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# Synthesis and Host−Guest Studies of Chiral *N*-Linked Peptidoresorc[4]arenes†

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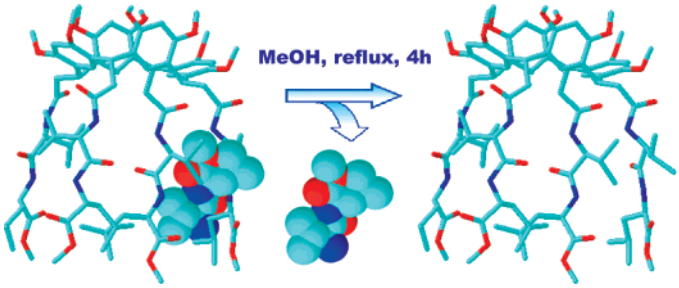
*Received July 30, 2007*




Four *cone* resorc[4]arene octamethyl ethers (**10**, **11**, *ent*-**10**, and *ent*-**11**) tetrafunctionalized at the feet with valyl-leucine [LL- (**6**); DD- (*ent*-**6**)] and leucyl-valine [LL- (**9**); DD- (*ent*-**9**)] methyl esters have been synthesized. These compounds, obtained by conjugation of macrocycle tetracarboxylic acid chlorides with the appropriate terminal amino groups of the above dipeptides, are *N*-linked peptidoresorc[4]arenes. We found that these macrocycles (M) are capable of recognizing the homologue dipeptides as guests (G), both in solution and in the gas phase, by forming relatively stable host−guest complexes ([M·G]), resistant to chromatographic purification but not to heating. Complexation phenomena between M and G in solution were investigated by NMR methods, including NMR DOSY experiments, for the detection of translational diffusion. Heteroassociation constants of 2030 and 186 $M^{-1}$ were obtained by the Foster−Fyfe method for the complexes [**10**·**6**] and [**10**·*ent*-**6**], respectively, the latter being comparable to the self-association constant of dipeptide itself. Conversely, the structural features of the proton-bound complexes $[M{\cdot}H{\cdot}G_n]^+$ ($n = 1, 2$), generated in the gas phase by electrospray ionization mass spectrometry (ESI-MS), were investigated by collision-induced dissociation (CID) experiments. In both cases, the four *N*-linked peptidoresorc[4]arenes were shown to act as synthetic receptors and to recognize the homologue dipeptide by means of hydrogen bonds.

## Introduction

Calixarenes have had an enormous impact in supramolecular chemistry,[1] as building blocks in the field of molecular recognition. The modulation of shape and size of their cavities led to the preparation of cavitands,[2,3] (hemi)carcerands,[4] and self-assembling capsules.[5] A number of calix[4]arenes with either α-amino acids or peptides attached to the upper rim are

† Dedicated to the memory of Dmitry Rudkevich, an enthusiastic, exceptional scientist, and friend who passed away too early.

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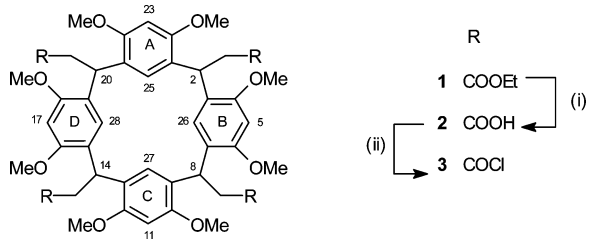
10.1021/jo7016636 CCC: $37.00 
Published on Web 10/25/2007

known,[6] and the first generation of calix[4]arene peptide dendrimers has recently been synthesized.[7] Mostly, the presence of a *N*- or *C*-linked peptide endows the macrocycle with novel properties, such as the recognition of protein surfaces[8] and carbohydrates,[9] the formation of stable inclusion complexes,[10] and self-assembly phenomena.[11] A four-armed hydrophilic peptidocalix[4]arene library consisting of 1000 members, suitable for peptide recognition in aqueous media, has recently been developed.[12] Only a few papers reported on cavitands equipped with dipeptide residues. For example, either peptides of helical conformation have been connected to a cavitand platform, using a thioether bridge at the external carbon of the aromatic rings,[13] or the C-termini of four short peptide chains have been linked to the aromatic amino methyl groups.[14]

We have previously functionalized resorc[4]arene octamethyl ethers at the feet with L- and D-valine ethyl ester units,[15] and later exploited their capability of enantiodiscriminating amino acidic guests[16] in the gas phase by mass spectrometry (MS). The limited body of work addressed to investigate by MS techniques the gas-phase stability of host−guest complexes formed by calixarenes and resorcarenes with organic molecular guests has been discussed in detail in a recent comprehensive review.[17] In this paper we report on the synthesis and host−guest properties of *N*-linked peptidoresorc[4]arene octamethyl ethers, obtained by conjugation of macrocycle tetracarboxylic acid chlorides in the *cone* conformation with the appropriate terminal amino groups of valyl-leucine and leucyl-valine methyl esters. Complexation phenomena between peptidoresorc[4]-arenes and the homologue dipeptides as guests were investigated in solution by NMR methods and in the gas phase by ESI-MS.

**SCHEME 1** [a]




[a] Reagents and conditions: (i) 2 N NaOH, EtOH, reflux, 4 h; glacial AcOH; (ii) thionyl chloride, THF, reflux, 4 h.

## Results and Discussion

**Synthesis of the *N*-Linked Peptidoresorc[4]arenes.** We chose for the synthesis resorc[4]arene octamethyl ether tetraester **1** (Scheme 1) in the *cone* conformation for the presence of four *all-cis* substituents that could preorganize the peptide chain on the same side of the scaffold. Resorcarene **1**, obtained as previously described,[18] was hydrolyzed to tetracarboxylic acid **2** (Scheme 1) and quantitatively converted into the corresponding acid chloride **3** by reaction with thionyl chloride in tetrahydrofuran (THF).

Standard peptide coupling was employed to gather the four dipeptide chains **6**, **9**, *ent*-**6**, and *ent*-**9**. Commercially available *N*-BOC-L-valine **4** (Figure 1) was coupled with L-leucine methyl ester hydrochloride **5** in the presence of HOBT and triethylamine to afford, after deprotection with TFA-DCM, dipeptide L-valyl-L-leucine methyl ester **6**, as TFA salt, in a 93% overall yield. Analogously, dipeptide L-leucyl-L-valine methyl ester **9** (TFA salt, 95% overall yield) was prepared from commercially available *N*-BOC-L-leucine **7** and L-valine methyl ester hydrochloride **8**. The same procedure was applied to the DD-dipeptides series, to afford the TFA salts of dipeptides *ent*-**6** and *ent*-**9** with comparable yields, as summarized in Figure 1. The conjugation of acid chloride **3** with the single dipeptides gave the final compounds. In a general reaction, diisopropylethylamine (DIPEA) was added, under nitrogen, to a dry THF solution of **3** and the reaction mixture was stirred at room temperature for 20 min. After the slow addition of the proper dipeptide (in a 1.5 excess for each acid chloride group), the mixture was held at reflux for 3 h. Chromatographic purification afforded the *N*-linked peptidoresorc[4]arenes **10**, **11,** *ent*-**10**, and *ent*-**11** in 48−51% yield, starting from dipeptides **6**, **9**, *ent*-**6**, and *ent*-**9**, respectively (Figure 2). The structures of compounds **10**, **11**, *ent*-**10**, and *ent*-**11** were confirmed by $^1$H and $^{13}$C NMR spectroscopy and by electrospray ionization mass spectrometry (ESI-MS).

The $^1$H and $^{13}$C NMR spectral data were consistent with the distribution pattern of a resorc[4]arene containing four identical side chains. The inner protons (i.e., H-25, H-26, H-27, and H-28) gave only one signal, as well as the outer ones (H-5, H-11,

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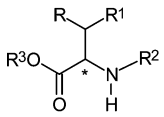
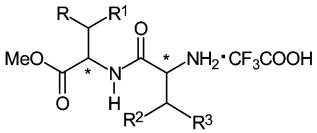




**4** R = $R^1$ = Me, $R^2$ = BOC, $R^3$ = H, * = L-

**5** R = $(Me)_2CH$, $R^1$ = H, $R^2$ = H·HCl, $R^3$ = Me, * = L-

**7** R = $(Me)_2CH$, $R^1$ = H, $R^2$ = BOC, $R^3$ = H, * = L-

**8** R = $R^1$ = Me, $R^2$ = H·HCl, $R^3$ = Me, * = L-

*ent*-**4** R = $R^1$ = Me, $R^2$ = BOC, $R^3$ = H, * = D-

*ent*-**5** R = $(Me)_2CH$, $R^1$ = H, $R^2$ = H·HCl, $R^3$ = Me, * = D-

*ent*-**7** R = $(Me)_2CH$, $R^1$ = H, $R^2$ = BOC, $R^3$ = H, * = D-

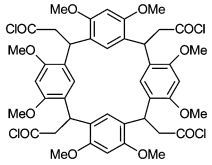
*ent*-**8** R = $R^1$ = Me, $R^2$ = H·HCl, $R^3$ = Me, * = D-

**6** R = $(Me)_2CH$, $R^1$ = H, $R^2$ = $R^3$ = Me, ** = LL-

**9** R = $R^1$ = Me, $R^2$ = $(Me)_2CH$, $R^3$ = H, ** = LL-

*ent*-**6** R = $(Me)_2CH$, $R^1$ = H, $R^2$ = $R^3$ = Me, ** = DD-

*ent*-**9** R = $R^1$ = Me, $R^2$ = $(Me)_2CH$, $R^3$ = H, ** = DD-



**3**

**FIGURE 1.** Building blocks for *N*-linked peptidoresorc[4]arenes.

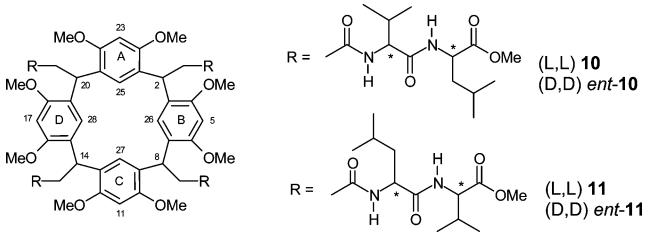


**FIGURE 2.** *N*-linked peptidoresorc[4]arene octamethyl ethers.

H-17, and H-23). The same was observed for the methine protons (H-2, H-8, H-14, and H-20) and for the elements of the four side chains. The methoxyl groups as well as those for $C_{Ar}$−C and $C_{Ar}$−O carbons are instead doubled, because, for instance, the 4-OMe is equivalent to the 22-OMe, but not to the 24-OMe. These findings are consistent with the introduction of stereogenic centers in the side chains, which destroyed all the symmetry planes of the resorc[4]arene skeleton. The above data are in agreement with a $C_4$ group of symmetry; in fact the molecule is superimposable to itself after a clockwise rotation of 90°. Finally, the broadening (sensitive to temperature increase) of the two methoxyl group signals was attributed to the influence of the peptide chain[19] on the interchange time between the two *flattened cone* forms, which becomes comparable to the NMR time scale. Surprisingly, all the synthesized *N*-linked peptidoresorc[4]arenes showed in the NMR spectra the presence of additional signals for each proton and carbon of the dipeptide chains. These minor peaks, still present after purification on Sephadex LH-20, were attributed to host−guest [M·G] complexes of peptidoresorc[4]arenes **10** and **11** (M), the guests (G) being the dipeptide reactants themselves (**6** and **9**, respectively). Only after heating at reflux in MeOH for 4 h and a further purification on Sephadex did we observe clean NMR spectra for compounds **10**, **11**, *ent*-**10**, and *ent*-**11**, the spectra of *ent*-**10** and *ent*-**11** being superimposable with those of the corresponding LL-forms (**10** and **11**, respectively). As expected, optical rotations with coincident absolute value and opposite sign were observed for each couple of enantiomers.

**Complexation Studies in Solution by NMR Spectroscopy.** For a deeper insight, we investigated by NMR spectroscopy

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**TABLE 1. Chemical Shifts (*δ*, ppm) and Chemical Shift Variation (Δ*δ*, ppm) of *ent*-6 (600 MHz, $CDCl_3$, 298 K)**

| protons | concn 0.1 mM | concn 10 mM | Δδ (ppm) $\delta_{10mM} - \delta_{0.1mM}$ |
|---|---|---|---|
| NH | 6.98 | 7.50 | 0.52 |
| CH−N (leucine) | 4.59 | 4.45 | 0.14 |
| CH−N (valine) | 3.76 | 3.93 | 0.17 |
| OMe | 3.74 | 3.72 | 0.02 |
| CH (valine) | 2.27 | 2.21 | 0.06 |
| Me (valine) | 1.06 | 1.07 | 0.01 |
| Me (valine) | 1.05 | 1.03 | 0.02 |
| Me (leucine) | 0.93 | 0.91 | 0.02 |
| Me (leucine) | 0.92 | 0.89 | 0.03 |

the interaction between peptidoresorc[4]arene **10** and dipeptide **6** or *ent*-**6** in $CDCl_3$ solutions. Because both resorcarene and dipeptide contain several hydrogen bond donor and acceptor groups, the analysis of self-association processes concerning the single **10** or **6**/*ent*-**6** compounds was mandatory prior to the analysis of heteroassociation processes. The self-association processes of **10** and **6** were thus investigated by the $^1$H NMR analysis of chemical shifts of progressively diluted $CDCl_3$ solutions. The low solubility in $CDCl_3$ of dipeptides **6**/*ent*-**6** allowed the use of a concentration range of 10.0−0.1 mM only. Within this concentration gradient, the signals for the amide NH proton and the two methine nuclei bonded to the two stereogenic centers of *ent*-**6** underwent remarkable chemical shift variations, whereas those for the methyl groups almost did not move (Table 1, Figure 3). The above trend strongly supports the self-aggregation of *ent*-**6** by hydrogen bond interactions.

On the hypothesis that dimerization would be more favored than the formation of higher order self-aggregates (at least within the low concentration ranges investigated), a self-association constant of 328 $M^{-1}$ was calculated by fitting chemical shifts versus concentration (Table 1) on the basis of eq 1 (see also Figure 4).

$$C = \frac{(\delta_{obs} - \delta_m)(\delta_d - \delta_m)}{2K(\delta_d - \delta_{obs})^2} \quad (1)$$

The presence of self-association equilibria was fully confirmed by NMR DOSY[20] measurement of diffusion coefficients (*D*)

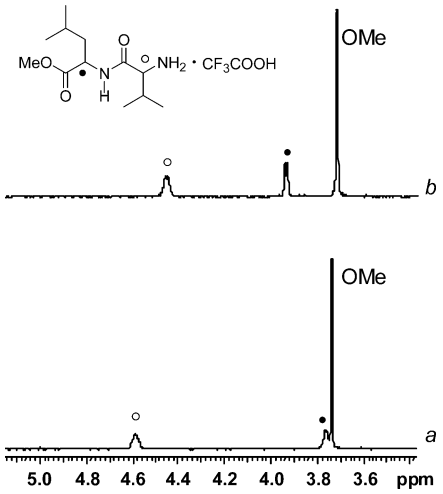


**FIGURE 3.** $^1$H NMR spectra (600 MHz, $CDCl_3$, 298 K) of a 0.1 mM (a) and a 10 mM (b) solution of dipeptide *ent*-**6**.

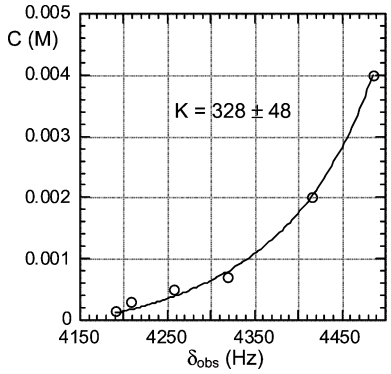


**FIGURE 4.** Self-association constant determination: $\delta_{obs}$ is the observed chemical shift variation of the NH proton of *ent*-**6**.

in solution, which are correlated by the hydrodynamic radius ($R_H$) to the molecular sizes by means of eq 2:

$$D = \frac{kT}{c\pi\eta R_H} \tag{2}$$

where $k$ is the Boltzmann constant, $T$ is the absolute temperature, $\eta$ is the solvent viscosity, and $c$ is a numerical factor[21] substantially dependent on the size of diffusing species.

The resort to NMR DOSY in the analysis of homo- and heteroassociation phenomena is justified by the fact that complexation processes originate species with increased sizes, and hence with lower diffusion coefficients. Notably, DOSY maps of compound *ent*-**6** (Figure 5, Table 2) at 0.1 mM revealed a diffusion coefficient of $8.1 \times 10^{-6}$ cm$^2$ s$^{-1}$, which decreased to $4.9 \times 10^{-6}$ cm$^2$ s$^{-1}$ at 10 mM, in agreement with an increase of molecular size due to self-aggregation processes.

Once having calculated the self-association constant of dipeptide *ent*-**6**, we obtained the dimer versus monomer molar fractions (Table 2) at each concentration value. Taking into account that in a fast exchange condition the observed diffusion coefficients ($D_{obs}$) represent the weighted average of their values

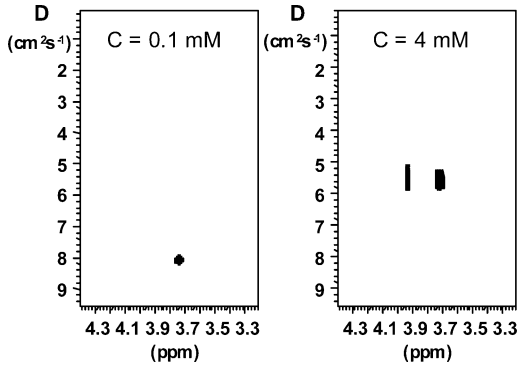


**FIGURE 5.** DOSY maps (600 MHz, $CDCl_3$, 298 K) of 0.1 ($D = 8.1 \times 10^{-6}$ cm$^2$ s$^{-1}$) and 4 mM ($D = 5.7 \times 10^{-6}$ cm$^2$ s$^{-1}$) solutions of dipeptide *ent*-**6**.

**TABLE 2. Diffusion Coefficients ($D$) of *ent*-6 at Variable Concentrations (600 MHz, $CDCl_3$, 298 K)**

| concn (mM) | $X_d$ | $D$ ($\times 10^{-6}$ cm$^2$ s$^{-1}$) |
|---|---|---|
| 10.0 | 0.68 | 4.9 |
| 4.0 | 0.54 | 5.7 |
| 2.0 | 0.43 | 6.1 |
| 0.1 | 0.06 | 8.1 |

in the dimer ($D_d$) and monomer ($D_m$), diffusion coefficients of $3.4 \times 10^{-6}$ and $8.3 \times 10^{-6}$ cm$^2$ s$^{-1}$, respectively, were calculated by eq 3, where $X_m$ and $X_d$ are the molar fractions of the monomer and the dimer forms of *ent*-**6**.

$$D_{obs} = D_m X_m + D_d X_d \tag{3}$$

The analogous NMR analysis of peptidoresorc[4]arene **10** in the concentration range 10.0−0.1 mM did not show significant variations both of the chemical shifts and of the diffusion coefficient which did not change from the initial value of $4.8 \times 10^{-6}$ cm$^2$ s$^{-1}$. This behavior can be attributed to the very close arrangement of the four dipeptide side chains that favors intramolecular interactions in place of intermolecular associative processes. The heteroassociation between **10** and **6**/*ent*-**6** was investigated in conditions where the self-aggregation of **6** was minimized, i.e., using very low concentrations of **6** (0.1 mM) versus a high molar excess of **10** (from 4 to 16 mM). By the Foster−Fyfe method,[22] the heteroassociation constant for the homochiral complex [**10**·**6**] was shown to be 2030 M$^{-1}$ (Figure 6 and Table 1S in the Supporting Information). For the heterochiral complex [**10**·*ent*-**6**], a significantly lower heteroassociation constant was obtained by the same method (186 M$^{-1}$), which is comparable to the self-association constant of dipeptide itself (see Table 2S in the Supporting Information).

The analysis of the diffusion coefficients of mixtures **10**·**6** gave further support to the tight interaction between guest **6** and host **10**. Because we could not obtain accurate diffusion coefficient measurements of **6** in the same experimental conditions employed for the determination of the stability constant, we analyzed the DOSY map of an equimolar mixture of **10**·**6** (0.1 mM), in which the molar fraction of **6** as dimer was quite low (about 6%). The diffusion coefficient of **6** in the mixture was shown to be $7.5 \times 10^{-6}$ cm$^2$ s$^{-1}$, which was lower than the value found for the pure dipeptide **6** ($8.1 \times 10^{-6}$ cm$^2$ s$^{-1}$) at the same concentration. The diffusion coefficient of **10**

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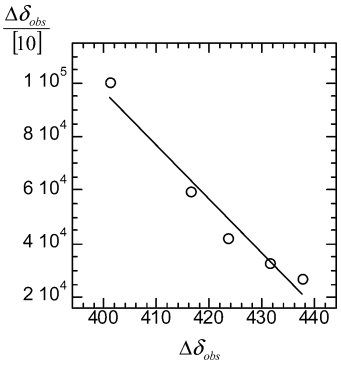


**FIGURE 6.** Heteroassociation constant determination by the Foster−Fyfe method: $\Delta\delta_{obs}$ is the observed chemical shift variation ($\delta_{mix} - \delta_f$, Hz) for the NH proton of **6** (0.1 mM) in the presence of an increasing molar excess of **10** (from 4 to 16 mM) and [**10**] is the total concentration of the peptidoresorc[4]arene.

remained $4.8 \times 10^{-6}$ cm$^2$ s$^{-1}$. The small difference of diffusion coefficients of **6** was unambiguously attributed to heteroassociation processes rather than to the viscosity gradient of the analyzed solution (see eq 2), as was confirmed by using tetramethylsilane (TMS) as the internal standard;[23] the TMS diffusion coefficient was the same in both a solution of pure **6** (0.1 mM) and in an equimolar mixture of **10**·**6** (0.1 mM).

In the rapid exchange conditions, the diffusion coefficient of **6** in the mixture **10**·**6** is the weighed average of the value in free ($D_f$) and complexed ($D_c$) forms (see eq 4):

$$D_{obs} = X_f D_f + X_c D_c \tag{4}$$

where $X_f$ and $X_c$ are the molar fractions of the free and the complexed forms of **6**, respectively.

Thus, by using the NMR approach proposed for the analysis of the diffusion of cyclodextrin inclusion complexes,[24] we applied the approximation that the diffusion coefficient of **6** in the bound state was equal to that of the complexing agent (resorcarene **10**), and we calculated by eq 5 the molar fractions of bound **6** ($X_c$) by a single-point measurement:

$$X_c = \frac{D_{obs} - D_f}{D_c - D_f} \tag{5}$$

A bound fraction value of 0.18 was obtained, in very good agreement with the value (0.15) expected (on the basis of the association constant) by linear fitting of chemical shift data.

Because the heteroassociation constant of the heterochiral complex [**10**·*ent*-**6**] was lower than the self-association of dipeptide *ent*-**6**, diffusion measurements did not allow heteroassociation phenomena to be shown unambiguously aside from self-aggregation processes for *ent*-**6**.

Information on the nature of heteroassociation processes involving the formation of the highly stable [**10**·**6**] complex was achieved by measuring intermolecular dipolar interactions by 1D ROESY of **6** (1 mM solutions) in the presence of resorcarene **10** (10 mM), thus providing a high bound molar fraction of the [**10**·**6**] complex, in spite of the autoaggregation tendency of dipeptide. In particular, perturbation of the leucine methine proton at $\delta$ 4.55 ppm of **6** produced detectable dipolar

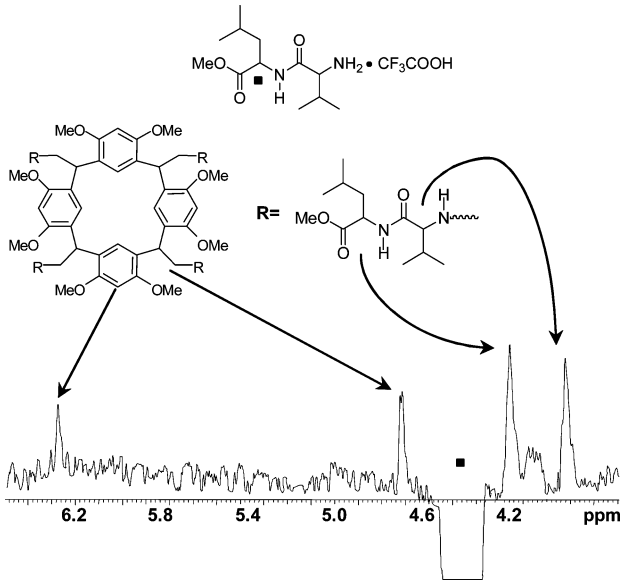


**FIGURE 7.** 1D ROESY spectrum (600 MHz, $CDCl_3$, 298 K, mixing time ranging 0.5 s) showing the perturbation of the leucine methine proton of **6** in the [**10**·**6**] complex (molar ratio 1:10).

interactions with valine and leucine methine protons of resorcarene dipeptide chains, in conjunction with an effect on the aromatic proton at $\delta$ 6.29 ppm that is located between the two methoxy groups (Figure 7). Therefore, we concluded that interaction between resorcarene **10** and its homologous dipeptide occurs at the external surface of resorcarene by means of attractive hydrogen bond interactions involving polar groups of both host and guest.

**Complexation Studies in the Gas Phase by ESI-MS**. We first run ESI-MS spectra of free peptidoresorc[4]arenes **10**−**11** and dipeptides **6**−**9** to investigate their mass spectral features. As already observed for tetraethylresorc[4]arenes,[25] compounds **10** and **11** (MW = 1737 amu) show a strong tendency to form complexes with sodium ($Na^+$) (*m*/*z* 1760), which is a ubiquitous contaminant present in glassy vessels (see Figure 8 for compound **10**). The most intense peak in the spectrum is that of the doubly charged disodium adduct $[M{\cdot}Na_2]^{2+}$, at *m*/*z* 891.5. The protonated molecule $[M{\cdot}H]^+$ at *m*/*z* 1738 was barely observable. The change of solvent from methanol to acetonitrile did not help in increasing the $[M{\cdot}H]^+$ signal, since essentially the same, though weaker spectrum was observed. Addition of acetic acid did not help either, because only extensive decomposition of the sample took place.

The ESI-MS spectrum of dipeptide **6** (L-valyl-L-leucine methyl ester, MW = 244 amu) is shown in Figure 9. The most abundant signal is due to the protonated molecule $[G{\cdot}H]^+$, at *m*/*z* 245, and to the proton-bound dimer $[G_2{\cdot}H]^+$, at *m*/*z* 489, which is accompanied by the sodium-bound analogue $[G_2{\cdot}Na]^+$, at *m*/*z* 511. Figure 10 presents the ESI-MS spectrum of the [resorcarene **10**]:[dipeptide **6**] = 1:7 mixture. Besides the above signals typical of free M and G, we observed the appearance of new signals corresponding to the complexes $[M{\cdot}H{\cdot}G]^+$ (*m*/*z* 1982), $[M{\cdot}H_2{\cdot}G]^{2+}$ (*m*/*z* 991.5), and $[M{\cdot}H{\cdot}Na{\cdot}G]^{2+}$ (*m*/*z* 1002.5). In spite of the sevenfold excess of G relative to M, the minor abundance of the *m*/*z* 489 ($[G_2{\cdot}H]^+$) and 511 ($[G_2{\cdot}$

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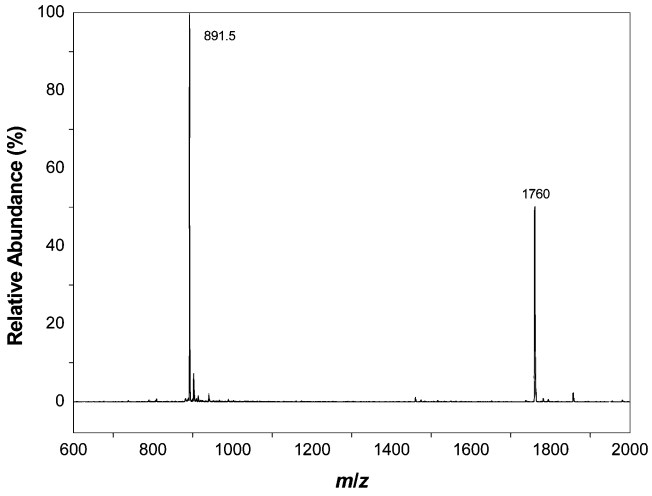



**FIGURE 8.** ESI mass spectrum of *N*-linked peptidoresorc[4]arene **10** (positive ion mode).

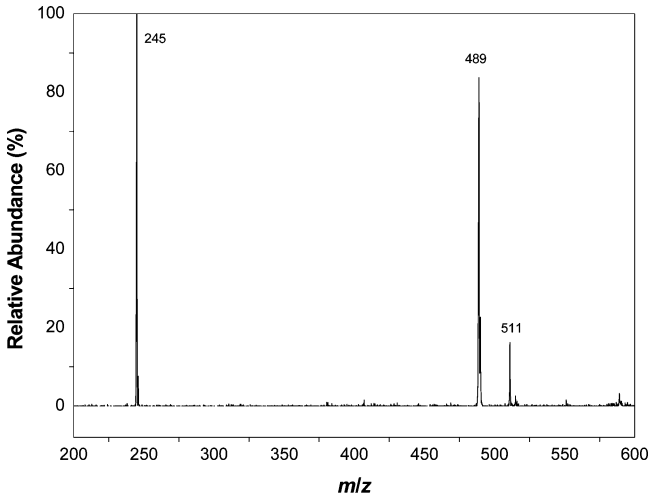



**FIGURE 9.** ESI mass spectrum of dipeptide **6** (positive ion mode).

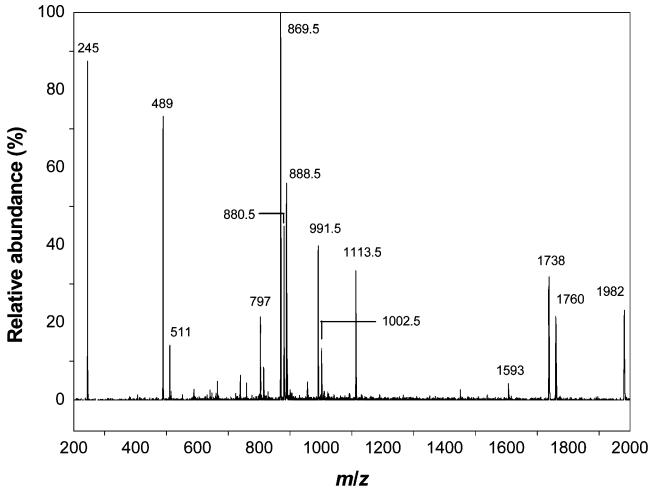



**FIGURE 10.** ESI mass spectrum of a 1:7 molar ratio solution of resorc[4]arene **10** and dipeptide **6**.

$Na]^+$) signals can only be attributed to the much larger affinity of the resorc[4]arene host M for the $H^+$, $Na^+$, and $K^+$ ions, as compared with the dipeptide guest G. This hypothesis is corroborated by the presence in the spectrum of Figure 10 of intense peaks at *m*/*z* 869.5 ($[M{\cdot}H_2]^{2+}$), 880.5 ($[M{\cdot}H{\cdot}Na]^{2+}$), and 888.5 ($[M{\cdot}H{\cdot}K]^{2+}$). The formation of the $[M{\cdot}H_2{\cdot}G_2]^+$ (*m*/*z* 2227) complex could not be directly observed in the ESI-MS spectrum because of mass range limitation of the instrument used (≤2000 amu). However, its formation is witnessed by the presence of a peak at *m*/*z* 1113.5, corresponding to the $[M{\cdot}H_2{\cdot}G_2]^{2+}$ ion. Notably, the ESI-MS of the M/G mixture revealed also the presence of small signals, absent in the spectrum of the pure host, at *m*/*z* 797 ($[(M - LeuOMe){\cdot}H_2]^{2+}$) and 1593 ($[(M - LeuOMe){\cdot}H]^+$), clearly deriving from the loss of the external amino acid of the dipeptide chain (leucine methyl ester, MW = 145 amu).

The much larger affinity of the resorc[4]arene host M for the $H^+$ cation, as compared with the dipeptide guest G, is further testified by collision-induced dissociation (CID) experiments on the $[M{\cdot}H{\cdot}G]^+$ (*m*/*z* 1982) and $[M{\cdot}H_2{\cdot}G_2]^{2+}$ (*m*/*z* 1113.5) complexes. CID of the isolated $[M{\cdot}H{\cdot}G]^+$ complex (isolation width ±10 amu, collision energy 20%) led to the exclusive loss of G to form the *m*/*z* 1738 ($[M{\cdot}H]^+$) ion. Similarly, CID of the isolated $[M{\cdot}H_2{\cdot}G_2]^{2+}$ complex (isolation width ±10 amu, collision energy 12%) gave rise exclusively to the $[M{\cdot}H_2{\cdot}G]^{2+}$ (*m*/*z* 991.5) fragment through the loss of a *neutral* G molecule. Formation of the $[M{\cdot}H_2]^{2+}$ fragment (*m*/*z* 869.5) by loss of the second *neutral* G required a significantly higher collision energy (35%). Under no condition did CID of the $[M{\cdot}H_2{\cdot}G_2]^{2+}$ and $[M{\cdot}H_2{\cdot}G]^{2+}$ complexes lead to the loss of a charged fragment in spite of the intense electrostatic repulsive forces operating in doubly charged species.

Similar ESI-MS spectral features were obtained with the [resorcarene **11**]:[dipeptide **9**] = 1:7 mixture, the only difference being the presence of the ions at *m*/*z* 804 ($[(M - ValOMe){\cdot}H_2]^{2+}$) and 1607 ($[(M - ValOMe){\cdot}H]^+$), due to the loss of valine methyl ester (MW = 131 amu), i.e., the external amino acid of the dipeptide chain. The heterologue M·G complexes (i.e., M = **10** and G = **9**; M = **11** and G = **6**) provided similar mass spectral patterns as well. Again, no differences were found in the ESI-MS spectra of the heterochiral complexes (i.e., M = **10** and G = *ent*-**6**; M = **11** and G = *ent*-**9**). On the ground of these observations as well as on the CID experiments, we concluded that (i) in all the examined cases, the dipeptide guest G is noncovalently bound to the peptidoresorc[4]arene M and (ii) the stereochemistry (LL versus DD) or the regioisomerism (leucyl-valine versus valyl-leucine sequences) of the dipeptide chain does not have any significant influence on the formation of the host−guest noncovalent complexes with the peptidoresorc[4]arenes.

## Conclusions

Four dipeptides, differing for composition and stereochemistry, have been attached to the feet of *cone* resorc[4]arene octamethyl ethers through their terminal amino groups to give *N*-linked peptidoresorc[4]arenes, which may represent a new class of synthetic receptors endowed with the ability of recognizing the homologue dipeptides, both in solution and in the gas phase. The resulting complexes appeared to be quite stable, since hosts and guests could not be separated by column chromatography. The NMR spectra revealed that hydrogen bonding interaction is at the basis of the guest recognition by the peptidoresorc[4]arenes. In particular, resorcarene **10** and its homologue and homochiral dipeptide **6** interact at the external surface of resorcarene by means of attractive hydrogen bond interactions involving polar groups of both host and guest, as

judged by intermolecular dipolar interactions measured by 1D ROESY. Heteroassociation constants of 2030 and 186 $M^{-1}$ were obtained for [**10**·**6**] and [**10**·*ent*-**6**] complexes, respectively, the latter being comparable to the self-association constant of dipeptide itself.

ESI mass spectrometry allowed detection of resorcarene/dipeptide complexes with 1:1 and 1:2 stoichiometry, which were shown to have different stabilities by the different collisional energies required to undergo dissociation. In the gas phase, no significant stereochemical (LL versus DD) or regioisomeric (leucyl-valine versus valyl-leucine sequences) influence was evidenced in the formation of host−guest complexes. The present results represent a very promising starting point for future studies on the intrinsic enantioselectivity of the new receptors toward small polyfunctional molecules or more complex frames such as dipeptide chains.

## Experimental Section

**2,8,14,20-Tetrakis(carboethoxymethyl)resorc[4]arene, Octamethyl Ether (1). 1** was synthesized as previously described.[18]

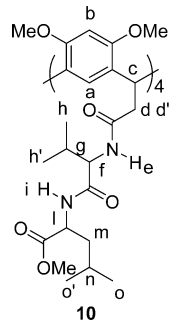
**2,8,14,20-Tetrakis(carboxymethyl)resorc[4]arene, Octamethyl Ether (2).** Compound **1** (1.6 g, 1.7 mmol) was dissolved in EtOH (10 mL), and 2 N NaOH (1.2 g, 30 mmol) was added (5 mL). The reaction mixture was stirred for 4 h at reflux. EtOH was removed under vacuo and the aqueous solution was acidified with glacial acetic acid. The precipitate was filtered, rinsed several times with water, and dried, giving **2** in a quantitative yield. $^1$H NMR (400 MHz, $CDCl_3$, 298 K, TMS): $\delta$ 9.57 (br s, 1H, COOH), 6.52 (s, 4H, H-i), 6.30 (s, 4H, H-e), 4.95 (t, 4H, CH, $J$ = 7.0 Hz), 3.63 (s, 24H, OMe), 2.78 (d, 8H, $CH_2$, $J$ = 7.0 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$, 298 K): $\delta$ 175.9 (COOH), 156.0 (C−O), 125.9 (CHi), 124.0 (C−C), 96.3 (CHe), 55.9 (OMe), 36.3 ($CH_2$), 33.0 (CH).

**2,8,14,20-Tetrakis(chlorocarboxymethyl)resorc[4]arene, Octamethyl Ether (3).** $SOCl_2$ (2.4 mL, 33 mmol) was added under nitrogen to a solution of **2** (150 mg, 0.18 mmol) in dry THF (15 mL). The reaction mixture was stirred for 4 h at reflux and allowed to stand at rt overnight. Final evaporation under vacuo gave **3** in a quantitative yield.

**General Procedure for the Preparation of Methyl Esters 5, 8, *ent*-5, and *ent*-8 as HCl Salts.** $CH_3COCl$ (5 mL) was added slowly to cooled dry MeOH (50 mL), in which the proper amino acid (1.7 mmol) has been dissolved. The mixture was heated at reflux for about 2 h, and then evaporated to dryness.

**General Procedure for the Preparation of Dipeptide Methyl Esters 6**, **9**, ***ent*-6, and *ent*-9 as TFA Salts.** The proper amino acid methyl ester hydrochloride (5.5 mmol), HOBT (0.75 g, 5.5 mmol), $Et_3N$ (0.96 mL, 7.1 mmol), and the proper commercially available *N*-BOC protected amino acid (5.5 mmol) were dissolved in $CH_2Cl_2$ (250 mL). DCC (1.14 g, 5.5 mmol) was added to the solution cooled in an ice bath. After 4 h at rt, the solvent was removed under vacuo and the residue was purified by silica gel column chromatography (hexane−EtOAc, 7.5:2.5) to yield the *N*-BOC protected dipeptide methyl esters as a white powder (yields 93−96%). The dipeptides (0.9 mmol) were deprotected by treatment with TFA−DCM (1:1, 25 mL). The reaction mixture was stirred for 1 h at rt, and then evaporated to dryness; the addition of $Et_2O$ (50 mL) under vigorous stirring yielded white solids that were filtered, washed several times with $Et_2O$, and dried under vacuo, to afford the pure salts **6**, **9**, *ent*-**6**, and *ent*-**9** in quantitative yields. For the characterization of the above compounds, see the Supporting Information.

**2,8,14,20-Tetrakis(L-valyl-L-leucinamido)resorc[4]arene (10).** DIPEA (0.42 mL, 2.5 mmol) was added under nitrogen to a dry THF solution (45 mL) of tetracarboxylic acid chloride **3** (150 mg, 0.15 mmol). The mixture was stirred 20 min at rt and a dry THF solution (30 mL) of **6** (322 mg, 0.9 mmol) was added dropwise in a 4 h period. The reaction mixture was held at reflux under nitrogen for 3 h. Evaporation of the solvent and purification by column chromatography ($CHCl_3$/MeOH mixtures) gave a crude fraction as a pale yellow powder, identified by ESI-MS as the complex [**10**·**6**]. This fraction was heated at reflux in MeOH for 4 h, evaporated to dryness, and afterward processed on Sephadex LH-20, to yield peptidoresorc[4]arene **10** as a white powder (127 mg, 49%). Mp: 220−221 °C.




**10**

$^1$H NMR (600 MHz, $CD_3CN$, 298 K): $\delta$ 0.60 (d, $J$ = 6.6 Hz, 3H, Me-h), 0.78 (d, $J$ = 6.6 Hz, 3H, Me-h′), 0.84 (d, $J$ = 6.5 Hz, 3H, Me-o), 0.88 (d, $J$ = 6.5 Hz, 3H, Me-o′), 1.50 (m, 2H, H-m), 1.59 (m, 1H, H-n), 1.83 (m, 1H, H-g), 2.55 (dd, $J$ = 14.2, 7.3 Hz, 1H, H-d), 2.77 (dd, $J$ = 14.2, 7.3 Hz, 1H, H-d′), 3.63 (s, 3H, COOMe), 3.66 (s, 3H, OMe), 3.71 (s, 3H, OMe), 4.20 (t, $J$ = 8.1 Hz, 1H, H-f), 4.27 (dt, $J$ = 8.4, 6.9 Hz, 1H, H-l), 4.84 (t, $J$ = 7.3 Hz, 1H, H-c), 6.44 (s, 1H, H-b), 6.84 (s, 1H, H-a), 6.85 (d, $J$ = 8.1 Hz, 1H, H-e), 7.34 (d, $J$ = 6.9 Hz, 1H, H-i). $^{13}$C NMR (150 MHz, $CD_3CN$, 298 K): $\delta$ 173.8 (CO), 173.2 (CO), 172.6 (CO), 157.2 (quaternary C), 156.9 (quaternary C), 128.7 (CH, Ar), 124.4 (quaternary C), 123.9 (quaternary C), 97.4 (CH, Ar), 59.1 (CH), 56.7 (OMe), 56.3 (OMe), 52.5 (OOMe), 52.1 (CH), 42.1 ($CH_2$), 40.9 ($CH_2$), 34.4 (CH), 31.9 (CH), 25.5 (CH), 22.9 (Me), 22.2 (Me), 19.3 (Me), 18.7 (Me). ESI-MS (pos.): *m*/*z* found 1760.7 ([$M$ + Na]$^+$), $C_{92}H_{136}N_8O_{24}$-Na requires 1761.12. Anal. Calcd for $C_{92}H_{136}N_8O_{24}$ (1738.10): C, 63.57; H, 7.89; N, 6.45. Found: C, 63.59; H, 7.91; N, 6.47. $[\alpha]^{20}_D$ −91.8 (*c* 0.66, MeOH).

**2,8,14,20-Tetrakis(L-leucyl-L-valinamido)resorc[4]arene (11). 11** was obtained as described for **10**, starting from tetracarboxylic acid chloride **3** and dipeptide methyl ester **9**. White powder, 49% overall yield. Mp: 248−249 °C. $^1$H and $^{13}$C NMR signals are reported in the Supporting Information. ESI-MS (pos.): *m*/*z* found 1760.7 ([$M$ + Na]$^+$), $C_{92}H_{136}N_8O_{24}$Na requires 1761.12. Anal. Calcd for $C_{92}H_{136}N_8O_{24}$ (1738.10): C, 63.57; H, 7.89; N, 6.45. Found: C, 63.53; H, 7.85; N, 6.41. $[\alpha]^{20}_D$ −86.8 (*c* 0.68, MeOH).

**2,8,14,20-Tetrakis(D-valyl-D-leucinamido)resorc[4]arene (*ent*-10). *ent*-10** was obtained as described for **10**, starting from tetracarboxylic acid chloride **3** and dipeptide methyl ester *ent*-**6**. White powder, 48% overall yield. $^1$H NMR, $^{13}$C NMR, ESI-MS data, and elemental analyses are coincident with those reported for **10**. $[\alpha]^{20}_D$ + 91.9 (*c* 0.20, MeOH).

**2,8,14,20-Tetrakis(D-leucyl-D-valinamido)resorc[4]arene (*ent*-11). *ent*-11** was obtained as described for **10**, starting from tetracarboxylic acid chloride **3** and dipeptide methyl ester *ent*-**9**. White powder, 51% overall yield. $^1$H NMR, $^{13}$C NMR, ESI-MS data, and elemental analyses are coincident with those reported for **11**. $[\alpha]^{20}_D$ +87.8 (*c* 1.00, MeOH).

**NMR Experiments.** $CDCl_3$ solution ranges of 0.1−0.3 and 4.0−16 mM for dipeptides and resorcarenes, respectively, were prepared for the Foster−Fyfe[22] determination of the association constants.

**ESI-MS Experiments.** Stock solutions of peptidoresorc[4]arenes **10**, **11**, *ent*-**10**, and *ent*-**11** ($0.6 \times 10^{-5}$ M) and dipeptides **6**, **9**, *ent*-**6**, and *ent*-**9** ($4 \times 10^{-5}$ M) were in MeOH. To achieve complex formation, all sample solutions contained a 7-fold excess of guest.

**Acknowledgment.** Financial support by Università "La Sapienza", Roma, Italy (Funds for selected research topics 2003−2005), and by FIRB 2003 is acknowledged. This work was partially supported by the Istituto Pasteur−Fondazione Cenci Bolognetti, Università "La Sapienza", Roma, Italy. G.U-B. is grateful to MIUR (Project "High performance separation systems based on chemo- and stereoselective molecular recognition", contract no. 2005037725), to University of Pisa, and to ICCOM-CNR (Italy).

**Supporting Information Available:** Data used for the determination of heteroassociation constants by the Foster−Fyfe method for complexes [**10**·**6**] and [**10**·*ent*-**6**] (Tables 1S and 2S); general experimental methods; characterization of dipeptide methyl esters **6**, **9**, *ent*-**6**, *ent*-**9**; and $^{1}H$ and $^{13}C$ NMR signals of peptidoresorc-[4]arene **11** and $^{1}H$ NMR spectra of peptidoresorc[4]arenes **10** and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO7016636