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Synthesis of a Gal-β-(1→4)-Gal disaccharide as a ligand for the fimbrial adhesin UcaD

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ABSTRACT

The disaccharide Gal-β-(1→4)-Gal was recently identified as a ligand for the adhesin UcaD, a fimbrial protein used by *Proteus mirabilis* to adhere to exfoliated uroepithelial cells and colonise the urinary tract. To facilitate further studies, Gal-β-(1→4)-Gal was synthesised as the α-methyl glycoside via glycosylation of methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside with 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl trichloroacetimidate, followed by deprotection. The disaccharide was fully characterised by NMR spectroscopy. Earlier attempts to use a thiogalactoside as the glycosyl acceptor were hindered by intermolecular aglycone transfer side reactions.

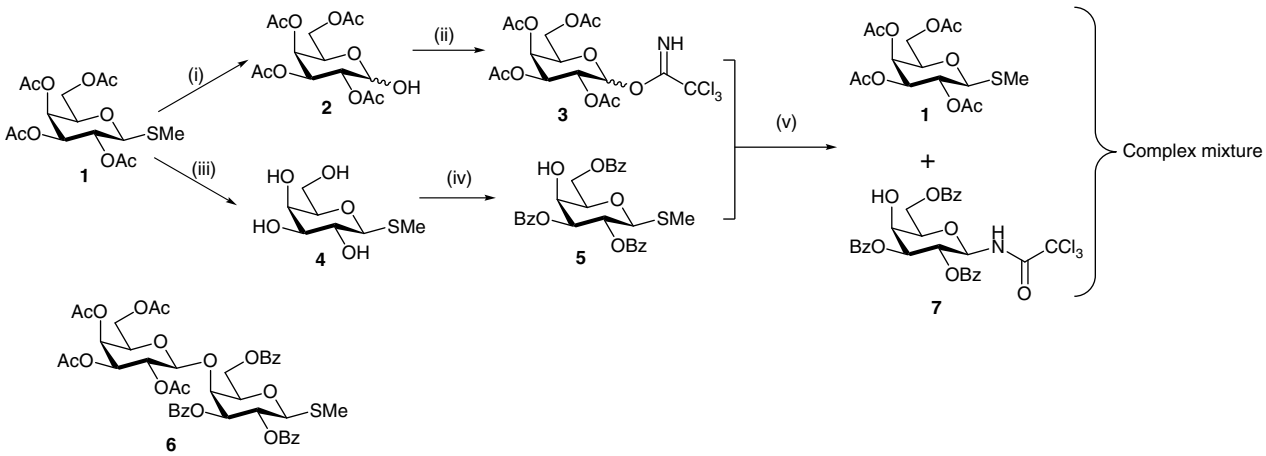
Keywords: bacterial adhesins, disaccharide synthesis, galactopyranoside, Gal-β-(1→4)-Gal, glycosylation, intermolecular aglycone transfer, *Proteus mirabilis*, uropathogenic *Escherichia coli*.

Introduction

Urinary tract infections (UTIs) affect over 150 million people globally per year[1,2] and are a major precursor to sepsis, a disease with a mortality rate of ~25%.[1] Despite effective antibiotic treatment, ~30% of patients who contract an initial UTI suffer a recurrent infection.[2] The major cause of UTI is uropathogenic *Escherichia coli* (UPEC), but additional pathogens also frequently cause this disease, including other Gram-negatives such as *Klebsiella pneumoniae*, *Enterobacter* spp. and *Proteus mirabilis*.[3] These pathogens are all associated with increasing antibiotic resistance, necessitating the urgent development of new therapeutics to treat and prevent UTI.[3,4] One emerging treatment aims to prevent the colonisation of UPEC by interrupting pathways associated with bacterial adhesion to the cell surface of the host.[2,3,5–7] Bacteria target and adhere to the extracellular matrix of host cells through recognition of specific structures, often specific carbohydrates, on the cell surface.[6,7] This recognition is mediated through binding interactions between adhesins (proteins on the pili/fimbriae of the bacteria) and the host glycans.[6,7] We initiated investigation of the receptor specificity of *P. mirabilis* UCA fimbriae, which mediate adherence to human exfoliated uroepithelial cells and colonisation of the bladder and kidneys in experimental mice.[8,9] Our analyses employing carbohydrate microarray screening revealed that the disaccharide Gal-β-(1→4)-Gal binds to the *P. mirabilis* fimbrial tip adhesin UcaD (dissociation constant, K_D 125.7 ± 7.4 nM).[10] Interestingly, Gal-β-(1→4)-Gal did not bind to the related UPEC Ucl fimbrial adhesin UclD.[2,10–13] The anomeric isomer Gal-α-(1→4)-Gal is known to bind to the UPEC fimbrial adhesin PapG, the receptor-binding component of P fimbriae that mediate colonisation of the upper urinary tract.[5] In order to confirm the binding results and to allow further study, we set out to synthesise milligram quantities of pure Gal-β-(1→4)-Gal disaccharide.

Results and discussion

We envisaged that synthesis of the target disaccharide would be readily achievable via glycosylation of a suitably protected D-galactosyl acceptor with a free 4-OH group with any number of D-galactosyl donors bearing participating protecting groups. Given the



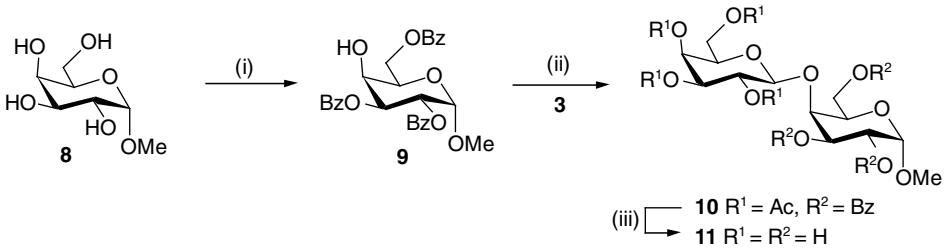
Scheme 1. Reagents and conditions: (i) NBS (*N*-bromosuccinimide), acetone/H_2O, −20°C, 25 min, 90%; (ii) Cl_3CCN, K_2CO_3, DCM, room temperature (rt), 16 h, 92%; (iii) NaOMe/MeOH/THF, 0°C → rt, 24 h, 99%; (iv) BzCl, pyridine, −78°C, 3 h, 30%; (v) $BF_3{\cdot}Et_2O$, DCM, 4 Å molecular sieves, −78°C.

availability of significant quantities of the protected thiogalactoside **1**, a synthetic route was devised to prepare both a glycosyl donor **3** and a glycosyl acceptor **5** from this same starting material (Scheme 1). A productive glycosylation between **3** and **5** would provide disaccharide **6**, which on deprotection would furnish the desired Gal-β-(1→4)-Gal disaccharide as the β-methyl thioglycoside. This route seemed attractive because the thioglycoside functionality could serve as a glycosyl donor for further transformations, if desired. For the glycosyl donor, the trichloroacetimidate was chosen, as this leaving group can be activated under mild Lewis acidic conditions that should not activate the thioglycoside moiety of the acceptor.

Thiogalactoside **1** was thus converted into trichloroacetimidate **3**[14] as a mixture of anomers (α/β = 2:1) in excellent yield following purification by flash chromatography. The thioglycoside was first hydrolysed with NBS in aqueous acetone and the resulting crude hemiacetal **2** was then treated with trichloracetonitrile and potassium carbonate to give **3**. To prepare the glycosyl acceptor, thiogalactoside **1** was deacetylated under Zemplén conditions to give the tetrol **4**[15,16] in essentially quantitative yield. Selective benzoylation of **4** with benzoyl chloride in pyridine at −78°C then gave the glycosyl acceptor **5**[17] in a modest but acceptable 30% yield, similarly to analogous selective benzoylations of D-galactosides.[18–20] Also isolated were the tetrabenzoate[21,22] (21%) and mixed fractions containing the isomeric 3,4,6-tribenzoate (16%).

The glycosylation of acceptor **5** with donor **3** was next attempted at −78°C with $BF_3{\cdot}Et_2O$ as the promoter. The reaction was monitored by TLC and was stopped on disappearance of the starting materials. Following workup, attempts were made to isolate the major product(s) by flash chromatography. ^{1}H NMR analysis indicated a complex mixture and the desired disaccharide **6** could not be detected. However, although no pure products could be isolated, mixed fractions of sufficient purity were obtained from which the products of intermolecular aglycone transfer,[23] **1** and **7**, were identified by 1D and 2D NMR spectroscopy. The NMR signals for compound **1** matched those of the starting material used at the beginning of the synthesis, while compound **7** showed a diagnostic doublet for the trichloroacetamide proton at 7.76 ppm (*J* 9.0 Hz) with a COSY correlation to a doublet of doublets at 5.39 ppm (H-1, $J_{1,2}$9.3 Hz), the latter also showing an HSQC (heteronuclear single-quantum correlation) correlation to C1 at 80 ppm. Intermolecular aglycone transfers of this type are fairly common side reactions of glycosylations involving thioglycosides[24,25] and occur when there is a mismatch in reactivity between the glycosyl donor and acceptor. However, these mismatches are difficult to predict *a priori*.

Intermolecular aglycone transfer side reactions can be minimised by changing the protecting groups[26] or the aglycone.[27] In this case, however, we decided to simply change the acceptor from a thiogalactoside (**5**) to an *O*-galactoside (**9**) in order to prepare the Gal-β-(1→4)-Gal disaccharide **11** as the target (Scheme 2). In the only previous chemical synthesis of **11**, Cox *et al.*[28] obtained an inseparable mixture of **11** and the isomeric Gal-α-(1→4)-Gal-α-OMe (ratio ~7:6) in a combined yield of only 31% during the attempted synthesis of the latter via a Koenigs–Knorr glycosylation. Subsequently, Fujimoto *et al.*[29] used a transglycosylation reaction catalysed by β-galactanase (from *Penicillium citrinium*) but obtained **11** in only 18.8% yield along with the corresponding trisaccharide (3.5%). Millqvist-Fureby *et al.*[30] used crude glycosidase preparations from barley and snail to obtain **11** along with the β-(1→6) isomer but no yield was reported. Yamamoto *et al.*[31] used the β-1,3-galactosidase (from *Bacillus circulans*) but obtained **11** in only 0.23% yield. All the previous methods for the preparation of **11** are unsatisfactory and, apart from the ^{13}C chemical shifts of the two anomeric carbons,[28] no characterisation data have been reported.



Scheme 2. Reagents and conditions: (i) BzCl, pyridine, 10–20°C, 5.5 h, 51%; (ii) TMSOTf, DCM, 4 Å molecular sieves, −20 to 0°C, 52%; (iii) NaOMe/MeOH, rt, 4.5 h, 68%.

Methyl α-D-galactopyranoside **8** was thus selectively benzoylated in a similar fashion to tetrol **4** to give the tribenzoate **9**[18,19] in good yield (51%) after purification by flash chromatography, along with the tetrabenzoate as the major by-product (29%). Glycosylation of acceptor **9** with trichloroacetimidate **3** proceeded smoothly at −20°C with TMSOTf as the promoter, to give the protected disaccharide **10**[32,33] in 52% yield after purification by flash chromatography. Zemplén deacylation then furnished the target disaccharide **11** in 68% yield following recrystallisation from methanol, which was fully characterised by 1D and 2D NMR spectroscopy and high-resolution mass spectrometry (HRMS). The ^{13}C NMR chemical shifts for the two anomeric carbons were in accordance with the literature.[28]

Conclusions

In conclusion, we synthesised a β-(1 → 4)-linked D-galabiose disaccharide as its α-methyl glycoside **11**, fully characterised by NMR spectroscopy, for further testing as a ligand for the fimbrial adhesin UcaD. The synthesis was achieved by glycosylation of a benzoyl-protected methyl α-D-galactopyranoside with a D-galactopyranosyl trichloroacetimidate donor followed by deprotection, and represents a significant improvement in yield over past syntheses. Earlier attempts at disaccharide synthesis using a D-thiogalactoside acceptor with the same donor were thwarted by intermolecular aglycone transfer. Binding studies with UcaD and other fimbrial adhesins will be reported elsewhere in due course.

Experimental

General methods

Melting points were determined on a DigiMelt MSRS apparatus. Optical rotations were determined on a JASCO P-2000 polarimeter at ambient temperature and are given in units of 10^{-1} degrees $cm^2\,g^{-1}$. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at 20°C. The residual solvent peaks ($CDCl_3$: δ_H 7.26 and δ_C 77.16 ppm or CD_3OD: δ_H 3.31 and δ_C 49.0 ppm) served as internal standards. Coupling constants in hertz (Hz) were measured from one-dimensional spectra. The analyses of ^1H and ^{13}C NMR spectra were assisted by gCOSY and HSQC experiments. Analytical low-resolution (LR)MS and HRMS were performed in positive or negative ion electrospray ionisation (ESI) mode on a Bruker HCT spectrometer and a Bruker micrOTOF$_Q$ spectrometer, respectively. All reagents and solvents were obtained from Merck, Australia, and were used without further purification, except EtOAc, *n*-hexane, MeOH and DCM, which were distilled prior to use. Reactions were monitored by analytical thin layer chromatography (TLC) on silica gel 60 F_{254} plates and visualised by charring with anisaldehyde/H_2SO_4 stain in ethanol. Flash chromatography was performed on silica gel under positive pressure with specified solvent systems.

2,3,4,6-Tetra-*O*-acetyl-D-galactopyranosyl trichloroacetimidate (3)

(a) Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **1** (1.20 g, 3.2 mmol) was dissolved in a mixture of acetone (30 mL) and water (2.5 mL) and was cooled to −20°C. A solution of *N*-bromosuccinimide (2.22 g, 12.5 mmol) in acetone (10 mL) was added dropwise and the mixture was stirred at −20°C for 25 min. Aqueous 20% NaS_2O_3/$NaHCO_3$ (1:1, 50 mL) was added, and then the mixture was diluted with EtOAc and washed with water (3 × 50 mL). The combined aqueous phase was extracted with EtOAc (3 × 50 mL) and the combined organic phase was washed with brine (1 × 100 mL), dried ($MgSO_4$), filtered and concentrated under vacuum to give the hemiacetal **2** as a clear syrup (1.00 g, 90%), used without further purification in the next step. The ^1H NMR spectrum was in accordance with the literature.[14] (b) The crude hemiacetal (865 mg, 2.4 mmol) was dissolved in DCM (10 mL) and trichloroacetonitrile (0.7 mL, 7.2 mmol, 3 equiv.) was added at rt with stirring. After 15 min, the mixture was cooled to 0°C, potassium carbonate (1.46 g, 10.6 mmol) was added and the reaction was stirred overnight at rt. The mixture was concentrated under vacuum and the residue was purified by flash chromatography (6:1 toluene/EtOAc → 3:1 toluene/EtOAc → EtOAc) to give the trichloroacetimidate **3** as a colourless oil (1.13 g, 92%) as a mixture of anomers (α/β = 2:1). The ^1H NMR spectrum was in accordance with the literature.[14] ^1H NMR (500 MHz, $CDCl_3$) α-anomer: δ 8.66 (s, 1H, NH), 6.60 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.56 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 1.4 Hz, H-4), 5.48 (dd, $J_{2,3}$ 10.9 Hz, H-3), 5.37 (dd, H-2), 4.46–4.42 (m, 1H, H-5),

4.17 (dd, 1H, A part of ABX, $J_{5,6a}$ 6.6 Hz, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.08 (dd, 1H, B part of ABX, $J_{5,6b}$ 6.7 Hz), 2.17, 2.03, 2.02, 2.01 (4s, 4 × 3H, 4 × Me); β-anomer: δ 8.71 (s, 1H, NH), 5.84 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.49 (dd, 1H, $J_{2,3}$ 10.4 Hz, H-2), 5.46 (dd, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 1.3 Hz, H-4), 5.12 (dd, H-3), 4.22–4.09 (m, 3H, H-5, 6a, 6b), 2.18, 2.04, 2.02, 2.00 (4s, 4 × 3H, 4 × Me).

Methyl 1-thio-β-D-galactopyranoside (4)

Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside **1** (1.11 g, 2.9 mmol) was dissolved in dry MeOH/THF (4:1, 50 mL). NaOMe in MeOH (0.7 M, 0.5 mL) was added dropwise, with stirring, at 0°C under a nitrogen atmosphere. The reaction was stirred at rt for 24 h and was then neutralised with Amberlite® IR120 (H^+ form). The suspension was filtered and the resin was washed with MeOH. The filtrate and washings were evaporated under vacuum to give the tetrol **4** as a fine, white powder (0.61 g, 99%), mp 173–174°C (lit.[15] 174–175°C), $[\alpha]_D$ +9.5 (*c* 0.9, H_2O; lit.[15] +10.7, H_2O). The ^{1}H NMR spectrum was in accordance with the literature.[16] ^{1}H NMR (500 MHz, CD_3OD): δ 4.21 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 3.89 (dd, 1H, $J_{4,5}$ 1.1 Hz, $J_{3,4}$ 3.3 Hz, H-4), 3.75 (dd, 1H, A part of ABX, $J_{5,6a}$ 6.8 Hz, $J_{6a,6b}$ 11.4 Hz, H-6a), 3.68 (dd, B part of ABX, 1H, $J_{5,6b}$ 5.2 Hz, H-6b), 3.58 (dd, 1H, $J_{2,3}$ 9.4 Hz, H-2), 3.53 (ddd, 1H, H-5), 3.47 (dd, 1H, H-3), 2.20 (s, 3H, SMe). ^{13}C NMR (125 MHz, CD_3OD): δ 87.9 (C-1), 80.7 (C-5), 76.2 (C-3), 70.8 (C-4), 70.6 (C-2), 62.7 (C-6), 12.0 (SMe). ESMS: *m/z* 233.0 $[M + Na]^+$.

Methyl 1-thio-2,3,6-tri-*O*-benzoyl-β-D-galactopyranoside (5)

The tetrol **4** (0.96 g, 4.5 mmol) was dissolved in anhydrous pyridine (4 mL), cooled to −78°C and stirred for 30 min. A solution of benzoyl chloride (1.57 g, 1.30 mL, 11.2 mmol, 2.5 equiv.) in anhydrous pyridine (7 mL) was added dropwise with stirring over 1 h, under an atmosphere of nitrogen. After 3 h, the reaction was quenched with water (10 mL). The mixture was diluted with DCM (25 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 25 mL). The combined aqueous phase was re-extracted with DCM (3 × 25 mL). The combined organic phase was then washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated under vaccuum. The residue was purified by flash chromatography (9:1 toluene/EtOAc → 5:1 toluene/EOAc → EtOAc) to give the tribenzoate **5** as a colourless solid (0.72 g, 30%); R_f = 0.43 (5:1 toluene/EtOAc); mp 136°C. $[\alpha]_D$ +60 (*c* 0.6, $CHCl_3$; lit.[17] +3.3). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–7.33 (m, 15H, Ph), 5.87 (dd, 1H, $J_{1,2}$ $J_{2,3}$ 9.9 Hz, H-2), 5.42 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 4.71 (dd, 1H, A part of ABX, $J_{5,6a}$ 6.7 Hz, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.65 (d, 1H, H-1), 4.47 (dd, 1H, B part of ABX, $J_{5,6b}$ 6.2 Hz, H-6b), 4.39–4.36 (m, 1H, H-4), 4.11 (bdd, 1H, H-5), 2.58 (bd, 1H, *J* 4.6 Hz, OH), 2.27 (s, 3H, Me). HRMS: *m/z* calcd for $C_{28}H_{26}O_8S$ $[M + H]^+$: 523.14267; found: 523.14007. Also isolated was the tetrabenzoate by-product, methyl 1-thio-2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside[21,22] (0.60 g, 21%), R_f 0.6 (5:1 toluene/EtOAc); and mixed fractions containing methyl 1-thio-3,4,6-tri-*O*-benzoyl-β-D-galactopyranoside (0.38 g, 16%), R_f 0.3 (5:1 toluene/EtOAc).

Attempted glycosylation of 3 and 5

The tribenzoate **5** (272 mg, 0.52 mmol) was dissolved in dry DCM (2 mL) and was stirred under an atmosphere of nitrogen for 2 h in the presence of 4 Å molecular sieves. The mixture was cooled to −78°C and $BF_3{\cdot}Et_2O$ (14 μL, 0.12 mmol, 0.23 equiv.) was added. After 15 min, a solution of the trichloroacetimidate **3** (390 mg, 0.79 mmol, 1.5 equiv.) in dry DCM (1 mL) and added dropwise over 5 min. After 30 min, the reaction was neutralised with triethylamine to pH 7. The reaction was diluted with chloroform (5 mL) and filtered to remove the molecular sieves. These were washed with chloroform (4 × 20 mL). The combined organic phase was washed with sat. aqueous $NaHCO_3$ (3 × 30 mL) and the combined aqueous phase was re-extracted with DCM (3 × 30 mL). The combined organic phase was washed with brine (50 mL), dried ($MgSO_4$), filtered and concentrated under vaccuum. The residue was purified by flash chromatography (7:1 toluene/EtOAc → 3:2 toluene/EtOAc) to give a white foam (718 mg) containing a mixture of products. Further purification allowed the identification of the thioglycoside **1** and the trichloroacetamide **7**. ^{1}H NMR data for 2,2,2-trichloro-*N*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)acetamide (**7**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07–7.86, 7.61–7.32 (m, 15H, Ph), 7.76 (d, 1H, $J_{1,NH}$ 9.0 Hz, NH), 5.82 (dd, 1H, $J_{1,2}$ 9.3 Hz, $J_{2,3}$ 10.2 Hz, H-2), 5.60 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 5.39 (dd, 1H, H-1), 4.76 (dd, 1H, A part of ABX, $J_{5,6a}$ 7.1 Hz, $J_{6a,6a}$ 11.4 Hz, H-6a), 4.53 (dd, 1H, B part of ABX, $J_{5,6b}$ 5.9 Hz, H-6b), 4.41–4.37 (m, 1H, H-4), 4.24–4.20 (m, 1H, H-5), 2.94 (d, 1H, $J_{4,OH}$ 3.3 Hz, OH). HRMS: *m/z* calcd for $C_{29}H_{24}Cl_3NO_9$ $[M + H]^+$: 636.05949; found: 636.05588. ^{1}H NMR data for thioglycoside **1** was identical to an authentic sample: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.43 (dd, 1H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 1.2 Hz, H-4), 5.26 (dd, 1H, $J_{1,2}$ 9.9 Hz, $J_{2,3}$ 10.1 Hz, H-2), 5.05 (dd, H-3), 4.39 (d, 1H, H-1), 4.16 (dd, 1H, A part of ABX, $J_{5,6a}$ 6.6 Hz, $J_{6a,6b}$11.3 Hz, H-6a), 4.12 (dd, 1H, B part of ABX, $J_{5,6b}$ 6.6 Hz, H-6b), 3.96 (ddd, 1H, H-5), 2.19 (s, 3H, SMe), 2.15, 2.08, 2.05, 1.99 (4 × s, 4 × 3H, 4 × Me).

Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (9)

Methyl α-D-galactopyranoside **8** (940 mg, 4.84 mmol) was suspended in anhydrous pyridine (6.3 mL) at 10°C and benzoyl chloride (2.43 mL, 20.9 mmol, 4.3 equiv.) was dropwise added over 2 h. The temperature was raised to 20°C and stirring was continued for 3.5 h. The reaction was quenched

by addition of water (7.5 mL) at 10°C over 10 min and left stirring overnight at rt. The suspension was diluted with toluene (40 mL) and the organic phase was washed with aqueous $NaHCO_3$ solution (8%, w/v, 2 × 12 mL), aqueous HCl (1 M, 2 × 12 mL), water (5 × 30 mL) and brine (2 × 30 mL) and dried ($MgSO_4$), and concentrated under vaccuum to give a colourless foam (2.33 g). Purification by flash chromatography (toluene/EtOAc 10:1→toluene/EtOAc 3:1) gave first the perbenzoylated by-product methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranoside as a white foam (850 mg, 29%; R_f 0.44, toluene/EtOAc, 10:1). Further elution then gave the tribenzoate **9** as white solid (1.26 g, 51%), R_f 0.18 (toluene/EtOAc, 3:1), mp 140–141°C (lit.[19] 139–140.5°C). $[\alpha]_D$ +126 (*c* 0.5, $CHCl_3$; lit.[18] +123). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.07–7.96, 7.60–7.34 (m, 15H, Ph), 5.76 (dd, 1H, $J_{2,3}$ 10.7 Hz, $J_{3,4}$ 3.1 Hz, H-3), 5.68 (dd, 1H, $J_{1,2}$ 3.6 Hz, H-2), 5.22 (d, 1H, H-1), 4.69 (dd, 1H, A part of ABX, $J_{5,6a}$ 5.9 Hz, $J_{6a,6b}$ 11.5, H-6a), 4.56 (dd, 1H, B part of ABX, $J_{5,6b}$ 6.8 Hz, H-6b), 4.42–4.39 (m, 1H, H-4), 4.36 (bdd, 1H, H5), 3.45 (s, 3H, Me), 2.48 (d, *J* 4.2 Hz, OH). HRMS: *m/z* calcd for $C_{28}H_{26}O_9$ $[M + H]^+$: 507.16551; found: 507.16252.

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside (10)

The alcohol **9** (103 mg, 203 μmol) was dissolved in dry DCM (1.5 mL) and stirred with freshly dried molecular sieves (4 Å) for 30 min, followed by dropwise addition of a stock solution of TMSOTf in dry DCM (36 μL/mL, 200 μL, 20 μmol, 0.2 equiv.) at rt. After 30 min, the mixture was cooled to −20°C and a stock solution of the trichloroacetimidate **3** in dry DCM (50 mg in 100 μL, 101 μmol) was added dropwise over 5 min. The mixture was then stirred at 0°C for 90 min. TLC indicated complete consumption of donor **3** and formation of product. To rearrange any possible orthoester, the suspension was again cooled to −20°C and more stock solution of TMSOTf in dry DCM (36 μL/mL, 400 μL, 40 μmol, 0.4 equiv. donor) was added dropwise over 5 min. The mixture was stirred at 0°C for 30 min, and then the reaction was neutralised by addition of Et_3N (5 μL) at 0°C and stirring was continued at rt for 10 min. The mixture was diluted with $CHCl_3$, centrifuged and the molecular sieves were washed with $CHCl_3$ (10 × 10 mL). The combined supernatant was washed with water (2 × 20 mL), brine (30 mL), dried ($MgSO_4$) and concentrated under vaccuum. The residue was purified by flash chromatography (hexane/EtOAc 2:1 → 3:2 → 1:1 → EtOAc, all containing 0.5% Et_3N) to give disaccharide **10** as an off-white foam (44 mg, 52%), R_f 0.24 (hexane/EtOAc 2:1). $[\alpha]_D$ +66 (*c* 0.4, $CHCl_3$; lit.[32] +56.6; lit.[33] +73). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.94–8.10, 7.34–7.59 (m, 15H, Ph), 5.84 (dd, 1H, $J_{2,3}$ 10.7 Hz, $J_{3,4}$ 2.9 Hz, H-3), 5.55 (dd, 1H, $J_{1,2}$ 3.6 Hz, H-2), 5.35 (dd, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.5 Hz, H-2′), 5.29 (dd, 1H, $J_{3',4'}$ 3.4 Hz, $J_{4',5'}$ 1.0 Hz, H-4′), 5.09 (d, 1H, H-1), 4.91(dd, 1H, H-3′), 4.67 (d, 1H, H-1′), 4.67 (dd, 1H, A part of ABX, $J_{5,6a}$ 4.6 Hz, $J_{6a,6b}$ 11.8 Hz, H-6a), 4.50 (dd, 1H, B part of ABX, $J_{5,6b}$ 7.2 Hz, H-6b), 4.45 (dd, 1H, $J_{4,5}$ 1.0 Hz, H-4), 4.36 (ddd, 1H, H-5), 4.04 (dd, 1H, A part of ABX, $J_{5',6a'}$ 6.9 Hz, $J_{6a',6b'}$ 11.4 Hz, H-6a′), 3.96 (dd, 1H, B part of ABX, $J_{5',6b'}$ 6.4 Hz, H-6b′), 3.70 (ddd, 1H, H-5′), 3.41 (s, 3H, OMe), 2.18, 2.16, 2.00, 1.95 (4 × s, 12H, 4 × OAc). HRMS: *m/z* calcd for $C_{42}H_{44}O_{18}$ $[M + Na]^+$: 859.24254; found: 859.23856.

Methyl β-D-galactopyranosyl-(1→4)-α-D-galactopyranoside (11)

A solution of NaOMe in MeOH (0.7 M, 100 μL, 70 μmol) was added dropwise to a solution of disaccharide **10** (40 mg, 47 μmol) in dry MeOH (2.5 mL) at 0°C. The mixture was stirred at rt for 4.5 h under sonication and then was neutralised with Amberlite IR 120 (H^+) to pH 7. The resin was removed by filtration and was washed under sonication with 50% aqueous MeOH (5 × 2 mL). The combined filtrate and washings were co-evaporated under vaccuum with 50% aqueous MeOH (3 × 5 mL). The residue was crystallised from MeOH to give *disaccharide* **11** as a solid (11.6 mg, 68%), R_f 0.18 (MeCN/H_2O, 85:15). $[\alpha]_D$ +118 (*c* 0.6, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 4.85 (bs, 1H, H-1), 4.58 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.22 (bs, 1H, H-4), 3.95 (bt, 1H, H-5), 3.89–3.92 (m, H-3, H-2, H-4′), 3.84 (dd, 1H, H-6′a or H-6a), 3.72–3.80 (m, 3H, 3 × H-6), 3.67 (ddd, $J_{4',5'}$ 0.8 Hz, H-5′), 3.66 (dd, $J_{3,4}$ 3.4 Hz, H-3′), 3.58 (dd, 1H, $J_{2,3}$ 9.9 Hz H-2′), 3.41 (s, 3H, Me). ^{13}C NMR (125 MHz, D_2O): δ 105.3 (C-1′), 100.4 (C-1), 79.2 (C-4), 76.1 (C-5′), 73.8 (C-3′), 72.4 (C-2′), 71.0, 70.9, 69.62, 69.58 (C-2, C-3, C-5, C-4′, assignments may be reversed), 61.9 (C-6 or C-6′), 61.8 (C-6 or C-6′), 56.1 (Me). The ^{13}C NMR chemical shifts for C-1 and C-1′ matched those in the literature.[28] HRMS: *m/z* calcd for $C_{13}H_{24}O_{11}$ $[M + Na]^+$: 379.12163; found: 379.11989.

Supplementary material

Supplementary data: copies of NMR spectra for compounds **1**, **3–5**, **7**, **9–11**. Supplementary material is available online.

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