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# Biological Detection and Tagging Using Tailorable, Reactive, Highly Fluorescent Chemosensors

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# Biological Detection and Tagging Using Tailorable, Reactive, Highly Fluorescent Chemosensors

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## Abstract

This program was focused on the development of a fluorogenic chemosensor family that could tuned for reaction with electrophilic (e.g. chemical species, toxins) and nucleophilic (e.g. proteins and other biological molecules) species. Our chemosensor approach utilized the fluorescent properties of well-known berberine-type alkaloids. *In situ* chemosensor reaction with a target species transformed two out-of-plane, weakly conjugated, short-wavelength chromophores into one rigid, planar, conjugated, chromophore with strong long wavelength fluorescence (530-560 nm,) and large Stokes shift (100-180 nm). The chemosensor was activated with an isourea group which allowed for reaction with carboxylic acid moieties found in amino acids.

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# Introduction

The purpose of this project was to create a versatile chemosensor family that enabled rapid, sensitive, and unambiguous labeling and detection of biological and chemical target molecules. Long-wavelength fluorogenic chemosensors that are reactively activated by biological agents would provide new opportunities in sensitivity and selectivity for bioscience applications. Other important fluorescent markers (e.g. green fluorescent protein and derivatives) in cell biology are costly and suffer from background fluorescence from unreacted probes in experiments designed to detect molecular interactions. Our programmatic objectives were focused on the development of a fluorogenic chemosensor family that could be tuned for reaction with electrophilic (e.g. chemical species, toxins) and nucleophilic (e.g. proteins and other biological molecules) species. Our chemosensor approach utilized the fluorescent properties of well-known berberine-type alkaloids. *In situ* chemosensor reaction with a target species transformed two out-of-plane, weakly conjugated, short-wavelength chromophores into one rigid, planar, conjugated, chromophore with strong long wavelength fluorescence (530-560 nm,) and large Stokes shift (100-180 nm). This approach was amenable to tuning fluorescence intensity, reactivity, wavelength, and Stokes shift through altering the substituents on the chemosensor molecule.

# Background

The detection of biomolecules and the fear of a terrorist bio-agent attack upon the US population has been the subject of increased study and debate. Specifically, the pursuit of fluorogenic sensors which become highly fluorescent upon reaction with biomolecules has become a research area of high importance. Indeed, only a handful of fluorogenic probes have been reported in the literature for use in the detection of biomolecules. One example is a coumarin based dye, 7-umbelliferylphospate, which is a non-flourescent phosphate ester which becomes highly fluorescent upon hydrolysis.[1] It is typically used to measure the activity of alkaline phosphatase. Another class of fluorogenic probes based on quinolinecarboxaldehydes become fluorescent upon reaction with amines through formation of isoindoles.[2] These probes have found application in protein labeling and sequencing, determination of protein concentration, and for the detection of low molecular weight amines.

Our biological detection method was based on the fluorescent properties of well-understood natural products based on berberine-type alkaloids. Berberine alkaloids, isolated from plants such as *Coptis japonica M.*, have been used in traditional and folk medicine around the world for centuries to treat a variety of ailments. More recently, berberine alkaloids have been studied in over 1000 cases for their anti-inflammatory, anti-fungicidal, and cardiovascular effects.[3] More than 20 chemical variations of berberine-type alkaloids exist.

Recently, Swager reported a novel fluorescent based chemical detection method for the detection of chemical warfare agents (CWAs), however, the chemosensors suffered from low Stokes shifts (65

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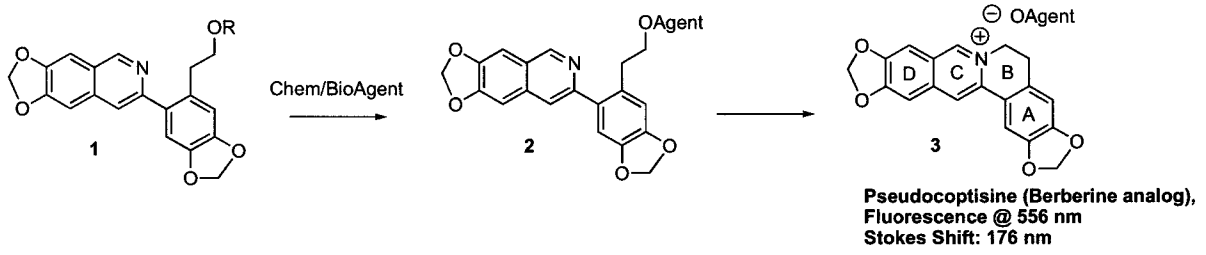
[1] Haugland, R.P.; Johnson, I. D. *J. Fluoresc.*. **1993**, *3*, 119.
[2] (a) Beale, S.C.; Savage, J.C.; Wiesler, D., Wietstock, S.M.; Novotny, M. *Anal. Chem.* **1988**, *60*, 1765. (b) Liu, J; Hsieh, Y-Z; Novotny, M. *Anal. Chem.* **1991**, *63*, 408.
[3] Kim, J-S., Tanaka, H.; Shoyama, Y. *Analyst*, **2004**, *129*, 87.

nm) with significant overlap of exciting light absorption and fluorescent emission.[4] This effect resulted in low detection sensitivity to CWA simulants. Our approach took advantage of Swager's ingenious ring-closing chemistry and the well characterized physical properties[5] (e.g. high fluorescence intensity, large Stokes shifts, etc.) of berberine derivatives and their *in situ* formation as fluorescent reporter molecules for the detection of bio-molecules. Important fluorescent bio-markers, such as green fluorescent protein (GFP) and its derivatives, are costly and suffer from background fluorescence from unreacted probes in experiments designed to detect molecular interactions. The creativity and innovation of this proposal resided in the fact that few fluorogenic sensors are commercially available and are only attainable through design and synthesis of appropriately functionalized precursors.

## Synthesis of Chemosensor

Our tagging and detection approach involved preparing appropriately functionalized precursors that were reactive toward nucleophilic bio-molecules. Reaction of **1** with these species was expected to result in formation of pseudocoptisine **3**, a highly fluorescent berberine analog. Pathways to the preparation of **1** and a family of related fluorogenic sensor molecules were well established in our laboratories. Compound **1** contained two, out-of-plane, weakly conjugated, short wavelength chromophores. Once **1** reacted with a target species, intermediate **2** formed, followed by an intramolecular cyclization reaction resulting in a rigid, planar, conjugated, highly delocalized chromophore, pseudocoptisine **3**.




**Scheme 1.2.1** Proposed reaction of chemosensor **1** with chem/bio agent to yield fluorescent pseudocoptisine **3**.

The key reaction step in the preparation of chemosensor **1** was based on a new isoquinoline ring forming reaction discovered by Larock in 1998.[6] The reaction involved the palladium/copper catalyzed cross coupling of terminal alkynes with aromatic haloimines to form iminoalkynes. Addition of electrophilic species such as $AgNO_3$, CuI, and $I_2$ to the tert-butyliminoalkyne resulted in electrophilic cyclization yielding a variety of substituted isoquinolines using mild conditions. The synthetic pathway to the preparation of isoquinoline based **1** using Larock's method is shown in Scheme 1.2.2.

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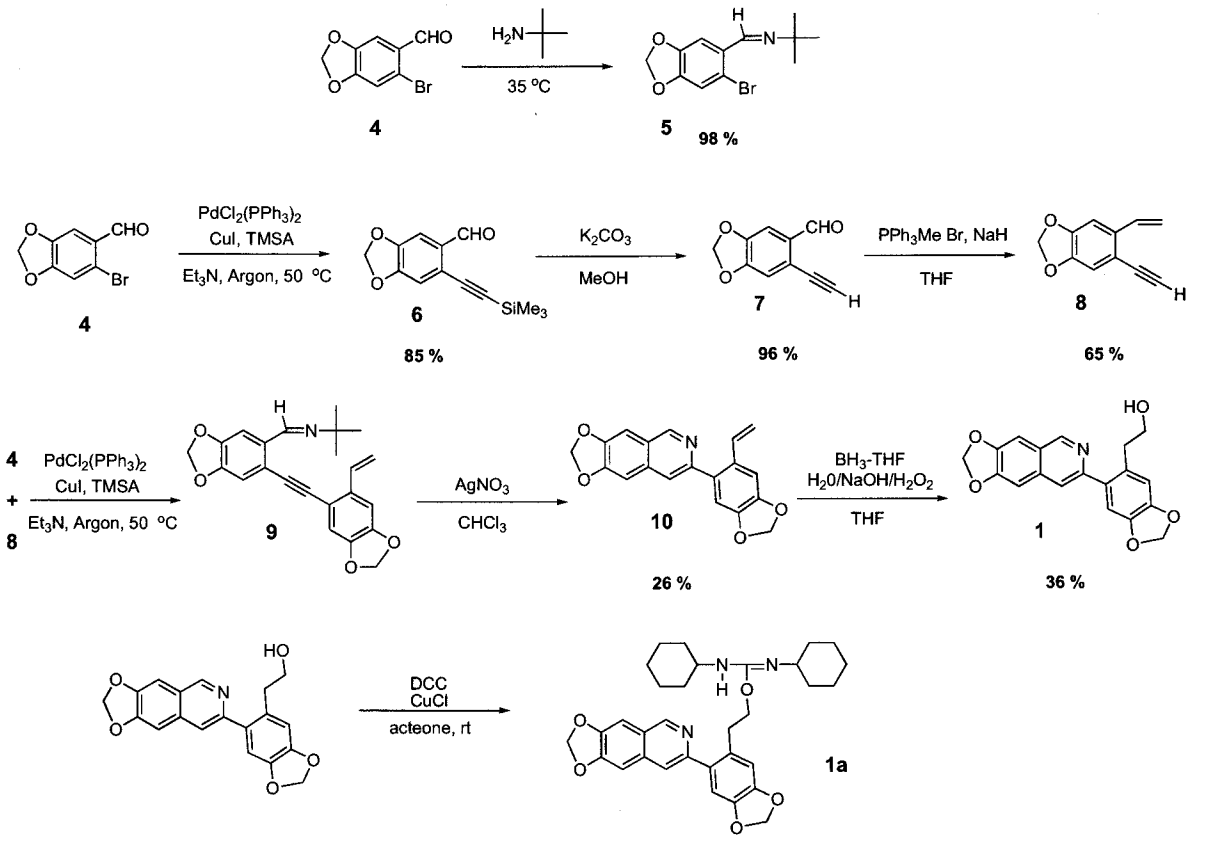
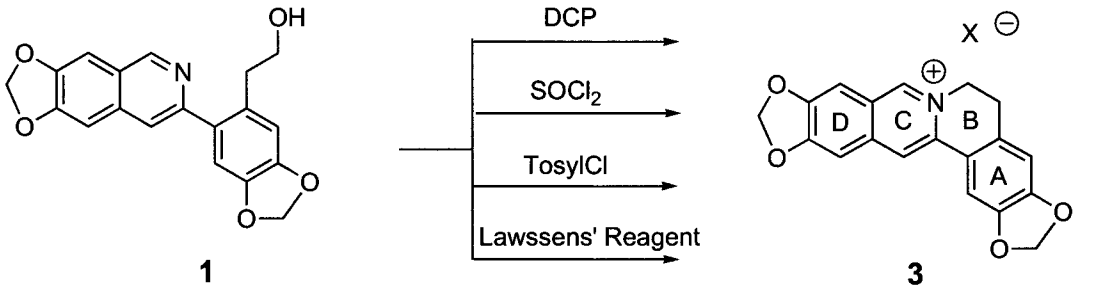
[4] Zhang, S-W.; Swager, T.M. *J. Am. Chem. Soc.* **2003**, *125*, 3420.
[5] Pavelka, S.; Smekal, E. *Collection Czechoslov. Chem. Commun.* **1976**, *41*, 3157.
[6] (a) Roesch K.R; Larock, R.C. *J. Org. Chem.* **1998**, *63*, 5306. (b) Roesch K.R; Larock, R.C. *J. Org. Chem.* **2001**, *66*, 8042.




**Scheme 1.2.2** Total synthesis of chemosensors **1** and **1a**.

An appropriately functionalized aromatic haloamine and terminal alkyne were required to take advantage of this new synthetic route to isoquinolines. Accordingly, 6-bromopiperanal **4** was reacted with large excess of *tert*-butylamine at 35°C to obtain *tert*-butylimine **5** in quantitative yield. 6-bromopiperanal **4** was also reacted with trimethylsilyl acetylene in a palladium/copper catalyzed coupling reaction to provide alkyne **6** in 85% yield. Alkyne **6** was deprotected in a $K_2CO_3$/methanol slurry to obtain terminal alkyne **7** in near quantitative yield. A Wittig reaction was performed on terminal alkyne **7** in NaH/methyltriphenylphosphonium bromide solution to yield ene-yne **8** in 65% yield. **Warning: ene-yne 8 may be an explosive hazard.** Ene-yne **8** was reacted with *tert*-butylamine **5** in a second palladium/copper catalyzed coupling reaction to yield di-aryl substituted alkyne **9**. **9** was not isolated by column chromatography as this would have lead to the acid hydrolysis of the *tert*-butyl imine back to the corresponding aldehyde . A solution of crude **9** in chloroform was heated to 50°C with a catalytic amount of $AgNO_3$ to effect the electrophilic cyclization reaction to yield substituted isoquinoline **10**. Hydroboration of **10** with $BH_3$-THF and treatment with alkaline peroxide provided alcohol **1** in 36% yield.

Reaction of alcohol **1** with electrophilic species to from psuedocoptisine **3** was first demonstrated with tosylchloride (Scheme 1.2.3). The toslylate group is a common protecting group used to protect alcohols in a variety of multi-step synthetic reactions. The tosylate group is also an excellent leaving group in nucleophilic substitution reactions. When **1** was reacted with tosylchloride in pyridine, a yellow precipitate formed within approximately one hour. The isolated solid was pseudocoptisine **3** obtained as a mixture of tosylate (75%) and chloride (25%) anions. Pseudocoptisine **3** could also be prepared by reaction of **1** with diisopropylchlorophosphate (DCP), thionyl chloride ($SOCl_2$) and Lawessons' reagent. All of these psuedocoptisine forming experiments involved the reaction of **1** with each electrophilic species to form intermediate **2** that then underwent a nucleophilic intramolecular substitution reaction to form **3**. **3** has been characterized by NMR, UV-vis, GC-MS, and fluorescence spectroscopies.




**Scheme 1.2.3.** Electrophilic species the are reactive with chemosensor **1** to form pseudocoptisine **3**.

Work was conducted at the Edgewood Chemical and Biological Center (ECBC) to evaluate this chemsensor in the detection of chemical warfare agents and non-traditional agents and is detailed in a previous report.

For nucleophilic reaction and detection of bio-molecules, **1** required activation of the alcohol functionality for reaction with such molecules. Carbonyldiimidizole (CDI) is a known linker for cross-linking biological molecules (proteins, antibodies, and DNA) and modifying polymeric substrates such as poly(vinyl alcohol) for chromatographic applications.[7] Other reports had suggested that the carbonyl group of carbamates similar to the carbamate of **1** were unreactive toward nucleophilic attack by the nitrogen of the quinoline group; therefore ring closing of the carbamate of **1** to form **3** wasn't anticipated.[8] **1** was reacted with CDI to generate the corresponding carbamate and was identified by thin layer chromatography. Unfortunately, this carbamate was a transient species and underwent the nucleophilic ring closing cyclization to pseudocoptisine **3** as observed by the formation of a fluorescent yellow spot at the origin of the thin layer chromatography plate.

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[7] Pathak, S.; Singh, A.K., McElhanon, J.R.; Dentinger, P.M. *Langmuir*, **2004**, *20*, 6075.
[8] Hartmann, R.W.; Frotscher, M. *Arch. Pharm. Pharm. Med. Chem.*, **1999**, *332*, 358.

Dicyclohexylcarbodiimide (DCC) can also be used for activation of **1** toward biomolecules and was pursued in parallel with CDI. Reaction of **1** with excess DCC and a catalytic amount of CuCl in acetone resulted in complete conversion to isourea **1a** as observed by $^1$H NMR. **1a** was found to be unstable in acetone solution when left for several days or when concentrated in vacuo and stored. Conversion to ring closed product **3** and degradation occurred when stored under these conditions. However, stable solutions of **1a** could be prepared through in vacuo concentration of the reaction mixture followed by immediate redissolving in petroleum ether.

## $^1$H NMR Studies

Isourea **1a** formed through the activation of **1** with DCC was expected to be reactive with molecules containing carboxylic acid residues, like those found in amino acids, proteins, and other biomolecules. Our initial studies involved reacting 1a with benzoic acid in DMSO-*d6* solution with subsequent monitoring of the reaction by $^1$H NMR. Indeed, reaction of **1a** with benzoic acid resulted in rapid and complete conversion to the ring closed product **3** as determined by NMR. Our studies revealed that 100% conversion to pseudocoptisine occurred in less than three minutes; this was the time required to perform the $^1$H NMR experiment.

Isourea **1a** was reacted with the amino acids aspartic acid and lysine in DMSO-*d6* solution and monitored by NMR. Complete conversion to psuedocoptisine **3** occurred in approximately six hours. Treatment of **1a** with lysine also required longer reactions times, requiring approximately 48 hours for complete conversion to psuedocoptisine **3**. The longer reaction times of the amino acids were attributed to their poor solubility in DMSO, unlike benzoic acid which was readily soluble. $^1$H NMR control studies were conducted with aspartic acid and lysine in DMSO-*d6*, verifying the insolubility of the amino acids under these experimental conditions. It is likely that mixtures of DMSO and water would improve solubility and reaction rate of the amino acids.

NMR solutions of **1a** were reacted with either ethanol amine, $D_2O$, aniline or benzyl amine to determine if amine functional groups, like those found in amino acids, or hydroxy containing compounds were responsible for the nucleophilic addition and the subsequent intramolecular ring closing reaction to form **3**. No reaction was observed by $^1$H NMR for any of these compounds. Thess results strongly indicated that aspartic acid and lysine were reactive with **1a** solely through the carboxylate addition to isourea **1a**.

## Absorption and Fluorescence Studies

The absorption spectra of **1a** and **3** were measured and are shown in Figure 1.4.1. **1a** exhibited absorption maxima in DMSO solution at 336 and 300 nm. Pseudocoptisine **3**, obtained as the tosylate salt isolated through reaction of **1** with tosyl chloride, exhibited absorption maxima in acetonitrile solution at 232, 264, 289, 315, 340, and 380 nm. The absorption spectra of **3** as the tosylate salt matched closely with reported values of **3** as the chloride salt in ethanol solution. Absorbance spectra of the NMR solutions of **1a** reacted with aspartic acid or lysine were measured to confirm the formation of **3**. Aspartic acid and lysine reaction with **1a** resulted in identical absorption maxima of 289, 317, 344, and 385 nm obtained in DMSO solution (Fig. 1.4.1). All of these results were consistent with the formation of pseudocoptisine **3**.

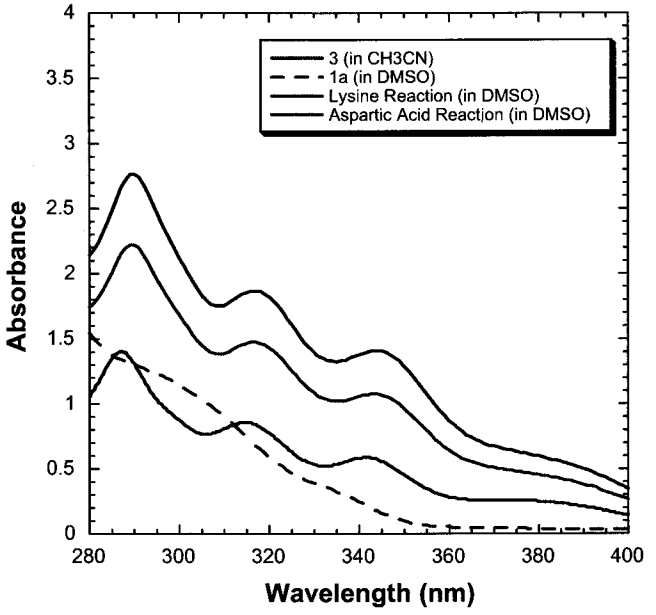


**Figure 1.4.1.** Absorbance spectra of solutions of **1a**, **3**, and reaction of **1a** with lysine and aspartic acid.

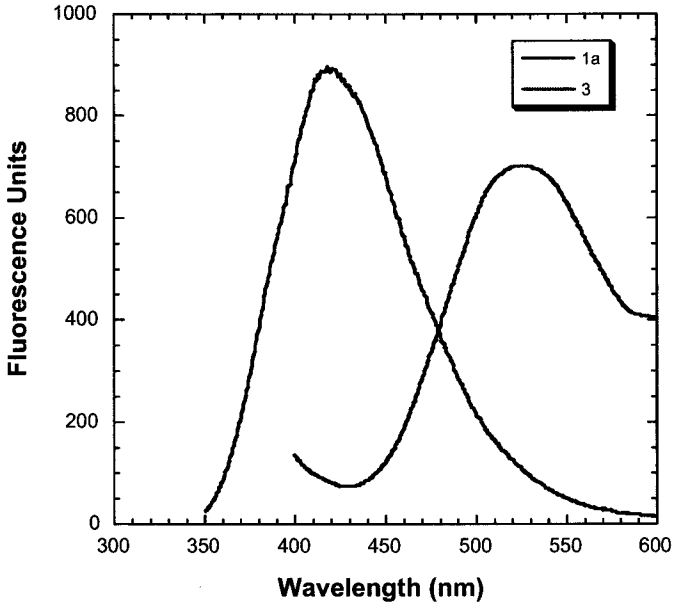


**Figure 1.4.2.** Fluorescence spectra of DMSO solutions of **1a** and **3**.

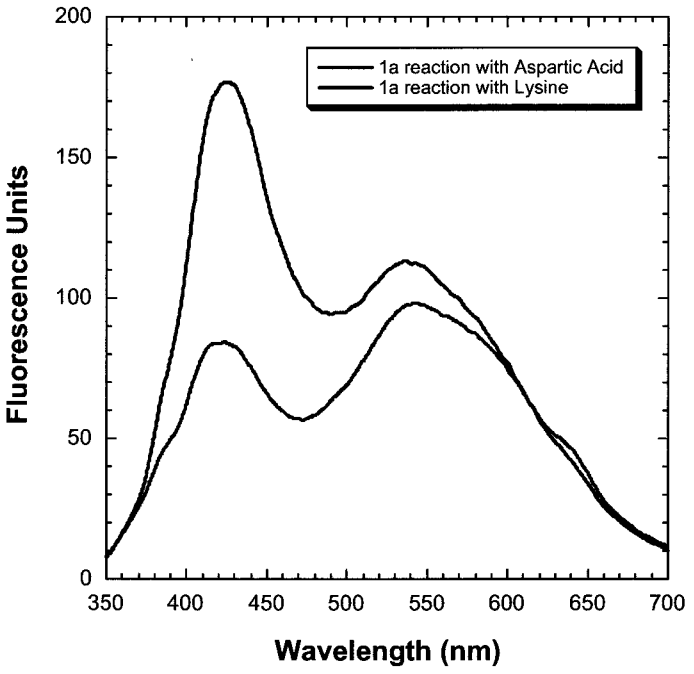


**Figure 1.4.3.** Fluorescence spectra of DMSO solutions of **1a** and **3**.

Fluorescence spectra were measured for **1a** and **3** through irradiation of solutions of each with 320 nm light (Fig. 1.4.2.). Fluorescence emissions were observed at 421 and 535 nm for DMSO solutions of **1a** and **3**, respectively. Fluorescence spectra of the NMR solutions of reacted **1a** with aspartic acid and lysine were also measured. Two fluorescence bands were observed for each reacted solution at 421 and 535 nm (Fig. 1.4.3.). Emission at 421 nm was suggestive that **1a** has not completely reacted with either of the amino acids, however, $^{1}$H NMR and absorption spectra had clearly demonstrated that complete conversion to pseudocoptisine **3** had occurred.

Solutions of **1a** were prepared and reacted with either benzoic acid, aspartic acid, or lysine and monitored by fluorescence spectroscopy. For each acid added to a solution of **1a**, immediate fluorescence quenching was observed at 421 nm and only a very small fluorescence increase was detected at 540 nm (Fig. 1.5.4). The observed quenching of fluorescence may be due to known collisional quenching of quinolinium compounds by halogen anions or amines.[9] Chemosensor **1a** is synthesized from **1** using CuCl and DCC. It is possible that some amount of chloride ion remains in solution with **1a** upon reaction workup and contributes to the fluorescence quenching observed. Additionally, amines are known quenchers of some fluorophores. DCC is present in large excess upon isolation of chemosensor **1a**. Also, dicyclohexylurea is formed upon the intramolecular ring

[9] Lakowicz, J.R. *Principles of Fluorescence Spectroscopy*; 3rd Ed.; Springer Science+Business Media, LLC: New York, 2006; pp 277-330.

closing reaction for form pseudocoptisine **3**. Fluorescence quenching did not occur for chemosensor **1** reaction with electrophilic reagents. Further study is required to elucidate why fluorescence quenching is occurring at 535 nm under these reaction conditions.

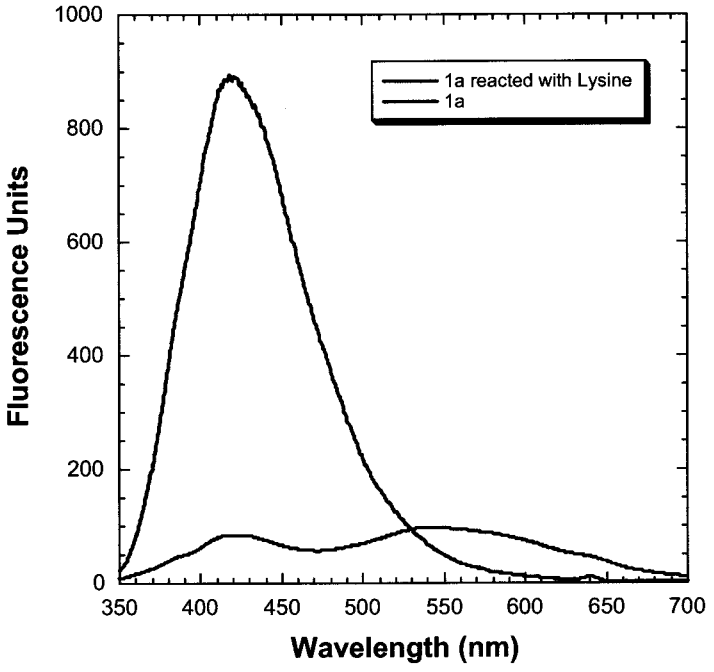


**Figure 1.5.4.** Fluorescence quenching of DMSO solutions of **1a** and **1a** reacted with lysine.

## Conclusions

This work has demonstrated the design, synthesis and function of a reactive chemosensor for the detection of biomolecules based on the formation of fluorescent berberine-based natural products. The chemosensor described has been tailored with a reactive isourea coupling group that is reactive with molecules containing carboxylic acid residues. Reaction of this fluorogenic sensor **1a** with benzoic acid resulted in a rapid (< 3 min) detection response time as observed by $^{1}$H NMR. Amino acids aspartic acid and lysine were also detected by $^{1}$H NMR and fluorescence spectroscopies, however, detection times were slower due to the solvent conditions employed. Significant fluorescence quenching was observed for all reactions of chemosensor **1a** with benzoic acid, lysine, and aspartic acid, possibly due to the presence of excess chloride ion, DCC, or dicyclohexylurea. Fluorescence quenching did not occur for chemosensor **1** reaction with electrophilic reagents. Further work is required to elucidate the fluorescence response of chemosensor **1a**. With further study, this work would likely lead to a rapid, sensitive, detection and discrimination method for the detection of a variety of chemical species, proteins and other biomolecules.

# Experimental

## Materials and Methods

All chemicals were purchased from Aldrich and used as received.

## NMR Experiments

All $^{1}H$ and $^{13}C$ experiments were performed on a 500 MHz Varian NMR spectrometer. Spectra were obtained in DMSO-*d6*, $CDCl_3$, Acetone-*d6*, or DMF-*d7* solutions.

## Reaction of Chemosensor 1a with Benzoic Acid, Aspartic Acid, and Lysine.

An aliquot of a solution of **1a** in petroleum ether was placed in an NMR tube and the solvent was removed in vacuo. The residue was immediately redissolved in DMSO-*d6* and $^{1}H$ NMR was performed to verify the purity of **1a**. Benzoic acid, aspartic acid, or lysine (ca. 5 mg) was added to the NMR tube and NMR spectra were immediately recorded. The extent of reaction and formation of pseudocoptisine **3** was monitored over time by observing the appearance of characteristic peaks for **3** while starting material peaks for **1a** decreased and completely disappeared.

## Reaction of Chemosensor 1a with D2O, Ethanolamine, Aniline, and Benzyl Amine.

NMR solutions of **1a** were prepared as described above and D2O, ethanolamine, aniline, or benzyl amine (ca. 5 mg) was added. No reaction or formation of **3** was observed by $^{1}H$ NMR.

## Fluorescence Experiments

Fluorescence spectra were obtained on a Perkin-Elmer Fluorescence Spectrometer (LS50B). The excitation wavelength for all experiments was 330 nm. All solutions were prepared in DMSO at a concentration of $1 \times 10^{-4}$ M unless otherwise stated. All fluorescence measurements were made at room temperature.

## UV-vis Experiments

UV-vis spectra of compounds were obtained on an Shimadzu UV-2501PC UV-vis spectrometer. Samples were prepared in acetonitrile or DMSO solution at a concentration sufficient enough to obtain a maximum absorbance no greater than 3 absorbance units.

**1,2-Methylenedioxy-5-alkynyl-6-vinylbenzene (8).** To a solution of NaH (380 mg, 15.8 mmol) in anhydrous THF (54 mL) was added methyltriphenylphosphonium bromide (4.48 g) and the reaction stirred for one hour under argon. 6-Ethynyl-1,3-benzodioxol-5-carbaldehyde **7** (1.40 g, 8.05 mmol) in anhydrous THF (7 mL) was added to the mixture and stirred under argon at room temperature until TLC ($SiO_2$, 1/9 ethyl acetate-petroleum ether) showed consumption of starting material. The reaction was quenched with methanol (5 mL) and water (10 mL). The reaction mixture was extracted with diethyl ether (3 x 75 mL), dried ($MgSO_4$), and concentrated in vacuo. **Warning: ene-yne 8 may be an explosive hazard.** Aromatic terminal bis-acetylenes are known to detonate upon shock when stored neat or at high temperature. Column chromatography ($SiO_2$, 1/9 ethyl acetate-petroleum ether) yielded ene-yne **8** in 65% yield as a colorless oil. $^{1}H$ NMR (500 MHz, $CDCl_3$) $\delta$ 7.15 (dd, $J$ = 17.5, 10.5 Hz, 1H), 7.02 (s, 1H), 6.88 (s, 1H), 5.95 (s, 2H), 5.62 (dd, $J$ = 17.5, 1.0 Hz, 1H), 5.24 (dd, J = 10.5, 1.0 Hz, 1H), 3.22 (s, 1H). $^{13}C$ NMR (125 MHz, $CDCl_3$) $\delta$ 148.52,

146.74, 135.07, 134.01, 114.14, 113.84, 111.66, 103.96, 101.24, 81.59, 80.30. Anal. Calcd for $C_{11}H_8O_2$: C, 76.73; H, 4.68. Found: C, 76.46; H, 4.56.

**6,7-Methylenedioxy-3-(3,4-methylenedioxy-6-vinylphenyl)-isoquinoline (10).** To a solution of 1,2-methylenedioxy-5-alkynyl-6-vinylbenzene in triethylamine (15 mL) was added Pd(PPh3)2Cl2 (18.5 mg, 0.26 mmol) and CuI (2.3 mg, 0.12 mmol) and the reaction was stirred under Ar for 15 minutes. N-(4-iodobenzo[1,3]dioxol-6-yl)methylene)-tert-butylamine was added and the reaction stirred at 50°C under Ar until TLC ($SiO_2$, 1/4 ethyl acetate-petroleum ether) showed consumption of starting material. The reaction was filtered, the filtrate washed with diethyl ether (20 mL), and concentrated in vacuo. The crude imine-alkyne was used without further purification or analysis. The residue was dissolved in $CHCl_3$, $AgNO_3$ (13.8 mg, 0.81 mmol) was added, and the reaction was heated and stirred under Ar at 50°C until TLC ($SiO_2$, 1/4 ethyl acetate-petroleum ether) showed consumption of starting material. The reaction was diluted with $CHCl_3$ and washed with brine. The organic layer was removed and the aqueous layer was extracted with $CHCl_3$, the organic layers combined, dried, and concentrated in vacuo. Column chromatography ($SiO_2$, 30/70 ethyl acetate-petroleum ether) of the residue yielded isoquinoline x in 26% yield as a yellow oil. $^1$H NMR (500 MHz, CDCl3) δ 9.08 (s, 1H), 7.63 (s, 1H), 7.41 (s, 1H), 7.26 (s, 1H), 7.20 (s, 1H), 7.04 (s, 1H), 6.87 (dd, J = 15.0, 10.0 Hz, 1H), 6.20 (s, 2H), 6.06 (s, 2H), 5.66 (dd, J = 15.0, 1.0 Hz, 1H), 5.08 (dd, J = 10.0, 1.0 Hz, 1H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 151.24, 150.90, 149.65, 148.49, 147.84, 147.38, 135.47, 134.46, 133.84, 130.53, 124.46, 121.03, 113.28, 110.11, 105.55, 103.03, 102.62, 101.66, 101.24. Anal. Calcd for $C_{19}H_{13}NO_4$: C, 71.47; H, 4.10; N, 4.39. Found: C, 71.07; H, 4.30; N, 4.18.

**6,7-methylenedioxy-3-(3,4-methylenedioxy-6-phenylethanol)-isoquinoline (1).** To a solution of 6,7-methylenedioxy-3-(3,4-methylenedioxy-6-vinylphenyl)-isoquinoline (750 mg, 2.35 mmol) in anhydrous THF was added 1.0 M $BH_3$-THF (4.70 mL, 4.70 mmol). The reaction stirred at RT for 1 hour under Ar after which water (230 mL) was added dropwise followed by 3N NaOH (320 mL). Hydrogen peroxide (37%, 320 mL) was added dropwise and the reaction stirred for 1 hour at room temperature. Ethyl acetate (150 mL) was added to the reaction mixture, which was then washed with water (100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$) and the solvent removed in vacuo. Column chromatography ($SiO_2$, 70/30 ethyl acetate-petroleum ether) yielded X in 36% yield as a light yellow solid. $^1$H NMR (500 MHz, $CDCl_3$) δ 9.00 (s, 1H), 7.63 (s, 1H), 7.22 (s, 1H), 7.10 (s, 1H), 6.85 (s, 1H), 6.12 (s, 2H), 5.98 (s, 2H), 3.97 (t, $J$ = 6.0 Hz, 2H), 5.08 (t, $J$ = 6.0 Hz, 2H). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 151.91, 150.95, 148.82, 148.24, 147.71, 146.16, 135.76, 133.04, 132.75, 124.38, 120.48, 110.09, 109.96, 103.18, 102.61, 101.89, 101.25, 63.79, 35.29. Anal. Calcd for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.48; N, 4.15. Found: C, 67.81; H, 4.58; N, 4.07.

**1a.** To a solution of 6,7-methylenedioxy-3-(3,4-methylenedioxy-6-vinylphenyl)-isoquinoline **1** in dry acetone-d6 (1 mL) was added CuCl (0.5 mg, 7 x $10^{-3}$ mmol), followed by DCC (51.7 mg, 0.250 mmol). After four hours the reaction was complete as evidenced by consumption of starting material **1** by $^1$H NMR. The reaction was dried in vacuo and immediately redissolved in petroleum ether and filtered. The solution of **1a** was used without any further purification. $^1$H NMR (500 MHz, Acetone-*d6*) δ 9.06 (s, 1H), 7.68 (s, 1H), 7.39 (s, 1H), 7.52 (s, 1H), 6.94 (s, 1H), 6.92 (s, 1H), 6.19 (s, 2H), 6.01 (s, 2H), 4.42 (m, 1H), 4.10 (t, J = 6.0 Hz, 2H), 3.25 (m, 1H), 3.04 (t, J = 6.0 Hz, 2H), 2.90 (m, 1H), 1.60-1.00 (m, 20H) overlapping with excess DCC.

**Pseudocoptisine (3).** To a solution of 6,7-methylenedioxy-3-(3,4-methylenedioxy-6-phenylethanol)-isoquinoline **1** (6.5 mg, 0.193 umol), in pyridine (1.5 mL) was added tosyl chloride (3.6 mg, 0.193 umol). The reaction stirred for 1 hour at room temperature and the resulting precipitate was filtered, washed with pyridine (2 mL) and dried *in vacuo* yielding pseudocoptisine (4.2 mg) as a mixture of the tosylate and chloride salt. $^{1}$H NMR (500 MHz, DMSO-d6) δ 9.48 (s, 1H), 8.73 (s, 1H), 7.74 (s, 1H), 7.70 (s, 1H), 7.51 (s, 1H), 7.45 (d, $J$ = 8.5 Hz, 2H), 7.09 (d, $J$ = 8.5 Hz, 2H), 7.09 (s, 1H), 6.52 (s, 2H), 6.25 (s, 2H), 4.74 (t, $J$ = 6.25, 2H), 3.18 (t, $J$ = 6.5 Hz, 2H), 2.28 (s, 3H) . $^{13}$C NMR (125 MHz, DMSO-d6) δ 155.98, 150.90, 150.00, 147.65, 145.88, 145.78, 138.76, 138.65, 137.44, 130.84, 127.97, 125.46, 123.51, 120.25, 118.91, 108.47, 105.38, 103.91, 103.69, 102.57, 102.10, 54.44, 26.35, 20.74. GCMS (EI) m/z (%) 320 (M+) (17), 319 (67), 318 (100), 260 (10), 203 (13), 202 (30), 160 (42), 131 (49), 88 (43).

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