Neutral Ditopic Receptors for Adenosine Monophosphate

Stephen M. Lacy, Dmitry M. Rudkevich, Willem Verboom, and David N. Reinholdt*

Laboratory of Organic Chemistry, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands.

Abstract: Novel neutral ditopic receptors for AMP², 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. Polyammonium¹ and other positively charged macrocycles and clefts² have been developed for the complexation of the nucleotide polyanions AMP³, ADP³ and ATP⁴ via electrostatic binding. Ditopic receptors have also been synthesised,²³ 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. 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 AMP², 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-propenylxylo)-3-hydroxybenzaldehyde 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 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 UO₃(OAc)₂·2H₂O gave 1 (red crystals, 74% yield) or 2 (brown powder, 66% yield), respectively. Hosts 1 and 2 were fully characterised by ¹H and ¹³C NMR spectroscopy, FAB mass spectrometry and elemental analysis. The elemental analysis and Karl Fischer titration of 1 and 2 are satisfactory for 1·H₂O and 2·H₂O, respectively. The positive and negative FAB mass spectra of 1 and 2, whilst supporting the proposed structures, 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(CH₂)₂Br, K₂CO₃, CH₂CN, reflux 20 h; ii) Thymine, K₂CO₃, DMSO, RT (Ar) 2 days; iii) Pd(OAc)₂, PPh₃, NEt₃, HCO₂H, 80:20 EtOH:H₂O, 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-butyrammonium salts. A preliminary ¹H NMR experiment in DMSO-d₆ of 1 with the two phosphates suggested that 3':5'-cAMP[²] was much more weakly bound by 1 (Kₘ <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 ³¹P 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.
In order to verify the bifunctional binding, we first studied the independent complexation of H$_2$PO$_4^-$ anion and 9-butyrladenine by receptor 1 in control experiments. From conductometry measurements (1% DMSO in CH$_3$CN, 295 K) an association constant of $K_{ass}$ 1.0 x 10$^5$ M$^{-1}$ for NBu$_4$H$_2$PO$_4$ with 1 was determined. $^1$H NMR titration of 9-butyrladenine with 1 in CDCl$_3$ yielded a $K_{ass}$ value of 1.0 x 10$^5$ M$^{-1}$ (295 K), confirming an interaction via base-pairing. Titrations of 3'-AMP$^+$ 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$^5$ M$^{-1}$ and $K_{ass}$ 1.2 x 10$^6$ M$^{-1}$, respectively at 295 K.

![Proposed structure of 1:1 2,3'-AMP$^+$.](image)

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$^+$ have been prepared as orange or red solids. The negative ion FAB mass spectra of both complexes have an ion corresponding to [1-3'-AMP$^+$-NBu$_4^+$] and [2-3'-AMP$^+$-NBu$_4^+$], with intensities of ~4% and ~55%, respectively. The $^1$H NMR spectra of both complexes in DMSO-d$_6$ show an imine singlet at δ 9.34 attributed to uranyl salphen-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$^+$ by 1 and 2 are currently in progress.
REFERENCES AND NOTES


10. Selected data for 1: m.p. 200-203 °C (methanol); 1H 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 (s, J=7.7 Hz, 2H, ArH), 4.65-4.60 (m, 2H, cycH), 4.28 (t, J=7.0 Hz, 4H, OCH$_2$), 3.96 (t, J=7.0 Hz, 4H, CH$_2$N), 2.40-2.35 (m, 2H, cycH), 1.9-1.8 (m, 1H, CH$_3$ + cycH), 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 decmp. (methanol); 1H 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, 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$_2$), 3.30-3.75 (m, 2H, CH$_2$N), 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.


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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