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Received 21 October 2009; accepted (revised) 3 June 2010

Novel double-armed peptido-conjugates of calix[4]arene have been developed on the lower rim of the macrocycle. The functional group pendants exhibit conformational bend through the involvement of 11-atom N-H…O hydrogen bond inscribed in a 14-atom O-H…O interaction. As a result, only the terminal -COOR and -COOH groups are exposed to the environment, but not the amide moiety. The cone-conformation of the calix[4]arene is further stabilized through the O-H…O interactions at the lower rim. In effect, the conjugates exhibit a binding core at the lower rim along with hydrophobic cavity formed by the enclosure of arene moieties. Conformational mobility induced by the replacement of lower rim phenolic–OH by –OCH₃ has also been demonstrated by variable temperature NMR studies in case of the corresponding –OCH₃ derivatives. Differential receptor binding characteristics of these conjugates towards phosphate are demonstrated using absorption spectroscopy. The negatively charged phosphate group is received preferentially by the carboxylic terminal over the ester terminal conjugate.

Keywords: Peptido-conjugates of calix[4]arene, HMQC, binding core, conformational mobility, phosphate binding

Calix[4]arene possesses ubiquitous properties of having both hydrophilic and hydrophobic compartments in the same molecule besides possessing an arene cavity, capable of trapping a variety of small species and hence are important in building model systems for biomimetic studies. Further, these molecules can be easily modified by derivatizing either at the hydrophilic lower rim or at the hydrophobic upper rim to result in the compounds of interest as receptors towards neutral as well as ionic species. Thus the resultant calix[4]arene-conjugates exhibit not only the characteristics of the individual partners present in it, but also several newer ones that requires the presence of both the units together. It is these properties that may provide novel characteristics to the resultant conjugates and thereby entice chemists to exploit such properties in order to answer several queries in the pertinent field. In this context it is intended to modify 1,3-positions of calix[4]arene to result in the conjugates of amino acids via amide linkage in order to explore their potential as receptors towards organic and inorganic guests and thereby act as bioorganic and bioinorganic models. In this regard, the present paper demonstrates the development of 1,3-disubstituted conjugates of Gly, Ala, Glu and Asp residues with calix[4]arene, and Gly, Ala and Glu residues with 1,3-di-OMe-calix[4]arene. The products have been characterized thoroughly, and the structure of the Gly-conjugate was established by single crystal X-ray diffraction. Further the conjugates were explored for their receptor properties towards organic phosphates.

Results and Discussion

A series of lower rim 1,3-di-amino acid conjugates of calix[4]arene have been synthesized either starting from p-tert-butyl-calix[4]arene or its di-OMe derivative in four or five steps respectively, to result in the products having terminal carboxylic ester (3e-6e and 7e-9e) or carboxylic acid (3a-6a and 7a-9a) as shown in Scheme I. All the compounds were characterized satisfactorily by FTIR, ¹H and ¹³C NMR, ¹H-¹H COSY, ¹H-¹³C HMQC and mass spectra (Experimental Section).

Characterization

Comparison of the melting points (m.p.) of the conjugates showed an increase in the m.p. for the -COOH conjugates 3a-6a as compared to the corresponding -COOR ones 3e-6e as expected.
because the former are involved in extensive H-bonding to raise the melting temperature. In fact the formation of the amide bond present in the peptido-
conjugates has been identified using FTIR spectra, wherein the amide I ($\nu_{C=O}$) and the amide II bands ($\delta_{NH}$) appear in 1659-1688 cm$^{-1}$ and 1531-1539 cm$^{-1}$ respectively. The $\nu_{C=O}$ vibration observed in the 1740-
1770 cm$^{-1}$ clearly differentiates the presence of ester moiety from that of the carboxylic moiety as the terminal groups of the arms, hence can be used as diagnostic to assess the end group of the arm. The $\delta_{OH}$ region showed a broad band comprising of both the phenolic $–OH$ and the amide $–NH$ vibrations.

$^1$H and $^{13}$C NMR studies

The proton and carbon resonances of amino acid portions of the conjugates were assigned based on $^1$H-$^1$H COSY and $^1$H-$^{13}$C HMQC. The positions of amide proton signals were confirmed through cross coupling peaks of $C^\alpha$H in COSY experiments. All the exchangeable proton resonances were cross checked from the spectra measured after D$_2$O addition. The chemical shifts observed in the present case were compared with those observed in case of the tetra-
substituted amino acid ester derivatives$^{13}$. The amide proton signals appeared downfield in the range 9.32 to 9.66 ppm (CDCl$_3$) for 3e-6e and 8.72 to 9.14 ppm for 3a-6a (DMSO-d$_6$), indicating the involvement of amide $–NH$ in an intramolecular hydrogen bonding. The phenolic $–OH$ protons were observed in the range, 7.73 to 7.97 ppm in case of 3e-6e and 8.19 to 8.43 ppm in case of 3a-6a.

The aromatic protons in 3e appear as two siglets whereas those in 4e-6e as two doublets of doublets. Similarly 3a showed a sharp singlet at 7.17 ppm, 4e and 6a showed two doublets, 5a showed one sharp doublet. This splitting is attributable to the presence of chiral $C^\alpha$ center present in the amino acid portion of the arms.$^{13}$ The axial and equatorial calixarene methylene bridged (CH$_2$) protons in 4e-6e showed two pairs of doublets, unlike 3e, due to the chiral nature of pendant amino acid groups. Similarly the diastereotopic nature of OCH$_3$H$_B$ appeared as two doublets in case of 4e-6e, but in case of 3e, it appeared as a singlet. The methyl protons of the tert-
butyl group in 3e-6e appears as two singlets in the range 1.19-1.30 and 0.96-1.04 ppm and not much shifted in comparison to the precursor diacid. Similar splitting was observed even in the spectra of 3a-6a. Thus the NMR spectral features were clearly indicative of the cone-conformation in all these conjugates.

The positions of the carbons arising from $C^\alpha$H, $C^\beta$H (4e-6e and 4a-6a), $C^\gamma$H (6e and 6a), OCH$_3$CO, Ar-CH$_2$-Ar were assigned by HMQC experiments. Results of $^1$H-$^1$H COSY and $^1$H-$^{13}$C HMQC for 6e and 6a are shown in Figure 1. The peak corresponding to the ester carbonyl carbon (COOR, R = Me or Et or CH$_2$Ph) appeared at 169.5-173.3 ppm in 3e-6e. An additional ester carbonyl peak appeared from side chain ester groups in case of 5e and 6e. The amide peak ($–NH$-CO) appeared at 168.7-169.2 ppm for 3e-6e. Similarly in case of 3a-6a the acid carbonyl (COOH) peak was found at 170.8-173.5 ppm and the

Scheme I - Schematic representation of the synthesis of the amino acid conjugates of p-tert-butyl-calix[4]arene and its $–$OMe analogue (a) BrCH$_2$COOEt, K$_2$CO$_3$, acetone, reflux 1.5 h (b)aq. NaOH, EtOH, reflux, 24 h (c) SOCl$_2$, benzene, reflux, 7 h (d) amino acid ester.
HCl, NEt$_2$, THF, RT, 24 h (e) LiOH, THF/H$_2$O, except in case of the reaction going from 5e to 5a where Pd/C, H$_2$ were used. The suffix, ‘e’ is for ester and ‘a’ is for acid.
amide carbonyl (-NH-CO-) peak at 167.7-168.4 ppm. The methylene group from OCH$_2$CO appeared at 74.8-74.9 ppm. Thus the structures of the conjugates shown in Scheme I were well supported by the NMR studies.

FAB MS studies: The mass spectra of all the conjugates showed molecular ion peaks corresponding to their molecular weights consistent with the formulae given in Scheme I based on the analytical data (Table I). Typical spectra for the glycine conjugates are shown in Figure 2. The spectra also exhibited some additional prominent peaks relevant to the fragments generated by the loss of arm(s) etc.

**Single crystal XRD studies of 3e**

The molecular structure of 3e has been established by single crystal XRD. Structural refinement and other data collection parameters for 3e are summarized in Table II. Molecular structure of 3e and its intra-molecular hydrogen bonding formed by the pendants is shown in Figure 3. As evident from the crystal structure, the calix[4]arene derivative is in cone conformation where the amide-NH is involved in hydrogen bonding with the ether oxygen (N-H…O$_{ether}$) of the calix[4]arene pendants. Similarly phenolic-OH also forms hydrogen bond with ether oxygen (O-H…O$_{ether}$, 7-atom H-bond) and the amide-NH (N-H…O$_{phenolic}$, 10-atom H-bond) intra-molecularly. Amide oxygen of the pendent arm is involved in an intermolecular hydrogen bond with the aromatic hydrogen (C=O…H-C$_{aromatic}$) of a neighbour molecule as shown in Figure 3b. Lattice structure of 3e is shown in Figure 4a. As evident from the figure that the structure resembles the cones arranged in antiparallel fashion and stacks one into the other as shown in line sketch of the lattice structure.
Thus the tert-butyl group of calix[4]-arene moieties protrude into the hydrophobic core of the neighboring calix[4]arene. Since the bond lengths and bond angles of the conjugate were found to be normal, only those corresponding to the arms were given in Table III.

Conformational mobility induced by the replacement of lower rim phenolic–OH by –OCH₃, 7e-9e

As understood from the NMR spectral studies of these conjugates and the crystal structure of 3e, the cone-conformation is stabilized via the conformational turns formed by the pendant arms built through 11-atom intramolecular N-H...O interaction in addition to the intra-rim O-H...O interactions, all operating at the lower rim. This observation entrusted us with the synthesis of the corresponding conjugates where the two phenolic –OH groups were modified to –OCH₃ resulting in the compounds 7e-9e as shown in Scheme I. The products were thoroughly characterized. In these –OCH₃ derivatized conjugates, the cone-conformation is less favoured owing to the breakage of such H-bond interactions resulting in conformational flexibility of the conjugates leading to the fluxional behaviour. While the RT ¹H NMR spectra were characteristic of dynamic phenomenon, the variable temperature study performed with 7e and 7a in the range -50°C to 110°C clearly indicated the fluxional behavior of these –OCH₃ derivatized conjugates. Selected portions of the spectra for 7a as a function of temperature are shown in Figure 5. The

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Table I — m/z Details for calix[4]arene derivatives

Figure 2: FAB mass spectra of (a) 7e and (b) 7a. M denotes the corresponding molecular ion.
Data is consistent with a mixture of at least two conformers at room and low temperatures, but fits well with the presence of cone-conformation predominantly at 110°C. Fluxional behavior of one of the early precursors of the –OCH₃ conjugates, viz., an ester precursor of 2a in Scheme I, is reported in the literature and the fluxional behavior seems to continue even when the two arms were extended by amino acid via amide bonds, as observed in the present case. All this is possible when the intra-molecular interactions are disrupted to induce the pseudo rotation about the calix[4]arene at the lower rim.

Differential binding property of the carboxylic-acid and ester-end conjugates of calix[4]arene towards phosphate: Differential recognition of the peptido-conjugates reported in this paper towards phosphate monoester was demonstrated by performing binding studies using spectrophotometric titrations. Addition of these conjugates, viz., 3a, 4a, 6a or 7a to disodium salt of 4-nitrophenyl phosphate (Na₂-npp) at various phosphate to conjugate ratios in 1% H₂O-DMSO resulted in dramatic change in the absorption spectrum wherein the absorbance of Na₂-npp at 433 nm decreases and that at 312 nm increases with an isosbestic point being observed at 370 nm. Spectra observed during the titration are shown in Figure 6a in case of the conjugate 6a. From the plot of absorbance of 433 nm band vs. concentration of the conjugate, the ratio of the resulting complex formed was determined. A typical plot for 6a was shown in Figure 6b. The results suggest the formation of 1:1 complex in case of 3a–4a, i.e., with those amino acids having one -COOH terminal group at each pendant and a 1:2 complex in case of 6a, viz., glu-conjugate possessing two -COOH functions at the terminal of each pendant.

Figure 3 — Molecular Structure of 3e (a) as an ORTEP plot, only pendant atoms are labeled and hydrogen atoms have been omitted for the sake of clarity and (b) intra and inter-molecular H-bonding in 3e.

Figure 4 — (a) Packing of the 3e in the lattice and (b) line diagram showing the stacking of calix[4]arene units in the lattice of 3e.
The binding constants were computed from the absorbance data\(^\text{15}\) and were found to be \((10\pm2) \times 10^5\), \((2.8\pm1) \times 10^5\), \((3.2\pm1) \times 10^5\) and \((3.3\pm1) \times 10^5\) M\(^{-1}\) for 3a, 4a, 6a and 7a respectively. Careful examination of the binding data, in comparison with that of the precursor calix-diacid, 1a\(_1\) \((5.5\pm1.2) \times 10^5\) M\(^{-1}\), is clearly suggestive of the interaction of the terminal –COOH group. The binding data is further influenced by the substitution at the C\(^\alpha\) center of the amino acid moiety. The binding constants observed in the present case are certainly higher by at least two orders of magnitude when compared to the reported calix-
phenolic -OH groups were modified to -OCH. This were to be true, in case of involving in an intramolecular H-bond interaction as explained based on the structure in this paper. If, as expected, the intramolecular H-bond of the amide-NH for interaction with the phosphate group. In contrast, the work reported in case of cyclic peptides possessing 6 to 14 peptide bonds exhibit low binding strength. Association between the phosphate and these conjugates is also evidenced from the $^1$H NMR spectrum of the reaction between Na$_2$-npp and 3a, where the phenyl protons of the phosphate moiety exhibit a down-field shift of 1.1 ppm upon binding. Comparison of these results with appropriate control experiments involving N-acetylglutamic acid and N-boc-glycine further supports the interaction of terminal carboxylic groups with the phosphate moiety. Thus the -COOH end conjugate recognizes the negatively charged phosphate group in preference over its -COOR counterpart, as the former is driven through the hydrogen bond formation.

Conclusions

The idea of building 1,3-di-functional pendants on the calix[4]arene lower rim to provide a binding core has been well demonstrated through the formation of the peptide- conjugates of calix[4]arene as reported in this paper. Indeed these conjugates yielded a variable-type binding site with a built-in flexibility where the core can act as either N$_2$O$_2$ or N$_2$O$_3$ or even as N$_2$O, that may be suitable for binding transition or lanthanide ions. The cone-conformation has been observed owing to the intra-rim O-H...O interactions operating at the lower rim. Breakage of such interactions indeed lead to the conformational characteristics. Indeed the observed binding constants are, (4.9±1.2) × 10$^5$ and (5.0±1.2) × 10$^5$ M$^{-1}$ respectively for 7e and 9e owing to the availability of amide-NH for interaction with the phosphate group. In case of the precursor ester-conjugates, viz., 3e, 4e, and 6e, there do not seem to be any specific binding present, indicating that the -COOH terminal is essential for recognition when the amide group is not accessible to the phosphate owing to its involvement in an intramolecular H-bond interaction as explained based on the structure in this paper. If this were to be true, in case of 7e and 9e where the phenolic -OH groups were modified to -OCH$_3$ (-OCH$_3$ counter parts for 3e and 6e), this results in the breakage of the intramolecular H-bond of the amide-NH and hence is expected to show good binding.

Figure 6 — (a) Absorption spectra of the titration between calix[4]arene-amino acid conjugate, 6a and the phosphate, Na$_2$-npp in 1% H$_2$O-DMSO. The upward and downward arrows indicate increase and decrease of the corresponding bands respectively. [Na$_2$-npp] = 4 × 10$^{-5}$ M, [6a]= 0.42 × 10$^{-5}$ to 7.04 × 10$^{-5}$ M; (b) Plot of absorbance of 433 nm band as a function of the concentration of the calix[4]arene-conjugate, 6a.

Figure 7 — Proposed model for phosphate binding with calix[4]arene-conjugate possessing end –COOH group

cyclic peptide. Based on these studies, the proposed model for phosphate binding with the –COOH end conjugate was shown in Figure 7.
flexibility as demonstrated by us in case of the conjugates where the two phenolic-OH groups were replaced by the –OMe groups at the lower rim. The arms adapt a conformational turn by extending 11-atom hydrogen bond interaction between the amide NH and the rim-phenolic oxygen. Differential recognition characteristics of the calix-peptide conjugates towards phosphate anion were demonstrated. This was attributed to the hydrogen-bond association between the carboxylic terminal groups and/or the amide moiety depending upon the availability of these groups for interaction. The phosphate did not show any preferential interaction with the ester group 3e-3a. Thus the present studies elicit an entry of this new series of calix[4]arene-peptido-conjugates into the field of molecular recognition.

**Experimental Section**

$^1$H and $^{13}$C NMR spectra were measured on a Varian Mercury NMR spectrometer working at 400 MHz. The mass spectra were recorded on Q-TOF micromass (YA-105) using electrospray ionization method. The absorption spectra were measured on JASCO V – 570. The elemental analyses were performed on ThermoQuest microanalysis. FT-IR spectra were measured on Perkin-Elmer spectrometer using KBr pellets. Single crystal X-ray diffraction data were measured on OXFORD XCALIBUR-S CCD machine. All the chemicals used were procured from Sigma Aldrich Chemical Co., U.S.A. All the solvents used were dried and distilled by usual procedures immediately before use. Distilled and deionized water was used in the studies.

*p*-tert-Butylcalix[4]arene conjugates, *viz.*, 3e-6e, 7e-9e, 3a-6a and 7a-9a shown in Scheme I, were synthesized using a reported procedure$^{11}$ with marginal modifications.

**Characterization data for the conjugates**

5,11,17,23-Tetra-tert-butyl-25,27-dihydroxy-26, 28-bis(O-methyl-R-α-alanylcarbonylmethoxy)calix[4]arene (3e). —Yield 70%; m.p. 182°C (decomposes). FTIR (KBr): 3321 (ν NH/ONH), 1754 (ν C=O, COOMe), 1685 (ν C=O, CONH) cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 1.03 (s, 18H, C(CH$_3$)$_3$), 1.27 (s, 18H, C(CH$_3$)$_3$), 3.43 (d, 4H, Ar-CH$_2$-Ar, $J = 13.4$ Hz), 3.72 (s, 6H, OCH$_3$), 4.14 (d, 4H, C$^6$H, $J = 4.95$ Hz), 4.23 (d, 4H, Ar-CH$_2$-Ar, $J = 13.3$ Hz), 4.63 (s, 4H, O-CH$_2$CO), 6.92 (s, 4H, Ar-H), 7.25 (s, 4H, Ar-H), 7.83 (s, 2H, OH), 9.27 (t, 2H, NH, $J = 4.6$, 4.7 Hz); $^{13}$C NMR (CDCl$_3$): δ 31.0, 31.6 (C(CH$_3$)$_3$), 32.1 (Ar-CH$_2$-Ar), 34.1, 33.9 (C(CH$_3$)$_3$), 41.2 (C$^6$H), 52.3 (OCH$_3$), 74.8 (OCH$_2$CO), 125.5, 126.2, 127.1, 132.4, 142.9, 148.2, 149.2, 149.8 (aromatic carbons), 169.4 (C=O, CONH), 169.5 (C=O, COOCH$_3$); MS-ES: m/z 906 ([M]$^+$, 100%). Anal. Calcd. for C$_9$H$_{29}$N$_2$O$_{10}$ (924): C, 70.13; H, 7.79; N, 3.03. Found: C, 70.66; H, 8.22; N, 2.87%.

5,11,17,23-Tetra-tert-butyl-25,27-dihydroxy-26, 28-bis(O-methyl-R-α-aspartyl-carbonylmethoxy)calix[4]arene (4e). —Yield: 65%; m.p. >240°C (decomposes). FTIR (KBr): 3337 (ν NH/ONH), 1749 (ν C=O, COOMe), 1680 (ν C=O, CONH) cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 0.97, 1.28 (s, 18H each, C(CH$_3$)$_3$), 1.47 (d, 6H, CH$_3$, $J = 7.21$ Hz), 3.40 (d, 2H, Ar-CH$_2$-Ar, $J = 13.3$ Hz), 3.46 (d, 2H, Ar-CH$_2$-Ar, $J = 13.5$ Hz), 3.69 (s, 6H, OCH$_3$), 4.18 (d, 2H, Ar-CH$_2$-Ar, $J = 13.2$ Hz), 4.31 (d, 2H, Ar-CH$_2$-Ar, $J = 13.5$ Hz), 4.53 (d, 2H, CH$_2$-O, $J = 15.2$ Hz), 4.74-4.49 (m, 2H of C$^6$H and 2H of O-CH$_2$CO), 6.93 (d, 4H, Ar-H), 7.08 (d, 4H, Ar-H), 7.5 (s, 2H, OH), 9.42 (d, 2H, NH, $J = 7.43$ Hz) ppm; $^{13}$C NMR (CDCl$_3$): δ 17.9 (CH$_3$ of ala), 30.9, 31.6 (C(CH$_3$)$_3$), 32.1, 32.3 (Ar-CH$_2$-Ar), 33.9, 34.1 (tert-C), 48.0 (C$^6$H), 52.3 (OCH$_3$), 74.8 (O-CH$_2$-CO), 125.4, 125.5, 126.2, 127.1,127.2, 134.2, 132.5, 142.8, 148.2, 149.60, 149.64 (aromatic carbons), 168.7 (C=O, CONH), 172.6 (C=O, COOMe); MS-ES: m/z 934 ([M]$^+$, 95%). Anal. Calcd. for C$_{56}$H$_{74}$N$_2$O$_{10}$ (934): C, 71.95; H, 7.92; N, 3.00. Found: C, 71.34; H, 7.71; N, 3.10%.
COOH), 170.0 (C=O, COOH); FABMS: m/z 1355 ([M+H]^+), 90%; 1377 ([M+Na]^+), 50%. Anal. Calcd. for C_{42}H_{38}N_{10}O_{12} (1354): C, 74.45; H, 6.94; N, 2.07. Found: C, 74.14; H, 7.34; N, 1.73%.

5.11,17,23-Tetra-tert-butyl-25,27-dihydroxy-26,28-bis(O-ethyl-R-α-glutyl-carbonylmethoxy)calix-[4]arene (6e). — Yield 84%; m.p. 168-170°C. FT-IR (KBr): 3362, 3312 (νOH), 1741 (νC=O, COOEt) 1685 (νC=O, CONH) cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): δ 0.96 (s, 18H, C(CH\(_3\))\(_2\)), 1.10 (t, 6H, CH\(_2\)-CH\(_3\), J = 7.1 Hz), 1.18 (t, 6H, CH\(_2\)-CH\(_3\)), 1.19 (s, 18H, C(CH\(_3\))\(_2\)), 1.97 (m, 2H, CH\(_2\)-CH\(_2\)), 2.10 (m, 2H, CH\(_2\)-CH\(_2\)), 2.29 (m, 4H, CH\(_2\)-CH\(_2\)), 3.26 (d, 2H, Ar-CH\(_2\)-Ar, J = 13.0 Hz), 3.40 (d, 2H, Ar-CH\(_2\)-Ar, J = 13.6 Hz), 3.97 (q, 4H, CH\(_2\)-CH\(_2\), J = 7.1 Hz), 4.13 (d, 2H, Ar-CH\(_2\)-Ar, J = 13.6 Hz), 4.24 (d, 2H, Ar-CH\(_2\)-Ar, J = 12.9 Hz), 4.31 (d, 2H, O-CH\(_2\)-CO, J = 15.2 Hz), 4.65 (q, 2H, C\(^{\text{H}}\)-glu, J = 7.0 Hz), 4.85 (d, 2H, O-CH\(_2\)-CO, J = 15.2 Hz), 6.83 (d, 4H, Ar-H), 6.99 (d, 4H, Ar-H), 7.15 (m, 8H, Ar-H), 7.78 (s, 2H, OH), 9.37 (d, 2H, NH-); \(^13\)C NMR (CDCl\(_3\)): δ 14.11, 14.13 (C(CH\(_3\))\(_2\)), 30.3 (t-CH\(_3\)), 30.9, 31.6 (C(CH\(_3\))\(_2\)), 32.2, 32.3 (Ar-CH\(_2\)-Ar), 33.8, 34.0 (t-CH\(_3\)), 60.4 (CH\(_2\)-CH\(_2\)), 61.3 (t-CH\(_3\)), 74.9 (O-CH\(_2\)-CO), 125.2, 125.6, 125.7, 126.5, 126.7, 127.3, 142.5, 148.0, 149.6, 150.0 (aromatic carbons), 169.0 (C=O, CONH), 171.2 (C=O, COOEt), 173.3 (C=O, COOEt); FABMS: m/z 1135 ([M+H]^+), 100%; 1157 ([M+Na]^+), 30%. Anal. Calcd. for C\(_{63}\)H\(_{68}\)N\(_{12}\)O\(_{19}\) (1134): C, 69.84; H, 7.94; N, 2.47. Found: C, 69.25; H, 8.46; N, 2.38%.

5.11,17,23-Tetra-tert-butyl-25,27-dihydroxy-26,28-bis(glycyl carbonylmethoxy)calix-[4]arene (3a). — Yield 89%; m.p. 210°C (decomposes). FTIR (KBr): 1743 (νC=O, COOH); 1668 (νC=O, CONH) cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\)): δ 1.13 (s, 18H, C(CH\(_3\))\(_2\)), 1.20 (s, 18H, C(CH\(_3\))\(_2\)), 3.47 (d, 4H, Ar-CH\(_2\)-Ar, J = 13.2 Hz), 4.03 (m, 4H, C\(^{\text{H}}\)), 4.23 (d, 4H, Ar-CH\(_2\)-Ar, J = 12.8 Hz), 4.53 (s, 4H, OCH\(_2\)CO), 7.17 (s, 8H, Ar-H), 8.41 (s, 2H, OH), 8.89 (t, 2H, HN) ppm; \(^13\)C NMR (DMSO-d\(_6\)): δ 30.8, 31.3 (C(CH\(_3\))\(_2\)), 33.6, 34.0 (C(CH\(_3\))\(_2\)), 40.6 (C\(^{\text{H}}\)), 67.0 (t-CH\(_3\)), 74.0 (OCH\(_2\)CO), 125.5, 125.9, 126.9, 132.8, 141.6, 147.5, 149.6, 149.9 (aromatic carbons), 168.4 (C=O, CONH), 170.8 (C=O, COOH); FABMS: m/z 885 ([M+Li]^+), 90%. Anal. Calcd. for C\(_{42}\)H\(_{38}\)N\(_{10}\)O\(_{12}\)H\(_2\)O (932): C, 66.93; H, 7.78; N, 3.00. Found: C, 66.71; H, 7.72; N, 2.86%.

5.11,17,23-Tetra-tert-butyl-25,27-dihydroxy-26,28-bis(R-α-alanyl-carbonylmethoxy)calix-[4]arene (4a). — Yield 93%; m.p. 196-198 °C. FTIR (KBr): 3343, 3343, 1741 (νC=O, COOH); 1660 (νC=O, CONH) cm\(^{-1}\); \(^1\)H NMR (DMSO-d\(_6\)): δ 1.11 (s, 18H, C(CH\(_3\))\(_2\)), 1.20 (s, 18H, C(CH\(_3\))\(_2\)), 1.99-2.08 (m, 4H, C\(^{\text{H}}\)), 2.32-2.36 (m, 4H, C\(^{\text{H}}\)), 3.44 (d, 2H, Ar-CH\(_2\)-Ar, J = 12.9 Hz), 3.51 (d, 2H, Ar-CH\(_2\)-Ar, J = 13.2 Hz), 4.21 (d, 2H, Ar-CH\(_2\)-Ar, J = 13.1 Hz), 4.40-4.43 (m, 6H, 2H each of OCH\(_2\)CO, C\(^{\text{H}}\), Ar-CH\(_2\)-Ar), 4.75 (d, 2H, OCH\(_2\)CO, J = 14.9 Hz), 7.20-7.12 (m, 8H, Ar-H), 8.17 (s, 2H, OH), 8.87 (d, 2H, NH, J = 7.2 Hz); \(^13\)C NMR (DMSO-d\(_6\)): δ 26.3, 29.9 (CH\(_2\)-CH\(_2\)), 30.7, 31.3
(C(CH3)3), 33.6, 33.9 (C-tert), 51.4 (C6H), 74.2 (OCH2CO), 125.4, 125.7, 126.2, 126.7, 127.2, 132.6, 132.7, 147.5, 149.4, 141.9, 150.2 (aromatic carbons), 168.0 (C=O, CONH), 172.6 (C=O, COOH), 173.5 (C=O, COOH); FABMS: m/z 1023 ([M+H]+, 50%), 1045 ([M+Na]+, 20%). Anal. Calcd. for C56H32N2O14.H2O (1040): C, 66.92; H, 7.31; N, 2.69. Found: C, 66.49; H, 7.18; N, 2.08%.

5.11,17,23-Tetra-tert-butyl-25,27-dimethoxy-26, 28-bis(O-methyl glycylicarbonylmethoxy)calix[4]arene (7e). —Yield: 51%; m.p. 220°C (decomposes). FT-IR (KBr): 3412 (v(NH)), 1754 (v(C=O), COOEt), 1688 (v(C=O), CONH) cm⁻¹; 1H NMR (CDCl3): δ 9.27 (t, J = 4.6, 4.7 Hz, 2H, NH), 7.25 (s, 4H, Ar-H), 6.92 (s, 4H, Ar-H), 4.63 (s, 4H, O-CH2CO), 4.23 (d, J = 13.3 Hz, 4H, Ar-CH2-CH2), 4.14 (d, J = 4.95 Hz, 4H, C6H), 3.85 (s, 6H, Ph-OCH3), 3.72 (s, 6H, OCH3), 3.43 (d, J = 13.4 Hz, 4H, Ar-CH2-Ar), 1.27 (s, 18H, C(CH3)3), 1.03 (s, 18H, C(CH3)3); 13C NMR (CDCl3): δ 31.6 (C(CH3)3), 32.1 (Ar-CH2-CH2), 33.9, 34.1 (C(CH3)3), 41.2 (C6H), 52.3 (OCH3), 74.8 (OCH2CO), 125.5 126.2, 127.1, 132.4, 142.9, 148.2, 149.2, 149.8 (Ar-C), 169.4 (CONH), 169.5 (COOMe); ESI MS: m/z 935 ([M+H]+, 60%), 957 ([M+Na]+, 10%). Anal. Calcd. for C56H32N2O14 (934): C, 71.95; H, 7.92; N, 3.00. Found: C, 71.52; H, 8.27; N, 2.73%.

5.11,17,23-Tetra-tert-butyl-25,27-methoxy-26, 28-bis(O-methyl-R-α-alanyl-carbonylmethoxy)calix[4]arene (8e). —Yield: 45%; m.p. >250°C. FTIR (KBr): 3421 (v(NH)), 1749 (v(C=O), COOEt), 1688 (v(C=O), CONH) cm⁻¹; ESI MS: m/z 985 ([M+Na]+, 100%). Anal. Calcd. for C62H68N2O16.H2O (980): C, 71.02; H, 8.16; N, 2.86. Found: C, 70.69; H, 7.92; N, 3.05%.

5.11,17,23-Tetra-tert-butyl-25,27-dimethoxy-26, 28-bis(O-ethyl-R-α-glutyl-carbonylmethoxy)calix[4]arene (9e). —Yield: 54%; m.p. 150°C. FTIR (KBr): 3414 (v(NH)), 1744 (v(C=O), COOEt), 1691 (v(C=O), CONH) cm⁻¹. FABMS: m/z 1163 ([M+H]+, 30%). Anal. Calcd. for C62H68N2O14 (1162): C, 70.22; H, 8.09; N, 2.41. Found: C, 69.77; H, 8.53; N, 2.99%.


5.11,17,23-Tetra-tert-butyl-25,27-dimethoxy-26, 28-bis(R-α-alanyl-carbonylmethoxy)-calix[4]arene (8a). —Yield: 29%; m.p. >250°C. FT-IR (KBr): 3421, 3353 (v(NH)), 1739 (v(C=O), COOH), 1601 (v(C=O), CONH) cm⁻¹; FABMS: m/z 934 ([M]+, 70%). Anal. Calcd. for C55H30N2O10 (924): C, 71.95; H, 7.92; N, 3.00. Found: C, 71.61; H, 7.48; N, 2.64%.

5.11,17,23-Tetra-tert-butyl-25,27-dihydroxy-26, 28-bis(R-α-glutyl-carbonylmethoxy)calix[4]arene (9a). —Yield: 85%; m.p. 200°C (decomposes). FTIR (KBr): 3404, 1739 (v(C=O), COOH), 1678 (v(C=O), CONH) cm⁻¹; 1H NMR (DMSO-d6): δ 1.11 (s, 18H, C(CH3)3), 1.20 (s, 18H, C(CH3)3), 1.99-2.08 (m, 4H, C6H), 2.32-2.36 (m, 4H, C6H), 3.44 (d, 2H, Ar-CH2-Ar, J = 12.9 Hz), 3.51 (d, 2H, Ar-CH2-Ar, J = 13.2 Hz), 3.85 (s, 6H, Ph-OCH3), 4.21 (d, 2H, Ar-CH2-Ar, J = 13.1 Hz), 4.40-4.33 (m, 6H, 2H each of OCH2CO, C6H, Ar-CH2-Ar), 4.75 (d, 2H, OCH2CO, J = 14.9 Hz), 7.20-7.12 (m, 8H, Ar-H), 8.17 (s, 2H, OH), 8.87 (d, 2H, NH, J = 7.2 Hz); 13C NMR (DMSO-d6): δ 26.3, 29.9 (CH2-CH2), 30.7, 31.3 (C(CH3)3), 33.6, 33.9 (C-tert), 51.4 (C6H), 74.2 (OCH2CO), 125.4, 125.7, 126.2, 126.7, 127.2, 132.6, 142.7, 147.5, 149.4, 141.9, 150.2 (aromatic carbons), 168.0 (C=O, CONH), 172.6 (C=O, COOH), 173.5 (C=O, COOH); FABMS: m/z 1052 ([M+H]+, 85%). Anal. Calcd. for C62H78N2O14.H2O (1068): C, 67.42; H, 7.49; N, 2.62. Found: C, 67.19; H, 6.98; N, 2.21%.

Phosphate binding studies

Binding of 4-nitrophenyl phosphate (Na2-npp) with the conjugates of calix[4]arene at 25°C has been studied by following the absorbance of the 433 nm band. In order to increase the solubility of Na2-npp, these experiments were carried out in 1% H2O-DMSO at a fixed concentration of Na2-npp. In a typical experiment, Na2-npp (4.0 × 10⁻⁵ M) and the calixarene-amino acid conjugate, (0.42 × 10⁻⁵ - 24.02 × 10⁻⁵ M) in 1% H2O-DMSO were mixed and the UV/Vis absorption spectra were measured at each addition of the conjugate (each ratio was made freshly) until no substantial decrease was observed in the absorbance of the 433 nm band. Simultaneously, the band at 312 nm increased with an isosbestic point being observed at 370 nm. The ratio of the resulting complex formed was determined from the plot of the measured absorbance at 433 nm versus concentration of calix-amino acid conjugate. The binding constants were then computed from the absorbance data using Benesi-Hildebrand method.17
Acknowledgements

One of the author CPR acknowledges the financial support from DST, CSIR and BRNS-DAE. MD, AA and AA acknowledge CSIR for their senior research fellowships. We thank SAIF, IIT Bombay for some spectral measurements.

References