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Design and Use of Fluorogenic Aldehydes for Monitoring the Progress of Aldehyde Transformations

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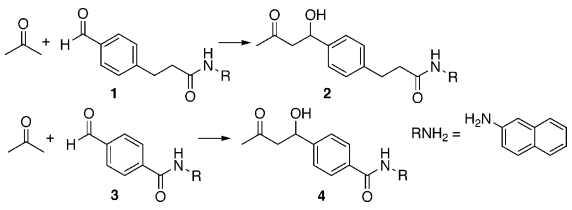
Simple and rapid methods for monitoring the progress of chemical reactions are critical for high-throughput screening of catalysts as well as for characterization of catalysts on a small scale.[1,2] Fluorogenic substrates that increase in fluorescence as reactions progress provide a straightforward method of reaction monitoring because reaction progress is directly observed as an increase in fluorescence.[2] We have previously developed fluorescent detection strategies to monitor Michael and Diels−Alder reactions using fluorogenic α,β-unsaturated carbonyl compounds[3] and have demonstrated that the system is useful for evaluation of catalysts and reaction conditions.[4] Aldehydes are versatile and are used for many types of reactions. To develop systems for monitoring the progress of aldehyde transformations, an entirely new approach was required. Here we report the first design, synthesis, and use of fluorogenic aldehydes for direct monitoring of aldehyde transformations by fluorescence growth.

Our design is based on resonance energy transfer[5] between a fluorophore and an aldehyde in a single molecule. The fluorogenic aldehydes are composed of a fluorophore and an aldehyde moiety coupled by a linker. When intact, the aldehyde moiety acts as a quencher of the fluorophore's fluorescence; however, the reaction product of the aldehyde moiety does not quench fluorescence and fluorescence is "turned-on" in the product. We reasoned that an arylaldehyde would quench the fluorescence of a proximal fluorophore, and that a simple aryl group without a carbonyl would not.[6] To test this hypothesis, we prepared the aldehyde **1** and aldol **2** shown in Scheme 1. As expected, aldol **2** showed a higher fluorescence than aldehyde **1** (Table 1). On the other hand, neither aldehyde **3** nor aldol **4** was fluorescent. Note that in **4**, the aryl group conjugated to the fluorophore via an amide bond quenched the fluorophore's fluorescence.

Scheme 1



We prepared candidate fluorogenic aldehydes and their aldols (**5**−**12**, Scheme 2) by using a series of fluorophores and compared their fluorescence (Table 1). Aldehyde **7**, prepared as the amide of 9-aminophenanthrene (**13**), was the most promising of the aldehydes prepared. The reaction product, aldol **8**, showed ∼80-fold higher fluorescence (λex 250 nm, λem 380 nm) than aldehyde **7** in aqueous buffer (pH 7.0) and ∼20-fold higher (λex 265 nm, λem 385 nm) in DMSO. Although the fluorescence intensity varied with solvent, aldol **8**/aldehyde **7** had an excellent fluorogenic range in aqueous buffer and in organic solvents, and the fluorescence intensity of **8** did not vary within the pH range of 5.3−8.0 in aqueous buffer. In addition, the fluorescence of aldol **8** differed from that of fluorophore **13** as shown in Figure 1. Aldol **10** showed ∼20-fold higher fluorescence than aldehyde **9** in DMSO. In contrast, aldehyde **11** showed higher fluorescence than aldol **12** at λex 260 nm and λem

Scheme 2

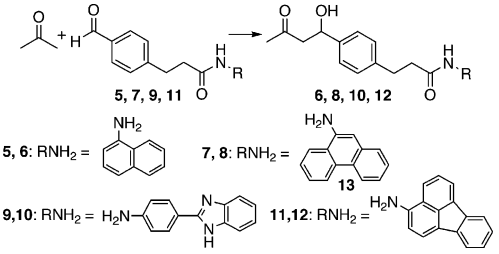


Table 1. Fluorescence of Aldehydes and Aldols[a]

		wavelength (nm)			fluorescence intensity		
	solvent	λex	λem	c[b]	aldehyde	aldol	fold[c]
1,**2**	DMSO	282	360	50	1.7×10^3	1.3×10^4	8
	DMF	282	360	50	1.3×10^3	1.2×10^4	9
	pH 7	250	352	50	74[d]	1.9×10^3 [d]	26
5,**6**	DMSO	300	360	50	1.1×10^2	5.7×10^2	5
7,**8**	DMSO	265	385	5	4.9×10^2	8.7×10^3	19
	DMF	265	385	5	4.6×10^2 [d]	4.2×10^3 [d]	9
	pH 7	250	380	5	57[d]	4.4×10^3 [d]	78
9,**10**	DMSO	315	360	5	2.5×10^3	4.5×10^4	18
	DMF	315	360	5	2.4×10^3	4.5×10^4	18
	pH 7	315	360	5	6.5×10^2	4.2×10^3	6
11,**12**	DMSO	260	380	25	2.5×10^2 [d]	8.6×10^2 [d]	3
	DMSO	260	450	25	1.5×10^4 [d]	8.2×10^3 [d]	0.5

[a] The fluorescence was recorded on a microplate spectrophotometer using 100 μL of solution composed of 0.5% CH_3CN, 0.5% 2-PrOH, and 99% of the indicated solvent in a 96-well polypropylene plate at 26 °C. Solvent pH 7 refers to 50 mM sodium phosphate, pH 7.0. The data are shown after background correction except where noted. [b] c = concentration of aldehyde or aldol (μM). [c] fold = fluorescence intensity of aldol/fluorescence intensity of aldehyde. [d] The data without background correction.

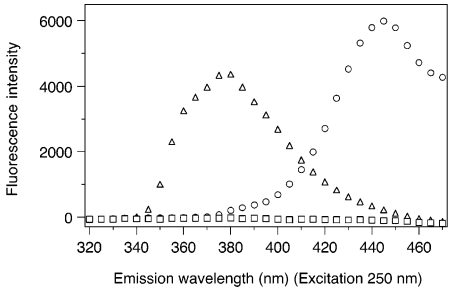


Figure 1. Fluorescence emission spectra (λex 250 nm) of aldehyde **7** (□), aldol **8** (△), and fluorophore **13** (○) at 5 μM in 0.5% CH_3CN−0.5% 2-PrOH−99% 50 mM sodium phosphate, pH 7.0.

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Scheme 3

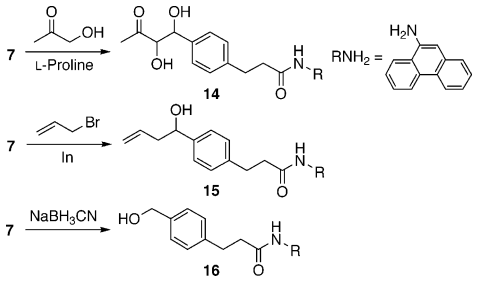

Table 2. Fluorescence of Compounds **14**−**16**[a]

	solvent	λex	λem	c[b]	fluorescence	fold[c]
14	DMSO	265	385	5	6.5×10^3	13
	DMF	265	385	5	2.6×10^3 [d]	6
	pH 7	250	380	5	4.4×10^3	77
15	DMSO	265	385	5	1.3×10^4	26
	DMF	265	385	5	5.6×10^3 [d]	12
	pH 7	250	380	5	3.2×10^3 [d]	57
16	DMSO	265	385	5	5.8×10^3	12
	DMF	265	385	5	2.7×10^3 [d]	6
	pH 7	250	380	5	3.0×10^3 [d]	53

[a,b] See Table 1 legend. [c] fold = fluorescence intensity of **14**, **15**, or **16**/ fluorescence intensity of aldehyde **7**. [d] See Table 1 legend.

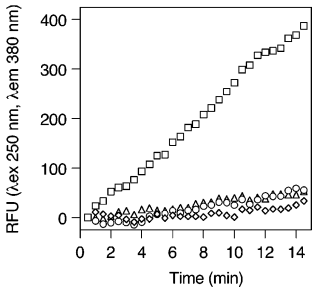

Figure 2. Fluorescence assay of antibody 38C2-catalyzed aldol reaction of acetone and aldehyde **7**. Conditions: [antibody] 2 μM (active site), [**7**] 50 μM, [acetone] 5%(v/v) (680 mM), 2.5% CH_3CN−2.5% 2-PrOH/PBS (pH 7.4). □: 38C2; ○: nonaldolase antibody IgG (control); ◇: reaction with 38C2 in the absence of acetone; △: reaction without antibody (blank). RFU = relative fluorescence intensity.

450 nm, although **12** showed a slightly higher fluorescence at λex 260 nm and λem 380 nm. These results indicate that the proper selection of fluorophores is important for the preparation of useful fluorogenic aldehydes.

To examine the applicability of the fluorogenic aldehydes to other reactions, aldehyde **7** was transformed to aldol **14** by aldol reaction with hydroxyacetone, to allyl alcohol **15** by In-mediated allylation,[7] and to alcohol **16** by reduction (Scheme 3). These products were all fluorescent (Table 2), indicating that the loss of π-conjugation between the aldehyde carbonyl and the aryl group is key to fluorescence and that aldehyde **7** can be used as a fluorogenic substrate for many reactions.

To monitor the time-course of an aldol reaction, we studied the reaction of acetone and aldehyde **7** catalyzed by aldolase antibody 38C2[8] (Figure 2). The reaction with antibody 38C2 showed a significant increase in fluorescence, while reaction with a control antibody, reaction without acetone, and reaction without antibody all showed little or no increase in fluorescence. Catalytic reduction of **7** with alcohol dehydrogenase in the presence of NADPH was successfully monitored by observing an increase in fluorescence (Figure 3). Although reactions with this enzyme can be monitored by changes in UV (340 nm) and fluorescence (λem 450 nm) of NADPH, fluorogenic aldehyde **7** can be used in a complementary

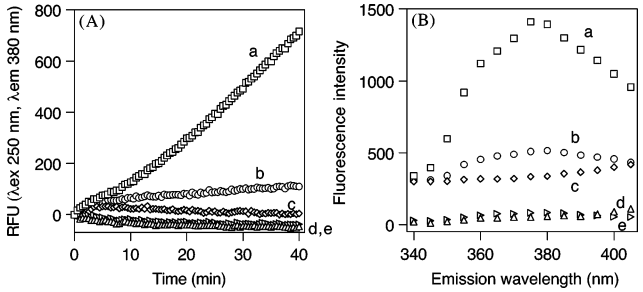

Figure 3. Fluorescence assay of reduction of aldehyde **7** with alcohol dehydrogenase (ADH) from *Thermoanaerobium brockii*.‡ (A) Time course, (B) emission spectra (λex 250 nm) at 50 min. Conditions: (a) [ADH] 0.235 unit/mL, [NADPH] 40 μM, [aldehyde **7**] 12.5 μM, 0.5% CH_3CN−0.5% 2-PrOH−99% 50 mM sodium phosphate, pH 7.0; (b) reaction without addition of NADPH; (c) reaction using **3** instead of **7**; (d) reaction without ADH; (e) reaction without ADH and NADPH. ‡The UV (340 nm) and fluorescence (λem 450 nm) studies suggested that this enzyme contained some reducing cofactor.

fashion to directly follow the reduction of the aldehyde. Formation of less than 0.2 μM of product **16** was readily detected in a 100 μL-scale reaction in a 96-well plate.

We have developed fluorogenic aldehydes that can be used for monitoring reactions through increased fluorescence. These fluorogenic aldehydes should be useful for screening of catalysts in approaches using libraries.[3,9,10] Our strategy for accessing fluorogenic aldehydes should also be applicable to the preparation of fluorogenic substrates that allow the transformations of other functional groups to be directly monitored.

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Supporting Information Available: Fluorescence spectra, graphs of standards of **8** and **16**, synthesis and characterization of compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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