# Experimental intrinsic barriers to amide–amide interaction. Transannular cyclolization in a cyclic diamide

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*N*-(2-Aminoacetyl)-ε-caprolactam (**1**) was synthesized. When **1** is dissolved in aprotic solvents such as chloroform or dichloromethane and water ($D_2O$), a *ca.* 1 : 1 equilibrium is established between two isomeric forms: *cyclol* **1c** and macrocyclic *diamide* **1d**. The methylene protons –$NHCH_2CON$– for both forms become diastereotopic. Therefore, the diastereotopic interconversion of **1c** and **1d** can be followed by dynamic $^1$H-NMR. In $D_2O$, specific base catalysis is observed for both interconversions. Since the equilibrium $K = k_{obs.f}/k_{obs.r}$ for the **1c** = **1d** transformation remains the same over the wide pD range studied (2 < pD < 11), a mechanism is proposed whereby the exchange occurs through the *cyclol* **1c** conjugate base. According to this mechanism, $k_{obs.f}$ and $k_{obs.r}$ can be measured by the diastereotopic interconversions of **1c** and **1d** respectively. Therefore, $K = k_{obs.f}/k_{obs.r} = k_2[H_2O]K_a/k_{-2}K_w$, where $k_2$ is the rate of cleavage of the cyclol **1c** (of acidity constant $K_a$) conjugate base toward the macrocyclic *diamide*, and $k_{-2}$ represents the reverse amino amide-to-carbonyl amide attack (transannular cyclolization). Values for $k_2$ of $1.8 \times 10^2$ $M^{-1}$ $s^{-1}$ ($\Delta G^{\ddagger} = 59.8$ kJ $mol^{-1}$ at 25 °C), and $k_{-2}$ of $1 \times 10^5$ $M^{-1}$ $s^{-1}$ ($\Delta G^{\ddagger} = 44.3$ kJ $mol^{-1}$ at 25 °C) were obtained. The uncatalyzed rates of $k_{obs.f} = k_{o1c}$ and $k_{obs.r} = k_{o1d}$ were also measured. These values are 2 $s^{-1}$ ($\Delta G^{\ddagger} = 71.1$ kJ $mol^{-1}$ at 25 °C) and 4 $s^{-1}$ ($\Delta G^{\ddagger} = 69.4$ kJ $mol^{-1}$ at 25 °C), respectively.

## Introduction

In continuation of our studies[1,2] on the reactivity of stable tetrahedral intermediates, compound **1** was prepared.[3,4] When **1** is dissolved in $CDCl_3$ or $D_2O$ an equilibrium (*ca.* 1 : 1) between cyclol **1c** and cyclic diamide **1d** is established. The $^1$H-NMR signals for the diastereotopic methylene protons in the α position (to the carbonyl group) of the cyclol **1c** are typical of an AB system centered at 4.3 ppm. Yet in cyclic diamide **1d** the same protons appear as two different signals (an AX system) at *ca.* 3.0 and 3.9 ppm. Dynamic $^1$H-NMR line shape analysis was used to measure the interconversion rates of the two diastereotopic protons of forms **1c** and **1d** ($H_a$ and $H_b$ in Fig. 1) at different pD ($D_2O$) values. The rates of cleavage of the tetrahedral intermediate toward the cyclic amide and the corresponding reverse reaction (Fig. 2) were obtained separately in this way. These values represent the intrinsic leaving ability and nucleophilicity of an amide group. By using different approaches,[5,6] they could be instrumental in the prediction of the most common non-thermoneutral amide–amide interactions. Also, the results obtained in this work might be of interest to peptide chemistry since the type of reaction under study occurs in the peptide cross-linking process. They are indeed particularly significant for the study of the synthesis[7] of medium-sized cyclic peptides and for the understanding of *cyclol* formation in alkaloids such as *rhetsinine*.[8]

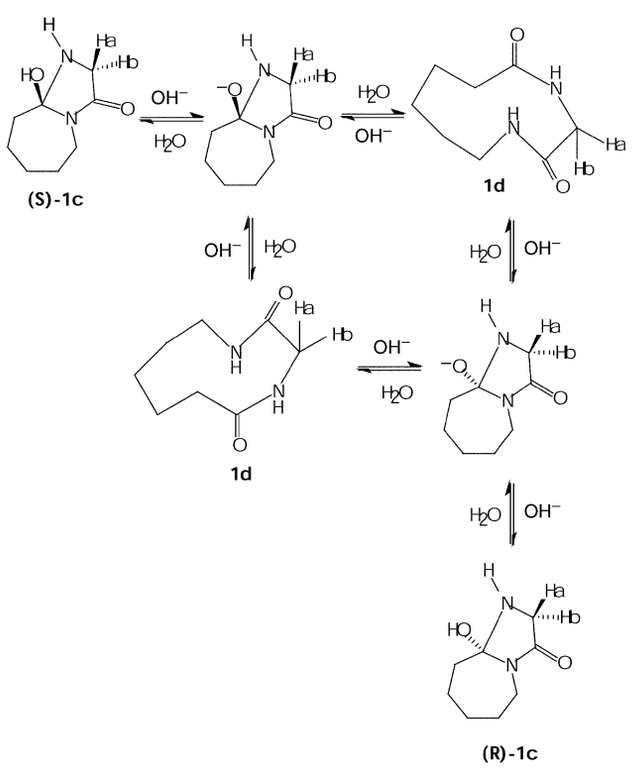


**Fig. 1** Equilibria established in water for compound **1**. The exchange of protons $H_a$ and $H_b$ occurs *via* reversible attack at the *Re vs. Si* face of the amide carbonyl of the atropisomers **1d** (based on preliminary mechanical calculations a *cis–trans* configuration has been used in the drawing).

## Experimental and results

### Synthesis, characterization and $^1$H-NMR signal identification

Compound **1** was prepared using the methodology described by Rapoport *et al.*[3] Only one product was detected in the GCMS.

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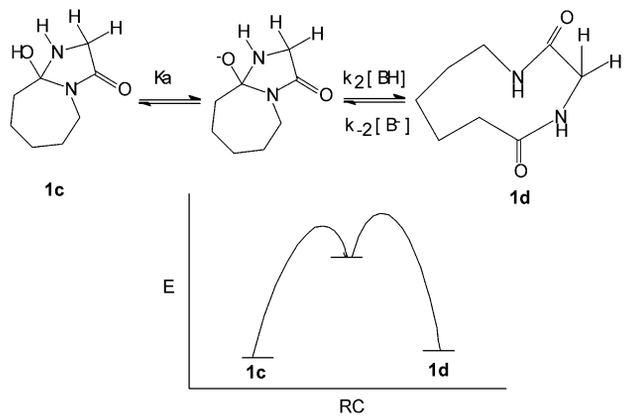
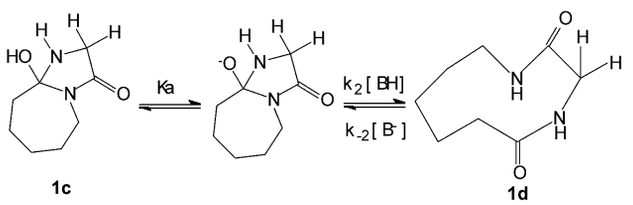



**Fig. 2** Reaction mechanism, energy profile and rate law. (For B = OH at pH < 12: $K = k_{obs.f}/k_{obs.r} = k_2[H_2O]K_a/k_{-2}K_w$.)




The mass spectrum showed a fragmentation pattern very similar to that reported[3] for the isomer 6,10-dioxo-1,5-diazacyclodecane, which displayed its main peaks at *m*/*z* 152 (molecular peak − 18), 124, 96, 68, 56 and 18. $^1$H-NMR ($CDCl_3$): 1.70 (m, 10.8H), 2.11 (m, 3.6H), 3.06 (AX, 0.80H), 3.19 (m, 3.6H), 3.81 (AX, 0.80H), 3.95 (m, 0.8H), 4.28 (AB, 2H). According to $^1$H-NMR two forms of **1**, **1c** and **1d**, are present in equilibrium. The $^1$H-NMR for **1c** coincides with the pattern previously reported[4] for other cyclols. In these cases the key signal corresponds to the methylene protons in the α position to the carbonyl group, which appears in $CDCl_3$ as an AB ($\nu_A$ = 1255 Hz, $\nu_B$ = 1307 Hz, $J$ = 13.7 Hz) system centered at 4.28 ppm. These values were used to perform simulations in $D_2O$ (slow exchange conditions). The two diastereotopic protons for **1d**, $H_a$ and $H_b$ (Fig. 1), do not form an AB system but an AX system. In fact, double irradiation experiments confirm that the signals at 3.81 and 3.06 ppm are coupled with a coupling constant value of 12 Hz. The unexpectedly large difference in chemical shift (0.75 ppm) for these two geminal protons is due to the anisotropic effect[9] of the carbonyl group directly attached to these methylene protons. Preliminary theoretical calculations[10] show that the optimal geometry for **1d** leaves one of these protons (A) aligned with the carbonyl plane while the other (X) is almost orthogonal to this plane. Each of these signals is additionally coupled to the hydrogens directly attached to the two amide nitrogens. The coupling constants obtained from the spectrum in $CDCl_3$ are $J_{AX}$ = 12, $J_{ANH}$ = 3, $J_{ACONH}$ = 3, $J_{XNH}$ = 9, $J_{XCONH}$ = 3 Hz ($\nu_A$ = 1145 and $\nu_X$ = 920 Hz). These values (slow exchange conditions) were used to perform simulations in $D_2O$. No other signals corresponding to a third form such as the *open form* (previously observed[2]) were detected in this work. The signals at 3.95 ppm observed in $CDCl_3$ correspond to protons directly attached to nitrogen (amide protons). These signals were assigned from low temperature experiments in $CDCl_3$ and $CD_2Cl_2$ and by their exchange in $D_2O$. For instance, at low temperatures (using aprotic solvents), the signals at 3.95 ppm become broader due to their more efficient relaxation which is induced by the $^{14}$N quadrupolar mechanism.

### NMR simulation and rate constants

As shown in Fig. 3 (left), specific base catalysis is observed in the diastereotopic interconversion of protons $H_a$ and $H_b$ (Fig. 1) of both forms **1c** and **1d**. Spectra were taken on a 300 MHz Bruker instrument using a probe at 25.0 °C. For **1c** (AB system) the observed rate constants at each pD ($D_2O$) were obtained by simulation using the equation of Fung and Olympia.[11] For **1d** the observed rate constants were obtained using the gamma program developed by Ernst *et al*.[12] In all cases, the simulated rate constants were varied until the best match with the experimental spectrum was obtained [Fig. 3 (right)]. Fig. 4 shows a

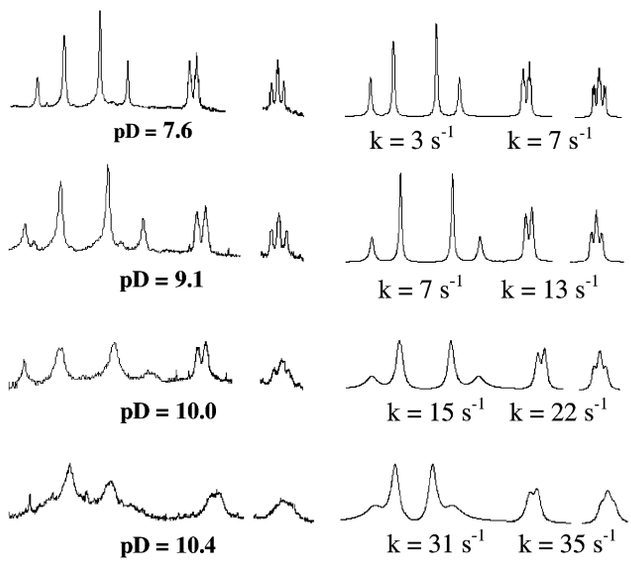



**Fig. 3** Experimental and simulated spectra at different pD ($D_2O$). Left side: experimental spectra at each pD. Right side: simulated spectra showing the observed rate for the diastereotopic interconversion in the AB and AX systems.

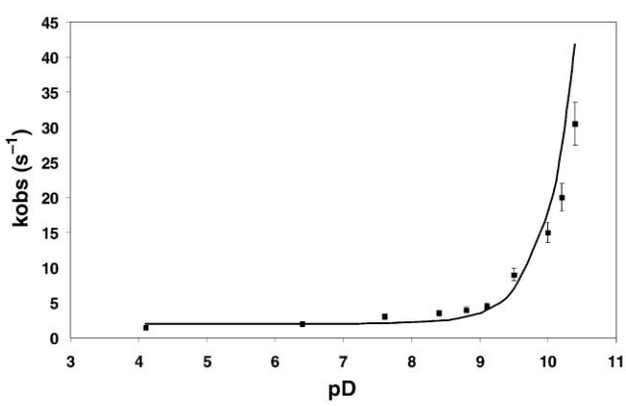



**Fig. 4** Plot of $k_{obs}$ *vs.* pD for **1c** (cyclol). $k_{obs}$ values determined at 25 °C. The experimental points and the best fit to the equation $k_{obs} = k_o + k/[H^+]$ with $k_o$ = 2 s$^{-1}$ and $k$ = 1.6 × 10$^{-9}$ M s$^{-1}$ are shown.

$k_{obs}$ *vs.* pD profile for the diastereotopic interconversion of the AB system (compound **1c**). A similar profile was obtained for compound **1d** (not shown). In both cases, the experimental points were fitted to the equation: $k_{obs} = k_o + k/[H^+]$, which gave $k_o$ and $k$ values of 2.0 s$^{-1}$ and 1.6 × 10$^{-9}$ M s$^{-1}$ for **1c**, and 4.0 s$^{-1}$ and 1.0 × 10$^{-9}$ M s$^{-1}$ for **1d**. Samples were prepared by dissolving compound **1** to a final concentration of approximately 0.05 M in a solution of 0.1 M phosphate buffer and 0.5 M of KCl in a total volume of $D_2O$ of 0.5 ml. Spectra were taken at 25.0 °C. No general base catalysis was observed except for in the case of **1c** at pD = 10.4 (pD = pH + 0.4).[13] From a plot of $k_{obs}$ *vs.* [phosphate]$t$ (5 points: 0.2–0.8 M, $r^2$ = 0.97), a value of $k_B$ = 12 M$^{-1}$ s$^{-1}$ was obtained.

## Discussion

Two isomeric forms have previously been reported[2] for *N*-(2-aminoacetyl)-2-pyrrolidone (a 5-membered ring lactam): the *open form* and the *cyclol*. For compound **1** (a 7-membered

lactam), however, the two forms observed by $^1$H-NMR (in $D_2O$ and $CDCl_3$) were the cyclol (**1c**) and the *cyclic diamide* (**1d**). No *open form* was detected at any pH. The *open form* is easily identified from the $^1$H-NMR spectra by means of the methylene protons attached to the α carbon of the exocyclic carbonyl. These protons appear as a singlet due to free rotation of the $–CO–CH_2–NH_2$ group. The formation of a cyclic diamide by cleavage of the cyclol of *N*-2-aminoethylphthalimide has also been observed and followed by UV spectroscopy[1] in our laboratory. However, in this last case the reaction is irreversible and occurs only at pH < 3. The high stability of the **1d** form as compared to the open form should be related to the size of the macrocycle involved. For instance, it has been previously reported[3,5] that a 10-membered ring size (as in **1d**) efficiently stabilizes the diamide macrocycle. The relative stability of **1d**, compared for instance to that of **1**, cannot be due to the tension released, since, in general, seven-membered rings are less strained than ten-membered rings, but is due instead to the difference in energy of the diamide structure of **1d** *vs.* the aminoimide of **1** (open form).

Fig. 1 shows the equilibria involved. Diastereotopic interconversion in **1c** occurs through **1d** and not through the *open form*. A sound rationale for the non-participation of the open form in the proposed mechanism is provided at the end of this section. As illustrated, it is proposed that **1c** is cleaved through its conjugate base. This proposal is indeed in agreement with the observed specific base catalysis. On the other hand, the diastereotopic interconversion of **1d** occurs through the conjugate base of **1c**. Therefore, specific base catalysis must be also involved in the last process (as is experimentally confirmed in Fig. 3). Slow ring inversion in ten-membered bislactam macrocycle has been previously reported[14] for the symmetric 1,6-diazacyclodecane-2,7-dione where coalescence of the NMR(H) axial and equatorial signals occurs in aprotic solvent ($CDBr_3$) at 345 K ($\Delta G^{\ddagger}$= 70 kJ mol$^{-1}$).

The proposed mechanism and the corresponding energy diagram are shown in Fig. 2. As shown, the equilibrium between **1c** and **1d** involves intramolecular aminoamide–carbonylamide interaction. This transannular bond formation is predictable in a medium-size macrocycle bislactam such as the ten-membered ring of **1d**. As depicted in Fig. 2, the equilibrium that is established (**1c** = **1d**) ought to be pH independent, since the 1/[H$^+$] dependence of $k_{obs.f}$ and $k_{obs.r}$ (f = forward, r = reverse) is cancelled out. In fact, in the proposed mechanism, $k_{obs.f} = k_2[H_2O]K_a/(K_a + [H^+])$, but since $K_a \ll [H^+]$ over the pH range studied, $k_{obs.f} = k_2[H_2O]K_a/[H^+]$. On the other hand, $k_{obs.r}$ is given by the observed rates of **1d** diastereotopic interconversion. According to the mechanism, $k_{obs.r} = k_{-2}K_w/[H^+]$. The equilibrium constant for the reaction **1c** = **1d** is then [H$^+$] independent: $K = k_{obs.f}/k_{obs.r} = k_2[H_2O]K_a/k_{-2}$Kw. The specific catalyzed $k_{-2}$ value can be obtained directly from the experimental *k* value for **1d** (see NMR simulation and rate constants). Since $k = k_{-2}K_w$, $k_{-2} = 1 \times 10^{-9}$ M s$^{-1}$/$10^{-14}$ M$^2$ = $1 \times 10^5$ M$^{-1}$ s$^{-1}$ (44.3 kJ mol$^{-1}$ at 25 °C). Similarly, $k_2$ can be obtained but the $K_a$ value is required. We have estimated[2] a p$K_a$ of 12.9 for the cyclol of *N*-(2-aminoacetyl)-2-pyrrolidone. Using this value and knowing that *K* is *ca.* 1 gives $k_2K_a = k_{-2}K_w/[H_2O]$ = $1.8 \times 10^{-11}$ s$^{-1}$ M$^{-2}$ and $k_2 = 1.8 \times 10^2$ M$^{-1}$ s$^{-1}$ (59.8 kJ mol$^{-1}$ at 25 °C). Both the uncatalyzed rates in $D_2O$ for the cleavage of **1c** into **1d** and the reverse reaction are given by the $k_o$ values obtained directly from $k_{obs}$ *vs.* pH plots (see NMR simulation and rate constants). These values are 2 s$^{-1}$ ($\Delta G^{\ddagger}$ = 71.1 kJ mol$^{-1}$ at 25 °C) for **1c**, and 4 s$^{-1}$ ($\Delta G^{\ddagger}$= 69.4 kJ mol$^{-1}$at 25 °C) for **1d**. These values ($k_{o1c}/k_{o1d}$ = 0.5) are in agreement with an experimental *K* value of *ca.* 0.8 measured directly from integration of the $^1$H-NMR signals for **1c** and **1d**.

General base catalysis was detected but only for **1c** at pD = 10.4. Most probably, general catalysis also occurs for **1d** at this pD value. However, due to the advanced coalescence of the signals at this pD, its detection becomes difficult. Bifunctional catalysis by $HPO_4^{2-}$ may well be the mechanism involved. Research is currently being conducted on this aspect.

Finally it is worth mentioning that the participation of the *open form* in the diastereotopic interconversion of **1c** was correctly excluded since a change in the $K = k_{obs.f}/k_{obs.r}$ value would otherwise have been observed. In fact, the p$K_a$ of the *open form* of **1** must be similar to that of *N*-(2-aminoacetyl)-2-pyrrolidone which has been estimated[2] to be 8.5. Therefore, at pH > 8.5 a *K* value inversely dependent on [H$^+$] would have been observed. Since this dependence is not observed experimentally, we can safely discard the participation of the *open form*.

An extension of the present work is the study of *N*-(2-aminoacetyl)-2-piperidone (similar to compound **1**, but with a six-membered cycle instead of seven) as well as of other exocyclic intramolecular nucleophiles such as O and S instead of NH in the *open form*. These aspects are currently under investigation in our laboratory.

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