Molecular Recognition

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Enantioselective Cooperativity Between Intra-Receptor Interactions and Guest Binding: Quantification of Reinforced Chiral Recognition**

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Cooperativity is one of the most fundamental concepts in molecular recognition and supramolecular self-assembly.[1] It explains how the behavior of a system as a whole is different from what is expected from every single isolated interaction and it lies at the heart of the development and regulation of collective properties in complex chemical and biological systems.[2–4] Cooperativity is considered positive if one interaction is favored by another and negative if it is disfavored. A very intriguing kind of positive cooperativity fairly frequent in proteins arises from the synergism between direct receptor–substrate interactions and intra-receptor interactions, also called primary and secondary interactions, respectively.[5] It is noteworthy that intra-receptor interactions are not directly involved in the binding event. Nonetheless they do affect the conformational landscape of the receptor and can impose the same local conformational restrictions as guest binding. If such is the case, then the adverse entropic cost of the binding is shared between secondary and primary interactions and therefore binding is enhanced.[6,7] Otto has proposed the term reinforced molecular recognition for the enforcement of guest binding by interactions within the receptor.[8] Both sets of interactions positively cooperate and therefore they are mutually reinforced.[9] However there are only a few reported precedents of reinforced molecular recognition in synthetic systems,[10] probably because it is hard to anticipate the effect, direction, and magnitude of the secondary interactions, thus making it tricky to design reinforced receptors. Moreover, chirality has not even been considered, although it is clear that reinforcement could be enantioselective, provided that at least one of the reinforced primary noncovalent interactions discriminates between both enantiomers of the guest. However, as far as we are aware, reinforced chiral recognition has not yet been reported. We describe herein a simple synthetic receptor that displays an enantioselective positive cooperativity between intra-receptor interactions and guest binding which leads to a reinforcement of the chiral recognition.

We have recently reported[11] how the high enantiodiscrimination displayed by receptor **1** (Scheme 1a) with aromatic amino acid ammonium salts is mainly caused by a single CH–π bond.[12] The free receptor is highly flexible and it can fold to one side or the other, thus generating two equivalent sets of folded conformers where the carbonyl groups are forced into a transoid conformation, one outside and the other inside the cavity (Scheme 1b).[13] Upon binding an amino acid, the receptor adopts again a folded conformation and one of the hydrogen atoms at position 13 interacts exclusively with the aromatic residue of the D enantiomer of the amino acid (Scheme 1c). The folded geometry seems to be relevant in the free state and in the complex, and therefore it is reasonable to think that folding of the receptor favors guest binding.[14] Folding is induced, among other things, by intra-receptor noncovalent interactions that could assist the binding event through two different mechanisms: either preorganizing the receptor or by cooperativity between secondary and primary interactions. However receptor **1** lacks any easily measured intra-receptor noncovalent interactions to help verify which of the mechanisms is taking place. Thus receptor **2** was preferred for the present work (Scheme 1d). Such a receptor incorporates an intra-receptor hydrogen bond that is not directly involved in guest binding and could favor the appropriate transoid folded conformations required for complexation.[15] The advantage of this system is that the secondary hydrogen bond can be qualitatively measured and therefore it can be confirmed whether secondary interactions are either preorganizing the system or reinforcing the primary interactions. Receptor **2** is perfectly suited for such a task as the effect of the secondary hydrogen bond can be cancelled out just by methylation. Indeed, receptor **3** displays a methoxy group which is unable to act as a hydrogen-bond donor (Scheme 1d). Moreover methoxy and hydroxy groups have identical Hammett σ_{meta} values ($\sigma_{meta} = +0.12$),[16] which ensures us that the electronic effects on the binding ability of the nitrogen atom of the pyridine are the same. It is well known that a single and subtle chemical modification can alter the conformational landscape even in complex biological molecules and therefore perturb their function.[17] Herein a single methylation is used in the same way, that is, to selectively favor or disfavor certain conformations and therefore tune the function of the system.

Association constants of both enantiomers of the picrate salts of an aliphatic ($LeuOMe^+$)[18] and an aromatic ($TrpOMe^+$) methyl ester with receptors **2** and **3** were determined (Tables 1 and 2). The effect of methylation on the D-$TrpOMe^+$ affinity is outstanding: The association

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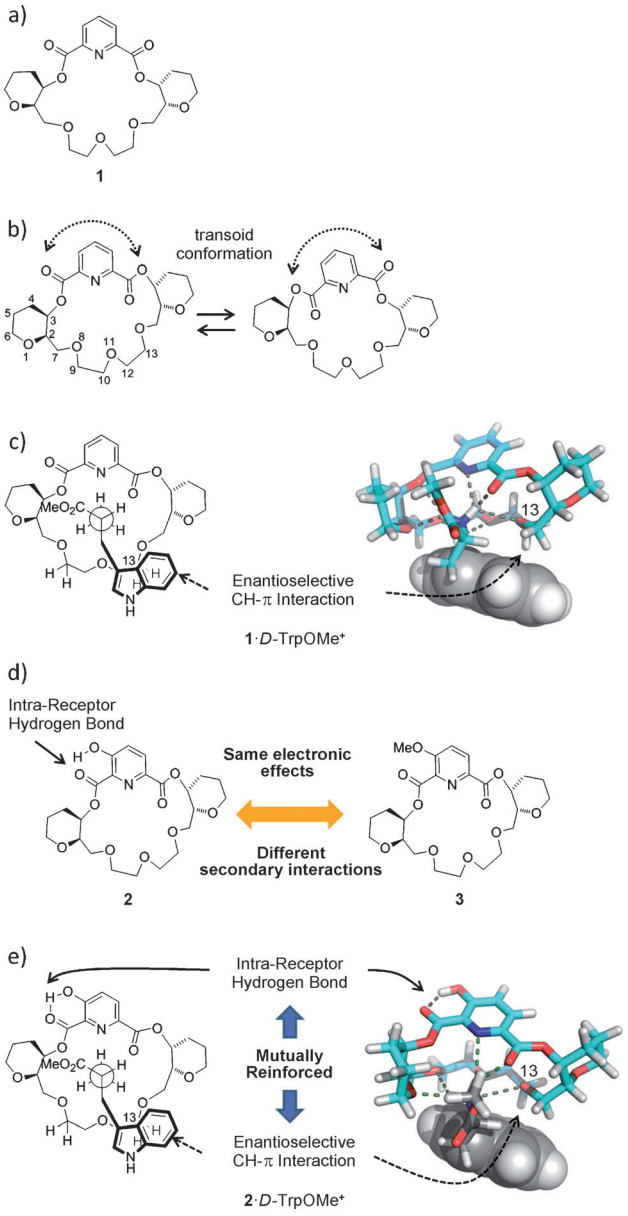


Scheme 1. a) Chemical structure of receptor **1**. b) Dynamic equilibrium between two sets of equivalent folded conformers of receptor **1**. c) The **1**·D-TrpOMe$^+$ complex is also folded and the CH–π interaction exclusively formed with the D enantiomer of aromatic guests plays a key role on chiral discrimination. d) Receptors **2** and **3** are employed to verify the presence of cooperativity; the electronic effects remain the same whilst a different pattern of secondary interactions is displayed. e) Enantioselective cooperativity arises from the interplay of the secondary hydrogen bond within receptor **2** and the CH–π interaction between host and guest. C cyan; H white; N blue; O red; indole groups are shown in grey and hydrogen bonds in green.

constant drops enormously upon methylation, and thus receptor **2** displays an association constant with D-TrpOMe$^+$ of more than 12 times higher than that of receptor **3** (Table 1, entries 1 and 2). For the rest of the guests the effect of methylation is much smaller. There is only a small drop in the association constants for L-TrpOMe$^+$ (1.99 times; entries 3 and 4), and the decrease in the associatetion constants for

Table 1: Association constants (K_a) for complexation of the hosts with chiral organic ammonium picrate salts in $CHCl_3$ at 298 K and the ratio of the association constants of receptor **2** versus receptor **3** (K_{OH}/K_{OMe}) for every guest.

Entry	Host	Guest[a]	K_a [M^{-1}][b][c]	K_{OH}/K_{OMe}
1	**2**	D-TrpOMe$^+$	40390 ± 1275	12.43
2	**3**	D-TrpOMe$^+$	3250 ± 9	
3	**2**	L-TrpOMe$^+$	5370 ± 239	1.99
4	**3**	L-TrpOMe$^+$	2705 ± 116	
5	**2**	D-LeuOMe$^+$	31720 ± 1322	1.37
6	**3**	D-LeuOMe$^+$	23100 ± 777	
7	**2**	L-LeuOMe$^+$	17200 ± 739	1.31
8	**3**	L-LeuOMe$^+$	13120 ± 586	

[a] The anion is always picrate. [b] The association constants were determined on the basis of differential UV/Vis spectroscopy at three wavelengths (λ = 380, 385, and 390 nm) by the typical nonlinear least squares method (1:1 simulation). [c] These values are the average of at least three independent measurements.

both enantiomers of LeuOMe$^+$ is almost the same (1.37 and 1.31 for the D and L enantiomers, respectively; entries 5–8).[19]

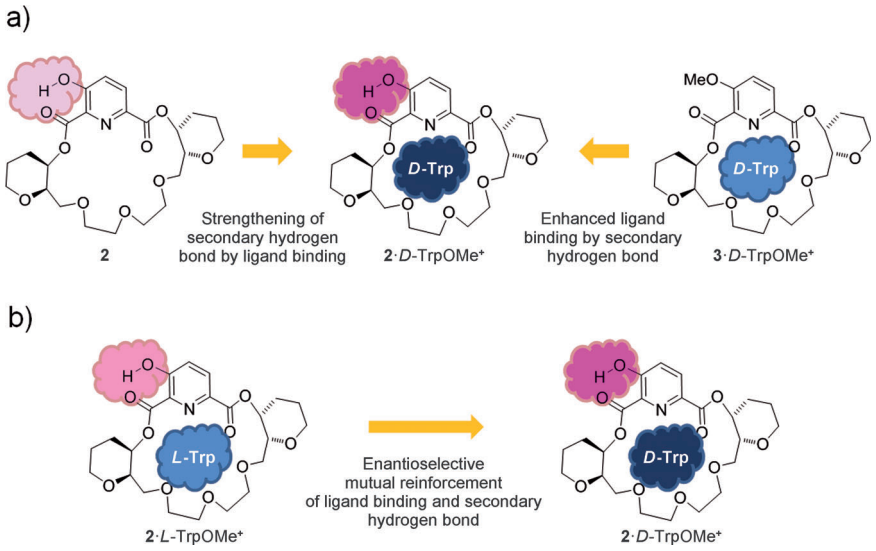
The effect of methylation on the enantiodiscrimination is also notable (Table 2). Thus, although the ability to discriminate between both enantiomers is almost unaffected by methylation when LeuOMe$^+$ is the guest, it drops more than six times for TrpOMe$^+$ upon methylation.

Table 2: Enantioselectivity (K_D/K_L), ratio between enantioselectivities of both receptors ($(K_D/K_L)_{OH}/(K_D/K_L)_{OMe}$), Gibbs free-energy of association ($-\Delta G_a$ in kJ mol^{-1}), and $\Delta\Delta G_a$ calculated from $-\Delta G_a$ for complexation of the hosts with chiral organic ammonium picrate salts in $CHCl_3$ at 298 K.

Host	Guest[a]	K_D/K_L	$(K_D/K_L)_{OH}/(K_D/K_L)_{OMe}$	$-\Delta G_a$ [kJ mol^{-1}]	$\Delta\Delta G_a$
2	D-TrpOMe$^+$	7.52	6.27	26.28 ± 0.08	5.00
2	L-TrpOMe$^+$			21.28 ± 0.11	
3	D-TrpOMe$^+$	1.20		20.04 ± 0.01	0.46
3	L-TrpOMe$^+$			19.58 ± 0.11	
2	D-LeuOMe$^+$	1.84	1.05	25.68 ± 0.10	1.52
2	L-LeuOMe$^+$			24.16 ± 0.11	
3	D-LeuOMe$^+$	1.76		24.90 ± 0.08	1.41
3	L-LeuOMe$^+$			23.49 ± 0.11	

[a] The anion is always picrate.

Such evidence of the effect of a secondary hydrogen bond on the binding ability of the receptor (Scheme 2a, right) is not enough to claim a cooperative mechanism between the binding and the secondary hydrogen bond. The opposite has also to be confirmed, that is, a strengthening of the secondary hydrogen bond upon binding (Scheme 2a, left).[20] Traditionally in proteins, a reduced rate of H/D exchange of amide protons upon guest binding is taken as definitive evidence of increased strength in secondary hydrogen bonds.[21,22,23] However in receptor **2** H/D exchange is too fast to be monitored accurately by NMR spectroscopy, even at low temperature, and therefore it is not possible to measure the rate of H/D exchange upon guest binding; this is probably due to the high acidity of the phenolic proton and the fact that it is exposed to the solvent. Nevertheless another good probe to follow the

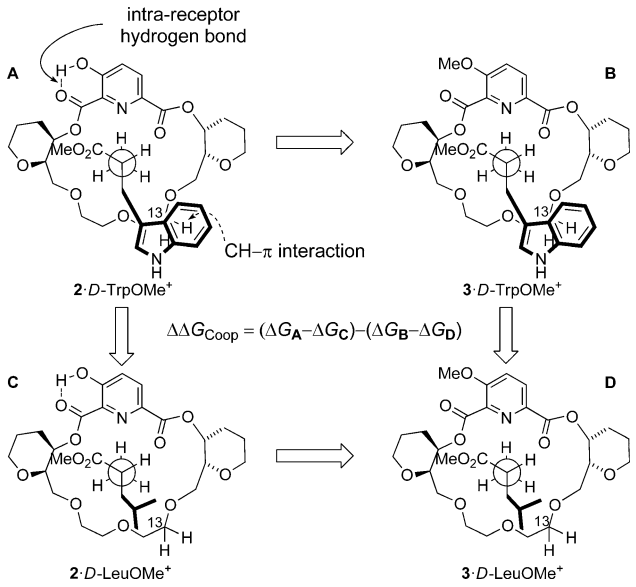


Scheme 2. Cooperativity between intra-receptor interaction and guest binding. The colored clouds symbolize an interaction: The darker the shade of color the stronger the interaction. a) Reinforced molecular recognition. The binding event of receptor **2** induces a strengthening of the secondary hydrogen bond. The presence of the secondary hydrogen bond in **2** induces an enhancement of the guest binding with respect to **3**, which is not able to form such a secondary interaction. Both behaviors converge on the **2**·D-TrpOMe$^+$ complex, thus supporting a reinforced molecular recognition event. b) Reinforced chiral recognition. Swapping from the L enantiomer of the guest to its antipode induces a strengthening of the secondary noncovalent interaction and a higher association constant.

intensification of the indicated hydrogen bond is the ^{1}H NMR chemical shift of the hydroxy proton.[24] Indeed, at the same temperature such a proton shifts downfield upon complexation with D-TrpOMe$^+$ ($\delta_{Free} = 11.18$ ppm; $\delta_D = 11.34$ ppm, both at 273 K), which suggests strengthening of the hydrogen bond in the complex with respect to the free receptor. Even better evidence comes from the temperature coefficients ($\Delta\delta/\Delta T$) for the hydroxy proton.[24,25] Thus a small value of $\Delta\delta/\Delta T$ has been related to protons completely locked into an intramolecular hydrogen bond.[26] The free receptor **2** shows a noticeable shift in the signal for its hydroxy proton as the temperature decreases, but in the complex with D-TrpOMe$^+$ the shift is much smaller,[13] which implies a much stronger hydrogen bond in the complexed receptor. Indeed $\Delta\delta/\Delta T$ was measured within the same temperature range (273 K–223 K) for the free receptor and its D-TrpOMe$^+$ complex to estimate the strengthening of the hydrogen bond upon guest binding. The value of $\Delta\delta/\Delta T$ of the free receptor **2** (-4.15 ppb K^{-1}) is almost three times higher than the value obtained for the complex (-1.41 ppb K^{-1}), which supports the presence of a stronger hydrogen bond in the complex. These results support a cooperative mechanism and exclude preorganization, because it is evident that in the free receptor **2** the secondary hydrogen bond is not fully formed and only upon guest binding does it get locked. Also notable is the downfield shift of the signal for the hydroxy proton of the free receptor as the temperature decreases, such that the shift is getting closer to the δ value of the complex. Such behavior not only provides evidence that the hydrogen bond is almost completely formed and locked for both the **2**·D-TrpOMe$^+$ complex and the cold, free receptor, but also that cooling has a similar effect as does guest binding upon the strengthening of certain secondary noncovalent interactions; such an effect has been reported before to explain reinforcement in proteins.[27]

All the data discussed thus far confirm a reinforced molecular recognition event (Scheme 2a), but additional analyses that take into account both enantiomers of the guest were carried out to prove the reinforced chiral recognition (Scheme 2b). From the NMR experiments it can be seen that at the same temperature (253 K) the chemical shift of the hydroxy proton in the complex of receptor **2** with D-TrpOMe$^+$ ($\delta_D = 11.37$ ppm) is shifted downfield with respect to that of the L-TrpOMe$^+$ complex ($\delta_L = 11.18$ ppm).[28] More revealing are the temperature coefficients ($\Delta\delta/\Delta T$) for the hydroxy proton within the same temperature range[29] (253 K–223 K). The temperature coefficient is greater in the L-TrpOMe$^+$ complex with **2** (-2.53 ppb K^{-1}) than in the D-TrpOMe$^+$ complex with **2** (-1.68 ppb K^{-1}), and both smaller than $\Delta\delta/\Delta T$ of the free receptor **2** (-3.35 ppb K^{-1}) within the same temperature range. These results imply that the secondary hydrogen bond in **2** is strengthened upon binding of L-TrpOMe$^+$ although to a smaller degree than that seen in the binding of D-TrpOMe$^+$. In summary, the absence of a secondary hydrogen bond makes the binding affinity and chiral discrimination smaller, and at the same time the strengthening of the intra-receptor hydrogen bond is much more evident with the binding of the D enantiomer. All the above-mentioned data provides good evidence for mutual enantioselective reinforcement of the CH–π interaction and the secondary hydrogen bond, and therefore supports the reinforced chiral recognition (Scheme 2b).

Finally a quantification of the reinforcement was performed through a thermodynamic double-mutant cycle (Scheme 3).[30,31] Two changes (mutations) on the original 2·D-TrpOMe$^+$ complex were performed, both independently and simultaneously, and therefore four complexes were compared: 1) the original **A** where all the interactions are present, two complexes (**B** and **C**) in which one interaction has been removed, and the double-mutant **D** where both interactions have been removed. If the change in free energy of upon removing the CH–π interaction from the complex ($\Delta\Delta G_{OH/Trp\rightarrow OH/Leu} = \Delta G_{\mathbf{A}} - \Delta G_{\mathbf{C}}$) differs from the free-energy change arising from the removal of both the CH–π interaction and the secondary hydrogen bond ($\Delta G_{OMe/Trp\rightarrow OMe/Leu} = \Delta G_{\mathbf{B}} - \Delta G_{\mathbf{D}}$), then there must be a cooperative relationship between both interactions. Thus, the difference between both energies [$\Delta\Delta G_{Coop} = (\Delta G_{\mathbf{A}} - \Delta G_{\mathbf{C}}) - (\Delta G_{\mathbf{B}} - \Delta G_{\mathbf{D}})$] provides a measure of the cooperativity or in other words, a quantification of the reinforcement of the CH–π interaction by the



Scheme 3. Schematic representation of the chemical double-mutant cycle used to quantify the cooperativity between the intra-receptor hydrogen bond and the intermolecular CH–π interaction.

secondary hydrogen bond (and vice versa). However two conditions must apply for the double-mutant cycle to be valid: 1) mutations do not interact and 2) the geometry of all the complexes is similar. The first condition is met as the methoxy group cannot be the donor of a hydrogen bond and D-Leu lacks the aromatic ring required for the CH–π interaction to occur.[32] Additionally, the geometry of all the complexes in solution was proven by NMR experiments to be quite similar.[13]

The final result of the calculation yields a notable $\Delta\Delta G_{Coop} = (-5.46 \pm 0.15)\ kJ\,mol^{-1}$, which explains the much better performance of the receptor **2** relative to receptor **3** regarding D-TrpOMe$^+$. It also explains why the nonaromatic guest does not show any remarkable response to the suppression of the secondary hydrogen bond, as they lack the CH–π interaction which would have been reinforced. Finally, as the reinforced CH–π bond is exclusively formed with the D enantiomer, it can be actually considered a reinforced chiral recognition.

The behavior of the receptor **2** proves that reinforced molecular recognition can be achieved by much simpler systems than was initially thought. In addition it can also be concluded that reinforcement is not only a valid tool to improve receptors but also for favoring chiral discrimination, thus paving the way to a novel concept for asymmetric catalysis.[33,34] It is worth mentioning that up until now all the reported methods for improved chiral recognition are based either on a more rigid receptor or on bulkier chiral auxiliaries. The approach described herein is more subtle: remote chemical modification tunes the conformational landscape of the receptor and induces a positive cooperativity between secondary and primary interactions, and provided that at least one of the primary interactions is enantioselective, chiral discrimination will be reinforced. Finally, the work presented herein shows how apparently subtle differences between two systems can be easily overlooked, even though they can also deeply affect the behavior of such systems. Intra-receptor interactions must be taken into account to correctly analyze the cooperativity even in simple synthetic systems.

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[19] The receptor **2** is better than **3** probably because of small electronic and steric effects of the substituent.
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[28] It is worth mentioning that such a hydroxy proton signal from the **2**·L-TrpOMe$^+$ complex is not shifted downfield relative to that of the free receptor, which excludes any inductive effect on the chemical shift of the hydroxy proton caused by pyridine complexation.
[29] The temperature range is different because complexes of the receptor **2** are in fast equilibrium with the uncomplexed species on the NMR time scale. Chemical exchange is observed between the hydroxy proton and the ammonium of the guest and therefore no hydroxy signal is distinguished at room temperature. Only at low temperature is the hydroxy peak perceptible. However the temperature at which this occurs for the D-TrpOMe$^+$ complex is different than that of the L-TrpOMe$^+$ complex. Thus the temperature range has to be chosen adequately (see the Supporting Information).
[30] It has been reported that there are triple-mutant boxes for the evaluation of cooperativity, however they not only involve a higher chance of experimental error because eight different complexes have to be measured, but they also are not even required as cooperativity can be quantified by a double-mutant cycle. See: a) C. A. Hunter, S. Tomas, *Chem. Biol.* **2003**, *10*, 1023 – 1032; b) S. L. Cockroft, C. A. Hunter, *Chem. Soc. Rev.* **2007**, *36*, 172 – 188.
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