

Kinetics of α -Amino Acids Acylation by *p*-Nitrophenyl *p*-Nitrobenzoate in Water-propan-2-ol mixtures

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Kinetic studies of α -amino acids acylation by *p*-nitrophenyl *p*-nitrobenzoate in water-propan-2-ol mixtures were carried out at different temperatures under pseudo-first conditions by keeping the concentration of α -amino acid in thirty fold excess over that of the *p*-nitrophenyl *p*-nitrobenzoate. We can put the investigated α -amino acids in the following order according to their reacting ability in the acylation reaction :

L-proline >> glycine > L- α -alanine > L-valine > L-serine > DL-threonine.

Increase of the water content in the binary water-propan-2-ol solvent leads to a decrease of energy, enthalpy and entropy of activation. The positive effect of water with its high dielectric constant on the rate of α -amino acids acylation was discussed.

Key Words: Acylation, Rate constant, Amino acid, Temperature.

INTRODUCTION

Amino acids represent for organism forerunners of essential biomolecules such as proteins, hormones, enzymes, *etc.* They also may serve as energy source, loosing their amino group by transamination or oxidative deamination¹. They are widely used in a broad variety of medical, industrial and environmental applications, *e.g.*, in synthesis of cosmetic and pharmaceutical preparations, pesticides, surfactants and textile products².

α -Amino acids is a subgroup of amino acids in which both the amino ($-\text{NH}_2$) and carboxylic ($-\text{COOH}$) functional groups are attached to the same carbon atom. One of the most important reactions which amino acids undergo is the acylation reaction, which can be considered as a nucleophilic substitution type accompanied with a formation of N-C bond between the amino compound and the acylating agent molecule. The most common used agents for amino acids acylation are 4-nitrophenylacetate, benzoyl chloride, benzoic, thiobenzoic and acetic anhydrides and complex esters^{3,4}.

The previous studies concerned with the acylation of amino compounds in general and amino acids in particular agreed with the fact that the rate and mechanism of such reactions depend mainly on structure and

concentration of aminocompound, nature of *p*-nitrophenyl *p*-nitrobenzoate, properties and composition of solvent and finally on temperature of the process^{4,7}.

We report herein the kinetic studies on α -amino acids acylation by *p*-nitrophenyl *p*-nitrobenzoate in water-propan-2-ol mixtures at different temperatures with a good evaluation of the rate constants and the activation parameters of the reaction taking into account the effect of reagents structure, concentrations, temperature and solvent properties.

EXPERIMENTAL

All kinetic measurements were made on Pye Unicam SP8000 spectrophotometer using 2 cm matched silica cells. Temperature control during measurement was achieved by connecting a thermostat of the type Julabo Paratherm PT40PS to the cell-housing of the spectrophotometer.

All the reagents used were highly pure certified analytical reagent grade of Fluka origin. Glycine (Gly), alanine (Ala), proline (Pro), serine (Ser), threonine (Thr) and valine (Val) before use were dried for 1 h at 373 K. Propan-2-ol, 4-benzoyl chloride, 4-nitrophenol, NaOH and HCl acid were used as received.

All water-containing solutions were prepared using doubly distilled water. Water-propan-2-ol mixtures were prepared by mixing the components in appropriate mass proportions.

Preparation of alkaline-organic solution (0.022 M): 0.88 g of solid sodium hydroxide was transferred into 1 L volumetric flask and completed to the mark by binary water-propan-2-ol solvent. The standardization of the resulting solution was done using potentiometric titration and HCl as titrant (pH = 8-9).

Preparation of α -amino acids working solutions (0.132 M): A weighed portion of α -amino acids (0.099-0.185 g) was transferred into a calibrated picnometer and completed to 10 mL by alkaline-organic mixture.

Ester synthesis: The *p*-nitrophenyl *p*-nitrobenzoate was obtained *via* reflux of 4-benzoyl chloride with 4-nitrophenol for 10 h at 393 K. Purification of product was performed by recrystallization from benzene. Its purity was checked by melting point (m.p. = 407 K), which agrees with a literature value^{9,10}.

Kinetic procedure

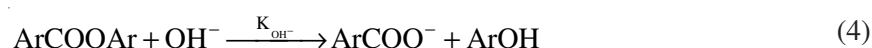
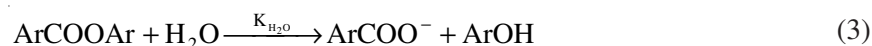
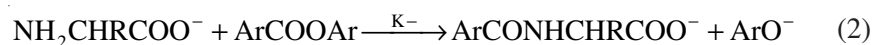
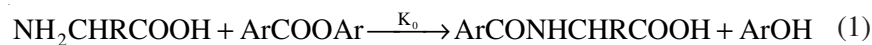
Kinetic measurements were done at different temperatures, with an excess of amino acid over the *p*-nitrophenyl *p*-nitrobenzoate. Before each the working solution (0.132 M) of a particular α -amino acid was prepared, placed in the cell holder and thermostated during 15 min. Then one drop of *p*-nitrophenyl *p*-nitrobenzoate solution in propan-2-ol was added to it. The

moment of adding the *p*-nitrophenyl *p*-nitrobenzoate was taken as initial time of the reaction. The rate of α -amino acids acylation were determined by monitoring the absorbance variation per time of the formation of yellow 4-nitrophenolate ion at 400 nm.

RESULTS AND DISCUSSION

All experiments were carried out under pseudo-first order conditions by keeping the concentration of α -amino acid in thirty fold excess over that of the *p*-nitrophenyl *p*-nitrobenzoate, so this fact will simplify the derivation of kinetic equation.

Interactions of α -amino acids with the *p*-nitrophenyl *p*-nitrobenzoate can be represented by the following steps:



where: Ar = 4-NO₂-C₆H₄; K₀, K⁻, K_{H₂O}, K_{OH⁻} are rate constants of processes (1-4), respectively.

The change of *p*-nitrophenyl *p*-nitrobenzoate concentration (C_{aa}) per time is given by eqn. 6

$$-d\text{C}_{aa}/dt = k_0\text{C}_0\text{C}_{aa} + K\text{-C-C}_{aa} + K_{\text{H}_2\text{O}}\text{C}_{\text{H}_2\text{O}}\text{C}_{aa} + K_{\text{OH}^-}\text{C}_{\text{OH}^-}\text{C}_{aa} = K_{\text{H}}\text{C}_{aa} \quad (6)$$

$$K_{\text{H}} = K_0\text{C}_0 + K\text{-C-} + K_{\text{H}_2\text{O}}\text{C}_{\text{H}_2\text{O}} + K_{\text{OH}^-}\text{C}_{\text{OH}^-} \quad (7)$$

where: K₀, K⁻ are the rate constants of the acylation of neutral and anionic α -amino acid forms, respectively; K_{H₂O}, K_{OH⁻} rate constants of the *p*-nitrophenyl *p*-nitrobenzoate hydrolysis; K_H effective (observed) rate constant of the reaction; C₀, C⁻, C_{H₂O}, C_{OH⁻} concentrations of neutral and anionic α -amino acid forms, water and hydroxide ions, respectively.

From the condition of the experiments, it follows that C₀ << C⁻ and K₀C₀ << K-C⁻; K₀C₀ and K_{H₂O}C_{H₂O} are too small and can be ignored, so expression (7) takes the form:

$$K_{\text{H}} = K\text{-C-} + K_{\text{OH}^-}\text{C}_{\text{OH}^-} \quad (8)$$

The C_{OH⁻} can be determined from the relations:

$$K_w = \text{CH} + \text{C}_{\text{OH}^-}\gamma^2 \quad (9)$$

$$K_3 = (\text{C} - \text{C}_{\text{OH}^+}/\text{C}_{\pm})\gamma^2 \quad (10)$$

where: C_± concentration of the α -amino acid zwitter ion form; γ activity coefficient.

From (9) and (10) it follows that

$$C_{\text{OH}^-} = K_w C^- / K_3 C_{\pm} \quad (11)$$

Substitution (11) in (8) we get the expression for effective rate constant of pseudo- first order α -amino acid acylation:

$$K_H = K \cdot C^- + K_{\text{OH}^-} (K_w C^- / K_3 C_{\pm}) \quad (12)$$

From (12), it is concluded that the decrease of the *p*-nitrophenyl *p*-nitrobenzoate hydrolysis rate and increase of α -amino acid acylation rate can be achieved by sharp increase of the zwitter ion form concentration comparing with that of anionic form of α -amino acid. In this case the second term in eqn. 12 can be neglected, so it becomes

$$K_H = K \cdot C^- \quad \text{or} \quad K^- = K_H / C^- \quad (13)$$

Results of kinetic investigation show that when $C^- / C_{\pm} = 1/2$ or $1/3$ the rate of hydrolysis is with that of the acylation process.

Rate constants

Rate constants were determined by the first order plot using the eqn.

$$Kt = 2.303 \log [A_{\infty} / (A_{\infty} - A_t)]$$

where: A_{∞} -final absorbance and A_t the absorbance at any time, after addition of *p*-nitrophenyl *p*-nitrobenzoate and appearance of yellow 4-nitrophenolate ion at 400 nm.

The validity of this interpretation was checked by plotting

$\log \left(\frac{A_{\infty}}{(A_{\infty} - A_t)} \right)$ against t , straight line was obtained and the pseudo first order rate constant is determined from the slope. As an illustration example such plot is represented in Fig. 1 for glycine acylation case.

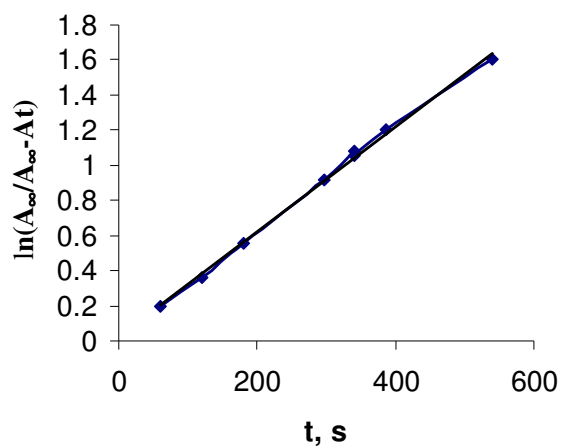


Fig. 1. Pseudo first order plot using absorbance change per time to determine the rate constant of glycine acylation by the acylating agent in water-propan-2-ol mixtures at 298 K illustration example

The values of the rate constants of the acylation of all investigated α -amino acids are summarised in Tables 1-6, from these data one can put the investigated α -amino acids in the following order according to their reacting ability in the acylation reaction:

L-proline >> glycine > L- α -alanine > L-valine > L-serine > DL-threonine
 the highest reacting ability of L-proline is due to its high basicity comparing with other amino acids. Fig. 2 shows a strong linear correlation between the rate constant of acylation and the basicity of investigated α -amino acids. These results are in a good agreement with previous studies concerning the acylation of some amino acids using benzoyl chloride and nitrophenylacetate as acylating agents^{8,11}.

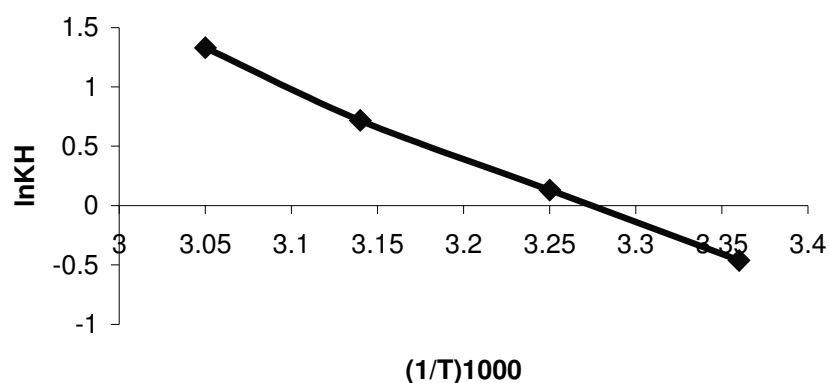


Fig. 2. Arrhenius plot for determination of the activation energy in glycine acylation by the acylating agent in water-propan-2-ol mixture (50:50 by mass)

Activation parameters

The experiments of α -amino acids acylation were carried out at different temperatures. So the rate constants dependence upon temperatures was also studied. Using Arrhenius equation the activation parameters were determined for each amino acid-*p*-nitrophenyl *p*-nitrobenzoate pair:

$$K = A \cdot \text{EXP}(-E_a/RT) \quad (14)$$

$$A = 2.73(kT/h) \text{EXP}(\Delta S^*/R) \quad (15)$$

$$E_a = \Delta H^* + RT \quad (16)$$

where: E_a is the activation energy; ΔS^* is the entropy of activation; ΔH^* is the enthalpy of activation; A -frequency factor; k -Boltzman constant; h -planck constant.

We determined the activation energy from plot of rate constant \log vs. reciprocal of temperature (Fig. 3), then eqn. 14 was used to calculate the value of pre-exponential factor, entropy of activation by eqn. 15 and at last eqn. 16 was used to calculate the enthalpy of activation.

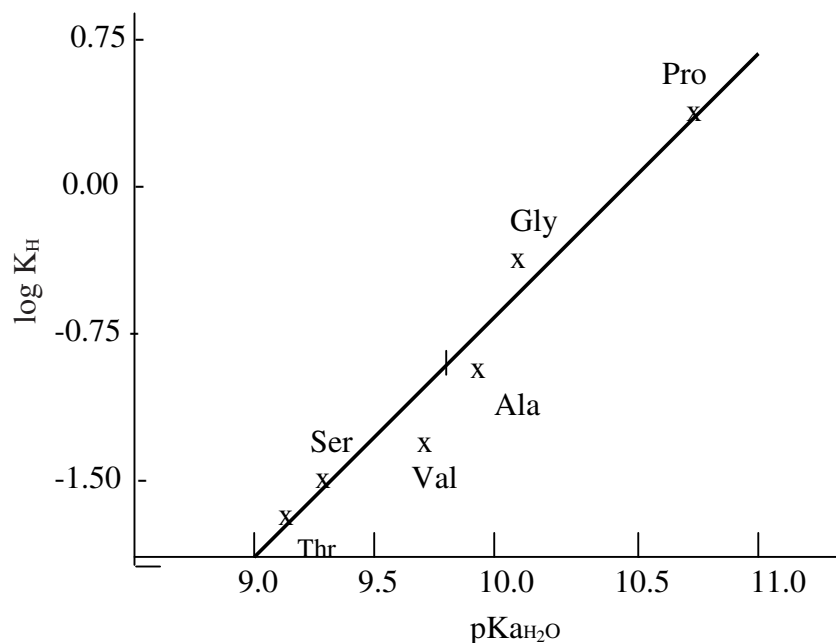


Fig. 3. Plot of the logarithms of α -amino acids acylation rate constants by acylating agent in water (50 % mass)-propan-2-ol on pK_a of investigated α -amino acids at 298 K

The values of different activation parameters are given in Tables 1-6. From these data it is easy to observe that increase of the water content in the binary water-propan-2-ol solvent leads to a decrease of energy, enthalpy and entropy of activation, which can be explained in terms of formation of more ordered solvated activated complexes between the water molecules and reagents molecules. This interpretation was also supported by other authors^{12,13}.

TABLE-1
KINETIC CHARACTERISTICS OF GLYCINE ACYLATION BY
p-NITROPHENYL *p*-NITROBENZOATE IN WATER-PROPAN-2-OL
MIXTURE AT 298 K

Mass of water (%)	K_H (s^{-1})	$\ln A$	E_a ($kJ\ mol^{-1}$)	ΔH^* ($kJ\ mol^{-1}$)	ΔS^* ($J\ (mol\ K)^{-1}$)
40	0.14	15.63	43.7	41.2	123
50	0.16	15.01	41.9	39.3	128
60	0.18	14.76	40.8	38.2	131
70	0.20	14.37	39.6	37.1	134
80	0.27	14.18	38.4	35.9	137

TABLE-2
KINETIC CHARACTERISTICS OF L- α -ALANINE ACYLATION BY
p-NITROPHENYL *p*-NITROBENZOATE IN WATER-PROPAN-2-OL
MIXTURES AT 298 K

Mass of water (%)	K_H (s ⁻¹)	ln A	Ea (kJ mol ⁻¹)	ΔH^* (kJ mol ⁻¹)	ΔS^* J (mol K) ⁻¹
40	0.60	16.01	42.3	39.8	116
50	0.63	15.96	40.8	38.3	120
60	0.69	15.20	38.6	36.1	127
70	0.77	14.31	36.1	33.6	135
80	0.94	13.82	34.4	31.9	148

TABLE-3
KINETIC CHARACTERISTICS OF L-SERINE ACYLATION BY
p-NITROPHENYL *p*-NITROBENZOATE IN WATER-PROPAN-2-OL
MIXTURES AT 298 K

Mass of water (%)	K_H (s ⁻¹)	ln A	Ea (kJ mol ⁻¹)	ΔH^* (kJ mol ⁻¹)	ΔS^* J (mol K) ⁻¹
40	3.38	18.69	48.6	46.1	119
50	3.50	16.99	45.2	42.6	130
60	3.75	16.11	43.2	43.2	136
70	4.09	15.59	42.1	42.1	139
80	4.58	14.98	40.9	40.9	142

TABLE-4
KINETIC CHARACTERISTICS OF DL-THREONINE ACYLATION BY
p-NITROPHENYL *p*-NITROBENZOATE IN WATER-PROPAN-2-OL
MIXTURES AT 298 K

Mass of water (%)	K_H (s ⁻¹)	ln A	Ea (kJ mol ⁻¹)	ΔH^* (kJ mol ⁻¹)	ΔS^* J (mol K) ⁻¹
40	1.89	20.47	52.3	49.9	111
50	2.44	19.61	50.8	48.2	116
60	3.54	18.46	48.9	46.4	120
70	4.09	17.72	47.4	44.8	123
80	4.75	17.09	46.2	43.7	127

Effect of solvent

The modern studies proved that the solvent in which the reaction takes place is not only a medium but also a reagent^{14,15}. There are some specific interactions between solvent molecules and α -amino acid molecules of a dipole-dipole types because both structures contain polar groups resulting in a formation of solvated activated complexes with hydrogen

TABLE-5
KINETIC CHARACTERISTICS OF L-VALINE ACYLATION BY
p-NITROPHENYL *p*-NITROBENZOATE IN WATER-PROPAN-2-OL
MIXTURES

Mass of water (%)	K_H (s ⁻¹)	ln A	Ea (kJ mol ⁻¹)	ΔH^* (kJ mol ⁻¹)	ΔS^* J (mol K) ⁻¹
40	7.78	16.31	45.5	42.9	125
50	7.99	16.01	45.0	42.4	128
60	8.13	15.34	43.2	40.6	131
70	9.79	14.79	42.3	39.8	134
80	11.23	14.05	40.8	38.3	138

TABLE-6
KINETIC CHARACTERISTICS OF L-PROLINE ACYLATION BY
p-NITROPHENYL *p*-NITROBENZOATE IN WATER-PROPAN-2-OL
MIXTURES AT 298 K

Mass of water (%)	K_H (s ⁻¹)	ln A	Ea (kJ mol ⁻¹)	ΔH^* (kJ mol ⁻¹)	ΔS^* J (mol K) ⁻¹
40	4.73	13.42	37.1	34.6	116
50	5.05	12.95	36.1	33.6	119
60	5.21	12.60	35.3	32.8	121
70	6.00	11.98	34.2	31.7	124
80	6.94	11.34	32.9	30.4	127

bonding. From data in Tables 1-6, it is concluded that as the water content in the binary water-propan-2-ol solvent increases, the rate of α -amino acids acylation also increases. This observed trend was also obtained in early studies dealing with different amino compounds acylation in protic and aprotic solvents¹²⁻¹⁵.

One of the most important solvent properties that can be used to estimate the quantitative affect on the rate of a reaction is a dielectric constant (ϵ), which is a measure of solvent polarity. But it is preferred to use the function $(\epsilon - 1/2 \epsilon + 1)$ versus the rate constant logarithm, which is in its turn proportional directly to water mass percent in water-propan-2-ol binary solvent. Figs. 4 and 5 show clearly the positive effect of water with its high dielectric constant on the rate of α -amino acids acylation¹⁶.

Increase in water content also affect the values of activation parameters by decreasing them because of the above mentioned reasons^{17,18}.

Effect of temperature

The effect of temperature on the kinetic characteristics of investigated α -amino acids was explored by performing the kinetic experiments at

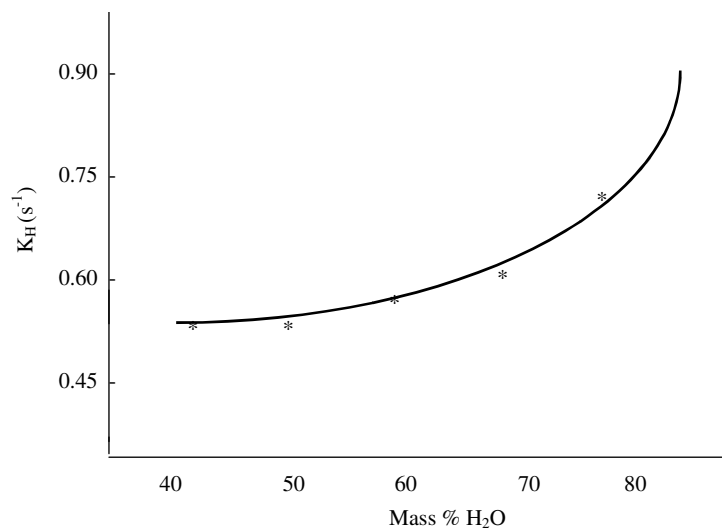


Fig. 4. Effect of water content in a binary water-propan-2-ol solvent on the rate constant of α -amino acids acylation by acylating agent at 298 K

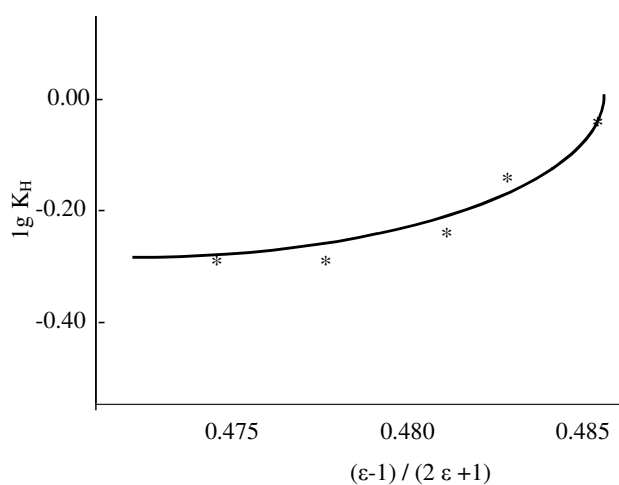


Fig. 5. Plot of the logarithms of glycine acylation rate constant by acylating agent in water (50 % mass)-propan-2-ol against properties of medium in term of the function $(\epsilon-1)/(2\epsilon+1)$ at 298 K

different temperatures namely: 298, 308, 318 and 328 K. The results for all acylated amino acids are summarized in Table-7.

It is concluded that as temperature increases the rate constants of the acylation reaction increase sharply and the activation energies slightly decrease. This trend can be explained in terms of kinetic theory and effective

TABLE-7
 RATE CONSTANTS AND ACTIVATION ENERGIES OF THE
 INVESTIGATED α -AMINO ACIDS ACYLATION BY
p-NITROPHENYL *p*-NITROBENZOATE IN WATER-PROPAN-2-OL
 MIXTURES (50:50 BY MASS) AT DIFFERENT TEMPERATURES

Amino acid	T (K)	K_H (s^{-1})	Ea ($kJ\ mol^{-1}$)
Glycine	298	0.63	40.8
	308	1.14	40.5
	318	2.05	40.3
	328	3.79	39.9
L- α -Alanine	298	0.16	41.9
	308	0.29	41.6
	318	0.54	41.3
	328	0.98	40.9
L-Serine	298	3.50	45.2
	308	6.58	44.9
	318	12.31	44.6
	328	22.70	44.2
DL-Threonine	298	2.44	50.8
	308	4.64	50.4
	318	8.76	50.1
	328	15.94	49.8
L-Valine	298	7.99	45.0
	308	14.46	44.7
	318	26.89	44.4
	328	48.96	44.1
L-Proline	298	5.05	36.1
	308	9.49	35.9
	318	18.04	35.7
	328	33.19	35.3

collisions theory according to which as temperature increases, the average molecular speed and kinetic energy increase and the fraction of reacting molecules possessing minimum energy, required to undergo a chemical change also increases.

Conclusions

The results of this work illustrate that spectrophotometric monitoring of the α -amino acids acylation is an effective method inspite of a complex set of factors that affect rate and mechanism of such reactions. The properties of medium such as polarity and dielectric constant are greatly influence the kinetic regularities of the reaction. But one must recognize that the specific structure effect of α -amino acids can be explained more clearly in term of quantum-chemical calculations.

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