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Highly regioselective synthesis of a 3-O-sulfonated arabino Lewis[a] asparagine building block suitable for glycopeptide synthesis

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Abstract—Using the stannylene method, the trisaccharide 2-acetamido-3-*O*-[6-*O*-benzyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide was regioselectively sulfonated and, after reduction of the anomeric azide, coupled to Fmoc α-allyl aspartate. After Pd(0)-catalyzed deallylation, the sulfatyl Lewis[a] asparagine building block was obtained, suitable for solid-phase glycopeptide synthesis applying the fluoride labile PTMSEL linker system.

Keywords: E-Selectin ligand; Sulfatyl Lewis[a]; Regioselective sulfonation; Glycopeptides

1. Introduction

Communication between different types of cells is of tremendous importance for human life, for example, in immuno differentiation and in inflammatory processes. At the beginning of inflammation, Ca^{2+}-dependent cell-surface receptors, so-called selectins, are expressed on the inner surface of blood vessels and on leukocytes.[1] Through recognition of specific carbohydrate epitopes of their ligands, the selectins mediate the rolling of leukocytes on endothelial cells. This is the first step of leukocyte adhesion on the endothelium which finally results in the leukocyte invasion into inflamed tissue. On the other hand, overexpression of selectins leads to pathological recruitment of endogenous leukocytes and can cause acute diseases such as reperfusion syndrome or rheumatoid arthritis. To prevent these pathological processes, soluble selectin ligands could be applied as antagonists binding competitively to the selectins and thus suppressing the adhesion cascade from the beginning. The tetrasaccharide sialyl-Lewis[a] (sLe[a]) **1** and the isomeric sialyl-Lewis[x] (sLe[x]) have been identified as lead compounds for binding to P- and E-selectin,[2] although the binding affinity for sLe[a/x] is rather low ($IC_{50} \approx 1$ mM) (Fig. 1).

In 1992, a mixture of sulfated Le[x] and Le[a] pentasaccharides was isolated from an ovarian cystoadenoma glycoprotein,[3] which turned out to be an even more potent inhibitor than the sialylated Lewis antigens.[4] In addition, previous studies have shown a distinct influence of the peptide backbone of sLe[x] glycopeptides upon receptor binding to P-selectin[5] and E-selectin,[6] respectively. An artificial glycoconjugate consisting of the highly conserved partial amino acid sequence 672–681 of the E-selectin-ligand **1** (ESL-1)[7] and arabino sLe[x] significantly amplified the binding to E-selectin compared to the tetrasaccharide **1** by a factor of 10.[8]

In this context, it appeared interesting to combine both aspects and to elucidate the potential of a sulfated glycopeptide containing the partial structure $I^{670}V\,G\,N\,L\,T\,E\,L\,E\,S\,E\,D\,I^{682}$ **2** of ESL-1, in which the sulfated arabino Lewis[a] is incorporated into the peptide via a *N*-glycosidic linkage to asparagine. Although the chemical syntheses of sialylated glycopeptides succeeded in a number of cases, the assembly of defined sulfated Lewis antigen glycopeptide analogues still is challenging due to the extreme sensitivity of their fucoside moiety to acidic conditions. Hence, instead of

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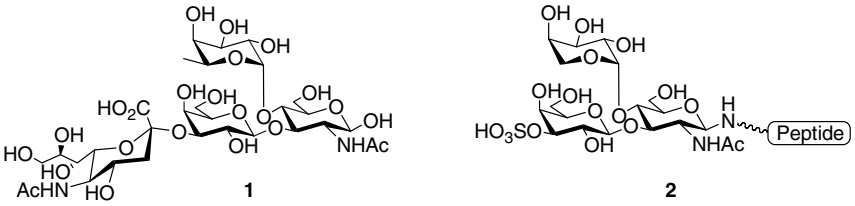


Figure 1. Natural ligand sLe[a] **1** and mimetic sulfonyl arabino Lewis[a] **2**.

acid-labile protecting groups and linkers commonly used for solid-phase glycopeptide synthesis, a new approach was chosen: exclusively hydrogenolytically cleavable benzyl ester[9] and ether[10] groups were utilized in combination with the fluoride labile PTMSEL[11] linker.

2. Results and discussion

For the preparation of a sulfated arabino Lewis[a] analogue conjugated to asparagine, the strategy efficient for the synthesis of sialylated analogues was appropriately modified.[12a,b] The key building blocks for the construction of the sulfatyl Lewis[a] mimic are *N*-acetylglucosaminyl azide **3**,[13] galactosyl bromide **4**[14] and the 1-thio-D-arabinoside **5**.[15] The latter was chosen instead of the natural L-fucose because of its assumed increased stability against enzymatic degradation[16] while it presents the three hydroxyl groups in a sterically identical arrangement.[17] The anomeric azido group of the *N*-acetylglucosamine receptor **3** acts as a temporary protecting group[18] throughout the synthesis and can finally be reduced to give the corresponding glycosylamine suitable for the conjugation with amino acids and peptides (Fig. 2).[19]

The glycosylation of acceptor **3** with **4** obtained from 1,2,3,4-tetra-*O*-acetyl-6-*O*-benzyl-galactopyranose by treatment with TMSBr/$BiBr_3$[20] was carried out using $Hg(CN)_2$ as the promoter,[21] because the corresponding trichloroacetimidate,[22] usually an excellent galactosyl donor, gave only moderate yields. By changing the solvent from dichloromethane/nitromethane (3:1) to dichloromethane/dichloroethane (1:1), the yield of β-galactoside **6** was increased from 63% to 97%. The regioselective opening of the benzylidene acetal using triethylsilane/trifluoromethanesulfonic acid[23] in dichloromethane at −78 °C is superior to the usually applied sodium cyano-borohydride/HCl[24] in THF at 0 °C. Fewer side reactions occur and, thus, purification was considerably simplified. Under these conditions, the 6-*O*-benzyl ether **7** was regioselectively formed in a yield of 76%. The per-O-benzylated ethylthio-arabinoside **5** was activated with copper(II)bromide[25] and tetrabutylammonium bromide[26] furnishing the desired β-stereochemistry of the arabino Lewis[a] trisaccharide **8** formed as a single isomer in a yield of 84%. Treatment of **8** with sodium methoxide in methanol yielded **9** (93%) suitable for subsequent sulfonation. The sulfonate was regioselectively introduced taking advantage of the stannylene procedure and sulfur trioxide–trimethylamine[27] complex in dimethylformamide at room temperature with a reproducible yield of 91%. For the exclusive formation of the 3-O-monosulfonated product, previous activation by dibutyltin oxide[28] is essential (Scheme 1).

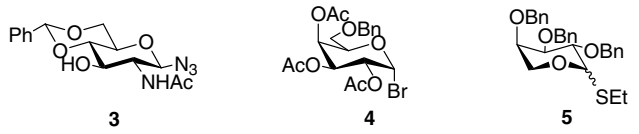
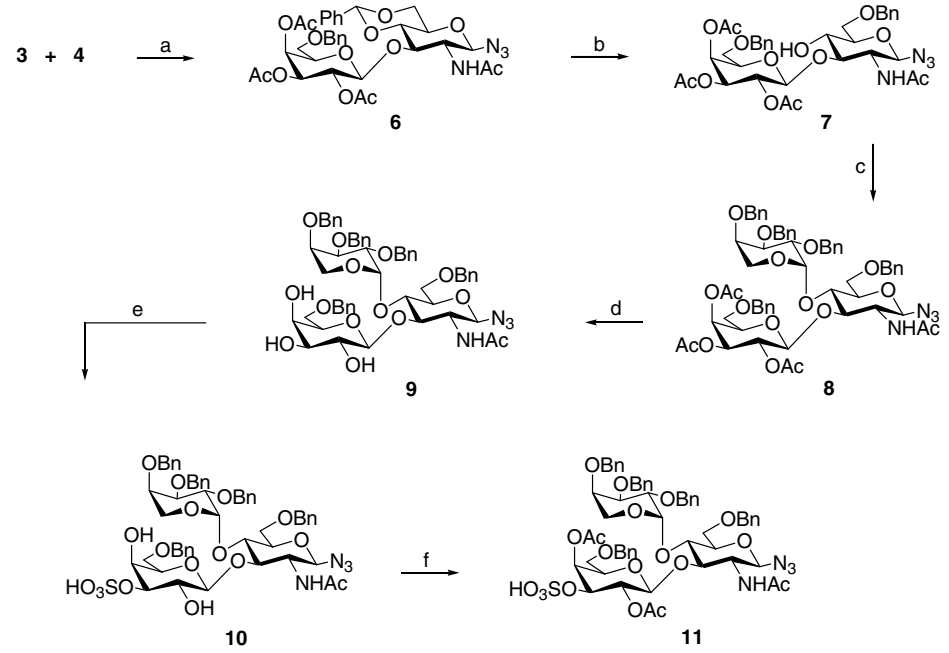


Figure 2. Carbohydrate building blocks.

Without pre-activation as a stannylene intermediate, mixtures of mono- and disulfonated products had been obtained.[29] The regioselectivity of the reaction was proven by NMR chemical shift differences for the signals of protons and carbon atoms in 2′-, 3′- and 4′-positions of the galactose residue in compounds **10**, **9** and **11** (Table 1).

The sulfate group in position 3′ of **10** caused downfield shifts of the carbon signal of 6 ppm and for the proton signal of 0.52 ppm, whereas the signals for carbon atoms carrying the remaining free hydroxyl groups at positions 2′ and 4′ occurred at almost unchanged values. After O-acetylation at these positions, downfield shifts of 0.14 and 2.94 ppm (^{13}C) and 1.26 and 1.55 ppm (^{1}H) gave evidence of the proposed structure **11**.

To find the optimal conditions for the reduction of the anomeric azido group and the subsequent conjugation with the Fmoc α-allyl aspartate **13**, the nonsulfated trisaccharide Le[a] **9** and the sulfated analogue **10** were investigated. Using commercially available Raney-nickel at pH 7,[30] both glycosyl azides were reduced to give the corresponding amines in nearly quantitative yield. Due to the increased solubility caused by the sulfate group, **10** reacted even faster and with complete retention of sulfate and benzyl protecting groups. The building blocks **12a/b** were coupled with *N*-Fmoc α-allyl aspartate[12,31] **13** using *O*-(7-aza-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate/1-hydroxy-7-aza-benzotriazole[32] (HATU/HOAt) and diisopropylethylamine (*i*-Pr_2NEt, Huenigs base) to furnish the



Scheme 1. Reagents and conditions: (a) **3** (1.0 equiv), **4** (2.8 equiv), $CH_2Cl_2/C_2H_4Cl_2$ (1:1), $Hg(CN)_2$ (2.8 equiv), 20 °C, 6 d, 97%; (b) Et_3SiH, TfOH, CH_2Cl_2, −78 °C, 3 h, 64%; (c) **5** (3 equiv), $CuBr_2$, Bu_4NBr, CH_2Cl_2, 20 °C, 6 d, 84%; (d) $NaOCH_3$, CH_3OH, 20 °C, 3 h, 93%; (e) (1) Bu_2SnO (1.1 equiv), CH_3OH, 65 °C, 4 h; (2) $SO_3/N(CH_3)_3$ (1.1 equiv), DMF, 20 °C, 14 h, 91% (two steps); (f) pyridine/Ac_2O, 20 °C, 15 h, quant.

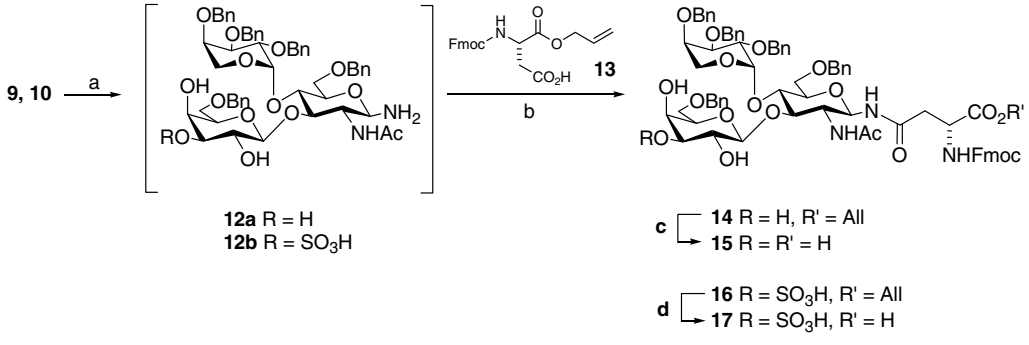
Table 1. NMR spectroscopy of trisaccharides **9**, **10** and **11**: chemical shift of protons and carbon atoms in positions 2′, 3′ and 4′ of the galactose residue

Position	9		10		11	
	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)
2′	70.34	3.35	69.09	3.51	69.23	4.77
3′	73.20	3.28	79.20	3.80	73.70	4.19
4′	68.01	3.61	66.29	3.85	69.23	5.40

arabino Lewis[a] asparagine conjugate **14** and its sulfated analogue **15** (Scheme 2).

Deprotection of the carboxylic acid by palladium(0) catalyzed[33] allyl transfer to *p*-toluenesulfinic acid[34] or *N*-methylaniline[35] as the allyl scavenger yielded the arabino Lewis[a] asparagine building block **15** and the sulfatyl arabino Lewis[a]-asparagine analogue **17** both suitable for solid-phase peptide synthesis.

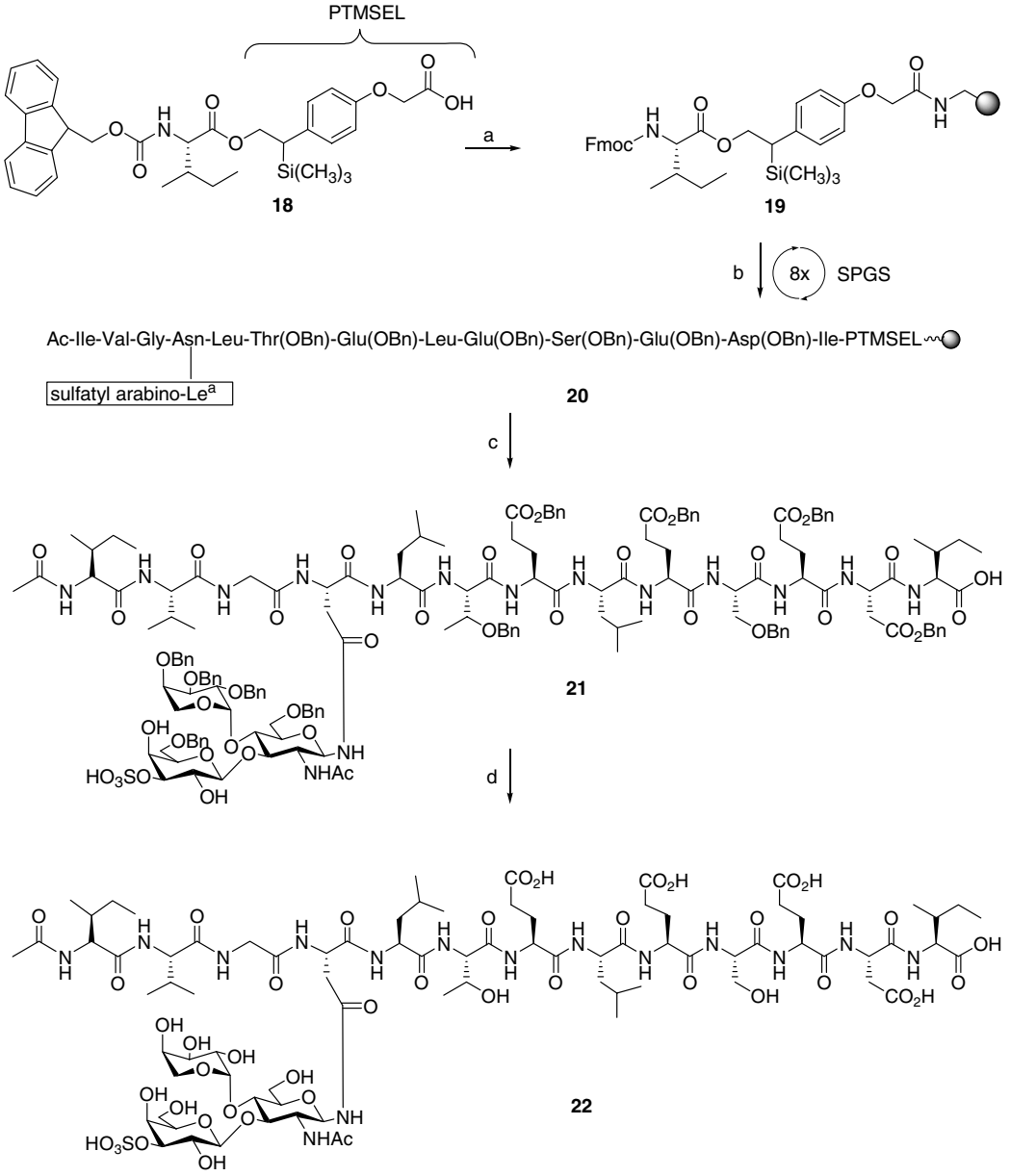
The solid-phase synthesis of the target sulfatyl arabino-Lewis[a] glycopeptide was performed in a peptide synthesizer according to the Fmoc strategy.[36] Amino-functionalized TentaGel®-resin was loaded with the



Scheme 2. Reagents and conditions: (a) Raney-nickel, H_2, 2-propanol/water, 5 h (**12a**), 3.5 h (**12b**), 20 °C, quant.; (b) Fmoc-Asp-OAll[12] (**13**), HATU/HOAt, DMF, 20 °C, 14 h, 82% (**14**), 66% (**16**); (c) $Pd(PPh_3)_4$, *N*-methylaniline, THF, 20 °C, 74%; (d) $Pd(PPh_3)_4$, *N*-methylaniline, THF, 20 °C, 5 h, 92%.

novel fluoride-labile PTMSEL-linker[11] **18**, carrying the C-terminal amino acid Fmoc isoleucine to give **19**. The subsequent eight Fmoc amino acids were coupled according to a standard Fmoc protocol. After removal of the Fmoc-protecting group from **19** with 30% of piperidine in *N*-methylpyrrolidone (NMP), the peptide chain was extended by iterative coupling using 10 equiv of each Fmoc amino acid activated by *O*-(1-hydroxybenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)[31] and 1-hydroxybenzotriazole (HOBt) as additive in *N*-methylpyrrolidone (NMP) (Scheme 3).

To avoid deletion sequences, unreacted amino groups were capped after each coupling step with Ac_2O/*i*-Pr_2NEt/HOBt (4:1:0.1) in NMP. The sulfated arabino Lewis[a] building block **17** was dissolved in NMP. For its coupling the reaction time was extended to 3 h, and the more reactive HATU/HOAt system[32] was used for activation. A large excess of the activating reagent was applied to complete the carboxy activation and to accelerate the coupling reaction of the sterically demanding building block. The final three amino acids were coupled according to the standard protocol. After exchanging the terminal Fmoc for an acetyl group to



Scheme 3. Reagents and conditions: (a) CH_2Cl_2/DMF, 20 °C, NovaSyn TG® HL, HBTU/HOBt/NMP, 98%; (b) solid-phase glycopeptide synthesis (SPGS): (1) *Fmoc cleavage*: piperidine/NMP (30%), (2) *coupling*: 2.-9., 11.-13.: Fmoc-amino acid (10 equiv), HBTU/HOBt/*i*-Pr_2NEt, DMF; 10.: **17** (1.6 equiv), HATU/HOAt/NMP, DMF, 3 h, (3) *capping*: Ac_2O/*i*-Pr_2NEt/HOBt (4:1:0.1); (c) (1) TBAF·$3H_2O$ (2 × 1 equiv), CH_2Cl_2, 20 min, (2) Amberlite® CG120 (Na^+), 15% (based on **19**); (d) H_2, $Pd(OH)_2$, 3 d, CH_3OH/dioxane (1:1), quant.

give **20**, the protected glycopeptide was detached from the resin by a twofold treatment with 1 equiv TBAF·$3H_2O$ in dichloromethane for 20 min. These conditions are almost neutral. The formation of isoaspartate structures by rearrangement via aspartimides was not observed. The sulfate group was kept intact as well. The glycoconjugate **21** was furnished in a yield of 15% based on the loaded resin **19**. The incomplete couplings of the glycosylated amino acid and of the N-terminal isoleucine as well as the required purification by a repeated chromatography are the major reasons for the low yield of isolated **21**. The cleavage of the anchor by TBAF·$3H_2O$ resulted in the formation of tetrabutylammonium salts of the negatively charged sulfonate and carboxylate moieties. Unfortunately, the ionic bonds between *O*-sulfonate and the tetrabutylammonium cations were too favoured to be completely exchanged for Na^+ by ion exchange chromatography and hence a precise analysis of **21** by NMR spectroscopy was not possible. Therefore, the use of the PTMSEL linker was also demonstrated in the synthesis of the glycopeptide **24** containing the nonsulfonated arabino Lewis[a] asparagine **15**. After fluoride-induced cleavage of the linker, the fully protected arabino Lewis[a] glycopeptide **24** was isolated in a yield of 43%, demonstrating the efficiency of this method. NMR analysis and mass spectroscopy of **24** proved its correct structure (Scheme 4).

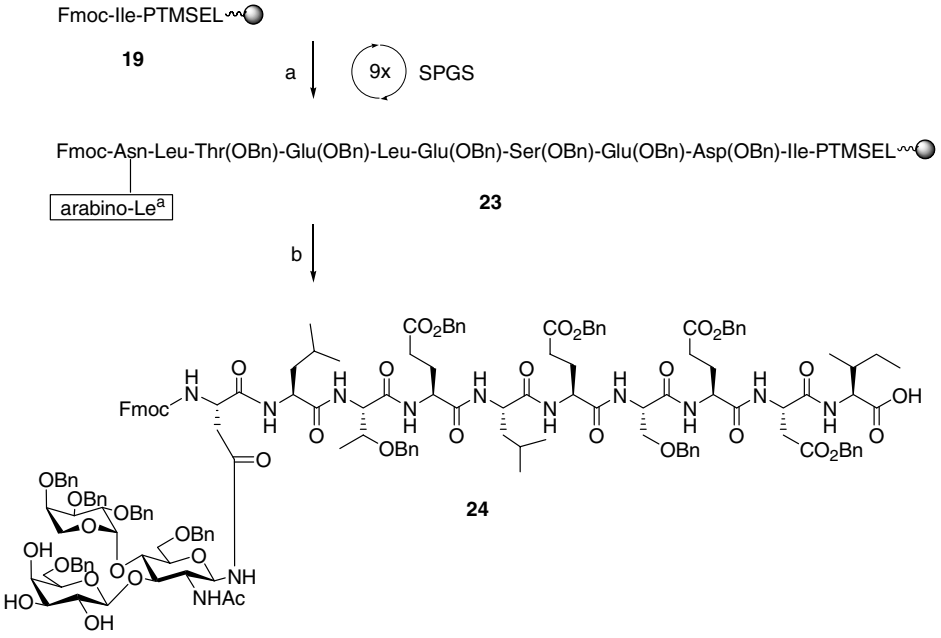
Because of the poor solubility of the protected sulfatyl glycopeptide **21** in solvents applicable to RP-HPLC, the benzyl ether and -ester protecting groups of the carbohydrate moiety and of the peptide side chains were simultaneously removed by hydrogenolysis catalyzed by $Pd(OH)_2$ in a mixture of methanol and 1,4-dioxane without previous purification to give **22**. The target compound was purified by preparative RP-thin-layer chromatography and identified by MALDI-TOF mass spectroscopy in negative ion mode. Its acid-rich nature is responsible for it retaining a mixture of ammonium and sodium ions, leading to broad signals in the NMR spectra.

3. Experimental

3.1. General methods

NMR-Spectra were recorded on a Bruker AC-300, Bruker AM-400 or Bruker DRX-600 spectrometer. The following abbreviations were used to explain multiplicities: s (singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were recorded on a ESI Navigator-1 (ThermoQuest) or a Tofspec E instrument (Micromass, 2,5-dihydroxy-benzoic acid (dhb) as the matrix). Optical rotations were recorded using a Perkin–Elmer 241 polarimeter. Elemental analyses were performed by the microanalytical laboratory of the Institut für 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 solutions and heat for developing. Silica gel 60 (particle size 0.04–0.0063, E. Merck, Darmstadt, Germany) was used for flash chromatography. All reactions were carried out



Scheme 4. Reagents and conditions: (a) solid-phase glycopeptide synthesis (SPGS): (1) *Fmoc cleavage*: piperidine/NMP (30%), (2) *coupling*: 2.-9.: Fmoc amino acid (10 equiv), HBTU/HOBt/*i*-Pr_2NEt, DMF; 10.: **15** (1.65 equiv), HATU/HOAt/NMP, DMF, 3 h; (3) *capping*: Ac_2O/*i*-Pr_2NEt/HOBt (4:1:0.1); (b) TBAF·$3H_2O$, CH_2Cl_2, 20 min, 43%.

under argon atmosphere with dried solvents. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials. Analytical RP-HPLC was performed on a Phenomenex Jupiter 300 A C18 5 μm column, 250 × 4.6 mm, flow 1 mL/min using a Knauer HPLC equipment (Maxistar K1000, DAD 2026 detector). Solvent: (CH_3CN/H_2O + 0.1% TFA); Gradient: (A) (20:80)→(20:80) [5 min]→(50:50) [20 min]→(80:20) [40 min]→(100:0) [45 min]→100:0 [60 min]; (B) (50:50)→(90:10) [5 min]→(0:100) [15 min]→(0:100) [25 min].

3.2. 2-Acetamido-3-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide (6)

To a suspension of 8.5 g (25.5 mmol) of glucosaminyl azide **3**, 21.5 g (85.1 mmol) of $Hg(CN)_2$ and 15 g of dried molecular sieves (3 Å) in CH_2Cl_2 (300 mL) was added 48.9 g (111.5 mmol) of galactosyl bromide[14] **4** in 1,2-dichloroethane (350 mL). The mixture was stirred for 6 d, filtered through Hyflo-Supercel® and washed with CH_2Cl_2. The combined organic layers were washed with 30% NaI in water and with water, then dried ($MgSO_4$), concentrated in vacuo and purified by flash chromatography (cyclohexane/EtOAc). Yield: 17.63 g (24.6 mmol, 97%; 63%[12a]); amorphous; $[\alpha]_D^{22}$ −8.7 (*c* 1, $CHCl_3$); −16.2 (*c* 1, $CHCl_3$[12b]); R_f = 0.67 (EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42–7.16 (m, 10H, $H_{arom.}$), 5.78 (d,1H, $J_{NH,2}$ = 7.0 Hz, NH), 5.40 (s, H, CH-aryl), 5.31–5.26 (m, 2H, $J_{1,2}$ = 9.2 Hz, H-1, H-4′), 5.09 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{2',1'}$ = 8.1 Hz, H-2′), 4.89 (d, 1H, H-3′), 4.70 (d, 1H, H-1′), 4.53–4.20 (m, 4H, H-6a/b, CH_2Ph), 3.71–3.50 (m, 4H, J_{vic} = 6.2 Hz, J_{vic} = 6.6, H-3, H-4, H-5, H-5′), 3.40–3.29 (m, 2H, H-6′a/b), 3.19 (dd, 1H, H-2), 2.05 (s, 3H, CH_3(NHAc)); 1.97, 1.95, 1.92 (3s, 9H, CH_3(OAc)); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 170.89, 170.14, 170.04, 169.59 (CO (OAc, NHAc)), 137.40, 137.05 (C_{ipso}-aryl), 129.30, 128.55, 128.47, 128.32, 127.99 ($C_{arom.}$), 126.14 (C_{ipso}-aryl), 99.84 (C-1′), 87.32 (C-1), 80.29 (C-4), 76.07 (C-5), 71.11 (C-3′), 69.67 (C-3), 68.38 (C-6), 68.14 (C-2′), 67.60 (C-6′), 67.42(C-4′), 57.63 (C-2), 23.55 (CH_3(NHAc)), 20.78, 20.63, 20.57 (CH_3(OAc)); ESIMS calcd: 712.33. Found: 735.61 $[M+Na]^+$. Anal Calcd for $C_{34}H_{40}N_4O_{13}\cdot 2H_2O$: C, 54.53; H, 5.65; N, 7.48. Found: C, 54.07; H, 5.69; N, 7.33. The lyophilized compound is hygroscopic.

3.3. 2-Acetamido-3-*O*-(2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl)-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide (7)

To a solution of 1 g (1.36 mmol) **6** and 3 g molecular sieves (4 Å) in CH_2Cl_2 (75 mL) at −78 °C was added 175 μL (2 mmol, 1.5 equiv) TfOH and 320 μL (2 mmol, 1.5 equiv) Et_3SiH. After 2 h of stirring, an additional 1.5 equiv of each reagent was added. For neutralization, 1.6 mL NEt_3 was added, the solution subsequently filtered through Hyflo-Supercel® and the filter washed with $CHCl_3$ (150 mL). The organic layer was washed with satd $NaHCO_3$ (3 × 150 mL), dried ($MgSO_4$), concentrated in vacuo and purified by flash chromatography (cyclohexane/EtOAc) on silica gel (50 g). Yield: 0.76 g (1.03 mmol, 76%; 78%[12a]); amorphous; $[\alpha]_D^{22}$ −11.4 (*c* 1, $CHCl_3$); −11.9 (*c* 1, $CHCl_3$[12b]); R_f = 0.54 (EtOAc); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.26 (m, 10H, $H_{arom.}$), 5.80 (d, 1H, $J_{NH,2}$ = 7.7 Hz NH), 5.34 (d, 1H, $J_{4',3'}$ = 3.0 Hz, H-4′), 5.16 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{2',1'}$ = 8.1 Hz, H-2′), 4.96 (m, 2H, H-3′, H-1), 4.62–4.54 (m, 1H, H-1′), 4.56 (s, 1H, OH), 4.49 (d, 2H, J_{gem} = 11.8 Hz, CH_2Ph), 4.38 (d, 2H, J_{gem} = 12.1 Hz, CH_2Ph), 4.10 (m, 2H, H-6a/b), 3.91 (dd, 1H, J_{vic} = 12.1 Hz, H-5′), 3.80 (d, 1H, $J_{4,3}$ = 8.6 Hz, H-4), 3.66 (dd, 1H, J_{gem} = 11.0 Hz, $J_{5,4}$ = 4.8 Hz, H-5), 3.48 (m, 3H, H-3, H-6′a/b), 3.21 (dd, 1H, H-2), 2.05, 2.04, 1.97, 1.94 (4s, 12H, CH_3OAc, CH_3NAc); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 170.59, 170.12, 169.96, 169.30 (CO (OAc, NHAc)), 138.21, 137.02 (C_{ipso}-aryl), 128.49, 128.32, 128.02, 127.96, 127.56, 127.53 ($C_{arom.}$), 101.03 (C-1′), 87.17 (C-1), 83.03 (C-4), 77.58 (C-5), 76.58 (C-5′), 72.41 (C-3′), 69.30 (C-3), 73.69, 73.57 (CH_2Ph), 70.93 (C-3′), 69.27, 67.34 (C-6, C-6′), 67.34 (C-4′), 56.31 (C-2), 23.59 (CH_3(NHAc)), 20.82, 20.58, 20.50 (CH_3(OAc)); ESIMS calcd: 714.35. Found: 737.3 $[M+Na]^+$. Anal Calcd for $C_{34}H_{42}N_4O_{13}\cdot 3H_2O$: C, 53,12; H, 6.29; N, 7.29. Found: C, 53.47; H, 5.87; N, 7.21.

3.4. 2-Acetamido-3-*O*-[2,3,4-tri-*O*-acetyl-6-*O*-benzyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide (8)

Disaccharide **7** (0.7 g,1.0 mmol) and 1.47 g (3.1 mmol) of ethyl 2,3,4-tri-*O*-acetyl-1-thio-α,β-D-arabinopyranoside **5** were dissolved in CH_2Cl_2 (50 mL) and stirred with molecular sieve (3 Å) for 30 min. After addition of 1.3 g (4 mmol) tetrabutylammonium bromide and 0.9 g (4 mmol) $CuBr_2$, the mixture was stirred under exclusion of light at rt for 6 d. The suspension was filtered through a thin layer of silica gel. The organic phase was washed with satd $NaHCO_3$ until the blue colour disappeared. After washing with brine, the solution was dried ($MgSO_4$), concentrated in vacuo and the residue purified by flash-chromatography (2:1, cyclohexane/EtOAc). Yield: 935 mg (0.84 mmol, 84%); amorphous; $[\alpha]_D^{22}$ −90.9 (*c* 1, CH_2Cl_2); R_f = 0.72 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.37–7.18 (m, 25H, $H_{arom.}$), 6.68 (d, 1H, $J_{NH,2}$ = 9.4 Hz, NH), 5.41 (d, 1H, $J_{4',3'}$ = 2.8 Hz, H-4′), 5.20 (d, 1H, $J_{1'',2''}$ = 3.5 Hz, H-1″), 5.14 (dd, 1H, $J_{2',1'}$ = 7.8 Hz, $J_{2',3'}$ = 10.6 Hz, H-2′), 4.99–4.95 (m, 3H, H3, H-1, H-3′), 4.71–4.31 (m, 11H, H-1′,

CH_2Ph), 4.09 (m, 1H, H-5), 4.04–3.97 (m, 4H, H-4′, H-2″, H-6a, H-3), 3.92 (m, 2H, H-2, H-4″), 3.82 (dd, 1H, $J_{vic} = 6.3$ Hz, $J_{vic} = 6.7$ Hz, H-5′), 3.69–3.63 (m, 6H, H-5″a/b, H6b, H-5, H-4, H-3″), 3.48 (dd, 1H, $J_{gem} = 17.6$ Hz, H-6′a), 3.41 (dd, 1H, $J_{vic} = 6.7$, H-6′b), 2.08 (s, 3H, CH_3NHAc), 1.95, 1.92, 1.53 (3s, 9H, CH_3(OAc)); ^{13}C NMR ($CDCl_3$, 100.9 MHz): δ 169.99, 169.92, 169.78, 169.62 (CO (OAc, NHAc)), 138.27, 138.15, 138.07, 137.65, 137.41 (C_{ipso}-aryl), 128.45, 128.43, 128.40, 128.30, 128.11, 127.90, 127.84, 127.69, 127.61, 127.57, 127.39, 127.30 ($C_{arom.}$), 99.23 (C-1′), 94.12 (C-1″), 87.84 (C-1), 77.22 (C-3″), 76.05 (C-2″), 73.92, 73.37, 73.31, 72.38, 71.46 (CH_2Ph), 73.84 (C-4″), 73.14 (C-4), 72.93 (C-5); 72.17 (C-5′), 70.88 (C-3′), 69.82 (C-3), 69.06 (C-6), 68.38 (C-2′), 67.40 (C-6′), 67.46 (C-4′), 60.58 (C-5″), 50.68 (C-2), 22.63 (CH_3(NHAc)), 21.02, 20.75, 20.53 (CH_3(OAc)); ESIMS calcd: 1117.19. Found: 1140.2 $[M+Na]^+$. Anal Calcd for $C_{60}H_{68}N_4O_{17}$: C, 64.50; H, 6.12; N, 5.01. Found: C, 63.65; H, 5.98; N, 5.05. The compound retains inorganic impurities.

3.5. 2-Acetamido-3-*O*-[6-*O*-benzyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide (9)

Lewis[a] derivative **8** (7.41 g, 6.64 mmol) was dissolved in CH_3OH (150 mL) and 200 mg Na was added. After 20 h of stirring at rt, 7.3 g of ion exchange resin Amberlyst® 15 was added to neutralize the reaction mixture. The solvent was decanted and the resin washed three times with CH_3OH (25 mL). The combined organic layers were dried ($MgSO_4$), concentrated in vacuo and the residue purified on silica gel (200 g) by flash-chromatography (20:1, CH_2Cl_2/CH_3OH). Yield: 5.83 g (5.88 mmol, 88%); $[\alpha]_D^{22}$ −60.9 (*c* 1, CH_2Cl_2); $R_f = 0.21$ (15:1, CH_2Cl_2/CH_3OH); ^{1}H NMR (DMSO-d_6, 600 MHz): δ 8.06 (d, $J_{NH,H2} = 9.1$ Hz, NH), 7.36–7.18 (m, 25H, $H_{arom.}$), 4.99 (d, 1H, $J_{1'',2''} = 3.6$ Hz, H-1″), 4.87 (d, 1H, $J_{3'\text{-OH},H\text{-}3'} = 5.3$ Hz, 3′-OH), 4.70–4.67 (m, 2H, H-5″a, CH_2Ph), 4.62–4.52 (m, 5H, CH_2Ph, H-1), 4.46–4.35 (m, 6H, CH_2Ph, H-1′), 3.99 (br s, 1H, H-4″), 3.94 (d, 1H, $J_{3'',2''} = 10.4$ Hz, H-3″), 3.87–3.78 (m, 4H, H-6′a, H-3, H-2″, H-2), 3.68–3.59 (m, 4H, H-6a, H-4′, H-6′b, H-4), 3.55–3.50 (m, 4H, H-5, H-6b, H-5′, H-5″e), 3.35–3.34 (m, 2H, H-2′, 2′-OH), 3.29–3.27 (m, 1H, H-3′), 1.83 (s, 3H, CH_3(NHAc)); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ 171.31 (CO(NHAc)), 138.31, 138.22, 138.05, 173.26 (C_{ipso}-Ar), 128.41, 128.38, 128.18, 127.98, 127.89, 127.76, 127.69, 127.62, 127.53 ($C_{arom.}$), 103.05 (C-1′), 97.42 (C-1″), 87.88 (C-1), 77.55 (C-3″), 76.72 (C-5), 75.76 (C-3), 75.72 (C-2″), 74.69 (C-4″), 73.46, 72.88, 72.16, 70.82, 70.48 (CH_2Ph), 73.20 (C-3′), 73.06 (C-5′), 72.42 (C-4), 70.36 (C-2′), 68.93 (C-6), 68.01 (C-4′), 67.41 (C-6′), 60.67 (C-5″), 54.74 (C-2), 23.23 (CH_3(NHAc)); ESIMS calcd: 990.43. Found: 1013.2 $[M+Na]^+$; 985.2 $[M-N_2+Na]^+$. Anal Calcd for $C_{54}H_{62}N_4O_{14}$: C, 65.44; H, 6.31; N, 5.65. Found: C, 65.40; H, 6.30; N, 5.49.

3.6. 2-Acetamido-3-*O*-[6-*O*-benzyl-3-*O*-sulfatyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl azide (10)

A mixture of 156 mg (0.158 mmol) **9** and 43 mg (0.173 mmol, 1.1 equiv) of dibutyltin oxide was dissolved in CH_3OH (10 mL) and heated under reflux. After 20 min, the suspension became clear and was kept at this temperature for additional 3.5 h. After concentration in vacuo and co-distillation twice with toluene (10 mL), the colourless residue was dried until constant weight was achieved. After dissolution of the residue in DMF (15 mL), 24 mg (0.173 mmol, 1.1 equiv) SO_3/NMe_3 complex was added and the mixture was stirred at rt for 14 h. The solvent was removed under high vacuum, and the residue was co-distilled twice with toluene (10 mL). The resulting colourless oil was purified by flash-chromatography (15:1, $CHCl_3/CH_3OH$) on silica gel (15 g). Yield: 0.144 mg (0.14 mmol, 91%); amorphous; $[\alpha]_D^{22}$ −83.5 (*c* 1, CH_3CN); $R_f = 0.17$ (10:1, $CHCl_3/CH_3OH$); $t_R =$ 38.3 min (A); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.01 (d, $J_{NH,H2} = 9.1$ Hz, NH), 7.47–7.14 (m, 25 H, $H_{arom.}$), 4.97 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1″), 4.78 (d, 1H, $J_{2'\text{-OH},H\text{-}2'} = 2.5$ Hz, 2′-OH), 4.70–4.59 (m, 4H, 4′-OH, CH_2Ph, H-5″a), 4.56–4.33 (m, 10H, CH_2Ph, H-1′, H-1), 3.97–3.73 (m, 8H, H-4″, H-4′, H-3′, H-3″, H-2″, H-6′a, H-3, H-2), 3.67–3.58 (m, 3H, H-6a, H-4, H-6′b), 3.54–3.47 (m, 5H, H-5′, H-5″e, H-5, H-6b, H-2′), 1.81 (s, 3H, CH_3(NHAc)); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 169.59 (CO(NHAc)), 139.03, 138.95, 138.71, 138.54, 138.27 (C_{ipso}-aryl), 128.38, 128.32, 128.23, 128.17, 127.77, 127.50, 127.44, 127.38, 127.30, 127.18, 127.08 ($C_{arom.}$), 102.92 (C-1′), 97.43 (C-1″), 88.01 (C-1), 79.12 (C-3′), 77.50 (C-3″), 76.82 (C-5), 75.82 (C-3), 75.44 (C-2″), 74.51 (C-4″), 73.45, 72.33, 72.22, 70.74, 70.34 (CH_2Ph), 72.78 (C-5′), 72.43 (C-4), 69.05 (C-2′), 68.63 (C-6), 67.44 (C-6′), 66.26 (C-4′), 60.23 (C-5″), 54.68(C-2), 22.95 (CH_3(NHAc)); ESIMS calcd: 1070.38. Found: 1115.35 $[M-H+2Na]^+$. Anal Calcd for $C_{54}H_{62}N_4O_{17}S{\cdot}2H_2O$: C, 58.58; H, 6.01; N, 5.06; S, 2.90. Found: C, 58.46; H, 6.38; N, 4.94; S, 3.24.

3.7. N^{α}-Fluorenylmethoxycarbonyl-N^{ω}-(2-acetamido-3-*O*-[6-*O*-benzyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine allyl ester (14)

To 280 mg (0.26 mmol) **9** in 75 mL of *i*-PrOH/water (9:1) was added a catalytic amount (10 mg) of freshly washed (pH 7) Raney-nickel. Hydrogen was bubbled through the stirred solution via a cannula. After

completion of the reaction (5 h, TLC), the catalyst was filtered off and washed thoroughly with *i*-PrOH and CH_3OH. The combined organic solutions were concentrated in vacuo, and the amine **12a** was used in the subsequent reaction without purification and characterization. Yield: 252 mg (quant.); amorphous; $R_f = 0.10$ (5:1, $CHCl_3/CH_3OH$); MS calcd for $C_{54}H_{64}N_2O_{14}$: 964.43.

To a mixture of 147 mg (0.37 mmol, 1.3 equiv) Fmoc-Asp-OAll[12] **13** with 51 mg (0.37 mmol, 1.3 equiv) HOAT in DMF (5 mL) were added 0.13 mL (0.74 mmol, 2.6 equiv) *i*-Pr_2NEt and 140 mg (0.37 mmol, 1.3 equiv) HATU. After keeping the solution at rt for 10 min, glycosylamine **12a** in DMF (5 mL) was added, and the solution was stirred for 18 h. After removal of the solvents in high vacuum, the residue was dissolved in CH_2Cl_2 (100 mL) and washed with satd $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo and the residue purified by flash-chromatography on silica gel (30 g) with CH_2Cl_2/CH_3OH (15:1). Lyophilization from benzene yielded a colourless amorphous solid. Yield: 313 mg (0.30 mmol, 82%); $[\alpha]_D^{22}$ −69.8 (*c* 1, CH_3CN); $R_f = 0.32$ (15:1, $CHCl_3/CH_3OH$); $t_R = 46.3$ min (A); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.49 (d, 1H, $J_{NH,H1} = 9.3$ Hz, $^{\omega}$NH-Asn), 7.96 (d, 1H, $J_{NH,H2} = 8.9$ Hz, NH), 7.88 (d, 2H, $J_{H4,H3=H5H6} = 7.9$ Hz, H4-, H5-Fmoc), 7.69 (d, 3H, $J_{H1,H2=H8,H7} = 7.9$ Hz, H1-, H8-Fmoc), 7.40 (t, 2H, $J_{H3,H2/4=H6,H5/7} = 7.9$ Hz, H3-, H6-Fmoc), 7.36–7.14 (m, 27H, H2-, H7-Fmoc, $H_{arom.}$), 5.89–5.79 (m, 1H, $^3J_{trans} = 17.2$ Hz, $^3J_{cis} = 10.6$ Hz, All-H2), 5.26 (dd, 1H, $^3J_{trans} = 17.3$ Hz, All-H3a), 5.14 (dd, 1H, $^3J_{cis} = 10.4$ Hz, All-H3b), 4.98 (d, 1H, $J_{1'',2''} = 3.5$ Hz, H-1″), 4.91 (t, 1H, H-1), 4.70–4.40 (m, 15H, H-5″a, CH_2Ph, α-CH-Asn), 4.31–4.17 (m, 5H, CH_2Ph, Fmoc-CH_2, H9-Fmoc), 4.00–3.97 (m, 2H, H-3″, H-4″), 3.86–3.78 (m, 4H, H-3, H-6′a, H-2″, H-2), 3.69–3.48 (m, 8H, H-6′, H-4′, H-4, H6b, H-6′b, H-5″e, H-5′), 3.36–3.29 (m, 3H, H-2′, 2′-OH, H-5), 3.23 (dd, 1H, $J_{3',2'} = 9.4$ Hz, H-3′), 2.65 (m, 1H, β-CH_2-Asn), 2.45 (m, 1H, β-CH_2-Asn), 1.73 (s, 3H, CH_3(NHAc)); ^{13}C NMR (DMSO-d_6, 100.6): δ 171.08 (CO-ester), 171.00, 169.23 (CO-NHAc, -amide), 155.79 (CO-urethane), 143.74 (C1a-, C8a-Fmoc), 140.69 (C4a-, C5a-Fmoc), 138.99, 138.87, 138.71, 138.66, 138.14 (C_{ipso}-aryl), 132.30 (All-C2), 128.21, 128.12; 127.46; 127.22, 128.02, 127.16, 127.05, 126.98 ($C_{arom.}$), 127.63 (C3-, C6-Fmoc), 127.33 (C2-, C7-Fmoc), 125.22 (C1-, C8-Fmoc), 120.11 (C4-, C5-Fmoc), 117.41 (All-C3), 102.85 (C-1′), 97.30 (C-1″), 78.31 (C-1), 77.49 (C-3″), 76.58 (C-3), 76.54 (C-5), 75.78 (C-2″), 74.67 (C-4″), 73.31 (CH_2Ph, C-3′), 72.11, 70.77 (CH_2Ph), 70.38 (CH_2Ph, C-2′), 73.03 (C-5′), 72.49 (C-4), 68.97 (C-6), 68.08 (C-4′), 67.62 (C-6′), 65.75 (CH_2-Fmoc), 64.89 (All-C1), 60.23 (C-5″), 54.45 (C-2), 50.21 (α-CH-Asn), 46.54 (C9-Fmoc), 36.82 (β-CH_2-Asn), 22.79 (CH_3(NHAc)); ESIMS calcd for $C_{76}H_{83}O_{19}N_3$: 1341.56. Found: 1364.97 $[M+Na]^+$.

3.8. N^{α}-Fluorenylmethoxycarbonyl-N^{ω}-(2-acetamido-3-*O*-[6-*O*-benzyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-L-asparagine (15)

To 390 mg (0.29 mmol) **14** in freshly degassed THF (30 mL) were added *N*-methylaniline (0.06 mL, 0.56 mmol) and a catalytic amount (about 10 mg) of $Pd(PPh_3)_4$. After 24 h, another 10 mg $Pd(PPh_3)_4$ and 0.2 mL (1.8 mmol) *N*-methylaniline were added, but total deallylation was not achieved. The solvents were removed in vacuo, the residue was dissolved in EtOAc (100 mL) and washed with 0.1 N HCl (50 mL). After drying ($MgSO_4$), the solvent was removed in vacuo, yielding a yellow oil of which the product was extracted with CH_3OH (5 mL) and filtered through a C18 cartridge. The solvent was removed in vacuo and the resulting colourless oil was purified by semi-preparative HPLC (Knauer Eurosphere C18). Yield: 279 mg (0.21 mmol, 74%); $[\alpha]_D^{22}$ −58.3 (*c* 1, CH_3CN); $R_f = 0.32$ (15:1, $CHCl_3/CH_3OH$); $t_R = 12.7$ min (B); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.61 (br s, 1H, acid); 8.41 (d, 1H, $J_{NH,H1} = 9.2$ Hz, $^{\omega}$NH-Asn), 7.96 (d, 1H, $J_{NH,H2} = 8.9$ Hz, NH-Glc), 7.87 (d, 2H, $J = 7.5$ Hz, H4-, H5-Fmoc), 7.70 (d, 2H, $J = 7.5$ Hz, H1-, H8-Fmoc), 7.48 (d, 1H, $J_{NH,\alpha CH} = 8.4$ Hz, NH-urethane), 7.40 (m, 3H, H3-, H6-Fmoc), 7.33–7.21 (m, 25H, $H_{arom.}$), 4.98 (d, 1H, $J_{1'',2''} = 3.44$ Hz, H-1″), 4.92 (pt, 1H, $J_{H1,NH} = 9.3$ Hz, $J_{H1,H2} = 9.4$ Hz, H-1), 4.86 (d, 1H, $J_{3'\text{-}OH,H3'} = 5.2$ Hz, 3′-OH), 4.68 (m, 2H, H-5″a, CH_2Ph), 4.62–4.19 (m, 14H, CH_2Ph, H-1′, α-CH-Asn, CH_2-Fmoc, H9-Fmoc), 4.02–3.97 (m, 2H, H-3″, H-4″), 3.86–3.77 (m, 4H, H-6′a, H-3, H-2″, H-2), 3.69–3.49 (m, 7H, H-6a, H-4′, H-4, H-6b, H-6′b, H-5′, H-5″e), 3.34 (m, 3H, H-2′, 2′-OH, H-5), 3.25–3.20 (m, 1H, $J_{H\text{-}3',3'\text{-}OH} = 5.0$ Hz, H-3′), 2.62 (m, 1H, β-CH_2-Asn), 2.45–2.41 (m, 1H, β-CH_2-Asn), 1.74 (s, 3H, CH_3(NHAc)); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 173.10 (CO-acid), 170.27, 169.61 (CO-amide, -NHAc), 155.89 (CO-urethane), 143.91, 143.87 (C1a-, C8a-Fmoc), 140.76 (C4a-, C5a-Fmoc), 139.08, 138.97, 138.79, 138.75, 138.28 (C_{ipso}-aryl), 128.43, 128.32, 128.23, 127.74, 127.57, 127.44, 127.27 ($C_{arom.}$), 127.30 (C3-,C6-Fmoc), 127.15 (C2-,C7-Fmoc), 125.36 (C1-, C8-Fmoc), 120.18 (C4-, C5-Fmoc), 102.94 (C-1′), 97.41 (C-1″), 78.41 (C-1), 77.59 (C-3″), 76.69 (C-3), 76.63 (C-5), 75.81 (C-2″), 74.81 (C-4″), 73.45, 72.23, 70.92, 70.53 (CH_2Ph), 73.45 (C-3′), 73.14 (C-5′), 72.62 (C-4), 70.46 (C-2′), 69.12 (C-6), 69.07 (C-6), 68.18 (C-4′), 67.69 (C-6′), 65.79 (CH_2-Fmoc), 60.33 (C-5″), 54.64 (C-2), 50.23 (α-CH-Asn), 46.79 (C9-Fmoc), 37.07 (β-CH_2-Asn), 22.89 (CH_3(NHAc)). ESIMS calcd for $C_{73}H_{80}N_3O_{19}$: 1302.5386 $[M+H]^+$. Found: 1302.5345 $[M+H]^+$.

3.9. N^{α}-Fluorenylmethoxycarbonyl-N^{ω}-(2-acetamido-3-*O*-[6-*O*-benzyl-3-*O*-sulfatyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-(2-deoxy-β-D-glucopyranosyl)-L-asparagine α-allyl ester (16)

To 196 mg (0.18 mmol) **10** in 47 mL of *i*-PrOH/water (9:1) was added a catalytic amount (about 5 mg) of freshly washed (pH 7) Raney-nickel. Hydrogen was bubbled through the stirred solution via a cannula. After completion of the reaction (3.5 h, TLC), the catalyst was filtered off and washed thoroughly with *i*-PrOH and CH_3OH. The combined organic solutions were concentrated in vacuo and the amine **12b** was used in the subsequent reaction without purification and characterization. Yield: 187 mg (quant); amorphous; $R_f = 0.09$ (5:1, $CHCl_3/CH_3OH$); MS calcd for $C_{54}H_{64}N_2O_{17}S$: 1044.40.

To a solution of 90 mg (0.23 mmol, 1.3 equiv) of Fmoc-Asp-OAll[12] **13** and 31 mg (0.194 mmol, 1.3 equiv) HOAt in DMF (10 mL) were added 0.08 mL (2.6 equiv) *i*-Pr_2NEt and 90 mg (0.194 mmol, 1.3 equiv) HATU. After keeping the mixture at rt for 10 min, glycosylamine **12b** in DMF (5 mL) was added and the solution was stirred for 17 h. After removal of the solvents in high vacuum, the residue was dissolved in CH_2Cl_2 (100 mL) and washed with satd $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Lyophilization from benzene yielded a colourless amorphous solid. Yield: 172 mg (0.12 mmol, 66%); $[\alpha]_D^{22}$ −46.1 (*c* 1, CH_3CN); $R_f = 0.49$ (5:1, $CHCl_3/CH_3OH$); $t_R = 45.5$ min (A); ^{1}H NMR DMSO-d_6, 600 MHz): δ 8.42 (d, 1H, $J_{NH,H1} = 9.3$ Hz, $^{\omega}$NH-Asn), 7.91 (d, 1H, $J_{NH,H2} = 9.8$ Hz, NH), 7.88 (d, 2H, $J_{H4,H3\,=\,H5,H6} = 7.8$ Hz, H4-, H5-Fmoc), 7.69 (d, 3H, $J_{H1,H2\,=\,H8,H7} = 7.8$ Hz, H1-, H8-Fmoc), 7.40 (t, 2H, $J_{H3,H2/4\,=\,H6,H5/7} = 7.5$ Hz, H3-, H6-Fmoc), 7.36–7.14 (m, 27H, H2-, H7-Fmoc, $H_{arom.}$), 5.84 (m, 1H, All-H2), 5.26 (dd, 1H, $^3J_{trans} = 17.2$ Hz, All-H3a), 5.14 (dd, 1H, $^3J_{cis} = 10.5$ Hz, All-H3b), 4.98 (d, 1H, $J_{1'',2''} = 3.40$ Hz, H-1″), 4.91 (t, 1H, $J_{1,NH} = 9.3$ Hz, H-1), 4.68–4.42 (m, 14H, CH_2Ph, H-5″a, AllH1a/b, α-CH-Asn), 4.31–4.18 (m, 4H, Fmoc-CH_2, H9-Fmoc), 3.97–3.84 (m, 6H, H-3″, H-4″, H-4′, H-3, H-3′,H-6′a), 3.79 (dd, 1H, $J_{2'',3''} = 9.$ 6 Hz, H-2″), 3.75 (d, 1H, $J_{2,1} = 9.6$ Hz, H-2), 3.65 (dd, 1H, $J_{gem} = 14.2$ Hz, H-6′b), 3.58 (d, 1H, $J_{4,3} = 9.2$ Hz, H-4), 3.55–3.47 (m, 7H, H-4, H-6b, H-6′b, H-5′, H-5″e, H-2), 3.30 (d, 1H, $J_{5,4} = 9.6$ Hz, H-5), 2.64 (m, 1H, β-CH_2-Asn), 2.46 (m, 1H, β-CH_2-Asn), 1.73 (s, 3H, CH_3(NHAc)); ^{13}C NMR (DMSO-d_6, 150.9 MHz): δ 171.11 (CO (ester)), 169.60, 169.11 (CO (NHAc), (amide)), 155.82 (CO-urethane), 143.75 (C1a-, C8a-Fmoc), 140.71 (C4a-, C5a-Fmoc), 138.98, 138.91, 138.65, 138.62, 138.18 (C_{ipso}-aryl), 132.83 (All-C2), 128.23, 128.14, 128.11, 127.66, 127.57, 127.05, 127.44, 127.08, 127.04 ($C_{arom.}$), 127.33 (C3-, C6-Fmoc), 127.23 (C2-, C7-Fmoc), 125.25 (C1-, C8-Fmoc), 120.12 (C4-, C5-Fmoc), 117.43 (All-C3), 102.76 (C-1′), 97.29 (C-1″), 79.24 (C-3′), 78.25 (C-1), 77.45 (C-3″), 76.68 (C-5), 75.96 (C-3), 75.79 (C-2″), 74.49 (C-4″), 73.33, 72.18, 72.11, 70.68, 70.29 (CH_2Ph), 72.72 (C-5′), 72.51 (C-4), 68.95 (C-2′), 68.64 (C-6), 67.58 (C-6′), 66.31 (C-4′), 65.77 (CH_2-Fmoc), 64.90 (All-C1), 60.12 (C-5″), 54.56 (C-2), 50.19 (α-CH-Asn), 46.55 (C9-Fmoc), 36.82 (β-CH_2-Asn), 22.76 (CH_3(NHAc)); ESIMS calcd for $C_{76}H_{83}N_3O_{22}S$: 1421.51. Found: 1466.41 $[M-H+2Na]^+$.

3.10. N^{α}-Fluorenylmethoxycarbonyl-N^{ω}-(2-acetamido-3-*O*-[6-*O*-benzyl-3-*O*-sulfatyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-(2-deoxy-β-D-glucopyranosyl)-L-asparagine (17)

To 165 mg (0.116 mmol) **16** in freshly degassed THF (15 mL) were added two drops of *N*-methylaniline and catalytic amounts (about 3 mg) of $Pd(PPh_3)_4$. After 6 h complete consumption of the starting material was detected by TLC. Purification was achieved by preparative RP-TLC in CH_2Cl_2/CH_3OH (2:1) as the eluent. Yield: 142 mg (0.103 mmol, 92%); $[\alpha]_D^{22}$ −51.9 (*c* 1, DMSO); $R_f = 0.78$ (RP-C_{18}; 2:1, CH_3CN/H_2O); $t_R = 41.7$ min (A); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.57 (br s, 1H, $^{\omega}$NH-Asn), 7.91 (d, 1H, $J_{NH,H2} = 9.0$ Hz, NH), 7.88 (d, 2H, $J_{H4,H3\,=\,H5,H6} = 7.5$ Hz, H4-, H5-Fmoc), 7.69 (d, 3H, $J_{H1,H2\,=\,H8,H7} = 7.768$ Hz, H1-, H8-Fmoc), 7.40 (t, 2H, $J_{H3,H2/4\,=\,H6,H5/7} =$ 7.4 Hz, H3-,H6-Fmoc), 7.36–7.14 (m, 27H, H2-, H7-Fmoc, $H_{arom.}$), 4.98 (d, 1H, $J_{1'',2''} = 3.3$ Hz, H-1″), 4.89 (t, 1H, $J_{1,2} = 9.0$ Hz, $J_{1,NH} = 9.1$ Hz, H-1), 4.69–4.60 (m, 5H, CH_2Ph, H-5″a, α-CH-Asn), 4.54–4.42 (m, 8H, H-1′, CH_2Ph), 4.33–4.20 (m, 4H, CH_2Ph, Fmoc-CH_2, H9-Fmoc), 4.01–3.72 (m, 8H, H-3″, H-4″, H-4′, H-3, H-3′, H-6′a, H-2″, H-2), 3.68–3.47 (m, 7H, H-6a, H-4, H-6b, H-6′b, H-5′, H-5″e, H-2′), 3.28 (m, 1H, H-5), 2.56 (d, 1H, J_{gem} 15.7 Hz, β-CH_2-Asn), 2.42 (d, 1H, $J_{gem} = 15.4$ Hz, β-CH_2-Asn), 1.73 (s, 3H, CH_3(NHAc)); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 171.11 (CO-ester), 169.60, 169.11 (CO-amide, -NHAc), 155.82 (CO-urethane), 143.75 (C1a-, C8a-Fmoc), 140.71 (C4a-, C5a-Fmoc), 138.98, 138.91, 138.65, 138.62, 138.18 (C_{ipso}-aryl), 132.83 (All-C2), 128.23, 128.14, 128.11, 127.66, 127.57, 127.05, 127.44, 127.08, 127.04 ($C_{arom.}$), 127.59 (C3-, C6-Fmoc), 127.21 (C2-, C7-Fmoc), 125.31 (C1-, C8-Fmoc), 120.10 (C4, C5-Fmoc), 102.54 (C-1′), 97.09 (C-1″), 79.14 (C-3′), 78.34 (C-1), 77.47 (C-3″), 76.32 (C-5), 75.97 (C-3), 75.58 (C-2″), 74.37 (C-4″), 73.18, 72.03, 70.78, 70.26, 70.15 (CH_2Ph), 72.62 (C-5′), 72.21 (C-4), 68.86 (C-2′), 68.44 (C-6), 67.40 (C-6′), 66.35 (C-4′), 65.38 (CH_2-Fmoc), 59.88 (C-5″), 54.56 (C-2), 50.19 (α-CH-Asn), 46.87 (C9-Fmoc), 36.82 (β-CH_2-Asn), 22.81 (CH_3(NHAc)); ESIMS calcd for $C_{73}H_{79}N_3O_{22}S$: 1382.4949 $[M+H]^+$. Found: 1383.4910 $[M+H]^+$.

3.11. Loading of NovaSyn TG® Amino Resin (HL) with 4-[2-(*N*-fluorenylmethoxycarbonyl-L-isoleucyloxy)-1-(trimethylsilyl)-ethyl]-phenoxy-acetic acid (19)

NovaSyn Tentagel Amino Resin HL® (2.7 g) was placed in a *Merrifield* solid-phase reactor and swollen in CH_2Cl_2 (20 mL) for 1 h. In a separate flask, 4-[2-(*N*-fluorenylmethoxycarbonyl-L-isoleucyloxy)-1-(trimethylsilyl)-ethyl]-phenoxyacetic acid **18** (561 mg, 0.93 mmol) was dissolved in CH_2Cl_2 (40 mL) and DMF (5 mL) and 127 mg of HOAT, 297 mg of HBTU and 0.21 mL *N*-methylmorpholine (NMM) were added. After 20 min, the solution was added to the resin via a cannula and shaken for 14 h. The solution was filtered off, the loaded polymer washed with DMF (5×10 mL) and CH_2Cl_2 (5×10 mL) and then dried in high vacuum. Yield: 3.07 g. Photometric determination of resin loading gave $c = 0.3$ mmol/g (this corresponds to a yield of 87%).

3.12. $^{\alpha}$*N*-Acetyl-L-isoleucinyl-L-valinyl-L-glycyl-N^{ω}-(2-acetamido-3-*O*-[6-*O*-benzyl-3-sulfatyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-(2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-leucyl-(3-*O*-benzyl)-L-threonyl-(5-*O*-benzyl)-L-glutamyl-L-leucyl-(5-*O*-benzyl)-L-glutamyl-(3-*O*-benzyl)-L-seryl-(5-*O*-benzyl)-L-glutamyl-(4-*O*-benzyl)-L-aspariginyl-L-isoleucine (21)

Resin **19** loaded with Fmoc isoleucine (0.5 g, 0.15 mM) was placed in a solid-phase reactor. The Fmoc group was removed with 30% piperidine in NMP. For sequential coupling reactions, Fmoc amino acids were used in an excess of 10 equiv and activated with HBTU/HOBt/*i*-Pr_2NEt in DMF/NMP 1:1 (4 mL) for 20 min. After each coupling, the resin was treated with 0.5 M Ac_2O, 0.125 M *i*-Pr_2NEt and 0.015 M HOBt for capping. The sulfated glycosyl amino acid **17** was coupled in an excess of 1.65 equiv and HATU/HOAt (10 equiv) as the coupling reagent. The coupling time was extended to 3 h. The final three Fmoc amino acids were coupled according to the standard protocol, and the glycopeptide was acetylated at the N-terminus to give **20**. Cleavage from the polymer support was achieved by shaking **20** with 40 mg (0.15 mmol, 1 equiv) of TBAF·$3H_2O$ in CH_2Cl_2 (20 mL) for 20 min at rt. This procedure was repeated once, and the collected organic solvents were washed twice with 50 mL of H_2O, dried with $MgSO_4$, concentrated in vacuo and the residue purified by preparative TLC (5:1, $CHCl_3/CH_3OH$) and ion exchange chromatography on Amberlite® CG120 (Na^+-form) (25 g; 5:1, $CHCl_3/CH_3OH$). Yield: 70 mg (0.023 mmol, 15%); amorphous: $R_f = 0.30$ (5:1, $CHCl_3/CH_3OH$). MALDI-TOF-MS (dhb, *negative ion mode*): calcd for $C_{154}H_{201}N_{15}O_{43}S$: 3042.43. Found: 3099.80 $[M-3H+Na+K]^-$. The compound retains tetrabutylammonium cations even after ion exchange chromatography.

3.13. $^{\alpha}$*N*-Acetyl-L-isoleucinyl-L-valinyl-L-glycyl-(2-acetamido-3-*O*-[3-sulfatyl-β-D-galactopyranosyl]-4-*O*-[β-D-arabinopyranosyl]-[2-deoxy-β-D-glucopyranosyl]-L-asparaginyl-L-leucyl-L-threonyl-L-glutamyl-L-leucyl-L-glutamyl-L-seryl-L-glutamyl-L-aspariginyl-L-isoleucine (22)

To 70 mg (0.023 mmol) of glycotridecapeptide **21** in CH_3OH/dioxane (10 mL, 1:1), 20 mg $Pd(OH)_2$ on charcoal was added. Hydrogen was bubbled through the solution until complete consumption of starting material (3 d) was detected by TLC. The solution was filtered through Hyflo-Supercel® and washed with CH_3OH (50 mL) and *i*-PrOH (50 mL). After evaporation of solvents, the product was purified by preparative RP-TLC with CH_3CN/CH_3OH (2:1). Yield: 48 mg (0.023 mmol, quant.); $[\alpha]_D^{22}$ −17.2 (*c* 0.5, DMSO); amorphous; $R_f = 0.20$–0.35 (RP-C18; 2:1, CH_3CN/CH_3OH); MALDI-TOF-MS (dhb, *negative ion mode*): calcd for $C_{82}H_{133}N_{15}O_{43}S$: 2049.85. Found: 2087.98 $[M-2H+K]^-$. The glycopeptide containing six acidic functions retains tetrabutylammonium cations obviously as a mixture of ammonium, sodium salts and free acids. Its complex NMR spectrum therefore shows broad signals.

3.14. *N*-Fluorenylmethoxycarbonyl-N^{ω}-(2-acetamido-3-*O*-[6-*O*-benzyl-β-D-galactopyranosyl]-4-*O*-[2,3,4-tri-*O*-benzyl-β-D-arabinopyranosyl]-6-*O*-benzyl-(2-deoxy-β-D-glucopyranosyl)-L-asparaginyl-L-leucyl-(3-*O*-benzyl)-L-threonyl-(5-*O*-benzyl)-L-glutamyl-L-leucyl-(5-*O*-benzyl)-L-glutamyl-(3-*O*-benzyl)-L-seryl-(5-*O*-benzyl)-L-glutamyl-(4-*O*-benzyl)-L-aspariginyl-L-isoleucine (24)

Resin **19** loaded with Fmoc isoleucine (0.165 g, 0.05 mM) was placed in a solid-phase reactor. The Fmoc group was removed with 30% piperidine in NMP. For sequential coupling reactions, Fmoc amino acids were used in an excess of 10 equiv and activated with HBTU (10 equiv)/HOBt (10 equiv)/*i*-Pr_2NEt (20 equiv) in DMF/NMP 1:1 (4 mL) for 20 min. After each coupling, the resin was treated with 0.5 M Ac_2O, 0.125 M *i*-Pr_2NEt and 0.015 M HOBt for capping. The glycosyl amino acid **15** was coupled in an excess of 1.65 equiv and HATU/HOAt (10 equiv) as the coupling reagent. The coupling time was extended to 3 h to give **23**. Cleavage from the polymer support was achieved by shaking of **23** with 38 mg (0.12 mmol, 2.4 equiv) of TBAF·$3H_2O$ in CH_2Cl_2 (20 mL) for 20 min at rt. This procedure was repeated once with 15 mg (0.9 equiv) TBAF·$3H_2O$. The polymer was washed with CH_2Cl_2 (3×10 mL), and the collected organic solvents were washed twice with H_2O (50 mL), dried with $MgSO_4$, concentrated in vacuo and the residue was purified by preparative TLC (15:1, $CHCl_3/CH_3OH$). Yield: 62 mg (0.022 mmol, 43%); amorphous: $R_f = 0.43$ (5:1, $CHCl_3/CH_3OH$). 15:1); $[\alpha]_D^{22}$ −74.9 (*c* 1, DMSO); ^1H NMR (DMSO-d_6, 400 MHz): δ 8.37–8.25 (m, 2H, NH-urethane, $^{\omega}$NH),

8.13–7.95 (m, 8H, NH), 7.86 (d, 2H, $J_{H4,H3=H5,H6} = 7.4$ Hz, H4-, H5-Fmoc), 7.68 (m, 2H, H1, H8-Fmoc), 7.40–7.15 (m, 59H, $H_{arom.}$, H3-, H6-, H2-, H7-Fmoc), 5.03–5.00 (m, 2H, H-1″, H-1), 4.71–4.66 (m, 3H, CH_2Ph, α-CH-Asn, H-5″e), 4.65–4.25 (m, 16H, CH_2Ph, CH_2-Fmoc, α-CH-(Glu, Ser, Thr, Asn, Leu)), 4.20–4.12 (m, 3H, H9-Fmoc, α-CH-Ile, CH_2-Fmoc), 4.09–3.73 (m, 7H, H-3″, H-4″, H-6′a, H-3, β-CH-Thr, H-2″, H-2), 3.61–3.39 (m, 9H, H-4, H-5′, H-4′, H6a/b, H-6′b, H-5″a, β-CH_2-Ser), 2.81–2.75 (m, 1H, β-CH_2-Asn), 2.59–2.52 (m, 1H, β-CH_2-Asn), 2.39–2.31 (m, 8H, γ-CH_2-Glu, β-CH_2-Asp), 1.96–1.74 (m, 10H, β-CH_2-Glu, β-CH-Ile, CH_3(NHAc)), 1.01–0.71 (m, 27H, γ-CH_3-Thr, δ-CH_3-Leu, γ-CH_3-Ile, δ-CH_3-Ile); ^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 173.08 (CO-acid), 172.19 (br s, CO-amide), 170.70, 169.97, 169.78, 169.12, 156.36 (CO-urethane), 143.75 (C1a-, C8a-Fmoc), 140.64 (C4a-, C5a-Fmoc), 138.99, 138.87, 138.61, 138.46, 138.09, 137.99 (C_{ipso}-aryl), 128.34, 128.10, 128.01, 127.87, 127.83, 127.60, 127.52, 127.46, 127.40, 127.30, 127.21, 127.13, 127.06, 126.98 (C3-, C6-, C2-, C7-Fmoc, $C_{arom.}$), 125.10 (C1-, C8-Fmoc), 120.06 (C4-, C5-Fmoc), 101.84 (C-1′), 97.33 (C-1″), 79.01 (C-1), 73.29 (C-3″), 76.47 (C-3, C-5), 75.82 (C-2″), 74.85 (β-CH_2-Thr), 74.61 (C-4″), 73.32 (C-3′), 73.32, 72.13, 70.44, 70.73 (CH_2Ph), 72.84 (C-5′), 72.35 (C-4), 70.50 (C-2′), 68.80 (C-6), 68.08 (C-4′), 67.59 (C-6′), 67.82 (CH_2-Fmoc), 60.57 (C-5″), 57.82 (β-CH_2-Ser), 56.28 (α-CH-Ile), 54.60 (C-2), 52.87 (α-CH-Glu), 51.66 (α-CH-Asn), 51.22 (α-CH-Thr), 50.94 (α-CH-Leu), 49.23 (α-CH-Asp), 46.59 (C9-Fmoc), 40.63 (β-CH_2-Leu), 37.99 (β-CH_2-Asp), 36.20 (β-CH-Ile), 35.25 (β-CH_2-Asn), 30.12 (δ-CH_2-Glu), 27.78 (β-CH_2-Glu), 24.81 (γ-CH_2-Ile), 23.04 (γ-CH-Leu), 23.01, 21.35 (δ-CH_3-Leu), 16.33 (γ-CH_3-Thr), 15.92 (γ-CH_3-Ile), 11.11 (δ-CH_3-Ile); MALDI-TOF-MS (dhb, positive *ion mode*): calcd for $C_{154}H_{186}N_{12}O_{38}$: 2873.23. Found: 2896.11 $[M+Na]^+$.

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