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Selective Amine Recognition: Development of a Chemosensor for Dopamine and Norepinephrine

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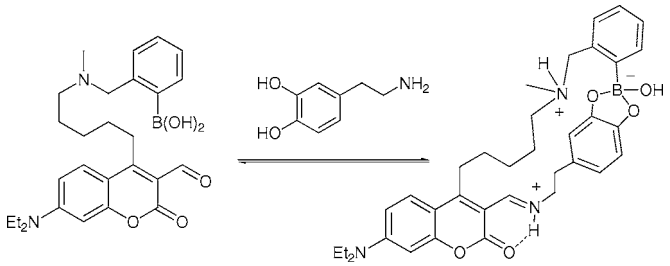
ABSTRACT



A boronic acid-containing coumarin aldehyde was designed and synthesized. The sensor binds to catecholamines such as dopamine and norepinephrine by forming an iminium ion with the amine as well as a boronate ester with the catechol. An internal hydrogen bond produces a colorimetric response to these analytes with good selectivity for catecholamines over simple amines. The fluorescence of the sensor is quenched by the catechol.

Catecholamines, including dopamine, are an important class of neurotransmitters that are involved in a variety of central nervous system functions. Malfunction of dopamine-responsive neurons has been implicated in a number of disease states including Parkinson's disease, sparking the development of tools to study these systems.[1] In particular, the recognition and sensing of dopamine in biological media has been of great interest. Direct dopamine detection has been accomplished using electrochemical techniques due to the favorable redox properties of the catechol.[2] A small-molecule chemosensor for dopamine would complement electrochemical techniques in a variety of experimental systems. Ideally, a chemosensor must bind dopamine in the presence of many diverse competitors. Thus, we have undertaken an effort to develop a chemical sensor that is selective for dopamine against other neurotransmitters and biological competitors.

The literature is replete with examples of dopamine recognition. These efforts include dopamine transport agents[3] and other receptors that function in organic solvents.[4] Aqueous recognition of dopamine has been accomplished using membrane-associated receptors,[5] sol gel films,[6] RNA aptamers,[7] and certain solid-state sensors.[8] Cyclophanes have been quite useful for selective dopamine recognition,[9]

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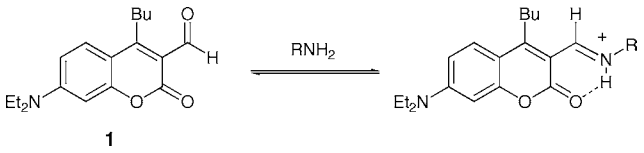
including a recent example that displays shape-selective recognition with only noncovalent interactions.[10]

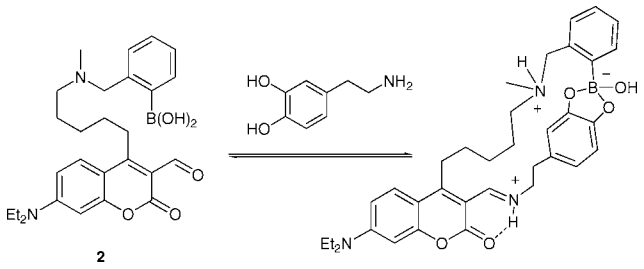
We have recently been investigating sensing of amine-containing analytes under physiological conditions using coumarin aldehydes.[11] Formation of an iminium ion between the aldehyde and an amine analyte produced a large chromophoric response due to an internal hydrogen bond with the coumarin carbonyl (Scheme 1). In addition, selective

Scheme 1



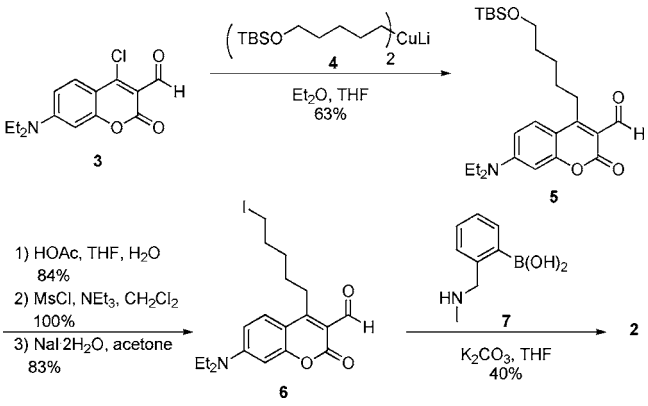
excitation of the iminium ion resulted in a substantial increase in fluorescent intensity upon titration with amines. Importantly, these studies showed that electron-poor aldehydes can serve as good recognition elements for amines under high-salt physiological conditions. However, aldehyde **1** showed little discrimination among various primary amine guests, giving weak associations with most primary amines. The coumarin aldehyde scaffold thus provides an ideal starting point for the development of sensors for analytes such as dopamine that contain primary amines.

Scheme 2



In this study, sensor **2** (Scheme 2) was designed to bind catecholamines under physiological conditions. A boronic acid[3a] was incorporated as an additional recognition element to enhance both selectivity and affinity for dopamine over other primary amine competitors. A flexible spacer between the coumarin and boronic acid was utilized to preserve the integrity of the internal hydrogen bond in the complex. A zwitterionic ammonium−boronate complex is postulated on the basis of recent evidence for such structures[12] as well as modeling (Macromodel 8.5), which indicates that a B−N

Scheme 3



bond is difficult to achieve in this system. The synthesis of sensor **2** is outlined in Scheme 3. TBS-protected alcohol **5** was produced from 4-chloro-7-diethylamino-3-formyl coumarin, **3**,[13] and cuprate **4**[14] in 63% yield. The TBS-protected alcohol was converted to iodide **6** in three steps via the mesylate. A final alkylation with aminomethyl phenylboronic acid **7**[15] gave **2** in 40% yield following purification by HPLC.

Sensor **2** was then examined spectrophotometrically by titration with dopamine under neutral aqueous conditions in which a reducing agent was included to suppress oxidation of the air-sensitive catechol group. By UV−vis absorption, sensor **2** gave a large red shift of the major visible absorption (Figure 1) similar to that seen for the simple coumarin butyl

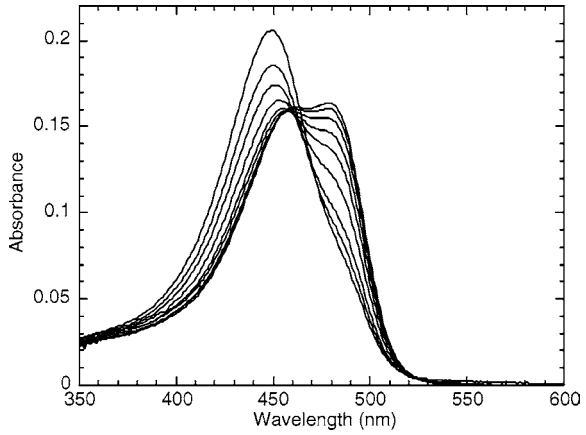


Figure 1. UV−vis absorption titration of sensor **2** with dopamine ([**2**] = 10 μM with 100 mM $Na_2S_2O_3$, 50 mM HEPES, 20 mM NaCl, pH = 7.0, 37 °C).

aldehyde **1**.[11] Table 1 lists changes in absorbance ($\Delta\lambda_{max}$) for a series of analytes. All primary amines gave similar

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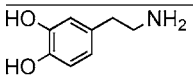
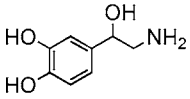
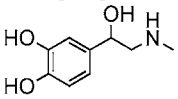
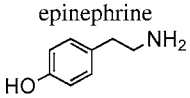
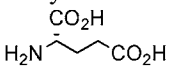
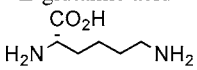
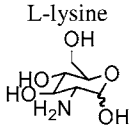
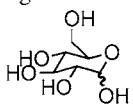
Table 1. Association Constants and Spectroscopic Parameters for Titration of Sensor **2** with Various Analytes (Conditions Listed in Figure 1)

guest	$\Delta\lambda_{max}$ (nm)	K_a (M^{-1})[a]	I_{sat}/I_0[b] (λ_{ex} = 446 nm)	I_{sat}/I_0[b] (λ_{ex} = 484 nm)	I_{sat}/I_0[b] (λ_{ex} = 495 nm)
 dopamine	30	3400	0.50	0.60	-[c]
 norepinephrine	24	6500	0.39	0.46	-[c]
 epinephrine	0	5000	0.73	0.76	0.77
 tyramine	31	250	0.41	8.0	52
 L-glutamic acid	35	6.8	0.87	7.0	25
 L-lysine	36	4.0	0.75	8.0	25
 D-glucosamine	39	5.0	0.55	5.9	27
 D-glucose	-	-	-	-	-

[a] K_a measured by fluorescence spectroscopy, λ_{ex} = 484 nm, λ_{em} = 505 nm. Error in K_a values are ±20% based on triplicate titration. [b] I_{sat} = fluorescence intensity at saturation taken from the theoretical fit to the binding isotherm. [c] Not determined.

shifts in absorbance. Glucose and epinephrine gave no shift in absorbance, as they do not interact with the aldehyde moiety.

Binding constants were determined by fluorescence titrations. In this case, exciting **2** at the 446 nm gave a decrease in fluorescence upon addition of all guests except glucose (which produced no chromophoric response). Fitting the decrease in fluorescence to a 1:1 binding isotherm gave the binding constants as shown in Table 1.[16] Excellent binding constants were observed for the three catechol-containing analytes. The sensor bound epinephrine and norepinephrine with similar efficiency even though the former does not form an iminium ion with the aldehyde, indicating that the boronic acid-catechol interaction was responsible for most of the affinity of the receptor. Tyramine, with only one phenolic hydroxyl group, had significantly reduced affinity. Other primary amines such as glutamate had low affinity similar to the binding constants observed with coumarin aldehyde **1**.[11] Even glucosamine, which possesses both an amine and a diol functionality, bound with affinity similar to the simple amines. Apparently, the geometry of sensor **2** is not suited to the compact aminosugar. Thus, the binding of analytes was quite selective for catecholamines. Sensor **2** exhibited both strong binding affinity and colorimetric response only to dopamine and norepinephrine. Epinephrine gave no color change, and other amines bound with 3 orders of magnitude lower affinity.

Table 1 lists the fluorescence response of the sensor as I_{sat}/I_0 (the maximum fluorescence change at saturation) for three different excitation wavelengths. By exciting sensor **2** at the aldehyde absorption maximum (446 nm), a decrease in fluorescence was observed for all analytes because the sensor shifted from the aldehyde to the iminium ion form. At higher wavelengths of excitation, simple amines gave the typical large increase in fluorescence since the iminium ion preferentially absorbs at these wavelengths.[11] Interestingly, the fluorescence of sensor **2** was quenched upon binding dopamine, giving an overall decrease in

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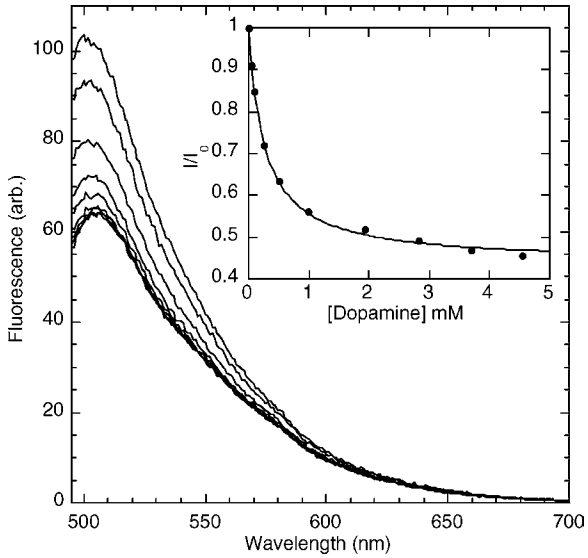

Figure 2. Fluorescence titration of sensor **2** with dopamine (λ_{ex} = 484 nm). Inset is the fit to a one-site binding isotherm.

emission when excited at 484 nm (Figure 2). The fluorescence quenching effect was found to be directly related to the catechol group. All of the catecholamines quenched the sensor regardless of excitation wavelength, which indicated that the bound complexes were not emissive in these cases. In contrast, even tyramine, with a phenol rather than a catechol group, gave a large fluorescence increase, particularly when excited at 495 nm. The electron-rich catechol is likely acting as a photoinduced electron transfer (PET) quencher of the coumarin under these conditions. This conclusion is supported by the fact that epinephrine quenches the sensor fluorescence even though its secondary amine does not make an imine with the aldehyde (vide infra).

In conclusion, compound **2** binds to primary catecholamines with good affinity and acts as an effective colorimetric sensor for dopamine and norepinephrine with excellent selectively over epinephrine, amino acids, and glucose. In the fluorescence manifold, sensor **2** responds differentially to catechol amines over simple amines, giving a fluorescence decrease in response to catechol-containing compounds and a fluorescence increase with other amines. Current efforts in this area include modification of the fluorescent core of the sensor to produce a fluorescent "on" sensor for the catecholamines.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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