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Molecular Discrimination of *N*-Protected Amino Acid Esters by a Self-Assembled Cylindrical Capsule: Spectroscopic and Computational Studies

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A self-assembled, cylindrical capsule was used to bind *N*-α-protected amino acid esters. The reversible encapsulation was studied using NMR spectroscopy in deuterated mesitylene solution and by computer-aided molecular modeling. BOC-L-alanine alkyl esters and BOC-*β*-alanine alkyl esters were tested as guests, and the relative binding affinities were established by direct competition experiments. A good correlation was found between the experimental and calculated relative binding affinities in these two series. Guests that were slightly longer than the internal dimensions of the cavity were accommodated by adopting compacted conformations.

Introduction

Self-assembled capsules[1] are current vehicles for exploring how molecules fit together.[2,3] These capsules, held together by hydrogen bonds or metal−ligand coordination,[4−6] are capable of providing well-defined inner spaces that more or less completely surround targets and detain them. The host/guest interactions are themselves molecular recognition phenomena[7] and offer other possibilities such as catalysis.[8] An example is the resorcin[4]arene-based cavitand **1** in its vase-shaped C_{4v} conformation. It forms a cylindrical capsule **1·1** in apolar organic solvents,[3e,9] and we earlier measured its inner space using rigid guest molecules of well-defined length and shape (molecular rulers).[9] The study revealed that *para*-substituted benzanilides about 14.7 Å long and 5.7 Å thick could be accommodated[9] (Figure 1). The current research was undertaken to answer the following questions: (1) Will the capsule accommodate longer guests that can adopt folded conformations? (2) What other factors, e.g., volume or shape, define an optimum guest? (3) What can be predicted by modeling of the host/guest complexes? We proceeded by interrogating a series of *N*-protected amino acid esters (**2**−**25**) as guest molecules. The guests could be easily obtained as a large family of related structures. These are known compounds, some of which are commercial products. The capsule is notorious for its ability to sequester trace impurities, and only through synthesis could we have a history of possible contaminants. The amino group of L-alanine was protected by *tert*-butoxycarbonyl (Boc), benzyloxycarbonyl (Z), and 9-fluorenylmethoxycarbonyl (Fmoc) groups; these groups are relatively hydrophobic, rigid, and bulky. The lengths of the guest molecules were defined by their ester

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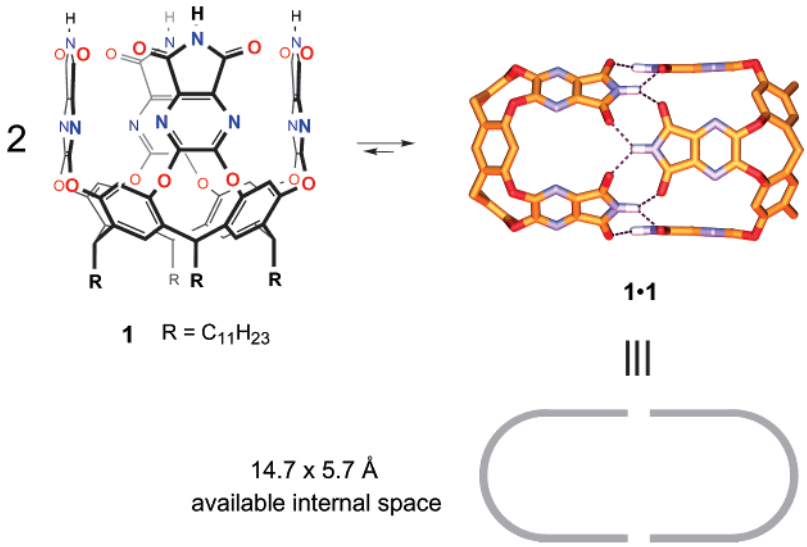
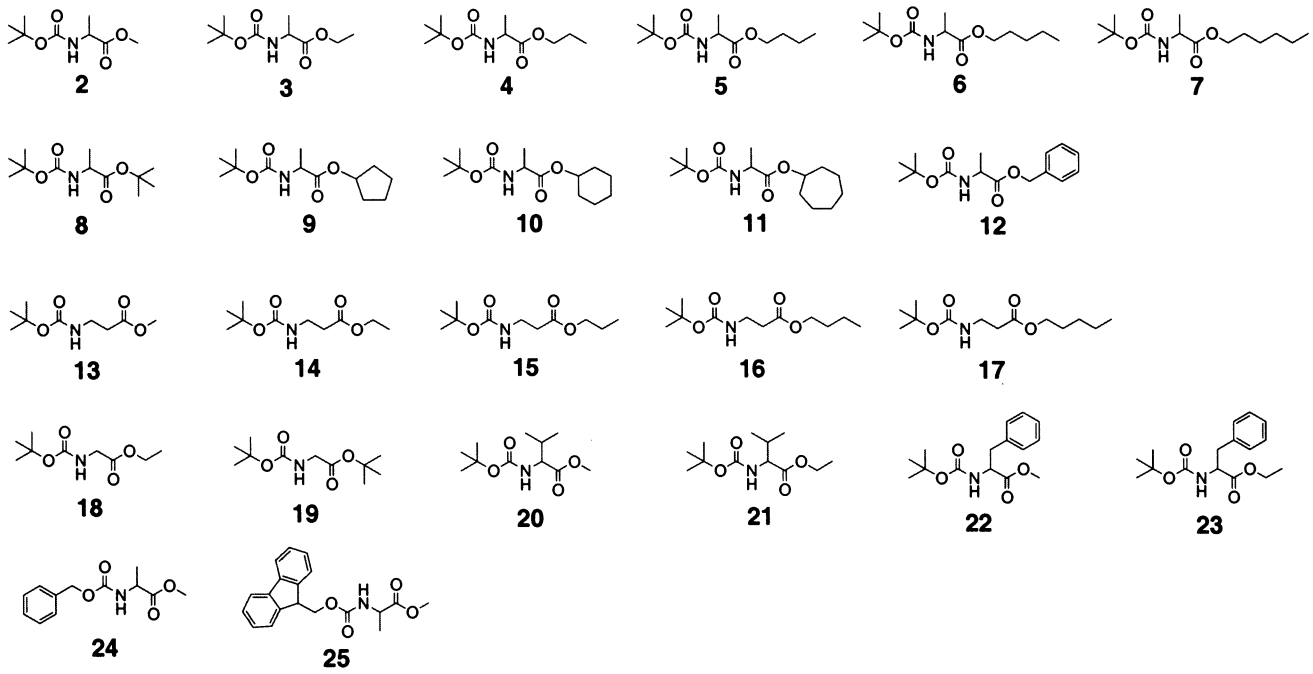


FIGURE 1. (Top) structure of **1** and the energy-minimized (Amber force field) presentation of self-assembled cylindrical capsule (**1·1**); the long alkyl chains and CH hydrogen atoms are omitted for clarity. (Bottom) the cartoon representation of the capsule. The dimension of internal space estimated experimentally is also shown.

CHART 1



groups: methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-hexyl, *tert*-butyl, cyclopentyl, cyclohexyl, cycloheptyl, and benzyl (**2**−**12**). Other amino acid esters: Boc-*β*-alanine, Boc-glycine, Boc-L-valine, and Boc-L-phenylalanine were prepared to probe additional specific molecular discrimination feature of the capsule (**13**−**25**) (Chart 1).

Results and Discussion

NMR Spectroscopic Study. Encapsulation of guests in mesitylene-d_{12} at 296 K was determined by ^{1}H NMR spectroscopy as previously described (Figure 2).[10] Selected chemical shifts (δ, ppm) and the change in the chemical shifts ($\Delta\delta$, ppm) for encapsulated guest molecules and δ for capsule N−H protons are summarized in Table 1. Among the series of Boc-L-alanine *n*-alkyl esters (**2**−**7**), the shortest guest **2**, with a molecular length of 10.9 Å,[11] was not encapsulated (Figure 2a), but the slightly longer guests (**3** and **4**) were. (Figure 2b,c). The large upfield shifts of the guest Boc and terminal alkyl groups ($\Delta\delta$ = −4.15 to −4.30, −4.40 to −4.43 ppm, respectively) place them near the ends of capsule **1·1**, while the methyl protons of the amino acid side chain showed relatively

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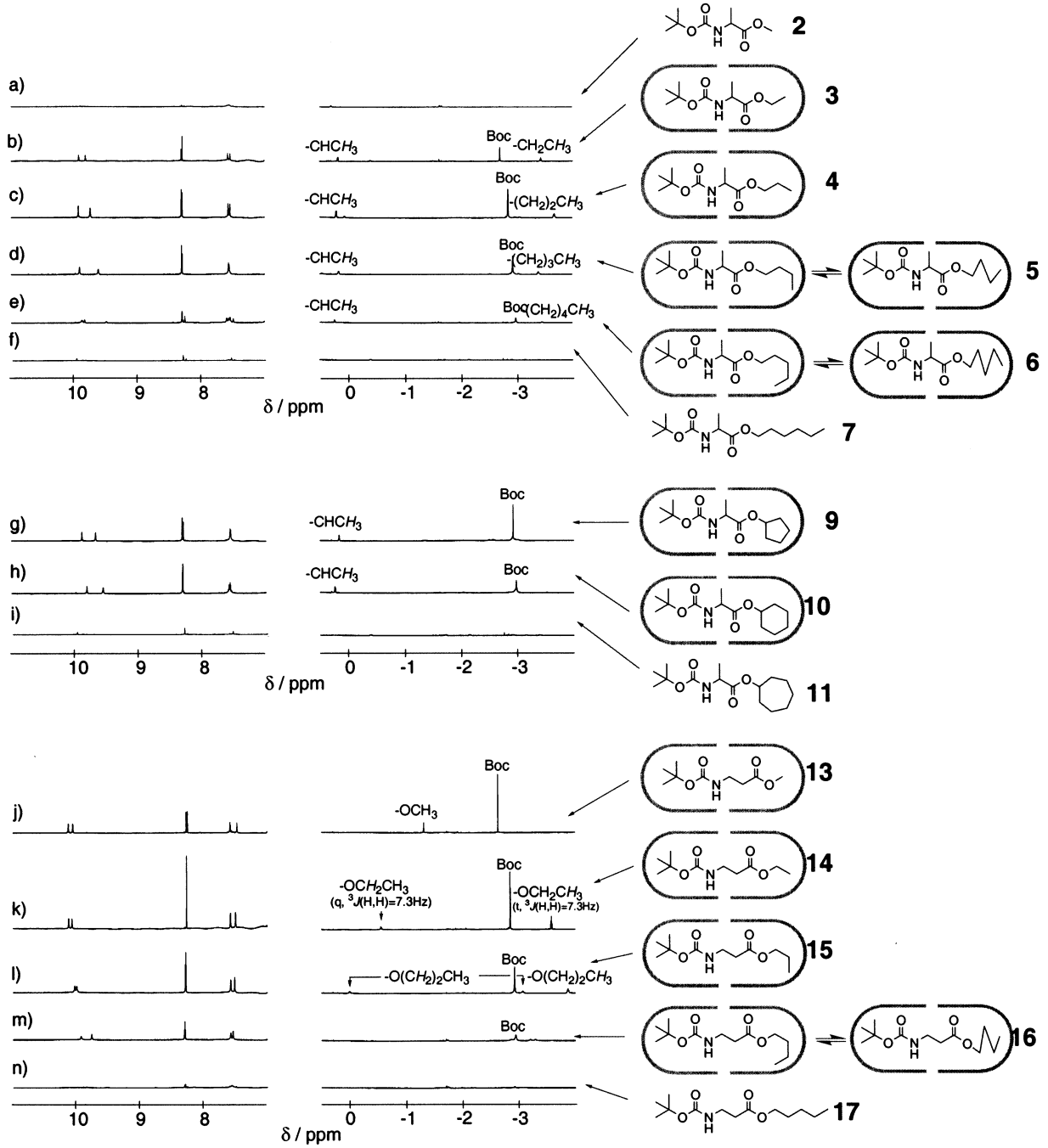

FIGURE 2. Downfield and upfield portions of ^{1}H NMR spectra (600 MHz, 296 K) of **1** in mesitylene-d_{12} in the presence of **2** (a), **3** (b), **4** (c), **5** (d), **6** (e), **7** (f), **9** (g), **10** (h), **11** (i), **13** (j), **14** (k), **15** (l), **16** (m), and **17** (n).

small upfield shifts ($\Delta\delta$ = −0.94 to −0.97 ppm) (Table 1). These locate them around the seam of hydrogen bonds in the middle of the capsule (Figure 2b, c). Intense intermolecular NOE contacts were observed between encapsulated guest Boc protons and the arene protons of capsule **1·1**, supporting these geometrical arrangements. Moreover, nonequivalent N−H signals for the two halves of the capsule were observed in the downfield region of the spectra (Figure 2b,c), indicating that the tumbling of the encapsulated guest molecules is restricted in the cavity, at least on the NMR time scale. Moreover, *n*-butyl and *n*-pentyl esters (**5** and **6**) were also encapsulated by **1·1**, even though their molecular lengths (14.6 and 15.9 Å, respectively, in their extended conformation) are equivalent to or even exceed the dimensions of **1·1**. In all cases the terminal methyl resonances of the esters were the highest upfield signals.[12] The guest molecules must reduce their lengths to accommodate themselves, but their contractions are not through folding as shown schematically in Figure 2d,e,m. Such folding would place methylene groups in the most shielded regions of the hosts. Rather, the contortions must take place elsewhere in the guest's structure. The $\Delta\delta$ values for Boc protons of **6** and **5** were larger than those of **3** and **4**, while the observed $\Delta\delta$ values for terminal methyl

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TABLE 1. Selected Chemical Shifts (δ, ppm) and the Change in Chemical Shifts (Δδ, ppm) for Encapsulated Guest Molecules and δ for Capsule

	δ (Δδ) for encapsulated species			
guest	Boc protons	terminal methyl protons of ester group	methyl protons of amino acid side chain	δ for capsule imide protons
2		n.d.[a]		
3	−2.71 (−4.15)	−3.43 (−4.40)	0.17 (−0.97)	9.93, 9.82
4	−2.86 (−4.30)	−3.67 (−4.43)	0.20 (−0.94)	9.93, 9.75
5	−2.94 (−4.38)	−3.39 (−4.22)	0.16 (−0.99)	9.92, 9.62
6	−2.99 (−4.43)	−3.45 (−4.23)	0.24 (−0.80)	9.88, 9.50
7		n.d.[a]		
8	−2.76 (−4.20)	−2.84 (−4.15)	0.27 (−0.88)	9.89, 9.72
9	−2.92 (−4.36)	-	0.15 (−0.97)	9.88, 9.67
10	−2.98 (−4.43)	-	0.23 (−0.95)	9.81, 9.56
11		n.d.[a]		
12		n.d.[a]		
13	−2.64 (−4.05)	−1.32 (−4.60)	-	10.12, 10.07
14	−2.85 (−4.26)	−3.58 (−4.57)	-	10.11, 10.07
15	−2.93 (−4.35)	−3.87 (−4.65)	-	10.03, 9.99
16	−2.95 (−4.37)		-	9.93, 9.76
17		n.d.[a]		
18	−2.73 (−4.16)	−3.57 (−4.50)	-	10.02, 10.17
19	−2.71 (−4.14)	−2.85 (−4.16)	-	10.00, 9.85
20		n.d.[a]		
21		n.d.[a]		
22		n.d.[a]		
23		n.d.[a]		
24	-	−1.27 (−4.57)	−0.47 (−1.55)	9.89, 9.82
25		n.d.[a]		

[a] No detectable signals of encapsulated guests.

proton of **6** and **5** were smaller than those of **3** and **4** (Table 1). Again, their positions near the capule's ends effect the shifts. Neither the longest ester guest **7** nor benzyl ester **12** were encapsulated; only solvent-filled capsules[10] were detected by ^{1}H NMR spectroscopy (for **7**, Figure 2f; for **12**, not shown). Encapsulation with conformational change of the guest molecules is a reasonable expectatition: The cyclic analogues (**9** and **10**) were also encapsulated (Figure 2g,h), and they resemble the tightly folded conformers of the longer guests mentioned above. However, guest **11**, bearing a slightly larger seven-membered ring was not encapsulated (Figure 2i). Similar molecular discrimination behavior was observed for the homologous series of Boc-*β*-alanine esters (**13**−**17**) (Figure 2j−n). Boc-glycine esters **18** and **19**, lacking the methyl group from **3** and **8**,[13] were also encapsulated, while more "stocky" amino acids such as **20**, **21**, **22**, and **23** were not. The Fmoc-protecting group of L-alanine methyl ester (**25**) was too large to be accommodated.

Relative binding affinities of the guests to the capsule were examined by ^{1}H NMR competition experiments; [**1·1**] = 1.0 mM, [$\mathbf{G_1}$] = [$\mathbf{G_2}$] = 5.0 mM. Integration of the corresponding peaks of the encapsulated guests after equilibrium was attained gave a direct readout of the relative binding affinities. For example, the ^{1}H NMR spectrum for the competition experiment between **14** and **15** was shown in Figure 3. Through this method the relative binding affinities of all of the guests (K_i/K_{14}) were determined and they are summarized in Figure 4. The Boc-L-alanine *n*-alkyl esters follow the sequence: **4**:**5**:**3**:**6** = 1.00:0.24:0.03:0.001, while Boc-*β*-alanine esters, **14**:**15**:**13**:**16** = 1.00:0.43:0.05:0.01. In short, the relative

(13) Boc-protected L-alanine *tert*-butyl ester (**8**) and Z-protected L-alanine methyl ester (**24**) can be also encapsulated.

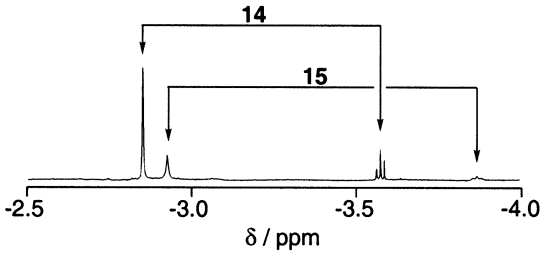

FIGURE 3. Upfield portion of ^{1}H NMR spectrum for the competition experiment with **1** (2.0mM) between **14** (5.0 mM) and **15** (5.0 mM) in mesitylene-d_{12}.

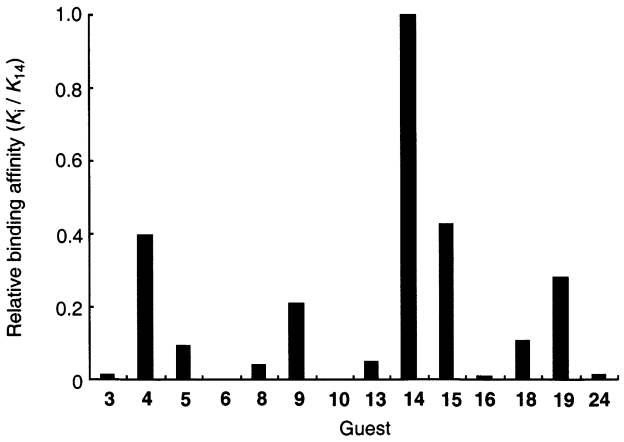

FIGURE 4. Relative binding affinities of *N*-α-protected amino acid esters to capsule **1·1**.

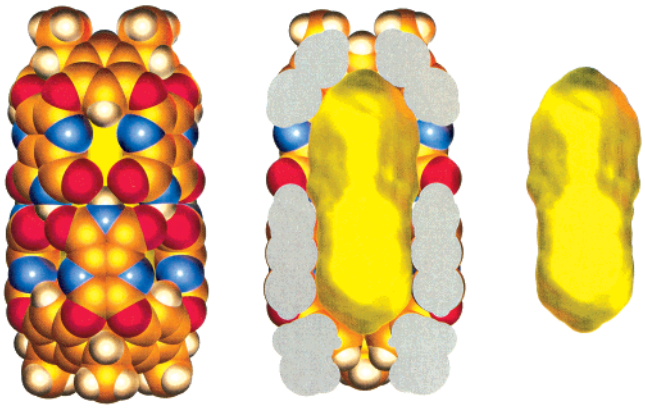

FIGURE 5. A space-filling representation of the empty cylindrical capsule (left), sliced in XY plane (middle), showing the capsule cavity (right).

binding affinities are subject to the length of the guest molecules. In case of the encapsulation with **6** and **16**, relatively upfield shifted δ values for the imide resonances of the hosts suggested loosely held dimeric capsules. Among these guests, Boc-*β*-alanine ethyl ester **14** with a molecular length of about 13.5 Å in the extended conformation fits the inner space of capsule **1·1** best. The cyclic esters proved better guests than their open-chain counterparts (e.g., **9** > **6**). Apparently, the binding affinity is also effected by preorganization of the guest.

Computational Study. The structure of the empty cylindrical capsule **1·1** was built and minimized using program Macromodel/Maestro[11] (AMBER* force-field,

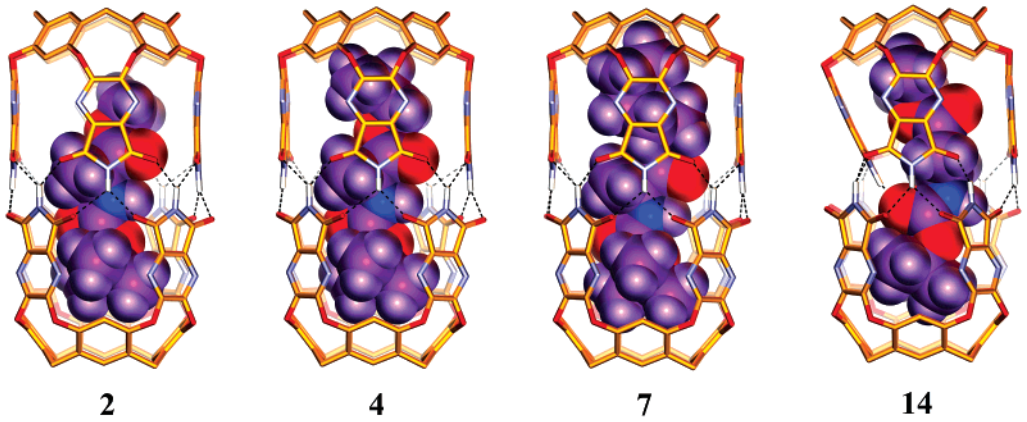

FIGURE 6. Representative lowest-energy structures of the complexes between the cylindrical capsule and guests (**2**, **4**, **7**, and **14**)) obtained from MCMM conformational search.

vacuum, user defined cutoff). The inner space of the capsule was examined using the program GRASP.[14] The cavity was generated by rolling a sphere of 1.4 Å around the inner surface. Its estimated size is 16.5 × 7.1 Å comprising a volume of ~420 Å^3 (Figure 5). The cavity has a (roughly) cylindrical shape tapered at both ends with a distance of ~16.5 Å, tip to tip. The tapered ends are best suited for complements that are thin, e.g., terminal acetylenes. The thick BOC groups must be lodged at some distance from these ends. Accordingly, the effective space for guests bearing this terminus is considerably shortened. Given the limits imposed by the BOC group, the longer derivatives cannot fit without some buckling along their length.

The size and the molecular volume of the encapsulated guests provide an indication of the capsule binding preferences. Acceptable guest volume ranges from 180 Å^3 to 240 Å^3. The length of the encapsulated guests is between 12 and 16 Å in the extended lowest-energy conformation. The flexible guests are compacted upon encapsulation, causing changes in the guests' lengths, but not their volume. At the same time, the cavity of the cylindrical capsule can adjust its shape and volume. All components of the encapsulation complex must be carefully analyzed in comparisons.

The initial structures of the cylindrical capsule with encapsulated guests were built and their local energy-minima were found using program Macromodel/Maestro (AMBER* force-field, vacuum, user defined cutoff). To find the global energy minima of the molecular complexes, Monte Carlo multiple minimum (MCMM) conformational searches, as implemented in Macromodel/Maestro, were used (3000 steps, AMBER* force-field, vacuum, user defined cutoff). In the case of guests **11**, **12**, **22**, **23**, and **25**, no global energy-minimum structure of the complex could be obtained because the MCMM conformational search provided only structures with guest located outside the cylindrical capsule. The representative lowest-energy structures are shown in Figure 6.

The volumes and length of free guests in the extended conformation, the length of encapsulated guests in the folded conformation, and change in the length upon encapsulation are summarized in Table 2. The cavity volume of the corresponding complexes were also calculated and summarized in Table 2 together with Packing Coefficient[15] (PC). The length of the capsule was found to be nearly constant (17.7 Å ± 1%) in all complexes examined. On the other hand, the capsule width reflected extremes in the guest size and led to changes in cavity volume. In the case of the small guests (**13**, **14**, and **18**), the relatively flexible walls of the capsule collapsed, probably in order to eliminate the residual empty space in the cavity (Figure 6). Another stabilizing factor for such a collapsed capsule structure may be a formation of new intermolecular hydrogen bonds between the guest and the capsule. The result is a reduced volume of the cavity (−13% for **13** and **14**, −18% for **18**) and increased PC. Therefore the cylindrical capsule can be seen as a container structure with rigid ends and a flexible center. The PC values of the five best guests (**14**, **15**, **4**, **19**, and **9**) range from 51 to 55%.

When a large flexible guest is encapsulated, its structure adopts the shape of the cavity. Often an energetically less favorable, but more compact gauche, conformation of the alkyl chain is preferred over the most stable trans conformation in the folded guest structure. A 20% length reduction (3.2 Å) was estimated for the largest encapsulated guest **16**. The length of the folded encapsulated guests ranges from 11.7 to 13.5 Å.

The lowest-energy structures obtained from MCMM conformational search were further refined and the energies of the complexes were estimated including a GB/SA solvation model for $CHCl_3$ in a final minimization (Macromodel/Maestro, AMBER* force-field, extended cutoff. Although this rough estimate neglects any entropic changes during the encapsulation, the structure-affinity relationship obtained may have value for the interpretation of the experimental data and permit prediction of the binding affinity for new guests.

In the series of Boc-L-alanine *n*-alkyl esters **2**−**7** the highest relative binding affinities were estimated for guest **4**; this is in agreement with experimental data. The same is true for the Boc-*β*-alanine series **13**−**17**, where both the calculation and the experiment identified **14** as the best guest (Figure 7).

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(15) Mecozzi, S.; Rebek, J. Jr. *Chem. Eur. J.* **1998**, *4*, 1016.

TABLE 2. Structural Changes upon Encapsulation

guest[a]	guest volume[b] (V_G), Å^3	cavity volume[c] (V_C), Å^3	cavity volume change,[d] %	packing coefficient (PC) (= V_G/V_C), %	free guest length (L_0), Å	folded guest length (L_f), Å	guest length change ($L_f - L_0$),[e] Å (%)
2	180	416	−1	43	10.9	10.9	0.0 (0)
3	194	411	−2	47	12.1	12.1	0.0 (0)
4	208	408	−3	51	13.4	12.5	−0.9 (−7)
5	222	417	−1	53	14.6	12.3	−2.3 (−16)
6	236	424	1	56	15.9	13.5	−2.4 (−15)
7	250	437	4	57	17.1	13.9	−3.2 (−18)
8	222	426	1	52	12.2	12.0	−0.2 (−2)
9	228	415	−1	55	13.3	12.8	−0.5 (−4)
10	241	415	−1	58	13.9	13.3	−0.6 (−4)
11	255	*f*	*f*	*f*	13.9	*f*	*f*
12	246	*f*	*f*	*f*	14.9	*f*	*f*
13	180	365	−13	49	12.2	11.7	−0.5 (−5)
14	194	365	−13	53	13.5	12.9	−0.6 (−5)
15	208	391	−7	53	14.7	12.8	−1.9 (−13)
16	222	418	0	53	16.0	12.8	−3.2 (−20)
17	237	428	2	55	17.1	13.2	−3.9 (−23)
18	180	346	−18	52	12.3	12.0	−0.3 (−2)
19	208	408	−3	51	12.3	11.8	−0.5 (−4)
20	208	438	4	47	10.3	10.9	0.6 (5)
21	222	436	4	51	11.5	12.1	0.6 (5)
22	246	*f*	*f*	*f*	12.9	*f*	*f*
23	261	*f*	*f*	*f*	12.9	*f*	*f*
24	204	388	−8	53	13.0	12.5	−0.5 (−4)
25	263	*f*	*f*	*f*	15.3	*f*	*f*

[a] Underlined = encapsulation observed. [b] GRASP. [c] GRASP, probe radius 1.4 Å. [d] (V_C − 420)/420. [e] ($L_f - L_0$)/L_0. [f] The guest was found outside the capsule in all obtained structures.

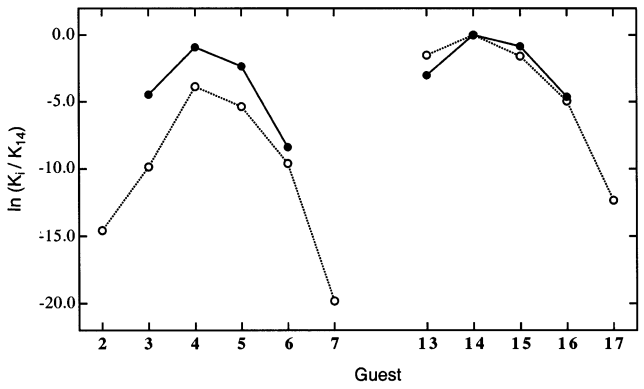

FIGURE 7. Comparison of experimental (filled circles) and estimated (empty circles) relative binding affinities ln(K_i/K_{14}) for the *N*-Boc-L-Ala (left) and *N*-Boc-*β*-Ala (right) ester series.

In conclusion, the size and shape selectivity for molecular encapsulation of *N*-α-protected amino acid esters in capsule **1**·**1** was revealed by NMR spectroscopy. The most strongly bound guest to the capsule was guest **14**. The capsule also accommodated guests that were slightly longer than the internal dimensions of the cavity, and these guests adopted compacted conformations. The conformational changes of the alkyl chains of these guests were also supported by the computer-aided molecular modeling analysis of the complexes. A good correlation was found between the experimental and calculated relative binding affinities in a homologous series of Boc-L-alanine and Boc-*β*-alanine *n*-alkyl esters. The computational analysis of the host/guest complexes may be useful in predicting relative binding affinities in other cases of molecules within molecules.

Experimental Section

General. ^{1}H NMR spectra were recorded on a 600 MHz spectrometer at 296 K. All commercially available chemicals were used without further purification. Compound **1** was prepared following literature procedures, after changing reaction conditions from 80 °C, 5 h to room temperature, 10 h.[9]

Encapsulation Studies. ^{1}H NMR experiments were carried out using a 600 MHz spectrometer at 296 K. Deuterated mesitylene was used as purchased from Aldrich. In encapsulation experiments, the concentration of **1** and individual guest was 2.0 and 5.0 mM, respectively. In a typical competition experiment, a solution of **1** (2.0 mM), **14** (5.0 mM), and **15** (5.0 mM) in mesitylene-d_{12} was placed in an NMR tube and allowed to stand at room temperature at least for 1 day and then analyzed by ^{1}H NMR spectroscopy. The relative binding affinities of the guests to the capsule were determined by direct integration of the corresponding peaks of the encapsulated guests. NOE experiment was carried out by using a GOESY sequence, with $d1$ =2.5 s and $d8$ = 400 ms.

General Procedure for the Synthesis of *N*-α-Protected Amino Acid Esters. Procedure A. *N*,*N*′-Dicyclohexylcarbodiimide (DCC) (1.1 equiv) and 1-hydroxybenzotriazole (HOBt) were added to a solution of *N*-α-*tert*-butoxycarbonylamino acid in dry dichloromethane at 0 °C, and the mixture was allowed to stand at the same temperature while being stirred for 20 min. Corresponding alcohol (1.0 equiv) was added to the mixture, and the resulting mixture was stirred for 4 h at 0 °C and then overnight at room temperature. Precipitates that formed (*N*,*N*′-dicyclohexylurea) were removed by filtration, the solvent was eliminated under reduced pressure, and the residue was dissolved in ethyl acetate. The solution was then washed with 10% aqueous citric acid, saturated aqueous sodium chloride, and 4% aqueous sodium hydrogen carbonate in this sequence. After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel with ethyl acetate−hexane as eluant. The product fraction was collected and dried in vacuo. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol−chloroform (1:1 v/v) as eluant. The product fraction was collected and dried in vacuo.

Procedure B. Amino acid ester hydrochloride (*p*-tosylate) and equivalent of triethylamine were dissolved in chloroform. One equivalent of di-*tert*-butyl dicarbonate [$(Boc)_2O$] in chloroform was added dropwise to the solution at room tempera-

ture, and the mixture was stirred for overnight at room temperature. The resulting mixture was evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate. After removal of an amount of insoluble materials by filtration, the filtrate was then washed with 10% aqueous citric acid, saturated aqueous sodium chloride, and 4% aqueous sodium hydrogen carbonate in this sequence. After being dried ($MgSO_4$), the solution was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel with ethyl acetate−hexane as eluant. The product fraction was collected and dried in vacuo. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol−chloroform (1:1 v/v) as eluant. The product fraction was collected and dried in vacuo.

***N*-α-*tert*-Butoxycarbonyl-L-alanine methyl ester (2):** prepared following the general procedure B on a 144 mM scale of L-alanine methyl ester hydrochoride. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.32 (3H, t, *J* 7.2 Hz, $CHCH_3$), 1.38 [9H, s, $(CH_3)_3CO$], 3.68 (3H, s, OCH_3), 4.26(1H, m, $CHCH_3$), 5.12 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.74.

***N*-α-*tert*-Butoxycarbonyl-L-alanine ethyl ester (3):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and ethanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.26 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.37 (3H, d, *J* 7.2 Hz, $CHCH_3$), 1.43 [9H, s, $(CH_3)_3CO$], 4.18 (2H, q, *J* 7.1 Hz, CH_2CH_3), 4.27 (1H, m, $CHCH_3$), 5.17 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.74.

***N*-α-*tert*-Butoxycarbonyl-L-alanine *n*-propyl ester (4):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and 1-propanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.88 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.32 (3H, d, *J* 7.2 Hz, $CHCH_3$), 1.37 [9H, s, $(CH_3)_3CO$], 1.60 (2H, q, *J* 7.1 Hz, $CH_2CH_2CH_3$), 4.04 (2H, m, $CH_2CH_2CH_3$), 4.23 (1H, m, $CHCH_3$), 5.15 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.74.

***N*-α-*tert*-Butoxycarbonyl-L-alanine *n*-butyl ester (5):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and 1-butanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.94 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.39 (3H, d, *J* 7.2 Hz, $CHCH_3$), 1.4 [2H, m, $O(CH_2)_2CH_2CH_3$], 1.45 [9H, s, $(CH_3)_3CO$], 1.64 (2H, m, $OCH_2CH_2CH_2CH_3$), 4.16 (2H, m, OCH_2CH_2), 4.3 (1H, m, $CHCH_3$), 5.06 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.74.

***N*-α-*tert*-Butoxycarbonyl-L-alanine *n*-pentyl ester (6):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and 1-pentanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.88 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.29−1.31 [7H, $O(CH_2)_2(CH_2)_2CH_3$, $CHCH_3$], 1.45 [9H, s, $(CH_3)_3CO$], 1.62 [2H, m, $OCH_2CH_2(CH_2)_2CH_3$], 4.11 [2H, m, $OCH_2(CH_2)_3CH_3$], 4.27 (1H, m, $CHCH_3$), 5.09 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.74.

***N*-α-*tert*-Butoxycarbonyl-L-alanine *n*-hexyl ester (7):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and 1-hexanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.84 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.2−1.6 (11H, $OCH_2(CH_2)_4CH_3$, $CHCH_3$), 1.39 [9H, s, $(CH_3)_3CO$], 1.59 (2H, m, OCH_2CH_2), 4.08 [2H, m, $OCH_2(CH_2)_4CH_3$], 4.24 (1H, m, $CHCH_3$), 5.12 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.71.

***N*-α-*tert*-Butoxycarbonyl-L-alanine *tert*-Butyl ester (8):** prepared following the general procedure B on a 110 mM scale of L-alanine *tertert*-butyl ester hydrochoride. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.30 (3H, d, *J* 7.0 Hz, $CHCH_3$), 1.40 [9H, s, $(CH_3)_3COCOCH$], 1.42 [9H, s, $(CH_3)_3COCONH$], 4.14 (1H, m, CH), 5.1 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.77.

***N*-α-*tert*-Butoxycarbonyl-L-alanine cyclopentyl ester (9):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and cyclopentanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.33 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.40 [9H, s, $(CH_3)_3CO$], 1.4−1.8 (8H, m, cyclic-CH_2), 4.22 (1H, m, OCH), 5.08 (1H, m, $CHCH_3$), 5.18 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.69.

***N*-α-*tert*-Butoxycarbonyl-L-alanine cyclohexyl ester (10):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and cyclohexanol. 1H NMR (600 MHz, $CDCl_3$, 296 K) ^{1}H 1.36 (3H, t, J 7.2 Hz, CH_2CH_3), 1.44 [9H, s, $(CH_3)_3CO$], 1.3−1.8 (10H, m, cyclic-CH_2), 4.27 (1H, m, OCH), 4.80 (1H, m, $CHCH_3$), 5.08 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.69.

***N*-α-*tert*-Butoxycarbonyl-L-alanine cycloheptyl ester (11):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-L-alanine and cycloheptanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.35 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.43 [9H, s, $(CH_3)_3CO$], 1.3−1.8 (12H, m, cyclic-CH_2), 4.24 (1H, m, OCH), 4.96 (1H, m, $CHCH_3$), 5.08 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.72.

***N*-α-*tert*-Butoxycarbonyl-L-alanine benzyl ester (12):** prepared following the general procedure B on a 114 mM scale of L-alanine benzyl ester p-tosylate. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.33 (3H, d, *J* 7.0 Hz, $CHCH_3$), 1.48 [9H, s, $(CH_3)_3$-COCONH], 4.4 (1H, m, $CHCH_2$), 5.1 (1H, m, NH), 5.2 (2H, m, CH_2), 7.39 (5H, m, Ar−H). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.82.

***N*-α-*tert*-Butoxycarbonyl-*β*-alanine methyl ester (13):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-*β*-alanine and methanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.42 [9H, s, $(CH_3)_3CO$], 2.52 (2H, t, *J* 6.0 Hz, $NHCH_2CH_2$), 3.39 (2H, m, $NHCH_2CH_2$), 3.69 (3H, s, OCH_3), 5.04 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3-OH:AcOH = 95:5:3 v/v) 0.68.

***N*-α-*tert*-Butoxycarbonyl-*β*-alanine ethyl ester (14):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-*β*-alanine and ethanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.26 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.42 [9H, s, $(CH_3)_3CO$], 2.51 (2H, t, *J* 6.0 Hz, $NHCH_2CH_2$), 3.39 (2H, m, $NHCH_2CH_2$), 4.15 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 5.03 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.68.

***N*-α-*tert*-Butoxycarbonyl-*β*-alanine *n*-propyl ester (15):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-*β*-alanine and 1-propanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.88 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.37 [9H, s, $(CH_3)_3CO$], 1.66 (2H, m, $OCH_2CH_2CH_3$), 2.47 (2H, t, *J* 6.4 Hz, $NHCH_2CH_2$), 3.33 (2H, m, $NHCH_2CH_2$), 4.00 (2H, q, *J* 6.4 Hz, $OCH_2CH_2CH_3$), 5.16 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.70.

***N*-α-*tert*-Butoxycarbonyl-*β*-alanine *n*-butyl ester (16):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-*β*-alanine and 1-butanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.88 (3H, t, *J* 7.3 Hz, CH_2CH_3), 1.30 [9H, s, $(CH_3)_3CO$], 1.2−1.5 [4H, m, $OCH_2(CH_2)_2CH_3$], 2.40 (2H, t, *J* 6.4 Hz, $NHCH_2CH_2$), 3.25 (2H, m, $NHCH_2CH_2$), 3.97 [2H, q, *J* 6.4 Hz, $OCH_2(CH_2)_2CH_3$], 5.21 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.71.

***N*-α-*tert*-Butoxycarbonyl-*β*-alanine *n*-pentyl ester (17):** prepared following the general procedure A on a 106 mM scale of *N*-α-*tert*-butoxycarbonyl-*β*-alanine and 1-pentanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.92 (3H, t, *J* 7.2 Hz, CH_2CH_3), 1.45 [9H, s, $(CH_3)_3CO$], 1.3−1.7 [6H, m, $OCH_2(CH_2)_3CH_3$], 2.53 (2H, t, *J* 6.4 Hz, $NHCH_2CH_2$), 3.41 (2H, m, $NHCH_2CH_2$), 4.10 [2H, q, *J* 6.4 Hz, $OCH_2(CH_2)_3CH_3$], 5.09 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.71.

***N*-α-*tert*-Butoxycarbonyl-glycine ethyl ester (18)**: prepared following the general procedure A on a 114 mM scale *N*-α-*tert*-butoxycarbonyl-glycine and ethanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.22 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.39 [9H, s, $(CH_3)_3CO$], 3.84 (2H, d, *J* 5.4 Hz, CH_2), 4.15 (2H, q, *J* 7.1 Hz, CH_2CH_3), 5.12 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.71.

***N*-α-*tert*-Butoxycarbonyl-glycine *tert*-Butyl ester (19):** prepared following the general procedure B on a 120 mM scale

of glycine *tert*-butyl ester hydrochoride. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.45 [18H, s, $(CH_3)_3COCOCH$, $(CH_3)_3COCONH$], 3.80 (2H, m, $NHCH_2$), 5.00 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.65.

***N*-α-*tert*-Butoxycarbonyl-L-valine methyl ester (20):** prepared following the general procedure B on a 120 mM scale of L-valine methyl ester hydrochoride. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.84 [nonequivalent, 3H, d, *J* 6.9 Hz, $CH(CH_3)_2$], 0.90 [nonequivalent, 3H, d, *J* 6.9 Hz, $CH(CH_3)_2$], 1.39 [9H, s, $(CH_3)_3CO$], 2.07 [1H, m, $CH(CH_3)_2$], 3.68 (3H, s, OCH_3), 4.17 (1H, m, $NHCH$), 5.03 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.78.

***N*-α-*tert*-Butoxycarbonyl-L-valine ethyl ester (21):** prepared following the general procedure A on a 92 mM scale of *N*-α-*tert*-butoxycarbonyl-L-valine and ethanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 0.87 [nonequivalent, 3H, d, *J* 6.8 Hz, $CH(CH_3)_2$], 0.94 [nonequivalent, 3H, d, *J* 6.8 Hz, $CH(CH_3)_2$], 1.26 (3H, d, *J* 7.1 Hz, $CHCH_3$), 1.42 [9H, s, $(CH_3)_3CO$], 2.11 [1H, m, $CH(CH_3)_2$], 4.17 (2H, m, CH_2CH_3), 4.17 (1H, m, $CHCH_3$), 5.02 (1H, m, NH). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.78.

***N*-α-*tert*-Butoxycarbonyl-L-phenylalanine methyl ester (22):** prepared following the general procedure B on a 76 mM scale of L-phenylalanine methyl ester hydrochoride. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.48 [9H, s, $(CH_3)_3CO$], 3.11 (2H, m, CH_2Ph), 3.73 (3H, s, OCH_3), 4.62 (1H, m, $CHCH_2$), 5.11 (1H, m, NH), 7.2 (5H, m, Ar−H). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.80.

***N*-α-*tert*-Butoxycarbonyl-L-phenylalanine ethyl ester (23):** prepared following the general procedure A on a 76 mM scale of *N*-α-*tert*-butoxycarbonyl-L-phenylalanine and ethanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.24 (3H, d, *J* 7.1 Hz, $CHCH_3$), 1.45 [9H, s, $(CH_3)_3CO$], 3.11 (2H, m, CH_2Ph), 4.18 (2H, q, *J* 7.1 Hz, CH_2CH_3), 4.59 (1H, m, $CHCH_2$), 5.12 (1H, m, NH), 7.2 (5H, m, Ar−H). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.80.

***N*-α-benzyloxycarbonyl-L-alanine methyl ester (24):** prepared following the general procedure A on a 90 mM scale of *N*-α-benzyloxycarbonyl-L-alanine and methanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.26 (3H, t, *J* 7.3 Hz, $CHCH_3$), 3.76 (3H, s, OCH_3), 4.40 (1H, m $CHCH_3$), 5.11 (2H, m, CH_2-Ph), 5.35 (1H, m, NH), 7.3−7.4 (5H, m, Ph-H). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.58.

***N*-α-9-fluorenylmethoxycarbonyl-L-alanine methyl ester (25):** prepared following the general procedure A on a 90 mM scale of *N*-α-9-fluorenylmethoxycarbonyl-L-alanine and methanol. ^{1}H NMR (600 MHz, $CDCl_3$, 296 K) δ_H 1.38 (3H, t, *J* 6.9 Hz, $CHCH_3$), 3.74 (3H, s, OCH_3), 4.25 (1H, m $CHCH_3$), 4.42 (3H, m, CH_2Ar, CO_2CH_2CH), 5.39 (1H, m, NH), 7.3−7.8 (8H, m, Ar−H). R_f (K6F Silica Gel; $CHCl_3$:CH_3OH:AcOH = 95:5:3 v/v) 0.58.

Molecular Modeling. For simplification, the C_{11} chains in **1** were replaced by Me groups. Initial structures of molecular complexes were generated by a conjugate gradient minimization with the AMBER* force-field and the BatchMin program implemented within Maestro/MacroModel *7.0*.[11] These structures were further refined by a 3000−4000 step Monte Carlo multiple minimum simulation *in* a vacuum using a user defined cutoffs: van der Waals 3.5 Å, Electrostatic 6.0 Å, H−Bond 4.0 Å. All conformations within 20 kJ mol^{-1} of the computed global minimum were stored, and the representative lowest-energy structure was analyzed.

The volume of cavities of the capsules and the volume of the guests were estimated using GRASP (probe radius 1.4 Å).[14] The length of the capsule was measured as a distance between the centroids of methine carbons of the capsule.

For energy estimation all structures were further minimized using the *GB/SA* solvation model for chloroform (Amber*, Macromodel/Maestro 7.0, extended cutoff). The guest binding affinities were estimated as a difference between the total energy of the complex and the energies of the free guest and the free capsule. The relative binding affinities were estimated from these guest binding affinities using guest **14** as a standard.

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Supporting Information Available: NOE data and Table with the comparison of experimental and estimated relative binding affinities calculated using AMBER* force-field. This material is available free of charge via the Internet at http://pubs.acs.org.

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