Research Article

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Acid catalyzed intramolecular attack of β-phenylthioureido group on amide function. Parallel formation of thiodihydrouracil and 4-iminothiodihydrouracil. Different pathways in the Edman degradation reaction in the formation of six- *versus* five-membered cyclic intermediates

Ivan G. Pojarlieff[a]*, Iva B. Blagoeva[a], Maria M. Toteva[b], Ergun Atay[a], Violina T. Angelova[c], Nikolay G. Vassilev[a] and Asen H. Koedjikov[a]

Contrary to the cleavage of α-phenylthioureido peptides 1 proceeding through intermediate 2-anilinothiazolinone 2, the β-analog *cis*-2-(3-phenylthioureido)cyclopentane-carboxamide 5 forms transiently 4-imino-2-thioxopyrimidine 6. Monitoring amide cyclization and hydrolysis of iminopyrimidine 6 in acid by UV showed that an equilibrium between 5 and 6 was reached followed by slower conversion of both compounds into 2-oxo-4-thioxopyrimidine 7. Both processes were characterized by isosbestic points, the first due to parallel conversion of 5 into 6 and 7 (or 6 into 5 and 7) at a constant ratio while the second identical for both reactants – to conversion of equilibrated 5 and 6 into 7. The special isosbestic points allowed the determination of the individual constants of Scheme 2. Further confirmation was obtained from NMR product analysis and following the cyclization of amide 5 in DMSO:DCl. Product 2-oxo-4-thioxopyrimidine 7 hydrolyzed reversibly to thioureido acid 8. The cyclization rate of 8 allowed the participation of 6-oxothiazine 10 formed by sulfur attack to be excluded. The absence of sulfur attack in the six-membered case is explained by the longer C—S bond bringing about greater bond angle strain at the tetrahedral ring atoms due to the geometrical characteristics of five- and six-membered rings with planar segments. The cyclizations of amide 5 to iminopyrimidine 6 and to thiodihydrouracil 7 are first order in [H^+], while the reactions of protonated imine $6H^+$ are zero order to amide and −1 to thiodihydrouracil. The reaction orders can be reconciled by assuming a rate determining proton transfer from the tetrahedral intermediate in amide cyclization. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: kinetics; mechanism; cyclization; β-phenylthioureido amide; 4-imino-2-thioxopyrimidine; 2-thiodihydrouracil

INTRODUCTION

The acid catalyzed intramolecular attack of an α-phenylthioureido group on a peptide bond, known as the Edman degradation reaction, serves to cleave the *N*-terminal amino acid of peptides and proteins[1] in automated sequenators[2] for determining the amino acid sequence. To avoid hydrolysis of the peptide, anhydrous acidic media are used such as trifluoroacetic acid. As established by Edman,[3] cleavage is achieved by a fast cyclization of the ureido peptide **1** giving anilinothiazolinone **2**.

In aqueous acid, **2** hydrolyzes rapidly to the α-thioureido acid **3** which then cyclizes to the more stable thiohydantoin **4** (Scheme 1) used to identify R_1 and thence the terminal amino acid.

Kinetic studies[4] by means of UV-spectra showed that the same reaction sequence is observed in aqueous acids. Several authors[5,6] recently found that in more dilute acids or in neutral and weakly alkaline media, the formation of thiohydantoin **4** from **1** takes place by direct attack of the anilino nitrogen.

We now report that in fairly concentrated aqueous acids, a simple β-thioureido amide, namely *cis*-2-(3-phenylthioureido) cyclopentanecarboxamide **5**, cyclizes through a pathway different from the Edman degradation. In the range 0.01–1 M HCl, the following steps are observed: (a) an equilibrium between

* *Institute of Organic Chemistry, Bulgarian Academy of Sciences, ul. Acad. G. Bonchev Block 9, Sofia 1113, Bulgaria.*
E-mail: ipojarli@orgchm.bas.bg

a *I. G. Pojarlieff, I. B. Blagoeva, E. Atay, N. G. Vassilev, A. H. Koedjikov*
Institute of Organic Chemistry, Bulgarian Academy of Sciences, ul. Acad. G. Bonchev Block 9, Sofia 1113, Bulgaria

b *M. Toteva*
Pharmaceutics, Amgen San Francisco, 1120 Veterans Blvd., South San Francisco, California 94080, USA

c *V. T. Angelova*
Faculty of Pharmacy, Medical University, ul. Dunav 2, Sofia 1000, Bulgaria

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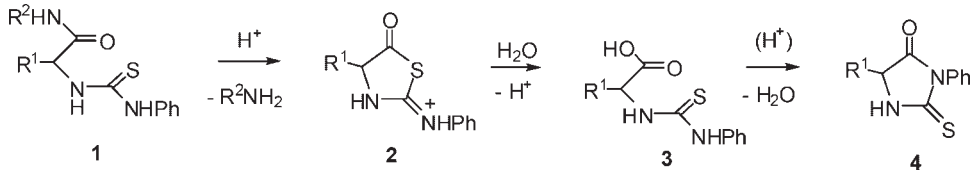


Scheme 1.

phenylthioureido amide **5** and the 4-imino-2-thiooxopyrimidine **6** is established faster than subsequent processes; (b) reactants **5** and **6** convert cleanly into *cis*-4-oxo-2-thiooxopyrimidine **7**; (c) product **7** equilibrates with *cis*-thioureido acid **8**.

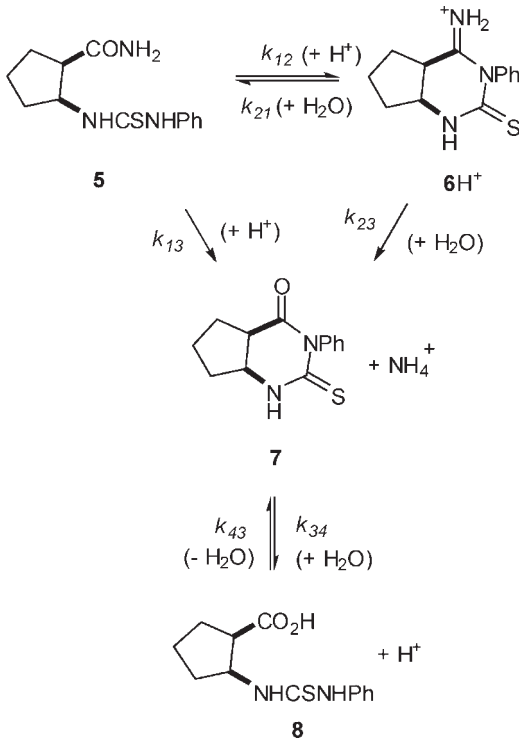
RESULTS

The synthesis of the compounds presented in Scheme 2 has already been described.[7]

Two sets of kinetic experiments were carried out; preliminary measurements were carried out in 0.125–1.5 M H_2SO_4 with the intent to use acidity functions as descriptor of acidity. However, the sensitivity to salt effects noticed also by previous investigators for the charged substrates[4] made us prefer 0.01–1 M HCl solutions where the ionic strength was kept at 1 M (KCl).

Reversible formation of 4-imino-2-thiooxopyrimidine 6 from phenylthioureido amide 5 in acid solutions

Bethell *et al.*[4] observed that the cleavage of phenylthioureido peptides **1** begins with a decrease in the UV absorption at 245 nm due to the phenylthiouredo chromophore with concurrent formation of a peak at 230 nm due to anilinothiazolinone **2**. Then



Scheme 2.

absorption at 245 nm began to increase due to the formation of the phenylthioureido acid **3** changing finally to the peak at 265 nm characteristic of 2-thiohydantoins **4**. Bethell *et al.*[4] supported Scheme 1 by measuring also the rate-pH profiles for the conversions of the intermediates **2** and **3**.

The course of spectral changes observed with the β-phenylthioureido amide **5** in acid are quite different to those of the α-analog. These proceeded in two stages. At first, the decrease in amide absorption at 244 nm was accompanied by a rapid increase in the absorption at longer wavelengths. This build up was greater at higher acid concentrations accompanied by the formation of an isosbestic point around 270 nm (Fig. 1a). The longer wavelength absorption reached a maximum and then began to decrease. In the second stage, a new isosbestic process was established yielding ultimately the spectrum of thiodihydouracil **7** (λ_{max} (H_2O) 278 nm, Fig. 1b). More detailed examination showed the final product to be an equilibrium mixture containing 15% phenylthioureido acid **8** (*v.i*) due to partial hydrolysis of **7**. Remarkably, the isosbestic process maintained for a considerable period of time during the final conversion into **7**. As is well known, an isosbestic point obtains upon conversion of one compound into another at the wavelength where the two compounds possess the same absorption coefficients. This is a common proof for a 'clean' process because the presence of an isosbestic point requires stoichiometric conversion. The pattern of behavior in our case proves that the transiently formed compound and the initial reactant are in a state of fast equilibrium both converting into the product **7** in parallel reactions because when the two compounds, the amide and the transient, convert into a third compound, the thiodihydrouracil, an isosbestic point can be formed only if the ratio of the two reactants is maintained constant by a fast equilibrium or stationary state and thus the spectrum of the mixture is maintained constant with time. The position of the two isosbestic points observed in the second stage shifted with acidity; for example, from 260.5 and 291 nm in 1.5 M H_2SO_4 to 264.5 and 303.5 nm in 0.25 M H_2SO_4. This is an expected result with a changing ratio of amide and intermediate depending on [H^+] (Alternatively, the isosbestic points could be shifted because of spectral dependencies on acidities. However, the chromophore phenylthioureido group is expected to be a too weaker base for any appreciable protonation in 1 M HCl (pK_{BH+} of $PhNHCSNH_2$ is −2.20[8]), while the product **7** should be a still weaker base. On the other hand, iminopyrimidine **10** has a $pK_{BH+} = 6.40$[7] and is fully protonated in the region of acidities studied.) according equilibrium K_{12} of Scheme 2.

The transiently formed compound was found to be the iminopyrimidine **6** obtained before from *cis*-2-(3-phenylthioureido)cyclopentane-carbonitrile **9** in aqueous KOH as an intermediate during its hydrolysis to amide **5**.[7] It could be isolated and its structure determined as **6**. A breakthrough in unraveling the transformations and the kinetics of the system studied occurred when the reaction could be studied starting

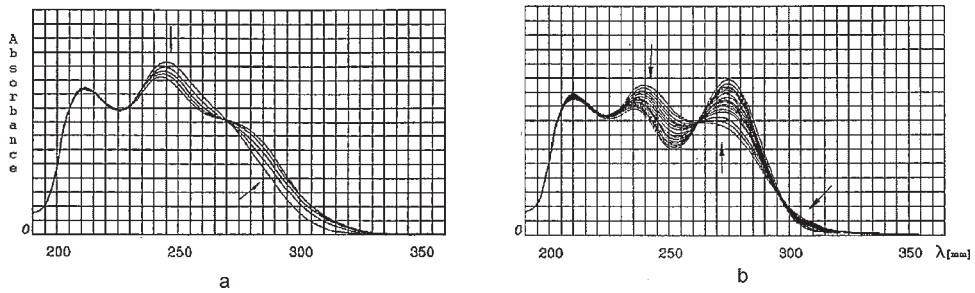


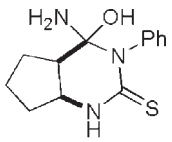
Figure 1. Transformation of UV spectra of amide **5** with time in 0.75 M H_2SO_4 at 50°C. (a) The first several minutes of the reaction. (b) Changes observed after the 17th minute of the reaction

from **6**. When iminopyrimidine **6** was used to initiate the reaction in acid, a peak at 293 nm was first observed due to its protonated form ($pK_{BH+} = 6.40$)[7] (Fig. 2b). The peak decreased rapidly through an isosbestic transformation (278 nm) reaching equilibrium with amide **5**. The spectral changes are more pronounced because the amide is preferred at equilibrium and hence the equilibrium composition is reached with a greater change in the spectra. Later, this isosbestic point is lost and then a second isosbestic process sets in which exactly repeats the second stage of the reaction initiated with amide **5** indicating that the same equilibrium amide–intermediate is reached (Fig. 2).

The observed spectral changes can be accommodated by Scheme 2.

Two pieces of kinetic evidence support Scheme 2. Iminopyrimidine **6** has the longest wavelength absorption so that its formation or depletion could be followed by the tail absorption at 305 nm at higher substrate concentration on a more precise instrument (Shimadzu UV 3000). In the initial stages of the reaction, the rise (fall) of absorbance could be described as a single exponential decay reaching a plateau. Actually for sulfuric acid solutions, the plato was more stable till $10\tau_{1/2}$ at 0.5 M. In more dilute solutions, a slow linear increase due to the conversion of **5** into **7** while in more concentrated ones the plato decreased linearly due to the predominant effect of conversion of **6** into **7**. These linear changes when extrapolated to time zero amounted to small (up to 5%) corrections in the end absorbances. The end absorbance increased with acidity. Table 1 lists the observed rates which are very similar in both directions as expected for an equilibrium process. Similarly the observed rates of formation of thiodihydrouracil in the second stage are practically the same for the reactions initiated with **5** or **6** (as described below).

The spectral changes described above leave little doubt that the product thiodihydrouracil **7** obtains in parallel from the two reactants, amide **5** and iminopyrimidine **6**, which establish a fast preliminary equilibrium. Scheme 2 assumes this to take place via direct conversion. Most likely, this could proceed by the loss of ammonia from a common unstable tetrahedral intermediate:



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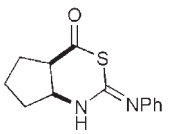
Is 6-oxothiazine 10 an intermediate in the elimination of ammonia?

Scheme 2 for the formation of thiodihydrouracil is contrary to the mechanism established for the Edman degradation of peptides depicted on Scheme 1. In our case, the latter would involve the formation of 6-oxothiazine **10**,



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the six-membered analog of anilinothiazolinone **2,** hydrolysis to acid **8** followed by cyclization to the product **7**. As long as **10** does not accumulate, the pattern of change of the UV-spectra will not

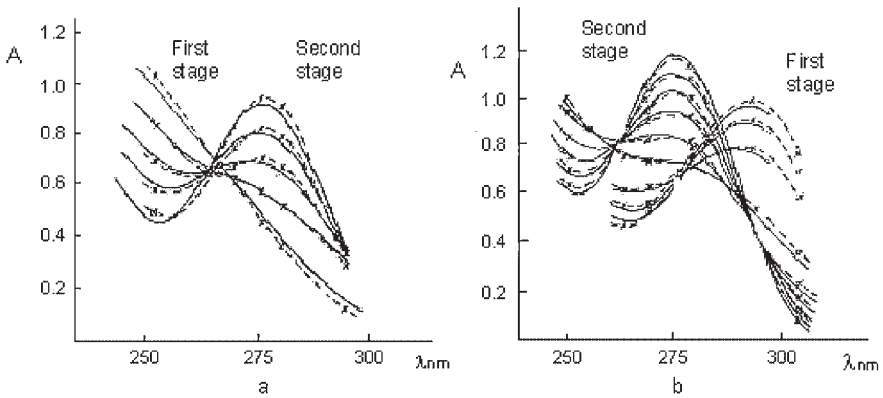

Figure 2. Spectral changes of reactants in 1 M HCl. Unbroken lines are experimental and dashed lines calculated as described in text. (a) Reaction initiated with amide **5**. (b) Reaction initiated with iminopyrimidine **6**. Calculated values denoted by *x*s

Table 1. Overall pseudo first order rates $10^4 \times k\ s^{-1}$ in sulfuric acid solutions at 50°C for reaching equilibrium between **5** and **6** $k_{(1)}$ and for the formation of **7** in the second phase of the reaction (K_2)

M H_2SO_4	$k_{(1)amide}$[a]	$k_{(1)imine}$[a]	A_∞[b]	$k_{(2)amide}$[c]	$k_{(2)imine}$[c]
0.125	34.4	32.8	0.285	1.59	1.70
0.255	32.6	31.0	0.350	—	—
0.510	30.5	28.0	0.488	—	—
0.755	32.6	28.3	0.640	3.49	3.39
1.025	33.3	31.8	0.795	3.98	3.93

[a] Calculated for *ca.* 50% conversion before reaching the plateau.
[b] Converted to initial A_o of amide 0.252 (3.57×10^{-4} M).
[c] Approximate values calculated by fitting A_∞ (as described below).

be altered because amide **5** and acid **8** have the same chromophore and to all purposes the same spectra. The thiazine mechanism requires cyclization of acid **8** to be faster than the overall conversion of amide **5** into thiodihydrouracil **7** in order for the second isosbestic phase of the reaction to be maintained. Otherwise acid **8** will accumulate and the total concentration of the mixture amide – imine in equilibrium will no longer correspond stoichiometrically to thiodihydrouracil **7** formed leading to loss of the isosbestic point.

To elucidate this possibility, the rate of cyclization of the acid was measured directly:

$$\mathbf{8} \underset{k_{34}}{\overset{k_{43}}{\rightleftharpoons}} \mathbf{7}$$

The reaction proceeded with a clean isosbestic point and was conveniently monitored at 280 nm. It was found to be reversible, the same equilibrium mixture being obtained starting either from the thioureido acid **8** or the thiodihydrouracil **7**. Making the end reaction mixture alkaline restored the initial phenylthioureido absorption thus showing the absence of side reactions.

At 50°C in 0.5 and 1 M HCl solutions ($I = 1$ M KCl) K_{43} was found to be 5.53. As can be seen on Table 2 (derivation of the remaining rate constants is described below), the observed rates, $k_{obs} = k_{34} + k_{43}$, are smaller than the rate of product formation in the second 'isosbestic' stage, $k_{13} + k_{23}$, but similar to k_{13}, the rate of amide cyclization. However, with consecutive reactions, the overall rate constant equals that of the last stage only when reactant and intermediates are rapidly and completely converted into the last intermediate. Product analysis (^{1}H NMR) of the reaction mixture after reaching the second isosbestic point showed that the amounts of thioureido acid **8** were less than 10%. Thus, it can be definitely concluded that cyclization via acid **5** and by implication via oxothiazine **10** is not the major route for the formation of thiodihydrouracil **7**.

Derivation of the rate constants of Scheme 2 for amide 5 cyclization and imine 6 hydrolysis

The pseudo first order rate constants of Scheme 2 listed in Table 2 could be obtained by suitable partitioning of the observed rates in the 'isosbestic' phases of the transformations using some additional information. The analysis is based on the requirement discussed above that in the case of three compounds with different spectra when one of them is converted into the other two an isosbestic point will be formed when the ratio of the latter remains constant. The same applies in the reverse situation when two compounds are converted into a third one. The estimate of the constants included the following steps:

(a) $k^{(1)} = k_{12} + k_{21} + k_{23}$ – the observed first order rate constant for the depletion of iminopyrimidine **6** during the first isosbestic phase.

As depicted on Scheme 2, imine **6** converts along two parallel reactions: reversibly into amide **5** with a rate $k_{12} + k_{21}$ and irreversibly into thiodihydrouracil **7** with a rate k_{23}. Accordingly, product analysis during the first isosbestic stage showed that a mixture of amide **5** and thiodihydrouracil **7** is formed. According to kinetic laws, the ratio of product concentrations is constant in parallel reactions of the same order. When one of the reactions is reversible, this will be valid for the initial stages of the reaction. Thus, the isosbestic point is obtained because when the imine is converted into amide and dihydrouracil the last two maintain a constant ratio. In time, this is upset when the amount of thiodihydrouracil formed from the amide along k_{13} changes the [amide]/[thiodihydrouracil] ratio significantly and also the effect of the equilibrium shows up. This takes some time because the concentration of amide is small in the first stage of the reaction. At lower acid concentrations, the first stage is prolonged because amide cyclization is slowed and equilibrium is strongly shifted toward the amide **5**.

Table 2. Pseudo first order rate constants $\times 10^4$, s^{-1}, for the cyclization of amide **5** and acid **8** and the hydrolysis of iminopyrimidine **6** and thiodihydrouracil **7** in HCl solutions, ionic strength $I = 1$ M (KCl) at 50.0°C. Constants are defined in Scheme 2

[HCl] M	k_{12}	k_{21}	K_{12}[a]	k_{13}	k_{23}	k_{34}	k_{43}
0.010	—	11.1	—	0.131	33.4	—	—
0.050	0.288	14.4	0.40	0.214	16.6	0.0233[a]	0.129[b]
0.10	0.679	13.6	0.50	0.414	9.97	0.0414[a]	0.257[b]
0.50	3.26	16.2	0.40	1.57	4.88	0.233	1.29
1.00	4.44	11.06	0.40	3.24	8.84	0.456	2.52

[a] $K = k^{12}/k^{21} \times [H^+]$.
[b] Calculated values from data in 0.5 and 1 M HCl ($I = 1$ M).

In order to calculate the pseudo first order rate constant of the first stage of the reaction from the UV spectral changes, the end absorbance for the parallel reactions is needed[9] – that of the equilibrium mixture **5/6** for amide formation ($k_{12}+k_{21}$) and of **7** for k_{23}. Wavelengths where these absorbances are equal present the best solution and these are available in our case as the isosbestic points in the second phase of the reaction when the equilibrium mixture **5/6** converts into **7**. At these points, of course, the absorbance will be the same both when only thiodihydrouracil or only the equilibrium mixture of **5** and **6** is present as well as any combination of these two of the same total concentration. The wavelength of the isosbestic point in the 290–300 nm region was suitable for measuring the decrease of the peak of **6** using A of the isosbestic point as A_∞.

(b) The $(k_{12}+k_{21})/k_{23}$ ratio is equal to $N_{\mathbf{5}}/N_{\mathbf{7}}$ in the first isosbestic stage.

For parallel reactions of the same order giving different products, the ratio of product concentrations is equal to the ratio of the rate coefficients. The ratio under consideration is readily obtained from the absorbance of the isosbestic point in the first stage of transformation of imine **6**. This isosbestic point is formed at the wavelength where the absorbance of imine **6** is equal to that of the mixture of amide **5** and thiodihydrouracil **7** produced. The ratio of the products remains constant at the beginning until enough **7** is obtained along the second pathway from amide to change the spectrum of the mixture appreciably. As already discussed, little of **7** is obtained from **5** at the beginning because sufficient amounts of **5** have not yet been formed and also this is a significantly slower reaction. The absorbance at the isosbestic point is equal either to $A_{\mathbf{7}}$ when $N_{\mathbf{7}}=1$, or to $A=A_{\mathbf{5}}N_{\mathbf{5}}+A_{\mathbf{7}}N_{\mathbf{7}}$ when $N_{\mathbf{5}}+N_{\mathbf{7}}=1$. With known absorbances of the two products at this wavelength, their ratio, $N_{\mathbf{5}}/N_{\mathbf{7}}$, is readily calculated from the absorbance of the isosbestic point. This ratio in turn is equal to $(k_{12}+k_{21})/k_{23}$. The concentration of initial **6** (as described in the section Experimental) was required to calculate the absorbances. Due to the instability of **6**, this was obtained from the end absorbance of product **7** which in turn was corrected for the presence of acid and/or unconverted amide from the peak to minimum ratio at 275 and 252 nm, respectively:

$$(A_{275}/A_{252})_{obs}=(A_{275}/A_{252})_{\mathbf{7}}N_7+(A_{275}/A_{252})_{(\mathbf{5}\text{ or }\mathbf{8})}N_{(\mathbf{5}+\mathbf{8})};$$

$$1=N_{\mathbf{7}}+N_{\mathbf{5}}+N_{\mathbf{8}}$$

(c) k_{12}/k_{21}.

Similar consideration show that at the isosbestic points during the second 'isosbestic' phase of the conversion starting either from amide or from iminopyrimidine, the absorbance equals $A_{\mathbf{5}}(N_{\mathbf{5}})_{eq}+A_{\mathbf{6}}(N_{\mathbf{6}})_{eq}$ in the absence of product **7**. This allowed the equilibrium ratio $(N_{\mathbf{6}})_{eq}/(N_{\mathbf{5}})_{eq}=k_{12}/k_{21}$ to be calculated in the same way as above from the absorbances of pure **5** and **6** and the total substrate concentration obtained as described above.

(d) k_{12}, k_{21}, and k_{23}.

Since $k^{(1)}=k_{12}+k_{21}+k_{23}$, known $k^{(1)}$ values and constant ratios yielded readily the individual rate constants.

(e) k_{13}.

This remaining constant from the triangle in Scheme 2 was determined from $k^{(2)}$, the pseudo first order rate constant calculated from the increase of absorbance at 278 nm due to the formation of thiodihydrouracil **7** in the second 'isosbestic' stage when equilibrium between **5** and **6** has been reached.

$$k^{(2)}=k_{13}(N_{\mathbf{5}})_{eq}+k_{23}(N_6)_{eq}$$

The kinetic analysis of the spectral changes with time yielding the constants pertaining to the triangle in Scheme 2 ignores the hydrolysis of the end product **7** to acid **8**, k_{34} and k_{43}, respectively. In the computer simulation of the spectra, these constants were included using the data from the separate cyclization experiments in 0.5 and 1 M HCl described above. For the more dilute acid solutions, values were extrapolated assuming first order dependence on [HCl].

The simulation of the spectra showed that the values of k_{13} obtained from $k^{(2)}$, the rate of thiodihydrouracil formation, were too high. Although good linear first order plots were obtained, the high values apparently were due to the lower infinity readings due to the equilibrium thiodihydrouracil–thioureido acid. Good agreement was achieved when the respective constants were uniformly decreased by 20%.

The constants were further refined by solving the corresponding differential equations,[10] and a computer program was written which allowed the spectral changes with time to be calculated according to the exact solution. This was done for several more important wavelengths and the results are illustrated as *xs* in Fig. 2. The values of the constants were then slightly adjusted manually to improve the fit with the experimental spectra which is illustrated in Fig. 2. The measurements were carried out on a UV-instrument with no digital output which precluded computer fitting of the spectral data set to the rate equations as suggested by one of the referees. For this reason, no statistical criteria for the fit could be given. Detailed product analysis was carried out at various stages of the reactions by quenching the reaction in ice and extracting the organic matter and taking the ^{1}H NMR of the dry residues. Imine **6** as base remains in the acid solution. This is a very unstable compound – apart from ready hydrolysis in acid and in base, in solutions containing organic solvents readily undergoes rearrangement to *cis*-4-phenylimino-1,2,3,4a,5,6,7,7a-octahydro-3-phenyl-2-thioxocyclopenta[d]pyrimidine.[7] The results agreed reasonably well with the compositions calculated from the spectra and from computer calculations of the exact solution of the rate equation with a tendency for higher thiodihydrouracil **7** in the extract. Exact agreement was difficult to expect taking into account possible transformations during work up. To avoid these complications at the price of different experimental conditions, the reaction was monitored by means of ^{1}H NMR in a 5:1 DMSO-d_6:6.5 M DCl solution. As depicted in Fig. 3, amide **5** transforms simultaneously into iminopyrimidine **6** and thiodihydrouracil **7**. Under the conditions of the experiment, the amount of amide at equilibrium appears to be quite small. At a later stage, there appears thioureido acid **8**. Thus, direct monitoring of product evolution confirms completely the veracity of Scheme 2.

DISCUSSION

Two sets of experiments, each starting with amide **5** or iminopyrimidine **6**, were carried out. The coincidence between

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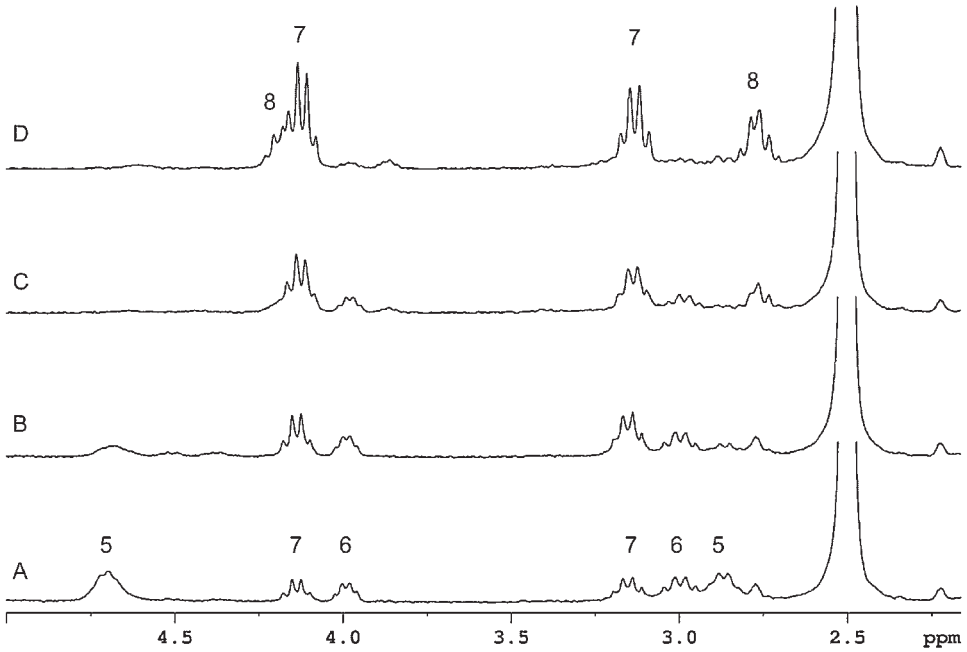


Figure 3. Selected scans of the conversion of amide **5** in 5:1 DMSO:6.5 M DCl at 25°C. A, 6 min after mixing; B, 26 min; C, 845 min; D, 5 days

the simulated and experimental spectra is shown in Fig. 2. The data for the rate constants are summarized in Table 2.

The pseudo first order rate constants listed in Table 2 indicate that the conversions of amide **5** are first order in [H^+]. Good linear plots of k_{obs} against [HCl] were obtained (Fig. 4) yielding second order constants $k_{12}^{(2)} = 10^{-4} \times (5.18 \pm 0.86)\,dm^3\ mol^{-1} \cdot s^{-1}$ and $k_{13}^{(2)} = 10^{-4} \times (.(2.98 \pm 0.19)$ and $k_{13}^{(1)} = 10^{-6} \times (9 \pm 10)s^{-1}$ (Fig. 4).

The rates for conversion of imine **6** into amide **5** show zero order in [HCl]. The average value of k_{21} equals 0.00132 s^{-1}. The conversion of **6** into thiodihydrouracil **7** decreases with acidity which can be accomodated by Scheme 3 involving the tetrahedral intermediate (only the overall processes are shown).

The attack of water on the protonated imine **6**H^+ produces an *O*-protonated **T** which rapidly sheds a proton being a strong acid to yield neutral $\mathbf{T}^0$. The scheme assumes that breakdown of $\mathbf{T}^0$ to amide is acid catalyzed while that to the thiodihydrouracil **11** is not (most likely through a zwitter ion obtained by a water-mediated proton switch[11]). The steady state approximation with respect to $\mathbf{T}^0$ for the reaction starting from the imine **6** postulates:

$$\frac{d[\mathbf{T}^0]}{dt} = 0 = k_{-2}[\mathbf{6}H^+] - k_2[\mathbf{T}^0][H^+] - k_{-1}[\mathbf{T}^0][H^+] + k_3[\mathbf{T}^0]$$
$$-\frac{d[\mathbf{6}H^+]}{dt[\mathbf{6}H^+]} = k_{21} + k_{23} = \frac{k_{-1}k_{-2}[H^+]}{(k_{-1}+k_2)[H^+]+k_3} + \frac{k_3 k_{-2}}{(k_{-1}+k_2)[H^+]+k_3} \quad (1)$$

The observed zero order of k_{21} in [HCl] (Fig. 2) obtains when $(k_{-1} + k_2)[H^+] > k_3$. Most probably, $k_{-1} < k_2$ because as discussed below k_2 is a rate limiting proton transfer, the neutral tetrahedral intermediate is then in a fast equilibrium with imine **6**H^+, k_{-2}/k_2, with rate limiting k_{-1}, acid catalyzed breakdown of $\mathbf{T}^0$, that is, $k_{21} = K_{\mathbf{6}^+}k_{-1} = 0.00132\ s^{-1}$ (the average cited above). With the same denominator, an inverse first order in [HCl] is predicted for k_{23}; the inverse dependence is observed but the slope is less than −1, apparently a change in the rate determining step takes place at lower acidity. Curve fitting by means of Eqn 1 produced the dotted line in Fig. 4; although it confirms the assumed mechanism, too few points were available, however, in order to obtain reliable values for the individual rate constants.

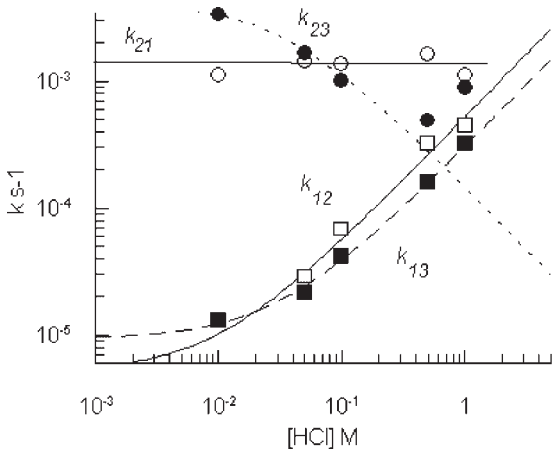
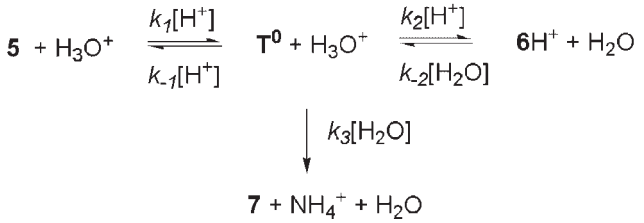


Figure 4. Logarithmic plots of the first order rate constants $k\ s^{-1}$ against [HCl] M: k_{12}, open squares; k_{13}, full squares; k_{21}, open circles; k_{23}, full circles. Lines present linear fits (k_{12} and k_{13}), average value (k_{21}), and nonlinear fit (k_{23})



Scheme 3.

For the conversion of amide **5** into imine **6** and thiodihydrouracil **7**, Scheme 3 gives Eqn 2:

$$-\frac{d[\mathbf{5}]}{dt[\mathbf{5}]} = k_{12} + k_{13} = \frac{k_1 k_2 [H^+]^2}{(k_{-1} + k_2)[H^+] + k_3} + \frac{k_1 k_3 [H^+]}{(k_{-1} + k_2)[H^+] + k_3} \quad (2)$$

Scheme 3 also predicts different orders for the conversion of amide into the two products; in the case when $(k_{-1} + k_2)[H^+] > k_3$, the production of iminopyrimidine **6** should be first order in [HCl] and the production of thiodihydrouracil **7** zero order. As shown in Fig. 4, this, however, is not the case because both reactions of the amide are first order in [HCl]. The conflict between the rate dependencies on acidities of the reactions of the amide on the one hand and those of the iminopyrimidine on the other cannot be solved within the framework of processes presented in Scheme 3 when they involve only heavy atom reorganization and fast equilibrium proton transfers. A common intermediate in equilibrium with the two reactants should impose rate laws that are consistent with each other. The situation closely resembles the 'thiazoline dilemma' encountered by Martin *et al.*[12] where acid inhibition of thiazoline hydrolysis could not be reconciled with acid catalysis of the S—N migration of product *S*-acetyl-2-aminoethanthiol. Barnett and Jencks[13] solved the dilemma by introducing an r.d. proton transfer step on the path from ester to the tetrahedral intermediate limiting the rate of ester migration while the ratio of *N*-acetylamino thiol and thiazoline produced are determined by partitioning of the intermediate after this step. In the present case, the discrepancy between predicted rate law of Eqn 2 and that experimentally observed can be removed by introducing a rate determining proton transfer step on the pathway between amide and $\mathbf{T}^0$. Most likely, this is deprotonation by a molecule of water of $\mathbf{T}^+$ resulting from the attack of the thioureido group on the protonated amide function (Scheme 4).

According to Scheme 4, the slow step is deprotonation at the nitrogen atom stemming from the thioureido group. Due to its low basicity, the protonated thioureido group has very strong fugacity thus probably enforcing catalysis by trapping of an unstable intermediate. Rate determining k_{H_2O} leads to the following rate law for the reaction of amide:

$$-\frac{d[\mathbf{5}]}{dt[\mathbf{5}]} = k_1[H^+] = k_{H_2O}\frac{K_{cyc}}{K_{BH^+}}[H^+] \quad (3)$$

Since the breakdown of $\mathbf{T}^0$ to iminopyrimidine and thiodihydrouracil occurs after the r.d.s., they should obtain not only by the same rate law but also with the same rates (Eqn 3); as the data on Table 2 shows the values for k_{12} and k_{13} are similar but the difference in the second order rates ($k_{13}^{(2)}$ is 40% smaller than $k_{12}^{(2)}$) is greater than their standard errors. However, taking into account the complicated fashion by which these individual constants were obtained the difference in rates is hardly significant and could be due to some systematic distortion.

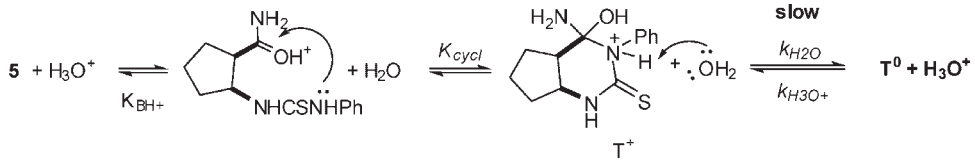
The product ratio is determined by the partitioning of $\mathbf{T}^0$ which according to Scheme 3 favors imine **6** with increased acidity:

$$\frac{[\mathbf{6}H^+]}{[\mathbf{6}H^+] + [\mathbf{7}]} = \frac{k_2[H^+]}{k_2[H^+] + k_3}$$

and

$$\frac{[\mathbf{7}]}{[\mathbf{6}H^+] + [\mathbf{7}]} = \frac{k_3}{k_2[H^+] + k_3}$$

The most interesting feature of the present investigation is that contrary to the case of α-*N*-phenylthiocarbamoylamino acid amides used in the Edman degradation of peptides where a anilinothiazolinone **2** (Scheme 1) is the first intermediate in acid catalyzed cyclization,[4] in the case of the β-*N*-phenylthiocarbamoylamino acid amide **5** the formation of 6-oxothiazine **9** has been ruled out. Obviously, the change in the mechanism is brought about by a change in the relative stabilities of the tetrahedral intermediates resulting from S or N attack in the five- and six-membered ring. Basically, this is a bond angle effect in 'normal' rings (five-, six-, or seven-membered rings are classed as normal as opposed to small, medium, and large) containing planar segments of trigonal atoms enforced by some geometrical requirements. In a regular pentagon, the angles are 108° matching the tetrahedral angles of 109° which is the basis of von Baeyer's theory why cyclopentane is strainless. In an irregular pentagon, the angles are different but the sum is fixed at 540° as long as it remains planar. When some of the ring atoms are sp^2 hybridized with intrinsic bond angles 120°, the sum exceeds 540°. As conjugation enforces planarity, resulting bond angle strain is accommodated by ring puckering and bond angle reduction. The opposite effect for the bond angles operates in six-membered rings. In a regular hexagon, the bond angles are 120° and the sum 720°. With a planar segment and part of the atoms tetragonal, the sum becomes less than 720° and partly the conflict is resolved by bond angle opening. We have recently studied[14] the extent of bond angle variation in hydantoins and dihydrouracils with a system four conjugated sp^2 atoms by the analysis of X-ray data from the Cambridge Crystal Structure Database. The average angle at the tetragonal atom of hydantoins (451) is $(101.2 \pm 1.1)°$ while those in dihydrouracils (456 and 561) are $(109.9 \pm 1.9)°$ and $(109.7 \pm 1.6)°$. This served to explain[14] the much stronger *gem*-dimethyl or dialkyl effect in five-membered rings with planar segments because the Thorpe–Ingold (The *gem*-dimethyl effect is exhibited in ring stabilization upon substitution in the chain; the Thorpe–Ingold effect refers to the reduction of adjacent bond angles upon substitution favoring small ring stability.) component helps to reduce the bond angles demanded in the formation of the five-membered rings. The effect can be quite large; an acceleration of 10^6 was observed in the acid catalyzed cyclization of hydantoic acid upon introduction of 2,2,3-trimethyl groups.[15]



Scheme 4.

Table 3. UV spectral data for compounds in water solutions

Compound	**5**	**6**H^{+a}	**7**	**8**
λ_{max} (nm)	245	290	278	245
ε	11 500	9500	11 700	1200

[a]Protonated form

A similar effect is expected to be exerted by difference in bond lengths. In irregular polygons, angles opposite longer sides are wider. Thus, the longer C—S bond 1.83 Å against 1.51 Å of the C—N will alleviate the strain of the reduced angle at 5-C of the thiazoline ring being formed under sulfur attack compared to the case of nitrogen attack while the opposite will be true in the formation of the six-membered thiazine and pyrimidine rings; the longer C—S will aggravate the strain at the tetragonal atoms 5-C and 6-C. Apparently, these opposite effects on bond angle strain determine the drastically different mechanisms in the acid catalyzed cyclization of α-thioureido and β-thioureido amides. Thiazine **9** proves to be so unstable that it is no longer a viable intermediate particularly in aqueous media. Our attempts to obtain anilinothiazine **9** using Edman's[3] procedure in dry nitromethane saturated with HCl failed in spite of repeated efforts and various modifications. In contact with moisture, it rapidly hydrolyzed the first product being the amide **5**.

EXPERIMENTAL

Instruments included a Bruker Spectrospin WM 250 NMR spectrometer (chemical shifts in ppm against TMS, couplings in Hz), a Unicam SP 800 UV spectrophotometer, wavelengths in nm.

Materials

Inorganic reagents for kinetic measurements were of analytical grade and were used without further purification. *cis*-2-(3-Phenylthioureido)cyclopentanecarboxamide (**5**), *cis*-2-(3-phenylthioureido)cyclopentanecarboxylic acid (**8**), *cis*-2-(-3-phenylthioureido)-cyclopentanecarbonitrile (**9**), and *cis*-4-oxo-1,2,3,4a,5,6,7,7a-octahydro-3-phenyl-2-thiooxocyclopenta[d]pyrimidine (**7**) were prepared as described before.[7] UV-spectral data of compounds studied are listed in Table 3.

cis-4-Imino-1,2,3,4a,5,6,7,7a-octahydro-3-phenyl-2-thiooxocyclopenta[d]pyrimidine (**6**) appears as a reactive intermediate upon hydrolysis of nitrile **9** in aqueous KOH. At 25°C in 0.01 M KOH, its maximum formation is at the 3rd minute. For the purposes of the kinetic runs, **6** was prepared in solution separately for each experiment adapting the previously described procedure.[7] 1.3 ml of a 0.01 M solution of nitrile **9** in MeCN are added to 10 ml 0.01 M KOH at room temperature, stirred, and allowed to stand 3 min. The solution is then added to a cooled funnel to which 0.32 ml of 1 M HCl and weighted amount of crushed ice have been added so that a final concentration of 0.01 M HCl was achieved. The contents are rapidly extracted with CH_2Cl_2 3 × 5 ml. The water layer is filtered through a fluted filter, transferred to a new funnel, and extracted with dichloromethane (2 × 5 ml) and filtered again. The whole procedure could be carried out for 9 min. Suitable aliquots were used for the kinetic experiments.

Kinetic measurements

The rates were measured under pseudo first order conditions in the thermostated cell compartment of a Unicam SP 800 spectrophotometer. In the case of amide **5**, acid **8**, and thiodihydrouracil **7**, the reactions were initiated by injecting 20–80 μl of a 10^{-2}–10^{-3} M stock solution of the substrate in MeCN to HCl solution of ionic strength 1 M (KCl) preheated to 50°C in the UV cell. Under an automatic program, whole spectra were scanned at suitable time intervals and readings taken at the wavelengths described in the Results section. In the case of imine **6**, 0.4 ml of its solution prepared as above (0.01 M in HCl) was added to 2.40 ml of HCl and KCl so that the final concentration was that desired at ionic strength of 1 M. The latter solution was preheated to 60°C so that temperature equilibrium at 50.0°C could be reached faster. Pseudo first order rate constants k_{obs} were calculated by the least squares procedure from plots against time of $\ln(A - A_t)$ or $\ln(A - A_t)$ as appropriate where A was the absorbance after 10 half-lives or that described in Results.

Product analyses

These were carried out by means of large-scale experiments; appropriate amounts of substrate solution in MeCN were added to HCl solutions ($I = 1$ M, KCl) preheated to 50°C in a flask whereby the final concentration matched that of the kinetic runs. At appropriate intervals, for example, during the first isosbestic point or during the second isobestic point 10 ml aliquots were withdrawn, extracted three times with CH_2Cl_2, the solvent of the organic layer removed, and the residue analyzed by means of t.l.c. and ^{1}H NMR. In the case of iminopyrimidine **6**, two sets of experiments were carried out; at 25°C which avoided the problem of attaining rapidly temperature equilibrium at 50°C and were compared with parallel kinetic experiments monitored by UV and a second set where the aqueous HCl solution of **6** with an ambient temperature was mixed with a HCl solution preheated at 60–70°C and then placed in bath at 50°C.

CONCLUSIONS

The kinetics of cyclization of *cis*-2-(3-phenylthioureido) cyclopentanecarboxamide **5** in 0.01–1 M HCl revealed that contrary to the case of α-phenylthioureido amides used in the Edman degradation the reaction of the β-analog proceeds by nitrogen attack as opposed to sulfur attack observed in the α-compounds. However, *N*-attack of the thioureido group does not proceed by direct displacement of amide amino group to *cis*-4-oxo-2-thiooxopyrimidine **7** but rather amide **5** forms reversibly the respective *cis*-4-imino-2-thiooxopyrimidine **6** followed by a slower conversion of both **5** and **6** into thiodihydrouracil **7**. Product **7** hydrolyses to an appreciable extent to the *cis*-2-(3-phenylthioureido)cyclopentanecarboxylic acid **8**. The reaction was followed more conveniently by UV when initiated with imine **6**. The two phases of the reaction formed isosbestic points in the UV scans due to the interconversion of three

compounds two of which remain in a constant ratio. This allowed an analysis to be made yielding the individual rate for the separate conversions on Scheme 2. Cyclizations of amide **5** to **6** and **7** showed first order in $[H^+]$ while hydrolysis to imine **6** to **5** and **7** showed 0 and −1, respectively. The kinetics indicates a rate determining proton transfer on the route from amide to neutral tetrahedral intermediate. The absence of sulfur attack in the six-membered case is explained by the longer C—S bond bringing about greater bond angle strain at the tetrahedral ring atoms due to the geometrical characteristics of five- and six-membered rings with planar segments.

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