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A convenient preparation of several *N*-linked glycoamino acid building blocks for efficient solid-phase synthesis of glycopeptides

Jeroen van Ameijde, H. Bauke Albada and Rob M. J. Liskamp *

Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, NL-3508 TB Utrecht, The Netherlands. E-mail: R.M.J. Liskamp@pharm.uu.nl; Fax: (+31)30-2536655

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A convenient, high yielding route for the preparation of several Boc- and Fmoc-protected *N*-linked glycopeptide monomers is presented. These building blocks can be used for the solid-phase synthesis of glycopeptides or glycopeptidomimetics, which is exemplified by the preparation of an *N*-linked dodecaglycopeptide Ac–(GlyProAsn[Gal])$_4$–NH$_2$, a potential collagen mimic.

Introduction

Among post-translational modifications of peptides and proteins, two are especially prominent, namely phosphorylation and glycosylation. We have a long-standing interest in phosphopeptides with respect to methods for their preparation and investigation of their behaviour.[1] Like phosphorylation, glycosylation has a profound influence on the properties of peptides and proteins. This is, for example, nicely illustrated by the 'anti-freeze' peptides[2] and collagen proteins containing carbohydrate moieties.[3]

As part of a programme towards inhibitors of the 'food channels' of pathogenic bacteria,[4] we were interested in finding efficient ways to connect carbohydrates to amino acids, leading to glycoamino acids. Such building blocks, especially when easily available, can be of great value for the solid-phase synthesis of glycopeptides and glycopeptidomimetics. Diversity of building blocks is also an interesting aspect, since this will open up possibilities for combinatorial chemistry applications.

We were especially interested in preparing building blocks containing '*N*-linked' carbohydrates, since this would avoid the sometimes relatively difficult glycosidic coupling reaction. In addition, as a result of the considerable similarity between an *O*-linked glycoamino acid/peptide and an *N*-linked amino acid/peptide (Fig. 1), the biological and structural properties of the resulting glycopeptides are likely to be maintained.

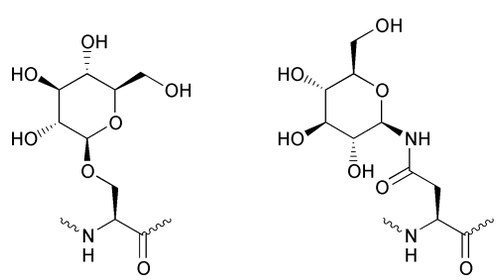

Fig. 1 An '*O*-linked' *vs.* an '*N*-linked' carbohydrate-containing amino acid.

N-Glycopeptides and *N*-glycoamino acids have been prepared before by various methods.[5] However, we present here a method in which *all* steps in the prepared glycoamino acids have high yields and a large variety of glycopeptide building blocks can be prepared. Convenient, high yielding methods are indispensable for solid-phase and combinatorial chemistry purposes, since an excess of building blocks is used. To demonstrate the suitability of the use of these glycoamino acid building blocks in solid-phase synthesis we have prepared a glycopeptide containing four carbohydrate moieties.

Results and discussion

Inspired by the excellent recent method of Soli *et al.*[6] for the preparation of carbohydrate derivatives containing an anomeric azide, the anomeric amine of glucose tetra-acetate (**4a**) obtained after reduction of the glucose azide **3a** was subjected to a BOP-coupling† with Boc-protected amino acid derivatives **5a**–**c** to furnish the glucose-amino acid adducts **6a**–**c** in excellent yields. Coupling of analogously prepared galactose and lactose (a disaccharide) amine derivatives worked equally well, giving rise to derivatives **6d**–**i** in good to very good yields (71–89%, Scheme 1).

These encouraging results suggested that this route could also be applied to the synthesis of Fmoc-protected building blocks. Moreover, coupling to the β- (Asp) or γ-carboxylic acid moiety (Glu) was now envisaged, so that the carbohydrate moiety will be attached at the side chain in order to obtain a true *N*-linked building block for glycopeptide synthesis (Fig. 1).

Starting from Fmoc-Asp(OBn)-OH (**7a**) and Fmoc-Glu(OBn)-OH (**7b**) the corresponding *tert*-butyl esters Fmoc-Asp(OH)-O-*t*Bu (**9a**) and Fmoc-Glu(OH)-O-*t*Bu (**9b**) were prepared (Scheme 2). Choice of the *tert*-butyl ester is mandatory because it can be hydrolyzed under acidic conditions which are tolerated by the acetate esters of the carbohydrate residue. Hydrogenolysis of the benzyl ester **8a** and **8b** was followed by coupling of the resulting side chain carboxylic acids with anomeric amino sugars to give the fully protected building blocks. Coupling of the amino sugars **4a**–**c** to the Fmoc amino acid derivatives **9** was more sluggish (24 hours) than coupling to the Boc amino acid derivatives **5** (3 hours). Despite this, the yields of products **10** were good (60–80%). Finally, treatment of the *tert*-butyl esters **10** with TFA in the presence of triisopropylsilane (TIS) as a scavenger afforded the *N*-glycopeptide building blocks **11a**–**h**, which can be directly used in a solid-phase synthetic procedure (Scheme 2).

In order to demonstrate the applicability of the Fmoc-protected *N*-linked glycopeptide building blocks in solid-phase

† BOP = (benzotriazol-1-yloxy)tris(dimethylamine)phosphonium hexafluorophosphate.

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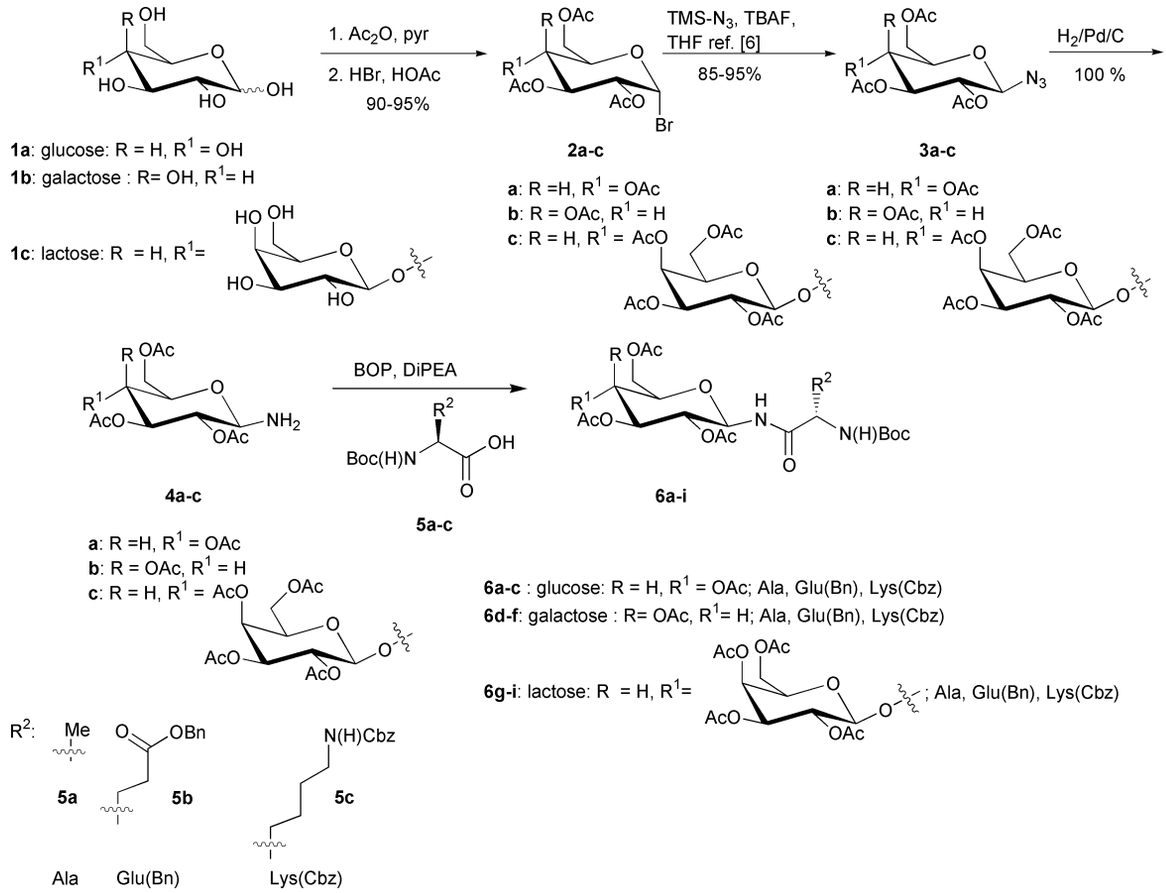


Scheme 1 Synthesis of Boc-protected glycoamino acids.

synthesis, a number of short glycopeptide sequences were prepared on Argogel® S RAM Fmoc resin. These glycopeptides, which are *N*-linked analogues of the glycopeptides prepared by Bächinger *et al.*,[3] will be investigated for collagen-like properties. An example of the synthesis of an *N*-linked glycopeptide (Ac-(GlyProAsn[Gal])$_4$NH$_2$) is depicted in Scheme 3. It was found that the coupling of the glycopeptide monomers proceeded with greater difficulty than that of ordinary amino acid derivatives. However, it turned out to be possible to perform these condensations using HATU,‡ combined with three equivalents of monomer. After completion of the desired sequences, the acetyl protecting groups on the carbohydrate moieties were removed on resin, using a 2% solution of sodium methoxide in methanol, followed by an aqueous washing step. Cleavage of the *N*-linked glycopeptides was carried out using TFA (Scheme 3) to afford **12** in reasonable purity. Complete purification of the glycopeptide could be achieved using preparative HPLC (Fig. 2).

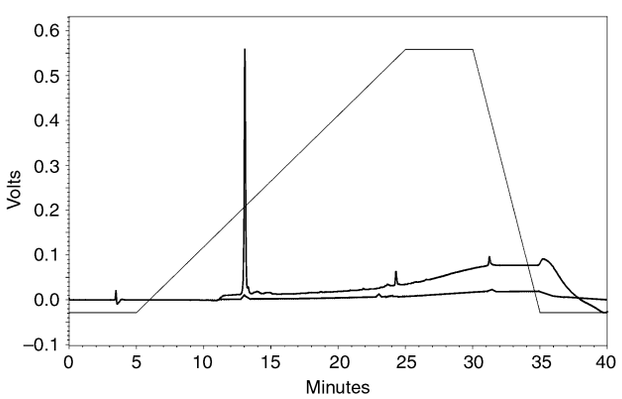


Fig. 2 HPLC chromatogram of the purified collagen mimic Ac–(GlyProAsn[Gal])$_4$–NH$_2$ (**12**).

Conclusions

Carbohydrate derivatives bearing an amino group at the anomeric center could be coupled to a carboxylic acid functionality of both Boc- and Fmoc-protected amino acids in good yields. This led to a considerable number of *N*-linked glycopeptide building blocks.

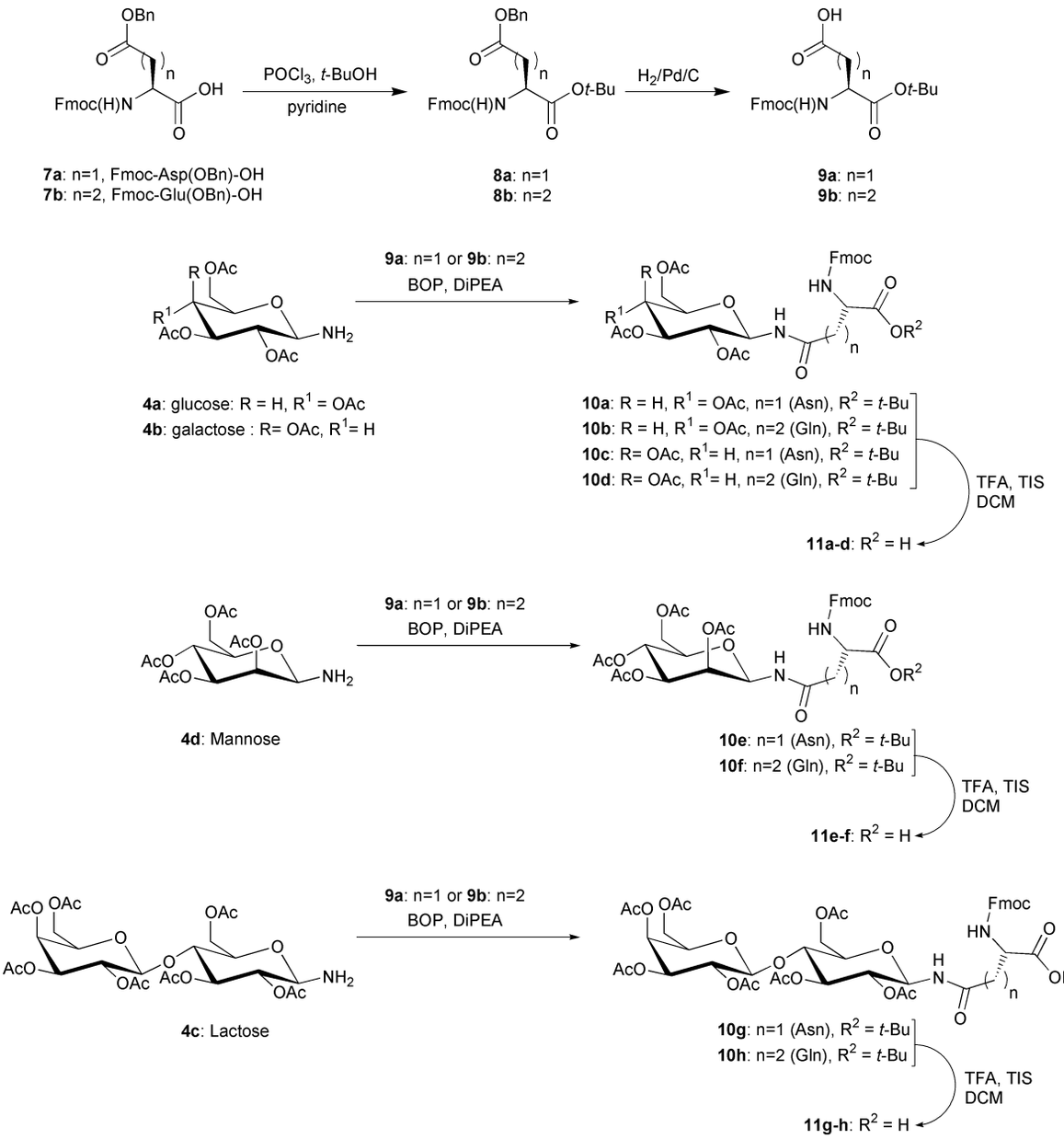
Fmoc-protected monomers can be used for the construction of glycopeptides or glycopeptidomimetics on a solid support. In order to demonstrate this applicability, a number of short glycopeptides – collagen mimics – was prepared. The protocol employed is expected to be suitable for the synthesis of any desired *N*-linked glycopeptide.

‡ HATU = *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

Experimental

General

Unless stated otherwise, chemicals were obtained from commercial sources and used without further purification. Argogel® S RAM Fmoc resin was purchased from Argonaut (Muttenz, Switzerland). All protected amino acids were purchased from Advanced Chemtech (Mechelen, Belgium). NMP (*N*-methylpyrrolidinone) and DCM were purchased from Biosolve (Valkenswaard, The Netherlands) and were stored on molecular sieves (4 Å). DIPEA (diisopropylethylamine) was distilled from ninhydrin and KOH prior to use. Pyridine was distilled from KOH. Column chromatography was performed on ICN Silica 60 Å, 32–100 μm. TLC was performed on Merck precoated Silica 60 plates. Spots were visualized using 10% sulfuric acid in methanol. NMR spectroscopy was performed on a Varian G-300 (300 MHz for ^{1}H, and 75.5 MHz for ^{13}C).



Scheme 2 Synthesis of Fmoc-protected glycoamino acids.

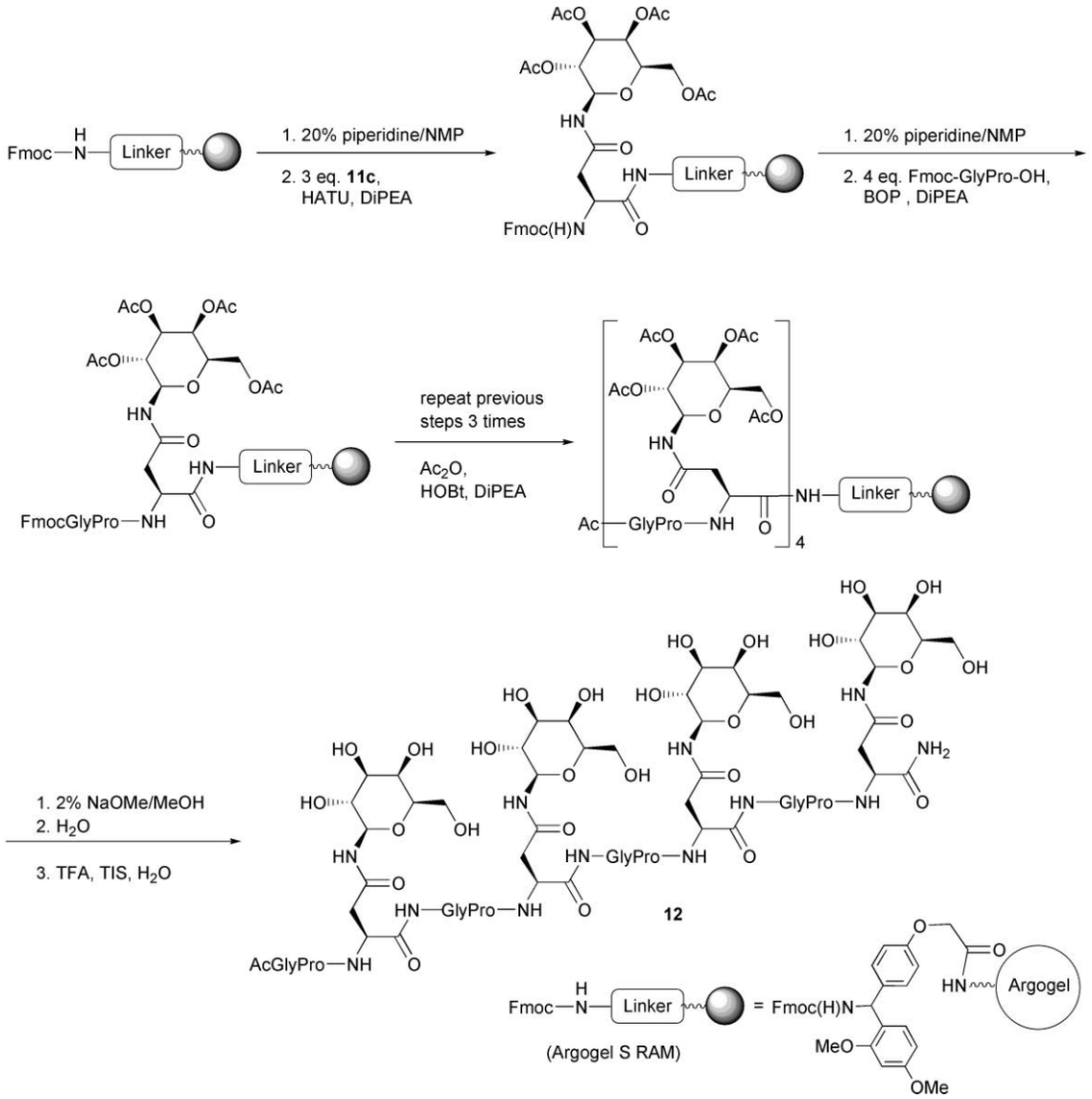
For ^{1}H experiments, TMS (δ 0.00) was used as internal reference; for ^{13}C experiments, $CDCl_3$ (δ_C 77.0) was used. ESI-MS experiments were performed on a Shimadzu LCMS QP8000 system. Analytical HPLC was performed on a Shimadzu Class-VP automated high-performance liquid chromatograph using an analytical reversed-phase column (Alltech Adsorbosphere C18, 5 μm, 250 × 4.6 mm) and a UV detector operating at 220 nm and 254 nm. Preparative HPLC was performed on a Gilson automated high-performance liquid chromatograph using a preparative reversed-phase column (Alltech Adsorbosphere C8, 10 μm, 250 × 22 mm) and a UV detector operating at 220 nm and 254 nm. Elution was effected using an appropriate gradient from 0.1% TFA in water to 0.085% TFA in acetonitrile–water (1 : 1, v/v) using a flow rate of 1 ml min^{-1} (analytical) or 11.5 ml min^{-1} (preparative). Combustion analyses were performed at Kolbe AG (Mülheim a.d. Ruhr, Germany).

General procedure I: Boc-protected *N*-linked glycopeptide monomers 6a–i

Hexose bromides **2a**–**c** were prepared according to standard methods. Conversion to hexose amines **4a**–**c** was accomplished in excellent yields *via* hexose azides **3a**–**c** analogously to the method of Soli *et al.*[6]

A Boc-protected amino acid **5a**, **b** or **c** (1.0 mmol), hexose amine **4a**, **b** or **c** (1.0 mmol), and (benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (2.0 mmol) were dissolved in DCM (10 ml), DIPEA (2.0 mmol) was added, and the resulting mixture was stirred at room temperature (3 h). Subsequently, the reaction mixture was washed successively with 1 M $KHSO_4$, 5% aq. $NaHCO_3$, water, and brine. The organic layer was dried over $NaSO_4$. After evaporation of the solvent the crude product was obtained, which was purified by column chromatography over silica.

Boc-Ala-NH-[Glc(OAc)$_4$] (6a). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6a** (435 mg, 84%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 50.86; H, 6.63; N, 5.32. $C_{22}H_{34}N_2O_{12}$ requires C, 50.96; H, 6.61; N, 5.40%); δ_H (300 MHz; $CDCl_3$) 1.33 (3H, d, C$^{\beta}H_3$, J = 7.0 Hz), 1.45 (9H, s, C(CH_3)$_3$), 1.94–2.10 (12H, 4 s, C(O)CH_3), 3.80–4.15 (4H, br m, C$^{\alpha}H$, C^5H, C$^6H_2$), 4.92–5.32 (5H, br m, NHBoc, C^1H, C^2H, C^3H, C^4H), 6.92 (1H, br d, NHC^1); δ_C (75.5 MHz; $CDCl_3$) 17.8 (C$^{\beta}$H$_3$), 20.5, 20.6, 20.6, 21.0 (C(O)CH_3), 28.2 (C(CH_3)$_3$), 50.3 (C$^{\alpha}$), 61.7 (C^6), 68.0, 70.7, 72.7, 73.6 (C^2, C^3, C^4, C^5), 78.1 (C^1), 80.3 (C(CH$_3$)$_3$), 155.2, 169.3, 169.9, 170.6, 170.8, 173.3 (C=O); *m/z* (ESI)



Scheme 3 Solid-phase synthesis of *N*-linked glycopeptides as exemplified by the synthesis of a collagen mimic: Ac–(GlyProAsn[Gal])$_4$–NH$_2$ (**12**).

519.25 ([M + H]$^+$. $C_{22}H_{35}N_2O_{12}$ requires *m*/*z*, 519.22), 541.25 ([M + Na]$^+$).

Boc-Glu(Bn)-NH-[Glc(OAc)$_4$] (6b). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6b** (557 mg, 84%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 55.68; H, 6.42; N, 4.14. $C_{31}H_{42}N_2O_{14}$ requires C, 55.85; H, 6.35; N, 4.20%); δ_H (300 MHz; $CDCl_3$) 1.37–1.60 (2H, m, C$^\beta H_2$), 1.44 (9H, s, C(CH_3)$_3$), 1.97–2.07 (12H, 4 s, C(O)CH_3), 2.44 (2H, m, C$^\gamma H_2$), 3.96–4.35 (4H, m, C$^\alpha H$, C$^5 H$, C$^6 H_2$), 5.05–5.54 (7H, m, ArCH_2, C$^1 H$, C$^2 H$, C$^3 H$, C$^4 H$, N*H*Boc), 5.85 (1H, br d, N*H*C^1), 7.33 (5H, m, Ar*H*); δ_C (75.5 MHz; $CDCl_3$) 20.5, 20.6 (C(O)*C*H$_3$), 27.2 (*C*$^\beta$), 28.2 (C(*C*H$_3$)$_3$), 30.2 (*C*$^\gamma$), 53.8 (*C*$^\alpha$), 61.7 (*C*^{6}), 68.1, 70.5, 72.7, 73.5 (*C*^{2}, *C*^{3}, *C*^{4}, *C*^{5}), 78.0 (*C*^{1}), 80.2 (*C*(CH$_3$)$_3$), 128.3, 128.3, 128.5, 135.6 (Ar*C*), 155.4, 169.4, 169.8, 170.5, 170.6, 172.3, 172.7 (*C*=O); *m*/*z* (ESI) 667.30 ([M + H]$^+$. $C_{31}H_{43}N_2O_{14}$ requires *m*/*z*, 667.27), 689.35 ([M + Na]$^+$), 567.30 ([M − Boc + H]$^+$).

Boc-Lys(Cbz)-NH-[Glc(OAc)$_4$] (6c). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6c** (619 mg, 87%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 55.72; H, 6.69; N, 5.83. $C_{33}H_{47}N_3O_{14}$ requires C, 55.84; H, 6.67; N, 5.92%); δ_H (300 MHz; $CDCl_3$) 1.36–1.81 (6H, m, C$^\beta H_2$, C$^\gamma H_2$, C$^\delta H_2$), 1.45 (9H, s, C(CH_3)$_3$), 2.03 (12H, 4 s, C(O)CH_3), 3.20 (2H, m, C$^\varepsilon H_2$), 3.79–4.28 (4H, m, C$^\alpha H$, C$^5 H$, C$^6 H_2$), 4.92–5.33 (7H, m, C$^1 H$, C$^2 H$, C$^3 H$, C$^4 H$, BocN*H*, ArCH_2), 7.02 (1H, br d, C^{1}N*H*), 7.30–7.40 (5H, m, Ar*H*); δ_C (75.5 MHz; $CDCl_3$) 20.5, 20.6, 20.9 (C(O)*C*H$_3$), 22.2 (*C*$^\beta$), 28.1 (C(*C*H$_3$)$_3$), 29.3 (*C*$^\gamma$), 31.4 (*C*$^\delta$), 40.2 (Ar*C*H$_2$), 54.4 (*C*$^\alpha$), 61.5 (*C*^{6}), 66.5 (*C*$^\varepsilon$), 68.1, 70.5, 72.7, 73.5 (*C*^{2}, *C*^{3}, *C*^{4}, *C*^{5}), 78.0 (*C*^{1}), 80.0 (*C*(CH$_3$)$_3$), 128.0, 128.0, 128.4, 136.5 (Ar*C*), 155.5, 156.5, 169.4, 170.5, 170.7, 172.8 (*C*=O); *m*/*z* (ESI) 710.35 ([M + H]$^+$. $C_{33}H_{48}N_3O_{14}$ requires *m*/*z*, 710.32), 732.45 ([M + Na]$^+$), 654.40 ([M − *t*-Bu + H]$^+$), 610.40 ([M − Boc + H]$^+$).

Boc-Ala-NH-[Gal(OAc)$_4$] (6d). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6d** (463 mg, 89%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 50.77; H, 6.70; N, 5.52. $C_{22}H_{34}N_2O_{12}$ requires C, 50.96; H, 6.61; N, 5.40%); δ_H (300 MHz; $CDCl_3$) 1.32 (3H, d, C$^\beta H_3$), 1.44 (9H, s, C(CH_3)$_3$), 1.97–2.16 (12H, 4 s, C(O)CH_3), 4.10 (4H, m, C$^5 H$, C$^6 H_2$, C$^\alpha H$), 5.10–5.27 (4H, m, BocN*H*, C$^1 H$, C$^2 H$, C$^3 H$), 5.43 (1H, d, C$^4 H$), 7.32 (1H, br d, C^{1}N*H*); δ_C (75.5 MHz; $CDCl_3$) 17.7 (*C*$^\beta$), 20.3, 20.3, 20.4 (C(O)*C*H$_3$), 28.0 (C(*C*H$_3$)$_3$), 50.2 (*C*$^\alpha$), 60.9 (*C*^{6}), 67.0, 68.2, 70.7, 72.1 (*C*^{2}, *C*^{3}, *C*^{4}, *C*^{5}), 78.1 (*C*^{1}), 80.1 (*C*(CH$_3$)$_3$), 155.1, 169.6, 169.8, 170.0, 170.1, 173.3 (*C*=O); *m*/*z* (ESI) 541.25 ([M + Na]$^+$. $C_{22}H_{34}N_2NaO_{12}$ requires *m*/*z*, 541.20), 485.20 ([M − *t*-Bu + Na]$^+$), 419.15 ([M − Boc + H]$^+$).

Boc-Glu(Bn)-NH-[Gal(OAc)$_4$] (6e). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6e** (560 mg, 84%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found:

C, 55.79; H, 6.30; N, 4.14. $C_{31}H_{42}N_2O_{14}$ requires C, 55.85; H, 6.35; N, 4.20%); δ_H (300 MHz; $CDCl_3$) 1.43 (9H, s, C(CH_3)$_3$), 1.91–2.27 (16H, m, C(O)CH_3, C$^{\beta}H_2$, C$^{\gamma}H_2$), 4.09 (3H, m, C^5H, C$^6H_2$), 4.32 (1H, m, C$^{\alpha}H$), 5.09–5.27 (5H, m, C^1H, C^2H, C^3H, ArCH_2), 5.33 (1H, br d, BocNH), 5.44 (1H, d, C^4H), 6.62 (1H, br d, C^{1}NH), 7.32 (5H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 20.4, 20.4, 20.5, 20.9 (C(O)CH$_3$), 27.9 (C$^{\beta}$), 28.1 (C(CH_3)$_3$), 32.0 (ArCH_2), 52.7 (C$^{\alpha}$), 61.0 (C^6), 67.1 (C$^{\gamma}$), 67.1, 68.1, 70.9, 72.1 (C^2, C^3, C^4, C^5), 78.3 (C^1), 80.0 (C(CH$_3$)$_3$), 128.3, 128.4, 128.5, 135.1 (ArC), 155.5, 169.7, 169.9, 170.3, 170.9, 171.8, 172.1 (C=O); *m*/*z* (ESI) 689.30 ([M + Na]$^+$. $C_{31}H_{42}N_2NaO_{14}$ requires *m*/*z*, 689.25), 611.50 ([M − *t*-Bu + H]$^+$, 567.30 ([M − Boc + H]$^+$).

Boc-Lys(Cbz)-NH-[Gal(OAc)$_4$] (6f). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6f** (585 mg, 82%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 55.55; H, 6.61; N, 5.74. $C_{33}H_{47}N_3O_{14}$ requires C, 55.84; H, 6.67; N, 5.92%); δ_H (300 MHz; $CDCl_3$) 1.33–1.85 (6H, m, C$^{\beta}H_2$, C$^{\gamma}H_2$, C$^{\delta}H_2$), 1.45 (9H, s, C(C$_3$)$_3$), 1.96–2.15 (12H, 4 s, C(O)CH_3), 3.17 (2H, m, C$^{\varepsilon}H_2$), 4.04–4.15 (4H, m, C^5H, C$^6H_2$, C$^{\alpha}H$), 5.10–5.30 (6H, m, C^1H, C^2H, C^3H, BocNH, ArCH_2), 5.43 (1H, d, C^4H), 6.99 (1H, br d, C^{1}NH), 7.33 (5H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 20.4, 20.5, 20.5 (C(O)CH$_3$), 22.3 (C$^{\beta}$), 28.1 (C(CH_3)$_3$), 29.3 (C$^{\gamma}$), 31.4 (C$^{\delta}$), 40.2 (ArCH_2), 54.5 (C$^{\alpha}$), 61.0 (C^6), 66.4 (C$^{\varepsilon}$), 67.0, 68.2, 70.7, 72.2 (C^2, C^3, C^4, C^5), 78.3 (C^1), 80.0 (C(CH$_3$)$_3$), 127.9, 128.0, 128.3, 136.5 (ArC), 155.4, 156.5, 169.7, 169.9, 170.2, 170.9, 172.7 (C=O); *m*/*z* (ESI) 732.55 ([M + Na]$^+$. $C_{33}H_{47}N_3NaO_{14}$ requires *m*/*z*, 732.30), 654.55 ([M − *t*-Bu + H]$^+$), 610.35 ([M − Boc + H]$^+$).

Boc-Ala-NH-[Lac(OAc)$_7$] (6g). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6g** (571 mg, 71%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 50.65; H, 6.22; N, 3.51. $C_{34}H_{50}N_2O_{20}$ requires C, 50.62; H, 6.25; N, 3.47%); δ_H (300 MHz; $CDCl_3$) 1.26 (3H, d, C$^{\beta}H_3$), 1.43 (9H, s, C(CH_3)$_3$), 1.97–2.16 (21H, 7 s, C(O)CH_3), 3.78–4.17 (7H, m, C$^{\alpha}H$, C^5H, C$^{5'}H$, C$^6H_2$, C$^{6'}H_2$), 4.47 (2H, m, C^4H, C$^{1'}H$), 4.83–5.36 (7H, m, NHBoc, C^1H, C^2H, C^3H, C$^{2'}H$, C$^{3'}H$, C$^{4'}H$), 7.00 (1H, br d, NHC^1); δ_C (75.5 MHz; $CDCl_3$) 17.7 (C$^{\beta}$), 20.3, 20.5, 20.6, 20.7 (C(O)CH$_3$), 28.1 (C(CH_3)$_3$), 50.0 (C$^{\alpha}$), 60.7, 61.8 (C^6, C$^{6'}$), 66.5, 68.8, 70.5, 70.8, 72.4, 74.3, 75.8 (C^2, C^3, C^4, C^5, C$^{2'}$, C$^{3'}$, C$^{4'}$, C$^{5'}$), 77.7 (C^1), 100.7 (C$^{1'}$), 155.2, 168.8, 169.3, 169.9, 170.0, 170.2, 170.7, 173.2 (C=O); *m*/*z* (ESI) 807.20 ([M + H]$^+$. $C_{34}H_{51}N_2O_{20}$ requires *m*/*z*, 807.31), 829.30 ([M + Na]$^+$), 751.45 ([M − *t*-Bu + H]$^+$), 707.25 ([M − Boc + H]$^+$).

Boc-Glu(Bn)-NH-[Lac(OAc)$_7$] (6h). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6h** (694 mg, 73%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 53.92; H, 5.98; N, 2.95. $C_{43}H_{58}N_2O_{22}$ requires C, 54.08; H, 6.12; N, 2.93%); δ_H (300 MHz; $CDCl_3$) 1.41 (9H, s, C(CH_3)$_3$), 1.96–2.14 (23H, m, C(O)CH_3, C$^{\beta}H_2$), 2.45 (2H, m, C$^{\gamma}H_2$), 3.80–4.13 (7H, m, C$^{\alpha}H$, C^5H, C$^6H_2$, C$^{5'}H$, C$^{6'}H_2$), 3.93–4.57 (2H, m, C$^{1'}H$, C^4H), 4.86–5.36 (8H, m, ArCH_2, C^1H, C^2H, C^3H, C$^{2'}H$, C$^{3'}H$, C$^{4'}H$), 5.54 (1H, br d, NHBoc), 7.34 (6H, m, ArH, NHC^1); δ_C (75.5 MHz; $CDCl_3$) 20.3, 20.5, 20.6, 20.6, 20.7, 20.9 (C(O)CH$_3$), 27.1 (C$^{\beta}$), 28.1 (C(CH_3)$_3$), 30.1 (ArCH_2), 53.8 (C$^{\alpha}$), 60.8, 61.8 (C^6, C$^{6'}$), 66.4 (C$^{\gamma}$), 66.5, 68.9, 70.7, 70.8, 71.2, 72.4, 74.3 (C^2, C^3, C^4, C^5, C$^{2'}$, C$^{3'}$, C$^{4'}$, C$^{5'}$), 75.8 (C^1), 80.1 (C(CH$_3$)$_3$), 100.7 (C$^{1'}$), 128.1, 128.2, 128.4, 135.5 (ArC), 155.5, 168.8, 169.3, 169.5, 169.9, 170.0, 170.7, 172.1, 172.7 (C=O); *m*/*z* (ESI) 955.25 ([M + H]$^+$. $C_{43}H_{59}N_2O_{22}$ requires *m*/*z*, 955.36), 977.35 ([M + Na]$^+$), 899.40 ([M − *t*-Bu + H]$^+$), 855.50 ([M − Boc + H]$^+$).

Boc-Lys(Cbz-NH)-[Lac(OAc)$_7$] (6i). After column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **6i** (737 mg, 74%) was obtained as a white foam. R_f (5% MeOH–DCM) 0.34 (Found: C, 54.20; H, 6.31; N, 4.11. $C_{45}H_{63}N_3O_{22}$ requires C, 54.16; H, 6.36; N, 4.21%); δ_H (300 MHz; $CDCl_3$) 1.35–1.53 (6H, m, C$^{\beta}H_2$, C$^{\gamma}H_2$, C$^{\delta}H_2$), 1.43 (9H, s, C(CH_3)$_3$), 1.96–2.15 (21H, 7 s, C(O)CH_3), 3.15 (2H, m, C$^{\varepsilon}H_2$), 3.75–4.17 (7H, m, C$^{\alpha}H$, C^5H, C$^6H_2$, C$^{5'}H$, C$^{6'}H_2$), 4.29 (2H, m, C^4H, C$^{1'}H$), 4.84–5.36 (9H, m, ArCH_2, NHBoc, C^1H, C^2H, C^3H, C$^{2'}H$, C$^{3'}H$, C$^{4'}H$), 7.12 (1H, br d, NHC^1), 7.34 (5H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 20.5, 20.5, 20.6, 20.6, 20.7, 20.8, 21.0 (C(O)CH$_3$), 22.2 (C$^{\beta}$), 28.2 (C$^{\gamma}$), 29.3 (C$^{\delta}$), 40.3 (ArCH_2), 54.3 (C$^{\alpha}$), 60.8, 61.7 (C^6, C$^{6'}$), 66.6 (C$^{\varepsilon}$), 69.0, 70.7, 70.9, 72.4, 74.5, 74.9, 75.9 (C^2, C^3, C^4, C^5, C$^{2'}$, C$^{3'}$, C$^{4'}$, C$^{5'}$), 77.9 (C^1), 80.2 (C(CH$_3$)$_3$), 101.0 (C$^{1'}$), 128.0, 128.5, 136.6 (ArC), 155.5, 156.5, 168.9, 169.3, 170.0, 170.0, 170.1, 170.3, 171.0, 172.6 (C=O); *m*/*z* (ESI) 998.30 ([M + H]$^+$. $C_{45}H_{64}N_3O_{22}$ requires *m*/*z*, 998.40), 1022.10 ([M + Na]$^+$), 942.24 ([M − *t*-Bu + H]$^+$), 898.30 ([M − Boc + H]$^+$).

General procedure II: Fmoc-protected *N*-linked glycopeptide monomers 10a–h

A *t*-Bu-protected amino acid **9a** or **9b** (1.0 mmol), hexoseamine **4a**, **4b**, **4c** or **4d** (1.0 mmol), and BOP (1.0 mmol) were dissolved in DCM (10 ml). DIPEA (2.0 mmol) was added. The resulting mixture was stirred at room temperature (24 h). Subsequently, the reaction mixture was washed successively with 1 M $KHSO_4$, 5% aq. $NaHCO_3$, water, and brine. The organic layer was dried over Na_2SO_4. Evaporation of the solvent yielded the crude product, which was purified using column chromatography over silica.

Fmoc-Asn[Glc(OAc)$_4$]-O*t*-Bu (10a). Reaction of **9a** and **4a** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **10a** (596 mg, 80%) as a white foam. R_f (5% MeOH–DCM) 0.43 (Found: C, 60.11; H, 5.98; N, 3.76. $C_{37}H_{44}N_2O_{14}$ requires C, 59.99; H, 5.99; N, 3.78%); δ_H (300 MHz; $CDCl_3$) 1.43 (9H, s, C(CH_3)$_3$), 1.75–2.10 (12H, 4 s, C(O)CH_3), 2.84 (2H, m, C$^{\beta}H_2$), 3.79 (1H, m, C^5H), 4.06 (1H, t, C^6H), 4.22 (3H, m, Fmoc-CH, C$^6H'$, C$^{\alpha}H$), 44.0–4.52 (2H, m, Fmoc-CH_2), 4.93 (1H, t, C^2H), 5.06 (1H, t, C^4H), 5.19–5.35 (2H, m, C^1H, C^3H), 5.91 (1H, br d, FmocNH), 6.42 (1H, br d, C^{1}NH), 7.26–7.78 (8H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 19.9, 20.3, 20.3 (C(O)CH$_3$), 27.8 (C(CH_3)$_3$), 37.8 (C$^{\beta}$), 47.1 (Fmoc-CH), 54.4 (C$^{\alpha}$), 67.2 (Fmoc-CH$_2$), 67.9, 70.5, 72.5, 73.6 (C^2, C^3, C^4, C^5), 78.0 (C^1), 82.5 (C(CH$_3$)$_3$), 120.0, 127.0, 125.6, 127.7, 141.3 (ArC), 156.1, 169.5, 169.9, 170.6, 171.2 (C=O); *m*/*z* (ESI) 741.75 ([M + H]$^+$. $C_{37}H_{45}N_2O_{14}$ requires *m*/*z*, 741.29), 763.25 ([M + Na]$^+$), 685.30 ([M − *t*-Bu + H]$^+$).

Fmoc-Gln[Glc(OAc)$_4$]-O*t*-Bu (10b). Reaction of **9b** and **4a** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **10b** (605 mg, 80%) as a white foam. R_f (5% MeOH–DCM) 0.41 (Found: C, 60.40; H, 6.22; N, 3.63. $C_{38}H_{46}N_2O_{14}$ requires C, 60.47; H, 6.14; N, 3.71%); δ_H (300 MHz; $CDCl_3$) 1.47 (9H, s, C(CH_3)$_3$), 1.82 (2H, m, C$^{\beta}H_2$), 1.90–2.06 (12H, 4 s, C(O)CH_3), 2.22 (2H, m, C$^{\gamma}H_2$), 3.81 (1H, m, C$^5H_2$), 4.05 (1H, d, C^6H), 4.20–4.32 (3H, m, Fmoc-CH, C$^{\alpha}H$, C$^6H'$), 4.42 (2H, m, Fmoc-CH_2), 4.95 (1H, t, C^2H), 5.08 (1H, t, C^4H), 5.29 (2H, m, C^1H, C^3H), 5.51 (1H, br d, FmocNH), 6.68 (1H, br d, C^{1}NH), 7.27–7.78 (8H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 20.5, 20.6 (C(O)CH$_3$), 27.9 (C(CH_3)$_3$), 28.6 (C$^{\beta}$), 47.2 (Fmoc-CH), 54.9 (C$^{\alpha}$), 60.4 (C$^{\gamma}$), 61.6 (Fmoc-CH$_2$), 67.0 (C^6), 68.0, 70.5, 72.9, 73.5 (C^2, C^3, C^4, C^5), 78.0 (C^1), 84.6 (C(CH$_3$)$_3$), 120.0, 125.1, 127.1, 127.7, 141.3, 144.1 (ArC), 156.8, 169.4, 169.5, 171.2, 172.5 (C=O); *m*/*z* (ESI) 755.40 ([M + H]$^+$. $C_{38}H_{47}N_2O_{14}$ requires *m*/*z*, 755.30), 777.35 ([M + Na]$^+$), 699.45 ([M − *t*-Bu + H]$^+$).

Fmoc-Asn[Gal(OAc)$_4$]-O*t*-Bu (10c). Reaction of **9a** and **4b** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **10c** (531 mg, 72 %) as a white foam. R_f (5% MeOH–DCM) 0.42 (Found: C, 59.77; H, 5.98; N, 3.76.

$C_{37}H_{44}N_2O_{14}$ requires C, 59.99; H, 5.99; N, 3.78%); δ_H (300 MHz; $CDCl_3$) 1.42 (9H, s, C(CH_3)$_3$), 1.90–2.17 (12H, 4 s, C(O)CH_3), 2.80 (2H, br m, C$^\beta H_2$), 4.01 (1H, t, C^5H), 4.11 (2H, m, C$^6H_2$), 4.20–4.34 (2H, m, Fmoc-CH, C$^\alpha H$), 4.45 (2H, m, Fmoc-CH_2), 5.10 (1H, m, C^2H, C^4H), 5.23 (1H, t, C^1H), 5.43 (1H, d, C^3H), 5.94 (1H, br d, FmocNH), 6.46 (1H, br d, C^{1}NH), 7.27–7.77 (8H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 19.1 (C(O)CH_3), 27.8 (C(CH_3)$_3$), 38.0 (C^β), 47.0 (Fmoc-CH), 53.9 (C^α), 61.0 (C^6), 67.1 (Fmoc-CH_2), 67.2, 68.4, 70.7, 72.3 (C^2, C^3, C^4, C^5), 78.3 (C^1), 119.9, 125.1, 127.0, 127.6, 141.2, 143.8 (ArC), 153.1, 169.7, 170.1, 170.3, 170.8 (C=O); *m*/*z* (ESI) 741.75 ([M + H]$^+$. $C_{37}H_{45}N_2O_{14}$ requires *m*/*z*, 741.29), 763.25 ([M + Na]$^+$), 685.30 ([M − *t*-Bu + H]$^+$).

Fmoc-Gln[Gal(OAc)$_4$]-O*t*-Bu (10d). Reaction of **9b** and **4b** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **10d** (528 mg, 70%) as a white foam. R_f (5% MeOH–DCM) 0.40 (Found: C, 60.11; H, 6.11; N, 3.66. $C_{38}H_{46}N_2O_{14}$ requires C, 60.47; H, 6.14; N, 3.71%); δ_H (300 MHz; $CDCl_3$) 1.46 (9H, s, C(CH_3)$_3$), 1.86–2.33 (16H, m, C(O)CH_3, C$^\beta H_2$, C$^\gamma H_2$), 3.99–4.15 (3H, m, C$^\alpha H$, C^5H, C^6H), 4.22 (2H, m, Fmoc-CH, C$^6H'$), 4.40 (2H, m, Fmoc-CH_2), 5.12 (2H, m, C^2H, C^4H), 5.26 (1H, m, C^1H), 5.44 (1H, d, C^3H), 5.56 (1H, br d, FmocNH), 6.57 (1H, br d, C^{1}NH), 7.27–7.78 (8H, ArH); δ_C (75.5 MHz; $CDCl_3$) 20.5, 20.5, 20.6, 20.7 (C(O)CH_3), 27.9 (C(CH_3)$_3$), 32.1 (C^β), 47.1 (Fmoc-CH), 53.5 (C^α), 60.3 (C^γ), 61.0 (Fmoc-CH_2), 66.9 (C^6), 67.1, 68.1, 70.9, 72.2 (C^2, C^3, C^4, C^5), 78.4 (C^1), 82.5 (C(CH$_3$)$_3$), 119.9, 125.0, 127.0, 127.6, 141.2, 143.7 (ArC), 156.1, 169.7, 170.0, 170.3, 170.8, 171.0, 172.2 (C=O); *m*/*z* (ESI) 755.40 ([M + H]$^+$. $C_{38}H_{47}N_2O_{14}$ requires *m*/*z*, 755.30), 777.35 ([M + Na]$^+$), 699.45 (M − *t*-Bu + H]$^+$).

Fmoc-Asn[Man(OAc)$_4$]-O*t*-Bu (10e). Reaction of **9a** and **4d** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **10e** (571 mg, 77%) as a white foam. R_f (5% MeOH–DCM) 0.43 (Found: C, 60.08; H, 6.04; N, 3.66. $C_{37}H_{44}N_2O_{14}$ requires C, 59.99; H, 5.99; N, 3.78%); δ_H (300 MHz; $CDCl_3$) 1.44 (9H, s, C(CH_3)$_3$), 1.98–2.28 (12H, 4 s, C(O)CH_3), 2.75–2.93 (2H, br m, C$^\beta H_2$), 3.75 (1H, m, C^5H), 4.03 (1H, d, C^6H), 4.20–4.43 (5H, m, Fmoc-CH, Fmoc-CH_2, C^{6}H′, C$^\alpha H$), 5.10 (1H, m, C^3H), 5.23 (1H, t, C^4H), 5.36 (1H, dd, C^2H), 5.54 (1H, d, C^1H), 5.92 (1H, br d, FmocNH), 6.61 (1H, br d, C^{1}NH), 7.27–7.77 (8H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 20.5, 20.7, 20.8 (C(O)CH_3), 27.8 (C(CH_3)$_3$), 47.0 (Fmoc-CH), 50.6 (C^α), 54.7 (C^β), 60.4 (C^6H_2), 62.0 (Fmoc-CH_2), 65.0, 69.8, 71.5, 74.1, 75.9 (C^1, C^2, C^3, C^4, C^5), 82.8 (C(CH$_3$)$_3$), 120.0, 125.1, 127.1, 127.7, 141.2, 143.8 (ArC), 155.3, 169.6, 169.9, 180.1 (C=O); *m*/*z* (ESI) 741.65 ([M + H]$^+$. $C_{37}H_{45}N_2O_{14}$ requires *m*/*z*, 741.29), 763.19 ([M + Na]$^+$), 685.32 ([M − *t*-Bu + H]$^+$).

Fmoc-Gln[Man(OAc)$_4$]-O*t*-Bu (10f). Reaction of **9b** and **4d** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 1 : 1 v/v), **10f** (559 mg, 74%) as a white foam. R_f (5% MeOH–DCM) 0.42 (Found: C, 60.27; H, 6.11; N, 3.66. $C_{38}H_{46}N_2O_{14}$ requires C, 60.47; H, 6.14; N, 3.71%); δ_H (300 MHz; $CDCl_3$) 1.43 (9H, s, C(CH_3)$_3$), 1.91–2.33 (16H, m, C(O)CH_3, C$^\beta H_2$, C$^\gamma H_2$), 3.77 (1H, m, C^5H), 4.08 (2H, m, C$^6H_2$), 4.20–4.49 (4H, m, Fmoc-CH, Fmoc-CH_2, C$^\alpha H$), 5.11 (1H, m, C^3H), 5.24 (1H, t, C^4H), 5.40 (1H, m, C^2H), 5.54 (2H, m, C^1H, FmocNH), 7.25–7.79 (9H, m, C^{1}NH, ArH); *m*/*z* (ESI) 755.30 ([M + H]$^+$. $C_{38}H_{47}N_2O_{14}$ requires *m*/*z*, 755.30), 777.30 ([M + Na]$^+$), 699.35 ([M − *t*-Bu + H]$^+$).

Fmoc-Asn[Lac(OAc)$_7$]-O*t*-Bu (10g). Reaction of **9a** and **4c** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 3 : 2 v/v), **10g** (617 mg, 60%) as a white foam. R_f (10% MeOH–DCM) 0.47 (Found: C, 57.28; H, 5.96; N, 2.78. $C_{49}H_{60}N_2O_{22}$ requires C, 57.19; H, 5.88; N, 2.72%); δ_H (300 MHz; $CDCl_3$) 1.44 (9H, s, C(CH_3)$_3$), 1.97–2.18 (21H, 7 s, C(O)CH_3), 2.71–2.99 (2H, m, C$^\beta H_2$), 3.70–4.51 (13H, m, C$^\alpha H$, Fmoc-CH, Fmoc-CH_2, C^4H, C^5H, C$^6H_2$, C$^{1'}H$, C$^{3'}H$, C$^{5'}H$, C$^{6'}H_2$), 4.80–5.39 (5H, m, C^1H, C^2H, C^3H, C$^{2'}H$, C$^{4'}H$), 6.00 (1H, br d, NHBoc), 6.62 (1H, br d, NHC^1), 7.28–7.77 (8H, m, ArH); δ_C (75.5 MHz; $CDCl_3$) 13.3 (C(CH_3)$_3$), 20.3, 20.4, 20.5, 20.6, 20.6 (C(O)CH_3), 37.7 (C^β), 46.9 (Fmoc-CH), 50.8 (C^α), 58.3, 60.7 (C^6, $C^{6'}$), 67.0 (Fmoc-CH_2), 66.5, 68.8, 70.5, 70.7, 70.8, 72.3, 74.4, 75.8 (C^2, C^3, C^4, C^5, $C^{2'}$, $C^{3'}$, $C^{4'}$, $C^{5'}$), 77.7 (C^1), 82.2 (C(CH$_3$)$_3$), 100.7 ($C^{1'}$), 119.8, 125.0, 126.9, 127.5, 141.1, 143.6 (ArC), 156.0, 169.3, 169.7, 169.9, 170.0, 170.2, 170.3, 170.5, 172.0 (C=O); *m*/*z* (ESI) 1029.15 ([M + H]$^+$. $C_{49}H_{61}N_2O_{22}$ requires *m*/*z*, 1029.37), 1051.20 ([M + Na]$^+$), 973.15 ([M − *t*-Bu + H]$^+$).

Fmoc-Gln[Lac(OAc)$_7$]-O*t*-Bu (10h). Reaction of **9b** and **4c** gave, after work-up and column chromatography (eluent: EtOAc–hexanes 3 : 2 v/v), **10h** (688 mg, 66%) as a white foam. R_f (10% MeOH–DCM) 0.51 (Found: C, 57.50; H, 6.22; N, 2.74. $C_{50}H_{62}N_2O_{22}$ requires C, 57.58; H, 5.99; N, 2.69%); δ_H (300 MHz; $CDCl_3$) 1.44 (9H, s, C(CH_3)$_3$), 1.90–2.20 (23H, m, C(O)CH_3, C$^\beta H_2$), 2.20–2.36 (2H, m, C$^\gamma H_2$), 3.76–4.50 (13H, C$^\alpha H$, Fmoc-CH, Fmoc-CH_2, C^4H, C^5H, C$^6H_2$, C$^{1'}H$, C$^{3'}H$, C$^{5'}H$, C$^{6'}H_2$), 4.85–5.39 (5H, m, C^1H, C^2H, C^3H, C$^{2'}H$, C$^{4'}H$), 5.89 (1H, br d, FmocNH), 7.27–7.77 (9H, m, NHC^1, ArH); δ_C (75.5 MHz; $CDCl_3$) 20.3, 20.4, 20.4, 20.6, 20.6 (C(O)CH_3), 27.8 (C(CH_3)$_3$), 31.2 (C^β), 46.8 (Fmoc-CH), 54.1 (C^α), 60.7 (C^6, $C^{6'}$), 61.8 (C$^\gamma$), 67.0 (Fmoc-CH_2), 66.5, 68.8, 70.4, 70.5, 70.8, 72.4, 74.3, 75.8 (C^2, C^3, C^4, C^5, $C^{2'}$, $C^{3'}$, $C^{4'}$, $C^{5'}$), 77.7 (C^1), 80.6 (C(CH$_3$)$_3$), 100.7 ($C^{1'}$), 119.8, 124.9, 126.9, 127.5, 141.0, 143.5 (ArC), 156.2, 168.9, 169.3, 169.9, 170.0, 170.2, 170.6, 171.9, 172.5 (C=O); *m*/*z* (ESI) 1043.23 ([M + H]$^+$. $C_{50}H_{63}N_2O_{22}$ requires *m*/*z*, 1043.39), 1009.18 ([M − *t*-Bu + Na]$^+$), 987.31 ([M − *t*-Bu + H]$^+$).

General procedure III: *tert*-butyl-deprotection of Fmoc-protected monomers

A pure *t*-Bu-protected monomer **10a**–**h** was dissolved in a mixture of TFA (5 ml), DCM (5 ml), and water (0.5 ml). The resulting mixture was stirred for 3 hours at room temperature. The solvents were evaporated off. After dissolution in water–acetonitrile 1 : 1 (v/v) and lyophlilization, Fmoc-protected *N*-linked glycopeptide monomers **11a**–**h** were obtained.

Fmoc-Asn[Glc(OAc)$_4$]-OH (11a). 560 mg (0.76 mmol) of *t*-Bu ester yielded **11a** (511 mg, 99%) as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.27; δ_H (300 MHz; $CDCl_3$) 1.96–2.10 (12H, 4 s, C(O)CH_3), 2.75–3.02 (2H, m, C$^\beta H_2$), 3.45 (1H, br s, OH), 3.83 (1H, m, C^5H), 4.07 (1H, t, C^6H), 4.19–4.41 (4H, m, Fmoc-CH, Fmoc-CH_2, C$^6H'$), 4.62 (1H, br s, C$^\alpha H$), 4.95 (1H, t, C^2H), 5.08 (1H, t, C^4H), 5.20–5.37 (2H, m, C^1H, C^3H), 6.22 (1H, br d, FmocNH), 6.89 (1H, br d, C^{1}NH), 7.26–7.77 (8H, m, ArH); *m*/*z* (ESI) 685.30 ([M + H]$^+$. $C_{33}H_{37}N_2O_{14}$ requires *m*/*z*, 685.23), 707.20 ([M + Na]$^+$).

Fmoc-Gln[Glc(OAc)$_4$]-OH (11b). 580 mg (0.77 mmol) of *t*-Bu ester yielded **11b** (531 mg, 99%) as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.31; δ_H (300 MHz; $CDCl_3$) 1.94–2.09 (12H, 4 s, C(O)CH_3), 2.23–2.35 (4H, m, C$^\beta H_2$, C$^\gamma H_2$), 3.82 (1H, m, C^5H), 4.09 (1H, d, C^6H), 4.23 (2H, m, C$^\alpha H$, C$^6H'$), 4.23–4.43 (3H, m, Fmoc-CH, Fmoc-CH_2), 4.95 (1H, t, C^2H), 5.07 (1H, t, C^4H), 5.24–5.35 (2H, m, C^1H, C^3H), 5.47 (1H, br s, OH), 5.88 (1H, br d, FmocNH), 6.98 (1H, br d, C^{1}NH), 7.26–7.77 (8H, m, ArH); *m*/*z* (ESI) 699.30 ([M + H]$^+$. $C_{34}H_{39}N_2O_{14}$ requires *m*/*z*, 699.24), 721.30 ([M + Na]$^+$).

Fmoc-Asn[Gal(OAc)$_4$]-OH (11c). 500 mg (0.67 mmol) of *t*-Bu ester yielded **11c** (452 mg, 98%) as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.28; δ_H (300 MHz; $CDCl_3$)

1.83–2.19 (12H, 4 s, C(O)CH_3), 2.76–3.04 (2H, m, C$^{\beta}H_2$), 4.12 (2H, C^5H, C^6H), 4.22 (1H, t, C$^6H'$), 4.28–4.44 (3H, Fmoc-CH, Fmoc-CH_2), 4.40 (1H, br s, OH), 4.60 (1H, m, C$^{\alpha}H$), 5.08–5.20 (2H, m, C^2H, C^4H), 5.32 (1H, m, C^1H), 5.47 (1H, t, C^3H), 6.27 (1H, br d, FmocNH), 6.62 (1H, br d, C^{1}NH), 7.26–7.77 (8H, m, ArH); *m*/*z* (ESI) 684.80 ([M + H]$^+$. $C_{33}H_{37}N_2O_{14}$ requires *m*/*z*, 685.23), 707.05 ([M + Na]$^+$).

Fmoc-Gln[Gal(OAc)$_4$]-OH (11d). 500 mg (0.66 mmol) of *t*-Bu ester yielded **11d** (459 mg, 99%) as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.32; δ_H (300 MHz; $CDCl_3$) 1.98–2.19 (13H, m, C(O)CH_3, C$^{\beta}H$), 2.23 (1H, m, C$^{\beta}H'$), 2.38 (2H, t, C$^{\gamma}H_2$), 3.14 (1H, br s, OH), 4.01–4.11 (3H, m, C^5H, C$^6H_2$), 4.22 (1H, t, Fmoc-CH), 4.35–4.42 (3H, m, Fmoc-CH_2, C$^{\alpha}H$), 5.07–5.18 (2H, m, C^2H, C^4H), 5.23 (1H, t, C^1H), 5.42 (1H, d, C^3H), 5.86 (1H, br d, FmocNH), 6.80 (1H, br d, C^{1}NH), 7.26–7.78 (8H, m, ArH); *m*/*z* (ESI) 698.95 ([M + H]$^+$. $C_{34}H_{39}N_2O_{14}$ requires *m*/*z*, 699.24), 721.25 ([M + Na]$^+$).

Fmoc-Asn[Man(OAc)$_4$]-OH (11e). 540 mg (0.73 mmol) of *t*-Bu ester yielded **11e** (494 mg, 99%) was obtained as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.27; δ_H (300 MHz; $CDCl_3$) 1.98–2.24 (12H, 4 s, C(O)CH_3), 2.74–2.99 (2H, m, C$^{\beta}H_2$), 3.79 (1H, m, C^5H), 4.08 (1H, d, C^6H), 4.21–443 (5H, m, Fmoc-CH, Fmoc-CH_2, C$^6H'$, OH), 4.61 (1H, m, C$^{\alpha}H$), 5.14 (1H, m, C^3H), 5.24 (1H, t, C^4H), 5.39 (1H, m, C^2H), 5.55 (1H, d, C^1H), 6.15 (1H, br d, FmocNH), 6.87 (1H, br s, C^{1}NH), 7.26–7.76 (8H, m, ArH); *m*/*z* (ESI) 684.90 ([M + H]$^+$. $C_{33}H_{37}N_2O_{14}$ requires *m*/*z*, 685.23), 707.30 ([M + Na]$^+$).

Fmoc-Gln[Man(OAc)$_4$]-OH (11f). 520 mg (0.69 mmol) of *t*-Bu ester yielded **11f** (473 mg, 98%) as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.30; δ_H (300 MHz; $CDCl_3$) 1.96–2.30 (16H, m, C(O)CH_3, C$^{\beta}H_2$, C$^{\gamma}H_2$), 3.78 (1H, m, C^5H), 4.09 (1H, m, C^6H), 4.19–4.30 (3H, m, Fmoc-CH, C$^6H'$, C$^{\alpha}H$), 4.38 (2H, m, Fmoc-CH_2), 4.77 (1H, br s, OH), 5.12 (1H, m, C^3H), 5.23 (1H, m, C^4H), 5.41 (1H, m, C^2H), 5.68 (1H, d, C^1H), 5.87 (1H, br d, FmocNH), 7.24–7.77 (9H, m, C^{1}NH, ArH); *m*/*z* (ESI) 698.90 ([M + H]$^+$. $C_{34}H_{39}N_2O_{14}$ requires *m*/*z*, 699.24), 721.85 ([M + Na]$^+$).

Fmoc-Asn[Lac(OAc)$_7$]-OH (11g). 580 mg (0.56 mmol) of *t*-Bu ester yielded **11g** (546 mg, 100%) as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.27; δ_H (300 MHz; $CDCl_3$) 1.97–2.15 (21H, 7 s, C(O)CH_3), 2.76–3.06 (2H, m, C$^{\beta}H_2$), 3.71–4.59 (12H, m, C$^{\alpha}H$, Fmoc-CH, Fmoc-CH_2, C^4H, C^5H, C$^6H_2$, C$^{1'}H$, C$^{5'}H$, C$^{6'}H_2$), 4.86–5.36 (6H, m, C^1H, C^2H, C^3H, C$^{2'}H$, C$^{3'}H$, C$^{4'}H$), 6.15 (1H, br d, FmocNH), 7.28–7.77 (9H, m, ArH, NHC^1), 8.60 (1H, br s, OH); *m*/*z* (ESI) 972.95 ([M + H]$^+$. $C_{45}H_{53}N_2O_{22}$ requires *m*/*z*, 973.31), 995.30 ([M + Na]$^+$).

Fmoc-Gln[Lac(OAc)$_7$]-OH (11h). 650 mg (0.62 mmol) of *t*-Bu ester yielded **11h** (605 mg, 98%) as a white solid. R_f (5% MeOH–DCM, 0.5% AcOH) 0.27; δ_H (300 MHz; $CDCl_3$) 1.91–2.14 (23H, m, C(O)CH_3, C$^{\beta}H_2$), 2.44 (2H, m, C$^{\gamma}H_2$), 3.69–4.48 (12H, m, C$^{\alpha}H$, Fmoc-CH, Fmoc-CH_2, C^4H, C^5H, C$^6H_2$, C$^{1'}H$, C$^{5'}H$, C$^{6'}H_2$), 4.86–5.35 (6H, m, C^1H, C^2H, C^3H, C$^{2'}H$, C$^{3'}H$, C$^{4'}H$), 6.08 (1H, br d, FmocNH), 7.25–7.75 (9H, m, ArH, NHC^1), 8.74 (1H, br s, OH); *m*/*z* (ESI) 987.25 ([M + H]$^+$. $C_{46}H_{55}N_2O_{22}$ requires *m*/*z*, 987.33), 1009.20 ([M + Na]$^+$).

Ac-[GlyProAsn(Gal)]$_4$-NH$_2$ (12)

The solid-phase synthesis of the glycopeptide was performed on 52 mg (containing 0.0187 mmol N(H)Fmoc) of Argogel® S RAM Fmoc which was washed with NMP (2 × 3 ml, each 2 min) prior to use.

1 Fmoc-deprotection. The resin was treated with 20% piperidine in NMP (2 × 3 ml, each 8 min), then was washed successively with NMP (3 × 3 ml, each 2 min) and DCM (3 × 3 ml, each 2 min).

2 Coupling of Fmoc-Asn[Gal(OAc)$_4$]-OH 11c. The glycopeptide monomer **11c** (38.5 mg, 0.056 mmol), HATU (21.4 mg, 0.056 mmol) and DiPEA (20 μl, 0.11 mmol) were dissolved in NMP (2 ml). This solution was added to the resin, after which the reaction was allowed to proceed (1 hour). Then, the resin was washed successively with NMP (3 × 3 ml, each 2 min) and DCM (3 × 3 ml, each 2 min).

3 Fmoc-deprotection. The resin was treated with 20% piperidine in NMP (2 × 3 ml, each 8 min), then was washed successively with NMP (3 × 3 ml, each 2 min) and DCM (3 × 3 ml, each 2 min).

4 Coupling of Fmoc-GlyPro-OH. Fmoc-GlyPro-OH (22 mg, 0.056 mmol), BOP (25 mg, 0.056 mmol), and DPEA (20 μl, 0.11 mmol) were dissolved in NMP (2 ml). This solution was added to the resin, after which the reaction was allowed to proceed (45 min). The resin was then washed successively with NMP (3 × 3 ml, each 2 min) and DCM (3 × 3 ml, each 2 min).

5 Completion of the resin-bound protected glycopeptide. Three-times repetition of steps 1–4 afforded the completely protected resin-bound glycopeptide.

6 Fmoc-deprotection. Treatment of the resin with 20% piperidine in NMP (2 × 3 ml, each 8 min) was followed by washing with NMP (3 × 3 ml, each 2 min) and DCM (3 × 3 ml, each 2 min).

7 Acetylation. After treatment of the resin with a solution of acetic anhydride (4.72 ml, 50 mmol), DiPEA (2.18 ml, 12.5 mmol), and HOBt (230 mg, 1.9 mmol) in NMP (100 ml) (2 × 2 mL, each 10 min), it was washed successively with NMP (3 × 3 ml, each 2 min) and DCM (3 × 3 ml, each 2 min).

8 Deacetylation of the carbohydrate moieties. Treatment of the resin with 2% NaOMe in MeOH (2 × 3 ml, each 30 min) was followed by washing with water (3 ml, each 3 min), diethyl ether (3 ml, each 3 min), NMP (3 × 3 ml, each 2 min), and DCM (3 × 3 ml, each 2 min).

9 Cleavage and purification. TIS (100 μl) and water (100 μl) were added to TFA (3 ml). The resulting mixture was added to the resin. The cleavage reaction was allowed to proceed for three hours. The resin was then removed by filtration, and the filtrate was evaporated to dryness. The crude product was then lyophilized and purified by preparative HPLC to yield 4.1 mg (12%) of **12**, δ_H (500 MHz; D_2O–H_2O 1 : 1 v/v) 1.86 (4H, m, Pro-C$^{\beta}H$), 1.94 (4H, m, Pro-C$^{\beta}H'$), 2.13 (4H, m, Pro-C$^{\gamma}H$), 2.39 (4H, m, Pro-C$^{\gamma}H'$), 2.72–3.04 (8H, m, Asp-C$^{\beta}H_2$), 3.40–3.80 (24H, m, C^2H, C^3H, C^4H, C^5H, C$^6H_2$), 3.59 (8H, m, Pro-C$^{\delta}H_2$), 4.06–4.22 (8H, m, Gly-C$^{\alpha}H_2$), 4.60 (4H, m, Pro-C$^{\alpha}H$), 4.87 (4H, m, Asn-C$^{\alpha}H$), 5.02 (4H, m, C^1H), 8.02–8.19 (4H, br, Gly-NH), 8.64–8.80 (4H, br m, Asn-NH), 8.80 (4H, br, C^1-NH). Exact mass (TOF MS ES) *m*/*z* 1802.7 ([M + Na]$^+$. $C_{70}H_{109}N_{17}NaO_{37}$ requires *m*/*z*, 1802.7.

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