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From Theozymes to Artificial Enzymes: Enzyme-Like Receptors for Michael Additions with Oxyanion Holes and Active Amino Groups

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Different artificial enzymes, based on the theozyme concept, have been designed for Michael additions of pyrrolidine to α,β-unsaturated lactams. These molecules each have skeleton able to mimic a structure called an "oxyanion hole", as is present in many enzymes. Amine groups are also responsible for the catalytic activities of these receptors, since they support the important proton-transport step. The requirement for the amine groups was established from the reaction mechanism and from theoretical calculations. The catalytic activities of the receptors are discussed, taking into account their relative association constants with the substrate: k_{cat}/k_{uncat} values of up to 10^4 were obtained. The catalytic activities of the receptors are compared with those found in natural enzymes and catalytic antibodies.

Introduction

Over the last two decades, the use of metal catalysts has afforded very promising results in chemistry, but their toxicities[1] and instabilities handicap their use in synthetic chemical and pharmaceutical processes.

Recently, much emphasis has been placed on asymmetric organocatalysts that covalently bind to substrates,[2] in some cases allowing high stereoselectivities to be obtained. Nevertheless, asymmetric organocatalysts usually lack the extraordinary turnover numbers and reaction rate enhancement that are observed in enzyme catalytic reactions. Recent advances in our knowledge of enzyme reaction mechanisms and the reliability of theoretical methods could be extremely useful tools in the search for new catalysts inspired by natural enzymes.

The Pauling hypothesis[3] relates enzyme catalytic activity to the existence of stronger interactions between the enzyme and the substrate's transition state than between the enzyme and the substrate itself. This hypothesis is confirmed by the study of enzyme proficiency – $K_{tx}^{-1} = k_{cat}/(K_M \times k_{uncat})$ – which is assumed to formally evaluate the association constant between an enzyme and the reaction transition state.[4] Houk et al.[5] have reported a comparative study of the affinities of several receptors (including organic receptors, proteins, antibodies and enzymes) for substrates, inhibitors and transition states. Their analysis yields extraordinarily high "binding affinities" of enzymes to transition states (typical values for enzymes are: $K_M = 10^{-3.7\pm1.3}$; $k_{cat}/k_{uncat} = 10^{12}$; $K_{tx}^{-1} = 10^{16\pm4.0}$). Truhlar and Karplus[6] have pointed out that, overall, the stabilization of transition states in enzymes contributes to factors of not less than 10^{11} in reaction rate enhancement. Although very important for understanding of the catalytic activities of enzymes, other effects (such as stabilization of a complete ensemble of transition states by the enzyme[7] and the favouring of quantum tunnelling effects in enzymatic proton transfer processes[8]) contribute by factors of less than 10^3.

Although catalytic antibodies are not very stable to modifications of pH, temperature etc., and their production and purification may, like those of natural enzymes, be very costly, they have been proposed as very useful candidates in regiochemical and stereochemical reactions, drug degradation, drug activation, environmental processes etc.[9] Nevertheless, the proficiencies of such catalytic antibodies are far from those shown by enzymes ($K_M = 10^{-3.5\pm1.0}$; $K_{tx}^{-1} = 10^{6.6\pm2.0}$; $k_{cat}/k_{uncat} = 10^3$).[5]

The literature contains many examples[10,11] of small organic molecules with supramolecular properties resembling enzymatic behaviour. A very promising tool in this design is the application of the "theozyme" concept, which Houk[12] has defined as "an array of functional groups in a geometry predicted by theory to provide transition state stabilization". Such models are useful not only for predic-

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ting the mechanisms of known enzymes, but also for the development of new catalysts, saving lots of time and experimental resources.

Oxyanion holes,[13] in which a negative charge is created on an oxygen as a consequence of a nucleophilic attack, are common structures in enzymes such as hydrolases, lipases, proteases, esterases, peroxidases and epoxide hydrolases. The role of the oxyanion hole is the H-bond stabilization of this accumulation of negative charge. In addition, these enzymes usually combine oxyanion holes with other groups to improve their catalytic activities. X-ray structures of diaminoxanthones (Figure 1) show that they could be good mimics of oxyanion holes.[14] In fact, xanthone receptors have previously been reported to show catalytic activity in α deuteration reactions[15] and Michael additions.[14,16] The receptors addressed in this paper are designed to exploit the similarity of the xanthone diamine with the oxyanion hole.

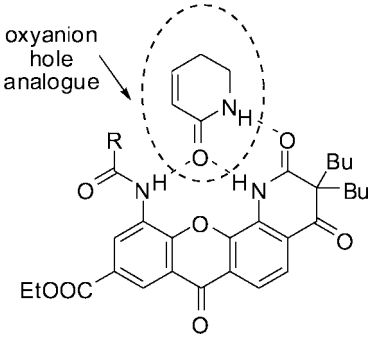


Figure 1. Structure of the complex of a diaminoxanthone receptor showing the α,β-unsaturated valerolactam in the oxyanion hole analogue.

In the first part of this paper, both computational chemistry tools and experimental methods are used to establish the need for this auxiliary group. The concept of a "theozyme" is also employed to explore different possibilities in the receptor design. In the last part, the catalytic activities of xanthone receptors containing α-amino acids or substituted ethylenediamine fragments are studied, and simple computational methods are used to predict their catalytic activities. These receptors have been reported to show good catalytic activities in the addition of nucleophiles to α,β-unsaturated lactams.[16] The addition of pyrrolidine to the α,β-unsaturated valerolactam (Figure 2) was chosen as a model reaction, since kinetic studies with this nucleophile are easier to perform than in the case of thiol addition.

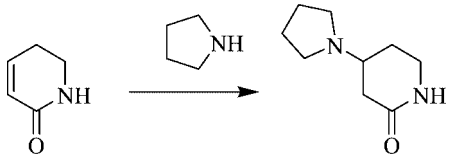


Figure 2. Reaction between the α,β-unsaturated valerolactam and pyrrolidine.

Results and Discussion

Reaction Mechanism – Experimental Studies

In enzymes, oxyanion holes are located inside hydrophobic pockets.[17] Oxyanion holes require an environment in which water molecules are unable to compete with the substrate for the establishment of hydrogen bonds. The mechanism of Michael addition was thus studied in the nonpolar solvent benzene, which was able to dissolve the compounds studied and which, through use of the perdeuterated form, allowed the use of ^{1}H NMR spectroscopy.

The reaction between pyrrolidine and the α,β-unsaturated valerolactam was too slow for results to be obtained in a reasonable time without use of a high concentration of the nucleophile. Therefore, to determine the reaction order with respect to the pyrrolidine, kinetic experiments were performed with the commercially available α,β-unsaturated valerolactone and α,β-unsaturated butyrolactone (for details on these and further kinetic experiments, see electronic supporting information). The results are summarized in Table 1.

Table 1. Rate constants for the reactions between pyrrolidine and α,β-unsaturated carbonyl compounds in benzene at 298 K.

Substrate	k (L^2 mol^{-2} s^{-1})
α,β-Unsaturated valerolactone	1.2×10^{-4}
α,β-Unsaturated butyrolactone	2.8×10^{-3}

The reactions studied here are second order in pyrrolidine. The nature of the catalytic effect of the second amine molecule was investigated by including other bases in the reaction mixture. Since the reaction with α,β-unsaturated valerolactam was very slow, we decided to study this effect in the reaction with the α,β-unsaturated butyrolactone. The results are summarized in Table 2. No general basic effect seems to be responsible for the catalysis driven by these groups, since a stronger correlation with the pK_a of the amines would be expected. Accordingly, DABCO, which should have a similar pK_a to pyrrolidine, showed almost no rate increase.

Table 2. Catalytic activities of some bases in the reaction between pyrrolidine and the α,β-unsaturated butyrolactone (k units are $L^2mol^{-2}s^{-1}$, and pK_a values are measured in THF,[18] except in the case of DABCO).

Base	k	pK_a
Pyrrolidine	2.8×10^{-3}	16.0
DABCO	4.2×10^{-4}	≈16.0
DBU	1.0×10^{-2}	19.1
Phosphazene	4.3×10^{-1}	21.7

Owing to its relevance in receptor design, the stereochemistry of this reaction was also investigated (Figure 3). Deuterium was used to label the α position of the α,β-unsaturated methylvalerolactam. Pyrrolidine was first added in deuterium oxide, but overlapping of the Michael adduct signals did not allow easy measurement of the *syn*/*anti* ratio by ^{1}H NMR spectroscopy.

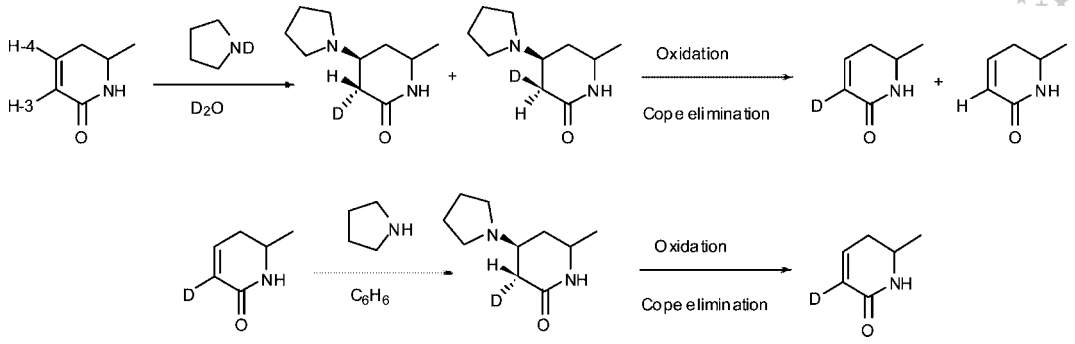


Figure 3. Stereochemical outcome of Michael addition in deuterium oxide and in benzene determined by integration of ^{1}H NMR signals in Cope *syn* elimination products.

The unsaturated lactam was regenerated by means of nitrogen oxidation followed by Cope elimination. A deuteration level of 50% was determined through integration of the ^{1}H NMR signals of the lactam 3-H and 4-H protons. This is probably the result of both *syn* and *anti* processes, due to rearrangement of the planar enol intermediate. The reaction of the deuterated lactam in benzene, followed by the same elimination sequence, showed no decline in the degree of deuteration, as revealed by the integrals of 3-H and 4-H in this lactam after the experiment. This suggests that both processes – addition and Cope elimination – take place with *syn* stereochemistry.

Reaction Mechanism – Molecular Modelling Studies

Pardo et al.[19] studied the mechanism of the addition of ammonia to acrolein in water. Their results suggested that a water molecule transports the proton of the nucleophile to the α carbon of the substrate. A similar mechanism, in which a second amine molecule could assist the proton transfer, can be invoked to explain our experimental results. To confirm this hypothesis, we performed similar modelling studies using the GAUSSIAN 98W[20] program (see details in the electronic supporting information).

Low-energy transition states (TS-1 and TS-2 in Figure 4) were obtained for the 1,2- and 1,4-additions of ammonia to acrolein when the reaction was assisted by a second ammonia molecule as shown in Table 3. The energy barriers were similar in both cases. Analysis of the imaginary frequency vibrational mode for these transition state structures revealed that both proton-transport processes were concerted. This kind of mechanism is usually known as a "proton-switch" in the literature.[19]

Tertiary amines such as DABCO showed catalytic activity in the reaction, but the proton-switch mechanism was not possible for this auxiliary amine. Quantum chemical calculations for the reaction mechanism of the trimethylamine-catalysed addition of ammonia to *s-trans*-acrolein afforded a stepwise mechanism (TS-3, Int-4 and TS-5, Figure 4, Table 3). This mechanism is related to the "proton-slide" mechanism suggested by Bruice et al.[21] Houk has also found a similar mechanism in the addition of hydroxy esters to ketenes catalysed by tertiary amines,[22] and a similar role for a trimethylamine catalyst in the base-assisted displacement of chloride by alcohol in sulfinyl derivatives has also been suggested.[23] In the initial step, a proton is transferred to the auxiliary trimethylamine molecule. This proton is then transferred to the α carbon in acrolein in the last step of the reaction.

Once it had been confirmed that the role of the water molecule can be performed by a second ammonia molecule, we decided to study the reaction on a more realistic model.

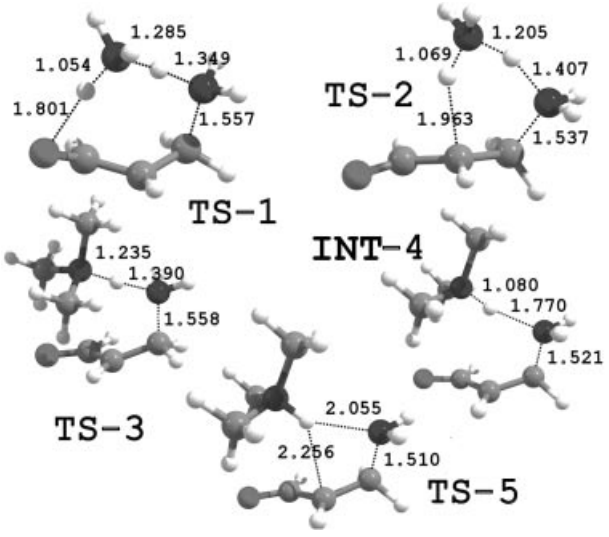


Figure 4. Transition state structures obtained for the Michael addition of ammonia to acrolein. Distances are expressed in Angstroms.

Table 3. Energy barriers (ΔE), zero-point energy barriers ($\Delta z.p.E$) and free energy barriers at 298.15 K (ΔG) for the addition of ammonia to acrolein. The structures of the transition states are shown in Figure 4. Energy units are kcal mol^{-1}.

Structure	ΔE	$\Delta z.p.E$	ΔG
TS-1 (1,4-addition)	10.81	15.00	35.61
TS-2 (1,2-addition)	12.35	16.16	36.71
TS-3 (addition and proton transfer to trimethylamine)	16.08	19.51	41.80
Int-4 (intermediate)	12.51	19.32	41.94
TS-5 (proton transfer to acrolein)	12.84	19.15	41.68

Modelling studies for the addition of dimethylamine to the unsaturated valerolactam were performed with use of the Gaussian 03W[24] program (see details in the electronic supporting information). The resulting structures are shown in Figure 5, and the results are given in Table 4.

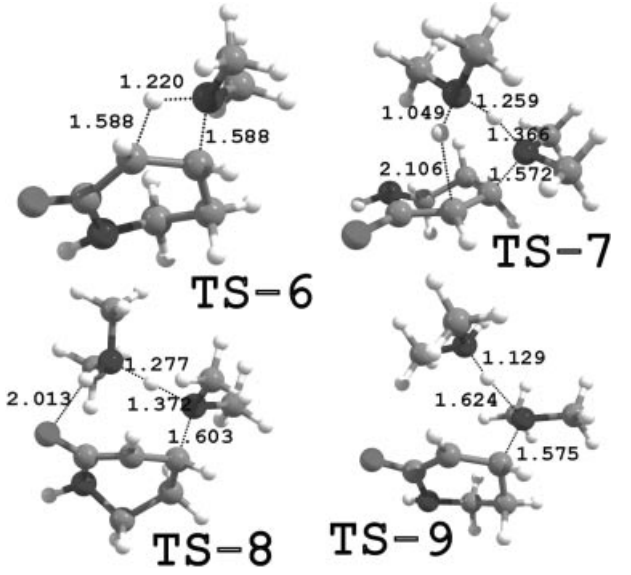


Figure 5. Transition state structures obtained for the reaction between dimethylamine and the unsaturated valerolactam.

Table 4. Energy barriers (ΔE), zero-point energy barriers ($\Delta z.p.E$) and free energy barriers at 298.15 K (ΔG) for the structures in Figure 5. Energy units are kcal mol^{-1}.

Structure	ΔE	$\Delta z.p.E$	ΔG
TS-6 (unimolecular in amine)	41.07	40.60	51.54
TS-7 (1,2-addition)	27.79	29.31	50.29
TS-8 (1,4-addition)	27.53	29.19	50.16
TS-9 (proton slide)	36.27	39.37	60.64

Results showed that mechanisms that were bimolecular in amine were energetically preferred over the unimolecular one (TS-6; see supporting information). The addition step of a proton-slide mechanism was investigated (TS-9), but the resulting structure showed a very high reaction barrier. Calculations show, nevertheless, that all mechanisms are possible, and hence the different mechanisms should be considered in the theozyme model.

Theozyme Models

H-bond stabilization of the transition state structures obtained for the addition of ammonia to lactam was investigated by use of Gaussian 98W[20] (see details in the supporting information). Transition state structures in which two formamide groups, which simulate the oxyanion hole, were bonded to the oxygen atom of the lactam were sought. The resulting structures are shown in Figure 6 and the results are summarized in Table 5. Comparison with activation energies obtained for identical calculations without the H-bond donors afforded slightly better stabilization for proton-slide (TS-13) and 1,2-addition (TS-11) than for 1,4-addition (TS-12). The energy differences between the most energetically favoured structures with and without formamide molecules (around 6.5 kcal mol^{-1}) suggested that the reaction rate could be enhanced by a factor of up to $10^{4.7}$ at room temperature in the presence of two amide H-bonding catalysts.

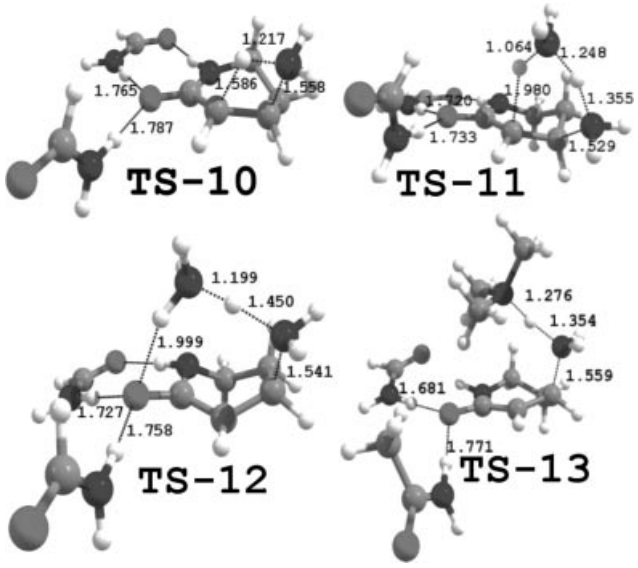


Figure 6. Theozymes for the addition of ammonia to the unsaturated valerolactam.

Table 5. Energy barriers (ΔE) for transition state structures and theozyme models and overall stabilization of the transition state structures in theozyme models. Structures are shown in Figure 6. Energy units are kcal mol^{-1}.

Structure	ΔE	$\Delta E(theozyme)$	$\Delta\Delta E$
TS-10 (unimolecular in amine)	43.36	38.86	4.50
TS-11 (1,2-addition)	23.43	15.95	7.48
TS-12 (1,4-addition)	22.47	16.29	6.18
TS-13 (proton-slide)	27.63	20.35	7.28

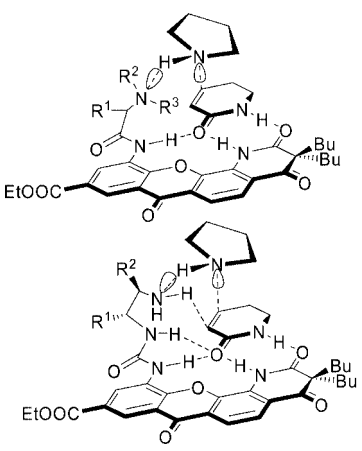
Receptor Catalytic Activities

Xanthone receptors 2 to 6 (Figure 7) were derived from α-amino acids. They have previously shown good catalytic activities in the conjugated additions of thiols to the unsaturated valerolactam.[16] These receptors combine structures capable of mimicking oxyanion holes with amino groups that can assist proton-transport processes. To study their catalytic activity in the reaction with pyrrolidine, kinetic experiments were carried out in benzene. Receptor 1, lacking the amino group, was also included in the study, in order to assess the relative importance of the amino groups with respect to the oxyanion holes. The resulting valerolactam half lives are shown in Table 6.

Table 6. Parameters obtained after analysis of the kinetic data for receptors 1 to 10 in benzene at 298 K.

Receptor	$t½$ (min)	k (k_{uncat})	K_{rel}	K_{ass}	k_{cat}	k_{cat}/k_{uncat}	K_{tx}^{-1}
–	2385	6.2×10^{-7} [a]					
1	84	1.7×10^{-5} [a]	1.000	11–40[c]	$10^{-3.43}$–$10^{-3.47}$ [a]	$10^{2.78}$–$10^{2.74}$	$10^{4.08\pm0.26}$ [e]
2	48	8.5×10^{-5} [b]	0.064	2.6–0.74[c]	$10^{-2.34}$–$10^{-2.59}$ [b]	$10^{3.86}$–$10^{3.61}$ [d]	$10^{3.87\pm0.40}$
3	85	4.8×10^{-5} [b]	0.007	7.7×10^{-2}–0.28[c]	$10^{-1.76}$–$10^{-2.28}$ [b]	$10^{4.44}$–$10^{3.92}$ [d]	$10^{3.34\pm0.54}$
4	76	4.9×10^{-5} [b]	0.118	4.3–1.3[c]	$10^{-2.71}$–$10^{-2.91}$ [b]	$10^{3.49}$–$10^{3.29}$ [d]	$10^{3.67\pm0.38}$
5	83	5.3×10^{-5} [b]	0.054	2.2–0.59[c]	$10^{-2.47}$–$10^{-2.79}$ [b]	$10^{3.73}$–$10^{3.41}$ [d]	$10^{3.62\pm0.44}$
6 (n = 1)	257	1.7×10^{-5} [b]	0.022	0.88–0.24[c]	$10^{-2.67}$–$10^{-3.10}$ [b]	$10^{3.52}$–$10^{3.10}$ [d]	$10^{2.98\pm0.54}$
6 (n = 2)		5.9×10^{-6} [a]			$10^{-3.13}$–$10^{-3.55}$ [a]	$10^{3.05}$–$10^{2.66}$ [d]	$10^{2.52\pm0.47}$ [e]
7	28	1.5×10^{-4} [a]	0.052	2.08–0.57[c]	$10^{-2.01}$–$10^{-2.36}$ [b]	$10^{4.20}$–$10^{3.85}$ [d]	$10^{4.49\pm0.46}$
8	40	1.0×10^{-4} [a]	0.310	12.4–3.4[c]	$10^{-2.53}$–$10^{-2.66}$ [b]	$10^{3.68}$–$10^{3.54}$ [d]	$10^{4.42\pm0.35}$
9 (n = 1)	76	5.4×10^{-5} [b]	0.930	37–10[c]	$10^{-2.90}$–$10^{-2.96}$ [b]	$10^{3.30}$–$10^{3.24}$ [d]	$10^{4.56\pm0.31}$
9 (n = 2)		2.0×10^{-5} [a]			$10^{-3.35}$–$10^{-3.55}$ [a]	$10^{2.87}$–$10^{2.80}$ [d]	$10^{4.12\pm0.32}$ [e]
10	33	1.2×10^{-4} [b]	0.071	2.8–0.78[c]	$10^{-2.19}$–$10^{-2.49}$ [b]	$10^{4.01}$–$10^{3.71}$ [d]	$10^{4.03\pm0.43}$

[a] $L^2mol^{-2}s^{-1}$. [b] $Lmol^{-1}s^{-1}$. [c] $Lmol^{-1}$. [d] $MolL^{-1}$. [e] $Lmol^{-1}$. For the remaining receptors, the k_{cat} values obtained were more than one order of magnitude higher than the k_{cat} of receptor 1. The k_{cat}/k_{uncat} and K_{tx}^{-1} values obtained for these receptors were very different from those obtained for enzymes[5] ($K_M = 10^{-3.7\pm1.3}$; $K_{tx}^{-1} = 10^{16\pm4.0}$; $k_{cat}/k_{uncat} = 10^{12}$). Nevertheless, the $k_{cat/uncat}$ values of these receptors were similar to the catalytic antibody values ($K_M = 10^{-3.5\pm1.0}$; $K_{tx}^{-1} = 10^{6.6\pm2.0}$; $k_{cat}/k_{uncat} = 10^3$). The small association constants (and not the low catalytic activities) prevent these receptors from reaching K_{tx}^{-1} values similar to those of catalytic antibodies.



Rec	R^1	R^2	R^3
2	Ph	H	H
3	$(CH_2)_3$		H
4	*i*Pr	H	H
5	*i*Bu	H	H
6	$(CH_2)_3$		Me

Rec	R^1	R^2
7	H	H
8	H	Me
9	Ph	Ph
10	$(CH_2)_4$	

Figure 7. Complexes of receptors 2 to 10 with the unsaturated valerolactam and the proton-transport mechanism.

The catalytic properties of ethylenediamine-derived receptors 7 to 10 were also tested (Figure 7). The kinetic data for all these compounds are also shown in Table 6. In comparison with the catalytic activity of the decanoyl receptor 1 ($t½$ = 84 min.), the half-lives of the receptors were surprisingly long. Nevertheless, competitive experiments aimed at measuring the relative association constants revealed smaller association constants for most receptors containing amino groups (Table 6).

In the case of the proline derivative receptor 3, DOSY experiments (see supporting information) discounted the existence of a dimer, so it is likely that the smaller association constants are a consequence of an intramolecular H-bond, which makes complex formation nonquantitative and increases the half lives. Thus, a more detailed study was performed, considering the affinities of the receptors for the reaction substrate. From the kinetic equations of the catalysed and uncatalysed reactions, one has [Equation (1)]:

$$\frac{d[lactam]}{dt} = k_{noncat}[lactam][pyrr]^2 \quad (1)$$

Equation (2) is the Michaelis–Menten equation for an enzymatic-like reaction considering competitive product inhibition. In this equation, K_M^{-1} and K_i^{-1} are the association constants with the receptor for the lactam and for the inhibitor, respectively, and n is the order of the reaction with respect to pyrrolidine. In the case of inhibition by the product it is reasonable to consider that $K_M = K_i$, since no additional interactions are expected between the product and the receptors than between the lactam and the receptors. Under this assumption, Equation (2) yields Equation (3), similar to the equation for the uncatalysed reaction (1):

$$\frac{d[lactam]}{dt} = \frac{k_{cat}[lactam][receptor]_0}{K_M(1+\frac{[inhibitor]}{K_i})+[lactam]}[pyrr]^n \quad (2)$$

$$\frac{d[lactam]}{dt} = \frac{k_{cat}[receptor]_0}{K_M+[lactam]_0}[lactam][pyrr]^n = k[lactam][pyrr]^n \quad (3)$$

Numerical integration of the kinetic equations was performed using the fourth-order Runge–Kutta method,[25] and optimization of the values of the rate constants yielded the values shown in Table 6. Although it would be desirable to measure the rate order of pyrrolidine for the catalysed reactions, this measurement is complicated since it requires kinetic experiments to be performed at different pyrrolidine concentrations, and at these different pyrrolidine concentrations, the receptors will probably show different association constants with the substrate, making the interpretation of the results very difficult. Therefore, it is simply assumed that for the uncatalysed reaction and for the reaction cata-

Table 7. Influence of the presence of δ-valerolactam on the catalytic activities of receptors **1** and **3** in benzene at 298 K.

Receptor	$t½$ (min.) with inhibitor	$t½$ (min.) without inhibitor	k with inhibitor	k without inhibitor	K_{ass} (calcd.)	K_{rel} (calcd.)
1	84	163	1.7×10^{-5} [a]	8.9×10^{-6} [a]	10.9–6.0	1
3	85	95	4.8×10^{-5} [b]	4.5×10^{-5} [b]	0.25–0.05	0.02–0.002

[a] $L^2 mol^{-2} s^{-1}$. [b] $L mol^{-1} s^{-1}$.

lysed by receptor 1, the reaction could be considered to be second-order in pyrrolidine ($n = 2$). For the other receptors, since the amine molecule is bound to the receptor structure it was initially considered that $n = 1$.

In order to calculate k_{cat} from Equation (3), it was necessary to measure K_M. In previous work[14] the association constant between receptor 1 and lactam under the reaction conditions was estimated to lie between 11 M^{-1} and 40 M^{-1}. The measurement of smaller association constants with receptors in 3.0 M pyrrolidine is problematic, so relative association constants with respect to receptor 3 were obtained from competitive titrations in deuterated chloroform and in the absence of pyrrolidine. These relative constants were used to approximate real association constants, assuming that the same ratio would be obtained under the reaction conditions. k_{uncat} was calculated from a kinetic study under similar conditions and with the assumption of a second-order reaction. The k_{cat}/k_{uncat} and $K_{tx}^{-1} = K_{ass} \times k_{cat}/k_{uncat}$ range of values for receptor catalysis are summarized in Table 6.

The k_{cat}/k_{uncat} value obtained for receptor 1 ($10^{2.76}$) is in agreement with the values obtained recently for the H-bond catalysis in the Michael addition of phenylthiol to a substituted maleimide by Philp et al.[11] ($10^{3.13}$). Catalysis is slightly lower than expected, indicating that the effect of the second H-bond probably cannot be considered additive.

For receptors 6 and 9 the k_{cat} values obtained were similar to the k_{cat} of receptor 1, and when the kinetic data for these receptors were fitted with $n = 2$, the k_{cat} values obtained were similar to that of receptor 1. This indicates that the efficiency of receptors 6 and 9 in catalysing the reaction in a mechanism unimolecular in amine was similar to or smaller than their efficiency in catalysing a second-order mechanism in amine. These results suggested that the amino groups in these receptors were not active in assisting proton transfer.

The relevance of the relative association constants and of complex formation in the catalytic activities of the receptors was confirmed by inhibition experiments with receptors 1 and 3. The reaction mixture was set up with the same amount of unsaturated valerolactam and δ-valerolactam, which lacks the conjugated double bond (0.8 M each). This saturated guest cannot react with pyrrolidine, but it will act as a competitive inhibitor of the artificial enzyme. If it is assumed that the association constant with the saturated inhibitor was similar to the substrate association constant, Equation (2) can be simplified to Equation (4) [which is analogous to Equation (3), provided that $(lactam)_0$ is substituted by $(lactam)_0$ + (inhibitor)]. Table 7 compares the rate constants k obtained in the presence and absence of inhibitor. The K_M values calculated from these data after substituting into Equation (4) are in the range of those used in the previous calculation of k_{cat} in Table 6.

$$\frac{d[lactam]}{dt} = \frac{k_{cat}[receptor]_0}{K_M + [lactam]_0 + [inhibitor]}[lactam][pyrr]^n = k[lactam][pyrr]^n \quad (4)$$

For receptor 1, the reaction rate constant in the experiment including the inhibitor was nearly half the value obtained when no δ-valerolactam was present. This strong sensitivity to the addition of inhibitor indicated that almost quantitative complex formation was occurring under the reaction conditions. For receptor 3, however, similar values for the reaction rate constant were obtained both in the presence and in the absence of δ-valerolactam. This type of behaviour would be expected for a receptor (or an enzyme) that showed only a small degree of complex formation under the reaction conditions: since the inhibitor had a large amount of the free receptor available, it scarcely inhibited the association of the substrate. In this case, the Michaelis constant term K_M ($= K_{ass}^{-1}$) dominates the denominator of Eq. (4). These experiments also confirm that the catalytic activity of the receptors must be due to their enzyme-like behaviour, since competitive inhibition processes are typical in this kind of catalyst.

Theoretical Studies on Receptor Catalytic Activities

The development of computational methods to help in the design of this kind of receptor is of great relevance since reliable theoretical tools will save time and effort and allow the less promising possibilities to be discarded. In addition to reliability, however, if these methods are to be applied to many possibilities for the design of receptors, it is also very important that they should have reasonable computational costs. We have investigated the ability of a simple computational method to reproduce the catalytic activity.

To reduce the computational cost of the calculations, the ONIOM[26] method, combining DFT and semiempirical methods (see supporting information for details and Figure 8 for layer distribution) was used in transition state searching. Single-point energies were evaluated on the resulting structures, using the same level of theory as employed before in mechanism computational studies (B3LYP/6-31g** PCM: Benzene).

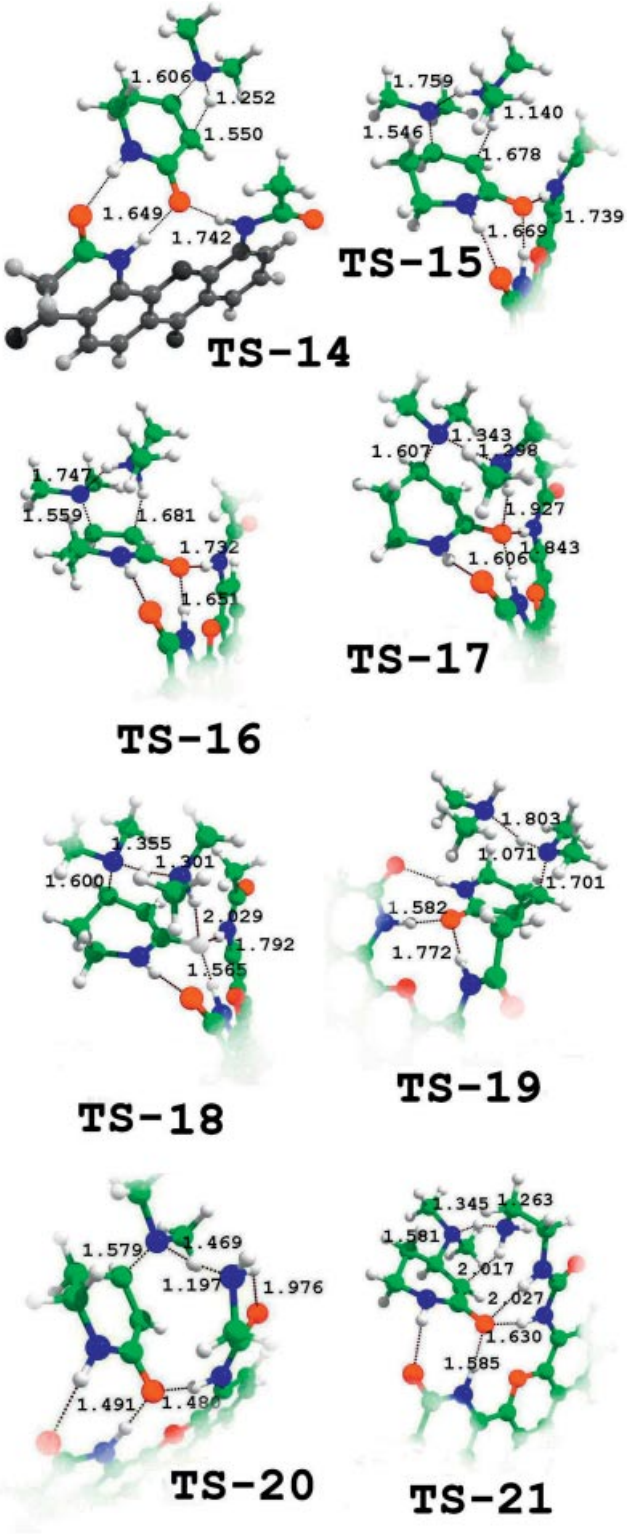


Figure 8. Top: transition state structures obtained for the addition of dimethylamine to the unsaturated valerolactam catalysed by a simplified model of receptor 1. For TS-14, the atoms included in the low-level layer in the ONIOM scheme are in grey. Bottom: Transition state structures obtained for the addition of dimethylamine to the unsaturated valerolactam catalysed by an α-amino acid and an ethylenediamine derivative receptor (TS-20 and TS-21).

Application of this method yielded six different transition state structures for the addition of dimethylamine to the unsaturated valerolactam catalysed by an acetamide xanthone derivative used as a simplified model of receptor 1 (Figure 8). These transition state structures variously correspond to a unimolecular mechanism in amine (TS-14), 1,2-addition (TS-15, TS-16), 1,4-addition (TS-17, TS-18) and a proton-slide mechanism (TS-19). Energy barriers, referenced to the complex between the simplified receptor and the lactam, are summarized in Table 8. The calculations indicated that 1,4-addition is disfavoured in the presence of the receptor and, unlike in the uncatalysed reaction, the proton-slide mechanism shows a smaller energy barrier. The difference with respect to the ΔG energy of TS-12 (the most energetically favoured transition state structure for the uncatalysed reaction) is also included in Table 8. In this regard, the $k_{cat}/k_{uncat} = 10^{2.76}$ value obtained for receptor 1 corresponds to $\Delta\Delta G = 3.81$ kcal mol^{-1}, which implies that our calculations overestimate the transition state stabilization by around 1.5 kcal mol^{-1}.

Table 8. Energy barriers (ΔE), zero-point energy barriers ($\Delta z.p.E$), free energy barriers at 298.15 K (ΔG) and difference in energy barriers ($\Delta\Delta G$) for the structures shown in Figure 8. Energy units are kcal mol^{-1}, $\Delta\Delta G$ are referenced to the ΔG energy of TS-12.

Structure	ΔE	$\Delta z.p.E$	ΔG	$\Delta\Delta G$
TS-14 (unimolecular in amine)	35.87	35.27	46.95	3.20
TS-15 (1,2-addition)	20.49	22.53	44.93	5.22
TS-16 (1,2-addition)	20.06	22.35	45.14	5.02
TS-17 (1,4-addition)	26.24	27.66	50.38	–0.21
TS-18 (1,4-addition)	27.98	29.21	51.87	–1.71
TS-19 (proton-slide)	19.02	22.79	44.91	5.25

The same search for transition-state structures was repeated for the reaction catalysed by a glycine-substituted receptor, used as a model for α-amino acid receptor derivatives. The proton-slide mechanism (TS-20) is the most energetically favoured; it is conceivable that an intramolecular H-bond between ammonium N and the O in the carboxamide (see Figure 8) might be responsible for further stabilization of this structure. Free energy barriers at 298.15 K are shown in Table 9. Other disfavoured transition state structures corresponding to 1,2- and 1,4-mechanisms are included in the supporting information.

Molecular modelling of the complete structures of α-amino acid derivative receptors is a time-consuming procedure. Instead, to estimate their catalytic activity we decided to develop a simple and fast procedure that could be applied to a large array of candidates in a reasonable period of time.

A detailed description of this procedure is included in the supporting information. Our approximation considers that the positions of all the atoms in transition state structures remain constant in all the amino acid derivatives, neglecting the possibility of a distortion of the positions of the atoms with respect to the positions in the glycine model receptor as those atoms "accommodate" to the new ones. This crude approximation was taken in view of the need to minimize computational costs. The rest of the atoms are

Table 9. ΔG values for the simplified glycine model receptor and the estimated (est.) ΔG for the α-amino acid derivative receptors 2–6 (above) and for an ethylenediamine receptor and estimated (est.) ΔG for urea-derived receptors 7–10. Energy units are kcal mol^{-1}.

Structure	ΔG	ΔG est. 2	ΔG est. 3	ΔG est. 4	ΔG est. 5	ΔG est. 6
TS-20 (proton-slide)	43.45	42.78	40.42	44.72	43.85	52.712
Structure	ΔG	ΔG est. 7	ΔG est. 8	ΔG est. 9	ΔG est. 10	
TS-21 (1,2-addition)	33.20	35.35	42.3	50.85	39.03	

optimized by using a cheap semiempirical level of theory. An ONIOM-like scheme for calculating the resulting electronic energy was applied, and the entropies associated with frozen rotations in the transition state structures were estimated by means of an approximate model.

The results are summarized in Table 9. As can be observed, the predicted free energy barrier for receptor 6 is higher than the free energy barrier observed for bimolecular mechanisms (such as TS-15, TS-17 or TS-19), indicating (in good agreement with the experimental results) that the amino groups in these receptors are probably not efficient in the proton-transfer step. For the other amino acid-derived receptors, although this method predicts a stronger stabilization of the transition state energy barriers than those observed experimentally, good qualitative results are obtained. For all the amino acid derivatives, the most favoured mechanism is the proton-slide mechanism.

In the ethylenediamine urea model, the NH of the urea group was able to establish a third H-bond with the lactam carbonyl oxygen, which further stabilized the transition state. The procedure described for α-amino acid derivatives was also used in the ethylenediamine urea. In this set of receptors, a stronger deviation between the calculated and the experimentally measured catalytic activities was observed. Nevertheless, as in the case of receptors derived from amino acids, the computational procedure was able to sort the catalytic activities of the receptors. In the case of receptor 9, for which no additional catalytic activity was observed, due to the presence of the amino group, the method predicted a very high activation free energy. This can be explained by considering that this receptor must adopt a very disfavoured conformation in order to fit the transition state.

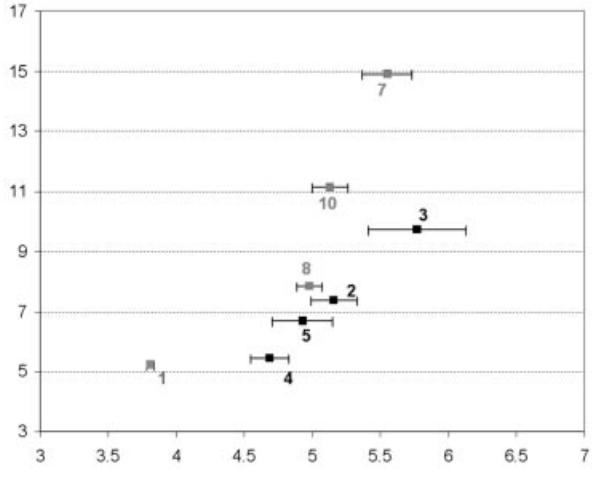


Figure 9. Graphic plot of experimentally determined (x axis) vs. calculated (y axis) $\Delta\Delta G$ values for receptor 1, receptors derived from α-amino acid (2–5), and ethylenediamine urea receptors (7–10).

Figure 9 shows a plot of the calculated vs. the experimentally determined k_{cat}/k_{uncat} values for the receptors (excluding receptors 6 and 9). Although our calculations are not able to sort the catalytic activity of receptors belonging to different sets correctly, it is possible to use a simple procedure such as the one reported to obtain a qualitative description of the catalytic activity of each receptor within each set of receptors. It is also interesting to bear in mind that a marked deviation was observed for the ethylenediamine-derived catalysts. The activation free energy calculated for this receptor was obtained by the ab initio procedure, so it is very likely that the level of theory used in the ab initio calculation would overestimate the effect of the third H-bond formed between the urea NH and oxygen atom in the lactam.

Conclusions

The mechanism of the conjugate addition of pyrrolidine to the α,β-unsaturated valerolactam has been studied both by kinetic experiments and by computational methods. The information obtained from these studies indicates that receptors combining oxyanion hole mimics with amino groups are promising candidates for obtaining good artificial enzymes for this reaction.

The catalytic activities of two different sets of receptors, derived from α-amino acids and from ethylenediamine ureas, have been studied, with the observation of increases in the reaction rates close to typical values observed for catalytic antibodies. Moreover, the catalytic activities of this set of receptors have been studied theoretically, and a simple computational method able to rank the receptor candidates qualitatively by their catalytic activity is described.

Experimental Section

The experimental procedures for the preparation of the unsaturated valerolactam and receptors 1–10 can be found in recent publications.[14,16] The unsaturated 5-methylvalerolactam was prepared according to the literature.[16]

Kinetics experiments were performed in benzene at 298 K with a lactam concentration of 0.8 M. A high concentration of pyrrolidine (3.0 M) was used to prevent catalytic activity by the reaction product, which could have complicated the interpretation of the process under study. Under these conditions, the half-life of the reaction in

the absence of catalyst was 2385 min. Receptors were added at 0.04 M concentration and the lactam concentration was deduced from the ^{1}H NMR spectra at different times.

Supporting Information (see also the footnote on the first page of this article): Description of quantum chemistry calculations, Cartesian coordinates of all structures optimized, colour figures of structures optimized, determination of rotational entropy for substituted receptors, kinetic experiments, competitive titration plots, DOSY experiments with receptor 3, determination of the absolute stereochemistry of the Michael addition of pyrrolidine to methylvalerolactam.

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