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Journal Name

COMMUNICATION

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

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A thiolated co-pillar[5]arene was attached to the surface of a gold electrode and shown to give an analyte-selective voltammetric response to linear biogenic amines.

The linear biogenic amines putrescine, spermine and spermidine have no intrinsic fluorescent or chromogenic properties so their detection, and particularly differentiation between them, is hard to achieve. Although not highly toxic in themselves, they are indicators of bacterial growth and are thus markers of food spoilage.¹ Elevated levels of these biogenic amines, particularly spermidine, are also often found in the urine of cancer patients.² Biogenic amine analysis therefore has applications in food security and clinical assessment of the efficacy of cancer treatment. They have been shown to interact favourably with macrocycles known as pillar[5]arenes which suggests that detection of biogenic amine complexes could be used to monitor their concentrations.³ Some progress has been made in this direction with water-soluble pillar[5]arene modified silver nanoparticles responding to spermine, tetraethylenepentamine and triethylenetetramine.⁴ Impedance measurements of a surface immobilized calix[4]crown-5 can differentiate between levels of spermidine in urine samples taken from cancerous and non-cancerous patients⁵ and gold nanoparticles coated with a calix[4]crown-5 give a distinctive pink to blue colour change in the presence of either spermine or spermidine.⁶ Here we describe a method which can discriminate between three biogenic diamines.

Since the first report of 1,4-dimethoxypillar[5]arene in 2008⁷ numerous applications have been found for members of the pillar[n]arene class of macrocycles.⁸⁻¹⁷ They have been employed to detect species as diverse as paraquat,¹⁸⁻²⁰ dopamine,²¹ phosphate²² and bacterial lectins²³ predominantly by fluorescent^{3,24-39} or electrochemical³⁰⁻³⁹ methods. However, the association constants between a pyrene labelled

pillar[5]arene and the three biogenic amines showed no significant differences when assessed by fluorescence.³ To exploit the known association between linear polyamines and pillar[5]arenes, co-pillar[5]arene dithiol derivative **1** (Fig. 1) was designed to adhere to gold while aligning the macrocyclic cavity parallel to the metal surfaces so that small molecules could be detected as they passed through.⁴⁰

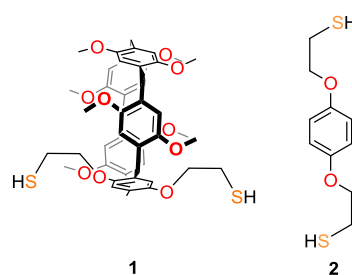


Fig. 1 Co-pillar[5]arene **1** and monomer **2**.

A gold electrode surface was treated with **1** over 4 h whereupon a combination of cyclic voltammetry and impedance was utilised to demonstrate successful binding. Fig 2A shows the voltammetric response of 1 mM ferrocyanide on the gold electrode before and after addition of **1**. The faradaic responses completely disappear, resulting in a purely capacitive signal which is suggestive of hindered mass transfer of ferrocyanide to the electrode surface consistent with the presence of a surface monolayer.

Fig 2B shows the real and imaginary impedance for the gold electrode before and after treatment with **1**. Charge transfer resistance (R_{ct}) significantly increased from $21 \pm 0.2 \Omega$ to $42.3 \pm 5.5 \Omega$ following addition of **1** ($P < 0.001$, $n = 3$) and consistent with surface attachment leading to hindered electron transfer for the redox couple. The ohmic resistance (R_Ω) significantly decreased following attachment of **1** ($p < 0.001$, $n = 3$) which is also indicative of a surface monolayer attached to the electrode surface.

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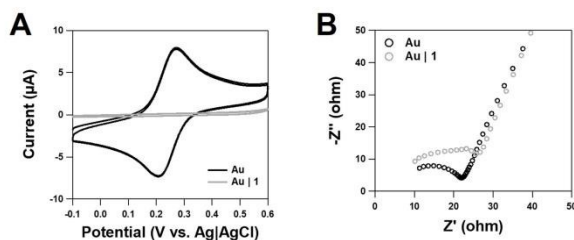


Fig 2. Evaluation of the attachment of **1** on to a gold surface through voltammetric (A) and electrochemical impedance spectroscopy (B) measurements.

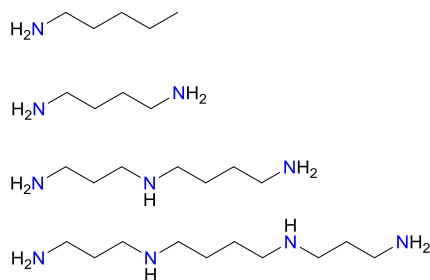


Fig 3. Amines tested: (from top) pentylamine, putrescine, spermidine and spermine

To understand how **1** detects the linear biogenic amines shown in Fig. 3, voltammetric responses were determined, initially with spermidine on the bare gold electrode then the electrodes surface-modified with monomer **2** or co-pillar[5]arene **1** (Fig 4A).

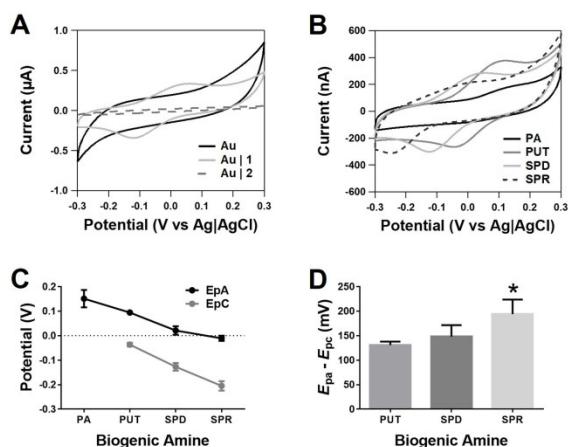


Fig. 4 Detection of linear biogenic amines: (A) response of spermidine on a gold electrode with and without attachment of **1** and **2**, (B) voltammetric responses of pentylamine (PA), putrescine (PUT) spermidine (SPD) and spermine (SPR) on a gold electrode with **1** attached, (C and D) responses of oxidation peak potential (EpA) and reduction peak potential (EpC) for the biogenic amines and difference between the EpA and EpC (mean \pm S.D., $n=3$, * $p < 0.05$)

No faradaic responses were observed for the bare gold electrode or with **2** attached, suggesting that oxidation under the small potential window was not facilitated. The capacitive current for the electrode with **2** attached was significantly lower than the gold electrode, suggesting successful attachment. The electrode modified with **1** showed faradaic responses for spermidine with oxidation and reduction peaks indicating that the electrochemical activity of spermidine is facilitated through the host-guest interaction with **1**. Consequently it is the presence of the macrocycle on the surface which facilitates amine binding and detection.

Fig 4B shows the voltammetric responses of four different linear biogenic amines of increasing complexity, putrescine, spermidine, spermine and *n*-pentylamine. Fig 4C shows the response of the oxidation and reduction peak potential (EpA and EpC, respectively) to the amines which reveals a linear relationship between the peak potential for oxidation and reduction decreasing with increasing numbers of amine groups present. This is consistent with the kinetics for electron transfer being most favourable for spermine, with four amine groups and a longer overall chain length, than for *n*-pentylamine or putrescine. Fig 4D shows the voltage difference between EpA and EpC an indicator of kinetic and mass transfer behaviour of the redox species which is significantly higher for spermine than putrescine or spermidine ($p < 0.05$, $n = 3$). This suggests that access to the electrode surface is most difficult for spermine even though it has excellent electron transfer kinetics. These findings indicate that host-guest interactions between the co-pillar[5]arene and the linear biogenic amines facilitate electron transfer from the amine group to the electrode surface.

A linear relationship between the concentration of spermine and current was observed on the gold electrode modified with **1** (Fig. S2). The current monitored at EpC was greater than that for EpA. The sensitivity was 57 nA mM^{-1} and the limit of detection was $113 \text{ }\mu\text{M}$. Repeated cycling of the modified electrodes and daily use over five days resulted in negligible attenuation of the response to analytes (Fig S3.)

A semiempirical computational investigation of biogenic amine complexation by 1,4-dimethoxypillar[5]arene, as a model for **1**, suggests that the amines approach the macrocycle before threading through (see ESI). As this process proceeds the number of intermolecular hydrogen bonds increases, leading to increasing $\Delta G_{\text{(binding)}}$ values and the formation of pseudorotaxanes analogous to those observed in other pillar[5]arene-amine complexes.^{3,27} We propose that reversible formation of rotaxanes between macrocycle **1** and linear amines generates the voltammetric signal and reversible peaks occur when the amine is bound by the macrocycle. A similar mechanism has been suggested for the complexation of amines by calix[4]crown-5.⁵

In conclusion, we have demonstrated that electrochemical detection of putrescine, spermidine and spermine is facilitated through complexation between the co-pillar[5]arene and the linear biogenic amine. The resultant faradaic response is dependent on the number of amine groups and carbon chain length within each biogenic amine. While other macrocyclic

systems have been used to detect linear polyamines,³⁻⁶ this approach is able to rapidly differentiate between biogenic amines.

Conflict of interest

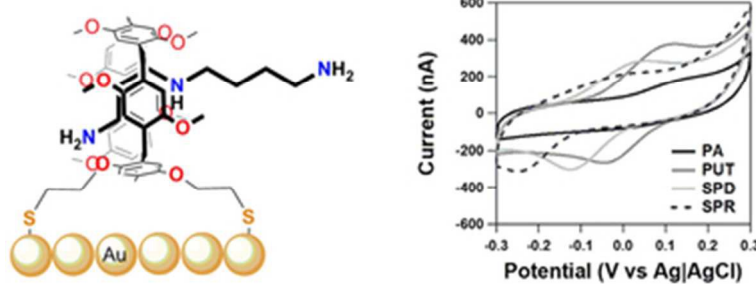
There are no conflicts to declare.

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