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Neutral Ditopic Receptors for Adenosine Monophosphate

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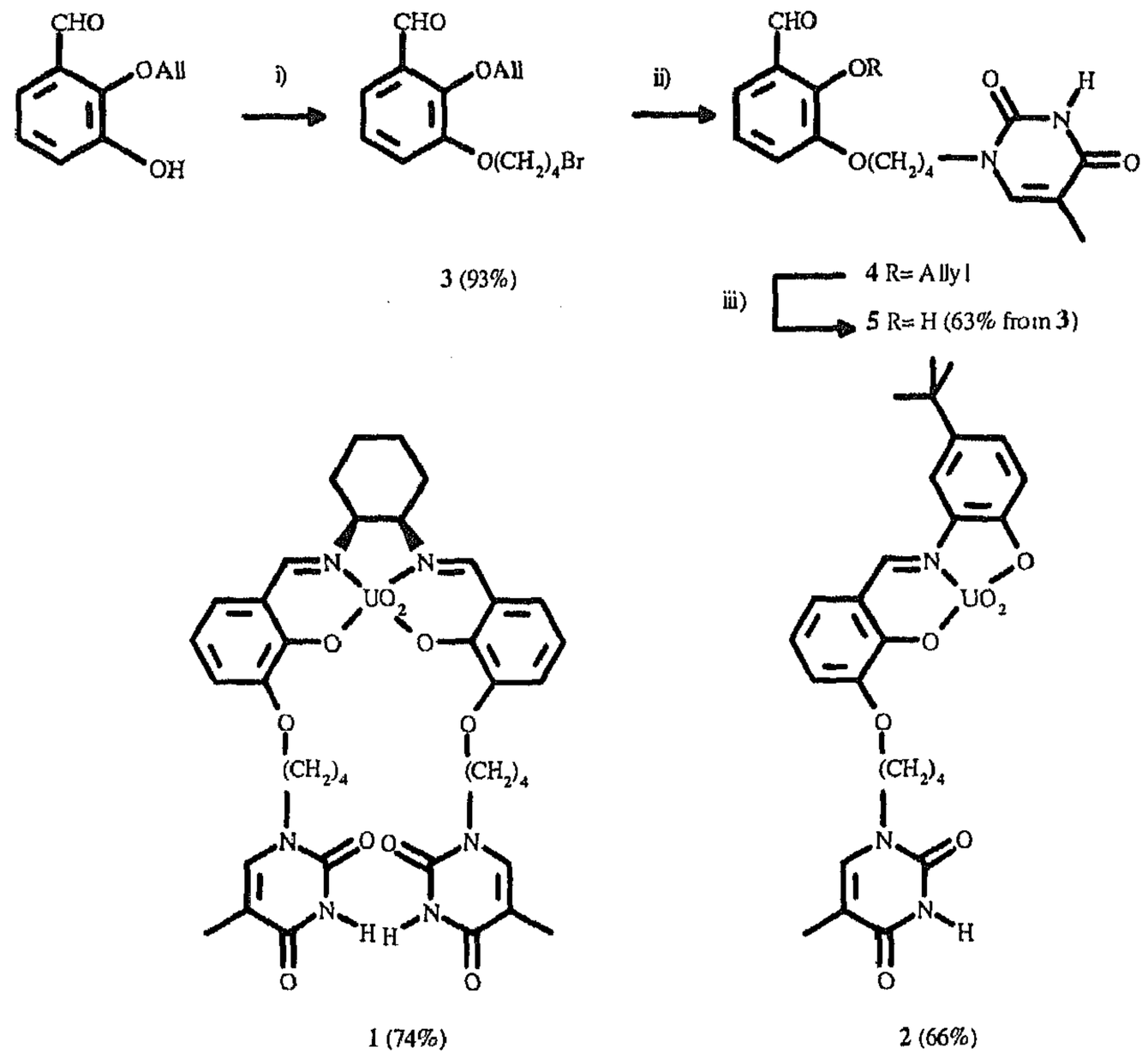
Abstract: Novel neutral ditopic receptors for AMP^{2-} consisting of an immobilised Lewis acidic uranyl centre covalently coupled to thymine are described.

The challenge of creating simple synthetic host molecules as receptors for the recognition and binding of nucleotides has recently received considerable attention.[1-5] Polyammonium[1] and other[2,3] positively charged macrocycles and clefts[4] have been developed for the complexation of the nucleotide polyanions AMP^{2-}, ADP^{3-} and ATP^{4-} *via* electrostatic binding. Ditopic receptors have also been synthesised,[2,5] consisting of one or more protonated nitrogen centres (e.g. guanidinium etc.), for electrostatic binding of the anionic phosphate group of the guest, coupled to a moiety that is complementary to a nucleotide base.

We report here the synthesis and preliminary complexation studies of the first *neutral ditopic receptors* for nucleotide binding. It has previously been demonstrated by our group that uranyl-salen-based hosts form strong complexes with the dihydrogenphosphate anion.[6,7] The coupling of a thymine unit to such a uranyl-salen group might therefore form the basis of some new ditopic receptors for the complexation of AMP^{2-}, employing the *combination of a Lewis acid-anion interaction and complementary base-pairing*.

The synthesis of hosts **1** and **2** was achieved in four steps (Scheme 1). The condensation of 2-(2-propenyloxy)-3-hydroxybenzaldehyde[8] with a ninefold excess of 1,4-dibromobutane in the presence of potassium carbonate in refluxing acetonitrile yielded compound **3** (93% yield). Alkylation of thymine by one equivalent of bromoaldehyde **3** in DMSO at ambient temperature, followed by palladium-catalysed[9] deallylation of product **4** led to the isolation of the aldehyde **5** (63% yield from **3**). Schiff base condensation in refluxing methanol of **5** with 0.5 equivalents of *cis*-1,2-cyclohexanediamine or one equivalent of 2-amino-4-*tert*-butylphenol followed by metallation with $UO_2(OAc)_2{\cdot}2H_2O$ gave **1** (red crystals, 74% yield) or **2** (brown powder, 66% yield), respectively. Hosts **1** and **2** were fully characterised[10] by ^1H and ^{13}C NMR spectroscopy, FAB mass spectrometry and elemental analysis. The elemental analysis and Karl Fischer titration of **1** and **2** are satisfactory for $1{\cdot}2H_2O$ and $2{\cdot}H_2O$, respectively. The positive and negative FAB mass spectra of **1** and **2**, whilst supporting the proposed structures,[10] also show strong ions for self-associated dimers, suggesting that the vacant uranyl binding site(s) may be occupied by one water and/or a carbonyl from the thymine unit.



Scheme 1. *Reagents and Conditions*: i) $Br(CH_2)_4Br$, K_2CO_3, CH_3CN, reflux 20 h; ii) Thymine, K_2CO_3, DMSO, RT (Ar) 2 days; iii) $Pd(OAc)_2$, PPh_3, NEt_3, HCO_2H, 80:20 EtOH:H_2O, reflux 1 h.

For the complexation studies, 3':5'-cyclic AMP^{2-} (3':5'-$cAMP^{2-}$) and 3'-AMP^{2-} were used as their tetra-*n*-butylammonium salts.[11] A preliminary ^1H NMR experiment in DMSO-d_6 of **1** with the two phosphates suggested that 3':5'-$cAMP^{2-}$ was much more weakly bound by **1** (K_{ass} <10 M^{-1}, 295 K) than 3'-AMP^{2-} and so the investigations were focussed on 3'-AMP^{2-} only. The inspection of CPK models confirmed that the bulky phosphate diester of 3':5'-$cAMP^{2-}$ is less complementary to **1** and **2** than 3'-AMP^{2-}, whose structure allows the formation of two base-pairing hydrogen bonds with each host.

The interactions of **1** and **2** with 3'-AMP^{2-} were first studied by ^{31}P NMR spectroscopy in DMSO. Two signals are consistently observed for 3'-AMP^{2-} in the presence of **1** or **2**; the first being a sharp singlet at δ 1.3, due to free 3'-AMP^{2-}. In the presence of **1** the second signal observed is a broad singlet at δ 9.0-7.0, tentatively assigned to complexed 3'-AMP^{2-}. In the case of **2**, a sharp singlet at δ -5.6 corresponds to the complex, the upfield position probably due to the chelation of the uranyl by the dianionic phosphate ester

(Figure 1).

In order to verify the difunctional binding,[6] we first studied the independent complexation of $H_2PO_4^-$ anion and 9-butyladenine[12] by receptor **1** in control experiments. From conductometry measurements (1% DMSO in CH_3CN, 295 K) an association constant of K_{ass} 1.0 x 10^4 M^{-1} for $NBu_4H_2PO_4$ with **1** was determined. ^1H NMR titration of 9-butyladenine with **1** in $CDCl_3$ yielded a K_{ass} value of 1.0 x 10^2 M^{-1} (295 K), confirming an interaction *via* base-pairing.[13] Titrations of 3'-AMP^{2-} with **1** or **2** in DMSO-d_6 exhibited the appearance of an upfield imine singlet at δ 9.34 at the expense of the free host singlet at δ 9.42 for **1** and δ 9.50 for **2** giving association constants of K_{ass} 7.5 x 10^1 M^{-1} and K_{ass} 1.2 x 10^3 M^{-1}, respectively at 295 K.

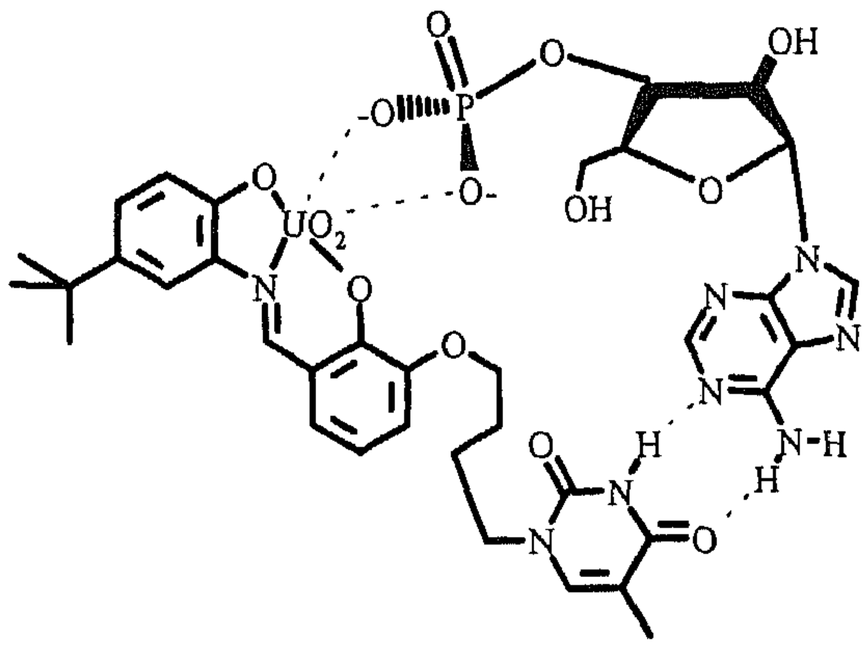


Figure 1. Proposed structure of 1:1 **2**:3'-AMP^{2-}.

This difference in K_{ass} values can be explained by the increased steric crowding, and the presence of only one vacant site, at the Lewis acid centre of **1** compared with **2**. The addition of the guest solution to a solution of the host also resulted in a downfield shift and broadening of the low field thymine N-H signal, suggesting that base-pairing interaction is occurring.

Solid 1:1 complexes of **1** and **2** each with 3'-AMP^{2-} have been prepared as orange or red solids.[14] The negative ion FAB mass spectra of both complexes have an ion corresponding to [**1**·3'-AMP^{2-}-NBu_4^+]$^-$ and [**2**·3'-AMP^{2-}-NBu_4^+]$^-$, with intensities of ~4% and ~55%, respectively. The ^1H NMR spectra of both complexes in DMSO-d_6 show an imine singlet at δ 9.34 attributed to uranyl salophen-phosphate complexation, though that of the complex containing **1** also exhibits a singlet at δ 9.42 implying the presence of free host in this case.

Studies into the membrane transport of 3'-AMP^{2-} by **1** and **2** are currently in progress.

REFERENCES AND NOTES

1. a) Dietrich, B.; Guilhem, J.; Lehn, J.-M.; Pascard, C.; Sonveaux, E. *Helv. Chim. Acta*, **1984**, *67*, 91; b) Dietrich, B. *Pure Appl. Chem.*, **1993**, *65*, 1457 and references therein.

2. Sessler, J. L.; Furuta, H.; Král, V. *Supramol. Chem.*, **1993**, *1*, 209 and references therein.

3. a) Schneider, H.-J.; Blatter, T.; Eliseev, A.; Rüdiger, V.; Raevsky, O. A. *Pure Appl. Chem.*, **1993**, *65*, 2329 and references therein; b) Schneider, H.-J.; Blatter, T. *Angew. Chem. Int. Ed. Engl.*, **1992**, *31*, 1207.

4. Schiessl, P.; Schmidtchen, F. P. *J. Org. Chem.*, **1994**, *59*, 509.

5. a) Kato, Y.; Conn, M. M.; Rebek, Jr., J. *J. Am. Chem. Soc.*, **1994**, *116*, 3279; b) Deslongchamps, G.; Galán, A.; de Mendoza, J.; Rebek, Jr., J. *Angew. Chem. Int. Ed. Engl.*, **1992**, *31*, 61.

6. Rudkevich, D. M.; Brzozka, Z.; Palys, M.; Visser, H. C.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem. Int. Ed. Engl.*, **1994**, *33*, 467.

7. a) Rudkevich, D. M.; Stauthamer, W. P. R. V.; Verboom, W.; Engbersen, J. F. J.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.*, **1992**, *114*, 9671; b) Rudkevich, D. M.; Verboom, W.; Brzozka, Z.; Palys, M.; Stauthamer, W. P. R. V.; van Hummel, G. J.; Franken, S. M.; Harkema, S.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.*, **1994**, *116*, 4341.

8. van Staveren, C. J.; van Eerden, J.; van Veggel, F. C. J. M.; Harkema, S.; Reinhoudt, D. N. *J. Am. Chem. Soc.*, **1988**, *110*, 4994.

9. Yamada, T.; Goto, K.; Mitsuda, Y.; Tsudi, J. *Tetrahedron Lett.*, **1987**, *28*, 4557.

10. Selected data for **1**: m.p. 200-203 °C (methanol); ^{1}H NMR (DMSO-d_6) δ 11.15 (bs, 2H, NH), 9.42 (s, 2H, CH=N), 7.59 (s, 2H, ThymH), 7.35 (2 x d, *J*=7.7 Hz, 4H, ArH), 6.56 (t, *J*=7.7 Hz, 2H, ArH), 4.65-4.60 (m, 2H, cyclH), 4.28 (t, *J*=7.0 Hz, 4H, OCH_2), 3.96 (t, *J*=7.0 Hz, 4H, CH_2N), 2.40-2.35 (m, 2H, cyclH), 1.9-1.8 (m, 14H, CH_2 + cyclH), 1.72 (s, 6H, ThymCH_3); FAB-MS *m/z* 983.5 ([M+H]$^+$, calc. 983.4). Selected data for **2**: m.p. 190 °C decomp. (methanol); ^{1}H NMR (DMSO-d_6) δ 11.25 (s, 1H, NH), 9.50 (s, 1H, CH=N), 7.60 (s, 1H, ThymH), 7.55 (s, 1H, ArH), 7.39 (d, *J*=7.6 Hz, ArH), 7.27 (d, 1H, *J*=8.5 Hz, ArH), 7.19 (d, 1H, *J*=7.2 Hz, ArH), 6.71 (d, *J*=8.4 Hz, 1H, ArH), 6.60 (t, 1H, *J*=7.7 Hz, ArH), 4.26-4.22 (m, 2H, OCH_2), 3.80-3.75 (m, 2H, CH_2N), 1.90-1.85 (m, 4H, CH_2), 1.72 (s, 3H, ThymCH_3), 1.35 (s, 9H, CH_3); FAB-MS *m/z* 734.0 (M$^+$, calc. 733.6).

11. These salts were prepared by the titration of 1M tetrabutylammonium hydroxide in methanol (Aldrich) to a slurry of the free acid in methanol. The solvent is then removed on a rotary evaporator without warming and the residue is dried in vacuo to give a colourless powder.

12. Leonard, N. J.; Carraway, K. L.; Helgeson, J. P. *J. Heterocyclic Chem.*, **1965**, *2*, 291.

13. This value is in agreement with those found for simple uracil-adenine systems, see: Kyogoku, Y.; Lord, R. C.; Rich, A. *J. Am. Chem. Soc.*, **1967**, *89*, 496.

14. Prepared by stirring acetonitrile solutions of equimolar amounts of host and guest followed by evaporation of the solvent and drying.

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