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A Versatile and Highly Selective Colorimetric Sensor for Detection of Amines

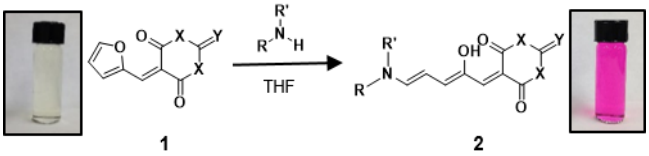
Yvonne J. Diaz, Zachariah A. Page, Abigail S. Knight, Nicolas J. Treat, James R. Hemmer, Craig J. Hawker,* and Javier Read de Alaniz*

Abstract: The utility of Meldrum's activated furan (MAF) for the colorimetric detection of sub ppm levels of amines in solution, on solid supports, and as vapors is described herein. MAF is synthesized in one step from inexpensive and commercially available starting materials and exhibits high selectivity for primary and secondary amines in the presence of competing nucleophiles. The reaction between activated furans and amines results in a distinct color change, discernable by the naked eye. UV-vis absorption spectroscopy was utilized to monitor reactions in solution and determine detection limits. Additionally, solutions of MAF were useful as stains for thin layer chromatography and for monitoring solid phase synthesis of peptides and peptidomimetics. Finally, MAF was used to detect volatile amines released from fish samples, demonstrating potential for food spoilage applications.

The ability to selectively sense amine-containing molecules is pertinent to a variety of research and commercial applications, including chemical synthesis,[1] drug detection,[2,3] and environmental monitoring.[4–12] Emerging methods that track changes in resistivity[13–15] and luminescence[2,7,16–18] have allowed increased sensitivity and selectivity with chemiresistors[13,15,19] and fluoresence-sensors[7,16,20–23] providing sub-ppm limits of detection. However these systems often require complex synthetic methods and the use of secondary equipment for amine identification. Additionally, the majority of these sensors are limited to detection of volatile and primary amines.

In addressing the challenges with these techniques, colorimetric sensors are attractive alternatives due to their simple and rapid detection of amines in solution, as vapors, and on solid supports.[24] Recent improvements have also provided competitive sensitivities (<1 ppm).[11,25,26] For example, polyacrylonitrile nanofibers have been used for the visible detection of ammonia vapors of <1 ppm.[11,25] Suslick and coworkers have achieved <1 ppm sensitivities by utilizing a combination of metalloporphyrins and pH acid/base indicators in colorimetric arrays with the additional benefit of merging modern digital imaging and pattern recognition techniques.[26] Although advances to colorimetric sensors have allowed for the identification and quantification of specific analytes, these methods often involve the use of specialized equipment and are restricted to the detection of volatile amines, limiting their use as a general amine colorimetric sensor. Recent investigation into new negative photoswitches, termed donor acceptor Stenhouse adducts (DASAs), revealed an avenue to increasing the versatility and selectivity of colorimetric detection of amines.[27,28] The ring opening of activated furans with secondary amines is a rapid and efficient reaction that produces DASAs, which possess high molar absorptivity values ($\varepsilon_{\lambda max} \approx 10^5$ $M^{-1}cm^{-1}$).[29–31] The facile synthesis of activated furans from commercially available and inexpensive starting materials coupled with the high ε of the DASA product indicated the potential of activated furans as colorimetric sensors.[30]

Herein, we present a simple methodology to monitor the presence of amines in solution, on solid supports, and as vapors that is available to non-experts (**Scheme 1**). These activated furan-based sensors combine the sensitivity of state-of-the-art detectors with the ability to distinguish various amines by the naked eye. In illustrating the utility of this novel system across different fields, applications including thin layer chromatography (TLC) staining, solid phase peptide/peptoid synthesis, and food spoilage detection were demonstrated.



Scheme 1. General reaction for activated furan-based amine sensor. Activated furan (1) reacts with ammonia, or a primary or secondary amine to form a colored DASA (2) . Inset: Photographs of Meldrum's activated furan in THF (20 mM, left) and 5 min after the addtion of diethylamine (200 ppm, right).

Activated furan-carbon acids (**1**) are rapidly synthesized by reacting furfural (≈$2/kg), a derivative of non-edible biomass, with cyclic 1,3-dicarbonyl compounds on water.[29,30] Conveniently, both Meldrum's acid activated furan (MAF, compound **3**) and 1,3-dimethyl barbituric acid activated furan (BAF, compound **5**) (**Figure 1**) precipitate out of the reaction mixture and can be isolated in near quantitative yields by filtration.[30] Additionally, MAF and BAF have excellent stability under ambient conditions as well as when dissolved in non-nucleophilic organic solvents (e.g., tetrahydrofuran and dichloromethane), as evidenced by monitoring the solution over time using ^{1}H NMR spectroscopy (**Figure S1**). These activated furans then react rapidly with amines to produce highly colored and thermodynamically stable triene forms of DASAs (**2**).[29–31] Although DASAs are light-responsive dyes, basic "donors", such as aliphatic amines, require a highly nonpolar matrix (e.g., toluene, hexanes) for photoswitching (color bleaching) to be observed, making them excellent candidates for amine sensing (**Figure S2**).[31–33]

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UV-Vis absorption spectroscopy was utilized to compare the optical properties and amine reactivities of MAF and BAF. For equimolar solutions (10 μM in THF) of the two activated furans, MAF solution was found to be colorless to the naked eye, while BAF solution was slightly yellow (**Figure 1a**, **1b**). The near colorless initial MAF solutions are favorable for sensing applications, given the more distinguishable "turn-on" of color that occurs upon amine exposure (**Figure S3-S5**). Introducing 0.6 ppm diethylamine (DEA) to MAF and BAF solutions (20 mM in THF), and monitoring the reactions *in situ*, revealed that MAF reacts ~2 times faster (**Figure 1c**). Given the beneficial optical properties and faster response time of MAF based sensor, it was selected for in-depth investigation. Initially, the limit of detection by eye for DEA in a 20 mM solution of MAF (in THF) was found to be ~0.4 ppm after 1 h of exposure (**Figure S6**). Significantly, other secondary amines, primary amines, and ammonia were all found to form the characteristic pink color (λ_{max} ≈ 532 nm), however, with varying reaction rates (**Figure S7**). After 5 min, the absorbance of **4** using DEA is 11-fold higher than the reaction with butylamine (1° amine), and 33-fold higher than the reaction with ammonia (**Figure S7**). The same trend was observed for other 2° and 1° amines; 10 ppm of dimethylamine and piperidine (2° amines) resulted in pink color after 5 min, while 100 ppm of cadaverine (a common biogenic 1° amine[34]) was required to turn the solution pink within 5 mins (**Figure S8**). This difference in reaction rates and absorbance provides a pathway to distinguish between primary and secondary amines in solution. To establish selectivity for amines, the MAF solution was reacted with other nucleophiles, such as water, alcohols, thiols, and organophosphorus compounds (**Figure S9**). As expected only amines were found to be reactive with the MAF solution, highlighting the amine-selectivity.

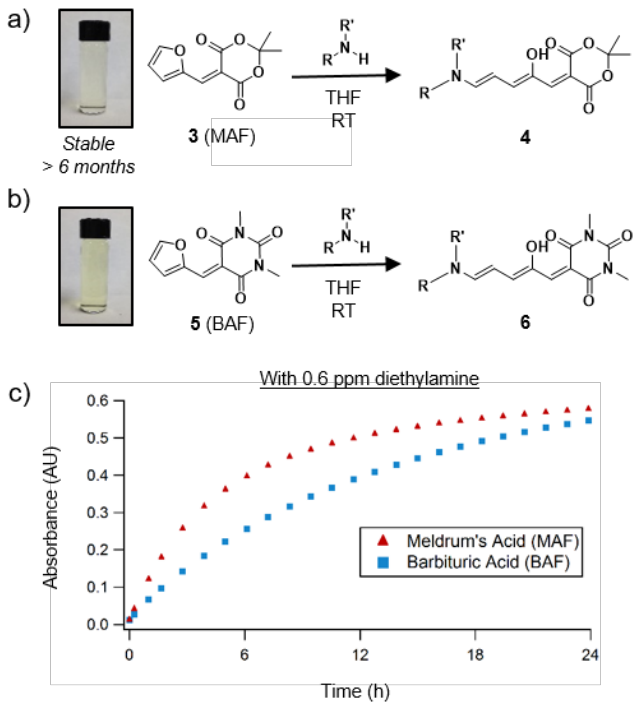

Figure 1. General reaction with amines for a) MAF, **3**, and b) BAF, **4** (photographs represent respective 20 mM solutions of MAF and BAF in THF). c) Reaction of MAF (red triangles and BAF (blue squares) with DEA (0.6 ppm).

To showcase MAF as an amine-selective stain for thin layer chromatography (TLC), a tryptamine-based synthetic sequence was monitored (**Figure 2**). TLC staining is one of the most common methods to track the progress of a chemical reaction and determine chromatographic purification conditions. Although the ability to rapidly distinguish different amine moieties by eye is beneficial for synthetic chemists, traditional stains, such as ninhydrin,[35] often do not provide this distinction. Tryptamine derivatives are prevalent in the pharmaceutical industry and natural product synthesis, making them exemplary candidates for TLC staining with MAF. Four derivatives, possessing various aliphatic and/or conjugated amines, were synthesized from tryptamine, spotted onto silica TLC plates, and stained with MAF (20 mM in THF). After staining for <1 min at room temperature, a wide range of vibrant colors became apparent, making it possible to clearly distinguish the different reaction stages by eye (**Figure 2**). To quantify the color difference between stained tryptamine derivatives, ΔE* values were determined following International Commission on Illumination (CIE) guidelines (**Figure S10-S14**).[36–38] All pairs had ΔE* values > 7, which is generally considered to be distinguishable by eye.[38–40] This supported that MAF stains are an effective method to distinguish between amine-containing compounds with subtle chemical differences (see SI for more details on color quantification).

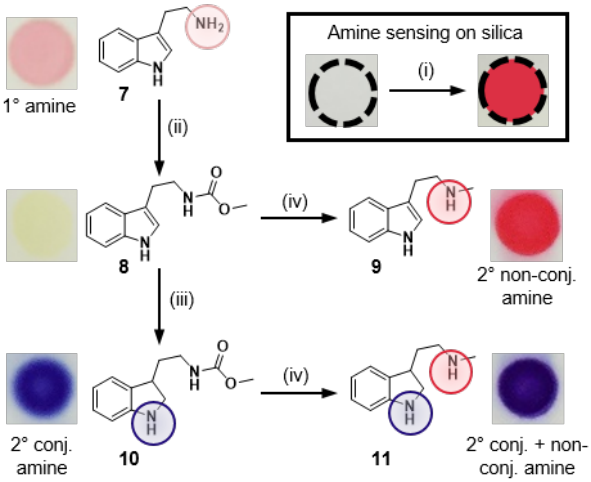

Figure 2. Tryptamine-based synthesis and TLC staining with MAF (20 mM in THF). Reagents and conditions: i) secondary amine. ii) methyl chloroformate, NaOH(aq):DCM (1:1), rt, >95%. iii) Et_3SiH, CF_3COOH, 60°C, 48%. iv) LAH, THF, 60°C, >95%.

MAF solutions were further used to stain amine-functional resins found in solid phase synthesis of peptides and peptidomimetics. For modular solid phase synthesis, the quantitative conversion at each synthetic step is critical for obtaining the desired product.[41] As such, stains have been

developed to identify the presence of 1° and 2° amines.[42–46] However, commonly used stains such as ninhydrin[42] (a.k.a., Kaiser test) and chloranil,[43] used for detection of 1° and 2° amines respectively, are unstable and require the use of toxic reagents such as acetaldehyde and potassium cyanide. To highlight the ease of reaction monitoring with MAF, peptide and peptoid-functionalized resins were tested. A stable stock solution of MAF in THF (200 mM) was prepared and diluted tenfold into methanol prior to adding 2-3 drops to functionalized resins. For peptides (1° amines), exposure to MAF resulted in beads with a light pink color in 5 min with heat (**Figure 3a**). Unlike the Kaiser test, the MAF solution is capable of sensing 1° and 2° amines, allowing for the detection of all amino acids and many peptidomimetics (**Figure S15**). The addition of 2-3 drops of our MAF solution to resin-bound peptoids, immediately turned the beads bright pink under ambient conditions (**Figure 3b**). These results confirmed that MAF stains are a simple and effective alternative to classic methods used to determine reaction completion in solid phase peptide and peptoid synthesis.

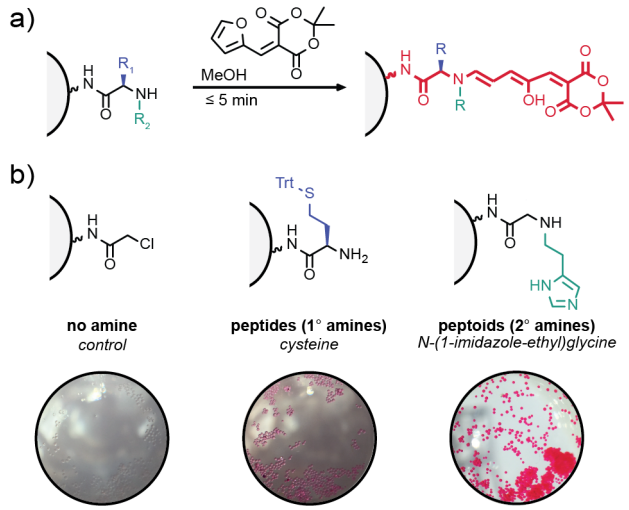

Figure 3. a) General reaction between MAF and resin bound peptides/peptoids. b) Images of resin beads 5 min after adding 2-3 drops of a MAF solution (20 mM methanol) where 1° amine containing beads (peptides) turn pale pink after heating and 2° amine containing beads (peptoids) turn bright pink under ambient conditions. Control image represents resin beads (no amine) after heating for 5 min.

Given the facile detection of amines in solution and on solid supports with MAF, we investigated its utility to sense amine vapors. Detection of amine vapors is important for various applications, including food spoilage[4–7,19,47,48], where volatile amines such as dimethylamine (DMA) and ammonia are released from aging meat.[34] To evaluate the sensitivity of our sensor toward volatile amines, MAF was exposed to known concentrations of amine vapors. First, nylon filter membranes were dipped into a 450 mM solution of MAF in THF, dried, and sealed in septa-capped scintillation vials. The MAF coated filters were then exposed to vapors of DMA and ammonia. After 5 min, the filters were removed from the vials and a digital image of the filter membrane was captured. Calculating ΔE* for each image revealed that filter membrane exposed to 0.5 ppm of DMA and ammonia vapors is distinguishable (ΔE* > 5)[38–40] from the MAF coated reference (no amine exposure) (**Figure 4**). Although MAF has a similar limit of detection for both vapors, the relative change in color for the 2° amine provides ΔE* values of 68 at 2 ppm DMA, as compared to 31 at 50 ppm ammonia. The more pronounced color change for the secondary amine is consistent with prior observations made for amines in solution and on solid supports. This demonstrated the ability to use MAF coated nylon filters for quick and sensitive detection of volatile amines.

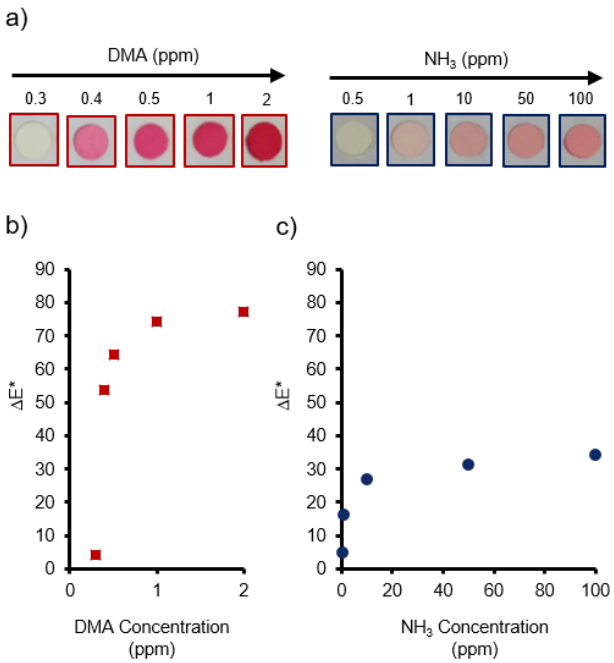

Figure 4. a) Images of nylon filters that correspond to data points from graphs b and c. Visual responses of MAF coated nylon filters after 5 minute amine exposures to b) 0.3, 0.4, 0.5, 1, and 2 ppm DMA (red squares, detection limit of ~0.4 ppm) and c) 0.5, 1, 10, 50, and 100 ppm ammonia (blue circles, detection limit of ~0.5 ppm)

To expand the utility of this sensor to applications in food spoilage, the release of volatile amines from two commercially important fish samples, cod and tilapia, was studied. Freshly thawed fish samples (~15 g) were sealed into separate glass jars containing five nylon filter membranes coated with MAF solutions in THF at concentrations ranging from 56 to 450 mM (see **Figure S16** for experimental setup). As a control, an analogous setup was constructed with no added fish samples. The sensors were monitored over the course of 48 h with time lapse imaging (**Figure 5**). Interestingly, the cod sample resulted in a noticeable change in color after ~8 h, while the tilapia had a significantly delayed response, changing color after ~24 h (**Figure 5**). Ultimately, ΔE* values of 60 and 38 were obtained for the cod and tilapia samples (respectively) after 48 h relative to the control sample, highlighting the very distinct color change for MAF samples upon exposure to volatile amines. These

results show the potential applicability for this type of sensor in real-time monitoring of food spoilage.

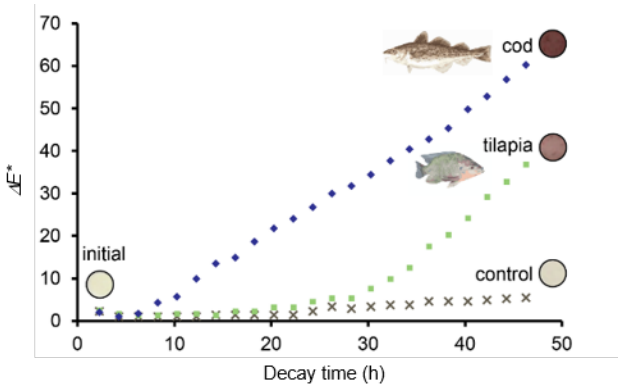

Figure 5. Change in color of MAF coated nylon filters over time in the presence of cod (blue diamonds), tilapia (green squares) and air only (black *x*'s, control) at room temperature.

In conclusion, Meldrum's activated furan (MAF) has been identified as a versatile and simple to use sensor for the selective detection of amines in solution, on solid supports, and in the vapor phase. With an amine detection limit <1 ppm MAFs are among the most sensitive, all organic, colorimetric tests for amines, highlighting the capacity of activated furans to be used as simpler alternatives to state-of-the-art sensors. Further utility of activated furans for sensing in organic synthesis, drug detection, and food spoilage applications are currently under investigation.

Acknowledgements

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Keywords: Activated furan • colorimetric sensor • stain • peptide • food spoilage

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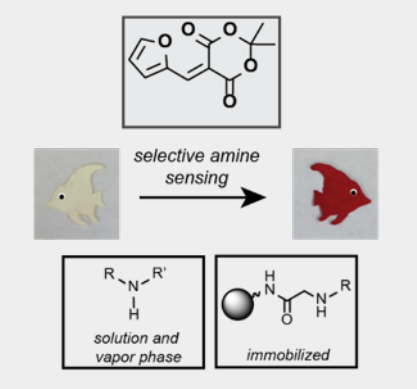

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