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Factors Controlling the Complex Architecture of Native and Modified Cyclodextrins with Dipeptide (Z-Glu-Tyr) Studied by Microcalorimetry and NMR Spectroscopy: Critical Effects of Peripheral Bis-trimethylamination and Cavity Size

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Abstract: Complex stability constant (K), standard free energy (ΔG°), reaction enthalpy (ΔH°), and entropy change ($T\Delta S^\circ$) for 1:1 inclusion complexation of the diastereomeric dipeptides Z-D/L-Glu-L-Tyr (Z = benzyloxycarbonyl) and its component amino acids (Z-D/L-Glu and *N*-Ac-Tyr) with native α-, β-, and γ-cyclodextrins (CDs) and A,X-modified bis(6-trimethylammonio-6-deoxy)-β-CDs (AX-TMA_2-β-CDs) were determined in buffer solution (pH 6.9) at $T = 298.15$ K by isothermal titration microcalorimetry. Concurrent NMR spectral examinations revealed that the penetration mode and the resulting complex architecture are dramatically altered by the peripheral modification and also by the CD's cavity size. Upon complexation of the ditopic Z-Glu-Tyr guest, native α- and β-CDs preferentially bind the Z's phenyl group, whereas AX-TMA_2-β-CDs predominantly include the Tyr's phenol moiety. In contrast, native γ-CD includes both of the aromatic moieties simultaneously in the same cavity. Furthermore, for isomeric AB-, AC, and AD-TMA_2-β-CDs, an inversion of enantioselectivity and a switching of the penetration mode were observed, critically depending on the position of TMA substituents.

Introduction

It is widely recognized[1−4] that the inclusion complexation by native and modified cyclodextrins (CDs) is driven by the insertion of the less hydrophilic part of guest molecule into the CD cavity, whereas the more hydrophilic, often charged, group stays just outside the primary or secondary opening of the cavity. Typical examples include the complexation of Z-D/L-Glu (Z = benzyloxycarbonyl) with native and A,X-modified bis(6-trimethylammonio-6-deoxy)-β-CDs (AX-TMA_2-β-CDs) reported recently.[5,6] Inclusion of Z-Glu's phenyl ring (as the most hydrophobic part) into native β-CD occurs from the wider secondary opening, leaving the two negatively charged carboxylate groups in the bulk solution. The introduction of two positively charged trimethylammonio (TMA) groups to the primary side of β-CD cavity switches the mode of Z-Glu penetration from the secondary to the primary side, where Z-Glu's phenyl is included deeply into the AX-TMA_2-β-CD cavity and the carboxylate groups are located closely to the positively charged TMA groups, maximizing the electrostatic interactions.

Ditopic guests, possessing two hydrophobic moieties, give two distinct 1:1 complexes with size-matched CDs.[7,8] The population of these two species, and the preferred mode of penetration, are thought to be determined essentially by the relative hydrophobicity of the two, thus making it difficult to dramatically alter, for example, by simple peripheral derivatization of CD rim. Probably, even more difficult is to switch the penetration mode and the resulting complex architecture by such a small difference as guest's chirality. Thus, the goal of our study is to explore the feasibility of these possibilities, and if possible, to elucidate the conditions for dynamically controlling the complex architecture of ditopic guests by peripheral modification of the host CD as well as by guest chirality. This sort of study is of particular interest and importance as a prototype model for polytopic guest complexation by a variety of natural and synthetic supramolecular hosts, and hence contributes to a deeper understanding and further application of the molecular recognition phenomena in chemistry and biology, including the creation of smart supramolecular nanomaterials, devices, and machines.

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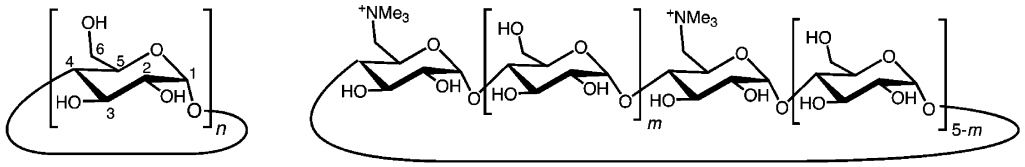
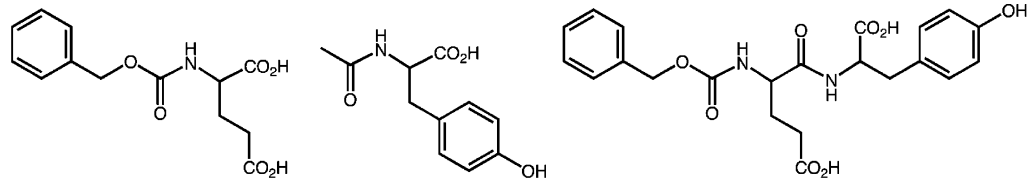
Chart 1



α-CD ($n = 6$)
β-CD ($n = 7$)
γ-CD ($n = 8$)

A,B-TMA_2-β-CD ($m = 0$)
A,C-TMA_2-β-CD ($m = 1$)
A,D-TMA_2-β-CD ($m = 2$)



N-Carboxybenzoyl-D/L-glutamic acid (Z-D/L-Glu)

N-Acetyl-D/L-tyrosine (*N*-Ac-D/L-Tyr)

N-Carboxybenzoyl-D/L-glutamyl-L-tyrosine (Z-D/L-Glu-L-Tyr)

For this purpose, we employed diastereomeric Z-D/L-Glu-L-Tyr as chiral ditopic guests and performed complexation thermodynamic and NMR spectral studies, using a systematic series of native and modified CDs, i.e., α-CD, *β*-CD, along with AB-, AC-, and AD-TMA_2-*β*-CD, and finally *γ*-CD (Chart 1), for the following reasons. First, the component amino acid residues of Z-Glu-Tyr, i.e., Z-D/L-Glu and *N*-Ac-D/L-Tyr (Chart 1), are known to exhibit comparable affinities toward *β*-CD.[5,9] Hence, it is a challenge to generate two Z-Glu-Tyr complexes of different architectures, where the Z's phenyl and Tyr's phenol compete for one CD cavity. Second, for a reliable elucidation of the complex structure by NMR, the two aromatic rings should be unambiguously distinguishable before and after complexation. Third, diastereomeric, rather than enantiomeric, guest pairs exhibit much larger differences in chiral discrimination upon complexation with *γ*-CD.[10]

Experimental Section

Materials. Commercially available samples of the highest purities were used in the microcalorimetric experiments as received. The vendors employed a variety of methods to determine and guarantee the purity of guest compounds as >98−99% by means of HPLC, LC, GC, titration, and/or elemental analysis. The commercially available α-, *β*-, and *γ*-CDs and synthesized *β*-CD derivatives[11] contained water of hydration or crystallization. The moisture contents were quantitatively determined by the vendors or by us using the Karl−Fisher technique and/or elemental analysis, for which appropriate corrections were made.

Microcalorimetric Measurements. An isothermal calorimeter (ITC), purchased from MicroCal Inc., MA, was used throughout the work. Titration microcalorimetry allows us to determine simultaneously the enthalpic change and equilibrium constant from a single titration curve. The ITC instrument was periodically calibrated electrically with an internal electric heater and chemically by using the enthalpy of neutralization of HCl with NaOH and the ionization enthalpy of TRIS buffer. These standard reactions gave excellent agreements (<±1−2%) with literature data.[12] The thermodynamic parameters obtained for the complexation reaction of cyclohexanol with *β*-CD were also in good agreement with our previous data.[9,13,14]

The ORIGIN software (OriginLab Co. Northampton, MA), which was used in the calculations of the equilibrium constant and the standard molar enthalpy of reaction from the titration curve, gave a standard deviation based on the scattering of the data points in a single titration curve. As we reported previously,[13,14] the accuracy of the thermodynamic quantities determined for 1:1 complexations was checked by performing several independent titration runs. The uncertainties of the thermodynamic quantities obtained for 1:1 complexations, shown in Table 1, are two standard deviations of the mean value unless otherwise stated.

Applicability of the 1:1 host−guest complex model was carefully checked for each complexation reaction. As we demonstrated in our previous study,[15] microcalorimetric titration curves for 1:1 and 1:2 complex stoichiometries are totally different in shape and can be distinguished unambiguously from the fitting curve. Nevertheless, computer simulations are usually employed in order to ensure the complex stoichiometry. It should be emphasized that, in addition to the calculation based on 1:1 stoichiometry, we also performed calculations assuming 1:*n* and *n*:1 binding models whenever such higher-order complexes were suspected to exist. However, such calculations did not lead to any appreciable improvement of the overall fit, rendering these more complicated models irrelevant in the present cases, and the assumption of the 1:1 model with a single binding site appears to be the only reasonable choice for all of the host−guest combinations examined.

In each microcalorimetric titration, a constant 5 *μ*L portion of a 0.05 M phosphate buffer solution (pH 6.9), containing an appropriate concentration of guest compound, was successively injected up to 20 times into the reaction cell (1.36 mL) charged with a CD solution in the same buffer; see Table 1, for the initial concentrations of guest and CD employed in each run. The heat of dilution of the guest buffer solution upon addition to the pure buffer solution (without CD) placed in the cell was determined in each run using the same number of

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Table 1. Complex Stability Constant (K), Standard Free Energy ($\Delta G°$), Enthalpy ($\Delta H°$), and Entropy Changes ($T\Delta S°$) for 1:1 Inclusion Complexation of Z-Glu-Tyr and Related Compounds with α-, β-, AB-TMA_2-β-, AC-TMA_2-β-, AD-TMA_2-β-, and γ-Cyclodextrin in Buffer Solutions (pH 6.9)[a] at $T = 298.15$ K

host	guest	stereo-chemistry	N [b]	K/M^{-1}	K_D/K_L or K_{DL}/K_{LL}	$\Delta G°$ /kJ mol^{-1}	$\Delta H°$ /kJ mol^{-1}	$T\Delta S°$ /kJ mol^{-1}	ref
α-CD	Z-Glu	D	2	15 ± 2	1.1	-6.7 ± 0.4	-19 ± 2	-12 ± 2	*c*
		L	2	14 ± 2		-6.4 ± 0.5	-21 ± 2	-15 ± 2	*c*
	N-Ac-Tyr	D	2	9 ± 2	1.3	-5.4 ± 0.6	-16 ± 3	-11 ± 3	*c*
		L	2	7 ± 2		-4.8 ± 0.7	-24 ± 3	-19 ± 3	*c*
	Z-Glu-Tyr	D,L	2	17 ± 2	1.0	-7.0 ± 0.3	-19 ± 2	-12 ± 2	*c*
		L,L	3	17.5 ± 1.5		-7.1 ± 0.2	-18.4 ± 1.5	-11.3 ± 1.5	*c*
β-CD	Z-Glu	D	2	84 ± 2	0.98	-10.98 ± 0.06	-11.20 ± 0.10	-0.22 ± 0.15	*d*
		L	2	86 ± 2		-11.04 ± 0.06	-10.49 ± 0.10	0.53 ± 0.15	*d*
	N-Ac-Tyr	D	2	125 ± 2	0.96	-11.97 ± 0.04	-16.7 ± 0.3	-4.7 ± 0.3	*e*
		L	2	130 ± 2		-12.07 ± 0.04	-17.1 ± 0.3	-5.0 ± 0.3	*e*
	Z-Glu-Tyr	D,L	3	116 ± 3	1.20	-11.78 ± 0.07	-14.8 ± 0.2	-3.0 ± 0.2	*c*
		L,L	3	97 ± 2		-11.34 ± 0.05	-13.3 ± 0.2	-2.0 ± 0.2	*c*
AB-TMA_2-β-CD	Z-Glu	D	2	135 ± 3	0.71	-12.16 ± 0.06	-10.00 ± 0.10	2.16 ± 0.15	*d*
		L	2	189 ± 3		-12.99 ± 0.04	-12.79 ± 0.10	0.20 ± 0.15	*d*
	N-Ac-Tyr	D	2	145 ± 2	1.12	-12.34 ± 0.04	-19.2 ± 0.2	-6.9 ± 0.2	*c*
		L	2	130 ± 3		-12.07 ± 0.06	-19.1 ± 0.2	-7.0 ± 0.2	*c*
	Z-Glu-Tyr	D,L	2	139 ± 3	1.26	-12.23 ± 0.06	-13.6 ± 0.2	-1.4 ± 0.2	*c*
		L,L	2	110 ± 3		-11.65 ± 0.07	-14.5 ± 0.2	-2.9 ± 0.2	*c*
AC-TMA_2-β-CD	Z-Glu	D	2	113 ± 3	0.85	-11.72 ± 0.07	-6.85 ± 0.15	4.9 ± 0.2	*d*
		L	3	133 ± 3		-12.12 ± 0.06	-8.90 ± 0.15	3.2 ± 0.2	*d*
	N-Ac-Tyr	D	2	206 ± 2	1.00	-13.21 ± 0.03	-19.7 ± 0.2	-6.5 ± 0.2	*c*
		L	2	207 ± 3		-13.22 ± 0.04	-19.5 ± 0.2	-6.3 ± 0.2	*c*
	Z-Glu-Tyr	D,L	2	157 ± 4	1.28	-12.53 ± 0.06	-13.1 ± 0.3	-0.6 ± 0.3	*c*
		L,L	2	123 ± 3		-11.93 ± 0.06	-13.5 ± 0.3	-1.6 ± 0.3	*c*
AD-TMA_2-β-CD	Z-Glu	D	2	94 ± 3	0.88	-11.26 ± 0.08	-7.52 ± 0.15	3.7 ± 0.2	*d*
		L	2	107 ± 3		-11.58 ± 0.07	-7.80 ± 0.15	3.8 ± 0.2	*d*
	N-Ac-Tyr	D	2	136 ± 2	0.80	-12.18 ± 0.04	-17.1 ± 0.2	-4.9 ± 0.2	*c*
		L	2	170 ± 3		-12.73 ± 0.04	-19.1 ± 0.2	-6.4 ± 0.2	*c*
	Z-Glu-Tyr	D,L	2	120 ± 3	1.10	-11.87 ± 0.07	-12.3 ± 0.2	-0.4 ± 0.2	*c*
		L,L	2	109 ± 2		-11.63 ± 0.05	-13.5 ± 0.2	-1.9 ± 0.2	*c*
γ-CD	Z-Glu	D	1	*f*					*c*
		L	1	*f*					*c*
	N-Ac-Tyr	D	1	*f*					*c*
		L	1	*f*					*c*
	Z-Glu-Tyr	D,L	2	97 ± 2	2.4	-11.34 ± 0.05	-25.6 ± 0.2	-14.3 ± 0.2	*c*
		L,L	2	40 ± 2		-9.14 ± 0.10	-22.3 ± 0.4	-13.2 ± 0.4	*c*

[a] Standard phosphate buffer at pH 6.9 [NaH_2PO_4 (0.025 M) + $NaHPO_4$ (0.025 M)]. [b] Number of titration microcalorimetric experiments performed. [c] This work. [d] ref 5. [e] ref 9. [f] Equilibrium constant and/or reaction enthalpy is too small to be determined by titration microcalorimetry.

injections of the guest solution at the same concentration employed in the titration experiment. The dilution enthalpy determined in such a control experiment was subtracted from the enthalpy obtained in each titration experiment. It should be emphasized that the enthalpies of dilution obtained in all runs were in the same order of magnitude as those of simple electrolytes such as NaCl at the same concentration. It is concluded therefore that there is no significant self-association of guest under the experimental conditions used. We have previously shown that the nonideality corrections are not necessary under the experimental conditions employed.[13,14]

NMR Measurements. 1D and 2D NMR spectra, including ROESY, COSY, and HOHAHA, were obtained at 600 MHz in D_2O at 25 °C on a Bruker Avance 600 instrument. HOHAHA experiments were performed by using the MLEV-17 pulse sequence with a mixing time of 120 ms, whereas ROESY spectra were recorded with a mixing time of 200 ms. An equimolar mixture (30 mM each) of CD and the sodium salt of each peptide were used for the NMR experiments.

Results and Discussion

Thermodynamic parameters obtained for complexation reactions of Z-D/L-Glu-L-Tyr and the related guests with α-CD, β-CD, AB-, AC-, and AD-TMA_2-β-CD, and γ-CD are presented in Table 1. In the following sections we will comparatively discuss these thermodynamic and relevant NMR data to first elucidate the effects of the cavity size and the peripheral TMA-modifications upon the mode of guest penetration and chiral discrimination, and then draw a comprehensive picture of the complex architecture controlled by these factors.

Mode of Guest Penetration into Native CDs. Comparison of the thermodynamic parameters for the complexation of Z-D/L-Glu-L-Tyr toward β-CD with those obtained for the component amino acid residues, i.e., Z-D/L-Glu and *N*-Ac-D/L-Tyr, reveals two intriguing features of the guest moieties' penetration

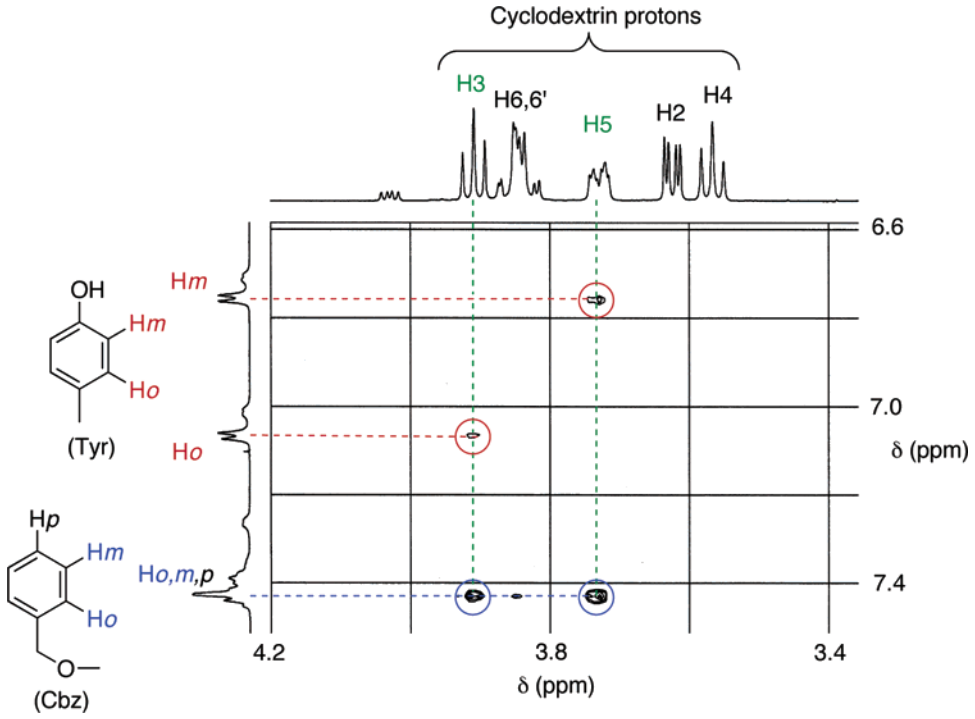

Figure 1. ROESY spectrum of Z-L-Glu-L-Tyr complex with native β-CD (20 mM each) in D_2O containing 40 mM NaOH.

behavior and therefore the resulting architecture of the CD-dipeptide complex. First, the two aromatic moieties of the guest, i.e., Z's phenyl and Tyr's phenol, can compete for the same interacting site, i.e., the CD cavity, with similar probabilities, giving a comparable population of two different 1:1 complexes, that are "phenol-in" and "phenyl-in." Through such a competing complexation model, one can explain why the reaction enthalpy for dipeptide complexation is less negative than that for *N*-Ac-D/L-Tyr, but is more negative than that for Z-D/L-Glu. Indeed, it is likely that the complexation enthalpy obtained for the dipeptide is an algebraic sum of the heat releases upon formation of the phenol-in and phenyl-in complexes, which coexist in comparable concentrations in the solution. Nevertheless, we cannot rigorously exclude the second possibility that the guest's phenyl, as the most hydrophobic group, is predominantly included into the cavity, since this type of inclusion is assisted by the phenol moiety located near the edge of the cavity like a lid, allowing the phenol to weakly interact with the host rim through the hydrogen-bonding and/or van der Waals interactions. This complex architecture can also explain why the reaction enthalpy and entropy for dipeptide complexation are less negative than those for *N*-Ac-D/L-Tyr but is more negative than that for Z-D/L-Glu.

NMR spectral examinations greatly contributed to the elucidation of precise complex architectures in solution. Interestingly, the ROESY spectra obtained show two sets of cross-peaks, as shown in Figure 1; i.e., very strong NOE signals for the CD's H3 and H5 with the phenyl's H*o* and H*m* and extremely weak ones with the phenol's H*o* and H*m*.

This observation unambiguously reveals that the guest's phenyl penetrates from the wider secondary opening of the CD, while the phenol moiety only weakly interacts with the cavity mostly staying in the bulk solution.A plausible dominant architecture of the Z-Glu-Tyr complex with β-CD, elucidated from the NMR data, is illustrated in Figure 2. No cross-peaks

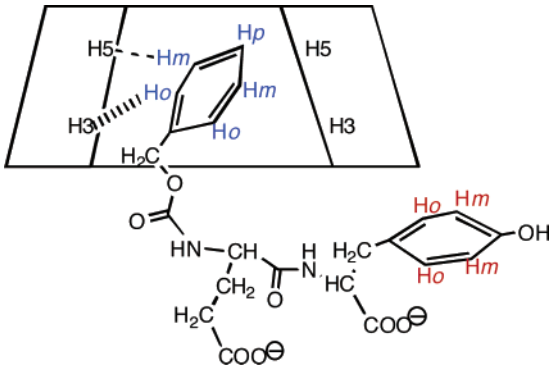

Figure 2. Plausible structure of Z-Glu-Tyr complex with β-CD, elucidated from the ROESY spectrum.

between the two aromatic rings of a single guest molecule were observed in ROESY spectra and probably the rings stay apart from each other as depicted in Figure 1. Nevertheless, interestingly the NMR data reveal coexistence of two (major and minor) conformers in equilibrium with each other (which was confirmed by COSY experiments). This complex structure is consistent with the second model anticipated from the thermodynamic parameters in the above discussion. This seems rather reasonable, since the Z's phenyl is more hydrophobic than the Tyr's phenol. Thus, it seems very difficult and hence challenging to switch the penetration mode from phenyl-in to phenol-in upon complexation of Z-Glu-Tyr with CDs, for which some invention should be made to facilitate the inclusion of the less-hydrophobic phenol into the CD's cavity, while simultaneously keeping the more-hydrophobic phenyl outside the cavity in the bulk water.

The affinities (K) of Z-D/L-Glu-L-Tyr and its components (Z-D/L-Glu and *N*-Ac-D/L-Tyr) toward α-CD are roughly 5−10 times lower than those toward β-CD, simply due to the α-CD's small cavity relative to the penetrating groups. As frequently observed, the tight packing of the penetrating group in the small cavity of α-CD, enhancing van der Waals interactions, leads to

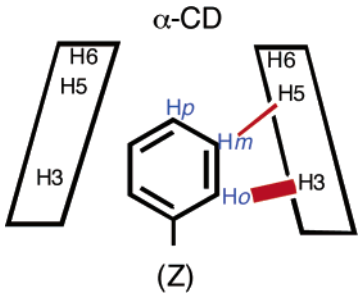

Figure 3. α-CD Complex of Z-Glu-Tyr, for which NOE signals were observed between H*o* and H3 and between H*m* and H5 in the ROESY spectrum.

relatively large enthalpic gains ($-\Delta H^\circ = 16-24$ kJ mol^{-1}), most of which are however canceled by equally large entropic losses ($-T\Delta S^\circ = 11-19$ kJ mol^{-1}), arising from the accompanying conformational fixation, to ultimately give the low affinities for these guests (Table 1). The equilibrium constants (K) as low as 7−18 M^{-1}, associated with the relatively large errors of ±2 M^{-1}, do not allow us to draw any sensible conclusion on the complex structure/architecture from the thermodynamic data. However, even for such a weak α-CD complex of Z-D/L-Glu-L-Tyr ($K = 17-18$ M^{-1}), careful ROESY experiments enabled us to observe relatively strong NOE signals between the Z's H*o* and H*m* protons of Z-Glu-L-Tyr and the H3 and H5 protons of α-CD (Figure 3). This result clearly reveals the complex architecture, in which practically only the Z's phenyl group penetrates into the α-CD cavity from the secondary side. Unfortunately, only extremely weak NOE signals were observed for the Tyr's phenol protons, although it is possible that the phenol residue is located in proximity of the cavity's rim, forming a hydrogen bond, and the whole complex architecture resembles the β-CD complex discussed above. Yet, we cannot strictly exclude the possibility that the more hydrophilic phenol ring stays totally outside the cavity in the bulk solvent. In any case, the more hydrophobic phenyl is preferentially accommodated in the cavity of both α- and β-CDs. Thus, changing the cavity size (α- versus β-CD) does not appear to switch the complex architecture or the penetration mode from phenyl-in to phenol-in.

The γ-CD cavity is large enough to comfortably accommodate the two aromatic rings of Z-Glu-Tyr simultaneously. Indeed, in our recent comparative NMR and microcalorimetric study,[10] we confirmed the synergetic inclusion of two aromatic rings into the γ-CD cavity upon complexation with various Z- and *N*-Ac-dipeptides containing Phe and/or Tyr residues. It was shown[10] that the large interior of the γ-CD cavity (8.3 Å) provides a space large enough to accommodate two aromatic rings, which are loosely packed in a slide fashion, allowing the coexistence of several guest conformers with different geometries and architectures inside the cavity.

The large negative enthalpies observed for the complexation of Z-D/L-Glu-L-Tyr with γ-CD (Table 1), exceed the heat effect obtained for β-CD by >10 kJ mol^{-1}, supporting the simultaneous inclusion model, which is accompanied by strong van der Waals and $\pi-\pi$ interactions between the guest's aromatic rings and the cavity walls. Such an inclusion model is supported also by the highly negative entropy of complexation, most probably arising from the severe conformational restriction of the guest molecule upon complexation. NMR examinations revealed the coexistence of at least two different conformers of

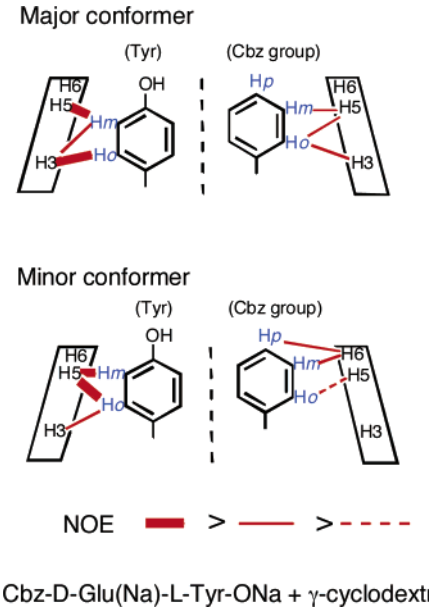

Figure 4. Plausible structures of the major and minor conformers of the 1:1 complex of Z-Glu-Tyr with γ-CD, elucidated from the ROESY spectrum.

a 1:1 complex of Z-Glu-Tyr with γ-CD (Figure 4). It is interesting that in the case of the major conformer the phenol's H*m* proton and the phenyl's H*o* proton exhibit strong NOE cross-peaks with both of the H3 and H5 protons of the γ-CD's interior. This observation is fully compatible with the loosely packed simultaneous inclusion model, where considerable sliding motions of the included aromatic rings are allowed to occur inside the cavity, which is in consistent with our previous observations.[10]

It is noted that the varying cavity sizes of native α- to γ-CDs allow us to investigate the two different (single versus dual) penetration modes of dipeptide Z-D/L-Glu-L-Tyr from thermodynamic and architectural points of view. At the same time, it has turned out that simple native CDs do not provide us with the opportunity to explore yet another mode of penetration, that involving the phenol moiety. Obviously, some invention should be made to achieve such a switching of the penetration mode from phenyl-in to less-favored phenol-in. For this purpose, we performed the chemical derivatization of CD to attract the less-hydrophobic phenol moiety into the CD cavity, although it was not immediately clear if this strategy would work or not.

Mode of Guest Penetration into Modified CDs. We have demonstrated recently that the introduction of two positively charged trimethylammonio (TMA) groups to the primary side of CD causes a switching of the penetration mode of Z-D/L-Glu from the secondary side (for native β-CD) to the primary side (for AB-TMA$_2$-β-CD).[5] There are two possibilities for TMA-modified CDs to affect the penetration mode. First, the strong electrostatic interactions between the two TMAs and the two carboxylates of Z-Glu-Tyr may lead to a switching of the penetrating direction from the secondary to primary side, as was the case with Z-Glu. If this effect is dominant, not the phenol but the phenyl ring should be included into the cavity as the most hydrophobic part of the guest molecule. However, there is the second possibility that the presence of TMA groups on the primary side facilitates the deep penetration of phenol group from the secondary side. This mode of penetration can be

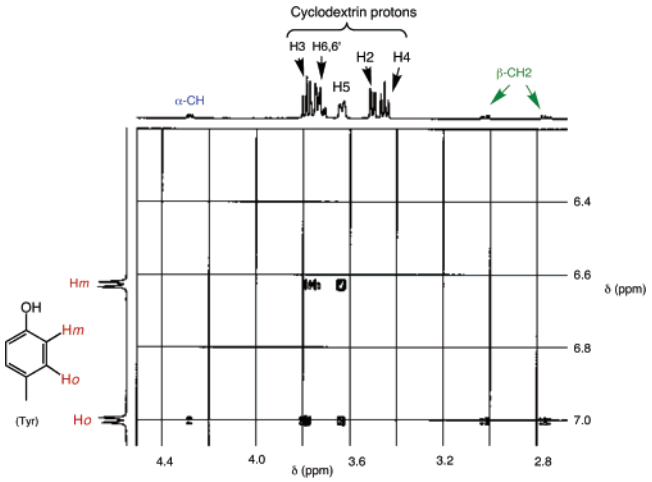

Figure 5. ROESY spectrum of *N*-Ac-L-Tyr complex with native β-CD (20 mM each) in D_2O containing 20 mM NaOH.

thermodynamically favored by the strong ion−dipole interactions between the host's TMAs and the phenolic hydroxyl. If this effect dominates the complexation behavior of Z-Glu-Tyr with TMA-modified CDs, we will be able to achieve the desired switching of the penetration mode and also to explore the effects of relative position of two TMAs on the rim upon thermodynamics and complex architecture. We first examine the effects of TMA modification on the penetration mode of *N*-Ac-D/L-Tyr as an essential component of the dipeptide guest Z-Glu-Tyr.

Complexation of *N*-Ac-D/L-Tyr toward β-CD and AX-TMA$_2$-β-CDs. As can be seen from Table 1, the higher affinities of *N*-Ac-D/L-Tyr toward AX-TMA$_2$-β-CDs, rather than native β-CD, are attained exclusively by enthalpic gains, which are however canceled to some extent by entropic losses. Upon complexation with *N*-Ac-D/L-Tyr, AB- and AC-TMA$_2$-β-CDs afford more negative $\Delta H°$ (19.1−19.7 kJ mol^{-1}) than those obtained for native β-CD (16.7−17.1 kJ mol^{-1}), but the originally poor enantioselectivity (K_D/K_L = 0.96) is not appreciably improved (K_D/K_L = 1.0−1.1). It is interesting to note that the increased $\Delta H°$ is almost totally canceled by the more negative $\Delta S°$ upon complexation with AB-TMA$_2$-β-CD, whereas the canceling effect is less extensive for AC-TMA$_2$-β-CD. From the viewpoint of enantioselectivity, AD-TMA$_2$-β-CD exhibits the most pronounced differences upon complexation with enantiomeric *N*-Ac-Tyr. Thus, the $\Delta H°$ value for the D-isomer becomes more exothermic by 0.4 kJ mol^{-1} (relative to β-CD) to give a slightly enhanced K of 136 M^{-1}. In contrast, the $\Delta H°$ for L-isomer becomes much more exothermic by 2.0 kJ mol^{-1} (relative to β-CD) and the K is increased from 130 to 170 M^{-1} to give a much higher enantioselectivity of K_D/K_L = 0.80. Clearly, the enthalpy-driven enhancement of the affinity cannot be taken as experimental evidence for or against any mode of penetration, and therefore we carried out the NMR experiments to obtain a comprehensive view of the complex architecture.

Figure 5 illustrates the ROESY spectrum of *N*-Ac-L-Tyr complex with native β-CD. The intense cross-peaks of Tyr's H*o* and H*m* with CD's H3 and H5 unambiguously indicates deep penetration of the phenol group into the CD cavity. It is noted also that only H*m* protons afford significant NOE signals with H6 protons with the strongest cross-peak with H5, while the H*o* protons exhibit the strongest NOE signal with H3. All

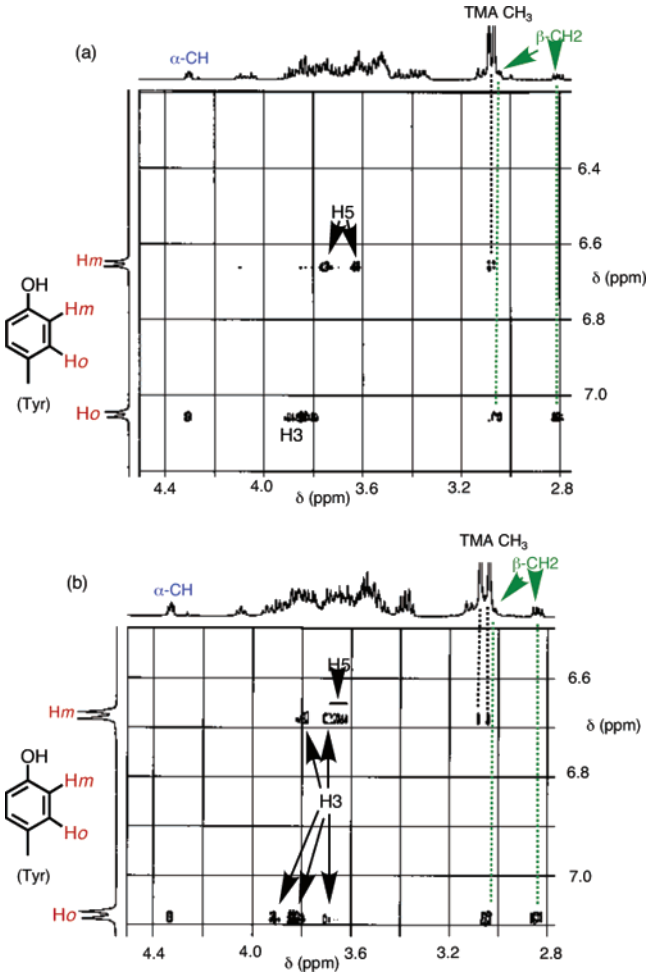

Figure 6. ROESY spectra of (a) *N*-Ac-D-Tyr and (b) *N*-Ac-L-Tyr complexes with AD-TMA$_2$-β-CD (20 mM each) in D_2O containing 20 mM NaOH.

of these features are fully compatible with the deep penetration of the phenol group from the secondary side of CD.

Similar ROESY examinations allowed us to definitely discriminate between the two possible modes of penetration (from the secondary versus primary side) upon complexation with *N*-Ac-D/L-Tyr with AD-TMA$_2$-β-CD. As shown in Figure 6a, *N*-Ac-D-Tyr only gave strong NOE signals between H*m* and H5 and between H*o* and H3. In contrast, the H*m* protons of *N*-Ac-L-Tyr displayed cross-peaks with both H5 and H3, whereas H*o* only gave NOE signals with H3; see Figure 6b. It is likely therefore that *N*-Ac-D-Tyr penetrates deeper than *N*-Ac-L-Tyr, or that *N*-Ac-L-Tyr significantly inclines inside the CD cavity, causing a close contact of H*m* with H3.

All of the above results unambiguously prove that the insertion of Tyr's phenol group occurs from the wider secondary side of the CDs. Hence, we may conclude that the two TMA groups introduced to the primary side of CD do not cause any significant changes in complex structure that lead to the switching of penetration mode of *N*-Ac-D/L-Tyr. This observation indicates that, at least for *N*-Ac-Tyr, weak ion−dipole interactions can surpass apparently stronger ion−ion interactions to dominate the complexation behavior of CDs. In this context, it is an intriguing logical extension to investigate the complexation behavior of Z-D/L-Glu-L-Tyr with AX-TMA$_2$-β-CDs, as stronger electrostatic interactions are expected to occur between a dicationic host and a dianionic Z-Glu-Tyr guest.

Complexation of Z-D/L-Glu-L-Tyr toward AX-TMA$_2$-β-CDs. Thermodynamic parameters obtained for the complexation of dipeptide Z-D/L-Glu-L-Tyr with AB-, AC-, and AD-TMA$_2$-β-CDs are listed in Table 1. In contrast to the seemingly random enantioselectivities exhibited by native and modified CDs for Z-Glu (K_D/K_L = 0.7−1.1) and *N*-Ac-Tyr (K_D/K_L = 0.8−1.1), the dipeptide guest Z-Glu-Tyr displayed consistent diastereoselectivities for the DL- over LL-isomer (K_{DL}/K_{LL} = 1.0−1.3), regardless of the host used (native or modified). It should be noted however that the thermodynamic origin of the DL-preference is totally different for native and modified CDs. Upon complexation with AX-TMA$_2$-β-CDs, the DL-preference is brought about by the less negative entropy change, but is enthalpic in origin in the case of native CDs. As demonstrated in our recent study on the complexation thermodynamics of various diastereomeric dipeptide pairs with γ-CD,[10] switching of the driving force (enthalpy versus entropy) is often caused by the alterations of penetration mode, or inserting the guest moiety. For instance, the DL-isomer of Z-Ala-Phe shows a 6 times larger affinity than the LL-isomer upon complexation with γ-CD (which includes two phenyls simultaneously) exclusively due to the entropy factor, whereas the affinity enhancement for the DL-isomer of *N*-Ac-Tyr-Tyr over the LL-isomer is entirely enthalpy driven. This is a general tendency observed for practically all examined dipeptides that contain only phenyl residues, but absolutely not for those dipeptides that possess at least one phenol residue.[10] However, since similar thermodynamic behavior of dipeptide has not been reported for β-CDs, the opposite driving force for affinity enhancement upon complexation with AX-TMA$_2$-β-CDs versus native β-CD cannot immediately be attributed to the switching of penetration mode (from phenyl to phenol). Hence, we carried out complimentary NMR studies to clarify this issue.

ROESY spectral examinations revealed that the guest's phenol ring is preferentially included in the CD cavity upon complexation of Z-D/L-Glu-L-Tyr with AD- and AC-TMA$_2$-β-CDs and of Z-D-Glu-L-Tyr with AB-TMA$_2$-β-CD. Although these five complexes possess slightly different architecture or location of phenol ring inside the cavity (Figure 7), all of them share one common feature: based on the ROESY spectra; the phenol's H*m* protons are located very closely to the CD's H5 proton and the H*o* proton to the H3 proton in perfect agreement with the phenol penetration from the secondary side. Interestingly, the ROESY spectra show two sets of cross-peaks, as exemplified for the Z-D-Glu-L-Tyr complex with AD-TMA$_2$-β-CD in Figure 8. The first one is related to the host−guest NOE between the CD protons inside the cavity and the guest's aromatic protons, while the second one is assigned to the intramolecular NOE between the two aromatic residues in the same guest molecule. Furthermore, the phenyl's H*m* and H*o* protons of Z-D/L-Glu-L-Tyr afford relatively strong NOE signals with CD's H3 protons, suggesting a very shallow penetration of the phenyl ring just above the cavity edge. Thus, the NMR data reveal coexistence of major and minor conformers in equilibrium (as further confirmed by COSY experiments). Combining all the experimental data, we propose the most plausible structure of Z-D/L-Glu-L-Tyr complex (major conformer) with AD- and AC-TMA$_2$-β-CDs in Figure 7. It should be emphasized that the ROESY experiments reveal the distinctly different penetration modes, guest conformation, and complex architecture for AB-TMA$_2$-β-CD complex with Z−L-Glu-L-Tyr. This is rather a special case, and will be discussed further in the next subsection in connection with the attained chiral discrimination.

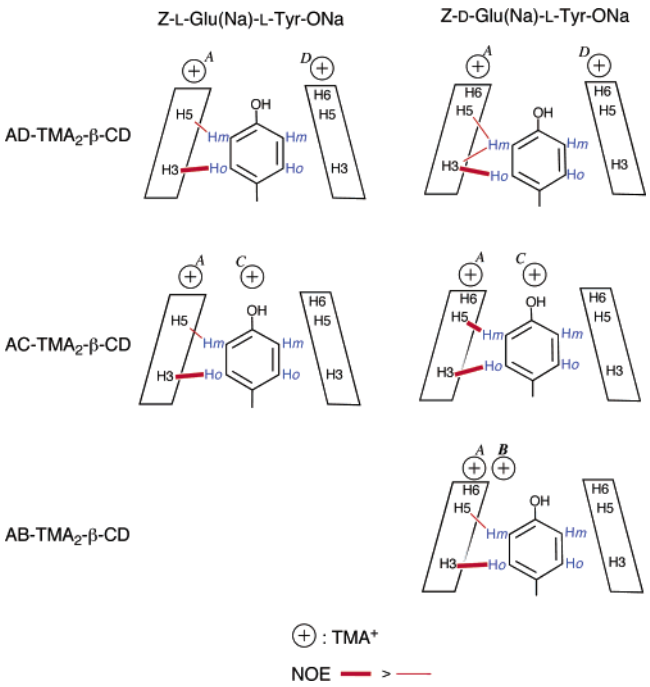

Figure 7. Tyr's phenol ring preferentially included in the CD cavity upon complexation of Z-D/L-Glu-L-Tyr with AD- and AC-TMA$_2$-β-CDs and of Z-D-Glu-L-Tyr with AB-TMA$_2$-β-CD, as indicated by the NOE signals.

Chiral Discrimination upon Complexation with Native and Modified CDs. In our previous study,[5] we showed that the enantioselectivity for Z-D/L-Glu increases in the order: AD-TMA$_2$-β-CD < AC-TMA$_2$-β-CD < AB-TMA$_2$-β-CD, with the L-isomer always preferred over the D-isomer to give a highest enantioselectivity of K_L/K_D = 1.41 (and the largest affinity) for the less-symmetrically substituted AB-TMA$_2$-β-CD. In this study, we have found quite different thermodynamic behavior upon complexation with *N*-Ac-D/L-Tyr. The symmetrically substituted AD-TMA$_2$-β-CD displays the largest enantioselectivity in favor of L-isomer upon complexation with *N*-Ac-D/L-Tyr; i.e., K_L/K_D = 1.25. AC-TMA$_2$-β-CD gives the largest affinity but shows virtually no chiral discrimination, while AB-TMA$_2$-β-CD prefers the antipodal D-isomer of *N*-Ac-Tyr with K_D/K_L = 1.12. To the best of our knowledge, this is the first case in which the enantioselectivity is switched by a simple rearrangement of the charged groups on the CD rim.

It is also interesting to compare the chiral recognition behavior of enantiomeric Z-D/L-Glu with that of diastereomeric Z-D/L-Glu-L-Tyr by AX-TMA$_2$-β-CDs. As stated above, all of the AX-TMA$_2$-β-CD hosts preferentially bind the L-isomer of Z-Glu and the enantioselectivity (K_L/K_D) increases from 1.14 for AD to 1.18 for AC and then to 1.41 for AB-TMA$_2$-β-CD. Similarly, the DL-isomer of Z-Glu-Tyr is consistently favored by the TMA-modified CDs, but no enhancement in chiral discrimination is obtained by TMA substitution to give the K_{DL}/K_{LL} ratios of 1.26, 1.28, and 1.10 for AB-, AC-, and AD-TMA$_2$-β-CD, respectively, which are comparable to or even less than that for native β-CD (K_{DL}/K_{LL} = 1.20). Such contrasting thermodynamic behavior observed for native and TMA-modified CDs may originate from the altered complex architectures of Z-Glu versus Z-Glu-Tyr with native and modified β-CDs. In general,

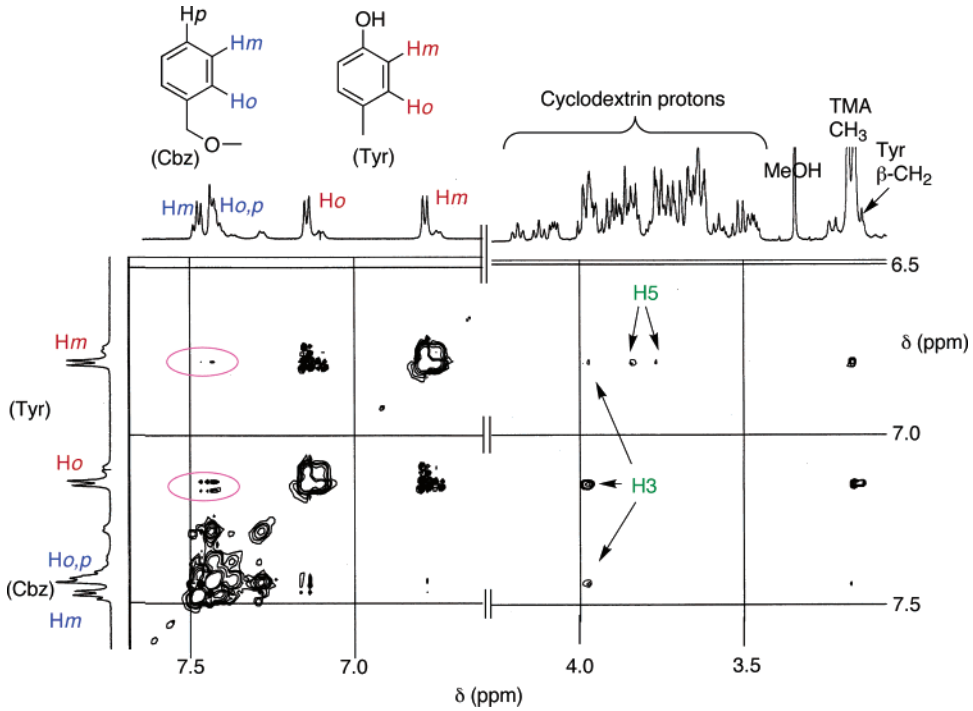

Figure 8. ROESY spectrum of D-Glu-L-Tyr complex with AD-TMA$_2$-β-CD (20 mM each) in D_2O containing 40 mM NaOH.

native β-CD is a poor chiral discriminator of enantiomeric guests as quantitatively demonstrated in our previous study,[9] and therefore it is not unexpected that native β-CD does not exhibit any appreciable enantioselectivities toward Z-D/L-Glu (K_D/K_L = 0.98). On the other hand, the complex architectures of Z-Glu with AX-TMA$_2$-β-CDs are typical examples of the three-point interaction model, in which the hydrophobic phenyl group, penetrating from the primary side, is fixed deeply in the cavity with the two negatively charged carboxylates positioned near the positively charged TMAs through electrostatic interactions. Due to such a strict three-point interaction between the host and guest that is less tolerant to structural variations, the stereochemical requirement becomes more demanding in particular for the less-symmetrically substituted AC- and AB-TMA$_2$-β-CD hosts, giving higher enantioselectivities of up to 1.41.

The architectures of the Z-Glu-Tyr complexes with AX-TMA$_2$-β-CDs are totally different from those of the Z-Glu complexes. Indeed, guest insertion occurs from the secondary side, leaving no chance for the electrostatic interactions to work between the carboxylates and TMAs. Consequently, the guest's phenol ring retains a reasonable degree of flexibility to adjust its position/location inside the cavity, minimizing the difference in affinity due to chirality. In addition, the Z's phenyl ring, perching on the cavity's edge (Figure 9), is not severely restricted in conformation upon complexation, which is the second very important structural feature that reduces the diastereoselectivity. As a result of the relatively large conformational freedom in the complex of symmetrically substituted AD-TMA$_2$-β-CD, the diastereoselectivity for Z-Glu-Tyr is virtually zero. In the case of the less-symmetrically substituted AB-TMA$_2$-β-CD, the closely located TMA groups may contribute to the fixation of the guest's phenol ring within the cavity through the ion−dipole interactions, which in turn restricts the guest conformation in the complex, eventually leading to an

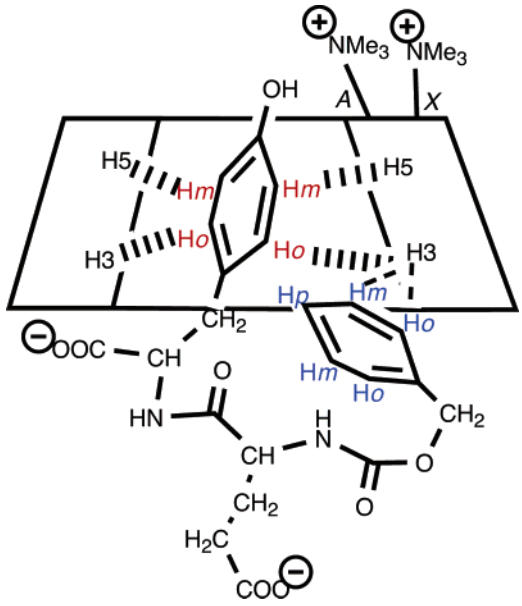

Figure 9. A plausible structure of Z-D/L-Glu-L-Tyr complex with AD- and AC-TMA$_2$-β-CDs.

enhancement of chiral discrimination. Nevertheless, Z-Glu-Tyr possesses more freedom in conformation and penetration mode to minimize the free energy differences between the opposite diastereomers to show a smaller chiral discrimination than the Z-Glu case. Thus, it is likely that the penetration mode is switched from phenol-in to phenyl-in, where the normal phenol-in mode becomes conformationally unfavorable. To prove this hypothesis, we examined the results of NMR studies on this system.

The complexation of Z-D-Glu-L-Tyr with AB-TMA$_2$-β-CD is caused predominantly by the deep insertion of the Tyr's phenol ring into the host's cavity (Figure 7). However, diastereomeric Z-L-Glu-L-Tyr guests exhibited totally different ROESY

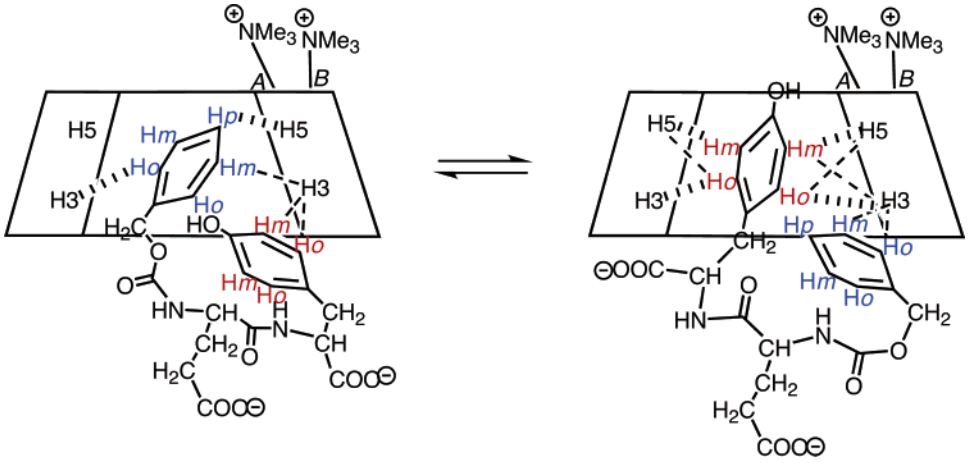

Figure 10. Equilibrating "phenyl-in" and "phenol-in" complexes of Z−L-Glu-L-Tyr with AB-TMA$_2$-β-CD.

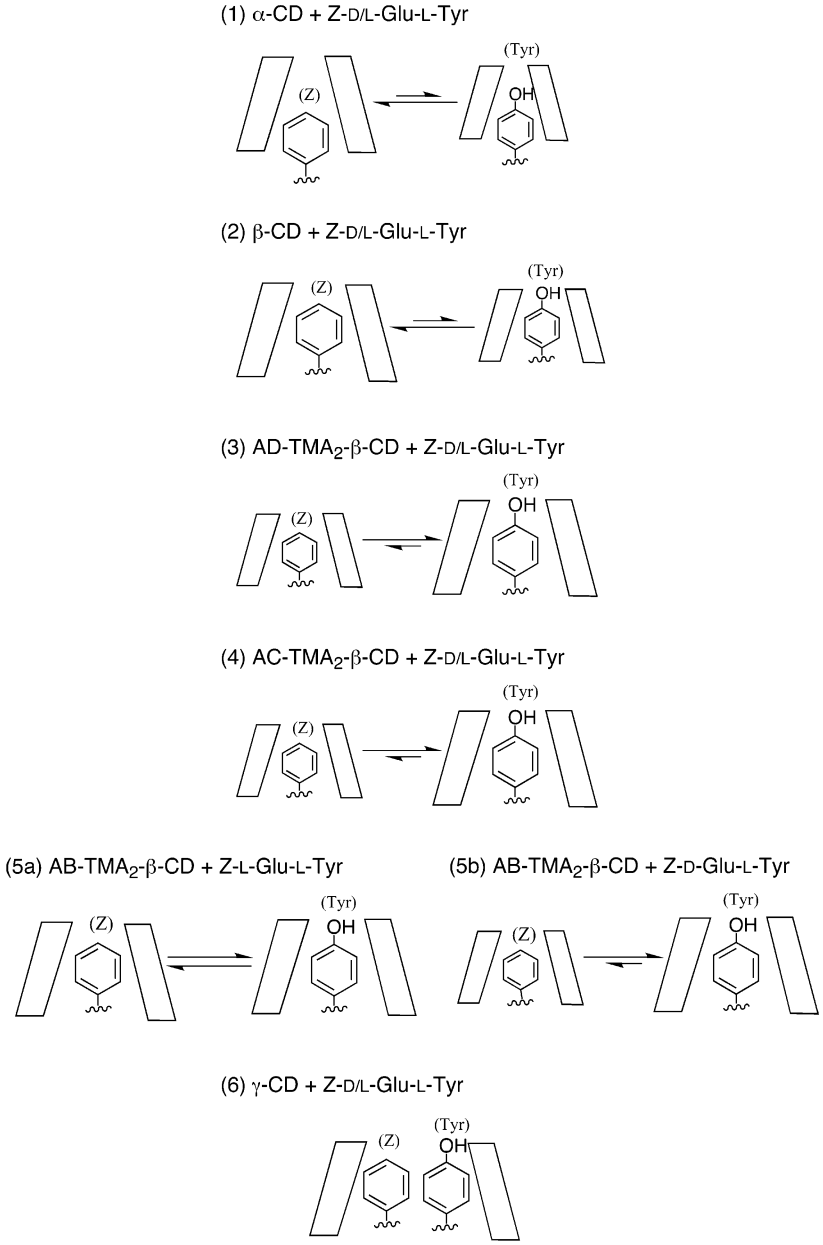

Figure 11. Critical control of the guest penetration mode and resulting complex architecture upon complexation of Z-D/L-Glu-L-Tyr with native and TMA-modified CDs.

patterns upon complexation with the same host. Thus, not only the Tyr's phenol but also the Z's phenyl protons gave cross-peaks of almost equal intensities with the CD's H3 and H5 protons. Such ROESY behavior is rationalized only by an alternating inclusion model, in which the CD cavity is time-shared by the two guest moieties with nearly equal probability. On the basis of these results, we propose the plausible complex structure of Z-L-Glu-L-Tyr with AB-TMA_2-β-CD as an equilibrating mixture of the phenol-in and phenyl-in complexes shown in Figure 10. This is the first reported example in which an inversion of stereochemistry at a single stereogenic center of guest causes a dramatic switching of the penetration mode.

From a chiral recognition point of view, it should be emphasized that the CD cavity size also plays a crucial role in determining the magnitude of chiral discrimination. As discussed above, native β-CD shows a modest diastereoselectivity (K_{DL}/K_{LL}) of 1.20 for the dipeptide Z-D/L-Glu-L-Tyr, which is better than the practically zero enantiodifferentiation ($K_D/K_L \approx 1$) observed for its component amino acid residues, i.e., Z-D/L-Glu and *N*-Ac-D/L-Tyr (Table 1). Obviously, γ-CD is a much better chiral discriminator for diastereomeric Z-D/L-Glu-L-Tyr, affording a high K_{DL}/K_{LL} ratio of 2.43. This result nicely coincides with our recent finding that diastereomeric pairs of dipeptides with two aromatic rings display good-to-excellent chiral discrimination, giving 3−7 times different *K*s upon complexation with native γ-CD.[10] It is likely that such an efficient chiral discrimination is realized by the severe conformational restriction of guest freedoms in the γ-CD cavity, which leads to a greater difference in complexation free energy by preferentially stabilizing one of the diastereomeric complexes and destabilizing the other one. Relatively large Z-D/L-Glu-L-Tyr cannot be fully accommodated in the very small cavity of α-CD, preventing the intermolecular approach of the chiral centers of the guest and the host. Consequently, Z-D/L-Glu-L-Tyr and its components, i.e., Z-D/L-Glu and *N*-Ac-D/L-Tyr, exhibit virtually no chiral discrimination upon complexation with α-CD.

Conclusions

This comprehensive NMR and microcalorimetric studies allowed us to fully characterize the detailed structural and thermodynamic features of the complexes of diastereomeric Z-D/L-Glu-L-Tyr and its components (Z-D/L-Glu and *N*-Ac-D/L-Tyr) with native α-, β-, and γ-CDs and AX-TMA_2-β-CDs. The most interesting findings obtained in this study may be summarized as follows:

(1) By using the native and TMA-modified CDs, we could critically control the mode of guest penetration and the resulting complex architecture (Figure 11). Upon complexation with native α- and β-CD, the Z's phenyl ring was preferentially included, while the Tyr's phenol ring predominantly penetrates into the cavity of AX-TMA_2-β-CDs. In contrast, γ-CD accommodates simultaneously the both aromatic rings in the same cavity.

(2) We demonstrated for the first time that the arrangement of charged groups on the CD rim can lead to a sudden change of the penetrating group in Z-L-Glu-L-Tyr, switching from the predominant phenol-in complex formation for AD- and AC-TMA_2-β-CDs to the statistical formation of both phenyl-in and phenol-in complexes for AB-TMA_2-β-CD.

(3) We also revealed a reversal of the enantiomeric preference from *N*-Ac-D-Tyr to *N*-Ac-L-Tyr by changing the position of the charged groups around the CD rim (AB-TMA_2-β-CDs versus AD-TMA_2-β-CD).

All of the above findings are crucial in discussing the mechanistic and structural aspects of chiral recognition of multi-chiral oligomeric guests by native and modified CDs, and further provide practical tools for controlling the chiral recognition ability of CDs. Finally, the combined use of thermodynamic and NMR techniques is highly recommended as a powerful method of choice for elucidating the comprehensive picture of global supramolecular interactions in chemistry and biology.

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Supporting Information Available: The enthalpy−entropy compensation plot obtained with the thermodynamic parameters for complexation of diastereomeric Z-D/L-Glu-L-Tyr and their component amino acids with native α-, β-, and γ-cyclodextrins and *A*,*X*-bis(trimethylammonio)-β-cyclodextrins. This material is available free of charge via the Internet at http://pubs.acs.org.

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