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Binding of acetylcholine and quaternary ammonium compounds to a C_3-symmetric bowl-shaped tripeptide of 2-(3-aminophenoxy) propanoic acids acting as a ditopic receptor

Motohiro Akazome *, Norihiro Hamada, Koji Takagi, Daisuke Yagyu, Shoji Matsumoto

Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University, 1-33 Yayoicho, Inageku, Chiba 263-8522, Japan

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ABSTRACT

The new tripeptide reported here is composed of (*R*)-2-(3-aminophenoxy)propionic acid and is a bowl-shaped receptor that simultaneously binds both cations and anions of acetylcholine chloride and benzyltrimethylammonium compounds. An intriguing conformational change of the host was observed in the complexation of the ionic pair, where anion-induced flipping of the amide group on the macrocycle occurred.

Ion-pair receptors are of great interest in the field of host–guest chemistry,[1] and we have found that the small C_3-symmetric bowl-shaped tripeptide **1** has properties suitable for binding quaternary ammonium compounds, including acetylcholine chloride. Since the binding of acetylcholine has been attracting considerable interest due to its biological importance as a neurotransmitter, we designed a model system using the cation–π interaction pointed out by Dougherty.[2] Many artificial acetylcholine receptors such as calixarenes (including resorcinarenes),[3] cavitands derived from resorcinarenes,[4] and other hosts[5] have been reported, but the only C_3-symmetric peptide receptors were Kubik's pioneer hosts: hexapeptides composed of 3-aminobenzoic acid (AB) and α-amino acids.[6] When the Kubik host contained glutamic acid 5-isopropyl ester [Glu(OiPr)-AB]$_3$, the K_a for its complex with *n*-butyltrimethylammonium iodide was 300 M^{-1}.[6a] Substituting proline for glutamic acid [yielding (Pro-AB)$_3$] increased the association constant by 2 orders of magnitude, to 21,100 M^{-1}.[6b]

Kubik's findings with hexapeptides prompted us to introduce 2-(3-aminophenoxy)propanoic acid instead of Kubik's dipeptide unit (*N*-3-aminobenzoylamino acid). The first reason that we use 2-(3-aminophenoxy)propanoic acids is that molecular modeling of the tripeptides of (*R*)-2-(3-aminophenoxy)propanoic acid shows a proper cavity size and restricted conformation, which will reduce the conformational entropy loss accompanying guest binding. Another reason is that the benzene ring of 2-(3-aminophenoxy)propanoic acid, which has two electron-donating groups (–NH– and –OCsp^3), seems to be an electron-enriched benzene ring suitable for cation–π interaction. The third reason is that (*S*)-2-hydroxypropanoic acid is one of the (*S*)-2-hydroxyalkanoic acids easily and enantiopurely prepared from natural amino acids via diazotization and displacement by water under acidic conditions (i.e., neighboring group participation reaction). Therefore several side chains of natural amino acids can be applied to this tripeptide. In the work reported here, we found the C_3-symmetric bowl-shaped tripeptide has an intriguing guest response using quaternary ammonium compounds such as acetylcholine, and we elucidated their binding properties.

The synthetic route of **1** is shown in Scheme 1. The Mitsunobu reaction[7] of methyl (*S*)-lactate and 3-nitrophenol afforded enatiopure methyl (*R*)-2-(3-nitrophenoxy)propanoate **4**, which was transformed into (*R*)-2-(3-nitrophenoxy)propanoic acid **5** and methyl (*R*)-2-(3-aminophenoxy)propanoate **6** by saponification and hydrogenation, respectively. For the coupling reaction, we utilized standard conditions with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) and 1-hydroxy-1*H*-benztriazole (HOBt).[8] After reduction of the nitro group of **7**, amino compound **8** was coupled with **5** by the same conditions. After final reduction and saponification of **9**, the desirable cyclic tripeptide **1** was obtained by the intramolecular coupling reaction of **11** using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 4-dimethylaminopyridine (DMAP).[9]

* Corresponding author. Tel.: +81 43 290 3388; fax: +81 43 290 3401.
E-mail address: akazome@faculty.chiba-u.jp (M. Akazome).

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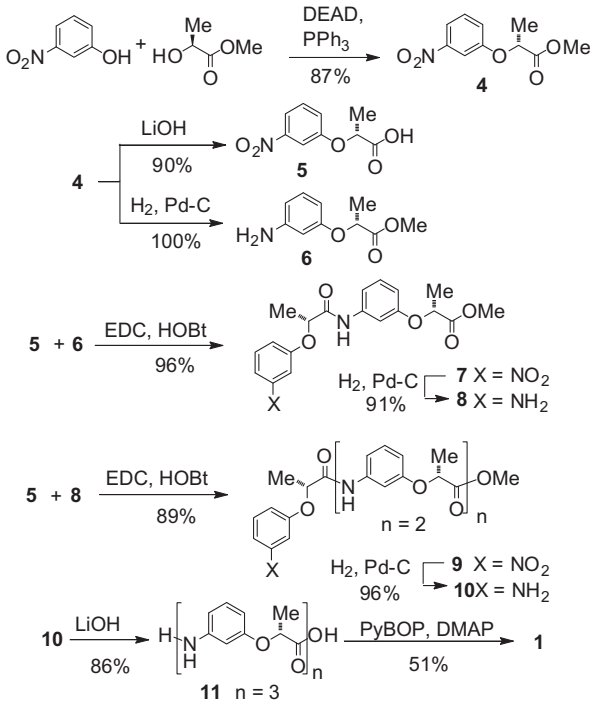

Scheme 1. Synthesis of compound **1**.

First, the binding of acetylcholine chloride (AChCl) with **1** was examined in $CDCl_3$ by using ^1H NMR spectra. In the presence of AChCl, ^1H NMR spectra of **1** showed only one third of the peaks of the whole host structure with AChCl, which indicates rapid equilibrium between free **1** and a host–guest complex (i.e., within the NMR time-scale). The NMR titration experiment showed up-field shift of guest protons (Fig. 2). The Job's plot shows 1:1 complexation (see Fig. S1 in Supporting information). The shifts of the methyl and methylene groups of the ammonium cation were greater than the shift of the acetyl moiety, which suggests that the ammonium cation is located in the bowl cavity throughout the cation–π interaction. Therefore association constants of cations were estimated from the up-field shift of cations by adding **1** to the guest solution (Method A). Nonlinear curve fitting gave an association constant of 1998 M^{-1} for the complex consisting of host **1** and AChCl. Host (**1**) also bound benzyltrimethylammoium (BnTriMA) compounds with K_a = 705–1750 M^{-1} (Method A in Table 1). During the NMR titration experiment, two protons of benzylic methylene groups of BnTriMA cation not only shifted up-field

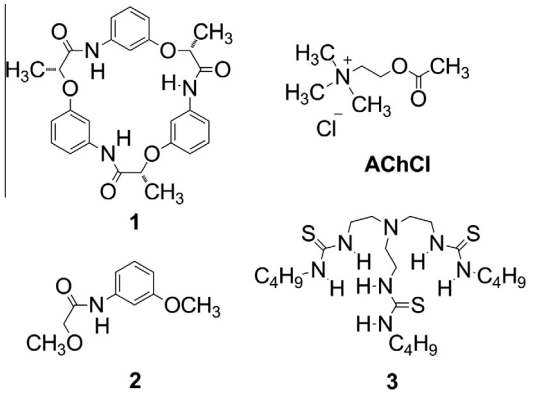

Figure 1. Structures of hosts (**1**, **2**, and **3**) and a guest (AChCl).

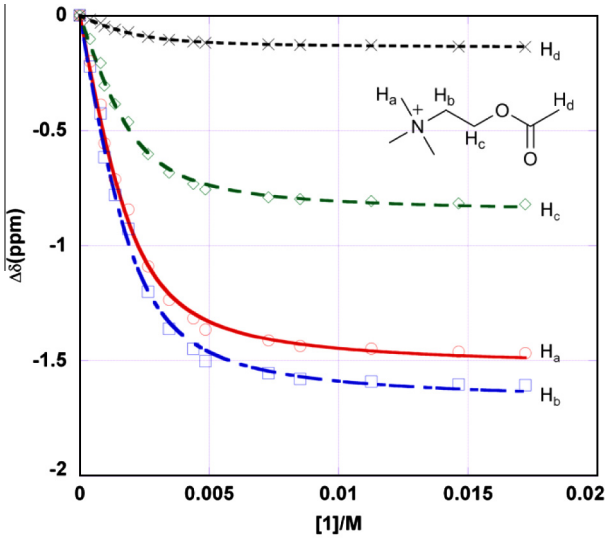

Figure 2. Titration curve for up-field shifts of AChCl upon addition of **1**.

but also showed diastereotopic germinal coupling by forming a complex with the chiral bowl of **1**.

In addition, the association constants of the BnTriMA cation depended on the counter anions and indicated that the anion binds simultaneously. In ^1H NMR spectra, NH of **1** caused down-field shifting by complexation and the amplitude of the shift depended on the counter anions. We therefore also estimated the association constants of counter anions by reversed ^1H NMR titration in $CDCl_3$ (Method B, in which the guest solution was added to a solution of **1** and the down-field shift of the amide protons was measured by the ^1H NMR titration curves). The association constant of chloride in AChCl was 1640 M^{-1}, which was estimated from nonlinear curve fitting. Using BnTriMAX gave the association constants for Cl, Br, and I anions that were 1284, 2725, and 2368 M^{-1}, respectively.

In addition, we estimated the simple anion binding ability of **1** by Method B using tetrabutylammonium halide (TBAX), which is bulky for the cavity of **1**. Since the association constants of Cl, Br, and I were respectively 252, 122, and 40 M^{-1}. It is noteworthy that these values were an order of magnitude smaller than those of BnTriMAX. These results suggest the binding of cations in the cavity of **1** facilitates the corresponding anion binding. Similar cooperative effects were observed with the ditopic receptors reported by Kubik[6], Sessler–Schmidtchen–Gale,[10] and Gibb.[11]

In a control experiment, where the association constants of monomeric structure **2** (shown in Fig. 1) against chloride were estimated, and they were 46 M^{-1} for BnTriMACl and 32 M^{-1} for TBACl. No up-field shift of BnTriMA and TBA cations binding with **2** was observed using Method A, however. Therefore the bowl-shaped structure of tripeptide **1** is essential for the complexation of **1** with cations.

We performed single-crystal X-ray analysis of **1** with AChCl and elucidated the bowl-shaped structure with the cations as a guest molecule (Fig. 3). As expected, the trimethylammonium part was settled in the bowl-shaped cavity and surrounded by three benzene rings with cation–π interaction via CH–π interaction. The chloride anion was located in the bottom of bowl and bound with an amide proton by a hydrogen bond. Water and ethanol molecules, which came from chloroform solvent for crystallization, bridged between the chloride and amide protons by two hydrogen bonds. It is noteworthy that all three amide protons were directed toward the bottom of the bowl structure and bound to anions through hydrogen bonds. At the same time, we noticed that the carbonyl oxygen of **1** was bound to the electro-deficient proton

Table 1
Association constants (K_a) between **1** and various quaternary ammonium compounds[a]

Guest	Cation binding K_a M^{-1} (Method A)	Anion binding K_a M^{-1} (Method B)
AChCl	1998 ± 213	1640 ± 189
BnTriMACl	1750 ± 66	1284 ± 216
BnTriMABr	1680 ± 87	2725 ± 329
BnTriMAI	705 ± 28	2368 ± 454
TBACl	—	252 ± 12
TBABr	—	122 ± 3
TBAI	—	40 ± 2

[a] K_a values were determined by fitting ^{1}H NMR spectroscopic titration curves. Detail conditions are shown in Supporting Information.

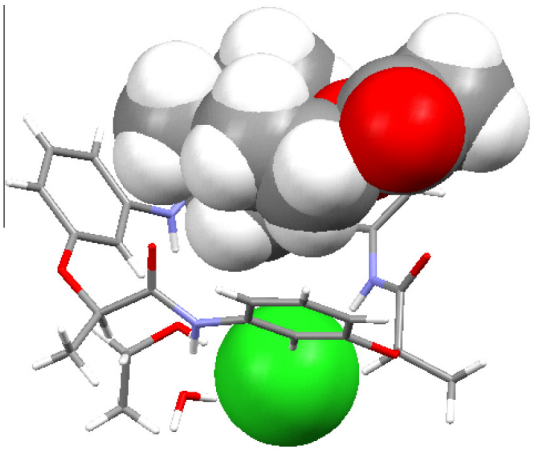

Figure 3. Single-crystal X-ray structure of 1 with AChCl (AChCl shown by space filling model).

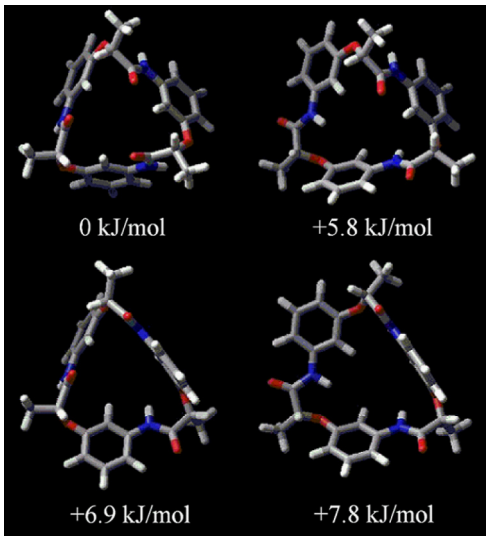

Figure 4. Molecular mechanics of **1**.

of methyl groups in AChCl by CH–O interaction. The shortest distance between the carbonyl oxygen of **1** and the electron-deficient proton of methyl group in AChCl was 2.60 Å, which is 0.3 Å shorter than the sum of the van der Walls radii of H (1.20 Å) and O (1.70 Å). A similar assist of electron-negative carbonyl oxygen atoms for the binding of cationic guests has already been reported.[5c,6b]

To study the driving force for the complexation of AChCl with **1** in $CDCl_3$ (K_a = 1998 M^{-1} at 298K, ΔG = −18.7 kJ mol^{-1}), we made a van't Hoff plot (see Supporting information) and found that ΔH = −17.5 kJ mol^{-1} and ΔS = 4.0 J mol^{-1} K^{-1}. This means that 94% of ΔG was gained from the enthalpy term, thus that the complexation was caused by the cation–π and CH–O interactions and the cooperative hydrogen bonding of the counter anion. Probably, a small positive value of entropy change (6% of ΔG) was gained through the desolvation process of **1** with chloroform. In our previous Letter, a bowl-shaped *ortho*-substituted analog of **1** was crystallized as a solvated structure with three chloroform molecules, where one was located inside the cavity and another two molecules were bound with **1** by weak CH–O hydrogen bonds.[12]

At this stage, we could not get crystal structure of guest free **1**. To estimate the preferred conformation, we performed a conformational search for **1** by doing molecular mechanics calculations with Monte Carlo minimization procedures. We did this using the MMFF94s force field[13] and GB/SA solvation treatment (chloroform)[14] implemented in the MacroModel program.[15] Figure 4 shows that the lowest-energy conformation of **1** is a C_3-symmetric bowl-shaped structure in which amide oxygen atoms are directed toward the bottom of the bowl. The calculations revealed that there are three other conformers within 7.8 kJ mol^{-1}: conformers with higher energies of +5.6, +6.9, and +7.8 kJ mol^{-1} (Fig. 4). We could see that in each of those structures two coplanar arrangements of the amide against the benzene ring are possible because the amide group of **1** conjugates with the benzene ring. In fact, the X-ray structure of **1** with acetylcholine chloride showed the guest binding was accompanied by flipping motion of all the amides of **1**.

Guest-induced relation of NOE's on ^{1}H NMR supported this conformational change of the amide group of **1** (Fig. 5). In addition, the flipping of the amide groups of **1** caused the chemical shifts of the benzene ring of **1** in 1H NMR spectra to move. Although Ha (in Fig. 5) of free **1** is 6.7 ppm, the presence of 15 equiv amount of AChCl moved the peak to 7.4 ppm (down-field shift). This significant change was caused by a deshielding effect from carbonyl groups of the flipped amide group.

To consider a binding effect of the chloride anion on this conformational change of the amide group, we performed an additional

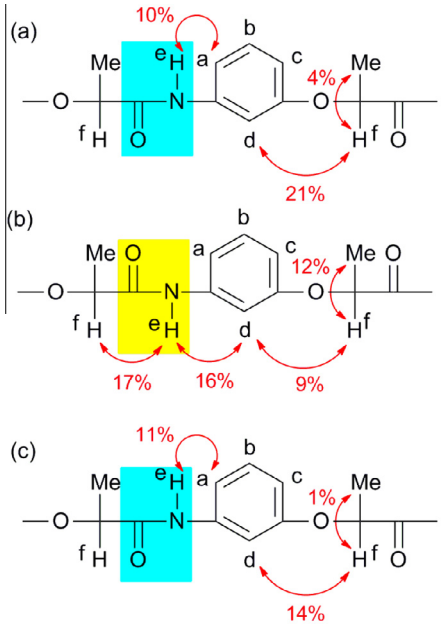

Figure 5. NOE correlations of (a) free **1**, (b) **1** with AChCl (1 equiv), (c) **1** with AChCl and **3** (1 equiv) in $CDCl_3$.

experiment using tris(thioura) (**3** in Fig. 1) as the specific receptor (K_a >10^4) for chloride anion.[16] In the presence of **3** (1 equiv of ACh-Cl), the NOE's on ^{1}H NMR is close to free host in Figure 5. It suggests that the presence of chloride anion is crucial for flipping of the amides of **1**. A similar anion-induced conformational reorganization of the macrocycles has been reported in the case of not only cyclic peptides[6] but also calixpyrroles.[10]

In this simultaneous binding of the ionic pair, anion-induced flipping of the amide group of **1** occurred. This guest response is a result of the flipping of the amide group causing dipole inversion that increases electron density on the rim of the bowl cavity. In the crystal structure, the amide oxygen atoms with negative charge are located in the rim of the bowl and complementarily interact with incoming ammonium cations. We conclude that this bowl-shaped tripeptide (**1**), which is composed of (*R*)-2-(3-aminophenoxy)propionic acid, has the properties of ditopic receptors for acetylcholine chloride and benzyltrimethylammoium compounds. The bowl-shaped tripeptide is a candidate pepitidomimetic receptor for molecular recognition of quaternary ammonium compounds.

Supplementary data

Crystallographic data for the structures in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 920352. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.02.066.

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