Synthesis of a series of phenylacetic acid 1-β-*O*-acyl glucosides and comparison of their acyl migration and hydrolysis kinetics with the corresponding acyl glucuronides

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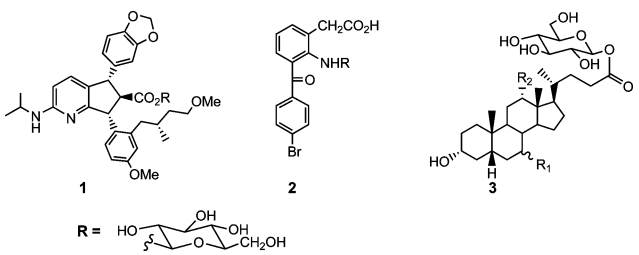
We report the synthesis of the 1-β-*O*-acyl glucoside conjugates of phenylacetic acid (PAA), R- and S-α-methyl-PAA and α,α′-dimethyl-PAA, and measurement of their transacylation and hydrolysis reactivity by NMR methods. These are analogues of acyl glucuronides, the transacylation kinetics of which could be important in adverse drug effects. One aim of this work was to investigate whether, as previously postulated, the free carboxylate group of the acyl glucuronides plays a part in the mechanism of the internal acyl migration. In addition, such acyl glucosides are known to be endogenous biochemicals in their own right and investigation of their acyl migration propensities is novel. Our previously described selective acylation procedure has proved highly successful for 1-β-*O*-acyl glucuronide synthesis and when subsequently applied to 6-*O*-trityl glucose, it gave good yields and excellent anomeric selectivity. Mild acidolysis of the *O*-trityl intermediates gave the desired acyl glucosides in excellent yield with essentially complete β-selectivity. Measurement of the acyl glucoside transacylation kinetics by ^{1}H NMR spectroscopy, based simply on the disappearance of the 1-β-isomer in aqueous buffer at pH 7.4, showed marked differences depending on the degree of methyl substitution. Further kinetic modelling of the isomerisation and hydrolysis reactions of the acyl glucosides showed considerable differences in kinetics for the various isomeric reactions. Reactions involving the –CH_2OH group, presumably *via* a 6-membered ring *ortho*-ester intermediate, are facile and the α-glucoside anomers are significantly more reactive than their β-counterparts. By comparison with degradation rates for the corresponding acyl glucuronides, it can be inferred that substitution of the carboxylate by –CH_2OH in the acyl glucosides has a significant effect on acyl migration for those compounds, especially for rapidly transacylating molecules, and that thus the charged glucuronide carboxylate is a factor in the kinetics.

INTRODUCTION

Although xenobiotic Phase 2 mammalian metabolic conjugation generally involves the formation of 1-β-D-glucuronides,[1] a significant number of glucoside metabolites have also been identified, including both *O*- and *N*-linked types. Examples include the *O*-acyl glucoside of a selective endothelin ET_A antagonist, [(+)-(5*S*,6*R*,7*R*)-2-isopropylamino-7-[4-methoxy-2-((2*R*)-3-methoxy-2-methylpropyl)-5-(3,4-methylenedioxyphenyl) cyclopenteno [1,2-*b*] pyridine 6-carboxylic acid][2] **1** and the *N*-glucoside of bromfenac[3] **2**, as well as sulfonamides,[4] 5-aminosalicylic acid[5] and 4-bromoaniline.[6] Interestingly a number of the *N*-glucosides found in biological samples exist as mixtures of both α– and β–anomers[6] and may owe their existence to simple chemical, rather than biochemical, reactions in the body. Endogenous mammalian glucoside metabolites, notably *O*-acyl glucosides (and galactosides) of bile acids such as compounds **3** (R_1, R_2 = H, OH) are also known.[7,8]



As with acyl glucuronides,[9,10] acyl glucosides are initially formed as 1-β-esters as a consequence of the biosynthetic pathway. By analogy with the corresponding glucuronides, it is anticipated that transacylation and acyl migration of the acyl group in these reactive metabolites will also take place (Scheme 1). Hydrolysis of all of the isomers to the aglycone and glucose is also possible. Similar effects have also recently been shown using NMR

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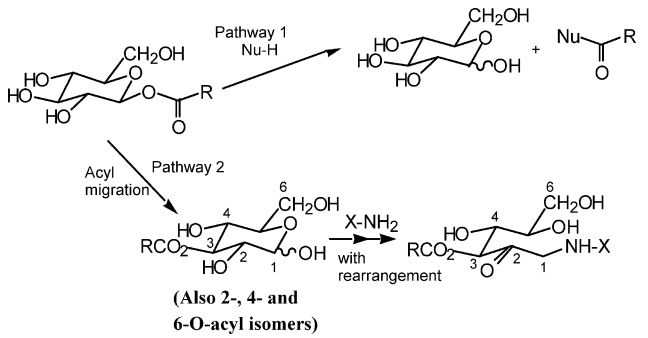
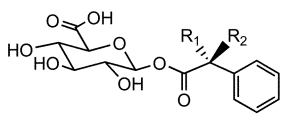


Scheme 1 Reactivity pathways of acyl glucosides by analogy with acyl glucuronides.

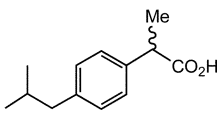
spectroscopy for acyl galactosides with the anomeric position blocked as a galactoside.[11] Clearly, for the normal hexopyranoses, migration to the $–CH_2OH$ group is also feasible, and in this case the cyclic *ortho*-ester intermediate will be a 6-membered ring and not a 5-membered ring as for all other transacylation reactions.[9] In their recent studies of acyl glucuronides[12] Baba and Yoshioka included some acyl glucoside examples which were prepared from fully benzylated glucose precursors. Other groups have reported syntheses from fully protected intermediates.[13]

The question of possible toxicity of acyl glucuronides has been much debated and is clearly of importance in the assessment of the safety of drugs such as ibuprofen,[14] mycophenolic acid[15] and diclofenac[16] which are extensively metabolised by glucuronidation, followed by acyl migration as in Scheme 1 (with $–CO_2^-$ replacing $–CH_2OH$ in the case of those drugs). It has been surmised that the transacylated positional isomers formed from 1-β-acyl glucuronides can react *via* Schiff base formation with endogenous proteins and hence cause toxicological and immunological adverse effects.[9,10]

We have probed the reactivity of the acyl glucuronides of phenylacetic acid and its derivatives with various degrees of α-methyl substitution (**4**–**7**) using NMR analysis of the time course of acyl migration. Additionally, using DFT-MO calculations of the transition states of the acyl migration step we inferred some structure-reactivity correlations and further elucidated the mechanism.[17]



4 $R_1 = R_2 = H$
5 R_1 = Me, R_2= H (R)
6 R_1 = H, R_2 = Me (S)
7 $R_1 = R_2$ = Me



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These results bear directly on the behaviour of ibuprofen-like NSAIDs (arylpropionates), where the structure-reactivity of their acyl glucuronides is more complex than for benzoic acids.[18–20] Interestingly, for the arylpropionate series, these results have already shown that both aryl substitution [even by a remote isobutyl group, as in ibuprofen (**8**)] and esterification of the carboxylate moiety of the glucuronic acid have significant effects on the degradation rates.[14]

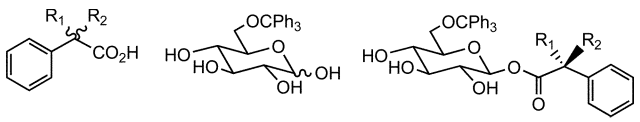
It was therefore of considerable interest to synthesise acyl glucosides and compare their reaction kinetics with those of acyl glucuronides. In this way, the aim was both to extend the understanding of the structure-reactivity relationships of the compounds, varying here the carbohydrate rather than the aglycone, and to extend the selective acylation method to other carbohydrates. In particular, the replacement of the $–COO^-$ moiety of the glucuronides with a neutral $-CH_2OH$ moiety could shed light on the potential involvement of the $–COO^-$ group in the transacylation and hydrolysis reactivity.

We have also extended the kinetic analysis by considering the full reaction scheme that involves transacylation, mutarotation and hydrolysis of all isomers. By computer modelling of these reactions, a highly detailed view of the reactivity can be determined.

EXPERIMENTAL METHODS

Synthesis

In our earlier studies[17] we showed that glucuronate monoesters were ideal substrates for selective 1-β-acylation, without the need for global protection. By analogy, we considered that a 6-protected glucose derivative would be a suitable, minimally-protected substrate and indeed 6-*O*-trityl glucose proved ideal. The more reactive primary alcohol is protected and the molecule is made soluble in organic media. Furthermore, the product ester is anticipated to have good stability under the mildly acidic conditions needed for detritylation.[21] When phenylacetic acid **9** was reacted with 6-*O*-trityl glucose **10** under previously defined conditions[22,23] (HATU, NMM, MeCN, 20 °C) a steady reaction occurred and after chromatography the product **11** was obtained as the β-anomer only in 55% yield. The racemic α–monomethyl acid **12** was likewise coupled and following diastereomer separation, the products **13** and **14** were obtained in similar yields, again as single β-anomers. We have assumed that, as in the acyl glucuronide series,[17] the (2*R*)-diastereoisomer is the less polar and this is consistent with its faster acyl migration, *vide infra*. Finally, the α,α–dimethyl acid **15** was also successfully reacted, giving **16** in 49% yield unoptimised.

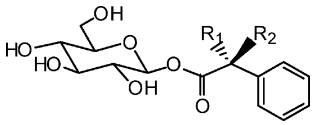


9 $R_1 = R_2 = H$
12 R_1 = Me, R_2= H (R, S)
15 $R_1 = R_2$ = Me

10

11 $R_1 = R_2 = H$
13 R_1 = Me, R_2 = H
14 R_1 = H, R_2 = Me
16 $R_1 = R_2$ = Me

Deprotection of the formed *O*-trityl adducts was conveniently achieved by brief acidolysis (TFA in a 5 : 1 mixture of CH_2Cl_2 and water: *cf.* the deprotection of PMB glucuronates[17]) to deliver the free acyl glucosides **17** to **20** in >88% yield in each case. All compounds were obtained in excellent purity although **20** was rather hygroscopic.



17 $R_1 = R_2 = H$

18 $R_1 = Me$, $R_2 = H$ (R)

19 $R_1 = H$, $R_2 = Me$ (S)

20 $R_1 = R_2 = Me$

RESULTS AND DISCUSSION

Assignment of ^{1}H NMR spectra

The assignment of the ^{1}H NMR peaks to the various hydrogens of each isomer follows from previous studies on acyl glucuronides, bearing in mind that the aglycone structure has little effect on the carbohydrate moiety chemical shifts.[24] In particular the chemical shifts of the 1-β isomers can be made by inspection based on spin-coupling patterns, and the use of a ^{1}H-^{1}H COSY spectrum. Selected assignments of anomeric protons and protons adjacent to the *O*-acyl group are given in Table 1, and also shown on Fig. 1. In particular the chemical shifts of the C6 protons in the 6-*O*-acyl isomers are determined as these are used to monitor the kinetics of the 4-*O*-acyl to 6-*O*-acyl reversible reactions, and hydrolysis of the 6-*O*-acyl isomers. The intensities of these peaks were then used for the subsequent kinetic analysis.

Table 1 ^{1}H NMR chemical shifts (δ, ppm) of selected protons of the isomers of the acyl glucoside compounds **17–20** obtained from ^{1}H NMR analysis of the equilibrium reaction mixtures

Positional isomer[a]	Proton	**17**[b]	**18**[b]	**19**[b]	**20**[b]
1-β	H1	5.59 (d)	5.56(d)	5.57(d)	5.57 (d)
1-α	H1	6.12 (d)	6.08(d)	6.11(d)	6.09 (d)
2-β	H1	4.79 (d)	—	4.81(d)	—
2-α	H1	5.36 (d)	5.25(d)	5.36 (d)	5.33 (d)
3-β	H3	5.00 (t)	4.99(t)	4.98 (t)	5.00 (t)
3-α	H3	5.20 (t)	5.18(t)	5.18 (t)	5.19 (t)
4-β	H1	5.17 (d)	—	—	—
4-α	H1	—	5.05(d)	5.14 (d)	—
6-β	H6	4.47 (dd)	4.50 (dd)	4.42 (dd)	—
	H6′	4.30 (dd)	4.26 (dd)	4.30 (dd)	
6-α	H6	4.46 (dd)	4.47 (dd)	4.37 (dd)	—
	H6′	4.34 (dd)	4.26 (dd)	4.35 (m)	
Glucose	H1 (α-isomer)	5.24 (d)	—	—	—
Aglycone methyl	CH_3	—	1.42 (d)	1.41 (d)	1.40 (2s)[c]

[a] 1-β denotes the 1-β-*O*-acyl-glucoside, and the other isomers are labeled by analogy. [b] d – doublet, dd – doublet of doublets: t – triplet; m – multiplet. [c] Methyl groups non-equivalent, two singlets

1-β- acyl glucoside degradation rates

The half-life degradation constants of **17–20** as measured by ^{1}H NMR spectroscopy in phosphate buffer at pH 7.4 are given in Table 2, where they are compared with the values for the

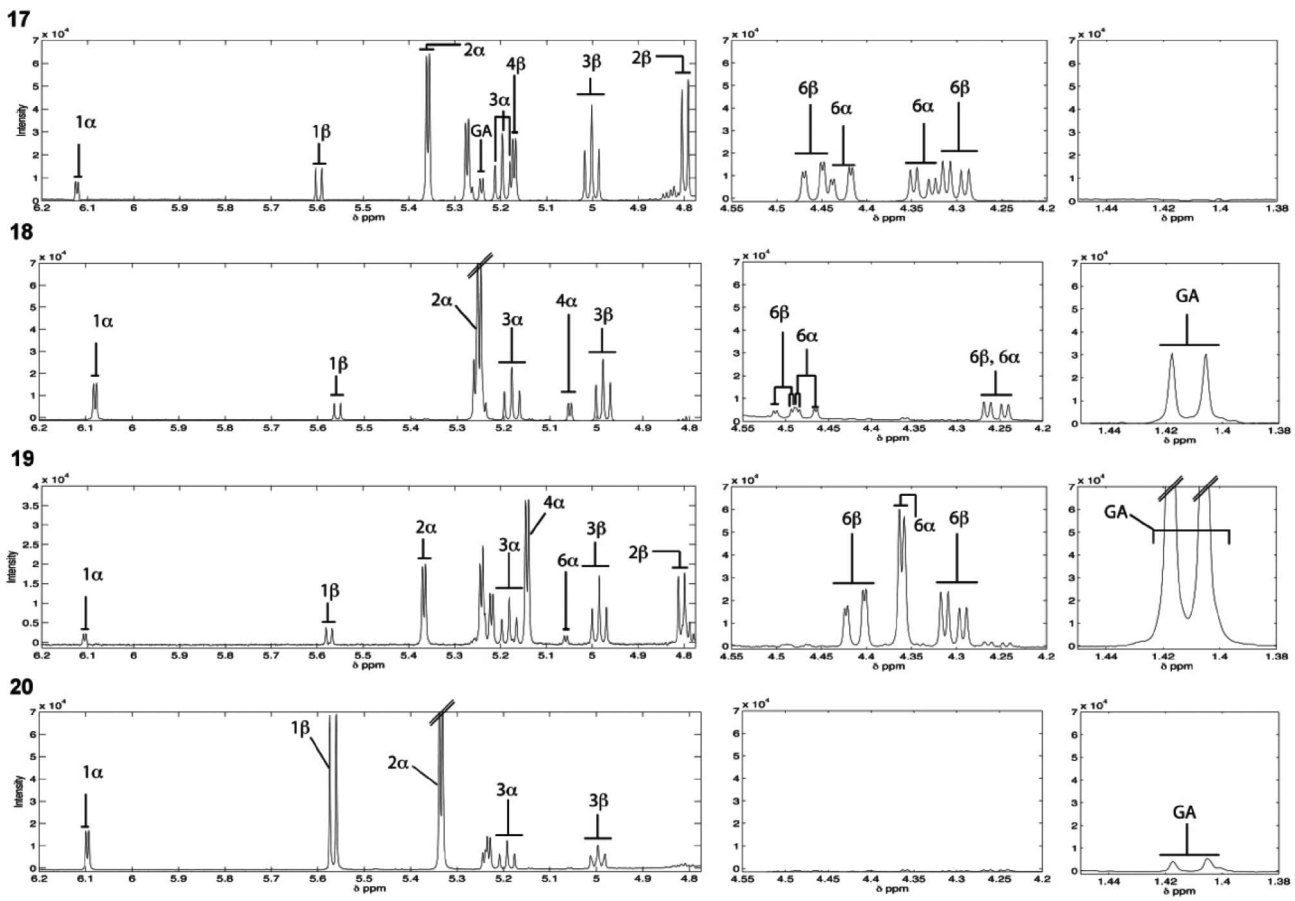


Fig. 1 600 MHz ^{1}H NMR spectra of an equilibrium mixture following the degradation of the acyl glucosides **17–20**. The assignments are as shown.

Table 2 Degradation rate constants and half-lives of 1-β-*O*-acyl glucuronides (**4–7**) and 1-β-*O*-acyl glucosides (**17–20**)

	Compound	k_d/h^{-1}[a]	$t_{1/2}$/h
Acyl glucuronides	**4**	2.353	0.29
	5	0.903	0.78
	6	0.405	1.71
	7	0.029	23.3
Acyl glucosides	**17**	1.419	0.49
	18	0.782	0.89
	19	0.476	1.46
	20	0.043	16

[a] The rate constant is a composite value reflecting direct hydrolysis and acyl migration; under the conditions employed, direct hydrolysis accounts for <10% of the loss of the initial 1-β-isomer.[17] The standard error for the least squares linear fit to the slope are all 0.003 or less.

corresponding acyl glucuronides.[17] The linearity of the data fits can be seen in Fig. 2. It will be seen immediately that the sets of values are overall quite similar, with increasing bulk at the α-position slowing the degradation and, as found in other series of compounds, the (2*R*) compound **18** reacting about twice as fast as the (2*S*) compound **19**. In addition there are some other interesting observations to be made. The degradation rate constants in the two series are not linearly related and thus there is a differential effect of the structural difference between a glucoside and the glucuronide as a function of reactivity. For the unsubstituted and hence fastest reacting compounds, the acyl glucoside has a half life around 65% longer that the glucuronide, clearly implying that the presence of the carboxylate function increases the reactivity, and this is in agreement with our previous finding in the glucuronide series in cases where the carboxylate function had been esterified.[14] Specifically, the ethyl and allyl esters of the1β-acyl glucuronide of (*S*)-ibuprofen had $t_{1/2}$ = 7.2 and 9.4 h respectively compared to $t_{1/2}$ = 3.8 h for the parent acyl glucuronide.[14] For the slowest reacting α,α′-dimethyl compounds where the half lives are many hours, the reverse was observed; now the glucuronide has the slower degradation kinetics. The carbohydrate structural effect is thus

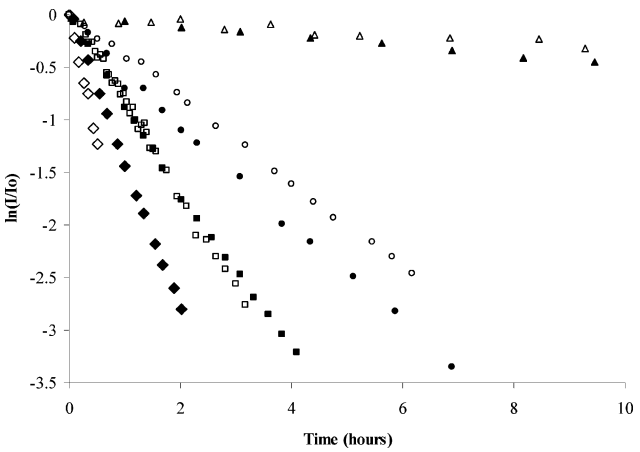


Fig. 2 The degradation of the 1-β- isomers of the acyl glucosides and glucuronides as a function of time. The various compounds are identified by number. Key to compound identification: ◇ - **4**; □ - **5**; ○ - **6**; △ -**7**; ◆ - **17**; ■ - **18**; ● - **19**; ▲ - **20**.

subtle and is less than that caused by relatively small changes in the aglycone, as observed by us and others.[12,17,20,25]

It is clear that, in the case of the glucuronides, carboxylate is not acting as a general base because the rate is concentration-independent as we noted previously.[17] In the case of the glucosides, no plausible general acid or base catalysis mechanism could be proposed involving another molecule. The direct comparison between glucuronide carboxylate (charged) and glucoside (neutral) is therefore important. It would appear from our results that it is the transacylation step that is relatively accelerated for CO_2^-, rather than direct hydrolysis, and this can hardly be attributed to a steric effect. We return to this point below.

Based on the appearance of hydrolysis products in the NMR spectra, the proportion of the reaction that can be attributed to hydrolysis varies only slightly with methyl substitution, being about 15%, 4%, 12% and 5% for **17–20** respectively.

Full kinetic modelling

The average rate constants, obtained through sequential perturbation of the best fit rate constants, with a *k* value start estimate of 1 h^{-1}, for each acyl migration reaction for each acyl glucoside compound, **17–20**, are presented in Table 3. A plot of the best-fit calculated intensities overlaid on the experimental ^{1}H NMR-determined concentration curves for acyl migration of the acyl glucoside compound **17** is shown in Fig. 3 for each migratory step. For certain isomers of some of the compounds, high fitting errors caused by a low abundance of a given isomer at a particular time point means that not all rate constants can be determined reliably.

It should be noted that there is good agreement between the full kinetic analysis and the simple degradation rates in those cases where both transacylation and hydrolysis can be modeled. For example, for the combined 1-β to 2-β transacylation and hydrolysis of the 1-β isomer, the rate constant is 1.42 h^{-1} from the simple degradation measurement and 1.46 h^{-1} by combining the rate constants from the detailed kinetic modeling (see Tables 3 and 4).

Other points to note are the generally more facile migration involving the primary alcohol because of the formation of a 6-membered, *trans*-decalin like ring intermediate, and the very fast formation of the 2-α isomer from the 1-α isomer (whilst noting the faster rate also for the reverse reaction), but of course only once the 2-β isomer has been formed and allowed to mutarotate to the 2-α form. In general the 1-α isomers all show rapid reaction rates, faster than their corresponding 1-β isomers. This can be attributed to the well-known *exo*-anomeric effect.[26] Additionally, for 1α→2 migration and the reverse reaction the transition state involves *cis*-fused five and six membered rings. In the case of 1β→2 migration, the transition state involves *trans* fusion and this requires higher activation energy. In Scheme 3 we summarise the three classes of transition state alluded to above; rearrangements between the 2 and 3, or 3 and 4 hydroxyls are essentially similar to the 1β→2 case, involving *trans*-6,5-fused rings.

It is also instructive to contrast our results with those for the series of acyl galactosides, referred to above,[11] where the anomeric position was permanently blocked as a β-benzyl galactoside. Although the authors do not comment in detail on the transition states involved, it is noticeable that at a pD = 8.0 (their closest value to our pH of 7.4) the fastest migrations of acetyl and

Table 3 Average rate constants (h^{-1}) for acyl migration of 1-β-*O*-acyl glucoside compounds (**17**–**20**)and their positional isomers calculated based on the kinetic model given in the Experimental Methods section and Scheme 2

	17		**18**		**19**		**20**	
Reaction	k ($\times 10^2$)	s.d.	k ($\times 10^2$)	s.d.	k ($\times 10^2$)	s.d.	k ($\times 10^2$)	s.d.
1β→2β	141.5	0.0	75.1	4.6	47.3	1.0	*	*
2β→3β	120.2	0.4	80.0	4.3	40.3	1.7	4.2	0.1
3β→2β	65.8	18.9	114.6	18.5	46.4	9.7	*	*
3β→4β	94.5	20.0	62.1	13.9	99.4	3.4	155.0	88.7
4β →3β	17.4	16.4	57.3	28.9	853.8	136.9	*	*
4β→6β	145.8	75.8	**	**	125.9	6.4	*	*
6β→4β	133.2	74.7	326.1	302.5	*	*	106.6	49.4
1α→2α	974.4	88.4	667.9	107.6	935.0	167.2	*	*
2α→1α	153.4	15.4	173.7	17.0	134.3	11.5	*	*
2α→3α	64.7	3.8	36.2	6.2	12.4	4.9	*	
3α→2α	48.8	17.9	51.8	12.1	*	*	*	*
3α→4α	70.1	20.3	65.3	16.9	22.3	1.5	*	*
4α→3α	58.3	52.4	*	*	*	*	*	*
4α→6α	692.7	248.6	663.4	140.0	936.8	130.8	*	*
6α→4α	*	*	747.8	133.9	519.7	125.6	*	*

Due to the fast rates of the mutarotation reactions, these were not modelled and fixed fast values were used with appropriate ratios of values based on the observed proportions of the various anomers. Isomer identification as for Table 2; * - denotes large uncertainty in the determination of k values; ** - denotes k values approximating zero

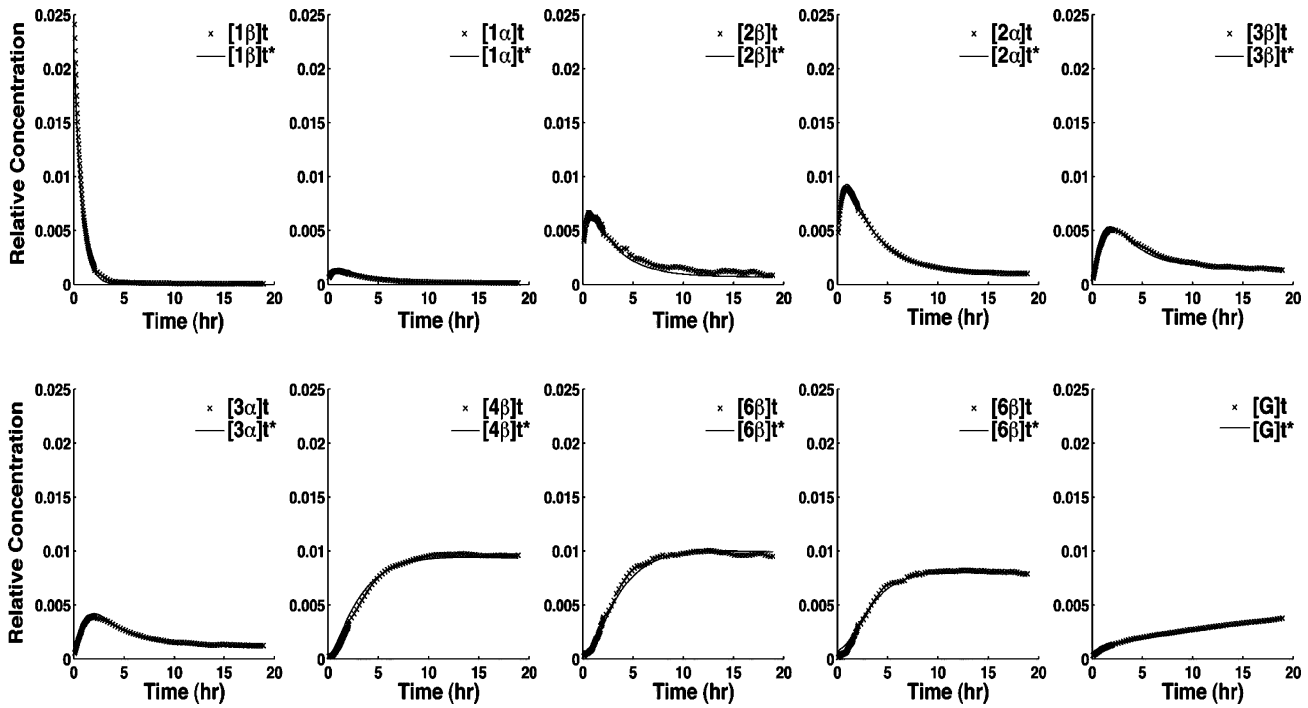


Fig. 3 Full kinetic modelling of [**17**]. The dashed lines are the peak areas (relative concentrations) of positional isomers observed from the NMR profiles. The solid lines are the peak areas (relative concentrations) predicted from the best-fit kinetic model.

benzoyl groups were to and from the 4-OH group, which is axial in galactose. Here again, *cis*-fused 5 and 6-membered rings are involved. Here, however, migrations to and from the anomeric position could not be observed and we suggest the particularly high value of k for the 1α→2 migration in our compounds reflects both the favourable *cis*-ring fusion in the transition state and the expulsion of a better leaving group, the anomeric OH being a significantly stronger acid.

In cases where the ester is no longer at O(1), the acyl migration rate differences between α/β hemiacetals are less predictable. For 2→3 and 3→4 rearrangement (forward or reverse) the differences are minor, but migration from 4→6 again shows a much higher rate for the α-anomers. Clearly the reaction intermediate's greater ring size has an effect on this particular migration but the stereoelectronic implications are not clear: also the standard deviations are much higher for these cases compared to those between O(1) and O(2).

Given the good agreement between the full analysis and the simple degradation measurement, it is possible to interpret the latter with some confidence. Thus, comparing compounds **4**–**7**

$$\frac{d[1\beta]}{dt} = -\left(k_{1\beta-2\beta} + k_{1\beta-G}\right)[1\beta]$$

$$\frac{d[1\alpha]}{dt} = -\left(k_{1\alpha-2\alpha} + k_{1\alpha-G}\right)[1\alpha] + k_{2\alpha-1\alpha}[2\alpha]$$

$$\frac{d[2\beta]}{dt} = k_{1\beta-2\beta}[1\beta] - \left(k_{2\beta-3\beta} + k_{2\beta-2\alpha} + k_{2\beta-G}\right)[2\beta] + k_{2\alpha-2\beta}[2\alpha] + k_{3\beta-2\beta}[3\beta]$$

$$\frac{d[2\alpha]}{dt} = k_{1\alpha-2\alpha}[1\alpha] + k_{2\beta-2\alpha}[2\beta] - \left(k_{2\alpha-1\alpha} + k_{2\alpha-3\alpha} + k_{2\alpha-2\beta} + k_{2\alpha-G}\right)[2\alpha] + k_{3\alpha-2\alpha}[3\alpha]$$

$$\frac{d[3\beta]}{dt} = k_{2\beta-3\beta}[2\beta] - \left(k_{3\beta-2\beta} + k_{3\beta-4\beta} + k_{3\beta-3\alpha} + k_{3\beta-G}\right)[3\beta] + k_{3\alpha-3\beta}[3\alpha] + k_{4\beta-3\beta}[4\beta]$$

$$\frac{d[3\alpha]}{dt} = k_{2\alpha-3\alpha}[2\alpha] + k_{3\beta-3\alpha}[3\beta] - \left(k_{3\alpha-2\alpha} + k_{3\alpha-4\alpha} + k_{3\alpha-3\beta} + k_{3\alpha-G}\right)[3\alpha] + k_{4\alpha-3\alpha}[4\alpha]$$

$$\frac{d[4\beta]}{dt} = k_{3\beta-4\beta}[3\beta] - \left(k_{4\beta-3\beta} + k_{4\beta-4\alpha} + k_{4\beta-6\beta} + k_{4\beta-G}\right)[4\beta] + k_{4\alpha-4\beta}[4\alpha] + k_{6\beta-4\beta}[6\beta]$$

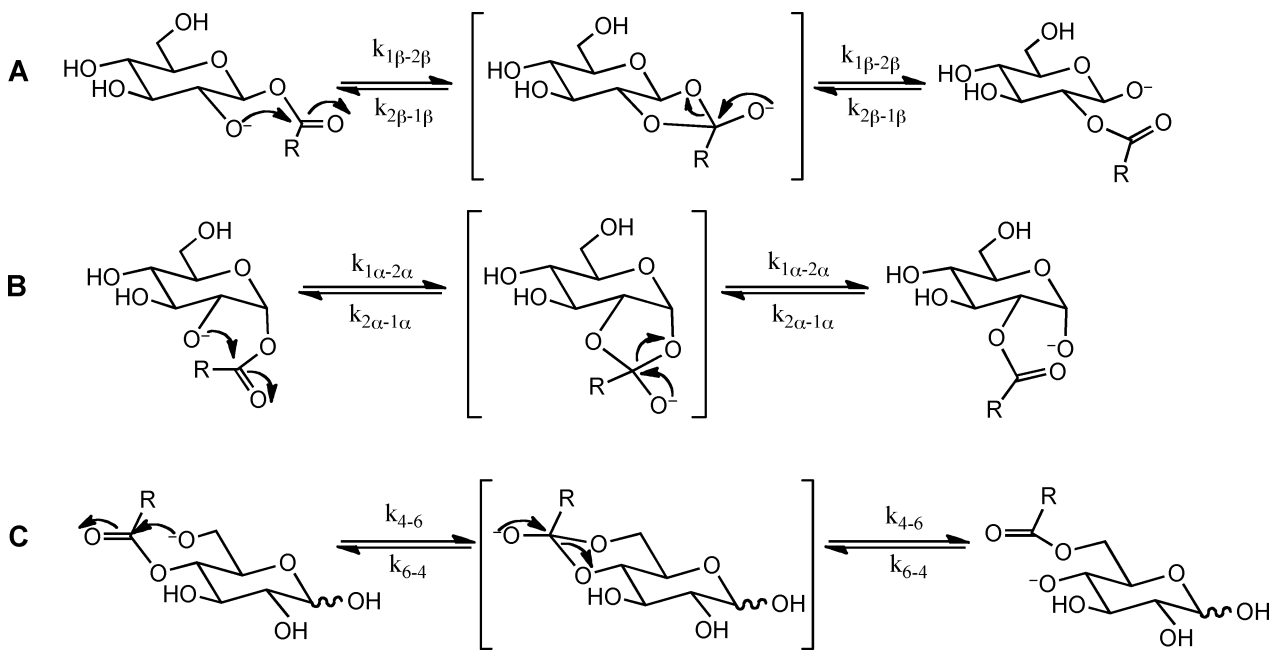
$$\frac{d[4\alpha]}{dt} = k_{3\alpha-4\alpha}[3\alpha] + k_{4\beta-4\alpha}[4\beta] - \left(k_{4\alpha-3\alpha} + k_{4\alpha-6\alpha} + k_{4\alpha-4\beta} + k_{4\alpha-G}\right)[4\alpha] + k_{6\alpha-4\alpha}[6\alpha]$$

$$\frac{d[6\beta]}{dt} = k_{4\beta-6\beta}[4\beta] - \left(k_{6\beta-4\beta} + k_{6\beta-6\alpha} + k_{6\beta-G}\right)[6\beta] + k_{6\alpha-6\beta}[6\alpha]$$

$$\frac{d[6\alpha]}{dt} = k_{4\alpha-6\alpha}[4\alpha] + k_{6\beta-6\alpha}[6\beta] - \left(k_{6\alpha-4\alpha} + k_{6\alpha-6\beta} + k_{6\alpha-G}\right)[6\alpha]$$

$$\frac{d[G]}{dt} = k_{1\beta-G}[1\beta] + k_{1\alpha-G}[1\alpha] + k_{2\beta-G}[2\beta] + k_{2\alpha-G}[2\alpha] + k_{3\beta-G}[3\beta] + k_{3\alpha-G}[3\alpha] + k_{4\beta-G}[4\beta] + k_{4\alpha-G}[4\alpha] + k_{6\beta-G}[6\beta] + k_{6\alpha-G}[6\alpha]$$

Scheme 2 The differential equations describing the various reaction rates (transacylation, mutarotation and hydrolysis) of the acyl glucosides. Here [1β], for example, denotes the concentration of the β-1-*O*-acyl glucoside isomer, G denotes free glucose, a hydrolysis product, and $k_{1\beta\text{-}2\beta}$ is, for example, the reaction rate constant for the internal transacylation reaction where the acyl group is transferred from the 1-β position to the 2-β position of the glucose moiety.



Scheme 3 The three classes of transition states appropriate to the acyl migration steps: 1β to 2 [*trans*-6,5-fused rings] **A**, 1α to 2 [*cis*-6,5-fused rings] **B**, and 4 to 6 [*trans*-decalin like] **C**. In **C** the anomeric configuration may be α or β.

Table 4 Selected average rate constants (h^{-1}) for hydrolysis of acyl glucoside compounds **17–19** and their positional isomers calculated based on the kinetic model given in the Experimental Methods and Scheme 2; s. d. = standard deviation. For compound **20** the rate (not shown) was very slow

	17		**18**		**19**	
	k (*10^2)	s.d.	k (*10^2)	s.d.	k (*10^2)	s.d.
1β	4.6	3.1	3.9	0.4	6.0	0.1
1α	—*	—*	53.2	10.8	88.9	6.8
6α	0.6	0.5	—*	—*	—*	—*

* denotes large uncertainty in the determination of k values.

with **17–20**, removal of the carboxylate function causes the overall reaction to slow considerably for the fast reacting compounds but to speed up for the slower reacting compounds. This can be interpreted as the loss of the carboxylate causing a slowing of the transacylation but having no effect on hydrolysis. A similar effect had been observed previously for the acyl glucuronide of ibuprofen where, if the free carboxylate is esterified, then the transacylation also slows.[14] However, in that case, it was not possible to exclude a possible steric as opposed to electronic effect on the reaction rate. In the acyl glucoside series, it is highly unlikely that a steric effect could be responsible.

Some selected hydrolysis rates could be extracted from the kinetic analyses, but in many cases the slow reactions meant that there was no significant variation in the concentration of the hydrolysis products over time. The determined values are given in Table 4. It can be seen that the hydrolysis rates for the various 1-β isomers are comparable (except for compound **20**, where the reaction was very slow and a value could not be measured). The hydrolysis rates of the 1-α isomers are considerably faster.

In summary, therefore, this study has shown that acyl glucoside conjugates of a series of phenylacetic acids with varying α-methylation can undergo acyl migration at rates comparable to their related acyl glucuronides. The reaction rate constants for a glucoside and a glucuronide of a given aglycone show marked differences and this implicates the carboxylate group of the glucuronic acid in the transacylation mechanism.

Experimental

Synthetic intermediates were purchased from Sigma–Aldrich group and used as supplied; the preparation of the known compound **10** is referenced. All organic solvents were anhydrous and of AR grade. Moisture sensitive reactions were carried out in anhydrous organic solvents, under a nitrogen atmosphere. Vacuum rotary evaporation was carried out at <35 °C. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX 400 spectrometer (at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectroscopy). Chemical shifts (δ) are given in ppm from internal tetramethylsilane (TMS) and coupling constants (J) are quoted in Hertz (Hz). Mass spectrometry was carried out on a VG analytical 7070E machine, using electron ionisation (EI). A Micromass LCT spectrometer was used for HRMS in the electrospray mode, and elemental analysis was performed using a Thermo Flash EA1112 analyzer, configured for automated elemental analysis. Thin layer chromatography was carried out using Merck 5 × 2 cm aluminium backed plates with a 0.2 mm layer of Kieselgel 60 F_{254}. They were visualised by UV (254 nm) and anisaldehyde stain where appropriate. Flash column chromatography was carried out using ICN (230–400 mesh) silica gel. Optical rotations ($[\alpha]_D$) were carried out on a PerkinElmer polarimeter 343 at a wavelength of 598 nm at 20 °C. Solvents and concentrations used were as quoted.

Acylation of 6-*O*-trityl glucose 10: General Method

To a solution of 6-*O*-trityl glucose **10**[27] (0.71 mmol) in MeCN (8 ml) was added HATU (0.78 mmol), and the respective carboxylic acid (0.78 mmol). The reaction was then flushed with N_2, then NMM (1.42 mmol) was added while stirring at 20 °C. After 4 h, the reaction was quenched by addition of Amberlite H^+ resin (1.42 mmol) and stirred for 30 min. The resin was filtered off and washed with MeCN, then the solvent was removed *in vacuo* and the residue purified by column chromatography using 5% EtOH–DCM. The α-dimethyl substituted carboxylic acid **15** required a longer reaction time (16 h).

6-*O*-Trityl 1-(2-phenyl)-acetyl-β-D-glucopyranose 11

Yield: 55%. Found: C, 72.9; H, 6.1; $[M+Na]^+$, m/z 563.205. $C_{33}H_{32}O_7$ requires C, 73.3; H, 6.0%; $C_{33}H_{32}O_7Na$ requires m/z 563.204; ^{1}H NMR δ_H [CD_3CN, 400 MHz]: 3.12–3.16 (1H, dd, 6-H, J = 10.2, 5.7 Hz), 3.22–3.25 (1H, dd, 6-H′, J = 10.2, 2.0 Hz), 3.34–3.38 (2H, m, 2-H, 4-H), 3.53-3.55 (2H, m, 3-H, 5-H), 3.77 (2H, s, CH_2Ph), 5.49–5.51 (1H, d, 1-H, J = 7.8 Hz), 7.22–7.34 (14H, m, ArH), 7.41–7.44 (6H, m, ArH); ^{13}C NMR δ_C [CD_3CN, 100 MHz]: 17.4, 40.1, 56.6, 62.8, 69.5, 72.2, 75.8, 76.2, 85.8, 95.3, 126.7, 127.4, 128.2, 129.1, 133.6, 143.7, 170.1; m/z (ES +ve ion mode) 563 ($[M+Na]^+$, 100%).

6-*O*-Trityl 1-([(2*R*)-phenyl]propanoyl)-β-D-glucopyranose 13

Yield: 53%. Found: C, 70.6; H, 6.6; $[M+Na]^+$, m/z 577.220. $C_{34}H_{34}O_7{\cdot}H_2O$ requires C, 71.3; H, 6.3%; $C_{34}H_{34}O_7Na$ requires m/z 577.220; ^{1}H NMR δ_H [$(CD_3)_2CO$, 400 MHz]: 1.54–1.56 (3H, d, CH(CH_3), J = 7.2 Hz), 3.24–3.28 (1H, dd, 6-H′, J = 9.9, 4.2 Hz), 3.38–3.41 (1H, dd, 6-H, J = 9.9, 2.0 Hz), 3.42–3.50 (2H, m, 2-H, 4-H), 3.57–3.64 (2H, m, 3-H, 5-H), 3.87–3.93 (1H, q, CH(CH_3)), 4.23 (1H, m, OH), 4.46 (1H, d, OH), 4.49 (1H, m, OH), 5.58–5.59 (1H, d, 1-H, J = 7.8 Hz), 7.21–7.39 (12H, m, ArH), 7.40–7.41 (2H, m, ArH), 7.50–7.52 (6H, m, ArH); ^{13}C NMR δ_C [$(CD_3)_2CO$, 100 MHz]: 19.9, 46.5, 64.7, 71.7, 74.2, 77.7, 78.7, 87.4, 96.2, 128.2, 128.2, 128.9, 128.9, 129.8, 130.1, 141.8, 145.6, 174.2; m/z (ES +ve ion mode) 577 ($[M+Na]^+$,100%).

6-*O*-Trityl 1-([(2*S*)-phenyl]propanoyl)-β-D-glucopyranose 14

Yield: 53%. ^{1}H NMR δ_H [$(CD_3)_2CO$, 400 MHz]: 1.49–1.50 (3H, d, CH(CH_3) J = 7.1 Hz), 3.16–3.20 (1H, dd, 6-H′, J = 9.9, 5.9 Hz), 3.34–3.37 (1H, dd, 6-H, J = 9.9, 2.0 Hz), 3.38–3.50 (2H, m, 2-H, 4-H), 3.55–3.62 (2H, m, 3-H, 5-H), 3.88–3.94 (1H, q, CH(CH_3)), 5.60–5.62 (1H, d, 1-H, J = 7.8 Hz), 7.20–7.38 (12H, m, ArH), 7.40–7.41 (2H, m, ArH), 7.50–7.52 (6H, m, ArH); ^{13}C NMR δ_C [$(CD_3)_2CO$, 100 MHz]: 20.1, 46.4, 64.6, 71.6, 74.2, 77.7, 78.6, 87.3, 96.2, 128.1, 128.2, 128.8, 128.9, 129.9, 130.0, 142.0, 145.6, 174.1.

6-*O*-Trityl 1-(2,2-dimethylphenyl)acetyl-β-D-glucopyranose 16

Yield: 49%. Found: $[M+Na]^+$, *m/z* 591.283; $C_{35}H_{36}O_7Na$ requires *m/z* 591.236; ^1H NMR δ_H (CD_3CN, 400 MHz): 1.58 (3H, s, C(CH_3)), 1.64 (3H, s, C(CH_3)), 3.0–3.14 (1H, dd, 6-H), 3.21–3.38 (3H, m, 6-H′, 2-H, 4-H), 3.51-3.56 (2H, m, 3-H, 5-H), 5.51–5.53 (1H, d, 1-H, J = 8.1 Hz), 7.22–7.32 (14H, m ArH), 7.40–7.45 (6H, m, ArH); ^{13}C NMR δ_C (CD_3CN, 100 MHz): 25.1, 26.3, 46.3, 62.7, 69.7, 72.1, 75.7, 76.3, 85.8, 94.3, 125.4, 126.5, 126.7, 127.5, 128.1, 128.3, 143.7, 144.1, 174.9; *m/z* (ES +ve ion mode) 591 ($[M+Na]^+$, 100%).

Deprotection of the 6-*O*-trityl group by acid hydrolysis: General method

To a solution of the trityl protected compound (0.4 mmol) in DCM (4 ml) was added water (0.8 mmol) followed by TFA (0.4 mmol) with vigorous stirring at RT. The reactions were monitored by TLC and showed completion in 30 min. The solvent was removed *in vacuo* and the residue purified by column chromatography using 10–20% EtOH–DCM.

1-(2-phenyl)acetyl-β-D-glucopyranose 17

Yield: 92%. Found: C, 56.3; H, 6.1; $[M+Na]$,$^+$ *m/z* 321.095. $C_{14}H_{18}O_7$ requires C, 56.4; H, 6.1%; $C_{14}H_{18}O_7Na$ requires *m/z* 321.095; $[\alpha]_D^{293\,K}$ = +1.8° (*c* = 0.011 gml^{-1}, MeOH); ^1H NMR δ_H (CD_3CN, 400 MHz): 3.26–3.31 (2H, m, 2-H, 4-H), 3.33–3.41 (2H, m, 3-H, 5-H), 3.56–3.60 (1H, dd, 6-H, J = 12.0, 5.2 Hz), 3.70–3.74 (1H, dd, 6-H′, J = 12.0, 2.5 Hz) 3.74 (2H, s, CH_2Ph), 5.45–4.47 (1H, d, 1-H, J = 8.1 Hz), 7.29–7.37 (5H, m, ArH); ^{13}C NMR δ_C (CD_3CN, 100 MHz): 40.0, 61.0, 69.5, 72.2, 75.9, 76.8, 94.1, 126.8, 128.2, 129.2, 133.6, 170.2; *m/z* (ES +ve ion mode) 321 ($[M+Na]^+$,100%).

1-(|(2*R*)-phenyl|propanoyl)-β-D-glucopyranose 18

Yield: 90%. Found: $[M+Na]^+$, *m/z* 335.1095; $C_{15}H_{20}O_7Na$ requires *m/z*, 335.1107; $[\alpha]_D^{293\,K}$ = −7.2° (*c* = 0.017 gml^{-1}, MeOH); ^1H NMR δ_H [(CD_3)$_2$CO, 400 MHz]: 1.47–1.49 (3H, d, CH(CH_3), J = 7.2 Hz), 3.32–3.36 (1H, t, 2-H, J = 8.6 Hz), 3.44–3.53 (3H, m, 3-, 4-, 5-H), 3.69–3.73 (1H, dd, 6-H, J = 11.8, 4.4 Hz), 3.80–3.86 (2H, m, 6-H′, CH(CH_3)), 5.51–5.53 (1H, d, 1-H, J = 8.2 Hz), 7.25–7.30 (5H, m, ArH); ^{13}C NMR δ_C [(CD_3)$_2$CO, 100 MHz]: 19.7, 46.4, 62.8, 71.5, 74.2, 78.4, 78.8, 96.0, 128.2, 128.9, 129.7, 141.8, 174.1; *m/z* (ES +ve ion mode) 335 ($[M+Na]^+$, 100%).

1-(|(2*S*)-Phenyl|propanoyl)-β-D-glucopyranose 19

Yield: 88%. Found: $[M+Na]^+$, *m/z* 335.111; $C_{15}H_{20}O_7Na$ requires *m/z* 335.1107; ^1H NMR δ_H [(CD_3)$_2$CO, 400 MHz]: 1.48–1.49 (3H, d, CH(CH_3), J = 7.15 Hz), 3.36–3.41 (2H, m, 2-H, 3-H), 3.43-3.48 (1H, m, 5-H), 3.52–3.57 (1H, t, 4-H, J = 8.95 Hz), 3.62–3.66 (1H, dd, 6-H, J = 12.1, 5.2 Hz), 3.78–3.81 (1H, dd, 6-H′, J = 12.1, 2.3 Hz), 3.88–3.92 (1H, q, CH(CH_3), J = 7.1 Hz), 5.51–5.53 (1H, d, 1-H, J = 8.1 Hz), 7.27–7.39 (5H, m, ArH); ^{13}C NMR δ_C [(CD_3)$_2$CO, 100 MHz]: 19.7, 46.3, 62.3, 70.9, 73.7, 77.6, 78.6, 96.0, 128.4, 128.9, 129.9, 141.6, 174.9.

1-(2,2-dimethylphenyl)acetyl-β-D-glucopyranose 20

Yield: 89%. Found: C, 58.71; H, 6.9; *m/z*, $[M+Na]^+$ 349.1266; $C_{16}H_{22}O_7$ requires C, 58.9; H, 6.8%; $C_{16}H_{22}O_7Na$ requires *m/z*, 349.1263; $[\alpha]_D^{293\,K}$ = +9.2° (*c* = 0.006 gml^{-1}, MeOH); ^1H NMR δ_H [(CD_3)$_2$CO, 400 MHz]: 1.55 (3H, s, C(CH_3)), 1.59 (3H, s, C(CH_3)), 3.29–3.34 (1H, m, 2-H), 3.38–3.42 (2H, m, 4-H, 5-H), 3.48–3.52 (1H, m, 3-H), 3.67–3.72 (1H, m, 6-H), 3.80–3.83 (1H, m, 6′-H), 5.54–5.56 (1H, d, 1-H, J = 8.1 Hz), 7.21–7.25 (1H, m, ArH), 7.30–7.35 (2H, m, ArH), 7.39–7.42 (2H, m, ArH); ^{13}C NMR δ_C [(CD_3)$_2$CO, 100 MHz]: 27.2, 27.8, 47.9, 63.0, 71.7, 74.2, 78.4, 78.8, 96.3, 127.1, 127.8, 129.5, 146.0, 176.2; *m/z* (ES +ve ion mode) 349 ($[M+Na]^+$, 100%).

Reactivity monitored by ^{1}H NMR spectroscopy

^1H NMR spectroscopy was carried out on the acyl glucosides at 310 K using a standard water peak presaturation pulse sequence.[28] A Bruker AVANCE600 spectrometer (Bruker Biospin, Rheinstetten, Germany) was used with a 5 mm broadband inverse (BBI) probe operating at a ^1H observation frequency of 600.13 MHz. Once the acquisition parameters were optimised using a standard sample, 550 μL of 100 mM sodium phosphate buffer (pH 7.4) was added quickly to one of the samples containing 3.5, 3.3, 3.0 and 3.3 mg of the acyl glucoside compounds **17–20** respectively. The sample was transferred to a 5 mm NMR tube containing 50 μL sodium trimethylsilyl[2,2,3,3-$^2H_4$]propionate (TSP) (0.5 mg mL^{-1} in ^2H_2O) and 16 scans were acquired with a ^1H spectral width of 20.02 ppm. The spectrum was phased manually, and an automated program was run by which 159 spectra were acquired sequentially. The first 61 experiments were acquired with a time delay of 37.8 s between experiments; the subsequent 95 experiments were acquired with a time delay of 812.9 s between each experiment. The total reaction times for the two segments are 1.92 and 23.45 h respectively. For **20**, the automated experiment was repeated as the reaction products had not appeared to any significant amount after 25 h. An exponential apodization function corresponding to a line-broadening factor of 0.3 Hz was applied to the FIDs. Spectra were chosen at different time points, and manually phase- and baseline-corrected. The anomeric proton doublet of the various acyl glucosides was chosen to monitor the reactions. Assignment of the NMR spectra of the positional isomers has been given previously for acyl glucuronides[24] and those for acyl glucosides follow closely except for the 5- and 6-position protons. The assignments for the C6 CH_2 protons and the C5-H proton were made by inspection, based on the known greater proportion of the β-anomer and were confirmed by measurement of 2-dimensional COSY spectra. Peak integration was used to measure the intensity of the NMR resonances which were referenced to the TSP peak intensity set at unity. The logarithms of the intensities were plotted against time, and the slope of the graph gave the degradation rate constant assuming first-order kinetics.

The % of the reaction due to hydrolysis was obtained by integration of the aglycone methyl doublets of **18** and **19** at ~ δ 1.41, and the singlet resonance from the two methyl groups of **20** at δ 1.47. For **17**, the free α-glucose anomeric proton resonance at δ 5.25 was integrated.

Detailed kinetic modelling of all reactions

The kinetic models used to describe the acyl migration, anomerization and hydrolysis reactions of the acyl glucoside compounds **17–20** are given in Scheme 2. First order irreversible reactions were

used to describe the 1-β to 2-β acyl migration and hydrolysis, and first order reversible reaction models were used to describe the remainder of the acyl migration and all anomerization reactions. Owing to the fast rate of the mutarotation reactions, these were not modelled and fixed values based on the observed β- to α-anomer ratios were used. Thus the calculated concentrations of all species at all time points were obtained by solving the differential equations given in Scheme 2.

The software GEPASI v.3[29] was used to solve the differential equations and estimate the rate constants by minimizing the residual sum of squares between observed and calculated peak intensities with the Levenberg–Marquardt gradient descent method. A 15-point Savitsky–Golay filtering[30] with a second-order polynomial was applied to the ^{1}H NMR reaction profiles of **17–20** to increase the signal-to-noise ratio prior to kinetic modeling. For each of the ^{1}H NMR spectra of **17–20** at each time point the water peak region between δ ~4.5–4.7 was removed to avoid the effects of imperfect water suppression. A weighted least squares 10th order polynomial baseline correction was applied between δ ~0.5–4.5 and δ ~4.7–7 respectively. The peak areas of selected resonances for each positional isomer were measured relative to that of the TSP peak set to unity, which was assumed to be constant through the time period followed.

Abbreviations

HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
NMM	*N*-methylmorpholine
PMB	4-methoxybenzyl.

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