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A Fluorogenic Aldehyde Bearing a 1,2,3-Triazole Moiety for Monitoring the Progress of Aldol Reactions

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Abstract

We have developed a new type of fluorogenic aldehyde bearing a 1,2,3-triazole moiety that is useful for monitoring the progress of aldol reactions through an increase in fluorescence. Whereas 6-methoxy-2-naphthaldehyde was highly fluorescent, the fluorogenic aldehyde, 4-formylbenzene connected to 6-methoxy-2-naphthyl group through 1,2,3-triazole moiety, was essentially non-fluorescent in aqueous solutions. We suggest that the 4-formylphenyl group acts as a quencher to suppress the fluorescence of the 6-methoxy-2-naphthyltriazole moiety. The product of the aldol reaction of this aldehyde does not have a quenching moiety and showed more than 800-fold higher fluorescence than the aldehyde. Assay systems using the fluorogenic aldehyde were validated by screening of aldol catalysts, ranking of the activities of the catalysts, and evaluation of reaction conditions.

Introduction

Fluorogenic substrates that afford fluorescent products can be used for monitoring the progress of chemical reactions by fluorescence growth and are useful for high-throughput screening and rapid characterization of catalysts.^{1,2,3} Assay systems using fluorogenic substrates are critical for measurements and ranking of catalytic activities in the creation of designer enzymes and in directed enzyme evolution in vitro.³ Fluorogenic substrates have also been employed in assessment of the catalytic activities of catalysts under different conditions and in screening of catalysts useful for synthetic organic chemistry.⁴ Detection of formation of fluorescent products from non-fluorescent fluorogenic substrates (i.e., detection of an increase in fluorescence) is typically more sensitive than detection of formation of non-fluorescent products from highly fluorescent reactants/substrates (i.e., detection of a decrease in fluorescence).² Here we report the development of a new type of fluorogenic aldehyde useful for monitoring the progress of aldol reactions through an increase in fluorescence.

The aldol reaction is an important C-C bond forming reaction in enzyme catalysis and synthetic organic chemistry.^{2b,3,4,5} The development of efficient aldol catalysts is the topic of a number of current research efforts.^{3,4,5} Because factors that affect the catalysis of aldol reactions are not completely understood and because design of aldol catalysts is often difficult, approaches that allow rapid screening of libraries are important for the development of aldol catalysts and for the investigation of aldol reactions.^{3,4,5} That is, there is a high demand for fluorogenic substrates that can be used for monitoring the progress of aldol reactions.^{2b,3,4,5} Whereas many fluorogenic substrates for bond cleaving reactions, including fluorogenic substrates for retro-aldol reactions, are available,^{1,3} few fluorogenic substrates for C-C bond forming reactions have been reported² and only our fluorogenic aldehydes have been reported for aldol reactions.^{2b} Because of the lack of the fluorogenic substrates for aldol reactions, catalysts of aldol reactions have often been identified or evaluated by retro-aldol reactions using fluorogenic and chromogenic substrates for retro-aldol reactions.^{3,6} Catalysts of aldol reactions have also been evaluated using a fluorescence-based assay method involving Michael addition to a fluorogenic maleimide.^{2a,4} However, aldol catalysts do not necessarily accelerate both aldol and retro-aldol reactions and retro-aldol catalysts or Michael catalysts may not catalyze aldol reactions. With appropriate fluorogenic aldehydes, aldol catalysts can be screened and evaluated based on aldol reactions.

For rapid evaluation of aldehyde transformations, including aldol reactions, we previously developed fluorogenic aldehydes and demonstrated their use in monitoring the progress of aldol reactions.^{2b} The design of our previous fluorogenic aldehydes was a conceptual advance. However, improved fluorogenic aldehydes are required. For example, the solubility of our fluorogenic aldehydes was low in aqueous solutions. For the fluorescence-based evaluation of aldol catalysts (such as designer protein and peptide catalysts and small organocatalysts) in water, high water solubility of the fluorogenic aldehydes is required. Ratios of fluorescence intensities of aldol/aldehyde in the assay systems using our previous fluorogenic substrates were up to 78-fold in aqueous solutions and 19-fold in organic solvents.^{2b} To efficiently detect the progress of aldol reactions using a fluorogenic aldehyde, the fluorogenic aldehyde should show little or no fluorescence and the aldol product should be highly fluorescent. The fluorogenic range (or ratio of fluorescence intensities of product/substrate) should be high enough to allow monitoring of product formation during the initial stages of the reactions; higher fluorogenic range provides better detection of the product formation in assays. Fluorogenic aldehydes and assay systems using fluorogenic aldehydes that allow efficient monitoring of the progress of aldol reactions are needed for creation and discovery of new aldol catalysts and for research into the chemistry of aldol reactions.

Results and Discussion

Strategy for Design of Fluorogenic Aldehydes

Many benzene and naphthalene derivatives that bear both electron-donating and electron-withdrawing groups are fluorescent.^{1d,f,7} For example, aldehyde **1** (Scheme 1), which is fluorescent in aqueous buffers,⁸ has a methoxy group (an electron-donating group) and an aldehyde group (an electron-withdrawing group) on the naphthalene ring. Aldol **2**, which does not have an electron-withdrawing group, is not fluorescent in aqueous buffers at the wavelengths used for the detection of fluorescence of aldehyde **1**.⁹ Because of the fluorescence features of aldehyde **1** and aldol **2**, aldol **2** has been used as a fluorogenic substrate for the retro-aldol reaction.^{1b,3a,c-e} On the other hand, arylaldehyde moieties can act as intramolecular quenchers to suppress fluorescence in some molecules and such arylaldehydes may be used as fluorogenic substrates for the aldehyde transformations.^{2b} Therefore, arylaldehydes within or connected to aromatic systems bearing electron-donating groups may be either fluorescent or non-fluorescent depending on the structure of the molecule and depending on wavelengths to

observe the fluorescence. Electron-donating groups attached to arylaldehydes often influence the fluorescence.¹⁰ Length of π -conjugation usually correlates with UV absorption and/or fluorescence emission wavelengths of fluorescent molecules.¹¹ Use of aromatic systems with electron-donating groups should provide absorption and fluorescence at longer wavelengths than those without electron-donating groups. In addition, attachment of oxygen- or nitrogen-containing groups (such as methoxy group or dialkylamino group) to aromatic hydrocarbons often increases the solubility of the compounds in aqueous solutions. We reasoned that arylaldehydes with fluorescent aromatic systems bearing electron-donating groups would be fluorogenic when there was no conjugation between the arylaldehyde moiety and the electron-donating group; in such a system, the arylaldehyde moiety would act as a quencher.¹² Although many fluorescent molecules are known, it is often difficult to predict fluorescence or fluorescence-quenching features of a given molecule in a particular solvent based on the chemical structure of the molecule. Therefore, to develop fluorogenic aldehydes that are useful for assays of aldol reactions in aqueous solutions, we synthesized a series of aldehydes with aromatic moieties bearing electron-donating groups, their aldols, and related compounds (compounds **1–23** in Schemes 1 and 2) and analyzed the fluorescence of these compounds. As described below, we found that aldehyde **20** was the most useful fluorogenic aldehyde of those tested for evaluation of the aldol reaction in aqueous solutions.

Fluorescence of Aldehydes, Aldols, and Related Compounds

Fluorescence of compounds **1** and **3–21** (Schemes 1 and 2) was analyzed in 5% DMSO/50 mM sodium phosphate, pH 7.0. The results, including excitation and emission wavelengths (λ_{ex} and λ_{em} , respectively), that provided a significant difference in fluorescence between aldehyde and the corresponding aldol are summarized in Table 1. Selected fluorescence spectra are shown in Figure 1. All of the aldehydes shown in Scheme 1 (6-oxygen-substituted-2-naphthaldehydes **1**, **4**, and **6**; 2-naphthaldehydes **7**, **9**, and **11** bearing 6-amino groups; aldehyde **15** containing a coumarin moiety with a diethylamino group; aldehyde **17** containing a coumarin moiety with a hydroxy group) were fluorescent (entries 1–8 and 10 and Figure 1A and B). For the tested 6-oxygen- or 6-nitrogen-substituted-2-naphthaldehydes and the corresponding aldols shown in Scheme 1 (aldehyde/aldol pairs **1/3**, **4/5**, **7/8**, **9/10**, and **11/12**), wavelengths that provided higher fluorescence of aldol than the corresponding aldehyde were not found. Aldols **8**, **10**, and **12** showed fluorescence at some excitation and emission wavelengths, but the corresponding aldehydes also showed fluorescence at these wavelengths. Thus, like aldol **2**, aldols **3**, **5**, **8**, **10**, and **12** may be used as fluorogenic substrates for the retro-aldol reaction to detect the formation of the aldehyde by fluorescence increase, but the corresponding aldehydes are not fluorogenic aldehyde substrates for aldol reactions in this buffer. Of the 6-nitrogen-substituted 2-naphthylaldehydes **7**, **9**, and **11**, 6-pyrrolidyl-2-naphthaldehyde (**7**) showed the highest fluorescence, although 6-methoxy-2-naphthaldehyde (**1**) showed higher fluorescence than aldehyde **7**. It has been reported that the nitrogen lone electron pair of the *N*-phenylpyrrolidine is conjugated with the phenyl group but there is no such favored conjugation in *N*-phenylpiperidine.¹³ Higher fluorescence of **7** than that of **9** or of **11** in the buffer may be explained by the efficient conjugation between the aryl π and the N lone pair in **7**. Features of the conjugation of the N lone pair with aryl π vary depending on solvent¹⁴ and thus fluorescence features of these molecules vary depending on solvent (see below). Enone **13** showed fluorescence (entry 5 and Figure 1A), as did **14**.¹⁵ Under some conditions, formation of **13** from aldol **8** may be detected by fluorescence.

For the aldehyde **15** and aldol **16** pair, aldol **16** showed only weak fluorescence under the excitation and emission wavelengths (λ_{ex} 390 nm, λ_{em} 485 nm) optimal for the fluorescence of **15** (entry 8 and Figure 1B). On the other hand, aldol **16** showed fluorescence higher than that of aldehyde **15** at λ_{ex} 390 nm and λ_{em} 430 nm (entry 9 and Figure 1B). Therefore, aldehyde

15 and aldol **16** may be used as fluorogenic substrates for aldol and retro-aldol reactions, respectively, when the reactions are monitored at optimized emission wavelengths.

In contrast to the aldehydes described above, aldehyde **18** was not fluorescent; aldol **19** showed high fluorescence at λ_{ex} 300 nm and λ_{em} 365 nm (entry 11 and Figure 1C). Fluorescence of aldol **19** was more than 140-fold higher than that of aldehyde **18**. Although fluorescence of **18** was analyzed at a number of excitation and emission wavelengths, wavelengths where aldehyde **18** showed high fluorescence were not found. Like the **18** and **19** pair, aldehyde **20** was not fluorescent and aldol **21** showed high fluorescence (entry 12 and Figure 1D). At λ_{ex} 260 nm and λ_{em} 375 nm, aldol **21** showed more than 170- and 800-fold higher fluorescence than aldehyde **20** at 5 μM and 50 μM , respectively (entries 12 and 15). Because aldehyde **20** was essentially non-fluorescent in aqueous solution, the fluorogenic range of >800 for **21/20** was achieved. Note that aldehyde **22** and aldol **23** were both non-fluorescent.

For aldehyde **20**, the arylaldehyde moiety may quench the fluorescence of the methoxynaphthyltriazole moiety. The methoxynaphthyl, 1,2,3-triazole, and formylphenyl groups may not be on a single plane because of steric interaction between the aryl groups attached to the triazole ring; that is, there may be no π -conjugation between the formylphenyl group and methoxynaphthyl group in **20** and this may be why the formylphenyl moiety quenches the fluorescence. In the reported X-ray crystal structures of 4,5-diphenyl-1,2,3-triazole derivatives, one phenyl ring and the triazole group are almost on a single plane but the other phenyl ring is not on the plane, indicating that there is no π -conjugation between the two phenyl groups.¹⁵ Aldehyde **20** and aldol **21** should have conformations similar to these crystal structures, supporting that the methoxynaphthyl group does not have π -conjugation with the formylphenyl moiety in aldehyde **20**. In aldol **21**, no moiety quenches the fluorescence and this aldol is fluorescent. The aldol reaction of aldehyde **20** proceeded efficiently. The aldol reaction of aldehyde **20** was faster than that of aldehyde **18** in aqueous buffer under the same conditions (see below). These results also support that there is no π -conjugation between the methoxynaphthyl group and the formylphenyl moiety in aldehyde **20**. Note that aldehyde **15**, in which formylphenyl group is connected to the coumarin moiety with a single bond, showed high fluorescence. For aldehydes **15** and **17**, π -conjugation between the coumarin moiety and formylphenyl group is possible and this is presumably why these compounds are fluorescent.

Use of the 1,2,3-triazole moiety to connect the methoxynaphthyl group and the formylphenyl group in aldehyde **20** may be the key for preventing π -conjugation between the two aryl groups attached on the 4- and 5-positions of the 1,2,3-triazole. If *cis* olefin is used instead of the triazole ring, the *cis* olefin may be isomerized to *trans* olefin under the UV required for the fluorescence measurements.¹⁶ In the resulting *trans* form, fluorescence may be different from that of the *cis* form,¹⁶ thus the *cis-trans* isomerization may complicate analysis of the fluorescence data. With the 1,2,3-triazole group, there is no opportunity of such isomerization.

For aldehyde **18**, the arylaldehyde group may act as a quencher of the fluorescence of the 6-methoxynaphthylalkynyl moiety or 6-methoxynaphthylalkynylphenyl moiety. We initially expected that aldehyde **18** might emit fluorescence at longer wavelengths than aldehyde **1** because π -conjugation in **18** is longer than that in **1**. Interestingly, aldehyde **18** differed from our expectation; aldehyde **18** was not fluorescent and aldol **19** was highly fluorescent, as described above. Although reported crystal structures of 1,2-diphenylacetylene suggested strong π -conjugation between the two phenyl groups, some of the structures were distorted from a single plane, suggesting flexible rotation of the single bonds.¹⁷ For aldehyde **18**, frequent disruption of the π -conjugation between the formylphenyl moiety and the 6-methoxynaphthylalkynyl moiety by rotation of the single bonds may lead the fluorescence quenching.

For aldol **21**, the λ_{em} max of the fluorescence slightly shifted depending on pH; λ_{em} max was 370 nm at pH 5, 375 nm at pH 7, and 385 nm at pH 9 at λ_{ex} 260 nm (entries 12–14 and Figure 2). This may be related to the dependence of deprotonation of the triazole moiety on pH. Aldehyde **20** showed no fluorescence at pH 5, pH 9, or pH 7.0. Note that the λ_{em} max of the fluorescence of aldehyde **1** was unchanged over the pH range from 5 to 9.³

Fluorogenic aldehyde **20** was soluble at 50 μM or 100 μM in aqueous buffers containing small amounts of water-miscible organic solvents. The triazole group of **20** may contribute to the water solubility as **20** was more soluble than **18** in aqueous buffer. Aldehyde **20** was also more soluble than fluorogenic aldehyde **24**, which we previously reported^{2b} (Scheme 3), in aqueous solutions. The ratio of fluorescence intensities of **21/20** was more than 170 at 5 μM and was more than 800 at 50 μM as described above, a higher ratio than that of **25/24** (~80). Thus, fluorogenic aldehyde **20** is superior to **24** for monitoring the progress of aldol reactions in aqueous solutions with respect to the fluorescence increase upon aldol reaction and to the solubility of the aldehyde.

Fluorescence of the compounds was also analyzed in organic solvents (Table 2). For aldehydes **7**, **9**, and **11** and aldols **8**, **10**, and **12**, either aldehyde or aldol was fluorescent at 5 μM in organic solvents, such as DMSO or CH_3CN , depending on excitation and emission wavelengths (entries 1–8). However, the fluorescence of aldols **8**, **10**, and **12** did not correlate with concentration; the fluorescence of these aldols at 100 μM was not higher than that at 5 μM in DMSO at λ_{ex} 260 nm. Thus, aldehydes **7**, **9**, and **11** may be used for the detection of formation of aldol products only under certain conditions. For aldehydes **7**, **9**, and **11**, a higher concentration of the compound provided higher fluorescence in DMSO and in CH_3CN at λ_{ex} 375 nm or at λ_{ex} 360 nm. Thus, retro-aldol reactions of aldols **8**, **10**, and **12** that afford aldehydes **7**, **9**, and **11**, respectively, may be monitored more reliably by fluorescence increase than aldol reactions using aldehydes **7**, **9**, and **11**. Whereas aldehydes **9** and **11** showed moderate fluorescence compared to aldehyde **1** in aqueous buffer (Table 1, entries 6 and 7 versus entry 1; approximately 10-fold difference), these compounds showed similar levels of fluorescence in DMSO at selected excitation and emission wavelengths (Table 2, entries 1, 5, and 7; within 2-fold difference). These results may be related to the efficient conjugation between the aryl π and the amine nitrogen lone pair in organic solvents including DMSO.¹⁴ Compound **13** was also fluorescent in DMSO and the fluorescence of **13** differed from the fluorescence of aldehyde **7** and of aldol **8** (Table 2, entry 2); thus formation of **13** may be detected by an increase in fluorescence. For aldehyde **15** and aldol **16**, aldehyde **15** was fluorescent (entries 9 and 10). For aldehyde/aldol pairs **18/19** and **20/21**, the aldols were highly fluorescent (entries 11–14). Aldol **21** showed approximately 50–70-fold higher fluorescence than aldehyde **20** in DMSO, CH_3CN , and EtOAc.

Monitoring of the Progress of Aldol Reactions Using the Fluorogenic Aldehyde

To demonstrate the use of fluorogenic aldehyde **20**, a time-course of the aldol reaction of this aldehyde with acetone was monitored by fluorescence. Aldol reactions (100 μL -scale) were performed using 12.5 mM of amine catalyst, 50 μM or 100 μM of aldehyde **20**, and 5% (v/v) acetone (680 mM) in 5% DMSO-170 mM Na_2HPO_4 , pH 9 and the fluorescence was recorded at λ_{ex} 260 nm and λ_{em} 370 nm.¹⁸ The assay results are shown in Figure 3. Reaction progress was detected by an increase in fluorescence (Figure 3A, B). Pyrrolidine, a known catalyst of aldol reactions in water,^{5b,19} catalyzed the aldol reaction of **20** at pH 9. Reaction in the presence of DBU²⁰ was slower than the pyrrolidine-catalyzed reaction; differences in reaction rate were detected by fluorescence measurements. By monitoring the fluorescence of the catalyzed-aldol reactions of fluorogenic aldehyde **20**, activities of catalysts were ranked within 10 min to 60 min.

The assay system using aldol reaction of acetone and aldehyde **20** was also employed for the evaluation of a set of amines as aldol catalysts. Reactions were monitored over 60 min and initial velocities were determined based on fluorescence increase (Table 3).²¹ When 50 μ M of aldehyde **18**, instead of **20**, was used for the evaluation of the same set of catalysts under the same conditions, reactions were slower and 60 min was not sufficient time to rank the catalysts or to determine the velocities. Although aldehyde **18** may be useful for special cases, aldehyde **20** was more suitable than **18** for evaluation of catalysts. When the same catalysts were evaluated in the reaction of **20** in 5% DMSO-170 mM NaH₂PO₄-Na₂HPO₄, pH 7.0 or in 5% DMSO-170 mM NaH₂PO₄, pH 5, no significant formation of **21** was detected for any of the amines listed in Table 3. When the pyrrolidine-catalyzed reaction was performed in 5% DMSO-170 mM NaH₂PO₄-Na₂HPO₄, pH 7.5, the velocity was approximately 50-fold lower than that of the reaction at pH 9. For pyrrolidine to efficiently catalyze the aldol reaction in water, alkaline conditions were required, consistent with reported observations.^{5b,19} These results indicate that the fluorescence assay system using fluorogenic aldehyde **20** is useful for identification of aldol catalysts, ranking of activities of catalysts, and evaluation of reaction conditions. Although the fluorescence assay system using aldehyde **20** does not allow determination of stereochemistries of the products, superior catalysts based on the catalytic activity can be rapidly identified using this assay system.²² Selected catalysts with high activities may be further analyzed in detail. Methods of the determination of stereoselectivities (such as chiral phase HPLC) are generally slower and generate more waste than the fluorescence assay with **20**.^{23,24} Thus, the assay system using aldehyde **20** will reduce the time and energy required for the development of aldol catalysts.

Although development of efficient aldol catalysts is a topic of interest, design of desired aldol catalysts is difficult.^{3,4,5} Factors that affect the catalysis of aldol reactions are not completely understood.^{3,4,5} The fluorogenic aldehyde and the assay system reported here will make discovery of new aldol catalysts more efficient and will contribute to the study of the chemistry of aldol reactions.

Fluorogenic aldehyde **24**, which we previously reported, was useful for fluorescence-based monitoring of the aldol reaction and both the reduction to form the alcohol and the formation of the thiazolidine with cysteine.^{2b,25} Thus, fluorogenic aldehyde **20** may also be useful for monitoring the progress of these reactions and of other aldehyde transformations^{24d,26} in aqueous solutions.

Conclusions

We have developed a new fluorogenic aldehyde bearing 1,2,3-triazole moiety and have demonstrated that the aldehyde is useful for monitoring the progress of aldol reactions in aqueous solutions through an increase in fluorescence. Fluorogenic aldehyde **20** was essentially non-fluorescent and the aldol product, **21**, showed more than 800-fold higher fluorescence than the aldehyde in aqueous solutions. The fluorogenic aldehyde was useful for evaluation of aldol catalysts and reaction conditions. Fluorescence assays of aldol reactions using the fluorogenic aldehyde allowed ranking of a set of aldol catalysts within a short time. The fluorescence assay system using the aldol reaction of the fluorogenic aldehyde will be useful for the development of aldol catalysts.

For fluorogenic aldehyde **20**, the methoxynaphthyl, triazole, and formylphenyl groups may not be on a single plane and the formylphenyl group efficiently quenches the fluorescence of the methoxynaphthyltriazole moiety. This type of system for preparation of a fluorogenic reactant should be useful for the creation of fluorogenic molecules for monitoring other chemical transformations.

Experimental Section

Aldehyde 18

A mixture of 2-bromo-6-methoxynaphthalene (1.02 g, 4.30 mmol), 3-ethynylbenzaldehyde (**22**) (560 mg, 4.30 mmol), Ph_3P (56 mg, 0.22 mmol), PdCl_2 (9 mg, 0.05 mmol), and $\text{Cu}(\text{OAc})_2$ (10 mg, 0.05 mmol) in anhydrous triethylamine (30 mL) was refluxed for 6 h.²⁷ After being cooled to room temperature, the precipitate was filtered off, and the filtrate was concentrated in vacuo. The crude mixture was purified by flash column chromatography to give aldehyde **18** (380 mg, 31%) as a yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 10.0 (s, 1H), 8.01 (s, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.75-7.73 (m, 2H), 7.70 (d, J = 8.1 Hz, 2H), 7.55 (dd, J = 8.7 Hz, 1.7 Hz, 1H), 7.18 (dd, J = 8.7 Hz, 2.1 Hz, 1H), 7.12 (d, J = 2.1 Hz, 1H), 3.93 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 191.3, 158.5, 135.1, 134.4, 131.9, 131.7, 129.7, 129.5, 129.3, 128.7, 128.3, 126.9, 119.5, 117.2, 105.7, 94.2, 88.2, 55.2. HRMS: calcd for $\text{C}_{20}\text{H}_{15}\text{O}_2$ (MH^+) 287.1072, found 287.1076.

Aldehyde 20

A mixture of **18** (56 mg, 0.2 mmol), NaN_3 (52 mg, 0.8 mmol), and DMF (10 mL) was stirred at 100 °C for 6 h. The mixture was concentrated in vacuo, added to water, and extracted with EtOAc. Organic layers were combined, dried over MgSO_4 , filtered, concentrated, and purified by flash column chromatography to afford **20** (44 mg, 67%) as a pale yellow solid. ^1H NMR (400 MHz, CD_3OD) δ 9.97 (s, 1H), 7.91-7.88 (m, 3H), 7.82-7.69 (m, 4H), 7.49-7.47 (m, 1H), 7.27 (d, J = 2.4 Hz, 1H), 7.15 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 193.5, 160.0, 137.4, 136.2, 131.0, 130.6, 130.1, 129.5, 128.7, 128.6, 127.2, 120.6, 106.7, 55.8. HRMS: calcd for $\text{C}_{20}\text{H}_{16}\text{N}_3\text{O}_2$ (MH^+) 330.1237, found 330.1239.

Aldol 21

Aldol **21** was synthesized using pyrrolidine as a catalyst.¹⁹ The reaction was performed using acetone (0.5 mL), aldehydes **20** (40 mg, 0.12 mmol), and pyrrolidine (3.0 μL , 0.04 mmol) in H_2O (0.5 mL) to afford **21** (26 mg, 55%). ^1H NMR (400 MHz, CD_3OD) δ 7.84 (s, 1H), 7.70 (d, J = 8.8 Hz, 1H), 7.61 (d, J = 8.8 Hz, 1H), 7.46-7.44 (m, 3H), 7.35-7.33 (m, 2H), 7.19 (d, J = 2.0 Hz, 1H), 7.08 (dd, J = 8.8 Hz, 2.0 Hz, 1H), 5.10 (dd, J = 8.8 Hz, 4.4 Hz, 1H), 3.85 (s, 3H), 2.86 (dd, J = 16.0 Hz, 8.8 Hz, 1H), 2.73 (dd, J = 16.0 Hz, 4.4 Hz, 1H), 2.12 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 209.1, 158.1, 142.9, 134.3, 129.7, 128.6, 128.2, 127.2, 127.1, 126.3, 125.9, 119.2, 105.6, 69.5, 55.3, 51.7, 30.7. HRMS: calcd for $\text{C}_{23}\text{H}_{22}\text{N}_3\text{O}_3$ (MH^+) 388.1656, found 388.1660.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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24. To our knowledge, there are no fluorescence- or color-based assay methods to detect enantiomers of simple β-hydroxycarbonyl compounds. Although methods for the determination of enantiomeric ratios of some compounds by fluorescence- or color-based assays using small molecule probes have been developed, these methods often require relatively high concentrations of compounds of interest (i.e., products) compared to our assay method in which reaction progress is directly observed as an increase in fluorescence. The fluorescence-based assay methods for the determination of enantiomeric ratios based on selective interactions with enantiomers are not usually suited for use during the course of the chemical reaction while concentrations of the products vary. For fluorescence- or color-based assays of enantiomeric compounds, see: (a) Leung D, Folmer-Andersen JF, Lynch VM, Anslyn EV. *J Am Chem Soc* 2008;130:12318. [PubMed: 18714996] (b) Leung D, Anslyn EV. *J Am Chem Soc* 2008;130:12328. [PubMed: 18714993] (c) Reetz MT, Sostmann S. *Tetrahedron* 2001;57:2515. A high-throughput method for the sequential determination of enantioselectivity, yield, and conversion of starting material in reactions to form acetylated cyanohydrins has been developed; in this method, three enzymes were employed, including one enantioselective enzyme used to analyze an enantiomer of the product: (d) Hamberg A, Lundgren S, Penhoat M, Moberg C, Hult K. *J Am Chem Soc* 2006;128:2234. [PubMed: 16478176]
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Figure 1.

Fluorescence emission spectra. Compound (5 μ M) in 5% DMSO/50 mM NaH_2PO_4 - Na_2HPO_4 , pH 7.0. Open square, aldehyde; solid circle, aldol. (A) Aldehyde **7**, aldol **8**, and enone **13**; λ_{ex} 380 nm. (B) Aldehyde **15**, aldol **16**; λ_{ex} 390 nm. (C) Aldehyde **18**, aldol **19**; λ_{ex} 300 nm. (D) Aldehyde **20**, aldol **21**; λ_{ex} 260 nm.

Figure 2. Fluorescence emission spectra of **21** (5 μ M) in 5% DMSO/50 mM NaH_2PO_4 , pH 5, in 5% DMSO/50 mM NaH_2PO_4 - Na_2HPO_4 , pH 7.0, and in 5% DMSO/50 mM Na_2HPO_4 , pH 9.

Figure 3.

Fluorescence assay of amine-catalyzed aldol reaction of acetone and aldehyde **20**.¹⁸ Conditions: [amine catalyst] 12.5 mM, [aldehyde **20**] 100 μ M for (A) or 50 μ M for (B) and (C), [acetone] 5% (v/v) (680 mM) in 5% DMSO-170 mM Na_2HPO_4 , pH 9 in a 96-well plate at 26 $^\circ\text{C}$; a, reaction in the presence of pyrrolidine; b, reaction in the presence of DBU; c, reaction without catalyst (blank). RFU = relative fluorescence intensity. (A) Time course for 12 min at λ_{ex} 260 nm and λ_{em} 370 nm. (B) Time course for 60 min. (C) Emission spectra (λ_{ex} 260 nm) of reactions at 60 min.

Scheme 1.
Fluorogenic aldols, fluorescent aldehydes, and related compounds.

Scheme 2.
Fluorogenic aldehydes, fluorescent aldols, and related compounds.

Scheme 3.

A fluorogenic aldehyde and its fluorescent aldol product.

Table 1

Fluorescence of aldehydes and aldols in aqueous buffer^a

entry	λ_{ex} (nm)	λ_{em} (nm)	aldehyde			aldol or enone		
			compound	fluorescence intensity ^b	ϕ^c	compound	fluorescence intensity ^b	ϕ^c
1	315	450	1	7.6×10^2	0.56	3	<5	0.00
2	315	445	4	1.6×10^2	0.12	5	<5	0.00
3	319	445	6	90	0.06			
4 ^d	380	450	7	3.0×10^2	0.22	8	<5	0.00
5 ^e	380	535	7	1.0×10^2	0.07	13	<5	0.00
						8	<5	0.00
						13	40	0.03
6	365	530	9	80	0.06	10	<5	0.00
7	355	520	11	55	0.04	12	<5	0.00
8	390	485	15	7.0×10^2	0.51	16	50	0.04
9	390	430	15	11	—	16	2.3×10^2	0.17
10	325	450	17	60	0.04			
11	300	365	18	<5	0.00	19	7.2×10^2	0.53
12	260	375	20	<5	0.00	21	8.8×10^2	0.64
13 ^f	260	370	20	<5	0.00	21	8.8×10^2	0.64
14 ^g	260	385	20	<5	0.00	21	8.8×10^2	0.64
15 ^h	260	375	20	<5	0.00	21	4.2×10^3	

^aThe fluorescence was recorded on a microplate spectrophotometer using 100 μL of a 5 μM solution in 5% DMSO/50 mM sodium phosphate, pH 7.0 in a 96-well plate at 26 °C.

^bRelative fluorescence intensity after background correction.

^cRelative to 2-aminopyridine in 0.1 N H₂SO₄ as a standard (λ_{ex} 300 nm, $\phi = 0.60$).

^dWavelengths for detection of formation of **7** from **8**.

^eWavelengths for detection of formation of **13** from **8**.

^fIn 5% DMSO/50 mM NaH₂PO₄, pH 5.

^gIn 5% DMSO/50 mM Na₂HPO₄, pH 9.

h_{C} Compound concentration 50 μM .

Table 2

Fluorescence of aldehydes and aldols in organic solvents^a

entry	solvent	λ_{ex} (nm)	λ_{em} (nm)	aldehyde			aldol or enone		
				compound	fluorescence intensity ^b	ϕ^c	compound	fluorescence intensity ^b	ϕ^c
1 ^d	DMSO	375	470	7	8.0×10^2	0.59	8	<5	0.00
2 ^e	DMSO	375	555	7	<5	0.00	13	<5	0.00
3	DMSO	260	420	7	<5	0.00	8	<10	<0.01
4	CH ₃ CN	375	475	7	1.2×10^3	0.88	13	1.1×10^3	0.81
5	DMSO	375	470	9	1.2×10^3	0.88	8	2.0×10^3	—
6	DMSO	260	420	9	1.2×10^2	0.09	13	<5	0.00
7	DMSO	360	475	11	6.2×10^2	0.45	8	15	0.01
8	DMSO	260	415	11	30	0.02	10	60	0.04
9	DMSO	375	460	15	1.5×10^3	1.1	10	2.2×10^3	—
10	CH ₃ CN	375	455	15	1.7×10^3	1.2	12	60	0.04
11	DMSO	275	370	18	<50	<0.04	12	1.3×10^3	—
12	DMSO	260	370	20	<15	<0.01	16	<5	0.00
13	CH ₃ CN	260	370	20	12	0.01	16	<10	<0.01
14	EtOAc	260	370	20	12	0.01	19	2.8×10^3	2.0
							21	7.0×10^2	0.51
							21	8.3×10^2	0.60
							21	7.5×10^2	0.55

^aThe fluorescence was recorded on a microplate spectrophotometer using 100 μL of a 5 μM solution in DMSO or in 5% DMSO/indicated solvent in a 96-well polypropylene plate at 26 $^\circ\text{C}$.

^bRelative fluorescence intensity after background correction.

^cRelative to 2-aminopyridine in 0.1 N H₂SO₄ as a standard (λ_{ex} 300 nm, $\phi = 0.60$).

^dWavelengths for detection of formation of 7 from 8.

^eWavelengths for detection of formation of 13.

Table 3Velocities of Aldol Reactions of Acetone and **20** Determined by Fluorescence Assays^a

entry	catalyst	velocity ($\mu\text{M}/\text{min}$)
1	pyrrolidine	0.10 (0.002 ^b)
2	prolinamide	0.08
3	2,3-diaminopropionic acid	0.04
4	proline	0.03
5	DBU	0.02
6	2-aminoethanol	0.01
7	each catalyst listed in footnote ^c	<0.01
8	– (blank)	0.004

^aConditions: [catalyst] 12.5 mM, [acetone] 5%(v/v) (680 mM), [aldehyde **20**] 50 μM in 5% DMSO-170 mM Na_2HPO_4 , pH 9. Fluorescence of 100 μL -scale reactions was analyzed at λ_{ex} 260 nm and λ_{em} 370 nm in a 96-well plate.²¹ Deviations of the velocities were $\pm 20\%$ in duplicated experiments.

^bData of the reaction performed in 5% DMSO-170 mM NaH_2PO_4 - Na_2HPO_4 , pH 7.5.

^cPiperidine, morpholine, pyridine, imidazole, Et_2NH , $n\text{-BuNH}_2$, Et_3N , $i\text{-Pr}_2\text{NEt}$, and DABCO.