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Carbohydrate Research 318 (1999) 75–81

CARBOHYDRATE RESEARCH

Active–latent glycosylation strategy toward Lewis X pentasaccharide in a form suitable for neoglycoconjugate syntheses☆

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Received 17 February 1999; accepted 29 March 1999

Abstract

Glycosylation of 4-nitrophenyl 2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-1-thio-β-D-glucopyranoside with phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside in the presence of NIS and TfOH as catalyst gave the lactosamine derivative regiospecifically in high yield. Further 3-*O*-fucosylation with phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside using DMTST as promoter afforded the Le^x trisaccharide intermediate. The latent glycosyl donor was transformed into its active form (*p*-acetamidothiophenyl) by reduction with zinc in acetic acid and N-acetylation. Glycosidation with *p*-nitrothiophenyl lactoside acceptor in the presence of NIS/TfOH as catalyst gave the Le^x pentasaccharide in 71% yield.

Keywords: Active–latent; Glycosylation; 4-Nitrophenyl thioglycoside; Lewis X; Pentasaccharide

1. Introduction

Earlier studies from our laboratory demonstrated that 'active–latent' para-substituted phenyl 1-thioglycosides could be used efficiently as glycosyl donors or acceptors depending on the electron density of the aryl substituents [1–4]. Thus, *p*-nitrophenyl thioglycosides are inert (latent) towards thiophilic promoters, but the electron-withdrawing *p*-nitro substituent can be 'turned on' into an electron-donating *N*-acetyl group which is active with suitable thiophilic promoters. The glycosylation of this 'active' thioglycoside with a 'latent' *p*-nitrophenyl thioglycoside should be possible under suitable conditions. The 'active–latent' glycosylation strategy was also exploited with aryl sulfoxides [5], allyl versus vinyl glycosides [6], and bulky alkyl thioglycosides [7] by other groups. In order to further study the usefulness of 'active–latent' glycosylation strategy, we choose the synthesis of Lewis X pentasaccharide [8], which is widely distributed in many different human and animal tissues, and also in human milk oligosaccharides.

2. Results and discussion

Zemplén deacetylation of 4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**1**) [4] and selective silylation of OH-6 with *tert*-butylchlorodiphenylsilane in pyridine gave 3,4-*O*-unprotected *p*-nitrophenyl acceptor **2** in 82% yield. Condensa-

☆ Active–latent glycosylation strategy, Part 5. For Part 4, see Ref [4].

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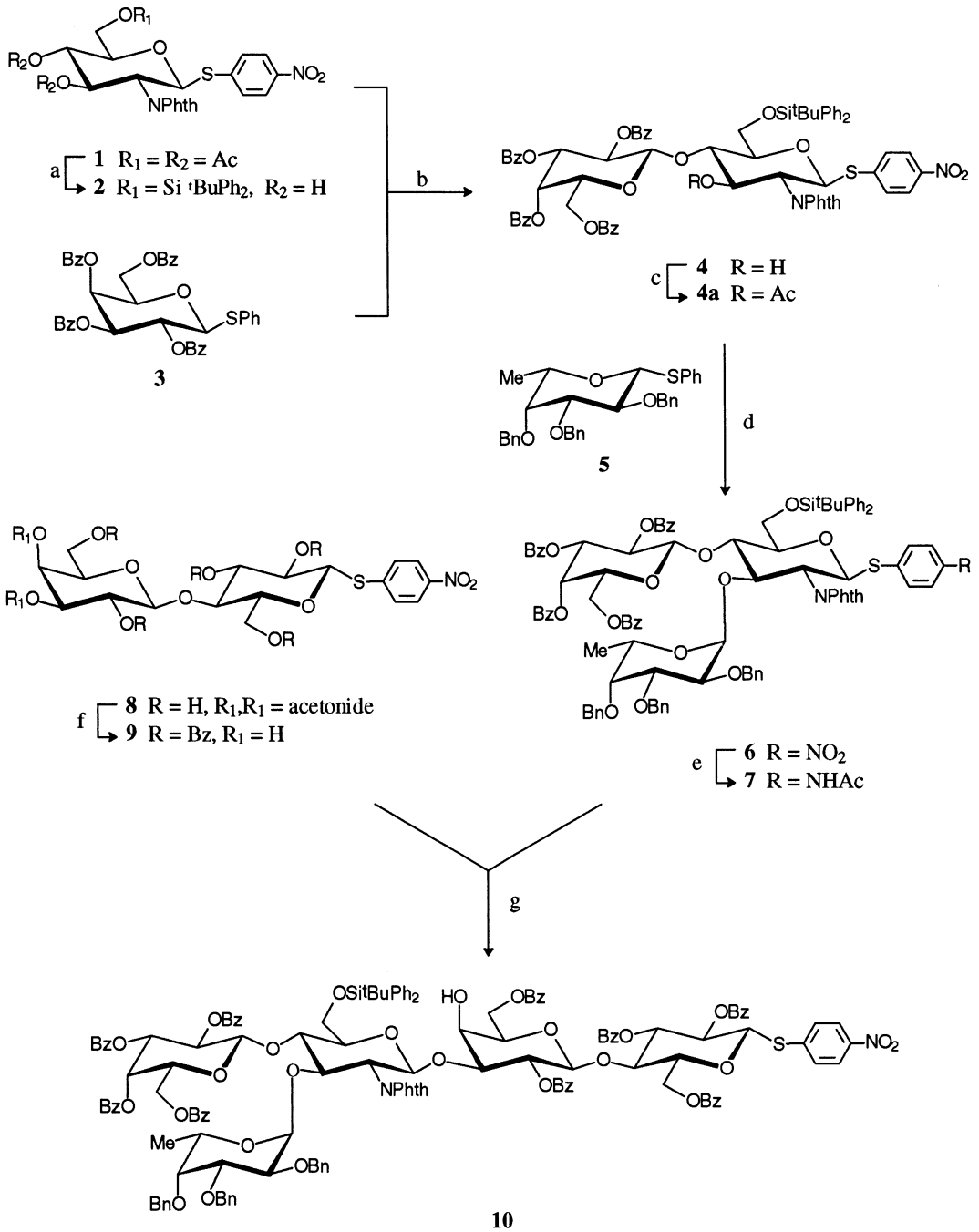
0008-6215/99/$ - see front matter
PII: S0008-6215(99)00080-4

tion of thiophenyl donor **3** with 6-*O*-*tert*-butyldiphenylsilyl acceptor **2** was performed using *N*-iodosuccinimide and trifluoromethanesulfonic acid as promoter at −30 °C. The reaction afforded exclusively the desired β-(1→4)-linked disaccharide **4** in 74% yield (Scheme 1). No (1→3)-linked regioisomer was detected during the coupling reaction. The β-configuration of the disaccharide **4** was deduced from the ^{1}H NMR spectrum, which showed H-1′ as a doublet at δ 5.09 ppm ($J_{1',2'}$ 8.1 Hz). The regiochemistry of the newly introduced glycosidic linkage of **4** was proved by converting **4** into its corresponding 4-acetate **4a**, which showed H-3 at δ 5.87 ppm ($\Delta\delta = +1.2$ ppm) as a



Scheme 1. Reagents and conditions: (a) [i] NaOMe–MeOH, [ii] TBDPSCl, pyridine, 82%; (b) NIS–TfOH, CH_2Cl_2, −30 °C, 74%; (c) Ac_2O–pyridine; (d) DMTST, C_6H_6–CH_2Cl_2, 0 °C; 72%; (e) [i] Zn–AcOH, [ii] Ac_2O–pyridine, 84%; (f) [i] BzCl–pyridine, [ii] *p*-TsOH, 40 °C, 92%; (g) NIS–TfOH, CH_2Cl_2, −45 °C, 71%.

downfield shift signal in its ^{1}H NMR spectrum.

It seemed that the bulky 6-*O*-*tert*-butyldiphenylsilyl protecting group in acceptor **2** played a key role for the regiospecificity. Under such reaction conditions, the very bulky protecting group could cover the top side (3,5-cis) of the OH-3 of the acceptor **2** and block the glycosyl donor from approaching the OH-3 [9]. This strategy, coupled with the steric hindrance imparted by the neighboring 2-phthalimido group on OH-3, concurred to the total regiospecificity of the glycosylation. Previous studies [10] have shown that the 2-phthalimido group alone could not be entirely responsible for the selectivity/specificity observed.

Further 3-*O*-fucosylation of disaccharide **4** with phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside (**5**) [11] using DMTST as promoter at 0 °C afforded Le^x trisaccharide **6** in good yield (72%). The α configuration of the newly introduced anomeric center in **6** was assigned from its ^{1}H NMR spectrum ($J_{1'',2''}$ 3.6 Hz). The *p*-nitrothiophenyl group of **6** was then reduced with zinc in acetic acid, and the resulting amine was N-acetylated to give the active *p*-acetamidothiophenyl donor **7** in 84% yield.

The latent diol lactoside acceptor **9** was obtained through benzoylation and hydrolysis from the known 4-nitrophenyl 3′,4′-*O*-isopropylidene-1-thio-β-D-lactopyranoside (**8**) [3]. Condensation of *p*-acetamidothiophenyl donor **7** with the *p*-nitrothiophenyl acceptor **9** was performed using NIS and TfOH as promoter at −45 °C. The reaction afforded β-(1→3)-linked pentasaccharide **10** in 71% yield. The stereochemistry of the newly introduced β-anomeric center in **10** was assigned from the ^{1}H NMR spectrum which showed a doublet for H-1″ at δ 5.15 ppm ($J_{1'',2''}$ 8.5 Hz). The ^{1}H NMR spectrum of **10** was less informative to confirm the regiochemistry of the newly introduced glycosidic linkage of **10**. However, the regioselectivity could be confirmed from the ^{13}C NMR spectrum, which showed a downfield shift signal for C-3′ at δ 80.1 ppm ($\Delta\delta = +7.5$ ppm) for pentasaccharide **10** compared to δ 72.6 ppm for C-3′ of the lactose acceptor **9**.

In conclusion, the 'active–latent' glycosylation strategy made it possible to manipulate the reactivity of both glycosyl donors and acceptors by means of changing the electron density of the aryl substituents at the anomeric center. This strategy provides a facile approach toward the synthesis of complex oligosaccharides in a highly convergent manner. Therefore, pentasaccharide **10** with its *p*-nitrothiophenyl aglycone can be reiteratively introduced into a new glycosylation cycle or simply transformed into various neoglycoconjugates using well-established strategy [12].

3. Experimental

General methods.—Melting points were determined on a Gallenkamp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 500 instrument or a Varian Gemini 200 MHz. Proton chemical shifts (δ) were given relative to internal $CHCl_3$ (7.24 ppm) for $CDCl_3$ solutions and to internal HOD (4.76 ppm) for D_2O solutions. Carbon chemical shifts were given relative to $CDCl_3$ (77.0 ppm). Mass spectra were obtained using a Kratos II H instrument (FAB-glycerol) or VG 7070-E spectrometer (CI). Optical rotations were measured at 23 °C on a Perkin–Elmer 241 polarimeter. Thin-layer chromatography (TLC) was performed on Silica Gel 60 F-254, and column chromatography was carried out on Silica Gel 60 (E. Merck).

4-Nitrophenyl 2-acetamido-6-O-tert-*butyldiphenylsilyl-2-deoxy-1-thio-β-*D-*glucopyranoside* (**2**).—To a suspension of 4-nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside **1** [4] (4.0 g, 6.99 mmol) in MeOH (100 mL), NaOMe in MeOH (0.3 mL, 1.0 M) was added. After stirring for 2 h at room temperature, the reaction was quenched with H^+ resin (Dowex 50W-X8) and then filtered. The filtrate was evaporated under reduced pressure. Toluene was added to and distilled from the residue. The crude product was then dissolved in dry pyridine (20 mL), and *tert*-butylchlorodiphenylsilane (2.1 mL, 8.20 mmol) was added in one portion. The mixture was stirred for 3 h at room temperature (rt)

and poured into ice water, extracted with CH_2Cl_2, and washed with satd aq $NaHCO_3$. The organic extract was dried (Na_2SO_4) and concentrated. The crude product was subjected to column chromatography (1:10 MeOH–CH_2Cl_2) to give pure **2** as a foamy solid (3.93 g, 82%): $[\alpha]_D$ +37.8° (*c* 1.0, $CHCl_3$); FABMS (glycerol) gave *m/z* (ion, relative intensity): 530.3 ($[M-SPhNO_2]^+$, 0.9%); ^{1}H NMR ($CDCl_3$) δ 7.87–7.18 (m, 18 H, aromatic H), 5.75 (d, 1 H, $J_{1,2}$ 10.3 Hz, H-1), 4.40 (dd, 1 H, $J_{3,4}$ 8.3 Hz, H-3), 4.23 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 4.05 (dd, 1 H, $J_{5,6a}$ 2.7, $J_{6a,6b}$ 10.3 Hz, H-6a), 3.94 (dd, 1 H, $J_{5,6a}$ 3.0 Hz, H-6b), 3.80–3.68 (m, 2 H, H-4, H-5), 3.45–3.25 (br, 2 H, OH) 1.06 (s, 9 H, SiC*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 168.2, 167.7 (2 × C=O), 148.8–123.3 (24 C, aromatic), 81.9 (C-1), 80.2 (C-5), 72.5 (C-3), 71.9 (C-4), 63.9 (C-6), 55.1 (C-2), 26.7 (SiC*Me*$_3$), 19.2 (Si*C*Me$_3$). Anal. Calcd for $C_{36}H_{36}N_2O_8SSi$: C, 63.14; H, 5.30; N, 4.09. Found: C, 63.09; H, 5.32; N, 4.13.

4-Nitrophenyl 2,3,4,6-tetra-O-*benzoyl-β*-D-*galactopyranosyl*-(1→4)-*2-acetamido-6*-O-tert-*butyldiphenylsilyl-2-deoxy-1-thio-β*-D-*glucopyranoside* (**4**).—To compound **2** (500 mg, 0.73 mmol) and phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-galactopyranoside **3** [9] (720 mg, 1.05 mmol) dissolved in dry dichloromethane (10 mL) under nitrogen, powdered 4 Å molecular sieves (1.2 g) were added. The mixture was stirred at rt for 2 h, and then cooled to −30 °C. *N*-Iodosuccinimide (330 mg, 1.46 mmol) and trifluoromethanesulfonic acid (40 μL, 0.44 mmol) were added. After stirring at −30 °C for 55 min, the mixture was diluted with dichloromethane (20 mL) and filtered through Celite. The filtrate was washed with 10% aq sodium thiosulfate (30 mL), sat aq $NaHCO_3$ (2 × 30 mL), and brine (30 mL). The solution was dried (Na_2SO_4) and concentrated. Column chromatography (1:49 MeOH–CH_2Cl_2) of the residue on silica gel afforded disaccharide **4** (681 mg, 74%): $[\alpha]_D$ +114.5° (c 1.0, $CHCl_3$); FABMS (glycerol) gave *m/z* (ion, relative intensity): 1108.4 ($[M-SPhNO_2]^+$, 0.5%); ^{1}H NMR (500 Hz, $CDCl_3$) δ 8.09–7.13 (m, 38 H, aromatic H), 5.95 (d, 1 H, H-4′), 5.84 (dd, 1 H, $J_{2',3'}$ 10.5 Hz, H-2′), 5.71 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.57 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 5.09 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.69 (dd, 1 H, $J_{5',6a'}$ 4.2, $J_{6a',6b'}$ 12.5 Hz, H-6a′), 4.67 (dd, 1 H, $J_{3,4}$ 8.9 Hz, H-3), 4.36 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 4.34 (dd, 1 H, $J_{5',6b'}$ 4.7 Hz, H-6b′), 4.30 (dd, 1 H, H-5′), 4.15 (dd,1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.85 (dd, 1 H, $J_{5,6a}$ 1.4, $J_{6a',6b'}$ 11.8 Hz, H-6a), 3.79 (dd, 1 H, $J_{5,6b}$ 2.5 Hz, H-6b), 3.57 (ddd, 1 H, H-5), 0.95 (s, 9 H, SiC*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 168.2, 167.2, 166.1, 165.4, 165.3, 165.0 (6 × C=O), 146.5–123.4 (48 C, aromatic), 101.0 (C-1′), 81.9 (C-1), 79.0 (C-4), 78.7 (C-5), 72.5 (C-5′), 71.4 (C-3′), 70.2 (C-3), 69.6 (C-2′), 68.1 (C-4′), 62.6 (C-6′), 61.5 (C-6), 55.0 (C-2), 26.8 (SiC*Me*$_3$), 19.4 (Si*C*Me$_3$). Anal. Calcd for $C_{70}H_{62}N_2O_{17}SSi$: C, 66.55; H, 4.95; N, 2.22. Found: C, 66.61; H, 4.81; N, 2.19.

4-Nitrophenyl 2,3,4,6-tetra-O-*benzoyl-β*-D-*galactopyranosyl*-(1→4)-*2-acetamido-3*-O-*acetyl-6*-O-tert-*butyldiphenylsilyl-2-deoxy-1-thio-β*-D-*glucopyranoside* (**4a**).—Acetic anhydride (150 μL) was added to a solution of compound **4** (20 mg, 0.016 mmol) in dry pyridine (1 mL) and the mixture was stirred at rt for 2 h. The solution was directly evaporated under reduced pressure to give compound **4a** (21 mg) in quantitative yield: $[\alpha]_D$ +90.8° (c 1.0, $CHCl_3$); ^{1}H NMR (500 Hz, $CDCl_3$) δ 8.11–7.14 (m, 38 H, aromatic H), 5.93 (d, 1 H, H-4′), 5.87 (dd, 1 H, $J_{3,4}$ 9.1 Hz, H-3), 5.82 (d, 1 H, $J_{1,2}$ 10.5 Hz, H-1), 5.75 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 5.50 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 5.20 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1′), 4.60 (dd, 1 H, $J_{5',6a'}$ 5.3, $J_{6a',6b'}$ 11.5 Hz, H-6a′), 4.43 (dd, 1 H, $J_{4,5}$ 9.9 Hz, H-4), 4.40 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 4.39 (dd, 1 H, $J_{5',6b'}$ 7.8 Hz, H-6b′), 4.30 (m, 1 H, H-5′), 3.92 (s, 2 H, H-6a, H-6b), 3.53 (d, 1 H, H-5), 1.93 (s, 3 H, OAc), 1.03 (s, 9 H, SiC*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 170.1, 167.8, 167.0, 166.0, 165.4, 165.3, 164.6 (7 × C=O), 146.7–123.7 (48 C, aromatic), 101.2 (C-1′), 81.6 (C-1), 79.3 (C-5), 73.9 (C-4), 71.9 (C-3′), 71.5 (C-5′), 71.4 (C-3), 69.8 (C-2′), 68.1 (C-4′), 62.0 (C-6′), 60.9 (C-6), 53.6 (C-2), 26.8 (SiC*Me*$_3$), 20.6 (OAc), 19.4 (Si*C*Me$_3$).

4-Nitrophenyl 2,3,4,6-tetra-O-*benzoyl-β*-D-*galactopyranosyl*-(1→4)-*[2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl*-(1→3)*]-2-phthalimido-6*-O-tert-*butyldiphenylsilyl-2-deoxy-1-thio-β*-D-*glu*-

copyranoside (**6**).— To a solution of phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside **5** (200 mg, 0.38 mmol) and compound **4** (328 mg, 0.26 mmol) in 10 mL of 5:1 benzene–CH_2Cl_2 were added powdered 4 Å molecular sieves (500 mg). The mixture was stirred for 2 h at rt under nitrogen. DMTST (156 mg, 0.76 mmol) was added to the stirred mixture at 0 °C. After 1 h, TLC (1:49 MeOH–CH_2Cl_2) showed complete conversion of the donor. Then triethylamine (200 μL) and MeOH (200 μL) were added to the reaction mixture, which was stirred for an additional 25 min. The mixture was then diluted with dichloromethane (15 mL) and filtered through Celite. The filtrate was washed with 10% aq sodium thiosulfate (10 mL), satd aq $NaHCO_3$ (2 × 10 mL), and brine (10 mL). The solution was dried (Na_2SO_4) and concentrated. Column chromatography (1:49 MeOH–CH_2Cl_2) of the residue on silica gel afforded **6** (314 mg, 72%): $[\alpha]_D$ +24.5° (c 1.0, $CHCl_3$); FABMS (glycerol) gave *m*/*z* (ion, relative intensity): 1678.7 (M^+, 0.1%); ^1H NMR (500 MHz, $CDCl_3$) δ 8.16–7.00 (m, 53 H, aromatic H), 5.89 (d, 1 H, $J_{4',5'}$ 3.6 Hz, H-4′), 5.83 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2′), 5.61 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.54 (dd, 1 H, $J_{3',4'}$ 3.6 Hz, H-3′), 5.39 (d, 1 H, $J_{1',2''}$ 8.3 Hz, H-1′), 5.02 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1″), 4.89 (dd 1 H, $J_{3,4}$ 9.3 Hz, H-3), 4.81 (m, 1 H, H-5′), 4.63 (dd, 1 H, $J_{5',6a'}$ 7.7, $J_{6a',6b'}$ 11.2 Hz, H-6a′), 4.62–4.11 (3 × AB pattern, 6 H, 3 × $PhCH_2$), 4.58 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 4.43 (dd, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 4.29 (dd, 1 H, $J_{5',6b'}$ 3.6 Hz, H-6b′), 4.18 (m, 1 H, H-5′), 4.02 (dd, 1 H, $J_{5,6a}$ 1.5, $J_{6a,6b}$ 12.0 Hz, H-6a), 3.97 (dd, 1 H, $J_{3'',4''}$ 2.7 Hz, H-3″), 3.88 (dd, 1 H, $J_{5,6b}$ 1.4 Hz, H-6b), 3.79 (dd, 1 H, $J_{2'',3''}$ 10.2 Hz, H-2″), 3.53 (d, 1 H, H-4′), 3.38 (m, 1 H, H-5), 1.43 (d, 3 H, $J_{5'',6''}$ 6.4 Hz, H-6″), 1.02 (s, 9 H, SiC*Me*$_3$); ^{13}C NMR ($CDCl_3$) δ 146.3–123.8 (66 C, aromatic), 100.2 (C-1′), 97.2 (C-1″), 82.2 (C-1), 79.6 (C-5), 79.4 (C-4″), 79.3 (C-3′), 75.3 (C-2′), 75.2 (CH_2), 74.9 (C-4), 73.0 (2 C, C-3, CH_2), 71.9 (C-3′), 71.8 (C-5′), 71.7 (CH_2), 69.7 (C-2′), 68.5 (C-4′), 66.9 (C-5″), 61.4 (C-6′), 61.0 (C-6), 55.0 (C-2), 26.8 (SiC*Me*$_3$), 19.3 (Si*C*Me$_3$), 16.8 (C-6′). Anal Calcd for $C_{97}H_{90}N_2O_{21}SSi$: C, 69.35; H, 5.40; N, 1.67. Found: C, 69.29; H, 5.47; N, 1.66.

4-Acetamidophenyl2,3,4,6-tetra-O-*benzyl*-β-D-*galactopyranosyl*-(1→4)-*[2,3,4-tri*-O-*benzyl*-α-L-*fucopyranosyl*-(1→3)]-*2-phthalimido-6*-O-tert-*butyldiphenylsilyl-2-deoxy-1-thio*-β-D-*glucopyranoside* (**7**).—To a solution of **6** (150 mg, 0.089 mmol) in CH_2Cl_2 (5 mL) was added acetic acid (1 mL) and zinc dust (75 mg). The mixture was stirred for 1 h at rt. TLC (1:99 EtOH–dichloromethane) indicated the transformation was completed. Triethylamine was then added (1.25 mL), and the mixture was concentrated under reduced pressure. The resulting crude *p*-aminophenyl thioglycoside was immediately treated overnight with pyridine (2 mL) and acetic anhydride (1 mL) at rt. The solution was thoroughly evaporated under reduced pressure. The crude residue was purified by silica gel chromatography using 0.5:99.5 MeOH–CH_2Cl_2 as eluent to give **7** (127 mg, 84%): $[\alpha]_D$ +28.6° (c 1.0, $CHCl_3$); FABMS (glycerol) gave *m*/*z* (ion, relative intensity): 1691.8 (M^+, 0.4%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.13–7.01 (m, 53 H, aromatic), 5.87 (d, 1 H, $J_{3',4'}$ 3.6 Hz, H-4′), 5.78 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, H-2′), 5.49 (dd, 1 H, H-3′), 5.37 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1), 5.35 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 5.04 (d, 1 H, $J_{1',2'}$ 3.6 Hz, H-1′), 4.81 (dd, 1 H, $J_{3,4}$ 9.8 Hz, H-3), 4.80 (m, 1 H, H-5′), 4.62–4.11 (3 × AB pattern, 6 H, 3 × $PhCH_2$), 4.56 (dd, 1 H, $J_{5',6a'}$ 6.6, $J_{6a',6b'}$ 11.2 Hz, H-6a′), 4.48 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-2), 4.42 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.32 (dd, 1 H, $J_{5',6b'}$ 7.4 Hz, H-6b′), 4.10 (m, 1 H, H-5′), 3.98–3.95(m, 2 H, H-3′, H-6a), 3.88 (d, 1 H, $J_{6a,6b}$ 10.1 Hz, H-6b), 3.79 (dd, 1 H, $J_{2',3'}$ 10.2 Hz, H-2′), 3.55 (d, 1 H, $J_{3'',4''}$ 1.7 Hz, H-4″), 3.24 (m, 1 H, H-5), 2.10 (s, 3 H, NHAc), 1.42 (d, $J_{5',6'}$ 6.5 Hz, H-6′), 1.05 (s, 9 H, SiCMe$_3$); ^{13}C NMR ($CDCl_3$) δ 139.0–119.3 (66 C, aromatic), 100.1 (C-1′), 97.0 (C-1″), 84.6 (C-1), 79.6 (2 C, C-5, C-4″), 79.3 (C-3″), 75.2 (2 C, C-2″, CH_2), 75.0 (C-4), 73.2 (C-3), 72.9 (CH_2), 72.0 (C-3′), 71.7 (CH_2), 71.6 (C-5′), 69.7 (C-2′), 68.5 (C-4′), 66.7 (C-5″), 61.4 (2 C, C-6′, C-6), 55.5 (C-2), 26.9 (SiC*Me*$_3$), 24.6 (OAc), 19.4 (Si*C*Me$_3$), 16.9 (C-6′). Anal Calcd for $C_{99}H_{94}N_2O_{20}SSi$: C, 70.28; H, 5.60; N, 1.66. Found: C, 70.10; H, 5.63; N, 1.70.

4-Nitrophenyl 2,6-di-O-*benzoyl*-β-D-*galactopyranosyl* (1→4)-*2,3,6-tri*-O-*benzoyl-1-*

*thio-β-*D*-glucopyranoside* (**9**).—4-Nitrophenyl 3′,4′-*O*-isopropylidene-β-lactoside derivative **8** [3] (250 mg, 0.481 mmol) was dissolved in dry pyridine (10 mL), the solution was cooled to 0 °C, and benzoyl chloride (0.5 mL) was added. The mixture was then allowed to reach rt and stir overnight. The reaction mixture was poured onto ice water and extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed successively with 5% HCl solution, satd aq $NaHCO_3$, and water. The solution was dried (Na_2SO_4) and concentrated to a foam, which was dissolved in 15 mL of a 1:1 mixture of CH_2Cl_2 and CH_3OH, to which *p*-toluenesulfonic acid (100 mg) was added. The mixture was stirred at 40 °C for 2 h. The reaction mixture was neutralized with triethylamine and concentrated under reduced pressure. The residue was purified by silica gel chromatography using 1:2 hexane–EtOAc as eluent to afford **9** (440 mg, 92%): mp 178–180 °C; $[\alpha]_D$ +11.8° (*c* 1.0, $CHCl_3$); FABMS (glycerol) gave *m*/*z* (ion, relative intensity): 1000.3 ($[M+1]^+$, 3%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01–7.27 (m, 29 H, aromatic H), 5.72 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 5.41 (dd, 1 H, $J_{2,3}$ 9.7 Hz, H-2), 5.31 (dd, 1 H, $J_{2',3'}$ 7.7 Hz, H-2′), 4.97 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 4.65 (dd, 1 H, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.62 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.51 (dd, 1 H, $J_{5,6b}$ 5.9 Hz, H-6b), 4.05 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.02 (dd, 1 H, $J_{6a',6b'}$ 11.3 Hz, H-6a′), 3.96 (ddd, 1 H, H-5), 3.84 (d, 1 H, H-4′), 3.73 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 3.64 (dd, 1 H, $J_{5',6b'}$ 6.5 Hz, H-6b′), 3.65 (dd, 1 H, $J_{5',6a'}$ 6.4 Hz, H-5′); ^{13}C NMR ($CDCl_3$) δ 166.4, 166.1, 165.7, 165.7, 165.2, (5 × C=O), 146.8–123.7 (36 C, aromatic), 100.9 (C-1′), 84.1 (C-1), 77.4 (C-5), 76.1 (C-4), 73.8 (C-3), 73.7 (C-2′), 72.7 (C-5′), 72.6 (C-3′), 70.1 (C-2), 68.5 (C-4), 62.7 (C-6), 61.9 (C-6′). Anal. Calcd for $C_{53}H_{45}NO_{17}S$: C, 63.66; H, 4.54; N, 1.40. Found: C, 63.71; H, 4.68; N, 1.30.

*4-Nitrophenyl 2,3,4,6-tetra-*O*-benzoyl-β-*D*-galactopyranosyl-(1→4)-[2,3,4-tri-*O*-benzyl-α-*L*-fucopyranosyl-(1→3)]-(2-acetamido-6-*O*-tert-butyldiphenylsilyl-2-deoxy-β-*D*-glucopyranosyl)-(1→3)-2,6-di-*O*-benzoyl-β-*D*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-1-thio-β-*D*-glucopyranoside* (**10**).—To compound **7** (81 mg, 0.048 mmol) and compound **9** (48 mg, 0.048 mmol) in dry CH_2Cl_2 (3 mL) were added powdered 4 Å molecular sieves (120 mg) under nitrogen. The mixture was stirred at rt for 3 h and then cooled to −30 °C. *N*-Iodosuccinimide (22 mg, 0.096 mmol) and trifluoromethanesulfonic acid (4.2 μL, 0.048 mmol) were added. After stirring at −45 °C for 45 min, the mixture was diluted with CH_2Cl_2 (12 mL) and filtered through Celite. The filtrate was washed with 10% aq sodium thiosulfate (15 mL), satd aq $NaHCO_3$ (2 × 15 mL), and brine (15 mL). The solution was dried (Na_2SO_4) and concentrated to a foam that was chromatographed on silica gel (1:99 ethanol–dichloromethane) to afford pentasaccharide **10** (86.2 mg, 71%): $[\alpha]_D$ +12.8° (c 1.0, $CHCl_3$); FABMS (glycerol) gave *m*/*z* (ion, relative intensity): 2524.9 ($[M]^+$, 0.1%); ^{1}H NMR (500 Hz, $CDCl_3$) δ 8.08–6.97 (m, 78 H, aromatic H), 5.78 (d, 1 H, H-4d), 5.67 (dd, 1H, $J_{2,3}$ 10.4 Hz, H-2d), 5.61 (dd, 1 H, $J_{3,4}$ 9.3 Hz, H-3a), 5.32 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2a), 5.31 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-3d), 5.18 (dd, 1 H, $J_{2,3}$ 9.6 Hz, H-2b), 5.15 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1c), 5.12 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1d), 4.96 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1e), 4.85 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1a), 3.46 (m, 1 H, H-5c), 1.32 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6e), 1.02 (s, 9 H, $SiCMe_3$); ^{13}C NMR ($CDCl_3$) δ 166.0, 165.9, 165.8, 165.6, 165.2, 165.1, 164.6, 164.1 (9 × C=O) 146.7–123.2 (96 C, aromatic), 100.5 (C-1b), 99.6 (C-1d), 99.0 (C-1c), 96.6 (C-1e), 84.0 (C-1a), 80.1 (C-3b), 79.3 (C-4e), 79.1 (C-3e), 77.3 (C-2e), 75.5 (2 C, C-5a, C-5c), 75.2 (CH_2), 75.0 (2 C, C-4C, C-5d), 74.7 (C-3a), 73.2 (CH_2), 72.8 (C-5b), 72.2 (C-3c), 71.9 (CH_2), 71.8 (C-3d), 71.7(C-4a), 71.4 (C-2b), 70.8 (C-2a), 70.0 (C-2d), 69.7 (C-4d), 68.2 (C-4b), 67.8 (C-5e), 66.7 (C-6b), 62.8 (C-6a), 61.5 (C-6c), 61.2 (C-6d), 55.9 (C-2c), 26.9 ($SiCMe_3$), 19.5 ($SiCMe_3$), 16.8 (C-6e). Anal. Calcd for $C_{144}H_{130}N_2O_{36}SSi$: C, 68.51; H, 5.19; N, 1.11. Found: C, 68.39; H, 5.23; N, 1.19.

Acknowledgements

The authors thank the Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support.

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