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Supporting Information

Synthesis and Immunochemical characterization of *S*-linked glycoconjugate vaccines against *Candida albicans*

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*Several monosaccharide derivatives that were used to prepare the thio-linked trisaccharides **12** and **22** were prepared according to literature methods or by methods reported in the Ph.D. thesis of M. Nitz.*[1] *To provide convenient access for interested readers the detailed procedures given below are taken from this Ph.D. thesis.*

Allyl 4,6-O-benzylidene-3-O-benzyl-D-glucopyranoside (1). This compound was synthesized according to the published literature.[2]

Allyl 2-O-acetyl-3,4,6-tri-O-benzyl-b-D-glucopyranoside (I). Allyl 3,4,6-tri-O-benzyl-b-D-glucopyranoside (II).

3,4,6 Tri-O-benzyl-1,2-*O*-(*exo*-ethoxyethylidene)-α-D-glucopyranose[3] (1.33 g, 2.6 mmol) was dissolved in allyl alcohol (25 mL) and cooled to 0 °C in an ice bath. $BF_3{\cdot}OEt_2$ (200μL, 1.6mmol) was added dropwise, the reaction was stirred at 0 °C for 30 min and then quenched with triethylamine. The solution was concentrated to a yellow oil and subject to column chromatography in toluene:ethylacetate (9:1) that gave **I** (646 mg, 43%) and **II** (670 mg, 53%) as white crystals that could be recrystallized from EtOAc-hexane mixtures. Data for **I** : m.p. 44-46 °C; $[\alpha]_D$ -8.3° (*c* 1.1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34-7.15 (15 H, Ar), 5.84 ($OCH_2C\underline{H}CH_2$), 5.24 ($OCH_2CHCHa\underline{Hb}$), 5.14 ($OCH_2CH\underline{CHa}Hb$), 5.02 (1 H, m, H-2), 4.78 (d, 2 H, J 11.1 Hz, $OC\underline{H_2}Ph$), 4.66 (d, 1 H, J 11.4 Hz, $OC\underline{H_2}Ph$), 4.58 (d, 1 H, J 10.4 Hz, $OC\underline{H_2}Ph$), 4.54 (d, 1 H, J 12.2 Hz, $OC\underline{H_2}Ph$), 4.40 (d, 1 H, H-1), 4.32 ($OC\underline{H_2}CHCH_2$), 4.06 ($OC\underline{H_2}CHCH_2$), 3.76-3.61 (4 H, H-3, H-4, H-6a, H-6b), 3.47 (ddd, 1 H, $J_{4\text{-}5}$ 9.3, $J_{5\text{-}6a}$ 2.3, $J_{5\text{-}6b}$ 4.6 Hz, H-5), 1.96 (s, 3 H, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 138.9, 138.8, 138.6, 134.6, 129.1-128.3, 117.7, 100.6, 83.7, 78.7, 77.9, 75.9, 75.7, 74.2, 73.8, 70.3, 69.5; Anal. Calcd. For $C_{32}H_{36}O_7$; C, 72.16; H, 6.81; O, 21.03; Found; C, 71.99; H, 6.94

Data for **II**: m.p. 41-43 ºC; $[\alpha]_D$ –3.0° (c 1.4, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35-7.14 (15 H, Ar), 5.92 ($OCH_2C\underline{H}CH_2$), 5.29 ($OCH_2CHC\underline{H_2}$), 5.19 ($OCH_2CHC\underline{H_2}$), 4.78 (d, 1 H, J 11.4 Hz, $OC\underline{H_2}Ph$), 4.82 (d, 1 H, J 11.4 Hz, $OC\underline{H_2}Ph$), 4.81 (d, 1 H, J 10.8 Hz, $OC\underline{H_2}Ph$), 4.59 (d, 1 H, J 12.3 Hz, $OC\underline{H_2}Ph$), 4.52 (d, 1 H, J 12.4 Hz, $OC\underline{H_2}Ph$), 4.52 (d, 1 H, J 10.8 Hz, $OC\underline{H_2}Ph$), 4.38 ($OC\underline{H_2}CHCH_2$), 4.28 (ABX, 1 H, $J_{1\text{-}2}$ 7.1 Hz, H-1), 4.10 ($OC\underline{H_2}CHCH_2$), 3.72 (dd, 1 H, J_{gem} 10.8, $J_{5\text{-}6a}$ 1.1 Hz, H-6a), 3.67 (dd, 1 H, $J_{5\text{-}6b}$ 4.8 Hz, H-6b), 3.60-3.55 (3 H, m, H-2, H-3, H-4), 3.45 (ddd, 1 H, H-5), 1.96 (s, 3 H, $OCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.3, 135.9, 135.8, 131.6, 126.1-125.3, 115.5, 99.4, 82.3, 75.3, 72.9, 72.8, 72.6, 72.4, 71.2, 67.9, 66.6; Anal. Calcd. For $C_{30}H_{34}O_6$; C, 73.45; H, 6.99; O, 19.57; Found; C, 73.09; H, 6.99.

Allyl 3,4,6 tri-O-benzyl-b-D-mannopyranoside (13).

Allyl glucopryanoside **II** (500 mg) was dissolved in freshly distilled dimethyl sulfoxide (8 mL) and acetic anhydride (4 mL) was added. The resulting solution was stirred for 18 h at room temperature and then concentrated under vacuo to give a yellow oil. This was dissolved in 1:1 dichloromethane:methanol (20 mL) and cooled to 0 ºC. Sodium borohydride (100 mg) was added and the suspension was stirred for 30 min at 0 ºC. The reaction was then diluted with dichloromethane and washed with 2% citric acid followed by brine. The organic layer was dried over sodium sulfate and concentrated to a yellow oil. Chromatography in toluene:EtOAc (4:1) on silica gel gave **13** (425 mg, 85%) as a white crystal; m.p. 52-53 ºC; $[\alpha]_D$ –21.5° (*c* 1.2, $CHCl_3$); ^{1} H NMR (600 MHz, $CHCl_3$) δ 7.36-7.18 (15 H, Ar), 5.90 ($OCH_2C\underline{H}CH_2$), 5.26 ($OCH_2CHCHa\underline{Hb}$), 5.19 ($OCH_2CHC\underline{Ha}Hb$), 4.87 (d, 1 H, J 11.0 Hz, $OC\underline{H}_2Ph$), 4.75 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.66 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.60 (d, 1 H, J 12.1 Hz, $OC\underline{H}_2Ph$), 4.54 (d, 1 H, J 11.9 Hz, $OC\underline{H}_2Ph$), 4.52 (d, 1 H, J 10.6 Hz, $OC\underline{H}_2Ph$), 4.44 (d, 1 H, J_{1-2} 0.7 Hz, H-1), 4.40 ($OC\underline{H}_2CHCH_2$), 4.11-4.08 (m, 2 H, H-2, $OC\underline{H}_2CHCH_2$), 3.85 (dd, J_{4-5}≈J_{3-4}≈9.3 Hz, H-4), 3.76 (dd, 1 H, J_{gem} 10.8 J_{5-6a} 2.2 Hz, H-6a), 3.70 (dd, J_{5-6b} 5.3 Hz, H-6b), 3.55 (1 H, dd, J_{2-3} 3.1 Hz, H-3), 3.41 (ddd, 1 H, H-5), ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.3, 137.9, 133.7, 128.5-127.6, 117.85, 98.6 (J^1_{c1-H1}158.6), 81.6, 75.4, 75.1, 74.3, 73.5, 71.4, 69.9, 69.3, 68.4.

4,6-O-benzylidene-3-O-benzyl-D-glucopyranose (III).

3-*O*-Benzyl-D-glucopyranose[4] (3.0 g, 110 mmol) was dissolved in DMF (20 mL) and benzaldehyde dimethyl acetal (2.0 mL, 130 mmol) followed by *p*TSA (110 mg) were added. The solution was subjected to rotary evaporation for 2 h at 25 °C with a standard water aspirator. The solution was quenched with pyridine (200 μL) and concentrated to a yellow oil under vacuum. The oil was chromatographed on silica gel in toluene:EtOAc (1:1) to yield a white solid (3.9 g, 94%) after concentration. This solid could be recrystallized from EtOAc hexane-mixtures. mp. 148-149 °C; $[\alpha]_D$ –20.5° (*c* 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, CH_3OD) δ 7.45-7.20 (m, 10 H, Ar), 5.59 (s, 1 H, $O_2C\underline{H}Ph$, β anomer), 5.58 (s, 1 H, $O_2C\underline{H}Ph$, α anomer), 5.13 (d, 1 H, J_{1-2} 3.7 Hz, H-1, α anomer), 4.87-4.80 (m, 4 H, 4(OC$\underline{H}_2$Ph)), 4.61 (d, 1 H, $J_{1\text{-}2}$ 7.7 Hz, H-1, β anomer), 4.25 (dd, 1 H, $J_{5\text{-}6}$ 5.1, J_{gem} 10.4 Hz, H-6eq, β anomer) 4.18 (dd, 1 H, $J_{5\text{-}6}$ 5.0, J_{gem} 10.3 Hz, H-6eq, α anomer), 3.99 (ddd, 1 H, $J_{4\text{-}5}$≈$J_{5\text{-}6ax}$ 10.1 Hz, H-5, α anomer), 3.84 (dd, 1 H, $J_{2\text{-}3}$≈$J_{3\text{-}4}$ 9.2 Hz, H-3, α anomer), 3.77 (dd, 1 H, H-6ax, β anomer), 3.73 (dd, 1-H, H-6ax, α anomer), 3.66-3.58 (m, 4H, H-2, H-4, α anomer, H-3, H-4, β anomer), 3.46 (1 H, ddd, $J_{4\text{-}5}$≈$J_{5\text{-}6}$ 9.5 Hz, H-5 β anomer), 3.37 (ddd, 1 H, $J_{2\text{-}3}$≈$J_{3\text{-}4}$ 8.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 129.8-128.4, 127.2, 102.6, 102.5, 99.0, 94.9, 83.5, 82.8, 82.5, 80.1, 76.8, 75.8, 75.6, 74.1, 70.4, 69.8, 67.6, 63.6; Anal. Calcd. For $C_{20}H_{22}O_6$; C, 67.03; H, 6.28; O, 26.79; Found; C, 66.82; H, 6.28.

1,2-di-O-acetyl-4-6-O-benzylidene-3-O-benzyl-b-D-glucopyranose (IV).

4,6-*O*-benzylidene-3-*O*-benzyl-D-glucopyranose (**III**) (3.84 g, 10.6 mmol) was dissolved in a solution of acetic anhydride (50 mL), acetic acid (10 mL) and sodium acetate (3.0 g) was added. The mixture was warmed to reflux over 20 minutes and allowed to cool to room temperature. The solvent was removed under vacuum and the colourless oil was taken up in dichloromethane (200 mL). The solution was washed repeatedly with water, dried over sodium sulfate and concentrated to yield a 6:1 β:α anomeric mixture (4.13g, 88%) from which the β anomer could be crystallized with EtOAc hexane mixtures; m.p. 162-164 °C $[\alpha]_D$ +16° (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CHCl_3$) δ 7.48-7.24 (m, 10 H, Ar), 5.68 (d, 1 H, $J_{1\text{-}2}$ 8.1 Hz, H-1), 5.56 (s, 1 H, $O_2C\underline{H}Ph$), 5.13-5.10 (2 H, m, H-2, H-3), 4.87 (d, 1 H, J 12.1 Hz, OC$\underline{H}_2$Ph), 4.66 (d, 1 H, OC$\underline{H}_2$Ph), 4.36 (dd, 1 H, $J_{4\text{-}5}$ 4.9, $J_{5\text{-}6}$ 10.4 Hz, H-6ax), 3.80 (m, 2 H, H-4, H-6eq), 3.57 (ddd, 1 H, $J_{4\text{-}5}$≈$J_{5\text{-}6ax}$ 9.7, $J_{5\text{-}6eq}$ 4.9 Hz, H-5), 2.07 (s, 3 H, $COCH_3$), 1.96 (s, 1 H, $COCH_3$) ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 169.0, 137.9, 136.9, 129.0, 128.3, 128.2, 127.8, 127.7, 126.0, 125.9, 101.3, 92.5, 81.2, 78.4, 74.3, 71.8, 68.4, 66.9, 20.9, 20.8. Anal. Calcd. For $C_{20}H_{22}O_6$; C, 65.15; H, 5.92; O, 28.93; Found; C, 64.97; H, 6.03.

2-O-Acetyl-4,6-O-benzylidene-3-O-benzyl-D-glucopyranose (V).

1,2-di-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-benzyl-β-D-glucopyranose (**IV**) (4.13g, 93.4 mmol) was dissolved in THF (40 ml) and benzyl amine (1.1mL, 101 mmol) was added. The mixture was stirred for 16 h at room temperature and concentrated to a yellow syrup under vacuum. Chromatography on silica gel using hexane:EtOAc (2:1) yielded a colourless syrup (2.9 g, 78%) which crystallized upon standing. m.p. 149-150 °C; $[\alpha]_D$ +21° (*c* 0.98, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.49-7.24 (10 H, m, Ar), 5.58 (1 H, s, O_2CHPh, α anomer), 5.57 (1 H, s, O_2CHPh, β anomer), 5.42 (d, 1 H, J_{1-2} 3.9 Hz, H-1, α anomer), 4.89-4.84 (m, 3 H, H-2 α anomer, 2($OC\underline{H}_2Ph$)), 4.80 (dd, 1 H, $J_{1-2}≈J_{2-3}$ 8.9 Hz, H-2 β anomer), 4.68 (d, 1 H, J 11.7, $OC\underline{H}_2Ph$), 4.66 (d, 1 H, J 11.9, $OC\underline{H}_2Ph$), 4.65 (d, 1 H, H-1, β anomer), 4.34 (dd, 1 H, J_{5-6} 5.0 , J_{gem} 10.5 Hz, H-6eq, β anomer), 4.26 (dd, 1 H, J_{5-6}, 5.0, J_{gem} 10.2 Hz, H-6eq α anomer), 4.09 (ddd, $J_{5-6ax}≈J_{4-5}$ 9.9 Hz, H-5 α anomer), 4.05 (dd, 1 H, $J_{2-3}≈J_{3-4}$ 9.5 Hz, H-3, α anomer), 3.80-3.68 (m, 4 H, H-6ax, α anomer, H-3, H-4, H-6ax, β anomer), 3.45 (ddd, 1 H, $J_{5-6ax}≈J_{4-5}$ 10.1 Hz, H-5 β anomer), 2.06 (s, 3 H, $COCH_3$, α anomer), 2.05 (s, 3 H, $COCH_3$, α anomer), ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 137.1, 128.9-125.9, 101.3, 101.3, 96.4, 91.2, 82.1, 81.6, 78.0, 75.8, 75.7, 74.8, 74.5, 73.1, 68.9, 68.6, 66.6, 62.6, 21.0. Anal. Calcd. For $C_{24}H_{24}O_7$; C, 65.99; H, 6.09; O, 27.97; Found; C, 65.79; H, 6.18

2-O-Acetyl-4,6-O-Benzylidene-3-O-benzyl-b-D-glucopyranosyl trichloroacetimidate (14).

2-O-Acetyl-4,6-*O*-benzylidene-3-*O*-benzyl-D-glucopyranose (**V**) (1.6 g, 4.0 mmol) was dissolved in dichloromethane (20 mL) and cooled to 0 °C. Trichloroacetonitrile (6.0 mmol, 600 μL) was added followed by DBU (20 μL, 0.001 mmol). The ice bath was removed, the reaction warmed to room temperature and stirring was continued for 2 h. Concentration gave a dark brown syrup which was purified by silica gel chromatography to give two products (1.86 g, 86%) in a 4:1 ratio which were identified as β and α anomers respectively. β anomer $[\alpha]_D$ +28.7 °(*c* 1.3, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.65 (1 H, s, NH), 7.49-7.24 (m, 10 H, Ar), 5.85 (d, 1 H, J_{1-2} 7.9 Hz, H-1), 5.58 (s, 1 H, $O_2C\underline{H}Ph$), 5.29 (dd, 1 H, $J_{1-2}≈J_{2-3}$ 8.5 Hz, H-2), 4.86 (d, 1 H, J 12.3 Hz, $OC\underline{H}_2Ph$) 4.69 (d, 1 H, $OC\underline{H}_2Ph$), 4.41 (dd, 1 H, J_{5-6} 5.0, J_{gem} 10.4 Hz, H-6eq), 3.89 (dd, 1 H, $J_{4-5}≈J_{3-4}$ 9.3 Hz, H-4), 3.82 (1 H, dd, J_{5-6} 10.8 Hz, H-6ax), 3.80 (dd, 1 H, H-3), 3.64 (ddd, 1 H, H-5), 1.95 (3H, s, $COCH_3$), ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 161.1, 129.0-125.9, 101.4, 96.2, 81.1, 81.5, 78.2, 74.0, 71.6, 68.6, 66.9, 20.8; ES HRMS Calcd. for $C_{24}H_{24}NO_7NaCl_3$ 566.0516 found 566.0521;

α anomer $[\alpha]_D$ +37.6 °(*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (s, 1 H, NH), 7.49-7.23 (m, 10 H, Ar), 6.49 (d, 1 H, J_{1-2} 3.7 Hz, H-1), 5.60 (s, 1 H, $O_2C\underline{H}Ph$), 5.07 (1 H, dd, J_{2-3} 9.7 Hz, H-2), 4.91 (d, 1 H, J 11.7 Hz, $OC\underline{H}_2Ph$), 4.73 (d, 1 H, $OC\underline{H}_2Ph$), 4.34 (dd, 1 H, J_{5-6} 4.9, J_{gem} 10.4 Hz, H-6eq), 4.12 (dd, 1 H, J_{3-4} 9.7 Hz, H-3), 4.04 (ddd, 1 H, $H_{4-5}\approx H_{5-6ax}$ 10.1 Hz, H-5), 3.81 (dd, 1 H, H-6eq), 3.77 (1 H, dd, H-4), 1.97 (s, 3 H, $COC\underline{H}_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 160.9, 138.1, 136.9, 129.0-125.9, 101.4, 93.9, 81.4, 75.6, 74.8, 68.6, 65.2, 20.6; ES HRMS for $C_{24}H_{24}NO_7NaCl_3$ 566.0516 found 566.0520

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