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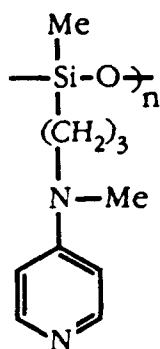


## POLYMERIC CATALYSTS WITH ENZYME-LIKE PROPERTIES

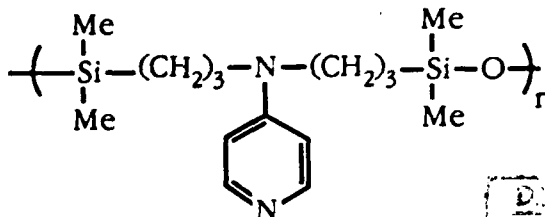
Wilmer K. Fife,<sup>1</sup> Martel Zeldin and Slawomir Rubinsztajn

### Abstract

New polymeric materials functionalized with the highly nucleophilic 4-(dialkyl-amino)pyridine group pendant to a polysiloxane chain, **1**, or incorporated within a



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bis-(trimethylene) amine-disiloxane backbone, **2**, have been prepared and evaluated as catalysts for hydrolysis of a series of p-nitrophenyl alkanoates. Polymer **2**, unlike **1** and 4-(dimethylamino)pyridine (DMAP), exhibits enzyme-like behavior in catalysis of hydrolysis of p-nitrophenyl dodecanoate in 1:1 methanol-water at pH 8.0, 30°C. Michaelis-Menten analysis gives values of  $V_{max} = 4.73 \times 10^{-7} \text{ M sec}^{-1}$  ( $t_{1/2} \cong 15 \text{ sec}$ ) and  $K_M = 3.99 \times 10^{-5} \text{ M}^{-1}$ . Furthermore, polymer **2** alone exhibits selectivity for substrate with hydrolysis of p-nitrophenyl palmitate fastest of the substrate hydrolyses studied to-date. The relative reactivity (catalytic efficiency) of DMAP, **1**, and **2** in hydrolysis of p-nitrophenyl dodecanoate at pH 8.0, 30°C is: DMAP:1:2 = 1.2:3.6:32.0; for hydrolysis of p-nitrophenyl palmitate: DMAP:1:2 = 1.2:21.0:152.0.

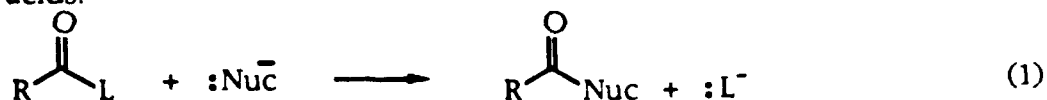
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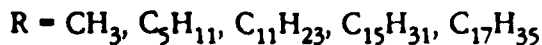
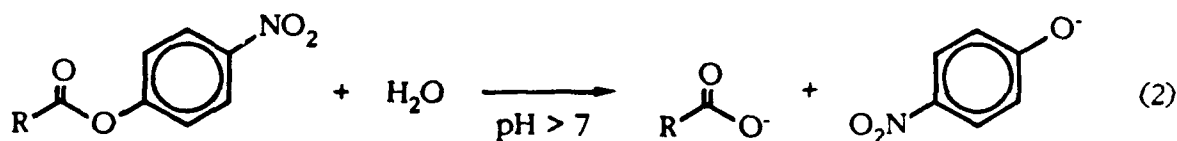
## Introduction

Enzymes are acknowledged to represent the highest expression of chemical catalysis. Duplication of enzymic catalysis in synthetic materials has been pursued by many investigators for several decades. The high levels of substrate selectivity and catalytic efficiency afforded by enzymes offer advantages of immense proportions for chemical synthesis, degradation and detection. Our work is based specifically on an interest in the design, synthesis and evaluation of synthetic polymers which emulate enzymes when serving as catalysts for transacylation reactions, e.g.; Eq. 1. In recent studies polymer-supported nucleophilic catalytic sites have exhibited remarkable selectivity and/or efficiency in promoting reactions of derivatives of carboxylic and phosphoric acids.<sup>2-5</sup>



The 4-(dialkylamino)pyridines, DAAP, are such highly reactive nucleophilic catalysts in aprotic solvents that they are often referred to as "super-nucleophiles."<sup>6-19</sup> Monomeric and polymeric forms of DAAP are increasingly utilized as catalysts in transacylation reactions. Recently, we have reported on the synthesis and catalytic efficiency in aprotic media of polysiloxanes functionalized with DAAP groups.<sup>20,21</sup> Two structurally different backbones have been explored: polymers **1** and **2** contain pendant and intra-chain DAAP moieties, respectively. Simple, monomolecular DAAP variants are known to be relatively ineffective nucleophilic catalysts of transacylation reactions in aqueous media. However, the recent report of Klotz and co-workers<sup>9</sup> confirms that polymeric DAAP's of suitable structure are capable of high reactivity in hydrolysis of p-nitrophenyl hexanoate. Rate enhancements of 50-2000-fold were reported for specific DAAP's when covalently linked to poly(ethylenimines) alkylated with dodecyl groups. A particular virtue of the DAAP catalytic site is its propensity for true catalytic behavior, i.e.; it exhibits turnover during reaction with its substrate.

Hydrolysis of a series of p-nitrophenyl alkanates, Eq. 2, catalyzed by polymers



**1** and **2** was investigated by methods widely used for evaluation of enzymic catalysis. The dependence of hydrolysis rates on catalyst and substrate concentrations, pH, temperature, reaction medium, and length of alkanate chain in substrate were determined. For comparison purposes, similar experiments were run with DMAP.

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## Experimental

**Materials.** Polymers **1** and **2** were prepared by methods reported previously.<sup>20,21</sup> Spectrophotometric grade methanol and dioxane, and twice-distilled water were used for stock solutions of polymers and substrates. Buffer solutions for pH 7.0 and 8.0 were made by adjusting the pH of 0.05 M  $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$  with aqueous NaOH. The pH 9.0 buffer utilized 0.05 M  $\text{Na}_2\text{B}_4\text{O}_7$ . Samples of the substrates, p-nitrophenyl acetate (Aldrich); p-nitrophenyl hexanoate (Sigma), p-nitrophenyl dodecanoate (Sigma), p-nitrophenyl palmitate (Sigma) and p-nitrophenyl stearate (Sigma), and 4-(dimethylamino)pyridine (Reilly Industries) were used without further purification.

**Kinetic Measurements.** Reaction mixtures were made up in a 1.00 cm quartz cuvet. The cuvet was filled with 2.97 mL of a 1:1 mixture of methanol and aqueous buffer. A stock solution of catalyst in methanol (usually 5  $\mu\text{L}$ ) was added by micro-syringe and the solution was equilibrated for 10 min. in the thermostatted cell compartment ( $30 \pm 1^\circ\text{C}$ ) of a Hewlett-Packard Model 8450 spectrophotometer. An appropriate aliquot (0.03 mL) of a stock solution of p-nitrophenyl alkanoate in dioxane was added by micro-syringe. The reaction mixture was quickly mixed by shaking, returned to the spectrophotometer cell compartment, and the absorbance at 400 nm was recorded as a function of time.

The pseudo apparent first-order rate constants ( $k$ ) were obtained as slopes of the plots of  $\ln [A_\infty/(A_\infty - A_t)]$  vs. time, where  $A_\infty$  and  $A_t$  are the absorbances at infinite time and time  $t$ , respectively. Duplicate runs generally showed a measurement error of less than 5%. The results of these experiments are displayed in Figures 1 and 2, and summarized in Table 1.

## Results and Discussion

The pH dependence of apparent rate constants ( $k$ ) for hydrolysis of p-nitrophenyl dodecanoate in 1:1 (v/v) methanol-water was measured in the presence of **1**, **2**, and DMAP at  $30^\circ\text{C}$ . The rate of reaction increases with pH over the interval 7.0 to 9.0 for all three catalysts with **2** clearly the most efficient one. The reaction rate continues to increase at higher pH due to uncatalyzed reaction with hydroxide ion. The apparent pKa values in water for **1** and **2** ( $\text{pKa}(\mathbf{1}) = 8.61$ ;  $\text{pKa}(\mathbf{2}) = 8.5_{\text{est}}$ ) are significantly lower than that of DMAP ( $\text{pKa}(\text{DMAP}) \cong 9.5$ ).<sup>22</sup> Therefore, the proportion of free base for **1** and **2** is much higher (approx. 50% at pH 8.0) than that of DMAP (approx. 10% at pH 8.0) over the pH interval used. Rates were routinely determined from reaction mixtures that contained 10-fold excess substrate over catalyst concentrations and were followed to complete reaction as indicated by absorbance values for reaction times of 4-5 half-lives. Thus, polymers **1** and **2** like DMAP were functioning as true catalysts.

Polymer **2**, in contrast to **1** and DMAP, exhibits a clear preference for p-nitrophenyl palmitate as substrate over the other p-nitrophenyl esters studied (Figure 1, Table 1). Thus, only **2** appears to function as an enzyme-like catalyst. The dependence of normalized apparent rate of p-nitrophenyl alkanoate hydrolysis on substrate concentration for the hexanoate and dodecanoate esters was examined under conditions of excess substrate. Significantly, the dodecanoate ester exhibited saturation kinetics, while the hexanoate ester did not.

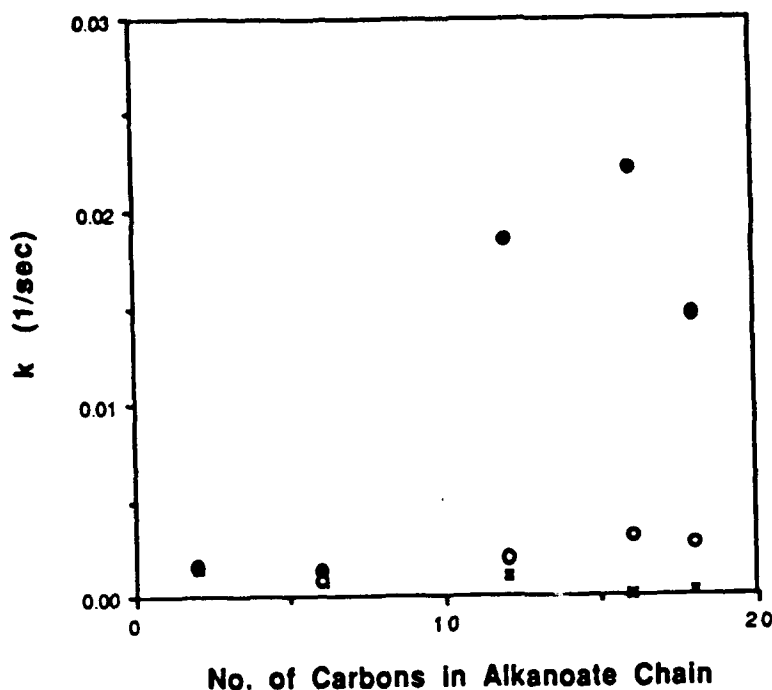


Fig 1. The dependence of apparent rate constants ( $k$ ) for p-nitrophenyl alkanolate hydrolysis on alkanolate chain length in 1:1 methanol-water in the presence of nucleophilic catalysts at pH 8.0, 30°C,  $[S] = 5.0 \times 10^{-5}$  M,  $[cat] = 1.0 \times 10^{-5}$  M. (DMAP - x, Polymer 1 - o, Polymer 2 - •).

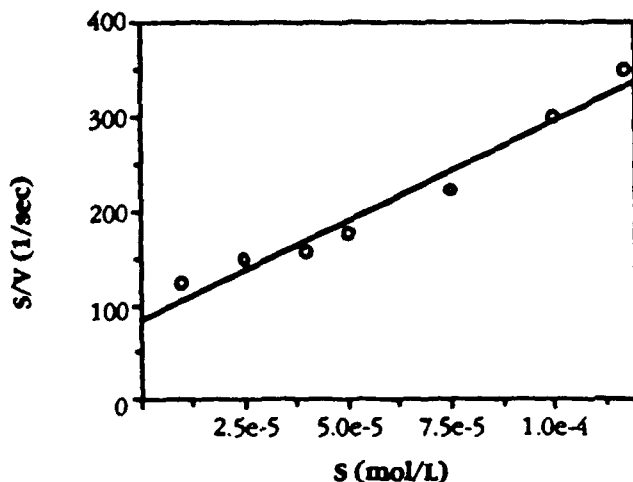
TABLE 1

Effect of nucleophilic catalysts on relative reactivity ( $R$ )<sup>a</sup> and reaction half time ( $t_{1/2}$ ) for hydrolysis of p-nitrophenyl alkanolate in 1:1 methanol-water at pH 8.0, 30°C,  $[S] = 5.0 \times 10^{-5}$  M,  $[cat] = 1.0 \times 10^{-5}$  M.

No carbons in alkanolate chain	DMAP (R), ( $t_{1/2}$ )	Polymer 1 (R), ( $t_{1/2}$ )	Polymer 2 (R), ( $t_{1/2}$ )
2 <sup>b</sup>	1.0, (450)	1.0, (441)	1.1, (425)
6	1.1, (884)	1.2, (834)	2.0, (502)
12	1.2, (1005)	3.6, (335)	32.0, (38)
16	1.2, (3938)	21.0, (220)	152.0, (31)
18	1.1, (4227)	19.0, (254)	97.0, (47)

- a)  $R$  = Rate constant of catalyzed hydrolysis/Rate constant of uncatalyzed hydrolysis  
 b)  $[S] = 1.0 \times 10^{-4}$  M.

As a further test of the ability of **2** to function as an enzyme mimic, the data from p-nitrophenyl dodecanoate hydrolysis were subjected to Michaelis-Menten analysis,<sup>23</sup> Figure 2. A plot of substrate concentration (S) divided by reaction velocity



**Fig 2.** Michaelis-Menten analysis of p-nitrophenyl dodecanoate hydrolysis in 1:1 methanol-water, pH 8, 30°C, in the presence of **2** ( $2.5 \times 10^{-6}$  M).

(V) vs. S is linear as predicted by the Michaelis-Menten model, Eq. 3, where  $V_{\max}$  = the maximum reaction velocity and  $K_M$  = the Michaelis constant. The values of  $V_{\max} = 4.73$

$$\frac{S}{V} = \frac{1}{V_{\max}} \cdot S + \frac{K_M}{V_{\max}} \quad (3)$$

$\times 10^{-7} \text{ M sec}^{-1}$  and  $K_M = 3.99 \times 10^{-5} \text{ M}^{-1}$  are consistent with effective and selective binding of substrate to the catalyst surface and efficient catalysis ( $t_{1/2} \cong 15 \text{ sec}$ ) of the hydrolysis reaction.

Activation parameters for hydrolysis of p-nitrophenyl dodecanoate in the presence of **2** were estimated from an Arrhenius plot. The uncatalyzed hydrolysis reaction is characterized by a large activation energy ( $\Delta H^\ddagger = 17.4 \text{ kcal/mol}$ ) and a relatively unordered transition state ( $\Delta S^\ddagger = -3.5 \text{ cal.mol deg}$ ). The reaction catalyzed by **2** is marked by a small activation barrier ( $\Delta H^\ddagger = 1.6 \text{ kcal/mole}$ ) and a highly ordered transition state ( $\Delta S^\ddagger = -61.1 \text{ cal/mole deg}$ ).

## CONCLUSIONS

The results document the importance of polymer structure in determining level of catalytic activity and substrate selectivity. Polymer **2** is a substantially more effective catalyst for long chain alkanooate esters than **1** which exhibits similar levels of activity to

that of DMAP, a widely-used transacylation catalyst. Polymer 2 also shows surprising selectivity for p-nitrophenyl palmitate. A plot of apparent reaction velocity vs. alkanolate chain length in the series of p-nitrophenyl esters studied (Figure 1) appears to reach a maximum at C<sub>16</sub>. Polymer 1 and DMAP show no substrate preference. Thus, 2 is the third type of DAAP functionalized polymer to exhibit high nucleophilicity in aqueous solution (the first reported examples of such catalysts were the DAAP derivatives of poly(ethylenimine) studied by Klotz and co-workers,<sup>9</sup> and the poly[4-(pyrrolidino)pyridines] of Vaidya and Mathias.<sup>14</sup>) More significantly, 2 is the first example of a synthetic linear polymer to exhibit enzyme-like specificity for a particular substrate.

Michaelis-Menten analysis is commonly used to evaluate activity and selectivity in enzymic catalysis. Figure 2 shows that the data from hydrolysis of p-nitrophenyl docoecanate in the presence of 2 in 1:1 methanol-water at pH 8.0, 30°C conforms well to the Michaelis-Menten model for enzyme catalyzed reactions. The values of  $V_{\max} = 4.73 \times 10^{-7} \text{ M sec}^{-1}$ , and  $K_M = 3.990 \times 10^{-5} \text{ M}^{-1}$  support the contention that 2 is functioning as a true enzyme-mimic.

#### ACKNOWLEDGMENT

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