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The synthesis of compatible solute analogues—solvent effects on selective glycosylation

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ABSTRACT

Ethyl 6-*O*-acetyl-2,3,4-tribenzyl-1-D-thioglucoside and ethyl 6-*O*-acetyl-2,3,4-tribenzyl-1-D-thiogalactoside, as a mixture of anomers, were employed in the study of the influence of solvent in the stereoselectivity of the glycosylation reaction with small and reactive acceptors. High α-selectivities were obtained in the glycosylation reactions using NIS/TfOH as activator and ethyl ether as the solvent at −60 °C. Other solvent mixtures such as dichloromethane, THF, THF/ethyl ether and toluene/dioxane were not nearly as selective. The corresponding thiogalactoside underwent similar glycosylations with the same solvents but with low anomer selectivity. These glycosides are key intermediates for the synthesis of new analogues of compatible solutes.

1. Introduction

In response to stressful conditions such as extreme temperature and high salt concentration many microorganisms accumulate compatible solutes.[1] A superior thermoprotecting ability was ascribed to 'hypersolutes', which are compatible solutes isolated from hyperthermophiles, and confirmed by in vitro protein stabilisation experiments.[2,3] In contrast to the solutes more commonly found in mesophiles, hypersolutes are generally negatively charged, and most fall into two categories: hexose derivatives such as α-D-mannosyl-(1→2)-D-glycerate (MG) and α-D-glucosyl-(1→2)-D-glycerate (GG), and polyol-phosphodiesters like di-*myo*-inositol phosphate (DIP). A new trisaccharide (2*R*)-2-*O*-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-2,3-dihydroxypropanoic acid, potassium salt [(α-D-glucosyl-(1→6)-α-D-glucosyl-(1→2)-D-glycerate (GGG)] isolated from the hyperthermophile *Persephonella marina*, has recently been synthesised, as well as the related α-D-glucosyl-(1→2)-D-glycerate (GG) (Fig. 1).[4] In another study,[5] several molecules chemically related to mannosyl glycerate (MG) were synthesised (Fig. 1). The effectiveness of the newly synthesised compounds for the protection of model enzymes against heat-induced denaturation, aggregation and inactivation was studied, revealing that α-D-mannosyl-(*S*)-lactate (ML), a synthetic solute, was superior to MG in several applications. This has stimulated the present study which is aimed towards the production of new synthetic analogues of GG and GGG. Although the observed *cis*-1,2 glycosylation selectivities for the synthesis of GG (>10:1 *cis*-1,2-/*trans*-1,2-isomers) and GGG (>10:1 *cis*-1,2-/*trans*-1,2-isomers) were very good,[4] they were much lower than for the corresponding *trans*-1,2 selectivity observed for mannosylation.[5] For the formation of glucosyl lactate (GL) using the same donor and conditions used for GG, a selectivity of only 4:1 (*cis*-1,2-/*trans*-1,2-isomers) was obtained. In order to optimise the glycosylation process, a study of the effect of solvent and temperature was carried out. The influence of solvent on the stereochemical outcome of glycosylations is well documented.[6] It is known that ethyl ether generally greatly favours the formation of the thermodynamically more stable axial glycoside, by formation of a diethyl oxonium-ion intermediate having the β-configuration, due to steric interactions. The 1,2-*cis*-glycoside is then obtained by nucleophilic displacement with inversion of configuration.[6]

2. Results and discussions

The main challenge for the synthesis of GG and GGG[4] was the stereoselective formation of the α-glucosidic bonds. Thioglucoside **1**, which is readily prepared in just three steps from the corresponding methyl glucoside, was used as the glucosidic donor and in both glycosylation reactions the α-anomer was the major product (>10:1 α/β) using the NIS/TfOH reagent combination. Besides the clear synthetic advantages in obtaining and using thioglucoside **1**,[4] this donor has an acetate group at the C-6 position which should favour the formation of the α-glucosides (1,2-*cis* glucosylation)[4] as it is a less reactive donor than the corresponding tetrabenzylated thioglucoside due to the electron-withdrawing substituent at C-6.[7] The effects of remote substituents on glycosylation are obviously less

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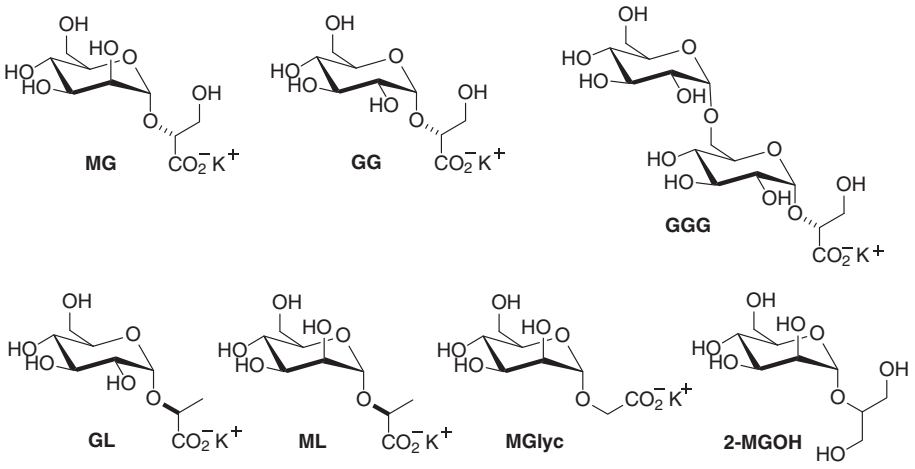


Figure 1. Compatible solutes and analogues.

important than those at C-2, however a substituent at the C-6 position should influence the stereochemical outcome of glycosylation reactions, by electronically shielding the upper face of the pyranose ring.[6,8]

Following the same line of research,[5] the corresponding analogues of GG, namely GL, α-D-glucosyl glycolate (GGlyc) and α-D-glucosyl-(1→6)-α-D-glucosyl lactate (GGL) were required. The obvious synthetic route would be to use the thioglucoside **1** as the donor. However, as was earlier reported,[4] transglycosylation of **1** with the less bulky, more reactive, methyl (*S*)-lactate produced the glucosyl lactate with an α/β ratio of only 4:1 under the glycosylation reaction conditions used (dichloromethane as solvent at 0 °C—Table 1, entry 1).

Boons and co-workers have reported that the best solvent mixture for obtaining α-glucosides from a tetrabenzylated β-thioglucoside was toluene/1,4-dioxane.[9] Ethyl ether was not tested alone, but only in mixtures with other solvents. Ethyl ether with dichloromethane afforded a lower α/β ratio and with toluene afforded a considerably higher α/β ratio but lower than that obtained with toluene/dioxane mixtures. Although no yields were presented, it was reported that glycosylations in ether/toluene were very sluggish and incomplete. Dichloromethane was the worst solvent affording more β anomer (0.7:1 α/β) and the glycosylation reaction in THF alone was not studied.

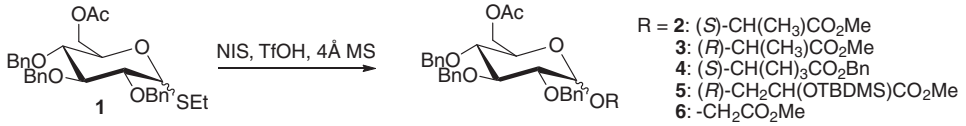
More recently,[10] Bonnaffé and co-workers have described optimisation studies for the glycosylation reaction of a 2-azido trichloroacetimidate disaccharide derived from glucose with several acceptors, also disaccharides, in order to improve the α-stereoselectivity. It was shown that dichloromethane was not the best reaction solvent in terms of both yield and diastereoselectivity and that by changing to ethyl ether, no improvement in the α/β ratio was observed but the yield was reduced. By performing the glycosylation reaction in THF, the α/β ratio was considerably better but the yield was even lower than that obtained in ethyl ether. After testing several solvent mixtures, they found that the best reaction solvent was a THF/Et_2O (9:1) mixture.

In another study, Ito and co-workers observed the effect of solvents during the construction of three sequential α-glucosidic bonds.[11] The thioglucosides used were obtained in several steps and possessed a 4,6-*O*-cyclohexylidene-protecting group, a donor similar to the 4,6-*O*-benzylidene thioglucoside used by Crich to obtain 1,2-*cis* glycosides.[12] The best solvent systems were found to be 1:1 mixtures of $CHCl_3/Et_2O$ and $CHCl_3$/cyclopentylmethylether (CPME) and the reagents used were 2,6-di-*tert*-butyl-4-methylpyridine and a large excess of MeOTf. In most of these studies the acceptors were other glycosides, mono and higher saccharides, and no smaller or more reactive alcohols were studied. The acetal-protecting group is not very stable under acidic glycosylation conditions and this accounted for the lower yields of the glycosylation reactions.

This problem prompted us to find a more stable and more easily prepared donor which we have found in thioglucoside **1**.[4] During attempts to optimise the α/β ratio for the formation of glucosyl lactates, we studied the glycosylation reaction between donor **1** and methyl (*S*)-lactate using NIS/triflic acid as the promoter and several solvents or solvent combinations at 0 °C, in the presence of 4 Å molecular sieves (MS) (Scheme 1, Table 1).

The toluene/dioxane (1:3) solvent combination employed by Boons and co-workers[9] afforded an improved 6:1 α/β ratio, but the donor was consumed much slower (80 min) and the yield was only 43% (Table 1, entry 6). The recovered byproduct was the corresponding reducing monosaccharide. This was formed particularly in the glycosylation reactions that were slower and incomplete (see also Table 2) and was probably formed by quenching of the activated donor during the aqueous work-up. Changing the promoter to NIS/TMSOTf increased the yield to 78% within a shorter reaction time (20 min), however, the anomeric ratio remained almost the same (α/β 6.3:1, Table 1, entry 7).

A THF/Et_2O (9:1) mixture, that had given good results in the work of Bonnaffé,[10] afforded a 5.8:1 α/β mixture but only 60% yield (Table 1, entry 3). Reversing the proportions of THF and Et_2O to 1:9 increased the yield considerably to 87%, however the anomeric ratio was only marginally improved (6:1 α/β, Table 1, entry 2). Finally, Et_2O was used as the sole solvent and pleasingly a higher anomeric ratio of 7.5:1 α/β and 85% yield was obtained (Table 1, entry 4). However, using neat THF as the solvent, the reaction



Scheme 1. Glycosylation reaction of thioglucoside **1**.

was slower and the yield and the selectivity were lower (Table 1, entry 5). Comparing the results obtained, ethyl ether alone was clearly the best solvent for this glycosylation reaction.

The same glycosylation reaction was conducted in dichloromethane and ethyl ether at −60 °C (Table 1). At this temperature, the anomeric selectivity increased in CH_2Cl_2 (7:1 α/β, entry 8) and even though the yield was slightly lower (83%), the reaction time remained the same (10 min). With Et_2O as the solvent, the donor was consumed more slowly (45 min) but this was largely compensated by improvement of the α/β ratio to 9:1 and the good yield (81%) (Table 1, entry 9).

Glycosylation with methyl (*R*)-lactate in CH_2Cl_2 and in Et_2O at both 0 and −60 °C (Table 1, entries 10–13) afforded an α/β ratio very similar to that observed with methyl (*S*)-lactate as the donor, indicating that double stereodifferentiation was probably involved but not very important. With the bulkier benzyl (*S*)-lactate, the anomeric selectivity was the highest obtained in Et_2O at −60 °C (10:1 α/β Table 1, entry 17), with the additional advantage of the benzyl ester being removable at the same time as the benzyl ether-protecting groups, thus avoiding the final basic ester hydrolysis to afford the acid salt of GL.[4,5] Interestingly, when employing dichloromethane as the solvent, at 0 °C, the stereoselectivity obtained with methyl (*S*)-lactate, methyl (*R*)-lactate and benzyl (*S*)-lactate was the same (4:1 α/β, Table 1, entries 1, 10 and 14), indicating that under these conditions the (*R*),(*S*)-stereochemistry of the lactate and the bulkiness of the ester moiety were not important for the anomeric selectivity.

Glycosylation with the primary hydroxyl of methyl glycerate[4] was also attempted (Table 1, entry 18). This alcohol **7** was obtained using standard protection reactions. Only the α-anomer was observed but the yield of the reaction was low (30%) and thioglucoside **1** was recovered. Attempts to carry out the glycosylation reaction of donor **1** with the small primary alcohol methyl glycolate in ethyl ether at −60 °C (Table 1, entry 22) resulted in an excellent yield (88%) and the α/β ratio was 13:1. The low yield obtained with the acceptor **7** was inherent to its protecting groups.

In the case of methyl glycolate, the anomeric stereoselectivity was not improved in CH_2Cl_2 at −60 °C (4.2:1 α/β, Table 1, entry 21) compared with the one obtained in the same reaction at 0 °C (4.2:1 α/β, Table 1, entry 19).

The rationale behind the design of the analogues synthesised so far[4,5] had been to vary the aglycone part of the molecule. Variation of the sugar is also of great interest, both for the possible stabilising properties and also for an understanding of the mechanism of action of these solutes. To our knowledge, galactose containing compatible solutes have not been isolated. Thus, the glycosylation of the thiogalactoside **8**, prepared in the same way as the thioglucoside **1**, was attempted. Glycosylation of thiogalactosides has been less studied than those of thioglucosides but in general produce lower *cis*-1,2- selectivities. Once again, the studies that we found referred to other sugars as the acceptor, not small unhindered alcohols.[6]

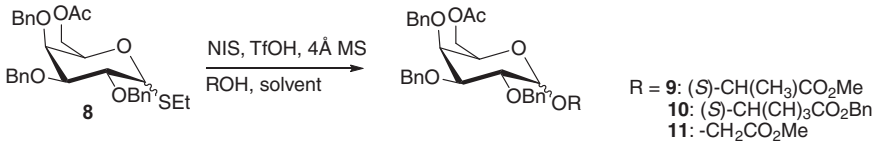
The results of the glycosylation reactions using thiogalactoside **8** as donor and methyl or benzyl (*S*)-lactate and methyl glycolate as acceptor are (Scheme 2) summarised in Table 2. The selectivities of the reactions were generally lower than for the corresponding glucoside donor. Notable also are the shorter reaction times (Table 2, entry 2), leaving the reaction longer decreased the yield and the anomeric selectivity (Table 2, entry 3). Thiogalactosides are known to be more reactive than thioglucosides. This tendency is observed in glycosylation reactions, for example, *p*-methylphenyl thiogalactoside is 6.4 times more reactive than the corresponding glucoside in the reaction with MeOH promoted by NIS/TfOH.[12] The concept of an axial polar substituent stabilising a positive charge to a higher degree than the corresponding equatorial equivalent explains this observation and supports the concept of very reactive super-armed thioglycosides.[13]

Changing the solvent to ethyl ether lowered the rate of reaction but did not increase the proportion of the α-anomer (α/β 3:1, Table 2, entry 5). This contrasts dramatically with the result obtained for the corresponding reaction with thioglucoside **1**. At −60 °C, in both dichloromethane and ethyl ether, the α/β ratio decreased to 1.5:1 and 2.2:1, respectively (Table 2, entries 4 and 6). Using bulkier benzyl (*S*)-lactate as the acceptor, in dichloromethane at 0 °C, no improvement on the anomeric selectivity was observed (Table 2, entry 8). However, when the acceptor was benzyl (*S*)-lactate and the solvent was ethyl ether at −60 °C, the α/β ratio increased to 4:1 (Table 2, entry 11). In CH_2Cl_2, the same was not observed, the anomeric selectivity at −60 °C was decreased to 1.7:1 α/β (Table 2, entry 10). The glycosylation reaction of **8** with methyl glycolate, in dichloromethane at 0 °C, also afforded a similar α/β ratio of 3:1 (Table 2, entry 12) and in the other conditions tested (Table 2, entries 13–15) the ratio was lower. The different behaviour of glucose and galactose donors under the same conditions appears to be related to conformational differences.

The selectivity of the glycosylation reaction is influenced by the reactivity of its components.[6,7] The more reactive galactose donors, with a non-participating protective group at C-2, afforded lower anomeric α selectivity,[7] however, the reactivity of the acceptor also plays an important role in the selectivity of the reaction. In most examples cited in the literature,[6,9–11] the acceptor is usually another sugar molecule having a large number of possible interactions with the donor, whereas in our case the acceptor is a relatively small alcohol with few functional groups.

The nature of the promoter has also been reported to have an influence on the stereoselectivity of the glycosylation reaction,[6,9] but studies under the Crich conditions for the glycosylation of thioglucoside **1** with methyl (*S*)-lactate did not improve the selectivity.[14]

The highest selectivity was obtained when THF was used as the solvent (α/β 5:1, Table 2, entry 1), however the reaction time was long, the yield was only 15%, 43% of starting material was recovered and the hydrolysis product was obtained in 38% yield. The toluene/dioxane mixture (1:3)[9] afforded a low selectivity (α/β 2.4:1, Table 2, entry 7). The best solvent for the glycosylation reaction between thiogalactoside **8** and methyl (*S*)-lactate was dichloromethane, affording a α/β ratio of 3:1 and 87% yield (Table 2, entry 2). Ethyl ether afforded the same α/β ratio of 3:1, however the yield was lower. The anomers of galactosides **9**, **10** and **11** were easier to separate than the corresponding glucosides, and the synthesis of α- and β-galactosyl-(*S*)-lactate and of α- and β-galactosylglycolate afforded, after separation, four new solute analogues to be tested for protein stabilisation properties.



Scheme 2. Glycosylation reactions of thiogalactoside **8**.

3. Conclusions

Ethyl ether was the solvent of choice for the glycosylation reaction of thioglucoside **1** with small, reactive lactate and glycolate esters using NIS/TfOH as the promoter. This result was expected, ethyl ether is considered a participating solvent and the diethyl oxonium-ion formed in situ preferentially adopts equatorial orientation, leading towards the axial glycosidic bond formation.[6] Generally, with dichloromethane as the solvent, performing the reaction at −60 °C improved the anomeric selectivity compared to the same reaction at 0 °C. Similar glycosylations with the thiogalactoside **8** did not afford such high stereoselectivity, and changing the solvent or lowering the reaction temperature did not improve the results. Comparing these results with those reported in the literature shows once more that the stereoselectivity of the glycosylation reaction depends on many factors and that it is difficult to find a general method to obtain 1,2-*cis* glycosides even when using donors and acceptors of similar structure. Most of the reported studies[6] describing solvent effects in glycosylation use bulky and much less reactive glycosides as acceptors and the results cannot be applied to simpler molecules.

The glucosides **2**, **3**, **4** and **6** and galactosides **9**, **10** and **11** were key intermediates in the synthesis of α-glucosyl-(*S*)-lactate, α-glucosyl-(*R*)-lactate, α-glucosylglycolate, α- and β-galactosyl-(*S*)-lactate and α- and α-galactosylglycolate for testing as protein stabilisers.

4. Experimental

4.1. General

^{1}H NMR spectra were obtained at 400 MHz in $CDCl_3$ with chemical shift values (δ) in ppm downfield from tetramethylsilane, and ^{13}C NMR spectra were obtained at 100.61 MHz in $CDCl_3$. Assignments are supported by 2D correlation NMR studies. Medium pressure preparative column chromatography: Silica Gel Merck 60 H. Analytical TLC: Aluminium-backed Silica Gel Merck 60 F254. Reagents and solvents were purified and dried according to Ref. 15. All the reactions were carried out under an inert atmosphere (argon). Thioglycosides **1** and **7** were prepared as described in Ref. 4.

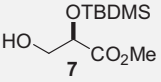
4.2. General glycosylation procedure

A suspension of thioglycoside donor (0.15 mmol), acceptor (0.15 mmol) and 4 Å MS in the solvent/mixture of solvents indicated in Tables 1 and 2 (1 mL) was stirred for 1 h at room temperature then cooled to 0 °C. *N*-Iodosuccinimide (0.19 mmol) and TfOH (0.9 μL) were added at 0 °C and when the reaction was complete (TLC), 10% $Na_2S_2O_3$ aqueous solution (2 mL) and satd aq $NaHCO_3$ (1 mL) were added and the mixture was extracted with CH_2Cl_2 (3 × 5 mL); the combined organic phases were dried ($MgSO_4$), filtered and the solvent was removed under vacuum. The crude product was purified by PLC (3:7 EtOAc/hexane). The α/β ratio of the isolated product was measured by comparison of the integral of the methyl group of the lactate moiety signals in ^{1}H NMR (400 MHz, $CDCl_3$) spectra. Yields and α/β ratio values are described in Tables 1 and 2.

4.2.1. Methyl (2*S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)propanoate 2

ν_{max} (film): 1743 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ 7.42–7.24 (m, 15H, Bn aromatic), 5.01 (d, *J* = 10.7 Hz, 1H, Bn), 4.89–4.76 (m, 3H, Bn), 4.75 (d, *J* = 3.0 Hz, 1H, H-1 (α)), 4.63 (d, *J* = 12.0 Hz, 1H, Bn), 4.57 (d, *J* = 10.9 Hz, 1H, Bn), 4.50 (d, *J* = 6.2 Hz, H-1 (β)), 4.27 (dd, *J* = 12.2 Hz, *J* = 3.9 Hz, 1H, H-6), 4.15–4.02 (m, 4H, H-3, H-5, H-6, C*H*(CH_3)CO_2Me), 3.71 (s, –OMe (β)), 3.70 (s, 3H, –OMe (α)), 3.55–3.48 (m, 2H, H-2, H-4), 2.03 (s, –OAc (β)), 2.00 (s, 3H, –OAc (α)), 1.50 (d, *J* = 5.5 Hz, CH(C*H*$_3$)CO_2Me (β)), 1.44 (d, *J* = 5.4 Hz, 3H, CH(C*H*$_3$)CO_2Me (α)). ^{13}C NMR ($CDCl_3$): δ 172.9, 172.7, 138.6,

Table 1
The effect of solvent and temperature on the stereoselectivity of the glycosylation of **1**

Entry	ROH	*T* (°C)	Solvent	*t* (min)	Yield (%)	α/β
1	Methyl (*S*)-lactate	0	CH_2Cl_2	10	91	4:1
2	Methyl (*S*)-lactate	0	THF/Et_2O (1:9)	10	87	6:1
3	Methyl (*S*)-lactate	0	THF/Et_2O (9:1)	10	60	5.8:1
4	Methyl (*S*)-lactate	0	Et_2O	10	85	7.5:1
5	Methyl (*S*)-lactate	0	THF	45	72	6.5:1
6	Methyl (*S*)-lactate	0	Toluene/dioxane (1:3)	80	43[a]	6:1
7	Methyl (*S*)-lactate	0	Toluene/dioxane (1:3) [b]	20	78	6.3:1
8	Methyl (*S*)-lactate	−60	CH_2Cl_2	10	83	7:1
9	Methyl (*S*)-lactate	−60	Et_2O	45	81	9:1
10	Methyl (*R*)-lactate	0	CH_2Cl_2	8	85	4:1
11	Methyl (*R*)-lactate	0	Et_2O	15	76	7.9:1
12	Methyl (*R*)-lactate	−60	CH_2Cl_2	10	82	6.5:1
13	Methyl (*R*)-lactate	−60	Et_2O	45	83	8.3:1
14	Benzyl (*S*)-lactate	0	CH_2Cl_2	10	93	4:1
15	Benzyl (*S*)-lactate	0	Et_2O	15	82	8.3:1
16	Benzyl (*S*)-lactate	−60	CH_2Cl_2	10	97	6.1:1
17	Benzyl (*S*)-lactate	−60	Et_2O	45	81	10:1
18	 **7**	−60	Et_2O	45	30[c]	1:0
19	Methyl glycolate	0	CH_2Cl_2	10	93	4.2:1
20	Methyl glycolate	0	Et_2O	45	85	4.4:1
21	Methyl glycolate	−60	CH_2Cl_2	10	96	4.2:1
22	Methyl glycolate	−60	Et_2O	45	88	13:1

[a] Hydrolysis product also recovered.
[b] TMSOTf was used instead of TfOH.
[c] Starting material was recovered.

Table 2
Effect of solvent and temperature on the stereoselectivity of the glycosylation of **8**

Entry	ROH	*T* (°C)	Solvent	*t* (min)	Yield (%)	α/β
1	Methyl (*S*)-lactate	0	THF	60	15[a]	5:1
2	Methyl (*S*)-lactate	0	CH_2Cl_2	5	87	3:1
3	Methyl (*S*)-lactate	0	CH_2Cl_2	30	68	2.6:1
4	Methyl (*S*)-lactate	−60	CH_2Cl_2	5	83	1.5:1
5	Methyl (*S*)-lactate	0	Et_2O	40	70	3:1
6	Methyl (*S*)-lactate	−60	Et_2O	40	86	2.2:1
7	Methyl (*S*)-lactate	0	Toluene/dioxane (1:3)	10	54[b]	2.5:1
8	Benzyl (*S*)-lactate	0	CH_2Cl_2	5	92	2.8:1
9	Benzyl (*S*)-lactate	0	Et_2O	40	84	2.7:1
10	Benzyl (*S*)-lactate	−60		10	82	1.7:1
11	Benzyl (*S*)-lactate	−60	Et_2O	40	86	4:1
12	Methyl glycolate	0	CH_2Cl_2	5	87	3:1
13	Methyl glycolate	0	Et_2O	15	92	2.2:1
14	Methyl glycolate	−60	CH_2Cl_2	5	96	1.2:1
15	Methyl glycolate	−60	Et_2O	15	95	1.4:1

[a] 43% of starting material and 38% the hydrolysis product were recovered.
[b] 18.2% of the hydrolysis product was recovered.

138.5, 138.0, 135.6, 135.5, 132.8, 129.9, 129.8, 128.5–127.4, 102.4 (C-1(β)), 97.8 (C-1(α)), 84.3, 82.0, 81.6, 80.1, 75.7, 75.6, 75.1, 75.0, 74.6, 74.4, 73.4, 72.8, 71.5, 62.0, 61.8, 52.1, 52.0, 19.1, 18.0.

4.2.2. Methyl (2*S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)propanoate 2—Crich conditions

To a solution of **1** (0.060 g, 0.11 mmol), 1-benzenesulfinyl piperidine (0.023 g, 0.11 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.045 g, 0.22 mmol) and 4 Å powdered molecular sieves in dichloromethane (1.5 mL) at −60 °C was added Tf_2O (0.021 mL, 0.12 mmol). After 5 min, a solution of methyl (*S*)-lactate (0.016 mL, 0.16 mmol) in dichloromethane (0.5 mL) was added. After 5 min at −60 °C, satd aq $NaHCO_3$ was added (5 mL) and the mixture extracted with dichloromethane (3 × 5 mL), dried with anhyd $MgSO_4$ and concentrated. Purification by preparative layer chromatography (30:70 EtOAc/hexane) afforded glycoside **2** (0.051 g, 77%, α/β 4:1) as a very viscous oil.

4.2.3. Methyl (2*R*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)propanoate 3

ν_{max} (film): 1742 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 7.43–7.25 (m, 15H, Bn aromatic), 5.10 (d, *J* = 3.4 Hz, 1H, H-1 (α)), 5.06 (d, *J* = 10.8 Hz, 1H, Bn), 4.89 (d, *J* = 11.9 Hz, 1H, Bn), 4.88 (d, *J* = 10.7 Hz, 1H, Bn), 4.81 (d, *J* = 10.8 Hz, 1H, Bn), 4.72 (d, *J* = 11.9 Hz, 1H, Bn), 4.55 (d, *J* = 10.7 Hz, 1H, Bn), 4.48 (d, *J* = 7.7 Hz, H-1 (β)), 4.37 (q, *J* = 7.0 Hz, 1H, C*H*(CH_3)CO_2Me), 4.26 (d, *J* = 3.4 Hz, 2H, H-6), 4.08 (t, *J* = 9.2 Hz, 1H, H-3), 3.83 (ddd, *J* = 10.0 Hz, *J* = 3.2 Hz, *J* = 3.2 Hz, 1H, H-5), 3.76 (s, 3H, OMe (α)), 3.72 (s, OMe (β)), 3.58 (dd, *J* = 9.6 Hz, *J* = 3.6 Hz, 1H, H-2), 3.48 (t, *J* = 9.7 Hz, 1H, H-4), 2.04 (s, OAc (β)), 2.02 (s, 3H, OAc (α)), 1.50 (d, *J* = 7.0 Hz, 3H, CH(C*H*$_3$)CO_2Me (α)), 1.46 (d, *J* = 6.8 Hz, CH(C*H*$_3$)CO_2Me (β)). ^{13}C NMR ($CDCl_3$): δ 172.9 (β), 172.5 (α), 170.7 (β), 170.6 (α), 138.6 (α), 138.4 (β), 138.2 (β), 137.9 (α), 137.6 (α), 137.5 (β), 128.5–127.6, 103.4 (C-1(β)), 95.3 (C-1(α)), 84.5 (β), 81.8 (β), 81.5 (α), 78.9 (α), 77.1 (α), 75.7 (α), 75.6 (β), 75.5 (β), 75.2 (α), 75.0 (β), 74.9 (β), 72.9 (β), 72.3 (α), 69.7 (α), 69.3 (α), 62.9 (α), 62.8 (β), 51.9 (α), 20.7 (α), 18.4 (α), 18.2 (β).

4.2.4. Benzyl (2*S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)propanoate 4

ν_{max} (film): 1739 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 7.34–7.24 (m, 20H, Bn aromatic), 5.18–5.09 (m, 2H, Bn ester), 4.99 (d, *J* = 10.7 Hz, 1H, Bn), 4.86 (d, *J* = 10.9 Hz, 1H, Bn), 4.82 (d, *J* = 11.0 Hz, 1H, Bn), 4.79 (d, *J* = 12.1 Hz, 1H, Bn), 4.77 (1H, d, *J* = 3.1 Hz, H-1 (α)), 4.63 (d, *J* = 12.0 Hz, 1H, Bn), 4.54 (d, *J* = 11.0 Hz, 1H, Bn), 4.51 (1H, d, *J* = 7.8 Hz, H-1 (β)), 4.20–4.09 (m, 3H, H-5, H-6, C*H*(CH_3)CO_2Bn), 4.06–3.99 (m, 2H, H-3, H-6), 3.54–3.47 (m, 2H, H-2, H-4), 2.01 (s, OAc (β)), 1.97 (s, 3H, OAc (α)), 1.51 (d, *J* = 6.8 Hz, CH(C*H*$_3$)CO_2Bn (β)), 1.45 (d, *J* = 6.8 Hz, 3H, CH(C*H*$_3$)CO_2Bn (α)). ^{13}C NMR ($CDCl_3$): δ 172.1 (β), 171.9 (α), 170.7 (β), 170.6 (α), 138.6 (α), 138.5 (β), 138.4 (β), 138.1 (α), 138.0 (α), 137.7 (β), 135.4 (α), 128.6–127.6, 102.4 (C-1(β)), 97.3 (C-1(α)), 84.4 (β), 81.8 (α), 79.7 (α), 77.1 (β), 77.0 (α), 75.7 (α), 75.0 (β), 74.8 (α), 74.5 (β), 73.8 (α), 73.3 (α), 72.9 (β), 72.8 (β), 69.1 (α), 66.7 (α), 63.0 (β), 62.7 (α), 20.8 (α), 19.1 (β), 17.8 (α).

4.2.5. Methyl (2*R*)-*tert*-butyldimethylsilyl-3-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)propanoate 5

ν_{max} (film): 1744 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): 7.38–7.25 (m, 15H, Bn aromatic), 4.95 (d, *J* = 10.8 Hz, 1H, Bn), 4.88 (d, *J* = 11.1 Hz, 1H, Bn), 4.87 (d, *J* = 3.5 Hz, 1H, H-1), 4.77 (d, *J* = 10.8 Hz, 1H, Bn), 4.72–4.66 (m, 2H, Bn), 4.56 (d, *J* = 11.1 Hz, 1H, Bn), 4.45 (dd, *J* = 4.4 Hz, *J* = 6.2 Hz, 1H, CH_2C*H*(OTBDMS)-CO_2Me), 4.24–4.22 (m, 2H, H-6), 4.00 (t, *J* = 9.2 Hz, 1H, H-3), 3.88 (dt, *J* = 10.0 Hz, *J* = 3.0 Hz, 1H, H-5), 3.81–3.75 (m, 1H, C*H*$_2$CH(OTBDMS)CO_2Me), 3.72–3.67 (m, 1H, C*H*$_2$CH(OTBDMS)-CO_2Me), 3.70 (s, OMe), 3.54–3.44 (m, 2H, H-2, H-4), 2.01 (s, 3H, OAc), 0.91 (s, 9H, *tert*-Butyl), 0.13 (s, 6H, $SiMe_2$). ^{13}C NMR ($CDCl_3$): δ 172.2, 170.7, 138.6, 138.3, 138.1, 128.6–127.6, 97.1 (C-1), 81.7, 79.2, 77.1, 75.7, 74.8, 72.6, 72.0, 68.8, 65.0, 63.1, 52.0, 25.7, 20.8, 18.3, −4.9.

4.2.6. Methyl 2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-glucopyranosyl)acetate 6

ν_{max} (film): 1742 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): 7.32–7.25 (m, 15H, Bn aromatic), 5.05 (d, *J* = 4.0 Hz, 1H, H-1 (α)), 5.03 (d, *J* = 11.2 Hz, 1H, Bn), 4.88 (d, *J* = 10.8 Hz, 1H, Bn), 4.86 (d, *J* = 11.9 Hz, 1H, Bn), 4.81 (d, *J* = 10.8 Hz, 1H, Bn), 4.77 (d, *J* = 11.9 Hz, 1H, Bn), 4.55 (d, *J* = 10.9 Hz, 1H, Bn), 4.51 (d, *J* = 7.7 Hz, H-1 (β)), 4.27 (d, *J* = 16.6 Hz, 1H, OC*H*$_2$$CO_2$Me), 4.25–4.24 (m, 2H, H-6), 4.15 (d, *J* = 16.6 Hz, 1H, OC*H*$_2$$CO_2$Me), 4.06 (t, *J* = 9.2 Hz, 1H, H-3), 3.91 (dt, *J* = 10.1 Hz, *J* = 3.1 Hz, 1H, H-5), 3.76 (s, 3H, CO_2*Me* (α)), 3.75 (s, CO_2*Me* (β)), 3.59 (dd, *J* = 9.6 Hz, *J* = 3.6 Hz, 1H, H-2 (α)), 3.54–3.50 (m, 1H, H-2 (β)), 3.48 (dd, *J* = 9.7 Hz, *J* = 9.2 Hz, 1H, H-4), 2.03 (s, OAc (β)), 2.02 (s, 3H, OAc (α)). ^{13}C NMR ($CDCl_3$): δ 170.7, 170.0, 138.6, 137.9, 137.8, 128.5, 128.47, 128.45, 128.3, 128.1, 128.0, 127.95, 127.92, 127.7, 103.2 (C-1(β)), 96.3 (C-1(α)), 81.6, 79.3, 77.0, 75.8, 75.0, 72.7, 69.3, 63.3, 62.9, 51.9, 20.8. Anal. Calcd for $C_{32}H_{36}O_9$: C, 68.07; H, 6.43. Found: C, 68.60, H, 6.54.

4.2.7. Ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-α/β−D-galactopyranoside 8

Alpha anomer (56% yield): $[\alpha]_D^{20}$ +113.8 (*c* 2.32, CH_2Cl_2). ν_{max} (film): 1743 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 7.40–7.27 (m, 15H, Bn aromatic), 5.49 (d, 1H, *J* = 3.5 Hz, H-1), 4.95 (d, 1H, *J* = 7.0 Hz, Bn), 4.86 (d, 1H, *J* = 7.5 Hz, Bn), 4.76–4.66 (m, 3H, Bn), 4.61 (d, 1H, *J* = 7.0 Hz, Bn), 4.31–4.25 (m, 2H, H-2, H-5), 4.18 (dd, 1H, *J* = 4.5 Hz, *J* = 2.8 Hz, H-6), 4.05 (dd, 1H, *J* = 3.3 Hz, *J* = 3.8 Hz, H-6), 3.85–3.84 (m, 1H, H-4), 3.79 (dd, 1H, *J* = 1.8 Hz, *J* = 4.5 Hz, H-3), 2.62–2.44 (m, 2H, SC*H*$_2$$CH_3$), 1.97 (s, 3H, OAc), 1.27 (t, 3H, *J* = 4.5 Hz, SCH_2C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 138.7, 138.2, 138.1, 128.5–127.5, 83.2 (C-1), 79.4, 76.4, 74.7, 74.5, 73.7, 72.5, 68.9, 63.4, 23.5, 20.8, 14.7. HR-MS: calcd for $C_{31}H_{36}O_6SH^+$ $[M]^+$: 537.23054; found: 537.23155. Beta anomer (23% yield): $[\alpha]_D^{20}$ +17.1 (*c* 1.18, CH_2Cl_2). ν_{max} (film): 1742 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 7.39–7.26 (m, 15H, Bn aromatic), 4.98 (d, 1H, *J* = 7.3 Hz, Bn), 4.89 (d, 1H, *J* = 6.3 Hz, Bn), 4.83–4.74 (m, 3H, Bn), 4.65 (d, 1H, *J* = 7.5 Hz, Bn), 4.41 (d, 1H, *J* = 6.0 Hz, H-1), 4.22 (dd, 1H, *J* = 4.3 Hz, *J* = 2.8 Hz, H-6), 4.05 (dd, 1H, *J* = 3.8 Hz, *J* = 3.3 Hz, H-6), 3.87–3.82 (m, 2H, H-2, H-4), 3.58–3.53 (m, 2H, H-3, H-5), 2.80–2.64 (m, 2H, SC*H*$_2$$CH_3$), 1.96 (s, 3H, OAc), 1.29 (t, 3H, *J* = 4.5 Hz, SCH_2C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 170.5, 138.28, 138.25, 138.22, 128.5–127.6, 85.4 (C-1), 84.1, 78.4, 75.9, 75.8, 74.3, 73.3, 73.1, 63.3, 24.9, 20.8, 15.1. HR-MS: calcd for $C_{31}H_{36}O_6SH^+$ $[M]^+$: 537.23054; found: 537.23002.

4.2.8. Methyl (2*S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)propanoate 9

ν_{max} (film): 1743 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): 7.45–7.25 (m, Bn aromatic, α and β), 5.12 (d, *J* = 10.8 Hz, 1H, Bn), 4.98–4.58 (m, Bn, α and β), 4.85 (s, 1H, H-1 α), 4.51–4.44 (m, 1H, C*H*(CH_3)CO_2Me β), 4.45 (d, *J* = 7.6 Hz, H-1 β), 4.21–3.95 (m, H-6 α, H-6 β, H-5 α, C*H*(CH_3)CO_2Me α, H-2 α, H-6 α, H-6 β, H-3 α), 3.93 (sl, 1H, H-4 α), 3.86 (dd, *J* = 7.6 Hz, *J* = 9.6 Hz, 1H, H-2 β), 3.75 (d, *J* = 2.2 Hz, H-4 β), 3.69 (s, 3H, –OMe β), 3.67 (s, 3H, –OMe α), 3.58–3.44 (m, 2H, H-3 β, H-5 β), 1.96 (s, 3H, –OAc β), 1.95 (s, 3H, –OAc α), 1.49 (d, *J* = 6.9 Hz, 3H, CH(C*H*$_3$)CO_2Me β), 1.43 (d, *J* = 6.8 Hz, 3H, CH(C*H*$_3$)CO_2Me α). ^{13}C NMR ($CDCl_3$): 173.0, 172.9, 170.5, 138.8, 138.7, 138.5, 138.3, 138.1, 128.6, 128.4, 128.37, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.57, 127.53, 127.45, 127.43, 102.6 (C-1 α), 98.0 (C-1 β), 81.9 (C-3 β), 79.1 (C-2 β), 78.9 (C-3 α), 76.3 (C-2 α), 74.9, 74.57, 74.53, 74.2, 73.7, 73.4, 73.2, 72.8, 72.5, 72.1 (C-5 β), 68.9 (C-5 α), 63.1 (C-6 α), 62.9 (C-6 β), 51.98, 51.94, 20.7, 19.1, 17.8.

4.2.9. Benzyl (2*S*)-2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)propanoate 10

ν_{max} (film): 1744 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): 7.37–7.23 (m, 20H, Bn aromatic), 5.15–5.07 (m, 2H, Bn ester), 4.97–4.89 (m, 3H, Bn), 4.86–4.84 (m, 1H, H-1(α)), 4.82–4.49 (m, 3H, Bn), 4.46 (d, J = 7.6 Hz, H-1 (β)), 4.19–4.12 (m, 2H, H-5, C*H*(CH_3)CO_2Bn), 4.10–3.96 (m, 4H, H-2, H-3, H-6), 3.87 (br s, 1H, H-4), 1.93 (s, OAc (β)), 1.92 (s, 3H, OAc (α)), 1.50 (d, J = 6.9 Hz, CH(C*H*$_3$)CO_2Bn (β)), 1.44 (d, J = 6.8 Hz, 3H, CH(C*H*$_3$)CO_2Bn (α)). ^{13}C NMR ($CDCl_3$): 172.2, 170.4, 138.7, 138.5, 138.2, 128.2–127.4, 102.6 (C-1(β)), 98.0 (C-1(α)), 81.9 (β), 79.1 (β), 78.9 (α), 76.3 (α), 74.9 (β), 74.6 (α), 74.5 (α), 74.2 (β), 73.7 (β), 73.5 (α), 73.2, 72.9 (β), 72.4 (β), 72.2 (β), 68.9, 66.6 (α), 66.5 (β), 63.0 (α), 62.9 (β), 20.8, 17.8.

4.2.10. Methyl 2-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α/β-D-galactopyranosyl)acetate 11

ν_{max} (film): 1743 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): 7.43–7.26 (m, Bn aromatic, α and β), 5.08 (d, J = 10.0 Hz, Bn), 5.05 (d, J = 3.6 Hz, H-1(α)) 4.99–4.91 (m, Bn), 4.85–4.72 (m, Bn), 4.67–4.60 (m, Bn), 4.48 (d, J = 7.6 Hz, 1H, H-1(β)), 4.39–4.26 (m, C*H*$_2$$CO_2$Me), 4.22–3.98 (m, H-6 α, H-6 β, C*H*$_2$$CO_2$Me, H-2 α, H-6 β, H-6 α, H-5 α, H-3 α), 3.93–3.88 (m, H-2 β, H-4 α), 3.77 (br s, H-4 β, CO_2*Me* β), 3.75 (br s, CO_2*Me* α), 3.56–3.48 (m, 2H, H-3 β, H-5 β), 1.98 (s, 3H, –OAc α), 1.97 (s, 3H, –OAc β). ^{13}C NMR ($CDCl_3$): 170.5, 170.2, 138.7, 138.3, 138.1, 138.0, 128.5–128.1, 103.3 (C-1(β)), 97.3 (C-1(α)), 81.8 (β), 79.0 (β), 78.6 (α), 76.0 (α), 75.0 (β), 74.7 (α), 74.6 (α), 74.3 (β), 73.7 (α), 73.6 (β), 73.1 (α), 72.9 (β), 72.2 (β), 69.1 (α), 65.5 (β), 63.6 (α), 63.4 (α), 63.0 (β), 51.9, 20.8.

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