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Fluorinated Glycosyl Amino Acids for Mucin-Like Glycopeptide Antigen Analogues

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Abstract: The aberrant glycosylation profiles of mucin glycoproteins on epithelial tumour cells represent attractive target structures for the development of immunotherapy against cancer. Mucin-type glycopeptides have been successfully investigated as molecularly defined vaccine prototypes for triggering humoral immunity but are susceptible to rapid in vivo degradation. As a potential means to enhance the bioavailabilities of the antigenic structures, hydrolysis-resistant carbohydrate analogues with fluorine substituents at positions C6, C2′ and C6′ were synthesised and incorporated into the tandem repeat sequence of the mucin MUC1. The resulting pseudo-glycopeptides can be used to elucidate the effects of chemically modified antibody determinants on metabolic and immunological properties.

Keywords: antigens · fluorine · glycopeptides · mucin-1 · solid-phase synthesis

Introduction

Malignancies are frequently associated with the over-expression of various cell-surface glycoproteins and glycosphingolipids.[1] In many carcinomas, the transmembrane glycoprotein mucin-1 (MUC1) is strongly over-expressed and characterised by the exposure of immunogenic peptide epitopes and truncated glycans, as a result of the down-regulation of certain glycosyltransferases.[2] Expression of various tumour-associated carbohydrate antigens (TACAs) has been found to correlate with cancer progression and metastasis,[3] and so these antigens are of particular interest for the development of cancer diagnostics and immunotherapy.[4] A severe drawback in the development of efficient carbohydrate-based vaccines is the low metabolic stabilities of their glycosidic bonds, which are easily cleaved by endogenous glycosidases, leading to reduced bioavailability of the antigens to the immune system. Moreover, loss of essential saccharide recognition elements would be expected to affect antigen presentation and specificity of the immune response. In attempts to circumvent hydrolytic degradation, a number of hydrolysis-resistant TACA mimics (e.g., C-glycosides, S-glycosides) have been incorporated into carbohydrate-based vaccines.[5] Deoxyfluoro carbohydrate analogues, however, have been less widely investigated as antigen mimetics.[6]

The substitution of a sugar hydroxy group by a fluorine atom is an established method for systematic examination of the specificity of carbohydrate binding to a variety of protein binding sites with minimal steric perturbation.[6b,7] The Pauling electronegativity and the van der Waals radius of fluorine (4.0, 1.47 Å) compare favourably with those of oxygen (3.5, 1.52 Å), allowing a C–F moiety to serve as an effective C–OH mimic.[8] Moreover, the electron-withdrawing nature of fluorine affects the reactivity of an adjacent glycosidic bond, enabling the use of fluorinated substrate analogues as mechanistic probes for glycosyl-processing enzymes and metabolic studies.[9]

The strong interest in fluorinated mono- and oligosaccharides has resulted in a number of methods for their synthesis,[10] including the syntheses of mucin-like core structures.[11] In contrast, only a few pseudo-glycopeptides containing fluorinated glycosyl amino acids are available from the literature.[6c,e] We have recently described the first examples of TACA-threonine conjugates containing one or two fluorine substituents in their glycan components, together with their incorporation into short mucin-type model glycopeptides by solid-phase peptide synthesis (SPPS).[12] Here we report the detailed syntheses of orthogonally protected deoxyfluoro analogues of the T_N, TF and 2,6-sialyl-TF antigens, as well as the assembly of pseudo-glycopeptides each containing a complete MUC1 tandem repeat sequence in-

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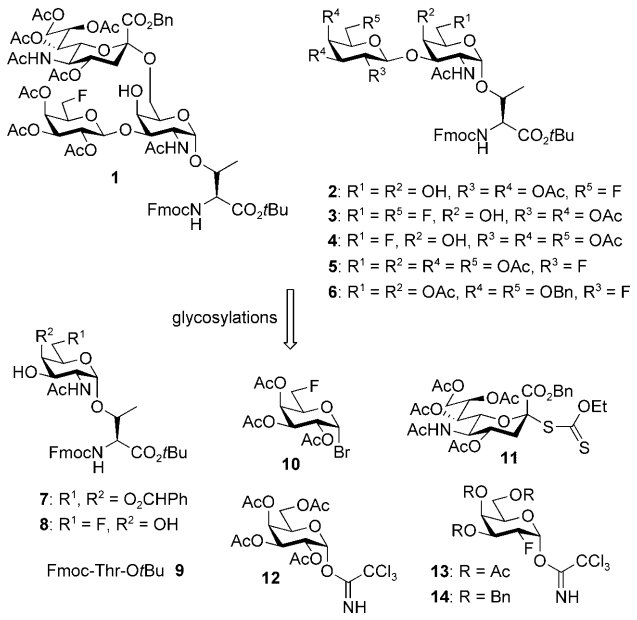
corporating a 2′-deoxy-2′-fluoro-TF or a 6,6′-dideoxy-6,6′-difluoro-TF antigen analogue. The resulting modified glycopeptide antigens might prove to be valuable cancer vaccine building blocks with enhanced immunogenicities and metabolic stabilities.

Results and Discussion

From the point of view of the desired chemical and metabolic stabilisation, TACA analogues with fluorine substituents at position C2 and C6 in the non-reducing glycan terminus are particularly interesting because *exo*-glycosidases operate by cleavage of these residues. In addition, the inductive destabilising effect on a transition state resembling an oxocarbenium ion should be at its maximum in close proximity to C1. To limit the number of synthetic precursors and protecting group manipulations, we decided to assemble the corresponding antigen analogues by stepwise extension of the saccharide chains on preformed GalNAc-threonine building blocks, rather than coupling the deoxyfluoro saccharides individually to suitably protected threonine derivatives. Our strategy for the synthesis of the fully protected TACA analogues **1**–**6** (Scheme 1) was therefore based on the T_N antigen derivatives **7**[13] and **8**, the fluorinated galactosyl donors **10**,[6a] **13**[14] and **14** and the sialyl donor **11**.[15]

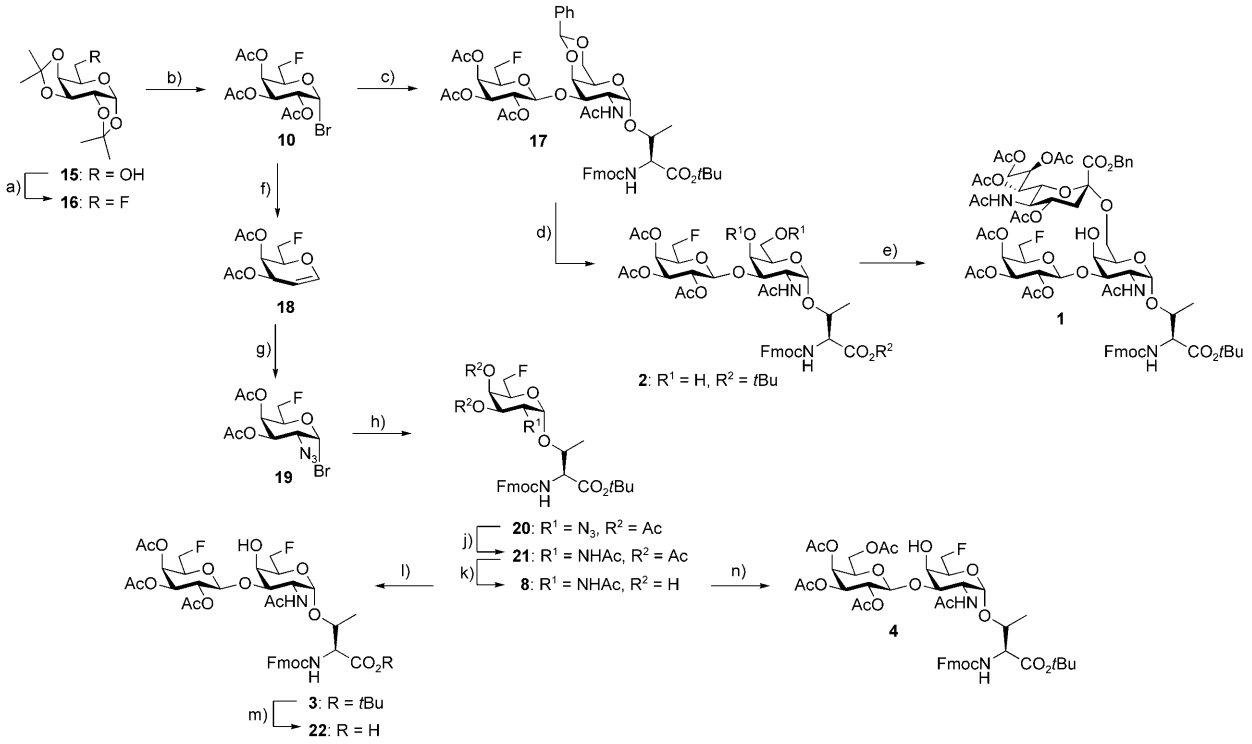


Scheme 1. Retrosynthetic plan for targeting the fluorinated TACA analogues **1**–**6**.

Synthesis of the 6-deoxy-6-fluoro-TACA analogues 1–4: The 6-deoxy-6-fluoro-glycosyl amino acid syntheses commenced with the preparation of the fluorinated precursor **16** (Scheme 2),[16] easily available through microwave-assisted deoxyfluorination of 1,2:3,4-di-*O*-isopropylidene galactose (**15**) with *N*,*N*-diethylaminosulfur trifluoride (DAST) in the presence of 2,4,6-collidine.[17] Subsequent acidolysis, acetylation and treatment with HBr in glacial acetic acid afforded the galactosyl donor **10** in 70% yield over three steps after column chromatography. Stereoselective coupling of **10** with Fmoc-Thr(α-4,6-*O*-Bzn-GalNAc)-O*t*Bu (**7**) was achieved most successfully by the Helferich method[18] with microwave assistance. Thus, on irradiation of the acceptor **7** with 6-deoxy-6-fluorogalactosyl bromide (**10**) and $Hg(CN)_2$ in $MeNO_2/CH_2Cl_2$ 3:2 at 100 W (80 °C) a clean conversion into the β-configured disaccharide **17** (98%) was observed without significant formation of orthoester or α-configured by-products. Remarkably, conventional heating of the reaction mixture only led to diminished yields of conjugate **17**, due to decomposition of the donor, although the corresponding galactosylation with the non-fluorinated donor Ac_4GalBr[19] had proceeded smoothly even at room temperature without microwave support.[20] Iodine-promoted cleavage[21] of the benzylidene acetal of **17** at 60 °C finally provided the 6′-deoxy-6′-fluoro-TF antigen-threonine analogue **2** (80%), which was further converted into **1**, the first known deoxyfluoro analogue of the 2,6-sialyl-TF antigen conjugate. The natural 2,6-sialyl-TF antigen, together with its regioisomeric 2,3-sialylated congener, represents an important TACA for breast cancer and leukaemia.[22]

Compound **1** was directly obtained from **2** through chemo- and stereoselective glycosylation at 6-OH with the sialyl xanthate **11**[15] as the donor (Scheme 2). Protection of the carboxy function of **11** as a benzyl ester has proven advantageous in this reaction, because this ester can readily be removed from the glycopeptide at a later stage under neutral conditions, avoiding epimerisation of the amino acids or β-elimination of the glycan.[20] Chemoselective sialylation of the disaccharide conjugate **2** was performed at −40 °C in a mixture of MeCN and CH_2Cl_2 as the solvents. Activation of the xanthate **11** was promoted with methylsulfenyl triflate generated in situ from methylsulfenyl bromide and silver triflate.[23] Under these conditions, formation of the α-sialoside is favoured and the desired conjugate **1** was obtained in a yield of 46% after flash chromatographic removal of concomitant sialic acid glycal and minor amounts of the corresponding β-anomer. In view of the planned glycopeptide synthesis we abstained from acetylation of the remaining axial 4-OH, because deacetylation of such sterically hindered *O*-acetyl groups has been found to be difficult.[24] Therefore, liberation of the carboxy terminus of **1** immediately provided a glycosyl amino acid building block suitable for SPPS.

The TF antigen analogues **3** and **4**, with fluorine substituents in their GalNAc moieties, were also accessible from the galactosyl bromide **10** (Scheme 2). In an approach similar to that used in the synthesis of the GalNAc-threonine conjugate **7**,[13] the building block **10** was transformed into the 6-deoxy-6-fluoro-galactal **18** by zinc-mediated reductive elimination of the 1-bromo and 2-acetoxy groups (89%). Subsequent azidonitration[25] and installation of the anomeric bromide furnished the azido bromide **19** (31%, two steps), which was used as the galactosyl donor in the Ag_2CO_3/



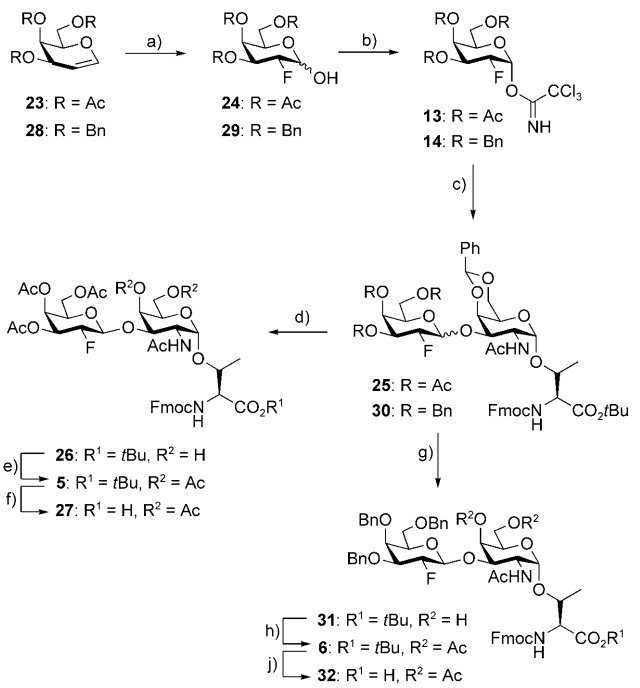
Scheme 2. a) DAST, 2,6-collidine, microwave irradiation 100 W, 80 °C, 1 h, 84 %; b) i) AcOH, reflux, then Ac_2O/pyridine 1:2, 99 %; ii) HBr/AcOH, CH_2Cl_2, 0 °C to RT, 71 %; c) **7**, $Hg(CN)_2$, $MeNO_2/CH_2Cl_2$ 3:2, MS (4 Å), microwave irradiation 100 W, 80 °C, 1 h, 98 % (β-**17**); d) I_2, MeOH, reflux, 80 %; e) **11**, MeSBr, AgOTf, MS (4 Å), $MeCN/CH_2Cl_2$ 2:1, −40 °C to RT, 46 % (α-anomer); f) Zn, *N*-methylimidazole, EtOAc, reflux, 89 %; g) i) CAN, NaN_3, MeCN, −18 °C, 47 %; ii) LiBr, MeCN, 67 %; h) **9**, Ag_2CO_3, $AgClO_4$, MS (4 Å), toluene/CH_2Cl_2 1:1, 0 °C to RT, 45 % (α-**20**); j) Zn, THF/Ac_2O/AcOH 3:2:1, 75 %; k) NaOMe (cat.), MeOH, pH 8.5, 78 %; l) **10**, $Hg(CN)_2$, $MeNO_2/CH_2Cl_2$ 3:2, MS (4 Å), microwave irradiation 100 W, 80 °C, 4 h, 73 % (β-**3**); m) TFA/anisole 10:1, 87 %; n) **12**, TMSOTf (cat.), MS (4 Å), CH_2Cl_2, −20 °C to RT, 54 % (β-**4**).

$AgClO_4$-promoted coupling to Fmoc-Thr-O*t*Bu (**9**).[26] The glycosylation proceeded stereoselectively at 0 °C in a mixture of toluene and CH_2Cl_2 as the solvents and led to the formation of the desired α-configured conjugate **20** in 45 % yield. Concomitant traces of the corresponding β-anomeric glycosyl amino acid were removed by flash column chromatography and the conjugate **20** was transformed into the acetamide **21** upon reductive acetylation (75 %). De-*O*-acetylation of **21** under Zemplén conditions at pH 8.5[27] provided the hitherto unknown 6-deoxy-6-fluoro-T_N antigen analogue **8** in 78 % yield, and this was regio- and stereoselectively β-galactosylated at 3-OH with the 6-deoxy-6-fluoro-galactosyl bromide **10** to yield the 6,6′-difluoro-conjugate **3**. As had also been the case for compound **17**, formation of the disaccharide **3** required microwave-assisted activation of the galactosyl donor with $Hg(CN)_2$ in a $MeNO_2/CH_2Cl_2$ 3:2 solvent mixture. Under these conditions, a clean conversion into β-**3** (73 %) was again observed, with the less reactive axial 4-hydroxy group being left untouched. Because protection of this free 4-OH had been found not to be essential for SPPS (vide infra), the target glycopeptide building block **22** was obtained through acidolysis of the *tert*-butyl ester (87 %).

Interestingly, the corresponding $Hg(CN)_2$-promoted 3-β-galactosylation of the monofluorinated T_N derivative **8** with Ac_4GalBr to yield compound **4** was found not to proceed satisfactorily either at ambient temperature or under microwave irradiation conditions. Instead, the use of the more reactive Ac_4Gal trichloroacetimidate **12**[28] was required. Thus, in the presence of catalytic amounts of trimethylsilyl triflate in CH_2Cl_2 the desired 6-deoxy-6-fluoro-TF conjugate **4** was isolated as the only glycosylated product in a moderate 54 % yield. It is interesting to note that no formation of the corresponding orthoester was observed, in contrast with the related glycosylation of the nonfluorinated T_N acceptor **7**.[13] However, the inherent low reactivity of the accepting 3-OH group resulting from negative inductive effects and/or the engagement of its lone pairs in an intramolecular hydrogen bond to the 2-acetamido group seemed to impede the complete conversion of acceptor **8**, which was always recovered from the reaction mixture to some extent.

Synthesis of the 2′-deoxy-2′-fluoro-TF analogues 5 and 6: In view of the microwave-supported activation of the 6-deoxy-6-fluoro-galactosyl donor **10**, which had proved to be essential for the successful glycosylation of the T_N acceptors **7** and **8**, we also expected the corresponding 2-deoxy-2-fluoro-galactosylations to be difficult, because anomeric reactivity is greatly suppressed by the inductive effect of a 2-fluoro group. Indeed, microwave-supported galactosylation of **7**

with 2F-Ac_3GalBr[29] proved unsuccessful for formation of the desired 2′-deoxy-2′-fluoro-TF antigen analogue **5**. We therefore turned our attention to the use of the more reactive 2-deoxy-2-fluoro trichloroacetimidate donor **13** (Scheme 3),[30] which can be conveniently prepared by Selectfluor-mediated electrophilic fluorination of 3,4,6-tri-*O*-acetylgalactal (**23**).[29–31] However, in our synthesis of the disaccharide **5**, a slightly modified microwave-assisted protocol for the preparation of **24** was used. Treatment of **23** with Selectfluor in MeCN/H_2O 4:1 under microwave irradiation conditions (100 W, 108 °C) provided 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluorogalactose (**24**) in 66% yield within minutes, in contrast with a 63% yield after 6.5 h at reflux without microwave support. Compound **24** was then transformed into the trichloroacetimidate **13** by treatment with DBU in CH_2Cl_2 (71%). The subsequent glycosylation of the acceptor **7** with the donor **13** afforded the desired disaccharide **25** in good yields (70–77%) only at temperatures above 0 °C. Under these conditions, however, poor stereoselectivity of the glycosylation reaction was observed, with predominant formation of the thermodynamically favoured α-glycoside of **25**. Because the formation of the desired β-anomeric product of **25** was impeded both by electronic effects and by the non-participating character of the fluorine substituent, we were not able to exceed a β/α ratio of 1:2, even after extensive optimisation studies with use of various promoters and solvents. Flash chromatographic separation of the anomeric mixture proved difficult at this stage, but was conveniently achieved after removal of the benzylidene acetal (**26**, 77%). Subsequent *O*-acetylation proceeded smoothly (89%) and afforded the pure conjugate β-**5** in 89%. Acidolytic cleavage of the *tert*-butyl ester finally provided the 2′-deoxy-2′-fluoro-TF-glycopeptide building block **27** (89%), which was directly subjected to the solid-phase glycopeptide synthesis.



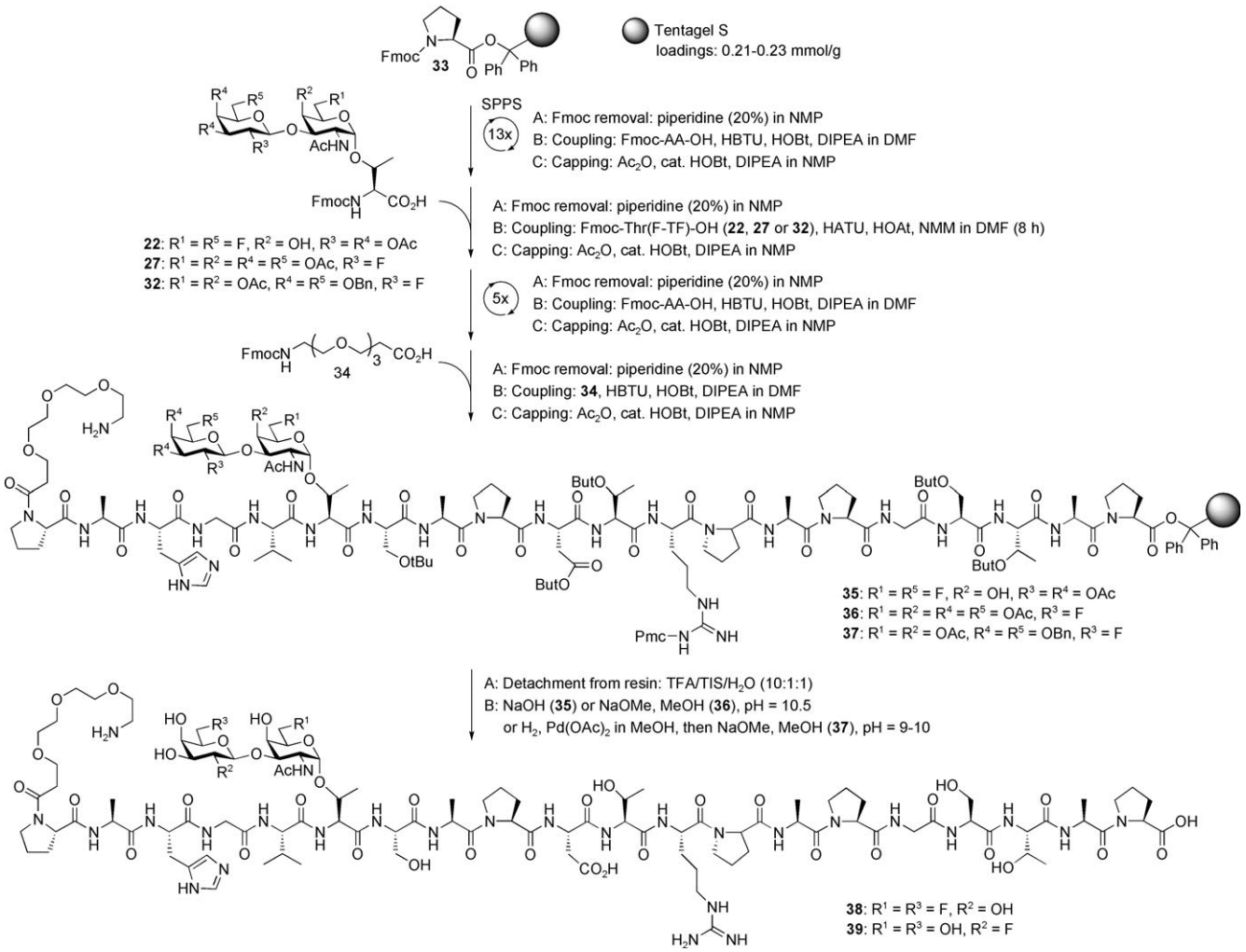
Scheme 3. a) R = Ac: Selectfluor, $MeNO_2/H_2O$ 4:1, microwave irradiation 100 W, 108 °C, 2 min, 66% (**24**), R = Bn: Selectfluor, $MeNO_2/H_2O$ 4:1, reflux, 66% (**29**); b) CCl_3CN, DBU, CH_2Cl_2, 71% (**13**), 84% (**14**); c) **7**, TMSOTf (cat.), MS (4 Å), CH_2Cl_2, 0 °C to RT, 12 h, 70% (**25**, α/β 2:1), 86% (**30**, α/β 1:10); d) R = Ac: $NaHSO_4/SiO_2$, CH_2Cl_2/MeOH 4:1, 77%; R = Bn: i) H_2, Pd/C, EtOH, 12 h; ii) FmocOSu, DIPEA, CH_2Cl_2; e) Ac_2O/pyridine 1:2, 89% from **26** and 30–90% from **30** over three steps; f) TFA/anisole 10:1, CH_2Cl_2, 89%; g) $NaHSO_4/SiO_2$, CH_2Cl_2/MeOH 4:1, 85%; h) Ac_2O/pyridine 1:2, 88%; j) TFA/anisole 10:1, CH_2Cl_2, 70%.

It is generally accepted that the natures of the carbohydrate protecting groups can modulate the efficiency of glycosylation by influencing both the reactivities of the coupling partners and the reaction stereoselectivity. Woerpel and co-workers,[32] for instance, have shown that the presence of electronegative substituents, in particular at C3 and C4 as in the galactosyl donor **13**, favours the pseudoaxial conformation of the intermediate oxocarbenium ion, which then leads to a 1,4-*trans*-selective glycosylation reaction. In contrast to this, the corresponding but more reactive benzylated trichloroacetimidate donor **14** should react preferably through the pseudoequatorially substituted oxocarbenium ion, thus favouring β-anomeric selectivity. 3,4,6-Tri-*O*-benzyl-2-deoxy-2-fluorogalactosyl trichloroacetimidate (**14**) was obtained from the starting 3,4,6-tri-*O*-benzylgalactal (**28**) over two steps and in 55% yield through Selectfluor-mediated fluorination by a literature protocol[6d] (**29**, 66%), followed by treatment with trichloroacetonitrile in the presence of DBU in CH_2Cl_2 (86%).

To our delight, the subsequent galactosylation of the acceptor **7** with the donor **14** in the presence of TMSOTf proceeded in an excellent yield of 86% and with a significantly increased β-selectivity of 10:1 (β/α). Again, as with donor **13**, elevated temperatures and long reaction times were required for the glycosylation to go to completion. Minor amounts of the corresponding α-anomer were separated from β-**30** by flash chromatography, and the hydroxy protecting groups of β-**30** were removed by hydrogenolysis in the presence of palladium on activated charcoal in EtOH. Unfortunately, this step was found not to be reliable under a wide range of reaction conditions, including the use of $Pd(OAc)_2$ and MeOH/AcOH as the solvents. Besides the sluggish course of the debenzylation reaction, partial loss of the Fmoc protecting group was observed; it therefore had to be reinstalled prior to *O*-acetyl protection, furnishing the 2′-deoxy-2′-fluoro-TF building block **5** in variable yields of 30–80% over three steps. In view of this tedious and inefficient protecting group transformation, we decided to refrain from global *O*-acetylation. Encouraged by literature precedence with regard to the use of benzylated glycosyl amino acids for SPPS[33] and in spite of the expected increase in acid-sensitivity of the resulting conjugate **6**, we exchanged only the labile benzylidene acetal for acetyl groups. Thus, upon selective removal[34] of the benzylidene acetal (**31**, 77%), followed by *O*-acetylation (**6**, 88%) and careful acidolysis of the *tert*-butyl ester, the alternative 2′-deoxy-2′-fluoro-TF-gly-

copeptide building block **32** was obtained in 47% yield over three steps.

Solid-phase synthesis of MUC1 glycopeptide antigen analogues: After liberation of their C termini, the glycosyl amino acids **22**, **27** and **32** were directly used in the sequential solid-phase synthesis of two glycopeptide antigen analogues, each containing a full tandem repeat (TR) sequence of the epithelial mucin MUC1 and an *N*-terminal triethylene glycol spacer. These can then be used for conjugation to immunostimulants such as BSA[35] or tetanus toxoid[36] and for immobilisation onto microarray platforms[37] in functional immunological studies. The MUC1 pseudo-glycopeptides were assembled with an automated synthesiser by the Fmoc strategy on a Tentagel S resin (**33**) modified with a bulky trityl linker[38] to avoid diketopiperazine formation and preloaded with Fmoc-proline (Scheme 4). The first 13 amino acids of the MUC1 sequence were coupled under standard conditions with use of piperidine in *N*-methylpyrrolidone (NMP) to remove the temporary Fmoc protecting group, followed by coupling of excess (10 equiv) Fmoc-amino acid activated by HBTU/HOBt[39] and diisopropylethylamine (DIPEA) in DMF. Unreacted amino acids were capped after each cycle with Ac_2O in the presence of DIPEA and catalytic amounts of HOBt in NMP. To allow for the sterically demanding natures of their carbohydrate moieties, the threonine conjugates **22** (1.3 equiv), **27** (2.0 equiv) and **32** (2.5 equiv) were each coupled with an extended reaction time of 8 h and with use of the more reactive reagents HATU/HOAt[40] with *N*-methylmorpholine (NMM) in NMP for activation. Subsequent Fmoc removal from the glycosylated peptides was again carried out with piperidine in NMP.[13,20] After the final five Fmoc-amino acids of the TR sequence had been coupled by the standard protocol, the triethylene glycol spacer **34** (10 equiv) was attached to each peptide, again by the standard coupling procedure. Simultaneous detachment of the glycopeptides from the resin and cleavage of the acid-labile amino acid side chain protecting



Scheme 4. Solid-phase synthesis of the tumour-associated MUC1 tandem repeat glycopeptide analogues **38** and **39** with fluorinated TF antigen glycan chains at Thr15. NMP = *N*-methylpyrrolidone, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOBt = *N*-hydroxybenzotriazole, DIPEA = diisopropylethylamine, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, HOAt = *N*-hydroxy-7-azabenzotriazole, NMM = *N*-methylmorpholine, TIS = triisopropylsilane.

groups was achieved by treatment with a mixture of TFA, triisopropylsilane and water. The resulting partially deblocked glycopeptides **35** and **36** were isolated after purification by semipreparative RP-HPLC in yields of 29 and 21%, respectively (based on the loaded resin **33**). The final de-*O*-acetylation of the saccharide moiety was accomplished by prolonged treatment either with aqueous NaOH (5 mM) solution (**38**, 55%) or with catalytic amounts of NaOMe in methanol (**39**, 60%) at pH 10–10.5. In each case, careful monitoring of the reaction by analytical RP-HPLC was necessary, in order to avoid epimerisation of amino acids and/or β-elimination of the glycan. Deprotection of the glycan part in the MUC1 pseudo-glycopeptide **37** was directly performed on the crude product in a two-step sequence after release from the resin. At first, debenzylation was achieved by hydrogenolysis in the presence of $Pd(OAc)_2$ as the catalyst, followed by de-*O*-acetylation through careful transesterification with NaOMe in MeOH at pH 9–10. Purification by RP-HPLC finally furnished the desired glycopeptide **39** with an improved overall chemical yield of 34% based on the loaded resin **33**.

Conclusion

A set of orthogonally protected fluorinated analogues of the tumour-associated carbohydrate antigens T_N, TF and 2,6-sialyl-TF with fluorine substituents at positions C6, C2′ and C6′ has been successfully prepared. In general, the regio- and stereoselective glycosylation reactions of fluorinated galactosyl donors were characterised by markedly reduced reactivities, in the case of glycosyl bromide **10** even requiring microwave-supported activation to provide the mono- and difluorinated TF antigen-threonine conjugates **17** and **3** in good chemical yields. In addition, use of the benzyl-protected 2-fluoro-substituted trichloroacetimidate donor **14** allowed β-selective formation of the 2′-deoxy-2′-fluoro-TF antigen analogue **5** despite the non-participating character of its 2-fluoro substituent. The orthogonally protected 2′-fluorinated TF antigen building blocks **27** and **32**, as well as the 6,6′-difluorinated derivative **22**, were incorporated into MUC1 glycopeptide sequences, each containing a non-immunogenic linker, for further conjugation to carrier proteins or microarray platforms. The presented antigen analogues are potentially interesting tools for the development of novel glycoconjugate mimics with increased biological half-lives and enhanced immunogenicities.

Experimental Section

General remarks: Solvents for moisture-sensitive reactions (toluene, MeCN, CH_2Cl_2, $MeNO_2$) were distilled and dried by standard procedures. Glycosylations were performed in flame-dried glassware under argon. DMF (amine-free, for peptide synthesis) and NMP were purchased from Roth, and Ac_2O and pyridine in p.a. quality from Acros. Reagents were purchased in the highest available commercial quality and were used as supplied except where noted. Fmoc-protected amino acids were purchased from Orpegen Pharma. For solid-phase syntheses, pre-loaded Tentagel S resins (Rapp Polymere) were employed. Reactions were monitored by TLC with pre-coated 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 and PerfectSil C18(2) (250×4.6 mm, 5 μm), Phenomenex Luna C18(2) (250×4.6 mm, 10 μm) or Phenomenex Jupiter C18(2) (250×4.6 mm, 10 μm) columns at a flow rate of 1 mL min^{-1}. Preparative HPLC separations were carried out with a JASCO-HPLC System and PerfectSil C18(2) (250×20 mm, 5 μm), Phenomenex Luna C18(2) (250×30 mm, 10 μm) or Phenomenex Jupiter C18(2) (250×30 mm, 10 μm) columns at a flow rate of 20 mL min^{-1} or 10 mL min^{-1}. H_2O/MeCN and H_2O/MeOH mixtures were used as solvents; if required TFA (0.1%) was added. ^1H, ^{13}C, ^{19}F and 2D NMR spectra were recorded with a Bruker AC 300 or a Bruker AM 400 spectrometer. The chemical shifts are reported in ppm relative to the signal of the deuterated solvent. Multiplicities are given as: s (singlet), br s (broad singlet), d (doublet), t (triplet) and m (multiplet). Assignment of proton and carbon signals was achieved by means of additional COSY, TOCSY, HMQC and HMBC experiments when noted. The signals of the saccharide portions were denoted as follows: *N*-acetyl-D-galactosamine (no prime), D-galactose (′) and *N*-acetyl-neuraminic acid (″). In the glycopeptide spectra, the glycosylated amino acid threonine is characterised by a star. ESI and HR-ESI mass spectra were recorded with a Micromass Q TOF Ultima 3 spectrometer, whereas MALDI-TOF mass spectra were acquired with a Micromass Tofspec E spectrometer and use of 2,5-dihydroxybenzoic acid as the matrix. Optical rotations were measured at 546 nm and 578 nm with a Perkin–Elmer polarimeter 241.

Detailed experimental procedures and characterisation data for compounds **5**, **10**, **13**, **14**, **16**, **18**, **19**, **24**–**26** and **29** are provided in the Supporting Information (see footnote at the first page of this article).

Fmoc-Thr(β-6F-Ac$_3$Gal-(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu (17): A solution of Fmoc-Thr-(α-4,6-*O*-Bzn-GalNAc)-O*t*Bu (**7**, 812 mg, 1.18 mmol) in dry $MeNO_2/CH_2Cl_2$ 3:2 (12.5 mL) was treated with activated powdered molecular sieves (4 Å, 800 mg) and $Hg(CN)_2$ (596 mg, 2.36 mmol) and was stirred for 30 min at room temperature under argon. The glycosyl donor **10** (876 mg, 2.36 mmol), dissolved in dry $MeNO_2/CH_2Cl_2$ 3:2 (12.5 mL), was added and the reaction mixture was irradiated in a CEM Discover microwave reactor at 100 W (80 °C) for 1 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through Hyflo Supercel into saturated aqueous $NaHCO_3$ (80 mL), and the aqueous phase was extracted with CH_2Cl_2 (2×80 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (40 mL) and brine (40 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc, 2:3) afforded **17** as a colourless amorphous solid (1.13 g, 1.15 mmol, 98%); R_f=0.29 (*c*Hex/EtOAc 2:3); analytical RP-HPLC (Luna, H_2O/MeCN 25:75→0:100, 30 min): t_R=6.89 min; $[\alpha]_D^{23}$= 64.7 (c=1.0 in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$, COSY): δ=7.79 (d, ^3J(4-H,3-H)=^3J(5-H,6-H)=7.5 Hz, 2H; 4-H-, 5-H-Fmoc), 7.63 (d, ^3J(1-H,2-H)=^3J(8-H,7-H)=6.8 Hz, 2H; 1-H-, 8-H-Fmoc), 7.57–7.53 (m, 2H; H_{ar}-Bzn), 7.45–7.31 (m, 7H; 2-H-, 3-H-, 6-H-, 7-H-Fmoc, H_{ar}-Bzn), 5.85 (d, ^3J(NH,2-H)=8.8 Hz, 1H; NH-Ac), 5.56 (s, 1H; CH-Bzn), 5.44 (d, ^3J-(NH,Tα)=9.0 Hz, 1H; NH-urethane), 5.40–5.37 (m, 1H; 4′-H), 5.21 (dd, ^3J(2′-H,1′-H)=7.4 Hz, ^3J(2′-H,3′-H)=9.8 Hz, 1H; 2′-H), 5.00–4.94 (m, 2H; 3′-H {4.99}, 1-H {4.94}), 4.76 (d, ^3J(1′-H,2′-H)=7.2 Hz, 1H; 1′-H), 4.67–4.61 (m, 1H; 2-H), 4.50–4.37 (m, 3H; CH_2-Fmoc {4.50}, 6a′-H {4.48}), 4.37–4.33 (m, 1H; 4-H), 4.30–4.21 (m, 4H; 6b′-H {4.30}, 9-H-Fmoc {4.25}, $T^α$ {4.23}, 6a-H {4.21}, $T^β$ {4.18}), 4.14–4.05 (m, 2H; 6b-H {4.10}, 5′-H {4.06}), 3.88 (d, ^3J(3-H,2-H)=8.7 Hz, 1H; 3-H), 3.70 (br s, 1H; 5-H), 2.15, 2.09, 2.00, 1.92 (4×s, 12H; CH_3-Ac), 1.46 (s, 9H; *t*Bu), 1.25 ppm (d, ^3J(Tγ,Tβ)=6.3 Hz, 3H; $T^γ$); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT, HMQC): δ=170.7, 170.2, 170.0, 169.9 (C=O), 156.4 (C=O-urethane), 143.7, 143.6 (C-1a-, C-8a-Fmoc), 141.3 (C-4a-, C-5a-Fmoc), 137.4 (C_q-Bzn), 128.8, 128.2, 128.1 (C_{ar}-Bzn), 127.8, 127.7 (C-3-, C-6-Fmoc), 127.1 (C-2-, C-7-Fmoc), 126.2 (C_{ar}-Bzn), 124.9 (C-1-, C-8-Fmoc), 120.1 (C-4-, C-5-Fmoc), 101.4 (C-1′), 100.6 (CH-Bzn), 100.2 (C-1), 83.2 (C_q-*t*Bu), 81.3 (d, ^1J(F,C-6′)=171.5 Hz; C-6′), 76.1 ($T^β$), 75.2 (C-4), 75.1 (C-3), 71.4 (d, ^2J(F,C-5′)=21.3 Hz; C-5′), 70.3 (C-3′), 69.3 (C-6), 68.7 (C-2′), 67.6 (d, ^3J(F,C-4′)=5.3 Hz; C-4′), 66.8 (CH_2-Fmoc), 63.7 (C-5), 58.9 ($T^α$), 47.9

(C-2), 47.3 (9-H-Fmoc), 28.0 (CH_3-*t*Bu), 20.8, 20.7, 20.6, 20.1 (CH_3-Ac), 18.9 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$), $\delta = -230.1$ (dt, ^3J(F,5′-H) = 13.3 Hz, ^2J(F,6′-H) = 46.7 Hz), −232.2 ppm (dt, ^3J(F,5-H) = 13.8 Hz, ^2J(F,6-H) = 46.2 Hz, rotamer); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{50}H_{59}FN_2O_{17}Na$: 1001.3698 $[M+Na]^+$; found: 1001.3681.

Fmoc-Thr(β-6F-Ac$_3$Gal-(1–3)-α-GalNAc)-O*t*Bu (2): Iodine (a few milligrams) and water (a few drops) were added to a solution of the disaccharide **17** (847 mg, 0.87 mmol) in methanol (90 mL). The reaction mixture was heated at reflux for 5 h and diluted with CH_2Cl_2 (100 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (60 mL) and brine (60 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc 2:3) afforded **2** as a colourless amorphous solid (617 mg, 0.69 mmol, 80%); $R_f = 0.13$ (*c*Hex/EtOAc 1:5); analytical RP-HPLC (Luna, H_2O/MeCN 50:50→15:85 (40 min)): t_R = 13.13 min; $[\alpha]_D^{23} = 43.2$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.79$ (d, ^3J(4-H,3-H) = ^3J(5-H,6-H) = 7.4 Hz, 2H; 4-H-, 5-H-Fmoc), 7.62 (d, ^3J(1-H,2-H) = ^3J(8-H,7-H) = 6.3 Hz, 2H; 1-H-, 8-H-Fmoc), 7.42 (t, ^3J(3-H,4-H) = ^3J(6-H,5-H) = 7.4 Hz, 2H; 3-H-, 6-H-Fmoc), 7.32 (t, ^3J(2-H,1-H) = ^3J(7-H,8-H) = 7.3 Hz, 2H; 2-H-, 7-H-Fmoc), 5.93 (d, ^3J(NH,2-H) = 9.5 Hz, 1H; NH-Ac), 5.44 (d, ^3J(NH,Tα) = 9.3 Hz, 1H; NH-urethane), 5.40 (d, ^3J(4′-H,3′-H) = 3.0 Hz, 1H; 4′-H), 5.21 (dd, ^3J(2′-H,1′-H) = 8.0 Hz, ^3J(2′-H,3′-H) = 10.3 Hz, 1H; 2′-H), 4.97 (dd, ^3J(3′-H,4′-H) = 3.1 Hz, ^3J(3′-H,2′-H) = 11.2 Hz, 1H; 3′-H), 4.83 (m, 1H; 1-H), 4.65 (d, ^3J(1′-H,2′-H) = 7.8 Hz, 1H; 1′-H), 4.57–4.45 (m, 4H; 2-H, CH_2-Fmoc, 6a′-H), 4.37–4.33 (m, 1H; 4-H), 4.27–4.20 (m, 2H; 6b′-H, 9H-Fmoc), 4.17–4.09 (m, 2H; T^{α}, T^{β}), 3.97–3.92 (m, 2H; 6a/b-H), 3.86–3.81 (m, 2H; 5′-H, 3-H), 3.72–3.69 (m, 1H; 5-H), 2.87 (brs, 1H; OH), 2.60 (brs, 1H; OH), 2.16, 2.09, 2.01, 1.94 (4 × s, 12H; 4 × CH_3-Ac), 1.45 (s, 9H; *t*Bu), 1.25 ppm (d, ^3J(Tγ,Tβ) = 6.6 Hz, 3H; T^{γ}); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.2$, 170.1, 170.0, 169.8 (C=O), 156.3 (C=O-urethane), 143.7 (C-1a-, C-8a-Fmoc), 141.3 (C-4a-, C-5a-Fmoc), 127.8 (C-3-, C-6-Fmoc), 127.1 (C-2-, C-7-Fmoc), 124.8 (C-1-, C-8-Fmoc), 120.0 (C-4-, C-5-Fmoc), 101.5 (C-1′), 100.1 (C-1), 83.1 (C_q-*t*Bu), 80.9 (d, ^1J(F,C-6′) = 172.6 Hz; C-6′), 77.3 (C-3), 76.2 (T^{β}), 71.7 (d, ^2J(F,C-5′) = 22.0 Hz; C-5′), 70.5 (C-3′), 69.9 (C-4), 69.6 (C-6), 68.4 (C-2′), 66.9 (d, ^3J(F,C-4′) = 6.5 Hz; C-4′), 66.7 (CH_2-Fmoc), 62.6 (C-5), 59.0 (T^{α}), 47.6 (C-2), 47.3 (C-9-Fmoc), 28.0 (CH_3-*t*Bu), 20.7, 20.5, 20.41 (CH_3-Ac), 18.7 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -230.7$ ppm (dt, ^3J(F,5′-H) = 12.4 Hz, ^2J(F,6′-H) = 46.1 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{43}H_{55}FN_2O_{17}Na$: 913.3385 $[M+Na]^+$; found: 913.3383.

Fmoc-Thr(β-6F-Ac$_3$Gal-(1–3)-[α-Ac$_4$NeuNAcCO$_2$Bn-(2–6)]-α-GalNAc)-O*t*Bu (1): A solution of Ac_4NeuNAcCO$_2$Bn-Xan (**11**, 566 mg, 0.84 mmol) in dry CH_2Cl_2 (3 mL) and dry MeCN (6 mL) was stirred with activated powdered molecular sieves (4 Å, 1 g) at room temperature for 16 h under argon. The glycosyl acceptor **2** (300 mg, 0.34 mmol), dissolved in dry CH_2Cl_2 (4 mL) and dry MeCN (7 mL), was added and the reaction mixture was stirred at room temperature for 1 h with protection from light. The suspension was cooled to −40 °C, and silver triflate (130 mg, 0.84 mmol) and a pre-cooled (0 °C) solution of methylsulfenyl bromide in dry 1,2-dichloroethane [527 μL of a 1.6 M solution in 1,2-dichloroethane; the methylsulfenyl bromide solution was prepared by addition of bromine (205 μL, 3.99 mmol) to a solution of dimethyl disulfide (354 μL, 3.99 mmol) in dry 1,2-dichloroethane (5 mL) and stirring at room temperature with exclusion of light for 13 h] were added slowly. The reaction mixture was stirred for 24 h at −45 to −30 °C under argon with exclusion of light. It was then neutralised with DIPEA and allowed to warm to room temperature. The suspension was diluted with CH_2Cl_2 (20 mL) and filtered through Hyflo Supercel, and the solvents were removed in vacuo. Purification by flash chromatography on silica gel (*c*Hex/EtOAc 5:1→EtOAc) afforded α-**1** as a colourless amorphous solid (216 mg, 0.15 mmol, 46%); $R_f = 0.12$ (*c*Hex/EtOAc 1:5); analytical RP-HPLC (Luna, H_2O/MeCN 70:30→23:77 (25 min)→0:100, 30 min): t_R = 27.79 min; $[\alpha]_D^{23} = 15.9$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, COSY): $\delta = 7.78$ (d, ^3J(4-H,3-H) = ^3J(5-H,6-H) = 7.4 Hz, 2H; 4-H-, 5-H-Fmoc), 7.62 (d, ^3J(1-H,2-H) = ^3J(8-H,7-H) = 6.3 Hz, 2H; 1-H-, 8-H-Fmoc), 7.43–7.32 (m, 9H; 2-H-, 3-H-, 6-H-, 7-H-Fmoc, H_{ar}-Bn), 5.89 (d, ^3J(NH,2-H) = 9.8 Hz, 1H; NH-Ac), 5.43 (d, ^3J(NH,Tα) = 9.1 Hz, 1H; NH-urethane), 5.43–5.41 (m, 1H; 4′-H), 5.33–5.28 (m, 2H; 8″-H {5.33}, 7″-H {5.30}), 5.23–5.18 (m, 3H; 2′-H {5.22}, CH_2-Bn {5.20}), 5.12 (d, ^3J(NH,5-H) = 8.7 Hz, 1H; NH-NeuNAc), 4.98 (dd, ^3J(3′-H,4′-H) = 2.7 Hz, ^3J(3′-H,2′-H) = 10.5 Hz, 1H; 3′-H), 4.87–4.80 (m, 1H; 4″-H), 4.73 (d, ^3J(1-H,2-H) = 3.2 Hz, 1H; 1-H), 4.66 (d, ^3J(1′-H,2′-H) = 7.7 Hz, 1H; 1′-H), 4.55–4.50 (m, 3H; CH_2-Fmoc {4.53}, 2-H {4.52}), 4.46–4.43 (m, 1H; 6a′-H), 4.36–4.25 (m, 3H; 6b′-H {4.33}, 9a″-H {4.30, dd, ^3J(9a″-H,8′-H′) = 2.1 Hz, ^2J(9a″-H,9b″-H) = 12.1 Hz}, 9H-Fmoc {4.25}), 4.16 (d, ^3J(Tα,Tβ) = 10.0 Hz, 1H; T^{α}), 4.11–4.01 (m, 4H; T^{β} {4.11}, 9a″-H {4.08}, 5″-H, 6″-H {4.05}), 3.98–3.89 (m, 3H; 5′-H {3.95}, 4-H {3.93}, 6a-H {3.89}), 3.81 (brs, 1H; 5-H), 3.55 (dd, ^3J(6b-H,5-H) = 5.1 Hz, ^2J(6-Ha,6b-H) = 10.0 Hz, 1H; 6b-H), 2.61 (dd, ^3J(3-H_{eq},4-H) = 4.4 Hz, ^2J(3-H_{eq},3-H_{ax}) = 12.7 Hz, 1H; 3″-H_{eq}), 2.48 (s, 1H; OH), 2.15, 2.11, 2.09, 2.00, 1.98, 1.95, 1.86 (7 × s, 27H; 9 × CH_3-Ac), 1.94–1.90 (m, 1H; 3″-H_{ax}), 1.45 (s, 9H; *t*Bu), 1.24 ppm (d, ^3J(Tγ,Tβ) = 6.2 Hz, 3H; T^{γ}); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT, HMQC): $\delta = 170.3$, 170.3, 170.2, 169.9, 169.8, 167.5 (C=O), 156.6 (C=O-urethane), 143.9, 143.8 (C-1a-, C-8a-Fmoc), 141.5 (C-4a-, C-5a-Fmoc), 135.0 (C_q-Bzn), 128.9, 128.6 (C_{ar}-Bzn), 128.5 (C-3-, C-6-Fmoc), 128.0 (C-2-, C-7-Fmoc), 127.3 (C_{ar}-Bzn), 125.0 (C-1-, C-8-Fmoc), 120.2 (C-4-, C-5-Fmoc), 101.8 (C-1′), 100.4 (C-1), 98.9 (C-2″), 83.2 (C_q-*t*Bu), 81.1 (d, ^1J(F,C-6′) = 171.0 Hz; C-6′), 77.4 (C-3), 77.2 (T^{β}), 72.9 (C-6″), 71.9 (d, ^2J(F,C-5′) = 23.4 Hz; C-5′), 70.8 (C-3′), 69.1 (C-8″), 69.1 (C-5), 69.0 (C-4″), 68.6 (C-2′), 67.6 (C-4), 67.5 (CH_2-Bn), 67.1 (d, ^3J(F,C-4′) = 6.2 Hz; C-4′), 67.0 (C-7″), 66.9 (CH_2-Fmoc, C-1″), 63.7 (C-6), 62.5 (C-9″), 59.2 (T^{α}), 49.2 (C-5″), 47.8 (C-2), 47.3 (C-9-Fmoc), 37.5 (C-3″), 28.2 (CH_3-*t*Bu), 23.5, 23.2 (CH_3-NHAc), 20.9, 20.8, 20.7 (CH_3-Ac), 18.8 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -230.4$ ppm (dt, ^3J(F,5′-H) = 12.8 Hz, ^2J(F,6′-H) = 46.2 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{69}H_{86}FN_3O_{29}Na$: 1462.5228 $[M+Na]^+$; found: 1462.5206.

Fmoc-Thr(α-6F-Ac$_2$GalN$_3$)-O*t*Bu (20): Fmoc-Thr-O*t*Bu (**9**, 283 mg, 0.71 mmol) was dissolved in a mixture of dry toluene and dry CH_2Cl_2 1:1 (6 mL) and stirred under argon with activated powdered molecular sieves (4 Å, 0.5 g) at room temperature for 45 min. The suspension was cooled to 0 °C, after which silver carbonate (206 mg, 0.75 mmol) and silver perchlorate (28.1 mg, 0.14 mmol), dissolved in anhydrous toluene (1 mL), were added. The glycosyl donor **19** (250 mg, 0.68 mmol), dissolved in a mixture of dry toluene and dry CH_2Cl_2 1:1 (6 mL), was added dropwise at 0 °C over a period of 25 min. The reaction mixture was stirred at 10 °C for 16 h, diluted with CH_2Cl_2 (50 mL), and filtered through Hyflo Supercel. The organic layer was washed with saturated aqueous $NaHCO_3$ (30 mL) and brine, (30 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc 3:1) afforded **20** as a colourless amorphous solid (212 mg, 0.32 mmol, 45%); $R_f = 0.27$ (*c*Hex/EtOAc 4:1); analytical RP-HPLC (Luna, H_2O/MeCN 50:50→25:75 (40 min)→0:100, 10 min): $t_R = 39.97$ min; $[\alpha]_D^{23} = 59.8$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.77$ (d, ^3J(4-H,3-H) = ^3J(5-H,6-H) = 7.0 Hz, 2H; 4-H-, 5-H-Fmoc), 7.63 (d, ^3J(1-H,2-H) = ^3J(8-H,7-H) = 7.4 Hz, 2H; 1-H-, 8-H-Fmoc), 7.40 (t, ^3J(3-H,4-H) = ^3J(6-H,5-H) = 7.4 Hz, 2H; 3-H-, 6-H-Fmoc), 7.32 (t, ^3J(2-H,1-H) = ^3J(7-H,8-H) = 7.4 Hz, 2H; 2-H-, 7-H-Fmoc), 5.66 (d, ^3J(NH,Tα) = 9.6 Hz, 1H; NH-urethane), 5.52 (d, ^3J(4-H,3-H) = 2.9 Hz, 1H; 4-H), 5.35 (dd, ^3J(3-H,4-H) = 2.9 Hz, ^3J(3-H,2-H) = 11.4 Hz, 1H; 3-H), 5.15 (d, ^3J(1-H,2-H) = 3.7 Hz, 1H; 1-H), 4.51–4.21 (m, 8H; 5-H, 6a/b-H, 9-H-Fmoc, CH_2-Fmoc, T^{α}, T^{β}), 3.66 (dd, ^3J(2-H,1-H) = 3.7 Hz, ^3J(2-H,3-H) = 11.4 Hz, 1H; 2-H), 2.15, 2.09 (2 × s, 6H; 2 × CH_3-Ac), 1.49 (s, 9H; *t*Bu), 1.25 ppm (d, 3H; ^3J(Tγ,Tβ) = 6.3 Hz, T^{γ}); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.1$, 170.0, 169.5 (C=O), 157.0 (C=O-urethane), 144.1, 144.0 (C-1a-, C-8a-Fmoc), 141.5 (C-4a-, C-5a-Fmoc), 127.9, 127.3 (C-3-, C-6-Fmoc), 127.3 (C-2-, C-7-Fmoc), 125.5, 125.3 (C-1-, C-8-Fmoc), 120.2, 120.1 (C-4-, C-5-Fmoc), 99.3 (C-1), 83.0 (C_q-*t*Bu), 81.6 (d, ^1J(F,C-6) = 173.9 Hz; C-6), 76.6 (T^{β}), 68.4 (d, ^2J(F,C-5) = 26.7 Hz; C-5), 68.0 (C-3), 67.7 (d, ^3J(F,C-4) = 6.2 Hz; C-4), 67.3 (CH_2-Fmoc), 59.4 (T^{α}), 58.0 (C-2), 47.4 (C-9-Fmoc), 28.2 (CH_3-*t*Bu), 20.9, 20.8 (CH_3-Ac), 19.2 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = -231.5$ ppm (dt, ^3J(F,5-H) = 14.8 Hz, ^2J(F,6-H) = 46.5 Hz); HRMS (ESI): *m*/*z*: calcd for $C_{33}H_{39}FN_4O_{10}Na$: 693.2550 $[M+Na]^+$; found: 693.2455.

Fmoc-Thr(α-6F-Ac$_2$GalNAc)-O*t*Bu (21): Activated zinc dust [783 mg, 11.3 mmol; activation was achieved by suspension in aqueous $CuSO_4$ solution (2%), followed by subsequent washings with water, EtOAc and Et_2O] was added to a stirred solution of **20** (760 mg, 1.13 mmol) in a THF/AcO_2/AcOH mixture 3:2:1 (48 mL). The reaction mixture was stirred for 18 h at room temperature, diluted with THF (30 mL) and fil-

tered through Hyflo Supercel. The filtrate was concentrated in vacuo and co-evaporated with toluene (5×20 mL) and CH_2Cl_2 (20 mL). The residue was dissolved in CH_2Cl_2 (40 mL), washed with saturated aqueous $NaHCO_3$ (3×20 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc 1:1) afforded **21** as a colourless amorphous solid (603 mg, 0.87 mmol, 75%); R_f=0.16 (*c*Hex/EtOAc 1:1); analytical RP-HPLC (Luna, H_2O/MeCN 70:30→25:75 (25 min)→0:100, 30 min): t_R=30.21 min; $[\alpha]_D^{23}$=35.6 (c=1.0 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.78 (d, ^3J(4-H,3-H)=^3J(5-H,6-H)=7.0 Hz, 2H; 4-H-, 5-H-Fmoc), 7.64 (d, ^3J(1-H,2-H)=^3J(8-H,7-H)=6.6 Hz, 2H; 1-H-, 8-H-Fmoc), 7.40 (t, ^3J(3-H,2-H)=^3J(6-H,7-H)=7.4 Hz, 2H; 3-H-, 6-H-Fmoc), 7.32 (t, ^3J(2-H,3-H)=^3J(7-H,6-H)=7.4 Hz, 2H; 2-H-, 7-H-Fmoc), 6.06 (d, ^3J(NH,2-H)=9.9 Hz, 1H; NH-Ac), 5.66 (d, ^3J(NH,Tα)=8.8 Hz, 1H; NH-urethane), 5.41 (brs, 1H; 4-H), 5.11 (dd, ^3J(3-H,4-H)=2.9 Hz, ^3J(3-H,2-H)=11.4 Hz, 1H; 3-H), 4.92 (d, ^3J(1-H,2-H)=2.9 Hz, 1H; 1-H), 4.63 (dt, ^3J(2-H,1-H)=3.0 Hz, ^3J(2-H,3-H)=9.9 Hz, 1H; 2-H), 4.53–4.39 (m, 3H; 5-H, 6a/b-H), 4.34–4.21 (m, 5H; 9-H-Fmoc, CH_2-Fmoc, $T^α$, $T^β$), 2.15, 2.00 (2×s, 6H; 2×CH_3-Ac), 2.15 (s, 3H; CH_3-NHAc), 1.46 (s, 9H; *t*Bu), 1.25 ppm (d, ^3J(Tγ,Tβ)=6.3 Hz, 3H; $T^γ$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.1, 170.6, 170.5, 170.2 (C=O), 156.7 (C=O-urethane), 144.0, 143.9 (C-1a-, C-8a-Fmoc), 141.5 (C-4a-, C-5a-Fmoc), 128.0, 127.3 (C-3-, C-6-Fmoc), 127.2 (C-2-, C-7-Fmoc), 125.3, 125.2 (C-1-, C-8-Fmoc), 120.3, 120.2 (C-4-, C-5-Fmoc), 100.0 (C-1), 83.4 (C_q-*t*Bu), 81.8 (d, ^1J(F,C-6)=171.0 Hz; C-6), 76.8 ($T^β$), 68.8 (C-3), 68.5 (d, ^2J(F,C-5)=22.5 Hz; C-5), 67.5 (d, ^3J(F,C-4)=6.7 Hz; C-4), 67.4 (CH_2-Fmoc), 59.1 ($T^α$), 52.1 (C-2), 47.4 (C-9-Fmoc), 28.3 (CH_3-*t*Bu), 23.5 (CH_3-NHAc), 20.9, 20.8 (CH_3-Ac), 18.8 ppm ($T^γ$); ^{19}F NMR (376 MHz, $CDCl_3$): δ=−231.1 ppm (dt, ^3J(F,5-H)=14.6 Hz, ^2J(F,6-H)=45.1 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{35}H_{43}FN_2O_{11}Na$: 709.2751 $[M+Na]^+$; found: 709.2744.

Fmoc-Thr(α-6F-GalNAc)-O*t*Bu (8): A solution of NaOMe in MeOH (1%) was added dropwise to a solution of Fmoc-Thr(α-6F-Ac_2GalNAc)-O*t*Bu (**21**, 573 mg, 0.83 mmol) in MeOH (10 mL) until pH 8.5 was reached. The reaction mixture was stirred for 4 d, during which the pH was carefully monitored and re-adjusted when necessary. After neutralisation with acidic cation exchanger (Amberlyst IR 120) and filtration, the solvent was removed at reduced pressure. Flash chromatography on silica gel (EtOAc/MeOH 20:1) afforded **8** as a colourless amorphous solid (394 mg, 0.66 mmol, 78%); R_f=0.10 (EtOAc/MeOH 20:1); analytical RP-HPLC (Luna, H_2O/MeCN 70:30→23:77 (25 min): t_R=21.71 min; $[\alpha]_D^{23}$=26.5 (c=1.0 in $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.77 (d, ^3J(4-H,3-H)=^3J(5-H,6-H)=7.4 Hz, 2H; 4-H-, 5-H-Fmoc), 7.61 (d, ^3J(1-H,2-H)=^3J(8-H,7-H)=7.4 Hz, 2H; 1-H-, 8-H-Fmoc), 7.40 (t, ^3J(3-H,2-H)=^3J(6-H,7-H)=7.3 Hz, 2H; 3-H-, 6-H-Fmoc), 7.32 (t, ^3J(2-H,3-H)=^3J(7-H,6-H)=6.9 Hz, 2H; 2-H-, 7-H-Fmoc), 6.89 (d, ^3J(NH,2-H)=7.6 Hz, 1H; NH-Ac), 5.61 (d, ^3J(NH,Tα)=9.4 Hz, 1H; NH-urethane), 4.86 (d, ^3J(1-H,2-H)=3.8 Hz, 1H; 1-H), 4.74 (m, 1H; 2-H), 4.59–4.38 (m, 3H; 5-H, 6a/b-H), 4.29–4.06 (m, 5H; 9-H-Fmoc, CH_2-Fmoc, $T^α$, $T^β$), 3.93 (brs, 1H; 4-H), 3.83 (dd, ^3J(3-H,4-H)=2.7 Hz, ^3J(3-H,2-H)=10.1 Hz, 1H; 3-H), 2.11 (s, 3H; CH_3-NHAc), 1.46 (s, 9H; *t*Bu), 1.32 ppm (d, ^3J(Tγ,Tβ)=6.2 Hz, 3H; $T^γ$); ^{13}C NMR (75 MHz, $CDCl_3$): δ=174.2, 171.5 (C=O), 156.6 (C=O-urethane), 144.0, 143.8 (C-1a-, C-8a-Fmoc), 141.5 (C-4a-, C-5a-Fmoc), 128.1, 128.0 (C-3-, C-6-Fmoc), 127.3 (C-2-, C-7-Fmoc), 125.2, 125.1 (C-1-, C-8-Fmoc), 120.3, 120.2 (C-4-, C-5-Fmoc), 99.7 (C-1), 83.7 (C_q-*t*Bu), 83.6 (d, ^1J(F,C-6)=167.8 Hz; C-6), 76.8 ($T^β$), 71.4 (C-3), 69.7 (d, ^2J(F,C-5)=20.7 Hz; C-5), 68.3 (d, ^3J(F,C-4)=7.2 Hz; C-4), 67.3 (CH_2-Fmoc), 59.1 ($T^α$), 51.3 (C-2), 47.4 (C-9-Fmoc), 28.3 (CH_3-*t*Bu), 23.0 (CH_3-NHAc), 18.9 ppm ($T^γ$); ^{19}F NMR (376 MHz, $CDCl_3$): δ=−230.68 ppm (m); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{31}H_{39}FN_2O_9Na$: 625.2540 $[M+Na]^+$; found: 625.2521.

Fmoc-Thr(β-6F-Ac_3Gal-(1–3)-α-6F-GalNAc)-O*t*Bu (3): A solution of the glycosyl acceptor **8** (150 mg, 0.25 mmol) in a mixture of dry $MeNO_2$ and dry CH_2Cl_2 3:2 (4 mL) was stirred under argon with activated, powdered molecular sieves (4 Å, 200 mg) and $Hg(CN)_2$ (125 mg, 0.49 mmol) for 30 min at room temperature. The glycosyl donor **10** (185 mg, 0.49 mmol), dissolved in a mixture of dry $MeNO_2$ and dry CH_2Cl_2 3:2 (4 mL), was added, and the reaction mixture was irradiated in a CEM Discover™ microwave reactor for 4 h at 100 W (80 °C). The reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through Hyflo Supercel into saturated aqueous $NaHCO_3$ (20 mL), and the aqueous phase was extracted with CH_2Cl_2 (2×30 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc 1:5) afforded **3** as a colourless amorphous solid (162 mg, 0.18 mmol, 73%); R_f=0.53 (*c*Hex/EtOAc 1:5); analytical RP-HPLC (Luna, H_2O/MeCN 70:30→23:77 (15 min)→0:100, 35 min): t_R=27.16 min; $[\alpha]_D^{23}$=59.8 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ=7.78 (d, ^3J(4-H,3-H)=^3J(5-H,6-H)=7.4 Hz, 2H; 4-H-, 5-H-Fmoc), 7.61 (d, ^3J(1-H,2-H)=^3J(8-H,7-H)=7.4 Hz, 2H; 1-H-, 8-H-Fmoc), 7.42 (t, ^3J(3-H,2-H)=^3J(6-H,7-H)=7.3 Hz, 2H; 3-H-, 6-H-Fmoc), 7.32 (t, ^3J(2-H,3-H)=^3J(7-H,6-H)=6.9 Hz, 2H; 2-H-, 7-H-Fmoc), 5.96 (d, ^3J(NH,2-H)=8.8 Hz, 1H; NH-Ac), 5.43 (d, ^3J(NH,Tα)=9.9 Hz, 1H; NH-urethane), 5.40 (d, ^3J(4′-H,3′-H)=2.7 Hz, 1H; 4′-H), 5.20 (dd, ^3J(2′-H,1′-H)=8.0 Hz, ^3J(2′-H,3′-H)=10.3 Hz, 1H; 2′-H), 4.97 (dd, ^3J(3′-H,4′-H)=3.0 Hz, ^3J(3′-H,2′-H)=10.5 Hz, 1H; 3′-H), 4.80 (d, ^3J(1-H,2-H)=3.0 Hz, 1H; 1-H), 4.71–4.63 (m, 2H; 1′-H {4.75}, 6a-H {4.66}), 4.57–4.54 (m, 3H; CH_2-Fmoc {5.57}, 2-H {4.55}, 6b-H {4.54}), 4.48–4.43 (m, 2H; 6a′-H{4.47}, CH_2-Fmoc {4.42}), 4.41–4.29 (m, 2H; 6b′-H {4.34}, 5-H {4.30}), 4.25–4.17 (m, 3H; 9-H-Fmoc {4.23}, $T^β$ {4.19}, $T^α$ {4.14}), 4.06 (s, 1H; 4-H), 4.00–3.92 (m, 1H; 5′-H), 3.71 (d, ^3J(3-H,2-H)=10.3 Hz, 1H; 3-H), 2.15, 2.08, 1.97 (3×s, 9H; 3×CH_3), 2.00 (s, 3H; CH_3-NHAc), 1.45 (s, 9H; *t*Bu), 1.25 ppm (d, ^3J(Tγ,Tβ)=6.3 Hz, 3H; $T^γ$); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT, HMQC): δ=170.2, 170.1, 170.1, 170.0 (C=O), 156.3 (C=O-urethane), 143.7, 143.6 (C-1a-, C-8a-Fmoc), 141.3 (C-4a-, C-5a-Fmoc), 127.8, 127.7 (C-3-, C-6-Fmoc), 127.0 (C-2-, C-7-Fmoc), 124.9, 124.8 (C-1-, C-8-Fmoc), 120.1, 120.0 (C-4-, C-5-Fmoc), 101.7 (C-1′), 100.0 (C-1), 83.4 (d, ^1J(F,C-6)=168.3 Hz; C-6), 83.1 (C_q-*t*Bu), 81.0 (d, ^1J(F,C-6′)=174.4 Hz; C-6′), 77.3 (C-3), 76.8 ($T^β$), 71.8 (d, ^2J(F,C-5′)=22.2 Hz; C-5′), 70.5 (C-3′), 69.2 (d, ^2J(F,C-5)=22.3 Hz; C-5), 68.3 (d, ^3J(F,C-4)=7.2 Hz; C-4), 68.2 (C-2′), 66.9 (d, ^3J(F,C-4′)=6.5 Hz; C-4′), 66.8 (CH_2-Fmoc), 59.0 ($T^α$), 47.5 (C-2), 47.2 (C-9-Fmoc), 28.0 (CH_3-*t*Bu), 23.2 (CH_3-NHAc), 20.9, 20.8, 20.6 (CH_3-Ac), 18.7 ppm ($T^γ$); ^{19}F NMR (376 MHz, $CDCl_3$): δ=−229.5 (dt, ^3J(F,5-H)=14.7 Hz, ^2J(F,6-H)=46.7 Hz), −230.6 ppm (dt, ^3J(F,5-H)=12.4 Hz, ^2J(F,6-H)=46.3 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{43}H_{54}F_2N_2O_{16}Na$ $[M+Na]^+$: 915.3338; found: 915.3334.

Fmoc-Thr(β-6F-Ac_3Gal-(1–3)-α-6F-GalNAc)-OH (22): The disaccharide **3** (130 mg, 0.15 mmol) was treated with anisole (0.4 mL, 3.66 mmol) and TFA (4 mL, 22.8 mmol). The reaction mixture was stirred for 4 h at room temperature and was subsequently co-evaporated with toluene (5×30 mL). The residue was purified by flash chromatography on silica gel (EtOAc/MeOH/AcOH 4:1:0.05) to afford **22** as a colourless amorphous solid (106 mg, 0.13 mmol, 87%), which was subjected to SPPS without further characterisation; R_f=0.43 (EtOAc/MeOH/AcOH 4:1:0.05); analytical RP-HPLC (Luna, H_2O/MeCN +0.1% TFA, 70:30→10:90, 25 min): t_R=20.18 min; HRMS (ESI-TOF): *m*/*z*: calcd for $C_{39}H_{46}F_2N_2O_{16}Na$: 859.2715 $[M+Na]^+$; found: 859.2726.

Fmoc-Thr(β-Ac_4Gal-(1–3)-α-6F-GalNAc)-O*t*Bu (4): A solution of the glycosyl acceptor **8** (200 mg, 0.33 mmol) and the donor **12** (145 mg, 0.29 mmol) in dry CH_2Cl_2 (8 mL) was stirred under argon with activated, powdered molecular sieves (4 Å, 260 mg) for 30 min at room temperature. The suspension was cooled to −20 °C and TMSOTf (16.3 μL, 0.09 mmol) in dry CH_2Cl_2 (1 mL) was added. After stirring for 25 h, the reaction mixture was neutralised with solid $NaHCO_3$ and filtered through Hyflo Supercel. The organic phase was washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. Purification by flash chromatography on silica gel (EtOAc/MeOH 20:1) afforded **4** as a colourless amorphous solid (170 mg, 0.18 mmol, 54%). R_f=0.49 (EtOAc/MeOH 20:1); analytical RP-HPLC (Luna, H_2O/MeCN 50:50→15:85, 40 min): t_R=18.79 min; $[\alpha]_D^{23}$=59.8 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, [D_6]DMSO, COSY): δ=7.89 (d, ^3J(4-H,3-H)=^3J(5-H,6-H)=8.6 Hz, 2H; 4-H-, 5-H-Fmoc), 7.61 (m, 2H; 1-H-, 8-H-Fmoc), 7.53 (d, ^3J(NH,2-H)=9.4 Hz, 1H; NH-Ac), 7.51 (d, ^3J(NH,Tα)=9.5 Hz, 1H; NH-urethane), 7.44–7.39 (m, 2H; 3-H-, 6-H-Fmoc), 7.34–7.27 (m, 2H; 2-H-, 7-H-Fmoc), 5.27 (d, ^3J(3′-H,4′-H)=3.5 Hz 1H; 4′-H), 5.02 (dd, ^3J(3′-H,4′-H)=3.5 Hz, ^3J(3′-H,2′-H)=10.4 Hz, 1H; 3′-H), 4.99–4.94 (m, 1H; 2′-H), 4.73 (d, ^3J(1′-H,2′-H)=7.7 Hz, 1H; 1′-H,), 4.62 (d, ^3J(1-H,2-H)=4.0 Hz, 1H; 1-H), 4.57–4.37 (m,

4H; 6a/b-H {4.53, 4.39}, CH_2-Fmoc {4.48, 4.38}), 4.32–4.29 (m, 1H; 9-H-Fmoc), 4.26–4.20 (m, 2H; 2-H {4.21}, T^{β} {4.19}), 4.15–4.11 (m, 2H; 6a′-H {4.13}, 5′-H {4.12}), 4.07 (dd, ^3J(Tα,Tβ) = 1.8 Hz, ^3J(Tα,NH) = 9.9 Hz, 1H; T^{α}), 4.00–3.95 (m, 2H; 5-H {3.92}, 6b′-H {3.91}), 3.92 (d, ^3J(4-H,3-H) = 2.6 Hz, 1H; 4-H), 3.60 (dd, ^3J(3-H,4-H) = 2.6 Hz, ^3J(3-H,2-H) = 11.2 Hz, 1H; 3-H), 2.18, 2.00, 1.97, 1.89 (4 × s, 12H; 4 × CH_3-Ac), 1.81 (s, 3H; CH_3-NHAc), 1.34 (s, 9H; *t*Bu), 1.12 ppm (d, ^3J(Tγ,Tβ) = 6.4 Hz, 3H; T^{γ}); ^{13}C NMR (100 MHz, [D$_6$]DMSO, DEPT, HMQC): δ = 170.1, 170.0, 169.8, 169.2, 169.1 (C=O), 156.9 (C=O-urethane), 143.8, 143.7 (C-1a-, C-8a-Fmoc), 140.9 (C-4a-, C-5a-Fmoc), 127.9, 127.8 (C-3-, C-6-Fmoc), 127.2 (C-2-, C-7-Fmoc), 125.4, 125.3 (C-1-, C-8-Fmoc), 120.3 (C-4-, C-5-Fmoc), 101.5 (C-1′), 99.4 (C-1), 83.7 (d, ^1J(F,C-6) = 165.5 Hz; C-6), 81.5 (C_q-*t*Bu), 76.9 (C-3), 74.4 (T^{β}), 70.6 (C-3′), 69.7 (C-5′), 69.8 (d, ^2J(F,C-5) = 18.8 Hz; C-5), 68.5 (C-2′), 67.5 (C-4′), 67.3 (C-4), 65.7 (CH_2-Fmoc), 61.2 (C-6′), 59.4 (T^{α}), 46.9 (C-2), 46.8 (C-9-Fmoc), 27.7 (CH_3-*t*Bu), 22.9 (CH_3-NHAc), 20.6, 20.5, 20.4 (CH_3-Ac), 19.0 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −229.8 ppm (dt, ^3J(F,5-H) = 14.3 Hz, ^2J(F,6-H) = 47.4 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{45}H_{57}FN_2O_{18}Na$ $[M+Na]^+$: 955.3490; found: 955.3470.

Fmoc-Thr(β-2F-Bn$_3$Gal-(1–3)-α-4,6-*O*-Bzn-GalNAc)-O*t*Bu (30): A solution of the glycosyl acceptor **7** (1.40 g, 2.35 mmol) and the donor **14** (1.02 g, 1.45 mmol) in dry CH_2Cl_2 (40 mL) was stirred under argon with activated, powdered molecular sieves (4 Å, 1.34 g) for 30 min at room temperature. The suspension was cooled to 0 °C and TMSOTf (50 μL, 0.277 mmol) in dry CH_2Cl_2 (1 mL) was added. After having been stirred for 20 h, the reaction mixture was diluted with CH_2Cl_2 (50 mL), neutralised with solid $NaHCO_3$ and filtered through Hyflo Supercel. The organic phase was washed with saturated aqueous $NaHCO_3$ (3 × 100 mL) and brine (2 × 100 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc 1:1) afforded **30** as a colourless amorphous solid (1.51 g, 1.35 mmol, 92%); analytical RP-HPLC (PerfectSil, H_2O/MeCN 25:75→0:100, 30 min): t_R = 16.64 min (β-anomer), t_R = 19.57 min (α-anomer); R_f = 0.33 (*c*Hex/EtOAc 1:2), $[\alpha]_D^{23}$ = 62.8 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ = 7.81–7.75 (m, 2H; 4-H-, 5-H-Fmoc), 7.65 (d, ^3J(1-H,2-H) = ^3J(8-H,7-H) = 7.4 Hz, 2H; 1-H-, 8-H-Fmoc), 7.55 (d, ^3J(3-H,4-H) = ^3J(6-H,5-H) = 7.3 Hz, 2H; 3-H-, 6-H-Fmoc), 7.44–7.22 (m, 22H; 2-H-, 7-H-Fmoc, H_{ar}), 5.75 (d, ^3J(NH,2-H) = 9.0 Hz, 1H; NH-Ac), 5.57 (d, ^3J(NH,Tα) = 10.9 Hz, 1H; NH-Fmoc), 5.51 (s, 1H; CH-Bzn), 5.03 (d, ^3J(1-H,2-H) = 3.3 Hz, 1H; 1-H), 4.92 (d, ^2J(H,H) = 11.6 Hz, 1H; CH_2-Bn), 4.89–4.70 (m, 1H; 2′-H), 4.81–4.70 (m, 2H; CH_2-Bn, 2-H {4.75}), 4.67 (d, ^2J(H,H) = 12.1 Hz, 1H; CH_2-Bn), 4.58 (d, ^2J(H,H) = 11.6 Hz, 1H; CH_2-Bn), 4.57 (s, 1H; 1′-H), 4.54–4.45 (m, 2H; CH_2-Fmoc), 4.44–4.40 (m, 2H; CH_2-Bn), 4.37–4.14 (m, 5H; 4-H {4.34}, T^{α} {4.23}, T^{β} {4.22}, 9-H-Fmoc {4.22}, 6a′/b′-H {4.18, d, ^2J(6a-H,6b-H) = 12.4 Hz}), 3.98–3.84 (m, 3H; 4′-H {3.89}, 3-H {3.88}, 6a′/b′-H {3.94, d, ^2J(6a′-H,6b′-H) = 12.1 Hz}), 3.65–3.50 (m, 5H; 5′-H {3.63}, 6a/b-H {3.62, 3.55}, 5-H {3.61}, 3′-H {3.58}), 2.00 (s, 3H; CH_3-NHAc), 1.46 (s, 9H; *t*Bu), 1.28 ppm (d, ^3J(Tγ,Tβ) = 6.3 Hz, 3H; T^{γ}); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT, HMQC): δ = 170.3, 169.9 (C=O), 156.5 (C=O-urethane), 143.8, 143.7 (C-1a-, C-8a-Fmoc), 141.3 (C-4a-, C-5a-Fmoc), 138.2, 137.9, 137.7, 137.6 (C_q-Bzn, C_q-Bn), 128.7, 128.4, 128.4, 128.2, 128.0, 127.7, 127.7 (C_{ar}-Bzn, C_{ar}-Bn), 127.6, 127.5 (C-2-, C-7-Fmoc), 127.2, 127.0 (C_{ar}-Bzn, C_{ar}-Bn), 126.4 (C-3-, C-6-Fmoc), 125.0 (C-1-, C-8-Fmoc), 120.0, 120.0 (C-4-, C-5-Fmoc), 102.8 (d, ^2J(C-1′,F) = 23.3 Hz; C-1′), 100.7 (CH-Bzn), 100.6 (C-1), 91.2 (d, ^1J(C-2′,F) = 183.4 Hz; C-2′), 83.0 (C_q-*t*Bu), 80.1 (d, ^2J(C-3′,F) = 15.8 Hz; C-3′), 76.6 (C-3), 76.4 (T^{β}), 75.8 (C-4), 74.6 (CH_2-Bn), 74.1 (d, ^3J(C-4′,F) = 8.8 Hz; C-4′), 73.7 (C-5), 73.5, 72.6 (CH_2-Bn), 69.0 (C-6′), 68.7 (C-6), 67.0 (CH_2-Fmoc), 63.7 (C-5′), 59.2 (T^{α}), 48.0 (C-2), 47.2 (C-9-Fmoc), 28.1 (CH_3-*t*Bu), 23.3 (CH_3-NHAc), 19.1 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −204.4 ppm (dd, ^2J(F,2-H) = 51.6 Hz, ^3J(F,3-H) = 12.2 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{65}H_{71}FN_2O_{14}Na$ $[M+Na]^+$: 1145.4787; found: 1147.4796.

Fmoc-Thr(β-2F-Ac$_3$Gal-(1–3)-α-Ac$_2$GalNAc)-OH (27): Anisole (0.4 mL, 3.66 mmol) and TFA (4.0 mL, 22.8 mmol) were added to a solution of the disaccharide **5** (194 mg, 0.20 mmol) in dry CH_2Cl_2 (20 mL) and the reaction mixture was stirred for 4 h at room temperature. The mixture was co-evaporated with toluene (5 × 30 mL) and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 5:0.3) to afford **27** as a colourless amorphous solid (163 mg, 0.177 mmol, 89%); analytical RP-HPLC (Luna, H_2O/MeCN 50:50→10:90, 30 min): t_R = 10.91 min; R_f = 0.77 (CH_2Cl_2/MeOH 5:0.3); $[\alpha]_D^{23}$ = 55.7 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, [D$_6$]DMSO, COSY): δ = 12.97 (br s, 1H; OH), 7.91 (d, ^3J(4-H,3-H) = ^3J(5-H,6-H) = 7.6 Hz, 2H; 4-H-, 5-H-Fmoc), 7.74 (dd, ^3J(1-H,2-H) = ^3J(8-H,7-H) = 7.4 Hz, ^3J(1-H,3-H) = ^3J(8-H,6-H) = 3.7 Hz, 2H; 1-H-, 8-H-Fmoc), 7.47 (d, ^3J(NH,2-H) = ^3J(NH,Tα) = 9.7 Hz, 2H; NH-Fmoc, NH-Ac), 7.43 (t, ^3J(3-H,4-H) = ^3J(6-H,5-H) = 7.5 Hz, 2H; 3-H-, 6-H-Fmoc), 7.33 (t, ^3J(2-H,1-H) = ^3J(7-H,8-H) = 6.8 Hz, 2H; 2-H-, 7-H-Fmoc), 5.35 (d, ^3J(4-H,3-H) = 3.1 Hz, 1H; 4-H), 5.25 (s, 1H; 4′-H), 5.21–5.18 (m, 1H; 3′-H), 4.87 (dd, ^3J(1′-H,2′-H) = 7.6 Hz, ^3J(1′-H,F) = 3.6 Hz, 1H; 1′-H), 4.72 (d, ^3J(1-H,2-H) = 3.9 Hz, 1H; 1-H), 4.58–4.39 (m, 2H; CH_2-Fmoc), 4.37–4.07 (m, 8H; 2′-H {4.34, 4.21}, 9-H-Fmoc {4.31}, T^{β} {4.25}, 2-H {4.20}, 5-H {4.18}, 5′-H {4.14}, T^{α} {4.12}, 6a′-H{4.11}), 4.01 (dd, ^2J(6a-H,6b-H) = 11.3 Hz, ^3J(6a-H,5-H) = 6.2 Hz, 1H; 6a-H), 3.96–3.79 (m, 2H; 6b-H {3.88}, 6b′-H {3.83}), 2.10, 2.06, 2.00, 1.99, 1.98 (5 × s, 15H; 5 × CH_3-Ac), 1.80 (s, 3H; CH_3-NHAc), 1.12 ppm (d, ^3J(Tγ,Tβ) = 6.4 Hz, 3H; T^{γ}); ^{13}C NMR (100 MHz, [D$_6$]DMSO, HMQC): δ = 171.7, 170.1, 170.0, 169.9, 169.8, 169.6, 169.2 (C=O), 156.9 (C=O-urethane), 143.8, 143.8 (C-1a-, C-8a-Fmoc), 140.9, 140.8 (C-4a-, C-5a-Fmoc), 127.7, 127.7 (C-3-, C-6-Fmoc), 127.1 (C-2-, C-7-Fmoc), 125.2, 125.1 (C-1-, C-8-Fmoc), 120.3, 120.2 (C-4-, C-5-Fmoc), 100.3 (d, ^2J(C-1′,F) = 22.8 Hz; C-1′), 99.2 (C-1), 88.1 (d, ^1J(C-2′,F) = 185.2 Hz; C-2′), 75.4 (C-3), 75.1 (T^{β}), 70.6 (d, ^2J(C-3′,F) = 18.5 Hz; C-3′), 69.6 (C-5), 69.6 (C-5′), 69.4 (C-4), 67.6 (d, ^3J(C-4′,F) = 8.4 Hz; C-4′), 65.4 (CH_2-Fmoc), 63.2 (C-6′), 60.7 (C-6), 58.5 (T^{α}), 47.5 (C-2), 46.9 (C-9-Fmoc), 22.7 (CH_3-NHAc), 20.8, 20.5, 20.5, 20.4, 20.3 (CH_3-Ac), 19.4 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −206.7 ppm (d, ^2J(F,2-H) = 49.4 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{43}H_{51}FN_2O_{19}K$: 957.2707 $[M+K]^+$; found: 957.2675.

Fmoc-Thr(β-2F-Bn$_3$Gal-(1–3)-α-GalNAc)-O*t*Bu (31): $NaHSO_4/SiO_2$ (760 mg) was added as the de-*O*-acetylation catalyst to a solution of the disaccharide **30** (991 mg, 0.883 mmol) in a mixture of CH_2Cl_2 and MeOH 4:1 (50 mL) and the suspension was stirred for 18 h at room temperature. The catalyst was filtered off and the filtrate was washed with saturated aqueous $NaHCO_3$ (3 × 70 mL) and brine (50 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc 1:10) afforded **31** as a colourless amorphous solid (775 mg, 0.75 mmol, 85); analytical RP-HPLC (Jupiter, H_2O/MeOH 20:80→0:100, 30 min): t_R = 13.25 min; R_f = 0.53 (*c*Hex/EtOAc 1:10); $[\alpha]_D^{23}$ = 33.4 (*c* = 1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ = 7.77 (d, ^3J(4-H,3-H) = ^3J(5-H,6-H) = 7.5 Hz, 2H; 4-H-, 5-H-Fmoc), 7.63 (d, ^3J(1-H,2-H) = ^3J(8-H,7-H) = 6.7 Hz, 2H; 1-H-, 8-H-Fmoc), 7.39 (t, ^3J(3-H,4-H) = ^3J(6-H,5-H) = 7.3 Hz, 2H; 3-H-, 6-H-Fmoc), 7.37–7.23 (m, 17H; 2-H-, 7-H-Fmoc, H_{ar}), 5.77 (d, ^3J(NH,2-H) = 9.5 Hz, 1H; NH-Ac), 6.14 (d, ^3J(NH,Tα) = 9.3 Hz, 1H; NH-Fmoc), 4.90 (d, ^2J(H,H) = 11.2 Hz, 1H; CH_2-Bn, 4.90 (d, ^3J(1-H,2-H) = 4.0 Hz, 1H; 1-H), 4.77 (d, ^2J(H,H) = 12.2 Hz, 1H; CH_2-Bn), 4.67 (d, ^2J(H,H) = 11.6 Hz, 1H; CH_2-Bn), 4.64–4.57 (m, 1H; 2-H), 4.55 (d, ^2J(H,H) = 11.4 Hz, 1H; CH_2-Bn), 4.52–4.45 (m, 2H; CH_2-Fmoc), 4.27 (t, ^3J(9-H,10-H) = 6.7 Hz, 1H; 9-H-Fmoc), 4.24–4.13 (m, 3H; T^{α} {4.23}, T^{β} {4.18}, 4-H {4.15}), 3.92–3.85 (m, 2H; 6a/b-H {3.90}, 3.84–3.79 (m, 1H; 5-H), 3.76–3.65 (m, 2H; 3-H {3.68}, 6a/b-H {3.72}), 3.61–3.52 (m, 3H; 3′-H {3.58}, 6a′/b′-H {3.56}, 5′-H {3.59}), 3.51–3.44 (m, 1H; 6a′/b′-H {3.49}), 2.01 (s, 3H; CH_3-Ac), 1.45 (s, 9H; *t*Bu), 1.28 ppm (d, ^3J(Tγ,Tβ) = 6.2 Hz, 3H; T^{γ}); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT, HMQC): δ = 170.4, 170.1 (C=O), 156.4 (C=O-urethane), 143.8, 143.7 (C-1a-, C-8a-Fmoc), 141.3 (C-4a-, C-5a-Fmoc), 137.9, 137.8, 137.6 (C_q-Bn), 128.5, 128.5, 128.3, 128.0, 127.8, 127.8, 127.6, 127.1 (C_{ar}-Bn, C-3-, C-6-Fmoc, C-2-, C-7-Fmoc), 125.0, 125.0 (C-1-, C-8-Fmoc), 120.0 (C-4-, C-5-Fmoc), 102.5 (d, ^2J(C-1′,F) = 22.6 Hz; C-1′), 100.3 (C-1), 91.4 (d, ^1J(C-2′,F) = 184.7 Hz; C-2′), 83.1 (C_q-*t*Bu), 79.8 (C-3), 74.0 (d, ^3J(C-4′,F) = 9.5 Hz; C-4′), 74.8 (CH_2-Bn), 74.0 (d, ^2J(C-3′,F) = 14.4 Hz; C-3′), 73.9 (C-5′), 73.6, 72.7 (CH_2-Bn), 69.7 (C-5), 69.6 (C-4), 69.4 (T^{β}), 68.8 (C-6′), 67.0 (CH_2-Fmoc), 63.1 (C-6), 59.0 (T^{α}), 47.5 (C-2), 47.2 (C-9-Fmoc), 28.1 (CH_3-*t*Bu), 23.3 (CH_3-NHAc), 18.9 ppm (T^{γ}); ^{19}F NMR (376 MHz, $CDCl_3$): δ = −205.1 ppm (dd, ^2J(F,2-H) = 51.6 Hz, ^3J(F,3-H) = 12.2 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{58}H_{67}FN_2O_{14}Na$: 1057.4474 $[M+Na]^+$; found: 1057.4476.

Fmoc-Thr(β-2F-Bn$_3$Gal-(1–3)-α-Ac$_2$GalNAc)-O*t*Bu (6): The disaccharide **31** (235 mg, 0.28 mmol), dissolved in a mixture of pyridine and Ac_2O (9 mL, 2:1), was stirred under argon for 20 h at room temperature. The

reaction mixture was poured into ice/water and was extracted with CH_2Cl_2 (5×50 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (2×70 mL) and brine (2×50 mL), dried ($MgSO_4$) and concentrated in vacuo. Flash chromatography on silica gel (*c*Hex/EtOAc 1:1) afforded **6** as a colourless amorphous solid (199 mg, 0.18 mmol, 78%). Analytical RP-HPLC (Jupiter, H_2O/MeOH 30:70→0:100, 40 min): t_R=21.00 min; R_f=0.37 (*c*Hex/EtOAc 1:1); $[\alpha]_D^{23}$=50.9 (c=1.0 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ=7.77 (d, ^3J(4-H,3-H)=^3J(5-H,6-H)=7.3 Hz, 2H; 4-H-, 5-H-Fmoc), 7.64 (d, ^3J(1-H,2-H)=^3J(8-H,7-H)=6.7 Hz, 2H; 1-H-, 8-H-Fmoc), 7.40 (t, ^3J(3-H,4-H)=^3J(6-H,5-H)=7.3 Hz, 2H; 3-H-, 6-H-Fmoc), 7.36–7.24 (m, 17H; 2-H-, 7-H-Fmoc, H_{Ar}), 5.83 (d, ^3J(NH,2-H)=7.4 Hz, 1H; NH-Ac), 5.51 (d, ^3J(NH,Tα)=9.2 Hz, 1H; NH-Fmoc), 5.43 (s, 1H; 5′-H), 4.90 (d, 3J(1′-H,2′-H)=4.0 Hz, 1H; 1′-H), 4.88 (d, ^2J(H,H)=11.6 Hz, 2H; CH_2-Bn, 4.76–4.70 (m, 3H; CH_2-Bn, 2′-H {4.75, 4.63}, CH_2-Bn {4.73}), 4.67–4.57 (m, 1H; 2-H, CH_2-Bn {4.64}), 4.55 (d, ^2J(H,H)=11.6 Hz, 1H; CH_2-Bn), 4.51–4.45 (m, 4H; CH_2-Bn {4.50}, CH_2-Fmoc {4.48}, 1-H {4.48}), 4.40 (d, ^2J(H,H)=11.6 Hz, 1H; CH_2-Bn), 4.27 (t, ^3J(9-H,10-H)=6.7 Hz, 1H; 9-H-Fmoc), 4.24–4.15 (m, 3H; T$^\alpha$ {4.23}, 6a/b-H {4.19}, T$^\beta$ {4.19}, 4.12–4.06 (m, 1H; 5-H), 3.94 (dd, ^2J(6a-H,6b-H)=11.1 Hz, ^3J(6a/b-H,5-H)=8.0 Hz, 1H; 6a/b-H), 3.90 (t, ^3J(4′-H,3′-H)=^4J(4′-H,F)=2.8 Hz, 1H; 4′-H), 3.80 (dd, ^3J(3-H,2-H)=11.1 Hz, ^3J(3-H,4-H)=2.8 Hz, 1H; 3-H), 3.60–3.52 (m, 4H; 6a′/b′-H {3.55}, 3′-H {3.54}, 4-H {3.53}), 2.10, 2.03 (2×s, 6H; CH_3-Ac), 1.92 (s, 3H; CH_3-NHAc), 1.46 (s, 9H; *t*Bu), 1.30 ppm (d, 3H; ^3J(Tγ,Tβ)=6.2 Hz, T$^\gamma$); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT, HMQC): δ=170.5, 170.4, 170.2, 169.9 (C=O), 156.5 (C=O-urethane), 143.7, 143.7 (C-1a-, C-8a-Fmoc), 141.3 (C-4a-, C-5a-Fmoc), 138.4, 137.9, 137.8 (C_q-Bn), 128.4, 128.2, 127.9, 127.9, 127.8, 127.7, 127.5, 127.1 (C_{ar}-Bn, C-3-, C-6-Fmoc, C-2-, C-7-Fmoc), 125.0, 125.0 (C-1-, C-8-Fmoc), 120.0 (C-4-, C-5-Fmoc), 102.2 (d, ^2J(C-1′,F)=24.0 Hz; C-1′), 100.2 (C-1), 91.5 (d, ^1J(C-2′,F)=182.3 Hz; C-2′), 83.1 (C_q-*t*Bu), 79.8 (d, ^2J(C-3′,F)=15.5 Hz; C-3′), 77.0 (T$^\beta$), 76.2 (C-3), 74.6 (CH_2-Bn), 74.0 (d, ^3J(C-4′,F)=8.7 Hz; C-4′), 73.6 (CH_2-Bn, C-4), 72.4 (CH_2-Bn), 69.4 (C-5′), 68.2 (C-6′), 68.2 (C-5), 67.1 (CH_2-Fmoc), 63.4 (C-6), 59.1 (T$^\alpha$), 48.4 (C-2), 47.2 (C-9-Fmoc), 28.1 (CH_3-*t*Bu), 23.3 (CH_3-NHAc), 20.9, 20.7 (CH_3-Ac), 18.7 ppm (T$^\gamma$); ^{19}F NMR (376 MHz, $CDCl_3$): δ=−204.4 ppm (dd, ^2J(F,2-H)=51.6 Hz, ^3J(F,3-H)=12.2 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{62}H_{71}FN_2O_{16}Na$: 1141.4685 [*M*+Na]$^+$; found: 1141.4691.

Fmoc-Thr(β-2F-Bn$_3$Gal-(1–3)-α-Ac$_2$GalNAc)-OH (32): Anisole (0.8 mL, 7.32 mmol) and TFA (8.0 mL, 45.6 mmol) were added to a solution of disaccharide **6** (282 mg, 0.25 mmol) in dry CH_2Cl_2 (20 mL) and the reaction mixture was stirred for 2 h at room temperature. The mixture was co-evaporated with toluene (5×30 mL) and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 5:0.3) to afford **27** as a colourless amorphous solid (188 mg, 0.18 mmol, 70%); analytical RP-HPLC (Luna, H_2O/MeCN 50:50→10:90, 30 min): t_R=26.07 min; $[\alpha]_D^{23}$=120.4 (c=1.0 in $CHCl_3$); R_f=0.09 (CH_2Cl_2/MeOH 5:0.3); ^{1}H NMR (400 MHz, $CDCl_3$, COSY): δ=12.94 (s, 1H; COOH), 8.32 (s, 1H; OH), 7.90 (d, ^3J(4-H,3-H)=^3J(4-H,6-H)=7.5 Hz, 2H; 4-H-, 5-H-Fmoc), 7.73 (d, ^3J(1-H,2-H)=^3J(8-H,7-H)=7.5 Hz, 2H; 1-H-, 8-H-Fmoc), 7.48–7.38 (m, 4H; 3-H-, 6-H-Fmoc, NH-Fmoc {7.45}, NH-Ac {7.40}), 7.38–7.25 (m, 17H; 2-H-, 7-H-Fmoc, H_{ar}), 5.31 (d, ^3J(4-H,3-H)=3.0 Hz, 1H; 4-H), 4.79–4.68 (m, 3H; CH_2-Bn {4.77}, CH_2-Bn {4.72}, 1-H {4.71}), 4.66–4.59 (m, 2H; 1′-H {4.71}, CH_2-Bn {4.62}), 4.55–4.41 (m, 5H; CH_2-Bn {4.53}, CH_2-Fmoc {4.51}, CH_2-Bn {4.52, 4.44, d, ^2J(H,H)=11.9 Hz}), 4.33–4.22 (m, 2H; 9-H-Fmoc {4.31}, T$^\beta$ {4.27}), 4.30–4.13 (m, 1H; 2′-H {4.28}, {4.15}), 4.19–4.11 (m, 2H; 2-H {4.18}, T$^\alpha$-{4.14}), 4.07–3.99 (m, 3H; 5-H {4.04}, 4′-H {4.03}, 6a-H {4.02}), 3.91–3.78 (m, 2H; 3-H {3.86}, 6b-H {3.81}), 3.76–3.67 (m, 2H; 5′-H {3.73}, 3′-H {3.71}), 3.55–3.46 (m, 2H; 6a′/b′-H {3.51}), 2.02, 1.97, (2×s, 6H; CH_3-Ac), 1.81 (s, 3H; CH_3-NHAc), 1.12 ppm (d, ^3J(Tγ,Tβ)=6.4 Hz, 3H; T$^\gamma$); ^{13}C NMR (100 MHz, $CDCl_3$, DEPT, HMQC): δ=171.7, 170.0, 169.8 (C=O), 156.9 (C=O-urethane), 143.8, 143.7 (C-1a-, C-8a-Fmoc), 140.8, 140.8 (C-4a-, C-5a-Fmoc), 138.6, 138.3, 138.2 (C_{Ar}-Bn), 128.3, 128.2, 127.6, 127.5, 127.4, 127.1 (C-2-, C-7-, C-3-, C-6-Fmoc, C_{Ar}-Bn), 125.2, 125.1 (C-1-, C-8-Fmoc), 120.2, 120.2 (C-4-, C-5-Fmoc), 101.2 (d, ^2J(C-1′,F)=23.2 Hz; C-1′), 99.3 (C-1), 90.8 (d, ^1J(C-2′,F)=183.4 Hz; C-2′), 79.1 (d, ^2J(C-3′,F)=12.1 Hz; C-3′), 75.1 (T$^\beta$), 75.0 (C-3), 74.1 (CH_2-Bn), 73.8 (C-5), 72.7 (CH_2-Bn), 72.6 (C-5′), 71.0 (CH_2-Bn), 67.9 (C-6′), 67.4 (C-4′), 65.4 (CH_2-Fmoc), 63.1 (C-6), 58.3 (T$^\alpha$), 47.1 (C-2), 46.8 (C-9-Fmoc), 22.7 (CH_3-NHAc), 20.7, 20.5 (CH_3-Ac), 18.5 ppm (T$^\gamma$); ^{19}F NMR (376 MHz, $CDCl_3$): δ=−204.8 ppm (d, ^2J(F,2-H)=44.4 Hz); HRMS (ESI-TOF): *m*/*z*: calcd for $C_{58}H_{63}FN_2O_{16}Na$: 1085.4060 [*M*+Na]$^+$; found: 1085.4034.

General procedure for the automated solid-phase glycopeptide synthesis: Peptide syntheses were carried out with an Applied Biosystems ABI 433 A peptide synthesiser (standard programme Fastmoc 0.1 mmol) with use of pre-loaded Fmoc-Pro-Trt-Tentagel S resin (0.10 mmol, loading: 0.21–0.23). For the coupling reactions, the amino acids Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Asp-OH, Fmoc-Gly-OH, Fmoc-His-(Trt)-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH and Fmoc-Val-OH were employed. 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 HBTU (1 mmol), HOBt (1 mmol) and DIPEA (2 mmol) in DMF (20–30 min 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 blocks was performed with the use of HATU (1.1 equiv with respect to the glycosyl amino acid), HOAt (1.1 equiv) and NMM (2.2 equiv) for activation (8 h vortex). After coupling of the remaining five amino acids by the standard procedure, the triethylene glycol spacer (1 mmol, 10 equiv based on the loaded resin) was coupled by use of HBTU (1 mmol), HOBt (1 mmol) and DIPEA (2 mmol) in DMF (20–30 min vortex) and the N-terminal Fmoc group was removed by treatment with piperidine (20%) in NMP. Detachment from the resins and simultaneous removal of all side chain protecting groups was performed by shaking with TFA (10 mL), TIS (1.0 mL) and H_2O (1.0 mL) in a Merrifield glass reactor for 3 h. The solutions were filtered, the resins were washed with TFA (3×5 mL) and CH_2Cl_2 (3×5 mL), and the combined TFA solutions were concentrated in vacuo to a volume of 0.5 mL. After co-evaporation with toluene (5×20 mL), the crude products were dissolved in H_2O and subjected to lyophilisation.

Glycopeptide 38: The MUC1 glycopeptide analogue **38** was prepared by the general procedure from Fmoc-Pro-Trt-Tentagel S resin (435 mg, 0.10 mmol; loading: 0.23 mmol g^{-1}) and the glycosylated threonine building block **22** (106.2 mg, 0.127 mmol). The crude product was purified by RP-HPLC (Luna, MeOH/H_2O +0.1% TFA, 10:90→100:0, 40 min, t_R=30.1 min) to afford the partially protected glycopeptide **35** as a colourless amorphous solid (77 mg, 0.03 mmol, 29%). Glycopeptide **35** (66 mg, 0.026 mmol) was dissolved in aqueous NaOH (5 mL, pH 10.5) and the solution was being stirred for 2 days at room temperature. The solution was neutralised by addition of AcOH (3 drops), concentrated in vacuo and co-evaporated with toluene (3×10 mL). Purification by RP-HPLC (Jupiter, MeOH/H_2O +0.1% TFA, 10:90→100:0, 30 min, t_R=26.6 min) and lyophilisation afforded the glycopeptide **37** as a colourless amorphous solid (39 mg, 0.016 mmol, 55%); analytical RP-HPLC (Luna, MeOH/H_2O +0.1% TFA, 10:90→100:0, 40 min): t_R=18.0 min; $[\alpha]_D^{23}$=−166.6 (c=1.0 in H_2O); ^{1}H NMR (400 MHz, D_2O, COSY): δ=8.62 (d, ^3J(Hε,Hδ)=1.4 Hz, 1H; H$^\varepsilon$), 7.32 (d, ^3J(Hδ,Hε)=1.2 Hz, 1H; H$^\delta$), 5.01 (d, ^3J(1-H,2-H)=3.8 Hz, 1H; 1-H), 4.83–4.36 (m, 7H; D$^\alpha$ {4.73}, H$^\alpha$ {4.69}, 6a′-H{4.68}, 6a-H {4.67}, T$^\alpha$* {4.66}, R$^\alpha$ {4.65}, A$_3^\alpha$ {4.64}), 4.61–4.51 (m, 5H; A$_2^\alpha$ {4.59}, 6b′-H {4.58}, A$_4^\alpha$ {4.57}, 6b-H {4.56}, S$_1^\alpha$ {4.52}), 4.48–4.19 (m, 17H; S$_2^\alpha$ {4.67}, 1′-H {4.47}, V$^\alpha$ {4.42}, P$_{1-5}^\alpha$ {4.41, 4.39, 4.38, 4.35, 4.33}, T$_{1-2}^\alpha$ {4.36, 4.31}, T$^\beta$* (4.31}, 5-H {4.28}, 2-H {4.24}, A$_1^\alpha$ {4.23}, 4-H {4.22}, T$_{1-2}^\beta$ {4.21, 4.20}), 4.03 (d, ^3J(3-H,4-H)=4.8 Hz, 1H; 3-H), 4.00–3.95 (m, 5H; G$^\alpha$ {3.99, 3.97}, 4′-H {3.95}), 3.93–3.74 (m 17H; 5′-H {3.91}, S$_{1,2}^\beta$ {3.87, 3.83}, P$_{1-4}^\delta$ {3.85, 3.81, 3.78, 3.75}, CH_2O-spacer {3.75, 3.74}), 3.69–3.65 (m, 10H; P$_5^\delta${3.69}, CH_2O-spacer {3.69, 3.68, 3.67, 3.64}, 3.62 (dd, ^3J(3′-H,4′-H)=3.2 Hz, ^3J(3′-H,2′-H)=10.1 Hz, 1H; 3′-H), 3.51 (dd, ^3J(2′-H,1′-H)=7.8 Hz, ^3J(2′-H,3′-H)=9.9 Hz, 1H; 2′-H), 3.31 (dd, ^3J(Hβa,Hα)=5.5 Hz, ^2J(Hβa,Hβb)=15.5 Hz, 1H; H$^{\beta a}$), 3.21–3.16 (m 5H; R$^\delta$ {3.2}, H$^\beta$ {3.19} CH_2NH_2-spacer {3.17}), 3.00–2.87 (m, 2H; D$^\beta$), 2.79–2.63 (m, 2H; CH_2CO-spacer), 2.38–2.21 (m, 6H; P$_{1-3}^\beta$ {2.34, 2.30, 2.24}, 2.12–1.84 (m, 16H; V$^\beta$ {2.10}, P$_{1-5}^\gamma$ {2.08, 2.05, 2.00, 1.94, 1.93}, CH_3-NHAc {2.00}, P$_{4,5}^\beta$ {1.92}, R$^{\beta a}$ {1.84}), 1.75–1.68 (m, 3H; R$^{\beta b}$ {1.72}, R$^\gamma$ {1.66}), 1.39–1.30 (m, 12H; A$_{1-4}^\beta$ {1.38, 1.36, 1.34, 1.32}), 1.26 (d, ^3J(Tγ,Tβ)=6.3 Hz, 3H; T$^\gamma$*), 1.20 (d, ^3J(Tγ,Tβ)=6.5 Hz, 3H; T$_1^\gamma$), 1.18

(d, ^3J(Tγ,Tβ) = 6.4 Hz, 3 H T_2^{γ}), 0.97 (d, ^3J(Vγ,Vβ) = 4.9 Hz, 3 H V^{γ}), 0.96 ppm (d, ^3J(Vγ,Vβ) = 4.8 Hz, 3 H V^{γ}); ^{13}C NMR (100 MHz, D_2O, DEPT, HMQC): δ = 176.5, 175.8, 174.9, 174.5, 174.4, 174.0, 173.7, 173.6, 173.5, 173.1, 173.0, 172.7, 172.5, 172.4, 172.0, 171.9, 171.5, 171.3, 171.1, 170.9, 170.7 (C=O), 156.7 (C=NH), 133.4 (H^{ε}), 128.4 (H^{γ}), 117.3 (H^{δ}), 104.5 (C-1′), 99.2 (C-1), 83.7 (d, ^1J(F,C-6) = 164.4 Hz; C-6), 83.2 (d, ^1J(F,C-6) = 165.1 Hz; C-6′), 77.5 (T^{β}*), 76.9 (C-3), 73.07 (d, ^2J(F,C-5′) = 20.4 Hz; C-5′), 72.3 (C-3′), 70.4 (C-2′), 69.5 (C-5), 69.6, 69.5, 69.5, 69.4 (CH_2O-spacer), 68.5 (d, ^3J(F,C-4) = 9.0 Hz; C-4), 68.1 (d, ^3J(F,C-4′) = 7.1 Hz; C-4′), 67.0 (T_1^{β}), 66.3 (T_2^{β}), 66.0 (CH_2-spacer), 61.4, 61.1 ($S_{1,2}^{\beta}$), 60.8, 60.5, 60.1, 60.0 (P_{1-5}^{α}), 59.4 (V^{α}), 59.9, 58.8 ($T_{1,2}^{\alpha}$), 57.0 (T^{α}*), 55.6, 55.0 ($S_{1,2}^{\alpha}$), 52.3 (H^{α}), 51.1 (R^{α}), 50.1 (D^{α}), 49.6 (C-2), 48.2, (A^{α}), 48.1, 47.9, 47.8, (P_{1-3}^{δ}), 47.7, 47.6 (A^{α}), 47.4, 47.3 ($P_{4,5}^{\delta}$), 42.4, 42.3 ($G_{1,2}^{\alpha}$), 40.5 (R^{δ}), 39.1 (CH_2NH_2-spacer), 34.9 (D^{β}), 34.0 (CH_2CONH-spacer), 30.1 (V^{β}), 29.6, 29.3, 29.2, 28.7 (P_{1-4}^{β}), 27.5 (R^{β}), 26.2 (H^{β}), 24.7, 24.6, 24.5, 24.3 (P_{1-5}^{γ}), 23.9 (R^{γ}), 22.3 (CH_3-NHAc), 18.8, 18.7 (T^{γ}), 18.5 (V^{γ}), 18.2 (T^{γ}*), 17.8 (V^{γ}), 16.3, 15.3, 15.2, 15.1 ppm (A_{1-4}^{β}); ^{19}F NMR (376 MHz, D_2O): δ = −229.4 ppm (m, 2F; {-229.4 (dt, ^3J(F,H) = 12.7 Hz, ^2J(F,H) = 48.5 Hz}); MALDI-TOF-MS (dhb, positive ion mode), *m*/*z*: calcd for $C_{103}H_{166}F_2N_{27}O_{40}$: 2460.57 $[M+H]^+$; found: 2460.90; HRMS (ESI-TOF), *m*/*z*: calcd for $C_{43}H_{55}FN_2O_{17}Na$: 913.3385 $[M+Na]^+$; found: 913.3383.

Glycopeptide 39: The MUC1 glycopeptide analogue **39** was prepared by the general procedure from Fmoc-Pro-Trt-Tentagel S resin (476 mg, 0.10 mmol; loading: 0.21 mmol g^{-1}) and the glycosylated threonine building block **27** (225.3 mg, 0.245 mmol). The crude product was purified by RP-HPLC (Jupiter, MeOH/H_2O + 0.1 % TFA, 10:90→100:0, 40 min, t_R = 24.4 min) to afford the partially protected glycopeptide **36** as a colourless amorphous solid (55.9 mg, 21 %); t_R = 21.3 min (Phenomenex Luna, grad.: CH_3CN/H_2O + 0.1 % TFA (5:95)→(40:60), 30 min →(100:0), 10 min). A solution of the partially protected glycopeptide **36** in dry MeOH (20 mL) was carefully adjusted to pH 9.5 by addition of small portions of NaOMe and was stirred for 16 h at room temperature. The solution was neutralised by addition of AcOH (3 drops), concentrated in vacuo and co-evaporated with toluene (3 × 10 mL). Purification by RP-HPLC (Luna, MeCN/H_2O + 0.1 % TFA, 5:95→40:60, 30 min→100:0, 10 min, t_R = 17.2 min) and lyophilisation afforded the glycopeptide **39** as a colourless amorphous solid (31 mg, 0.012 mmol, 60 %);

The MUC1 glycopeptide analogue **39** was also prepared by the general procedure from Fmoc-Pro-Trt-Tentagel S resin (454 mg, 0.10 mmol; loading: 0.22 mmol g^{-1}) and the glycosylated threonine building block **32** (208 mg, 0.195 mmol). Without further purification the crude product was dissolved in degassed MeOH (30 mL) under argon and treated with a catalytic amount of $Pd(OAc)_2$. The reaction flask was purged with hydrogen and the reaction mixture was stirred under hydrogen for 12 h. The mixture was filtered through Hyflo Supercel, which was afterwards washed with MeOH (5 × 50 mL). The solvent was evaporated in vacuo, the residue was again dissolved in dry MeOH (30 mL), and the pH of the solution was adjusted to pH 9 with a freshly prepared NaOMe solution (1 %). After the mixture had been stirred for 18 h at room temperature, the reaction was stopped by addition of AcOH (5 drops) and the solvents were evaporated in vacuo. Purification by RP-HPLC (Luna, CH_3CN/H_2O + 0.1 % TFA, 5:95→40:60, 30 min→100:0, 10 min, t_R = 17.2 min) and lyophilisation afforded the glycopeptide **39** as a colourless amorphous solid (83.1 mg, 0.033 mmol, 34 %); analytical RP-HPLC (Luna, MeCN/H_2O + 0.1 % TFA, 5:95→40:60, 30 min→100:0, 10 min): t_R = 13.2 min; $[\alpha]_D^{23} = -89.4$ (*c* = 1.0 in H_2O); ^{1}H NMR (400 MHz, [D_6]DMSO, COSY, TOCSY, NOESY): δ = 12.44 (br s, 2H; COOH), 8.96 (s, 1H; H^{δ}), 8.34–8.18 (m, 4H; A_1^{NH} {8.31, d, ^3J(NH,Aα) = 7.0 Hz}, D^{NH} {8.25, d, ^3J(NH,Dα) = 7.7 Hz}, $G_{1,2}^{NH}$ {8.21}), 8.14 (d, ^3J(NH,Aα) = 6.8 Hz, 3H; $A_{(2,3)}^{NH}$), 8.13 (d, ^3J(NH,Aα) = 7.0 Hz, 1H; A_4^{NH}), 8.07 (d, ^3J(NH,Hα) = 7.2 Hz, 1H; H^{NH}), 8.04–7.88 (m, 4H; T^{NH}* {8.00}, R^{NH} {7.97}, V^{NH} {7.91, d, ^3J(NH,Vα) = 7.2 Hz}, R^{NH} {7.91}), 7.86–7.72 (m, 3H; $S_{1,2}^{NH}$ {7.78}, T_1^{NH} {7.75, d, ^3J(NH,Tα) = 7.0 Hz}), 7.52 (t, ^3J(NH,Hδ) = 5.5 Hz, ^3J(NH,Hε) = 5.5 Hz, 1H; $NH^{imidazole}$), 7.38 (s, 1H; $R^{NH-guanidine}$), 7.34 (d, ^3J(NH,Tα) = 8.0 Hz, 1H; T_2^{NH}), 6.99 (d, ^3J(NH,2-H) = 9.3 Hz, 1H; NH-Ac)), 4.72 (d, ^3J(1-H,2-H) = 2.5 Hz, 1H; 1-H), 4.60–4.35 (m, 10H; 1′-H {4.56}, H^{α} {4.55}, $A_{2,3}^{\alpha}$ {4.51, 4.48}, D^{α} {4.50}, R^{α} {4.49}, V^{α} {4.48}, T^{α}* {4.41}, S_1^{α} {4.37}, S_2^{α} {4.36}), 4.34–4.01 (m, 13H; 2-H {4.23}, 2′-H {4.20, 4.07}, T_1^{α} {4.18}, T_2^{α} {4.17}, T^{β}* {4.09}, A_1^{α} {4.24}, $P_{(1-5)}^{\alpha}$ {4.27, 4.25, 4.21}, A_4^{α} {4.15}, T_1^{β} {4.04}), 3.97–3.91 (m, 2H; T_2^{β} {3.93}, 4-H {3.92}), 3.89–3.30 (m, 34H; G_1^{α} {3.85}, G_2^{α} {3.83}, 5-H {3.70}, 4′-H {3.69}, 3-H {3.66}, 3′-H {3.51}, 6a/b-H {3.51}, P_{1-5}^{δ} {3.62, 3.55, 3.50, 3.45}, CH_2-spacer {3.60, 3.58}, CH_2O-spacer {3.54, 3.48}, 6a′/b′-H {3.45}), 3.18–2.92 (m, 6H; $H^{\beta a}$ {3.14, dd, ^2J(Hβa,Hβb) = 15.9 Hz, ^3J(Hβa,Hα) = 5.0 Hz}, R^{δ} {3.09}, $H^{\beta a}$ {2.97}, CH_2NH_2-spacer {2.97}), 2.73 (dd, ^2J(Dβa,Dβb) = 16.1 Hz, ^3J(Dβa,Dα) = 5.3 Hz, 1H; $D^{\beta a}$), 2.62–2.52 (m, 3H; $D^{\beta b}$ {2.53}, CH_2CO-spacer {2.52}), 2.19–1.66 (m, 24H; P_{1-5}^{β} {2.13, 1.99}, P_{1-5}^{γ} {1.92, 1.89, 1.86, 1.82}, CH_3-NHAc {1.79}, $R^{\beta a}$ {1.69}), 1.52 (br s, 1H; R^{γ}), 1.21 (d, ^3J(A1β,A1α) = 7.2 Hz, 3H; A_1^{β}), 1.19 (d, ^3J(A2–4β,A2–4α) = 7.0 Hz, 9H; $A_{(2-4)}^{\beta}$), 1.13 (d, ^3J(Tγ,Tβ) = 6.2 Hz, 3H; T$^{\gamma}$*), 1.03 (d, ^3J(T1γ,T1β) = 6.4 Hz, 3H; T_1^{γ}), 1.00 (d, ^3J(T2γ,T2β) = 6.3 Hz, 3H; T_2^{γ}), 0.90 (d, ^3J(Vγa,Vβ) = 6.6 Hz, 3H; $V^{\gamma a}$), 0.84 ppm (d, ^3J(Vγb,Vβ) = 6.6 Hz, 3H; $V^{\beta b}$); ^{13}C NMR (100 MHz, [D_6]DMSO, HMQC): δ = 173.2, 172.5, 172.2, 172.0, 171.9, 171.9, 171.7, 171.3, 171.1, 170.9, 170.4, 170.3, 170.2, 170.0, 169.5, 169.4, 169.3, 168.8 (C=O), 156.7 (C=NH), 133.8 (H^{ε}), 129.2 (H^{γ}), 117.1 (H^{δ}), 102.2 (C-1′), 98.6 (C-1), 91.6 (d, ^1J(C-2′,F) = 175.3 Hz; C-2′), 77.9 (C-3), 75.2 (C-5′), 74.4 (T^{β}*), 71.6 (C-5), 71.5 (d, ^2J(C-3′,F) = 15.1 Hz; C-3′), 68.7 (C-4′), 67.9 (C-4), 67.0, 69.7, 69.6 (CH_2O-spacer), 66.8 (T_2^{β}), 66.7, 66.3 (CH_2-spacer), 66.2 (T_1^{β}), 61.8 ($S_{1,2}^{\beta}$), 60.8 (C-6′), 60.2 (C-6), 59.8, 59.5, 59.2, 59.1 (P_{1-4}^{α}), 59.3 ($T^{\alpha*}$), 58.5 (P_5^{α}), 58.0 (T_2^{α}), 57.7 (T_1^{α}), 57.2 (V^{α}), 54.9 (S_1^{α}), 54.7 (S_2^{α}), 51.4 (H^{α}), 50.1 (D^{α}), 49.5 (R^{α}), 48.4 (A_1^{α}), 47.1 (C-2), 47.0, 46.8, 46.7, 46.5 (P_{1-5}^{δ}), 46.3, 46.3 (A_{2-4}^{α}), 42.0 ($G_{1,2}^{\alpha}$), 40.6 (R^{δ}), 38.6 (CH_2NH_2-spacer), 35.5 (D^{β}), 34.3 (CH_2-spacer), 31.0 (V^{β}), 29.3, 29.1, 29.1, 29.0, 28.6 (P_{1-5}^{β}), 28.4 (R^{β}), 26.9 (H^{β}), 24.6, 24.5, 24.4, 24.3 (P_{1-5}^{γ}), 24.4 (R^{γ}), 22.8 (CH_3-NHAc), 19.8 (T_1^{γ}), 19.6 (T_2^{γ}), 19.2 ($V^{\gamma a}$), 18.1 ($V^{\gamma b}$), 18.1 (T$^{\gamma}$*), 17.4 (A_1^{β}), 16.9, 16.8, 16.6 ppm (A_{2-4}^{β}); ^{19}F NMR (376 MHz, [D_6]DMSO): δ = −205.4 ppm (dd, ^2J(F,2-H) = 51.9 Hz, ^3J(F2,3-H) = 14.6 Hz); MALDI-TOF-MS, (dhb, positive ion mode): *m*/*z*: calcd for $C_{103}H_{167}FN_{27}O_{41}$: 2458.58 $[M+H]^+$; found: 2458.29; HRMS (ESI-TOF): *m*/*z*: calcd for $C_{103}H_{168}FN_{27}O_{41}$ $[M+2H]^{2+}$: 1229.0890; found: 1229.0933.

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