{\beta,\alpha}$ 4.7 Hz, (Asp-β-H)$_b$); 4.21 (t, 1H, J_{vic} 7.0 Hz, (H-9)$_{Fmoc}$); 4.35 (dd, 1H, J_{vic} 7.3 Hz, J_{gem} 10.6 Hz, –C*H*$_2$–Fmoc); 4.42 (dd, 1H, J_{vic} 7.3 Hz, J_{gem} 10.6 Hz, –C*H*$_2$–Fmoc); 4.65–4.70 (m, 3H, Asp-α-H, –O–C*H*$_2$–CH=CH$_2$); 5.24 (d, 1H, J_{vic} 10.3 Hz, –CH$_2$–CH=C*H*$_{2cis}$); 5.31 (d, 1H, J_{vic} 17.0 Hz, –O–CH$_2$–CH=C*H*$_{2trans}$); 5.84 (d, 1H, $J_{NH,\alpha\text{-Asp}}$ 8.8 Hz, Asp–N*H*); 5.84–5.92 (m, 1H, –O–CH$_2$–C*H*=CH$_2$); 7.29 (t, 2H, J_{gem} 7.3 Hz, (H-2)$_{Fmoc}$, (H-7)$_{Fmoc}$); 7.38 (t, 2H, J_{gem} 7.3 Hz, (H-3)$_{Fmoc}$, (H-6)$_{Fmoc}$); 7.58 (d, 2H, J_{gem} 7.3 Hz, (H-1)$_{Fmoc}$, (H-8)$_{Fmoc}$); 7.74 (d, 2H, J_{gem} 7.3 Hz, (H-4)$_{Fmoc}$, (H-5)$_{Fmoc}$). ^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 36.42 (C-β Asp); 47.10 (C-9 Fmoc); 50.28 (C-α Asp); 66.62 (CH$_2$=CH–*C*H$_2$–O–); 67.43 (–*C*H$_2$–Fmoc); 119.10 (*C*H$_2$=CH–CH$_2$–O–); 120.04, 125.13, 127.14, 127.79 (C–Ar); 131.31 (CH$_2$=*C*H–CH$_2$–O–); 141.33, 143.67, 143.79 (C$_{ipso}$); 156.11 (C-γ Asp); 170.3 (–*C*OOAll); 175.93 (–*C*OOH). Anal. Calcd for $C_{22}H_{21}NO_6$: C, 66.83; H, 5.35; N, 3.54. Found: C, 66.79; H, 5.32; N, 3.48.

3.1.16. Allyl N^2-fluorenylmethoxycarbonyl-N^4-{14-*tert*-butyloxycarbonyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginate 20 (Fmoc-Asn(glycol$_4$-(CH$_2$)$_2$COO*t*-Bu)-OAll). To a soln of Fmoc-Asp-OAll **19** (2.28 g, 5.77 mmol) in 50 mL of dry CH_2Cl_2, isobutyl (2-isobutoxy)-1,2-dihydroquinoline-1-carboxylate[27] (IIDQ, 2.63 g, 8.66 mmol) was added, and the mixture was stirred for 15 min at room temperature. Subsequently, amino-spacer component **17** (1.55 g, 4.81 mmol) was added and stirring was continued for additional 14 h. After dilution with CH_2Cl_2 (100 mL) and washing with brine (50 mL) and water (50 mL), the organic soln was dried with $MgSO_4$ and the solvent was evaporated under diminished pressure. The oily yellow crude product was purified by column chromatography in 60:1 CH_2Cl_2–MeOH. Yield: 2.85 g (85%), colourless oil, which solidifies to a waxy mass within some days. $[\alpha]_D^{22}$ 15.3 (*c* 1, $CHCl_3$); $R_f = 0.44$ (100:1 ethyl acetate–acetic acid). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.41 (s, 9H, –C(C*H*$_3$)$_3$); 2.47 (t, 2H, J_{vic} 6.6 Hz, –C*H*$_2$–COO*t*-Bu); 2.72 (dd, 1H, J_{gem} 15.9 Hz, J_{vic} 4.3 Hz, (Asn-β-H)$_a$); 2.96 (dd, 1H, J_{gem} 15.9 Hz, J_{vic} 4.7 Hz, (Asn-β-H)$_b$); 3.38–3.45 (m, 2H, –O–CH$_2$–C*H*$_2$–NH–); 3.47–3.64 (m, 12H, –C*H*$_2$–O–); 3.67 (t, 2H, J_{vic} 6.5 Hz, –C*H*$_2$–CH$_2$–COO*t*-Bu); 4.21 (t, 1H, J_{gem} 7.2 Hz, (H-9)$_{Fmoc}$); 4.27 (dd, 1H, J_{gem} 10.3 Hz, J_{vic} 7.6 Hz, –C*H*$_2$–Fmoc); 4.41 (dd, 1H, J_{gem} 10.3 Hz, J_{vic} 7.0 Hz, –C*H*$_2$–Fmoc); 4.59–4.65 (m, 3H, Asp-α-H, –O–C*H*$_2$–CH=CH$_2$); 5.20 (d, J_{cis} 10.6 Hz, –O–CH$_2$–CH=C*H*$_{2cis}$); 5.31 (d, J_{trans} 17.3 Hz, –O–CH$_2$–CH=C*H*$_{2trans}$); 5.92–5.84 (m$_c$, 1H, –O–CH$_2$–C*H*=CH$_2$); 6.22 (d, 1H, $J_{NH,H\alpha}$ 8.8 Hz, Asn-α-NH); 6.65 (t, J_{vic} 1.6 Hz, –N*H*–CH$_2$–); 7.24–7.39 (m, 2H, H–Ar$_{Fmoc}$); 7.55–7.61 (m, 2H, H–Ar$_{Fmoc}$); 7.74 (d, 2H, J_{vic} 7.3 Hz, (H-4)$_{Fmoc}$, (H-5)$_{Fmoc}$). ^{13}C NMR ($CDCl_3$, 50.3 MHz), GASPE: δ 28.09 (–C(CH$_3$)$_3$); 36.18 (–*C*H$_2$–COO*t*-Bu); 37.45 (C-β Asn); 39.34 (–*C*H$_2$–NH–); 47.12 (C-9 Fmoc); 51.00 (C-α Asn); 66.17 (–*C*H$_2$–CH$_2$–NH–); 66.88 (–*C*H$_2$–CH$_2$–COO*t*-Bu); 67.15 (–*C*H$_2$–Fmoc); 69.70 (CH$_2$=CH–*C*H$_2$–O–); 70.19, 70.32, 70.37 (–*C*H$_2$–O–); 80.62 (–*C*(CH$_3$)$_3$); 118.44 (*C*H$_2$=CH–CH$_2$–O–); 119.96, 125.21, 125.29, 127.11, 127.70 (C–Ar Fmoc); 131.77 (CH$_2$=*C*H–CH$_2$–O–); 141.28, 143.83, 143.96 (C–Ar *ipso*); 156.27 (Fmoc–*C*O–NH–); 169.90, 170.89, 171.06 (–*C*OO*t*-Bu, C-γ Asn, C-1 Asn). Anal. Calcd for $C_{37}H_{30}N_2O_{11}$: C, 63.59; H, 7.21; N, 4.01. Found: C, 63.65; H, 7.28; N, 4.14.

3.1.17. N^2-Fluorenylmethoxycarbonyl-N^4-{14-*tert*-butyloxycarbonyl-3,6,9,12-tetraoxatetradecyl}-L-asparagine 21 (Fmoc-Asn(glycol$_4$-(CH$_2$)$_2$COO*t*-Bu)-OH). To a soln of asparagine-spacer-conjugate **20** (2.04 g, 2.92 mmol) in dry tetrahydrofuran (60 mL) under an argon atmosphere, 476 μL of *N*-methylaniline and 40 mg of $(Ph_3P)_4Pd^0$ were added. After stirring for 5 h at room temperature under exclusion of light, THF was distilled off. The residue was dissolved in 150 mL of CH_2Cl_2 and the soln extracted with 50 mL of each 0.25 N HCl and water. After drying with $MgSO_4$, the solvent was evaporated under diminished pressure and the residue purified by column chromatography (200:5:1 CH_2Cl_2–MeOH–AcOH). Yield: 1.353 g (70%), yellow oil, which solidifies to a waxy mass within some days. $[\alpha]_D^{24}$ 40.5 (*c* 1, $CHCl_3$); $R_f = 0.35$ (10:1 CH_2Cl_2–MeOH).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.41 (s, 9H, –C(C*H*$_3$)$_3$); 2.47 (t, 2H, J_{vic} 6.6 Hz, –C*H*$_2$COO*t*-Bu);

2.71 (dd, 1H, J_{gem} 15.3 Hz, J_{vic} 7.6 Hz, Asn-Hβ_a); 2.92 (dd, 1H, J_{gem} 15.3 Hz, J_{vic} 3.2 Hz, Asn-Hβ_b); 3.30–3.34 (m, 1H, –O–CH$_2$–CH_2–NH–); 3.52–3.64 (m, 17H, –O–CH_2–); 3.65 (t, 2H, J_{vic} 6.5 Hz, –CH_2–CH$_2$–COO*t*-Bu); 4.20 (t, 1H, J_{vic} 7.2 Hz, H-9 Fmoc); 4.30 (dd, 1H, J_{gem} 10.6 Hz, J_{vic} 7.3 Hz, –CH_2–Fmoc); 4.37 (dd, 1H, J_{gem} 10.3 Hz, J_{vic} 7.3 Hz, –CH_2–Fmoc); 4.55 (td, 1H, $J_{\text{Asn-H}\alpha,\text{NH}} \approx J_{\text{Asn-H}\alpha,\text{Asn-H}\beta a}$ 7.0 Hz, $J_{\text{Asn-H}\alpha,\text{Asn-H}\beta b}$ 3.2 Hz, Asn-Hα); 6.20 (d, 1H, $J_{\text{NH,Asn-H}\alpha}$ 7.0 Hz, Asn-N$H\alpha$); 7.06 (m, 1H, Asn-NHβ); 7.27–7.31 (m, 2H, H–Ar$_{Fmoc}$); 7.35–7.39 (m, 2H, H–Ar$_{Fmoc}$); 7.57–7.61 (m, 2H, H–Ar$_{Fmoc}$); 7.73–7.74 (d, 2H, J_{vic} 7.3, (H-4)$_{Fmoc}$, (H-5)$_{Fmoc}$).

^{13}C NMR ($CDCl_3$, 50.3 MHz): δ 28.09 (–C(CH$_3$)$_3$); 36.15 (–*C*H$_2$–COO*t*-Bu); 37.88 (C-β Asn); 39.78 (–*C*H$_2$–NH–); 47.09 (C-9 Fmoc); 50.76 (C-α Asn); 66.84 (–*C*H$_2$–CH$_2$–COO*t*-Bu); 67.26 (–*C*H$_2$–Fmoc); 69.22, 70.05, 70.27, 70.34, 70.43, 70.67 (–CH$_2$–O–); 80.74 (–*C*(CH$_3$)$_3$); 119.97, 125.27, 127.14, 127.73 (C–Ar$_{Fmoc}$); 141.26, 143.93 (C–Ar *ipso*); 155.96 (Fmoc–*C*O–NH–); 170.98, 171.94 (–COO*t*-Bu, C-γ Asn). Anal. Calcd for $C_{34}H_{46}N_2O_{11}$: C, 61.99; H, 7.04; N, 4.25. Found: C, 61.76; H, 7.17; N, 4.27.

3.1.18. Allyl [N^4-{14-*N*-(2-acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-4-*O*-(2″,4″-di-*O*-acetyl-6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosyl)-carbamoyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginate] 23 (Fmoc-Asn(glycol$_4$-(CH$_2$)$_2$CO-β-Ac$_6$Bn$_5$sLe^x-COOMe)-OAll). Fmoc-Asn(glycol$_4$-(CH$_2$)$_2$COO*t*-Bu)-OAll **20** (56 mg, 80.5 μmol) was reacted with 10 mL of 1:1 CH_2Cl_2–TFA for 2 h at room temperature. The solvents were evaporated under diminished pressure and the residue co-distilled three times with toluene (10 mL). The quantitatively obtained Fmoc-Asn(Glycol$_4$-(CH$_2$)$_2$COOH)-OAll **22** (54.0 mg, 80 μmol) was dissolved in dimethylformamide (8 mL). PyBOP[29] (83.2 mg, 160 μmol), diisopropylethylamine (68.5 μL, 393 μmol), HOBt[33] (12.3 mg, 80.3 μmol) and sLe^x amine **13** (156.3 mg, 100 μmol) were added, and the soln was stirred at room temperature for 15 h. DMF was evaporated in high vacuum and the residue co-distilled three times with 5 mL of toluene, then dissolved in 100 mL of CH_2Cl_2 and washed with 15 mL of each satd aq $NaHCO_3$ and water. After evaporation of CH_2Cl_2 under diminished pressure the remaining residue was dissolved in 0.4 mL of acetonitrile. Insoluble material was removed by filtration through a syringe and the remaining clear yellow soln was purified by preparative RP-HPLC (column: Eurospher C-18, Knauer, Berlin, Germany; gradient: 70% H_2O/30% $H_3CCN \rightarrow$ 100% CH_3CN in 140 min; $t_R = 63.4$ min). Yield 125.0 mg (72%), colourless amorphous solid; $R_f = 0.46$ (6:1 CH_2Cl_2–MeOH); $[\alpha]_D^{22}$ −34.2 (*c* 1, $CHCl_3$); Anal. HPLC: $t_R = 31.5$ min (Chromasil C-18, Knauer, gradient: 50% H_2O/50% $H_3CCN \rightarrow$ 100% CH_3CN in 40 min). ^{1}H NMR, ^{1}H, ^{1}H-COSY ($CDCl_3$, 400 MHz): δ 0.94 (d, 1H, $J_{6',5'}$ 6.2 Hz, H-6′); 1.70 (t, 1H, $J_{3'''a,3'''e}$ $J_{3'''a,4'''}$ 12.3 Hz, H-3‴a); 1.82, 2 × 1.90, 1.96, 1.98, 2.04, 2.05, 2.20 (8 × s, 3H, –CH_3 Acetyl); 2.17 (m, 2H, –CH_2–CO–); 2.55 (dd, 1H, $J_{3'''e,4'''}$ 4.4 Hz, $J_{3'''e,3'''a}$ 12.6 Hz, H-3‴e); 2.69 (dd, 1H, J_{gem} 15.8 Hz, J_{vic} 4.1 Hz, H-β_a-Asn); 2.93 (dd, 1H, J_{gem} 15.8 Hz, J_{vic} 4.4 Hz, H-β_b-Asn); 3.31 (m, H-6″a); 3.37 (m, –NH–CH_2–); 3.40 (m, –CH_2–CH$_2$–NH–); 3.42 (m, H-6″b); 3.43–3.57 (m, 16H, –O–CH_2-Glycol); 3.48 (m, H-4′); 3.58 (m, H-6a); 3.60 (m, H-6‴); 3.63 (m, H-6b); 3.68 (m, H-3′); 3.71 (m, H-5″); 3.80 (m, H-5); 3.81 (s, 3H, –OCH_3); 3.88 (m, H-5′); 3.92 (m, H-3); 3.94 (m, H-9‴a); 4.01 (m, H-4, H-5‴); 4.02 (m, H-2′); 4.13 (m$_c$, H-2); 4.20 (t, 1H, J_{gem} 6.9 Hz, H-9 Fmoc); 4.24 (m, –CH$_{2a}$–Fmoc); 4.28 (m, –CH_2Bn); 4.32 (m, H-9‴b); 4.38 (m, –CH_2Bn); 4.40 (m, –CH$_{2b}$–Fmoc); 4.50–4.78 (m, 7 × –CH_2–Bn); 4.60 (m, H-3″, H-α Asn); 4.62 (m, –O–CH_2–CH=CH$_2$); 4.70 (m, 1H, H-1″); 4.83–4.98 (m, H-2″, H-4‴, –CH_2–Bn); 5.02–5.14 (m, 5H, H-1, H-1′, H-4″, C5‴-NH); 5.19 (d, 1H, J_{vic} 10.6 Hz, –CH=CH$_{2cis}$); 5.29 (d, 1H, J_{vic} 17.0 Hz, –CH=CH$_{2trans}$); 5.35 (dd, 1H, $J_{7''',6'''}$ 2.6 Hz, $J_{7''',8'''}$ 8.8 Hz, H-7‴); 5.52 (m$_c$, 1H, H-8‴); 5.87 (m$_c$, 1H, –C*H*=CH$_2$); 6.23 (d, 1H, α-N*H* Asn); 6.57 (s, 1H, –N*H*–CH$_2$–); 6.80 (d, 1H, $J_{2,NH}$ 8.5 Hz, C2–N*H*); 7.11–7.42 (m, 29H, H–Ar); 7.52–7.64 (m, 3H, C1–N*H*, H-1 Fmoc, H-8 Fmoc); 7.73 (d, 2H, J_{vic} 7.6 Hz, H-4 Fmoc, H-5 Fmoc). ^{13}C NMR ($CDCl_3$, 100.6 MHz-), DEPT: δ 16.49 (C-6′); 20.52, 20.71, 20.95, 21.24, 22.88, 23.08 (–*C*H$_3$Ac); 36.74 (–CH$_2$–*C*H$_2$–CO–); 37.52 (C-β Asn); 37.63 (C-3‴); 39.36 (–*C*H$_2$–NH–); 47.17 (C-9 Fmoc); 49.19 (C-5‴); 49.60 (C-2); 51.05 (C-α Asn); 53.08 (–O*C*H$_3$); 62.35 (C-9‴); 66.11 (–*C*H$_2$–CH=CH$_2$); 66.80 (C-6); 67.13 (–*C*H$_2$–Fmoc); 67.30 (C-7‴); 67.39 (C-5); 67.59 (C-6″); 67.94 (C-4″); 68.07 (C-8‴); 69.41, 69.61, 69.69, 70.21, 70.26, 70.34, 70.39, 70.74 (C-2″, C-3″, C-4‴, –O–*C*H$_2$-Glycol); 71.26, 71.88, 72.16, 72.40, 72.75, 72.86, 73.25 (C-4, C-5′, C-5″, C-6‴, 3 × –*C*H$_2$Bn); 74.73, 74.61, 73.99 (C-3, 2 × –*C*H$_2$–Bn); 76.48 (C-2′); 77.28 (C-4′); 78.01 (C-1); 79.48 (C-3′); 96.97 (C-2‴); 97.81 (C-1′); 99.05 (C-1″); 118.36 (–O–CH$_2$–CH=*C*H$_2$); 119.89 (C-4 Fmoc, C-5 Fmoc); 125.15, 125.22 (C-1 Fmoc, C-8 Fmoc); 127.01, 127.06, 127.17, 127.37, 127.59, 127.63, 128.20, 128.24, 128.46, 128.65 (C–Ar); 131.77 (–O–CH$_2$–*C*H=CH$_2$); 137.44, 137.82, 138.41, 138.50, 138.57 (C-*ipso*); 141.27 (C-4a Fmoc, C-4b Fmoc); 143.97, 143.85 (C-8a Fmoc, C-9a Fmoc); 156.20 (–NH–*C*O–Fmoc); 167.87, 169.65, 169.80, 169.94, 170.17, 2 × 170.33, 170.52, 170.75, 170.85, 170,94, 171.18 (–*C*=O). MALDIMS: calcd for $C_{112}H_{137}N_5O_{38}$: 2161.3. Found: 2185.0 $[M+Na]^+$, 2200.8 $[M+K]^+$. Anal. Calcd for $C_{112}H_{137}N_5O_{38}\cdot 3H_2O$: C, 60.72; H, 6.56; N, 3.18. Found: C, 60.72; H, 6.71; N, 3.09.

In addition, 43.0 mg (25%) of the α-configured anomer of **23** was isolated. Anal. HPLC: $t_R = 31.5$ min (Chromasil C-18, Knauer, gradient: 50% H_2O/50% $H_3CCN \rightarrow$ 100% CH_3CN in 40 min).

^1H NMR ($CDCl_3$, 400 MHz): characteristic signals: δ 0.81 (d, 1H, $J_{6',5'}$ 6.5 Hz, H-6′); 1.71 (t, 1H, $J_{3'''a,3'''e}$ $J_{3'''a,4'''}$ 12.3 Hz, H-3‴a); 1.83, 1.90, 1.98, 2.03, 2.06, 2.10, 2.12, 2.19 (8 × s, 3H, –CH_3Ac); 2.58 (dd, 1H, $J_{3'''e,3'''a}$ 12.5 Hz, $J_{3'''e,4'''}$ 4.2 Hz, H-3‴e); 2.71 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$ 15.6 Hz, $J_{H\beta a,H\text{-}\alpha}$ 3.9 Hz, H-β_a Asn); 2.96 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$ 15.8 Hz, $J_{H\beta b,H\text{-}\alpha}$ 4.4 Hz, H-β_b Asn); 3.84 (s, 3H, –OCH_3); 5.04 (d, 1H, $J_{4'',3''}$ 2.9 Hz, H-4″); 5.10 (d, 1H, $J_{5''',NH}$ 10.6 Hz, C5‴–NH); 5.19 (d, 1H, J_{vic} 10.6 Hz, H_2C═CH–CH_2–O–$_{cis}$); 5.29 (d, 1H, J_{vic} 17.3 Hz, H_2C═CH–CH_2–O–$_{trans}$); 5.35 (dd, 1H, $J_{7''',6'''}$ 2.3 Hz, $J_{7''',8'''}$ 8.5 Hz, H-7‴); 5.51 (m_c, 1H, H-8‴); 5.73 (d, 1H, $J_{1,NH}$ 8.5 Hz, $J_{1,2} \ll 1$ Hz, H-1); 5.87 (m_c, 1H, H_2C═CH–); 6.21 (d, 1H, $J_{H\text{-}\alpha,NH}$ 8.8 Hz, α–NH Asn); 6.63 (s, 1H, –NH–CH_2–); 6.71 (d, 1H, $J_{2,NH}$ 9.7 Hz, C2–NH); 7.03 (d, 1H, $J_{1,NH}$ 8.5 Hz, C1–NH); 7.53–7.62 (m, 2H, H-1 Fmoc, H-8 Fmoc); 7.73 (d, 2H, H-4 Fmoc, H-5 Fmoc). MALDIMS calcd for $C_{112}H_{137}N_5O_{38}$:2161.3, Found: 2184.7 $[M+Na]^+$, 2200.8 $[M+K]^+$.

3.1.19. N^2-Fluorenylmethoxycarbonyl-N^4-{14-*tert*-butyloxycarbonyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginyl-L-valyl-L-prolyl-L-glycyl-N^4-{14-*tert*-butyloxycarbonyl-3,6,9,12-tetraoxatetradecyl}-L-valyl-L-prolyl-L-glycine 25 (Fmoc-Asn(Glycol$_4$-$(CH_2)_2$COO*t*-Bu)-Val-Pro-Gly-Asn-(Glycol$_4$-$(CH_2)_2$COO*t*-Bu)-Val-Pro-Gly-OH).

The solid-phase synthesis of the octapeptide containing two asparagine units equipped with a spacer-acid side chain was carried out using an aminopolystyrene resin loaded with Fmoc glycine through an allyl HYCRON anchor **24** according to a procedure described earlier.[26,34] Loading of **24** was ca. 0.6 mmol/g. The reactions on Fmoc-Gly-HYCRON resin **24** (1.41 g, 0.84 mmol) were performed in a solid-phase reactor. *Fmoc removal*: 1:1 DMF–morpholine, 80 mL, 30–45 min, washing DMF (3 × 20 mL); *Boc removal*: 1:1 CH_2Cl_2–TFA, 30 mL, washing CH_2Cl_2 (6 × 20 mL); neutralization with 10:1 CH_2Cl_2–diisopropylethylamine, 10 min, then washing CH_2Cl_2 (6 × 20 mL) and DMF (20 mL). *Capping* was carried out after each coupling reaction: 3:1 pyridine–Ac_2O, 24 mL, 15 min, washing DMF (6 × 20 mL). *Coupling reactions*: Suspension of the resin in DMF (20 mL), after each coupling washing with DMF (6 × 20 mL); applied amounts (a) Boc-Pro-OH (0.85 g, 3.95 mmol), HOBt (0.61 g, 3.95 mmol), *N*-methylmorpholine (0.87 mL, 7.40 mmol), TBTU[32] (1.27 g, 3.95 mmol) in DMF (10 mL, 15 h); (b) Fmoc-Val-OH (1.46 g, 4.31 mmol), HOBt (0.66 g, 4.31 mmol), *N*-methylmorpholine (0.95 mL, 8.61 mmol), TBTU (1.38 g, 4.31 mmol) in DMF (10 mL), 17 h; (c) Fmoc-Asn(spacer)-OH **21** (1.24 g, 1.9 mmol), HOBt (286 mg, 1.9 mmol), *N*-methylmorpholine (0.45 mL, 3.78 mmol), TBTU (607 mg, 1.9 mmol) in DMF (10 mL), 17 h; (d) Fmoc-Gly-OH (944 mg, 3.18 mmol), HOBt (488 mg, 3.18 mmol), *N*-methylmorpholine (0.7 mL, 6.35 mmol), TBTU (1.02 g, 3.18 mmol) in DMF (10 mL), 18 h; (e) Fmoc-Pro-OH (1.072 g, 3.18 mmol), HOBt (488 mg, 3.18 mmol), *N*-methylmorpholine (0.7 mL, 6.35 mmol), TBTU (1.02 g, 3.18 mmol) in DMF (10 mL), 18 h; (f) Fmoc-Val-OH (950 mg, 2.80 mmol), HOBt (430 mg, 2.39 mmol), *N*-methylmorpholine (616 μL, 4.78 mmol), TBTU (0.90 mg, 2.80 mmol) in DMF (10 mL), 16 h; (g) Fmoc-Asn(spacer)-OH **21** (1.57 g, 2.39 mmol), HOBt (367 mg, 3.18 mmol), *N*-methylmorpholine (526 μL, 4.78 mmol), TBTU (767 mg, 2.39 mmol) in DMF (10 mL), 18 h.

3.1.19.1. Detachment of linear peptide 25 from resin. After the final coupling reaction and consecutive washings, the resin was suspended in 1:1 DMF–Me_2SO (30 mL). *N*-Methylaniline (1.4 mL) was added, and air was exchanged for an argon atmosphere. $[Ph_3P]_4Pd(0)$ (40 mg) was added and the mixture shaken for 15 h under an argon atmosphere and exclusion of light. The resin was filtered off and washed with DMF (6 × 20 mL). The combined filtrate and washing solns were concentrated under diminished pressure at 30 °C. The residue was co-distilled under diminished pressure with toluene (3 × 50 mL). The yellow residue was dissolved in CH_2Cl_2 (250 mL) and the soln washed with 0.05 N HCl (50 mL) and brine (2 × 40 mL). After drying with $MgSO_4$, the soln was concentrated under diminished pressure to a volume of 5mL and kept in a refrigerator at 4 °C for 16 h. The soln was separated from precipitating amounts of the palladium catalyst, which were washed with cold CH_2Cl_2 (3 mL). The combined solns were evaporated under diminished pressure, dried in high vacuum, dissolved in 8:1 CH_2Cl_2–MeOH and filtered through a short column (3 cm) of silica gel. After evaporation of the solvent, the oily residue was purified by preparative RP-HPLC (Eurosphere C-18, Knauer, gradient: 30% CH_3CN/70% $H_2O \rightarrow$ 75% CH_3CN/25% H_2O in 90 min). The fractions containing product **25** (anal. HPLC) were combined and evaporated under diminished pressure. The remaining peptide was lyophilized. Yield: 892 mg (61%); colourless amorphous solid; $[\alpha]_D^{22}$ −47.3 (*c* 1, MeOH); $R_f = 0.67$ (5:1 CH_2Cl_2–MeOH); anal. HPLC (Eurosphere C-18, Knauer): gradient 25% H_3CCN/75% $H_2O \rightarrow$ 75% H_3CCN/25% H_2O, $t_R = 32.2$ min. ^1H NMR (Me_2SO-d_6, 400 MHz), ^1H, ^1H-COSY: δ 0.81 (d, 6H, $J_{H\text{-}\beta,H\text{-}\gamma a}$ 4.7 Hz, 2 × H-γ_a Val); 0.87 (d, 6H, $J_{H\text{-}\gamma b,H\text{-}\beta}$ 5.1 Hz, 2 × H-γ_b Val); 1.39 (s, 18H, 2 × –C(CH_3)$_3$); 1.68–2.11 (m, 10H, 2 × H-β Val (1.99, 2.00), 4 × H-β Pro (1.82), 4 × H-γ Pro (2.00)); 2.38 (t, 2H, J_{vic} 6.3 Hz, –O–CH_2CH_2–COO*t*-Bu); 2.39 (t, 2H, J_{vic} 6.3 Hz, –O–CH_2CH_2–COO*t*-Bu); 2.35–2.44 (m, 3H, H-β_a Fmoc-Asn, H-β_b Fmoc-Asn, H-β_a –Asn–); 2.51 (m, 1H, H-β_b –Asn–); 3.17 (t, 4H, J_{vic}

5.1 Hz, 2 × –O–CH_2CH_2–NH–); 3.36 (t, 4H, J_{vic} 5.9 Hz, 2 × –O–CH_2CH_2–NH–); 3.55 (t, 2H, J_{vic} 6.3 Hz, –CH_2CH_2–COO*t*-Bu); 3.56 (t, 2H, J_{vic} 6.3 Hz, –CH_2CH_2–COO*t*-Bu); 3.59 (m, 2H, 2 × H-α_a Gly); 3.77 (m, 2H, 2 × H-α_b Gly); 4.14–4.26 (m, 3H, H-9 Fmoc, –CH_2–Fmoc); 4.26–4.37 (m, 4H, 2 × H-α Val, 2 × H-α Pro); 4.38 (q, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a} \approx J_{H\text{-}\alpha,H\text{-}\beta b} \approx {}_{H\text{-}\alpha,\text{-}NH\text{-}}$ 5.5 Hz, H-α Fmoc-Asn); 4.57 (q, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a} \approx J_{H\text{-}\alpha,H\text{-}\beta b} \approx {}_{H\text{-}\alpha,\text{-}NH\text{-}}$ 6.6 Hz, H-α –Asn–); 7.31 (t, 2H, J_{vic} 7.4 Hz, H-2 Fmoc, H-7 Fmoc); 7.40 (t, 2H, J_{vic} 7.4 Hz, H-3, H-6 Fmoc); 7.61 (d, 1H, $J_{NH,H\text{-}\alpha}$ 8.2 Hz, –NH–α Fmoc-Asn); 7.67 (m, 1H, –NH– Val); 7.68 (d, 2H, J_{vic} 7.1 Hz, H-1 Fmoc, H-8 Fmoc); 7.78 (d, 1H, $J_{NH,H\text{-}\alpha}$ 8.2 Hz, –NH– Val); 7.87 (d, 2H, J_{vic} 7.4 Hz, H-4 Fmoc, H-5 Fmoc); 7.91 (t, 1H, J_{vic} 5.1 Hz, –O–CH_2–CH_2–N*H*–); 7.95 (t, 1H, J_{vic} 5.1 Hz, –O–CH_2–CH_2–N*H*–); 8.04 (d, 1H, $J_{NH,H\text{-}\alpha}$ 7.4 Hz, –NH– Val); 8.14 (t, 2H, $J_{NH,H\text{-}\alpha}$ 5.0 Hz, 2 × –NH– Gly). ^{13}C NMR (MeOD, 100.6 MHz), DEPT: δ 18.66, 19.89, 19.93 (C-γ Val); 25.92, 26.14 (C-γ Pro); 28.39 (–C(CH_3)$_3$); 30.35 (C-β Pro); 31.78, 31.68 (C-β Val); 37.27 (–CH_2–COO*t*-Bu); 38.48, 38.60 (C-β Asn); 40.50 (–O–CH_2CH_2–NH–); 43.72 (C-α Gly); 48.33 (C-9 Fmoc); 49.60 (C-δ Pro); 51.81 (C-α Asn); 53.34 (C-α Val); 61.78, 58.06 (C-α Pro); 67.86, 68.15 (–CH_2–Fmoc, –CH_2CH_2–COO*t*-Bu); 81.68 (–C(CH_3)$_3$); 120.92, 126.17, 128.17, 128.77 (C–Ar Fmoc); 142.50, 145.16 (C-*ipso* Fmoc); 158.02 (–NH–CO–O); 171.36, 172.07, 172.29, 172.65, 173.21, 173.96, 174.64 (–CO–). MALDIMS (dhb) calcd for $C_{77}H_{118}N_{10}O_{25}$: 1583.9. Found: 1606.9 $[M+Na]^+$, 1622.9 $[M+K]^+$, 1628.9 $[M+2Na-H]^+$; Anal. Calcd for $C_{77}H_{118}N_{10}O_{25}\cdot 3H_2O$: C, 56.47; H, 7.63; N, 8.55. Found: C, 56.35; H, 7.55; N, 8.44.

3.1.20. *cyclo*-[N^4-{14-*tert*-Butyloxycarbonyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginyl-L-valyl-L-prolyl-L-glycyl-N^4-{14-*tert*-butyloxycarbonyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginyl-L-valyl-L-prolyl-L-glycine] 26 (*cyclo*-[Asn(Glycol$_4$-$(CH_2)_2$COO*t*-Bu)-Val-Pro-Gly-Asn(Glycol$_4$-$(CH_2)_2$COO*t*-Bu)-Val-Pro-Gly-])

3.1.20.1. Fmoc-removal. A soln of octapeptide **25** (300 mg, 0.19 mmol) in 8:1 DMF–morpholine (14 mL) was stirred at room temperature for 1 h. The solvents were evaporated in high vacuum, the residue co-distilled with toluene (10 mL) and the pale yellow residue purified by column chromatography in 5:2 acetonitrile–water on 50 g of reversed phase C18 silica gel.

3.1.20.2. Cyclization. The deprotected peptide obtained (250 mg, 0.184 mmol, $R_f = 0.29$, reversed phase TLC (C18), 3:2 acetonitrile–water was dissolved in 200 mL of 1:1 CH_2Cl_2–DMF. To this soln HOAt[31] (50.3 mg, 0.367 mmol), diisopropylethylamine (94.3 μL, 0.551 mmol) and HATU[31] (104.6 mg, 0.275 mmol) were added, and the soln was stirred at room temperature for 2 h. The solvents were evaporated in high vacuum, the residue was co-distilled with toluene (5 mL), dissolved in acetonitrile (5 mL) and filtered through a C18 RP-silica gel cartridge, which was washed with 20 mL of acetonitrile. The combined solns were concentrated to a volume of 2 mL, and cyclopeptide **26** was purified by preparative RP-HPLC (column Eurospher C18; Knauer, gradient: 30% H_3CCN/70% H_2O → 100% H_3CCN in 90 min, $t_R = 51.8$ min). Yield: 176 mg (71%), colourless amorphous solid; $R_f = 0.29$ (RP C-18), $CH_3CN/H_2O = 2:3$. ^{1}H NMR (CD_3OD, 400 MHz): characteristic signals: δ 0.83 (d, 3H, $J_{H\text{-}\gamma,H\text{-}\beta}$ 6.2 Hz, H-γ Val); 0.99–1.08 (m, 6H, H-γ Val); 1.14 (d; 3H, $J_{H\text{-}\gamma,H\text{-}\beta}$ 5.9 Hz, H-γ Val); 1.49 (s, 18H, 2 × –C(CH_3)$_3$); 2.52 (t, 4H, J_{gem} 6.2 Hz, 2 × –CH_2–COO*t*-Bu); 3.74 (t, 4H, J_{gem} 6.2 Hz, 2 × –CH_2–CH_2–COO*t*-Bu). In addition in Me_2SO-d_6 the following NH-signals are visible: δ 7.24–7.42 (m, 2H, 2 × –NH_γ–), 7.82–7.93 (m, 1H, –NH_α–); 7.97–8.07 (m, 1H, 1H, –NH_α–); 8.08–8.17 (m, 1H, –NH_α–); 8.19–8.30 (m, 1H, –NH_α–); 9.02–9.15 (m, 1H, –NH_α–). ^{13}C NMR (CD_3OD, 100.3 MHz): δ 17.77, 19.64, 20.51, 21.66 (2 × C-β Val, 2 × C-γ Val); 26.71, 29.50, 29.87, 32.84 (2 × C-β Pro, 2 × C-γ Pro); 37.27 (–CH_2COO*t*-Bu); 28.38 (–C(CH_3)$_3$); 40.26, 40.44 (2 × –CH_2–NH–); 43.49, 43.69 (2 × C-α Gly); 47.91 (C-δ Pro); 50.38, 51.38, 52.78, 56.60 (2 × C-α Val, 2 × C-α Asn); 63.03, 64.56 (2 × C-α Pro); 67.88 (–CH_2–CH_2–COO*t*-Bu); 70.42, 71.27, 71.37, 71.47, 71.53 (–CH_2–O–); 171.39, 171.52, 173.36, 174.72, 175.29 (–CO–NH–); 172.74 (–COO*t*-Bu). MALDIMS (dhb) calcd for $C_{62}H_{106}N_{10}O_{22}$: 1344.6. Found: 1366.9 $[M+Na]^+$, 1382.7 $[M+K]^+$.

3.1.21. *cyclo*-[N^4-{14-*N*-(2-Acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-4-*O*-(2″,4″-di-*O*-acetyl-6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosyl)-carbamoyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginyl-L-valyl-L-prolyl-L-glycinyl-N^4-{14-*N*-(2-acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-4-*O*-(2″,4″-di-*O*-acetyl-6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosyl)-carbamoyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginyl-L-valyl-L-prolyl-L-glycine] 27 (*cyclo*-[Asn(Glycol$_4$-$(CH_2)_2$CO-α/β-Ac$_6$Bn$_5$sLe^{x}COOMe)-Val-Pro-Gly-Asn(Glycol$_4$-$(CH_2)_2$-CO-β-Ac$_6$Bn$_5$sLe^{x}COOMe)-Val-Pro-Gly-])

3.1.21.1. Side chain deprotection. The side chain-protected *cyclo*-octapeptide **26** (174 mg, 0.13 mmol) was stirred in a mixture of TFA (5 mL), CH_2Cl_2 (5 mL), ethylmethylsulfide (0.4 mL) and ethanedithiole (0.1 mL) at room temperature for 2 h. After evaporation of the solvents, the remaining residue was co-distilled with toluene (4 × 10 mL), dried in high vacuum and purified by preparative RP-HPLC (column Eurospher, 100-C18, Knauer, gradient: 99% H_2O → 35% H_2O/

65% H_3CN in 60 min, $t_R = 41.3$ min). Yield: 156 mg (0.127 mmol, 97%); colourless amorphous solid; $[\alpha]_D^{26}$ 5.9 (*c* 1, CH_3CN); anal. RP-HPLC (column Eurospher 100 C_{18} Knauer): gradient: 1% CH_3CN/99% $H_2O \rightarrow$ 100% CH_3CN in 50 min, $t_R = 19.7$ min; MALDIMS calcd for $C_{54}H_{90}N_{10}O_{22}$: 1231.4. Found: 1233.0 (100%) $[M+H]^+$, 1254.6 (18%) $[M+Na]^+$, 1270.7 (7%) $[M+K]^+$.

3.1.21.2. Conjugation with sLeX amine 17. To a soln of the deprotected cyclooctapeptide (67 mg, 0.054 mmol) in DMF (5 mL), HOBt (16.9 mg, 0.11 mmol), diisopropylethylamine (92 μL, 0.54 mmol) and PyBOP[29] (114.5 mg, 0.22 mmol) were added. sLeX amine **17** (221 mg, 0.144 mmol) was then added and the soln stirred at room temperature for 22 h. The solvent was distilled off under diminished pressure, and the residue was co-distilled with toluene (3 × 5 mL). The crude product was dissolved in CH_2Cl_2 (120 mL), washed with water (80 mL), satd aq $NaHCO_3$ (80 mL) and water again. After drying with $MgSO_4$ and evaporation under diminished pressure, the remaining brown amorphous solid (362 mg) was purified by preparative RP-HPLC to give the two diastereomers **27ββ** and **27αβ**: (column LUNA, C18, Phenomenex): gradient 75% CH_3CN/25% $H_2O \rightarrow$ 100% CH_3CN in 90 min, $t_R = 59.0$ min (**27ββ**) and 68.4 min (**27αβ**).

Compound **27ββ**: Yield: 89.4 g (38.5%); colourless amorphous solid; Anal. RP-HPLC (column Eurospher 100, C8, Knauer): $t_R = 22.6$ min, gradient: 50% CH_3CN/50% $H_2O \rightarrow$ 100% CH_3CN/0% H_2O in 50 min. MALDIMS calcd for $C_{212}H_{280}N_{16}O_{76}$: 4268.6. Found: 4292.8 $[M+Na]^+$, 4308.7 $[M+Ka]^+$.

Compound **27αβ**: Yield: 43.0 mg (18.5%); colourless amorphous solid; Anal. RP-HPLC (column Eurospher 100, C8, Knauer): $t_R = 27.4$ min, gradient: 50% CH_3CN/50% $H_2O \rightarrow$ 100% CH_3CN in 50 min. MALDIMS calcd for $C_{212}H_{280}N_{16}O_{76}$: 4268.6 Found: 4292.3 $[M+Na]^+$, 4308.2 $[M+Ka]^+$.

3.1.22. *cyclo*-[N^4-{14-*N*-(2-Acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-4-*O*-(2″,4″-di-*O*-acetyl-6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosyl)-carbamoyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginyl-L-valyl-L-prolyl-L-glycinyl-N^4-{14-*N*-(2-acetamido-3-*O*-(2′,3′,4′-tri-*O*-benzyl-α-L-fucopyranosyl)-2-deoxy-4-*O*-(2″,4″-di-*O*-acetyl-6″-*O*-benzyl-3″-*O*-[methyl 5‴-acetamido-4‴,7‴,8‴,9‴-tetra-*O*-acetyl-3‴,5‴-dideoxy-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-β-D-glucopyranosyl)-carbamoyl-3,6,9,12-tetraoxatetradecyl}-L-asparaginyl-L-valyl-L-prolyl-L-glycine] 28ββ (*cyclo*-[Asn(Glycol$_4$-(CH$_2$)$_2$CO-α/β-sLe^{x}COOH)-Val-Pro-Gly-Asn(Glycol$_4$-(CH$_2$)$_2$CO-sLe^{x}COOH)-Val-Pro-Gly-]). To a soln of the cyclic glycopeptide **27ββ** (76 mg, 0.0178 mmol) in MeOH (30 mL), water (0.2 mL) and acetic acid (3 mL) Pearlman's catalyst (10 mg, $Pd(OH)_2$ 20% on charcoal) was added. After hydrogenation for 25 h at atmospheric pressure, the catalyst was filtered off through *Hyflo* and washed with MeOH (150 mL). The combined filtrate solns were evaporated under diminished pressure, the residue was co-distilled with toluene (10 mL) and dried in high vacuum. The debenzylated product was dissolved in MeOH (30 mL). Freshly prepared 0.1 N sodium methoxide in MeOH was added until a drop of the soln on a moist pH-paper showed pH 10 (about 2.4 mL). After stirring for 20 h at room temperature, solid CO_2 was added for neutralization, and the solvent was evaporated under diminished pressure. The remaining solid was dissolved in aq NaOH of pH 10–10.5 and stirred at room temperature for 20 h. After neutralization with solid CO_2, the water was evaporated under diminished pressure and the residue purified by gelpermeation chromatography on Sephadex LH 15 in water (column: 80 × 2 cm; eluent: water).

Compound **28ββ**: Yield: 45.0 mg (89%); $[\alpha]_D^{23}$ −66.6 (*c* 1, H_2O); ^1H NMR (D_2O, 400 MHz): characteristic signals: δ 0.94 (d, 12H, $J_{H\gamma,H\beta}$ 6.5 Hz, H-γ Val); 1.15 (d, 6H, $J_{6',5'}$ 6.5 Hz, H-6′); 1.77 (t, 2H, $J_{3'''a,3'''e} \approx J_{3'''a,4'''}$ 11.9 Hz, H-3‴a); 1.96 (s, 6H, –NHAc); 2.00 (s, 6H, –NHAc); 2.57 (t, 4H, J_{vic} 5.9 Hz, –C*H*$_2$–CO–); 2.73 (d, 2H, $J_{3'''e,3'''a}$ 10.7 Hz, H-3‴e); 3.32–3.42 (m, 4H, –NH–C*H*$_2$–); 3.56 (t, 2H, $J_{2'',1''} \approx J_{2'',3''}$ 8.5 Hz, H-2″); 4.11 (d, 2H, $J_{3'',2''}$ 8.8 Hz, H-3″); 4.52 (d, 2H, $J_{1'',2''}$ 7.6 Hz, H-1″); 4.88 (m_c, 2H, H-5′); 5.08 (d, 2H, $J_{1',2'}$ 3.5 Hz, H-1′); 5.11 (d, 2H, $J_{1,2}$ 9.7 Hz, H-1). ^{13}C NMR (D_2O, 100.6 MHz): δ 15.44 (C-6′); 18.85 (C-γ Val); 22.24 (–*C*H$_3$Ac); 22.33 (–*C*H$_3$Ac); 25.36; 28.71; 36.27 (–CO–*C*H$_2$–); 39.06 (–*C*H$_2$–NH–); 40.15 (C-3‴); 42.57; 47.87; 51.98 (C-5‴); 54.86 (C-2); 59.84 (C-6″); 61.65; 62.92 (C-9‴); 66.54; 66.85 (C-5′); 67.55; 67.94; 68.39; 68.91; 69.46; 69.60; 69.66; 69.75; 72.12; 73.13; 73.29; 75.11; 75.44; 75.94; 77.35; 78.33 (C-1); 98.66 (C-1′); 101.87 (C-1″, C-2‴); 170.97, 171.53, 172.57, 174.30, 174.80, 175.28 (–*C*O–NH–). ESI-MS calcd for ($C_{116}H_{192}N_{16}O_{64}$): 2834.9. Found: 1448.3 (27%) $[(M+Na+K)/2]^+$, 1451.1 (18%) $[(M-H+3Na)/2]^+$, 1456.1 (45%) $[(M+2K)/2]^+$, 1459.3 (100%) $[(M-H+2Na+K)/2]^+$, 1462.1 (53%) $[(M-2H+4Na)/2]^+$, 1467.3 (28%) $[(M-H+Na+2K)/2]^+$, 1478.8 (16%) $[(M-2H+2Na+2K)/2]^+$.

In an analogous preparation, **27αβ** (37.1 mg, 0.0087 mmol) was converted to **28αβ**: Yield: 22.7 mg (92%); colourless amorphous solid. $[\alpha]_D^{23}$ −12.6 (*c* 1, H_2O); ^1H NMR (D_2O, 400 MHz): characteristic signals: δ 0.94 (d, 12H, $J_{H\gamma,H\beta}$ 6.4 Hz, H-γ Val); 1.13 (d, 3H, $J_{5',6'}$ 5.6 Hz, H-6′); 1.15 (d, 3H, $J_{5',6'}$ 5.6 Hz, H-6′); 1.76 (t, 2H, $J_{3'''a,3'''e}$ 11.7 Hz, H-3‴a); 1.95 (s, 3H, –NHAc); 1.97 (s, 3H, –NHAc); 1.99 (s, 6H, –NHAc); 2.04–2.13 (m, 2H, H-γ Pro); 2.13–2.23 (m, 2H, H-β Val); 2.24–2.38 (m, 2H, H-γ Pro); 2.52 (t, 2H, J_{gem} 13.9 Hz, –C*H*$_2$–CO–); 2.58–2.67 (m, 2H, –C*H*$_2$–CO–);

2.73 (d, 2H, $J_{3'''e,3'''a}$ 11.2 Hz, H-3‴e); 3.30–3.42 (m, 4H, –CH_2–NH–); 4.51 (d, 2H, $J_{1'',2''}$ 7.6 Hz, H-1″); 5.06 (d, 1H, $J_{1',2'}$ 4.1 Hz, H-1′); 5.07 (d, 1H, $J_{1',2'}$ 4.1 Hz, H-1′); 5.11 (d, 1H, $J_{1\beta,2}$ 9.7 Hz, H-1β); 5.58 (d, 1H, $J_{1\alpha,2}$ 4.4 Hz, H-1α). MALDIMS calcd for ($C_{116}H_{192}N_{16}O_{64}$): 2834.9. Found: 2564.8 $[M-NeuNAc+Na]^+$, 2900.7 $[M-2H+3Na]^+$, 2894.9 $[M-H+Na+K]^+$, 2878.7 $[M-H+2Na]^+$, 2872.8 $[M+K]^+$, 2856.9 $[M+Na]^+$.

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