Red Algal Molecules - Synthesis of Methyl Neo-β-carrabioside and Its S-Linked Variant via Two Synthetic Routes: A Late Stage Ring Closure and Using a 3,6-Anhydro-d-galactosyl Donor

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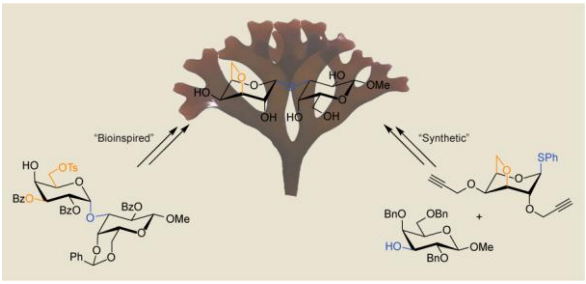


Graphic for table of contents only

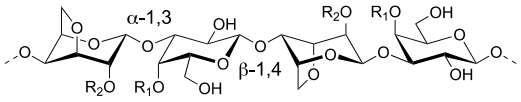
Abstract

Methyl neo-β-carrabioside has been synthesised for the first time, employing either a late stage ring closure to install the required 3,6-anhydro-bridge or by utilising a suitable 3,6-anhydro-galactosyl donor to form the unfavoured 1,2-*cis*-equatorial α-linkage. Using the late stage ring closure approach an *S*-linked analogue of methyl neo-β-carrabioside was also realised. These compounds have applications in the identification and characterisation of marine bacterial *exo*-α-3,6-anhydro-D-galactosidases that have specific activity on red algal neo-carrageenan oligosaccharides, such as those found in both family 127 and 129 of the glycoside hydrolases. In addition a biochemical assay using the synthesised methyl neo-β-carrabioside and the marine bacterial *exo*-α-3,6-anhydro-D-galactosidase *Zg*GH129 demonstrates that the minimum substrate unit for the enzyme is neo-β-carrabiose.

Introduction

Carrageenans are complex sulfated polysaccharides and one of the major cell wall components of carrageenophyte red macroalgae (Rhodophyta).[1] The structure is comprised primarily of repeating units of D-galactose and the bicyclic carbohydrate 3,6-anhydro-D-galactose (some variants contain 6-*O*-sulfo-D-galactose in place of 3,6-anhydro-D-galactose), containing alternating β-(1,4) and α-(1,3) linkages (Figure 1). The core carrageenan backbone is further decorated with different levels of sulfation and may also be substituted with methyl and pyruvate groups. The major 3,6-anhydro-D-galactose containing variants of carrageenans are κ- and ι-carrageenan, and the desulfated, β-carrageenan (Figure 1); however, natural carrageenans are hybrid structures that contain diverse carrabiose motifs within the polymer.[2]

Carrageenans are hydrocolloids; highly hydrated molecules with gelling capabilities and the ability to increase viscosity.[3] Both the 3,6-anhydro-bridge, which locks the corresponding pyranose ring in the $^1C_4$ conformation, and the sulfate groups are responsible for carrageenans' rheological properties. This is also observed with agars, the other major red macroalgal polysaccharides, which encompass agarose (unsulfated) and sulfated derivatives. Agars have a similar repetitive disaccharide motif to carrageenans but contain 3,6-anhydro-L-galactosyl residues rather than 3,6-anhydro-D-galactosyl residues. Due to the rheological properties and natural abundance of carrageenans, they have been utilised in food, personal care and cosmetic products. Some carrageenans have also been shown to exhibit bioactivities, such as anti-tumour or anti-viral activity[3] presumably due to the sulfations that mimic the sulfations on animal glycans.[2]



β-carrageenan	$R_1=R_2=H$
κ-carrageenan	$R_1=SO_3^-$, $R_2=H$
ι-carrageenan	$R_1=R_2=SO_3^-$

Figure 1. Major variants of 3,6-anhydro-D-galactose containing carrageenan units, β-, κ- and ι-carrageenan, with different levels of sulfation.

Some marine heterotrophic bacteria use carrageenans as a carbon source.[4,5] Recently a polysaccharide utilisation locus and regulon from *Zobellia galactanivorans* dedicated to the catabolism of carrageenans was discovered.[4] As part of the study, four genes encoding *exo*-α-

3,6-anhydro-D-galactosidase activity were described, an enzyme class which had been predicted to exist in Nature but had not been elucidated. The biochemical function of these enzymes is to cleave the 3,6-anhydro-D-galactosyl residue from the non-reducing end of neo-carrageenan oligosaccharides.* Three of the enzymes are classified as belonging to family GH127 of the glycoside hydrolases, whereas the fourth falls into family GH129 (http://www.cazy.org/).[6] Gene deletion experiments abolished growth for the bacterium when the *Zg*GH127-3 and *Zg*GH129 double mutant was grown on carrageenan substrates demonstrating their importance in carrageenan catabolism and to the biology of the marine bacterium.[4] An in-depth biochemical investigation into *Zg*GH129 found it to be inactive on neo-κ-carrageenan oligosaccharide substrates[4] but active on oligosaccharides with the neo-β-carrageenan motif at the non-reducing end.[7] Furthermore, the enzyme demonstrated activity on a novel synthetic substrate allowing a fine kinetic characterisation of its enzymatic properties.[7]

Despite the biological and industrial importance of carrageenans there are currently only limited molecular tools to study the biochemistry of the enzymes that possess *exo*-α-3,6-anhydro-D-galactosidase activity.[4,7] Here, we describe the synthesis of two new chemical tools to aid in the further study of this class of enzymes, the disaccharide methyl neo-β-carrabioside (methyl 3-*O*-(3,6-anhydro-α-D-galactopyranosyl)-β-D-galactopyranoside) **1** and the non-hydrolysable *S*-linked variant **2** as a potential inhibitor of *exo*-α-3,6-anhydro-D-galactosidases (Figure 2). As part of our investigation into the synthesis, we also examined the use of a 3,6-anhydro-galactosyl donor capable of directly forming the required 1,2-*cis*-equitorial α-linkage. The exploration and development of the successful synthetic routes described, will also aid in the synthesis of related analogues and putative inhibitors.

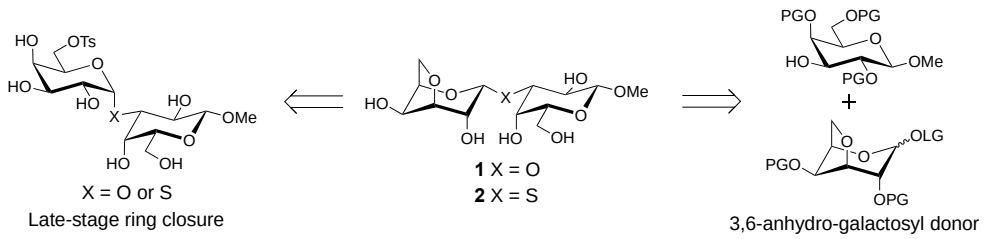


Figure 2. Two approaches for the synthesis of methyl neo-β-carrabioside **1** and the *S*-linked variant **2**. PG = protecting group, LG = leaving group.

*'Neo' denotes that the non-reducing end residue of the carrageenan oligosaccharide is either a 3,6-anhydro-D-galactosyl or 6-*O*-sulfo-D-galactosyl residue.

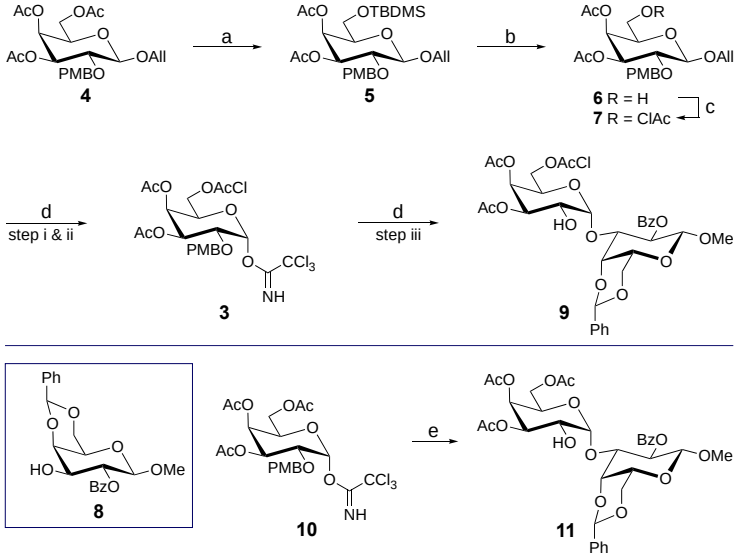
Results and Discussion

Two synthetic strategies were considered for the preparation of both **1** and **2** (Figure 2). The first strategy was to form the desired α-(1,3) glycoside and then install a suitable leaving group (e.g. sulfonate) at the C6 position of the non-reducing galactosyl residue, which would allow for late-stage 3,6-anhydro-bridge formation. Interestingly this method has a biosynthetic inspiration as galactose-sulfurylases,[†] which are unique red algal enzymes, utilise a 6-*O*-sulfo-D-galactosyl residue as a substrate to form the 3,6-anhydro-bridge and release sulfate.[8-11] In addition, this synthetic methodology has been used in the synthesis of methyl β-carrabioside and the unnatural derivative methyl 3-*O*-(3,6-anhydro-β-D-galactopyranosyl)-α-D-galactopyranoside.[12] The second strategy was to explore the use of 3,6-anhydro-galactosyl donors that would be suitable for α-glycosylation (Figure 2). We were buoyed by previous studies which utilised orthoester[13] and cyanoethylidene[14] 3,6-anhydro-galactose derivatives as glycosyl donors to prepare β-glycosides. Moreover, Christina *et al.*[15] whilst exploring galacturonic acid lactone thioglycoside donors for use in making α-glycosides demonstrated the possible use of a 3,6-anhydro-galactosyl-type donor in this regard. Overall though, the α-glycoside is inherently more difficult to form as it is a 1,2-*cis*-equitorial product, which is disfavoured by a combination of both neighbouring group participation and the anomeric effect. With the goal of synthesising **1** and **2** the biosynthetic-inspired route was first explored.

In the first instance the galactosyl trichloroacetimidate **3** was trialled (Scheme 1), as a similar donor has been used with a thiol acceptor,[16] which was important in developing a shared method to synthesise both **1** and **2**. By including a chloroacetyl protecting group at the C6 position, selective removal could be achieved followed by tosylation for the subsequent ring-closure. Thus Zemplén deacetylation of the triacetate **4**[16], followed by selective protection of the C6 hydroxy group with the TBDMS group and acetylation of the remaining hydroxy groups yielded the diacetate **5**. The TBDMS group was then removed via treatment with TBAF and the subsequent alcohol **6** was protected with a chloroacetyl protecting group to obtain the triester **7**. The allyl group was then removed via a Pd(II)-mediated deprotection[17] to give the presumed hemiacetal, which was then treated with trichloroacetonitrile and DBU to afford the trichloroactimidate **3**. With **3** in hand glycosylation with the known acceptor **8**[18] was then

[†] Despite being known in Nature biochemical studies on recombinant galactose-sulfurylase enzymes have not yet been described in the literature and these enzymes remain one of red algae's most fascinating, yet least understood, family of enzymes.

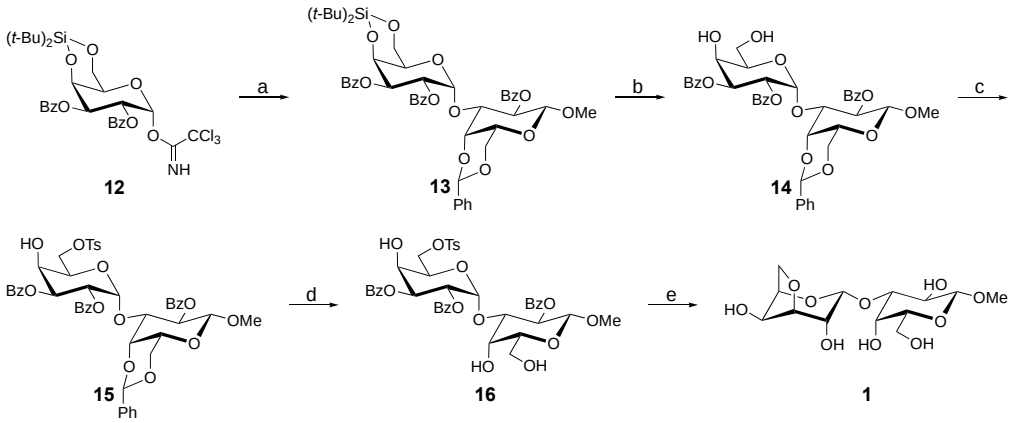
attempted, using TMSOTf as a catalyst and Et_2O as an additive to promote α-selectivity.[19] Unfortunately, the only product isolated in low yield (35%) was the disaccharide **9** which had the desired α-(1,3)-linkage but had lost the 4-methoxybenzyl (PMB) group at C2. Due to this unexpected result the reaction was reattempted using the donor **10** which was previously employed by Xia *et al.*[16] (Scheme 1), to synthesise both *O*- and *S*-linked isoglobotrihexosylceramides. However, in our hands only the disaccharide **11** was obtained in a low yield (38%), again with loss of the PMB group. Due to this result another donor was sourced.



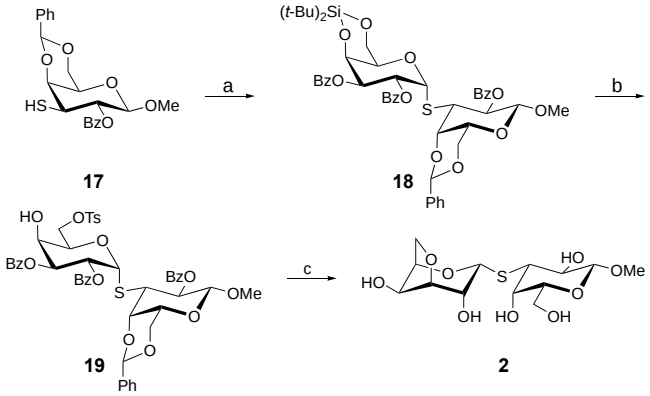
Scheme 1. a) i. NaOMe, MeOH, r.t.; ii. TBDMSCl, imidazole, DMF, r.t.; iii. Ac_2O, pyridine, CH_2Cl_2, r.t., 73% over 3 steps; b) TBAF, AcOH, THF, r.t. 95%; c) $ClAc_2O$, pyridine, CH_2Cl_2, 0 °C, 95%; d) i. $PdCl_2$, NaOAc, AcOH:H_2O 9:1, EtOAc, r.t.; ii. Cl_3CCN, DBU, CH_2Cl_2, r.t.; iii. **8**, TMSOTf, 4 Å MS, Et_2O, CH_2Cl_2, -20 °C, 20% over three steps; e) **8**, TMSOTf, 4 Å MS, Et_2O, CH_2Cl_2, -20 °C, 38%.

The galactosyl donor **12** developed by Kiso and co-workers[20,21] has been shown to be an excellent glycosyl donor that gives exclusively α-anomeric products, despite the participating benzoyl group at C2 (Scheme 2). Gratifyingly, glycosylation of **12** with the acceptor **8** resulted in formation of the disaccharide **13** in excellent yield (82%) and with no observable formation of the undesired β-glycoside. The di-*tert*-butylsilylene group was then selectively removed with 70% HF-pyridine to obtain the diol **14**, which was selectively tosylated to furnish the tosylate **15**. Aqueous acid-mediated hydrolysis of the 4,6-*O*-benzylidene acetal afforded the triol **16**, and finally treatment with methanolic NaOMe removed both the benzoate protecting

groups and concurrently formed the desired 3,6-anhydro-bridge to give **1**. With this successful route now developed it was then applied, using instead the thiol acceptor **17**,[22] to the synthesis of **2** in good yield (Scheme 3).



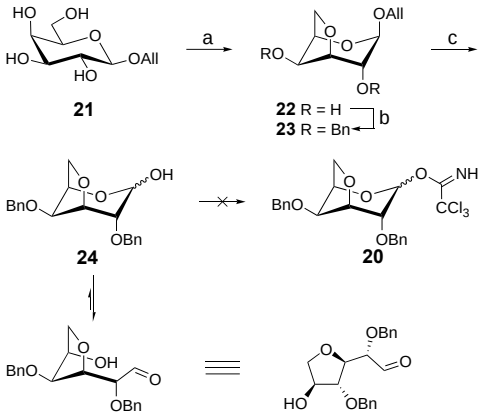
Scheme 2. a) **8**, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 88%; b) 70% w/w HF-pyridine, THF, r.t., 90%; c) TsCl, DMAP, pyridine, CH_2Cl_2, r.t., 87%; d) 80% aq. AcOH, r.t., 91%; e) NaOMe, MeOH, r.t., 82%.



Scheme 3. a) **12**, TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C, 23%; b) i. 70% w/w HF-pyridine, THF, r.t.; ii. TsCl, DMAP, pyridine, CH_2Cl_2, r.t., 61% over two steps; d) i. 80% aq. AcOH, r.t.; ii. NaOMe, MeOH, r.t., 65% over two steps.

Expediting the synthesis of **1** would be useful in the synthesis of related compounds. Thus, with **1** and **2** in hand via the late-stage ring closure method, we now looked into the application of using a 3,6-anhydro-galactosyl donor that would allow for the formation of α-glycosides (Figure 2). Indeed, it has been suggested that molecules of this type would be highly armed glycosyl donors.[23,24] In the first instance we wanted to explore the possible formation and use of a glycosyl imidate[25] based donor, as these types of donors are common throughout synthetic carbohydrate chemistry, so the synthesis of the 3,6-anhydro-galactosyl-based

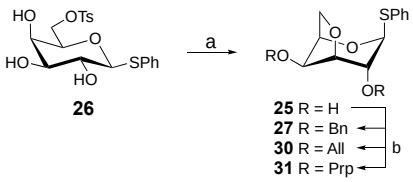
trichloroacetimidate **20** was attempted (Scheme 4). Treatment of allyl β-D-galactopyranoside **21** using Appel reaction conditions, which have previously been used in the synthesis of other 3,6-anhydro-galactosides,[7,26,27] successfully installed the 3,6-anhydro-bridge yielding the diol **22**. Protection of the C2 and C4 hydroxy groups with armed non-participating benzyl groups,[28] yielded the allyl ether **23**. Removal of the allyl glycoside using a Pd(II)-mediated deprotection[17] to obtain the hemiacetal **24**, looked successful by TLC but upon ^{1}H NMR analysis the material was determined to be a complex mixture of products with distinctive aldehyde signals, although these did not seem to relate to the major component (see Supporting Information). Despite this the material was taken forward to attempt the preparation of **20**. Attempts at preparing the trichloroacetimidate using standard conditions did not result in any observable formation of **20**, but only again gave a complex mixture of products. We presumed that inability to form **20** was due to 3,6-anhydro-galactose preferring to exist in the open aldehyde form rather than the bicyclic pyranose form (Scheme 4), which is required for the successful reaction. This preference has been shown through the study of 3,6-anhydro-D-galactose, which was found to have aldehydic character due to the added ring strain caused by the 3,6-anhydro-bridge.[29] Another possible reason is that this system could also be too armed to be isolated with standard conditions, which additionally would not make it a desirable glycosyl donor.



Scheme 4. a) CBr_4, PPh_3, pyridine, 60 °C, 91%; b) BnBr, NaH, DMF, r.t., 95%; c) $PdCl_2$, NaOAc, AcOH:H_2O 9:1, EtOAc, r.t.

Based on these results, in order to avoid the 3,6-anhydro-galactose hemiacetal, a glycosyl donor needed to be prepared where the activatable leaving group was in place before formation of the 3,6-anhydro-bridge. Indeed, this result highlights the benefit of a thioglycoside, used previously in this regard,[15] where the activatable group is stable to many different chemistries

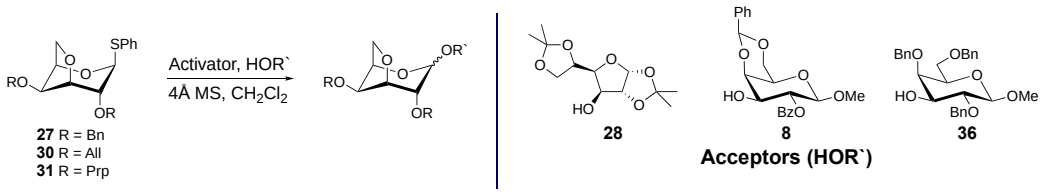
and this stability allows for manipulation of the other hydroxy groups to generate molecules of interest.[30] Thus the diol **25** was prepared from the 6-*O*-tosylate **26**[31] via treatment with methanolic NaOMe (Scheme 5), and protection of **25** yielded the benzyl protected putative donor **27**. For α-selectivity, formation of the heavily disfavoured 1,2-*cis*-equatorial bond was required. We were drawn to the methodologies used in β-D-mannosyl-, β-L-rhamnosyl- and uronic acid 6,3-lactone-based glycosylations, as these have the desired 1,2-*cis*-equatorial system. The pre-activation strategy pioneered by Crich and co-workers[32] has been utilised for these difficult glycosylations, which entails pre-activation of an appropriate thioglycoside with an activator and Tf_2O. Previously Christina *et al.*,[15] applied this system to a comparable 3,6-anhydro-galactosyl donor to study the reactivity and selectivity of a galacturonic acid 6,3-lactone thioglycoside as a glycosyl donor. However, they did not explore the utility of 3,6-anhydro-galactosyl donors in great detail.[15]

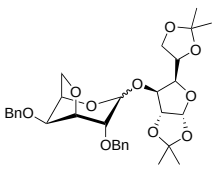
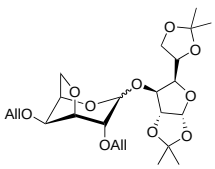
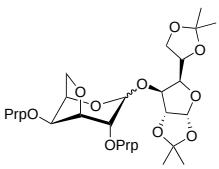
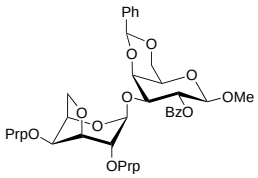
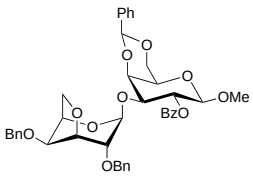
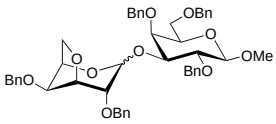
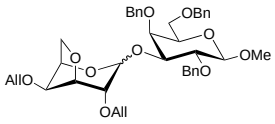
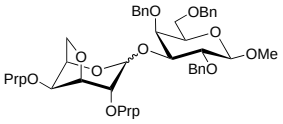


Scheme 5. a) NaOMe, MeOH, r.t., 91%; b) R-Br, NaH, DMF, r.t., 95-99%.

In the first instance, the benzyl protected **27** was glycosylated with a test acceptor **28**, firstly using the common NIS/TfOH promotor system for comparison. Pleasingly, the disaccharide **29** was obtained in good yield (Table 1), however, the β-glycoside was heavily favoured. Turning attention to the pre-activation methodology it was decided to employ the less reactive 1-benzenesulfinylpiperidine (BSP)/Tf_2O activation system first as the donor **27** does not have an electron withdrawing 6,3-lactone functionality, which was present in the donors utilised by Christina *et al.*,[15] which require the more reactive Ph_2SO/Tf_2O system.[33-35] Treatment of **27** with BSP and Tf_2O at -60 °C gave full activation within 5 min. Subsequent addition of the test acceptor **28**, bearing a secondary hydroxy group, gave the disaccharide **29** in an 86% overall yield and a shift in selectivity to an α:β ratio of 0.8:1 (Table 1). To explore this result further we modified the glycosyl donor to have the mildly disarming and sterically minimal allyl and propargyl (Prp) protecting groups which Crich and co-workers found can provide better 1,2-*cis*-equitorial selectivity.[36] We again tested these glycosyl donors with the test acceptor **28** and found a slight shift in selectivity for **30** (**32**, Table 1), and to a further extent for **31**, which now favoured the formation of the α-glycoside with a 2:1 α:β ratio (**33**, Table 1).

Table 1. Explorations into the use of suitable thiophenyl 3,6-anhydro-D-galactosides as glycosyl donors.



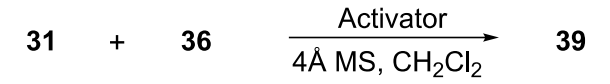
Donor	**27**	**30**	**31**	**27**
Acceptor	**28**	**28**	**28**	**8**
Product	 **29**	 **32**	 **33**	 **34**
Yield (α:β)	63% (0.1:1)[a,c] 86% (0.8:1)[a,d]	89% (1:1)[a,d]	89% (2:1)[a,d]	76% (β only)[b,d]
Donor	**31**	**27**	**30**	**31**
Acceptor	**8**	**36**	**36**	**36**
Product	 **35**	 **37**	 **38**	 **39**
Yield (α:β)	71% (β only)[b,d]	91% (1:1)[b,d]	87% (1:1)[b,d]	75% (2.8:1)[b,d]

[a]α/β ratio determined by ^{1}H NMR of anomeric mixture. [b]α/β ratio determined by isolation of individual anomers. [c]Reaction conditions: NIS, TfOH, CH_2Cl_2, -40 °C. [d]Reaction conditions: BSP, TTBP, Tf_2O, 4Å MS, CH_2Cl_2, -60→0 °C.

With these results in hand, attention was now directed towards suitable acceptors to prepare methyl neo-β-carrabioside **1**. Initially we used the acceptor **8**, but unfortunately only the β-glycosides **34** and **35** were observed using both the benzyl **27** and propargyl **31** protected glycosyl donors, respectively (Table 1). This result is potentially due to the weak nucleophilicity and low solubility of the acceptor **8**.[37] Therefore the more nucleophilic acceptor **36** was prepared from methyl 3-*O*-(4-methoxybenzyl)-β-galactopyranoside[38] via benzylation, followed by selective deprotection of the PMB with DDQ. Gratifyingly, using **36** resulted in good yields and selectivity towards the α-glycoside (α:β 2.8:1) when using the donor **31**, while

a 1:1 ratio was obtained for the benzyl- **27** and allyl-protected **30** donors (giving **37**-**39**, Table 1).

Table 2. Effect of activator and additives on the glycosylation of thiophenyl 3,6-anhydro-D-galactoside donor.



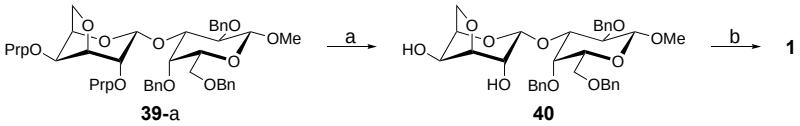
Entry	Activator	Additive	Yield (α:β)[a]
1[b]	BSP, Tf_2O	–	75% (2.8:1)
2[b]	Ph_2SO, Tf_2O	–	78% (2.8:1)
3[b]	BSP, Tf_2O	CH_3CN[c]	83% (2.5:1)
4[b]	BSP, Tf_2O	DMF[d]	18% (2:1)

[a]Yield and α/β ratio determined by isolation of individual anomers. [b]Reaction conditions: activator, TTBP, 4Å MS, CH_2Cl_2, -60→0 °C. [c]CH_3CN:CH_2Cl_2 5:95. [d]16 equivalents.

In an effort to try and improve upon the glycosylation selectivity results obtained we explored some variations of the reaction conditions. Firstly, the more powerful activator Ph_2SO, as used by Christina[15] was used instead of BSP, however, this resulted in no improvements in selectivity (Entry 2, Table 2). Two additives were also trialled, acetonitrile[39] which has been found to slightly favour the formation of β-L-rhamnosides through the nitrile effect, and DMF[40,41] which has been used to selectively form *cis*-1,2-glycosides. However, acetonitrile had little effect (Entry 3, Table 2) whereas DMF resulted in a poor yield and favoured the β-product (Entry 4, Table 2), suggesting it is incompatible with this type of glycosylation system.

With the improvement in stereoselectivity observed for the BSP/Tf_2O pre-activation methodology compared to the NIS/TfOH activation (**29**, Table 1) we attempted to investigate whether the presumed intermediate covalent glycosyl triflate could be observed using NMR, which has been identified as the intermediate species driving the stereoselectivity in the β-D-mannosyl-,[32,42] β-L-rhamnosyl-[32] and uronic acid 6,3-lactone-based[15,34] glycosylations. Attempts at this using the NMR techniques previously described[15,43] we were unable to observe a discernible glycosyl triflate, rather a complex mixture of species. This result highlights the armed nature of the 3,6-anhydro-galactosyl donor and suggests a less armed donor may allow for visualisation of this interesting intermediate.

With the result of using a suitable 3,6-anhydro-D-galactoyl donor to form the desired glycosidic bond with sufficient selectivity, the final desired product **1** was sought. Although propargyl groups are not common in synthetic carbohydrate chemistry some deprotection methods are known, which include conversion to the allene motif followed by cleavage with acid[44] or OsO_4 in the presence of NMNO,[36,44] low-valent titanium,[45,46] benzyltriethylammonium tetrathiomolybdate,[47] nickel-catalysed electrochemical protocols,[48] palladium-mediated,[49,50] and treatment with a SmI_2-amine-water system.[51] With consideration of the protecting groups on **39-α** and simplicity of reagents, we chose the two-step allene methodology. Conversion of the propargyl ethers to the corresponding allenyl ethers by treatment with *t*-BuOK in THF, followed by acid catalysed cleavage in 5% TFA, gave the diol **40** in excellent yield (Scheme 6). Subsequent Pd-mediated hydrogenolysis of the benzyl protecting groups on **40** yielded the desired methyl neo-β-carrabioside **1**.



Scheme 6. a) i. *t*-BuOK, THF, r.t.; ii. 5% TFA in H_2O:acetone (1:1), r.t., 90%; b) Pd/C (10% w/w), MeOH, H_2 atm, r.t., 96%.

With both methyl neo-β-carrabioside **1** and the *S*-linked variant **2** available we evaluated their use directly against the *exo*-α-3,6-anhydro-D-galactosidase *Zg*GH129. After incubation of the compounds with the enzyme, TLC analysis demonstrated that the *O*-linked disaccharide **1** was hydrolysed (Lane 2, Figure 3) and, as predicted, the *S*-linked disaccharide **2** was not (Lane 6, Figure 3). This demonstrates, for the first time, that the minimum unit for hydrolysis by *Zg*GH129 is neo-β-carrabiose. In addition as a control both compounds were incubated with a *exo*-α-3,6-anhydro-L-galactosidase from *Z. galactanivorans* (*Zg*3165)[52] which is an enzyme involved in agarose degradation. As expected, both of the compounds were not substrates for the enzyme (Lanes 4 and 8, Figure 3).

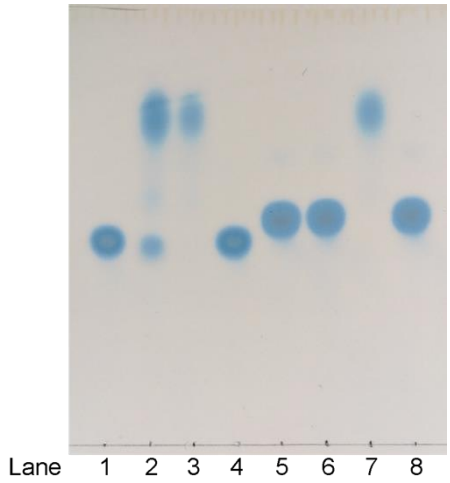


Figure 3. TLC assay of the reaction of **1** and **2** with *Zg*GH129. Lane 1) **1**; 2) **1** + *Zg*GH129; 3) 3,6-anhydro-D-galactose; 4) **1** + *Zg*3165; 5) **2**; 6) **2** + *Zg*GH129; 7) 3,6-anhydro-D-galactose; 8) **2** + *Zg*3165. The reactions were visualised with a 1,3-dihydroxynaphthalene stain, which colours the 3,6-anhydro-galactose residue after heating.[53]

Conclusion

Overall, we have used a biosynthesis inspired late stage ring closure for the successful synthesis of methyl neo-β-carrabioside **1** and the *S*-linked variant **2**. In addition, we have shown that 3,6-anhydro-galactosyl based compounds can be used as glycosyl donors to synthesise the required α-(1,3)-glycoside found in carrageenans, utilising the BSP/Tf_2O pre-activation system. The disaccharides prepared will be useful in the further study of *exo*-α-3,6-anhydro-D-galactosidases, in areas such as X-ray crystallography, but also will aid in the discovery and characterisation of enzymes with similar activity. Furthermore, the synthetic methodologies presented have the benefit of having a variety of protecting groups and modifications which could allow for the installation of different substituents, such as sulfate groups, which will expand the utility of the procedures we have developed.

Experimental Section

General experimental: All reagents and materials were purchased from commercial suppliers. Thin layer chromatography (TLC) was affected on Merck silica gel 60 F254 aluminium-backed plates and spots stained by heating with 5% conc. H_2SO_4 in ethanol, unless stated otherwise. Flash column chromatography was performed on Merck silica gel using the specified solvents. NMR spectra were obtained on a Bruker Avance IIIHD 400, 500 or 600 spectrometers. The solvents used were $CDCl_3$, D_2O or DMSO-d_6 with $CHCl_3$ (^1H, δ 7.26 ppm),

$CDCl_3$ (^{13}C, δ 77.16 ppm), DHO (^1H, δ 4.49 ppm), CH_3OH in D_2O (^{13}C, δ 49.5 ppm), $CD_3S(O)CD_2H$ (^1H, δ 2.50 ppm) or $(CD_3)_2SO$ (^{13}C, δ 39.52 ppm) used as an internal standard. Infrared spectra were obtained with neat samples on a PerkinElmer spectrum one FT-IR spectrometer fitted with a PerkinElmer Universal Attenuated Total Reflectance (ATR) sampling accessory. Wave numbers annotated with peak intensity; w = weak, m = medium, s = strong. High resolution mass spectra (HR-MS) were obtained on a Waters LCT Premier XE TOF spectrometer, run in W-mode, using either the ESI or APCI equipped ion source, in positive or negative mode.

Allyl 3,4-di-*O*-acetyl-6-*O*-(*tert*-butyl-di-methylsilyl)-2-*O*-(4-methoxybenzyl)-β-D-galactopyranoside 5

To a solution of allyl 2-O-(4-methoxylbenzyl)-3,4,6-O-tri-acetyl-β-D-galactopyranoside **4**[16] (1.3 g, 2.8 mmol) in MeOH (15 ml) was added NaOMe (50 mg) at 0 °C and the solution stirred at room temperature for 2 h. The mixture was then neutralized with Amberlite IR-120 (H^+ form), filtered and concentrated to give a colourless oil presumably the crude triol (920 mg). To a portion of the crude triol (700 mg) in DMF (16 ml) was added TBDMSCl (374 mg, 2.5 mmol) and then imidazole (340 mg, 4.9 mmol), at 0 °C. The solution was left overnight at room temperature, quenched with MeOH at 0 °C and concentrated. The residue was diluted with EtOAc and washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 3:7) to obtain a colourless oil (930 mg), which was dissolved in CH_2Cl_2 (6 ml) and pyridine (10 ml), and Ac_2O (2.5 ml, 26 mmol) was added at 0 °C. The solution was left for 24 h, quenched with MeOH and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 3:22) to obtain **5** as a colourless oil (1.1 g, 73%, over three steps). R_f = 0.70 (EtOAc:hexanes 3:7). ^1H NMR (500 MHz, $CDCl_3$): δ 7.24-7.19 (m, 2H), 6.88-6.82 (m, 2H), 6.01-5.89 (m, 1H), 5.42 (d, *J* = 3.4 Hz, 1H), 5.35 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.22 (dq, *J* = 10.4, 1.4 Hz, 1H), 4.96 (dd, *J* = 10.2, 3.5 Hz, 1H), 4.80 (d, *J* = 11.1 Hz, 1H), 4.56 (d, *J* = 11.1 Hz, 1H), 4.50 (d, *J* = 7.8 Hz, 1H), 4.44 (ddt, *J* = 12.9, 5.2, 1.5 Hz, 1H), 4.16 (ddt, *J* = 12.9, 6.1, 1.4 Hz, 1H), 3.79 (s, 3H), 3.73-3.57 (m, 4H), 2.09 (s, 3H), 1.96 (s, 3H), 0.86 (s, 9H), 0.02, (s, 3H), 0.01 (s, 3H); $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): δ 170.2, 159.4, 133.9, 130.7, 129.6, 117.8, 113.8, 102.9, 76.5, 74.6, 73.4, 72.7, 70.6, 67.7, 60.9, 55.4, 25.9, 20.9, 18.3, -5.4, -5.5; FT-IR (ATR): ν = 2929 (w), 1748 (s), 1514 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+Na]^+$ calcd. for $C_{27}H_{42}NaO_9Si$: 561.2496, found: 561.2498.

Allyl 3,4-di-*O*-acetyl-2-*O*-(4-methoxybenzyl)-β-D-galactopyranoside 6

To a solution of **5** (1.10 g, 2.42 mmol) and AcOH (2.5 ml) in THF (10 ml) was added TBAF in THF (1 M, 2.5 ml, 2.5 mmol) at 0 °C and the solution was then left at room temperature for 24 h. The mixture was then concentrated and the resultant residue purified by flash column chromatography (EtOAc:hexanes 22:28→1:1) to obtain **6** as a colourless oil (820 mg, 95%). R_f = 0.25 (EtOAc:hexanes 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24-7.19 (m, 2H), 6.89-6.83 (m, 2H), 6.02-5.91 (m, 1H), 5.40-5.33 (m, 1H), 5.32 (d, *J* = 3.4 Hz, 1H), 5.27-5.20 (m, 1H), 4.98 (dd, *J* = 10.2, 3.5 Hz, 1H), 4.81 (d, *J* = 11.1 Hz, 1H), 4.58 (d, *J* = 11.1 Hz, 1H), 4.53 (d, *J* = 7.8 Hz, 1H), 4.47-4.40 (m, 1H), 4.23-4.16 (m, 1H), 3.80 (s, 3H), 3.75-3.63 (m, 2H), 3.56-3.46 (m, 1H), 2.14 (s, 3H), 1.99 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 171.4, 170.1, 159.4, 133.8, 130.5, 129.6, 117.9, 113.8, 103.2, 76.5, 74.7, 73.4, 72.4, 71.0, 68.6, 60.9, 55.4, 20.9; FT-IR (ATR): ν = 3479 (br), 2963 (w), 1744 (s), 1514 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_{21}H_{29}O_9$: 425.1812, found: 425.1811.

Allyl 3,4-di-*O*-acetyl-6-*O*-chloroacetyl-2-*O*-(4-methoxybenzyl)-β-D-galactopyranoside 7

To a solution of **6** (850 mg, 2.00 mmol) and pyridine (3 ml) in CH_2Cl_2 (12 ml), was added $ClAc_2O$ (400 mg, 3.54 mmol) at 0 °C. The solution was stirred at 0 °C for 0.5 h, then quenched with MeOH and concentrated, co-evaporating with toluene. The residue was purified by flash column chromatography (EtOAc:hexanes 3:7) to obtain **7** as a colourless oil (950 mg, 95%). R_f = 0.28 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24-7.19 (m, 2H), 6.90-6.82 (m, 2H), 6.01-5.91 (m, 1H), 5.40-5.32 (m, 2H), 5.27-5.21 (m, 1H), 4.95 (dd, *J* = 10.2, 3.5 Hz, 1H), 4.80 (d, *J* = 11.1 Hz, 1H), 4.57 (d, *J* = 11.1 Hz, 1H), 4.52 (d, *J* = 7.8 Hz, 1H), 4.46-4.40 (m, 1H), 4.28 (dd, *J* = 11.2, 6.8 Hz, 1H), 4.22 (dd, *J* = 11.2, 6.6 Hz, 1H), 4.20-4.14 (m, 1H), 4.05 (s, 2H), 3.92-3.86 (m, 1H), 3.80 (s, 3H), 3.65 (dd, *J* = 10.2, 7.8 Hz, 1H), 2.12 (s, 3H), 1.97 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 170.4, 170.2, 167.0, 159.4, 133.7, 130.4, 129.6, 118.0, 113.8, 102.9, 76.1, 74.7, 72.3, 70.8, 70.4, 67.5, 63.1, 55.4, 40.7, 20.8; FT-IR (ATR): ν = 2974 (w), 1744 (s), 1513 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{23}H_{29}O_{10}{}^{35}ClNa$: 523.1347, found: 523.1345.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(3,4-di-*O*-acetyl-6-*O*-chloroacetyl-α-D-galactopyranosyl)-β-D-galactopyranoside 9

A suspension of **7** (152 mg, 0.303 mmol), NaOAc (160 mg, 1.95 mmol) and $PdCl_2$ (85 mg, 0.48 mmol) in aq. 90% AcOH:EtOAc (5:2, 5 ml) was stirred at room temperature for 24 h. The suspension was filtered through celite, washing with EtOAc. The filtrate was washed with

water, saturated aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 2:3) to obtain a colourless oil (128 mg), which was dissolved in CH_2Cl_2 (2 ml). Trichloroacetonitrile (0.27 ml, 2.7 mmol) followed by DBU (20 µl, 0.13 mmol) were added at 0 °C and the solution was stirred at 0 °C for 2 h. The solution was concentrated and the residue purified by flash column chromatography (EtOAc:hexanes 3:7) to obtain the trichloroacetimidate **3** as a colourless oil (105 mg). R_f = 0.47 (EtOAc:hexanes 2:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.24-7.19 (m, 2H), 6.89 (m, 2H), 6.49 (d, *J* = 3.6 Hz, 1H), 5.54-5.50 (m, 1H), 5.34 (dd, *J* = 10.6, 3.3 Hz, 1H), 4.60 (s, 2H), 4.47-4.40 (m, 1H), 4.20 (d, *J* = 2.1 Hz, 1H), 4.19 (d, *J* = 2.9 Hz, 1H), 4.03-3.98 (m, 1H), 4.01 (s, 2H), 3.80 (s, 3H), 2.13 (s, 3H), 2.01 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 170.2, 170.1, 167.0, 161.3, 159.6, 129.7, 129.3, 114.0, 94.2, 91.1, 72.8, 72.3, 69.5, 68.8, 67.9, 63.2, 55.4, 40.6, 20.9, 20.8. To a suspension of the trichloroacetimidate **3** (100 mg, 0.165 mmol), the alcohol **8**[18] (58 mg, 0.15 mmol) and 4 Å molecular sieves (210 mg) in CH_2Cl_2 (3.5 ml) and Et_2O (1 ml) was added TMSOTf (2 drops) at -20 °C and stirred for 2 h at this temperature. The suspension was neutralised with Et_3N and filtered through celite, washing with CH_2Cl_2. The filtrate was washed with sat. aq. $NaHCO_3$, water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 3:2) to obtain **9** as an off-white solid (38 mg, 20%, over 3 steps). R_f = 0.24 (EtOAc:hexanes 3:2). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15-8.06 (m, 2H), 7.63-7.56 (m, 1H), 7.56-7.50 (m, 2H), 7.49-7.42 (m, 2H), 7.42-7.34 (m, 3H), 5.60 (s, 1H), 5.57-5.49 (m, 1H), 5.14 (d, *J* = 4.0 Hz, 1H), 5.07 (d, *J* = 2.4 Hz, 1H), 4.86 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.62 (d, *J* = 8.1 Hz, 1H), 4.44 (d, *J* = 4.6 Hz, 1H), 4.41 (d, *J* = 3.6 Hz, 1H), 4.18-4.10 (m, 2H), 3.92-3.77 (m, 4H), 3.75 (d, *J* = 15.1 Hz, 1H), 3.70 (dd, *J* = 10.9, 6.3 Hz, 1H), 3.56 (s, 1H), 3.54 (s, 3H), 2.55 (d, *J* = 12.1 Hz, 1H), 2.04 (s, 3H), 1.96 (s, 3H); ^{13}C{^{1}H} NMR (100.6 MHz, $CDCl_3$): δ 170.2, 170.0, 166.5, 166.2, 137.1, 133.6, 129.9, 129.6, 129.4, 128.7, 128.5, 126.3, 101.8, 101.4, 96.4, 76.0, 72.1, 70.5, 70.1, 69.2, 67.6, 66.9, 66.6, 66.5, 62.5, 56.7, 40.6, 20.8, 20.6; FT-IR (ATR): ν = 3515 (br), 1728 (s) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{33}H_{37}O_{15}{}^{35}ClNa$: 731.1719, found: 731.1717.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(3,4,6-tri-*O*-acetyl-6-α-D-galactopyranosyl)-β-D-galactopyranoside 11

To a suspension of 3,4,6-tri-*O*-acetyl-2-*O*-(4-methoxybenzyl)-α-D-galactopyranosyl trichloroacetimidate **10**[16] (150 mg, 0.263 mmol), alcohol **8**[18] (85 mg, 0.22 mmol) and 4 Å molecular sieves (300 mg) in CH_2Cl_2 (5 ml) and Et_2O (2.5 ml) was added TMSOTf (3 drops)

at -20 °C and stirred for 2 h at this temperature. The suspension was neutralised with Et_3N and filtered through celite, washing with CH_2Cl_2. The filtrate was washed with sat. aq. $NaHCO_3$, water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 3:2) to obtain **11** as a white foam (56 mg, 38%). R_f = 0.28 (EtOAc:hexanes 3:2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.11-8.06 (m, 2H), 7.59-7.54 (m, 1H), 7.54-7.50 (m, 2H), 7.47-7.41 (m, 2H), 7.41-7.34 (m, 3H), 5.60 (s, 1H), 5.57-5.50 (m, 1H), 5.13 (d, *J* = 4.0 Hz, 1H), 5.12-5.09 (m, 1H), 4.87 (dd, *J* = 10.4, 3.2 Hz, 1H), 4.62 (d, *J* = 8.0 Hz, 1H), 4.46-4.42 (m, 1H), 4.41 (d, *J* = 3.6 Hz, 1H), 4.17-4.09 (m, 2H), 3.90-3.82 (m, 2H), 3.76 (dd, *J* = 10.8, 8.1 Hz, 1H), 3.58-3.52 (m, 5H), 2.59 (d, *J* = 11.9 Hz, 1H), 2.04 (s, 3H), 1.96 (s, 3H), 1.82 (s, 3H); ^{13}C{^{1}H} NMR (150.9 MHz, $CDCl_3$): δ 170.3, 170.1, 170.0, 165.2, 137.1, 133.5, 129.9, 129.6, 129.5, 128.6, 128.5, 126.4, 101.8, 101.5, 96.6, 76.1, 72.2, 70.7, 70.0, 69.2, 67.7, 67.2, 66.7, 66.5, 60.9, 56.6, 20.9, 20.7; FT-IR (ATR): ν = 3402 (br), 1717 (s) cm^{-1}; HR-MS (APCI): *m/z* $[M+H]^+$ calcd. for $C_{33}H_{39}O_{15}$: 675.2289, found: 675.2284.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilanediyl)-α-D-galactopyranosyl)-β-D-galactopyranoside 13

To a mixture of the alcohol **8**[18] (175 mg, 0.453 mmol), trichloroacetimidate **12**[20] (383 mg, 0.569 mmol) and 4 Å molecular sieves (600 mg) in CH_2Cl_2 (10 ml) at 0 °C, was added TMSOTf (6 µl, 33 µmol). The mixture was stirred at 0 °C for 1 h, then neutralised with addition of Et_3N and filtered through celite, washing with CH_2Cl_2. The filtrate was washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 2:23 → 3:7), to obtain **13** as a white foam (360 mg, 88%). R_f = 0.47 (EtOAc:hexanes 2:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12-8.05 (m, 2H), 7.97-7.92 (m, 2H), 7.82-7.77 (m, 2H), 7.54-7.58 (m, 1H), 7.53-7.44 (m, 3H), 7.37-7.27 (m, 3H), 7.24-7.17 (m, 3H), 7.16-7.10 (m, 2H), 7.04-6.97 (m, 2H), 5.68 (dd, *J* = 10.2, 7.9 Hz, 1H), 5.64 (d, *J* = 3.9 Hz, 1H), 5.52 (dd, *J* = 10.6, 3.9 Hz, 1H), 5.42 (dd, *J* = 10.6, 3.1 Hz, 1H), 5.10 (s, 1H), 4.57 (d, *J* = 7.9 Hz, 1H), 4.55 (d, *J* = 2.9 Hz, 1H), 4.27 (dd, *J* = 12.2, 1.5 Hz, 1H), 4.16 (d, *J* = 3.0 Hz, 1H), 4.06 (dd, *J* = 10.2, 3.3 Hz, 1H), 3.96 (dd, *J* = 12.2, 1.4 Hz, 1H), 3.82 (dd, *J* = 12.8, 1.4 Hz, 1H), 3.71-3.67 (m, 1H), 3.57 (dd, *J* = 12.7, 2.0 Hz, 1H), 3.48 (s, 3H), 3.45-3.42 (m, 1H), 1.03 (s, 9H), 0.84 (s, 9H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 166.9, 165.9, 165.2, 137.4, 133.5, 133.3, 133.1, 130.1, 130.07, 129.9, 129.8, 129.7, 129.0, 128.7, 128.6, 128.42, 128.39, 128.0, 126.1, 102.1, 100.5, 95.4, 76.8, 72.7, 70.8, 70.0, 69.2, 68.9, 67.3,

66.7, 66.6, 56.3, 27.5, 27.3, 23.3, 20.8; FT-IR (ATR): ν = 1723 (s), 1602 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_{49}H_{57}O_{14}Si$: 897.3518, found: 897.3522.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3-di-*O*-benzoyl-α-D-galactopyranosyl)-β-D-galactopyranoside 14

Hydrogen fluoride in pyridine (70% w/w, 0.15 ml) was added to a solution of **13** (340 mg, 0.379 mmol) in THF (6 ml), at 0 °C. The solution was stirred at room temperature for 2 h and then neutralised with addition of sat. aq. $NaHCO_3$. The mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 4:1) to obtain **14** as a white foam (256 mg, 90%). R_f = 0.21 (EtOAc:hexanes 7:3). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.17-7.12 (m, 2H), 7.95-7.91 (m, 2H), 7.81-7.76 (m, 2H), 7.64-7.59 (m, 1H), 7.54-7.45 (m, 3H), 7.67-7.26 (m, 3H), 7.26-7.22 (m, 2H), 7.22-7.17 (m, 1H), 7.15-7.09 (m, 2H), 7.06-7.01 (m, 2H), 5.69 (dd, *J* = 10.1, 8.0 Hz, 1H), 5.66 (d, *J* = 3.1 Hz, 1H), 5.54-5.48 (m, 2H), 5.18 (s, 1H), 4.59 (d, *J* = 7.9 Hz, 1H), 4.28 (dd, *J* = 12.1, 1.5 Hz, 1H), 4.19 (d, *J* = 3.1 Hz, 1H), 4.17-4.15 (m, 1H), 4.06 (dd, *J* = 10.1, 3.3 Hz, 1H), 3.97 (dd, *J* = 12.1, 1.5 Hz, 1H), 3.85 (dd [appt t], *J* = 4.1, 4.1 Hz, 1H), 3.58-3.49 (m, 2H), 3.48 (s, 3H), 3.46-3.43 (m, 1H), 2.87 (d, *J* = 2.4 Hz, 1H), 1.98 (dd, *J* = 7.2, 5.6 Hz, 1H); ^{13}C{^{1}H} NMR (150.9 MHz, $CDCl_3$): δ 166.7, 165.5, 165.4, 137.3, 133.5, 133.41, 133.38, 130.1, 129.9, 129.8, 129.7, 129.5, 128.9, 128.7, 128.6, 128.5, 128.4, 128.0, 126.1, 102.0, 100.6, 95.2, 76.6, 72.6, 70.8, 70.2, 69.8, 69.20, 69.17, 68.9, 66.6, 63.2, 56.4; FT-IR (ATR): ν = 3441 (br), 1723 (s), 1602 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_{41}H_{41}O_{14}$: 757.2496, found: 757.2498.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2,3-di-*O*-benzoyl-6-*O*-tosyl-α-D-galactopyranosyl)-β-D-galactopyranoside 15

Tosyl chloride (75 mg, 0.39 mmol) and DMAP (4 mg, 0.03 mmol) were added to a solution of **14** (250 mg, 0.330 mmol) and pyridine (1 ml) in CH_2Cl_2 (3 ml), at 0 °C. The resulting solution was left to stir at room temperature for 24 h, with additions of further tosyl chloride (35 mg, 0.18 mmol) after 3 h and 9 h each with cooling to 0 °C. After this time the reaction was quenched with MeOH and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 2:3) to obtain **15** as a white foam (260 mg, 87%). R_f = 0.43 (EtOAc:hexanes 1:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19-7.14 (m, 2H), 7.90-7.86 (m, 2H), 7.81-7.73 (m, 4H), 7.60-7.54 (m, 1H), 7.51-7.44 (m, 3H), 7.39-7.30 (m, 5H), 7.24-7.19 (m,

2H), 7.16-7.11 (m, 1H), 7.10-7.05 (m, 2H), 7.03-6.97 (m, 2H), 5.71 (dd, *J* = 10.2, 8.0 Hz, 1H), 5.56 (d, *J* = 3.7 Hz, 1H), 5.45 (dd, *J* = 10.6, 3.7 Hz, 1H), 5.38 (dd, *J* = 10.6, 3.1 Hz, 1H), 5.30 (s, 1H), 4.59 (d, *J* = 8.0 Hz, 1H), 4.32 (dd, *J* = 12.2, 1.4 Hz, 1H), 4.27 (d, *J* = 3.2 Hz, 1H), 4.16 (dd [appt t], *J* = 6.3, 6.3 Hz, 1H), 4.09 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.05-3.98 (m, 3H), 3.89-3.85 (m, 1H), 3.51 (s, 3H), 3.50-3.48 (m, 1H), 2.46 (s, 3H), 2.07-2.03 (m, 1H); $^{13}C\{^{1}H\}$ NMR (125.8 MHz, $CDCl_3$): δ 166.5, 165.3, 165.1, 145.2, 137.3, 133.5, 133.4, 133.3, 132.9, 130.1, 129.83, 129.82, 129.3, 128.9, 128.7, 128.59, 128.56, 128.4, 128.1, 128.0, 126.0, 102.0, 100.6, 93.2, 74.6, 71.6, 70.4, 69.8, 69.1, 68.5, 68.1, 67.8, 67.4, 66.5, 56.5, 21.8; FT-IR (ATR): ν = 1726 (s), 1601 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_{48}H_{47}O_{16}S$: 911.2585, found: 911.2586.

Methyl 2-*O*-benzoyl-3-*O*-(2,3-di-*O*-benzoyl-6-*O*-tosyl-α-D-galactopyranosyl)-β-D-galactopyranoside 16

A solution of **15** (260 mg, 0.285 mmol) and 80% aq. AcOH (5 ml) was stirred at room temperature for 1.5 h. The solution was then concentrated, co-evaporating with toluene. The residue was purified by flash column chromatography (EtOAc:hexanes 3:2) to obtain **16** as a white foam (213 mg, 91%). R_f = 0.44 (EtOAc:hexanes 7:3). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.14-8.08 (m, 2H), 7.97-7.90 (m, 4H), 7.75-7.70 (m, 2H), 7.59-7.54 (m, 1H), 7.53-7.48 (m, 2H), 7.47-7.43 (m, 2H), 7.40-7.32 (m, 6H), 5.60 (dd, *J* = 10.7, 3.7 Hz, 1H), 5.55-5.50 (m, 1H), 5.49 (dd, *J* = 10.7, 3.1 Hz, 1H), 5.39 (d, *J* = 3.7 Hz, 1H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.10 (dd [appt t], *J* = 6.4, 6.4 Hz, 1H), 4.02-3.98 (m, 1H), 3.97-3.92 (m, 3H), 3.92-3.86 (m, 1H), 3.81 (dd, *J* = 10.1, 6.6 Hz, 1H), 3.78-3.71 (m, 1H), 3.55 (dd [appt t], *J* = 5.7, 5.7 Hz, 1H), 3.49 (s, 3H), 2.58 (*br* s, 1H), 2.46 (s, 3H), 2.19 (d, *J* = 3.7 Hz, 1H), 2.06-2.01 (m, 1H); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, $CDCl_3$): δ 166.2, 165.43, 165.38, 145.3, 133.9, 133.6, 133.5, 132.7, 130.0, 129.88, 129.86, 129.5, 129.2, 128.82, 128.78, 128.6, 128.1, 102.2, 94.5, 77.7, 74.1, 70.3, 70.2, 68.4, 68.2, 67.4, 67.3, 66.3, 62.3, 56.9, 21.8; FT-IR (ATR): ν = 3510 (br), 1726 (s), 1601 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_{41}H_{43}O_{16}S$: 823.2272, found: 823.2275.

Methyl 3-*O*-(3,6-anhydro-α-D-galactopyranosyl)-β-D-galactopyranoside 1

From **16**: A 1.83 M solution of NaOMe in MeOH (0.35 ml, 0.65 mmol) was added to a solution of **16** (105 mg, 0.128 mmol) in MeOH (5 ml) at 0 °C. The solution was left at room temperature for 24 h, then neutralised with addition of AcOH and concentrated. The residue was purified by flash column chromatography (MeOH:$CHCl_3$ 1:4) to obtain **1** as a white solid

(36 mg, 82%). R_f = 0.55 (MeOH:$CHCl_3$ 3:7). ^{1}H NMR (600 MHz, D_2O): δ 5.07 (d, *J* = 2.5 Hz, 1H), 4.50 (d, *J* = 1.9 Hz, 1H), 4.44-4.42 (m, 1H), 4.38 (d, *J* = 5.4 Hz, 1H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.20 (d, *J* = 10.7 Hz, 1H), 4.14 (d, *J* = 3.0 Hz, 1H), 4.08-4.02 (m, 2H) 3.85 (dd, *J* = 9.8, 3.4 Hz, 1H), 3.81 (dd, *J* = 11.7, 7.8 Hz, 1H), 3.76 (dd, *J* = 11.7, 4.4 Hz, 1H), 3.68 (ddd, *J* = 7.8, 4.4, 0.8 Hz, 1H), 3.61 (dd, *J* = 9.8, 8.0 Hz, 1H), 3.58 (s, 3H); ^{13}C{^{1}H} NMR (150.9 MHz, D_2O): δ 104.2, 94.5, 81.2, 80.5, 77.7, 75.6, 70.3, 70.2, 69.8, 69.3, 66.4, 61.6, 57.8; FT-IR (ATR): ν = 3337 (br) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{13}H_{22}O_{10}Na$: 361.1111, found: 361.1111.

From **40**: A mixture of **40** (28 mg, 0.046 mmol) and Pd/C (10% w/w, 7 mg) in MeOH (3 ml) was stirred under an atmosphere of H_2 for 5 h. The mixture was filtered through celite, washed with MeOH:H_2O (1:1), and concentrated to obtain **1** as a white solid (15 mg, 96%). The ^{1}H and ^{13}C spectra with that above.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*S*-(2,3-di-*O*-benzoyl-4,6-*O*-(di-*tert*-butylsilanediyl)-α-D-galactopyranosyl)-3-thio-β-D-galactopyranoside 18

To a mixture of the thiol **17**[22] (210 mg, 0.522 mmol), trichloroacetimidate **12**[20] (436 mg, 0.648 mmol) and 4 Å molecular sieves (700 mg) in CH_2Cl_2 (11 ml) at 0 °C, was added TMSOTf (6 µl, 33 µl). The mixture was stirred at 0 °C for 1 h, then neutralised with addition of Et_3N and filtered through celite, washing with CH_2Cl_2. The filtrate was washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 1:4) to obtain **18** as a colourless oil (110 mg, 23%). R_f = 0.31 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08-8.03 (m, 2H), 8.00-7.95 (m, 2H), 7.95-7.91 (m, 2H), 7.63-7.57 (m, 1H), 7.53-7.46 (m, 3H), 7.45-7.33 (m, 4H), 7.26-7.14 (m, 6H), 5.99 (d, *J* = 5.8 Hz, 1H), 5.73 (dd, *J* = 10.6, 5.8 Hz, 1H), 5.34 (dd, *J* = 11.4, 7.6 Hz, 1H), 5.24 (dd, *J* = 10.6, 3.1 Hz, 1H), 5.23 (s, 1H), 4.61 (d, *J* = 3.0 Hz, 1H), 4.57 (d, *J* = 7.7 Hz, 1H), 4.32 (dd, *J* = 12.3, 1.2 Hz, 1H), 4.03-3.95 (m, 2H), 3.94-3.88 (m, 1H), 3.90 (s, 1H), 3.85-3.79 (m, 1H), 3.54 (s, 1H), 3.46 (s, 3H), 3.32 (dd, *J* = 11.3, 3.1 Hz, 1H), 1.06 (s, 9H), 0.88 (s, 9H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 166.4, 165.9, 165.3, 137.4, 133.6, 133.34, 133.30, 130.1, 129.9, 129.8, 129.3, 128.6, 128.58, 128.50, 128.0, 126.1, 103.1, 101.0, 83.6, 74.6, 71.3, 70.8, 69.4, 69.0, 68.8, 68.78, 68.1, 66.9, 56.4, 49.6, 27.6, 27.3, 23.3, 20.8; FT-IR (ATR): ν = 1725 (s) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_{49}H_{57}O_{13}SSi$: 913.3289, found: 913.3288.

Methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*S*-(2,3-di-*O*-benzoyl-6-*O*-tosyl-α-D-galactopyranosyl)-3-thio-β-D-galactopyranoside 19

Hydrogen fluoride in pyridine (70% w/w, 0.04 ml) was added to a solution of **18** (100 mg, 0.110 mmol) in THF (2 ml), at 0 °C. The solution was stirred at room temperature for 1 h and neutralised with addition of sat. aq. $NaHCO_3$. The mixture was diluted with EtOAc, and washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 4:1 → 1:0) to obtain a colourless oil (70 mg), which was dissolved in CH_2Cl_2 (1 ml) and pyridine (0.3 ml). Tosyl chloride (21 mg, 0.11 mmol) and DMAP (1 mg, 8 µmol) were added to the solution at 0 °C. The resulting solution was stirred at room temperature for 24 h, with additions of further tosyl chloride (9.5 mg, 0.05 mmol) after 3 h, 9 h and 28 h, each with cooling to 0 °C. After this time the reaction was quenched with MeOH and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 2:3) to obtain **19** as a white foam (62 mg, 61%, over two steps). R_f = 0.42 (EtOAc:hexanes 1:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.13-8.08 (m, 2H), 7.95-7.90 (m, 2H), 7.90-7.86 (m, 2H), 7.84-7.79 (m, 2H), 7.58-41 (m, 6H) 7.41-7.34 (m, 4H), 7.33-7.20 (m, 4H), 7.17-7.12 (m, 2H), 5.98 (d, *J* = 5.9 Hz, 1H), 5.71 (dd, *J* = 10.5, 5.9 Hz, 1H), 5.42 (s, 1H), 5.41-5.35 (m, 2H), 4.62 (d, *J* = 7.7 Hz, 1H), 4.44 (dd [appt t], *J* = 6.1, 6.1 Hz, 1H), 4.35 (d, *J* = 12.4 Hz, 1H), 4.17 (dd, *J* = 10.5, 7.6 Hz, 1H), 4.11-4.02 (m, 4H), 3.59 (s, 1H), 3.51-3.45 (m, 1H), 3.49 (s, 3H), 2.47 (s, 3H), 2.13 (d, *J* = 3.9 Hz, 1H); ^{13}C{^{1}H} NMR (150.9 MHz, $CDCl_3$): δ 166.0, 165.5, 165.3, 145.4, 137.5, 133.7, 133.6, 133.3, 132.9, 130.2, 130.1, 130.04, 130.01, 129.9, 129.1, 128.9, 128.7, 128.6, 128.1, 128.0, 127.8, 126.1, 103.3, 101.0, 80.6, 73.2, 71.0, 69.2, 69.1, 68.7, 68.3, 68.2, 67.5, 56.5, 47.3, 21.8; FT-IR (ATR): ν = 1728 (s), 1601 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_{48}H_{47}O_{15}S_2$: 927.2356, found: 927.2360.

Methyl 3-*S*-(3,6-anhydro-α-D-galactopyranosyl)-3-thio-β-D-galactopyranoside 2

A solution of **19** (60 mg, 0.065 mmol) and 80% aq. AcOH (2 ml) was stirred at room temperature for 2 h. The solution was then concentrated, co-evaporating with toluene. The residue was dissolved in MeOH (2 ml) and a 1.69 M solution of NaOMe in MeOH (0.15 ml, 0.25 mmol) was added at 0 °C. The solution was left at room temperature for 30 h, neutralised with AcOH and concentrated. The residue was purified by flash column chromatography (MeOH:$CHCl_3$ 1:4) to obtain **2** as an off-white foam (15 mg, 65%, over two steps). R_f = 0.58 (MeOH:$CHCl_3$ 3:7). ^{1}H NMR (600 MHz, D_2O): δ 5.13 (d, *J* = 2.5 Hz, 1H), 4.47-4.43 (m, 2H),

4.38 (d, *J* = 7.7 Hz, 1H), 4.36 (d, *J* = 5.3 Hz, 1H), 4.24 (d, *J* = 10.9 Hz, 1H), 4.06 (dd, *J* = 5.3, 2.9 Hz, 1H), 4.05 (dd, *J* = 11.0, 2.9 Hz, 1H), 4.01 (d, *J* = 2.8 Hz, 1H), 3.79-7.72 (m, 3H), 3.58 (s, 3H), 3.49 (dd, *J* = 11.2, 7.7 Hz, 1H), 3.19 (dd, *J* = 11.2, 2.9 Hz, 1H); $^{13}C\{^{1}H\}$ NMR (150.9 MHz, D_2O): δ 105.5, 81.9, 79.8, 79.6, 77.9, 72.0, 70.3, 69.9, 69.1, 68.9, 61.7, 57.7, 53.4; FT-IR (ATR): ν = 3420 (br) cm^{-1}; HR-MS (ESI+): *m/z* $[M+AcCN+Na]^+$ calcd. for $C_{15}H_{25}NO_9SNa$: 418.1148, found: 418.1150.

Allyl 3,6-anhydro-β-D-galactopyranoside 22

To a solution of allyl β-D-galactopyranoside **21** (500 mg, 2.27 mmol) in pyridine (15 ml) at 0 °C, was added CBr_4 (753 mg, 2.27 mmol) and PPh_3 (1.19 g, 4.54 mmol). The yellow solution was then stirred at 60 °C with a heating mantle for 1.5 h, quenched with MeOH at 0 °C and concentrated. The residue was purified by flash column chromatography (MeOH:$CHCl_3$ 2:23) to obtain **22** as a white solid (418 mg, 91%). R_f = 0.47 (MeOH:$CHCl_3$ 4:21). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 5.93-5.83 (m, 1H), 5.44 (d, *J* = 4.6 Hz, 1H), 5.24 (dq, *J* = 17.2, 1.8 Hz, 1H), 5.17 (d, *J* = 3.9 Hz, 1H), 5.16-5.12 (m, 1H), 4.44 (s, 1H), 4.16-4.13 (m, 1H), 4.11 (dt, *J* = 5.1, 1.6 Hz, 1H), 4.09-4.06 (m, 1H), 3.93 (d, *J* = 5.1 Hz, 1H), 3.92 (s, 1H), 3.86 (ddt, *J* = 13.2, 5.8, 1.5 Hz, 1H), 3.74 (dd, *J* = 9.5, 4.6 Hz, 1H), 3.72 (dd, *J* = 9.1, 3.2 Hz, 1H); $^{13}C\{^{1}H\}$ NMR (125.8 MHz, $(CD_3)_2SO$): δ 134.8, 116.5, 101.1, 80.8, 77.4, 72.5, 69.5, 69.4, 67.6; FT-IR (ATR): ν = 3342 (br), 2936 (w) cm^{-1}; HR-MS (APCI+): *m/z* $[M+H]^+$ calcd. for $C_9H_{15}O_5$: 203.0919, found: 203.0924.

Allyl 3,6-anhydro-2,4-di-*O*-benzyl-β-D-galactopyranoside 23

Sodium hydride (60% dispersion in oil, 213 mg, 5.33 mmol) was added to a solution of **22** (415 mg, 2.05 mmol) and benzyl bromide (0.25 ml, 2.1 mmol) in DMF (11 ml) at 0 °C. A further amount of benzyl bromide (0.33 ml, 2.8 mmol) was then added. The resultant suspension was then stirred at room temperature for 1 h, quenched with MeOH at 0 °C and concentrated. The residue was diluted with EtOAc and washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 15:85) to obtain **23** as a colourless oil (745 mg, 95%). R_f = 0.44 (EtOAc:hexanes 1:4). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.26 (m, 10H), 5.95-5.81 (m, 1H), 5.28 (dq, *J* = 17.2, 1.6 Hz, 1H), 5.18 (dq, *J* = 10.4, 1.6 Hz, 1H), 4.68 (s, 1H), 4.62 (d, *J* = 12.0 Hz, 1H), 4.58 (d, *J* = 11.9 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.51 (d, *J* = 11.9 Hz, 1H), 4.40 (d, *J* = 4.8 Hz, 1H), 4.33-4.29 (m, 1H), 5.27-4.17 (m, 3H), 4.01-3.91 (m, 2H), 3.83 (d, *J* = 4.8 Hz, 1H); $^{13}C\{^{1}H\}$ NMR (125.8 MHz, $CDCl_3$): δ 138.0, 137.7, 134.0, 128.61,

128.59, 128.1, 128.0, 127.9, 127.8, 117.5, 98.8, 80.4, 78.1, 77.3, 76.1, 72.7, 71.2, 70.8, 68.7; FT-IR (ATR): ν = 1497 (w), 1455 (m) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{23}H_{26}O_5Na$: 405.1678, found: 405.1669.

3,6-anhydro-1-*S*-phenyl-1-thio-β-D-galactopyranoside 25

A 1.3 M solution of NaOMe in MeOH (0.85 ml, 1.1 mmol) was added to 1-*S*-phenyl-1-thio-6-tosyl-*β*-D-galactopyranoside **26**[31] (350 mg, 0.821 mmol) in MeOH (3.4 ml) at 0 °C. The solution was left at room temperature for 24 h, neutralised with AcOH and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 1:1→7:3) to obtain **25** as a white solid (190 mg, 91%). R_f = 0.28 (EtOAc:hexanes 1:1). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ 7.44-7.39 (m, 2H), 7.35-7.30 (m, 2H), 7.26-7.21 (m, 1H), 5.85 (d, *J* = 3.8 Hz, 1H), 5.35 (d, *J* = 3.7 Hz, 1H), 5.18 (s, 1H), 4.56 (d, *J* = 9.4 Hz, 1H), 4.27-4.24 (m, 1H), 4.21-4.18 (m, 1H), 4.09-4.03 (m, 2H), 3.80 (dd, *J* = 9.4, 2.8 Hz, 1H); $^{13}C\{^1H\}$ NMR (125.8 MHz, $(CD_3)_2SO$): δ 136.6, 129.6, 129.0, 126.5, 86.9, 81.1, 78.6, 74.7, 69.3, 68.9; FT-IR (ATR): ν = 3246 (br) cm^{-1}; HR-MS (ESI-): *m/z* $[M+HCOO]^-$ calcd. for $C_{13}H_{15}O_6S$: 299.0589, found: 299.0579.

3,6-Anhydro-2,4-di-*O*-benzyl-1-*S*-phenyl-1-thio-β-D-galactopyranoside 27

Sodium hydride (60% dispersion in oil, 272 mg, 6.80 mmol) was added to a solution of **25** (665 mg, 2.62 mmol) and benzyl bromide (0.40 ml, 3.4 mmol) in DMF (14 ml) at 0 °C. A further amount of benzyl bromide (0.35 ml, 2.9 mmol) was then added. The resultant suspension was then stirred at room temperature for 1 h, quenched with MeOH at 0 °C and concentrated. The residue was diluted with EtOAc and washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 5:95→1:9) to obtain **27** as a colourless oil (1.13 g, 99%). R_f = 0.38 (EtOAc:hexanes 1:9). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.40 (m, 2H), 7.37-7.20 (m, 13H), 5.35 (s, 1H), 4.84 (d, *J* = 9.7 Hz, 1H), 4.63 (d, *J* = 11.9 Hz, 1H), 4.60-5.52 (m, 2H), 4.48 (d, *J* = 11.9 Hz, 1H), 4.45 (d, *J* = 4.9 Hz, 1H), 4.40 (t, *J* = 2.1 Hz, 1H), 4.31 (d, *J* = 1.7 Hz, 1H), 4.11 (d, *J* = 4.9 Hz, 1H), 3.98 (dd, *J* = 9.7, 2.7 Hz, 1H); $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): δ 137.8, 137.4, 136.5, 130.5, 129.1, 128.63, 128.61, 128.1, 128.0, 127.9, 128.8, 127.0, 84.6, 82.5, 78.1, 77.8, 77.2, 72.6, 71.3, 70.1; FT-IR (ATR): ν = 1584 (w), 1496 (w), 1481 (w), 1454 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+H]^+$ calcd. for $C_{26}H_{27}O_4S$: 435.1630, found: 435.1621.

2,4-di-*O*-Allyl-3,6-anhydro-1-*S*-phenyl-1-thio-β-D-galactopyranoside 30

Sodium hydride (60% dispersion in oil, 77 mg, 1.9 mmol) followed by allyl bromide (0.15 ml, 1.8 mmol) was added to a solution of **25** (188 mg, 0.739 mmol) in DMF (3 ml) at 0 °C. The resultant suspension was then stirred at room temperature for 1 h, quenched with MeOH at 0 °C and concentrated. The residue was diluted with EtOAc and washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 4:46) to obtain **30** as a colourless oil (235 mg, 95%). R_f = 0.53 (EtOAc:hexanes 1:4). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51-7.44 (m, 2H), 7.34-7.28 (m, 2H), 7.26-7.21 (m, 1H), 5.96-5.81 (m, 2H), 5.34 (s, 1H), 5.31 (ddd [appt dq], *J* = 12.0, 1.6 Hz, 1H), 5.27 (ddd [appt dq], *J* = 12.1, 1.6 Hz, 1H), 5.23-5.18 (m, 2H), 4.85 (d, *J* = 9.7 Hz, 1H), 4.46-4.41 (m, 2H), 4.21 (d, *J* = 1.6 Hz, 1H), 4.14-4.02 (m, 4H), 3.99 (dddd [appt ddt], *J* = 12.9, 5.8, 1.4 Hz, 1H), 3.95 (dd, *J* = 9.7, 2.6 Hz, 1H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 136.5, 134.4, 133.9, 130.5, 129.1, 127.0, 117.9, 117.7, 84.6, 82.2, 77.90, 77.88, 77.1, 71.5, 70.3, 70.0; FT-IR (ATR): ν = 3078 (w), 1646 (w), 1584 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+H]^+$ calcd. for $C_{18}H_{23}O_4S$: 335.1317, found: 335.1321.

3,6-Anhydro-2,4-di-*O*-propargyl-1-*S*-phenyl-1-thio-β-D-galactopyranoside 31

Sodium hydride (60% dispersion in oil, 143 mg, 3.59 mmol) was added to a solution of **25** (350 mg, 1.38 mmol) in DMF (5.5 ml) at 0 °C. After the suspension was stirred at 0 °C for 15 min, propargyl bromide (80% in toluene, 0.37 ml, 3.3 mmol) was added. The resultant suspension was then stirred at room temperature for 1 h, quenched with MeOH at 0 °C and concentrated. The residue was diluted with EtOAc and washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 15:85) to obtain **31** as a colourless oil (274 mg, 99%). R_f = 0.35 (EtOAc:hexanes 1:4). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52-7.44 (m, 2H), 7.34-7.27 (m, 2H), 7.26-7.21 (m, 1H), 5.41 (s, 1H), 4.89 (d, *J* = 9.8 Hz, 1H), 4.57-4.54 (m, 1H), 4.53 (d, *J* = 5.1 Hz, 1H), 4.34 (d, *J* = 1.7 Hz, 1H), 4.30-4.16 (m, 5H), 3.95 (dd, *J* = 7.8, 2.8 Hz, 1H), 2.47 (t, *J* = 2.4 Hz, 1H), 2.45 (t, *J* = 2.4 Hz, 1H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 136.4, 130.6, 129.1, 127.1, 84.6, 82.2, 79.5, 79.0, 78.1, 77.6, 77.0, 75.6, 75.2, 70.0, 58.1, 56.8; FT-IR (ATR): ν = 3285 (m), 2118 (w), 1584 (w) cm^{-1}; HR-MS (ESI-): *m/z* $[M-H]^-$ calcd. for $C_{18}H_{17}O_4S$: 329.0848, found: 329.0840.

Methyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 36

Sodium hydride (60% dispersion in oil, 232 mg, 5.80 mmol) was added to a solution of methyl 3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside[38] (505 mg, 1.61 mmol) and benzyl bromide

(0.30 ml, 2.5 mmol) in DMF (6.5 ml) at 0 °C. A further amount of benzyl bromide (0.35 ml, 2.9 mmol) was then added. The resultant suspension was then stirred at room temperature for 1 h, quenched with MeOH at 0 °C and concentrated. The residue was diluted with EtOAc and washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 1:4) to obtain methyl 2,4,6-tri-*O*-benzyl-3-*O*-(4-methoxybenzyl)-β-D-galactopyranoside as a colourless oil (922 g, 98%). R_f = 0.30 (EtOAc:hexanes 1:4). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46-7.27 (m, 17H), 6.92-6.83 (m, 2H), 4.97 (d, *J* = 11.7 Hz, 1H), 4.92 (d, *J* = 11.0 Hz, 1H), 4.79 (d, *J* = 11.0 Hz, 1H), 4.71 (d, *J* = 11.5 Hz, 1H), 4.67 (d, *J* = 11.6 Hz, 1H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.48 (d, *J* = 11.8 Hz, 1H), 4.44 (d, *J* = 11.8 Hz, 1H), 4.30 (d, *J* = 7.8 Hz, 1H), 3.91-3.87 (m, 1H), 3.85-3.78 (m, 1H), 3.82 (s, 3H), 3.66-3.60 (m, 2H), 3.60-3.50 (m, 2H), 3.57 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 159.3, 139.0, 138.8, 138.0, 130.7, 129.3, 128.5, 128.39, 128.36, 128.3, 128.2, 128.0, 127.9, 127.6, 113.9, 105.1, 81.9, 79.7, 75.2, 74.5, 73.7, 73.6, 73.5, 72.8, 69.0, 57.1, 55.4; FT-IR (ATR): ν = 1584 (w), 1496 (w), 1481 (w), 1454 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+H]^+$ calcd. for $C_{26}H_{27}O_4S$: 435.1630, found: 435.1621. The colourless oil (922 mg, 1.58 mmol) was then dissolved in a mixture of in CH_2Cl_2 (30 ml) and H_2O (3 ml), and cooled to 0 °C. To this mixture was added DDQ (430 mg, 1.90 mmol). The mixture was stirred at room temperature for 1 h, then diluted with CH_2Cl_2 and washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 1:4) to obtain **36** as a white solid (631 mg, 86%). The ^{13}C{^{1}H} NMR spectrum was consistent with that reported in the literature.[54]

General procedure for pre-activation based glycosylation using 3,6-anhydro-galactosyl thiophenyl donors. The donor (1 eq.), BSP (1.1 eq.), TTBP (2 eq.) and 4 Å molecular sieves in CH_2Cl_2 (0.03 M donor concentration) were stirred at room temperature for 15 min, before being cooled to -60 °C. After further stirring for 15 min at -60 °C, Tf_2O (1.1 eq.) was added and the mixture stirred at -60 °C for 5 min. The acceptor (1.5 eq.) was then added and the mixture stirred at -60 °C for 1 h, before being allowed to slowly warm to 0 °C, after which it was quenched with addition of Et_3N. The mixture was filtered through celite, washing with CH_2Cl_2, the filtrate was then washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography. Yields and anomeric ratios detailed in Table 1.

General procedure for pre-activation based glycosylation using 3,6-anhydro-galactosyl thiophenyl donors and additives. The donor **31** (1 eq.), BSP or Ph_2SO (1.1 eq.), TTBP (2 eq.), 4 Å molecular sieves and additive (none, 5% CH_3CN or 16 eq. DMF) in CH_2Cl_2 (0.03 M donor concentration) were stirred at room temperature for 15 min, before being cooled to -60 °C. After further stirring for 15 min at -60 °C, Tf_2O (1.1 eq.) was added and the mixture stirred at -60 °C for 5 min. The acceptor **36** (1.5 eq.) was then added and the mixture stirred at -60 °C for 1 h, before being allowed to slowly warm to 0 °C, after which it was quenched with addition of Et_3N. The mixture was filtered through celite, washing with CH_2Cl_2, the filtrate was then washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 1:4 → 3:7) first eluted **39-β** as a colourless oil followed by **39-α** as a colourless oil. Yields and anomeric ratios detailed in Table 2.

3-*O*-(3,6-anhydro-2,4-di-*O*-benzyl-D-galactopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 29

Using donor **27** (130 mg, 0.300 mmol) and acceptor **28** (117 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 1:4) yielded **29** as a colourless oil and mixture of inseparable anomers (151 mg, 86%). R_f = 0.41 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.26 (m, 10H-β, 10H-α), 5.87 (d, *J* = 3.6 Hz, 1H-α, H1-α), 5.62 (d, *J* = 3.7 Hz, 1H-β, H1-β), 5.00 (d, *J* = 2.3 Hz, 1H-α, H1`-α), 4.80-7.75 (m, 2H-α), 4.67-4.50 (m, 3H-β, 3H-α), 4.64 (s, 1H-β, H1`-β), 4.48-4.42 (m, 2H-β), 4.40 (d, *J* = 1.8 Hz, 1H-α), 4.39-4.35 (m, 1H-β, 1H-α), 4.35-4.26 (m, 3H-β, 1H-α), 4.22 (d, *J* = 1.7 Hz, 1H-β), 4.19-3.98 (m, 3H-β, 6H-α), 3.96 (dd, *J* = 8.4, 5.1 Hz, 1H-α), 3.91 (dd, *J* = 8.6, 6.4 Hz, 1H-β), 3.84 (dd, *J* = 9.8, 3.0 Hz, 1H-β), 3.76-3.70 (m, 1H-β, 1H-α), 1.49 (s, 3H-α), 1.46 (s, 3H-β), 1.41 (s, 3H-α), 1.40 (s, 3H-β), 1.34 (s, 3H-β), 1.32-1.27 (m, 3H-β, 6H-α); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 138.3, 138.0, 137.9, 137.8, 128.7, 128.64, 128.62, 128.5, 128.33, 128.31, 128.1, 128.00, 127.97, 127.94, 127.89, 111.93, 111.88, 109.43, 109.36, 105.6, 105.0, 99.9, 97.3, 84.2, 84.0, 81.3, 81.2, 81.0, 80.7, 78.3, 78.2, 78.0, 77.0, 76.6, 76.1, 75.8,74.0, 73.4, 72.6, 71.6, 71.5, 71.2, 70.4, 69.6, 68.1, 67.9, 27.0, 26.93, 26.88, 26.5, 26.4, 25.5; FT-IR (ATR): ν = 1584 (w), 1497 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{32}H_{40}O_{10}Na$: 607.2519, found: 607.2512.

Method using NIS/TfOH: The donor **27** (130 mg, 0.300 mmol), acceptor **28** (117 mg, 0.450 mmol) and 4 Å molecular sieves in CH_2Cl_2 (6 ml) were stirred at room temperature for 15 min, before being cooled to -40 °C. After further stirring for 15 min at -40 °C, NIS (81 mg,

0.36 mmol) and TfOH (2 drops) were added and the mixture stirred at -40 °C for 30 min, before being allowed to slowly warm to 0 °C, after which it was quenched with addition of Et_3N. The mixture was filtered through celite, washing with CH_2Cl_2, the filtrate was then washed with sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:hexanes 1:4) yielded **29** as a colourless oil and mixture of inseparable anomers (111 mg, 63%).

3-*O*-(3,6-anhydro-2,4-di-*O*-allyl-D-galactopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 32

Using donor **30** (100 mg, 0.300 mmol) and acceptor **28** (117 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 1:4) yielded **32** as a colourless oil (129 mg, 89%). R_f = 0.43 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.96-5.80 (m, 6H), 5.33-5.15 (m, 8H), 4.99 (d, *J* = 2.3 Hz, 1H, H1`-α), 4.80 (s, 1H, 1H`-β), 4.78 (d, *J* = 3.6 Hz, 1H), 4.50 (d, *J* = 3.7 Hz, 1H), 4.47 (d, *J* = 9.7 Hz, 1H), 4.42-4.32 (m, 6H), 4.30-4.25 (m, 2H), 4.21-3.92 (m, 18H), 3.82 (dd, *J* = 9.8, 2.9 Hz, 1H), 3.72 (dd, *J* = 5.5, 2.3 Hz, 1H), 3.68 (d, *J* = 4.7 Hz, 1H), 1.50 (s, 3H), 1.49 (s, 3H), 1.42 (s, 3H), 1.41 (s, 3H), 1.35 (s, 3H), 1.33 (s, 3H), 1.31 (s, 3H), 1.30 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 134.5, 134.14, 134.10, 133.99, 117.5, 117.4, 117.3, 117.0, 111.7, 111.6, 109.1, 109.0, 105.2, 104.7, 99.6, 96.8, 83.9, 83.7, 80.94, 80.90, 80.2, 78.1, 76.9, 76.7, 76.6, 76.4, 75.8, 75.4, 73.0, 72.3, 71.5, 71.3, 70.1, 70.0, 69.9, 69.2, 67.7, 67.5, 26.64, 26.60, 26.57, 26.1, 25.18, 25.16; FT-IR (ATR): ν = 3539 (w), 1721 (m), 1602 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{24}H_{36}O_{10}Na$: 507.2206, found: 507.2209.

3-*O*-(3,6-anhydro-2,4-di-*O*-propargyl-D-galactopyranosyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 33

Using donor **31** (99 mg, 0.300 mmol) and acceptor **28** (117 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 1:4) yielded **33** as a colourless oil (128 mg, 89%). R_f = 0.50 (EtOAc:hexanes 2:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.92 (d, *J* = 3.6 Hz, 1H-α, H1-α), 5.86 (d, *J* = 3.8 Hz, 1H-β, H1-β), 5.05 (d, *J* = 2.3 Hz, 1H-α, H1`-α), 4.93 (s, 1H-β, H1`-β), 4.80 (d, *J* = 3.6 Hz, 1H-α), 4.59 (d, *J* = 3.7 Hz, 1H-β), 4.57-4.50 (m, 1H-α, 1H-β), 4.50-3.92 (m, 14H-α, 12H-β), 3.90 (d, *J* = 4.7 Hz, 1H-β), 3.85 (dd, *J* = 9.8, 3.1 Hz, 1H-β), 2.52-2.44 (m, 2H-α, 2H-β), 1.46 (s, 3H-α, 3H-β), 1.39 (s, 3H-α, 3H-β), 1.32 (s, 3H-α, 3H-β), 1.27 (s, 3H-α, 3H-β); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 111.92, 111.88, 109.4,109.3, 105.4, 105.0, 99.8, 96.9,

84.2, 84.1, 81.2, 81.1, 80.5, 79.6, 79.52, 79.46, 79.4, 78.2, 78.0, 77.9, 77.3, 76.7, 75.9, 75.6, 75.5, 75.4, 75.2, 75.1, 72.6, 71.6, 70.2, 69.4, 68.0, 67.8, 59.2, 58.2, 56.9, 56.6, 26.87, 26.85, 26.3; FT-IR (ATR): ν = 3269 (w), 2109 cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{24}H_{32}O_{10}Na$: 503.1893, found: 503.1892.

Methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-benzyl-β-D-galactopyranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 34

Using donor **27** (130 mg, 0.300 mmol) and acceptor **8** (174 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 35:65) yielded **34** as a white solid (162 mg, 76%). R_f = 0.23 (EtOAc:hexanes 2:3). ^1H NMR (500 MHz, $CDCl_3$): δ 8.14-8.05 (m, 2H), 7.62-7.50 (m, 3H), 7.46-7.26 (m, 10H), 7.25-7.15 (m, 3H), 6.85-6.76 (m, 2H), 5.61-5.52 (m, 2H), 4.82 (s, 1H, H1`), 4.59 (d, *J* = 8.1 Hz, 1H), 4.56 (d, *J* = 12.0 Hz, 1H), 4.51-4.42 (m, 3H), 4.40 (d, *J* = 12.3 Hz, 1H), 4.28-4.24 (m, 1H), 4.22 (d, *J* = 4.3 Hz, 1H), 4.16-4.10 (m, 2H), 3.99 (d, *J* = 11.7 Hz, 1H), 3.94 (dd, *J* = 10.1, 3.0 Hz, 1H), 3.84 (d, *J* = 11.7 Hz, 1H), 3.81-3.76 (m, 1H), 3.67 (d, *J* = 4.6 Hz, 1H), 3.57-3.54 (m, 1H), 3.52 (s, 3H); $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): δ 164.9, 137.8, 137.6, 137.4, 133.3, 130.1, 129.8, 129.1, 128.6, 128.5, 128.33, 128.31, 127.92, 127.85, 127.8, 127.3, 126.4, 102.9, 102.0, 101.2, 80.6, 79.5, 78.0, 76.7, 76.3, 76.0, 72.3, 71.0, 69.9, 69.2, 66.8, 56.3; FT-IR (ATR): ν = 1721 (s), 1603 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{41}H_{42}O_{11}Na$: 733.2625, found: 733.2637.

Methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-propargyl-β-D-galactopyranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside 35

Using donor **31** (99 mg, 0.30 mmol) and acceptor **8** (174 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 45:55) yielded **35** as a white solid (129 mg, 71%). R_f = 0.36 (EtOAc:hexanes 1:1). ^1H NMR (500 MHz, $CDCl_3$): δ 8.10-8.00 (m, 2H), 7.62-7.50 (m, 3H), 7.46-7.40 (m, 2H), 7.40-7.32 (m, 3H), 5.57-5.54 (m, 1H), 5.52 (dd, *J* = 10.2, 8.1 Hz, 1H), 4.77 (s, 1H, H1`), 4.56 (d, *J* = 8.1 Hz, 1H), 4.49 (d, *J* = 9.5 Hz, 1H), 4.44 (d, *J* = 3.5 Hz, 1H), 4.39-4.33 (m, 2H), 4.29 (d, *J* = 4.7 Hz, 1H), 4.18 (dd, *J* = 15.9, 2.4 Hz, 1H), 4.15-4.07 (m, 3H), 3.90 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.73 (d, *J* = 4.7 Hz, 1H), 3.71-3.63 (m, 2H), 3.56-3.47 (m, 2H), 3.50 (s, 3H), 2.43 (t, *J* = 2.4 Hz, 1H), 2.10 (t, *J* = 2.4 Hz, 1H); $^{13}C\{^1H\}$ NMR (125.8 MHz, $CDCl_3$): δ 164.9, 137.6, 133.2, 130.0, 129.8, 129.1, 128.5, 128.3, 126.4, 102.6, 102.0, 101.1, 80.1, 79.8, 79.4, 78.6, 77.8, 76.3, 76.0, 75.9, 75.02, 74.98, 70.7, 69.7, 69.1, 66.6, 57.5, 56.4, 56.3; FT-IR

(ATR): ν = 3538 (w), 3286 (w), 1721 (s), 2121 (w), 1602 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{33}H_{34}O_{11}Na$: 629.1999, found: 629.2011.

Methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-benzyl-β-D-galactopyranosyl)-2,4,6-*O*-benzyl-β-D-galactopyranoside 37-β and methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-benzyl-α-D-galactopyranosyl)-2,4,6-*O*-benzyl-β-D-galactopyranoside 37-α

Using donor **27** (130 mg, 0.300 mmol) and acceptor **36** (209 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 15:85 → 1:3) first eluted **37-β** as a colourless oil (109 mg, 46%). R_f = 0.41 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41-7.16 (m, 23H), 7.08-7.00 (m, 2H), 5.21 (s, 1H, H1`), 5.02 (d, *J* = 11.8 Hz, 1H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.67 (d, *J* = 9.7 Hz, 1H), 4.65 (d, *J* = 10.2 Hz, 1H), 4.59 (d, *J* = 12.0 Hz, 1H), 4.52 (d, *J* = 12.0 Hz, 1H), 4.47 (d, *J* = 11.8 Hz, 1H), 4.43 (d, *J* = 11.8 Hz, 1H), 4.38 (d, *J* = 4.6 Hz, 1H), 4.35-4.26 (m, 4H), 4.26-4.20 (m, 2H), 3.95 (dd, *J* = 9.2, 2.9 Hz, 1H), 3.89 (d, *J* = 4.7 Hz, 1H), 3.88-3.85 (m, 1H), 3.85-3.80 (m, 2H), 3.65-3.58 (m, 3H), 3.51 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 139.0, 138.8, 138.0, 137.9, 137.8, 128.6, 128.5, 128.39, 128.36, 128.2, 128.1, 128.0, 127.93, 127.88, 127.7, 127.5, 127.44, 127.40, 127.37, 105.3, 100.4, 80.9, 80.1, 78.0, 77.8, 77.2, 75.9, 75.5, 74.4, 74.1, 73.70, 73.66, 72.3, 71.1, 70.9, 69.0, 57.1; FT-IR (ATR): ν = 1497 (w), 1454 (m) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{48}H_{52}O_{10}Na$: 811.3458, found: 811.3456. Next to elute was **37-α** as a colourless oil (107 mg, 45%). R_f = 0.27 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44-7.38 (m, 2H), 7.38-7.26 (m, 15H), 7.22-7.16 (m, 6H), 7.11-7.05 (m, 2H), 5.11 (d, *J* = 2.2 Hz, 1H, H1`), 4.87-4.77 (m, 3H), 4.73 (d, *J* = 10.9 Hz, 1H), 4.62 (d, *J* = 12.1 Hz, 1H), 4.59-4.53 (m, 2H), 4.49-4.40 (m, 4H), 4.39-4.34 (m, 2H), 4.27 (d, *J* = 7.6 Hz, 1H), 4.05-3.96 (m, 2H), 3.96-3.90 (m, 2H), 3.74-3.56 (m, 5H), 3.54 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 139.1, 138.50, 138.46, 137.9, 137.7, 128.6, 128.34, 128.29, 128.2, 128.1, 128.01, 127.96, 127.87, 127.6, 127.5, 127.3, 127.2, 104.6, 94.9, 79.6, 78.5, 78.4, 77.9, 77.4, 77.2, 75.6, 75.3, 75.0, 73.7, 73.2, 73.0, 71.3, 69.6, 68.4, 57.3; FT-IR (ATR): ν = 1496 (w), 1454 (m) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{48}H_{52}O_{10}Na$: 811.3458, found: 811.3472.

Methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-allyl-β-D-galactopyranosyl)-2,4,6-*O*-benzyl-β-D-galactopyranoside 38-β and methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-allyl-α-D-galactopyranosyl)-2,4,6-*O*-benzyl-β-D-galactopyranoside 38-α

Using donor **30** (100 mg, 0.300 mmol) and acceptor **36** (209 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 1:4 → 3:7) first eluted **38-β** as a colourless oil (89 mg, 43%). R_f = 0.60 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39-7.24 (m, 15H), 5.95-5.84 (m, 1H), 5.69-5.59 (m, 1H), 5.29 (ddd [appt dq], *J* = 17.2, 1.6 Hz, 1H), 5.19 (ddd [appt dq], *J* = 10.4, 1.3 Hz, 1H), 5.13 (s, 1H, H1`), 5.12-5.04 (m, 2H), 5.02 (d, *J* = 11.8 Hz, 1H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.66 (d, *J* = 9.7 Hz, 1H), 4.64 (d, *J* = 9.3 Hz, 1H), 4.47 (d, *J* = 11.8 Hz, 1H), 4.43 (d, *J* = 11.8 Hz, 1H), 4.35 (d, *J* = 4.6 Hz, 1H), 4.34-4.26 (m, 3H), 4.12 (d, *J* = 1.6 Hz, 1H), 4.09 (dddd [appt ddt], *J* = 12.8, 5.5, 1.4 Hz, 1H), 4.03 (dddd [appt ddt], *J* = 12.8, 5.7, 1.4 Hz, 1H), 3.91 (dd, *J* = 9.3, 3.0 Hz, 1H), 3.89-3.72 (m, 6H), 3.65-3.59 (m, 3H), 3.51 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 139.0, 138.9, 138.0, 134.5, 134.2, 128.6, 128.4, 128.2, 128.07, 128.05, 127.9, 127.50, 127.47, 127.45, 117.6, 116.9, 105.3, 100.5, 80.7, 80.2, 77.90, 77.86, 77.3, 75.8, 75.6, 74.5, 74.1, 73.8, 73.7, 71.1, 70.9, 70.1, 69.0, 57.2; FT-IR (ATR): ν = 1646 (w), 1497 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{40}H_{48}O_{10}Na$: 711.3145, found: 711.3141. Next to elute was **38-α** as a colourless oil (91 mg, 44%). R_f = 0.33 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52-7.47 (m, 2H), 7.38-7.25 (m, 13H), 5.97-5.86 (m, 1H), 5.81-5.70 (m, 1H), 5.33-5.26 (m, 1H), 5.25-5.18 (m, 1H), 5.13-5.06 (m, 2H), 5.05-4.99 (m, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.82 (d, *J* = 11.6 Hz, 1H), 4.76 (d, *J* = 10.8 Hz, 1H), 4.56 (d, *J* = 11.6 Hz, 1H), 4.46 (d, *J* = 11.6 Hz, 1H), 4.42 (d, *J* = 11.6 Hz, 1H), 4.40-4.33 (m, 3H), 4.33-4.28 (m, 1H), 4.27 (d, *J* = 7.7 Hz, 1H), 4.13-3.99 (m, 3H), 3.99-3.88 (m, 4H), 3.73-3.55 (m, 5H), 3.54 (s, 3H); ^{13}C{^{1}H} NMR (125.8 MHz, $CDCl_3$): δ 139.2, 138.5, 137.8, 134.9, 134.4, 128.6, 128.5, 128.4, 128.2, 128.11, 128.06, 127.7, 127.4, 117.6, 116.5, 104.7, 94.9, 79.7, 78.6, 78.5, 77.9, 77.1, 75.5, 75.4, 75.1, 73.8, 73.2, 73.0, 72.9, 70.4, 69.5, 68.5, 57.4; FT-IR (ATR): ν = 1646 (w), 1497 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{40}H_{48}O_{10}$: 711.3145, found: 711.3142.

Methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-propargyl-β-D-galactopyranosyl)-2,4,6-*O*-benzyl-β-D-galactopyranoside 39-β and methyl 3-*O*-(3,6-anhydro-2,4-di-*O*-propargyl-α-D-galactopyranosyl)-2,4,6-*O*-benzyl-β-D-galactopyranoside 39-α

Using donor **31** (99 mg, 0.300 mmol) and acceptor **36** (209 mg, 0.450 mmol) and flash column chromatography (EtOAc:hexanes 1:4 → 3:7) first eluted **39-β** as a colourless oil (41 mg, 20%). R_f = 0.48 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43-7.25 (m, 15H), 5.12 (s, 1H, H1`), 5.03 (d, *J* = 11.7 Hz, 1H), 4.97 (d, *J* = 11.3 Hz, 1H), 4.67 (d, *J* = 5.7 Hz, 1H), 4.64 (d, *J* = 5.1 Hz, 1H), 4.51-4.41 (m, 4H), 4.32 (d, *J* = 9.3 Hz, 1H), 4.29 (d, *J* = 6.6 Hz, 1H), 4.28-

4.22 (m, 2H), 4.20 (dd, *J* = 16.0, 2.3 Hz, 1H), 3.99 (d, *J* = 4.7 Hz, 1H), 3.97-3.90 (m, 2H), 3.90-3.87 (m, 1H), 3.87-3.82 (m, 2H), 3.80 (dd, *J* = 16.0, 2.4 Hz, 1H), 3.67-3.60 (m, 3H), 3.52 (s, 3H), 2.47 (t, *J* = 2.4 Hz, 1H), 2.42 (t, *J* = 2.4 Hz, 1H); $^{13}C\{^{1}H\}$ NMR (125.8 MHz, $CDCl_3$): δ 139.0, 138.9, 138.0, 128.6, 128.4, 128.3, 128.10, 128.08, 128.0, 127.7, 127.6, 127.5, 105.3, 100.2, 80.2, 80.0, 79.6, 79.4, 78.02, 77.96, 77.1, 75.7, 75.6, 75.2, 75.1, 74.6, 74.2, 73.74, 73.73, 70.9, 69.0, 57.5, 57.2, 56.7; FT-IR (ATR): ν = 3285 (w), 2117 (w), 1497 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{40}H_{44}O_{10}Na$: 707.2832, found: 707.2841. Next to elute was **39-α** as a colourless oil (113 mg, 55%). R_f = 0.24 (EtOAc:hexanes 3:7). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56-7.49 (m, 2H), 7.39-7.22 (m, 13H), 5.10 (d, *J* = 2.4 Hz, 1H, H1`), 4.86-4.78 (m, 3H), 4.58 (d, *J* = 11.5 Hz, 1H), 4.52-4.40 (m, 5H), 4.34 (dd, *J* = 16.1, 2.2 Hz, 1H), 4.32-4.23 (m, 3H), 4.21 (dd, *J* = 15.9, 2.4 Hz, 1H), 4.04 (d, *J* = 10.1 Hz, 1H), 3.99-3.88 (m, 4H), 3.71-3.60 (m, 3H), 3.60-3.53 (m, 4H), 2.50-2.46 (m, 1H), 2.36-2.32 (m, 1H); $^{13}C\{^{1}H\}$ NMR (125.8 MHz, $CDCl_3$): δ 139.1, 138.4, 137.7, 128.6, 128.40, 128.37, 128.20, 128.19, 128.1, 127.9, 127.8, 127.5, 104.8, 94.9, 79.8, 79.7, 79.6, 78.43, 78.39, 78.1, 75.6, 75.42, 75.35, 75.2, 75.1, 75.0, 73.8, 73.2, 73.0, 69.5, 68.5, 58.9, 57.4, 57.0; FT-IR (ATR): ν = 3282 (w), 2116 (w) cm^{-1}; HR-MS (ESI+): *m/z* $[M+Na]^+$ calcd. for $C_{40}H_{44}O_{10}Na$: 707.2832, found: 707.2830.

Methyl 3-*O*-(3,6-anhydro-α-D-galactopyranosyl)-2,4,6-*O*-benzyl-β-D-galactopyranoside 40

A solution of *t*-BuOK in THF (1 M, 0.14 ml, 0.14 mmol) was added to a solution of **39-α** (40 mg, 0.058 mmol) in THF (1 ml). The solution was left at room temperature for 0.5 h before being diluted with EtOAc and washed with brine, dried over $MgSO_4$, filtered and concentrated. The resultant residue was then diluted with acetone (0.5 ml) and 10% aq. TFA (0.5 ml) was added. The solution was stirred at room temperature for 2 h before being concentrated, co-evaporating with toluene. The residue was purified by flash column chromatography (EtOAc:hexanes 55:45) to obtain **40** as a colourless gum (32 mg, 90%). R_f = 0.32 (EtOAc:hexanes 3:2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.26 (m, 15H), 4.95 (d, *J* = 2.7 Hz, 1H), 4.90 (d, *J* = 11.3 Hz, 1H), 4.70 (d, *J* = 4.7 Hz, 1H), 4.67 (d, *J* = 4.2 Hz, 1H), 4.60-4.54 (m, 2H), 4.48 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 11.7 Hz, 1H), 4.31-4.25 (m, 2H), 4.23 (d, *J* = 4.23 Hz, 1H), 4.07 (dd, *J* = 10.1, 3.0 Hz, 1H), 4.03-3.95 (m, 2H), 3.87 (d, *J* = 2.9 Hz, 1H), 3.76-3.67 (m, 2H), 3.63-3.56 (m, 3H), 3.55 (s, 3H), 3.33 (*br* s, 1H), 2.05 (*br* s, 1H); $^{13}C\{^{1}H\}$ NMR (100.6 MHz, $CDCl_3$): δ 138.5, 138.2, 137.8, 128.6, 128.52, 128.46, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 105.0, 93.7, 81.2, 78.0, 77.5, 77.2, 75.2, 75.1, 74.7, 73.8,

73.1, 71.4, 70.7, 69.3, 68.6, 57.2; FT-IR (ATR): ν = 3399 (br), 1497 (w), 1454 (w) cm^{-1}; HR-MS (ESI-): *m/z* $[M+HCOO]^-$ calcd. for $C_{35}H_{41}O_{12}$: 653.2598, found: 653.2595.

TLC cleavage assay

The *exo*-3,6-anhydro-α-D-galactosidase[4,7] from *Z. galactanivorans* (*Zg*GH129) and the *exo*-3,6-anhydro-α-L-galactosidase[52] from *Z. galactanivorans* (Zg3615) were produced as previously described. The disaccharides **1** and **2** at a concentration of 10 mM were incubated with *Zg*GH129 (500 nM) or *Zg*3615 (500 nM) in 1x PBS pH 7.4 (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4), at room temperature overnight. TLC analysis was then performed with a 1-2 µL aliquot of reaction solutions, and the unreacted disaccharides **1** and **2** and 3,6-anhydro-D-galactose as controls. The mobile phase used was water:ethanol:*n*-butanol (1:1:3) and the plate, after development, was stained with 5% 1,3-dihydroxynaphthalene in ethanol:10% sulfuric acid in ethanol (1:2) and placed in an incubator at 60 °C for 1 h to visualise 3,6-anhydro-D-galactose containing materials.

Supporting Information

^1H and ^{13}C NMR of synthesised novel compounds.

Acknowledgements

The authors acknowledge the facilities, and the scientific and technical assistance of the Microscopy Australia at the Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, a facility funded by the University, State and Federal Commonwealth Governments. MDW is supported by a Research Training Program Scholarship provided by the Australian Federal Government and The University of Western Australia. MDW also thanks the School of Molecular Sciences for the Dr Wayne Best Travel Award, and Campus France and the Franco-Australian Hubert Curien Programme (FASIC) for funding, which together funded the travel of MDW to the Station Biologique de Roscoff. KAS also thanks the Australian Research Council for funding (FT100100291). EF-B acknowledges support from the Agence National de la Recherche (ANR) for the "Blue Enzymes" project (reference ANR-14-CE19-0020-01).

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