

## **Recognition of lysine, arginine and histidine by novel *para*-sulfonatocalix[4]arene thiol functionalized gold nanoparticles in aqueous solution**

Gaurang Patel, Shobhana Menon\*

Department of Chemistry, School of Sciences, Gujarat University, Ahmedabad, Gujarat, India  
Fax: +91 79 26308545; Tel: +91 79 26302286  
E-mail: shobhanamenon07@gmail.com

### **Synthetic procedure:**

All chemicals used are of analytical grade or of the highest purity available. All solutions were prepared with double-distilled, deionized water. H<sub>2</sub>SO<sub>4</sub> was purchased from Aldrich. Silica gel (Merck, 0.040-0.063mm) was used for column chromatography. Amino acids were obtained from Himedia (Mumbai, India). All amino acids standards are of 98–99% purity and were dissolved in deionized water.

UV–vis absorption spectra were acquired on a Jasco V-570 UV–vis spectrometer. IR spectra were measured with a Bruker Tensor 27 FT-IR spectrometer. Transmission electron micrograph (TEM) was recorded by a ZEISS EM-900 electron microscope operating at 100 kV. The samples are dropped onto a small formvar coated copper grid (200 mesh) and left at room temperature so that the samples precipitate homogeneously on the carbon films among the tiny pores of the copper mesh. <sup>1</sup>H NMR was carried out in CDCl<sub>3</sub> (TMS for internal standard) or D<sub>2</sub>O on a Varian Spectrometer at 400 MHz.

### **Synthesis of 25, 27-bis(12-bromo-1-oxododecane)-26,28-(dihydroxy)calix[4]arene (3)**

A mixture of calix[4]arene (3.0 g, 7.0 mmol), K<sub>2</sub>CO<sub>3</sub> (1.9 g, 14.0 mmol) and the dibromododecane **2** (14.0 mmol) in dry acetone (150 ml) was stirred for two days. The solvent was then evaporated under vacuum and the residue taken up with CH<sub>2</sub>Cl<sub>2</sub>. The organic phase was washed with 0.1 M HCl up to neutrality and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After complete evaporation of the solvent, the resulting crude product was purified by column chromatography (silica gel, hexane 9: ethyl acetate 1).

65% yield;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 400 MHz, ppm):  $\delta$ 1.30-1.41(24H, m), 1.65-1.73(4H, m), 2.09-2.29(8H, m), 3.31(4H, d), 3.89(4H, t), 4.30(4H, d), 4.90-5.12(4H, m), 5.75-5.95(4H, m), 6.61(4H, t), 6.81(4H, d), 7.14(4H, d), 8.27(2H, s). IR: (C-Br) 667; (-OH) 3350; ( $\text{CH}_2\text{-CH}_2$ ) 2841  $\text{cm}^{-1}$  m.p.: 220-225  $^\circ\text{C}$ . Elemental analysis for  $\text{C}_{52}\text{H}_{70}\text{Br}_2\text{O}_4$ : calcd. C: 65.35%, H: 7.68%; found C: 65.20%, H: 7.50%

#### Synthesis 25, 27-bis(12-thiol-1-oxydodecane)-26,28-dihydroxycalix[4]arene (4)

To 40-mL of acetonitrile (Merck) containing 1.0 g of **3** and 0.28 g of thiourea (3.65 mmol) was added and the resultant mixture was heated under reflux overnight. Acetonitrile was removed via reduced pressure. The resulting solid product was mixed with 0.37 g of KOH (6.59 mmol) and an aliquot of 40 mL of deionized water. The mixture was refluxed for 2 h. Compound 25, 27-bis(12-thiol-1-oxydodecane)-26, 28-dihydroxycalix[4]arene was extracted with 1 M HCl and  $\text{CH}_2\text{Cl}_2$ , dried with  $\text{MgSO}_4(\text{s})$ , and purified with column chromatography ( $\text{SiO}_2$ , hexane/EtOAc 1:3)

85% yield;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 400 MHz, ppm):  $\delta$ 1.20-1.40(24H, m), 1.55-1.73(8H, m), 2.00-2.19(8H, m), 3.33(2H, d), 3.80(4H, t), 4.32(4H, d), 4.92-5.15(4H, m), 5.75-5.91(4H, m), 6.60(4H, t), 6.71(4H, d), 7.14(4H, d), 8.17(2H, s). IR: (S-H) 2580; (C-S) 1041; (-OH) 3350; ( $\text{CH}_2\text{-CH}_2$ ) 2841  $\text{cm}^{-1}$  m.p.: 200-205  $^\circ\text{C}$ . Elemental analysis for  $\text{C}_{52}\text{H}_{72}\text{O}_4\text{S}_2$ : calcd. C: 75.68%, H: 8.79%; found C: 75.52%, H: 8.50%

#### Synthesis of 25, 27-bis(12-thiol-1-oxydodecan)-26,28-dihydroxy sulfonatocalix[4]arene (5)

The procedure according to Shinkai's method<sup>2</sup> was slightly modified as follows. **4** (0.5 g, 0.65 mmol) in 5 mL of sulphuric acid (96%) was stirred at 50 $^\circ\text{C}$  for 5 h. An aliquot was withdrawn from the solution and poured in to water to determine the progress of the reaction. The reaction was completed when water insoluble material was not detected. After cooling the precipitate was recovered by filtration. The product was obtained after evaporation of water. The product is dried by high vacuum overnight. The  $^1\text{H}$  NMR of **5** shows the same pattern as for **4** confirming that the *p*-sulfonatocalix[4]arene derivatives are present in the cone conformation.

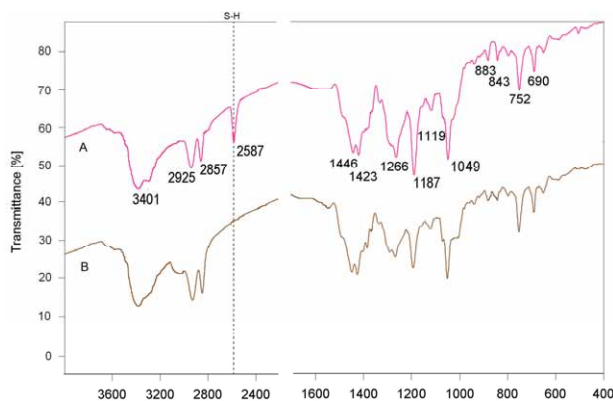
87% yield;  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ , 400 MHz, ppm):  $\delta$ 1.22-1.41(24H, m), 1.45-1.63(8H, m), 2.11-2.28(8H, m), 3.70(4H, t), 4.33(2H, d), 4.42(4H, d), 4.82-5.05(4H, m), 5.79-5.90(4H, m), 6.61(4H, t), 6.75(4H, d), 7.18(4H, d), 8.19(2H, s). IR: (S-H) 2579; (C-S) 1040; (S=O) 1052; (-OH) 3431 ( $\text{CH}_2\text{-CH}_2$ ) 2839  $\text{cm}^{-1}$  m.p.: 205-210  $^\circ\text{C}$ . Elemental analysis for  $\text{C}_{52}\text{H}_{72}\text{O}_{16}\text{S}_6$ : calcd. C: 52.92%, H: 5.92%; found C: 52.14%, H: 5.36%

### Synthesis of Gold nanoparticles

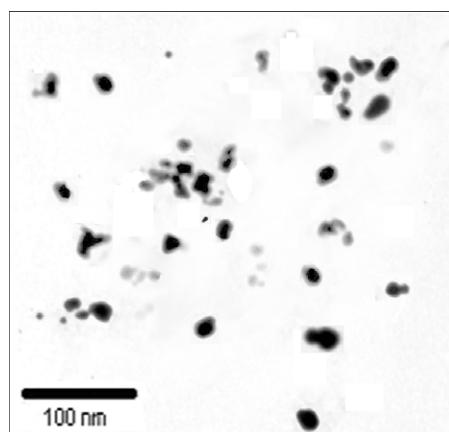
The procedures were essentially the same as those developed by Turkevich-Frens.<sup>1</sup> with difference only in the molar ratio of  $\text{HAuCl}_4$  to sodium citrate, where higher  $\text{HAuCl}_4$  concentration resulted in larger particles. All glassware was thoroughly cleaned with freshly prepared 3:1  $\text{HCl}/\text{HNO}_3$  (aqua regia) and rinsed thoroughly with Millipore-Q water prior to use. The 18  $\text{M}\Omega\text{ cm}^{-1}$  water was used to prepare all solutions in this study. To synthesize 18-nm-diameter colloids, in a round bottom flask equipped with a condenser, 180 mL of 0.8 mM  $\text{HAuCl}_4$  was brought to a boil with vigorous stirring, and 10 mL of 19.4 mM sodium citrate was rapidly added to the vortex of the solution. The solution changed from pale yellow to burgundy. The solution was boiled for additional 10 min and then cooled with stirring for another 15 min. After the solution reached room temperature, it was filtered through a 0.45 $\mu$  filter (Millipore, Nylon membrane).

### Synthesis of *p*-sulphonato calix capped gold nanoparticles

Modification of nanoparticles preceded by adding **5** to colloidal gold in water and stirring in the dark for 3 h.<sup>3</sup> Specifically, an aqueous solution of **5** (3 ml, 0.25 mM) containing equivalent of sodium hydroxide was added to an aqueous solution of citrate stabilized gold nanoparticles (10 mL, 0.8 mM) under stirring. It was stirred at room temperature and filtered (0.45  $\mu\text{m}$  millipore filter). The particles were purified by repeated centrifugation (3 times) at 5000 rpm and re-dispersion in water. The final concentration obtained 0.65mM based on the pure gold nanoparticle concentration.



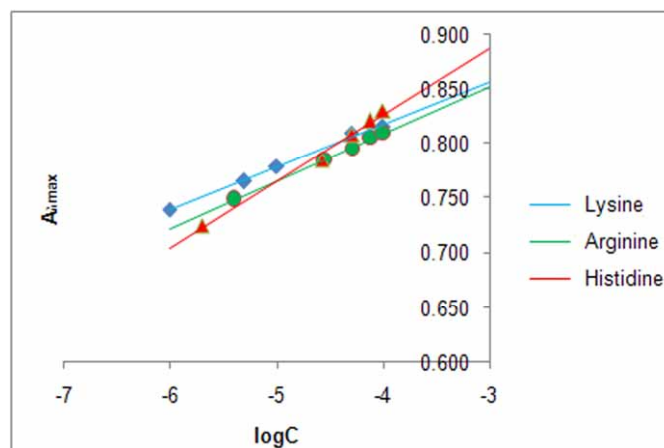
**Fig. S1** Transmission IR spectra of (A) pSC4T (B) pSC4T modified gold nanoparticle Note: the arrow is pointing to the disappearance of S–H stretching in the pSC4T-Au Np



**Fig. S2** TEM micrographs of calix functionalized gold nanoparticles after addition of 0.0001M alanine into 0.62 mM calix-gold nanoparticles solution.

**Table S1.**

<b>Solution pH</b>	2	4	6	8	10
<b>Stability of Au Np-Calix</b>	1 h	2 h	Week	Months	Months



**Figure S2.** Linear correlations between  $A_{\lambda_{\max}}$  and the logarithm of Lys, Arg and His concentration

### References:

- [1] Enustun B V, Turkevich J, *J. Am. Chem. Soc.*, 1963, **85**, 3317
- [2] S. Shinkai, S. Mori, H. Koreishi, T. Tsubaki and O. Manabe, *J. Am. Chem. Soc.*, 1986, **108**, 2409
- [3] Y. Kim, R. C Johnson, J. T Hupp, *Nano Lett.*, 2001, **1**, 165.