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Synthesis of the tetrasaccharide related to the repeating unit of the antigen from *Shigella dysenteriae* type 5☆

Indrani Mukherjee, Saibal Kumar Das, Ali Mukherjee, Nirmolendu Roy *

Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Calcutta 700 032, India

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Abstract

Starting from L-rhamnose, D-mannose and 2-amino-2-deoxy-D-glucose hydrochloride, two disaccharide blocks, namely, ethyl 2,4-di-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside and 2-(trimethylsilyl)ethyl 2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside, were synthesised and then allowed to react in the presence of *N*-iodosuccinimide and trifluoromethane sulfonic acid to give a tetrasaccharide derivative. This compound was converted into 2-(trimethylsilyl)ethyl 2,4-di-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-L-rhamno-pyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside, which on hydrogenolysis, afforded the methyl ester 2-(trimethylsilyl)ethyl glycoside of the tetrasaccharide related to the repeating unit of the O-antigen from *Shigella dysenteriae* type 5.

Keywords: Synthesis; Tetrasaccharide repeating unit; *Shigella dysenteriae* type 5

1. Introduction

Shigella dysenteriae is the most virulent among the pathogenic bacilli of the genus *Shigella* [1]. Enterobacteria from the *Shigella* family are responsible for intestinal diseases including dysentery, and have the potential for causing catastrophic public health problems in developing countries [2]. They are very resistant to antimicrobial drugs. This resistance, therefore, necessitates the exploration of other medical approaches for control of diseases caused by this pathogen [3]. It has been suggested [4,5] that circulating antibodies to the O-specific polysaccharide of *Shigella* may protect the host against shigellosis, and that conjugate vaccines consisting of the O-specific polysaccharide (O-SP) of *Shigella* covalently attached to an immunogenic protein could, indeed, confer protective immunity to humans against shigellosis. Much work on the synthesis of oligosaccharides related to *Shigella flexnery* variant Y [6] and *Shigella dysenteriae* types 1 [7] and 2 [8] has been reported. Pozsgay [9] prepared a glycogonjugate vaccine against *Shigella dysenteriae* type 1 that claimed to have better antigenicity than the native O-SP. It is, therefore, probable that the O-SP from *Shigella dysenteriae* type 5 may also play a protective role against shigellosis and bacillary dysentery in human. We report herein the total synthesis of tetrasaccharide **II** related to the repeating unit (**I**) of *Shigella*

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* Corresponding author. Fax: +91-33-4732805.

E-mail address: bcnr@mahendra.iacs.res.in (N. Roy)

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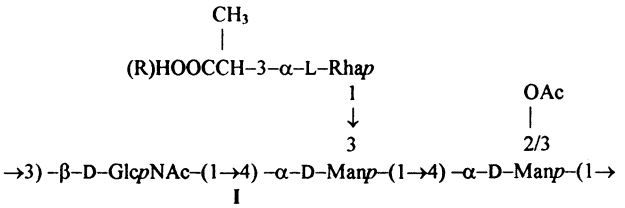
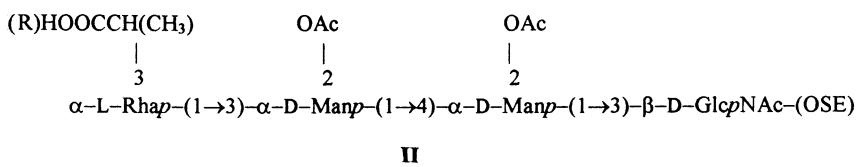
dysenteriae type 5 [10]. The synthesised oligosaccharide can also be utilised as a molecular probe for studying the immunochemical behaviour of the antigen.



I

The position of the *O*-acetyl groups on the mannose moiety in **I** was not unequivocally established and was arbitrarily assigned to either the C-2 or C-3 position. The possibility that the importance of the *R*-lactic acid substituted L-rhamnose component at the non-reducing end might play a role in the immune response is also a reason for our decision to synthesise tetrasaccharide **II** related to *Shigella dysenteriae* type 5.

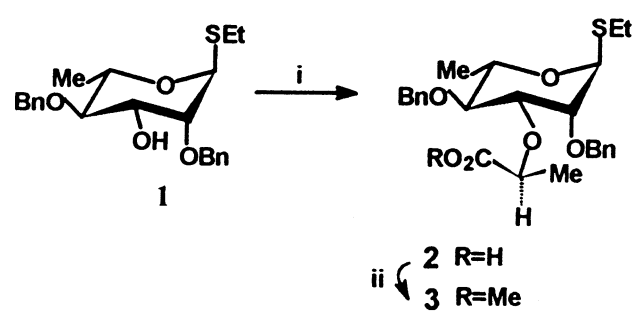


II

2. Results and discussion

The known ethyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside [11] (**1**), prepared from L-rhamnose, was allowed to react with (*S*)-2-bromopropionic acid and sodium hydride to give **2**, which was esterified with diazomethane [12] to afford the donor ethyl 2,4-di-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-1-thio-α-L-rhamnopyranoside (**3**) (Scheme 1). The structure of **3** was confirmed by its ^{1}H NMR spectrum, which showed a broad singlet at δ 5.29 for the anomeric proton, and peaks at δ 4.10, 3.63 and 1.37 for C*H*(CH$_3$)COOCH$_3$, CH(CH$_3$)COOC*H*$_3$ and CH(C*H*$_3$)COOCH$_3$, respectively.

In another experiment, 2-(trimethylsilyl)-ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside [13] (**4**) was allylated [14] with allyl bromide and sodium hydride to afford 2-(trimethylsilyl)ethyl 3-*O*-allyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**5**). Removal of the benzylidene group from **5** followed by benzylation of product **6** with benzyl bromide and sodium hydride in *N*,*N*-dimethylformamide gave 2-(trimethylsilyl)ethyl 3-*O*-allyl-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**7**). Removal of the allyl group from **7** with palladium chloride [15] in methanol afforded 2-(trimethylsilyl)ethyl 4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**8**) (Scheme 2).



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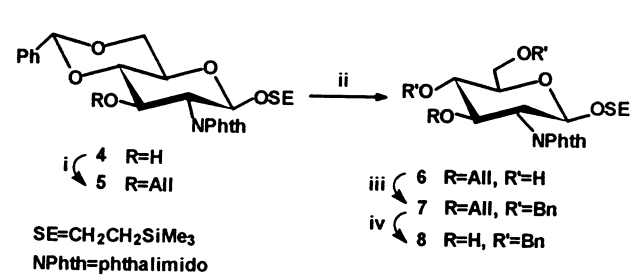
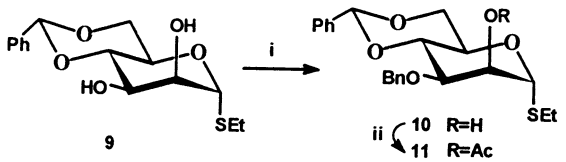
Scheme 1. (i) NaH, (*S*)-2-bromopropionic acid, dioxane; (ii) CH_2N_2, Et_2O.



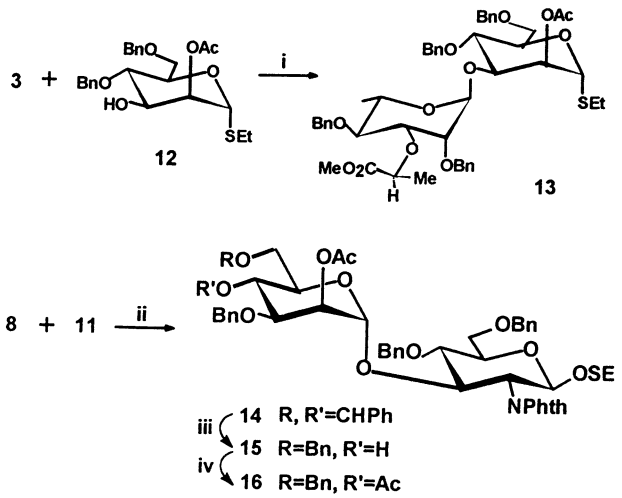
Scheme 2. (i) AllBr, DMF, NaH; (ii) 80% ACOH; (iii) BnBr, NaH, DMF; (iv) $PdCl_2$, MeOH.

Ethyl 4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside [16] (**9**) was converted into ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**10**) via its stannylene derivative [17]. Acetylation of **16** afforded ethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (**11**) (Scheme 3). Compound **17** has signals for C*H*Ph (δ 5.63), acetyl, benzyl and thioethyl groups in the ^{1}H NMR spectrum.

Donor **3** was then allowed to react with the acceptor ethyl 2-*O*-acetyl-4,6-di-*O*-benzyl-1-



Scheme 3. (i) Bu_2SnO, C_6H_6, BnBr, Bu_4NBr; (ii) Ac_2O, pyridine.



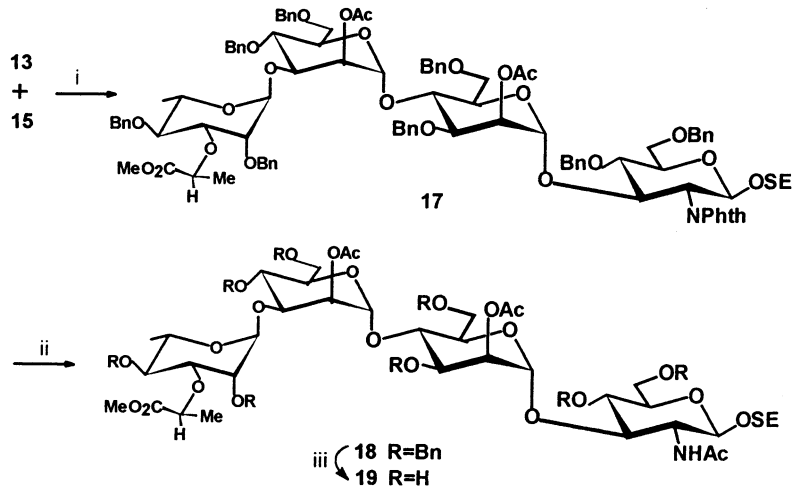
Scheme 4. (i) IDCP, CH_2Cl_2, 4 Å MS; (ii) NIS, TfOH, CH_2Cl_2; (iii) $NaBH_3CN$, HCl–ether, THF; (iv) Ac_2O, pyridine.

thio-α-D-mannopyranoside (**12**) [18] in the presence of iodonium dicollidine perchlorate (IDCP) [19] in dichloromethane to give the disaccharide ethyl 2,4-di-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (**13**) in 71% yield. The prevalence for the reaction [20,21] of the ethyl thioglycoside **6** as donor, in the presence of the ethyl thioglycoside **12** as acceptor, is obviously ascribed to the activation of **6** by the benzyl substituent at O-2 while **12** is deactivated by the acetyl substituent at O-2 (Scheme 4).

In a separate experiment, donor **11** was allowed to react with acceptor **8** in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) [21] in dichloromethane to afford the disaccharide 2-(trimethylsilyl)ethyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranosy-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**14**) in 90% yield. Treatment of **14** with sodium cyanoborohydride and hydrogen chloride in ether and tetrahydrofuran gave 2-(trimethylsilyl)ethyl 2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**15**) in 68% yield with a free OH group at its C-4^{II} position (Scheme 4). The structure of **15** was confirmed from its signals at δ 5.44 (H-2^{II}), 5.23 (H-1^{II}), 5.17 (H-1^{I}), 4.23 (H-2^{I}), 3.63 (H-4^{II}), 1.91 (CH_3CO), 0.78 (OCH_2CH_2Si) and −0.13 ($Si(CH_3)_3$) in the ^{1}H NMR spectrum and at δ 169.8 ($COCH_3$), 99.6 (C-1^{I}), 97.5 (C-1^{II}), (C-2^{I}), 20.7 ($COCH_3$), 17.7 ($CH_2Si(CH_3)_3$) and −1.5 ($Si(CH_3)_3$) in the ^{13}C NMR spectrum. The presence of an OH group at C-4^{II} of **15** was confirmed by acetylation with acetic anhydride and pyridine as described above. In the ^{1}H NMR spectrum of the resulting acetate **16**, the signal of H-4^{II} had shifted downfield to δ 5.17 compared with its position at δ 3.63 in the spectrum of **15**.

Reaction of donor **13** with acceptor **15** in the presence of NIS and TfOH in dichloromethane afforded 2-(trimethylsilyl)ethyl 2,4-di-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**17**) in 79% yield. Compound **17** was characterised by its signals at δ 5.43, 5.28 (2 bs, 2 H, H-2^{II}, H-2^{III}), 5.25 (2 bs, 2 H, H-1^{II}, H-1^{III}), 5.16 (d, 2 H, *J* 8.4 Hz, H-1^{I}, H-1^{IV}), 3.41 ($CH(CH_3)COOCH_3$), 2.06, 1.94 (2 $COCH_3$), 1.38 ($CH(CH_3)COOMe$), 1.36 (H-6^{IV}), 0.83 (OCH_2CH_2Si), −0.13 ($Si(CH_3)_3$) and at δ 173.3, 169.8, 169.7 (3 CO), 99.2 (C-1^{I}), 99.1, 97.6 (C-1^{II}, C-1^{III}), 93.4 (C-1^{IV}), 55.6 (C-2^{I}), 51.7 ($COOCH_3$), 20.9, 20.7 (2 $COCH_3$), 18.8 ($CH(CH_3)COOMe$), 18.2 (C-6^{IV}), 17.7 ($CH_2Si(CH_3)_3$) and −1.5 ($Si(CH_3)_3$) in the ^{13}C NMR spectrum.

Dephthaloylation of **17** with ethylene diamine [22] in butanol followed by N-acetylation of the product with acetic anhydride and triethylamine gave 2-(trimethylsilyl)ethyl 2,4-di-*O*-benzyl-3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→4)-2-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**18**) in



Scheme 5. (i) NIS–TfOH, CH_2Cl_2, 4 Å MS; (ii) ethylene diamine, butanol, Ac_2O, triethyl amine; (iii) 10% Pd–C, H_2, AcOH.

79% yield. The presence of the acetamido group in **18** was confirmed by its signal at δ 1.74 ($NHCOCH_3$) in the ^1H NMR spectrum and at δ 23.0 ($NHCOCH_3$) in the ^{13}C NMR spectrum. Hydrogenolysis [23] of **18** gave 2-(trimethylsilyl)ethyl 3-*O*-[(*R*)-1-(methoxycarbonyl)ethyl]-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-α-D-mannopyranosyl-(1→4)-2-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**19**) in 73% yield (Scheme 5). Compound **19** had signals at δ 5.47–5.37 (H-2^{II}, H-2^{III}, H-1^{II}, H-1^{III}), 5.15 (H-1^{IV}), 5.14 (H-1^{I}), 3.41 ($CH(CH_3)COOCH_3$), 2.03, 2.02 (2 OAc), 1.92 ($NHCOCH_3$), 0.76 (OCH_2CH_2Si) and −0.13 ($Si(CH_3)_3$) in the ^1H NMR spectrum and at δ 174.4, 174.1, 173.8, 173.8 (4 *C*O), 100.8 (C-1^{I}), 100.3, 99.9 (C-1^{II}, C-1^{III}), 97.6 (C-1^{IV}), 54.7 (C-2^{I}), 51.9 ($COOCH_3$), 23.7 ($NCOCH_3$), 21.4, 21.1 (2 $COCH_3$), 19.7 ($CH(CH_3)$COOMe), 17.9 (C-6^{IV}), 17.6 ($CH_2Si(CH_3)_3$) and −1.5 ($Si(CH_3)_3$) in the ^{13}C NMR spectrum.

3. Experimental

General.—All reactions were monitored by thin-layer chromatography (TLC) on Silica Gel G (E. Merck). Column chromatography was performed on 100–200 mesh Silica Gel (SRL, India). All solvents were distilled and/or dried before use and all evaporations were conducted below 40 °C under reduced pressure unless stated otherwise. Optical rotations were measured with a Perkin–Elmer model 241 MC polarimeter. The ^1H and ^{13}C NMR spectra were recorded on a Varian A-60 or Bruker DPX 300 spectrometer using $CDCl_3$ as solvent (internal standard TMS) unless otherwise stated. Melting points were determined on a paraffin oil bath and are uncorrected.

*Ethyl 2,4-di-*O*-benzyl-3-*O*-[(*R*)-2-(methoxycarbonyl)ethyl]-1-thio-α-L-rhamnopyranoside* (**3**).—To a solution of ethyl 2,4-di-*O*-benzyl-1-thio-α-L-rhamnopyranoside [11] (**1**, 1.6 g, 4.09 mmol) in dioxane (42 mL) was added NaH (60% oil coated, 883 mg, 22.09 mmol). The solution was then stirred for 1 h at 95 °C. The mixture was cooled to 65 °C and a solution of (*S*)-2-bromopropionic acid (2.2 mL, 24.1 mmol) in dioxane (10 mL) was added with vigorous stirring. After 1.5 h, a suspension of NaH (3.5 g) in dioxane was added and stirring was continued overnight at 65 °C. The mixture was then cooled to room temperature (rt) and MeOH (12 mL) was added to decompose the excess of NaH. The reaction mixture was then diluted with CH_2Cl_2, washed with water, dried (Na_2SO_4) and concentrated to a syrup. Column chromatography with 0.01% AcOH in 3:1 toluene–EtOAc gave **2** (1.2 g, 64%). This acid (**2**) was dissolved in ether (10 mL) and an ethereal diazomethane solution (20 mL) was added dropwise. The reaction was completed within 1 min and the excess of CH_2N_2 was decomposed by adding dilute AcOH. The solvents were evaporated off and traces of acid and water were removed by co-evaporation with toluene. Chromatography

with 3:1 toluene–EtOAc gave pure **3** (1.2 g, 97%); $[\alpha]_D^{25}$ −77.23° (*c* 0.3, $CHCl_3$). ^1H NMR: δ 7.43–7.19 (m, 10 H, aromatic protons), 5.29 (bs, 1 H, H-1), 5.04, 4.59 (2 d, 2 H, *J* 10.8, CH_2Ph), 4.75, 4.67 (2 d, 2 H, *J* 12.3 Hz, CH_2Ph), 4.10 (q, 1 H, *J* 6.6 Hz, CH(CH_3)-$COOCH_3$), 4.00 (m, 1 H, H-5), 3.84 (bs, 1 H, H-2), 3.76 (m, 1 H, H-3), 3.63 (s, 3 H, COOCH_3), 3.58 (t, 1 H, *J* 9.0 Hz, H-4), 2.58 (m, 2 H, SC$H_2$$CH_3$), 1.37 (d, 3 H, *J* 6.6 Hz, CH(CH_3)COOMe), 1.31 (d, 3 H, *J* 6.3 Hz, H-6), 1.24 (t, 3 H, *J* 8.0 Hz, SCH_2CH_3). Anal. Calcd for $C_{26}H_{34}O_6S$: C, 65.79; H, 7.22. Found: C, 65.68; H, 7.36.

*2-(Trimethylsilyl)ethyl 3-*O*-allyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**5**).—To a solution of 2-(trimethylsilyl)ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside [13] (**4**, 4.6 g, 9.2 mmol) in DMF (37 mL) was added NaH (60% oil coated, 552 mg, 13.8 mmol) at 0 °C with stirring. After 30 min, allyl bromide (1.1 mL, 12.9 mmol) was added and stirring was continued at rt for 7 h. Excess NaH was decomposed by the addition of MeOH (2.3 mL). The reaction mixture was then diluted with CH_2Cl_2. The organic layer was washed with water, dried (Na_2SO_4) and concentrated. Column chromatography of the syrupy product with 3:1 toluene–Et_2O gave **5** (1.44 g, 29%); $[\alpha]_D^{25}$ −10.5° (*c* 1.2, $CHCl_3$). ^1H NMR: δ 8.05–7.23 (m, 9 H, aromatic protons), 5.56 (s, 1 H, C*H*Ph), 5.38 (m, 1 H, CH_2=C*H*–CH_2), 5.28 (d, 1 H, *J* 8.4 Hz, H-1), 5.14 (m, 2 H, CH_2=CH–CH_2), 4.70 (t, 1 H, *J* 9.6 Hz, H-3), 4.46 (dd, 1 H, $J_{3,4}$ 10.5, $J_{4,5}$ 4.5 Hz, H-4), 4.30 (dd, 1 H, $J_{1,2}$ 9.0, $J_{2,3}$ 10.5 Hz, H-2), 4.11 (m, 2 H, CH_2=CH–CH_2), 3.90 (m, 2 H, OC$H_2$$CH_2$Si), 3.80 (m, 1 H, H_{eq}-6), 3.65 (m, 1 H, H_{ax}-6), 3.50 (m, 1 H, H-5), 0.80 (m, 2 H, OCH_2CH_2Si), −0.13 (s, 9 H, Si(CH_3)$_3$). Anal. Calcd for $C_{29}H_{35}O_7NSi$: C, 64.78; H, 6.56. Found: C, 64.64; H, 6.77.

*2-(Trimethylsilyl)ethyl 3-*O*-allyl-4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**7**).—Compound **5** (1.4 g, 2.6 mmol) was dissolved in AcOH (23.4 mL) and water (5.9 mL) and stirred at 80 °C for 2 h. The mixture was concentrated to dryness and co-evaporated with toluene to remove traces of acid and water to give **6** (1.2 g, 95%). Compound **6** (1.2 g, 2.7 mmol) was benzylated according to the conventional method [24]. Column chromatography of the product with 4:1 toluene–Et_2O gave pure **7** (0.90 g, 55%); $[\alpha]_D^{25}$ +10.67° (*c* 1.0, $CHCl_3$). ^1H NMR: δ 8.02–7.23 (m, 14 H, aromatic protons), 5.28 (d, 1 H, *J* 8.4 Hz, H-1), 5.11 (m, 2 H, CH_2=CH–CH_2), 4.70 (m, 4 H, 2 CH_2Ph), 4.50 (t, 1 H, *J* 9.6 Hz, H-3), 4.45 (dd, 1 H, $J_{3,4}$ 10.5, $J_{4,5}$ 4.5 Hz, H-4), 4.20 (dd, 1 H, $J_{1,2}$ 9.0, $J_{2,3}$ 10.5 Hz, H-2), 4.17 (m, 2 H, CH_2=CH–CH_2), 4.0 (m, 2 H, OC$H_2$$CH_2$Si), 3.80 (m, 1 H, H_{eq}-6), 3.70 (m, 1 H, H_{ax}-6), 3.40 (m, 1 H, H-5), 0.80 (m, 2 H, OCH_2CH_2Si), −0.13 (s, 9 H, Si(CH_3)$_3$). Anal. Calcd for $C_{36}H_{43}O_7NSi$: C, 68.65; H, 6.88. Found: C, 68.53; H, 7.10.

*2-(Trimethylsilyl)ethyl 4,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-*D*-glucopyranoside* (**8**).—The allyl derivative **7** (0.8 g, 1.3 mmol) was dissolved in dry MeOH (10 mL) and $PdCl_2$ (114 mg, 0.6 mmol) was added. The mixture was stirred at 25 °C for 4 h, then filtered through a Celite bed and concentrated to dryness. Column chromatography with 9:1 toluene–Et_2O gave pure **8** (417 mg, 54%); $[\alpha]_D^{25}$ −6.85° (*c* 2.0, $CHCl_3$). ^1H NMR: δ 8.02–7.20 (m, 14 H, aromatic protons), 5.22 (d, 1 H, *J* 8.4 Hz, H-1), 4.80–4.60 (m, 4 H, 2 CH_2Ph), 4.40 (t, 1 H, *J* 9.6 Hz, H-3), 4.35 (dd, 1 H, $J_{3,4}$ 10.5, $J_{4,5}$ 4.5 Hz, H-4), 4.10 (dd, 1 H, $J_{1,2}$ 9.0, $J_{2,3}$ 10.5 Hz, H-2), 3.90 (m, 2 H, OC$H_2$$CH_2$Si), 3.80 (m, 1 H, H_{eq}-6), 3.60 (m, 1 H, H_{ax}-6), 3.40 (m, 1 H, H-5), 0.80 (m, 2 H, OCH_2-CH_2Si), −0.13 (s, 9 H, Si(CH_3)$_3$). Anal. Calcd for $C_{33}H_{39}O_7NSi$: C, 67.21; H, 6.66. Found: C, 67.33; H, 6.78.

*Ethyl 3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-*D*-mannopyranoside* (**10**).—To a solution of **9** (5.32 g, 17.0 mmol) in benzene (150 mL), Bu_2SnO (5.1 g, 20.4 mmol) was added and the mixture was refluxed with azeotropic removal of water for 6 h. The reaction mixture was concentrated to dryness. Fresh benzene (60 mL), benzyl bromide (12 mL, 102.2 mmol) and Bu_4NBr (2.7 g, 8.5 mmol) were added and stirred overnight at 65 °C. The solvent was evaporated off, MeOH was added and the mixture was cooled. Solids that separated were filtered off and the filtrate was concentrated to a syrup. Column chromatography with 3:1 toluene–EtOAc gave pure **10** (5.53 g, 81%); $[\alpha]_D^{25}$ +73.64° (*c* 0.7, $CHCl_3$). ^1H NMR:

δ 7.53–7.31 (m, 10 H, aromatic protons), 5.62 (s, 1 H, C*H*Ph), 5.38 (s, 1 H, H-1), 486, 4.71 (2 d, 2 H, *J* 11.7 Hz, CH_2Ph), 4.29–4.18 (m, 3 H, H-2, H-3, H-4), 4.13 (m, 1 H, H_{eq}-6), 3.90 (m, 1 H, H_{ax}-6), 3.70 (m, 1 H, H-5), 2.61 (m, 2 H, SCH_2CH$_3$), 1.30 (t, 3 H, *J* 7.5 Hz, SCH$_2$CH_3). Anal. Calcd for $C_{22}H_{26}O_5S$: C, 65.65; H, 6.51. Found: C, 65.76; H, 6.71.

Ethyl 2-O-*acetyl-3*-O-*benzyl-4,6*-O-*benzylidene-1-thio*-α-D-*mannopyranoside* (**11**).—To a solution of **10** (5.5 g, 13.7 mmol), pyridine (30 mL) and Ac_2O (15 mL) were added. After 3 h at rt, the reaction mixture was evaporated and then co-evaporated with toluene. Column chromatography with 3:1 toluene–Et_2O gave pure **11** (6.0 g, 98.5%) as a syrup; $[\alpha]_D^{25}$ +58.69° (*c* 0.2, $CHCl_3$). ^{1}H NMR: δ 7.52–7.26 (m, 10 H, aromatic protons), 5.63 (s, 1 H, C*H*Ph), 5.45 (dd, 1 H, H-2), 5.26 (s, 1 H, H-1), 4.68 (m, 2 H, CH_2Ph), 4.22 (m, 2 H, H-3, H-5), 4.09 (t, 1 H, H-4), 3.96 (dd, 1 H, H_{eq}-6), 3.87 (t, 1 H, H_{ax}-6), 2.63 (m, 2 H, SCH_2CH$_3$), 2.17 (s, 3 H, COCH_3), 1.29 (t, 3 H, *J* 7.5 Hz, SCH$_2$CH_3). Anal. Calcd for $C_{24}H_{28}O_6S$: C, 64.84; H, 6.35. Found: C, 64.96; H, 6.51.

Ethyl 2,4-di-O-*benzyl-3*-O-*[(*R*)-1-(methoxycarbonyl)ethyl]*-α-L-*rhamnopyranosyl-(1→3)-2*-O-*acetyl-4,6-di*-O-*benzyl-1-thio*-α-D-*mannopyranoside* (**13**).—A solution of **3** (360 mg, 0.8 mmol) and ethyl 2-*O*-acetyl-4,6-di-*O*-benzyl-1-thio-α-D-mannopyranoside (**12**) [18] (395 mg, 0.9 mmol) in CH_2Cl_2 (15 mL) containing MS 4 Å (1 g) was stirred at rt for 12 h under N_2. Freshly prepared IDCP (750 mg, 1.6 mmol) was added at a time to the solution at −10 °C. The mixture was stirred for 2 h at 24 °C. The mixture was then filtered and the filtrate was washed with 10% aq $Na_2S_2O_3$, sat aq $NaHCO_3$ and water in succession, dried (Na_2SO_4) and concentrated to a syrup. Column chromatography with 8:1 toluene–Et_2O gave pure **13** (550 mg, 71%); $[\alpha]_D^{25}$ −38.97° (*c* 1.5, $CHCl_3$). ^{1}H NMR: δ 7.48–7.12 (m, 20 H, aromatic protons), 5.44 (bs, 1 H, H-2^I), 5.30 (bs, 1 H, H-1^{II}), 5.12 (bs, 1 H, H-1^I), 5.09–4.34 (m, 9 H, 4 CH_2Ph, H-2^{II}), 4.12 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.6 Hz, H-3^{II}), 3.97 (q, 1 H, *J* 6.6 Hz, C*H*(CH$_3$)COOMe), 3.89 (dd, 1 H, H-4^I), 3.86 (dd, 1 H, H-3^I), 3.84 (dd, 1 H, H-4$^{II'}$), 3.61 (m, 4 H, H-5^I, H-5^{II}, H-6^I), 3.37 (s, 3 H, COOCH_3), 2.61 (m, 2 H, SCH_2CH$_3$), 2.16 (s, 3 H, COCH_3), 1.33 (d, 3 H, *J* 6.9 Hz, CH(CH_3)COOMe), 1.26 (t, 3 H, *J* 6.3 Hz, SCH$_2$CH_3), 1.25 (d, 3 H, *J* 6.3 Hz, H-6^{II}). ^{13}C NMR: δ 173.3, 170.1 (2 carbonyl carbons), 139.3–127.2 (aromatic carbons), 93.0 (C-1^{II}), 82.3 (C-1^I), 79.7, 78.6, 75.8, 75.0, 73.9, 73.5, 73.4, 72.9, 72.6, 71.9, 71.7, 69.1, 68.6, 68.1, 51.7 (CH(CH$_3$)COO*C*H$_3$), 25.3 (CH(*C*H$_3$)COOMe), 21.1 (CO*C*H$_3$), 18.8 (S*C*H$_2$CH$_3$), 18.0 (C-6^{II}), 14.8 (SCH$_2$*C*H$_3$). Anal. Calcd for $C_{48}H_{58}O_{12}S$: C, 67.11; H, 6.81. Found: C, 66.96; H, 7.00.

2-(Trimethylsilyl)ethyl 2-O-*acetyl-3*-O-*benzyl-4,6*-O-*benzylidene*-α-D-*mannopyranosyl-(1→3)-4,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**14**).—A solution of donor **11** (225.5 mg, 0.5 mmol) and acceptor **8** (250 mg, 0.4 mmol) in CH_2Cl_2 (2 mL) containing 4 Å MS (200 mg) was stirred at rt for 14 h under N_2. NIS (158 mg, 0.7 mmol) and TfOH (6.2 μL, 0.07 mmol) were then added at −20 °C. After 4 h, the reaction mixture was diluted with CH_2Cl_2, filtered through a Celite bed, washed with 5% $Na_2S_2O_3$, sat aq $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography with 15:1 toluene–Et_2O gave pure **14** (350 mg, 90%) as a white foam; $[\alpha]_D^{25}$ +13.5° (*c* 0.3, $CHCl_3$). ^{1}H NMR: δ 7.83–7.17 (m, 24 H, aromatic protons), 5.55 (s, 1 H, C*H*C$_6$H$_5$), 5.38 (bs, 1 H, H-2^{II}), 5.27 (bs, 1 H, H-1^{II}), 5.18 (d, 1 H, *J* 8.7 Hz, H-1^I), 3.30 (m, 2 H, OCH_2CH$_2$Si), 1.95 (s, 3 H, COCH_3), 0.78 (m, 2 H, OCH$_2$CH_2Si), −0.13 (s, 9 H, Si(CH_3)$_3$). ^{13}C NMR data ($CDCl_3$): δ 169.7 (*C*OCH$_3$), 138.8–126.2 (aromatic carbons), 101.1 (Ph*C*H), 99.8 (C-1^I), 97.7 (C-I^{II}), 79.8, 77.5, 76.8, 74.9, 74.3, 73.8, 73.5, 72.2, 69.5, 68.1, 66.9, 64.7, 55.4 (C-2^I), 20.8 (CO*C*H$_3$), 17.8 (*C*H$_2$SiMe$_3$), −1.5 (Si(*C*H$_3$)$_3$). Anal. Calcd for $C_{55}H_{61}O_{13}NSi$: C, 67.95; H, 6.32. Found: C, 67.82; H, 6.50.

2-(Trimethylsilyl)ethyl 2-O-*acetyl-3,6-di*-O-*benzyl*-α-D-*mannopyranosyl-(1→3)-4,6-di*-O-*benzyl-2-deoxy-2-phthalimido*-β-D-*glucopyranoside* (**15**).—To a solution of **14** (54.9 mg, 0.06 mmol) and $NaBH_3CN$ (32 mg, 0.5 mmol) in THF (9 mL) containing MS 3 Å (100 mg) was added saturated HCl gas in ether at 0 °C until the solution was acidic or the evolution

of H_2 ceased. After 5 min, TLC indicated complete reaction. The mixture was diluted with CH_2Cl_2, washed in succession with water, sat aq $NaHCO_3$, water, then dried (Na_2SO_4) and concentrated. Column chromatography with 3:1 toluene–Et_2O gave **15** (40 mg, 68%) as white foam; $[\alpha]_D^{25}$ +19.5° (*c* 0.3, $CHCl_3$). ^{1}H NMR: δ 7.76–7.16 (m, 24 H, aromatic protons), 5.44 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.0 Hz, H-2^{II}), 5.23 (d, 1 H, *J* 1.2 Hz, H-1^{II}), 5.17 (d, *J* 8.4 Hz, H-1^{I}), 4.77–4.16 (m, 8 H, 4 CH_2Ph), 4.23 (dd, 1 H, $J_{1,2}$ 10.5, $J_{2,3}$ 8.4 Hz, H-2^{I}), 3.94 (m, 1 H, H-3^{I}), 3.93 (m, 2 H, OCH_2CH$_2$Si), 3.84 (dd, 1 H, H-4^{I}), 3.78 (m, 3 H, H$_b$-6^{I}, H-6′^{II}), 3.63 (m, 2 H, H$_a$-6^{I}, H-4^{II}), 3.49 (m, 2 H, H-3^{II}, H-5^{II}), 3.20 (m, 1 H, H-5^{I}), 1.91 (s, 3 H, CH_3CO), 0.78 (m, 2 H, OCH$_2$CH_2Si), −0.13 (s, 9 H, Si(CH_3)$_3$). ^{13}C NMR: δ 169.8 (*C*OCH$_3$), 137.9–127.1 (aromatic carbons), 99.6 (C-1^{I}), 97.5 (C-1^{II}), 79.6, 76.5, 73.4, 73.3, 71.9, 71.7, 67.9, 66.4, 55.5 (C-2^{I}), 20.7 (CO*C*H$_3$), 17.7 (*C*H$_2$Si(CH$_3$)$_3$), −1.5 (Si-(*C*H$_3$)$_3$). Anal. Calcd for $C_{55}H_{63}O_{13}NSi$: C, 67.81; H, 6.52. Found: C, 67.94; H, 6.65. Compound **15** (10 mg) was acetylated in the usual way with acetic anhydride and pyridine to give the acetate **16** (9 mg), ^{1}H NMR: δ 7.73–7.19 (m, 24 H, aromatic protons), 5.39 (t, 1 H, *J* 2.7 Hz, H-2^{II}), 5.24 (d, 1 H, *J* 1.5 Hz, H-1^{II}), 5.18 (d, 1 H, *J* 8.1 Hz, H-1^{I}), 5.17 (t, 1 H, *J* 9.3 Hz, H-4^{II}), 4.76–4.26 (m, 8 H, 4 CH_2PH), 4.22 (dd, 1 H, $J_{1,2}$ 10.8, $J_{2,3}$ 8.4 Hz, H-2^{I}), 3.92 (m, 2 H, OCH_2CH$_2$Si), 3.78 (m, 2 H, H-6^{II}), 3.67 (m, 2 H, H-6^{I}), 3.47 (m, 1 H, H-5^{II}), 3.24 (m, 1 H, H-5^{I}), 1.94, 1.65 (2 S, 6 H, 2 COCH_3), 0.78 (m, 2 H, OCH$_2$CH_2Si), −0.14 (s, 9 H, Si(CH_3)$_3$).

2-(Trimethylsilyl)ethyl 2,4-di-O-*benzyl-3*-O-*[*(R)*-1-(methoxycarbonyl)ethyl]-α*-L-*rhamno-pyranosyl-(1→3)-2*-O-*acetyl-4,6-di*-O-*benzyl-α*-D-*mannopyranosyl-(1→4)-2*-O-*acetyl-3,6-di*-O-*benzyl-α*-D-*mannopyranosyl-(1→3)-4,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**17**).—A solution of donor **13** (80 mg, 0.09 mmol) and acceptor **15** (65.0 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) containing 4 Å MS (200 mg) was stirred for 2 h under N_2. The temperature was then lowered to −20 °C. *N*-Iodosuccinimide (28 mg, 0.12 mmol) and TfOH (3.5 μL, 0.04 mmol) were then added. After 2 h, the reaction was completed and then the reaction mixture was diluted with CH_2Cl_2, filtered through Celite and washed with 5% aq $Na_2S_2O_3$, sat aq $NaHCO_3$ and water, respectively, dried (Na_2SO_4) and concentrated. Column chromatography with 10:1 toluene–ether gave pure **17** (98 mg, 79%) as a syrup; $[\alpha]_D^{25}$ +31.4° (*c* 0.07, $CHCl_3$). ^{1}H NMR: δ 7.56–7.01 (m, 44 H, aromatic protons), 5.43, 5.28 (2 bs, 2 H, H-2^{II}, H-2^{III}), 5.25 (2 bs, 2 H, H-1^{II}, H-1^{III}), 5.16 (d, 2 H, *J* 8.4 Hz, H-1^{I}, H-1^{IV}), 3.91 (m, 2 H, OCH_2CH$_2$Si), 3.41 (s, 3 H, CH(CH$_3$)COOCH_3), 2.06, 1.94 (2 s, 6 H, 2 COCH_3), 1.38 (d, 3 H, *J* 6 Hz, CH(CH_3)COOMe), 1.36 (d, 3 H, *J* 6.6 Hz, H-6^{IV}), 0.83 (m, 2 H, OCH$_2$CH_2Si), −0.13 (s, 9 H, Si(CH_3)$_3$). ^{13}C NMR: δ 173.3, 169.8, 169.7 (3 carbonyl carbons), 138.4–127.2 (aromatic carbons), 99.2 (C-1^{I}), 99.1, 97.6 (C-1^{II}, C-1^{III}), 93.4 (C-1^{IV}), 79.8, 79.7, 78.7, 78.0, 75.6, 75.1, 74.9, 74.5, 73.9, 73.5, 73.3, 72.8, 72.6, 72.3, 72.0, 71.9, 71.6, 71.1, 68.8, 66.1, 55.6 (C-2^{I}), 51.7 (COO*C*H$_3$), 20.9, 20.7 (2 CO*C*H$_3$), 18.8 (CH(*C*H$_3$)COOMe), 18.2 (C-6^{IV}), 17.7 (*C*H$_2$Si(CH$_3$)$_3$), −1.5 (Si(*C*H$_3$)$_3$). Anal. Calcd for $C_{101}H_{115}O_{25}NSi$: C, 68.49; H, 6.54. Found: C, 68.60, H, 6.74.

2-(Trimethylsilyl)ethyl 2,4-di-O-*benzyl-3*-O-*[*(R)*-1-(methoxycarbonyl)ethyl]-α*-L-*rhamno-pyranosyl-(1→3)-2*-O-*acetyl-4,6-di*-O-*benzyl-α*-D-*mannopyranosyl-(1→4)-2*-O-*acetyl-3,6-di*-O-*benzyl-α*-D-*mannopyranosyl-(1→3)-2-acetamido-4,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**18**).—Compound **17** (80 mg, 0.04 mmol) in butanol (10 mL) was added to ethylenediamine (1.2 mL) under Ar. The solution was stirred for 20 h at 90 °C. The solvents were then removed under reduced pressure by evaporation twice with toluene and once with ethanol to give a yellow syrup. The mixture was then treated with Ac_2O (1.3 mL) and Et_3N (125 μL). After stirring for 14 h at rt, EtOH (25 mL) and water (1.3 mL) were added, and the solution was concentrated to dryness. The residue was purified by column chromatography using 1:1 toluene–EtOAc to give **18** (60 mg, 79%); $[\alpha]_D^{25}$ +21.94° (*c* 2.4, $CHCl_3$). ^{1}H NMR: δ 7.23–7.01 (m, 40 H, aromatic protons), 6.12, 5.32 (2 bs, 2 H, H-2^{II}, H-2^{III}), 5.19, 5.11 (2 bs, 2 H, H-1^{II}, H-1^{III}), 5.17 (d, 1 H, *J* 10.2, H-1^{I}), 4.99 (bs, 1 H, H-1^{IV}), 3.91 (m, 2 H, OCH_2CH$_2$Si), 3.52 (s, 3

H, $CH(CH_3)COOCH_3$), 1.94, 1.90, 1.74 (3 s, 9 H, 2 OAc, 1 $NHCOCH_3$), 0.83 (m, 2 H, OCH_2CH_2Si), −0.13 (s, 9 H, $Si(CH_3)_3$). ^{13}C NMR data ($CDCl_3$): δ 175.6, 170.5, 169.8, 169.6 (4 carbonyl carbons), 137.0–123.3 (aromatic carbons), 99.9 (C-1^{I}), 99.3, 98.6 (C-1^{II}, C-1^{III}), 93.1 (C-1^{IV}), 80.4, 78.3, 76.0, 75.4, 74.5, 73.8, 73.5, 73.3, 72.8, 72.2, 72.1, 71.3, 68.1, 66.7, 56.8 (C-2^{I}), 51.7 ($COOCH_3$), 23.0 ($NCOCH_3$), 21.0, 20.6 (2 $COCH_3$), 19.7 ($CH(CH_3)COOMe$], 18.1 (C-6^{IV}), 17.9 (CH_2-$Si(CH_3)_3$], −1.5 ($Si(CH_3)_3$) Anal. Calcd for $C_{95}H_{115}O_{24}NSi$: C, 67.79; H, 6.89. Found: C, 67.66; H, 7.09.

*2-(Trimethylsilyl)ethyl 3-*O*-[(*R*)-1-methoxycarbonyl]ethyl-α-*L*-rhamnopyranosyl-(1→3)-2-*O*-acetyl-α-*D*-mannopyranosyl-(1→4)-2-*O*-acetyl-α-*D*-mannopyranosyl-(1→3)-2-acetamido-2-deoxy-β-*D*-glucopyranoside* (**19**).—Compound **18** (50 mg, 0.03 mmol) was dissolved in AcOH (4 mL) and stirred under H_2 for 2 days in the presence of 10% Pd–C (95 mg). The reaction mixture was then filtered through a Celite bed and concentrated. Column chromatography with 10:5:1 $CHCl_3$–MeOH–H_2O gave **19** as a glassy syrup (21 mg, 73%); $[\alpha]_D^{25}$ −30.57° (*c* 0.4, H_2O). ^1H NMR: δ 5.47–5.37 (m, 4 H, H-2^{II}, H-2^{III}, H-1^{II}, H-1^{III}), 5.15 (bs, 1 H, H-1^{IV}), 5.14 (d, 1 H, *J* 8.7 Hz, H-1^{I}), 3.41 (s, 3 H, $CH(CH_3)$-$COOCH_3$), 3.90 (m, 2 H, OCH_2CH_2Si), 2.03, 2.02, 1.92 (3 s, 9 H, 2 OAc, 1 $NHCOCH_3$), 0.76 (m, 2 H, OCH_2CH_2Si), −0.13 (s, 9 H, $Si(CH_3)_3$). ^{13}C NMR: δ 174.4, 174.1, 173.8, 173.8 (4 carbonyl carbons), 100.8 (C-1^{I}), 100.3, 99.9 (C-1^{II}, C-1^{III}), 97.6 (C-1^{IV}), 78.9, 76.5, 74.8, 74.1, 72.5, 71.8, 71.7, 69.8, 69.1, 68.1, 65.5, 61.4, 54.7 (C-2^{I}), 51.9 ($COOCH_3$), 23.7 ($NCOCH_3$), 21.4, 21.1 (2 $COCH_3$), 19.7 ($CH(CH_3)COOMe$), 17.9 (C-6^{IV}), 17.6 (CH_2-$Si(CH_3)_3$), −1.5 ($Si(CH_3)_3$). Anal. Calcd for $C_{39}H_{67}O_{24}NSi$: C, 48.69; H, 7.02. Found: C, 48.57; H, 7.19.

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