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# *Proximal* and *distal N,C*-linked tetra-peptidocalix[4]arenes as bifunctional receptors: synthesis, conformation and preliminary binding studies†

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New 1,2-*proximal N,N,C,C*-linked and 1,3-*distal N,C,N,C*-linked tetra-peptidocalix[4]arenes have been synthesised by functionalisation of the corresponding calixarene bis-amino acids. Their conformational properties in solution, which have been studied by $^1$H NMR, are determined by the presence of an extensive network of intramolecular hydrogen bonds. Electrospray ionisation mass spectrometry studies show that the 1,2-*proximal* peptidocalix[4]arene **19** is a more efficient receptor than the 1,3-*distal* isomer **8** in the gas phase and that it is able to recognise aromatic amino acids both as methyl esters and N-acetyl derivatives. Interestingly, these observations indicate that **19** is a bifunctional receptor since it displays both the selectivity of *N*-linked peptidocalixarenes for ammonium groups and that of *C*-linked peptidocalixarenes for carboxylates.

**Keywords:** peptidocalixarenes; calixarene amino acid; ESI-MS binding studies; amino acid receptors; chiral host



## Introduction

Since the past few years, we and others (*1*) have been interested in the synthesis and study of upper rim peptidocalixarenes, a class of synthetic receptors obtained by the conjugation of α-amino acids or small peptides to the aromatic *para*-positions of calix[4]arenes. The properties of these hybrid derivatives originate from the combination of the hydrophobic cavity of the calixarene with the polar, hydrogen bonding and chiral groups of the amino acids. By appropriately choosing the number and type of the amino acids or peptides, efficient biomimetic receptors for a wide range of targets (from small molecules of biological interest (*2–6*) to protein surfaces involved in clinically important protein–protein interactions (*7*)) or self-assembled architectures held together by hydrogen bonds (*8–10*) have been prepared. In addition to the synthesis of the first examples of peptidocalixarenes, our contribution to this research field, in particular, has dealt with the study of the relationship between the type of linkage of the amino acids to the calixarene and the supramolecular properties of these derivatives. The *N*-linked peptidocalix[4]arenes (i.e. **Ia**,**b**) (*11*), obtained by reaction of the calixarene di- or tetra-carboxylic acid with the amino groups of the amino acids, are able to complex carboxylic acids and ammonium cations, but not anionic guests. On the contrary, the *C*-linked derivatives (i.e. **IIa**,**b**) (*12*), prepared by condensing the di- or tetra-aminocalixarene with the carboxylic acid group of the amino acids, selectively recognise carboxylate anions. Moreover, the *N*-linked peptidocalix[4]arenes do not show any evidence of intra- or intermolecular hydrogen bonding, while the amino acids of the *C*-linked analogues are involved in intra- or intermolecular hydrogen-bonding interactions (*13*). On the other hand, the *N,C*-linked peptidocalix[4]arenes (i.e. **IVa**,**b**) (*14*, *15*), which we consider as members of a 'second generation' of peptidocalixarenes, are indeed peptides that incorporate in their sequence an unnatural monomer, the calix[4]arene amino acid (**IIIa**,**b**) (*14*, *15*). They can be synthesised either by solution (*15*) or solid-phase (*16*) peptide synthesis protocols as linear or cyclic oligomers of different lengths, and their conformational properties can be tuned by the rigidity of the calixarene scaffold. The linear derivatives based on the rigid 1,3-dipropoxycalix[4]arene (**IVa**) self-assemble into dimers (the shorter peptides) (*15*) or long helical ribbons (the 11-mer) (*16*) by a β-sheet-like hydrogen-bonding motif, while the flexible tetrapropoxycalix[4]arene scaffold of **IVb** behaves as a loop region favouring the formation of intramolecular hydrogen bonds (*15*).

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†Dedicated to the memory of Dmitry (Prof. Dmitry M. Rudkevich, 1963–2007).

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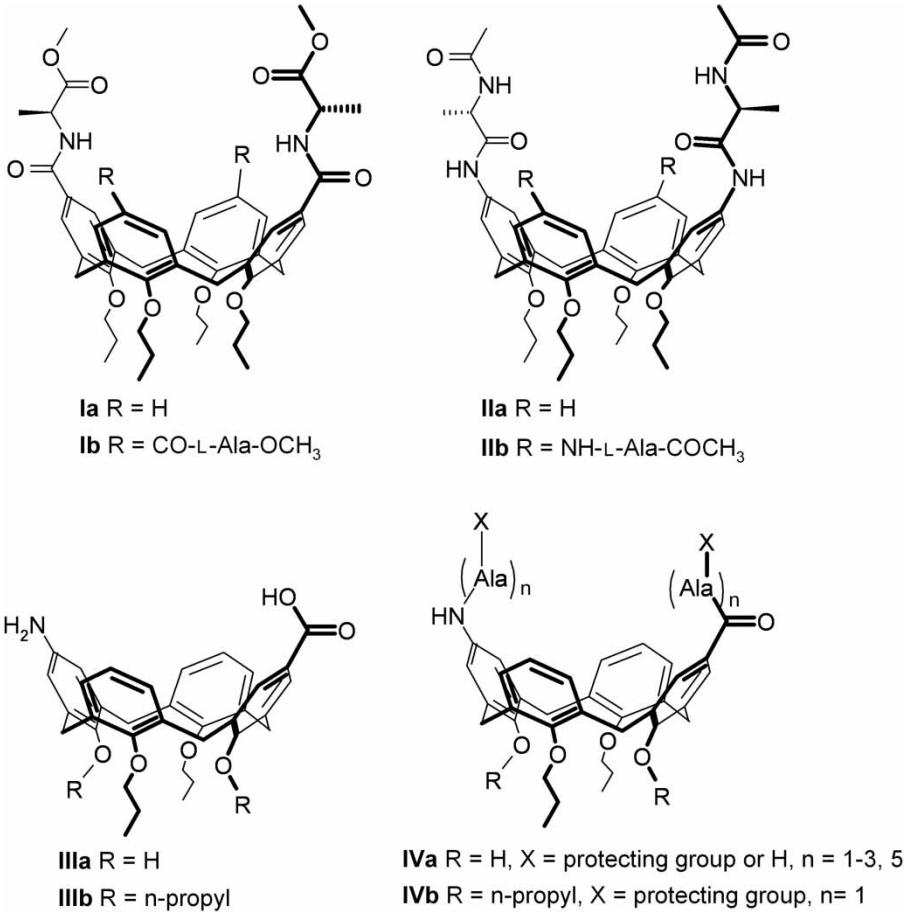



Compounds **I**–**IV**.



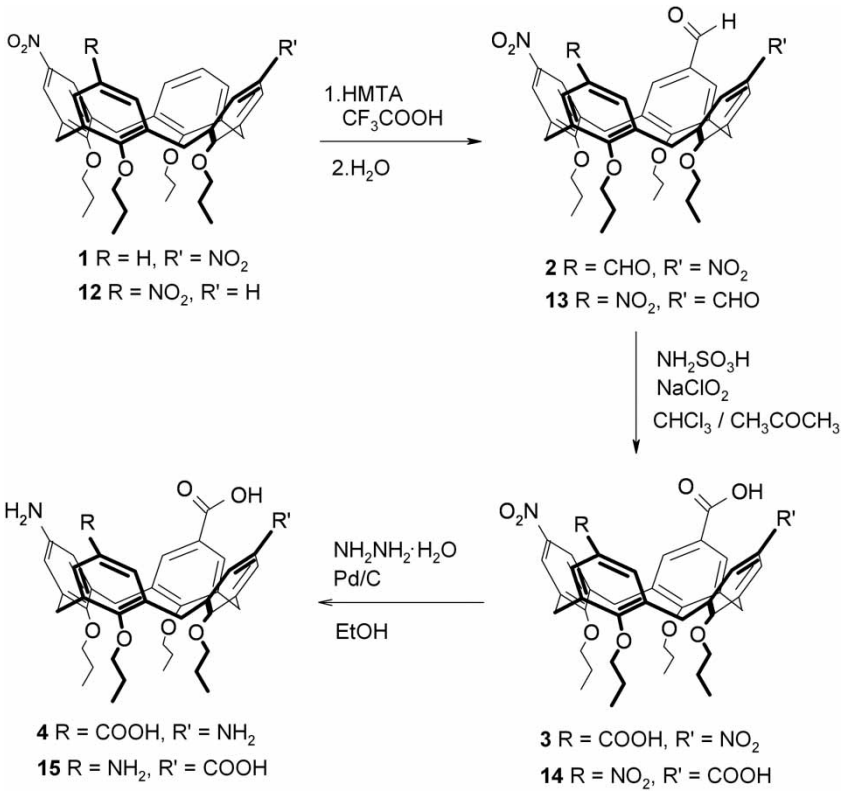
In order to expand the scope of peptidocalixarenes and ideally complete the family, we report herein the synthesis of the new *N*,*C*,*N*,*C*-linked (**8**–**11**) and *N*,*N*,*C*,*C*-linked (**19**) tetra-peptidocalix[4]arenes, which are based on the two isomeric bis-amino acids, the *distal* 1,3-diamino-2,4-dicarboxylic acid calix[4]arene (**4**) and the *proximal* 1,2-diamino-3,4-dicarboxylic acid calix[4]arene (**15**) (*17*). In particular, we were interested in studying their complexation properties and in investigating whether the presence of four amino acids would lead to the formation of self-assembled capsules of higher stability than those formed by the difunctionalised analogues **IVa.**

## Results and discussion

### *Synthesis and self-assembly properties of* distal N,C,N,C-*linked and* proximal N,N,C,C-*linked peptidocalix[4]arenes*

Tetrapropoxycalix[4]arene diamino diacids **4** and **15** were obtained in high yields from 1,3-*distal* (**1**) and 1,2-*proximal* (**12**) dinitro derivatives (*18*), respectively, following the synthetic pathway depicted in Scheme 1.

This methodology had been developed for the synthesis of calixarene amino acids **IIIa** and **IIIb** and consists in the formylation of the dinitro compounds followed by the oxidation of the aldehyde functionalities and the reduction of the nitro groups. We found that, in this case, the Duff formylation gives higher yields and cleaner products than the Gross reaction with titanium or tin tetrachloride and α,α-dichloromethyl methyl ether that we (*15*) and others (*19*) had often used. Both the *distal* 1,3-diamino-2,4-dicarboxylic acid calix[4]arene (**4**) and the *proximal* 1,2-diamino-3,4-dicarboxylic acid calix[4]arene (**15**) show broad $^1$H NMR spectra in $CDCl_3$ and sharp signals in DMSO-$d_6$. This indicates the presence of an irregular intermolecular aggregation through hydrogen bonding in the non-polar solvent. As previously observed for calixarene amino acids **IIIa**,**b** (*15*), we collected evidence that also **4** and **15** do not exist as zwitterions. The aromatic protons *ortho* to the amino groups, in fact, resonate at high fields in DMSO-$d_6$ (5.76 ppm for **4** and 5.91 and 5.89 ppm for **15**) and move to lower fields after the addition of a drop of trifluoroacetic acid (TFA) (6.33 ppm for **4**), indicating that the amino group is initially neutral and gets protonated only in the presence of



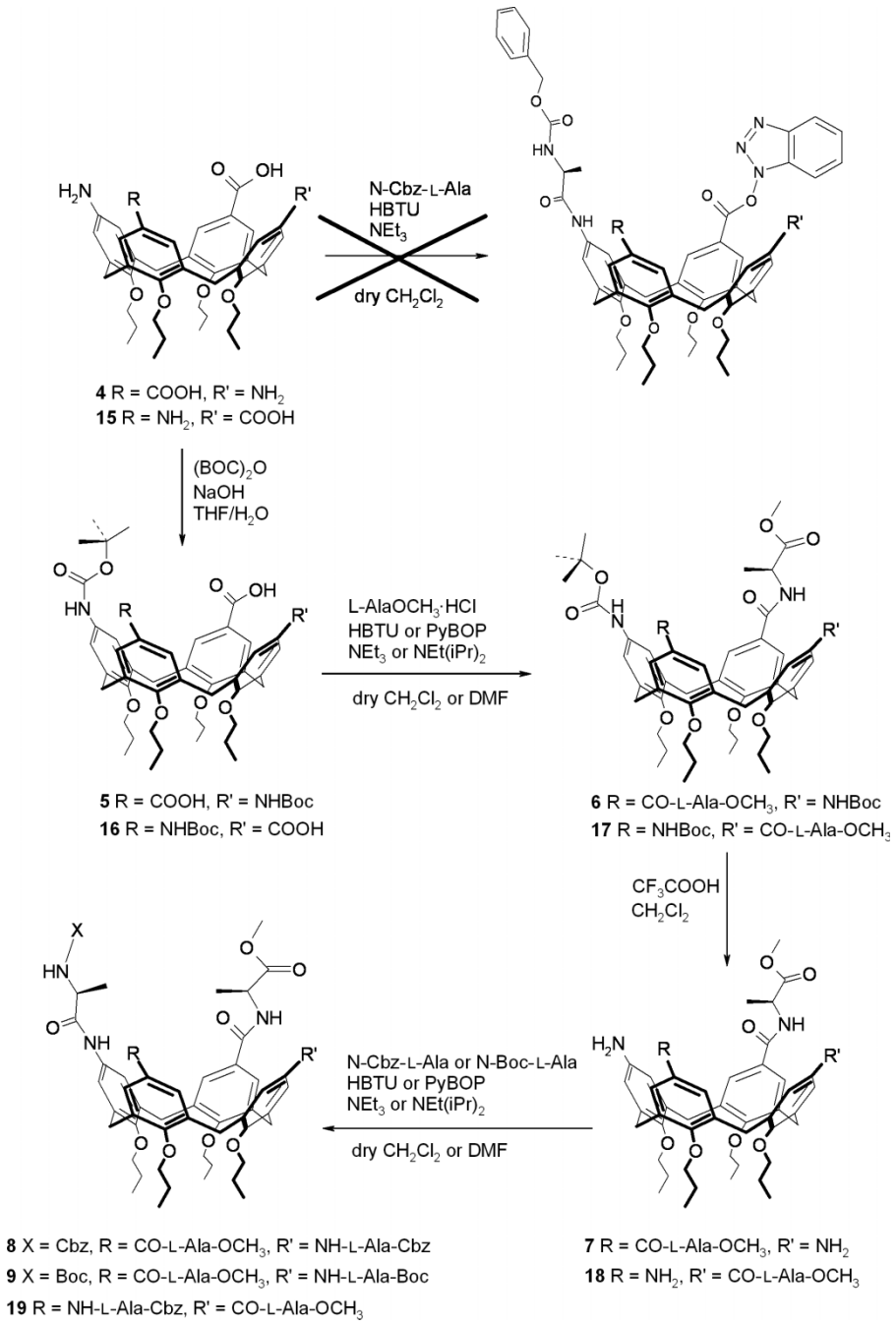

Scheme 1 . Synthesis of calix[4]arene diamino diacids **4** and **15.**



an acid stronger than the COOH group present on the calixarene. Moreover, the IR spectra of both compounds show the bands around 1690 and 3415 $cm^{-1}$ due to the carbonyl group of carboxylic acid and to a neutral $NH_2$ group, respectively. Due to the lower basicity of the $NH_2$ groups linked to the calixarene aromatic nuclei compared to the aliphatic amine of α-amino acids, the formation of zwitterions does not take place in the calixarene amino acids.

To functionalise **4** and **15** with amino acids, we tried at first the procedure developed for the synthesis of *N*,*C*-linked peptidocalix[4]arenes **IVa**, which starts with the reaction of the calixarene amino acid with Cbz-protected L-alanine in the presence of the coupling reagent HBTU and of triethylamine, to obtain an intermediate bearing benzotriazolyl-active esters on the calixarene carboxylic acids in addition to the *C*-linked amino acids (Scheme 2). However, this reaction yielded a complex mixture of compounds where the desired product could be detected neither by $^1$H NMR nor by electrospray ionisation mass spectrometry (ESI-MS). The target compounds **8** and **19** were instead synthesised following a protection–coupling–deprotection procedure typical of peptide synthesis (Scheme 2), using the Boc-protecting group for the amino functionalities of **4** and **15** and the HBTU-triethylamine (or, alternatively, the PyBOP-diisopropylethylamine) coupling conditions. The reaction of **4** and **15** with Boc anhydride required the use of NaOH to prevent the formation of products of condensation between $(Boc)_2O$ and the carboxylic acid groups, which formed along with the desired compound if triethylamine was used. The condensation of intermediates **5** and **16** with L-alanine methyl ester and the subsequent deprotection of the amino groups with TFA to yield compounds **7** and **18** proceeded smoothly and in high yields. Reaction of these compounds with Cbz-L-alanine yielded protected tetraalanine derivatives **8** and **19** in 40 and 44% yield, respectively, after column chromatography. All the compounds were fully characterised by 1D and 2D NMR spectroscopy and ESI-MS.

In the $^1$H NMR spectrum of **8** in $CDCl_3$, which shows slightly broad peaks, both the signals of the four aromatic protons of the ArCO rings and the signals of the four aromatic protons of the ArNH rings are split into two peaks, with a $\Delta\delta$ of 0.06 and 0.56 ppm, respectively. A similar splitting is observed in the $^1$H NMR spectrum of **6** ($\Delta\delta = 0.06$ and 0.13 ppm for the ArCO and the ArNH aromatic protons, respectively), while no splitting is shown in the spectrum of **7**, where two singlets for the two different aromatic rings are present. We attribute this behaviour to the combination of the chiral centres on the alanine units and the presence of intramolecular hydrogen bonds between the four chains at the upper rim that rigidify molecules **6** and **8**, while in **7** the two *N*-linked alanine




Scheme 2 . Synthesis of protected *N,C*-linked tetra-peptidocalix[4]arenes **8**, **9** and **19.**

chains, in 1,3 position, do not significantly interact. This compound, in fact, presents an open flattened cone conformation, in analogy with the *N*-linked peptidocalix[4]arene **Ia** (*11*), as evidenced by the upfield shift of the aromatic protons *ortho* to the $NH_2$ group (5.58 vs. 6.07 ppm in tetraamino tetrapropoxycalix[4]arene (*10*)). Interestingly, in methanol-$d_4$ and DMSO-$d_6$, all the signals are sharp, the ArCO aromatic protons give a singlet and the splitting of the two signals corresponding to the ArNH aromatic protons is smaller than in $CDCl_3$ ($\Delta\delta = 0.35$ and 0.31 ppm in methanol-$d_4$ and DMSO-$d_6$, respectively).

The $^1$H NMR spectra of **19** in methanol-$d_4$ and DMSO-$d_6$ show sharp signals, but are very difficult to explain. Due to the chiral centres on the alanine units and the 1,2-*proximal* substitution pattern on the calixarene scaffold, the molecule lacks any element of symmetry, and therefore every proton of the molecule gives a different signal. On the contrary, the $^1$H NMR spectrum of **19** in

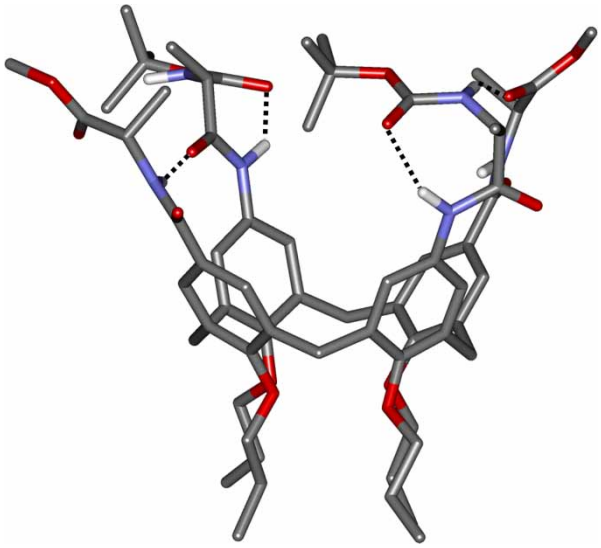


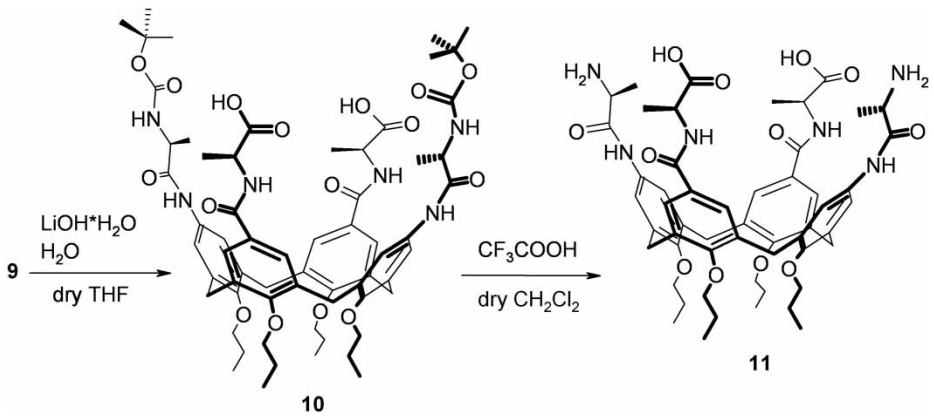
Figure 1. Energy-minimised (molecular mechanics) structure of compound **9**. The non-amide hydrogen atoms have been omitted for clarity. The dotted lines indicate hydrogen bonding.

$CDCl_3$ shows an apparently too high number of slightly broad peaks. These can be attributed to the presence of strong intramolecular hydrogen bonds between the amino acid chains, which do not take place in polar solvents like methanol.

Next, we had planned to unmask first the amino groups and subsequently the carboxylic acid functionalities to obtain the fully deprotected peptidocalixarenes. The removal of the Cbz groups from **8** by hydrogenation, however, afforded a complex mixture of different products. We, therefore, synthesised the analogous derivative **9**, having Boc instead of Cbz-protecting groups, by the condensation reaction of intermediate **7** with Boc-L-alanine (Scheme 2). The $^1$H NMR spectrum of **9** in $CDCl_3$, in analogy with that of **8**, shows the splitting of the signals of the ArCO aromatic protons ($\Delta\delta = 0.05$ ppm) and an even larger splitting than **8** ($\Delta\delta = 1.01$ ppm) for the ArNH aromatic protons. Also, in methanol-$d_4$ and acetone-$d_6$, the aromatic protons of the two different nuclei are split into two pairs of signals ($\Delta\delta = 0.03$ and 0.41 ppm in methanol-$d_4$ and 0.06 and 0.53 ppm in acetone-$d_6$). This seems to indicate the persistence of intramolecular hydrogen bonds between the alanine chains, also in the polar solvents. Moreover, the NOESY spectrum of **9** in $CDCl_3$ shows correlation peaks of the Boc protons with one of the ArCO and with one of the ArNH aromatic protons, indicating that at least one of the *t*-butyl groups is projected inwards towards the aromatic cavity of the calixarene. An energy-minimised structure that reflects the NMR data is reported in Figure 1.

The fully deprotected peptidocalix[4]arene **11** was obtained in high yield by reaction of compound **9** first with LiOH to hydrolyse the ester groups and subsequently with TFA to remove the Boc-protecting groups (Scheme 3). As expected, this compound is scarcely soluble in chloroform, while it dissolves well in methanol. The $^1$H NMR spectrum of **11** in methanol-$d_4$ shows sharp peaks, which do not change their position upon varying the sample concentration, ruling out the formation of aggregates. Intramolecular hydrogen bonding, also in this case, leads to the splitting of the aromatic signals ($\Delta\delta = 0.03$ and 0.15 ppm for the ArCO and the ArNH aromatic protons, respectively). Unfortunately, the solubility of **11** in water is poor and a $^1$H NMR spectrum of a diluted solution of **11** in $D_2O$ shows broad signals, indicating a large degree of disordered aggregation, mainly due to the hydrophobic interactions of the lipophilic part of the molecule, as frequently found also for guanidino- (*20*) and glucocalixarenes (*21*).

In order to verify if peptidocalix[4]arenes **8**, **9**, and **19** show self-assembly properties analogous to those observed for the *N,C*-linked peptidocalixarenes **IVa**, we measured $^1$H NMR spectra of these compounds in $CDCl_3$ at different concentrations. We found that in the concentration range 0.1–10 mM, the $^1$H NMR spectra of the three compounds do not undergo any modification.




Scheme 3. Synthesis of deprotected 1,3-*distal N,C,N,C*-linked tetra-peptidocalix[4]arene **11**.

Moreover, the $^1$H NMR spectrum of an equimolar mixture of **8** and **9** in $CDCl_3$ is the exact superposition of the spectra of the two compounds, ruling out the formation of intermolecular hetero-aggregates. Also, the ESI-MS spectra of **8** and **9** do not show the presence of peaks corresponding to self-assembled dimers or higher-order aggregates. From these data, we conclude that these compounds do not display any tendency to self-aggregate under the same conditions where the *N*,*C*-linked peptidocalix[4]arenes **IVa** formed dimeric capsules (*15*). Subsequently, with the aim of promoting the formation of self-assembled capsules by the template effect of a suitable solvent, the $^1$H NMR spectrum of **8** in toluene-$d_8$ was measured. At room temperature, however, **8** shows scarce solubility and it was necessary to heat the sample to achieve the complete dissolution. The $^1$H NMR spectrum measured at 363 K is quite sharp and very similar to the room temperature spectrum of **8** in $CDCl_3$. By cooling the sample to room temperature, no precipitation was observed, but the $^1$H NMR spectrum showed very broad peaks. Moreover, the ArNH signal, which resonates at 8.08 ppm at 363 K, was shifted downfield upon cooling the sample ($\delta = 8.90$ at 300 K). Taken together, these results indicate a tendency of **8** to give irregular aggregates at room temperature through hydrogen bonds, involving at least the ArNH group, which are broken at high temperature.

### *Gas-phase complexation properties*

Mass spectrometric investigations exploiting soft ionisation techniques, such as ESI, provide a fast and simple screening method of non-covalent recognition events with minimal sample consumption (*22*). A preliminary study of the gas-phase complexation properties of peptidocalix[4]arenes **8** and **19** towards selected amino acid derivatives was performed by ESI-MS competitive experiments. A methanol solution of the *N*,*C*,*N*,*C*-linked peptidocalix[4]arene **8** containing an equimolar concentration of two amino acid derivatives (L-alanine methyl ester and L-phenylalanine methyl ester as hydrochloride salts) was subjected to ESI-MS analysis in positive ion mode. The spectrum reported in Figure 2, however, shows as main signals the double- and single-charged ions formed by the interaction of **8** with two or one sodium cations that are present in trace in solution, and, with a lower intensity, the peaks corresponding to the 1:1 complexes of **8** with the protonated amino acids. No peaks corresponding to complexes of higher stoichiometry were observed. Even if the affinity of **8** for these guests in the experimental conditions is not very high, it is noteworthy that phenylalanine is complexed more efficiently than alanine. The preference for amino acids with aromatic side chains, in fact, had been previously observed by our group in gas-phase complexation experiments of glucocalixarenes (*23*).

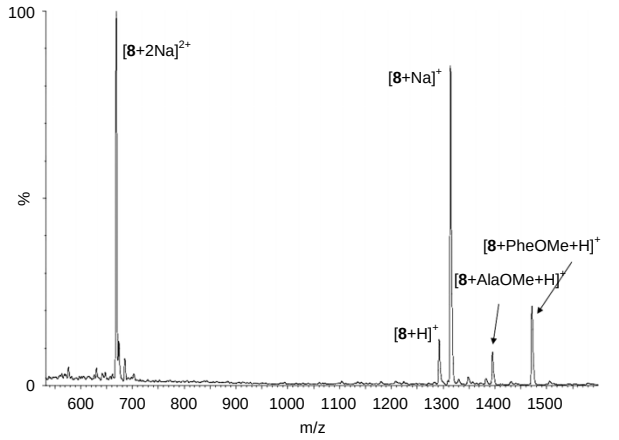



Figure 2. ESI-MS spectrum of a mixture containing receptor **8**, AlaOMe·HCl and PheOMe·HCl (each $1 \times 10^{-5}$ M) in methanol.

Interestingly, the ESI-MS spectrum of an equimolar mixture of **19** and five different amino acid methyl ester hydrochlorides in methanol suggests that 1,2-*proximal* *N*,*N*,*C*,*C*-linked peptidocalix[4]arene **19** is a more efficient receptor for protonated amino acid methyl esters in the gas phase than **8**. The choice of the amino acids was dictated by the purpose of studying the effect on the host–guest interaction of a different side chain, in particular an aromatic group (Phe and Trp), an additional hydrogen-bonding group (Tyr), and a lipophilic aliphatic residue (Leu), with respect to alanine. This competitive experiment showed a higher affinity of **19** for the three aromatic amino acids and a lower one for leucine and alanine (TrpOMe $>$ PheOMe $\approx$ TyrOMe $>$ LeuOMe $>$ AlaOMe) (Figure 3(a)). The additional hydrogen bonding group of tyrosine, in this case, did not improve the host–guest interaction, as instead observed for glucocalixarenes (*23*). Also for peptidocalix[4]arene **19**, no complexes of higher stoichiometry than 1:1 were observed in the ESI-MS spectrum. Moreover, the ESI-MS spectrum in negative ion mode of an equimolar mixture of **19** and the N-acetyl derivatives of Ala, Leu, Phe and Trp showed as the most intense peak at $m/z > 600$ the 1:1 complex of **19** with N-acetyl tryptophan, followed by the 1:1 complex of **19** with N-acetyl phenylalanine (Figure 3(b)). Lower intensity peaks corresponding to the complex of **19** with a chloride ion, to the negative ion of **19** and to the complexes of **19** with N-acetyl leucine and N-acetyl alanine, were also observed. In addition to the high affinity of **19** for N-acetyl tryptophan, it is noteworthy that the efficiency in the complexation of the N-acetyl amino acids (AcTrp $\gg$ AcPhe $>$ AcLeu $>$ AcAla) follows exactly the same order as the efficiency in the complexation of the amino acid methyl esters (Trp $>$ Phe $>$ Leu $>$ Ala). Because in the positively charged complexes, most likely, the protonated residue is the amino group of the amino acid, while in the negatively charged complexes the highest acidity is displayed by the carboxylic

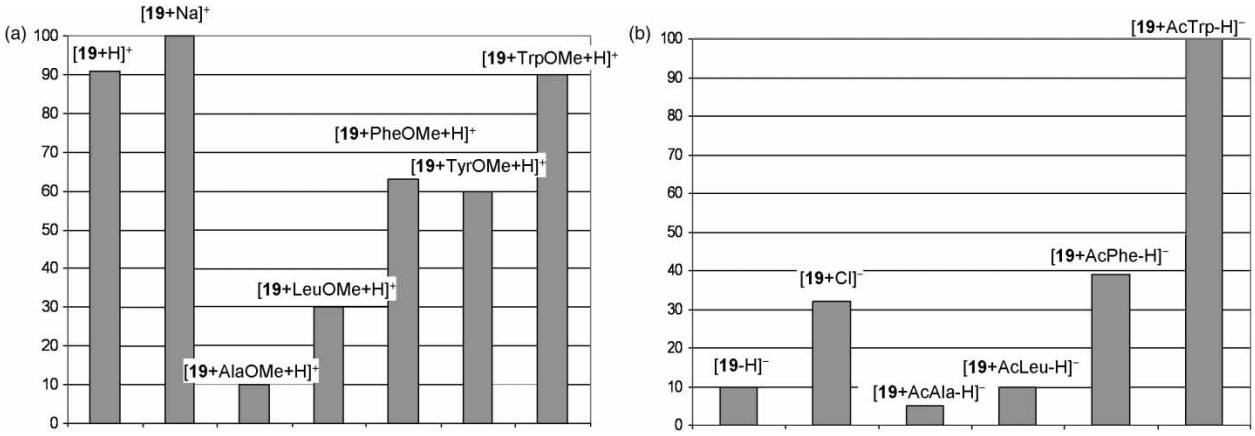



Figure 3. Competitive complexation experiment of (a) α-amino acid methyl ester hydrochlorides and (b) N-acetyl α-amino acids in the presence of **19**.



acid group of the guest, we conclude that the simultaneous presence of *C*-linked and *N*-linked amino acids gives to this class of receptors the ability to act as bifunctional receptors, complexing both carboxylate anions and ammonium cations. The amino acid side chains provide the additional interactions that account for the observed selectivity.

The affinity of **19** for tryptophan was confirmed in solution by $^1$H NMR titration experiments with the tetrabutylammonium salt of N-acetyl tryptophan. Upon addition of a concentrated solution of the guest to a 0.002 M solution of the host in $CDCl_3$, complexation-induced shifts were observed for all the resonances of Trp and for several signals of the host. Yet, these shifts are very difficult to analyse because of the complexity of the spectrum of **19** in this solvent and due to the superposition of the signals of the guest to those of the host. By fitting the few signals that could be unequivocally identified to a 1:1 binding model, a stability constant ($K_a$) in the order of $200-500\,M^{-1}$ was obtained. In DMSO-$d_6$, the signals appeared sharp and easier to follow during the titration. As expected, however, due to the polarity of the solvent that reduces the strength of hydrogen-bonding interactions, the measured binding constant resulted much lower ($K_a = 40\,M^{-1}$).

## Conclusions

The synthesis of the new 1,2-*proximal N*,*N*,*C*,*C*-linked and 1,3-*distal N*,*C*,*N*,*C*-linked tetra-peptidocalix[4]arenes completes the family of upper rim peptidocalixarenes. The 1,3-*distal* derivatives **8** and **9**, despite the possible self-complementarity in terms of hydrogen-bonding groups, do not show any tendency to self-assemble, as was indeed observed for some *N*,*C*-linked peptidocalixarenes. The flexibility of the tetrapropoxycalixarene scaffold is the determining factor for the conformational properties of these derivatives, allowing the amino acid chains to move closer and form an extensive network of intramolecular hydrogen bonds that disfavours the formation of intermolecular interactions. ESI-MS preliminary studies indicate that the 1,2-*proximal* peptidocalix[4]arene **19** is a more efficient receptor than its 1,3-*distal* isomer **8** in the gas phase and that it is able to recognise aromatic amino acids both as methyl ester hydrochlorides and as N-acetyl derivatives. The presence of two *N*-linked and two *C*-linked amino acids in 1,2-*proximal* positions, therefore, imparts to the *N*,*N*,*C*,*C*-linked peptidocalix[4]arene **19** the complexation properties of both the *N*-linked and the *C*-linked derivatives, i.e. the ability to recognise both ammonium groups and carboxylate anions, respectively. Accordingly, **19** can be considered as a bifunctional receptor.

In conclusion, from these results, we believe that the receptors of this class have the potential to give interesting results in the field of enantioselective recognition of organic substrates (*24*, *25*) and as multivalent ligands in the inhibition of certain protein functions (*26*, *27*). Moreover, the two novel calix[4]arene bis-amino acids (**4** and **15**) herein reported provide interesting platforms for the synthesis of *de novo* template-assembled synthetic proteins (*28*), allowing the linkage on the same scaffold of peptides both through their N- and C-terminus (anti-parallel caviteins) (*29*).

## Experimental procedures

All moisture-sensitive reactions were carried out under nitrogen atmosphere. All dry solvents were prepared according to the standard procedures and stored over molecular sieves. Melting points were determined on an electrothermal apparatus in capillaries sealed under nitrogen. $^1$H and $^{13}$C NMR spectra (300 and 75 MHz, respectively) were recorded on Bruker AV300 and AC300 spectrometers (partially deuterated solvents were used as internal standards). Mass spectra were recorded in ESI

mode on a single quadrupole Micromass ZMD instrument (capillary voltage = 3 kV, cone voltage = 30–160 V, extractor voltage = 3 V, source block temperature = 80° C, desolvation temperature = 150°C, cone and desolvation gas ($N_2$) flow rates = 1.6 and 8 l/min, respectively). High-resolution mass spectra were recorded in ESI mode on the LTQ ORBITRAP XL Thermo instrument. Thin-layer chromatography (TLC) was performed on silica gel Merck (Darmstadt, Germany) 60 F254 and flash chromatography using 32–63 mm on 60 Å Merck silica gel. 5,17-Dinitro-25,26,27,28-tetrapropoxycalix[4]arene (**1**) and 5,11-dinitro-25,26,27,28-tetrapropoxycalix[4]arene (**12**) were synthesised according to the literature procedures (*18*). Other reagents were purchased from Sigma-Aldrich (St Louis, MO, USA).

### *5,17-Dicarbaldehyde-11,23-dinitro-25,26,27,28-tetrapropoxycalix[4]arene (2)*

A TFA solution (40 ml) of 5,17-dinitro-25,26,27,28-tetrapropoxycalix[4]arene (**1**) (0.50 g, 0.73 mmol) and hexamethylenetetramine (1.64 g, 11.71 mmol) was refluxed for 4 h. After TLC control (hexane/ethyl acetate, 5:2 (v/v)), the reaction was quenched by the addition of water and the mixture was vigorously stirred for 30 min. The aqueous phase was then extracted with $CH_2Cl_2$, the organic phase was washed three times with water and evaporated to dryness to obtain the product as a light grey solid that did not require any further purification. Yield 0.51 g, 0.69 mmol, 95%. All the spectroscopic data resulted in agreement with those previously reported (*30*).

### *5,17-Dicarboxylic acid-11,23-dinitro-25,26,27,28-tetrapropoxycalix[4]arene (3)*

The product was synthesised according to the literature procedure (*30*).

### *5,17-Diamino-11,23-dicarboxylic acid-25,26,27,28-tetrapropoxycalix[4]arene (4)*

Hydrazine hydrate (1.1 ml, 18.8 mmol) and a catalytic amount of Pd/C (10%) were added to a suspension of **3** (358 mg, 0.46 mmol) in ethanol (35 ml). The mixture was refluxed for 6 h, and then the catalyst was filtered off. The organic solvent was evaporated under reduced pressure to obtain the product as a white solid, to be used without further purification. Yield 311 mg, 0.44 mmol, 95%. Mp >200°C (decomp.). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 7.46 (s, 4H, Ar*H*-COOH), 5.76 (s, 4H, Ar*H*-$NH_2$), 4.27 (d, 4H, $J$ = 12.7 Hz, $H_{ax}$ of $ArCH_2Ar$), 3.91 (t, 4H, $J$ = 7.6 Hz, $OCH_2CH_2CH_3$), 3.61 (t, 4H, $J$ = 7.2 Hz, $OCH_2CH_2CH_3$), 3.05 (d, 4H, $J$ = 12.7 Hz, $H_{eq}$ of $ArCH_2Ar$), 1.95–1.78 (m, 8H, $OCH_2CH_2CH_3$), 0.98 (t, 6H, $J$ = 7.3 Hz, $OCH_2CH_2CH_3$), 0.91 (t, 6H, $J$ = 7.5 Hz, $OCH_2CH_2CH_3$). $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ = 167.5 (COOH), 161.8 and 151.4 (Ar *ipso*), 136.3, 134.1, 130.4, 124.2 and 118.4 (Ar), 76.6 and 76.3 ($OCH_2CH_2CH_3$), 30.3 ($ArCH_2Ar$), 23 and 22.8 ($OCH_2CH_2CH_3$), 10.7 and 9.8 ($OCH_2CH_2CH_3$). ESI-MS: $m/z$ 733.4 [100%, $(M + Na)^+$].

### *5,17-Bis(Boc-amino)-11,23-dicarboxylic acid-25,26,27,28-tetrapropoxycalix[4]arene (5)*

A solution of NaOH (10 mg, 0.24 mmol) in $H_2O$ (2 ml) and di-*tert*-butyl dicarbonate (62 mg, 0.28 mmol) was added to a solution of **4** (84 mg, 0.12 mmol) in a mixture of freshly distilled THF (10 ml). The mixture was stirred for 20 h at room temperature. The organic solvent was evaporated under reduced pressure and the residue was taken up in $CH_2Cl_2$ (10 ml). The organic layer was separated, the aqueous phase was acidified until pH 5–6 with 1 M HCl and extracted with $CH_2Cl_2$ (4 × 10 ml). The combined organic layers were washed with $H_2O$ (20 ml), and evaporated to dryness to obtain the product as a white solid to be used without further purification. Yield 87 mg, 0.096 mmol, 80%. Mp > 200°C (decomp.). $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.17 (s, 4H, Ar*H*-COOH), 6.79 (s, 4H, Ar*H*-NHBoc), 6.52 (s, 2H, N*H*-Boc), 4.37 (d, 4H, $J$ = 13.5 Hz, $H_{ax}$ of $ArCH_2Ar$), 3.92 (t, 4H, $J$ = 8.1 Hz, $OCH_2CH_2CH_3$), 3.63 (t, 4H, $J$ = 6.6 Hz, $OCH_2CH_2CH_3$), 3.1 (t, 4H, $J$ = 13.8 Hz, $H_{eq}$ of $ArCH_2Ar$), 1.89–1.79 (m, 8H, $OCH_2CH_2CH_3$), 1.59 (s, 18H, $C(CH_3)_3$), 1.08 (t, 6H, $J$ = 7.3 Hz, $OCH_2CH_2CH_3$), 0.82 (t, 6H, $J$ = 7.3 Hz, $OCH_2CH_2CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 172.1 (COOH), 159.8 and 153.1 (Ar *ipso*), 153.4 (CO-Boc), 136.8, 133.5, 132.3, 129.6, 123.2 and 119.9 (Ar), 76.6 ($C(CH_3)_3$), 76.8 and 76.4 ($OCH_2CH_2CH_3$), 31.0 ($ArCH_2Ar$), 28.5 ($C(CH_3)_3$), 23.4 and 22.7 ($OCH_2CH_2CH_3$), 10.7 and 9.7 ($OCH_2CH_2CH_3$). ESI-MS: $m/z$ 933.6 [100%, $(M + Na)^+$].

### *5,17-Bis(Boc-amino)-11,23-bis[(methoxy-L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (6)*

Triethylamine (0.075 ml, 0.54 mmol), L-alanine methyl ester hydrochloride (33 mg, 0.23 mmol) and HBTU (117 mg, 0.31 mmol) were added to a solution of **5** (70 mg, 0.08 mmol) in dry $CH_2Cl_2$ (10 ml). The mixture was stirred for 6 h at room temperature, then $H_2O$ (10 ml) was added and the mixture was vigorously stirred for 0.5 h. The organic layer was separated, washed with $H_2O$ (2 × 5 ml) and evaporated to dryness. The crude was purified by flash column chromatography (eluent: hexane–ethyl acetate, 3:2 (v/v)). Yield 73 mg, 0.07 mmol, 85%. Mp 204–206°C. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.43 (d, 2H, $J$ = 2 Hz, Ar*H*-COAla), 7.37 (d, 2H, $J$ = 2 Hz, Ar*H*-COAla), 6.61 (d, 2H, $J$ = 7.1 Hz, AlaNH), 6.31 (d, 2H,

$J = 2.5$ Hz, Ar*H*-NHBoc), 6.20 (d, 2H, $J = 2.4$ Hz, Ar*H*-NHBoc), 5.98 (s, 2H, NH-Boc), 4.72 (quin, 2H, $J = 7.1$ Hz, AlaCH), 4.41 (d, 4H, $J = 13.4$ Hz, $H_{ax}$ of $ArCH_2Ar$), 3.97 (t, 6H, $J = 7.4$ Hz, $OCH_2CH_2CH_3$), 3.78 (s, 6H, $AlaOCH_3$), 3.69 (t, 6H, $J = 7.0$ Hz, $OCH_2CH_2CH_3$), 3.19 (d, 4H, $J = 13.5$ Hz, $H_{eq}$ of $ArCH_2Ar$), 1.93–1.80 (m, 8H, $OCH_2CH_2CH_3$), 1.5 (d, 6H, $J = 7.1$ Hz, $AlaCH_3$), 1.44 (s, 18H, $C(CH_3)_3$), 1.03 (t, 6H, $J = 7.4$ Hz, $OCH_2CH_2CH_3$), 0.91 (t, 6H, $J = 7.5$ Hz, $OCH_2CH_2CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 173.7$ (Ala-*C*$OOCH_3$), 167.2 (Ar-CO-Ala), 160.5 (Ar-CO-Ala *ipso*), 153.6 (Ar-NHBoc *ipso*), 152.3 (CO-Boc), 136.2, 133.8, 132.2, 127.9, 127.7, 127.4, 120.8 (Ar), 79.9 ($C(CH_3)_3$), 76.8 ($OCH_2CH_2CH_3$), 52.4 (AlaCH), 48.6 ($AlaOCH_3$), 31.0 ($ArCH_2Ar$), 28.4 ($C(CH_3)_3$), 23.3 and 23.1 ($OCH_2CH_2CH_3$), 18.4 ($AlaCH_3$), 10.6 and 10.0 ($OCH_2CH_2CH_3$). ESI-MS: *m/z* 1103.5 [100%, $(M + Na)^+$].

### *5,17-Diamino-11,23-bis[(methoxy-L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (7)*

A sample of **6** (70 mg, 0.065 mmol) was dissolved in a $CH_2Cl_2$–TFA–triethylsilane–$H_2O$ (5 ml, 47.5:47.5:2.5:2.5, v/v/v/v) solution and the mixture was stirred for 2 h at room temperature. The solvents were evaporated under reduced pressure and the residue was dissolved in $CH_2Cl_2$ (5 ml). The organic phase was washed with 5% $NaHCO_3$ (5 ml) and $H_2O$ (5 ml) and evaporated to dryness. The product was obtained as a white solid and used without further purification. Yield 49 mg, 0.056 mmol, 86%. Mp >180°C (decomp.). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.50$ (s, 2H, Ar*H*-COAla), 6.76 (d, 2H, $J = 6.9$ Hz, Ar-CO-NH-Ala), 5.58 (s, 4H, Ar*H*-$NH_2$), 4.80 (quin, 2H, $J = 7.1$ Hz, AlaCH), 4.4 (d, 4H, $J = 13.4$, $H_{ax}$ of $ArCH_2Ar$), 4.02 (t, 4H, $J = 7.9$ Hz, $OCH_2CH_2CH_3$), 3.80 (s, 6H, $OCH_3$), 3.63 (t, 4H, $J = 6.8$ Hz, $OCH_2CH_2CH_3$), 3.12 (d, 4H, $J = 13.5$ Hz, $H_{eq}$ of $ArCH_2Ar$), 1.95–1.78 (m, 8H, $OCH_2CH_2CH_3$), 1.55 (d, 6H, $J = 7.1$ Hz, $AlaCH_3$), 1.06 (t, 6H, $J = 7.4$ Hz, $OCH_2CH_2CH_3$), 0.87 (t, 6H, $J = 7.4$ Hz, $OCH_2CH_2CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 175.1$ (Ala-*C*$OOCH_3$), 170.1 (Ar-CO-Ala), 162.8 (Ar-CO-Ala *ipso*), 150.6 (Ar-NHBoc *ipso*), 141.8, 138.3, 134.5, 129.5, 128.1 and 117.6 (Ar), 78.3 and 78.0 ($OCH_2CH_2CH_3$), 52.8 (AlaCH), 50.2 ($AlaOCH_3$), 32.2 ($ArCH_2Ar$), 24.7 and 24.4 ($OCH_2CH_2CH_3$), 17.2 ($AlaCH_3$), 11.5 and 10.4 ($OCH_2CH_2CH_3$). ESI-MS: *m/z* 903.6 [100%, $(M + Na)^+$].

### *5,17-Bis[(N-Cbz-L-alanyl)amino]-11,23-bis[(methoxy-L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (8)*

Triethylamine (0.021 ml, 0.15 mmol), N-Cbz-L-alanine (20 mg, 0.09 mmol) and HBTU (46 mg, 0.121 mmol) were added to a solution of **7** (26 mg, 0.03 mmol) in dry $CH_2Cl_2$ (10 ml). The mixture was stirred for 3 h at room temperature, then $H_2O$ (10 ml) was added and the mixture was vigorously stirred for 0.5 h. The organic layer was separated, washed with $H_2O$ (2 × 5 ml) and evaporated to dryness. The crude was purified by flash column chromatography (eluent: hexane–ethyl acetate, 2:3 (v/v)). Yield 15 mg, 0.01 mmol, 40%. Mp 140–143°C. $^1$H NMR (300 MHz, $CDCl_3$, $10^{-2}$ M): $\delta = 8.06$ (s, 2H, Ar-NH-CbzAla), 7.53 (d, 2H, Ar*H*-$COAlaOCH_3$), 7.47 (d, 2H, Ar*H*-$COAlaOCH_3$), 7.29 (bs, 10H, ArCbz), 6.86 (bs, 2H, N*H*-$AlaOCH_3$), 6.61 (d, 2H, Ar*H*-NH-CbzAla), 6.05 (d, 2H, Ar*H*-NH-CbzAla), 5.79 (bs, 2H, NH-CbzAla), 5.14 (d, 2H, $J = 12$ Hz, $OCH_2Ph$), 4.91 (d, 2H, $J = 12$ Hz, $OCH_2Ph$), 4.74–4.72 (m, 2H, C*H*$AlaOCH_3$), 4.43 (d, 4H, $J = 12$ Hz, $H_{ax}$ of $ArCH_2Ar$), 4.05–4.01 (m, 6H, C*H*AlaCbz and $OCH_2CH_2CH_3$), 3.75 (s, 6H, $OCH_3$), 3.70–3.65 (m, 4H, $OCH_2CH_2CH_3$), 3.26–3.17 (m, 4H, $H_{eq}$ of $ArCH_2Ar$), 1.95–1.84 (m, 8H, $OCH_2CH_2CH_3$), 1.51 (d, 6H, $J = 6$ Hz, $CH_3AlaOCH_3$), 1.26 (d, 6H, $J = 9$ Hz, $CH_3AlaCbz$), 1.07 (t, 6H, $J = 6$ Hz, $OCH_2CH_2CH_3$), 0.90 (t, 6H, $J = 6$ Hz, $OCH_2CH_2CH_3$). $^1$H NMR (300 MHz, $CD_3OD$): $\delta = 7.60$ (s, 4H, Ar*H*-$COAlaOCH_3$), 7.30 (bs, 10H, ArCbz), 6.74 (bs, 2H, Ar*H*-NH-CbzAla), 6.39 (s, 2H, Ar*H*-NH-CbzAla), 5.08 (d, 2H, $J = 12.4$ Hz, $OCH_2Ph$), 4.96 (d, 2H, $J = 12.4$ Hz, $OCH_2Ph$), 4.57 (m, 2H, C*H*$AlaOCH_3$), 4.52 (d, 4H, $J = 13.2$ Hz, $H_{ax}$ of $ArCH_2Ar$), 4.15–4.02 (m, 6H, C*H*CbzAla and $OCH_2CH_2CH_3$), 3.77–3.72 (m, 4H, $OCH_2CH_2CH_3$), 3.72 (s, 6H, $OCH_3$), 3.31–3.24 (m, 4H, $H_{eq}$ of $ArCH_2Ar$), 2.05–1.9 (m, 8H, $OCH_2CH_2CH_3$), 1.47 (d, 6H, $J = 7.3$ Hz, $CH_3AlaOCH_3$), 1.22 (d, 6H, $J = 7$ Hz, $CH_3AlaCbz$), 1.10 (t, 6H, $J = 7.5$ Hz, $OCH_2CH_2CH_3$), 0.96 (t, 6H, $J = 7.4$ Hz, $OCH_2CH_2CH_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 175.1$ (Ala-*C*O), 173.4 (Ala-CO), 169.8 (Ar-*C*O), 162.0 (Ar-CO-$AlaOCH_3$ *ipso*), 158.3 (NH-*C*$OOCH_2Ph$), 154.0 (ArC-NH-CbzAla *ipso*), 137.5, 134.7, 133.3, 129.5, 129.04, 122.7 and 121.9 (Ar), 78.6 and 78.1 ($OCH_2CH_2CH_3$), 67.9 ($OCH_2Ph$), 52.8 ($CHAlaOCH_3$), 52.4 (*C*HCbzAla), 50.2 ($AlaOCH_3$), 32.2 ($ArCH_2Ar$), 24.6 and 24.4 ($OCH_2CH_2CH_3$), 18.5 ($CH_3AlaOCH_3$), 17.3 ($CH_3CbzAla$), 11.2 and 10.4 ($OCH_2CH_2CH_3$). ESI-MS: *m/z* 668.7 [100%, $(M + 2Na)^{2+}$], 1314.0 [95%, $(M + Na)^+$]. HR-ESI-MS *m/z*: calcd for $C_{72}H_{86}N_6O_{16}$ $[M + Na]^+$1313.5998, found 1313.6055.

### *5,17-Bis[(N-Boc-L-alanyl)amino]-11,23-bis[(methoxy-L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (9)*

The product was prepared as described for compound **8** starting from calix[4]arene **7** (43 mg, 0.042 mmol) and *N*-Boc-L-alanine (24 mg, 0.126 mmol). The crude was purified by flash column chromatography (eluent: hexane–ethyl acetate, 7:3 (v/v)). Yield 31 mg, 0.025 mmol, 60%.

Mp 152–155°C. $^1$H NMR (300 MHz, $CDCl_3$, $c = 10^{-2}$ M): $\delta = 8.53$ (bs, 2H, Ar-NH), 7.50 (bd, 2H, Ar*H*-COAlaOCH$_3$), 7.45 (bd, 2H, Ar*H*-COAlaOCH$_3$), 7.01 (bs, 2H, N*H*-AlaOCH$_3$), 6.85 (bd, 2H, Ar*H*-NH-AlaBoc), 5.84 (bd, 2H, Ar*H*-NH-AlaBoc), 5.26 (s, 2H, AlaNHBoc), 4.79 (m, 2H, C*H*AlaOCH$_3$), 4.41 (d, 4H, $J = 13.6$ Hz, $H_{ax}$ of ArCH$_2$Ar), 4.12–4.02 (m, 6H, C*H*AlaBoc and OC*H*$_2$-CH$_2$CH$_3$), 3.81 (s, 6H, OCH$_3$), 3.65–3.62 (m, 4H, OC*H*$_2$CH$_2$CH$_3$), 3.25 (d, 2H, $J = 13.6$ Hz, $H_{eq}$ of ArCH$_2$-Ar), 3.14 (d, 2H, $J = 13.6$, $H_{eq}$ of ArCH$_2$Ar), 1.98–1.62 (m, 8H, OCH$_2$C*H*$_2$CH$_3$), 1.54 (d, 6H, $J = 7$ Hz, C*H*$_3$-AlaOCH$_3$), 1.39 (s, 18H, C(CH$_3$)$_3$), 1.21 (d, 6H, $J = 6.9$ Hz, C*H*$_3$AlaBoc), 1.06 (t, 6H, $J = 7.4$ Hz, OCH$_2$-CH$_2$*CH*$_3$), 0.86 (t, 6H, $J = 7.4$ Hz, OCH$_2$CH$_2$*CH*$_3$). $^1$H NMR (300 MHz, $CD_3OD$): $\delta = 7.62$ (bs, 2H, Ar*H*-COAlaOCH$_3$), 7.59 (bs, 2H, Ar*H*-COAlaOCH$_3$), 6.69 (bs, 2H, Ar*H*-COAlaBoc), 6.28 (bs, 2H, Ar*H*-COAlaBoc), 4.60–4.55 (m, 2H, C*H*AlaOCH$_3$), 4.52 (d, 4H, $J = 13.3$ Hz, $H_{ax}$ of ArCH$_2$Ar), 4.12 (t, 4H, $J = 7.8$ Hz, OC*H*$_2$CH$_2$CH$_3$), 4.00 (m, 2H, C*H*AlaBoc), 3.74 (bs, 10H, OC*H*$_2$CH$_2$CH$_3$ and OCH$_3$), 3.35–3.18 (m, 4H, $H_{eq}$ of ArCH$_2$Ar), 2.05–1.87 (m, 8H, OCH$_2$C*H*$_2$CH$_3$), 1.50 (d, 6H, $J = 7.3$ Hz, C*H*$_3$AlaOCH$_3$), 1.42 (s, 18H, C(CH$_3$)$_3$), 1.20 (d, 6H, $J = 7.2$ Hz, C*H*$_3$AlaBoc), 1.09 (t, 6H, $J = 7.4$ Hz, OCH$_2$CH$_2$*CH*$_3$), 0.94 (t, 6H, $J = 7.1$ Hz, OCH$_2$CH$_2$*CH*$_3$). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 173.8$ (Ala-*C*O), 171.4 (Ala-CO), 167.3 (Ar-*C*O), 160.7 (Ar*C*-CO-AlaOCH$_3$ *ipso*), 156.2 (*C*O-Boc), 152.3 (ArC-NH-AlaBoc *ipso*), 136.7, 136.4, 133.2, 132.8, 131.8, 127.9, 127.7, 121.8 and 118.8 (Ar), 80.1 (*C*(CH$_3$)$_3$), 52.4 (*C*HAla), 50.3 (*C*HAla), 48.6 (AlaOCH$_3$), 31.2 and 30.9 (Ar*C*H$_2$Ar), 28.1 (C(*C*H$_3$)$_3$), 23.3 and 22.8 (OCH$_2$-*C*H$_2$CH$_3$), 18.7 (*C*H$_3$AlaOCH$_3$), 17.6 (*C*H$_3$AlaBoc), 10.7 and 9.8 (OCH$_2$CH$_2$*C*H$_3$). ESI-MS: *m/z* 634.7 [100%, $(M + 2Na)^{2+}$], 642.6 [<20%, $(M + Na + K)^{2+}$], 1246.0 [<50%, $(M + Na)^+$], 1261.8 [<10%, $(M + K)^+$]. HR-ESI-MS *m/z*: calcd for $C_{66}H_{90}N_6O_{16}$ $[M + Na]^+$ 1245.6311, found 1245.6331.

### *5,17-Bis[(N-Boc-L-alanyl)amino]-11,23-bis[(L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (10)*

A solution of LiOH·H$_2$O (3.5 mg, 0.084 mmol) in H$_2$O (1 ml) was added to a solution of **9** (25.8 mg, 0.021 mmol) in distilled THF (5 ml) and the mixture was stirred at room temperature for 4 h. The organic solvent was evaporated, then H$_2$O (5 ml), a few drops of 1 M HCl (until pH 4–5) and CH$_2$Cl$_2$ (5 ml) were added and the mixture was vigorously stirred for 0.5 h. The organic layer was separated, the aqueous phase was extracted with CH$_2$Cl$_2$ (2 × 5 ml) and the combined organic layers were evaporated to dryness to obtain a white solid that was used without further purification. Yield 21 mg, 0.018 mmol, 85%. Mp >200°C (decomp.). $^1$H NMR (300 MHz, $CD_3OD$): $\delta = 7.65$ (s, 2H, Ar*H*-COAla), 7.62 (s, 2H, Ar*H*-COAla), 6.64 (s, 2H, Ar*H*-NHAla), 6.28 (s, 2H, Ar*H*-NHAla), 4.57 (q, 2H, $J = 8.7$ Hz, C*H*AlaOH), 4.52 (d, 4H, $J = 13.5$ Hz, $H_{ax}$ of ArCH$_2$Ar), 4.13 (t, 4H, $J = 8.1$ Hz, OC*H*$_2$CH$_2$CH$_3$), 3.98 (q, 2H, $J = 6.6$ Hz, C*H*AlaBoc), 3.73 (t, 4H, $J = 5.4$ Hz, OC*H*$_2$CH$_2$CH$_3$), 3.25 (d, 4H, $J = 13.5$ Hz, $H_{eq}$ of ArCH$_2$-Ar), 2.04–1.89 (m, 8H, OCH$_2$C*H*$_2$CH$_3$), 1.52 (d, 6H, $J = 6.6$ Hz, AlaCH$_3$), 1.42 (s, 18H, C*H*$_3$Boc), 1.19 (d, 6H, $J = 6.6$ Hz, AlaCH$_3$), 1.11 (t, 6H, $J = 6.9$ Hz, OCH$_2$CH$_2$-C*H*$_3$), 0.93 (t, 6H, $J = 6.7$ Hz, OCH$_2$CH$_2$C*H*$_3$). ESI-MS(+): *m/z* 1239.6 [10%, $(M + 2Na - H)^+$], 1261.6 [100%, $(M + 3Na - 2H)^+$] ESI-MS(−): *m/z* 1193.5 [20%, $(M - H)^-$], 1215.7 [50%, $(M + Na - 2H)^-$].

### *5,17-Bis[(L-alanyl)amino]-11,23-bis[(L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (11)*

TFA (0.6 ml, 8.1 mmol) was added to a solution of **10** (21 mg, 0.018 mmol) in dry CH$_2$Cl$_2$ (5 ml) and the mixture was stirred for 4 h at room temperature and evaporated to dryness to obtain a white solid that was used without further purification. Yield 17 mg, 0.018 mmol, >95%. Mp >250 °C (decomp.). $^1$H NMR (300 MHz, $CD_3OD$): $\delta = 7.48$ (d, 2H, $J = 2.2$ Hz, Ar*H*-COAla), 7.45 (d, 2H, $J = 2.2$ Hz, Ar*H*-COAla), 6.93 (d, 2H, $J = 2.4$ Hz, Ar*H*-NHAla), 6.78 (d, 2H, $J = 2.4$ Hz, Ar*H*-NHAla), 4.53 (d, 4H, $J = 12.6$ Hz, $H_{ax}$ of ArCH$_2$Ar), 4.52 (q, 2H, $J = 7.5$ Hz, C*H*AlaOH), 4.05 (t, 4H, $J = 7.3$ Hz, OC*H*$_2$CH$_2$CH$_3$), 3.87 (q, 2H, $J = 6.9$ Hz, CHAla), 3.82 (t, 4H, $J = 7.2$ Hz, OC*H*$_2$CH$_2$-CH$_3$), 3.27 (d, 4H, $J = 12.6$ Hz, $H_{eq}$ of ArCH$_2$Ar), 2.08–1.93 (m, 8H, OCH$_2$C*H*$_2$CH$_3$), 1.49 (d, 6H, $J = 7.5$ Hz, AlaCH$_3$), 1.47 (d, 6H, $J = 6.9$ Hz, AlaCH$_3$), 1.07 (t, 6H, $J = 7.5$ Hz, OCH$_2$CH$_2$C*H*$_3$), 1.01 (t, 6H, $J = 7.5$ Hz, OCH$_2$CH$_2$C*H*$_3$). $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta = 176.5$ (COOH), 169.6 and 168.7 (Ala-CO), 161.2 and 154.4 (Ar *ipso*), 136.8, 136.7, 135.6, 135.5, 132.9, 129.4, 129.1, 129.0 and 122.1 (Ar), 78.6 and 78.2 (OCH$_2$*C*H$_2$CH$_3$), 50.7 and 49.9 (CHAla), 32.1 and 32.0 (Ar*C*H$_2$Ar), 24.5 and 24.4 (OCH$_2$*C*H$_2$CH$_3$), 17.6 and 17.5 (AlaCH$_3$), 10.9 and 10.5 (OCH$_2$CH$_2$*C*H$_3$). ESI-MS(+): *m/z* 520.4 [100%, $(M + 2Na)^{2+}$], 995.5 [10%, $(M + H)^+$], 1017.6 [60%, $(M + Na)^+$], 1039.5 [50%, $(M + 2Na - H)^+$], 1061.6 [10%, $(M + 3Na - 2H)^+$]. ESI-MS(−): *m/z* 993.7 [90%, $(M - H)^-$], 1015.5 [20%, $(M + Na - 2H)^-$].

### *5,11-Dicarbaldehyde-17,23-dinitro-25,26,27,28-tetrapropoxycalix[4]arene (13)*

The product was prepared as described for compound **2** starting from 5,11-dinitro-25,26,27,28-tetrapropoxycalix[4]arene (**12**) (0.50 g, 0.73 mmol). The crude was purified by flash column chromatography (hexane–ethyl acetate, 3:1 (v/v)). Yield 0.44 g, 0.60 mmol, 71%. $^1$H NMR (300 MHz,

$CDCl_3$): $\delta = 9.34$ (s, 2H, CHO), 7.53 and 7.50 (2d, 2H each, $J = 2.7$ Hz, Ar*H*), 7.20 and 7.18 (2d, 2H each, $J = 2.7$ Hz, Ar*H*), 4.51 and 4.50 (2d, 2H each, $J = 13.9$ Hz, $H_{ax}$ of $ArCH_2Ar$), 3.98–3.88 (m, 8H, $OCH_2CH_2CH_3$), 3.69–3.62 (m, 4H, $OCH_2CH_2CH_3$), 3.36 (d, 4H, $J = 13.9$ Hz, $H_{eq}$ of $ArCH_2Ar$), 1.99–1.84 (m, 8H, $OCH_2CH_2CH_3$), 1.01 and 1.00 (2t, 6H each, $J = 6.5$ Hz, $OCH_2CH_2CH_3$). ESI-MS(+): *m/z* 761.1 [100%, $(M + Na)^+$].

### *5,11-Dicarboxylic acid-17,23-dinitro-25,26,27,28-tetrapropoxycalix[4]arene (14)*

A solution of **13** (440 mg, 0.60 mmol) in $CHCl_3$–acetone (40 ml, 1:1, v/v) was cooled to 0°C and treated with an aqueous solution (2 ml) of $H_2NSO_3H$ (462 mg, 4.76 mmol) and $NaClO_2$ (380 mg, 4.20 mmol). The mixture was vigorously stirred at room temperature for 18 h and the solvent was evaporated to dryness at reduced pressure. Then, 1 M HCl (20 ml) was added to give **14** as a pale yellow solid, which was crystallised from ethyl acetate–hexane. Yield 430 mg, 0.56 mmol, 93%. $^1$H NMR (300 MHz, $CDCl_3/CD_3OD = 9/1$): $\delta = 7.47$ and 7.26 (2d, 4H each, $J = 5.4$ Hz, Ar*H*), 4.45, 4.40 and 4.36 (3d, 1H, 2H, 1H, $J = 12.8$ Hz, $H_{ax}$ of $ArCH_2Ar$), 3.90–3.75 (m, 8H, $OCH_2CH_2CH_3$), 3.23–3.28 (m, 4H, $H_{eq}$ of $ArCH_2Ar$), 1.97–1.72 (m, 8H, $OCH_2CH_2CH_3$), 1.09 and 1.07 (2t, 6H each, $J = 6.5$ Hz, $OCH_2CH_2CH_3$). ESI-MS(+): *m/z* 793.5 [100%, $(M + Na)^+$], 1563.7 [25%, $(2M + Na)^+$].

### *5,11-Diamino-17,23-dicarboxylic acid-25,26,27,28-tetrapropoxycalix[4]arene (15)*

The product was prepared as described for compound **4** starting from calix[4]arene **14** (260 mg, 0.34 mmol). Yield 230 mg, 0.32 mmol, 95%. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 7.36$ and 7.34 (2d, 2H each, $J = 2.1$ Hz, Ar*H*-CO), 5.93 and 5.89 (2d, 2H each, $J = 2.7$ Hz, Ar*H*-$NH_2$), 4.38, 4.27, 4.15 (3d, 1H, 2H, 1H, $J = 13.1$, 12.8, 12.8 Hz, $H_{ax}$ of $ArCH_2Ar$), 3.92–3.81 (m, 4H, $OCH_2CH_2CH_3$), 3.69–3.62 (m, 4H, $OCH_2CH_2CH_3$), 3.37, 3.07, 2.81 (3d, 1H, 2H, 1H, $J = 12.8$, 13.1, 12.8 Hz, $H_{eq}$ of $ArCH_2Ar$), 1.90–1.77 (m, 8H, $OCH_2CH_2CH_3$), 0.98–0.90 (m, 12H, $OCH_2CH_2CH_3$). ESI-MS(+): *m/z* 733.5 [100%, $(M + Na)^+$].

### *5,11-Bis(Boc-amino)-17,23-dicarboxylic acid-25,26,27,28-tetrapropoxycalix[4]arene (16)*

The product was prepared as described for compound **5** starting from calix[4]arene **15** (240 mg, 0.34 mmol). Yield 217 mg, 0.24 mmol, 70%. $^1$H NMR (300 MHz, $(CD_3)_2CO$): $\delta = 7.75$–7.35 (bs, 6H, Ar*H*-CO and COOH), 7.15 (d, 2H, $J = 8.7$ Hz, N*H*Boc), 7.0–6.7 (bs, 4H, Ar*H*-NHBoc), 4.55, 4.49, 4.44 (3d, 1H, 2H, 1H, $J = 13.6$, 13.4, 13.4 Hz, $H_{ax}$ of $ArCH_2Ar$), 3.98 and 3.85 (2bs, 4H each, $OCH_2CH_2CH_3$), 3.38, 3.23, 3.08 (3d, 1H, 2H, 1H, $J = 13.6$, 13.4, 13.4 Hz, $H_{eq}$ of $ArCH_2Ar$), 2.08–1.93 (m, 8H, $OCH_2CH_2CH_3$), 1.42 (s, 18H, $CH_3Boc$), 1.02–0.98 (m, 6H, $OCH_2CH_2CH_3$). ESI-MS(+): *m/z* 933.7 [100%, $(M + Na)^+$], 949.8 [20%, $(M + K)^+$].

### *5,11-Bis(Boc-amino)-17,23-bis[(methoxy-L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (17)*

$NEt(iPr)_2$ (96 µl, 0.55 mmol), PyBOP (172 mg, 0.33 mmol) and L-alanine methyl ester hydrochloride (46 mg, 0.33 mmol) were added to a solution of **16** (100 mg, 0.11 mmol) in dry DMF (15 ml). The mixture was stirred for 5 h at room temperature. Upon addition of $H_2O$ (10 ml), the product was obtained as a white precipitate which was collected by Büchner filtration, washed with $H_2O$ (2 × 2 ml) and used without further purification. Yield 99 mg, 0.09 mmol, 83%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.10$ (d, 1H, $J = 2.0$ Hz, Ar*H*-COAla), 7.08 (d, 2H, $J = 2.0$ Hz, Ar*H*-COAla), 6.56 (bs, 2H, Ar*H*-NHBoc), 6.45 (bs, 1H, Ar*H*-NHBoc), 6.43 (d, 2H, $J = 7.4$ Hz, N*H*-Ala$OCH_3$), 6.39 (bs, 1H, Ar*H*-NHBoc), 5.99 (bs, 2H, N*H*Boc), 4.74 (quin, 2H, $J = 5.7$ Hz, C*H*Ala$OCH_3$), 4.46, 4.39, 4.33 (3d, 1H, 2H, 1H, $J = 13.8$, 13.5, 13.5 Hz, $H_{ax}$ of $ArCH_2Ar$), 3.9–3.7 (m, 8H, $OCH_2CH_2CH_3$), 3.76 (s, 6H, Ala$OCH_3$), 3.22, 3.21, 3.17, 3.12 (4d, 1H each, $J = 13.5$ Hz, $H_{eq}$ of $ArCH_2Ar$), 1.85 (m, 8H, $OCH_2CH_2CH_3$), 1.47–1.44 (m, 24H, $CH_3Boc$ and Ala$CH_3$), 0.97–0.94 (m, 6H, $OCH_2CH_2CH_3$). ESI-MS(+): *m/z* 1104.1 [100%, $(M + Na)^+$], 2184.9 [5%, $(2M + Na)^+$].

### *5,11-Diamino-17,23-bis[(methoxy-L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (18)*

The product was prepared as described for compound **7** starting from calix[4]arene **17** (139 mg, 0.34 mmol). Yield 195 mg, 0.22 mmol, 65%. $^1$H NMR (300 MHz, $CDCl_3/CD_3OD = 9/1$): $\delta = 7.14$ (bs, 3H, Ar*H*-COAla), 7.07 (bs, 1H, Ar*H*-COAla), 6.62 (d, 2H, $J = 6.3$ Hz, N*H*-Ala$OCH_3$), 6.04, 6.01 and 5.96 (bs, 4H, Ar*H*-NHBoc), 4.65 (m, 2H, C*H*Ala$OCH_3$), 4.46, 4.39, 4.33 (3d, 1H, 2H, 1H, $J = 13.5$ Hz, $H_{ax}$ of $ArCH_2Ar$), 3.82–3.89 (m, 8H, $OCH_2CH_2CH_3$), 3.75 (s, 6H, Ala$OCH_3$), 3.23, 3.07, 2.90 (3d, 1H, 2H, 1H, $J = 13.5$ Hz, $H_{eq}$ of $ArCH_2Ar$), 1.92–1.78 (m, 8H, $OCH_2CH_2CH_3$), 1.56 (d, 6H, Ala$CH_3$), 0.91–0.96 (m, 6H, $OCH_2CH_2CH_3$). ESI-MS(+): *m/z* 881.7 [100%, $(M + H)^+$].

### *5,11-Bis[(N-Cbz-L-alanyl)amino]-17,23-bis[(methoxy-L-alanyl)carbonyl]-25,26,27,28-tetrapropoxycalix[4]arene (19)*

$NEt(iPr)_2$ (108 µl, 0.62 mmol), PyBOP (190 mg, 0.37 mmol) and N-Cbz-L-alanine (85 mg, 0.37 mmol)

were added to a solution of **18** (110 mg, 0.12 mmol) in dry DMF (15 ml). The mixture was stirred overnight at room temperature. Upon addition of $H_2O$ (10 ml), the product was obtained as a white precipitate which was collected by Büchner filtration, washed with $H_2O$ (2 × 2 ml) and purified by flash column chromatography (eluent: hexane–ethyl acetate, 1:2 (v/v)). Yield 71 mg, 0.05 mmol, 44%. Mp 137–140°C. $^1$H NMR (300 MHz, $CD_3OD$): $\delta = 7.37–7.28$ (m, 10H, ArCbz, Ar*H*-COAla), 6.89, 6.87, 6.78 and 6.74 (4bs, 1H each, Ar*H*-N*H*-Ala), 5.06–5.00 (m, 2H, $CH_2$-OPh), 4.57–4.43 (m, 6H, C*H*AlaOCH$_3$, $H_{ax}$ of ArCH$_2$Ar), 4.18 (q, 2H, $J = 7.5$ Hz, C*H*AlaCbz), 3.95 and 3.87 (2t, 4H each, $J = 7.0$ Hz, OC$H_2$CH$_2$CH$_3$), 3.65 (s, 6H, AlaOCH$_3$), 3.32, 3.24, 3.12 (3d, 1H, 2H, 1H, $J = 13.5$ Hz, $H_{eq}$ of ArCH$_2$Ar), 2.03–1.93 (m, 8H, OCH$_2$C$H_2$CH$_3$), 1.47 and 1.39 (d, 6H each, $J = 7.3$ Hz, AlaCH$_3$), 1.03 (t, 12H, $J = 7.4$ Hz, OCH$_2$-CH$_2$C$H_3$). $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta = 175.1$ (Ala-*C*O), 173.7 (Ala-*C*O), 170.3 (Ar-*C*O), 161.1 (Ar-CO-AlaOCH$_3$ *ipso*), 158.4 (NH-*C*OOCH$_2$Ph), 154.9 (ArC-NH-CbzAla *ipso*), 138.3, 136.5, 136.4, 136.2, 136.0, 135.9, 133.3, 129.6, 129.3, 129.1, 123.2 (Ar), 78.4 and 78.3 (O*C*H$_2$CH$_2$CH$_3$), 67.9 (O*C*H$_2$Ph), 52.9 (*C*HAlaOCH$_3$), 52.4 (*C*HCbzAla), 50.1 (AlaOCH$_3$), 32.2 (Ar*C*H$_2$Ar), 24.6 and 24.5 (OCH$_2$*C*H$_2$CH$_3$), 18.9 (*C*H$_3$AlaOCH$_3$), 17.3 (*C*H$_3$CbzAla), 10.9 and 10.8 (OCH$_2$CH$_2$*C*H$_3$). ESI-MS(+): *m/z* 1314.2 [100%, $(M + Na)^+$], 2604.8 [5%, $(2M + Na)^+$]. HR-ESI-MS *m/z*: calcd for $C_{72}H_{86}N_6O_{16}$ $[M + Na]^+$1313.5998, found 1313.5989.

### *ESI-MS complexation studies*

A methanol solution containing an equimolar amount of the receptor and the amino acid derivatives (AlaOMe·HCl and PheOMe·HCl, all $1 \times 10^{-5}$ M, for **8** and AlaOMe·HCl, PheOMe·HCl, LeuOMe·HCl, TyrOMe·HCl and TrpOMe·HCl or N-AcAla, N-AcLeu, N-AcPhe and N-AcTrp, all $5 \times 10^{-5}$ M, for **19**) was analysed in positive or negative ionisation mode by direct perfusion in ESI-MS interface; injection flow rate = 20 μl/min; desolvation temperature = 150°C; source block temperature = 80°C; cone and desolvation gas flow rates = 1.6 and 8 l/min, respectively; capillary = 3.6–3.9 kV; cone = 64 V in positive ionisation mode and −187 V in negative ionisation mode; extractor = 3 V.

### *Tetrabutylammonium salt of N-acetyl triptophane*

This guest was prepared by dissolving N-acetyl triptophane (145.7 mg, 0.591 mmol) in 1 equiv. of a freshly titrated solution of tetrabutylammonium hydroxide in 2-propanol/methanol. The mixture was stirred at room temperature for 7 h and evaporated to dryness under reduced pressure. The resulting deliquescent solid was dried at high vacuum for 24 h, examined by NMR and stored in a desiccator.

### *NMR titration experiments*

Titration experiments were performed in $CDCl_3$ or DMSO-$d_6$ by titrating a $2.0 \times 10^{-3}$ M solution of **19** with a $1.76 \times 10^{-2}$ M solution of the tetrabutylammonium salt of N-acetyl triptophane, varying the guest/host ratio from 0 to 16. To estimate the binding constants ($K_a$), nonlinear regression analysis was performed with the Gauss–Newton–Marquardt method, using the HypNMR2004 tools (*31*).

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