Tetrahedron Letters, Vol. 34, No. 49, pp. 7935-7938, 1993
Printed in Great Britain

0040-4039/93 $6.00 + .00
Pergamon Press Ltd

Preparation and Characterization of a Water-soluble Peptide Cyclophane as a Cationic Host for Various Hydrophobic Guest Molecules

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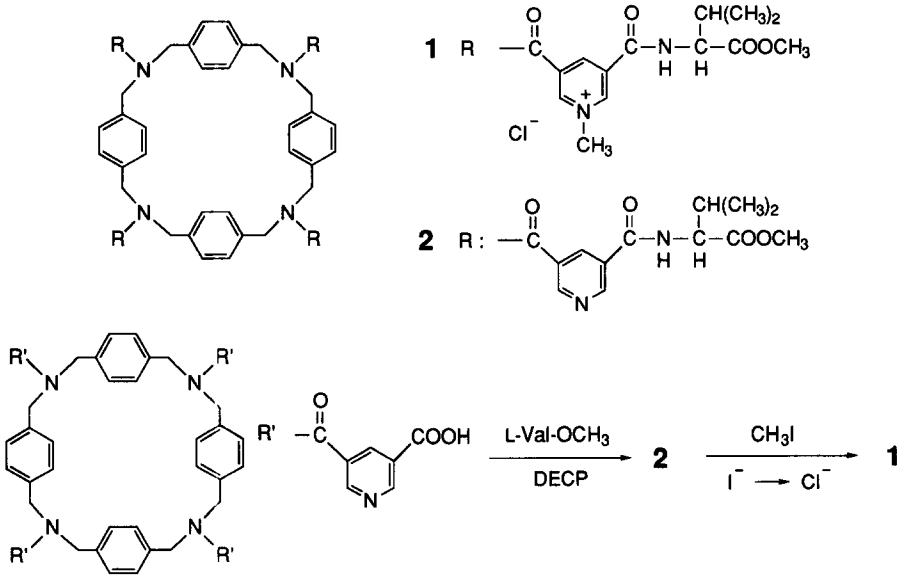
Abstract: A novel water-soluble peptide cyclophane was prepared by condensation of *N,N',N'',N'''*-tetrakis(5-carboxynicotinoyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane with L-valine methyl ester, followed by quaternization with methyl iodide, and its guest-binding behavior was examined by means of electronic absorption spectroscopy and electrospray ionization (ESI) mass spectrometry.

Various artificial receptors, such as octopus-type and cage-type cyclophanes, have been designed and prepared by appropriate modifications of cyclophane skeletons to mimic specific functions performed by naturally occurring receptors toward guest molecules.[1] We now designed a novel water-soluble peptide cyclophane bearing L-valine residues of hydrophobic nature and hydrophilic pyridinium moieties (**1**). In this communication, we are to report on the preparation of host **1** and its unique guest-recognition ability toward various hydrophobic guest molecules, with emphasis on successful observations of the host–guest complexes by means of electrospray ionization (ESI[2]) mass spectrometry.

A precursor of the water-soluble peptide cyclophane (**2**) was synthesized by condensation of *N,N',N'',N'''*-tetrakis(5-carboxynicotinoyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane[3] with the hydrochloride adduct of L-valine methyl ester in the presence of diethyl cyanophosphonate (DECP) and triethylamine in dry *N,N*-dimethylformamide (DMF) at 0 °C by following the reaction sequence given in Scheme 1. The product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluent to afford a pale yellow solid: yield 85%, mp 158–160 °C; 500 MHz ^{1}H NMR (CD_3OD, 27 °C, TMS) δ = 0.96 [24H, m, $CH(C\underline{H}_3)_2$], 2.20 [4H, m, $C\underline{H}(CH_3)_2$], 3.69 (12H, s, $COOCH_3$), 4.4–4.7 (16H, m, $ArCH_2N$), 4.48 (4H, d, J = 6.4 Hz, COCH), 6.9–7.2 (16H, m, ArH), 8.30 (4H, m, Py-H4), 8.78 (4H, m, Py-H2), and 9.00 (4H, m, Py-H6); IR (KBr) 1740 (ester C=O) and 1660 (amide C=O) cm^{-1}. Found: C, 65.60; H, 6.08; N, 10.79%. Calcd for $C_{84}H_{92}N_{12}O_{16}\cdot 1/2H_2O$: C, 65.76; H, 6.06; N, 10.96%.

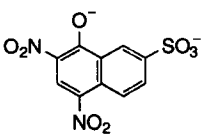
Host **1** was obtained by quaternization of **2** with methyl iodide in dry DMF and the subsequent replacement of the counterion iodide with chloride. The product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. Evaporation of the solvent under reduced pressure gave a pale yellow solid: yield 97%, mp 193–194 °C; 500 MHz ^{1}H NMR (CD_3OD, 27 °C, TMS) δ = 1.02 [24H, m, $CH(C\underline{H}_3)_2$], 2.27 [4H, m, $C\underline{H}(CH_3)_2$], 3.74 (12H, s, $COOCH_3$), 4.4–4.7 (16H, m, $ArCH_2N$), 4.42 (4H, m, COCH), 4.51 (12H, s, NCH_3), 6.9–7.2 (16H, m, ArH), 8.95 (4H, m, Py-H4), and 9.4 (8H, m, Py-H2 and Py-H6); IR(KBr) 1735 (ester C=O) and 1640 (amide C=O) cm^{-1}; MS (electrospray ionization MS; ESI), m/z 828

$(M - 2Cl)^{2+}$, 540 $(M - 3Cl)^{3+}$, and 396 $(M - 4Cl)^{4+}$; calcd M for $C_{88}H_{104}Cl_4N_{12}O_{16}$, 1726. Found: C, 61.17; H, 6.00; N, 9.63%. Calcd for $C_{88}H_{104}Cl_4N_{12}O_{16}$: C, 61.17; H, 6.06; N, 9.73%.

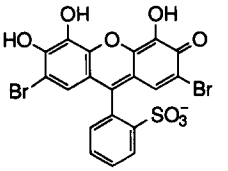


Scheme 1.

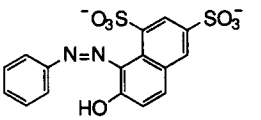
Anionic guests



NYS

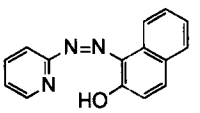


BPR

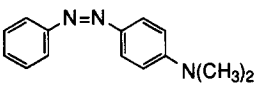


OG

Nonionic guests

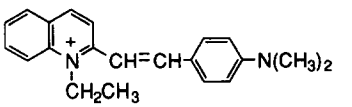


PAN



MY

Cationic guest



QR

The guest-binding behavior of host **1** toward various hydrophobic molecules was examined by electronic absorption spectroscopy in aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethansulfonate (HEPES) buffer (0.01 mol dm^{-3}, pH 7.0, μ 0.10 with KCl) at 30.0 °C. The following dyes were adopted as hydrophobic guest molecules: 2,4-dinitro-1-naphthol-7-sulfonate (NYS), 5,5'-dibromopyrogallolsulfonphthalein (BPR), 1-phenylazo-2-naphthol-6,8-disulfonate (OG), 1-(2-pyridylazo)-2-naphthol (PAN), *N*,*N*-dimethyl-*p*-phenylazo-

aniline (MY), and 2-[4-(dimethylamino)styryl]-1-ethylquinolinium (QR). An absorption intensity originated from each of the guest molecules, except for QR, decreased along with a concomitant red shift of its absorption maximum upon addition of host **1**, reflecting formation of the host–guest complexes. The stoichiometry for the complexes formed with the host and the guest was investigated by the Job's continuous variation methods.[4] The resulting Job's plot for an NYS complex of **1** is shown in Fig. 1 as a typical example. This reveals that host **1** forms a complex with NYS in a 1:1 molar ratio of host to guest. The same 1:1 stoichiometry was confirmed for the other host–guest complexes. The binding constants (K) for 1:1 host–guest complexes were calculated on the basis of spectroscopic data obtained at various concentrations of the host molecule in a manner as described previously;[5] 2.7 x 10^4, 1.5 x 10^4, 1.5 x 10^4, 1.2 x 10^4, and 6.9 x 10^3 mol^{-1} dm^3 for inclusion of NYS, , BPR, OG, MY, and PAN, respectively. The complexation behavior of host **1** toward the guests was also evidenced in D_2O–CD_3OD (4:1 v/v) by means of ^1H NMR spectroscopy. Proton signals for the guests showed upfield shifts upon complexation in a manner similar to those reported for a variety of host–guest systems.[6] On the other hand, host **1** showed no capacity of binding a cationic guest, QR. Consequently, the water-soluble peptide cyclophane recognizes hydrophobic guests in aqueous media through hydrophobic and electrostatic interactions in a manner as observed with other water-soluble cyclophanes.[7] Peptide cyclophane **2** is also expected to behave as a polycationic host toward these guest molecules in acidic aqueous media. However, the K values for **2** with the present guests were not accurately determined owing to its very limited solubility in aqueous media.

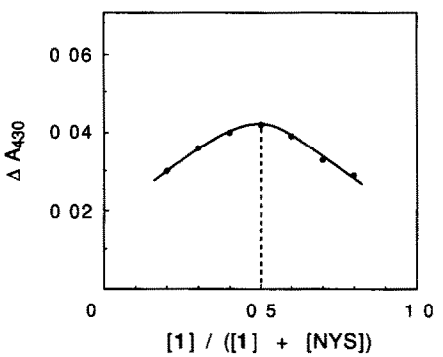


Fig. 1. Job's plot for the **1**•NYS system in aqueous HEPES buffer (pH 7, μ 0.10 with KCl) at 30.0 °C with attention to absorbance change at 430 nm (ΔA_{430}); total concentration of **1** and NYS, [**1**] + [NYS], being maintained at 8.0 x 10^{-5} mol dm^{-3}.

The inclusion behavior of host **1** toward the anionic and cationic guest molecules cited above was also examined by ESI mass spectrometry. The mass spectrum of the OG complex of host **1** obtained by using water–methanol (1:1 v/v) as a mobile phase provides m/z 996 $(M_{complex} - 2Cl)^{2+}$; calcd $M_{complex}$[8] for $C_{104}H_{114}Cl_2N_{14}O_{23}S_2$, 2062 (Fig. 2). Similar mass spectra were also observed with the other complexes of **1** holding anionic guests such as NYS and BPR: m/z 949 $(M_{complex} - 2Cl)^{2+}$ and 714 $(M_{complex} - 3Cl)^{3+}$ for NYS and BPR complexes, respectively; calcd $M_{complex}$ for the NYS and BPR complexes of host **1** are 1968 ($C_{98}H_{108}Cl_2N_{14}O_{24}S$) and 2248 ($C_{107}H_{113}Br_2Cl_3N_{12}O_{24}S$), respectively. However, any ESI peak originating from a QR complex of **1** was not detected. Therefore, the electrostatic interaction between host and guest molecules having opposite charges to each other is an effective recognition factor. To the best of our knowledge,

the present finding can be cited as the first successful detection of the host–guest complexes by means of ESI mass spectrometry.

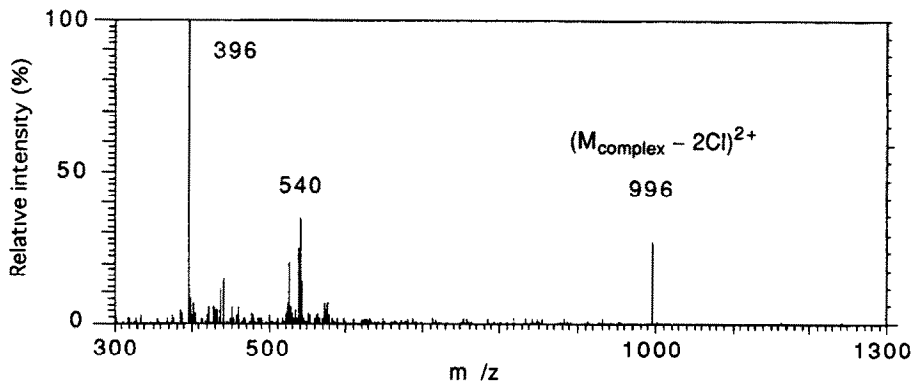


Fig. 2. ESI mass spectrum for OG complex of **1**; mobile phase, water–methanol (1:1 v/v)

In conclusion, the present water-soluble peptide cyclophane is capable of performing molecular discrimination toward various guest molecules through hydrophobic and electrostatic interactions as confirmed by ESI mass spectrometry and electronic absorption spectroscopy, and is expected to be utilized as a multifunctional receptor model.

The present work was supported by a Special Distinguished Grant for Scientific Research No. 02102006 from the Ministry of Education, Science and Culture of Japan.

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8. $M_{complex}$ stands for a molecular mass of a 1:1 host–guest complex bearing no overall charge.

(Received in Japan 19 August 1993)