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Synthesis of Mannosyl and Oligomannosyl Threonine Building Blocks Found on the *Mycobacterium tuberculosis* 45 kDa MPT 32 Glycoprotein*

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Mycobacterium tuberculosis secretes a 45 kDa glycoprotein (MPT 32) carrying mannosylated threonine residues. A general strategy was developed to couple peracetylated mannose, α-(1,2)-linked mannobiose or mannotriose to Fmoc-threonine-benzyl ester. Activation of mannosyl acetates or fluorides by borontrifluoride etherate gave the desired Fmoc-glycosyl amino acid building blocks in good yields. The synthesis leads to stable compounds and can easily be scaled up.

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Introduction

During the invasion of phagocytic cells by *Mycobacterium tuberculosis*, the bacterial surface carbohydrates, especially mannose residues, play an important role in the receptor-mediated binding and internalization process.[1] Mannose has been found to be a component of various molecules generated by mycobacteria, the most important being lipoarabinomannan (LAM) and secreted glycoproteins.[2] The only *M. tuberculosis* glycoprotein whose glycosylation pattern has been completely elucidated up to now is the 45 KDa MPT32.[3] In this glycoprotein, four threonine residues are *O*-glycosidically modified by α-D-mannose, an α-(1,2)-linked dimannoside, or the corresponding trimannoside. The structures of these oligomannosides are similar to the capping motifs reported for lipoarabinomannan.[4] Pure carbohydrates have long been known not to induce T cell responses, however, carbohydrates conjugated to lipids[5] or peptides[6] can stimulate T cells. Thus, synthetic *M. tuberculosis* glycopeptides appear to be good candidates for the development of molecules capable of stimulating T cell response, which is required for protection against tuberculosis.[7] In order to assemble *M. tuberculosis* MPT32 glycopeptides in a stepwise manner on a solid support,[8] we developed a general strategy for the synthesis of the appropriately protected glycosyl amino acid building blocks **A–C** (Fig. 1).

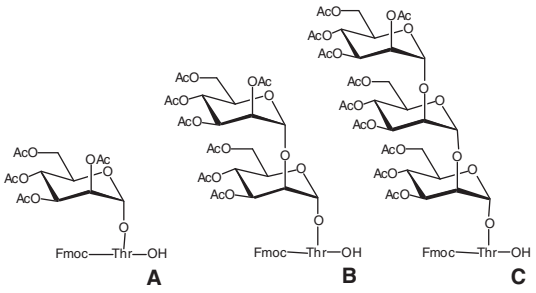

Fig. 1. Glycosyl amino acid building blocks **A–C** derived form the oligomannoside structures found on *Mycobacterium tuberculosis* MPT32 glycoprotein.

Results and Discussion

There are several reports on the synthesis of *O*-mannosylated amino acids,[9] however, our attempts to utilize the previously disclosed strategies revealed some drawbacks. In particular, the difficult purification of larger quantities of C-terminally unprotected threonine derivatives[10] or the latent instability of threonine pentafluorophenyl esters[11] prompted us to develop a more robust strategy that could be conveniently scaled up. Our interest turned to Fmoc-threonine-benzyl ester (3)[12] which had been successfully glycosylated using glycosyl halides and silver triflate.[13] To avoid unstable glycosyl halides and silver salts, the mannosylation of (3) was investigated using the mannosylperacetate[14] (1) or the fluoride[14] (2). When activated with $BF_3{\cdot}Et_2O$† both donors gave the protected α-mannosyl threonine (4)[13,17] in yields of 51 and 75%, respectively. This compound is stable and can easily be purified in large quantities. In a previous report an α-carboxy unprotected threonine was reacted with the peracetate (1) under similar conditions[10] with nearly identical yields, suggesting that the benzyl ester moiety of

* Dedicated to Professor Peter Köll on the occasion of his 60th birthday.
† For activation of glycosyl fluorides using $BF_3{\cdot}Et_2O$ see reference 15. For activation of glycosyl acetates using $BF_3{\cdot}Et_2O$ see reference 16.

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compound (3) was not influencing the course of the reaction. The mannosyl fluoride (2) turned out to be a far more reactive donor, which possibly explains the significantly higher yields. Catalytic hydrogenation[13] of the benzyl ester (4) furnished the monosaccharide–threonine building block A[13] in high yield (Fig. 2).

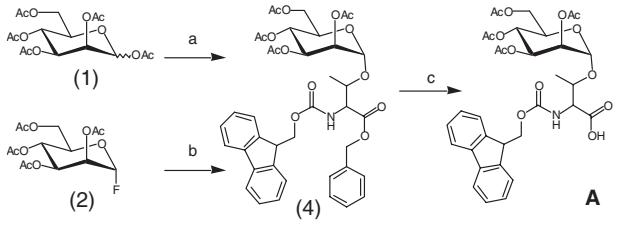

Fig. 2. Glycosylation of Fmoc-Thr-OBzl (3). (a) Fmoc-Thr-OBzl (3), $BF_3{\cdot}Et_2O$, absolute CH_2Cl_2, 24 h, (52%). (b) Fmoc-Thr-OBzl (3), $BF_3{\cdot}Et_2O$, absolute CH_2Cl_2, 2 h, (75%). (c) Pd/C 10%, H_2, MeOH, 2 h (93%).

To obtain the oligosaccharide–amino acid building blocks **B** and **C**, containing α-(1,2)-linked mannobiose and mannotriose, the peracetylated oligomannosides were synthesized initially (Fig. 3). Glycosylation of 1,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose (6)[18] with the thiomannoside (5)[14] using *N*-iodosuccinimide (NIS) and triflic acid (TfOH) as activants[14] gave the dimannoside (7)[19] in 67% yield. Subsequently, the disaccharide (7) was converted into the corresponding glycosyl fluoride (8) by reaction with the HF–pyridine complex.[20] The disaccharide fluoride (8) proved to be a valuable donor in the glycosylation of (6), furnishing the trimannoside (9) in 60% yield. Subsequent acidolysis of the trisaccharide (9) with HF–pyridine led to the trisaccharide fluoride (10).

With the four oligomannosyl building blocks (7)–(10) in hand, the glycosylation of Fmoc-Thr-OBzl (3) was investigated. Reaction of peracetyldimannoside (7) with Fmoc-Thr-OBzl (3) was performed as elaborated for

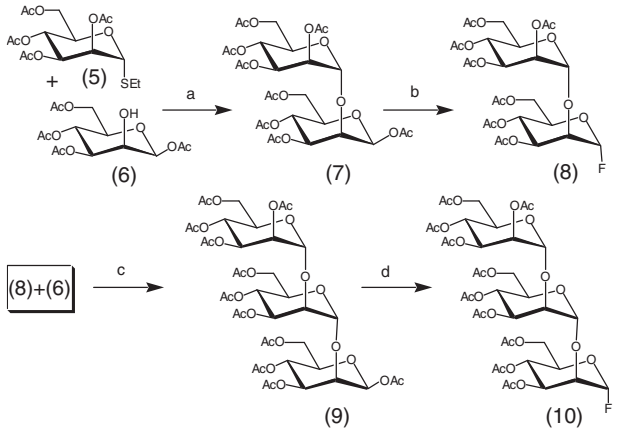

Fig. 3. Synthesis of oligomannosyl donors. (a) NIS, TfOH, 4 Å molecular sieves, 0°C, 30 min (67%). (b) HF–pyridine, CH_2Cl_2, 2 h (70%). (c) $BF_3{\cdot}Et_2O$, 4 Å molecular sieves, CH_2Cl_2, 3 h (60%). (d) HF–Pyridine, CH_2Cl_2, 3 h (55%).

peracetylmannose (1). After 24 hours of reaction time, a 55% yield of the disaccharide–threonine derivative (11) was obtained. Prolonged reaction time (36 hours) led to a maximum yield of 61%. Using the dimannosyl fluoride (8) as an alternative donor improved the yield in the glycosylation step to 70%. Despite the lack of neighboring group participation in donors (7) and (8), only α-linked product was obtained.

Surprisingly, the reaction of Fmoc-Thr-OBzl (3) with the peracetylated trimannoside (9) could not be accomplished. However, the trimannosylated threonine building block (12) was obtained in 61% yield using the more reactive trisaccharide fluoride (10) (Fig. 4). Liberation of the free carboxylic acid was effected by catalytic hydrogenation affording the target oligomannosyl–threonines **B** and **C** in high yields.

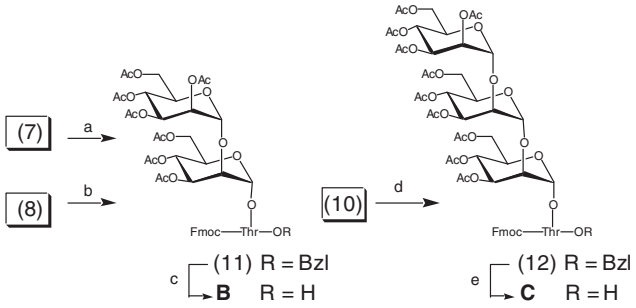

Fig. 4. Glycosylation of Fmoc-Thr-OBzl (3) with oligomannosyl donors. (a) Fmoc-Thr-OBzl (3), $BF_3{\cdot}Et_2O$, absolute CH_2Cl_2, 36 h, (61%). (b) Fmoc-Thr-OBzl (3), 4 Å molecular sieves, $BF_3{\cdot}Et_2O$, absolute CH_2Cl_2, 2 h, (70%). (c) Pd/C 10%, H_2, MeOH, 2 h (95%). (d) $BF_3{\cdot}Et_2O$, absolute CH_2Cl_2, 3 h, (61%). (e) Pd/C 10%, H_2, MeOH, 2 h (91%).

Conclusion

In conclusion we have devized a general strategy to obtain mannosylated threonines as valuable building blocks in solid-phase glycopeptide synthesis. Mannose and oligomannosides can conveniently be coupled to Fmoc-Thr-OBzl (3) by use of borontrifluoride etherate activating either a mannosyl acetate or fluoride. The reactions are useful for large-scale synthesis and provide stable compounds that can be easily purified.

Experimental

Materials and Methods

Reagents for synthesis were obtained from Fluka, Aldrich, Sigma or Acros. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F254 plates; compounds were visualized by heating with a 1 : 1 mixture of 0.2% 3-methoxyphenol in ethanol and 1 M H_2SO_4. Flash chromatography was carried out using Merck silica gel 60 (0.040–0.063 mm). Nuclear magnetic resonance (NMR) spectra (^1H, ^{13}C, H–H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), HMQC-COSY, and total correlation spectroscopy (TOCSY)) were recorded on a Bruker DRX-500 spectrometer. The chemical shifts are expressed in δ (ppm) relative to tetramethylsilane. Electrospray-ionization time-of-flight (ESI-TOF) mass spectra (MS) were recorded on a Micromass LCT spectrometer.

N-*(9-Fluorenylmethoxycarbonyl)-L-threonine Benzyl Ester (3)*[12]

To a stirred solution of 8.31 g (32.7 mmol) of H-Thr-OBzl hemioxalate in 120 mL of dry dichloromethane were added 12.5 g (36 mmol) of 9-fluorenylmethyloxycarbonyl-*N*-hydroxysuccinimide (Fmoc-OSu) and 5.5 mL (40 mmol) of triethylamine. After TLC (AcOEt/cyclohexane, 1 : 1) indicated the reaction was complete, the organic phase was washed twice with 1 N HCl and 2 M $KHCO_3$. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The remainder was purified by flash chromatography using AcOEt/cyclohexane (1 : 1) as eluent. Yield: 12.83 g (28.7 mmol, 84%) of (3) as a white solid. $[\alpha]_D^{20}$ –6.94 (*c,* 0.5 in AcOEt) (lit.[12a] $[\alpha]_D^{20}$ +6.65 in AcOEt; lit.[12b] $[\alpha]_D^{20}$ –4.0 (*c,* 1.0 in $CHCl_3$)); R_F 0.56 (AcOEt/cyclohexane, 1 : 1); ESI-TOF MS: Found 432.3 $[M+1]^+$. Calc. for $C_{26}H_{25}NO_5$: 431.17. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 7.90, d, *J* 7.5 Hz, 2H, Ar-Fmoc; 7.88, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.46–7.28, m, 10H, Ar-Fmoc, Ar-Bzl, and NH; 5.16, s, 2H, CH_2-Bzl; 4.87, d, *J* 7 Hz, 1H, OH; 4.35–4.12, m, 5H, H9-Fmoc, CH_2-Fmoc, Hα, Hβ; 1.11, d, *J* 6 Hz, 3H, Hγ. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 170.5–169.1, C=O; 157.1, C=O; 156.7, C=O; 128.9–120.8, Ar-Fmoc and Ar-Bzl; 66.5, Cβ; 66.1, CH_2-Bzl and CH_2-Fmoc; 59.7, Cα; 45.4, CH-Fmoc; 16.5, Cγ.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-L-threonine Benzyl Ester (4)*[13,17]

(a) From pentaacetate (1). Fmoc-Thr-OBzl (3) (3.83 g, 8.90 mmol) was added to a solution of pentaacetylmannose (1) (3.81 g, 9.90 mmol) in 180 mL of absolute dichloromethane. The atmosphere in the flask was replaced by argon and 10.1 mL (80.1 mmol) of $BF_3{\cdot}Et_2O$ was slowly added. When maximum conversion was observed (TLC, AcOEt/cyclohexane, 1 : 1; 24 h) the mixture was diluted with 180 mL of dichloromethane and washed with 1 M $KHCO_3$. The organic phase was dried over $MgSO_4$ and concentrated. Purification of the remainder by flash chromatography (AcOEt/cyclohexane, 1 : 1) gave (4) (4.02 g, 5.17 mmol, 51% yield) as a white solid. $[\alpha]_D^{20}$ +31.7 (*c,* 0.1 in $CHCl_3$) (lit.[17] $[\alpha]_D^{20}$ +34.0 (*c*, 1.1 in $CHCl_3$)); R_F 0.58 (AcOEt/cyclohexane, 1 : 1).

(b) From fluoride (2). A suspension of fluoride (2) (150 mg, 0.43 mmol)**,** Fmoc-Thr-OBzl (3) (220 mg, 0.50 mmol) and freshly ground and heated 4 Å molecular sieves (400 mg) in 5 mL of absolute dichloromethane was stirred for 30 min at ambient temperature. After addition of $BF_3{\cdot}Et_2O$ (55 μL, 0.43 mmol), TLC analysis (AcOEt/cyclohexane, 1 : 1) revealed that the reaction was complete within 1 h. The molecular sieves were removed by filtration over celite and the diluted filtrate was washed with 1 N HCl and 2 M $KHCO_3$. The organic phase was dried over $MgSO_4$, concentrated and purified by flash chromatography (AcOEt/cyclohexane, 1 : 1.2). Yield: 240 mg (0.32 mmol) 75% of compound (4). ESI-TOF MS: Found: 762.5 $[M+1]^+$. Calc. for $C_{40}H_{43}NO_{14}$: 761.27. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 8.20, d, *J* 6 Hz, 1H, NH; 7.89, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.82, t, *J* 7 Hz, 2H, Ar-Fmoc; 7.50–7.32, m, 9H, Ar-Fmoc and Ar-Bzl; 5.20, dd, *J* 10 Hz, 1H, H4; 5.15–5.00, m, 4H, H2, H3, and $PhCH_2$; 4.91, d, $J < 1$ Hz, 1H, H1; 4.30–4.00, m, 8H, H5, H6a, H6b, CH_2-Fmoc, CH-Fmoc, Hα, and Hβ; 2.05–1.92, 4 × s, 12H, Ac; 1.20, d, *J* 6 Hz, 3H, Hγ. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 170.9–169.0, C=O. 157.6, C=O; 146.7, C=O; 128.3–120.1, Ar-Fmoc and Ar-Bzl; 97.8, C1; 76.0, Cβ; 68.9, C2; 68.3, C5; 68.0, C3; 66.2, CH_2-Bzl; 65.9, CH_2-Fmoc; 65.8, C4; 61.5, C6; 58.8, Cα; 46.5, CH-Fmoc; 20.6–20.3, 4 × C; 17.4, Cγ.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-L-threonine **A***[13]

Compound (4) (3.5 g, 4.5 mmol) was dissolved in 300 mL of absolute MeOH. After addition of 620 mg of palladium on activated carbon (5% Pd) the reaction was stirred under a hydrogen atmosphere for 90 min. The removal of the benzyl group was monitored by TLC (dichloromethane/MeOH, 10 : 1). Following complete deprotection, the catalyst was filtered off and washed repeatedly. The organic phase was concentrated and purified by flash chromatography using dichloromethane/MeOH (10 : 1). Yield: 2.80 g (4.01 mmol, 93%). $[\alpha]_D^{20}$ +30.4 (*c*, 0.3 in CH_2Cl_3); R_F 0.53 (dichloromethane/MeOH, 10 : 1); ESI-TOF MS: Found: 672.4 $[M+1]^+$. Calc. for $C_{33}H_{37}NO_{14}$: 671.22. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 7.90, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.77, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.48–7.30, m, 5H, Ar-Fmoc and NH; 5.30, dd, *J* 10 Hz, 1H, H4; 5.12–4.92, m, 3H, H1, H2, and H3; 4.32–4.00, m, 8H, H5, H6a, H6b, CH_2-Fmoc, CH-Fmoc, Hα, and Hβ; 2.12–1.88, 4 × s, 12H, Ac; 1.22, d, *J* 6 Hz, 3H, H_3γ. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 170.6–169.9, C=O; 157.0, C=O; 128.1–120.6, Ar-Fmoc; 98.5, C1; 76.5, Cβ; 69.0, C2; 68.5, C5; 66.8, C3; 66.4, C4; 66.3, CH_2-Fmoc; 62.6, C6; 60.3, Cα; 47.2, CH-Fmoc; 21.1–20.9, Ac; 18.5, Cγ.

Acetyl-O-*(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)-3,4,6-tri*-O-*acetyl*-β-*D-mannopyranose (7)*[19]

A 5 g portion of powdered and activated 4 Å molecular sieves was added to a solution of thioglycoside (5) (12.1 g, 30.9 mmol) and tetraacetyl mannose (6) (9.75 g, 28 mmol) in 150 mL of absolute dichloromethane. After stirring the suspension at 0ºC for 15 min, *N*-iodosuccinimide (15.77 g, 70.11 mmol) was added. The reaction was started by the dropwise addition of triflic acid (1.95 mL, 22.4 mmol). When the reaction was terminated (TLC; AcOEt/cyclohexane, 2 : 1), the reaction mixture was diluted with 200 mL of dichloromethane, filtered over Celite and washed with 2 M $KHCO_3$, a 10% solution of $Na_2S_2O_3$ (× 3) and water. The organic phase was dried over $MgSO_4$ and concentrated. Flash chromatography (AcOEt/cyclohexane, 2 : 1) of the remainder yielded 12.7 g (18.8 mmol, 67%) of (7). $[\alpha]_D^{20}$ +5.0 (*c*, 0.5 in $CHCl_3$); (lit.[19a] $[\alpha]_D^{20}$ +1.2 (*c*, 1.0 in $CHCl_3$); lit.[19b] $[\alpha]_D^{20}$ +5.7 (*c*, 0.78 in $CHCl_3$); lit.[19c] $[\alpha]_D^{20}$ +0.7 (*c*, 0.6 in $CHCl_3$); lit.[19d] $[\alpha]_D^{20}$ +9.0 (*c*, 0.25 in $CHCl_3$)); R_F 0.42 (AcOEt/cyclohexane, 2 : 1); ESI-TOF MS: Found: 701.2 $[M+Na]^+$. Calc. for $C_{28}H_{38}O_{19}$: 678.20. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 6.15, d, $J < 1$ Hz, 1H, H1; 5.31, dd, 1H, *J* 10 Hz, H4; 5.26–5.14, m, 4H, H3, H1′, H2′, and H3′; 5.07, dd, *J* 10 Hz, 1H, H4′; 4.19, m, 1H, H2; 4.16–3.98, m, 6H, H5, H6a, H6b, H5′, H6a′, and H6b′; 2.15–1.95, 8 × s, 24H, Ac. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 98.1, C1′; 90.5, C1; 75.4, C2; 69.7, C5; 68.9, C3; 68.8, C2′; 68.7, C5′; 68.0, C3′; 65.5, C4′; 64.7, C4; 62.0, C6′; 61.2, C6; 20.8–20.3, Ac.

O-*(2,3,4,6-Tetra*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)-3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl Fluoride (8)*

To a stirred solution of disaccharide (7) (5.20 g, 7.7 mmol) in 20 mL of dry dichloromethane was added 20 mL of HF–pyridine (65% HF) at 0°C. Once the reaction was complete (3 h, TLC; AcOEt/cyclohexane, 2 : 1), the mixture was diluted with 150 mL of dichloromethane and poured onto a mixture of cold water and ice in a Teflon separatory funnel. The organic phase was washed three times with 1 N HCl and 2 M $KHCO_3$, dried over $MgSO_4$ and concentrated. After flash chromatography (AcOEt/cyclohexane, 2 : 1) 3.4 g of fluoride (8) was obtained (5.32 mmol, 70%). $[\alpha]_D^{20}$ +13.7 (*c*, 0.4 in CH_2Cl_2); R_F 0.48 (AcOEt/cyclohexane, 2 : 1); ESI-TOF MS: Found: 661.3 $[M+Na]^+$. Calc. for $C_{26}H_{35}FO_{17}$: 638.19. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 5.92, dd, *J* 49 Hz, 1H, H1; 5.30, dd, *J* 10 Hz, 1H, H4; 5.22–5.15, m, 4H, H3, H1′, H2′, and H3′; 5.12, dd, *J* 10 Hz, 1H, H4′; 4.34, m, 1H, H2; 4.19–4.05, m, 6H, H5, H6a, H6b, H5′, H6a′, and H6b′; 2.12–1.96, 7 × s, 21H, Ac. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 105.5, d, C1; 98.1, C1′; 74.0, d, C2; 70.3, C5; 68.6, C3; 68.6, C2′; 68.3, C5′; 68.0, C3′; 65.3, C4′; 64.3, C4; 61.8, C6′; 61.1, C6; 2.12–1.96, Ac.

Acetyl-O-*(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)*-O-*(3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)-3,4,6-tri*-O-*acetyl*-β-*D-mannopyranose (9)*

A suspension of fluoride (8) (1.6 g, 2.5 mmol), tetraacetyl mannose (6) (830 mg, 2.38 mmol) and powdered and activated 4 Å molecular sieves (3.2 g) in 50 mL of absolute dichloromethane was stirred for 30 min. The reaction was started by slow addition of BF_3Et_2O (750 μL, 5.96 mmol). After 3 h complete reaction was observed (TLC; AcOEt/cyclohexane, 1.5 : 1). The mixture was diluted with 100 mL of dichloromethane and the molecular sieves were filtered off over celite. The combined organic phases were extracted with 2 M $KHCO_3$, dried over $MgSO_4$ and concentrated. Flash chromatography of the remainder using AcOEt/cyclohexane (1.5 : 1) gave 1.45 g (1.50 mmol, 60%) of trisaccharide (9). $[\alpha]_D^{20}$ +17.9 (*c*, 0.5 in CH_2Cl_2); R_F 0.29 (AcOEt/

cyclohexane, 1.5 : 1); ESI-TOF MS: Found: 989.2 $[M+Na]^+$. Calc. for $C_{40}H_{54}O_{27}$: 966.29. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 6.15, d, *J* 1.5 Hz, 1H, H1; 5.32, dd, *J* 10 Hz, 1H, H4; 5.25–5.02, m, 9H, H3, H1′, H2′, H3′, H4, H1′′, H2′′, H3′′, and H4′′; 4.20, m, 1H, H2; 4.15–4.01, m, 9H, H5, H6a, H6b, H5′, H6a′, H6b′, H5′′, H6a′′, and H6b′′; 2.16–1.95, 33H, Ac. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 170.6–169.1, C=O; 98.6, C1′; 92.6, C1′′; 90.5, C1; 75.4, C2; 69.7, C5; 68.9, C3; 68.8, C5′′; 68.5, 2 × C, C2′′, and C3′; 68.4, 2 × C, C2′, and C3′′; 68.0, C5′; 65.5, C4′′; 65.4, C4′; 68.4, C4; 62.0, C6′′; 61.6, C6′; 61.2, C6; 20.4–19.0, Ac.

(2,3,4,6-Tetra-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)*-O-*(3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)-3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl Fluoride (10)*

To a stirred solution of trisaccharide (9) (1.1 g, 1.14 mmol) in 10 mL of absolute dichloromethane at 0°C were added 10 mL of HF–pyridine (65%). The biphasic reaction was complete after 3 h (TLC; acetone/cyclohexane, 1.5 : 1). The mixture was diluted with 100 mL of dichloromethane and added to a mixture of ice and water in a Teflon separatory funnel. The organic phase was washed with 1 N HCl (× 3), 2 M $KHCO_3$, dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography using AcOEt/cyclohexane (2 : 1) to obtain 0.580 g (0.63 mmol, 55%) of a white solid. $[\alpha]_D^{20}$ +13.7 (*c*, 0.4 in CH_2Cl_2); R_F 0.56 (acetone/cyclohexane, 1.5 : 1). ESI-TOF MS: Found: 949.2 $[M+Na]^+$. Calc. for $C_{38}H_{51}FO_{25}$: 926.27. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 5.90, dd, *J* 49 Hz, 1H, H1; 5.31, dd, *J* 10 Hz, 1H, H4; 5.23–5.15, m, 5H, H3, H3′, H3′′, H2′, and H1′; 5.13, dd, *J* 10 Hz, 1H, H4′; 5.09, dd, *J* 10 Hz, 1H, H4′′; 5.07–5.00, m, 2H, H1′′ and H2′′; 4.34, m, 1H, H2; 4.18–3.99, m, 9H, H5, H6a, H6b, H5′, H6a′, H6b′, H5′′, H6a′′, and H6b′′; 2.13–1.93, 30H, Ac. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 170.2–169.1, C=O; 106.4, d, C1; 98.0, C1′; 91.0, C1′′; 73.8, d, C2; 70.3, C5; 70.1, C2′; 68.7, C5′; 68.6, C2′′; 68.6, C3; 68.4, C5′′; 68.3, C3′; 68.0, C3′′; 65.8, C4′; 65.3, C4′′; 64.3, C4; 62.4, C6′′; 62.8, C6′; 61.1, C6; 20.8–20.3, Ac.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*[(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)-(3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl)]-L-threonine Benzyl Ester (11)*

(a) From acetate (7). A portion of disaccharide (7) (9.42 g, 13.88 mmol) and amino acid (2) (5.17 g, 11.55 mmol) were dissolved in 150 mL of absolute dichloromethane. The flask was flushed with argon and $BF_3{\cdot}Et_2O$ complex (5.3 mL, 41.7 mmol) was added dropwise. After 24 h the reaction mixture was diluted and washed with 2 M $KHCO_3$. The organic phase was dried over $MgSO_4$, concentrated and purified by flash chromatography with acetone/cyclohexane (1 : 1.2) to obtain 6.76 g (6.5 mmol, 55%) of glycosyl amino acid (11). R_F 0.52 (acetone/cyclohexane, 1 : 1.2).

(b) From fluoride (8). A suspension of fluoride (8) (200 mg, 0.32 mmol), Fmoc-Thr-OBzl (3) (150 mg, 0.34 mmol) and 400 mg of ground and preactivated 4 Å molecular sieves in 6 mL of absolute dichloromethane was stirred for 20 min. Subsequently, $BF_3{\cdot}Et_2O$ (80 µL, 0.63 mmol) was added. After 2 h (TLC; acetone/cyclohexane, 1 : 1.2) the reaction was completed and the solids were removed by filtration over Celite. The diluted filtrate was washed with 2 M $KHCO_3$, dried over $MgSO_4$ and concentrated. The remainder was subjected to flash chromatography (acetone/cyclohexane 1 : 1), yielding 235 mg (0.22 mmol, 70%) of compound (11). $[\alpha]_D^{20}$ +22.4 (*c*, 0.4 in CH_2Cl_2); R_F 0.52 (acetone/cyclohexane, 1 : 1.2). ESI-TOF MS: Found: 1050.7 $[M+1]^+$. Calc. for $C_{52}H_{59}NO_{22}$: 1049.35. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 8.1, d, *J* 9 Hz, 1H, NH; 7.82, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.67, t, *J* 7 Hz, 2H, Ar-Fmoc; 7.38–7.25, m, 9H, Ar-Fmoc and Ar-Bzl; 5.23–5.04, m, 8H, H1, H3, H4, H2′, H3′, H4′ and H_2-Bzl; 4.89, s, 1H, H1′; 4.32–4.20, m, 5H, CH_2-Fmoc, CH-Fmoc, Hα, and Hβ; 4.10–3.97, m, 6H, H5, H6a, H6b, H5′, H6a′, H6b′; 3.77, s, 1H, H2; 2.09–1.92, 7 × s, 21H, Ac; 1.20, d, *J* 6 Hz, 3H, Hγ. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 170.80–170.1, C=O; 157.4, C=O; 141.5, C=O; 129.1–126.0, Ar-Fmoc; 99.5, C1; 98.8, C1′; 77.0, C2; 76.8, Cβ; 70.1, C3; 69.6, C2′; 69.2, C5; 68.8, C3′; 67.4, CH_2-Bzl; 67.0, C4′; 66.8, CH_2-Fmoc; 66.6, C4; 62.7, C6′; 62.4, C6; 59.6, Cα; 47.4, CH-Fmoc; 21.2–21.1, Ac; 18.3, Cγ.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*[(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)-(3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl)]-L-threonine **B***

To a solution of benzyl ester (11) (5.23 g, 4.98 mmol) in 150 mL absolute MeOH was added 44 mg of palladium on activated carbon (10%). The suspension was stirred under a hydrogen atmosphere for 3 h (TLC; acetone/cyclohexane, 2 : 1). Removal of the catalyst by filtration and evaporation of the solvent gave a crude product, which was purified by flash chromatography using acetone/cyclohexane (2 : 1). Yield 4.87 g (4.98 mmol, 95%) of compound **B**. $[\alpha]_D^{20}$ +48.4 (*c*, 0.4 in CH_2Cl_2); R_f 0.33 (acetone/cyclohexane, 2 : 1); ESI-TOF MS: Found: 960.4 $[M+1]^+$. Calc. for $C_{45}H_{53}NO_{22}$: 959.31. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 7.85, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.75, d, *J* 9 Hz, 1H, NH; 7.70, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.38, t, *J* 7 Hz, 2H, Ar-Fmoc; 5.22, dd, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, 1H, H3; 5.18, dd, $J_{2',3'}$ 3, $J_{3',4'}$ 10 Hz, 1H, H3′; 5.14–5.06, m, 4H, H1, H4, H2′, and H4′; 4.99, d, $J < 1$ Hz, 1H, H1′; 4.23–3.98 m, 11H, H5, H6a, H6b, H5′, H6a′, H6b′, Hα, Hβ, CH_2-Fmoc, and CH-Fmoc; 3.90, s, 1H, H2; 2.07–1.91, 7 × s, 21H, Ac; 1.21, d, *J* 6 Hz, 3H, Hγ. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 171.2–170.5, C=O; 157.6, C=O; 128.1–126.4, Ar-Fmoc; 120.2, Ar-Fmoc; 99.6, C1; 98.8, C1′; 76.9, C2; 76.7, Cβ; 70.2, C3; 69.6, C2′; 69.2, C5; 68.9, C5′; 68.8, C3′; 66.8, C4′; 66.7, CH_2-Fmoc; 66.0, C4; 62.6, C6′; 62.5, C6; 59.6, Cα; 47.4, CH-Fmoc; 21.4–21.0, Ac; 18.8, Cγ.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*[*O-*(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)*-O-*(3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)-(3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl)]-L-threonine Benzyl Ester (12)*

A suspension of fluoride (10) (460 mg, 0.45 mmol), Fmoc-Thr-OBzl (1) (220 mg, 0.50 mmol) and 1 g of powdered and activated 4 Å molecular sieves in 10 mL of absolute dichloromethane was stirred for 20 min. Subsequently, $BF_3{\cdot}Et_2O$ (110 µL, 0.9 mmol) was added. After 4 h the reaction was complete (TLC; acetone/cyclohexane, 1 : 1.5). The mixture was diluted with 25 mL of dichloromethane and the molecular sieves were filtered off over Celite. The organic phase was extracted with 2 M $KHCO_3$, dried over $MgSO_4$ and concentrated. Flash chromatography of the remainder using acetone/cyclohexane (1 : 4) yielded 370 mg (0.28 mmol, 61%) of compound (12). $[\alpha]_D^{20}$ +27.0 (*c*, 1.0 in CH_2Cl_2); R_F 0.61 (acetone/cyclohexane, 1 : 1.5); ESI-TOF MS: Found: 1338.4 $[M+1]^+$. Calc. for $C_{64}H_{75}NO_{30}$: 1337.44. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 8.12, d, *J* 9 Hz, 1H, NH; 7.89, d, *J* 7.5 Hz, 2H, Ar-Fmoc; 7.70, t, *J* 7 Hz, 2H, Ar-Fmoc; 7.45–7.25, m, 9H, Ar-Fmoc and Ar-Bzl; 5.29–5.07, m, 12H, H1, H3, H4, H1′, H2′, H3′, H4′, H2′′, H3′′, H4′′, and H_2-Bzl; 4.92, d, $J < 1$ Hz, 1H, H1′′; 4.37–3.98, m, 14H, H5, H6a, H6b, H5′, H6a′, H6b′, H5′′, H6a′′, H6b′′, CH-Fmoc, CH_2-Fmoc, Hα, and Hβ; 3.81, br s, 1H, H2; 2.14–1.93, 30H, Ac; 1.24, d, *J* 6 Hz, 3H, Hγ. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 170.4–169.2, C=O; 154.2, C=O; 100.1, C1; 98.9, C1′′; 91.8, C1′; 77.3, C2; 77.1, Cβ; 70.8, C3; 70.4, C2′; 69.7, C5; 69.5, C3′; 69.4, C2′; 69.2, 2 × C, C5′ and C3′′; 69.0, C5′′; 67.6, CH_2-Bzl; 67.2, C4′′; 66.9, CH_2-Fmoc; 66.4, 2 × C, C4′ and C4; 62.9, C6′′; 62.5, C6′; 62.3, C6; 58.6, Cα; 47.6, CH-Fmoc; 20.6–19.7, Ac; 18.6, Cγ.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*[*O-*(2,3,4,6-tetra*-O-*acetyl*-α-*D-mannopyranosyl)-(1→2)*-O-*(3,4,6-tri*-O-*acetyl*-α-*D-mannopyrano-syl)-(1→2)-(3,4,6-tri*-O-*acetyl*-α-*D-mannopyranosyl)]-L-threonine **C***

A suspension of compound (12) (0.35 g, 0.26 mmol) and 40 mg of palladium on carbon (10% Pd) in 5 mL of absolute MeOH was stirred under a hydrogen atmosphere. After 3 h (TLC; acetone/cyclohexane, 2 : 1) the catalyst was removed by filtration. The combined washings were evaporated and purified by flash chromatography using acetone/cyclohexane (2 : 1). Yield 0.30 g (0.24 mmol, 91%) of glycosyl amino acid **C**. $[\alpha]_D^{20}$ +41.4 (*c*, 0.5 in CH_2Cl_3); R_F 0.25 (acetone/cyclohexane, 3 : 1). ESI-TOF MS: Found: 1248.5 $[M+1]^+$. Calc. for $C_{57}H_{69}NO_{30}$: 1247.39. ^{1}H NMR (500 MHz, (D_6)DMSO) δ 7.91, d, *J* 7.5 Hz, 2H, Ar-Fmoc; 7.88, d, *J* 9 Hz, 1H, NH; 7.80, t, *J* 7 Hz, 2H, Ar-Fmoc; 7.77, d, *J* 7 Hz, 2H, Ar-Fmoc; 7.45, t, *J* 7.5 Hz, 2H, Ar-Fmoc; 5.30–5.08, m, 10H, H1, H3, H4, H1′, H2′, H3′, H2′′, H3′′, and H4′′; 4.88, d, $J < 1$ Hz, 1H, H1′′; 4.28–3.96, m, 14H, H5, H6a, H6b, H5′, H6a′, H6b′, H5′′, H6a′′,

H6b′′, Hα, Hβ, CH-Fmoc, and CH_2-Fmoc; 3.88, s, 1H, H2; 2.12–1.94, 30H, Ac; 1.23, d, *J* 6 Hz, 3H, Hγ. ^{13}C NMR (125 MHz, (D_6)DMSO) δ 99.9, C1; 99.0, C1′′; 92.0, C1′; 77.0, C2; 76.9, Cβ; 70.9, C3; 70.4, C2′; 69.8, C5; 69.7, C3′; 69.3, C2′′; 69.2, C5′; 69.0, C3′′; 68.9, C5′′; 67.5, C4′′; 67.0, CH_2-Fmoc; 66.8, C4′; 66.6, C4; 62.8, C6′′; 62.4, C6′; 62.3, C6; 57.9, Cα; 47.5, CH-Fmoc; 20.2–19.1, Ac; 19.0, Cγ.

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References

[1] B. K. Kang, L. S. Schlesinger, *Infect. Immun.* **1998**, *66*, 2769.
[2] S. Nagai, H. G. Wiker, M. Harboe, M. Kinomoto, *Infect. Immun.* **1992**, *60*, 4781.
[3] K. M. Dobos, K. H. Khoo, K. Swiderek, P. J. Brennan, J. T. Belisle, *J. Bacteriol.* **1996**, *178*, 2498.
[4] (*a*) A. Vennise, M. Rivière, J. Vercauteren, G. Puzo, *J. Biol. Chem.* **1995**, *270*, 15012; (*b*) M. Gilleron, J. Nigou, B. Cahuzac, G. Puzo, *J. Mol. Biol.* **1999**, *285*, 2147.
[5] L. Brossay, M. Chioda, N. Burdin, Y. Koeyuka, G. Casorati, P. Dellabona, M. Kronenberg, *J. Exp. Med.* **1998**, *188*, 1521.
[6] (*a*) C. Ronin, *Clin. Chem. Lab. Med.* **1998**, *36*, 373; (*b*) A. Corthay, J. Backlund, J. Broddefalk, E. Michaelsson, T. J. Goldschmidt, J. Kihlberg, R. Holmdahl, *Eur. J. Immunol.* **1998**, *28*, 2580.
[7] M. G. Bonecini-Almeida, S. Chitale, I. Boutsikakis, J. Geng, H. Doo, S. He, J. L. Ho, *J. Immunol.* **1998**, *160*, 4490.
[8] H. Herzner, T. Reipen, M. Schultz, H. Kunz, *Chem. Rev.* **2000**, *100*, 4495.
[9] G. Arsequell, G. Valencia, *Tetrahedron: Asymmetry* **1997**, *8*, 2839.
[10] L. A. Salvador, M. Elofsson, J. Kihlberg, *Tetrahedron* **1995**, *51*, 5643.
[11] A. M. Jansson, M. Meldal, K. Bock, *J. Chem. Soc., Perkin Trans. 1* **1992**, 1699.
[12] (*a*) C.-D. Chang, M. Waki, M. Ahmad, J. Meienhofer, J. D. Lundell Haug, *Int. J. Pept. Protein Res.* **1980**, *15*, 59; (*b*) J. M. Lacombe, A. A. Pavia, *J. Org. Chem.* **1983**, *48*, 2557.
[13] H. Vegad, C. J. Gray, P. J. Somers, A. S. Dutta, *J. Chem. Soc., Perkin Trans. 1* **1997**, 1429.
[14] K. Toshima, K. Tatsuta, *Chem. Rev.* **1993**, *93*, 1503.
[15] (*a*) K. C. Nicolaou, A. Chuchulowsky, R. E. Dolle, J. L. Randall, *J. Chem. Soc., Chem. Commun.* **1984**, 1155; (*b*) S. Hashimoto, M. Hayashi, R. Noyori, *Tetrahedron Lett.* **1984**, *25*, 1379; (*c*) H. Kunz, W. Sager, *Helv. Chim. Acta* **1985**, *68*, 283; (*d*) Y. A. Vozny, A. A. Galoyan, O. S. Chizhov, *Bioorg. Khim.* **1985**, *11*, 276.
[16] (*a*) R. T. Lee, Y. C. Lee, *Carbohydr. Res.* **1974**, *37*, 193; (*b*) J. Dahmen, G. Frejd, G. Magnusson, *Carbohydr. Res.* **1983**, *114*, 328.
[17] A. Helander, L. Kenne, S. Oscarson, T. Peters, J.-R. Brisson, *Carbohydr. Res.* **1992**, *230*, 299.
[18] J. Deferari, E. G. Gros, I. O. Mastronardi, *Carbohydr. Res.* **1967**, *4*, 432.
[19] (*a*) P. L. Durette, T. Y. Shen, *Carbohydr. Res.* **1979**, *69*, 316; (*b*) C. M. Reichert, *Carbohydr. Res.* **1979**, *77*, 141; (*c*) A. M. Jansson, M. Meldal, K. Bock, *J. Chem. Soc., Perkin Trans. 1* **1992**, 1699; (*d*) M. Upreti, D. Ruhela, R. A. Vishwakarma, *Tetrahedron* **2000**, *56*, 6577.
[20] M. Yokoyama, *Carbohydr. Res.* **2000**, *327*, 5.