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Hydrophilic photolabelling of glycopeptides from the murine liver–intestine (LI) cadherin recognition domain

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Abstract—LI-Cadherin is a transmembrane glycoprotein involved in cell adhesion of epithelial cells. Its supposed recognition domain contains the peptide motif AAL and is distinctly hydrophobic. In order to obtain sufficiently soluble model compounds, glycan side chains of T-antigen, (2,6)sialyl T-antigen and sialyl T_N-antigen structure were linked to the serine located in the supposed turn sequence of the LI-cadherin recognition domain. A quinic acid-glycine-7-amino-coumarine (Quiglac) chromophore was constructed in order to enhance the solubility of labelled LI-cadherin (glyco)peptides in water.

1. Introduction

Cadherins constitute a family of transmembrane glycoproteins which exert decisive functions in cell adhesion phenomena.[1,2] In their extracellular portion they contain consensus repeat units of about 110 amino acids. According to the number of these repeats cadherins are subdivided in different classes.[3] Epithelial cadherin (E-cadherin) is the most intensely investigated cadherin.[4,5] It belongs to the classical cadherins containing five extracellular consensus repeat (EC) units. It is strongly expressed on polarized, differentiated epithelial cells, however, down-regulated on tumour cells. Its Ca^{2+}-dependent interaction is homotypic and homophilic and proceeds via a cis dimer (on the same cell) which binds to a trans dimer on the neighbouring cell.[6] The homophilic recognition site of E-cadherin has been identified in consensus repeat 1 (EC 1).[1–5] According to NOE NMR spectroscopic analyses, the homophilic recognition domain is formed by three β-sheets (βC, βF, and βG) and includes the recognition motif His^{79}-Ala^{80}-Val^{81} in β-sheet F close to a turn motif Ser^{83}-Asn^{84}-Gly^{85} between β-sheets F and G.[7] Synthetic glycopeptides glycosylated at Ser^{83} and adopting a turn-type conformation induced cell differentiation[8] in transformed basal keratinocytes of the HaCat[9] cell line. This effect turned out to be dependent upon the conformation of these (glyco)peptides.[8]

Liver intestine (LI) cadherin has been found as a member of a new class of cadherins consisting of seven extracellular EC domains.[10] Instead of the tripeptide motif HAV of E-cadherin, the tripeptide AAL in the furthermost consensus repeat EC1 was identified as the recognition motif of LI-cadherin.[11]

Whereas the classical cadherins are involved in important cellular processes such as differentiation[12] or growth regulation and morphogenesis,[2,4] there are indications that murine LI-cadherin during differentiation influences transcription factors CDx1 and CDx2[13] important for intestine formation. In addition, a connection between LI-cadherin expression and the differentiation of pancreas carcinoma cells was found.[14]

Except for the amino acid sequence and the AAL recognition motif[10,11] not much is known about the detailed structure of LI cadherin. A comparison of the amino acid sequence around the AAL motif for human (A), rat (B) and murine (C) LI cadherin with the related area of E cadherin suggests that the recognition region of LI cadherins also should preferably adopt a turn-type conformation.

Keywords: Glycopeptides; LI-cadherin; Coumarine chromophore; Sialyl-T antigen; Solid-phase synthesis.
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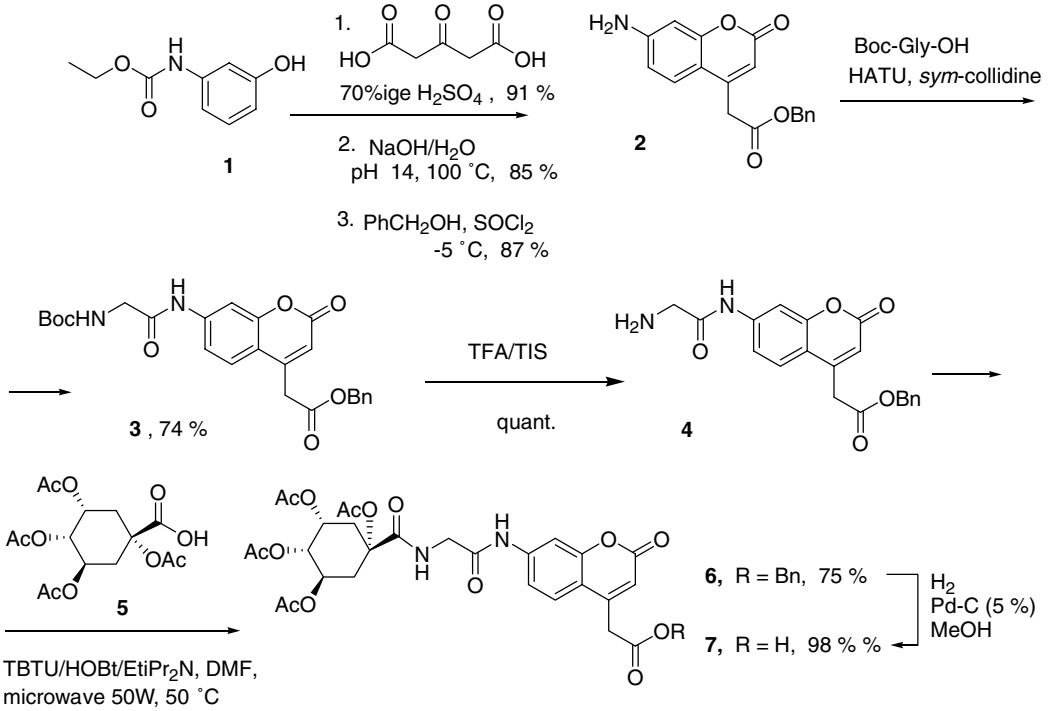
human	LI-CAD	Q^{95}	$VAALD^{100}$	$ANGII^{105}$	VEGPV	A
rat	LI-CAD	QL^{95}	$AALDS^{100}$	$QGAIV^{105}$	DGPV	B
mouse	LI-CAD	QL^{95}	$AALDS^{100}$	$HGAIV^{105}$	DGPV	C

Conformational analysis of a glycodecapeptide containing the amino acid sequence L^{95}-I^{104} of rat-LI-cadherin and an α-*O*-galactosaminyl side chain at S^{100} by NOESY NMR spectroscopy in DMSO-d_6 showed that this molecule actually prefers a turn-type conformation.[15] The interesting question is whether this result indicates a similar homophilic recognition site conformation as was found for E-cadherin.[7] To answer this question, it requires to investigate the compound and its biological effects in water. However, the insufficient water-solubility of this molecule opposed all these efforts. Therefore, we turned our interest to the supposed recognition region of murine LI-cadherin containing a histidine in the assumed turn sequence. Introduction of sialic acid containing saccharides at serine[100] should further enhance the water-solubility. As localization of the binding site of such a LI-cadherin glycopeptide on a cell is of particular interest, the labelling of the glycopeptides with a fluorophore appeared highly desirable. Fluorescent chromophores usually are hydrophobic. Therefore, it was a first aim to construct a fluorophore which promotes water-solubility of hydrophobic LI-cadherin glycopeptides.

2. Construction of a novel chromophore promoting solubility in water

Coumarine derivatives are fluorescent chromophores frequently used for fluorescent resonance energy transfer (FRET) spectroscopy.[16] In order to synthesize a suitable coumarine chromophore, ethoxycarbonyl-protected *m*-amino-phenol[17] **1** was subjected to a Pechmann condensation reaction[18] with acetone 1,3-dicarboxylic acid. After removal of the ethoxycarbonyl group, Brenner ester formation[19] with benzylic alcohol in $SOCl_2$ at −5 °C gave 7-aminocoumarine derivative **2** in high yield. Its amino function turned out to be a poor nucleophile. Therefore, its acylation with Boc-glycine to give **3** was promoted by the efficient reagent *O*-(7-azabenzotriazol-1-yl-)-*N*,*N*,*N*′,*N*′,-tetramethyluronium-hexafluorophosphate (HATU)[20] in combination with collidine. After acidolytic removal of the Boc group, coupling of **4** with tetra-*O*-acetyl quinic acid[21] **5** was achieved by activation with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluroniumtetrafluoroborate (TBTU)[22]/1-hydroxybenzotriazol (HOBt) under microwave irradiation (50 W, 50 °C). The hydrogenolytic cleavage of the benzylic ester of **6** requires selective conditions. Hydrogenation catalyzed by Pd/C (10%) produced the coumarine carboxylic acid **7** in a yield of only 22%, but the analogous compound saturated in the pyrone ring as the major product. In contrast, hydrogenation over Pd/C (5%) and careful monitoring of the reaction by TLC yielded the fluorophoric carboxylic compound **7** almost quantitatively (Scheme 1).

During coupling reactions of the fluorescent amino acid **7**, decarboxylation occurred as a notable side reaction. The amount of the decarboxylated methyl derivative depended upon the chosen reaction conditions. There-



Scheme 1.

fore, it appeared advisable to couple the N-terminal amino acid of the glycopeptide to be synthesized prior to the solid-phase synthesis. In coupling reactions with leucine *tert*-butyl ester best results were obtained with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and HOBt (Scheme 2). The chromophore-leucine ester conjugate **8** and 4-methyl-coumarine **9** derivative were formed in a ratio of 2.8:1. The mixture of by-product and product could only be separated by HPLC.

However, after treating the mixture of **8** and **9** with TFA/triisopropylsilane(TIS)/H_2O (14:1:1), the free carboxylic acid **10** was separable by flash-chromatography and isolated in an overall yield of 63% after the two steps.

For fluorescence spectroscopy, the *O*-acetyl groups were removed from **10** by Zemplén transesterification[23] with NaOMe in methanol to give **11**.

3. Syntheses of *O*-glycosyl serine building blocks

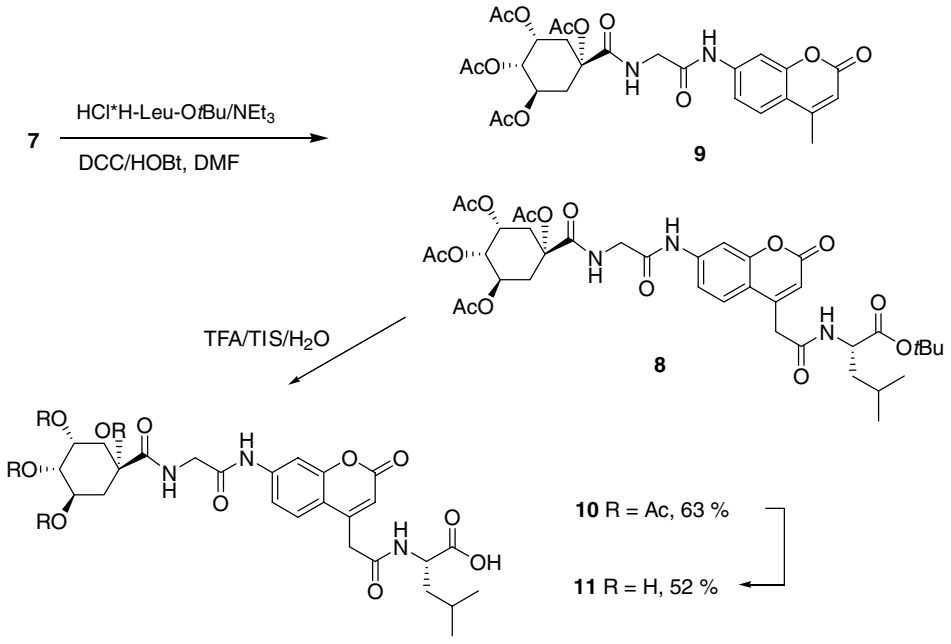
The glycosylated serine building blocks were synthesized from Fmoc serine *tert*-butyl ester[24] as is shown for the example of the sialyl T_N derivative in Scheme 3. Fmoc-protected 2-azido-galactosyl serine *tert*-butyl ester[25] **12** was carefully *O*-deacetylated by Zemplén transesterification at pH 8.5.[26]

By these conditions, the base-labile Fmoc group remained stable. Regioselective and stereoselective sialylation of **13** was achieved using the xanthate[27] **14** of sialic acid benzyl ester promoted by methylsulfenyl trifluoromethylsulfonate[28] in dichloromethane/acetonitrile at −65 °C[29] to yield the desired sialyl T_N-serine conjugate **15**. After purification by flash-chromatography, the hydroxy groups in 3- and 4-position were acetylated to give **16** which is sufficiently stable to acids. Cleavage of its *tert*-butyl ester with trifluoroacetic acid and anisole furnished the sialyl T_N-serine building block **17** suitable for solid-phase glycopeptide synthesis. The corresponding T-antigen (**18**) and 2,6-sialyl T-antigen (**19**) serine conjugates obtained by a similar pathway have already been described.[25]

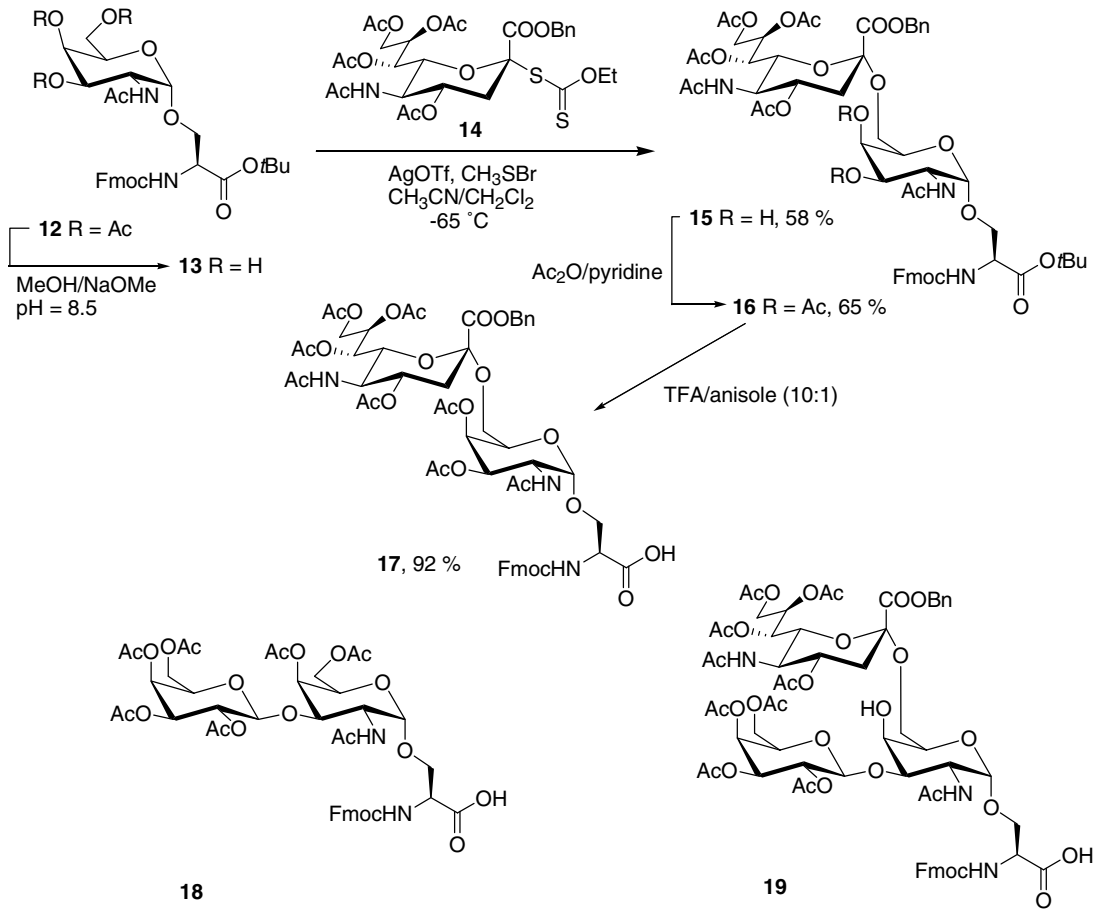
4. Solid-phase synthesis of LI-cadherin glycopeptides

The peptide and glycopeptide sequences of the supposed homophilic recognition site of the murine LI-cadherin were synthesized on solid-phase using a peptide synthesizer. Tentagel® resin[30] preloaded with Fmoc valine via the acid-labile Wang anchor[31] **20** was used. The syntheses were carried out according to the Fmoc protocol.[32] The Fmoc amino acids were used in an excess of 20 equiv. HBTU/HOBt[22] served as coupling reagent (coupling time 20–30 min). The Fmoc groups were removed by treatment with piperidine (20%) in *N*-methyl-pyrrolidinone (NMP). The glycosylated amino acid building blocks were coupled to the resin-linked peptides manually using HATU/HOAt[20] as the coupling reagent.

The synthesis of the pentadecapeptide Leu^{95}-Val^{109} of murine LI-cadherin (see introduction) attempted at first suffered from the insolubility of the product. It could not be purified by chromatography and was only detectable by mass spectroscopy. In order to overcome the low solubility rendering any purification and characterization of the product impossible, a glycopeptide containing the shortened undecapeptide sequence Leu^{95}-Val^{105} and carrying the disaccharide T-antigen side chain (from **18**) was constructed (Scheme 4).



Scheme 2.



Scheme 3.

Detachment from the resin **21** and simultaneous removal of the amino acid side-chain-protecting groups were achieved with TFA/triisopropylsilane(TIS)/water. Addition of diethyl ether to the reaction solution resulted in the precipitation of crude glycopeptide **22**. After removal of the *O*-acetyl groups from **22** by Zemplén reaction at pH 9.5, a product of good solubility in water was obtained (Scheme 5). Its purification by preparative HPLC yielded the desired T antigen LI-cadherin glycopeptide **23** (45%) and two deletion sequences **23a** (13%) and **23b** (16%).

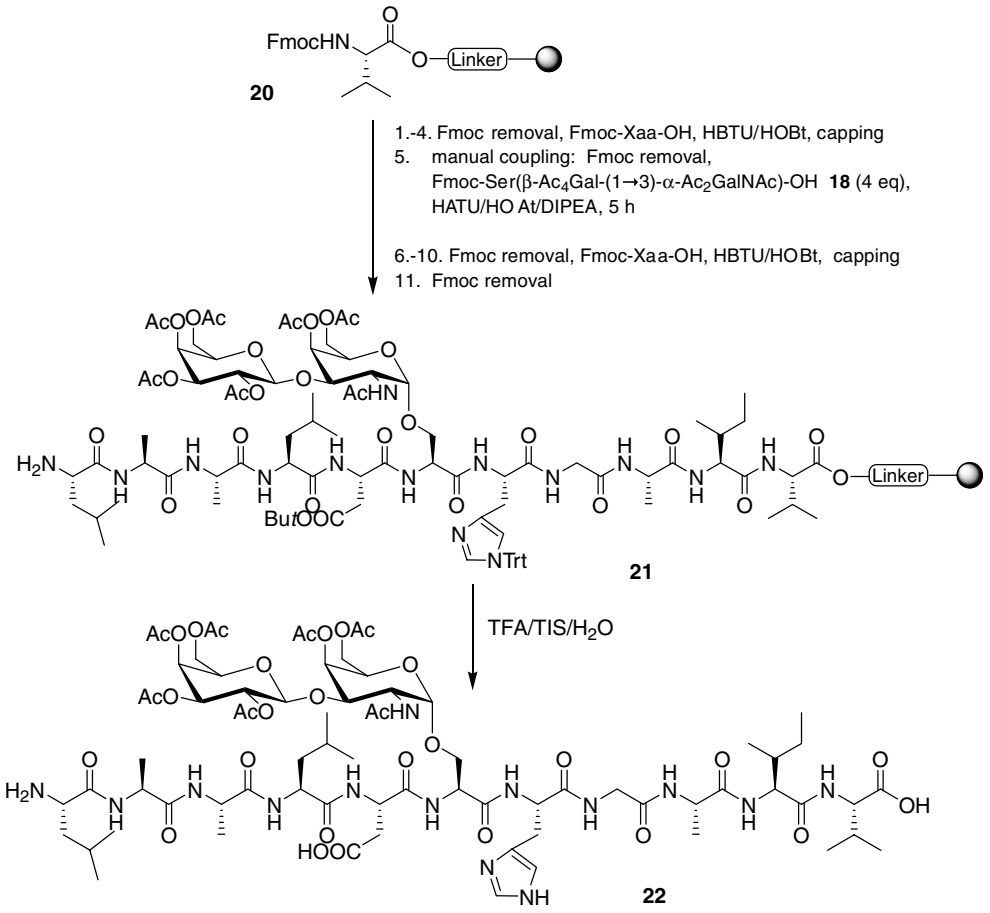
Encouraged by the good solubility and successful purification of **23** an extended partial sequence of the supposed recognition domain of LI-cadherin, the glycohexadecapeptide (Gln^{94}-Val^{109}) containing 2,6-sialyl-T-antigen **19** as a larger saccharide, was synthesized according to an analogous solid-phase synthesis protocol (Scheme 6). Again, the glycosyl amino acid was coupled manually to the resin-linked peptide using an excess of 2.34 equiv and HATU/HOAt as the activating reagent.

Detachment of the product from the resin-linked form **24** using trifluoroacetic acid, triisopropylsilane and water gave crude, partially protected glycopeptide **25**. It was sufficiently soluble for purification by preparative HPLC to afford **25** in a yield of 42%. Removal of the benzyl ester by hydrogenation using Pd/C (10%) as the catalyst and of the *O*-acetyl groups under Zemplén conditions (pH 9) gave the water-soluble 2,6-sialyl T-glycohexapeptide **26** which was isolated after purification by preparative HPLC in an overall yield of 23% relative to **20** (Scheme 7).

5. Synthetic LI-cadherin glycopeptide containing the amino-coumarine chromophore

According to the good solubility of the LI-cadherin glycopeptide **26**, a fluorescent-labelled partial sequence of the LI-cadherin recognition domain was designed as a pentadecapeptide containing the solubilizing quinic acid–coumarine residue (Quiglac) **11** instead of the N-terminal glutamine of **26** and the sialyl T_N-antigen saccharide considered necessary as the minimal hydrophilizing saccharide side chain. The solid-phase assembly was carried out in analogy to the protocol described for **24**.

The 2,6-sialyl-T_N-antigen **17** was coupled manually using an excess of 1.02 equiv and activation by HATU/HOAt. The coupling of the chromophore **10** was accomplished likewise by activation with HATU/HOAt and an excess of **10** of 2 equiv (Scheme 8). Release of the glycopeptide from the resin **27** was achieved by treatment with trifluoroacetic acid, triisopropylsilane and water. After purification by



Scheme 4.

preparative HPLC, fluorescent-labelled glycopeptide **28** was isolated in a yield of only 10% (Scheme 8).

The main reasons for the poor yield are the two manual couplings and only equimolar amounts of the sialyl T_N serine building block available for the coupling reaction. Accordingly, the major product of the synthesis was deletion sequence **29**. Nevertheless, the structure **28** could be proven by NMR spectroscopy.

Benzyl ester and *O*-acetyl groups were removed from **28** by treatment with sodium methanolate in methanol at pH 9.5 and, subsequently, with aqueous sodium hydroxide at pH 10.5. After acidification with acetic acid, purification of the product was carried out by preparative HPLC to yield the desired water-soluble glycopentadecapeptide **30** equipped with the fluorescent chromophore Quiglac (**11**) in an overall yield of 4%. Due to the small amount of **30**, its characterization could only be performed by mass spectroscopy.

6. Fluorescence and UV spectroscopic data

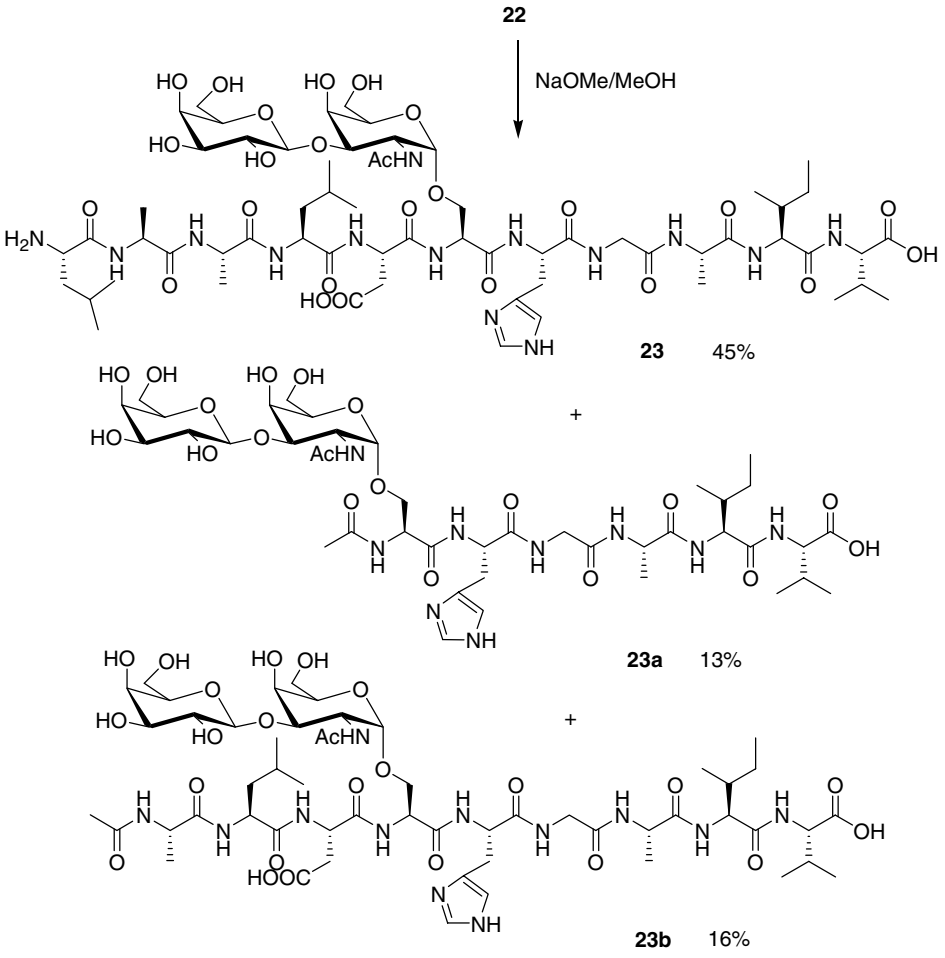
To analyze the spectroscopic behaviour of compound **11**, the UV absorption and the fluorescence spectra were measured on a Zeiss MCS 320/340 diode-array spectrometer and on a Perkin-Elmer LS 50B spectrometer at an exciting wavelength of 330 nm. In UV, λ_{max} was found at 328 nm, whereas the fluorescence spectrum has its maximum at λ_{max} 404.5 nm. The flank which can be seen in the UV absorption spectrum of compound **11** is responsible for difficulties using the new chromophore in a FRET experiment with tryptophane as the donor (Fig. 1). Because of this flank, an overlap of the absorption of tryptophane and compound **11** does occur.

Accordingly, a sufficiently separated excitation of donor and acceptor as would be necessary for FRET experiments cannot reliably be achieved.

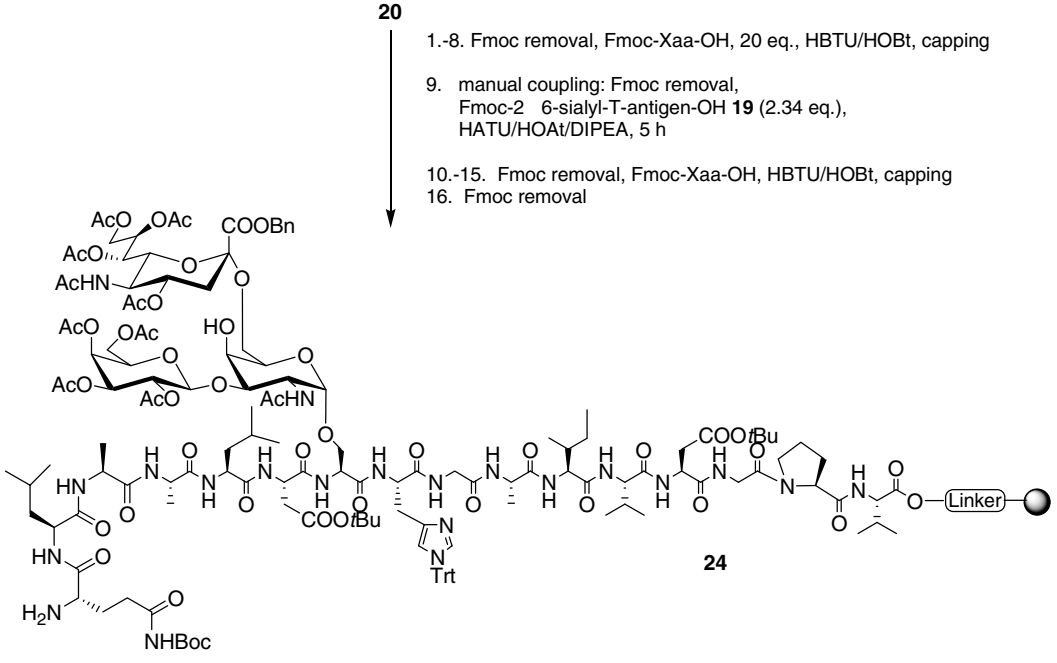
7. Experimental

7.1. General instrumentation

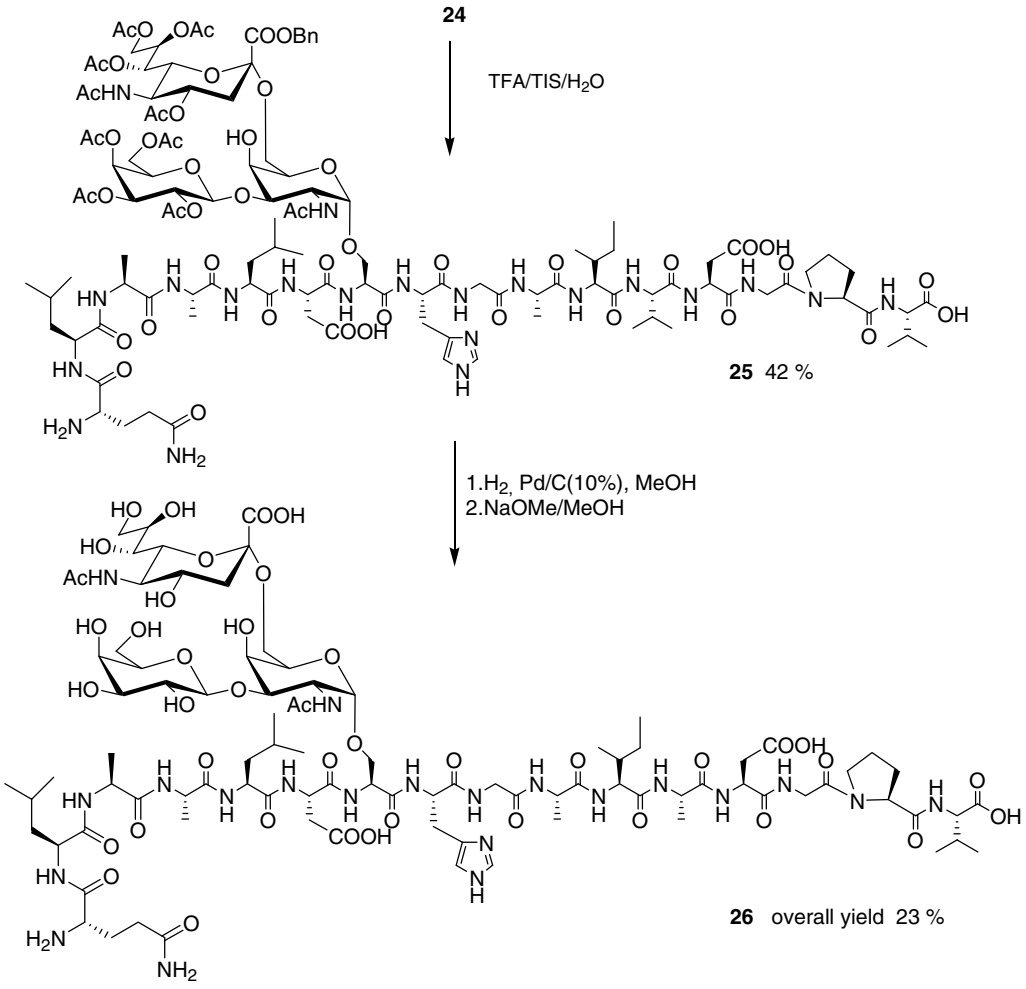
Solvents were dried and distilled according to standard procedures. Fmoc amino acids were purchased from Novabiochem. The Tentagel-PHB resin preloaded with valine was supplied by Rapp Polymere (Tübingen, Germany). Reactions were monitored on TLC aluminium plates coated with silica gel $60F_{254}$ (Merck, Darmstadt, Germany). Purification by flash-chromatography was performed with silica gel (32–63 μm) from Merck, Darmstadt. For microwave heating a CEM Discover System was used. RP-HPLC analyses were performed by application of a Knauer HPLC system on a Phenomenex



Scheme 5.



Scheme 6.



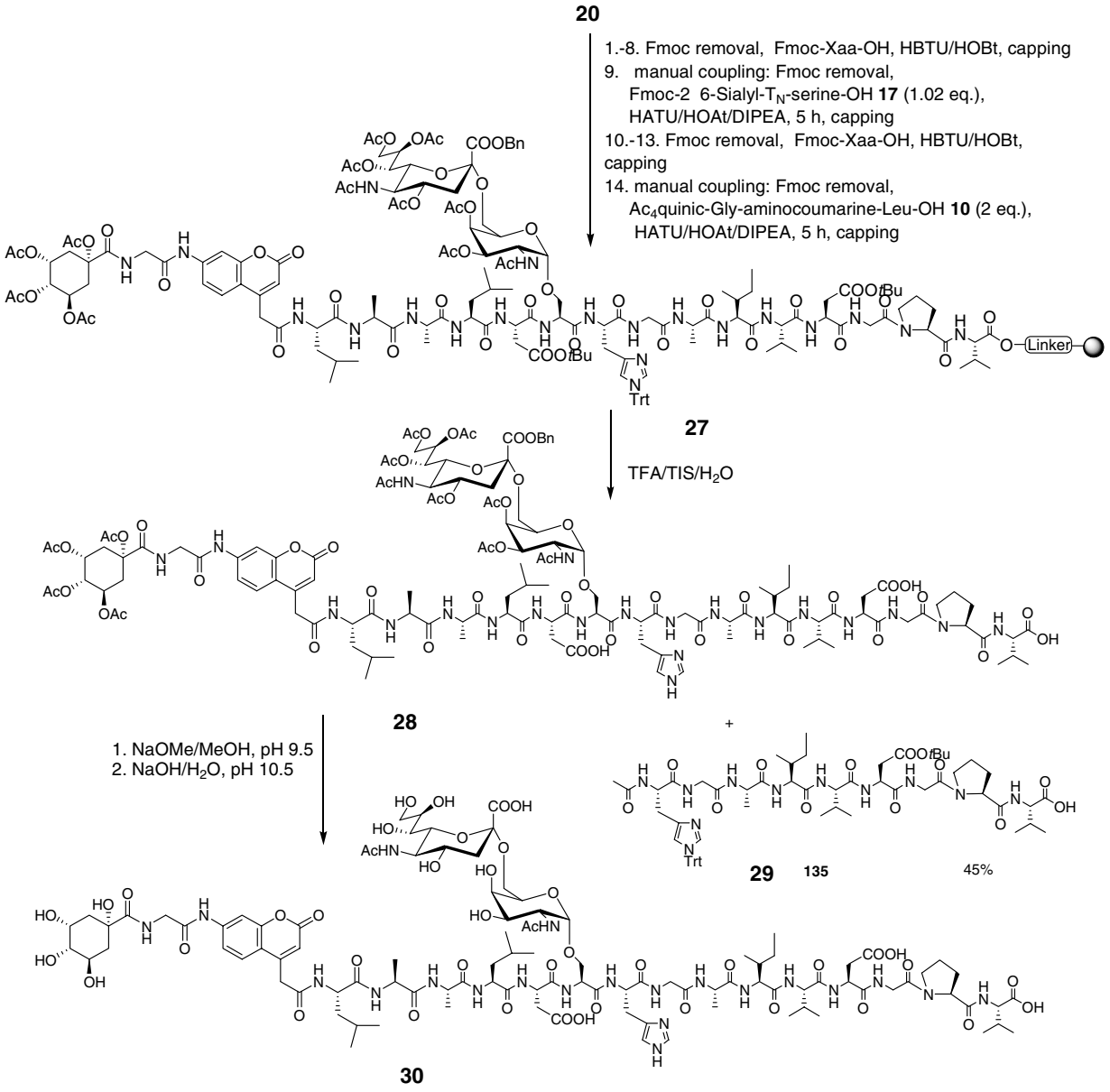
Scheme 7.

Luna C18 column (5 μm, 250 × 4.6 mm) at a pump rate of 1 mL/min. Semi-preparative HPLC were carried out on a Knauer HPLC system on a Phenomenex Luna C 18 column at a pump rate of 15 mL/min. Preparative RP-HPLC purifications were carried out on a Knauer HPLC system on a Phenomenex Luna C18 column (10 μm, 250.0 × 50.0 mm) at a pump rate of 30 mL/min. MeCN and H_2O were used (+0.1% TFA) as the solvents.

Gradients used for analytical RP-HPLC: gradient a (SH76): MeCN/H_2O (+0.1% TFA) (30:70) → MeCN/H_2O (+0.1% TFA) (80:20) in 40 min; gradient b (SH80): MeCN/H_2O (+0.1% TFA) (5:95) → MeCN/H_2O (+0.1% TFA) (100:0) in 40 min; gradient c (SH132): MeCN/H_2O (+0.1% TFA) (3:97) → MeCN/H_2O (+0.1% TFA) (100:0) in 40 min; gradient d (SH133): MeCN/H_2O (+0.1% TFA) (10:90) → MeCN/H_2O (+0.1% TFA) (50:50) in 40 min; gradient e (Sh150): MeCN/H_2O (+0.1% TFA) (10:90) → MeCN/H_2O (+0.1% TFA) (100:0) in 40 min; gradient f (SH151): MeCN/H_2O (+0.1% TFA) (10:90) → MeCN/H_2O (+0.1% TFA) (50:50). Gradients used for semi-preparative HPLC: gradient g (SH80) MeCN/H_20 (+0.1% TFA) (5:95) → MeCN/H_2O (+0.1% TFA) (100:0) in 60 min.

Gradients used for preparative HPLC: gradient h (SH132): MeCN/H_2O (+0.1% TFA) (25:75) → MeCN/H_2O (+0.1% TFA) (100:0) in 120 min; gradient i (SH133): MeCN/H_2O (+0.1% TFA) (20:80) → MeCN/H_2O (+0.1% TFA) (30:70) in 120 min; gradient j (SH150): MeCN/H_2O (+0.1% TFA) (40:60) → MeCN/H_2O (+0.1% TFA) (90:10) in 90 min; gradient k (SH151): MeCN/H_2O (+0.1% TFA) (10:90) → MeCN/H_2O (+0.1% TFA) (50:50) in 120 min.

^1H, ^{13}C and 2 D NMR spectra were recorded on a Bruker Ac-300 or a Bruker AM-400 spectrometer. The chemical shifts are correlated to the signal of the deuterated solvent. Multiplicities are given as s (singlet), s_b (broad singlet), d (doublet), t (triplet) or m (multiplet). In case of complex structures the protons were assigned by additional COSY and HMQC experiments. In the synthesized complex carbohydrate structures the incorporated carbohydrate structures are named as follows: *N*-acetyl-D-galactoseamine: normal, D-galactose: one apostrophe and D-neuraminic acid: two apostrophes. As abbreviations for quinic acid Qui and for the coumarine-core Cou were choosen. MALDI-TOF mass spectra were recorded on a Micromass Tofpec E spectrometer, ESI-mass spectra on a ThermoQuest Navigator spec-



Scheme 8.

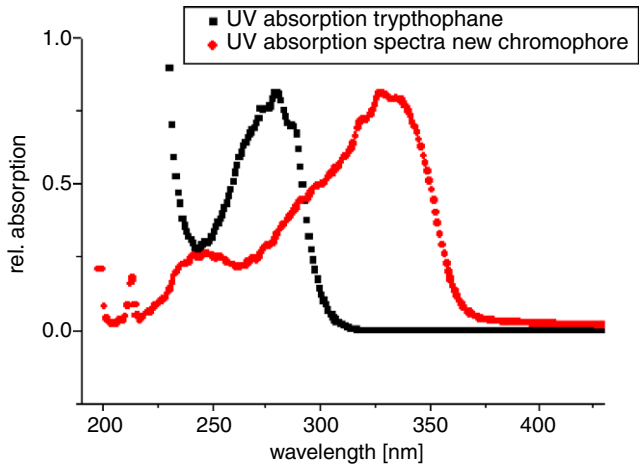


Figure 1. UV absorption spectra of tryptophane and chromophore **11**.

trometer. Optical rotations $[\alpha]_D$ were measured on a Perkin Elmer polarimeter 241.

7.2. Benzyl 2-(7-aminocoumarin-4-yl)-acetate (2)

To 10 mL of benzyl alcohol cooled to −5 °C, thionyl chloride (0.36 mL, 5.02 mmol) was added dropwise. Subsequently 2-(7-aminocoumarin-4-yl)acetic acid[17] (1.00 g, 4.56 mmol) was added. The mixture was stirred at −5 °C for 15 min and subsequently heated to 55 °C for 1 h. While cooling to room temperature a yellow solid precipitated. It was washed with diethyl ether. Yield: 1.36 g (87%); mp 170 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ: 7.41 (d, 1H, H5-Cou, $J_{H4,H5}$ = 8.82 Hz); 7.31 (s_b, 5H, $H_{arom.}$-Bn); 6.74–6.68 (m, 2H, H6-, H8-Cou); 6.13 (s, 1H, H3-Cou); 5.12 (s, 2H, CH_2-Bn); 3.93 (s, 2H, CH_2-Cou); ^{13}C NMR (DMSO-d_6), δ: 169.27, 160.38 (CO); 155.20 (C4-Cou); 149.59 (C9-Cou); 149.30 (C7-Cou);

135.88 (C_{ipso}-Bn); 128.51, 128.22, 128.01 ($C_{arom.}$-Bn); 126.54 (C5-Cou); 113.35 (C6-Cou); 110.92 (C3-Cou); 110.44 (C10-Cou); 101.73 (C8-Cou); 66.37 (CH_2-Bn); 36.88 (CH_2-Cou); FD-MS, *m*/*z*: 309.5 $(M)^+$, calcd: 309.1.

7.3. Benzyl 2-(7-(*N*-*tert*-butyloxycarbonyl-glycyl)-amino-coumarin-4-yl)-acetate (3)

In dry DMF (1.5 mL) Boc-Gly-OH (57 mg, 0.32 mmol, 2 equiv), HATU (123 mg, 0.32 mmol, 2 equiv) and 2,4,6-collidine (43.1 μL) were dissolved. After 5 min of stirring, benzyl 2-(7-aminocoumarin-4-yl)-acetate **2** (50 mg, 0.16 mmol) was added and the mixture was stirred at room temperature for 42 h. After evaporation of the solvent in vacuo, the residue was diluted by 20 mL EtOAc, washed with 1 N HCl, satd aq $NaHCO_3$ solution, satd aq NaCl solution, dried over $MgSO_4$ and evaporated in vacuo. Purification by flash-chromatography on silica gel (EtOAc/cyclohexane 2:1) yielded a colourless, amorphous solid. Yield: 56 mg (74%); R_f = 0.5 (cyclohexane/EtOAc 1:2); ^{1}H NMR (DMSO-d_6), δ: 10.40 (s, 1H, NH); 7.75 (d, 1H, H8-Cou, $J_{H6,H8}$ = 1.83 Hz); 7.61 (d, 1H, H5-Cou, $J_{H5,H6}$ = 8.82 Hz); 7.43–7.38 (m, 6H, H6-Cou, H_{arom}-Bn); 7.12 (t, 1H, NH^G, $J_{NH,G\alpha}$ = 5.88 Hz); 6.39 (s, 1H, H3-Cou); 5.14 (s, 2H, CH_2–O); 4.04 (s, 2H, CH_2-Cou); 3.27 (d, 2H, G^α, $J_{G\alpha,NH}$ = 5.88 Hz); 1.39 (s, 9H, CH_3-*t*-Bu); ^{13}C NMR (DMSO-d_6), δ: 170.56, 169.26 (CO); 159.96 (C2-Cou); 156.08 (CO-Urethan); 153.97 (C9-Cou); 149.21 (C4-Cou); 142.45 (C7-Cou); 135.85 (C_{ipso}-Bn); 128.52, 128.25, 128.08 ($C_{arom.}$-Bn); 126.26 (C5-Cou); 115.24 (C6-Cou); 114.35 (C10-Cou); 114.22 (C3-Cou); 105.73 (C8-Cou); 78.27 (C_q-*t*-Bu); 66.45 (CH_2-Bn); 44.04 (G^α); 36.71 (CH_2-Cou); 28.31 (CH_3-*t*-Bu); ESI-MS, *m*/*z*: 499.3 $(M+Na)^+$, calcd: 499.2.

7.4. (1*S*,3*R*,4*S*,5*R*)-1,3,4,5-Tetraacetoxycyclohexane carboxylic acid (5)[21]

Compound **5** was obtained from quinic acid by treatment with acetic acid/aceticanhydride (2:1) and two drops of 70% H_2SO_4. Yield 88%; ^{1}H NMR (^{1}H, COSY) ($CDCl_3$), δ: 9.59 (s_b, 1H, COOH); 5.52 (q, 1H, H3-Qui, $J_{H3,H4}$ = 3.66 Hz); 5.39 (m, 1H, H5-Qui); 4.99 (dd, 1H, H4-Qui, $J_{H3,H4}$ = 3.66 Hz, $J_{H4,H5}$ = 9.93 Hz); 2.67–2.51 (m, 2H, H2a-, H6a-Qui); 2.33 (dd, 1H, H2b-Qui, $J_{H2b,H3}$ = 3.69 Hz, $J_{H2a,b}$ = 15.81 Hz); 2.10, 2.03, 2.00, 1.96 (4× s, 12H, CH_3-Ac); 1.96–1.86 (m, 1H, H6b-Qui); ^{13}C NMR (^{13}C, HMQC) ($CDCl_3$), δ: 175.32 (COOH); 170.13, 169.95, 169.86, 169.81 (CO); 78.26 (C1-Qui); 71.37 (C4-Qui); 67.51 (C3-Qui); 66.34 (C5-Qui); 36.60 (C2-Qui); 31.58 (C6-Qui); 20.92, 20.88, 20.67, 20.62 (CH_3-Ac).

7.5. Benzyl 2-(7-{*N*-[1*S*,3*R*,4*S*,5*R*]-1,3,4,5-tetraacetoxy-cyclohexanoyl-glycyl}-amino-coumarin-4-yl)-acetate (6)

A solution of 2-(7-(*N*-*tert*-butyloxycarbonyl-glycyl)-aminocoumarin-4-yl)-acetic acid benzyl ester**3** (0.99 g, 2.12 mmol), 2.0 mL trifluoroacetic acid and 0.2 mL H_2O was stirred at room temperature for 1 h. Subsequently, the solution of **4** was concentrated in vacuo, the residue codistilled with toluene (2×) and dried in vacuo. (1*S*,3*R*,4*S*,5*R*)-1,3,4,5-tetra-*O*-acetyl-quinic acid **5** (1.53 g, 4.24 mmol, 2 equiv), TBTU (1.36 g, 4.24 mmol, 2 equiv) and diisopropylethylamine (DIPEA, 741 μL) were dissolved in 10 mL dry DMF and stirred for 15 min at room temperature. After adding DIPEA (525 μL), the two solutions were combined and brought into the microwave system for 20 min (50 °C, 50 W). DMF was removed, the residue diluted with EtOAc (100 mL) and washed with aq citric acid solution (5%, 2×), satd aq $NaHCO_3$ solution, satd aq NaCl solution, dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash-chromatography on silica gel (EtOAc/cyclohexane 4:1). Yield: 984 mg (75%); colourless, amorphous solid; R_f = 0.20 (EtOAc/cyclohexane 4.1); $[\alpha]_D^{22}$ −10.0 (*c* 1.00, DMSO); ^{1}H NMR (^{1}H, COSY) (CD_3OD), δ: 7.79 (d, 1H, H8-Cou, $J_{H6,H8}$ = 1.96 Hz); 7.53–7.51 (m, 1H, H5-Cou); 7.41 (dd, 1H, H6-cou, $J_{H6,H8}$ = 2.36 Hz, $J_{H5,H6}$ = 9.00 Hz); 7.33 (s, 5H, $H_{arom.}$-Bn); 6.33 (s, 1H, H3-Cou); 5.61 (m, 1H, H3-Qui); 5.50 (m, 1H, H5-Qui); 5.18 (s, 2H, CH_2-Bn); 5.12 (dd, 1H, H4-Qui, $J_{H3,H4}$ = 3.92 Hz, $J_{H4,H5}$ = 10.56 Hz); 4.02 (s, 2H, CH_2-Cou); 3.93 (d, 2H, G^α, $J_{NH,G\alpha}$ = 7.04 Hz); 2.76 (m, H2a-Qui); 2.68–2.64 (m, 1H, H6a-Qui); 2.55 (1H, H2b-Qui, $J_{H2a,H3}$ = 3.52 Hz, $J_{H2a,b}$ = 16.04 Hz); 2.29, 2.15, 2.07, 2.02 (4× s, 12H, CH_3-Ac); 2.07–2.04 (m, 1H, H6b-Qui); ^{13}C NMR (^{13}C, HMQC) (CD_3OD), δ: 173.31, 172.67, 171.83, 171.70, 171.62, 170.47, 169.72 (CO); 162.65 (C2-Cou); 155.48 (C9-Cou); 150.63 (C4-Cou); 143.10 (C7-Cou); 137.04 (C_{ipso}-Bn); 129.55, 129.36 ($C_{arom.}$-Bn); 126.82 (C5-Cou); 116.79 (C6-Cou); 116.15 (C10-Cou); 115.68 (C3-Cou); 107.75 (C8-Cou); 82.27 (C1-Qui); 73.27 (C4-Qui); 69.46 (CH_2-Bn); 68.20 (C3-Qui); 67.78 (C5-Qui); 44.39 (G^α); 38.57, 38.32 (C2-Qui, CH_2-Cou); 31.97 (C6-Qui); 21.77, 20.82, 20.64, 20.58 (CH_3-Ac); ESI-MS, *m*/*z*: 731.1 $(M+Na)^+$, calcd: 731.2.

7.6. 2-(7-{*N*-[1*S*,3*R*,4*S*,5*R*]-1,3,4,5-Tetraacetoxycyclo-hexanoyl-glycyl}-amino-coumarin-4-yl)-acetic acid (7)

In MeOH (15 mL) compound **6** (778 mg, 1.11 mmol) was dissolved and repeatedly degassed. Subsequently 8 mg Pd/C (5%) was added and the argon atmosphere was exchanged for hydrogen. After stirring for 45 min, the mixture was filtered through Hyflo Supercel and evaporated. Purification by flash-chromatography on silica gel (EtOAc/EtOH/AcOH 6:1:0.1) gave a colourless, amorphous solid. Yield: 667 mg (98%); $[\alpha]_D^{22}$ −21.7 (*c* 1.00, DMSO); ^{1}H NMR (DMSO-d_6), δ: 10.21 (s, 1H, NH); 8.44 (t, 1H, NH^G, $J_{NH,G\alpha}$ = 5.52 Hz); 7.74 (m, 2H, H5-, H8-Cou); 7.44–7.39 (m, 1H, H6-Cou); 6.34 (s, 1H, H3-Cou); 5.40–5.28 (m, 2H, H3-, H5-Qui); 5.05 (dd, 1H, H4-Qui, $J_{H3,H4}$ = 3.66 Hz, $J_{H4,H5}$ = 10.29 Hz); 3.92 (dd, 1H, G^αa, $J_{NH,G\alpha}$ = 5.52 Hz, $J_{G\alpha a,b}$ = 16.53 Hz); 3.83 (s, 2H, CH_2-Cou); 3.75 (dd, 1H, G^αb, $J_{NH,G\alpha}$ = 5.52 Hz, $J_{G\alpha a,b}$ = 16.53 Hz); 2.63–2.58 (m, 1H, H2a-Qui); 2.49–2.38 (m, 3H, H2b-, H6-Qui); 2.05, 2.01, 1.93, 1.90 (4× s, 12H, CH_3-Ac); ^{13}C NMR (DMSO-d_6), δ: 172.25, 170.43, 169.93, 179.87, 169.84, 169.66, 168.29 (CO); 160.09 (C2-Cou); 153.77 (C9-Cou); 153.20 (C4-Cou); 142.11 (C7-Cou); 126.17 (C5-Cou); 115.19 (C6-Cou); 115.08 (C10-Cou); 112.44

(C3-Cou); 105.56 (C8-Cou); 80.50 (C1-Qui); 71.36 (C4-Qui); 68.05 (C3-Qui); 66.52 (C5-Qui); 44.39 (G^{α}); 39.12 (CH_2-Cou); 37.37 (C2-Qui); 30.32 (C6-Qui); 21.76, 20.82, 20.58, 18.08 (CH_3-Ac); ESI-MS, *m/z*: 641.2 $(M+Na)^+$, calcd: 641.2.

7.7. 2-(7-{*N*-[1*S*,3*R*,4*S*,5*R*]-1,3,4,5-Tetraacetoxycyclohexanoyl-glycyl}-amino-coumarin-4-yl)-acetyl-L-leucine *tert*-butyl ester (8)

A mixture of compound **7** (541 mg, 0.88 mmol), DCC (181 mg, 0.88 mmol) and HOBt (134 mg, 0.88 mmol) was stirred in 5 mL of dry DMF at 0 °C for 15 min. Subsequently, H-Leu-O-*t*-Bu hydrochloride(196 mg, 0.88 mmol) and NEt_3 (123 μL, 0.88 mmol) were added. After 1.5 h at 0 °C, the mixture was stirred at room temperature for 17 h. The precipitated urea was filtered off and the solvent evaporated in vacuo. The residue was dissolved in EtOAc and the precipitating urea filtered off. The organic layer was washed with 1 N HCl, satd aq $NaHCO_3$ solution, satd aq NaCl solution and dried with $MgSO_4$. The solvent was removed in vacuo. An analytical probe was isolated by HPLC [LUNA C 18, gradient (% CH_3CN): 30–80 (120 min)]: $[\alpha]_D^{22}$ −18.0 (*c* 0.50, DMSO); t_R = 27.12 min [LUNA C 18 gradient (% CH_3CN): 30–80 (40 min)]; ^1H NMR (DMSO-d_6) (^1H, COSY), δ: 10.19 (s, 1H, NH); 8.59 (d, 1H, NH^L, $J_{NH,L\alpha}$ = 7.71 Hz); 8.43 (t, 1H, NH^G, $J_{NH,G\alpha}$ = 5.88 Hz); 7.74 (d, 1H, H8-Cou, $J_{H6,H8}$ = 1.83 Hz); 7.71 (d, 1H, H5-Cou, $J_{H5,H6}$ = 8.82 Hz); 7.40 (dd, 1H, H6-Cou, $J_{H6,H8}$ = 1.83 Hz, $J_{H5,H6}$ = 8.82 Hz); 6.31 (s, 1H, H3-Cou); 5.39–5.27 (m, 2H, H3-, H5-Qui); 5.05 (dd, 1H, H4-Qui, $J_{H3,H4}$ = 3.66 Hz, $J_{H4,H5}$ = 10.29 Hz); 4.14–4.09 (m, 1H, L^{α}); 3.93 (dd, 1H, $G^{\alpha}a$, $J_{NH,G\alpha}$ = 5.97 Hz, $J_{G\alpha a,b}$ = 16.65 Hz); 3.80–3.73 (m, 3H, $G^{\alpha}b$, CH_2-Cou); 2.62–2.57 (m, 1H, H2a-Qui); 2.52–2.46 (m, 2H, H2b-, H6a-Qui); 2.12, 2.04, 2.01, 1.94 (4× s, 13H, CH_3-Ac, H6b-Qui); 1.70–1.41 (m, 3H, L^{β}, L^{γ}); 1.35 (s, 9H, CH_3-*t*-Bu); 0.89 (d, 3H, L^{δ}, $J_{L\gamma,\ L\delta}$ = 6.24 Hz); 0.80 (d, 3H, L^{δ}, $J_{L\gamma,L\delta}$ = 6.60 Hz); ^{13}C NMR (DMSO-d_6) (^{13}C, HMQC), δ: 171.64, 170.41, 169.93, 169.86, 169.81, 169.63, 168.32, 167.85 (8C, CO); 159.99 (1C, C2-Cou); 153.92 (1C, C9-Cou); 150.82 (1C, C4-Cou); 142.09 (1C, C7-Cou); 126.14 (1C, C5-Cou); 114.92 (1C, C6-Cou); 114.58 (1C, C10-Cou); 113.84 (1C, C3-Cou); 105.68 (1C, C8-Cou); 80.71 (1C, C_q-*t*-Bu); 80.52 (1C, C1-Qui); 71.36 (1C, C4-Qui); 68.09 (1C, C3-Qui); 66.53 (1C, C5-Qui); 51.34 (1C, L^{α}); 43.02 (1C, G^{α}); 40.61 (1C, L^{β}); 38.45 (1C, CH_2-Cou); 37.36 (1C, C6-Qui); 30.38 (1C, C2-Qui); 27.66 (3C, CH_3-*t*-Bu); 24.46 (1C, L^{γ}); 21.78, 21.37, 20.87, 20.82, 20.58 (6C, CH_3–CO, L^{δ}); ESI-MS, *m/z*: 810.4 $(M+Na)^+$; HR-ESI-MS, *m/z*: 810.3078 $(M+Na)^+$.

7.8. 7-{*N*-(1*S*,3*R*,4*S*,5*R*)-1,3,4,5-Tetraacetoxycyclohexanoyl-glycyl}-amino-4-methyl-coumarine (9)

The compound was separated by analytical HPLC as an analytical probe (see, **8**): Colourless solid; t_R = 17.0 min; $[\alpha]_D^{22}$ −16.7 (*c* 1.00 MeOH); ^1H NMR (DMSO-d_6), δ: 10.18 (s, 1H, NH); 8.43 (t, 1H, NH^G, $J_{NH,G\alpha}$ = 5.52 Hz); 7.72–7.69 (m, 2H, H5-, H8-Cou); 7.40 (m, 1H, H6-Cou); 6.26 (s, 1H, H3-Cou); 5.40–5.27 (m, 2H, H3-, H5-Qui); 5.05 (dd, 1H, H4-Qui, $J_{H3,H4}$ = 3.66 Hz, $J_{H4,H5}$ = 10.29 Hz); 4.14–4.09 (m, 1H, L^{α}); 3.93 (dd, 1H, $G^{\alpha}a$, $J_{NH,G\alpha}$ = 5.97 Hz, $J_{G\alpha a,b}$ = 16.65 Hz); 3.76 (dd, 1H, $G^{\alpha}b$, $J_{NH,G\alpha}$ = 5.97 Hz, $J_{G\alpha a,b}$ = 16.65 Hz); 2.62–2.57 (m, 1H, H2a-Qui); 2.52–2.46 (m, 2H, H2b-, H6a-Qui); 2.38 (s, 3H, CH_3-Cou); 2.13, 2.05, 2.02, 1.94 (4× s, 13H, CH_3-Ac, H6b-Qui); ^{13}C NMR (DMSO-d_6) (^{13}C, HMQC), δ: 170.42, 169.93, 169.87, 169.83, 169.65, 168.27 (CO); 160.09 (C2-Cou); 153.79 (C9-Cou); 153.19 (C4-Cou); 142.09 (C7-Cou); 126.17 (C5-Cou); 115.21 (C6-Cou); 115.10 (C10-Cou); 112.44 (C3-Cou); 105.58 (C8-Cou); 80.51 (C1-Qui); 71.37 (C4-Qui); 68.09 (C3-Qui); 66.53 (C5-Qui); 43.00 (G^{α}); 37.37 (C6-Qui); 30.36 (C2-Qui); 21.76, 20.87, 20.82, 20.58 (CH_3–CO); 18.08 (CH_3-Cou); ESI-MS, *m/z*: 597.2 $(M+Na)^+$.

7.9. 2-(7-{*N*-[1*S*,3*R*,4*S*,5*R*]-1,3,4,5-Tetraacetoxycyclohexanoyl-glycyl}-aminocumarin-4-yl)-acetyl-L-leucine (10)

The crude product **8/9** (0.5 g) was dissolved in trifluoroacetic acid (7 mL), triisopropylsilane (0.5 mL), H_2O (0.5 mL) and stirred at room temperature for 1 h. The solvent was evaporated in vacuo and the residue codistilled with toluene. Purification by flash-chromatography on silica gel (EtOAc/EtOH/AcOH 6:1:0.1) yielded a colourless solid. Yield: 400 mg (63%, over two steps); R_f = 0.28 (EtOAc/EtOH/AcOH 6:1:0.1); $[\alpha]_D^{22}$ −16.5 (*c* 0.5, DMSO); ^1H NMR (DMSO-d_6), δ: 12.44 (s_b, 1H, COOH); 10.23 (s, 1H, NH); 8.61 (d, 1H, NH^L, $J_{NH,L\alpha}$ = 7.71 Hz); 8.44 (t, 1H, NH^G, $J_{NH,G\alpha}$ = 5.49 Hz); 7.73–7.70 (m, 2H, H5-Cou, H8-Cou); 7.40 (d, 1H, H6-Cou, $J_{H5,H6}$ = 8.82 Hz); 6.32 (s, 1H, H3-Cou); 5.39–5.27 (m, 2H, H3-, H5-Qui); 5.05 (dd, 1H, H4-Qui, $J_{H3,H4}$ = 3.69 Hz, $J_{H4,H5}$ = 10.29 Hz); 4.14–4.09 (m, 1H, L^{α}); 3.93 (dd, 1H, $G^{\alpha}a$, $J_{NH,G\alpha}$ = 5.88 Hz, $J_{G\alpha a,b}$ = 16.53 Hz),; 3.80–3.73 (m, 3H, $G^{\alpha}b$, CH_2-Cou); 2.62–2.57 (m, 1H, H2a-Qui); 2.52–2.46 (m, 2H, H2b-, H6a-Qui); 2.12, 2.04, 2.01, 1.94 (4× s, 13H, CH_3-Ac, H6b-Qui); 1.70–1.41 (m, 3H, L^{β}, L^{γ}); 0.88, 0.80 (2× d, 6H, L^{δ}, $J_{L\gamma,L\delta}$ = 6.24 Hz).

^{13}C NMR (DMSO-d_6), δ: 174.00, 170.41, 169.91, 169.83, 169.63, 168.32, 167.93 (CO); 160.02 (C2-Cou); 153.89 (C9-Cou); 150.91 (C4-Cou); 142.11 (C7-Cou); 126.17 (C5-Cou); 114.92 (C6-Cou); 114.58 (C10-Cou); 113.90 (C3-Cou); 105.65 (C8-Cou); 80.52 (C1-Qui); 71.37 (C4-Qui); 68.09 (C3-Qui); 66.53 (C5-Qui); 50.58 (L^{α}); 43.02 (G^{α}); 40.61 (L^{β}); 38.50 (CH_2-Cou); 37.36 (C2-Qui); 30.39 (C6-Qui); 24.48 (L^{γ}); 21.77, 21.23, 21.17, 20.85, 20.82, 20.58 (CH_3-Ac, L^{δ}); ESI-MS, *m/z*: 732.3 $(M+H)^+$; HR-ESI-MS, *m/z*: 732.2633 $(M+H)^+$, calcd: 732.2616.

7.10. 2-(7-{*N*-[1*S*,3*R*,4*S*,5*R*]-1,3,4,5-Tetrahydroxycyclohexanoyl-glycyl}-aminocoumarin-4-yl)-acetyl-L-leucine (11)

Compound **10** (20 mg, 0.027 mmol) was dissolved in 2 mL MeOH. NaOMe/MeOH (0.1 M) was added dropwise until a pH of 10.5 was reached. After 19 h stirring at room temperature, the solution was neutralized by addition of AcOH, and the sovent was evaporated in vac-

uo. Purification was performed by semi-preparative HPLC [LUNA C-18, gradient (% CH_3CN+0.1% TFA): 5–100, 60 min]. Yield: 11 mg (73%); $[\alpha]_D^{22}$ −16.7 (*c* 1, MeOH); t_R = 14.80 min [LUNA C 18, gradient (% CH_3CN+0.1% TFA): 5–100, 40 min]; ^1H NMR (CD_3OD), *δ*: 7.81–7.75 (m, 2H, H5-Cou-, H8-Cou); 7.48 (d, 1H, H6-Cou, $J_{H5,H6}$ = 8.43 Hz); 6.40 (s, 1H, H3-Cou); 4.51–4.48 (m, 1H, L^{α}); 4.20–4.03 (m, 4H, H3-Qui, G^{α}, H5-Qui); 3.85 (s, 2H, CH_2-Cou); 3.48–3.43 (m, 1H, H4-Qui); 2.10–1.97 (m, 4H, H2-, H6-Qui); 1.73–1.65 (m, 3H, L^{β}, L^{γ}); 1.00 (d, 3H, L^{δ}, $J_{L\gamma,\ L\delta}$ = 5.52 Hz); 0.95 (d, 3H, L^{δ}, $J_{L\gamma,L\delta}$ = 5.61 Hz); ^{13}C NMR (CD_3OD), *δ*: 178.05, 170.91, 169.71 (4C, CO); 162.90 (1C, C2-Cou); 155.62 (1C, C9-Cou); 151.92 (1C, C4-Cou); 143.47 (1C, C7-Cou); 126.94 (1C, C5-Cou); 116.86 (1C, C6-Cou); 116.38 (1C, C10-Cou); 115.50 (1C, C3-Cou); 107.87 (1C, C8-Cou); 78.07 (1C, C4-Qui); 77.03 (1C, C1-Qui); 72.21 (1C, C3-Qui); 68.19 (1C, C5-Qui); 42.44 (1C, G^{α}); 43.89 (1C, L^{β}) 41.47 (1C, C2-Qui); 39.74 (1C, C6-Qui); 38.83 (1C, CH_2-Cou); 26.16 (1C, L^{γ}); 23.42 (1C, L^{δ}); 21.67 (1C, L^{δ}); ESI-MS, *m*/*z*: 564.3 $(M+H)^+$; HR-ESI-MS, *m*/*z*: 586.2040 $(M+Na)^+$.

7.11. *N*-(9*H*-Fluoren-9-yl-methoxycarbonyl)-*O*-(2-acetamido-2-desoxy-α-D-galacto-pyranosyl)-L-serine *tert*-butyl ester (13) [Fmoc-Ser(α-GalNAc)-O-*t*-Bu]

Fmoc-Ser(α-Ac_3GalNAc)-O-*t*-Bu[25] **12** (500 mg, 0.70 mmol) was dissolved in 15 mL dry MeOH. Fresh NaOMe/MeOH solution (0.1 mol) was added until a pH of 8.5 was reached. The reaction was terminated after 3 h by addition of ion exchange resin Amberlite® IR-120. After filtration, evaporation of the solvent in vacuo gave the crude product which was purified by flash-chromatography on silica gel (EtOAc → EtOH). Yield: 275 mg (67%) R_f = 0.16 (toluene/EtOH 5:1). ^1H NMR (CD_3OD), *δ*: 7.81 (d, 2H, H4-, H5-Fmoc, $J_{H3,H4}$ = $J_{H5,H6}$ = 7.71 Hz); 7.69 (d, 2H, H1-, H8-Fmoc, $J_{H1,H2}$ = $J_{H7,H8}$ = 6.63 Hz); 7.44–7.31 (m, 4H, H2-, H3-, H6-, H7-Fmoc); 4.83 (d, 1H, H1, $J_{H1,H2}$ = 3.69 Hz); 4.48–4.22 (m, 5H, CH_2-Fmoc, S^{β}a, H2, H9-Fmoc); 3.92–3.76 (m, 7H, S^{β}b, S^{α}, H3, H4, H5, H6); 1.99 (s, 3H, CH_3–NHAc); 1.49 (s, 9H, CH_3-*t*-Bu).

^{13}C NMR (CD_3OD), *δ*: 173.79, 171.06 (CO); 158.59 (CO-urethane); 145.24 (C1a-, C8a-Fmoc); 142.63 (C4a-, C5a-Fmoc); 128.86 (C3-, C6-Fmoc); 128.23 (C2-, C7-Fmoc); 126.16 (C1-, C8-Fmoc); 121.00 (C4-, C5-Fmoc); 100.03 (C1); 83.43 (C_q-*t*-Bu); 72.92 (S^{β}); 70.28, 69.73, 68.90 (C3, C4, C5); 67.99 (CH_2-Fmoc); 62.79 (C6); 56.63 (S^{α}); 51.20 (C2); 47.25 (C9-Fmoc); 28.36 (CH_3-*t*-Bu); 23.03 (CH_3-Ac); ESI-MS, *m*/*z*: 609.2 $(M+Na)^+$, calcd: 609.2.

7.12. *N*-(9*H*-Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-2-deoxy-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-serine *tert*-butyl ester [Fmoc-Ser(α-NeuAc$_4$5NAcCOOBn-(2,6)-α-GalNAc)-O-*t*-Bu] (15)

To a solution of Fmoc-Ser(α-GalNAc)-O-*t*-Bu **13** (259 mg, 0.44 mmol) and xanthate of acetylated sialic acid benzyl ester[29] (α-NeuAc$_4$5NAcCOOBnXan) **14** (773 mg, 1.15 mmol) in 15 mL dry CH_3CN/CH_2Cl_2 (2:1), 1 g molecular sieve (4Å) was added. The mixture was stirred at room temperature for 1 h and subsequently cooled to −65 °C. Anhydrous silver trifluoromethanesulfonate (AgOTf) (299 mg, 1.17 mmol) was added under exclusion of light. Over a period of 20 min, 719 μL methylsulfenyl bromide [MSB, prepared by addition of Br_2 (205 μL, 4 mmol) to a solution of dimethyl disulfide (355 μL, 4 mmol) in 1,2-dichlorethane (5 mL) after stirring for 15 h under exclusion of oxygen and light and cooled to 0 °C] was added dropwise.

After stirring for 4.5 h, *N*-ethyldiisopropylamine (0.39 mL) was added. The stirring was continued at −65 °C for another 15 min. Then the mixture was warmed to room temperature, diluted with CH_2Cl_2 (20 mL) and filtered through Hyflo Supercel. The filtrate was evaporated in vacuo and the residue purified by flash-chromatography on silica gel (EE → EE/EtOH 50:1 → EE/EtOH 40:1). Yield: 290 mg (58%); R_f = 0.15 (CH_2Cl_2/MeOH 20:1); $[\alpha]_D^{22}$ 4.0 (*c* 1.0, CH_2Cl_2); ^1H NMR (^1H, COSY) ($CDCl_3$), *δ*: 7.72 (d, 2H, H4-, H5-Fmoc, $J_{H3,H4}$ = $J_{H5,H6}$ = 7.44 Hz); 7.56 (d, 2H, H1-, H8-Fmoc, $J_{H1,H2}$ = $J_{H7,H8}$ = 7.04 Hz); 7.40–7.28 (m, 9H, H2-, H3-, H6-, H7-Fmoc, $H_{arom.}$-Bn); 6.51 (d, 1H, NHAc, $J_{NH,H2}$ = 7.35 Hz); 5.83 (d, 1H, NH–Fmoc, $J_{NH,S\alpha}$ = 9.45 Hz); 5.32–5.25 (m, 2H, H7″ (5.31), H8″ (5.28)); 5.22–5.14 (m, 2H, CH_2–Bn); 4.83–4.72 (m, 2H, H4″ (4.80), H1 (4.73)); 4.41–4.20 (m, 6H, S^{α} (4.38), CH_2–Fmoc (4.34), H9a″ (4.31), H2 (4.23), H9-Fmoc (4.21)); 4.07–4.00 (m, 3H, H5″ (4.05), H6″ (4.04), H9b″ (4.02)); 3.92–3.50 (m, 7H, S^{β}a (3.89), H6a (3.84), S^{β}b (3.73), H4 (3.76), H5 (3.69), H3 (3.68), H6b (3.51)); 2.58 (dd, 1H, $H3''_{eq}$, $J_{H3''eq,H4''}$ = 4.72 Hz, $J_{H3''eq,H3''ax}$ = 12.92 Hz); 2.11, 2.03, 2.01, 1.98 (4× s, 18H, CH_3-Ac); 1.91 (m, 1H, $H3''_{ax}$); 1.44 (s, 9H, CH_3-*t*-Bu); ^{13}C NMR (^{13}C, HMQC) ($CDCl_3$), *δ*: 173.39, 170.95, 170.71, 170.25, 170.07, 169.59 (CO); 167.41 (C1″); 155.90 (CO-urethane); 143.63 (C1a-, C8a-Fmoc); 141.23 (C4a-, C5a-Fmoc); 134.76 (C_{ipso}-Bn); 128.72, 128.56, 128.42, 127.74, 127.06 (C3-, C6-, C2-, C7-Fmoc, $C_{arom.}$-Bn (5C)); 124.97 (C1-, C8-Fmoc); 119.98 (C4-, C5-Fmoc); 98.90 (C1); 98.84 (C2″); 82.98 (C_q-*t*-Bu); 72.83 (C6″); 70.50 (C3); 69.30 (C5, C7″); 68.90 (C4″); 68.81 (S^{β}); 68.25 (C4); 67.87 (CH_2–Bn); 67.44 (C8″); 67.32 (CH_2-Fmoc); 63.80 (C6); 62.65 (C9″); 54.90 (S^{α}); 50.64 (C2); 49.16 (C5″); 47.21 (C9-Fmoc); 37.50 (C3″); 27.96 (CH_3-*t*-Bu); 23.08, 22.85, 21.03, 20.75 (CH_3-Ac, CH_3–NHAc); ESI-MS, *m*/*z*: 1158.6 $(M+Na)^+$, 1174.6 $(M+K)^+$, calcd: 1158.4, 1174.5.

7.13. *N*-(9*H*-Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-serine *tert*-butyl ester [Fmoc-Ser(α-NeuAc$_4$5NAcCOOBn-(2,6)-α-Ac$_2$GalNAc)-O-*t*-Bu] (16)

At 0 °C Fmoc-Ser(α-NeuAc$_4$5NAcCOOBn-(2,6)-α-GalNAc)- O-*t*-Bu **15** (290 mg, 0.26 mmol) was dissolved in 3.5 mL of pyridine. Ac_2O (2 mL) was added dropwise.

After stirring for 19 h, the mixture was diluted with 30 mL CH_2Cl_2. Ice was added, and the mixture was extracted three times with 90 mL CH_2Cl_2. The combined organic layers were washed with satd aq $NaHCO_3$ solution (2×) and satd aq NaCl solution, dried ($MgSO_4$) and the solvent was evaporated in vacuo. The crude product was purified by flash-chromatography on silica gel (EtOAc). Yield: 203 mg (65%); R_f = 0.15 (EtOAc); $[\alpha]_D^{22}$ 23.2 (*c* 1, $CHCl_3$); ^{1}H NMR (^{1}H, COSY) ($CDCl_3$), δ: 7.73 (d, 2H, H4-, H5-Fmoc, $J_{H3,H4} = J_{H5,H6}$ = 7.44 Hz); 7.59 (d, 2H, H1-, H8-Fmoc, $J_{H1,H2} = J_{H7,H8}$ = 7.04 Hz); 7.39-7.27 (m, 9H, H2-, H3-, H6-, H7-Fmoc, $H_{arom.}$-Bn); 5.79, 5.65 (2× d, 2H, NH-Fmoc, NHAc, $J_{NH,S\alpha}$ = 9.76 Hz, $J_{NH,H2}$ = 8.24 Hz); 5.33–5.24 (m, 2 H, H7″ (5.30), H8″ (5.26)); 5.18–5.11 (m, 3H, H4 (5.16), CH_2-Bn (5.12)); 5.00 (dd, 1H, H3, $J_{H3,H4}$ = 2.58 Hz, $J_{H2,H3}$ = 11.40 Hz); 4.80–4.72 (m, 2H, H1 (4.78), H4″ (4.77)); 4.59–4.49 (m, 1H, H2); 4.45–4.32 (m, 3H, CH_2a-Fmoc (4.41), S^{α} (4.38), CH_2b-Fmoc (4.35)); 4.25–4.20 (m, 2H, H9a″ (4.23), H9-Fmoc (4.22)); 4.05–3.87 (m, 5H, H5″ (4.03), H9b″ (4.03), H6″ (4.01), S^{β}a, H5 (3.90)); 3.82–3.74 (m, 2H, S^{β}b (3.80), H6a (3.77)); 3.12 (dd, 1H, H6b, $J_{H5,H6b}$ = 5.48 Hz, $J_{H6a,b}$ = 10.56 Hz); 2.55 (dd, 1H, $H3''_{eq}$, $J_{H3''eq,H4''}$ = 4.68 Hz, $J_{H3''eq,H3''ax}$ = 12.88 Hz); 2.06, 2.01, 1.98, 1.96, 1.91 (5× s, 24H, CH_3-Ac); 1.88 (m, 1H, $H3''_{ax}$.); 1.45 (s, 9H, CH_3-*t*-Bu); ^{13}C NMR (^{13}C, HMQC) ($CDCl_3$), δ: 170.90, 170.82, 170.65, 170.30, 170.12, 169.89, 169.70, 169.59 (CO); 167.30 (C1″); 155.60 (CO-urethane); 143.71 (C1a-, C8a-Fmoc); 141.15 (C4a-, C5a-Fmoc); 134.56 (C_{ipso}-Bn); 128.62, 128.42, 128.40, 127.84, 127.12 (C3-, C6-, C2-, C7-Fmoc, $C_{arom.}$-Bn (5C)); 125.07 (C1-, C8-Fmoc); 120.00 (C4-, C5-Fmoc); 98.88 (C1); 98.58 (C2″); 82.87 (C_q-*t*-Bu); 72.70 (C6″); 69.30 (C5); 68.10 (C7″); 68.87 (S^{β}); 68.85 (C4″); 68.70 (C3); 68.00 (CH_2–Bn); 67.35 (CH_2-Fmoc); 67.31 (C4); 67.10 (C8″); 63.52 (C6); 62.43 (C9″); 54.82 (S^{α}); 49.12 (C5″); 47.31 (C2); 47.16 (C9-Fmoc); 37.60 (C3″); 27.98 (CH_3-*t*-Bu); 23.20, 23.14, 20.99, 20.75, 20.67, 20.62 (CH_3-Ac, CH_3–NHAc); ESI-MS, *m*/*z*: 1254.7 $(M+Na)^+$, calcd: 1254.5.

7.14. *N*-(9*H*-Fluoren-9-ylmethoxycarbonyl)-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-didesoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-serine [Fmoc-Ser(α-NeuAc$_4$5NAcCOOBn-(2,6)-α- Ac$_2$GalNAc)-OH] (17)

Compound **16** (135 mg, 0.11 mmol) was dissolved in 5 mL of dichloromethane, 3 mL of trifluoroacetic acid and 0.5 mL of anisole and stirred at room temperature for 6 h. Subsequently, the solvents were evaporated in vacuo and the crude product codistilled with toluene (3×). Purification was accomplished by flash-chromatography on silica gel (EE → EE/MeOH 2:1). Yield: 119 mg (92%); R_f = 0.28 (EtOAc/MeOH 2:1); $[\alpha]_D^{22}$ 24.2 (*c* 1, $CHCl_3$); ^{1}H NMR (^{1}H, COSY) (DMSO-d_6), δ: : 8.11 (s_b, 1 H, NH); 7.78 (d, 2H, H4-, H5-Fmoc, $J_{H3,H4} = J_{H5,H6}$ = 7.43 Hz); 7.75 (d, 1H, NHAc, $J_{H2,NH}$ = 9.78 Hz); 7.60 (d, 2H, H1-, H8-Fmoc, $J_{H1,H2} = J_{H7,H8}$ = 7.40 Hz); 7.39–7.27 (m, 9H, H2-, H3-, H6-, H7-Fmoc, $H_{arom.}$-Bn); 5.30–5.11 (m, 5H, H4, H7″, H8″, CH_2–Bn); 4.98 (dd, 1H, H3, $J_{H3,H4}$ = 2.65 Hz, $J_{H2,H3}$ = 11.51 Hz); 4.85 (d, 1H, H1, $J_{H1,H2}$ = 2.45 Hz); 4.68 (dt, 1H, H4″, $J_{H3''eq,H4''}$ = 2.60 Hz, $J_{H3''ax,H4'}$ = 11.58 Hz); 4.33 (d, 2H, S^{β}, $J_{S\alpha,S\beta}$ = 6.50 Hz); 4.28–3.65 (m, 6H, CH_2-Fmoc, H9-Fmoc, H9″, S^{α}); 4.20–4.15 (m, 1H, H2); 4.05–3.96 (m, 2H, H5, H6″); 3.90–3.82 (m, 1H, H5″); 3.82–3.68 (m, 1H, H6a); 3.10 (dd, 1H, H6b, $J_{H5,H6b}$ = 5.52 Hz, $J_{H6a,b}$ = 8.70 Hz); 2.53 (dd, 1H, $H3''_{eq}$, $J_{H3''eq,H4''}$ = 4.20 Hz, $J_{H3''eq,H3''ax}$ = 12.30 Hz); 2.04, 2.01, 1.98, 1.95, 1.91, 184, 1.80 (7× s, 25H, CH_3-Ac, $H3''_{ax}$); ^{13}C NMR (^{13}C, HMQC) (DMSO-d_6), δ: 170.20, 169.79, 169.53, 169.00 (CO, C1″); 158.85 (CO-urethane); 143.80 (C1a-, C8a-Fmoc); 141.00 (C4a-, C5a-Fmoc); 134.87 (C_{ipso}-Bn); 129.02, 128.63, 128.20, 127.53, 127.10 (C3-, C6-, C2-, C7-Fmoc, $C_{arom.}$-Bn (5C)); 125.89, 125.07 (C1-, C8-Fmoc); 120.10 (C4-, C5-Fmoc); 98.57 (C1); 98.09 (C2″); 72.53 (C6″); 69.35 (C5); 68.00 (C7″); 68.89 (S^{β}); 68.76 (C4″); 68.52 (C3); 68.10 (CH_2-Bn); 67.40 (CH_2-Fmoc); 67.20 (C4); 66.95 (C8″); 63.00 (C6); 62.10 (C9″); 58.54 (S^{α}); 49.00 (C5″); 47.21 (C2); 47.10 (C9-Fmoc); 38.40 (C3″); 23.20, 23.14, 20.99, 20.75, 20.67, 20.62 (CH_3-Ac, CH_3–NHAc); ESI-MS, *m*/*z*: 1168.7 $(M+Na)^+$, 1202.9 $(M+K)^+$, calcd: 1186.4, 1202.4.

7.15. General procedure for the automated solid-phase glycopeptide synthesis

The solid-phase syntheses were carried out in a Perkin Elmer ABI 433A peptide synthesizer using Tentagel® resin (263 mg, 0.05 mmol) equipped with Wang linker[31] and loaded with Fmoc valine **20** as the C-terminal amino acid. Removal of the Fmoc group was performed with piperidine (20%) in NMP in cycles of 4 min. As a rule, this treatment was repeated. The coupling reactions were carried out with Fmoc amino acids (1 mmol, 20 equiv) in a solution of 1 mmol of HBTU, 1 mmol of HOBt and 2 mmol ethyl-diisopropylamine (DIPEA) in NMP/DMF (1:1) within 15–17 min. For glycosylated Fmoc amino acids less excess and extended reaction times were applied. Unreacted amino groups were acetylated using a solution of acetic anhydride (0.5 M), DIPEA (0.125 M) and HOBt (0.015 M) in NMP. After each cycle, the resin was washed with NMP and dichloromethane.

7.16. L-Leucyl-L-alanyl-L-alanyl-L-leucyl-L-aspartyl-L-seryl-*O*-(2-acetamido-2-deoxy-3-*O*-[-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-histidyl-L-glycyl-L-alanyl-L-isoleucyl-L-valine (23)

7.16.1. (H-Leu-Ala-Ala-Leu-Asp-Ser(β-Gal-(1,3)-α-GalNAc)-His-Gly-Ala-Ile-Val-OH). The synthesis was carried out according to the general protocol using 0.263 mg (0.05 mmol) of resin **20**. After Fmoc-removal from histidine, the resin was treated with a freshly prepared solution of 95 mg (0.20 mmol, 4 equiv) of Fmoc-Ser(β-Ac$_4$Gal-(1 → 3)-α- Ac$_2$GalNAc)-OH **18**, 30 mg HOAt (0.19 mmol), 82 mg HATU (0.21 mmol) and 48 μL (0.29 mmol) DIPEA in NMP under vigorous orbital shaking for 5 h. The coupling reactions of the further Fmoc amino acids (20 equiv) again were performed according to the standard protocol. After coupling of the N-terminal leucine and Fmoc removal,

no acetylation was performed. Release of the glycopeptide from resin **21** was accomplished with 15 mL TFA, 0.9 mL of H_2O and 0.9 mL TIS and orbital shaking for 2 h. After filtration and washing three times with THF (10 mL each) the solvents were evaporated in vacuo. The residue was washed with cold diethyl ether (30 mL) and the crude **22** lyophilized. Yield: 81 mg.

ESI-MS (positive), *m/z*: 1707.0 ($[M+Na]^+$, calcd: 1706.7).

After mass-spectrometric investigation, the crude product **23** was dissolved in 20 mL MeOH. Freshly prepared NaOMe-solution was added until a pH of 9.5 was reached. The solution was stirred for 68 h and then neutralized by addition of a few drops of acetic acid. Evaporation of the solvent yielded crude **23** (92 mg). Purification by preparative HPLC [LUNA C-18, gradient (% CH_3CN+0.1% TFA): 3–20 (75 min)–20(140 min)] gave pure **23**: Yield: 32 mg (45%); colourless amorphous solid; t_R = 38.55 min [LUNA C-18, gradient (% CH_3CN+0.1% TFA): 3–21(40 min)–21(60 min)]; $[\alpha]_D^{22}$ −32.4 (*c* 1.4, H_2O).

ESI-MS (positive), *m/z*: 1432.2 ($[M+H]^+$, calcd: 1432.5); 1454.5 ($[M+Na]^+$, calcd: 1454.5); 1470.3 ($[M+K]^+$, calcd: 1470.6); 1492.5 ($[M+Na+K-H]^+$, calcd: 1492.6).

HR-ESI-MS (positive), *m/z*: 1431.7188 ([M+H$^+$], calcd: 1431.7219).

^{1}H NMR (400 MHz, D_2O) (^{1}H, COSY), δ: 8.61 (s, 1H, $H^{\varepsilon 1}$); 7.31 (s, 1H, $H^{\delta 2}$); 4.85–4.64 (m, 3H, H1 (4.83), D^{α} (4.72), H^{α} (4.71), under H_2O-signal); 4.54 (t, 1H, S^{α}, $J_{S\alpha,S\beta}$ = 5.28 Hz); 4.44 (d, 1H, H1′, $J_{H1',H2'}$ = 7.44 Hz); 4.37–4.16 (m, 8H, 3× A^{α} (4.36–4.26), H2 (4.31), L^{α} (4.29), V^{α} (4.22), I^{α} (4.19), H4 (4.19)); 4.00–3.82 (m, 7H, H3 (3.98), G^{α} (3.99, 3.90), L^{α} (3.96), $S^{\beta a}$ (3.93), H5 (3.89), H4′ (3.85)); 3.80–3.66 (m, 5H, $S^{\beta b}$ (3.78), C6 (3.75–3.66), C6′ (3.75–3.66)); 3.64–3.57 (m, 2H, H5′ (3.62), H3′ (3.58)); 3.51–3.47 (m, 1H, H2′); 3.31–3.28 (m, 1H, H^{β}a); 3.22–3.15 (m, 1H, H^{β}b); 2.96–2.78 (m, 2H, D^{β}); 2.17–2.10 (m, 1H, V^{β}); 1.99 (2× s, 6H, CH_3-Ac); 1.87–1.80 (m, 1H, I^{β}); 1.66–1.40 (m, 7H, 2× L^{γ} (1.71–1.58), 2× L^{β} (1.75–1.55), I^{γ}a (1.51)); 1.36 (m, 9H, A^{β}); 1.23–1.13 (m, 1H, I^{γ}b); 0.92–0.80 (m, 18H, 4× L^{δ} (0.94, 0.85), I^{γ} (0.89), V^{γ} (0.90), I^{δ} (0.83)).

^{13}C NMR (100.6 MHz, D_2O) (^{13}C, HMQC), δ: 104.98 (1C, C1′); 98.27 (1C, C1); 77.40 (1C, C3); 75.11 (1C, C5′); 72.71 (1C, C3′); 71.21 (1C, C4′); 70.80 (1C, C2′); 68.81 (1C, C4); 68.80 (1C, C5); 67.27 (1C, S^{β}); 61.41 (2C, C6, C6′); 58.60 (1C, V^{α}); 58.42 (1C, I^{α}); 53.80 (1C, S^{α}); 52.74 (1C, H^{α}); 51.90 (1C, L^{α}); 50.09 (1C, D^{α}); 49.81 (2C, A^{α}); 49.70 (1C, L^{α}); 48.51 (1C, C2); 42.31 (1C, G^{α}); 39.85 (2C, L^{β}); 36.12 (1C, I^{β}); 35.60 (1C, D^{β}); 30.11 (1C, V^{β}); 26.78 (1C, H^{β}); 24.23 (2C, L^{γ}); 22.39 (2C, CH_3-Ac); 21.67, 21.03 (4C, L^{δ}); 18.37 (2C, V^{γ}); 16.78 (3C, A^{β}); 14.80 (1C, I^{γ}); 10.11 (1C, I^{δ}).

7.17. *N*-Acetyl-L-seryl-*O*-(2-acetamido-2-deoxy-3-*O*-[β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-histidyl-L-glycyl-L-alanyl-L-isoleucyl-L-valine (23a)

7.17.1. (Ac-Ser(β-Gal-(1,3)-α-GalNAc)-His-Gly-Ala- Ile-Val-OH). Yield 7 mg (13%, from 0.05 mmol resin **20**).

MALDI-TOF-MS (positive, dhb), *m/z*: 1013.7 ($[M+Na]^+$, calcd: 1013.0).

^{1}H NMR (400 MHz, D_2O) (^{1}H, COSY), δ: 8.60 (s, 1H, $H^{\varepsilon 1}$); 7.31 (s, 1H, $H^{\delta 2}$); 4.86–4.70 (m, 2H, H1 (4.84), H^{α} (4.75), under H_2O-signal); 4.53 (t, 1H, S^{α}, $J_{S\alpha,S\beta}$ = 6.28 Hz); 4.43 (d, 1H, H1′, $J_{H1',H2'}$ = 7.40 Hz); 4.33–4.25 (m, 2H, A^{α}, H2); 4.23–4.17 (m, 3H, V^{α}, I^{α}, H4); 3.97–3.82 (m, 5H, H3, H5, H4′, G^{α}, S^{β}a); 3.78–3.65 (m, 5H, C6, C6′, S^{β}b); 3.64–3.57 (m, 2H, H3′, H5′); 3.32–3.28 (m, 1H, H^{β}a); 3.22–3.15 (m, 1H, H^{β}b); 2.18–2.10 (m, 1H, V^{β}); 2.02, 1.98 (2× s, 6H, CH_3-Ac); 1.88–1.80 (m, 1H, I^{β}); 1.53–1.45 (m, 1H, I^{γ}a); 1.36 (d, 3H, A^{β}, $J_{A\beta,A\alpha}$ = 7.44 Hz); 1.22–1.14 (m, 1H, I^{γ}b); 0.92 (d, 6H, V^{γ}, $J_{V\gamma,V\beta}$ = 7.04 Hz); 0.83 (t, 3H, I^{γ}, $J_{I\gamma,I\beta}$ = 7.44 Hz).

^{13}C NMR (100.6 MHz, D_2O) (^{1}H, HMQC), δ: 104.94 (1C, C1′); 98.41 (1C, C1); 77.33 (1C, C3); 75.22 (1C, C5′); 72.81 (1C, C3′); 71.23 (1C, C4′); 70.83 (1C, C2′); 68.77 (2C, C4, C5); 67.20 (1C, S^{β}); 61.22 (2C, C6, C6′); 58.71 (1C, V^{α}); 54.13 (1C, S^{α}); 52.62 (1C, H^{α}); 49.90 (1C, A^{α}); 48.51 (1C, C2); 42.38 (1C, G^{α}); 36.20 (1C, I^{β}); 30.24 (1C, V^{β}); 26.75 (1C, H^{β}); 24.60 (1C, I^{γ}); 22.40, 21.95 (2C, CH_3-Ac); 18.10 (2C, V^{γ}); 16.72 (1C, A^{β}); 14.80 (1C, I^{γ}); 10.15 (1C, I^{δ}).

7.18. *N*-Acetyl-L-alanyl-L-leucyl-L-aspartyl-L-seryl-*O*-(2-acetamido-2-deoxy-3-*O*-[β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-histidyl-L-glycyl-L-alanyl-L-isoleucyl-L-valine (23b)

7.18.1. (Ac-Ala-Leu-Asp-Ser(β-Gal-(1,3)-α-GalNAc)-His-Gly-Ala-Ile-Val-OH). Yield: 10 mg (16%, from 0.05 mmol resin).

MALDI-TOF (positive, dhb), *m/z*: 1289.7 ($[M+H]^+$, calcd: 1289.6); 1312.0 ($[M+Na]^+$, calcd: 1311.6); 1334.0 ($[M+2Na–H]^+$, calcd: 1333.6).

^{1}H NMR (400 MHz, D_2O) (^{1}H, COSY), δ: 8.60 (s, 1H, $H^{\varepsilon 1}$); 7.30 (s, 1H, $H^{\delta 2}$); 4.85–4.64 (m, 3H, H1 (4.83), H^{α} (4.71), D^{α} (4.67), under H_2O-signal); 4.54 (t, 1H, S^{α}, $J_{S\alpha,S\beta}$ = 5.28 Hz); 4.44 (d, 1H, H1′, $J_{H1',H2'}$ = 7.84 Hz); 4.37–4.26 (m, 3H, A^{α} (4.31), L^{α} (4.31), H2 (4.29)); 4.22–4.15 (m, 4H, A^{α} (4.19), H4 (4.19), V^{α} (4.18), I^{α} (4.17)); 3.97–3.82 (m, 6H, H3 (3.98), G^{α} (3.98, 3.90), S^{β}a (3.93), H5 (3.89), H4′ (3.86)); 3.80–3.67 (m, 5H, S^{β}b (3.79), C6 (3.71–3.67), C6′ (3.71–3.67)); 3.64–3.57 (m, 2H, H5′ (3.62), H3′ (3.57)); 3.52–3.48 (m, 1H, H2′); 3.31–3.28 (m, 1H, H^{β}a); 3.22–3.15 (m, 1H, H^{β}b); 2.91–2.78 (m, 2H, D^{β}); 2.15–2.10 (m, 1H, V^{β}); 1.98 (2× s, 6H, CH_3-Ac); 1.87–1.80 (m, 1H, I^{β}); 1.66–1.43 (m, 4H, L^{γ} (1.63), L^{β} (1.58), I^{γ}a (1.48)); 1.33 (m, 6H, A^{β}); 1.20–1.15 (m, 1H, I^{γ}b); 0.92–0.80 (m, 18H, 2× L^{δ} (0.92, 0.85), I^{γ}(0.90), V^{γ} (0.90), I^{δ} (0.84)).

^{13}C NMR (100.6 MHz, D_2O) (^{13}C, HMQC), δ/: 104.94 (1C, C1′); 98.31 (1C, C1); 77.50 (1C, C3); 75.24 (1C, C5′); 72.80 (1C, C3′); 71.25 (1C, C4′); 70.84 (1C, C2′); 68.80 (1C, C5); 68.75 (1C, C4); 67.20 (1C, S^{β}); 61.20 (2C, C6, C6′); 58.80 (1C, V^{α}); 58.71 (1C, I^{α}); 53.94 (1C, S^{α}); 52.81 (1C, H^{α}); 50.27 (1C, D^{α}); 49.90 (2C, A^{α}); 49.81 (1C, L^{α}); 48.50 (1C, C2); 42.29 (1C, G^{α}); 39.71 (1C, L^{β}); 36.10 (1C, I^{β}); 35.90 (1C, D^{β}); 30.06 (1C, V^{β}); 26.81 (1C, H^{β}); 24.41 (1C, L^{γ}); 22.41, 20.90 (2C, L^{δ}); 21.80 (2C, CH_3-Ac); 18.49 (2C, V^{γ}); 16.78 (2C, A^{β}); 14.90 (1C, I^{γ}); 10.21 (1C, I^{δ}).

7.19. L-Glutamyl-L-leucyl-L-alanyl-L-alanyl-L-leucyl-L-aspartyl-L-seryl-*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-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-histidyl-L-glycyl-L-alanyl-L-isoleucyl-L-valyl-L-aspartyl-L-glycyl-L-prolyl-L-valine (25)

7.19.1. H-Gln-Leu-Ala-Ala-Leu-Asp-Ser(β-Ac$_4$Gal-(1,3)-[α-NeuAc$_5$NAcCOOBn-(2,6)]-α-GalNAc)-His-Gly-Ala-Ile-Val-Asp-Gly-Pro-Val-OH. Using 227 mg (0.05 mmol) of Fmoc-Val-Tentagel® resin **20**, the coupling reactions of the initial eight Fmoc amino acids (20 equiv) were performed according to the general protocol. For coupling of sialyl T serine conjugate **19** the Fmoc histidine-terminated resin was transferred into separate reaction flask and treated with a freshly prepared solution of 165 mg (0.12 mmol, 2.34 equiv) of Fmoc-Ser(β-Ac$_4$Gal-(1 → 3)-[α-NeuAc$_5$NAcCOOBn-(2 → 6)]-α-GalNAc)-OH **19**, 18 mg (0.12 mmol) HOAt, 47 mg HATU (0.12 mmol) and 25 μL (0.15 mmol) DIPEA in NMP. After orbital shaking for 5 h, the resin was filtered off, washed with NMP and subjected to the capping reaction. Coupling reactions for the six N-terminal Fmoc amino acids were carried in the automated synthesizer again. After removal of the Fmoc group from the N-terminal glutamine, no capping reaction was carried out.

The detachment of the glycopeptide from resin **24** was performed with 10 mL TFA, 0.9 mL H_2O and 0.9 mL TIS under orbital shaking within 2 h. The resin was filtered off and washed twice with 10 mL TFA. After concentration of the filtrate in vacuo, 30 mL of cold diethyl ether was added, and the solvent was removed by centrifugation. The remaining sialyl T glycopentadecapeptide **25** was lyophilized and purified by preparative HPLC [LUNA C-18, gradient (% CH_3CN+ 0.1%TFA): 25–100 (120 min)].

Yield: 55 mg (42%); colourless amorphous solid; t_R = 20.20 min [LUNA C-18, gradient (% CH_3CN+ 0.1% TFA): 3–100 (40 min)].

ESI-MS (positive), *m*/*z*: 1323.7 ([M+2H]$^{2+}$/2, calcd: 2647.8).

^{1}H NMR (400 MHz, CD_3OD) (^{1}H, COSY), δ: 8.86 (s, 1H, $H^{\varepsilon 1}$); 7.52–7.40 (m, 6H, $H_{arom.}$-Bn, $H^{\delta 2}$); 5.46–5.33 (m, 2H, H4′ (5.35), H8″ (5.34)); 5.30–5.10 (m, 4H, CH_2-Bn (5.21), H7″ (5.14), H2′ (5.12)); 4.87–4.71 (m, 3H, H1′ (4.85), H3′ (4.83), H1 (4.74)); 4.68–4.45 (m, 7H, D^{α} (4.66), H^{α} (4.66), P^{α} (4.57), S^{α} (4.53), A^{α} (4.48), A^{α} (4.46), L^{α} (4.46)); 4.44–3.80 (m, 24H, H2 (4.43), A^{α} (4.34), A^{α} (4.33), L^{α} (4.33), CH_2-Fmoc (4.30), V^{α} (4.29), I^{α} (4.29), H9″ (4.27, 4.09), V^{α} (4.24), G^{α} (4.19, 4.14), H6″ (4.12), H5′ (4.11), Q^{α} (4.00), H4 (4.00), G^{α} (3.99, 3.90), H9-Fmoc (3.98), H5 (3.87), H6a (3.84), H3 (3.83)); 3.70–3.40 (m, 4H, P^{δ} (3.62, 3.58), C6b (3.53), H^{β}a (3.41)); 3.30–3.20 (m, 1H, H^{β}b (3.21)); 3.05–2.75 (m, 2H, D^{β} (2.93)); 2.57–2.50 (m, 1H, $H3''_{eq}$); 2.30–1.98 (m, 34H, P^{β} (2.19, 2.02), V^{β} (2.18), $H3''_{ax}$ (2.14), Q^{β} (2.13), V^{β} (2.08), P^{γ} (2.02), CH_3-Ac); 1.90 (s, 3H, CH_3-Ac); 1.84–1.58 (m, 7H, I^{β} (1.84), 2× L^{γ} (1.73), 2× L^{β} (1.63)); 1.58–1.38 (m, 10H, I^{γ}a (1.57), 3× A^{β}); 1.30–1.15 (m, 1H, I^{γ}b); 1.10–0.88 (m, 30H, 4× V^{γ} (0.99, 0.91), 4× L^{δ} (0.95, 0.91, 0.97), I^{γ} (0.90), I^{δ} (0.87)).

^{13}C NMR (100.6 MHz, CD_3OD) (^{13}C, HMQC), δ: 102.68 (1C, C1′); 99.21 (1C, C1); 78.19 (1C, C3); 73.12 (1C, C6″); 72.00 (1C, C2′); 71.52 (1C, C5′); 70.22 (1C, C3′); 70.11 (2C, C7″, C5); 69.59 (1C, C8″); 68.91 (1C, C4); 68.52 (1C, CH_2-Bn); 68.18 (1C, C4′); 64.05 (1C, C6); 63.03 (1C, C9″); 63.00 (1C, CH_2-Fmoc); 60.93 (1C, P^{α}); 59.59 (1C, V^{α}); 59.05 (1C, V^{α}); 59.00 (1C, I^{α}); 53.82 (2C, 2× L^{α}); 53.51 (1C, D^{α}); 53.42 (1C, Q^{α}); 51.51 (1C, H^{α}); 50.96, 50.29, 49.04 (4C, A^{α}); 50.35 (1C, C2); 49.80 (1C, C9-Fmoc); 47.42 (1C, P^{δ}); 43.00, 42.67 (1C, G^{α}); 41.41 (2C, 2× L^{β}); 37.44 (1C, I^{β}); 36.12 (1C, D^{β}); 31.89, 31.46 (2C, 2× V^{β}); 31.31 (1C, C3″); 30.25 (1C, P^{β}); 27.73 (1C, Q^{β}); 27.52 (1C, H^{β}); 25.79 (1C, I^{γ}); 25.52 (2C, 2× L^{γ}); 25.31 (1C, P^{γ}); 22.92–20.71 (9C, CH_3-Ac); 23.09, 21.97, 21.69 (4C, L^{γ}); 19.50, 18.30 (2C, 2× V^{γ}); 17.65 (3C, A^{β}); 15.68 (1C, I^{γ}); 10.90 (1C, I^{δ}).

7.20. L-Glutamyl-L-leucyl-L-alanyl-L-alanyl-L-leucyl-L-aspartyl-L- seryl-*O*-{2-acetamido-2-deoxy-3-*O*-[-β-D-galactopyranosyl]-6-*O*-[(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2- nonulopyranosyl)onate]-α-D-galactopyranosyl}-L-histidyl-L-glycyl- L-alanyl-L-isoleucyl-L-valyl-L-aspartyl-L-glycyl-L-prolyl-L-valine (26)

7.20.1. H-Gln-Leu-Ala-Ala-Leu-Asp-Ser(β-Gal-(1,3)-[α-Neu5NAc-(2,6)]-α-GalNAc)-His-Gly-Ala-Ile-Val-Asp-Gly-Pro-Val-OH. Glycopeptide **25** was hydrogenated in 15 mL MeOH within 18 h using catalytic amounts of Pd/C (10%) as the catalyst. After filtration, freshly prepared solution of NaOMe in MeOH was added until a pH of 9.0–9.5 was reached. The solution was stirred for 72 h. A few drops of acetic acid were added for neutralization, and the solvents were evaporated in vacuo. Purification of the glycopeptides **26** was performed by preparative HPLC [LUNA C-18, gradient (% CH_3CN+0.1% TFA): 20–30(120 min)].

Yield: 26 mg (23%, from **20**); colourless amorphous solid; t_R = 19.50 [(% CH_3CN+0.1% TFA): 10–50 (40 min)]; $[\alpha]_D^{22}$ −19.3 (*c* 1, H_2O).

ESI-MS (positive), *m*/*z*: 2218.2 ([M]$^+$, calcd: 2218.0).

^{1}H NMR (600 MHz, D_2O) (^{1}H, COSY), δ: 8.52 (d, 1H, $H^{\varepsilon 1}$, $J_{H\varepsilon 1,H\delta 2}$ = 7.04 Hz); 7.22 (d, 1H, $H^{\delta 2}$,

$J_{H\varepsilon1,H\delta2}$ = 7.04 Hz); 4.69–4.58 (m, 2× D^{α}, H1); 4.47–4.42 (m, H1′, H^{α}); 4.41–4.31 (m, 3× A^{α}, P^{α}, 2× L^{α}); 4.28–4.11 (m, G^{α}, I^{α}); 4.10–4.00 (m, G^{α}, Q^{α}); 3.98–3.70 (m, H4′ (3.88), H6′ (3.73)); 3.68–3.54 (m, H5′, H3′, P^{δ}); 3.53–3.38 (m, H2′); 3.35–3.11 (m, H^{β}); 3.11 (m, H^{β}); 2.91–2.69 (m, 2× D^{β}); 2.22–2.13 (m, V^{β}, Q^{β}, P^{β}); 2.11–2.00 (m, V^{β}, P^{γ}); 1.98–1.82 (m, NH-Ac, P^{β}); 1.78–1.69 (m, 1 H, I^{β}); 1.60–1.45 (m, 3H, L^{β}, L^{γ}); 1.42–1.35 (m, 1H, I^{γ}); 1.30–1.22 (m, 9H, A^{β}); 1.17–1.12 (m, 1H, I^{γ}); 0.88–0.70 (m, 30H, 4× V^{γ}, I^{γ}, I^{δ}, 4× L^{δ}).

^{13}C NMR (150.9 MHz, D_2O) (HMQC), δ: 104.58 (1C, C1′); 74.81 (1C, C5′); 72.43 (1C, C3′); 71.14 (1C, C4′); 70.40 (1C, C2′); 60.73 (1C, C6′); 60.11 (1C, P^{α}); 59.05 (1C, I^{α}); 53.71 (1C, H^{α}); 52.50 (2C, D^{α}); 52.37 (2C, L^{α}); 52.02 (1C, Q^{α}); 49.62, 49.58, 48.06 (3C, A^{α}); 46.81 (1C, P^{δ}); 41.96, 41.68 (2C, G^{α}); 39.48 (2C, L^{β}); 35.66 (1C, I^{β}); 35.18 (2C, D^{β}); 29.96, 29.71 (2C, V^{β}); 29.18 (1C, P^{β}); 26.38 (1C, Q^{β}); 26.32 (1C, H^{β}); 24.47 (1C, I^{γ}); 24.23 (1C, P^{γ}); 24.11 (2C, L^{γ}); 21.48 (1C, CH_3–NHAc); 20.57, 21.94 (4C, L^{δ}); 18.26, 17.83, 17.11 (4C, V^{γ}); 16.34 (3C, A^{β}); 14.51 (1C, I^{γ}); 9.74 (1C, I^{δ}).

7.21. 2-(7-{*N*-[1*S*,3*R*,4*S*,5*R*]-1,3,4,5-Tetraacetoxycyclohexanoyl-glycyl}-aminocumarin-4-yl)-acetyl-L-leucyl-L-alanyl-L-alanyl-L-leucyl-L-aspartyl-L-seryl-*O*-(2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-[benzyl-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-histidyl-L-glycyl-L-alanyl-L-isoleucyl-L-valyl-L-aspartyl-L-glycyl-L-prolyl-L-valine (28)

7.21.1. Quiglac-Gly-Aca-Leu-Ala-Ala-Leu-Asp-Ser(α-NeuAc$_4$5NAcCOOBn-(2,6)-α-Ac$_2$GalNAc)-His-Gly-Ala-Ile-Val-Asp-Gly-Pro-Val-OH. According to the general protocol solid-phase synthesis was performed starting with Fmoc-Val-loaded resin **20** (227 mg, 0.05 mmol). Coupling reactions for the next 8 Fmoc amino acids (20 equiv) were carried out as described above. For coupling of the sialyl T_N serine **17** the histidine-terminated resin was transferred to a separate reaction flask. After removal of the Fmoc group, a freshly prepared solution of 60 mg (0.05 mmol, 1.02 equiv) of Fmoc-Ser(α-NeuAc$_4$5NAcCOOBn-(2 → 6)-α-Ac$_2$GalNAc)-OH **17**, 8 mg (0.05 mmol) HOAt, 20 mg (0.05 mmol) HATU and 10.7 μL DIPEA in NMP was added. The mixture was vigorously shaken for 6 h and filtered. After washing with NMP, a capping reaction was carried out. The coupling reactions for the further Fmoc amino acids were performed according to the general protocol. For coupling of the N-terminal quinic acid–coumarine chromophore the resin-linked glycopeptide was placed in flask, 73 mg (0.10 mmol, 2 equiv) of Ac$_4$Quiglac-Gly-Aca-Leu-OH **10**, 15 mg (0.10 mmol) HOAt, 38 mg (0.10 mmol) HATU and 21 μL of DIPEA in 2 mL of NMP were added, and the mixture was vigorously shaken for 6 h. After a final capping acetylation, the loaded resin **27** was filtered off and washed with NMP and dichloromethane.

Release of the labelled glycopeptide from resin **27** was performed by treatment with 10 mL TFA, 0.9 mL H_2O and 0.9 mL TIS and orbital shaking for 2 h. After filtration and washing of the resin three times with 10 mL of TFA, the filtered solutions were combined, the solvent was evaporated, and diethyl ether (30 mL) was added to the remaining residue. The solvent was removed by centrifugation and the crude product containing the desired glycopeptide **28** and the deletion peptide **29** was lyophilized. Separation and purification was carried out by preparative HPLC [LUNA C-18, gradient (% MeOH+0.1TFA): 40–90 (90 min)].

Yield of **28**: 15 mg (10%); colourless amorphous solid; t_R = 22.0 min [LUNA C-18, gradient (% CH_3CN+0.1% TFA) 10–100 (40 min)].

ESI-MS (positive), *m*/*z*: 1447.8 ($[M+Na+H]^{2+}$/2, calcd: 1448.0).

^{1}H NMR (400 MHz, CD_3OD) (^{1}H, COSY), δ/ppm: 8.83 (m, 1H, $H^{\varepsilon1}$); 7.89–7.78 (m, 1H, H8-Cou); 7.77–7.74 (m, 1H, H5-Cou); 7.63–7.58 (m, 1H, H6-Cou); 7.50–7.38 (m, 6H, $H^{\delta2}$, $H_{arom.}$-Bn); 6.40 (s, 1H, H3-Cou); 5.63–5.61 (m, 1H, H3-Qui); 5.53–5.48 (m, 1H, H5-Qui); 5.44–5.35 (m, 2H, H7″ (5.41), H8″ (5.37)); 5.33–5.27 (m, H4 (5.31), H9″a (5.28)); 5.26–5.22 (m, 1H, H9″b); 5.18–5.09 (m, 2H, H3 (5.15), H4-Qui (5.11)); 4.90–4.80 (m, H4″ (4.83), D^{α} 4.82); 4.72–4.60 (m, D^{α} (4.65), H^{α} (4.64), P^{α} (4.58)); 4.47–3.92 (m, A^{α} (4.42, 4.24, 4.18), V^{α} (4.31, 4.22), G^{α} (4.17, 4.01, 3.97)); 3.70–3.60 (m, P^{δ}); 3.50–3.40 (m, H^{β}a); 3.31–3.20 (m, H^{β}b); 3.10–2.88 (m, D^{β}a (2.99, 2.90, 2.80)); 2.84–2.75 (m, 2H, D^{β}b, H2a-Qui); 2.59–2.55 (m, 1H, H2b-Qui); 2.29 (s, 3H, CH_3-Ac); 2.24–1.58 (m, CH_3-Ac, P^{β} (2.22, 2.06), V^{β} (2.20, 2.12), P^{γ} (2.02), I^{β} (1.90), I^{γ}a (1.61)); 1.45–1.38 (m, 9H, A^{β}); 1.36–1.14 (m, L^{γ} (1.30, 1.22, 1.16), I^{γ}b (1.22)); 1.08–0.90 (m, 30H, L^{δ} (1.04, 1.00, 0.94, 0.94), V^{γ} (1.01, 0.97, 0.95), I^{γ} (0.94), I^{δ} (0.90)).

^{13}C NMR (100.6 MHz, CD_3OD) (HMQC), δ/ppm: 73.33 (1C, C4-Qui); 70.81 (1C, C4″); 69.73 (1C, C3); 69.48 (1C, C3-Qui); 69.43 (1C, C7″); 69.12 (1C, C9″); 68.66 (1C, C4); 68.41 (1C, C8″); 67.91 (1C, C5-Qui); 61.42 (1C, P^{α}); 60.38, 59.09 (2C, V^{α}); 54.95, 54.83, 50.82 (3C, A^{α}); 59.73, 52.66 (3C, L^{α}); 59.41 (1C, I^{α}); 52.63 (1C, H^{α}); 52.58, 51.33 (2C, D^{α}); 47.71 (1C, P^{δ}); 44.41, 43.02 (2C, G^{α}); 40.84 (3C, L^{β}); 37.75 (1C, I^{β}); 36.71 (1C, D^{β}); 36.69 (1C, C2-Qui); 36.22 (1C, D^{β}); 31.96 (1C, C6-Qui); 31.87, 31.67 (2C, V^{β}); 30.63 (1C, P^{β}); 27.71 (1C, H^{β}); 27.46 (3C, L^{γ}); 26.23 (1C, I^{γ}); 25.61 (1C, P^{γ}); 23.37, 23.17, 22.30, 22.02 (4C, L^{δ}); 19.91, 19.64, 18.81, 18.56 (4C, V^{γ}); 18.26–17.04 (3C, A^{β}); 16.01 (1C, I^{γ}); 11.31 (1C, I^{δ}).

7.22. 2-(7-{*N*-[1*S*,3*R*,4*S*,5*R*]-1,3,4,5-Tetraacetoxycyclohexanoyl-glycyl}-aminocumarin-4-yl)-acetyl-L-leucyl-L-alanyl-L-alanyl-L-leucyl-L-aspartyl-L-seryl-*O*-(2-acetamido-2-deoxy-6-*O*-[(5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranosyl)onate]-α-D-galactopyranosyl)-L-histidyl-L-glycyl-L-alanyl-L-isoleucyl-L-valyl-L-aspartyl-L-glycyl-L-prolyl-L-valine (30)

7.22.1. Quiglac-Gly-Aca-Leu-Ala-Ala-Leu-Asp-Ser(α-Neu5NAcCOOH-(2,6)-α-GalNAc)-His-Gly-Ala-Ile-Val-Asp-Gly-Pro-Val-OH. To a solution of glycopeptide **28**

(15 mg, 5.23×10^{-6} mol) in 15 mL of dry MeOH was added freshly prepared NaOMe solution until a pH of 9.5 is reached. The solution was stirred at room temperature for 48 h, neutralized by addition of a drop of acetic acid, and the solvent was removed in vacuo. The crude product was dried in high vacuum, dissolved in water (5 mL), and 0.1 N NaOH solution was added until a pH of 10.0 was reached. The solution was stirred at room temperature for 24 h, neutralized by addition of a few drops of acetic acid and lyophilized. Purification was performed by preparative HPLC [LUNA C 18 (% CH_3CN+0.1% TFA) 10–50 (120 min)].

Yield of **30**: 5 mg (4%, overall from **20**); t_R = 11.20 min [LUNA C-18, (% CH_3CN+0.1% TFA) 10–50 (40 min)]; $[\alpha]_D^{22}$ −12.1 (*c* 0.3, H_2O).

MALDI-TOF-MS (positive), *m*/*z*: 2363.6 ($[M]^+$, calcd: 2360.0); 2385.5 ($[M+Na]^+$, calcd: 2383.0).

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