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Solid phase peptide templated glycosidic bond formation

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Abstract—Glycosylation reactions performed between a glycosyl donor and acceptor covalently linked to a peptide template both in the solution and solid phase give similar yields and product distributions. The adoption of a solid phase approach opens the way for the synthesis of libraries of peptide templates in an attempt to screen for particular peptide sequences that effect complete regio-and stereochemical control during glycosidic bond formation, whilst the use of second generation donors allows the possibility of an iterative approach.

1. Introduction

Despite enormous efforts over the course of the last century, the stereo- and regioselective construction of glycosidic bonds remains an unsolved problem.[1] Although there have been some very impressive recent advances[2] no reliable, generally applicable processes have yet been developed to enable the construction of any oligosaccharide in an automated fashion, particularly with complete control of anomeric stereochemistry. Moreover, regiocontrol is currently only achieved in all but a few cases by extensive protecting group manipulations of the glycosyl acceptor. This generally means that protracted reaction sequences are required to construct the individual monosaccharide building blocks. In fact it is almost certainly true that when undertaking the construction of an oligosaccharide, the majority of time is spent synthesising the building blocks, and *not* actually performing the individual glycosylation steps.

The development of an automated approach for the synthesis of oligosaccharides is clearly highly desirable, particularly since oligonucleotides and oligopeptides are readily constructed in this manner. Ready access to even short oligosaccharides would be extremely beneficial to the further unravelling of the important roles these materials play in an enormous range of biological processes.[3]

Recently there has been considerable interest in the development of intramolecular approaches in order to achieve better stereocontrol during glycosylation reactions.[4] Temporary linking of glycosyl donor and acceptor is subsequently followed by intramolecular glycosylation, or intramolecular aglycon delivery (IAD).[5] Elegant as many of these approaches are,[6] they are mainly limited in that each such sequence of tethering and intramolecular glycosylation can really only effect the construction of one particular type of glycosidic linkage. In addition the majority of these studies have focussed on the stereocontrol of glycosylation, and regiocontrol has not been a major consideration.

It may be reasoned that the inherent increased regio- and stereochemical control of an intramolecular glycosylation reaction may be further exploited by the use of a more flexible approach. To this end we recently disclosed[7] our initial studies into a novel method of intramolecular glycosylation whereby we abandoned the classical philosophy of the search for a 'generalised glycosylation reaction', and proposed the use of combinatorial techniques to search for separate synthetic solutions to the construction of each individual glycosidic bond. In these studies we detailed the use of short peptide templates[8] which were able to affect the regio- and stereochemical outcome of glycosylation reactions preformed between donors and acceptors that were covalently linked to them. In particular it was demonstrated that the product distributions observed for intramolecular glycosylation reactions performed between the glycosyl acceptor **1** and the glycosyl donor **2**, when incorporated into the short peptides **3**, were to an extent dependent on the nature of the peptide sequence (Fig. 1).

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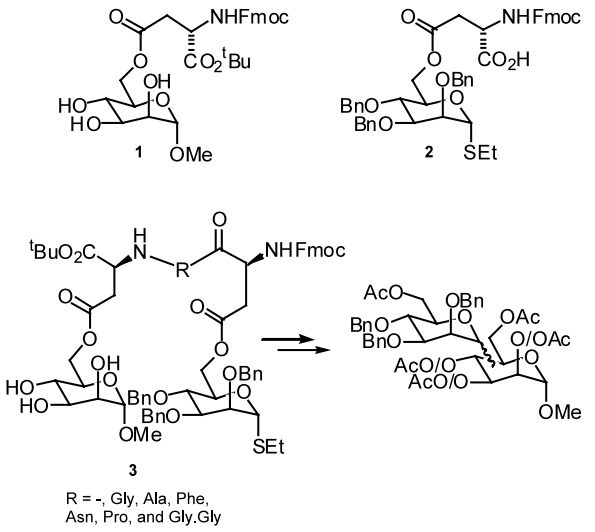


Figure 1.

Armed with the knowledge that the peptide sequence of such templates could influence the regio- and stereochemical outcome of glycosylation reactions, the next logical step involved screening libraries of templates for their potential to promote the formation of particular glycosidic linkages with much higher degrees of regio- and stereocontrol. However, in order to access such template libraries for screening, it was first necessary to adapt the approach to the solid phase. In addition, to open up the possibility of an iterative process, whereby further glycosylation reactions may be performed following peptide coupling of more donors, it was necessary to synthesise a second generation of glycosyl donors and acceptors.[9] Herein, we describe the synthesis of a novel glycosyl amino acid donor **4** and acceptor **5**, elaboration of these monosaccharides into a dipeptide **6a**, and its resin bound derivative **6b**, and the outcome of intramolecular glycosylation reactions of **6a** and **6b** (Fig. 2).

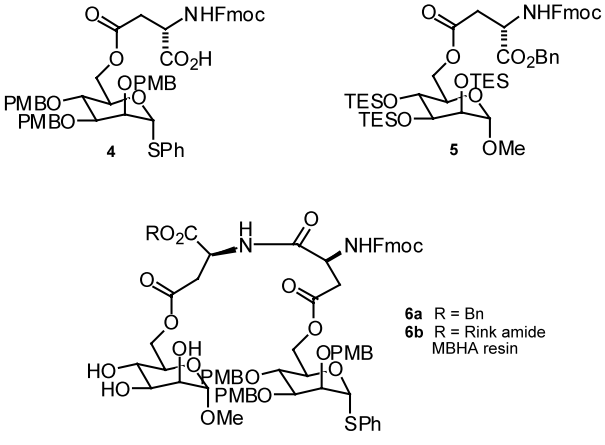
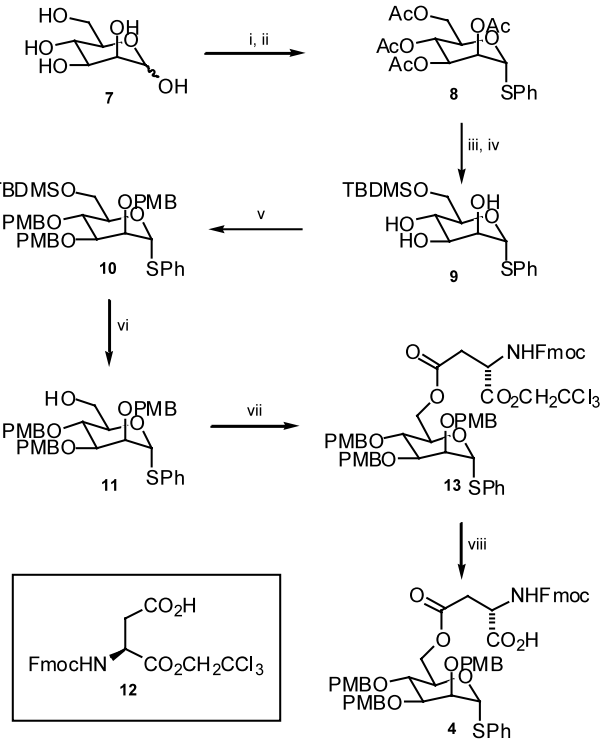


Figure 2.

2. Results and discussion

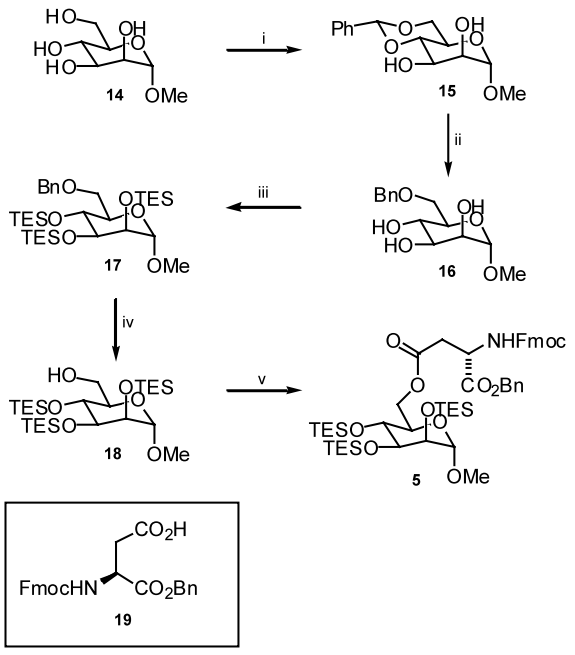
Glycosyl amino acid **4**, which is the glycosyl donor, was synthesised from mannose in the following manner. Peracetylation of mannose **7**, by treatment with acetic anhydride and sodium acetate, gave the α-acetate, which was then treated with thiophenol and boron trifluoride etherate to afford the known α-thioglycoside **8**.[10] The thioglycoside **8** was deprotected by treatment with sodium methoxide in methanol, and the crude product was then treated with *tert*-butyldimethylsilyl (TBDMS) chloride and imidazole in DMF to afford the triol **9**. Subsequent protection of **9** with *para*-methoxybenzylchloride (PMBCl) and sodium hydride in THF gave the fully protected thioglycoside **10** which was then desilylated by treatment with tetrabutylammonium fluoride (TBAF) in THF to afford the alcohol **11**. Dicyclohexylcarbodiimide (DCC) mediated coupling of **11** with aspartic acid derivative **12**[11] gave the ester **13**, which was finally deprotected by treatment with zinc in acetic acid to afford the desired glycosyl amino acid donor **4** (Scheme 1).



Scheme 1. *Reagents and conditions*: (i) Ac_2O, NaOAc, reflux; (ii) PhSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt; (iii) Na, MeOH, 0°C to rt; (iv) TBDMSCl, imidazole, DMF, −20°C; (v) PMBCl, Bu_4NI, THF, NaH, reflux; (vi) Bu_4NF, Et_2O, 0°C; (vii) DCC, CH_2Cl_2, DMAP, **12**, rt; (viii) AcOH, Zn, rt.

Glycosyl amino acid acceptor **5**[12] was synthesised from methyl mannopyranoside **14** by initial treatment with benzaldehdye dimethylacetal and camphorsulphonic acid in DMF to give the monobenzylidene derivative **15**.[13] Subsequent regioselective reductive cleavage of the benzylidene ring was achieved by treatment with TFA and triethylsilane to give the triol **16**. Treatment of **16**

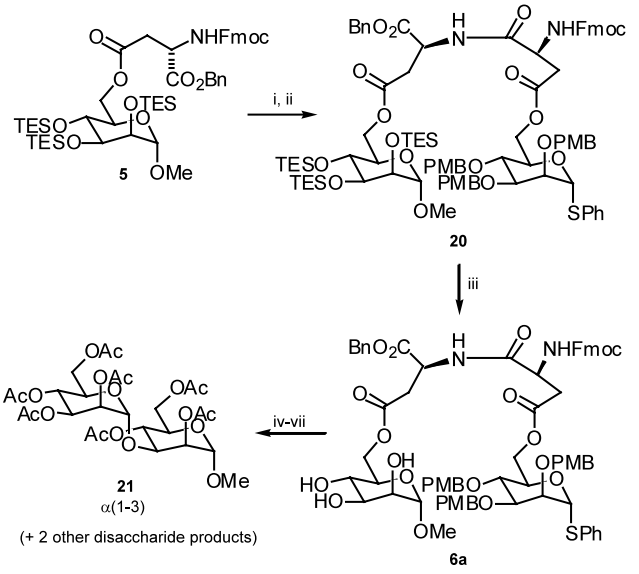
with triethylsilyltriflate then gave the completely protected glycosyl acceptor **17**, which was hydrogenated in the presence of palladium black to afford the alcohol **18**. Finally DCC mediated coupling of **18** with aspartic acid derivative **19**[14] gave the desired protected glycosyl amino acid acceptor **5** (Scheme 2).



Scheme 2. *Reagents and conditions*: (i) $PhCH(OMe)_2$, CSA, DMF, 60°C; (ii) TFA, Et_3SiH, CH_2Cl_2, 0°C; (iii) TESOTf, CH_2Cl_2, pyridine, –40°C; (iv) H_2, Pd, EtOAc, EtOH, rt; (v) DCC, CH_2Cl_2, DMAP, **19**, rt.

The glycosyl amino acid donor **4** and acceptor **5** were then elaborated into dipeptide **6a**. Treatment of **5** with piperidine in DMF was followed by EEDQ mediated coupling with carboxylic acid **4** to give the protected dipeptide **20**. Removal of the TES protecting groups with formic acid in ethyl acetate afforded the deprotected dipeptide **6a**. Intramolecular solution phase glycosylation of **6a**, promoted by NIS and AgOTf, afforded three separable disaccharide glycopeptide products in a 1:2:3.5 ratio in an overall yield of 65% (based on recovered starting material). The major reaction product was identified as follows; stirring with potassium carbonate in methanol cleaved the disaccharide from the peptide template, treatment with TFA removed the PMB protection and finally complete acetylation gave the known α-(1–3)-linked disaccharide **21**[15] (Scheme 3).

With the solution phase glycosylation in hand, our attention turned to the development of a solid phase approach and the construction of resin bound dipeptide **6b**. A polystyrene resin with a Rink amide linker[16] was chosen as the solid support since the solvents used in the construction and glycosylation of **6b** (namely DCM, DMF and THF) have favourable swelling properties. Further to this the Rink linker would withstand the

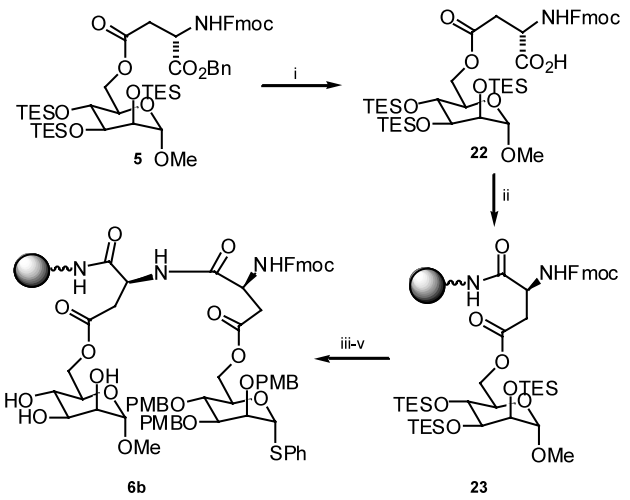


Scheme 3. *Reagents and conditions*: (i) piperidine, DMF, rt; (ii) **4**, EEDQ, EtOH, benzene, rt; (iii) EtOAc, HCO_2H, rt; (iv) NIS, AgOTf, CH_2Cl_2, rt; (v) K_2CO_3, MeOH, rt; (vi) TFA, CH_2Cl_2, rt; (vii) Ac_2O, pyridine, rt.

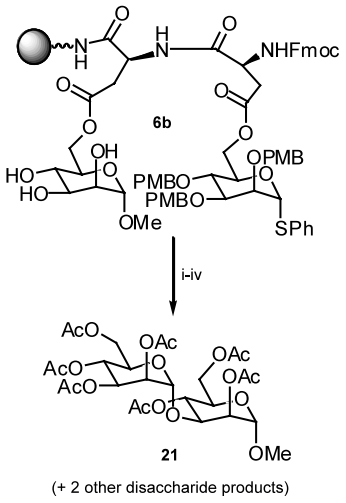
basic conditions employed to cleave the disaccharides from their peptide templates thus aiding product purification.

Glycosyl amino acid acceptor **5** was attached to the resin: catalytic hydrogenation afforded the free acid **22**, which was then coupled to deprotected Rink amide resin with TBTU to give glycosyl amino acid acceptor **23**. A notable point is that since the hydroxyls of **23** are temporarily TES protected it is possible to cap any free amino groups between successive peptide coupling steps by treatment with acetic anhydride and pyridine, a procedure which is useful for the synthesis of longer peptides. Next **23** was treated with piperidine in DMF to remove the Fmoc protection, and then coupled with glycosyl amino acid donor **4** (mediated by EEDQ) to give the solid supported dipeptide, after which any amino groups were again capped by acetylation. Finally removal of the temporary TES protection was achieved by treatment with DMF and formic acid to give the desired deprotected dipeptide **6b** (Scheme 4).

With the resin bound dipeptide **6b** in hand the efficiency of the coupling reactions was ascertained. Thus a sample of **6b** was shaken with *n*-propylamine in a mixture of THF and methanol, which resulted in cleavage of the sugar-peptide ester linkages and yielded the free carbohydrate glycosyl acceptor **14** and donor **11** in 76 and 47% yields, respectively. The high yield of recovered acceptor averaged over five steps (coupling of glycosyl amino acid acceptor **5**, removal of Fmoc, coupling of glycosyl amino acid donor **4**, removal of TES and basic cleavage from the template) equates to an average yield of 95% per step. However, the yield of the recovered glycosyl donor **11** is considerably lower and averaged over three steps (coupling of glycosyl amino acid donor **4**, removal of TES and basic cleavage



Scheme 4. *Reagents and conditions*: (i) H_2, Pd, EtOH, rt; (ii) deprotected Rink amide MBHA resin, DMF, TBTU, rt; (iii) DMF, piperidine, rt; (iv) **4**, EEDQ, DMF, rt; (v) DMF, HCO_2H, rt.



Scheme 5. *Reagents and conditions*: (i) NIS, AgOTf, CH_2Cl_2, rt; (ii) THF, MeOH, $PrNH_2$, rt; (iii) TFA, CH_2Cl_2, rt; (iv) Ac_2O, pyridine, rt.

from the template) only equates to an average yield of 77% per step. Presumably the low yielding step is the second peptide coupling reaction which was not driven to completion through the repeated use of an excess of reagents.

Undeterred by the yield of recovered glycosyl donor, **6b** was glycosylated under identical conditions as those used for the solution phase glycosylation of **6a**. The products were then cleaved from the resin under basic conditions (*n*-propylamine and methanol in THF) and purified. Together with the glycosyl acceptor **14** (which was recovered in 53% yield) three disaccharide products were isolated in a total yield of 26% (55% based on quantity of donor bound to the resin). The major disaccharide product (which constituted about 50% of this total yield, 28% overall yield) was identified by deprotection with TFA and then acetylation which again afforded the α-(1–3)-linked disaccharide **21**, i.e. the same major product as for the solution phase glycosylation of dipeptide **6a** (Scheme 5). The large amount of recovered glycosyl acceptor **12** is to be expected in the light of the relative amounts of donor and acceptor recovered from the resin before glycosylation (vide supra).

Several notable observations can be made from the glycosylation of resin bound dipeptide **6b**. The yield for the glycosylation was comparable to that of the solution phase reaction (55 and 65% total yields), the product distributions of the two glycosylations were similar (three products from a possible six), and in both cases the major product, which constitutes about half of the total yield of disaccharide, was identified as the α-(1–3)-linked disaccharide **21**.

3. Conclusions

In conclusion, the use of the second generation donor and acceptor both in solution and pleasingly on the solid phase produces results similar to those obtained previously. At this stage it is clear that the resin bound glycosylation strategy has yet to be optimised, particularly in terms of the efficiency of the peptide coupling steps. In addition the regio- and stereocontrol observed in both solution phase and solid supported glycosylations is still very modest, but it is perhaps unrealistic to hope that such a simple peptide template could completely control the regio- and stereochemical outcome of such a glycosylation reaction and result in the formation of a single product. However, the development of the solid supported approach detailed herein is crucial since it now paves the way for the rapid solid-supported construction of many peptide templates. In addition the use of a second generation donor, which is itself easily deprotected under mild conditions will also allow iteration of the whole process in order to access higher oligosaccharides. The synthesis of solid-supported libraries of glycopeptides, the screening of these libraries for particular peptide sequences that result in the high yielding formation of single products, and attempts at iteration, are all currently in progress and the results will be published in due course.

4. Experimental

4.1. General

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 Bruker DPX 400 (100.6 MHz) or a Varian Gemini 200

(50.3 MHz) spectrometer. 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 g/100 ml. Hydrogenations were run under an atmosphere of hydrogen gas maintained by inflated balloon. Microanalyses were performed by the microanalytical services of the Inorganic Chemistry Laboratory, Oxford. Thin layer chromatography (TLC) was carried out on Merck glass backed sheets, pre-coated 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 between 40 and 60°C before use to remove involatile fractions.

4.2. General pre-washing and deprotection of Rink amide MBHA resin (loading 0.65 mmol/g)

The resin was placed in a 'Reacti-vial' and DMF (4 ml) added. After approximately 20 s the resin had swelled and the solvent was drained. The vial was stoppered and 20% piperidine in DMF (4 ml) added. The vial was capped and shaken on a flat bed shaker for 15 min. The solvents were removed and the resin washed with DMF (6×2 ml). The above process was then repeated two more times.

4.3. General bromophenol blue indicator test

A small sample of resin (a few beads) was removed after the resin had been washed and treated with a few drops of bromophenol blue (0.01 M in DCM). Retention of the resin's yellow colour indicated the absence of amine functionality. Conversion of the resin to a deep blue colour indicated the presence of amine functionality.

4.4. General TNBSA (tri-nitrobenzene sulfonic acid) indicator test

A small sample of resin (a few beads) were removed after the resin had been washed and treated with a two drops of DMF, one drop of TNBSA (1% in DMF) and two drops of di-*iso*-propylethylamine. Retention of the resin's yellow colour indicated the absence of amine functionality. Conversion of the resin to a red colour indicated the presence of amine functionality.

4.5. Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside, 8

A stirred solution of sodium acetate (15 g) in acetic anhydride (500 ml) was heated under reflux for 15 min. D-Mannose **7** (50 g, 278 mmol) was then added portionwise over a period of 30 min. After a further 10 min the reaction mixture was allowed to cool to room temperature and was poured into iced water (500 ml). Ethyl acetate (1000 ml) was added and the organic phase was extracted, washed with brine (2×500 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 3:1) to give mannose α-pentacetate (80.92 g, 75%) as a clear oil a portion of which was used directly without characterisation. Boron trifluoride etherate (8.52 ml, 67.2 mmol) was added to a stirred solution of mannose α-pentacetate (17.48 g, 44.8 mmol) and thiophenol (13.8 ml, 134.5 mmol) in DCM (80 ml) at 0°C, under an atmosphere of argon. The reaction mixture was allowed to equilibrate to room temperature. After 17 h, TLC (hexane:ethyl acetate, 1:1) indicated complete conversion of the starting material (R_f 0.3) to a major product (R_f 0.5). The reaction mixture was diluted with DCM (300 ml) and washed with saturated aqueous sodium bicarbonate solution (200 ml) and brine (200 ml). The combined aqueous phases were re-extracted with DCM (200 ml) and then the combined organic phases were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 3:1) to give the thioglycoside **8** (17.46 g, 88%) as a white solid; mp 83–85°C (methanol/water) [lit.[10] mp 87°C (hexane/diethyl ether)]; $[\alpha]_D^{22}$ +74.4 (*c*, 1.17 in $CHCl_3$) [lit.[17] $[\alpha]_D^{27}$ +88.6 (*c*, 2.0 in DCM)]; δ_H (500 MHz, $CDCl_3$): 2.02, 2.06, 2.08, 2.16 (12H, 4×s, 4×CH_3CO_2), 4.11 (1H, dd, $J_{6,5}$ 2.2 Hz, $J_{6,6'}$ 12.2 Hz, H-6), 4.31 (1H, dd, $J_{6',5}$ 5.9 Hz, H-6′), 4.55 (1H, m, H-5), 5.33 (2H, m), 5.51 (2H, m), 7.31–7.50 (5H, m, Ar).

4.6. Phenyl 6-*O*-*tert*-butyldimethylsilyl-1-thio-α-D-mannopyranoside, 9

A solution of sodium (13.8 mg) in methanol (3 ml) was added via cannula, to a stirred solution of thioglycoside **8** (300 mg, 0.68 mmol) in methanol (3 ml) at 0°C, under an atmosphere of argon. The reaction mixture was then allowed to equilibrate to room temperature. After 1 h, TLC (ethyl acetate:methanol, 9:1) indicated complete conversion of the starting material (R_f 0.7) to a major product (R_f 0.3). Aqueous HCl (1 M, 0.6 ml) was added and the solvents removed in vacuo to give phenyl 1-thio-α-D-mannopyranoside which was used without characterisation. To this residue were added *tert*-butyldimethylsilylchloride (123 mg, 0.82 mmol) and imidazole (93 mg, 1.36 mmol) in DMF (3 ml) at −20°C, under an atmosphere of argon. After 25 min, TLC (ethyl acetate:methanol, 9:1) indicated complete conversion of the starting material (R_f 0.3) to a single product (R_f 0.6). The reaction mixture was concentrated in vacuo and the resulting residue was purified by flash column chromatography (hexane:ethyl acetate, 1:1) to give the triol **9** (197 mg, 75%) as a clear oil; $[\alpha]_D^{23}$ +179.8

(*c*, 0.97 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3443 (br, OH) cm^{-1}; δ_H (400 MHz+COSY, $CDCl_3$): 0.09 (6H, s, $(CH_3)_2Si$), 0.91 (9H, s, $(CH_3)_3CSi$), 3.34, 3.62, 3.77 (3H, 3×s, 3×OH), 3.85–3.93 (4H, m, H-3, H-4, H-6, H-6′), 4.12 (1H, m, H-5), 4.21 (1H, s, H-2), 5.54 (1H, d, $J_{1,2}$ 1.1 Hz, H-1), 7.24–7.32 (3H, m, Ar), 7.45–7.48 (2H, m, Ar); δ_C (50.3 MHz, $CDCl_3$):−5.5 (q, $(CH_3)_2Si$), 18.2 (s, $(CH_3)_3\underline{C}Si$), 25.7 (q, $(\underline{C}H_3)_3CSi$), 64.1, 69.5, 72.2, 72.3, 72.9 (4×d, 1×t, C-2, C-3, C-4, C-5, C-6), 88.1 (d, C–1), 127.4, 129.1, 131.6, 134.6 (4×d, Ar-CH); *m*/*z* (ES^+): 425 ($M+K^+$, 22), 409 ($M+Na^+$, 100), 404 ($M+NH_4^+$, 11%); HRMS calcd for $C_{18}H_{34}O_5SNSi$ ($M+NH_4^+$) 404.1927. Found 404.1923.

4.7. Phenyl 6-*O*-*tert*-butyldimethylsilyl-2,3,4-tri-*O*-4-methoxybenzyl-1-thio-α-D-mannopyranoside, 10

para-Methoxybenzyl chloride (12.12 ml, 89.3 mmol) was added to a stirred solution of the triol **9** (8.617 g, 22.3 mmol) in THF (80 ml). The reaction mixture was cooled to 0°C and sodium hydride (60% dispersion in silicon oil, 2.679 g, 111.6 mmol) was added portionwise. Tetrabutylammonium iodide (247 mg, 0.67 mmol) was added and the mixture was heated under reflux. After 18 h, TLC (hexane:ethyl acetate, 1:1) indicated the complete conversion of starting material (R_f 0.3) to a single product (R_f 0.7). The reaction mixture was quenched with methanol, diluted with DCM (500 ml) and washed with distilled water (3×200 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 5:1) to give the thioglycoside **10** (10.02 g, 60%) as a clear oil; $[\alpha]_D^{23}$ +46.6 (*c*, 0.7 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): no significant peaks; δ_H (400 MHz +COSY, $CDCl_3$): 0.09, 0.12 (6H, 2×s, $(CH_3)_2Si$), 0.89 (9H, s, $(CH_3)_3CSi$), 3.81, 3.82, 3.83 (9H, 3×s, 3×$\underline{CH_3}OAr$), 3.77–3.89 (2H, m, obs), 3.91–3.95 (3H, m), 4.06 (1H, m, H-5), 4.47–4.63 (5H, m, $\underline{CH_2}Ar$), 4.86 (1H, d, *J* 10.5 Hz, $\underline{CH_2}Ar$), 5.50 (1H, d, $J_{1,2}$ 1.2 Hz, H-1), 6.82–6.93 (6H, m, Ar), 7.01–7.30 (9H, m, Ar), 7.41–7.44 (2H, m, Ar); δ_C (50.3 MHz, $CDCl_3$): −5.3, −5.5 (2×q, $(CH_3)_2Si$), 18.4 (s, $(CH_3)_3\underline{C}$), 25.9 (q, $(\underline{C}H_3)_3CSi$), 55.3 (q, 3×$\underline{C}H_3OAr$), 62.8, 71.4, 71.8, 74.9 (4×t, C-6, 3×$\underline{C}H_2Ar$), 74.3, 74.6, 76.0, 79.8 (4×d, C-2, C-3, C-4, C-5), 95.7 (d, C-1), 113.9, 127.5, 129.1, 129.5, 129.8, 129.9, 131.8 (7×d, Ar-CH), 130.2, 131.2, 159.5 (3×s, Ar-C); *m*/*z* (FAB^+): 769.6 ($M+Na^+$, 15), 121.1 (100%). (Found: C, 67.34; H, 7.41. $C_{42}H_{54}O_8SSi$ requires C, 67.53; H, 7.29%.)

4.8. Phenyl 2,3,4-tri-*O*-4-methoxybenzyl-1-thio-α-D-mannopyranoside, 11

Tetrabutylammonium fluoride (16.11 ml, 1.0 M solution in THF) was added to a stirred solution of thioglycoside **10** (10.02 g, 13.4 mmol) in diethyl ether (50 ml) at 0°C. The mixture was stirred under an atmosphere of argon for 10 h, when TLC indicated complete conversion of starting material (R_f 0.8) to a single product (R_f 0.5). The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography (hexane:ethyl acetate, 2:1) to give the alcohol **11** (6.77 g, 79%) as a clear oil; $[\alpha]_D^{23}$ +55.6 (*c*, 0.78 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3500 (br, OH) cm^{-1}; δ_H (400 MHz+COSY, $CDCl_3$): 1.87 (1H, s, OH), 3.77–3.86 (3H, m, H-3, H-6, H-6′), 3.81, 3.85 (9H, 2×s, 3×$\underline{CH_3}OAr$), 3.94 (1H, m, H-2), 3.98 (1H, d, *J* 9.3 Hz, H-4), 4.07 (1H, m, H-5), 4.53–4.60 (3H, m, $\underline{CH_2}Ar$), 4.62 (2H, s, $\underline{CH_2}Ar$), 4.87 (1H, d, *J* 10.8 Hz, $\underline{CH_2}Ar$), 5.44 (1H, d, $J_{1,2}$ 1.5 Hz, H-1), 6.84–6.91 (6H, m, Ar), 7.24–7.31 (9H, m, Ar), 7.36–7.39 (2H, m, Ar); δ_C (50.3 MHz, $CDCl_3$): 55.3 (q, 3×$\underline{C}H_3OAr$), 72.0, 72.0, 73.4, 74.9 (4×t, C-6, 3×$\underline{C}H_2Ar$), 73.4, 74.5, 76.0, 79.8 (4×d, C-2, C-3, C-4, C-5), 99.2 (d, C-1), 114.0, 127.9, 129.3, 129.7, 129.9, 131.9, 132.1 (7×d, Ar-CH), 130.5, 130.7, 134.2, 159.5 (4×s, Ar-C); *m*/*z* (FAB^+): 655.3 ($M+Na^+$, 25) 121.1 (100%). (Found: C, 67.91; H, 6.68. $C_{36}H_{40}O_8S$ requires C, 68.33; H, 6.37%.)

4.9. α-2,2,2-Trichloroethyl-β-carboxy-(phenyl 2,3,4-tri-*O*-4-methoxybenzyl-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 13

Dimethylaminopyridine (29 mg, 0.24 mmol) and dicyclohexylcarbodiimide (0.979 g, 4.74 mmol) were added to a stirred solution of the alcohol **11** (1.499 g, 2.37 mmol) in DCM (20 ml). After 1 h, α-2,2,2-trichloroethyl-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid **12**[11] (1.731 g, 3.56 mmol) was added and the reaction mixture was stirred for 7 h when TLC (hexane:ethyl acetate, 2:1) indicated no further conversion of the starting material (R_f 0.2) to the major product (R_f 0.3). The reaction mixture was filtered through Celite® (eluting with DCM) and the solvent removed in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 2:1) to give the ester **13** (1.881 g, 72% (87% over recovered starting material)) as a white foam; $[\alpha]_D^{24}$ +46.7 (*c*, 1.0 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3339 (br, NH), 1729 (st, C=O) 1520 (N–CO) cm^{-1}; δ_H (400 MHz+COSY, C_6D_6): 2.70 (1H, dd, $J_{CH2,CH}$ 4.5 Hz, $J_{CH2,CH2'}$ 17.3 Hz, $\underline{CH_2}CH$), 2.84 (1H, dd, $J_{CH2',CH}$ 5.3 Hz, $\underline{CH_2'}CH$), 3.29, 3.30, 3.31 (9H, 3×s, 3×$\underline{CH_3}OAr$), 4.00–4.05 (3H, m, CH(Fmoc), H-2, H-3), 4.14 (1H, a-t, *J* 9.2 Hz, H-4), 4.26–4.29 (2H, m, CH_2(Fmoc)), $\underline{CH_2}Ar$), 4.34–4.50 (9H, m, H-5, H-6, H-6′, CH_2'(Fmoc), CH_2CCl_3, $\underline{CH_2}Ar$), 4.58 (1H, d, *J* 10.9 Hz, $\underline{CH_2}Ar$), 4.69 (1H, m, $CH_2\underline{CH}$), 4.97 (1H, d, *J* 10.8 Hz, $\underline{CH_2}Ar$), 5.68 (1H, s, H-1), 5.79 (1H, d, $J_{NH,CH}$ 8.7 Hz, NH), 6.74–6.83 (6H, m, Ar), 6.98–7.29 (13H, m, Ar), 7.41–7.55 (6H, m, Ar); δ_C (50.3 MHz+DEPT, $CDCl_3$): 36.2 (t, $\underline{C}H_2CH$), 47.1, 50.4 (2×d, CH(Fmoc), $CH_2\underline{C}H$), 55.3 (q, 3×$\underline{C}H_3OBn$), 64.2, 67.4, 71.8, 74.8, 75.0 (5×t, CH_2(Fmoc), C-6, $\underline{CH_2}CCl_3$, 3×$\underline{CH_2}Ar$), 70.9, 74.3, 75.5, 79.8, 85.8 (5×d, C-1, C-2, C-3, C-4, C-5), 94.6 (s, CCl_3), 114.1, 120.3, 125.4, 127.5, 128.0, 129.4, 129.8, 130.1, 131.9 (9×d, Ar-CH), 141.6, 144.0, 156.4, 159.7 (4×s, C=O(Fmoc), Ar-C), 169.6, 170.9 (2×s, 2×C=O); *m*/*z* (ES^+): 1119 ($M+NH_4^+$, 100), 692 (55%). (Found: C, 62.05; H, 5.25; N; 1.27. $C_{57}H_{56}O_{13}NSCl_3$ requires C, 62.15; H, 5.12; N, 1.27%.)

4.10. β-Carboxy-(phenyl 2,3,4-tri-*O*-4-methoxybenzyl-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 4

Ester **13** (1.484 g, 1.35 mmol) and zinc powder (529 mg, 8.09 mmol) were stirred in acetic acid:water (9:1, 10 ml)

for 4 h when TLC (hexane:ethyl acetate, 1:9) indicated no further conversion of starting material (R_f 0.8) into a single product (R_f 0.6). The reaction mixture was filtered (eluting with toluene) and concentrated in vacuo. The residue was dissolved in DCM (200 ml), washed with saturated aqueous sodium bicarbonate solution (100 ml) and brine (100 ml), dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 1:9) to give the acid **4** (1.049 g, 80%) as a white foam; $[\alpha]_D^{24}$ +79.1 (*c*, 0.53 in $CHCl_3$); ν_{max} ($CHCl_3$, thin–film): 3401 (br, NH, CO_2H), 1736 (st, C=O) 1513 (N–C=O) cm^{-1}; δ_H (400 MHz+COSY, $CDCl_3$): 2.87, 3.02 (2H, 2×s, $\underline{CH_2}CH$), 3.77, 3.82 (9H, 2×s, 3×$\underline{CH_3}OAr$), 3.86–3.95 (3H, m), 4.11–4.19 (3H, m), 4.29–4.32 (3H, m), 4.51–4.62 (6H, m), 4.82 (1H, d, *J* 8.3 Hz), 5.44 (1H, s, H-1), 5.94 (1H, s, NH), 6.81–6.89 (6H, m, Ar), 7.00–7.44 (15H, m, Ar), 7.52 (2H, s, Ar), 7.71 (2H, s, Ar); δ_C (50.3 MHz, $CDCl_3$): 36.7 (t, $\underline{C}H_2CH$), 47.1, 50.3 (2×d, $CH_2\underline{C}H$, CH(Fmoc)), 55.3 (q, 3×$\underline{C}H_3OAr$), 63.6, 67.3, 71.9, 72.1, 75.1 (4×t, 1×d, C-1, C-2, C-3, C-4, C-5), 114.1, 120.2, 125.1, 125.1, 125.4, 127.4, 127.9, 129.4, 130.2, 130.3, 132.0 (11×d, Ar-CH), 134.0, 141.5, 143.9, 144.1, 156.4, 159.7, 159.8 (7×s, C=O (Fmoc), Ar-C), 170.9, 173.7 (2×s, 2×C=O); *m*/*z* (APCI$^+$): 993.9 (M+Na$^+$, 5), 225.2 (28), 120.9 (100%); (ES$^-$): 968.4 (M–H$^-$, 100%); HRMS calcd for $C_{55}H_{54}O_{13}NS$ (M+H$^-$) 968.3316. Found 968.3326.

4.11. Methyl 4,6-*O*-benzylidene-α-D-mannopyranoside, 15

Camphorsulphonic acid (263 mg, 1.13 mmol) was added to a stirred solution of methyl α-D-mannopyranoside **14** (2.2 g, 11.34 mmol) and benzaldehyde dimethylacetal (1.872 ml, 12.47 mmol) in DMF (15 ml). The reaction mixture was then put on a rotary evaporator at a pressure of 260 mbar and a temperature of 60°C. After 32 h 45 min, TLC (hexane:ethyl acetate, 1:3) indicated the presence of starting material (R_f 0.0) a minor product (R_f 0.9) and a major product (R_f 0.4). The remaining solvents were removed in vacuo. The residue was dissolved in DCM (200 ml) and washed with distilled water (100 ml) and brine (100 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 1:1) to give the benzylidene **15** (1.186 g, 37%) as a white solid a portion of which was crystallised; mp 141–143°C (ethanol/hexane) [lit.[13] mp 143–144°C]; $[\alpha]_D^{23}$ +70.1 (*c*, 1.07 in $CHCl_3$) [lit.[13] $[\alpha]_D^{19}$ +63.2 (*c*, 1.0 in $CHCl_3$)]; δ_H (200 MHz, $CDCl_3$): 3.41 (3H, s, CH_3O), 3.81–4.03 (3H, m), 4.07 (2H, m), 4.29 (1H, d, *J* 5.4 Hz), 5.58 (1H, s, $\underline{CH}Ar$), 4.77 (1H, s, H-1), 7.36–7.52 (5H, m, Ar).

4.12. Methyl 6-*O*-benzyl-α-D-mannopyranoside, 16

TFA (1.011 ml, 13.12 mmol), was added dropwise to a stirred solution of the benzylidene **15** (740 mg, 2.62 mmol) and triethylsilane (2.096 ml, 13.12 mmol) in DCM (20 ml), at 0°C under an atmosphere of argon. After 45 min, TLC (hexane:ethyl acetate, 1:5) indicated complete conversion of the starting material (R_f 0.5) to a major product (R_f 0.1). The reaction mixture was diluted with ethyl acetate (200 ml), washed with saturated aqueous sodium bicarbonate solution (100 ml) and brine (100 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 1:5–1:7) to give the triol **16** (412 mg, 55%) as a clear oil; $[\alpha]_D^{23}$ +69.7 (*c*, 0.71 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3392 (s, OH) cm^{-1}; δ_H (400 MHz+COSY, $CDCl_3$): 3.29 (3H, s, CH_3O), 3.62 (1H, m, H-5), 3.71–3.77 (4H, m, H-3, H-4, H-6, H-6′), 3.84 (1H, s, H-2), 4.55 (2H, ABq, *J* 12.0 Hz, $\underline{CH_2}Ar$), 4.65 (1H, d, $J_{1,2}$ 1.0 Hz, H-1), 4.79–4.80 (2H, m, 2×OH), 4.99 (1H, s, OH), 7.22–7.33 (5H, m, Ar); δ_C (100.6 MHz+DEPT, $CDCl_3$): 54.8 (q, CH_3O), 67.3, 70.4, 71.1, 71.5 (4×d, C-2, C-3, C-4, C-5), 69.5, 73.5 (2×t, C-6, $\underline{C}H_2Ar$), 101.0 (d, C-1), 127.7, 127.8, 128.2, 128.4 (4×d, Ar-CH), 137.8 (s, Ar-C); *m*/*z* (APCI$^+$): 307.1 (M+Na$^+$, 8), 285.1 (M+H$^+$, 6), 253.2 (28), 161.0 (34), 133.1 (100%). HRMS calcd for $C_{14}H_{24}O_6N$ (M+NH$_4^+$) 302.1604. Found 302.1596.

4.13. Methyl 2,3,4-tri-*O*-triethylsilyl-6-*O*-benzyl-α-D-mannopyranoside, 17

TESOTf (1.161 ml, 5.38 mmol) was added to a stirred solution of the triol **16** (254 mg, 0.89 mmol), anhydrous pyridine (0.724 ml, 8.96 mmol) and 3 Å molecular sieves in anhydrous DCM (12 ml) at–40°C under an atmosphere of argon. After 25 min, TLC (hexane:ethyl acetate, 10:1) indicated complete conversion of starting material (R_f 0.0) to a major product (R_f 0.7). The solvents were removed in vacuo and the residue poured into a mixture of DCM (150 ml) and saturated aqueous sodium bicarbonate solution (150 ml). The organic phase was washed with distilled water (150 ml), dried ($MgSO_4$), filtered and concentrated in vacuo. The resulting residue was purified by flash column chromatography (hexane:ethyl acetate, 20:1) to yield the fully protected glycoside **17** (521 mg, 93%) as a clear oil; $[\alpha]_D^{23}$ +35.3 (*c*, 1.68 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): no significant peaks; δ_H (500 MHz+HSQC, d^8-toluene at 363 K): 0.72–0.82 (18H, m, 3×($CH_3\underline{CH_2}$)$_3$Si), 1.03–1.12 (27H, m, 3×($\underline{CH_3}CH_2$)$_3$Si), 3.29 (3H, s, CH_3O), 3.77 (1H, a-dt, $J_{6,6'}$ 10.6 Hz, *J* 1.6 Hz, *J* 2.8 Hz, H-6), 3.80 (1H, a-ddd, *J* 6.0 Hz, *J* 2.0 Hz, H-6′), 3.86 (1H, m), 4.05–4.08 (2H, m), 4.18 (1H, a–t, *J* 6.3 Hz), 4.56 (2H, ABq, *J* 11.7 Hz, $\underline{CH_2}Ar$), 4.69 (1H, m, H-1), 6.99–7.01 (2H, m, Ar), 7.10 (1H, s, Ar), 7.19 (1H, m, Ar), 7.33 (1H, d, *J* 7.6 Hz, Ar); δ_C (125.3 MHz+HSQC, d^8-toluene at 363 K): 5.6 (t, 3×($CH_3\underline{C}H_2$)$_3$Si), 6.9 (q, 3×($\underline{C}H_3CH_2$)$_3$Si), 54.5 (q, CH_3O), 70.5 (t, C-6), 70.9, 73.4, 75.5, 76.6 (4×d, C-2, C-3, C-4, C-5), 73.4 (t, $\underline{C}H_2Ar$), 101.9 (d, C-1), 125.0, 125.9, 127.5, 127.8, 128.1 (5×d, Ar-CH); *m*/*z* (APCI$^+$): 649.4 (M+Na$^+$, 10), 595.5 (28), 463.4 (26), 121.9 (100%). HRMS calcd for $C_{32}H_{62}O_6Si_3Na$ (M+Na$^+$) 649.3753. Found 649.3752.

4.14. Methyl 2,3,4-tri-*O*-triethylsilyl-α-D-mannopyranoside, 18

A solution of benzyl ether **17** (483 mg, 0.77 mmol) in ethanol:ethyl acetate (2:1, 9 ml) was stirred under an

atmosphere of hydrogen in the presence of a catalytic amount of palladium black (50 mg). After 16 h 45 min, TLC (hexane:ethyl acetate, 10:1) indicated complete conversion of the starting material (R_f 0.7) to a single product (R_f 0.3). The reaction mixture was filtered through Celite® and concentrated in vacuo to give the alcohol **18** (408 mg, 99%) as a clear oil; $[\alpha]_D^{23}$ +38.5 (*c*, 1.38 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3521 (br, OH) cm^{-1}; δ_H (500 MHz+HSQC, $CDCl_3$): 0.68–0.81 (18H, m, 3×($CH_3\underline{CH_2}$)$_3$Si), 0.98–1.10 (27H, m, 3×($\underline{CH_3}CH_2$)$_3$Si), 3.21 (3H, s, CH_3O), 3.62 (1H, m), 3.78 (2H, a–d, *J* 2.3 Hz), 3.99–4.03 (2H, m), 4.63 (1H, d, $J_{1,2}$ 3.2 Hz, H-1); δ_C (125.7 MHz, $CDCl_3$): 5.5 (t, 3×($CH_3\underline{C}H_2$)$_3$Si), 7.1 (q, 3×($\underline{C}H_3CH_2$)$_3$Si), 54.5 (q, CH_3O), 62.1, 68.5, 74.0, 75.1 (4×d, 1×t, C-2, C-3, C-4, C-5, C-6), 103.3 (d, C-1); *m*/*z* (APCI$^+$): 559.4 (M+Na$^+$, 7), 373.5 (100), 259.2 (63), 122.0 (96%). HRMS calcd for $C_{25}H_{57}O_6Si_3$ (M+H$^+$) 537.3463. Found 537.3454.

4.15. α-Benzyl-β-carboxy-(methyl 2,3,4-tri-*O*-triethylsilyl-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 5

DMAP (9 mg, 0.08 mmol) and DCC (314 mg, 1.52 mmol) were added to a stirred solution of the alcohol **18** (408 mg, 0.76 mmol) and α-benzyl-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid **19**[14] (508 mg, 1.14 mmol) in DCM (8 ml) under an atmosphere of argon. After 5 h 20 min, TLC (hexane:ethyl acetate, 3:1) indicated conversion of the starting material (R_f 0.6) to a major product (R_f 0.5). The reaction mixture was filtered through Celite® (eluted with DCM) and the solvent removed in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 4:1) to give the glycosyl amino acid **5** (682 mg, 93%) as a clear oil; $[\alpha]_D^{23}$ +35.9 (*c*, 0.57 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 1734 (st, C=O) cm^{-1}; δ_H (500 MHz+HSQC, d^8-toluene at 363 K): 0.70–0.81 (18H, m, 3×($CH_3\underline{CH_2}$)$_3$Si), 1.02–1.10 (27H, m, 3×($\underline{CH_3}CH_2$)$_3$Si), 2.79 (1H, dd, $J_{CH2,CH2'}$ 16.6 Hz, $J_{CH2,CH}$ 5.0 Hz, $\underline{CH_2}$CH), 2.88 (1H, dd, $J_{CH2',CH}$ 5.2 Hz, $\underline{CH_2'}$CH), 3.29 (3H, s, CH_3O), 3.87 (1H, m), 4.03–4.08 (4H, m, CH(Fmoc), 3H), 4.28 (1H, dd, $J_{6,6'}$ 11.8 Hz, $J_{6,5}$ 7.0 Hz, H-6), 4.33–4.39 (2H, m, H-6′, CH_2(Fmoc)), 4.49 (1H, d, *J* 11.8 Hz, CH_2'(Fmoc)), 4.65–4.69 (2H, m, H-1, $CH_2\underline{CH}$), 5.00 (2H, ABq, *J* 12.5 Hz, $\underline{CH_2}$Ar), 5.56 (1H, d, $J_{NH,CH}$ 7.5 Hz, NH), 6.99–7.21 (9H, m, Ar), 7.46 (2H, s, Ar), 7.56 (2H, d, *J* 7.5 Hz, Ar); δ_C (500 MHz+HSQC, d^8-toluene at 363 K): 5.5 (t, 3×($CH_3\underline{C}H_2$)3Si), 6.6 (q, 3×($\underline{C}H_3CH_2$)$_3$Si), 36.8 (t, $\underline{C}H_2$CH), 47.7 (d, CH(Fmoc)), 51.3 (d, $CH_2\underline{C}H$), 54.8 (q, CH_3O), 64.7 (t, CH_2(Fmoc)), 67.1 (t, $\underline{C}H_2$Ar), 67.2 (t, C-6), 71.1, 73.1, 74.1, 75.2 (4×d, C-2, C-3, C-4, C-5), 101.5 (d, C-1), 119.8, 125.0, 125.1, 126.9, 127.3, 127.9, 128.2 (7×d, Ar-CH); *m*/*z* (ES$^+$): 981.5 (M+NH$_4^+$, 51), 932.4 ([M−OCH$_3$]$^+$, 18), 800.2 ([M−OCH$_3$−OTES]$^+$, 100%). HRMS calcd for $C_{51}H_{78}O_{11}NSi_3$ (M+H$^+$) 964.4882. Found 964.4882.

4.16. α-Benzyl-β-carboxy-(methyl 2,3,4-tri-*O*-triethylsilyl-α-D-mannopyranos-6-*O*-yl)-L-aspartyl-β-carboxy-(phenyl 2,3,4-tri-*O*-4-methoxybenzyl-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 20

Piperidine (1.0 ml) was added dropwise to a stirred solution of carbamate **5** (423 mg, 0.44 mmol) in DMF (4 ml). After 17 min, TLC (hexane:ethyl acetate, 1:1) indicated complete conversion of starting material (R_f 0.9) to a single product (R_f 0.5). The solvents were removed in vacuo (co-evaporation with toluene) and the residue dissolved in benzene:ethanol (1:1, 6 ml) and EEDQ (217 mg, 0.88 mmol) and carboxylic acid **4** (512 mg, 0.53 mmol) were added. After 23 h, TLC (hexane:ethyl acetate, 1:1) indicated the formation of a major product (R_f 0.7). The mixture was concentrated in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 5:2) to give the protected dipeptide (687 mg, 92%) as a white foam; $[\alpha]_D^{24}$ +43.7 (*c*, 1.32 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3358 (br, NH), 1736 (st, C=O), 1513 (N–CO) cm^{-1}; δ_H (500 MHz+HSQC, d^8-toluene at 363 K): 0.70–0.82 (18H, m, 3×($CH_3\underline{CH_2}$)$_3$Si), 1.01–1.11 (27H, m, 3×($\underline{CH_3}CH_2$)$_3$Si), 2.43 (1H, dd, $J_{CH2,CH2'}$ 17.1 Hz, $J_{CH2,CH}$ 9.8 Hz, $\underline{CH_2}CH^A$), 2.56 (1H, dd, $J_{CH2,CH2'}$ 17.3 Hz, $J_{CH2,CH}$ 9.9 Hz, $\underline{CH_2}CH^B$), 2.94 (1H, dd, $J_{CH2',CH}$ 3.2 Hz, $\underline{CH_2'}CH^A$), 3.07 (1H, dd, $J_{CH2',CH}$ 3.5 Hz, $\underline{CH_2'}CH^B$), 3.30 (3H, s, CH_3O), 3.44, 3.47, 3.48 (9H, 3×s, 3×$\underline{CH_3}$OAr), 3.82–3.92 (3H, m), 4.02–4.10 (8H, m), 4.35–4.42 (6H, m), 4.51–4.55 (6H, m), 4.60 (1H, d, *J* 11.2 Hz, $\underline{CH_2}$Ar), 4.73 (1H, d, $J_{1,2}$ 3.3 Hz, H-1), 4.89 (1H, d, $\underline{CH_2'}$Ar), 5.74 (1H, d, $J_{1,2}$ 2.0 Hz, H-1′), 6.77–7.61 (24H, m, Ar), 7.62–7.63 (2H, m, Ar), 8.16 (2H, d, *J* 8.2 Hz, Ar), 8.73–8.74 (2H, m, Ar); δ_C (125.3 MHz+HSQC, d^8-toluene at 363 K): 5.4 (t, 3×($CH_3\underline{C}H_2$)$_3$Si), 6.7 (q, 3×(CH_3CH_2)$_3$Si), 37.8 (t, $\underline{C}H_2CH^A$), 37.9 (t, $\underline{C}H_2CH^B$), 54.7 (q, 3×CH_3OAr), 54.9 (q, CH_3O), 51.5, 64.1, 64.9, 70.9, 71.6, 72.1, 73.3, 73.7, 74.8, 74.9, 77.1, 80.8 (12×d/t, 2×C-2, 2×C-3, 2×C-4, 2×C-5, 2×C-6, 3×$\underline{C}H_2$Ar, CH_2(Fmoc), CH^A, CH^B, CH(Fmoc)), 74.7 (t, $\underline{C}H_2$Ar), 86.4, 101.6 (2×d, 2×C-1), 114.1, 120.9, 125.2, 126.2, 128.0, 128.7, 128.8, 129.6, 132.0, 135.1, 150.8, 150.9, 151.9 (13×d, Ar-CH), 149.3, 150.5 (2×s, Ar–C), 159.9, 165.7, 165.9, 170.6, 171.0 (5×s, 5×C=O); *m*/*z* (ES$^+$): 1472.7 (M+H$^+$−Fmoc, 100%). (Found: C, 64.74; H, 7.55; N, 1.34. $C_{30}H_{31}O_6N$ requires C, 64.51; H, 7.14; N, 1.65%.)

4.17. α-Benzyl-β-carboxy-(methyl α-D-mannopyranos-6-*O*-yl)-L-aspartyl-β-carboxy-(phenyl 2,3,4-tri-*O*-4-methoxybenzyl-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 6a

Protected dipeptide **20** was stirred in ethyl acetate:formic acid (3:2, 5 ml) for 9 h, when TLC (hexane:ethyl acetate, 1:3) indicated complete conversion of starting material (R_f 0.8) to a single product (R_f 0.2). The solvents were removed in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 1:3) to yield the triol **6a** (19 mg, 72%) as a clear oil which was used directly without characterisation.

4.18. 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranose-(1–3)-methyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside, 21

Triol **6a** (150 mg, 0.11 mmol) was stirred with powdered 3 Å molecular sieves in DCM (8 ml) for 1 h. NIS (125 mg, 0.56 mmol) and silver trifluoromethanesulfonate (31 mg, 0.12 mmol) were added. After 45 min, TLC (hexane:ethyl acetate, 1:3) indicated no further conversion of starting material (R_f 0.1) to three major products (R_f 0.8, 0.7 and 0.5). A single drop of 2,4,6-collidine was added and the mixture was filtered through Celite® (eluting with DCM), diluted with DCM (100 ml) and washed with 10% sodium thiosulfate solution (50 ml). The aqueous phase was extracted with DCM (100 ml) and the combined organic phases dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 1:1–1:2) to give three glycopeptide disaccharide products: **A** (19 mg, 13%), **B** (9 mg, 7%), **C** (34 mg, 25%) together with recovered starting material (27 mg, 20%) as clear oils. The major product **C**, was stirred in methanol (3 ml) in the presence of K_2CO_3 (20 mg) for 1 h then the solvents removed in vacuo. It was then stirred in DCM:TFA (9:1) for 16 h. The solvents were then removed in vacuo and the residue dissolved in pyridine:acetic anhydride (1:1, 2 ml). After 23 h, TLC (hexane:ethyl acetate, 3:2) indicated the formation of a single product. The solvents were removed in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 3:2) to give α-(1,2)-linked disaccharide **21** (12 mg, 69%) as a clear oil; $[\alpha]_D^{23}$ +30.4 (*c*, 0.26 in $CHCl_3$) [lit.[15] $[\alpha]_D^{22}$ +34.9 (*c*, 1.1 in $CHCl_3$)]; δ_H (200 MHz, $CDCl_3$): 1.97–2.21 (21H, m, 7×CH_3CO_2), 3.38 (3H, s, CH_3O), 3.73–3.90 (2H, m, H-5, H-5′), 4.03–4.30 (5H, m), 4.72 (1H, s, H-1), 4.96–5.03 (2H, m), 5.20–5.35 (4H, m).

4.19. β-Carboxy-(methyl 2,3,4-tri-*O*-triethylsilyl-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 22

A solution of benzyl ester **5** (19 mg, 0.02 mmol) in ethanol (2 ml) was stirred under an atmosphere of hydrogen in the presence of a catalytic amount of palladium black (5 mg). After 30 min, TLC (ethyl acetate) indicated complete conversion of the starting material (R_f 0.8) to a single product (R_f 0.6). The reaction mixture was filtered through Celite® (eluted with ethanol) and concentrated in vacuo. The residue was purified by flash column chromatography (hexane:ethyl acetate, 1:1 to ethyl acetate) to give carboxylic acid **22** (14 mg, 79%) as a clear oil; $[\alpha]_D^{23}$ +31.0 (*c*, 0.93 in $CHCl_3$); ν_{max} ($CHCl_3$, thin-film): 3430, 3348 (br, NH), 1734 (st, C=O), 1508 (N–CO) cm^{-1}; δ_H (500 MHz+HMBC, d^8-toluene at 363 K): 0.72–0.80 (18H, m, 3×($CH_3\underline{CH_2}$)$_3$Si), 1.03–1.09 (27H, m, 3×($\underline{CH_3}CH_2$)$_3$Si), 2.82 (1H, dd, $J_{CH2,CH2'}$ 16.7 Hz, $J_{CH2,CH}$ 5.1 Hz, $\underline{CH_2}$CH), 2.92 (1H, dd, $J_{CH2',CH}$ 5.0 Hz, $\underline{CH_2'}$CH), 3.31 (3H, s, CH_3O), 3.87 (1H, s), 4.03 (3H, s), 4.08 (1H, a–t, *J* 6.8 Hz, CH(Fmoc)), 4.29–4.42 (3H, m, CH_2(Fmoc), H-6, H-6′), 4.50 (1H, d, *J* 11.2 Hz, CH_2'(Fmoc)), 4.62–4.70 (2H, m, H-1, $CH_2\underline{CH}$), 5.73 (1H, d, $J_{NH,CH}$ 8.4 Hz, NH), 6.99–7.22 (4H, m, Ar), 7.49–7.57 (4H, m, Ar); δ_C (125.3 MHz+HMBC, d^8-toluene at 363 K): 5.5 (t, 3×($CH_3\underline{C}H_2$)$_3$Si), 6.7 (q, 3×($\underline{C}H_3CH_2$)$_3$Si), 36.7 (t, $\underline{C}H_2CH$), 54.9 (q, CH_3O), 47.7 (d, CH(Fmoc)), 50.9 (d, $CH_2\underline{C}H$), 71.0, 75.2, 74.1, 75.2 (4×d, C-2, C-3, C-4, C-5), 67.5 (t, C-6), 64.6 (t, CH_2(Fmoc)), 101.6 (d, C-1), 119.5, 119.7, 125.2, 127.1, 127.6, 128.8 (6×d, Ar-CH); *m*/*z* (ES^+): 891.5 ($M+NH_4^+$, 13), 532.1 (100%). HRMS calcd for $C_{44}H_{75}O_{11}N_2Si_3$ ($M+NH_4^+$) 891.4678. Found 891.4665.

4.20. Solid supported β-carboxy-(methyl 2,3,4-tri-*O*-triethylsilyl-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 23

A solution of TBTU (59 mg, 0.183 mmol) and carboxylic acid **22** (104 mg, 0.120 mmol) in DMF (1 ml) were added to pre-washed Rink amide MBHA resin (141 mg, loading 0.65 mmol/g) in a reacti-vial and the mixture shaken for 24 h. The vial was then drained and the resin washed with DMF (6×1 ml), DCM (6×1 ml) and diethyl ether (6×1 ml). A further solution of TBTU (29 mg, 0.092 mmol) and acid **22** (56 mg, 0.064 mmol) in DMF (1 ml) were again added to the Rink amide MBHA resin in the reacti-vial and the mixture shaken for 24 h. The vial was then drained and the resin washed with DMF (6×1 ml), DCM (6×1 ml) and diethyl ether (6×1 ml). Bromophenol blue and TNBSA tests indicated the absence of any amine functionality.

4.21. Solid supported β-carboxy-(methyl α-D-mannopyranos-6-*O*-yl)-aspartyl-β-carboxy-(phenyl 2,3,4-tri-*O*-4-methoxybenzyl-1-thio-α-D-mannopyranos-6-*O*-yl)-*N*-9-fluorenylmethoxycarbonyl-L-aspartic acid, 6b

Carbamate **23** (0.19 mmol) was pre-washed to remove Fmoc protection on nitrogen according to the general procedure. A solution of EEDQ (97 mg, 0.39 mmol) and carboxylic acid **4** (246 mg, 0.25 mmol) in DMF (2 ml) were then added in a reacti-vial and the mixture shaken for 24 h. The vial was then drained and the resin washed with DMF (6×1 ml), DCM (6×1 ml) and diethyl ether (6×1 ml). Bromophenol blue and TNBSA tests indicated the absence of any amine functionality. The resin was then shaken in DMF:formic acid (3:2, 2 ml) for 48 h. The vial was then drained and the resin washed alternately with DMF (6×1 ml), DCM (6×1 ml) and diethyl ether (6×1 ml).

4.22. Phenyl 2,3,4-tri-*O*-4-methoxybenzyl-1-thio-α-D-manno-pyranoside 11 and methyl α-D-manno-pyranoside, 14

Resin **6b** (0.056 mmol) in THF:methanol:propylamine (3:1:1, 2.5 ml) was shaken in a reacti-vial for 48 h. The reacti-vial was drained and the solution retained. The resin was washed with DCM (2×1 ml) and these washings were combined with the original solution and concentrated in vacuo. The crude mixture was purified by flash column chromatography (hexane:ethyl acetate, 1:1 then methanol:ethyl acetate, 1:9) to yield glycosyl donor **11** (12.2 mg, 47%) and glycosyl acceptor **14** (6.1 mg, 76%).

4.23. Solid supported glycosylation

NIS (93 mg, 0.41 mmol) and AgOTf (22 mg, 0.08 mmol) were added to resin bound glycopeptide **6b** (0.08 mmol) and DCM (2 ml) in a reacti-vial. The vial was shaken under an atmosphere of argon for 1 h. The vial was then drained and the resin washed with DMF (6×1 ml), DCM (6×1 ml) and diethyl ether (6×1 ml). A solution of THF:methanol:propylamine (3:1:1, 2 ml) was then added to the resin and the reacti-vial shaken for 48 h. The reacti-vial was drained and the solution retained. The resin was washed with DCM (2×1 ml) and these washings were combined with the original solution and concentrated in vacuo. The crude mixture was purified by flash column chromatography (ethyl acetate to ethyl acetate:methanol, 9:1) to give **A′** (7.4 mg, 13%) and a mixture of two other products **B′** and **C′** (7.7 mg, 13%) and unreacted acceptor **14** (8.6 mg, 53%), as clear oils. The major product **A′**, was stirred in DCM:TFA (9:1) for 22 h. The solvents were then removed in vacuo and the residue dissolved in pyridine:acetic anhydride (1:1, 2 ml). After 24 h, TLC (hexane:ethyl acetate, 3:2) indicated the formation of a single product. The solvents were removed in vacuo and the residue purified by flash column chromatography (hexane:ethyl acetate, 3:2) to give the α-(1–3)-linked disaccharide **21** (5.8 mg, 86%) as a clear oil, identical to the material described previously.

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