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Structure-reactivity relationships for β-galactosidase (*Escherichia coli, lac Z*): a second derivative effect on β_{nuc} for addition of alkyl alcohols to an oxocarbenium ion reaction intermediate

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Velocities for the synthesis of trifluoroethyl 2-deoxy-β-D-galactopyranoside by transfer of the 2-deoxygalactosyl group from β-galactosidase to trifluoroethanol were determined from studies of the β-galactosidase-catalyzed cleavage of 4-nitrophenyl-2-deoxy-β-D-galactopyranoside as the difference in rates of appearance of 4-nitrophenoxide anion and 2-D-deoxygalactose. These data were used to calculate a rate constant ratio of $k_{ROH}/k_s = 2.3\ M^{-1}$ for partitioning of the intermediate between addition of trifluoroethanol and solvent water. Velocities for the synthesis of other alkyl 2-deoxy-β-D-galactopyranosides by transfer of the 2-deoxygalactosyl group from β-galactosidase to alkyl alcohols were determined from the effect of alkyl alcohols on the velocity of β-galactosidase-catalyzed cleavage of 4-nitrophenyl-2-deoxy-β-D-galactopyranoside in a reaction where breakdown of the intermediate is rate determining. These data were used to calculate rate constant ratios k_{ROH}/k_s for the reactions of eight alkyl alcohols. Absolute rate constants k_{ROH} ($M^{-1}\,s^{-1}$) were calculated from k_{ROH}/k_s and $k_s = 0.002\ s^{-1}$ for the addition of water. A Brønsted coefficient of $\beta_{nuc} = -0.07 \pm 0.08$ was determined as the slope of a logarithmic correlation of k_{ROH} against alcohol pK_a. The change from a 2-OH to a 2-H substituent at the β-D-galactopyranosyl intermediate causes a 0.12 ± 0.04 increase in the value of β_{nuc} for alcohol addition. This anti-Hammond effect provides evidence that general base-catalyzed addition of alcohols to an enzyme-bound β-D-galactopyranosyl oxocarbenium ion intermediate proceeds along a reaction coordinate in which there is strong coupling between carbon–oxygen bond formation and proton transfer from the alcohol to a basic residue at the enzyme. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: glycosyl transfer; Brønsted coefficient; structure-reactivity effects; nucleophile addition; substituent effects

INTRODUCTION

Brønsted coefficients α and β, and Hammett coefficients ρ for the reactions of small organic molecules in solution may be conveniently determined as the slopes of linear logarithmic free energy relationships between rate and equilibrium constants. These first-derivative parameters provide a wealth of useful information about the *position* of the transition state along a free energy reaction diagram.[1–5] The specificity of enzymes for their substrates is generally too narrow to allow for the types of structural variations required for the determination of Brønsted and Hammett reaction coefficients. When such variation is possible, the quality of the derived Brønsted or Hammett correlation for the enzymatic reaction seldom match those for organic reactions. Much valuable information about enzyme mechanisms has been obtained in the relatively rare cases where good Brønsted correlations are observed for the appropriate kinetic parameter.[6–10]

β-Galactosidase catalyzes the hydrolysis of lactose and other β-D-galactopyranosyl derivatives by a two-step mechanism. The first step is the readily reversible transfer of the β-D-galactopyranosyl group from substrate to the carboxylate side-chain of Glu-537 to form a covalent reaction intermediate (Scheme 1).[11] This is followed by transfer of the sugar from the enzyme to water to form β-D-galactose.

β-Galactosidase shows a high specificity for the β-D-galactopyranosyl group of substrate (X = OH, Scheme 1). It is tolerant of variations in the leaving group and has been found to catalyze the cleavage of β-D-galactopyranosyl derivatives with pyridine,[12] ring-substituted phenol,[11] alkyl alcohol,[13] azide anion,[14,15] and fluoride anion[16] leaving groups. We have reported good linear Brønsted correlations of kinetic parameters for enzyme-catalyzed cleavage and synthesis of alkyl β-D-galactopyranosides with slopes $[(\beta_{nuc})_{k_{cat}/k_m} = -0.75 \pm 0.14]$ and $[\beta_{nuc} = -0.19 \pm 0.10]$ for the cleavage and synthesis reactions, respectively.[13,14] These results are consistent with participation by the carboxylic acid side-chain of Glu-461 in concerted general acid catalysis of the cleavage of alkyl β-D-galactopyranosides to form an enzyme-

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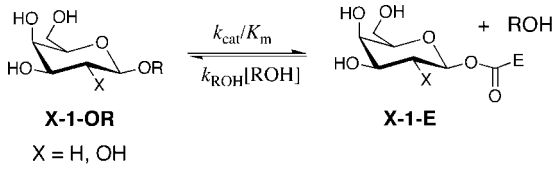

X-1-OR **X-1-E**

X = H, OH

Scheme 1.

bound glycosyl oxocarbenium ion that is trapped by Glu-537 to form the reaction intermediate (Scheme 1); and, with general base catalysis of alcohol addition to the enzyme-bound glycosyl oxocarbenium ion for the reaction in the reverse direction.[17,18]

Brønsted coefficients are first-derivative structure-reactivity effects that provide a measure of the development of transition state "effective charge" at the reaction center.[5] Changes in first-derivative correlations with changing substrate structure have been characterized to give second-derivative structure-reactivity parameters. The interpretation of these second-derivative effects has the potential to provide deep insight into reaction mechanisms.[3,4] There have been many determinations of Brønsted coefficients β_{nuc} for reactions similar to that in Scheme 1. These reactions show changes in second-derivative structure-reactivity effects that provide evidence for the following changes in transition state structure with changing electrophile reactivity.[4,19]

(1) The Brønsted coefficient β_{nuc} for the addition of alkyl alcohols to unstable ring-substituted 1-phenylethyl carbocations *decreases* with increasing carbocation reactivity.[20,21] This corresponds to a Hammond-type shift from a transition state with a large degree of C—O bond formation and positive charge development at the alkoxy oxygen, to an early transition state with little bond formation to the nucleophile.[22] This change may be illustrated on a one-dimension reaction coordinate diagram for nucleophile addition; or, on a two-dimension reaction coordinate diagram that assigns separate coordinates to changes in bonding to carbon and the transferred proton.[3,4] In the latter case, the dominant atomic motion at the transition state is C—O bond formation, and the proton is stationary at the oxygen nucleophile.[20,21,23]

(2) Values of β_{nuc} for general base-catalyzed addition of alkyl alcohols to formaldehyde[24] and acetaldehyde[25] *increase* with increasing electrophile reactivity. This corresponds to anti-Hammond movement of the position of the transition state.[4] The result cannot be explained on a one-dimension reaction diagram, but can be rationalized on a two-dimension diagram by a reaction coordinate in the region of the transition state where movement toward C—O bond formation on one axis is strongly coupled to movement on the second axis of the proton from the alkoxy oxygen to the base catalyst.[3,24,25]

We recently characterized the effects of a 2-H for 2-OH substitution on the kinetic mechanism for β-galactosidase-catalyzed cleavage of **HO-1-OC$_6$H$_4$-4-NO$_2$**.[26] This conservative substitution causes a large 3.2×10^5-fold decrease in the rate constant k_s (s^{-1}) for transfer of the reaction intermediate from enzyme to water.[26] We now report that the 2-H for 2-OH substitution at **HO-1-E** (Scheme 1) causes a change to a more positive value of β_{nuc} for the addition of alkyl alcohols to an enzyme-bound glycosyl oxocarbenium ion. This result is consistent with an anti-Hammond shift in the position of the transition state for enzyme-catalyzed β-D-galactopyranosyl group transfer that is similar to the shift observed for the addition of alcohols to simple aldehydes.[24,25]

EXPERIMENTAL SECTION

Reagent grade organic chemicals and inorganic salts from commercial sources were used without further purification. Water was distilled and then passed through a Milli-Q water purification system. β-D-Nicotinamide adenine dinucleotide (NAD^+), 4-nitrophenyl β-D-galactopyranoside, and β-galactosidase from *Escherichia coli* (*E. coli*, Grade VIII) were purchased from Sigma. Galactose dehydrogenase from *E. coli* that contains the gene for the *Pseudomonas fluorescens* enzyme on a plasmid was purchased from Boehringer Mannheim or Sigma. The commercial preparation of galactose dehydrogenase was freed of ammonium sulfate by dialysis against 25 mM sodium pyrophosphate at pH 8.6 that contained 1 mM EDTA and the enzyme was assayed as described in earlier work.[14] 4-Nitrophenyl 2-deoxy-β-D-galactopyranoside (**H-1-OC$_6$H$_4$-4-NO$_2$**) was prepared by a published procedure.[27]

The solution pH was determined at the end of each kinetic experiment on β-galactosidase using an Orion Model 601A pH meter equipped with a Radiometer GK2321C combination electrode that was standardized at pH 7.00 and 10.00.

Enzyme assays

The activity of β-galactosidase was routinely determined at 25°C by monitoring the formation of 4-nitrophenoxide anion at 405 nm for reactions at pH 8.6 (25 mM sodium pyrophosphate) in solutions that contain 1.0 mM $MgCl_2$ and 0.5 mM **HO-1-OC$_6$H$_4$-4-NO$_2$**. β-Galactosidase-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** (0.1 mM) at pH 8.6 (25 mM sodium pyrophosphate) in solutions that contain 1.0 mM $MgCl_2$ was followed by monitoring the formation of 4-nitrophenoxide anion at 405 nm.[26] Control experiments for reactions in the presence of alkyl alcohols were performed which showed that the presence of the highest concentrations of alkyls alcohols used in these experiments caused a $\leq$5% reduction in the velocity of enzyme-catalyzed cleavage of **HO-1-OC$_6$H$_4$-4-NO$_2$**.

The initial velocities of the formation of 4-nitrophenoxide anion and of 2-deoxygalactose in the β-galactosidase-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** were determined at 25°C in a single assay solution that contained 25 mM sodium pyrophosphate (pH 8.6), 1.0 mM $MgCl_2$, 0.7 mM NAD^+, 0.1 mM **H-1-OC$_6$H$_4$-4-NO$_2$**, *ca.* 1 μM subunits β-galactosidase, and 2.3 units of galactose dehydrogenase in a total volume of 1.0 ml. The absorbance at 340 nm was monitored until it was constant with time (*ca.* 3 min) and β-galactosidase was added. The formation of 2-deoxygalactose was then monitored by following the increase in absorbance at 340 nm (ΔA_{340}), and the formation of 4-nitrophenoxide anion was monitored by following the increase in absorbance at 405 nm (ΔA_{405}). The enzyme-catalyzed reaction was allowed to reach steady-state over a period of several minutes[26]; and, the initial velocities for the formation of 2-deoxygalactose and 4-nitrophenoxide anion were then determined by following the reaction for an additional 10 min as described in earlier studies.[14]

$$(\Delta A_{340})_{Gal} = (\Delta A_{340})_{obsd} - \frac{\Delta A_{405}}{82} \quad (1)$$

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The initial velocity (<10% consumption of substrate) of formation of 4-nitrophenoxide anion (v_{PNP}) was calculated from ΔA_{405} using $\Delta\varepsilon = 18\,300\ M^{-1}\,cm^{-1}$ at pH 8.6.[26] The initial velocity of the formation of 2-deoxygalactose (v_{gal}) was calculated from the observed change in absorbance at 340 nm $(\Delta A_{340})_{obsd}$, with a small correction for the contribution to this absorbance change from the formation of 4-nitrophenoxide anion (Eqn (1)).

RESULTS

The initial velocity of β-galactosidase-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** (0.1 mM) to give 4-nitrophenoxide anion (v_{PNP}) and D-galactose (v_{gal}) was determined at pH 8.6 (25 mM pyrophosphate) in a single cuvette by monitoring: (a) the formation of 4-nitrophenoxide anion at 405 nm; and, (b) the formation of 2-deoxygalactose by trapping this sugar with NAD^+ in a reaction catalyzed by galactose dehydrogenase.[14,28] The UV spectra of 4-nitrophenoxide anion and NADH are well resolved, but there is overlap between the spectra of 4-nitrophenol and NADH. Therefore, these experiments were conducted at pH 8.6 where 4-nitrophenol is largely ionized.[14]

Control experiments showed the following: (1) there is no significant change in absorbance at 405 nm associated with the reduction of NAD^+; (2) the change in absorbance at 340 nm due to the formation of 4-nitrophenoxide anion is 82-fold smaller than the change in absorbance at 405 nm. (3) The ratio of the velocities for formation of 4-nitrophenoxide anion and 2-deoxygalactose, v_{PNP}/v_{gal}, is independent of the concentration of the galactose dehydrogenase coupling enzyme.

A ratio of $v_{gal}/v_{PNP} = 1.0$ is observed for stoichiometric formation of 4-nitrophenoxide anion and 2-deoxygalactose in water. This ratio increases when the intermediate is trapped by added alcohols to form **H-1-OR** at the expense of D-2-deoxygalactose (**H-1-OH**). The velocity of formation of **H-1-OR** is equal to the difference between the total velocity of cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** (v_{PNP}) and the velocity of formation **H-1-OH** (v_{gal}).[14]

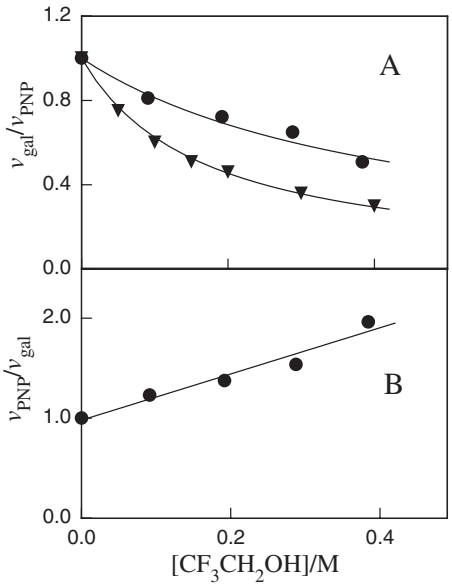


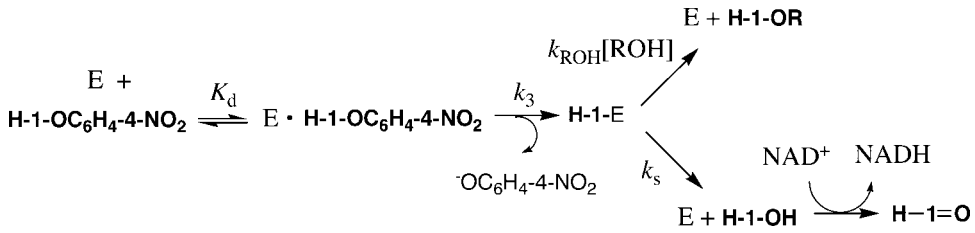
Figure 1. (A) The effect of increasing initial concentration of trifluoroethanol on the ratio of the velocities for the formation of 4-nitrophenoxide anion (v_{PNP}) and of 2-deoxygalactose (v_{gal}) in the β-galactosidase-catalyzed reactions of 4-nitrophenyl 2-deoxy-β-D-galactopyranoside at pH 8.6 (●); and, 4-nitrophenyl β-D-galactopyranoside at pH 8.6 (▼).[14] (B) A linear replot of data from Fig. 1(A) for the reaction of 4-nitrophenyl 2-deoxy-β-D-galactopyranoside

Figure 1(A) shows the decrease in v_{gal}/v_{PNP} for the reaction of **H-1-OC$_6$H$_4$-4-NO$_2$** (●) in the presence of increasing initial con-

Table 1. Rate constant ratios for partitioning of **X-1-E** (Scheme 2) between reaction with alkyl alcohols and water and, absolute reaction rate constants[a]

		H-1-OC$_6$H$_4$-4-NO$_2$		**HO-1-OC$_6$H$_4$-4-NO$_2$**[c]	
ROH	pK_a[b]	k_{ROH}/k_s[d] (M^{-1})	k_{ROH} ($M^{-1}\,s^{-1}$)[e]	k_{ROH}/k_s (M^{-1})	k_{ROH} ($M^{-1}\,s^{-1}$)
CH_3CH_2OH	16.0	1.02 ± 0.07	0.0020	1.1	780
CH_3OH	15.5	1.40 ± 0.05	0.0028	2.3	1600
$HOCH_2CH_2OH$	15.1	4.4 ± 0.2	0.0088	4.9	3500
$CH_3OCH_2CH_2OH$	14.8	3.05 ± 0.08	0.0061	2.9	2060
$ClCH_2CH_2OH$	14.3	6.1 ± 0.2	0.013	11.7	8300
FCH_2CH_2OH	14.2	1.8 ± 0.2	0.0036	2.0	1420
Cl_2CHCH_2OH	12.9	3.3 ± 0.2	0.0066	10.2	7200
F_3CCH_2OH	12.4	2.2 ± 0.2	0.0044	6.0	4200
		2.3 ± 0.2[f]	0.0046		

[a] At 25°C in 25 mM sodium pyrophosphate buffer (pH 8.6) containing 1.0 mM $MgCl_2$.
[b] Reference [34].
[c] Data from Reference [14].
[d] Rate constant ratios for partitioning of **H-1-E** (Scheme 2) between addition of ROH and water, determined as the slopes of the correlations shown in Fig. 2. The quoted errors are standard deviations.
[e] Calculated from k_{ROH}/k_s and $k_s = 0.002$.[26]
[f] Determined by analysis of data from Fig. 2 as described in the text.



Scheme 2.

centrations of trifluoroethanol; and, earlier data for the reaction of **HO-1-OC$_6$H$_4$-4-NO$_2$ (▼)**.[14] Figure 1(B) shows the linear increase in v_{PNP}/v_{gal}. The rate constant ratio $k_{ROH}/k_s = 2.3\ M^{-1}$ (Table 1) for partitioning of the 2-deoxy-β-D-galactopyranosyl reaction intermediate between reaction with trifluoroethanol and with water was obtained as the slope of this linear correlations using Eqn (2) derived for Scheme 2.

$$v_{PNP}/v_{gal} = 1 + k_{ROH}[ROH]/k_s \quad (2)$$

Figure 2 shows linear plots of normalized initial steady-state velocities v_{obsd}/v_o for the β-galactosidase-catalyzed reaction of **H-1-OC$_6$H$_4$-4-NO$_2$** (0.1 mM) for reactions carried out in the presence of increasing initial concentrations of alkyl alcohols at pH 8.6, where v_0 is the velocity of the reaction when no alcohol is present. The addition of ROH to **H-1-E** to form **H-1-OR** causes an increase in the observed velocity (v_{obsd}) for cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** to form 4-nitrophenoxide anion. This is because the breakdown of **H-1-E** is the rate-determining for enzyme-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$**.[26] Downward curvature (not shown) was observed for some plots as [ROH] was increased to 1.0 M. We attribute this to: (1) a partial change in rate-determining step for the enzyme-catalyzed reactions when the value of ($k_s + k_{ROH}$[ROH]) approaches that for k_3 (Scheme 2);

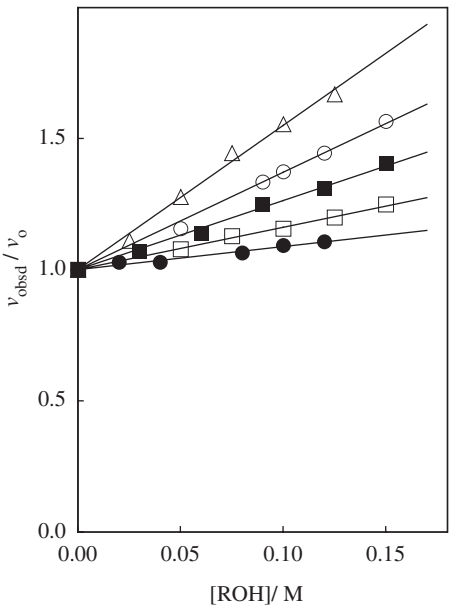


Figure 2. The effect of increasing initial concentrations of alkyl alcohols on the initial velocity of β-galactosidase-catalyzed cleavage of 0.10 mM **H-1-OC$_6$H$_4$-4-NO$_2$** at pH 8.6, monitored by following the formation of 4-nitrophenoxide anion at 405 nm

and, (2) a medium effect that causes a decrease in the rate constant k_3 for the formation of the reaction intermediate (Scheme 2). Control experiments performed for each alcohol showed that the highest [ROH] used in the "trapping" studies (Fig. 2) has no effect ($\leq$5%) on the initial velocity of cleavage of **HO-1-OC$_6$H$_4$-4-NO$_2$**, a substrate for which k_3 (Scheme 2) is the rate-determining step.[14]

$$\upsilon_{obsd} = (k_{cat})_{app}[E][S] = \frac{k_3(k_s + k_{ROH}[ROH])[E][S]}{(k_3 + k_s + k_{ROH}[ROH])} \quad (3)$$

$$\frac{\upsilon_{obsd}}{\upsilon_0} = 1 + \frac{k_{ROH}[ROH]}{k_s} \quad (4)$$

Equation (3) derived for Scheme 2 shows the dependence on [ROH] of the observed initial velocity of β-galactosidase-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$**.[29] Equation (3) simplifies to Eqn (4) under the conditions of the experiments reported in Fig. 2 because, $k_3 \gg k_s + k_{ROH}$[ROH].[26] The solid lines through the experimental data in Fig. 2 show the theoretical fit using the slopes k_{ROH}/k_s (M^{-1}) reported in Table 1, determined by least-squares analysis.

DISCUSSION

A simple method (Scheme 2) was used to monitor the initial velocity of β-galactosidase-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** to form 4-nitrophenoxide anion (v_{PNP}) and of the hydrolysis reaction to form D-2-deoxygalactose (v_{gal}).[14] A rate constant ratio of $k_{ROH}/k_s = 2.3\ M^{-1}$ was determined from the linear increase in v_{PNP}/v_{gal} for reactions in the presence of increasing initial concentration of trifluoroethanol (Fig. 1(B)). Rate constant ratios for partitioning of **H-1-E** (Scheme 2) between addition of alkyl alcohols and water were also determined by examining the effect of increasing [ROH] on the initial velocity of β-galactosidase-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** (v_{obsd}) when the breakdown of **H-1-E** is rate determining for turnover (Fig. 2). There is good agreement between $k_{ROH}/k_s = 2.3\ M^{-1}$ determined for the reaction of trifluoroethanol by monitoring the relative yields of **H-1-OH** and of **H-1-OCH$_2$CF$_3$**, and $k_{ROH}/k_s = 2.2\ M^{-1}$ determined by examining the effect of trifluoroethanol on the rate of breakdown of **H-1-E** (Table 1).

Figure 1(A) shows that increasing [CF_3CH_2OH] has a smaller effect on the yields of 2-deoxygalactose from β-galactosidase-catalyzed cleavage of **H-1-OC$_6$H$_4$-4-NO$_2$** than on the yields of galactose from enzyme-catalyzed cleavage of **HO-1-OC$_6$H$_4$-4-NO$_2$**.[14] We conclude that the 2-H for 2-OH substitution causes a *decrease* in the selectivity **X-1-OH** for the addition of weakly nucleophilic trifluoroethanol. A similar decrease in selectivity is

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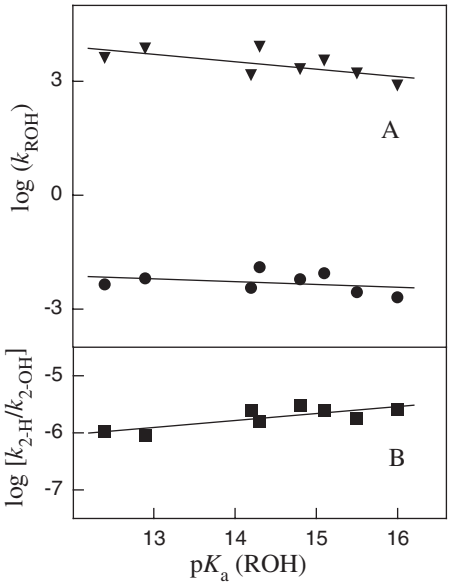


Figure 3. (A) Brønsted correlations with slope $\beta_{nuc} = -0.19 \pm 0.10$ and -0.07 ± 0.08 for the addition of alkyl alcohols to the β-D-galactopyranosyl enzyme intermediate (**HO-1-E,** ▼)[14] and to the 2-deoxy-β-D-galacto-pyranosyl intermediate (**H-1-E,** ●) of β-galactosidase-catalyzed cleavage reactions. (B) Brønsted correlation of the ratio of rate constants for the addition of alcohols to **H-1-E** ($k_{2\text{-H}}$) and to **HO-1-E** ($k_{2\text{-OH}}$) with slope $(\Delta\beta)_{nuc} = 0.12 \pm 0.04$

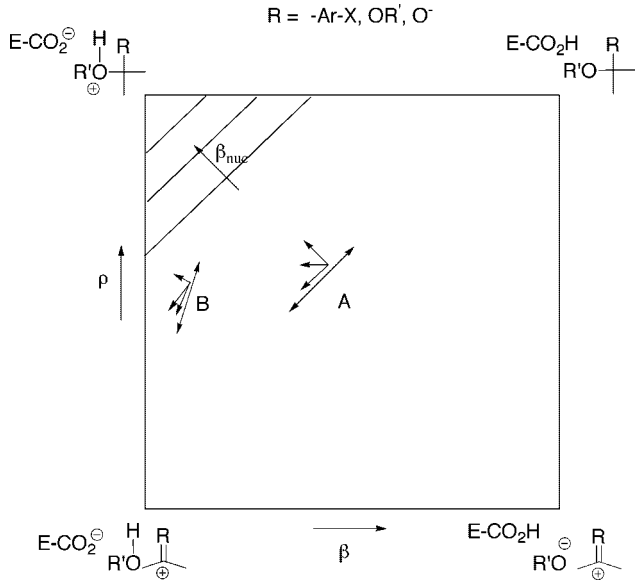


Figure 4. Reaction coordinate diagram for general base catalysis of the addition of alcohols to electrophilic trivalent carbon. The *x*- and *y*-axes represent proton transfer and C–O bond formation, respectively. The values of β_{nuc} are indicated by diagonal lines of constant change at the alcohol oxygen. The reaction coordinates *A* and *B* are discussed in the text

also observed for the reactions of the other alkyl alcohols examined in this work (Table 1).

Absolute rate constants k_{ROH} ($M^{-1}\,s^{-1}$) for the addition of ROH to **H-1-E** (Table 1) were calculated from the alcohol selectivity and $k_s = 0.002\,s^{-1}$ for the reaction of solvent water.[26] Figure 3(A) shows Brønsted plots of the rate constants k_{ROH} ($M^{-1}\,s^{-1}$) against the pK_a of the alkyl alcohol for the reactions of **H-1-E**. Also shown in Fig. 3(A) is the Brønsted correlation for the addition of ROH to **HO-1-E** reported in earlier work.[14] These data are correlated by slopes of $\beta_{nuc} = -0.07 \pm 0.08$ and -0.19 ± 0.010 for the addition of ROH to **H-1-E** and **HO-1-E**,[14] respectively.

The significant deviations from the Brønsted correlations shown in Fig. 3(A) cause a relatively large uncertainty in the Brønsted parameters β_{nuc}. A better logarithmic correlation is observed for the rate constant ratio, $k_{2\text{-H}}/k_{2\text{-OH}}$, against alcohol p$K_a$ (Fig. 3(B)). The slope of this correlation, $\Delta\beta_{nuc} = 0.12 \pm 0.04$, is equal to the difference in the Brønsted coefficients for alcohol addition to **H-1-E** and to **HO-1-E**. This shows that similar deviations from Brønsted correlations are observed for rate constants for the addition of individual alcohols to **H-1-E** and **HO-1-E** and that the deviations cancel in the ratio $k_{2\text{-H}}/k_{2\text{-OH}}$. The deviations from the correlations shown Fig. 3(A) have been proposed to result from specific binding interactions between β-galactosidase and the alkyl groups of ROH.[13,14]

Figure 4 shows a structure-reactivity diagram for the addition of alcohols to electrophilic trivalent carbon, where alcohol addition is assisted by general base catalysis.[18] This diagram assigns separate reaction coordinates to the bond formation between carbon and the oxygen nucleophile (*y*-axis) and to proton transfer from the alcohol to the base catalyst (*x*-axis). The third *z*-axis is for energy, but the energy contour lines are not shown in order to simplify the diagram. The value of β_{nuc} for the addition of alcohols determined in this work provides a measure of the change in the *effective* charge at the alcohol oxygen.[5] This depends upon the extent of C—O bond formation, and of proton transfer to the base catalyst.[3] The values of β_{nuc} are indicated in Fig. 4 by diagonal lines of constant charge at the alcohol oxygen.

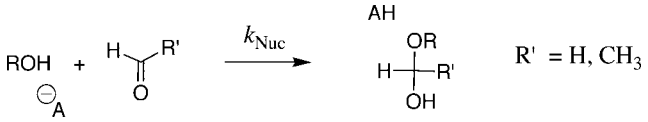
Systematic variations in reactant structure that cause the driving force for a chemical reaction to change may cause one of two types of changes in transition state structure.

(1) *A Hammond-type shift*. Variations in structure that cause the energy of a species to increase in a direction that is *parallel* to that of the reaction coordinate will cause the structure of the transition state to move toward that of the species of increasing energy.[22] This change in transition state structure results in the decrease in nucleophile selectivity with increasing carbocation reactivity associated with the reactivity-selectivity principal.[30]

(2) *An anti-Hammond-type shift*. Variations in structure that cause the energy of a species to increase in a direction that lies *perpendicular* to that of the reaction coordinate will cause the structure of the transition state to move away from that of the species of increasing energy.[3,4]

The direct addition of alcohols to carbocations is described by a one-dimension reaction coordinate, where changes in the relative energy of reactants and products must lie *parallel* to the reaction coordinate and cause Hammond-type shifts in transition state structure. For example, changes from electron-donating to electron-withdrawing ring-substituents that destabilize ring-substituted 1-phenylethyl carbocations cause a shift to an earlier transition state for alcohol addition that more closely resembles the reactant 1-phenylethyl carbocation.[31]

General base catalysis of the addition of alcohols to relatively stable carbonyl electrophiles proceeds by a fully concerted mechanism with a diagonal reaction coordinate (*A*, Fig. 4).[24]



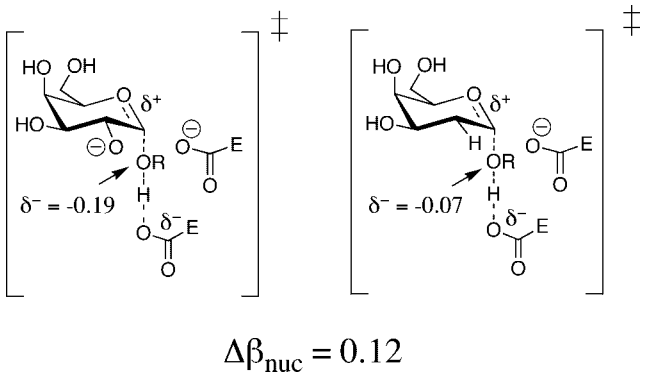
Scheme 3.

Substituents that destabilize the electrophile reactant increase the energy of the bottom edge of the reaction diagram relative to the top edge. This causes both Hammond and anti-Hammond shifts in the position of the transition state. For example, formaldehyde is more reactive than acetaldehyde and might therefore be expected to react through an earlier transition state for addition of ROH, and show a less positive value of β_{nuc} for alcohol addition. Instead, the value of β_{nuc} is 0.17 units more positive for general base-catalyzed addition of alkyl alcohols to formaldehyde compared to acetaldehyde (Scheme 3).[25] This result is consistent with a diagonal reaction coordinate (*A*, Fig. 4), where movement of the proton toward the base catalyst is tightly coupled to C—O bond formation. Increasing electrophile reactivity by replacing the methyl group of acetaldehyde with hydrogen causes an increase in the energy of the bottom edge of Fig. 4 relative to the top edge, and results in both Hammond and anti-Hammond shifts in the position of the transition state. The vector sum of these two shifts is in accord with the observed increase in β_{nuc} for the addition of ROH (*A*, Fig. 4).

The value of β_{nuc} for uncatalyzed addition of alcohols to ring-substituted 1-phenylethyl carbocations increases with decreasing carbocation reactivity, as expected for a reaction that can be treated by a one-dimension reaction coordinate diagram.[31] There is no general base catalysis of the addition of alkyl alcohols to the 1-(4-methylphenyl)ethyl carbocation (Scheme 4).[23] General base catalysis by carboxylate anions *appears* for the addition of alcohols to the more stable 1-(4-methoxyphenyl)ethyl carbocation,[23] and becomes more pronounced for addition to the more stable 1-(4-dimethylaminophenyl)ethyl carbocation.[32]

There is little difference in the values of β_{nuc} for acetate anion-catalyzed addition of alkyl alcohols to the 1-(4-methoxyphenyl)-ethyl carbocation and to the much more stable 1-(4-dimethyl-aminophenyl)-ethyl carbocation.[32] This result is consistent with a reaction coordinate that has been rotated in a clockwise direction from the vertical coordinate for a fully stepwise reaction, but is not strongly diagonal (coordinate *B*, Fig. 4). The small change in β_{nuc} for alcohol addition shows that the shift in the position of the transition state for nucleophile addition with changing electrophile reactivity, which is obtained as the vector sum of changes in the position of the transition state caused by parallel and perpendicular substituent effects, occurs along a diagonal line of constant charge at the alcohol nucleophile.

The large selectivity observed for the transfer of the enzyme-bound β-D-galactopyranosyl group to *nucleophilic anions* provides good evidence for a stepwise reaction through an enzyme-bound galactosyl oxocarbenium ion intermediate in which there are differing degrees of stabilization of the transition

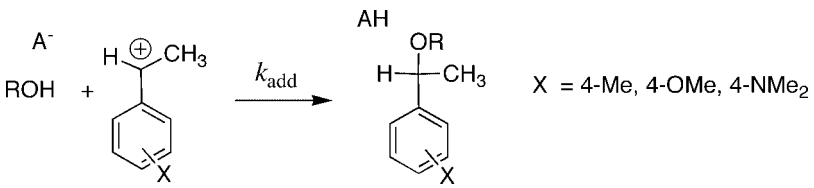


Scheme 5.

state for its capture by interactions with anions of differing nucleophilic reactivity.[17] It has been proposed, by analogy, that the transfer of the enzyme-bound β-D-galactopyranosyl group to alcohols also proceeds by a stepwise mechanism through an oxocarbenium ion intermediate, with assistance from general base catalysis by the carboxylate anion side chain of Glu-461.[17,33]

The 2-H for 2-OH substitution at **HO-1-E** causes a 320 000-fold decrease in k_s for hydrolysis to form the sugar product.[26] This effect on k_s for transfer of the sugar from β-galactosidase to water or alkyl alcohols is due to the loss of specific interactions (*ca.* 7.5 kcal/mol) that stabilize the transition state for cleavage of the covalent intermediate to form the enzyme-bound sugar oxocarbenium ion. We have proposed that these stabilizing interactions result because of the ionization of the 2-OH group to form an alkoxide anion (2-O^-) which interacts strongly and favorably with the neighboring positive charge at the oxocarbenium ion.[26]

The 2-H for 2-OH substitution destabilizes the transition state for cleavage of **X-1-E** to form the putative glycosyl oxocarbenium ion and should destabilize the fully formed cation. This corresponds to an increase in the energy of the bottom edge of Fig. 4 relative to the top edge. This change results in a 0.12 unit *increase* in β_{nuc} (5)for alcohol addition to **X-1-E** that resembles the *increase* in β_{nuc} with increasing electrophile reactivity observed for the addition of alcohols to simple aldehydes (Scheme 3), where there is a high degree of coupling of proton transfer to C—O bond formation (coordinate *A*, Fig. 4).[24,25] These results provide evidence that the reaction coordinate for general base-catalyzed alcohol addition to an enzyme-bound sugar oxocarbenium ion shows a more strongly diagonal component than the coordinate for general base-catalyzed addition of alcohols to ring-substituted 1-phenylethyl carbocations (*B*, Fig. 4). The proposed diagonal reaction coordinate might be caused by a high pK_a for the Brønsted base catalyst for the breakdown of **X-1-E**,[33] and/or reflect a particularly large stabilization of the enzyme-bound glycosyl oxocarbenium ion by interaction with the enzyme catalyst.[17,32]



Scheme 4.

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