Aldol sensors for the rapid generation of tunable fluorescence by antibody catalysis

BENJAMIN LIST, CARLOS F. BARBAS III*, AND RICHARD A. LERNER*

The Skaggs Institute for Chemical Biology and the Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

ABSTRACT The synthesis of novel fluorogenic *retro*-aldol substrates for aldolase antibody 38C2 is described. These substrates are efficiently and specifically processed by antibody aldolases but not by natural cellular enzymes. Together, the fluorogenic substrates and antibody aldolases provide reporter gene systems that are compatible with living cells. The broad scope of the antibody aldolase allows for the processing of a range of substrates that can be designed to allow fluorescence monitoring at a variety of wavelengths. We also have developed the following concept in fluorescent protein tags. β-Diketones bearing a fluorescent tag are bound covalently by the aldolase antibody and not other proteins. We anticipate that proteins fused with the antibody can be tagged specifically and covalently within living cells with fluorophores of virtually any color, thereby providing an alternative to green fluorescent protein fusions.

Fluorescent molecules have long played a key role in chemical, biological, and medical sciences (1). Their use ranges from the localization of molecules in cells and tissues to the quantitation of divalent cation fluctuations in metabolism. In the age of genomics there is a renewed search for genes whose products will catalyze the formation of multiple fluorophores from substrates introduced into tissue and cellular compartments or whose products carry a bound fluorophore, as does green fluorescent protein (2). The general idea is to introduce these genes into cellular genomes as reporters so that the cells are rendered constitutively "prefluorescent." Such reporter functions are now widely used in the study of transcription and in temporal and spatial studies involving the expression of particular proteins. Further, when the reporter gene is an enzyme, such systems can be use to localize ligand binding when the ligand is appended to a fluorogenic substrate.

Some of the practical considerations in the search for optimal fluorogenic systems include the rate of fluorophore generation, the quantum yield, penetration of cellular membranes and/or compartments by the substrate, and the "tuneability" of the substrate so as to yield multiple colors for simultaneous observations. Finally, in an ideal system the enzyme that catalyzes the generation of fluorescence should not be present in normal cells so as to preclude background reactions.

Catalytic antibodies as "artificial enzymes" offer the opportunity to design such systems, because chemical reactions catalyzed by antibodies are programmable. Recently, we have described the generation, scope, structure, and mechanism of two aldolase antibodies, 38C2 and 33F12 (3–7). Unlike most antibody or enzyme catalysts, these aldolase antibodies maintain catalytic efficiency with a broad range of substrates and have been applied to the enantioselective synthesis of a wide variety of aldols. The substrate specificity of these antibodies

contrasts with that of the natural aldolase enzymes, which use highly polar sugar derivatives as substrates. Typical antibody substrates are cell-permeant nonpolar organic molecules that are not substrates for the natural enzymes. These properties make the aldolase antibodies ideal candidates for cellular systems if one uses nonnatural fluorogenic substrates or antibody-specific fluorescent tags.

MATERIALS AND METHODS

Synthesis of the Aldol Sensors

Synthesis of Cynol (1) (8). Acetone (294 μl, 4 mmol) was added dropwise to a stirred solution of dicyclorodicyclohexylborane chloride (Cy$_2$BCl; 894 μl, 4.08 mmol) and Et$_3$N (567 μl, 4.08 mmol) in diethyl ether (15 ml) cooled to 0°C. After 1 h the mixture was cooled to −78°C and 4-dimethylaminocinnamaldehyde (**3**, 2 mmol) was added in portions. The mixture was stirred for 2 h at −78°C and for 1 h at room temperature (RT). Methanol (8 ml), phosphate-buffered saline (PBS; pH 7.4, 4 ml), and 30% hydrogen peroxide solution (1.36 ml) were added at 0°C, and the mixture was stirred for 45 min. Extraction with ether, drying (MgSO$_4$), filtration, concentration, and crystallization from diethyl ether gave pure cynol (**1**) as slightly yellow solid in 92% yield. ^{1}H NMR (250 MHz, CDCl$_3$): δ 2.20 (s, 3 H), 2.74 (d, J = 6.2, 2 H), 2.95 (s, 6 H), 4.71 (q, J = 6.2, 1 H), 5.99 (dd, J = 15.9, 6.7, 2 H), 6.52 (d, J = 15.9, 1 H), 6.67 (d, J = 8.8, 2 H), 7.27 (d, J = 8.8, 2 H). ^{13}C NMR (63 MHz, CDCl$_3$): δ 30.9, 40.5, 50.2, 69.0, 112.3, 126.0, 127.5, 130.8.

Synthesis of Aldol 2. Twenty millimoles [2 equivalents (eq)] of the lithium enolate of acetone (prepared from freshly synthesized lithium diisopropylamide under standard conditions) in 50 ml of dry tetrahydrofuran (THF) was treated at −78°C with 1.76 g (10 mmol) of 4-methoxy-α-methylcinnamaldehyde (**4**) in 5 ml of dry THF. After 7 min at −78°C, 2 ml of a saturated ammonium chloride solution and 50 ml of ether were added. The mixture was warmed to RT, dried (MgSO$_4$), filtered, concentrated, and purified by flash chromatography hexanes/ethyl acetate (3:1 to 2:1) to give 1.71 g (7.3 mmol, 73%) of aldol **3** as a colorless liquid. ^{1}H NMR (250 MHz, CDCl$_3$): δ 1.87 (s, 3 H), 2.24 (s, 3 H), 4.81 (s, br, 1 H), 2.76 (m, 2 H), 3.04 (d, J = 2.9, 1 H), 3.81 (s, 3 H), 4.62 (m, 1 H), 6.50 (s, 1 H), 6.87 (d, J = 8.7, 2 H), 7.22 (d, J = 8.7, 2 H). ^{13}C NMR (63 MHz, CDCl$_3$): δ 13.8, 29.2, 30.8, 48.7, 55.2, 73.2, 113.5, 125.3, 130.1, 136.6, 209.4.

Synthesis of Methodol (5). Twenty millimoles (2 eq) of the lithium enolate of acetone (prepared from freshly synthesized lithium diisopropylamide under standard conditions) in 50 ml of dry THF was treated at −78°C with 1.86 g (10 mmol) of 6-methoxy-2-naphthaldehyde (**9**) in 5 ml of dry THF. After 7 min at −78°C, 2 ml of a saturated ammonium chloride solution and 50 ml of ether were added. The mixture was warmed to

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Abbreviations: THF, tetrahydrofuran; RT, room temperature.
*To whom reprint requests should be addressed at: The Scripps Research Institute, BCC-515, 10550 North Torrey Pines Road, La Jolla, CA 92037. e-mail: carlos@scripps.edu.

RT, dried (MgSO$_4$), filtered, concentrated, and crystallized from hexanes/ethyl acetate to give 1.83 g (7.5 mmol, 75%) of methodol (**5**) as a colorless solid. ^{1}H NMR (250 MHz, CDCl$_3$): δ 2.19 (s, 3 H), 2.91 (m, 2 H), 3.91 (s, 3 H), 5.27 (m, 1 H), 7.16 (m, 2 H), 7.40 (m, 1 H), 7.73 (m, 3 H). ^{13}C NMR (63 MHz, CDCl$_3$): δ 30.7, 51.9, 55.2, 69.9, 105.6, 119.0, 124.2, 127.1, 129.4, 134.0, 137.8; high-resolution mass spectroscopy calculated for C$_{15}$H$_{16}$O$_3$Na: 267.0997, observed 267.1002.

Synthesis of Aldol 6. 6′-Methoxy-2′-acetonaphthone (**10**, 801 mg, 4 mmol, 1 eq) in 10 ml of dry THF was treated at −78°C with 2-methylallylmagnesium chloride (10 ml of a 0.5 M solution in THF, 5 mmol, 1.25 eq) and stirred for 5 min. The mixture was warmed to −30°C and treated with 0.75 ml of a saturated ammonium chloride solution and 15 ml of ether, warmed to RT, dried (MgSO$_4$), filtered over silica, and concentrated to give a colorless product, which was used without further purification in the next step.

The product was dissolved in 20 ml of acetone and treated with 2.47 ml of a 50% *N*-methylmorpholine *N*-oxide solution in water and 1.2 ml of a 2.5% OsO$_4$ solution in *t*-BuOH. After 1 h the mixture was treated with saturated sodium metabisulfite solution and extracted five times with EtOAc. After evaporation of the dried organic layers, the colorless triol was isolated. This material was pure (TLC) and was used without further purification in the next step.

The product was dissolved in 60 ml of methylene chloride and treated with 1.05 eq of Pb(OAc)$_4$. After 5 min, solid sodium carbonate was added and the mixture was stirred for 30 min. Filtration and flash chromatography (25% EtOAc/hexane) gave 778 mg (3.02 mmol, 75% for the three steps) of a colorless solid. ^{1}H NMR (250 MHz, CDCl$_3$): δ 1.60 (s, 3 H), 2.07 (s, 3 H), 2.90 (d, *J* = 16.9, 1 H), 3.27 (d, *J* = 16.9, 1 H), 3.91 (s, 3 H), 4.67 (s, 1 H), 7.13 (m, 2 H), 7.47 (m, 1 H), 7.84 (m, 1 H). ^{13}C NMR (63 MHz, CDCl$_3$): δ 30.6, 31.9, 53.8, 55.2, 105.4, 118.9, 122.7, 123.4, 126.8, 128.6, 129.6, 142.3, 210.7.

Synthesis of Dimedol (7). Prodan (9) (**11**, 200 mg, 0.88 mmol, 1 eq) in 15 ml of dry THF was treated at −78°C with 2-methylallylmagnesium chloride (2.64 ml of a 0.5 M solution in THF, 1.32 mmol, 1.5 eq) and stirred for 5 min. The mixture was warmed to −30°C and treated with 0.75 ml of a saturated ammonium chloride solution and 15 ml of ether, warmed to RT, dried (MgSO$_4$), filtered, and concentrated to give a colorless product, which was used without further purification in the next step.

The product was dissolved in 10 ml of acetone and treated with 0.66 ml of a 50% *N*-methylmorpholine *N*-oxide solution in water and 0.65 ml of a 2.5% OsO$_4$ solution in *i*-PrOH. After 1 h the mixture was treated with saturated sodium metabisulfite solution and extracted five times with EtOAc. After evaporation of the dried organic layers, 218 mg (0.688 mmol, 78%) of the triol was isolated. This material was pure (TLC) and was used without further purification in the next step.

The product was dissolved in 20 ml of chloroform and treated with 1.05 eq of Pb(OAc)$_4$. After 5 min, solid sodium carbonate was added and the mixture was stirred for 30 min. Filtration and flash chromatography (17% EtOAc/hexane) gave 120 mg (0.421 mmol, 48% for the three steps) of dimedol as a colorless solid. ^{1}H NMR (250 MHz, CDCl$_3$): δ 0.76 (t, *J* = 7.4, 3 H), 1.87 (m, 2 H), 2.03 (s, 3 H), 2.86 (d, *J* = 16.5, 1 H), 3.03 (s, 6 H), 3.27 (d, *J* = 16.5, 1 H), 4.57 (s, 1 H), 6.91 (m, 1 H), 7.17 (m, 1 H), 7.34 (m, 1 H), 7.61–7.72 (m, ³H). ^{13}C NMR (63 MHz, CDCl$_3$): δ 7.7, 35.7, 40.9, 75.9, 100.1, 126.2, 128.9, 133.6, 148.7; high-resolution mass spectroscopy calculated for C$_{18}$H$_{23}$NO$_2$: 285.1729, observed 285.1737.

Synthesis of Aldol 8 (10). Gaseous dimethylamine was introduced in a mixture of 2.4 ml of dry benzene and 2.4 ml of hexamethylphosphoramide (HMPA) until 750 mg (16.7 mmol, 8 eq) was dissolved. To this was added at 0°C under argon 10.43 ml of *n*-BuLi (1.6 M in hexanes, 16.7 mmol, 8 eq) and, after 15 min, 390 mg (2.09 mmol, 1 eq) of 6-methoxy-2-naphthaldehyde

(**9**). The mixture was stirred at RT for 14 h, poured into cold PBS, and extracted with ether. After drying (MgSO$_4$), filtering, and evaporation, the resulting solid was purified by flash chromatography (14% EtOAc/hexane + 10% CH$_2$Cl$_2$) to give 350 mg (1.76 mmol, 84%) of 6-dimethylamino-2-naphthaldehyde (**12**) as yellow crystals. ^{1}H NMR (250 MHz, CDCl$_3$): δ 3.10 (s, 6 H), 6.85 (m, 1 H), 7.15 (m, 1 H), 7.64 (m, 1 H), 7.81 (m, 1 H), 8.12 (m, 1 H), 9.99 (s, 1 H). ^{13}C NMR (63 MHz, CDCl$_3$): δ 40.3, 105.3, 116.1, 123.4, 124.9, 126.8, 130.5, 130.7, 134.8, 138.6, 191.8.

The above aldehyde (**12**, 25 mg, 0.126 mmol) in 0.7 ml of acetone at 0°C was treated with 0.05 ml of 2 M NaOH. After 3 h at this temperature, the mixture was treated with 0.1 ml of a saturated aqueous solution of NH$_4$Cl and purified by TLC (hexanes/ethyl acetate = 2:1) to give 18 mg (0.07 mmol, 56%) of crystalline aldol **8**. ^{1}H NMR (250 MHz, CDCl$_3$): δ 2.20 (s, 3 H), 2.91 (m, 2 H), 3.00 (s, 6 H), 5.27 (m, 1 H), 6.90 (m, 1 H), 7.17 (m, 1 H), 7.36 (m, 1 H), 7.66 (m, 3 H).

Antibody-Catalyzed *Retro*-Aldol Reactions

Aldols **1**, **2**, **5–8**, and aldol **14** in acetonitrile (5 mM) were diluted with PBS to the required concentration and then treated with antibody 38C2 (67 μM in PBS, pH 7.4, commercially available from Aldrich, no. 47,995-0) to give a final antibody concentration of 500 nM (10 μM with substrate **14**). To obtain kinetic data, the reactions were followed either with fluorescence spectroscopy (aldols **5–8** and **14**) or UV spectroscopy (aldols **1** and **2**). Product formation was followed with a fluorescence plate-reader by monitoring at λ_{abs} = 355 nm and λ_{em} = 460 nm, both with aldols **5** and **6**, and by monitoring at λ_{abs} = 355 nm and λ_{em} = 538 nm, with aldols **7** and **8**. Formation of resorufin (**15**) from mol-red (**14**) and from ketone **17** was followed by monitoring at λ_{abs} = 544 nm and λ_{em} = 590 nm. Product formation in the reactions with aldols **1** and **2** was followed with a UV plate-reader by monitoring absorption at the λ_{max} of the aldehyde formed in the *retro*-aldol reactions. Formation of 4-methoxy-α-methylcinnamaldehyde (**4**) was monitored at 315 nm (ε = 21,722 M^{-1}·cm^{-1}), formation of 4-dimethylaminocinnamaldehyde (**3**) was followed at 400 nm (ε = 23,000 M^{-1}·cm^{-1}), and formation of 6-methoxy-2-naphthaldehyde was followed at 314 nm (ε = 8,128 M^{-1}·cm^{-1}). The experimental data were plotted by using nonlinear regression analysis with GRAFIT software to give k_{cat} and K_m of the reactions. All data are reported per antibody active site.

RESULTS AND DISCUSSION

Design, Synthesis, and Antibody-Catalyzed Reactions of Aldol Sensors. The present study was initiated with a search for sensitive sensor systems for the aldol reaction. These would allow for a more convenient detection of catalysis from individual aldolase catalytic antibodies as well as for a possible rapid high-throughput screening of combinatorial antibody or other catalyst libraries. Libraries of either chemical or biolog-
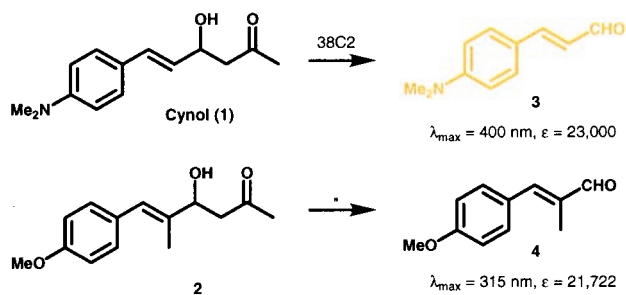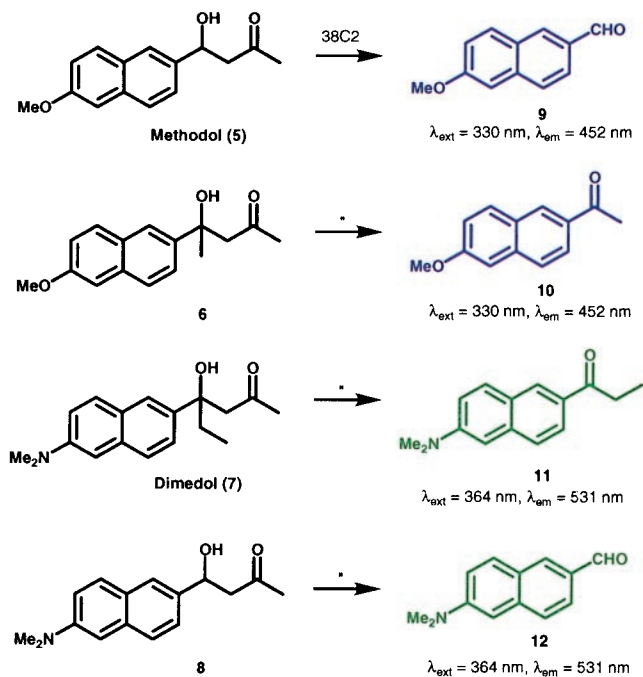

FIG. 1.   Chromogenic aldol sensors.

FIG. 2. Fluorogenic aldol sensors.

Aldols **1** and **2** were prepared by using standard techniques, and the λ_{max} were determined to be 283 nm ($\varepsilon = 18,400$ $M^{-1} \cdot cm^{-1}$) and 255 nm ($\varepsilon = 20,000$ $M^{-1} \cdot cm^{-1}$), respectively. Both aldols were found to be very efficient substrates for antibody 38C2-catalyzed *retro*-aldol fragmentation, after Michaelis—Menten kinetics with $k_{cat} = 5.0$ min^{-1} ($K_m = 25$ μM) for cynol (**1**) and $k_{cat} = 2.2$ min^{-1} ($K_m = 16$ μM) for aldol **2** (Fig. 1). The reaction employing cynol (**1**) as substrate can be followed visually, and solutions containing low concentrations of the aldehyde product (≈ 100 nM) appear yellow. Antibody concentrations below 10 nM are easily detectable.

Fluorogenic Aldol Sensors. We reasoned that the detection limit could be decreased further by applying fluorescence instead of simple absorption spectroscopy. Fluorescence tech-

ical origin could be studied. Although a number of sensors for hydrolytic reactions are available, there are no known systems for C—C bond forming or cleaving reactions. Surprisingly, we found only one practical sensor system for reactions based on the formation or cleavage of the carbonyl group (11).

Our solution to this problem lay in the reversibility of the aldol reaction in that any catalyst that catalyzes the aldol reaction catalyzes the *retro*-aldol reaction, as well. Thus, we chose systems based on *retro*-aldolization. These have the advantage that only one substrate is required. Initially, we looked at aldols **1** (cynol) and **2** because it was anticipated that their UV absorption would be distinguishable from their aldehyde precursors, dimethylaminocinnamaldehyde (**3**, $\lambda_{max} = 400$ nm, $\varepsilon = 23,000$ $M^{-1} \cdot cm^{-1}$) and 4-methoxy-α-methylcinnamaldehyde (**4**, $\lambda_{max} = 315$ nm, $\varepsilon = 21,722$ $M^{-1} \cdot cm^{-1}$, in PBS/10% acetonitrile, pH 7.4), respectively (Fig. 1). These aldehydes possess extended π-systems with a donor and an acceptor group. Since this conjugation is interrupted in the aldols, one expects absorption at a shorter wavelength for the aldol substrates as opposed to the aldehyde products.
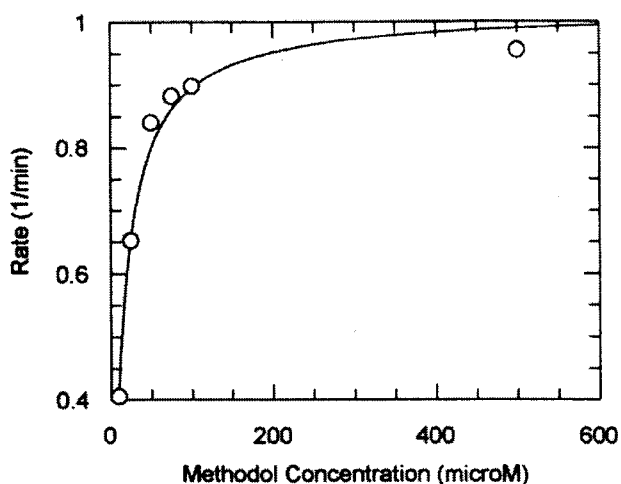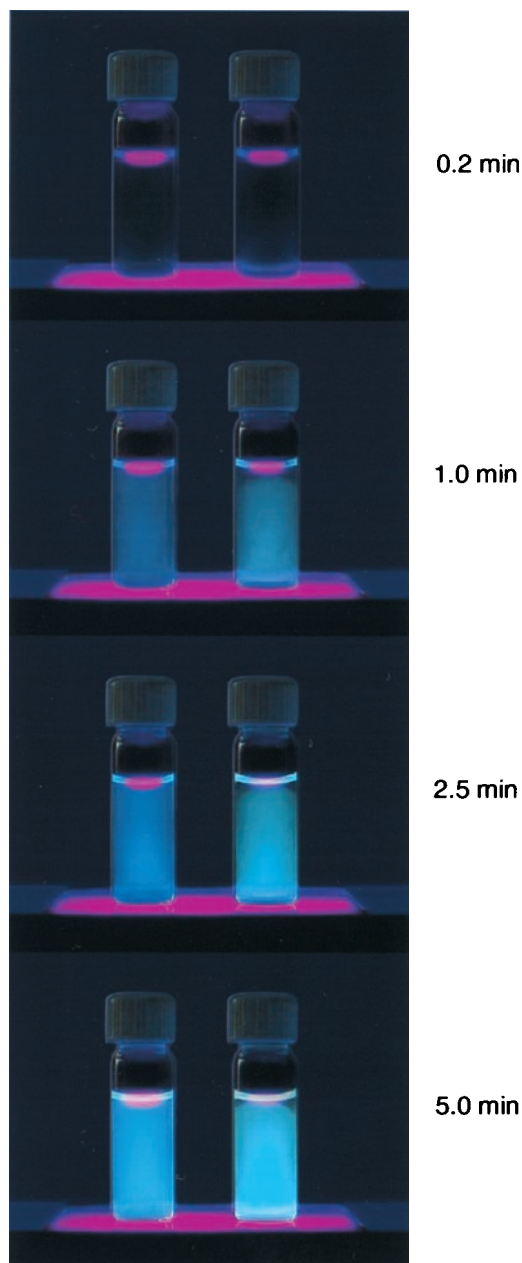

FIG. 3. Michaelis—Menten kinetics for methodol; $k_{cat} = 1$ min^{-1}, $K_m = 14$ μM.

FIG. 4. Development of fluorescence in the antibody-catalyzed *retro*-aldol reaction of methodol (*Left*) and dimedol (*Right*). Conditions: 200 μM methodol, 6 μM antibody 38C2 in PBS, pH 7.4, and 10% CH_3CN; 200 μM dimedol, 10 μM antibody 38C2 in PBS, pH 7.4, and 10% CH_3CN. The pictures were taken after the indicated time intervals. The wells were irradiated with a standard long-wave UV lamp.
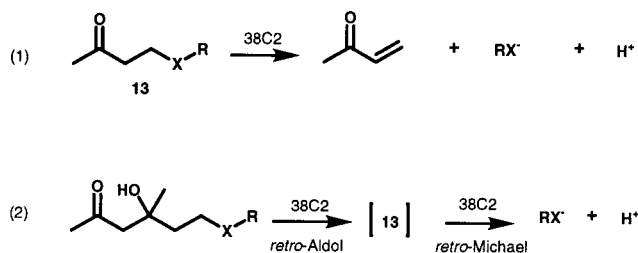
FIG. 5. (1) The antibody-catalyzed *retro*-Michael reaction. (2) The antibody-catalyzed tandem *retro*-aldol–*retro*-Michael reaction.

niques also offer attractive possibilities for cell-based assays. The design of methodol (**5**), aldol **6**, dimedol (**7**), and aldol **8** was based on the known fluorescence of 6-methoxy-2-naphthaldehyde [**9**, blue, absorption (abs) 287 nm, emission (em) 450 nm in PBS at pH 7.4) (11, 12) and 2-propionyl-6-dimethylaminonaphthalene [prodan (**11**), green, abs 364, em 531 in PBS at pH 7.4] (9) (Fig. 2). Again, interruption of the π-conjugation within these molecules was anticipated to greatly lower their fluorescence. This conjugation then could be restored by *retro*-aldolization, yielding the fluorescent product. Methodol and **8** were synthesized by using standard techniques. Dimedol and **6**, as tertiary aldols, were synthesized from commercially available prodan (**11**) and 6′-methoxy-2′-acetonaphthone (**10**), respectively, by Grignard reaction with 2-methylallylmagnesium chloride and oxidative cleavage of the resulting olefins. All four aldols (**5**–**8**) show very low fluorescence emission upon irradiation with long-wavelength UV. They are efficient substrates for antibody 38C2-catalyzed *retro*-aldol reactions, with $k_{cat} = 1.0$ min^{-1} ($K_m = 14$ μM) for methodol (**5**) (Fig. 3), $k_{cat} = 3.3$ min^{-1} ($K_m = 150$ μM) for **6**, $k_{cat} = 0.15$ min^{-1} ($K_m = 37$ μM) for dimedol (**7**), and $k_{cat} = 0.3$ min^{-1} ($K_m = 7$ μM) for aldol **8** (Figs. 2–4).

An important feature of these reactions is their sensitivity. In the case of methodol, we found that antibody concentrations of less than 0.5 nM are detectable over the background by using a commercially available fluorescence plate-reader. The detection limit for aldols **4** and **5** lies within the same range.

Second-Generation Aldol Sensors. In the fluorogenic aldol sensors described above, the fluorescence is based on the formation of a carbonyl group. However, the number of fluorescent molecules in which this is the case is relatively small and the wavelength range of these molecules is limited. Thus, it was desirable to develop red-shifted substrates that are useful for screens based on cell sorting (13). We found that our aldolase antibodies catalyze the β-elimination (or *retro*-Michael reactions) of β-heterosubstituted ketones **13** (Fig. 5, Eq. **1**) (C.F.B., B.L., R.A.L., D. Shabat, R. D. Lewis II, and H. Almer, unpublished data). These reactions are generally fast and, in principal, allow us to use many known fluorogenic, chromogenic, and luminogenic substrates, where the detect-
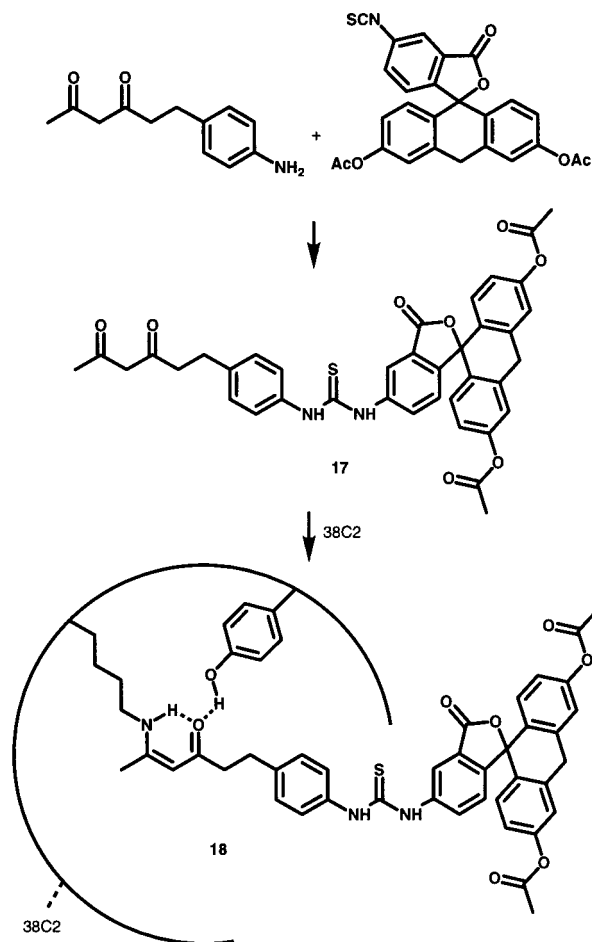

FIG. 7. Covalent labeling of aldolase antibody 38C2 with a fluoresceine diketone.

able property is based on an ionizable group (*p*-nitrophenol, umbelliferone, etc.). Unfortunately, the *retro*-Michael reaction has a relatively high background. We reasoned that a tandem reaction should give a much decreased background rate of fluorescence generation in that two or more reactions would be required to generate the fluorophore. Thus, a tandem *retro*-aldol–*retro*-Michael sequence would show a strongly decreased background. This sequence then would be compatible with a virtually unlimited number of sensors (Fig. 5, Eq. **2**) (14). In this scheme, the actual carbon—carbon-bond-cleaving event of the *retro*-aldol reaction is "translated" into a carbon—heteroatom-bond cleavage, which, in turn, leads to a detectable signal. In fact, it is likely that most (if not all) catalysts of the (*retro*-) aldol reaction are catalysts for the *retro*-Michael
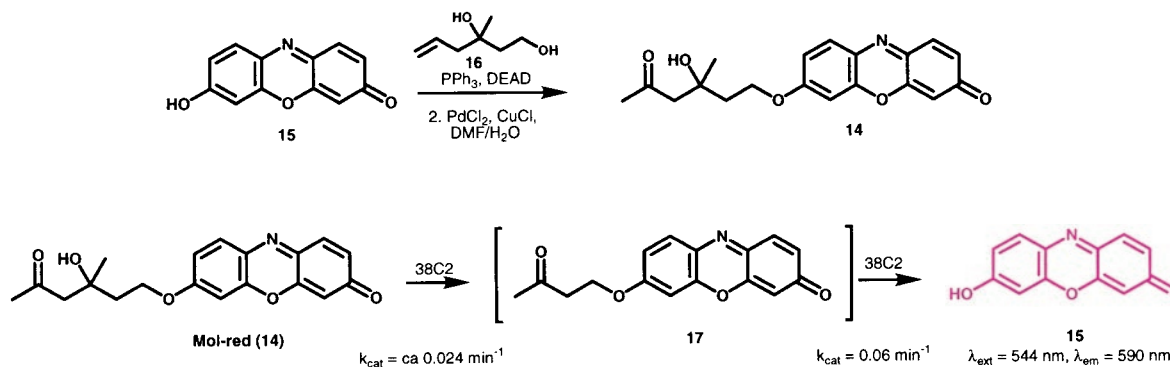

FIG. 6. Synthesis of mol-red (**14**) and antibody-catalyzed tandem *retro*-aldol–*retro*-Michael reaction of **14** to give red fluorescent resorufin.

reaction as well. To test this concept we prepared a variety of chromogenic, fluorogenic, and luminogenic *retro*-aldol–*retro*-Michael sensors, which, upon treatment with 38C2, specifically and efficiently give the corresponding reporter molecules. We focused on resorufin (**15**) (Fig. 6) since its red fluorescence emission (590 nm) is well beyond the autofluorescence exhibited by most biological samples. This feature, along with its nontoxicity, allows for its use in flow cytometry and other assays within living cells (15, 16). The synthesis of *retro*-aldol–*retro*-Michael sensor **14** (mol-red) is shown in Fig. 6. Thus, Mitsunobu etherification (17) of resorufin with diol **16**, followed by a Wacker oxidation (18), gave mol-red (**14**). mol-red is an efficient substrate for 38C2, although the rate at which it is processed is somewhat lower than aldols **1** and **2** and **5**–**8**. The k_{cat} of the *retro*-aldol–*retro*-Michael tandem reaction has been estimated to be around 0.024 min^{-1} ($K_m = 70$ μM). However, the transformation is very specific, and k_{cat}/k_{uncat} is $>10^5$. The k_{cat} for the *retro*-Michael reaction of ketone **17** alone was determined to be 0.06 min^{-1} ($K_m = 13$ μM). Here the background reaction in PBS ($k_{uncat} = 0.0009$ min^{-1}) is considerably higher ($k_{cat}/k_{uncat} = 67$). This observation applies for all *retro*-aldol–*retro*-Michael sensors we have prepared to date.

Fluorescent Tagging of the Aldolase Antibody. The aldolase antibody 38C2 was raised against a β-diketone hapten that served as a chemical trap to imprint the lysine-dependent class I aldolase mechanism in the active site of the antibody (3). The ε-amino group of a lysine residue within the binding pocket of this antibody reacts with a carbonyl function of the β-diketone moiety of the hapten to form a β-keto hemiaminal that dehydrates to give a β-keto imine that finally tautomerizes into a stable enaminone. Consequently, the hapten becomes covalently bound in the binding pocket. We have shown that the antibody 38C2 reacts covalently with a variety of β-diketones to form enaminones that are observable by using UV spectroscopy (3–5). The unique chemical reactivity of this lysine could allow for the specific fluorescent labeling of the antibody if the fluorescent molecule was simply appended to a β-diketone. To test this concept we synthesized a cell-permeant fluoresceine β-diketone **17** (Fig. 7). Incubation of the antibody with this compound resulted in specific covalent labeling of its active site. The enaminone **18** was formed rapidly upon addition of **17** to a solution of antibody. Enaminone **18** was observed by UV spectroscopy, with $\lambda_{max} = 318$ nm. β-Diketone **17** did not label other proteins or antibodies. Given the ability of the antibody to react with a wide variety of β-diketones, the antibody could be specifically labeled with a large number of fluorescent molecules. Reactive immunization allows for the chemistry of covalent labeling to be programmed; thus, antibodies reactive with defined functionalities other than β-diketones should be accessible.

Applications. Our aldol sensors are now routinely used in catalysis screens of new antibodies with potential aldolase activity. Moreover, we are screening libraries of biological origin with these substrates, with the goal of finding antibody aldol catalysts that are more efficient than the ones now available. Studies of fluorogenic substrates **5**–**8** and mol-red in living cells are ongoing. Initial results indicate that neither the substrates nor their fluorescent products are toxic to cells

expressing the antibody (N. B. Gilula, personal communication). These reactions have very low backgrounds in cells that do not express antibody 38C2. Furthermore, these aldol sensors are not restricted to biological systems. One application lies in the use of our aldol sensors as detection systems in synthetic libraries of possible enantioselective catalysts for the aldol reaction. Since antibody 38C2 can be specifically and covalently labeled with fluorescent β-diketones, it should be possible to study protein localization and interactions within living cells with antibody fusion proteins in much the same way that green fluorescent proteins have been used (2, 19). The antibody-based approach has the advantage that it is adaptable to fluorescent tags of any color.

Note. While we were preparing this manuscript an assay based on an enzyme-mediated oxidation followed by a β-elimination was described (14).

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