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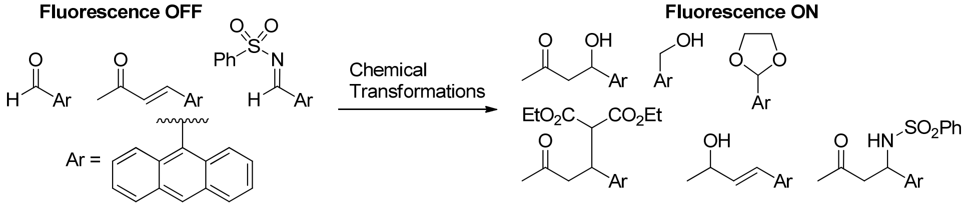

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Fluorogenic probes for chemical transformations: 9-anthracene derivatives for monitoring reaction progress by an increase in fluorescence

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ABSTRACT

The development of fluorogenic probes for chemical transformations bearing anthracene as a fluorescent core moiety is reported. Fluorogenic probes were designed by linking anthracene with functional groups used for reactions of interest. Each fluorogenic probe, possessing a reaction group such as aldehyde, α,β-unsaturated ketone, or imine at the 9-position of the anthracene, showed no or very low fluorescence. Reaction products of the probes, including aldol and addition products, were highly fluorescent. The products showed more than 1000-fold higher fluorescence than did the fluorogenic probes under the same conditions. The utility of the fluorogenic probes was demonstrated in monitoring the progress of a catalyzed aldol reaction.

Fluorogenic substrates or probes for chemical transformations, molecules that show no or very low fluorescence but show high fluorescence upon chemical transformations, are useful for monitoring the progress of chemical transformations.[1-5] Analyses of the fluorescence increase correlated to the product formation under no or very weak fluorescence conditions are highly sensitive to detect the formation of low concentrations of the product, compared to the analysis of the fluorescence decrease related to the consumption of fluorescent substrates.[1,3e] Thus, when a fluorogenic probe is used in a reaction, formation of the fluorescent product can be evaluated at initial stages of the reaction.[1-3] Assays using fluorogenic substrates accelerate rapid identification of superior catalysts and reaction conditions in high-throughput formats as well as characterization of catalysis on a small scale. Here, we report the development of 9-anthracene-derived fluorogenic probes (Figure 1) and their use in monitoring various chemical transformations, including C–C bond-forming reactions.

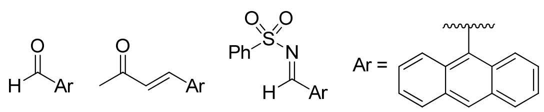

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Figure 1. Fluorogenic substrate probes developed in this study

Many 9-anthracene derivatives are highly fluorescent; however, the fluorescence depends on the substituents.[6] For example, aminomethylanthracenes are weakly fluorescent or non-fluorescent when the amino group is not protonated; upon protonation, the compounds become highly fluorescent.[7] Because aldehydes conjugated with aryl groups and α,β-unsaturated compounds often quench fluorescence,[2,3] we reasoned that anthracene with one of these functional groups should be a candidate for fluorogenic substrates. When the reacting functional group of the fluorogenic substrate candidate is transformed to a non-quenching group, an increase in fluorescence will be observed as the reaction progresses. We reasoned that by linking a highly fluorescent anthracene moiety to reacting functional groups that quench the anthracene fluorescence, fluorogenic probes with great fluorogenic ranges for chemical transformations would be generated. Note that although many fluorescence-based sensors derived from 9-anthracene have been developed for detecting certain molecules through noncovalent binding and changes in protonated stages,[8] no examples of anthracene-based fluorogenic probes have been reported to detect covalent bond-formations and other chemical transformations.

First, 9-anthraldehyde (**1**) was evaluated as a fluorogenic aldehyde candidate (Scheme 1). Aldehyde **1** is non-fluorescent or very weakly fluorescent in many solvents. Reactions of **1**

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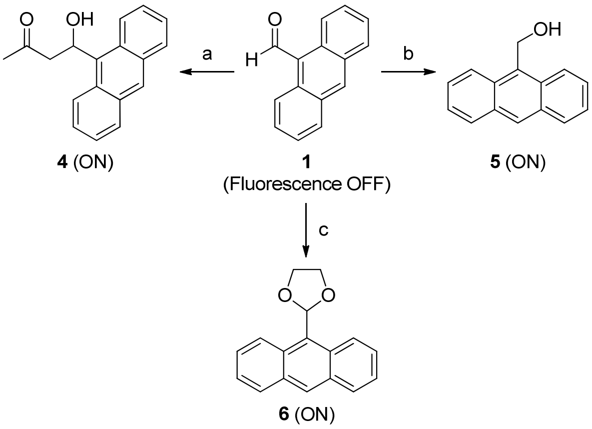
involving aldol reaction, carbonyl reduction, and acetal formation were performed and fluorescence of the products were analyzed. Aldol **4** was highly fluorescent in aqueous buffers (λ_{ex} 250 nm, λ_{em} 415 nm) as well as in organic solvents (Figure 2 and Table 1). Fluorescence intensity of **4** varied with solvent, but did not vary within the pH range 5.3–8.4 in aqueous buffer. Ratios of fluorescence of aldol **4**/aldehyde **1** were more than 1000-fold under the conditions shown in Table 1. Thus, aldehyde **1** is an excellent fluorogenic aldehyde substrate for the aldol reaction. In addition, alcohol **5** and acetal **6** were also highly fluorescent (Table 1), suggesting that aldehyde **1** could be used for monitoring not only the aldol reaction but also other aldehyde transformations.



Scheme 1. Reagents and conditions: (a) acetone, L-proline, rt, 4 d, 23%; (b) $NaBH_4$, EtOH, rt, 30 min, 99%; (c) ethylene glycol, *p*-TsOH, toluene, reflux, 2 h, 14%.

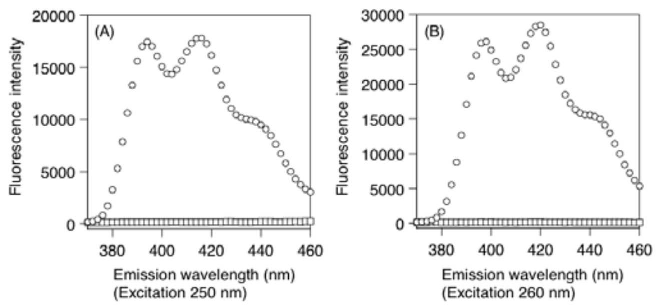


Figure 2. Fluorescence emission spectra of **1** (5 μM; square) and **4** (2.5 μM; circle) (A) in 1% DMSO-99% 50 mM Na phosphate, pH 7.0 (λ_{ex} 250 nm) (B) in DMSO (λ_{ex} 260 nm).

To examine the utility of **1** for monitoring reaction progress in real time, the aldol reaction of acetone and aldehyde **1** catalyzed by aldolase peptide FT-YLK25[9] was performed and the fluorescence was analyzed (Figure 3). The reaction in the presence of this peptide showed a significant increase in fluorescence, whereas reaction in the presence of control peptide (nonaldolase peptide) FT-YLK3-R5,[9] reaction without acetone, and reaction without peptide showed little or no increase in fluorescence. Whereas peptide FT-YLK25 catalyzed the reaction, aldolase antibody 38C2[10] did not. Antibody 38C2 has a narrow active site cavity[10] and aldehyde **1** was too bulky to be accepted into the cavity for the reaction. Note that phenanthrene-based fluorogenic aldehyde **7** (Figure 4) was a substrate for the reaction of antibody 38C2.[2h] Thus, the size of the catalytic active sites can be discriminated by analyses using the fluorogenic aldehydes **1** and **7**. In most solvents, aldehyde **1** showed no fluorescence at 5 μM and aldehyde **7** was weakly fluorescent. Because fluorescence intensity of **4** was approximately 3–8-fold higher (depending on solvent) than that of the corresponding aldol product of **7** at the optimized excitation and emission wavelengths, use of **1** allowed more sensitive detection of reaction progress than the use of **7**. Formation of less than 0.04 μM of **4** was detected in a 100 μL-scale reaction in a 96-well plate.

Table 1. Fluorescence of **1** and derivatives from **1**[a]

	Solvent	Wavelength (nm) λ_{ex}	λ_{em}	Conc. (μM)	Fluorescence intensity
1	pH 7	250	415	5	[b]
	pH 7	250	415	50	2.0×10^2
	DMSO	260	420	5	[b]
	DMSO	260	420	50	1.1×10^2
	DMF	260	420	5	[b]
	2-PrOH	250	420	5	[b]
4	pH 7	250	415	2.5	1.8×10^4
	DMSO	260	420	2.5	2.8×10^4
	DMF	260	420	2.5	5.6×10^3
	2-PrOH	250	420	2.5	1.2×10^4
5	pH 7	250	415	2.5	1.1×10^4
	DMSO	260	420	2.5	9.2×10^3
6	DMSO	260	420	2.5	1.2×10^4

[a]The fluorescence was recorded on a microplate spectrophotometer using 100 μL of solution composed of 1% DMSO and 99% of the indicated solvent in a 96-well polypropylene plate at 26 °C. Solvent pH 7 refers to 50 mM Na phosphate, pH 7.0.

[b]No fluorescence was detected after background correction.

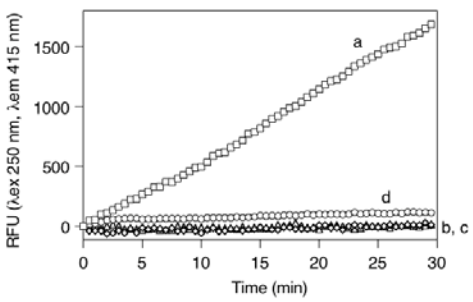


Figure 3. Fluorescence assay of peptide FT-YLK25-catalyzed aldol reaction of acetone and aldehyde **1**. Conditions: [peptide] 100 μM, [**1**] 50 μM, [acetone] 5% (v/v) (680 mM), 5% DMSO–40 mM Na phosphate, pH 7.0, total volume 100 μL in a 96-well plate at 26 °C; (a) reaction with aldolase peptide FT-YLK25; (b) reaction with FT-YLK25 in the absence of acetone; (c) reaction with nonaldolase peptide FT-YLK3-R5; (d) reaction without peptide. RFU = relative fluorescence intensity. FT-YLK25, $SPFLGQYKLLKELLAKLKWLLRKL-NH_2$ (C-terminal amide); FT-YLK3-R5, $YRLLRELLARLRWLLRRLLGPTCL-NH_2$ (C-terminal amide).[9]

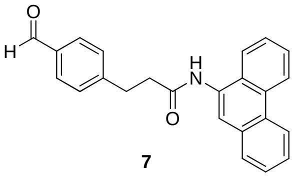


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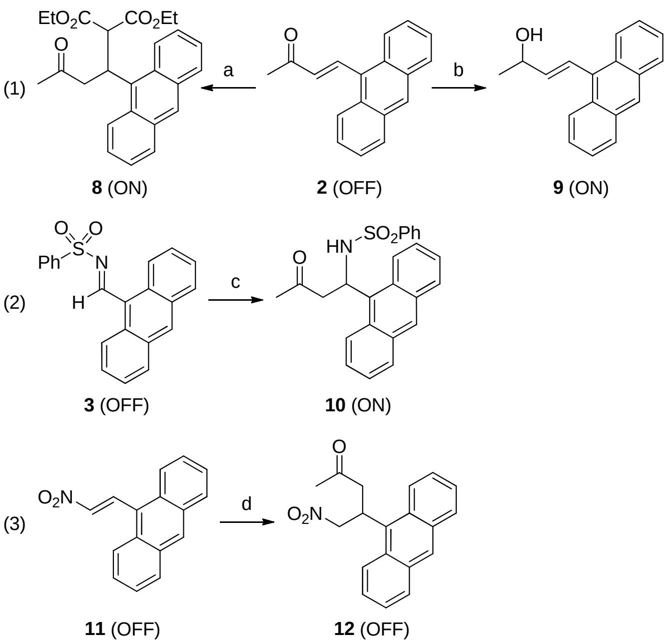
Figure 4. Previously reported fluorogenic aldehyde[2h]

Next, candidate fluorogenic substrates and possible products were synthesized and their fluorescence was analyzed (Scheme 2 and Table 2). Michael adduct **8** and alcohol **9** were highly fluorescent, whereas α,β-unsaturated ketone **2** showed no fluorescence at 2.5 μM under the same conditions. As observed for **4**/**1**, the fluorescence ratios of **8**/**2** and **9**/**2** were more than 1000-fold, suggesting that compound **2** is a useful fluorogenic substrate. Sulfonylimine **3** was weakly fluorescent and its addition product **10** was highly fluorescent. The fluorescence ratio of **10**/**3** was 84-fold in aqueous buffer, pH 7, and was 22-fold in DMSO. Loss of π-conjugation between the carbonyl or phenylsulfonyl group and anthryl group or vinylanthryl group restored the fluorescence. On the other hand, a nitro group quenched the fluorescence in both conjugated and non-conjugated forms. Fluorescence intensity of nitroolefin **11** and of Michael product **12** was less than 1% of that of **1**, and the ratio of the fluorescence of **12**/**11** was only 2-fold.

In summary, we have developed 9-anthracene-derived fluorogenic probes for chemical transformations. We have demonstrated that these probes are useful for detecting reaction progress on a small scale through an increase in fluorescence. These developed fluorogenic probes should be useful for rapid identification and characterization of catalysts and catalyzed reactions.



Scheme 2. Reagents and conditions: (a) diethyl malonate, pyrrolidine, AcOH, rt, 24 h, 85%; (b) $NaBH_4$, MeOH, rt, 30 min, 99%; (c) acetone, pyrrolidine, AcOH, DMF/$CHCl_3$ 1:1, rt, 1 d, 21%; (d) acetone, pyrrolidine, AcOH, $CHCl_3$, rt, 3 d, 76%.

Table 2. Fluorescence of compounds[a]

	Solvent	Wavelength (nm) λ_{ex}	λ_{em}	Fluorescence intensity
2	pH 7	250	420	[b]
	pH 7	250	420	43[c]
8	pH 7	250	420	2.1×10^4
	DMSO	260	420	3.9×10^4
	DMF	260	420	8.9×10^3
	2-PrOH	250	420	1.9×10^4
9	pH 7	255	435	3.5×10^4
	DMSO	260	435	2.8×10^4
	DMF	260	435	9.1×10^3
	2-PrOH	250	420	1.8×10^4
3	pH 7	255	420	1.9×10^2
	DMSO	260	420	9.1×10^2
10	pH 7	255	420	1.6×10^4
	DMSO	260	420	2.0×10^4
11	DMSO	260	400	50
12	DMSO	260	400	1.1×10^2

[a,b]See Table 1 legend. Concentration of compound was 2.5 μM except where noted.

[c]Concentration of **2** was 50 μM.

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Supplementary Material

Supplementary data of fluorescence spectra and synthesis and characterization of compounds associated with this article can be found, in the online version, at doi:10.1016/XX.

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