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A 'naked-eye' chemosensor system for phytate

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Abstract—We report the synthesis of two new anion receptors of a covalently linked 1,3,5-triarylbenzoamido-crown ether. Our results show that combined with a picrate salt they act by means of an intermolecular charge transfer process (EDA complex), as naked-eye sensors for basic anions, especially for sodium phytate in DMSO/H_2O (1:1).

In the field of supramolecular chemistry the use of naked-eye anion chemosensors capable of the simultaneous complexation of cationic and anionic guests has recently experienced a considerable advance due to the great advantages the use of these sensors presents in relevant fields such as biology, the environment or food technology.[1] These chemosensors are constructed according to the receptor–chromophore general binomial, covalently binding a specific anion receptor with another unit, a chromophore, responsible for translating the former receptor–anion association into an optical signal.[2,3] This colour variation can be related to either structural or conformational changes in the receptor structure when the complex is formed[4] or even to the formation of a charge transfer complex.[5]

Myo-Inositolhexakis(dihydrogenphosphate) dodecasodium salt, a compound known as phytate, present in blood, urine and interstitial and intracellular fluids, is also known to be an important constituent of the human diet[6] (Fig. 1). From several studies, in vitro and in vivo, the results prove that phytate plays an important role as a crystallisation inhibitor of calcium salts in biological fluids,[7] becoming a clear alternative in the treatment of calcium oxalate renal lithiasis. In addition, phytate presents other therapeutic properties as an anticancerogenic [8] or an antioxidant agent.[9] Nevertheless, to our knowledge and despite the great interest, there is no evidence of the existence of naked-eye chemosensing systems for the determination of phytate.[10]

Herein we report two new anion sensing systems, chemosensors, based on 1,3,5-triarylbenzoamidobenzo-15-crown-5, **6a**, and 1,3,5-triarylbenzoamido-18-crown-6, **6c**, receptors (Scheme 1), which in addition to a chromophore not covalently coordinated to the receptor, that is, a picrate salt (ammonium, sodium or potassium), act together as a colorimetric anion sensor capable of initiating a visible to the naked eye colour change, which is specific for basic anions and especially for sodium phytate (Fig. 1).

Receptors **6a** and **6c** were synthesised from the previously reported triester.[11] As illustrated in Scheme 1, tris ester **1** was protected as the corresponding tris benzyl **2a** or tris allyl ethers **2b**. Hydrolysis of triesters **2a**–**b** afforded the triacids **3a** and **3b**, respectively, which were converted to triacyl chlorides **4a**–**b** and coupled with 4′-aminobenzo-15-crown-5 and 2-(aminomethyl)-18-crown-6, in dichloromethane, respectively, to produce receptors **5a**, **5b**, **5c** and **5d** in 85%, 52%, 77% and 48% yields, respectively. Debenzylations were observed with compounds **5a** and **5c** using Me_3SiI/CH_2Cl_2 providing yields of 63% and 44% for **6a** and **6c**, respectively. ^1H, ^{13}C NMR and mass spectra were consistent with the proposed structures.[12]

The receptors **6a** and **6c** showed a high affinity for picrate salts. In the ^1H NMR spectra, the proton signals of the crown ether ethylenes of **6a** and **6c** shifted downfield upon complexation with ammonium, sodium and potassium picrate, indicating the presence of a

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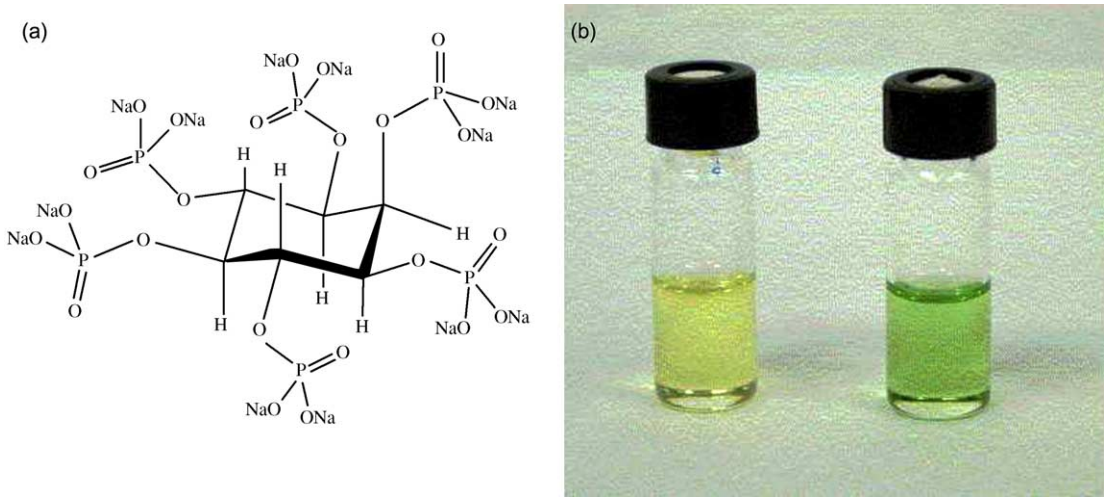
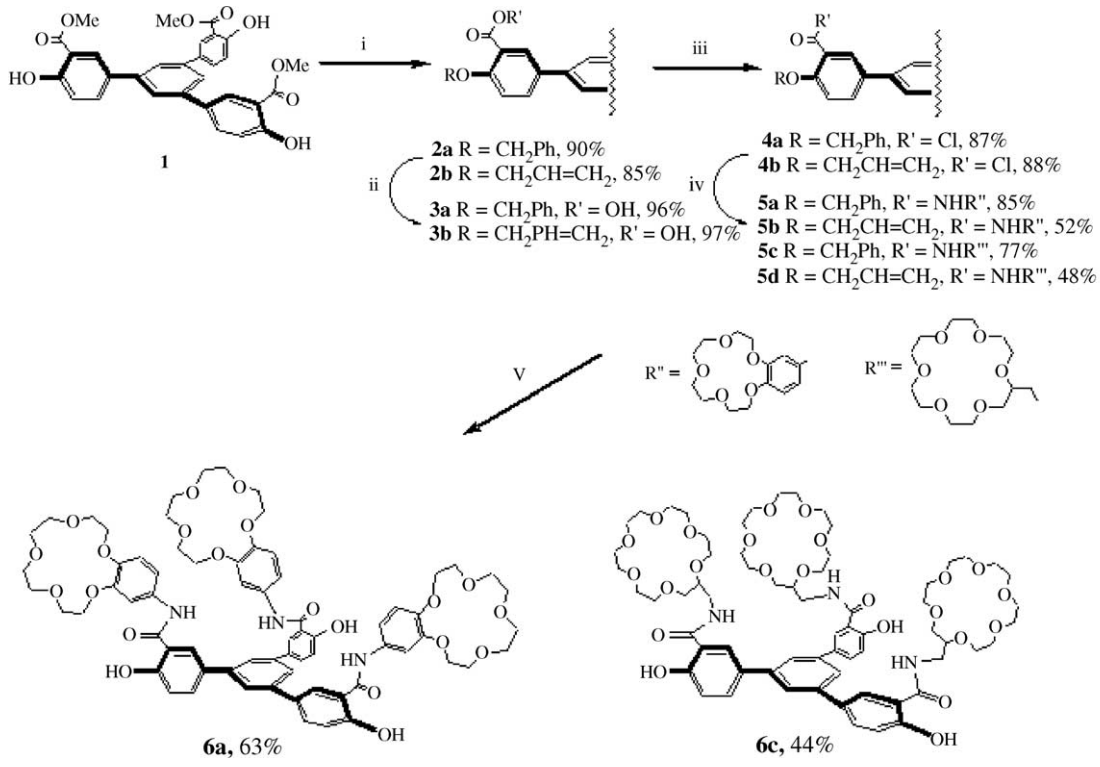


Figure 1. Sodium phytate. Colour change in the presence of **6a**/potassium picrate in DMSO/H_2O (1:1): from left to right: no anion, sodium phytate.



Scheme 1. Reagents and conditions: (i) K_2CO_3, RBr; (ii) NaOH, H_2O/THF, reflux; (iii) $SOCl_2/CH_2Cl_2$; (iv) $R''NH_2$, CH_2Cl_2; (v) Me_3SiI, CH_2Cl_2.

cation–dipole interaction between the metallic and the Lewis basic crown ether groups. The extractabilities ([complexed]/[free + complexed] × 100%) of **6c** in CH_2Cl_2 were estimated to be sodium picrate, 51%, ammonium picrate, 87% and potassium picrate, 99%.

The chemosensor systems were prepared by mixing equal volumes of a receptor solution, **6a** or **6c**, 10^{-3} M, with a 10^{-4} M picrate salt solution and an anion solution of 10^{-2} M in DMSO. Thus, for example, upon addition to the **6a**/potassium picrate system, which is initially pale yellow due to the presence of the picrate ion, a basic anion in the form of a tetrabutylammonium acetate, citrate, trimesoate, isophthalate, F^- or $H_2PO_4^-$ salt, the solution changes from yellow to dark green in a few seconds. No colour variation was observed for tetrabutylammonium salts of the following anions: Cl^-, Br^-, I^-, NO_3^- and HSO_4^- under the same experimental conditions. In the case of insoluble salts in DMSO, such as potassium acetate, KF, $NaHPO_4$ and sodium phytate, a mixture in DMSO/H_2O (1:1 v/v) was employed, the same colour variation being observed. The **6a**/potassium picrate system has proved to be very useful with both simple monoanionic salts and more complex poly-

anionic salts, GMP and ADP. On the contrary, no colour variation was observed when using the **6a** and **6c**/potassium picrate systems, respectively, with ATP.

In the same way, we have proved that our chemosensor systems formed independently with receptors **6a** or **6c**, with either ammonium picrate, sodium picrate or potassium picrate showing the same colouration change when adding a TBA acetate solution. However, in the absence of the picrate ion there was no colour variation detected. The formation of the **6a**/picrate-anion complex, implies the change of colour from yellow to green, which usually takes a few seconds to occur, the final green colour being very stable (more than 4 h). Nevertheless, in the sensor developed from **6c**/picrate, colour changes were always slower than those in the **6a**/picrate system, recovering the initial yellow colour in a few minutes. Unfortunately, calculation of the stability constants of the complexes from the linear titration curves happened to be unsuccessful, presumably, due to the coexistence of different species at equilibrium with either the formation of weak complexes or due to the existence of slow reactions, as suggested by the colour variation delay present in several anions. The study of the complexation process of the receptor system **6a**, by means of the UV–vis absorption method (Fig. 2), or **6c**/potassium picrate shows two maxima: one located at 264 nm corresponding to the absorption of the receptor, and the second at 374 nm assigned to picrate. When treating a solution of receptors **6a** and **6c**, independently, with an increasing amount of NaOH (1.0 M) in DMSO the disappearance of the 264 nm band due to the receptor and the appearance of a new band due to phenolate, caused by the deprotonation of receptor **6a**/**6c** located at about 320 nm, was observed. In this way, when adding aliquots of a sufficiently basic anion in DMSO or a DMSO/H_2O mixture, a gradual decrease of the maximum at 264 nm (phenol) and the gradual appearance of a new band at 316 nm (phenolate) together with the strengthening of the band assigned to picrate at 374 nm was detected. In several cases, the appearance of a new absorption band in the region of 618 nm, usually very weak and broad was observed, which in the case of receptor **6c** disappears very quickly and is ascribed to the charge transfer complexes,[13] TBA fluoride (615 nm), TBA acetate (625 nm) and TBA citrate (618 nm) (Fig. 3). All the anions presenting a positive change show similar behaviour. However, anions which do not alter the colour of the system do not usually show changes in the UV–vis spectrum of the chemosensor system.

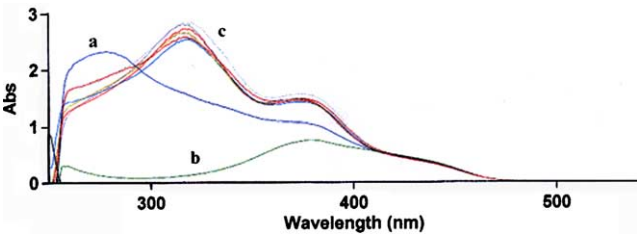
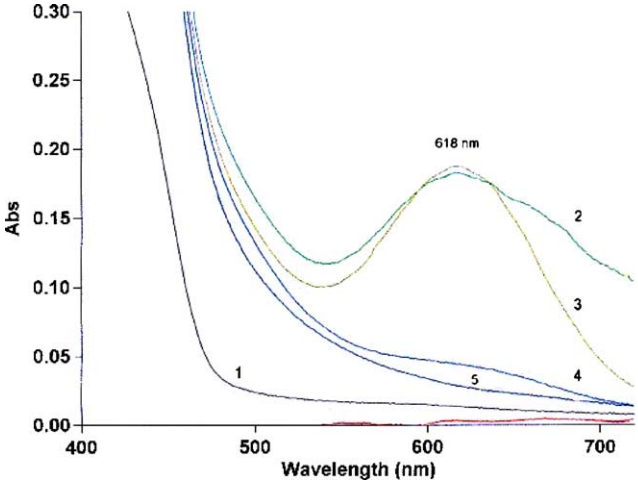


Figure 2. (a) Absorption spectra of **6a** + potassium picrate. (b) Absorption spectra of potassium picrate. (c) Absorption spectra changes of **6a** (4.5×10^{-5} M) + potassium picrate (4.5×10^{-5} M) with trimesoate *n*-Bu_4N^+, F^-*n*-Bu_4N^+, AcO^-*n*-Bu_4N^+, citrate *n*-Bu_4N^+, isophthalate *n*-Bu_4N^+, $H_2PO_4^-$*n*-Bu_4N^+, respectively, in DMSO. [X^-*n*-Bu_4N^+] = 1.70×10^{-4} M.

Most probably, colour variation, is due to a charge transfer process between the receptor–phenolate, and the picrate, electron donor–acceptor (EDA) complex. After the attack of an anion on the phenolic OH of the receptor, the formation of phenolate takes place, usually occurring at an apparent pH of 8. Due to the aromatic nature of receptors **6a** and **6c**, this negative charge is delocalised in the π system of the receptor, conferring electron donating properties of negative charge to the receptors. The spatial proximity of the electron deficient picrate molecule, coordinated to the crown ether ring, allows the charge transfer, within the EDA complex, which is responsible for the visual colour change from



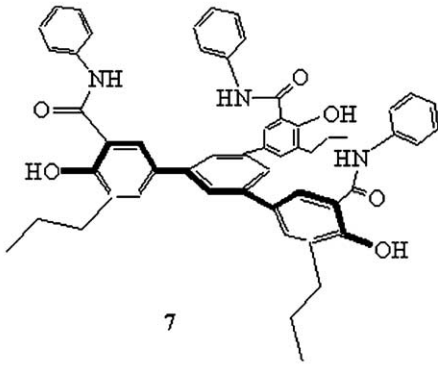


Figure 3. Absorption spectral changes of **6c** (4.7×10^{-4} M) + potassium picrate (4.7×10^{-5} M) with citrate *n*-Bu_4N^+ (4.7×10^{-3} M) in DMSO. 1 at 0 min, 2 at 1 min, 3 at 2 min, 4 at 3 min and 5 at 5 min.

yellow to dark green. In this way, the ability to intercept a phenolic proton of the receptor by a strong basic anion (derived from a weak acid) is the first cause of the charge transfer.

Several complementary tests support, to a certain extent, this suggested process. For example, if the formation of phenolate was hindered, by protecting the phenolic OH group with allyl ethers, which is the case in the system formed by receptor **5b** or **5d**/potassium picrate, no change in colour is observed. Accordingly, all the assays carried out with receptor **7**,[13c] (Fig. 3), which lacks the crown ether units but possesses the ability to form the corresponding phenolate, did not show a colour change in the presence of an anion giving a positive result, for example, phytate, even when an adequate ratio of benzo-15-crown-5 was added to this, **7**/picrate system. This, reveals the importance of the crown ether subunits, which allow the receptor–chromophore to be found, not covalently coordinated, but at an adequate distance so as to produce the intermolecular charge transfer.

Summing up, we have synthesised two new ditopic receptors (**6a** and **6c**) capable of complexing a cation (picrate salt) and reacting with a strong basic anion or polyanion, which caused a colour change in an aqueous solution of DMSO. This colour change can be utilised for the easy detection of sodium phytate.

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12. All new compounds gave spectroscopic data in agreement with the structures indicated. Selected data for compound **6a**: ^{1}H NMR δ (DMSO-d_6, 300 MHz, ppm): 12.30 (s, 3H, OH), 10.52 (s, 3H, NH), 8.52 (d, 3H, $J = 1.1$ Hz), 8.10 (dd, 3H, $J = 8.4$, 1.1 Hz), 8.04 (s, 3H), 7.48 (s, 3H), 7.28 (d, 3H, $J = 6.6$ Hz), 7.22 (d, 3H, $J = 6.6$ Hz), 7.04 (d, 3H, $J = 8.4$ Hz), 4.13–3.51 (m, 48H); ^{13}C NMR δ (DMSO-d_6 75.4 MHz, ppm): 170.96, 163.10, 152.39, 149.62, 144.83, 135.41, 135.18, 131.04, 122.02, 121.03, 118.31, 117.96, 112.49, 74.40, 73.81, 72.90, 72.78, 72.51; IR (KBr): ν 3330, 2923, 2871, 1662, 830 cm^{-1}. MS (m/z) MALDI TOF 1304 (M+Na) $C_{69}H_{75}N_3O_{21}$ requires $M^+ = 1281$. Mp = 93 °C. Compound **6c**: ^{1}H NMR δ (DMSO-d_6, 300 MHz, ppm):

12.78 (s, 3H, NH), 9.13 (s, 3H, OH), 8.39 (d, 3H, $J = 1.2$ Hz), 8.00 (dd, 3H, $J = 8.6$, 1.2 Hz), 7.92 (s, 3H), 7.17 (d, 3H, $J = 8.6$ Hz), 3.79–3.57 (m, 75H); ^{13}C NMR δ (DMSO-d_6 75.4 MHz, ppm): 172.82, 163.70, 144.83, 135.03, 130.35, 127.30, 121.97, 119.56, 81.11, 75.39, 74.19, 74.10, 73.89, 73.83, 73.72, 72.91, 44.30; IR (KBr): ν 2902, 2872, 1642, 1246, 1098, 830 cm^{-1}. MS (m/z) MALDI TOF 1312 (M^+) $C_{66}H_{93}N_3O_{24}$ requires $M^+ = 1312$. Mp = 100 °C.

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