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Complexation and Chiral Recognition Thermodynamics of γ-Cyclodextrin with *N*-Acetyl- and *N*-Carbobenzyloxy-dipeptides Possessing Two Aromatic Rings

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The stability constants (K) and the standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy changes ($\Delta S°$) for the complexation of γ-cyclodextrin with 34 enantiomeric and diastereomeric *N*-acetyl- and *N*-carbobenzyloxy-D/L-dipeptides with two aromatic moieties were determined in aqueous buffer solution at 298.15 K by titration microcalorimetry. Chiral recognition of the enantiomeric dipeptide pairs by γ-cyclodextrin was found to be fairly poor, exhibiting only small percentage differences in K, while the diastereomeric dipeptides were discriminated to much greater extent with affinity differences of up to 6−7 times. The complex structures of several selected pairs were elucidated by NMR techniques. Combining the microcalorimetric and NMR data, the complexation and chiral recognition behavior of γ-cyclodextrin is discussed in particular in terms of the length, bulkiness, and flexibility of the tether connecting the two aromatic moieties in a guest.

Introduction

The symmetrical arrangement of glucopyranose units in native cyclodextrins (CDs) and the symmetrical distribution of chiral centers in the cavity are thought to be the intrinsic origin of generally poor chiral discrimination by native CDs.[1−4] Correspondingly, modified CDs are known to exhibit significantly higher chiral recognition abilities. Mono- and diaminated β-CDs discriminate a wide variety of chiral guest pairs much better than native β-CD, for which the reduced molecular symmetry of modified CDs and the electrostatic and/or hydrogen-bonding interactions are thought to be responsible.[2,5−10] The high chiral recognition ability of diaminated β-CDs, which generally exceeds that of monoaminated ones, is attributed to the "classical" three-point interaction.[2,8,9] For instance, trimethylamino(TMA)-β-CD, as well as native β-CD, affords virtually the same affinity toward both the D- and L-isomers of *N*-carbobenzyloxy(Cbz)-Glu, while *A*,*B*-TMA_2-β-CD exhibits a 40% difference in equilibrium constants between the enantiomers.[8,9] Nevertheless, we have demonstrated in our recent microcalorimetric study that even monoaminated β-CD can exhibit appreciable chiral discrimination, provided that the chiral guest possesses a hydrophobic moiety of appropriate size and shape to penetrate into the CD cavity.[10] Thus, monoaminated β-CD binds (*R*)-α-methoxyphenylacetic acid 2.8 times stronger than the (*S*)-isomer. Such a large chiral discrimination by monosubstituted CD is considered to be realized through the critical balance between the van der Waals and electrostatic interactions of the hydrophobic and cationic moieties of the guest with the cavity and anionic substituent of the host, respectively. Eventually, the (*R*)-isomer energetically more efficiently optimizes its conformation and location upon interaction with monoaminated-β-CD than the (*S*)-isomer.[10]

In view of the generally low ability of native CDs to recognize guests with point chirality, it is intriguing to elucidate the structural features of chiral guests that are discriminated by native CDs with high efficiency. Some clues may be found in our recent work;[11] when the hydrophobic moiety (of a chiral guest) to be accommodated in the CD cavity is achiral (e.g. phenyl or *tert*-butoxycarbonyl), only poor chiral discrimination is obtained. Even if the hydrophobic moiety contains chiral center(s) (e.g. camphor derivatives), no significant discrimination is observed. Furthermore, noncovalently interacting hydrophobic moieties, which are formed in situ within the cavity upon complexation through stacking of two aromatic rings of such guests as *N*-carboben-

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zoyloxy(Cbz)-protected Phe, Tyr, Trp, and His, do not improve the chiral recognition by native γ-CD. Nevertheless, our finding[8,9] that an apparently trivial difference in guest structure, such as an extra methylene or methyl group, can lead to a large enhancement in chiral discrimination prompted us to further examine the effects of tether type and length between the two stacking groups in a guest.

A noncovalently interacting hydrophobic moiety formed tentatively upon complexation is quite different from a conventional covalently bonded, structurally well-defined hydrophobic group of similar size, since the latter, without any tethered burden, can *freely* adjust its lateral and longitudinal positions inside the cavity to find the optimal stabilizing conformation spot. Upon chiral recognition by CD, such an energy-optimizing process often leads to minimal differences in complexation thermodynamic parameters for the enantiomeric guest pairs, which, however, affords an excellent enthalpy−entropy compensation relationship for almost all chiral guests, as demonstrated in our previous study.[4,10] In contrast, the in situ formation of the noncovalently interacting hydrophobic moiety requires a more precise fit to the CD cavity and is easily hindered by small size/shape alterations in guest structure. Such a critical requirement should be rather advantageous for the discrimination of chiral guest pairs. Indeed, we have shown in our preliminary study that chiral guests such as Cbz-Ala-Phe, Cbz-Ala-Tyr, and Cbz-Ala-Trp exhibit highly efficient chiral discrimination of up to 7 times between the diastereomeric D,L- and L,L-isomers upon stacking complexation with native γ-CD.[11]

In this paper, we present our results of comparative microcalorimetric and NMR spectral studies on the chiral discrimination by γ-CD of several *N*-acetyl(Ac)- and *N*-carbobenzyloxy-dipeptides bearing two aromatic rings. Careful choice of the guest peptides was made to investigate systematically the steric, electronic, and stereochemical effects of the chiral tether and aromatic groups on the formation of a noncovalently interacting hydrophobic moiety inside the cavity upon complexation with γ-CD. The first guest set employed is a series of Ac-dipeptides that contain Phe and/or Tyr residues, which are structural homologues of previously investigated Cbz-Ala-Phe, Cbz-Ala-Tyr, and Cbz-Ala-Trp[11] with a very different tether group. The second set, i.e., Cbz-Phe, Cbz-Phe-Val, and Cbz-Phe-Pro, enables us to elucidate the effect of nonaromatic chiral "attachments" (Val or Pro) on chiral discrimination. The third set, i.e., Cbz-$(Gly)_n$-Phe and Cbz-$(Gly)_n$-Trp ($n = 0-2$) with a varying number of Gly residues between the Cbz and aromatic amino acid residues, will reveal the effect of distance between the two aromatic rings in a guest. Finally, we can also discuss the effects of length, bulkiness, and rigidity of the tether upon complexation and chiral recognition thermodynamics by using several guest series.

Results and Discussion

The stability constant (K), standard free energy ($\Delta G°$), enthalpy ($\Delta H°$), and entropy changes ($T\Delta S°$) determined for the complexation reactions of γ-CD with 34 chiral dipeptide guests are listed in Table 1, along with the relevant data reported for the complexation reactions of γ-CD with Cbz-D/L-Phe, Cbz-D/L-Tyr, and Cbz-D/L-Trp. The enthalpy and entropy changes obtained for dipeptide guests are consistently negative (-68 kJ mol^{-1} < $\Delta H°$ < -12 kJ mol^{-1}; -62 kJ mol^{-1} < $T\Delta S°$ < -0.1 kJ mol^{-1}) with the exception of Cbz-D-Phe, which gives a slightly positive entropy (Table 1). As have been demonstrated amply in our previous studies,[3,4,12] large negative enthalpy changes are accounted for in general terms by the pronounced van der Waals interactions arising from the precise matching in size and shape between the host and guest involved. Additional negative enthalpy gains in the case of the above dipeptide guests are expected to arise from $\pi-\pi$ interactions between the guest's aromatic rings upon insertion into the cavity. The large negative entropy changes usually arise from the significantly reduced translational and conformational freedoms of the host and guest upon complexation.[3,4,12] Additional loss of entropy is expected to occur from the translational restrictions of the two aromatic rings upon insertion into the cavity. This simple rationale is in good agreement with the experimental results (Table 1).

It may be reasonable to classify the obtained thermodynamic data into a few categories in terms of the nature (flexibility, bulkiness, and length) of the tether connecting the two aromatic rings in a guest, since the tether's properties intrinsically determine the inclusion mechanism. Thus, the thermodynamic consequence of a short bulky tether, which causes steric hindrance in adjusting the relative location/position of two aromatic rings in the CD cavity, should completely differ from that of a long and flexible tether, which allows full conformational optimization of the two aromatic rings in the cavity without seriously reducing the tether's freedom. Accordingly, the experimental results are separately presented and discussed in several subsections classified by the properties of the tether group.

Prior to the discussion of the detailed structure of the γ-CD complexes of dipeptides, we summarize the available experimental evidence in support of the folding of the dipeptide guests and the simultaneous inclusion of their two aromatic rings into the γ-CD cavity. First, in contrast to the complexation behavior of dipeptides, the lower analogues with only one aromatic ring, such as Ac-Phe, Ac-Tyr, Ac-Trp, and Gly-Phe, show no appreciable affinity toward γ-CD under identical conditions. Second, the aromatic protons of dipeptides show large upfield shifts in the NMR spectra upon complexation with γ-CD most probably as a result of the ring current of the co-included aromatic moieties. Third, the observed NOE patterns of ROESY spectra unambiguously prove the penetration of the aromatic rings from the wider opening of the cavity, which facilitates simultaneous inclusion. It should be emphasized that the large interior of the γ-CD cavity (9.4 Å in diameter) provides space enough to comfortably accommodate two aromatic rings probably in a loosely packed, slide fashion (see Supporting Information for an MM2-optimized molecular model of Cbz-D-Ala-Phe + γ-CD complex), which keeps the inter-aromatic-proton distance larger than that required for observing appreciable NOEs (ca. 3.5 Å). This is why the coexistence of several guest conformers with different geometry and architecture is possible inside the γ-CD cavity as proved by our NMR study discussed below.

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 *N*-Ac- and *N*-Cbz-mono and -dipeptides Containing Aromatic Amino Acids with γ-Cyclodextrin in Buffer Solutions (pH 6.9) at $T = 298.15$ K

guest	stereo-chemistry	guest concn/mM	γ-CD concn/mM	pH	N^a	K/M^{-1}	$\Delta G°$/ kJ mol^{-1}	$\Delta H°$/ kJ mol^{-1}	$T\Delta S°$/ kJ mol^{-1}	footnote
Ac-Phe-Tyr	D,D	125	0.98−1.49	6.9[b]	2	24 ± 5	−7.9 ± 0.6	−12.0 ± 1.0	−4.1 ± 1.2	*c*
	L,L	92−106	2.08−2.28	6.9[b]	2	29 ± 2	−8.3 ± 0.2	−12.6 ± 0.4	−4.3 ± 0.5	*c*
	D,L	25−91	1.28−2.08	6.9[b]	3	87.5 ± 1.5	−11.08 ± 0.05	−16.0 ± 0.2	−4.9 ± 0.2	*c*
	L,D	82	0.98	6.9[b]	2	82.4 ± 1.5	−10.94 ± 0.05	−16.4 ± 0.2	−5.5 ± 0.2	*c*
Ac-Tyr-Phe	D,D	133	1.16	6.9[b]	2	36 ± 2	−8.88 ± 0.15	−12.5 ± 0.3	−3.6 ± 0.3	*c*
	L,L	151	1.49	6.9[b]	2	40 ± 2	−9.14 ± 0.15	−13.5 ± 0.3	−4.4 ± 0.3	*c*
	D,L	117	1.41	6.9[b]	2	65.7 ± 1.5	−10.37 ± 0.06	−18.7 ± 0.2	−8.3 ± 0.2	*c*
	L,D	118	1.35	6.9[b]	2	65.5 ± 1.5	−10.37 ± 0.06	−17.7 ± 0.2	−7.3 ± 0.2	*c*
Ac-Tyr-Tyr	D,D	133	1.55	6.9[b]	2	30 ±2	−8.4 ± 0.2	−17.5 ± 0.3	−9.1 ± 0.4	*c*
	L,L	152	1.65	6.9[b]	2	37 ± 2	−8.95 ± 0.15	−18.7 ± 0.3	−9.8 ± 0.3	*c*
	D,L	83	1.33	6.9[b]	2	66.5 ± 1.5	−10.40 ± 0.06	−21.7 ± 0.3	−11.3 ± 0.3	*c*
	L,D	146	1.45	6.9[b]	2	70.1 ± 1.5	−10.53 ± 0.06	−20.7 ± 0.3	−10.2 ± 0.3	*c*
Ac-Phe-Phe[d]	D,D	146	6.73	10.0[e]	1	*f*		*f*		*c*
	L,L	136	1.48−6.73	10.0[e]	2	*f*		*f*		*c*
Cbz-Tyr	D	34	1.02	6.9[b]	2	175 ± 4	−12.80 ± 0.05	−19.2 ± 0.3	−6.4 ± 0.3	*g*
	L	44	0.86	6.9[b]	2	192 ± 3	−13.03 ± 0.04	−20.4 ± 0.2	−7.4 ± 0.2	*g*
Cbz-Phe	D	51	1.12	6.9[b]	2	175 ± 3	−12.80 ± 0.04	−11.9 ± 0.2	0.9 ± 0.2	*g*
	L	52	0.89	6.9[b]	2	185 ± 4	−12.94 ± 0.05	−12.8 ± 0.2	−0.1 ± 0.2	*g*
Cbz-Homophe	D	110	2.14	6.9[b]	2	38.5 ± 1.5	−9.05 ± 0.10	−20.8 ± 0.2	−11.8 ± 0.3	*c*
	L	51−110	0.99−2.14	6.9[b]	4	24 ± 2	−7.9 ± 0.2	−27.3 ± 0.6	−19.4 ± 0.6	*c*
Cbz-Gly-Phe	D	123	2.22	6.9[b]	2	56.0 ± 1.5	−9.98 ± 0.09	−18.5 ± 0.3	−8.5 ± 0.3	*h*
	L	137	2.15	6.9[b]	2	65 ± 2	−10.35 ± 0.08	−18.7 ± 0.3	−8.4 ± 0.3	*h*
Cbz-Gly-Gly-Phe	L	144	1.61	6.9[b]	2	13 ± 2	−6.4 ± 0.5	−35 ± 3	−29 ± 3	*c*
Cbz-Trp	D	47	0.51−1.40	6.9[b]	2	53 ± 5	−9.8 ± 0.3	−31 ± 2	−21 ± 2	*h*
	L	31	0.51−1.40	6.9[b]	2	57 ± 5	−10.0 ± 0.2	−31 ± 2	−21 ± 2	*h*
Cbz-Gly-Trp	D	111	1.60	6.9[b]	2	49 ± 2	−9.65 ± 0.09	−30.6 ± 0.5	−21.0 ± 0.5	*h*
	L	120	1.65	6.9[b]	2	53 ± 2	−9.84 ± 0.09	−29.7 ± 0.5	−19.9 ± 0.5	*h*
Cbz-Gly-Gly-Trp	L	135	1.98	6.9[b]	2	16 ± 2	−6.9 ± 0.4	−48 ± 5	−41 ± 5	*c*
Cbz-Ala-Phe	D,L	112	1.81	6.9[b]	2	73 ± 2	−10.64 ± 0.07	−19.3 ± 0.2	−8.7 ± 0.2	*h*
	L,L	111	1.76	6.9[b]	2	12 ± 2	−6.2 ± 0.5	−33 ± 4	−27 ± 4	*h*
Cbz-Ala-Tyr	D,L	104	1.34	6.9[b]	2	184 ± 2	−12.93 ± 0.03	−27.1 ± 0.3	−14.2 ± 0.3	*h*
	L,L	156	1.63	6.9[b]	2	60.8 ± 1.5	−10.18 ± 0.06	−20.1 ± 0.2	−9.9 ± 0.2	*h*
Cbz-Ala-Trp	D,L	113	1.76	6.9[b]	2	73.8±1.5	−10.66±0.05	−28.0 ± 0.3	−17.3 ± 0.3	*h*
	L,L	139	1.88	6.9[b]	2	10 ± 4	−6 ± 2	−68 ± 20	−62 ± 20	*h*
Cbz-Phe-Val	D,L	84	0.84−1.40	6.9[b]	2	51 ± 4	−9.7 ± 0.2	−19.8 ± 0.7	−10.1 ± 0.7	*c*
	L,L	72	0.84	6.9[b]	2	59 ± 4	−10.1 ± 0.2	−17.6 ± 0.6	−7.5 ± 0.6	*c*
Cbz-Phe-Pro	D,L	124	1.41	6.9[b]	2	29 ± 3	−8.3 ± 0.2	−22.5 ± 0.6	−14.2 ± 0.6	*c*
	L,L	52−118	0.99−1.45	6.9[b]	4	78 ± 8	−10.8 ± 0.3	−15.3 ± 0.8	−4.5 ± 0.8	*c*
Cbz-Pro-Phe	D,L	102	1.28	6.9[b]	2	138 ± 3	−12.21 ± 0.05	−12.78 ± 0.15	−0.6 ± 0.2	*c*
	L,L	138	1.90	6.9[b]	2	26.1 ± 1.5	−8.09 ± 0.15	−21.8 ± 0.4	−13.7 ± 0.4	*c*

[a] Number of titration microcalorimetric experiments performed. [b] Standard phosphate buffer at pH 6.9 [NaH_2PO_4 (0.025 M) + $NaHPO_4$ (0.025 M)]. [c] This work. [d] No measurable complexation was detected in microcalorimetric experiments even at high concentration of γ-CD up to 6.7mM. [e] Titration performed with a 0.05 M carbonate buffer at pH 10.0 [$NaHCO_3$ (0.025 M) + Na_2CO_3 (0.025 M)], since the guest is practically insoluble at pH 6.9. [f] Equilibrium constant and/or reaction enthalpy is too small to be determined by titration microcalorimetry. [g] Reference 15. [h] Reference 11.

Critical Effect of the Methyl Position at the Chiral Centers on Chiral Discrimination of Cbz-dipeptides. As reported in our recent paper,[11] the relatively long tether of Cbz-Gly-Phe is in a fairly restricted conformation upon complexation and does not allow the guest to greatly alter the location/position of the aromatic rings inside the cavity. Similarly, it is deduced that the tether of Cbz-Gly-Trp is conformationally restricted upon complexation, as judged from the observed large negative entropy of complexation.

If we accept the above assumption of highly restricted tether conformations for Cbz-Gly-D/L-Phe and Cbz-Gly-D/L-Trp, we may expect large steric hindrance by making the tether bulkier. Furthermore, when a substitution makes the tether chiral, the magnitude of steric hindrance upon guest insertion is expected to critically affect the induced chirality. Indeed, native γ-CD showed an intriguing chiral recognition behavior upon complexation with diastereomeric guest pairs: Cbz-D/L-Ala-L-Phe, Cbz-D/L-Ala-L-Tyr, and Cbz-D/L-Ala-L-Trp. The D,L-isomers of these dipeptides exhibit 3−7 times higher affinity toward γ-CD than the corresponding L,L-isomers. Affinity enhancement is entropy driven in the case of Cbz-D/L-Ala-L-Phe and Cbz-D/L-Ala-L-Trp, but is enthalpy driven for Cbz-D/L-Ala-L-Tyr. To the best of our knowledge, this is the first time that the difference of one methyl group has been shown to have a pronounced effect on the overall chiral recognition by γ-CD or by any native CDs.

Our previous NMR study[11] revealed the coexistence of two slowly interconverting conformers of D,L- and L,L-Cbz-Ala-Tyr upon complexation with γ-CD in solution. In the present study, similar slow interconversions were demonstrated to occur between two coexisting conformers of Cbz-D-Ala-L-Phe and Cbz-D-Ala-L-Trp in the γ-CD cavity (Figures 1 and 2), although the very low affinities of Cbz-L-Ala-L-Phe and Cbz-L-Ala-L-Trp did not allow elucidation of their complex structures by NMR spectroscopy. In principle affinity of each of these conformers toward γ-CD can be characterized by microscopic binding constants.[13a] However, only macroscopic binding constants, e.g. deter-

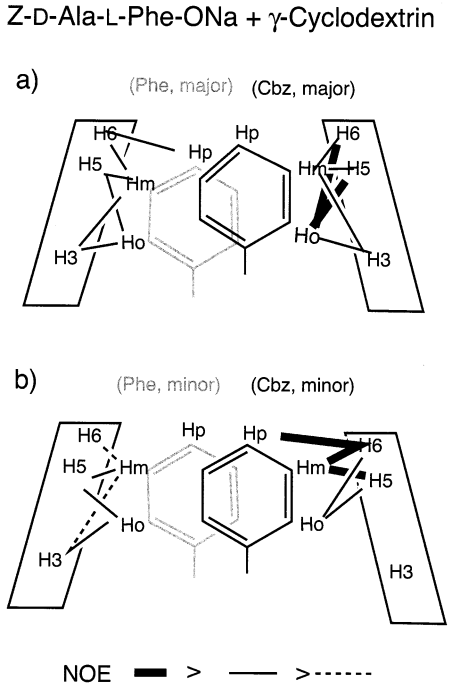

FIGURE 1. Plausible complex structures of (a) major and (b) minor conformers of Cbz-D-Ala-L-Phe with γ-CD.

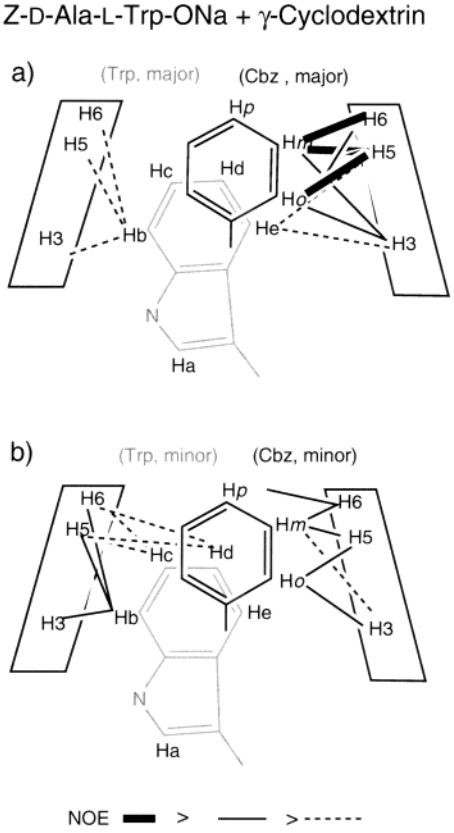

FIGURE 2. Plausible complex structures of (a) major and (b) minor conformer of Cbz-D-Ala-L-Trp with γ-CD.

mined by microcalorimetry, are the thermodynamic characteristics of the total complexation reaction and only these thermodynamic quantities are presented in Table 1.

Upon stacking complexation of Cbz-D-Ala-L-Trp with γ-CD, the large indole ring will not have enough space for rotation inside the cavity, but is still allowed to slide parallel to the cavity axis changing the depth of penetration. This picture is in good agreement with the NOE results, which show NOE cross-peaks of the indole's H*b* proton with the CD's H3, H5, and H6. The stronger NOE intensity of H*b* obtained for the minor, rather than major, conformer and the NOE signals between indole's H*c* and H*d* with CD's H5 and H6 observed only for the minor conformer jointly indicate the more intimate interactions of indole and cavity walls in the minor conformer. Usually, the more closely located host−guest pair leads in general to stronger van der Waals interactions, affording a more negative enthalpy. In addition, the observed NOE patterns of Cbz's H*o*, H*m*, and H*p* with selected CD protons (Figure 2; see Supporting Information for the ROESY spectrum) are compatible with the simultaneous translation of stacked indole/Cbz rings inside the cavity, rather than the independent motions of the two rings.

Effect of Flexibility of Amino Acid Residues. Comparison of the thermodynamic behavior of Cbz-Phe, Cbz-Phe-Val, and Cbz-Phe-Pro is insightful. The more negative enthalpy of Cbz-Phe-Val versus Cbz-Phe is readily accounted for in terms of additional van der Waals interactions of the Val residue with the CD cavity walls. Simultaneously, the Val residue suffers conformational fixation and causes steric hindrance upon stacking complexation with γ-CD, both of which should cause a large entropic loss, as observed (Table 1). It should be emphasized that the flexible aliphatic chain of Val allows fine-tuning of the complex structure of the D,L- and L,L-isomers with the accompanying frequently observed enthalpy−entropy compensation leading to low chiral discrimination.

Clearly, the rigid Pro residue has less freedom to alter its complex structure and consequently balances the entropy and enthalpy changes between the D,L- and L,L-isomers resulting in similar affinities. Due to its rigidity, Pro is already conformationally restricted in bulk water and the loss of entropy upon stacking complexation is relatively small if the shape/size of the stacked moiety is well fitted to the cavity. Indeed, the Cbz-L-Phe-L-Pro guest exhibits less unfavorable entropy of complexation compared with the D,L- and L,L-isomers of Cbz-Phe-Val. Furthermore, Cbz-L-Phe-L-Pro gives much smaller negative enthalpy of complexation than that for both isomers of Cbz-Phe-Val. The enthalpy of complexation of Cbz-L-Phe-L-Pro is quite close to that of Cbz-D/L-Phe, and hence it is inferred that the originally existing van der Waals interaction pattern for the simpler guest (Cbz-Phe) is not significantly altered by the presence of one extra Pro residue, or alternatively, that the additional Pro does not make any strong direct contacts with the cavity walls upon complexation. In contrast, the Pro of the D,L-isomer behaves as a large bulk and leads to a severely restricted complex structure, as judged from the largest negative entropy among the above guests. It is also amply observed in CD complexation thermodynamics that restricted conformation often leads to a large negative

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enthalpy arising from extensive van der Waals interactions. As expected, the rigid structure of Pro does not allow precise cancellation of the entropy and enthalpy changes between the two isomers of Cbz-Phe-Pro through the entropy−enthalpy compensation effect. Indeed, the affinity difference between L,L- and D,L-Cbz-Phe-Pro amounts to ca. 2.5 times, which is much higher than that for the diastereomeric Cbz-Phe-Val.

For even higher chiral discrimination, it is reasonable to introduce a rigid Pro residue in the tether connecting Cbz and Phe. As discussed above, the long flexible tether of Cbz-Gly-Phe is conformationally restricted upon stacking complexation. Thus, if we fix the conformation of the tether in bulk water by introducing a rigid Pro, instead of Gly, we can expect that only one of the diastereomeric Cbz-peptides achieves precise fitting to the cavity, giving high chiral discrimination. Indeed, the D,L-isomer of Cbz-Pro-Phe exhibits more than 5 times higher affinity than the corresponding L,L-isomer. The reaction entropy is almost zero and thus it may be concluded that the D,L-isomer is not severely restricted upon complexation. In contrast, the L,L-Cbz-Pro-Phe affords totally different thermodynamic parameters. As judged from its low affinity and large negative entropy of complexation, the L,L-isomer does not properly fit into the cavity. As usual, the severally restricted conformation of L,L-Cbz-Pro-Phe is confirmed not only by the large negative entropy but also by the large negative enthalpy of complexation.

It should be noted that the close to zero reaction entropy, which was obtained for the complexation of the D,L-isomer of Cbz-Pro-Phe, is not an exception. Similar thermodynamic behavior is frequently observed for complexation reactions where severe conformational restriction upon complexation is not expected,[4,10] for instance in the case of Cbz-D(L)-Phe (Table 1).

Further NMR studies of the complexation of D,L- and L,L-Cbz-Pro-Phe are useful in elucidating the molecular mechanisms leading to the above-mentioned global discussion based solely on the overall thermodynamic parameters. Judging from the observed NOE signals shown in Figure 3, parts a and b (see Supporting Information for the ROESY spectra), Cbz-L-Pro-L-Phe is included deeper into the cavity than the diastereomeric Cbz-D-Pro-L-Phe. Thus, only in the case of Cbz-L-Pro-L-Phe can the Phe's H*m* and H*p* penetrate deep enough to cause NOEs not only with H5 but also with H6 of γ-CD. Deeper penetration results in more pronounced van der Waals interactions and thus a more favorable reaction enthalpy. This complex structure picture nicely coincides with the microcalorimetric data, which show almost doubled reaction enthalpy for L,L- in comparison to D,L-Cbz-Pro-Phe.

It is interesting that two diastereomeric pairs of dipeptide guests of inverted sequence, i.e., Cbz-D/L-Pro-L-Phe and Cbz-D/L-Phe-L-Pro, exhibit the opposite chiral selectivity upon complexation with γ-CD. Thus, the D,L-Cbz-Pro-Phe shows more than 5 times higher affinity than the L,L-isomer, whereas the L,L-Cbz-Phe-Pro is preferred by γ-CD. It should be emphasized that in both cases the simultaneous inclusion of stacked Cbz and phenyl rings is expected to fill all the inner space of the cavity, thus making a reasonably stable complex. In our previously work,[4] we established a direct correlation between the mode of guest penetration into the CD cavity

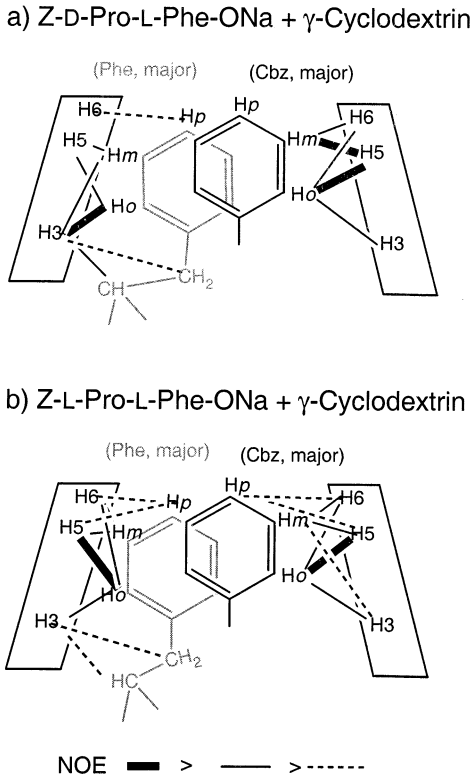

FIGURE 3. (a) Plausible complex structure of the major conformer of Cbz-D-Pro-L-Phe with γ-CD (for the minor conformer, the only clear NOEs observed were between H*o* of Phe and H3 of γ-CD and between H*o* of Cbz and H5 of γ-CD; Obviously this information is not enough to elucidate the complete complex structure). (b) Plausible complex structure of the major conformer of Cbz-L-Pro-L-Phe with γ-CD (for the minor conformer, only two clear NOEs were observed between α-CH and β-CH_2 of Phe and H3 of γ-CD; obviously this information is not enough to elucidate the complete complex structure).

and the chiral discrimination. For instance, L-isomers of Ac-Phe, Ac-Trp, Ac-Tyr, Gly-Phe, and phenylalanine amide (Phe-am) consistently show higher affinities toward β-CD than the corresponding D-isomers. In contrast, D-isomers of *N*-Boc-Ala, *N*-Boc-Ser, and *N*-Boc-Ala methyl ester are better bound to β-CD. Indeed, the introduction of a Boc group to alanine or serine at the amino terminus switches the hydrophobicity order of the guest substituents through the conversion of the originally most hydrophilic group (NH_3^+) to the most hydrophobic group (*t*-BuOCON). Consequently, the guests with the same absolute configuration are consistently preferred, as long as the hydrophobicity order of the substituents around the stereogenic center is preserved throughout the enantiomeric guest pairs. Thus, if one alters the hydrophobicity order of the relevant substituents around the stereogenic center, the antipodal guest is favored. However, in the present case two aromatic rings attached to different amino acid residues are simultaneously included in the same γ-CD cavity upon complexation. Hence, the mode of penetration of the Cbz- and Ac-dipeptides, possessing two aromatic rings, into the γ-CD cavity cannot be compared directly with those of other amino acid derivatives possessing only one aromatic ring.[12]

To establish the rules governing chiral discrimination upon stacking complexation of two simultaneously included guest aromatic rings with γ-CD it is crucial to elucidate the roles of the tether connecting the two aromatic rings, as the shape of the hydrophobic moiety formed in situ upon complexation is significantly affected by the tether. Tether length is thought to be the most important factor that determines the ability of two aromatic rings to adjust their location/position within the cavity. In good agreement with this idea, L-isomers of Cbz-Phe and Cbz-Tyr are preferred by γ-CD, while the introduction of one extra methylene into the tether, i.e., Cbz-Homophe, immediately leads to the opposite selectivity in favor of the D-isomer. In the case of the guests with two chiral centers, the effect of tether length upon chiral recognition is even more profound. Indeed, Cbz-Ala-Phe, Cbz-Ala-Tyr, Cbz-Ala-Trp, and Cbz-Pro-Phe, having the same tether length, exhibit the same preference for the D,L-isomer upon complexation with γ-CD. However, Cbz-Phe-Val and Cbz-Phe-Pro with a shorter tether show the opposite L,L-preference. Certainly, the various tethers of the same length may lead to a different chiral preference, as exemplified by a series of Ac-dipeptides (Ac−Phe-Tyr, Ac-Tyr-Phe, Ac-Tyr-Tyr, and Ac−Phe-Phe). Possessing virtually the same tether length as Cbz-Phe-Val and Cbz-Phe-Pro, the Ac-dipeptides display a consistent preference for heterochiral D,L- or L,D-isomers than for homochiral D,D- or L,L-isomers, which is in sharp contrast to the L,L-preference observed for Cbz-Phe-Val and Cbz-Phe-Pro. At present, our knowledge is obviously limited and is not enough to comprehensively predict the chiral recognition of a wide variety of dipeptides by γ-CD simply from the chemical structure.

Tether Length Effect on Complexation Thermodynamics. The effect of tether length can be elucidated by systematically comparing the thermodynamic parameters for two series of guests, in which the same aromatic groups are connected by a tether of varying length: (1) Cbz-Phe, Cbz-Homophe, Cbz-Gly-Phe, and Cbz-Gly-Gly-Phe and (2) Cbz-Trp, Cbz-Gly-Trp, and Cbz-Gly-Gly-Trp.

As can be seen from Table 1, the introduction of one methylene to Cbz-Phe causes drastic changes in the thermodynamic parameters for the complexation of Cbz-Homophe with γ-CD. The affinities of D- and L-Cbz-Homophe were decreased by a factor of 4−7, despite the doubled enthalpic gains, for which the much greater entropic losses are solely responsible. The longer tether of Cbz-Homophe, versus Cbz-Phe, provides the two guest phenyls with additional freedom to precisely adjust the shape/size of the stacked hydrophobic moiety to the γ-CD cavity. The strong well-optimized stacking of aromatic rings inside the cavity is the origin of the much larger entropic losses observed upon complexation of Cbz-Homophe.

What would be anticipated if we further extend the tether length? If the above logic is correct, both the enthalpy and entropy changes will become more negative and the complex stability will decrease by inserting Gly unit(s) between the two stacking phenyls of Cbz-Phe as a tether. As shown in Table 1, this is exactly the case with the Cbz-$(Gly)_n$-Phe series ($n = 0-2$), where the $\Delta H°$ value decreases from −12 to −19 and then to −35 kJ mol^{-1} and the $T\Delta S°$ from 0 to −8 and then to −29 kJ mol^{-1}, with concomitant decreases in K or $\Delta G°$ as the number of Gly residues increases from 0 to 2. It is somewhat unexpected that the observed decreases in both $\Delta H°$ and $T\Delta S°$ are smaller than the corresponding values obtained for Cbz-Homophe in view of the much longer tethers of Cbz-$(Gly)_n$-Phe ($n = 1-2$). Although we could not find any convincing rationales for this apparent discrepancy, the nature of the tether group should affect the thermodynamic behavior, probably through the tether hydration/dehydration and flexibility differences. This explanation seems reasonable, since there are intrinsic differences between the poorly hydrated flexible hydrophobic aliphatic tether and the more rigid hydrophilic peptide tether capable of relatively strong interactions with water molecules.

It is also interesting to compare the complexation thermodynamic behavior of the Cbz-$(Gly)_n$-Trp series ($n = 1-2$). Cbz-Trp and Cbz-Gly-Trp guests exhibit virtually the same affinity toward γ-CD, as well as comparable reaction enthalpies and entropies. Probably, the larger loss of conformational/translational entropy arising from the restriction of the longer tether of Cbz-Gly-Trp is compensated for by the extra entropy gain arising from thorough desolvation of the Trp moiety stacked within the γ-CD cavity, which is not available to the Phe group of Cbz-$(Gly)_n$-Phe. On the other hand, the van der Waals interactions are not appreciably disturbed by the extensive guest/host desolvation as can be judged from the same reaction enthalpy for both Cbz-Trp and Cbz-Gly-Trp. Nevertheless, the insertion of two Gly units between the Cbz and Trp groups leads to a much decreased affinity toward γ-CD and highly negative $\Delta H°$ and $T\Delta S°$ values for Cbz-Gly-Gly-Trp. It may be concluded therefore that the affinity for the stacking guest toward γ-CD consistently decreases (or is at least unchanged) with increasing tether length: Cbz-Phe > Cbz-Homophe; Cbz-Phe > Cbz-Gly-Phe > Cbz-Gly-Gly-Phe; and Cbz-Trp ≈ Cbz-Gly-Trp > Cbz-Gly-Gly-Trp. However, it should be emphasized that in both cases of Cbz-Phe versus Cbz-Gly-Phe and of Cbz-Trp versus Cbz-Gly-Trp, the entropy losses (if any) resulting from the tether elongation are much smaller than that for the Cbz-Phe versus Cbz-Homophe case, despite the larger tether elongation magnitude for the former two guest pairs. The most likely explanation for this is the more extensive desolvation upon complexation of the heavily hydrated hydrophilic peptide tether, which is not available for the highly hydrophobic aliphatic chain.

This observation, if general, is of particular interest in view of biological supramolecular recognition involving peptide chains. This is because the interfaces of interacting proteins "are more likely to incorporate nonpolar amino acids than the remainder of the protein exterior"[13b] and hence desolvation from these relatively strongly hydrated nonpolar amino acids should make a significant impact on the overall thermodynamics of protein−protein interactions. Our findings suggest that the entropic advantages upon interaction may arise not only from the desolvation of nonpolar amino acids residues but also from the desolvation of the peptide chain itself. If a peptide tether is able to "entropically" mediate specific interactions of amino acid residues separated in space, then very extensive structural changes in proteins, induced by apparently trivial local interactions, become sensible. Indeed, the drastic but smooth conformational

changes of proteins, e.g. upon interaction with another protein or substrate, or in different solvents, are reasonably understood by assuming that the free energy difference between the relevant protein structures before and after interaction is minimized by the enthalpy−entropy compensation effect through the solvation/desolvation of peptide chains. This is why the interaction of proteins with small ligands such as glutamate leads to the re-organization of whole subunits in the dimeric matabotropic glutamate receptor.[14]

Complexation Thermodynamics and Chiral Recognition of *N*-Acetyl-dipeptides. Comparison of complexation thermodynamics of Ac-dipeptides containing two aromatic rings with that of Cbz-Phe and Cbz-Tyr is interesting, since both classes of guest are very similar in tether length, separating the two aromatics by 7 single bonds, and also in the number of C−C or C−N bonds. The only exception is that the tethers of Ac-dipeptides contain the more bulky Ac group, instead of the oxygen found in Cbz-amino acids. This difference in the tether leads to very profound thermodynamic consequences. Thus, Cbz-Phe exhibits a moderate affinity of around 175−185 M^{-1} toward γ-CD, whereas Ac-Phe-Phe does not show any appreciable affinity under similar complexation conditions. The most likely reason is the steric hindrance caused by the Ac group, which completely disrupts complex formation. The negative effect of the steric bulk of Ac on complex formation is supported by other observations as well. First, the D,D- and L,L-isomers of Ac-Phe-Tyr and Ac-Tyr-Phe exhibit 4−8 times lower affinity than Cbz-Tyr, despite possessing the same phenyl and phenol rings for stacking complexation. This reduction in stability is ascribed solely to the enthalpic losses for Ac-Phe-Tyr and Ac-Tyr-Phe as compared with Cbz-Tyr, which most probably originates from the loss of optimal van der Waals and/or hydrogen-bonding interactions due to the steric hindrance of the Ac group. This question will be discussed in more detail below in connection with the NMR study. Second, Ac-Tyr-Tyr, possessing two phenol moieties for intracavity hydrogen bonds, gives almost the same complexation enthalpy as Cbz-Tyr, with only one hydrogen-bonding phenol moiety; again, the steric bulk of Ac is likely to be responsible.

It is noted, however, that the steric effect of Ac strongly depends on the stereochemistry of the guest. Indeed, the heterochiral D,L- and L,D-isomers of Ac-Phe-Tyr, Ac-Tyr-Phe, and Ac-Tyr-Tyr are consistently more favored by γ-CD by a factor of 2−3 than the corresponding homochiral D,D- and L,L-isomers. In contrast to the good diastereomeric discrimination, the enantiomeric discrimination of each dipeptide guest, i.e., D,L versus L,D and D,D versus L,L, is poor, giving only several percentage differences in *K*. This chiral recognition behavior may also be attributed to the constraint of the bulky tether upon complexation, as the original conformational differences in diastereomeric pairs are likely to be exaggerated upon complexation with γ-CD.

Interestingly, diastereomeric Ac-D-Phe-L-Tyr and Ac-L-Phe-L-Tyr guests show significantly different thermodynamic parameters with relatively high chiral discrimination, but give quite similar NOE patterns (Figure 4, parts a and b; see Supporting Information for the ROESY spectra). The same NOE patterns indicate that the two aromatic moieties of the diastereomeric guest pair are stacked in similar shape and position inside the cavity of γ-CD, as a result of analogous van der Waals contact patterns. However, such a situation inevitably leads to very similar reaction enthalpies for both diastereomers. Consequently, the NMR spectral examinations cannot explain the enthalpic difference due to chirality as large as 3.4 kJ mol^{-1}. A probable answer to this puzzle may be found in the different intracavity hydrogen-bonding behavior for Ac-D-Phe-L-Tyr versus Ac-L-Phe-L-Tyr. Indeed, Ac-Phe-Phe, lacking the ability to form intracavity hydrogen bonds, cannot produce an appreciably stable complex with γ-CD (see Table 1). Furthermore, the complexation enthalpy of Ac-D/L-Phe-Tyr with γ-CD is the smallest among the Tyr-containing dipeptides. Thus, the existence of intracavity hydrogen bonds is absolutely crucial for the stability of the Ac-D/L-Phe-L-Tyr complex with γ-CD. Taking into account the substantial heat effect of phenolic hydrogen-bond formation inside the CD cavity in the range of 6−8 kJ mol^{-1}, it is not unrealistic to ascribe the thermodynamic differences observed for the diastereomeric Ac-D/L-Phe-L-Tyr guests to the change in intracavity hydrogen bonding of Tyr, which is not appreciably detected by the ROESY spectra.

In the present study with γ-CD as a model receptor, we have demonstrated that a simple truncated cone-shaped chiral cavity ca. 8 Å in diameter and in depth can effectively recognize peptide sequence, e.g. Ac-Phe-Tyr versus Ac-Phe-Phe, and unambiguously favors the heterochiral rather than homochiral dipeptides. Since a pair of diastereomers are chemically different species, we do not need a chiral receptor to discriminate them. Thus, it is not unrealistic to anticipate in general that similar

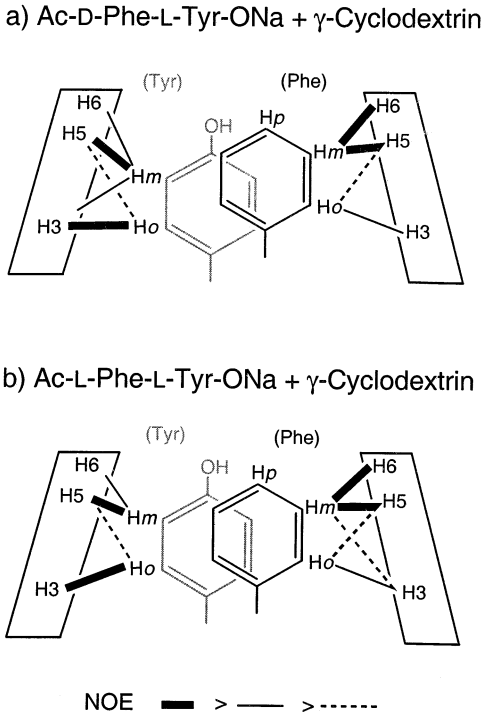

FIGURE 4. Plausible complex structures of (a) Ac-D-Phe-L-Tyr with γ-CD and (b) Ac-L-Phe-L-Tyr with γ-CD.

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differentiating binding of heterochiral versus homochiral peptides takes place upon inclusion complexation or surface adsorption by other concaved/porous, homogeneous/heterogeneous, organic/inorganic, and molecular/supramolecular/polymeric receptors with an interacting site of similar size and shape, and also that such a diastereomeric differentiation mechanism would play some role on prebiotic Earth, ultimately leading to the homochirality in the biosphere.

Experimental Section

Materials. Commercially available samples of the highest purity were used in the microcalorimetric experiments without further purification. The vendors employed a variety of methods to determine and guarantee the purity of the guests: >98−99% (HPLC, LC, GC, titration, and/or elemental analysis). The β-CD, monoamino-β-CD, and some of the guest compounds contained water of hydration or crystallization, and therefore appropriate corrections were made for the moisture content, based on values determined by the vendors and/or by us using the Karl-Fisher technique.

Microcalorimetric Measurements. An isothermal calorimeter (ITC) was used in all microcalorimetric experiments. Titration microcalorimetry allows us to determine simultaneously the enthalpy and equilibrium constant from a single titration curve. The ITC instrument was periodically calibrated electrically with use of an internal electric heater. The instrument was also calibrated chemically by using the neutralization enthalpy of the reaction of HCl with NaOH, and the ionization enthalpy of TRIS buffer. These standard reactions gave excellent agreement (±1−2%) with the literature data.[15] The thermodynamic parameters for the complexation reaction of cyclohexanol with β-CD were also in good agreement with our previous results.[4,16,17]

The ORIGIN software (Microcal), which was used to calculate the equilibrium constant and standard molar enthalpy of reaction from the titration curve, gave a standard deviation based on the scatter of the data points in a single titration curve. As we reported previously,[16,17] the accuracy of the calculated thermodynamic quantities for 1:1 complexations was checked by performing several independent titration runs. The uncertainties in the obtained thermodynamic quantities 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,[12] the microcalorimetric titration curve for 1:1 and 1:2 complex stoichiometry shows a totally different shape and can be distinguished unambiguously from the fitting curve. Nevertheless, computer simulations are usually employed to ensure the complex stoichiometry. It should be emphasized that in addition to the calculations based on the 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 experiment, a constant volume of guest solution in 0.05 M standard phosphate buffer usually at pH 6.9 was injected into the reaction cell (1.36 mL) charged with a CD solution in the same buffer (5 μL/injection; 20 injections in total), except for the Ac-Phe-Phe case, where 0.05 M standard carbonate buffer at pH 10.0 was used (as the guest is not soluble at pH 6.9) to satisfy the requirement $|pK_a(\text{guest}) - pH| > 2$. The initial concentrations of guest and CD in each run are indicated in Table 1. The heat of dilution of the guest solution upon addition into the buffer solution in the absence of CD was determined in each run by using the same number of injections of guest solution at the same concentration employed in the titration experiments. The dilution enthalpies determined in these control experiments were subtracted from the enthalpies obtained in the titration experiments. It should be emphasized that the enthalpies of dilution obtained in all runs were in the same order of magnitude as the enthalpies of dilution of simple electrolytes such as NaCl at the same concentration. Thus, it is concluded that there is no significant self-association of any guest under the experimental conditions used. We have previously shown that the nonideality corrections are not necessary under the experimental conditions employed.[16,17]

NMR Measurements. 1D and 2D NMR spectra, including ROESY, COSY, and HOHAHA, were obtained at 600 MHz in D_2O at 25 °C. HOHAHA experiments were performed by using the MLEV-17 pulse sequence with a mixing time of 120 ms, while ROESY spectra were recorded with a mixing time of 200 ms. An equimolar mixture (30 mM each) of γ-CD and the sodium salt of each peptide (Cbz-D/L-Ala-L-Phe, Cbz-D/L-Ala-L-Trp, Cbz-D/L-Pro-L-Phe, and Ac-D/L-Phe-L-Tyr) was used for the NMR experiments.

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Supporting Information Available: ROESY spectrum of the mixture of (a) Ac-D-Ala-L-PheONa and γ-CD (Figure S1), (b) Cbz-D-Ala-L-TrpONa and γ-CD (Figure S2), (c) Cbz-D-Pro-L-PheONa and γ-CD (Figure S3a), (d) Cbz-L-Pro-L-PheONa and γ-CD (Figure S3b), (e) Cbz-D-Phe-L-TyrONa and γ-CD (Figure S4a), and (f) Cbz-L-Phe-L-TyrONa and γ-CD (Figure S4b), as well as formulas, molecular weights, and CAS Registry Numbers for the chemical compounds used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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