Synthesis of Peptide and Glycopeptide Partial Structures of the Homophilic Recognition Domain of Epithelial Cadherin

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Received 15 July 2003; revised 27 August 2003

Dedicated to Professor Gunter Fischer on the occasion of his 60th birthday.

Abstract: Peptide and glycopeptide sequences of the homophilic recognition site of epithelial cadherin (E-cadherin) were synthesized by solid-phase technique based on acid-sensitive Wang anchor according to Fmoc strategy. Fmoc serine building blocks with T_N-, T-, (2-6)-sialyl T-antigen and β-*N*-acetylglucosamine side chains were prepared for the construction of E-cadherin glycopeptides. The T- and (2-6)-sialylT-serine derivatives have been obtained by chemical glycosylations of the T_N-antigen serine derivative carrying Fmoc/*tert*-butyl ester protecting group combination. According to NOESY and ROESY NMR experiments, E-cadherin(glyco)peptides not acylated at the N-terminus prefer turn-type conformations in water.

Key words: peptides, glycopeptides, solid-phase synthesis, glycosylation, oligosaccharides

Introduction

Cadherins constitute a class of recently discovered calcium-dependent cell adhesion molecules which play crucial roles in cell adhesion processes.[1] Their interactions result in homophilic cell adhesion in tissues between cells expressing the same type of cadherin on their surfaces. Cadherins are also involved in other important cellular processes including differentiation,[2] growth regulation and morphogenesis.[3–5] A down-regulation of cadherins can cause invasiveness of cancer cells,[6–10] while CD44-mediated adhesion is increased in tumours.[6,7]A member of the classical cadherin family is epithelial cadherin (E-cadherin).

Its extracellular portion consists of five domains of which the terminal E-CAD1 is considered responsible for the homotypic and homophilic cell adhesion activity.[11,12] The recognition between E-cadherin molecules proceeds via formation of dimers.[12,13] The proposed specific binding site is embedded into a surface formed by three antiparallel β-sheets including the cell adhesion motif His-Ala-Val presented in a well-defined steric arrangement (Figure 1).[11] Two of these antiparallel β-sheets are linked by a β-turn-type structure (Ser^{83}- Gly^{85}) (Figure 2). As recently reported, biological evaluation showed that synthetic (glyco)peptides of this region induce differentiation in transformed HaCaT keratinocytes.[14]

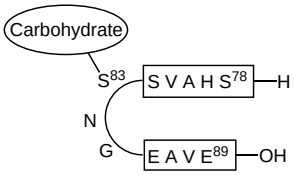


Figure 2 Partial stucture from the β-turn region of the homophilic recognition site of E-CAD1

In this case the differentiation marker proteins involucrin and contactinhibin receptor were up-regulated after treatment of the transformed cells with a synthetic glycopeptide from the proposed homophilic binding site whereas the expression of the tumour-associated receptor CD44 was down-regulated at the same time. However, to date it has not been possible to exert influence on the cell adhe-

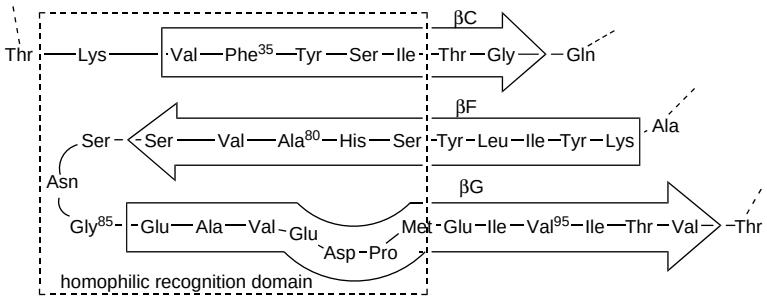


Figure 1 Homophilic recognition domain of E-cadherin in E-CAD1[11]

SYNTHESIS 2003, No. 16, pp 2487–2502
Advanced online publication: 14.10.2003
DOI: 10.1055/s-2003-42429; Art ID: T06903SS

sion processes. Thus, our goal was to synthesize a variety of partial structures from the β-turn region of the homophilic recognition site of E-CAD1 (Figure 2) in order to investigate structural details of E-cadherin-mediated cell adhesion. Results of such experiments are interesting because of the importance of E-cadherin in processes like morphogenesis, tumour development and self-recognition. Glycosylation of the recognition sequence on serine 83 with carbohydrate residues of different sterical demand should induce or support the formation of β-turn-type structures and, thus, influence the cell biological effects.[14]

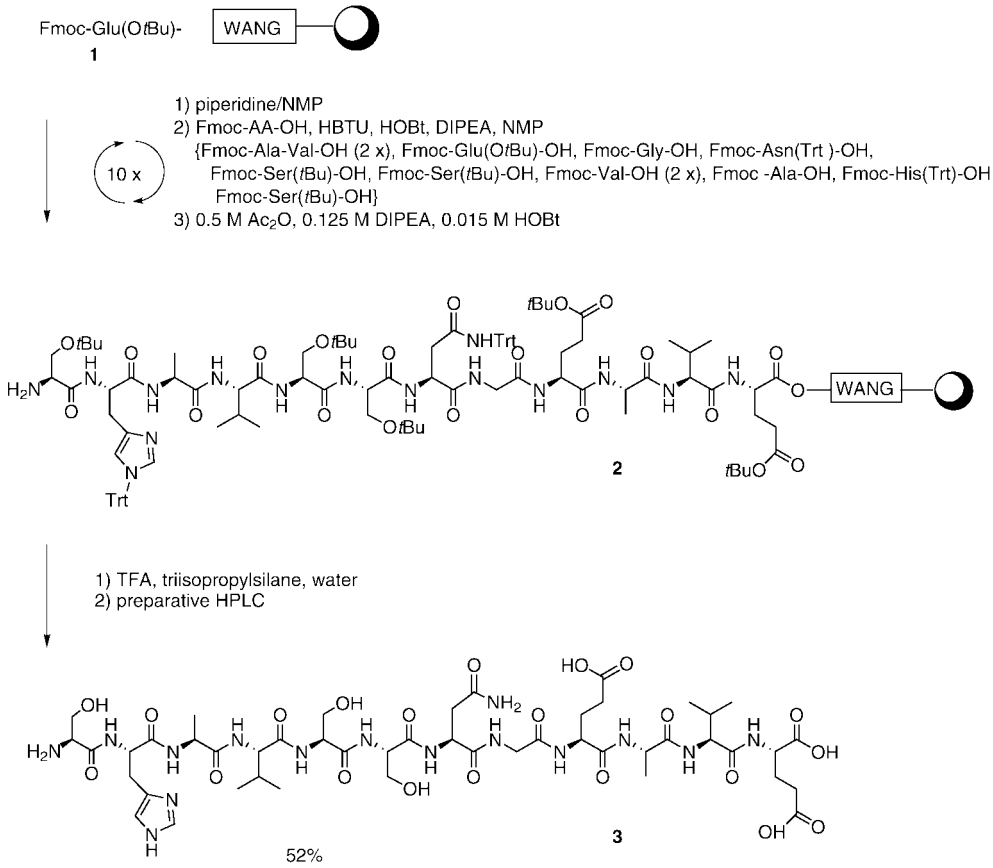
Results and discussion

The peptide and glycopeptide sequences of the homophilic recognition site of E-cadherin were synthesized by automated solid-phase methods. As the solid support either Tentagel® or polystyrene equipped with the acid-labile Wang anchor and loaded with the Fmoc-protected starting amino acid were used. The syntheses were carried out in a peptide synthesizer. The Fmoc-protected amino acids were used in an excess of 10 equivalents, HBTU/HOBt served as coupling reagent (coupling time 20–30 min). Fmoc groups were removed with piperidine in 1-methyl-2-pyrrolidinone (NMP) (20%). The glycosylated amino acid building blocks were synthesized in multi-step reactions and manually coupled to the resin-linked peptide.

Peptide Syntheses

The first synthesis of the dodecapeptide Ser[78]-Glu[89] suffered from low coupling yields from the eighth coupling step onward (Val[81]) obviously as a result of backfolding phenomena.[14] It was possible to overcome these difficulties by utilizing double coupling steps and extended coupling times during the automated synthesis.

For the synthesis of the resin-linked dodecapeptide Ser[78]-Glu[89] (**2**), the standard protocol was modified (Scheme 1). In order to avoid the formation of diketopiperazines, the second and third amino acids (Ala-Val) were introduced as a dipeptide building block in a double coupling. UV-monitoring of the fluorenylmethylpiperidine indicated difficulties in the Fmoc-removal from the ninth amino acid onward. Due to these difficulties, the valine residue 81 was also introduced in a double coupling step. The desired peptide **3** was obtained in an overall yield of 52% after treatment of the resin-linked form **2** with a mixture of trifluoroacetic acid, triisopropylsilane and water (15:0.9:0.9) and subsequent purification by preparative RP-HPLC. For further investigations of the adhesion



Scheme 1 Wang resin: *p*-hydroxybenzyl (PHB) ester; HBTU: *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,N′-tetramethyluronium hexafluorophosphate; HOBt: 1-hydroxybenzotriazole; DIPE: diisopropylethylamine (Hünig's base)

properties of the synthetic structures, a peptide Ser[78]-Glu[89] **4** containing a fluorescent label was synthesized (Figure 3). The dansyl-lysine conjugate[15] was coupled to the *N*-terminus of the solid-phase-bound dodecapeptide. The condensation gave a *N*-terminally labelled structure still carrying a free *N*-terminus considered essential for the biological activity of the synthetic peptides.[14] The synthesis was carried out as described for **3**. The ε-dansyl-lysine was reacted with immobilized dodecapeptide in a threefold excess using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate/1-hydroxy-7-azabenzotriazole (HATU/HOAt) as the activating reagent.[16] After acidolytic release from the resin, the peptide **4** was isolated by preparative RP-HPLC in an overall yield of 75%. In addition, the non-labelled tridecapeptide Lys-Ser[78]-Glu[89] **5** was also synthesized (yield: 84%) in order to assure that potential activity in biological assays is independent of the fluorescent dye.

Synthesis of the Glycosylated Building Blocks

The synthesis of the glycosylated amino acids started from Fmoc serine *tert*-butyl ester [Fmoc-Ser(OH)-O-*t*-Bu] **6** obtained from Fmoc serine in one step (Scheme 2).[17] Glycosylation of **6** was achieved using the 2-azidogalactosyl bromide (**7**) of Paulsen and Hölck.[18] The lacking neighboring group-participation favors the formation of the desired α-glycoside **8**. Subsequently, the azide **8** was reduced and N-acetylated with activated zinc in a mixture of THF, acetic anhydride and acetic acid (3:2:1),[19] to give **9**. The synthesis of the monosaccharide T_N-antigen serine building block **10** was completed by the acidolysis of the *tert*-butyl ester.

In order to furnish a suitably protected acceptor for the synthesis of the Thomsen–Friedenreich (T)-antigen disaccharide,[20] the acetyl groups in positions 3, 4, and 6 of **9** were selectively removed by careful Zemplén transesterification (pH 8.5).[21] An accurate adjustment of the pH is mandatory to avoid simultaneous cleavage of the base-labile Fmoc-group. The crude product was then reacted with *p*-anisaldehyde dimethylacetal to yield compound **11**. Several glycosylation methods had to be examined for the generation of the β-linked T-antigen disaccharide. Koenigs–Knorr glycosylation[22] and reactions with trichloroacetimidate[23] resulted in the formation of complex mixtures and undesired ortho ester, respectively. The Helferich glycosylation[24] with glycosyl bromide **12** and mercury cyanide as the activator in a mixture of dichloromethane and nitromethane proved most effective. The disaccharide **13** was isolated in a yield of 80%. Subsequently, the *p*-methoxybenzylidene acetal was removed by treatment with ceric ammonium nitrate[25] in acetonitrile to furnish the disaccharide **14** with deprotected 4- and 6-position. Acetylation with acetic anhydride and pyridine furnished the completely protected T-antigen unit **15**. *C*-Terminal deprotection was carried out in a mixture of trifluoroacetic acid and anisole to afford the T-antigen building block **16** suitable for solid-phase synthesis.

The disaccharide serine conjugate **14** was also used for the synthesis of the demanding 2,6-sialyl-T-antigen building block (Scheme 3). In contrast to another strategy,[26] the complex sialic acid was introduced at a late stage of the synthesis. Sialylation reactions still are demanding as they often suffer from low yields and low stereoselectivity. Using the xanthate **17** of sialic benzyl ester as donor[27] at low temperature in the presence of acetonitrile proved successful.

The xanthate **17** was applied in an excess of 2.5 equivalents and activated by methylsulfenyl triflate[28] formed in situ from methylsulfenyl bromide[29] and silver triflate at –62 °C. Due to the sterically demanding structure of the xanthate and the enhanced reactivity of the primary hydroxy group the sialylation proceeded regioselectively at OH-6. In addition to the desired α-glycoside **18**, the β-

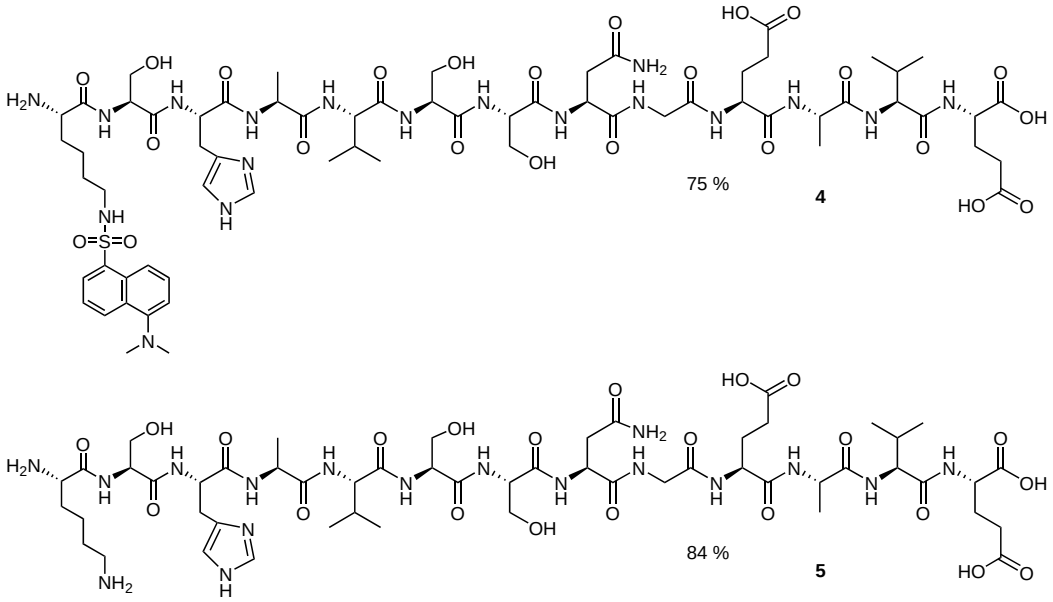
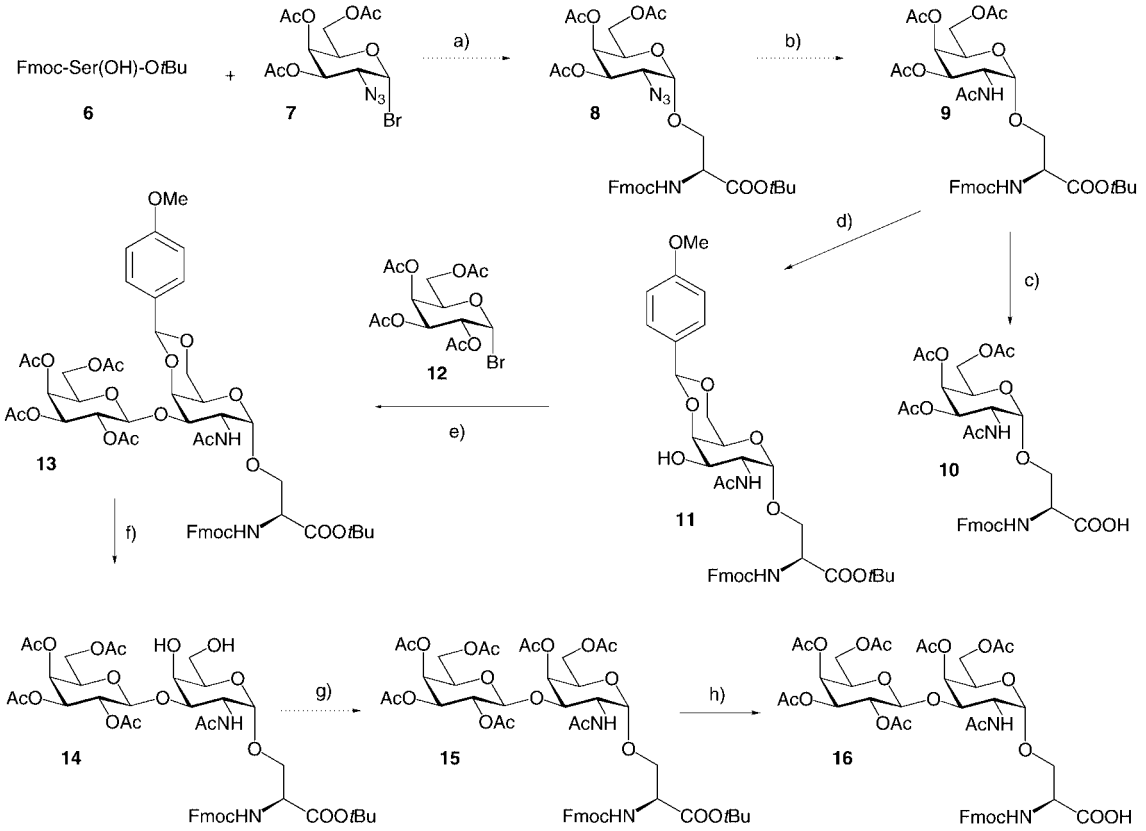
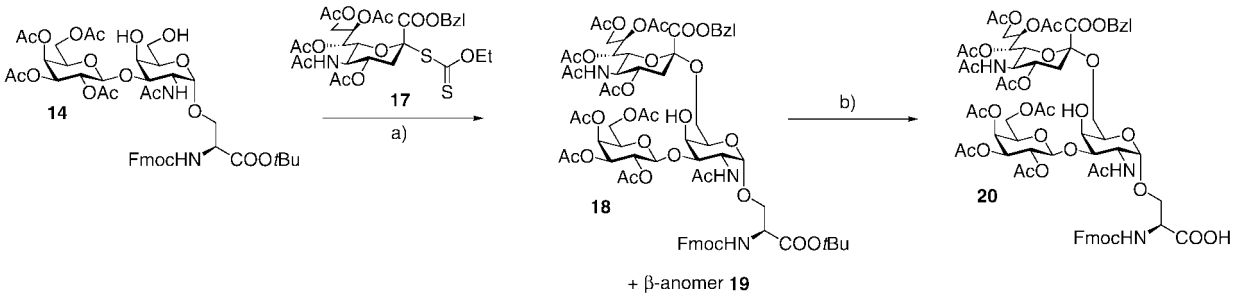


Figure 3 Structures of peptides **4** and **5**



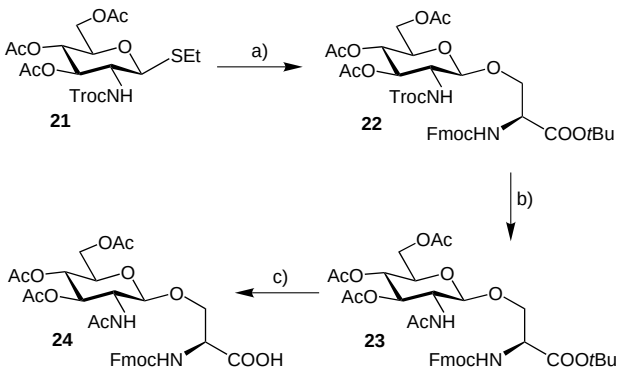
Scheme 2 a) Fmoc-Ser-*t*-BuO, Ag_2CO_3, $AgClO_4$, toluene, CH_2Cl_2; 54%; b) Zn, THF–Ac_2O, AcOH (3:2:1); 92%; c) TFA–anisole (10:1); 89%; d) 1. NaOMe, MeOH, pH 8.5; 2. *p*-anisaldehyde dimethyl acetal, *p*-TosOH, DMF; 52%; e) $Hg(CN)_2$, nitromethane, CH_2Cl_2; 80%; f) $Ce(NH_4)_2(NO_3)_6$, MeCN–H_2O (9:1); 76%; g) Ac_2O–pyridine (1:3); 88%; h) TFA–anisole (10:1); 84%



Scheme 3 a) AgOTf, MeSBr, CH_2Cl_2–MeCN (1:2), 28%; b-anomer: 7%; b) TFA–anisole (10:1), 94%

anomer **19** was also formed in the ratio of 1:4. Purification of the trisaccharide turned out difficult. The sialic glycal also formed as a by-product could only be removed by careful column chromatography. The α- and β-sialyl-T antigens were separated by preparative RP-HPLC yielding the pure α-glycoside **18** in a yield of 28%. Trisaccharide threonine conjugate **18** was deprotected with trifluoroacetic acid and anisole to furnish the building block **20** in a yield of 94%.

As a further variation, a β-linked glucosamine serine conjugate was incorporated into the peptide chain (Scheme 4). To this end, glucosamine hydrochloride was transformed into the β-thioglycoside **21**[30] which was reacted with Fmoc-Ser(OH)-O-*t*-Bu[17] **6** under activation

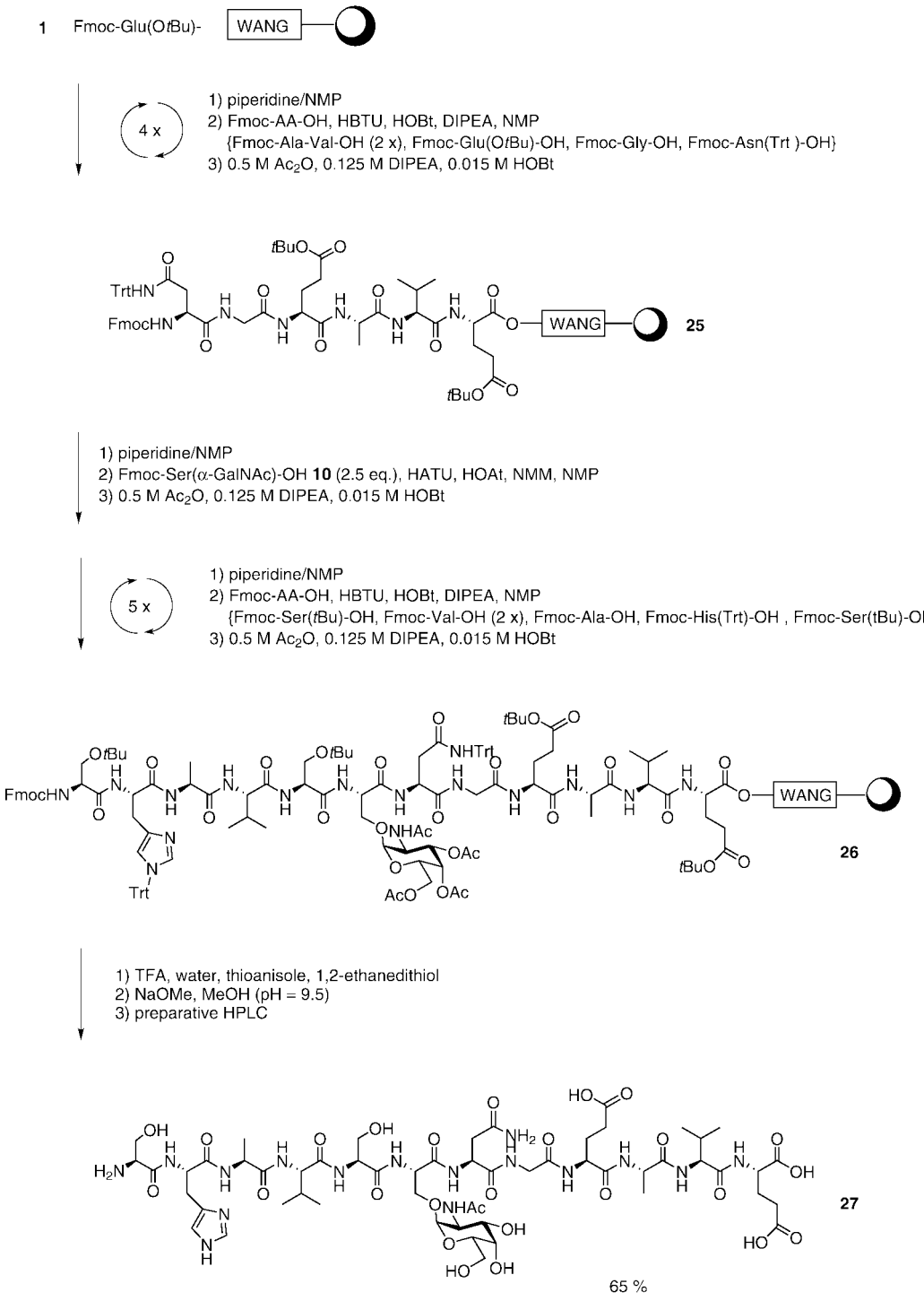


Scheme 4 a) Fmoc-Ser-*t*-BuO, NIS, TfOH, CH_2Cl_2, 80%; b) 1. Zn, AcOH; 2. pyridine, Ac_2O; 86%; c) TFA, anisole; 87%

with *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid[31,32] to furnish the β-glycoside **22** in a yield of 80%. The Troc group was cleaved with zinc in acetic acid, and the resulting glucosamine subsequently acetylated with acetic anhydride to give **23**. Finally, acidolysis of the *tert*-butyl ester was accomplished to give **24** in a yield of 87%.

Glycopeptide Synthesis

The preformed glycosylated amino acid building blocks **10**, **16**, **20** and **24** were incorporated into dodecapeptide sequences derived from the homophilic recognition domain of epithelial cadherin. The first glycopeptide synthesized for testing in biological assays was the glycosylated dodecapeptide **27** (Ser^{78}-Glu^{89}) (Scheme 5).[14]



Scheme 5

The synthesis was carried out in analogy to the procedure described for the non-glycosylated peptides. The glycosylated amino acid **10** was manually coupled to the resin-linked peptide **25** after *N*-terminal deprotection. The T_N-antigen building-block **10** was used in an excess of 2.5 equivalents and activated with HATU/HOAt[16] within 5 hours. The detachment from the resin-linked form **26** was carried out by treatment with trifluoroacetic acid, water, thioanisole and 1,2-ethanedithiol in order to furnish a selectively deprotected glycopeptide. Due to its insolubility, the crude product was further deprotected under Zemplén conditions (pH 9.5) to yield the water-soluble glycododecapeptide **27** in a yield of 65% after purification by preparative RP-HPLC.

For the synthesis of the glycopeptide containing the β-linked serine-glucosamine conjugate, the building block **24** was applied in an excess of two equivalents to couple with N-deprotected **25**. After acidolytic release from the resin, the crude glycopeptide was deacetylated (pH 9.5) to yield the glycopeptide **28** in an overall yield of 51% (Figure 4).

The disaccharide T-antigen serine conjugate **16** (1.3 equiv) was applied to the synthesis of glycopeptide **29** carried out in analogy to the other glycopeptide syntheses. Coupling time for the reaction with the N-deprotected immobilized peptide **25** was extended to 4 hours. The subsequent Fmoc amino acids were coupled according to the standard protocol, but also for an extended reaction time. Due to the low solubility of the resulting glycopeptide, it was deacetylated without further purification. After stirring with a catalytic amount of NaOMe in methanol for 48 hours (monitoring by analytical RP-HPLC), the pure product **29** (Figure 4) was isolated by preparative RP-HPLC in an overall yield of 32%.

In order to further modify the glycan, the sterically demanding 2,6-sialyl-T-antigen serine **20** was incorporated into the dodecapeptide sequence according to the procedure described above (Scheme 6). After release from the solid support, the partially protected glycopeptide **30** was obtained which showed low solubility in common solvents making purification by preparative HPLC impossible. Thus, the benzyl ester protecting group was directly cleaved by hydrogenolysis using palladium on charcoal. After three days the reaction was terminated. The crude product **31** could hence be purified by preparative RP-HPLC. After lyophylization, the desired compound **31** was isolated in a yield of 33%. In the subsequent deprotection, the *O*-acetyl groups were removed by transesterification (pH 9.5). The solution was stirred for 18 hours, carefully monitored by analytical RP-HPLC. The target molecule **32** was obtained in a yield of 61% (overall yield: 20%) after purification by preparative RP-HPLC.

Synthesis of a Dimeric Peptide

To impose influence on cell adhesion phenomena in cells carrying E-cadherin molecules on their surfaces, a dimeric structure of the recognition peptide sequence simulating the native dimerization process was synthesized. To this end, two monomeric nonapeptide units were connected via a disulfide bridge (Scheme 7). The octapeptide Ser^{78}-Gly^{85} was synthesized by solid-phase peptide synthesis as described above and subsequently N-terminally acylated with a cysteine residue.

Polystyrene resin preloaded with Fmoc-Gly-O-PHB was used as solid support. Since cysteine residues are known to be sensitive,[33] the coupling of Fmoc-Cys(Trt)-OH was carried out manually with *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) and HOBt without preactivation in dichloromethane. *sym*-Collidine was used as the base. Subsequently, the peptide was released from the solid support with a mixture of trifluoroacetic acid, triisopropylsilane and water. According

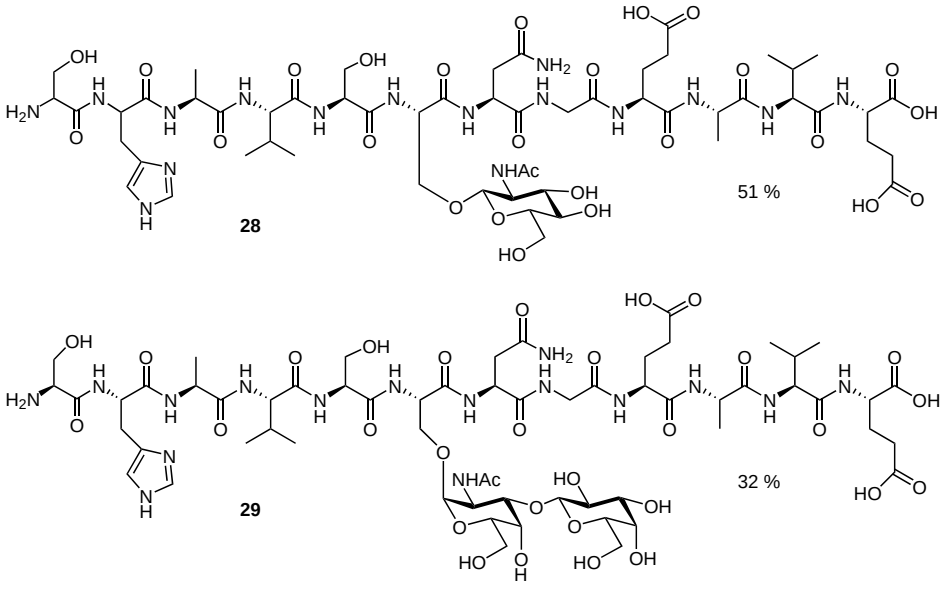
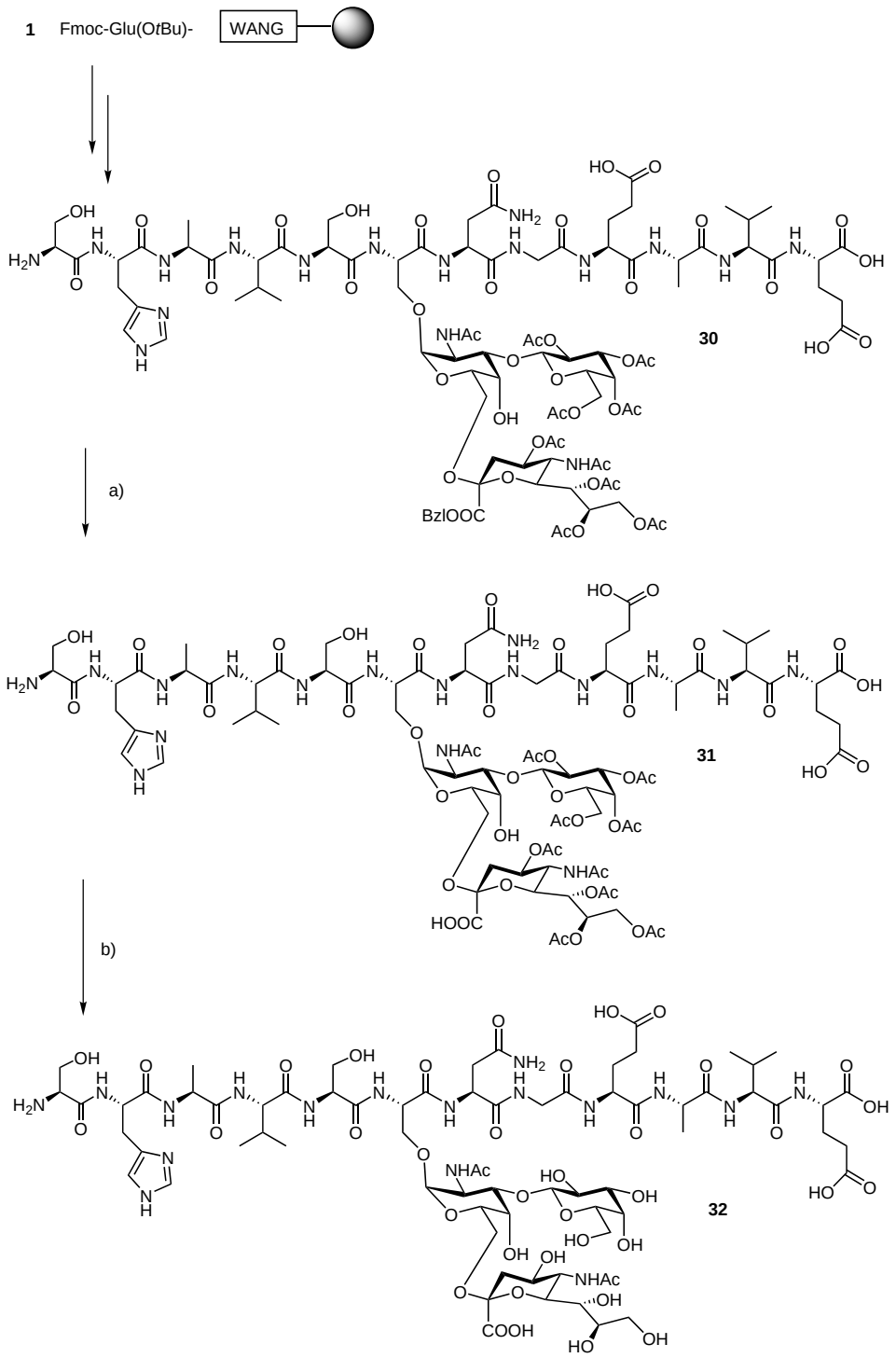


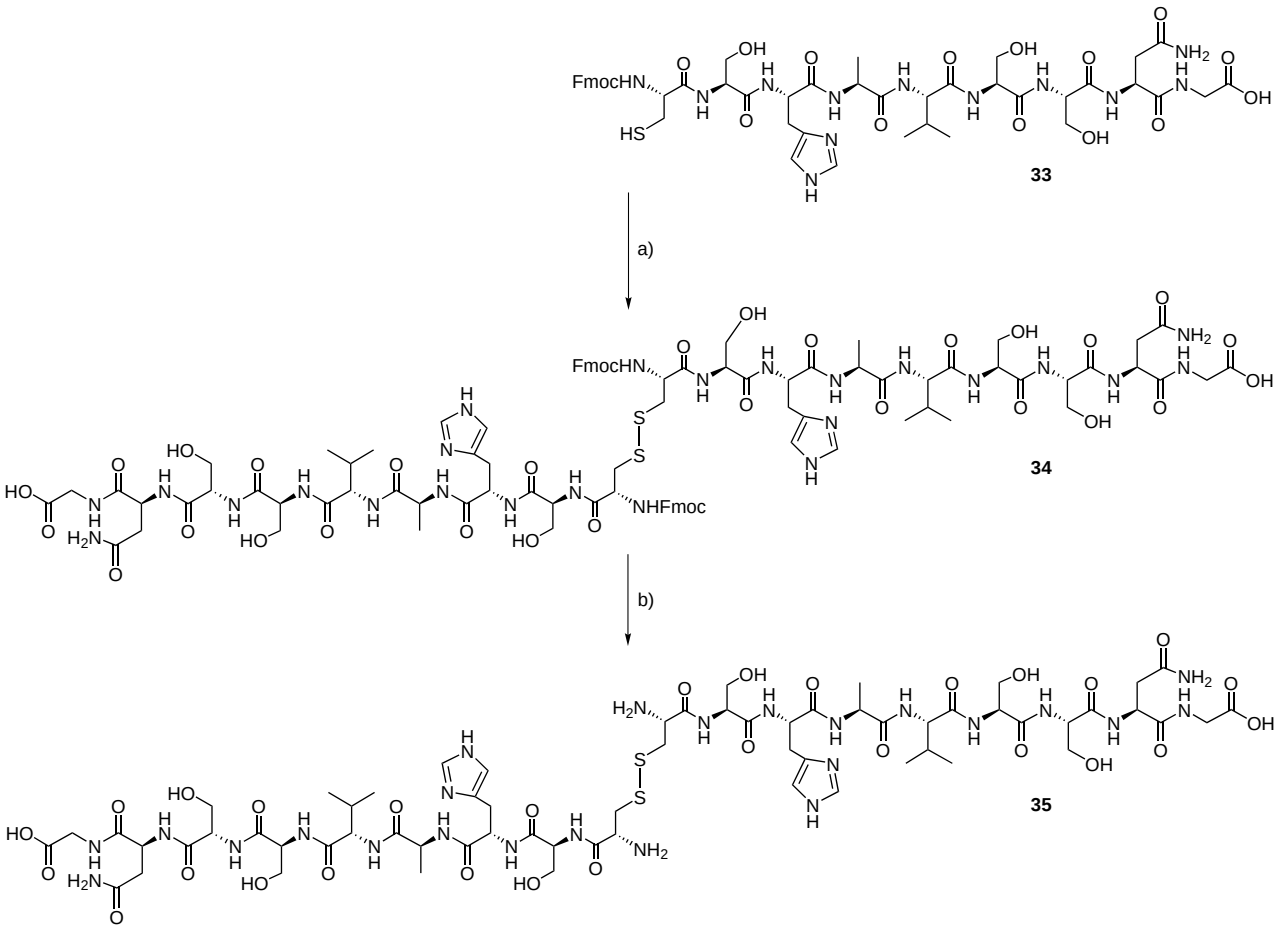
Figure 4 Structures of peptide **28** and **29**



Scheme 6 a) H_2, Pd-C, MeOH; overall yield: 33%; b) NaOMe, MeOH, pH 9.5; 61%

to the analytical RP-HPLC, the crude product **33** was of sufficient purity to be directly subjected to dimerization.

To achieve this reaction, a number of conditions were examined. As the most common reagent, iodine was utilized at first,[34] but no conversion to the dimeric structure was observed. Alternatively, compound **33** treated with a mixture of DMSO and trifluoroacetic acid (10%) within 3 hours led to quantitative conversion.[35] As the resulting dimer **34** was completely insoluble in common solvents, it was directly deprotected with DMF and piperidine (1:1) to give the water-soluble dimer **35** in an overall yield of 68% after purification by gel permeation chromatography.

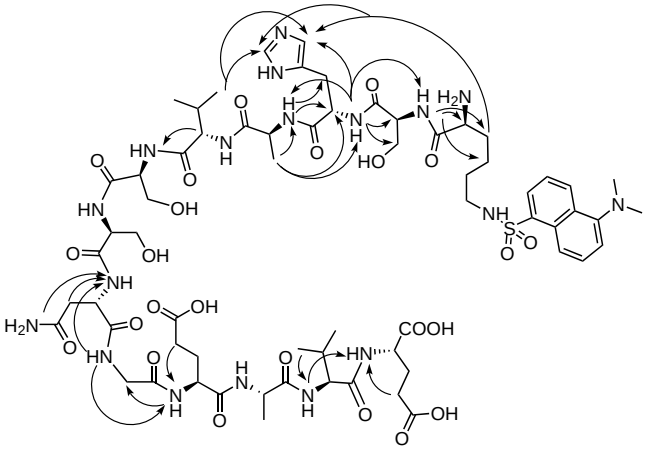


Scheme 7 a) DMSO–TFA (10%); b) DMF–piperidine (1:1); overall yield: 68%

Preliminary Evaluation of Structural Properties and Biological Activity

Preliminary results of conformational analysis by high-resolution NOESY and ROESY experiments showed that all synthesized E-cadherin peptides and glycopeptides adopt preferred turn-type conformations in DMSO solution. Similar CH,NH- and NH,NH-contacts have been found for protons within the turn sequence Ser-Asn-Gly and in the *C*-terminal region as are shown in Figure 5 for compounds **4** and **5**. Arrows in Figure 5 indicate contacts of protons in distances between 235 pm (strong, for example, NH,NH of Ser-Asn and Asn-Gly in the turn and Glu-Val in the C-terminal region) and 400 pm (weak, for example, CH,CH of His_{imid}-Val^{γ} and His_{imid}-Lys^{β}). Glycosylation might support, but is not essential for the formation of these turn conformations, whereas no turn conformation is found for *N*-terminally protected peptides. In the case of the E-cadherin peptides carrying an additional *N*-terminal ε-dansyl-labelled **4** or free lysine residue **5**, additional intensified contacts of protons in the *N*-terminal region have been observed (Figure 5), for example, NH,NH of Ser-His and NH,CH between Ser_{NH}- Lys^{β}. This suggests that the *N*-terminal region of these peptides containing the recognition motif His-Ala-Val is presented in a preferred geometry. It is interesting to note that these particular E-cadherin peptides **4** and **5** showed the strongest anti-adhesive effects on cell cultures of E-cadherin expressing cells. Detailed description of conformational analysis and biological evaluation of the synthetic E-cadherin (glyco)peptides will be reported elsewhere in due course.

THF, Et_2O, light petroleum (bp 50–70 °C), MeOH, CH_2Cl_2, nitromethane, and MeCN were distilled and dried according to standard procedures.[36] DMF (amine free, for peptide synthesis) was purchased from Roth, Ac_2O and pyridine in p.a. quality from Acros. Reagents were purchased at highest commercially available quality and used without further purification. Fmoc-protected amino acids were purchased from Novabiochem. As the resins for solid-phase synthesis preloaded Tentagel® resins (Rapp Polymers) or preloaded polystyrene resins (Novabiochem) were used. Reactions were monitored by TLC with aluminum-backed silica gel 60 F_{254} plates (Merck KGaA, Darmstadt). Flash column chromatography was performed with silica gel (40–63 μm) from Merck KGaA, Darmstadt. Optical rotations $[\alpha]_D$ were measured with a Perkin Elmer polarimeter 241. RP-HPLC analyses were carried out on a Knauer HPLC system with a Eurospher C8 column (5 μm, 250 × 4 mm) or a Phenomenex Luna C18-2 (5 μm, 250 × 4.6 mm) and a pump rate of 1 mL/min. Preparative HPLC separations were carried out on a Knauer HPLC system with a Eurospher C8 column (10 μm, 250 × 20 mm) and a pump rate of 10 mL/min or with a Phenomenex Luna C18-2 (10 μm, 250 × 50 mm) at a pump rate of 20 mL/min.



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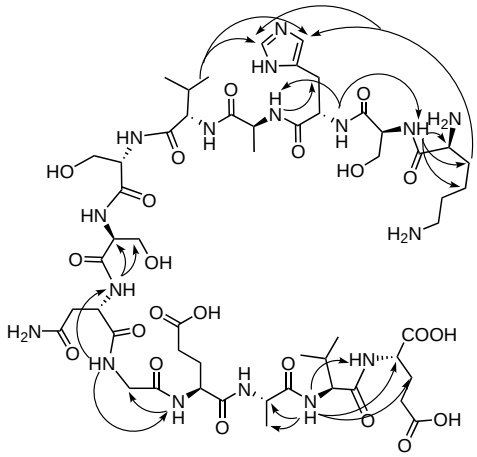

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Figure 5 NH,NH, NH,CH and CH,CH contacts determined by 600 MHz NOESY and ROESY NMR spectroscopy

Mixtures of H_2O and MeCN were used as the solvents, if required 0.1% TFA were added.

^1H, ^{13}C, and 2D-NMR spectra were recorded on Bruker AM-400, ARX-400 or DRX-600 spectrometers. Proton chemical shifts are given in ppm relative to $CHCl_3$ (δ = 7.24) or DMSO (δ = 2.49). ^{13}C chemical shifts are reported relative to $CDCl_3$ (δ = 77.0), DMSO (δ = 39.5). Assignments of proton and carbon signals were achieved by COSY, TOCSY and HMQC experiments when noted. MALDI-TOF mass spectra were recorded on a Micromass Tofspec E spectrometer, ESI-mass spectra were recorded on ThemoQuest Navigator spectrometer.

L-Seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-L-seryl-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic Acid (3, [H_2N-SHAVSSNGEAVE-OH])

For the synthesis of the dodecapeptide **3**, Tentagel® resin preloaded with Fmoc-Glu(O-*t*-Bu)-O-PHB **1** (455 mg, loading: 0.22 mmol/g) was used. Fmoc-groups were removed by treatment with 20% piperidine in NMP (3–5 × 2.5 min). Peptide couplings were carried out by reaction with Fmoc-protected amino acids (10 equiv), HBTU (10 equiv), HOBt (10 equiv) and DIPEA (20 equiv) within 20–30 min. After cleavage of the first Fmoc group, the dipeptide Fmoc-Ala-Val-OH was coupled to the resin in a double coupling (coupling time: 2 × 20 min). Free amino groups were capped with Ac_2O/DIPEA/HOBt after every coupling step. The following amino acids, Fmoc-Glu(O-*t*-Bu)-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH and 2 × Ser(*t*-Bu)-OH, were reacted with the resin-linked peptide chain according to the standard protocol. The ninth amino acid Fmoc-Val-OH was coupled twice. The coupling times of the following amino acids, Fmoc-Ala-OH, Fmoc-His(Trt)-OH und Fmoc-Ser(*t*-Bu)-OH, were extended to 30 min. The resin was transferred into a solid-phase reactor and thoroughly washed with CH_2Cl_2. The immobilized peptide was released from the resin by treatment with trifluoroacetic acid (15 mL), triisopropylsilane (0.9 mL) and H_2O (0.9 mL) for 2 h. The acid-labile protecting groups were removed simultaneously. Afterwards, the supernatant was filtered and the solid support was washed with trifluoroacetic acid (3 × 10 mL). The filtrates were combined and concentrated in vacuo. The liquid residue was poured into cold Et_2O (50 mL). The colorless precipitate was centrifuged and washed with cold Et_2O (2 ×). The crude product was dissolved in H_2O and purified by preparative RP-HPLC (Luna C18, 1% MeCN in H_2O → 100% MeCN in H_2O + 0.1% TFA in 60 min). The completely deprotected peptide **3** was obtained as a colorless lyophilisate (62 mg, 52%); $[\alpha]_D^{23}$ –40.9 (*c* = 0.97, H_2O); R_t 11.58 min (RP-HPLC, C8-Eurospher, 215 nm, 1% MeCN in H_2O → 100% MeCN + 0.1% TFA in 42 min).

^1H NMR (400 MHz, D_2O, ^1H-COSY): δ= 8.52 (s, 1 H, Im^2), 7.21 (s, 1 H, Im^4), 4.70–4.60 (m, 2 H, N^α, H^α), 4.42–4.23 (m, 6 H, 2 × A^α, 2 × E^α, 2 × S^α), 4.10–3.97 (m, 3 H, 2 × V^α, S^α), 3.85–3.74 (m, 8 H, G^α, 3 × S^β), 3.29 (dd, 1 H, $H^{\beta a}$, $J_{H\beta a,H\beta b}$ = 15.76 Hz, $J_{H\beta a,H\alpha}$ = 6.36 Hz), 3.14 (dd, 1 H, $H^{\beta b}$, $J_{H\beta b,H\beta a}$ = 15.56 Hz, $J_{H\beta b,H\alpha}$ = 7.44 Hz), 2.73 (m, 2 H, N^β), 2.35 (m, 4 H, E^γ), 1.98–1.88 (m, 6 H, 2 × E^β, 2 × V^β), 1.25 (2 d, 6 H, 2 × A^β, $J_{A\beta,A\alpha}$ = 6.90 Hz), 0.83 (d, 12 H, 2 × V^γ, $J_{V\gamma,V\beta}$ = 6.74 Hz).

^{13}C NMR (100.6 MHz, D_2O, HMQC): δ = 176.93, 174.63, 173.39, 173.29, 172.99, 172.86, 171.69, 170.79, 167.79, 163.02 (C = O), 133.70 (N=CN-Im), 128.14 (NC=CH-Im), 117.47 (HNCH=CR-Im), 61.28, 61.01, 60.14 (3 × S^β), 59.43 (2 × V^α), 55.70, 55.23, 54.46 (3 × S^α), 52.83, 52.54 (2 × E^α, H^α), 50.62 (N^α), 49.73 (2 × A^α), 42.83 (G^α), 36.09 (N^β), 30.04, 30.23 (2 × V^β, 2 × E^γ), 26.51, 26.30, 25.86 (2 × E^β, H^β), 18.47, 18.40, 17.70, 17.62, 16.65, 16.47 (2 × A^β, 2 × V^γ).

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{47}H_{76}N_{15}O_{21}$: 1187.2; found: 1187.8 (M + H^+); *m*/*z* calcd for $C_{47}H_{75}N_{15}NaO_{21}$: 1209.2, found: 1209.7 (M + Na^+).

***N*$^\varepsilon$-(5-Dimethylaminonaphthaline-1-sulfonyl)-L-lysyl-L-seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-L-seryl-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic Acid (4, [H_2N-K(dansyl)SHAVSSNGEAVE-OH])**

The synthesis of the fluorescent-labelled peptide was carried out according to the preparation of the dodecapeptide **3** (see above). Resin preloaded with Fmoc-Glu(O-*t*-Bu)-O- PHB **1** (500 mg, loading: 0.2 mmol Fmoc-Glu(O-*t*-Bu)/g) was utilized. After formation of the resin-linked dodecapeptide **2** and Fmoc removal, the fluorescent-labelled building block[15] was manually coupled. To this end, Fmoc-Lys(dansyl)-OH (180 mg, 0.3 mmol, 3.0 equiv), HATU (114 mg, 0.3 mmol, 3.0 equiv), HOAt (40 mg, 0.3 mmol, 3.0 equiv) and NMM (66 µL, 0.6 mmol, 6.0 equiv) were dissolved in NMP (2 mL) and added to the immobilized peptide and vortexed for 2 h. After removal of the *N*-terminal Fmoc group, the polymer was treated with trifluoroacetic acid to release the peptide **4** from the resin as described above. The crude fluorescent-labelled peptide **4** was purified by preparative RP-HPLC (C8-Eurospher, 1% MeCN in H_2O → 100% MeCN in H_2O + 0.1% TFA in 60 min). The completely deprotected dansylpeptide **4** was obtained as a slightly yellow lyophilisate (115 mg, 75%); $[\alpha]_D^{23}$ –25.2 (*c* = 1.00, H_2O); R_t 12.35 min

(RP-HPLC, Luna C18, 215 nm, 1% MeCN in H_2O → 100% MeCN + 0.1% TFA in 42 min).

^{1}H NMR (400 MHz, D_2O, ^{1}H-COSY): δ = 8.73 (d, 1 H, CH-dansyl, J = 9.00 Hz), 8.58 (s, 1 H, Im^2), 8.42 (d, 1 H, CH-dansyl, J = 8.60 Hz), 8.33 (d, 1 H, CH-dansyl, J = 7.44 Hz), 8.07 (d, 1 H, CH-dansyl, J = 7.84 Hz), 7.19–7.83 (m, 2 H, CH-dansyl), 7.29 (s, 1 H, Im^4), 4.70 (m, 2 H, N$^\alpha$, H$^\alpha$), 4.50–4.44 (m, 3 H, 3 × S$^\alpha$), 4.41–4.31 (m, 4 H, 2 × A$^\alpha$, 2 × E$^\alpha$), 4.17–4.06 (m, 2 H, 2 × V$^\alpha$), 4.01 (t, 1 H, K$^\alpha$, $J_{K\alpha,K\beta}$ = 6.24 Hz), 3.91–3.81 (m, 8 H, G$^\alpha$, 3 × S$^\beta$), 3.49 (s, 6 H, 2 × CH_3-dansyl), 3.30 (dd, 1 H, H$^{\beta a}$, $J_{H\beta a,H\beta b}$ = 15.64 Hz, $J_{H\beta a,H\alpha}$ = 5.88 Hz), 3.14 (dd, 1 H, H$^{\beta b}$, $J_{H\beta b,H\beta a}$ = 15.64 Hz, $J_{H\beta b,H\alpha}$ = 8.24 Hz), 2.91 (m, 2 H, K$^\varepsilon$), 2.81 (m, 2 H, N$^\beta$), 2.43 (m, 4 H, 2 × E$^\gamma$), 2.17–1.78 (m, 6 H, 2 × E$^\beta$, 2 × V$^\beta$), 1.79 (m, 2 H, K$^\beta$), 1.43–1.31 (m, 10 H, K$^\gamma$, K$^\delta$, 2 × A$^\beta$), 0.91 (t, 12 H, 2 × V$^\gamma$).

^{13}C NMR (100.6 MHz, D_2O, HMQC): δ = 173.17, 172.85, 172.76, 172.68, 171.80, 171.71, 171.53, 171.30, 171.17, 171.03, 170.82, 169.69, 162.90, 162.55 (C=O), 138.40 (C_{quart}-dansyl), 135.20 (C_{quart}-dansyl), 133.22 (N=CN-Im), 130.01 (C_{tert}-dansyl), 128.52 (C_{quart}-dansyl), 128.09 (NC=CH-Im), 127.85, 126.84, 125.26 (C_{tert}-dansyl), 125.50 (C_{quart}-dansyl), 125.40, 119.82 (C_{tert}-dansyl), 117.21 (HNCH=CR-Im), 61.00, 60.82, 60.69 (3 × S$^\beta$), 59.21 (2 × V$^\alpha$), 55.59, 55.45, 55.24, 55.01 (3 × S$^\alpha$), 52.63 (K$^\alpha$), 52.49 (E$^\alpha$), 52.19 (H$^\alpha$), 51.68 (E$^\alpha$), 50.37 (N$^\alpha$), 49.78, 49.50 (2 × A$^\alpha$), 46.57 (CH_3-dansyl), 42.55 (G$^\alpha$), 41.94 (K$^\varepsilon$), 35.78 (N$^\beta$), 30.03, 29.97, 29.87, 29.67, 28.20 (2 × V$^\beta$, 2 × E$^\gamma$, K$^\delta$, K$^\gamma$), 26.15, 25.97, 25.91, 25.45, 25.35 (H$^\beta$, 2 × E$^\beta$), 20.64 (K$^\beta$), 18.23, 18.14, 17.55, 17.43, 17.40, 16.99, 16.43, 16.37, 16.17 (2 × A$^\beta$, 2 × V$^\gamma$).

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{65}H_{99}N_{18}O_{24}S$: 1548.7; found: 1549.4 (M + H$^+$); *m*/*z* calcd for $C_{65}H_{98}N_{18}NaO_{24}S$: 1570.6; found: 1571.5 (M + Na$^+$).

L-Lysyl-L-seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-L-seryl-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic Acid (5, [H_2N-KSHAVSSNGEAVE-OH])

The automated synthesis of the tridecapeptide was carried out in analogy to the one of the labelled peptide **4**. Tentagel® resin preloaded with Fmoc-Glu(O-*t*-Bu)-O-PHB **1** (455 mg, loading: 0.22 mmol Fmoc-Glu(O-*t*-Bu)/g) served as the solid support, the standard coupling time was 20 min. Fmoc-Lys-OH was finally coupled to **2** after Fmoc removal. The peptide was detached from the solid support and isolated as described above. The crude product was purified by RP-HPLC (Luna C18, 5% MeCN in H_2O → 5% MeCN in H_2O in 15 min → 20% MeCN in H_2O in 50 min → 100% MeCN + 0.1% TFA in 60 min). The completely deprotected glycopeptide **5** was obtained as a colorless lyophilisate (110 mg, 84%); $[\alpha]_D^{23}$ –47.9 (*c* = 1.00, H_2O); R_t 20.71 min (RP-HPLC, Luna C18, 215 nm, 5% MeCN in H_2O → 5% MeCN in H_2O in 10 min → 60% MeCN in H_2O in 40 min → 100% MeCN + 0.1% TFA in 60 min).

^{1}H NMR (400 MHz, D_2O, ^{1}H-COSY): δ = 8.61 (d, 1 H, Im^2, J = 1.16 Hz), 7.29 (s, 1 H, Im^4), 4.71 (m, 2 H, N$^\alpha$, H$^\alpha$), 4.53–4.40 (m, 4 H, 1 × E$^\alpha$, 3 × S$^\alpha$), 4.38–4.32 (m, 3 H, 2 × A$^\alpha$, E$^\alpha$), 4.19–4.02 (m, 2 H, 2 × V$^\alpha$), 3.92–3.81 (m, 8 H, G$^\alpha$, 3 × S$^\beta$), 3.29 (dd, 1 H, H$^{\beta a}$, $J_{H\beta a,H\beta b}$ = 15.24 Hz, $J_{H\beta a,H\alpha}$ = 5.84 Hz), 3.14 (dd, 1 H, H$^{\beta b}$, $J_{H\beta b,H\beta a}$ = 15.24 Hz, $J_{H\beta b,H\alpha}$ = 8.24 Hz), 2.91 (t, 2 H, K$^\varepsilon$, $J_{K\varepsilon,K\delta}$ = 7.44 Hz), 2.82 (m, 2 H, N$^\beta$), 2.44 (m, 4 H, 2 × E$^\gamma$), 2.08–1.88 (m, 8 H, 2 × E$^\beta$, K$^\beta$, 2 × V$^\beta$), 1.69 (m, 2 H, K$^\delta$), 1.45–1.33 (m, 8 H, K$^\gamma$, 2 × A$^\beta$), 0.92 (d, 12 H, 2 × V$^\gamma$, $J_{V\gamma,V\beta}$ = 6.64 Hz).

^{13}C NMR (100.6 MHz, D_2O, HMQC): δ = 133.27 (NC=CH-Im), 117.22 (HNCH=CR-Im), 60.99, 60.77 (3 × S$^\beta$), 59.24, 59.09 (2 × V$^\alpha$), 55.51, 55.39, 55.27, 55.00 (3 × S$^\alpha$), 52.55 (K$^\alpha$, E$^\alpha$), 52.18 (H$^\alpha$), 51.66 (E$^\alpha$), 50.36 (N$^\alpha$), 49.79, 49.45 (2 × A$^\alpha$), 42.48 (G$^\alpha$), 38.77 (K$^\varepsilon$), 35.72 (N$^\beta$), 30.17 (K$^\beta$), 29.97 (2 × V$^\beta$), 29.72, 29.65 (2 × E$^\gamma$), 26.12, 25.96, 25.47, 25.36 (K$^\delta$, H$^\beta$, 2 × E$^\beta$), 20.98 (K$^\gamma$), 18.22, 18.13, 17.55, 17.40, 17.01, 16.43, 16.38, 16.15 (2 × A$^\beta$, 2 × V$^\gamma$).

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{53}H_{88}N_{17}O_{22}$: 1315.4; found: 1549.4 (M + H$^+$); *m*/*z* calcd for $C_{53}H_{87}N_{17}NaO_{22}$: 1337.4; found: 1337.9 (M + Na$^+$).

N-(9*H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-α-D-galactopyranosyl)-L-serine *tert*-Butyl Ester (11, [Fmoc-Ser(α-4,6-*O*-Pmb-GalNAc)-O-*t*-Bu])

To a solution of Fmoc-Ser(α-Ac_3GalNAc)-O-*t*-Bu (**9**;[14,37] 2.0 g, 2.8 mmol) in anhyd MeOH (50 mL) was added a 1% solution of NaOMe in MeOH dropwise until a pH of 8.5 is reached. The mixture was stirred for 7 h. After every 30 min, the pH was adjusted to 8.5. Finally, ion exchange resin Amberlyst IR 120 was added for neutralization. After filtration, evaporation of the solvent in vacuo and drying the remaining product in high vacuum, the product was dissolved in DMF (100 mL). *p*-Methoxybenzylaldehyde dimethyl acetal (0.96 mL, 5.6 mmol) and catalytic amounts of *p*-TsOH (pH 4) were added. The mixture was kept at 50 °C and 25 mbar (rotavapor) for 2 h. After neutralization with Hünig's base, the solvent was evaporated in vacuo, the remaining residue dissolved in EtOAc (100 mL) and washed with aq sat. $NaHCO_3$ solution (3 ×) and brine, and dried ($MgSO_4$). The solvent was evaporated, and the crude product was purified by flash chromatography on silica gel (light petroleum–EtOAc, 2:1) to give **11** as a colorless amorphous product (1.03 g, 52%); $[\alpha]_D^{23}$ +60.1 (*c* = 1, CH_2Cl_2); R_f 0.20 (light petroleum–EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY): δ = 7.84 (d, 2 H, H4-, H5-Fmoc, $J_{H3,H4}$ = $J_{H5,H6}$ = 7.44 Hz), 7.67 (m, 2 H, H1-, H8-Fmoc), 7.49–7.37 (m, 6 H, H2-, H3-, H6-, H7-Fmoc, H$_{meta}$-, H$_{para}$-Pmb), 6.95 (d, 2 H, H$_{ortho}$-Pmb, $J_{Hortho,Hmeta}$ = 9.00 Hz), 6.21 (d, 1 H, NH-Ac, $J_{NH,H2}$ = 8.96), 6.08 (d, 1 H, NH-Fmoc, $J_{NH,S\alpha}$ = 8.24 Hz), 5.52 (s, 1 H, CH-Pmb), 4.97 (d, 1 H, H1-Gal, $J_{H1,H2}$ = 1.96 Hz), 4.57–4.40 (m, 4 H, H2-Gal, S$^\alpha$, CH_2-Fmoc), 4.31–4.16 (m, 3 H, H6a-, H6b-Gal, H9-Fmoc), 4.03–3.89 (m, 4 H, H5-, H3-Gal, S$^\beta$), 3.86 (s, 3 H, OCH_3-Pmb), 2.08, (s, 3 H, NHCOCH_3), 1.54 (s, 9 H, *t*-C_4H_9).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 171.60, 169.36 (C=O), 160.15 (C_{para}-Pmb), 155.98 (CO-urethan), 143.72, 143.64 (C4a-, C4b-Fmoc), 141.30 (C8a-, C9a-Fmoc), 130.10 (C_{ipso}-Pmb), 127.82, 127.71, 127.11 (C2-, C3-, C6-, C7-Fmoc, C_{tert}-Pmb), 125.05, 124.94 (C1-, C8-Fmoc), 120.9 (C4-, C5-Fmoc), 114.31, 113.56 (C_{tert}-Pmb), 101.11, 99.55 (CH-Pmb, C1-Gal), 83.00 [$C(CH_3)_3$], 75.80 (C3-Gal), 69.09 (S$^\beta$), 68.73 (C4-Gal), 67.18 (CH_2-Fmoc), 63.38 (C5-Gal), 63.30 (C6-Gal), 55.28 (OCH_3-Pmb), 54.94 (S$^\alpha$), 50.15, 47.08 (C2-Gal, C9-Fmoc), 28.01 [$C(CH_3)_3$], 23.22 (NHCOCH_3).

ESI-MS: *m*/*z* calcd for $C_{38}H_{45}N_2O_{11}$: 705.3, found: 705.3 (M + H$^+$).

Anal. Calcd for $C_{38}H_{44}N_2O_{11}$ + H_2O: C, 63.15; H, 41.41; N, 3.88. Found: C, 62.81; H, 6.99; N, 4.12.

N-(9*H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-2-deoxy-4,6-*O*-*p*-methoxybenzylidene-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-serine *tert*-Butyl Ester (13, [Fmoc-Ser(Ac_4Gal-(β1→3)-α-4,6-*O*-Pmb-GalNAc)-O-*t*-Bu])

To Fmoc-protected *O*-(methoxybenzylidene-*N*-acetylgalactosaminyl)threonine *tert*-butyl ester **11** (750 mg, 1.1 mmol, 1.0 equiv) and molecular sieves (1.5 g, 4 Å) in CH_2Cl_2–nitromethane (20 mL, 3:1) was added $Hg(CN)_2$ (555 mg, 2.2 mmol, 2.0 equiv). The suspension was stirred at r.t. for 1 h. After cooling to 0 °C, a solution of 2,3,4,6-tetra-*O*-acetyl-α-galactopyranosyl bromide (**12**; 1.36 g, 3.3 mmol, 3.0 equiv) in CH_2Cl_2–nitromethane (12 mL, 3:1) was added dropwise. Finally, an additional portion of $Hg(CN)_2$ (555 mg, 2.2 mmol, 2.0 equiv) was added and the mixture was allowed to warm up to r.t. The suspension was stirred for 15 h, diluted with CH_2Cl_2 (20 mL) and filtered through Hyflo Supercel into a sat. aq solution of $NaHCO_3$ (30 mL). The phases were separated, and the organic layer

was washed with aq $NaHCO_3$ (2 × 30 mL), aq NaI (30 mL), and with brine (30 mL). After drying ($MgSO_4$), the solvents were removed in vacuo. Purification was performed by flash chromatography on silica gel (light petroleum–EtOAc, 1:2) to yield **13** as a colorless amorphous solid (880 mg, 80%); $[\alpha]_D^{23}$ +53.4 (*c* = 1.00, CH_2Cl_2); R_f 0.51 (light petroleum–EtOAc, 1:4).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY): δ = 7.76 (d, 2 H, H4-, H5-Fmoc, $J_{H3,H4} = J_{H5,H6}$ = 7.44 Hz), 7.61 (m, 2 H, H1-, H8-Fmoc, $J_{H1,H2} = J_{H8,H7}$ = 9.26 Hz), 7.45–7.27 (m, 6 H, H2-, H3-, H6-, H7-Fmoc, H_{meta}-Pmb), 6.87 (2 d, 2 H, H_{ortho}-Pmb, $J_{Hortho,Hmeta}$ = 8.80 Hz), 5.77 (d, 1 H, N*H*Ac, $J_{NH,H2\text{-}Gal}$ = 7.84 Hz), 5.79 (d, 1 H, N*H*-Fmoc, $J_{NH,S\beta}$ = 8.64 Hz), 5.46 (s, 1 H, C*H*–Pmb), 5.34 (d, 1 H, H4′-Gal, $J_{H4,H3}$ = 2.76 Hz), 5.12 (dd, 1 H, H2′-Gal, $J_{H2',H3'}$ = 10.16 Hz, $J_{H2',H1'}$ = 7.80 Hz), 4.96–4.92 (m, 2 H, H1-, H3′-Gal), 4.51–4.48 (m, 2 H, H1′-, H2-Gal, $J_{H1',H2'}$ = 7.80 Hz), 4.46–4.33 (m, 3 H, S^{α}, CH_2-Fmoc), 4.23–3.86 (m, 9 H, H5-, H5′-, H6a-, H6b-, H6′a-, H6′b-Gal, S^{β}, H9-Fmoc), 3.79, 3.77 (2 s, 3 H, OCH_3-Pmb), 3.70–3.60 (m, 2 H, H3-, H4-Gal), 2.12 (s, 3 H, NHCOCH_3), 2.04, 2.01, 1.98, 1.95, 1.92 (5 s, 12 H, 4 × $COCH_3$), 1.46, 1.45 (2 s, 9 H, *t*-C_4H_9).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.28, 170.16, 169.64, 169.48 (C=O), 159.97 (C_{para}-Pmb), 155.89 (CO-urethane), 143.62, 141.31 (C4a-, C4b-, C8a-, C9a-Fmoc), 130.12 (C_{ipso}-Pmb), 127.89, 127.50, 127.35, 127.12, 124.86, 120.13 (C1-, C2-, C3-, C4-, C5-, C6-, C7-, C8-Fmoc, C_{tert}-Pmb), 113.51 (C_{tert}-Pmb), 100.99, 100.64 (CH–Pmb, C1′-Gal), 99.17 (C1-Gal), 83.08 [*C*$(CH_3)_3$], 77.80 (C3-Gal), 75.42, 75.36, 73.99, 70.96, 70.82, 69.12, 68.79 (C2′-, C3′-, C4-, C5-, C5′-Gal), 69.03 (S^{β}), 67.14 (CH_2-Fmoc), 66.94 (C4′-Gal), 63.44, 61.36 (C6-, C6′-Gal), 55.28, 54.97 (OCH_3-Pmb), 28.01 [C(*C*$H_3)_3$], 23.32 (NHCO*C*H_3), 20.75, 20.58 (4 × CO*C*H_3); doubled signals due to different conformers.

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{52}H_{62}N_2NaO_{20}$: 1058.0; found: 1058.9 (M + Na^+); *m*/*z* calcd for $C_{52}H_{62}KN_2O_{20}$: 1074.2; found: 1074.9 (M + K^+).

N-(9*H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-2-deoxy-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-serine *tert*-Butyl Ester (14, [Fmoc-Ser(Ac_4Gal-(β1→3)-α-GalNAc)-O-*t*-Bu])

To a solution of disaccharide **13** (494 mg, 0.48 mmol, 1.0 equiv) in MeCN–H_2O (20 mL, 9:1) was added $Ce(NH_4)_2(NO_2)_6$ (390 mg, 0.72 mmol, 1.5 equiv). The mixture was stirred for 45 min at r.t., diluted with H_2O (20 mL) and neutralized by dropwise addition of sat. aq $NaHCO_3$. MeCN was removed in vacuo, and the remaining aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (2 × 30 mL) and subsequently dried ($MgSO_4$). After removal of the solvents in vacuo, the crude product was purified by flash chromatography (EtOAc) and subsequent preparative RP-HPLC (Luna C18, 45% MeCN in H_2O) to give **14** as a colorless amorphous solid (332 mg, 76%); $[\alpha]_D^{23}$ +50.6 (*c* = 1.00, CH_2Cl_2); R_f 0.17 (EtOAc); R_t 8.41 min (Luna C18, 45% MeCN in H_2O).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY): δ = 7.73 (d, 2 H, H4-, H5-Fmoc, $J_{H3,H4} = J_{H5,H6}$ = 7.84 Hz), 7.55 (d, 2 H, H1-, H8-Fmoc, $J_{H1,H2} = J_{H8,H7}$ = 7.04 Hz), 7.38–7.25 (m, 4 H, H2-, H3-, H6-, H7-Fmoc), 5.93 (d, 1 H, N*H*Ac, $J_{NH,H2\text{-}Gal}$ = 9.92 Hz), 5.85 (d, 1 H, NHFmoc, $J_{NH,S\alpha}$ = 8.64 Hz), 5.31 (d, 1 H, H4′-Gal, $J_{H4,H3}$ = 2.72 Hz), 5.12 (t, 1 H, H2′-Gal, $J_{H2',H3'} = J_{H2',H1'}$ = 10.16 Hz), 4.92 (dd, 1 H, H3′-Gal, $J_{H3',H2'}$ = 10.16 Hz, $J_{H3',H4'}$ = 3.12 Hz), 4.84 (br s, 1 H, H1-Gal), 4.51–4.48 (m, 2 H, H1′-, H2-Gal), 4.42–4.36 (m, 3 H, S^{α}, CH_2-Fmoc), 4.18 (t, 1 H, H9-Fmoc, *J*(H9, CH_2) = 6.64 Hz), 4.12–4.00 (m, 4 H, H4-, H6a-Gal, S^{β}), 3.93–3.91 (m, 1 H, H6b-Gal), 3.89–3.71 (m, 4 H, H5-, H5′-, H6′a-, H6′b-Gal), 3.67 (dd, 1 H, H3-Gal, $J_{H3,H2}$ = 10.56 Hz, $J_{H3,H4}$ = 2.76 Hz), 2.10 (s, 3 H, NHCOCH_3), 2.01 (s, 3 H, $COCH_3$), 1.96 (s, 3 H, $COCH_3$), 1.93 (s, 3 H, $COCH_3$), 1.89 (s, 3 H, $COCH_3$), 1.43 (s, 9 H, *t*-C_4H_9).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.39, 169.84, 169.51, 169.39 (4 × CO), 156.04 (CO-urethane), 143.68 (C4a-, C4b-Fmoc), 141.29, 141.24 (C8a-, C9a-Fmoc), 127.83 (C3-, C6-Fmoc), 127.10 (C2-, C7-Fmoc), 124.95 (C1-, C8-Fmoc), 120.10 (C4-, C5-Fmoc), 101.47 (C1′-Gal), 99.05 (C1-Gal), 83.07 [*C*$(CH_3)_3$], 77.94 (C3-Gal), 70.84, 70.60, 70.13 (C3′-, C4-, C5-, C5′-Gal), 68.96 (S^{β}), 68.58 (C2′-Gal), 67.02, 66.91 (CH_2-Fmoc, C4′-Gal), 62.53 (C6-Gal), 61.35 (C6′-Gal), 55.04 (S^{α}), 47.65 (C2-Gal), 47.08 (C9-Fmoc), 27.97 [C(*C*$H_3)_3$], 23.17 (NHCO*C*H_3), 20.66, 20.60, 20.55, 20.52 (4 × CO*C*H_3).

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{44}H_{57}N_2O_{19}$: 917.9; found: 916.3 (M + H^+); *m*/*z* calcd for $C_{44}H_{56}N_2NaO_{19}$: 939.9; found: 939.4 (M + Na^+).

Anal. Calcd for $C_{44}H_{56}N_2O_{19}$ (916.92): C, 57.64; H, 6.16; N, 3.06. Found: C, 57.96; H, 6.07; N, 2.86.

N-(9*H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-serine *tert*-Butyl Ester (15, [Fmoc-Ser(Ac_4Gal-(β1→3)-α-Ac_2GalNAc)-O-*t*-Bu])

A solution of partially deprotected disaccharide **14** (210 mg, 0.23 mmol) in pyridine–Ac_2O (9 mL, 3:1) was stirred for 17 h at r.t. The solution was poured onto ice and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with sat. aq $NaHCO_3$ solution (2 × 20 mL) and with brine (2 × 20 mL), dried ($MgSO_4$), and the solvents were evaporated in vacuo. The product **15** was isolated by flash chromatography on silica gel (EtOAc) as a colorless amorphous solid (201 mg, 88%); $[\alpha]_D^{23}$ 53.0 (*c* = 1.00, CH_2Cl_2); R_f 0.52 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY): δ = 7.77 (d, 2 H, H4-, H5-Fmoc,

$J_{H3,H4} = J_{H5,H6}$ = 7.84 Hz), 7.66 (d, 2 H, H1-, H8-Fmoc, $J_{H1,H2} = J_{H8,H7}$ = 7.04 Hz), 7.50–7.38 (m, 4 H, H2-, H3-, H6-, H7-Fmoc), 5.78 (m, 2 H, NHAc, NHFmoc), 5.42 (m, 2 H, H4-, H4′-Gal), 5.18 (dd, 1 H, H2′-Gal, $J_{H2',H3'}$ = 10.56 Hz, $J_{H2',H1'}$ = 8.25 Hz), 5.01 (dd, 1 H, H3′-Gal, $J_{H3',H2'}$ = 10.56 Hz, $J_{H3',H4'}$ = 3.16 Hz), 4.94 (br s, 1 H, H1-Gal), 4.63–4.45 (m, 5 H, H1′-, H2-Gal, CH_2-Fmoc, S^{α}), 4.31 [t, 1 H, H9-Fmoc, *J*(H9,CH_2) = 6.64 Hz), 4.22–4.17 (m, 3 H, H6a-, H6b-, H6′a-Gal), 4.12–4.10 (m, 1 H, H6′b-Gal), 4.03–4.00 (m, 2 H, S^{β}), 3.93–3.90 (m, 2 H, H5-, H5′-Gal), 3.84 (dd, 1 H, H3-Gal, $J_{H3,H2}$ = 10.56 Hz, $J_{H3,H4}$ = 2.72 Hz), 2.23 (s, 3 H, NHCOCH_3), 2.19 (s, 3 H, $COCH_3$), 2.13 (s, 3 H, $COCH_3$), 2.09 (s, 6 H, 2 $COCH_3$), 2.04 (s, 3 H, $COCH_3$), 2.01 (s, 3 H, $COCH_3$), 1.56 (s, 9 H, *t*-C_4H_9).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.48, 170.36, 170.33, 170,12, 169.72, 169.67, 168.96 (C=O), 155.80 (CO-urethane), 143.65 (C4a-, C4b-Fmoc), 141.32, 141.28 (C8a-, C9a-Fmoc), 127.85 (C3-, C6-Fmoc), 127.10 (C2-, C7-Fmoc), 124.86 (C1-, C8-Fmoc), 120.11 (C4-, C5-Fmoc), 100.52 (C1′-Gal), 98.55 (C1-Gal), 83.10 [*C*$(CH_3)_3$], 77.38 (C3-Gal), 73.12, 70.87, 70.70 (C3′-, C4-, C5-, C5′-Gal), 69.09 (S^{β}), 68.65 (C2′-Gal), 67.14 (CH_2-Fmoc), 66.71 (C4′-Gal), 62.65 (C6-Gal), 61.05 (C6′-Gal), 54.84 (S^{α}), 48.66 (C2-Gal), 47.08 (C9-Fmoc), 28.00 [C(*C*$H_3)_3$], 23.24 (NHCO*C*H_3), 20.70, 20.66, 20.63, 20.52 (6 × CO*C*H_3).

ESI-MS: *m*/*z* calcd for $C_{48}H_{61}N_2O_{21}$: 1001.4; found: 1001.3 (M + H^+); *m*/*z* calcd for $C_{48}H_{60}N_2NaO_{21}$: 1023.4; found: 1023.2 (M + Na^+).

***N*-(9*H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-serine (16, [Fmoc-Ser(Ac_4Gal-(β1→3)-α-Ac_2GalNAc)-OH])**

Disaccharide conjugate **15** (190 mg, 0.19 mmol, 1.0 equiv) was dissolved in trifluoroacetic acid (10 mL) and anisole (1 mL). After stirring at r.t. for 1 h, the solvents were removed in vacuo. The residue was repeatedly coevaporated with toluene. Subsequently, the crude product was purified by flash chromatography on silica gel (EtOAc–EtOH–AcOH, 10:1:0.1) to give **16** as a colorless amorphous solid (150 mg, 84%); $[\alpha]_D^{23}$ +102.9 (c = 1.00, CH_2Cl_2); R_f 0.23 (light petroleum–EtOH–HOAc, 10:1:0.1).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY): δ = 7.73 (m, 2 H, H4-, H5-Fmoc), 7.61 (m, 2 H, H1-, H8-Fmoc), 7.40–7.25 (m, 4 H, H2-, H3-, H6-, H7-Fmoc), 6.14 (m, 1 H, N*H*Ac), 5.90 (m, 1 H, NHFmoc), 5.34 (br s, 1 H, H4-Gal), 5.31, 5.15 (2 d, 1 H, H4′-Gal, $J_{H4',H3'}$ = 3.12 Hz), 5.05 (dd, 1 H, H2′-Gal, $J_{H2',H1'}$ = 7.8 Hz, $J_{H2',H3'}$ = 9.80 Hz), 5.01 (d, 1 H, H1-Gal, $J_{H1,H2}$ = 2.72 Hz), 5.02, 4.95 (2 dd, 1 H, H3′-Gal, $J_{H3',H2'}$ = 9.80 Hz, $J_{H3',H4'}$ = 3.12 Hz), 4.82, 4.66 (2 d, 1 H, H1′-Gal, $J_{H1',H2'}$ = 7.80 Hz), 4.49–4.35 (m, 3 H) and 4.26–3.74 (m, 11 H) (H2-, H3-, H5-, H-5′, H6-, H6′, H9-Fmoc, CH_2-Fmoc, S^{α}, S^{β}), 2.11, 2.09, 2.07, 2.06, 2.04, 2.02, 2.00, 1.99, 1.98 (9 s, 21 H, 6 $COCH_3$ and NHCOCH_3).

^{13}C NMR (100.6 MHz, $CDCl_3$, HMQC): δ = 170.07, 169.92, 169.78, 169.31 (C=O), 155.92 (CO-urethane), 143.90, 143.58 (C4a-, C4b-Fmoc), 141.30, 141.21 (C8a-, C9a-Fmoc), 127.95, 127.85, 127.19, 127.15, 127.07 (C3-, C6-, C2-, C7-Fmoc), 125.36 (C1-, C8-Fmoc), 120.10 (C4-, C5-Fmoc), 100.94 (C1′-Gal), 97.74 (C1-Gal), 77.56 (C3-Gal), 72.72, 70.76, 70.64, 70.46 (C3′-, C4-, C5-, C5′-Gal), 69.29 (S^{β}), 68.91, 68.58, 68.35, 67.06 (CH_2-Fmoc, C2′-Gal), 66.74 (C4′-Gal), 62.88 (C6-Gal), 61.10 (C6′-Gal), 54.14 (S^{α}), 49.21 (C2-Gal), 47.24, 47.07 (2 C9-Fmoc), 23.40, 22.76 (NHCOCH_3), 20.73, 20.62, 20.56, 20.47, 20.40 (6 × COCH_3), doubled signals due to conformers.

ESI-MS: m/z calcd for $C_{44}H_{52}N_2NaO_{21}$: 967.3; found: 967.5 (M + Na^+); m/z calcd for $C_{44}H_{51}N_2Na_2O_{21}$: 989.9; found: 989.4 (M + 2 $Na^+ - H^+$).

***N*-(9*H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-2-deoxy-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α/β-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-serine *tert*-Butyl Ester (18/19, [Fmoc-Ser({Ac_4Gal-(β1→3)}[Ac_4Neu5AcCOOBzl-(α/β2→6)]-α-Ac_2GalNAc)-O-*t*-Bu])**

To a mixture of glycosyl acceptor **14** (530 mg, 0.58 mmol, 1.0 equiv) and xanthate donor **17**[27] (974 mg, 1.45 mmol, 2.5 equiv) dissolved in MeCN–CH_2Cl_2 (2:1) under argon were added molecular sieves (1.50 g, 3 Å). After stirring for 1 h, anhyd AgOTf (373 mg, 1.45 mmol, 2.5 equiv) was added under the exclusion of light, and the mixture was cooled to –62 °C. During a period of 15 min, methylsulfenyl bromide [MSB, prepared by adding Br_2 (410 μL, 8.0 mmol, 1.0 equiv) to a solution of dimethyl disulfide (709 μL, 8.0 mmol, 1.0 equiv) in 1,2-dichloroethane (10 mL) and stirring for 12 h under exclusion of oxygen and light; 0.91 mL, 1.6 M] cooled to 0 °C, was added dropwise. After addition of MSB, the suspension was stirred at –62 °C for 4 h. Diisopropylamine (0.2 mL) was added, and the suspension was stirred for 30 min. Subsequently, the mixture was allowed to warm up to 10 °C, diluted with CH_2Cl_2 (50 mL) and filtered through Hyflo Supercel. After washing with CH_2Cl_2 (20 mL), the solvents of the filtrate were evaporated in vacuo. The glycal formed as a by-product was removed by flash chromatography on silica gel (EtOAc). Separation of **18** from β-anomer **19** and educt **14** was achieved by preparative RP-HPLC (Luna C18, 55% MeCN in H_2O → 55% MeCN in H_2O in 15 min → 70% MeCN in H_2O in 20 min → 70% MeCN in H_2O in 60 min → 100% MeCN in 80 min). The α-anomer **18** (240 mg, 28%) and the β-anomer **19** (61 mg, 7%) were obtained as colorless amorphous solids.

α-Anomer **18**

$[\alpha]_D^{23}$ +24.6 (c = 1.00, CH_2Cl_2); R_f 0.52 (EtOAc–MeOH, 10:1); R_t 26.57 min (Luna C18, 55% MeCN in H_2O → 55% MeCN in H_2O in 10 min → 70% MeCN in H_2O in 60 min → 100% MeCN in 80 min).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY, TOCSY): δ = 7.73 (d, 2 H, H4-, H5-Fmoc, $J_{H3,H4}$ = $J_{H5,H6}$ = 7.44 Hz), 7.55 (m, 2 H, H1-, H8-Fmoc, $J_{H1,H2}$ = $J_{H8,H7}$ = 7.44 Hz), 7.38–7.26 (m, 9 H, H2-, H3-, H6-, H7-Fmoc, C_{tert}-benzyl), 5.83–5.76 (m, 2 H, N*H*Fmoc, N*H*Ac_{GalNAc}), 5.32–5.28 [m, 4 H, H4′-Gal (5.32), H7′′-, H8′′-Neu5Ac, N*H*Ac_{Neu5Ac}], 5.17–5.11 [m, 3 H, H2′-Gal (5.13), CH_2-benzyl (5.16)], 4.91 (d, 1 H, H3′-Gal, $J_{H3',H2'}$ = 10.20 Hz), 4.77 (m, 1 H, H4′′-Neu5Ac), 4.68 (br s, 1 H, H1-Gal), 4.51–4.43 [m, 3 H, H1′- (4.53), H2-Gal (4.49), S^{α} (4.37)], 4.35 (m, 2 H, CH_2-Fmoc), 4.26 (dd, 1 H, H9′′a-Neu5Ac, $J_{H6a,H5}$ = 2.36 Hz, $J_{H6a,H6b}$ = 12.52 Hz), 4.19 [t, 1 H, H9-Fmoc, J(H9,CH_2) = 6.64 Hz], 4.07–3.95 [m, 5 H, H6′-Gal (4.07, 3.99), H5′′- (4.05), H6′′- (4.06), H9′′b- Neu5Ac (4.05)], 3.89 (m, 1 H, $S^{\beta a}$), 3.84–3.66 [m, 6 H, H4-, H5-Gal, H6a- (3.84), H5′-Gal, $S^{\beta b}$ (3.67)], 3.61 (m, 1 H, H3-Gal), 3.52 (m, 1 H, H6b-Gal), 2.62 (dd, 1 H, H3′′*eq*-Neu5Ac, $J_{H3eq,H4}$ = 3.52 Hz, $J_{H3eq,H3ax}$ = 12.52 Hz), 2.10, 2.06, 2.05, 2.00, 1.96, 1.95, 1.93, 1.89, 1.81 (9 s, 30 H, 8 × $COCH_3$, 2 × NHCOCH_3), 1.88 (m, 1 H, H3′′*ax*-Neu5Ac), 1.48 (s, 9 H, *t*-C_4H_9).

^{13}C NMR (100.6 MHz, $CDCl_3$, HMQC): δ = 170.78, 170.69, 170.25, 170.19, 170.170.16, 170.08, 169.51, 169.40 (C=O), 167.22 (C1′′-Neu5Ac), 155.99 (CO-urethane), 143.69 (C4a-, C4b-Fmoc), 141.30, 141.24 (C8a-, C9a-Fmoc), 134.88 (C_{quart}-benzyl), 128.74, 128.62, 128.32, 127.84, 127.12 (C3-, C6-, C2-, C7-Fmoc, C_{tert}-benzyl), 124.92 (C1-, C8-Fmoc), 120.08 (C4-, C5-Fmoc), 101.46 (C1′-Gal), 98.94, 98.82 (C1-Gal, C2′′-Neu5Ac), 82.99 [$C(CH_3)_3$], 77.28 (C3-Gal), 72.83, 70.58, 69.13, 68.76, 67.68 (C4-, C5-Gal, C5′-Gal, C6′′-, C7′′-, C8′′-Neu5Ac), 70.70 (C3′-Gal), 68.99 (H4′′-Neu5Ac), 68.44 (C2′-Gal), 68.37 (S^{β}), 67.76 (CH_2-benzyl), 67.03 (CH_2-Fmoc), 66.74 (C4′-Gal), 63.24 (C6-Gal), 62.51 (C9′′-Neu5Ac), 60.81 (C6′-Gal), 54.94 (S^{α}), 49.09 (C5′′-Neu5Ac), 47.63, 47.09 (C2-Gal, C9-Fmoc), 37.67 (C3′′-Neu5Ac), 27.96 [C(CH_3)$_3$], 23.19, 23.11, 21.02, 20.78, 20.73, 20.62, 20.58, 20.53 (8 × COCH_3, 2 × NHCOCH_3).

ESI-MS: m/z calcd for $C_{70}H_{88}N_3O_{31}$: 1466.5; found: 1466.6 (M + H^+); m/z calcd for $C_{70}H_{87}N_3NaO_{31}$: 1488.5; found: 1488.6 (M + Na^+).

β-Anomer **19**

$[\alpha]_D^{23}$ = 29.3 (c = 1.00, CH_2Cl_2); R_f 0.52 (EtOAc–MeOH 10:1); R_t 34.54 min (Luna C18, 55% MeCN in H_2O → 55% MeCN in H_2O in 10 min → 70% MeCN in H_2O in 60 min → 100% MeCN in 80 min).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY): δ = 7.74 (d, 2 H, H4-, H5-Fmoc, $J_{H3,H4}$ = $J_{H5,H6}$ = 7.44 Hz), 7.60 (m, 2 H, H1-, H8-Fmoc, $J_{H1,H2}$ = $J_{H8,H7}$ = 7.04 Hz), 7.40–7.26 (m, 9 H, H2-, H3-, H6-, H7-Fmoc, C_{tert}-benzyl), 6.71 (d, 1 H, N*H*Ac_{Neu5Ac}, $J_{NH,H5''}$ = 9.76 Hz), 6.23 (d, 1 H, N*H*Fmoc, $J_{NH,S\alpha}$ = 7.44 Hz), 5.53 (d, 1 H, N*H*Ac_{GalNAc}, $J_{NH,H2}$ = 8.60 Hz), 5.40–5.34 (m, 3 H, H4′-Gal, H4′′-, H7′′-Neu5Ac), 5.25–5.11 (m, 4 H, H2′-Gal, H8′′-Neu5Ac, CH_2-benzyl), 4.96 (d, 1 H, H3′-Gal, $J_{H3',H2'}$ = 8.65 Hz), 4.80–4.73 (m, 2 H, H1-, H9′′a-Neu5Ac), 4.61–4.45 (m, 3 H, H1′-, H2-Gal, S^{α}), 4.37 (m, 2 H, CH_2-Fmoc), 4.20 [t, 1 H, H9-Fmoc, J(H9,CH_2) = 7.44 Hz], 4.13–3.98 (m, 7 H, H4-, H5′-, H6′a, H6′b-Gal, H5′′-, H6′′-, H9′′b-Neu5Ac), 3.87 (m, 1 H, $S^{\beta a}$), 3.81–3.78 (m, 2 H, H5-, H6a-Gal), 3.68–3.62 (m, 3 H, $S^{\beta b}$, H3-, H6b-Gal), 2.46 (dd, 1 H, H3′′*eq*-Neu5Ac, $J_{H3eq,H4}$ = 4.72 Hz, $J_{H3eq,H3ax}$ = 12.96 Hz), 2.13, 2.12, 2.06, 1.96, 1.95, 1.94, 1.78 (7 s, 30 H, 8 × $COCH_3$, 2 × NHCOCH_3), 1.88 (m, 1 H, H3′′*ax*-Neu5Ac), 1.48 (s, 9 H, *t*-C_4H_9).

^{13}C NMR (100.6 MHz, $CDCl_3$, HMQC): δ = 170.93, 170.63, 170.31, 170.19, 170.12, 169.52 (C=O), 166.09 (C1″-Neu5Ac), 156.04 (CO-urethane), 143.62 (C4a-, C4b-Fmoc), 141.30, 141.24 (C8a-, C9a-Fmoc), 134.84 (C_{quart}-benzyl), 128.63, 128.56, 128.33, 127.86, 127.15, 127.11 (C3-, C6-, C2-, C7-Fmoc, C_{tert}-benzyl), 125.04 (C1-, C8-Fmoc), 120.07 (C4-, C5-Fmoc), 101.26 (C1′-Gal), 98.32, 98.26 (C1-Gal, C2″-Neu5Ac), 83.23 [*C*(CH_3)$_3$] 77.21 (C3-Gal), 72.96, 70.61, 69.05, 68.76, 68.72, 67.56 (C4-, C5-, C2′-, C5′-Gal, C4″-, C6″-, C7″-, C8″-Neu5Ac), 70.70 (C3′-Gal), 68.67 (S^{β}), 67.62 (CH_2-benzyl), 67.33 (CH_2-Fmoc), 66.62 (C4′-Gal), 63.10 (C6-Gal), 62.44 (C9″-Neu5Ac), 60.62 (C6′-Gal), 54.58 (S^{α}), 48.94 (C5″-Neu5Ac), 47.51, 47.00 (C2-Gal, C9-Fmoc), 37.25 (C3″-Neu5Ac), 28.14 [C(*C*H_3)$_3$], 22.88, 20.98, 20.90, 20.76, 20.72, 20.56, 20.53 (CH_3 – OAc, NHCO*C*H_3).

ESI-MS: *m*/*z* calcd for $C_{70}H_{88}N_3O_{31}$: 1466.5; found: 1466.6 (M + H^+); *m*/*z* calcd for $C_{70}H_{87}N_3NaO_{31}$: 1488.5; found: 1488.6 (M + Na^+).

N-(9*H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-2-deoxy-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-6-*O*-[benzyl(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α/β-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-serine (20, [Fmoc-Ser({Ac_4Gal-(β1→3)}[Ac_4Neu5AcCOOBzl-(α2→6)]-α-Ac_2GalNAc)-OH])

A solution of sialyl-T conjugate **18** (220 mg, 0.15 mmol, 1.0 equiv) in trifluoroacetic acid (5 mL) and anisole (0.5 mL) was stirred at r.t. for 2 h. After evaporation of the solvents in vacuo, the residue was repeatedly coevaporated with toluene. The crude product was purified by flash chromatography on silica gel (EtOAc → EtOAc–EtOH–AcOH, 10:1:0.1) to yield an amorphous colorless solid **20** (200 mg, 94%); $[\alpha]_D^{23}$ +38.2 (*c* = 1.00, CH_2Cl_2); R_f 0.31 (EtOAc–EtOH–AcOH, 10:1:0.1).

^{1}H NMR (400 MHz, $CDCl_3$, ^{1}H-COSY, TOCSY): δ = 7.71 (m, 2 H, H4-, H5-Fmoc), 7.57 (m, 2 H, H1-, H8-Fmoc), 7.38–7.22 (m, 9 H, H2-, H3-, H6-, H7-Fmoc, C_{tert}-benzyl), 7.15 (m, 1 H, N*H*Fmoc), 5.35 (m, 1 H, H8″-Neu5Ac), 5.40–5.09 [m, 5 H, H2′- (5.09), H4′-Gal (5.26), H7″-Neu5Ac (5.28), CH_2-benzyl (5.15)], 4.94–4.87 [m, 2 H, H1-Gal (4.93), H3′-Gal (4.88)], 4.80–4.69 [m, 2 H, H4″-Neu5Ac (4.76), S^{α} (4.70)], 4.38–4.24 [m, 4 H, H1′-Gal (4.24), H9″a-Neu5Ac (4.31), CH_2-Fmoc], 4.10–3.95 [m, 6 H, H9-Fmoc (4.06), H5″- (4.06), H-6″- (4.00), H9″b-Neu5Ac (4.03), S^{β} (4.06)], 3.89–3.67 [m, 6 H, H2- (3.83), H4- (3.80), H5- (3.69), H6a-Gal (3.89), H6′-Gal (3.81)], 3.63 (m, 1 H, H3-Gal), 3.39–3.42 [m, 2 H, H6b-Gal (3.41), H5′-Gal (3.39)], 2.58 (dd, 1 H, H3″*eq*-Neu5Ac, $J_{H3eq,H4}$ = 4.28 Hz, $J_{H3eq,H3ax}$ = 12.52 Hz), 2.09, 2.07, 2.02, 2.01, 1.98, 1.97, 1.95, 1.93, 1.83, 1.79 (10 s, 30 H, 8 × $COCH_3$, 2 × NHCOCH_3), 1.89 (m, 1 H, H3″*ax*-Neu5Ac).

^{13}C NMR (100.6 MHz, $CDCl_3$, HMQC): δ = 170.09, 170.02, 169.97, 169.69, 167.35 (C=O), 167.35 (C1″-Neu5Ac), 156.01 (CO-urethane), 143.92, 143.62 (C4a-, C4b-Fmoc), 141.20 (C8a-, C9a-Fmoc), 134.87 (C_{quart}-benzyl), 128.76, 128.73, 128.59, 128.27, 127.94, 127.89, 127.27, 127.09 (C3-, C6-, C2-, C7-Fmoc, C_{tert}-benzyl), 125.34 (C1-, C8-Fmoc), 120.07 (C4-, C5-Fmoc), 102.05, 101.26 (C1′-Gal), 98.72, 98.63, 98.03 (C1-Gal, C2″-Neu5Ac), 80.59 (C3-Gal), 72.89, 69.18, 69.07, 68.91, 68.82, 67.56, 67.49, 66.69 (C4-, C5-, C2′-, C5′-Gal, C4″-, C6″-, C7″-, C8″-Neu5Ac), 70.67 (C3′-Gal), 67.65 (CH_2-benzyl), 67.38 (CH_2-Fmoc), 66.97 (S^{β}), 66.68 (C6″-Gal), 63.68 (C6-Gal), 62.56, 62.48 (C9″-Neu5Ac), 60.80 (C6′-Gal), 54.28 (S^{α}), 49.13 (C2-Gal), 47.22, 47.00 (C5″-Neu5Ac, C9-Fmoc), 37.67 (C3″-Neu5Ac), 23.13, 21.05, 20.80, 20.70, 20.62, 20.51, 20.40 (8 × $COCH_3$, 2 × NHCOCH_3); doubled signals due to conformers.

MALDI-TOF-MS: *m*/*z* calcd for $C_{66}H_{79}N_3NaO_{31}$: 1433.3; found: 1433.1 (M + Na^+); *m*/*z* calcd for $C_{66}H_{79}KN_3O_{31}$: 1449.4; found: 1449.2 (M + K^+).

L-Seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-L-seryl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic Acid (27) [H_2N-SHAVSS(αGalNAc)NGEAVE-OH])

The solid-phase glycopeptide synthesis was carried out according to the standard protocol described above employing a Perkin Elmer A433 peptide synthesizer. Starting from Rapp Tentagel® resin **1** preloaded with Fmoc-Glu(O-*t*-Bu)-O-PHB (500 mg, loading: 0.20 mmol/g), the protected fragment Fmoc-N(Trt)GE(O-*t*-Bu)AVE(O-*t*-Bu)-PHB-TentaGel **25** was prepared by iterative coupling of amino acids with coupling times of 30 min. In this course, the dipeptide Fmoc-Ala-Val-OH was introduced by a double coupling procedure. The glycosylated building block **10** was introduced in a manual coupling step. To this end, a solution of Fmoc-Ser(α-Ac_3GalNAc)-OH (**10**; 160 mg, 0.25 mmol, 2.5 equiv), HATU[16] (106 mg, 0.28 mmol, 2.8 equiv), HOAt (38 mg, 0.28 mmol, 2.8 equiv) and *N*-methylmorpholine (NMM) (60 μL, 0.56 mmol, 5.6 equiv) in DMF (2 mL) was added to the resin and vortexed for 5 h. The subsequent five amino acids were attached according to the standard protocol. Among these, Fmoc-Val-OH was coupled twice. The resin was washed with NMP and CH_2Cl_2. For the detachment, the resin was transferred into a solid-phase reactor and treated with a mixture of trifluoroacetic acid (13.13 mL), H_2O (0.75 mL), thioanisole (0.75 mL) and 1,2-ethanedithiol (0.25 mL) for 90 min. The crude glycopeptide was isolated as described above and subsequently dissolved in MeOH (50 mL). A 0.1 M solution of NaOMe in MeOH was added until pH 9.5 was reached. After stirring for 18 h, the reaction mixture was neutralized by addition of AcOH. The solvent was evaporated in vacuo, the residue dissolved in H_2O and purified by preparative RP-HPLC (Eurospher C8, 1% MeCN in H_2O → 100% MeCN in H_2O + 0.1% TFA in 60 min). The completely deprotected glycopeptide **27** was obtained as a colorless lyophilisate (90 mg, 65%); $[\alpha]_D^{23}$ –16.3 (*c* = 1.00, H_2O) {Lit.[38] $[\alpha]_D^{24}$ –14.0 (*c* = 0.8, H_2O)}; R_t 12.56 min (RP-HPLC, Eurospher C8, 215 nm, 1% MeCN in H_2O → 100% MeCN + 0.1% TFA in 42 min).

^{1}H NMR (400 MHz, D_2O, ^{1}H-COSY, TOCSY): δ = 8.51 (s, 1 H, Im^2), 7.20 (s, 1 H, Im^4), 4.88 (d, 1 H, H1-Gal, $J_{H1,H2}$ = 3.82 Hz), 4.70–4.60 (m, 2 H, N^{α}, H^{α}), 4.61 (m, 1 H, S^{α}), 4.46 (m, 1 H, S^{α}), 4.38 (m, 1 H, E^{α}), 4.31–4.22 (m, 3 H, 2 × A^{α}, E^{α}), 4.11–4.00 [m, 4 H, H2-Gal (4.10), S^{α} (4.07), 2 × V^{α} (4.10, 4.04)], 3.90–3.86 [m, 13 H, H3- (3.79), H4- (3.89), H5- (3.81), H6-Gal, G^{α}, 3 × S^{β}], 3.22 (dd, 1 H, $H^{\beta a}$, $J_{H\beta a,H\beta b}$ = 15.55 Hz, $J_{H\beta a,H\alpha}$ = 6.16 Hz), 3.17 (dd, 1 H, $H^{\beta b}$, $J_{H\beta a,H\beta b}$ = 15.85 Hz, $J_{H\beta a,H\alpha}$ = 7.62 Hz), 2.83–2.71 (m, 2 H, N^{β}), 2.42 (m, 4 H, 2 × E^{γ}), 2.12 (m, 1 H, $E^{\beta a}$), 2.06–1.88 (m, 8 H, E^{β}, $E^{\beta b}$, 2 × V^{β}, NHCOCH_3), 1.30 (m, 6 H, 2 × A^{β}), 0.86 (d, 12 H, 2 × V^{γ}, $J_{V\gamma,V\beta}$ = 6.74 Hz).

^{13}C NMR (100.6 MHz, D_2O, HMQC): δ = 176.91, 174.65, 173.32, 172.84, 172.78, 170.80, 170.79, 167.79, 163.02, 162.68 (C=O), 133.70 (N=CN-Im), 128.14 (NC=CH-Im), 117.47 (HNCH=CR-Im), 97.97 (C1-Gal), 71.40 (C5-Gal), 68.56 (C4-Gal), 67.82 (C3-Gal, S^{β}), 61.27, 60.14 (C6-Gal, 2 × S^{β}), 59.41 (2 × V^{α}), 54.46, 52.95, 52.84 (3 × S^{α}), 52.53, 51.92 (2 × E^{α}, H^{α}), 50.65 (N^{α}), 49.81, 49.71 (2 × A^{α}, C2-Gal), 42.69 (G^{α}), 36.29 (N^{β}), 30.34, 30.23, 30.00, 29.96 (2 × V^{β}, 2 × E^{γ}), 26.52 (H^{β}), 25.79, 25.69 (2 × E^{β}), 22.18 (NHCO*C*H_3), 18.50, 18.40, 17.72, 17.63 (2 × V^{γ}), 16.67, 16.48 (2 × A^{β}).

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{55}H_{89}N_{16}O_{26}$: 1390.4; found: 1391.0 (M + H^+); *m*/*z* calcd for $C_{55}H_{88}N_{16}NaO_{26}$: 1412.4; found: 1413.1 (M + Na^+); *m*/*z* calcd for $C_{55}H_{87}N_{16}Na_2O_{26}$: 1434.4; found: 1435.0 (M + 2Na^+ – H^+).

L-Seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-seryl-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic Acid (28, [H_2N-SHAVSS(β-GlcNAc)NGEAVE-OH])

The synthesis of **28** was carried out according to the described standard procedure. Tentagel® resin preloaded with Fmoc-Glu(O-*t*-Bu)-O-PHB (loading 500 mg, 0.20 mmol/g) **1** served as starting material. In order to react the glycosylated building block with the N-terminally deblocked resin-linked peptide **25**, Fmoc-Ser(β-Ac_3GlcNAc)-OH (**24**; 130 mg, 0.2 mmol, 2.0 equiv), HATU (83 mg, 0.22 mmol, 2.2 equiv), HOAt (30 mg, 0.22 mmol, 2.2 equiv) and NMM (47 μL, 0.44 mmol, 4.4 equiv) were dissolved in DMF (2 mL) and added to the resin. After 4 h, the reaction was terminated by filtration. The subsequent amino acids were then coupled according to the standard protocol. The acidolytic detachment from the resin was carried out by treatment with trifluoroacetic acid (15 mL), triisopropylsilane (0.9 mL) and H_2O (0.9 mL) within 2.5 h. The solution of the crude product was poured into cold Et_2O resulting in precipitation of the crude glycopeptide. Subsequently, the partially protected glycopeptide was deprotected with 0.1 M NaOMe in MeOH as described above (reaction time: 15 h). The crude deprotected glycopeptide **28** was dissolved in H_2O and purified by preparative RP-HPLC (Luna C18, 1% MeCN in H_2O → 100% MeCN in H_2O + 0.1% TFA in 60 min) to furnish the completely deprotected glycopeptide **28** as a colorless lyophilisate (70 mg, 51%); $[\alpha]_D^{23}$ –55.6 (*c* = 2.50, H_2O); R_t 11.27 min (RP-HPLC, Luna C18, 215 nm, 1% MeCN in H_2O → 100% MeCN + 0.1% TFA in 42 min).

^{1}H NMR (400 MHz, D_2O, ^{1}H-COSY, TOCSY): δ = 8.47 (s, 1 H, Im^2), 7.17 (s, 1 H, Im^4), 4.60–4.57 (m, 2 H, N^α, H^α), 4.41–4.30 (m, 3 H, H1-Glc, 2 × S^α), 4.25& ndash;4.12 (m, 3 H, 2 × A^α, E^α), 4.11–3.91 (m, 5 H, 2 × V^α, S^α, $S^{\beta a}$, E^α), 3.90–3.70 (m, 8 H, G^α, 2 × S^β, $S^{\beta b}$, H6a-Glc), 3.61–3.52 (m, 2 H, H2-Glc, H6b-Glc), 3.39 (t, 1 H, H3-Glc, $J_{H3,H2}$ = $J_{H3,H4}$ = 8.60 Hz), 3.29 (m, 2 H, H4-, H5-Glc), 3.13 (dd, 1 H, $H^{\beta a}$, $J_{H\beta a,H\beta b}$ = 15.68 Hz, $J_{H\beta a,H\alpha}$ = 6.28 Hz), 3.04 (dd, 1 H, $H^{\beta b}$, $J_{H\beta a,H\beta b}$ = 15.64 Hz, $J_{H\beta a,H\alpha}$ = 7.84 Hz), 2.74–2.65 (m, 2 H, N^β), 2.15 (m, 4 H, 2 × E^γ), 2.14–1.77 (m, 9 H, 2 × E^β, 2 × V^β, NHCOCH_3), 1.25 (m, 6 H, 2 × A^β), 0.78 (m, 12 H, 2 × V^γ).

^{13}C NMR (100.6 MHz, D_2O, HMQC): δ = 174.12, 173.18, 172.81, 170.77, 167.66 (C=O), 133.52 (NC=CH-Im), 127.98 (NC=CH-Im), 117.32 (HNCH=CR-Im), 100.87 (C1-Glc), 73.85 (C3-Glc), 75.77, 69.75 (C4-, C5-Glc), 67.88 (S^β), 60.59 (S^β), 60.56 (C6-Glc), 60.01 (S^β), 59.25, 59.12 (2 × V^α), 55.22, 54.25 (2 × S^α, 2 × E^α), 53.44 (S^α), 52.38 (H^α), 50.36 (N^α), 49.50 (2 × A^α), 42.63 (G^α), 37.06 (N^β), 32.69 (2 × E^γ), 30.10 (V^β), 27.51 (2 × E^β), 26.51 (H^β), 22.18 (NHCOCH_3), 18.43, 18.35, 17.56, 17.47, 17.38, 17.32, 16.70, 16.54, 16.44, 16.29 (2 × A^β, 2 × V^γ).

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{55}H_{89}N_{16}O_{26}$: 1389.6; found: 1389.3 (M + H^+); *m*/*z* calcd for $C_{55}H_{88}N_{16}NaO_{26}$: 1411.6; found: 1411.5 (M + Na^+); *m*/*z* calcd for $C_{55}H_{88}KN_{16}O_{26}$: 1427.6; found: 1427.5 (M + K^+).

L-Seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-*O*-(2-acetamido-2-deoxy-3-*O*-[β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-seryl-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic Acid (29, [H_2N-SHAVSS(Gal-(β1→3)-α-GalNAc)NGEAVE-OH])

Glycopeptide **29** was synthesized according to the described protocol. As starting material Tentagel® resin preloaded with Fmoc-Glu(O-*t*-Bu)-O-PHB **1** (455 mg, loading 0.22 mmol/g) was used. The coupling of the glycosylated building block was carried out by dissolving **16** (125 mg, 0.13 mmol, 1.3 equiv), HATU (152 mg, 0.4 mmol, 4.0 equiv), HOAt (54 mg, 0.4 mmol, 4.0 equiv) and NMM (88 μL, 0.8 mmol, 8.0 equiv) in DMF (2 mL). The mixture was added to the resin and kept vortexing for 5 h. After coupling of the following amino acids, the partially protected glycopeptide was isolated as described above. The acetyl groups were removed within 48 h, and the crude product was purified by preparative RP-RP-HPLC (Luna C18, 5% MeCN in H_2O → 5% MeCN in H_2O in 15 min → 20% MeCN in H_2O in 50 min → 100% MeCN + 0.1% TFA in 60 min). The completely deprotected glycopeptide **29** was obtained as a slightly yellowish lyophilisate (50 mg, 32%); $[\alpha]_D^{23}$ –9.4 (*c* = 1.00, H_2O); R_t 23.40 min (RP-HPLC, Luna C18, 215 nm, 5% MeCN in H_2O → 5% MeCN in H_2O in 10 min → 60% MeCN in H_2O in 40 min → 100% MeCN + 0.1% TFA in 60 min).

^{1}H NMR (400 MHz, D_2O, ^{1}H-COSY, TOCSY): δ = 8.62 (s, 1 H, Im^2), 7.32 (s, 1 H, Im^4), 4.91 (1 H, H1-Gal), 4.78 (1 H, H^α), 4.76 (1 H, N^α), 4.67 (m, 1 H, S^α), 4.54 (m, 1 H, S^α), 4.48 (d, 1 H, H1′-Gal, $J_{H1',H2'}$ = 7.44 Hz), 4.40–4.29 [m, 5 H, H2-Gal (4.36), 2 × A^α (4.38), 2 × E^α (4.33)], 4.25–4.08 [m, 4 H, H4-Gal (4.24), 2 × V^α (4.20, 4.15), S^α (4.17)], 4.05–3.85 [m, 11 H, H3- (4.02), H5-Gal (3.91), H4′-Gal (3.92), G^α, 3 × S^β], 3.74 (br s, 4 H, H6-Gal, H6′-Gal), 3.63–3.59 [m, 2 H, H3′-Gal (3.62), H5′-Gal (3.65)], 3.51 (m, 1 H, H2′-Gal), 3.21 (m, 2 H, H^β), 2.83 (m, 2 H, N^β), 2.32 (br s, 4 H, E^γ), 2.15–1.90 (m, 9 H, 2 × V^β, 2 × E^β, NHCOCH_3), 1.38 (m, 6 H, A^β), 0.94 (br s, 12 H, V^γ).

^{13}C NMR (100.6 MHz, D_2O, HMQC): δ = 104.25 (C1′-Gal), 97.49 (C1-Gal), 76.83 (C3-Gal), 74.51 (C5′-Gal), 72.02 (C3′-Gal), 70.56 (C4′-Gal), 70.20 (C2′-Gal), 68.17 (C4-Gal), 68.06 (C5-Gal), 60.60 (C6-Gal, C6′-Gal), 66.46, 60.80, 59.71 (3 × S^β), 59.01, 58.87 (2 × V^α), 54.43, 53.43 (2 × S^α), 54.00 (2 × E^α), 53.13 (S^α), 52.02 (H^α), 50.19 (N^α), 49.48 (2 × A^α), 42.36 (C2-Gal), 42.14 (G^α), 35.72 (N^β), 31.86 (2 × E^γ), 29.79 (2 × V^β), 27.02, 26.63 (2 × E^β), 26.33 (H^β), 21.62 (NHCOCH_3), 18.10, 17.09 (2 × V^γ), 16.04 (2 × A^β).

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{61}H_{99}N_{16}O_{31}$: 1552.5; found: 1552.3 (M + H^+); *m*/*z* calcd for $C_{61}H_{98}N_{16}NaO_{31}$: 1574.5; found: 1574.4 (M + Na^+); *m*/*z* calcd for $C_{61}H_{98}KN_{16}O_{31}$: 1590.6; found: 1590.3 (M + K^+).

L-Seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-*O*-(2-acetamido-2-deoxy-3-*O*-[β-D-galactopyranosyl]-6-*O*-{(5-acetamido-3,5-dideoxy-a-glycero-d-galacto-2-nonulopyranosyl)onate}-a-d-galacto-pyranosyl)-L-seryl-L-asparaginyl-glycyl-L-glutamyl-L-alanyl-L-valyl-L-glutamic Acid (32, [H_2N-SHAVSS({Gal-(β1→3)}[Neu5Ac-(α2→6)]-α-GalNAc)NGEAVE-OH])

This glycopeptide was synthesized according to the procedure described for **28**. Tentagel® resin preloaded with Fmoc-Glu(O-*t*-Bu)-O-PHB **1** (455 mg, loading 0.22 mmol/g) was used. The glycosylated building block was coupled manually by addition of a mixture of **18** (190 mg, 0.13 mmol, 1.3 equiv), HATU (148 mg, 0.39 mmol, 3.9 equiv), HOAt (53 mg, 0.39 mmol, 3.9 equiv) and NMM (86 μL, 0.78 mmol, 7.8 equiv) in DMF (2 mL) to the resin (coupling time: 4 h). The crude product was isolated as described above.

MALDI-TOF-MS (DHB): *m*/*z* calcd for $C_{95}H_{138}N_{17}O_{47}$: 2270.2; found: 2270.5 (M + H^+); *m*/*z* calcd for $C_{95}H_{137}N_{17}NaO_{47}$: 2292.2; found: 2292.2 (M + Na^+).

The benzyl ester was subsequently removed by hydrogenation. For this purpose, the partially protected glycopeptide **30** was dissolved in MeOH (30 mL). After addition of a catalytic amount of 10% Pd/C, the mixture was hydrogenated for 3 d. The hydrogenation was terminated by flooding the flask with argon and filtration through Hyflo Supercel. After removal of the solvents in vacuo, the crude product **31** was dissolved in MeOH, and purified by preparative RP-HPLC (Luna C18, 5% MeCN in H_2O → 5% MeCN in H_2O in 15 min → 20% MeCN in H_2O in 50 min → 100% MeCN + 0.1% TFA in 60 min) and obtained as a colorless lyophilisate (71 mg, 33%).

31

$[\alpha]_D^{23}$ –1.7 (*c* = 1.00, MeOH); R_t 28.95 min (RP-HPLC, Luna C18, 215 nm, 5% MeCN in H_2O → 5% MeCN in H_2O in 10 min → 60% MeCN in H_2O in 40 min → 100% MeCN + 0.1% TFA in 60 min).

^{1}H NMR (600 MHz, DMSO-d_6, ^{1}H-COSY, TOCSY): δ = 8.98 (s, 1 H, Im^2), 8.69 (d, 1 H, H^{NH}, $J_{NH,H\alpha}$ = 7.92 Hz), 8.31 (d, 1 H, A^{NH}, $J_{NH,A\alpha}$ = 6.60 Hz), 8.23–8.13 (m, 5 H, E^{NH}, G^{NH}, 2 × S^{NH}, N^{NH}), 8.03 (d, 1 H, A^{NH}, $J_{NH,A\alpha}$ = 6.60 Hz), 8.00–7.93 (m, 2 H, E^{NH}, S^{NH}), 7.82–7.72 (m, 3 H, 2 × V^{NH}, NH$_{Neu5NAc}$), 7.46 (s, 1 H, NH$_2$-N) 7.36 (s, 1 H, Im^4), 7.28 (d, 1 H, NH$_{GalNAc}$, $J_{NH,H2}$ = 7.44 Hz), 6.93 (s, 1 H, NH$_2$-N), 5.22 (m, 2 H, H4′-Gal, H8″-Neu5Ac), 5.15 (d, 1 H, H7″-Neu5Ac, $J_{H7'',H8''}$ = 8.52 Hz), 5.01 (dd, 1 H, H3′-Gal, $J_{H3',H2'}$ = 10.44 Hz), 4.95 (t, 1 H, H2′-Gal, $J_{H2',H3'}$ = 10.14 Hz), 4.81 (d, 1 H, H1′-Gal, $J_{H1',H2'}$ = 7.68 Hz), 4.72 (m, 1 H, H4″-Neu5Ac), 4.66 (m, 1 H, H$^\alpha$), 4.62 (d, 1 H, H1-Gal, $J_{H1,H2}$ = 2.20 Hz), 4.53 (m, 1 H, N$^\alpha$), 4.46–4.27 [m, 7 H, 2 × A$^\alpha$ (4.36, 4.31), 2 × S$^\alpha$ (4.46, 4.43), V$^\alpha$ (4.27), E$^\alpha$ (4.27), H2-Gal (4.24)], 4.18–4.09 [m, 5 H, H6a-Gal (4.14), H5′- (4.11), H6′a-Gal (4.12), E$^\alpha$ (4.18), V$^\alpha$ (4.15)], 4.06–3.93 [m, 3 H, H6b-Gal (4.03), H6′β-Gal (3.98), H6″-Neu5Ac (4.02)], 3.90–3.76 [m, 4 H, S$^\alpha$ (3.87), G$^{\alpha a}$ (3.80), H4-Gal (3.81), H5″-Neu5Ac (3.83)], 3.74–3.53 [m, 10 H, H3- (3.62), H5-Gal (3.69), H9″α-Neu5Ac (3.69), G$^{\alpha b}$ (3.62), 3 × S$^\beta$ (3.72, 3.61; 3.70, 3.65; 3.66, 3.58), 3.55 (m, H9″β-Neu5Ac)], 3.07 (dd, 1 H, H$^{\beta a}$, $J_{H\beta a,H\beta b}$ = 15.68 Hz, $J_{H\beta a,H\alpha}$ = 6.28 Hz), 3.01 (dd, 1 H, H$^{\beta b}$, $J_{H\beta a,H\beta b}$ = 15.64 Hz, $J_{H\beta a,H\alpha}$ = 7.84 Hz), 2.53–2.44 (m, 3 H, H3″$_{eq}$-Neu5Ac, N$^\beta$), 2.24 (m, 4 H, E$^\gamma$), 2.09–1.74 (10 s, 30 H, 8 × COC*H*$_3$, 2 × NHCOC*H*$_3$), 1.99 (m, 2 H, V$^\beta$), 1.95, 1.76 (m, 2 H, E$^\beta$), 1.89, 1.73 (m, 2 H, E$^\beta$), 1.61 (m, 1 H, H3″$_{ax}$-Neu5Ac), 1.23 (m, 6 H, 2 × A$^\beta$), 0.85, 0.81 (m, 12 H, 2 × V$^\gamma$).

^{13}C NMR (150.9 MHz, DMSO-d_6, HMQC): δ = 132.81 (N=CN-Im), 117.43 (HNCH=CR-Im), 100.98 (C1′-Gal), 97.96 (C1-Gal), 77.10 (C3-Gal), 71.52 (C6″-Neu5Ac), 70.56 (C3′-Gal), 69.89 (C5′-Gal), 69.41 (C4″-Neu5Ac), 69.13 (C5-Gal), 68.26 (C2′-Gal), 67.59, 67.20 (C4′-Gal, C8″-Neu5Ac), 67.30 (C4-Gal), 67.02 (C7″-Neu5Ac), 66.25 (S$^\beta$), 63.28 (C9″-Neu5Ac), 61.83 (S$^\beta$), 61.84 (C6-Gal), 60.94 (C6′-Gal), 60.48 (S$^\beta$), 57.57, 57.41 (V$^\alpha$), 54.07 (H5″-Neu5Ac), 54.34, 52.81 (S$^\alpha$), 51.73, 50.97 (E$^\alpha$), 51.43 (H$^\alpha$), 49.89 (N$^\alpha$), 48.41, 48.31 (2 × A$^\alpha$), 47.16 (C2-Gal), 42.18 (G$^\alpha$), 37.91 (C3″-Neu5Ac), 37.33 (N$^\beta$), 30.78 (V$^\beta$), 29.83 (E$^\gamma$), 27.26 (H$^\beta$), 27.35, 26.20 (E$^\beta$), 22.83, 22.70, 22.64, 20.86, 20.67, 20.64, 20.42, 20.41 (8 CH$_3$, 8 × COCH$_3$, 2 × NHCOC*H*$_3$), 19.08, 17.68 (2 × V$^\gamma$), 17.75 (2 × A$^\beta$).

Subsequently, removal of *O*-acetyl groups was carried out as described above. Glycopeptide **31** (68 mg, 0.03 mmol) was treated with 0.1 M NaOMe in MeOH for 18 h. The crude glycopeptide was purified by preparative RP-HPLC (Luna C18, 5% MeCN in H$_2$O → 5% MeCN in H$_2$O in 15 min → 20% MeCN in H$_2$O in 50 min → 100% MeCN + 0.1% TFA in 60 min) in order to obtain **32** as a colorless lyophilisate (34 mg, 61%).

32

$[\alpha]_D^{23}$ +13.9 (*c* = 1.0, H$_2$O); R$_t$ 22.83 min (RP-HPLC, Luna C18, 215 nm, 5% MeCN in H$_2$O → 5% MeCN in H$_2$O in 10 min → 60% MeCN in H$_2$O in 40 min → 100% MeCN + 0.1% TFA in 60 min).

^{1}H NMR (600 MHz, DMSO-d_6, ^{1}H-COSY, TOCSY, NOESY, ROESY): δ = 8.83 (s, 1 H, Im^2), 8.67 (d, 1 H, H^{NH}, $J_{NH,H\alpha}$ = 7.44 Hz), 8.36 (d, 1 H, A^{NH}, $J_{NH,A\alpha}$ = 5.48 Hz), 8.37–7.91 (m, 9 H, 2 × E^{NH}, N^{NH}, A^{NH}, 2 × S^{NH}, V^{NH}, G^{NH}, NH$_{Neu5NAc}$), 7.92 (d, 1 H, E^{NH}, $J_{NH,E\alpha}$ = 7.44 Hz), 7.77 (d, 1 H, V^{NH}, $J_{NH,V\alpha}$ = 8.60 Hz), 7.47 (s, 1 H, NH$_2$-N), 7.28 (m, 2 H, NH$_{GalNAc}$, Im^4), 6.93 (s, 1 H, NH$_2$-N), 4.66 (m, 2 H, H1-Gal, H$^\alpha$), 4.52 (m, 1 H, N$^\alpha$), 4.46–4.25 [m, 6 H, 2 × A$^\alpha$ (4.30, 4.33), 2 × S$^\alpha$ (4.45, 4.40), V$^\alpha$ (4.22), E$^\alpha$ (4.26)], 4.18–4.09 [m, 3 H, H2-Gal (4.18), E$^\alpha$ (4.17), V$^\alpha$ (4.15)], 3.90–3.87 (m, 2 H, H5-Gal, S$^\alpha$), 3.76–3.25 [m, 24 H, G$^\alpha$ (3.76, 3.61), 3 × S$^\beta$ (3.70, 3.64; 3.73, 3.57; 3.65, 3.58), H3- (3.67), H4- (3.61), H6-Gal (3.67, 3.55), H2′- (3.32), H3′- (3.25), H4′- (3.61), H6′-Gal (3.48), H4″- (3.54), H5″- (3.44), H6″-, H7″-, H8″-, H9″a-Neu5Ac (3.61), H9″b-Neu5Ac (3.37))], 3.10–2.96 (m, 2 H, H$^\beta$), 2.57–2.44 (m, 3 H, H3″$_{eq}$-Neu5Ac, N$^\beta$), 2.27 (m, 4 H, 2 × E$^\gamma$), 1.96–1.73 [m, 12 H, 2 × NHCOC*H*$_3$ (1.86, 1.81), 2 × V$^\beta$ (1.94), 2 × E$^\beta$ (1.94, 1.76; 1.87, 1.71)], 1.47 (m, 1 H, H3″$_{ax}$-Neu5Ac, $J_{H3''eq,H3''ax}$ = $J_{H3''eq,H4''}$ = 11.32 Hz), 1.23 (d, 3 H, A$^\beta$, $J_{A\beta,A\alpha}$ = 6.68 Hz), 1.17 (d, 3 H, A$^\beta$, $J_{A\beta,A\alpha}$ = 6.64 Hz), 0.83 (m, 12 H, 2 × V$^\gamma$).

^{13}C NMR (150.9 MHz, DMSO-d_6, HMQC): δ = 104.54 (C1′-Gal), 98.04 (C1-Gal), 76.94 (C3-Gal), 75.04 (C5′-Gal), 73.02 (C3′-Gal), 71.21 (C4-Gal), 70.78 (C2′-Gal), 69.79 (C4′-Gal), 67.53 (C5-Gal), 73.04, 69.07, 68.51 (H6″-, H7″-, H8″-Neu5NAc), 66.24 (S$^\beta$), 63.16 (C6-Gal), 62.88 (C9″-Neu5Ac), 61.58 (S$^\beta$), 60.26 (C6′-Gal), 60.21 (S$^\beta$), 57.57, 57.26 (2 × V$^\alpha$), 54.86, 53.97, 52.77 (3 × S$^\alpha$), 52.25 (C5″-Neu5Ac), 51.51 (H$^\alpha$, E$^\alpha$), 50.90 (E$^\alpha$), 49.70 (N$^\alpha$), 48.29, 48.04 (A$^\alpha$), 47.70 (C2-Gal), 42.09 (G$^\alpha$), 41.13 (C3″-NeuNAc), 36.97 (N$^\beta$), 30.72 (2 × V$^\beta$), 29.98 (E$^\gamma$), 27.47 (H$^\beta$), 27.39, 26.17 (E$^\beta$), 22.84, 22.50 (NHCO*C*H$_3$), 19.10, 17.88 (2 × V$^\gamma$), 17.64 (2 × A$^\beta$).

MALDI-TOF-MS: *m*/*z* calcd for C$_{61}$H$_{98}$N$_{16}$NaO$_{31}$: 1574.5; found: 1576.0 (M – sialic acid + H$^+$ + Na$^+$); *m*/*z* calcd for C$_{61}$H$_{98}$KN$_{16}$O$_{31}$: 1590.6; found: 1590.3 (M – sialic acid + H$^+$ + K$^+$).

L-Cystyl-di(L-seryl-L-histidyl-L-alanyl-L-valyl-L-seryl-L-seryl-L-asparaginyl-glycine) (35)

For automated peptide synthesis, polystyrene resin preloaded with Fmoc-Gly-O-PHB (164 mg, loading 0.61 mmol/g) was used. The subsequent seven amino acids were then coupled according to the standard protocol. The last amino acid, Fmoc-Cys(Trt)-OH, was coupled manually to the resin. For that purpose, a mixture of Fmoc-Cys(Trt)-OH (293 mg, 0.5 mmol, 5 equiv), TBTU (161 mg, 0.5 mmol, 5 equiv), HOBt (77 mg, 0.5 mmol, 5 equiv) was dissolved in CH_2Cl_2 (2 mL) and rapidly added to the resin. Finally, *sym*-collidine (67 μL, 1.0 mmol, 10 equiv) was transferred into the reaction vessel. After 20 min, the resin was washed thoroughly. The crude peptide **33** was isolated by acidolysis with trifluoroacetic acid, H$_2$O, and triisopropylsilane as described above (90 mg). As the compound showed sufficient purity according to RP-HPLC (R$_t$ 14.56 min, Luna C18, 215 nm, 1% MeCN in H$_2$O → 100% MeCN + 0.1% TFA in 42 min), a portion of the crude peptide (73 mg) was directly oxidized by a mixture of trifluoroacetic acid (5 mL) and DMSO (0.5 mL) at r.t. within 3 h to give **34** (RP-HPLC, R$_t$ 15.39 min, Luna C18, 215 nm, 1% MeCN in H$_2$O → 100% MeCN + 0.1% TFA in 42 min). Finally, the solvent was removed in vacuo, and the residue was coevaporated efficiently with DMF and toluene. The Fmoc-protected dimer **34** was treated with a mixture of DMF and piperidine (1:1, 15 mL) for 2 h. After evaporation of the solvents in vacuo and coevaporation with toluene, the dimeric peptide **35** was dissolved in H$_2$O and purified by gel permeation chromatography (Sephadex G-25, 0.4 mL/min, fraction size: 15 min). Lyophilization yielded **35** as a slightly yellowish amorphous solid. (40 mg, 68% overall yield); $[\alpha]_D^{23}$ –31.4 (*c* = 1.00, H$_2$O).

^{1}H NMR (600 MHz, D$_2$O, ^{1}H-COSY): δ = 8.40 (m, 2 H, 2 × Im^2), 7.24 (m, 2 H, 2 × Im^4), 4.85 (m, 2 H, 2 × N$^\alpha$), 4.70 (m, 2 H, 2 × H$^\alpha$), 4.54–4.46 (m, 6 H, 6 × S$^\alpha$), 4.34 (q, 2 H, 2 × A$^\alpha$, $J_{A\alpha,A\beta}$ = 7.04 Hz), 4.15 (dd, 2 H, 2 × V$^\alpha$, $J_{V\alpha,V\beta a}$ = 7.04 Hz, $J_{V\alpha,V\beta b}$ = 2.76 Hz), 3.92–3.81 (m, 12 H, 6 × S$^\beta$), 3.75 (m, 4 H, 2 × G$^\alpha$), 3.30–3.09 (m, 4 H, 2 × H$^\beta$), 2.86 (dd, 2 H, 2 × N$^{\beta a}$, $J_{N\beta a,N\beta b}$ = 15.64 Hz, $J_{N\beta a,N\alpha}$ = 5.48 Hz), 2.71 (dd, 2 H, 2 × N$^{\beta b}$, $J_{N\beta b,N\beta a}$ = 15.64 Hz, $J_{N\beta b,N\alpha}$ = 8.60 Hz), 2.07 (m, 2 H, 2 × V$^\beta$), 1.35 (m, 6 H, 2 × A$^\beta$), 0.94 (d, 12 H, 2 × V$^\gamma$, $J_{V\gamma,V\beta}$ = 7.04 Hz); not visible: C$^\alpha$, C$^\beta$.

^{13}C NMR (150.9 MHz, DMSO-d_6, HMQC): δ = 177.31, 177.22, 175.97, 174.35, 174.22, 173.97, 173.81 (C=O), 119.8 (NCH=CRIm), 63.72, 63.47 (S$^\beta$), 62.01 (V$^\alpha$), 58.19, 57.71 (S$^\alpha$), 55.06 (H$^\alpha$), 53.04 (N$^\alpha$), 52.21 (A$^\alpha$), 45.92 (G$^\alpha$), 38.99 (N$^\alpha$), 32.70 (V$^\beta$), 29.45 (H$^\beta$), 20.93, 20.17 (V$^\beta$), 19.01 (A$^\beta$); not visible: C$^\alpha$, C$^\beta$.

MALDI-TOF-MS: *m*/*z* calcd for C$_{64}$H$_{103}$N$_{24}$O$_{28}$S$_2$: 1720.8; found: 1720.7 (M + H$^+$); *m*/*z* calcd for C$_{64}$H$_{102}$N$_{24}$NaO$_{28}$S$_2$: 1742.8; found:

1742.6 ($M + Na^+$); *m/z* calcd for $C_{64}H_{102}KN_{24}O_{28}S_2$: 1758.9; found: 1758.8 ($M + K^+$).

Acknowledgment

This work was supported by the Volkswagen-Stiftung and by the Fonds der Chemischen Industrie.

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