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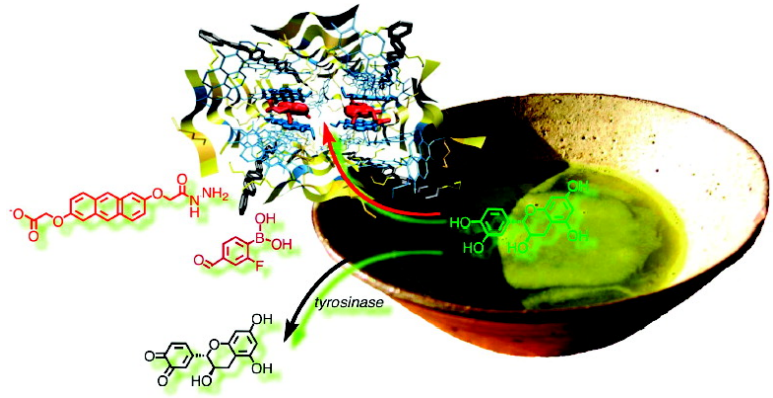
*J. Am. Chem. Soc.*, **2008**, 130 (17), 5656-5657 • DOI: 10.1021/ja801094p • Publication Date (Web): 09 April 2008



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# Boronic Acid Converters for Reactive Hydrazide Amplifiers: Polyphenol Sensing in Green Tea with Synthetic Pores

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Synthetic ion channels and pores[1] attract current scientific attention as multicomponent sensors[2] in complex matrices from the supermarket or the hospital.[3] For this purpose, stimuli-responsive pores that can discriminate between substrate and products serve as general optical transducers of reactions (Figure 1). Enzymes are used as signal generators to select for a specific analyte. Reactive signal amplifiers have been introduced to covalently capture otherwise elusive analytes after enzymatic signal generation and drag them into the pore for signal transduction. All amplifiers known today are hydrazides such as anthracene $\mathbf{A_1}$ that can block pores and react in situ with aldehydes and ketones to form hydrazones.[3,4] To expand amplifier technology beyond the limitations of signal generation with aldehydes and ketones, we here introduce converters that can transform hydrazide amplifiers in situ to capture otherwise inaccessible analytes with orthogonal functional groups.

We selected boronic acids such as benzaldehyde $\mathbf{C_1}$ as model converters. Boronic acids react in neutral water with catechols and, less preferred, also with vicinal diols or α-hydroxyacids to give boronic esters.[5] This characteristic is routinely used for the creation of chemosensors, particularly for carbohydrates.[2,5] In the following, we demonstrate that conversion of the reactivity of hydrazide amplifers such as $\mathbf{A_1}$ with converters such as $\mathbf{C_1}$ makes synthetic pore transducers **T** responsive to chemoorthogonal analytes such as (+)-catechin $\mathbf{S_1}$. The compatibility of converters with complex matrices is demonstrated with polyphenol sensing in green tea.[6]

Synthetic pores **T** and their response to amplifiers $\mathbf{A_1}-\mathbf{A_4}$ have been described (Figure S1).[4,7] Pore **T** is a rigid-rod $\beta$-barrel composed of *p*-octiphenyl staves that are brought together by short $\beta$-sheets. The peptide sequence of the $\beta$-sheets ($L\pi_ALKL\text{-}NH_2$) is designed to place hydrophobic leucine residues at the outer surface for maximal interactions with the surrounding lipid bilayer. The artificial, $\pi$-acidic naphthalenediimide (NDI) amino acids ($\pi_A$) serve as internal $\pi$-clamps that can catch $\pi$-basic dialkoxynaphthalene (DAN) and -anthracene (DAA) amplifiers $\mathbf{A}_n$ together with captured analytes $\mathbf{S}_n$ to form inclusion complexes $\mathbf{T}\supset\mathbf{A}_n\mathbf{S}_n$ assisted by ion pairing with the adjacent lysines (K) (Figure 1).

The boronate amplifiers $\mathbf{A}_n\mathbf{C}_n$ were prepared by incubating hydrazides $\mathbf{A}_n$ with aldehydes $\mathbf{C}_n$ in DMSO and used directly without further workup. The response of pore **T** to the obtained boronate amplifiers $\mathbf{A}_n\mathbf{C}_n$ was determined under standard conditions.[3,4] Namely, egg yolk phosphatidylcholine large unilamellar vesicles (EYPC LUVs) loaded with 5(6)-carboxyfluorescein (CF) were used to report the activity of pore **T** as increase in CF emission with CF efflux. Changes in pore activity in response to the presence of amplified converters (or converted amplifiers) $\mathbf{A}_n\mathbf{C}_n$ were then recorded. The found pore inactivation, due to blockage of CF efflux, was reported as dose response

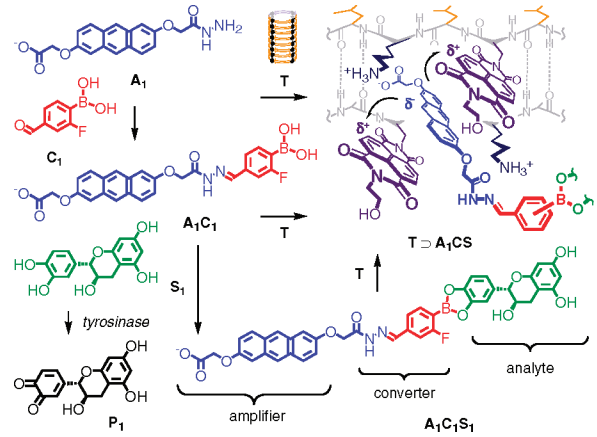


***Figure 1.*** Sensing system with converter $\mathbf{C_1}$ for amplifier $\mathbf{A_1}$ to covalently capture the otherwise undetectable analyte $\mathbf{S_1}$ (before but not after enzymatic signal annihilation with tyrosinase) and drag it into pore **T** for signal transduction. See ref 4 for details on pore **T**. The tetrahedral conjugate bases of the hydrated trigonal boronic acids and esters, and possible degradation products of $\mathbf{P_1}$ are not shown for clarity.

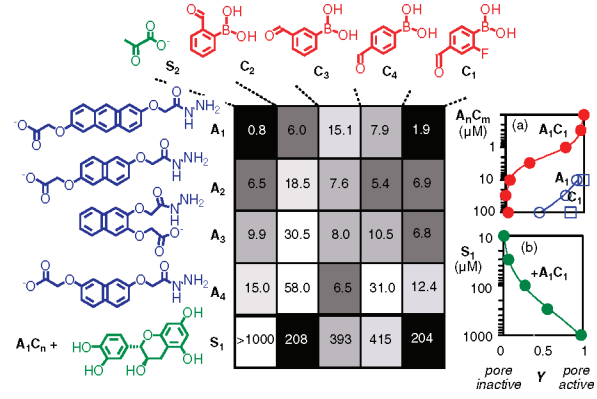


***Figure 2.*** Inactivation of pores **T** by boronate amplifiers $\mathbf{A}_{1\sim4}\mathbf{C}_{1\sim4}$ ($IC_{50}$'s in $\mu$M, rows 1−4, compared to $\mathbf{A}_n\mathbf{S}_2$, see ref 4a) and their formal reactivation by amplified catechol analyte $\mathbf{A_1}\mathbf{C}_n\mathbf{S_1}$ ($EC_{50}$ values in $\mu$M, row 5). Representative dose response curves show change of fractional pore activity *Y* with increasing concentration of (a) $\mathbf{A_1C_1}$ (●), $\mathbf{A_1}$ (○), and $\mathbf{C_1}$ (□) and (b) $\mathbf{S_1}$ with constant $\mathbf{A_1C_1}$.

curve to reveal the $IC_{50}$ (inhibitory concentration needed to reduce pore activity to 50%). Converters $\mathbf{C}_n$ without amplifiers and amplifiers $\mathbf{A}_n$ without converters failed to inactivate pore **T** under the same conditions (Figures 2a, Supporting Information Figures S2−S4).

For *ortho*- and *para*-formylphenylboronic acid amplifiers $\mathbf{A}_n\mathbf{C_1}$, $\mathbf{A}_n\mathbf{C_2}$, and $\mathbf{A}_n\mathbf{C_4}$, the dependence of blocker efficiency on the structure of hydrazinoboronic acid amplifiers $\mathbf{A}_n\mathbf{C}_n$ roughly followed the series found previously for hydrazinopy-


10.1021/ja801094p CCC: $40.75 

ruvates $\mathbf{A}_n\mathbf{S}_2$ ($\mathbf{A}_1 > \mathbf{A}_2 > \mathbf{A}_3 > \mathbf{A}_4$). These findings supported the formation of inclusion complex $\mathbf{T}\supset\mathbf{A}_n\mathbf{C}_n$ with operational aromatic electron donor−acceptor (AEDA) interactions as described.[4a] The fluorinated $\mathbf{A}_1\mathbf{C}_1$ was better than the fluorine-free $\mathbf{A}_1\mathbf{C}_4$ and almost as good as the dianionic $\mathbf{A}_1\mathbf{S}_2$. These findings suggested that the p$K_a$ of boronic acid in $\mathbf{A}_1\mathbf{C}_1$ is sufficiently low to enable contributions from ion pairing with the tetrahedral boronate anion[2,5] to pore inactivation at pH 7.4 (Figure S5). All amplified converters $\mathbf{A}_n\mathbf{C}_n$ were membrane active at high concentrations.

The ability of boronate amplifier $\mathbf{A}_1\mathbf{C}_1$ to inactivate pore **T** decreased in the presence of increasing concentrations of (+)-catechin $\mathbf{S}_1$ (Figures 2b, S6). The dose response curve for the formal liberation of pore **T** from inactivator $\mathbf{A}_1\mathbf{C}_1$ revealed an $EC_{50} = 204\ \mu M$ (Figure 2, row 5). This sensitivity range was as expected for the covalent capture of catechol analytes with strong boronic acids.[5] Pore activation presumably occurs either because obtained esters such as $\mathbf{A}_1\mathbf{C}_1\mathbf{S}_1$ are too large to bind within the synthetic $\beta$-barrel pore **T** or because they prefer to partition into the bilayer membrane (Figure 1). Highest sensitivity was found with fluorinated $\mathbf{A}_1\mathbf{C}_1$ and ortho-converter $\mathbf{A}_1\mathbf{C}_2$. This trend suggested that the tetrahedral boronate anion, either in the conjugate base of the acidic $\mathbf{A}_1\mathbf{C}_1\mathbf{S}_1$ and from the coordinate covalent N−B bond of $\mathbf{A}_1\mathbf{C}_2\mathbf{S}_1$, contributes to the stability of $\mathbf{A}_1\mathbf{C}_n\mathbf{S}_1$ (Figure S5).[2,5] Pore activation was not observed at similar concentrations with $\mathbf{A}_1\mathbf{S}_2$ because it lacks the boronic acid to react with catechol $\mathbf{S}_1$.

The compatibility of the overall most efficient boronate amplifier $\mathbf{A}_1\mathbf{C}_1$ with multicomponent sensing in complex matrices was explored next, using polyphenols as example. This family of natural product antioxidants includes flavonoids such as flavan-3-ols (*e.g.*, (+)-catechin $\mathbf{S}_1$), their gallate esters, and their higher oligomers, and occurs in green tea, red wine, fruits, vegetables, and chocolate.[6] The numerous beneficial health effects of polyphenols against aging, cardiovascular diseases, neurodegenerative diseases, and cancer are under intense scientific investigation.

One or more catechols in their polyphenolic structure makes many polyphenols accessible for boronic acid amplifiers. Green tea extracts did activate blocked pores $\mathbf{T}\supset\mathbf{A}_1\mathbf{C}_1$ as expected, although competing blockage by the crude extract and membrane activity of some amplified polyphenol at increasing concentration required careful attention to sample preparation, calibration, and controls. To eliminate eventual contributions from unrelated compounds, green tea extracts were incubated with *mushroom* tyrosinase, which oxidizes catechols and other phenolics in polyphenols. The produced *ortho*-quinones were removed by filtration through a thiol-rich resin. The obtained mixtures were unable to activate blocked pores $\mathbf{T}\supset\mathbf{A}_1\mathbf{C}_1$ under relevant conditions. This finding demonstrated that all pore activators have a phenolic structure and enzymatic treatment to correct for not-phenolic activators is not necessary to determine green tea polyphenol content (Figure S7). As mixtures of polyphenols with different molecular weight and different $EC_{50}$ to activate blocked pore $\mathbf{T}\supset\mathbf{A}_1\mathbf{C}_1$, polyphenol sensing remains necessarily qualitative. Polyphenon, a commercially available polyphenol extract from green tea containing at least 60% polyphenol, was used for calibration. Application of the calibration parameters obtained with (+)-catechin to the dose response with polyphenon (Figure 2b) gave a 5.8-times higher polyphenol content than expected (Table 1, entry 1). This suggested that either the average molecular weight of polyphenol mixtures is higher or their average $EC_{50}$ is lower than that of (+)-catechin. Contributions from both effects are likely considering the existence of higher oligomers as well as multivalent monomers such as the powerful antioxidant epigallocatechin gallate (Figure S7).[6] The dose response obtained from green tea bags calibrated for (+)-catechin gave a comparably high polyphenol content. Calibration with polyphenon, however, gave values that were in good agreement with expectations (Table 1, entry 2). The polyphenol content in high quality ("Shincha") Japanese green tea leaves was more than double compared to that in the standard green tea bags sold in local supermarkets (Table 1, entries 2 and 3).

***Table 1.*** Polyphenol Sensing with Pore **T** and amplifier $\mathbf{A}_1\mathbf{C}_1$[a]

| entry | sample | expected (mg/g)[b] | found (mg/g as (+)-catechin)[c] | found (mg/g as polyphenon)[d] |
|---|---|---|---|---|
| 1 | polyphenon | ≥600 | 3500 ± 200 | ≥600 |
| 2 | green tea bags | 72.2 ± 5.3 | 286.4 ± 4.7 | ≥49.1 |
| 3 | Shincha leaves | | 649.6 ± 54.7 | ≥111.4 |

[a] Determined from dose response as activation of complex $\mathbf{T}\supset\mathbf{A}_1\mathbf{C}_1$ in fluorogenic vesicles (as in Figure 2b).[7] [b] From supplier/literature (total catechins).[6b,7] [c] Calibrated against the dose response curve of $\mathbf{S}_1$ (Figure 2b). [d] Calibrated against polyphenon (entry 1).

From a fundamental point of view, these results are important because they advance our ability to create increasingly complex systems that do well what they are asked to do. Namely, formylphenylboronic acid converters of hydrazide amplifiers are shown to shift the compatibility of pore sensors in situ from aldehydes and ketones to catechols, α-hydroxy acids and diols. The compatibility of converters with covalent capture of chemoorthogonal analytes in complex matrices is demonstrated with polyphenol sensing in green tea of different quality, using tyrosinase as specific signal generator.

**Acknowledgment.** We thank D.-H. Tran and L. Gremaud for contributions to synthesis of pore and amplifiers, T. D. James (University of Bath) for advise, and the University of Geneva and the Swiss NSF for financial support.

**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) See Supporting Information.

JA801094P