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THE EFFECT OF HYDROPHOBIC–LIPOPHILIC INTERACTIONS ON CHEMICAL REACTIVITY. 12. MECHANISTIC INVESTIGATIONS OF NaCMA-CATALYZED HYDROLYSIS OF ESTERS

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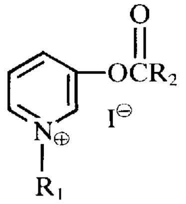
ABSTRACT

The dependence of the catalytic efficiencies of sodium carboxymethylamylose (NaCMA) for the hydrolysis of N-lauryl-3-acetoxypyridinium iodide (**1**) and *p*-nitrophenyl dodecanoate (**3**) on its degree of substitution (D.S. = 0·00, 0·12, 0·18, 0·24, 0·29 and 0·35) and on the pH values of the solutions (pH = 7·32, 7·80, 8·10 and 9·30) have been studied. At fixed D.S. values, the observed hydrolysis rates of **1** and **3** increase with increasing concentrations of NaCMA and follow saturation kinetics. At fixed concentration of NaCMA, the rates increase with decreasing D.S. values until they reach maxima at D.S. = 0·00. Furthermore, at any D.S. value the catalytic efficiency increases with increasing pH values of the solutions. All these results indicate that the hydroxyl groups are actually the principal catalyzing groups.

Recently, it has been reported that sodium carboxymethylamylose (NaCMA) in aqueous solution (pH = 7·80) can effectively catalyze the hydrolysis of *N*-lauryl-3-acetoxypyridinium iodide (**1**) and *N*-methyl-3-dodecanoyloxypyridinium iodide (**2**) by first wrapping up these long-chain substrates in its helical cavities.[1] The formation of helical inclusion complexes as a prerequisite for catalysis has also been established by kinetic studies of the hydrolysis of substituted phenol esters of carboxylic acids with different chain lengths [2,3,4] in $Me_2SO—H_2O$ binary systems (pH = 12·60 to > 14) with various volume-fractions (ϕ) of the organic cosolvent (Me_2SO). In the former case (NaCMA in H_2O), it was proposed that the pendant carboxylate groups were the catalyzing groups,[1] mainly because it was believed that the OH groups were essentially un-ionized at pH = 7·80 and that the carboxylate ion ($CH_3CO_2^-$) could catalyse the hydrolysis of **1** with a measureable rate constant ($k_2 = 0·67 \times 10^{-3}\ s^{-1}\ M^{-1}$).



1 $R_1 = C_{12}H_{25}$, $R_2 = CH_3$
2 $R_1 = CH_3$, $R_2 = C_{11}H_{23}$

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Obviously, the aforesaid proposition was not established in this previous work, particularly in consideration of the fact that the NaCMA used only had one fixed value of the degree of substitution (D.S. = 0·24), which is defined as the number of carboxylate groups per glucose residue. Thus it would be of great mechanistic interest to find out whether the carboxylate groups were indeed the catalyzing groups, or whether the OH groups, probably mainly the ionized ones, were actually performing the catalytic job. At first sight, it might appear that the catalytic efficiency of NaCMA should increase with the D.S. values if carboxylate groups were truly able to catalyze the hydrolysis. Having had second thoughts, one would immediately think of the possibility that substitution might impair the capability of amylose to encapsulate its substrates and thus reduce its catalytic efficiency. At the time of the previous work, none of the answers to the two aforementioned questions were known, and it was not possible to make a simple prediction of the effect of increasing D.S. values on the catalytic efficiency of NaCMA. Therefore, we adopted a two-pronged approach to address this mechanistic problem. In a companion paper[5], the dependency of the wrapping capability of NaCMA on its D.S. is examined. In the present work, the catalytic efficiencies of NaCMA with graded D.S. values (0·00, 0·12, 0·18, 0·24, 0·29 and 0·35) at different pH values (7·32, 7·80, 8·10 and 9·30) of the aqueous solutions have been evaluated. The pyridinium iodide **1** and *p*-nitrophenyl dodecanoate (**3**) were used as the substrate esters.

EXPERIMENTAL SECTION

Preparation of substrates

N-Lauryl-3-acetoxypyridinium iodide (**1**). An equimolar mixture of 3-acetoxypyridine and lauryl iodide[6] was refluxed with appropriate amounts of anhydrous benzene for 12 h. The reaction mixture was cooled, diluted with anhydrous ethanol and then poured gradually into a large amount of anhydrous ether. The lower layer was separated and the solvent was removed on a rotatory evaporator to yield a red oil, which was identified by H-NMR and elemental analysis to be the desired product **1**.

Calculated for $C_{19}H_{32}O_2NI$. C: 52·67; H: 7·39; N: 3·23. Found. C: 52·48; H: 7·53; N: 3·03. NMR: ($CDCl_3$): 0·2–2(m, 23H); 2·5(s, 3H); 5·0(t, 2H); 8·65(m, 2H); 9·65(m, 2H).

p-Nitrophenyl laurate (**3**) was prepared according to the method previously reported.[7]

Solvents. The following aqueous solutions were used as the buffer solutions: Na_2HPO_4–NaH_2PO_4 0·01 M, pH = 7·32; Na_2HPO_4–NaH_2PO_4, 0·01 M, pH = 7·80; H_2BO_3–NaOH–KCl, 0·01 M, pH = 8·10; Na_2CO_3–$NaHCO_3$, 0·01 M, pH = 9·30. To all of these aqueous solutions 2% (wt) of sodium chloride was added.

Amylose solutions. The aqueous solution of amylose was prepared by dissolving it in 0·05 N NaOH followed by neutralizing with dilute HCl to the desired pH. This solution was then mixed with appropriate amounts of the buffer solution just before use.

The NaCMA solutions were prepared by dissolving the NaCMA directly in the buffer solutions, and diluting it to the desired concentrations. NaCMA with D.S. = 0·12, (Molecular weight $\langle M\rangle_n = 5{\cdot}1 \times 10^4$); 0·18 ($\langle M\rangle_n = 5{\cdot}1 \times 10^4$); 0·24, ($\langle M\rangle_n = 5{\cdot}15 \times 10^4$); 0·29, ($\langle M\rangle_n = 5{\cdot}15 \times 10^4$) and 0·35, ($\langle M\rangle_n = 5{\cdot}2 \times 10^4$) were used.[5]

Kinetics

Kinetic measurements were performed on a Perkin–Elmer 559 Spectrophotometer equipped with a thermostated cell as previously described.[1] Rate constants for the hydrolysis of **1** and **3**

Table 1. Hydrolysis rate constants k_{ob} ($10^{-3}s^{-1}$) of **1** in the presence of increasing amounts of NaOH and NaOAc[a], [**1**] = 8 × 10^{-5} M

[NaOH] (10^{-4} M)		0·05	0·76	1·38	2·45	3·09	4·17
k_{ob}		1·67	3·50	13·4	23·3	33·5	43·3
[CH_3CO_2Na] (M)	0·00	0·10	0·30	0·40	0·60	0·80	1·00
k_{ob}	0·81	0·82	0·95	1·08	1·37	1·60	1·83

[a] experimental uncertainty: ± 4%

were determined by following the change in absorbance at 320 nm for **1** and at 410 nm for **3**, respectively. The kinetics was shown to be first-order.

The concentration of **1** was fixed at 8 × 10^{-5} M, below its critical aggregate concentration (CAgC)[8], which is *ca.* 15 × 10^{-5} M in the presence of NaCMA, and 10 × 10^{-5} M in the absence of NaCMA. For similar reasons, **3** was fixed at 2 × 10^{-6} M.

The catalytic capabilities of hydroxide and acetate ions were compared by measuring the rate constants (k_{ob}) of the hydrolysis of **1** in Na_2HPO_4–NaH_2PO_4 buffer solutions containing different amounts of NaOH or sodium acetate (cf. Table 1). From the slopes of the linear portion of the k_{ob} vs. [CH_3CO_2Na] and k_{ob} vs. [NaOH] curves the second-order rate constants (k_2) were evaluated.

RESULTS AND DISCUSSION

The observed hydrolytic rate constants (k_{ob}) of the substrate **1** in the presence of increasing amounts of NaCMA with graded D.S. values, in aqueous solutions with pH values of 7·32, 7·80 and 8·10 are summarized in Tables 2, 3 and 4 respectively. Similarly, the k_{ob} for **3** in solutions with pH = 9·30 are listed in Table 5. The k_{ob} in each table can be plotted against [NaCMA] to yield six curves, which correspond to the six D.S. values chosen (0·00, 0·12, 0·18, 0·24, 0·29 and 0·35). For illustration, the data from Table 2 is plotted to give Figure 1 and 2. Similar figures were obtained for data from Tables 3, 4 and 5. The curves in Figure 1 show that the rates increase with the amount of NaCMA. Furthermore, they display saturation behaviour, in

Table 2. Hydrolytic rate constants k_{ob} ($10^{-3}s^{-1}$) of **1**[a] at pH = 7·32, as catalyzed by increasing concentrations of NaCMA ([NaCMA]) with graded D.S. values at 35 °C[b]

D.S	[NaCMA]%								
	0·00	0·05	0·10	0·15	0·20	0·30	0·40	0·50	0·60
0·00	0·47	2·87	4·58	5·83	7·0				
0·12	0·47		2·38		3·67	4·58	5·24	6·00	6·27
0·18	0·47		1·73		2·58	3·20	3·63	4·17	4·33
0·24	0·47		1·03		1·42	1·67	1·83	1·98	2·12
0·29	0·47		0·95		1·17	1·42	1·61	1·83	1·94
0·35	0·47		0·65		0·79	0·89	0·98	1·04	

[a] [**1**] is 8 × 10^{-5} M

[b] uncertainty: ± 5%

Table 3. Hydrolytic rate constants k_{ob} ($10^{-3}s^{-1}$) of **1**[a] at pH = 7·80, as catalyzed by increasing [NaCMA] with graded D.S. values at 35 °C[b]

D.S	[NaCMA]%									
	0·00	0·025	0·05	0·10	0·15	0·20	0·30	0·40	0·50	0·60
0·00	0·87	3·30	5·50	9·28	12·9	15·3				
0·12	0·87			4·80		7·33	9·33	10·8	11·7	12·5
0·18	0·87			3·20		5·00	6·25	7·17	7·83	8·27
0·24	0·87			1·88		2·47	2·93	3·27	3·40	3·57
0·29	0·87			1·48		1·94	2·19	2·53	2·80	
0·35	0·87			1·28		1·57	1·75	1·90	2·00	2·07

[a] [**1**] is 8×10^{-5} M

[b] uncertainty is ± 5%

Table 4. Hydrolytic rate constants k_{ob} ($10^{-3}s^{-1}$) of **1**[a] at pH = 8·10, as catalyzed by increasing [NaCMA] with graded D.S. values at 35 °C[b]

D.S.	[NaCMA]%								
	0·00	0·05	0·10	0·15	0·20	0·30	0·40	0·50	0·60
0·12	1·30	7·67	13·1	15·7	19·3	24·0	27·6	30·7	31·6
0·18	1·30		8·07		12·3	15·6	18·7	20·7	22·4
0·24	1·30		4·53		6·53	8·00	8·87	9·33	9·87
0·29	1·30		3·43		4·93	6·00	6·93	7·50	
0·35	1·30		2·57		3·47	4·13	4·73	5·07	5·13

[a] [**1**] is 8×10^{-5} M

[b] uncertainty is ± 3%

Table 5. Hydrolytic rate constants k_{ob} ($10^{-4}s^{-1}$) of **3**[a] at pH = 9·30, as catalyzed by increasing [NaCMA] with graded D.S. values at 35 °C[b]

D.S.	[NaCMA]%						
	0·00	0·10	0·20	0·40	0·60	0·80	1·00
0·12	0·51	2·81	4·60	7·81	10·3	11·6	12·3
0·18	0·51		2·60	4·79	6·31	7·11	7·51
0·24	0·51		1·31	2·01	2·75	3·30	3·69
0·35	0·51		0·81	0·99	1·10	1·25	1·40

[a] [**3**] is 2×10^{-6} M.

[b] uncertainty: 8%.

accordance with the fact that the substrate molecules are encapsulated inside the helical cavities of NaCMA.[1–4,9] Here an observation of crucial importance is (e.g., see Figure 1): at any host concentration ([NaCMA]) and at any pH, the rate of catalyzed hydrolysis of **1** or **3** decreases with the increasing degree of substitution (D.S.) of the host. This observation

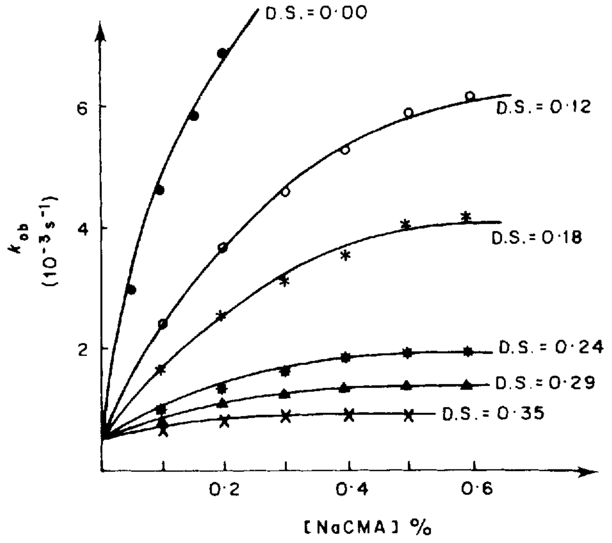

Figure 1. Observed rate constants at 35 °C, pH = 7·32 for the hydrolysis of **1** as a function of NaCMA concentration.

dovetails with an observation of the companion work,[5] which has established that the largest number of binding sites (*n*) of NaCMA decreases with increasing D.S. e.g., at D.S. = 0·12, *n* = 33·3; D.S. = 0·18, *n* = 15·9; D.S. = 0·35, *n* = 3·9. Taking both these observations into consideration and disregarding the nature of the catalyzing groups for the time being, we can reasonably postulate that substitution with carboxylate groups will reduce the catalytic efficiency of NaCMA because the number (*n*) as well as the stability of the helical inclusion

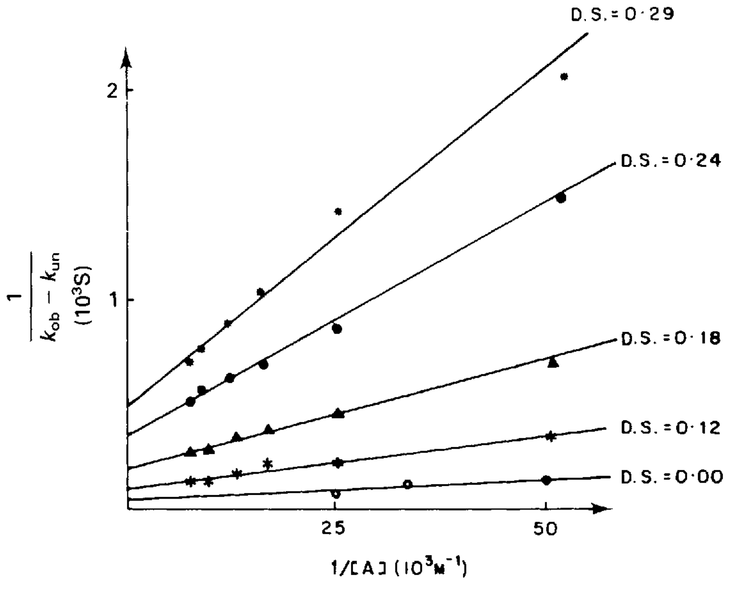

Figure 2. Lineweaver–Burk plot with the data from Table 2.

Table 6. Dissociation constants of the inclusion complex of **1** and NaCMA and catalytic rate constants of the hydrolysis of **1** at different pH and 35 °C

pH	measured constants	D.S. 0·00	0·12	0·18	0·24	0·29	0·35
7·32	$k_c \times 10^3$	15·2	10·4	6·38	3·16	2·67	
	k_c/k_{un}	32·3	22·1	13·6	6·72	5·68	
	$K'_d \times 10^5$	5·1	8·24	6·6	7·3	7·3	
	k_c/K'_d	298	126	96·7	43·3	36·6	
7·80	$k_c \times 10^3$	52·4	20·3	15·0	5·03	4·70	2·90
	k_c/k_{un}	60·2	23·3	17·2	5·78	5·40	3·33
	$K'_d \times 10^5$	10·1	7·74	9·81	6·08	10·3	7·51
	k_c/K'_d	519	262	153	82·7	45·6	38·6
8·10	$k_c \times 10^3$		47·6	36·9	14·6	13·3	8·46
	k_c/k_{un}		36·6	28·4	11·2	10·2	6.51
	$K'_d \times 10^5$		6·11	8·46	6·01	9·02	8·88
	k_c/K'_d		779	436	243	147	95·3

[a] The uncertainties are: for k_c, ± 5%; for K'_d, ± 8%.

complexes formed between NaCMA and the substrate are reduced. The validity of the aforesaid postulate should be tested by examining the k_c, K'_d, k_c/k_{un} and k_c/K'_d values summarized in Table 6. The corresponding values for substrate **3** were not tabulated because the rate constants for **3** are less accurate than those for **1**.

$$\begin{tikzcd}
\mathrm{S + NaCMA} \arrow[r, shift left, "k_1"] \arrow[d, "k_{un}"] & \mathrm{NaCMA \cdot S} \arrow[l, shift left, "k_{-1}"] \arrow[r, "k_c"] & \mathrm{product} \\
\mathrm{product} & &
\end{tikzcd}$$

$$\frac{1}{k_{ob} - k_{un}} = \frac{K'_d}{(k_c - k_{un})[\mathrm{A}]} + \frac{1}{k_c - k_{un}} \qquad (1)$$

The k_c and K'_d values were obtained from Lineweaver–Burk plots using equation (1),[1] where $K'_d = (k_{-1} + k_c) / k_1$ and [A] is the molar concentration of the NaCMAs.

Table 6 reveals that although the K'_d values are not very sensitive to changes in D.S., the trends of change of the k_c / k_{un} and k_c / K'_d values, both indicators of catalytic efficiency, are clear-cut; they decrease with increasing D.S. values. At this point, one may already argue that since the catalytic efficiency decreases with the degree of carboxylate substitution, and particulary, it is highest at D.S. = 0·00, then the carboxylate groups can not be the main catalyzing groups as previously suggested. However, the above argument is not completely on safe ground because all data can be rationalized on the following assumptions: (1) Both the hydroxyl (—OH and —O$^-$) and carboxylate groups can catalyze the hydrolysis, although their relative importance may vary with changing circumstances. (2) The predominant factors which determine the catalytic efficiency are the number and the stability of helical inclusion complexes which decrease with increasing D.S. In fact, exceedingly strong support for the last statement can be obtained from plotting either k_c / k_{un} or k_c / K'_d against the number of binding sites (n) evaluated from the companion work,[5] because all such plots turned out to be straight lines, as shown in Figures 3 and 4. Therefore, in order to assess the importance or unimportance of the carboxylate ions as catalyzing groups, we should look into the effect of pH on the catalytic efficiency.

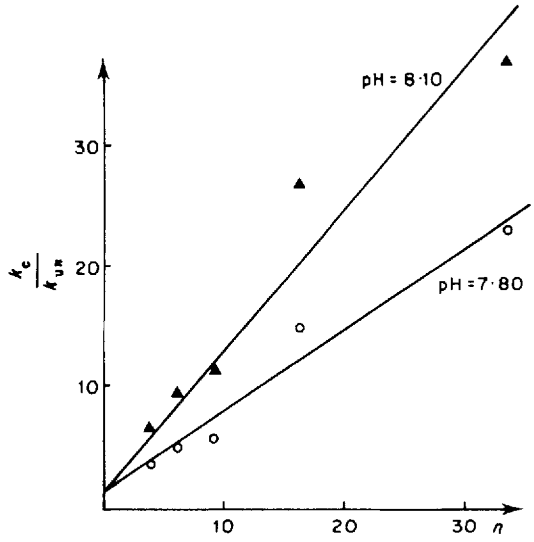

Figure 3. Correlation of k_c/k_{un} with the largest number of binding sites (n) at pH = 7·80 and 8·10.

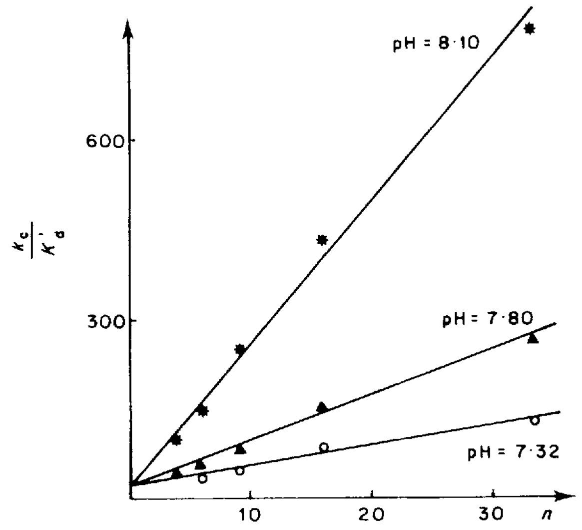

Figure 4. Correlation of k_c/K'_d with the largest number of binding sites (n) at pH = 7·32, 7·80 and 8·10.

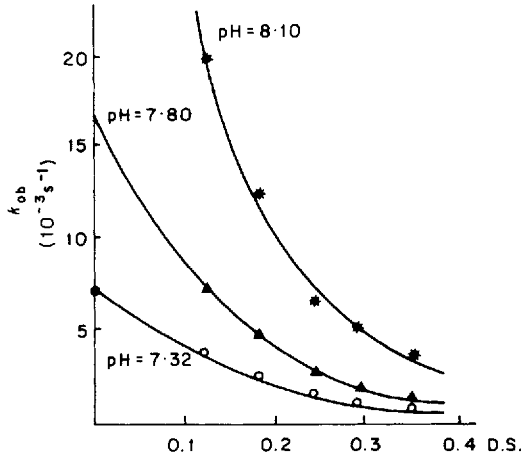

Figure 5. Correlation of k_{ob} of **1** with the D.S. values of NaCMA at different pH values. [**1**] = 8×10^{-5} M. [NaCMA] = 0·2% (wt).

The carboxylic groups of NaCMA has a pK_a value of *ca.* 4·2,[7] in other words, at pH 7·3, they are already 99·5% ionized, and at pH 7·8, essentially 100%. Therefore, if the carboxylate groups are mainly responsible for the catalysis, then at any specific D.S. value, the observed rate constants should be relatively insensitive to small pH changes. This is evidently not so, as shown by Figure 5 in which k_{ob} is plotted against D.S. at pH values of 7·32, 7·80 and 8·10. At any D.S. value, the rates increase with increasing range of pH to a much greater extent than would be expected from the pH change itself. Furthermore, both k_c / k_{un} and k_c / K_d' also increase with pH, as shown in Figures 3 and 4. All these facts can only be reconciled with the proposition that the ionized, and probably to a much lesser extent un-ionized, hydroxyl groups are actually the principal catalyzing groups.[10,11]

In order to obtain supporting evidence for the above conclusion, rate constants (Table 1) for the hydroxide-catalyzed and acetate-catalyzed hydrolysis of **1** have been measured. Second-order rate constants (k_2) for the acetate- and hydroxide-catalyzed hydrolysis are $(1{\cdot}28 \pm 0{\cdot}02) \times 10^{-3} s^{-1} M^{-1}$ and $120 \pm 2\ s^{-1} M^{-1}$ respectively. The hydroxide ion is thus four to five orders of magnitude more effective than the acetate ion and thereby can operate at much lower concentrations. Bearing in mind that: (1) the OH groups of glucose are slightly more acidic than the OH of water, [7,12] (2) in NaCMA the OH groups outnumber the carboxylate groups, and (3) in the helical microenvironment intramolecular hydrogen-bonding may get in the game in some subtle ways, it is entirely reasonable to conclude that the OH groups are the principal catalyzing groups for the hydrolysis of long-chain esters wrapped-up inside the helical cavities of NaCMA. Finally, it is worthwhile to recall that substitution of the OH by other groups will impair the capability of amylose to form helical inclusion complexes which are essential to the realization of catalytic hydrolysis of the long-chain esters.

REFERENCES

1. Hui Yongzheng, Gu Jianhua and Jiang Xikui, *Scientia Sinica B*, **26**, 225 (1983).
2. Yongzheng Hui, Shujing Wang and Xikui Jiang, *J. Am. Chem. Soc.* **104**, 347 (1982).
3. Xi-Kui Jiang, Yong-Zheng Hui and Wei-Quiang Fan, *J. Am. Chem. Soc.* **106**, 3839 (1984).
4. Xi-Kui Jiang, Yong-Zheng Hui and Wei-Quiang Fan, *Acta Chimica Sinica*. **43**, 57 (1985), *Eng. Ed.*, 217 (1986).

5. Xi-Kui Jiang, Xing-Ya Li and Bang-Zhou Huang, *Journal of Physical Organic Chemistry*, **1**, 133–141 (1988).
6. H. M. Wuest and H. C. Huffman *J. Am. Chem. Soc.* **63**, 1210 (1951).
7. Hui Yongzheng, Wang Shujing and Xikui Jiang, *Acta Chimica Sinica*, **40**, 1148 (1982).
8. Xi-Kui Jiang, Xing-Ya Li and Bang-Zhou Huang, *Proc. Indian Acad. Sci. (Chem. Sci.)*, **98**, (5), 423–434 (1987).
9. Hui Yongzheng, Wang Shujing, Shen Youdi and Jiang Xikui, *Acta Chimica Sinica* **42**, 1161 (1984).
10. R. Breslow *et. al*, *J. Am. Chem. Soc.* **103**, 154 (1981).
11. Cheng Xian-En, Jiang Xi-Kui, Gu Jian-Hua and Shen You-Di, *Acta Chimica Sinica* **43**, 99 (1985); *Eng. Ed.*, 84 (1985).
12. H. A. Sober (ed), *Handbook of Biochemistry, Selected Data for Molecular Biology*, 2nd ed., Cleveland, The Chemical Rubber Co, 1970.