Is acyl migration to the aglycon avoidable in 2-acyl assisted glycosylation reactions?[1]

Attila Bérces, Dennis M. Whitfield, Tomoo Nukada, Iwona do Santos Z., Agnes Obuchowska, and Jiri J. Krepinsky

Abstract: This report unequivocally separates orthoester formation from acyl transfer for the first time and indicates possible routes to eliminate 2-*O*-acyl transfer during glycosylation reactions. Experimental evidence is shown that acyl transfer from 2-*O*-acyl-3,4,6-tri-*O*-benzyl-D-galactopyranose-derived glycosyl donors decreases in the order formyl > acetyl > pivaloyl. The 2-*O*-benzoyl derivatives are more variable, in some cases transferring easily, and in others not at all. Density functional theory (DFT) calculations of the structure and energetics of dioxolenium ion and related intermediates suggest that a proton transfer pathway from the nucleophile to O-2 provides an explanation for the observed trends. These DFT calculations of the proton transfer pathway support a mechanism in which a relay molecule is involved. Further DFT calculations used a constraint based on linear combinations of six bond lengths to establish the sequence of bond breaking and bond forming. The calculated anomeric carbon to former carbonyl oxygen bond that breaks during acyl transfer is the longest in the formyl case and shortest in those that exhibit little or no acyl transfer. Rotation about the aromatic to carbonyl Ph—C(=O) bond is different from the alkyl series. Analysis of this proposed TS led to the postulate that 2,6-substitution may hinder rotation even more. Thus, the 2,6-dimethylbenzoyl analogue was synthesized and it does not transfer directly or by rearrangement of its readily formed orthoester. DFT calculations suggested that 2,6-dimethoxybenzoyl should also not transfer easily. Experimentally, this proved to be the case and this new 2-*O*-acyl protecting group cleaves at 50 °C with a 1 mol/L solution of LiOH in methanol. Thus, a calculated transition state has led to a prototype of a protecting group that solves a major problem in oligosaccharide synthesis.

Key words: glycosylation, carbohydrates, quantum chemistry, reaction mechanism, neighboring-group effects.

Résumé : Dans ce travail, on sépare sans équivoque et pour la première fois la formation d'orthoester des transferts d'acyles et on suggère des voies possibles pour éliminer le transfert de 2-*O*-acyle lors des réactions de glycosylation. On présente des données expérimentales montrant que le transfert d'acyle de donneurs glycosyles dérivés du 2-*O*-acyl-3,4,6-tri-*O*-benzyl-D-galactopyranose diminue dans l'ordre formyle > acétyle > pivaloyle. Les dérivés 2-*O*-benzoyles sont plus variables; dans certains cas, le transfert se fait facilement alors que dans d'autres il ne se fait pas du tout. Des calculs basés sur la théorie de la densité fonctionnelle (THF) de la structure et des énergies de l'ion dioxolénium et d'intermédiaires apparentés suggèrent qu'un transfert de proton du nucléophile vers O-2 s'avère la meilleure explication pour les tendances observées. Ces calculs de THF de la voie de transfert de proton appuient un mécanisme dans lequel une molécule relais serait impliquée. Des calculs supplémentaires de THF ont été réalisés en appliquant une contrainte basée sur des combinaisons linéaires de six longueurs de liaisons pour déterminer la séquence de bris et de formation de la liaison. La longueur calculée pour la liaison entre le carbone anomérique et l'oxygène du carbonyle antérieur qui se brise au cours du transfert d'acyle est la plus longue dans le cas du formyle et elle est la plus courte dans les cas de ceux qui ne donnent que peu ou pas de transfert d'acyle. La rotation autour de la liaison aromatique vers le carbonyle de Ph—C(=O) est différente de celle observée dans la série alkyle. L'analyse de cet état de transition proposé conduit au postulat que la substitution en 2 et 6 peut empêcher la rotation encore plus. On a donc réalisé la synthèse de l'analogue 2,6-diméthoxybenzoyle et on a observé qu'il n'y a pas de transfert direct ou par le biais d'un réarrangement de la part de cet orthoester facilement formé. Des calculs de TDF suggèrent que le 2,6-diméthoxybenzoyle ne devrait pas donner lieu à des transferts. D'un point de vue expérimental, cette prédiction s'est avérée juste et ce nouveau groupe protecteur 2-*O*-acyle peut être clivé à 50 °C, avec du 1 mol/L LiOH dans du méthanol. Ainsi, un état

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de transition calculé a conduit à un prototype de groupe protecteur qui résout un problème majeur dans la synthèse d'oligosaccharides.

Mots clés : glycosylation, carbohydrates, chimie quantique, mécanisme réactionnel, effets de groupes voisins.

[Traduit par la Rédaction]

Introduction

Many biologically active molecules contain sugar molecules with acetal or ketal linkages. The study of the diastereomeric complexities of joining two sugar molecules together to form such linkages is an active area of research. Most linkages are formed by glycosylation reactions between an activated electrophilic glycosyl donor and a weakly nucleophilic alcoholic acceptor. This necessity to use highly reactive donors makes these reactions prone to side reactions. Minimizing side reactions and achieving stereochemical control are the two main objectives of research into glycosylation reaction mechanisms (1).

The commonest approach to stereochemical control of glycosylation reactions is to use neighboring group participation, typically of an ester at C-2 of a hexopyranosyl donor. After activation by a promoter in the absence of nucleophiles such species form dioxolenium ions by nucleophilic attack of the ester carbonyl oxygen (O-7) on C-1 (2). It has been shown theoretically that such cations have a LUMO predominantly situated on the former carbonyl carbon (C-7) and therefore C-7 should be the site of nucleophilic attack. In the presence of nucleophiles, calculations suggest that stable intermediates that have long C-7—O_{nuc} bonds are formed (3). After shortening of this bond, hydroxylic proton transfer and pyranose ring conformational change stable orthoesters can be formed. A common side reaction long associated with orthoester formation is acyl transfer. This unwanted side reaction is the transfer of the O-2 acyl protecting group (4) to the nucleophilic acceptor to form a new ester linkage and a free OH group at O-2 of the sugar (5). This side reaction is the focus of this study.

Acyl transfer from the glycosyl donor to the sugar acceptor has been noted numerous times over many years (6). This side reaction has been observed with many different glycosyl donors (7) under many different glycosylation reaction conditions. Besides the acylated acceptor alcohols, glycosides often of both anomers with a 2-OH group, for example **5**, are frequently formed as well (8). For "conventional" solution-phase chemistry this side reaction is only a problem of yield and adds extra complexity to the reaction mixture to be purified. For solidphase methodologies, where the acceptor is bound to the polymeric support, it is particularly troublesome (9). If the acyl group is used as the cleavable group then two or more hydroxyl species instead of one will be available for the subsequent glycosylation. If the acyl group is not the cleavable protecting group then the acyl transfer itself essentially caps the polymer bound acceptor and it is the formation of polymer bound donor derived glycosides with free OH groups that can undergo further glycosylation, which is the problem.

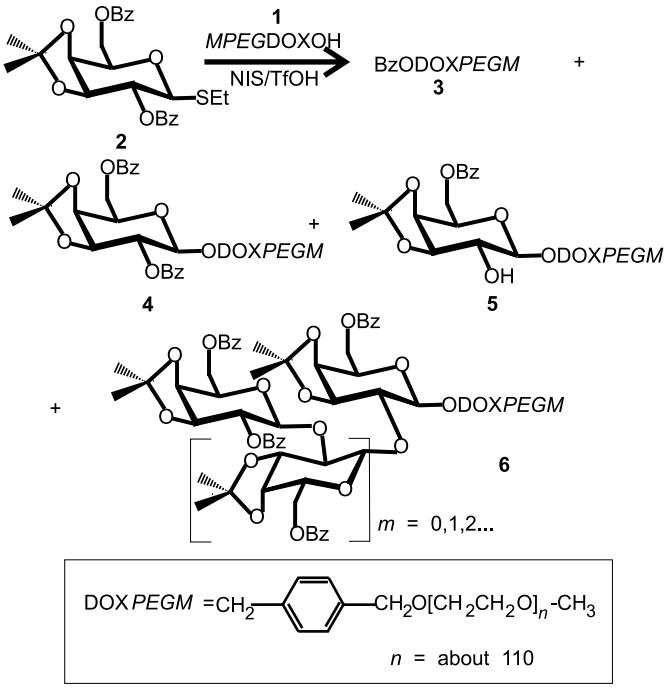
Such a reaction was observed between the linker-polymer combination MPEG-DOXOH (**1**) and the donor ethyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (**2**) under a wide variety of reaction conditions. Donor **2** was designed to allow for subsequent branching at O-3 and O-4. Among the reaction products were: **3** the result of benzoyl transfer to the nucleophile, **4** the expected product, **5** a 2-OH glycoside, and **6** β(1 → 2)-linked oligomers that accounted for up to half the polymer bound products (see Scheme 1 (10)). Previously (1 → 2)-linked disaccharides have been isolated as side products in glycosylation reactions that also exhibited acyl transfer (11). Also, some groups have independently developed protocols to preparatively prepare (1 → 2)-linked disaccharides based on this reaction type (12). Since preparative polymer-supported chemistry requires very high yields with no side products, we adopted a program to develop strategies towards the elimination of the acyl transfer side reaction.

It has long been known that increasing the steric bulk about the acyl substituent minimizes acyl transfer. For example, the suppression of acyl transfer during the preparation of multigram quantitities of glucosylated sapogenins was achieved by switching from peracetyl- to perbenzoyl-substituted donors (13). Another group has shown the utility of isobutyryl to minimize acyl transfer with glucuronyl donors (14). Several groups have used pivaloyl esters to suppress acyl transfer (15). Most of these reports discuss the use of pivaloyl in terms of suppressing orthoester formation and do not discuss acyl transfer (16). Similarly, the mesitoyl (2,4,6-trimethylbenzoyl) group has also been used to suppress orthoester formation (17). Orthoesters can be rearranged to glycosides by acid catalysis (18) but acyl transfer is frequently observed in this reaction (19). As we will show below, the propensity to form orthoesters and the propensity to give acyl transfer are not directly related (20).

Results and discussion

Few studies have attempted to systematically study the effect of the acyl group on acyl transfer. We had previously studied a series of 2,3,4,6-tetra-*O*-acyl-substituted galactopyranosyl trichloroacetimidate donors (**7ab** and **8**), and shown that the order of acyl transfer was benzoyl (**12b**) << isobutyryl (**12c**) ≈ acetyl (**12a**) (8). This study also showed that trifluoromethanesulfonic anhydride as promoter with the peracetate **7a** (21) led to 1,2 linked-oligomers, whereas using silver or copper(II) triflate as promoter eliminated the 1,2-oligomers but not acetate transfer (see Scheme 2). We now show that the powerful promoter triethylsilyltriflate also leads to acetate transfer in a ratio of 40:60 (glycoside **11a** to acetate transfer **12a**) for the α-trichloroacetimidate **7a** and 33:67 for the β-trichloroacetimidate **9a**. Changing to the perbenzoyl trichloroacetimidate **9b** improves the ratio to 93:7 (see *Experimental*). This matches the previously reported result with the perbenzoyl 1-thio-galactoside donor

Scheme 1. Acyl transfer side reaction during a polymer-supported glycosylation reaction. Even after extensive optimization desired glycoside **4** could only be formed in <40% yield (10).



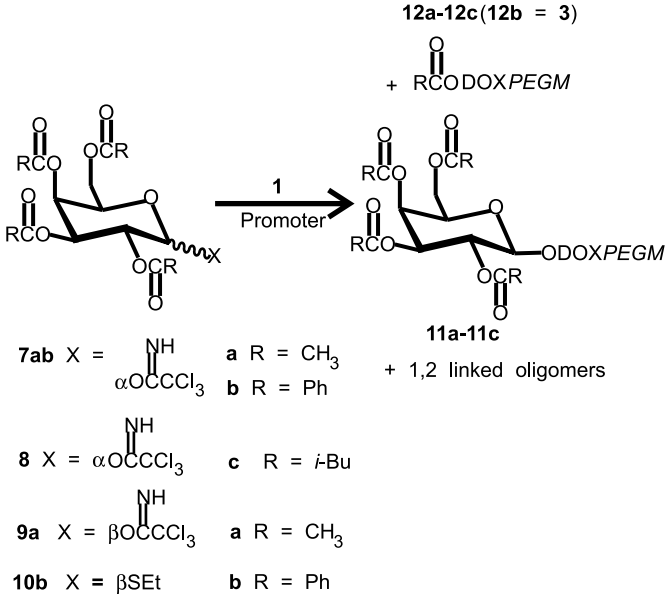
10b, where a ratio of 93:7 (**11b**:**12b**) was also found (10). We interpret these results to indicate that one or more intermediates are formed in such glycosylation reactions that eventually lead to acyl transfer or glycosylation (cf. **D** and **E** in Scheme 3).

Returning to 3,4-*O*-isopropylidene galactose derivatives, we showed that even pivaloyl esters transfer to form MPEG-DOXOPiv although 1,2-oligomer formation was suppressed.

Following a literature precedent (22), increasing the steric bulk about the alcohol acceptor by forming the α-methyl benzyl derivative MPEG-MDOXOH was undertaken for the preparation of synthetically useful amounts of MPEG-MDOXyl glycosides. Two other factors, namely operating at the highest possible concentration of TfOH and the highest possible temperature that did not lead to decomposition reactions, were also necessary to suppress acyl transfer. These last two observations can be interpreted to indicate that acyl transfer gives the apparent kinetic product and glycosylation the apparent thermodynamic product (10). The importance of the acid concentration also suggests that proton transfer is integral to the mechanism as previously proposed (11*a*).

Given the high propensity to undergo acyl transfer of the 3,4-isopropylidene galactose derivatives, we undertook to study the mechanism of acyl transfer with the 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-D-galactopyranosyl cations. The structures and energetics of the following intermediates were calculated using density functional theory (DFT) with a continuum solvent correction (see Scheme 3). The "parent" cat-

Scheme 2. Effect of acyl groups on acyl transfer with galactose donors (8, 10).



ion **B** is derived by ionization of donor **A**. Monocyclic **B** after ring inversion can cyclize to the bicyclic dioxolenium ion **C** associated with neighboring group participation with a calculated barrier of 34 kJ mol^{-1} (23). This ion has a vacant *p*-like orbital centered on the former carbonyl carbon (C-7) (3). This reacts with methanol to form an ion–dipole complex **D** with a C-7—O-8 (O-8 from MeOH) bond length of 2.81 Å. After considerable search, a pathway involving proton transfer to O-2 from **D** spontaneously led to acyl transfer and the 2-OH ion **E**.[4] Ion **E** is presumably the precursor to 2-OH glycosides and 1,2-oligomer formation but its reactivity has not been studied further.

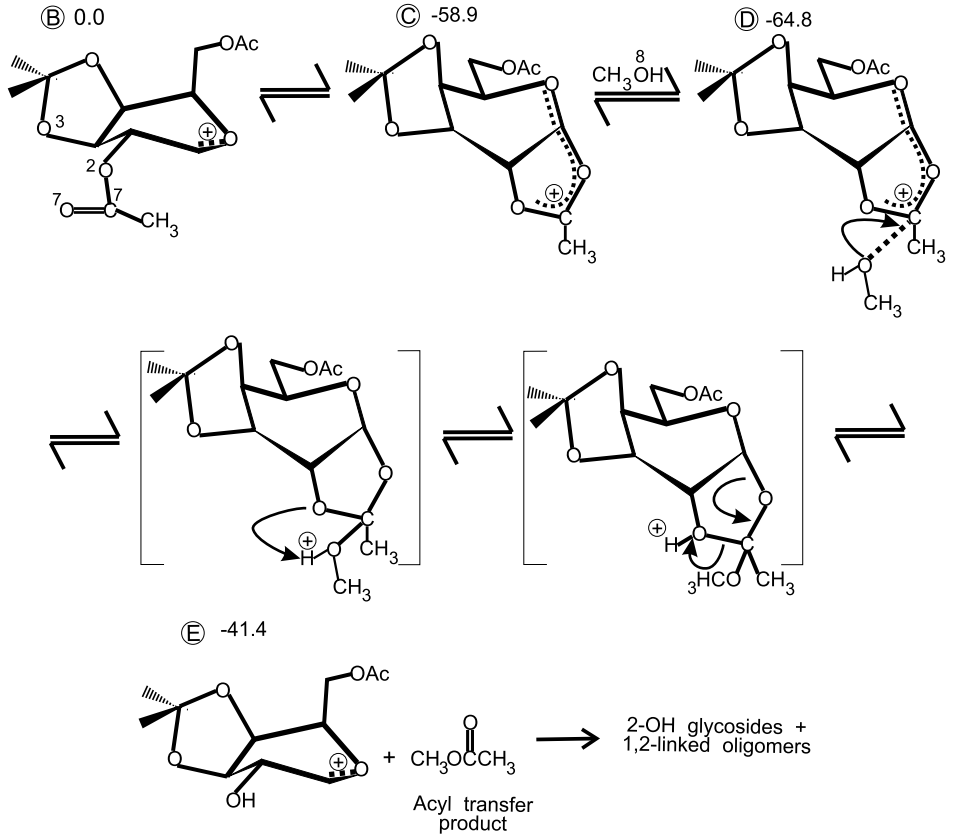
To further study the acyl transfer pathway, we decided to study the effect of acyl substituents. For this purpose, a series of 2-*O*-acyl 3,4,6-tri-*O*-benzyl-D-galactopyranosyl donors (**13a**–**13e**), where the acyl groups are formyl, acetyl, benzoyl, pivaloyl, and levulinoyl, were prepared and reacted with **1** (see *Supplementary material*[5] and *Experimental*. The ease of acyl transfer during the preparation of glycosides **14a**–**14e** followed the order formyl > acetyl > levulinoyl >> benzoyl = pivaloyl in the amounts 56%, 5%, 2%, and 0% (cf. **15a**-**15e**, see Scheme 4). This compares to the 3,4-isopropylidene donors where benzoyl transfers more than pivaloyl with the same acceptor suggesting that the more reactive 3,4,6-tri-*O*-benzyl donors suppress acyl transfer (24).

To study the acyl group effect theoretically we calculated the intermediates **B**, **C**, **D**, and **E′** for the series 2-*O*-acyl 3,4-*O*-isopropylidene-6-*O*-acetyl-D-galactopyranosyl cation plus MeOH where the acyl is formyl, acetyl, benzoyl, and pivaloyl (see Table 1). Note as shown below, **E′** in this series is an ion–dipole complex of the ion **E** and the appropriate acyl methyl ester. The pyranose ring in all **C** and **D**s is in the

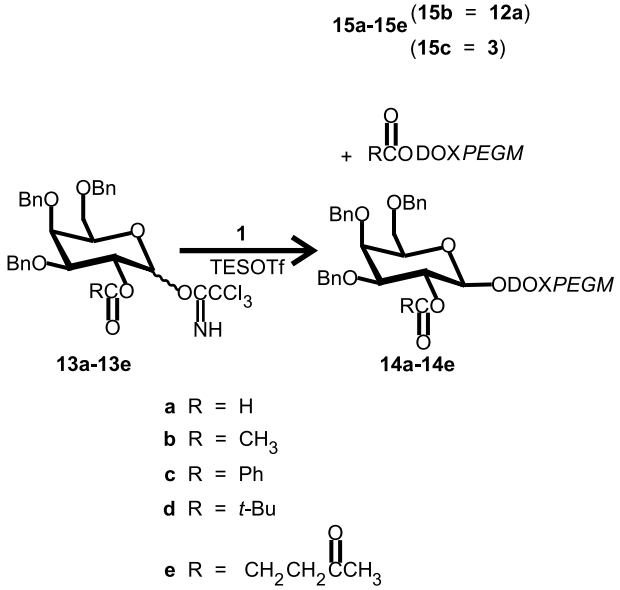
[4] In the original report of ref. 3 other ion–dipole complexes resulting from the methanol approach to the anomeric center of **C**, to the β-face of **B**, and the α-face of **B**, respectively, as well as a plausible intermediate with a tetrahedral carbon were considered.

[5] Supplementary data may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada (http://www.nrc-cnrc.gc.ca/cisti/irm/unpub_e.shtml for information on ordering electronically).

Scheme 3. Previously proposed mechanism for acyl transfer via proton transfer to O-2. Intermediates **B**–**E** derived by the ionization of donor **A** (10).



Scheme 4. 2-*O*-Acyl-substituted galactose donors used to assess the substituent effect on acyl transfer.



$B_{2,5}$ conformation and in all **B**s is in an ^2S_O conformation. Figure 1 shows partial ball and stick representations of the structures of the **D** complexes. It is readily apparent that the C-1—O-7 bond length is the longest for the formyl derivative among the series formyl (1.810 Å), acetyl (1.640 Å), and pivaloyl (1.618 Å). These trends fit the observation of acyl transfer diminishing in the series formyl > acetyl > pivaloyl (see Figs. 1*a*–1*c*). The benzoyl **D** has the shortest calculated C-1—O7 bond length (1.607 Å) but is quite close to pivaloyl (cf. Figs. 1*d* and 1*c*). These results suggest some π-resonance contribution, which is not unexpected given the similarity to a substituted benzyl cation. Similarly, the benzoyl complex **D** is the most stable relative to isolated **B** and MeOH as shown in Table 1.

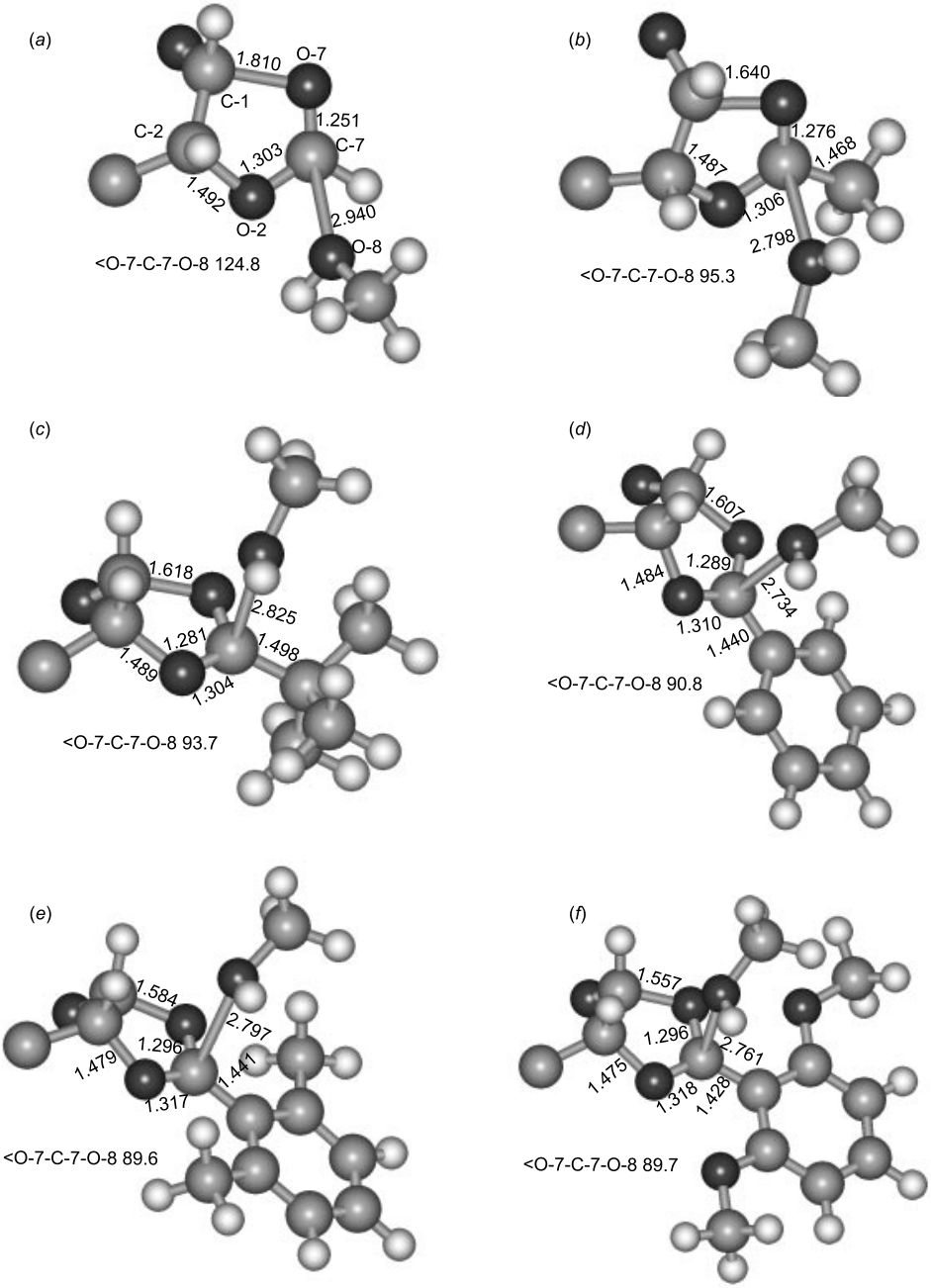
From our previous calculations we know that proton transfer triggers the acyl transfer and for this reason, the proton transfer is probably the rate-determining step (10). We first studied the direct transfer of the H-(O-8) to O-2 using constrained linear transit calculations explained in *Computational details*. We found that the direct proton transfer involves a 170 kJ mol^{-1} barrier, which is clearly too high and this mechanism is therefore unrealistic. For a proton transfer to take place with a reasonably low energy barrier, the donor, the acceptor, and the proton should ideally be situated on a straight line. In contrast, the angle between the H—O-8 bond and the H—O-2 hydrogen bond is 78° in the formyl case. Consequently, the proton transfer does not take place directly but likely involves a relay molecule. We note that these reactions normally take place in aprotic solvent but a small amount of water or other proton relay molecules cannot be ruled out. The counteranions or leaving-group derived entities as well as added bases and molecular sieves etc. are all possible relay molecules. To keep the model as simple as possible we considered a second methanol to be involved as

Table 1. Relative energies in kJ mol^{-1} for cations **B**–**E′** derived from 2,6-di-*O*-acyl-3,4-*O*-isopropylidene-D-galactopyranosyl donors.

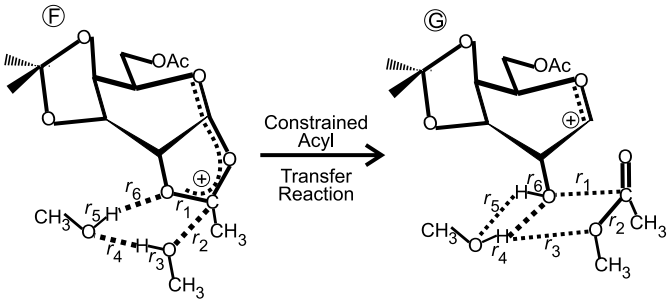
	Formyl	Acetyl	Pivaloyl	Benzoyl	2,6-Dimethylbenzoyl	2,6-Dimethoxybenzoyl
B	0.0[a]	0.0	0.0	0.0	0.0	0.0
C	–34.9	–51.6	–48.7	–56.0	–65.9	–82.6
D	–35.9	–57.7	–56.1	–62.6	–70.0	–85.2
E′	–44.6	–46.2	–16.6	–30.8	–46.9	–57.6
ME (*c*)	0.0[b]	0.0	0.0	0.0	0.0	0.0
ME (*t*)	–10.7	–18.3	–44.8	–33.5	–15.7	–12.8

[a]Energy including solvated methanol for **B** and **C** at the same level of theory.
[b]Energy for the solvated cis (*c*) ester set to 0.0 for comparison to the trans ester (*t*) reaction (25).

Fig. 1. Partial ball and stick representations of ion–dipole complexes **D** for: (*a*) formyl, (*b*) acetyl, (*c*) pivaloyl, (*d*) benzoyl, (*e*) 2,6-dimethylbenzoyl, and (*f*) 2,6-dimethoxybenzoyl.



Scheme 5. The constraint based on r_1r_6 used for the mechanism calculation (see eq. [2]).



a relay molecule in the proton transfer. Similar relay molecules have been used in theoretical studies of the mutarotation reaction (25).

To understand the mechanism of acyl transfer we carried out calculations mapping the reaction path leading from **F** to **G** as shown in Scheme 5. General methods of reaction path following usually consider elementary reaction steps. The application of these methods becomes intractable for multistep reactions such as acyl transfer. Consequently, we customized our approach to this reaction to gain insight into the mechanism. We carried out linear transit calculations using a constraint that ensures that the reactant and the product states correspond to **F** and **G**.

This reaction involves the formation and breaking of seven bonds as well as hybridization changes such as C-1 going from sp^2 in **F** to sp^3 in neutral products. Two hydrogen bonds become covalent bonds and two covalent bonds involving hydrogen break to become hydrogen bonds. In addition, the formation of the O-8—C-7 bond and the breaking of O-2—C-7 and O-7—C-1 are key features in the mechanism. Since the O-7—C-1 bond breaking is the consequence of the rest of the rearrangements, we do not have to consider this bond as part of our linear transit constraints. To consider the breaking and formation of six separate bonds individually requires assessing the $6 \times 5 \times 4 \times 3 \times 2 = 720$ possible sequences of events. For this reason, we have to define a constraint that brings the reactants to product state without predefining the sequence of events. We shall explain how this can be carried out on a simpler example and extend the results to acyl transfer. Lets consider the transfer of H—(O-R) to O-2. There are two bonds involved in this transfer: the forming H—O-2 bond and the breaking H—(O-R) bond. A simple constraint

$$[1] \qquad Q = \mathrm{R[H—(O\text{-}R)]} - \mathrm{R[H—(O\text{-}2)]}$$

where R[H—(O-R)] and R[H—(O-2)] are the H—(O-R) and H—(O-2) bond lengths, respectively, can be used to study this mechanism. At the reactant state, the value of Q is negative while it is positive in the product state. To study this reaction by quantum mechanical methods, we can carry out constrained geometry optimization at varying values of Q representing the progress of the reaction. This procedure is called linear transit. The application of Q as a constraint has some distinct advantages compared to using either the forming or the breaking bond distance as constraint alone. Most importantly, this constraint does not specify the sequence of events, whether the bond breaking or the formation takes place first or if the two steps are synchronized. In addition, it is straightforward to extend this constraint to the case described in Scheme 5. For the exploration of the reaction path from **F** to **G** we used the following definition of the constraint:

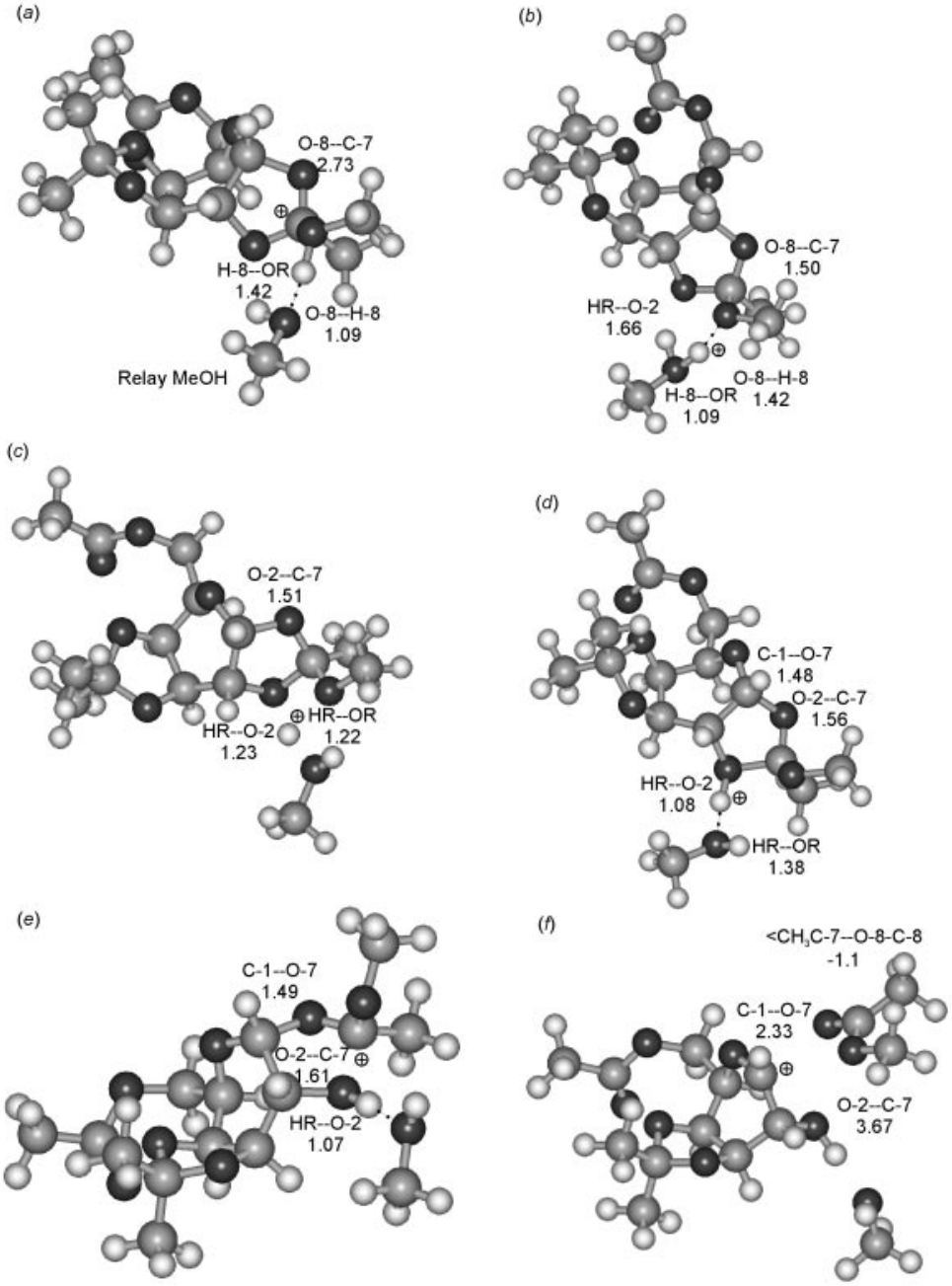
$$[2] \qquad Q = r_1 - r_2 + r_3 - r_4 + r_5 - r_6$$

where r_1 through r_6 are defined in Scheme 5. In our calculations, we varied Q stepwise from a negative value to a positive one in 50–60 steps. At each value of the constraint the geometry was optimized subject to the constraint. Consequently, going from one value to the next of Q in the reaction path, the bond that changes its length is always whichever bond involves the least increase in the energy. These calculations enable one to find out the sequence of events without any assumption. However, this constraint has its own limitations. If the mechanism is stepwise, after the first proton transfer the broken bond involving the transferred hydrogen becomes the softest coordinate. Consequently, the lowest energy path involving the increase of Q is the increase of the distance of the broken bond, which may be irrelevant to the mechanism. For this reason, in a clearly stepwise reaction, once the first step is explored Q may be modified to the exploration of the next step to exclude the coordinates irrelevant for the rest of the reaction.

In addition, in the definition of Q we assumed that all conformational changes are the consequence of the formation and breaking of the bonds. As we found in the calculations, the products often involve different conformations from the reactants. To explore the importance of conformational change in the mechanism in a quantitative manner is a subject for future research. Even with its limitations, these calculations gave some significant insight into the reaction mechanism. It is remarkable that out of the 720 theoretically possible reaction mechanisms, all studied reactions follow essentially the same steps with four different protecting groups (see Fig. 2*a*–2*f* for the acetyl example). The potential energy surface involves an initial barrier followed by a high energy intermediate in a plateau region (see Fig. 3). The plateau is followed by a second barrier, which is higher than the first barrier except for the pivaloyl case.

The mechanism starts with the shortening of the O-8—C-7 bond length, which markedly increases the acidity of the nucleophilic hydroxlic proton. This charge movement presumably triggers transfer of this hydroxylic proton from O-8 to the relay molecule (see Fig. 2*b*) followed immediately by the transfer of the proton from the relay molecule to O-2. The first barrier (TS1) on all calculated potential surfaces corresponds to the second proton transfer step to O-2 (see Fig. 4). The first proton transfer causes the formation of a shoulder on the potential surface but no barrier can be associated with it. The intermediate is not clearly defined due to the shallow nature of the plateau region. The second barrier (TS2) involves the breaking of the O-2—C-7 bond and charge transfer to C-7 associated with the change of sp^3 to sp^2 hybridization of C-7. Subsequently the C-1—O-7 bond breaks to give **G**. This mechanism resembles the intuitive proposal of Bochkov et al. (26) except that their model has

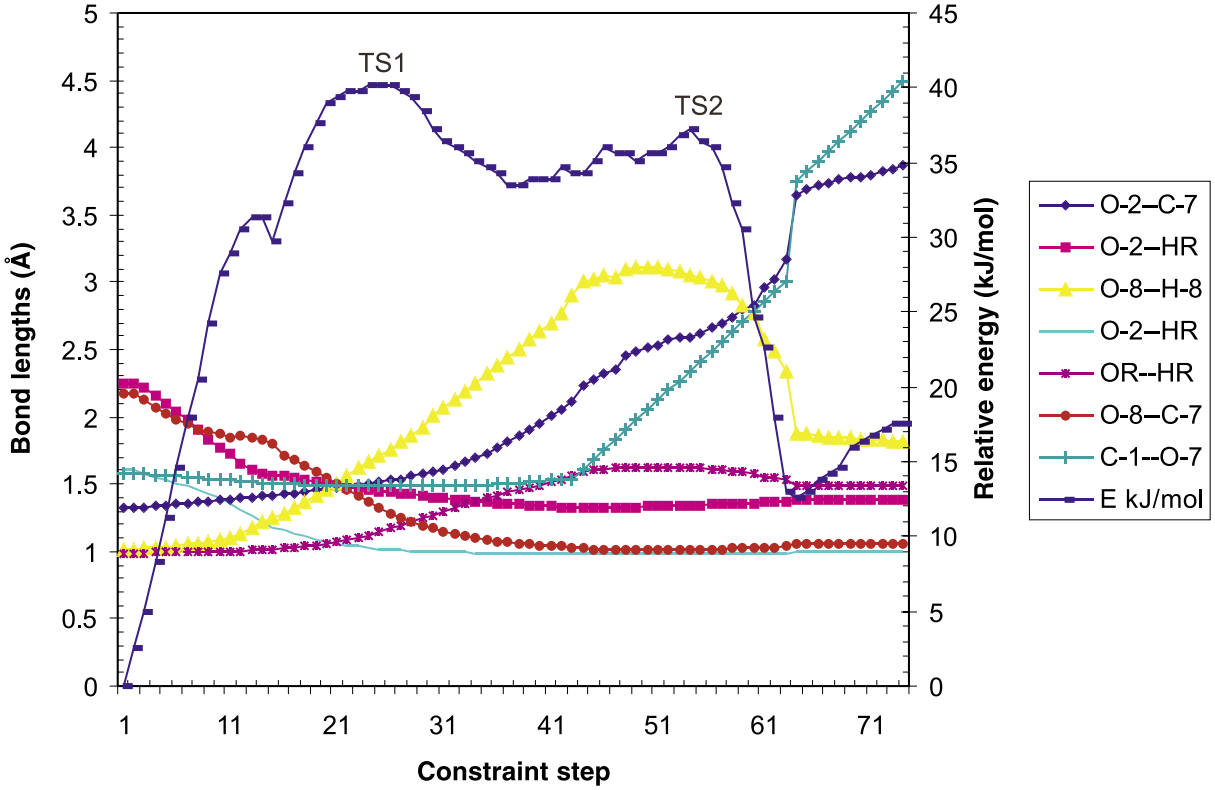
Fig. 2. Ball and stick representations of the constrained acyl transfer reaction for the acetyl case. (*a*) Initial structure showing relay methanol (OR and OHR). (*b*) Proton transfer to the relay methanol. (*c*) Proton transferring from the relay methanol to O-2 (1st TS). (*d*) Proton transferred to O-2. (*e*) Breaking of bond O-2—C-7 (2nd TS). (*f*) Final structure with bond C-1—O-7 broken also.



an equivalent to **E′** with a covalent O-7—C-1 bond and no proton relay.

The first barrier is between 30 and 78 kJ mol^{-1}, the formyl being the lowest and the benzoyl the highest. The second barrier is between 40 and 92 kJ mol^{-1}, the pivaloyl being the lowest and the benzoyl the highest. The second barrier appears as a sharp peak in all potential surfaces except the pivaloyl (see Fig. 3). We found that the lack of the large peak at the second barrier in the case of the pivaloyl is due to a qualitatively different pathway. The difference is best understood by considering the difference in the conformation of the final ester product. Methyl pivalate is found in the preferred trans conformation, while all other esters were found in the undesirable cis conformation at the end of the reaction path (see Figs. 2*f* and 5). The steric repulsion of the bulky pivaloyl group prevented the rotation of the methyl group towards the cis orientation. For all other systems, the rotation of the methyl group is easier towards the cis position at the beginning of the reaction. Consequently, we consider the large second barrier an artifact of the conformational orientation. This clearly points to the need to improve the reaction path following method by enabling the

Fig. 3. Bond lengths (r_1–r_6) used for the constrained acyl transfer reaction plus C-1—O-7 and the relative energy vs. the constraint step. The acyl group is pivaloyl.



involvement of conformational as well as bond distance coordinates in the constraints. Such a development is for future studies.

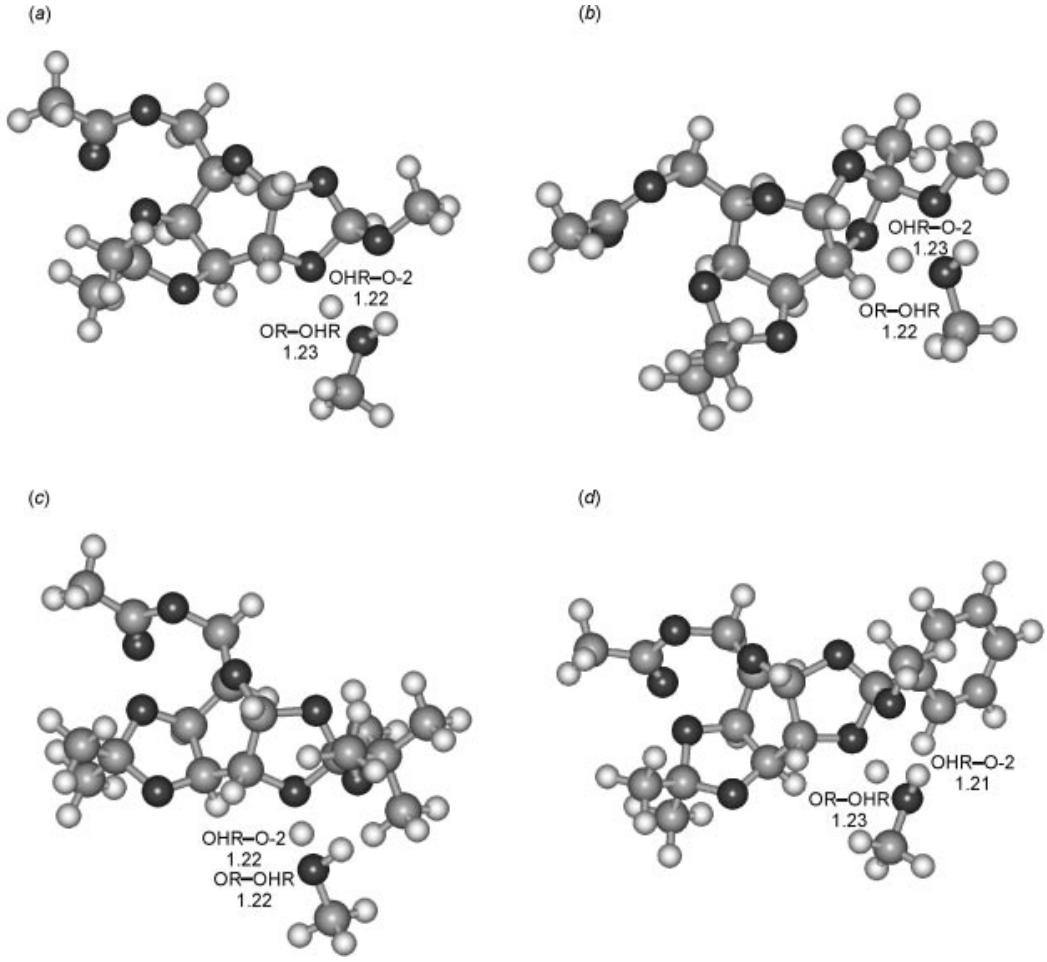
Taking all these finding into consideration, the rate-determining barrier is the first barrier on the calculated reaction surface associated with proton transfer to O-2. The height of this barrier is 30, 40, 40, and 78 kJ mol^{-1} for formyl, acetyl, pivaloyl, and benzoyl, respectively. Some contribution from the undesirable conformation can be attributed to the first barrier in all cases except the pivaloyl. The largest difference between the cis and trans product was found in the case of benzoyl ester, which is 33.5 kJ mol^{-1}, while it is 10.7 and 18.3 in the case of formyl and acetyl (see Table 1). Considering the relative magnitudes of these undesirable contributions, our calculated barriers are consistent with the experimentally found order of propensity to acyl transfer.

From these four trajectories we can draw some important conclusions. The conformational reorganization is driven by the change in the hybridization of C-7, which goes from sp^3 to sp^2, and consequently from tetrahedral to a planar conformation. In addition to the conformational change leading to either cis or trans in the final ester product, the protecting groups also play a role in determining the conformation around the C-7—O-7 bond, which is crucial in the reaction mechanism (see Fig. 5). In this regard, the benzoyl stands out as different from the rest and especially different from pivaloyl. The flat benzoyl group can accommodate a conformation helping the C-7—O-2 bond breaking by simply rotating around the C-7—phenyl bond.

The above analysis suggested to us that in the benzoyl case 2,6-disubstitution may inhibit this rotation and hence acyl transfer. The DFT-calculated intermediates **B**–**E′** are shown in Table 1 and Fig. 1*e*. Like the parent benzoyl, the 2,6-dimethylbenzoyl **D** is predicted to be stable with a short C-1—O-7 bond length. Thus, we synthesized the 2,6-dimethylbenzoic acid analogue in the 3,4-isopropylidene series and tested its reactivity (see *Experimental* and Scheme 6). This derivative **17** synthesized from known diol **16** (27) exhibited no acyl transfer giving an almost quantitative yield of glycoside **18**. The structure of **18** was confirmed by cleaving from the polymer to give **19** (28). Furthermore, if less triflic acid was added (0.7 equiv. vs. 1.4 equiv.) then a mixture of orthoester **20** and **18** was obtained. The orthoester **20** in the mixture could be smoothly isomerized to **18** with more triflic acid without acyl transfer. To the best of our knowledge this is the first experimental result that uncouples orthoester formation and acyl transfer.

Orthoesters (29) have been previously suggested to be intermediates to glycosides and acyl transfers (30). The acid-catalyzed isomerization of **20** to **18** without acyl transfer strongly supports a mechanism for acyl transfer that does not involve neutral orthoesters. Note that our **D** ion–dipole complexes have long C-7—O-8 bond lengths (see Figs. 1*a*–1*f*), and must undergo three major changes to become neutral orthoesters: the O-8—C-7 bond must shorten, the pyranose ring conformation must change, and the alcoholic proton must be transferred. DFT calculations (not shown) of this process suggest that proton transfer is the last of these three steps. This uncoupling of orthoester formation and acyl

Fig. 4. Ball and stick representations of putative TS1: (*a*) formyl, (*b*) acetyl, (*c*) pivaloyl, (*d*) benzoyl.



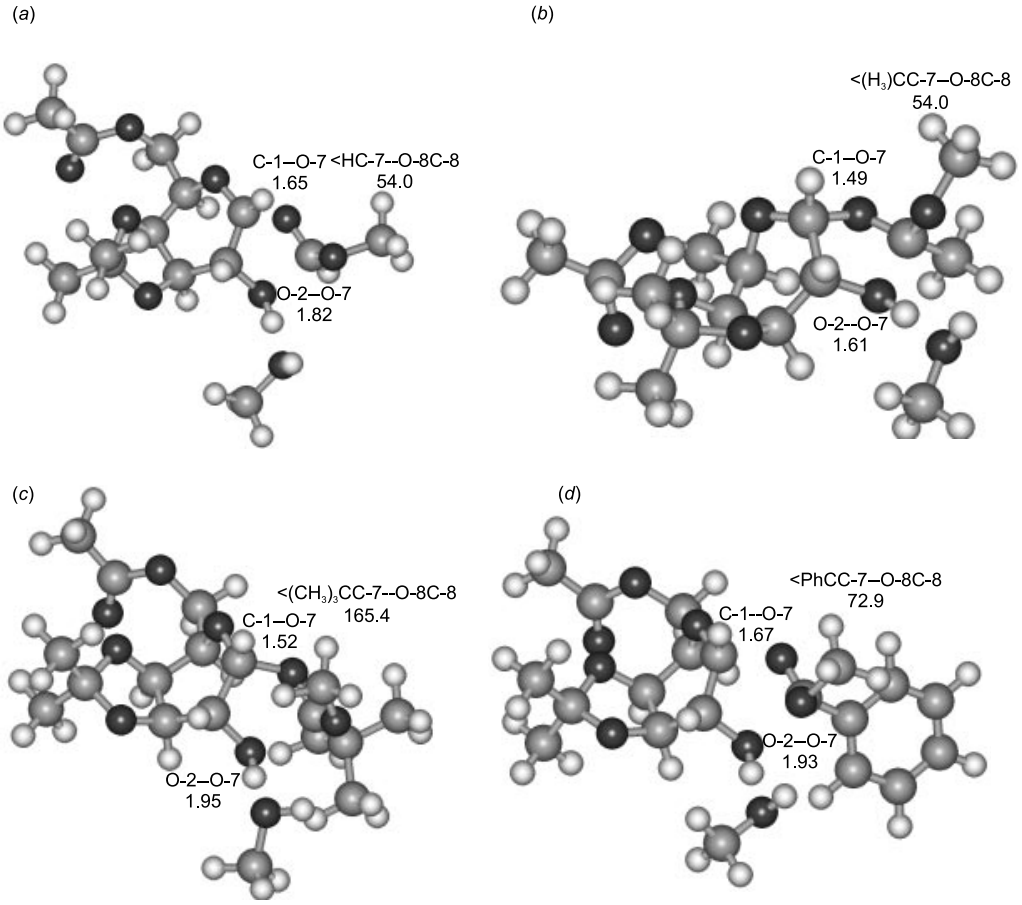
transfer is in agreement with the ease of formation of pivaloyl orthoester (31) but their reluctance to undergo acyl transfer. What the mechanism is for proceeding from **D** to glycosides is not known (32). This is a subject of current research for us (33).

Our previous results had shown that even benzoate is difficult to cleave from polymer-supported Gal C-2 (10) and it is well-known that 2,6-dimethylbenzoyl esters are difficult to hydrolyse (34). Therefore, it was not surprising that under a variety of vigorous conditions (for example $LiAlH_4$ at reflux in THF, 1 mol/L KOHaq at 50 °C, etc.), we could not cleave the 2,6-dimethylbenzoate group from **18** without decomposition or recovery of intact **18**. Eventually we discovered that 1 mol/L LiOH in methanol at 70 °C for 48–60 h did cleave this group (see Scheme 7). This led us back to the results in Figs. 1*e* and 1*d* where the C-1—O-7 bond length (1.584 Å) in complex **D** of the 2,6-dimethylbenzoate is shorter than in **D** of the benzoate (1.607 Å). This suggested to us that the difference in this bond length may be at least in part a π-electronic effect. Therefore, we calculated the parameters for the more electron-donating 2,6-dimethoxybenzoate analogue, and the C-1—O-7 bond length (1.557 Å) is even shorter (see Fig. 1*f*). Subsequently, we synthesized this analogue **21** from **16** and reacted it with **1** (see Scheme 7). The reaction proceeded smoothly to give glycoside **22** contaminated with only a small amount of acyl transfer product **23** (**22**:**23**, 92%:8%). We had also envisaged that the 2,6-dimethoxy analogue would cleave easier than the 2,6-dimethyl one due to the possibility of chelation to the methoxy oxygens. The 2,6-dimethoxybenzoyl group is smoothly cleaved with 1 mol/L LiOH in methanol at 50 °C for 16 h to diol **24**. To improve the ease of removal, we are examining 2,6-dimethoxy-4-X-benzoyl esters, where X is an electron-withdrawing group. Thus, we have progressed from a calculated TS to a prototype of a protecting group that minimizes acyl transfer. Since the 3,4-isopropylidene galactose donors are the most prone to acyl transfer among those that we have studied, this new protecting group should be a general solution to acyl transfer.

Conclusions

Computational evidence is presented that ion–dipole complexes like **D** are formed under glycosylation reaction conditions. A pathway from **D** that most likely involves a proton relay from the nucleophile's proton to O-2 of the electrophilic sugar with accompanying formation of the O-8—O-7 bond, O-2—C-7 bond rupture, and finally O-7—C-1 bond breaking leads to an ion–dipole complex of a 2-OH glycosyl cation and the ester by-product **E′**. This order of bond forming and bond breaking was determined by computations using a complex constraint (see Scheme 5). In the model, the

Fig. 5. Ball and stick representations of putative TS2: (*a*) formyl, (*b*) acetyl, (*c*) pivaloyl, (*d*) benzoyl.



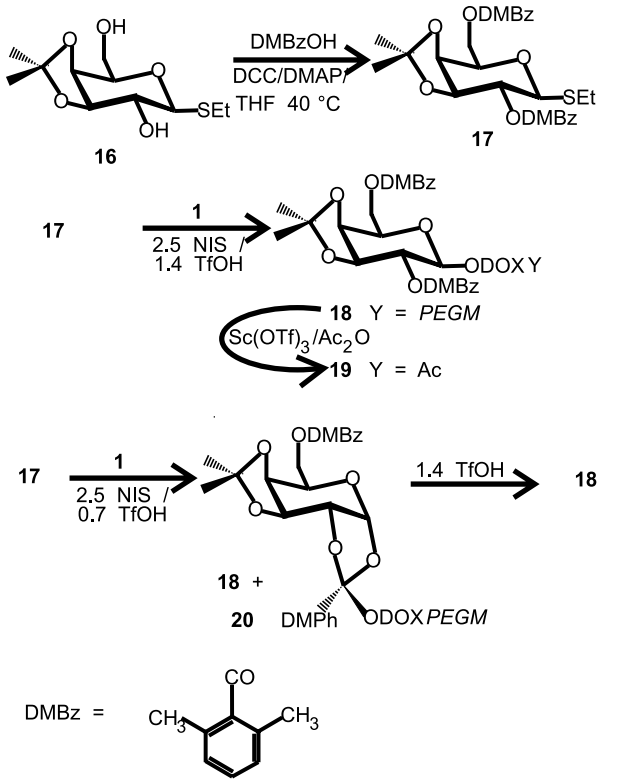
ester is formed in the unfavorable cis conformation except the pivaloyl, which perhaps explains the strong sensitivity to steric bulk of the acyl transfer side reaction. The O-7—C-1 bond in the **D** complexes is the longest in the formyl case and the shortest in the pivaloyl case along the series formyl, acetyl, pivaloyl. It is even shorter in the benzoyl case even though benzoyl still transfers in some cases suggesting an electronic component besides a steric component to this side reaction. The steric effect led us to use the 2,6-dimethyl- and 2,6-dimethoxybenzoyl groups as neighboring groups, which eliminate or greatly minimize acyl transfer especially compared to benzoate where >50% of the reaction products were 1,2-linked oligomers under the same reaction conditions. The 2,6-dimethoxybenzoyl is easier to remove and its use is under investigation in other oligosaccharide syntheses. Orthoester formation with the 2,6-dimethylbenzoyl protecting group is facile but its rearrangement to glycoside is not accompanied by acyl transfer. Although these two side reactions are probably related, this result clearly shows that the two reactions are distinct. This separation of reaction mechanisms strongly suggests that acyl transfer can be eliminated by the judicious choice of protecting groups and reaction conditions like the ones described here.

Methods

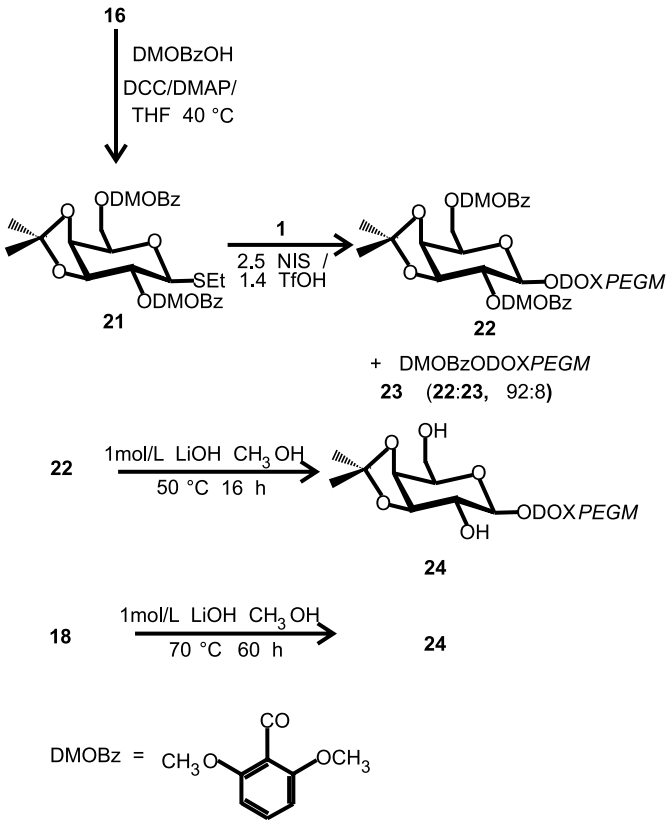
All starting materials were dried overnight in vacuo at 10^{-3} mmHg (1 mmHg = 133.322 4 Pa). All reactions were done under argon. The progress of the reactions was monitored by thin layer chromatography (TLC) on silica gel. The TLC results were visualized under UV light (254 nm) and by spraying with 50% sulphuric acid in methanol and heating at 200 °C. The ^{1}H and ^{13}C NMR spectra were recorded in deuteriochloroform solution at 500.1 MHz and 125.8 MHz, respectively, with Varian Unity Plus spectrometers. For polymer-bound samples, the *MPEG* methylenes were saturated and quantitation was made by comparing integrals to the terminal methyl of the MPEG. Assignments were made by comparison to the spectra of building blocks and cleaved compounds. ^{1}H NMR spectra in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm, and ^{13}C NMR spectra to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made by standard ^{1}H^{-1}H-COSY and ^{13}C-^{1}H-COSY experiments. The mass spectral analysis was done on a forward mass spectrometer. Fast atom bombardment (FAB) MS was performed using xenon atom at 6 kV as the source. Thioglycerol or a mixture of glycerol and thioglycerol was used as FAB matrix. Typically, 10–15 full-range, low-resolution MS scans were averaged to yield a low-resolution mass spectrum. For high-resolution MS, the electric sector was scanned over the range of interest. Typically, polyethylene glycol or polypropylene glycol was used as an internal mass standard and between 75 and 150 scans were averaged. Some MS were done by ion spray with a PerkinElmer/Sciex API triple quadrupole mass spectrometer.

The ^{1}H NMR spectrum of the acylated MPEGDOXOH

Scheme 6. 2,6-Dimethylbenzoyl as a protecting group to eliminate acyl transfer and to separate acyl transfer from orthoester formation.



Scheme 7. 2,6-Dimethoxybenzoyl as a protecting group for minimizing acyl transfer.



were obtained by acylating the polymer i.e., either formyl, acetyl, or benzoyl. The ^{1}H NMR spectrum of MPEGDOX-*O*-formyl shows that the CHO-O-CH_2-C_6H_4-CH_2-$(OCH_2CH_2)_nOCH_3$ peak is shifted to 5.19 ppm from 4.68 ppm in MPEGDOXOH. This same peak appears at 5.09, 5.05, 5.10, 5.35, and 5.34 ppm in the acetylated (**12a**), pivaloylated (**15d**), levulinoylated (**15e**), benzoylated (**3**), and 2,6-dimethoxybenzoylated (**23**), MPEGDOXOH, respectively.

Computational details

The Amsterdam density functional (ADF) calculations use the methods described in ref. 3 and include a continuum dielectric solvent term. The basis set used was a double ζ basis set with a single polarization function. The conformational description based on the IUPAC nomenclature is described in detail in ref. 35. A fully working version of the program is available at http://www.sao.nrc.ca/ibs/6ring.html.

To enable the exploration of the reaction pathways we incorporated some changes in the ADF program. ADF enables the user to follow the reaction pathway by slowly varying a constrained coordinate from the value representative of the reactant state to the product state with geometry optimization subject to this constraint at given values of the constraint. This procedure is called a linear transit calculation. The original implementation of linear transit allows only individual internal coordinates such as bond length, bending angles, and torsion angles to be defined as a constraint. However, it is not possible to define linear combinations of individual internal coordinates, which is desirable for complicated reaction profiles such as acyl transfer. To facilitate the exploration we extended the definition of constraint by implementing a general constraint of the following form:

$$Q = l_1{*}q_1 + l_2{*}q_2 \ \ldots \ + l_nq_n$$

where q can be any bond length, bending angle, or torsion angle.

The coordinates are kept constant in the geometry optimization by simply projecting out the contribution of the Cartesian forces to the force on the constrained coordinate followed by a Cartesian geometry optimization step. After the Cartesian optimization step, the constraints are exactly imposed by an iterative correction of the coordinates (36).

MPEGDOXOH (**1**) was synthesized according to ref. 37. The synthesis of donors **13a**–**13e** is described in the *Supplementary material*.[5]

MPEGDOXyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside 11a (38)

Experiment I (using α-imidate)

A solution of TESOTf in CH_2Cl_2 (2.0 µL, 8.8 µmol) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (39) (**8a**) (27 mg, 55 µmol) and **1** (57 mg, 27 µmol) in dry CH_2Cl_2 (2 mL) at room temperature. After 1 h of stirring, TLC (hexanes : ethyl acetate, 2:1, 2% diisopropylethylamine (DIPEA)) indicated complete disappearance of the starting imidate. The reaction was stopped 3 h after the addition of TESOTf by addition of

$NaHCO_3$ (and stirring for ~10 min), filtered, and the volume was reduced to ~0.5 mL. Dry *tert*-butylmethylether (TBME) (30 mL) was then added and the polymer was precipitated at 4 °C overnight. The polymer (64 mg) was then isolated by filtration. The ^{1}H NMR spectrum indicates that 40% yield of the β-glycosylated product **11a** and 63% yield of acyl transfer product **12a** were obtained. ^{1}H NMR (ppm) δ: 5.39 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.0 Hz, H-4), 5.27 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 4.98 (dd, 1H, H-3), 4.51 (d, 1H, H-1), 4.21 (dd, 1H, $J_{5,6}$ = 6.4 Hz, $J_{6,6'}$ = 11.3 Hz, H-6), 4.16 (dd, 1H, $J_{5,6'}$ = 6.8 Hz, H-6′), 3.89 (m, 1H, H-5), 2.16 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO).

Experiment II (using β-imidate)

TESOTf (3.9 μL, 17 μmol) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl trichloroacetimidate **9a** (43 mg, 87 μmol) and **1** (92 mg, 43 μmol) and were reacted as above to yield 33% of the β-glycosylated product **11a** and 67% of acyl transfer product **12a**.

MPEGDOXyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside (11b)

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate (39) (**10b**) (40 mg, 54 μmol) and **1** (57 mg, 27 μmol) were reacted as for **11a** to yield 93% of the β-glycosylated product **11b** and 7% of acyl transfer product **3**. ^{1}H NMR (ppm) δ: 8.10 (d, 2H, J = 7.5 Hz, Bz_o), 8.05 (d, 2H, J = 7.5 Hz, Bz_o), 7.92 (d, 2H, J = 7.7 Hz, Bz_p), 7.78 (d, 2H, J = 7.7 Hz, Bz_p), 5.98 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 5.86 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 5.54 (dd, 1H, H-3), 4.29 (m, 1H, H-5).

MPEGDOXyl 3,4,6-tri-*O*-benzyl-2-*O*-formyl-β-D-galactopyranoside (14a)

TESOTf (12 μL, 53 μmol) was added to a solution of compound **13a** (0.169 g, 0.271 μmol) and **1** (0.275 g, 0.130 μmol) in dry CH_2Cl_2 (3 mL). After 3 h of stirring at room temperature, TLC (hexanes : ethyl acetate, 3:1) indicated absence of the imidate. The reaction was stopped by the addition of 2 drops of DIPEA and the volume was reduced to ~0.5 mL. Dry TBME (50 mL) was added and the polymer was allowed to precipitate at 4 °C overnight. The polymer (0.278 g) was then isolated by filtration. The ^{1}H NMR spectrum indicated that ~25% yield of the β-glycosylated product **14a**, ~4% yield of the orthoester, and 56% yield of acyl transfer product **15a** were obtained. **14a**: ^{1}H NMR (ppm) δ: 8.14 (s, 1H, HCOO), 5.10 (dd, 1H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 9.0 Hz, H-2), 3.96 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-4).

MPEGDOXyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (14b)

Compound **13b** (27 mg, 42 μmol) was treated as for **14a**. The ^{1}H NMR spectrum indicated that 64% yield of the β-glycosylated product **14b**, 13% yield of the orthoester, and 5% yield of acyl transfer product **15b** were obtained. **14b**: ^{1}H NMR (ppm) δ: 7.36–7.22 (m, aromatic), 5.43 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.37 (d, 1H, H-1), 3.94 (m, 1H, H-4), 1.99 (s, 3H, CH_3CO).

MPEGDOXyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside (14c)

Compound **13c** (47 mg, 67 μmol) was treated as for **14a**. The ^{1}H NMR spectrum indicated that 66% yield of the β-glycosylated product **14c**, 11% yield of the orthoester, and no detectable acyl transfer product were obtained. **14c**: ^{1}H NMR (ppm) δ: 7.98–7.96 (m, 2H, Bz_o), 7.59 (m, 1H, Bz_o), 7.47–7.43 (m, 2H, Bz_p), 5.71 (dd, 1H, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 4.00 (m, 1H, H-4). Orthoester: 5.25 (d, 1H, H-1).

MPEGDOXyl 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranoside (14d)

Compound **13d** (17 mg, 25 μmol) was treated as for **14a**. The ^{1}H NMR spectrum indicated that 47% yield of the β-glycosylated product **14d**, 14% yield of the orthoester, and no detectable acyl transfer product were obtained. **14d**: ^{1}H NMR (ppm) δ: 5.49 (dd, 1H, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 4.42 (d, 1H, H-1), 3.94 (m, 1H, H-4).

MPEGDOXyl 3,4,6-tri-*O*-benzyl-2-*O*-levulinoyl-β-D-galactopyranoside (14e)

Compound **13e** (23 mg, 33 μmol) was treated as for **14a**. The ^{1}H NMR spectrum indicated that 49% yield of the β-glycosylated product **14e**, 7% yield of the orthoester, and 2% yield of acyl transfer product **15e** were obtained. **14e**: ^{1}H NMR (ppm) δ: 5.42 (dd, 1H, $J_{2,1}$ = 7.9 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.93 (m, 1H, H-4), 2.71–2.66 (m, 2H, CH_2 Lev), 2.53–2.49 (m, 2H, CH_2 Lev).

Ethyl 2,6-di-*O*-(2,6-dimethylbenzoyl)-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (17)

2,6-Diol (27, 40) (**16**) (1.5 g, 5.71 mmol) was dissolved in THF (30 mL) and 2,6-dimethylbenzoic acid (3.43 g, 4 equiv.), dicyclohexylcarbodiimide (5.3 g, 4.5 equiv.), and DMAP (50 mg) were added. After stirring at room temperature (RT) for 1 day, the mixture was heated to 40 °C and the temperature maintained for 1 week. Each day, further aliquots of DCC (0.5 equiv.) and DMAP (50 mg) were added until the starting material disappeared. After filtration and concentration, the residue was purified by MPLC eluting with hexanes : ethyl acetate : dichloromethane (8:1:1) to yield slightly impure material, which was recrystallized from ethanol:water about 1:1 to form colorless needles of **17** (750 mg, 25%); mp 159 to 160 °C. $[\alpha]_D$ = 35.5 (*c* 0.84, $CHCl_3$). ^{1}H NMR (ppm) δ: 7.22 (m, 2H, Bz), 7.06 (m, 4H, Bz), 5.37 (brt, 1H, H-2), 4.65 (m, 2H, H-6ab), 4.45 (d, 1H, $J_{1,2}$ = 10.3 Hz, H-1), 4.31 (m, 2H, H-3, H-4), 4.17 (t, 1H, $J_{4,5+5,6}$ = 12.2 Hz, H-5), 2.78 (m, 2H, CH_3CH_2S), 2.43, 2.37 (2s, 12H, $ArCH_3$), 1.66, 1.39 (2s, 6H, $O_2C(CH_3)_2$), 1.28 (t, 3H, J = 7.3 Hz, CH_3CH_2S). ^{13}C NMR (ppm) δ: 169.6, 168.6 (2*C*OAr), 135.3, 135.1 ($2Bz_{ip}$), 133.4 ($2Bz_o$), 129.5, 129.4 ($2Bz_p$), 127.5, 127.5 ($2Bz_m$), 110.8 ($O_2C(CH_3)_2$), 82.2 (C-1),

77.2 (C-3), 74.3 (C-5), 73.8 (C-4), 71.6 (C-2), 64.1 (C-6), 27.1, 26.0 ($2O_2C(CH_3)_2$), 23.7 (CH_3CH_2S), 19.9, 19.8 ($2ArCH_3$), 14.9 (CH_3CH_2S). FAB-MS calcd. for $C_{29}H_{36}O_7S$: 528.2181; found *m/z*: 529.1 (m + H^+), 467.1 (M – Set^+) 133 (DiMeBz$^+$). HR-MS calcd. for $C_{29}H_{36}O_7SNa$: 551.2079; found: 551.2065.

MPEGDOXyl 2,6-di-*O*-(2,6-dimethylbenzoyl)-3,4-*O*-isopropylidene-β-D-galactopyranoside (18)

Method 1

Donor **17** (77 mg, 0.15 mmol), **1** (512 mg, 0.1 mmol), and activated 4 Å molecular sieves (about 300 mg) were dried together overnight at high vacuum. Dichloromethane (3 mL) was added and the mixture cooled in an ice–salt bath. After stirring for 0.5 h, NIS (112 mg, 0.5 mmol) was added followed by TfOH (12 μL, 0.14 mmol). After stirring for 30 min, the reaction was quenched with DIPEA (50 μL) and the polymer precipitated with TBME (40 mL). The solid was recovered by filtration and after rinsing with TBME was reprecipitated from absolute ethanol (25 mL). The resulting solid was collected by filtration and after washing with cold ethanol and diethyl ether was taken up in CH_2Cl_2, filtered, and evaporated to yield **18** (490 mg, 88%). Partial ^{1}H NMR (ppm) δ: 7.31 (m, 4H, Ar*H*-DOX), 7.24 (t, 1H, *J* = 7.8 Hz, Bz_p), 7.20 (t, 1H, *J* = 7.8 Hz, Bz_p), 7.08 (d, 2H, *J* = 7.8 Hz, Bz_m), 7.03 (d, 2H, *J* = 7.8 Hz, Bz_m), 5.37 (brt, 1H, H-2), 4.95 (d, 1H, *J* = 11.7 Hz, C*H*HC$_6$H$_4$-DOX), 4.70 (m, 1H, H-6a), 4.65 (m, 1H, H-6b), 4.58 (d, 1H, *J* = 11.7 Hz, CH*H*C$_6$H$_4$-DOX), 4.56 (s, 2H, CH_2C$_6$H$_4$-DOX), 4.54 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.29 (m, 2H, H-3, H-4), 4.17 (t, 1H, $J_{4,5+5,6}$ = 9.8 Hz, H-5), 2.39, 2.29 (2s, 12H, ArCH_3), 1.68, 1.40 (2s, 6H, $O_2C(CH_3)_2$).

Method 2

Orthoester **20** was treated as for **18** above except no NIS, extra donor, or **1** were added.

6-*O*-(2,6-Dimethylbenzoyl)-3,4-*O*-isopropylidene-β-D-galactopyranosyl-1,2-*O*-MPEGDOXyl-*ortho*-(2,6-dimethylbenzoate) (20)

Orthoester **20** was prepared as for **18** except 0.7 equiv. of TfOH was used. Partial ^{1}H NMR (ppm) δ: 5.80 (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1), 4.38 (m, 1H, H-2), 1.68, 1.40 (2s, 6H, $O_2C(CH_3)_2$).

[4-*O*-Acetoxymethyl]benzyl 2,6-di-*O*-(2,6-dimethylbenzoyl)-3,4-*O*-isopropylidene(-β-D-galactopyranoside (19)

Compound **18** (480 mg, 0.087 mmol) was dissolved in CH_2Cl_2 (2.0 mL) and Ac_2O (2.0 mL) under an atmosphere of argon at RT. To this solution was added $Sc(OTf)_3$ (25 mg, 0.02 mmol) and the stirring continued for 4 h. After cooling with an ice bath, the polymer was precipitated with TBME (60 mL) and collected by filtration. The filtrate was evaporated. The solid was reprecipitated from ethanol (40 mL) and collected by filtration. The filtrate was combined with the residue from TBME and evaporated. The residue was purified by preparative TLC eluting with hexanes : ethyl acetate (80:20) to yield a viscous oil **19** (12 mg, 22% assuming 100% yields for all polymer steps including linker attachment). $[\alpha]_D$ –75.0 (*c*, 0.06, $CHCl_3$). ^{1}H NMR (ppm) δ: 7.27 (q_{AB}, 4H, *J* = 8.1 Hz, Ar*H*-DOX), 7.22 (t, 1H, *J* = 7.3 Hz, *J* = 8.0 Hz, Bz_p), 7.18 (t, 1H, *J* = 7.3, *J* = 8.0 Hz, Bz_p), 7.06 (d, 2H, *J* = 8.0 Hz, Bz_m), 7.01 (d, 2H, *J* = 7.3 Hz, Bz_m), 5.39 (brt, 1H, H-2), 5.08 (s, 2H, CH_2C$_6$H$_4$-DOX), 4.94 (d, 1H, *J* = 11.7 Hz, C*H*HC$_6$H$_4$-DOX), 4.67 (m, 2H, H-6ab), 4.57 (d, 1H, *J* = 11.7 Hz, CH*H*C$_6$H$_4$-DOX), 4.52 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 4.28 (m, 2H, H-3, H-4), 4.15 (t, 1H, $J_{4,5+5,6}$ = 10.2 Hz, H-5), 2.36, 2.26 (2s, 12H, ArCH_3), 2.11 (s, 3H, COCH_3), 1.66, 1.38 (2s, 6H, $O_2C(CH_3)_2$). ^{13}C NMR (ppm) δ: 170.8 (*C*OCH$_3$), 169.6, 168.4 (2*C*OAr), 136.7, 135.5 ($2CH_2$-*C*Ar-DOX), 135.0, 135.1 ($2Bz_{ip}$), 133.6, 133.3 ($2Bz_o$), 129.6, 129.3 ($2Bz_p$), 128.2, 128.1 (*C*HAr-DOX), 127.6, 127.4 ($2Bz_m$), 110.9 ($O_2C(CH_3)_2$), 99.0 (C-1), 77.2 (C-3), 73.8 (C-4), 73.0 (C-2), 71.1 (C-5), 70.1 (CH_2C$_6$H$_4$), 66.0 (CH_2C$_6$H$_4$-DOX), 64.1 (C-6), 27.7, 26.6, ($2O_2C(CH_3)_2$), 21.0 (CO*C*H$_3$), 19.8, 19.6 ($2ArCH_3$). FAB-MS: +ve 669 (M + Na^+), 467 (M – DOX^+), 133 (DiMeBz$^+$). HR-MS calcd. for $C_{37}H_{42}O_{10}Na$: 669.2676; found: 669.2715.

Ethyl 2,6-di-*O*-(2,6-dimethoxylbenzoyl)-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (21)

Diester **21** was made from diol **16** as for **17** (750 mg, 25%). $[\alpha]_D$ = 18.2 (*c* 0.2, $CHCl_3$). ^{1}H NMR (ppm) δ: 7.27 (m, 2H, Bz), 6.53 (m, 4H, Bz), 5.34 (brt, 1H, *J* = 6 Hz, H-2), 4.73 (d, 1H, $J_{1,2}$ = 7.0 Hz, H-1), 4.57 (m, 2H, H-6ab), 4.41 (brt, 1H, *J* = 5.7 Hz, H-3), 4.31 (brd, 1H, $J_{3,4}$ = 4.5 Hz, H-4), 4.14 (t, 1H, $J_{4,5+5,6}$ = 12.2 Hz, H-5), 3.80, 3.76 (2s, 12H, ArOCH_3), 2.76 (m, 2H, CH_3CH_2S), 1.61, 1.39 (2s, 6H, $O_2C(CH_3)_2$), 1.27 (t, 3H, *J* = 7.3 Hz, CH_3CH_2S). ^{13}C NMR (ppm) δ:, 166.2, 164.7 (2*C*OAr), 157.6, 157.2 ($2Bz_o$), 131.3, 131.0 ($2Bz_p$), 112.8, 112.2 ($2Bz_{ip}$), 110.5 ($O_2C(CH_3)_2$), 103.9, 103.8 ($2Bz_m$), 82.6 (C-1), 75.6 (C-3), 73.2 (C-5), 72.8 (C-4), 72.3 (C-2), 64.0 (C-6), 56.0, 55.9 ($2ArOCH_3$), 27.8, 26.6 ($2O_2C(CH_3)_2$), 23.7 (CH_3CH_2S), 14.8 (CH_3CH_2S). FAB- MS calcd. for $C_{29}H_{36}O_{11}S$: 592; found *m/z*: 615.2 (M + Na^+), 531.2 (M – SEt^+), 165 (DiMeOBz$^+$). HR-MS calcd. for $C_{29}H_{36}O_{11}S$: 592.2115; found: 592.2128.

MPEGDOXyl 2,6-di-*O*-(2,6-dimethoxylbenzoyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (22)

Donor **21** was reacted with **1** as for **18** to yield **22** and **23**. **22**: partial ^{1}H NMR (ppm) δ: 7.32 (m, 2H, Bz), 7.30 (d, 2H, *J* = 8.1 Hz, Ar*H*-DOX), 7.22 (d, 2H, *J* = 8.1 Hz, Ar*H*-DOX), 6.54 (m, 4H, Bz), 5.27 (brt, 1H, *J* = 6.1 Hz, H-2), 4.93 (d, 1H, *J* = 12.0 Hz, C*H*H-DOX), 4.38 (brt, 1H, H-3), 4.30 (dd, 1H, $J_{3,4}$ = 5.8 Hz, $J_{4,5}$ = 1.9 Hz, H-4), 4.11 (brt, 1H, *J* = 4.4 Hz, H-5), 1.60, 1.36 (2s, 6H, $O_2C(CH_3)_2$).

MPEGDOXyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (24)

Diester **22** (98 mg, 0.017 mmol) was dissolved in 1 mol/L LiOH in methanol (2 mL) and heated at 50 °C for 16 h un-

der an atmosphere of argon. The heat was removed and the mixture cooled in an ice bath. The polymer was recovered by precipitation with TBME and filtration followed by dissolution in warm absolute ethanol, filtration while warm, and cooling. The resulting solid was collected by filtration and after rinsing with ethanol and diethyl ether was dissolved in CH_2Cl_2, filtered, and evaporated to yield **24** (90 mg, 97%). Partial ^{1}H NMR (ppm) δ: 7.32 (s, 4H, Ar*H*-DOX), 4.89 (d, 1H, J = 11.6 Hz, C*H*H-DOX), 4.25 (brt, 1H, H-3), 4.14 (dd, 1H, $J_{3,4}$ = 5.6 Hz, $J_{4,5}$ = 1.9 Hz, H-4), 4.06 (brt, 1H, J = 5.9 Hz, H-5), 3.97 (m, 1H, H-6a), 1.52, 1.34 (2s, 6H, $O_2C(CH_3)_2$).

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