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Two Polymerizable Derivatives of 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)

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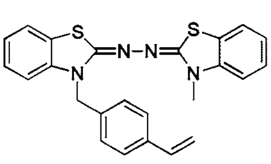
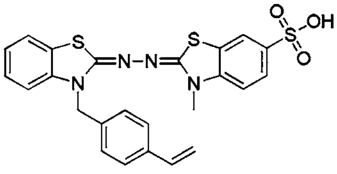
ABSTRACT



sABT



sABTS

A versatile strategy for the synthesis of polymerizable derivatives of the redox-active indicator dye 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) is reported. Two products are shown to illustrate how the final step in the synthetic strategy can be used to alter the physical properties of the product. Both products were characterized spectroscopically and electrochemically. The hydrophilic monomer (sABTS) was polymerized, and the utility of this polymer (polyABTS) is demonstrated in the context of bioelectrocatalysis.

The molecule 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) is an important redox-active compound with broad chemical, material, and biomedical applications. For example, ABTS and its derivatives have been used as the electrochromic component in smart windows,[1] as a chromogenic substrate in assays for enzymatic activity,[2] and as a mediator for electron transfer in bioelectrocatalysis.[3] Interest in the use of ABTS in the bioelectrocatalytic reduction of oxygen to water has increased primarily because its redox potential is near that of oxygen under mildly acidic conditions.[4]

Interest in redox-active polymers stems from their application in electrochromic devices,[5] biofuel cells,[6] and biosensors, all of which require a high concentration of electron mediators that are nonleachable.[7] Herein we describe the synthesis of two derivatives of ABTS that can be polymerized or copolymerized with other vinyl monomers to tune the physical properties of the resulting low potential, electrochromic, redox-active polymer.

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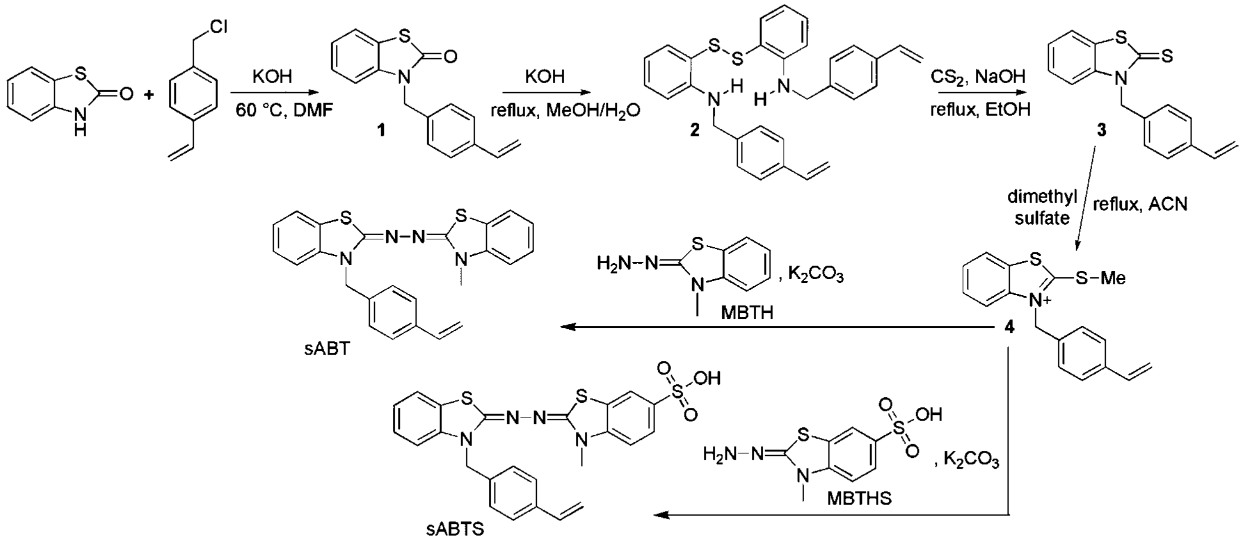
Scheme 1. Synthetic Route to sABT and sABTS



The synthesis and characterization of two polymerizable derivatives of ABTS are described: *N*-(3-methyl-3*H*-benzothiazol-2-ylidene)-*N*′-[3-(4-vinyl-benzyl)-3*H*-benzothiazol-2-ylidene]-hydrazine (sABT) and 3-methyl-2-{[3-(4-vinyl-benzyl)-3*H*-benzothiazol-2-ylidene]-hydrazono}-2,3-dihydro-benzothiazole-6-sulfonic acid (sABTS).

A five-step synthesis affords polymerizable derivatives of ABTS (Scheme 1). The first four steps result in a common intermediate (**4**) that can be used to manipulate the solubility of the target molecules and the hydrophilicity of their corresponding polymers. Reaction of **4** with MBTH results in a hydrophobic product (sABT), whereas reaction of **4** with MBTHS results in the hydrophilic product (sABTS). Both products yield redox-active polymers that are hydrophobic, hydrophilic, or amphiphilic depending on the ratio of sABT to sABTS. The first step in the synthetic sequence reacts 2-benzothiazolinone with 4-vinylbenzyl chloride under basic conditions using methods similar to that described by J. D'Amico et al. to yield the *N*-styryl-substituted benzothiazolinone (**1**) as a white crystalline solid in 91−93% yield.[8] Optimal reaction conditions were found at 60 °C for 1 h in a DMF solution containing 9 M KOH. Higher temperatures or longer reaction times led to more side products and a lower yield.

Hydrolysis of benzothiazolinone[9] and thiazolinone[10] can be accomplished using different approaches. The hydrolysis of **1** was accomplished by purging a mixture of methanol and water containing a high concentration of KOH with nitrogen to give an aminothiol product. Chromatographic isolation of the aminothiol product from several side products, however, proved difficult. Therefore, the hydrolysis reaction was performed in the presence of oxygen to give the corresponding disulfide (**2**) in 53−55% yield. The disulfide, which is a yellow crystalline solid, is easier to isolate because its R_f value is higher than that of **1** or the aminothiol product obtained in the absence of oxygen. Both the aminothiol product or **2** will react with carbon disulfide when refluxed in a mixture of ethanol and NaOH to give **3** as a white crystalline solid.[11] Choosing the disulfide product instead of the aminothiol product simplified the isolation process to give **3** in 82−84% yield. Diethyl ether was the solvent of choice for removal of **2** from the reaction mixture.

Benzothiazolinone or thiazolinone are known to react with the corresponding benzothiazolinethione or thiazolinethione via phosphorus pentasulfide thiation reaction or Lawesson's reagents without an intermediate hydrolysis step.[12] Compound **1**, however, does not react with those reagents using either method. Moreover, the R_f values of both benzothiazolinone and benzothiazolinethione are identical, making it difficult to isolate the product from the starting material using liquid chromatography.

Sulfonation of 3-methyl-benzothiazolinone-(2)-hydrazone (MBTH), available from Aldrich Chemical Co., Milwaukee, WI) yields 3-methyl-6-(M sulfonate)-benzothiazolinone-(2)-hydrazone (MBTHS) according to the published patent.[13] Both MBTH and MBTHS react with **4** to give the respective target compounds, sABT and sABTS (Scheme 1).

Methylation of **3** was achieved with dimethyl sulfate[14] as all attempts to methylate **3** with methyl iodide were unsuc-

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cessful. The derivatives of hydrazone, MBTH and MBTHS, were reacted with the methylated product (**4**) to obtain sABT (50%) and sABTS (63%), respectively. The isolation of product (**4**) was not necessary,[15] and thus, the next step in the reaction scheme was performed in the same reaction flask. The conversion from **3** and **4** was ~95% as indicated by TLC. Both sABT and sABTS are white powders. It should be noted that the sodium salt of sABTS is difficult to dissolve in most organic solvents. Therefore, this product was prepared for chromatographic isolation and polymerization by washing with 1 M HCl. After chromatographic isolation, the purified product was neutralized with an organic base (i.e., tetrabutylammonium hydroxide) to obtain a product that exhibits good solubility in both organic solvents and water for subsequent polymerization.

The tetrabutylammonium salt of sABTS was polymerized in ethanol for 1 day at 65 °C in the absence of oxygen using 2,2′-azo-bis(isobutyronitrile) (AIBN) (50:1) as the radical initiator. Cooling the reaction to room temperature subsequently stopped polymerization. The resulting viscous product was dialyzed (MWCO 3,500) against deionized water for 1 day. Polymerization of sABTS was indicated by peak broadening between 5 and 8 ppm in the ^{1}H NMR spectrum of the dialyzed product and the absence of sharp peaks corresponding to the vinyl protons in sABTS (see Supporting Information). The aromatic protons of the styryl substituent are represented by a broad peak at 7 ppm, which is similar to what is observed in the ^{1}H NMR spectrum of polystyrene sulfonate (see Supporting information) and other derivatives of polystyrene.[16] After dialysis, a solution containing polyABTS (~28 μmol of sABTS monomer, as calculated from the absorption spectrum using the extinction coefficient of sABTS) was dried on the surface of an electrode ($d = 4$ mm) for cyclic voltammetry and bioelectrocatalysis experiments. A second solution containing polyABTS (~56 μmol of sABTS monomer) was dried on the surface of an ITO electrode (1 cm^2) for electrochromic experiments.

Values for $E_{1/2}$ of sABT, sABTS and ABTS were obtained from the cyclic voltammograms (CVs) shown in Figure 1. All potentials are reported vs SCE. For sABT, $E_{1/2}$ is 613 mV when dissolved in a DMSO solution containing in 0.1 M tetrabutylammonium tetrafluoroborate. Under identical conditions, $E_{1/2}$ of sABTS is 620 mV. The peak to peak separation of 76 mV for sABT and 78 mV for sABTS indicates reversible electrochemical reactions.[17] The diffusion coefficients of sABT and sABTS are 1.21×10^{-6} and 8.1×10^{-7} cm^2 s^{-1}, respectively. For comparison, the value for $E_{1/2}$ of ABTS (commercially available) is 587 mV in DMSO and 440 mV in sodium acetate buffer (pH 4).[4] The diffusion coefficient of ABTS in DMSO is 1.39×10^{-6} cm^2 s^{-1}, and in sodium acetate buffer (pH 4) the value is 3.22×10^{-6} cm^2 s^{-1}. Also shown in Figure 1 is the cyclic voltammogram of a film of polyABTS prepared by drying a drop of a 25 μM solution of polyABTS on a glassy carbon electrode. $E_{1/2}$ of the polyABTS film is 500 mV in 0.2 M KCl solution, which is 120 mV negative to that of its monomer in DMSO. The peak to peak separation is 168 mV, indicating poor self-exchange kinetics in a pure film of polyABTS.[18]

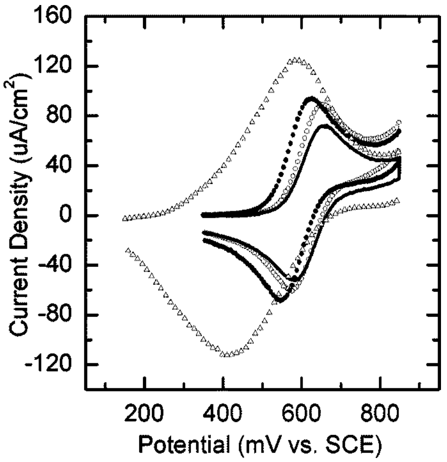

Figure 1. CVs of commercially available ABTS (closed circles), sABT (open circles), sABTS (solid line), and film of polyABTS (open triangles). The concentration of all three monomers was 2.5 mM, and the electrolyte was 0.1 M tetrabutylammonium tetrafluoroborate in DMSO. Also shown is the CV of an electrode coated with polyABTS immersed in an aqueous solution of 0.2 M KCl. The scan rate was 10 mV s^{-1} for all CVs.

The method of Nicholson was used to determine the rate constant (k_h) for heterogeneous electron transfer between a glassy-carbon electrode and the polymerizable monomers.[19] For sABT, $k_h = 1.47 \times 10^{-3}$ cm s^{-1}, and for sABTS $k_h = 2.18 \times 10^{-3}$ cm s^{-1}. For comparison, $k_h = 2.02 \times 10^{-3}$ cm s^{-1} for ABTS in 0.1 M tetrabutylammonium tetrafluoroborate DMSO solution or 4.54×10^{-3} cm s^{-1} in sodium acetate buffer (pH 4).

The compound *N*,*N*′-bis(3-methyl-3*H*-benzothiazol-2-ylidene)-hydrazine (mABT) has been used as the electroactive component in an electrochromic device.[1] Both mABT and sABTS have similar chemical structures; however, sABTS possesses an *N*-styryl group to render the monomer polymerizable and a sulfonate group to make it and its corresponding polymer water soluble. Shown in Figure 2 are the UV−vis spectra of an ITO electrode coated with a film of polyABTS (~56 μmol sABTS) while immersed in an aqueous solution of 0.2 M KCl. Spectra correspond to the film before (solid line) and after (dotted line) poising the electrode at 600 mV for 10 s. Application of an oxidizing potential converts polyABTS (transparent in the visible region of the absorption spectrum) to polyABTS$^{\bullet+}$, which is blue-green in color.

Shown in the inset of Figure 2 are the UV−vis spectra of 20 μM solutions of sABT, sABTS, and ABTS in DMSO.

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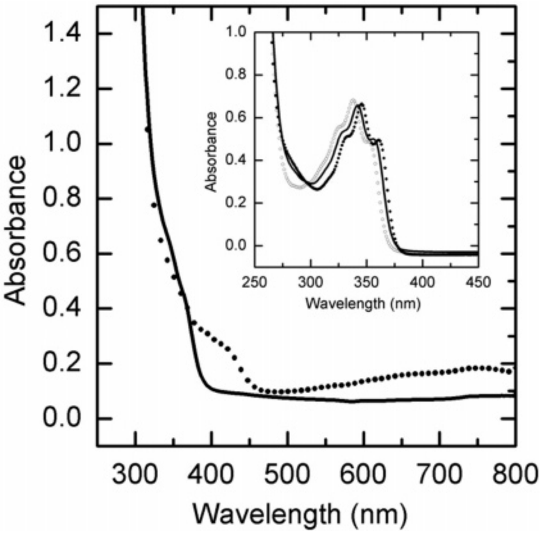

Figure 2. Spectroelectrochemistry of polyABTS/ITO electrode immersed in 0.2 M KCl. The spectra correspond to before (solid line) and after (dotted line) application of potential. Inset: UV−vis spectra of sABT (open circles), sABTS (solid line), and ABTS (closed circles) in DMSO.

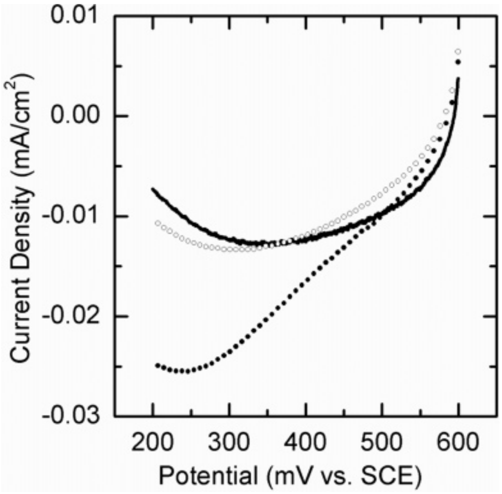

Figure 3. LSVs of a polyABTS/glassy carbon electrode. The electrode was immersed in 0.2 M KCl purged with either nitrogen or oxygen (solid line) or immersed in 0.2 M sodium acetate buffer (pH 4) containing 1 mg/mL laccase and purged with nitrogen (open circles) or oxygen (closed circles). The scan rate for all LSVs was 1 mV s^{-1}.

Both sABT and sABTS have absorption peaks at ∼255 nm, which corresponds to electronic transitions in the styrene ring, and at ∼340 nm, which corresponds to electronic transitions in the conjugated system that include the four nitrogen atoms. This absorption band is observed in the absorption spectrum of commercial ABTS and therefore confirms the presence of the same chromophore in both sABT and sABTS. The extinction coefficients of the reduced forms of sABT and sABTS dissolved in DMSO were determined to be 3.06×10^4 M^{-1} cm^{-1} at 338 nm and 2.96×10^4 M^{-1} cm^{-1} at 341 nm, respectively. We previously reported the extinction coefficient (3.45×10^4 M^{-1} cm^{-1}) of ABTS at 340 nm in 0.2 M sodium acetate buffer (pH 4).[4] Similar to polyABTS, a color change is observed upon electrochemical oxidation of solutions containing the monomers sABT, sABTS, and ABTS (data not shown).

We have used ABTS to facilitate electron transport between the cathode of a biofuel cell and the active site of laccase to generate electrical power.[4] Shown in Figure 3 are linear sweep voltammograms (LSVs) that demonstrate that polyABTS participates in the bioelectrocatalytic reduction of oxygen to water. The LSVs were collected under four different conditions: a polyABTS-coated electrode was immersed in 0.2 M KCl that was purged with either (1) nitrogen or (2) oxygen, or the polyABTS-coated electrode was immersed in 0.2 M sodium acetate buffer (pH 4) containing 1 mg/mL laccase that was purged with either (3) nitrogen or (4) oxygen. In the absence of oxygen or laccase or both, reductive current is not observed. This result indicates that polyABTS itself does not catalyze the electrochemical reduction of oxygen. When dioxygen and laccase are both present, however, reductive current is observed. It should be noted that reductive current is not observed at an uncoated electrode in the presence of both dioxygen and laccase. These results confirm that polyABTS facilitates the transport of electrons from the working electrode to the active site of laccase in solution and also confirm that polyABTS can be used in electrochemical sensors that detect dioxygen or laccase.

In conclusion, a simple synthetic route was developed that affords two polymerizable derivatives of the redox-active dye ABTS. The spectroscopic and electrochemical properties of both derivatives are reported. The polymerization of one of the derivatives (sABTS) into a water-soluble, redox-active polymer (polyABTS) is demonstrated and shown to be useful in applications such as biofuel cells, enzyme assays, biosensors, and electrochromic devices by demonstrating its ability to transfer electrons between an electrode and the active site of an oxidoreductase. Studies on copolymers of these derivatives and their applications will be reported elsewhere.

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

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