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Novel ester linked glycosyl amino acids: convenient building blocks for the synthesis of glycopeptide libraries

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Abstract

The completely orthogonally protected aspartic acid derivative FmocAsp(OBn)O^{t}Bu is readily synthesized on a large scale. Deprotection of the β-carboxylic acid allows coupling to various sugar derivatives via free hydroxyl groups to produce novel glycosyl amino acids. Subsequent deprotection of either the α-acid or nitrogen is achieved cleanly to allow elaboration into an oligopeptide, whilst selective deprotection of PMB protected sugar hydroxyls is also readily achievable. Such novel glycosyl amino acid building blocks may be useful for the combinatorial synthesis of novel glycopeptide libraries.

1. Introduction

The oligosaccharide chains of naturally occurring glycoproteins are almost invariably either N- or O-linked to the anomeric position of the terminal sugar residue.[1] Thus the sugars of N-linked glycoproteins are bound to the peptide backbone via an asparagine residue, which is β-linked through nitrogen to C-1 of a terminal *N*-acetylglucosamine residue. The synthesis of N-linked glycopeptides,[2] therefore, usually requires the availability of a suitably protected aspartic acid residue which is most commonly coupled to an *N*-acetylglucosamine residue which possesses a β-nitrogen at the anomeric position. In contrast, the oligosaccharides of naturally occurring O-linked glycoproteins are usually linked α to the hydroxyl of a serine or threonine residue. Synthetic approaches to such materials usually commence with an α-glycosidation of a serine or threonine hydroxyl.[3]

The biological activity of glycopeptides has aroused considerable synthetic interest, both in the construction of naturally occurring materials themselves and also in the synthesis of analogues; a particularly recent trend is the synthesis of C-linked versions.[4] Consideration of analogue synthesis by structural variation reveals myriad potential targets. This molecular diversity, taken hand in hand with

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the potential biological activity, makes the synthesis of libraries of novel glycopeptides an attractive proposition for the combinatorial chemist.

This possibility of structural variation immediately opens up other modes of linking of the oligosaccharide chain to the peptide backbone. Perhaps the most obvious modification of the naturally occurring type of structure is that rather than to link the sugar chain to an amino acid via the anomeric position, that it could be simply linked via a hydroxyl group of one of the sugar units — conveniently via an ester bond. Since it is well documented that glycosylation can affect both the biological activity[5] and hydrolytic stability[6] of peptides it would also be interesting to investigate any effects that such forms of non-natural glycosylation may have.

Although studies on the acylation of sugar hydroxyls with the free carboxylic acids of amino acids were first undertaken nearly 40 years ago,[7] and the chemical[8] or enzymatic[9] synthesis of this type of compound has subsequently been reported many times, there are only a very few reports in the literature where sugars have been linked via ester bonds to amino acid side chains.[10] The synthesis of novel glycopeptides containing sugar units which are linked through their hydroxyl groups to the peptide backone via ester bonds therefore requires a suitably protected aspartic or glutamic acid residue as a linker unit. This paper details the facile and large scale synthesis of a new completely orthogonally protected aspartic acid residue and details subsequent manipulations which will allow the synthesis of novel types of O-linked glycopeptides. In particular, selective deprotection of the β-carboxyl allows standard diimide mediated coupling to free hydroxyl groups of selectively protected sugars to produce novel O-linked glycosyl amino acids. Subsequent selective deprotection of either the α-carboxylic acid or nitrogen will allow extension of the peptide chain in either direction. Selective deprotection of the sugar hydroxyl groups by oxidative means may also be readily accomplished. All of these transformations can be easily performed without cleavage of the sugar–amino acid linkage. It is envisaged that these building blocks may be used for the solid phase combinatorial synthesis of libraries of novel glycopeptides, using standard procedures.

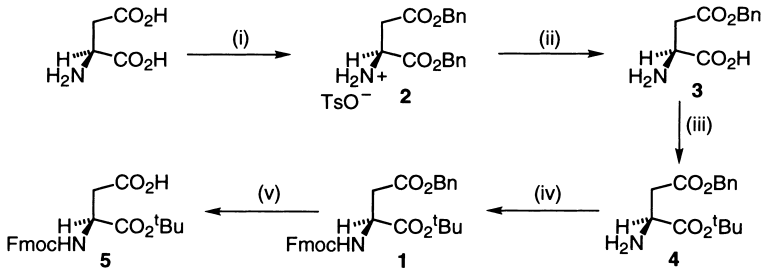
2. Results and discussion

2.1. Synthesis of protected amino acids

Although many protected aspartic acid derivatives are commercially available they are generally very expensive compared to the parent amino acid.[11] Since large amounts of orthogonally protected material were required for use in many later subsequent synthetic routes the synthesis of the protected amino acid **1** was undertaken directly from L-aspartic acid, following standard procedures. A literature search rather surprisingly revealed that the aspartic acid derivative **1** has not been previously described, and its synthesis is therefore detailed as follows. Refluxing L-aspartic acid with benzyl alcohol and tosic acid in benzene produced the known dibenzyl ester salt **2**.[12] Selective hydrolysis of the α-ester yielded the acid **3**,[13] which was then converted to the *t*-butyl α-ester **4**.[14] Finally, simple Fmoc protection of the nitrogen produced the orthogonally protected material **1** (Scheme 1). Simple catalytic hydrogenation allows deprotection of the β-carboxyl in very high yields to yield the free acid **5**,[15] a standard material used in *N*-glycopeptide synthesis. It should be noted that hydrogenation of the benzyl ester is fast and no competitive loss of the Fmoc nitrogen protection was observed during this reaction (vide infra).

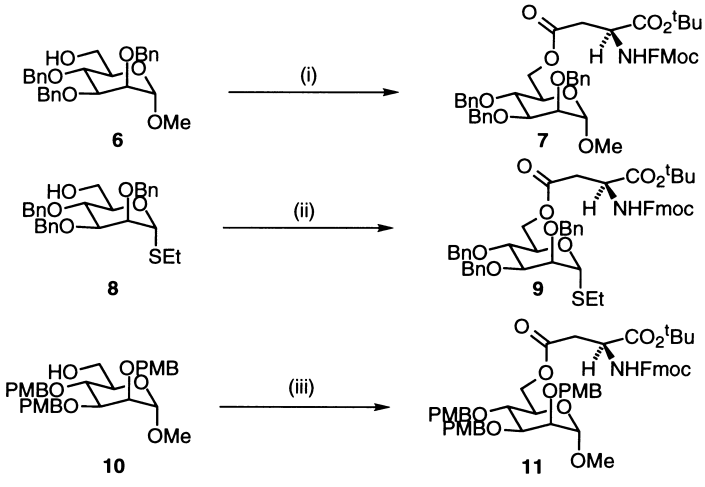
2.2. Coupling reactions to sugar hydroxyls

Initial studies were carried out in the *manno* series. The known[16] selectively protected methyl mannoside **6** was coupled to the β-carboxyl of **5** with DCC and DMAP in CH_2Cl_2 to yield the glycosyl



Scheme 1. Reagents: (i) TsOH, BnOH, benzene, reflux, 67% (ii) $CuSO_4$, NaOH, EtOH, H_2O, 32°C then EDTA, 100°C, 63% (iii) conc.H_2SO_4, isobutylene dioxan, 53% (iv) FmocCl, Na_2CO_3, dioxan, H_2O, 95% (v) H_2, Pd, EtOH, 95%

amino acid **7** in an acceptable 72% yield (Scheme 2). No resort to more expensive water soluble coupling reagents was necessary as purification of **7** by standard flash chromatography allowed straightforward removal of any urea impurities. Since it was envisaged that further sugar units may be added by glycosidation of an anomerically activated glycopeptide a similar coupling reaction was performed on thioglycoside **8**.[17] This proceeded directly analogously to give the thioglycosyl amino acid **9** in 75% yield. Finally, since it was envisaged that selective deprotection of the sugar hydroxyls may be desirable, and that problems may be encountered during hydrogenation of benzyl protecting groups on sugars linked to Fmoc protected amino acids (or indeed benzyl groups of thioglycosides), then similar coupling was undertaken for the PMB protected methyl glycoside **10**.[18] This again proceeded cleanly to produce the PMB protected derivative **11** in 72% yield.

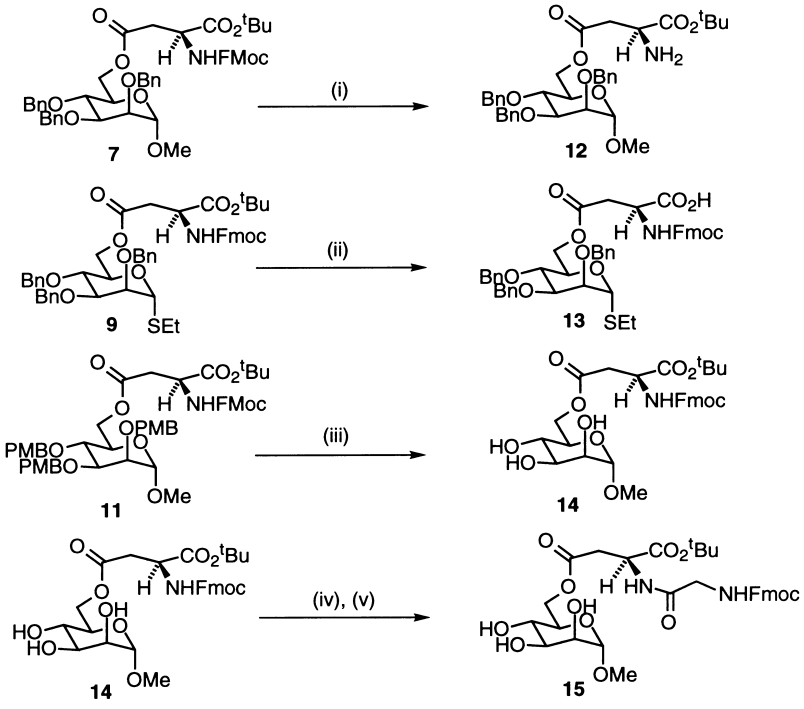


Scheme 2. Reagents (i) **5**, DCC, DMAP, CH_2Cl_2, 72% (ii) **5**, DCC, DMAP, CH_2Cl_2, 75% (iii) **5**, DCC, DMAP, CH_2Cl_2, 72%

2.3. Subsequent protecting group manipulations and peptide bond formation

In all cases removal of either the Fmoc or *t*-butyl ester amino acid protecting groups was achieved in high yield under standard conditions, with no loss of the sugar unit via hydrolysis of the ester linker. Thus treatment of **7** with piperidine in DMF gave the free amine **12** in 88% yield. Treatment of **9** with TFA/water resulted in quantitative hydroylsis of the *t*-butyl ester to give the free acid **13** (Scheme 3).

Selective deprotection of benzylated sugar hydroxyls by simple hydrogenation proved problematic. In all cases not only was loss of the Fmoc nitrogen protection competitive but also considerable amounts of cleavage of the sugar–acid ester bond were observed, even under slightly acidic conditions. However,



Scheme 3. Reagents (i) piperidine, DMF, 83% (ii) CF_3CO_2H, CH_2Cl_2, quantitative (iii) CAN, CH_3CN:H_2O, 9:1, CH_2Cl_2, 80% (iv) piperidine, DMF (v) Fmoc-glycine, EEDQ, benzene, 95% over two steps

oxidative deprotection of PMB groups was found to occur rapidly and cleanly. Thus treatment of **11** with ceric ammonium nitrate in acetonitrile/water yielded the triol **14** in 80% yield.

Finally, in order to demonstrate that peptide bond formation of such glycosyl amino acids is completely straightforward, the triol amino acid **14** was coupled with Fmoc protected glycine. The two step coupling of **14**, involving initial piperidine induced Fmoc removal and subsequent EEDQ mediated coupling with the Fmoc protected glycine, yielded the dipeptide **15** in 95% overall yield (Scheme 3).

3. Summary and conclusion

To summarize, we have synthesized several novel glycosyl amino acids, in which the sugar residue is linked to the amino acid via an ester bond of one of the sugar hydroxyls; in this particular instance mannose linked to aspartic acid. Also investigated and detailed are the necessary subsequent selective protecting group manipulations which will allow further elaboration of these materials in any of three senses: namely removal of nitrogen or carboxyl protection of the amino acid to allow peptide bond formation, or removal of the sugar hydroxyl group protection to allow further functionalization and/or glycosidation. In all cases it is possible to achieve these transformations without cleavage of the sugar–amino acid linkage. Finally, the two step EEDQ mediated coupling to Fmoc-glycine is found to proceed in high yield, demonstrating the feasibility of incorporation of these ester-linked glycosyl amino acids into synthetic oligopeptides.

Details of further investigations, both on the use of such building blocks for the assembly of libraries of novel glycopeptides, and of other potential applications of these materials, will be published in due course.

4. Experimental

Melting points were recorded on a Kofler hot block. Proton nuclear magnetic resonance (δ_H) spectra were recorded on Varian Gemini 200 (200 MHz), Bruker AC 200 (200 MHz), Bruker DPX 400 (400 MHz) or Bruker AMX 500 (500 MHz) spectrometers. Carbon nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50.3 MHz). Multiplicities were assigned using DEPT sequence. All chemical shifts are quoted on the δ scale. Infrared spectra were recorded on a Perkin–Elmer 150 Fourier Transform spectrophotometer. Mass spectra were recorded on VG Micromass 30F, ZAB 1F, Masslab20-250, Micromass Platform 1 APCI, or Trio-1 GCMS (DB-5 column) spectrometers, using desorption chemical ionization (NH_3 DCI), electron impact (EI), chemical ionization (NH_3 CI), atmospheric pressure chemical ionization (APCI), and fast atom bombardment (FAB) techniques as stated. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1 dm. Concentrations are given in g/100 ml. Hydrogenations were run under an atmosphere of hydrogen gas maintained by inflated balloon. Microanalyses were performed by the microanalytical service of the Dyson Perrins laboratory. Thin layer chromatography (TLC) was carried out on Merck glass-backed sheets, pre-coated with $60F_{254}$ silica. Plates were developed using 0.2% w/v cerium (IV) sulfate and 5% ammonium molybdate in 2 M sulfuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and available reagents were dried and purified before use according to standard procedures; methanol was distilled from magnesium methoxide, dichloromethane was distilled from calcium hydride, pyridine was distilled from calcium hydride and stored over potassium hydroxide, and tetrahydrofuran was distilled from a solution of sodium benzophenone ketyl immediately before use. Hexane was distilled at 68°C before use to remove involatile fractions.

4.1. L-*Aspartic acid-dibenzyl-ester*-p-*toluenesulphonate* ***2***

L-Aspartic acid (50 g, 376 mmol), *p*-toluenesulphonic acid monohydrate (82.2 g, 432 mmol) and benzyl alcohol (90.2 ml, 872 mmol) were refluxed in benzene (60 ml) with a Stark–Reed condensor. After 3 h the reaction mixture was allowed to cool to room temperature and diethyl ether (600 ml) was added. The mixture was kept in a refrigerator overnight. The resulting crystals were filtered and washed with cold diethyl ether (200 ml) to give the tosylate salt **2** (121.9 g, 67%) as a white waxy solid; m.p. 152–154°C (hexane/ethyl acetate) [lit.[12] m.p. 157–158°C]; $[\alpha]_D^{22}$ +7.04 (*c*, 1.25 in $CHCl_3$) [lit.[12] $[\alpha]_D^{20}$ +0.9 (*c*, 1.0 in MeOH)]; δ_H (200 MHz, $CDCl_3$): 2.27 (3H, s, *Me*PhSO$_2$), 3.13 (2H, m, CH_2), 4.49 (1H, m, CH), 4.89–5.01 (4H, m, 2×C*H*$_2$Ph), 7.01, 7.72 (4H, 2×d, Me*Ph*SO$_2$), 7.14–7.37 (10H, m, Ar).

4.2. L-*Aspartic acid-β-benzyl-ester* ***3***

The tosylate salt **2** (121.9 g, 251 mmol) and copper sulphate (251 g in 800 ml water, 1.004 mol) were stirred in ethanol (450 ml). The pH of the reaction mixture was raised to pH 8 by addition of 5 M NaOH, and then stirred at 32°C for 1 h. 3 M HCl was then added until the mixture reached pH 3. The resulting precipitate was filtered and washed with distilled water, ethanol and diethyl ether and then dissolved in distilled water (1000 ml). EDTA (103 g, 277 mmol) was added and the solution boiled for 10 min, filtered and allowed to cool to room temperature. The precipitated solid was then filtered and washed with distilled water (100 ml), ethanol (100 ml) and diethyl ether (100 ml) to give the α acid **3** (35.5 g, 63%) as a white solid; m.p. 209°C (water) [lit.[13] m.p. 218–219°C]; $[\alpha]_D^{22}$ +6.9 (*c*, 0.89 in H_2O) [lit.[13] $[\alpha]_D^{20}$ +7.73 (*c*, 1.0 in $MeCO_2H$)]; δ_H (200 MHz, DMSO): 2.47 (2H, s, NH_2), 2.58 (1H, dd, $J_{CH_2,CH}$ 5.2

Hz, $J_{CH_2,CH_2'}$ 16.8 Hz, CH_2), 2.88 (1H, dd, $J_{CH_2',CH}$ 7.6 Hz, CH_2'), 3.48 (1H, m, CH), 5.07 (2H, s, C*H*$_2$Ph), 7.35 (5H, m, Ar).

4.3. L-*Aspartic acid-β-benzyl-α*-tert-*butyl ester* **4**

A mixture of the α acid **3** (28.79 g, 124 mmol) and concentrated sulphuric acid (12 ml) in 1,4-dioxane (120 ml) was stirred in a pressure bottle at −25°C. Isobutylene gas (56 ml, 618 mmol) was then condensed into the reaction vessel which was immediately stoppered tightly and allowed to equilibrate to room temperature. After 20 h the reaction vessel was cooled to −25°C and the pressure released. The reaction mixture was diluted with 1 M sodium hydroxide (600 ml) and diethyl ether (200 ml). The aqueous phase was extracted with diethyl ether (3×200 ml) and the combined organic extracts dried ($MgSO_4$), filtered and concentrated in vacuo to give the α-*tert*-butyl ester **4** (18.01 g, 53%) as a clear oil; $[\alpha]_D^{22}$ +1.4 (*c*, 0.29 in $CHCl_3$), [lit.[14], $[\alpha]_D^{22}$ +7 (*c*, 1.0 in ethyl acetate)]; δ_H (200 MHz, $CDCl_3$): 1.44 (9H, s, $(CH_3)_3C$), 2.74 (1H, dd, $J_{CH_2,CH}$ 5.0 Hz, $J_{CH_2,CH_2'}$ 7.2 Hz, CH_2), 2.79 (1H, dd, $J_{CH_2',CH}$ 5.0 Hz, CH_2'), 3.73 (1H, dd, CH), 5.13 (2H, s, C*H*$_2$Ph), 7.37 (5H, m, Ar).

4.4. N-*(9-fluorenylmethoxycarbonyl)*-L-*aspartic acid-β-benzyl-α*-tert-*butyl ester* ***1***

9-Fluorenylmethoxychloroformate (4.437 g, 17 mmol) was added to a stirred solution of the α-*tert*-butyl ester **4** (4.785 g, 17 mmol) and sodium carbonate (9.089 g, 86 mmol) in 1,4-dioxane:water (3:5, 80 ml), at 0°C. The reaction mixture was allowed to equilibrate to room temperature. After 16 h, TLC (hexane:ethyl acetate, 1:1), indicated complete conversion of the starting material (R_f 0.1) to a single product (R_f 0.8). The reaction mixture was diluted with ethyl acetate (400 ml) and acidified to congo red with saturated potassium hydrogensulphate solution. The aqueous layer was extracted with ethyl acetate (200 ml) and the combined organic extracts washed with brine (200 ml) and distilled water (200 ml), then dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 3:1) to give the fully protected amino acid **1** (8.18 g, 95%) as a white crystalline solid, m.p. 82–85°C (diethyl ether/hexane); $[\alpha]_D^{26}$ +26.9 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr disk): 3355 (b, NH), 1721 (s, 3×C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.44 (9H, s, $(CH_3)_3C$), 2.90 (1H, dd, $J_{CH_2,CH}$ 4.5 Hz, $J_{CH_2,CH_2'}$ 17.0 Hz, CH_2), 3.05 (1H, dd, $J_{CH_2',CH}$ 4.6 Hz, CH_2'), 4.23 (1H, ap-t, $J_{CH[Fmoc],CH_2[Fmoc]}$ 7.2 Hz, $CH_{[Fmoc]}$), 4.39 (2H, m, $CH_{2[Fmoc]}$), 4.56 (1H, m, $J_{CH,NH}$ 8.4 Hz, CH), 5.16 (2H, ABq, J 12.2 Hz, C*H*$_2$Ph), 5.78 (1H, d, NH), 7.27–7.43, 7.60, 7.72 (13H, m, 2×d, CH_2Ph, $Ar_{[Fmoc]}$); δ_C (50.3 MHz, $CDCl_3$): 27.8 (q, $(CH_3)_3C$), 36.9 (t, CH_2), 47.1, 50.9 (2×d, CH, $CH_{[Fmoc]}$), 66.8, 67.2 (2×t, CH_2Ph, $CH_{2[Fmoc]}$), 120.2, 125.4, 127.4, 127.9, 128.6, 128.9 (6×d, Ar), 135.7, 141.6, 144.0, 144.2 (4×s, Ar), 156.3, 169.9, 171.1 (3×s, 3×C=O); m/z (CI^+): 502.2 ($M+H^+$, 15), 224.1 (35), 178.1 (100%). (Found: C, 71.88; H, 6.24; N, 2.78. $C_{30}H_{31}O_6N$ requires C, 71.84; H, 6.23; N, 2.79%.)

4.5. N-*(9-fluorenylmethoxycarbonyl)*-L-*aspartic acid-α*-tert-*butyl ester* ***5***

A solution of the fully protected amino acid **1** (18.84 g, 37.0 mmol) in ethanol (200 ml) was stirred under an atmosphere of hydrogen in the presence of palladium black (200 mg). After 8 h, TLC (hexane:ethyl acetate, 1:3) indicated complete conversion of the starting material (R_f 0.7) to a single product (R_f 0.6). The reaction mixture was filtered through Celite and concentrated in vacuo to give the mono acid **5** (15.225 g, 99%) as a white foam; $[\alpha]_D^{22}$ +13.8 (*c*, 1.0 in $CHCl_3$), [lit.[15], $[\alpha]_D^{25}$ −16.9 (*c*, 1.0 in DMF)]; δ_H (200 MHz, $CDCl_3$): 1.48 (9H, s, $(CH_3)_3C$), 2.91 (1H, dd, $J_{CH_2,CH}$ 4.4 Hz, $J_{CH_2,CH_2'}$

17.3Hz, CH_2), 3.04 (1H, dd, $J_{CH'_2,CH}$ 4.2 Hz, CH'_2), 4.26 (1H, d, $J_{CH[Fmoc],CH_2[Fmoc]}$ 7.6 Hz, $CH_{[Fmoc]}$), 4.39 (2H, m, $CH_{2[Fmoc]}$), 4.56 (1H, m, CH), 5.32 (1H, d, NH), 7.28–7.45 (8H, m, Ar).

*4.6. Methyl-6-*O*-carboxy-(*N*-(9-fluorenylmethoxycarbonyl)-*L*-aspartic acid-α-*tert*-butyl ester)-2,3,4-tri-*O*-benzyl-α-*D*-manno-pyranoside **7***

DMAP (40 mg) and DCCI (775 mg, 3.8 mmol) were added to a stirred solution of the methyl pyranoside **6** (1.743 g, 3.8 mmol) and the amino acid **5** (1.543 g, 3.8 mmol) in DCM (20 ml) at 0°C. The reaction mixture was allowed to equilibrate to room temperature. After 17 h, TLC (hexane:ethyl acetate, 1:1) indicated conversion of starting materials (R_f 0.5, 0.1 respectively) to a major product (R_f 0.7). The reaction mixture was filtered through Celite (eluted with DCM) and the solvent removed in vacuo. Purification by flash column chromatography (hexane:ethyl acetate, 5:1) gave the ester **7** (2.308 g, 72%) as a white foam; $[\alpha]_D^{22}$ +35.1 (*c*, 0.91 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 2910 (br, NH), 1731 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.51 (9H, s, ^{t}Bu), 2.91 (1H, dd, $J_{CH_2,CH}$ 4.5 Hz, J_{CH_2,CH'_2} 17.1 Hz, CH_2), 3.09 (1H, dd, $J_{CH'_2,CH}$ 4.5 Hz, CH'_2), 3.35 (3H, s, MeO), 3.38 (2H, m, H-2, H-5), 3.94 (2H, m, H-3, H-4), 4.25 (1H, apt, $J_{CH[Fmoc],CH_2[Fmoc]}$ 7.3 Hz, $CH_{[Fmoc]}$), 4.35 (2H, m, H-6, $CH_{2[Fmoc]}$), 4.42 (1H, dd, $J_{CH_2[Fmoc],CH[Fmoc]}$ 10.4 Hz, $CH_{2[Fmoc]}$), 4.48 (1H, dd, $J_{5,6'}$ 1.5Hz, $J_{6',6}$ 11.7 Hz, H-6′), 4.57 (1H, dd, $J_{CH,NH}$ 8.3 Hz, CH), 4.64 (3H, m, Ph*CH*$_2$), 4.76 (3H, m, H-1, Ph*CH*$_2$), 4.99 (1H, d, J 10.8 Hz, Ph*CH*$_2$), 5.87 (1H, d, NH), 7.30–7.45, 7.62–7.65, 7.80 (23H, 3×m, Ar); δ_C (50.3 MHz, $CDCl_3$): 27.8 (q, (*C*H_3)$_3$C), 38.7 (t, CH_2), 47.1, 50.9 (2×d, CH, $CH_{[Fmoc]}$), 54.9 (q, MeO), 64.2, 67.2, 72.1, 72.7, 75.2 (5×t, C-6, $CH_{2[Fmoc]}$ 3×*C*H_2Ph), 69.9, 74.4, 74.7, 80.3 (4×d, C-2, C-3, C-4, C-5), 82.7 (s, (CH$_3$)$_3$*C*), 99.2 (d, C-1), 120.2, 125.4, 127.6, 127.8, 127.9, 128.0, 128.3, 128.6, 129.1, 129.1 (10×d, Ar), 138.4, 138.5, 141.5, 144.1 (4×s, Ar), 156.4, 169.9, 171.2 (3×s, 3×C=O); m/z (APCI$^+$): 636.6 (M+H$^+$–Fmoc, 7), 770 (29), 548 (25), 243 (100%). (Found: C, 71.45; H, 6.62; N, 1.76. $C_{51}H_{55}O_{11}N$ requires C, 71.39; H, 6.46; N, 1.63%.)

*4.7. Ethyl-6-*O*-carboxy-(*N*-(9-fluorenylmethoxycarbonyl)-*L*-aspartic acid-α-*tert*-butyl ester)-2,3,4-tri-O-benzyl-1-thio-α-*D*-manno-pyranoside **9***

DMAP (40 mg) and DCCI (2.01 g, 9.7 mmol) were added to a stirred solution of the thioglycoside **8** (2.4 g, 4.9 mmol) and the amino acid **5** (2.6 g, 6.3 mmol) in DCM (40 ml) at 0°C. The reaction mixture was allowed to equilibrate to room temperature. After 7 h, TLC (hexane:ethyl acetate, 1:1) indicated complete conversion of the sugar starting material (R_f 0.2) to a major product (R_f 0.4). The reaction mixture was filtered through Celite (eluting with DCM) and the solvent removed in vacuo. Purification by flash column chromatography (hexane:ethyl acetate, 4:1) gave the ester **9** (3.24 g, 75%) as a white foam; $[\alpha]_D^{24}$ +59.0 (*c*, 0.4 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 1732 (s, 3×C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.24 (3H, t, J 7.4Hz, *Me*CH_2S), 1.47 (9H, s, ^{t}Bu), 2.57 (2H, dq, J 7.3 Hz, J 12.9 Hz, Me*CH*$_2$S), 2.85 (1H, dd, $J_{CH_2,CH}$ 4.4 Hz, J_{CH_2,CH'_2} 17.2 Hz, CH_2), 3.04 (1H, dd, $J_{CH'_2,CH}$ 4.6 Hz, CH'_2), 3.84 (2H, m), 3.92 (1H, ap-t, J 9.5 Hz), 4.19 (2H, m), 4.29 (1H, dd, $J_{CH[Fmoc],CH_2[Fmoc]}$ 7.3 Hz, $J_{CH[Fmoc],CH'_2[Fmoc]}$ 10.4 Hz, $CH_{[Fmoc]}$), 4.38 (3H, m, 1H, $CH_{2[Fmoc]}$), 4.53 (1H, m, CH), 4.59 (3H, m, Ph*CH*$_2$), 4.70 (2H, q, J 12.3 Hz, Ph*CH*$_2$), 4.95 (1H, d, J 10.8 Hz, Ph*CH*$_2$), 5.34 (1H, s, H-1), 5.82 (1H, d, J 8.3 Hz, NH), 7.25–7.42, 7.59, 7.76 (19H, m, 2×d, Ar); δ_C (50.3 MHz, $CDCl_3$): 14.9 (q, *Me*CH_2S), 25.4 (t, Me*CH*$_2$S), 27.8 (q, (*CH*$_3$)$_3$C), 36.6 (t, CH_2), 47.1, 50.9 (2×d, CH, $CH_{[Fmoc]}$), 64.1, 67.3, 72.1, 75.3 (4×t, C-6, $CH_{2[Fmoc]}$ 3×Ph*CH*$_2$), 70.2, 74.8, 76.1, 80.4, 82.1 (5×d, C-1, C-2, C-3, C-4, C-5), 82.7 (s, (CH$_3$)$_3$*C*), 120.2, 125.4, 127.1, 127.3, 128.1, 128.3, 128.7 (7×d, Ar), 138.2, 138.3, 141.5, 144.1 (4×s, Ar), 156.3, 169.9, 171.2

(3×s, 3×C=0); m/z (APCI$^+$): 666 (M+H$^+$–Fmoc, 20), 162 (79), 121 (52%). (Found: C, 70.21; H, 6.50; N,1.92. $C_{52}H_{57}O_{10}NS$ requires C, 70.33; H, 6.47; N, 1.58%.)

*4.8. Methyl-6-*O*-carboxy-(*N*-(9-fluorenylmethoxycarbonyl)-*L*-aspartic acid-α-*tert*-butyl ester)-2,3,4-tri-*O*-4-methoxybenzyl-α-*D*-manno-pyranoside **11***

DMAP (131 mg, 2.15 mmol) and DCCI (4.431 g, 21.47 mmol) were added to a stirred solution of the PMB protected methyl glycoside **10** (5.948 g, 10.74 mmol) and the amino acid **5** (6.619 g, 16.10 mmol) in DCM (80 ml) at 0°C. The reaction mixture was allowed to equilibrate to room temperature. After 8 h 30 min, TLC (hexane:ethyl acetate, 1:1) indicated conversion of the sugar starting material (R_f 0.3) to a major product (R_f 0.7). The reaction mixture was filtered through Celite (eluted with DCM) and the solvent removed in vacuo. Purification by flash column chromatography (hexane:ethyl acetate, 3:1 to 1:1) gave the ester **11** (8.559 g, 84%, 98% based on recovered starting material) as a white foam; $[\alpha]_D^{26}$ +28.3 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr disk): 3356 (br, NH), 1732 (3×C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.47 (9H, s, ^{t}Bu), 2.88, (1H, dd, $J_{CH_2,CH_2'}$ 17.4 Hz, $J_{CH_2,CH}$ 4.5 Hz, CH_2), 3.05 (1H, dd, $J_{CH_2',CH}$ 4.7 Hz, CH_2'), 3.28 (3H, s, MeO), 3.74–3.84 (13H, m, H-2, H-3, H-4, H-5, 3×*Me*OBn), 4.19 (1H, apt, $J_{CH[Fmoc],CH_2[Fmoc]}$ 7.2 Hz, $CH_{[Fmoc]}$), 4.27–4.38 (3H, m, H-6′, $CH_{2[Fmoc]}$), 4.40 (1H, dd, $J_{6,6'}$ 11.6 Hz, $J_{6,5}$ 1.8 Hz, H-6), 4.49–4.54 (4H, m, CH, Ph*C*H_2), 4.62–4.68 (3H, m, H-1, Ph*C*H_2), 4.86 (1H, d, J 10.8 Hz, Ph*C*H_2), 5.85 (1H, d, $J_{NH,CH}$ 8.3 Hz, NH), 6.82–6.87, 7.19–7.22, 7.25–7.31, 7.39, 7.58, 7.75 (20H, 3×m, 3×d, Ar); δ_C (50.3 MHz, $CDCl_3$): 27.9 (q, $(CH_3)_3C$), 36.7 (t, CH_2), 47.1, 50.9 (2×d, CH, $CH_{[Fmoc]}$), 54.8 (q, MeO), 55.2 (q, 3×*Me*OBn), 64.2, 67.2, 69.9, 71.7, 72.3, 73.9, 74.4, 74.8, 79.8 (4×d, 5×t, C-2, C-3, C-4, C-5, C-6, $CH_{2[Fmoc]}$ 3×MeOPh*C*H_2), 82.5 (s, $(CH_3)_3C$), 99.1 (d, C-1), 113.8, 113.8, 119.9, 125.2, 127.1, 127.7, 129.2, 129.5, 129.7, 130.2, 130.4, 130.5 (12×d, Ar), 141.3, 143.9 (2×s, Ar), 156.0, 169.6, 170.8 (3×s, 3×C=O); m/z (APCI$^+$): 726.8, (M+H$^+$–Fmoc, 91), 121.9 (100%). (Found: C, 68.23; H, 6.64; N, 1.62. $C_{54}H_{61}O_{14}N$ requires C, 68.36; H, 6.48; N, 1.48%.)

*4.9. Methyl-6-*O*-carboxy-(*L*-aspartic acid-α-*tert*-butyl ester)-2,3,4-tri-*O*-benzyl-α-*D*-manno-pyranoside **12***

Piperidine (1 ml) was added dropwise to a stirred solution of the Fmoc protected ester **7** in DMF (6 ml). After 1 h, TLC (ethyl acetate:methanol, 9:1) indicated complete conversion of starting material (R_f 0.7) to a single product (R_f 0.2). The solvents were removed in vacuo (co-evaporation with toluene) and the residue purified by flash column chromatography (ethyl acetate:methanol, 9:1) to give the free amine **12** (300 mg, 83%) as a clear oil; $[\alpha]_D^{22}$ +17.5 (*c*, 2.5 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 2914 (br, NH), 1732 (C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.49 (9H, s, ^{t}Bu), 2.68 (1H, dd, $J_{CH_2,CH}$ 4.4 Hz, $J_{CH_2,CH_2'}$ 16.3 Hz, CH_2), 2.84 (1H, dd, $J_{CH_2',CH}$ 8.2 Hz, CH_2'), 2.93, 3.00 (2H, 2×s, NH_2), 3.37 (3H, s, MeO), 3.77 (1H, dd, CH), 3.83 (2H, m, H-2, H-5), 3.95 (2H, m, H-3, H-4), 4.44 (2H, 2×d, $J_{5,6}$ 3.3 Hz, $J_{5,6'}$ 5.5 Hz, H-6, H-6′), 4.67 (3H, m, Ph*C*H_2), 4.76 (3H, m, H-1, Ph*C*H_2), 4.99 (1H, d, J 10.7 Hz, Ph*C*H_2), 7.32–7.43 (15H, m, Ar); δ_C (50.3 MHz, $CDCl_3$): 27.9 (q, $(CH_3)_3C$), 39.1 (t, CH_2), 51.8 (d, CH), 54.8 (s, MeO), 63.7, 72.1, 72.7, 75.2 (4×t, C-6, 3×*C*H_2Ph), 69.9, 74.3, 74.5, 80.2 (4×d, C-2, C-3, C-4, C-5), 81.7 (s, $(CH_3)_3C$), 99.0 (d, C-1), 127.8, 128.0, 128.1, 128.3, 128.6 (5×d, Ar), 138.4 (s, Ar), 171.5 (s, C=O); m/z (APCI$^+$): 636.7 (M+H$^+$, 8), 548.2 (100), 181.0 (26%). HRMS calcd. For $C_{36}H_{45}NO_9$ (M+H$^+$) 636.3173. (Found 636.3192.)

*4.10. Ethyl-6-*O*-carboxy-(*N*-(9-fluorenylmethoxycarbonyl)-*L*-aspartic acid)-2,3,4-tri-*O*-benzyl-1-thio-α-*D*-manno-pyranoside **13***

TFA (5 ml) was added to a stirred solution of the *tert*-butyl ester **9** (1.171 g, 1.3 mmol) in DCM (5 ml) at 0°C, then allowed to equilibrate to room temperature. After 3 h 30 min, TLC (ethyl acetate:methanol, 9:1) indicated complete conversion of starting material (R_f 0.9) to a single product (R_f 0.4).The reaction mixture was diluted with DCM (50 ml), and the solvents removed in vacuo to give the free acid **13** (1.604 g, quantitative yield) as a pale pink hygroscopic foam; $[\alpha]_D^{24}$ +58.4 (*c*, 1.0 in $CHCl_3$); ν_{max} (KBr disk): 3406 (br, NH), 1684 (s, 3×C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.23 (3H, t, J 6.9 Hz, *Me*CH_2S), 2.54 (2H, m, Me*C*H_2S), 2.82, (1H, m, CH_2), 3.07 (1H, m, CH_2'), 3.89 (3H, m), 4.07 (1H, m), 4.19 (1H, s), 4.38 (4H, m), 4.75 (2H, m), 4.92 (1H, d, J 10.8 Hz, Ph*C*H_2), 5.29 (1H, s, H-1), 5.94 (1H, s, NH), 7.16–7.59, 7.70, 7.75 (23H, m, 2×d, Ar); δ_C (50.3 MHz, $CDCl_3$): 15.3 (q, *Me*CH_2S), 25.1 (t, MeCH_2S), 36.6 (t, CH_2), 47.0, 51.3 (2×d, CH, $CH_{[Fmoc]}$), 63.8, 66.2, 70.9, 71.5, 74.7 (5×t, C-6, $CH_{2[Fmoc]}$ 3×Ph*C*H_2), 70.3, 74.7, 76.1, 79.9, 81.7 (5×d, C-1, C-2, C-3, C-4, C-5), 120.8, 125.9, 127.7, 128.2, 128.3, 128.9 (6×d, Ar), 138.8, 139.1, 141.4, 141.5 (4×d, Ar), 156.6, 171.1 (2×s, 2×C=O); m/z ($APCI^+$): 854.6 ($M+Na^+$, 41), 548.0 (44), 181.0 (87), 121.8 (100%); m/z (FAB^+): 854.4 ($M+Na^+$, 10), 179.1 (30), 91.0 (100%).

*4.11. Methyl-6-*O*-carboxy-(*N*-(9-fluorenylmethoxycarbonyl)-*L*-aspartic acid-α-*tert*-butyl ester)-α-*D*-manno-pyranoside **14***

The PMB protected ester **11** (1.819 g, 1.9 mmol) and CAN (6.74 g, 11.5 mmol) were stirred in acetonitrile:water (9:1, 18 ml) for 20 min when TLC (hexane:ethyl acetate, 1:1) indicated complete conversion of starting material (R_f 0.7) to a single product (R_f 0.1). The reaction mixture was diluted with DCM (250 ml) and washed with saturated sodium bicarbonate solution (150 ml). The aqueous layer was extracted with DCM (250 ml) and the combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 5:1) to yield the triol **14** (897 mg, 80%) as a white foam; $[\alpha]_D^{22}$ +50.1 (*c*, 0.9 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3414 (br, OH, NH), 1731 (3×C=O) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.47 (9H, s, ^{t}Bu), 2.49, 2.59, 2.87 (3H, 3×s, 3×OH), 2.91 (1H, dd, $J_{CH_2,CH_2'}$ 11.9Hz, $J_{CH_2,CH}$ 4.8 Hz, CH_2), 2.99 (1H, dd, $J_{CH_2',CH}$ 4.9 Hz, CH_2'), 3.26 (3H, s, MeO), 3.60 (1H, m, H-5), 3.68–3.81 (3H, m, H-2, H-3, H-4), 4.21 (1H, ap-t, $J_{CH[Fmoc],CH_2[Fmoc]}$ 6.8 Hz, $CH_{[Fmoc]}$), 4.29 (1H, dd, $J_{6,6'}$ 11.9 Hz, $J_{5,6}$ 1.5 Hz, H-6), 4.35 (1H, s, H-1), 4.45–4.57 (3H, m, H-6′, $CH_{2[Fmoc]}$), 4.59 (1H, m, CH), 6.13 (1H, d, $J_{NH,CH}$ 8.9 Hz, NH), 7.32, 7.42, 7.61, 7.78 (8H, 4×m, Ar); δ_C (50.3 MHz, $CDCl_3$): 27.8 (q, (*C*H_3)$_3$C), 37.7 (t, CH_2), 47.0, 51.0 (2×d, CH, $CH_{[Fmoc]}$), 55.0 (q, MeO), 63.1, 66.7, 67.4, 64.9, 70.6, 71.5 (4×d, 2×t, C-2, C-3, C-4, C-5, C-6, $CH_{2[Fmoc]}$), 83.1 (s, (CH$_3$)$_3$*C*), 100.9 (d, C-1), 120.0, 125.0, 125.0, 127.2, 127.8 (5×d, Ar), 141.2, 143.7, 143.7 (3×s, Ar), 156.2, 170.1, 170.3 (3×s, 3×C=O); m/z ($APCI^+$): 610.3 ($M+Na^+$, 9), 482.1 (57), 366.2 ($M+H^+$–Fmoc, 47), 310.2 (100%). (Found: C, 60.99; H, 6.21; N, 2.12. $C_{30}H_{37}O_{11}N$ requires C, 61.32; H, 6.35; N, 2.38%.)

*4.12. Methyl-6-*O*-carboxy-(*N*-(glycine-*N*-(9-fluorenylmethoxycarbonyl))-*L*-aspartic acid-α-*tert*-butyl ester)-α-*D*-manno-pyranoside **15***

Piperidine (0.5 ml) was added dropwise to a stirred solution of the triol **14** (37 mg, 0.06 mmol) in DMF (2.5 ml). After 30 min, TLC (hexane:ethyl acetate, 1:9) indicated complete conversion of starting material (R_f 0.3) to a single product (R_f 0.1). The solvents were removed in vacuo (co-evaporation with toluene) and the residue dissolved in benzene:ethanol (1:1, 2 ml). EEDQ (31 mg, 0.13 mmol) and *N*-(9-

fluorenylmethoxycarbonyl)-glycine (28 mg, 0.09 mmol) were added and the solution stirred under argon for 14 h when TLC (hexane:ethyl acetate, 1:9) indicated conversion of starting material (R_f 0.1) to a major product (R_f 0.2). The solvents were removed in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 1:9) to give the dipeptide **15** (39 mg, 95%) as a white foam; $[\alpha]_D^{24}$ +41.2 (c, 1.03 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3399 (br, OH, CO_2H), 1732 (3×C=O), 1529 (s, NC=O, Amide II) cm^{-1}; δ_H (400 MHz, $CDCl_3$): 1.45 (9H, s, ^tBu), 2.99 (2H, m, $CH_{2[Asp.]}$), 3.31 (3H, s, MeO), 3.65 (1H, d, J 8.5 Hz, H-5), 3.76–3.82 (2H, m, H-3, H-4), 3.90–4.05 (3H, m, H-2, $CH_{2[Gly.]}$), 4.22 (1H, apt, J 6.8 Hz, $CH_{[Fmoc]}$), 4.32–4.43 (3H, m, H-6, $CH_{2[Fmoc]}$), 4.50 (1H, d, $J_{6,6'}$ 11.8 Hz, H-6′), 4.73 (1H, s, H-1), 4.78 (1H, m, CH), 5.97 (1H, s, NH), 7.30, 7.39, 7.60, 7.75 (8H, 2×t, 2×d, Ar); δ_C (50.3 MHz, $CDCl_3$): 27.7 (q, $(CH_3)_3C$), 36.8, 44.0 (2×t, $CH_{2[Asp]}$ $CH_{2[Gly]}$), 46.9, 49.3 (2×d, CH, $CH_{[Fmoc]}$), 55.0 (q, MeO), 63.4, 67.4 (2×t, C-6, $CH_{2[Fmoc]}$), 67.2, 69.8, 70.6, 71.7 (4×d, C-2, C-3, C-4, C-5), 83.2 (s, $(CH_3)_3C$), 107.3 (d, C-1), 120.2, 125.4, 127.3, 127.9 (4×d, Ar), 141.4, 144.0 (2×s, Ar), 157.3, 170.0, 170.9 (3×s, 4×C=O); m/z ($APCI^+$): 645.7 ($M+H^+$, 82), 589.7 (100%).

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