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Cholic-Acid-Based Fluorescent Sensor for Dicarboxylates and Acidic Amino Acids in Aqueous Solutions

Shun-Ying Liu,[†,‡] Lei Fang,[†,‡] Yong-Bing He,[†] Wing-Hong Chan,*[,‡] Kai-Tai Yeung,[‡] Yuen-Kit Cheng,[‡] and Rong-Hua Yang[§]

Department of Chemistry, Wuhan University, Hubei, China, Department of Chemistry and Central Laboratory of the Institute of Molecular Technology for Drug Discovery and Synthesis, Hong Kong Baptist University, Hong Kong SAR, China, and College of Chemistry and Molecular Engineering, Peking University, Beijing, 100871, China

whchan@hkbu.edu.hk

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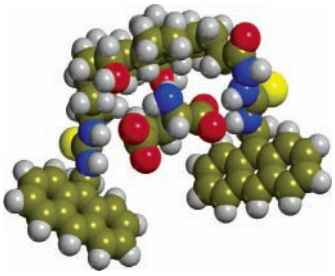
ABSTRACT



The binding affinities of a cholic-acid-based fluorescent neutral receptor toward dicarboxylate anions and amino acids have been investigated in a CH_3OH/H_2O system (1:1, 0.01 M HEPES buffer, pH = 7.4) by fluorescence titration experiments. The synthetic host bearing four convergent functionalities strongly binds glutamate via multiple hydrogen bonds with a binding constant of $(5.57 \pm 0.88) \times 10^6$.

The recognition and sensing of anionic substrates by positively charged or neutral synthetic receptive molecular systems have continued to attract much attention because of the fundamental roles of anions in many chemical and biological processes.[1] Dicarboxylates are among the most attractive targets for anion recognition and sensing because they are the key structural moieties of many bioactive molecules such as amino acids and proteins.[2] Dicarboxylates are biologically important because of their considerable roles in numerous metabolic processes such as the generation of high-energy phosphate bonds and the biosynthesis of important intermediates.[3] For example, the glutamate anion, an amino acid bearing two carboxylate groups, is the major excitatory neurotransmitter in the central nervous system.[4] Its sensitive determination is of great interest in a variety of areas of bioanalytical and biomedical research. Considerable effort has been devoted to the development of ditopic anion receptors with pertinent signal subunits (chromophore or fluorophore) as sensing probes for dicarboxylates.[5]

However, most known dicarboxylate sensors only function in aprotic solvents,[6] such as DMSO, $CHCl_3$, and CH_3CN,

[†] Wuhan University.
[‡] Hong Kong Baptist University.
[§] Peking University.

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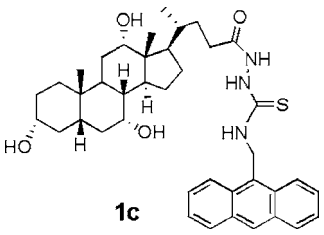
and they are inappropriate to be used in biochemical or physiological investigations. Thus, the design and synthesis of water-soluble artificial anion receptors is a challenging and attractive task in supramolecular chemistry. Recently, several excellent receptors have been strategically developed to satisfy the geometrical requirements of dicarboxylate and are amenable to fluorescent measurements in aqueous solutions. To overcome the competition from protic solvents for binding sites, positively charged receptors based on polyammonium,[7] guanidinium,[8] polyprotonated azacrown ether,[9] imidazolium,[10] or metal-containing species were developed.[11] In contrast, only fair binding affinity to dicarboxylates by neutral receptors in protic solvents was reported.[3b,12] In this letter, we report the synthesis of fluorescent dicarboxylate receptor **1a** developed from cholic acid. In CH_3OH/H_2O (1:1), **1a** showed good binding ability toward a variety of dicarboxylates and demonstrated outstanding affinity to glutamate.

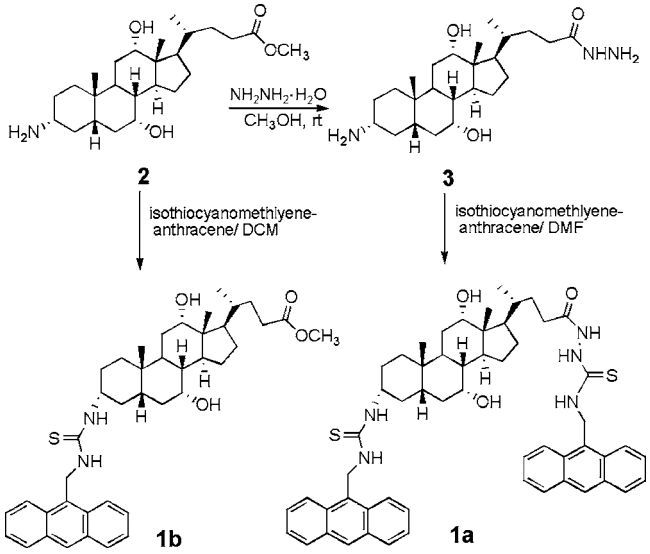


1c

Pioneered by the seminal works of Davis and others, cholic acid has proven to be a promising building block to prepare supramolecular systems for molecular recognition.[13] To obtain high affinity and selectivity, the three axially oriented functional groups at the C3, C7, and C12 of cholic acid after proper modifications could be assembled in such a way to confer cooperative binding interactions to a specific guest anion. We have shown for the first time that the flexible C17 side chain of cholic acid can be exploited to introduce a ligating group onto the C24 as an additional binding site for complexing anions.[14] To capitalize our finding in sensor design, we envision that, by proper alignment of the pendant functionalities and the flexible side chain present in the cholic acid scaffold, a highly convergent multibinding cavity can be designed. To create the ditopic receptor for binding dicarboxylates, we call for the incorporation of aminothiourea and amidothiourea groups to the C3 and the C24, respectively. The chemical environment of the cholic acid scaffold should make the sensor selective toward guest molecules. The hydroxyl pendant groups at the C7 and C12 of the host could provide additional binding sites for substituted dicarboxylates such as glutamate or aspartate. We envisage that, owing to the three point interactions between the neutral host and the guests, molecular recognition could be observed even in protic solvents. To complete the construction of fluorescent sensors **1a**, methylene anthracene groups were appended onto the receptor to make the corresponding Photo-induced Electron Transfer (PET) chemosensing.[15]

Treatment of **2**[13b] with hydrazine in methanol gave the corresponding hydrazide **3**. Anthracene moieties were appended onto the C3 and C24 of **3** via the formation of aminothiourea and amidothiourea, respectively, to give rise to the sensor **1a** (Scheme 1). For conducting control

Scheme 1. Synthetic Route for **1a** and **1b**



experiments, **1b** and **1c**,[14] analogues of **1a** with the presence of only one anthracenylmethyl group at the C3 and C24, respectively, were synthesized accordingly. **1a** is soluble in DMSO, CH_3OH, and CH_2Cl_2. Furthermore, its solubility in the CH_3OH/H_2O system is good even when the water content

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in the mixed solvent is as high as 90%. To investigate the binding properties of neutral receptor **1a** toward dicarboxylate anions in aqueous solution, the CH_3OH/H_2O system (1:1, 0.01 M HEPES buffer, pH = 7.4) was employed to ensure that the receptors are completely dissolved in the titration experiments.

In fluorimetric study, when the solution of **1a** (5.0×10^{-6} M) in CH_3OH/H_2O (1:1, 0.01 M HEPES buffer, pH = 7.4) was excited at 366 nm, **1a** gave a characteristic emission spectrum with a monomeric anthracene maximum at ca. 413 nm. Subsequently, the binding behaviors of **1a** with different dicarboxylate anions in the CH_3OH/H_2O system (1:1, 0.01 M HEPES buffer, pH = 7.4) were investigated by means of titration fluorimetry (vide supra).

With a gradual increase in the concentration of aliphatic dicarboxylate anions, malonate, succinate, glutarate, adipate, suberate, and sebrate (as tetrabutylammonium salts), the fluorescent emission of **1a** can be quenched by about 10%. The extent of quenching though small is of great significance. Much stronger quenching occurred when the titrations were performed in aprotic solvents. The aminothiourea and amidothiourea receptive sites of the host can complex the competitive methanol and water molecules via hydrogen bonding, triggering considerable PET fluorescent quenching on anthracene moieties. The suppressive effect by solvent molecules renders a relatively low monomeric emission peak even in the absence of guest molecules (see Supporting Information). In this regard, we are particularly gratified to find that, when treated with L-asparate, glutamate, *N*-acetyl-L-asparate (**4**), and *N*-acetyl-L-glutamate (**5**) (also in the form of tetrabutylammonium salts), the emission spectrum of **1a** can be quenched by ca. 20%. The typical change of emission spectra of **1a** caused by **5** is shown in Figure 1. On the basis

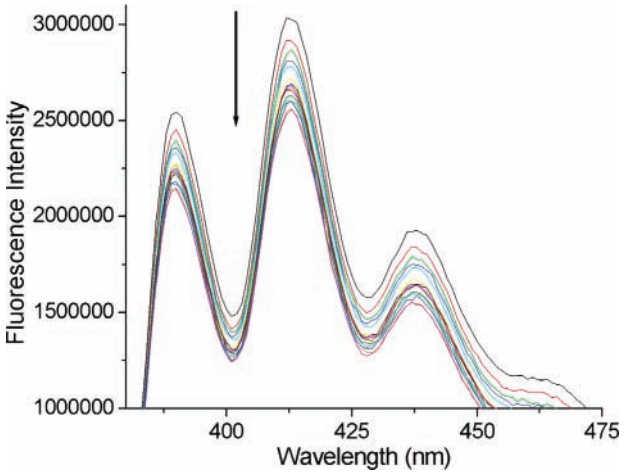

Figure 1. Emission spectra of **1a** (5.0×10^{-6} M) with different concentrations of *N*-acetyl-L-glutamate (**5**) in aqueous solution (CH_3OH/H_2O = 1:1, 0.01 M HEPES buffer, pH = 7.4). λ_{ex} = 366 nm.

of the change of fluorescent intensity associated with the stepwise addition of guest dicarboxylates, the complex

Table 1. Association Constants of **1a** with Guest Anions

anion[a]	K_a[b]	$-\Delta G$ (kcal/mol)
malonate	$(9.91 \pm 1.92) \times 10^4$	6.72
succinate	$(1.73 \pm 0.42) \times 10^5$	7.05
glutarate	$(2.93 \pm 0.61) \times 10^5$	7.35
adipate	$(5.61 \pm 1.43) \times 10^5$	7.73
suberate	$(1.89 \pm 0.33) \times 10^5$	7.10
sebate	$(6.53 \pm 0.91) \times 10^4$	6.48
isophthalate	$(2.20 \pm 0.49) \times 10^4$	5.84
terephthalate	$(1.37 \pm 0.29) \times 10^5$	6.91
citrate	$(1.48 \pm 0.20) \times 10^5$	6.96
4	$(4.21 \pm 0.51) \times 10^5$	7.57
5	$(1.04 \pm 0.26) \times 10^6$	8.10
6	$(5.45 \pm 0.97) \times 10^5$	7.72
L-aspartate	$(8.53 \pm 1.63) \times 10^4$	6.63
L-glutamate	$(5.57 \pm 0.88) \times 10^6$	9.08

[a] Anions were used as their tetrabutylammonium salts. [b] K_a is the apparent constant for the equilibrium of the 1:1 stoichiometric ratio between **1a** and dicarboxylate in aqueous solution, 0.01 M HEPES buffer solution, pH=7.4.

association constants (K_a) were calculated using nonlinear least-squares curve fitting and are compiled in Table 1. Excellent fitting ($R > 0.99$) of the titration data points shown in Figure 2 demonstrated that **1a** formed 1:1 complexes with

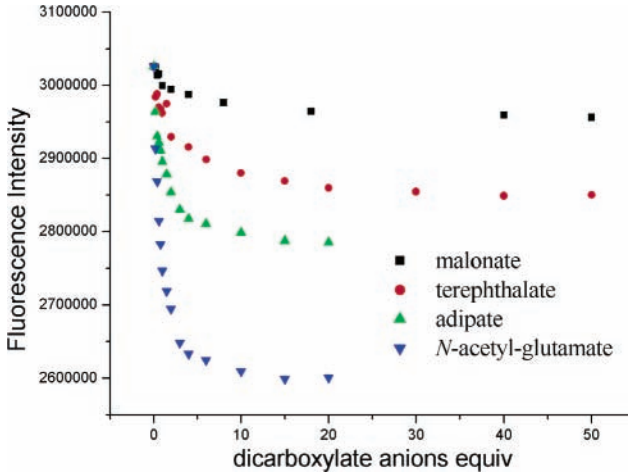

Figure 2. Changes of fluorescence intensity of **1a** at 413 nm upon addition of given anions in aqueous solution (CH_3OH/H_2O = 1:1, 0.01 M HEPES buffer, pH = 7.4). λ_{ex} = 366 nm.

aliphatic dicarboxylates.[16] Job plots of **1a** with manolate and adipate further confirmed the 1:1 stoichiometric ratios of the receptor and the dicarboxylates (see Supporting Information).

For the aliphatic dicarboxylates, the respective binding constants of **1a** exhibit a modest dependence on the chain

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length of the dicarboxylates. Receptor **1a** showed a slightly higher affinity to adipate ($K_a = 5.61 \times 10^5$) among other dicarboxylates. Conceivably, the flexible amidothiourea sidearm appended at the C17 of the semirigid cholic acid scaffold exercises some spatial constraints in complexing the dicarboxylate guests. The less complementary match between dicarboxylates which possess too short (malonate and succinate, $K_a = 9.91 \times 10^4$ and 1.73×10^5, respectively) or too long chain lengths (sebrate, $K_a = 6.53 \times 10^4$) and the receptor results in weaker binding. To provide more insight into the nature of the binding site, some more rigid aromatic dicarboxylates were chosen for comparative studies (see Table 1). The chain length between two terminal carboxylate groups of adipate and terephthalate is comparable; however, the association constant of the former with **1a** is substantially greater than that of the latter with **1a** (i.e., $K_a = 5.61 \times 10^5$ vs $K_a = 1.37 \times 10^5$). Furthermore, the association constant between **1a** and terephthalate was ca. 5-fold stronger than that with isophthalate. Apparently, the cavity defined by the host in an aqueous methanol solvent system is rather distinct in size and electronic environment and is able to bind specific guests with modest selectivity.

To extend the binding capacity of **1a**, other than the exploitation of two anion receptors appended at the C3 and the C24 of the host, the two axial hydroxyl groups at the C7 and C12 can be harnessed for complexing trifunctional guest molecules. Glutamate, aspartate, and their N-protected derivatives were added stepwise into the solution of **1a** under the same experimental conditions. Binding experiments revealed that receptor **1a** showed a much stronger affinity to L-glutamate and its N-protected derivatives than their simple dicarboxylate counterpart. **1a** binds glutamate with ca. a 10-fold stronger association constant than that of the **1a** and glutarate complex. In accord with the "tritopic" nature of the host, the association constant of **1a** and 1,3,5-benzenetricarboxylate (**6**) was found to be substantially greater than that of **1a** and isophthalate. In contrast, other trifunctional guest molecules such as L-aspartate, **4**, and citrate interacted with **1a** with the extent of binding affinity comparable to that of the aliphatic dicarboxylates, as evidenced by their respective association constants. This suggested that the preorganization of the receptive sites of the host fully utilizes the three point interactions only with structurally complementary guests (i.e., **5** and L-glutamate).[17] Such a presumption corroborates with modeling studies. Brief molecular dynamics in an explicit water solvent followed AM1 charge fitting for the host−guest complex of **1a**, and L-glutamate indicated that the calculated distance from the C12 oxygen of the host to the amino nitrogen of glutamate in the optimized conformation is 3.19 Å, which is a moderate H-bond (graphical abstract).[18] On the other hand, control binding experiments of **1b** and **1c** with glutamate, aspartate, and their *N*-acetyl derivatives under the same conditions revealed that no appreciable quenching was observed. Because of the solubility problems, ^{1}H NMR study of the binding of **1a** and guest molecules was not feasible. However, slight changes of the chemical shifts of the *N*-acetyl methyl protons of **4** and the anthracenylmethylene protons of **1a** were observed in DMSO-d_6 for the complex (see Supporting Information).

In conclusion, neutral fluorescent receptors **1a** and **1b** for dicarboxylates in a protic solvent system (CH_3OH/H_2O = 1:1) have been designed and synthesized. **1a**, adequately functionalized at the C3 and C24 of cholic acid, showed high affinities to dicarboxylate anions resulting from multiple hydrogen-bonding interactions in aqueous solution. The preorganization of **1a** permits three points binding with the guests leading to a more selective complexation with acidic amino acids than their corresponding dicarboxylates. **1a** is a promising chemosensor for the glutamate anion in aqueous solution, and the exploration of its use in chiral recognition of amino acids is underway.

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Supporting Information Available: Synthesis and characterization spectra of **1a** and **1b**, the fluorometric titration experiment, the ^{1}H NMR study, Job plots of **1a** with malonate and adipate, the fluorescence intensity of **1a** in different solvents, and computation data. This material is available free of charge via the Internet at http://pubs.acs.org.

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