Luminescent assays for ketones and aldehydes employing catalytic signal amplification†‡

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Herein we report the first use of transition metal catalytic signal enhancement for the analysis of small organic analytes. Two assays using Sonogashira and Suzuki cross-couplings have been used in the detection of ketones and aldehydes produce highly luminescent markers. The latter analysis utilizing the Suzuki coupling demonstrates the first use of peroxyoxalate initiated chemiluminescence in a sensing application. Chemiluminescent measurement revealed much higher sensitivity than fluorescence.

Introduction

The trend in supramolecular chemistry, as with many other application driven fields, is to generate the greatest output with the minimum size. In molecular recognition, size translates to detection limit. For example, when attempting to sense a disease marker, the earlier it can be discovered, the better. When attempting to decrease the limit of detection for an assay, two main questions must be addressed. The first is whether or not the recognition element has sufficient affinity for the target to attain favorable association at the desired detection level. Supramolecular chemists have become very proficient at dealing with this problem, and most assays today do not suffer from a lack of binding affinity.[1,2] The second question that must be answered is whether one will be able to observe the desired recognition event. Reconciling low analyte concentration with detectable signal output has received a good deal of attention in recent years.[3]

One way that organic chemists have begun to deal with this problem is through the incorporation of a catalytic process to increase signal output in an assay. To this end, biochemists and microbiologists have been exploiting enzymatic processes for a number of years. Perhaps the most famous use of this paradigm is the ELISA assay.[4] Many standard disease screenings utilize this powerful method, which makes use of the strong binding interactions between antibodies and their antigens. Though very powerful, these types of hybridization assays are only useful for bio-available analytes.

Recently, however, chemists have begun to exploit the vast wealth of transition metal catalysts for sensing purposes. The first instance of a metal catalyst used in a recognition assay for the detection of transition metal analytes was reported by our group.[5] Using EDTA as the host, a competition assay between Cu(II) and various other transition metals was performed. When the Cu(II) was released from EDTA by a stronger binding metal, it was reduced *in situ* to a Cu(I) species. The Cu(I) then catalysed a Huisgen cyclization in which the azide and the alkyne precursors each contained one member of a FRET pair. Upon cyclization, the FRET pairs would be brought together and the resulting emission shift was monitored. Sensitivity was reported for Pb(II) at millimolar concentrations. Though not terribly sensitive, the assay demonstrated the first use of a transition metal-catalyzed reaction being exploited to amplify signal output upon analyte recognition. Further studies in our group for the detection of copper and cadmium using a Heck cross-coupling reaction were later reported.[6,7]

Mirkin and coworkers have reported an assay for chloride anions through the clever use of their signature "weak-link" approach to allosteric macrocyclic ring expansion/constriction.[8] In this assay, a heterometallic macrocycle containing two zinc(II) and two ruthenium(I) centers was created. In the presence of chloride and CO the macrocycle adopts an open conformation, which fostered facile conversion of acetic anhydride and 4-hydroxymethylpyridine to 4-acetylmethylpyridine and acetic acid *via* the catalytic zinc centers. In the presence of 9-(aminomethyl)anthracene, the creation of acetic acid protonated the appended amine and disrupted the PET quenching mechanism. This led to a turn on of the anthracene fluorescence.

Several recent reports have been published for the detection of nucleic acids using transition metal based catalytic signal amplification as well.[9,10] As yet, however, no instances have been reported using transition metal catalysis to detect small organic analytes. In this paper, we discuss the first such assay to utilize these methods in the analysis of ketones and aldehydes. We also report the first instance of peroxyoxalate initiated chemiluminescence as a signaling motif in a supramolecular assay.[11,12]

Results and discussion

Target choice and assay design

All previously reported attempts to use transition metal catalysis for signal enhancement describe analyses of inorganic

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‡ Dedicated to Professor George Gokel on the occasion of his 60th birthday.

or large biological substrates.[13] The quandary which arises when dealing with a small organic analyte is that the strength of organic intermolecular noncovalent binding forces is generally much weaker than those associated with metal–organic interactions. Hence, a competition assay between a specific organic analyte and a metal based catalyst for an organic host/inhibitor scaffold is difficult to design. To this end we realized that a covalent interaction of our analyte with our host might better suit our needs than a purely supramolecular approach.

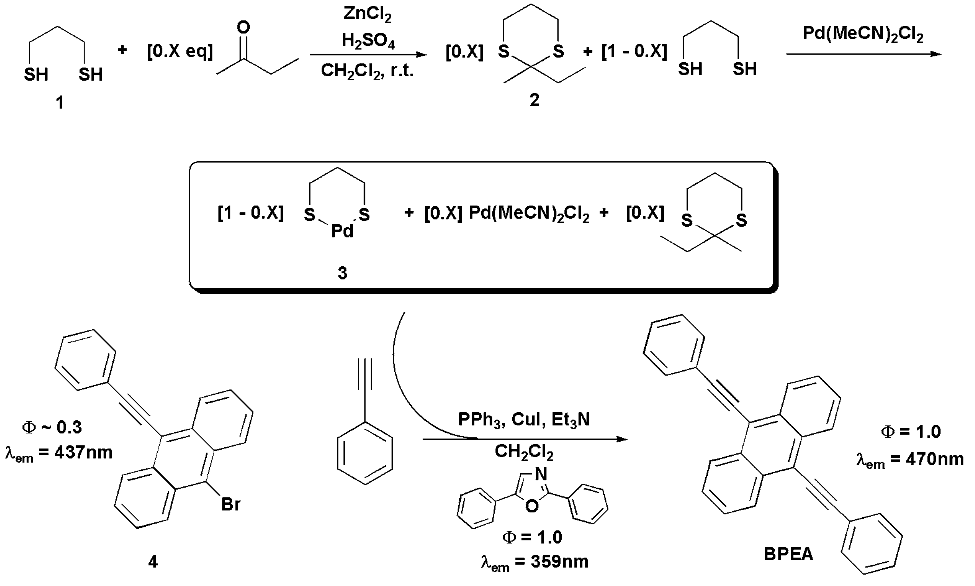
The poisoning interaction between thiols and palladium catalysts is well known.[14] For process scale heterogeneous hydrogenation catalysts, sulfur containing dopants are sometimes added to fresh catalysts to control the activity. The porous palladium surface acts as a sponge for the soft sulfur nucleophiles, which form very strong metal–sulfide bonds. The usefulness of dithiols has long been known to organic chemists. Dithiols represent one of the first protecting groups a student learns in introductory organic chemistry. When 1,3-propanedithiol is condensed with aldehydes to form 1,3-dithiolanes, so called "umpolung" reactions can be performed by deprotonation of the otherwise intractable aldehyde proton.[15] With both processes well established, we designed an assay for ketones and aldehydes based on the thioacetal condensation with a palladium-catalyzed Sonogashira cross-coupling reaction as a signal amplification method (Scheme 1).

Sonogashira assay

1,3-Propanedithiol is condensed with an analytical amount of a representative ketone, 2-butanone, in acidic dichloromethane with the Lewis acid catalyst zinc(II) chloride to give a mixture of the dithiolane and a remainder of free dithiol. After quenching with triethylamine, one equivalent of bis(acetonitrile)-palladium(II) chloride *vs.* original dithiol is added. The residual dithiol chelates and poisons some of the palladium precatalyst while the rest remains unspoiled. Hence, the unpoisoned amount of palladium catalyst is the same as the initial amount of 2-butanone. To this mixture, triphenylphosphine is added to displace the acetonitrile ligands for more effective catalysis. To commence the Sonogashira cross-coupling reaction, phenylacetylene and 9-bromo-10-(phenylethynyl)anthracene are added and the mother liquor is brought to a gentle reflux. Copper(I) iodide is added as a final step along with 2,5-diphenyloxazole (PPO), an internal fluorescence standard.

The Sonogashira coupling described in the final step of Scheme 1, produces the well known lumophore 9,10-bis(phenylethynyl)anthracene (BPEA). As a fluorophore it has been reported to have $\Phi_o = 1.0$.[16] At concentrations lower than 10^{-6} M, monomer λ_{max}em occurs near 470 nm in most common organic solvents. At higher concentrations, BPEA shows a strong tendency towards excimer formation with a λ_{max}em near 500 nm. The excimer emits the characteristic lime green color found in many commercially available glowsticks. For this assay, we chose to focus mainly on the fluorescence properties of BPEA rather than its chemiluminescent effects.

Due to PET quenching from the large amount of triethylamine present in the reaction, we were unable to perform direct fluorescence measurements on the system. Thus, the assay was conducted on a semi-preparative scale with aliquots taken at precise timed intervals over a two hour period. The aliquots were acid washed to remove the offending amine base and diluted in ethyl acetate to less than 10^{-6} M of fluorophore, assuring a safe range of emission even at full conversion to BPEA. Due to potential imprecision in aliquot acquisition and solvent evaporation over the two hour reaction time at reflux, we added the soluble internal standard 2,5-diphenyloxazole (PPO). PPO has been shown to have extremely high quantum efficiency and was an attractive choice as its λ_{max}ex overlays well with the troughs in the anthracene-dye excitation profiles (see Supplementary Information†). The use of this non-reactive internal standard negates the need for rigorous attention to volumetrics concerning aliquot acquisition (see derivation



Scheme 1 Description of the ketone assay based on a Sonogashira cross-coupling reaction. Pd(MeCN)$_2$ and **1** are added in equamolar amounts, and depending on the amount of 2-butanone present, a greater or lesser fraction of Pd(II) will remain unpoisoned towards the conversion of **4** to BPEA.

below). The following discussion of the fluorescence based kinetics relies heavily on the use of PPO in this assay.

Derivation of kinetics

Determination of the sensitivity of this detection method depends upon a good understanding of the kinetics of the Sonogashira coupling. A proposed rate law is given by eqn (1) where A is phenylacetylene, B is **4**, P is BPEA, and "cat" represents the Pd-catalyst or alternatively the ketone/aldehyde concentration.

$$\frac{\mathrm{dP}}{\mathrm{d}t} = k[\mathrm{cat}]^x[\mathrm{A}][\mathrm{B}] \tag{1}$$

If we assume initial rate kinetics we can make the simplification given in eqn (2). As mentioned above, a work-up step prior to fluorescence measurement results in an unknown amount of fluorophore loss. To solve this problem and eliminate the need for any volumetric congruity between aliquots, an internal standard was added to the reaction, represented here as S. If we divide both sides of eqn (2) by the concentration of the internal standard we arrive at a rate law based on the change in the ratio of P to S, eqn (3).

$$\frac{\mathrm{dP}}{\mathrm{d}t} = k'[\mathrm{cat}]^x \tag{2}$$

where $k' = [\mathrm{A}][\mathrm{B}]$ and $[\mathrm{A}][\mathrm{B}] = [\mathrm{A}]_0[\mathrm{B}]_0$

$$\frac{\frac{\mathrm{dP}}{\mathrm{d}t}}{[\mathrm{S}]} = \frac{\mathrm{d}\left(\frac{\mathrm{P}}{\mathrm{S}}\right)}{\mathrm{d}t} = \frac{k'[\mathrm{cat}]^x}{[\mathrm{S}]} \tag{3}$$

However, since neither concentration is absolutely known, the rate law needs to be expressed in terms of fluorescence intensity. Eqn (4) represents the Beer–Lambert Law for fluorescence where I_0 is the intensity of the incident light, ϕ is the quantum yield of the fluorophore, ε is the molar absorptivity, c is the concentration, and b is the path length.

$$F = I_0\,\phi(1\text{–}10^{-\varepsilon cb}) \tag{4}$$

$$F = I_0\,\phi\; 2.3\varepsilon cb \tag{5}$$

At low concentrations, only the first term of the expanded Taylor series for eqn (4) remains important, and the Law can be simplified to eqn (5). A ratio of the fluorescence of P to S is given by eqn (6). Eqn (6) can then be reduced to eqn (7)

$$\frac{F_\mathrm{P}}{F_\mathrm{S}} = \frac{I_0\phi_\mathrm{P}2.3\varepsilon_\mathrm{P}c_\mathrm{P}b}{I'_0\phi_\mathrm{S}2.3\varepsilon_\mathrm{S}c_\mathrm{S}b} = \frac{I_0\phi_\mathrm{P}\varepsilon_\mathrm{P}}{I'_0\phi_\mathrm{S}\varepsilon_\mathrm{S}} \times \frac{[\mathrm{P}]}{[\mathrm{S}]} \tag{6}$$

$$\frac{F_\mathrm{P}}{F_\mathrm{S}} = \kappa\frac{[\mathrm{P}]}{[\mathrm{S}]} \tag{7}$$

where κ is a constant representing the ratio of $I_0\phi\varepsilon$.

$$\frac{\mathrm{d}\left(\frac{\mathrm{P}}{\mathrm{S}}\right)}{\mathrm{d}t} = \frac{\mathrm{d}\left(\frac{F_\mathrm{P}}{F_\mathrm{S}}\right)}{\mathrm{d}t}\kappa = \frac{k'[\mathrm{cat}]^x}{[\mathrm{S}]} \tag{8}$$

Substituting eqn (7) into eqn (3) gives eqn (8).

We now have a rate law expressed in terms of fluorescence instead of concentration. Separating the derivative and combining the constants sets up the integrated rate law given in eqn (9) with the new apparent rate constant, k'', given by eqn (10).

$$\int \mathrm{d}\left(\frac{F_\mathrm{P}}{F_\mathrm{s}}\right) = \int \frac{k'[\mathrm{cat}]^x}{[\mathrm{S}]\kappa}\mathrm{d}t = \int k''[\mathrm{cat}]^x\mathrm{d}t \tag{9}$$

$$k'' = k\frac{[\mathrm{A}]_0[\mathrm{B}]_0}{[\mathrm{S}]\frac{I_0\phi_\mathrm{P}\varepsilon_\mathrm{P}}{I'_0\phi_\mathrm{S}\varepsilon_\mathrm{S}}} \tag{10}$$

The integrated rate law, eqn (11), shows that the rate can be sufficiently described by a plot of the ratio of fluorescence intensities of P to S *vs.* time.

$$\frac{F_\mathrm{P}}{F_\mathrm{S}} = k''[\mathrm{cat}]^x t \tag{11}$$

Finally, a plot of the rates *vs.* catalyst/ketone concentration will elucidate the order in catalyst and the apparent rate constant, k''.

Rate studies

Rate studies were conducted by varying the amount of 2-butanone from 0% to 100% *vs.* 1,3-propanedithiol. This represents a concentration range of roughly 1–10 mM of 2-butanone. Theoretically, a 0% assay should show no formation of BPEA whereas a 100% assay should give very fast conversion. A plot of the rate determinations for representative concentrations as a function of the ratio of product fluorescence to PPO emission *vs.* time is shown in Fig. 1. PPO is excited at 316 nm and read at 357 nm and BPEA is excited at 451 nm and read at 472 nm. As expected with no ketone present, no increase in product emission is observed over the 2 hour reaction time. As the amount of analyte increases so too does the rate of conversion to BPEA. At 85% ketone loading and above, the rates begin to plateau, indicating a departure from initial rate kinetics at a time dependent on the amount of catalyst present.

The rate profile is shown in Fig. 2. The plot of rate *vs.* ketone concentration, which directly translates to catalyst concentration, shows a marked nonlinearity. This deviation from linearity can be explained by the complex equilibrium shown in Scheme 2, which can occur for the palladium–dithiol interaction. It can be assumed that the strength of the initial Pd–S bond, K_1, will be very large due to the electron deficiency of the Pd(II)Cl_2 center. Upon binding the first sulfur, however, the palladium center becomes much less electrophilic and the

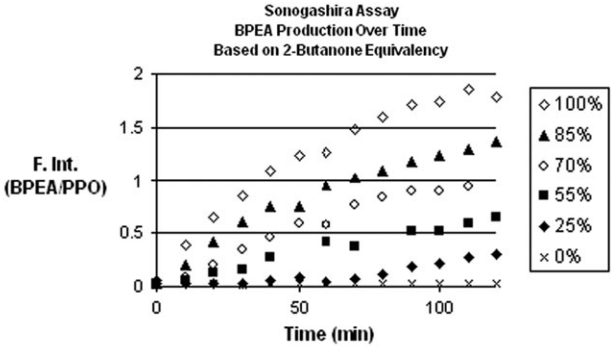


Fig. 1 Rate studies over several different equivalencies of 2-butanone.

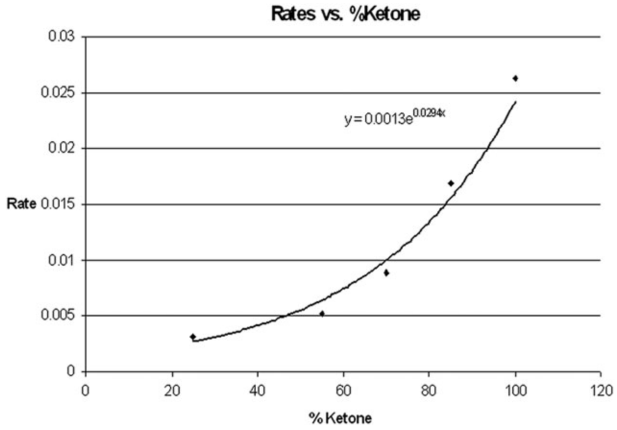


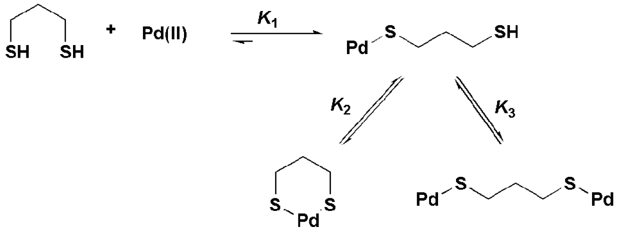
Fig. 2 A rate profile for the Sonogashira based assay shows a marked nonlinearity with respect to catalyst concentration.

second bond formation occurs less readily, allowing a more balanced equilibrium described by K_2. It is proposed that K_2 still favors the bidentate chelation such that at the high concentrations of residual dithiol brought about by low ketone equivalency, the equilibrium is driven towards **5**. As the concentration of dithiol drops relative to palladium, a second equilibrium begins to build up in which the Pd : dithiol binding becomes 2 : 1 as with **6**. We propose that **6** still retains a modest amount of catalytic activity.

Since this phenomenon occurred for both the Sonogashira assay above and the Suzuki assay described below, we wanted to test this theory. To that end, a test Suzuki reaction using 1 mmol of 9-bromoanthracene, 1.5 mmol phenylboronic acid, 2 mmol sodium carbonate, and 4.5 × 10^{-3} mmol each of $Pd(OAc)_2$ and 1-propanethiol, which can only monothiolate one palladium, was run in a 3 : 3.5 acetone–water mix. The reaction shows moderate conversion to 9-phenylanthracene over a 2 hour period based on TLC and CI-MS analysis. Thus, as the concentration of **6** increases due to decreasing dithiol concentration, the fraction of active palladium increases non-stoichiometrically.

Suzuki based assay

As a proof of concept, the Sonogashira assay worked well. However, several pragmatic issues arise from the use of this particular catalytic process. The first problem is the reaction's intolerance to water which makes the assay rather impractical to apply. Another issue is the necessity of an amine base to facilitate reduction of the Pd(II) salt to the active Pd(0) species. The side effect of PET quenching prohibits the direct monitoring of the Sonogashira by fluorescence again making the assay less applicable. Collisional quenching also occurs from the chloride anions released upon reduction of the palladium catalyst. The catalyst itself is also less stable than some other non-chloride palladium salts, introducing an unpredictable source of error. Also, the ultimate goal of generating a chemiluminescent sensing motif is impaired by the need for an extended work up of the aliquots. All of these concerns lead us to ponder a new potential catalytic system.



5

6

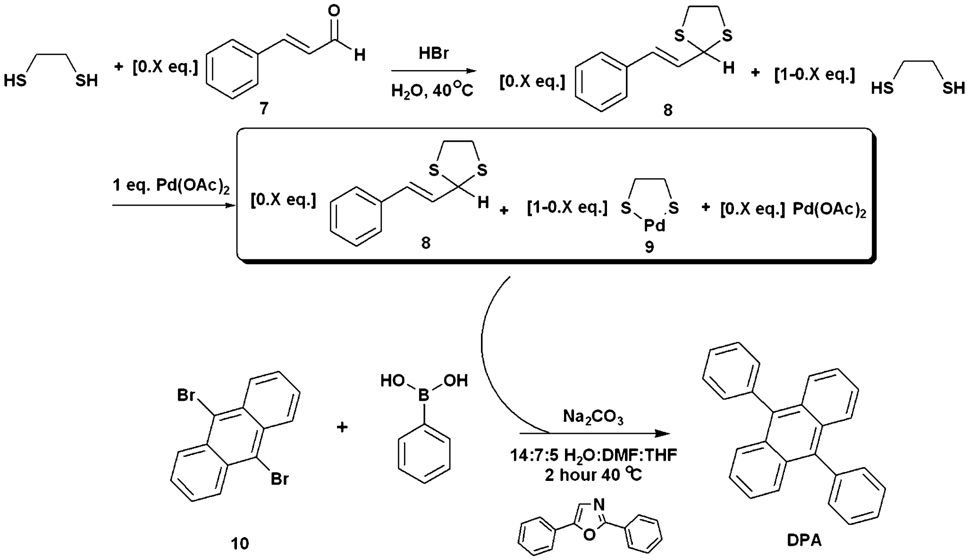
Scheme 2 The complex equilibrium of Pd(II)–dithiol coordination.

The Suzuki cross-coupling reaction solved many of the problems associated with the previous assay. Suzuki's are often run in water, they require only a carbonate base, and the catalyst, $Pd(OAc)_2$ is comparatively cheap and very stable. The product, 9,10-diphenylanthracene (DPA), of the Suzuki coupling shown in Scheme 3, is similar to its counterpart, BPEA, in many of its optical properties. A blue emitter with Φ_0 = 1.0, DPA is the lumophore found in most commercial blue glowsticks. Unlike BPEA, however, DPA shows very little tendency towards excimer formation and maintains emission near 410 nm over a wide concentration range. Unfortunately, the precursor **10**, and the intermediate 9-bromo-10-phenylanthacene also both emit near 410 nm, however, their apparent quantum efficiencies are much lower than that of DPA which allows for the determination of emission increase.

The Suzuki based assay is described by Scheme 3. Using an extremely simplified dithiane formation procedure reported by Wakharkar, 1,2-ethanedithiol was condensed with cinnamaldehyde in water in the presence of hydrobromic acid.[17] The reaction is extremely fast and forms a white precipitate which at low analyte concentrations, redissolves in the remaining immiscible ethanedithiol over the course of the reaction time. Upon completion, the reaction is quenched with 6N NaOH to sequester the hydrobromic acid and deprotonate the dithiol to allow homogenious mixing. Basifying the mixture also causes any dissolved dithiane product, **8**, to precipitate again.

Unfortunately, the low concentrations of the extremely efficient fluorophore product required for safe fluorescence determination again prevented this assay from direct monitoring. At nanomolar catalyst concentrations, the Suzuki reaction proceeds extremely slowly if at all. Thus, the reaction was again run on a semi-prep scale. Analogous to the previous assay, initial attempts were preformed using 9-bromo-10-phenylanthracene as a starting material, however, results for this reaction were erratic and inconclusive. 9,10-Dibromoanthracene, **10**, gave much better results for both fluorescence and chemiluminescence detection. The insolubility of the anthracenes also prevented accurate aliquot measurement. To overcome these difficulties two identical reactions were set up in parallel, one as a control and one as the sample.

Both reaction vessels were charged with palladium acetate in DMF and a small aliquot of the completed dithiane mixture was added such that the palladium to original dithiol ratio was unity. Then both flasks were given equal amounts of **10**, PPO, and sodium carbonate. The control flask was allowed to stir for 20 minutes at 40 °C. The sample flask was charged with 3 eq. of phenylboronic acid and allowed to react for 2 hours at 40 °C. The Suzuki reaction conditions were modified from the literature and run in a 7 : 5 : 14 mixture of DMF : THF :



Scheme 3 Description of the Suzuki based assay. Analogous to the Sonogashira assay, the rate of DPA production increases based on the concentration of cinnamaldehyde.

H_2O.[18] THF was added for initial solubility of **10** before the addition of water. Upon completion, the reactions were directly diluted with ethyl acetate to partition the solvents and dissolve the anthracenes. The organic layer was then diluted for fluorescence and chemiluminescence measurements.

Fluorescence *vs.* chemiluminescence

The solution resulting from the control experiment where no phenylboronic acid was added was used as a time zero measurement. A 120 minute measurement was taken with the solution garnered from the full reaction flask. For fluorescence measurements, the samples were diluted to 5 μM of total anthracene species. Chemiluminescence samples were prepared at 200 μM of total anthracene containing species. Data was observed over aldehyde loading from 0–90% *vs.* 1,2-ethanedithiol. This represents a concentration range starting at 10 mM. The results are plotted as a percent increase in fluorescence (Fig. 3) and chemiluminescence (Fig. 4).

Though single point measurements are insufficient to fully characterize the kinetics of the reaction, a similar nonlinear dependence on the catalyst concentration appears to be present in this assay as well. The reasons for this observation have already been discussed. Fluorescence data was taken in THF with an excitation of 393 nm and read at 407 nm and shows a modest 13% intensity increase for the lowest concentration up to nearly 200% increase for the highest concentration studied. Perhaps even more interesting is the chemiluminescence data.

Chemiluminescence studies were performed in a fluorometer with the excitation shutter closed. Using the Chemically Initiated Electron Exchange Luminescence (CIEEL) mechanism of initiation with bis(2-carbopentyloxy-3,5,6-trichlorophenyl)oxalate and hydrogen peroxide, we were able to obtain the data shown in Fig. 4. The increase in chemiluminescence is nearly an order of magnitude higher than that detected by fluorescence, with the lowest concentration tested giving an outstanding 150% intensity increase. It is interesting to note that even though **10**, and the mono-coupled intermediate have roughly the same excitation and emission profiles as DPA, their chemiluminescence efficiency is essentially non-existant as is evidenced by the large difference in intensity changes

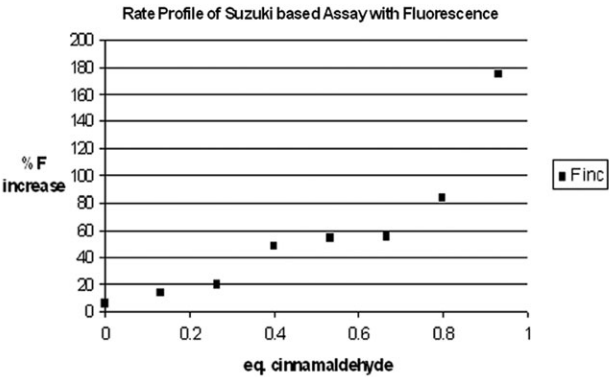


Fig. 3 Rate profile for the Suzuki assay measured from fluorescence.

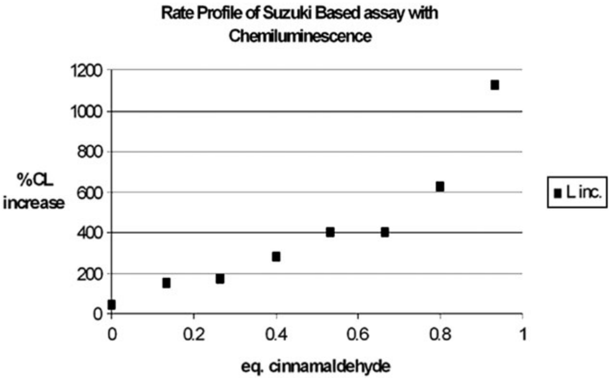


Fig. 4 Chemiluminescence measurements show a much greater turn on for the Suzuki based assay.

between fluorescence and chemiluminescence. This assay demonstrates the power of using chemiluminescent sensors for high sensitivity outside the biological arena.

Conclusions

We have reported the first use of catalytic signal amplification in the detection of a small organic analyte. Though these particular assays may not be extremely sensitive, the concept presented herein paves the road for the exploitation of transition metal catalysis for sensing purposes. Furthermore, these assays demonstrate the use and power of peroxyoxalate–CIEEL chemiluminescence in a sensing system, which to our knowledge has not been reported to date.

Experimental

Materials and methods

All reagents were obtained through Sigma-Aldrich or Acros Fine Organics and used without further purification unless noted. Bis(acetonitrile)palladium(II) chloride, 9,10-dibromoanthracene, and 9-bromo-10-phenylanthracene were prepared as described in the literature.[19–21] Triethylamine and dichloromethane were distilled over calcium hydride prior to use. Fuorescence and chemiluminescence measurements were performed on a Photon Technology International QuantaMaster Cuvet-Based spectrofluoromter.

9-Bromo-10-(phenylethynyl)anthracene (4). 4.0 g (12 mmol) 9,10-dibromoanthracene, 83.5 mg (0.12 mmol) bis(triphenyphosphine)palladium(II) chloride, and 23 mg (0.12 mmol) copper(I) iodide were added to 250 mL of freshly distilled triethylamine and brought to 40 °C. To this solution was added dropwise over 6 hours 12.7 mmol phenylacetylene in 50 mL dry triethylamine. The reaction was allowed to stir overnight at 40 °C after addition was complete. Upon completion of the reaction by TLC, the mother liquor was filtered with hexanes through celite and partitioned into 2N HCl. The organic extract was washed again with 2N HCl until slightly acidic by pH paper. The organic layer was then washed with water, saturated NaCl, and dried (Na_2SO_4). 100 mg of chromatography grade silica gel was added to the dried organic layer and the solvent was removed. The silica-adsorbed residue was chromatographed on silica gel with 100% hexanes to give 2.2 g (6.1 mmol, 52% yield). Characterization is reported in the literature and is concomitant with our analysis.[22]

Sonogashira assays

The trial using 55% 2-butanone to catalyst is used as an example procedure.

Dithiolane formation

Under inert argon atmosphere, 2.5 mL of an 8.60×10^{-3} M solution of 1,3-propanedithiol in dichloromethane is mixed with 2.5 mL of a 4.73×10^{-3} M solution of 2-butanone in dichloromethane. To the solution is added 10 μL concentrated sulfuric acid and 10 mg zinc chloride. The reaction is stirred under argon at room temperature for 1 hour and then quenched by adding 5 mL dichloromethane and 5 mL triethylamine.

Palladium poisoning and sonogashira

Then 2.5 mL of an 8.60×10^{-3} M solution of $Pd(MeCN)_2Cl_2$ in dichloromethane is added to the quenched solution and stirred for five minutes under argon. A 12.5 mL solution containing 0.19 mmol 9-bromo-10-(phenylethynyl)anthracene and 0.12 mmol 2,5-diphenyloxazole along with 5 mg neat CuI are then added and the 30 mL solution is brought to gentle reflux. Finally, 50 μL of phenylacetylene is added and 250 μL aliquots are taken and worked up every 10 min for 2 h.

Aliquot work-up

250 mL of the reaction liquor is partitioned between 4 mL ethyl acetate and 2 mL 2N HCl. For fluorimetry, the organic layer is then directly diluted by roughly 10^{-3} in ethyl acetate. Preparation of the aliquot for chemiluminescence requires further washing of the organic layer with 2M NaOH followed by drying (Na_2SO_4).

Fluorescence

Spectra were taken for 2,5-diphenyloxazole, 9-bromo-10-(phenylethynyl)anthracene, and 9,10-bis(phenylethynyl) anthracene in ethyl acetate exciting at 316 nm, 406 nm, and 451 nm, respectively for each aliquot. 2,5-Diphenylaoxazole was read at 357 nm, **4** at 432, and BPEA at 471. Slit widths were set at 2 nm for excitation and emission giving average fluorescence intensities of $>10^5$ counts per s. Data from each spectrum was collected *via* integration from -5 to $+5$ nm about the respective emission λ_{max} using the Felix32 v1.0 software package.

Suzuki assay

The trial with 13.3% cinnamaldehyde is used as an example.

Dithiane condensation

To a 5 mL roundbottom flask are added 1 mL thoroughly degassed water, 50 μL (0.6 mmol) ethanedithiol, 10 μL (0.08 mmol) cinnamaldehyde, and 25 μL 48% hydrobromic acid in water. The reaction is stirred at 40 °C for 2 h during which time a small amount of white precipitate forms and is redissolved in the remaining insoluble ethanedithiol. The reaction is quenched with 3 mL of 6N sodium hydroxide which reforms the white precipitate as the ethanedithiol is deprotonated and dissolved in the aqueous layer. At this time the total concentration of disulfur species (ethanedithiol and the condensed dithiolane) is 0.147 M. This solution is labeled solution **A**.

Suzuki coupling

In a 10 mL volumetric flask was placed 101.3 mg (0.3 mmol) 9,10-dibromoanthracene and 25.3 mg (0.1 mmol) 2,5-diphenyloxazole (PPO), which were then diluted to 10 mL with a 1 : 1 DMF : THF solution. The resulting solution was labeled solution **B**. 83.5 mg (0.37 mmol) of palladium(II) acetate was diluted to 50 mL in DMF in a separate volumetric flask and labeled solution **C**. To each of *two parallel reaction* flasks were added 3.5 mL degassed water, 0.5 mL of solution **C**, and 25 μL of solution **A**. The solution turned from a light brown to burnt orange instantaneously. To each of these solutions was then added 2.5 mL of solution **B** which precipitated upon contact with water. The reaction vessels were then brought to 40 °C. To the first reaction flask labeled **time 120**, was added 31.6 mg (0.3 mmol) sodium carbonate and 25.3 mg (0.2 mmol) phenylboronic acid and the timer was started. To the second flask labeled **time 0**, was added only 31.5 mg (0.3 mmol) sodium carbonate. The **time 0** flask was stirred for 20 min and the **time 120** flask was stirred for 2 h. Final concentrations in the reaction flask prior to reaction were as follows: 9,10-dibromoanthracene 0.012 M; PPO 0.004 M; palladium (II) acetate 5.7×10^{-4} M; phenylboronic acid 0.032 M; ethanedithiol based on no dithiolane formation 5.7×10^{-4} M; sodium carbonate 0.046 M. Upon completion of the reaction both flasks were treated in the following manner. 7 mL of ethyl acetate were added to quench the reaction giving a 10 mL organic layer on the assumption that all DMF was partitioned. From the organic layers, a 0.25 mL aliquot was diluted to 10 mL with THF and labeled **Lum0** and **Lum120**, respectively.

Fluorescence

Fluorescence measurements were taken by diluting 50 μL of **Lum0** or **Lum120**, respectively to 2 mL with THF in a quartz fluorescence cuvet. This dilution gives a maximum 4.9×10^{-6} M solution of fluorophore based on the initial amount of 9,10-dibromoanthracene. Scans of each sample were taken with excitations at 316 nm for PPO and 393 nm for the anthracene fluorophores. The emission outputs at 357 and 407, respectively were integrated as with the previous method and taken as a ratio of product emission : PPO emission. The percent fluorescence increase from time 0 to time 2 h was then calculated and plotted *vs.* the initial concentration of cinnamaldehyde.

Chemiluminescence

Chemiluminescence measurements were conducted using undiluted portions of **Lum0** and **Lum120** using the same fluorometer. To a cuvet equipped with a magnetic stirring rod was added 1.89 mL of the, respective sample, 50 mg (0.075 mmol) bis(2-carbopentyloxy-3,5,6-trichlorophenyl)oxalate, and 10 μL of 0.1 N sodium hydroxide. With the shutter to the excitation lamp closed 100 μL of 30% hydrogen peroxide solution was added followed quickly by initiation of a time-based data collection protocol. When luminescence levels plateaued, a spectrum scan was initiated, which took a 3-fold average at each wavelength. The data was integrated as before and the percent increase from time 0 to 2 h was calculated and plotted *vs.* the initial concentration of cinnamaldehyde.

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