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Effect of Heteroatom Insertion at the Side Chain of 5-Alkyl-1*H*-tetrazoles on Their Properties as Catalysts for Ester Hydrolysis at Neutral pH

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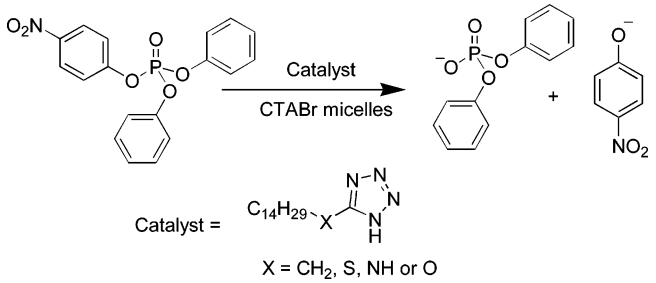

Herein we introduce tetrazole and its suitably designed derivatives as powerful ester-cleaving reagents. By first performing a detailed ab initio computational study, we found that, in the side chain of 5-alkyl-1*H*-tetrazoles, introduction of a heteroatom (e.g., N, O, or S at the α-position of the tetrazole ring) raises the charge on the tetrazole nucleus significantly. All calculations have been performed using restricted Hartree−Fock (RHF) and hybrid ab initio/DFT (B3LYP) methods employing 6-31G* and 6-31+G* basis sets. To estimate the nucleophilicity of these reagents, the charges on conjugate bases of various tetrazole derivatives have been calculated using natural population (NBO) analysis in gas phase and in water. Free energy of protonation (fep) of the 1*H*-tetrazole derivatives (**1**−**7**), free energy of solvation, ΔG_{aq}, and the corresponding pK_a values have been calculated by self-consistent reaction field (SCRF) methods applying the polarized continuum model (PCM). Since the calculation indicates that incorporation of heteroatom leads to enhanced nucleophilicity in their deprotonated anionic tetrazole forms, a series of 5-substituted 1*H*-tetrazole derivatives have been synthesized. These compounds indeed catalyze the hydrolysis of *p*-nitrophenyl diphenyl phosphate (PNPDPP) and *p*-nitrophenyl hexanoate (PNPH) efficiently in cationic cetyl trimethylammonium bromide (CTABr) micelles at pH 7.0 and 25 °C. The pseudo-first-order rate constants (k_{obs}) were determined for each catalyst against both substrates. The experimental and theoretical results show that, to achieve better k_{obs} values for the cleavage of PNPDPP and PNPH under micellar conditions, charge on the N^- atom (nucleophile) of conjugate base is important. Replacing the α-CH_2 in alkyl substituent with S (**3**), NH (**4**), or O (**5**) enhances the accumulation of charge on N^- in conjugate bases of tetrazoles and subsequently increases their intrinsic nucleophilic reactivity toward hydrolytic reactions. Significantly large rate enhancements were observed for the cleavage of PNPDPP and PNPH at pH 7.0 in the presence of catalytic system **5**/CTABr over background (only CTABr). Tetrazole **4** (α-isomer) showed 4−5-fold superior reactivity over **6** (β-isomer) under identical conditions. Natural charges obtained from NBO analysis (B3LYP/6-31+G*) are −0.94 and −0.852 on N^- in the conjugate bases of **4** and **6**, respectively. This also predicts that **4** is a better nucleophile than **6**. All the newly synthesized tetrazole derivatives in micellar media display true catalytic properties by cleaving several fold excess of substrates.

Introduction

There is continuous interest in the catalytic hydrolysis of phosphate esters since highly toxic organophosphates (OP) belong to this class of compounds (Chart 1). The chemical transformation of such toxic organophosphorus compounds is also highly relevant,[1] as there is continuing threat of the usage of chemical warfare. This mandates discovery of new catalytic systems for the destruction of chemical weapon stockpiles. From the work of several

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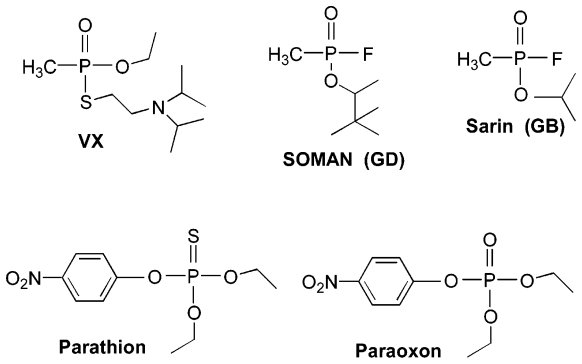
CHART 1. Structures of Some Toxic Organophosphorus Compounds

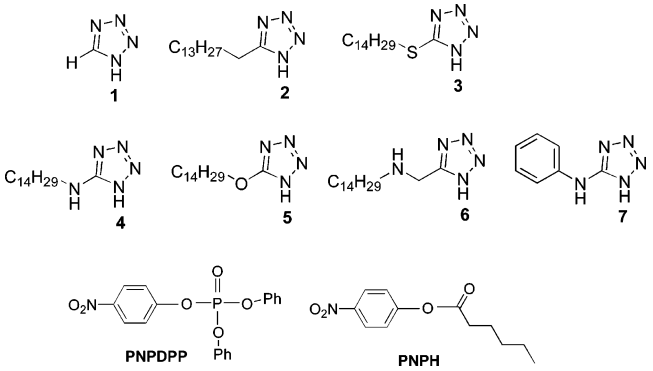


research groups over the past many years, a number of reagents have been developed for dephosphorylation.[2−4] However, there is still urgent need to design and develop new reagents that are inexpensive and biodegradable and show the catalytic turnover in dephosphorylation or deacylation reactions at near neutral or physiological pH.

Earlier we reported the catalytic hydrolysis of phosphoric and carboxylic acid esters using 4-(dialkylamino)-pyridine in various micellar aggregates.[5] We also examined the esterolytic properties of monoperoxypthalates[2a] and 1-hydroxybenzotriazoles[6] in various micellar aggregates.

Published reports indicate that imidazole derivatives act as potent nucleophiles in esterolytic reactions.[7−11] Since the chemical structure of imidazole is similar to that of tetrazole, it occurred to us that the latter may be worth examining as a nucleophile toward esterolysis reactions. Compared to imidazoles, tetrazoles have significantly lower pK_a values. Thus, tetrazole has a pK_a of ~4.9, while imidazole ionizes into its anionic form at pH ~14.5.[12a] It is the ionic tetrazolide species that are formed at pH 7 that make them potent nucleophiles even at neutral pH. In fact, a number of drugs that contain a

CHART 2. Structures of Tetrazoles (1−7) and Substrates (PNPDPP and PNPH) Used in This Work



tetrazole ring, which acts as a metabolically stable surrogate for carboxylic acid function, are known.[9,12−13] Tetrazole is important in coordination chemistry as a ligand as well. However, nothing is known about the esterolytic potency of tetrazoles, although the literature on tetrazoles is expanding rapidly.[14]

To explore the potential of the catalytic activity of 5-substituted 1*H*-tetrazoles (**2**−**6**, Chart 2) toward esterolytic reactions, we first performed a detailed ab initio and B3LYP density functional hybrid calculations. Since conjugate bases (N^- form) of these tetrazoles are nucleophilic and should mediate such hydrolytic reactions, detailed computational analyses of charge localization on N^- of conjugate bases of each of **1**−**6** in gas phase and in water were performed. The corresponding free energies of protonation (fep) were also computed.

In this report, we present the optimized geometries, fep values, computed pK_a values, and natural charges of each tetrazole to rationalize the intrinsic nucleophilicity of these reagents using ab initio restricted Hartree−Fock and hybrid ab initio DFT (B3LYP) methods. To examine the effectiveness of such computational predictions and to develop catalysts for the hydrolysis of *p*-nitrophenyl diphenyl phosphate (PNPDPP) and *p*-nitrophenyl hexanoate (PNPH), we also synthesized **2**−**6**. We present the results of the experimental kinetic studies at pH 7.0 for the hydrolysis of PNPDPP and PNPH performed under pseudo-first-order conditions in the presence of excess of each of **2**−**6** in cationic cetyltrimethylammonium bromide (CTABr) micelles. The results of the hydrolysis mediated by these reagents in the cleavage of excess substrates at pH 7.0 demonstrating their true catalytic properties are also included.

Results and Discussion

Computational Methods. All the ab initio Hartree−Fock and B3LYP density functional hybrid method[15] calculations reported in this work were performed using the Gaussian 98 suite program.[16] The geometries of 5-substituted 1*H*-tetrazoles (**1**−**6**) have been located and optimized at the level of restricted Hartree−Fock (RHF)

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and DFT (B3LYP) using the 6-31+G* basis set.[17] All structures were completely optimized without any symmetry restrictions. For all systems studied, vibrational frequency calculations were carried out to confirm that they converged to true minima. The conformers were defined as true minima by diagonalization of their Hessian (force constant) matrixes at the same level and making sure that all frequencies are real. Optimized geometries from the RHF level were taken as initial geometries to minimize at the B3LYP level. In general, the fully optimized geometries from prior calculations were used as starting points for calculations using the higher-level basis sets. Natural charges were computed based on the natural bond orbital (NBO) localization procedures developed by Weinhold et al.[18]

For the sake of simplicity, we define the deprotonation enthalpy of the neutral protonated forms of tetrazoles as free energy of protonation of the negatively charged species. Fep values were obtained from the difference between the calculated total energies of the neutral molecule and that of the derived anion, following Dewar's definition.[19] Essentially, we consider the cases as in the instances when we deal with isodesmic processes (e.g., $ROH + MeO^- \rightarrow RO^- + MeOH$) and internal comparisons of the energy differences.[20]

To assess the relative validity of the basis sets and to evaluate the influence of electron correlation and diffuse functions, we calculated the pK_a of **7**, for which the experimental pK_a in water is known.[21] We used the B3LYP/6-31+G* level to calculate the gas-phase deprotonation free energy and solvation free energies by self-consistent reaction field (SCRF) methods applying the polarized continuum model (PCM). This method requires less CPU time than the MP2 method. The calculated pK_a is found to be 5.47, and the experimental pK_a is 5.49, which shows a deviation of −0.02.[21] Recently, Friesner has used similar DFT and SCRF methods to calculate the pK_a's of various tetrazole derivatives. This enabled an accurate computation of the pK_a values, which showed only a mean observed deviation of +0.6 from the measured pK_a values.[22]

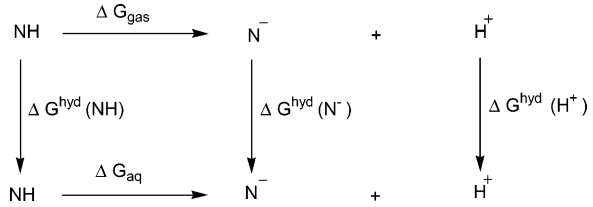
To calculate the pK_a values of molecules **2**−**6**, we have considered the thermodynamic cycle shown below. The thermodynamic cycle yields eq 1, in which the aqueous pK_a for the acid N−H is given by

$$\Delta G_{aq} = \Delta G_{gas} + \Delta G^{hyd}(N^-) + \Delta G^{hyd}(H^+) - \Delta G^{hyd}(NH) \quad (1)$$

At a given temperature T, the pK_a is then given by

$$pK_a = \Delta G_{aq}/2.303RT + 2.36 \quad (2)$$

The gas phase fep value and solvation free energy were calculated by using the same level of theory. The free energy of solvation in water can be obtained with reasonable accuracy using SCRF methods.[23] In this study, the solvent effects were estimated using Tomasi's SCRF procedure.[24] The effect of solvation in water on the relative free energies was estimated by using the polarized continuum model (PCM option of Gaussian 98).[25] A dielectric constant (ϵ) of 78.39 (water) was used in the solvation calculations, at the B3LYP/6-31+G* level. The solvation-free energy of the proton taken from the experimental $\Delta G^{hyd}(H^+)$ is equal to −254 kcal/mol.[26] Since the kinetic experiments were performed in water, we computed the natural charges in water with the NBO localization procedures.



Design. To develop highly potent reagents for the cleavage of organophosphorus compounds, several research teams have designed a number of catalysts by modifying the solubility and binding efficiency toward the micellar aggregates. However, usage of quantum mechanical calculations in the design of novel catalysts is indeed rare. Previously, Moss et al. developed[27] various iodosoarene carboxylates as efficient reagents in hydrolysis of PNPDPP in cationic micelles. Modifications such as inclusion of carbonyl group, alteration of the ring size, etc., allowed development of better catalyst for the cleavage of PNPDPP.[27] However, to date there have been no reports that describe the design of catalyst based on the charge on active nucleophile as predicted from

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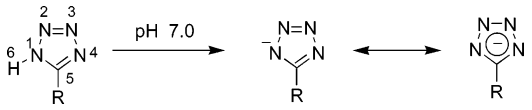
computational studies for such hydrolysis reactions. Here, for the first time we report design of improved catalysts based on analyzing the natural charge on active nucleophile (N^-) by ab initio and density functional methods.

Published reports show that imidazole derivatives act as nucleophiles in hydrolytic reactions.[8] Since the chemical structure of tetrazole is similar to that of imidazole, we thought it might act as a potent nucleophile toward esterolysis reactions. Tonellato has shown[8] that imidazole deprotonation in cationic micelles occurs at pH > 11. Therefore, the hydrolysis is only effective at high pH conditions. It is well-known that the acidity of the tetrazoles falls in the range of that of a carboxylic acid.[28] Accordingly, one can expect that due to lower pK_a values, tetrazole can act as a better nucleophile at neutral pH.



In this report, we utilize 5-substituted tetrazoles (**2**−**6**) as catalysts in the hydrolysis of PNPDPP and PNPH. Since ester hydrolysis experiments have been carried out in cationic micellar aggregates, it is important to design catalysts that can bind to micelles more efficiently. Tetrazole (**1**) is a highly polar water-soluble compound. Accordingly, to enhance their binding affinity toward the micelles in water, a long hydrophobic chain in **2**−**6** is attached. The electronic factor was also considered in this design. If esterolytic activity stems from tetrazolyl anion, then electronic variation of net charge on the tetrazole ion (conjugate base) should influence the nucleophilicity of these tetrazole derivatives.[28] By performing quantum mechanical calculations, we have found that insertion of a heteroatom such as S, NH, or O at the α-position of the side chain of 5-alkyl-1*H*-tetrazole enhances the charge on the tetrazole ring. Analysis of theoretical and experimental kinetic data enables us to draw a comparison on the effect of charge on the nucleophilicity of these reagents. This in turn allows a prediction of the reactivity of the tetrazole derivatives toward hydrolysis of PNPDPP and PNPH. To test the effect of the location of heteroatom in the 5-alkyl side chain of tetrazoles, the corresponding *β*-isomer (**6**) of **4** was also prepared. Synthetic routes of these reagents **2**−**6** are given in Scheme S1 (Supporting Information).

Theoretical Calculations. Accounting for solvent effects computationally is a challenging task. Rate constants are sensitive to small free energy changes. There are uncertainties in heats of formation from even high-level calculations, which apply to isolated molecules and themselves do not provide evidence on structural effects on entropy. However, these concerns should not preclude computational studies in this direction. Especially with the development of statistical Monte Carlo and quantum mechanical methods, it is possible to gauge the role of solvents with significant accuracy.[29] Although continuum models may not be the best in analyzing esterolysis reactions, there is an advantage in using continuum models, in that it speeds up computation considerably. Accordingly, we have applied quantum mechanical methods to predict the experimental results.

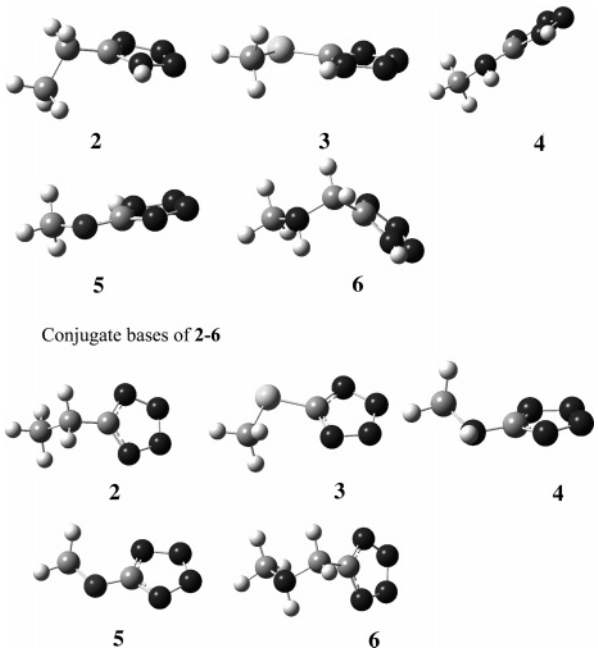


FIGURE 1. Fully optimized structures of various tetrazole derivatives, **2**−**6**, and their conjugate bases with B3LYP/6-31+G* method in gas phase.

Ab Initio and DFT Calculations. Various 5-substituted tetrazoles (**2**−**6**) have been optimized at RHF/6-31+G* and B3LYP/6-31+G* levels of theory. Fully optimized geometries of these compounds are shown in Figure 1. A preliminary guess for all calculations was taken from geometries optimized by using RHF/6-31G*. All molecules have been optimized without any symmetry constraints. To analyze the reliability of the level of theory and basis sets to optimize tetrazole derivatives, initially we optimized 1*H*-tetrazole (**1**) with RHF, MP2, and DFT (B3LYP) by using two different basis sets, 6-31G* and 6-31+G*. The calculated and experimental (from crystal structure) geometry parameters for **1** and its conjugate base tetrazole ion are given in Tables S1 and S2 (Supporting Information), respectively. It is known that HF-predicted bond lengths are usually underestimated[30] and the MP2 predicted bond lengths are usually overestimated.[30−32] The B3LYP-predicted geometries manifest best agreement with the experimental geometries.[32,33] The data in Table S1 and S2 disclose that MP2-predicted geometries are closer to the B3LYP geometries than the RHF geometries. The basis set effects on the calculated geometries are generally small. These results are consistent with the results observed

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TABLE 1. Calculated Free Energies of Protonation and pK_a Values for 2−6 at B3LYP/6-31+G* Levels in Water

amphiphile	free energy of protonation[a]	pK_a[b]
2	328	4.95
3	321	4.21
4	330	5.83
5	322	4.36
6	324	5.31

[a] Energies are in kcal/mol. [b] Calculated in water by using dielectric constant (∈) of the solvent, 78.39.

by El-Azhary et al. in the case of the tetrazole geometry optimization.[33d] We observed closer resemblance in the geometries with the results obtained at the B3LYP/6-31+G* level, and hence we used the same method for further calculations.

We calculated fep values for **2**−**6** at B3LYP method with 6-31+G* basis sets. Calculated fep values were 328, 321, 330, 322, and 324 kcal/mol for **2**, **3**, **4**, **5**, and **6**, respectively (Table 1). Among this series, **4** has the highest and **3** has the lowest fep value. In general, the inclusion of a lipophilic chain ($C_nH_{2n+1}X-$) bearing a heteroatom (X) at the 5-position lowers the fep values with the exception of **4**. This implies that deprotonation is more favorable for **3**, **5**, and **6** compared to **2**. Catalytic strengths of individual tetrazoles also depend on their pK_a values. It is therefore worthwhile to compute the pK_a values for **2**−**6**, as these are important data for predicting their catalytic potencies toward hydrolytic cleavage of PNPDPP and PNPH in micelles. For this purpose, the computation of the relative pK_a for these molecules provides a better comparison.

Calculation of pK_a Values. Computing the absolute pK_a from first-principle theory is tough, in part because of the difficulty in computing accurate gas-phase deprotonation free energies and also because of the large magnitude of solvent effects for charged ions.[34] Fortunately, trends in pK_a values for similar compounds suggest more accurate predictions,[34b,e−g] although rationalizing differences in some simple series (e.g., the methylated amines) continues to be challenging.[34,35] Several attempts have been made to predict the exact pK_a value for systems of particular interest by developing new parameters and by fitting the computational results with experimental ones using linear regression analysis.[36]

In this report, we compare the trends of calculated pK_a values for **2**−**6** using the thermodynamic cycle described in the Computational Methods section. It is important to note that the aim here is not to calculate the absolute pK_a values alone. Instead, we are interested in finding the trend in this series of compounds. We computed the pK_a value for **2**−**6** at the B3LYP/6-31+G* level (gas-phase deprotonation free energies and solvation free energies were computed at this level of theory). To find the reliability of this level of computation of the pK_a of different tetrazole derivatives, we compared the calculated pK_a values with the experimental ones known for **1**[22] and **7**.[21] By using the B3LYP/6-31+G* level of theory, we obtained theoretical pK_a values that are in excellent agreement with the experimentally determined pK_a values for these two molecules. Computed pK_a values of **1** and **7** are 4.96 and 5.47, respectively, while their experimental pK_a values are 4.9 and 5.49, respectively.

TABLE 2. Computed Natural Charges on N^- Atom in Conjugate Bases of 2−6 at Different Levels of Theory

	RHF/6-31+G*		B3LYP/6-31+G*	
molecule	gas phase	in water	gas phase	in water
2	−0.917	−0.967	−0.823	−0.858
3	−0.941	−0.978	−0.846	−0.878
4	−1.032	−1.074	−0.908	−0.94
5	−1.038	−1.07	−0.914	−0.94
6	−0.92	−0.962	−0.818	−0.852

The computed pK_a values for **2**−**6** are presented in Table 1. The data in Table 1 reveal that replacing the α-CH_2 at the side chain of 5-alkyl-1*H*-tetrazoles with S (**3**) or O (**5**) leads to lowering of the pK_a compared to 5-tetradecyl-1*H*-tetrazole. On the other hand, replacing α- or β-CH_2 with -NH (**4** and **6**, respectively) leads to an increase in the pK_a compared to **2**. Although the electronegativity of N is higher than that of S, it is not clear why the pK_a of **4** is higher than that of **3**. The hydrogen bonding involving -NH in **3** may be responsible for this. It should be noted that these pK_a values are calculated in bulk water. Generally, in cationic micellar aggregates, the pK_a values are lower than those in water.[8] Since these tetrazoles have low pK_a values, at pH 7.0 all of them should be in their deprotonated, conjugate base forms where the experiments have been performed. Therefore, at pH 7.0 the effective ionization of tetrazoles is complete and then the concentration of the nucleophilic species (N^-) of the tetrazoles would be almost comparable. It may be emphasized that pK_a calculation here aims to guarantee the presence of ionic tetrazolate species at neutral pH and there is no apparent correlation of that with the nucleophilicity. This implies that estimation of intrinsic nucleophilicity of these conjugate bases is important, which contributes to the differences in the catalytic strengths of **2**−**6**.

Since N^- is the reactive nucleophilic species, the charge on the N^- atom[37] gives an idea of the intrinsic nucleophilicity of the conjugate bases. To find out the extent of charge accumulation on the N^- of **2**−**6** conjugate bases, natural population analysis was performed. Relative natural charges calculated at RHF/6-31+G* and B3LYP/6-31+G* levels in gas phase and in water are given in Table 2. Since kinetic experiments were carried out in

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(37) Since charge generated upon deprotonation is localized between N_1 and N_4 due to resonance form, cumulative charge on these atoms is taken as N^- charge.

aqueous media, it is more realistic to compare charges obtained from the NBO analysis. Therefore, throughout our discussion we use these data. It is clear that replacing the α-CH_2 at the side chain of 5-alkyl-1*H*-tetrazoles with electron-donating heteroatoms such as S, NH, or O leads to increase in the charge on N^- in tetrazole rings. On the other hand, replacing β-CH_2 with NH does not alter the charge on N^- significantly. Similar trends were observed when gas-phase computations were considered as well. Order of charge accumulation on N^- is **2** ≈ **6** < **3** < **4** ≈ **5**. Our experimental kinetic data (Table 3) indeed show that the observed pseudo-first-order rate constants, k_{obs} for each **2**−**6**, are in excellent agreement with theoretical predictions (see below).

Experimental Study. Synthesis. Different 5-substituted 1*H*-tetrazoles were synthesized following the procedure outlined in Scheme S1 (Supporting Information). First, *n*-tetradecyl bromide was converted to the corresponding nitrile (**8**) or thiocyanate (**9**) by nucleophilic substitution reaction with NaCN and KSCN, respectively, in aqueous ethanol under refluxing conditions. Then **8** and **9** were converted to the corresponding 5-substituted 1*H*-tetrazoles, **2** and **3**, in 78 and 81% yields, respectively, using a modified procedure developed by Sharpless.[38] Compound **5** was synthesized in 63% yield starting from *n*-tetradecyl cyanate employing a similar procedure as adopted for **2** and **3** (Scheme S1). The tetrazole **4** was synthesized from *n*-tetradecyl aldehyde (**11**) via Schiff base formation with 5-aminotetrazole hydrochloride followed by reduction with sodium borohydride. Compound **6** was synthesized from *n*-tetradecyl aldehyde via Schiff base formation with 5-aminoacetonitrile followed by reduction with sodium borohydride. Then the product, **12**, was converted to the target tetrazole by a similar procedure as used for the preparation of **2**. The final products and intermediates were characterized by different spectroscopic methods and elemental analysis (Supporting Information).

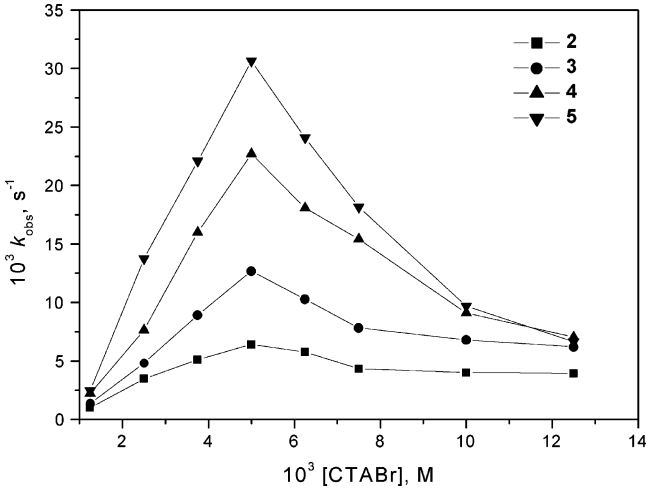

FIGURE 2. Plots of k_{obs} for the hydrolysis of PNPDPP (1.25×10^{-5} M) by catalysts **2**−**5** (1.25×10^{-4} M) as a function of [CTABr] at pH 7.0 (0.05 M HEPES) and 25 °C. Lines were drawn to show the trend.

Kinetic Studies in Micelles. Because it is important to determine not only the rate constants (k_{obs}) for hydrolytic reactions on the substrates in micellar conditions under pseudo-first-order conditions, but also the intrinsic reactivity of each tetrazole, the second-order rate constants, k_2, had to be determined. However, the determination of the k_2 values for a given micelle-bound catalyst and substrate is less obvious. Therefore, we first present the rate constants obtained under pseudo-first-order conditions and then address the determination of k_2 values in a micelle-mediated reaction.

Kinetics were performed under pseudo-first-order conditions, monitoring the appearance of *p*-nitrophenoxide ion by UV−visible spectroscopy at 400 nm, in 0.05 M HEPES buffer, pH 7.0, 25 ± 0.1 °C, using [substrate] = 1.25×10^{-5} M and [catalyst] = 1.25×10^{-4} M at different CTABr concentrations. The buffer solutions were also contained 1.0 vol % CH_3CN. Pseudo-first-order rate constants, k_{obs}, were determined for the cleavage of both PNPH and PNPDPP at each combination of [CTABr]/[catalyst]. The k_{obs} values were generally within 3% relative deviation from their mean value for all the catalytic formulations. Determination of full rate constant versus [CTABr] profiles for the cleavage reactions of PNPH and PNPDPP in micellar aggregates allowed assessment of the ester cleavage abilities of **2**−**5**. The plots of k_{obs} versus [CTABr] for the cleavage of PNPDPP by **2**−**5** gave maxima for all the catalysts at [CTABr] = 5 mM (Figure 2). These kinetic rate profiles obtained are characteristic of micelle-catalyzed reactions in aqueous solutions. Addition of CTABr to reaction medium causes progressive increase in the rate of hydrolysis up to a point, where the concentration of catalyst per micelle reaches a maximum. Subsequent addition of the CTABr causes a decrease in the observed reaction rate. This may be due to dilution of the catalyst in the Stern layer of micelles.

TABLE 3. Rate Constants (k_{obs}, s^{-1}) for the Hydrolysis of PNPH and PNPDPP Catalyzed by Various 5-Substituted 1*H*-Tetrazoles, 1−6 in CTABr Micelles[a]

			PNPDPP		PNPH	
entry	catalyst	10^3 [CTABr], M[b]	10^3 k_{obs}, s^{-1} [c]	k_{rel}[d]	10^3 k_{obs}, s^{-1} [c]	k_{rel}[d]
1	none	10.0	0.01	1	0.007	1
2	**1**	none[e]	0.04	–	0.056	–
3	**1**	10.0	0.2	20	0.3	43
4	**2**	5.0	6.4	640	8.1	1157
5	**3**	5.0	12.7	1270	16.0	2290
6	**4**	5.0	22.7	2270	32.5	4643
7	**5**	5.0	30.7	3070	36.5	5215
8	**6**	5.0	6.0	600	7.9	1129

[a] Conditions: 0.05 M HEPES buffer, pH = 7.0; 25 ± 0.1 °C; [substrates] = 1.25×10^{-5} M, [catalyst] = 1.25×10^{-4} M. [b] The CTABr concentration at which maximum k_{obs} was observed. [c] k_{obs} was taken from the k_{obs} vs [CTABr] profiles. All kinetic runs were carried out with freshly made solutions. Each kinetic experiment was carried out thrice, and the values of k_{obs} were within 3% relative deviation from the value reported. Average values of k_{obs} are given in the table. [d] $k_{rel} = k_{obs}/k_0$, where k_0 is the rate constant for the hydrolysis of substrates in the CTABr micelles at pH 7.0 buffer. [e] This kinetic study was done in 0.05 M HEPES buffer, pH = 7.0.

The values of k_{obs} for the cleavages of PNPDPP and PNPH by each catalyst are shown in Table 3. When the molar ratio of catalyst to CTABr was fixed at 1:40, we observed the best k_{obs} values for each catalyst, **2**−**6**. Table

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3 provides a comparison of the cleavage abilities of all the catalysts under pseudo-first-order conditions. The ratio k_{obs}/k_{CTABr} (k_{rel}) reveals the catalytic capacities of the tetrazoles compared to background cleavage rates at identical pH and temperature in CTABr micelles alone. Tetrazoles **3**, **4**, and **5** are found to be highly potent catalysts in CTABr micelles affording more than 3 orders of magnitude of rate accelerations. When compared to the hydrolysis rates at pH 7.0 alone (in the absence of CTABr), the relative rate accelerations were even larger. Comicellar **5**/CTABr displayed the highest esterolytic activity among all the catalytic systems toward both PNPDPP and PNPH.

The ~30-fold superiority of the catalytic system **2**/CTABr (entry 4, Table 3) over **1**/CTABr (entry 3, Table 3) for the cleavage of both substrates indicates that the tetradecyl chain incorporated in **2** significantly promotes its transfer into micelles compared to **1**.[39] The rate accelerations could be due to the enhanced concentration of ionized nucleophilic groups and substrate molecules at the micellar surfaces.[6−9]

The k_{obs} values increased in the order **2** < **3** < **4** < **5** although all catalysts have the same alkyl chain and hence comparable hydrophobicity. Indeed the inherent nucleophilic reactivities of **2**−**5** also increase in the same order as predicted by computation. However, it is possible that the rate differences in Table 3 could be due to differences in transfer effects and not due to inherent reactivities. Precise delineation of these factors requires determination of the binding affinities (transfer from water into micelles) of these lipophilic catalysts into CTABr micelles.

The data of Table 4 show that for ester hydrolysis reaction in comicelles there is no significant difference in the binding constant (K_b) values of a particular substrate. The substrate partitioning is not influenced by the changes in the molecular structure of the catalysts **2**−**5** in comicelles. However, the k_{obs} values are very different for a given substrate under identical reaction conditions as a different catalyst is employed. Hence, the increase in k_{obs} values is not due to differences in their substrate binding affinities, but can be attributed to the differences in intrinsic reactivities of the catalysts. Calculated natural charges on N^- of **2**−**5** clearly indicate that replacement of α-CH_2 in the side chain of 5-alkyl-1*H*-tetrazole with a heteroatom increases the charge accumulation on N^- species in tetrazole, which results in enhanced nucleophilicity. However, the k_{obs} values for the cleavage of PNPDPP by **4**/CTABr and **5**/CTABr are 2.27×10^{-2} s^{-1} and 3.07×10^{-2} s^{-1}, respectively. Though the conjugate bases of **4** and **5** carry equal charges, such a difference is not very surprising, since **4** can associate through hydrogen bonding, which may alter the nucleophilicity of N^- in **4**. To test whether the position of heteroatom in the substituted chain affects the intrinsic reactivity of the tetrazoles, we prepared **6** in which the β-CH_2 of the alkyl chain was substituted by a NH. **4** (α-isomer) shows superior reactivity over **6** (β-isomer) toward the cleavage of both substrates in CTABr micelles, which can be explained by the different natural charges on N^- of their conjugate bases: −0.94 and −0.852, respectively.

Inherent Reactivity. To understand the reasons for rate enhancements of these esterolytic reactions in CTABr micelles, additional kinetic experiments were necessary. Since we wanted to compare the second-order rate constants k_2 in aqueous phase and in micellar phase, we calculated the k_2 values in bulk water simply by dividing the k_{obs} values by the total concentration of the catalyst. In the case of catalytic homomicelles, only one type of surfactant is present, but comicelles bring out the problem of dilution of the catalyst in the micelles made of cosurfactant (e.g., CTABr). Therefore, the relative ratio of [cosurfactant]/[catalyst] does influence the rate of these hydrolytic reactions.

TABLE 4. Kinetic and Thermodynamic Parameters for the Cleavage of PNPDPP and PNPH by 1 in Water and by Comicelles of 2−5 with CTABr[a]

	PNPDPP			PNPH		
catalyst	$10^3 k_{lim}$, s^{-1} [b]	K_b, M^{-1}	$10^2 k_2$, M^{-1} s^{-1} [b]	$10^3 k_{lim}$, s^{-1} [b]	K_b, M^{-1}	$10^2 k_2$, M^{-1} s^{-1} [b]
1[c]	–	–	3.2	–	–	4.5
2	7.76	3718	11.5	9.44	4791	14.0
3	15.65	3408	23.2	19.1	3993	28.3
4	33.88	3450	50.1	38.45	4580	56.9
5	37.54	3547	55.6	43.2	4405	63.9

[a] Conditions: Kinetic cleavage experiment at pH 7.0, 0.05 M, HEPES buffer using solutions containing increasing amounts of catalyst and CTABr with [catalyst]/[CTABr] ratios of 1:40, 1:30, and 1:20 for each catalyst **2**−**5**, using either PNPH or PNPDPP as a substrate. [b] k_{lim} and k_2 were calculated by fitting with the Michaelis−Menten equation. See the text for details. [c] The k_2 was determined in bulk water (k_{obs}/[catalyst]), [catalyst] = 1.25×10^{-4} M at pH 7.0 (0.05 M HEPES buffer).

To determine the second-order rate constants in micellar pseudophase, further kinetic experiments were carried out at pH 7.0 in 0.05 M of HEPES buffer with solutions containing increasing amounts of catalyst and CTABr with molar ratios to the catalyst 40, 30, and 20 using either PNPH or PNPDPP as substrate. The corresponding rate-concentration profiles showing saturation behavior are shown in Figure 3. Analysis of the curves using the Michaelis−Menten equation[40] by fitting the k_{obs} versus [catalyst] data allows the estimation of (i) the k_{lim}, the rate constants for the substrate being fully incorporated into the micellar aggregates and (ii) the apparent binding constants, K_b, for PNPH or PNPDPP in different comicelles. The k_2 values for these reactions in micellar pseudophase were calculated[41] using the equation

$$k_2 = k_{lim} \cdot V_M \cdot [D^t]_m / [D^f]_m \, (1 + [H^+]/K_a) \qquad (3)$$

where V_M is the molar volume, $[D^t]_m$ is the total concentration of the micellized CTABr, $[D^f]_m$ is the concentration of the tetrazole amphiphile, and K_a is the dissociation constant for the catalyst at pH 7.0 at which kinetic reactions were performed. We have used a V_M value 0.37 dm^3 mol^{-1} for CTABr micelles.[40] The $[D^t]_m/[D^f]_m$ takes into

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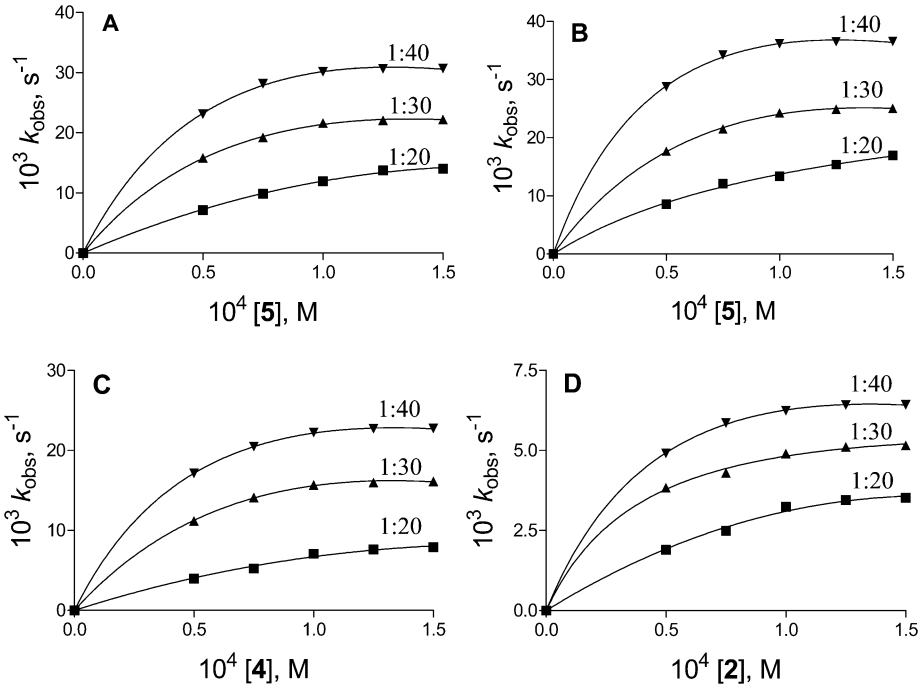

FIGURE 3. Hydrolysis of substrates at pH 7.0, 0.05 M HEPES buffer solutions containing various amounts of catalyst and CTABr with [catalyst]/[CTAB] ratios of 1:40, 1:30, and 1:20. (A) PNPDPP cleavage catalyzed by **5**. (B) PNPH cleavage catalyzed by **5**. (C) PNPDPP cleavage catalyzed by **4**. (D) PNPDPP cleavage catalyzed by **2**.

account the dilution of the reactive tetrazole amphiphile in the CTABr comicelles, and the term $(1 + [H^+]/K_a)$ denotes the fraction of the dissociated catalyst at pH 7.0. The k_{lim} and K_b values for cleavage reactions of both substrates by these reagents have been tabulated in Table 4.

For calculation of k_2, we used **1** in bulk water and more hydrophobic tetrazoles **2**−**6** in comicellar pseudophase. Data in Table 4 show that, although there are some differences in the reactivity of 5-substituted tetrazole derivatives toward both substrates, they actually fall in the same order. There is 1 order of magnitude enhancement in the k_2 values in micellar conditions compared to the k_2 value obtained in bulk water. This behavior is obvious, because acceleration of k_2 values in micelles is largely due to a high local concentration of the catalyst in the relatively small aggregate volume. This finding is consistent with earlier reports.[41] It is noteworthy that k_2 of **4** is ∼5-fold greater than **6** toward the cleavage of both substrates. This acceleration must originate due to increase in the intrinsic reactivity, which can be due to enhanced localization of charge on the N^- of conjugate bases of **4** compared to that of **6**. Thus, experimental and theoretical results are in good agreement with each other.

Examination of True Catalytic Behavior: Turnover Experiments. To examine the true catalytic efficiencies of **2**−**6**, kinetic runs were carried out in the presence of excess substrate using either PNPDPP or PNPH in CTABr micelles. At pH 7.0 and 25 ± 0.1 °C, [catalyst] = 2.5×10^{-6} M, [CTABr] = 1×10^{-3} M, and 20- to 30-fold excess substrate over catalyst concentration, we observed a nearly quantitative monoexponential release of *p*-nitrophenoxide ion with no indication of "burst" kinetics.[5b] Figure 4 shows the time course of cleavages of 30-fold excess PNPDPP and 20-fold excess PNPH by catalyst **3**. Both the plots confirm fast turnover.

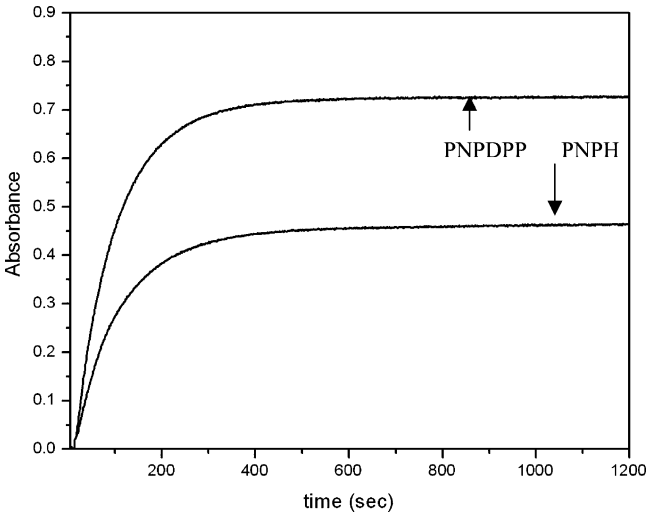

FIGURE 4. Time-dependent release of *p*-nitrophenoxide upon hydrolysis of PNPH or PNPDPP by catalyst **3**/CTABr in the presence of excess substrate over catalyst. Conditions: 0.05 M HEPES buffer (pH 7.0), 25 ± 0.1 °C, [**3**] = 2.5×10^{-6} M, [CTABr] = 1×10^{-3} M, and [PNPDPP] = 7.5×10^{-5} M or [PNPH] = 5×10^{-5} M.

Notably, the catalytic effectiveness is still intact after the hydrolysis of excess substrates. Analysis of time-dependent formation of *p*-nitrophenoxide ion for the cleavage of excess substrate reveals pseudo-first-order character of the reaction in both cases.

On the basis of the monoexponential time course that was observed, the following mechanism for the cleavage of these substrate esters by tetrazole nucleophiles is proposed. Since we did not observe "burst"-type kinetics, there is no accumulation of *N*-phosphoryl or *N*-acyl species in the reaction media. The hydroxide ion attack

on to the *N*-phosphorylated or *N*-acylated species at the cationic micellar surfaces must be fast, and it cleaves the intermediate species rapidly to furnish hydrolyzed products diphenyl phosphate or hexanoate, concomitantly regenerating the catalyst in the reaction media to be available to react with a fresh substrate molecule.

Conclusions

In summary, the theoretical and experimental investigations together provide insights to understand the catalytic abilities of the 5-substituted tetrazoles as nucleophiles in the hydrolytic reaction of PNPH or PNPDPP. By calculating the free energy of protonation, pK_a values, and charge accumulation on the reactive N^- of the conjugate bases of these tetrazoles, we find the potential of these reagents. Attaching a hydrocarbon chain with the tetrazole at the 5-position promotes their binding to CTABr micelles efficiently, increasing the k_{obs} values. More importantly, replacing the α-CH_2 in the hydrocarbon chain with either S, NH, or O enhances the accumulation of charge on N^- in conjugate bases of these tetrazoles significantly. This in turn leads to increases in intrinsic reactivity of these nucleophiles toward hydrolytic reactions of PNPDPP and PNPH. Position of insertion of heteroatom in the chain is also important. These amphiphilic 5-substituted tetrazoles are truly catalytic in nature and have proven to be extremely efficient for the cleavage of organophosphate and carboxylate esters.

In the present work it has been possible to isolate the factors that influence deprotonation and reactivity in water and in micelles. Both the hydrophobic substrate esters and the tetrazoles (**2**−**6**) should be confined almost exclusively in the micelles. This allows the theoretical prediction of the experimental outcome with significant accuracy. The findings of this investigation should therefore be useful for many research groups.

Experimental Section

General Methods. Melting points were determined using open capillary tubes and are uncorrected. ^{1}H NMR spectra were obtained on a 300 MHz instrument. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS). Double-distilled water was used for kinetic studies.

Materials. All the chemicals, reagents, and solvents were of the highest grade available commercially and were purified, dried, or freshly distilled as required. PNPDPP was prepared and purified by literature methods.[42]

Kinetic Studies. Reaction mixtures were generated in a quartz cuvette of 1-cm path length. The cuvette was filled with 0.5 mL of aqueous buffer (0.05 M HEPES, pH 7.0) containing a known concentration of the catalytic system. The micellar solution was equilibrated for 10 min in a thermostated cell compartment (25 ± 0.1 °C) of the UV−visible spectrophotometer. An appropriate aliquot (2.5 μL) of a stock solution of PNPH or PNPDPP in CH_3CN was added using a Hamilton syringe. The reaction mixture was initiated by quick but careful stirring, and the absorbance at 400 nm was recorded as a function of time. The esterolysis obeyed pseudo-first-order kinetics, and the rate constants were obtained by nonlinear fit of the equation $(A_\infty - A_o)/(A_\infty - A_t) = e^{kt}$ where A_∞ and A_t are the absorbances at infinite time and time t, respectively. All kinetic experiments were carried out with freshly made solutions. Each kinetic experiment was carried out thrice, and we observed that the k_{obs} values were within 3% relative deviation from the value reported. Reactions involving excess substrates were carried out, and these micellar reactions were followed beyond 95% completion and each showed good first-order kinetics ($r > 0.999$).

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Supporting Information Available: Synthesis and characterization of **2**−**6**. Geometrical parameters of tetrazole and tetrazole ion. Cartesian coordinates of full-optimized geometries of **1**−**6** and their conjugate bases. This material is available free of charge via the Internet at http://pubs.acs.org.

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