Inorganic Chemistry

Communication

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A Sensitive and Selective Fluorescence Sensor for the Detection of Arsenic(III) in Organic Media

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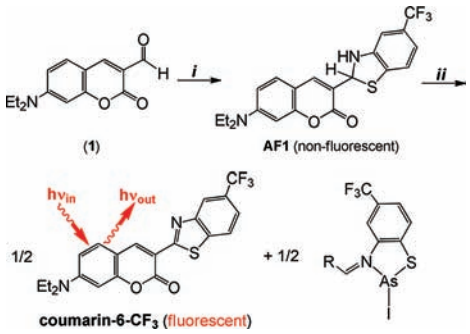
Supporting Information

ABSTRACT: Arsenic contamination is a leading environmental problem. As such, levels of this toxic metalloid must be constantly monitored by reliable and low-cost methodologies. Because the currently accepted upper limit for arsenic in water is 10 ppb, very sensitive and selective detection strategies must be developed. Herein we describe the synthesis and characterization of a fluorescent chemical probe, namely, ArsenoFluor1, which is the first example of a chemosensor for As^{3+} detection in organic solvents at 298 K. AF1 exhibits a 25-fold fluorescence increase in the presence of As^{3+} at λ_{em} = 496 nm in THF, which is selective for As^{3+} over other biologically relevant ions (such as Na^+, Mg^{2+}, Fe^{2+}, and Zn^{2+}) and displays a sub-ppb detection limit.

The abundance of arsenic (As) compounds in the environment poses a global public health concern. These compounds are introduced through the mining of sulfide ores,[1] industrial operations,[2] and agricultural activities such as the use of roxarsone (an antimicrobial additive in poultry feed, which was only recently discontinued).[3] Thus, exposure to As is quite varied. Arsenic oxidation states range from 3− to 5+, with the trivalent As^{3+} state being the most toxic of the environmentally accessible compounds.[4] In mammals, As^{3+} compounds like arsenite = $As(OH)_3$ show a strong affinity for thiol biomolecules like cysteine and glutathione [K_f = 32.0 for $As(SG)_3$],[5] which lead to the disruption of key enzymes such as pyruvate dehydrogenase,[6] whereas the As^{5+} compound arsenate = $HAsO_4^{2-}$ interrupts the Kreb's cycle by acting as a phosphate mimic.[7] Human exposure to As is primarily through drinking water and contaminated food[8] and leads to an increased risk of liver, bladder, and lung cancer.[2,9] Additionally, chronic As exposure causes a skin condition known as arsenicosis.[2,8]

Concerns over As exposure caused the U.S. EPA and the WHO to lower the maximum contaminant level (MCL) for As in drinking water from 50 to 10 ppb in 2001.[10] This lower MCL also stimulated research to develop new methods for monitoring As. Current methodologies for As detection either generate or use toxic chemicals or require sophisticated equipment and a long analysis time.[11] For example, the colorimetric Gutzeit method utilizes a strong reductant to reduce As compounds to AsH_3 (arsine gas) coupled with its subsequent reaction with mercuric bromide to afford a colored salt. This method is inexpensive and can be performed with ease; however, the Gutzeit reaction produces more toxic byproducts (>0.05 ppmv AsH_3 and mercury waste).[11] Instrumental methods like X-ray fluorescence and atomic absorption have excellent sensitivity; however, extensive sample preparation is required, and the instruments are expensive to maintain and operate. Fluorescence detection offers a promising approach for fast and simple tracking of As ions for environmental monitoring. Indeed, fluorescence sensors have been widely applicable in the detection of biologically relevant analytes such as Zn^{2+},[12] Hg^{2+},[13] and NO,[14] but no such methodology exists for As^{3+}. Some bacterial- and peptide-based biosensors exist that exhibit bioluminescence in the presence of $As(OH)_3$; however, it is difficult to engineer the bacteria to detect at the required ppb levels.[15] We have thus designed the small-molecule probe ArsenoFluor1 (AF1; Scheme 1) inspired from our previous work on the As^{3+}-promoted redox rearrangement of the benzothiazoline functional group.[16] We hypothesized that the reaction of these molecules with As^{3+} would afford the oxidized and highly fluorescent benzothiazole molecule, which is a common laser dye known as coumarin-6 (CF_3 analogue or C6-CF_3; see Scheme 1).

Scheme 1. Synthesis of AF1 and Proposed As^{3+} Response[a]



[a](i) 4-(Trifluoromethyl)-2-aminothiophenol·HCl, EtOH, Et_3N, 298 K, 6 h. (ii) AsI_3, THF, Et_3N, 298 K. R in the As^{3+} complex represents the (diethylamino)coumarin moiety.

AF1 combines a coumarin fluorescent reporter with excellent optical properties with an N,S-chelate for the thiophilic As^{3+} cation.[5] AF1 was synthesized by a modified literature procedure that we describe in Schemes 1 and S1 in the Supporting Information (SI). The reaction of diethyl malonate with 4-(diethylamino)salicylaldehyde affords the fused-ring structure of the coumarin containing an ethyl ester functionality in the

Received: November 1, 2011
Published: January 19, 2012

dx.doi.org/10.1021/ic2023715 | Inorg. Chem. 2012, 51, 1213−1215

third position. Conversion to 7-(diethylamino)coumarin and eventually 7-(diethylamino)coumarin-3-aldehyde (**1**) occurs via base-catalyzed hydrolysis of the ester group followed by a formylation reaction with $POCl_3$ and DMF (Vilsmeier–Haack reaction) in 79% and 72% yield, respectively.[17] The sensor was finally obtained by condensation of the aldehyde **1** with 4-(trifluoromethyl)-2-aminothiophenol to afford the pale-yellow AF1 in 88% yield. The chemical structure and purity of AF1 was confirmed by $^1H/^{13}C$ NMR, ESI-MS, UV–vis, fluorescence, and X-ray crystallography (vide infra). The X-ray structure of AF1 reveals specific features that give insight into the photophysical properties of this system (Figure 1). First,

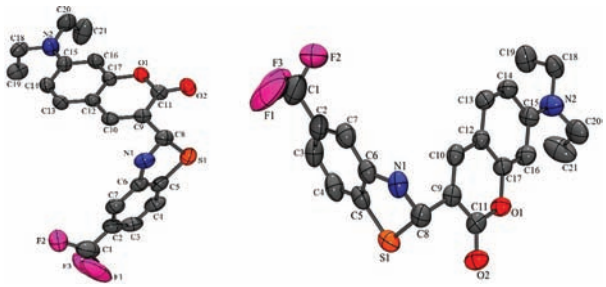


Figure 1. ORTEP diagrams (different views) of AF1 with the atom-labeling scheme. Thermal ellipsoids are shown at the 50% probability level. Hydrogen atoms are omitted for clarity. Selected bond distances (Å) and angles (deg) for AF1: C8–N1 1.439(3), C8–S1 1.869(3), C11–O2 1.221(2), C11–O1 1.366(3); N1–C8–S1 107.09(18), O1–C11–O2 116.0(2), C8–S1–C5 88.48(13), C8–N1–C6 110.6(2).

the planes that define the coumarin and benzothiazoline moieties are nearly perpendicular to each other. Second, the C–N bond distance of 1.439(3) Å is consistent with the sp^3-hybridized C in the C8–N1 single bond (Figure 1), which is substantially longer than the same distance (now sp^2-hybridized C=N) in the structure of the oxidized and highly fluorescent coumarin-6–benzothiazole (1.307 Å),[18] where the heterocyclic rings are coplanar. Furthermore, AF1 is thermally stable in both the solid and solution states when stored in the dark. AF1 also does not spontaneously oxidize to the benzothiazole (a common reaction[17a]) even after long exposure to pure O_2(g) or air, establishing the robustness of this platform for As monitoring.

The optical properties of AF1 reveal an intense broad absorption band centered at 385 nm (ε = 29 000 M^{-1} cm^{-1}) in THF that is primarily dominated by the coumarin chromophore (Figure S2 in the SI). The corresponding fluorescence emission maximum at 496 nm (λ_{em} of the benzothiazole C6-CF_3) displays an extremely low quantum yield (Φ_f) of 0.004 (Figures 2 and S4 in the SI) due to efficient quenching by the thiazoline N lone pair via a photoinduced-electron-transfer mechanism.[17,19] The nonconjugated AF1 is essentially nonfluorescent. The addition of As^{3+} (as AsI_3, although $AsCl_3$ yields similar results) leads to an approximate 25-fold increase in the fluorescence intensity of AF1 (Φ_f = 0.101; no change after 30 min; Figures 2 and S4 in the SI). This dramatic turn-ON response is accompanied by red-shifts in the absorption maxima from 385 to 464 nm, indicative of benzothiazole C6-CF_3 formation (Scheme 1 and Figure S4 in the SI). Coumarin–benzothiazole compounds such as C6-CF_3, the commercially available coumarin-6 (analogue of C6-CF_3 with hydrogen replacing the CF_3 group), and other C6 derivatives generally display fluorescence via an internal-charge-transfer (ICT)

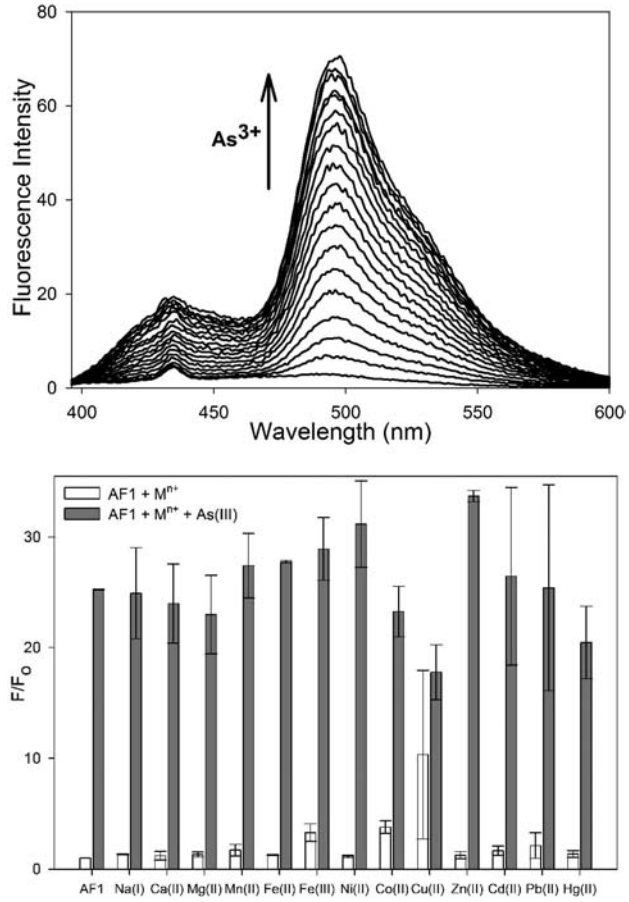


Figure 2. (Top) Fluorescence response of 0.45 μM AF1 in THF at 298 K (λ_{ex} = 385 nm). Spectra shown are for [As^{3+}] of 0, 0.26, 0.53, 0.79, 1.05, 1.31, 1.56, 1.84, 2.10, 2.36, 2.62, 2.88, 3.15, 3.41, 3.93, 4.45, 4.97, 5.49, and 6.78 nM. Each reading was obtained 30 min after the addition of As^{3+}. The arrow shows the direction of change. (Bottom) Fluorescence responses of AF1 to various ions (average of three trials) under the same conditions. Bars represent the final integrated fluorescence response (F) over the initial integrated emission (F_0). White bars represent the addition of the appropriate ion (4.5 μM) to a 0.45 μM solution of AF1. Gray bars represent the addition of 4.5 μM As^{3+} to the AF1 + ion solutions. The sharp peak at 435 nm could be due to scattering artifacts.

pathway.[20] Thus, AF1 performs as an effective OFF–ON fluorescence sensor for As^{3+} in organic media at 298 K.[21] The sensing mechanism likely involves bis-coordination to the Schiff-base thiolate form of AF1 followed by attack of the thiolate anion on the C–N carbon and loss of a proton to form the benzothiazole (C6-CF_3), which was confirmed by ^1H NMR of the reaction (Figure S9 in the SI). This proposal is reminiscent of the well-known Cu^+-catalyzed benzothiazole–benzothiazoline disproportionation products of Schiff-base-coordinated disulfides.[22] The turn-ON response is also sensitive to low [As^{3+}]. The detection limit was determined by measuring [As^{3+}], which gave a signal-to-background ratio ≥3, a widely supported method of determination.[23] The detection limit was estimated to be 0.53 nM (0.24 ppb). This figure of merit reveals that AF1 can potentially be used to monitor As^{3+} levels well below the EPA-mandated MCL standard of 10 ppb.

AF1 is also selective for As^{3+} over competing ions in THF. Figure 2 depicts the fluorescence responses of a 0.45 μM solution of AF1 in the presence of a 10-fold excess of a variety

dx.doi.org/10.1021/ic2023715 | Inorg. Chem. 2012, 51, 1213–1215

of metal ions. The emission profiles of AF1 + 10 mol-equiv (with respect to AF1) M^{n+} or AF1 + 10 mol-equiv M^{n+} + 10 mol-equiv AsI_3 are largely unperturbed with alkali and alkali-earth metals such as Na^+, Mg^{2+}, and Ca^{2+}, indicating no reaction and excellent selectivity over these common environmentally encountered cations. Furthermore, AF1 is selective for As^{3+} over common first-row transition-metal ions like Mn^{2+}, $Fe^{2+/3+}$, Ni^{2+}, and Zn^{2+}. Of the first-row metals tested, only Cu^{2+} interferes with the As^{3+}-induced turn-ON, which is due to copper-promoted oxidation of AF1 to its Schiff-base form and Cu^+.[24] Overall, the chemoselectivity of this first-generation As-sensor is quite remarkable, especially over other heavy metal toxic ions such as Hg^{2+}, Pb^{2+}, and Cd^{2+} (Figure 2).

In conclusion, the designed chemodosimeter compound, AF1, was synthesized and completely characterized by various spectroscopic and structural methods. This compound demonstrates the first selective (25-fold) and sensitive (low-ppb) fluorescence turn-ON response sensor for As^{3+} cations in organic media and ambient conditions to form the fluorescent benzothiazole compound C6-CF_3. This method is advantageous over common As-sensing strategies such as the Gutzeit colorimetric technique, which is far less sensitive (high ppb) and requires the use and generation of toxic chemicals (vide supra).[11] We are presently expanding this platform and methodology as the present systems do not operate under aqueous conditions.

■ ASSOCIATED CONTENT

Supporting Information

Details of the syntheses, reactivity, spectroscopic, and crystallographic (CIF) results. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENTS

T.C.H. thanks the Department of Chemistry at the University of Georgia (UGA) for start-up funds and the UGA Research Foundation (UGARF) for a junior faculty research grant. We also acknowledge partial support of this work from the National Science Foundation. We thank Jessica Simpson from Fort Valley State University (Fort Valley, GA) for experimental assistance.

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(21) The AF1 platform does not result in a turn-ON fluorescence response in water or in organic solvent/water combinations. AF1 does, however, operate in a variety of organic solvents in addition to THF such as MeCN, CH_2Cl_2, and $CHCl_3$.

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(24) The formation of the Schiff-base was deduced from ESI-MS and EPR, which cannot verify Cu^+ coordination. The emission maximum is centered at 433 nm instead of 496 nm as in As^{3+}, which should allow for ion differentiation.