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# Selectivity via Cooperative Interactions: Detection of Dicarboxylates in Water by a Pinwheel Chemosensor

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The recognition properties of a cooperative pinwheel chemosensor for dicarboxylates are described. The sensor possesses four guanidinium recognition elements to cooperatively bind two dicarboxylates of varying size. The effect of cooperativity and the read-out mechanism contributes to favorable binding constants for dicarboxylates in water, as well as a high degree of selectivity over monocarboxylates. Appropriate methods of reporting affinity for cooperative systems are discussed.

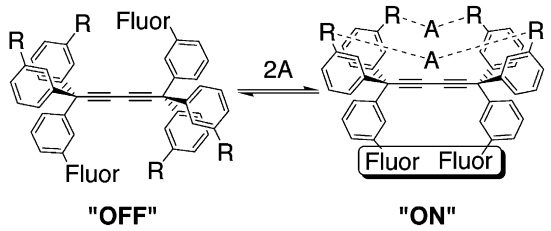
## Introduction

Chemical sensors can play a critical role in the elucidation of intracellular chemistry by giving real-time information about the environment of a cell in a nondestructive manner.[1] Several biologically useful fluorescent sensors for metal ions have been prepared, yet *practical* sensors for organic compounds are less well developed as a result of the difficulty in obtaining appropriate affinity and selectivity under physiologically relevant (aqueous) conditions.[2] As part of an ongoing project directed toward preparation of chemical sensors with useful recognition properties, we have developed a class of cooperative receptors based on bis-tritylacetylenes, termed pinwheel receptors.[3] We postulated that cooperative binding of multiple analytes can impart a higher affinity and greater selectivity to a given sensor relative to a similar noncooperative system. The first premise was initially verified using a pinwheel sensor that cooperatively bound three metal ions with higher affinity than a similar noncooperative analogue.[3]

More recently, we described a second generation design for sensing metal ions (Scheme 1). In this series, the fluorescent groups are appended directly to the sensor framework but held separate from the recognition elements, giving 2-fold cooperative recognition of metal ions.[4] Using this class of sensor, we herein document the advantages of cooperative recognition with respect to *selectivity* issues using a pinwheel sensor with strong affinity for dicarboxylates in water.

**Design Considerations.** Aqueous recognition of dicarboxylates has been studied by several groups[5,6] and provides a convenient benchmark for selectivity and affinity against which a cooperative sensor can be tested. Our cooperative dicarboxylate sensor (**1**, Scheme 2) contains four guanidinium groups such that one dicarboxylate will be bound between two pairs of guanidinium groups. Two anthracene sulfonanilide groups serve as the fluorescent read-out. The buytadiyne spacer of our previous system was not sufficiently long to accommodate dicarboxylate guests; thus the phenyldiyne was necessary for this application.[7] Clearly, any dicarboxylate could be bound by this sensor; however, models indicate that dicarboxylates longer than six carbons (adipate) could potentially bridge two guanidinium groups on the same trityl unit. Thus, the binding mode described in Scheme 2 is the only one accessible to small dicarboxylate guests. In the absence of analyte, the trityl groups enjoy free rotation about the phenyldiyne axis. Binding of the first dicarboxylate freezes out this rotational freedom and preorganizes the receptor for a stronger (cooperative) second binding event.[4,8] Restricted rotation also enforces an interaction between the pendant fluorophores leading to the fluorescent "ON" response (right in Scheme 2). This system requires that the analyte bind two opposing recognition elements simultaneously to give a fluorescent

**SCHEME 1. General Sensor Design**[a]




[a] R = recognition element; A = analyte; Fluor = fluorophore.

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(1) (a) *Chemosensors of Ion and Molecule Recognition*. Desvergne, J. P., Czarnik, A. W., Eds.; NATO ASI Series C: 492; Kluwer Academic Press: Dordrecht, 1997. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson T.; Huxley, A. J. M.; McCoy, C. P.; Radermacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.

(2) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423.

(3) Glass, T. E. *J. Am. Chem. Soc.* **2000**, *122*, 4522−4523.

(4) Raker, J.; Glass, T. E. *J. Org. Chem.* **2001**, *66*, 6505−6512.

(5) Fitzmaurice, R. J.; Kyne, G. M.; Douheret, D.; Kilburn, J. D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 841−864.

(6) For recent reviews of anion recognition, see: (a) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40* 486−516. (b) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609−1646.

(7) Garcia-Garibay has used bis-tritylphenyldiynes for a "molecular compass": Dominguez, Z.; Dang, H.; Strouse, M. J.; Garcia-Garibay, M. A. *J. Am. Chem. Soc.* **2002**, *124*, 2398−2399.

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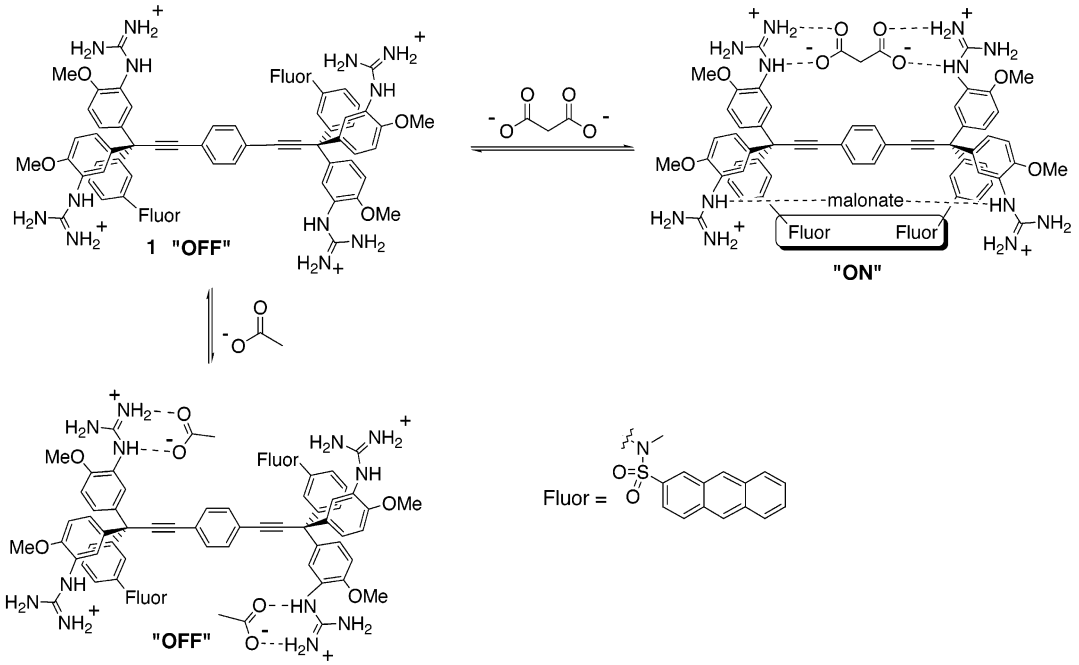
**SCHEME 2. Cooperative Recognition of a Dicarboxylate by Sensor 1**




response and should therefore be quite selective. Compounds that bind to only one guanidinium (e.g., acetate, bottom in Scheme 2) will not produce a fluorescent response since the response is coupled to the cooperative binding event rather than to binding to one of the recognition elements. Therefore, to the extent that monocarboxylates bind to the sensor, they should not turn the sensor "ON". Furthermore, guests that do not bridge a pair of guanidinium groups will not enjoy the cooperative binding interaction since they cannot freeze the rotational freedom of the trityl system. Thus, cooperatively bound guests will have a higher affinity than the noncooperatively bound guests. Because of this effect, high background concentrations of monocarboxylates should not interfere with the ability of the sensor to recognize the intended guest, a dicarboxylate.[9] For these reasons we anticipate that sensors based on this design will be very selective for their target analytes over other potential (monofunctional) contaminants.

## Results and Discussion

Sensor **1** was prepared as the tetra-trifluoroacetate salt as described previously.[10] In buffered water (10 mM TRIS, pH 7.5), sensor **1** had a broad emission spectrum typical of anthracene sulfonates in water[11] (Figure 1).

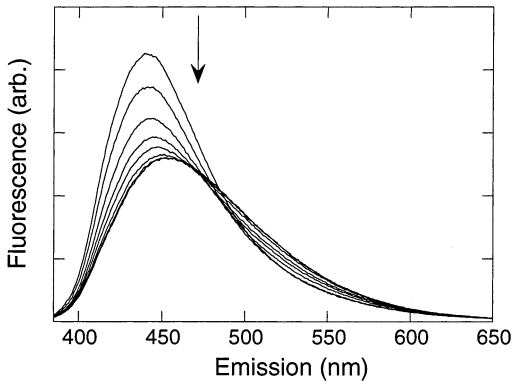



**FIGURE 1.** Fluorescence emission ($\lambda_{exc}$ = 376 nm) of sensor **1** as a function of added disodium malonate ([**1**] = 10 $\mu$M, [malonate] = 0−480 $\mu$M in 10 mM TRIS buffer, pH = 7.5).

Titration of **1** with malonate resulted in a decrease in fluorescence and a corresponding red shift of 23 nm.[12] As in previous examples,[4] the UV/vis spectrum of **1** did not change upon titration of malonate, indicating that the fluorescence response is not a function of changes in the ground state. Confirmatory NMR analysis of binding was impossible as compound **1** aggregates at concentrations above $1 \times 10^{-4}$ M in aqueous solution. Plotting the ratio of the fluorescence at 535 and 443 nm as a function of added dicarboxylate results in a binding isotherm (Figure 2) that is sigmoidal, indicative of cooperative binding. Such binding isotherms are commonly evaluated using the Hill equation (eq 1)[13] where $Y$ is the fractional saturation of the receptor, [guest] is the concentration

(8) Shinkai first used this method of freezing rotational freedom to produce cooperative interactions in his porphyrin double-decker allosteric receptors, as well as other systems that have been utilized for cooperative recognition of a range of analytes. For comprehensive reviews, see: Shinkai, S.; Ikeda, M.; Sugasaki, A.; Takeuchi, M. *Acc. Chem. Res.* **2001**, *34*, 494−503. Takeuchi, M.; Ikeda, M.; Sugasaki, A.; Shinkai, S. *Acc. Chem. Res.* **2001**, *34*, 865−873.

(9) The advantages of cooperative binding for selectivity of a divalent analyte over a monovalent analyte have been recently demonstrated by Shinkai in a cleverly devised tetraporphyrin array: Kubo, Y.; Ikeda, M.; Sugasaki, A.; Takeuchi, M.; Shinkai, S. *Tetrahedron Lett.* **42**, 7435−7438. See also: Takeuchi, M.; Shioya, T.; Swager, T. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 3372−3376. Sugasaki, A.; Sugiyasu, K.; Ikeda, M.; Takeuchi, M.; Shinkai, S. *J. Am. Chem. Soc.* **2001**, *123*, 10239−10244.

(10) Raker, J.; Glass, T. E. *Tetrahedron* **2001**, *57*, 10233−10240.

(11) (a) Richards, T. C.; Bard, A. J. *Anal. Chem.* **1995**, *67*, 3140−3147. (b) Hubbard, B. K.; Beilstein, L. A.; Heath, C. E.; Abelt, C. J. *J. Chem. Soc., Perkin Trans. 2* **1996**, 1005−1009.

(12) The shift in fluorescence can be attributed to changes in solvent exposure: (a) Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartor, G. *J. Org. Chem.* **1997**, *62*, 6283−6289. (b) Lu, L.; Lachicotte, R. J.; Penner, T. L.; Perlstein, J.; Whitten, D. G. *J. Am. Chem. Soc.* **1999**, *121*, 8146−8156.

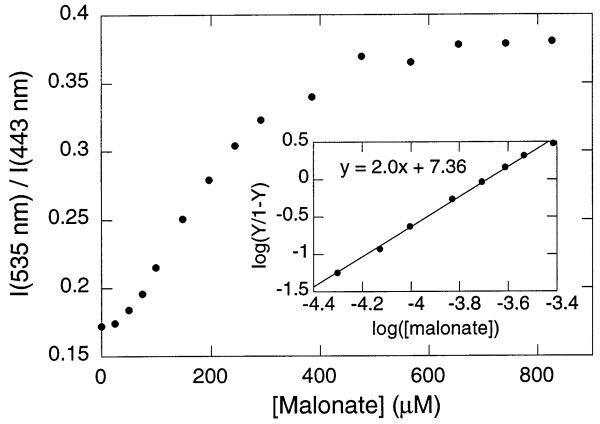



**FIGURE 2.** Plot of ratio of fluorescent emission of compound **1** at 535 and 443 nm as a function of added malonate. Inset is the Hill plot of the data using eq 1.

$$\log\left(\frac{Y}{1-Y}\right) = n\cdot\log[\text{guest}] + \log K_a \qquad (1)$$

of free malonate, $n$ is the Hill coefficient, and $K_a$ is the binding constant. In this case, it is appropriate to assume that the concentration of free malonate is the same as the concentration of added malonate as very little malonate is bound at any point in the titration.

Fitting the data to the Hill equation (inset in Figure 2) yields a $K_a$ of $2.3 \times 10^7$ $M^{-2}$ and a Hill coefficient of 2.0, which supports 2-fold cooperative binding of the guest.[14] Previous studies of dicarboxylate binding to polyammonium cations have shown that receptors with higher positive charge have stronger affinities for the anionic guests.[5b,15] The observation of positive cooperativity in this system underscores the importance of preorganization for enhancing the binding interaction. If electrostatic interactions were the only driving force, it would be expected that the second binding event would be weaker than the first, since the overall charge on the sensor is +4 in the unbound state but +2 in the singly bound state. A Hill coefficient of 2.0 in this case implies that the second binding event is stronger than the first.

Table 1 contains the Hill coefficients and association constants ($K_a$) derived from the Hill equation for a series of dicarboxylates in buffered water. The binding stoichiometry was determined by Job analysis.[16] All simple dicarboxylates tested had sigmoidal binding isotherms with Hill coefficients of 1.7−2.0.

**Analysis of Binding Affinity.** For typical chemical sensors, the associate constant is useful for determining the range of concentrations over which the sensor can detect the analyte.[2] The association constants derived from the Hill analysis are not useful in this regard. Furthermore, comparisons of the Hill derived $K_a$ values in Table 1 are complicated by the fact that not all of the

**TABLE 1. Hill Coefficients and Affinity Values for Various Analytes in Buffered Solution**

| | Tris buffer (10 mM, pH 7.5) | | | sodium acetate (10 mM) | | |
|---|---|---|---|---|---|---|
| dicarboxylate | Hill coeff | $K_a$ ($M^{-2}$) | $K_{0.5}$ ($\mu$M) | Hill coeff | $K_a$ ($M^{-2}$) | $K_{0.5}$ ($\mu$M) |
| oxalate | 2.0 | $7.1 \times 10^7$ | 111 | 1.9 | $1.8 \times 10^7$ | 150 |
| malonate | 2.0 | $2.3 \times 10^7$ | 196 | 1.9 | $7.6 \times 10^6$ | 219 |
| succinate | 1.9 | $8.3 \times 10^6$ | 229 | 2.0 | $1.4 \times 10^7$ | 271 |
| maleate | 2.0 | $6.3 \times 10^7$ | 108 | 2.0 | $5.3 \times 10^7$ | 134 |
| fumarate | 1.9 | $1.8 \times 10^7$ | 171 | 1.8 | $2.6 \times 10^6$ | 232 |
| glutarate | 1.7 | $1.2 \times 10^6$ | 264 | 1.7 | $8.9 \times 10^5$ | 375 |
| phthalate | 2.0 | $1.3 \times 10^9$ | 23 | 2.0 | $1.2 \times 10^9$ | 27 |
| aspartate | | | >4000 | | nd[a] | nd |
| *N*-acetyl aspartate | 2.0 | $7.8 \times 10^6$ | 437 | | nd | nd |
| glutamate | | | >4000 | | nd | nd |
| *N*-acetyl glutamate | 1.7 | $1.3 \times 10^6$ | 288 | | nd | nd |

[a] nd = not determined.

Hill coefficients are rigorously identical. The underlying assumption of the Hill equation is that of "all or none" binding. For situations in which the Hill coefficient is noninteger, this assumption is not rigorously satisfied and the $K_a$ is a poor indication of binding affinity.[17] As a more useful measure of affinity, we also report the half-saturation value of each analyte, which is the concentration of analyte required to saturate 50% of the sensor in solution ($Y = 0.5$). This value ($K_{0.5}$) is obtained by fitting the binding isotherm to eq 2. The half saturation value

$$Y = \frac{[\text{guest}]^n}{[\text{guest}]^n + K_{0.5}^n} \qquad (2)$$

is a useful indicator of affinity since it describes the median concentration of analyte that can be detected by the sensor. Furthermore, this value is directly comparable among other analytes and indeed among other sensors. For malonate, $K_{0.5} = 1.96 \times 10^{-4}$ M, indicating that the sensor can quantify malonate in the high micromolar range.

The data in Table 1 show that the cooperative receptor has high affinity for dicarboxylates in water, comparing very favorably to other noncooperative guanidinium-containing receptors.[18] The general trend in selectivity between dicarboxylates is that rigid dicarboxylates bind better (i.e., lower half saturation value) as expected on the basis of entropic considerations.[19] Thus, maleate binds better than succinate and the series oxalate, malonate, succinate, glutarate decreases in affinity. Interestingly, glutarate also has a depressed Hill coefficient, which may indicate that, with the long chain, preorganization of the sensor does not enhance the second binding event to the same extent as the smaller, more rigid dicarboxylates. Of the dicarboxylates tested, ph-

(13) Levitizki, A. Quantitative Aspects of Allosteric Mechanisms. *Mol. Biol. Biochem. Biophys.* **1978**.
(14) Control compounds having only two guanidiniums on one trityl unit did not show significant binding of dicarboxylates, indicating that the mode of binding is across the acetylene axis as indicated in Scheme 2. See also ref 4.
(15) Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1988**, *71*, 749−756.
(16) Connors, K. A. *Binding Constants*; John Wiley: New York, 1987.
(17) .Weiss, J. N. *FASEB J.* **1997**, *11*, 835−841.
(18) (a) Linton, B. R.; Goodman, M. S.; Fan, E.; van Arman, S. A.; Hamilton, A. D. *J. Org. Chem.* **2001**, *66*, 7313−7319. (b) Sebo, L.; Diederich, F.; Gramlich, V. *Helv. Chim. Acta* **2000**, *83*, 93−113. (c) Shao, Y.; Linton, B.; Hamilton, A. D.; Weber, S. G. *J. Electroanal. Chem.* **1998**, *441*, 33−37. (d) Metzger, A.; Lynch, V. M.; Anslyn E. V. *Angew. Chem., Int. Ed. Eng.* **1997**, *36*, 862−865.
(19) Hossain, M. A.; Schneider, H.-J. *Chem. Eur. J.* **1999**, *5*, 1284−1290.

thalate has the highest affinity. Given the similarity in length and geometry between phthalate and maleate, the 5-fold difference in affinity is rationalized in terms of hydrophobicity.

Since the core of the sensor is very hydrophobic, it is likely that the phenyl ring of the phthalate can juxtapose itself near the phenylbisacetylene while the carboxylates associate with the guanidinium groups. Finally, there is no apparent correlation between the p$K_a$'s of this series of carboxylic acids and their binding constants. Notably, the second p$K_a$ of succinate (5.72)[20] is intermediate between maleate (6.58) and fumarate (4.54), yet the binding constant of succinate is much lower.

Titration of the sensor with large amounts of sodium acetate (up to 100 mM) did not elicit any response from the fluorophores as anticipated from the receptor design. Moreover, titration of the sensor with dicarboxylates in a solution containing 10 mM acetate (1000-fold excess relative to the sensor concentration) gave only slightly lower binding constants compared to the noncoordinating buffer (right columns in Table 1). This result supports our contention that cooperativity combined with the method of fluorescent read-out imparts a high degree of selectivity for the intended guest.

**Amino Acids.** Two important neurotransmitters, glutamate and aspartate, were also tested as they possess dicarboxylate functionality similar to those described above. In fact, these compounds did not give rise to any response from the sensor (up to 2.0 mM), indicating that either the amino acids did not bind to the sensor or they associate with only one of the recognition elements and do not give an "ON" response. This result can be rationalized on an electrostatic basis since the amino acids have an overall unit negative charge compared to the −2 charge on the dicarboxylates. This rationale is supported by the fact that both *N*-acetyl glutamate and *N*-acetyl aspartate elicit a fluorescent response from the sensor with half saturation values that are somewhat higher than those of the corresponding dicarboxylates (glutarate and succinate) as a result of the more polar nature of the *N*-acetyl amino acids.

(20) *Data for Biochemical Research*, 3rd ed.; Dawson, R. M. C., Elliott, D. C., Elliott, W. H., Jones, K. M., Eds.; Clarendon Press: Oxford, 1986.

## Conclusion

In conclusion, a cooperative chemical sensor for dicarboxylates in aqueous media has been developed. The sensor affinity was analyzed in terms of half-saturation values ($K_{0.5}$). On the basis of the cooperative recognition capability of the sensor and the mechanism of fluorescent read-out, the sensor is quite selective for dicarboxylates, even in the presence of high backgrounds of monocarboxylates. Combined with previous results, these data indicate that the pinwheel framework constitutes an effective platform for the construction of a variety of sensors.

## Experimental Section

Sensor **1** was prepared according to the published procedure.[10] Fluorescence measurements were recorded on a spectrofluorimeter in a 1 cm quartz cuvette ($\lambda_{ex}$ = 376 nm, excitation and emission slit width = 5 nm). Solutions were prepared using filtered deionized deoxygenated water using the indicated buffer and pH adjusted with HCl. In a typical experiment, sensor **1** (10 $\mu$M in buffer) was titrated with the dicarboxylate (10 mM in buffer) containing 10 $\mu$M sensor **1** (in order to prevent dilution of the sensor).

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