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A Water Soluble Multisite Receptor: Synthesis, Cu(II) and Organic Molecule Complexation*

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Abstract. The synthesis of the new receptor, **1a**, containing two pyridino- and two diphenylmethane subunits is reported. It is remarkably soluble in water (up to 0.1M) and can bind metal ions such as Cu(II) and organic molecules such as *p*-nitrophenol and 1,8-ANS. The complexation constant for Cu(II) is rather modest whereas the binding of the organic molecules is comparable to that of the other receptors such as cyclodextrins or other cyclophanes.

Key words. 1,8-Anilinonaphthalene sulfonic acid (1,8-ANS), *p*-nitrophenol, Cu(II), association constants, synthetic receptor, large ring compound, macrocyclic compound, cationic surfactant.

1. Introduction

In the last few years the field of biomimetic chemistry has been growing rapidly. The design of artificial enzymes featuring selective binding ability of substrates as well as catalytic properties is the ultimate aim of many scientists [1].

Cyclophane type molecules constitute an interesting class of receptors [2]. Pioneering work by Koga and his co-workers [3] showed the ability of cyclophane hosts containing 4,4′-diaminodiphenylmethane units to bind apolar guests in acidic aqueous solutions. Diederich then synthesized a series of such cyclophanes which are soluble in water, due to the presence of quaternary ammonium groups [4] and are excellent receptors for naphthalenoid substrates. More recently Vögtle and his co-workers reported on the synthesis of a cyclophane in which the two diphenylmethane units are coupled by two 2,6-pyridinecarboxylate moieties [5].

Following a different approach, Lehn and his co-workers designed dinuclear cryptates [6] able to chelate two metal ions to properly connected subunits. His work is promising in biomimesis of metallo-proteins as well as of biological redox processes.

Here we report the synthesis of the new macrocyclic receptor **1a**, of the azacyclophane type, which is water soluble at neutral pH, and contains two chelating subunits and a hydrophobic cavity for the complexation of both metal ions and organic species within the same superstructure.

2. Experimental

2.1. GENERAL

NMR spectra were recorded on a Bruker WP 200 SY spectrometer operating at 200 MHz, UV spectra on a Perkin-Elmer Lambda 5 instrument, and fluorescence spectra on a Perkin

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* Dedicated to the memory of Prof. Iwao Tabushi.

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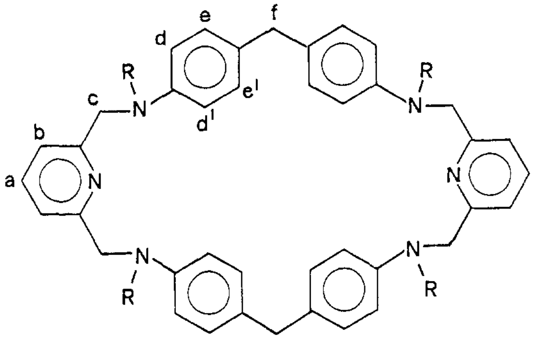
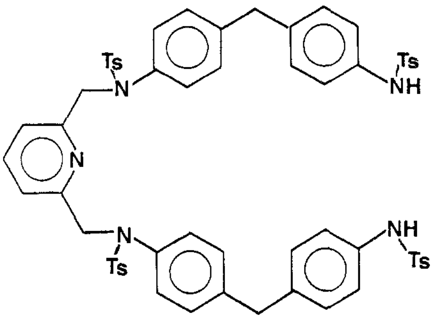


1a : R = $COCH_2N^+(CH_3)_3Cl^-$
1b : R = *p*-toluensulfonyl, (Ts)
1c : R = H
1d : R = $COCH_2Cl$



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Elmer MPF 66 fluorimeter. $Cu(NO_3)_2$ solutions were titrated according to standard procedures [7]. Dry DMF (distillation over 4A molecular sieves) was used throughout. 4,4'-ditosylamino-diphenylmethane was synthesized as described [8].

2.2. SYNTHESES

2,6-diaminomethyl-[N-tosyl-N(4-diphenylmethane-4'-tosylamino)]pyridine (**2**). To a dry DMF (20 mL) solution of 4,4'-ditosylaminodiphenylmethane [8] (1.2 g, 2.37 mmol) K_2CO_3 (0.45 g, 3.2 mmol) was added and the slurry heated to 80°C. 2,6-Dibromomethylpyridine [9] (0.2 g, 0.754 mmol) dissolved in 20 mL of DMF was then slowly added under vigorous stirring during a period of 3 h. Stirring was then continued for 1 more hour and after cooling to room temperature the reaction mixture was neutralized with 1N HCl and poured into ice. The white precipitate was eluted in a medium pressure (5 at) SiO_2 column (ethyl acetate/toluene (1:6)) to give **2** in 90% yield. ^{1}H-NMR ($CDCl_3$) δ: 2.36, 2.41 (2s, 12H, $4CH_3$); 3.8 (s, 4H, $2CH_2$); 4.7 (s, 4H, $2NCH_2$); 6.7 (br s, 2H, 2NH); 6.91, 6.94 (2s, 16H, 2Ar); 7.1–7.8 (m, 19H, 2Ts and Py).

7,15,25,33-Tetratosyl-7,15,25,33,45,46-hexaazaheptacyclo $[32.2.2^{3,6}.2^{16.19}.2^{21,24}.1^{9,13}.1^{27,31}]$ *hexatetraconta-3,5,9,11,16,18,21,23,27,29,34,36,37,39,41,43,45,46-octadecaene* (**1b**). To a stirred suspension of K_2CO_3 (2 mmol) in 50 mL of dry DMF, kept at 90°C a solution of **2** (1.12 g, 1 mmol) and 2,6-dibromomethylpyridine in DMF (100 mL) was added during an 8 h period. After 1 additional h at 90°, the slurry was cooled to room temperature and neutralized with 1N HCl. After removal of the solvent, the solid residue was washed with H_2O to remove the inorganic salts and finally chromatographed over $SiO_2(CHCl_3)$. The first eluted product was compound **1b** obtained as a white solid: 731 mg, 60% yield. ^{1}H-NMR ($CDCl_3$) δ: 2.44 (s, 12H, $4CH_3$); 3.81 (s, 4H, H_f); 4.71 (s, 8H, H_c); 6.76 (s, 16H, $H_{d.d'}$ and $H_{e,e'}$); 7.21–7.56 (m, 22H, 2Ts and 2Pyr).

7,15,25,33,45,46-hexaazaheptacyclo $[32.2.2.2^{3,6}.2^{16,19}.2^{21,24}.1^{9,13}.1^{27,31}]$*hexatetraconta-3,5,9,11,16,18,21,23,27,29,34,36,37,39,41,43,45,46-octadecaene* (**1c**). 730 mg (0.6 mmol) of cyclophane **1b** were dissolved in the minimum amount of 90% H_2SO_4 (ca. 10 mL) and stirred for 18 h at room temperature. The temperature was then raised to 50°C during a 3 h period. After cooling, the mixture was (**cautiously!**) neutralized with concentrated NaOH and the final pH adjusted to *ca.* 8 with $NaHCO_3$. The white precipitate was filtered off and purified by column chromatography (SiO_2, $CHCl_3$/MeOH (20 : 1)) to afford 252 mg (70%) of cyclophane **1c**.

Anal. Calcd. for $C_{40}H_{38}N_6$: C, 79.70; H, 6.35; N, 13.94. Found: C, 79.68; H, 6.29; N, 13.87%.

^{1}H-NMR (D_2O/D_2SO_4, pD ca. 1; reference to internal acetone = 2.17) δ: 3.83 (s, 4H, H_f); 4.64 (s, 8H, H_c); 6.86, 6.90, 7.08, 7.12 (AA′BB′ system, 16H, $H_dH_{d'}$ and $H_eH_{e'}$); 7.34 (d, J = 7.8 Hz, 4H, H_b); 7.75 (t, J = 7.8 Hz, 2H, H_a).

7.15.25.33-Tetra(2-trimethylammoniumchloride)acetyl-7,15,25,33,45,46-hexaazaheptacyclo [$32.2.2.2^{3,6}.2^{16,19}.2^{21,24}.1^{9,13}.1^{27,31}$]hexatetraconta-3,5,9,11,16,18,21,23,27,29,34,36,37, 39,41,43,45,46-octadecaene (**1a**). 290 mg (0.49 mmol) of cyclophane **1c** and 0.5 mmol of triethylamine were dissolved in DMF (10 mL) and chilled to −40°C. 2-Chloracetyl chloride (0.5 mmol) dissolved in 2 mL of DMF was added dropwise with stirring. The temperature was allowed to reach room temperature, water (30 mL) was added, and the mixture extracted (5 × 30 mL) with $CHCl_3$. The chloroform layer was washed twice with H_2O (10 mL), dried, and, after evaporation of the organic solvent, the crude material purified by preparative TLC (SiO_2, MeOH/$CHCl_3$ (1 : 5)) to give 185 mg of pure **1d** (56%).

^{1}H-NMR ($CDCl_3$) δ: 3.74 (s, 8H, CH_2Cl); 3.91 (s, 4H, H_f); 4.88 (s, 8H, H_c); 6.95, 6.99, 7.06, 7.11 (AA′BB′ system, 16H, $H_dH_{d'}$ and $H_eH_{e'}$); 7.30 (d, J = 7.63 Hz, 4H, H_b); 7.64 (t, J = 7.63 Hz, 2H, H_a).

This material was then dissolved in 10 ml of a 30% methanolic solution of trimethylamine and stirred overnight at 40°C. Evaporation of the solvent left a crude solid that was purified with a Sephadex G15 column (H_2O). The material obtained after freeze-drying was analytically pure **1a** (251 mg, 80%).

Anal. Calcd. for $C_{60}H_{78}N_{10}O_4Cl_4$: C, 62.93; H, 6.86; N, 12.23; Found: C, 63.22; H, 6.60; N, 12.18%.

^{1}H-NMR (D_2O, external TMS) δ: 3.09 (s, 36H, $4N(CH_3)_3$); 3.79 (s, 4H, H_f); 3.87 (s, 8H, $4CH_2N$); 4.75 (s, 8H, H_c); 6.66, 6.70, 6.77, 6.81 (AA′BB′ system, 16H, $H_dH_{d'}$, $H_eH_{e'}$); 7.25 (d, J = 8.24 Hz, 4H, H_b); 7.70 (t, J = 8.24 Hz, 2H, H_a).

2.3. BINDING CONSTANTS DETERMINATION

(a) UV: Changes of optical density were followed at 250–280 nm or 320 nm (competition experiment, see below) using as a medium a pH = 6.2 aqueous solution of morpholinoethanesulfonate (MES 0.05M). Changes in absorbance due to addition of **1a** or Cu(II) were recorded as already reported [10]. In competition experiments spectra were recorded for a 1 : 1 solution of $Cu(NO_3)_2$ and 2-hydroxymethyl-6-(methylthio)methylpyridine (1.0×10^{-4} M), after addition of varying amounts (0–1.7×10^{-3} M) of **1a**. (b) NMR: Resonance frequencies shifts were monitored in D_2O versus external TMS. Conditions [**1a**] = 7.45×10^{-4} M; [*p*-nitrophenol] = 0–3.72×10^{-2}. (c) Fluorescence: Excitation wavelength was 375 nm, emission wavelength was 463 nm. To an aqueous solution of 1,8-ANS ammonium salt, 2.0×10^{-6} M, varying amounts of **1a** were added; ([**1a**] = 0–4.36×10^{-4} M).

The binding constants were evaluated by means of standard Scatchard plots [11].

3. Results and Discussion

3.1. GEOMETRY OF RECEPTOR **1a**

An analysis of a CPK model of **1a** supports the hypothesis of the orientation of the pyridine and benzene rings as shown in Figure 1: the two pyridines lie in a plane almost perpendicular to that of the four benzene rings of the two diphenylmethane units. The dimensions of the cavity, as estimated from this model are about 11 Å between the two pyridine nitrogens and about 8 Å between the two diphenylmethane methylenes. Upon binding of two Cu(II) ions the dimension of the cavity reduces considerably and the distance between these metals is about 5.5 Å. The four ammonium groups appear unable to penetrate inside the cavity although their bulkiness may either cause deviation from coplanarity of the three nitrogens of the metal binding sites or a slightly difficult access to them.

3.2. SOLUBILITY AND AGGREGATION IN AQUEOUS MEDIA OF **1a**

The solubility of **1a** increases on going from non polar to polar solvents. It is remarkably soluble in water, up to 0.1 M, even at neutral pH. The results of a NMR analysis of **1a** in D_2O at 25°C as a function of its concentration are shown in Figure 2. In the concentration range 7.45×10^{-4}–2.0×10^{-2} M the chemical shifts of all protons are almost independent of the concentration, while at higher concentration they move upfield, the largest shift (ca. 0.2 ppm) being that of the H_b hydrogens of the two pyridine rings. No significant shift is observed for the other protons not indicated in Figure 2. From the data in Figure 2 an apparent 'critical aggregate concentration' of 2.4×10^{-2} M can be evaluated*. A similar behavior has been observed by Diederich for his water soluble macrocyclic hosts [4a].

3.3. BINDING ABILITY OF MACROCYCLE **1a** IN AQUEOUS SOLUTIONS

In 0.05M MES buffer at pH = 6.2 the complexation of Cu(II) is highlighted by a band at ca. 250 nm attributed to the charge-transfer transition pyridine → Cu(II) [13], observed either keeping constant [**1a**] and varying [Cu(II)] or operating in the opposite way. From the analysis of the spectral changes, a binding constant, K_b, of $5 \times 10^2\ M^{-1}$ is evaluated, assuming the formation of a 1 : 1 complex. Furthermore, addition of excess **1a** to a MES solution of the Cu(II) complex of 2-hydroxymethyl-6-(methylthio)methylpyridine [14]

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* Similar changes are diagnostic of the critical micelle concentration (c.m.c.) observed for surfactants [12] though in this case it is difficult to say which kind of aggregate is being formed.

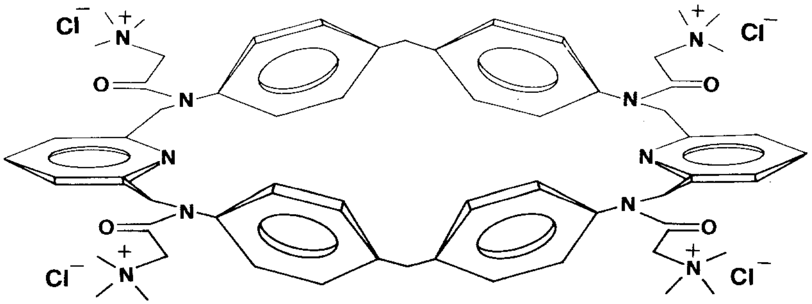

Fig. 1. Stereo drawing of receptor **1a**.

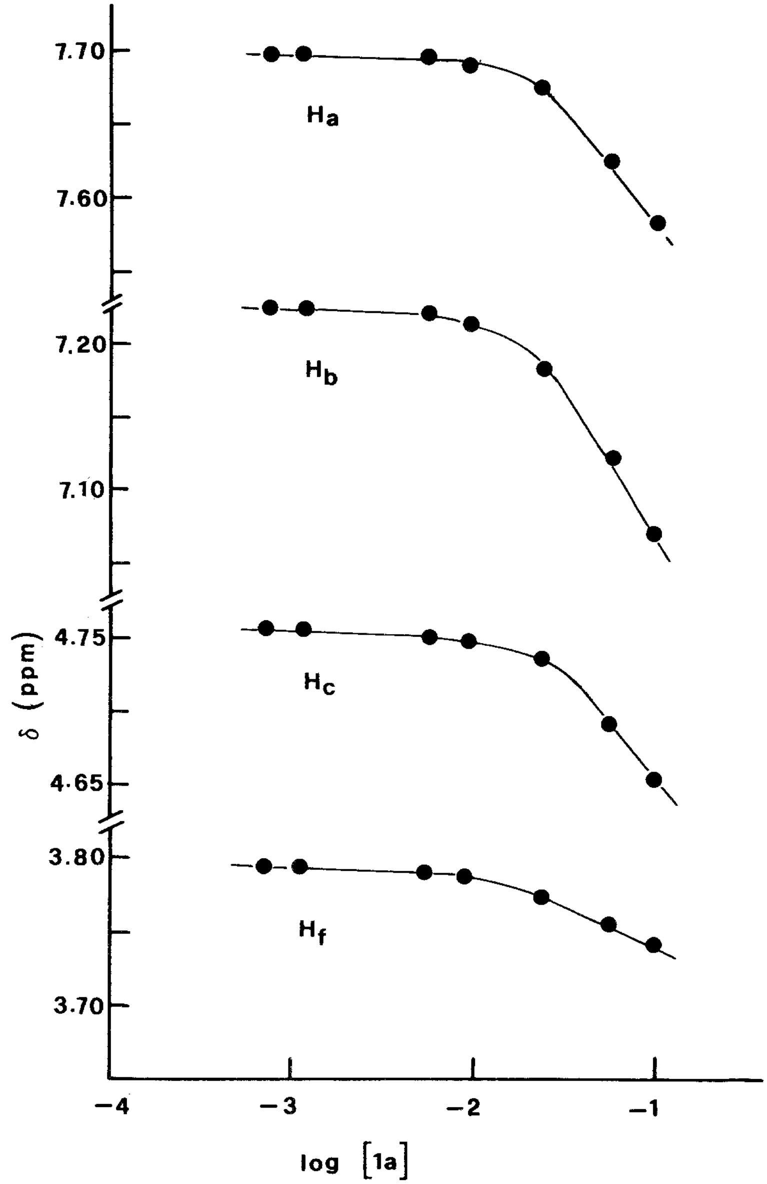

Fig. 2. Chemical shift changes of selected protons of receptor **1a** as a function of its concentration. (D_2O, 25°C, referred to external TMS).

causes the decrease of the 320 nm band associated with the S → Cu(II) charge-transfer transition [13] of this ligand, thus showing the ability of **1a**, albeit modest, to compete for Cu(II). We were unable, under the present conditions, to substantiate the possible formation of a 2 : 1 complex (Cu(II) : ligand). A possible explanation for the rather low complexation ability of Cu(II) to **1a** could call for the tertiary amide functions partially deactivating the aniline nitrogens or to a partial steric strain of the binding site due to interference from the ammonium groups.

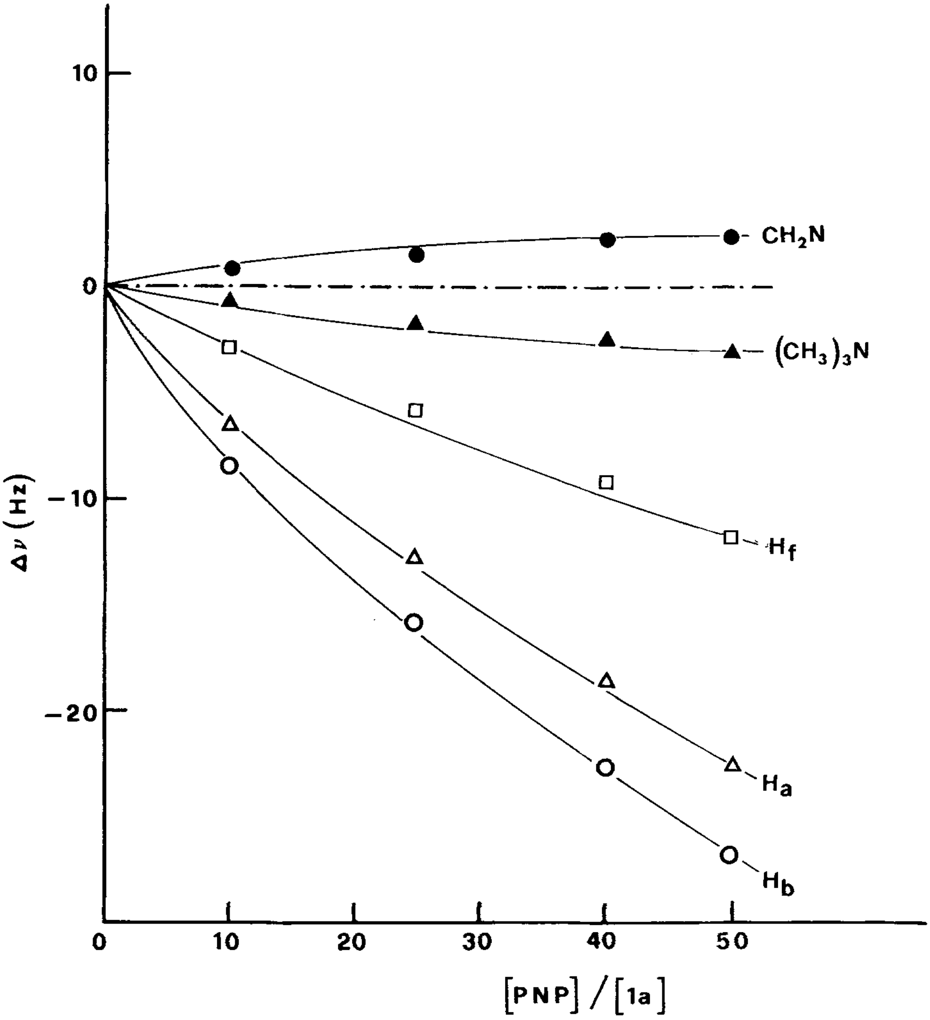

Fig. 3. Chemical shift changes experienced from the different protons of **1a** ([**1a**] = 7.45 × 10^{-4} M) with increasing amounts of added *p*-nitrophenol. (D_2O, 25°C, referred to external TMS).

The binding of *p*-nitrophenol within the host **1a** cavity has been evaluated in D_2O at 25°C by an NMR technique. Under the conditions described above changes of the chemical shift of selected protons of receptor **1a** were observed, as shown in Figure 3★. A similar behavior has been reported for other receptors such as cyclodextrins [15]. From the shifts of the H_a and H_b protons a binding constant, K_b, of 20 ± 2 M^{-1} can be evaluated for a 1 : 1 complex. As a comparison the binding constant of *p*-nitrophenol in α-CD is 18.9 M^{-1} [16].

The binding of 1,8-ANS to receptor **1a** has been observed by means of fluorescence spectroscopy [17] and from the changes observed a K_b value of 2.2×10^3 M^{-1} was

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★ As pointed out by a referee the upfield shifts of the pyridine protons could be explained by an orientation of the two heterocycles *in the complex* similar to the aromatic rings of the diphenylmethane unit, i.e. perpendicular to a mean plane of the macrocyclic receptor.

calculated. This is larger than that reported for β-cyclodextrin [18] although lower than that observed by Diederich ($K_b \sim 10^6$) for his 'naphthalenoid specific' receptors [4a].

Overall, the present pyridine cyclophane fulfills the tasks for which it has been designed: water solubility, ability to bind either a transition metal cation like Cu(II) and hydrophobic organic molecules. This is quite remarkable for a single receptor molecule although it may be pointed out that less eclectic hosts reported in the literature proved better in the complexation of metal cations [19] or in the binding of organic molecules [4]. The synthesis of a more efficient, water soluble, multisite receptor (especially for divalent metal cations) is in progress in our laboratory with the final goal of obtaining co-operativity between the different receptive sites.

Acknowledgments

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