Complexation of a Peptidocalix[4]arene, a Vancomycin Mimic, with Alanine-Containing Guests by NMR Diffusion Measurements

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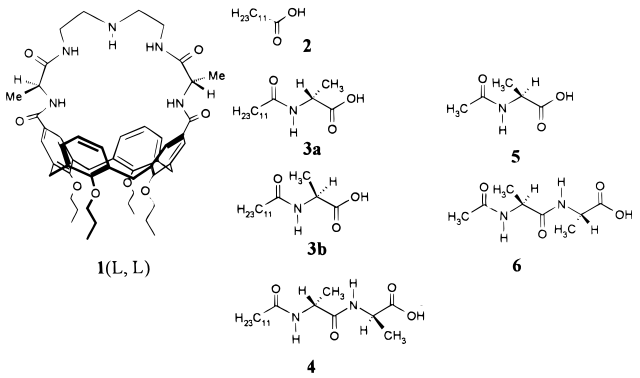
Introduction

Molecular recognition is a general principle in nature, and the design of artificial receptors for specific target molecules based on molecular recognition is an important theme in bioorganic[1] and supramolecular[2] chemistry. The vancomycin group of antibiotics is an example of biologically active molecules, which act through a relatively simple molecular recognition process.[3] Their mode of action has been studied in recent years, and it is now believed that these antibiotics bind to the cell wall mucopeptide precursors through the terminal -D-alanyl-D-alanine sequence. This results in the inhibition of cell wall growth, leading to lysis. Recently, however, resistance toward this class of antibiotics has been reported,[4] and the obvious need for synthetic analogues of vancomycin has emerged.

Calixarenes have been extensively used in the past few years as platforms for binding groups for selective complexation with several guest molecules.[5] Recently, the macrobicyclic peptidocalixarene **1** was synthesized as a receptor for the -D-alanyl-D-alanine residue. Interestingly, the in vitro behavior of compound **1** was similar to that of the vancomycin group antibiotics, and its selective antimicrobial activity toward Gram-positive bacteria was only slightly inferior to that of vancomycin.[6]



Recently, we demonstrated the applicability of NMR diffusion measurements, as obtained by the pulse gradient spin−echo (PGSE) technique,[7] to organic supramolecular chemistry.[8] We showed that this method is suitable for determining the association constants (K_as) of organic complexes in organic solvents. It was also pointed out that when complexation is associated with proton exchange, ^{1}H NMR diffusion measurements might be superior to the ^{1}H NMR chemical shift techniques for K_a determination.[8a] In addition, this method could be used to study the interaction of water molecules with macrocyclic systems in organic solvents,[8d] to probe the inclusion of guests in molecular capsules,[8e] and to evaluate the enantioselective recognition of lipophilic cyclodextrins.[8f] In recent years diffusion-ordered NMR spectroscopy (DOSY), which is based on the PGSE[7] technique, was developed,[9] and the potential of this approach for the analysis of ligand−receptor interactions by affinity NMR has been demonstrated.[10]

Therefore, we decided to explore the binding affinity of vancomycin mimic **1** with several guests, i.e., **2**−**6**, in different solvent mixtures in order to further our understanding of the ability of **1** to bind to guests containing carboxylic acid and alanine residues and to possibly correlate the binding properties with the observed biological activity. Following the widely accepted notation (J.-M. Lehn, *Struct. Bonding* **1973**, *16*, 1−68) we indicate the formation of inclusion complexes of host **1** with the different guests (**2**−**6**) as **2**−**6**⊂**1**.

Experimental Section

General. Diffusion experiments were carried out on a 500 MHz ARX Bruker (Karlshrue, Germany) NMR spectrometer

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Table 1: Diffusion Coefficients ($\times 10^5$ in $cm^2\ s^{-1}$) and the Computed Association Constants (log K_as) of Host 1 and Guests 2, 3a, 4, and 5 in Dry $CDCl_3$

system	*D* (chloroform)	*D* (host)	*D* (guest)	*X*	log *K*
1 in $CDCl_3$	2.36(±0.01)	0.61(±0.01)			
2 in $CDCl_3$	2.36(±0.02)		1.00(±0.07)		
2⊂**1** in $CDCl_3$	2.44(±0.01)	0.60(±0.01)	0.78(±0.02)	0.55(±0.09)	2.7(±0.2)
				[0.58(±0.08)][a]	[2.8(±0.2)][a]
3a in $CDCl_3$	2.47(±0.01)		0.94(±0.01)		
3a⊂**1** in $CDCl_3$	2.47(±0.02)	0.57(±0.01)	0.57(±0.01)	>0.99	>4.0
4 in $CDCl_3$	2.31(±0.04)		0.65(±0.02)		
4⊂**1** in $CDCl_3$	2.35(±0.02)	0.53(±0.01)	0.56(±0.01)	0.8(±0.1)	3.4(±0.4)[b]
				[0.8(±0.1)][a]	[3.5(±0.4)][a,b]
5 in $CDCl_3$	2.44(±0.02)		1.29(±0.01)		
5⊂**1** in $CDCl_3$	2.20(±0.05)	0.44(±0.02)	0.58(±0.01)	0.84(±0.02)	3.8(±0.1)
				[0.81(±0.03)][a]	[3.7(±0.2)][a]

[a] As the diffusion coefficient of $CDCl_3$ in the 1:1 host/guest solution was different from the value obtained in the solution containing the host in the free state, the ratios of the two values were used to correct the experimental values giving rise the corrected values in the parenthesis. [b] The relative error in this value might be even larger than depicted.

equipped with a B-AFPA10 pulsed gradient unit capable of producing magnetic field pulse gradients of about 50 G cm^{-1} in the *z*-direction. All experiments were carried out using a 5 mm inverse probe. The pulse gradient separation was 62 ms (for measurements of **2**, **3**, **4**, **2**⊂**1**, **3**⊂**1**, and **4**⊂**1**) or 42 ms (for measurements of **5**, **6**, **5**⊂**1**, and **6**⊂**1**). The pulsed gradients were incremented from 0 to 46.8 G cm^{-1} in 10 steps, and their duration in all experiments was 2 ms. The experiments were performed at least three times and only data for which the correlation coefficient (*R*) was higher than 0.999 were included. The measurements were all preformed at 298 K. In these experiments, 5 mM solutions were used.

Materials. Compound **1** was synthesized according to ref 6. All deuterated solvents were purchased from Aldrich (USA) and were used as supplied. Only $CDCl_3$ was used in three different conditions: as is, dried on alumina, and equilibrated with D_2O (3:2 (v/v) $CDCl_3/D_2O$). Compound **2** was purchased from Aldrich (USA), and compounds **3**−**6** were synthesized according to standard methods.[11]

Determination of Association Constants. Association constants were determined by evaluating the changes in the diffusion coefficients of the guests upon addition of compound **1**. The diffusion coefficients were determined by the PGSE technique according to which the ratio between the echo intensity in the presence (*I*) and in the absence of pulsed gradient (I_0) is given by the eq 1[7]

$$\ln(I/I_0) = -\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D = -bD \qquad (1)$$

in which γ is the magneticgyro ratio, *g* is the pulsed gradient strength, Δ and δ are the time separation between the pulsed-gradients and their duration, respectively, and *D* is the diffusion coefficient. For an isotropic solution, a plot of $\ln(I/I_0)$ vs *b* should give a straight line, whose slope is equal to $-D$. From the changes in the diffusion coefficients of the guests upon addition of host **1** the bound fractions were calculated and then translated to association constants (K_as) as described previously.[8]

Results

Figure 1 depicts the natural log of the normalized signal attenuation as a function of *b* for host **1** and guests **2** and **3a** in the free state and in their equimolar dry $CDCl_3$ solutions with **1**. As expected, host **1**, being the highest molecular weight molecule, shows the slowest signal attenuation indicating that this compound has the smallest diffusion coefficient. In addition, Figure 1 also reveals that guests **2** and **3a** behave differently upon the addition of **1**, with the change in the signal attenuation and hence in the computed diffusion coefficient being much less pronounced for guest **2**. In the equimolar

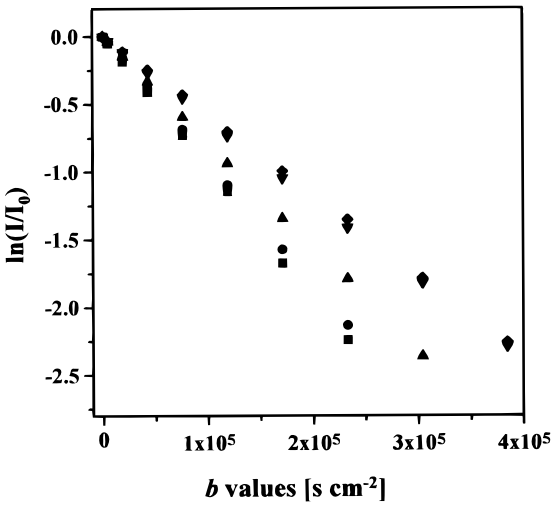

Figure 1. The natural log of the normalized signal attenuation ($\ln I/I_0$) as a function of the *b* values for **1** (◆), **2** (■), and **3a** (●) in the free state and of **2** (▲) and **3a** (▼) in chloroform solutions containing equimolar concentrations of **1** and **2** or **3a**.

$CDCl_3$ solution of **1** and **3a**, the signal attenuation for **1** and **3a** is identical (within the margin of experimental error). The smaller decrease in the diffusion coefficient of **2** as compared with that of **3a** upon addition of **1** indicates that the association constant of **2** to **1** is much lower than that between **1** and **3a**.

Table 1 depicts the diffusion coefficients of host **1** and guests **2**−**5** in the free state and in their equimolar dry $CDCl_3$ solutions with **1**, along with the association constants (log K_as) computed from these values. The data show that there is significant binding between **1** and lauric acid (**2**) and that the addition of the alanine moiety to obtain guest **3a** increases the binding to **1** by more than 1 order of magnitude. The same results were obtained with the L enantiomer **3b** (data not shown), indicating that receptor **1** is not capable of enantioselective recognition. This result was not surprising given that the biological activity of the DD isomer of **1** was shown to be very similar to that of the LL isomer.[6] Moreover, chiral discrimination could be obtained for complexes of **1** only on the basis of steric effect; however, the methyl groups of the alanine residues at the upper rim loop of the host are too small to create an efficient steric barrier.[6] Therefore, depending on their availability L and D isomers were used for the binding studies.

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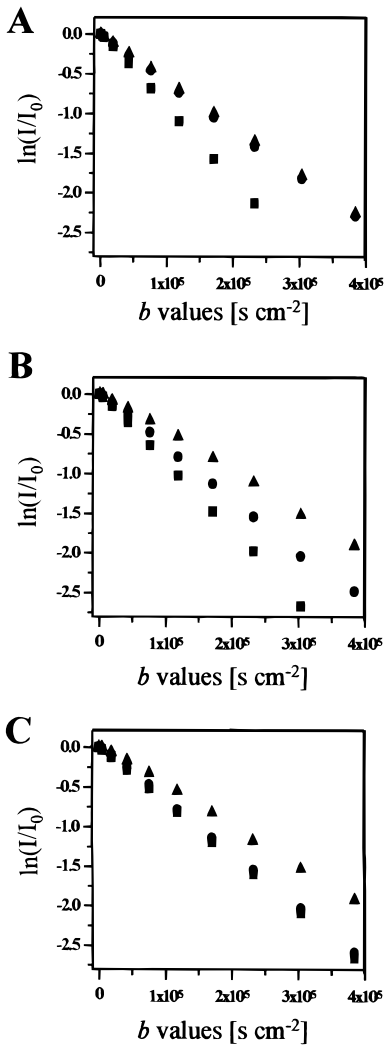

Figure 2. The natural log of the normalized signal attenuation (ln I/I_0) as a function of the b values for **3a** (■) in the free state and of **1** (▲) and **3a** (●) in equimolar solutions of (A) dry $CDCl_3$, (B) CD_3OD, and (C) $CDCl_3$ containing 10% of DMSO-d_6.

However, the direct measurements on *N*-lauroyl-D-alanyl-D-alanine (**4**), which contains two alanine residues reminiscent of the terminal mucopeptide percusor, failed to demonstrate that its binding affinity to **1** is larger than that of **3a**. However, as can be seen in Table 1, the diffusion coefficients of **1** and **4** in the free state are very similar, implying that in this case the calculated association constant may not be very accurate. Therefore, we tried to perform the above measurements on *N*-acetyl-L-alanine (**5**) and *N*-acetyl-L-alanyl-L-alanine (**6**), which, disregarding their stereochemistry, are the acetyl analogues of **3a** and **4**, respectively. The data in Table 1 clearly demonstrate that indeed the diffusion coefficient of **5** in the free state is, as expected, much higher than that of **3a**. In this system, the value of log K_a was also around 4. However, we could not perform these measurements for **6** in $CDCl_3$ because of its low solubility in this solvent.

To obtain additional information on the nature of the interactions involved in the complexation between host **1** and the different guests, we also studied their binding in different solvents and solvent mixtures. Figure 2 shows the semilogarithmic plots of the normalized signal attenuation as a function of b for host **1** and guest **3a** in the free state and for their equimolar solutions in dry $CDCl_3$, in methanol-d_4, and in $CDCl_3$ containing 10% DMSO-d_6 (Figures 2A, 2B, and 2C, respectively). From Figure 2A it is clear that the signal attenuation and, hence, the diffusion coefficients of **1** and **3a** in their equimolar solution are nearly identical and very different from that of **3a** in the free state. However, the opposite is true for the $CDCl_3$ solution containing 10% DMSO-d_6. Here the signal attenuation for **3a** in the free state and in the equimolar solution with **1** are very similar and differ considerably from that of **1**, implying the existence of a very weak interaction between **1** and **3a** under these conditions. In Figure 2B an intermediate situation is observed.

The diffusion coefficients and the computed association constants of **1** with the various guests in different solvents are summarized in Tables 2−4. The cumulative data in these tables show the following:

(1) Changing the solvent from chloroform to a protic solvent such as methanol significantly lowers the association constants.

(2) The decrease in K_as is more pronounced in the complex of **1** and **3a** than that for the complex between **1** and **2**. Compound **4** exhibits the same trend as **3a** (data not shown).

(3) The results for dry $CDCl_3$ and untreated $CDCl_3$ (data not shown) are similar. However, the association constants are significantly lower in D_2O saturated $CDCl_3$ solutions. Here the decrease in the association constants is more pronounced for the **3a**⊂**1** system than for the **2**⊂**1** system.

(4) The addition of 2−3% DMSO-d_6 to the chloroform solution lowers the association constants of the complexes of **1** with **3a** and **5** by about 1 order of magnitude. This decrease in the K_as is dependent on the concentration of DMSO-d_6 in the chloroform solution. The addition of 10%

Table 2: Diffusion Coefficients ($\times 10^5$ in $cm^2\ s^{-1}$) and the Computed Association Constants (log K_as) of Host 1 and Guest 2 in Different Solvents

system	D (solvent)	D (host)	D (guest)	X	log K_a
2 in $CDCl_3$[b]	2.36(±0.02)		1.00(±0.07)		
2⊂**1** in $CDCl_3$[b]	2.44(±0.01)	0.60(±0.01)	0.78(±0.02)	0.55(±0.09)	2.7(±0.2)
				[0.58(±0.08)][a]	[2.8(±0.2)][a]
2 in MeOD	2.30(±0.03)		1.16(±0.03)		
2⊂**1** in MeOD	2.10(±0.03)	0.53(±0.01)	0.89(±0.01)	0.43(±0.03)	2.4(±0.1)
				[0.32(±0.04)][a]	[2.1(±0.1)][a]
2 in $CDCl_3$c	2.51(±0.02)		1.18(±0.03)		
2⊂**1** in $CDCl_3$[c]	2.53(±0.06)	0.60(±0.01)	1.03(±0.01)	0.26(±0.04)	2.0(±0.1)

[a] As the diffusion coefficient of $CDCl_3$ in the 1:1 host/guest solution was different from the value obtained in the solution containing the host in the free state, the ratios of the two values were used to correct the experimental values giving rise the corrected values in the parenthesis. [b] In dry $CDCl_3$. [c] In $CDCl_3$ equilibrated with D_2O.

Table 3: Diffusion Coefficients ($\times 10^5$ in $cm^2\ s^{-1}$) and the Computed Association Constants (log K_as) of Host 1 and Guest 3a in Different Solvents

system	*D* (solvent)	*D* (host)	*D* (guest)	*X*	log K_a
3a in $CDCl_3$[b]	2.47(±0.01)		0.94(±0.01)		
3a⊂**1** in $CDCl_3$[b]	2.47(±0.02)	0.57(±0.01)	0.57(±0.01)	>0.99	>4.0
3a in $CDCl_3$ +2% DMSO-d_6	2.26(±0.04)		0.74(±0.01)		
3a⊂**1** in $CDCl_3$ +2% DMSO-d_6	2.30(±0.01)	0.51(±0.01)	0.60(±0.01)	0.61(±0.05) [0.63(±0.05)][a]	2.9(±0.1) [3.0(±0.2)][a]
3a in $CDCl_3$[c]	2.60(±0.01)		1.06(±0.01)		
3a⊂**1** in $CDCl_3$[c]	2.59(±0.06)	0.65(±0.01)	0.86(±0.01)	0.49(±0.03)	2.6(±0.1)
3a in MeOD	2.17(±0.01)		0.86(±0.01)		
3a⊂**1** in MeOD	2.06(±0.02)	0.51(±0.01)	0.70(±0.02)	0.46(±0.06) [0.39(±0.07)][a]	2.5(±0.2) [2.3(±0.2)][a]
3a in $CDCl_3$ +10% DMSO-d_6	2.04(±0.07)		0.72(±0.02)		
3a⊂**1** in $CDCl_3$ +10% DMSO-d_6	2.09(±0.04)	0.52(±0.01)	0.67(±0.01)	0.25(±0.09) [0.32(±0.08)][a]	1.9(±0.3) [2.1(±0.2)][a]

[a] As the diffusion coefficient of $CDCl_3$ in the 1:1 host/guest solution was different from the value obtained in the solution containing the host in the free state, the ratios of the two values were used to correct the experimental values giving rise the corrected values in the parenthesis. [b] In dry $CDCl_3$. [c] In $CDCl_3$ equilibrated with D_2O.

Table 4: Diffusion Coefficients ($\times 10^5$ in $cm^2\ s^{-1}$) and the Computed Association Constants (log K_as) of Host 1 and Guests 5 and 6 in Chloroform Solution and in Chloroform:DMSO Mixtures

system	*D* (chloroform)	*D* (host)	*D* (guest)	*X*	log K_a
5 in $CDCl_3$	2.44(±0.02)		1.29(±0.01)		
1⊂**5** in $CDCl_3$	2.20(±0.05)	0.44(±0.02)	0.58(±0.01)	0.84(±0.02) [0.81(±0.03)][a]	3.8(±0.1) [3.7(±0.2)][a]
5 in $CDCl_3$ +3% DMSO-d_6	2.40(±0.06)		1.19(±0.04)		
1⊂**5** in $CDCl_3$ +3% DMSO-d_6	2.24(±0.03)	0.55(±0.01)	0.90(±0.01)	0.44(±0.04) [0.38(±0.05)][a]	2.4(±0.1) [2.3(±0.1)][a]
6 in $CDCl_3$ +3% DMSO-d_6	2.38(±0.03)		0.95(±0.01)		
1⊂**6** in $CDCl_3$ +3% DMSO-d_6	1.99(±0.01)	0.45(±0.01)	0.57(±0.01)	0.75(±0.03) [0.65(±0.04)][a]	3.4(±0.1) [3.0(±0.1)][a]

[a] As the diffusion coefficient of $CDCl_3$ in the 1:1 host/guest solution was different from the value obtained in the solution containing the host in the free state, the ratios of the two values were used to correct the experimental values giving rise the corrected values in the parenthesis.

DMSO-d_6 to the chloroform solution lowers K_as by about 2 orders of magnitude in the **3a**⊂**1** system (Table 3).

(5) The log K_as for **3a**⊂**1** and **5**⊂**1** in dry $CDCl_3$ were around 4, but in a $CDCl_3$ solution containing 2−3% DMSO-d_6 these values were only 2.9 ± 0.1 and 2.4 ± 0.1 for **3a**⊂**1** and **5**⊂**1**, respectively. Interestingly, under these conditions, log K_a for the complex between **1** and **6**, which contains two alanine residues, was found to be 3.4 ± 0.1 (Table 4).

Discussion

The vancomycin analogue **1** was found to have a significant antibiotic activity that may originate from the ability of this compound to interact with the ala-ala residue. As compound **1**, like vancomycin, contains several functional groups of various types, the nature of their interactions with the terminal -D-alanyl-D-alanine sequence of cell wall mucopeptide precursors may be complex. The interaction might involve electrostatic, π-hydrogen, hydrophobic, and dipole−dipole interactions as well as hydrogen bonds. We therefore decided to study the complexation of host **1** with a selected series of guests of similar structure without and with one or two alanine residues.

Our data show that, in $CDCl_3$, **1** forms with lauric acid (**2**) a complex of moderate stability whose log K_a does not decrease significantly upon increasing the ability of the solvent to compete for hydrogen bonds. Addition of the alanine moiety to the guest increases the association constant by at least 1 order of magnitude, and log K_a for the **3a**⊂**1** system was found to be higher than 4.0. On the basis of these findings, one would expect the association constant between **1** and **4**, which contains two alanine residues, to be even higher. The data presented in Table 1 seem to contradict this expectation as we found that log K_a for the **3a**⊂**1** and **4**⊂**1** systems was >4.0 and 3.4 ± 0.4, respectively. However, it should be noted that the experimental error in the association constant computed for the **4**⊂**1** system may be very large due to the similarity between the diffusion coefficients of **1** and **4** in the free state. As we were interested in the association constant between **1** and guests containing two alanine residues, we decided to determine the association constant between **1** and guests **5** and **6**. The rationale was that because of the lower molecular weights of compounds **5** and **6** they would have a much higher diffusion coefficient than **1** in the free state, thereby increasing the accuracy in determining the association constants by NMR diffusion measurements. Unfortunately, compound **6** exhibited a marginal solubility in $CDCl_3$, preventing direct determination of its association constant to host **1**. However, the association constants for the various guests in the different media provided a means to obtain an indirect estimation of the association constants of host **1** to guests **4** and **6** in $CDCl_3$. The data in Table 3 show that the addition of ∼2% DMSO-d_6 to a solution of **3a**⊂**1** in $CDCl_3$ causes a decrease in log K_a by more than 1 order of magnitude and that the addition of ∼10% lowers log K_a by at least 2 orders of magnitude. Also, as can be seen in Table 4 the addition of ∼3% DMSO-d_6 to a solution of **5**⊂**1** in $CDCl_3$ lowers log K_a from 3.8 ± 0.1 to 2.4 ± 0.1. However, it was found that log K_a for the **6**⊂**1** system containing 3% DMSO-d_6 was 3.4 ± 0.1. Assuming that 3% DMSO-d_6 decreases the association constant by more than 1 order of magnitude, as compared with chloroform,

one can extrapolate that the log K_a for the **6**⊂**1** system in chloroform is significantly higher than 4.0. A comparison of the association constants between **1** and guests **3a** and **5** reveals that the alkyl chains (C1 for **5** and C12 for **3a**) have only a marginal effect on the stability of these complexes. If there is any effect it seems that the C12 group increases the stability of the complex. As the extrapolated value of log K_a for the **6**⊂**1** system in $CDCl_3$ is higher than 4.0, it seems reasonable to assume that for guest **4**, which is the C12 analogue of **6**, log K_a is also greater than 4.0. This value is somewhat higher than the value obtained by our direct measurements that, as explained, may not be very accurate.

As the macrobicyclic peptidocalixarene **1** contains several binding groups, many interactions may be involved in the formation of the supramolecular complexes of **1** and guests **2**−**6**. Our experimental results are all consistent with the fact that, inter alia, hydrogen bonds play a significant role in these complexes in chloroform solutions. This is manifested by the decrease in the association constants of the complexes of **1** by the addition of DMSO-d_6 to the chloroform solutions or when the complexes are studied in a protic solvent such as methanol. As observed in other cases in which hydrogen bonding is involved,[12] even equilibration of the $CDCl_3$ solution with D_2O significantly reduces the association constants. It should be noted, however, that the addition of solvents that form or break hydrogen bonds causes a more pronounced decrease in the association constants of the alanine-containing guests (i.e., **3**−**6**) as compared with lauric acid (**2**), where only elecrostatic interactions are involved. In guests **3**−**6**, the NH moiety of the alanine residues can also participate in hydrogen bonding to **1**, so it is likely that weakening the hydrogen bonds between **1** and these guests will have a more dramatic effect on the association constants.

The most important results which may be particularly relevant for a possible correlation between the antimicrobial activity of the peptidocalix[4]arene **1** are the stability of its complexes with ala-ala-containing dipeptides. In this context, the log K_a value observed for **6**⊂**1** in $CDCl_3$ + 3% DMSO-d_6 (Table 4) is of importance. This is the first direct quantitative evaluation of the binding constant between **1** and a dipeptide, since it was not possible to determine it by classical ^{1}H NMR experiments.[6] A comparison between this value and that of the system **5**⊂**1** under the same conditions shows an increase of 1 order of magnitude in the association constant on going from the simple *N*-acetyl-L-alanine (**5**) amino acid to the *N*-acetyl-L-ala-L-alanine (**6**) dipeptide. This suggests that an additional hydrogen bond or other solvophobic interactions contribute to the stabilization of the inclusion complex of **1** and **6** containing two alanine residues. These results demonstrate unequivocally that host **1**, being a vancomycin analogue, has a significant affinity to the ala-ala moiety which is believed to be the action site of the vancomycin antibiotics.

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