The Gas-Phase Chemistry of Cyclodextrin Inclusion Complexes

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ABSTRACT
Complexes of cyclodextrins with amino acid analytes are produced in the gas phase and are ideal for the study of molecular recognition. In this Account, we discuss the evidence for the presence of gas-phase inclusion complexes and the nature of the interaction. The use of cyclodextrins as chiral selectors in gas-phase guest-exchange reactions is illustrated, and the nature of the enantioselectivity is discussed. The development of the enantioselective reaction into an analytical method for determining enantiomeric excess is also described.

Introduction

Complexes of cyclodextrins are studied in the gas phase to obtain fundamental insight into the nature of molecular recognition. Cyclodextrins are a group of cyclic oligosaccharides composed of α(1,4)-linked glucopyranose units.[1,2] The most common have six, seven, and eight units with the common names α-, *β*-, and *γ*-cyclodextrin, respectively. The utility of cyclodextrins stems from their molecular shapes, which are often described as "turos-like" (see, for example, *β*-cyclodextrin, Scheme 1). The wide rim is composed of (carbon 2) C2−OH and C3−OH groups, while the narrow rim is composed of C6−OH groups. The molecules, in their most symmetrical forms, resemble truncated cones with a sizable inner cavity. In solution, compounds that are more hydrophobic than cyclodextrins insert into the cavity to produce *inclusion complexes*. This feature makes them suitable for an extremely large number of applications from drug delivery devices to enzyme mimics.[1,2] Cyclodextrins also provide simple but highly specific systems for the study of host−guest interactions.

Complexes of protonated amino acids and cyclodextrins [CD:AA+H$^+$] are readily produced in the gas phase using electrospray ionization (ESI).[3,4] In the Fourier transform mass spectrometer, the ions persist until they are energetically activated or reacted with neutral reagent gases. The gas phase offers the opportunity to observe the intrinsic properties of the complexes without the complicating effect of solvent molecules. This makes it easier, for example, to identify specific host−guest interactions. However, the driving force for the formation of inclusion complexes in solution is the so-called "hydrophobic effect".[5] It is caused by a combination of factors, including the liberation of water from the cyclodextrin cavity after the inclusion of a guest compound. In the gas phase where the hydrophobic effect is absent, it was not apparent that inclusion complexes could persist. There was a general concern that the corresponding mass, observed in the mass spectra, belonged simply to nonspecific ion−dipole complexes.[6]

Cyclodextrins are commonly used as selectors for chiral separation in high-performance liquid chromatography and capillary electrophoresis.[7] In the gas phase, the complexes undergo guest-exchange reactions whereby the analyte is replaced by a gaseous reagent base such as *n*-propylamine.[8] The rates of the reactions differed for each enantiomer, making cyclodextrin also a selector in the gas phase. The exchange reaction was common to the amino acids and selected pharmaceutical compounds. It has been developed into an analytical method for quantifying enantiomeric excesses.[9] The reactions are also ideal for studying chiral recognition in small well-defined systems, thereby allowing the investigation of the role of the "three-point" interaction in gas-phase enantioselective reactions.

Enantioselective Reactions with *β*-Cyclodextrin Hosts

Protonated cyclodextrin:amino acid complexes [CD:AA+H]$^+$ react with gaseous alkylamines to undergo a gas-phase guest-exchange reaction.[8] The reaction, outlined in reaction 1, involves essentially a proton transfer between the amino acid (AA) and the alkylamine (B). The resulting

$$[\text{CD:AA} + \text{H}]^+ + \text{B} \xrightarrow{k_{\text{L/D}}} [\text{CD:B} + \text{H}]^+ + \text{AA} \qquad (1)$$

protonated species remains coordinated to the permethylated cyclodextrin while the neutral species leaves. All the work discussed in this Account, unless specified, will refer to permethylated derivatives of cyclodextrin. However, exchange reactions with complexes of ammonia and underivatized *β*-cyclodextrins have also been observed.[10] It was found that the rate of the reaction was sensitive to the chirality of the amino acid, making cyclodextrin a gas-phase chiral selector. The enantioselectivity (*S*, defined by the ratio $S = k_L/k_D$) increased from Ala, the smallest ($S = 1.6$), to Val (3.1), Ile (3.8), and Leu (3.6) when *n*-propylamine was used as the gaseous reagent (B) (Table 1).[8,11] However, Phe and Tyr with relatively large alkyl groups exhibited significantly smaller enantioselectivities, 0.82 and 0.67, respectively. Similar trends were obtained when more basic alkylamines were used, including 2-butylamine and 1-amino-2-propanol.[12] These results indicated that increasing the size of the alkyl side chain increased

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Scheme 1

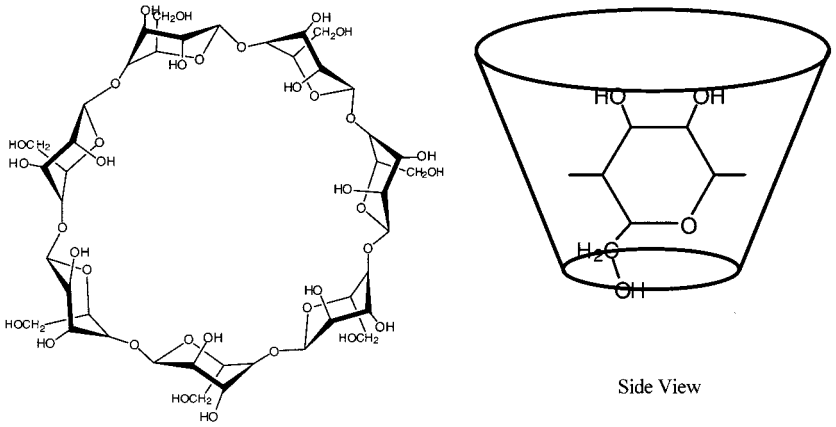


Table 1. Reaction Selectivity (Defined by the Ratio k_L/k_D) of Various Amino Acid Complexes Reacting with *n*-Propylamine and Ethylenediamine (Italics)[a]

amino acid	k_L	S (k_L/k_D)
Ala	2.4	1.6
Asn	*0.13*	*0.93*
Asp	0.02	2.2
Cys	3.4	2.2
Glu	0.011	1.9
His	*0.019*	*2.3*
Ile	1.0	3.8
Leu	0.50	3.6
Met	0.014	0.37 (2.70)
Phe	1.4	0.82 (1.2)
Pro	1.2	1.5
Ser	0.064	1.2
Thr	0.12	0.63 (1.6)
Trp	*0.019*	*2.1*
Tyr	*0.019*	0.67
Val	3.1	3.1

[a] Permethylated β-CD was used as hosts. The inverse of some selectivity values are given in parentheses. The list was compiled from refs 8, 9, 11, and 12. Values of selectivities (k_L/k_D) are averages, with multiple values within ±5%. Rate constants for L enantiomers are $k_L \times 10^{-11}$ cm^3/molecule·s.

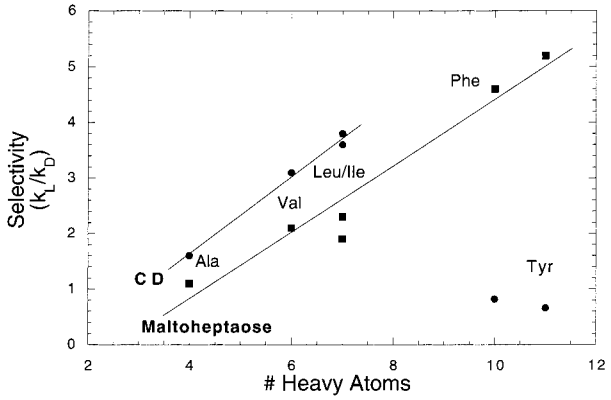


FIGURE 1. Plot of the enantioselectivity ($S = k_L/k_D$) as a function of the number of non-hydrogen atoms on the amino acid side chain. The hosts are permethylated β-cyclodextrin (solid circles) and the linear analogue, permethylated maltoheptaose (solid triangle). *n*-Propylamine was used as the reagent base.

enantioselectivity to a point (Figure 1, solid circles). Leu and Ile with four carbons in the side chain had the optimal size to yield high enantioselectivity with permethylated β-cyclodextrin, while Phe and Tyr with seven carbons were too large and yielded smaller enantioselectivities.

Molecular modeling simulations predicted the formation of inclusion complexes and provided insight into the differences in the reactivity of the different enantiomers.[11] The most stable conformation for D- and L-Val interacting with permethylated β-cyclodextrin is shown in Figure 2. To obtain these structures, calculations commenced with both included and nonincluded geometries using the Insight II program from Biosym Technologies and the consistent valence force field (CVFF). The structures were obtained from 50 heating and annealing cycles. Both sets of calculations yielded similar results, with the inclusion structure being the most favored. In the illustration (Figure 2), the narrow (lower) rim is composed of carbon 6 (C6) methoxyl groups, while the wide (upper) rim is composed of C2 and C3 methoxyl groups. The amino acid is shown in space-filling while the oligosaccharide is shown in wire frame. Both D- and L-amino acids are fully included. Examination of the hydrogen-bonding interactions in the valine complexes indicated that the ammonium group in the L-isomer binds primarily with the narrow rim (NH···O, 2.09 and 1.90 Å) and the glycosidic bond oxygen (NH···O, 2.15 Å). The carboxylic hydrogen interacted with the glycosidic bond oxygen (OH···O, 2.32 Å). In the D-isomer, the ammonium group interacted with the narrow rim (1.96 Å), the glycosidic bond (2.24 Å), and the wide rim (2.12 Å). The carboxylic acid interacted with the wide rim (1.84 Å). The interaction of the two groups with the cyclodextrin further helped orient the alkyl side chain. In the L-complex it was oriented toward the wide rim, while in the D-complex it was oriented more toward the side of the cavity. There were noticeable differences in the interactions of the two isomers with the cyclodextrin host. Chiral differentiation occurs when the access to the protonated ammonium group is limited either by the alkyl side chains or by the methoxyl groups on the host that are drawn in by hydrogen-bonding interactions. Thus, the differences in binding translate to differences in reaction rates.

The behavior of Phe under molecular dynamics calculations differed from that of Val. With Phe, both L- and

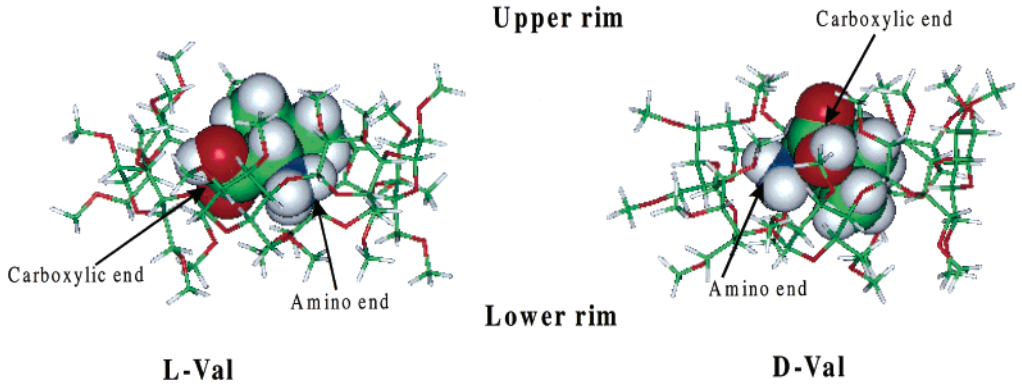


FIGURE 2. Lowest energy structure of protonated valine complexed to permethylated β-cyclodextrin obtained from molecular modeling calculations. The calculations were started with both included and nonincluded structures. The results were nearly identical, with the included structure being the most favorable. With L-Val (left), the attractive interactions occur along the lower rim, while with D-Val (right), the ammonium interacts with the lower rim and the carboxylic acid interacts with the upper rim.

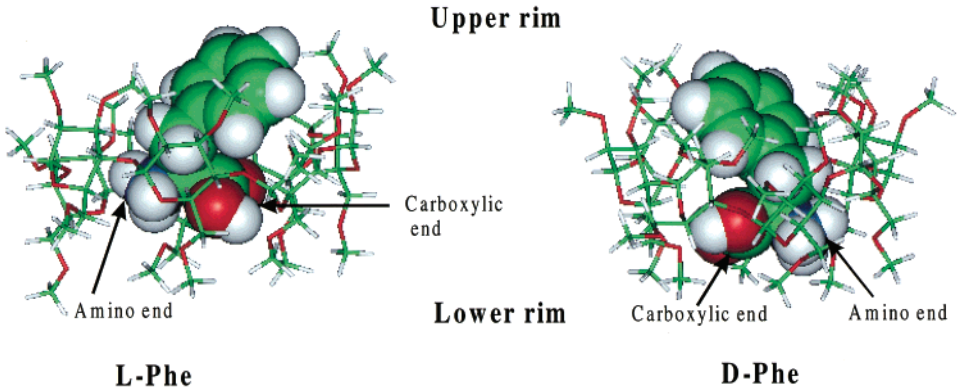


FIGURE 3. Lowest energy structure of protonated phenylalanine complexed to permethylated β-cyclodextrin obtained from molecular modeling calculations. Both included and nonincluded initial geometries yielded the same result. The L- and D-isomers interact in the same manner, with the attractive interactions occurring along the lower rim.

D-isomers interacted in similar ways. That is, both ammonium and carboxylic acid groups interacted predominantly with the narrow rim (Figure 3). Apparently, the cavity constrained the phenyl group, forcing both the amine and carboxylic acid groups to interact primarily with the narrow rim. The phenyl group remained inside the cavity but was shifted slightly higher to increase the interaction of the protonated amine with the narrow rim. The similarities in the binding of the L- and D-isomers are consistent with the small enantioselectivity ($S = 0.8$) observed between L-Phe and D-Phe.

Gas-Phase Species Are Inclusion Complexes

It was initially believed that the presence of specific mass-to-charge ratios corresponding to the expected value for the inclusion complexes meant that an inclusion complex was produced in the gas phase.[3,4] A study by Prokai employing in-source CID on cyclodextrin−amino acid complexes produced results that were consistent with gas-phase inclusion complexes.[13] Vouros and co-workers reported a study involving a large number of guest molecules including amino acids with aromatic and nonaromatic side chains and peptides. They concluded, based on the ESI-MS of reference compounds that were believed not to form inclusion complexes in solution, that the protonated complexes observed in the gas phase were merely the products of electrostatic interactions and not inclusion.[6]

Experimental results obtained from the guest-exchange reaction point to a cooperation between the size of the host cavity and the size of the guest. A maximum guest size exists for optimal enantioselectivity with β-cyclodextrin. This correspondence between the guest size and the cavity dimension is a necessary consequence of inclusion. Nonspecific complexes involving simple ion−dipole interactions may show either a gradual increase in the enantioselectivity or no change when the size of the amino acid is increased for a specific host.

Additional experiments were performed to examine further the role of molecular size in the selectivity by varying the size of the host. Increasing the extent of methyl derivatization in β-cyclodextrin from 14 to 21 methyl groups decreased the effective size of the cavity, as each additional methyl group orients itself toward the center of the cavity.[11] Because the amino acids Val, Ile, and Leu have high enantioselectivity with β-cyclodextrins, it was believed that varying the degree of methylation would affect the enantioselectivity of these compounds the most. Indeed, increasing the derivatization, for example with Val, decreased the selectivity from 5.5 for the 14 methyl

derivative to 3.1 for the 21-methyl (or the permethyl) derivative.[11] The enantioselectivity of alanine, a relatively small amino acid, fluctuated between 0.6 and 1.6 with increasing methyl derivatization but stayed generally low. Ala is a sufficiently small amino that minor changes in the cavity size do not affect its enantioselectivity.

We also increased the size of the cavity further by studying the exchange reactions with γ-cyclodextrins.[11] The size of the internal cavity increases with the number of glucose units, with diameters ranging from 4.7 to 5.3 Å for α-, 6.0 to 6.5 Å for β-, and 7.5 to 8.3 Å for γ-cyclodextrin.[2] The larger cavity size decreased *S* for Val (from 3.1 to 0.71 for γ-CD), Ile, and Leu (3.6 to 1.4), consistent with the notion that the three amino acids have optimal enantioselectivity with β-cyclodextrin. Conversely, Phe increased in selectivity (or rather 1/*S*) from 1.2 to 1.8. Presumably, the larger cavity size allowed each enantiomer of the larger amino acid to find more distinct interactions with the larger host.

We may conclude from the experimental and theoretical data that protonated amino acid−cyclodextrin complexes are inclusion complexes in the gas phase. The question now is whether gas-phase inclusion complexes come directly from solution. One of the main points of Vouros's study was that the presence of a gas-phase complex does not necessarily confirm the presence of solution-phase inclusion complexes.[6] Indeed, molecular modeling calculations suggest that nonspecific complexes in the solution phase may convert to inclusion complexes in the gas phase. Calculations that were initiated with the analyte either on the rim or on the outer surface of the cyclodextrin yielded annealed structures where the analyte migrated into the inner cavity.[11,12] This event could occur during electrospray ionization.

Linear Oligosaccharides Produce Quasi-Inclusion Complexes

The maltose-based oligomers are exact linear analogues of the cyclodextrins. For example, maltoheptaose is composed of seven α(1−4)-linked glucose units such as β-cyclodextrin. We initially believed that the linear oligosaccharides would allow us to compare the behavior of the included versus nonincluded guest. Instead, experimental and theoretical results indicate that linear oligosaccharides are sufficiently flexible to wrap around guest molecules and form "quasi-inclusion" complexes in the gas phase.[12]

Exchange experiments of amino acids were repeated with the linear hosts. Table 2 lists the enantioselectivities ($S = k_L/k_D$) for the exchange reactions of a selected group of amino acids complexed to permethylated maltoheptaose and reacted with *n*-propylamine.[12] The enantioselectivities of the exchange reactions were slightly less than those with the cyclodextrins for most of the amino acids. However, the same trend was obtained for the amino acids with alkyl side chains, i.e., *S* increased with the increasing size of the alkyl side chain. Remarkably, the reactivity of both Tyr and Phe complexed to maltoheptaose exhibited significantly greater enantioselectivity relative to β-cyclodextrin. For example, the value *S* for Phe increased from 0.8 for β-CD to 4.6 for maltoheptaose. A plot with maltoheptaose and the amino acids with alkyl side chains shows that Phe and Tyr are now in linear agreement with the other amino acids (Figure 1, solid squares).

Table 2. Enantioselectivity (k_L/k_D) of Selected Amino Acids with Linear Oligosaccharide Hosts (Maltoheptaose = Heptaose, etc.)[12] [a]

amino acids	heptaose S (k_L/k_D)	hexaose S (k_L/k_D)	pentaose S (k_L/k_D)
Ala	1.1		1.1
Val	2.1		1.0
Ile	2.3		1.5
Leu	1.9	1.4	1.8
Phe	4.6	1.2	1.2
Tyr	4.9		

[a] All hosts compounds are permethylated

Molecular modeling calculations again give important insight into the nature of the interaction. Figure 4 shows the result of a simulation with the complexes of maltoheptaose and Phe. The maltoheptaose hosts are oriented as similarly as possible in both structures, with the C2 and C3 (wide rim) again on top and the C6 on the bottom. The appearance of the complex is consistent with an inclusion-type interaction where maltoheptaose fully envelops the protonated phenylalanine. More importantly, the orientation and hence the bonding interaction of L- and D-Phe appear different, even with simple visual inspection. In the L form, the phenyl group is oriented down, while in the D form it is oriented up. The differences in the binding interaction readily support the large experimental *S* value obtained.

Smaller linear hosts such as maltohexaose and maltopentaose were examined, but lower enantioselectivities were obtained (Table 2).[12] For Phe, *S* decreased considerably from 4.6 for maltoheptaose to 1.2 for hexaose and 1.2 for pentaose. Molecular modeling calculations predicted that these hosts were too small to fully envelop the guest and create an environment for high enantioselectivity.

Enantioselectivity and the Three-Point Interaction Model

In the study of solution-phase cyclodextrin inclusion complexes, the "three-point attachment"[14,15] model is often invoked as the putative mode of interaction that governs enantioselectivity. It is composed of hydrogen bonding, inclusion, and steric hindrance.[16] In the gas phase an analogous three-point attachment, or rather a more general three-point interaction,[17] can be formulated to understand enantioselectivity in the guest-exchange reactions. For α-amino acids, the three-point interaction is illustrated in Scheme 2, where the ammonium group (the protonated terminal amine) and the carboxylic acid group induce points of attraction, via hydrogen bonding, and the side chain induces either repulsion or attraction depending on the functional groups. In our molecular modeling calculations, we generally assign the terminal

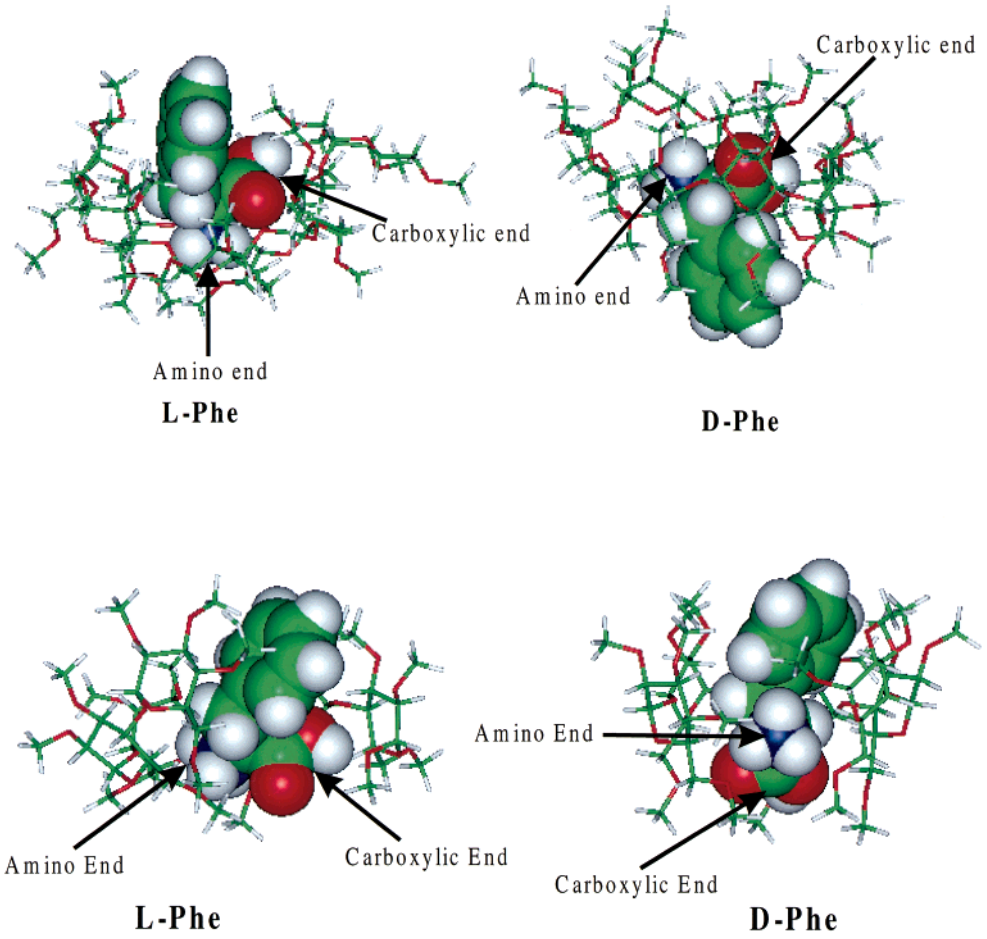
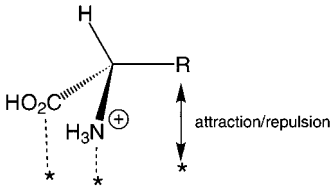


FIGURE 4. Lowest energy structures of protonated phenylalanine complexed to (a) permethylated maltoheptaose and (b) maltopentaose obtained from molecular modeling calculations. Unlike the cyclic analogue (β-CD), the linear maltoheptaose allows each enantiomer to find its most favorable conformation. The large selectivity obtained with maltoheptaose is consistent with the large difference in binding interactions of the two enantiomers.

Scheme 2

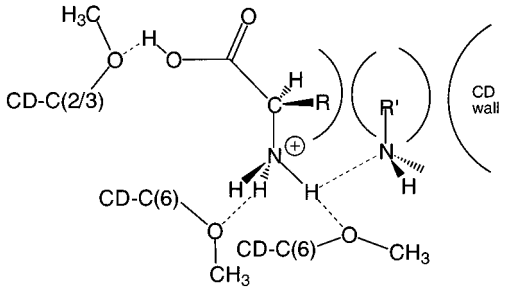


amine as the site of protonation. It is the most basic site in complexes with amino acids having alkyl side chains. Furthermore, CID experiments of the gas-phase complex indicate that the proton that induces fragmentation originates from the protonated terminal amine.[18]

For optimum enantioselectivity, there is a preference for two attractive and one repulsive over three attractive interactions. For example, Asp and Glu, with three attractive hydrogen-bonding interactions, exhibit only moderate selectivity (S = 2.2 and 1.9, respectively) compared to similarly sized amino acids such as Val (3.1), with only two attractive interactions (Table 1).

Two attractive interactions also yield better selectivity than a single attractive one. The conversion of the amino acids to the corresponding methyl esters decreases the number of attractive interactions. For the amino acids

Scheme 3



with alkyl side chains, esterification decreased the enantioselectivity.[12] For example, the esters of both valine (S = 0.90) and Leu (S = 0.76) exhibited significantly less selectivity than the native amino acids.

The nature of the interaction between the protonated amino acid and the cyclodextrin determines the rate of proton transfer to the reagent amine. This is conceptually easier to understand in the case of the two-point attraction, where the analytes are the amino acids with alkyl side chains. For exchange to occur, an incoming base must enter the cavity and compete for the proton as shown in Scheme 3. The extent to which the R group interferes with the incoming base depends on the chirality

of the guest, the size of R, and the size of the cavity. When the size of R is increased, enantioselectivity increases. When R is too large, as with Phe and Tyr, the bulky side chains are forced by the fixed cavity size of the β-CD host to adopt similar configurations, thereby decreasing selectivity.

Linear oligosaccharides provide "cavities" with adjustable sizes that expand to provide the optimal size for enantioselectivity with amino acids such as phenylalanine, tyrosine, and tryptophan. However, for smaller molecules, enantioselectivity is generally greater with cyclodextrin because it is more rigid than its linear analogue. It is known that some rigidity is important for enhancing enantioselectivity. Still et al. pointed out that the limited conformational flexibility of the macrocycles they studied was a key element in obtaining enantioselectivity.[19] Rigidity of the host is consistent with the "three-point interaction model", as it ensures that the diastereomeric complexes are structurally and thermodynamically different. Therefore, a more rigid host usually exhibits higher selectivity than a less rigid one.

Probing the Strength of the Host−Guest Interactions

Heated capillary dissociation (HCD)[20] was employed to probe the interactions in the cyclodextrin−amino acid complexes (reaction 2). A heated capillary is used in the

$$[\mathrm{CD{:}AA} + \mathrm{H}]^+ \xrightarrow{\Delta} \mathrm{CD} + [\mathrm{AA} + \mathrm{H}]^+ \qquad (2)$$

ionization source to desolvate analyte ions during electrospray ionization.[21] By increasing the temperature beyond that used for desolvation, complexes were dissociated to their molecular constituents, providing a relative measure of binding strengths. Although HCD may be used to determine the activation barrier and the pre-exponential factor of the dissociation reaction,[22,23] it must first be calibrated with another experimental method such as blackbody infrared radiative dissociation (vide infra).[24] The dissociation temperatures of various cyclodextrin−amino acid complexes were determined. Although the activation barrier for the dissociation of the complex cannot be accurately determined at this time, the dissociation temperatures still provided the relative dissociation threshold of the complex (Table 3).[25] The dissociation temperature did not necessarily correlate with the proton affinity (or gas-phase basicity) of the amino acid but rather with the number of possible hydrogen-bonding interactions. It was found that the dissociation temperature depended on the types of functional groups on the side chain.[20] The dissociation temperature was the lowest when R = alkyl, higher when R = hydroxyl and carboxylic, and highest when R = amine. Lys had the highest dissociation temperatures of all the amino acids. There was no correlation between the selectivity and the dissociation temperature.

The behaviors of the smallest amino acids do not follow the apparent trend. Gly and Pro formed more stable complexes than some amino acids with polar side chains. Similarly, the Ala complex was more stable than the amino acids with larger alkyl side chains. These effects are further evidence of inclusion. The methyl derivatives on the rims of cyclodextrins retain the smaller amino acids better than the larger ones. Decreasing the degree of derivatization decreases the relative stability of the small amino acids, as removing the methyl groups makes it easier for the amino acid to escape the cavity.[25]

Table 3. Dissociation Temperatures (T_d) of Various Protonated Amino Acids Complexed to Tri-*O*-methyl-β-cyclodextrin[a]

amino acid	T_d (average) (°C)	GB (kJ/mol)	PA (kJ/mol)
Lys	460	951	996
His	457	950.2	988
Arg	441	1006.6	1051
Gly	415	852.2	886.5
Pro	413	886	920.5
Ser	413	880.7	914.6
Gln	406	900	937.8
Trp	403	915	948.9
Thr	403	888.5	922.5
Ala	396	867.7	901.6
Tyr	392	892.1	926
Phe	386	888.9	922.9
Leu	386	880.6	914.6
Val	378	876.7	910.6
Ile	368	883.5	917.4

[a] The gas-phase basicity (GB) and proton affinity (PA) are obtained from the literature.[29]

Blackbody infrared radiation emanating from the wall of the vacuum chamber was used to dissociate the complexes of cyclodextrins. This method was developed by Williams and co-workers to obtain activation barriers in the gas-phase dissociation of peptides and proteins.[26,27] Blackbody infrared radiative dissociation (BIRD) has not yet been used to dissociate the amino acid complexes, but it has been used on a selected group of cyclodextrin−peptide complexes.

Interestingly, the reaction products of BIRD do not correspond to the simple dissociation of the complex but rather the dissociation of the peptide. Two major products are observed in the BIRD of $[\mathrm{CD{:}BK} + 2\mathrm{H}]^{2+}$) corresponding to $[\mathrm{CD{:}Arg\text{-}Pro} + \mathrm{H}]^+$ and [Pro-Gly-Phe-Ser-Pro-Phe-Arg + H]$^+$ (reaction 3). Analogous fragments were ob-

$$[\mathrm{CD{:}RPPGFSPFR} + 2\mathrm{H}]^{2+} \xrightarrow{h\nu} [\mathrm{CD{:}RP} + \mathrm{H}]^+ + [\mathrm{PGFSPFR} + \mathrm{H}]^+ \qquad (3)$$

tained in the BIRD of uncomplexed BK ($[\mathrm{BK} + 2\mathrm{H}]^{2+}$), namely $[\mathrm{Arg\text{-}Pro} + \mathrm{H}]^+$ and [Pro-Gly-Phe-Ser-Pro-Phe-Arg + H]$^+$.[27] The cyclodextrin appeared to have little influence on the type of fragments produced, suggesting a passive role for cyclodextrin as "solvent cage" in the peptide fragmentation reaction.

From the rate constants at various temperatures, an Arrhenius plot was constructed to yield an E_a of 1.07 ± 0.09 eV and an A of 2.3×10^{11}. Several other cyclodextrin−peptide complexes were examined, including the peptide GGBK, where the two Phe residues were replaced with Gly—the putative position of inclusion. The photodissociation of the complex $[\mathrm{CD{:}GGBK} + 2\mathrm{H}]^{2+}$ produces fragments that are analogous to the BK complex, corresponding to the loss of water and peptide bond cleavage

to yield the analogous products with the shorter fragment also coordinated to CD. The GGBK complex has a slightly smaller activation barrier (0.79 eV).

Molecular modeling calculations are consistent with the coordination of the CD to the Arg-Pro fragment rather than to the longer piece. The calculations of the complex $[CD:BK + 2H]^{2+}$ initiated with the phenyl groups inside the cavity produced structures where the Arg group migrated into the cyclodextrin cavity to allow the guanidino group to interact with the narrow rim.

Analytical Applications of Guest-Exchange Reactions in the Determination of Enantiomeric Excess in Mixtures of Amino Acids and Pharmaceutical Compounds

A mass spectrometry-based method for the quantitative determination of enantiomeric excess has many attractive features including speed, sensitivity (in terms of amounts of total analyte rather than enantiomeric compositions), and the capability for obtaining structural information. The latter is particularly important in heterogeneous mixtures, where several components are present. The enantioselective guest-exchange reaction was further investigated to determine its analytical potential.

The most sensitive and simplest method for employing the guest-exchange reaction is to exploit the differences in peak heights, rather than obtaining rate plots for each mixture and deconvoluting the apparent rate constants. This has the advantage that the analysis of the mixture will require only a single mass spectrum, in contrast to the alternative method of determining rate constants for mixtures.

Peak height analysis involved the creation of a calibration curve consisting of the relative intensity of reactant and product ions at a specified reaction time. A calibration curve was produced by measuring the peak heights of various mixtures. A two-point calibration would be sufficient for quantitative determination of enantiomeric excess, but more points produced more accurate numbers. A calibration curve is shown for phenylalanine ($S = 4.6$) reacting with *n*-propylamine in maltoheptaose (Figure 5c). A reaction time of 25 s was found to be optimal, and six points (mixtures) were obtained to produce a coefficient of determination (r^2) of 0.999 (Figure 5c, solid circles).

The quality of the calibration curve is governed by the enantioselectivity of the reaction. Low enantioselectivity produces the largest scatter and hence the largest error. For example, in the reaction of Ala with *n*-propylamine, the low selectivity (1.6) yields a calibration curve with $r^2 = 0.961$ (Figure 5a). This r^2 value increases for Leu ($S = 3.2$, Figure 5b).

Test mixtures were prepared and analyzed days and even months after the calibration curve was constructed. To determine the enantiomeric excess of an unknown mixture, its mass spectrum was measured at the same reaction time as the calibration curve. The test mixtures (solid triangles) distribute appropriately around the calibration curve (Figure 5). The results are tabulated (Table 4). As expected, the precision increases with increasing selectivity. In systems where the selectivity is lowest ($S = 1.6$), the average absolute difference between the known and the determined values is 8%, while for systems with high selectivity (e.g., $S = 4.6$) the difference is as little as 2.3%.

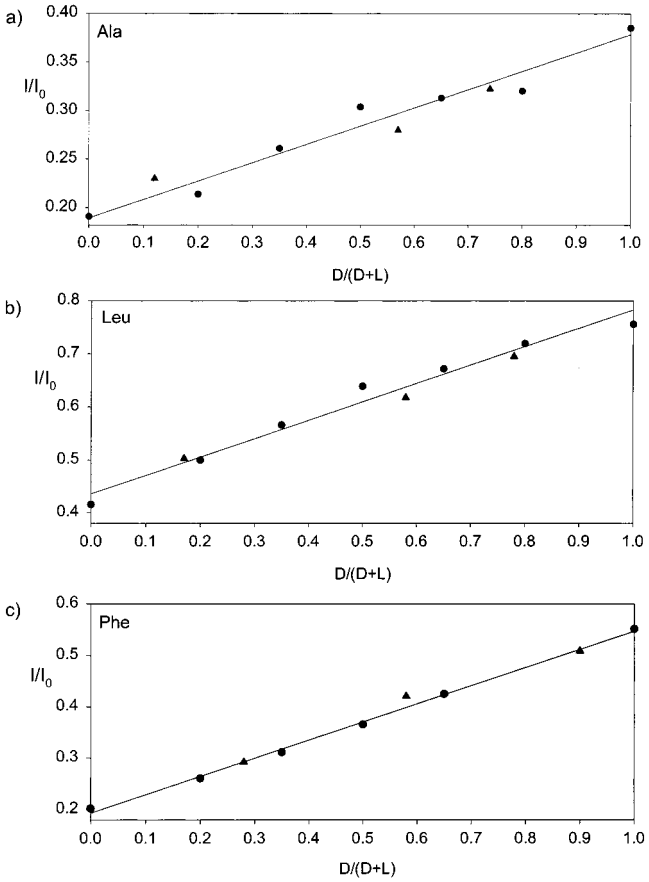


FIGURE 5. Calibration curves of (a) Ala, (b) Leu, and (c) Phe. The calibration points (solid circle) were obtained in the same day. The test points (solid triangles) were obtained from new mixtures prepared weeks and even months after the calibration curve was produced.

Table 4. Enantiomeric Excess and the Determined Values of Mixtures of L- and D- Amino Acids[a]

	prepared value D/[D + L] (×100)	determined value D/[D + L] (×100)	difference
		Ala	
test 1	12.0	22.2	12.2
test 2	57.0	48.1	8.9
test 3	74.0	70.9	3.1
average absolute difference			8.1
		Leu	
test 1	17.0	19.3	2.3
test 2	58.0	52.4	5.6
test 3	78.0	74.6	3.4
average absolute difference			3.8
		Phe	
test 1	28.0	27.9	0.1
test 2	58.0	64.2	6.2
test 3	90.0	89.3	0.7
average absolute difference			2.3

[a] The determined values were obtained directly from the calibration curves.

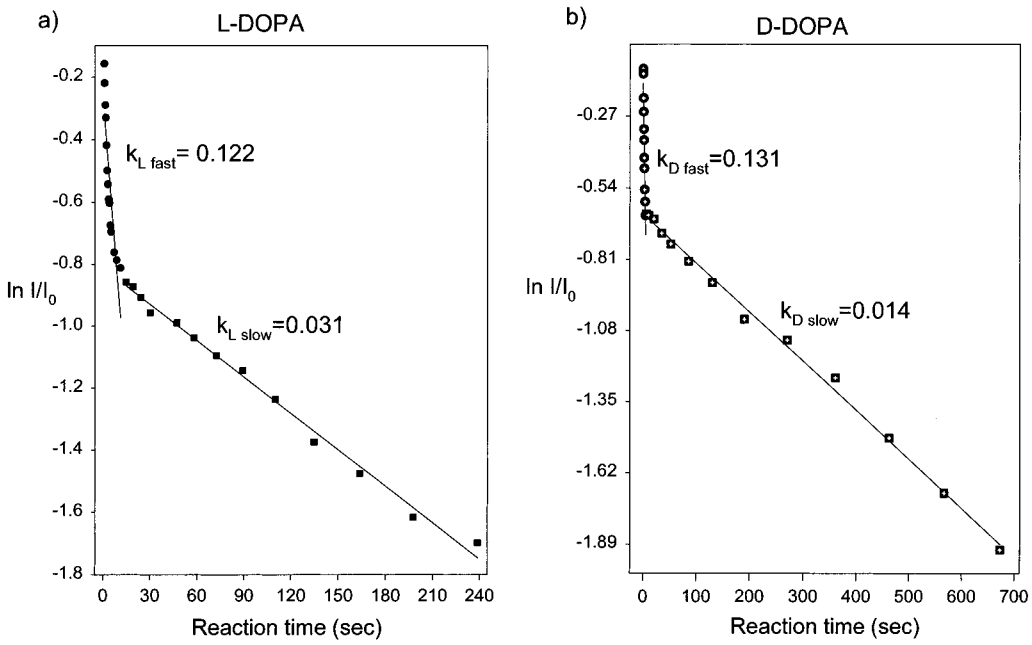


FIGURE 6. Rate plot of DOPA reacting with 1,3-diaminopropane. Two rates were obtained for each enantiomer corresponding to two different ion populations. A fast reaction exhibited little enantioselectivity, while the slow reaction yielded a selectivity of 2.1.

This method for determining enantiomeric excess has several advantages over existing methods. It has high detection limits; samples as little as picomole and femtomole quantities can be analyzed. Furthermore, once the calibration curve is established, the method is fast, requiring only a few seconds for each mixture. The calibration curve appears to remain accurate for a long period of time. We have used the same calibration curve for over a year.

Pharmaceutical compounds were also examined including DOPA, amphetamine, ephedrine, and penicillamine.[28] Enantioselective reactions were obtained for all compounds, but DOPA yielded some intriguing results. DOPA is structurally similar to phenylalanine and tyrosine; however, the guest-exchange reaction of DOPA is significantly slower than that of Phe. The reaction of the complex with β-CD is hardly observed with *n*-propylamine, making the rate constant for the reaction at least 3 or 4 orders of magnitude less. The difference in gas-phase basicities between *n*-propylamine (889.0 kJ/mol) and DOPA is part of the reason for the weak reactivity. The gas-phase basicity of DOPA has not been determined, but it is expected to be only slightly more than that of Tyr (892.1 kJ/mol).

To obtain a rate plot, the more basic amine diaminopropane (DAP) was used. The rate plots of both D and L differed from those of the amino acids by the presence of a break in the curve, indicating two reacting species (Figure 6). The rate constants of the fast-reacting component were over 10 times greater than those of the slow-reacting ones. The enantioselectivity of the fast reaction was low ($S = 0.93$), while for the slow reaction it was significantly larger (2.2). A calibration curve was constructed on the basis of the slow reaction ($S = 2.2$) with $r^2 = 0.996$. Molecular modeling calculations have been performed that suggest two reacting species, where the position of the analyte relative to the cyclodextrin differs.

Conclusion

The versatility and usage of cyclodextrins extend even into the gas phase. As in the solution phase, gas-phase complexes of analytes and cyclodextrins also involve inclusion structures. The majority of the work presented here, particularly with regard to chiral recognition, involved primarily amino acids; however, these studies are currently being extended to include peptides and other compounds of pharmaceutical importance. Other hosts, such as calixarenes, are also being studied. The resulting complexes should also undergo similar guest-exchange reactions with enantioselectivity in host compounds with appropriate derivatives. The method of BIRD appears promising as well and is highly suited for probing further the interactions between amino acids and their hosts.

Unfortunately, due to space limitations, it was not possible to include in this Account the works of others who have studied chiral recognition in the gas phase and those who have developed other mass spectrometry methods for determining enantiomeric excess. The reader is referred to the articles cited herein for more extensive discussions on these topics.

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References

(1) Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akadémiai Kiadó: Budapest, 1982.
(2) Szejtli, J. Introduction and General Overview of Cyclodextrin Chemistry. *Chem. Rev.* **1998**, *98*, 1743−1753.
(3) Camilleri, P.; Haskins, N. J.; New, A. P.; Saunders, M. R. Analysing the Complexation of Amino Acids and Peptides with β-Cyclodextrin Using Electrospray Ionization Mass Spectrometry. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 949−952.

(4) Selva, A.; Redenti, E.; Zanol, M.; Ventura, P.; Caseta, B. A Study of Beta-Cyclodextrin and Its Inclusion Complexes with Piroxicam and Terfenadine by Ionspray Mass Spectrometry. *Org. Mass Spectrom.* **1993**, *28*, 983−986.
(5) Rekharsky, M. V.; Inoue, Y. Complexation Thermodynamics of Cyclodextrins. *Chem. Rev.* **1998**, *98*, 1875−1917.
(6) Cunniff, J. B.; Vouros, P. False Positives and the Detection of Cyclodextrin Inclusion Complexes by Electrospray Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 437−447.
(7) Wan, H.; Blomberg, L. G. Chiral Separation of Amino Acids and Peptides by Capillary Electrophoresis. *J. Chromatogr. A* **2000**, *875*, 43−88.
(8) Ramirez, J.; He, F.; Lebrilla, C. B. Gas-Phase Chiral Differentiation of Amino Acid Guests in Cyclodextrin Hosts. *J. Am. Chem. Soc.* **1998**, *120*, 7387−7388.
(9) Grigorean, G.; Ramirez, J.; Ahn, S. H.; Lebrilla, C. B. A Mass Spectrometry Method for the Rapid Determination of Enantiomeric Excess in Mixtures of D,L-Amino Acids. *Anal. Chem.* **2000**, *72*, 4275−4281.
(10) Kellersberger, K. A.; Dejsupa, C.; Liang, Y.; Pope, R. M.; Dearden, D. V. Gas-Phase Studies of Ammonium−Cyclodextrin Compounds Using FT-ICR. *Int. J. Mass Spectrom.* **1999**, *193*, 181−195.
(11) Ramirez, J.; Ahn, S.; Grigorean, G.; Lebrilla, C. B. Evidence for the Formation of Gas-Phase Inclusion Complexes with Cyclodextrins and Amino Acids. *J. Am. Chem. Soc.* **2000**, *122*, 6884−6890.
(12) Ahn, S.; Ramirez, J.; Grigorean, G.; Lebrilla, C. B. Chiral Recognition in Gas-Phase Cyclodextrin: Amino Acid Complexes—Is the Three Point Interaction Still Valid in the Gas Phase? *J. Am. Soc. Mass Spetrom.* **2000**, in press.
(13) Ramanathan, R.; Prokai, L. Electrospray Ionization Mass Spectrometric Study of Encapsulation of Amino Acids by Cyclodextrins. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 866−871.
(14) Easson, E. H.; Stedman, E. Studies on the Relationship between Chemical Constitution and Physiological Action. Molecular Dissymmetry and Physiological Activity. *Biochem. J.* **1933**, *27*, 1257.
(15) Dalgliesh, C. E. The Optical Resolution of Aromatic Amino Acids on Paper Chromatograms. *J. Chem. Soc.* **1953**, 3940−3952.
(16) Kitae, T.; Nakayama, T.; Kano, K. Chiral Recognition of Alpha-Amino Acids by Charged Cyclodextrins through Cooperative Effects of Coulomb Interaction and Inclusion. *J. Chem. Soc., Perkin Trans. 2* **1998**, 207−212.
(17) Davankov, V. The Nature of Chiral Recognition: Is It a Three Point Interaction? *Chirality* **1997**, *9*, 99−102.
(18) He, F.; Ramirez, J.; Lebrilla, C. B. Evidence for an Intermolecular Proton-Transfer Reaction Induced by Collision in Gas-Phase Non-Covalently Bound Complexes. *J. Am. Chem. Soc.* **1999**, *121*, 4726−4727.
(19) Still, W. C.; Kilburn, J. D.; Sanderson, P. E. J.; Liu, R.; Wiley, M. R.; Hollinger, F. P.; Hawley, R. C.; Nakamima, M.; Bernardi, A.; Hong, J. I.; Namgoong, S. K. Enantioselective Hosts for Neutral Derivatives of Amino Acids. *Isr. J. Chem.* **1992**, *32*, 41−45.
(20) Penn, S. G.; He, F.; Green, M. K.; Lebrilla, C. B. The Use of Heated Capillary Dissociation and Collision-Induced Dissociation to Determine the Strength of Noncovalent Bonding Interactions in Gas-Phase Peptide−Cyclodextrin Complexes. *J. Am. Soc. Mass Spectrom.* **1997**, *8*, 244−252.
(21) Chowdhury, S. K.; Katta, V.; Chait, B. T. An Electrospray-Ionization Mass Spectrometer with New Features. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 81−87.
(22) Busman, M.; Rockwood, A. L.; Smith, R. D. Activation Energies of Gas-Phase Dissociations of Multiply Charged Ions from Electrospray Ionization Mass Spectrometry. *J. Phys. Chem.* **1992**, *96*, 2397−2400.
(23) Meot-Ner (Mautner), M.; Dongré, A. R.; Somogyi, A.; Wysocki, V. H. Thermal Decomposition Kinetics of Protonated Peptides and Peptide Dimers, and Comparison with Surface-Induced Dissociation. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 829−836.
(24) He, F.; Ramirez, J.; Garcia, B. A.; Lebrilla, C. B. Differentially Heated Capillary for Thermal Dissociation of Noncovalently Bound Complexes Produced by Electrospray Ionization. *Int. J. Mass Spectrom. Ion Processes* **1999**, *182*/*183*, 261−273.
(25) Garcia, B.; Ramirez, J.; Wong, S.; Lebrilla, C. B. Thermal Dissociation of Protonated Cyclodextrin−Amino Acid Complexes in the Gas Phase. *Int. J. Mass Spectrom.* **2001**, in press.
(26) Jockush, R. A.; Schnier, P. D.; Price, W. D.; Strittmatter, E. F.; Demirev, P. A.; Williams, E. R. Fragmentation Pathways, Dynamics and Activation Energies of Ubiquitin Ions Measured by Blackbody Infrared Radiative Dissociation. *Anal. Chem.* **1997**, *69*, 1119−1126.
(27) Schnier, P. D.; Price, W. D.; Jockusch, R. A.; Williams, E. R. Blackbody Infrared Radiative Dissociation of Bradykinin and Its Analogues: Energetics, Dynamics, and Evidence for Salt-Bridge Structures in the Gas Phase. *J. Am. Chem. Soc.* **1996**, *118*, 7178−7189.
(28) Grigorean, G.; Lebrilla, C. B. Enantiomeric Analysis of Pharmaceutical Compounds by Ion/Molecule Reactions. *Anal. Chem.* **2001**, *73*, 1684−1691.
(29) Hunter, E. P. L.; Lias, S. G. Evaluated Gas-Phase Basicities and Proton Affinities of Molecules: An Update. *J. Phys. Chem. Ref. Data* **1998**, *27*, 413−656.

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