Electronic Supplementary Material (ESI) for ChemComm.


# A co-pillar[5]arene sensor for linear biogenic amines

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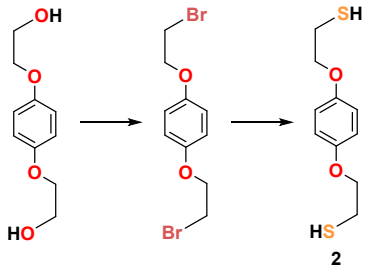
**Supplementary Information**

**Synthesis of 2**




2

1,4-Bis(bromoethyoxy)benzene was synthesised according previously reported procedure.[1] 1,4-Bis(hydroxyethoxy)benzene (5.00 g, 25.2 mmol) was mixed with triphenylphosphine (15 g, 60 mmol) in CH3CN. The white mixture was stirred and cooled to 0°C. Carbon tetrabromide (20.00 g, 60 mmol) was slowly added in small portions to a solution with stirring, at 0°C. The reaction mixture was left to warm to room temperature and the resulting clear solution was stirred for another 4 h under $N_2$ gas. Then cold water (100 ml) was added to precipitate a white solid which was collected by vacuum filtration. The solid was then recrystallised from hot methanol (200 ml) producing white needle-crystals. 35 formed as white flake-like crystals following vacuum filtration.

1,4-Bis(bromoethyoxy)benzene: yield 6.54 g (20 mmol, 80%); m. p. 112.1-113.0°C; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.85 (s, 4H, ArH), 4.23 (t, J = 6.35 Hz, 4H, -CH2O), 3.59 (t, 4H, J = 6.4 Hz, -CH2Br); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 152.89 (Ar-C-0), 116.49( Ar), 116.17 (Ar), 115.8 (Ar), 68.81 (OCH2), 29.38 CH2Br); HRMS (m/z): calcd for $[M + Na]^+$, $C_{10}H_{12}Br_2O_2{\cdot}NaC_2HO_2$, 391.9063; found, 391.9195. Note: the molecular ion could not be detected for this compound and was always observed as the sodium orthoformate complex.

1,4-Bis(thioethoxy)benzene (**2**) was synthesised using a previously reported thiolation method.[2] 1,4-Bis(bromoethyoxy)benzene (0.897 g, 3.25 mmol) was dissolved in dry THF (30 ml) at -30°C under a nitrogen atmosphere. To this, $(SiMe_3)_2S$ (1.64 ml, 7.78 mmol) was added followed by addition of tetrabutylammonium fluoride trihydrate, TBAF·3$H_2O$, (2.26 g, 7.16 mmol). The resulting light green reaction mixture was stirred overnight and allowed to warm to room temperature. The solvent was evaporated and deionised water (50 ml), and DCM (50 ml) were added to the resulting residue. The organic layer was separated and washed three times with water before the organic layer was dried with anhydrous $Na_2SO_4$ and filtered. Solvent was removed by rotary evaporation to give 1,4-bis(bromoethyoxy)benzene, **2**, as a colourless oil mixture following an ethanol wash.

1,4-Bis(thioethyoxy)benzene (**2**): yield 0.56 g (2.43 mmol, 78%); m.p. 64–66°C; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.84 (s, 4H, ArH), 4.06 (t, J = 8, 4 Hz , H, -$CH_2O$), 2.89 (dt, $J_1$ = 8, $J_2$ = 8.5 Hz, 4 H, -$CH_2S$), 1.67 (t, J = 8 Hz, 2 H, SH); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm): 153.11 (Ar-C-O), 116.49 ( Ar),

116.51 (Ar), 70.11 ($OCH_2$), 24.31 ($CH_2S$). Analytical data are consistent with literature values for this compound prepared by an alternative route.[3]

**Preparation of gold electrodes**

The gold electrode (2.0 mm diameter) was mechanically polished with two micropolish alumina suspensions of 0.3 µm followed by 0.05 µm for 3 min each and washed with distilled water. Subsequent ultrasonic cleaning with absolute ethanol removed the residual alumina powder.

After mechanical cleaning, the gold electrode was immersed in piranha solution ($H_2SO_4/H_2O_2$, 3:1 v/v) for 10 min at room temperature and finally washed with distilled water. Prior to sensor fabrication, a gold working electrode was electrochemically cleaned by cycling between -0.3 and 1.5 V at a scan rate 0.1 $Vs^{-1}$ versus Ag|AgCl in 0.5 M sulfuric acid until gold oxide formation was detected in the voltammogram. The electrodes were washed thoroughly with distilled water and dried in a nitrogen stream to obtain a clean gold surface.

**Attachment of 1 and 2 to gold electrodes**

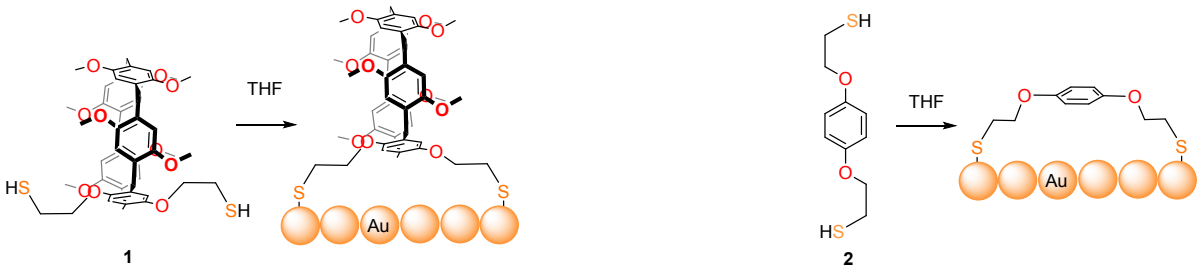



**Fig. S1** Surface modification by **1** and **2**

The procedure for the attachment of **1** has been previously described.[4] Briefly, the 2.0 mm gold electrode was placed in a solution of **1** (2 ml of a 10 mM solution in THF) from 20 min to 6 h at ambient temperature. Surface attachment time was found to be complete at 4 h. The modified gold electrodes were characterized by cyclic voltammetry and electrochemical impedance spectroscopy (EIS). All cyclic voltammetric measurements were conducted using a 3M KCl Ag|AgCl reference electrode and platinum wire as a counter electrode. A solution of 10 mM potassium ferrocynaide in 0.1 M KCl (pH 7.0) was used as a redox probe and scanned at 0.1 $Vs^{-1}$ from -0.1 to +0.6V on bare and modified gold electrodes. Electrochemical impedance spectroscopy (EIS) was performed at 270 mV with an amplitude of 10 mV at a frequency from $1x10^6$ Hz to 1 Hz in an aqeuous solution of 0.5 mM

potassium ferricyanide and 0.5 mM potassium ferrocyanide in 1 M KCl. The same methods were used to coat electrodes with monomer **2**.

**Electrochemical apparatus**

All the measurements were made using a conventional three-electrode system comprising reference, counter and working electrodes. Platinum wire acted as the auxiliary electrode. All the potentials are referred to an Ag|AgCl/KCl saturated reference electrode. The gold electrode (2.0 mm diameter) was used as working electrode. All the electrochemical experiments were carried out at room temperature (25°C) without. The potential scan was carried out between -0·3 and +0·3 V at 100 $mVs^{-1}$.

**Determination of biogenic amines on surface modified gold electrodes**

Voltammetric scans were carried out in 1 mM solutions of pentylamine (PA), putrescine (PUT), spermidine (SPD) and spermine (SPR) freshly prepared in HPLC grade water. Recordings were carried out between -0.3 and +0.3 V at a 0.1 $Vs^{-1}$ scan rate. All cyclic voltammetric measurements were carried outing a Ag|AgCl reference electrode and platinum wire as a counter electrode. Measurements were carried out using a bare gold electrode and gold electrodes with **1** or **2** attached. Electrodes were rinsed in deionized water between each recording. The anodic (EpA) and cathodic (EpC) peak potential were recorded from measurements.

**Calibration of spermine on gold electrode modified with 1**

The cyclic voltammograms of various concentration of spermine on a gold electrode modified with **1** is shown in Fig S3. Clear increases in the current can be observed with increasing concentration, however the sensitivity was far greater for the EpC than the EpA. The EpC responses for varying concentrations of sperime (0.1 to 1 MM) are shown in Fig S3B. The calibration response for both the EpA and EpC are shown in Fig S3C. The sensitivity of the sensor was 23.5 nA $mM^{-1}$ for the EpA and 57.8 nA $mM^{-1}$ for the EpC. For the response at the EpC, the limit of detection of 113 µM and the limit of quantification was 376 µM.

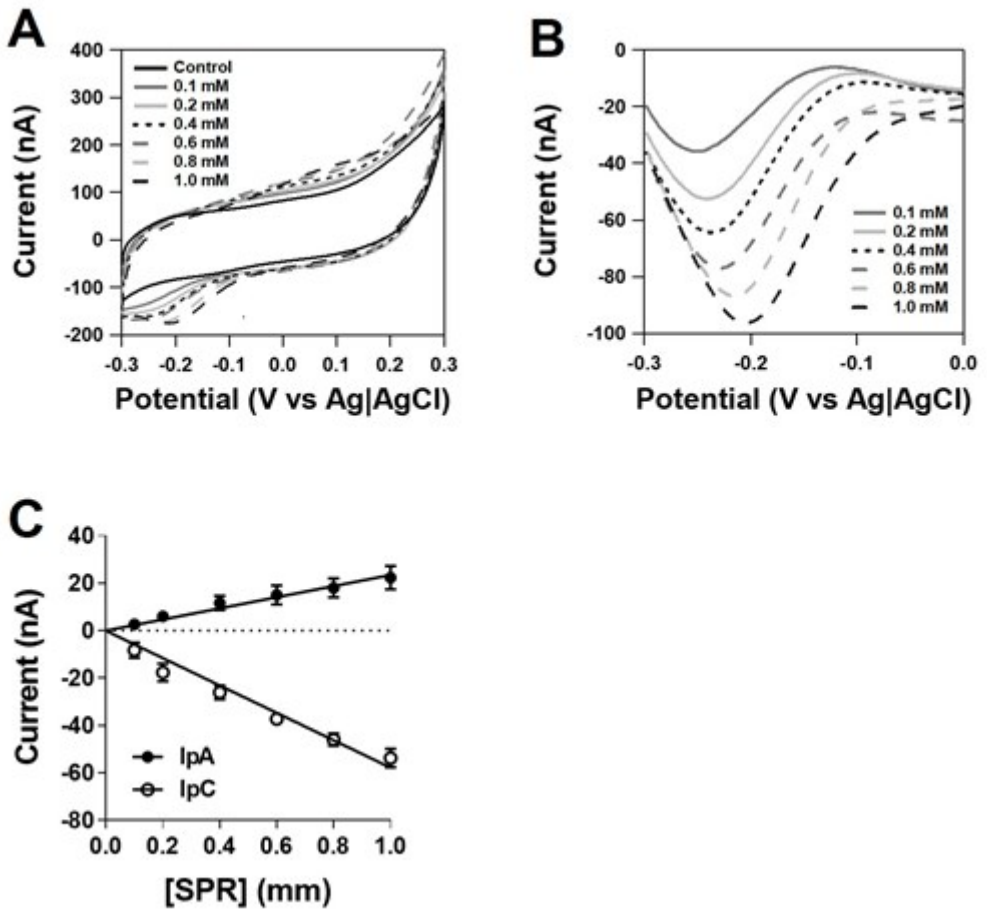



**Fig. S2** Relationship between the concentration of spermine and current. (A) shows cyclic voltammograms on an electrode modified with **1** for varying concentration of spermine, (B) shows the EpC response at varying concentrations following subtraction from the blank and (C) gives calibration curves.

**Electrode stability**

When running 20 sequential scans of 1 mM spermine on the gold electrode with **1** attached we observed a 3.4 % decrease in the response (n = 3). There was also no significant difference in the responses after 5 days were the signal decreased by 5.4 % (n = 3).

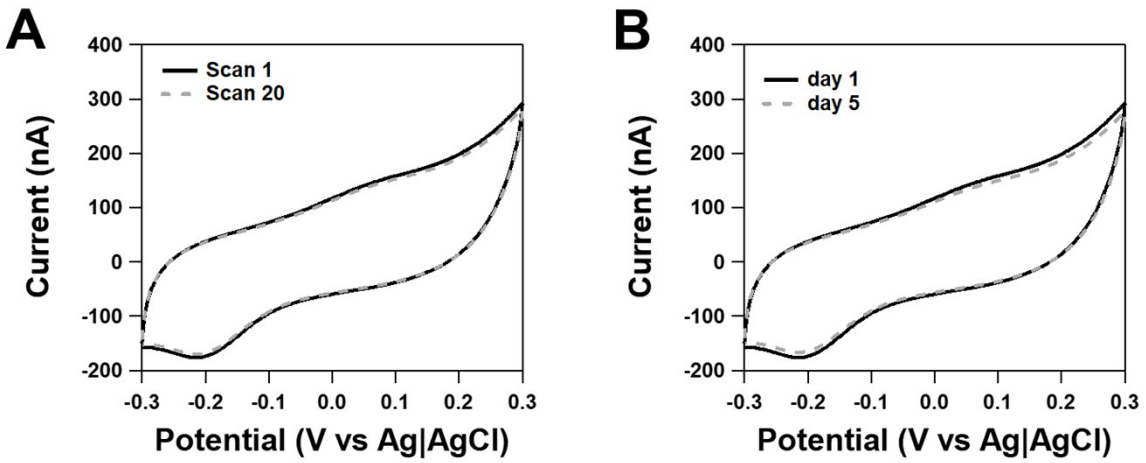



**Fig. S3** Electrode stability over (A) 20 scans and (B) five days.

**Potential binding orientations**

To visualise how the biogenic amines may be binding within the macrocyclic cavity a series of gas phase calculations were undertaken in which each amine was threaded into 1,4-dimethoxypillar[5]arene. Calculations were undertaken using *Spartan '16 Parallel Suite*[5] running on a Mac Pro with 3.5 GHz 6-Core Intel Xenon E5 processors and two threads per core. An initial molecular geometry was generated using molecular mechanics energy minimization methods (Merck Molecular Force Field). The resulting atomic coordinates were used as the input to a semiempirical (PM6) calculation. The effects of threading are apparent when analysing the ΔG of binding as greater levels of encapsulation are experienced by the amine. The structures are shown in Figure S4 with the ΔG values tabulated as complexation evolves.

**Table S1** Predicted $\Delta G_{(binding)}$ for 1,4-dimethoxypillar[5]arene-amine complexes and hydrogen bonds formed

| **Complex** | **PM6 predicted binding energy (kJmol$^{-1}$)/number of hydrogen bonds** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **$\Delta G_0$** | **H-bond** | **$\Delta G_I$** | **H-bond** | **$\Delta G_{II}$** | **H-bond** | **$\Delta G_{III}$** | **H-bond** |
| Pentylamine | 0.0 | 0 | -4.0 | 1 | -28.0 | 1 | - | - |
| Putrescene | 0.0 | 0 | -8.1 | 0 | -33.3 | 4 | - | - |
| Spermine | 0.0 | 0 | +6.5 | 0 | -24.4 | 2 | -30.3 | 2 |
| Spermidine | 0.0 | 0 | -5.0 | 1 | -32.1 | 1 | -39.1 | 2 |

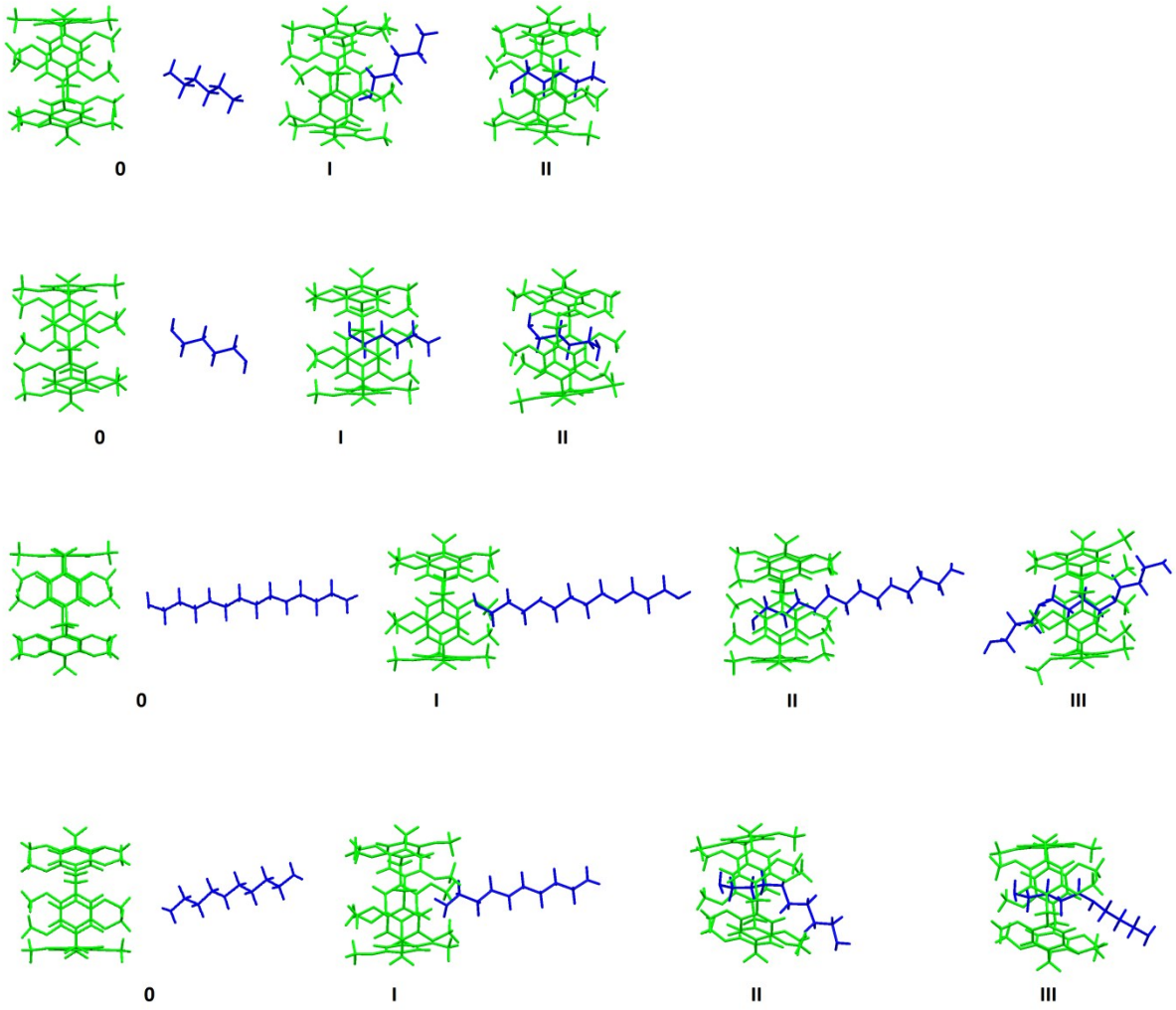



**Fig. S4** Complexes formed with 1,4-dimethoxypillar[5]arene: (from top) pentylamine, putrescene, spermine and spermidine

## References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