Article

REMARKABLE REACTIVITY DIFFERENCES

BETWEEN GLUCOSIDES WITH IDENTICAL LEAVING GROUPS

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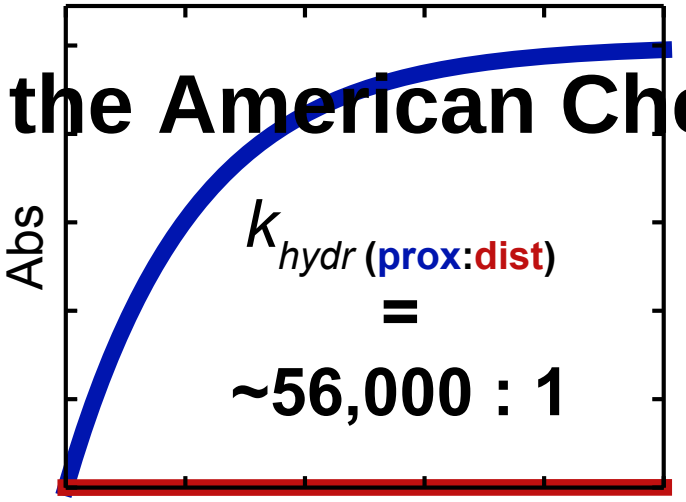
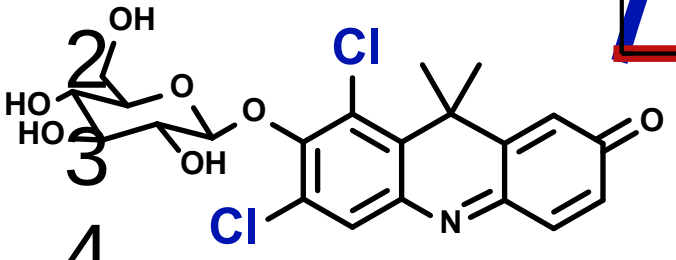


DDAOprox-Glc



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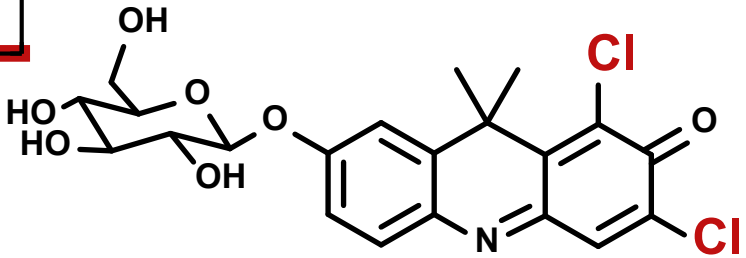
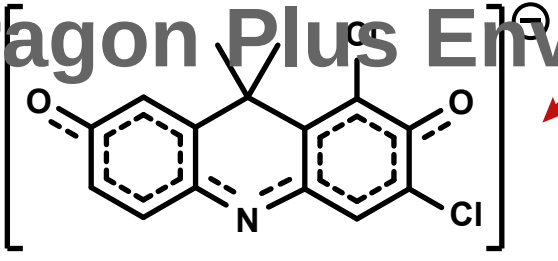


k_{hydr} = 0.46 h^{-1}

DDAO



+ Glucose

k_{hydr} = 8.2 x 10^{-6} h^{-1}

REMARKABLE REACTIVITY DIFFERENCES BETWEEN GLUCOSIDES WITH IDENTICAL LEAVING GROUPS

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ABSTRACT

Two isomeric aryl 2-deoxy-2-fluoro-β-glucosides react with a β-glucosidase at rates differing by 10^6 fold, despite the fact that they release the same aromatic aglycone. In contrast the equivalent glucoside substrates react with essentially identical rate constants. Insight into the source of these surprising rate differences was obtained through a comprehensive study of the non-enzymatic (spontaneous) hydrolysis of these same substrates, wherein an approximate 10^5 fold difference in rates was measured, clarifying that the differences were inherent, rather than being due to specific interactions with the enzyme. The possibility that an alternate nucleophilic aryl substitution mechanism was responsible for the rapid reaction of the faster substrate was excluded through ^{18}O-labelling studies. Further exploration of the origins of these rate differences involved analysis of X-ray crystal structures as well as quantum chemical calculations, which surprisingly revealed that ground state destabilization and transition state stabilizing effects contribute almost equally to the observed reactivity differences. These studies

highlight the dangers of using simple reference equilibria such as pK_a values, as measures of leaving group ability.

INTRODUCTION

Much of our understanding of the mechanisms of enzymatic and non-enzymatic glycoside hydrolysis has come from kinetic studies of aryl glycosides.[1] The ability to manipulate reactivity through the incorporation of different substituents into the phenolic moiety allows the construction of Hammett and Brønsted relationships, which in turn inform on rate-limiting steps and transition state structures.[2-4] In such studies leaving group ability is generally quantified through Hammett σ values, or more intuitively through the pK_a values of the corresponding phenols. In this way, spontaneous hydrolysis of glycosides under neutral to acidic conditions has been shown to proceed *via* a dissociative mechanism involving a late, oxocarbenium ion-like transition state.[5-10] Likewise, mechanisms of the vast majority of glycosidases feature highly developed oxocarbenium ion-like transition states, with departure of the less-acidic aglycones assisted by acid catalysis from suitably positioned carboxylic acid residues in the active site.[11-13] This is true both for inverting glycosidases, which effect catalysis through a single displacement mechanism, and for retaining glycosidases, which employ a double-displacement mechanism *via* two oxocarbenium ion-like transition states.[11] Accordingly, Brønsted plots of log k_{cat} or log k_{cat}/K_m versus aglycone pK_a typically feature negative slopes with β_{lg} values often approaching -1, indicating substantial negative charge development on the glycosidic oxygen at the reaction transition state.[2, 14] This is also true for spontaneous (non-enzymatic) aryl glycoside hydrolysis since β_{lg} values determined in four independent, spontaneous hexopyranoside hydrolysis studies[15] fall in a tightly clustered range with an average β_{lg} = 1.25 ± 0.02. The increase in rate constant with decreasing phenol pK_a is typically ascribed to increased transition state stabilisation.

As part of a recent study aimed at generating active site titrating agents for retaining glycosidases we synthesised 2-deoxy-2-fluoro-glycosides bearing fluorogenic leaving groups of low pK_a.[16-17] The fluorine at C-2 inductively destabilises both transition states, slowing both the formation and the hydrolysis of the glycosyl enzyme intermediate. Incorporation of a good leaving group of low pK_a ensures that intermediate formation is fast and thus that the glycosyl enzyme accumulates, with stoichiometric release of the fluorophore. One of the most sensitive such reagents we synthesized was the DDAO-glucoside **1** (Figure 1), which includes a dichloro-dimethylacridinone (DDAO) leaving group having a pK_a value of 5.3.[16] This fluorogenic glycoside proved very useful for selective quantitation of retaining β-glucosidases down to sub-nanomolar levels according to the process shown in Figure 2.

Reaction of **1** with the *Agrobacterium sp.* β-glucosidase (Abg) was very fast with a second order rate constant of k_i/K_i = 1.38 × 10^6 mM^{-1} min^{-1}, which is equivalent to 2.2 × 10^7 M^{-1} s^{-1} thus approaching diffusion control.[16] Indeed, stopped-flow techniques were needed to measure this value. Synthesis of **1** had been achieved by coupling DDAO with the protected 2-fluoroglycosyl bromide, yielding both **1** and its regioisomer **2,** in which the aglycone is attached by the other oxygen of the aryl moiety in its alternative tautomeric form, thus will release the same phenolic leaving group. Henceforth this will be referred to as the DDAOdist glycoside wherein the two chlorines are distal to the sugar. Likewise, **1** will be referred to as the DDAOprox glycoside where the two chlorines are proximal to the sugar. These two isomers have distinctly different absorption properties, where **1** appears yellow in colour (λ_{max} = 403 nm) and **2** appears red (λ_{max} = 460 nm). Under the synthetic conditions employed they were formed in a ratio of 85:15 (**1**:**2**). It was therefore of interest to explore the utility of the distal isomer as a potential mechanism-based inhibitor, but surprisingly, when tested as a titrating agent for Abg, the distal isomer **2** proved to react at a rate over 10^6 times lower than does **1**, despite the fact that both

release the same leaving group. This report describes a detailed experimental and computational analysis of the underlying basis for this reactivity difference, through an experimental and computational exploration of the reactivity of the corresponding (non-fluorinated) glucosides towards both enzymatic and non-enzymatic hydrolysis.

RESULTS AND DISCUSSION

The two isomeric acridinoyl 2-deoxy-2-fluoro glycosides **1** and **2** were synthesised as described previously[16] and characterised as described in Supplementary Information. Incubation of the *Agrobacterium sp* β-glucosidase (Abg) with the distal isomer (**2**) of the acridinoyl β-2-deoxy-2-fluoro-glucoside resulted in only extremely slow inactivation of the enzyme activity compared to the rapid inactivation afforded by **1.** Much higher concentrations (60-120 μM *vs*. 0.1 μM) as well as longer reaction times, of up to 60 minutes (*vs.* 10 s), to obtain complete inactivation of Abg. Indeed, since **1** and **2** were products of the same synthetic reaction there was a concern that even this slow observed inactivation might arise largely – or perhaps only – from small amounts of contaminating **1** at levels too low to be detected by NMR but, given the reactivity difference, still sufficient to effect inactivation. To address this concern a 100 μM solution of **2** was treated with a 300 nM solution of Abg as a "reagent" to purify **2** by destruction of potentially contaminating **1,** and the remaining enzymatic activity was monitored for 60 minutes until a low, constant rate was observed. The sample was then loaded onto a C18 SepPak reverse phase column to remove the Abg and any released DDAO. Purified **2** still inactivated Abg, but with an apparent rate constant that was 2-fold lower than that seen prior to purification, confirming the initial presence and subsequent removal of contaminating **1**. Kinetic parameters for this purified sample of **2** were determined by conventional inactivation kinetic analysis and found to be

k_i = 4.8 x 10^{-2} min^{-1}, K_i = 100 μM and k_i/K_i = 0.5 mM^{-1} min^{-1}. Thus, remarkably, the second order rate constants for inactivation by **1** and **2** differ by over six orders of magnitude!

Such a huge difference in inactivation behaviour was particularly surprising since the aglycones released by **1** and **2** are identical, as is seen in Figure 2. Further, since glycosidase transition states are late, it is expected that there should be almost full charge development on the aglycone, and electron reorganisation therein via resonance should be complete. A first concern was that the assumed structures for **1** and/or **2** were incorrect, despite our detailed NMR characterisation. To explore this possibility we set up crystallization trials for **1** and **2** and for DDAO glycosides of other 2-fluorosugars, and were successful in establishing accurate x-ray structures for protected forms of the two *galacto* derivatives, **3** and **4** as shown in Figure 3. These structures fully confirmed the presumed connectivity of the aglycone to the sugar moiety in each case. Importantly **3** was also shown to be a potent inactivator of Abg (which has both *β*-glucosidase and *β*-galactosidase activities[14]), reacting with a rate constant of k_i/K_i = 6180 $mM^{-1}min^{-1}$. Meanwhile, **4** was a very weak inactivator, requiring 1000-fold higher concentrations than were employed for **3**, and even then reacting with a rate constant of only 2 min^{-1}.

In order to determine whether these differences in inactivation rate constant are also reflected in differing hydrolytic rate constants for the corresponding glucoside substrates, the DDAO glucosides **5** and **6** were synthesized *via* standard Koenigs-Knorr chemistry followed by deprotection using HCl/methanol. Kinetic parameters for hydrolysis of **5** and **6** by Abg were then determined and, surprisingly, very similar values of k_{cat}, K_m and k_{cat}/K_m were found, as seen in Table 1 and Supplemental Table 1. Thus, while the enzyme discriminates by approximately 10^6-fold between the pairs of DDAO 2-fluoroglycosides **1** / **2** and **3** / **4**, there is essentially no discrimination between the pair of glucoside substrates. Importantly however, the second order rate constants for the two substrates (k_{cat}/K_m) are both very large and, at ~ 2 x 10^7 M^{-1} s^{-1}, similar to those previously measured for hydrolysis of 2,4-

dinitrophenyl β-glucoside and 2,4,6-trichlorophenyl β-glucoside by Abg.[14] In that previous study, the Brønsted plot of log (k_{cat}/K_m) *vs*. aglycone pK_a had shown pronounced downward curvature and this was attributed as being most likely due to diffusion control becoming rate-limiting for substrates with the lowest aglycone pK_a values. Since **5** and **6** have similar k_{cat}/K_m values it is probable that diffusion control is rate-limiting for both these substrates, hence the lack of observed kinetic discrimination. Indeed, even the non-chlorinated glucosyl acridinone DAO glucoside **7**, (DAO pK_a = 7.0) is hydrolysed by Abg at similar rates, approximating diffusion control (Table 1, Supp Table 1). By contrast, within the 2-fluoroglycoside series, only the proximal derivative **1** has a rate constant in that range, while the distal derivative **2** has a much slower, rate-limiting, chemical step. The inductive destabilisation afforded by the fluorine has brought rates down to below diffusion control.

A key question then was whether the surprising differences in inactivation rates seen between **1** and **2** (but masked for substrates **5**, **6** and **7**) were due to differences in enzyme recognition and catalysis, or whether they had their origins in fundamental reactivity differences. To address this question we measured rates of non-enzymatic hydrolysis for **5** and **6** at 50 °C, but otherwise under the conditions used in the enzymatic studies (pH 7, phosphate buffer). DDAOprox glucoside **5** hydrolysed completely within 10 hours, allowing determination of its solvolytic rate constant by direct fit of the time course data to a first order expression, k_{solv} = 0.46 h^{-1}. Hydrolysis of **6** and **7**, by contrast, was extremely slow. Thus, their respective rate constants were determined by measurement of initial rates at a series of substrate concentrations. Since possible contamination by small amounts of **5** could confound initial rate measurements, aglycone release was monitored for over 14 hours, during which any contaminating **5** would have hydrolysed ($t_{½}$ = 1.9 h). Results are shown in Table 1, alongside a literature value for hydrolysis of 2,4-dinitrophenyl β-glucoside.[18] As is evident, under these conditions **5** hydrolyses nearly 1,000 fold more rapidly than **6** and **7**, and at approximately the same rate as 2,4-dinitrophenyl β-glucoside. Clearly, placement of the two chlorine atoms proximal to the glycosidic bond

has major consequences, thus the differences in inactivation rate observed with Abg have their origins, at least partially, in fundamental reactivity differences.

Since these hydrolytic rate constants may well be differentially pH sensitive – thereby containing variable contributions from various pathways – a quantitative comparison required a more careful study. Accordingly, rate constants for spontaneous hydrolysis of **5** and **6** at a series of pH values ranging from approximately pH 1 to pH 11 were measured and the data and resultant plots are shown in Figure 4 and Supplemental Table 3. As can be seen, rate constants for hydrolysis of **5** are larger than those for **6** at each of the pH values studied. The profile observed for **5** includes a pH-independent region from approximately pH 4 to 8.5 with flanking pH-dependent arms in which rate constants increase as pH moves away from neutrality. As shown previously for solvolysis of 2,4-dinitrophenyl β-glucoside, this pH-independent region corresponds to simple heterolysis of the glycosidic bond while the flanking limbs contain additional pathways involving acid or base assistance.[18-19] The pH profile for hydrolysis of **6** is similar, but with a narrower pH-independent regime from approximately pH 4 to 5. The presence of this pH-independent region for both substrates allows direct comparison of rate constants for simple heterolysis of the glycosidic bond in the two cases: an approximate 10^5 fold difference in rate constants is seen. This enormous difference closely reflects the large (~10^6 fold) difference in rates of Abg inactivation by the pair of 2-fluoroglucosides. Thus it appears that a large portion of the difference in enzymatic inactivation rates resides in fundamental reactivity differences between the two isomers rather than being due to different substrate-specific interactions with the enzyme.

One possible explanation for this vast difference in fundamental reactivity, despite their apparently very similar transition states, is that a different mechanism is followed in the two cases. The most probable alternative would be one involving *ipso*-attack of water on carbon-1 of the aryl moiety of

the proximally substituted derivative **5**, since this carbon, situated between the two chlorines, is very electron deficient. To investigate this possibility, **5** was incubated in $H_2{}^{18}O$ phosphate buffer at 50 °C and the composition of the hydrolysis products over time was determined to explore possible ^{18}O incorporation into the DDAO product. The resulting products – ^{16}O-DDAO, single ^{18}O-substituted DDA(^{18}O) and double ^{18}O-substituted DDA($^{18}O)_2$ – were monitored by mass spectrometry and their ratios were calculated (taking into account the chlorine isotopes). The relative concentration of each species as a function of time was then determined by substitution into a first order equation representing decay, $y = Ae^{k_{hydr}t}$, using the rate constant of k_{hydr} = 0.46 h^{-1} measured previously. This value represents the combined rates of hydrolysis from attack at both the anomeric carbon and the C-1 aryl carbon.

As is shown in Figure 5, the analysis of the resultant data is rendered somewhat more complex by the possibility of "wash-in" of ^{18}O into the carbonyl group of the DDAO moiety, either into the glycoside **5** or into the released DDAO. To simplify the analysis, rates of these processes were measured in simple parallel experiments, yielding a rate constant for "wash-in" of ^{18}O into DDAO and the DDAOprox-glucoside **1** of k_5 = 0.02 h^{-1}, and k_2 = 0.23 h^{-1} respectively.

The resulting percentages as a function of time are shown in Figure 6. These progress curves were fit globally using the KinTek Explorer software[20-21] (Supp Info Table 2), revealing that the *ipso* attack mechanism contributes a maximum of 24% to the overall rate of hydrolysis, which is clearly not sufficient to explain the enormous rate differences observed between **5** and **6**. These results therefore confirm that, at pH 7, no significant change of mechanism has occurred between substrates and that the primary pathway for hydrolysis of **5** is that of the originally anticipated heterolysis of the glycosidic bond. These enormous (>10^5 fold) differences in reactivity of two isomeric glycosides that hydrolyse through very similar dissociative transition states were very surprising and are the underlying reason for the differences in glycosidase inactivation efficiency observed for **1** and **2**.

Given the essentially electronically-identical transition state structures, some of the difference in reactivity must have its origins in ground state structural differences, be they steric effects arising from the relatively bulky *ortho*-chloro substituents, or electronic due to the inductive effects of the two halogens. Closer inspection of the X-ray crystal structures obtained for the per-*O*-acetylated versions of **3** and **4** (**S3** and **S4**) reveals somewhat different conformations of the two glycosides. The plane of the aromatic ring is tilted 50^{o} away from the plane of the sugar ring in **S4**, while in **S3**, with its proximal chlorine substituents, the aromatic ring is almost perpendicular to the sugar ring plane (75^{o}). This displacement appears to be a response to steric constraints imposed by these halogens. Perhaps more importantly, there are small but very significant differences in the glycosidic bond lengths in the two cases, with that for **S3** being 0.009 Å longer than that for **S4**. This longer bond length for **S3** is consistent with inductive effects that favour charge development on the glycosidic oxygen in the early stages of ionisation/heterolysis of the glycosidic bond towards the oxocarbenium ion/phenolate pair. Such behaviour has been investigated and quantitated in some detail by Kirby *et al.* through determination of x-ray crystal structures of several series of axial and equatorial aryl acetals bearing different aryl substituents within both a tetrahydropyranyl series and a glycosyl series.[18, 22-24] Within each series Kirby *et al.* observed a linear correlation of the acetal carbon-aryl oxygen bond length with the pK_a of the phenol from which it had been derived. The sensitivity (slope) of that correlation varied for each system, being higher for axial than equatorial systems, consistent with expectations of stereoelectronic theory.[22] The sensitivity was decreased by electronegative substituents alpha to the acetal centre, with β-glucosides (OH at C2) showing a smaller dependence (0.00285 Å / pK_a) than the equatorial tetrahydropyranyl (H at C2) system (0.00476 Å / pK_a).

The most directly comparable data from the Kirby studies are for the (equatorial) β-glucosides. Applying the value of bond length *vs.* pK_a dependence observed in that case to the bond length difference measured between **S3** and **S4** (0.009 Å) suggests a difference in effective pK_a of the phenols of **1** and **2** of

a little over 3 units. However, this is undoubtedly an underestimate given the abovementioned dependence of this value on electronegative substituents and the presence, in **1** and **2**, of a fluorine substituent at C-2. Based upon the σ_I values for H, OH and F of 0, 0.25 and 0.50, substantial inductive effects could be expected, as was seen in our previous data on the pH-independent heterolysis of 2,4-dinitrophenyl glycosides, where the solvolysis rate constant correlated strongly (ρ_I = -9)with the σ_I value of the 2-substituent.[18] This would suggest a difference of effective pK_a of the phenols in the two isomers of at least 3 to 4 units. Using the average β_{lg} value of -1.25 ± 0.02 determined within four independent studies of spontaneous hexopyranoside hydrolysis[15] this pK_a difference should translate into a rate difference of approximately 10^4 fold. Thus it would seem that much of the rate difference may be explained by different effective leaving group pK_a values.

To probe the relative contributions of transition state and ground state energetic effects, computational models for the spontaneous hydrolysis of the proximal and distal glucosides **5** and **6** were constructed using the SMD/M06-2X/6-31+G(d,p) quantum chemical methods (see Supporting Information). These models predicted that the ground state structure of **5** was indeed 5.3 kcal mol^{-1} less stable than the ground state structure of **6**, as suggested from the measured bond length data above. Somewhat surprisingly, they also suggested that the transition state structure of **5** was 4.7 kcal mol^{-1} more stable than the corresponding structure for **6**. These differences in energy are reflected in key transition state structure bond lengths: the anomeric carbon – leaving group oxygen bond length for DDAOprox-Glc **5** is 2.276 Å, with an associated anomeric carbon – nucleophilic water oxygen bond length of 2.596 Å, while the C–O_{lg} bond length for DDAOdist-Glc **6** is 2.360 Å with an associated C–O_{water} bond length of 2.529 Å. These observed bond lengths are consistent with DDAOprox being a better leaving group than DDAOdist, yielding an earlier transition state, i.e. both bond fission and bond formation are less far advanced in the transition state for DDAOprox than for DDAOdist due to the better stabilisation of the charge at the leaving group oxygen. In addition to these differences in key

transition state bond lengths, the conformational searches carried out in determining the lowest energy ground state and transition state structures (*supplementary info*) reveal that the dispositions of the aglycone with respect to the glycone in both ground state and transition state structures are quite distinct for DDAOprox and DDAOdist. The models, in combination with transition state theory, predict that **5** would be spontaneously hydrolyzed some 6×10^6-fold faster than **6** at the experimental temperature of 50 °C. These results are consistent with the experimentally observed difference in spontaneous rates of hydrolysis and, perhaps surprisingly, indicate that both ground state and transition state energy differences contribute almost equally to the overall difference in rates of hydrolysis. The primary source of these effects is likely electronic, due to through-bond inductive effects from the proximal chlorines, though steric contributions to the ground state destabilisation cannot be discounted at this stage.

CONCLUSIONS

The surprisingly large differences in reaction rate of two isomeric glycosides that release the same aglycone were explored. Through the use of a pseudo-symmetric aglycone the resonance contributions to transition state stabilisation in the reactions of the two glycosides are made largely equivalent, such that rate differences arise primarily from inductive or steric effects on the ground state. These effects are surprisingly large, and do not truly reflect the measured pK_a values of DDAO (5.3) and DAO (7.0); rather, they must reflect values of effective pK_a at the reaction transition state. The study therefore serves as a reminder of the limitations (hazards) of relating effects on rate to thermodynamic parameters such as pK_a values.

MATERIALS AND METHODS

All experimental protocols plus product characterisations are provided as Supplementary Information.

Acknowledgments

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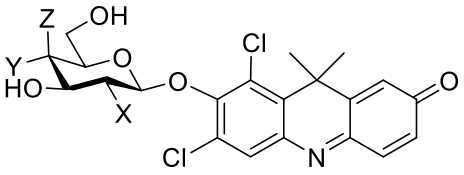
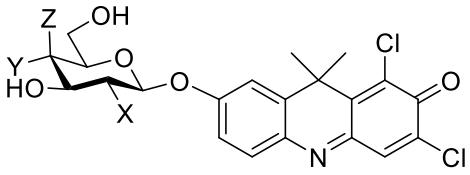
Figure 1.

DDAOprox-glycoside



DDAOdist-glycoside



1 X = F, Y = OH, Z = H

3 X = F, Y = H, Z = OH

5 X = OH, Y = OH, Z = H

2 X = F, Y = OH, Z = H

4 X = F, Y = H, Z = OH

6 X = OH, Y = OH, Z = H

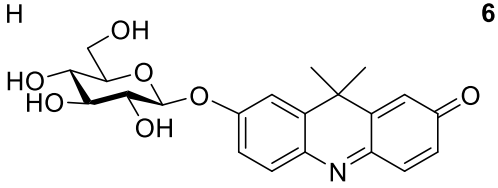


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Figure 2. Inactivation of Abg by DDAO 2-fluoroglucosides

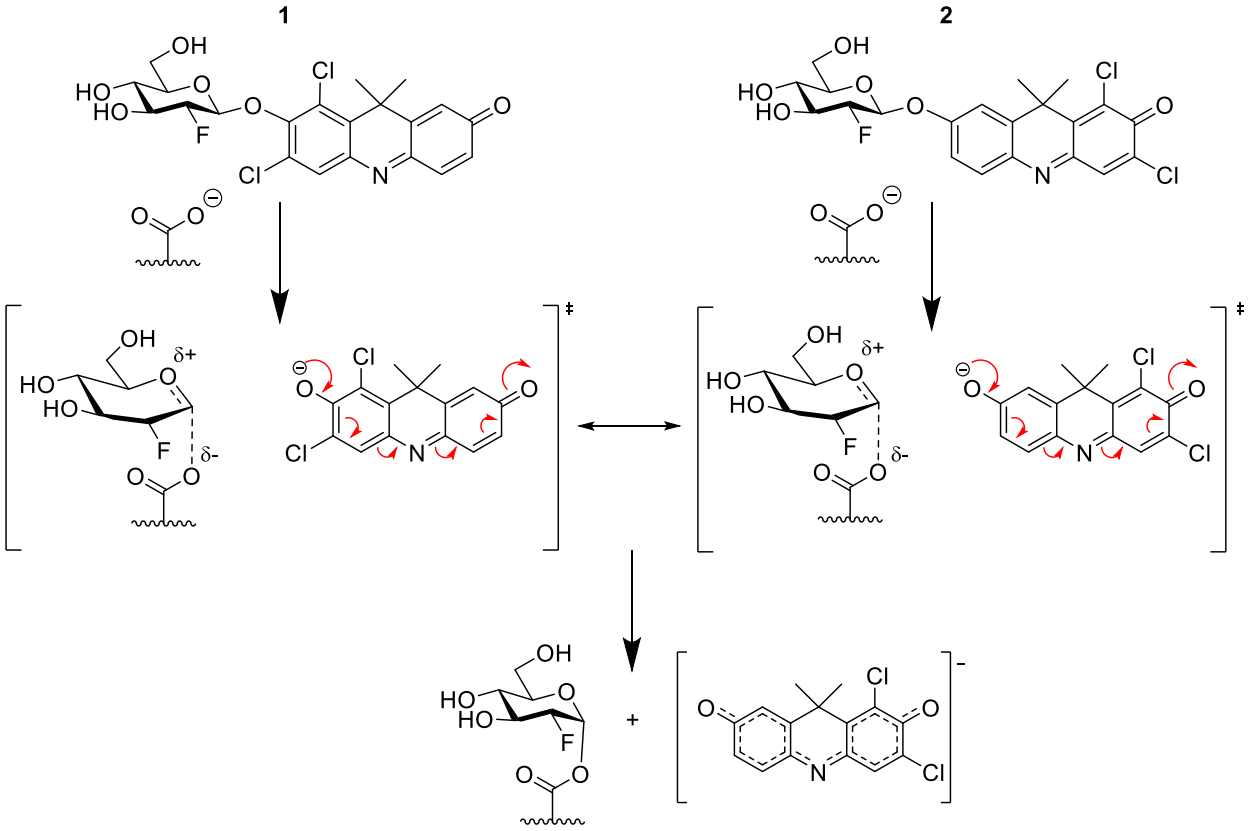

Figure 3.

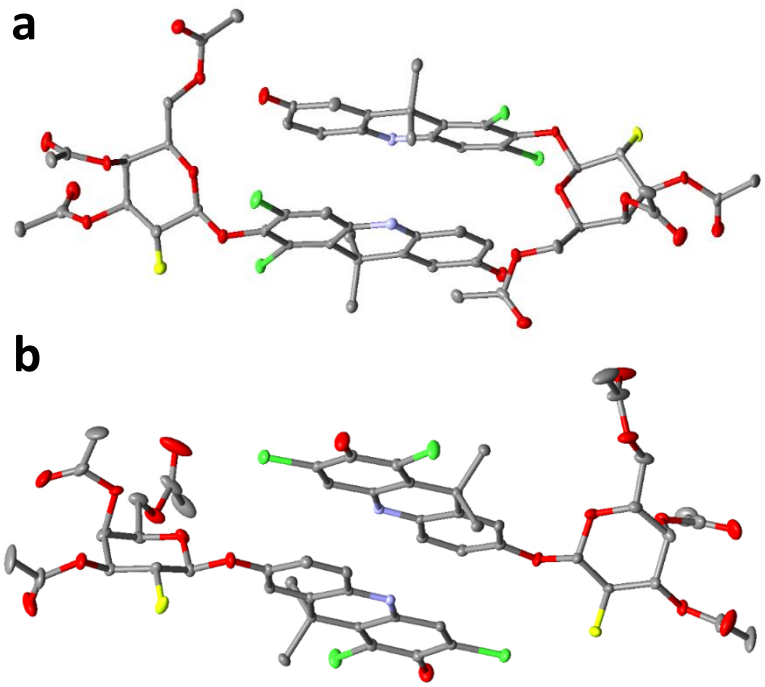

Figure 4.

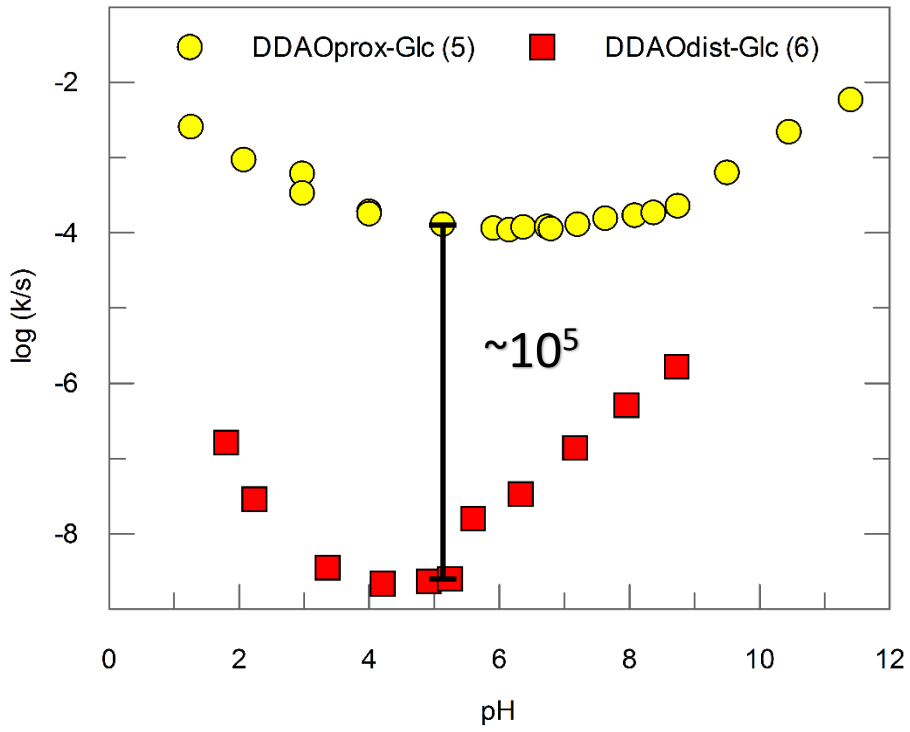

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Figure 5.

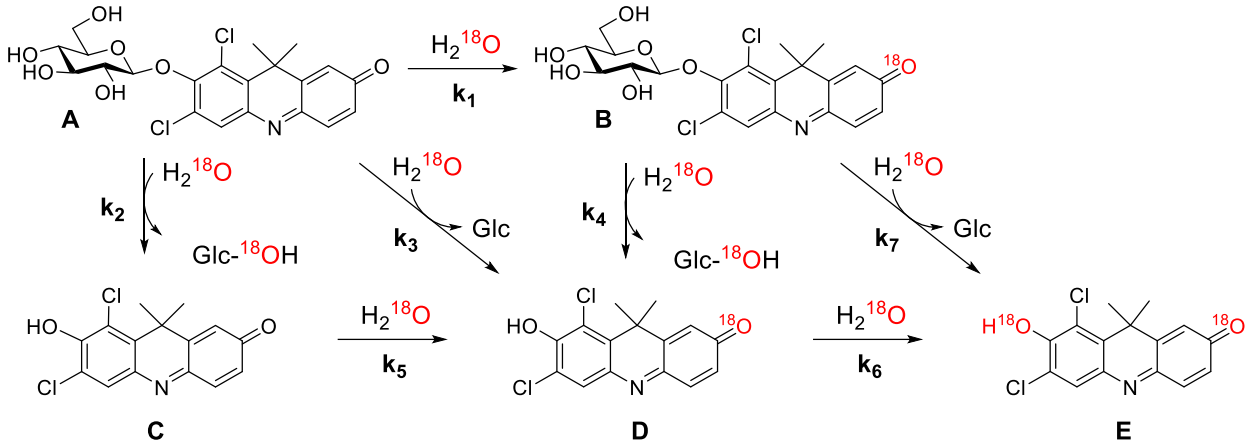

Figure 6.

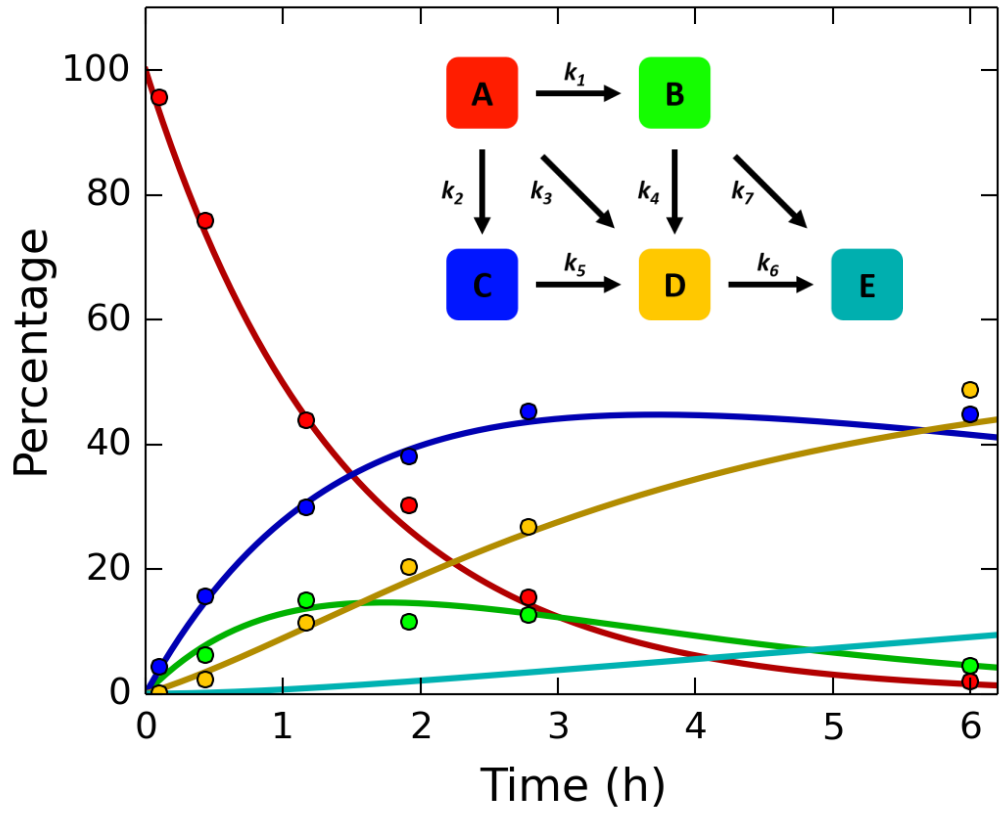
A
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Figure captions

Figure 1. Aryl glycosides (**1-7**) synthesized in this study. DDAO-proximal glycosides (**1**,**3**,**5**) have the Cl atoms of DDAO adjacent to the anomeric center, while DDAO-distal glucosides (**2**,**4**,**6**) have the Cl atoms on the opposite side of the aglycone. DDAO was dechlorinated and coupled with the glucosyl bromide to form DAO-glucoside after deprotection (**7**).

Figure 2. Mechanistic depiction of β-glucosidase-mediated hydrolysis of DDAO-2FGlc. DDAOprox (**1**) and DDAOdist (**2**) 2F-glucosides are regioisomers that, when cleaved, release the same aglycone (DDAO).

Figure 3. a) Crystal structure of per-*O*-acetylated DDAOprox-2F-Gal (**S3**) b) Crystal structure of per-*O*-acetylated DDAOdist-2F-Gal (**S4**) Colour code: gray – carbon; red - oxygen; blue - nitrogen; yellow - fluorine; green - chlorine. Hydrogens have been omitted for clarity.

Figure 4. pH-Dependent hydrolysis of DDAO glucosides (**5**,**6**). Logarithm of spontaneous hydrolysis rate (50 mM buffer, 50 °C) versus pH of buffer. At neutral pH comparison of hydrolytic rates show **5** cleaves >10^5-fold faster than regioisomer **6**.

Figure 5. Potential breakdown pathways of DDAOprox-Glc (**5**) in heavy water ($H_2{}^{18}O$) at 50 °C. **A** to **B** (k_1) occurs via 'wash-in' of water to the distal carbonyl of the aglycone; **A** to **C** (k_2) and **B** to **D** (k_4) occurs via classical heterolysis of water to the anomeric carbon of glucose; **A** to **D** (k_3) and **B** to **E** (k_7) hydrolysis occurs via attack of water on the ipso carbon of the aglycone; **C** to **D** (k_5) and **D** to **E** (k_6) occurs via 'wash-in' of water to the distal carbonyl of DDAO. Product m/z values of **A** – **E** were monitored over time via mass-spectrometry.

Figure 6. Monitoring DDAOprox-Glc cleavage over time in $H_2{}^{18}O$ at 50 °C. Inset model - **A** = DDAOprox-Glc; **B** = DDA^{18}Oprox-Glc; **C** = DDAO; **D** = DDA^{18}O; **E** = DDA($^{18}O)_2$. Best fit curves are generated by KinTek Explorer[20] to determine rates of each pathway. Constraints employed to fitting parameters: $k_2 = k_4$; $k_3 = k_7$; $k_6 = k_5/2$; $k_2 + k_3 = 0.46$ h^{-1}.

Table 1. Kinetic parameters for Abg-catalysed, and spontaneous hydrolysis of aryl glycosides

Compound	k_i/K_i (mM^{-1} min^{-1})	k_{cat}/K_m (s^{-1} mM^{-1})	**Spontaneous hydrolysis** (s^{-1})
2F-DDAOprox-Glc (**1**)[16]	1.3×10^6	-	-
2F-DDAOdist-Glc (**2**)	0.5	-	-
2F-DDAOprox-Gal (**3**)	6180	-	-
2F-DDAOdist-Gal (**4**)	<20	-	-
DDAOprox-Glc (**5**)	-	28000	1.27×10^{-4} (pH 5) 50 °C
DDAOdist-Glc (**6**)	-	17000	2.29×10^{-9} (pH 5) 50 °C
DAO-Glc (**7**)	-	17000	2.54×10^{-8} (pH 5) 50 °C
2,4-dinitrophenyl β-glucoside[18]	-	5960	5.58×10^{-6} (pH 6.5) 37 °C
2,4,6-trichlorophenyl β-glucoside[14]	-	26000	-

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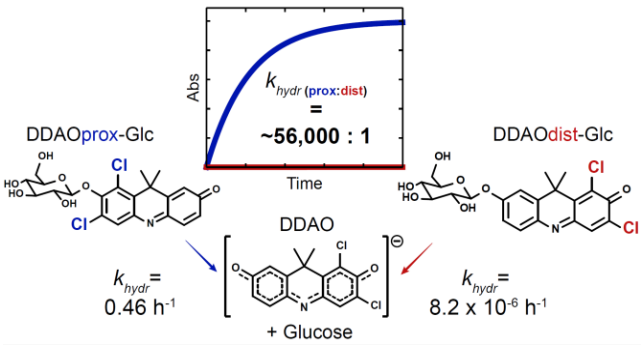
TOC Graphic


Abs
Time
k_{hydr} (prox:dist)
=
~56,000 : 1
DDAOprox-Glc
DDAOdist-Glc
DDAO
k_{hydr} = 0.46 h^{-1}
k_{hydr} = 8.2 x 10^{-6} h^{-1}
+ Glucose