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Synthesis of D-Galactopyranosides of *trans*-4-Hydroxy-L-proline Utilizing the Sulfoxide Glycosylation Method

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Phenyl 1-thio-β-D-galactopyranoside (1) was converted into two sulfoxide glycosyl donors: phenyl (2,3,4,6-tetra-*O*-benzyl)-1-thio-β-D-galactopyranoside *S*-oxide (3) and phenyl (2,3,4,6-tetra-*O*-pivaloyl)-1-thio-β-D-galactopyranoside *S*-oxide (5). These glycosyl donors were then each reacted with *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline allyl ester (7). The glycosylation reactions were conducted at –70°C with triflic anhydride as promotor, in the presence of 2,6-di-*tert*-butyl-4-methylpyridine. In the case of the perbenzylated sulfoxide donor (3), the major product was *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-benzyl)-α-D-galactopyranosyl]-L-proline allyl ester (8α). In dichloromethane, the α-to-β ratio was 3:1 and in toluene this improved to 5:1, with a combined yield of 41%. In the case of the perpivaloylated sulfoxide donor (5), *N*α-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-4-*O*-[(2,3,4,6-tetra-*O*-pivaloyl)-β-D-galactopyranosyl]-L-proline allyl ester (9) was obtained as the sole glycoside product in 46% yield.

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Introduction

Glycosides of *trans*-4-hydroxy-L-proline (Hyp) are widespread in the plant kingdom (Fig. 1).[1] In 1969, Lamport proposed that the role of Hyp in extensin was to provide a site for a covalent linkage between the protein and the cell wall.[2] Hydroxyproline-rich glycoproteins (HRGPs) are glycosylated by saccharides ranging from a single arabinose or galactose residue, up to a 75-residue arabinogalactan.[3] A β-D-xyloside was isolated from the leaves of sugar beet, *Beta vulgaris*.[4] Two discoveries of Hyp glycosides have been made outside the plant kingdom: a β-D-galactoside from an algae, *Chlamydomonas reinhardtii*,[5] and a novel, linear hexasaccharide, linked via an *N*-acetylglucosamine (GlcNAc) residue, was recently identified in SKP1, a glycoprotein of the slime mould *Dictyostelium discoideum*.[6]

The synthesis of glycosides of Hyp has attracted considerable attention. This is due to the potential of peptide glycosylation to modulate biological activity rather than because of their occurrence in nature. For example, H–Tyr–D–Met–Gly–Phe–(β-D-Galp–Hyp)–NH_2 is a particularly potent enkephalin analogue.[7]

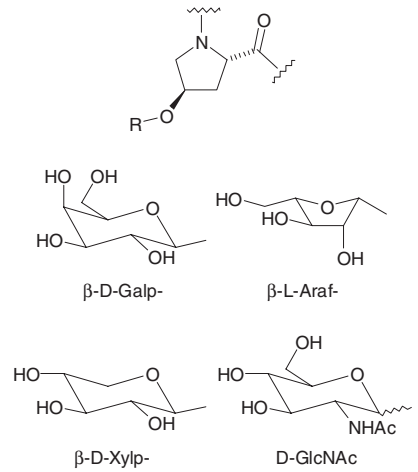

Fig. 1. Glycosides of *trans*-4-hydroxy-L-proline.

We chose to focus on the synthesis of galactosides. Such Hyp derivatives have been synthesized enzymatically using β-galactosidases from *E. coli* and *Achatina achatina*, but the chemical yields were less than 30%.[8,9] Several chemical methods have been employed for the synthesis of Hyp

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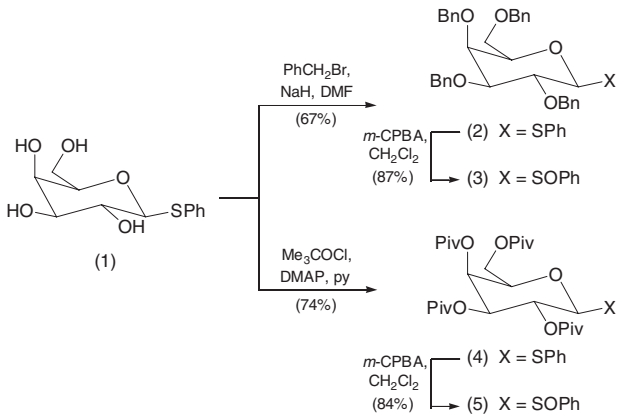
glycosides: the Helferich reaction,[10–17] the Koenigs–Knorr reaction,[12,17–21] the use of anomeric triflates,[22] and the trichloroacetimidate method.[17,23] Glycosides have rarely been obtained in greater than 60% yield.

The sulfoxide glycosylation method was introduced by Kahne and coworkers in 1989.[24,25] A sulfoxide glycosyl donor is activated with triflic anhydride, in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), to give a species which is highly reactive at low temperatures.[26,27] The method has found wide application in the construction of glycosidic linkages to sterically hindered alcohols.[28] Glycosides of Thr have been synthesized and the resulting building blocks incorporated successfully into glycopeptides.[29,30] We sought to apply the sulfoxide glycosylation method to the synthesis of galactosides of Hyp. We felt that the high reactivity of the glycosylating species, coupled with the ability to control the stereochemistry of the glycosidic linkage by an appropriate choice of protecting groups, boded well for the synthesis of both α- and β-galactosides. The synthesis of both is ultimately important for diversity when studying structure–activity relationships (SARs).

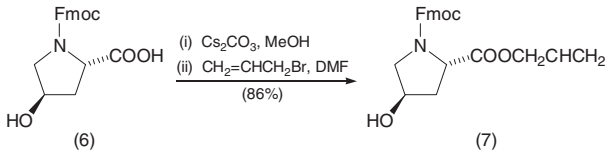
Results and Discussion

Phenyl 1-thio-β-D-galactopyranoside (1) used to be commercially available at reasonable cost; this is no longer the case. This starting material was therefore prepared in two steps from 1,2,3,4,6-penta-*O*-acetyl-D-galactose. [31]* Sulfoxides (3)[32] and (5),[33] intended to lead to α- and β-glycosides, respectively (see above), were both prepared in two steps from compound (1) (Scheme 1).



Scheme 1

Our choice of a *trans*-4-hydroxy-L-proline (Hyp) glycosyl acceptor involved temporary Nα-protection by the fluorenylmethyl carbamate (Fmoc) group and subsequent masking of the carboxylic acid as its allyl ester. This choice was influenced by protecting groups employed in other projects ongoing in our laboratory,[34] and the experience of Kunz and coworkers.[35] The allyl ester (7) was synthesized from Fmoc–Hyp–OH (6) according to Scheme 2.



Scheme 2

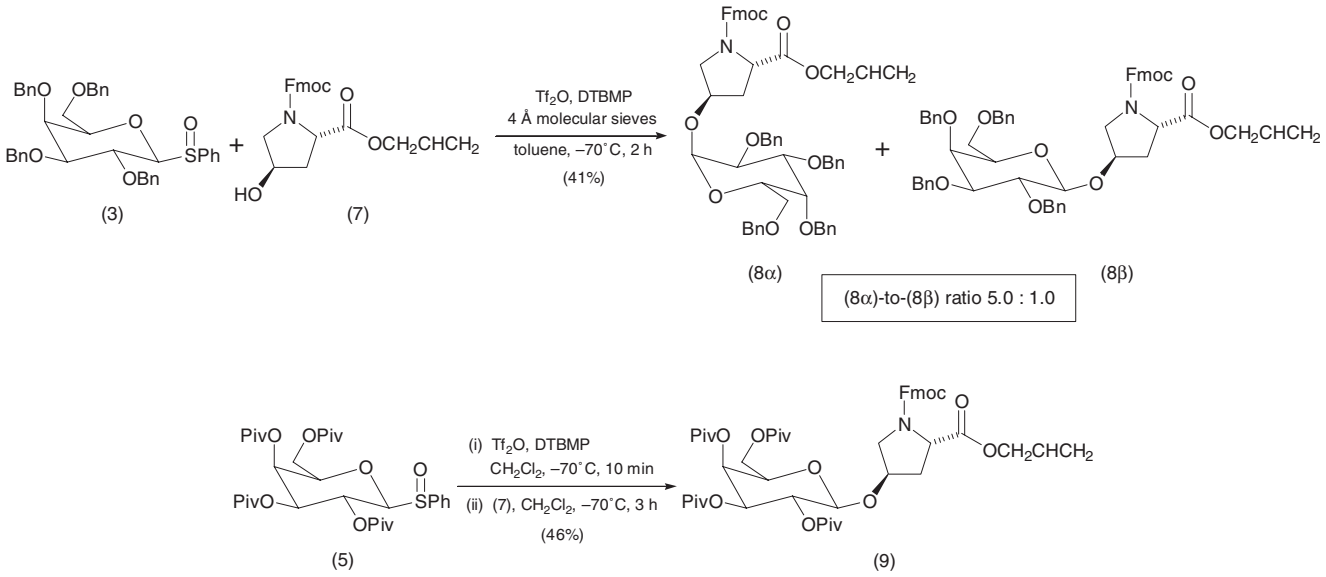
The glycosylation reactions are depicted in Scheme 3. Sulfoxide (3) has been reported previously and employed in the synthesis of oligosaccharides containing an α-D-galactopyranosyl group.[32] In the absence of a 'neighbouring group' at C2, the anomeric effect is normally the dominant force in determining the stereochemistry of the glycosidic linkage. A mixture of anomers of (8) was obtained, favouring the α-glycoside. When the reaction was conducted in dichloromethane a ratio of 3 : 1 was obtained; in toluene this improved to 5 : 1. The glycosides each existed as mixtures of two rotamers about the C–N bonds of their carbamate functionality, and most signals in the nuclear magnetic resonance (NMR) spectra were doubled up. In the case of the α-glycoside, a pair of signals was observed at δ 96.9 and 97.7 in the ^{13}C NMR spectrum, which was assigned to the anomeric carbon. These chemical shifts are consistent with an α-galactoside.[22] The minor glycoside product had a ^{13}C signal at δ 103.3, supporting the presence of a β-glycosidic linkage. Increasing the amount of donor (3) or acceptor (7) did not improve the yield; in fact the resulting increase in by-products made it more difficult to purify the desired products.

Sulfoxide (5), bearing a pivaloate protecting group at C2, has been used by Kahne and coworkers to generate β-galactosides.[33,36,37] The high stereoselectivity observed in these reactions is attributed to neighbouring group participation by the bulky pivaloate ester,[38] which discourages approach of the glycosyl acceptor from the α-face. The steric bulk of the pivaloyl group can also impede glycosylation reactions, perhaps accounting for the modest yield of β-glycoside (9) in the present case. A single stereoisomer was obtained, which appeared as a mixture of two rotamers about the carbamate C–N bond in the NMR spectra. Assignment of the stereochemistry of the glycosidic linkage was based on literature precedent,[33,36,37] and the ^{13}C NMR spectrum, in which two signals were observed at δ 100.3 and 100.5. These signals were attributed to the anomeric carbon and are consistent with a β-galactoside.

Conclusion

In summary, glycosides (8) and (9) have been synthesized using the sulfoxide glycosylation method in yields of 41 and 46%, respectively. By comparison, Andreotti and Kahne reported a 70% yield for the glycosylation of Fmoc–L-Thr–OBn with phenyl 3,4,6-tri-*O*-acetyl-2-azido-

*1,2,3,4,6-Penta-*O*-acetyl-galactopyranoside can be prepared according to this reference. It is also commercially available from Aldrich (Catalogue No. 13,403-1).



Scheme 3

2-deoxy-1-thio-galactopyranosyl *S*-oxide (1:1 ratio of anomers).[29] Similar results were reported for the attachment of a disaccharide.[30] Their lack of stereocontrol is a consequence of the azido substitutent at C2. The lower chemical yield of our reactions may be attributable to steric hinderance associated with the Hyp–OH glycosyl acceptor, and complications arising from the conformation of the pyrrolidine ring.

It may be possible to enhance the nucleophilicity of the hydroxy group by altering the conformation of the pyrrolidine ring, although the attempts of Burger et al. were clearly a move in the wrong direction.[17] It may also be possible to increase the reactivity of the glycosyl donor in the formation of β-glycosides by utilizing electron-donating *p*-methoxybenzyl (PMB) ether protecting groups, instead of bulky, electron-withdrawing pivaloate esters. PMB ethers have been demonstrated to lead to β-glycosides with good stereoselectivity,[28*a*,39] although the mechanistic basis for this is unclear.

While we will seek to improve the efficiency with which we can produce Hyp glycosides, we already have sufficient quantities of these glycosylated hydroxyproline building blocks to incorporate them into peptides and to investigate the effect of glycosylation on structure and function, relative to the parent peptides.

Experimental

General

Optical rotations were determined on either a Perkin Elmer 341 or an Optical Activity Ltd AA-10 polarimeter. NMR spectra were recorded on one of the following NMR spectrometers: Bruker DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C), Bruker Avance 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C), Bruker AM-200 (200 MHz for ^{1}H and 50 MHz for ^{13}C) or JEOL 270 JNM-GX270W (270 MHz for ^{1}H and 67.5 MHz for ^{13}C). NMR data for compounds (2), (3A,B), (4), (5A,B), (7), (8α,β) and (9) are available as supplementary material (website: http://www.publish.csiro.au/journals/ajc/AccessMat.cfm). Thin-layer chromatography (TLC) was performed on Kieselgel 60 F254 (E-Merck) and spots were visualized by ultraviolet (UV) light or by staining with anisaldehyde. Flash chromatography was conducted on silica gel 60 (230–400 mesh) from Scharlau. High-performance liquid chromatography (HPLC) was conducted on a Waters 600 semi-preparative system, equipped with a Waters 2487 dual wavelength UV detector. Methanol was dried and distilled from magnesium turnings and stored over 4 Å molecular sieves. *N*,*N*-Dimethylformamide (DMF) was dried and distilled from BaO and stored over 4 Å molecular sieves. Dichloromethane was freshly distilled from CaH_2. Pyridine was dried and distilled from CaH_2 and stored over KOH pellets. Toluene was freshly distilled from Na. Allyl bromide and pivaloyl chloride were obtained from Acros. Thiophenol was purchased from Riedel-de-Häen. Sodium methoxide, Amberlite-IR H^+ resin, sodium hydride (as a 60% dispersion in mineral oil) and 1,6-di-*tert*-butyl-4-methylpyridine (DTBMP) were purchased from Aldrich. Benzyl bromide and *N*,*N*-dimethylaminopyridine (DMAP) were obtained from Merck. *m*-Chloroperbenzoic acid (*m*-CPBA) and caesium carbonate were obtained from Janssen. *trans*-4-Hydroxyproline (Hyp) was purchased from Lancaster. All other reagents were used without further purification.

Phenyl 1-Thio-β-D-galactopyranoside (1)

$BF_3{\cdot}OEt_2$ (13.08 mL, 15.09 g, 0.106 mol, 5 equiv.) was added dropwise to a solution of 1,2,3,4,6-penta-*O*-acetyl-D-galactose[31] (8.30 g, 0.021 mol, 1.0 equiv.) and thiophenol (2.62 mL, 2.81 g, 0.026 mol, 1.2 equiv.) in CH_2Cl_2 (85 mL) at 0°C. The mixture was warmed to room temperature and then heated at reflux for 5.5 h. The mixture was cooled, quenched with water and diluted with CH_2Cl_2 (100 mL), washed with water (200 mL), sat. aq. $NaHCO_3$ (200 mL), water (200 mL) and brine (200 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography, with 3:1 to 1:1 hexanes/EtOAc as eluent, to give mixed fractions of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-galactopyranoside and phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (4.59 g, 49%), and pure phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (4.62 g, 49%). Total yield 98%.

*Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside*: (Found [HRMS–chemical ionization (CI)]: $[M+NH_4]^+$, 458.1485. Calc. for $C_{20}H_{28}NO_9S$: $[M+NH_4]^+$, 458.1484). R_F 0.53 (1:1 hexanes/EtOAc). $[\alpha]_D^{20}$ +5.3° (*c*, 1.35 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.98, s, 3H, Ac; 2.05, s, 3H, Ac; 2.10, s, 3H, Ac; 2.12, s, 3H, Ac; 3.94, app. t, *J* 6.5 Hz, 1H, H5; 4.11, dd, *J* 11.3, 6.1 Hz, 1H, H6a; 4.20, dd, *J* 11.3, 7.2 Hz, 1H, H6b; 4.72, d, *J* 9.9 Hz, 1H, H1; 5.05, dd, *J* 9.9, 3.2 Hz, 1H, H3;

5.25, app. t, *J* 9.9 Hz, 1H, H2; 5.42, d, *J* 3.2 Hz, 1H, H4; 7.30–7.41, m, 3H, ArH; 7.49–7.54, m, 2H, ArH. ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.1, 20.4, 61.3, 66.9 (2C), 71.5, 74.0, 85.9, 127.7, 128.5, 132.1, 168.9, 169.5, 169.7, 169.8.

Sodium methoxide (518 mg, 9.12 mmol, 3.3 equiv.) was added to a solution of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (1.217 g, 2.76 mmol, 1.0 equiv.) in dry methanol (25 mL). The solution was stirred at room temperature, under nitrogen for 2 h. Amberlite-IR120 H^+ resin (4 g) was added and the suspension stirred for 15 min. The resin was removed by filtration and washed well with methanol. The filtrate was concentrated and the residue purified by flash chromatography, with 9:1 to 17:3 CH_2Cl_2/MeOH as eluent, to give (1) as a colourless solid (681 mg, 91%) (Found [HRMS–electron impact (EI)]: M^+, 272.0713. Calc. for $C_{12}H_{16}O_5S$: M^+, 272.0718). R_F 0.11 (9:1 CH_2Cl_2/MeOH). ^{1}H NMR (CD_3OD, 200 MHz) δ 3.35–3.77, m, 5H, H2, H3, H5, H6a, H6b; 3.95, d, *J* 2.6 Hz, 1H, H4; 4.62, d, *J* 9.2 Hz, 1H, H1; 7.17–7.33, m, 3H, ArH; 7.53–7.57, m, 2H, ArH. ^{13}C NMR (CD_3OD, 50 MHz) δ 62.5, 70.4, 70.9, 76.1, 80.3, 90.1, 128.0, 129.9, 132.0, 135.8.

Phenyl (2,3,4,6-Tetra-O-*benzyl)-1-thio*-β-*D-galactopyranoside (2)*

Benzyl bromide (1.93 mL, 2.78 g, 16.3 mmol, 6.5 equiv.) was added to a solution of phenyl 1-thio-β-D-galactopyranoside (1) (681 mg, 2.50 mmol, 1.0 equiv.) in DMF (7.0 mL) at 0°C. Sodium hydride (651 mg, 16.3 mmol, 6.5 equiv.) was added over 1.75 h. The mixture was warmed at 40°C for 5 h, and then left to stir at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (75 mL) and washed with water (75 mL). The aqueous layer was further extracted with CH_2Cl_2 (75 mL). The organic extracts were combined and washed with sat. aq. $NaHCO_3$ (150 mL), water (150 mL) and brine (150 mL), dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography, with 5:1 hexanes/EtOAc as eluent, to afford *phenyl (2*,*3*,*4*,*6-tetra*-O-*benzyl)-1-thio*-β-*D-galactopyranoside* (2) as a colourless solid (1.37 g, 87%) (Found [HRMS–fast atom bombardment (FAB)]: $[M+H]^+$, 633.2655. $C_{40}H_{41}O_5S$ requires $[M+H]^+$, 633.2674). R_F 0.51 (2:1 hexanes/EtOAc). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.56–3.67, m, 4H, H3, H5, H6a, H6b; 3.91, app. t, *J* 9.4 Hz, 1H, H2; 3.97, d, *J* 2.7 Hz, 1H, H4; 4.37–4.95, m, 9H, CH_2Ph, H1; 7.15–7.39, m, 23H, ArH; 7.53–7.58, m, 2H, ArH. ^{13}C NMR ($CDCl_3$, 50 MHz) δ 68.7, 72.7, 73.5 (2C), 74.4, 75.6, 77.3 (2C), 84.1, 87.7, 126.9, 127.4, 127.5, 127.7, 127.8, 127.9, 128.1, 128.3, 128.4, 128.7, 129.3, 129.7, 129.9, 131.4, 131.9, 134.1, 137.8, 138.1, 138.2, 138.7.

Phenyl (2,3,4,6-Tetra-O-*benzyl)-1-thio*-β-*D-galactopyranoside* S-*Oxide (3)*

m-Chloroperbenzoic acid (166 mg, 0.65 mmol, 67% w/w, 1.2 equiv.) was added to a solution of (2,3,4,6-tetra-*O*-benzyl)-1-phenylthio-β-D-galactoside (2) (340 mg, 0.54 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at –30°C. After stirring for 20 min at –30°C under N_2, the reaction mixture was quenched by the addition of dimethyl sulfide (3 drops), and warmed to room temperature. The solution was diluted with CH_2Cl_2 (40 mL), washed with water (40 mL), sat. aq. $NaHCO_3$ (40 mL), and water again (40 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography, with 5:1 then 1:1 hexanes/EtOAc as eluent, to afford the first-eluting diastereoisomer (3A) as a colourless solid (76 mg), fractions containing a mixture of (3A) and the second-eluting diastereoisomer (3B) (129 mg), and (3B) as a colourless solid (100 mg). Total yield: 305 mg (87%) (Found (HRMS–FAB, of mixture): $[M+H]^+$, 649.2642. Calc. for $C_{40}H_{41}O_6S$: $[M+H]^+$, 649.2623).

First-eluting diastereoisomer (3A). R_F 0.29 (2:1 hexanes/EtOAc). ^{1}H NMR [(D_6)acetone, 200 MHz] δ 3.32, dd, *J* 9.9, 5.9 Hz, 1H, H6a; 3.55, dd, *J* 9.9, 5.9 Hz, 1H, H6b; 3.66, td, *J* 5.9, 1.1 Hz, 1H, H5; 3.96, dd, *J* 9.6, 2.9 Hz, 1H, H3; 4.14, dd, *J* 2.9, 1.1 Hz, 1H, H4; 4.23, d, *J* 9.6 Hz, 1H, H1; 4.38, app. t, *J* 9.6 Hz, 1H, H2; 4.61–5.08, m, 8H, CH_2Ph; 7.14–7.24, m, 2H, ArH; 7.27–7.39, m, 14H, ArH; 7.43–7.54, m, 7H, ArH; 7.64–7.69, m, 2, ArH. ^{13}C NMR [(D_6)acetone, 50 MHz] δ 69.8, 72.9, 73.6, 74.9, 75.2, 75.5, 76.1, 79.6, 84.9, 94.4, 126.0, 128.2, 128.4, 128.9, 129.2, 129.5, 131.2, 139.6.

Second-eluting diastereoisomer (3B). R_F 0.22 (2:1 hexanes/EtOAc). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.51, dd, *J* 8.9, 7.3 Hz, 1H, H6a; 3.61, dd, *J* 8.9, 5.5 Hz, 1H, H6b; 3.65–3.70, m, 2H, H3, H5; 3.92, d, *J* 1.3 Hz, 1H, H4; 4.03, app. t, *J* 9.0 Hz, 1H, H2; 4.38–4.87, m, 9H, CH_2Ph, H1; 7.07–7.12, m, 2H, ArH; 7.24–7.36, m, 21H, ArH; 7.56–7.59, m, 2H, ArH. ^{13}C NMR ($CDCl_3$, 50 MHz) δ 68.1, 72.3, 72.6, 73.5, 73.6, 74.0, 74.4, 77.4, 83.8, 95.3, 125.9, 127.1, 127.6, 127.8, 128.1, 128.2, 128.4, 130.9, 137.7, 137.9, 138.5, 140.2.

Phenyl (2,3,4,6-Tetra-O-*pivaloyl)-1-thio*-β-*D-galactopyranoside (4)*

Pivaloyl chloride (2.10 mL, 2.05 g, 17.1 mmol, 8 equiv.) and 4-dimethylaminopyridine (131.5 mg, 1.08 mmol, 0.5 equiv.) were added to a solution of phenyl 1-thio-β-D-galactopyranoside (1) (580.6 mg, 2.13 mmol, 1.0 equiv.) in pyridine (10 mL). The solution was heated at 100°C overnight, cooled, diluted with CH_2Cl_2 (120 mL), washed with water (120 mL) and brine (120 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography, with 9:1 hexanes/EtOAc as eluent, to afford *phenyl (2*,*3*,*4*,*6-tetra*-O-*pivaloyl)-1-thio*-β-*D-galactopyranoside* (4) (1.09 g, 84%) (Found (HRMS–CI): $[M+NH_4]^+$, 626.3377. $C_{32}H_{52}NO_9S$ requires $[M+NH_4]^+$, 626.3362). R_F 0.55 (5:1 hexanes/EtOAc). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.09, s, 9H, Piv; 1.17, s, 9H, Piv; 1.19, s, 9H, Piv; 1.22, s, 9H, Piv; 3.97–4.21, m, 2H, H5, H6a; 4.19, dd, *J* 13.7, 9.6 Hz, 1H, H6b; 4.73, d, *J* 9.6 Hz, 1H, H1; 5.13, dd, *J* 9.6, 2.8 Hz, 1H, H3; 5.22, app. t, *J* 9.6 Hz, 1H, H2; 5.42, d, *J* 2.8 Hz, 1H, H4; 7.29–7.34, m, 3H, ArH; 7.50–7.54, m, 2H, ArH. ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.9, 27.0, 38.6, 38.8, 61.3, 66.5, 66.8, 72.0, 74.6, 85.7, 128.2, 128.7, 131.2, 133.5, 176.2, 176.6, 177.0, 177.7.

Phenyl (2,3,4,6-Tetra-O-*pivaloyl)-1-thio*-β-*D-galactopyranoside* S-*Oxide (5)*

m-Chloroperbenzoic acid (180 mg, 67% w/w, 0.69 mmol, 1.2 equiv.) was added to a solution of phenyl (2,3,4,6-tetra-*O*-pivaloyl)-1-thio-β-D-galactopyranoside (4) (354 mg, 0.58 mmol, 1.0 equiv.) in CH_2Cl_2 (10 mL) at –30°C. After stirring for 30 min at –30°C under N_2, the reaction mixture was quenched by the addition of dimethyl sulfide (3 drops), and warmed to room temperature. The solution was diluted with CH_2Cl_2 (50 mL), washed with water (50 mL), sat. aq. $NaHCO_3$ (50 mL), and water again (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography, with 5:1 then 1:1 hexanes/EtOAc as eluent, to afford (5A) as a colourless solid (106 mg), fractions containing a mixture of (5A) and (5B) (32 mg), and (5B) as a colourless solid (168 mg). Total yield: 307 mg (84%) (Found (HRMS–DEI, of mixture): M^+, 624.2959. Calc. for $C_{32}H_{48}O_{10}S$: M^+, 624.2968).

First-eluting diastereoisomer (5A). R_F 0.38 (3:1 hexanes/EtOAc). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.08, s, 18H, Piv; 1.12, s, 9H, Piv; 1.21, s, 9H, Piv; 3.87–4.09, m, 3H, H5, H6a, H6b; 4.42, d, *J* 9.8 Hz, 1H, H1; 5.14, dd, *J* 9.8, 3.0 Hz, 1H, H3; 5.34, d, *J* 3.0 Hz, 1H, H4; 5.52, app. t, *J* 9.8 Hz, 1H, H2; 7.46–7.55, m, 3H, ArH; 7.59–7.65, m, 2H, ArH. ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.9, 38.6, 38.7, 38.8, 38.9, 61.0, 64.4, 66.5, 72.1, 75.6, 89.5, 125.8, 128.8, 131.5, 138.8, 176.0, 176.7, 177.3, 177.7.

Second-eluting diastereoisomer (5B). R_F 0.25 (3:1 hexanes/EtOAc). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.95, s, 9H, Piv; 1.09, s, 9H, Piv; 1.16, s, 9H, Piv; 1.25, s, 9H, Piv; 3.67–3.80, m, 1H, H5; 4.00–4.14, m, 2H, H6a, H6b; 4.65, dd, *J* 8.8, 2.0 Hz, 1H, H3; 5.04–5.19, m, 2H, H1, H2; 5.31, d, *J* 2.0 Hz, 1H, H4; 7.50–7.57, m, 3H, ArH; 7.74–7.81, m, 2H, ArH. ^{13}C NMR ($CDCl_3$, 50 MHz) δ 26.8, 26.9, 38.6, 38.9, 60.2, 64.8, 65.9, 71.7, 74.9, 92.2, 127.1, 128.5, 131.9, 137.1, 176.2, 176.8, 176.9, 177.6.

Nα-*Fluorenylmethoxycarbonyl*-trans-*4-hydroxy-L-proline Allyl Ester (7)*

Caesium carbonate (82 mg, 0.25 mmol, 0.5 equiv.) was added to a suspension of *N*-fluorenylmethoxycarbonyl-*trans*-4-hydroxy-L-proline (6) (179 mg, 0.51 mmol, 1.0 equiv.) (prepared according to the general procedure of Carpino and Han[40]) in dry methanol (2.5 mL). The homogeneous reaction mixture was left to stir at room temperature under N_2 for 2 h. The methanol was removed under reduced pressure

and the colourless foam was dissolved in dry DMF (2.5 mL) and treated with allyl bromide (53 µL, 0.61 mmol, 1.2 equiv.). The resulting colourless suspension was stirred at room temperature overnight. The reaction mixture was diluted with EtOAc (30 mL), washed with water (30 mL), brine (30 mL), dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography, with 1:1 hexanes/EtOAc as eluent, to obtain Nα-*fluorenylmethoxycarbonyl*-trans-*4-hydroxy-L-proline allyl ester* (7) as a colourless oil (199 mg, 86%) (Found (HRMS–CI): $[M+H]^+$, 394.1646. $C_{23}H_{24}NO_5$ requires $[M+H]^+$, 394.1654). R_F 0.34 (2:1 hexanes/EtOAc). ^{1}H NMR ($CDCl_3$, 200 MHz) (mixture of rotamers) δ 2.10, ddd, *J* 12.7, 7.6, 5.0 Hz, 1H, Hβ; 2.28–2.45, m, 1H, Hβ′; 3.54, d, *J* 11.4 Hz, Hδ; 3.68, s, 1H, OH; 3.75, dd, *J* 11.4, 4.4 Hz, 1H, Hδ′; 4.13–4.66, m, 5H, Fmoc CH and CH_2, C**H**$_2$CH=CH_2, Hα, Hγ; 5.14–5.35, m, 2H, CH=C**H**$_2$; 5.75–5.96, m, 1H, C**H**=CH_2; 7.28–7.41, m, 4H, ArH; 7.51–7.61, m, 2H, ArH; 7.73, d, *J* 7.4 Hz, 2H, ArH. ^{13}C NMR ($CDCl_3$, 50 MHz) (mixture of rotamers) δ 38.4 and 39.3, 47.1, 54.6 and 55.3, 57.6 and 57.9, 65.8, 67.6 and 67.8, 69.2, 70.0, 118.5 and 118.9, 119.9, 124.9, 125.1, 127.0, 127.6, 131.5 and 131.6, 141.2, 143.5, 143.7, 143.9, 154.4 and 154.9, 172.2.

4-O-*[(2,3,4,6-Tetra*-O-*benzyl)*-Nα-*fluorenylmethoxycarbonyl-D-galactopyranosyl]*-trans-*4-hydroxy-L-proline Allyl Ester (8)*

Molecular sieves (1 g) were added to a solution of (2,3,4,6-tetra-*O*-benzyl)-1-thio-β-D-galactopyranoside *S*-oxide (3B) (204.5 mg, 0.315 mmol, 1.2 equiv.), *N*-fluorenylmethoxycarbonyl-*trans*-4-hydroxyproline allyl ester (7) (103.8 mg, 0.264 mmol, 1.0 equiv.) and DTBMP (233 mg, 1.13 mmol, 3.6 equiv.) in toluene (15 mL). The suspension was stirred at room temperature under N_2 for 1 h and then cooled to –70°C. Triflic anhydride (42 µL, 71 mg, 0.252 mmol, 0.8 equiv.) was added and the mixture was stirred at –70°C for 2 h. Sat. aq. $NaHCO_3$ (5 mL) was added, the suspension warmed to room temperature and then filtered through a pad of Celite, washing well with EtOAc. The filtrate was washed with sat. aq. $NaHCO_3$ (50 mL), water (50 mL), brine (50 mL), and then dried ($MgSO_4$), filtered and concentrated. The residue was partially purified by flash chromatography, with 3:1 to 1:1 hexanes/EtOAc as eluent, to give the α-anomer (8α) (74.9 mg) and mixed fractions (63.8 mg). The latter was subjected to HPLC to give more α-anomer (8α) (7.4 mg, for a total of 82.3 mg, 34%) and the β-anomer (8β) (17.8 mg, 7%).

Nα-*Fluorenylmethoxycarbonyl*-trans-*4-hydroxy-4*-O-*[(2,3,4,6-tetra*-O-*benzyl)*-α-*D-galactopyranosyl]-L-proline allyl ester (8α).* (Found (HRMS–FAB): $[M+H]^+$, 916.4036. Calc. for $C_{57}H_{58}NO_{10}$: $[M+H]^+$, 916.4060). R_F 0.35 (2:1 hexanes/EtOAc). HPLC: R_t 21.9 min (3:1 hexanes/EtOAc; 0.6 mL min^{-1}, 4.6 by 250 mm silica column). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.13–2.22, m, 1H, Hyp Hβ; 2.47–2.55, m, 1H, Hyp Hβ′; 3.44–3.56, m, 3H, Gal H5, H6a, H6b; 3.68–3.76, m, 1H, Hyp Hδ; 3.80, dd, *J* 11.2, 5.2 Hz, 1H, Hyp Hδ′; 3.89, dd, *J* 10.1, 2.8 Hz, 1H, Gal H3; 3.91–3.97, m, 2H, Gal H2, H4; 4.02–4.05, m, 1H, Hyp Hα/Hγ; 4.15, dd, *J* 13.0, 6.3 Hz, 1H, Hyp Hα/Hγ; 4.21–4.63, m, 8H, Gal H1, Fmoc CH, CH_2, C**H**$_2$CH=CH_2, CH_2Ph; 4.69–4.95, m, 6H, CH_2Ph; 5.15–5.35, m, 2H, CH=C**H**$_2$; 5.76–5.92, m, 1H, C**H**=CH_2; 7.21–7.44, m, 24H, ArH; 7.51–7.63, m, 2H, Fmoc ArH; 7.75, d, *J* 7.5 Hz, 2H, Fmoc ArH. ^{13}C NMR [(D_6)acetone, 67.5 MHz] (mixture of rotamers) δ 37.7 and 38.2, 47.7 and 47.9, 52.7 and 52.8, 56.7 and 59.2, 65.7 and 66.0, 67.9 and 68.0, 70.1, 70.8, 72.8, 73.1, 73.2, 73.6, 75.3, 75.5, 76.4, 77.0, 79.3, 97.4 and 97.9, 117.8 and 118.2, 120.6, 125.8, 125.9, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.6, 128.7, 128.8, 133.0 and 133.1, 129.3, 139.8, 129.9, 141.8, 144.6, 144.7, 144.8, 154.7 and 154.9, 172.3 and 172.6.

4-O-*[(2,3,4,6-Tetra*-O-*benzyl)*-β-*D-galactopyranosyl]*-Nα-*fluorenylmethoxycarbonyl*-trans-*4-hydroxy-L-proline allyl ester (8β).* (Found (HRMS–FAB): $[M+H]^+$, 916.4072. Calc. for $C_{57}H_{58}NO_{10}$: $[M+H]^+$, 916.4060). R_F 0.20 (2:1 hexanes/EtOAc). HPLC: R_t 30.9 min (3:1 hexanes/EtOAc; 0.6 mL min^{-1}, 4.6 by 250 mm silica column). ^{1}H NMR [(D_6)acetone, 270 MHz] δ 2.09–2.28, m, 1H, Hyp Hβ; 2.47–2.60, m, 1H, Hyp Hβ′; 3.60–3.82, m, 7H, Hyp Hδ, Hδ′, Gal H2, H3, H5, H6, H6′; 4.07–4.64, m, 11H, Hyp Hα, Hγ, Fmoc CH, CH_2, OC**H**$_2$CH=CH_2, Gal H1, H4, CH_2Ph; 4.73–5.00, m, 6H, CH_2Ph; 5.17, d, *J* 10.6 Hz, 1H, CH=C**H**$_A$; 5.35, ddd, *J* 17.4, 6.6, 1.5 Hz, 1H, CH=C**H**$_B$; 5.85–5.99, m, 1H, C**H**=CH_2; 7.21–7.39, m, 24H, ArH; 7.52–7.61, m, 2H, ArH; 7.73–7.76, m, 2H, ArH. ^{13}C NMR [(D_6)acetone, 67.5 MHz] δ 36.1 and 37.3, 47.8 and 48.0, 53.9 and 54.3, 58.3 and 58.7, 65.8 and 66.0, 67.9, 69.5, 73.1, 73.7, 73.9, 75.0, 75.1, 75.3, 75.4, 75.5, 76.9 and 77.7, 80.0 and 80.1, 83.0, 103.3, 117.8 and 118.2, 125.8, 125.9, 126.0, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.8, 128.9. 133.1, 139.3, 139.7, 139.9, 140.0, 141.8, 144.7, 154.6 and 155.0, 172.3 and 172.5.

Nα-*Fluorenylmethoxycarbonyl*-trans-*4-hydroxy-4*-O-*[(2,3,4,6-tetra-*|O-*pivaloyl)*-β-*D-galactopyranosyl]-L-proline Allyl Ester (9)*

Phenyl (2,3,4,6-tetra-*O*-pivaloyl)-1-thio-β-D-galactopyranoside *S*-oxide (5) (64 mg, 0.10 mmol, 1.0 equiv.) and DTBMP (42 mg, 0.20 mmol, 2.0 equiv.) were azeotroped with toluene three times, dissolved in dry CH_2Cl_2 (3 mL) and cooled to –70°C. Triflic anhydride (35 mg, 21 µL, 0.12 mmol, 1.2 equiv.) was added and the solution was left to stir for 10 min at –70°C. *N*-Fluorenylmethoxycarbonyl-L-*trans*-4-hydroxyproline allyl ester (7) (87 mg, 0.22 mmol, 2.2 equiv.) was azeotroped with toluene three times, dissolved in dry CH_2Cl_2 (2 mL) and added dropwise to the glycosyl donor mixture. The resulting mixture was stirred at –70°C under N_2 for 3 h. The reaction mixture was quenched by the addition of sat. aq. $NaHCO_3$ (0.5 mL) and left to warm to room temperature. The mixture was diluted with EtOAc (35 mL) and washed with water (15 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash column chromatography, with 5:1 then 3:1 hexanes/EtOAc as eluent. This afforded Nα-*fluorenylmethoxycarbonyl*-trans-*4-hydroxy-4*-O-*[(2,3,4,6*-tetra-O-pivaloyl)-β-D-galactopyranosyl]-L-proline allyl ester (9) as a colourless oil (42 mg, 46%) (Found (HRMS–FAB+): [M+H]+, 892.4496. $C_{49}H_{66}NO_{14}$ requires [M+H]+, 892.4483). R_F 0.21 (3:1 hexanes/EtOAc). $[\alpha]_D^{20}$ –23.3° (*c*, 0.70 in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) (mixture of rotamers) δ [0.95, s, 3H; 1.09, s, 3H; 1.11, s, 3H; 1.12, s, 3H; 1.16, s, 6H; 1.17, s, 3H; 1.18, s, 3H; 1.19, s, 3H; 1.25, s, 3H; 1.26, s, 3H; 1.28, s, 3H] total 36H, 4 × Piv; 2.13–2.19, m, 1H, Hyp Hβ; 2.30–2.41, m, 1H, Hyp Hβ′; 3.68–3.88, m, 1H, Hyp Hδ, Hδ′; 3.94–4.11, m, 2H, Gal H5, H6a; 4.15, dd J 10.8, 6.7 Hz, 1H, H6b; 4.25–4.67, m, 7H, Fmoc CH and CH_2, C**H**$_2$CH=CH_2, Gal H1, Hyp Hα, Hγ; 5.08–5.40, m, 5H, CH_2CH=CH_2, Gal H2, H3, H4; 5.78–5.96, m, 1H, CH_2C**H**=CH_2; 7.28–7.34, m, 2H, ArH; 7.39, t, *J* 7.4 Hz, 2H, ArH; 7.52–7.55, m, 2H, ArH; 7.59, t, *J* 6.8 Hz, 2H, ArH; 7.74–7.79, m, 2H. ^{13}C NMR ($CDCl_3$, 100 MHz) (mixture of rotamers) δ 26.8, 27.0 (2C), 27.1, 35.2 and 36.6, 38.7 (2C), 38.9 and 39.1, 47.1 and 47.2, 52.5 and 52.9, 57.4 and 57.7, 60.2 and 61.0, 64.9 and 65.9, 66.0 and 66.6, 67.7, 68.5 and 68.6, 70.9, 71.2 and 71.8, 75.0 and 76.1, 92.2, 100.3 and 100.5, 118.6 and 118.9, 119.9, 124.9 and 125.1, 127.1 and 125.1, 127.1 and 127.2, 127.7, 128.6, 131.4 and 131.6, 132.0, 141.3 (2C), 143.6 and 143.8, 143.9 and 144.1, 154.1 and 154.7, 171.8 and 172.0, 176.6, 176.8, 177.3, 177.8.

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