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Artificial Receptors Involved in Enolization and pK_a Shifts

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We dedicate this manuscript in honor of Professor Peter Dervan, a pioneer in the field of bioorganic chemistry, and a masterful teacher and mentor for students in the chemical sciences

Abstract—Abiotic receptors used to enolize carbonyl compounds or to shift substrate pK_a values are reviewed. These systems exhibit disparate frameworks and several approaches to binding and anion stabilization. Detailed emphasis is placed on a bicyclic cyclophane that induces pK_a shifts in active methylene compounds through NH–π hydrogen bonding with the resultant enolates.

Introduction

Few synthetic receptors have been evaluated as enolase mimics or for pK_a shift studies. This may largely be due to the intrinsic thermodynamic difficulty in deprotonating weakly acidic substrates with functional groups that are basic near neutral pH. Also, intramolecular bifunctional acid-base catalysts can pose a synthetic challenge, because such systems require a rigid structural architecture to restrain the acid and base from strongly associating and the binding cavity from collapsing. Receptors necessitate some type of electrophilic catalysis such as metal–ion, charge pairing, or hydrogen bonding interactions to dramatically increase the acidity of the substrate. Incorporation of a hydrophobic binding pocket into the receptor surrounding the region of electrophilic interaction, as in enzyme active sites, may enhance substrate acidity and lead to more competent artificial enolase, racemase, aldolase, or isomerase mimics. These features are manifest in the following examples that may set the stage for meliorated enolase mimics and for delineating the roles of specific active site residues in enzyme reactions involving pK_a shifts or the stabilization of otherwise high-energy enolate intermediates.

Herein, we review the use of synthetic receptors for two related uses: as enolization catalysts and for shifting carbon acid pK_a values. This review sets the stage for an expanded analysis that we give of a cyclophane receptor we have previously reported can shift the pK_a's of active methylene compounds. Therefore, this paper delineates the current stage of development of receptors, from our labs and others, for the study of enolase mimics.

Artificial Receptors in Enolization Reactions

Cyclodextrins exhibit several characteristics that make them attractive potential enzyme mimics (Fig. 1). They possess hydrophobic interiors allowing them to sequester organic substrates from aqueous or polar organic solutions, while remaining water-soluble. Hydroxyl groups line the interior wall (secondary face) of the cyclodextrin cavity and extend away from the exterior surface (primary face) allowing selective functionalization at strategic positions within or outside the cavity. This allows simple preparation of potential multi-functional catalysts. For example, by appending an electrophile to one face and a Brønsted base to the other, a bifunctional molecule with a hydrophobic core can be constructed. Cyclodextrins do suffer from disadvantages, however. They are flexible, making complexation geometries difficult to predict. Also, being oligosaccharides, cyclodextrins are prone to acid decomposition, limiting reaction conditions and choices of Lewis or Brønsted acids that may be used as catalytic functional groups or in the synthesis of a potential catalyst.[1]

Breslow and coworkers have pioneered the use of modified β-cyclodextrins, (β-CDs or cycloheptaamyloses) as

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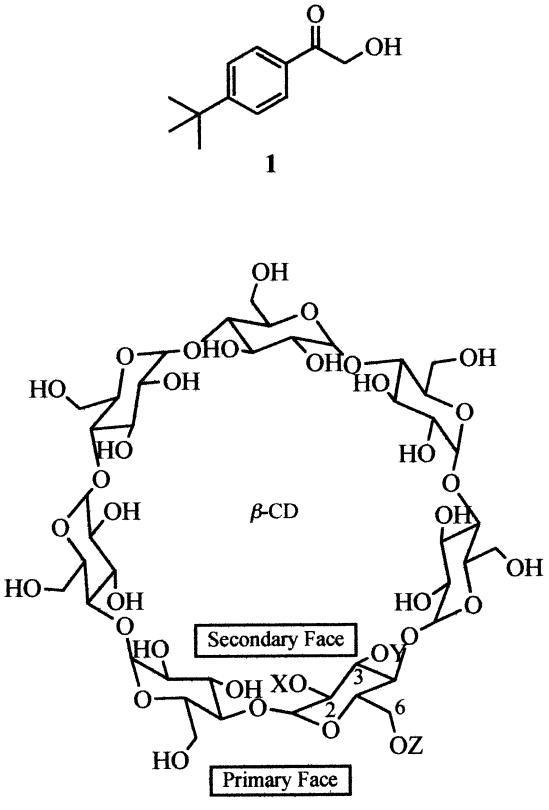
templates for enolization reactions. Their first functionalized β-cyclodextrin for this purpose was used in the base-catalyzed enolization of *p-tert*-butylphenacyl alcohol (**1**) (Fig. 1).[2] Tritium–hydrogen exchange in **1** shows both specific base (hydroxide ion) and general base catalysis (HCO_3^-/CO_3^{-2}). Introduction of β-CD (**2**) resulted in enhanced catalysis by complexation of the α-hydroxyarylketone, $K_a = 8.3\times10^5\ M^{-1}$, with a rate constant for isotope exchange 3.2 times greater than that for the buffer solution alone at pH 12.03. Phosphate disodium salt derivatives of β-CD, **3**, **4**, and **5**, gave comparable association constants to **2** and showed saturation kinetics in the tritium-hydrogen exchange near pH 9.0. Dianionic **3**, **4**, and **5** provided 430-, 690-, and 650-fold catalysis compared to k_{OH}-, respectively. These derivatives were only effective in their dianionic form, as monoanionic, monoprotic **4** showed no rate enhancement compared to β-CD. Also, the dianion of *trans*-cyclohexane-1,2-diol monophosphate showed no detectable catalysis of exchange with **1**.

Catalyst **4**, functionalized at C-3, was thought to be more effective than **3** because substitution at C-2 might crowd the cavity resulting in competitive external binding to the primary face, away from the catalytic phosphate and the hydrophobic core. Because the observed catalytic rate constants are actually the rate constants for productive binding to the appropriate edge of the β-CD where the catalytic phosphate is attached multiplied by the fraction of the complex having this binding relationship, crowding at C-2 could lead to substantial unproductive binding to the primary face even though the actual rate constant for exchange is greater with the phosphate positioned at C-2 than at C-3. The authors suggest that capping the cyclodextrin or incorporating additional binding interactions could lead to enhanced productive binding at the appropriate edge and therefore larger observed catalytic rate constants.

More recently, Breslow and Graf examined bifunctional β-cyclodextrin C-6 functionalized bis(imidazole) receptors as catalysts in the enolization of *p-tert*-butylacetophenone (Fig. 2).[3] Deuterium–hydrogen exchange was followed by GC/MS using CI (CH_4). The catalyst concentration was 6 mM with the ketone at 2 mM in D_2O (170 mM phosphate buffer) containing 14% CD_3OD, conditions leading to saturation binding. At pD 6.2, the approximate pK_a of imidazolium, catalysis was not observed by buffer or by β-CD and imidazole. Catalyst **6**, however, showed catalysis ($k_{obs} = 2.0\times10^{-6}\ s^{-1}$ at pD 5.95) and a bell-shaped k_{obs} versus pD profile between pD 4.5 and 7.5. This suggests **6** acts as a bifunctional general acid-general base catalyst (Fig. 3). Other



2 X = Y = Z = H
3 X = PO_3H_2, Y = Z = H
4 Y = PO_3H_2, X = Z = H
5 Z = PO_3H_2, X = Y = H

Figure 1. Diagram showing β-cyclodextrin (**2**) and Breslow et al.'s phosphate derivatives (**3**–**5**) used in the enolization of *para-tert*-butylphenacyl alcohol (**1**).

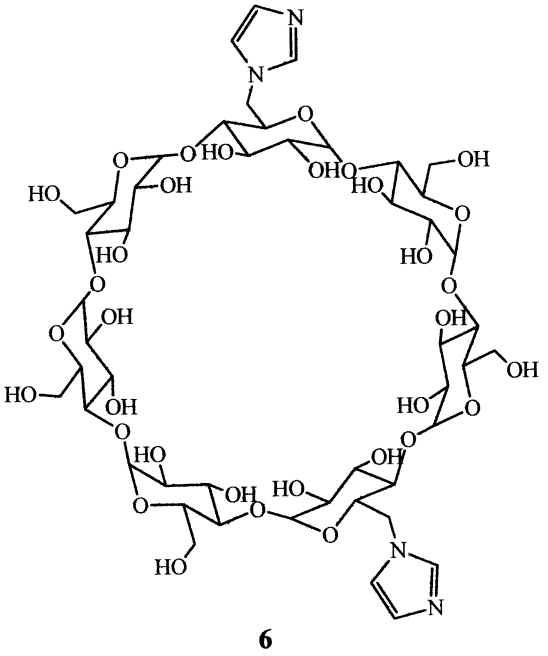


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Figure 2. Bifunctional β-cyclodextrin C-6 functionalized bis(imidazole) receptors.

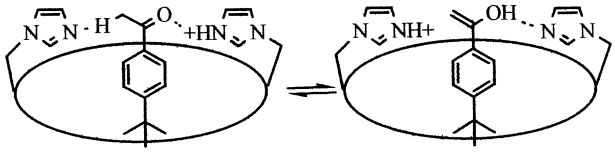


Figure 3. Schematic of β-CD catalyst **6** acting as a bifunctional general acid–general base catalyst in the enolization of *para-tert*-butylacetophenone.

isomers bearing only one imidazole or two imidazoles attached to different amylose subunits of β-CD also displayed catalysis, but to a lesser extent than **6**. These isomers showed sigmoidal rate versus pD profiles indicative of only monofunctional general base catalysis.[4]

Although molecule **6** was the first example of an abiotic bifunctional catalyst for enolization in water, Rebek et al. evaluated molecule **7**, a bis(Kemp's triacid) acridine derivative, earlier in $CHCl_3$ (Fig. 4).[5] The receptor's carboxylic acids are arranged orthogonal to the acridine's basic lone pair electrons, allowing complexation of quinuclidinone (**8**) and ensuant deprotonation to catalyze substrate enolization (Fig. 2.4). Titration of **7** into a solution of **8** (dry $CDCl_3$) gave a K_d of $10^{-3}\,M^{-1}$. Hydrogen/deuterium exchange of the complexed enol of **8** (in $CDCl_3$ saturated with D_2O beginning with 10 mol% of **7**) showed saturation kinetics with a $k_{obs} = 1.7\times10^{6}\,s^{-1}$ and a $k_{cat} = 2.5\times10^{-3}\,s^{-1}$. It is proposed that the acridine nitrogen acts as a general base to deprotonate the substrate while a proton is transferred from a carboxylic acid (complex **9**), perhaps giving concerted general acid-base catalyzed enolization of **8** and thereby allowing rapid deuteration during ketonization of the substrate.

Lehn et al. have used aza-crowns to catalyze H/D exchange in malonate (Fig. 5).[6] Macrocyclic polyamines combine several properties that make them potentially suitable as general acid-base catalysts. These include carrying multiple protonated amines while simultaneously offering unprotonated nucleophilic sites and serving as a proton reservoir for a complexed substrate. Aza-crown **10**, in its tetraprotonated form near pH 7, is known to bind malonate dianion with a $K_a = 2.4\times10^{2}\,M^{-1}$.[7] Isotope exchange of the methylene protons was followed by

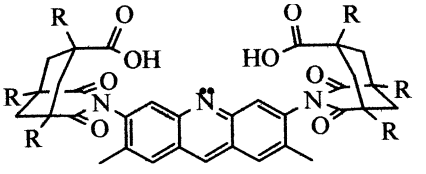


Figure 4. Bis(Kemp's triacid) acridine derivative (**7**) used in the bifunctional enolization of quinuclidinone (**8**) shown in complex **9**.

^1H NMR spectroscopy in D_2O at variable pD, ionic strength, and temperature values in the presence and absence of **10**. Malonate displayed saturation kinetics with a $K_m = 3.8\times10^{-3}\,M$, and $k_{cat} = 1.75\times10^{-3}\,s^{-1}$ at pD = 7 and 25 °C giving a rate enhancement of 1.4×10^{3} relative to the uncatalyzed H/D exchange. Diethylenetriamine only gave a rate enhancement near a factor of 6. The reaction requires a pH near 7 in order to allow complex formation between malonate dianion and protonated **10** (malonic acid pK_a's = 2.63, 5.27[8] and the polyamine is unprotonated near pH = 10).

Macrocycle **11** was also examined, showing a $K_m = 7\times10^{-2}\,M$ and an improved rate enhancement of 1.8×10^{5} relative to the uncatalyzed exchange, despite its much larger cavity than **10**. The authors suggest **11** may form a pocket-shaped conformation, based upon crystallographic data,[9] into which malonate might fit. Experiments could not be run under saturation conditions because k_{obs} was too fast to follow by NMR spectroscopy.

Acceleration of H/D exchange using **10** and **11** likely results from combined effects including: neutralization of the dianion by the polyammonium receptor, electrostatic attraction of a base from the reaction medium by the polycationic complex, and sites for proton transfer and proton abstraction within the receptor framework. The protonated aza-crowns would likely lead to electrophilic catalysis. Protonation of a mandelic acid carbonyl can reduce the α-CH pK_a by 15 units.[10] Proton abstraction from the methylene and proton transfer to one of the carboxylates would produce a dianionic intermediate [$^-O_2CCH{=}C(O^-)OH$] that would remain tightly bound with the polyammonium receptor, existing along a lower energy reaction path for deprotonation and subsequent H/D exchange.[11] The precise mechanism is conjectural, nonetheless these aza-crown macrocycles show catalysis of enolization competitive with, or superior to, more elaborate receptors described herein.

Perhaps inspired by the successes of receptor **10** and the functionalized β-cyclodextrins in enolization reactions, Menger and Binder investigated heptakis-6-amino-6-deoxy-β-cyclodextrin (**12**) as a catalyst for H/D exchange (Fig. 6).[12] They examined an assortment of 12 substrates with the catalyst at 0.4 mM and the substrate

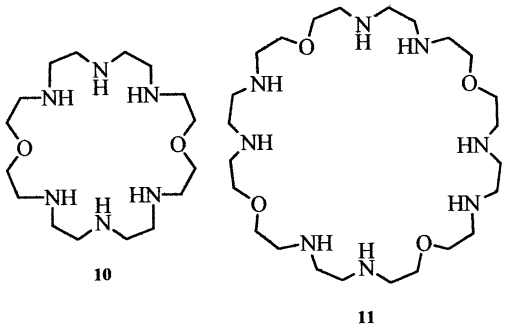


Figure 5. Aza-crowns used by Lehn et al. to catalyze H/D exchange in malonate.

at 5.0 mM concentrations in D_2O at constant pD values by NMR spectroscopy, following the disappearance of the proton(s) alpha to the carbonyl. Reactions went to completion, despite the excess substrate, thereby showing turnover with **12**. The rate of hydrogen exchange in malonate was accelerated by a factor of 150 ($k_{obs} = 7.6\times10^{-4}\,s^{-1}$) at pD = 6.5 and, as with catalyst **6**, was strongly pD-dependant (maximized near pD 6.5–7.0) perhaps registering bifunctional catalysis in the macrocycle.

Six 2-oxo-1-carboxylic acid derivatives were also investigated with **12** present. Exchange in pyruvate and its *n*-alkyl derivatives was accelerated by a factor of 1.6×10^3–3.8×10^3 at pD 6.5 (k_{obs} values ranging from $3.1\times10^{-4}\,s^{-1}$ to $1.6\times10^{-4}\,s^{-1}$), whereas 2-oxo-3-methyl-1-butanoic acid exhibited a rate acceleration of 300 ($k_{obs} = 3.8\times10^{-5}\,s^{-1}$) and cyclopropylglyoxylic acid showed no H/D exchange. Macrocycle **12** was also capable of catalyzing exchange in acetaldehyde ($k_{obs} = 1.7\times10^{-4}\,s^{-1}$), but not in acetone or 2-hexanone, or in glycolate or phenylacetate. Meanwhile, a monomeric control, methyl-*O*-6-amino-6-deoxy-α-D-glucopyranoside, resulted in virtually no rate acceleration for H/D exchange with pyruvate at pD = 6.5. This suggests the amine of the monomer is protonated near neutral pH, while **12** retains some free amino groups along with the ammoniums. Hence, **12** is a more competent general acid/general base catalyst for enolization than the non-macrocyclic amino/ammonium control near neutral pH.

Isied et al. have prepared a receptor for binding electron-withdrawing aryl substituted barbituric acid enolates and barbituric acids attached to ruthenium polypyridine moieties (complex **13** in Fig. 7).[13] However, they have not investigated rates of enolization or H/D exchange, or pK_a shifts to date. The receptor is capable of forming six hydrogen-bonding contacts with a suitable substrate and is selective for barbituric acid enolates relative to the corresponding enols, which disrupt the complimentary hydrogen bonding between the receptor and the enols.

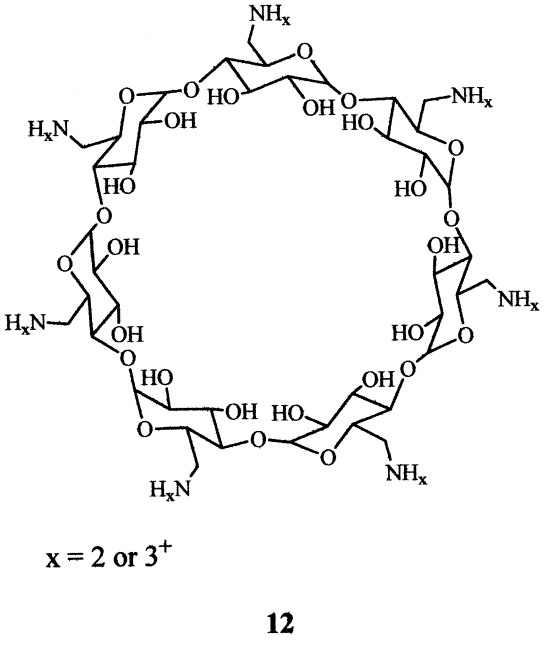


Figure 6. Heptakis-6-amino-6-deoxy-β-cyclodextrin (**12**) used by Menger and Binder to catalyze H/D exchange in a variety of carbonyl substrates.

Artificial Receptors used in pK_a Shift Experiments

Cyclen **14a** and its Zn(II) complex **14b** (Fig. 8) were prepared by Kimura and coworkers to investigate pK_a shifts in the N(1)H position of uracil,[14] the site of deglycosylation by uracil–DNA glycosylase.[15] It was thought that factors leading to increased acidity at the N(1) position might explain the stabilization of the uracil $N(1)^-$ anion in the transition state during hydrolysis of the glycosidic bind at physiological pH, and in its selective activation for phosphoribosylation in the presence of orotate and metal ions.[16] The pK_a value determined for the N(1)H of **14a** by potentiometric, UV and ^{13}C NMR measurements was 7.14, 2.73 units lower than that of free 3-*N*-methyluracil.[17] The stabilization of the resulting anion arises from an electrostatic field effect from the attached dicationic cyclen. As the solution pH is elevated and the cyclen deprotonates, the uracil anion

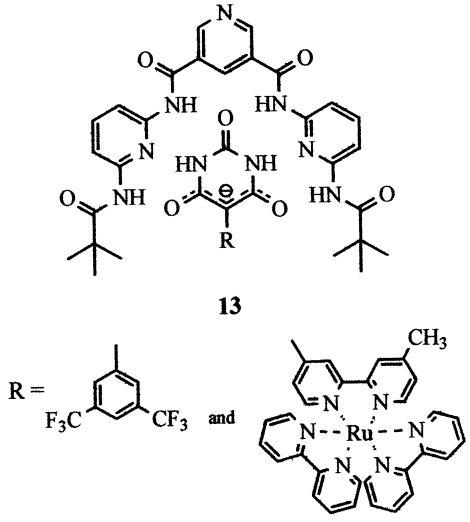


Figure 7. Polycyclic receptor for binding electron-withdrawing aryl substituted barbituric acid enolates and barbituric acids attached to ruthenium polypyridine moieties.

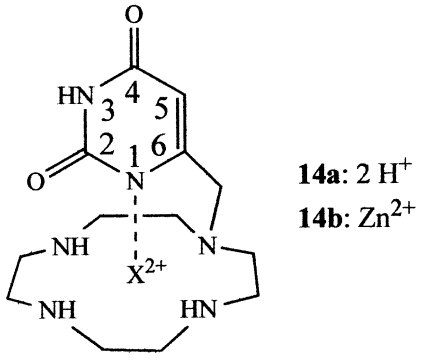


Figure 8. Cyclen **14a** and its Zn(II) complex **14b** prepared by Kimura and coworkers to investigate pK_a shifts in the N(1)H position of uracil.

delocalizes to N(3) as well, eliminating any preference for electrophilic attack (glycosidic bond formation) or nucleophilic displacement (glycosidic bond hydrolysis) at N(1).

The introduction of divalent Zn(II) into the cyclen has an enhanced effect on the stabilization of the $N(1)^-$ anion, decreasing the N(1)H pK_a value to ~4, as estimated by UV spectrophotometry. This results primarily from coordination of the $N(1)^-$ anion with the Zn^{2+}, as supported by an X-ray crystal structure and the large stability constant of complex **14b** [log $K_{(Zn\text{-}L\text{-})} = 20.2$].

A 4-bromophenacyl-pendant cyclen (**15a** in Fig. 9) was synthesized by Kimura et al. to evaluate the potential role of the zinc cation in the reversible aldol condensation catalyzed by class II aldolases.[18] The primary goal was to determine how much the pK_a value of the active methylene of the dihydroxyacetone phosphate substrate is lowered by the proximal zinc (II) ion in class II aldolases. The carbonyl-bound Zn^{2+} complex **15a** was determined to have an α-methylene pK_a value of 8.41. This is nearly 10 units lower than that of the acetophenone methyl group ($pK_a = 18.3$),[19] although the inductively electron withdrawing cyclen likely makes the 4-bromophenacyl-pendant substituent inherently more acidic than acetophenone. Potentiometric titration of **15a** yielded a 3:1 mixture of hydroxide-bound complex (**15b**) and enolate-bound complex (**15c**), with the ratio being temperature and solvent polarity dependant. The proportion of enolate accrued with increasing temperature and decreasing solvent dielectric constant. One of the equilibrium deprotonation products, **15c**, was separately isolated and characterized in acetonitrile. Complex **15a** was also used in a H/D exchange experiment. The alpha methylene hydrogens were rapidly exchanged in 10% (v/v) CD_3CN/D_2O, pD 7.0 MOPS buffer (20 mM) with a $k_{obs} = 2.8 \times 10^{-2}\,min^{-1}$. This is 100 times faster than with the diprotonated cyclen alone (i.e., without Zn^{2+} present). Thus, it was found that enolates could be readily formed under physiological conditions in the presence of chelated Zn^{2+}. This model may help clarify the roles played by zinc (II) in class II aldolase reactions.

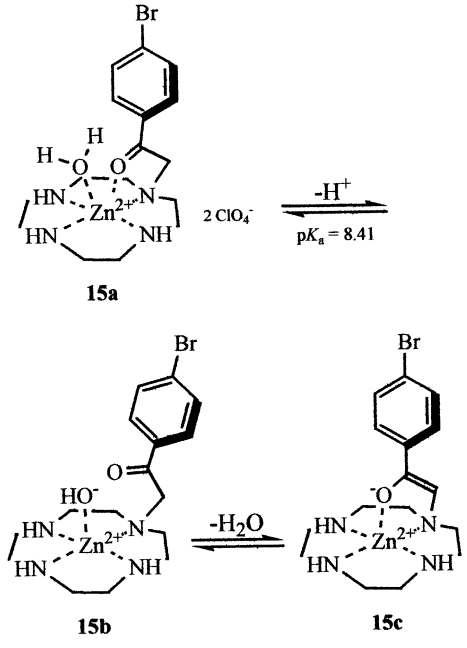


Figure 9. A 4-bromophenacyl-pendant cyclen (**15a**) used to evaluate the pK_a shift produced by the zinc cation in the reversible aldol condensation catalyzed by class II aldolases.

Anslyn et al. have synthesized and evaluated receptor **16** (Fig. 10) as a model for enolase and racemase enzymes.[20] The receptor (**16**) possesses four amide-like hydrogen bond donors on the concave face of a crescent shaped cavity. Placing electron-withdrawing ethylcarboxyl groups on the β-carbons increased the hydrogen bond donating ability. Compound **16** binds conformationally biased active methylene enolates, but not more fluxional enolate substrates. The sodium 15-crown-5 enolate of 1,3-cyclohexanedione showed the largest association constant of the 10 active methylene enolates reported ($K_a = 1.35 \times 10^4\,M^{-1}$ in CD_3CN) and was presumed to be most complementary to **16**. Comparative NMR binding studies with control **17** implicate cooperative binding of 1,3-cyclohexanedione- and 2-nitrocyclohexanone enolates from both pairs of hydrogen bond donors in receptor **16**. Despite the cooperative binding by four hydrogen bonds and the significant association constants between the enolates and the receptor in CD_3CN, potentiometric titrations in anhydrous acetonitrile showed that **16** is only capable of lowering the pK_a value of 1,3-cyclohexanedione by a minimum of 1.0 unit in that solvent. The authors concluded from these studies that the electrostatic interactions derived from normal hydrogen bonds are insufficient to produce the pK_a shifts engendered by enolase and racemase enzymes, which catalyze pK_a shifts of ca. 20–25 units with certain substrates.[21]

The receptors and experiments highlighted in the preceding sections demonstrate the significance and influences of general acid-base, metal–ion, and hydrogen bonding interactions on substrate enolization. They also help define the importance of individual and cooperative interactions in more complex biological systems. This not only leads to a better understanding of relevant enzyme mechanisms, but also to improved models for the study of additional and multiple interactions or for application as increasingly efficient artificial catalysts for reactions in which no viable enzyme is available.

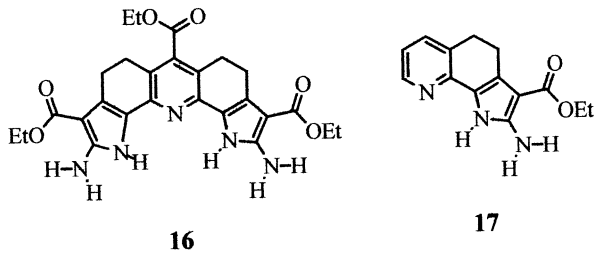


Figure 10. Receptor **16** used to bind active methylene enolates through hydrogen bonding to the enolate lone-pair electrons, and a control (**17**) used to evaluate cooperativity in **16**.

Design and Evaluation of a Cyclophane Intended to Induce pK_a Shifts in Active Methylene Compounds[22]

Receptor **16** directs its hydrogen atoms toward the lone pair electrons of the enolate substrates, thereby modestly increasing the acidities of the parent active methylene compounds by stabilizing the delocalized anionic charge of the enolate. Our intention was to evaluate a receptor that could direct its hydrogens toward the π-*electron system* of a bound enolate, so that the effect different hydrogen bond acceptor orbitals have on the acidity constants of carbon acids could be compared. The C_3-symmetric bicyclic cyclophane **18** seemed an attractive receptor for such an evaluation. It had previously been used to selectively bind nitrate and acetate over cyanide, chloride, dihydrogen phosphate, bromide and hydrogen sulfate in 25% CD_2Cl_2/CD_3CN (v/v).[23] It was thought the enhanced binding of nitrate and acetate was attributed to multiple hydrogen bonding contacts from above and below the plane of their respective π-systems while encapsulated within the receptor. Note that both substrates have planar geometries that should allow hydrogen bond donation by the convergent intracavity amide hydrogen bonds from two arms of the C_3-symmetric receptor. The lone pair on the nitrogens of the pyridine rings may present repulsions with bound anions, but they make weak hydrogen bonds with the NH groups of the adjacent amides, assisting in the synthesis of the cyclophane.

The findings related to acetate and nitrate association with **18** lead us to expect similar binding of planar enolate substrates. A distance of 7.0 Å separates the aromatic caps within the receptor, forcing the enolates to be sandwiched parallel between the aromatic rings as previously found for acetate and prohibiting rotation within the cavity.[23] Molecular modeling[24] supports encapsulation of active methylene enolates with *two pairs* of hydrogen bonds directed toward the π-system of the substrate *trans*-oriented carbonyl groups as shown in Figure 11. We view these as simple electrostatic interactions, as with most normal hydrogen bonds.

Hydrogen bonding plays a crucial role in stabilizing reactive intermediates in enzyme active sites. For example, the acidity of carbon acids is increased when hydrogen bonds are present to stabilize the corresponding enolates. The strength of the hydrogen bonds should depend upon the hydrogen bond acceptor orbitals. Hydrogen bond donation to the π-system of an enolate rather than to the oxygen lone pairs may enhance stabilization, because the negative charge delocalization occurs within the π-system. With this in mind, we determined whether larger pK_a shifts of carbon acids might be obtained when the hydrogen bonds are oriented toward the enolate π-system rather than the lone pair electrons.

An examination of the crystal structure of 4-chlorobenzoyl-coenzyme A dehalogenase, an enzyme that catalyzes a nucleophilic aromatic substitution via a Meisenheimer intermediate (Fig. 12), revealed that hydrogen bonds from the amide backbone of Gly 114 and Phe 64 are oriented toward the π-system of a thioester carbonyl enolate (Fig. 13).[25] These hydrogen bonds likely consist of relatively weak contacts to the carbonyl lone pair electrons in the ground state but increase in strength upon formation of the Meisenheimer complex, leading to differential binding. Importantly, a G114A mutation reduces catalytic activity 300-fold, thus displaying the significance of this hydrogen bond to catalysis.[26] It is thought the NH bonds from Gly 114 and Phe64, along with the dipole from a nearby α-helix, polarize the thioester carbonyl to facilitate the unfavorable nucleophilic aromatic substitution at the distal end of the aromatic ring. Raman and heteronuclear NMR evidence also indicate the enolate intermediate

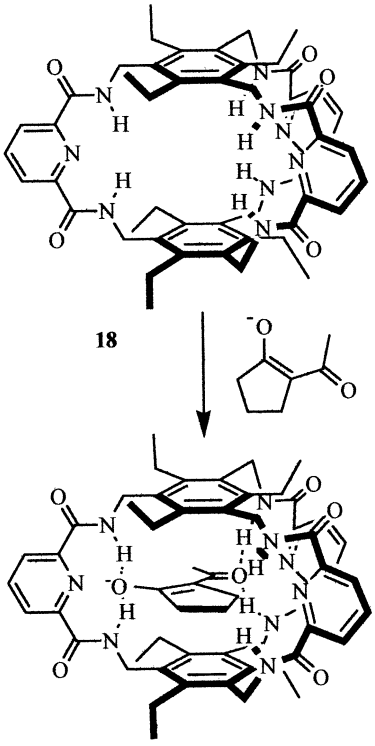


Figure 11. Complexation between receptor **18** and 2-acetylcyclopentanone.

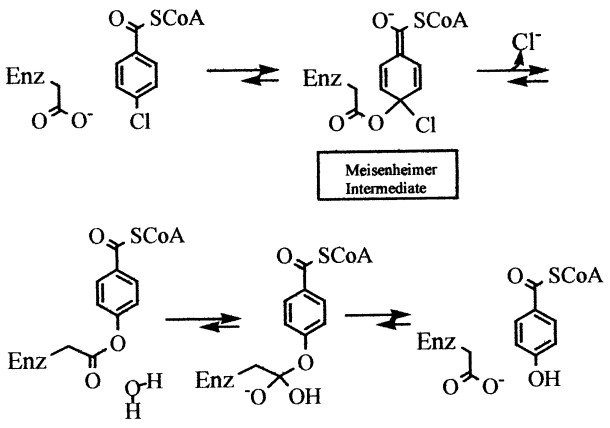


Figure 12. Mechanism of 4-chlorobenzoyl-coenzyme A dehalogenase wherein the Meisenheimer intermediate is stabilized by NH–π hydrogen bonding interactions.

maintains a more quinoidal- than benzenoidal-type structure.[27,28] This could result from NH–π hydrogen bonds contributing to thioester enolate stabilization of the former valence bond structure, whereas they are precluded from doing so with the latter.

Other members of the enoyl-CoA hydratase/isomerase superfamily share a highly conserved active site design with 4-chlorobenzoyl-Co A dehalogenase.[29,30] These additional members catalyze a variety of reactions (e.g., olefin isomerization, hydration, ester hydrolysis, and carbon–carbon bond formation and cleavage) that involve a polarized thioester or amide unit. Considering the conserved nature of the active site structures and the importance of the Gly 114 and Phe 64 backbone amide hydrogen bonds to the mechanism of 4-chlorobenzoyl-Co A dehalogenase, it seems plausible that NH–π hydrogen bonds participate in similar thioester enolate stabilization in those reactions involving an enolate. X-ray crystallographic analysis and site-directed mutagenesis studies are still required for many of these enzymes to assess the tenability of this proposal.

Results

Binding and deprotonation studies

Before examining the pK_a shift potential of **18**, we determined which enolates were most complementary to the receptor. The association constants of **18** with various enolates were determined by 300 MHz ^{1}H NMR titration experiments in 95:5 CD_3CN/CD_2Cl_2 by following the deshielding of the amide protons of **18** with increasing enolate concentration (Table 1a).[31] In cases where there was a methyl group adjacent to a carbonyl,

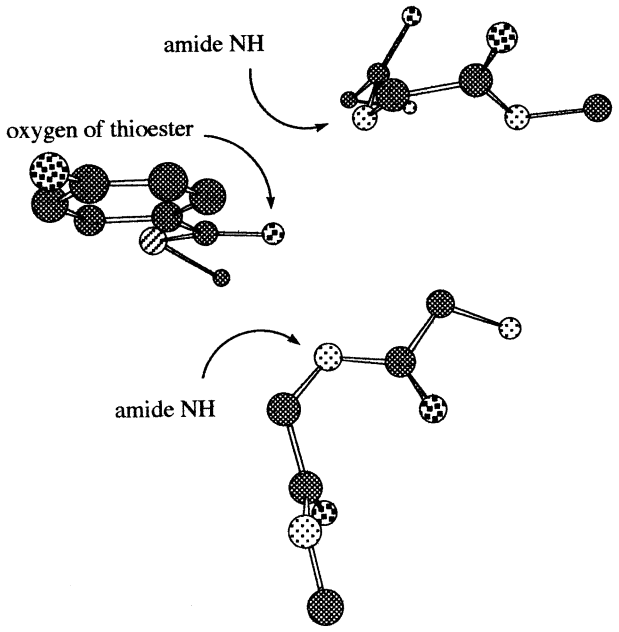
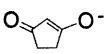
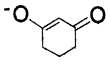
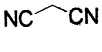
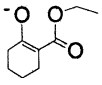
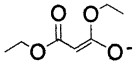
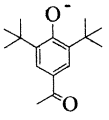
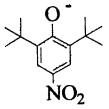
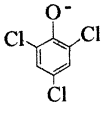
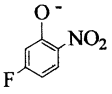


Figure 13. Crystal structure of 4-chlorobenzoyl-coenzyme A dehalogenase emphasizing the NH–π hydrogen bonding interactions from two amino acid backbone amides to the acyl thioster enolate intermediate.

Table 1. Sodium 15-crown-5 (a) enolates and (b) phenoxides, the anion numbers, association constants in (a) CD_3CN/CD_2Cl_2 (95:5) and (b) CD_2Cl_2, aqueous p$K^{a,39}$ and determined nonaqueous pK_a values in MeCN of the conjugate acids

Anion	Number	K_{assoc} (M^{-1})	pK_a^{Water}	pK_a^{MeCN}
(a)				
	**19**	3060±180	7.8	25.4
	**20**	1500±65	10.6	
	**21**	1360±200		
	**22**	1060±134	9.0	
	**23**	440±15		
	**24**	350±35	13.0	
	**25**	100±10	10.1	
	**26**	95±10	5.25	
	**27**	90±90	11.2	
	**28**	80±10	11.5	
	**29**	75±15	13.3	
(b)				
	**30**	Negligible	8.9	
	**31**	Negligible	8.05	
	**32**	730±30	6.65	
	**33**	Negligible	6.23	21.2
	**34**	Negligible	6.07	

the methyl protons were shielded relative to their chemical shift in the absence of the cyclophane, typically on the order of 0.2 ppm. We ascribe these upfield shifts to the anisotropy of the aromatic rings of **18**, suggesting that these substrates are positioned within the cavity. However, the best evidence for encapsulation in the cavity derives from a previous crystal structure of **18** with acetate.[22] Molecular modeling where the acetate is replaced with enolate gives a similar structure.

Table 1 displays the discrimination of anions toward receptor **18**. This selectivity appears to arise from steric and geometric restrictions to association. The tightest binding enolates are the smaller cyclopentyl and aliphatic enolates. Cyclohexyl and bulky enolates display poorer binding. The cavity of **18** is quite confined, occluding many substrates from association. None of the examined phenoxides bind with the cyclophane except **32**. This base is likely bound by the unhindered *para*-nitro substituent not by hydrogen bonding to the oxyanion. This interpretation is consistent with the association of nitrate reported previously.[23]

Enolates **19** and **20** were chosen for the pK_a shift experiments because of their high affinities for **18**. A ^{1}H NMR technique was employed to probe the change in pK_a values of 2-acetylcyclopentanone, and ethyl acetoacetate, the parent active methylene compounds of **19** and **20**, respectively, in the presence of **18**. Bases were added to deprotonate the active methylene compounds in the presence of 1.0 equivalent of **18** and induce enolate binding. The acid-base reaction was monitored by observing the complexation-induced change in the N*H* chemical shift of **18** (Figs 14 and 15). Although CD_3CN/CD_2C1_2 (95:5) was used to measure the association constants, CD_2C1_2 was used in these studies to ensure that **19** and **20** were completely encapsulated in **18** upon generation ($K_a > 10^5\,M^{-1}$ in CD_2C1_2). Importantly, ^{1}H NMR titration experiments showed that the parent active methylene compounds displayed negligible binding to **18** in CD_2C1_2 ($K_a < 10\,M^{-1}$). However, an association constant for 2-acetylcyclopentanone was obtained by titrating an 11 mM solution of **18** with up to 200 equivalents of the parent active methylene compound in CD_3CN/CD_2Cl_2 (4:1), providing $K_a = 4.9 \pm 1.2\,M^{-1}$.[32]

The bases employed to deprotonate 2-acetylcyclopentanone and ethyl acetoacetate were 15-crown-5 sodium phenoxides of differing base strength. Each phenoxide used in the deprotonation study showed negligible binding to **18** in CD_2C1_2 (Table 1b) with the exception of **32**, as noted above. Titrating a solution of the active methylene compound with the phenoxides in CD_2C1_2 showed that in the absence of **18** no acid/base chemistry occurred, except with **30,** which has a higher conjugate acid pK_a value in water than 2-acetylcyclopentanone. This base did not deprotonate ethylacetoacetate in the absence of **18**, as expected from the β-ketoester's higher pK_a value.

However, in the presence of **18**, six equivalents of 2,4,6-trichlorophenoxide (**33**), whose conjugate acid has a pK_a value nearly 1.6 units lower than that of 2-acetylcyclopentanone in water, deprotonated 40% of this diketone (Fig. 14). More impressively, less than six equivalents of 3-iodophenoxide (**30**), whose conjugate acid has a pK_a value 1.7 units lower than that of ethyl acetoacetate in water, fully formed **20** from the parent conjugate acid (Fig. 15) in the presence of the cyclophane. Thus, the receptor proved its capability to increase substrate acidities in low dielectric media as hoped. Nuclear magnetic resonance and molecular modeling suggest this occurs through NH–π hydrogen bond stabilization of the resulting enolate that arises after deprotonation of the parent active methylene compounds.

Nonaqueous photometric titrations

The pK_a shift of 2-acetylcyclopentanone induced by **18** was quantified and compared to the result found with **16** and 1,3-cyclohexanedione. Although the deprotonation studies were conducted in CD_2Cl_2, it was necessary to determine the pK_a shift of 2-acetylcyclopentanone induced by **18** in acetonitrile so that the result could be compared to the original study using **16**. Employing external indicators (HI), photometric titrations were carried out on 2-acetylcyclopentanone and 2,4,6-trichlorophenol in anhydrous acetonitrile. Plots of [HI]/[I$^-$] versus the volume of Bu_4NOH (0.1 N in 10:1 isopropyl alcohol/methyl alcohol) titrant allowed the calculation of pK_a values for the conjugate acids of the

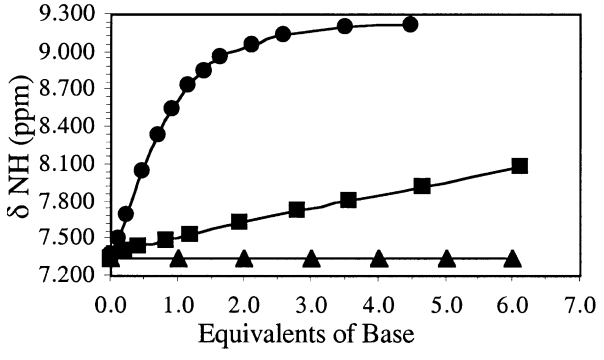


Figure 14. Change in the amide N*H* chemical shift of 1.8×10^{-2} M **18** with increasing base concentration in the deprotonation of 1.8×10^{-2} M 2-acetylcyclopentanone in CD_2Cl_2. (●) is with **31**, (■) with **33**, and (▲) with **34**.

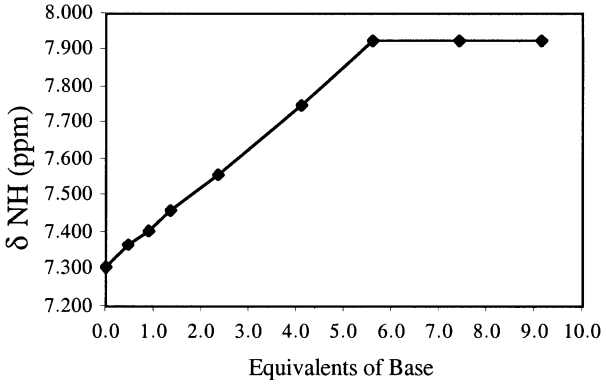


Figure 15. Change in the amide ^{1}H chemical shift of **18** with increasing **30** concentration in the deprotonation of ethylacetoacetate in CD_2Cl_2.

enolates using pK_a values for the indicators. A pK_a value of 25.4 was found for 2-acetylcyclopentanone using thymol blue sodium salt as the indicator (Fig. 16).[33] Similarly, a pK_a value of 21.2 was determined for 2,4,6-trichlorophenol with α-naphtholbenzein (Fig. 17). Using the extent of deprotonation of 2-acetylcyclopentanone induced by 2,4,6-trichlorophenoxide along with the phenol's pK_a value allowed us to calculate the pK_a of the active methylene structure as 22.5 in the presence of **18**. This value is 2.9 pK_a units lower than in the absence of **18**.

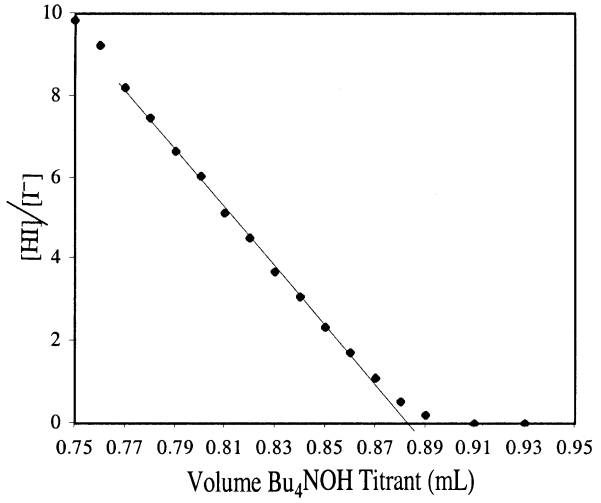


Figure 16. Plot of [IH]/[I$^-$] versus volume of titrant added in the nonaqueous pK_a determination of 2-acetylcyclopentanone using thymol blue, sodium salt as indicator.

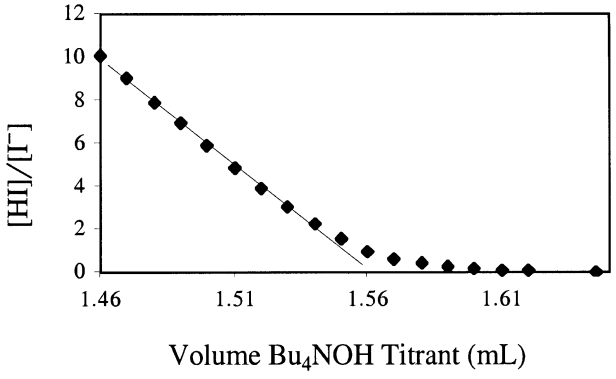


Figure 17. Plot of [IH]/[I$^-$] versus volume of titrant added in the nonaqueous pK_a determination of 2,4,6-trichlorophenol using α-naphtholbenzein as indicator.

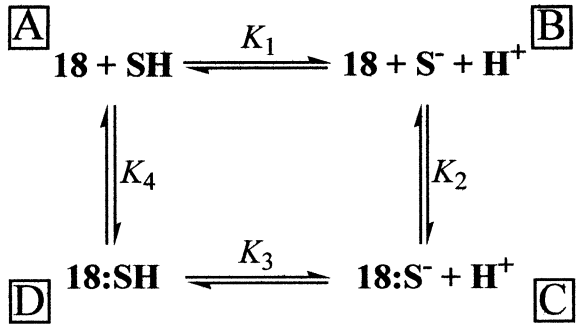


Figure 18.

Unfortunately, we were not able to titrate ethyl acetoacetate in anhydrous acetonitrile because of its high pK_a value. We were unable to find a suitable indicator with an established pK_a value in acetonitrile that was sufficiently basic to use in the photometric titration of the β-ketoester. A similar problem was encountered in the photometric titration of 3-iodophenol (the conjugate acid of the base used to form **20** in the presence of **18**). Attempts at potentiometric titration of **30** with picric acid proved problematic and unsatisfactory, perhaps partly due to homoconjugation of the titrand.

Discussion

Cyclophane **18** increases the acidities of particular active methylene compounds as evidenced by the outlined deprotonation studies and photometric titrations. The receptor reduced the pK_a of 2-acetylcyclopentanone by 2.9 units in acetonitrile, while compound **16** only gave a 1.0 unit pK_a shift to 1,3-cyclohexanedione. The increased acidity of 2-acetylcyclopentanone is likely a conservative pK_a shift value. The deprotonation studies were conducted in CD_2Cl_2, a solvent with a lower relative permittivity and a larger autoprotolysis constant than acetonitrile (the study in which the pK_a values were determined). Thus CD_2Cl_2 has a broader pH scale than acetonitrile and should result in a larger pK_a shift than that measured.

The cyclic equilibrium depicted in Figure 18 represents the steps involved in the association of substrate (SH) with receptor **18** and proton dissociation from the substrate to give the enolate (S$^-$). Equilibrium constant K_1 denotes the acid dissociation constant of the free parent active methylene compound, while K_3 signifies that of the substrate bound with **18**. Equilibrium constants K_2 and K_4 represent the association constants of **18** with the enolate (Table 1a) and the parent active methylene compound, respectively.

From Figure 18, it is evident that $K_1K_2 = K_3K_4$, and thus the ratio of the acid dissociation constants is equal to the ratio of the binding constants, that is $K_3/K_1 = K_2/K_4$. Because K_3/K_1 is the pK_a shift ($\Delta pK_a = pK_3 - pK_1$), the greater the differential binding of the enolate versus the parent carbon acid, the larger the anticipated ΔpK_a. Indeed, substituting the experimentally determined values for each of the equilibrium constants in Figure 18 demonstrates this to be experimentally valid ($K_3 = 2.86\times10^{-23}$; $K_1 = 3.71\times10^{-26}$; $K_2 = 3.06\times10^{3}\,M^{-1}$, $K_4 \approx 5\,M^{-1}$). Hence, the free energy of differential binding in CD_3CN/CD_2Cl_2 (95:5) at 283 K ($\Delta G = -4.5$ kcal/mol) correlates with the witnessed increase in substrate acidity ($\Delta pK_a \times 1.37$ kcal/mol $= -4.0$ kcal/mol).

It is unclear what sequence of steps is involved in the pK_a decrease effected by **18**, that is which route in Figure 18 (or to what extent each route) is involved in proceeding from A to complex C, although the fundamental interaction in C is well-defined. The stability of complex C arises from the NH–π hydrogen bonds formed between the receptor and the enolate. This effectively increases the parent compound's acidity in

nonaqueous media independent of whether the sequence involves proton dissociation from the free carbon acid (A to B) followed by binding of the resulting enolate to **18** (B to C), or rather weak binding of the carbon acid by the receptor (A to D) succeeded by carbon acid ionization while associated with **18**. Neither going from A to B nor from A to D is facile, as deprotonation of 2-acetylcyclopentanone by **33** is undetectable in the absence of **18**, while the K_a of 2-acetylcyclopentanone and **18** is $<10\,M^{-1}$.

The important factor for increasing carbon acidity is the differential binding of the conjugate acid and the enolate by the receptor. We are not stating that NH–π hydrogen bonds are stronger than NH–lone pair hydrogen bonds. This has yet to be determined, and we suspect it not to be the case. Instead, initially weak hydrogen bonding interactions between the neutral amide NHs and the parent active methylene carbonyl oxygens are promoted to stronger interactions with the establishment of the electron-rich enolate π-system. Upon formation of the enolate, there is a significantly smaller increase in charge density on the oxygen lone pairs relative to the change that occurs in the π-system. Since there is a larger change in the π-system, there is a larger difference in the hydrogen bond strengths. Therefore, to better stabilize an enolate relative to its conjugate acid, it is more effective to employ a receptor that preferentially targets the substrate π-system.

Differential binding of substrates is not restricted to reactions involving carbon acid proton dissociation. Such preferential binding is a basic principle behind the catalytic efficiency of enzymes, and has been proposed as a driving force in the nucleophilic aromatic substitution catalyzed by the aforementioned 4-chlorobenzoyl-CoA dehalogenase.[34] The NH–π stabilization of **19** by **18** likely mirrors transition state interactions in several enzymatic reactions, for example those furnished by the 2-enoyl-CoA hydratase/isomerase superfamily, wherein metastable delocalized anions are formed.

Conclusion

Bicyclic cyclophane **18** is capable of increasing the acidity of select active methylene compounds through differential substrate binding brought about by NH-π hydrogen bonding. This process shifts the pK_a of 2-acetylcyclopentanone by at least 2.9 units in acetonitrile. Thus, NH–π hydrogen bonding proves superior to the enolate lone pair hydrogen bonding exhibited by receptor **16** with 1,3-cyclohexanedione. Differential binding via NH–π hydrogen bonds may also be an important feature of the catalytic efficiency of several enzymes. A well-characterized example is 4-chlorobenzoyl-CoA dehalogenase, where amide backbone residues preferentially stabilize the developing thioester enolate relative to the ground state carbonyl group, thereby polarizing the substrate through NH–π interactions similar to those described herein.

Experimental

The synthesis of **18** has been reported elsewhere.[35]

Photometric titrations

General considerations. All Hamilton gas-tight syringes, cuvettes and volumetric flasks were dried in a vacuum dessicator for at least 24 h prior to use. Anhydrous Omnisolv acetonitrile (less than 30 ppm water) was obtained from EM Science (Gibbstown, NJ) and bottles were fitted with a rubber septum in a glove box before use. Tetrabutylammonium hydroxide for titration in nonaqueous medium (2-propyl alcohol/methyl alcohol 10:1 v/v) was obtained from Fluka (Milwaukee, WI). All indicators and acids were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used as received. Reagents and solutions were stored in a dessicator. A Beckman DU-640 spectrophotometer was used to record absorbance readings during the titrations. A 1-cm cell fitted with a teflon septum was filled with argon during solution transfers. All volumetric flasks were fitted with a rubber septum, wrapped in parafilm, and maintained under an argon atmosphere during use. All solutions except indicator stock solutions were freshly prepared prior to titration.

Procedure. Nonaqueous photometric titrations were conducted following the procedure of Higuchi, Rehm, and Barnstein.[36] An accurately weighed quantity of acid or phenol (generally $\sim 1\times10^{-4}$ mol s) was dissolved in approximately 50 mL of anhydrous acetonitrile containing an appropriate quantity of saturated indicator stock solution (previously prepared in anhydrous acetonitrile) to give an absorbance value below 1.0 at its λ_{max}. Then a measured volume of 0.1 M Bu_4NOH calculated to deprotonate 85–90% of the acid was added. The resulting solution was brought to volume with acetonitrile in a dry 100 mL volumetric flask, thoroughly mixed, and split into 25 mL aliquots to allow multiple titrations.

After each 0.010 mL addition of 0.1 M Bu_4NOH containing the same concentration of indicator as the titrand solution, and intimate mixing, the absorbance of the titrated solution was measured at the λ_{max} of the indicator. After each measurement, the titrated solution in the cuvette was returned to the 25 mL bulk solution and the process repeated until no change in absorbance was witnessed upon addition of titrant.

Calculation of pK_a values from photometric titration data

The recorded absorbance values were applied using the following approach to obtain pK_a values. In the titration of 2-acetylcyclopentanone, the line from the plot in Figure 16 is fit by the first order equation

$$y = -66.2x + 58.7$$

The exchange equilibrium constant (K_{ex}), defined as the equilibrium constant for proton exchange between the

conjugate acid form of the indicator and the weakly acidic titrand, is determined from the inverse y-intercept of plots of the type shown in Figure 16:[37]

$$K_{ex} = 1/b = 1.70 \times 10^{-2}$$

Using this K_{ex} value and the known acid dissociation constant of the indicator thymol blue, sodium salt ($K_I = 6.31 \times 10^{-28}$),[38] the desired acid dissociation constant of the titrand (K_a) may be calculated according to the definition of the exchange constant:

$$K_a = K_I/K_{ex} = 6.31 \times 10^{-28}/1.70 \times 10^{-2} = 3.71 \times 10^{-26}$$

Thus, the experimentally determined pK_a of 2-acetylcyclopentanone is

$$-\log K_a = -\log 3.71 \times 10^{-26} \text{ or } 25.4 \text{ p}K_a \text{ units}$$

The same approach was taken for 2,4,6-trichlorophenol using α-naphtholbenzein[38] as the indicator (Fig. 17):

$$y = -98.633x + 153.95$$
$$-\log ((K_I)(b)) = -\log ((3.98 \times 10^{-24})(153.95))$$

gives a p$K_a = 21.2$

In each case, three runs were conducted and the values averaged to determine the reported pK_a values. Hence, the ΔpK_a in anhydrous acetonitrile between 2-acetylcyclopentanone and the base capable of deprotonating **19** in the presence of **18**, 2,4,6-trichlorophenol, is 4.2 units.

Calculation of effective pK_a shift incorporating extrapolation of ^{1}H NMR deprotonation data

The following treatment was applied to calculate the pK_a shift induced by **18** using the data depicted in Figure 14. The equilibrium expression between the active methylene compound (AH) and the phenoxide base (P^-) is

$$K_{eq} = ([A^-][PH])/([AH][P^-]) = Ka_{AH}/Ka_{PH}$$

The initial concentration of the 2-acetylcyclopentanone was 0.0184 M. Upon addition of 6.12 equivalents of 2,4,6-trichlorophenoxide, the initial concentration of the diketone was 40% deprotonated as indicated by the amide proton chemical shift change shown in Figure 14. Thus, the equilibrium expression at this point becomes

$$K_{eq} = ((0.40)\ [0.0184\text{M}]\ (0.40)[0.0184\text{M}])\ /((0.60)\ [0.0184\text{M}])((6.12 \text{ equiv} - 0.40)\ [0.0184\text{M}])$$
$$= 4.66 \times 10^{-2}$$

Using the determined K_a value for 2,4,6-trichlorophenol in acetonitrile described above allows us to estimate the effective pK_a of 2-acetylcyclopentanone from the equilibrium constant evaluated in the previous equation.

$$Ka_{AH} = (Ka_{PH})\ (K_{eq}) = (6.13 \times 10^{-22})\ (4.66 \times 10^{-2})$$
$$= 2.86 \times 10^{-23}$$

Thus, the pK_a of 2-acetylcyclopentanone in the presence of **18** is 22.5, 2.9 pK_a units lower than in the absence of the cyclophane.[39]

Acknowledgements

We wish to thank Dr. John Gerlt, Professor of Biochemistry at the University of Illinois—Urbana-Champaign for bringing the crystal structure of 4-chlorobenzoyl-CoA dehalogenase to our attention and Dr. Gunther Hennrich for determining the association constant of 2-acetylcyclopentanone. We greatly appreciate research funding provided by the National Institutes of Health and the Welch Foundation.

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