Using a Synthetic Receptor to Create an Optical-Sensing Ensemble for a Class of Analytes: A Colorimetric Assay for the Aging of Scotch

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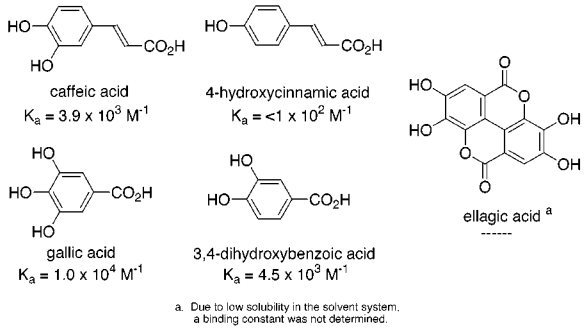
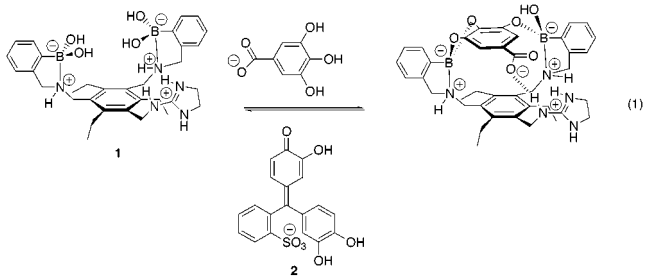
One driving force behind the creation of synthetic receptors is to demonstrate that humankind can, by design, create receptors with affinities and selectivities that rival natural receptors such as antibodies and enzymes.[1] Some excellent selectivities can indeed be achieved with synthetic systems.[2] Although this is a very worthwhile endeavor that our group[3] and many others are pursuing,[4] it is still true that the relative simplicity of synthetic receptors render most of these less selective than natural receptors. However, this lack of selectivity can, for some applications, make synthetic receptors more desirable than natural ones. Herein, we report that a receptor with broad recognition properties for a class of analytes can create a sensing system that has a practical application.

Fine scotches are required to age for a period of time in oak barrels or casks before they are ready for consumption. This aging process is known to affect the flavor and color of the beverage through extraction of phenolic acids from the wood.[5] Some examples are ellagic acid, protocatechuic acid, caffeic acid, and gallic acid (shown below). The amount of gallic acid present in scotch is considered to be an indication of age, since it is generated through the hydrolysis of tannins over time.[5a] However, the concentration of these other "gallate-like" compounds can also act as an indicator of the age of the scotch. Other factors that determine the amount of gallic acid and analogues incorporated into the spirit depend on the type of wood, how many times the casks have been used, and what they were used for. Therefore, the exact level of any specific compound can only be roughly related to age. Yet, gallate is currently the compound most often quantitated. We now report that synthetic receptors can be used when it is desired that a class of structures be collectively analyzed.



To determine the age of the scotch we synthesized a receptor that would bind a class of molecules, meaning that the receptor has selectivity for a series of similar structures, but is not highly selective for one analyte. Many of the age-related molecules possess diols and carboxylates, and thus we designed a receptor around the binding of gallate, which contains a carboxylate and a 3,4,5-trihydroxy phenyl group, with the expectation that the very similar molecules shown above would bind as well. To complex these functional groups, a guanidinium imbedded in an aminoimidazoline group and two boronic acids were chosen as recognition units (receptor **1**). Phenyl boronic acids with an aminomethyl group in the ortho position, are known to form boronate esters with 1,2- and 1,3-diols in aqueous media at neutral pH,[6] and guanidiniums are known to complex carboxylates.[7] A 1,3,5-trisubstituted-2,4,6-triethylbenzene unit was used as the base of the receptor, where the binding sites are sterically preorganized to one face of the aromatic ring. Similar structures have been found to bind citrate,[3a] tartrate, and mallate,[3b] but these compounds are not found at significant levels in scotch.



Indicator displacement from the host upon guest binding can provide a facile method for quantification assays.[3b,e,8] This method of detection involves a noncovalently attached indicator that is associated with the host. Upon the addition of an analyte, the indicator is displaced from the host cavity, causing a signal modulation. Pyrocatechol violet (**2**), a colorimetric indicator commonly used for the determination of tin and bismuth,[9] was

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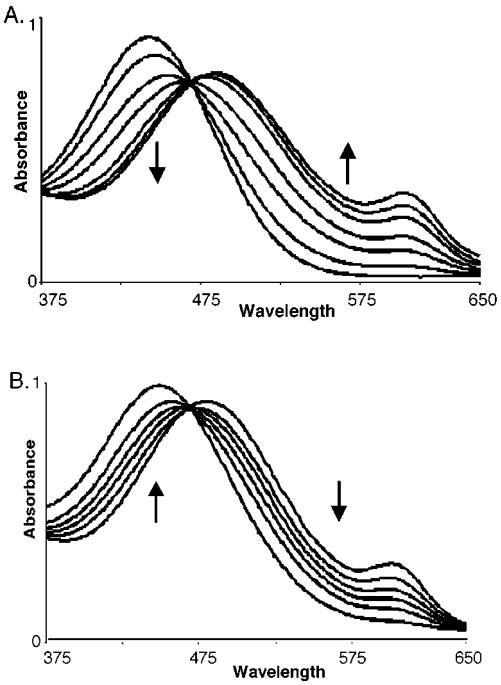

Figure 1. UV/vis spectra of **2** (25% water in methanol (v/v), 10 mM HEPES, pH 7.0). (A) Absorbance decrease at $\lambda = 442$ nm and increase at $\lambda = 488$ nm as **1** is added to a solution of **2** at constant concentration. (B) Absorbance decrease at $\lambda = 488$ nm and increase at $\lambda = 442$ nm as gallic acid is added to a solution of **1** and **2** at constant concentration.

chosen for the analysis of gallic acid (eq 1), due to its similar structural features to the analyte. It was expected that the microenvironment change upon binding the indicator to the host would cause a change in its absorbance in a manner similar to that caused by the increasing of the pH. Upon addition of **1** to a solution of **2** (0.06 mM), the color of the indicator changed from yellow to maroon (Figure 1A) in a 25% water in methanol mixture (v/v). A binding constant of 6.1×10^4 M^{-1} using a 1:1 binding algorithm[10] was obtained.

Upon addition of a stock solution of gallate to a sensing ensemble of **1** (0.26 mM) and **2** (0.06 mM), the color returned to a similar hue of yellow (Figure 1B) as the indicator was displaced from the binding pocket. A binding constant of 1.0×10^4 M^{-1} was determined for the association between **1** and gallate using algorithms for competitive equilibria.[10] Other analytes tested using this sensing ensemble included ellagic acid, 3,4-dihyroxybenzoic acid, caffeic acid, 4-hydroxycinnamic acid, fructose, and acetate. Some of their calibration curves are shown in Figure 2, and the obtained K_a values are noted under their previously shown structures. The sensing ensemble shows selectivity for the analytes that possess both diols and carboxylates, but shows little response to fructose (4×10^2 M^{-1}), 4-hydroxycinnamic acid, and acetate.

The sensing ensemble of **1** and **2** was used to evaluate several different scotches that had been aged between 5 and 16 years. To have the scotches as similar as possible in regards to production and materials, Islay scotches were chosen, which refers to a Scottish island. It was expected that the sensor response would correlate with the age of the scotch due to an overall response to the related class of compounds that contain diols and carboxylates. Upon addition of microliter quantities of scotch, a "response

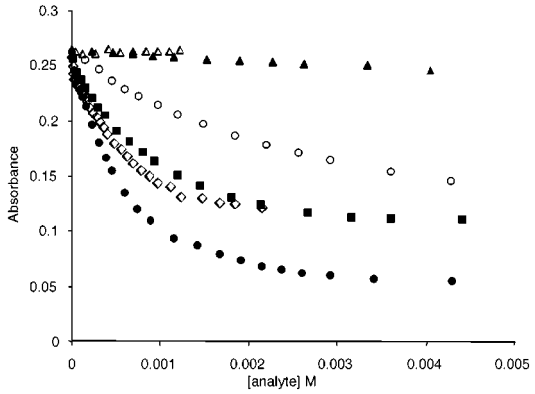

Figure 2. UV/vis calibration curves at 605 nm for the ensemble of **1** (0.26 mM) and **2** (0.06 mM) upon addition of the analytes: gallate (●), 3,4-dihydroxybenzoic acid (◇), 3,4-dihydroxycinnamic acid (■), 4-hydroxycinnamic acid (△), fructose (○), and acetate (▲) (25% water in methanol (v/v), 10 mM HEPES, pH 7.0).

Table 1. Sensing Ensemble (**1** and **2**): Analysis and HPLC Analysis of Scotches

single Islay malt whiskies	age (years)	UV/vis analysis "response number" (mM)	HPLC analysis gallate only (mM)
Vintage Islay	5	0.69	0.03
Caol Ila	7	0.89	0.02
Laphroaig	10	1.85	0.04
Macallan	12	2.5	0.09
Lagavulin	16	3.23	0.06

number" was determined from the single calibration curve of gallate (Table 1). This means the response of the sensing ensemble to the class of similar analytes was correlated as if the entire response was from gallate, which is not the case. Hence, our "response number" should be much higher than the real gallate concentration.

Indeed, as the age of the scotch increased, there was an increase in the "response number." To show that the concentration obtained was more than just gallate, HPLC analysis[11] was performed to quantify gallate. As expected, the concentration of gallate was significantly lower than the "response" obtained through absorption analysis of the sensing ensemble. Either there are tannin hydrolysis products in much higher concentrations than gallate, or there are some which bind significantly better than gallate. Importantly, targeting the entire class of gallate analogues with our sensing ensemble shows a better correlation with age than quantitating gallate alone. It will be interesting to discover if the correlation holds for scotches from other regions of Scotland, and for blends.

In summary, we have shown that a sensing ensemble of **1** and **2** was able to correlate an increase in the age of scotches to a class of compounds that increase in concentration during the maturation process. This shows that the inherent low selectivities of some synthetic systems can actually be an advantage for certain applications and that such applications can be attractive targets for supramolecular and analytical chemists to contemplate.

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