ELSEVIER

Carbohydrate Research 337 (2002) 1313–1317

CARBOHYDRATE RESEARCH

www.elsevier.com/locate/carres

Note

Concise synthesis of a buffalo milk pentasaccharide derivative

Guofeng Gu,[a] Yuguo Du,[a,*] Jingqi Pan[b]

[a]*Research Center for Eco-Environmental Sciences, Academia Sinica, PO Box 2871, Beijing 100085, PR China*
[b]*College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, PR China*

Received 14 May 2002; accepted 5 June 2002

Abstract

An efficient synthesis of buffalo milk pentasaccharide derivative via a 3 + 2 strategy is described. The use of a trisaccharide isopropyl thioglycoside as a latent glycosyl donor and the application of two well-defined regioselective glycosylations significantly simplified the target preparation. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Glycosylation; Antigens; Thioglycosides; Oligosaccharides; Regioselectivity

Milk, which contains numerous complex carbohydrate components, is known as a good source of oligosaccharide antigens and bifidus factors for breast-fed newborns.[1] Monoclonal antibodies of several tumour cell lines or carbohydrate antigens have provided evidence that membrane glycoproteins or glycolipids, which may function as differentiation antigens or tumour-associate antigens, also occur as free oligosaccharides in human milk.[2] Different types of milk since then have been analyzed for their oligosaccharides that have immunological properties.[3] Recently, Deepak and co-workers[4] isolated a novel pentasaccharide from immunostimulant oligosaccharide fraction of buffalo milk with the following structure: β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-Gal-(1→4)-D-Glc. A six-fold increase in haemagglutinating antibody (HA) titre and a two-fold increase in plaque-forming cell (PFC) count were reported in treated animals as compared with untreated controls. To investigate structure–activity relationships, we planned to prepare a monomer and a trimer of the above-mentioned pentasaccharide. We present here the concise chemical synthesis of this pentasaccharide derivative.

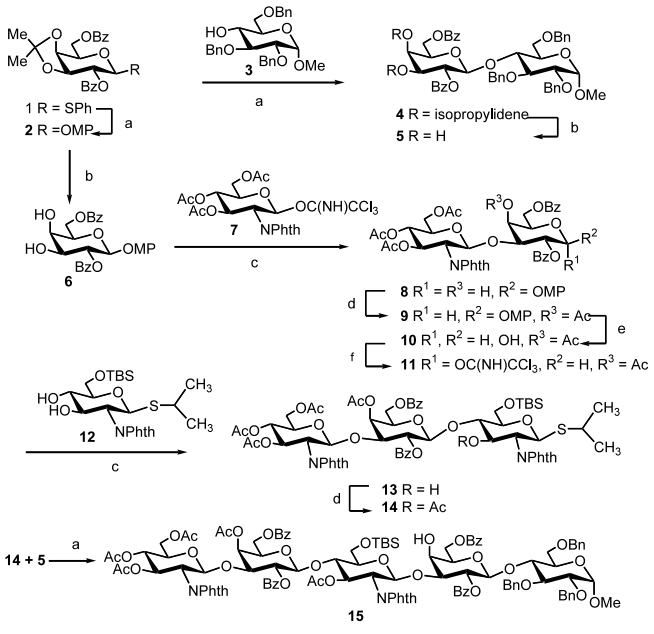
The efficient chemical synthesis of complex oligosaccharides requires highly convergent strategies in which well-designed glycosyl donors and acceptors are assembled involving a minimum of steps of reactions. Accordingly, the target molecule was retrosynthetically disconnected into a disaccharide acceptor **5** and a trisaccharide donor **14** as shown in Scheme 1. Thus, compound **1**[5] was condensed with **3**[6] in the presence of *N*-iodosuccinimide (NIS) and catalytic amount of trimethylsilyl triflate (TMSOTf) to afford disaccharide **4** in 89% yield. The stereochemical outcome is induced by the neighbouring-group participation of donor **1** and confirmed by the ^{1}H NMR spectroscopy of **4** (H-1: δ 4.49 ppm, $J_{1,2}$ 8.0 Hz). Hydrolysis of **4** in aqueous 90% trifluoroacetic acid (TFA) furnished disaccharide acceptor **5** in good yield (84.5%). Convergently, **1** was reacted with *p*-methoxyphenol (MPOH) with the promotion of NIS–TMSOTf in dry CH_2Cl_2 to give **2**, which was treated with 90% TFA to generate diol **6** (63.7% for two steps). Regioselective coupling of **6** with glucosamine imidate **7** in anhydrous CH_2Cl_2 in the presence of TMSOTf produced β-(1→3)-linked disaccharide **8** in 72% yield. Acetylation of **8** with acetic anhydride in pyridine gave **9** showing H-4 at δ 5.63 ppm ($J_{4,3}$ 3.4 Hz), which confirmed the correct regioselectivity. Ceric ammonium nitrate (CAN) promoted cleavage of anomeric MP of **9** was carried out smoothly in toluene–MeCN–H_2O co-solvent system (→**10**), followed by Schmidt activation using trichloroacetonitrile and DBU, to afford imidate **11** in 72.3% yield (based on **8**).

* Corresponding author. Tel.: +86-10-62914475; fax: +86-10-62923563
E-mail address: ygdu@mail.rcees.ac.cn (Y. Du).

0008-6215/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0008-6215(02)00131-3

To save steps in protection-group manipulation on the anomeric centre, the isopropyl thioglycoside[7] was designed as a latent glycosyl donor for the glucosamine residue. It was also reported[8] that the 6-OH benzoylated glucosamine unit decreased the activity of the group 4-OH while the ether-protected counterparts improved the yields greatly. As we expected, coupling of isopropyl thioglycoside **12**[9] and **11** at 0 °C using TMSOTf as catalyst gave β-(1→4)-linked trisaccharide **13** (71%), which was further acetylated with acetic anhydride in pyridine to furnish trisaccharide donor **14**. Neither α nor β product with 1→3 linkage[10] was detected in our experiments. 2D NMR experiments showed that the chemical shift of H-3^{I} moved downfield from 4.39 ppm (in **13**) to 5.64 ppm (in **14**), together with H-1^{II} at δ 4.74 ppm ($J_{1,2}$ 8.1 Hz), confirming the formation of a β-(1→4) linkage. Glycosylation of isopropyl thioglycoside **14** and disaccharide diol **5** in the presence of NIS–TMSOTf at −15 °C completed the synthesis of pentasaccharide derivative **15** (66%). ^{1}H–^{1}H and ^{1}H–^{13}C COSY analyses assigned the peak at δ 81.25 ppm (^{13}C NMR spectroscopy) as C-3^{II}. This downfield movement of C-3^{II}, compared with signals of C-3^{V} (70.14 ppm), C-4^{II} (67.67 ppm) and C-4^{IV} (69.29 ppm), indicated the (1→3)-bond formation in the last glycosylation step. H-1^{III} appeared at δ 5.31 ppm in the ^{1}H NMR spectrum ($J_{1,2}$ 8.4 Hz), which confirmed the β stereoselectivity.

In conclusion, we have described the concise synthesis of the methyl glycoside derivative corresponding to the bioactive buffalo milk pentasaccharide. Two well-defined regioselective glycosylation steps and the use of isopropyl thioglycoside as the latent donor simplified the protecting-group manipulations in the synthesis and the strategic principle described here is currently employed in the exploring of trimeric analogue preparation.



Scheme 1. Reagents and conditions: (a) NIS, TMSOTf; 89.1% for **4**; 65.5% for **15**; (b) 90% TFA; 84.5% for **5**; 63.7% for **6** (from **1**); (c) TMSOTf, CH_2Cl_2, 71.9% for **8**; 71.2% for **13**; (d) Ac_2O, Pyr; (e) CAN, 3:4:3 Toluene–MeCN–water; (f) Cl_3CCN, DBU, 78.7% from **9**.

1. Experimental

General methods.—Optical rotations were determined at 20 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. Melting points were determined with a "Mel-Temp" apparatus. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with ARX 400 spectrometers for solutions in $CDCl_3$. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using MALDI-TOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH, or in some cases by a UV detector.

Methyl 2,6-di-O-*benzoyl-3,4-di*-O-*isopropylidene-β*-D-*galactopyranosyl-(1→4)-2,3,4-tri*-O-*benzyl-α*-D-*glucopyranoside* (**4**).—To a solution of **1** (290 mg, 0.558 mmol) and **3** (246 mg, 0.526 mmol) in anhyd CH_2Cl_2 (4 mL) was added NIS (310 mg, 1.38 mmol) and Me_3SiOTf (20 μL, 0.11 mmol) under an N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 1 h, then at rt for another 30 min, at the end of which time TLC (6:1 petroleum ether–EtOAc) indicated that starting material **1** was completely consumed. The reaction mixture was neutralized with Et_3N and concentrated. Column chromatography (5:1 petroleum ether–EtOAc) of the residue gave **4** (410 mg, 89.1%) as a syrup: $[\alpha]_D$ +18° (*c* 0.75, $CHCl_3$); ^{1}H NMR: 1.34, 1.60 (2 s, 2 × 3 H, 2 CH_3), 3.27 (s, 3 H, OCH_3), 3.35–3.52 (m, 3 H, H-6a, H-6b, H-5), 3.67 (dd, 1 H, $J_{2,1}$ 2.5, $J_{2,3}$ 9.2 Hz, H-2), 3.80–3.92 (m, 3 H, H-3, H-4, H-5′), 4.04 (dd, 1 H, $J_{3',4'}$ 4.7, $J_{3',2'}$ 7.5 Hz, H-3′), 4.16 (d, 1 H, H-4′), 4.29 (d, 1 H, *J* 12.2 Hz, one proton of PhCH_2), 4.36 (dd, 1 H, $J_{6a',6b'}$ 11.3, $J_{6a',5'}$ 7.0 Hz, H-6a′), 4.49 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.52–4.58 (m, 2 H, H-6b, H-1), 4.60, 4.68, 4.77, 4.78, 5.03 (5 d, 5 H, *J* 12.2, 10.7 Hz, PhCH_2), 5.21 (t, 1 H, $J_{2',3'}$ 7.5, $J_{2',1'}$ 8.0 Hz, H-2′), 7.25–8.04 (m, 30 H, Ph). MALDITOF-MS Calcd for $C_{51}H_{54}O_{13}$: 874 [M]; Found: 897.3 $[M + Na]^+$.

Methyl 2,6-di-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-2,3,4-tri*-O-*benzyl-α*-D-*glucopyranoside* (**5**).—A solution of compound **4** (400 mg, 0.458 mmol) in 90% TFA (5 mL) was stirred at rt for 15 min, then neutralized with aq $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 20 mL). The organic phases were combined and concentrated. The residue was purified on a silica gel column

using 2:1 petroleum ether–EtOAc as eluent to give foamy **5** (323 mg, 84.5%): $[\alpha]_D$ +6° (*c* 2.4, $CHCl_3$); ^1H NMR: 3.29 (s, 3 H, OCH_3), 3.45–3.58 (m, 5 H, H-6a, H-6b, H-5, H-3′, H-5′), 3.70 (dd, 1 H, $J_{2,1}$ 3.4, $J_{2,3}$ 10.8 Hz, H-2), 3.80–3.96 (m, 3 H, H-3, H-4, H-4′), 4.17 (dd, 1 H, $J_{6a',6b'}$ 11.2, $J_{6a',5'}$ 5.7 Hz, H-6a′), 4.31 (d, 1 H, *J* 12.8 Hz, one proton of $PhCH_2$), 4.52 (dd, 1 H, $J_{6b',5'}$ 7.8 Hz, H-6b′), 4.56 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.59 (d, 1 H, $J_{1',2'}$ 8.2 Hz, H-1′), 4.63, 4.76, 4.78, 4.84, 4.98 (5 d, 5 H, *J* 12.3, 12.8, 10.7 Hz, $PhCH_2$), 5.17 (t, 1 H, $J_{2',3'}$ 9.2, $J_{2',1'}$ 8.2 Hz, H-2′), 7.25–8.04 (m, 30 H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): 55.31 (OCH_3), 62.03 (C-6′), 67.90 (C-6), 68.34 (C-4′), 69.56 (C-3′), 71.97 (C-2′), 72.68 (C-5′), 73.49 ($PhCH_2$), 73.50 ($PhCH_2$), 74.27 (C-5), 75.28 ($PhCH_2$), 76.48 (C-3), 79.02 (C-2), 79.98 (C-4), 98.34 (C-1), 100.10 (C-1′), 166.50, 166.65 (2 C, PhCO); MALDITOF-MS Calcd for $C_{48}H_{50}O_{13}$: 834 [M]; Found 857.4 $[M+Na]^+$, 873.4 $[M+K]^+$. Anal. Calcd for $C_{48}H_{50}O_{13}$: C, 69.06; H, 6.04. Found: C, 68.84; H, 6.13.

p-*Methoxyphenyl 2,6-di*-O-*benzoyl-β*-D-*galactopyranoside* (**6**).—To a solution of **1** (1.46 g, 2.81 mmol) and *p*-methoxyphenol (418 mg, 3.37 mmol) in anhyd CH_2Cl_2 (10 mL) was added NIS (948 mg, 4.22 mmol) and Me_3SiOTf (31 μL, 0.17 mmol) under N_2 atmosphere at −15 °C. The mixture was stirred under these conditions for 1 h, at the end of which time TLC (5:1 petroleum ether–EtOAc) indicated that starting material **1** was completely consumed. The reaction mixture was neutralized with Et_3N and concentrated. The above residue was dissolved into 90% TFA (10 mL), stirred at rt for 20 min, then co-evaporated with toluene under diminished pressure to dryness. Purification of the residue on a silica gel column using 1:1 petroleum ether–EtOAc as eluent gave **6** (884 mg, 63.7%) as a white solid: $[\alpha]_D$ −9° (*c* 0.9, $CHCl_3$); ^1H NMR: 3.71 (s, 3 H, OCH_3), 3.94 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 3.5 Hz, H-3), 4.01 (dd, 1 H, $J_{5,6a}$ 7.6, $J_{5,6b}$ 5.4 Hz, H-5), 4.11 (d, 1 H, H-4), 4.65 (dd, 1 H, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.75 (dd, 1 H, H-6b), 5.03 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.44 (dd, 1 H, H-2), 6.65 (d, 2 H, Ph), 6.95 (d, 2 H, Ph), 7.40–8.08 (m, 10 H, Ph).

p-*Methoxyphenyl 3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-2,6-di*-O-*benzoyl-β*-D-*galactopyranoside* (**8**).—Compound **6** (900 mg, 1.82 mmol) and **7** (1.10 g, 1.90 mmol) were pre-dried in one flask under vacuum at 60 °C for 2 h. The mixture was then dissolved in CH_2Cl_2 (10 mL). To the solution was added Me_3SiOTf (36 μL, 0.20 mmol) at rt under an N_2 atmosphere. The mixture was stirred for 1.5 h, then neutralized with Et_3N, concentrated under reduced pressure, and purified on a silica gel column with 1:1 petroleum ether–EtOAc as the eluents to give **8** (954 mg, 71.9%, based on recovered **5**) as crystals: mp 203–205 °C; $[\alpha]_D$ +50° (*c* 0.75, $CHCl_3$); ^1H NMR: 1.79, 2.05, 2.13 (3 s, 3 × 3 H, 3 CH_3CO), 3.65 (s, 3 H, OCH_3), 3.85–4.08 (m, 3 H, H-3, H-5, H-5′), 4.16–4.34 (m, 3 H, H-4, H-6a′, H-6b′), 4.38 (t, 1 H, $J_{2',3'}$ 10.7, $J_{2',1'}$ 8.2 Hz, H-2′), 4.68 (dd, 1 H, $J_{6a,6b}$ 11.4, $J_{6a,5}$ 7.9 Hz, H-6a), 4.73 (dd, 1 H, $J_{6b,5}$ 4.3 Hz, H-6b), 4.83 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.14 (t, 1 H, $J_{4',3'} = J_{4',5'} = 9.5$ Hz, H-4′), 5.54–5.72 (m, 3 H, H-2, H-1′, H-3′), 6.53 (d, 2 H, Ph), 6.76 (d, 2 H, Ph), 7.17–8.09 (m, 14 H, Ph). Anal. Calcd for $C_{47}H_{45}NO_{18}$: C, 69.91; H, 4.97. Found: C, 70.12; H, 5.09.

p-*Methoxyphenyl 3,4,6-tri*-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-4*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*galactopyranoside* (**9**).—To a solution of compound **8** (915 mg, 1.00 mmol) in pyridine (4 mL) was added Ac_2O (2 mL). The mixture was stirred at rt for about 12 h, then co-evaporated with toluene under diminished pressure to remove pyridine. Column chromatography (2:1 petroleum ether–EtOAc) of the residue gave crystalline **9** (879 mg, 91.8%): mp 210–212 °C; $[\alpha]_D$ +53° (*c* 1.4, $CHCl_3$); ^1H NMR: 1.78, 2.03, 2.12, 2.23 (4 s, 4 × 3 H, 4 CH_3CO), 3.64 (s, 3 H, OCH_3), 3.80–3.84 (m, 1 H, H-5′), 4.03–4.14 (m, 2 H, H-3, H-5), 4.18 (dd, 1 H, $J_{6a',6b'}$ 11.9, $J_{6a',5'}$ 4.2 Hz, H-6a′), 4.25 (dd, 1 H, $J_{2',3'}$ 10.7, $J_{2',1'}$ 8.2 Hz, H-2′), 4.33 (dd, 1 H, $J_{6b',5'}$ 5.7 Hz, H-6b′), 4.41 (dd, 1 H, $J_{6a,6b}$ 11.4, $J_{6a,5}$ 7.9 Hz, H-6a), 4.52 (dd, 1 H, $J_{6b,5}$ 4.3 Hz, H-6b), 4.87 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.16 (t, 1 H, $J_{4',3'} = J_{4',5'} = 9.3$ Hz, H-4′), 5.48–5.57 (m, 2 H, H-2, H-1′), 5.63 (d, 1 H, $J_{4,3}$ 3.4 Hz, H-4), 5.67 (t, 1 H, $J_{3',4'}$ 9.3, $J_{3',2'}$ 10.7 Hz, H-3′), 6.50 (d, 2 H, Ph), 6.74 (d, 2 H, Ph), 7.26–8.08 (m, 14 H, Ph). Anal. Calcd for $C_{49}H_{47}NO_{19}$: C, 61.70; H, 4.97. Found: C, 61.64; H, 5.13.

3,4,6-Tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-4*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*galactopyranosyl trichloroacetimidate* (**11**).—Ceric ammonium nitrate (CAN; 1.22 g, 2.23 mmol) was added to a solution of **9** (850 mg, 0.892 mmol) in 3:4:3 toluene–MeCN–H_2O (30 mL), and the mixture was stirred at rt for 2 h. An additional amount of CAN (734 mg, 1.34 mmol) was added, and the stirring was continued for another 2 h, at the end of which time TLC (1:1 petroleum–EtOAc) indicated the completion of the reaction. The resulting mixture was diluted with EtOAc, washed successively with water, satd aq $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated, then subjected to silica gel column chromatography (1:1 petroleum ether–EtOAc). The product generated above was dissolved in anhydrous CH_2Cl_2 (5 mL). To the solution was added trichloroacetonitrile (0.45 mL, 4.46 mmol) and DBU (45 μL, 0.45 mmol) at rt. The mixture was stirred for 2 h, then concentrated, and the residue was subjected to a silica gel column (1.5:1 petroleum ether–EtOAc) to afford syrupy **11** (696 mg, 78.7% for two steps): $[\alpha]_D$ +96° (*c* 1.5, $CHCl_3$); ^1H NMR: 1.80, 2.02, 2.12, 2.20 (4 s, 4 × 3 H, 4 CH_3CO), 3.85–3.92 (m, 1 H, H-5′), 4.15–4.25 (m, 2 H, H-3, H-2′), 4.32–4.44 (m, 4 H, H-5, H-6a, H-6a′, H-6b′), 4.55 (dd, 1 H, $J_{6b,6a}$ 12.3,

$J_{6b,5}$ 5.0 Hz, H-6b), 5.17 (t, 1 H, $J_{4',3'}$ 9.2, $J_{4',5'}$ 9.5 Hz, H-4′), 5.46 (dd, 1 H, $J_{2,3}$ 10.5, $J_{2,1}$ 3.8 Hz, H-2), 5.52 (d, 1 H, $J_{1',2'}$ 3.8 Hz, H-1′), 5.70–5.77 (m, 2 H, H-4, H-3′), 6.52 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 7.28–8.01 (m, 14 H, Ph), 8.34 (s, 1 H, NH). Anal. Calcd for $C_{44}H_{41}Cl_3N_2O_{18}$: C, 53.25; H, 4.17. Found: C, 53.08; H, 4.22.

Isopropyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-4*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-6*-O-tert-*butyldimethylsilyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* (**13**).—To a solution of compound **11** (660 mg, 0.666 mmol) and **12** (300 mg, 0.623 mmol) in anhyd CH_2Cl_2 (5 mL) was added Me_3SiOTf (12 μL, 0.067 mmol) under an N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 1 h, neutralized with Et_3N and concentrated under reduced pressure. The residue was purified on a silica gel column with 1.3:1:0.1 petroleum ether–EtOAc–toluene as the eluent to give **13** (582 mg, 71.2%) as a syrup: $[\alpha]_D$ +47° (*c* 1, $CHCl_3$); ^{1}H NMR: −0.17, −0.16 (2 s, 2 × 3 H, 2 $SiCH_3$), 0.73 (s, 9 H, $C(CH_3)_3$), 1.10, 1.12 (2 d, 2 × 3 H, *J* 6.6, 6.9 Hz, $CH(CH_3)_2$), 1.76, 2.00, 2.13, 2.18 (4 s, 4 × 3 H, 4 CH_3CO), 3.00 (m, 1 H, $CH(CH_3)_2$), 3.24–3.42 (m, 3 H, $J_{6a,6b}$ 10.4 Hz, H-6a^I, H-6b^I, H-5^I), 3.57 (t, 1 H, $J_{4,3} = J_{4,5} = 8.7$ Hz, H-4^I), 3.82 (m, 1 H, H-5^{III}), 3.88–4.25 (m, 6 H, H-5^{II}, H-6a^{III}, H-6b^{III}, H-2^I, H-2^{III}, H-3^{II}), 4.34 (br d, 1 H, $J_{6a,6b}$ 11.6 Hz, H-6a^{II}), 4.39 (t, 1 H, $J_{3,4}$ 8.7, $J_{3,2}$ 10.1 Hz, H-3^I), 4.61–4.72 (m, 2 H, H-1^{II}, H-6b^{II}), 5.14 (t, 1 H, $J_{4,3} = J_{4,5} = 9.3$ Hz, H-4^{III}), 5.26 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1^I), 5.34 (br t, 1 H, $J_{2,3} = J_{2,1} = 8.7$ Hz, H-2^{II}), 5.47 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1^{III}), 5.57 (br s, 1 H, H-4^{II}), 5.64 (t, 1 H, $J_{3,2}$ 10.6, $J_{3,4}$ 9.3 Hz, H-3^{III}), 7.26–8.05 (m, 18 H, Ph). Anal. Calcd for $C_{65}H_{74}N_2O_{23}SSi$: C, 59.54; H, 5.69. Found: C, 59.21; H, 5.50.

Isopropyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-4*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-3*-O-*acetyl-6*-O-tert-*butyl-dimethylsilyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* (**14**).—To a solution of compound **13** (560 mg, 0.427 mmol) in pyridine (2 mL) was added Ac_2O (1 mL). The mixture was stirred at 40 °C for about 20 h, then co-evaporated with toluene under diminished pressure to remove pyridine. The residue was purified by silica-gel column chromatography (2:1 petroleum ether–EtOAc) to give syrupy **14** (550 mg, 95.2%): $[\alpha]_D$ +41° (*c* 1.3, $CHCl_3$); ^{1}H NMR: −0.06, 0.13 (2 s, 2 × 3 H, 2 $SiCH_3$), 0.81 (s, 9 H, $C(CH_3)_3$), 1.10, 1.11 (2 d, 6 H, *J* 6.1, 6.5 Hz, $CH(CH_3)_2$), 1.76, 1.88, 2.00, 2.10, 2.18 (5 s, 5 × 3 H, 5 CH_3CO), 2.97 (m, 1 H, $CH(CH_3)_2$), 3.20 (m, 1 H, H-5^I), 3.47–3.55 (m, 2 H, H-6a^I, H-6b^I), 3.79–3.90 (m, 4 H, H-4^I, H-3^{II}, H-5^{II}, H-5^{III}), 4.08–4.22 (m, 4 H, H-2^I, H-2^{III}, H-6a^{III}, H-6b^{III}), 4.30 (dd, 1 H, $J_{6a,6b}$ 11.4, $J_{6a,5}$ 2.1 Hz, H-6a^{II}), 4.55 (t, 1 H, $J_{6b,5}$ 4.5 Hz, H-6b^{II}), 4.74 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1^{II}), 5.13 (t, 1 H, $J_{4,3}$ 9.4, $J_{4,5}$ 9.8 Hz, H-4^{III}), 5.19 (t, 1 H, $J_{2,3}$ 9.8, $J_{2,1}$ 8.1 Hz, H-2^{II}), 5.34 (d, 1 H, $J_{1,2}$ 10.6 Hz, H-1^I), 5.44 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^{III}), 5.55 (d, 1 H, $J_{4,3}$ 3.6 Hz, H-4^{II}), 5.64 (t, 2 H, $J_{3,2}$ 10.5, $J_{3,4}$ 9.4 Hz, H-3^I, H-3^{III}), 7.11–8.15 (m, 18 H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): −5.47, 0.95 (2 C, $SiCH_3$), 20.25, 20.44, 20.53, 20.65, 20.68 (5 C, CH_3CO), 23.74, 24.14 (2 C, $CH(CH_3)_2$), 25.71 (4 C, $C(CH_3)_3$), 34.81 ($CH(CH_3)_2$), 54.27 (C-2^{III}), 54.43 (C-2^I), 60.88 (C-6^I), 61.43 (C-6^{III}), 62.45 (C-6^{II}), 68.68 (C-4^{III}), 69.24 (C-4^{II}), 70.15 (C-3^{III}), 71.01 (C-2^{II}), 71.34 (C-3^I), 71.36 (C-5^{III}), 71.77 (C-5^{II}), 74.57 (C-5^I), 77.76 (C-3^{II}), 78.83 (C-4^I), 79.99 (C-1^I), 98.39 (C-1^{III}), 100.21 (C-1^{II}), 163.83, 166.16 (2 C, PhCO), 166.45, 167.26, 167.61 (4 C, CO of Phth, some overlapped), 169.29, 169.91, 170.00, 170.65 (5 C, CH_3CO, some overlapped); MALDITOF-MS Calcd for $C_{67}H_{76}N_2O_{24}SSi$: 1352 [M]. Found: 1375.6 $[M + Na]^+$, 1391.6 $[M + K]^+$.

Methyl 3,4,6-tri-O-*acetyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-4*-O-*acetyl-2,6-di*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-3*-O-*acetyl-6*-O-tert-*butyldimethylsilyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1→3)-2,6-di*-O-*benzoyl-β*-D-*galactopyranosyl-(1→4)-2,3,4-tri*-O-*benzyl-α*-D-*glucopyranoside* (**15**).—To a solution of compound **14** (530 mg, 0.392 mmol) and **5** (327 mg, 0.392 mmol) in anhyd CH_2Cl_2 (4 mL) was added NIS (220 mg, 0.98 mmol) and Me_3SiOTf (21 μL, 0.12 mmol) under an N_2 atmosphere at −15 °C. The mixture was stirred under these conditions for 1 h. TLC (1:1 petroleum ether–EtOAc) indicated the completion of the reaction. The mixture was neutralized with Et_3N and concentrated. Purification of the residue on a silica gel column with 1:1.3 petroleum ether–EtOAc as eluent gave **15** (542 mg, 65.5%) as a syrup: $[\alpha]_D$ +34° (*c* 1, $CHCl_3$); ^{1}H NMR: −0.10, −0.03 (2 s, 2 × 3 H, 2 $SiCH_3$), 0.80 (s, 9 H, $C(CH_3)_3$), 1.76, 1.79, 1.99, 2.10, 2.16 (5 s, 5 × 3 H, 5 CH_3CO), 3.09–3.19 (m, 4 H, H-6a^I, OCH_3), 3.19–3.27 (m, 2 H, H-5^I, H-5^{III}), 3.28–3.39 (m, 3 H, H-2^I, H-3^{II}, H-6b^I), 3.42 (dd, 1 H, $J_{6a,6b}$ 11.4 Hz, H-6a^{III}), 3.48–3.57 (m, 2 H, H-5^{II}, H-6b^{III}), 3.67–3.80 (m, 6 H, H-3^I, H-4^I, H-4^{III}, H-3^{IV}, H-5^{IV}, H-5^V), 3.94 (d, 1 H, $J_{4,3}$ 4.6 Hz, H-4^{II}), 4.01–4.26 (m, 6 H, H-2^{III}, H-2^V, H-6a^{II}, H-6a^{IV}, H-6a^V, one proton of $PhCH_2$), 4.26–4.31 (m, 2 H, $J_{1,2}$ 8.1 Hz, H-1^{II}, H-6b^V), 4.44 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1^I), 4.46–4.61 (m, 4 H, H-6b^{II}, H-6b^{IV}, two protons of $PhCH_2$), 4.64 (d, 1 H, *J* 10.8 Hz, one proton of $PhCH_2$), 4.72 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{IV}), 4.73, 4.94 (2 d, 2 H, *J* 12.3, 10.8 Hz, $PhCH_2$), 5.07–5.24 (m, 3 H, H-2^{II}, H-2^{IV}, H-4^V), 5.31 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1^{III}), 5.43 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^V), 5.51 (t, 1 H, $J_{3,2}$ 10.7, $J_{3,4}$ 9.1 Hz, H-3^{III}), 5.53 (d, 1 H, $J_{4,3}$ 4.5 Hz, H-4^{IV}), 5.64 (t, 1 H, $J_{3,2}$ 10.7, $J_{3,4}$ 9.1 Hz, H-3^V), 7.09–8.10 (m, 43 H, Ph); ^{13}C NMR (100 MHz, $CDCl_3$): −5.45, −5.07 (2 C, $SiCH_3$), 20.27, 20.55, 20.67 (5 C, CH_3CO, some overlapped), 25.63 (4 C, $C(CH_3)_3$), 54.43 (C-2^V), 54.70

(C-2^{III}), 55.12 (OCH_3), 60.45 (C-6^{III}), 60.47 (C-6^{V}), 61.41 (C-6^{IV}), 63.19 (C-6^{II}), 67.38 (C-6^{I}), 67.67 (C-4^{II}), 68.62 (C-4^{V}), 69.17 (C-5^{III}), 69.29 (C-4^{IV}), 69.99 (C-3^{III}), 70.14 (C-3^{V}), 70.85 (C-2^{II}), 70.98 (C-2^{IV}), 71.45 (C-5^{II}), 71.55 (C-5^{V}), 71.80 (C-5^{IV}), 73.29 ($PhCH_2$), 73.42 ($PhCH_2$), 74.00 (C-4^{III}), 75.01 (C-5^{I}), 75.38 ($PhCH_2$), 76.04 (C-3^{I}), 77.76 (C-3^{IV}), 78.65 (C-2^{I}), 79.76 (C-4^{I}), 81.25 (C-3^{II}), 98.37 (2 C, C-1^{I}, C-1^{V}), 98.57 (C-1^{III}), 99.81 (C-1^{II}), 100.11 (C-1^{IV}), 163.80, 164.09, 166.13, 166.17 (4 C, $PhCH_2$), 166.60, 167.68, 167.72 (4 C, CO of Phth, some overlapped), 169.29, 169.91, 170.01, 170.65 (5 C, CH_3CO, some overlapped); MALDITOF-MS Calcd for $C_{112}H_{118}N_2O_{37}Si$: 2110 [M]. Found: $[M + Na]^+$ 2133.98; $[M + K]^+$ 2148.96. Anal. Calcd for $C_{112}H_{118}N_2O_{37}Si$: C, 63.70; H, 5.63. Found: C, 64.02; H, 5.57.

Acknowledgements

This work was supported by NNSF of China (Projects 29972053 and 39970179) and RCEES of CAS.

References

[1] Strecker, G.; Fievre, S.; Wieruszeski, J. M.; Michalski, J. C.; Montreuil, J. *Carbohydr. Res.* **1992**, *226*, 1–14 and the references cited therein.
[2] (a) Feizi, T. *Nature* **1985**, *314*, 53–57;
(b) Wieruszeski, J. M.; Chekkor, A.; Bouquelet, S.; Montreuil, J.; Strecker, G.; Peter-Katalinic, J.; Egge, H. *Carbohydr. Res.* **1985**, *137*, 127–138;
(c) Dua, V. K.; Goso, K.; Dube, V. E.; Bush, C. A. *J. Chromatogr.* **1985**, *328*, 259–269.
[3] Yamashita, K.; Mizuochi, T.; Kobata, A. *Methods Enzymol.* **1982**, *83*, 105–126.
[4] Saksena, R.; Deepak, D.; Khare, A.; Sahai, R.; Tripathi, L. M.; Srivastava, V. M. L. *Biochem. Biophys. Acta* **1999**, *1428*, 433–445.
[5] Catelani, G.; Colonna, F.; Marra, A. *Carbohydr. Res.* **1988**, *182*, 297–300.
[6] Garegg, P. J.; Hultberg, J.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97–102.
[7] Du, Y.; Zhang, M.; Yang, F.; Gu, G. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3122–3127.
[8] Aguilera, B.; Fernandez-Mayoralas, A. *J. Org. Chem.* **1998**, *63*, 2719–2723.
[9] Du, Y.; Yang, F. *Chinese Patent Appl.* 01136484.x, **2001**.
[10] Sato, S.; Ito, Y.; Nukada, T.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *167*, 197–210.