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Noncovalent Interactions between ([18]Crown-6)-Tetracarboxylic Acid and Amino Acids: Electrospray-Ionization Mass Spectrometry Investigation of the Chiral-Recognition Processes

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Abstract: Chiral recognition of enantiomers by host compounds is one of the most challenging topics in modern host–guest chemistry. Amongst the well-established methods, mass spectrometry (MS) is increasingly used nowadays, due to its low detection limit, short analysis time, and suitability for analyzing mixtures and for studying chiral effects in the gas phase. The development of electrospray-ionization (ESI) techniques provides an invaluable tool to study, in the gas phase, diastereoisomeric complex ions prepared from enantiomer ions and a chiral selector. This paper reports on an ESIMS and ESIMSMS study of the molecular mechanisms that intervene in the chiral-recognition phenomena observed between amino acids and a chiral crown ether. The modified crown ether, namely (+)-([18]crown-6)-2,3,11,12-tetracarboxylic acid, is used as the chiral selector when covalently bound on a stationary phase in liquid chromatography. This study was stimulated by the fact that, except with threonine and proline, consistent elution orders were observed, which indicates that the D enantiomers interact more strongly with the chiral selector than the L enantiomers. For proline, the lack of a primary amino group is likely to be responsible for the nonresolution of the two forms, whereas the second stereogenic center on threonine could explain the reversed elution order. In light of those observations, we performed mass spectrometry experiments to understand more deeply the enantiomeric recognition phenomena, both in solution by the enantiomer-labeled guest method and in the gas phase by gas-phase ligand-exchange ion/molecule reactions. The results have been further supported by quantum chemical calculations. One of the most interesting features of this work is the identification of a nonspecific interaction between proline and the crown ether upon ESIMS analysis.

Keywords: amino acids · chirality · crown compounds · mass spectrometry · noncovalent interactions

Introduction

Chiral recognition is nowadays an active area of research in mass spectrometry (MS).[1] Actually, it has long been considered that, because mass spectrometry is intrinsically a nonchiral methodology, it is insensitive to chiral differences. Indeed, chiral discrimination is a challenging endeavor in mass spectrometry investigations because enantiomeric isomers present, by definition, identical mass to charge ratios (m/z) and similar dissociation pathways. Consequently, they are indistinguishable by either MS or MSMS experiments. The formation of diastereoisomeric noncovalent complexes in the gas phase is currently an elegant methodology to access the configuration of chiral molecules by taking advantage of the potential differences in the relative stability of the generated stereoisomeric complexes.[2] In this context, the study of chiral recognition by MS requires the use of an optically pure host molecule to play the role of chiral selector. When both enantiomeric compounds under investigation are threaded in the host molecule, two diastereoisomeric complexes will be produced.

Chiral crown ethers as chiral macrocyclic receptors have been extensively reported to be very efficient for the enan-

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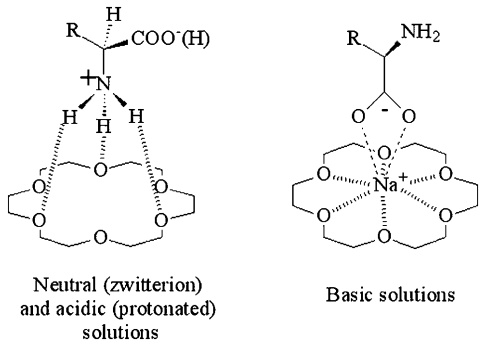
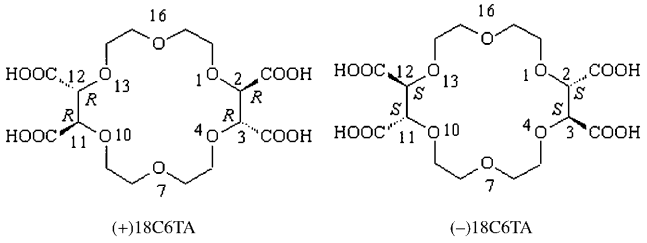
tiomeric distinction of primary amine compounds, such as amino acids in solution, for instance, by NMR spectroscopy or UV/Vis measurements.[3] The fundamental binding interaction between an amino acid and a crown ether in either acidic or neutral solutions is a tripod hydrogen bonding, which, for example, in the case of [18]crown-6 (18C6), involves three oxygen atoms of the crown ether ring and three hydrogen atoms of the ammonium group (Scheme 1, left).[4]



Scheme 1. Fundamental binding interactions between an amino acid and a crown ether in different types of solution.

On the other hand, it is thought that the amino acid is complexed through the carboxylate function in basic media (Scheme 1, right).[5] In this case, ion-pair receptors exhibiting cooperative and allosteric effects were proposed to account for the formation of the supramolecular structure.[5]

Chiral crown ethers are so efficient for the chiral recognition of primary amines and amino acids that they have been extensively developed as chiral selectors in chromatographic techniques with chiral stationary phases (CSP). Such CSPs achieve good enantiomer separation for analytical and preparative purposes.[6] For instance, (+)-([18]crown-6)-2,3,11,12-tetracarboxylic acid ((+)-18C6TA; Scheme 2), when cova-



Scheme 2. Structure of (+)-([18]crown-6)-2,3,11,12-tetracarboxylic acid ((+)-18C6TA).

lently bound to aminopropyl silica gel, is employed for the resolution of racemic α-amino acids and several drugs containing primary amines (such as DOPA and baclofen) by liquid chromatography.[7] (+)-18C6TA is typically prepared from L-tartaric acid.[8] It was demonstrated that all 27 natural and unnatural racemic α-amino acids could be resolved from acidic solutions on a CSP prepared from (+)-18C6TA and that reasonable separation factors were measured, except with proline, which lacks a primary amino group. This study also revealed that, except for the threonine analyte, consistent elution orders of the examined amino acids were observed; this result showed that the D enantiomers always interact more strongly with the chiral selector than the L enantiomers.[9] In a search for the origin of the chiral recognition of α-amino acids in the presence of 18C6TA as a chiral selector, the interactions responsible for the differential affinities towards enantiomers were investigated for the specific case of phenylglycine by NMR spectroscopy and molecular dynamics calculations.[10] It was revealed that the polyether ring forms a bowl that is shaped by intramolecular hydrogen bonding involving the carboxylic groups located under the plane of the ring and situated on carbon atoms 3 and 12 of the polyether ring (Scheme 2). As a consequence, the upper face is open to allow intermolecular hydrogen bonding between the hydrogen atoms of the ammonium group and three oxygen atoms of the crown ether. The key features for enantiomeric discrimination are 1) the three $^{+}$NH···O hydrogen bonds in a tripod arrangement between polyether oxygen atoms and the ammonium moiety of the L or D enantiomer, 2) a hydrophobic interaction between the polyether ring of the host molecule and the phenyl moiety of the enantiomers, and 3) a hydrogen bond between the carboxylic acid of the crown ether and the carbonyl oxygen atom for the D enantiomer only.[10] This additional hydrogen bond is considered to be crucial for effective chiral discrimination because, as a consequence, the D isomer of phenylglycine would form a more favorable complex with chiral (+)-18C6TA than the L isomer.[10] Chiral discrimination requires the cooperative interaction of several weak forces, such as dipole–dipole, hydrophobic, electrostatic, van der Waals, and hydrogen-bonding interactions. The three-point interaction model was developed to describe more generally the nature of the interactions and relies on the combination of two attractive forces and one repulsive interaction to efficiently achieve enantioselectivity.[11]

Electrostatic noncovalent associations can be transferred to the gas phase of a mass spectrometer by the intermediacy of a soft-ionization methodology, such as electrospray ionization.[12] The first prerequisite for mass spectrometry characterization of a supramolecular entity is that the structure can be successfully charged and transferred into the gas phase without destruction. This requirement is totally met in the present case because the noncovalent interaction is between an ammonium group and the crown ether, at least if the entity is sprayed from an acidic or neutral solution. The aim of the present work is to investigate, by electrospray-ionization mass spectrometry, the 18C6TA/amino acid system to shed light on the different behaviors observed with phenylglycine, threonine, and proline. Results in the literature revealed that, with (+)-18C6TA as the chiral selector, the elution orders were reversed between threonine and phenylglycine, with D-phenylglycine interacting more strongly with the 18C6TA-derived CSP than the L enantiomer. On the other hand, a racemic mixture of proline cannot be re-

solved with this kind of chiral-phase liquid chromatography and this was explained by the fact that no primary amino moiety is present in proline. Our experimental results are discussed in the light of the literature results[10] and are further supported by the results of quantum chemical calculations.

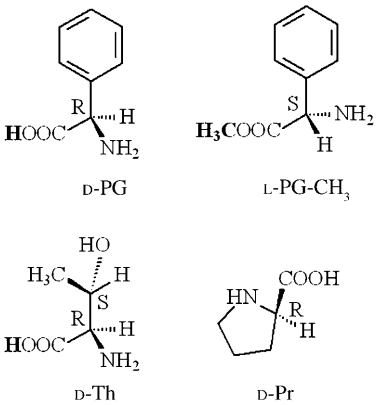
Results and Discussion

When the MS experiments on the amino acid (ester)/chiral crown ether systems were started, the dextrogyre optical isomer of 18C6TA used in the literature[9,10] was no longer commercially available, unlike the levogyre enantiomer ((−)-18C6TA; Scheme 2). As a consequence, the expected enantioselectivity for our measurements should be reversed relative to the literature results. Table 1 summarizes the binding constants in methanol determined by NMR spectroscopy for the enantiomers of phenylglycine (PG) and phenylglycine methyl ester (PG-CH_3; Scheme 3).[10]

Table 1. NMR-determined binding constants (K_A [mol^{-1}]) for phenylglycine (PG) and phenylglycine methyl ester (PG-Me) enantiomers with 18C6TA.

	(+)-18C6TA[a]			(−)-18C6TA		
	K_A(D)	K_A(L)	α(D/L)	K_A(D)	K_A(L)	α(D/L)
PG	1034	238	4.34	238	1034	0.23
PG-Me	1992	1159	1.72	1159	1992	0.58

[a] Values reported in the literature.[10]



Scheme 3. Structures of some of the α-amino acids and α-amino esters used in this work.

Preliminary ESI time-of-flight (TOF) measurements: A typical spectrum from an ESI-TOF mass spectrometry analysis of a solution containing 10^{-5} M amino acid and 10^{-5} M (−)-18C6TA in methanol is presented in Figure 1a. This spectrum, obtained from an L-phenylglycine/(−)-18C6TA mixture, features intense signals at *m*/*z* 152, 463, and 479, which correspond to protonated phenylglycine and the sodium and potassium adducts of the crown ether, respectively. More interestingly, the base peak of the spectrum is observed at *m*/*z* 592 and confirms that the noncovalent complex between protonated phenylglycine and the crown ether can be transferred from the solution in methanol to the gas phase of the mass spectrometer by the electrospray process. The key parameter for the detection of such a signal is the cone voltage, which must be kept below 20 V to avoid collision-induced decomposition of the fragile ions during the flight between the sample and the extractor cones. It must be emphasized that, under the conditions used for the ESIMS measurements, chiral recognition cannot be achieved. Indeed, the spectrum obtained with the D enantiomer of phenylglycine is extremely similar to the spectrum presented in Figure 1a, even though the complex between D-PG and the chiral selector is, according to the literature, less stable.[10] The important piece of information is that both (protonated) diastereoisomeric complexes can be detected by mass spectrometry upon electrospray ionization. The same conclusion was obtained for the enantiomers of threonine, for which the protonated diastereoisomeric complexes

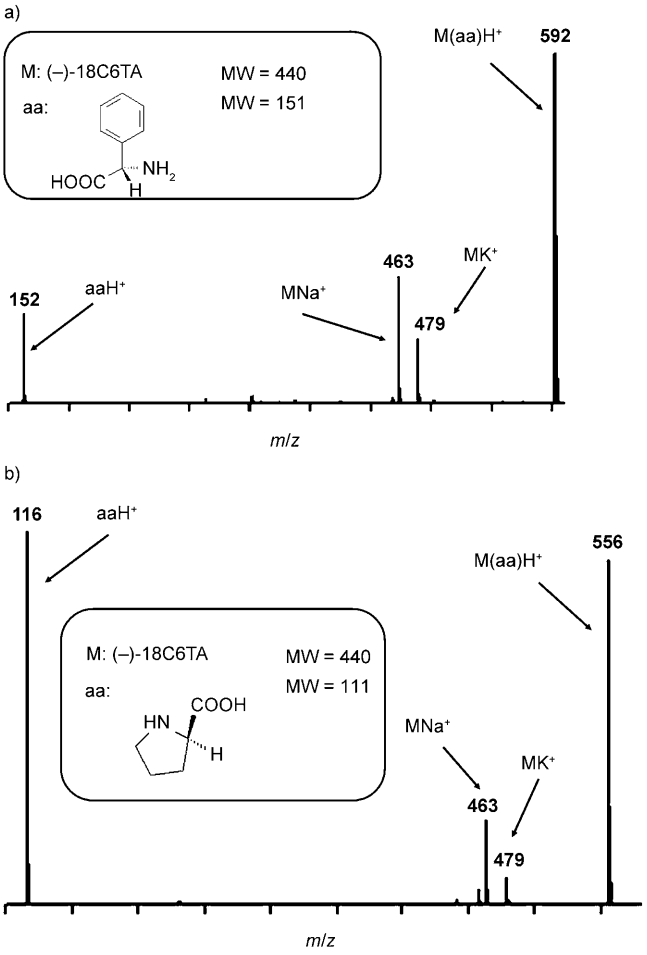


Figure 1. ESI-TOF MS analysis of an equimolar solution (10^{-5} M in methanol) of a) L-phenylglycine (aa) and b) D-proline (aa) with (−)-18C6TA (M).

with the chiral crown ether could also be detected in the gas phase of the mass spectrometer.

More surprisingly, as revealed by the ESIMS spectrum shown in Figure 1b, protonated noncovalent complexes associating protonated proline and 18C6TA were also detected (*m*/*z* 556) in quite good yield, whatever the configuration of proline. Such an observation was definitely unexpected, given the fact that no resolution is achieved for racemic proline by CSP liquid chromatography with 18C6TA as the chiral selector.[9] In other words, the lack of a primary amino group in proline is likely to prevent this amino acid from associating with the crown ether, thereby ruling out any chiral discrimination. The absence of a noncovalent complex between proline and 18C6TA in solution rationalizes the nonresolution of a racemic mixture on the CSP. We tentatively suggest that the observed complexes between protonated proline and 18C6TA are electrostatic adducts formed during the electrospray process and, as a consequence, can be classified amongst the nonspecific noncovalent complexes.[13]

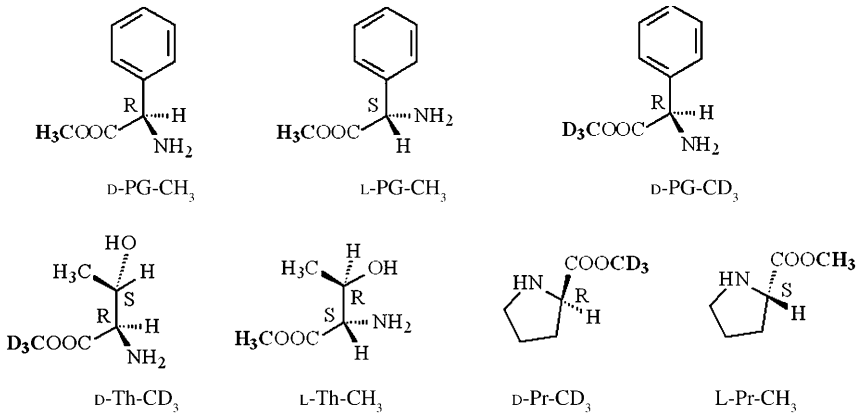
Probing chiral recognition in solution: Enantiomer-labeled (EL) guest method: Sawada et al. described an elegant method for the determination of the chiral-recognition ability of chiral organic compounds (hosts) towards chiral guests. This methodology is based on the study of the host–guest complexation by fast-atom-bombardment (FAB) MS[14] or ESIMS.[15] The method relies on the preparation of a 1:1 mixture of unlabeled (G_R) and deuterium-labeled (G_S–D_n) guest enantiomers in the presence of a large deficit of chiral host (H). Such conditions were chosen to induce competition between the two complexation reactions in solution [Eq. (1),(2)]. Isotope labeling is, of course, useful to create a mass difference between the diastereoisomeric complexes, namely G_R·H and G_S–D_n·H. Sawada et al. demonstrated that the relative peak intensity (*I*) in the measured ESI spectrum is a measure of the chiral-recognition ability.[13,14] In this paper, we will use the terminology of Sawada et al. by introducing the IRIS value, which corresponds to the relative peak intensity [Eq. (3)].

$$G_R + H \rightleftharpoons G_R \cdot H \; K_A(R) \quad (1)$$

$$G_S - D_n + H \rightleftharpoons G_S - D_n \cdot H \; K_A(S) \quad (2)$$

$$IRIS = \frac{I(R)}{I(S)} = \frac{I(G_R \cdot H)}{I(G_S - D_n \cdot H)} = \frac{K_A(R)}{K_A(S)} \quad (3)$$

To investigate our amino acid/crown ether systems by the EL-guest methodology, we prepared several labeled and unlabeled amino esters, with the isotope labeling introduced by replacement of CH_3 by CD_3 in the enantiomers (see the Experimental Section for details). The prepared molecules are shown in Scheme 4. The phenylglycine methyl ester enantiomers were particularly interesting because the binding constants in methanol were determined by NMR spectroscopy to be 1159 and 1992 M^{-1} for K_A(D) and K_A(L), respectively (Table 1).[10] Before starting to assess the chiral-recognition ability of (−)-18C6TA towards the phenylglycine methyl ester enantiomers, we wanted to determine first whether the introduction of the deuterium atoms would play a role in the chiral-recognition process. That would be the case if the methyl group of the amino ester were to participate in the noncovalent association. The fact that the isotope labeling does not affect the complexation was unambiguously demonstrated by electrospray analysis of a 1:10:10 ternary solution containing (−)-18C6TA, D-PG-CH_3, and D-PG-CD_3. No discrimination is observed in the recorded ESIMS spectrum (Figure 2a): the intensities of the signals corresponding to the noncovalent association between both protonated isotopomers and the chiral selector are similar.



Scheme 4. Structures of some of the labeled and unlabeled α-amino acids and α-amino esters used for the enantiomer-labeled-guest methodology.

On the other hand, in the ESI-TOF mass spectrum (Figure 2b) obtained from a 1:10:10 ternary solution prepared with (−)-18C6TA, L-PG-CH_3, and D-PG-CD_3, which incorporates both pseudoenantiomers, chiral discrimination is immediately observed and the measured IRIS value amounts to 0.61. This result reveals that L-PG-CH_3 is more strongly bound to the chiral crown ether than the D enantiomer, a fact that is in complete agreement with the literature data. The IRIS value obtained here, IRIS = 0.61, compares remarkably well to the binding constant ratio in Table 1 (α(D/L) = α(*R*/*S*) = 0.58).

Quantum chemical calculations were performed at the AM1 level on the isolated molecules, as well as on the charged noncovalent complexes. We focus here exclusively on phenylglycine because this is the system that is the most

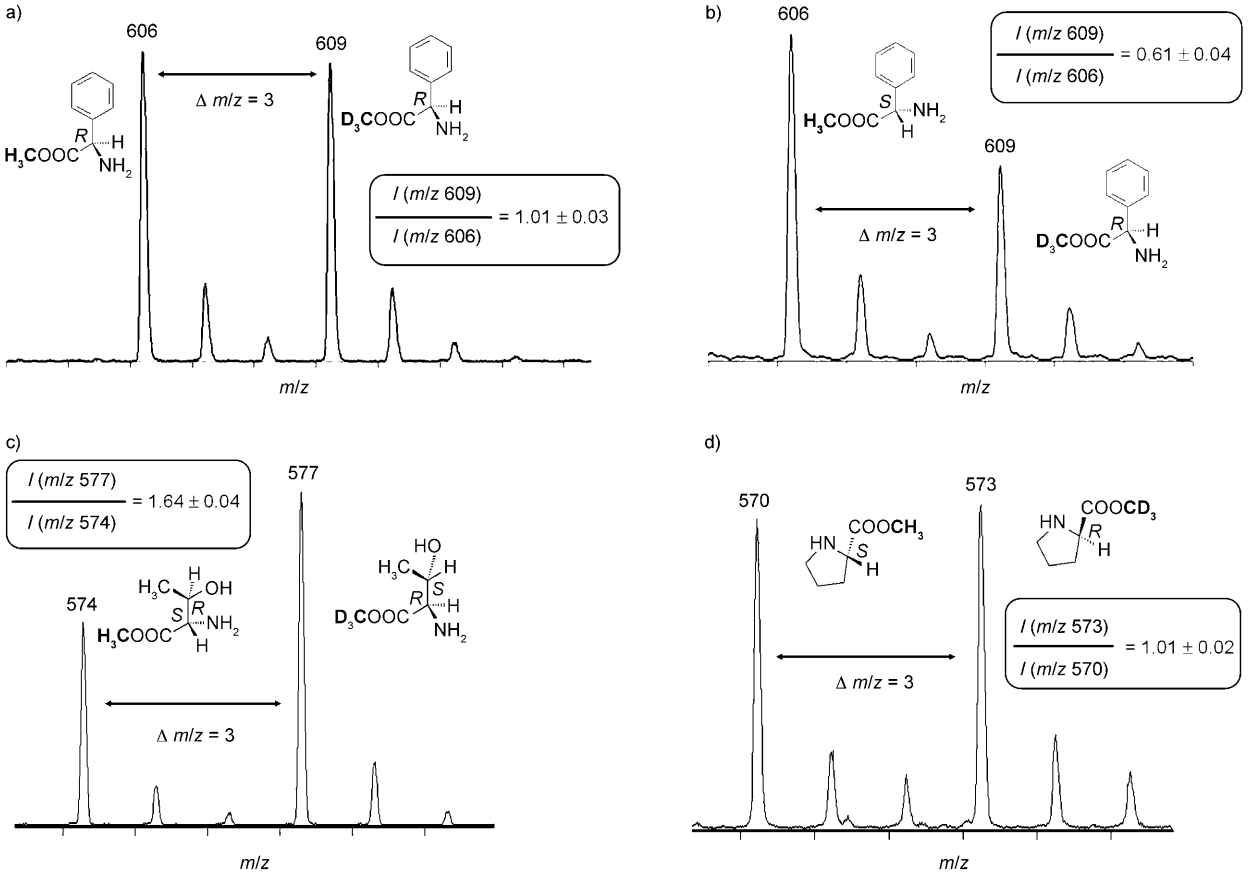


Figure 2. ESI-TOF analysis of 1:10:10 ternary solutions in methanol containing a) (−)-18C6TA, D-PG-CH_3, and D-PG-CD_3; b) (−)-18C6TA, L-PG-CH_3, and D-PG-CD_3; c) (−)-18C6TA, L-Thr-CH_3, and D-Thr-CD_3; and d) (−)-18C6TA, L-Pro-CH_3, and D-Pro-CD_3.

characterized at the experimental level; moreover, the rigidity of the system introduced by the hydrogen bonds and the benzene ring of the backbone facilitates the search of the complex geometry to yield the global energy minimum. Table 2 shows the total energies of the different species involved and Figure 3 exhibits some representative structures. The geometry optimization of the crown ether (18C6TA in Figure 3b) yields a structure in agreement with that previously reported from molecular mechanics calculations.[10] In particular, the polyether ring is found to adopt a bowl shape due to the intramolecular hydrogen bonding O30−H···O23 (Figure 3a). As a consequence, the upper face of the polyether ring is open, which allows additional hydrogen bonds with the ammonium group of a (protonated) amino acid (Figure 3b).

Figure 3c presents the AM1 structure of the noncovalent complex associating protonated D-phenylglycine and the chiral crown ether. During complexation, three hydrogen bonds are created between the ammonium group of the D enantiomer and three oxygen atoms of the crown ether ring. An additional hydrogen bond is also observed between a carboxylic acid group of the crown ether and the carboxylic acid group of the protonated amino acid. The AM1 geometry obtained from optimization of the noncovalent complex associating the L enantiomer and the crown ether fea-

Table 2. Total energies obtained by using the AM1 methodology.

Structure	Geometry	AM1 calculations [eV]
isolated molecules and ions:		
(+)18C6TA	Figure 3a and b	−6867.139
D-phenylglycine (D-PG)		−1996.164
protonated D-phenylglycine (D-PGH)		−2003.280
protonated L-phenylglycine (L-PGH)		−2003.280
ammonia		−248.591
noncovalent complexes:		
[D-PG·(+)-18C6TA]H^+	Figure 3c	−8872.762
[L-PG·(+)-18C6TA]H^+	Figure 3d	−8872.740
ligand-exchange-process products and intermediates:		
[D-PG·(+)-18C6TA·NH_3]H^+	Scheme 5	−9121.333
[(+)-18C6TA·NH_3]H^+	Scheme 5	−7122.754
[(+)-18C6TA·NH_4^+]	Scheme 5	−7125.283

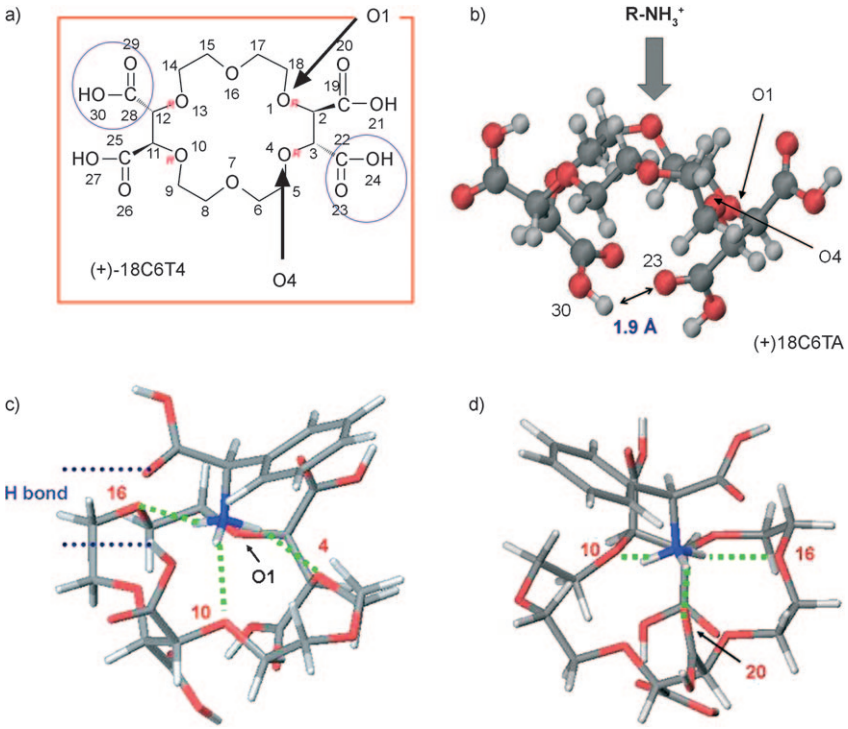


Figure 3. Optimized geometries obtained at the semiempirical Hartree–Fock AM1 level of theory: a) Structure of the (+)-18C6TA crown ether; b) the (+)-18C6TA crown ether; c) noncovalent complex between protonated D-phenylglycine and the crown ether; and d) noncovalent complex between protonated L-phenylglycine and the crown ether.

tures a smaller number of hydrogen bonds (three hydrogen bonds; Figure 3d) than the previous diastereoisomeric complex (four hydrogen bonds; Figure 3c). As a consequence, the stabilization of the system upon complexation is calculated to be larger in the case of the D enantiomer [Eq. (4),(5)]. At variance with the optimized structure previously reported,[10] we observe from our quantum chemical calculations that the three hydrogen bonds linking the ammonium group of the L enantiomer to the crown ether involve two oxygen atoms of the crown ether ring and a carboxylic acid group of the crown ether (Figure 3c). The difference in the stabilization energies, $\Delta\Delta E = 2.1\ \mathrm{kJ\,mol^{-1}}$, from Equations (4) and (5) and Table 2 can be used to estimate the binding-constant ratio in a first approximation by inserting the energy difference into the well-known relationship $\Delta\Delta E = \Delta E_L - \Delta E_D = RT\ln(K_D/K_L)$. The obtained value of $K_D/K_L = 2.33$ is fully consistent with the value of 4.34 determined by NMR spectroscopy for a solution in methanol (Table 1 and reference [10]).

The EL-guest methodology was then applied to the pseudoenantiomers of threonine, D-Thr-CD_3 and L-Thr-CH_3 (Scheme 4; see Figure 2c for the ESIMS spectrum). As expected, chiral recognition (IRIS = 1.64) is reversed relative to the phenylglycine methyl ester. This observation is also in line with the reversed order of elution in the CSP liquid chromatography experiments.[9]

As far as proline is concerned, we have already mentioned in this paper that observations based upon electrospray of the noncovalent complexes between protonated proline and the crown ether are quite tricky. The formation of electrostatic adducts during the ESI desolvation process is likely to be responsible for the detection of nonspecific interactions. We submitted an equimolar solution of pseudoenantiomers of proline methyl ester, L-Pro-CH_3 and D-Pro-CD_3, and (−)-18C6TA to electrospray ionization. The recorded spectrum is presented in Figure 2d; the signals corresponding to the diastereoisomeric complexes are detected in the same intensity. At first sight, this finding could match the CSP chromatography results.[9] However, given the fact that both noncovalent complexes associating protonated proline methyl ester and 18C6TA are diastereoisomeric, the observation of an IRIS value of one, from an equimolar solution of both enantiomers, is just statistical and our feeling that we are dealing with nonspecific interactions is reinforced.

$$\Delta E_D = -2.343\ \mathrm{eV} = -226.0\ \mathrm{kJ\ mol^{-1}}$$

$$\text{D-PG H}^+ + (+)18C6TA \longrightarrow [\text{D-PG H}^+ \bullet (+)18C6TA] \quad (4)$$

$$-2003.280\ \mathrm{eV} \qquad -6867.139\ \mathrm{eV} \qquad -8872.762\ \mathrm{eV}$$

$$\Delta E_L = -2.321\ \mathrm{eV} = -223.9\ \mathrm{kJ\ mol^{-1}}$$

$$\text{L-PG H}^+ + (+)18C6TA \longrightarrow [\text{L-PG H}^+] \bullet (+)18C6TA] \quad (5)$$

$$-2003.280\ \mathrm{eV} \qquad -6867.139\ \mathrm{eV} \qquad -8872.740\ \mathrm{eV}$$

Probing chiral recognition in the gas phase: Collision-induced dissociation (CID) experiments: As already explained in the Introduction, chiral recognition between the enantiomers of phenylglycine and (−)-18C6TA originates from an additional hydrogen bond that binds the L enantiomer to the crown ether.[10] It is well known that electrostatic interactions are strengthened upon evaporation from methanolic or aqueous solutions as the competitive effect of the solvent is removed. Therefore, we decided to probe the presence of this additional hydrogen bond by collision-induced dissociation experiments. In the particular case of protonated diastereoisomeric complexes, the sole expected difference would

be the dissociation threshold. This value can be approached by performing a kinetic-energy-resolved CID experiment, that is, by measuring the signal-intensity ratio between the parent and fragment ions at various center-of-mass energies (E_{com}). When the kinetic energy of the incident ions prior to the collision step is increased, competitive, as well as consecutive, decomposition reactions are expected. We thus decided to quantify the decomposition extent of the parent ions by the parameter $I_{parent}/\Sigma I$. In this relationship, I_{parent} stands for the intensity of the parent-ion signal and ΣI corresponds to the sum of the intensities of all peaks in the CID spectrum. Unfortunately, as shown in Figure 4, this experiment

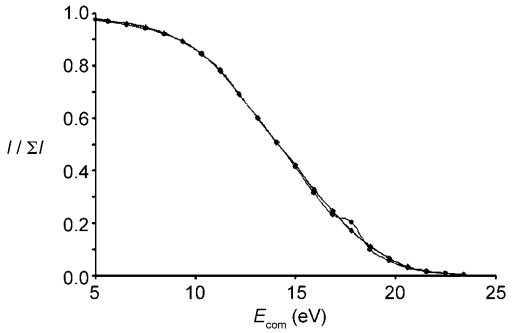


Figure 4. Kinetic-energy-resolved CID experiments: Breakdown curves presenting the ion survival yield versus the center-of-mass energy for the [D-PG·(−)-18C6TA]H$^+$ (◆) and [L-PG·(−)-18C6TA]H$^+$ (●) diastereoisomeric complexes.

is totally unsuccessful for distinction between the two protonated diastereoisomeric complexes.

Probing chiral recognition in the gas phase: Gas-phase ligand-exchange reactions: Molecular recognition of enantiomers by a chiral receptor has been probed on several occasions by investigating ligand-displacement reactions in the gas phase by ESI-FT-ICR mass spectrometry.[16] This peculiar ion/molecule reaction between noncovalent diastereoisomeric complexes and a nonchiral reagent can be used to reveal distinct enantioselectivities.[16] Indeed, when submitted to ion/molecule reactions toward a selected nonchiral reactant G2, the noncovalent complex [H·G1]$^+$ can undergo exchange of its ligand molecule with the nonchiral guest according to the reactions described by Equations (6) and (7), in which H stands for the host receptor and G1(R), G1(S), and G2 are the R and S enantiomers of guest molecule 1 and the nonchiral guest molecule, respectively. The relative pseudo-first-order rate constants measured with the FT-ICR mass spectrometer can be exploited for the description of the enantioselectivity of chiral host H towards both enantiomeric guest molecules G1(R) and G1(S).

$$[H \cdot G1(R)]^+ + G2 \xrightarrow{k(R)} [H \cdot G2]^+ + G1(R) \quad (6)$$

$$[H \cdot G1(S)]^+ + G2 \xrightarrow{k(S)} [H \cdot G2]^+ + G1(S) \quad (7)$$

The interaction between mass-selected m/z 592 cations, in which protonated L-phenylglycine is associated to (−)-18C6TA, and ammonia in the hexapole cell of our QToF instrument led to different ion/molecule reaction products. In particular, as presented in Figure 5a, this low-kinetic-energy

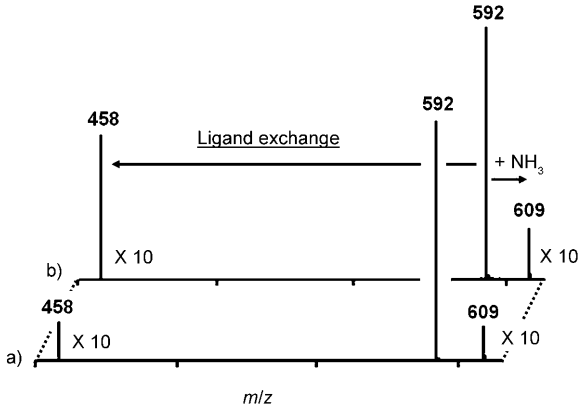


Figure 5. Associative ion/molecule reaction between ammonia and the protonated noncovalent complex associating the phenylglycine enantiomers and the chiral crown ether (m/z 592): ESIMSMS analysis from initial a) [L-PG·(−)-18C6TA]H$^+$ and b) [D-PG·(−)-18C6TA]H$^+$ diastereoisomeric complexes.

interaction affords m/z 458 cations in quite a good yield. These ions, easily identified as a noncovalent complex between the crown ether and the ammonium cation, result from exchange of the amino acid with ammonia in the mass-selected m/z 592 ions. Interestingly (see below), addition of neutral ammonia to the noncovalent complex also occurs and the corresponding ions are observed at m/z 609. These ions are likely to be the intermediate species in the gas-phase ligand-exchange process.

When reacting with the neutral reagent, noncovalent complex ions incorporating the D enantiomer of phenylglycine also undergo the ligand-exchange reaction (indicated by the m/z 458 peak in Figure 5b) and neutral ammonia addition (to give ions at m/z 609). The m/z 458 signal is far more intense in Figure 5b (D enantiomer) than in Figure 5a (L enantiomer); this observation is a clear-cut demonstration that the protonated L enantiomer of phenylglycine is more strongly bound to (−)-18C6TA than the D enantiomer. These results are again fully consistent with the NMR-determined binding constants (see Table 1) and with the previous data derived from the EL-guest method (see above).

The same set of experiments was then performed with the enantiomers of threonine. As expected on the basis of the already mentioned inversion in enantioselectivity relative to that of phenylglycine, the exchange reaction between the noncovalent complex associating protonated L-threonine and the crown ether with ammonia is significantly faster. This is readily observed by comparing Figure 6a (L enantiomer) with Figure 6b (D enantiomer). Ammonia addition to the mass-selected complex ions is once again detected.

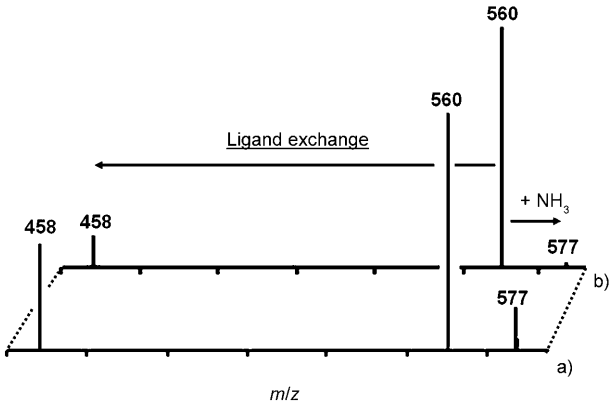


Figure 6. Associative ion/molecule reaction between ammonia and the protonated noncovalent complex associating the threonine enantiomers and the chiral crown ether (*m*/*z* 560): ESIMSMS analysis from initial a) [L-Thr•(−)-18C6TA]H^+ and b) [D-Thr•(−)-18C6TA]H^+ diastereoisomeric complexes.

The experiments involving proline as the guest molecule are very informative. Indeed, chiral discrimination can be clearly measured on the basis of the spectra presented in Figure 7a (L enantiomer) and Figure 7b (D enantiomer). It is

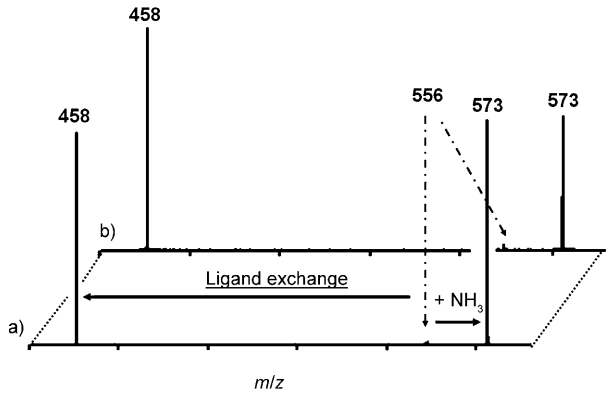


Figure 7. Associative ion/molecule reaction between ammonia and the protonated noncovalent complex associating the proline enantiomers and the chiral crown ether (*m*/*z* 556): ESIMSMS analysis from initial a) [L-Pro•(−)-18C6TA]H^+ and b) [D-Pro•(−)-18C6TA]H^+ diastereoisomeric complexes.

interesting to note that, under the same experimental conditions as those used for phenylglycine and threonine, the exchange reaction is far more efficient, whatever the configuration of proline. Both spectra are dominated by the associative ion/molecule reaction product signals at *m*/*z* 458 (exchange process) and *m*/*z* 573 (ammonia addition on the complex ion). The really small intensity of the signal corresponding to the mass-selected precursor ions unambiguously reveals that the noncovalent interactions inside the proline complex are different from those prevailing in the complex between protonated threonine or phenylglycine and the crown ether. The enantioselectivity of the exchange reaction is measured by calculating the $I_{458}/(I_{458}+I_{556}+I_{573})$ intensity ratio, for which $I_{458}+I_{556}+I_{573}=\Sigma I$. The $I/\Sigma I$ value amounts to 62 and 49% for D- and L-proline, respectively. In other words, the D-amino acid is more rapidly exchanged than the L enantiomer. The observation of such a difference can be attributed to the fact that both noncovalent complexes are diastereoisomers and, as a consequence, their chemical reactivities are likely to be different. This experimental observation has to be considered in respect of the EL-guest experiment results, which did not allow a distinction between the diastereoisomeric complexes.

The next step was to measure the gas-phase ligand-exchange-reaction efficiency, $I/\Sigma I$, with solutions in methanol containing a mixture of both enantiomers in different ratios. To do this, we decided to use two different solutions: 1) solutions in methanol with a large excess of amino acids to induce competition for binding to the crown ether, and 2) solutions containing a large deficit of amino acids relative to the crown ether. In the first case, the ratio between the diastereoisomeric noncovalent complex ions in the ion beam must be correlated to the relative binding constants because competition conditions are created. On the other hand, for the solutions with a deficit of amino acids, it is expected that the ion-beam composition is determined by the enantiomeric excess of the prepared solution. Indeed, under such conditions, all amino acids (D or L) present in the solution are likely to be bound to the crown ether.

Gas-phase ligand-exchange reactions with solutions containing an excess of amino acids: Figure 8 presents the results of

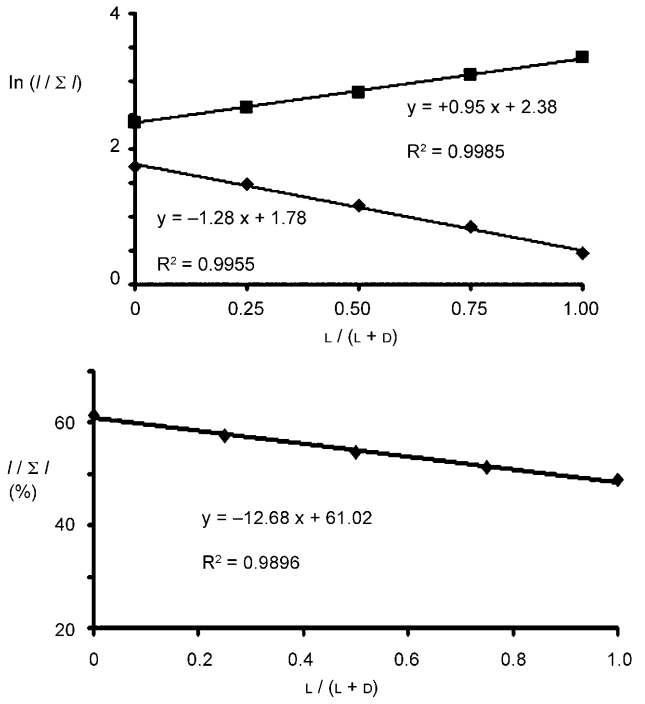


Figure 8. Gas-phase ligand-exchange reactions between protonated noncovalent complexes and ammonia: Reaction efficiency ($I/\Sigma I$) versus enantiomeric excess for complexes associating a) phenylglycine (◆) and threonine (■) and b) proline, from initial solutions containing an excess of amino acids.

the gas-phase ligand-exchange reactions realized for the three amino acids of interest, phenylglycine, threonine, and proline. These plots present the evolution of the ligand-exchange-reaction efficiency ($I_{458}/\Sigma I$) as a function of the molar ratios (L/L+D) of the initial solutions in methanol.

In the case of phenylglycine and threonine, linear relationships are obtained by plotting the natural logarithm of the intensity ratio ($I/\Sigma I$) versus the molar ratio between the enantiomers in the starting solutions (L/L+D). The enantioselectivity is immediately described by the slope of the linear relationship. For instance, the negative slope for phenylglycine (Figure 8a) reveals that the exchange reaction is faster for the D enantiomer. The opposite conclusion is derived from Figure 8a for threonine and this confirms the reversed enantioselectivity for this amino acid.

Proline once again presents a different behavior because a linear relationship is immediately obtained by plotting the intensity ratio ($I/\Sigma I$) versus the molar ratio (L/L+D, Figure 8b). As discussed below, this points once more towards a nonspecific interaction. Nevertheless, the negative slope also confirms that enantioselectivity occurs between both diastereoisomeric complex ions. Further confirmation of the nonspecific nature of the noncovalent interaction between protonated proline and the crown ether was obtained by analyzing the gas-phase exchange propensity of the noncovalent complexes generated by electrospray ionization of solutions containing an excess of the crown ether.

Gas-phase ligand-exchange reactions with solutions containing a deficit of amino acids: As already explained, these conditions were selected to prepare ion beams, the compositions of which are determined by the enantiomeric excess of the prepared solution, without the intermediacy of the binding constants. As a consequence, the relationship between the intensity ratio ($I/\Sigma I$) versus the molar ratio (L/L+D) is expected to be linear (Figure 9), whereas logarithm functions were previously observed (Figure 8). From an analytical point of view, such plots can be used to measure the enantiomeric excess of an unknown solution.[16a]

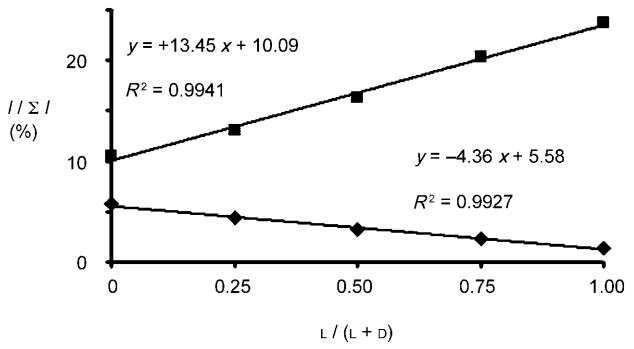


Figure 9. Gas-phase ligand-exchange reactions between protonated noncovalent complexes and ammonia: Reaction efficiency ($I/\Sigma I$) versus enantiomeric excess for complexes associating phenylglycine (◆) and threonine (■), from initial solutions containing a deficit of amino acids.

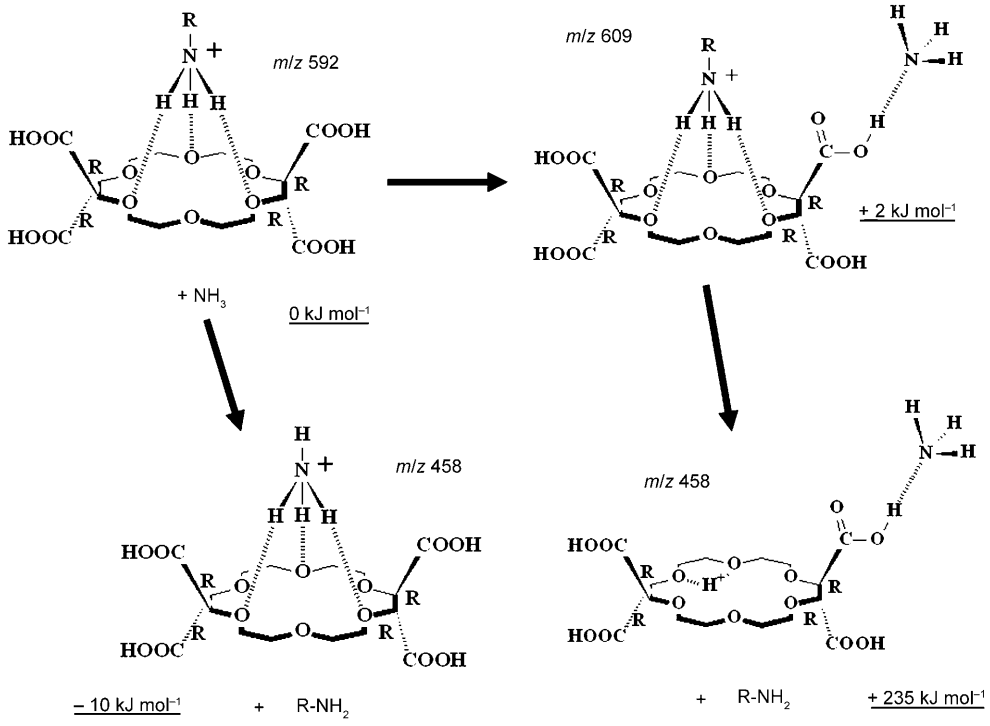
As far as proline is concerned, the obtained results (not shown) are similar to those previously determined with a solution containing an excess of amino acids. The observation of a linear relationship whatever the initial solution represents definitive proof that the protonated proline–crown ether noncovalent interaction is nonspecific.

It is also possible to characterize the intrinsic enantioselectivity by measuring the slope of the obtained linear relationship. For phenylglycine, threonine, and proline, this value is −4.36, +13.45, and −12.68, respectively. These results clearly demonstrate the reversed enantioselectivity between phenylglycine and threonine. It is also revealed that, whereas enantioselectivity is not observed for proline in solution, the enantioselectivity detected for this amino acid in the gas phase is even more pronounced than that for phenylglycine.

The mechanism leading to the gas-phase exchange of the neutral amino acid by ammonia inside the protonated noncovalent complex deserves further attention. We have decided to focus our discussion and our calculations on the associative ion/molecule reaction between the [D-PGH$^+$•(+)-18C6TA] mass-selected ion (m/z 592) and ammonia to give [NH_4^+•(+)-18C6TA] plus neutral D-phenylglycine. Different ionic products are observed when m/z 592 ions react with neutral ammonia (Figure 5). Besides the mass-selected cations detected at m/z 592, signals are also observed at m/z 458 and 609, which correspond to the exchange-reaction product and to the addition of neutral ammonia to the mass-selected cations, respectively. At first sight, this terbody species can be considered as the key intermediate along the way to the exchange process [Eq. (8)].

$$\begin{array}{l}[\text{D-PGH}^+ \cdot (+)\text{-18C6TA}]+\text{NH}_3 \rightarrow \\ m/z\ 592 \\ [\text{D-PGH}^+ \cdot (+)\text{-18C6TA} \cdot \text{NH}_3] \rightarrow \\ m/z\ 609 \\ [(+)\text{-18C6TA} \cdot \text{NH}_4^+]+\text{D-PG} \\ m/z\ 458\end{array} \quad (8)$$

Scheme 5 schematically presents the key structures that are expected to play a significant role during the gas-phase exchange reaction. Just after the low-kinetic-energy collision, a terbody complex associating protonated D-phenylglycine, the chiral crown ether, and a molecule of ammonia in the vicinity of one of the carboxylic acid groups of the crown ether is created (Scheme 5). This process is calculated to be endothermic by 2 kJ mol^{-1} at the AM1 level. Therefore, because this reaction can be considered as a thermoneutral process, the observation of the corresponding signal at m/z 609 amongst the ion/molecule reaction products (Figure 5) is consistent. Nevertheless, direct elimination of neutral phenylglycine from this complex is not likely to occur, given the high energy requirement calculated for this process (about 235 kJ mol^{-1} from the terbody species). This reaction will afford a noncovalent complex associating the



Scheme 5. Gas-phase ligand-exchange reaction: proposed reaction mechanism and intermediates. Energies obtained at the semiempirical Hartree–Fock AM1 level of theory (see Table 2).

ring-protonated crown ether with a molecule of ammonia located close to one carboxylic acid group (Scheme 5). On the other hand, the production of a noncovalent complex that binds the ammonium cation with the crown ether ring with three hydrogen bonds appears to be a favorable process and the inherent exothermicity, which amounts to 10 kJ mol^{-1}, explains the production of the *m*/*z* 458 cations.

Conclusion

ESIMS and ESIMSMS analyses of the amino acid/18C6TA system were performed to gain information on the molecular mechanisms that drive the chiral-recognition process. Phenylglycine, threonine, and proline as amino acids and amino esters were selected in this work because qualitative and quantitative results have been reported in recent articles. Experiments were performed to probe the chiral-recognition ability of the chiral selector towards the selected amino acids, both in solution (by the enantiomer-labeled-guest method) and in the gas phase (by kinetic-energy-resolved collision-induced dissociation methodology or by gas-phase ligand-exchange ion/molecule reactions). All of the experimental results were in close agreement with the previously reported data as far as the reverse enantioselectivities of phenylglycine and threonine were concerned. For proline, comparison of the obtained experimental data points to a nonspecific electrostatic interaction because the noncovalent complexes observed by ESIMS are likely to be generated during the electrospray ionization process. Semiempirical Hartree–Fock calculations were also performed to ascertain the structures of the noncovalent complexes based on phenylglycine and to propose a mechanism for the gas-phase ligand-exchange reaction; the good agreement observed between theory and experiment encourages further calculations to elucidate the structural and energetic properties of other complexes.

Experimental Section

Mass spectrometry investigations: All experiments were performed on a Waters QToF2 mass spectrometer. The analyte solutions were delivered to the ESI source by a Harvard Apparatus syringe pump at a flow rate of 5 μL min^{-1}. Typical ESI conditions were: capillary voltage 3.1 kV, cone voltage 15 V, source temperature 80 °C, and desolvation temperature 120 °C. Dry nitrogen was used as the ESI gas. For the recording of single-stage ESIMS spectra, the quadrupole (rf-only mode) was set to pass ions from 50 to 800 Th, and all ions were transmitted into the pusher region of the time-of-flight analyzer where they were mass analyzed with a 1 s integration time. Data were acquired in continuum mode until acceptable averaged data were obtained. For the ESIMSMS CID experiments, the ions of interest were mass selected by the quadrupole mass filter. The precursor ion resolution was adjusted to select only the monoisotopic signal amongst the complete isotopic cluster. The selected ions were then submitted to collision against argon in the rf-only hexapole collision cell (pressure estimated at 0.9–1 mBar), and the laboratory-frame kinetic energy was selected to afford sufficiently intense fragment-ion signals. All ions coming out of the hexapole cell, either fragments or the nondissociated precursor ions, were finally mass measured with the oa-ToF analyzer. As far as the ligand-exchange experiments are concerned, the rf-only hexapole cell was pressurized with ammonia (see below) to reach a constant and reproducible pressure of approximately 1.0×10^{-4} mBar on the penning gauge situated outside the cell. The kinetic energy of the in-

cident ions was then fixed at approximately 5 eV to maximize the yield of the associative ion/molecule reaction processes and to avoid any dissociation processes. Again, all ions coming out of the hexapole cell, either the ion/molecule reaction products or the unreacted precursor ions, were finally mass measured with the oa-ToF analyzer.

Materials: All samples used in the present work were commercially available, except for the α-amino esters, which were prepared according to a procedure described in the literature.[17] D- and L-phenylglycine and (−)-([18]crown-6)-2,3,11,12-tetracarboxylic acid were purchased from Sigma–Aldrich, whereas D-and L-proline and D-and L-threonine were purchased from Acros Organics. Ammonia used as the ligand-exchange reagent was a synthetic mixture of 1% ammonia in argon obtained from Praxair Belgium.

Calculations: The geometry of the isolated molecules was optimized at the semiempirical Hartree–Fock Austin Model 1 (AM1) level with the AMPAC package.[18] This method is well adapted to describe the geometry of systems containing hydrogen bonds. (+)-([18]Crown-6)-2,3,11,12-tetracarboxylic acid was fully optimized in its neutral conformation, whereas all amino acids were optimized in their protonated form (charge = +1).

The complexation energies were determined from the difference between the total AM1-calculated energies of 1) the fully optimized complex with the amino acid initially positioned near the top of the (+)-18C6TA host (so that the global minimum is reached and is ensured by a frequency analysis) and 2) the two isolated molecules in their equilibrium geometry. The choice of the AM1 method is validated by the fact that the formation of the complexes is mostly driven by electrostatic interactions that are properly described at this level of theory.

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