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(*p*-Sulfomethyl)phenylalanine as a mimic of *O*-sulfatyl-tyrosine in synthetic partial sequences of P-Selectin glycoprotein ligand 1 (PSGL-1)

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Abstract—Fmoc-L-(*p*-sulfomethyl)phenylalanine, a bioisosteric mimic of acid-sensitive *O*-sulfatyl tyrosine, was synthesized from L-tyrosine according to a novel route. Partial sequences of the recognition site of P-Selectin glycoprotein ligand **1** (PSGL-1), which contain (sulfomethyl)phenylalanine were synthesized on solid-phase. By fragment condensation, a sialyl Lewis^x peptide conjugate containing a (sulfomethyl)-phenylalanine mimic of *O*-sulfatyl tyrosine was prepared without destruction of the acid-sensitive fucoside bond within the saccharide side chain. Compounds of this type are of interest as sufficiently acid-stable potential inhibitors of P-Selectin in inflammatory processes.

1. Introduction

The recruitment of leukocytes into inflamed tissues is directed by an intercellular communication cascade between membrane-located cell adhesion molecules and their ligands expressed on endothelial cells or leukocytes. Carbohydrate-recognizing Selectins constitute an important class of cell adhesion molecules.[1] At the site of inflammation, signal molecules (chemokines) are produced, which stimulate endothelial cells of venules in the neighborhood to expose P-Selectin[2] and E-Selectin[3] on their luminal surface.[4] Few minutes after the stimulation, P-Selectin is presented on the endothelial surface.[5] It is a long protein extending its carbohydrate recognition domain about 40 nm above the cell surface.[6] Its natural ligand on the leukocytes is P-Selectin glycoprotein ligand **1** (PSGL-1), a disulfide-linked homodimer.[7] A 19 amino acid sequence in the N-terminus has been identified as the binding site to P-Selectin.[8] It involves an *O*-glycan side chain terminating in sialyl Lewis^x[9] and three tyrosines (Tyr^{46}, Tyr^{48}, and Tyr^{51}). At least one of the tyrosines must be *O*-sulfated to guarantee a strong binding to P-Selectin.[10] A crystal structure analysis of a complex of PSGL-1 and the carbohydrate recognition domain of P-Selectin confirmed these requirements for binding.[11] Glycopeptide partial structures of PSGL-1 containing this recognition site have been synthesized by solid-phase synthesis of the peptide sequence, subsequent enzymatic glycan formation and sulfation of the tyrosines.[12] These compounds showed particularly strong binding to P-Selectin. A chemoenzymatic synthesis of such PSGL-1 substructures containing *O*-sulfatyl tyrosine and the core 6 saccharide not very sensitive to acids has also been reported.[13] However, the chemical synthesis of such molecules with sialyl Lewis^x side chains has not been achieved so far because of the acid-sensitivity of the tyrosine *O*-sulfate structure[14] and the high acid-sensitivity of the fucoside bond within the sialyl-Lewis^x saccharide.[15] In order to overcome these problems caused by the acid-sensitivity, the synthesis of glycopeptide partial structures of the recognition site of PSGL-1 containing (sulfomethyl)phenylalanine as a mimic of *O*-sulfatyl tyrosine has been investigated.

2. Results and discussion

The preparation of (*p*-sulfomethyl)-phenylalanine can be achieved according to the procedure described by Roques et al.[14] Starting from *p*-methyl-benzonitrile, *N*-acetyl-D,L-(*p*-hydroxymethyl)phenylalanine **1** was synthesized in five steps.[14] Enzymatic deacetylation using pig-kidney acylase I[16] gave L-(*p*-hydroxymethyl)phenylalanine **2** in a yield of 54%. Brenner esterification[17] of **2** with thionyl chloride in methanol gave the methyl ester **3**, which was *N*-acylated with 9-fluorenyl-succinimidyl-carbonate[18] (Fmoc–ONSu) to furnish Fmoc protected (*p*-hydroxylmethyl)phenylalanine **4**.

While **4** did not react with triphenylphosphine and tetrabromomethane under Appel conditions, the *p*-bromomethyl-phenylalanine derivative **5** was formed by treatment of **4**

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with NBS/triphenylphosphine. Nucleophilic substitution with sodium sulfite quantitatively gave the (sulfomethyl)-phenylalanine derivative **6**. Due to the high polarity of **6**, chromatographic purification turned out impossible. Therefore, the sodium salt of **6** was extracted with ethanol from the mixture. Finally, the methyl ester was hydrolyzed by enzymatic catalysis with subtilisin to give the Fmoc–(*p*-sulfomethyl)phenylalanine **7** useful for application to solid-phase synthesis according to Fmoc strategy.

The synthesis described in Scheme 1 has the disadvantage that 50% of compound **1** is lost during separation of enantiomers, which itself needs a long reaction time.

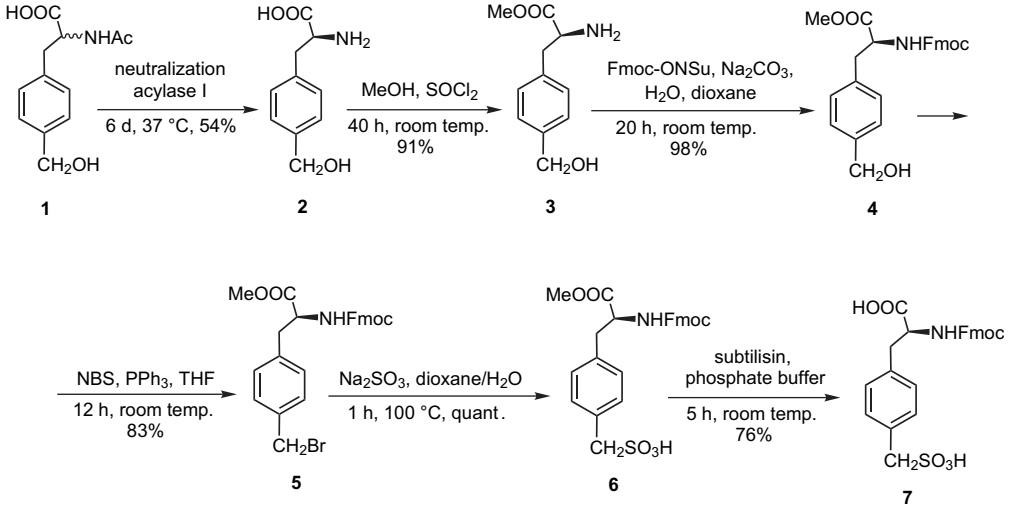
An alternative synthesis involving the chloromethylation of *N*-acetyl-L-phenylalanine[19] did not work in our hands. Therefore, a synthesis of **7** starting from Boc-L-*O*-trifluoromethanesulfonyl-tyrosine methyl ester[20] **8** was developed (Scheme 2). Nickel(0) catalyzed reaction[21] of **8** with potassium cyanide furnished Boc(*p*-cyano)phenylalanine ester **9** (Scheme 2). Hydrogenation of **9** with Raney nickel afforded the corresponding benyzlamine derivative, which after treatment with sodium nitrite gave the Boc-L-(*p*-hydroxymethyl)phenylalanine methyl ester **10**.

As described for the analogous Fmoc derivative **4**, **10** was converted via the bromomethyl derivative **11** into the (sulfomethyl)phenylalanine **12**. Hydrolysis of the methyl ester, cleavage of the Boc group with trifluormethanesulfonic acid in dichloromethane and introduction of the Fmoc group[18] gave **7** of identical data as the one synthesized according to Scheme 1.

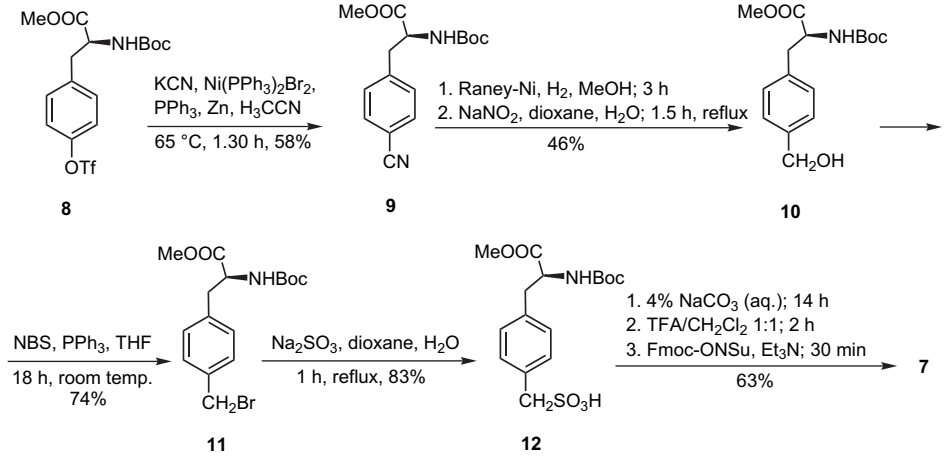
The still long way to the (*p*-sulfomethyl)phenylalanine building block **7** can be shortened by transforming Fmoc tyrosine methyl ester to its *O*-triflate **13** (Scheme 3).

In analogy to a reaction on the corresponding *N*-Boc derivative,[22] **13** was subjected to a palladium(0)-catalyzed reductive carbonylation reaction to give (*p*-formyl)phenylalanine **14** (Scheme 3). Reduction with sodium borohydride furnished **4** (see, Scheme 1) in good yield.

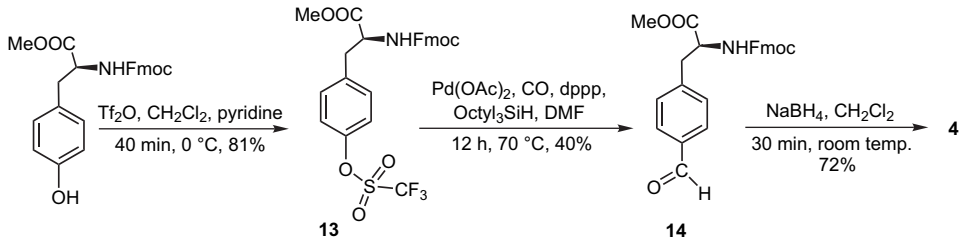
In order to explore the properties of the Fmoc–(*p*-sulfomethyl)phenylalanine building block in solid-phase syntheses, the octapeptide sequence Ala[43]-Asp[50] of PSGL-1 was assembled. To this end, aminomethyl polystyrene resin equipped with the Wang anchor[23] was loaded with Fmoc–Asp(OBn)–OH using 1-mesitylenesulfonyl-3-nitro-^{1}H-



Scheme 1.



Scheme 2.



Scheme 3.

1,2,4-triazole (MSNT)[24] as the condensing reagent to give the starting resin **15** (Scheme 4). The solid-phase synthesis was carried out in a Merrifield reaction vessel equipped with a frit and a valve at the bottom. Coupling reactions were performed using 2-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)[25] and 1-hydroxy-benzotriazol (HOBt). The Fmoc groups were removed with morpholine/dimethylformamide without affecting side-chain benzylester functions. Photometric determination of *N*-fluorenyl morpholine at λ=300.5 nm on analytical probes served for monitoring of the coupling reactions. After each coupling step, unreacted amino groups were capped using acetic anhydride/pyridine in order to prevent the formation of failure sequences during the continued solid-phase synthesis.

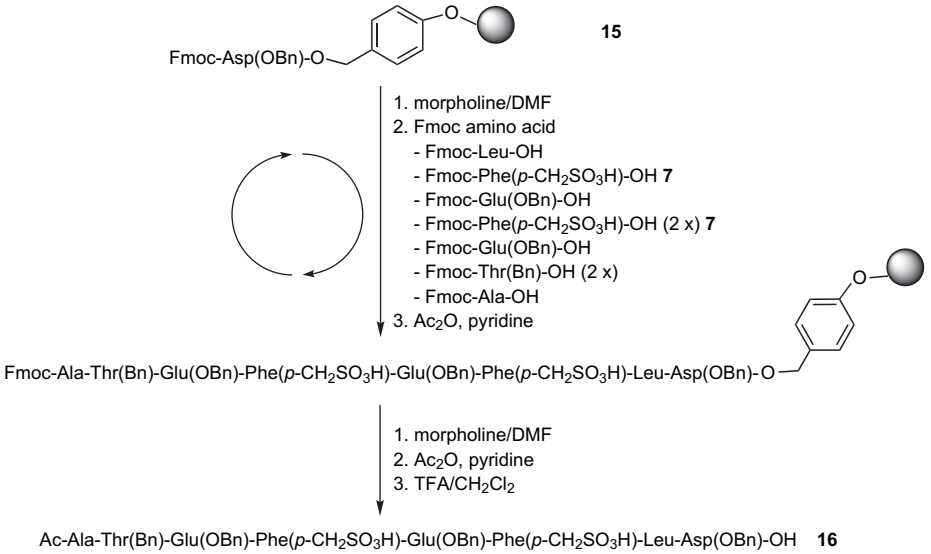
Coupling of the first three amino acids proceeded smoothly, while coupling of the fifth amino acid (Fmoc–Phe(*p*-CH_2SO_3H)–OH to H–Glu(OBn)–) even after repetition resulted in a reduced yield of less than 50%. In addition, coupling of the seventh amino acid Fmoc–Thr(Bn)–OH (again double coupling) only gave a yield of 34% (according to photometric monitoring, see above). In contrast, the eighth amino acid Fmoc–Ala–OH was coupled in almost quantitative yield. After Fmoc removal and N-acetylation, detachment of the target peptide **16** selectively deprotected at the C-terminal carboxy group was achieved with TFA/dichloromethane. Purification of **16** turned out difficult even by preparative RP-HPLC in acetonitrile/water. Only the use of a buffered eluant (acetonitrile/0.1 ammonium acetate) resulted in the separation of the mixture by RP-HPLC. The target substance **16** was identified by MALDI mass spectrometry. Due to the two incomplete coupling reaction and the difficult purification, only 5.5% of pure **16** was isolated.

In the solid-phase synthesis of hexapeptide sequence Phe[53]-Glu[58] of PSGL-1 containing a spacer-equipped asparagine instead of Thr[57] as the linking site for a sialyl Lewis^x saccharide, the Rink amide linker[26] was used (Scheme 5).

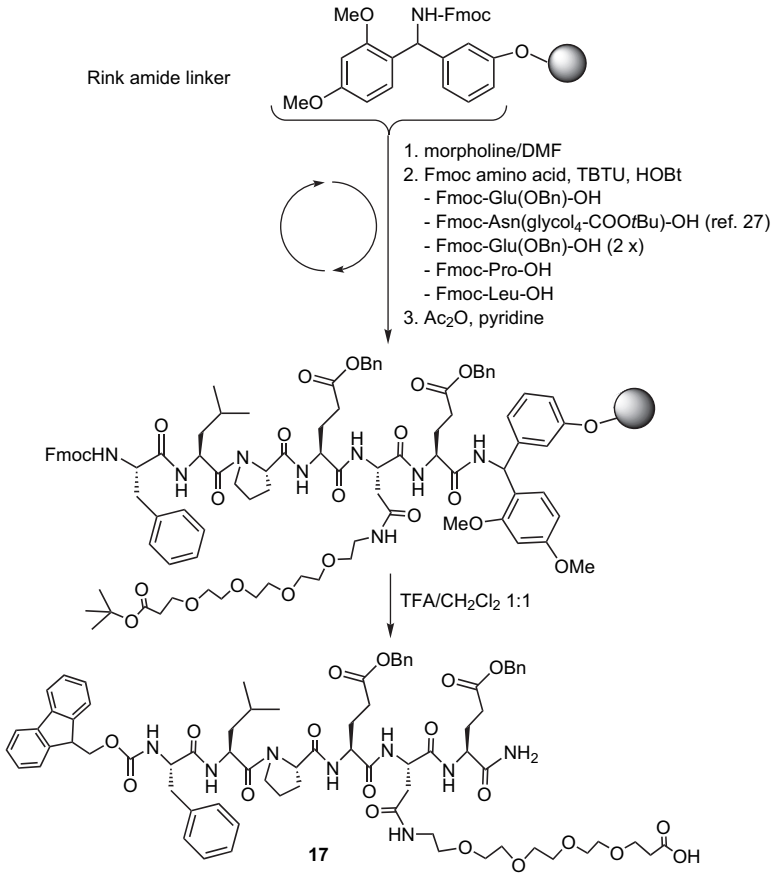
The synthesis was carried out in analogy to the one shown in Scheme 4 and gave the Fmoc protected hexapeptide amide **17** after acidolytic cleavage of the Rink linker and simultaneous cleavage of the spacer-side-chain *tert*-butyl ester. Purification by RP-HPLC gave **17** in an overall yield of 45%. Monitoring of the solid-phase synthesis had revealed that the coupling of proline to Glu(OBn) remained incomplete, what suggests that some formation of pyroglutamate had taken place.

Condensation of the spacer carboxylic function with freshly prepared sialyl Lewis^x amine **18** according to a procedure efficient in syntheses of spacer-separated sialyl Lewis^x cyclopeptides[27] gave sialyl-Lewis^x peptide conjugate **19** (Scheme 6).

For peptide chain extension on **19**, a dipeptide was prepared from aspartic acid α-*tert*-butyl β-benzyl diester **20** and Fmoc (*p*-sulfomethyl)phenylalanine **7** using benzotriazol-1-yloxy-tris-pyrrolidinophosphonium hexafluorophosphate



Scheme 4.

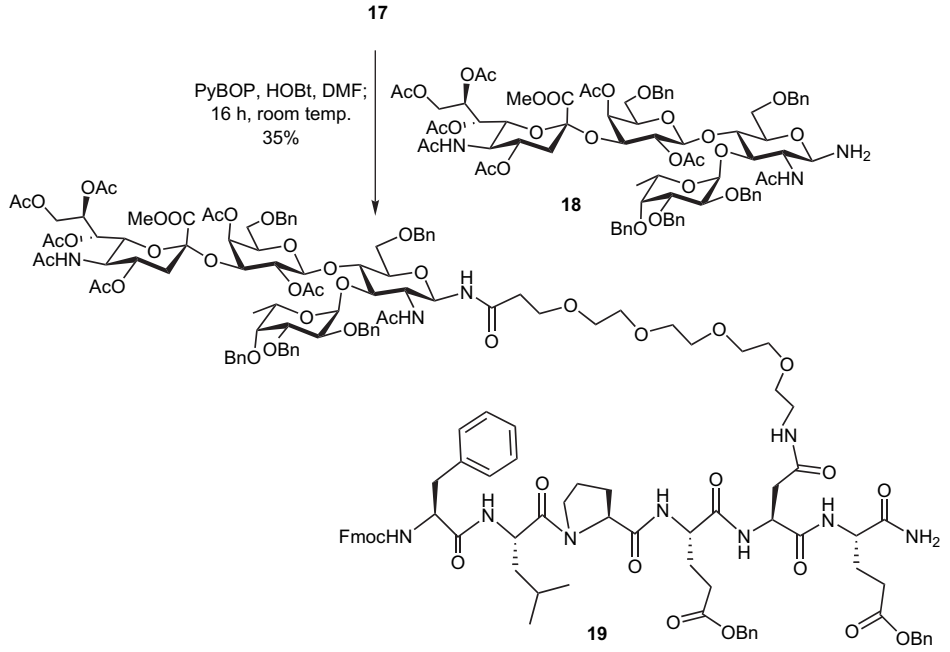


Scheme 5.

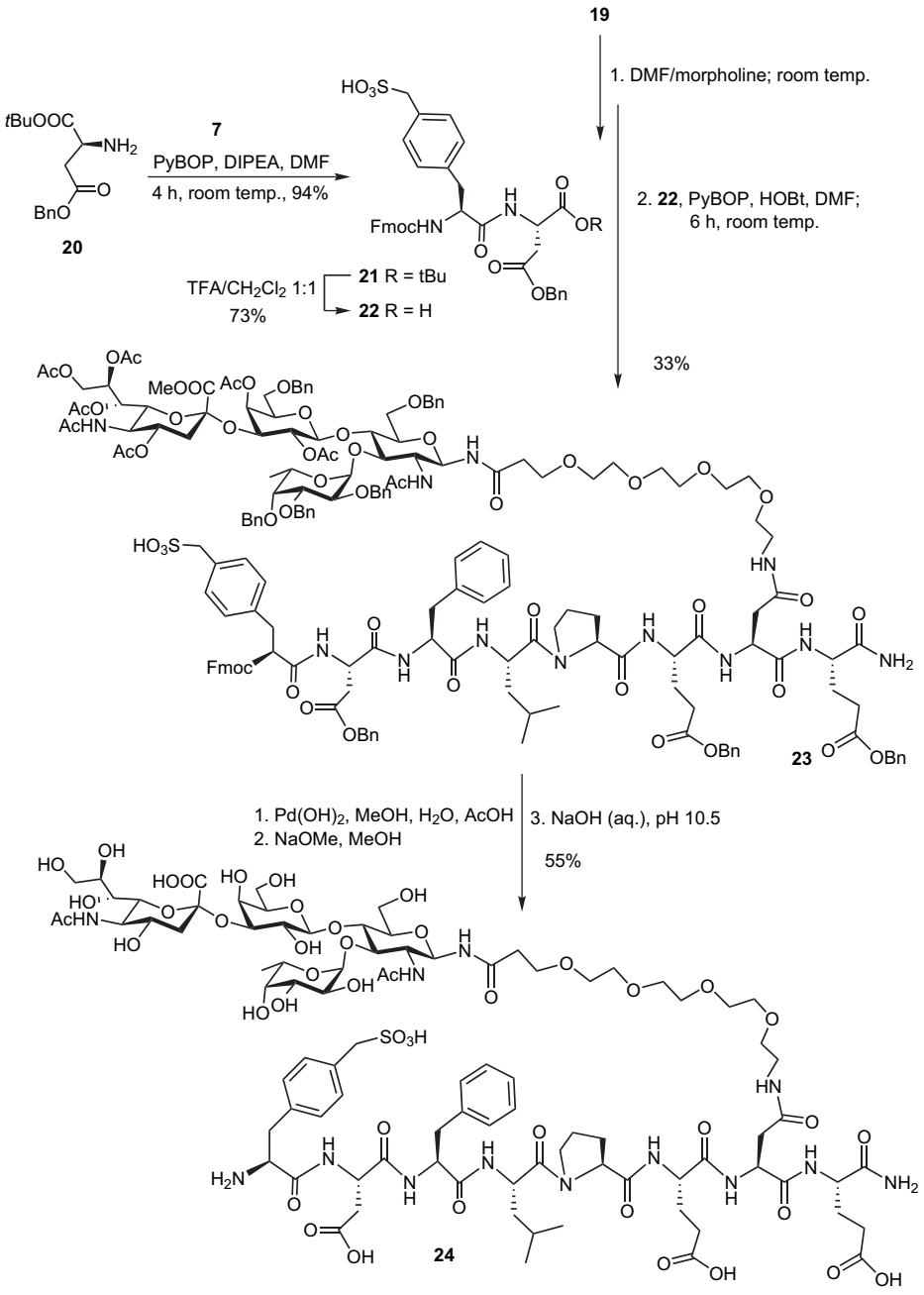
(PyBOB)[28] as the condensing reagent. Subsequent cleavage of the *tert*-butyl ester of **21** gave the carboxy-deblocked building block **22** (Scheme 7).

Removal of the Fmoc group from sialyl Lewis^x peptide conjugate **19** followed by coupling of the Fmoc dipeptide **22** again using PyBOP furnished the glycooctapeptide conjugate **23**.

Successive removal of the protecting groups by hydrogenation of the Fmoc and benzyl groups, Zemplén transesterification, mild saponification of the sialic acid methyl ester,[29] and



Scheme 6.



Scheme 7.

neutralization with solid CO_2 gave the free sialyl Lewis^x peptide conjugate **24** containing (*p*-sulfomethyl)phenylalanine as a mimic of tyrosine sulfate (Scheme 7).

Purification of **24** was achieved by gelpermation chromatography through a Sephadex LH15 column. It turned out that the coupling of **19** and **22** remained incomplete. Almost 50% of **23** was recollected. Monitoring of the condensation reaction was difficult because the amino component **23** and the product **24** showed very similar behavior in TLC analysis.

3. Conclusion

Although optimization of the described processes certainly will afford higher yield, the strategy applied in the synthesis of **24** provides access to PSGL-1 glycopeptide partial sequences, which contain the very acid-sensitive fucoside bond as well as the *p*-(sulfomethyl)phenylalanine as an acidic, but acid-stable mimic of *O*-sulfatyl tyrosine. These types of molecules are considered as sufficiently stable ligands of P-Selectin.

4. Experimental

4.1. General

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 heating for

visualization. Silica gel 60 (particle size 0.04–0.063 mm, E. Merck, Darmstadt, Germany) was used for flash-chromatography, silica particle size 0.063–0.2 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 homogenous materials. Analytical RP-HPLC was performed on a Knauer HPLC system with a Phenomenex LUNA C_{18} (5 μ column, 250×4.6 mm), flow rate 1 mL/min, solvent: (CH_3CN/H_2O+0.1% TFA); semi-preparative (flow rate10 mL/min) and preparative HPLC (flow 20 mL/min) were carried out on a Knauer HPLC equipment with columns specified for the different compounds.

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), and m (multiplet). Indication of ^1H 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-cyano cinnamic acid (cca) as the matrix) or a Finnigan MAT 95 (FD and FAB) instrument. Optical rotation values were recorded using a Perkin Elmer 241 polarimeter. Elemental analyses were performed by the microanalytical laboratory of the Institut fuer Organische Chemie, Universitaet Mainz.

4.1.1. L-(*p*-Hydroxymethyl)phenylalanine 2. A solution of racemic *N*-acetyl-(*p*-hydroxyethyl)phenylalanine[14b] **1** (20.0 g, 102 mmol) in 250 mL of water was degassed in vacuo, and neutralized under an argon atmosphere by dropwise addition of 0.5 N KOH. After addition of acylase I[16] (50 mg, 2000–3000 units/mg), the solution was shaken at 37 °C for 2 d and neutralized. Another 20 mg of the acylase was added, and the shaking was continued for 24 h. This procedure was repeated twice. After an overall reaction time of 6 d, the solution was acidified with 1 N HCl and heated with 200 mg of charcoal to 70 °C for 30 min. After filtration the solution was concentrated to a volume of 200 mL and passed through an ion-exchange column (Dowex 50 W-X8, 20–50 mesh), which was washed with water until the pH of the eluate was 6.5. The product **2** was then eluted using 1 N aq ammonia solution. This eluate was evaporated to dryness. The remaining crude product was dissolved in boiling methanol (150 mL) by dropwise addition of water. Dioxane (50 mL) was added. Cooling in an ice-bath resulted in the precipitation of crystalline **2**. Yield: 5.3 g (colorless crystals); mp 232 °C; $[\alpha]_D^{23}$ −28.1 (*c* 1, H_2O); R_f=0.36 ($CHCl_3$/MeOH/AcOH 5:5:0.5). ^1H NMR (200 MHz, D_2O), δ 2.99 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=14.7 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=7.8 Hz, H-$\beta_a$); 3.16 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=14.6 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=4.9 Hz, H-$\beta_b$); 3.86 (dd, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a}$=7.8 Hz, $J_{H\text{-}\alpha,H\text{-}\beta b}$=4.9 Hz, H-α); 4.50 (s, 2H, –CH_2OH); 7.18 (d, 2H, J_{vic}=8.3 Hz, H–Ar); 7.27 (d, J_{vic}=8.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, D_2O), δ 36.15 (C-β); 56.21 (C-α); 63.85 (–CH_2OH); 128.81, 130.32 (C–Ar); 135.26 (C-*ipso*$_{C\text{-}\beta}$); 140.26 (C-*ipso*$_{-CH2OH}$); 174.84 (–*C*OOH). Anal. Calcd for $C_{10}H_{13}NO_3$ (195.22): C, 61.53; H, 6.71; N, 7.17. Found: C, 61.48; H, 6.74; N, 7.29.

4.1.2. L-(*p*-Hydroxymethyl)phenylalanine methyl esterhydrochloride 3. Thionyl chloride (2.30 mL, 30.80 mmol) was added dropwise to dry methanol (35 mL) at −10 °C. To the solution L-Phe(*p*-CH_2OH)–OH **2** (5.10 g, 26.12 mmol) was added, and the suspension was stirred at room temperature for 40 h giving a clear colorless solution. The solvent was evaporated in vacuo; traces of thionyl chloride were removed by co-distillation with methanol. The residue was dissolved in boiling methanol (25 mL) and the solution cooled to 0 °C. The amino acid esterhydrochloride **3** precipitated as colorless crystals by dropwise addition of diethyl ether (50 mL). Yield: 5.85 g (91%); mp 166 °C; R_f=0.60 (CH_2Cl_2/MeOH/AcOH 5:5:0.5); $[\alpha]_D^{22}$ 12.9 (*c* 1, MeOH). ^1H NMR (200 MHz, CD_3OD), δ 3.12–3.33 (m, 2H, H-β_a, H-β_b); 3.36 (t, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a} \approx J_{H\text{-}\alpha,H\text{-}\beta b}$=6.6 Hz, H-α); 4.64 (s, 2H, –C*H*$_2$OH); 7.28 (d, 2H, J_{vic}=8.3 Hz, H–Ar); 7.41 (d, J_{vic}=7.8 Hz, H–Ar). ^{13}C NMR (50.3 MHz, CD_3OD), δ 37.30 (C-β); 53.88 (*C*H_3–O–); 55.54 (C-α); 65.02 (–*C*H_2OH); 128.98, 130.75 (C–Ar); 134.47 (C-*ipso*$_{C\text{-}\beta}$); 142.74 (C-*ipso*$_{-CH2OH}$); 170.67 (–*C*OOH). Anal. Calcd for $C_{11}H_{15}NO_3 \cdot HCl$ (245.71): C, 51.87; H, 7.12; N, 5.50. Found: C, 52.15; H, 7.05; N, 5.63.

4.1.3. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-hydroxymethyl)phenylalanine methyl ester 4. To a solution of **3** (5.80 g, 23.61 mmol) in dioxane (22 mL) and 46.5 mL of 10% aq Na_2CO_3 at 0 °C, a solution of Fmoc–ONSu[18] (8.77 g, 26.00 mmol) in 45 mL of dioxane was added dropwise. While warming up to room temperature, the solution was stirred for 20 h and then diluted with ice-water (300 mL). After extraction with diethyl ether (three times 150 mL) and drying with $MgSO_4$, the solvent was evaporated in vacuo. The residue was dissolved in boiling ethyl acetate (80 mL), and the product **4** was precipitated by dropwise addition of light petroleum ether (300 mL). Yield: 9.96 g (98%); colorless crystals; mp 124 °C; R_f=0.36 (light petroleum ether/ethyl acetate 1:1); $[\alpha]_D^{24}$ 38.3 (*c* 1, $CHCl_3$). ^1H NMR (200 MHz, $CDCl_3$), δ 3.05 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=13.7 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=5.9 Hz, H-$\beta_a$); 3.14 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=13.7 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=5.4 Hz, H-$\beta_b$); 3.72 (s, 3H, –O–C*H*$_3$); 4.18 (t, 1H, J_{vic}=6.8 Hz, H-9 Fmoc); 4.32 (dd, 1H, J_{vic}=6.5 Hz, J_{gem}=10.5 Hz, –C*H*$_2$–Fmoc); 4.43 (dd, 1H, J_{vic}=7.0 Hz, J_{gem}=10.5 Hz, –C*H*$_2$–Fmoc); 4.63 (s, 2H, –C*H*$_2$OH); 4.64–4.71 (m, 1H, H-α); 5.26 (d, 1H, $J_{NH,H\text{-}\alpha}$=6.4 Hz, –NH–); 7.05 (d, 2H, J_{gem}=7.8 Hz, H–Ar); 7.24–7.43 (m, 6H, H–Ar); 7.51–7.57 (m, 2H, H–Ar); 7.75 (d, J_{gem}=7.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, $CDCl_3$), δ 37.91 (C-β); 47.18 (C-9 Fmoc); 52.43 (*C*H_3–O–); 54.82 (C-α); 64.89 (–*C*H_2OH); 66.98 (–*C*H_2–Fmoc); 120.03 (C-4 Fmoc, C-5 Fmoc); 125.07, 127.10, 127.26, 127.77, 129.48 (C–Ar); 135.09 (C-*ipso*$_{C\text{-}\beta}$); 139.90 (C-*ipso*$_{-CH2OH}$); 141.33, 143.78, 143.84 (C-4a$_{Fmoc}$, C-4b$_{Fmoc}$, C-8a$_{Fmoc}$, C-9a$_{Fmoc}$); 155.65 (–O–CO–NH–); 171.99 (–*C*OOMe). EIMS: 454.3 (100%) $[M+Na]^+$, calcd: $C_{26}H_{25}NO_5$ 431.2.

4.1.4. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-bromomethyl)phenylalanine methyl ester 5. To a solution of **4** (9.70 g, 22.48 mmol) in 500 mL of dry tetrahydrofuran (THF) at 0 °C, triphenylphosphine (17.69 g, 67.44 mmol) was added. After stirring for 5 min *N*-bromo-succinimide (12.00 g, 67.44 mmol) was added, and the stirring was continued at room temperature for another 12 h. The precipitate (hygroscopic) was filtered off and immediately washed with

THF. The brownish filtrate was concentrated in vacuo to a volume of 80 mL, diluted with water (500 mL) and extracted with diethyl ether (five times 150 mL). The combined ether solutions were dried with $MgSO_4$, and the solvent was evaporated in vacuo. The crude product was purified by flash-chromatography in light petroleum ether/ethyl acetate (2:1) and subsequently re-crystallized from ethyl acetate/light petroleum ether. Yield: 9.22 g (83%), colorless crystals; mp 120 °C; R_f=0.45 (light petroleum ether/ethyl acetate 5:2); $[\alpha]_D^{22}$ 40.9 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$), δ 3.05 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=14.2 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$= 5.4 Hz, H-$\beta_a$); 3.14 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=14.2 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$= 5.9 Hz, H-$\beta_b$); 3.72 (s, 3H, –O–C$H_3$); 4.19 (t, 1H, J_{vic}=6.8 Hz, H-9 Fmoc); 4.31–4.48 (m, 2H, –CH_2–Fmoc); 4.45 (s, 2H, –CH_2Br); 4.65 (q, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a} \approx J_{H\text{-}\alpha,H\text{-}\beta b} \approx J_{H\text{-}\alpha,NH}$=5.9 Hz, H-α); 5.25 (d, 1H, $J_{NH,H\text{-}\alpha}$=8.3 Hz, –NH–); 7.04 (d, 2H, J_{gem}=7.8 Hz, H–Ar); 7.24–7.57 (m, 10H, H–Ar); 7.76 (d, J_{gem}=7.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, $CDCl_3$), δ 33.17 (–*C*H_2Br); 37.95 (C-β); 47.21 (C-9 Fmoc); 52.44 (*C*H_3–O–); 54.69 (C-α); 66.97 (–*C*H_2–Fmoc); 120.03 (C-4 Fmoc, C-5 Fmoc); 125.04, 127.10, 127.77, 129.30, 129.76 (C–Ar); 136.16, 136.69 (C-*ipso*$_{C\text{-}\beta}$, C-*ipso*$_{-CH2Br}$); 141.35, 143.76, 143.82 (C-4a$_{Fmoc}$, C-4b$_{Fmoc}$, C-8a$_{Fmoc}$, C-9a$_{Fmoc}$); 155.54 (–O–*C*O–NH–); 171.78 (–*C*OOMe). Anal. Calcd for $C_{26}H_{24}NO_4Br$ (494.40): C, 63.16; H, 4.89; N, 2.83. Found: C, 63.09; H, 4.81; N, 2.88.

4.1.5. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-sulfomethyl)phenylalanine methyl ester 6. To a stirred solution of **5** (2.90 g, 5.87 mmol) in dioxane (30 mL) was added a solution of Na_2SO_3 (3.70 g) in 30 mL of water. The mixture was gently refluxed for 1 h, the solvents were evaporated in vacuo, and the solid residue was dried in high vacuum. The powdered residue was extracted several times with dry ethanol (80 mL, each). After filtration, the combined solutions were evaporated in vacuo and the product **6** was purified by column chromatography in CH_2Cl_2/MeOH (6:1). Yield: 3.03 g (quant.), colorless crystals; mp 243 °C (decomp.); R_f=0.36 (CH_2Cl_2/MeOH 5:1); $[\alpha]_D^{22}$ −0.2 (*c* 0.5, MeOH). ^{1}H NMR (400 MHz, MeOD), δ 2.98 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=13.8 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=9.4 Hz, H-$\beta_a$); 3.17 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=13.5 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=5.3 Hz, H-$\beta_b$); 3.74 (s, 3H, –O–C$H_3$); 4.06 (s, 2H, –$CH_2SO_3H$); 4.19 (t, 1H, J_{vic}=6.9 Hz, H-9 Fmoc); 4.31 (d, 2H, J_{vic}=7.1 Hz, –CH_2–Fmoc); 4.65 (dd, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a}$=9.4 Hz, $J_{H\text{-}\alpha,H\text{-}\beta b}$=5.3 Hz, H-α); 7.21 (d, 2H, J_{gem}=7.9 Hz, H–Ar); 7.31–7.44 (m, 8H, H–Ar); 7.65 (d, J_{vic}=7.3 Hz, H–Ar); 7.83 (d, J_{vic}=7.6 Hz, H–Ar). ^{13}C NMR (50.3 MHz, MeOD), δ 35.95 (C-β); 46.49 (C-9 Fmoc); 51.90 (*C*H_3–O–); 55.54 (C-α); 57.07 (–*C*H_2SO_3H); 65.62 (–*C*H_2–Fmoc); 120.02 (C-4 Fmoc, C-5 Fmoc); 125.20, 127.00, 127.56, 128.20, 130.06 (C–Ar); 133.38, 135.30 (C-*ipso*$_{C\text{-}\beta}$, C-*ipso*$_{-CH2SO3H}$); 140.63, 143.61, 143.76 (C-4a$_{Fmoc}$, C-4b$_{Fmoc}$, C-8a$_{Fmoc}$, C-9a$_{Fmoc}$); 155.87 (–O–*C*O–NH–); 172.32 (–*C*OOMe). MALDIMS (dhb): *m*/*z*=518.1 $[M+Na]^+$, 540.1 $[M-H+2Na]^+$, 1057.9 $[M-2H+3Na]^+$, 1575.3 $[3M-3H+4Na]^+$.

4.1.6. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-sulfomethyl)phenylalanine 7. Method (a) from **6**: Amino acid methyl ester **6** (1.047 g, 2.113 mmol) was stirred in 25 mL of water and 2.2 mL of phosphate buffer (0.01 M KCl, 10^{-4} M KH_2PO_4). Subtilisin Carlsberg (8 mg, Sigma) was added and the pH was kept constant by dropwise addition of 0.3 N NaOH (monitoring by pH meter). After about 4 h the hydrolysis was complete. The solution was centrifuged and evaporated to dryness. The crude product **7** was dissolved in 150 mL of methanol and purified by flash-chromatography in CH_2Cl_2/MeOH/AcOH/H_2O (50:8:1:1). Yield: 805 mg (76%); colorless amorphous solid; mp>270 °C; R_f=0.64 (CH_2Cl_2/MeOH/H_2O/AcOH 7:3:0.6:0.3); analyt. HPLC (column: VYDAC C18, eluant with 0.1% TFA): gradient: 1% H_3CCN/99% $H_2O \rightarrow$ 100% CH_3CN within 40 min, t_R=20.2 min; $[\alpha]_D^{20}$ −3.7 (*c* 0.5, H_2O). ^{1}H NMR (400 MHz, DMSO), δ 2.90 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=13.8 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$= 7.0 Hz, H-$\beta_a$); 3.08 (d, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=9.4 Hz, H-$\beta_b$); 3.63 (s, 2H, –$CH_2SO_3H$); 3.97–4.07 (m, 1H, H-α); 4.10 (dd, 1H, J_{vic}=7.33 Hz, J_{gem}=10.0 Hz, –CH_2–Fmoc); 4.20 (t, 1H, J_{vic}=6.8 Hz, H-9 Fmoc); 4.35 (dd, 1H, J_{vic}=6.8 Hz, J_{gem}= 10.0 Hz, –CH_2–Fmoc); 7.04–7.61 (m, 8H, H–Ar); 7.66 (t, 2H, J_{vic}=7.3 Hz, H–Ar); 7.87 (d, 2H, J_{vic}=7.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, DMSO-d_6), δ 36.78 (C-β); 46.63 (C-9 Fmoc); 56.61 (C-α); 57.16 (–*C*H_2SO_3H); 65.33 (–*C*H_2–Fmoc); 119.99 (C-4 Fmoc, C-5 Fmoc); 125.12, 125.34 (C-1 Fmoc, C-8 Fmoc); 127.03, 127.53, 128.58, 129.76 (C–Ar); 132.76, 136.61 (C-*ipso*$_{C\text{-}\beta}$, C-*ipso*$_{-CH2SO3H}$); 140.59, 140.64 (C-4a$_{Fmoc}$, C-4b$_{Fmoc}$); 143.80, 143.85 (C-8a$_{Fmoc}$, C-9a$_{Fmoc}$); 155.42 (–O–*C*O–NH–); 175.50 (–*C*OOH). MALDIMS (dhb): *m*/*z*=504.2 $[M+H]^+$, 526.4 $[M+Na]^+$, 542.2 $[M+K]^+$, 1029.8 $[2M+Na]^+$, 1051.8 $[2M-H+2Na]^+$, 1067.7 $[2M-H+Na+K]^+$.

Method (b) from **12**: Boc-Phe(*p*-CH_2SO_3H)-OMe **12** (124 mg, 0.332 mmol) was dissolved in 10 mL of CH_3CN and 15 mL of 4% aq Na_2CO_3 and stirred for 16 h at room temperature. After neutralization with acidic ion-exchange resin, the solvent was evaporated in vacuo. The carboxy-deblocked amino acid (112.6 mg, R_f=0.55, CH_2Cl_2/MeOH/AcOH 5:2:0.1) was dissolved in 3 mL of dichloromethane, 3 mL of TFA, and 0.1 mL of water. After stirring at room temperature for 2 h the solvents were removed in vacuo. The remaining residue was co-distilled three times with 10 mL of toluene. The hydrotrifluoroacetate of Phe(*p*-CH_2SO_3H)–OH **7** (R_f=0.07 CH_2Cl_2/MeOH/AcOH 5:2:0.2) was dried in vacuo and dissolved in water (2 mL) and 48.8 μL (0.35 mmol) of triethylamine. To this solution, a solution of Fmoc–ONSu[18] (118.1 mg, 0.35 mmol) in 2 mL of CH_3CN was added and the mixture stirred for 30 min at room temperature. The pH of the mixture was kept at 8.5–9.0 by occasional addition of triethylamine. The clear yellowish solution was concentrated in vacuo and the crude product **7** purified by chromatography in CH_2Cl_2/MeOH/AcOH/H_2O (50:8:1:1). Yield: 100.0 mg (63%), colorless amorphous solid of identical data as the compound obtained by method (a).

4.1.7. *N*-*tert*-Butyloxycarbonyl-L-*p*-cyano-phenylalanine methyl ester 9. In a Schlenk vessel Boc-Tyr(OTf)-OMe[20] **8** (3.17 g, 7.42 mmol) was dissolved in dry acetonitrile (3.5 mL). Bis(triphenylphosphine)nickel bromide (276 mg, 0.37 mmol), triphenylphosphine (195 mg, 0.74 mmol), potassium cyanide (146 mg, 2.23 mmol), and zinc powder (146 mg, 2.23 mmol) were added under an argon atmosphere. The mixture was stirred at 65 °C for 1.5 h. The solution was filtered through *Hyflo*, diluted with dichloromethane (200 mL) and washed with 70 mL of 1 N aq $NaHCO_3$ and water. The organic layer was dried with $MgSO_4$ and the

solvent evaporated in vacuo. The crude product **9** was purified by flash-chromatography in light petroleum ether/ethyl acetate (4:1). Yield: 1.30 g (58%), colorless crystals; mp 110 °C; R_f=0.70 (light petroleum ether/ethyl acetate 3:2); $[\alpha]_D^{22}$ 56.7 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$), δ 1.38 (s, 9H, –C(C*H*$_3$)$_3$); 3.03 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=13.7 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=6.8 Hz, H-$\beta_a$); 3.20 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=13.7 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=5.4 Hz, H-$\beta_b$); 3.70 (s, 3H, –OC*H*$_3$); 4.58 ($m_c$, 1H, H-$\alpha$); 5.02 (d, 1H, $J_{NH,H\text{-}\alpha}$=7.3 Hz, –NH–); 7.23 (d, 2H, J_{vic}=8.3 Hz, H–Ar); 7.56 (d, 2H, J_{vic}=8.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, $CHCl_3$), δ 28.22 (–*C*(*C*H$_3$)$_3$); 38.62 (C-β); 52.44 (–O–*C*H$_3$); 54.10 (C-α); 80.21 (–*C*(CH$_3$)$_3$); 110.96 (C-*ipso*$_{-CN}$); 118.70 (–*C*N); 130.16 (C–Ar); 132.21 (C–Ar); 141.97 (C-*ipso*$_{C\text{-}\beta}$); 154.91 (–NH–*C*OCH$_3$); 171.72 (–*C*OOMe). Anal. Calcd for $C_{16}H_{20}N_2O_4$ (304.4): C, 63.14; H, 6.62; N, 9.20. Found: C, 63.40; H, 6.63; N 8.95.

4.1.8. *N-tert*-Butyloxycarbonyl-L-(*p*-hydroxymethyl)phenylalanine methyl ester 10. To a solution of nitrile **9** (1.33 g, 4.36 mmol) in 10 mL of MeOH, concd aq NH_3 (3 mL) and catalytic amounts of Raney-nickel (pH 9) were added. After vigorous stirring under hydrogen atmosphere for 3 h, the solvent was evaporated in vacuo. The formed amine (R_f=0.56, CH_2Cl_2/MeOH/AcOH 5:1:0.1) was dissolved in 20 mL of dioxane and 50 mL of water, and carefully acidified with 1 N HCl (about 3.2 mL) to pH 3. Sodium nitrite (400 mg, 5.80 mmol) was added, and the solution was refluxed for 1.5 h. After neutralization by addition of satd aq $NaHCO_3$ and concentration in vacuo to a volume of 10 mL, dichloromethane (150 mL) was added. The solution was washed twice with water (50 mL) and dried with $MgSO_4$. The solvent was evaporated in vacuo. The residue was purified by column chromatography in light petroleum ether/ethyl acetate 5:2. Yield: 618 mg (46%), colorless, highly viscous oil, which crystallizes within 2 d to form **10** as long needles; mp 81 °C; R_f=0.48 (light petroleum ether/ethyl acetate 1:1); $[\alpha]_D^{23}$ 50.8 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$), δ 1.40 (s, 9H, –C(CH$_3$)$_3$); 2.18 (br s, 1H, –CH$_2$OH); 3.02 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=13.7 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=6.8 Hz, H-$\beta_a$); 3.75 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=13.2 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=5.4 Hz, H-$\beta_b$); 3.70 (s, 3H, –OC*H*$_3$); 4.55 ($m_c$, 1H, H-α); 4.65 (s, 2H, –C*H*$_2$OH); 4.95 (d, 1H, $J_{NH,H\text{-}\alpha}$=7.8 Hz, –NH–); 7.09 (d, 2H, J_{vic}=7.8 Hz, H–Ar); 7.27 (d, 2H, J_{vic}=8.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, $CDCl_3$), δ 28.28 (–C(*C*H$_3$)$_3$); 37.99 (C-β); 52.22 (–O*C*H$_3$); 54.45 (C-α); 64.84 (–*C*H$_2$OH); 80.00 (–*C*(CH$_3$)$_3$); 127.21, 128.81 (C–Ar); 135.30 (C-*ipso*$_{C\text{-}\beta}$); 139.77 (C-*ipso*$_{-CH2OH}$); 155.12 (–NH–*C*O–O); 172.34 (–*C*OOCH$_3$). FDMS: *m*/*z*=309.4 (100%) $[M]^+$.

4.1.9. *N-tert*-Butyloxycarbonyl-L-(*p*-bromomethyl)phenylalanine methyl ester 11. To Boc-Phe(*p*-CH$_2$OH)–OMe **10** (225 mg, 0.73 mmol) dissolved in 5 mL of dry THF at 0 °C, triphenylphosphine (571 mg, 2.18 mmol) was added. After 5 min NBS (388 mg, 2.18 mmol) was added. The solution stirred at room temperature for 18 h. After dilution with diethyl ether (30 mL) and washing with aq sodium thiosulfate and water (twice), the organic layer was dried with $MgSO_4$ and the solvent distilled off. The crude **11** was purified by flash-chromatography in light petroleum ether/ethyl acetate 8:1. Yield: 202 mg (74%), colorless crystals; mp 115 °C; R_f=0.42 (light petroleum ether/ethyl acetate 5:1); $[\alpha]_D^{22}$ 53.1 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$), δ 1.39 (s, 9H, –C(C*H*$_3$)$_3$); 3.00 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=13.7 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=6.4 Hz, H-$\beta_a$); 3.10 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=13.7 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=5.9 Hz, H-$\beta_b$); 3.69 (s, 3H, –O–C*H*$_3$); 4.45 (s, 2H, –C*H*$_2$Br); 4.56 (q, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a} \approx J_{H\text{-}\alpha,H\text{-}\beta b} \approx J_{H\text{-}\alpha,NH}$=6.4 Hz, H-α); 4.96 (d, 1H, $J_{NH,H\text{-}\alpha}$=8.3 Hz, –NH–); 7.07 (d, 2H, J_{vic}=7.8 Hz, H–Ar); 7.29 (d, 2H, J_{vic}=7.8 Hz, (H–Ar). ^{13}C NMR (50.3 MHz, $CDCl_3$), δ 28.28 (–C(*C*H$_3$)$_3$); 33.21 (–*C*H$_2$Br); 38.10 (C-β); 52.27 (*C*H$_3$–O–); 54.33 (C-α); 80.00 (–*C*(CH$_3$)$_3$); 129.23, 129.75 (C–Ar); 136.48 (C-*ipso*$_{-CH2Br}$); 155.03 (–O–*C*O–NH–); 172.18 (–*C*OOMe). FDMS: *m*/*z*=371.4 (100%), 373.4 (99.9%) $[M]^+$. Anal. Calcd for $C_{16}H_{22}NO_4Br$ (372.26): C, 51.62; H, 5.96; N, 3.76. Found: C, 51.66; H, 5.91; N, 3.81.

4.1.10. *N-tert*-Butyloxycarbonyl-L-(*p*-sulfomethyl)phenylalanine methyl ester 12. To a solution of **11** (47.0 mg, 0.126 mmol) in 2 mL of dioxane a solution of sodium sulfite (65.4 mg, 0.76 mmol) in water (2.3 mL) was added. The mixture was stirred under reflux for 1 h. The solvents were removed in vacuo, and the crude product **12** was purified by column chromatography in dichloromethane/methanol 7:1. Yield: 39.0 mg (83%), colorless amorphous solid; R_f=0.25 (CH_2Cl_2/MeOH 6:1). ^{1}H NMR (200 MHz, MeOD), δ 1.43 (s, 9H, –C(C*H*$_3$)$_3$); 2.94 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=13.7 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=8.8 Hz, H-$\beta_a$); 3.11 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=13.7 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=5.4 Hz, H-$\beta_b$); 3.73 (s, 3H, –O–C*H*$_3$); 4.07 (s, 2H, –C*H*$_2SO_3$H); 4.37 (q, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a}$=8.8 Hz, $J_{H\text{-}\alpha,H\text{-}\beta b}$= 5.4 Hz, H-α); 7.20 (d, 2H, J_{vic}=8.3 Hz, H–Ar); 7.39 (d, 2H, J_{vic}=7.8 Hz, H–Ar). ^{13}C NMR (50.3 MHz, MeOD), δ 28.94 (–C(*C*H$_3$)$_3$); 38.43 (C-β); 52.92 (*C*H$_3$–O); 56.82 (C-α); 58.33 (–*C*H$_2$SO$_3$H); 80.91 (–*C*(CH$_3$)$_3$); 130.31, 131.97 (C–Ar); 133.18 (C-*ipso*$_{-CH2SO3H}$); 137.74 (C-*ipso*$_{C\text{-}\beta}$); 158.07 (–O–*C*O–NH–); 174.50 (–*C*OOMe). FDMS: *m*/*z*=318.5 (58%) $[M-BocO+2Na]^+$, 418.6 (100%) $[M-H+2Na]^+$, 813.9 $[2M-2H+3Na]^+$.

4.1.11. *N*-Fluorenyl-9-methoxycarbonyl-L-*O*-trifluoromethylsulfonyl-tyrosine methyl ester 13. In a mixture of dry dichloromethane (12 mL) and pyridine (3.04 mL), *N*-(fluorenyl-9-methoxycarbonyl)-L-tyrosine methyl ester (3.20 g, 7.67 mmol) was dissolved. After cooling to 0 °C–5 °C trifluormethanesulfonic anhydride (1.60 mL, 9.51 mmol) was added. The mixture was stirred under an argon atmosphere for 40 min, diluted with 100 mL of dichloromethane, and washed quickly with 1 N aq $NaHCO_3$ (30 mL), 10% aq citric acid, and water. The organic layer was dried with $MgSO_4$ and the solvent evaporated in vacuo to give **13** as a highly viscous brownish oil, which was purified by flash-chromatography in light petroleum ether/ethyl acetate (3:1). Yield: 3.42 g (81%), colorless amorphous solid; R_f=0.67 (light petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{23}$ 38.0 (*c* 1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$), δ 3.11 (m_c, 2H, H-β_a, H-β_b); 3.70 (s, 3H, –O–CH$_3$); 4.18 (t, 1H, $J_{H\text{-}9,-CH2a-} \approx J_{H\text{-}9,-CH2b-}$=6.6 Hz, H-9 Fmoc); 4.30–4.54 (m, 2H, –C*H*$_2$–Fmoc); 4.63 (q, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a} \approx J_{H\text{-}\alpha,H\text{-}\beta b} \approx J_{H\text{-}\alpha,NH}$=6.8 Hz, H-α); 5.25 (d, 1H, $J_{NH,H\text{-}\alpha}$= 7.8 Hz, –NH–); 7.14–7.44 (m, 8H, H–Ar); 7.55 (d, 2H, J_{vic}=7.3 Hz, H–Ar); 7.46 (d, 2H, J_{vic}=7.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, $CDCl_3$), δ 37.60 (C-β); 47.23 (C-9 Fmoc); 52.51 (–OCH$_3$); 54.65 (C-α); 66.84 (–CH$_2$–Fmoc); 118.75 (q, *J*=321.2 Hz, –SO$_2$*C*F$_3$); 120.06, 121.40, 124.93, 127.10, 127.80, 130.13, 131.14, 132.29 (C–Ar); 136.59 (C-*ipso*$_{C\text{-}\beta}$); 141.40 (C-4a Fmoc, C-4b Fmoc);

143.66, 143.76 (C-8a Fmoc, C-9a Fmoc); 148.64 (C-*ipso*$_{-OSO2CF3}$); 155.49 (–O–*C*O–NH–); 171.45 (–*C*OOMe). Anal. Calcd for $C_{26}H_{22}NO_7SF_3$ (549.47): C, 56.63; H, 4.03; N, 2.54. Found: C, 56.67; H, 4.03; N, 2.53.

4.1.12. *N*-Fluorenyl-9-methoxycarbonyl-L-*p*-formyl-phenylalanine methyl ester 14. To a solution of **13** (200 mg, 0.363 mmol) in 3 mL of dry dimethylformamide, $Pd(OAc)_2$ (7 mg, 31.18 μmol), 1,3-bis-(diphenylphosphino)-propane (dppp, 12 mg, 22.14 μmol), and 171.2 μL (0.726 mmol) of triethylamine were added. Carbon monoxide (toxic! all reactions and work up in an efficient fume hood) was bubbled through the solution via a cannula for 10 min, while the solution was turning brownish. While bubbling of carbon monoxide was continued, trioctylsilane (326 μL, 0.726 mmol) was added via a syringe. The mixture was warmed up to 70 °C and stirred for 12 h. The solvent was evaporated in vacuo, and the crude **14** was purified by column chromatography in light petroleum ether/ethyl acetate (4:1). Yield: 62.9 mg (40%), colorless crystals; mp 111 °C; R_f=0.39 (light petroleum ether/ethyl acetate 2:1); $[\alpha]_D^{21}$ 41.9 (*c* 1, $CHCl_3$). Starting material 59.0 mg (107 μmol, 29.5%) was recovered. ^{1}H NMR (200 MHz, $CDCl_3$), δ 3.07–3.28 (m, 2H, H-βa, H-β_b); 3.72 (s, 3H, –OCH_3); 4.18 (t, 1H, $J_{H\text{-}9,-CH2a-} \approx J_{H\text{-}9,-CH2a-}$= 6.6 Hz, H-9 Fmoc); 4.27–4.52 (m, 2H, –C*H*$_2$–Fmoc); 4.69 (q, 1H, $J_{H\text{-}\alpha,NH} \approx J_{H\text{-}\alpha,H\text{-}\beta a} \approx J_{H\text{-}\alpha,H\text{-}\beta b}$=7.1 Hz, H-α); 5.27 (d, 1H, $J_{NH,H\text{-}\alpha}$=7.8 Hz, –NH–); 7.20–7.57 (m, 8H, H–Ar); 7.74–7.80 (m, 4H, H–Ar); 9.96 (s, 1H, –C*H*O). ^{13}C NMR (50.3 MHz, $CDCl_3$), BB, δ 38.43 (C-β); 47.19 (C-9 Fmoc); 52.55 (–O*C*H$_3$); 54.58 (C-α); 66.89 (–*C*H$_2$–Fmoc); 120.04 (C-4 Fmoc, C-5 Fmoc); 124.96, 125.02 (C-1 Fmoc, C-8 Fmoc); 127.10, 127.80, 129.97, 130.03 (C–Ar); 135.38 (C-*ipso*$_{C\text{-}\beta}$); 141.38 (C-*ipso*$_{-CHO}$); 141.32, 143.12, 143.65, 143.78 (C-8a Fmoc, C-9a Fmoc, C-4a Fmoc, C-4b Fmoc); 191.83 (–CHO). Anal. Calcd for $C_{26}H_{23}NO_5$ (429.29): C, 72.71; H, 5.40; N, 3.26. Found C, 72.54; H, 5.52; N, 3.19.

4.1.13. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-hydroxymethyl)phenylalanine methyl ester 4. To a solution of **14** (45.0 mg, 0.105 mmol) in 4 mL of CH_2Cl_2, $NaBH_4$ (9.5 mg, 0.250 mmol) was given. Within 10 min, MeOH (0.8 mL) was added dropwise. After stirring for 30 min at room temperature, the starting material was completely consumed. The solution was diluted with 40 mL of CH_2Cl_2 and extracted with 30 mL of 10% aq acetic acid. The organic layer was washed with 10 mL of water, dried with $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by flash-chromatography in light petroleum ether/ethyl acetate (2:1) to give pure **4**. Yield: 32.7 mg (72%), R_f=0.22 (light petroleum ether/ethyl acetate 3:2); $[\alpha]_D^{22}$ 39.3 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{26}H_{25}NO_5$ (431.49): C, 72.37; H, 5.84; N, 3.25. Found: C, 72.45; H, 5.83; N, 3.30.

The NMR-spectroscopic data are identical with those of compound **4** obtained from **3**.

4.1.14. *N*-Acetyl-L-alanyl-L-(*O*-benzyl)-threonyl-L-(5-*O*-benzyl)-glutamyl-L-(*p*-sulfomethyl)phenylalanyl-L-(5-*O*-benzyl)-glutamyl-L-(*p*-sulfomethyl)phenylalanyl-L-leucyl-L-(4-*O*-benzyl)-aspartate 16. (Ac-Ala-Thr(Bn)-Glu(OBn)-Phe(*p*-CH_2SO_3H)-Glu(OBn)-Phe(*p*-CH_2SO_3H)-Leu-Asp(OBn)–OH).

Loading of the polymeric support in a solid-phase reaction vessel (100 mL) equipped with a frit and a valve at the bottom. Wang resin[23] (470 mg, 1 mmol/g, 100–200 mesh, Rapp Polymere, Tübingen, Germany) was swollen in DMF (10 mL) for 30 min and washed three times with 10 mL of DMF and four times with 10 mL of dichloromethane. A solution of Fmoc–Asp(OBn)–OH (670 mg, 1.50 mmol), *N*-methylimidazole (89.5 μL), and 445 mg of 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT) in 10 mL of dry dichloromethane was added. The suspension was shaken under an argon atmosphere for 30 min. After filtration the resin was washed successively three times with 10 mL of CH_2Cl_2, three times with 10 mL of DMF and five times with 10 mL of CH_2Cl_2 and dried in high vacuum. After treatment of a probe with morpholine/DMF, photometric determination of fluorenyl methyl morpholine indicated a loading of 0.63 mmol/g. The loaded resin (140 mg, 0.09 mmol) was used for the synthesis of the PSGL 1 peptide.

Fmoc removal was carried out by shaking with morpholine/DMF (1:1, 10 mL) as long as no more fluorenyl methyl morpholine was formed according to photometrical determination at λ=300.5 nm (as a rule 30 min–45 min). Subsequently, the resin was washed with DMF (six times with 10 mL). Capping was performed with pyridine/Ac_2O (3:1, 16 mL) for 30 min followed by washing (again six times with 10 mL of DMF).

Coupling of Fmoc amino acids was carried out in DMF (5 mL) with TBTU[25] (161 mg, 0.50 mmol), HOBt (76.8 mg, 0.50 mmol), and diisopropyl-ethylamine (DIPEA, 171 μL, 1.00 mmol), except for the coupling reactions with Fmoc–Phe(*p*-CH_2SO_3H)–OH **7**, which were conducted with 385 mg (1.20 mmol) of TBTU, 184 mg (1.20 mmol) of HOBt, and 264 μL (2.40 mmol) of *N*-methyl-morpholine.

Applied amounts of amino acids and coupling times: Fmoc–Leu–OH: 76.8 mg (0.50 mmol), 15 h; Fmoc–Phe(*p*-CH_2SO_3H)–OH **7**: 201 mg (1.20 mmol), 16 h; Fmoc–Glu(OBn)–OH: 230 mg (0.50 mmol), 17 h; Fmoc–Phe(*p*-CH_2SO_3H)–OH **7**: 201 mg (1.20 mmol), 16 h, this coupling was repeated; Fmoc–Glu(OBn)–OH: 230 mg (0.50 mmol), 17 h; Fmoc–Thr(Bn)–OH: 216 mg (0.50 mmol), 16 h, this coupling was repeated; Fmoc–Ala–OH: 156 mg (0.50 mmol), 15 h. Photometric determination shows a loading of 0.11 mmol.

The Fmoc group was removed and N-acetylation was carried out with pyridine/Ac_2O 3:1.

For detachment of the peptide, the resin was suspended in 20 mL of CH_2Cl_2/TFA/H_2O (10:10:0.4) and shaken at room temperature for 1.5 h. The solution containing the product was filtered, concentrated in vacuo, and co-distilled several times with toluene to dryness. The crude product was dissolved in acetonitrile/water 2:1, filtered, and purified by preparative RP-HPLC (column: Eurospher C8, eluent: acetonitrile/water (0.1 M NH_4OAc): gradient: 1% CH_3CN/99% $H_2O \rightarrow$ 60% CH_3CN/40% H_2O within 100 min, t_R=69.6 min). Yield: 7.8 mg (5.5%); colorless amorphous solid; $[\alpha]_D^{22}$ 1.1 (*c* 0.5, DMSO); analyt. HPLC (column: VYDAC C18): gradient: 10% CH_3CN/90% H_2O (0.1 N

NH_4OAc)→40% CH_3CN/60% H_2O (0.1 N NH_4OAc) in 50 min, t_R=31.8 min. ^1H NMR (600 MHz, DMSO), ^1H–^1H COSY, ^1H–^1H NOESY: δ 0.77 (d, 3H, $J_{H\text{-}\delta a,H\text{-}\gamma}$=6.8 Hz, H-$\delta_a$ Leu); 0.83 (d, 3H, $J_{H\text{-}\delta b,H\text{-}\gamma}$=6.5 Hz, H-$\delta_b$ Leu); 1.01 (d, 3H, $J_{H\text{-}\gamma,H\text{-}\beta}$=6.2 Hz, H-γ Thr); 1.16 (d, 3H, $J_{H\text{-}\beta,H\text{-}\alpha}$=7.1 Hz, H-β Ala); 1.42 (t, 2H, $J_{H\text{-}\alpha,H\text{-}\beta}$=$J_{H\text{-}\beta,H\text{-}\gamma}$=7.2 Hz, H-$\beta_a$ Leu, H-β_b Leu); 1.58 (m_c, 1H, H-γ Leu); 1.77 (m, 2H, H-β_b Glu_1, H-β_b Glu_2); 1.81 (s, 3H, CH_3CO–); 1.89 (m, 2H, H-β_a Glu_1, H-β_a Glu_2); 2.37 (m, 4H, H-γ Glu_1, H-γ Glu_2); 2.67 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=14.1 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=10.3 Hz, H-$\beta_b$ Phe_2); 2.70 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=16.4 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=6.8 Hz, H-$\beta_b$ Asp); 2.76 (dd, 1H, $J_{H\text{-}\beta b,H\text{-}\beta a}$=13.2 Hz, $J_{H\text{-}\beta b,H\text{-}\alpha}$=8.8 Hz, H-$\beta_b$ Phe_1); 2.83 (dd, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=16.1 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=6.2 Hz, H-$\beta_a$ Asp); 2.95 (d, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=11.2 Hz, H-$\beta_a$ Phe_2); 3.02 (d, 1H, $J_{H\text{-}\beta a,H\text{-}\beta b}$=10.9 Hz, H-$\beta_a$ Phe_1); 3.60 (s, 2H, –CH_2SO_3H); 3.62 (s, 2H, –CH_2SO_3H); 3.92 (m_c, 1H, H-β Thr); 4.25 (q, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a}\approx J_{H\text{-}\alpha,H\text{-}\beta b}\approx J_{H\text{-}\alpha,NH}$=7.1 Hz, H-α Glu_1); 4.28–4.35 (m, 4H, H-α Leu, H-α Ala, H-α Thr, H-α Glu_2); 4.36 (d, 1H, J_{gem}=11.7 Hz, –CH_2Bn ether); 4.43 (d, 1H, J_{gem}=11.7 Hz, –CH_2Bn ether); 4.47 (m_c, 1H, H-α Phe_2); 4.53 (m, 2H, H-α Asp, H-α Phe_1); 4.98–5.11 (m, 6H, –CH_2Bn ester); 7.77 (d, 1H, $J_{H\text{-}\alpha,NH}$=7.6 Hz, –NH–); 7.90 (d, 2H, $J_{H\text{-}\alpha,NH}$=8.5 Hz, –NH–); 8.06 (d, 1H, $J_{H\text{-}\alpha,NH}$=7.1 Hz, –NH–Phe_2); 8.11 (d, 1H, $J_{H\text{-}\alpha,NH}$=7.1 Hz, –NH–); 8.20 (d, 1H, $J_{H\text{-}\alpha,NH}$=8.2 Hz, –NH–); 8.25 (d, 1H, $J_{H\text{-}\alpha,NH}$=7.9 Hz, –NH–Glu_1). ^{13}C NMR (100.6 MHz, DMSO), ^1H–^{13}C COSY, ^1H–^{13}C HSQC: δ 17.1 (C-γ Thr); 18.7 (C-β Ala); 22.3 (C-δ_a Leu); 23.0 (CH_3CO–); 23.7 (C-δ_b Leu); 24.8 (C-γ Leu); 28.4 (C-β Glu); 30.5 (C-γ Glu); 36.9 (C-β Asp); 37.6 (C-β Phe); 41.8 (C-β Leu); 49.0, 51.7 (C-α Leu, C-α Ala); 49.5 (C-α Asp); 52.1 (C-α Glu_2); 52.5 (C-α Glu_1); 54.2 (C-α Phe_1); 54.5 (C-α Phe_2); 57.3 (C-α Thr); 58.1 (–CH_2SO_3H); 2×66.1, 66.6 –CH_2Bn ester); 71.1 (–CH_2Bn ether); 75.3 (C-β Thr).

MALDIMS (dhb, $C_{77}H_{92}N_8O_{23}S_2$): m/z=1584.9 $[M+Na]^+$, 1606.9 $[M+2Na–H]^+$, 1629.0 $[M+3Na–2H]^+$, 1650.9 $[M+4Na–3H]^+$, calcd: 1561.7.

4.1.15. *N*-Fluorenyl-9-methoxycarbonyl-L-phenylalanyl-L-leucyl-L-prolyl-L-(5-*O*-benzyl)-glutamyl-L-N^4-(14-hydroxycarbonyl-3,6,9,12-tetra-oxa-tetradecyl)asparaginyl-L-(5-*O*-benzyl)-glutaminic acid amide 17. (Fmoc–Phe-Leu-Pro-Glu(OBn)-Asn(glycol$_4$-COOH)-Glu(OBn)–NH_2.

Rink-amide AM resin (200–400 mesh, Novabiochem) containing 0.64 mmol/g Fmoc Rink-linker (520 mg, 0.333 mmol) was swollen in DMF (10 mL) in a 100-mL Merrifield solid-phase reactor. The procedures for Fmoc removal, coupling reactions and capping reactions were carried out as described for the synthesis of **16**.

Applied amounts of amino acids and coupling times: Fmoc–Glu(OBn)–OH: 651 mg (1.42 mmol), 15 h; Fmoc–Asn(glycol$_4$-COO-*t*-Bu)–OH:[27] 751 mg (1.14 mmol), 16 h; Fmoc–Glu(OBn)–OH: 643 mg (1.40 mmol), 16 h, this coupling was repeated; Fmoc–Pro–OH: 388 mg (1.15 mmol), 16 h; according to photometric determination, the remaining content of Fmoc amounted to 0.267 mmol at this stage of the synthesis; Fmoc–Leu–OH: 406 mg (1.15 mmol), 15 h; Fmoc–Phe–OH: 445.5 mg (1.15 mmol), 15 h.

For detachment of the peptide, the resin was shaken with a mixture of CH_2Cl_2 (8 mL), TFA (8 mL), and water (0.2 mL) for 1 h at room temperature. After a few minutes a ruby color occurred. After filtration, the resin was washed with TFA/CH_2Cl_2 (1:9), the combined organic layers were concentrated in vacuo and co-distilled several times with toluene to dryness. The crude product was dissolved in 4 mL of dichloromethane and precipitated by dropwise addition of 20 mL of dry diethyl ether. Purification of the precipitate was achieved by preparative RP-HPLC (column: Eurospher C8, gradient: 40% H_3CCN/60% H_2O→90% CH_3CN/10% H_2O in 70 min, t_R=46.6 min). Yield: 207 mg (0.148 mmol, 45% related to the loading glutamic acid), colorless amorphous solid; R_f=0.47 (CH_2Cl_2/MeOH/AcOH 8:1:0.1); $[\alpha]_D^{22}$ −24.9 (*c* 1, $CHCl_3$); analyt. HPLC: t_R=30.9 min (column: Eurospher C8, gradient: 1% CH_3CN/99% H_2O→100% CH_3CN in 40 min). ^1H NMR (400 MHz, $CHCl_3$), δ 0.80 (d, 3H, $J_{H\text{-}\delta a,H\text{-}\gamma}$=5.3 Hz, H-$\delta_a$ Leu); 0.94 (d, 3H, $J_{H\text{-}\delta b,H\text{-}\gamma}$=5.9 Hz, H-$\delta_b$ Leu); 1.46–1.67 (m, 3H, H-β_a Leu, H-β_b Leu, H-γ Leu); 1.72–1.94 (m, 4H, H-β_a Pro, H-β_b Pro, H-γ_a Pro, H-γ_b Pro); 1.98–2.49 (m, 8H, H-β_a Glu, H-β_b Glu, H-β_a Glu–NH_2, H-β_b Glu–NH_2, H-γ_a Glu, H-γ_b Glu, H-γ_a Glu–NH_2, H-γ_b Glu–NH_2); 2.50–2.63 (m, 2H, –CH_2COOH); 2.75–2.84 (m, 2H, Hβ_a Asn, H-β_b Asn); 2.90–3.01 (m, 1H, H β_a Phe); 3.02–3.11 (H-β_b Phe); 3.20–3.39 (m, 2H, H-δ_a Pro, H-δ_b Pro); 3.40–3.47 (m, 2H, –NH–C$H_2$$CH_2$–O–); 3.47–3.63 (m, 14H, –CH_2–O–); 3.64–3.75 (m, 2H, –O–CH_2–CH_2–COOH); 3.97–4.22 (m, 3H, H-α Pro, H-9 Fmoc, –CH_{2a}–Fmoc); 4.28–4.42 (m, 3H, –CH_{2b}–Fmoc, H-α Glu, H-α Glu–NH_2); 4.53–4.69 (m, 2H, H-α Phe, H-α Asn); 4.78–4.88 (m, 1H, H-α Leu); 4.99–5.10 (m, 2H, –CH_2Bn); 5.66 (α-NH Phe); 6.81 (s, 2H, –CO–NH_2); 7.05–7.40 (m, 19H, H–Ar); 7.43–7.51 (m, 2H, H–Ar); 7.60–7.92 (m, 4H, 2×H–Ar, 2×–*NH*–); 8.02–8.11 (s, 1H, –NH–). ^{13}C NMR (100.3 MHz, $CHCl_3$), BB, DEPT, δ 21.58 (C-δ_a Leu); 23.27 (C-δ_b Leu); 24.63 (C-γ Leu); 25.26 (C-γ Pro); 25.91, 26.39 (C-β Glu, C-β Glu–NH_2); 28.38 (C-β Pro); 30.89, 31.12 (C-γ Glu, C-γ Glu–NH_2, –O–CH_2–*C*H_2–CO–); 36.83, 38.67 (C-β Asn, C-β Phe); 39.40 (–*C*H_2–NH–); 40.98 (C-β Leu); 47.15 (C-9 Fmoc); 47.32 (C-δ Pro); 49.46 (C-α Leu); 51.96, 53.04, 55.48, 55.71, 61.24 (C-α Asn, C-α Glu, C-α Glu–NH_2, C-α Pro, C-α Phe); 66.18, 66.80, 66.99 (–O–*C*$H_2$$CH_2$COOH, –*C*$H_2$Bn, –*C*$H_2$–Fmoc); 69.43, 70.13, 70.34, 70.45, 70.49 (–O–CH_2–glycol); 119.84 (C-4 Fmoc, C-5 Fmoc); 125.02, 125.11, 126.78, 126.97, 127.59, 127.90, 128.01, 128.34, 128.43, 128.56, 129.38 (C–Ar); 135.43, 135.98, 136.41 (C-*ipso* Bn); 143.77, 141.19 (C-4a Fmoc, C-4b Fmoc, C-8a Fmoc, C-9a Fmoc); 155.72 (–O–*C*O–NH–Fmoc); 170.60, 170.96, 171.31, 172.06, 172.62, 172.91, 173.32, 173.97 (–*C*O–). MALDIMS: m/z=1398.1 $[M+H]^+$, 1420.3 $[M+Na]^+$, 1436.3 $[M+K]^+$, 1442.1 $[2M-H+2Na]^+$, 1458.1 $[M-H+Na+K]^+$, 1474.1 $[M-2H+2\ K]^+$, calcd: 1397.6. Anal. Calcd for $C_{74}H_{92}N_8O_{19}\cdot H_2O$ (1397.6·H_2O): C, 62.83; H, 6.70; N, 7.92. Found: C, 62.49; H, 6.69; N, 7.91.

4.1.16. *N*-Fluorenyl-9-methoxycarbonyl-L-phenylalanyl-L-leucyl-L-prolyl-L-(5-*O*-benzyl)-glutamyl-N^4-{14-(2-acetamido-2-deoxy-6-*O*-benzyl-3-*O*-(α-L-tri-*O*-benzyl-fucopyranosyl)-4-*O*-(2,4-di-*O*-acetyl-6-*O*-benzyl-[methyl 5‴-acetamido-4,7,8,9,-tetra-*O*-acetyl-3‴,5‴-dideoxy-α-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-α/β-D-glucopyranosyl)-aminocarbonyl-3,6,9,12-

tetra-oxa-tetradecyl}-L-asparaginyl-L-(5-*O*-benzyl)-glutaminic acid amide 19. (Fmoc–Phe-Leu-Pro-Glu(OBn)-Asn(glycol$_4$-$(CH_2)_2$CO-β-Ac_6Bn_5sLe^{x}COOMe)-Glu(OBn)–NH_2).

To a solution of **17** (180 mg, 0.129 mmol) in 8 mL of DMF were added 19.8 mg (0.129 mmol) of HOBt, 22 μL (0.129 mmol) of DIPEA, and 201 mg (0.386 mmol) of PyBOP.[28] Subsequently, 397 mg (0.258 mmol) of sialyl Lewis^x-amine[27,30] **18** was added, and the mixture was stirred at room temperature for 16 h. DMF was distilled off in vacuo, and the residue was co-distilled three times with toluene (20 mL), dissolved in 100 mL of dichloromethane, and extracted twice with 40 mL of water. The organic solution was dried with $MgSO_4$, and the solvent was evaporated in vacuo. A slightly brownish solid was obtained (620 mg), which was purified by flash-chromatography in CH_2Cl_2/MeOH (14:1). From the isolated product (259 mg, HPLC-purity: 78%), impurities were separated by preparative HPLC (column: Eurospher C8, gradient: 40% H_3CCN/60% $H_2O \rightarrow 100\%$ CH_3CN in 110 min). Since the glycopeptide showed tailing on the column, only 132 mg of the pure product **19** were isolated. Yield: 132 mg (35%); colorless amorphous solid; R_f=0.50 (CH_2Cl_2/MeOH 10:1); analyt. HPLC (column: Eurospher C-8): gradient 50% CH_3CN/50% $H_2O \rightarrow 100\%$ CH_3CN in 40 min, t_R=25.8 min; $[\alpha]_D^{22}$ −39.0 (*c* 0.5, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$), ^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY: δ 0.83 (d, 3H, $J_{H\text{-}\delta a,H\text{-}\gamma}$= 6.2 Hz, H-$\delta_a$ Leu); 0.94 (d, 3H, $J_{H\text{-}\delta b,H\text{-}\gamma}$=6.2 Hz, H-$\delta_b$ Leu); 0.99 (d, 3H, $J_{6',5'}$=6.5 Hz, H-6′); 1.48 (H-β_a Leu); 1.58 (H-β_b Leu); 1.59 (H-γ Leu); 1.67 (t, 1H, $J_{3'''a,3e'''} \approx J_{3'''a,4'''}$=12.3 Hz, H-3‴a); 1.71 (C-γ_a Pro); 1.83 (s, 3H, CH_3CO–NH–(C-2); 1.84 (C-γ_b Pro); 1.88 (H-β_a Glu, H-β_a Glu–NH_2); 1.91 (s, 6H, 2×CH_3CO–); 1.97 (s, 3H, CH_3CO–); 1.98 (s, 3H, CH_3CO–); 1.99 (C-β_a Pro, C-β_b Pro); 2.03 (s, 3H, CH_3CO–); 2.04 (s, 3H, CH_3CO–); 2.06 (H-β_b Glu, H-β_b Glu–NH_2); 2.19 (–O–CH_2CH_2CO–NH–); 2.20 (s, 3H, CH_3CO–); 2.25 (–O–CH_2CH_2CO–NH–); 2.40 (H-γ_a Glu, H-γ_a Glu–NH_2); 2.54 (dd, 1H, $J_{3'''e,3'''a}$= 12.3 Hz, $J_{3'''e,4'''}$=4.4 Hz, H-3‴e); 2.56 (H-γ_b Glu, H-γ_b Glu–NH_2); 2.68 (H-β_a Asn); 2.77 (H-β_b Asn); 2.93 (H-β_a Phe); 3.05 (H-β_b Phe); 3.27 (–NH–CH_2CH_2–O–); 3.31 (H-6″a); 3.42 (H-6″b); 3.48 (H-4′); 3.51 (H-δ_a Pro); 3.52 (16H, –CH_2–O–glycol); 3.53 (H-6a); 3.63 (H-6‴); 3.64 (H-δ_b Pro); 3.65 (H-6b); 3.72 (H-3′); 3.74 (H-5″); 3.75 (H-5); 3.80 (s, 3H, –COOCH_3); 3.93 (H-3); 3.96 (H-9‴a); 4.01 (H-α Glu); 4.02 (H-2′, H-5‴); 4.03 (H-4); 4.04 (H-5′); 4.10 (H-9 Fmoc); 4.14 (H-2); 4.15 (–CH_{2a}–Fmoc); 4.27 (1H, –CH_2Bn); 4.29 (1H, –CH_2Bn); 4.30 (H-9‴b); 4.31 (H-α Glu–NH_2); 4.32 (H-α Pro); 4.36 (–CH_{2b}–Fmoc); 4.38 (–CH_2Bn); 4.54 (H-α Asn); 4.57 (–CH_2Bn); 4.58 (1H, –CH_2Bn); 4.61 (H-α Phe, –CH_2Bn); 4.62 (H-3″); 4.70 (2H, –CH_2Bn); 4.71 (H-1″); 4.72 (1H, –CH_2Bn); 4.80 (H-α Leu); 4.89 (1H, –CH_2Bn); 4.91 (H-2″); 4.94 (H-4‴); 5.03 (4H, 2×–CH_2Bn); 5.04 (H-4″); 5.06 (H-1); 5.16 (H-1′); 5.37 (dd, 1H, $J_{7''',6'''}$=2.6 Hz, $J_{7''',8'''}$=9.1 Hz, H-7‴); 5.52 (H-8‴); 5.68 (–N$H_{-C\text{-}5'''}$); 5.69 (–NH–Phe); 7.06 (–NH–$_{C\text{-}2}$); 7.10 (β-NH–Asn); 7.12–7.41 (48H, H–Ar); 7.55 (α-NH–Glu–NH_2, –NH–$_{C\text{-}1}$); 7.80 (–NH–Glu); 8.03 (α-NH–Asn). ^{13}C NMR (100.6 MHz, $CDCl_3$), DEPT, ^{1}H–^{13}C COSY: δ 16.42 (C-6′); 20.42, 20.55, 2×20.61, 20.84, 21.45, 22.84, 22.97 (*C*H_3CO–); 21.13 (C-δ_a Leu); 23.20 (C-δ_b Leu); 24.53 (C-γ Leu); 25.18, 25.70, 26.43, 28.31 (C-β Glu, C-β Glu–NH_2, C-β Pro, C-γ Pro); 30.80, 31.04 (C-γ Glu, C-γ Glu–NH_2); 2×36.70 (C-β Asn, –O–CH_2CH_2CO–); 37.55 (C-3‴); 38.58 (C-β Phe); 39.24 (–O–CH_2CH_2–NH–); 40.89 (C-β Leu); 47.06 (C-9 Fmoc); 47.16 (C-δ Pro); 48.99 (C-5‴); 49.46 (C-α Leu); 50.32 (C-2); 51.77 (C-α Asn); 52.82, 55.56 (C-α Glu, C-α Glu–NH_2); 52.95 (–O*C*H_3); 55.57 (C-α Phe); 61.18 (C-α Pro); 62.27 (C-9‴); 66.04 (–*C*H_2Bn); 66.67 (C-6); 66.70 (–*C*H_2–Fmoc); 66.85 (C-4); 67.08 (C-7‴); 67.32 (C-8‴); 67.45 (C-6″); 67.89 (C-4″); 68.27, 69.42, 70.07, 70.11, 70.25, 70.31, 71.18, 71.68, 71.99, 72.45, 72.61, 72.85, 73.10, 73.56 (–O–*C*H_2–glycol, 5×–*C*H_2Bn, C-5, C-2″, C-3″, C-4‴, C-6‴, C-5′); 2×74.59, 74.75 (C-3, C-5″, –*C*H_2Bn); 76.31 (C-2′); 77.51 (C-4′); 78.24 (C-1); 79.34 (C-3′); 96.86 (C-2‴); 97.41 (C-1′); 99.04 (C-1″); 119.80 (C-4 Fmoc, C-5 Fmoc); 124.95, 125.04 (C-1 Fmoc, C-8 Fmoc); 126.71, 126.92, 127.03, 127.22, 127.44, 127.55, 127.83, 127.93, 127.99, 128.09, 128.29, 128.32, 128.38, 128.45, 128.49, 129.31 (C–Ar); 135.37, 135.94, 136.36, 137.48, 137.72, 138.36, 138.43, 138.56 (C-*ipso*); 141.14 (C-4a Fmoc, C-4b Fmoc); 143.71 (C-8a Fmoc, C-9a Fmoc); 155.69 (–O–*C*O–NH–Fmoc); 167.75 (–*C*O–NH_2 Glu–NH_2); 169.50, 169.73, 170.03, 170.25, 170.28, 170.42, 170.62, 170.74, 170.88, 171.00, 171.27, 171.79, 172.53, 172.75, 173.35, 173.73, 173.92 (–*C*O–). MALDIMS: *m*/*z*= 2938.6 (100%) $[M+Na]^+$, 2954.3 $[M+K]^+$, calcd: 2916.2. Anal. Calcd for $C_{153}H_{187}N_{11}O_{46}\cdot 6H_2O$ ($2916.2\cdot 6H_2O$): C, 60.77; H, 6.63; N 5.09. Found: C, 60.89; H, 7.16; N, 5.25.

4.1.17. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-sulfomethyl)phenylalanyl-L-(4-*O*-benzyl)-aspartic acid-α-*tert*-butyl ester 21. To a solution of Fmoc–Phe(*p*-CH_2SO_3Na)–OH **7** (300 mg, 0.596 mmol) in dry DMF (12 mL), dry diisopropyl-ethylamine (122 μL, 0.715 mmol) and PyBOP[29] (403 mg, 0.775 mmol) were added. After stirring for 15 min H–Asp(OBn)–O-*t*-Bu[31] **20** (167 mg, 0.596 mmol) was added, and stirring was continued for 4 h at room temperature. DMF was distilled off in high vacuum. After co-distillation of toluene (three times 10 mL) from the crude product, purification was achieved by column chromatography in CH_2Cl_2/MeOH/AcOH (12:0.5:0.1). Yield: 431 mg (94%), colorless amorphous solid; R_f=0.33 (CH_2Cl_2/MeOH/AcOH/H_2O 50:8:1:1); analyt. HPLC (column: Eurospher C-8): gradient: 10% CH_3CN/90% $H_2O \rightarrow$ 100% CH_3CN in 40 min, t_R=17.9 min; $[\alpha]_D^{21}$ −2.8 (*c* 2, MeOH). ^{1}H NMR (400 MHz, MeOD), δ 1.42 (s, 9H, –C(CH_3)$_3$); 2.83–2.96 (m, 3H, H-β_a Phe, H-β_b Asp, H-β_a Asp); 3.16 (dd, 1H, $J_{H\beta a,H\text{-}\beta b}$=14.0 Hz, $J_{H\text{-}\beta a,H\text{-}\alpha}$=4.7 Hz, H-$\beta_b$ Phe); 4.03 (s, 2H, –CH_2SO_3H Phe); 4.17 (t, 1H, J_{vic}=6.8 Hz, H-9 Fmoc); 4.23–4.33 (m, 2H, –CH_2–Fmoc); 4.45 (dd, 1H, $J_{H\text{-}\alpha,H\text{-}\beta a}$=9.7 Hz, $J_{H\text{-}\alpha,H\text{-}\beta b}$=4.7 Hz, H-α Phe); 4.67–4.76 (m, 1H, H-α Asp); 5.10 (d, 1H, J_{gem}=12.3 Hz, –CH_2Bn); 5.16 (d, 1H, J_{gem}=12.3 Hz, –CH_2Bn); 7.22–7.45 (m, 13H, H–Ar); 7.59–7.65 (m, 2H, H–Ar); 7.81 (d, 2H, J_{vic}=7.3 Hz, H–Ar). ^{13}C NMR (50.3 MHz, $CDCl_3$), DEPT, δ 28.41 (–C(*C*H_3)$_3$); 37.41 (C-β Phe); 38.78 (C-β Asp); 48.55 (C-9 Fmoc); 51.22 (C-α Asp); 57.89 (C-α Phe); 58.43 (–*C*H_2SO_3H); 67.99, 68.39 (–O–*C*H_2Bn, –*C*H_2–Fmoc); 83.75 (–*C*(CH_3)$_3$); 121.16 (C-4 Fmoc, C-5 Fmoc); 126.52, 128.48, 129.05, 129.57, 129.81, 130.43, 131.97 (C–Ar); 133.26 (C-*ipso*$_{-CH2SO3H}$); 137.49, 137.84 (C-*ipso*$_{-CH2-Bn}$, C-*ipso*$_{C\text{-}\beta Phe}$); 142.79 (C-4a Fmoc, C-4b Fmoc); 145.45 (C-8a Fmoc, C-9a

Fmoc); 158.46 (–NH–*C*O–O–Fmoc); 171.10, 172.18, 174.18 (C-γ Asp, C-1 Asp, C-1 Phe). ESIMS ($C_{40}H_{41}N_2O_{10}SNa$): *m*/*z*=731.3 (100%) [M−isobutene+Na]$^+$, 765.4 (18%) [M+H]$^+$, 787.4 (51%) [M+Na]$^+$, calcd: 764.3. Anal. Calcd for $C_{40}H_{41}N_2O_{10}SNa\cdot 2.5H_2O$ (764.8·2.5H_2O): C, 59.33; H, 5.73; N, 3.45. Found: C, 59.21; H, 5.88; N, 3.55.

4.1.18. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-sulfomethyl)phenylalanyl-L-(4-*O*-benzyl)-aspartic acid 22. Dipeptide **21** (451 mg, 0.590 mmol) was dissolved in 20 mL of TFA/CH_2Cl_2 (1:1). Water (200 μL) was added, and the solution stirred for 1 h at room temperature. The solvents were removed in vacuo by repeated co-distillation with toluene. The crude **22** was purified by column chromatography in CH_2Cl_2/MeOH/AcOH/H_2O 60:8:1:1.Yield: 296 mg (73%), colorless amorphous solid; R_f=0.15 (CH_2Cl_2/MeOH/AcOH/H_2O 50:8:1:1); analyt. HPLC (column: VYDAC C18): gradient: 1% CH_3CN/99% H_2O→50% CH_3CN/50% H_2O in 50 min, t_R=32.2 min; $[\alpha]_D^{22}$ −7.3 (*c* 1, H_3CCN/H_2O 1:2). ^{1}H NMR (400 MHz, D_2O/CD_3CN 1:2), δ 2.85–3.40 (m, 4H, H-β_a Phe, H-β_b Phe, H-β_a Asp, H-β_b Asp); 4.21–4.62 (m, 6H, –CH_2–Fmoc, –CH_2SO_3H, H-9 Fmoc, H-α Phe); 4.72–4.88 (m, 1H, H-α Asp); 5.10–5.26 (m, 2H, –CH_2Bn); 7.30–8.00 (m, 17H, H–Ar). ^{13}C NMR (100.6 MHz, D_2O/CD_3CN 1:2), BB, δ 37.71 (C-β Asp); 38.19 (C-β Phe); 47.61 (C-9 Fmoc); 52.41 (C-α Asp); 56.99 (C-α Phe); 57.57 (–CH_2SO_3H); 67.55, 67.64 (–O–CH_2Bn, –CH_2–Fmoc); 120.99 (C-4 Fmoc, C-5 Fmoc); 126.01 (C-1 Fmoc, C-8 Fmoc); 128.30, 128.88, 128.92, 129.17, 129.55, 130.19, 131.46 (C–Ar); 131.95 (C-*ipso*$_{-CH2SO3H}$); 136.72, 137.47 (C-*ipso*$_{-CH2-Bn}$, C-*ipso*$_{C-\beta Phe}$); 141.97 (C-4a Fmoc, C-4b Fmoc); 144.68 (C-8a Fmoc, C-9a Fmoc); 157.69 (–NH–*C*O–O–Fmoc); 172.92, 173.42 (C-γ Asp, C-1 Phe), 176.52 (C-1 Asp). ESIMS (negative mode, $C_{36}H_{34}N_2O_{10}S$): *m*/*z*=685.3 (20%) [M−H]$^-$, 707.2 (100%) [M+Na−2H]$^-$; calcd: 686.3. MALDIMS (dhb): *m*/*z*= 709.7 [M+Na]$^+$, 731.7 [M−H+2Na]$^+$, 753.7 [M−2H+3Na]$^+$, 1418.5 [2M−H+2Na]$^+$, 1440.9 [2M−2H+3Na]$^+$, 1463.2 [2M−3H+4Na]$^+$, 1485.4 [2M−4H+5Na]$^+$ calcd: 686.7.

4.1.19. *N*-Fluorenyl-9-methoxycarbonyl-L-(*p*-sulfomethyl)phenylalaninyl-L-(4-*O*-benzyl)-aspartyl-L-phenylalanyl-L-leucyl-L-prolyl-L-(5-*O*-benzyl)-glutamyl-*N*^{4}-{14-(2-acetamido-2-deoxy-6-*O*-benzyl-3-*O*-(α-L-tri-*O*-benzyl-fucopyranosyl)-4-*O*-(2,4-di-*O*-acetyl-6-*O*-benzyl-[methyl 5‴-acetamido-4,7,8,9,-tetra-*O*-acetyl-3‴,5‴-dideoxy-α-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-α/β-D-glucopyranosyl)-aminocarbonyl-3,6,9,12-tetra-oxa-tetradecyl}-L-asparaginyl-L-(5-*O*-benzyl)-glutaminic acid amide 23. (Fmoc–Phe(*p*-CH_2SO_3H)-Asp-Phe-Leu-Pro-Glu(OBn)-Asn(glycol$_4$-$(CH_2)_2$CO-β-$Ac_6Bn_5sLe^xCOOMe$)-Glu(OBn)–NH_2).

Fmoc protected glycohexapeptide **19** (108 mg, 0.037 mmol) was stirred at room temperature in a mixture of DMF (8 mL) and morpholine (8 mL) for 1 h. The solvents were evaporated in vacuo, and the residue was co-distilled with toluene (three times 10 mL) and lyophilized from benzene. The remaining colorless solid (131 mg) was immediately used for coupling with dipeptide **22**.

To this end, **22** (45.3 mg, 0.066 mmol), HOBt (10.1 mg, 0.066 mmol), diisopropyl-ethylamine (45.2 μL, 0.264 mmol), and PyBOP[29] (34.3 mg, 0.066 mmol) in DMF (3 mL) were added to the solution of the amino-deblocked glycohexypeptide (88.9 mg, 0.025 mmol) in 3 mL of DMF. The mixture was stirred at room temperature for 6 h, DMF was distilled off in vacuum and the residue co-distilled three times with 10 mL of toluene. The crude product was purified by preparative HPLC (column: Eurospher C18, gradient: 30% H_3CCN/70% H_2O→70% H_3CN/30% H_2O in 140 min, t_R=88.3 min). Yield: 27.6 mg (33%); colorless amorphous solid; R_f=0.46 (CH_2Cl_2/MeOH/AcOH 8:1:0.1); analyt. HPLC (column: VYDAC C18): gradient: 40% CH_3CN/60% H_2O→60% CH_3CN/40% H_2O in 50 min, t_R= 33.8 min; $[\alpha]_D^{22}$ −28.4 (*c* 1, DMSO); amino-deblocked hexapeptide (40.0 mg,470.014 mmol, 47%) was re-collected. ^{1}H NMR (600 MHz, DMSO), ^{1}H–^{1}H COSY: δ 0.78–0.85 (m, 6H, H-δ_a Leu, H-δ_b Leu); 1.18 (d, 3H, $J_{H\text{-}6',H\text{-}5'}$= 5.9 Hz, H-6′); 1.34 (t, 1H, $J_{H3'''a,H\text{-}3'''e} \approx J_{H\text{-}3'''a,H\text{-}4'''}$= 11.4 Hz, H-3‴a); 1.38–1.46 (m, 2H, H-β_a Leu, H-β_b Leu); 1.59 (m$_c$, 1H, H-γ Leu); 1.67 (s, 3H, –COCH_3); 1.70–2.09 (m, 8H, H-γ_a Pro, H-γ_b Pro, H-β_a Pro, H-β_b Pro, H-β_a Glu–NH_2, H-β_b Glu–NH_2, 2×H-γ Glu/Glu–NH_2); 1.81 (s, 3H, –COCH_3); 1.88 (s, 3H, –COCH_3); 1.91 (s, 3H, –COCH_3); 1.93 (s, 3H, –COCH_3); 1.96 (s, 3H, –COCH_3); 1.98 (s, 3H, –COCH_3); 2.20 (s, 3H, –COCH_3); 2.28–2.42 (m, 5H, 2×H-γ Glu/Glu–NH_2, –O–CH_2CH_2–CO–NH–, H-β_a Glu); 2.45 (m$_c$, 1H, 3‴e); 2.51–2.62 (m, 3H, H-β_b Glu, 2×H-β Asp/Asn); 2.66–2.74 (m, 1H, H-β Phe/Phe(*p*-CH_2SO_3H)); 2.74–2.82 (m, 2H, H-β Asp/Asn, H-β Phe/Phe(*p*-CH_2SO_3H)); 2.83–2.90 (m, 1H, H-β Phe/Phe(*p*-CH_2SO_3H)); 1.94–3.02 (m, 1H, H-β Phe/Phe(*p*-CH_2SO_3H)); 3.08–3.21 (m, 2H, –O–CH_2CH_2–NH–); 3.30–3.38 (m, 3H, H-6″a, –O–C$H_2$$CH_2$–NH–); 3.38–4.43 (m, 1H, H-δ_a Pro); 3.42–3.50 (m, 13H, H-4′, –O–CH_2–glycol); 3.50–3.65 (m, 7H, –CH_2SO_3H, H-6″b, H-δ_b Pro, H-6‴, –CH_2–CH_2–CO–NH–); 3.65–3.90 (m, 9H, H-5‴, H-5″, H-2′, H-3′, H-3, H-4, H-5, H-6a, H-6b); 3.76 (s, 3H, –COOCH_3); 3.97 (dd, 1H, $J_{H\text{-}9'''a,H\text{-}9'''b}$=12.6 Hz, $J_{H\text{-}9'''a,H\text{-}8'''}$=4.8 Hz, H-9‴a); 4.03 (dd, 1H, J_{vic}=7.0 Hz, J_{gem}=9.2 Hz, –CH_{2a}–Fmoc); 4.07–4.25 (m, 6H, H-9 Fmoc, –CH_{2b}–Fmoc, H-9‴b, H-α Glu–NH_2; H-α Phe/Phe(*p*-CH_2SO_3H), H-2); 4.27 (d, 1H, J_{gem}=13.9 Hz, –CH_2–Bn); 4.31–4.46 (m, 5H, 3×–CH_2Bn, H-α Asp/Asn, H-α Pro); 4.48–4.58 (m, 3H, H-α Phe/Phe(*p*-CH_2SO_3H), H-α Leu, –CH_2Bn); 4.59–4.72 (m, 6H, H-α Asp/Asn, H-α Glu, H-3″, 3×–CH_2Bn); 4.73–4.82 (m, 4H, H-5′, H-4‴, H-1″, H-2″); 4.85 (d, 1H, J_{gem}=13.4 Hz, –CH_2Bn); 4.93 (s, br, 1H, H-1); 4.99–5.08 (m, 7H, 6×–CH_2Bn, H-4″); 5.21 (dd, 1H, $J_{H\text{-}7''',H\text{-}6'''}$=2.6 Hz, $J_{H\text{-}7''',H\text{-}8'''}$=9.1 Hz, H-7‴); 5.33 (s, br, 1H, H-1′); 5.47 (m$_c$, 1H, H-8‴); 7.08–7.41 (m, 53H, H–Ar); 7.58–7.67 (m, 4H, 2×–NH–, 2H–Ar); 7.82–7.99 (m, 4H, 2×–NH–, 2H–Ar); 8.05–8.32 (m, 6H, –NH–); 8.42–8.50 (m, 4H, –NH–).

Characteristic peaks in the ^{13}C NMR (100.6 MHz, $CDCl_3$), ^{1}H–^{13}C COSY: δ 16.2 (C-6′); 20.1, 20.2, 20.3, 2×20.4, 20.8, 22.3, 22.9 (–CO*C*H_3); 21.7 (C-δ Leu); 22.9 (C-δ Leu); 23.8 (C-γ Leu); 24.5 (C-γ Pro); 26.6, 27.0 (C-β Glu, C-β Glu–NH_2); 29.0 (C-β Pro); 30.0 (–O–CH_2*C*H_2–CO–, C-γ Glu/Glu–NH_2); 36.9 (C-β Phe/Phe(*p*-CH_2SO_3H)); 37.7 (C-3‴); 38.6 (–O–CH_2*C*H_2–NH–); 40.0 (C-β Leu); 46.3 (C-9 Fmoc); 46.7 (C-δ Pro); 47.2 (C-5‴); 48.5 (C-α Leu); 50.00 (C-α Asp/Asn); 52.8 (–O*C*H_3); 57.2 (–*C*H_2SO_3H); 59.0 (C-α Pro); 61.9 (C-9‴); 66.7 (C-7‴); 66.8 (C-8‴); 67.1 (C-4″); 69.6 (C-4‴); 71.1 (C-6‴); 75.9

(C-2′); 76.0 (C-4′); 78.5 (C-3′); 78.8 (C-1); 96.0 (C-1′); 99.2 (C-1″). MALDIMS ($C_{174}H_{209}N_{13}O_{53}S$): *m/z*=3384.9 (100%) $[M+Na]^+$, 3405.9 $[M-H+2Na]^+$, 3421.8 $[M-H+Na+K]^+$, calcd: 3362.6.

4.1.20. L-(*p*-Sulfomethyl)phenylalaninyl-L-aspartyl-L-phenylalanyl-L-leucyl-L-prolyl-L-glutamyl-*N*^{4}-{14-(2-acetamido-3-*O*-(α-L-fucopyranosyl)-2-deoxy-4-*O*-([5‴-acetamido-3‴,5‴-dideoxy-α-D-galacto-2‴-nonulopyranosylate]-β-D-galactopyranosyl)-α/β-D-glucopyranosyl)-aminocarbonyl-3,6,9,12-tetra-oxa-tetradecyl}-L-asparaginyl-L-(5-*O*-benzyl)-glutaminic acid amide 24. (H–Phe(*p*-CH_2SO_3H)-Asp-Phe-Leu-Pro-Glu-Asn(glycol$_4$-$(CH_2)_2$CO-β-sLe^{x}COOH)-Glu–NH_2).

Glycooctapeptide conjugate **23** (16.1 mg, 4.79 μmol) was dissolved in a mixture of 25 mL of methanol, 0.5 mL of acetic acid, and 0.2 mL of water. Catalytic amounts (10 mg) of Pearlman's catalyst ($Pd(OH)_2$) were added. After evacuation the stirred mixture was hydrogenated (balloon) for 48 h. According to MALDIMS analysis of a probe, the compound was completely debenzylated (*m/z* calcd: 2419.1, found: 2464.1 $[M-H+2Na]^+$) after this time. The mixture was filtered through Celite, the solvent evaporated and the residue co-distilled with toluene (three times with 5 mL). The debenzylated product was dissolved in methanol (30 mL). To the solution 0.1 M NaOMe in MeOH was added until a pH of 10 was reached (about 2 mL). The solution was stirred at room temperature for 15 h, neutralized by addition of solid carbon dioxide and the solvent was evaporated in vacuo. The residue was dissolved in 10 mL of water and adjusted to pH 10–10.5 by dropwise addition of diluted aq NaOH. After stirring for 16 h at room temperature, solid carbon dioxide was added for neutralization, and the solvent was evaporated in vacuo. The crude product was purified by chromatography in water through a Sephadex LH-15 column and lyophilized. Yield: 5.7 mg (55%); colorless amorphous solid; R_f=0.75 (MeOH/H_2O 1:1, RP C18-DC); $[\alpha]_D^{22}$ −42.4 (*c* 0.5, H_2O). ^{1}H NMR (600 MHz, D_2O), ^{1}H–^{1}H COSY: δ 0.87–0.98 (m, 6H, H-δ_a Leu, H-δ_b Leu); 1.17 (d, 3H, $J_{6',5'}$=6.8 Hz, H-6′); 1.48–1.72 (m, 3H, H-β_a Leu, H-β_b Leu, H-γ Leu); 1.79 (t, 1H, $J_{3'''a,3'''e} \approx J_{3'''a,4'''}$=12.2 Hz, H-3‴a); 1.98 (s, 3H, –COC*H*$_3$); 2.02 (s, 3H –COC*H*$_3$); 1.86–2.19 (m, 7H, H-β_a Glu, H-β_b Glu, H-β_a Glu–NH_2, H-β_b Glu–NH_2, H-β_a Pro, H-γ_a Pro, H-γ_b Pro); 2.25–2.50 (m, 4H, H-γ_a Glu, H-γ_b Glu, H-γ_a Glu–NH_2, H-γ_b Glu–NH_2, H-β_b Pro); 2.51–2.58 (m, 2H, –O–CH_2C*H*$_2$–CO–); 2.76 (dd, 1H, $J_{3'''e,3'''a}$=12.2 Hz, $J_{3'''e,4}$=4.3 Hz, H-3‴e); 2.71–2.86 (m, 2H, H-β_a Asn, H-β_b Asn); 2.88–3.22 (m, 4H, H-β_a Phe/Phe(*p*-CH_2SO_3H), H-β_a Phe/Phe(*p*-CH_2SO_3H), H-β_a Asp, H-β_b Asp); 3.30–3.47 (m, 2H, –O–CH_2C*H*$_2$–NH–); 3.53 (t, 1H, $J_{2'',3''} \approx J_{2'',1''}$=8.8 Hz, H-2″); 3.55–3.80 (m, 28 H, H-δ_a Pro, H-δ_b Pro, –O–C*H*$_2$–glycol, H-5, H-5″, H-6″a, H-6″b, H-2′, H-4′, H-4‴, H-6‴, H-7‴, H-9‴a); 3.80–3.99 (m, 9H, H-3, H-4, H-6a, H-6b, H-3′, H-4″, H-5‴, H-8‴, H-9‴b); 4.03 (m, 1H, H-2); 4.08 (dd, 1H, $J_{3'',4''}$=2.4 Hz, $J_{3'',2''}$=9.8 Hz, H-3″); 4.14–4.47 (m, 5H, H-α Phe, H-α Phe(*p*-CH_2SO_3H), –C*H*$_2$$SO_3H$, H-α Pro); 4.54 (d, 1H, $J_{1'',2''}$=7.9 Hz, H-1″); 4.60–4.77 (m, 3H, H-α Asp, H-α Asn, H-α Leu); 4.85 (m_c, 1H, H-5′); 5.11 (d, 1H, $J_{1',2'}$=3.8 Hz, H-1′); 5.13 (d, 1H, $J_{1,2}$=9.7 Hz, H-1); 7.17–7.54 (m, 10H, H–Ar). Characteristic peaks of the ^{13}C NMR (150.9 MHz, D_2O), ^{1}H–^{13}C COSY: δ 15.3 (C-6′); 22.0, 22.1 (*C*H_3CO–); 24.5 (C-γ Leu); 26.9 (C-γ Glu, Glu–NH_2); 28.8 (C-β Pro); 32.2 (C-γ Glu, Glu–NH_2); 36.2 (–O–CH_2–*C*H_2–CO–); 36.9 (C-β Asn), 37.0 (C-β Phe/Phe(*p*-CH_2SO_3H); 39.0 (–O–CH_2–*C*H_2–NH–); 39.7 (C-β Leu); 40.01 (C-3‴); 48.1 (C-γ Pro); 50.1, 50.9, 55.0 (C-α Asp, C-α Asn, C-α Leu); 51.9 (C-5‴); 53.2, 53.7 (C-α Phe, C-α Phe(*p*-CH_2SO_3H)); 55.0 (C-2); 56.7 (–*C*H_2SO_3H); 59.9 (C-6); 60.6 (C-α Pro); 61.7 (C-6″); 62.8 (C-9‴); 67.0 (C-5′); 69.5 (C-2″); 75.9 (C-3′); 78.4 (C-1); 99.1 (C-1′); 102.0 (C-1″). MALDIMS ($C_{90}H_{137}N_{13}O_{45}S$): *m/z*=2198.0 $[M-H+2Na]^+$, 2220.0 $[M-2H+3Na]^+$, calcd: 2151.9.

References and notes

1. For review see: (a) Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833; (b) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **2001**, *57*, 207; (c) Ehrhardt, C.; Kuener, C.; Bakowsky, U. *Adv. Drug Delivery Rev.* **2004**, *56*, 527.
2. McEver, R. P.; Beckstead, J. H.; Moore, K. L.; Marshal-Carlson, L.; Bainton, D. F. *J. Clin. Invest.* **1989**, *84*, 92.
3. Bevilacqua, M. P.; Stengelin, S.; Gimbrone, M. A.; Seed, B. *Science* **1989**, *243*, 1160.
4. Philips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. *Science* **1990**, *250*, 1130.
5. Tedder, T. F.; Steeber, T. A.; Chen, A.; Engel, P. *FASEB J.* **1995**, *9*, 866.
6. Pahl, K. D.; Nollert, M. U.; McEver, R. P. *J. Cell Biol.* **1995**, *131*, 1893.
7. (a) Moore, K. L.; Stults, N. L.; Diaz, S.; Smith, D. F.; Cummings, R. D.; Varki, A.; McEver, R. P. *J. Cell. Biol.* **1992**, *118*, 445; (b) McEver, R. P.; Cummings, R. D. *J. Clin. Invest.* **1997**, *100*, 485.
8. (a) Sako, D.; Comess, K. M.; Barone, K. M.; Camphausen, R. T.; Cummings, B. A.; Shaw, G. D. *Cell* **1995**, *83*, 232; (b) Ponyami, T.; Seed, B. *Cell* **1995**, *83*, 333.
9. Kumar, R.; Camphausen, R. T.; Sullivan, F. X.; Cumming, D. A. *Blood* **1996**, *88*, 3872.
10. Ramachandran, V.; Nollert, M. U.; Qui, H.; Liu, W.-J.; Cummings, R. D.; Zhu, C.; McEver, R. P. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13771.
11. Somers, W. S.; Tang, J.; Shaw, G. D.; Camphausen, R. T. *Cell* **2000**, *103*, 467.
12. (a) Leppänen, A.; Mehta, P.; Onyang, Y.-B.; Ju, T.; Helin, J.; Moore, K. L.; van Die, J.; Canfield, W. M.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **1999**, *274*, 24838; (b) Koeller, K. M.; Smith, M. E. B.; Huang, R.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 4241.
13. Koeller, K. M.; Smith, M. E. B.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 742.
14. (a) Gonzalez-Muniz, R.; Cornille, F.; Bergeron, F.; Ficheux, D.; Pothier, J.; Durieux, C.; Roques, B. P. *Int. J. Pept. Protein Res.* **1991**, *37*, 331; (b) Marseigne, I.; Roque, B. P. *J. Org. Chem.* **1988**, *53*, 3621.
15. Rösch, A.; Kunz, H. *Carbohydr. Res.* **2006**, *341*, 1597.
16. (a) Smith, S. C.; Sloane, N. H. *Biochim. Biophys. Acta* **1967**, *148*, 414; (b) Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354.
17. Brenner, M.; Huber, W. *Helv. Chim. Acta* **1953**, *36*, 1109.

18. ten Kortenaar, P. B. W.; van Dijk, B. G.; Peters, J. M.; Raaben, B. J.; Hans, P. J.; Adams, M.; Tesser, G. F. *Int. J. Pept. Protein Res.* **1986**, *27*, 398.
19. Machini Miranda, M. T.; Liddle, R. A.; Rivier, J. E. *J. Med. Chem.* **1993**, *36*, 1681.
20. Shieh, W.-C.; Carlson, J. A. *J. Org. Chem.* **1992**, *57*, 379.
21. Takagi, K.; Sakakibara, Y. *Chem. Lett.* **1989**, 1957.
22. Morera, E.; Ortar, G.; Varani, A. *Synth. Commun.* **1998**, *28*, 4279.
23. Wang, S.-W. *J. Am. Chem. Soc.* **1973**, *95*, 1328.
24. Blankemeyer-Menge, B.; Nimtz, M.; Frank, R. *Tetrahedron Lett.* **1990**, *31*, 1701.
25. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927.
26. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.
27. Herzner, H.; Kunz, H. *Carbohydr. Res.* **2007**, *342*, 541.
28. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205.
29. Liebe, B.; Kunz, H. *Angew. Chem., Int. Ed.* **1997**, *36*, 618.
30. Sprengard, U.; Kretzschmar, G.; Bartnik, E.; Hüls, C.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 990.
31. Yang, C. C.; Merrifield, R. B. *J. Org. Chem.* **1976**, *51*, 1032.