Supporting Information

Monitoring Solid-Phase Glycoside Synthesis with ^{19}F NMR Spectroscopy

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General methods and materials. – All reactions were carried out in room temperature under an inert nitrogen atmosphere using dry, freshly distilled solvents under anhydrous conditions, unless otherwise stated. MeCN and CH_2Cl_2 were distilled from calcium hydride. Diethyl ether and THF were distilled from sodium benzophenone. MeOH and pyridine were dried over 3 Å and 4 Å molecular sieves, respectively. Organic solutions were dried over Na_2SO_4 before being concentrated. Solid phase synthesis was performed on ArgoGel-NH_2 resin (151 μm, loading capacity: 0.38 mmol/g) using a semi - automatic Quest 210 synthesizer, which agitates the resin by moving the magnetic stirring bar vertically in the reactor with an external magnet.

TLC was performed on Silica Gel F_{254} (Merck) and detection was carried out by examination under UV light and by charring with 10% sulfuric acid. Flash column chromatography was performed on Silica Gel (Matrex, 60 Å, 35 – 70 μm, Grace Amicon). Preperative HPLC separations were performed on a Beckman System Gold

HPLC, using a Kromasil C-8 column (250×20 mm, 5 μm, 100 Å) with a flowrate of 11 mL/min, detection at 214 nm, and the following eluent systems: *A*, aq. 0.1% CF_3CO_2H; and *B*, 0.1% CF_3CO_2H in MeCN. Analytical HPLC were performed on a Beckman System Gold HPLC, using a Kromasil C-8 column (250×4.6 mm, 5 μm, 100 Å) with a flowrate of 1.5 mL/min, detection at 214 nm, and the following eluent systems: *A*, aq. 0.1% CF_3CO_2H; and *B*, 0.1% CF_3CO_2H in MeCN.

^1H and ^{13}C NMR spectra were recorded with a Bruker DRX-400 spectrometer for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26 ppm), $CDCl_3$ (δ_C 77.0 ppm) as internal standard] or CD_3OD [residual CD_2HOD (δ_H 3.35 ppm), CD_3OD (δ_C 49.0 ppm) as internal standard] at 300 K. First order chemical shifts and coupling constants were determined from one-dimensional spectra and proton resonances were assigned from COSY and HETCOR experiments. Proton resonances that could not be assigned are not reported. Proton decoupled gel phase ^{19}F NMR spectra were recorded with a Bruker DRX-400 spectrometer for resin suspensions in $CDCl_3$ [$CFCl_3$ (δ_F 0.00 ppm) as internal standard] at 300 K. Two peaks appear in the spectra around 0.00 ppm, one resonance originates from $CFCl_3$ inside the polymer the other resonance from $CFCl_3$ outside the polymer. The peak with highest shift was used as internal standard.

Resin 3

HOBt (185 mg, 1.37 mmol) and DIC (138 μl, 0.89 mmol) were added to a solution of 3-fluoro-4-hydroxybenzoic acid **2** (142 mg, 0.91 mmol) in DMF (4 mL). The mixture was stirred for 20 min and then transferred to resin **1** (0.228 mmol) that had been preswollen in DMF and briefly washed with 20% piperidine in DMF (3 mL, 1 min agitation) and with DMF (5×3 mL). Bromophenol Blue (0.001 equiv) was added as

indicator of free amino groups and the mixture turned blue. After 12 h of vertical agitation the color of the reaction turned yellow, indicating that the reaction had reached completion. The solution was removed by filtration. The resin was washed with DMF (3×3 mL), 20% piperidine in DMF (3 mL, 1 min agitation), DMF (3×3 mL), and CH_2Cl_2 (5×3 mL). A solution of CH_2Cl_2 (5 mL) and NaOMe in MeOH (2 M, 0.4 mL) were added to the resin and after 2 h agitation the resin was washed with CH_2Cl_2, MeOH, CH_2Cl_2 containing 3% HOAc, CH_2Cl_2, DMF and CH_2Cl_2 (3×5 mL each). Resin **3** had: ^{19}F NMR data ($CDCl_3$): δ -137.9 (s, 1F).

4-Hydroxybut-1-yl 4-fluorobenzoate (4)

4-Fluorobenzoyl chloride (1.18 mL, 10.0 mmol) was added dropwise over 10 min to a solution of 1,4-butanediol (5.32 mL, 60 mmol) in pyridine (30 mL). After stirring for 18 h the mixture was concentrated and residual pyridine was removed by co-evaporation with toluene. The residue was chromatographed (heptane/EtOAc 5:1 → 2:1) to give **4** (1.55 g, 73%). Compound **4** had: ^{1}H NMR data (CD_3Cl_3): δ 8.05 (m, 2 H, ArH), 7.10 (t, 2 H, *J* 8.7 Hz, ArH), 4.35 (t, 2 H, *J* 6.5 Hz, CH_2), 3.73 (t, 2 H, *J* 6.4 Hz, CH_2), 1.87 (m, 2 H, CH_2), 1.80 (br s, 1 H, OH) 1.72 (m, 2 H, CH_2). ^{19}F NMR data ($CDCl_3$): δ -106.2 (s, 1 F).

Resin 5

DEAD (0.538 mL, 3.42 mmol, in 1.5 mL THF) was added dropwise during 30 min to a mixture of resin **3** (0.228 mmol, prewashed with 2×8 mL THF), PPh_3 (90 mg, 3.43 mmol) and **4** (0.73 g, 3.42 mmol) in THF (1.5 ml) at -5 °C. After 3 h of vertical agitation the temperature was raised to 0 °C. After 21 h the resin was washed with THF, DMF,

20% piperidine in DMF, DMF and CH_2Cl_2 (5×5 mL each). Resin **5** had: ^{19}F NMR data ($CDCl_3$): δ -106.3, -134.4 (2s each 1F).

Resin 6

Sodium methoxide in methanol (0.2 M, 1.2 mL) was added to a suspension of resin **5** (0.228 mmol) in CH_2Cl_2 (4.8 mL). After 3 h of vertical agitation the resin was washed with CH_2Cl_2, MeOH, CH_2Cl_2 containing 3% HOAc, CH_2Cl_2, DMF and CH_2Cl_2 (3×5 mL each). Resin **6** had: ^{19}F NMR data ($CDCl_3$): δ -134.5 (s, 1F).

Resin 7

MSNT (0.27 g, 0.91 mmol) and Fmoc-Ser(*t*Bu)-OH (0.35 g, 0.91 mmol) were dissolved in CH_2Cl_2 (2.5 mL) and added to resin **6** (0.228 mmol). Methyl imidazole (55 µl, 0.69 mmol) was added and the mixture was agitated vertically during 4 h. The resin was washed with CH_2Cl_2, DMF and CH_2Cl_2 (6×6 mL each). The coupling was repeated once. Resin **7** had: ^{19}F NMR data ($CDCl_3$): δ -134.3 (s, 1F).

Resin 8

Resin **7** (0.228 mmol) was treated with 20% piperidine in DMF (2×6 mL, 5 min vertical agitation) and washed with DMF and CH_2Cl_2 (5×6 mL each). DIC (137.6 µl, 0.89 mmol) was added to a solution of 4-fluorobenzoic acid (128 mg, 0.91 mmol) and HOBt (185 mg, 1.37 mmol) in DMF (5 mL). The mixture were transferred to the resin and Bromophenol Blue was added (0.001 equiv.) as indicator of free amino groups. After 20 h agitation the resin was washed with DMF and CH_2Cl_2 (5×6 mL each). The resin was suspended in CH_2Cl_2 (3.5 mL) and Ac_2O (1.5 mL) was added. After 5 h agitation

the resin was washed CH_2Cl_2, DMF and CH_2Cl_2 (5×6 mL each). TFA/H_2O (9:1, 5 mL) was added to the resin and after 2 h of agitation the resin was washed with HOAc (5×6 mL), CH_2Cl_2 (5×6 mL), 20% piperidine in DMF (6 mL), DMF (5×6 mL), CH_2Cl_2 (5×6 mL) and then dried under vacuum. Resin **8** had: ^{19}F NMR data ($CDCl_3$): δ -108.4, -134.3 (2s each 1F).

4-Methylphenyl 2,3,4,6-tetra-*O*-(4-fluorobenzoyl)-1-thio-β-D-galactopyranoside (9)

Sodium methoxide in methanol (0.2 M, 2mL) was added to a solution of 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (3.75 g, 8.25 mmol) in methanol (38 mL). After stirring for 2 h the mixture was neutralized with Amberlite-H IR-120, filtered and co-concentrated with toluene. The crude product and DMAP (0.4 g, 3.3 mmol) were dissolved in pyridine (25 mL), and 4-fluorobenzoyl chloride (5.85 mL, 49.5 mmol) was added dropwise over 10 min. The mixture was stirred for 18 h and then diluted with CH_2Cl_2 (200 mL), washed with sat. aq. $NaHCO_3$ (3×70 mL) and H_2O (100 mL). The combined organic phases were concentrated and residual pyridine was removed by co-evaporation with toluene. The residue was re-crystallized from EtOAc to give **9** (6.1 g, 95%) as white crystals. Compound **9** had: ^{1}H NMR data (CD_3Cl_3): δ 8.02 (m, 4 H, ArH), 7.89 (m, 2 H, ArH), 7.75 (m, 2 H, ArH), 7.47 (d, 2 H, *J* 8.1 Hz, ArH), 7.10 (m, 8 H, ArH), 6.91 (t, 2 H, *J* 8.7 Hz, ArH), 5.94 (d, 1 H, *J* 3.0 Hz, H-4), 5.67 (t, 1 H, *J* 9.0 Hz, H-2), 5.55 (dd, 1 H, *J* 9.9, 3.2 Hz, H-3), 4.97 (d, 1 H, *J* 9.8 Hz, H-1), 4.64 (dd, 1 H, *J* 10.8, 6.2 Hz, H-6), 4.37 (m, 2 H, H-5, H-6), 2.39 (s, 3 H, $PhCH_3$). ^{19}F NMR data ($CDCl_3$): δ -104.5, -104.9, -105.0, -105.3 (4s each 1 F).

4-Methylphenyl 2,4-di-*O*-(4-fluorobenzoyl)-3,6-di-*O*-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (10)

Sodium methoxide in methanol (1 M, 6.7 mL) was added to a solution of 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (4.98 g, 11.0 mmol) in MeOH (84 mL) at 0 °C. The solution was stirred for 1 h 15 min before being neutralized with Amberlite-H IR-120, filtered and concentrated. The crude product was dissolved in DMF (30 mL) and *tert*-butyldimethylsilyl chloride (3.38 g, 22.5 mmol) in DMF (7 mL) was added at 0 °C. The solution was allowed to attain room temperature and after stirring for 7 h the mixture was diluted with CH_2Cl_2 (500 mL) and washed with sat. aq. NH_4Cl (500 mL). The aqueous phase was extracted with CH_2Cl_2 (2×200 mL) and the combined organic phases were dried and concentrated. The residue was flash chromatographed twice (heptane/EtOAc 5:1 and heptane/EtOAc 6:1) to give 4-methylphenyl 3,6-di-*O*-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (3.04 g, 54%). 4-Methylphenyl 3,6-di-*O*-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (1.02 g, 1.98 mmol) and DMAP were dissolved in pyridine and 4-fluorobenzoyl chloride (1.52 mL, 5.57 mmol) was added. The mixture was stirred for 24 h and then diluted with CH_2Cl_2 (150 mL), washed with sat. aq. $NaHCO_3$ (2×50 mL) and H_2O (50 mL). The organic phase was concentrated and residual pyridine was removed by evaporation with toluene. Flash chromatography of the residue (heptane/EtOAc 12:1) gave **10** (0.99 g, 79%). Compound **10** had: ^{1}H NMR data (CD_3Cl_3): δ 8.07 (m, 2 H, ArH), 7.94 (m, 2 H, ArH), 7.44 (d, 2 H, *J* 8.0 Hz, ArH), 7.12 (m, 6 H, ArH), 5.61 (s, 1 H, H-4), 5,42 (t, 1 H, *J* 8.9 Hz, H-2), 4.77 (d, 1 H, *J* 9.7 Hz, H-1), 4.04 (d, 1 H, *J* 8.5 Hz, H-3), 3.82 – 3.66 (m, 3 H, H-5, 2 H-6), 2.38 (s, 3 H, $PhCH_3$), 1.55 (s, 9 H,

*t*Bu), 1.26 (s, 9 H, *t*Bu), 0.05 (s, 3 H, $SiCH_3$), 0.02 (s, 3 H, $SiCH_3$), -0.01 (s, 3 H, $SiCH_3$), -0.17 (s, 3 H, $SiCH_3$). ^{19}F NMR data ($CDCl_3$): δ -106.0, -106.3 (2s each 1 F).

4-Methylphenyl 2,3-di-*O*-(4-fluorobenzoyl)-4,6-*O*-(4-fluorobenzylidene)-1-thio-β-D-galactopyranoside (11)

Metanolic sodium methoxide (2 mM, 50 mL) was added to crystals of 4-methylphenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside (7.0 g, 9.96 mmol). After stirring for 19 h the mixture was neutralized with Amberlite-H IR-120, filtered and co-concentrated with toluene. The crude product and α,α-dimethoxy-4-fluorotoluene (2.02 mL, 13.0 mmol) were dissolved in MeCN (15 mL) and *para*-toluenesulphonic acid (284 mg, 1.49 mmol) was added. After stirring for 20 h the mixture was neutralized with Et_3N (0.4 mL) and the solvent was evaporated. Flash chromatography of the residue (heptane/EtOAc 3:1) gave 4-methylphenyl 4,6-*O*-(4-fluorobenzylidene)-1-thio-β-D-galactopyranoside (3.50 g, 90%).

4-Fluorobenzoyl chloride (0.78 mL, 6.60 mmol) was added to a solution of 4-methylphenyl 4,6-*O*-(4-fluorobenzylidene)-1-thio-β-D-galactopyranoside (1.00 g, 2.55 mmol) in pyridine (10 mL). The mixture was stirred for 17 h and then diluted with CH_2Cl_2 (150 mL), washed with sat. aqueous $NaHCO_3$ (3×50 mL) and H_2O (100 mL). The organic phase was concentrated and residual pyridine was removed by co-evaporation with toluene. Flash chromatography of the residue (heptane/EtOAc 5:1 → 3:1) gave **11** (1.28 g, 79%). Compound **11** had: ^1H NMR data (CD_3Cl_3): δ 8.00 (m, 2 H, ArH), 7.93 (m, 2 H, ArH), 7.49 (d, 2 H, *J* 8.1 Hz, ArH), 7.35 (m, 2 H, ArH), 7.05 (m, 8 H, ArH), 5.69 (t, 1 H, *J* 9.9 Hz, H-2), 5.47 (s, 1 H, 4-FPhCH), 5.31 (dd, 1 H, *J* 10.0, 3.3 Hz, H-3), 4.87 (d, 1 H, *J* 9.8 Hz, H-1), 4.55 (d, 1 H, *J* 3.2 Hz, H-4), 4.43 (dd, 1 H, *J* 12.4, 1.2 Hz,

H-6), 4.08 (dd, 1 H, *J* 12.4, 1.2 Hz, H-6), 3.74 (s, 1 H, H-5), 2.36 (s, 3 H, $PhCH_3$). ^{19}F NMR data ($CDCl_3$): δ -105.1, -105.5, -113.2 (3s each 1 F).

Resin 12, glycosylation promoted by DMTST

Dimethyl(methylthio)-sulphonium triflate (174 mg, 0.67 mmol), **9** (0.17 mmol) and resin **8** (0.057 mmol) were dried under vacuum for 4 h, after which CH_2Cl_2 (2.5 mL) was added. After 20 h of vertical agitation the resin was washed with CH_2Cl_2 (6×3 mL), 20% piperidine in DMF (3×3 mL), DMF (3×3 mL) and CH_2Cl_2 (6×3 mL). Resin **12** had: ^{19}F NMR data ($CDCl_3$): δ -104.2 (s, 0.23 F), -104.3 (s, 0.16 F), -104.6 (s, 0.27 F), -104.7 (s, 0.29 F), -104.8 (s, 0.08 F), -105.1 (s, 0.31 F), -105.3 (s, 0.07 F), -107.6 (s, 0.67 F), -107.7 (s, 0.07 F), -107.8 (s, 0.25 F), -134.3 (s, 1 F).

Resin 12 , glycosylation with triflic acid and *N*-iodosuccinimide (NIS)

Triflic acid (4 µl, 46 µmol) was added to a solution of resin **8** (0.11 mmol), NIS (128 mg, 0.57 mmol) and **9** (442 mg, 0.57 mmol) in CH_2Cl_2 (3 mL) and the mixture was agitated vertically in the absence of light. After 3 h the resin was washed with CH_2Cl_2 (5×3 mL), THF (5×3 mL), 20% piperidine in DMF (5×3 mL), DMF (5×3 mL), CH_2Cl_2 (5×3 mL) and then dried under vacuum. Resin **12** had**:** ^{19}F NMR data ($CDCl_3$): δ -104.1, -104.5, -104.6, -105.0, -107.8, -134.5 (6s each 1 F).

Resin 14, glycosylation with triflic acid and *N*-iodosuccinimide (NIS)

Triflic acid (2.1 µl, 24 µmol) was added to a solution of resin **8** (0.12 mmol), NIS (134 mg, 0.60 mmol) and **9** (452 mg, 0.60 mmol) in CH_2Cl_2 (3 mL) and the mixture was agitated vertically in the absence of light at. After 3 h the resin was washed with

CH_2Cl_2 (5×3 mL), THF (5×3 mL), 20% piperidine in DMF (5×3 mL), DMF (5×3 mL) and CH_2Cl_2 (5×3 mL) and then dried under vacuum. Resin **14** had: ^{19}F NMR data ($CDCl_3$): δ -105.2, -105.4, -105.9, -106.0 (4s, 2 F), -108.0 (s, 0.15 F), -108.2 (s, 0.84 F), -134.3 (s, 1 F).

N^{α}-(4-Fluorobenzoyl)-3-O-(β-D-galactopyranosyl)-L-serine (13)

From resin 12: A solution of LiOH in H_2O (30 mM, 4 mL) and THF (2 mL) was added to resin **12** (40 μmol). After 3 h of vertical agitation, more LiOH in H_2O (2 M, 0.12 mL) was added. The mixture was agitated for an additional 2 h and then filtered. The resin was washed with HOAc (2×3 mL) and H_2O (2×3 mL). The combined filtrates were freeze dried and the residue was purified with reversed-phase HPLC (gradient: 100% *A* → 100% *B* during 60 min) to give **13** (6 mg, 39%). Gel-phase ^{19}F NMR spectroscopy revealed complete cleavage of **13** from the resin.

From resin 14: Resin **14** (54 μmol) was agitated with TFA/H_2O (9:1, 4 mL) for 4 h and then washed with THF (3×3 mL), 20% piperidine in DMF (3×3 mL), DMF (5×3 mL) and CH_2Cl_2 (6×3 mL). The resin was then treated as in the cleavage of resin **12**. The crude product was purified with reversed phase HPLC (gradient: 100% *A* → 100% *B* during 60 min) to give **13** (4 mg, 19%). Gel-phase ^{19}F NMR spectroscopy revealed complete cleavage of **13** from the resin.

Compound **13** had: ^{1}H NMR data (CD_3OD): δ 7.96 (m, 2 H, ArH), 7.19 (t, 2 H, *J* 6.83 Hz, ArH), 4.45 (dd, 1 H, *J* 10.4, 4.7 Hz, Ser-Hα), 4.28 (d, 1 H, *J* 7.6 Hz, H-1), 3.93 (dd, 1 H, *J* 10.4, 3.5 Hz, Ser-Hβ), 3.82 (d, 1 H, *J* 2.9 Hz, H-4), 3.47 (dd, 1 H, *J* 9.8, 3.2 Hz, H-3). ^{13}C NMR data (CD_3OD): δ 169.08, 167.60, 165.10, 131.71, 131.27, 116.36, 105.55, 76.94, 74.88, 72.55, 70.77, 70.35, 62.52, 55.06. ^{19}F NMR data ($CD_3OD/CDCl_3$): δ -108.5 (s, 1 F).

Resin 15, glycosylation with triflic acid and *N*-iodosuccinimide (NIS)

Triflic acid (2.3 µl, 25 µmol) was added to a solution of resin **8** (0.17 mmol), NIS (190 mg, 0.84 mmol) and **9** (0.54 g, 0.84 mmol) in CH_2Cl_2 (3 mL) and the mixture was agitated vertically in the absence of light at ambient temperature. After 3h the resin was washed with CH_2Cl_2 (5×3 mL), THF (5×3 mL), 20% piperidine in DMF (5×3 mL), DMF (5×3 mL), CH_2Cl_2 (5×3 mL) and then dried under vacuum. Resin **15** had: ^{19}F NMR data ($CDCl_3$): δ -105.0, -105.3, -108.5, -113.2, -134.8 (5s, each 1 F).

N^{α}-(4-Fluorobenzoyl)-3-O-[(4,6-*O*-4-fluorbenzylidene)-β-D-galactopyranosyl]-L-serine (16)

Resin **15** (54 µmol) was treated as in the cleavage of resin **12**. The residue was purified with reversed-phase HPLC (gradient: 100% *A* → 100% *B* in 60 min) and flash chromatography (CH_2Cl_2/MeOH 10:1 → 3:1) to give **16** (10 mg, 37%). Gel-phase ^{19}F NMR spectroscopy revealed complete cleavage of **16** from the resin. Compound **16** had: ^{1}H NMR data (CD_3OD): δ 7.90 (dd, 2 H, *J* 8.7, 5.3 Hz, ArH), 7.48 (dd, 2 H, *J* 8.6, 5.5 Hz, ArH), 7.02 (m, 4 H, ArH), 5.55 (s, 1 H, 4-FPhC*H*), 4.43 (dd, 1 H, *J* 10.4, 4.0 Hz, Ser-Hα), 4.33 (d, 1 H, *J* 6.9 Hz, H-1), 4.21 (d, 1 H, *J* 12.5 Hz, H-6), 4.16 (d, 1 H, *J* 2.7 Hz, H-4), 4.08 (dd, 1 H, *J* 12.4, 1.1 Hz, H-6), 3.92 (dd, 1 H, *J* 10.1, 2.2 Hz, Ser-Hβ), 3.62 (m, 2 H, H-3, H-2), 3.49 (s, 1 H, H-5). ^{13}C NMR data (CD_3OD): δ 168.14, 165.63, 165.02, 163.18, 136.00, 131.76, 131.25, 129.64, 116.38, 115.65, 105.50, 101.59, 77.46, 73.55, 72.07, 71.50, 69.84, 68.14, 56.23. ^{19}F NMR data ($CD_3OD/CDCl_3$): δ -108.46, -113.44 (2s each 1 F).