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Synthesis of a fully protected glycooctaosyl serine isolated from blood group A human ovarian mucin

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Abstract

N-(9-Fluorenylmethoxycarbonyl)-*O*-{[*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)]-*O*-(4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 6)]-*O*-[(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)]-*O*-(4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-(1 → 3)-L-serine allyl ester, a protected glycosylserine identified as a blood group A mucin-type determinant, was synthesized for the first time in an efficient and stereocontrolled manner. Ethyl *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1 → 3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)]-*O*-(4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside and *N*-(9-fluorenylmethoxycarbonyl)-*O*-{*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-(1 → 3)-*O*-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 2)]-*O*-(4,6-di-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranosyl}-(1 → 3)-L-serine allyl ester were the key intermediates for the crucial glycosylation to afford the title compound.

Keywords: Serine; Protected glycooctaosyl; Mucin, human ovarian

1. Introduction

The carbohydrate structures responsible for blood group activity have been identified by immunological and chemical studies of the oligosaccharides isolated from mucin

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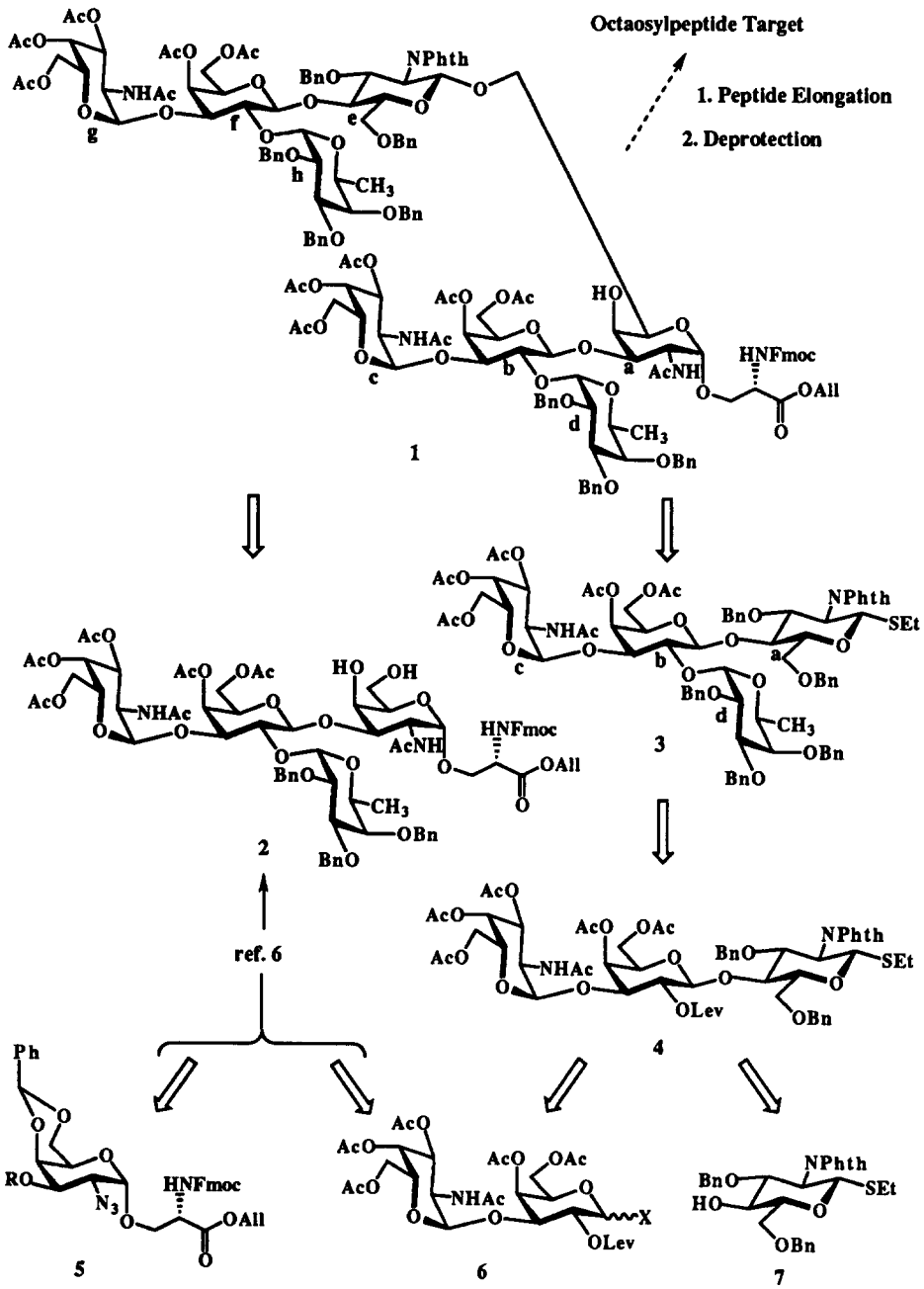
glycoproteins of various organs and species [1]. Bush and coworkers [2] could identify 14 different reduced alditols, ranging in size from monosaccharide to decasaccharide, all obtainable by Carlson degradation [3] of blood group A ovarian cyst mucin glycoproteins. Elucidation of these alditol structures was possible by a combination of NMR spectral methods and enzymatic degradations, with β-Gal, α-Fuc, α-GalNAc and β-GlcNAc being the characteristic terminal units. All larger oligosaccharides had at their nonreducing termini the α-D-GalNAc(1 → 3)(α-L-Fuc(1 → 2))-β-D-Gal substructure; however, structure **1** was a particularly attractive synthetic target on the basis of it possessing two such substructures interconnected by the characteristic core II sequence [4], β-D-Gal(1 → 3)(β-D-GlcNAc(1 → 6))-α-D-GalNAc. It is thus consistent with the corresponding octaosyl glycoprotein having high blood-group activity [5]. Recently we have reported on the synthesis of the glycotetraosyl serine, the deprotected form of synthon **2** [6], and in the same work it was demonstrated that the *O*-4a,6a-benzylidene group in **15** could be efficiently removed leaving the acid-sensitive fucosyl glycoside intact. These observations prompted us to construct the more complex octaosyl serine derivative **1**, whereby the C-2e phthalimido group was a necessary design feature to attain solely the β-glycosylation product. However, in view of the forcing conditions required for conversion of a phthalimido group to an acetamido functionality [1], a deprotection protocol for **1** may rely on accomplishing condensation of an amino acid protected by base-stable groups to the amino terminus of serine in **1** prior to attaining acetamido conversion (see retrosynthetic scheme). Nevertheless, the merit of the work described herein is that it represents an efficient and stereocontrolled synthetic route towards a protected octaosyl serine glycopeptide, whereas isolation from the natural source merely generates the equivalent reduced octasaccharide alditol, only.

2. Results and discussion

Since we have recently established the stereocontrolled synthesis of the tetrasaccharide-linked to serine **15** [6], the synthon **2** equivalent, retrosynthetic analysis for the target octaosyl serine led us to design another tetrasaccharide half on the basis of the convergent strategy shown. Numerous examples appear in the literature of employing thioglycosides as efficient glycosylation agents [8], whereby such thioglycosides are often used as the stable reducing end unit during a variety of transformations involving protective-group manipulations as well as glycosylations. Thus the choice of the thioglycoside **7** as the building block for constructing the key tetrasaccharide donor **3** would meet our requirements.

Benzylation of the known compound **8** [9] (**8** → **9**, 82%) and reductive benzylidene ring opening [10] produced **7** (81%).

[1] A model study, carried out in an attempt to evaluate the removal of the phthalimido function from compound **7** in the presence of a base-sensitive substrate **5** (R = *tert*-BuMe$_2$Si) using a mild sodium borohydride reductive method [7], led to extensive decomposition of **5**.


Octaosylpeptide Target
1. Peptide Elongation
2. Deprotection
1
2
3
4
5
6
7
ref. 6

Scheme 1.

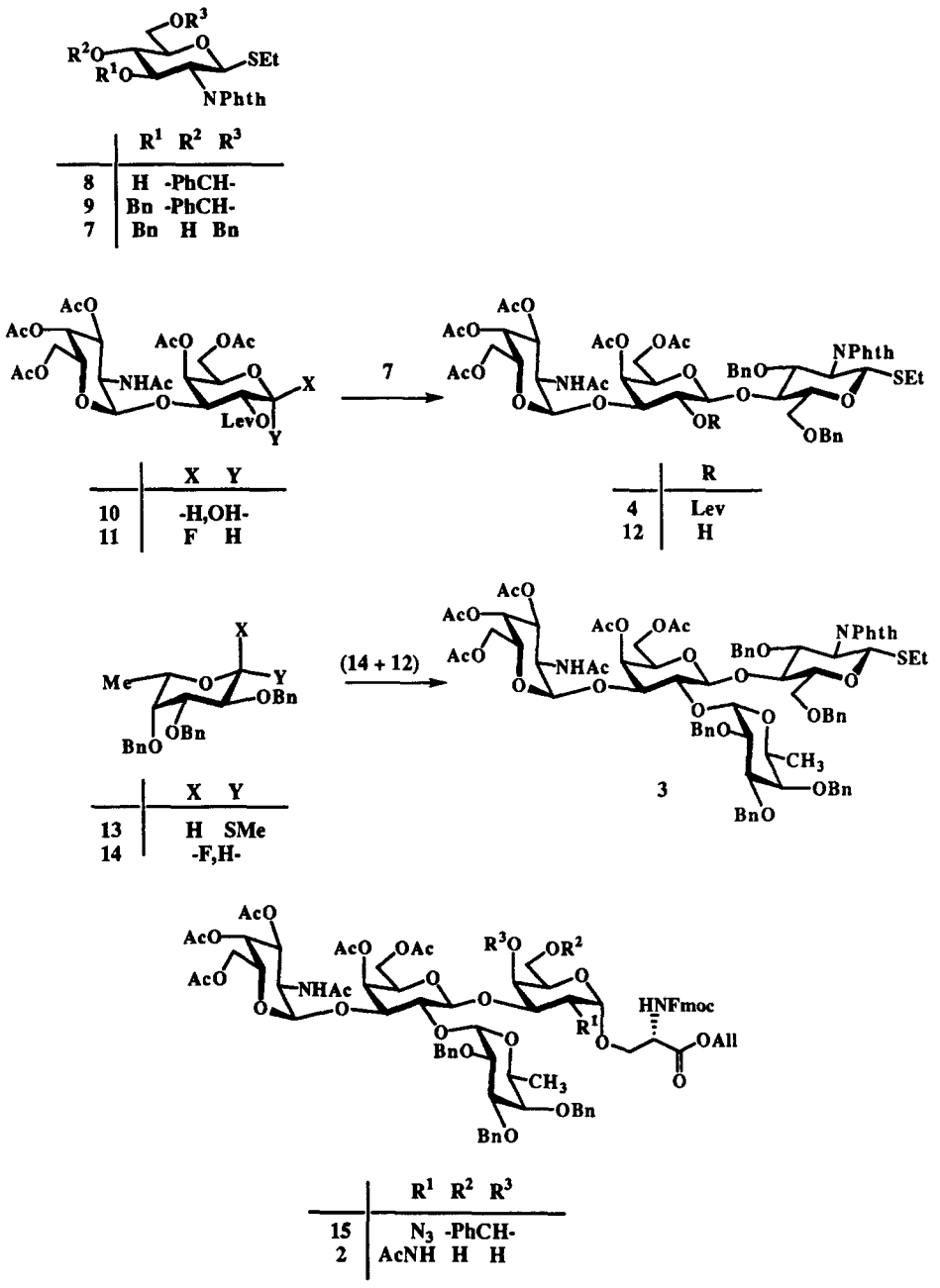
An efficient β-(1 → 3) glycosylation of a 2-azido galactose derivative **5** (R = H) with the α-imidate derived from the hemiacetal **10** using $(CH_3)_3SiOSO_2CF_3$ as a promoter was demonstrated in our previous work [6]. However, attempts to apply the latter coupling method to generate the β-(1 → 4) glycosylation product **4** using acceptor **7**, including use of boron trifluoroetherate in lieu of $(CH_3)_3SiOSO_2CF_3$ as a promoter, were met with failure. The fluoride **11**, derived from the hemiacetal **10** [6] in 85% yield by treatment with diethylaminosulfur trifluoride, proved to be the more suitable donor using a Mukaiyama-type promoter (silver triflate–stannous chloride) [11]. Based on consumption of acceptor **7**, β-(1 → 4) thioglycoside **4** ($\delta_{H\text{-}1b}$ 4.58, *J* 8.24 Hz) was obtained in 72% yield. Selective cleavage of the levulinoyl group from **4** without affecting the base-labile phthalimido group was successfully performed with three equivalents of hydrazine acetate in a solution of ethyl acetate–methanol affording **12** in 84%. Having the trisaccharide acceptor **12** at our disposal, we then proceeded towards coupling the latter to 2,3,4-tri-*O*-benzyl-L-fucosyl fluoride (**14**), which had been derived from **13**, based on the method of Nicolaou et al. [12]. After much experimentation with solvent, temperature and donor equivalence, it was observed that use of a solvent mixture of low polarity (5 : 1 Et_2O–toluene) in the presence of silver perchlorate and stannous chloride afforded exclusively the fucosylated product **3** of the desired α-configuration ($\delta_{H\text{-}1d}$ 5.57, *J* 3.66 Hz) in 80% yield. No formation of the corresponding β-fucosylated product was detected employing these glycosylation conditions.

Towards the synthesis of the tetraosyl serine acceptor **2**, the known compound **15** was converted to an acetamido equivalent using thioacetic acid in pyridine, and then acetic acid cleavage of the benzylidene group furnished the requisite acceptor **2** in 62% yield. A crucial glycosylation of the glycotetraosyl serine acceptor **2** with the thioglycoside **3** in the presence of a hypervalent iodine species prepared from iodosobenzene (PhIO) and trifluoromethanesulfonic anhydride [13] proceeded to give the fully protected target **1** as the sole glycosylation product, in 60% yield based on consumption of donor **3**. ^{1}H NMR and FAB-mass spectral data of the synthetic sample were in good agreement with those for the postulated structure **1**.

In conclusion, the protected glycooctaosyl-L-serine derivative **1** has been synthesized by employing glycotetraosyl thioglycoside **3** and glycotetraosyl-L-serine **2** as key intermediates. The synthetic steps leading up to these intermediates and the crucial glycosylation of the latter are all examples of efficient synthetic methodology.

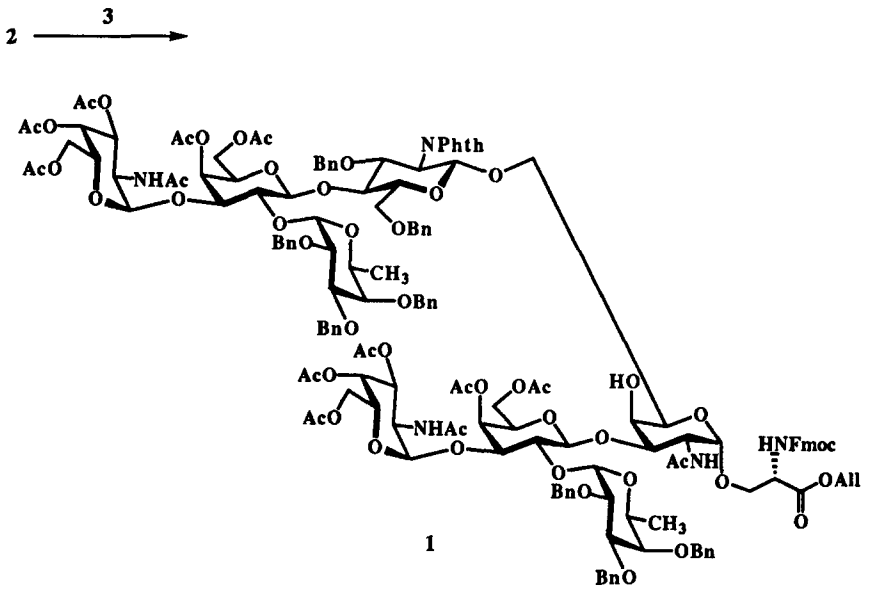
3. Experimental

General. — Optical rotations were determined with a JASCO DIP 370 polarimeter for solutions in $CHCl_3$. Column chromatography was performed on Silica Gel-60 (E. Merck 70–230 mesh). Flash chromatography was performed on Wako Gel C-300 (200–300 mesh). Thin-layer (TLC) and high-performance thin-layer chromatography (HPTLC) were performed on Silica Gel-60 F_{254} (E. Merck). NMR spectra were recorded with a JEOL GX 500 [^{1}H (500 MHz)] or as indicated with a JEOL GX 270 [^{1}H (270 MHz)] spectrometer. Chemical shifts are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$. FAB-mass spectra and SIMS-mass spectra were



Scheme 2.

obtained with a JEOL HX110(HF) and Hitachi M80 mass spectrometers using glycerol and 3-nitrobenzylalcohol, respectively, as matrices. Solvents were dried and purified using standard methods [14].



Scheme 3.

Ethyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**9**). — A mixture of compound **8** (1.31 g, 2.97 mmol) and 60% NaH in oil (300 mg, 7.48 mmol) in dry THF (26 mL) was stirred at 60°C for 1 h. Benzyl bromide (798 mL, 6.68 mmol) was added, and stirring was continued at 60°C overnight. Small pieces of ice were then added for quenching, and the solution was evaporated in vacuo, extracted with 1:1 ether–EtOAc, washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography of the residue on silica gel (65 g) in 7:3 hexane–EtOAc afforded **9** (1.30 g, 82.5%); $[\alpha]_D^{28} + 47.2$ (*c* 0.8); R_f 0.37 (7:3 hexane–EtOAc); NMR data: δ_H (270 MHz) 7.85–6.87 (m, 14 H, Ar), 5.631 (s, 1 H, PhC*H*), 5.344 (d, 1 H, *J* 10.56 Hz, H − 1), 4.758 and 4.548 (2d, 2 H, *J* 12.2 Hz, CH_2Ph), 4.440 (d, 1 H, *J* 8.58 Hz, H-4), 4.426 (m, 1 H, H-2), 4.297 (t, 1 H, *J* 10.23 Hz, H-5), 3.87–3.66 (m, 3 H, H-6, H-6′ and H-3), 2.652 (m, 2 H, SCH_2CH_3), 1.165 (t, 3 H, *J* 7.26 Hz, SCH_2CH_3); MS (SIMS): *m/z* 531 $[C_{30}H_{29}NO_6S]^+$.

Ethyl 3,6-di-O-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**7**). — To a stirred mixture of **9** (1.29 g, 2.42 mmol), $NaBH_3CN$ (1.37 g, 21.8 mmol) and crushed 3 Å molecular sieves (2.18 g) in THF (14.5 mL) at room temperature was added HCl-saturated ether until the reaction mixture was acidic at pH 3. The mixture was left to stir for 4 h. Addition of triethylamine (2.0 mL) neutralized the mixture, which was then filtered through Celite. The filtrate washed with water, dried, and evaporated in vacuo. The resultant syrup was purified twice by column chromatography (7:3 hexane–EtOAc) to give **7** (1.04 g, 80.8%); $[\alpha]_D^{25} + 41.9°$ (*c* 1.2); R_f 0.36 (5:3 hexane–EtOAc); NMR data: δ_H (270 MHz) 7.82–6.93 (m, 14 H, Ar), 5.271 (d, 1 H, *J* 8.91 Hz, H-1), 4.748 and 4.537 (ABq, 2 H, *J* 12.21 and 57.41 Hz, CH_2Ph), 4.66–4.60 (m, 3 H, H-3 and CH_2Ph), 4.253 (t, 2 H, H-2 and H-4), 3.87–3.64 (m, 3 H, H-6, H-6′

and H-5), 2.985 (s, 1 H, OH), 2.70–2.59 (m, 2 H, SCH_2CH_3), 1.160 (t, 3 H, *J* 7.43 Hz, SCH_2CH_3); MS (SIMS): *m/z* 533 $[C_{30}H_{31}NO_6S]^+$; 556 $[M + Na]^+$; 572 $[M + K]^+$.

O-(2-*Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-(1 → 3)-4,6-di*-O-*acetyl-2*-O-*levulinoyl*-D-*galactopyranosyl fluoride* (**11**). — To a stirred solution of the hemiacetal **10** [6] (90.0 mg, 0.130 mmol) in CH_2Cl_2 (1 mL), was added diethylaminosulfur trifluoride (27.5 mL, 0.208 mmol) at 0°C, and the mixture was left stirring for 30 min at room temperature. The reaction was quenched by adding MeOH (1 mL), diluted with EtOAc, washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography of the syrup (20:20:3 toluene–EtOAc–MeOH, 5 g of silica gel) gave the fluoride **11** (76.4 mg, 84.6%, α-F: β-F = 3:7); $[\alpha]_D^{28}$ + 64.5° (*c* 0.4); R_f 0.29 (20:20:3 toluene–EtOAc–MeOH); NMR data: δ_H 6.019 (d, 1 H, 0.7 H, *J* 9.46 Hz, βNH), 5.827 (d, 0.3 H, *J* 9.77 Hz, αNH), 5.738 (dd, 0.3 H, *J* 2.75, 53.4 Hz, αH-1a), 5.523 (d, 0.3 H, *J* 2.13 Hz, αH-4b), 5.505 (d, 0.7 H, *J* 2.13 Hz, βH-4b), 5.102 (d, 0.3 H, *J* 3.67 Hz, αH-1b), 5.071 (dd, 1 H, *J* 3.05, 11.6 Hz, H-3b), 4.67–4.59 (m, 1 H, α- and βH-2b), 4.37–3.96 (m, 7 H, H-6a, H-6'a, H-5a, H-6b, H-6b', H-5b and H-2), 2.94–2.53 (m, 4 H, CH_2CH_2 of Lev), 2.061, 2.058, 2.052, 1.969, 1.954, 1.953 [6s, 21 H (2.061, 1s, 6 H), 6Ac and CH_3CO of Lev]. Anal. Calcd for $C_{29}H_{40}FNO_{17}$: C, 50.22; H, 5.81; N, 2.02. Found: C, 49.75; H, 5.79; N, 2.04.

Ethyl O-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-(1 → 3)*-O-*(4,6-di*-O-*acetyl-2*-O-*levulinoyl-β*-D-*galactopyranosyl)-(1 → 3)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* (**4**). — Under an atomsphere of argon, a mixture of $AgOSO_2CF_3$ (65 mg, 0.252 mmol) and $SnCl_2$ (48 mg, 0.252 mmol) was added to 4 Å molecular sieves (368 mg) and stirred in 2:1 dichloroethane–toluene (0.3 mL) for 20 min at room temperature. A mixture of the donor **11** (175 mg, 0.252 mmol) and acceptor **7** (45 mg, 0.084 mmol) in the same solvent (1.0 mL) was added thereafter at room temperature and left to stir for 7 h. The reaction mixture was diluted with EtOAc, filtered over Celite, and the filtrate was washed with water, brine, dried (Na_2SO_4), and concentrated in vacuo. Column chromatography of the residue on silica gel (11 g) with 2:1 toluene–EtOAc gave **4** (72 mg, 72.0%); recoveries of **11** and **7** were 20.0% and 11.5%, respectively.

Compound **4** had $[\alpha]_D^{23}$ + 50.0° (*c* 1.2); R_f 0.37 (20:20:3 toluene–EtOAc–MeOH). NMR data: δ_H 7.80–6.87 (m, 14 H, Ar), 6.170 (d, 1 H, *J* 9.46 Hz, NH), 5.450 (d, 1 H, *J* 2.14 Hz, H-4), 5.243 (brs, 1 H, H-4), 5.230 (d, 1 H, *J* 7.63 Hz, H-1a), 5.080 (dd, 1 H, *J* 7.93, 9.76 Hz, H-2b), 4.999 (d, 1 H, *J* 3.66 Hz, H-1c), 4.939 (dd, 1 H, *J* 3.05, 11.06 Hz, H-3c), 4.813 (t, 2 H, *J* 11.90 Hz, CH_2Ph), 4.578 (d, 1 H, *J* 7.94 Hz, H-1b), 4.471 (dd, 2 H, *J* 11.90 Hz, CH_2Ph), 2.820 (t, 2 H, CH_2CH_2 of Lev), 2.70–2.54 (m, 4H, CH_2CH_2 of Lev and SCH_2CH_3), 2.224, 2.145, 2.079, 2.020, 2.009, 1.972, 1.967 (7s, 21 H, Ac), 1.176 (t, 3 H, *J* 7.33 Hz, SCH_2CH_3). δ_C (100.4 MHz) 100.16 (βC-1b), 98.03 (αC-1c), 81.05 (βC-1a). Anal. Calcd for $C_{59}H_{70}N_2O_{23}S$: C, 58.70; H, 5.84; N, 2.32. Found: C, 58.81;H, 5.81; N, 2.20.

Ethyl O-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-(1 → 3)*-O-*(4,6-di*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 3)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* (**12**). — Hydrazine acetate (5 mg, 0.052 mmol) was added to a stirred solution of the trisaccharide **4** (21 mg, 0.018 mmol), in 1:1 EtOAc–MeOH (0.2 mL) under argon at room temperature and left to stir for 2 h. The reaction mixture

was evaporated in vacuo, diluted with EtOAc, washed with water, brine, dried (Na_2SO_4) and evaporated in vacuo to a residue. Flash chromatography with 20:20:3 toluene–EtOAc–MeOH furnished compound **12** (16 mg, 83.5%); $[\alpha]_D^{25}$ + 68.5° (*c* 0.81); R_f 0.43 (20:20:3 toluene–EtOAc–MeOH). NMR data: δ_H 7.76–6.85 (m, 14 H, Ar), 6.168 (d, 1 H, *J* 10.07 Hz, NH), 5.362 (dd, 1 H, *J* 1.83 Hz, H-4), 5.219 (d, 1 H, *J* 9.47 Hz, H-1a), 5.193 (dd, 1 H, *J* 2.44 Hz, H-4), 5.021 (dd, 1 H, *J* 3.66, 11.60 Hz, H-3c), 4.940 (d, 1 H, *J* 3.66 Hz, H-1c), 4.796 (2d, 2 H, *J* 12.21, 37.54 Hz, CH_2Ph), 4.664 (ddd, 1 H, *J* 3.66, 10.07, 11.60 Hz, H-2c), 4.608 (d, 1 H, *J* 7.62 Hz, H-1b), 4.455 (d, 1 H, *J* 12.2 Hz, CH_2Ph), 3.469 (dd, 1 H, *J* 3.35, 9.46 Hz, H-2a), 2.70–2.58 (m, 2 H, SC$H_2$$CH_3$), 2.169, 2.143, 2.055, 2.003, 1.996, 1.937 (6s, CH_3CO of OAc, NHAc), 1.174 (t, 3 H, *J* 7.63 Hz, SCH_2CH_3). Anal. Calcd for $C_{54}H_{64}N_2O_{21}S$: C, 58.47; H, 5.82; N, 2.52. Found: C, 58.18; H, 5.82; N, 2.36.

Ethyl O-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl*)-(*1 → 3*)-O-[(*2,3,4-tri*)-O-*benzyl-α*-L-*fucopyranosyl*)-(*1 → 2*)]-O-(*4,6-di*-O-*acetyl-β*-D-*galactopyranosyl*)-(*1 → 3*)-*3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-1-thio-β*-D-*glucopyranoside* (**3**). — $AgClO_4$ (62 mg, 0.297 mmol) and $SnCl_2$ (56 mg, 0.297 mmol) were added to molecular sieves 4 Å (390 mg) contained in a dark 10 mL 2-neck round-bottom flask under argon. A mixture of 5:1 Et_2O–toluene (1.3 mL) was added to the reaction vessel and the mixture was allowed to stir for 30 min at room temperature, it was then cooled in an ice–MeOH bath. At −20 °C, a mixture of donor **14** (130 mg, 0.297 mmol) and acceptor **12** (66.0 mg, 0.059 mmol) in 5:1 Et_2O–toluene (4.0 mL) was added to the reaction vessel and stirred for 8 h with the temperature rising gradually to room temperature. The reaction mixture was diluted with EtOAc, filtered through Celite, washed with aq $NaHCO_3$, brine, dried ($MgSO_4$) and evaporated in vacuo. The crude residue was purified by gel-permeation chromatography (Biobeads S-X4; 300 mL, toluene) then further purified by preparative TLC (10:10:1 toluene–EtOAc–MeOH) to afford **3** (73 mg, 80.5%).

Compound **3** had $[\alpha]_D^{25}$ + 17.8° (*c* 1.63); R_f 0.47 (10:10:1 toluene–EtOAc–MeOH). NMR data: δ_H 7.81–6.89 (m, 29 H, Ar), 5.966 (d, 1 H, *J* 9.15 Hz, NH), 5.566 (d, 1 H, *J* 3.66 Hz, H-1d), 5.235 (d, 1 H, *J* 2.74 Hz, H-4c), 4.918 (dd, 1 H, *J* 2.75, 11.60 Hz, H-3c), 4.824 (dd, 2 H,*J* 11.9, 15.26 Hz, CH_2Ph), 4.664 (d, 1 H, *J* 11.29 Hz, CH_2Ph), 4.530 (ddd, 1 H, *J* 3.36, 11.60 Hz, H-2a), 4.502 and 4.404 (2d, 2 H, *J* 11.9 Hz, CH_2Ph), 4.454 (d, 1 H, *J* 7.63 Hz, H-1b), 3.797 (t, 1 H, *J* 11.9 Hz, H-4d), 2.72–2.62 (m, 2 H, SC$H_2$$CH_3$), 2.096, 2.022, 1.977, 1.969, 1.940, 1.825 (6s, 18 H, Ac), 1.360 (d, 3 H, *J* 6.15 Hz, CH_3 of Fuc), 1.220 (t, 3 H, *J* 7.44 Hz, SCH_2CH_3). Anal. Calcd for $C_{81}H_{92}N_2O_{25}S$: C, 63.76; H, 6.08; N, 1.84. Found: C, 63.73; H, 6.12; N, 1.79.

N-(*9-Fluorenylmethoxycarbonyl*)-O-{O-(*acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl*-(*1 → 3*)-O-[(*2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl*)-(*1 → 2*)]-O-(*4,6-di*-O-*acetyl-β*-D-*galactopyranosyl*-(*1 → 3*)}-*2-acetamindo-2-deoxy-α*-D-*galactopyranosyl*-(*1 → 3*)-L-*serine allyl ester* (**2**). — To compound **15** (125 mg, 0.0767 mmol) was added freshly distilled thioacetic acid (2.3 mL), followed by dry pyridine (1.2 mL). The reaction mixture was left to stir at room temperature for 24 h, then evaporated several times with toluene in vacuo. To the resulting residue was added 80% aq AcOH (11.2 mL), and the mixture was stirred for 12 h at 60°C. The reaction mixture was diluted with EtOAc, washed with aq $NaHCO_3$, 1% NaClO solution, and brine, dried (Na_2SO_4) and

evaporated in vacuo. Purification of the crude product by preparative TLC (10:10:2 toluene–EtOAc–MeOH) gave **2** (74 mg, 62.0%); $[\alpha]_D^{25}$ + 51.8° (*c* 0.38); R_f 0.21 (10:10:2 toluene–EtOAc–MeOH). NMR data: δ_H (270 MHz) 7.746 (d, 1 H, *J* 7.26 Hz, Ar of Fmoc), 7.42–7.26 (m, 15 H, Ar of Bn), 5.808 (d, 1 H, *J* 7.92 Hz, NH), 5.402 (d, 1 H, *J* 3.67 Hz, H-1d), 5.90–5.70 (m, 1 H, $CH_2CH:CH_2$), 5.250 (m, 1 H, $CH_2CH:CH_2$), 5.134 (d, 1 H, *J* 3.63 Hz, H-1c), 5.020 and 4.756 (2d, 2 H, *J* 11.22 Hz, CH_2Ph), 4.953 and 4.660 (2d, 2 H, J 12.2 Hz, CH_2Ph), 2.200, 2.171, 2.164, 2.045, 1.976, 1.911, 1.894 (7s, 21H, CH_3CO of OAc and NHAc), 1.246 (d, 3 H, *J* 6.27 Hz, CH_3 of Fuc). MS: *m/z* 1584 $[M + Na]^+$.

N-*(9-Fluorenylmethoxycarbonyl)*-O-*[{*O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-(1 → 3)*-O-*[(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1 → 2)]*-O-*(4,6-di*-O-*acetyl-β*-D-*galactopyranosyl)-(1 → 6)}*-O-*{*-O-*(2-acetamido 3,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*galactopyranosyl)-(1 → 3)*-O-*[(2,3,4-tri*-O-*benzyl-α*-L-*fucopyranosyl)-(1 → 2)]*-O-*(4,6-di*-O-*acetyl-β*-D-*galactopyranosy-(1 → 4)l*-O-*3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl-(1 → 3)}-2-acetamido-2-deoxy-α*-D-*galactopyranosyl]-(1 → 3)*-L-*serine allyl ester* (**1**). — PhIO (2 mg, 9.3 μmol) and $(CF_3SO_2)_2O$ (1.53 mL, 9.0 μmol) in sequence were added to 4 Å molecular sieves (47 mg) contained in a two-neck round-bottom flask. 1,2-Dichloroethane (0.1 mL) was added, and the mixture was stirred for 1 h at room temperature under argon. Upon cooling to −18°C, a solution of **3** (12 mg, 7.6 μmol) and **2** (24 mg, 0.015 mmol) dissolved $(CH_2Cl)_2$ (0.2 mL) was added to the stirred mixture. The reaction mixture was left to stir for 24 h with the temperature gradually rising to room temperature. The mixture was then diluted with EtOAc, filtered through Celite, washed with brine,dried ($MgSO_4$) and evaporated in vacuo. Purification by preparative TLC (10:10:2 toluene–EtOAc–MeOH) isolated target **1** (14 mg, 60.0%), 4 mg of decomposed donor **3** and 7 mg of recovered acceptor **2**.

Compound **1** had $[\alpha]_D^{22}$ + 13.5° (*c* 0.68); R_f 0.41 (10:10:2 toluene–EtOAc–MeOH). NMR data: δ_H 7.744 (d, 2 H, *J* 7.32 Hz, Ar of Fmoc), 7.652 (brs, 2 H, Ar of Fmoc), 7.554 (d, 2 H, *J* 7.33 Hz, Ar of Fmoc), 7.48–7.43 (m, 4 H, Ar of Fmoc and Bn), 7.38–7.21 (m, 38 H, Ar of Bn), 6.987 and 6.865 (2m, 4 H, Ar of NPhth), 6.004 (d, 1 H, *J* 8.85 Hz, NH), 5.808 (m, 1 H, $CH_2CH:CH_2$), 5.559 (d, 1 H, *J* 3.96 Hz, H-1h), 5.530 (brs, 1 H, H-4), 4.776 (2d, 2 H, *J* 10.38 Hz, CH_2Ph), 2.155 (6 H), 2.099, 2.002, 1,974 (6 H), 1.972 (6 H), 1.964, 1.950, 1.898, 1.874, 1,797 (10s, 39 H, CH_3CO of OAc, NHAc), 1.355 (d, 3 H, *J* 6.41 Hz, CH_3 of Fuc), 1.190 (d, 3 H, *J* 6.10 Hz, CH_3 of Fuc). MS: *m/z* 3025 $[M + 1]^+$, 3048 $[M + Na]^+$.

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