Noncovalent Interactions

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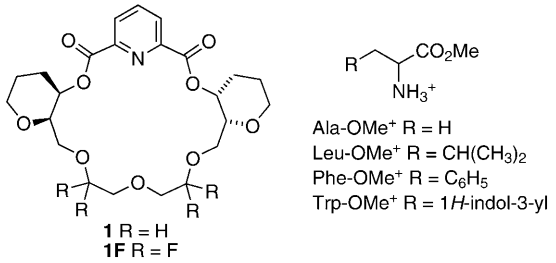
Quantification of a CH–π Interaction Responsible for Chiral Discrimination and Evaluation of Its Contribution to Enantioselectivity**

*Romen Carrillo, Matías López-Rodríguez, Victor S. Martín, and Tomás Martín**

Dedicated to Professor Julius Rebek, Jr. on the occasion of his 65th birthday

The CH–π interaction, in which the CH group is aliphatic, is one of the weakest known interactions. However, this interaction is very important,[1] as it contributes significantly to the overall stability of protein structures,[2] the selective recognition and binding affinity between proteins and ligands,[3] the conformational preference of DNA,[4] the stereoselectivity of organic reactions,[5] and molecular recognition.[6] Therefore, the measurement of its strength and scope is very important. Such CH–π interactions have been investigated qualitatively through protein mutation studies,[7] IR[8] and NMR[9] spectroscopy, X-ray crystallographic analysis,[10] and by computational methods.[11] However, the quantification of such a weak interaction is usually not easy and thus there are only a few reports of studies in this area.[12] Remarkable contributions to this field are Wilcox's torsion balance,[13] the carbohydrate–π interaction within a β-hairpin-peptide[14] and dangling-ended DNA[15] model systems, and cyclohexylphenyl recognition in the center of a DNA duplex.[16] It is even more difficult to evaluate the contribution of a single noncovalent interaction that is involved in a specific biological or chemical event, particularly for the weak interactions. Therefore, the development of simple molecular models in order to assess the contribution of a CH–π interaction in chiral recognition processes is extremely important. Herein, we show the key role of a CH–π interaction in a remarkably high chiral discrimination displayed by a new synthetic receptor. Furthermore, we use this new receptor as a useful model for the quantification of the interaction and for an unprecedented evaluation of its contribution to the whole chiral recognition process.

During our search for new chiral cation receptors using the *cis*-2-oxymethyl-3-oxy-tetrahydropyran unit as a key motif,[17] we found that receptor **1**[18] displayed high association constants with ammonium salts of α-amino acid methyl esters (Scheme 1).[19] The association constants were distinctively higher with the D enantiomers of the amino acids, and chiral discrimination was conserved using either different solvents or different anions (Table 1 and Table S1 in the Supporting Information). Particularly high enantioselectivity was shown for those amino acids that bear aromatic side chains. Tryptophan (Trp), which has the most effective donor aromatic side chain, displays the highest association constant for the D enantiomer and the best chiral discrimination, which reached values of up to $K_D/K_L = 33.78 \pm 0.90$ ($\Delta\Delta G_0 = (8.72 \pm 0.07)$ kJ mol^{-1}) for Trp-OMeNO_3 in CD_3CN (Table S1 in the Supporting Information). These data suggest that the aromatic side chain is likely to be involved in the origin of the enantioselectivity.[20]



Scheme 1. Chemical structures of receptors **1**, **1F**, and the ammonium salts of α-amino acid methyl esters.

To gain insight into the causes of this remarkably high chiral recognition, a detailed NMR-based study of the geometry of the complexes in solution was carried out. Complexation-induced chemical shifts (CISs) derived from ^{1}H NMR titration of the receptor **1** with both enantiomers of alanine (Ala), leucine (Leu), phenylalanine (Phe), and tryptophan picrate (Pic) salts[21] in CD_3CN were analyzed, and a great deal of information about their binding was obtained (Figure 1). The signals for protons in positions 3, 6 (equatorial), 7, 11, and 12 are shifted markedly downfield

[*] Dr. T. Martín
Instituto de Productos Naturales y Agrobiología, CSIC
Francisco Sánchez, 3, 38206 La Laguna, Tenerife (Spain)
Fax: (+34) 922-260-135
E-mail: tmartin@ipna.csic.es
Dr. R. Carrillo, Prof. Dr. M. López-Rodríguez, Prof. Dr. V. S. Martín, Dr. T. Martín
Instituto Universitario de Bio-Orgánica "Antonio González"
Universidad de La Laguna
Francisco Sánchez, 2, 38206 La Laguna, Tenerife (Spain)

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Table 1: Association constants (K_a), enantioselectivity, Gibbs free-energy changes ($-\Delta G_0$), and $\Delta\Delta G_0$ calculated from $-\Delta G_0$ for the complexation of the host **1**.

Guest (G^+Pic^-)	K_a [M^{-1}][b,c]	K_D/K_L[d]	$-\Delta G_0$ [kJ mol^{-1}]	$\Delta\Delta G_0$
D-Ala-OMe$^+$	25990 ± 1050	1.80	25.19 ± 0.10	1.46
L-Ala-OMe$^+$	14410 ± 990		23.73 ± 0.17	
D-Leu-OMe$^+$	29100 ± 950	3.32	25.47 ± 0.08	2.97
L-Leu-OMe$^+$	8760 ± 220		22.50 ± 0.06	
D-Phe-OMe$^+$	30230 ± 2100	4.55	25.56 ± 0.17	3.75
L-Phe-OMe$^+$	6640 ± 680		21.81 ± 0.25	
D-Trp-OMe$^+$	76190 ± 6260	10.39	27.85 ± 0.20	5.80
L-Trp-OMe$^+$	7330 ± 230		22.05 ± 0.08	

[a] Recorded in $CHCl_3$ at 298 K. [b] The association constants were determined on the basis of differential UV/Vis spectroscopy at three wavelengths (380, 385, and 390 nm) by the typical nonlinear least-squares method (1:1 simulation) and the uncertainty is given by the standard deviation. [c] These values are the average of at least three independent measurements. [d] K_D/K_L = enantioselectivity.

compared with their respective chemical shifts in the spectrum of free receptor **1**. These shifts imply that O-1, O-8, the oxygen atom from the ester, and the nitrogen atom from pyridine participate in the complex formation.[22] Also, the downfield shifts observed for the pyridine proton signals in all complexes indicates that the aromatic residue in the amino acids that bear an aromatic side chain (indole for Trp or phenyl for Phe) does not overlap the pyridine ring. Thus, the possibility of a π–π interaction between the aromatic side chain and the pyridine on the receptor was discarded. Conversely, significant upfield shifts of protons 9 and 10 from the diethyleneglycol spacer can be observed, and these chemical shifts are quite similar for both enantiomer of all guests except from complexes with D-Phe-OMe$^+$ and D-Trp-OMe$^+$, which showed larger upfield shifts than the rest of the complexes for these proton signals (Figure 1d,e).

This behavior can easily be explained when it is considered that the complexed structure of **1** is folded. Indeed, the X-ray structure of receptor **1** shows that it is already folded in its free form (Figure 2).[23] Several important features can be found by analyzing this structure. Firstly, the *cis* tetrahydropyran units are in the optimal conformation for complexation (with the 3-oxy group in the axial position).[17] Secondly, one of the carbonyl groups is directed outward from the cavity and the other is directed inward. As a consequence of this orientation, the pyridine ring is quite close to the diethylenglycol spacer. Finally, the distance between one proton in position 10 and the pyridine aromatic plane is around 2.73 Å. This observation clearly indicates the presence of an intramolecular CH–π interaction in the solid phase.[24] Additionally, the temperature-dependent ^{1}H NMR spectrum of the complex **1** with D-Trp-OMePic in $CDCl_3$ was also recorded (Figure S1 in the Supporting Information). At room temperature, some signals are broad, which suggests a dynamic effect. However, at low temperatures, these signals split into two peaks of equal intensity, which indicates a slow intermolecular face-to-face guest exchange on the NMR timescale.[18] In order to assign all the signals, COSY, HSQC, and HMBC experiments were performed at 223 K, and 1D and 2D ROESY were performed at 223 K. The intramolecular ROEs were observed between protons in positions 10 and 10′ with pyridine protons 11 and 11′, respectively. The ROE cross peak between pyridine proton 11′ and axial proton 5′ is in agreement with the folded structure found in the X-ray crystal structure of the free receptor **1** (Figure 3a,b), which is quite similar to the structure in solution of its complex with D-Trp-OMe$^+$. However, complexation induces conformational changes in the receptor **1**. The intramolecular ROEs between protons in positions 3 and 3′ with protons 7 and 7′, respectively, indicate that the oxygen

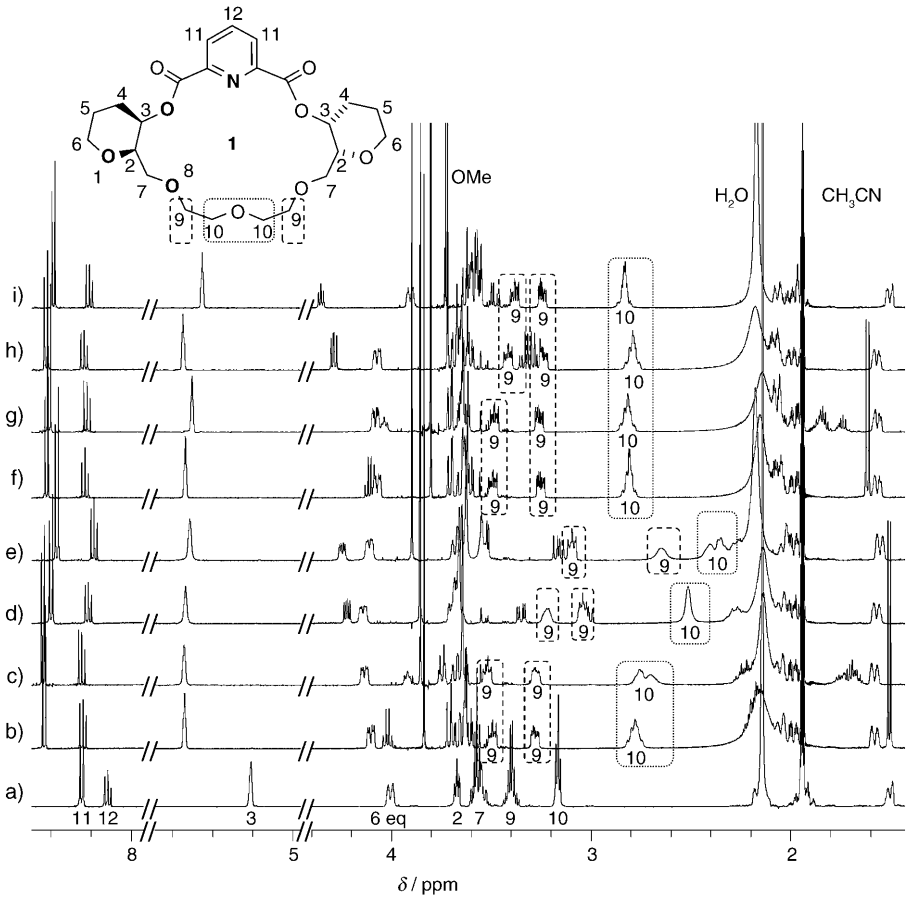


Figure 1. Sections of ^{1}H NMR spectra (500 MHz) of the complexes of host **1** with various chiral α-amino acid methyl ester ammonium picrate salts in CD_3CN (9.1 mM) at 298 K. a) Host **1**, b) D-Ala-OMePic·**1**, c) D-Leu-OMePic·**1**, d) D-Phe-OMePic·**1**, e) D-Trp-OMePic·**1**, f) L-Ala-OMePic·**1**, g) L-Leu-OMePic·**1**, h) L-Phe-OMePic·**1**, i) L-Trp-OMePic·**1**.

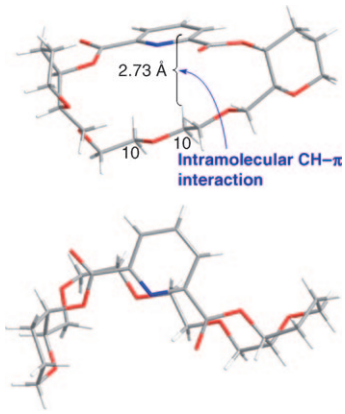


Figure 2. Front and top view of the X-ray structure of receptor **1**. C gray; H white; N blue; O red.

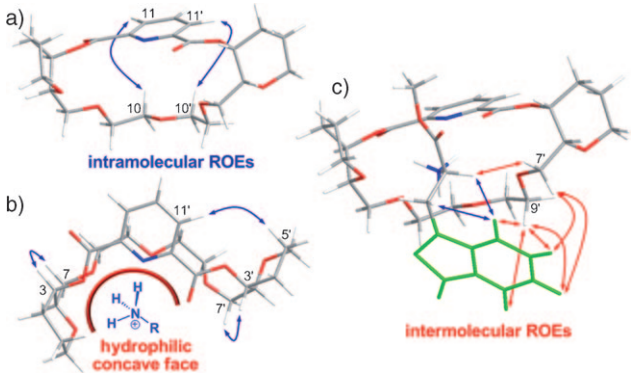


Figure 3. Three-dimensional structure of complex **1** with D-Trp-OMe$^+$ based on NMR studies. Intramolecular ROEs shown in blue and intermolecular ROEs shown in red. a) Front view without D-Trp-OMe$^+$, b) top view without D-Trp-OMe$^+$, and c) structure of complex **1** with D-Trp-OMe$^+$. The indole group is shown in green.

atoms in positions 8 and 8′ move into the cavity to create a hydrophilic concave face with six oxygen atoms and the nitrogen atom directed toward the center of the cavity (Figure 3b). This geometry is ideally suited for the complexation of ammonium salts and the folding of the receptor increases once the guest is complexed. This extra folding makes the pyridine ring lie closer to the diethylenglycol chain, and thus induces an upfield shift of these protons in all complexes. Moreover, the further upfield shifts for the D enantiomers of the amino acids that bear an aromatic side chain suggest that the phenyl and indole groups are situated over the diethylenglycol spacer. The intermolecular ROE effect on complex D-Trp-OMePic·**1** confirm that the indole group overlaps the diethyleneglycol spacer (Figure 3c). Additionally, in the ^{1}H NMR spectrum of the complex D-Trp-OMePic·**1** at 223 K (Figure S1 in the Supporting Information), the protons in positions 9 and 9′ are shifted upfield, which is consistent with the expected chemical shielding effect from the π electron clouds of the pyridine and the indole residue. Both protons in position 9 and one proton in position 9′ show a moderate shift ($\delta_9 = 2.78$ ppm, 3.29 ppm and $\delta_{9'} = 2.70$ ppm). A particularly remarkable upfield shift was observed for the other proton in position 9′ ($\delta =$ 1.35 ppm), which is the proton that displays intermolecular ROEs with the indole ring of the guest (Figure 3c). All the above data is clear evidence that this proton lies especially close to the indole group. Alternatively, the intramolecular ROEs in ROESY experiments in $CDCl_3$ at room temperature of complex L-TrpOMePic·**1** reveal a folded receptor geometry, which is similar to that found for complex D-Trp-OMePic·**1**, however, intermolecular ROEs were not observed.[18,22]

All these geometrical data suggest an appropriate conformation for a putative intermolecular CH–π interaction between one proton in position 9 and the aromatic side chain of Trp. The interaction occurs only in the D-enantiomer of the Trp, therefore it could be responsible for the great enantioselectivity displayed. Moreover, the chiral discrimination increased when the solvent was changed from $CHCl_3$ to CD_3CN (Table 1 and Table S1 in the Supporting Information). The biggest enhancement was for Phe-OMePic (2.54 times), whilst the enhancement was less than twofold for Trp-OMePic (1.73), Leu-OMePic (1.70), and Ala-OMePic (1.56).[25] These results support the putative intermolecular CH–π interaction. Based on the molecular surface electrostatic potential, the hydrogen-bond donor character for acetonitrile is less than that for chloroform, so acetonitrile is a less competitive solvent than chloroform for CH–π interactions.[26] A stronger interaction in acetonitrile for the D enantiomers of Phe-OMePic and Trp-OMePic, and therefore a better chiral recognition, is expected. Nonetheless, this behavior is only clearly observed for Phe-OMePic, which is in agreement with the presence of the best donor aromatic residue in the Trp-OMePic and therefore the CH–π interaction with the indole group is less sensitive to solvent changes.

However, further evidence is needed to support the existence of a putative intermolecular CH–π interaction. One such proof is to exchange the hydrogen atom thought to be involved in the interaction by a fluorine atom. The fluorine atom has a similar size to the hydrogen atom, so there will be no steric effects, but has a high electronegativity that inhibits the interaction with the aromatic chain.[27] With that aim in mind, we synthesized receptor **1F** in which the protons in position 9 had been replaced by fluorine atoms.[18] If our hypothesis of a CH–π interaction is correct, a decrease in chiral discrimination for **1F** would be expected. The association constants for this receptor are shown in Table 2.[28] At first glance, we can see how the value of the association constants decreased drastically as a consequence of the inductive effect exerted by the fluorine atoms on the adjacent oxygen atoms.[29] However, the most important information displayed in Table 2 is that, even though a slight preference for the D series still remained, the chiral discrimination was reduced for all substrates especially for tryptophan. We have verified that the structures of the complexes with the fluorinated receptor **1F** are quite similar to those found with receptor **1** (see below), thus, these results confirm that the CH–π interaction does not occur in the fluorinated receptor.

By considering all the above evidence, it can be confirmed that a CH–π interaction occurs between one proton in

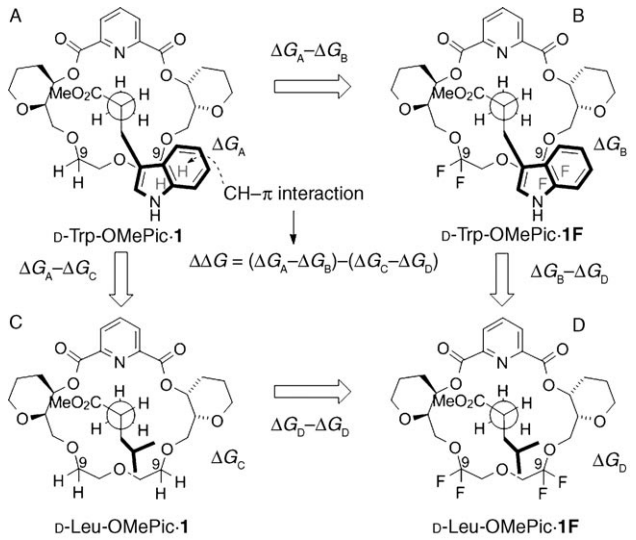
Table 2: Association constants (K_a) and enantioselectivity for complexation of the receptor **1F**.[a]

Guest	K_a [M^{-1}]	K_D/K_L
D-Trp-OMePic	400 ± 30	1.29
L-Trp-OMePic	310 ± 40	
D-Leu-OMePic	800 ± 60	1.25
L-Leu-OMePic	640 ± 20	
D-Ala-OMePic	1030 ± 60	1.47
L-Ala-OMePic	700 ± 20	

[a] Calculated from UV/Vis titrations in $CHCl_3$ at 298 K. Values are the average of at least three independent measurements and the uncertainty is given by the standard deviation.

position 9 and the aromatic ring of the amino acids, and that the interaction is especially strong for Trp. It has also been shown that the interaction is exclusive for the D enantiomers, and consequently it plays a key role in the chiral discrimination process.

At this stage, a quantification of the CH–π interaction is desirable. As receptor **1F** lacks this interaction, we could tentatively measure the strength of the interaction as the difference between the stabilities of complexes A and B (Scheme 2). However the value is perturbed by changes in the



Scheme 2. Schematic representation of the chemical double-mutant cycle.

ammonium-hydrogen-bond strength and in other secondary interactions associated with the change made from complex A to complex B, which will be referred to as the A→B mutation. In these cases, a double-mutant cycle is very useful because it overcomes this problem as the secondary free-energy effects of the mutations cancel in a pairwise fashion in the thermodynamic cycle.[30] The secondary effects can be quantified by using complexes C and D where the CH–π interaction has been removed by means of a mutation in the guest (D-Trp-OMe^+→D-Leu-OMe^+) and both in the host (**1**→**1F**) and the guest (D-Trp-OMe^+→D-Leu-OMe^+) respectively. Thus, the difference between the stabilities of complexes C and D provides a direct measurement of the changes in the ammonium-hydrogen-bond strength and in other secondary interactions associated with the A→B mutation, and it is possible to extract the thermodynamic contribution of the CH–π interaction from all of the other interactions present in complex A. This thermodynamic relationship can be expressed as:

$$\Delta\Delta G_{CH-\pi} = (\Delta G_A - \Delta G_B) - (\Delta G_C - \Delta G_D) \quad (1)$$

In order to apply the proposed chemical double-mutant cycle, two assumptions have to be made: 1) The substituents on the mutants do not interact. This assumption is valid as the fluorine atoms should not interact either with the indole ring in complex B or with the isopropyl group in complex D, and there should not be any interaction between diethylenglycol and the D-Leu-OMe^+ side chain in complex C;[31] and 2) there are no major conformational changes in the structures of all used complexes. This assumption has to be verified.

The X-ray analysis of the free fluorinated receptor **1F** reveals a structure that is very similar to that of the free receptor **1** (Supporting Information, Figure S2). For complex B, the ROESY experiment displays only intramolecular ROEs, which confirm the correct conformation for the complexation of the *cis* tetrahydropyran units. Also, some very important information can be obtain by comparing the ^{1}H NMR spectra of the free fluorinated receptor **1F** and the complex D-Trp-OMePic·**1F** (complex B), where downfield shifts of the pyridine proton signals and the upfield shifts of protons in on C-10 indicate that the indole group overlaps the diethylenglycol spacer.[18] The structure of complex C was confirmed by ROESY experiments. Intramolecular ROEs reveal a folded geometry of the receptor skeleton and the intermolecular ROEs show the proximity between the leucine residue and the diethylenglycol spacer.[18] For complex D, ROESY experiments confirm that the *cis* tetrahydropyran units are in the correct conformation. By comparing the ^{1}H NMR spectra of the free fluorinated receptor **1F**, complex D-Leu-OMePic·**1F** (D), and the guest alone, a pattern of chemical shift changes that is similar to those observed for the rest of the complexes is revealed, thus indicating that all complexes adopt similar three-dimensional structures.[18] The slight downfield shifts of the leucine residue with respect to the free substrate reveal that the isopropyl group is situated away from the pyridine ring most of the time.[32]

Subsequently, by using the association constants obtained in $CHCl_3$ by UV/Vis spectroscopic titration, we calculated the free-energy values for each complex involved in the chemical double-mutant cycle (Figure S3 in the Supporting Information). By applying Equation (1), we determined that the energy value of (−4.09 ± 0.34) kJ mol^{-1} for the CH–π interaction between the indole group of D-Trp-OMePic and the hydrogen atom in position 9. This value is in agreement with theoretical and experimental reported data.[1]

We next tried to evaluate the contribution of the CH–π interaction to the chiral discrimination. We can consider that the association constant for each complex with a different

enantiomer as guest is composed of the sum of different attractive and repulsive terms, so that the chiral discrimination is the sum of all these energetic differences between both complexes. In this study, we have already calculated the value of (-5.80 ± 0.22) kJ mol^{-1} (Table 1) for the chiral discrimination as the difference in free energy between complexes of **1** with D-Trp-OMePic and L-Trp-OMePic. But, even more importantly, we have also specifically measured one of the energetic differences between the complexes of both enantiomers to be (-4.09 ± 0.34) kJ mol^{-1} for a CH–π interaction exclusive to the D enantiomer. Thus, a remarkable (70.5 ± 6.4) % of this chiral recognition process is the consequence of a single CH–π interaction. Only one CH–π interaction is mainly responsible for the chiral discrimination observed.

In summary, we have shown that a CH–π interaction is involved in the remarkably high chiral recognition displayed by a new synthetic receptor based on *cis*-2-oxymethyl-3-oxytetrahydropyran. We also quantified the interaction through a chemical double-mutant cycle that provides a value of −4.09 kJ mol^{-1} for the interaction with tryptophan, which is in agreement with theoretical and experimental reported data. Finally, an assessment of the contribution of this single interaction to the whole chiral discrimination process was performed. This evaluation yielded that a single CH–π interaction causes a 70 % of the enantioselection. Thus, for every selective recognition process or asymmetric reaction where aromatic and aliphatic groups are involved, we should take into account that a weak noncovalent interaction, such as the CH–π bond, is likely to contribute to or even govern the whole process.

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[21] The choice of picrate salts is due to their higher solubility in CD_3CN and because their complexation can be followed by ^{1}H NMR spectroscopy. We are currently investigating the causes of the different enantioselectivity when different anions are used. However, preliminary studies reveal that the anions appear to play no role in the structure of the complexes as we can infer from the similarities in the ^{1}H NMR spectra for all complexes (see the Supporting Information). The observed differences could be related to the ion-pair strength of the salts employed.

[22] In the complex L-Trp-OMePic·**1**, the signals of the equatorial protons in position 6 are shifted upfield, which may be interpreted in terms of magnetic anisotropy induced by the indole group. This result clearly indicates that the indole group in the complex L-Trp-OMePic·**1** is in a different orientation that in the complex D-Trp-OMePic·**1**.

[23] Unfortunately all attempts to crystallize complexes of receptor **1** with any ammonium salts were not successful.

[24] In a CH–π interaction, the distance between the hydrogen atom and the π surface is around 2.5–2.9 Å. See Reference [10].

[25] The lower association constants recorded in CD_3CN can be explained because CD_3CN better solvates the ammonium cation, which competes much more than $CHCl_3$ with receptor **1** for the guest. A lower association constant is expected for both guest enantiomers, and an even lower value is expected for the less-preferred guest, because its complex is more sensitive to changes in the conditions. Thus, association constants for the L series decreased even more than for the D series, and therefore a general increase of the chiral discrimination was observed.

[26] Hunter has calculated a series of parameters, for common functional groups and solvents, based on the molecular surface electrostatic potential, which allow them to obtain a scale of hydrogen-bond strength. The values of hydrogen-bond-donor constant (α) for acetonitrile and chloroform are 1.7 and 2.2, respectively. C. A. Hunter, *Angew. Chem.* **2004**, *116*, 5424 – 5439; *Angew. Chem. Int. Ed.* **2004**, *43*, 5310 – 5324.

[27] a) A. Matsushima, T. Fujita, T. Nose, Y. Shimohigashi, *J. Biochem.* **2000**, *128*, 225 – 232; b) H. Adams, S. L. Cockroft, C. Guardigli, C. A. Hunter, K. R. Lawson, J. Perkins, S. E. Spey, C. J. Urch, R. Ford, *ChemBioChem* **2004**, *5*, 657 – 665; c) see reference [7a]; d) K. Müller, C. Faeh, F. Diederich, *Science* **2007**, *317*, 1881 – 1886.

[28] In order to obtain highly reliable binding constants with the receptor **1F**, UV/Vis titrations in $CHCl_3$ were carried out instead of ^{1}H NMR titrations in CD_3CN, because only very small changes in the chemical shifts were observed.

[29] T.-Y. Lin, W.-H. Lin, W. D. Clark, R. J. Lagow, S. B. Larson, S. H. Simonsen, V. M. Lynch, J. S. Brodbelt, S. D. Maleknia, C.-C. Liou, *J. Am. Chem. Soc.* **1994**, *116*, 5172 – 5179.

[30] S. L. Cockroft, C. A. Hunter, *Chem. Soc. Rev.* **2007**, *36*, 172 – 188.

[31] Dispersion interactions are always possible; however these interactions are similar on both sides of the cycle, therefore, the free energy differences must be canceled out in the analysis.

[32] We have verified that use of either $CDCl_3$ or CD_3CN as solvent makes no difference to the structure of the complexes. However the NMR structural studies of complexes involved in the chemical double-mutant cycle were performed in CD_3CN at 298 K because receptor **1F** displays low association constants and therefore it is unable to sufficiently solubilize the ammonium picrate salts of the α-amino acid methyl esters in $CDCl_3$ to obtain a suitable concentration for the NMR studies.

