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Synthesis and Antibody Binding of Highly Fluorinated Amphiphilic MUC1 Glycopeptide Antigens

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The analysis of humoral immune responses is of great importance for basic and clinical research. Mapping the structural requirements of epitope recognition with modified tumor-associated carbohydrate antigens allows both the development of biomarkers and the design of synthetic anticancer vaccines. For this purpose, double-tailed hydrocarbon/fluorocarbon membrane anchors have been prepared and conjugated to a T_N dipeptide. Furthermore, a novel hydrophobized MUC1 tandem repeat glycopeptide antigen was fully assembled on a solid support and its specific binding to different mouse anti-MUC1 antibodies was demonstrated through ELISA, QCM, and SPR measurements. Such functional fluorous MUC1 antigens are of great interest for specific glycan (micro-)array formats and allow a detailed analyses of serum antibodies obtained from immunization studies. In addition, the intriguing characteristics of fluorous surfactants, for example, their strong self-association tendency, might stimulate the use of novel fluorous-tagged antigen conjugates in the development of multivalent micellar glycopeptide vaccines.

Introduction

Glycoproteins are fundamental for a variety of biological events including fertilization, neuronal development, immune surveillance, and inflammatory responses.[1] As a consequence, glycoconjugates have become highly attractive targets for medical applications and important tools for chemical glycobiology. Alterations in cell surface carbohydrate structures have been found to affect normal cellular interactions and contribute to the pathogenesis and progression of neoplasia.[2] For example, most carcinoma express abnormal forms of the transmembrane glycoprotein mucin-1 (MUC1) characterized by the exposure of immunogenic peptide epitopes and truncated glycan chains.[3] Hence, mucin-type glycopeptides decorated with tumor-associated saccharide antigens have been shown to be important tools for the development of cancer immunotherapy and diagnostics.[4] With regard to vaccine design, the traditional approach of coupling glycopeptide antigens to carrier proteins[5] has been expanded by attaching multiple copies of MUC1 epitopes to peptide templates, dendrimers, nanoparticles, and liposomes.[6] To enhance the stability of liposomal vaccine formulations and to limit undesired biological side-effects, self-assembled delivery systems based on fluorinated analogues of amphiphilic compounds have recently been developed.[7]

Fluorocarbon moieties are characterized by very strong intramolecular bonds and very weak intermolecular interactions. In addition, perfluoroalkyl chains are larger and more rigid than their hydrocarbon counterparts and confer on fluorinated surfactants a strong tendency to collect at interfaces and self-associate into supramolecular assemblies.[8] Because fluorocarbon amphiphiles are at the same time hydrophobic, lipophobic, and fluorophilic, they do not accumulate in lipid membranes and hence are less haemolytic and less detergent than hydrocarbon amphiphiles.[9] Moreover, partially fluorinated surfactants show intriguing characteristics with regard to miscibility, phase separation, and compartmentalization that might stimulate novel applications in materials science and the field of medicine.[10]

In addition, the temporary attachment of fluorocarbon linkers and fluorous tags is an attractive strategy for facilitating solution-phase organic synthesis by fluorous separation and purification techniques.[11] The use of fluorocarbon tags for specific immobilization within fluorous (micro-)array formats has also expanded their application.[12] For example, fluorous-tagged small molecules,[13] carbohydrates,[14] and glycosphingolipids[15] have been used in this context. Recently, the first examples of fluorous-tagged tumor-associated MUC1 glycopeptide antigens and their successful recognition by specifically induced anti-MUC1 antibodies were described.[16] These hydrophobized glycoconjugates were based on tris(hydroxymethyl)aminomethane onto which three perfluoroundecyl chains were crafted. In this study, however, a modular approach towards

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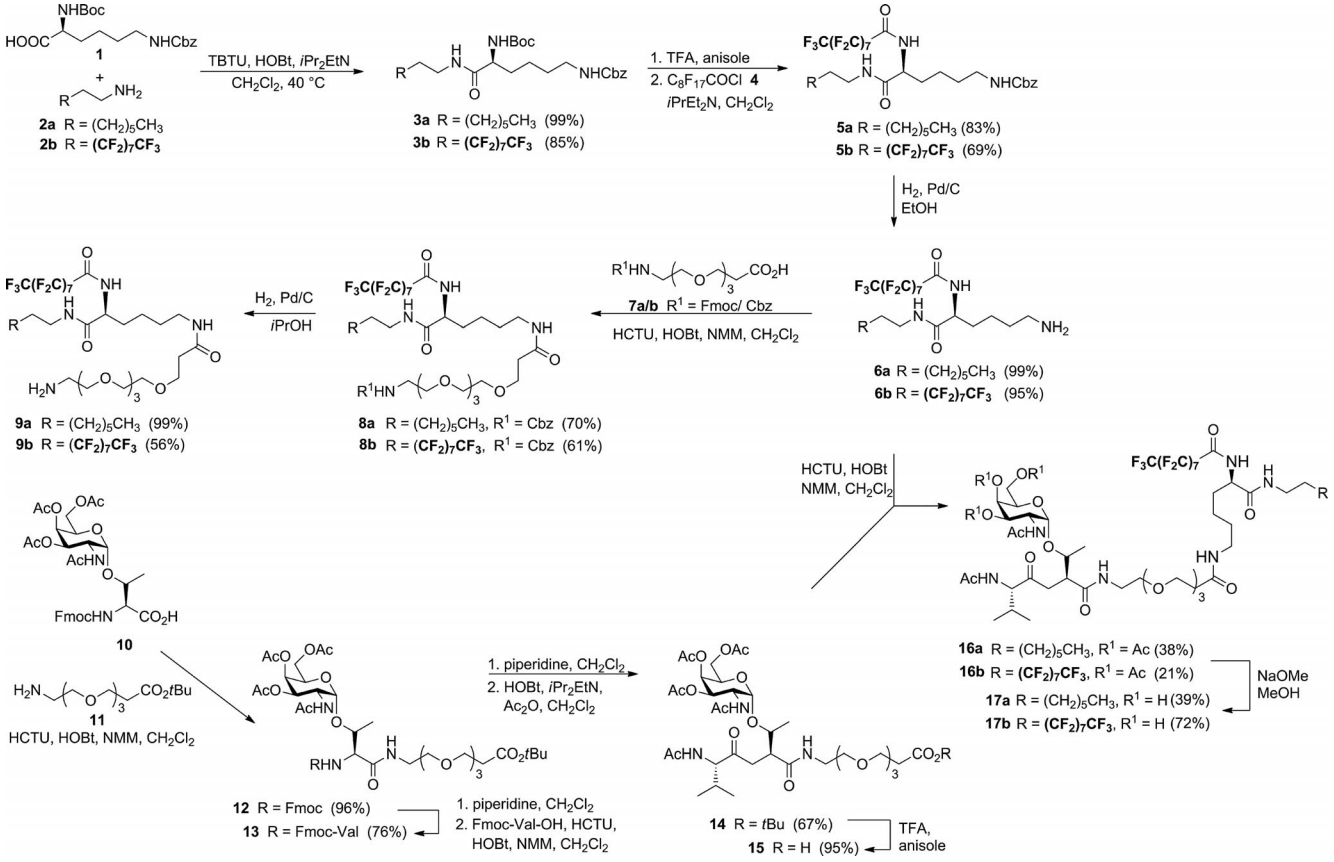
the preparation of novel MUC1 glycopeptide conjugates is presented based on a trifunctional chiral lysine core[17] with single- or double-chain fluorinated tails that could serve as potential building blocks in cancer immunotherapy and diagnostics.

Results and Discussion

Synthesis of Lysine-Based Fluorinated Amphiphiles

The synthetic route to the target double-tailed surfactants is depicted in Scheme 1 and starts from commercially available N^{α}-Boc-N^{ε}-Cbz-L-lysine (**1**), which was coupled to hydrocarbon and fluorocarbon amines **2a** and **2b**. Although the coupling reaction to **3a** has already been performed with dicyclohexylcarbodiimide (DCC),[18] in our hands, the best results were obtained by using TBTU/HOBt[19] and diisopropylethylamine (DIPEA) in CH_2Cl_2 at 40 °C. Subsequent Boc deprotection with trifluoroacetic acid (TFA) and anisole[20] then set the stage for further attachment of the perfluoroalkylated fatty acid. Because of the low solubility of the reactants and the reduced reactivity of the L-lysine α-amino group, this step required the use of the more reactive perfluoro acyl chloride **4**, accessible by treatment of the corresponding acid with $SOCl_2$/pyridine.[21] Hence, coupling of **4** to the deprotected amines **3a**,**b** was accomplished in the presence of DIPEA in CH_2Cl_2,[22] furnishing the desired Cbz-protected derivatives **5a** and **5b** in 83 and 69% yields, respectively, after column chromatography or recrystallization. The Cbz groups were then removed by hydrogenolysis with Pd/C in EtOH to yield amphiphiles **6a** and **6b** in nearly quantitative yields. Notably, MeOH is not a suitable solvent for this reaction because it provides *N*-methylated products almost exclusively.[23]

To install the tumor-associated carbohydrate antigen (TACA) head group, the hydrophilic *N*-protected ω-spacer amino acid **7a**[24] was attached to amines **6a**,**b** by using HCTU/HOBt[25] in the presence of *N*-methylmorpholine (NMM) in CH_2Cl_2 under sonication. Column chromatography or fluorous solid-phase extraction[26] (F-SPE) in the case of the corresponding double-tailed fluorocarbon derivative provided the desired surfactants in good yields. Subsequent N-terminal deprotection of the corresponding Fmoc-functionalized conjugate was attempted by treatment with either 20% piperidine in CH_2Cl_2 or tetrabutylammonium fluoride (TBAF) in *i*PrOH/DMF. Although TLC and HPLC indicated complete conversion in both cases, the low solubility and high aggregation tendency of the deprotected



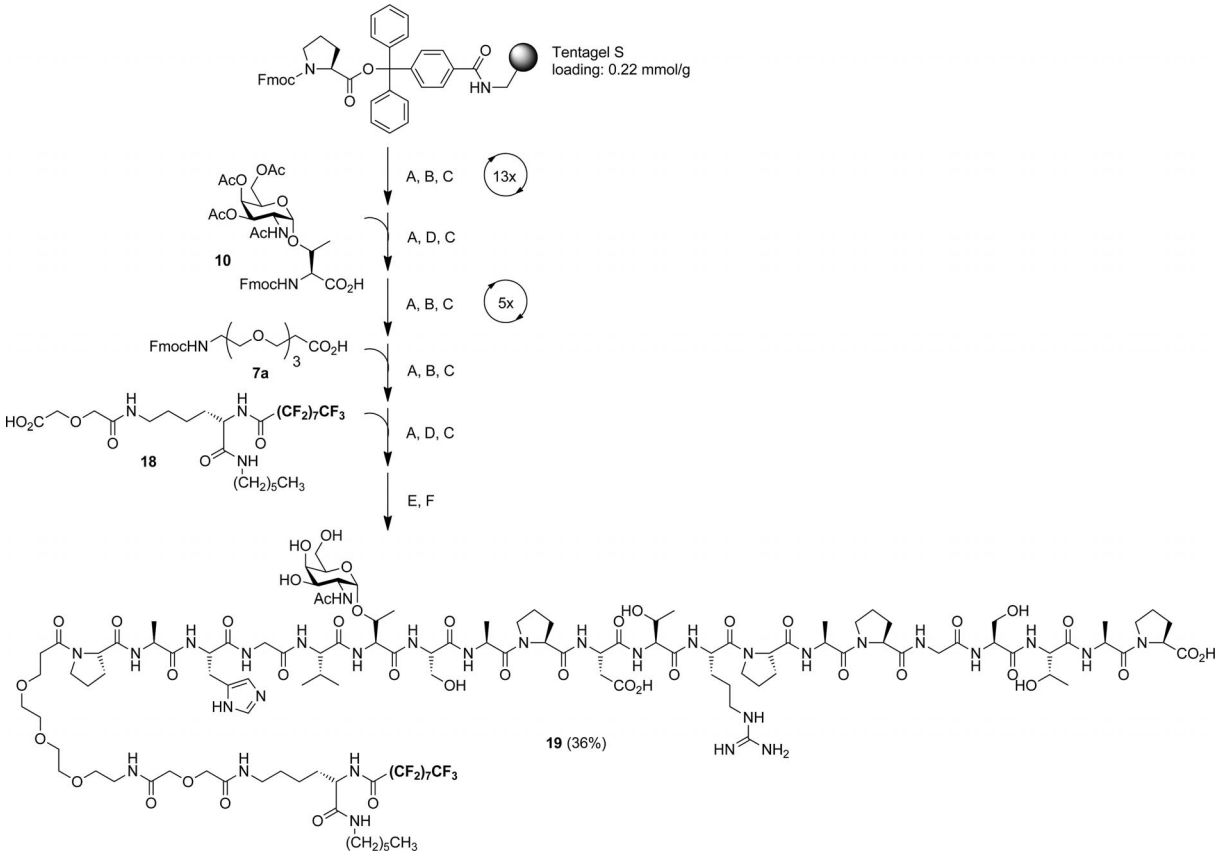
Scheme 1. Synthesis of the double-tailed fluorocarbon and hydrocarbon/fluorocarbon membrane anchors and their incorporation into a T_N antigen-Thr-Val dipeptide. TBTU = *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate, HOBt = 1-hydroxybenzotriazole, DIPEA = diisopropylethylamine, HCTU = *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, NMM = *N*-methylmorpholine, TFA = trifluoroacetic acid.

surfactants always impeded the isolation of significant amounts of pure material. Fortunately though, after attachment of the corresponding Cbz-protected spacer **7b** to **6a**,**b** and subsequent hydrogenolysis, the desired products **9a**,**b** were accessible in good chemical yields and sufficient purities upon simple filtration.

Synthesis of F-Amphiphilic TACA Conjugates

With regard to the low solubility of compounds **9a**,**b**, the initial synthetic strategy for their incorporation into tumor-associated MUC1 glycopeptide conjugates was slightly modified and T_N antigen building block **10**[27] was first coupled to the spacer amino acid *tert*-butyl ester **11**[24] by using HCTU/HOBt with NMM in CH_2Cl_2. To enhance the chemical stability of the antigen derivative, conjugate **12** was then further condensed to Fmoc-protected L-valine under similar coupling conditions (76% over two steps). The labile Fmoc protecting group was exchanged for a permanent acetyl protecting group and the *tert*-butyl ester of the resulting dipeptide **14** was cleaved under acidic conditions (TFA, H_2O) to afford building block **15** in 64% yield over three steps. Final attachment of **15** to the surfactants **6a**,**b** was again accomplished in the presence of HCTU/HOBt with NMM in CH_2Cl_2 at 40 °C, providing the desired conjugates **16a**,**b** together with unreacted starting material that could not be completely removed by RP-HPLC at this stage. However, separation of the latter was possible after *O*-deacetylation of the carbohydrate moiety. Thus, after careful Zemplén transesterification with NaOMe in MeOH at pH 9.5[28] and semipreparative RP-HPLC, glycolipid analogues **17a**,**b** were isolated in 15% yield over the two steps. Notably, the relatively low yield of **17a** was not a result of significant β-elimination of the glycan but mainly due to solubility problems.

For potential immunotherapeutic and diagnostic applications, that is, to map antibody specificities of humoral immune responses and elucidate the corresponding binding epitopes, it would be desirable to attach larger tumor-associated antigen structures to the fluorous anchors. Towards this end, a mixed hydrocarbon/fluorocarbon double-tailed glycolipopeptide carrying a complete MUC1 tandem repeat domain with a T_N determinant at Thr6 as TACA was prepared by solid-phase glycopeptide synthesis (SPPS). Thus, with the aid of automated solid-phase peptide chemistry, the MUC1 glycopeptide **19**, equipped with a hydrophilic triethylene glycol spacer and a fluorous anchor, was assembled on a Fmoc-Pro-trityl-TentaGel resin (Scheme 2).



Scheme 2. Solid-phase synthesis of the double-tailed fluorocarbon/hydrocarbon MUC1 glycopeptide antigen **19**. A) Fmoc removal: 20% piperidine in NMP; B) coupling: Fmoc-aa-OH, HBTU, HOBt, DIPEA, DMF; C) capping: cat. HOBt, Ac_2O, DIPEA, NMP; D) coupling: **10** or **18**, HATU, HOAt, NMM, NMP, 8 h; E) cleavage: TFA, TIS, H_2O (10:1:1); F) deprotection: NaOMe, MeOH, pH 9.5. HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, NMP = *N*-methylpyrrolidone, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt = *N*-hydroxy-7-azabenzotriazole, TIS = triisopropylsilane.

The first 13 amino acids of the tandem repeat sequence were attached under standard conditions (Fmoc protocol) with piperidine in *N*-methylpyrrolidone (NMP) for Fmoc deprotection and HBTU, HOBt, and DIPEA[29] in DMF to couple the amino acids (10 equiv. each). To minimize the amount of byproducts, unreacted amino groups were also capped with Ac_2O, HOBt, and DIPEA in NMP after each cycle. In the case of the sterically hindered glycosylated threonine derivative **10** (2.5 equiv.), successful attachment was ensured by using extended reaction times (8 h) and the more reactive reagents HATU/HOAt[30] with *N*-methylmorpholine (NMM) in NMP for activation. After coupling the final five Fmoc-amino acids of the tandem repeat sequence and the hydrophilic spacer **7a** according to the standard protocol, the fluorinated amphiphile **18**[31] (2.0 equiv.) was attached over 8 h by using the reagent cocktail of HATU, HOAt, and NMM in NMP. Simultaneous detachment of the glycopeptide from the resin and cleavage of the acid-labile amino acid side-chain protective groups was achieved upon treatment with a mixture of TFA, triisopropylsilane (TIS), and water. The subsequent de-*O*-acetylation of the saccharide moiety under Zemplén conditions at pH 9.5 was directly performed on the crude product and required stringent pH control to avoid epimerization of amino acids and/or β-elimination of the glycan. Purification of the resulting hydrophobized glycoconjugate by RP-HLPC was impossible due to its limited solubility. Fortunately though, precipitation from Et_2O provided **19** (36% based on the loaded resin) without concomitant deletion sequences, as indicated by analytical RP-HPLC and MALDI-TOF mass spectrometry.

Antibody Binding of Glycolipopeptide Analogue 19

With the mixed hydrocarbon/fluorocarbon glycolipopeptide conjugate **19** in hand, a preliminary study towards its use as an antibody profiling tool was conducted. Because antibodies possess multiple binding sites, the presentation, orientation, and multivalent architecture of epitopes all contribute to the recognition event. Hence, upon careful analysis of humoral responses, different antibodies or subpopulations of antibodies recognizing variations in binding epitopes and/or their spatial orientations might be distinguished to help fine-tune vaccine design and biomarker development. Towards this end, conjugate **19** was coated onto polystyrene microtiter plates and incubated with serum anti-MUC1 antibodies raised after immunization of mice with a structurally related glyco-^{6}Thr(TF)-MUC1-TTox vaccine.[32] Although this glycopeptide vaccine does not contain the exact TACA of the amphiphile **19**, the induced antibodies show relatively broad specificities and bind to various MUC1 peptide antigens. Hence, not unexpectedly, specific binding of these serum antibodies to the immobilized fluorous amphiphile **19** was also proven in a corresponding ELISA test (see Figure 1).

The binding of the commercially available monoclonal anti-MUC1 antibody SM3[33] to **19** was confirmed by using a quartz crystal microbalance[34] (QCM) and surface plasmon resonance (SPR) spectroscopy. The SM3 antibody was immobilized onto freshly cleaned SPR glass slides successively sputtered with 1.5 nm chromium and 55 nm gold films. Similarly, QCM measurements were performed by using commercially available quartz crystals coated with 100 nm gold layers. Because all cysteine residues are engaged in disulfide bridges inside the protein, binding of the antibody to the gold surface is assumed to take place through peripheral methionine residues, for example, Met18 and Met85.[35] Both residues are readily accessible and should not interfere with the antigen binding-site (see the Supporting Information). Thus, after addition of the antigenic amphiphile **19** to the QCM chamber, a typical Langmuir adsorption isotherm was obtained indicating a mass

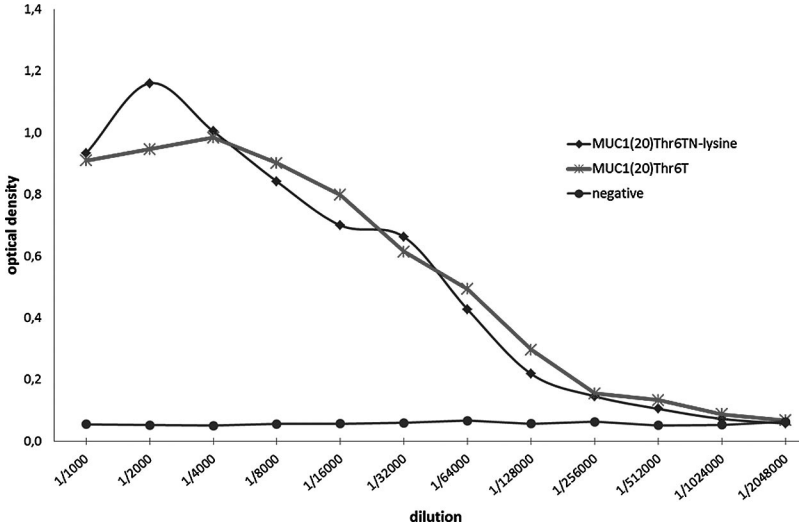


Figure 1. ELISA of the anti-serum induced by a structurally related glyco-^{6}Thr(TF)-MUC1-TTox vaccine demonstrates specific binding of amphiphile **19** to the mouse serum antibodies. Optical density at λ = 410 nm.

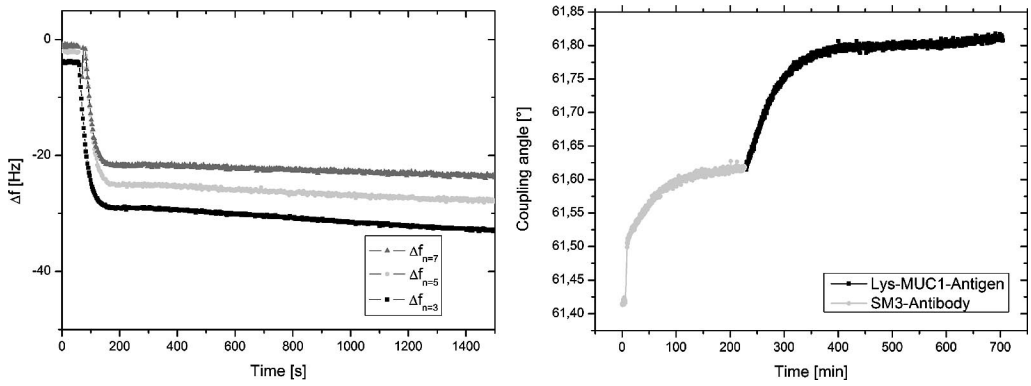


Figure 2. Specific binding of the MUC1 amphiphile **19** to the commercially available anti-MUC1 antibody SM3 shown by QCM and SPR spectroscopy. Left: QCM frequency shift vs. time for the binding of **19** to the preadsorbed antibody. Right: Coupling angle vs. time in the SPR experiment. Before the injection of the antigen, the SM3-coated surface was washed three times with PBS buffer.

increase upon antigen binding (Figure 2, left). Similarly, the corresponding SPR experiment reflects the recognition of the pre-adsorbed SM3 antibody through an increase of the layer thickness (Figure 2, right).

Conclusions

Fluorous double-tailed surfactants carrying one or two perfluoroalkyl chains and based on a lysine core have been assembled. Attachment of these amphiphiles to T_N antigen-Thr-Val dipeptides provided novel hydrophobized TACAs **17a** and **17b** that are of interest as building blocks for fluorous microarrays and/or multivalent cancer vaccines. With regard to the latter, the first hydrocarbon/fluorocarbon double-tailed MUC1 glycolipopeptide **19** was prepared by solid-phase glycopeptide chemistry and its specific recognition by different anti-MUC1 mouse antibodies was demonstrated through ELISA, SPR, and QCM measurements. Owing to the strong hydrophobizing influence of the fluorous tag, it is anticipated that such amphiphilic glycoconjugates might represent a novel potent type of self-aggregated multivalent cancer vaccine for future immunizations.

Experimental Section

General: Reagents were purchased in the highest available commercial quality and used as supplied, except where noted. DMF (amine-free, for peptide synthesis) and NMP were purchased from Roth. Fmoc-protected amino acids were purchased from Orpegen Pharma. For solid-phase syntheses, preloaded TentaGel S resin (Rapp polymere) was employed. Reactions were monitored by TLC using precoated silica gel 60 F_{254} aluminium plates (Merck KGaA, Darmstadt). Flash column chromatography was performed with silica gel (230–400 mesh) from Merck. RP-HPLC analyses were performed with a Jasco HPLC system with Phenomenex Luna-PFP(2) (250 × 4.6 mm, 5 μm) and Phenomenex Jupiter C18(2) (250 × 4.6 mm, 5 μm) columns at flow rates of 1 mL min^{-1}. Preparative HPLC separations were carried out with a Jasco HPLC system with Phenomenex Luna PFP(2) (250 × 30 mm, 5 μm) columns at a flow rate of 20 mL min^{-1}. Mixtures of MeCN/H_2O and MeOH/H_2O were used as solvents. If required, 0.1% TFA was added. ^{1}H, ^{13}C, ^{19}F, and 2D NMR spectra were recorded with a Bruker AC-300 or AM-400 spectrometer. The chemical shifts are reported in ppm relative to the signal of the deuteriated solvent. Multiplicities are given as s (singlet), br. s (broad singlet), d (doublet), t (triplet), and m (multiplet). The proton and carbon signals were assigned by additional COSY, HSQC, and HMBC experiments as noted. The signals of molecule fragments are denoted as follows: amino acids (Greek indices), octyl fragment ($_O$), decyl (F_{17}) fragment ($_D$) and oligo(ethylene glycol) fragment ($_{OEG}$). ESI-MS and ESI-HRMS were recorded with a Micromass Q TOF Ultima 3 spectrometer and MALDI-TOF MS were acquired with a Micromass Tofspec E spectrometer using 2,5-dihydroxybenzoic acid as the matrix. Optical rotations were measured at 546 and 578 nm with a Perkin–Elmer 241 polarimeter.

Boc-Lys(Z)-*O*-octyl (3a): Diisopropylethylamine (DIPEA; 3.45 mL, 20.87 mmol) was added to a solution of N$^{\alpha}$-Boc-N$^{\varepsilon}$-Cbz-L-lysine (**1**; 3.80 g, 9.98 mmol), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU; 3.50 g, 10.89 mmol), and 1-hydroxybenzotriazole (HOBt; 1.47 g, 10.89 mmol) in dry CH_2Cl_2 (30 mL). The reaction mixture was stirred for 60 min at room temperature under argon before octylamine (1.5 mL, 9.08 mmol) was added. The solution was heated at reflux for 6 h and then stirred for a further 13 h at room temperature. After dilution with CH_2Cl_2 (120 mL), the organic phase was washed with 1 M aq. HCl (2× 150 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, *c*Hex/EtOAc, 3:1→1:1) afforded **3a** as a colorless amorphous solid (4.42 g, 8.99 mmol, 99%). R_f = 0.10 (*c*Hex/EtOAc, 3:1). Analytical RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min): R_t = 8.7 min. $[\alpha]_D^{23}$ = –9.5 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ = 7.32–7.27 (m, 5 H, H_{ar}), 6.49 (br. s, 1 H, NH_{amide}), 5.35 (d, $J_{NH,K\alpha}$ = 7.6 Hz, 1 H, NH_{Boc}), 5.10 (br. s, 1 H, $NH_{carbamate}$), 5.06 (s, 2 H, CH_2-ar), 4.07–3.97 (m, 1 H, K^{α}), 3.20–3.13 (m, 4 H, K^{ε}, 1_O-H), 1.81–1.73 (m, 1 H, K_a^{β}), 1.63–1.54 (m, 1 H, K_b^{β}), 1.51–1.41 (m, 4 H, K^{δ}, 2_O-H), 1.40 [s, 9 H, C(CH$_3$)$_3$], 1.35–1.33 (m, 2 H, K^{γ}), 1.23 (br. s, 10 H, 3_O–7_O-H), 0.85 (t, 3 H, $J_{8O\text{-}H,7O\text{-}H}$ = 6.9 Hz, 8_O-H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, HSQC): δ = 172.1 (C=O$_{amide}$), 156.7, 155.9 (C=O$_{carbamate}$), 136.7 (C$_q$-ar), 128.5, 128.1 (5× CH$_{ar}$), 79.9 (C$_q$-*t*Bu), 66.6 (CH$_2$-ar), 54.4 (K^{α}), 40.5, 39.5 (K^{ε}, C-1_O), 32.1 (K^{β}), 31.8 (C-6_O), 29.6, 29.5 (K^{δ}, C-2_O), 29.3 (C-4_O, C-5_O), 28.4 [3× C(CH$_3$)$_3$], 26.9 (C-3_O), 22.7, 22.6 (K^{γ}, C-7_O) 14.2 (C-8_O) ppm. HRMS (ESI-TOF): calcd. for $C_{27}H_{45}N_3O_5Na$ [M + Na]$^+$ 514.3257; found 514.3234.

R$_F$-Lys(Z)-*O*-octyl (5a): A solution of **3a** (1.74 g, 3.54 mmol) and anisole (0.5 mL) in CH_2Cl_2 (30 mL) was treated with trifluoroacetic acid (TFA, 5.0 mL) and stirred for 15 h at room temperature. The

reaction mixture was concentrated in vacuo and co-evaporated with toluene (2 × 20 mL). The crude product was cooled in an ice bath, treated with aq. NH_4OH (50 mL), and extracted with Et_2O/CH_2Cl_2 (10:1, 3 × 30 mL). The combined organic phases were washed with H_2O (40 mL) and brine (40 mL), dried ($MgSO_4$), and concentrated in vacuo to yield the free amine H_2N-Lys(*Z*)-*O*-octyl as a colorless amorphous solid (1.37 g, 3.50 mmol, 99%). R_f = 0.37 (CH_2Cl_2/MeOH/NEt_3, 19:1:0.1). Analytical RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min): R_t = 3.4 min. $[\alpha]_D^{23}$ = –10.0 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ = 7.35–7.28 (m, 5 H, H_{ar}), 7.26 (br. s, 1 H, NH_{amide}), 5.08 (s, 2 H, CH_2-ar), 4.92–4.84 (m, 1 H, $NH_{carbamate}$), 3.31 (dd, $J_{K\alpha,Ka\beta}$ = 7.9, $J_{K\alpha,Kb\beta}$ = 4.3 Hz, 1 H, K^{α}), 3.25–3.14 (m, 4 H, K^{ε}, 1_O-H), 1.87 (br. s, 2 H, NH_2), 1.89–1.76 (m, 1 H, $K_a{}^{\beta}$), 1.57–1.45 (m, 5 H, $K_b{}^{\beta}$, K^{δ}, 2_O-H), 1.45–1.33 (m, 2 H, K^{γ}), 1.33–1.20 (m, 10 H, 3_O–7_O-H), 0.87 (t, $J_{8O\text{-}H,7O\text{-}H}$ = 6.9 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, HSQC): δ = 174.9 ($C{=}O_{amide}$), 156.6 ($C{=}O_{carbamate}$), 136.8 (C_q-ar), 128.6, 128.2 (5× CH_{ar}), 68.7 (CH_2-ar), 55.2 (K^{α}), 40.8, 39.2 (K^{ε}, C-1_O), 34.8 (K^{β}), 31.9 (C-6_O), 29.9, 29.8 (K^{δ}, C-2_O), 29.4, 29.3 (C-4_O, C-5_O), 27.1 (C-3_O), 23.0, 22.8 (K^{γ}, C-7_O) 14.2 (C-8_O) ppm. HRMS (ESI-TOF): calcd. for $C_{22}H_{37}N_3O_3H$ [M + H]$^+$ 392.2913; found 392.2925.

Acyl chloride **4** (2.06 g, 4.22 mmol) was prepared according to a known procedure[21] and added dropwise to H_2N-Lys(*Z*)-*O*-octyl (1.00 g, 2.55 mmol) and DIPEA (0.52 mL, 3.06 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C. After stirring at room temperature for 15 h, the solvent was removed in vacuo and the residue was purified by flash chromatography (SiO_2, *c*Hex/EtOAc, 2:1) to provide **5a** as a colorless amorphous solid (1.80 g, 2.14 mmol, 84%). R_f = 0.71 (*c*Hex/EtOAc, 1:1). Analytical RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min): R_t = 18.7 min. $[\alpha]_D^{23}$ = –1.3 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46 [d, $J_{NH,K\alpha}$ = 7.4 Hz, 1 H, $NH_{amide(F)}$], 7.39–7.29 (m, 5 H, H_{ar}), 6.13 (br. s, 1 H, NH_{amide}), 5.08 (s, 2 H, CH_2-ar), 4.88 (t, $J_{NH,K\varepsilon}$ = 5.9 Hz, 1 H, $NH_{carbamate}$), 4.40 (dd, $J_{K\alpha,Ka\beta}$ = 12.7, $J_{K\alpha,Kb\beta}$ = 7.2 Hz, 1 H, K^{α}), 3.31–3.11 (m, 4 H, K^{ε}, 1_O-H), 1.96–1.87 (m, 1 H, $K_a{}^{\beta}$), 1.81–1.71 (m, 1 H, $K_b{}^{\beta}$), 1.57–1.47 (m, 4 H, K^{δ}, 2_O-H), 1.38–1.30 (m, 2 H, K^{γ}), 1.30–1.26 (m, 10 H, 3_O–7_O-H), 0.87 (t, $J_{8O\text{-}H,7O\text{-}H}$ = 6.6 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 169.7 ($C{=}O_{amide}$), 156.9 ($C{=}O_{carbamate}$), 136.6 (C_q-ar), 128.7, 128.3, 128.1 (5× CH_{ar}), 66.9 (CH_2-ar), 53.7 (K^{α}), 40.2, 40.0 (K^{ε}, C-1_O), 32.0, 31.9 (K^{β}, C-6_O), 29.5 (K^{δ}, C-2_O), 29.3 (C-4_O, C-5_O), 27.0 (C-3_O), 22.7, 22.1 (K^{γ}, C-7_O) 14.2 (C-8_O) ppm. ^{19}F NMR (376.4 MHz, $CDCl_3$), δ = –81.12 (t, $J_{F,F}$ = 9.8 Hz, 3 F, CF_3), –119.92 (m, 2 F), –121.86 (br. s, 2 F), –122.22 (br. s, 4 F), –122.69 (br. s, 2 F), –123.05 (br. s, 2 F), –126.45 (m, 2 F, CF_2CF_3) ppm. HRMS (ESI-TOF): calcd. for $C_{31}H_{36}F_{17}N_3O_4H$ [M + H]$^+$ 838.2513; found 838.2523.

R_F-Lys-*O*-octyl (6a): Pd/C (10%, 200 mg) in a flask was activated through three cycles of a vacuum/H_2 flush. A solution of **5a** (1.50 g, 1.79 mmol) in EtOH (25 mL) was then added and the reaction mixture was stirred under H_2 for 12 h. The catalyst was removed by filtration through a Hyflo Super Cel® and washed with EtOH (4× 50 mL). The filtrate was concentrated in vacuo to give **6a** as a colorless viscous oil (1.25 g, 1.77 mmol, 99%). R_f = 0.06 (CH_2Cl_2/MeOH/NEt_3, 19:1:0.1). Analytical RP-HPLC (Luna-PFP, MeCN/H_2O, 20:80→100:0, 60 min): R_t = 25.3 min. $[\alpha]_D^{23}$ = –10.3 (*c* = 1.00, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.70 (t, $J_{NH,1OH}$ = 5.4 Hz, 1 H, NH_{amide}), 4.46 (t, $J_{K\alpha,Ka/b\beta}$ = 6.7 Hz, 1 H, K^{α}), 3.30–3.15 (m, 2 H, 1_O-H), 2.81–2.63 (m, 2 H, K^{ε}), 1.96–1.82 (m, 1 H, $K_a{}^{\beta}$), 1.79–1.69 (m, 1 H, $K_b{}^{\beta}$), 1.57–1.44 (m, 4 H, K^{δ}, 2_O-H), 1.44–1.35 (m, 2 H, K^{γ}), 1.30–1.23 (m, 10 H, 3_O–7_O-H), 0.86 (t, $J_{8O\text{-}H,7O\text{-}H}$ = 6.6 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 169.8 ($C{=}O_{amide}$), 157.6 [t, ^2J(C,F) = 26.5 Hz, $C{=}O_{RF}$], 53.8 (K^{α}), 41.4, 39.9 (K^{ε}, C-1_O), 32.3 (K^{δ}), 32.0, 31.9 (K^{β}, C-6_O), 29.5 (C-2_O), 29.3 (C-4_O, C-5_O), 27.0 (C-3_O), 22.7, 22.3 (K^{γ}, C-7_O) 14.2 (C-8_O) ppm. ^{19}F NMR (376.4 MHz, $CDCl_3$), δ = –81.20 (t, $J_{F,F}$ = 9.7 Hz, 3 F, CF_3), –119.20 (m, 2 F), –120.06 (br. s, 2 F), –121.91 (br. s, 2 F), –122.23 (br. s, 2 F), –122.76 (br. s, 2 F), –123.09 (br. s, 2 F), –126.50 (m, 2 F, CF_2CF_3) ppm. HRMS (ESI-TOF): calcd. for $C_{23}H_{30}F_{17}N_3O_2H$ [M + H]$^+$ 704.2145; found 704.2134.

R_F-Lys(OEG-Cbz)-*O*-octyl (8a): *N*-Methylmorpholine (NMM; 30 μL, 0.273 mmol) was added to a solution of CbzHN-OEG-CO_2H (**7b**;[24] 56 mg, 0.156 mmol), *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HCTU; 71 mg, 0.171 mmol), and HOBt (26 mg, 0.171 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred for 60 min at room temperature under argon, before **6a** (100 mg, 0.142 mmol), dissolved in dry CH_2Cl_2/DMF (1:1, 10 mL), was added. The reaction mixture was stirred at 40 °C for 15 h, diluted with CH_2Cl_2 (20 mL), washed with 1 M aq. HCl (2× 15 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min) afforded **8a** as a colorless lyophilisate (103 mg, 0.099 mmol, 70%). R_f = 0.66 (EtOAc). Analytical RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min): R_t = 18.0 min. $[\alpha]_D^{23}$ = –7.2 (*c* = 1.00, MeOH). ^{1}H NMR (400 MHz, CD_3OD, COSY): δ = 7.35–7.28 (m, 5 H, H_{ar}), 5.07 (s, 2 H, CH_2-ar), 4.37 (dd, $J_{K\alpha,Ka\beta}$ = 8.8, $J_{K\alpha,Kb\beta}$ = 6.1 Hz, 1 H, K^{α}), 3.69 (t, $J_{H3,H2}$ = 6.2 Hz, 2 H, 3_{OEG}-H), 3.61–3.56 (m, 8 H, 5,6,8,9_{OEG}-H), 3.53 (t, $J_{H11,H12}$ = 5.5 Hz, 2 H, 11_{OEG}-H), 3.30–3.28 (m, 2 H, 12_{OEG}-H), 3.24–3.10 (m, 4 H, 1_O-H, K^{ε}), 2.40 (t, $J_{H2,H3}$ = 6.2 Hz, 2 H, 2_{OEG}-H), 1.88–1.71 (m, 2 H, K^{β}), 1.56–1.48 (m, 4 H, K^{δ}, 2_O-H), 1.40–1.33 (m, 2 H, K^{γ}), 1.32–1.29 (m, 10 H, 3_O–7_O-H), 0.89 (t, $J_{8O\text{-}H,7O\text{-}H}$ = 6.9 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR (100.6 MHz, CD_3OD, HSQC): δ = 173.9, 172.6 (2× $C{=}O_{amide}$), 159.1 ($C{=}O_{amide(F)}$), 158.9 ($C{=}O_{carbamate}$), 138.4 (C_q-ar), 129.5, 129.0, 128.9 (5× CH_{ar}), 71.6, 71.5, 71.3 (C-5,6,8,9_{OEG}), 70.9 (C-11_{OEG}), 68.3 (C-3_{OEG}), 67.4 (CH_2-ar), 55.5 (K^{α}), 41.8 (C-12_{OEG}), 40.5, 40.0 (K^{ε}, C-1_O), 37.7 (C-2_{OEG}), 33.0 (C-6_O), 32.2 (K^{β}), 30.4, 30.3, 29.9 (K^{δ}, C-2_O, C-4_O, C-5_O), 27.9 (C-3_O), 24.2 (K^{γ}), 23.7 (C-7_O), 14.4 (C-8_O) ppm. ^{19}F NMR (376.4 MHz, CD_3OD): δ = –83.78 (t, $J_{F,F}$ = 10.1 Hz, 3 F, CF_3), –121.99 (t, $J_{F,F}$ = 12.8 Hz, 2 F), –123.87 (br. s, 2 F), –124.25 (br. s, 4 F), –124.89 (br. s, 2 F), –125.14 (br. s, 2 F), –128.64 to –128.73 (m, 2 F, CF_2CF_3) ppm. HRMS (ESI-TOF): calcd. for 1063.3490 $C_{40}H_{53}F_{17}N_4O_8Na$ [M + Na]$^+$; found 1063.3468.

R_F-Lys(OEG-NH_2)-*O*-octyl (9a): Pd/C (10%, 10 mg) in a flask was activated through three cycles of a vacuum/H_2 flush. A solution of **8a** (50 mg, 0.05 mmol) in *i*PrOH (10 mL) was then added and the reaction mixture was stirred under H_2 for 12 h. The catalyst was removed by filtration through Hyflo Super Cel® and washed with *i*PrOH (50 mL). The filtrate was concentrated in vacuo to give **9a** as a colorless amorphous solid (43 mg, 0.048 mmol, 99%). R_f = 0.05 (CH_2Cl_2/MeOH/NEt_3, 19:1:0.1). $[\alpha]_D^{23}$ = –8.1 (*c* = 1.00, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 4.37 (dd, $J_{K\alpha,Ka\beta}$ = 8.8, $J_{K\alpha,Kb\beta}$ = 6.0 Hz, 1 H, K^{α}), 3.72 (t, $J_{H3,H2}$ = 6.2 Hz, 2 H, 3_{OEG}-H), 3.64–3.59 (m, 8 H, 5,6,8,9_{OEG}-H), 3.56 (t, $J_{H11,H12}$ = 5.2 Hz, 2 H, 11_{OEG}-H), 3.22–3.12 (m, 4 H, 1_O-H, K^{ε}), 2.87 (t, $J_{H12,H11}$ = 5.2 Hz, 2 H, 12_{OEG}-H), 2.43 (t, $J_{H2,H3}$ = 6.2 Hz, 2 H, 2_{OEG}-H), 1.89–1.73 (m, 2 H, K^{β}), 1.55–1.48 (m, 4 H, K^{δ}, 2_O-H), 1.42–1.29 (m, 12 H, K^{γ}, 3_O–7_O-H), 0.90 (t, $J_{8O\text{-}H,7O\text{-}H}$ = 6.9 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR (75.5 MHz, CD_3OD): δ = 173.9, 172.6 (2× $C{=}O_{amide}$), 159.1 ($C{=}O_{amide(F)}$), 72.0 (C-11_{OEG}), 71.6, 71.5, 71.3 (C-5,6,8,9_{OEG}), 68.3 (C-3_{OEG}), 55.5 (K^{α}), 41.7 (C-12_{OEG}), 40.5, 40.0 (K^{ε}, C-1_O), 37.6 (C-2_{OEG}), 33.0 (C-6_O), 32.2 (K^{β}), 30.4, 30.3, 29.9 (K^{δ}, C-2_O, C-4_O, C-5_O), 27.9 (C-3_O), 24.2 (K^{γ}), 23.7 (C-7_O) 14.4 (C-8_O) ppm. ^{19}F NMR (376.4 MHz, CD_3OD), δ = –81.15 (t, $J_{F,F}$

= 10.0 Hz, 3 F, CF_3), –119.74 to –119.82 (m, 2 F), –121.90 (br. s, 2 F), –122.24 (br. s, 4 F), –122.62 (br. s, 2 F), –123.09 (br. s, 2 F), –126.49 (m, 2 F, CF_2CF_3) ppm. HRMS (ESI-TOF): calcd. for $C_{32}H_{47}F_{17}N_4O_{86}H$ $[M + H]^+$ 907.3302; found 907.3338.

Fmoc-Thr(αAc$_3$GalNAc)-OEG-O*t*Bu (12): The active ester was prepared by adding NMM (0.8 mL, 7.26 mmol) to a solution of Fmoc-Thr(αAc$_3$GalNAc)-OH (**10**; 2.44 g, 3.63 mmol), HCTU (1.64 g, 3.96 mmol), and HOBt (0.61 g, 3.96 mmol) in dry CH_2Cl_2 (35 mL). The reaction mixture was stirred at room temperature under argon for 60 min and H_2N-OEG-CO_2tBu (**11**; 0.916 g, 3.30 mmol), dissolved in dry CH_2Cl_2 (15 mL), was added. The reaction mixture was stirred at room temperature for 15 h, diluted with CH_2Cl_2 (50 mL), washed with 1 M aq. HCl (2× 50 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, CH_2Cl_2/MeOH, 39:1) afforded **12** as a yellow oil (2.96 g, 3.18 mmol, 96%). R_f = 0.39 (CH_2Cl_2/MeOH, 39:1). Analytical RP-HPLC (Jupiter C18, CH_3CN/H_2O, 50:50, 5 min, then 50:50→100:0, 25 min): R_t = 22.9 min. $[\alpha]_D^{23}$ = 36.5 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, $J_{H4,H3(Fmoc)}$ = $J_{H5,H6(Fmoc)}$ = 7.6 Hz, 2 H, 4-H-, 5-H-Fmoc), 7.63 (d, $J_{H1,H2(Fmoc)}$ = $J_{H8,H7(Fmoc)}$ = 7.4 Hz, 2 H, 1-H-, 8-H-Fmoc), 7.41–7.36 (m, 2 H, 3-H-, 6-H-Fmoc), 7.34–7.27 (m, 2 H, 2-H-, 7-H-Fmoc), 7.12 (t, $J_{NH,CH2}$ = 5.0 Hz, 1 H, NH_{OEG}), 6.62 (d, $J_{NH,H2}$ = 9.4 Hz, 1 H, NH_{GalNAc}), 5.90 (d, $J_{NH,T\alpha}$ = 9.1 Hz, 1 H, NH_T), 5.39 (d, $J_{H4,H5}$ = 2.2 Hz, 1 H, 4-H), 5.06 (dd, $J_{H3,H2}$ = 11.4, $J_{H3,H4}$ = 3.0 Hz, 1 H, 3-H), 4.91 (d, $J_{H1,H2}$ = 3.4 Hz, 1 H, 1-H), 4.60–4.53 (m, 1 H, 2-H), 4.46–4.38 (m, 2 H, CH_2-Fmoc), 4.35–4.16 (m, 4 H, 5-H, T^α, T^β, 9-H-Fmoc), 4.10–4.01 (m, 2 H, 6a/b-H), 3.67 (t, $J_{CH2,CH2}$ = 6.4 Hz, 2 H, 3_{OEG}-H), 3.60–3.57 (m, 8 H, 5,6,8,9_{OEG}-H), 3.55–3.51 (m, 2 H, 11_{OEG}-H), 3.47–3.42 (m, 2 H, 12_{OEG}-H), 2.47 (t, $J_{CH2,CH2}$ = 6.4 Hz, 2 H, 2_{OEG}-H), 2.15 [s, 3 H, CH_3(Ac)], 2.01 [s, 6 H, 2× CH_3(Ac)], 1.97 [s, 3 H, CH_3(Ac)], 1.41 [s, 9 H, $C(CH_3)_3$], 1.29 (d, $J_{T\gamma,T\beta}$ = 6.2 Hz, 3 H, T^γ) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 171.2, 171.1, 170.9, 170.5, 170.4, 170.3 (6× C=O), 156.8 ($C=O_{carbamate}$), 143.8 (C-1a-, C-8a-Fmoc), 141.4 (C-4a-, C-5a-Fmoc), 127.9 (C-3-, C-6-Fmoc), 127.2 (C-2-, C-7-Fmoc), 125.3 (C-1-, C-8-Fmoc), 120.1 (C-4-, C-5-Fmoc), 100.2 (C-1), 80.8 (C_q-*t*Bu), 78.1 (T^β), 70.5, 70.4, 70.3, 70.2 (C-5,6,8,9_{OEG}), 69.4 (C-11_{OEG}), 69.0 (C-3), 67.5, 67.4, 67.3 (C-4, C-5, CH_2-Fmoc), 66.9 (C-3_{OEG}), 62.2 (C-6), 58.6 (T^α), 47.6, 47.3 (C-2, C-9-Fmoc), 39.6 (C-12_{OEG}), 36.3 (C-2_{OEG}), 28.2 [$C(CH_3)_3$], 23.1, 20.9, 20.8, 20.7 [4× CH_3(Ac)], 18.0 (T^γ) ppm. HRMS (ESI-TOF): calcd. for $C_{46}H_{63}N_3O_{17}Na$ $[M + Na]^+$ 952.4055; found 952.4088.

Fmoc-Val-Thr(αAc$_3$GalNAc)-OEG-O*t*Bu (13): A solution of **12** (5.62 g, 6.04 mmol) in CH_2Cl_2 (80 mL) was treated with piperidine (4.19 mL, 42.3 mmol) and stirred at room temperature for 14 h. The solution was diluted with toluene (80 mL) and concentrated in vacuo. The residue was purified by flash chromatography [SiO_2, CH_2Cl_2/MeOH/NEt_3, 19:1:0.1. R_f = 0.36 (CH_2Cl_2/MeOH/NEt_3, 19:1:0.1)], dissolved in dry CH_2Cl_2 (25 mL), and added to the freshly prepared active ester of Fmoc-Val [obtained by treatment of Fmoc-Val (2.25 g, 6.64 mmol) with HCTU (3.00 g, 7.25 mmol), HOBt (1.11 g, 7.25 mmol), and NMM (1.46 mL, 13.29 mmol) in dry CH_2Cl_2 (100 mL)]. The resulting reaction mixture was stirred at room temperature for 15 h, diluted with CH_2Cl_2 (100 mL), washed with 1 M aq. HCl (2× 100 mL), dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography (SiO_2, CH_2Cl_2/MeOH, 39:1) yielded **13** as a brown amorphous solid (4.72 g, 4.59 mmol, 76%). R_f = 0.11 (CH_2Cl_2/MeOH, 39:1). Analytical RP-HPLC (Jupiter C18, CH_3CN/H_2O, 50:50, 5 min, then 50:50→100:0, 25 min): R_t = 23.5 min. $[\alpha]_D^{23}$ = 28.3 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, $J_{H4,H3(Fmoc)}$ = $J_{H5,H6(Fmoc)}$ = 7.5 Hz, 2 H, 4-H-, 5-H-Fmoc), 7.60 (d, $J_{H1,H2(Fmoc)}$ = $J_{H8,H7(Fmoc)}$ = 7.4 Hz, 2 H, 1-H-, 8-H-Fmoc), 7.41–7.36 (m, 2 H, 3-H-, 6-H-Fmoc), 7.32–7.27 (m, 2 H, 2-H-, 7-H-Fmoc), 7.08–7.00 (m, 2 H, NH_T, NH_{OEG}), 6.87 (d, $J_{NH,H2}$ = 8.7 Hz, 1 H, NH_{GalAc}), 5.61 (d, $J_{NH,V\alpha}$ = 7.9 Hz, 1 H, NH_V), 5.33 (d, $J_{H4,H5}$ = 3.0 Hz, 1 H, 4-H), 5.07 (dd, $J_{H3,H2}$ = 10.9, $J_{H3,H4}$ = 3.1 Hz, 1 H, 3-H), 4.93 (d, $J_{H1,H2}$ = 3.4 Hz, 1 H, 1-H), 4.57–4.49 (m, 2 H, 2-H, T^α), 4.46–4.35 (m, 2 H, CH_2-Fmoc), 4.29–4.20 (m, 3 H, 5-H, T^β, 9-H-Fmoc), 4.07–3.98 (m, 3 H, V^α, 6a/b-H), 3.70 (t, $J_{CH2,CH2}$ = 6.5 Hz, 2 H, 3_{OEG}-H), 3.60–3.56 (m, 8 H, 5,6,8,9_{OEG}-H), 3.53–3.49 (m, 2 H, 11_{OEG}-H), 3.47–3.38 (m, 2 H, 12_{OEG}-H), 2.49 (t, $J_{CH2,CH2}$ = 6.4 Hz, 2 H, 2_{OEG}-H), 2.25–2.16 (m, 1 H, V^β), 2.13 [s, 3 H, CH_3(Ac)], 2.01 [s, 3 H, CH_3(Ac)], 1.93 [s, 3 H, CH_3(Ac)], 1.91 [s, 3 H, CH_3(Ac)], 1.43 [s, 9 H, $C(CH_3)_3$], 1.24 (d, $J_{T\gamma,T\beta}$ = 6.2 Hz, 3 H, T^γ), 0.99 (d, $J_{V\gamma,V\beta}$ = 6.8 Hz, 3 H, V^γ), 0.97 (d, $J_{V\gamma,V\beta}$ = 6.8 Hz, 3 H, V^γ) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.0, 171.1, 170.8, 170.5, 169.9 (7× C=O), 156.4 ($C=O_{carbamate}$), 144.0 (C-1a-, C-8a-Fmoc), 141.4 (C-4a-, C-5a-Fmoc), 127.9 (C-3-, C-6-Fmoc), 127.2 (C-2-, C-7-Fmoc), 125.3, 125.2 (C-1-, C-8-Fmoc), 120.1 (C-4-, C-5-Fmoc), 99.9 (C-1), 80.8 (C_q-*t*Bu), 77.3 (T^β), 70.6, 70.5, 70.4, 70.3 (C-5,6,8,9_{OEG}), 69.4 (C-11_{OEG}), 68.9 (C-3), 67.5, 67.3, 67.2 (C-4, C-5, CH_2-Fmoc), 67.0 (C-3_{OEG}), 62.2 (C-6), 61.1 (V^α), 56.7 (T^α), 47.8, 47.3 (C-2, C-9-Fmoc), 39.6 (C-12_{OEG}), 36.3 (2_{OEG}-H), 30.7 (V^β), 28.2 [$C(CH_3)_3$], 23.1, 20.9, 20.7 [4× CH_3(Ac)], 19.5 (2× V^γ), 18.1 (T^γ) ppm. HRMS (ESI-TOF): calcd. for $C_{51}H_{72}N_4O_{18}Na$ $[M + Na]^+$ 1051.4739; found 1051.4749.

Ac-Val-Thr(αAc$_3$GalNAc)-OEG-O*t*Bu (14): Piperidine (1.35 mL, 13.6 mmol) was added to a solution of **13** (2.00 g, 1.94 mmol) in CH_2Cl_2 (30 mL) and the solution was stirred at room temperature for 5 h, concentrated in vacuo, and co-evaporated with toluene (3× 20 mL). The crude product was dissolved in dry CH_2Cl_2 (30 mL) and successively treated with HOBt (89 mg, 0.583 mmol), DIPEA (0.64 mL, 3.89 mmol), and Ac_2O (1.29 mL, 13.60 mmol). After stirring at room temperature for 15 h, the organic phase was washed with 1 M aq. HCl (2× 15 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH, 39:1→19:1) to give **14** as an yellow amorphous solid (1.10 g, 1.30 mmol, 67%). R_f = 0.33 (CH_2Cl_2/MeOH, 19:1). Analytical RP-HPLC (Jupiter C18, CH_3CN/H_2O, 50:50, 5 min, then 50:50→100:0, 25 min): R_t = 12.4 min. $[\alpha]_D^{23}$ = 38.1 (*c* = 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ = 7.27 (d, $J_{NH,T\alpha}$ = 8.0 Hz, 1 H, NH_T), 7.13 (t, $J_{NH,CH2}$ = 5.0 Hz, 1 H, NH_{OEG}), 6.87 (d, $J_{NH,H2}$ = 9.2 Hz, 1 H, NH_{GalNAc}), 6.60 (d, $J_{NH,V\alpha}$ = 7.9 Hz, 1 H, NH_V), 5.35 (d, $J_{H4,H5}$ = 2.3 Hz, 1 H, 4-H), 5.08 (dd, $J_{H3,H2}$ = 11.4, $J_{H3,H4}$ = 3.2 Hz, 1 H, 3-H), 4.94 (d, $J_{H1,H2}$ = 3.6 Hz, 1 H, 1-H), 4.55–4.48 (m, 2 H, 2-H {4.53}, T^α {4.50}), 4.31–4.25 (m, 2 H, 5-H {4.30}, T^β {4.26}), 4.22 (pseudo-t, $J_{V\alpha,NH}$ = $J_{V\alpha,V\beta}$ = 7.7 Hz, 1 H, V^α), 4.09–4.00 (m, 2 H, 6a/b-H), 3.69 (t, $J_{CH2,CH2}$ = 6.4 Hz, 2 H, 3_{OEG}-H), 3.60–3.56 (m, 8 H, 5,6,8,9_{OEG}-H), 3.55–3.47 (m, 2 H, 11_{OEG}-H), 3.46–3.38 (m, 2 H, 12_{OEG}-H), 2.49 (t, $J_{CH2,CH2}$ = 6.4 Hz, 2 H, 2_{OEG}-H), 2.20–2.14 (m, 1 H, V^β), 2.13 [s, 3 H, CH_3(Ac)], 2.03 [s, 3 H, CH_3(Ac)], 2.00 [s, 6 H, 2× CH_3(Ac)], 1.95 [s, 3 H, CH_3(Ac)], 1.42 [s, 9 H, $C(CH_3)_3$], 1.22 (d, $J_{T\gamma,T\beta}$ = 6.4 Hz, 3 H, T^γ), 0.97 (d, $J_{V\gamma,V\beta}$ = 6.4 Hz, 3 H, V^γ), 0.95 (d, $J_{V\gamma,V\beta}$ = 6.4 Hz, 3 H, V^γ) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, HSQC): δ = 174.2, 172.1, 171.3, 171.2, 170.8, 170.7, 170.5, 169.9 (8× C=O), 99.6 (C-1), 80.9 (C_q-*t*Bu), 76.6 (T^β), 70.5, 70.4, 70.3, (C-5,6,8,9_{OEG}), 69.5 (C-11_{OEG}), 68.7 (C-3), 67.5 (C-4), 67.2 (C-5), 66.4 (C-3_{OEG}), 62.3 (C-6), 59.6 (V^α), 56.9 (T^α), 47.8 (C-2), 39.6 (C-12_{OEG}), 36.3 (C-2_{OEG}), 30.1 (V^β), 28.2 [$C(\mathbf{C}H_3)_3$], 23.1, 20.9, 20.8 [5× CH_3(Ac)], 19.5 (V^γ), 18.4 (V^γ), 18.2 (T^γ) ppm. HRMS (ESI-TOF): calcd. for $C_{38}H_{64}N_4O_{17}Na$ $[M + Na]^+$ 871.4164; found 871.4149.

Ac-Val-Thr(αAc$_3$GalNAc)-OEG-OH (15): TFA (10.0 mL) was added to a solution of **14** (1.00 g, 1.18 mmol) and anisole (1.0 mL)

in CH_2Cl_2 (30 mL) and the solution was stirred at room temperature for 15 h. The solvent was removed in vacuo and the residue was co-evaporated with toluene (2 × 20 mL). Purification by flash chromatography (SiO_2, CH_2Cl_2/MeOH/HOAc, 19:1:0.1) yielded **15** as a brown oil (889 mg, 1.12 mmol, 95%). R_f = 0.07 (CH_2Cl_2/MeOH, 19:1). Analytical RP-HPLC (Jupiter C18, CH_3CN/H_2O, 50:50, 5 min, then 50:50→100:0, 25 min): R_t = 4.5 min. $[\alpha]_D^{23}$ = 36.0 (c = 1.00, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ = 7.72–7.68 (m, 1 H, NH_{OEG}), 7.54 (d, $J_{NH,T\alpha}$ = 9.0 Hz, 1 H, NH_T), 7.07 (d, $J_{NH,V\alpha}$ = 9.1 Hz, 1 H, NH_{GalNAc}), 6.56 (d, $J_{NH,H2}$ = 8.3 Hz, 1 H, NH_V), 5.35 (d, $J_{H4,H5}$ = 2.5 Hz, 1 H, 4-H), 5.10 (dd, $J_{H3,H2}$ = 11.4, $J_{H3,H4}$ = 3.2 Hz, 1 H, 3-H), 4.92 (d, $J_{H1,H2}$ = 3.5 Hz, 1 H, 1-H), 4.66 (dd, $J_{T\alpha,NH}$ = 8.9, $J_{T\alpha,T\beta}$ = 1.9 Hz, 1 H, T^α), 4.57–4.50 (m, 1 H, 2-H), 4.41 (pt, $J_{V\alpha,NH}$ = $J_{V\alpha,V\gamma}$ = 8.0 Hz, 1 H, V^α), 4.31–4.27 (m, 2 H, T^β {4.31}, 5-H {4.29}), 4.06–4.04 (m, 2 H, 6a/b-H), 3.76–3.71 (m, 2 H, 3_{OEG}-H), 3.62–3.56 (m, 8 H, 5,6,8,9_{OEG}-H), 3.50–3.43 (m, 4 H, 11_{OEG}-H {3.49}, 12_{OEG}-H {3.44}), 2.62 (t, $J_{CH2,CH2}$ = 5.4 Hz, 2 H, 2_{OEG}-H), 2.14 [s, 3 H, CH_3(Ac)], 2.12–2.08 (m, 1 H, V^β), 2.05 [s, 3 H, CH_3(Ac)], 2.03 [s, 3 H, CH_3(Ac)], 2.01 [s, 3 H, CH_3(Ac)], 1.95 [s, 3 H, CH_3(Ac)], 1.26 (d, $J_{T\gamma,T\beta}$ = 6.4 Hz, 3 H, T^γ), 0.97 (d, $J_{V\gamma,V\beta}$ = 6.8 Hz, 3 H, V^γ), 0.95 (d, $J_{V\gamma,V\beta}$ = 6.8 Hz, 3 H, V^γ) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$, HSQC): δ = 175.1, 172.5, 171.4, 171.3, 170.8, 170.7, 170.6, 169.9 (8 × C=O), 99.8 (C-1), 77.0 (T^β), 70.7, 70.6, 70.2 (C-5,6,8,9_{OEG}), 69.7 (C-11_{OEG}), 68.8 (C-3), 67.7 (C-4), 67.2 (C-5), 66.7 (C-3_{OEG}), 62.3 (C-6), 59.2 (V^α), 57.4 (T^α), 47.8 (C-2), 39.7 (C-12_{OEG}), 35.0 (C-2_{OEG}), 30.8 (V^β), 23.2, 23.1, 20.9, 20.8 [5 × CH_3(Ac)], 19.5 (V^γ), 18.4 (V^γ), 18.2 (T^γ) ppm. HRMS (ESI-TOF): calcd. for $C_{34}H_{56}N_4O_{17}Na$ $[M + Na]^+$ 815.3538; found 815.3541.

Ac-Val-Thr(αAc$_3$GalNAc)-OEG-R$_F$-Lys-*O*-octyl (16a): A solution of **15** (100 mg, 0.126 mmol), HCTU (57 mg, 0.138 mmol), and HOBt (221 mg, 0.138 mmol) in dry CH_2Cl_2 (5 mL) was treated with NMM (30 μL, 0.273 mmol) and stirred for 60 min at room temperature under argon. Compound **6a** (81 mg, 0.115 mmol), dissolved in dry CH_2Cl_2/DMF (1:1, 10 mL), was added to this solution and stirring was continued at 40 °C for 15 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with 1 M aq. HCl (2 × 15 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification by RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min) afforded **16a** as a colorless lyophilisate (64 mg, 0.043 mmol, 38%). Analytical RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min): R_t = 16.9 min. $[\alpha]_D^{23}$ = 10.5 (c = 1.00, MeOH). ^{1}H NMR [400 MHz, $(CD_3)_2O$, COSY]: δ = 8.71 (d, $J_{NH,CH}$ = 7.8 Hz, 1 H, NH_K), 7.74–7.69 (m, 2 H, NH_{OEG}, NH_T), 7.61–7.56 (m, 1 H, NH_V), 7.52 (t, $J_{NH,CH2}$ = 5.0 Hz, 1 H, NH_O), 7.34 (t, $J_{NH,CH2}$ = 5.3 Hz, 1 H, $NH_{K(\varepsilon)}$), 7.00 (d, $J_{NH,H2}$ = 9.4 Hz, 1 H, NH_{GalNAc}), 5.36 (d, $J_{H4,H5}$ = 2.2 Hz, 1 H, 4-H), 5.04 (dd, $J_{H3,H2}$ = 11.5, $J_{H3,H4}$ = 3.2 Hz, 1 H, 3-H), 4.99 (d, $J_{H1,H2}$ = 3.6 Hz, 1 H, 1-H), 4.60 (dd, $J_{T\alpha,NH}$ = 9.0, $J_{T\alpha,T\beta}$ = 2.1 Hz, 1 H, T^α), 4.50–4.29 (m, 5 H, K^α {4.45}, 2-H {4.43}, T^β {4.39}, 5-H {4.36}, V^α {4.30}), 4.13–4.03 (m, 2 H, 6a/b-H), 3.73 (t, $J_{CH2,CH2}$ = 6.1 Hz, 2 H, 3_{OEG}-H), 3.60–3.57 (m, 8 H, 5,6,8,9_{OEG}-H), 3.55–3.49 (m, 3 H, 11_{OEG}-H, 12_{OEG}-H), 3.30–3.13 (m, 5 H, 1_O-H, K^ε, 12_{OEG}-H), 2.41–2.39 (m, 2 H, 2_{OEG}-H), 2.14–2.09 (m, 1 H, V^β), 2.11 [s, 3 H, CH_3(NHAc)], 1.98 [s, 6 H, CH_3(NHAc), CH_3(Ac)], 1.90–1.80 (m, 2 H, K^β), 1.90, 1.85 (s, 6 H, OAc), 1.53–1.41 (m, 4 H, K^δ, 2_O-H), 1.45–1.36 (m, 2 H, K^γ), 1.32–1.25 (m, 13 H, 3_O–7_O-H, T^γ), 0.96 (d, $J_{V\gamma,V\beta}$ = 6.9 Hz, 3 H, V^γ), 0.94 (d, $J_{V\gamma,V\beta}$ = 6.9 Hz, 3 H, V^γ), 0.87 (t, $J_{CH3,CH2}$ = 6.0 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR [100.6 MHz, $(CD_3)_2O$, HSQC]: δ = 172.8, 171.7, 171.1, 170.9, 170.8, 170.7, 170.6, 170.3 (9 × C=O), 157.7 (t, ^2J = 26.1 Hz, $C{=}O_{amide(F)}$), 100.3 (C-1), 77.6 (T^β), 71.2, 71.1, 70.9 (C-5,6,8,9_{OEG}), 70.1 (C-11_{OEG}), 69.5 (C-3), 68.4 (C-4), 68.0 (C-3_{OEG}), 67.9 (C-5), 62.9 (C-6), 59.3 (V^α), 57.5 (T^α), 54.8 (K^α), 48.2 (C-2), 40.1 (C-12_{OEG}), 40.0 (C-1_O), 39.2 (K^ε), 37.5 (C-2_{OEG}), 32.5 (C-6_O), 32.2 (K^β), 31.1 (V^β), 27.9 (C-3_O), 23.4 (K^γ), 23.3 (C-7_O), 22.9, 22.7 [2 × CH_3(NHAc)], 20.7, 20.6, 20.5 [3 × CH_3(Ac)], 19.9 (V^γ), 19.0 (T^γ), 18.6 (V^γ), 14.3 (C-8_O) ppm. ^{19}F NMR [376.4 MHz, $(CD_3)_2O$]: δ = –81.96 (t, $J_{F,F}$ = 9.8 Hz, 3 F, CF_3), –119.90 to –120.20 (m, 2 F), –122.35 (br. s, 2 F), –122.70 (br. s, 4 F), –123.22 (br. s, 2 F), –123.57 (br. s, 2 F), –126.99 to –127.09 (m, 2 F, CF_2CF_3) ppm. HRMS (ESI-TOF): calcd. for $C_{57}H_{84}F_{17}N_7O_{18}Na$ $[M + Na]^+$ 1500.5499; found 1500.5535.

Ac-Val-Thr(αGalNAc)-OEG-R$_F$-Lys-*O*-octyl (17a): NaOMe (2.5% in MeOH) was added dropwise to a solution of **16a** (64 mg, 0.043 mmol) in MeOH (HPLC grade, 20 mL) until a pH of 9.5 was reached. After stirring for 18 h at room temperature, the solution was neutralized with HOAc and concentrated in vacuo. Purification by RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min) afforded **17a** as a colorless lyophilisate (23 mg, 0.017 mmol, 39%). Analytical RP-HPLC (Luna-PFP, MeOH/H_2O, 80:20→100:0, 20 min): R_t = 14.4 min. $[\alpha]_D^{23}$ = 24.2 (c = 1.00, MeOH). ^{1}H NMR (400 MHz, CD_3OD, COSY): δ = 4.88 (s, 10 H, all OH and NH), 4.84 (d, $J_{H1,H2}$ = 3.8 Hz, 1 H, 1-H), 4.52 (d, $J_{T\alpha,T\beta}$ = 2.3 Hz, 1 H, T^α), 4.37 (dd, $J_{K\alpha,K\alpha\beta}$ = 8.8, $J_{K\alpha,Kb\beta}$ = 6.1 Hz, 1 H, K^α), 4.26–4.22 (m, 2 H, V^α {4.24}, 2-H {4.24}), 4.20–4.14 (m, 1 H, T^β), 3.91–3.89 (m, 2 H, 5-H {3.90}, 4-H {3.90}), 3.79 (dd, $J_{H3,H2}$ = 10.9, $J_{H3,H4}$ = 3.0 Hz, 1 H, 3-H), 3.74–3.70 (m, 4 H, 3_{OEG}-H {3.73}, 6a/b-H {3.71}), 3.64–3.57 (m, 8 H, 5,6,8,9_{OEG}-H), 3.55–3.51 (m, 2 H, 11_{OEG}-H), 3.31 (m, 2 H, 12_{OEG}-H), 3.20–3.13 (m, 4 H, 1_O-H, K^ε), 2.43 (t, $J_{CH2,CH2}$ = 6.3 Hz, 2 H, 2_{OEG}-H), 2.08 [s, 3 H, CH_3(NHAc)], 2.12–2.07 (m, 1 H, V^β), 2.01 [s, 3 H, CH_3(NHAc)], 1.87–1.72 (m, 2 H, K^β), 1.57–1.41 (m, 4 H, 2_O-H, K^δ), 1.38–1.35 (m, 2 H, K^γ), 1.30 (m, 10 H, 3_O–7_O-H), 1.26 (d, $J_{T\gamma,T\beta}$ = 6.4 Hz, 3 H, T^γ), 1.00 (d, $J_{V\gamma,V\beta}$ = 6.8 Hz, 3 H, V^γ), 0.99 (d, $J_{V\gamma,V\beta}$ = 6.8 Hz, 3 H, V^γ), 0.90 (t, $J_{CH3,CH2}$ = 6.9 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR (100.6 MHz, CD_3OD, HSQC): δ = 174.3, 174.1, 173.9, 173.5, 172.6, 171.9 (6 × C=O), 100.9 (C-1), 78.2 (T^β), 72.9 (C-5 or C-4), 71.5, 71.4, 71.3, 71.2 (C-5,6,8,9_{OEG}), 70.4 (C-3, C-11_{OEG}), 70.3 (C-4 or C-5), 68.3 (C-3_{OEG}), 62.7 (C-6), 60.7 (V^α), 58.0 (T^α), 55.5 (K^α), 51.5 (C-2), 40.5, 40.5, 40.0 (C-12_{OEG}, C-1_O, K^ε), 37.7 (C-2_{OEG}), 33.0 (C-6_O), 32.2 (K^β), 31.3 (V^β), 30.4, 30.4, 30.3, 29.9 (C-2_O, K^δ, C-4_O, C-5_O), 27.4 (C-3_O), 24.2 (K^γ), 23.7 (C-7_O), 23.2, 22.4 [2 × CH_3(NHAc)], 19.9 (V^γ), 19.3 (T^γ), 19.0 (V^γ), 14.4 (C-8_O) ppm. ^{19}F NMR (376.4 MHz, CD_3OD): δ = –82.69 (t, $J_{F,F}$ = 9.8 Hz, 3 F, CF_3), –120.88 (t, $J_{F,F}$ = 12.6 Hz, 2 F), –122.78 (br. s, 2 F), –123.18 (br. s, 4 F), –123.80 (br. s, 2 F), –124.06 (br. s, 2 F), –127.55 to –127.63 (m, 2 F, CF_2CF_3) ppm. HRMS (ESI-TOF): calcd. for $C_{51}H_{78}F_{17}N_7O_{15}Na$ $[M + Na]^+$ 1374.55182; found 1374.5153.

Glycolipopeptide 19: Starting from Fmoc-Pro-Trt-TentaGel S resin (455 mg, 0.22 mmol g^{-1}, 0.10 mmol), the solid phase glycopeptide synthesis was conducted on a Perkin–Elmer ABI 433A peptide synthesizer according to the Fastmoc protocol. In every coupling cycle, the N-terminal Fmoc group was removed by treatment of the resin with a solution of piperidine (20%) in NMP for at least 3 × 2.5 min. The coupling of the amino acids (1 mmol or 10 equiv. based on the loaded resin) was carried out with *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 1 mmol), HOBt (1 mmol), and DIPEA (2 mmol) in DMF under 20–30 min of vortex. After every coupling step, unreacted amino groups were capped by treatment with a mixture of Ac_2O (0.5 M), DIPEA (0.125 M), and HOBt (0.015 M) in NMP (10 min vortex). Coupling of the glycosylated threonine building block **10** (168 mg, 0.25 mmol) was performed using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU; 105 mg, 0.275 mmol), *N*-hydroxy-7-azabenzotriazole (HOAt, 36 mg, 0.275 mmol), and NMM (45 μL, 0.44 mmol) in *N*-methyl-

pyrrolidone (NMP, 2.0 mL) for activation (8 h vortex). After attachment of the remaining five amino acids and the spacer **7a** (1 mmol, 10 equiv. based on the loaded resin) using the standard coupling procedure, again, the N-terminal Fmoc group was removed by piperidine (20%) in NMP. The coupling of amphiphile **18** (164 mg, 0.20 mmol) required the use of HATU (84 mg, 0.22 mmol), HOAt (30 mg, 0.22 mmol), and NMM (45 μL, 0.44 mmol) in NMP (2.0 mL) under extended coupling times of 8 h. Detachment from the resin and simultaneous removal of all side-chain protecting groups was performed in a Merrifield glass reactor by shaking with TFA (10 mL), triisopropylsilane (TIS, 1.0 mL), and H_2O (1.0 mL) for 3 h. The solution was filtered and the resin was washed with TFA (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL). The combined TFA solutions were concentrated in vacuo and lyophilized. The crude product was dissolved in MeOH (HPLC-grade, 40 mL) and treated dropwise with NaOMe (2.5% in MeOH) until a pH of 9.5 was reached. After stirring at room temperature for 18 h, the solution was neutralized with HOAc and concentrated in vacuo. The crude product was precipitated twice from cold Et_2O (40 mL) to furnish **19** (110 mg, 0.036 mmol, 36%) as a colorless foam after lyophilization. Analytical RP-HPLC (Luna-PFP, MeOH/H_2O, 70:30→100:0, 20 min): R_t = 15.4 min. $[\alpha]_D^{23}$ = –52.7 (c = 1.00, H_2O + TFA). ^{1}H NMR (400 MHz, CD_3OD + [D_1]TFA, COSY): δ = 8.79 (d, $J_{H\varepsilon,H\delta}$ = 1.3 Hz, 1 H, H^{ε}), 7.43 (s, 1 H, H^{δ}), 5.02 (d, $J_{H1,H2}$ = 3.6 Hz, 1 H, 1-H), 4.71–4.23 (m, 22 H, R^{α} {4.69}, H^{α} {4.67}, D^{α} {4.62}, $A_4{}^{\alpha}$ {4.61}, $A_{(2,3)}{}^{\alpha}$ {4.57}, $S_1{}^{\alpha}$ {4.52}, $S_2{}^{\alpha}$ {4.47}, K^{α} {4.40}, $T_{Tn}{}^{\alpha}$, V^{α} {4.39}, $P_{(1\text{-}5)}{}^{\alpha}$ {4.36}, $T_{(1,2)}{}^{\alpha}$ {4.35, 4.32}, $A_1{}^{\alpha}$ {4.31}, $T_{Tn}{}^{\beta}$ {4.30}, $T_2{}^{\beta}$ {4.29}, $T_1{}^{\beta}$ {4.25}), 4.18 (dd, $J_{H2,H3}$ = 3.6, $J_{H2,H1}$ = 3.6 Hz, 1 H, 2-H), 4.09–4.04 (m, 5 H, $G_1{}^{\alpha a}$ {4.08}, 2 × -C_qCH_2O- {4.05}), 3.98–3.56 (m, 34 H, $G_1{}^{\alpha b}$ {3.96}, 5-H {3.94}, $S_2{}^{\beta}$ {3.89}, 3-H {3.83}, 6a/b-H {3.81}, $P_{(1\text{-}2)}{}^{\delta}$ {3.80}, $G_2{}^{\alpha}$ {3.79}, $S_1{}^{\beta}$ {3.77}, 4-H {3.69}, 3_{OEG}-H {3.68}, $P_{(3\text{-}5)}{}^{\delta}$ {3.65}, 5,6,8,9_{OEG}-H {3.62}, 11_{OEG}-H {3.58}), 3.45 (t, $J_{CH2,CH2}$ = 5.3 Hz, 2 H, 12_{OEG}-H), 3.39 (d, $J_{H\beta,H\alpha}$ = 5.1 Hz, 1 H, $H^{\beta a}$), 3.28–3.14 (m, 8 H, K^{ε} {3.24}, $H^{\beta b}$ {3.21}, R^{δ} {3.20}, 1_O-H {3.19}), 2.95–2.92 (m, 3 H, $D^{\beta a}$ {2.94}, 2_{OEG}-H {2.93}), 2.72–2.68 (m, 1 H, $D^{\beta b}$), 2.29–1.83 (m, 25 H, $P_{(1\text{-}5)}{}^{\beta a}$ {2.25}, V^{β} (2.13), CH_3(NHAc) {2.08}, $P_{(1\text{-}5)}{}^{\gamma}$ {2.03, 1.99}, $P_{(1\text{-}5)}{}^{\beta b}$ {1.97}, $R^{\beta a}$ {1.90}), 1.82–1.77 (m, 2 H, K^{β}), 1.74–1.68 (m, 3 H, $R^{\beta b}$ {1.72}, R^{γ} {1.68}), 1.61–1.55 (m, 2 H, K^{δ}), 1.53–1.45 (m, 2 H, 2_O-H), 1.42 (d, $J_{A2/3\beta,A2/3\alpha}$ = 6.9 Hz, 6 H, $A_{(2,3)}{}^{\beta}$), 1.38–1.26 (m, 21 H, $A_{(1,4)}{}^{\beta}$ {1.37}, K^{γ} {1.35}, 3_O–7_O-H {1.29}, $T_{Tn}{}^{\gamma}$ {1.27}), 1.20 (d, $J_{T1/2\gamma,T1/2\beta}$ = 6.4 Hz, 6 H, $T_{(1,2)}{}^{\gamma}$), 1.00 (d, $J_{V\gamma a,V\beta}$ = 6.5 Hz, 3 H, $V^{\gamma a}$), 0.98 (d, $J_{V\gamma b,V\beta}$ = 6.6 Hz, 3 H, $V^{\gamma b}$), 0.89 (t, $J_{CH3,CH2}$ = 6.9 Hz, 3 H, 8_O-H) ppm. ^{13}C NMR (100.6 MHz, CD_3OD + [D_1]TFA, HSQC, HMBC): δ = 174.1, 174.0, 173.9, 173.2, 173.0, 172.9, 172.7, 172.4, 172.3, 172.2, 172.1, 171.7, 171.6, 171.4, 171.2, 171.0, 170.7, 170.6, 170.5, 170.2 (26 × C=O), 157.1 (C=NH), 133.7 (H^{ε}), 129.4 (H^{γ}), 117.5 (H^{δ}), 99.1 (C-1), 76.4 ($T_{Tn}{}^{\beta}$), 71.4 (C-5), 70.0 (2 × -C_qCH_2O-), 69.9 (C-3), 69.8 (C-4), 69.6 (C-5,6,8,9_{OEG}), 69.1 (C-11_{OEG}), 69.0 (C-3_{OEG}), 66.9, 66.4 ($T_{(1,2)}{}^{\beta}$), 61.9 ($S_1{}^{\beta}$), 61.7 (C-6), 61.6 ($S_2{}^{\beta}$), 61.6, 61.5, 61.4, 61.1, 60.9 ($P_{(1\text{-}5)}{}^{\alpha}$), 59.4 ($T_2{}^{\alpha}$), 59.1 ($V^{\alpha}$), 59.0 ($T_1{}^{\alpha}$), 58.8 ($T_{Tn}{}^{\alpha}$), 55.8 ($S_2{}^{\alpha}$), 55.2 ($S_1{}^{\alpha}$), 54.2 ($K^{\alpha}$), 52.4 ($R^{\alpha}$), 50.8 ($H^{\alpha}$), 50.5 ($D^{\alpha}$), 50.0 (C-2), 42.4 ($G_2{}^{\alpha}$), 42.4 ($G_1{}^{\alpha}$), 40.7 ($K^{\varepsilon}$), 39.1 (C-$1_O$), 38.4 (C-$12_{OEG}$), 34.4 (C-$2_{OEG}$), 34.3 ($D^{\beta}$), 31.5 (C-$6_O$), 30.8 ($K^{\beta}$), 30.0 ($V^{\beta}$), 29.4, 29.2 (2 × $P_{(1\text{-}5)}{}^{\beta}$), 29.1, 29.0 (C-$4_O$, C-$5_O$), 28.9, 28.8 (3 × $P_{(1\text{-}5)}{}^{\beta}$), 28.7 (C-$2_O$), 28.5 ($K^{\delta}$), 27.8 ($R^{\beta}$), 26.5 (C-$3_O$), 26.4 ($H^{\beta}$), 24.9, 24.7, 24.6, 24.5, 24.4 ($P_{(1\text{-}5)}{}^{\gamma}$), 24.3 ($R^{\gamma}$), 22.8 ($K^{\gamma}$), 22.3 (C-$7_O$), 21.8 [$CH_3$(NHAc)], 18.8 ($T_{(1,2)}{}^{\gamma}$), 18.4 ($V^{\gamma a}$), 17.9 ($T_{Tn}{}^{\gamma}$), 17.6 ($V^{\gamma b}$), 15.5, 15.3, 15.1 ($A_{(1\text{-}4)}{}^{\beta}$), 12.9 (C-$8_O$) ppm. ^{19}F NMR (376.4 MHz, CD_3OD + [D_1]TFA), δ = –84.26 (t, $J_{F,F}$ = 10.2 Hz, 3 F, CF_3), –122.43 (t, $J_{F,F}$ = 13.0 Hz, 2 F), –124.32 (br. s, 2 F), –124.71 (br. s, 4 F), –125.32 (br. s, 2 F), –125.60 (br. s, 2 F), –129.11 to –127.22 (m, 2 F, CF_2CF_3) ppm. MS (MALDI-TOF; DHB, positive): calcd. for $C_{124}H_{189}F_{17}N_{30}O_{42}H$ $[M + H]^+$ 3094.34; found 3094.08.

Surface Plasmon Resonance Spectroscopy: SPR was conducted on a customized Kretschmann-type spectrometer. For this purpose, glass slides were cleaned with piranha solution and water prior to sequentially sputtering with 1.5 nm chromium and 55 nm gold films. The slides were rinsed with doubly deionized H_2O and PBS buffer (pH 7.5), attached to the SPR spectrometer, and immersed in PBS buffer. The kinetic measurements were started with injection of the SM3 antibody or amphiphilic MUC1 antigen into the measurement chamber, both at a concentration of 2 μg mL^{-1} in PBS. All measurements were repeated three times.

Quartz Crystal Microbalance: QCM measurements were performed on a Qsense© D3000 microbalance. Quartz crystals with a resonance frequency of 5 MHz and with a 100 nm thick gold layer were used from Qsense. All measurements were made at T = 25 °C. The crystal was attached to the measurement chamber and immersed in PBS buffer. The respective antibody or antigen PBS solution (2 μg mL^{-1}) was injected upon a constant frequency shift, followed by washing steps until the frequency shifts remained constant. All measurements were repeated three times.

Supporting Information (see footnote on the first page of this article): Experimental procedures for compounds **3b**, **5b**, **6b**–**9b**, **16b**, **17b**, and **18** as well as the ^{1}H and ^{19}F NMR spectra of all new compounds.

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