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ACRIDONE HETEROCYCLES AS FLUORESCENT SENSORS FOR ANIONS

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Abstract - An acridone heterocycle with a suitable functionalization can be used as a fluorescent sensor for anions. Large differences in fluorescence efficiency are observed when chlorides are associated in the receptor cavity. Chlorides form two different complexes in chloroform, with 2:1 and 1:1 stoichiometries. The combined presence of receptor (**1**) and 18-crown-6 ether allows the extraction of aqueous α-amino acids to the chloroform phase. This extraction is very sensitive to temperature.

The development of efficient sensors is a challenging topic in organic chemistry.[1] Most sensors include a receptor unit linked to a fluorescent fragment capable of signalling the recognition event.[2] The acridone skeleton of receptor (**1**) can perform both tasks on its own. It shows strong fluorescence and the geometry of its 1,8-diamino substituted anthracene-like skeleton[3] is especially suitable for the simultaneous formation of several H-bonds with a guest (Figure 1).

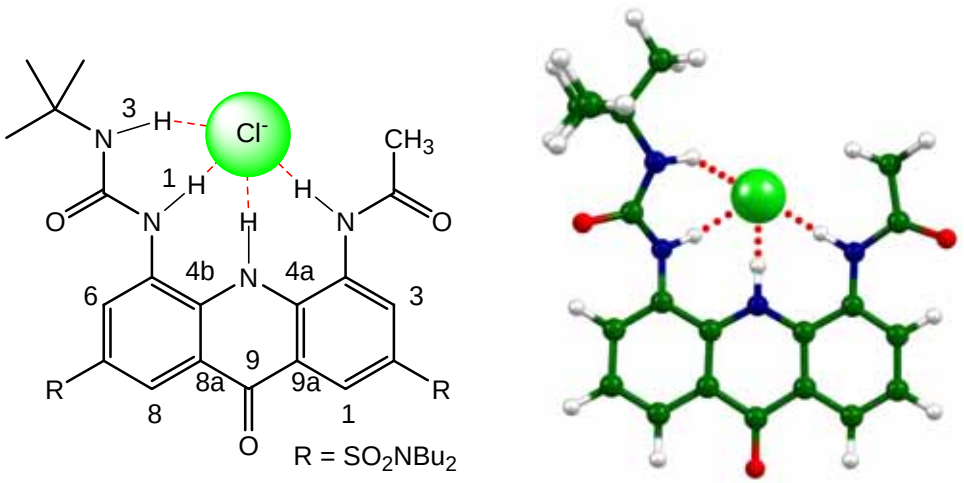
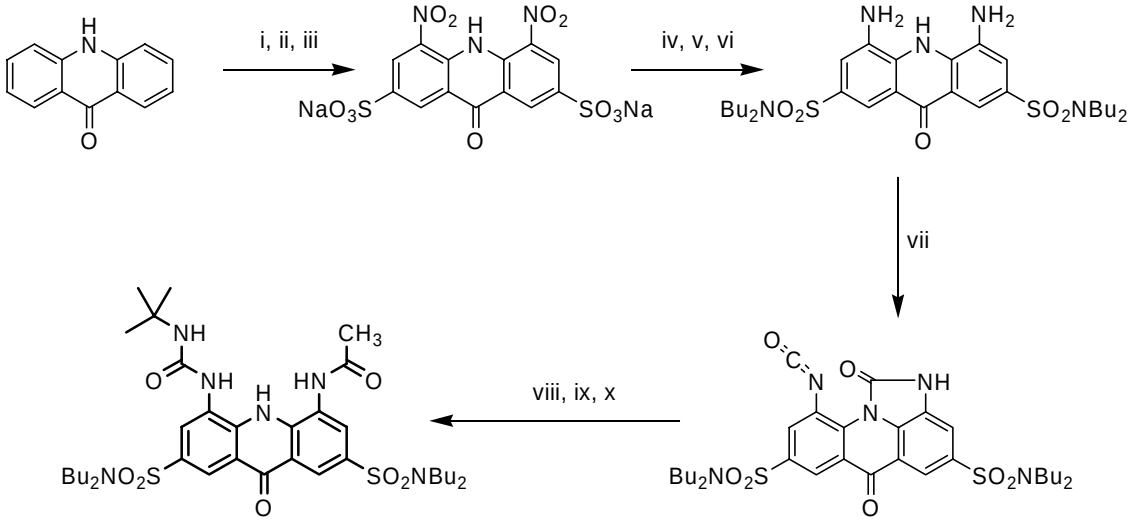


Figure 1. Proposed complex of receptor (**1**) with chloride and modelling of an analogue.

The preparation of receptor (**1**) is outlined in Figure 2. The large sulfonamide groups were chosen to increase the solubility of the receptor in non-polar solvents. The main merit of this procedure is the possibility of obtaining acridones with different functionalizations in carbons 4 and 5, starting from the commercially available symmetric acridone (Figure 2). The key step is the reaction of the 4,5 diaminoacridone with phosgene. The amino groups afford different functional groups: one yields an isocyanate while the other forms an imidazolone. Both groups react readily with nucleophiles, but the isocyanate is by far the most reactive one.



i) SO_3/H_2SO_4 20%, 80ºC; ii) HNO_3 , 50ºC; iii) NaCl aq.; iv) PCl_5, $POCl_3$, reflux; v) $BuNH_2$, THF, -20ºC; vi) Na_2S, $(NH_4)_2S$, EtOH, reflux; vii) phosgene, CH_2Cl_2 , reflux; viii) *t*-butylamine, THF, 0ºC; ix) KOH, H_2O, *t*-BuOH, reflux; x) acetyl chloride, THF.

Figure 2. Preparation of receptor (**1**) from the commercially available acridone.

Long range H-C correlations allow the assignment of all NMR signals shown in Table 1. Nevertheless, the ^{1}H-NMR spectrum of receptor (**1**) shows the four expected aromatic signals (Table 1) at a higher field

than expected. In our opinion this would be due to the presence of a dimer in the chloroform solution. A signal in the ESI mass spectrum at 1495 amu ($(2M-H)^-$, 20%) supports the presence of this aggregate. The large diffusion coefficient in DOSY experiments[4] (two-fold larger than for the reference 2,7-di-*t*-butyl-9,9-dimethyl-9-*H*-xanthene[5]) also confirms the dimer structure.

	C-1	C-2	C-3	C-4	C-4a	C-4b	C-5	C-6	C-7	C-8	C-8a	C9	C9-a	acet.	urea
(**1**)	122.9	132.1	126.9	126.9	136.5	137.3	128.1	126.8	132.5	122.0	120.8	177.2	120.8	171.3	156.2
0.5 eqvs.	122.6		126.7					123.9		119.4					
1.05 eqvs.	122.3	132.5	126.9	127.3	136.2	135.3	129.9	123.4	133.2	119.4	121.1	177.2	120.9	170.7	154.7

	H-1	H-3	H-6	H-8	NH acridone	NH acet.	NH urea-1	NH urea-3
(**1**)	7.841	7.841	7.494	7.672	10.715	8.865	7.373	5.701
0.5 eqvs.	8.185	8.185	7.690	7.759	10.984	9.496	9.099	6.426
1.05 eqvs.	8.585	8.377	8.516	8.424	10.200	10.848	9.948	6.515

Table 1. NMR aromatic signals of receptor (**1**) and its complexes with chloride; eqvs: equivalents of tetraethylammonium chloride added to a $5x10^{-2}$M solution of receptor (**1**) in $CDCl_3$ at 293K.

Attempts to establish the dimerization constant of the aggregate making use of dilution experiments[6] by NMR failed, since constant chemical shifts were obtained in the usual concentration range. Only at very diluted solutions (10^{-4}M) was it possible to observe the beginning of the expected low field shift of the signals. The presence of methanol, which strongly competes for the H-bonds of the receptor, yielded chemical shifts closer to the expected ones for the free receptor, and hence the aggregate may be stabilized through hydrogen bond formation. Receptor (**1**) fluorescence can be used to establish the dimerization constant. UV shows absorptions at 331 nm ($\varepsilon=1.7x10^4$) and 400 nm ($\varepsilon=6.5x10^3$) and a emission at 450 nm. Quantum efficiency was determined by the well-known method of Williams, with quinine sulphate as reference. In the concentration range of 3.7x10-5M to 1.5x10-6M the values were 0.09 (331nm) and 0.24 (400nm). Therefore the longer wave absorption was selected for irradiation. Since the free receptor showed greater fluorescence than the dimer it was possible to estimate[7] the dimerization constant as $8x10^5M^{-1}$.

Comparison with similar xanthone molecules whose X-Ray structures are known,[8] and which show similar deshielding effects in the NMR spectrum upon methanol addition, suggests the structure shown in Figure 3 for this dimer. Modelling studies (using the semiempirical AM1[9] method implemented in Gaussian98W[10]) fully support this kind of structure.

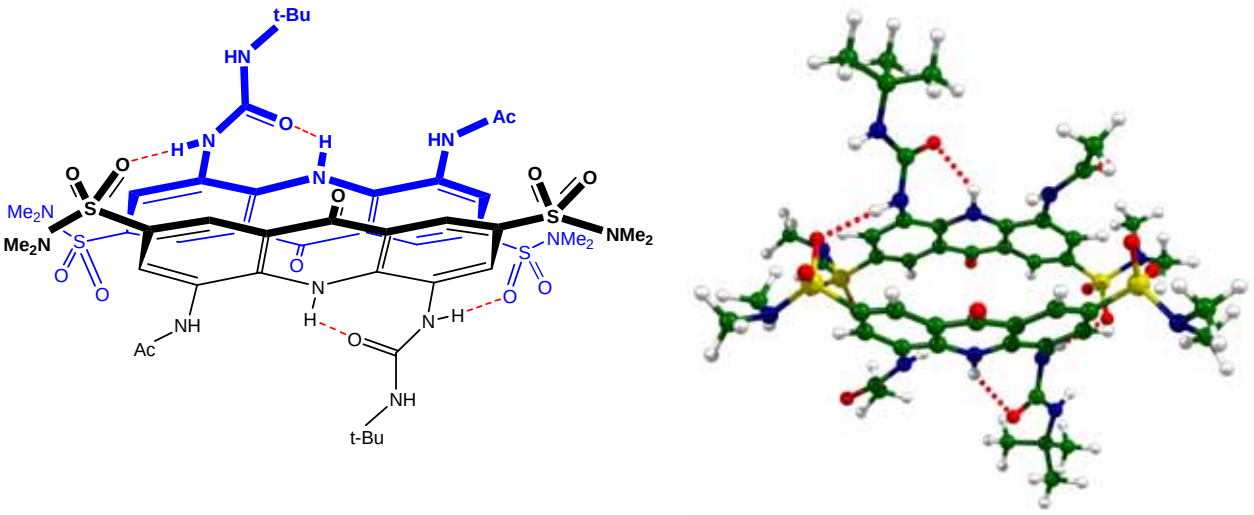


Figure 3. Dimer structure proposed for the analogue of receptor (**1**).

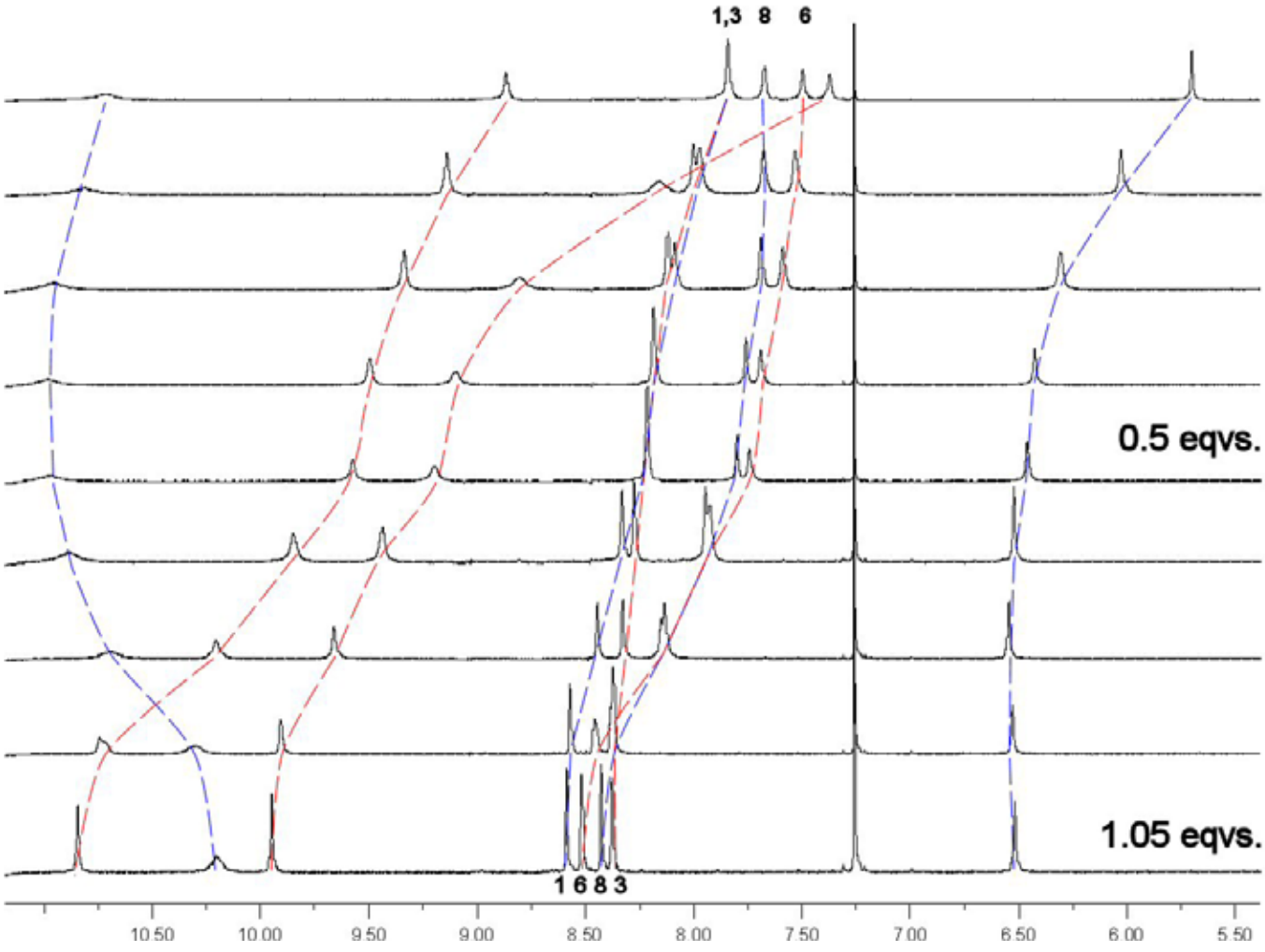


Figure 4. Downfield segment of receptor (**1**) ^{1}H-NMR spectra in the presence of increasing amounts of tetraethylammonium chloride.

The addition of suitable guests such as halogenides also afforded large downfield shifts (Table 1). Formation of the complexes probably breaks down the dimer, and the observed chemical shifts were again closer to those expected for the free receptor. The association constants of the halogenides with receptor (**1**) were high in chloroform, and could not be measured easily with the standard NMR procedure. Moreover, as shown in Figure 4, tetraethylammonium chloride yielded anomalous shifts during the titration, which cannot be interpreted as being only to the presence of the dimer receptor[11], and the presence of a further species in solution is required.[12] As a general trend, the change of the chemical shifts of the protons was very different before and after 0.5 equivalents of chloride had been added (Figure 4). A good explanation for this behaviour is the formation of a complex in which two receptors would hold a single chloride ion. ESI mass spectrometry supported the presence of this aggregate at 1531 amu ($(2M+Cl)^-$, 9%).

Especially interesting was the behaviour of H-10 (acridone NH) in ^{1}H-NMR which started at 10.715, deshielded to 10.984 in the middle of the titration and ended upfield at 10.200 ppm. C-6 (126.8, 123.9 and 123.4 ppm), and C-8 (122.0, 119.4 and 119.4 ppm) were also very revealing. The downfield position of these carbons at the beginning of the titration is consistent with the conformation shown in the dimer, in which only a weak conjugation between the nitrogen on C-5 and the aromatic ring is present. After the addition of 0.5 equivalents of chloride these carbon atoms (C-6 and C-8) were shielded 2.9 and 2.6 ppm, reflecting a better conjugation. Modelling studies (*vide infra*, Figure 5) suggest a 20º increase in the dihedral angle between the aromatic ring and the urea plane due to 2:1 complex formation. After this point, C-6 and C-8 only shifted 0.5 ppm and 0.0 ppm until the end of the titration. The proton spectra showed a similar change in the trends. H-6 (7.494, 7.690 and 8.516 ppm) showed a weak shift up to the middle of the titration, while the addition of more chloride yielded a strong deshielding, since in the final complex H-6 lay in the anisotropic cone of the urea carbonyl group.
Finally, DOSY experiments revealed a 15% decrease in the diffusion coefficient when 0.5 equivalents of chloride were added, which corresponds to a 60% increase in the molecular volume. Adding more than one half of the chloride reduced the molecular size, in agreement with the expectations.

Modelling studies of a simplified system were performed using the semiempirical AM1[9] method implemented in Gaussian 98W[10], revealing an interesting structure in which the Watson-Crick H-bonds between the urea groups stabilized the dimer receptor, in which a cavity was created to accommodate a chloride ion (Figure 5). The *syn/anti* conformation of the urea provides a good explanation for the shielded position of H-6 in this aggregate.

The efficiency of the fluorescence of receptor (**1**) changed in the presence of halogenides. For example, a 10^{-5}M solution of receptor (**1**) in chloroform emitted light with an intensity of 69 units.

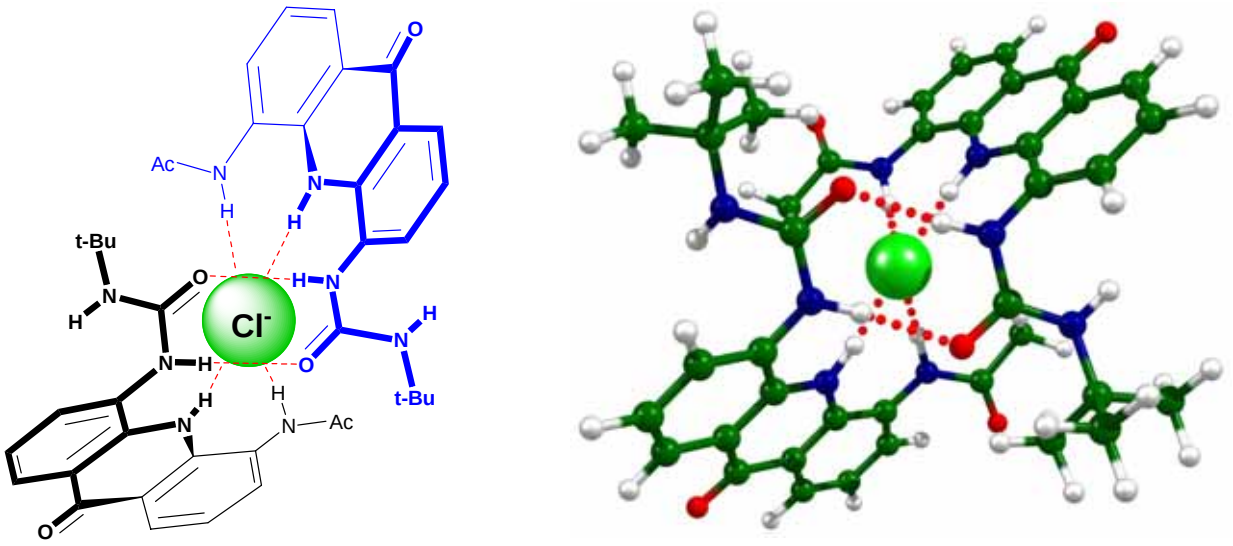


Figure 5. Proposed structure for the 2:1 aggregate formed between an analogue of receptor (**1**) and tetraethylammonium chloride.

Once the complex with chloride had been formed the intensity increased up to 100 units. A conventional titration keeping the receptor concentration constant at 10^{-5}M and increasing the concentrations of tetraethyl ammonium chloride allowed us to establish the stability of the complex at Kass= $2.7x10^{6}M^{-1}$. A similar procedure allowed the determination of the Kass of the bromide ($1.0x10^{6}M^{-1}$) and iodide ($5.9x10^{4}M^{-1}$). These latter two anions decreased the fluorescence efficiency to 34 and 23 units respectively. In order to detect the presence of chlorides, carbon tetrachloride was the solvent of choice. Probably due to the presence of higher aggregates, the fluorescence of receptor (**1**) was very inefficient in this solvent: only 3 units at 10^{-5}M (^{1}H-NMR spectrum of receptor (**1**) in carbon tetrachloride showed very broad signals, while higher aggregates would be expected in non-polar solvents since two H-bonds are not saturated in the dimer in Figure 3). The presence of chloride strongly increased the emission up to 54 units (Figure 6).

Figure 6. Fluorescence of a 10^{-5}M solution of receptor (**1**) in carbon tetrachloride in the presence (left) and absence (right) of chloride $2x10^{-5}$M.

Carboxylates were also studied as suitable guests for receptor (**1**). However, both acetate and benzoate failed to provide the expected results, due to the basicity of these anions, which abstract the acidic proton of the acridone. Zwitterionic amino acids are known to be less basic than conventional carboxylates,[13] and they seem to be suitable guests in the presence of 18-crown-6 ether.[14] Phenylalanine formed a complex in chloroform in which the aromatic acridone signals showed the expected downfield shifts. The acetate group of receptor (**1**) was, however, shielded in this complex. The aromatic ring of phenylalanine seems to be responsible for this effect since neither alanine nor leucine changed the field in which the acetate group showed up to any significant extend. These observations are in agreement with the proposed structure for the ternary complex of receptor (**1**), 18-crown-6 ether and phenylalanine (Figure 7).

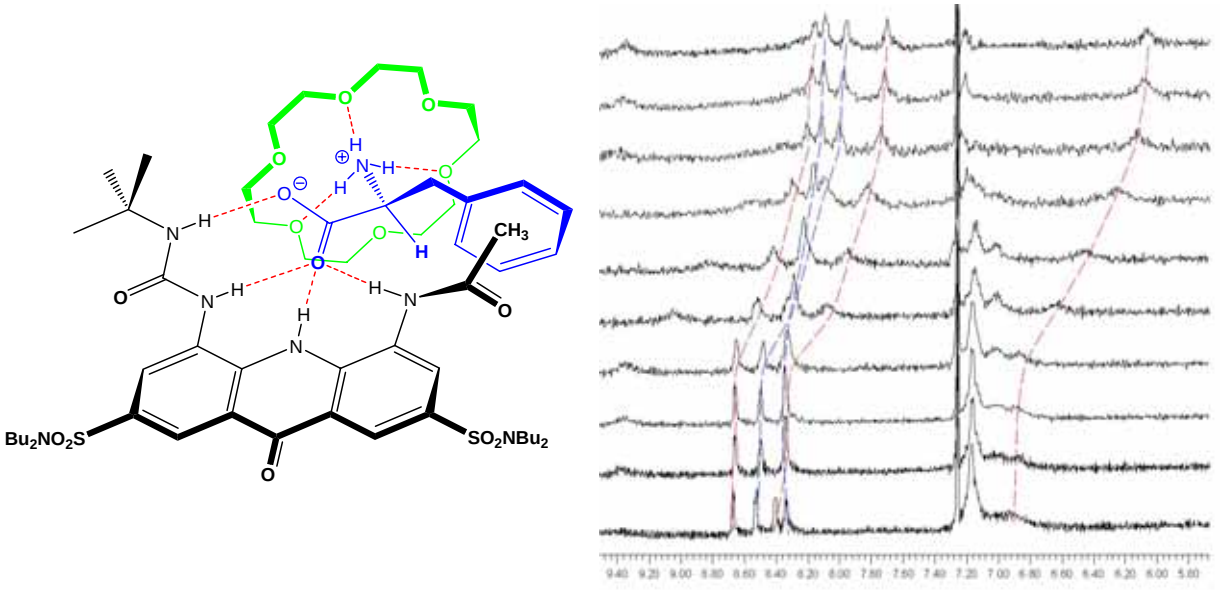


Figure 7. Proposed structures of receptor (**1**), phenylalanine, and 18-crown-6 ether complex (left). Downfield segments of receptor (**1**) (10^{-3}M) ^{1}H-NMR spectra in $CDCl_3$ at 293K in the presence of 18-crown-6 ether ($2x10^{-3}$M) and increasing amounts of phenylalanine (right).

Extraction experiments of aqueous phenylalanine to the chloroform phase were also carried out. The degree of extraction could readily be established through integration of the phenylalanine aromatic signals. The combination of receptor (**1**) (10^{-3}M) and 18-crown-6 ether ($2x10^{-3}$M) in chloroform (1 mL) was very effective for extracting phenylalanine ($2x10^{-3}$M) from the aqueous phase (1 mL) at 293K. Complex formation accounted for more than 95% of the acridone receptor (**1**). Interestingly, at higher temperatures extraction was only weak. At 333K, the same experiment revealed only 50% of complex formation. High temperatures disfavoured the formation of a highly ordered ternary complex.

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