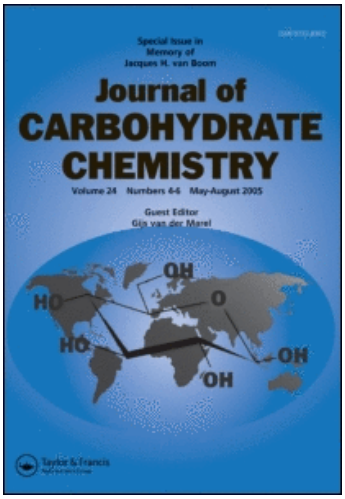

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Chemical Synthesis of (4,6-Pyr)-Gal β1→4GlcNAcβ1→3Fucβ1→OMe: A Pyruvated Trisaccharide Related to the Cell Aggregation of the Sponge *Microciona Prolifera*

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To cite this Article Deng, Shaojiang , Yu, Biao , Guo, Zhongwu and Hui, Yongzheng(1998) 'Chemical Synthesis of (4,6-Pyr)-Gal β1→4GlcNAcβ1→3Fucβ1→OMe: A Pyruvated Trisaccharide Related to the Cell Aggregation of the Sponge *Microciona Prolifera*', Journal of Carbohydrate Chemistry, 17: 3, 439 — 452
To link to this Article: DOI: 10.1080/07328309808002904
URL: http://dx.doi.org/10.1080/07328309808002904

J. CARBOHYDRATE CHEMISTRY, 17(3), 439-452 (1998)

CHEMICAL SYNTHESIS OF (4,6-Pyr)-Gal β1→4GlcNAcβ1→3Fucβ1→OMe: A PYRUVATED TRISACCHARIDE RELATED TO THE CELL AGGREGATION OF THE SPONGE *MICROCIONA PROLIFERA*

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Received July 20, 1997 - Final Form December 31, 1997

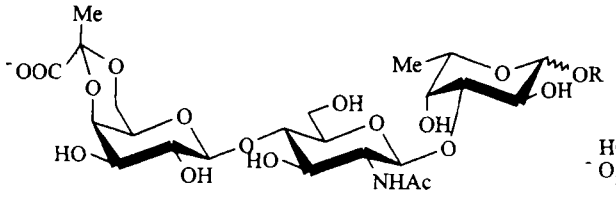
ABSTRACT

4,6-*O*-[(*R*)-1-carboxylethylidene]Galβ1→4GlcNAcβ1→3Fucβ1→OMe, a pyruvated trisaccharide unit involved in the aggregation factor of the marine sponge *Microciona prolifera*, was synthesized stereospecifically and unambiguously employing thioglycosides as glycosyl donors to construct glycosidic bonds.

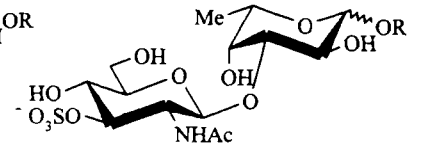
INTRODUCTION

Species-specific reaggregation of dissociated cells from the marine sponge *Microciona prolifera* has been used for a long time as a simple model to study the molecular interactions involved in tissue development and organization of higher animals.[1-4] Recent research has shown that the reaggregation of dissociated sponge cells mainly depends on a large extracellular adhesion proteoglycan, named marine cell aggregation factor (MAF).[5-6] The multiple low affinity carbohydrate-carbohydrate interactions between the MAFs and the interactions between MAF and the proteoglycan on the outer integument of the cells form the basis for cell reaggregation in the sponge system.[7-8] Two monoclonal antibodies, Block 1 and Block 2, raised against the native

Microciona aggregation factor can selectively inhibit the aggregation of the MAF.[3-4] The corresponding carbohydrate epitopes of the MAF have been characterized to be a pyruvated trisaccharide (4,6-Pyr)Galβ1→4GlcNAcβ1→3Fuc for Block 1,[3] and a sulfated disaccharide, (3-SO_3Na)GlcNAcβ1→3Fuc, for Block 2. [4] We have reported the chemical



1a R = H (reacted with Block1)
1b R = Me (β)

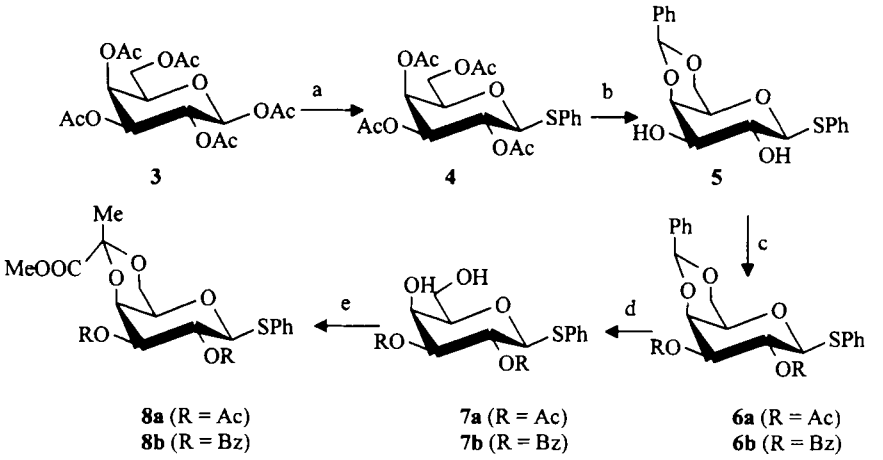


2a R = H (reacted with Block 2)
2b R = Me (β)

synthesis of the sulfated disaccharide unit (**2b**).[9] Recently, Ziegler reported a synthesis toward an aminopentyl trisaccharide of **1a**.[10] His synthesis employed lactose as a starting material to bypass the construction of the glycosidic bond between a pyruvated galactose and the 4-OH of a glucosamine unit. Herein, we wish to report a convergent synthesis of the methyl glycoside of this trisaccharide (**1b**) by using thioglycosides (**8b**, **14**) as glycosyl donors to build the glycosidic bonds.

RESULTS AND DISCUSSION

The pyruvated phenyl thiogalactosides (**8a** and **8b**) were prepared as outlined in Scheme 1. β-Phenyl thioglycoside **4**,[11] readily prepared from β-D-peracetyl galacto-pyranose (**3**), was deacetylated followed by protection of the 4,6-OH with a benzylidene group to furnish the diol **5**. Compound **5** was acetylated to give **6a**, which was treated with 80% HOAc at 80 °C to give **7a** with 4,6-OH free. Methyl pyruvate was then reacted with **7a** to bridge the 4,6-OH groups according to Ziegler's method.[12,13] However, treatment of the acetylated diol **7a** with methyl pyruvate in the presence of BF_3OEt_2 (2 equiv) in CH_3CN resulted in very complex products and the expected **8a** was isolated



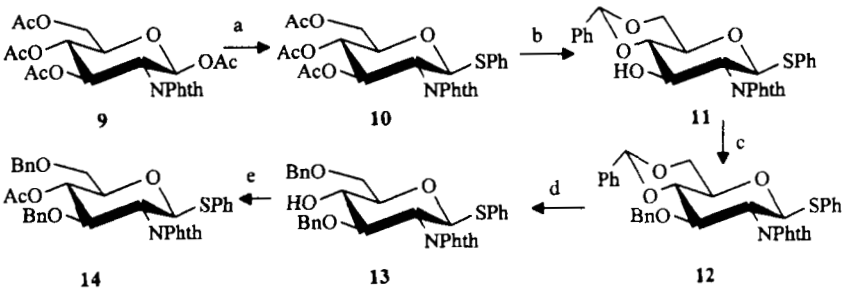
Reagents and Conditions: (a) PhSH, BF_3-OEt_2, CH_2Cl_2, rt, 86%; (b) 1) MeONa, MeOH, rt; 2) $PhCH(OMe)_2$, DMF, TsOH, 50 °C, 85%; (c) Ac_2O, Py, rt, 92% for **6a**; BzCl, Py, rt, 90% for **6b**; (d) 80% HOAc, 80 °C, 95% for **7a**; 80% for **7b**; (e) $CH_3COCOOMe$, BF_3-OEt_2, MeCN, rt, 30% for **8a**; 64% for **8b**.

Scheme 1

only in a poor yield (30%). Therefore, benzoylated diol **7b** was prepared from **5** and, as Ziegler reported, [13] converted into the desired **8b** in good yield (64%).

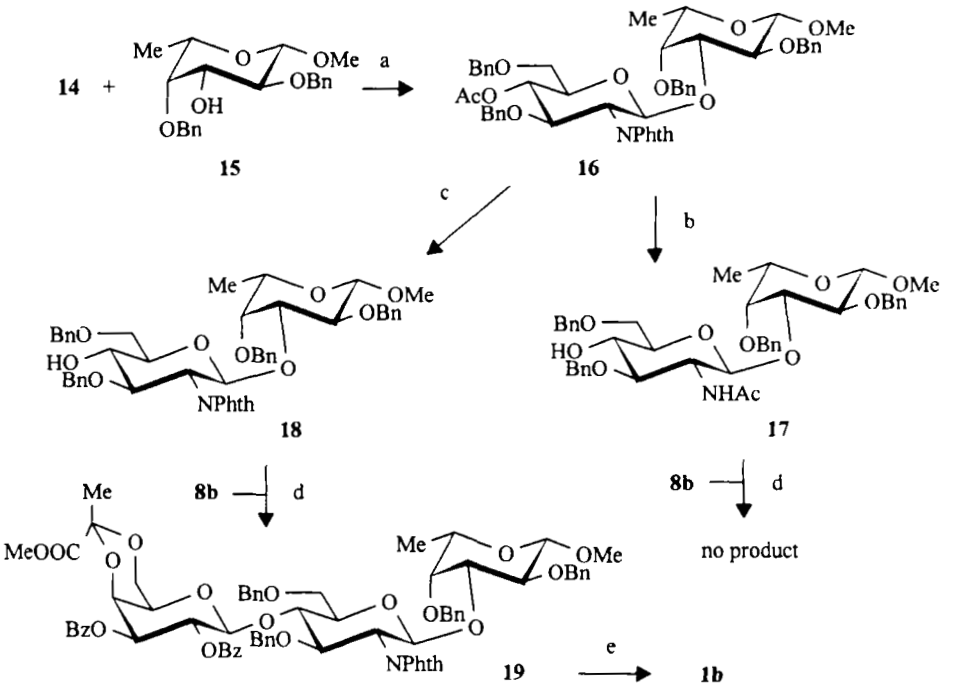
The phenyl thioglycoside **14** was prepared as shown in Scheme 2. β-Phenyl thioglycoside **10**, readily prepared from tetraacetate **9**, was deacetylated and benzylidinated to give **11**. Protection of the 3-OH of **11** with a benzyl group afforded **12**, which was converted to **13** quantitatively by regioselective ring-opening of the benzylidene group by treatment with $NaBH_3CN$ and HCl in ether.[14] Compound **13** was then acetylated to give **14**. The ^1H NMR signal for 4-H of **14** was found to be shifted downfield to 5.12 ppm compared with that for 4-H of **13** (3.95 ppm).

Scheme 3 depicts construction of the target molecule (**1b**). 2,4-Di-*O*-benzyl-β-L-fucopyranoside (**15**), readily prepared according to our previous procedure,[9] was efficiently glycosylated with phenyl thioglucoside **14** under promotion of NIS and AgOTf [15,16] to generate the disaccharide **16** in 72% yield. The newly formed glycosidic bond was proved to be β-form from the coupling constant value ($J_{1',2'}$ = 8.2 Hz). Originally, it was



Reagents and Conditions: (a) PhSH, BF_3-OEt_2, CH_2Cl_2, rt, 75%; (b) 1) MeONa, MeOH, rt; 2) $PhCH(OMe)_2$, DMF, TsOH, 50 °C, 80%; (c) BnBr, NaH, DMF, rt, 84%; (d) $NaBH_3CN$, THF, then HCl/Et_2O, rt, 100%; (e) Ac_2O, Py, rt, 100%.

Scheme 2



Reagents and Conditions: (a) NIS, AgOTf, CH_2Cl_2, 4ÅMS, -20 °C to rt, 72%; (b) 1) NH_2NH_2, 95% EtOH, reflux; 2) Ac_2O, Py, rt; 3) NaOMe, MeOH, rt, 74%; (c) NaOMe, MeOH, rt, 100%; (d) **8b**, NIS, AgOTf, CH_2Cl_2, 4ÅMS, -20 °C to rt, 71%; (e) 1) $NaBH_4$, *i*-PrOH-H_2O, rt, then AcOH (pH 5), 80 °C; 2) Ac_2O, Py, rt; 3) NaOMe, MeOH, rt; 4) NaOH, H_2O, rt; 5) 10% Pd-C, H_2, 95% EtOH, 44%.

Scheme 3

planned to convert the *N*-Phth to NHAc before introduction of the pyruvated galactose unit. Therefore, 4'-OH free disaccharide acceptor **17** was prepared by removal of the Phth group followed by *N*-acetylation and *O*-deacetylation. Unexpectedly, **17** was found to have very poor solubility in the usual solvents for glycosidation reactions, such as CH_2Cl_2, $ClCH_2CH_2Cl$, CH_3CN, THF, Et_2O, $PhCH_3$, etc.. Therefore, keeping the Phth protecting group intact, **16** was deacetylated to give **18**. Compound **18**, with 4'-OH free, was readily glycosylated with the pyruvated phenyl thiogalactoside **8b** in the presence of NIS and AgOTf [15,16] to furnish the fully protected trisaccharide **19** in 71% yield. The final protecting group manipulation was successfully performed in a straightforward manner (6 steps, 44% yield). Compound **19** was treated with $NaBH_4$ in *i*-PrOH and H_2O followed by being heated in the acidified solution (acetic acid was added till pH to 5) at 80 °C to remove the Phth group.[17,18] The crude product was then *N*-acetylated, debenzoylated, saponified, and hydrogenolyzed sequentially to yield the target **1b**. The structure of **1b** was further ascertained from 1D ^{1}H NMR, DQFCOSY, and ESIMS spectral results. The three anomeric protons of **1b** were determined to be at 4.86 ppm (H-1', $J_{1',2'}$= 8.5 Hz), 4.53 ppm (H-1'', $J_{1'',2''}$= 7.8 Hz), and 4.36 ppm (H-1, $J_{1,2}$ = 7.8 Hz).

In summary, the pyruvated trisaccharide, 4,6-*O*-[(*R*)-1-carboxyethylidene]Gal β1→4GlcNAcβ1→3Fucβ1→OMe (**1b**), which is related to the species-specific reaggregation of dissociated cells of *Microciona prolifera*, was unambiguously synthesized in a convergent and stereospecific manner.

EXPERIMENTAL

General methods. Melting points were uncorrected. Optical rotations were measured with a Perkin-Elmer Model 241 MC Polarimeter at 25 °C. TLC analyses were performed on precoated plates of Silica Gel HF_{254} (0.5 mm, Qingdao, China) and detected by 10% sulfuric acid in MeOH. Flash column chromatography was performed on Silica Gel H (400 mesh). ^{1}H NMR spectra were recorded on a Bruker AM-300 or a Bruker AM-600 spectrometer with Me_4Si as an internal standard. IR spectra were recorded with a Shimadzu IR-440 spectrometer. Mass spectra were recorded on a VG QUATTRO or a HP5989A mass instrument.

Phenyl 4,6-*O*-Benzylidene-1-thio-β-D-galactopyranoside (5). To a solution of **4**[11,19] (4.49 g, 10.2 mmol) in MeOH (100 mL) and CH_2Cl_2 (100 mL) was added a catalytic amount of NaOMe. After being stirred at rt for 2 h, the mixture was neutralized with Dowex-50WX8 (H^+ form), filtered, and concentrated *in vacuo*. The residue was dissolved in dry DMF (30 mL), then $PhCH(OMe)_2$ (3.1 mL) was added followed by camphorsulphonic acid (100 mg). After being stirred at 50 °C under diminished pressure for 2 h, the mixture was quenched with Et_3N (1mL), and extracted with EtOAc. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (CH_2Cl_2 : MeOH 100:1→ 50:1→20:1) to afford **5**[20] (3.13 g, 85%): mp 119~120 °C; $[\alpha]_D$ -37.4° (*c* 1.2 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.7-7.29 (m, 10H, $2C_6H_5$), 5.52 (s, 1H, PhCH), 4.52 (d, 1H, H-1, $J_{1,2}$ = 9.2 Hz), 4.40 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 1.1 Hz), 4.23 (m, 1H, H-4), 4.04 (dd, 1H, H-6b, $J_{5,6b}$ = 1.5 Hz), 3.71 (m, 2H, H-2, H-3), 3.57 (m, 1H, H-5); IR (cm^{-1}): 3430 (OH), 1580, 1100-950; HREIMS: Cald for $C_{19}H_{20}O_5S$: 360.1032. Found: 360.1048.

Phenyl 2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (6a). A solution of **5** (137 mg, 0.38 mmol) in dry pyridine (2 mL) and Ac_2O (2 mL) was stirred overnight. The mixture was quenched with MeOH (1 mL), diluted with EtOAc. The organic layer was washed with 5% HCl solution, saturated $NaHCO_3$ solution, and brine respectively, and then dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether:EtOAc 3:1) to afford **6a**[21] (147 mg, 92%) as a white amorphous solid: $[\alpha]_D$ +5.8° (*c* 0.33 CH_2Cl_2) [Lit[21] +12° (*c* 1.0 $CHCl_3$)]; ^1H NMR (300 MHz, $CDCl_3$) δ 7.65-7.2 (m, 10H, $2C_6H_5$), 5.47 (s, 1H, PhCH), 5.34 (t, 1H, H-2, $J_{1,2}$ = $J_{2,3}$ = 9.8 Hz), 5.00 (dd, 1H, H-3, $J_{3,4}$ = 3.5 Hz), 4.71 (d, 1H, H-1), 4.37 (d, 1H, H-4), 4.37 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.5 Hz, $J_{5,6a}$ = 1 Hz), 4.02 (dd, 1H, H-6b, $J_{5,6b}$ = 1 Hz), 3.58 (m, 1H, H-5), 2.07, 2.02 (2s, 2×3H, $2CH_3CO$); EIMS (*m/z*): 335 (M-PhS), 291, 275, 43 (base); IR (cm^{-1}): 1735, 1580, 1440, 1380, 1250, 1220, 1100, 1050.

Anal. Calcd for $C_{23}H_{24}O_7S$ (444.50): C, 62.15; H, 5.44. Found: C, 62.11; H, 5.46.

Phenyl 2,3-Di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (6b). A procedure similar to that for the preparation of **6a** was employed. Compound **5**

(4.5 g) was treated with BzCl (6.3 mL) to afford **6b** (6.4 g, 90%): mp 169-170 °C (Lit[22] 163 °C); $[\alpha]_D$ +64.2° (*c* 1.1 $CHCl_3$) [Lit[22] +61.1° (*c* 1.2 $CHCl_3$)]; ^1H NMR (300 MHz, $CDCl_3$) δ 8.0-7.2 (m, 20H, $4C_6H_5$), 5.81 (dd, 1H, H-2, $J_{1,2}$ = 9.8 Hz, $J_{2,3}$ = 9.9 Hz), 5.52 (s, 1H, PhCH), 5.36 (dd, 1H, H-3, $J_{3,4}$ = 3.4 Hz), 4.97 (d, 1H, H-1), 4.60 (bd, 1H, H-4), 4.46 (dd, 1H, H-6a, $J_{5,6a}$ = 1.3 Hz, $J_{6a,6b}$ = 12.3 Hz), 4.10 (dd, 1H, H-6b, $J_{5,6b}$ = 1.4 Hz), 3.77 (m, 1H, H-5); EIMS (*m/z*): 459 (M-PhS), 337, 105 (base).

Anal. Calcd for $C_{33}H_{28}O_7S$ (568.64): C, 69.70; H, 4.96. Found: C, 69.57; H, 4.86.

Phenyl 2,3-Di-*O*-acetyl-1-thio-β-D-galactopyranoside (7a). A solution of **6a** (703 mg, 1.60 mmol) in 80% HOAc (65 mL) was stirred for 2 h at 80 °C, then concentrated *in vacuo*. The residue was purified by flash chromatography (CH_2Cl_2 : MeOH 30 : 1) to afford **7a** (536 mg, 95%) as a colorless syrup: $[\alpha]_D$ +13.3° (*c* 0.23 CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.6-7.3 (m, 5H, C_6H_5), 5.32 (t, 1H, H-2, $J_{1,2}$ = $J_{2,3}$ = 9.9 Hz), 5.00 (dd, 1H, H-3, $J_{3,4}$ = 2.9 Hz), 4.75 (d, 1H, H-1), 4.19 (dd, 1H, H-4, $J_{4,5}$ = 0.7 Hz), 3.97 (dd, 1H, H-6a, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 5.9 Hz), 3.88 (dd, 1H, H-6b, $J_{5,6b}$ = 4.3 Hz), 3.66 (m, 1H, H-5); EIMS (*m/z*): 247 (M-PhS), 229, 187, 169, 145, 43 (base).

Phenyl 2,3-Di-*O*-benzoyl-1-thio-β-D-galactopyranoside (7b). A procedure similar to that for the preparation of **7a** was employed. Compound **6b** (1.0 g, 1.76 mmol) afforded **7b** (673 mg, 80%): mp 183-184 °C; $[\alpha]_D$ +94.5° (*c* 1.6 $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.1-7.3 (m, 15H, $3C_6H_5$), 5.80 (dd, 1H, H-2, $J_{1,2}$ = 10.0 Hz, $J_{2,3}$ = 9.9 Hz), 5.34 (dd, 1H, H-3, $J_{3,4}$ = 2.9 Hz), 4.98 (d, 1H, H-1), 4.43 (bd, 1H, H-4), 4.05 (dd, 1H, H-6a, $J_{5,6a}$ = 5.8 Hz, $J_{6a,6b}$ = 11.9 Hz), 3.94 (dd, 1H, H-6b, $J_{5,6b}$ = 3.8 Hz), 3.83 (m, 1H, H-5); EIMS (*m/z*): 371 (M-PhS), 365, 249, 105 (base).

Anal. Calcd for $C_{26}H_{24}O_7S$ (480.53): C, 64.99; H, 5.03. Found: C, 64.52; H, 4.84.

Phenyl 2,3-Di-*O*-acetyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-1-thio-β-D-galactopyranoside (8a). To a solution of **7a** (400 mg, 1.12 mmol) in dry MeCN (3 mL) was added methyl pyruvate (250 mg, 2.45 mmol) and BF_3-OEt_2 (0.28 mL, 2.19 mmol). After being stirred for 5 h at rt under N_2, the mixture was quenched with saturated $NaHCO_3$ solution, then extracted with EtOAc. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography of the residue (petroleum ether:EtOAc 8:1→6:1→4:1→2:1) afforded **8a** (146 mg, 30%) and recovered **7a** (122 mg, 30%). **8a**: mp 72-73 °C; $[\alpha]_D$ +2.1° (*c* 0.29 CH_2Cl_2); ^1H NMR

(300 MHz, $CDCl_3$) δ 7.6-7.3 (m, 5H, C_6H_5), 5.30 (t, 1H, H-2, $J_{1,2} = J_{2,3} = 9.9$ Hz), 4.85 (dd, 1H, H-3, $J_{3,4} = 3.4$ Hz), 4.64 (d, 1H, H-1), 4.32 (d, 1H, H-4), 4.12 (dd, 1H, H-6a, $J_{6a,6b} = 12.8$ Hz, $J_{5,6a} = 2.5$ Hz), 3.93 (dd, 1H, H-6b, $J_{5,6b} = 1.7$ Hz), 3.75 (s, 3H, CH_3O), 2.09, 2.07 (2s, 2×3H, $2CH_3CO$), 1.51 (s, 3H, CH_3); EIMS (*m/z*): 381 (M-Ac), 331 (M-PhS), 271, 229, 201, 43 (base).

Phenyl 2,3-Di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-1-thio-β-D-galactopyranoside (8b). A procedure similar to that for the preparation of **8a** was employed. Compound **7b** (673 mg, 1.4 mmol) was treated with methyl pyruvate (286 mg, 2.8 mmol) and BF_3-OEt_2 (0.35 mL, 2.8 mmol) to afford **8b** (505 mg, 64%): mp 81-83 °C; $[\alpha]_D$ +45.4° (*c* 0.6 $CHCl_3$) [Lit[12] +44° (*c* 1.0 $CHCl_3$)]; ^1H NMR (300 MHz, $CDCl_3$) δ 8.1-7.3 (m, 15H, $3C_6H_5$), 5.77 (dd, 1H, H-2, $J_{1,2} = 9.8$ Hz, $J_{2,3} = 10.0$ Hz), 5.22 (dd, 1H, H-3, $J_{3,4} = 3.5$ Hz), 4.92 (d, 1H, H-1), 4.57 (dd, 1H, H-4), 4.22 (dd, 1H, H-6a, $J_{6a,6b} = 12.4$ Hz, $J_{5,6a} = 1.5$ Hz), 4.04 (dd, 1H, H-6b, $J_{5,6b} = 1.7$ Hz), 3.66 (s, 3H, CH_3O), 3.45 (bs, H-5), 1.54 (s, 3H, CH_3); EIMS (*m/z*): 505, 455, 333, 105 (base).

Anal. Calcd for $C_{30}H_{28}O_9S$ (564.60): C, 63.82; H, 5.00. Found: C, 63.68; H, 4.99.

Phenyl 4,6-*O*-Benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (11). To a solution of **9**[18](9.0 g, 18.9 mmol) in dry CH_2Cl_2 (75 mL) was added PhSH (2.52 mL, 24.5 mmol) and BF_3-OEt_2 (23.2 mL, 184 mmol). After being stirred for 15 h at rt under N_2, the reaction was quenched with saturated $NaHCO_3$ solution (200 mL), then extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo.* Flash chromatography of the residue (petroleum ether:EtOAc 4:1→3:1→2:1) afforded **10** (7.5 g, 75%).

To a solution of **10** (2.76 g, 5.24 mmol) in MeOH (100 mL) and CH_2Cl_2 (100 mL) was added a catalytic amount of NaOMe. The solution was stirred for 2 h at rt, then neutralized with Dowex-50WX8 (H^+ form), filtered, and concentrated *in vacuo.* The residue was dissolved in dry MeCN (20 mL), then $PhCH(OMe)_2$ (3.1 mL) was added followed by TsOH-H_2O (100 mg). After being stirred for 15 h at rt, the mixture was quenched with Et_3N, and extracted with EtOAc. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo.* Flash chromatography of the residue (petroleum ether:EtOAc 3:1) afforded **11** (1.89 g, 80%) as a white syrup: $[\alpha]_D$ +41.2° (*c* 0.8 CH_2Cl_2) [Lit[23] +34.2° (*c* 1.3 $CHCl_3$); ^1H NMR (300

MHz, $CDCl_3$) δ 8.0-7.2 (m, 14 H, $2C_6H_5$, C_6H_4), 5.70 (d, 1H, H-1, $J_{1,2}$ = 10.4 Hz), 5.57 (s, 1H, PhCH), 4.64 (dd, 1H, H-3, $J_{2,3}$ = 9.0 Hz, $J_{3,4}$ = 10.0 Hz), 4.41 (dd, 1H, H-6a, $J_{6a,6b}$ = 10.1 Hz, $J_{5,6a}$ = 4.5 Hz), 4.34 (dd, 1H, H-6b, $J_{5,6b}$ = 9.4 Hz), 3.83 (dd, 1H, H-4, $J_{4,5}$ = 9.9 Hz), 3.72 (ddd, 1H, H-5), 3.61 (dd, 1H, H-2); EIMS (*m/z*): 380 (M-PhS), 362, 316, 256, 149, 91 (base).

Phenyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (12). To a solution of **11** (900 mg, 1.94 mmol) in dry DMF (9 mL) was added NaH (110 mg, 60% in mineral oil, 2.75 mmol). After being stirred for 30 min at 0 °C under N_2, BnBr (0.26 mL, 2.17 mmol) was added dropwise, and the reaction mixture was then stirred overnight at rt. The mixture was diluted with MeOH (2 mL), then extracted with EtOAc. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo*. Flash chromatography of the residue (petroleum ether:EtOAc 5:1→3:1) afforded **12**[23] as a colorless syrup (1.0 g, 84%) and recovered **11** (100 mg). **12**: $[\alpha]_D$ +73.2° (*c* 4.2 CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 8.0-6.8 (m, 19H, $3C_6H_5$, C_6H_4), 5.62 (s, 1H, PhCH), 5.61 (d, 1H, H-1, $J_{1,2}$ = 10.4 Hz), 4.78 and 4.49 (AB, 2H, $PhCH_2$, J = 12.3 Hz), 4.5-4.4 (m, 2H, H-3, H-6a), 4.29 (t, 1H, H-6b, $J_{6a,6b}$ = 10.1 Hz, $J_{5,6b}$ = 10.1 Hz), 3.86 (dd, 1H, H-4, $J_{3,4}$ = 9.9 Hz, $J_{4,5}$ = 9.5 Hz), 3.80 (dd,1H, H-2, $J_{2,3}$ = 8.8 Hz), 3.71 (ddd, 1H, H-5, $J_{5,6a}$ = 4.6 Hz); EIMS (*m/z*): 470 (M-PhS), 362, 256, 91 (base).

Phenyl 3,6-Di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (13). A mixture of **12** (888 mg, 1.53 mmol) and 4Å MS (1 g) in dry THF (10 mL) was stirred for 3 h, then $NaBH_3CN$ (960 mg) was added followed by addition of saturated HCl in dry Et_2O until gas evolution ceased. After 5 min the suspension was filtered and diluted with EtOAc. The organic layer was washed with brine, dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography to afford **13**[16,24] (888 mg, 100%) as a colorless syrup: $[\alpha]_D$ +63.3° (*c* 5.2 CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.9-6.8 (m, 19H, $3C_6H_5$, C_6H_4), 5.55 (d, 1H, H-1, $J_{1,2}$ = 10.2 Hz), 4.73 and 4.53 (AB, 2H, $PhCH_2$, J = 12.1Hz), 4.62 and 4.57 (AB, 2H, $PhCH_2$, J = 11.8 Hz), 4.3-4.2 (m, 2H, H-6a, H-6b), 3.9-3.8 (m, 3H, H-2, H-3, H-4), 3.70 (m, 1H, H-5); EIMS (*m/z*): 472 (M-PhS), 364, 226, 91 (base).

Phenyl 4-*O*-Acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (14). A solution of **13** (1.041 g, 1.79 mmol) in dry pyridine (2 mL) and Ac_2O (2 mL) was stirred overnight. The solution was quenched with MeOH, then diluted with EtOAc. The organic layer was washed with 5% HCl solution, saturated $NaHCO_3$ solution, and brine respectively, and then dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether:EtOAc 4:1) to afford **14**[16,24] (1.04 g, 100%) as a white syrup: $[\alpha]_D$ +96.4° (*c* 3.9 CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.9-6.8 (m, 19H, $3C_6H_5$, C_6H_4), 5.55 (d, 1H, H-1, $J_{1,2}$ = 10.4 Hz), 5.12 (dd, 1H, H-4, $J_{3,4}$ = 9.2 Hz, $J_{4,5}$ = 9.7 Hz), 4.60 and 4.31 (AB, 2H, $PhCH_2$, J = 12.0 Hz), 4.54 (s, 2H, $PhCH_2$), 4.45 (dd, 1H, H-3, $J_{2,3}$ = 10.0 Hz), 4.32 (dd, 1H, H-2), 3.81 (m, 1H, H-5), 3.68-3.60 (m, 2H, H-6a, H-6b), 1.96 (s, 3H, CH_3CO); EIMS (*m/z*): 530, 454, 91.

Anal. Calcd for $C_{36}H_{33}NO_7S$ (623.72): C, 69.33; H, 5.33. Found: C, 69.37; H, 5.28.

Methyl 2,4-Di-*O*-benzyl-3-*O*-(4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-L-fucopyranoside (16). A suspension of donor **14** (1.09 g, 1.73 mmol), acceptor **15** (517 mg, 1.44 mmol) and 4ÅMS (0.5 g) in dry CH_2Cl_2 (10 mL) was stirred for 1 h at rt under Ar, then cooled to -30 °C, NIS (520 mg, 2.31 mmol) was added followed by immediate addition of a solution of AgOTf (130 mg, 0.51 mmol) in dry PhMe (8 mL). The mixture was stirred for 15 min, diluted with saturated $Na_2S_2O_3$ solution (2mL), filtered, and then diluted with EtOAc. The organic layer was washed with saturated $Na_2S_2O_3$ solution and brine respectively, then dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether:EtOAc 6:1→5:1→3:1) to afford **16** (912 mg, 72%): mp 55-56 °C; $[\alpha]_D$ -15.5° (*c* 0.4 CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$, DQFCOSY, TOCSY) δ 7.5-6.8 (m, 24H, $4C_6H_5$, C_6H_4), 5.48 (d, 1H, H-1', $J_{1',2'}$ = 8.2 Hz), 5.16 (dd, 1H, H-4', $J_{3',4'}$ = 9.6 Hz, $J_{4',5'}$ = 9.2 Hz), 4.80 and 4.72 (AB, 2H, $PhCH_2$, J = 11.3 Hz), 4.60 and 4.31 (AB, 2H, $PhCH_2$, J= 12.1 Hz), 4.51 and 4.40 (AB, 2H, $PhCH_2$, J= 11.8 Hz), 4.43 (dd, 1H, H-3', $J_{2',3'}$ = 9.0 Hz), 4.37 (dd, 1H, H-2'), 4.36 and 4.10 (AB, 2H, $PhCH_2$, J= 11.2 Hz), 4.12 (d, 1H, H-1, $J_{1,2}$ = 7.5 Hz), 3.90 (dd, 1H, H-3, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 2.5 Hz), 3.76 (ddd, 1H, H-5', $J_{5',6a'}$ = 2.0 Hz, $J_{5',6b'}$ = 6.0 Hz), 3.595 (dd, 1H, H-2), 3.586 (dd, 1H, H-6a', $J_{6a',6b'}$ =

10.8 Hz), 3.51 (dd, 1H, H-6b'), 3.46 (s, 3H, CH_3O), 3.4-3.3 (m, 2H, H-4, H-5), 1.95 (s, 3H, CH_3CO), 1.115 (d, 3H, CH_3-6, $J_{5,6}$ = 6.4 Hz). FABMS (*m/z*): 872 (M+1), 871 (M).

Anal. Calcd for $C_{51}H_{53}NO_{12}$ (871.99): C, 70.25; H, 6.13; N, 1.61. Found: C, 70.44; H, 6.25; N, 1.68.

Methyl 2,4-Di-*O*-benzyl-3-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-β-L-fucopyranoside (17). A solution of **16** (600 mg, 0.688 mmol) and hydrazine monohydrate (1 mL) in 95% EtOH (20 mL) was refluxed for 10 h, then concentrated *in vacuo*. Traces of water and ethanol were removed by coevaporating with toluene several times. The residue was stirred overnight in dry pyridine (4 mL) and Ac_2O (4 mL), then the solvent was removed by coevaporating with toluene several times. The resulting crude product was dissolved in MeOH (10 mL) and CH_2Cl_2 (10 mL), then a catalytic amount of NaOMe was added. The solution was stirred overnight, neutralized with Dowex-50WX8 (H^+ form), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether:EtOAc 1.5:1) to give **17** (377 mg, 74%): mp 162-164 °C; $[\alpha]_D$ -106.6° (*c* 0.63 CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.5-7.1 (m, 20H, $4C_6H_5$), 5.43 (d, 1H, NHAc, J = 6.9 Hz), 5.28 (d, 1H, H-1', $J_{1',2'}$ = 8.0 Hz), 4.93 and 4.79 (AB, 2H, $PhCH_2$, J= 11.6 Hz), 4.85 and 4.62 (AB, 2H, $PhCH_2$, J= 11.8 Hz), 4.70 and 4.63 (AB, 2H, $PhCH_2$, J= 11.7 Hz), 4.48 (s, 2H, $PhCH_2$), 4.22 (dd, 1H, H-4', $J_{3',4'}$ = 9.0 Hz, $J_{4',5'}$ = 9.9 Hz), 4.20 (d, 1H, H-1, $J_{1,2}$ = 7.5 Hz), 3.85 (dd, 1H, H-3, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 2.9 Hz), 3.68 (dd, 1H, H-6a', $J_{5',6a'}$ = 2.5 Hz, $J_{6a',6b'}$ = 10.0 Hz), 3.65-3.64 (m, 2H, H-6b', H-2), 3.60-3.51 (m, 3H, H-2', H-3', H-4'), 3.50 (s, 3H, CH_3O), 3.46 (q, 1H, H-5, $J_{5,6}$ = 6.4 Hz), 3.12 (ddd, 1H, H-5', $J_{5',6b'}$ = 7.5 Hz), 1.49 (s, 3H, CH_3CO), 1.17 (d, 3H, CH_3-6); FABMS (*m/z*): 765 (M+1+Na), 760 (M+1+H_2O), 742 (M+1), 384.

Anal. Calcd for $C_{43}H_{51}NO_{10}$ (741.87): C, 69.62; H, 6.93; N, 1.89. Found: C, 69.36; H, 6.84; N, 1.60.

Methyl 2,4-Di-*O*-benzyl-3-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-L-fucopyranoside (18). To a solution of **16** (190 mg, 0.218 mmol) in MeOH (2 mL) and CH_2Cl_2 (2 mL) was added a catalytic amount of NaOMe. The solution was stirred for 1 h at rt, neutralized with Dowex-50WX8 (H^+ form), filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether:EtOAc 3:1) to afford **18** (180 mg, 100%): mp 62-63 °C; $[\alpha]_D$ -34.64° (*c* 0.6 $CHCl_3$);

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.6-6.7 (m, 24H, $4C_6H_5$, C_6H_4), 5.46 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.77 and 4.53 (AB, 2H, $PhCH_2$, J = 14.5 Hz), 4.75 (m, 2H, $PhCH_2$), 4.50 (s, 2H, $PhCH_2$), 4.39 and 4.05 (AB, 2H, $PhCH_2$, J = 11.2 Hz), 4.24 (m, 2H, H-2', H-3'), 4.16 (d, 1H, H-1, $J_{1,2}$ = 7.4 H), 3.86 (dd, 1H, H-4', $J_{3',4'}$ = 7.3 Hz, $J_{4',5'}$ = 8.7 Hz), 3.85 (dd, 1H, H-3, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 2.4 Hz), 3.70-3.56 (m, 4H, H-2, H-5', H-6a', H-6b'), 3.45 (s, 3H, CH_3O), 3.39 (q, 1H, H-5, $J_{5,6}$ = 6.4 Hz), 3.33 (bd, 1H, H-4), 1.10 (d, 3H, CH_3-6); FABMS (*m/z*): 852 (M+Na), 830 (M+1), 829 (M).

Anal. Calcd for $C_{49}H_{51}NO_{11}$ (829.94): C, 70.91; H, 6.19; N, 1.69. Found: C, 70.51; H, 6.10; N, 1.91.

Methyl 2,3-Di-*O*-benzoyl-4,6-*O*-[(*R*)-1-methoxycarbonylethylidene]-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzyl-β-L-fucopyranoside (19). A suspension of **8b** (150 mg, 0.266 mmol), **18** (110 mg, 0.133 mmol), and 4Å MS (0.1 g) in dry CH_2Cl_2 (3 mL) was stirred for 1 h at rt under Ar, then cooled to -20 °C. NIS (45 mg, 0.2 mmol) was added followed by immediate addition of a solution of AgOTf (17 mg, 0.066 mmol) in dry PhMe (1 mL). The reaction mixture was stirred for 30 min, quenched with saturated $Na_2S_2O_3$ solution (2 mL), filtered, and then diluted with EtOAc. The organic layer was washed with saturated $Na_2S_2O_3$ solution and brine respectively, dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (petroleum ether:EtOAc 6:1→5:1→3:1) to afford **19** (120 mg, 71%): mp 94-95 °C; ; $[\alpha]_D$ -35.7° (*c* 0.5 CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, DQFCOSY, TOCSY) δ 8.0-6.7 (m, 34H, $6C_6H_5$, C_6H_4), 5.80 (dd, 1H, H-2'', $J_{1'',2''}$ = 8.1 Hz, $J_{2'',3''}$ = 10.2 Hz), 5.33 (d, 1H, H-1', $J_{1',2'}$ = 8.0 Hz), 5.05 (dd, 1H, H-3'', $J_{3'',4''}$ = 3.6 Hz), 5.02 and 4.55 (AB, 2H, $PhCH_2$, J = 12.3 Hz), 4.86 (d, 1H, H-1''), 4.78 and 4.69 (AB, 2H, $PhCH_2$, J = 11.8 Hz), 4.59 and 4.31 (AB, 2H, $PhCH_2$, J = 12.2 Hz), 4.44 (d, 1H, H-4''), 4.39 and 4.03 (AB, 2H, $PhCH_2$, J = 11.3 Hz), 4.26 (dd, 1H, H-2'), 4.20 (m, 1H, H-5'), 4.16 (m, 1H, H-6a''), 4.12 (d, 1H, H-1, $J_{1,2}$ = 7.5 Hz), 3.95 (d, 1H, H-6b'', $J_{6a'',6b''}$ = 11.3 Hz), 3.86 (dd, 1H, H-4', $J_{3',4'} = J_{4',5'}$ = 9.1 Hz), 3.77 (dd, 1H, H-3, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 2.6 Hz), 3.66 (dd, 1H, H-3', $J_{2',3'}$ = 7.0 Hz), 3.63 (s, 3H, CH_3O), 3.57 (dd, 1H, H-2), 3.42 (s, 3H, CH_3O), 3.35-3.27 (m, 4H, H-5, H-5'', H-6a', H-6b'), 3.24 (d, 1H, H-4), 1.43 (s, 3H, CH_3), 1.05 (d, 3H,

CH_3-6, $J_{5,6}$ = 6.4 Hz); FABMS (*m/z*): 1284 (M+1), 456, 105 (base); IR (KBr, cm^{-1}): 2935, 2871, 1777, 1715, 1602, 1585, 1110, 1092, 1071.

Anal. Calcd for $C_{73}H_{73}NO_{20}$ (1284.37): C, 68.27; H, 5.73; N, 1.09. Found: C, 68.34; H, 5.96; N, 1.19.

Methyl 4,6-*O*-[(*R*)-1-Carboxyethylidene]-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-L-fucopyranoside (1b). To a solution of **19** (62 mg, 0.048 mmol) in *iso*-PrOH (5 mL) and H_2O (0.8 mL) was added $NaBH_4$ (18 mg, 0.26 mmol). After being stirred overnight at rt, the solution was acidified by AcOH to pH 5, then heated to 50 °C for 5 h. The solution was concentrated *in vacuo* and traces of water and HOAc were removed by coevaporating with toluene several times. The residue was dissolved in dry pyridine (1 mL) and Ac_2O (1 mL). After being stirred overnight, the mixture was diluted with MeOH (2 mL) then with EtOAc. The organic layer was washed with 5% HCl solution, saturated $NaHCO_3$, and brine respectively, and then dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was debenzoylated with a catalytic amount of NaOMe in MeOH (2 mL) at rt for 2 h, saponified with aq NaOH (13 mg, 1mL H_2O and 1mL MeOH, 2 h at rt). The mixture was neutralized with Dowex-50WX8 (H^+ form), filtered, and concentrated *in vacuo*. The residue was hydrogenolyzed with a catalytic amount of 10% Pd/C (20 mg, 1 atm H_2 for 2 days), then the suspension was filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2:MeOH 3:1→2:1→1:1) to give **1b** (14 mg, 44% from **19**): $[\alpha]_D$ -58° (*c* 0.3 H_2O); ^1H NMR (600 MHz, D_2O, DQFCOSY) δ 4.86 (d, 1H, H-1', $J_{1',2'}$ = 8.5 Hz), 4.53 (d, 1H, H-1'', $J_{1'',2''}$ = 7.8 Hz), 4.36 (d, 1H, H-1, $J_{1,2}$ = 7.8 Hz), 3.58 (s, 3H, CH_3O), 1.93 (s, 3H, CH_3CO), 1.42 (s, 3H, CH_3), 1.27 (d, 3H, CH_3-6); ESIMS (*m/z*): 699 (M+K-1), 662 (M+1).

ACKNOWLEDGMENT

This work was supported by the State Science and Technology Committee of China. We thank Prof. Houming Wu for kind assistance in recording NMR spectra.

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