Biomimetic Protein-Harpooning Surfaces

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Electronic Supplementary Information

GENERAL INFORMATION

Reagents were purchased from Sigma Aldrich (St.Louis, MO, USA) and used as received. All reactions were carried out under a nitrogen atmosphere unless stated otherwise. Thin layer chromatography (TLC) was carried out on silica gel plates (Merck 60, F254), column chromatography was carried out on silica gel 60 (Merck, 0.063–0.200 mm).

NMR experiments were carried out in $CDCl_3$ on a 500 MHz spectrometer (^1H at 499.88 MHz, ^{13}C at 125.7 MHz) equipped with a pulse field gradient module (Z axis) and a tunable 5 mm Varian inverse detection probe (ID-PFG); chemical shifts (δ) are expressed in ppm and are referenced to residual deuterated solvent. Two-dimensional experiments were performed using Varian standard pulse sequences. NMR data were processed using the MestReC software.

MS-ESI spectra were recorded with an Electrospray ionization mass spectra (ESI-MS) were recorded on a Finnigan LCQ Deca XP ion trap (Thermo Fischer Scientific, USA) using electrospray ionization (ESI) interface.

SYNTHESIS OF TCC5

Calix[4]arene derivative **1**[1] and Tetraethylene glycole ditosylate[2] were obtained according to literature procedures; their reaction afforded the Calix[4]crown-5 derivative 2 fixed in 1,3-alternate conformation. Subsequent Claisen transposition allowed the migration of the allyl groups from the lower rim to the upper rim, while the calixarenic scaffold re-adopts the cone conformation. The conversion of the allyl derivative **3** to the thioester **TACC5** was achieved by the radical addition of thioacetic acid on the terminal olefin. The structures and the conformations of all the intermediates and **TACC5** were confirmed by NMR spectroscopy and MS spectrometry.

25,27-DIALLYLOXY-26,28-CROWN[5]-CALIX[4]ARENE 2

Tetraethyleneglycol-di-p-toluenesolfonate (0.35 g, 0.70 mmol) was added to a suspension of 25,27-Di(1-Allyloxy)-26,28-Dihydroxy-Calix[4]Arene **1** (0.35 g, 0.70 mmol) and Cs_2CO_3 (2.71 g, 8.3 mmol) in 100 ml of acetonitrile. The reaction mixture is refluxed for 5 h under nitrogen atmosphere. The solvent was removed under reduced pressure and 50 ml of 10% acqueous HCl solution and 50 ml of CH_2Cl_2 were added. The organic layer was separated and washed with water (2x20ml). Organic phase was dried over anhydrous $MgSO_4$ and the CH_2Cl_2 was removed in vacuo. The mixture was purified by column chromatography (eluent CH_2Cl_2/AcOEt=80/20) to gave 0.24 g (52%) of the desidered product **2**.

^{1}H-NMR (500 MHz, 300 K, $CDCl_3$) δ: 7,13 (4H, d, J= 7.33 Hz, ArH_m); 6,98 (4H, d, J= 7.60 Hz, ArH_m); 6,91 (2H, t, J= 7.33 Hz, ArH_p); 6,73 (2H, t, J= 7.60 Hz, ArH_p); 5,58 (2H, m, -OCH_2CHCH_2); 4,92 (2H, dd, J_1= 10.64 Hz, J_2= 1.66 Hz, -OCH_2CHCH_2); 4,76 (2H, dd, J1= 17.28 Hz, J2= 1.80 Hz, -OCH_2CHCH_2); 3,99 (4H, m, -OCH_2CHCH_2); 3,83 (8H,s, ArCH_2Ar); 3,58 (8H, s, -OCH_2); 3,39 (4H, t, J= 6.22 Hz, -OCH_2); 3,29 (4H, t, J= 6.22 Hz, -OCH_2). ^{13}C-NMR (125 MHz, 300 K, $CDCl_3$) δ: 156.43, 155.72, 134.63, 133.88, 133.53, 130.31, 129.61, 122.70, 122.59, 115.74, 72.30, 70.67, 70.47, 70.25, 69.04, 38.07. MALDI-MS: m/z = 685.20 $[M+Na]^+$.

11,23-BIS-ALLYL-26,28-CROWN[5]-CALIX[4]ARENE 3

0,24 g (0.37 mmol) of **2** were suspended in 5ml of N,N-dimethylaniline. The mixture was refluxed for 3 h under nitrogen atmosphere. The solution was cooled at room temperature and poured into 25 ml of ice-water, stirred with 25 ml of HCl 37% and filtered to yield 0.19 g of product **3** (77%), that was used without further purification for the next step.

^{1}H-NMR (500 MHz, 300 K, CDCl3) δ: 7,63 (2H, s, ArO*H*); 6,89 (4H, s, ArH_m); 6,88 (4H, d, J= 7.34 Hz, ArH_m); 6,74 (2H, t, J= 7.34 Hz, ArHp); 5,98 (2H, m, ArCH_2CHCH_2); 5,06 (4H, m, ArCH_2CHCH_2); 4,42 (4H,d, J= 13.02 Hz, ArCH_2Ar); 4,10 (8H, s, -OCH_2); 3,96 (4H, t, J= 7.34 Hz, -OCH2); 3,87 (4H, t, J= 7.34 Hz, -OCH2); 3,34 (4H, d, J= 13.02 Hz, ArCH_2Ar); 3,29 (4H, d, J= 6.60 Hz, ArCH_2CHCH_2). ^{13}C-NMR (125 MHz, 300 K, CDCl3) δ: 152.07, 151.63, 138.33, 133.29, 129.97, 128.91, 128.46, 127.98, 125.27, 115.09, 76.72, 71.11, 71.07, 70.22, 39.41, 31.18. MALDI-MS: m/z = 701.07 (100) $[M+K]^+$.

11,23-BIS-(3-PROPYL-THIOACETATE)-CROWN[5]-CALIX[4]ARENE TACC5

To a solution of **3** (0.16 g, 0.27 mmol) and thioacetic acid (0.08 g, 1.1 mmol) in toluene dry (8 mL) is added a catalytic amount of AIBN. The solution is refluxed for 3 h under nitrogen atmosphere, so the solvent is removed by evaporation under reduced pressure. The organic phase is washed with H_2O and saturated $NaHCO_3$. The solvent was removed under reduced pressure and the mixture is purified by column chromatography (eluent Hexane/EthylAcetate 60/40) giving 0.16 g (74%) of the desidered product **TACC5**.

^{1}H-NMR (500 MHz, 300 K, CDCl3) δ: 7,66 (2H, s, ArO*H*); 6,89 (4H, d, J= 7.48 Hz, ArH_m); 6,87 (4H, s, ArH_m); 6,75 (2H, t, J= 7.48 Hz, ArH_p); 4,41 (4H, d, J= 12.97 Hz, ArCH_2Ar); 4,10 (8H, s, -OCH_2); 3,96 (4H, t, J= 5.49 Hz, -OCH2); 3,87 (4H, t, J= 5.49 Hz, -OCH2); 3,33 (4H, d, J= 12.97 Hz, ArCH_2Ar); 2,88 (4H, t, J= 7.02 Hz, Ar$CH_2CH_2CH_2$S): 2,58 (4H, t, J= 7.02 Hz, Ar$CH_2CH_2CH_2$S); 2,34 (6H, s, -SCOCH3); 1,87 (4H, m Ar$CH_2CH_2CH_2$S). ^{13}C-NMR (125 MHz,

300 K, CDCl3) δ: 195.88, 152.05, 151.56, 133.26, 131.14, 128.91, 128.33, 127.94, 125.28, 71.10, 71.08, 70.20, 33.99, 31.33, 31.19, 30.63, 28.66, 26.91. MALDI-MS: m/z = 837.27 (100) $[M+Na]^+$.

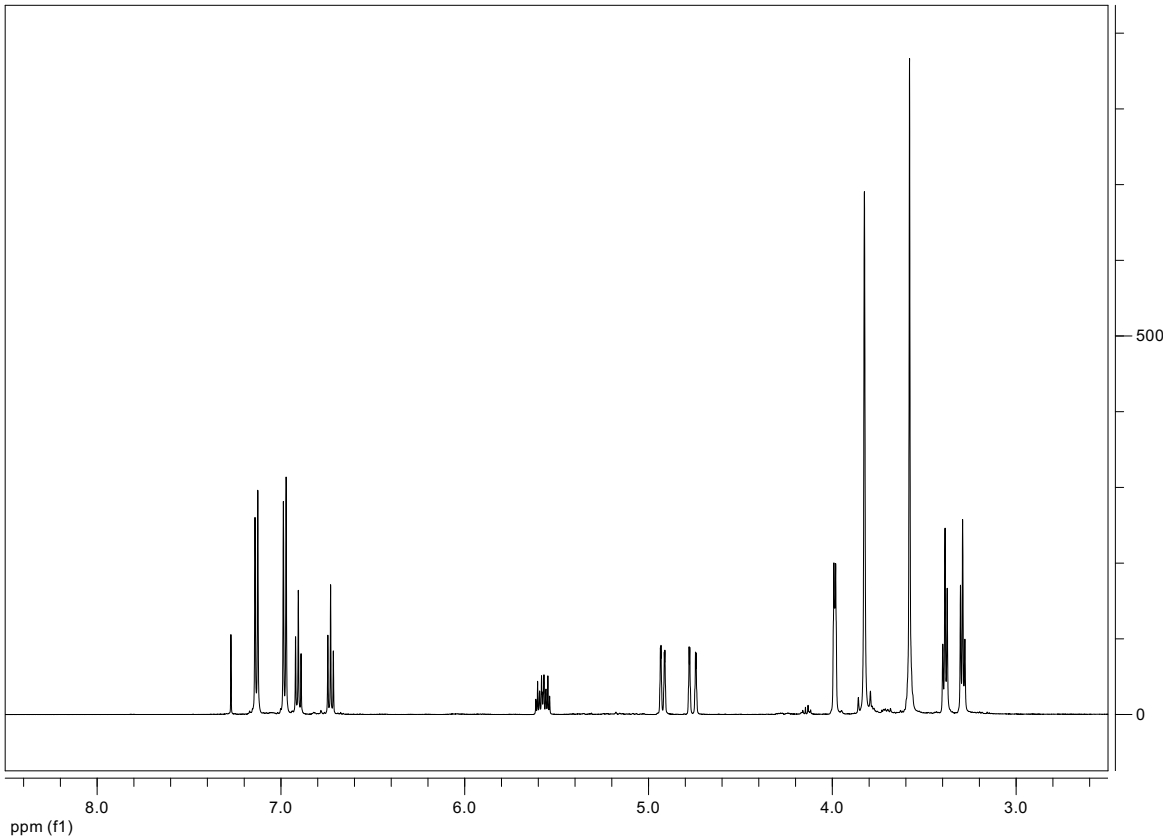

Figure S1 ^{1}H-NMR spectrum of **2** ($CDCl_3$, 300K)

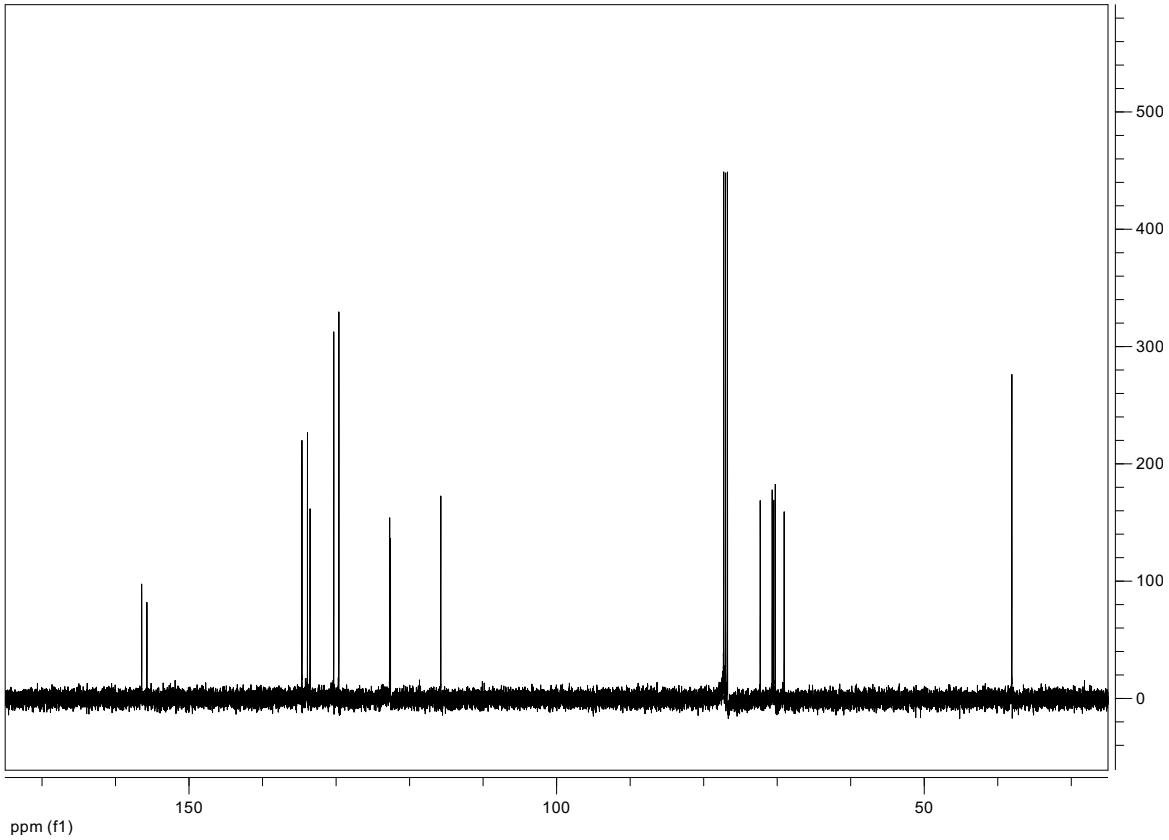

Figure S2 ^{13}C-NMR spectrum of **2** ($CDCl_3$, 300K)

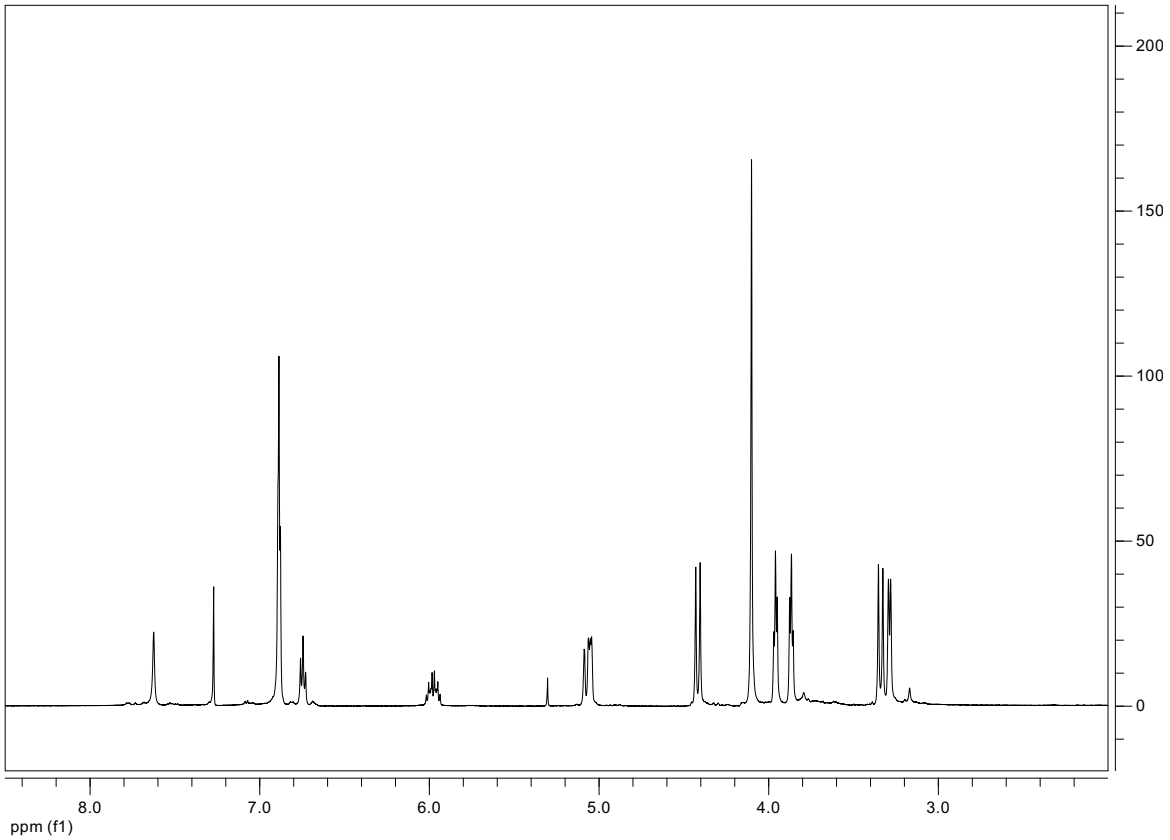

Figure S3 ^{1}H-NMR spectrum of **3** ($CDCl_3$, 300K)

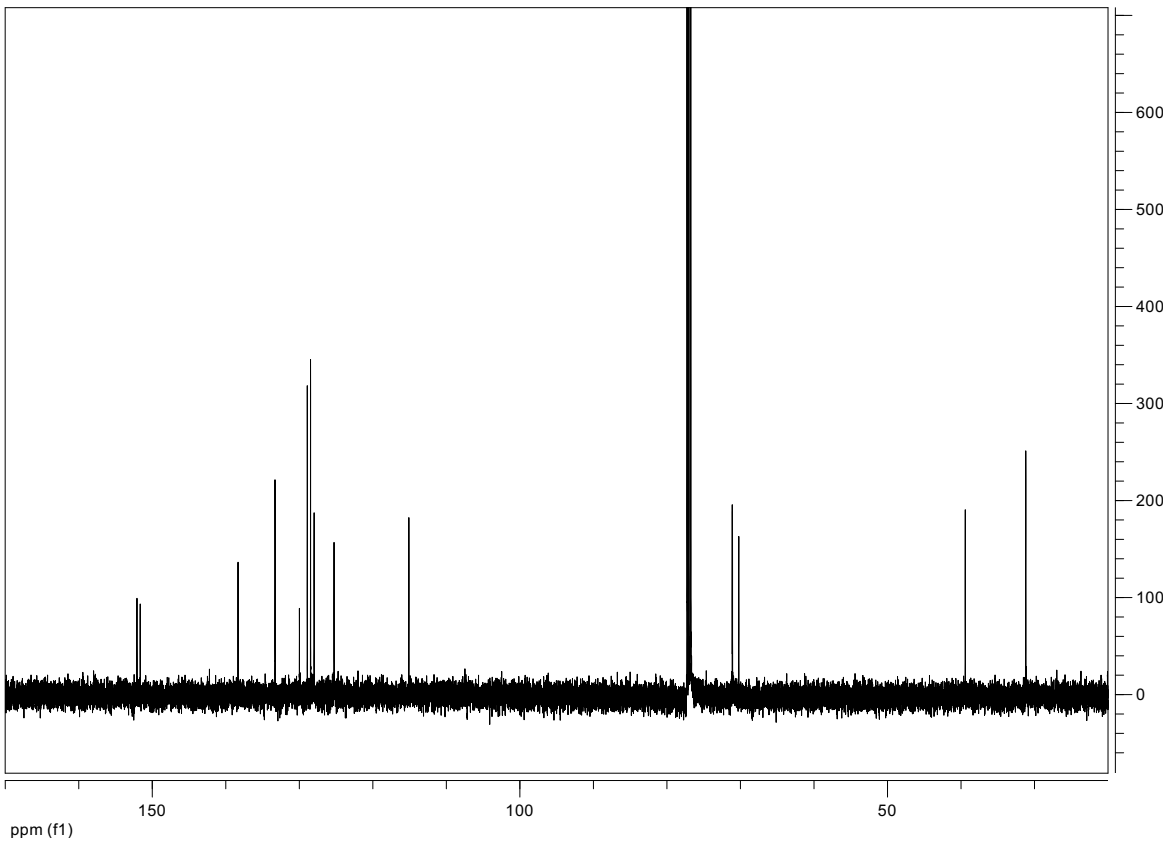

Figure S4 ^{13}C-NMR spectrum of **3** ($CDCl_3$, 300K)

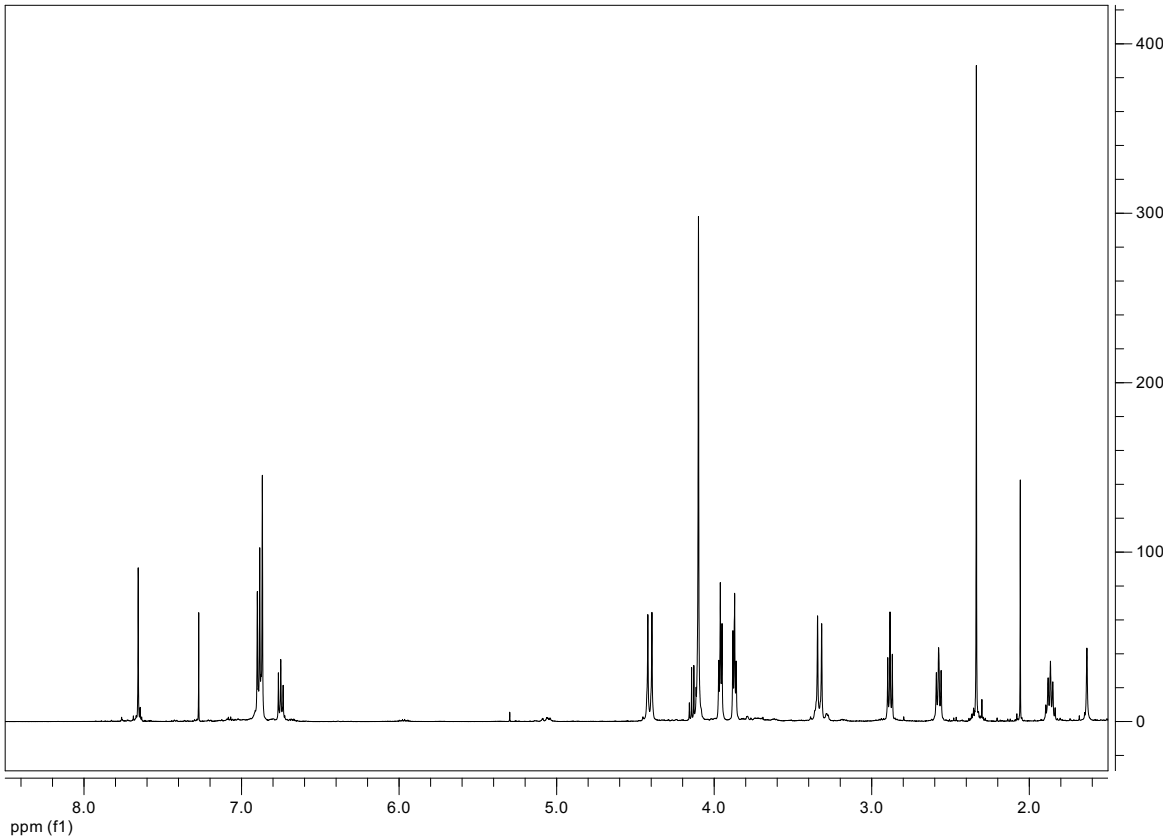

Figure S5 ^{1}H-NMR spectrum of **TCC5** ($CDCl_3$, 300K)

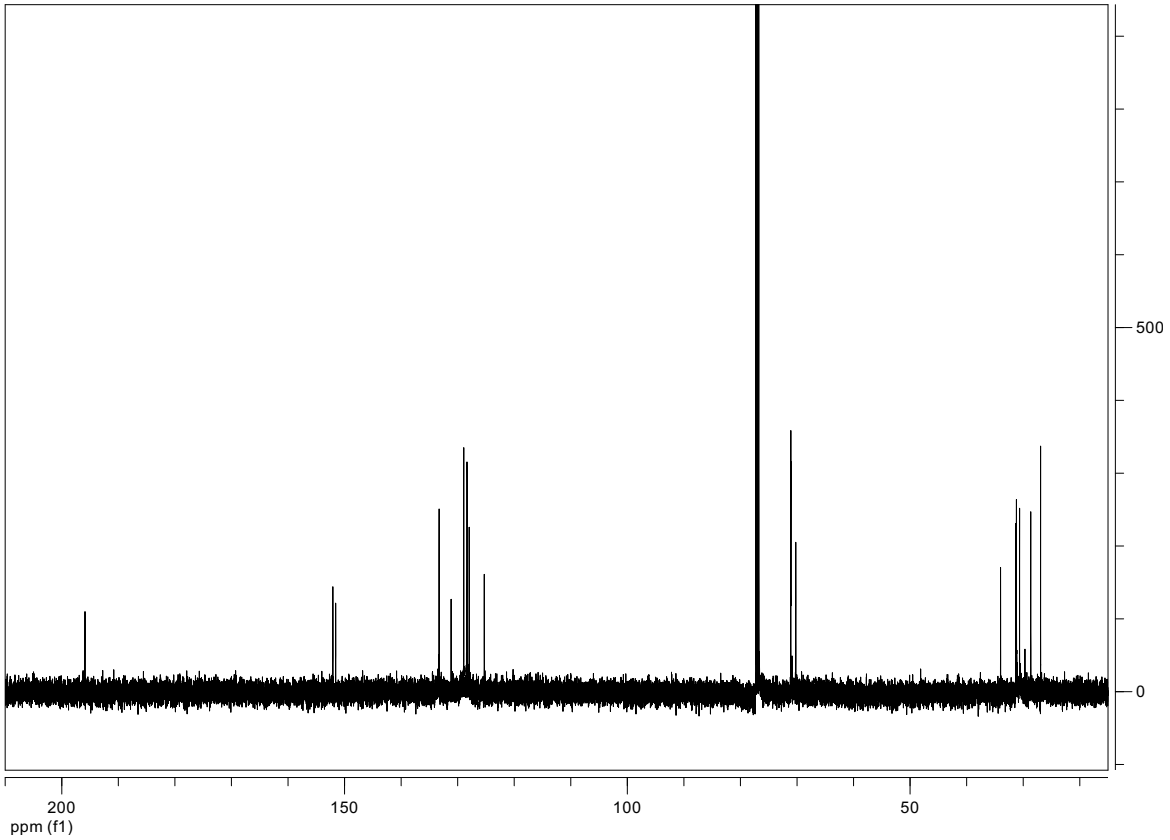

Figure S6 ^{13}C-NMR spectrum of **TCC5** ($CDCl_3$, 300K)

PREPARATION OF CALIX[4]CROWN-5 SAM

The gold sensors were cleaned by exposure to (UV)/ozone for 10 min at atmospheric pressure in a Jeligth Instr. apparatus (λexc of 185 nm and 254 nm). By this treatment, the surface is cleaned from traces of organic contaminants. The sensors were washed with ethanol and were dried under a stream of N_2 gas. A 0.5 mM solution of **TCC5** ($C_2H_4Cl_2/CH_3OH$=1:1) was prepared and an equimolar amount of KOH was added to this solution to convert the acetate group in thiol group. After 60 minute, the clean Au sensor was immersed in the solution of **TACC5** for 18 hours to form a calix monolayer on gold surface. Finally, the samples were rinsed with the solvent mixture and dried under nitrogen flux.

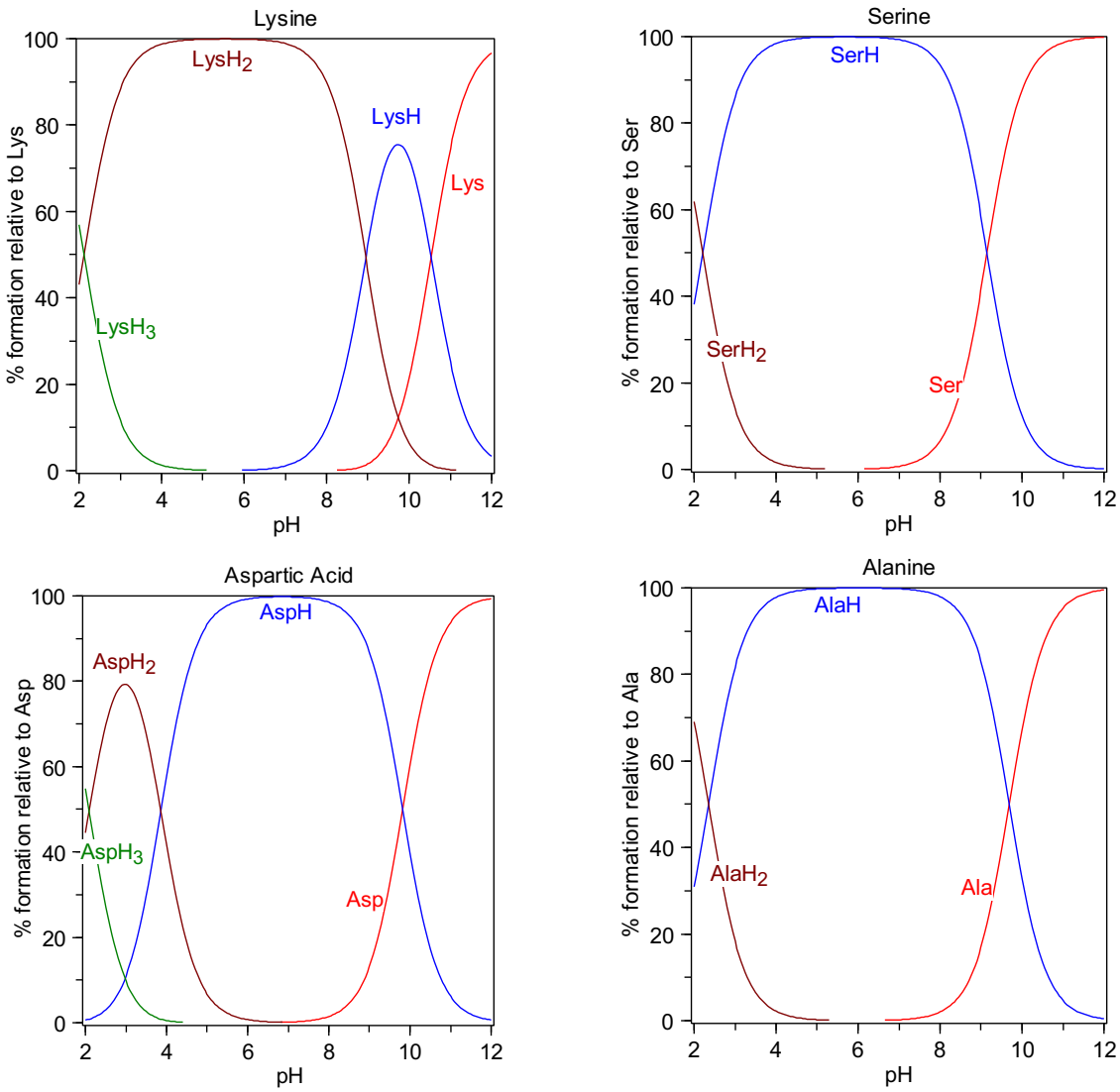

Figure S7. Species distribution diagrams for the investigated amino acids at different pH values.

NOTES AND REFERENCES

1 Jan Dirk Van Loon , Arturo Arduini , Laura Coppi , Willem Verboom , Andrea Pochini , Rocco Ungaro , Sybolt Harkema , David N. Reinhoudt J. Org. Chem., 1990, 55 (21), pp 5639–5646.