ORGANIC
LETTERS

2013
Vol. 15, No. 19
4944–4947

Red-Hair-Inspired Chromogenic System Based on a Proton-Switched Dehydrogenative Free-Radical Coupling

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Received July 30, 2013

ABSTRACT

In the presence of micromolar peroxides or biometals (Fe(III), Cu(II), V(V) salts), and following a strong acid input, the stable 3-phenyl-2*H*-1,4-benzothiazine is efficiently converted to a green-blue $\Delta^{2,2'}$-bi(2*H*-1,4-benzothiazine) chromophore via dehydrogenative coupling of a 1,4-benzothiazinyl radical. The new system is of potential practical interest for colorimetric peroxide and redox biometal detection.

$\Delta^{2,2'}$-Bi(2*H*-1,4-benzothiazine) is a unique photochromic and acidichromic four-state system which provides the core π-structure of red human hair pheomelanin and trichochrome pigments.[1] In vivo, the $\Delta^{2,2'}$-bi(2*H*-1,4-benzothiazine) system arises by a mutation-related deviation of the pathway of melanogenesis involving the oxidative cyclization of cysteinyldopas followed by self-coupling of the resulting highly unstable 2*H*-1,4-benzothiazine intermediates.[1d,e] Despite the growing interest in nature-inspired functional systems for biomedical and technological applications, the detailed mechanism of the key self-coupling step leading to the formation of the $\Delta^{2,2'}$-bi(2*H*-1,4-benzothiazine) system has remained unknown, due to the high instability of the 2*H*-1,4-benzothiazine precursors.[2] Herein, we report the results of an integrated experimental and computational approach to this issue using 3-phenyl-2*H*-1,4-benzothiazine (**1**) as a model compound because of its unusual stability and facile access in relatively large amounts.[3]

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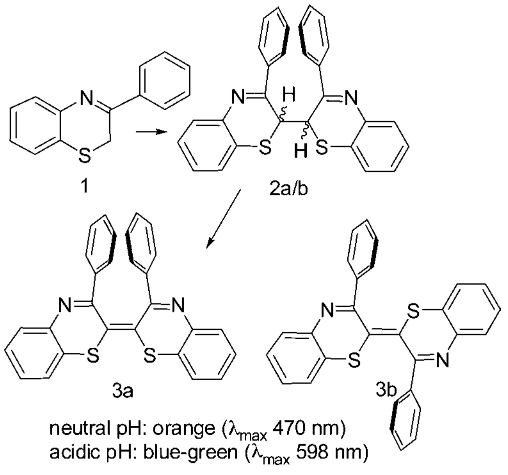
10.1021/ol402161j

In organic solvents (e.g., methanol) or in a neutral aqueous medium, **1** was fairly stable to a broad range of chemical and enzymatic oxidants, including potassium ferricyanide, hydrogen peroxide, or, notably, peroxidase/H_2O_2, remaining virtually unchanged over prolonged periods of time up to several days.

Remarkably, however, exposure of **1** to H_2O_2 in a strongly acidic medium (i.e., methanol/conc aq HCl 3:1 at room temperature) resulted in a fast and efficient reaction leading to a stable blue-green chromophore (λ_{max} 598 nm) in a few minutes. In the absence of H_2O_2, no detectable chromophore formation was observed over the time scale of 1 h. HPLC analysis of the reaction mixture revealed the very rapid accumulation in the initial stages of the reaction of colorless intermediates that were eventually converted to the green chromophore, with no other detectable intermediate/reaction product (see Supporting Information (SI)). This was also confirmed by proton NMR monitoring of the reaction course (SI). Workup of the mixtures allowed isolation and spectral characterization of the colorless intermediates as the *meso*/DL pair of diastereoisomers of the single-bonded dimers **2a**/**b** which were amply investigated by computational analysis (SI), whereas the chromophoric species proved to be a mixture of $\Delta^{2,2'}$-bi(3-phenyl-2*H*-1,4-benzothiazine) *Z*/*E* isomers **3a**/**b** (Scheme 1) in an approximate ratio of 3:1 (SI).

Assignment of the *Z*-configuration to the major component of the mixture (**3a**) was made possible by comparison of the experimental and computed ^{1}H NMR spectra (SI). For the mixture of isomeric **3**, an overall ε_{598} = 5700 ± 45 M^{-1} cm^{-1} in methanol/36% HCl 3:1 v/v was determined, which suggested a potential application for colorimetric determination of H_2O_2.

Scheme 1. Structures of Dimeric Oxidation Products of **1**



The mechanism by which H_2O_2 mediates conversion of **1** to **3** under acid conditions was then investigated. Figure 1 shows the spectrophotometric course of the oxidation of **1** with acidic H_2O_2 (left panel) leading to formation of the green chromophore. Data showed the relatively fast conversion of **1** to the final product following the addition of acid. The chromophore of dimers **2** was closely similar to that of **1**. The conversion of dimers **2a/b** to **3** was therefore separately followed (right panel). The pseudo-first-order rate constant of 0.089 ± 0.005 min^{-1} was determined with **1** at 50 μM and H_2O_2 at 500 μM based on the formation of **3** at 598 nm and decay of **2** at 320 nm (SI). Oxygen proved to be critical for chromophore formation from both **1** and **2a/b** since no significant reaction occurred under an argon atmosphere.

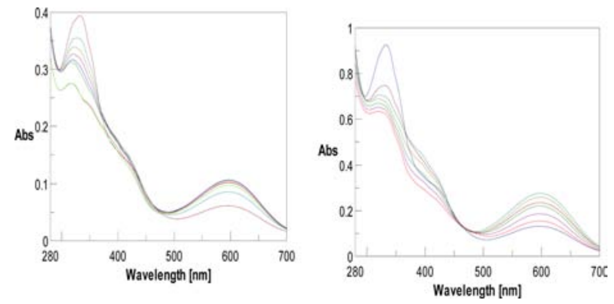


Figure 1. Monitoring of chromophore formation by oxidation of **1** (left) and dimers **2** (right) at 50 μM in air-equilibrated methanol/36% HCl 3:1 v/v solutions by 500 μM H_2O_2 at 5 min intervals over 30 min.

In another series of experiments, the ability of a series of acid-compatible oxidants to bring about conversion of **1** to **3** was investigated. Besides H_2O_2, other peroxides such as *t*-BuOOH, *m*-chloroperbenzoic acid (MCPBA), and benzoyl peroxide also induced acid-dependent chromophore formation, whereas persulfate at the same concentration proved ineffective.

In a screening aimed at identifying other oxidants capable of promoting the oxidative coupling reaction, it was found that some redox-active transition metal ions (e.g., Fe(III), V(V), and Cu(II)) were also able to generate the chromophore (Figure 2), whereas Fe(II) was inactive even in the presence of peroxides (i.e., under Fenton-type conditions, both at neutral and acidic pH; see also Figure 2).

A possible mechanism accounting for the reported observations is given in Scheme 2. In this scheme, peroxides or metal ions induce conversion of protonated **1** to the resonance-stabilized benzothiazinyl radical **1**. This conversion may be the result of two alternative, not mutually exclusive, routes, that is, direct H-atom abstraction and electron transfer with concomitant/sequential proton transfer. The former path is likely to be mediated mainly

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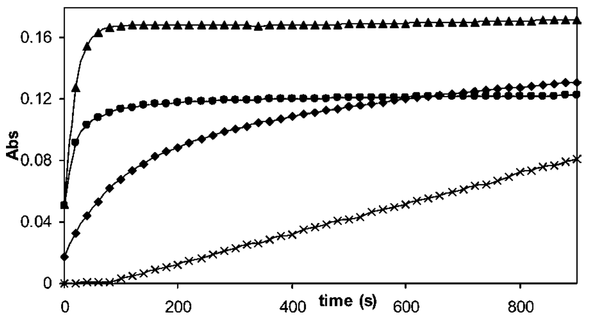
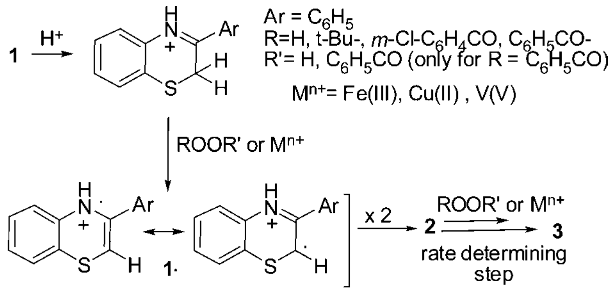


Figure 2. (a) Kinetic analysis of chromophore formation at 598 nm by reaction of 50 μM **1** with 100 μM V^{5+} (triangles), Fe^{3+} (squares), Cu^{2+} (circles), and H_2O_2 (crosses) in methanol/36% HCl 3:1 v/v solutions at 25 °C.

Scheme 2. Proposed Mechanism of Formation of Blue-Green Dimers **3** by Oxidation of **1** in Acidic Medium (All Formula Numbers Refer to Protonated Products)



by oxygenated species on the protonated benzothiazine, while the latter would occur only on the enamine tautomer of **1** and would be prevalent in the presence of metal ions alone. The equilibrium of the imine and enamine forms of **1** in an acidic medium should be considered in this regard (SI). Further work is necessary to clarify this issue. Self-coupling of **1** would then give dimer **2**, which would eventually be converted to **3** by acid-assisted dehydrogenation. Spectrophotometric and HPLC analyses concurred to indicate that conversion of **2** to **3** represents the rate determining step of the process.

In support of the proposed scheme, the acid-promoted (3 M HCl) oxidation of **1** in the presence of H_2O_2 and atmospheric oxygen in MeOH was monitored by electron paramagnetic resonance (EPR) spectroscopy, which revealed the generation of a signal (Figure 3) attributed to a C/N-centered radical ($g = 2.0051$) with hyperfine features fully consistent with the structure of protonated radical **1•**.[4] Assignment was unambiguously confirmed by comparison of the experimental data with simulated EPR spectra for the neutral and protonated radical form, using calculated (B3LYP[5]/EPR-II[6]//B3LYP/N07D[7]) hyperfine constants further optimized with the Monte Carlo method.[8]

EPR spectra consistently gave good agreement with those calculated for protonated **1•** but not for the neutral species, for which large coupling (~10 G) with (C2)H is predicted as a consequence of the larger spin density in position 2 (SI).

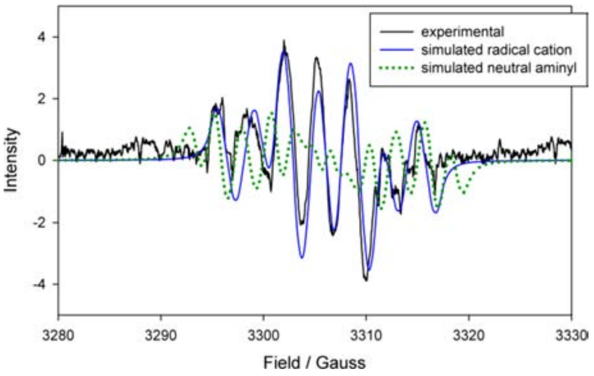


Figure 3. EPR spectrum of a solution of **1** in acidic MeOH and simulated spectra of the neutral and protonated free-radical **1•**.

The role of acids in triggering the H-atom abstraction from **1** was then investigated at the PBE0[9]/6-31+G(d,p) level. The unrestricted formulation was used to describe radicals, and for each species, different conformers were explored. Computations were performed either in vacuo or by adoption of a polarizable continuum medium.[10] Predicted variations in free-energy changes associated with H-atom abstraction from **1** and, for comparison, from the parent 2*H*-1,4-benzothiazine are reported in Table 1.

Consistent with the experimental observations, calculations predicted that protonation results in a marked decrease in free-energy changes for H-atom abstraction from both **1** and the parent benzothiazine. This effect would reflect a potentiation of the push−pull stabilization of the carbon-centered free radical at C2 induced by protonation at the imine group. The role of oxygen in the proposed free-radical coupling/dehydrogenation scheme deserves further investigation. Since product analysis did not reveal formation of other products besides **2** and **3**, and the mass balance was completely accounted for by these species, it follows that any oxygenated intermediate, such as a peroxyl radical, must eventually be converted to the final products with loss of oxygen.

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Table 1. Computed Free Energy (kcal mol^{-1}) of Radical Formation from **1**[a]

product/conditions	$\Delta\Delta_{rxn}G°$
benzothiazine, neutral form, in vacuo	0.0
benzothiazine, neutral form, MeOH	0.5
benzothiazine, monoprotonated form, MeOH	−7.2
1, neutral form, in vacuo	2.5
1, neutral form, MeOH	3.1
1, monoprotonated form, MeOH	−2.8

[a] All figures refer to the computed free energy of C2 radical formation from the parent neutral benzothiazine in vacuo, taken as zero. For each species, only the most stable tautomer/conformer was considered.

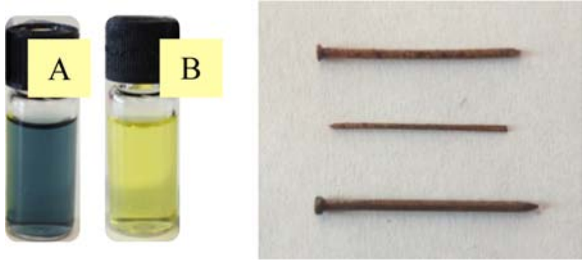

Figure 4. (A) Development of the chromophore of **3** on addition of 200 μM **1** in 3:1 methanol/36% HCl to an ethyl ether sample kept 1 month in air on the laboratory bench at a 3:1 v/v ratio before (left vial) and after (right vial) passage through a basic alumina column. (B) Rusty iron nails before (bottom) and after (middle) immersion in 36% HCl overnight and in the presence of 6 mM **1** (top).

Based on these results, the scope of the new chromogenic system was briefly assessed. Interestingly, **1** proved to be useful for the visual detection of peroxides in aged ethereal solvents such as THF, ethyl ether, dioxane (Figure 4A). Typically, the solvent to be tested was mixed with a solution of 200 μM **1** in 3:1 methanol/36% HCl (3:1 v/v), and the absorbance at 598 nm was measured spectrophotometrically. The method can be conveniently used for routine peroxide quantitations also on a visual basis.

As a curious aside to these experiments, it was noticed that addition of rusty iron objects to the typical mixture promoted oxidation of as high as 6 mM **1** in a very fast and efficient manner. Under these conditions, **1** was found to serve as an efficient inhibitor against corrosion of the rusty iron objects induced by concentrated HCl (Figure 4B). On average, **1** could decrease weight loss from rusty iron nails or staples immersed in concentrated HCl by ∼50% over 24 h. Though the mechanism by which **1** inhibits corrosion is at present unclear, it is conceivable that the effect is related in some way to the reduction of Fe(III) to Fe(II), slowing down rust generation or passivating the metal surface with Fe(II) oxides.[11]

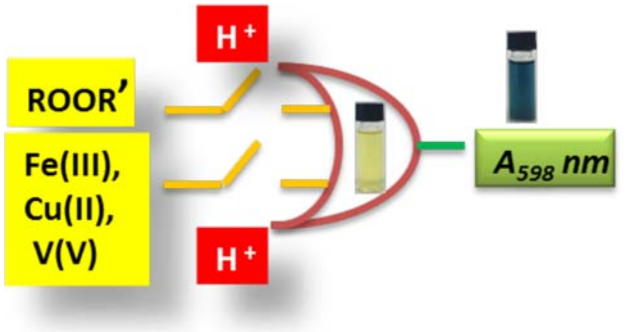

Figure 5. Proton switched "OR" logic gating with peroxide and biometal inputs and colometric output based on the oxidation of **1**.

An experimental protocol was also developed for detection of peroxide or metal-containing samples involving addition of the solutions to be tested to 200 μM **1** in methanol followed by the immediate addition of concentrated HCl. After careful mixing, spectrophotometric reading at 598 nm after 10 min at room temperature against the blank solution in the absence of added oxidant revealed the presence of peroxides or metal salts. Minimal detection limits with this protocol, as set by a ΔA_{598} of 0.05, were 50 μM for H_2O_2, 100 μM for *t*-BuOOH, 100 μM for MCPBA, 20 μM for benzoylperoxide, and 30−50 μM for metal ions.

From the above data, a two-input logic gate "OR" system can be represented with the extra value of the proton switch (Figure 5). Biologically and technologically relevant inputs such as peroxides and redox-active biometals lead to a colorimetric output at 598 nm, completing the H^+-switched OR logic gate. We plot the "truth table" for the response of these inputs in which output "1" is a ΔA_{598} of at least 0.05 and "0" is no change in A_{598} (SI).

In conclusion, we have disclosed a unique acid-induced free-radical coupling pathway inspired by red hair pigment synthesis, which has a potential for routine colorimetric detection of micromolar peroxides as well as of redox-active metal ions. The recognition process involves color changes from yellow to green that are clearly visible to the naked eye. To our knowledge, this is the first probe system for both peroxides and Fe(III), Cu(II), and V(V) detection regulated by a proton switch. These results would expand the scope of benzothiazine chemistry beyond main current applications as photoinitiator dyes[12] and in medicinal chemistry.[13]

Acknowledgment. This work was supported by Italian MIUR, PRIN 2010-11 (PROxi project).

Supporting Information Available. Experimental procedures, spectral and computational data for **1**−**3**, complete computational and EPR data for 3-phenyl-1,4-benzothiazinyl radical. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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