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Electrostatic interactions effect in the aminolysis of some β-lactams in the presence of poly(ethyleneimine):structure-reactivity

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The aminolysis reaction of a series of β-lactams in the presence of poly(ethyleneimine) (PEI) at 30°C and pH = 8.40 has been studied. The substrates investigated follow a pseudo first order rate, except two β-lactams which show a two step consecutive reaction. Increasing the polyelectrolyte concentration, Michaelis–Menten type kinetics are been observed and for four substrates a more complex rate behaviour was verified owing to the polyelectrolyte inhibition effect. Both the binding constant K_1 between polyelelectrolyte and β-lactam and the first order rate constant of the reactive complex decomposition k_{cat} were calculated. The substituent effect at C-6′ or C-7′ position of β-lactam on the aminolysis rate does not correlate with the σ_1 value (Taft plot). Most probably, steric and electronic effects are important, but the electrostatic ones are determining factors for the relevant acceleration attributable to both the binding phenomena and the increased reactivity of the substrate–polyelectrolyte complex. The comparison between poly(ethyleneimine) and Human Serum Albumin (HSA) is also discussed. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: β-lactams; aminolysis; poly(ethylenimine); polyelectrolytes

INTRODUCTION

In a previous paper[1] we reported the study of polyelectrolyte effect on the decomposition of benzylpenicillin in the presence of poly(ethyleneimine) (PEI). The substrate is electrostatically attracted on the polymer surface and it undergoes the nucleophilic attack of amino groups of PEI which is penicilloylated. Kinetic experiments and estimations of microscopic rate constants allowed us to hypothesize the reaction mechanism.[1]

In this paper, we extend the investigation to a series of β-lactams (six penicillins and one cephalosporin) in order to estimate the relationship between the nature of the substrate binding and the reactivity towards the aminolysis in the presence of polymeric amine in aqueous solution.

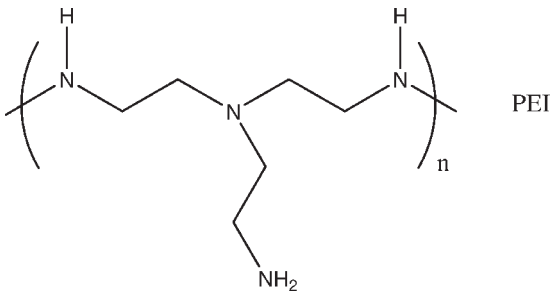
This study is interesting not only to understand the mechanism of β-lactam aminolysis in the presence of polymeric amine, but also to shed light on the phenomena of allergies caused by β-lactams.[2]

The binding of penicillins and cephalosporins to Human Serum Albumin (HSA) is a very complex process not yet completely understood.[2d] In fact, it seems that the major determinant in the penicillin allergy is the penicilloyl group bound to the amino groups of *L*-lysine residue present in HSA. Some studies identified peptides containing benzylpenicilloyl moieties in different regions of HSA involving several *L*-lysine residues.[3–5]

Many theoretical and experimental studies showed that the affinity of albumin for ligands depends on the charge and on the hydrophobic character of the molecules. The binding of short chain molecules positively charged are less firmly bound than those negatively charged having long alkyl chain.[6a,b],7 It seems that halogens, aromatic rings, methylene and —N═N— groups increase the binding while the amino group influences negatively.[8a] In addition, both in penicillins and cephalosporins analogous, the hydrophobic interactions of the substituent at C-6′ or C-7′ position, respectively, with the aminoacids of HSA would play a relevant role in the binding.[8b]

The PEI choice was guided by the simplicity of the model taking into account the macroion character of the human protein and the presence of primary amino groups. In fact, the branched polymeric structure of PEI, which has an average M.W. 60000[9] close to that of HSA, 66411,[10] contains beyond secondary (50%) and tertiary amino groups (25%), also primary (25%) ones which can simulate the *L*-lysine site binding of HSA (six different *L*-lysine residues of the total 56 can react with penicillins[4]).

Moreover, PEI is interesting in biological and medicinal field because it is a highly efficient vector for delivering gene and oligonucleotides transfer into cells in culture and *in vivo*.[11]



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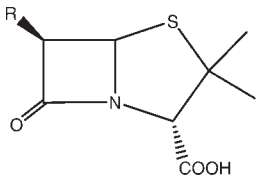
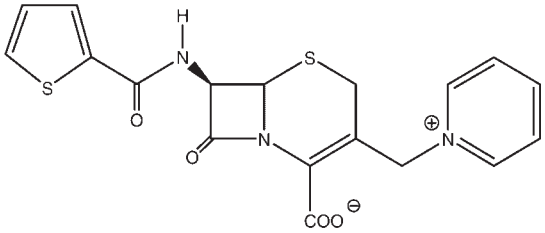
RESULTS AND DISCUSSION

Six penicillins, i.e. ticarcillin (TI), carbenicellin (CA), 6-α-chloropenicillanic acid (6-CLAPA), 6-β-aminopenicillanic acid (6-APA), ampicillin (AM), penicillin V (PV), and one cephalosporin, that is cephaloridin (CE), were submitted to kinetic investigations.



R

2-thienyl-CH(COOH)CONH (TI)
PhCH(COOH)CONH (CA)
Cl (6-CLAPA)
NH_2 (6-APA)
$PhCH(NH_2)CONH$ (AM)
$PhOCH_2CONH$ (PV)



(CE)

The aminolysis reactions were performed in the presence of PEI at pH = 8.40 and 30°. In these conditions all β-lactams are totally in anionic form. In fact, the pK_a of carboxylic group, which is expected to decrease (0.6–0.9 pK units) in the presence of the polyelectrolyte,[12] is in the range 2.3–3.0 and pK_{NH2} of AM and 6-APA are 7.22 and 4.90, respectively.[13]

The attack on the β-lactams occurs mainly by primary amino groups which are more nuclephilic, being the intrinsic ionization constant p$K_i = 9.5$[14a] in comparison with the secondary ones (p$K_i = 8.5$),[14a] and does not show a significant steric hindrance, since they are bound at the end of the branched chain.

In the presence of an excess of PEI, the β-lactams follow a pseudo first order rate constant (k_{obs}) for at least then half-lives, giving the corresponding poly(ethylenimine)penicilloylamides. The substrates CE and 6-CLAPA show a more complex behaviour typical of two consecutive first order reactions, as shown in Fig. 1.

In fact, the optical density against the time after an initial decrease reaches a minimum and then increases again. The rate constants k_1 and k_2 were calculated according to the following Equation 1[15] by using the FigP programme, a nonlinear last squares routine, and by fitting OD values vs time:

$$OD_t = A_o\{\varepsilon_3 + (\varepsilon_1 - \varepsilon_3)\exp(-k_1 t) + k_1(\varepsilon_2 - \varepsilon_3) \times [\exp(-k_2 t) - \exp(-k_1 t)]/(k_1 - k_2) \quad (1)$$

where A_o is the initial concentration of CE and 6-CLAPA, $A_o\varepsilon_2$ is the optical density of the intermediate (I) (Scheme 1), while $A_o\varepsilon_1 = OD_o$ and $A_o\varepsilon_3 = OD_\infty$ are the optical density at $t = 0$ and $t = \infty$, respectively.

Since the reaction products recognition is not easy owing to the polymeric structure of PEI, in large excess with respect to the β-lactam, we performed the reaction in the presence of the primary amine buffer, CH_3ND_2/CH_3ND_3Cl, in D_2O at pD = 10.0.[16] Thus, on the basis of the ^{1}HNMR analysis, it was possible to assign the rate constant k_1 to the first step, that is the β-lactam opening which gives the intermediates (I) for CE (Scheme 1) and (I′) for 6-CLAPA (Scheme 2). In the case of CE, the second step (k_2) occurs with the leaving group expulsion at the C-3′ of enamine (I) giving rise to the imine (II) (Scheme 1), as observed for the reaction with propylamine[17] or in the case of β-lactamase catalyzed hydrolysis,[18] confirming that the 3′-elimination is not concerted with β-lactam C—N bond cleavage when cephalosporin reacts with nucleophiles.[19a]

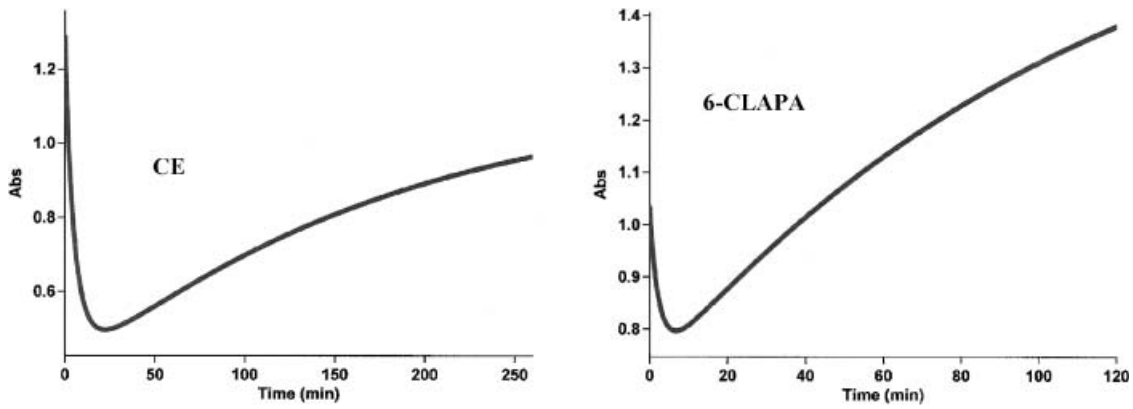
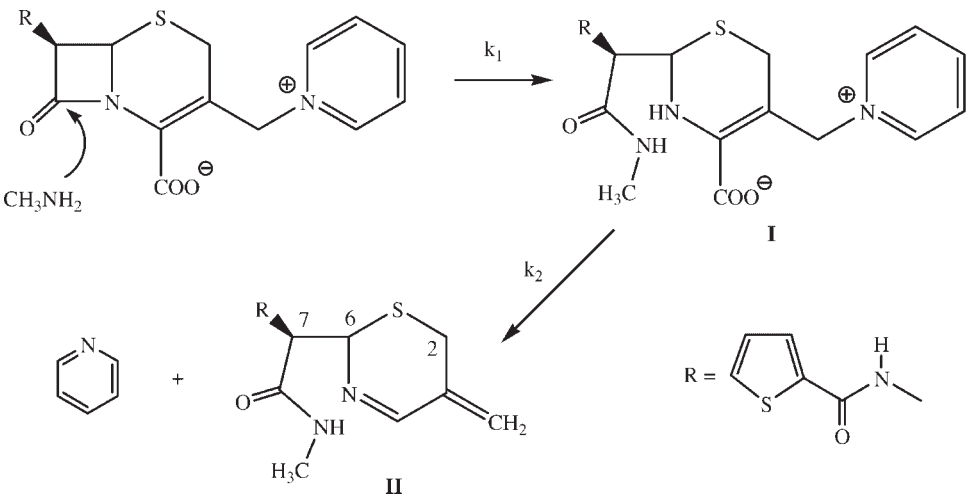


Figure 1. Dependence of absorbance vs time for the aminolysis in the presence of 0.064 monomer mol L^{-1} PEI at pH = 8.40 at 30°C for CE ($\lambda = 260$ nm) and for 6-CLAPA ($\lambda = 240$ nm). Experimental points are hidden by the solid line calculated by using Eqn 1



Scheme 1. Mechanism of CE aminolysis by methylamine

Also the structure of (II) was confirmed by HPLC-MS and HNMR analysis (see experimental part).

In the case of 6-CLAPA the second step (k_2) involves most probably the rearrangement of intermediate (I′) to an unstable imine (II′), which then quickly rearranges to the enamine (III′) (Scheme 2), the structure of which was deduced by HPLC-MS and HNMR analysis (see experimental part).

The kinetics of aminolysis were performed in the presence of increasing quantities of PEI. In the case of CE and 6-CLAPA, the rate constants k_1 vs [PEI] show a hyperbolic behaviour (Fig. 2).

For CE, the rate constant k_2 ($9.50 \times 10^{-5}\,s^{-1}$) does not depend on the [PEI] in agreement with the monomolecular decomposition of the intermediate (I) to the immine (II). Conversely, in the case of 6-CLAPA, the rearrangement of intermediate (I′) to immine (II′) is catalyzed by the [PEI] by a general base catalysis ($k = 2.69 \times 10^{-4}\,s^{-1}\,M^{-1}$, see Fig. 2 and footnote in Table 1).

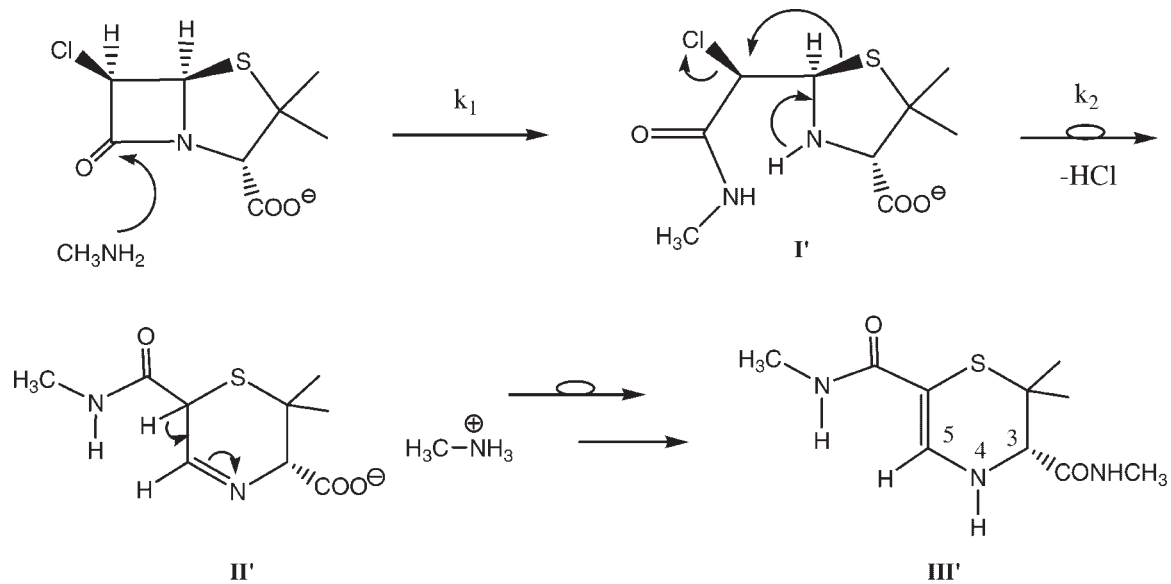
The nonlinear dependence of k_1 for CE and 6-CLAPA and of k_{obs} for TI on [PEI] is consistent with a polyelectrolyte–substrate association to give a complex [βL–PEI] which evolves to products, amides (II) or (III′) (Scheme 3):

The kinetics have been analyzed assuming the treatment previously reported for the aminolysis of some phenylacetates,[20] according to the following Michaelis–Menten type Eqn 2:

$$k_{obs} = k_{cat}K_1[PEI]/(1 + K_1[PEI]) \qquad (2)$$

where $K_1 = k_a/k_{-a}$ is the substrate polyelelectrolyte binding constant and k_{cat} is the first order rate constant of the reactive complex [βL–PEI] decomposition to (II) or to (III′).

The other penicillins AM, CA, PV and in a lesser extent 6-APA show a more complex behaviour. Indeed, as shown in Fig. 2, on increasing the PEI concentration, the k_{obs} initially increases, reaches a maximum and then decreases suggesting an apparent polyelectrolyte inhibition, as previously observed for the benzylpenicillin.[1]



Scheme 2. Mechanism of 6-CLAPA aminolysis by methylamine

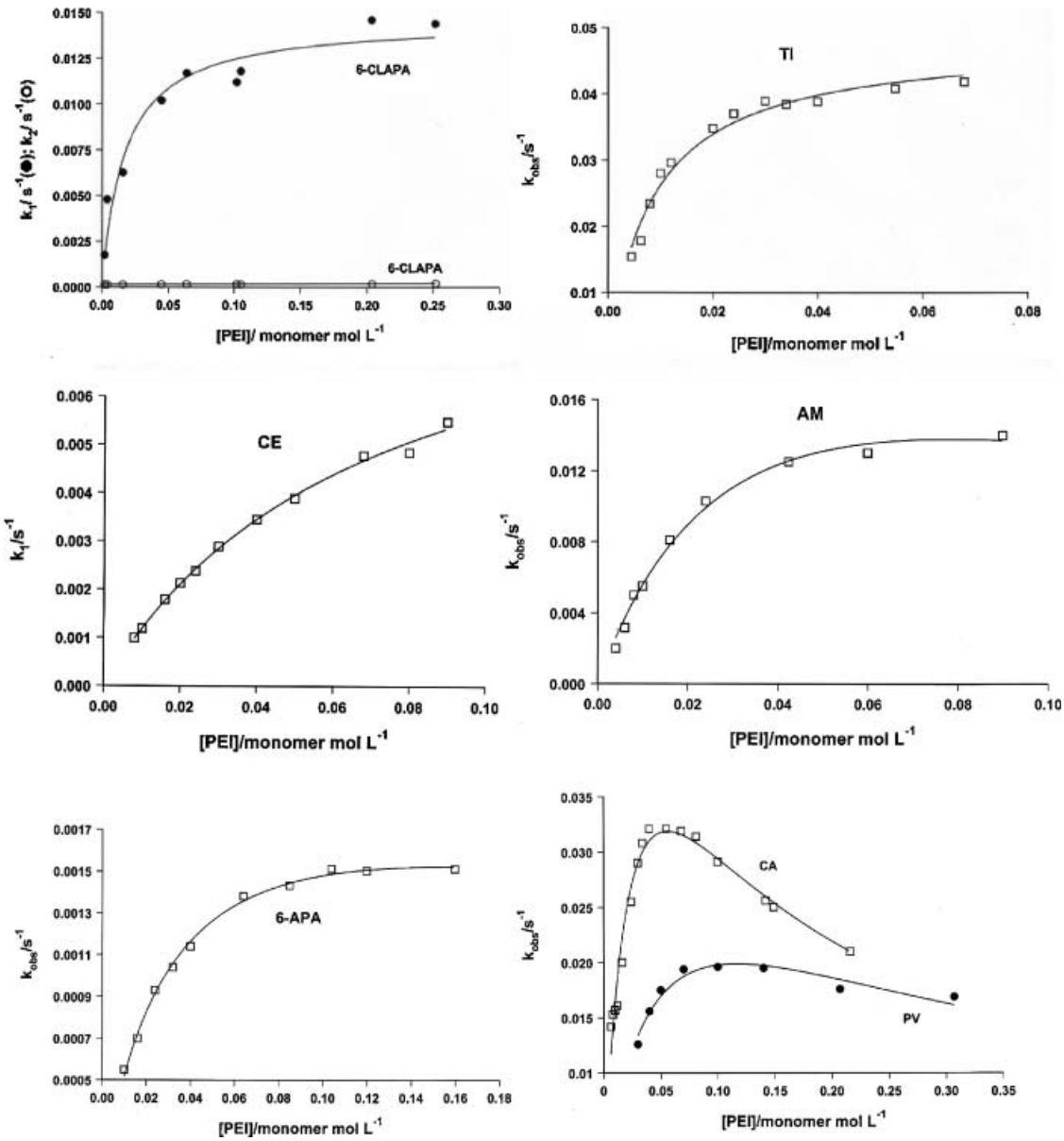


Figure 2. Dependence of pseudo first order rate constants on PEI concentration at pH = 8.40 at 30°C for various β-lactams. Points are experimental and solid curves are the best fit obtained using Eqn 2 or 3 and the parameters given in Table 1

These results can be interpreted by assuming that the aminolysis reaction involves the formation of a reactive complex [βL–PEI] between β-lactams (βL) and PEI, which can evolve to the product (penicilloylamide), or associate with an another PEI macroin to give an unreactive complex [βL–PEI]‡ according to Scheme 4.

By using the treatment already reported,[20] this kinetic behaviour can be explained by the following equation:

$$k_{obs} = k_{cat}K_1[PEI]/(1 + K_1[PEI] + K_1K_2[PEI]^2) \qquad (3)$$

where $K_1 = k_a/k_{-a}$ (binding constant), $K_2 = k_b/k_{-b}$ (inhibition constant) and k_{cat} is the first order rate constant of the reactive complex [βL–PEI] decomposition.

The estimation of the best values of K_1, K_2 and k_{cat} (reported in Table 1) was performed by using the FigP programme, a nonlinear curve fitting of k_{obs} (or k_1 for CE and 6-CLAPA) vs PEI concentration. The good agreement between experimental data and curve calculated by Eqns 2 and 3 suggests the effectiveness of the proposed model.

These results can be qualitatively interpreted in terms of polyelectrolyte-substrate interactions and can be ascribed to the molteplicity of catalytic sites on the macroion.[1,20] In fact, at pH = 8.40 a large fraction of free amino groups is present. Increasing the PEI concentration, the rate constant increases because the electrostatic field created by positive charges, although not relevant (the ionization degree being $\alpha = 0.18$), attracts the anionic substrates on the polymer surface and then the rate increases, reaching the maximum. Other interactions such as polar, hydrophobic, Wan der Walls and/or specific contribute to the substrate–pylelectrolyte binding. Although, the charge doublets are negligible in the region of low α,[14a] the interactions favour also the binding of the substrate at unproductive sites, far away from the nucleophilic amino groups responsible for the penicilloylation reaction and then the rate

Table 1. Aminolysis of β-lactams in the presence of PEI at pH = 8.40 and 30°C

β-lactams	[PEI]/monomer (mol L^{-1})[a]	$10^3 k_{cat}$ (s^{-1})[b]	K_1 (M^{-1})[b]	K_2 (M^{-1})[b]	$10^3 K_1 k'_{cat}$[c]	σ_l[d]
Ampicillin	0.004–0.09	27 ± 5.6	26.5 ± 7.7	6.1 ± 3.6	870	0.30
Carbenicellin	0.0063–0.216	63 ± 6.2	36.8 ± 6.1	8.6 ± 1.6	2820	0.30
Ticarcillin[e]	0.0045–0.068	48 ± 1.3	120 ± 11	—	7020	0.30
6-Aminopenicillanic acid	0.0096–0.16	2.12 ± 0.12	32.4 ± 3.3	1.3 ± 0.4	83	0.17[f]
6-α-Chloropenicillanic acid[eg]	0.002–0.252	14.6 ± 0.86	58 ± 16	—	1032	0.47[h]
Cephaloridin[e]	0.008–0.09	9.5 ± 0.39	14.3 ± 1.05	—	166	0.31
Benzylpenicillin[i]	—	28.8	21.0	7.0	730	0.31
Penicillin V	0.03–0.307	36.5 ± 5	20.5 ± 6	3.6 ± 1.2	918	0.33

[a] Total polyamine concentration.
[b] Values calculated from Eq 3, standard errors are reported.
[c] $k'_{cat} = k_{cat}/(1-\alpha)$.
[d] See Appendix.
[e] Values calculated from Eqn 2.
[f] Reference [41].
[g] Calculated from k_1 values obtained from Eqn 1; from k_2 values, $k = (2.69 \pm 0.3) \times 10^{-4}\ s^{-1}\ M^{-1}$ is calculated for general-base catalysis rearrangement of I′ to II′ (Scheme 2).
[h] Reference [40].
[i] Data from Reference [1].

decreases, increasing the macroin concentration.[20] Most probably, this behaviour causes the polyelectrolyte inhibition, as observed for AM, CA, PV and 6-APA, but not in the case of TI which does not give inhibition at this pH value.

The highest K_1 values were found for anionic TI ($K_1 = 120\ M^{-1}$) and 6-CLAPA ($K_1 = 58\ M^{-1}$), while the other penicillines investigated show K_1 values in the range 14–37 M^{-1}.When the substrate is in zwitterionic form, as CE, the interactions are loosen because the anionic carboxylate group favours the electrostatic interaction, but the positively charged pyridine moiety is repulsed by the cationic macroin and consequently the binding constant K_1 is lower.

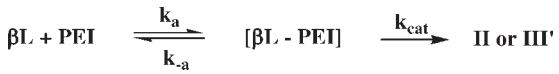
The inhibition binding constants are detectable only for β-lactams AM ($K_2 = 6.1\ M^{-1}$), CA ($K_2 = 8.6\ M^{-1}$), PV ($K_2 = 3.6\ M^{-1}$) and barely visible for 6-APA ($K_2 = 1.3\ M^{-1}$).

A more careful analysis of these results can be made on the basis of the linear free energy relationships. The reactivity of the [βL–PEI] complex decomposition can be expressed by the value $k'_{cat} = k_{cat}/(1-\alpha)$, where $(1-\alpha)$ is the fraction of free amino groups of PEI which attack the β-lactam[1,20] and α is the mean ionization degree at pH = 8.40 in the range of PEI investigated concentration.

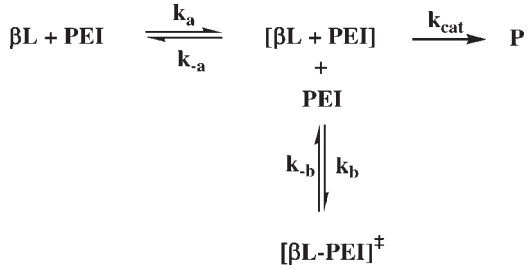
A dual logarithmic plot of log k'_{cat} vs log K_1 does not provide a visible information of the polyelectrolyte effect about the binding and the mechanism involved in the [βL–PEI] complex decomposition because no relationship was found. However, it can be observed that, except for 6-APA and 6-CLAPA, the rate of decomposition of [βL–PEI] complex (k'_{cat}) increases increasing the binding constant (Table 1).

Also in the case of Taft-plot no dependence of log k'_{cat} vs σ_l value was observed (Fig. 3). From the plot it is evident that the [βL–PEI] complex decomposition is affected by other effects in addition to the inductive and/or steric hindrance of the substituent at C′-6 or C′-7. The failure of correlation can be explained considering that the aminolysis reaction of the complex [βL–PEI] is very complicated because the transition states are considerably more crowded than, for instance, the simple bimolecular mechanism such as the alkaline hydrolysis of penicillins which is correlated by Hammett $\rho_l + 2.0$ (Reference [21]) and usually show quite small steric effects.[22] The polymeric structure could accommodate the various substrates in such way that the interactions could favour the binding of the substrate to the polymer overwhelming the electronic and/or steric effect of the substituent, altering the reactivity of the substrate which in the complex can be different from that of the unassociated substrate.[23]

Then, the greater reactivity of all anionic β-lactams complexes (3–7-fold) in comparison to CE could be ascribed to electrostatic phenomena, the σ_l values being practically coincident (Table 1). The lower reactivity of [CE–PEI] complex is a consequence of the weak substrate–polyelectrolyte interactions. The macroin influences the nucleophilic attack. Actually, while the CE is about 5-times more reactive in a bimolecular reaction than BP with a simple monomeric amine, as the propylamine[17] (or PEI with added KCl see below), in the presence of the polymeric amine,



Scheme 3. β-lactam-PEI reactive complex formation



Scheme 4. β-lactam-PEI reactive complex formation in equilibrium with the unreactive complex

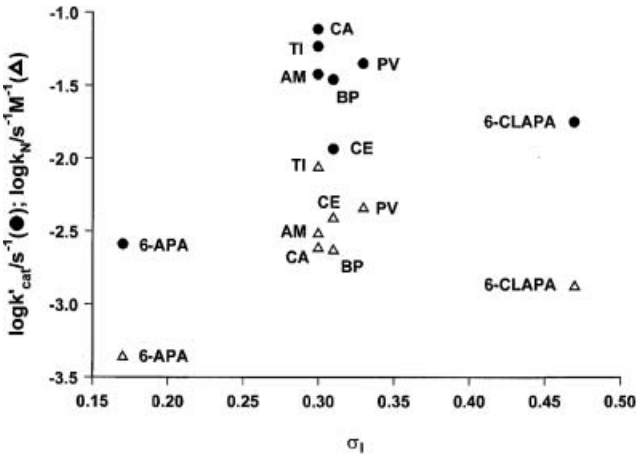


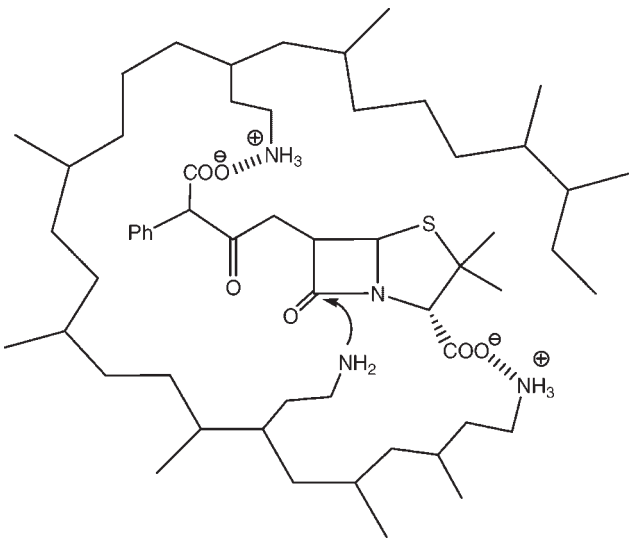
Figure 3. Plots of log k'_{cat}(●) or log k_N(Δ) vs σ_I for the aminolysis of some β-lactams in presence of PEI at 30°C. Data from Tables 1 and 2

the reactivity is reversed, the [BP–PEI] complex decomposition becomes 3-fold faster than [CE–PEI] complex. In addition, the rate decomposition of the [6-APA–PEI] complex is 14-fold lower than [BP–PEI] (Table 1). This result partially reflect the higher value of σ_I for BP which is responsible for *c.a.* 4-fold rate increase as found for simple nucleophiles amines,[24] but indicates that the nature of the interactions determinate the complex reactivity.

We note that σ_I, being equal, CA and TI have a greater bulky steric hindrance than BP, but the nucleophilic attack on the doubly charged TI and CA, is 1.7–2-fold faster. Most probably, the small but meaningful greater rate of TI and CA can be ascribed to a greater binding affinity.

At least for CA, the kinetics measurements followed in the presence of KCl (see below) agree with this hypothesis. A reasonable interpretation is that the substrate, as dianion, is attracted on the macroion chain to more than one site, its freedom degrees being reduced, as shown in Scheme 5.

Such a constrained structure increases the collision frequency between the substrate and the —NH_2 groups on the polymeric chain.[23,25] However, it is not possible to exclude that the



Scheme 5. Aminolysis of [CA-PEI] complex

electrostatic field could cause a strong desolvation of substrate–polyelectrolyte complex and/or transition state, due to the strong affinity of binding which sweep the water molecules away increasing the reaction rate.[1,26]

The importance of the electrostatic nature of this phenomenon is further supported by the effect on the decomposition of β-lactams in the presence of an added strong electrolyte.

As shown in Fig. 4, in the presence of 0.5 M KCl, the dependence of pseudo first order rate constant vs [PEI], becomes linear and follows the Eqn 4:[1]

$$k_{obs} = k_0 + (k'_{OH}[OH^-] + k_N)[PEI]_{tot}(1 - \alpha) \qquad (4)$$

where k_0 is the spontaneous hydrolysis, k'_{OH} is the hydroxide catalyzed aminolysis and k_N is the second order rate constant for the uncatalyzed aminolysis reaction. The term k_o is negligible and k'_{OH} is considered not contribuent,[19b] the PEI being not sufficiently nucleophile at pH = 8.40 ($pK_N = 7.80$).[1] The linearity of the plots, in a large PEI concentration (0–0.49 monomer mol L^{-1}), excludes the presence of the intermolecular second order terms in polyamine due to the general base and/or general acid catalysis, and we hypothesize that their contribution also in the absence of KCl is unimportant (data in Table 2). Under these conditions, CE is more reactive (2-fold) than BP in agreement with the bimolecular aminolysis with simple amines.[17]

The k_N value calculated for TI has been determined in a very narrow [PEI] range (Fig. 4) owing to the strong UV absorbance of KCl and probably it is overestimated because the substrate could be still associated.

We believe that the addition of a strong electrolyte screens the charged sites and reduces the effect on ligand binding in competitive way. So, in the presence of KCl the chloride ion, which possesses a greater charge density than β-lactams anion, is preferentially attracted to the surface of the macroion and then the reaction occurs out of the polymer surface by a bimolecular process and consequently the rate decreases. However, under these conditions the dianion CA is still bounded to the PEI ($K_1 = 1.85\ M^{-1}$, $k_{cat} = 4.71 \times 10^{-3}\ s^{-1}$) (see Fig. 4 and Table 2). At 1 M KCl the positive charges are shielded and the β-lactam is removed from the polyelectrolyte surface, suggesting that the Coulomb forces between the CA and PEI are stronger.

No Taft-correlation was observed by plotting the log k_N vs σ_I (Fig. 3). This result suggests that also in the absence of polyelectrolyte–substrate coulombic interactions, beyond steric and electronic effects, the solvation–desolvation of transition state is important also in the bimolecular process.

At this point the polyelectrolyte catalysis can be estimated by the $K_1 \times k'_{cat}/k_N$ ratio. All negatively charged substrates display a relevant reaction rate increase: for istance, the reaction rate for the monoanionic PV increases to 2.0×10^2-fold, while for dianionic CA, *c.a.* 1.2×10^3-fold, 8.1×10^2 for TI indicating that the coulombic effect appears predominant and only 43-fold for the zwitterionic CE owing to weaker substrate macroin interactions of not specific nature (see Tables 1 and 2).

The PEI reveals its catalytic efficiency not only accumulating the β-lactam near to the chain (K_1), but also by increasing the reactivity of the [βL–PEI] complex, as shown by the 'effective molarities' (EM).[27] Indeed, the EM values calculated by the ratio k'_{cat}/k_N are: 15 for BP, 6 for 6-APA, 11 for AM, 13 for 6-CLAPA, 32 for CA, 3 for CE, 10 for PV and 7 for TI. These values are too large to be ascribed to an 'effective concentration', because the reactants occupy a definite exclusion volume.[28] These findings suggest that the nature of the polymer domain modifies the reactivity of

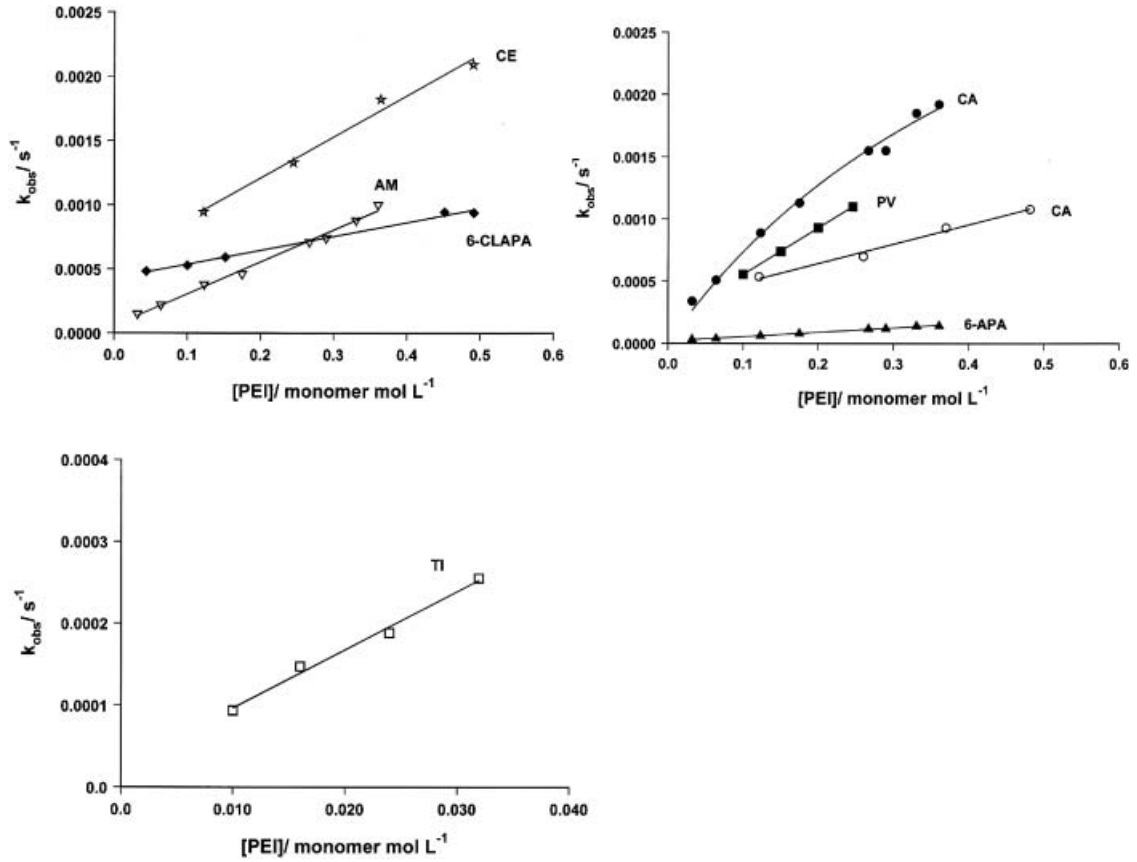


Figure 4. Dependence of the aminolysis of some β-lactams on [PEI] in the presence of 0.5 M KCl at 30°C. (O) CA in 1 M KCl

the [βL–PEI] complex, as observed in the aminolysis of phenylacetates with PEI.[20]

With regard to the mechanism involved, a dual logarithm plot, log k'_{cat} vs log k_N, was found with slope 1.04 ± 0.15 as shown in Fig. 5. The existence of satisfactory linear free energy relationship indicates that a single mechanism is operating[29] for the nucleophilic attack. The positive deviation of CA probably reflects the increase in the collision frequency, and the negative deviation of CE is ascribable to a different substrate–macroin interaction. The point for TI does not show positive deviation probably because the binding causes an unfavourable [TI–PEI] complex decomposition.

On these basis, we suggest that the nucleophilic attack of aminolysis can proceed stepwise with the formation of a zwitterionic tetraedrahedral $T^{\pm}$ intermediate which evolves to poly(ethyleneimine) penicilloylamide, as observed for BP[1]

Table 2. Aminolysis of β-lactams in the presence of PEI and KCl = 0.5 M at pH = 8.40 and 30°C

β-lactams	[PEI]/Monomer mol (L^{-1})[a]	$10^3 k_N$ $(s^{-1} M^{-1})$[b]
Ampicillin	0.032–0.361	3.02 ± 0.1
Carbenicellin	0.00034–0.00192	2.41 ± 0.19[c]
6-Aminopenicillanic acid	0.032–0.361	0.433 ± 0.01
Ticarcillin	0.01–0.032	8.66 ± 0.03
6-α-Chloropenicillanic acid	0.044–0.492	1.32 ± 0.06
Cephaloridin	0.122–0.492	3.89 ± 0.31
Benzylpenicillin	—	1.91[d]
Penicillin V	0.100–0.246	4.54 ± 0.24

[a] Total polyamine concentration.
[b] Values calculated from Eqn 4.
[c] Determined in 1 M KCl where the trend is linear; in 0.5 M KCl the substrate is still associated, $K_1 = 1.85 \pm 0.41\ M^{-1}$ and $k_{cat} = (4.71 \pm 0.7) \times 10^{-3}\ s^{-1}$ (see Fig. 4).
[d] In 1 M KCl from Reference [1].

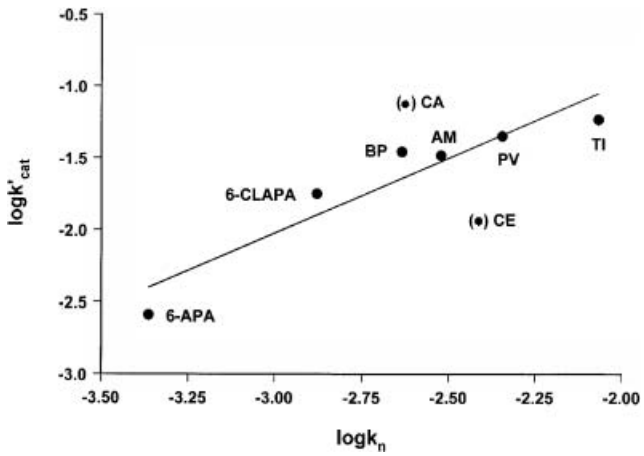
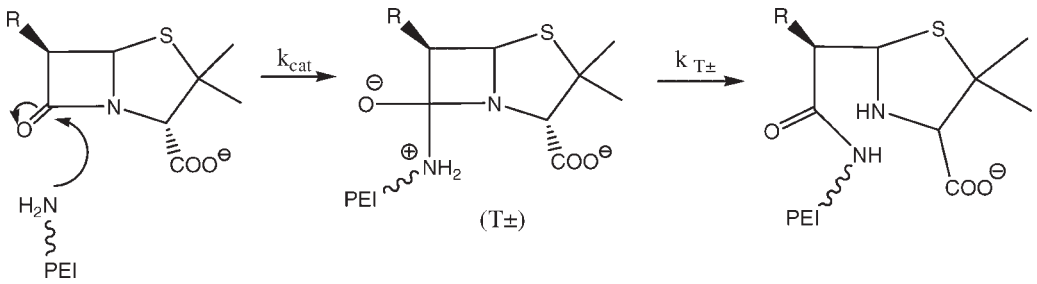


Figure 5. Plot of log k'_{cat} for the [β-lactams–PEI] complexes aminolysis vs log k_N for the second order reaction of various substrates. Points in parenthesis are excluded



Scheme 6. Mechanism of β-lactam ring cleavage by primary amino group of PEI

(Scheme 6) or to other intermediates (I) or (I′) reported in Schemes 1 and 2.

Most probably the β-lactams reacting with amino groups in the less polar environment of the polymer surface give a tetrahedral intermediate $T^{\pm}$ which is stabilized by electrostatic and/or hydrogen bond interactions.[1] This stabilization which is responsible of the increased reactivity of the β-lactam–PEI complex can be explained by a 'solvation substitution', as hypothesized also for enzyme catalysis.[30]

CONCLUSIONS

The obtained data show that the electrostatic effect is the determining factor for the binding of the β-lactam to the polyelectrolyte. The most important feature which emerges from this study is that the catalytic effect produced by substrate–polyelectrolyte association increases the reactivity of all β-lactams, especially for the dianionic substrates which show the highest accelerating effect.

Although, it is not possible to compare the behaviour of these β-lactams with HSA, some interesting similarities between the protein and the poly(ethyleneimine) can be found. Monoanion β-lactams are less bounded than dianion ones and a binding decrease has been found on going from 6-CLAPA to 6-APA, as expected for the HSA binding.[8a] Both the esterification of BP carboxylic group and the presence of KCl, even if in very low concentration, remove the binding on the BSA[31] indicating that the electrostatic interactions are very important.

The zwitterionic cephaloridin binds to the macroion in a lower extent in comparison to the other β-lactams and it seems that it is quite not bounded to HSA.[32] Similar binding to PEI were found for PenV and BP, while PenV binds to HSA better than BP.[33]

It has been reported from NMR studies[34] and X-ray structure[6b,35] that the penicillins bind to HAS, in *domains* labelled II and III, by electrostatic and hydrophobic interactions and the L-Lys-199 is the most important group for the nucleophilic attack of BP.[5] The pairs L-Lys-195 and L-Lys-199 which correspond to the protonated and neutral form of ε-amino group of protein are considered responsible for the aminolysis reaction of BP.[7c] Then we believe that the anionic β-lactams are electrostatically attracted by the protonated L-Lys-195 residue and undergoes the nucleophilic attack by near free —NH_2 group of L-Lys-199, giving the HSA-penicilloyl products.[7c]

In the case of dianionic CA and TI, both carboxylate groups could favour a better binding to the protein by the Coulomb forces between ionic compound and HSA. This is a possibility since electrostatic potential calculated from HSA crystal structure[35] in the neighbourhood of L-Lys-199 and L-Lys-195 is positive, favouring stronger Coulomb interactions between HSA and the carboxylate groups of CA and TI.

We envisaged and designed the present study as a purely kinetic investigation of aminolysis reaction of β-lactams on polymeric matrix. These results would suggest that the electrostatic effect appears to be more important in the allergy phenomena than other interactions of the substituent at C-6′ and C-7′.

Then, most probably, the allergy phenomena can be ascribed to both the binding ability of β-lactams to human protein and the reactivity of the complex substrate–HSA. To the best of our knowledge it was not reported that the penicilloylation is dependent on the binding of β-lactams to the protein.[36] However, on the basis of these results this possibility cannot be excluded.

EXPERIMENTAL

Materials

Ampicillin sodium (AM), Carbenicellin disodium (CA), Ticarcillin disodium salts (TI) were purchased from Sigma, while the β-Aminopenicillanic acid (sodium salt) (6-APA) from Lancaster, and Cephaloridin (CE) from Aldrich and Phenoxymethylpenicillin potassium salt (PV) was from Fluka. α-Chloropenicillanic acid (6-CLAPA) was synthesized according to the Reference [37] and isolated as benzylamonium salt. PEI was 'Polymin P' 47.6% by weigh was purchased from BDH. A monomer molecular weigh of 59 was determined by titration.[20] Other reagents were of analytical grade from Merck or Aldrich. Water was deionized and redistilled from $KMnO_4$.

Kinetic procedure

Buffer solutions at pH = 8.40 and at various concentrations (both in the absence and in the presence of KCl) were prepared by adding diluted HCl to the PEI. The pH of the solution, stored under nitrogen atmosphere, was measured at 30°C and the solutions were used during a day.

The kinetics were performed at 30°C by adding 10–30 μL of a stock solution of β-lactam (0.10–0.04 M) to 3 mL of buffer solutions contained in 1 cm cell placed in the thermostated compartment of a Varian Cary 100 spectrophotometer. The β-lactam ring opening was followed at various wavelengths: 6-CLAPA at $\lambda = 240$ (or 244 in the presence of KCl); CE at 260; CA at 240 (or 244 in KCl); AM at 240; 6-APA at 242; TI at 232 nm.

The kinetics were followed for at least ten half lives. For more diluted solutions of PEI 0.05–0.1 pH decrease was observed at the end of the reactions.

Pseudo first order rate constants were calculated on the basis of the equation $OD_t = (OD\infty - OD_0)(1 - \exp(-kt))$ by using the

Marquardt nonlinear regression analysis (enclosed on the software of Carry 100). In all cases investigated, the experimental curves and the best computed ones are indistinguishable. The pseudo first order rate constant values are reported in Figs. 2 and 4.

The kinetics of PV aminolysis were followed by HPLC. Actually, during the aminolysis, the absorbance at $\lambda = 240$ nm did not change, while at $\lambda = 270$ nm the increase vs time did not follow a first order. Most probably, also in this case a consecutive reaction occurs, as ascertained by HNMR analysis following the reaction in the presence of 1.1 M CH_3ND_2/CH_3ND_3Cl buffer at pD = 10.0[16] but further investigations were not done.

50 mL of PEI buffer solution thermostated at 30°C were added to the PV potassium salt (7.3×10^{-5}–1.32×10^{-4} moles) contained in a flash. At suitable times, 3 mL of the solution were taken and added to 2 mL of diluted HCl till pH = 6.0. The various solutions, frozen at −70°C, were brought again at room temperature and the β-lactam was analyzed on Hewlett-Packard HPLC series 1100 equipped with an Agilent Zorbax XDB C8 4.6 × 150 mm, 5 μm column, eluting with CH_3CN/0.025 M phosphate buffer pH = 3.1. The rate constants were calculated by a nonlinear regression analysis using the equation $h_t = (h_\infty - h_0)(1 - \exp(-kt))$ where h_∞ is the peak height at t_∞, h_o at t_0 and h_t at various times.

Reaction products

The aminolysis reaction with PEI of the β-lactams investigated yielded the corresponding penicilloyl amides recognized by penamaldate analysis.[38] The maximum absorbance of the penamaldates are at: $\lambda = 284$ nm for AM and CA; $\lambda = 283$ for TI; $\lambda = 287$ for CE; $\lambda = 291$ for PV; $\lambda = 279$ for 6-APA; $\lambda = 281$ for 6-CLAPA.

The reaction product (II) obtained from CE in the presence of CH_3ND_2 buffer (Scheme 1) was recognized through the ^{1}H-NMR and HPLC-MS. The CE (2.4×10^{-5} moles) was added to 1 mL of $CH_3ND_2/CH_3ND_3^+Cl^-$ buffer 2.2×10^{-3} M in D_2O at pD = 10.0,[16] and the reaction was monitored at room temperature by ^{1}H-NMR. The meaningful signals are: the q_{AB} at $\delta = 3.8$ ppm ($J = 15.9$ Hz) ascribable to the (C-2)-CH_2, the multiplet at $\delta = 4.6$ ppm ascribable to the (C-6)-H, the multiplet at $\delta = 5.29$ ppm ascribable to the (C-7)-H, two singlettes at $\delta = 5.5$ and 5.58 ppm ascribable to the $=CH_2$ The HPLC-MS spectrum shows the M + 1 peak at 368.

The reaction product recognition in the case of the 6-CLAPA was achieved by performing the experiment in the presence of CH_3NH_2 buffer. The 6-CLAPA (1.8×10^{-3} moles) were dissolved in 25 mL of 1.1 M buffer at pH = 10.0 and the solution, after stirring at 30°C for about one day, was then frozen and the water lyophilized. The residue, containing the ammonium carboxylate, was then converted into the corresponding methylamide (III′) (Scheme 2), according to the procedure already described.[39] The reaction product, after purification by silica gel chromatography eluting with hexane/ethyl acetate, was submitted to HPLC-MS and NMR analysis (Gemini spectrometer at 300 MHz using $CDCl_3$ as solvent) .The reaction product (III′) was recognized on the basis of the ^{1}H-NMR meaningful signals: the multiplet at $\delta = 5.61$ ppm attributable to the proton (N-4)-H which is coupled with the doublet at $\delta = 7.67$ ppm ($J = 6.3$ Hz) ascribable to the vinylic proton (C-5)-H and with the doublet of the (C-3)-H at $\delta = 3.82$ ppm. In addition, by irradiating the (N-4)-H proton a nOe was observed on the protons (C-3)-H and (C-5)-H. The reaction product structure deduced by ^{1}H-NMR analysis is coherent with the MS spectrum which shows the following peaks: 244 (M + 1), 266 (M + Na) and 282 (M + K).

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REFERENCES

[1] A. Arcelli, R. Cecchi, G. Porzi, M. Sandri, *J. Phys. Org. Chem.* **2005**, *18*, 255–263.
[2] a) A. L. De Weck, G. Blun, *Internat. Arch. Allergy Appl. Immunol.* **1965**, *27*, 221–226; b) F. R. Batchelor, J. M. Dewdney, D. Gazzard, *Nature* **1965**, *206*, 362–364; c) C. H. Schneider, A. L. De Weck, *Nature* **1965**, *208*, 57–59; d) P. Demoly, A. Romano, *Curr. Allergy Asthma Rep.* **2005**, *5*, 9–14.
[3] B. Nerli, D. Romanini, G. Pico, *Chem. Biol. Interact.* **1997**, *104*, 179–202.
[4] M. Yvon, P. Anglade, J. M. Wal, *Febs. Lett.* **1990**, *263*, 237–240.
[5] M. Yvon, P. Anglade, J. M. Wal, *Febs. Lett.* **1989**, *247*, 273–278.
[6] a) T. J. Peters, in *All about biochemistry, genetics and medical applications*, Academic Press, New York **1996**; b) He. Xiao Min, D. C. Carter, *Nature.* **1992**, *358*, 209–215.
[7] a) S. Tawara, S. Matsumoto, Y. Matsumoto, T. Kamimura, S. Goto, *J. Antibiotics* **1992**, *45*, 1346–1357; b) H. Zia, E. Malaz, J. K. H. Ma, L. A. Luzzi, *Can. J. Pharm. Sci.* **1980**, *15*, 14–16; c) N. Diaz, D. Suarez, T. L. Sordo, K. M. Merz, *J. Am. Chem. Soc.* **2001**, *123*, 7574–7583.
[8] a) L. M. Hall, L. H. Hall, L. B. Kier, *J. Comput. Aided mol. des.* **2003**, *17*, 103–118; b) T. Terasaki, H. Nouda, A. Tsuji, *J. Pharmacobio-Dynamics* **1992**, *15*, 99–106.
[9] J. Suh, Y-s. Lee, S. Han, *Bioorg. Med. Chem. Lett.* **1998**, 1331–1336.
[10] S. Barbosa, P. Taboada, D. Attwood, V. Mosquera, *Langmuir* **2003**, *19*, 1446–1448.
[11] B. Otmane, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix, J. P. Behr, *Proc. Natl. Sci. USA* **1995**, *92*, 7297–7301.
[12] A. Arcelli, C. Concilio, *J. Chem. Soc. Perin 2* **1989**, 887–891.
[13] a) V. G. Alekseev, I. A. Volkova, *Russian J. Gen. Chem.* **2003**, *73*, 1616–1618; b) H. D. C. Rapson, A. E. Bird, *J. Pharm. Pharmacol.* **1963**, *15* (Suppl.), 222–231.
[14] a) C. J. Bloys van Treslong, *J. Roy. Neth. Chem. Soc.* **1978**, *97*, 13–21; b) C. J. Bloys van Treslong, A. J. Staverman, *J. Roy. Neth. Chem. Soc.* **1974**, *93*, 171–178.
[15] A. Arcelli, R. Cecchi, G. Porzi, S. Rinaldi, S. Sandri, *Tetrahedron* **2001**, 4039–4043.
[16] J. F. Coetzee, C. D. Ritchie, *Solute-Solvent Interactions*, Marcell Dekker, New York **1969**, p. 45.
[17] M. I. Page, P. Proctor, *J. Am. Chem. Soc.* **1984**, *106*, 3820–3825.
[18] S. W. Faraci, R. F. Pratt, *J. Am. Chem. Soc.* **1984**, *106*, 1489–1490.
[19] a) M. I. Page, *Adv. Phys. Org. Chem.* **1987**, *23*; 250–252; b) M. I. Page, *Adv. Phys. Org. Chem.* **1987**, *23*; 233–250.
[20] A. Arcelli, *Macromolecules* **1999**, *32*, 2910–2919.
[21] P. Proctor, N. P. Gensmantel, M. I. Page, *J. Chem. Soc., Perkin Trans. 2* **1982**, 1185–1192.
[22] S. Nagaraja Rao, O' Farrall. More, *J. Am. Chem. Soc.* **1990**, *112*, 2729–2735.
[23] H. Morawetz, *Macromolecules in Solutions*, Interscience Publisher, NY, **1966**, p. 434.
[24] N. Diaz, D. Suarez, T. L. Sordo, R. Mendez, J. M. Villacorta, *Eur. J. Org. Chem.* **2003**, 4161–4172.
[25] E. Baumgartner, R. Fernandez-Prini, *Polyelectrolytes*, (Ed.: K. C. Frisch, D. Klempner, A. V. Patsis,), Technomic Publish., Wesport, **1976**, p. 1.
[26] a) A. Enokida, T. Okubo, N. Ise, *Macromolecules* **1980**, *13*, 49–453; b) N. Ise, T. Maruno, T. Okubo, *Proc. R. Soc. London* **1980A**, *370*, 485–500.
[27] A. J. Kirby, *Adv. Phys. Org. Chem.* **1980**, *17*, 183–278.
[28] a) T. Okubo, N. Ise, *Adv. Polym. Sci.* **1977**, *25*, 135–181; b) H. Morawets, G. Gordimer, *J. Am. Chem. Soc.* **1970**, *92*, 7532–7536.
[29] M. J. Page, *The Chemistry of Enzyme Action*, Elsevier, NY, **1984**, p. 127.
[30] A. Warshel, J. Aqvist, S. Creighton, *Proc. Natl. Acad. Sci USA* **1989**, 5820–5824.
[31] P. G. Owen, D. M. Power, C. Robinson, J. V. Davies, *Biochim. Biophys. Acta* **1970**, *215*, 491–502.

[32] R. W. Joos, W. H. Hall, *J. Pharmacol. Exp. Ther.* **1969**, *166*, 113–118.
[33] C. I. Smith, J. D. Levin, D. K. Embody, *Antibiot. Ann.* **1957**, 306–310.
[34] M. Landau, *Russian J. Org. Chem.* **1998**, *34*, 615–628.
[35] S. Sugio, A. Kashima, S. Mochizuki, M. Noda, K. Kobayashi, *Protein Eng.* **1999**, *12*, 439–446.
[36] H. Bundgaard, *Acta Pharm. Suec.* **1977**, *14*, 391–402.
[37] N. P. Gensmantel, P. Proctor, M. I. J. Page, *J. Chem. Soc., Perkin 2* **1980**, 1725–1732.
[38] Tsuji. Akira, Yamana. Tsukinaka, Miyamoto. Etsuko, Kiya. Emi, *J. Pharm. Pharmac.* **1975**, *27*, 580–587.
[39] M. Kunishima, C. Kawachi, J. Monta, K. Terao, F. Iwasaki, S. Tani, *Tetrahedron* **1999**, *55*, 13159–13170.
[40] M. Charton, *J. Org. Chem.* **1964**, *29*, 1222–1227.
[41] P. J. Taylor, *J. Chem. Soc., Perkin Trans. 2* **1993**, 1423–1427.
[42] D. D. Perrin, B. Dempsey, E. P. Serjeant, *pK_a Prediction for Organic Acids and Bases*, Chapman and Hall, NY, **1981**, p. 109.

APPENDIX

σ_I Estimation

Except Cl, $\sigma_I = 0.47$,[40] and NH_2, $\sigma_I = 0.17$[41] the σ_I values for the other substitutes are not available, but calculated values can be obtained as follows. The σ_I values are from σ^* (Reference [42]).

For BP: $\sigma^*(NHCOCH_2C_6H_5) \approx \sigma^*(NH_2)\text{-}\sigma^*H + \sigma^*(COCH_2C_6H_5)$; $\sigma^*(CO—CH_2C_6H_5) \approx \sigma^*(COCH_3)$. Then $\sigma^* = 0.62–0.49–1.81 = 1.94$ and from the equation $\sigma_I = \sigma^*/6.23$ (Reference[40]), $\sigma_I = 0.31$.

For Pen V: considering that $\sigma^*(CH_2OC_6H_5) = 0.87 \approx \sigma^*(CH_2Cl) = 0.94$, we assume that $\sigma^*(NHCOCH_2OC_6H_5) \approx \sigma^*(NHCOCH_2Cl) = 2.06$. From the equation $\sigma_I = \sigma^*/6.23$, $\sigma_I = 0.33$.

For CA: $\sigma^*(NHCOCH(CO_2^-)C_6H_5 \approx \sigma^*(NH_2)\text{-}\sigma^*(H) + \sigma^*(COCH(COO^-)C_6H_5)$; $\sigma^*(COCH(CO_2^-)\ C_6H_5) \approx \sigma^*(COCH_3) + \sigma^*(CH_2CO_2^-)$. Then $\sigma^* = 0.62–0.49 + 1.81–0.06 = 1.88$ and from the equation $\sigma_I = \sigma^*/6.23 = 1.88/6.23$, $\sigma_I = 030$.

For CE: $\sigma^*(NHCOCH_2C_4H_3S) \approx \sigma^*(NH_2)\text{-}\sigma^*(H) + \sigma^*(COCH_2C_4H_3S)$; $\sigma^*(COCH_2C_4H_3S) \approx \sigma^*(COCH_3)$. Then $\sigma^* = 0.62–0.49 + 1.81 = 1.94$ and from equation $\sigma_I = \sigma^*/6.23$, $\sigma_I = 0.31$.

For TI: $\sigma^*(NHCOCH(CO_2^-)C_4H_3S \approx \sigma^*(NH_2)\text{-}\sigma^*(H) + \sigma^*(COCH(CO_2^-)C_4H_3S)$; $\sigma^*(COCH(CO_2^-)\ C_4H_3S) \approx \sigma^*(CO—CH_3) + \sigma^*(CH_2CO_2^-)$. Then $\sigma^* = 0.62–0.49 + 1.81–0.06 = 1.88$ and from the equation $\sigma_I = \sigma^*/6.23$, $\sigma_I = 0.30$.

For AM: we assume that $\sigma_I(NHCOCH(NH_2)C_6H_5 \approx \sigma_I(NHCOCH_2Cl)\text{-}\sigma_I\ (CH_2Cl) + \sigma_I\ (CH(NH_2)C_6H_5)$, being $\sigma_I(CH(NH_2)C_6H_5) \approx \sigma_I(CH_2NH_2) + \sigma_I\ (CH_2C_6H_5) + \sigma_I(CH_3)$, then from the values of $\sigma^*$and from the equation $\sigma_I = \sigma^*/6.23$, $\sigma_I = 0.08 + 0.04 + 0 = 0.12$. Then $\sigma_I = 0.33–0.15 + 0.11 = 0.30$.