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Fluorescent Detection of Carbon−Carbon Bond Formation

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Abstract: We have developed a new spectroscopic system for detecting carbon−carbon bond formation by fluorescence to enhance high-throughput catalyst screening and rapid characterization of catalysts on a small scale. Fluorogenic substrates composed of a fluorophore possessing an amino group are readily prepared as amides of α,β-unsaturated carbonyl compounds and generally exhibit low fluorescence, while Michael or Diels−Alder reactions of these fluorogenic substrates provide products of significantly increased fluorescence. The product's fluorescence is approximately 20- to 100-fold higher than that of the substrate. The assay system was validated by screening potential catalysts of the Michael reaction and in solvent optimization experiments. The covalent combination of fluorophores possessing an amino group with α,β-unsaturated carbonyl compounds should provide a diverse range of fluorogenic substrates that may be used to rapidly screen catalysts and to optimize reaction conditions.

Introduction

Spectroscopic monitoring of the progress of chemical reactions enhances high-throughput catalyst screening and rapid characterization of catalysts on a small scale.[1] Although many fluorogenic or chromogenic substrates exist to monitor cleavage reactions including retro-aldol reactions[2] (cleavage of C−C bonds), the development of spectroscopic methods to monitor the progress of C−C bond formation has lagged behind.[3] Fluorogenic substrates for cleavage reactions often possess masked fluorophores with a cleavable moiety, after which the cleavage reactions restore the fluorescence of fluorophores by removing that masking moiety. Such substrates typically are not available for the reverse reactions, that is, bond forming reactions in solution. This is because when a bond forming reaction is performed by using the fluorophore as the substrate, the resulting small decrease in fluorescence (consumption of the fluorophore) is difficult to detect at the initial stage of the reaction because of the fluorophore's intense fluorescence. Therefore, alternative strategies are necessary for detection of bond formation by fluorescence. Here, we report a spectroscopic system for detecting C−C bond formation that results in an increase in fluorescence and apply this system to key reactions in synthetic organic chemistry, Michael and Diels−Alder reactions. Because fluorogenic thiol reagents, for example, *N*-[*p*-(2-benzimidazolyl)phenyl]maleimide (BIPM) (**1**), have been reported as useful for analyzing carbon−sulfur bond formation,[4] and BIPM is nonfluorescent whereas its thiol-Michael adducts are fluorescent, we used these types of compounds to develop systems to detect C−C bond formation in solution.

Results and Discussion

In our initial experiment, we performed the Michael reaction of **1** with acetone using proline as the catalyst in DMSO (Scheme 1).[5] Product **2** was generated, and the fluorescence of **2** was compared to that of **1** (Figures 1−3). We found that C−C bond formation with the maleimide moiety of **1** provided a remarkable increase in fluorescence. When the fluorescence was analyzed in 5% CH_3CN−47.5 mM Na phosphate (pH 7.0) by using an excitation wavelength of 315 nm (λex 315 nm), the fluorescence intensity of **2** (1 μM) was about 100-fold higher than that of **1** (1 μM) at the emission wavelength of 365 nm (λem 365 nm).[6] No variation of the fluorescence of **2** was

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(2) (a) Zhong, G.; Shabat, D.; List, B.; Anderson, J.; Shinha, S. C.; Lerner, R. A.; Barbas, C. F., III. *Angew. Chem., Int. Ed.* **1998**, *37*, 2481. (b) List, B.; Barbas, C. F., III; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *95*, 15351. (c) Jourdain, N.; Perez Carlson, R.; Reymond, J.-L. *Tetrahedron Lett.* **1998**, *39*, 9415. (d) Carlson, R. P.; Jourdain, N.; Reymond, J.-L. *Chem.-Eur. J.* **2000**, *6*, 4154. (e) Tanaka, F.; Lerner, R. A.; Barbas, C. F., III. *J. Am. Chem. Soc.* **2000**, *122*, 4835. (f) Tanaka, F.; Kerwin, L.; Kubitz, D.; Lerner, R. A.; Barbas, C. F., III. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2983.

(3) (a) Fluorescence-based assay for C−C bond formation using a solid support: Shaughnessy, K. H.; Kim, P.; Hartwig, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 2123. (b) C−C bond formation based on fluorescence resonance energy transfer: Stauffer, S. R.; Beare, N. A.; Stambuli, J. P.; Hartwig, J. F. *J. Am. Chem. Soc.* **2001**, *123*, 4641. Because decreases in fluorescence were monitored, an aliquot from the reaction mixture was diluted, and the yield was determined after a measurable amount of the product was generated (>10%). (c) Examples of spectroscopic and visual catalyst screening for bond forming reactions (other than C−C bond formation): Cooper, A. C.; McAlexander, L. H.; Lee, D.-H.; Torres, M. T.; Crabtree, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 9971. Taylor, S. J.; Morken, J. P. *Science* **1998**, *280*, 267. Reetz, M. T.; Becker, M. H.; Kuhling, K. M.; Holzwarth, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647. Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 4306. Harris, R. F.; Nation, A. J.; Copland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 11270. Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 6496. Francis, M. B.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 937. Yeung, E. S.; Su, H. *J. Am. Chem. Soc.* **2000**, *122*, 7422.

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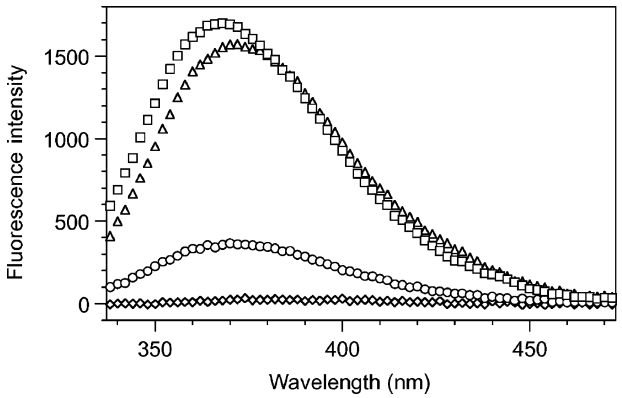
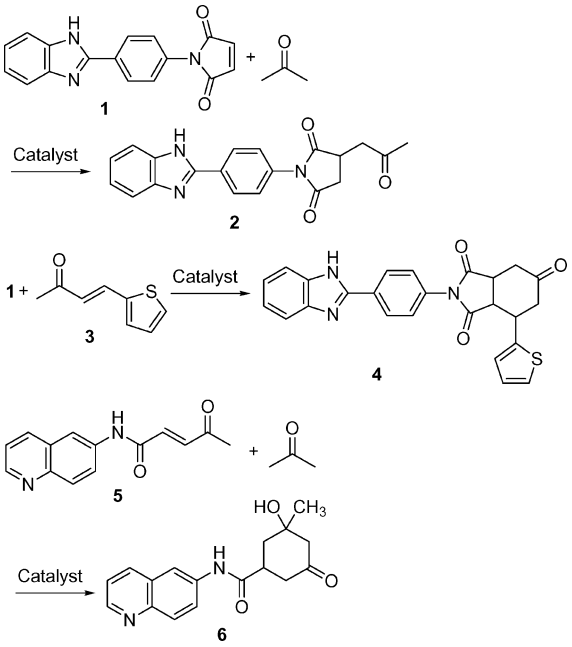


Figure 1. Fluorescence emission spectra (λex 315 nm) of **1**, **2**, and **4** in 5% CH_3CN−47.5 mM Na phosphate (pH 7.0).[6] ◇, **1** (1 μM), □, **2** (1 μM); △, **4** (5 μM); ○, **4** (1 μM).

Scheme 1



observed upon a change in pH, between pH 6.5 and 8.0. This difference in fluorescence between **1** and **2** was also observed in organic solvents, for example, DMSO, DMF, 2-PrOH, CH_2Cl_2, Et_2O, and EtOAc.

To analyze the reliability of this fluorescence detection system, the reaction of **1** with acetone to provide **2** was performed with a set of catalysts and reaction conditions. The

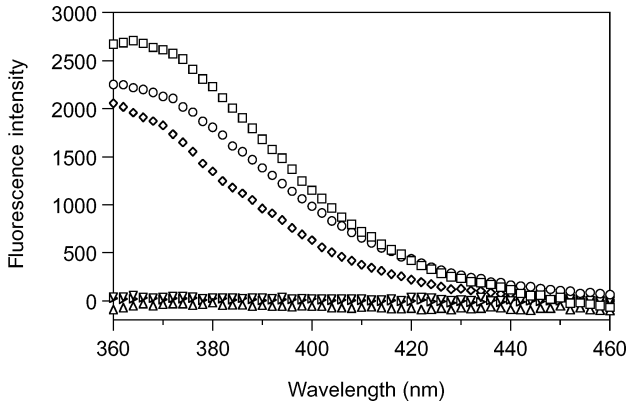


Figure 2. Fluorescence emission spectra (λex 315 nm) of **1** (1 μM) and **2** (1 μM) in organic solvents.[6] □, **2** in 1% CH_3CN/DMSO; ○, **2** in 1% CH_3CN/DMF; ◇, **2** in 1% CH_3CN/2-PrOH; △, **1** in the same solvents as those for **2**.

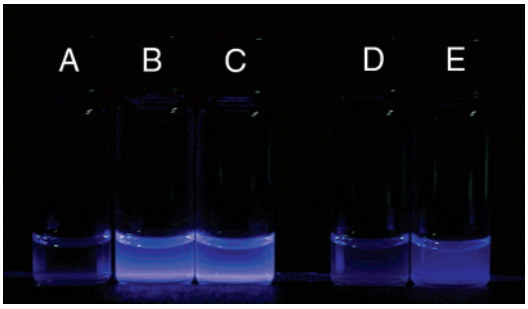


Figure 3. Photograph of solutions of **1**, **2**, **4**, **5**, and **6** (250 μM in 5% CH_3CN/2-PrOH) irradiated with a UV lamp (312 nm). A, **1**; B, **2**; C, **4**; D, **5**; E, **6**.

reaction was followed by both fluorescence and HPLC. Because proline catalyzed the reaction in DMSO, a series of amino acids were used for the reaction. The reactions were performed with amino acid (5 mM) and **1** (50 μM) in 20% acetone−0.5% CH_3CN−5% H_2O−74.5% DMSO, and the initial velocities were determined by HPLC (formation of **2**) and by the increase in fluorescence (λex 315 nm, λem 365 nm). Because free amino acids are not soluble in DMSO, amino acid solutions in H_2O were added to the reaction mixture to employ the same conditions for all amino acids. The results are shown in Figure 4. The reaction with arginine showed the highest initial velocity among the 12 reactions analyzed by both HPLC and fluorescence. The second group of catalysts resulting in an increase in fluorescence included glycine, proline, or lysine, although these reactions showed a much slower fluorescence increase than that of the reaction with arginine. The HPLC assay also ranked glycine and lysine in the second most active group of amino acid catalysts for the formation of **2**. The HPLC assay indicated that the velocity of the reaction with proline was slower than that of the reactions with glycine or lysine. Although these reaction profiles in DMSO−H_2O were different from those of the reactions in DMSO in the absence of H_2O, that is, arginine was a poor catalyst in neat DMSO, the fluorescence assays closely correlated with the HPLC assays. These results indicate that the fluorescence assay is useful in ranking the relative velocities with which a family of molecules catalyzes a particular reaction. In addition, this fluorescence assay is significantly faster than the HPLC assay. The fluorescence assay requires 10−20 min to determine the initial velocities of an entire set of

(5) Examples of amine-catalyzed Michael reactions of carbonyl compounds: Yamaguchi, M.; Shiraishi, T.; Hirama, M. *J. Org. Chem.* **1996**, *61*, 3520. Yamaguchi, M.; Shiraishi, T.; Hirama, M. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1176. Yamaguchi, M.; Shiraishi, T.; Igarashi, Y.; Hirama, M. *Tetrahedron Lett.* **1994**, *35*, 8233. Yamaguchi, M.; Igarashi, Y.; Reddy, R. S.; Shiraishi, T.; Hirama, M. *Tetrahedron* **1997**, *53*, 11223. Hanessian, S.; Pham, V. *Org. Lett.* **2000**, *2*, 2975. Sakthivel, K.; Notz, W.; Bui, T.; Barbas, C. F., III. *J. Am. Chem. Soc.* **2001**, *123*, 5260. Bui, T.; Barbas, C. F., III. *Tetrahedron Lett.* **2000**, *41*, 6951. Betancort, J. M.; Barbas, C. F., III. *Org. Lett.* **2001**, *3*, 3737. Betancort, J. M.; Sakthivel, K.; Thayumanavan, R.; Barbas, C. F., III. *Tetrahedron Lett.* **2001**, *42*, 4441. List, B.; Pojarliev, P.; Martin, H. J. *Org. Lett.* **2001**, *3*, 2423. Enders, D.; Seki, A. *Synlett* **2002**, 26. Halland, N.; Hazell, R. G.; Jorgensen, K. A. *J. Org. Chem.* **2002**, *67*, 8331. Alexakis, A.; Andrey, O. *Org. Lett.* **2002**, *4*, 3611. Paras, N. A.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2002**, *124*, 7894. Brown, S. P.; Goodwin, N. C.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2003**, *125*, 1192. Halland, N.; Aburel, P. S.; Jorgensen, K. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 661.

(6) Fluorescence spectra were recorded on Spectra Max Gemini (Molecular Devices) using 100 μL of the solution in a 96-well plate (Costar 3915) at 25 °C. The data are shown after background correction.

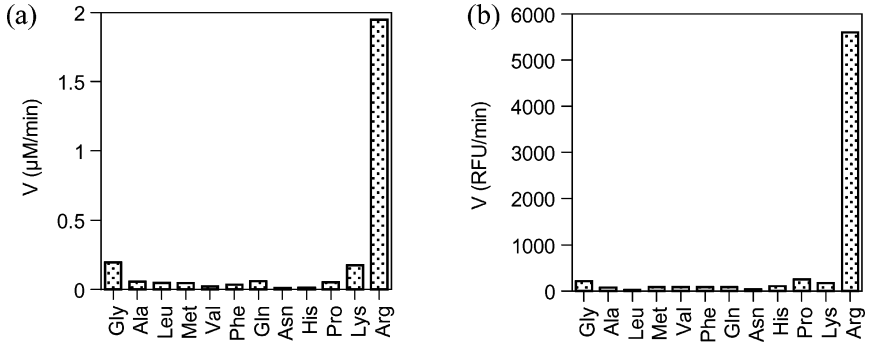


Figure 4. Comparison of the initial velocities of the reaction of **1** and acetone in the presence of amino acids. (a) HPLC assay (analysis of formation of **2**) and (b) fluorescence assay (λex 315 nm, λem 365 nm). Reaction conditions: [amino acid] 5 mM, [acetone] 20%(v/v) (2.7 M), [**1**] 50 μM in 0.5% CH_3CN−5% H_2O−74.5% DMSO. RFU = relative fluorescence unit.

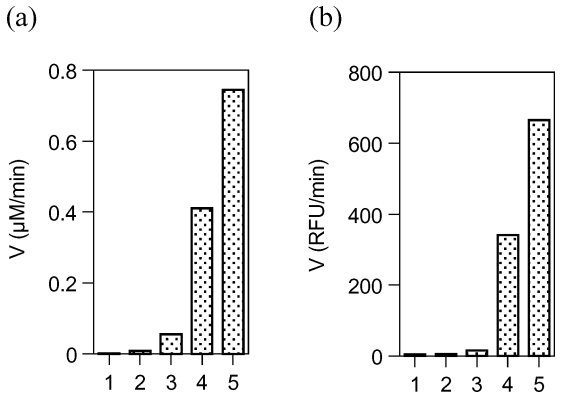


Figure 5. Comparison of the initial velocities of the reaction of **1** and acetone with glycine in H_2O−DMSO. (a) HPLC assay (analysis of formation of **2**) and (b) fluorescence assay (λex 315 nm, λem 365 nm). Reaction conditions: [glycine] 2 mM, [acetone] 20%(v/v) (2.7 M), [**1**] 50 μM in 0.5% CH_3CN−79% H_2O (column 1), in 0.5% CH_3CN−52% H_2O−27.5% DMSO (column 2), in 0.5% CH_3CN−22% H_2O−57.5% DMSO (column 3), in 0.5% CH_3CN−7% H_2O−72.5% DMSO (column 4), in 0.5% CH_3CN−2% H_2O−77.5% DMSO (column 5).

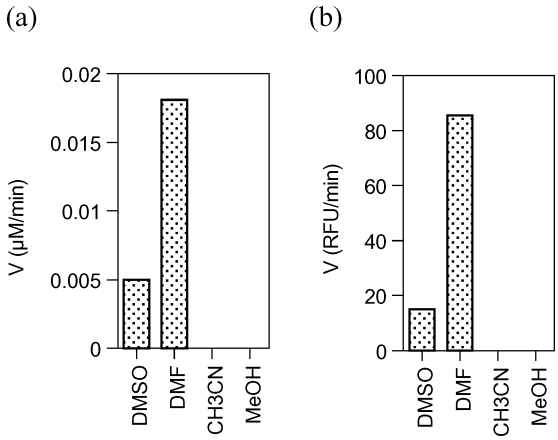


Figure 6. Comparison of the initial velocities of the reaction of **1** and acetone with L-proline in a set of solvents. (a) HPLC assay (analysis of formation of **2**) and (b) fluorescence assay (λex 315 nm, λem 365 nm). Reaction conditions: [L-proline] 25 μM, [acetone] 10%(v/v) (1.36 M), [**1**] 50 μM in DMSO, DMF, CH_3CN, or MeOH.

reactions, while the HPLC assay requires 1.5−2 h to acquire similar data for a single reaction.

Because the reaction medium had an effect on the velocity of these reactions, the reaction with glycine was performed under a variety of mixed solvent conditions, H_2O−DMSO (Figure 5). Reactions in DMSO with 7% and 2% H_2O (columns 4 and 5) were dramatically faster than the reactions in DMSO with 22% (column 3) or 52% H_2O (column 2). These reactions were also faster than reactions in H_2O without DMSO (column 1). Results in the fluorescence assay were again correlated to those of the HPLC assay. Although the fluorescence intensity of product **2** in DMSO is ∼1.5-fold higher than that in buffer, the fluorescence assay allowed the reaction velocities to be quickly ranked. The standardization of the fluorescence in a variety of solvents is simple and provides for a more accurate ranking. Thus, solvent conditions can also be optimized using this approach. When the reaction with proline was performed in a set of solvents, the fluorescence assay showed that the reaction was faster in DMF (Figure 6).

The reaction of **1** with acetone to provide **2** was also catalyzed by small amines, 2-pyrrolidinemethanol and 1-(2-pyrrolidinylmethyl)pyrrolidine in DMSO. The fluorescence assay of the reactions with these amines provided an increase in fluorescence when the reaction was performed by using **1** (25 μM) and amine (50 mM) in 10% acetone/DMSO. On the other hand, the reactions with pyrrolidine, triethylamine, and proline methyl ester hydrochloride showed no increase in fluorescence, and these amines did not catalyze the reaction.

Aldolase peptides[7] and nonaldolase peptides were also used for the reaction of **1** with acetone to assess the fluorescence assay. The reactions were performed by using peptide (100 μM) and **1** (400 μM) in 5% acetone (680 mM)−1% CH_3CN−1% DMSO−40 mM Na phosphate (pH 7.0) and were analyzed by both HPLC and fluorescence. The results are shown in Figure 7. Peptides FT-YLK3, FT-YLK3-NAc, FT-YLK3-23S, and FT-YLK25 catalyzed the reaction efficiently, and the acceleration of the reactions was detected by both HPLC and fluorescence. The low activity of FT-YLK3-R5 observed by HPLC was not detected by the fluorescence assay. In the peptide-catalyzed reactions, the order of the velocities observed in HPLC assays did not exactly match the results of the fluorescence assays. Noncovalent binding interactions between the peptides and **1** may result in fluorescence changes in either direction. Additionally, peptides FT-YLK3, FT-YLK3-NAc, and FT-YLK3-R5 have a cysteine residue in their sequences. The thiol group of peptide FT-YLK3 reacts to form a disulfide bond in solution, but the reaction is incomplete and some free thiol groups remain.[7a] The reaction of **1** with the remaining thiols may occur, and the fluorescence assay cannot discriminate between C−C bond formation and C−S bond formation. Although the

(7) (a) Tanaka, F.; Barbas, C. F., III. *Chem. Commun.* **2001**, 769. (b) Tanaka, F.; Barbas, C. F., III. *J. Am. Chem. Soc.* **2002**, *124*, 3510.

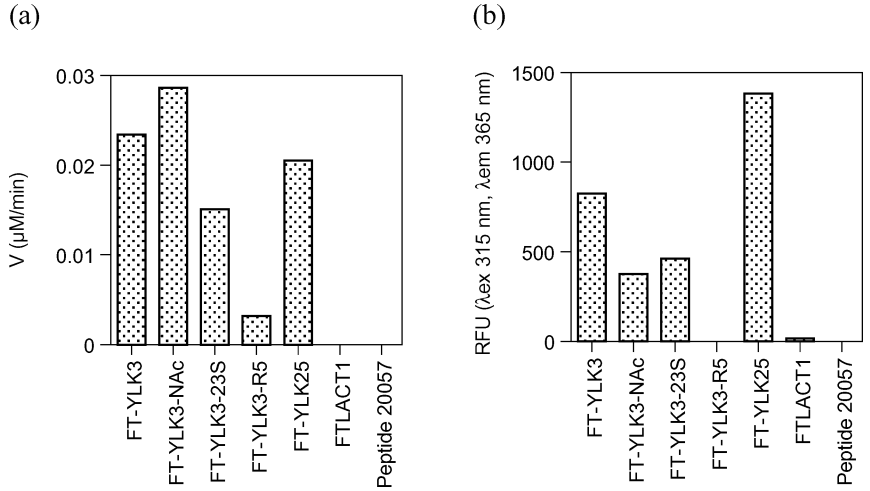


Figure 7. Peptide-catalyzed reaction of **1** and acetone. (a) HPLC assay (analysis of formation of **2**) and (b) fluorescence assay (relative fluorescence unit above the background at 25 min). Reaction conditions: [peptide] 100 μM, [acetone] 5%(v/v) (680 mM), [**1**] 400 μM in 1% CH_3CN−1% DMSO−40 mM Na phosphate (pH 7.0). Peptide sequences are following: FT-YLK3, YKLLKELLAKLKWLLRKLLGPTCL-NH_2; FT-YLK3-NAc, Ac-YKLLKELLAK-LKWLLRKLLGPTCL-NH_2; FT-YLK3-23S, YKLLKELLAKLKWLLRKLLGPTSL-NH_2; FT-YLK3-R5, YRLLRELLARLRWLLRRLLGPTCL-NH_2; FT-YLK25, SPFLGQYKLLKELLAKLKWLLRKL-NH_2; FTLACT1, MSTFLVL-NH_2; Peptide 20057, CPEKSKLQEIYQELTQLKAAVGEL.

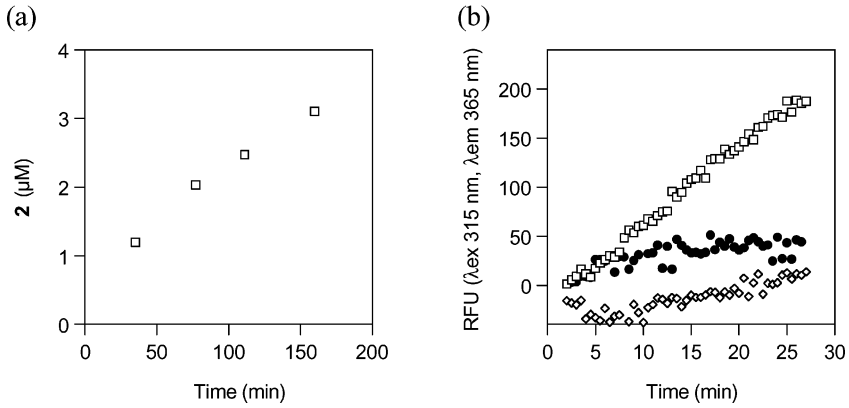


Figure 8. Antibody 38C2-catalyzed Michael addition to form **2**.[9] (a) HPLC assay (analysis of formation of **2**). Conditions: [antibody] 20 μM (active site), [**1**] 500 μM, [acetone] 5%(v/v) (680 mM), 5% CH_3CN/PBS (pH 7.4). (b) Fluorescence assay (λex 315 nm, λem 365 nm).[6] The same reaction conditions were used except the concentration of **1** (50 μM). □, 38C2; ◇, nonaldolase antibody IgG (control); ●, the reaction with 38C2 in the absence of acetone.

observed increase in fluorescence may include components corresponding to side reactions or noncovalent binding interactions, the fluorescence assay was useful to approximately estimate the rate of the peptide-catalyzed reactions. When a catalyst containing (a) thiol group(s) is studied, fluorescence contributed by C−S bond formation must be taken into account. Fluorescence contributed by the reaction of the thiol is, however, significantly lower than that contributed by catalytic C−C bond formation reactions because only an equivalent of fluorescent product is provided by the stoichiometric reaction of the catalyst's thiol group(s). Controlling for fluorescence increases attributed by such artifacts may be a key to developing more accurate rankings of catalysts in some systems.

To test the application of our system with a third class of catalysts, aldolase antibody 38C2[8] was studied. Antibody 38C2 was examined as a catalyst in the reaction of **1** with acetone to provide **2**, and the reaction was followed by HPLC and fluorescence (Figure 8). An increase in fluorescence was observed for the catalyzed reaction, and HPLC assay showed that antibody 38C2 catalyzed the desired reaction.[9] This constitutes a new reaction for catalytic antibody 38C2.

Next, we examined the applicability of the fluorescent detection system by using **1** for other reactions. The Diels−Alder reaction of **1** with α,β-unsaturated ketone **3** was performed in the presence of proline,[10] and the fluorescence of Diels−Alder product **4** was analyzed. Like the Michael product **2**, the Diels−Alder product **4** showed significant fluorescence, indicating that this assay can be used to continuously monitor C−C bond formation in the Diels−Alder reaction (Figures 1 and 3, and Supporting Information). Thus, fluorescence assays based on compound **1** are useful for reactions that use carbon nucleophiles in addition to the in situ-generated enamine of acetone.

(8) (a) Wagner, J.; Lerner, R. A.; Barbas, C. F., III. *Science* **1995**, *270*, 1797. (b) Barbas, C. F., III; Heine, A.; Zhong, G.; Hoffmann, T.; Gramatikova, S.; Bjornestedt, R.; List, B.; Anderson, J.; Stura, E. A.; Wilson, I. A.; Lerner, R. A. *Science* **1997**, *278*, 2085. (c) Hoffmann, T.; Zhong, G.; List, B.; Shabat, D.; Anderson, J.; Gramatikova, S.; Lerner, R. A.; Barbas, C. F., III. *J. Am. Chem. Soc.* **1998**, *120*, 2768. (d) Tanaka, F.; Barbas, C. F., III. *J. Immunol. Methods* **2002**, *269*, 67.

(9) Hydrolysis of **1** to generate 4-(1*H*-benzimidazol-2-yl)aniline, a fluorophore, contributes to an increase of fluorescence in the background reaction. In the HPLC assay of these reactions without antibody (background) and with nonaldolase antibody (control), no generation of **2** was detected (below the detection limit).

(10) Thayumanavan, R.; Dhevalapally, B.; Sakthivel, K.; Tanaka, F.; Barbas, C. F., III. *Tetrahedron Lett.* **2002**, *43*, 3817. Ramachary, D. B.; Chowdari, N. S.; Barbas, C. F., III. *Tetrahedron Lett.* **2002**, *43*, 6743.

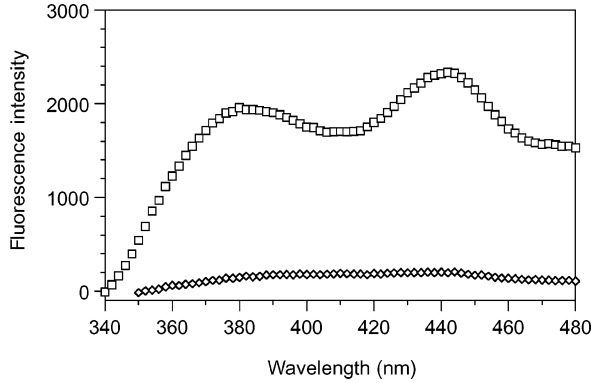


Figure 9. Fluorescence emission spectra (λex 254 nm) of **5** and **6** in 5% CH_3CN−47.5 mM Na phosphate (pH 7.0).[6] ◇, **5** (10 μM); □, **6** (10 μM).

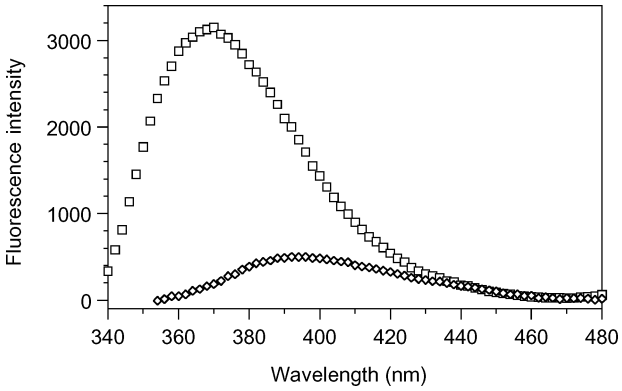
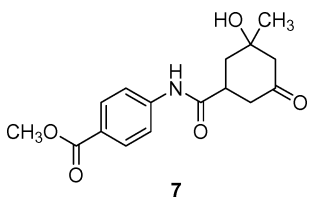


Figure 10. Fluorescence emission spectra (λex 254 nm) of **5** and **6** in 5% CH_3CN/2-PrOH.[6] ◇, **5** (10 μM); □, **6** (10 μM).

To examine the scope of our design to other fluorogenic substrates, an additional fluorogenic substrate **5**, which includes the fluorophore 6-aminoquinoline, was synthesized and studied. We found that the system was readily expanded to other fluorogenic substrates. The reaction of **5** with acetone in the presence of (2-pyrrolidinylmethyl)pyrrolidine in 2-PrOH provided **6** as the main product, formed by a Michael addition followed by an intramolecular aldol reaction. Compound **6** showed 19- and 28-fold higher fluorescence intensity than **5** at λem 364 nm (λex 254 nm) in Na phosphate buffer (pH 7.0) and in 2-PrOH, respectively (Figures 9 and 10). The fluorescence of **6** was different from that of 6-aminoquinoline.[11,12] Compound **7**, which does not include the quinoline structure, showed no fluorescence. When the mixture of **5** (500 μM) and amine (2 mM) in 10% acetone/2-PrOH was monitored by fluorescence (λex 254 nm, λem 365 nm), the intensity of fluorescence increased during the reaction catalyzed by 1-(2-pyrrolidinylmethyl)pyrrolidine. No fluorescence increase was observed when triethylamine was studied as a potential catalyst, indicating that triethylamine is not a catalyst for this reaction.



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Conclusion

We have demonstrated direct fluorescent monitoring of the progress of C−C bond forming reactions in solution. Fluorogenic substrates composed of a fluorophore possessing an amino group are readily prepared as amides of α,β-unsaturated carbonyl compounds and are generally poorly fluorescent, while the Michael or Diels−Alder reaction products of these substrates exhibit substantially enhanced fluorescence. Our system is applicable to reactions with protein catalysts, peptide catalysts, small molecule catalysts, and possibly with other types of catalysts.[13] The covalent combination of fluorophores possessing an amino group with α,β-unsaturated carbonyl compounds should provide a diverse range of fluorogenic substrates for the spectroscopic detection of designer reactions and catalysts.

Experimental Section

Fluorescence Spectra. Fluorescence spectra were recorded on Spectra Max Gemini (Molecular Devices) using 100 μL of the solution in a 96-well plate (Costar 3915) at 25 °C. The data are shown after background correction.

Assay for Figure 4. Reactions were initiated by adding 1 μL of a stock solution of substrate **1** (5 mM) in CH_3CN−DMSO (1:1) to a mixture of acetone (20 μL), 5 μL of L-amino acid solution (100 mM) in H_2O, and DMSO (74 μL) at 25 °C. Final concentrations: [amino acid] 5 μM, [acetone] 20%(v/v) (2.7 M), [**1**] 50 μM in 0.5% CH_3CN−5% H_2O−74.5% DMSO. Product **2** was measured by HPLC detection with a 10 μL injection of the reaction mixture. The analytical HPLC was performed on a Hitachi L-7100 equipped with an L-7400 UV detector, using a Microsorb-MV C18 analytical column (Varian) eluted with CH_3CN/0.1% aqueous TFA (15:85) at a flow rate of 1.0 mL/min, with detection at 254 nm. The retention times of **1** and **2** are 16.3 and 13.4 min, respectively. Fluorescence assay was performed at an excitation wavelength of 315 nm and an emission wavelength of 365 nm in a 96-well plate (Costar 3915) on Spectra Max Gemini (Molecular Devices).

Assay for Figure 5. Reactions were initiated by adding 1 μL of a stock solution of substrate **1** (5 mM) in CH_3CN−DMSO (1:1) to a mixture of acetone (20 μL), 2 μL of glycine solution (100 mM) in H_2O, and H_2O (77 μL) at 25 °C for column 1 experiments. For column 2 experiments, a mixture of H_2O (50 μL) and DMSO (27 μL) was used instead of H_2O (77 μL) for column 1 experiments. For column 3 experiments, a mixture of H_2O (20 μL) and DMSO (57 μL) was used; for column 4 experiments, a mixture of H_2O (5 μL) and DMSO (72 μL) was used; for column 5 experiments, DMSO (77 μL) was used instead of H_2O (77 μL) for column 1 experiments. Final concentrations: [glycine] 2 mM (active site), [acetone] 20%(v/v) (2.7 M), [**1**] 50 μM.

Assay for Figure 6. Reactions were initiated by adding 5 μL of a stock solution of substrate **1** (1 mM) in CH_3CN to a mixture of acetone

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(12) The fluorescence emission of 6-aminoquinoline is 400−650 nm (ref 11 and Supporting Information). When the formation of **6** is detected by fluorescence measurement at emission wavelengths shorter than 390 nm, the increase in fluorescence can exclude the effect of 6-aminoquinoline that may be generated by aryl amide hydrolysis of **5** in buffer.
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(10 μL), 0.5 μL of L-proline (5 mM in CH_3CN−MeOH (1:1)), and 81.5 μL of solvent indicated (DMSO, DMF, CH_3CN, or MeOH). Final concentrations: [L-proline] 25 μM, [acetone] 10%(v/v) (1.36 M), [**1**] 50 μM in 5.25% CH_3CN−0.25% MeOH−81.5% solvent indicated in the graph.

Assay for Figure 7. Reactions were initiated by adding 2 μL of a stock solution of substrate **1** (20 mM) in CH_3CN−DMSO (1:1) to a mixture of acetone (5 μL), 5 μL of peptide (1 mM) in H_2O, H_2O (3 μL), and 50 mM Na phosphate (pH 7.0) (80 μL) at 25 °C. Final concentrations: [peptide] 100 μM, [acetone] 5%(v/v) (680 mM), [**1**] 400 μM in 1% CH_3CN−1% DMSO−40 mM Na phosphate (pH 7.0).

Assay for Figure 8. (a) HPLC assay. Reactions were initiated by adding 5 μL of a stock solution of substrate **1** (10 mM) in CH_3CN to a mixture of acetone (5 μL) and 90 μL of an antibody solution in PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) at 25 °C. Final concentrations: [antibody] 20 μM (active site), [**1**] 500 μM, [acetone] 5%(v/v) (680 mM), 5% CH_3CN/PBS (pH 7.4). Product **2** was measured by HPLC detection with a 5 μL injection of the reaction mixture. (b) Fluorescence assay. Reactions were initiated by adding 5 μL of a stock solution of substrate **1** (1 mM) in CH_3CN to a mixture of acetone (5 μL) and 90 μL of an antibody solution in PBS at 25 °C. Final concentrations: [antibody] 20 μM (active site), [**1**] 50 μM, [acetone] 5%(v/v) (680 mM), 5% CH_3CN/PBS (pH 7.4). For the reaction in the absence of acetone, H_2O was added instead of acetone.

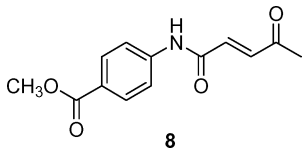
1-[4-(1*H*-Benzoimidazol-2-yl)phenyl]-3-(2-oxopropyl)pyrrolidine-2,5-dione (2). A mixture of **1** (14.5 mg, 0.050 mmol), acetone (0.2 mL, 2.72 mmol), L-proline (2.3 mg, 0.02 mmol), and DMSO (0.1 mL) was stirred at room temperature for 45 h. The reaction mixture was diluted with EtOAc, then poured into saturated NH_4Cl, and extracted with EtOAc. The organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated in vacuo, and flash chromatographed (EtOAc) to give **2** (9.6 mg, 55%). Colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10 (d, J = 8.5 Hz, 2H), 7.67−7.63 (m, 2H), 7.45 (d, J = 8.5 Hz, 2H), 7.29−7.26 (m, 2H), 3.20−3.05 (m, 4H), 2.59 (dd, J = 5.0 Hz, 18.2 Hz, 1H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 205.8, 178.5, 175.4, 150.5, 133.5, 129.8, 127.3, 127.0, 123.1, 43.4, 35.6, 34.6, 29.7. MALDI-FTMS: calcd for $C_{20}H_{18}O_3N_3$ (MH^+) 348.1343, found 348.1341.

2-[4-(1*H*-Benzoimidazol-2-yl)phenyl]-7-thiophen-2-yltetrahydroisoindole-1,3,5-trione (4). A mixture of **1** (14.5 mg, 0.050 mmol), *trans*-4-(2-thienyl)-3-buten-2-onee (**3**) (47.5 mg, 0.30 mmol), L-proline (2.5 mg, 0.02 mmol), and DMSO (0.2 mL) was stirred at room temperature for 45 h. The reaction mixture was diluted with EtOAc, then poured into saturated NH_4Cl, and extracted with EtOAc. The organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated in vacuo, and flash chromatographed (EtOAc/hexane = 4:1 and EtOAc). Additional purification with prep-TLC (EtOAc/hexane = 4:1) afforded **4** (8.0 mg, 36%). Colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (d, J = 8.5 Hz, 2H), 7.63 (brs, 2H), 7.60−7.29 (m, 2H), 7.24 (d, J = 5.3 Hz, 1H), 7.19 (d, J = 8.5 Hz, 2H), 6.96 (dd, J = 3.5 Hz, 5.3 Hz, 1H), 6.86 (d, J = 3.5 Hz, 1H), 4.20 (m, 1H), 3.69 (dd, J = 5.9 Hz, 9.7 Hz, 1H), 3.61 (dt, J = 4.1 Hz, 8.8 Hz, 1H), 3.18 (dd, J = 4.1 Hz, 17.6 Hz, 1H), 2.94 (dd, J = 9.7 Hz, 18,2 Hz, 1H), 2.89 (dd, J = 4.7 Hz, 13.2 Hz, 1H), 2.79 (dd, J = 9.1 Hz, 18.2 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$−CD_3OD): δ 206.3, 176.6, 174.6, 150.5, 141.0, 132.3, 130.2, 127.2, 127.1, 126.5, 125.8, 125.0, 122.9, 44.6, 43.0, 37.8, 36.5, 35.3. MALDI-FTMS: calcd for $C_{25}H_{20}O_3N_3S$ (MH^+) 442.1220, found 442.1230.

4-Oxopent-2-enoic Acid Quinolin-6-ylamide (5). A mixture of 6-aminoquinoline (579 mg, 4.02 mmol), *trans*-β-acetylacrylic acid[14] (464.3 mg, 4.06 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (942.6 mg, 4.92 mmol), and 4-(dimethylamino)-pyridine (2.0 mg, 0.016 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 20 h. The generated solid of **5** was filtered and washed with CH_2Cl_2. The filtrate was purified by flash chromatography (EtOAc/hexane = 4:1 and EtOAc) to give **5**. Combined yield of **5** (619.8 mg, 64%). Pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$−CD_3OD): δ 8.73 (dd, J = 1.9 Hz, 4.4 Hz, 1H), 8.54 (d, J = 2.2 Hz, 1H), 8.15 (dd, J = 1.9 Hz, 8.4 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.63 (dd, J = 2.2 Hz, 8.8 Hz, 1H), 7.38 (dd, J = 4.4 Hz, 8.4 Hz, 1H), 7.17 (d, J = 15.4 Hz, 1H), 6.95 (d, J = 15.4 Hz, 1H), 2.37 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$−CD_3OD): δ 198.7, 162.9, 148.8, 144.7, 136.9, 136.8, 136.3, 134.6, 128.9, 128.8, 123.5, 121.5, 116.5, 28.7. MALDI-FTMS: calcd for $C_{14}H_{13}O_2N_2$ (MH^+) 241.0971, found 241.0965.

3-Hydroxy-3-methyl-5-oxocyclohexanecarboxylic Acid Quinolin-6-ylamide (6). A mixture of **5** (95.7 mg, 0.398 mmol), acetone (1.0 mL, 13.6 mmol), (*S*)-(+)-1-(2-pyrrolidinylmethyl)pyrrolidine (12.9 μL, 0.0791 mmol), and 2-PrOH (3.0 mL) was stirred at 37 °C for 45 h. Purification with flash chromatography (EtOAc/hexane = 4:1 and EtOAc) and prep-TLC (EtOAc/hexane = 4:1) afforded **6** (44.1 mg, 37%). Colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$−CD_3OD): δ 8.76 (dd, J = 1.9 Hz, 4.0 Hz, 1H), 8.41 (d, J = 2.6 Hz, 1H), 8.28 (d, J = 8.1 Hz, 1H), 7.99 (d, J = 9.2 Hz, 1H), 7.84 (dd, J = 2.6 Hz, 9.2 Hz, 1H), 7.51 (dd, J = 4.0 Hz, 8.1 Hz, 1H), 3.32 (m, 1H), 2.73 (dd, J = 13.2 Hz, 14.0 Hz, 1H), 2.63 (d, J = 14.3 Hz, 1H), 2.54 (m, 1H), 2.43 (d, J = 14.3 Hz, 1H), 2.14−2.07 (m, 2H), 1.42 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$−CD_3OD): δ 211.4, 174.8, 149.8, 145.7, 138.1, 137.8, 130.1, 129.5, 125.1, 122.7, 117.3, 73.7, 54.2, 43.6, 42.3, 41.3, 30.6. MALDI-FTMS: calcd for $C_{17}H_{19}O_3N_2$ (MH^+) 299.1390, found 299.1379.

4-(4-Oxopent-2-enoylamino)benzoic Acid Methyl Ester (8). A mixture of methyl 4-aminobenzoate (1.5276 g, 10.1 mmol), *trans*-β-acetylacrylic acid1 (1.1477 g, 10.1 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (2.4969 g, 1.30 mmol), and 4-(dimethylamino)pyridine (31.5 mg, 0.258 mmol) in CH_2Cl_2 (20 mL) was stirred at room temperature for 2 h. The reaction mixture was added to 0.5 N HCl and extracted with CH_2Cl_2. The organic layers were washed with brine and dried over $MgSO_4$, filtered, and concentrated in vacuo. The generated solid was filtered and washed with CH_2Cl_2 to give **8** (975.9 mg). Pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$−CD_3OD):



8

δ 7.99 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 15.6 Hz, 1H), 6.89 (d, J = 15.6 Hz, 1H), 3.87 (s, 3H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$−CD_3OD): δ 198.6, 166.8, 162.7, 142.3, 137.0, 134.4, 130.6, 125.6, 119.1, 52.0, 29.1. MALDI-FTMS: calcd for $C_{13}H_{14}O_4N$ (MH^+) 248.0917, found 248.0920.

4-[(3-Hydroxy-3-methyl-5-oxocyclohexanecarbonyl)amino]-benzoic Acid Methyl Ester (7). Compound **7** was synthesized from **8** as described for **6**. Colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.05 (d, J = 18.3 Hz, 1H), 7.99 (d, J = 8.6 Hz, 2H), 7.63 (d, J = 8.6 Hz, 2H), 3.89 (s, 3H), 3.11 (m, 1H), 2.71 (dd, J = 12.9 Hz, 14.3 Hz, 1H), 2.56 (d, J = 14.3 Hz, 1H), 2.55 (m, 1H), 2.42 (d, J = 14.0 Hz, 1H), 2.10−2.07 (m, 2H), 1.66 (br, 1H), 1.42 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 209.6, 171.8, 166.5, 142.0, 130.8, 125.8, 118.9, 74.0, 54.1, 52.1, 43.0, 42.0, 40.4, 30.9. MALDI-FTMS: calcd for $C_{16}H_{19}O_5NNa$ (MNa^+) 328.1155, found 328.1163.

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Supporting Information Available: Additional fluorescence spectra and NMR hard copy of compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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