A simple access to 3,6-branched oligosaccharides: Synthesis of a glycopeptide derivative that relates to *Lycium barbarum* L.

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An efficient method is described for the synthesis of galactopyranosyl-containing 3,6-branched oligosaccharides using isopropyl thiogalactopyranoside as starting material. This method is successfully applied to the preparation of a glycopeptide derivative that relates to *Lycium barbarum* L. The potential application of isopropyl thioglycoside in glycosylation is also investigated.

Introduction

The availability of oligosaccharides and their analogues as substrates can provide insight into their biological roles and might lead to the discovery of novel carbohydrate-based therapeutics.[1] Despite many advances in the chemical synthesis of oligosaccharides, it is still time-consuming and often difficult to synthesize these highly asymmetric and densely functionalized molecules. Among the glycosylation methodologies currently used in carbohydrate chemistry, those using glycosyl trichloroacetimidate and thioglycosides are the most attractive choices for oligosaccharide assembly.[2] We have recently disclosed a new method for one-pot synthesis of 3,6-differentially protected carbohydrate building blocks and have successfully applied this method to natural oligosaccharides' preparation.[3] *Lycium barbarum* L., a famous traditional Chinese herbal medicine, has been widely used in China as a health-protection agent for 2300 years.[4] We here report the first example of a simple synthesis of a glycopeptide derivative that relates to *Lycium barbarum* L. using isopropyl thiogalactopyranosides as practical glycosylation building blocks.

Results and discussion

In our previous communication,[3] we have described a procedure for one-pot synthesis of 3,6-differentially protected carbohydrate building blocks. Thus, commercially available isopropyl 1-thio-β-D-galactopyranoside **1** was selectively tritylated on the primary hydroxy group with triphenylmethyl chloride (TrCl) in pyridine, silylated with *tert*-butylchlorodimethylsilane (TBDMSCl) in DMF on C-3 and then benzoylated with benzoyl chloride in one pot to afford isopropyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-1-thio-β-D-galactopyranoside **2** in 76% isolated overall yield (Scheme 1). Treatment of compound **2** with 90% trifluoroacetic acid (TFA) gave 3,6-diol **3** in 80% yield. Interestingly, treatment of **2** with 50% aq. TFA furnished 3-*O*-silylated product **4** as a major component. It is noteworthy that attempted preparation of the 3,6-diol *via* acidic hydrolysis of acetylated analogue isopropyl 2,4-di-*O*-acetyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-1-thio-β-D-galactopyranoside[3] **5** gave exclusively acetyl-migrated product isopropyl 2,6-di-*O*-acetyl-1-thio-β-D-galactopyranoside **6** (85%). Compared with **3**, chemical shifts of H-6a and H-6b in **6** moved downfield to δ 4.26 and 4.37 from δ 3.58 and 3.77, respectively, confirming the occurrence of this migration. Coupling reaction of 3,6-diol **3** and 2.1 equiv. of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate **7** in anhydrous CH_2Cl_2 using TMSOTf as catalyst gave trisaccharide **8** in 90% yield. No intermolecular aglycone transfer[5] was observed in this case. Convergently, fully acetylated isopropyl β-D-galactopyranoside (IPTG) **9** was condensed with L-serine derivative **10**[6] in methylene dichloride in the presence of *N*-iodosuccinimide (NIS) and TMSOTf to afford glycopeptide derivative **11**, which was subjected to a sequence of protecting-group manipulations to give desired acceptor **14** in a total yield of 45% from **10**. Coupling reaction of **8** and **14** likewise gave glycopeptide **15** in excellent yield.

Encouraged by the above result, we also tried the coupling reaction of glucosamine derivative **16** with 3,6-diol **3**, and the trisaccharide derivative **17**, corresponding to human blood-group substrates, was obtained in 53.6% yield (Scheme 2). The fair yield of this reaction may be ascribed to the quick consumption of imidate **16** and about 20% of isopropyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,4-di-*O*-benzoyl-1-thio-β-D-galactopyranoside was also isolated after separation on a column.

Condensation of fully benzylated isopropyl thioglycoside **18** and acceptor **19**[7] in the presence of NIS and TMSOTf in anhydrous methylene dichloride at 0 °C achieved α-linked disaccharide **20** in 80% yield. A singlet at δ 4.91 in the ^{1}H NMR spectrum, that was further determined as the signal for H-1′ by 2D spectroscopy, confirmed the presence of an α glycosidic linkage in **20**. Similar reaction using MeOTf as promoter gave an inseparable mixture of 1,2-*cis*- and 1,2-*trans*-linked disaccharides in 56–70% yield. The yields seem dependent on the amount of MeOTf used in the reactions (we used 2–5 equiv.) and part of the acceptor **19** could be recovered.

The armed–disarmed strategy[8] was next investigated using isopropyl thioglactopyranoside glycosyl donor **18** and NIS–TMSOTf as catalyst. When armed donor **18** was regioselectively glycosylated with disarmed acceptor thioglycoside **21**[9] in anhydrous methylene dichloride–diethyl ether (1 : 1, v/v) co-solvent, α-linked disaccharide **22** was obtained in an isolated yield of 62% after recovery of 60 mg of **21** (see Experimental section). Glycosylation of **18** and acceptor **23** in diethyl ether, on the other hand, furnished disaccharide **24** in 96% yield as a mixture of the α- and β-anomer in the ratio of 2 : 1. Reaction of **18** and **25** was not straightforward. This may be caused by the lower activity of 4-OH in **25**.

In summary, we present here a highly efficient and practical method for the preparation of 3,6-branched galactopyranosyl oligosaccharides. This method is successfully applied to the

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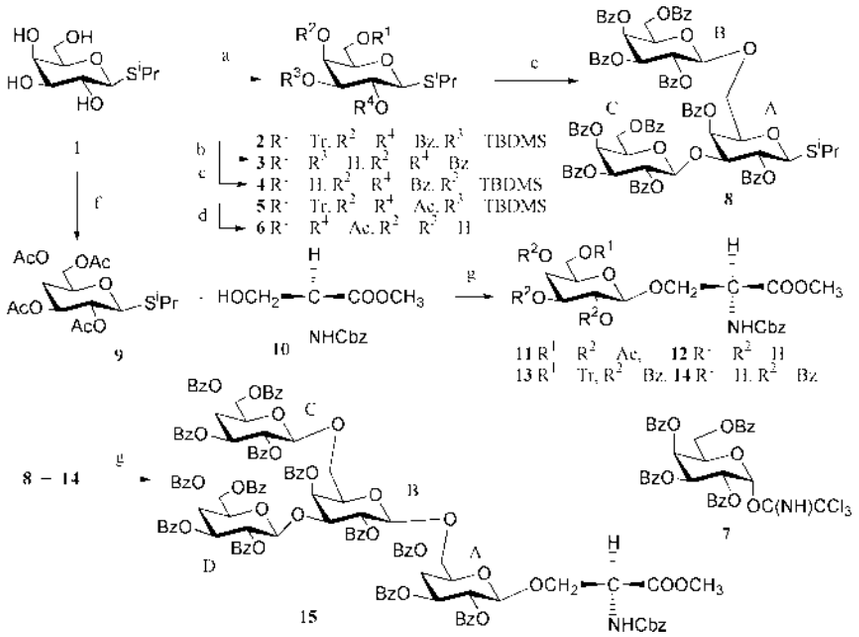
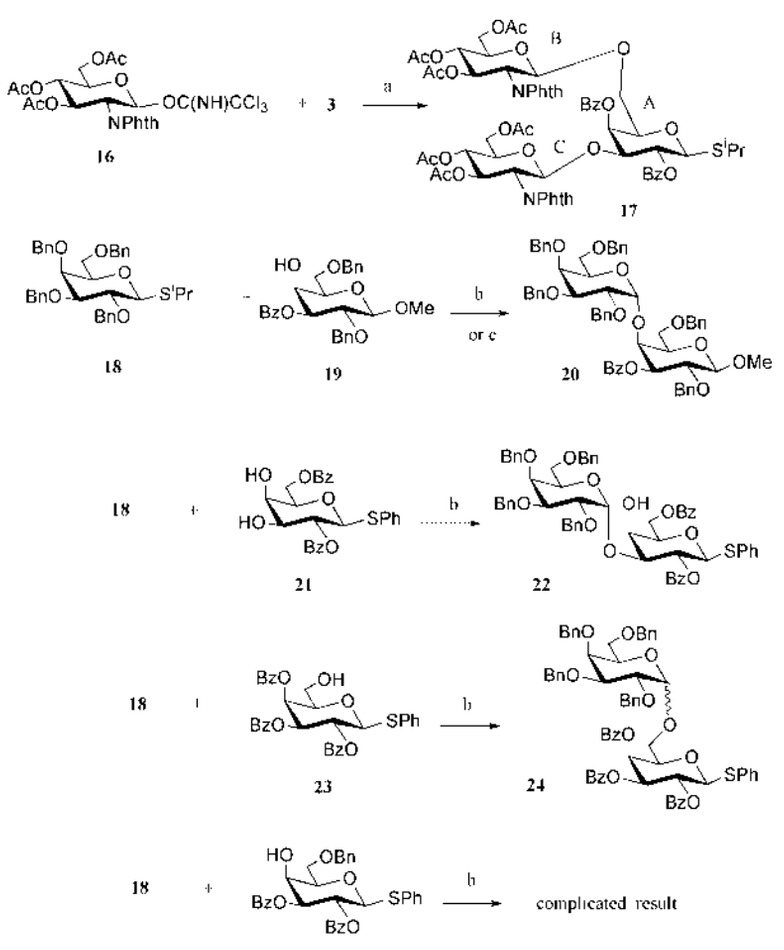
synthesis of a glycopeptide derivative that relates to *Lycium barbarum* L. This is the first report of using isopropyl thioglycosides as glycosyl donors. Its applications in α-glycosidic bond formation and the potential usage in the armed–disarmed glycosylation strategy were also investigated.

Experimental

Optical rotations were determined at 25 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter and $[\alpha]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}. Melting points were determined with



Scheme 1 *Reagents and conditions (yields)*: a) TrCl, pyridine; TBDMSCl, imidazole; BzCl (76%); b) 90% TFA (80%); c) Method A: $FeCl_3 \cdot 6H_2O$ (50%); Method B: 50% TFA (86.3%); d) 90% TFA (85%); e) TMSOTf (90%); f) Ac_2O, pyridine (100%); g) NIS, TMSOTf (87% for **11**, 91% for **15**).



Scheme 2 *Reagents and conditions (yields)*: a) TMSOTf, CH_2Cl_2 (54%); b) NIS, TMSOTf, Et_2O (80% for **20**, 62% for **22**, 96% for **24**); c) MeOTf, CH_2Cl_2 (56–70%).

a 'Mel-Temp' apparatus and are uncorrected. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with a Bruker ARX 400 spectrometer for solution 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 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 UV detector. Column chromatography was conducted on columns (16 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (100–200 mesh) using EtOAc–petroleum ether (60–90 °C) as eluent. Solutions were concentrated at <60 °C under diminished pressure.

Isopropyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-trityl-1-thio-β-D-galactopyranoside 2

To a solution of **1** (710 mg, 2.98 mmol) in pyridine (2 mL) were added TrCl (1.05 g, 3.7 mmol) and DMAP (20 mg). The mixture was stirred at 80 °C for 16 h then cooled to 0 °C. To the above reaction mixture was added imidazole (408 mg, 6.0 mmol) in one portion. A solution of TBDMSCl (450 mg, 3.0 mmol) in DMF (3 mL) was added portionwise during 1.5 h. The mixture was stirred at room temperature for 4 h, then premixed benzoyl chloride (1.05 g, 7.46 mmol) and pyridine (1 mL) was added. The reaction mixture was stirred at 50 °C for 24 h, then poured into ice-cold water, and extracted with EtOAc. The organic phase was concentrated to dryness by co-evaporation with toluene. The residue was subjected to column chromatography on silica gel using petroleum ether–EtOAc (3 : 2) as eluent to give **2** (1.65 g, 76%) as a white foam; $[\alpha]_D^{25}$ +17 (*c* 1, $CHCl_3$); ^{1}H NMR δ −0.12, 0.11 [2 s, 6 H, $Si(CH_3)_2$], 0.61 (s, 9 H, *t*-Bu), 1.26, 1.32 [2 d, 6 H, *J* 6.8 Hz, $CH(CH_3)_2$], 3.18 (dd, 1 H, $J_{5,6a}$ 6.6 Hz, $J_{6a,6b}$ 9.6 Hz, H-6a), 3.22–3.31 (m, 1 H, SCH), 3.44 (dd, 1 H, $J_{5,6b}$ 6.6 Hz, H-6b), 3.80 (t, 1 H, H-5), 4.06 (dd, 1 H, $J_{2,3}$ 9.2 Hz, $J_{3,4}$ 3.2 Hz, H-3), 4.68 (d, 1 H, $J_{1,2}$ 9.2 Hz, H-1), 5.46 (t, 1 H, H-2), 5.71 (d, 1 H, H-4), 7.14–8.17 (m, 25 H, Ph) (Calc. for $C_{48}H_{54}O_7SSi$: C, 71.79; H, 6.78. Found: C, 71.96; H, 6.65%).

Isopropyl 2,4-di-*O*-benzoyl-1-thio-β-D-galactopyranoside 3

Compound **2** (1.65 g, 2.07 mmol) was dissolved in 90% aq. TFA (15 mL) and the solution was stirred at room temperature for 2 h. Toluene (50 mL) was added and the solvents were evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 2 : 1) to give **3** (765 mg, 80%) as a syrup; $[\alpha]_D^{25}$ +15 (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.34, 1.28 [2 d, 6 H, *J* 6.8 Hz, $CH(CH_3)_2$], 3.23–3.30 (m, 1 H, SCH), 3.58 (dd, 1 H, $J_{5,6a}$ 7.2 Hz, $J_{6a,6b}$ 12 Hz, H-6a), 3.77 (dd, 1 H, $J_{5,6b}$ 6.4 Hz, H-6b), 3.86–3.90 (m, 1 H, H-5), 4.14 (dd, 1 H, $J_{2,3}$ 10.0 Hz, $J_{3,4}$ 3.4 Hz, H-3), 4.80 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.43 (t, 1 H, H-2), 5.62 (d, 1 H, H-4), 7.26–8.14 (m, 10 H, Ph) (Calc. for $C_{23}H_{26}O_7S$: C, 61.87; H, 5.87. Found: C, 61.53; H, 5.91%).

Isopropyl 2,4-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galactopyranoside 4

Method A. To a solution of **2** (1 g, 1.47 mmol) in methylene dichloride (10 mL) was added $FeCl_3$ hexahydrate (947 mg, 3.50 mmol) and the mixture was stirred at room temperature for 3 h. It was then diluted with CH_2Cl_2 (50 mL) and washed with cold water (× 3). The water phase was re-extracted with CH_2Cl_2 (20 mL) and the organic phases were combined and concentrated to dryness, and the residue was subjected to silica gel column chromatography (petroleum ether–EtOAc, 2 : 1) to give crystalline compound **4** (322 mg, 50%).

Method B. To a solution of **2** (420 mg, 0.52 mmol) in methylene dichloride (0.5 mL) was added 50% aq. TFA (3 mL). The mixture was stirred at room temperature for 30 min, then neutralized with sodium bicarbonate and extracted with CH_2Cl_2 (2 × 20 mL). The organic phases were combined and concentrated to dryness, and the residue was purified on a silica gel column (petroleum ether–EtOAc, 2 : 1) to give **4** (253 mg, 86.3%) as crystals: mp 110–112 °C; $[\alpha]_D^{25}$ +76 (*c* 1, $CHCl_3$); ^{1}H NMR δ 0.01, 0.16 [2 s, 6 H, $Si(CH_3)_2$], 0.74 (s, 9 H, *t*-Bu), 1.41, 1.44 [2 d, 6 H, *J* 6.6 Hz, $SCH(CH_3)_2$], 3.35–3.41 (m, 1 H, SCH), 3.72 (dd, 1 H, $J_{5,6a}$ 6.3 Hz, $J_{6a,6b}$ 11.4 Hz, H-6a), 3.93 (dd, 1 H, $J_{5,6b}$ 5.7 Hz, H-6b), 3.98–4.06 (m, 1 H, H-5), 4.28 (dd, 1 H, $J_{2,3}$ 9.6 Hz, $J_{3,4}$ < 1.0 Hz, H-3), 4.90 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 5.63 (d, 1 H, H-4), 5.73 (t, 1 H, H-2), 7.55–8.29 (m, 10 H, Ph) (Calc. for $C_{29}H_{40}O_7SSi$: C, 62.11; H, 7.19. Found: C, 62.39; H, 7.05%).

Isopropyl 2,6-di-*O*-acetyl-1-thio-β-D-galactopyranoside 6

Compound **5**[3] (200 mg, 0.29 mmol) was dissolved in 90% aq. trifluoroacetic acid (5 mL). The solution was stirred at room temperature for 2 h and then co-evaporated with toluene to dryness under diminished pressure. Column chromatography using 1 : 1, petroleum ether–EtOAc as eluent gave syrupy **6** (80 mg, 85%); $[\alpha]_D^{25}$ +4 (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.30, 1.31 [2 d, 6 H, *J* 5.4 Hz, $SCH(CH_3)_2$], 2.08, 2.13 (2 s, 6 H, 2 × $COCH_3$), 3.16–3.20 (m, 1 H, SCH), 3.65–3.68 (m, 2 H, H-5, H-3), 3.93 (s, 1 H, H-4), 4.26 (dd, 1 H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.37 (dd, 1 H, $J_{5,6b}$ 5.6 Hz, H-6b), 4.48 (d, 1 H, $J_{1,2}$ 9.6 Hz, H-1), 4.98 (t, 1 H, $J_{2,3}$ 11.6 Hz, H-2) (Calc. for $C_{13}H_{22}O_7S$: C, 48.43; H, 6.88. Found: C, 48.30; H, 6.99%).

2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate 7

1,2,3,4,6-Penta-*O*-benzoyl-D-galactopyranose (6 g, 8.57 mmol) was dissolved in ammonia-saturated THF–MeOH (6 : 4, 100 mL). The mixture was stirred at room temperature for 15 h, then concentrated to dryness, and purified on a silica gel column with 3 : 1, petroleum ether–EtOAc as eluent to yield 2,3,4,6-tetra-*O*-benzoyl-D-galactose (4.08 g, 80%). To a solution of 2,3,4,6-tetra-*O*-benzoyl-D-galactose (4 g, 6.71 mmol) in anhydrous CH_2Cl_2 (25 mL) were added trichloroacetonitrile (2 mL, 20 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), (0.45 mL, 3.0 mmol), and the mixture was stirred at room temperature for 4 h. TLC (3 : 1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated and purified on a silica gel column using 2 : 1, petroleum ether–EtOAc as eluent to give foamy **7** (4.47 g, 90%); $[\alpha]_D^{25}$ +122 (*c* 1, $CHCl_3$); ^{1}H NMR δ 4.49 (dd, 1 H, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.66 (dd, 1 H, $J_{5,6b}$ 6.9 Hz, $J_{6a,6b}$ 11.2 Hz, H-6b), 4.92 (dd, 1 H, H-5), 6.03 (dd, 1 H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.5 Hz, H-2), 6.14 (dd, 1 H, $J_{3,4}$ 2.6 Hz, H-3), 6.22 (d, 1 H, H-4), 6.98 (d, 1 H, H-1), 7.22–8.13 (m, 20 H, Ph), 8.69 (s, 1 H, NH) [MALDI-TOF-MS Calc. for $C_{36}H_{28}Cl_3NNaO_{10}$ $(M + Na)^+$: 762. Found: *m*/*z*, 762 $(M + Na)^+$].

Isopropyl *O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1 → 6)]-*O*-[2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-1-thio-β-D-galactopyranoside 8

To a cooled solution (−15 °C) of **3** (300 mg, 0.67 mmol) and **7** (1.06 g, 1.43 mmol) in dry CH_2Cl_2 (5 mL) was added TMSOTf (12 μL, 0.06 mmol) under N_2, and the mixture was stirred at this temperature for 2 h, then neutralized with triethylamine. The reaction mixture was concentrated to a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc, 2 : 1) to give **8** (966 mg, 90%) as a white foam; $[\alpha]_D^{25}$ +14 (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.01, 1.13 [2 d, 6 H, *J* 6.5 Hz, $SCH(CH_3)_2$], 2.97–3.01 (m, 1 H, SCH), 3.88 (dd, 1 H, *J* 8.4 Hz, 10.8 Hz, H-6a), 4.04–4.06 (m, 1 H, H-6), 4.19–4.49 (m, 5 H), 4.50 (dd, 1 H, *J* 6.0 Hz, 11.4 Hz, H-6), 4.64 (d, 1 H, $J_{1,2}$ 10.2 Hz,

H-1^{A}), 4.69–4.63 (m, 1 H, H-6), 4.75 (dd, 1 H, *J* 5.7 Hz, 10.8 Hz, H-6), 5.00 (dd, 1 H, *J* 7.8, 9.6 Hz, H-2^{A}), 5.00 (d, 1 H, *J* 7.8 Hz, H-1^{C}), 5.43 (dd, 1 H, *J* 10.5, 3.3 Hz, H-3^{C}), 5.61 (distorted t, 1 H, *J* 8.7, 9.9 Hz, H-2^{B}), 5.58–5.64 (m, 2 H, H-3, H-1^{B}), 5.91 (distorted t, 1 H, *J* 9.0, 9.6 Hz, H-2^{C}), 5.92 (d, 1 H, *J* 3.0 Hz, H-4^{B}), 5.98 (d, 1 H, *J* 3.0 Hz, H-4^{C}), 6.06 (d, 1 H, *J* 3.3 Hz, H-4^{A}), 7.05–8.23 (m, 50 H, Ph) (Calc. for $C_{91}H_{78}O_{25}S$: C, 68.16; H, 4.90. Found: C, 68.01; H, 5.02%).

Isopropyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside 9

Standard acetylation of IPTG (2.0 g, 8.4 mmol) with acetic anhydride (8 mL) in pyridine (10 mL) gave a quantitative yield of crystalline **9**, mp 70–72 °C; ^{1}H NMR δ 1.31, 1.32 [2 d, 6 H, CH(CH_3)$_2$], 1.99, 2.04, 2.06, 2.15 (4 s, 12 H, 4 × Ac), 3.19 [m, 1 H, CH(CH$_3$)$_2$], 3.93 (br t, 1 H, $J_{5,6a}$ 6.4, $J_{5,6b}$ 6.9 Hz, H-5), 4.10 (dd, 1 H, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.18 (dd, 1 H, H-6b), 4.58 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.06 (dd, 1 H, $J_{3,4}$ 3.6 Hz, H-3), 5.22 (t, 1 H, $J_{2,3}$ 10.0 Hz, H-2), 5.43 (d, 1 H, H-4) (Calc. for $C_{17}H_{26}O_9S$: C, 50.24; H, 6.45. Found: C, 50.37; H, 6.55%).

O-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-serine methyl ester 11

To a pre-cooled solution (0 °C) of compound **10**[6] (708 mg, 2.8 mmol) and **9** (1.2 g, 2.95 mmol) in CH_2Cl_2 (20 mL) were added NIS (900 mg, 4 mmol) and TMSOTf (90 μL, 0.5 mmol) under a nitrogen atmosphere. The mixture was stirred at this temperature for 2 h, TLC (2 : 1, petroleum ether–EtOAc) showed the starting material had disappeared. The reaction mixture was quenched with Et_3N, then concentrated, and the residue was subjected to flash chromatography with 2 : 1, petroleum ether–EtOAc as eluent to give **11** (1.42 g, 87%) as a syrup; $[a]_D^{25}$ +6 (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.95, 2.00, 2.01, 2.11 (4 s, 4 × 3 H, 4 × Ac), 3.74 (s, 3 H, OCH_3), 3.76–3.82 (m, 3 H, H-5, H-6a, H-6b), 3.84–3.88 (m, 1 H, one proton of OCH_2), 4.20–4.24 (m, 1 H, other proton of OCH_2), 4.41 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.44–4.46 (m, 1 H, OCH), 4.94 (dd, 1 H, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.3 Hz, H-3), 5.11–5.14 (m, 3 H, OCH_2, H-2), 5.33 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.55 (d, 1 H, *J* 8.1 Hz, NH), 7.23–7.33 (m, 5 H, Ph) (Calc. for $C_{26}H_{33}NO_{14}$: C, 53.51; H, 5.70. Found: C, 53.69; H, 5.61%).

O-(2,3,4-Tri-*O*-benzoyl-6-*O*-trityl-β-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-serine methyl ester 13

A solution of **11** (3.2 g, 5.5 mmol) in MeOH (50 mL) was treated with NaOMe (2.5 mL; 0.5 M in MeOH) at room temperature for 4 h. The mixture was neutralized with Amberlite 120 (H^+) resin, then filtered, and the filtrate was evaporated to give *O*-(β-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-serine methyl ester **12** (2.2 g) as a syrup. Compound **12** (2.2 g, 5.3 mmol) was dissolved in pyridine (10 mL), and trityl chloride (2.57 g, 9.54 mmol) and DMAP (20 mg) were added. The mixture was stirred at room temperature for 48 h, then cooled to 0 °C. To the above reaction mixture was added premixed benzoyl chloride (2.2 mL, 18.5 mmol) and pyridine (3 mL), and the mixture was stirred vigorously at room temperature for 48 h, and then poured into ice-cold water, and extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was concentrated to dryness by repeated co-evaporation with toluene. The residue was subjected to column chromatography on silica gel with toluene–petroleum ether–EtOAc as eluent (0.2 : 1.5 : 1) to give **13** (3.1 g, 58%); $[a]_D^{25}$ +8 (*c* 1, $CHCl_3$); ^{1}H NMR δ 3.24 (dd, 1 H, $J_{5,6a}$ 8.7, $J_{6a,6b}$ 12 Hz, H-6a), 3.42 (dd, 1 H, $J_{5,6b}$ 5.7 Hz, H-6b), 3.57 (s, 3 H, OCH_3), 3.87–3.91 (m, 1 H, one proton of OCH_2), 3.96 (dd, 1 H, $J_{5,6a}$ 7.5, $J_{5,6b}$ 6.0 Hz, H-5), 4.28–4.30 (m, 1 H, other proton of OCH_2), 4.40–4.43 (m, 1 H, OCH), 4.67 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 4.96, 5.04 (2 d, 2 H, *J* 12.3 Hz, OCH_2), 5.44 (d, 1 H, *J* 7.5 Hz, NH), 5.56–5.62 (m, 2 H, H-2, H-3), 6.02 (s, 1 H, H-4), 7.05–8.05 (m, 35 H, Ph) (Calc. for $C_{58}H_{51}NO_{13}$: C, 71.81; H, 5.30. Found: C, 71.57; H, 5.38%).

O-(2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-serine methyl ester 14

To a solution of **13** (600 mg, 0.62 mmol) in methylene dichloride (10 mL) was added $FeCl_3$ hexahydrate (406 mg, 1.5 mmol). The mixture was stirred at room temperature for 3 h and then diluted with more CH_2Cl_2 (50 mL). The above mixture was washed with cold water three times and the combined water phase was re-extracted with CH_2Cl_2 (30 mL). The organic phase were combined and concentrated to dryness, and the residue was subjected to silica gel column chromatography (petroleum ether–EtOAc, 3 : 2) to give **14** (400 mg, 89%) as an amorphous solid; $[a]_D^{25}$ +1 (*c* 1, $CHCl_3$); ^{1}H NMR δ 3.35 (s, 3 H, OCH_3), 3.56–3.62 (m, 1 H, H-5), 3.77 (dd, 1 H, $J_{5,6a}$ 7.2 Hz, $J_{6a,6b}$ 11.7 Hz, H-6a), 3.99 (dd, 1 H, $J_{5,6b}$ 5.7 Hz, H-6b), 4.09–4.12 (m, 2 H, OCH_2), 4.50–4.53 (m, 1 H, OCH), 4.74 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 5.02 (d, 1 H, *J* 8.4 Hz, NH), 5.09 (s, 2 H, OCH_2), 5.51–5.55 (m, 2 H, H-2, H-3), 5.73 (br s, 1 H, H-4), 7.20–8.09 (m, 20 H, Ph) (Calc. for $C_{39}H_{37}NO_{13}$: C, 64.37; H, 5.12. Found: C, 64.25; H, 5.20%).

O-{2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1 → 6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-β-D-galactopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl)}-*N*-benzyloxycarbonyl-L-serine methyl ester 15

A solution of compound **8** (368 mg, 0.23 mmol) and **14** (153 mg, 0.21 mmol) in anhydrous CH_2Cl_2 (3 mL) was treated with NIS (140 mg, 0.62 mmol) and TMSOTf (37 μL, 0.2 mmol) under N_2 at 0 °C. The mixture was stirred under these conditions for 2 h, then neutralized with Et_3N and concentrated. Column chromatography (2 : 1, petroleum ether–EtOAc) of the residue gave glycopeptide **15** (430 mg, 91%) as a syrup; $[a]_D^{25}$ +87 (*c* 1, $CHCl_3$); ^{1}H NMR δ 3.50 (s, 3 H, OCH_3), 3.50–3.65 (m, 3 H, H-5, H_2-6), 3.89–3.95 (m, 4 H, H_2-6, H-5, one proton of OCH_2), 4.08–4.11 (m, 2 H, H-3^{B}, other proton of OCH_2), 4.24–4.40 (m, 5 H, 3 × H-6, 2 × H-5), 4.53 (d, 1 H, *J* 8.0 Hz, H-1^{A}), 4.52–4.55 (m, 2 H, H-1^{B}, OCH), 4.70 (dd, 1 H, *J* 6.4, 11.4 Hz, H-6b), 4.80 (d, 1 H, *J* 7.6 Hz, H-1^{D}), 4.93 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1^{C}), 4.94, 5.01 (2 d, 2 H, *J* 12.4 Hz, OCH_2), 5.37 (d, 1 H, *J* 7.8 Hz, NH), 5.37 (dd, 1 H, *J* 9.2, 3.2 Hz, H-3^{D}), 5.42 (dd, 1 H, *J* 9.6, 3.2 Hz, H-3^{A}), 5.51–5.55 (m, 3 H, H-2^{A}, H-2^{B}, H-2^{D}), 5.64 (dd, 1 H, *J* 9.6, 3.2 Hz, H-3^{C}), 5.70 (d, 1 H, *J* 3.2 Hz, H-4^{A}), (5.75 (dd, 1 H, *J* 8.0, 9.2 Hz, H-2^{C}), 5.84 (d, 1 H, *J* 3.2 Hz, H-4^{D}), 5.89 (d, 1 H, *J* 2.8 Hz, H-4^{B}), 5.99 (d, 1 H, *J* 3.2 Hz, H-4^{C}), 7.03–8.15 (m, 70 H, Ph); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.00, 165.95, 165.92, 165.86, 165.54, 165.44 (2 C), 165.36, 165.19 (2 C), 165.15 (2 C), 164.39 (2 C), 154 (BnO*C*O), 100.55, 101.40, 101.40, 101.65 (4 C-1), 73.95, 72.89, 71.62, 71.48, 71.29, 71.04, 70.92, 70.61, 69.87, 69.49, 69.35, 68.63, 68.31, 68.12, 67.55, 66.88, 66.69, 61.93, 61.46, 54.12, 53.74, 52.46. [MALDI-TOF-MS Calc. for $C_{127}H_{107}NNaO_{38}$ (M + Na)$^+$: 2276.6. Found: *m/z*, 2276.9 (M + Na)$^+$] (Calc. for $C_{127}H_{107}NO_{38}$: C, 67.64; H, 4.78. Found: C, 67.85; H, 4.70%).

Isopropyl *O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 6)]-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1 → 3)]-2,4-di-*O*-benzoyl-1-thio-β-D-galactopyranoside 17

To a mixture of **3** (200 mg, 0.45 mmol), **16** (577 mg, 1.00 mmol) and 4 Å molecular sieves in anhydrous CH_2Cl_2 (8 mL) was added TMSOTf (12 μL, 0.06 mmol) under N_2 at −15 °C. The mixture was stirred these conditions for 2 h, at the end of which time TLC (1 : 1, petroleum ether–EtOAc) indicated that starting material **16** had disappeared. The reaction mixture was neutralized with Et_3N, then filtered, and the filtrate was

concentrated. The residue was purified by silica gel column chromatography (petroleum ether–EtOAc, 1 : 1) to give **17** (308 mg, 53.6%) as a syrup; $[\alpha]_D^{25}$ +58 (*c* 1, $CHCl_3$); ^{1}H NMR δ 0.91, 0.99 [2 d, 6 H, $CH(CH_3)_2$], 1.69, 1.83 (2 s, 6 H, 2 × Ac), 1.96 (s, 6 H, 2 × Ac), 2.03, 2.15 (2 s, 6 H, 2 × Ac), 2.79–2.87 (m, 1 H, SCH), 3.56 (dd, 1 H, *J* 8.6, 10.8 Hz, H-6a^A), 3.75 (m, 1 H, H-5^A), 3.85–3.91 (m, 2 H, H-3^A, H-6b^A), 4.00–4.09 (m, 2 H, H-5^B, H-6a^B), 4.12 (dd, 1 H, *J* 8.4 Hz, H-2^C), 4.13–4.25 (m, 3 H, H-6b^B, H-5^C, H-6a^C), 4.29 (dd, 1 H, *J* 8.5 Hz, H-2^B), 4.35 (dd, 1 H, *J* 9.6, 12.6 Hz, H-6b^C), 4.52 (d, 1 H, *J* 10.1 Hz, H-1^A), 5.02 (t, 1 H, *J* 10.5 Hz, H-4^C), 5.16 (t, 1 H, *J* 10.5 Hz, H-4^B), 5.35 (t, 1 H, *J* 10.1 Hz, H-2^A), 5.40 (d, 1 H, *J* 8.5 Hz, H-1^B), 5.51 (d, 1 H, *J* 8.4 Hz, H-1^C), 5.55 (dd, 1 H, H-3^C), 5.63 (d, 1 H, *J* 3.4 Hz, H-4^A), 5.72 (dd, 1 H, H-3^B), 7.22–8.02 (m, 18 H, Ph) (Calc. for $C_{63}H_{64}N_2O_{25}S$: C, 59.06; H, 5.03. Found: C, 59.27; H, 5.11%).

Isopropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside 18

To a cold (0 °C) solution of IPTG (1.0 g, 4.20 mmol) in DMF (10 mL) were added NaH (50%; 1.66 g, 34.6 mmol) and BnBr (2.3 mL, 18.9 mmol) cautiously. The reaction mixture was stirred at room temperature for 6 h, then poured into cold water, extracted with CH_2Cl_2 (2 × 30 mL), and the organic layer was dried over Na_2SO_4, and concentrated. Column chromatography (5 : 1, petroleum ether–ethyl acetate) of the residue gave **18** (2.1 g, 85%) as a syrup; $[\alpha]_D^{25}$ −5 (*c* 1, $CHCl_3$); ^{1}H NMR δ 1.28, 1.30 [2 d, 6 H, $SCH(CH_3)_2$], 3.14–3.23 (m, 1 H, SCH), 3.51–3.57 (m, 4 H, H-3, H-5, H-6a, H-6b), 3.77 (t, 1 H, *J* = 9.6 Hz, H-2), 3.91 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4), 4.35 (d, 1 H, *J* 11.4 Hz, CH_2), 4.42 (d, 1 H, *J* 11.4 Hz, CH_2), 4.46 (d, 1 H, $J_{1,2}$ 9.6 Hz, H-1), 4.58 (d, 1 H, *J* 11.7 Hz, CH_2), 4.69 (s, 2 H, CH_2), 4.73 (d, 1 H, *J* 9.9 Hz, CH_2), 4.85 (d, 1 H, *J* 10.2 Hz, CH_2), 4.91 (d, 1 H, *J* 11.7 Hz, CH_2), 7.18–7.36 (m, 20 H, Ph) (Calc. for $C_{37}H_{42}O_5S$: C, 74.22; H, 7.07. Found: C, 74.54; H, 6.96%).

Methyl *O*-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1 → 4)]-3-*O*-benzoyl-2,6-di-*O*-benzyl-β-D-galactopyranoside 20

To a solution of compound **18** (300 mg, 0.5 mmol) and **19**[7] (240 mg, 0.50 mmol) in anhydrous CH_2Cl_2 (6 mL) were added NIS (282 mg, 1.25 mmol) and Me_3SiOTf (27 μL, 0.18 mmol) under N_2 at 0 °C. The mixture was stirred under these conditions for 2 h, at which time TLC (2 : 1, petroleum ether–EtOAc) indicated that starting material **18** was consumed completely. The reaction mixture was neutralized with Et_3N, then concentrated. Column chromatography (5 : 2, petroleum ether–EtOAc) of the residue gave **20** (400 mg, 80%) as a syrup; $[\alpha]_D^{25}$ +44 (*c* 1, $CHCl_3$); ^{1}H NMR δ 3.08 (dd, 1 H, $J_{5,6a}$ 8.4, $J_{6a,6b}$ 5.4 Hz, H-6a), 3.40 (dd, 1 H, *J* 9.6, 8.4 Hz, H-5), 3.63 (s, 3 H, OCH_3), 3.67–3.83 (m, 4 H, H-2′, H-5, H-6a, H-6b), 3.89 (dd, 1 H, $J_{5,6b}$ 9.6 Hz, H-6b), 3.99–4.03 (m, 2 H, H-2, H-3′), 4.04 (s, 1 H, H-4), 4.11 (s, 1 H, H-4′), 4.26 (s, 2 H, CH_2), 4.31 (s, 2 H, CH_2), 4.42 (d, 1 H, $J_{1,2}$ 7.2 Hz, H-1), 4.50 (d, 1 H, *J* 11.7 Hz, CH_2), 4.60 (d, 1 H, *J* 11.7 Hz, CH_2), 4.61 (d, 1 H, *J* 11.7 Hz, CH_2), 4.75 (s, 2 H, CH_2), 4.80 (d, 1 H, *J* 11.7 Hz, CH_2), 4.82 (d, 1 H, *J* 11.7 Hz, CH_2), 4.89 (d, 1 H, *J* 11.4 Hz, CH_2), 4.91 (s, 1 H, H-1′), 5.11 (dd, 1 H, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.0 Hz, H-3), 7.06–8.09 (m, 35 H, Ph) [Calc. for $C_{62}H_{64}NaO_{12}$ $(M + Na)^+$: 1023.4. Found: *m/z*, 1023.1 $(M + Na)^+$] (Calc. for $C_{62}H_{64}O_{12}$: C, 74.38; H, 6.44. Found: C, 74.70; H, 6.32%).

Phenyl *O*-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1 → 3)]-2,6-di-*O*-benzoyl-1-thio-β-D-galactopyranoside 22

To a solution of compound **18** (145 mg, 0.24 mmol) and **21** (97 mg, 0.2 mmol) in anhydrous Et_2O–CH_2Cl_2 (1: 1; 3 mL) were added NIS (63 mg, 0.28 mmol) and Me_3SiOTf (13 μL, 0.07 mmol) under N_2 at −42 °C. The mixture was stirred under these conditions for 1 h, at which time TLC (2 : 1, petroleum ether–EtOAc) indicated that starting material **18** was consumed completely. The reaction mixture was neutralized with Et_3N, then concentrated. Column chromatography (4 : 1, petroleum ether–EtOAc) of the residue gave the pure product **22** (48 mg, 62% based on 60 mg of recovered acceptor **21**) as a syrup; $[\alpha]_D^{25}$ + 5 (*c* 1, $CHCl_3$); ^{1}H NMR δ 2.85 (dd, 1 H, $J_{5,6a}$ 8.4 Hz, $J_{6b,6a}$ 5.6 Hz, H-6a′), 3.28 (t, 1 H, $J_{5,6b}$ 8.4 Hz, H-5′), 3.76 (dd, 1 H, H-6b′), 3.74–3.99 (m, 9 H), 4.01 (dd, 1 H, *J* 10, 2.8 Hz, H-2′), 4.39–4.89 (m, 8 H, 4 × PhCH_2), 4.77 (d, 1 H, *J* 2.8 Hz, H-1′), 4.80 (d, 1 H, *J* 10 Hz, H-1), 5.52 (t, 1 H, *J* 10 Hz, H-2), 7.04–7.63 (m, 30 H, Ph), 8.06–8.92 (m, 5 H, Ph) (Calc. for $C_{60}H_{58}O_{12}S$: C, 71.84; H, 5.83. Found: C, 72.03; H, 5.92%).

Phenyl *O*-[2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl-(1 → 6)]-2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside 24

To a mixture of compound **18** (470 mg, 0.786 mmol) and **23** (330 mg, 0.56 mmol) in anhydrous Et_2O (5 mL) were added NIS (174 mg, 0.78 mmol) and Me_3SiOTf (7 μL, 0.04 mmol) under N_2 at −42 °C. The mixture was stirred under these conditions for 1 h, at which time TLC (2 : 1, petroleum ether–EtOAc) indicated that starting material was consumed completely. The reaction mixture was neutralized with Et_3N, then concentrated. Column chromatography (2 : 1, petroleum ether–EtOAc) of the residue gave **24** (600 mg, 96%; β : α = 1 : 2) as a syrup; ^{1}H NMR δ 3.47–3.52 (m, 3 H), 3.55 (dd, 0.33 H), 3.68 (dd, 0.33 H), 3.81–4.05 (m, 4.33 H), 4.07 (t, 0.33 H, *J* 9.8 Hz, H-2′ of β isomer), 4.31–5.00 (m, 10.67 H), 5.51 (dd, 0.67 H, *J* 9.8, 3.2 Hz, H-3 of α isomer), 5.55 (dd, 0.33 H, *J* 9.8, 3.2 Hz, H-3 of β isomer), 5.69 (t, 0.33 H, *J* 9.8 Hz, H-2 of β isomer), 5.70 (t, 0.67 H, *J* 9.8 Hz, H-2 of α isomer), 5.88 (d, 0.67 H, *J* 3.1 Hz, H-4 of α isomer), 5.91 (d, 0.33 H, *J* 3.1 Hz, H-4 of β isomer), 7.22–7.97 (m, 40 H, Ph) (Calc. for $C_{67}H_{62}O_{13}S$: C, 72.68; H, 5.64. Found: C, 72.91; H, 5.57%).

Phenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactopyranoside 25

1.04 g of phenyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside[9] (2.86 mmol) was dissolved in 10 mL of pyridine, then premixed benzoyl chloride (2.2 mL, 18.5 mmol) and pyridine (3 mL) was added; the mixture was stirred vigorously at 50 °C overnight, and then poured into ice-cold water, and extracted with CH_2Cl_2 (2 × 30 mL). The organic phase was concentrated to dryness by repeated co-evaporation with toluene. The residue was subjected to column chromatography on silica gel with petroleum ether–EtOAc as eluent (2 : 1) to give phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (1.5 g, 92%); $[\alpha]_D^{25}$ +6 (*c* 1, $CHCl_3$); ^{1}H NMR δ 3.76 (br s, 1 H, H-5), 4.10 (dd, 1 H, $J_{5,6a}$ 1.2 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4,45 (dd, 1 H, $J_{5,6b}$ 1 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.60 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 4.97 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 5.36 (dd, 1 H, *J* 9.8, 3.2 Hz, H-3), 5.51 (s, 1 H, PhCH), 5.81 (t, 1 H, *J* 9.8 Hz, H-2), 7.24–7.98 (m, 20 H, Ph).

129 mg of phenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside (0.225 mmol) and 311 mg of sodium cyanoborohydride (4.5 mmol) were dissolved in 3 mL of dry THF (distilled from sodium), then 5 mL of hydrogen chloride saturated diethyl ether was added with vigorous stirring at room temperature. A second portion of hydrogen chloride in diethyl ether (2 mL) was added dropwise during 1.5 h until evolution of the gas ceased. The reaction mixture was diluted with CH_2Cl_2 (50 mL), then washed with saturated aq. $NaHCO_3$. The organic phase was dried over Na_2SO_4 and concentrated to dryness. The residue was subjected to column chromatography on silica gel with petroleum ether–EtOAc as eluent (4 : 1) to give **25** (106 mg, 82%) as a syrup; $[\alpha]_D^{25}$ +85 (*c* 1, $CHCl_3$); ^{1}H NMR δ 2.72 (br s, 1 H, 4-OH), 3.84–3.92 (m, 3 H, H-5, H_2-6), 4.42 (d, 1 H, $J_{3,4}$ 2.7 Hz, H-4), 4.60 (2 d, 2 H, *J* 12.0

Hz, $PhCH_2$), 4.94 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 5.32 (dd, 1 H, *J* 9.8, 3.0 Hz, H-3), 5.79 (t, 1 H, *J* 9.8 Hz, H-2), 7.25–7.98 (m, 20 H, Ph) (Calc. for $C_{33}H_{30}O_7S$: C, 69.46; H, 5.30. Found: C, 69.71; H, 5.39%).

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