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Note

A comparative study of different glycosylation methods for the synthesis of D-mannopyranosides of *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester

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Abstract—The synthesis of *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-acetyl)-α-D-mannopyranosyl]-L-proline allyl ester and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-benzoyl)-α-D-mannopyranosyl]-L-proline allyl ester is described. Glycosylation using Königs–Knorr conditions with a benzoyl protected glycosyl donor provided the optimum method. Removal of the allyl ester gave two mannosylated building blocks suitable for solid phase glycopeptide synthesis.

Keywords: Sugar amino acids; *trans*-4-Hydroxy-proline; Mannose; O-Glycosylation; Vaccines and C-lectins

There is current interest in the use of mannosylated peptides as recognition units for synthetic vaccines. Synthetic vaccines based on peptide sequences are only mildly immunogenic and generally need an adjuvant for optimal effect.[1,2] Mannosylation of synthetic peptide-based vaccines can enable the vaccines to be transported efficiently into the cytoplasm of antigen presenting cells (APCs) via mannose receptors on the surface of the APCs that bind molecules containing mannose, fucose or *N*-acetyl glucosamine ligands.[3,4]

One method to incorporate carbohydrate ligands into peptide based vaccines to enable uptake by APCs, is to synthesize glycosylated amino acid building blocks suitable for solid phase peptide synthesis. These glycosylated moieties can then be sequentially inserted into a peptide chain. Due to our interest in designing synthetic vaccines, we desired to introduce mannose residues into glycopeptides in this manner. There are numerous examples in the literature of mannosylated serine and threonine constructs.[5] However, hydroxy-proline (Hyp) offers another avenue to incorporate mannose into a peptide and is of particular interest due to the conformational effects that incorporation of a Hyp residue could introduce (Fig. 1).

There are many examples of glycosylated Hyp in the literature but most feature carbohydrate residues other than mannose. A variety of coupling methods and different Hyp protecting groups have been used for the synthesis of these glycosylated Hyp units including use of boron trifluoride etherate,[6–11] Königs–Knorr

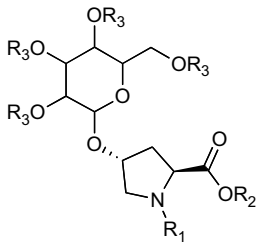

Figure 1. *trans*-4-Hydroxy-L-proline glycoconjugate.

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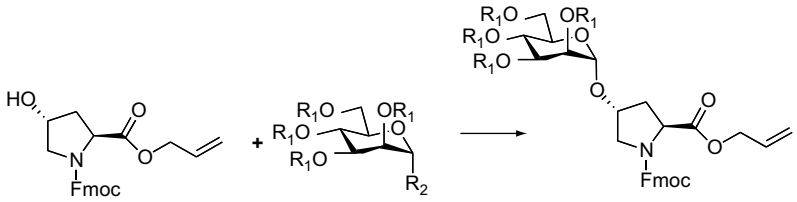
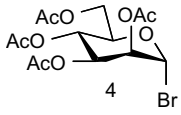
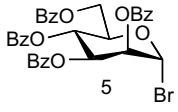
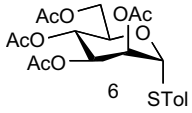
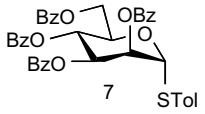
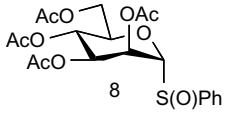
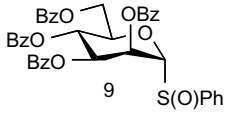
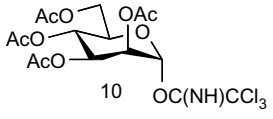
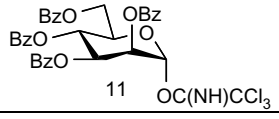
conditions under Helferich conditions using $Hg(CN)_2$,[12–14] standard Königs–Knorr conditions using silver triflate[9] and trifluoromethanesulfonic acid anhydride.[15,16] Silver zeolite promoted glycosylation,[17] enzymatic glycosylation,[18,19] the sulfoxide glycosylation method[20] and use of hexafluoroacetone as a protecting and activating reagent,[21,22] have also been used to glycosylate Hyp.

The synthesis of glycosylated Hyp derivatives with a single α-linked mannose is not well documented. Kessler has described the synthesis of 2-deoxy-2-iodo-α-*O*-glycopeptides including mannose derivatives of Fmoc-Hyp-OBn and Boc-Hyp-OBn[23] but the synthesis of the deiodinated compound is not described. A β-mannoside derivative of Fmoc-Hyp-OAllyl has been reported using an Umpolong approach[24] and Meldal and co-workers[25] have prepared α(1,2), α(1,3) and α(1,6) mannose disaccharides attached to Fmoc-Hyp-OPfp using the trichloroacetimidate method. The lack of information on the mannosylations of Hyp coupled with the often poor yields observed for Hyp glycosylations prompted detailed investigation of this reaction. In particular,

Table 1. Glycosylation of Fmoc-Hyp-OAllyl



Glycosyl donor	Promoter	Solvent	Conditions	Product	Yield (%)
 4	AgOTf	CH_2Cl_2	0 °C to rt	**2**	52
 5	AgOTf	CH_2Cl_2	0 °C to rt	**3**	89
 6	NIS/AgOTf	CH_2Cl_2	0 °C	**2**	41
 7	NIS/AgOTf	CH_2Cl_2	0°C	**3**	30
 8	Tf_2O	CH_2Cl_2	−40 °C to rt	**2**	54
 9	Tf_2O	CH_2Cl_2	−40 °C to rt	**3**	80
 10	Me_3SiOTf	Toluene	−78 °C	**2**	37
 11	Me_3SiOTf	Toluene	−78 °C	**3**	73

our attention was directed towards the facile synthesis of a mannosylated Hyp building block suitable for use in solid phase peptide synthesis.

N-α-Fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester (Fmoc-Hyp-OAllyl) **1**, synthesized according to Taylor et al.,[20] was chosen as the glycosyl acceptor due to the compatibility of the Fmoc group with solid phase peptide synthesis using Wang resin. The carboxylic acid was protected as the allyl ester as experience within our group and others,[20,26] attests to its stability and ease of removal. The result of four methods investigated to effect the key glycosylation are shown in Table 1.

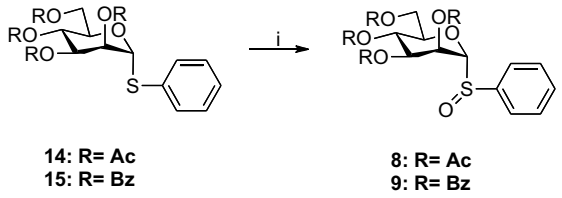
Königs–Knorr glycosylation[27] using silver triflate as the promoter has been used by several groups to glycosylate Fmoc-Hyp-OPfp.[9,25] Glycosylation of Fmoc-Hyp-OAllyl with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **4** gave the desired glycoside **2** in 52% yield (Table 1). As expected, use of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide **5** gave the corresponding product **3** in a superior 89% yield.

N-Iodosuccinimide (NIS) activation of thioglycoside donors was next investigated as a glycosylation technique.[28] Donors **6** and **7** with thiotolyl leaving groups were synthesized by addition of *p*-thiocresol to the fully protected sugars in the presence of boron trifluoride etherate.[29] Thioglycosides **6** and **7** were then reacted with Fmoc-Hyp-OAllyl **1** in the presence of NIS and catalytic silver triflate to give the glycosides **2** and **3** in 41% and 30% yield, respectively (Table 1).

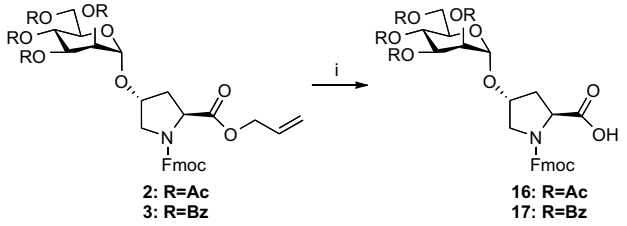
The sulfoxide glycosylation (Kahn) method[30] has been used with moderate success for the synthesis of galactosides of N^{α}-Fmoc-Hyp-OAllyl.[20] Thiophenyl ethers **14**[31] and **15**[32] were synthesized by addition of thiophenol under Lewis acid conditions. Oxidation with *m*-chloroperbenzoic acid gave sulfoxide donors **8**[33] and **9** (Scheme 1).[20] Glycosyl sulfoxides **8** and **9** were coupled with Fmoc-Hyp-OAllyl **1** in the presence of triflic anhydride to give glycosides **2** and **3** in 54% and 80% yield, respectively (Table 1).

The Schmidt procedure for glycosylation[34–36] has been used for the synthesis of Hyp glycoconjugates.[21,22,37] Use of trimethylsilyl triflate as a promoter with the acetyl and benzoyl protected trichloroacetimidate donors **10** and **11** gave glycosides **2** and **3** in 37% and 73% yield, respectively (Table 1).

Glycosides **2** and **3** were present as a mixture of rotamers of a single stereoisomer as determined by NMR analysis. In the ^{13}C NMR spectra two signals were observed indicating the presence of rotamers about the carbamate C–N bond. Both compounds were present as α-anomers, as to be expected with an axial hydroxyl at C-2 and neighbouring group participation preventing nucleophilic attack at the β-face. The NMR data was consistent with the literature examples of α-mannosides[25] with the small ($J = 1.3$–1.9 or unresolved) anomeric coupling constants supporting this assignment.



Scheme 1. Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, −78 °C, N_2, overnight, then Me_2S, 81% (**8** and **9**).



Scheme 2. Reagents and conditions: (i) CH_2Cl_2, $PhSiH_3$, $Pd(PPh_3)_4$, 2 h, rt, Ar, **16** (61%), **17** (50%).

In most cases use of benzoyl protecting groups resulted in higher yields due to competing formation of orthoesters[38] being observed with acetate groups.[39] Königs–Knorr glycosylation using bromide **5** resulted in the best yield of 89%, with satisfactory yields being obtained using the benzoyl protected sulfoxide and trichloroacetimidate donors **9** and **11**. Interestingly, triflic anhydride promoted sulfoxide glycosylation went in significantly higher yield than found by Taylor et al.[20] who reported yields of 41% and 46% for benzyl and pivaloyl protected galactose. Finally, removal of the allyl ester from mannosides **2** and **3** using tetrakis-(triphenylphosphine) palladium(0) gave the desired building blocks **16** and **17** (Scheme 2) ready for incorporation into solid phase peptide synthesis.

1. Experimental

1.1. General methods

High resolution mass spectra were recorded using a VG-70SE spectrometer at a nominal resolution of 5000–10,000 as appropriate. NMR spectra were recorded as indicated on either a Bruker DRX300 spectrometer operating at 300 MHz for ^1H nuclei and 75 Hz for ^{13}C nuclei or on a Bruker DRX400 spectrophotometer operating at 400 MHz for ^1H nuclei and 100 MHz for ^{13}C nuclei. Chemical shifts are reported in parts per million (ppm) relative to the tetramethylsilane peak recorded as ppm in $CDCl_3/Me_4Si$ solvent. Optical rotations were determined on a Perkin–Elmer 341 polarimeter.

1.2. Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside *S*-oxide (8)[33]

m-Chloroperbenzoic acid (1.2 equiv) was added to a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside **14** (490 mg, 1.11 mmol) in dichloromethane (10 mL) at −30 °C. After stirring overnight at −30 °C under N_2, the reaction mixture was quenched by the addition of dimethyl sulfide (0.1 mL), and warmed to room temperature. The reaction mixture was diluted with dichloromethane (30 mL) washed with water (15 mL), satd aq $NaHCO_3$, water (15 mL) and brine (15 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated in vacuo. Flash column chromatography using hexane–ethyl acetate (1:1) afforded sulfoxide **8** (409 mg, 81%) as an oil. (Found: MH^+, 457.1167, $C_{20}H_{25}NO_{10}S$ requires 457.1168); $[\alpha]_D^{20}$ −41.4 (*c* 2.95, $CHCl_3$); ν_{max} (film)/cm^{-1}3060 (w, ArC–H), 2961 (s, C–H alkane), 1748 (s, C=O ester), 1445 (w, ArC=C), 1370 (s, S=O), 1225 (s, C–O), 732 (m, C–S); δ_H (400 Hz, $CDCl_3$) 2.00, 2.04, 2.08, 2.12 (4 × 3H, s, $COCH_3$), 4.17 (1H, dd, *J* 12.5, 2.4 Hz, H-6_A), 4.29 (1H, dd, *J* 12.5, 5.5 Hz, H-6_B), 4.58 (1H, d, *J* 1.9 Hz, H-1), 4.60–4.64 (1H, m, H-5), 5.35 (1H, t, *J* 9.6 Hz, H-4), 5.64 (1H, dd, *J* 3.7, 2.1 Hz, H-2), 5.74 (1H, dd, *J* 9.4, 3.6 Hz, H-3), 7.53–7.75 (5H, m, ArH). δ_C (100 MHz, $CDCl_3$) 20.5, 20.6, 20.6, 20.7 ($COCH_3$), 60.3 (C6), 65.6, 65.8, 69.4 (C3, C4, C5), 74.5 (C2), 94.6 (C1), 125.0, 129.5, 131.8 (ArCH), 139.8 (quat., ArC), 169.4, 169.5, 169.6, 169.7 (quat., $COCH_3$); *m*/*z* (FAB, %) 457 (MH^+, 3), 331 (98), 169 (100), 127 (19), 109 (52).

1.3. Phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside *S*-oxide (9)

Using a similar procedure to that described above, oxidation of phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside **15** (519 mg, 0.75 mmol) with *m*-chloroperbenzoic acid gave, after flash column chromatography using hexane–ethyl acetate (3:1), sulfoxide **9** (430 mg, 81%) as an oil. (Found: MH^+, 705.1792, $C_{40}H_{33}O_{10}S$ requires 705.1794); $[\alpha]_D^{20}$ −85.6 (*c* 0.97, $CHCl_3$); ν_{max} (film)/cm^{-1} 3063 (w, ArC–H), 2960 (s, C–H alkane), 1728 (s, C=O ester), 1491 (w, ArC=C), 1315 (s, S=O), 1267 (s, C–O), 732, 709 (m, C–S); δ_H (400 Hz, $CDCl_3$) 4.56 (1H, dd, *J* 12.5, 4.4 Hz, H-6_A), 4.81 (1H, d, *J* 12.9 Hz, H-6_B), 4.91 (1H, s, H-2), 5.15–5.17 (1H, m, H-5), 6.10 (1H, s, H-1), 6.25 (1H, t, *J* 9.9 Hz, H-4), 6.38 (1H, dd, *J* 9.8, 3.3 Hz, H-3), 7.22–8.13 (25H, m, ArH). δ_C (100 MHz, $CDCl_3$) 62.6 (C6), 66.1, 67.0, 70.3 (C3, C4, C5), 74.7 (C2), 94.6 (C1), 128.2, 128.3, 128.4, 128.5, 129.4, 129.5, 129.6, 129.6, 129.8, 131.8, 133.1, 133.4, 133.5 (ArCH), 139.6 (quat., ArC), 164.7, 165.0, 165.3, 165.9 (quat., CO_2Bz); *m*/*z* (FAB, %) 457 (MH^+, 3), 331 (98), 169 (100), 127 (19), 109 (52); *m*/*z* (FAB, %) 705 (MH^+, 1), 579 (12), 154 (7), 105 (100), 77 (11).

1.4. General procedures for the synthesis of *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-α-D-mannopyranosyl]-L-proline allyl esters

1.4.1. Procedure A using glycosyl bromides. α-D-Mannopyranosyl bromide (2–3 equiv) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (1 equiv) were pre-dried under high vacuum in the presence of P_2O_5 for 6 h prior to reaction. To a solution of bromide and **1** in anhydrous dichloromethane (4 mL) at 0 °C was added powdered 4 Å molecular sieves (0.25 g). The reaction mixture was stirred at 0 °C for 10 min under argon and silver triflate (2.5 equiv) in dry toluene (2 mL) was added. After stirring for 3 h, *N*-methylmorpholine (2.5 equiv) was added to quench the reaction and the mixture stirred for 10 min. The reaction mixture was diluted with dichloromethane (20 mL), filtered through Celite and concentrated in vacuo. The crude product was purified by flash chromatography using hexane–ethyl acetate to afford the glycoside.

1.4.2. Procedure B using thiotolyl glycosyl ethers. *p*-Tolyl 1-thio-α-D-mannopyranoside (1.3 equiv) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (1 equiv) were pre-dried under high vacuum in the presence of P_2O_5 for 6 h prior to reaction. To a solution of thiotolyl glycosyl ether and **1** in anhydrous dichloromethane at 0 °C was added powdered 4 Å molecular sieves. The reaction mixture was stirred at 0 °C for 10 min under argon, then *N*-iodosuccinimide (1.3 equiv) and silver triflate (0.3 equiv) were added. The reaction mixture was stirred for 3 h then neutralized with Et_3N. The reaction mixture was diluted with dichloromethane (20 mL) and filtered through Celite. The organic layer was subsequently concentrated in vacuo and the resulting crude product was purified by flash chromatography using hexane–ethyl acetate to afford the glycoside.

1.4.3. Procedure C using glycosyl sulfoxides. Phenyl 1-thio-α-D-mannopyranoside *S*-oxide (1.2 equiv) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (1 equiv) were pre-dried under high vacuum in the presence of P_2O_5 for 6 h prior to reaction. These reagents were dissolved in dry toluene (5 mL), and 2,6-di-*tert*-butyl-4-methylpyridine (3.5 equiv) and powdered 4 Å molecular sieves (0.25 g) were added. After stirring for 1 h at room temperature, the reaction mixture was cooled to −78 °C (CO_2/acetone), trifluoromethanesulfonic anhydride (0.9 equiv) was added and the mixture stirred for 4 h. Satd aq $NaHCO_3$ (2 mL) was added to quench the reaction. The reaction mixture was diluted

with ethyl acetate (20 mL), filtered through Celite, washed with water (20 mL), satd aq $NaHCO_3$ (10 mL), water (20 mL) and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated in vacuo. The crude product was purified by flash chromatography using hexane–ethyl acetate to afford the glycoside.

1.4.4. Procedure D using glycosyl trichloroacetimidates. α-D-Mannopyranosyl trichloroacetimidate (2 equiv) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (1 equiv) were pre-dried under high vacuum in the presence of P_2O_5 for 6 h prior to reaction. To a solution of trichloroacetimidate and **1** in anhydrous dichloromethane (5 mL) at −78 °C (CO_2/acetone) was added powdered 4 Å molecular sieves (0.25 g). After stirring for 10 min, trimethylsilyltriflate (1 equiv) was added and the reaction mixture was left stirring at − 78 °C for 4 h. Et_3N (1 equiv) was added to quench the reaction, the mixture was stirred for 10 min, diluted with dichloromethane (20 mL), filtered through Celite and concentrated in vacuo. The crude product was purified by flash chromatography using hexane–ethyl acetate to afford the glycoside.

1.5. *N*α-Fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-acetyl)-α-D-mannopyranosyl]-L-proline allyl ester (2)

Following procedure A, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **4** (440 mg, 1.07 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (147 mg, 0.37 mmol) gave, after flash chromatography using hexane–ethyl acetate (1:1), glycoside **2** (140 mg, 52%).

Following procedure B, *p*-tolyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside **6** (1.05 g, 2.31 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (680 mg, 1.73 mmol) gave, after flash chromatography using hexane–ethyl acetate (1:1), glycoside **2** (513 mg, 41%).

Following procedure C, phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside *S*-oxide **8** (207 mg, 0.45 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (144 mg, 0.37 mmol) gave, after flash chromatography using hexane–ethyl acetate (1:1), glycoside **2** (143 mg, 54%).

Following procedure D, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate **10** (320 mg, 0.65 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (120 mg, 0.31 mmol) gave, after flash chromatography using hexane–ethyl acetate (1:1), glycoside **2** (82.3 mg, 37%).

Glycoside **2** was obtained as an oil. (Found: MH^+, 724.2601, $C_{37}H_{42}NO_{14}$ requires 724.2601); $[\alpha]_D^{20}$ 12.4 (*c* 1.95, $CHCl_3$); ν_{max} (film)/cm^{-1} 3064 (m, C–H alkene), 3018 (m, ArC–H), 2954 (m, C–H alkane), 1751 (s, C=O ester), 1648 (w, C=C alkene), 1451, 1420 (m, ArC=C), 1224 (s, C–O), 1133 (w, C–N); δ_H (300 Hz, $CDCl_3$) (mixture of rotamers) 2.00, 2.06, 2.10, 2.15 ($4 \times 3H$, s, $COCH_3$), 2.20–2.27 (1H, m, Hypβ-H_AH_B), 2.48–2.57 (1H, m, Hypβ-H_{AHB}), 3.60–3.83 (2H, m, Hypδ-H_2), 4.00–4.65 (10H, m, H-5, H-6_A, H-6_B, Hypα-H, Hypγ-H, $CH_2CH=CH_2$, Fmoc CH_2, Fmoc CH), 4.92 (1H, d, *J* 1.9 Hz, H-1), 5.18–5.35 (5H, m, H-2, H-3, H-4, $CH=CH_2$), 5.76–5.96 (1H, m, $CH=CH_2$), 7.29–7.78 (8H, m, Fmoc ArH). δ_C (75 Hz, $CDCl_3$) (mixture of rotamers) 20.4, 20.5, 20.6, 20.8 ($4 \times COCH_3$), 35.9, 37.0 (Hypβ-C), 46.9, 47.0 (Fmoc CH), 51.3, 51.3 (Hypδ-C), 57.4, 57.9 (Hypα-C), 62.4, 62.5 (C6), 65.7, 65.7 ($CH_2CH=CH_2$), 66.0, 66.0 (C5), 67.6 (Fmoc CH_2), 68.5 (C4'), 69.0, 69.1, 69.2, 69.4 (C3, C2), 74.6, 76.7 (Hypγ-C), 96.8, 97.4 (C1), 118.5, 118.8 ($CH=CH_2$), 119.7, 119.8, 124.8, 124.9, 126.9, 127.5 (Fmoc ArCH), 131.2, 131.4 ($CH=CH_2$), 141.1, 143.6, 143.7 (quat., Fmoc C), 154.1, 154.5 (quat., Fmoc C=O), 169.5, 169.7, 169.8, 170.3 (quat., $COCH_3$), 171.5, 171.6 (quat., COOH); *m*/*z* (FAB, %) 724 (MH^+, 5), 331 (97), 178 (54), 169 (100), 89 (32).

1.6. *N*α-Fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-benzoyl)-α-D-mannopyranosyl]-L-proline allyl ester (3)

Following procedure A, 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide **5** (600 mg, 0.91 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (168 mg, 0.43 mmol) gave, after flash chromatography using hexane–ethyl acetate (2:1), glycoside **3** (370 mg, 89%).

Following procedure B, *p*-tolyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside **7** (390 mg, 0.56 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (175 mg, 0.44 mmol) gave, after flash chromatography using hexane–ethyl acetate (2:1), glycoside **3** (128 mg, 30%).

Following procedure C, phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-α-D-mannopyranoside *S*-oxide **9** (296 mg, 0.42 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (141 mg, 0.36 mmol) gave, after flash chromatography using hexane–ethyl acetate (2:1), glycoside **3** (280 mg, 80%).

Following procedure D, 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate **11** (620 mg, 0.84 mmol) and *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester **1** (170 mg, 0.43 mmol) gave, after flash chromatography using hexane–ethyl acetate (2:1), glycoside **3** (306 mg, 73%).

Glycoside **3** was obtained as a colourless solid. (Found: MH^+, 972.3219, $C_{57}H_{50}NO_{14}$ requires 972.3231); mp 83–85 °C; $[\alpha]_D^{20}$ −40.8 (*c* 1.21, $CHCl_3$); ν_{max} (film)/cm^{-1} 3065 (m, ArC–H), 2954 (m, C–H

alkane), 1725 (s, C=O ester), 1601, 1451 (m, ArC=C), 1265, 1108 (s, C–O), 1176 (w, C–N); δ_H (400 Hz, $CDCl_3$) (mixture of rotamers) 2.30–2.36 (1H, m, Hypβ-H_AH_B), 2.58–2.63 (1H, m, Hypβ-H_AH_B), 3.73–3.91 (2H, m, Hypδ-H_2), 4.44–4.73 (10H, m, H-5, H-6_A, H-6_B, Hypα-H, Hypγ-H, $CH_2CH=CH_2$, Fmoc CH_2, Fmoc CH), 5.20–5.36 (3H, m, H-3, $CH=CH_2$), 5.91 (1H, s, H-1), 5.78–5.92 (2H, m, H-2, $CH=CH_2$), 6.12 (1H, q, *J* 9.8 Hz, H-4), 7.25–8.10 (28H, m, Bz ArH, Fmoc ArH). δ_C (100 Hz, $CDCl_3$) (mixture of rotamers) 36.4, 37.5 (Hypβ-C), 47.1, 47.2 (Fmoc CH), 51.4, 51.36 (Hypδ-C), 57.8, 58.2 (Hypα-C), 62.8 (C6), 65.9 ($CH_2CH=CH_2$), 66.8, 66.9 (C5), 67.8 (Fmoc CH_2), 69.6 (C4), 70.3, 70.5 (C3, C2), 74.8, 76.7 (Hypδ-C), 96.8, 97.5 (C1), 118.7, 119.9 ($CH=CH_2$), 119.9, 125.0, 127.1, 127.7 (Fmoc ArCH), 128.3, 128.4, 128.5, 128.6, 129.7, 129.8 (Bz ArCH), 131.4, 131.6 ($CH=CH_2$), 133.2, 133.2, 133.5, 133.6 (Bz ArCH), 141.3, 143.7, 143.9, 144.0 (quat., Fmoc C), 154.3, 154.7 (quat., Fmoc C=O), 165.2, 165.4, 166.1 (quat., CO_2Bz), 171.7, 171.8 (quat., COOH); *m*/*z* (FAB, %) 972 (MH^+, 1), 579 (9), 178 (19), 154 (100), 105 (57).

1.7. *N*α-Fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-acetyl)-α-D-mannopyranosyl]-L-proline (16)

*N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-acetyl)-α-D-mannopyranosyl]-L-proline allyl ester **2** (350 mg, 0.48 mmol) was dissolved in distilled dichloromethane (8 mL) and degassed with argon for 15 min. Tetrakis(triphenylphosphine)palladium(0) (0.025 equiv) and phenylsilane (2 equiv) were added and the reaction mixture was left to stir for 2 h. The reaction mixture was concentrated in vacuo and the crude product was purified by flash chromatography using hexane–ethyl acetate (1:1) + 10% acetic acid to afford glycoside **16** (203 mg, 61%) as a thick, viscous oil: (Found: MH^+, 684.2288, $C_{34}H_{38}NO_{14}$ requires 684.2288); $[\alpha]_D^{20}$ −2.85 (*c* 1.35, $CHCl_3$); υ_{max} (film)/cm^{-1} 3478 (br s, O–H), 3060 (s, ArC–H), 2956 (s, C–H alkane), 1755 (s, C=O ester, acid), 1435, 1477 (m, ArC=C), 1227, 1132 (s, C–O), 1047 (s, C–N); δ_H (300 Hz, $CDCl_3$) (mixture of rotamers) 1.99, 2.05, 2.08, 2.14 (4 × 3H, s, $COCH_3$), 2.28–2.32 (1H, m, Hypβ-H_AH_B), 2.48–2.53 (1H, m, Hypβ-H_AH_B), 3.57–3.79 (2H, m, Hypδ-H_2), 4.04–4.53 (8H, m, H-5, H-6_A, H-6_B, Hypα-H, Hypγ-H, Fmoc CH_2, Fmoc CH), 4.91 (1H, s, H-1), 5.23–5.36 (3H, m, H-2, H-3, H-4), 7.22–7.75 (8H, m, Fmoc ArH). δ_C (75 Hz, $CDCl_3$) (mixture of rotamers) 20.3, 20.5, 20.5, 20.7 (4 × $COCH_3$), 35.6, 36.9 (Hypβ-C), 46.7, 46.8 (Fmoc CH), 51.2, 51.3 (Hypδ-C), 57.3, 57.7 (Hypα-C), 62.4 (C6), 65.9, 66.0 (C5), 67.6, 67.7 (Fmoc CH_2), 68.5 (C4), 68.8, 69.0 (C3), 69.2, 69.3 (C2), 74.7, 76.6 (Hypγ-C), 96.6, 97.2 (C1), 119.6, 119.7, 124.8, 126.9, 127.3, 127.4 (Fmoc ArCH), 140.9, 141.0, 143.4, 143.5 (quat., Fmoc C), 154.3, 154.8 (quat., Fmoc C=O), 169.6, 169.7 (quat., $COCH_3$), 169.8 (quat. $COCH_3$), 170.5 (quat., $COCH_3$), 175.1, 175.4 (quat., COOH), 175.1 (quat., $COCH_3$); *m*/*z* (FAB, %) 684 (MH^+, 3), 331 (3), 178 (15), 154 (100), 89 (20).

1.8. *N*α-Fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-benzoyl)-α-D-mannopyranosyl]-L-proline (17)

Using the procedure described above, *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-benzoyl)-α-D-mannopyranosyl]-L-proline allyl ester **3** (370 mg, 0.38 mmol) was treated with tetrakis(triphenylphosphine)palladium(0) (0.025 equiv) and phenylsilane (2 equiv) to give after flash chromatography using hexane–ethyl acetate (2:1) + 10% acetic acid, glycoside **17** (179 mg, 50%) as a thick, viscous oil: (Found: MH^+, 932.2928, $C_{54}H_{46}NO_{14}$ requires 932.2918); $[\alpha]_D^{20}$ −32.8 (*c* 0.90, $CHCl_3$); υ_{max} (film)/cm^{-1} 3438 (br s, O–H), 3063 (s, ArC–H), 2959 (s, C–H alkane), 1728 (s, C=O ester, acid), 1601, 1451 (m, ArC=C), 1265, 1108 (s, C–O), 1068 (s, C–N); δ_H (300 Hz, $CDCl_3$) (mixture of rotamers) 2.38–2.44 (1H, m, Hypβ-H_AH_B), 2.61–2.67 (1H, m, Hypβ-H_AH_B), 3.73–3.90 (2H, m, Hypδ-H_2), 4.16–4.76 (8H, m, H-5, H-6_A, H-6_B, Hypα-H, Hypγ-H, Fmoc CH_2, Fmoc CH), 5.23 (1H, d, *J* 1.3 Hz, H-1), 5.73 (1H, d, *J* 1.5 Hz, H-2), 5.94 (1H, dd, *J* 10.1, 2.7 Hz, H-3), 6.12–6.20 (1H, m, H-4), 7.14–8.11 (28H, m, Bz ArH, Fmoc ArH). δ_C (75 Hz, $CDCl_3$) (mixture of rotamers) 36.1, 37.3 (Hypβ-C), 46.9, 47.0 (Fmoc CH), 51.3, 51.5 (Hypδ-C), 57.4, 57.6 (Hypα-C), 62.8 (C6), 66.4, 66.8 (C5), 67.9 (Fmoc CH_2), 69.4 (C4), 69.5, 69.6 (C3), 70.2, 70.4 (C2), 75.0, 76.7 (Hypγ-C), 96.9, 97.4 (C1), 119.7, 119.8, 124.9, 126.9, 127.0, 127.5, 127.6 (Fmoc ArCH), 128.1, 128.3, 128.4, 128.4, 128.5, 128.5, 132.9, 133.0, 133.4, 133.5 (Bz ArCH), 141.0, 141.1, 143.6, 143.7 (quat., Fmoc C), 154.5, 155.0 (quat., Fmoc C=O), 165.1 (quat., *C*OBz), 165.2, 165.3 (quat., *C*OBz), 166.0 (quat., *C*OBz), 176.0, 176.3 (quat., *C*OBz), 176.6 (quat., COOH); *m*/*z* (FAB, %) 932 (MH^+, 2), 579 (4), 178 (19), 154 (100), 105 (45).

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