

Fluorescence Spectroscopy Studies of Crossed Aldol Reactions: A Reactive Nile Red Dye Reveals Catalyst-Dependent Product Formation

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ABSTRACT

The synthesis of a new Nile Red derivative incorporating a reactive aldehyde moiety (NR-CHO) is reported and its use in spectroscopic studies of heterogeneous catalyst activity in crossed aldol reactions is demonstrated. ^1H and ^{13}C NMR, and high-resolution mass spectrometry confirmed the desired NR-CHO was obtained. Mg-Zr-Cs doped silica ($\text{Cs}(\text{Zr,Mg})\text{-SiO}_2$) was employed as the catalyst and its performance was compared to that of commercially available MgO. Fumed silica was used as a control. Aldol reactions with acetone and acetophenone were run in 4:1 (v/v) DMSO:ketone solutions in the presence of both dilute (1 μM) and concentrated (1 mM) NR-CHO. NR-CHO fluorescence spectra were acquired as the reactions progressed. Shifts in its emission spectrum are used to distinguish the products formed and to characterize the reaction rate. The dye exhibits different behavior that defines whether the reaction stops at the addition (alcohol) product, or forms both addition and condensation (olefin) products, providing valuable initial information on catalyst activity. The assignment of addition and condensation products is supported by thin layer chromatography, high performance liquid chromatography (HPLC), and HPLC-mass spectrometry data. Product formation is shown to depend upon the catalyst employed, with the $\text{Cs}(\text{Zr,Mg})\text{-SiO}_2$ yielding both addition and condensation products, while MgO yields primarily addition products. The advantages of NR-CHO in spectroscopic studies of aldol reactions are also demonstrated relative to commercially available 3-perylene-carboxaldehyde. The NR-CHO reported here and the results obtained will facilitate a broad range of both ensemble

and single molecule spectroscopic investigations of heterogeneous catalysis in crossed aldol reactions in the future.

KEYWORDS

Crossed aldol reactions, heterogeneous catalysis, in-situ investigations, fluorescence detection.

INTRODUCTION

Aldol reactions represent common and widely used synthetic routes to carbon-carbon bond formation via the coupling of, for example, an aldehyde and a ketone. The synthesis of pharmaceuticals, industrial feedstocks, commodity chemicals, and biomass-derived renewable liquid fuels frequently involve aldol reactions. These reactions are most often catalyzed by homogeneous catalysts comprising solutions of strong base (i.e., hydroxide)¹ or transition-metals.² However, homogeneous catalysts can be difficult to recover and reuse, producing excess waste and leading to increased cost. Heterogeneous catalysts are now being widely explored as a means to overcome these difficulties.³⁻⁵

Silica-based materials form an important class of porous solids now being investigated for use in heterogeneous catalysis of aldol reactions.⁶⁻⁸ Catalytic sites are readily incorporated into porous silica by addition of appropriate transition metals⁹⁻¹⁰ or acidic and basic moieties¹¹⁻¹³ during materials synthesis. For example, alkali or alkaline earth metals may be incorporated into the silica to form Brønsted base sites,^{9-10, 14} while the silanol groups intrinsic to the silica act as Brønsted acid sites.¹⁵⁻¹⁶ Bifunctional materials that incorporate both acid and base sites have been found to be particularly effective in catalyzing aldol condensation reactions.^{9-10, 17} The base sites appear to best catalyze the initial aldol addition step, forming an alcohol product, while a combination of base and weak acid sites in close proximity to each other catalyze the subsequent dehydration to obtain the final olefin product.^{15, 18}

It is generally accepted that both the concentration and strength of acid and base sites define the catalyst activity.¹⁹ These parameters are most commonly determined *ex situ* by methods such as temperature programmed desorption of ammonia and carbon dioxide.²⁰⁻²³ The results of such studies afford little direct information on the *in situ* acidity and basicity of the catalytic sites, which

are certain to vary with the solvent employed and the temperature at which the reaction is run, among other factors. Furthermore, the properties of the individual catalyst particles are expected to be intrinsically variable. To overcome some of the limitations of *ex situ* studies, fluorogenic reactions have been employed previously as a means to characterize catalyst activity *in situ* for a number of processes.²⁴⁻²⁶ Many such studies have been performed by ensemble spectroscopic methods, but when highly fluorescent reactants and products are involved, the reactions can even be followed at the single molecule/single turnover level.²⁷⁻³³ While fluorescence has been used previously to follow aldol reactions,³⁴⁻³⁵ no such studies have been reported at the single molecule level to date. The dearth of single molecule studies likely stems from the lack of suitable dyes designed to undergo aldol reactions while also remaining sufficiently fluorescent for detection at the single molecule level.

In this paper, we report the synthesis of a new derivative of Nile Red that incorporates a reactive aldehyde moiety (NR-CHO). NMR spectroscopy and high-resolution mass spectrometry were used to confirm that NR-CHO was obtained, and its use in solution-phase ensemble spectroscopic studies of the heterogeneous catalysis of crossed aldol reactions was demonstrated. The Nile Red chromophore was selected for use because it is now widely employed in single molecule detection, spectroscopy, and tracking studies.³⁶⁻³⁹ Because its lowest energy electronic transition involves an intramolecular charge transfer process,⁴⁰ its spectrum is highly dependent on its surroundings.⁴¹ Thus, we hypothesize that subtle changes to its structure occurring during an aldol reaction will also lead to detectable changes in its fluorescence spectrum. We compare its performance to that of a commercially available 3-perylenecarboxaldehyde. We employ Mg-Zr-Cs doped silica (Cs(Zr,Mg)-SiO₂)⁴² as the heterogeneous catalyst and compare its performance to that of commercially available MgO. The activities of these two catalysts are compared to that

of fumed silica. The occurrence of an aldol reaction and the assignment of the products formed are supported by thin layer chromatography, high performance liquid chromatography (HPLC), and HPLC-mass spectrometry (HPLC-MS) data. Changes in dye fluorescence observed during the reaction are recorded and used as a simple, real-time, *in situ* method for following reaction progress. The new NR-CHO dye reported and the results obtained in ensemble studies of aldol reactions will be useful to others seeking *in situ* methods to characterize the activity of heterogeneous catalysts. They will also facilitate future studies of catalyst activity at the single particle and single reaction event level via the implementation of single molecule spectroscopic methods. Such studies are now underway in our labs.

EXPERIMENTAL SECTION

Materials

Except as indicated below, all solvents, starting materials, and reagents employed in the synthesis of NR-CHO and Cs(Zr,Mg)-SiO₂ were obtained from commercial sources and used as received.

Methods for Heterogeneous Catalysts

Preparation of Cs(Zr,Mg)-SiO₂ catalyst. Fumed silica (Sigma-Aldrich, 99.8%) was calcined in flowing air at 500 °C for 3 h in a furnace (Barnstead F1500). The silica was then impregnated with aqueous solutions of Mg(NO₃)₂ · 6H₂O, ZrO(NO₃)₂ · xH₂O and CsNO₃ (Sigma-Aldrich) to yield particles incorporating theoretical loadings of magnesium, zirconium, and cesium at 0.025 wt.%, 0.033 wt.% and 13 wt.%, respectively. The aqueous mixture was subsequently immersed and sonicated at 40 °C for 24 h, followed by drying in an oven overnight at 90 °C. The catalyst was calcined in flowing air at 500 °C for 3 h prior to use.

Catalyst structural characterization. X-Ray diffraction (XRD) patterns of the as-synthesized Cs(Zr,Mg)-SiO₂, the commercially available MgO (Nanoscale Materials), and fumed silica were obtained using a desktop X-ray diffractometer (Rigaku Miniflex II). Transmission electron microscopy (TEM) images were acquired using a FEI Tecnai G2 Spirit BioTWIN microscope operating at 120 kV. Samples were prepared by dispersing them in ethanol, sonicating them, and then depositing them on a microgrid before imaging. Scanning electron microscopy (SEM) images were obtained using a Hitachi S-3500N microscope operating at 20 kV. The catalyst samples were sputter coated with palladium before imaging.

BET surface area and pore size distribution measurements were made on a Micromeritics ASAP 2020 Surface Area and Porosity Analyzer. Samples were first dehydrated in a 1430 series VWR vacuum oven for 24 h at 150 °C and < 77 Torr. To avoid undesirable interactions with atmospheric moisture, samples were kept in a humidity-free environment in a Sanplatec Dry Keeper Auto Desiccator Cabinet. Prior to surface area and porosity measurements, all samples went through an outgassing step for 12 h at 350 °C under vacuum. Nitrogen sorption isotherms were collected at 77 K over a relative pressure range of 0.05 to 0.995 (p/p₀), measured every 120 min. Mesopore-size distribution was evaluated by the nitrogen desorption isotherm and by the BJH method.⁴³ Total surface area was calculated by the BET method.⁴⁴ Total pore volume was determined by the amount of nitrogen adsorbed at a relative pressure of 0.99 (p/p₀).

Catalyst chemical characterization. Elemental analysis was accomplished by energy-dispersive X-ray spectroscopy (EDS) employing an Oxford detector (< 135 eV). The elemental composition of the catalysts was also determined using a Varian 720-ES inductively coupled plasma-optical emission spectrometer (ICP-OES). Samples were digested in nitric acid for this analysis. X-ray photoelectron spectroscopy (XPS) data were acquired using a Thermo Scientific

K-alpha⁺ XPS with a monochromatic Al K α X-ray source (1486.6 eV). XPS spectra were acquired using a 400 μ m spot size, 50.0 eV pass energy, and 0.100 eV step size. The binding energies were corrected for charge shifts using the C (1s) peak of adventitious carbon at 284.8 eV as a reference.¹⁸ Deconvolution of the XPS data was done using OriginPro software.

The acid-base properties of the catalysts were determined by temperature-programmed desorption (TPD) of CO₂ and NH₃ (Altamira Instruments AMI-200). For CO₂ TPD, 150 mg of catalyst was placed in a quartz tube and treated in helium flow at 450 °C for 1 h, with a heating rate of 10 °C/min. After cooling to 50 °C, a 10% CO₂-He mixture was passed over the samples for 30 min followed by a purge with pure helium until the baseline became stable. Desorption of CO₂ was accomplished by exposing the sample to a helium flow while heating to 500 °C at a rate of 10 °C/min. The same process was employed for NH₃ TPD using a 1% NH₃-He mixture. The desorption profile for both gases was recorded using a thermal conductivity detector.

Methods for Aldol Reactions

General procedure for crossed aldol reactions. The experimental conditions for aldol reactions were obtained from Sakthivel et al.⁴⁵ A stock solution of NR-CHO (7 mM) was prepared in DMSO (Fisher) and later used in the preparation of diluted solutions for aldol reactions. All experiments employed either ACS grade acetone (Fisher) or GC grade acetophenone (Sigma-Aldrich, > 99%) as the ketone. The ketones were each dissolved in DMSO in a 1:4 (v/v) ratio. Fluorescence measurements of the reaction kinetics employed low (1 μ M) NR-CHO concentrations, while HPLC and HPLC-MS analyses were performed for reactions using high (1 mM) NR-CHO concentrations. The performance of the Cs(Zr,Mg)-SiO₂ catalyst was compared to that of commercially available MgO, and fumed silica. The latter served as a control. Aldol reactions and controls employed a catalyst loading of 3.07 mg/mL and reaction volumes of either

3.250 mL or 1.625 mL. The reactions were stirred at room temperature for various times, as specified below. Aldol reactions are known to depend on temperature, but such effects were excluded from the present investigations. Therefore, heat transfer from the magnetic stir plate into the reaction mixture was prevented by placing an insulating material between the stir plate and the reaction cell.

Crude products from the aldol reactions employing 1 mM NR-CHO were prepared for HPLC-MS characterization by adding one volume equivalent of saturated aqueous NH₄Cl to the mixture and then extracting 2-3 times with ethyl acetate (Fisher). The combined organic fractions were dried over anhydrous sodium sulfate (Fisher) and concentrated under vacuum. The products were dissolved in 3:2 (v/v) HPLC grade acetonitrile (Fisher) and HPLC grade water (Fisher) and analyzed using an Acquity Ultra Performance HPLC-MS (Waters). The HPLC employed a water-acetonitrile mobile phase gradient and a C18 reversed phase silica column (Cortecs). Products eluting from the column were detected by their UV absorbance at 230-300 nm. MS detection employed an electrospray ionization source. An Ultimate 3000 HPLC (Thermo Fisher) with absorbance detection at 575 nm was employed for quantitative analysis of the aldol products. A water-acetonitrile mobile phase gradient and a C18 reversed phase silica column (Thermo Fisher) were also employed here.

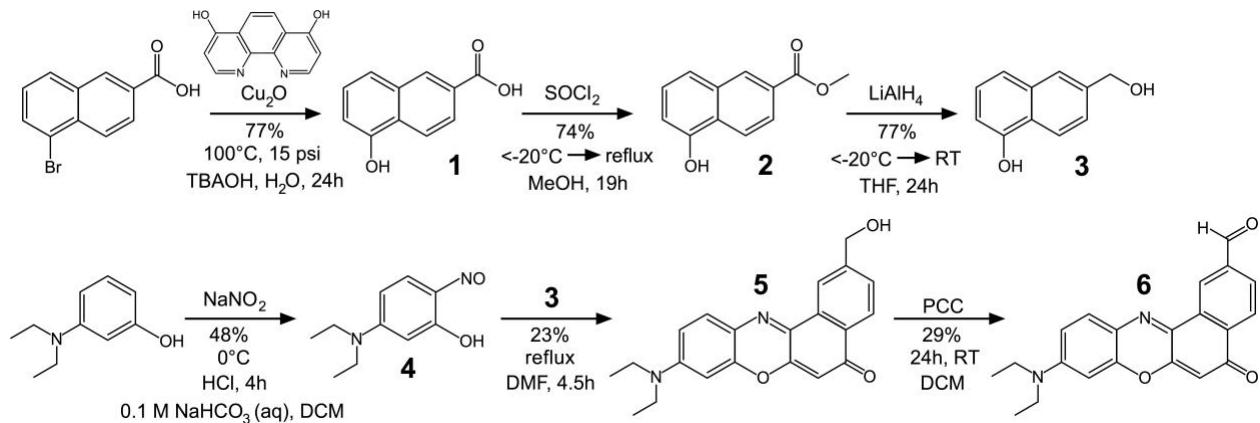
Fluorescence studies of crossed aldol reactions. Fluorescence spectra were acquired on a FluoroMax-2 spectrophotometer (Jobin Yvon Spex) using a Xenon lamp (150 W) as the light source. Aldol reactions were run in a quartz fluorimeter cell (Starna Cells) with a 10 mm pathlength. The cell was sealed with a Teflon cap and parafilm to prevent solvent evaporation during the reaction. Excitation and emission spectra were acquired primarily from 1 μ M NR-CHO solutions. While spectra were also acquired from 1 mM NR-CHO solutions, these were severely

distorted by inner filter effects and were not used in any spectroscopic analyses. The emission spectrum of NR-CHO was recorded from 578 to 800 nm, while exciting at 575 nm. The progress of the aldol reactions was monitored in real time by recording fluorescence spectra at various time intervals during the reaction.

As described in Supplementary Information, the utility of the newly synthesized NR-CHO dye in studies of crossed aldol reactions was compared to that of a commercially available 3-perylenecarboxaldehyde dye (TCI, > 95%). In this case, 1 μ M 3-perylenecarboxaldehyde solutions were employed. The 3-perylenecarboxaldehyde emission spectrum was recorded from 470 to 800 nm, while exciting at 465 nm.

Synthesis of NR-CHO

Synthesis of the aldol-reactive derivative of Nile Red, 9-diethylamino-5*H*-benzo[α]phenoxazine-5-one-2-carboxaldehyde (NR-CHO), required six steps and is based upon a previously reported synthesis of Nile Red.⁴⁶ The steps employed are shown in Scheme 1. Analytical thin layer chromatography (TLC) was employed to track reaction progress using silica gel 60 F₂₅₄ (Merck) TLC plates. Column chromatography was employed for product purification and employed silica gel 60 (230-400 mesh) as the stationary phase. ¹H and ¹³C NMR spectra were acquired on either 600 MHz (Bruker Avance III HD) or 400 MHz (Bruker Avance NEO) spectrometers. Fourier transform infrared (FTIR) spectra were taken using disposable polyethylene IR cards (Aldrich) as the sample support. Spectra were recorded in transmission mode on a Cary 630 FTIR (Agilent). High-resolution mass spectra were acquired on a LCT Premier Mass Spectrometer (Waters), using electron impact ionization.



Scheme 1. Synthesis of 9-diethylamino-5*H*-benzo[α]phenoxazin-5-one-2-carboxaldehyde (NR-CHO), **6**. Product yields are given below each reaction arrow.

Synthesis of 5-hydroxy-2-naphthoic acid (1). The synthetic procedure reported by Wang et al. was modified to prepare **1**.⁴⁷ A 50 mL reactor (Parr 5500 series) equipped with a mechanical mixer was charged with 5-bromo-2-naphthoic acid (1 g, 3.98 mmol, Combi-Blocks, 98%), tetrabutylammonium hydroxide (19 mL, 40% in H₂O, Sigma-Aldrich), Cu₂O (0.028 g, 0.195 mmol, Alfa Aesar, 97%), 4,7-dihydroxy-1,10-phenanthroline (0.085 g, 0.401 mmol, Alfa Aesar), and deionized water (10 mL). The reactor was degassed with Argon for 5 min prior to pressurizing to 15 psi. The reaction temperature was gradually increased to 100°C and the starting materials allowed to react for 24 h. Afterward, the reaction was cooled to room temperature prior to venting to the atmosphere. The solution was then acidified to pH 2-3 with 2N HCl and extracted with ethyl acetate. The combined organic fractions were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was further purified by column chromatography on silica gel using 1:1 hexane:ethyl acetate to obtain compound **1** as an off-white solid (0.581 g) in 77% yield: ¹H NMR (400 MHz, DMSO-d6): δ = 13.02 (s, 1H), 10.33 (s, 1H), 8.51 (d, J = 2 Hz, 1H), 8.21 (d, J = 9 Hz, 1H), 7.92 (dd, J = 2 Hz, 1H), 7.53 (d, J = 8 Hz, 1H), 7.40 (t, J = 8 Hz, 1H), 7.01 (d, J = 7.5 Hz, 1H). ¹³C NMR (400 MHz, DMSO-d6): δ = 168.00, 153.59, 134.01, 130.60, 128.66, 127.82, 126.80, 124.25, 122.94, 120.18, 110.77.

Synthesis of 5-hydroxy-2-naphthoic acid methyl ester (2). Compound **1**, was dissolved in methanol (30 mL, Fischer, ACS grade) and cooled to < -20 °C in a dry ice/isopropanol (IPA) bath for 10 min. Thionyl chloride (3 eq, Aldrich, 97%) was then slowly added. The reaction was subsequently allowed to warm to room temperature and then was heated under reflux for 19h. Afterward, the solvent was removed by rotary evaporation. The product was dissolved in ethyl acetate (30 mL) and washed with brine (12 mL). The organic phase was collected and dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was further purified by column chromatography on silica gel using 2:1 hexane:ethyl acetate to obtain **2** as a pale yellow solid (0.510 g) in 74% yield, post lyophilization (stored under Argon until use): ¹H NMR (400 MHz, DMSO-d6): δ = 10.39 (s, 1H), 8.53 (d, J = 2 Hz, 1H), 8.23 (d, J = 9 Hz, 1H), 7.92 (dd, J = 2 Hz, 1H), 7.56 (d, J = 8 Hz, 1H), 7.41 (t, J = 8 Hz, 1H), 7.02 (dd, J = 1 Hz, 1H), 3.91 (s, 3H). ¹³C NMR (400 MHz, DMSO-d6): δ = 166.90, 153.60, 133.94, 130.62, 128.04, 127.49, 126.89, 123.85, 123.20, 120.24, 111.02, 52.67. MS calculated for C₁₂H₁₀O₃: 202.06299, found: 202.06294.

Synthesis of 5-(hydroxymethyl)naphthalen-2-ol (3). To an Argon-charged round bottom flask equipped with an addition funnel was added **2** (510 mg, 2.52 mmol) and dry THF (25 mL, Acros). The solution was then cooled to -20 °C for 10 min in a dry ice/IPA bath. A slurry of lithium aluminum hydride (LAH, 191 mg, 5.03 mmol, Alfa Aesar, 97%) in dry THF (15 mL) was subsequently added dropwise over a period of 40 min while maintaining a temperature of < 0 °C. The mixture was then allowed to warm to RT and stirring continued overnight, under Argon. The reaction was quenched once reduction of the ester was observed by thin layer chromatography. Quenching of the reaction was accomplished by first cooling to 5 °C using an ice water bath. For 1 mg of LAH used in the reaction, 1 μ L of deionized water, 1 μ L of 15% KOH (aq), and 3 μ L of deionized water were added, in that order, to quench the reaction. Afterward, the reaction mixture

was stirred for an additional 5 h. The crude product was then filtered, and the filtrate was concentrated under vacuum to give **3** as a sticky green solid (0.340 g, 77% yield): ^1H NMR (600 MHz, DMSO-d6): δ = 10.52 (s, broad, 1H), 8.08 (d, J = 9 Hz, 1H), 7.71 (d, J = 1.5 Hz, 1H), 7.37 (dd, J = 2 Hz, 1H), 7.27 (m, 2H), 6.83 (dd, J = 2 Hz, 1H), 5.33 (s, broad, 1H), 4.64 (s, 2H). ^{13}C NMR (600 MHz, DMSO-d6): δ = 154.04, 140.63, 134.85, 127.01, 124.42, 124.21, 124.10, 122.41, 118.30, 108.14, 63.48. MS calculated for $\text{C}_{11}\text{H}_{10}\text{O}_2$: 174.06808, found: 174.06811.

Synthesis of 5-diethylamino-2-nitrosophenol (4). The synthetic procedure reported by Ramm, et al. was followed in this process.⁴⁸ 3-Diethylaminophenol (6 g, 0.0363 mol, Aldrich, 97%) was dissolved in 13 mL of 12 N HCl and 8 mL water and was then cooled to 0 °C using an ice water bath. Next, a solution of NaNO₂ (2.5 g, 0.036 mol) in 18 mL water was added dropwise over a period of 1 h while keeping the reaction temperature between 0 and 5 °C. The resulting brown slurry was stirred for an additional 4 h and the crude product was collected by filtration, yielding the hydrochloride salt as a yellow precipitate. The hydrochloride salt was subsequently converted to the free amine by diluting in 300 mL 0.1 M aqueous NaHCO₃ and extracting with dichloromethane. The combined organic fractions were dried over anhydrous sodium sulfate and concentrated under vacuum to give **4** as a brick-red solid (3.405 g) in 48% yield: ^1H NMR (600 MHz, DMSO-d6): δ = 7.31 (d, J = 10 Hz, 1H), 6.91-6.89 (m, 1H), 5.75 (d, J = 2.5 Hz, 1H), 3.61 (m, 4H), 1.19 (t, J = 7 Hz, 6H). ^{13}C NMR (600 MHz, DMSO-d6): δ = 169.26, 157.58, 149.59, 134.88, 115.84, 95.51, 46.1, 14.5. IR (polyethylene/cm⁻¹): 1629, 1514. MS calculated for $\text{C}_{10}\text{H}_{14}\text{O}_2\text{N}_2$: 194.10553, found: 194.10558.

Synthesis of 9-diethylamino-2-hydroxymethyl-5H-benzo[a]phenoxazine-5-one (5). To a round bottom flask was added **3** (0.340 g, 1.95 mmol), **4** (0.417 g, 2.15 mmol) and dry dimethylformamide (DMF) (45 mL). The flask was equipped with a condenser and the mixture

was stirred at reflux for 4.5 h, under an Argon atmosphere. The solvent was removed with a stream of nitrogen and mild heating. The crude product was purified by column chromatography on silica gel, using a 1:2 hexane:ethyl acetate mobile phase to give **5** as a dark violet solid (0.160 g) in 23% yield: ^1H NMR (400 MHz, DMSO-d6): δ = 8.50 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.60 (dd, J = 9.4, 2.4 Hz, 2H), 6.79 (dd, J = 9.1, 2.8 Hz, 1H), 6.61 (d, J = 2.7 Hz, 1H), 6.22 (s, 1H), 5.50 (t, J = 5.7 Hz, 1H), 4.71 (d, J = 5.6 Hz, 2H), 3.47 (q, J = 6.9 Hz, 4H), 1.15 (t, J = 7.0 Hz, 6H). ^{13}C NMR (400 MHz, DMSO-d6): δ = 182.33, 152.17, 151.15, 146.89, 146.76, 138.87, 131.94, 131.28, 128.32, 124.60, 125.41, 124.60, 121.04, 110.61, 104.91, 96.39, 63.10, 44.90, 12.91.

Synthesis of 9-diethylamino-5H-benzo[a]phenoxyazine-5-one-2-carboxaldehyde (6), NR-CHO. Compound **5** (0.160 g, 0.458 mmol) was dissolved in dry dichloromethane (70 mL) in a round bottom flask. A magnetic stir bar was added and the solution was placed under an Argon atmosphere. Pyridinium chlorochromate (0.197 g, 0.916 mmol, Frontier, 98%) (PCC) was next added portion-wise over 1 h. The reaction mixture was stirred at room temperature overnight under Argon and quenched when thin layer chromatography showed complete oxidation of the alcohol. Excess PCC was quenched by slowly adding isopropanol (17 eq) and stirring for an additional 30 min. The solvent was removed by rotary evaporation and the resulting solid was dissolved in deionized water and extracted with ethyl acetate. The combined organic fractions were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography on silica gel using 1:1 hexane:ethyl acetate as the mobile phase, giving **6** as a purple solid (0.041 g) in 29% yield: ^1H NMR (400 MHz, DMSO-d6): δ = 10.27 (s, 1H), 9.07 (dd, J = 2 Hz, 1H), 8.30 (d, J = 8 Hz, 1H), 8.15 (dd, J = 2 Hz, 1H), 7.71 (d, J = 9 Hz, 1H), 6.91 (dd, J = 3 Hz, 1H), 6.72 (d, J = 3 Hz, 1H), 6.39 (s, 1H), 3.54 (q, J = 7 Hz, 4H), 1.19 (t, J = 7 Hz, 3H). ^{13}C NMR (400 MHz, DMSO-d6): δ = 193.72, 181.51, 152.91, 151.79,

147.20, 138.15, 137.62, 134.74, 132.54, 131.70, 129.00, 126.54, 125.15, 111.35, 105.53, 96.49, 45.06, 29.48, 12.94. MS calculated for $C_{21}H_{18}O_3N_2$: 346.37922, found: 346.37920.

RESULTS AND DISCUSSION

Cs(Zr,Mg)-SiO₂ catalyst structural characterization. The XRD patterns obtained from fumed silica, and the Cs(Zr,Mg)-SiO₂ and MgO catalysts are shown in Figure 1. Broad peaks centered near $2\theta = 21^\circ$ were observed for the Cs(Zr,Mg)-SiO₂ catalyst and SiO₂, as is characteristic of silica-based materials;¹⁰ the weaker broad peak of the former confirms that the silica structure has been modified by impregnation of the metal nitrates.^{10, 14, 49} Hexagonal cesium nitrate was also observed in the Cs(Zr,Mg)-SiO₂ pattern, as evidenced by the sharp peaks centered at $2\theta = 19.9, 28.2, 34.8, 40.3, 45.4$ and 50° , while no magnesium nitrate or zirconium nitrate species were detected. The latter suggests these components are well dispersed in the silica matrix.¹⁴ Sharp peaks at $2\theta = 42.8$ and 62.1° , and weaker peaks at $2\theta = 37, 74.7$ and 78.7° were observed in the MgO XRD pattern and were attributed to the presence of its cubic phase.⁵⁰

SEM and TEM images of the catalysts are shown in Figure 2. SEM images of the MgO catalyst and fumed silica (see Figures 2A,C) show agglomerated spherical particles of various sizes. When Mg, Zr, and Cs were loaded into the silica, the catalyst became more aggregated, as evidenced by the TEM image in Figure 2F. The surface area and porosity results for these materials are summarized in Table 1. The corresponding nitrogen sorption isotherms and pore size distributions are shown in Figures S1, S2.

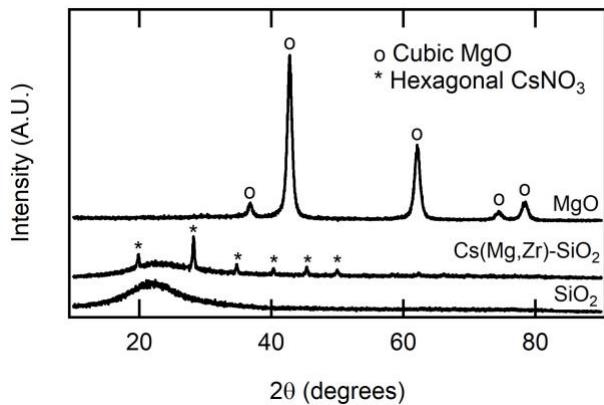


Figure 1. X-Ray diffraction patterns from fumed silica along with Cs(Zr,Mg)-SiO₂ and MgO catalysts.

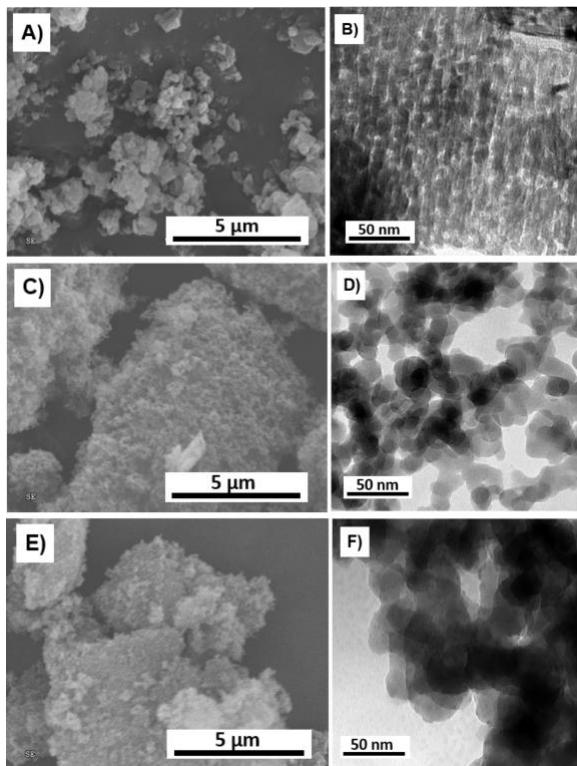


Figure 2. **A), B)** SEM and TEM images of commercial MgO catalyst particles. **C), D)** SEM and TEM images of SiO₂ particles. **E), F)** SEM and TEM images of Cs(Zr,Mg)-SiO₂ particles.

Catalyst chemical composition. EDS did not detect Mg or Zr on the Cs(Zr,Mg)-SiO₂ catalyst due to their low loadings of 0.025 wt.% and 0.033 wt.%, respectively, while a Cs content of 12.93 wt.% was measured, in agreement with the theoretical loading of 13 wt.%. ICP-OES

measurements detected all three metals. In this case, the metal contents were determined to be 0.021 ± 0.001 wt.% for Mg, 0.0051 ± 0.0003 wt.% for Zr, and 11.4 ± 0.4 wt.% for Cs. The error bars give the 95% confidence-intervals with $n = 6$ for Mg and Zr, and $n = 9$ in the case of Cs. While the values obtained for Cs and Mg were close to their theoretical loadings, Zr was lower than expected. This may be due to competition between the metals for active sites on the silica.⁴² The Mg content in commercial MgO was 53.18 wt.%.

XPS spectra (see Figure S2) show the Cs (3d) peaks for the Cs(Zr,Mg)-SiO₂ catalyst, but Mg and Zr were not detected due to their low loadings. The Si (2p) peak is also observed for fumed silica, the Mg (1s) peak for MgO, and the O (1s) peak for both catalysts and pure silica. The binding energies for the active components in the catalysts were 724.6 eV - 742.7 eV for suboxides of Cs in Cs(Zr,Mg)-SiO₂ and 1304.2 eV for Mg-O in MgO.

The density of base and acid sites on the catalysts were quantified by stepwise temperature-programmed desorption (TPD) of CO₂ and NH₃. The results of these experiments are shown in Figure S3. A higher desorption temperature indicates stronger basicity or acidity, while the area under each desorption peak is proportional to the number of active sites.⁵¹ The desorption profile for SiO₂ showed no base or acid sites while that of the Cs(Zr,Mg)-SiO₂, and MgO were found to have base and acid sites of various strengths.

The CO₂ desorption profile for Cs(Zr,Mg)-SiO₂ showed prominent peaks in the 75 - 175 °C range (weak base sites) and the 275 - 475 °C range (medium strength base sites), while for MgO, a broad CO₂ desorption peak in the 75 - 475 °C range (weak-medium base sites) was observed. The NH₃ desorption profile for Cs(Zr,Mg)-SiO₂ also showed one peak in the 75 - 175 °C range (weak acid sites) and a second, broader peak in the 250 - 475 °C range (medium strength acid sites). The commercial MgO catalyst showed a prominent desorption peak in the 200 - 300

°C range, corresponding to medium strength acid sites. The corresponding base and acid site densities determined from Figure S3 are shown in Table 1.

Elemental analyses and TPD data suggest that the metal nitrates in the Cs(Zr,Mg)-SiO₂ catalyst aid in enhancing the base and acid properties of the silica support. The differences in strength and quantity of these sites within the catalyst led to differences in aldol product formation compared to the MgO catalyst, as will be discussed below.

Table 1. Chemical and structural properties of the catalysts

Catalysts	Basic site density ($\mu\text{mol CO}_2/\text{g}$)	Acid site density ($\mu\text{mol NH}_3/\text{g}$)	Metal content (%)	Total Surface Area _{BET} (m^2/g)	Average Pore Diameter _{BJH} (nm)	Total Pore Volume (cm^3/g) at $p/p_0=0.99$
MgO	820	791	Mg=53	119	8	0.32
SiO ₂	0	0	-	194	12	0.50
Cs(Mg,Zr)-SiO ₂	252	143	Cs=13, Mg<0.1 Zr<0.1	87	30	0.94

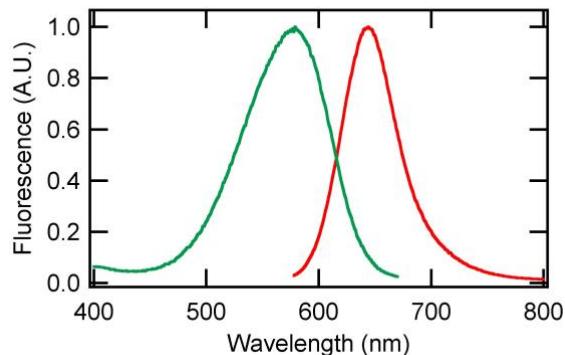
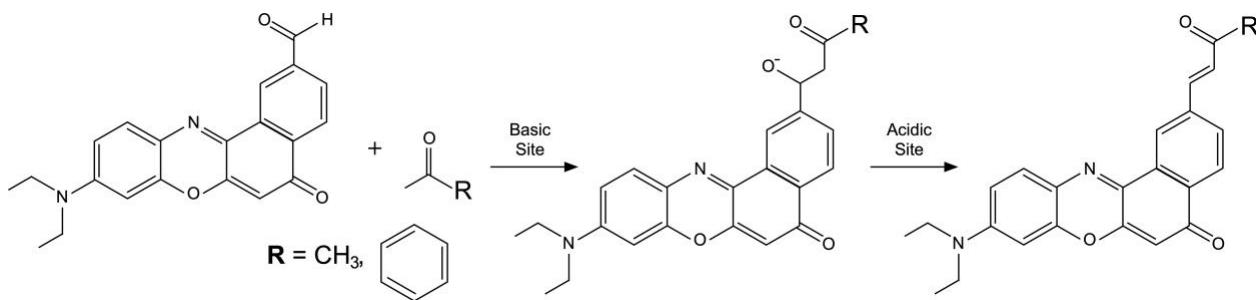


Figure 3. Fluorescence excitation (green) and emission (red) spectra of 1 μM NR-CHO in DMSO.



Scheme 2. Base-catalyzed aldol addition reactions, followed by acid-catalyzed dehydration reactions to form the aldol condensation products for reactions of NR-CHO (**6**) with ketones having **R** = methyl or phenyl.

Ensemble Fluorescence Studies of Aldol Reactions

Static spectra. Static fluorescence excitation and emission spectra of NR-CHO (1 μ M) in DMSO are shown in Figure 3. The dye is most efficiently excited near 580 nm and emits most strongly near 645 nm. Static spectra of the newly synthesized NR-CHO were compared to that of commercial Nile Red under identical experimental conditions (Figure S4). The emission spectrum of NR-CHO is shifted \sim 18 nm to the red of commercial Nile Red.

Aldol reactions. Aldol reactions may be catalyzed by either base or acid.^{12, 52-53} In this work, Cs(Zr,Mg)-SiO₂ having substantial base and acid sites was employed to catalyze reactions of NR-CHO (1 μ M) in DMSO with acetone or acetophenone. The results obtained were compared to those of reactions catalyzed by commercially available MgO, while fumed silica served as a control. All reactions were conducted by stirring at room temperature.

Cs(Zr,Mg)-SiO₂ was chosen due to the extensive literature showing that aldol reactions are significantly enhanced when bifunctional catalysts incorporating both weak acid and base sites are employed.^{15, 18, 45} It is believed that basic sites catalyze the aldol addition step, forming an alcohol, while acidic sites catalyze subsequent condensation to form the olefin product, as shown in Scheme 2. In Cs(Zr,Mg)-SiO₂, the cesium nitrate is believed to provide the base sites and some acid sites,

while SiOH groups on the silica likely provide additional acid functionality. The magnesium and zirconium aid in improving and maintaining the performance of the catalyst.^{9, 42}

The progress of crossed aldol reactions was monitored in real time by recording a series of fluorescence spectra at different time intervals. Figure 4 depicts the fluorescence spectra obtained. These data show notable differences in how the spectra evolve in time when either Cs(Zr,Mg)-SiO₂ or MgO was used as the catalyst.

When Cs(Zr,Mg)-SiO₂ was employed, a progressive blue shift in the emission spectrum of the dye was observed as the reaction proceeded. Aldol reactions with acetone and acetophenone showed gradual blue shifts of 16 nm and 8 nm, respectively, over the time the data were acquired (see Figures 4A,D). The gradual shift in fluorescence exhibited by the dye is concluded to result from formation of two different products, the addition and condensation products, in the presence of residual starting material, as confirmed by HPLC-MS, see below. Note that the reaction between NR-CHO and acetone catalyzed by Cs(Zr,Mg)-SiO₂ was faster than the others. The spectral shift to the blue appeared to be complete by 2 h in this case. In contrast, the reaction between NR-CHO and acetophenone took at least 8 h for the full spectral shift to develop. The differences in rates for aldol reactions using acetone and acetophenone is attributable to structural differences in these ketones. The reaction with acetone may be faster due to the presence of two alpha carbons, providing two acidic hydrogens that may make the first step of the reaction more kinetically favored. Alternatively, the incorporation of the phenyl group on the acetophenone precursor may make its reaction with NR-CHO more sterically hindered and slower.

When MgO served as the catalyst, NR-CHO reactions with both ketones led to the appearance of a new peak that grew in time as the reaction progressed, giving an apparent isoemissive point in the spectra (see Figures 4B, E). In both cases, the original fluorescence peak

centered at \sim 642 nm subsided, while a new peak centered at \sim 615 nm grew over time. The appearance of two peaks and an isoemissive point are consistent with the presence of two different species in the reaction mixture, likely NR-CHO and a new product, as confirmed by HPLC-MS, see below. As in the case of reactions catalyzed by $\text{Cs}(\text{Zr,Mg})\text{-SiO}_2$, the reaction with acetone was quicker than when acetophenone was employed. Reaction mixtures with SiO_2 resulted in no apparent fluorescence shift, as shown in Figures 4C, F, suggesting no reaction occurred. This may be attributed to insufficient base or acid sites in the SiO_2 to catalyze the reaction.⁵⁴ Note that the TPD analysis shown in Figure S3 revealed no detectable base or acid sites on the silica. The stability of the NR-CHO dye in the presence of the active heterogeneous catalysts employed in this work was confirmed using solutions prepared without the ketone, see Figure S6.

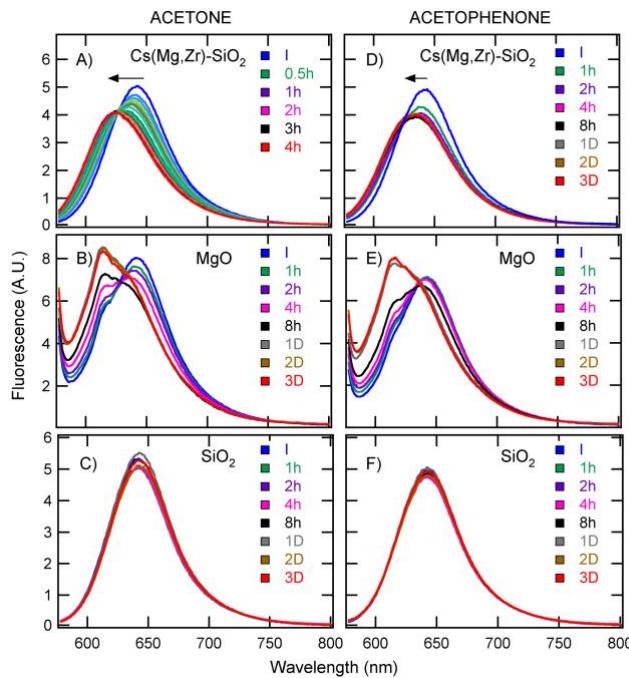


Figure 4. **A) - C)** Fluorescence from NR-CHO as a function of time in the presence of the catalysts listed with acetone employed as the ketone. **D) - F)** Fluorescence from NR-CHO as a function of time in the presence of the catalysts listed with acetophenone employed as the ketone. Fluorescence was excited at 575 nm in each case. DMSO was employed as the solvent. All reactions were stirred at room temperature.

HPLC and LC-MS Studies of Aldol Product Formation

The differences in spectral behavior for the Aldol reactions catalyzed by Cs(Zr,Mg)-SiO₂ and MgO were attributed to the formation of different products. Analytical HPLC and HPLC-MS were employed to confirm that different products were formed in each case and to help identify these products. Representative chromatograms are provided in Figure 5 and the mass spectra used to assign each peak to the different products are given in Figure S5. As noted above, these reactions employed a higher concentration of NR-CHO (1mM) to facilitate product recovery and detection. The aldol reactions in this case were run for an arbitrary time of 3 days (see *kinetic measurements* below for further discussion).

Chromatograms obtained from aldol reactions employing Cs(Zr,Mg)-SiO₂ as the catalyst with acetone as the reactant were dominated by two peaks (see Figure 5A). HPLC-MS data allowed these two peaks to be assigned to the addition and condensation products (see Figure S5). The aldol addition product for this reaction gave a peak at 405 atomic mass units (amu, m/z) in the MS data, as expected, while the aldol condensation product gave a peak at 387 amu, as expected. Note that the mass resolution in this instrument is \pm 1 amu. Separations performed using starting material alone revealed that one of the minor peaks was produced by residual NR-CHO. When acetophenone was employed as the reactant, the chromatograms obtained incorporated three peaks (see Figure 5C). HPLC-MS results (see Figure S5) allowed assignment of these peaks to the addition and condensation products, and residual NR-CHO. The chromatograph peak assigned to the aldol addition product gave a MS peak at 467 amu, while the condensation product produced a MS peak at 449 amu. In contrast, when MgO was employed as the catalyst, chromatograms obtained from both the acetone and acetophenone reaction mixtures were dominated by a single peak (see Figures 5B,D). HPLC-MS data obtained revealed these peaks were due to predominant

formation of the addition products and very little of the condensation products. The acetone addition product gave a MS peak at 405 amu, with the aldol condensation product yielding a MS peak at 387 amu. With acetophenone, the aldol addition product gave MS peaks at 467 amu and 449 amu for the addition and condensation products, respectively. These results provide strong support for the conclusions drawn from the spectroscopic data. The relatively large amount of unreacted NR-CHO found in the reaction mixture with acetophenone when Cs(Zr,Mg)-SiO₂ was employed as the catalyst provides support for the conclusion that the kinetics are slowed as a result of ketone structure. The much smaller NR-CHO peak found when MgO was employed suggests it may be better than Cs(Zr,Mg)-SiO₂ at catalyzing the first step of the aldol reaction, while the Cs(Zr,Mg)-SiO₂ is better able to catalyze the subsequent condensation step. A more detailed discussion is given below.

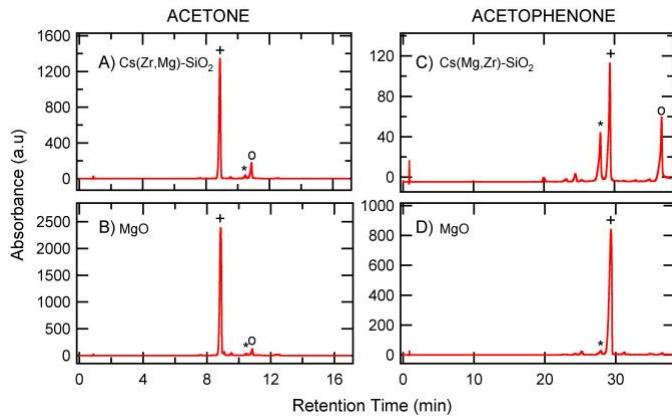


Figure 5. HPLC chromatograms (575 nm absorbance) of aldol products from 3-day reactions of NR-CHO (1mM) with acetone (**A**, **B**), and acetophenone (**C**, **D**) in the presence of Cs(Zr,Mg)-SiO₂ or MgO at room temperature. The chromatograms show the un-reacted NR-CHO (*), the aldol addition product (+), and the aldol condensation product (o). For **A**, **B**, the mobile phase gradient comprised H₂O and acetonitrile mixtures at 0.800 mL/min flow rate with the following compositions: 0-10 min: 95-40% H₂O; 10-15 min: 40-20% H₂O; 15-17 min: 20% H₂O. For **C**, **D** the mobile phase compositions were: 0-35 min: 95-45% H₂O; 35-36 min: 45-20% H₂O; 36-38 min: 20% H₂O. A Thermo Scientific Hypersil GOLD C18 column (100 X 3 mm², 3 μ m particle size) was employed.

It is believed that the HPLC and HPLC-MS results for aldol reactions run at the higher NR-CHO concentration (1 mM) also reflect the products formed at the lower NR-CHO concentration (1 μ M) employed in the spectroscopic studies. Therefore, the isoemissive points observed in aldol reactions catalyzed by MgO are attributed to the presence of primarily the un-reacted NR-CHO and the newly formed aldol addition product. Likewise, the gradual shifts in the spectra observed from aldol reactions employing the Cs(Zr,Mg)-SiO₂ catalyst (see Figures 4A, C) are attributed to the presence of time varying concentrations of un-reacted NR-CHO, the aldol addition product, and the aldol condensation product. Furthermore, spectra of the pure aldol products showed that both are blue shifted (alcohol by \sim 13-14 nm and olefin by \sim 2-4 nm), see Figure S7. The relatively small spectral blue shift of the olefin suggests that its fluorescence spectrum might be masked by unreacted NR-CHO in the reaction mixture. Since MgO primarily catalyzes aldol addition product formation, with its larger spectral shift, a clear peak at \sim 615 nm is distinguished in that mixture.

Aldol Reaction Kinetics

The kinetics of the crossed aldol reactions were followed using the fluorescence data shown in Figure 4. For this purpose, the fluorescence signals in two spectral bands spanning 642 ± 5 nm and 615 ± 5 nm were obtained and plotted as a function of time. This method was chosen over the usual procedure of fitting the data to multiple Gaussian functions because the latter did not provide robust, easily interpreted results on the disappearance of NR-CHO and the formation of products. Figure 6 shows the results of this analysis. These data show a time-dependent decrease in the 642 nm band that is concluded to reflect consumption of NR-CHO. Similarly, a time-dependent increase in the 615 nm band is observed that is interpreted to reflect appearance of the product(s). Note that control experiments employing fumed silica showed neither evidence for consumption of starting material nor appearance of product(s) (see Figure 6C,F).

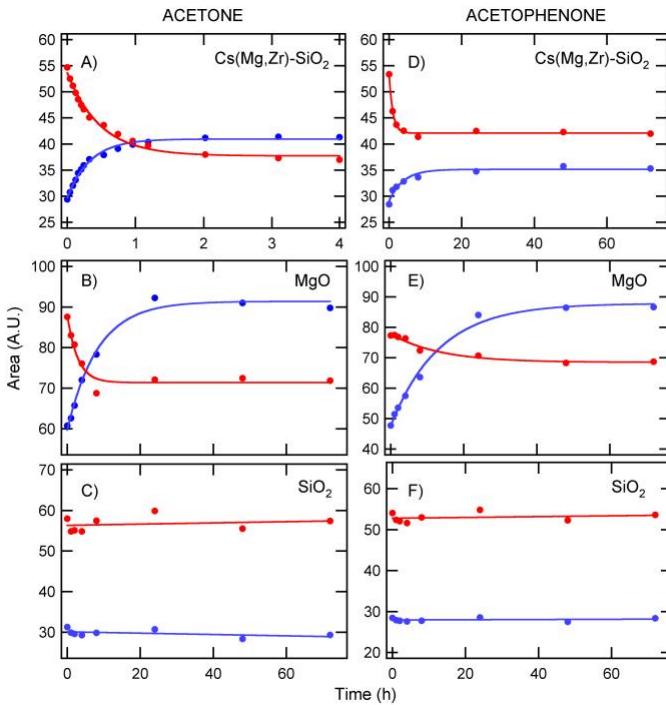


Figure 6. Fluorescence signals in bands spanning 642 ± 5 nm (red data points) and 615 ± 5 nm (blue data points) plotted as a function of time for crossed aldol reactions with acetone, **A)-C**), and acetophenone, **D)-F**). These data were obtained from Figure 4A-F. Data are given for reactions catalyzed by Cs(Zr,Mg)-SiO₂, **A**, **D**), as well as MgO, **B**, **E**), and the silica control, **C**, **F**). The solid lines have been added to better depict the trends in the data. Note that the NR-CHO reaction with acetone catalyzed by Cs(Zr,Mg)-SiO₂ is significantly faster than the others.

The data in Figures 6A,B and 6D,E were employed to determine the rate of disappearance of NR-CHO and the rate of formation of aldol product(s). These rates were obtained by fitting the curves to single exponentials. Estimated rates of disappearance of NR-CHO in the reactions with acetone employing Cs(Zr,Mg)-SiO₂ and MgO catalysts were 2.1 ± 0.2 h⁻¹ and 0.4 ± 0.1 h⁻¹, respectively, while the rates of aldol product formation were found to be 3.0 ± 0.2 h⁻¹ and 0.12 ± 0.02 h⁻¹, respectively. Similarly, the estimated rates of disappearance of NR-CHO in the reactions with acetophenone catalyzed by Cs(Zr,Mg)-SiO₂ and MgO were 1.0 ± 0.1 h⁻¹ and 0.08 ± 0.02 h⁻¹, respectively, while the rates of aldol product formation were 0.3 ± 0.1 h⁻¹ and 0.08 ± 0.01 h⁻¹,

respectively. Since the ketones were present at much higher concentrations than the NR-CHO, these rates represent estimates of the pseudo-first-order rate constants for these reactions. It is noteworthy that HPLC-MS did not reveal significant self-condensation of acetone. The above results show some complexities in interpreting the rates of aldol reactions obtained using fluorescence spectroscopy.

Catalyst Dependent Product Formation

The results given above show that the aldol reactions catalyzed by Cs(Zr,Mg)-SiO₂ produced both addition products (alcohols) and condensation products (olefins). In contrast, those catalyzed by MgO produced primarily addition products and little condensation products. The fluorescence results shown in Figure 4, demonstrate that the nature of the NR-CHO blue shift as the reaction progresses is diagnostic for catalyst activity. Catalysts that form a single product, the alcohol, produce spectra incorporating an isoemissive point. Catalysts that produce both addition and condensation products yield fluorescence spectra that gradually shift to the blue. The observed differences in the two catalysts are attributed to differences in surface area, as well as differences in acid and base site densities and strengths. Since aldol condensation reactions involve two distinct reaction steps, these will be addressed separately below.

The likely mechanism for the addition reaction and the factors governing its rate will be discussed first. Strong bases are commonly used as homogeneous catalysts in aldol reactions.⁵⁵ Therefore, strong base sites are likely to be important in initiating the reactions explored here on heterogeneous catalysts. Indeed, the results shown in Table 1 and in Figure S3 demonstrate that MgO has a much greater density of base sites (820 vs. 252 μmol CO₂/g in TPD experiments) and that the density of the stronger sites (CO₂ desorbed at 250 - 475 °C) is much greater than is the case for Cs(Zr,Mg)-SiO₂ (326.7 vs. 86.6 μmol CO₂/g in TPD experiments). The strong base sites

are best at abstracting acidic hydrogens on the alpha carbon(s) of the ketone. The nucleophilic enolate carbanion formed then attacks the electron-poor carbon of the aldehyde, forming the addition product. The literature on heterogeneous inorganic catalysts also suggests that the metal centers (M) can polarize the precursor carbonyls, increasing their reactivity.^{17, 56} The greater density of strong base sites and the greater metal content of the MgO is thus consistent with the predominant formation of addition product.

The MgO catalyst was also found to have greater surface area than the Cs(Zr,Mg)-SiO₂ materials (see Table 1). The greater base strength, greater base site density, and greater surface area of the MgO suggests product formation should be faster in reactions where it is employed as the catalyst. However, the results shown in Figure 6 indicate these reactions are actually slower. The apparently slower kinetics of the addition reaction in the case of MgO may be due to the reversibility of this reaction, with the retro-Aldol reaction also being relatively fast.

A common theme in the modern literature on heterogeneous aldol reactions is that bifunctional catalysts are best suited to formation of the condensation products.^{9, 16, 54, 56} This prior work also demonstrates that weaker acid sites better catalyze condensation than do strong acid sites.^{15, 17} Strong acid sites are believed to react with basic sites in close proximity, forming catalytically inactive ion pairs.^{8, 13} In contrast, weak acid sites are believed to remain active and can interact with the aldol addition products. The results shown in Figure S3 demonstrate that Cs(Zr,Mg)-SiO₂ incorporates more catalytically active weak acid sites (NH₃ desorbed at 75-175 °C) than does the MgO (NH₃ desorbed at 200-300°C). The improved catalytic activity of Cs(Zr,Mg)-SiO₂ toward aldol condensation may be due to surface silanol groups intrinsic to the silica support as well as the presence of some water, which together facilitate proton exchange in the bifunctional catalyst.^{15, 18} These weak acid sites act to protonate the products of the addition

reaction, catalyzing irreversible dehydration to form the condensation product (the olefin).^{5, 56} It is this final step that is believed to lead to relatively fast and irreversible conversion of the addition products in the Cs(Zr,Mg)-SiO₂ catalyzed reactions to condensation products.

While the model given above is most consistent with the present results, alternatives have also been discussed in the literature. For example, recent work claims that proton equilibration with the anion is fast and that addition product formation is the rate-limiting step.⁵⁵ While the addition reaction may be rate limiting, the present results are consistent with the conclusion that formation of the condensation product is critical to preventing the retro-aldol addition reaction from occurring. An alternative mechanism that has also been proposed is that the carbonyl on the aldehyde can be activated by protonation by the weak acid (Si-OH) sites. Nucleophilic attack of the enolate formed by interaction of the ketone with the basic sites then forms the addition product which then loses water to yield the condensation product.¹⁷ The authors state that by this acid-base cooperative mechanism, a strong base may be replaceable by a weaker base as long as the acid sites are strong enough to activate one of the reactants.¹⁷ This mechanism does not provide a satisfactory explanation for the present results because the MgO catalyst, which contains stronger acid sites, forms little of the condensation products. The exact mechanism of aldol product formation in the present studies has not yet been fully elucidated and will require further work.

Comparison of NR-CHO to a Commercially Available Dye

As a final demonstration of the utility of the NR-CHO dye in fluorescence studies of aldol reactions, its performance was compared to that of commercially available 3-perylene-carboxaldehyde. These studies are described in Supplementary Information. While the NR-CHO dye in the presence of catalyst produced spectral shifts of 27 nm upon formation of the addition product and smaller shifts of 16 nm and 8 nm when the condensation products were also formed,

the commercial dye showed little or no change in its fluorescence emission spectrum, as shown in Figure S8. It should be noted that coumarin dyes incorporating either aldehyde or ketone moieties also show unique spectral shifts in response to aldol product formation.⁵⁷ However, these dyes are not well suited to single molecule studies, and the reactions reported were also performed at higher temperatures. In contrast, the aldol reactions presented here employed the NR chromophore widely used in single molecule studies,³⁶⁻³⁷ and were run at room temperature, a condition more amenable to optical microscopic experiments. As a final caveat, it should be noted that spectral shifts similar to or larger than those exhibited by NR-CHO during addition product formation will be required for selective product detection in single molecule studies. The results obtained here suggest that it would be challenging to distinguish the condensation products from starting NR-CHO in single molecule studies. Further investigations using other ketones may afford greater spectral shifts.

CONCLUSIONS

In summary, we have synthesized a new form of the Nile Red dye, NR-CHO, incorporating a reactive aldehyde moiety to probe aldol reactions in real time through ensemble fluorescence measurements. Fluorescence from the dye exhibits a measurable blue shift when aldol addition and condensation products are formed. Catalysts that lead to formation of the addition product yield time-variant NR-CHO fluorescence spectra incorporating an isoemissive point, consistent with formation of a single product. When the condensation product is also formed, the fluorescence spectra instead exhibit a gradual shift to the blue, due to the presence of addition and condensation products, along with residual starting material. These observations allow for preliminary conclusions on catalyst activity to be drawn.

The NR-CHO fluorescence results in model reactions showed that product formation was dependent upon the type of catalyst employed. These observations were confirmed by HPLC and HPLC-MS characterization of the reaction mixtures. Reactions catalyzed by MgO yielded predominantly aldol addition products, while those catalyzed by Cs(Zr,Mg)-SiO₂ produced both addition (alcohol) and condensation (olefin) products. The Cs(Zr,Mg)-SiO₂ was shown to be more catalytically active towards olefin formation than MgO according to both fluorescence studies of the reaction kinetics and HPLC data. Detailed analysis of the base and acid site densities and strengths demonstrated that the MgO catalyst contained the strong base sites necessary to catalyze the addition reaction, but that the acid sites also present were likely too strong to give the condensation product. In contrast, the Cs(Zr,Mg)-SiO₂ catalyst was found to incorporate both the basic sites needed to initiate the addition reaction and weak acid sites of appropriate strength to drive dehydration and irreversible formation of the olefin. Fumed silica was employed as a control and showed no activity due to the absence of active basic and acidic sites. The newly synthesized NR-CHO was shown to be much more useful in following crossed aldol reactions than a commercially available 3-perylene carboxaldehyde, which showed little change in its fluorescence spectra during these reactions. The results presented here show that the NR-CHO dye is an effective fluorescent probe for *in situ* studies of aldol reactions of heterogeneous catalysts at the ensemble level. The dye may also have promising future applications in studies of these same reactions at the single molecule level. Similar single molecule studies have been published describing investigations of other molecular transformations in organic chemistry.⁵⁸⁻⁶⁰

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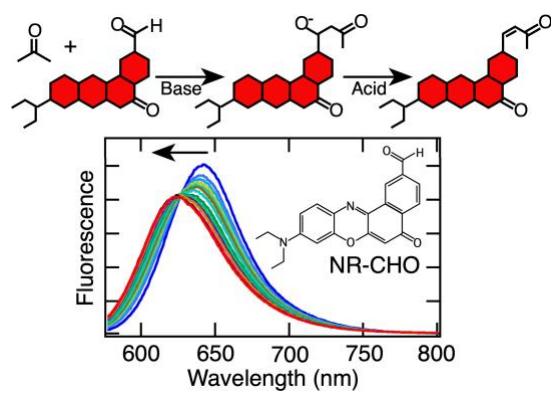
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TOC Art



A highly fluorescent, aldol-reactive derivative of the dye Nile Red is synthesized and evaluated as an *in situ* probe of crossed aldol reactions.

SUPPLEMENTARY INFORMATION

Fluorescence Spectroscopy Studies of Crossed Aldol Reactions: A Reactive Nile Red Dye Reveals Catalyst-Dependent Product Formation

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Additional data showing the characterization of the two catalysts employed, Cs(Mg,Zr)-SiO₂, and MgO, as well as fumed silica (SiO₂) are provided. The data show nitrogen adsorption and desorption isotherms and mesopore size distributions (**Figure S1**), XPS spectra (**Figure S2**), and CO₂ and NH₃ temperature programmed desorption curves (**Figure S3**). Data showing fluorescence spectra of NR-CHO compared to commercial Nile Red (**Figure S4**) are also given. MS data from aldol products produced in 3-day reactions of NR-CHO with acetone and acetophenone on Cs(Mg,Zr)-SiO₂ and MgO catalysts are included (**Figure S5**). The stability of NR-CHO in contact with the active heterogeneous catalysts is investigated (**Figure S6**). Fluorescence spectra of the pure aldol products from NR-CHO reactions with acetone or acetophenone are provided (**Figure S7**). HPLC chromatograms were also obtained to show the ratio of aldol products produced in reactions employing Cs(Mg,Zr)-SiO₂ catalysts (**Figure S8**). Finally, additional data showing results from similar aldol reactions run with commercially available 3-perylene carboxaldehyde are provided. The data show the static fluorescence spectra of the dye in DMSO (**Figure S9**), as well as fluorescence spectra of aldol reactions comparing Cs(Mg,Zr)-SiO₂ and MgO catalysts (**Figure S10**).

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Nitrogen Sorption Isotherms

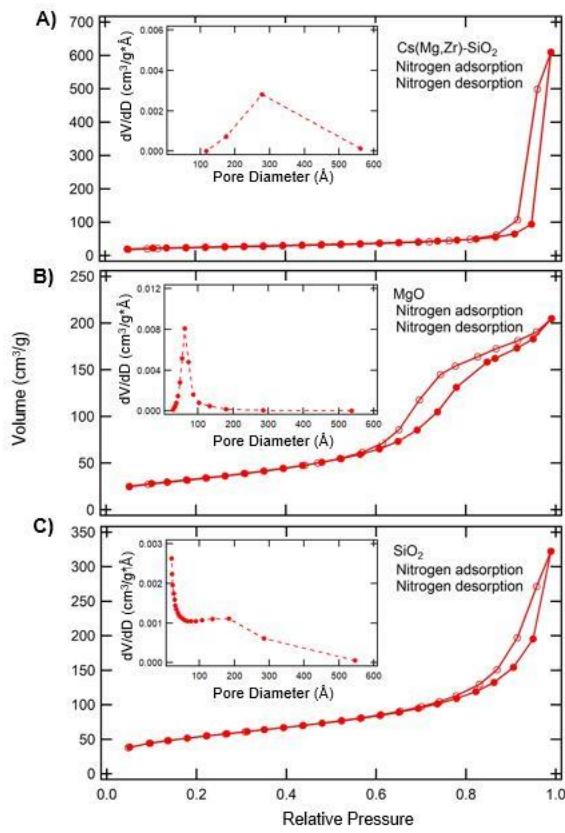


Figure S1. Nitrogen adsorption (filled circles) and desorption (open circles) isotherms of mesoporous catalysts Cs(Mg,Zr)-SiO₂ (A), MgO (B), and fumed silica (C). The insets denote the pore-size distribution of each and were obtained from the respective desorption isotherm.

Catalyst Composition

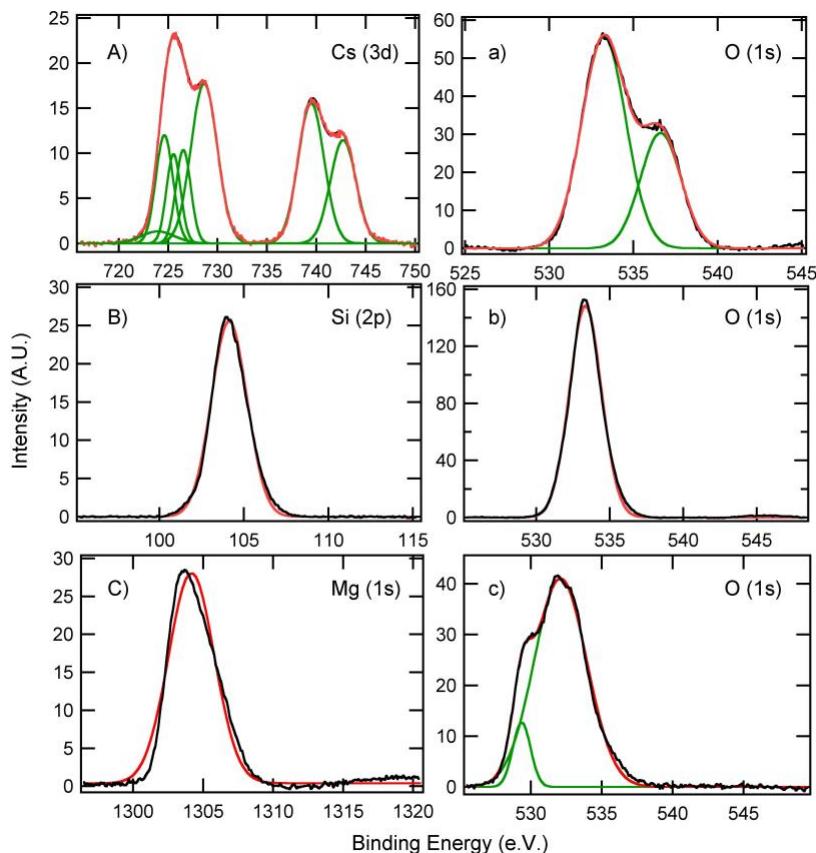


Figure S2. XPS spectra of the Cs(Mg,Zr)-SiO₂ catalyst. The Cs (3d) spectrum **(A)** includes peaks at 725.6 and 739.8 eV corresponding to Cs 3d_{5/2} and Cs 3d_{3/2} from the Cs⁺ ion.¹ The peaks at 724.6 eV, 725.5 eV are attributed to Cs₂O₂,² and Cs₂O,³ while those at > 726 eV are attributed to suboxides of Cs.⁴ The O (1s) spectrum **(a)** includes a peak at 533.2 eV due to Si-O (Si-O-Si, Si-O-H) from the SiO₂ support.⁵ XPS spectra of SiO₂ **(B)** reveal the Si (2p) peak at 104.1 eV due to Si-O₂,⁶ while its O (1s) spectrum **(b)** gives a peak 533.2 eV due to Si-O (Si-O-Si, Si-O-H) from the SiO₂ support.⁵ XPS spectra of the MgO catalyst **(C)** include the Mg (1s) peak at 1304.2 eV due to Mg-O,⁷ with its O (1s) spectrum **(c)** incorporating a peak at 529.4 eV attributable to lattice oxygen O²⁻. The higher binding energy peak at 532.0 eV can be attributed to adsorbed oxygen, weakly bound oxygen, or to surface hydroxyl species.⁸⁻⁹

Catalyst Basicity and Acidity

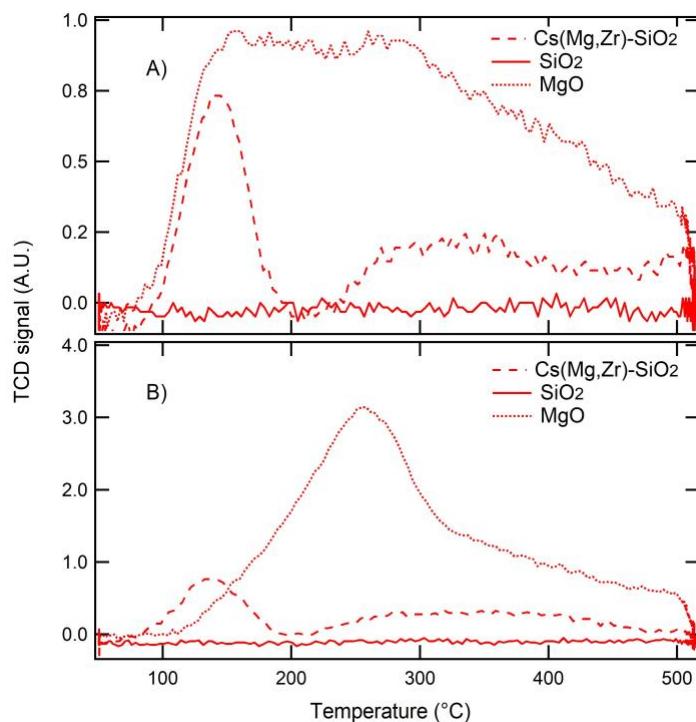


Figure S3. **(A)** CO₂ and **(B)** NH₃ temperature programmed desorption curves of Cs(Mg,Zr)-SiO₂, SiO₂ and MgO catalysts. Higher desorption temperatures indicate stronger basicity or acidity while the area under the desorption peaks provides the number of active sites.¹⁰ The desorption profile for SiO₂ showed no detectable basic or acid sites. The CO₂ desorption profile for Cs(Mg,Zr)-SiO₂ showed two prominent peaks at 75-175°C (weak strength basic sites) and 275-475°C (medium strength basic sites) while for MgO, a broad CO₂ desorption peak at 75-475°C (weak-medium strength sites) was observed. The NH₃ desorption profile for Cs(Mg,Zr)-SiO₂ also showed peaks at 75-175°C (weak strength acid sites) and at 250-475°C (medium strength acid sites). The commercial MgO catalyst showed a prominent desorption peak at 200-300°C, corresponding to medium strength acid sites.

NR and NR-CHO Fluorescence Spectra

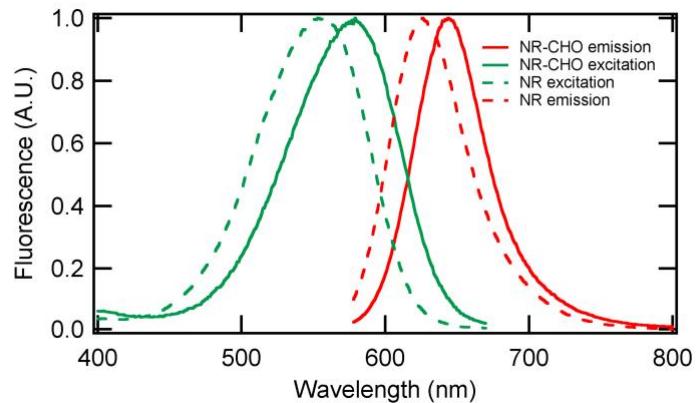
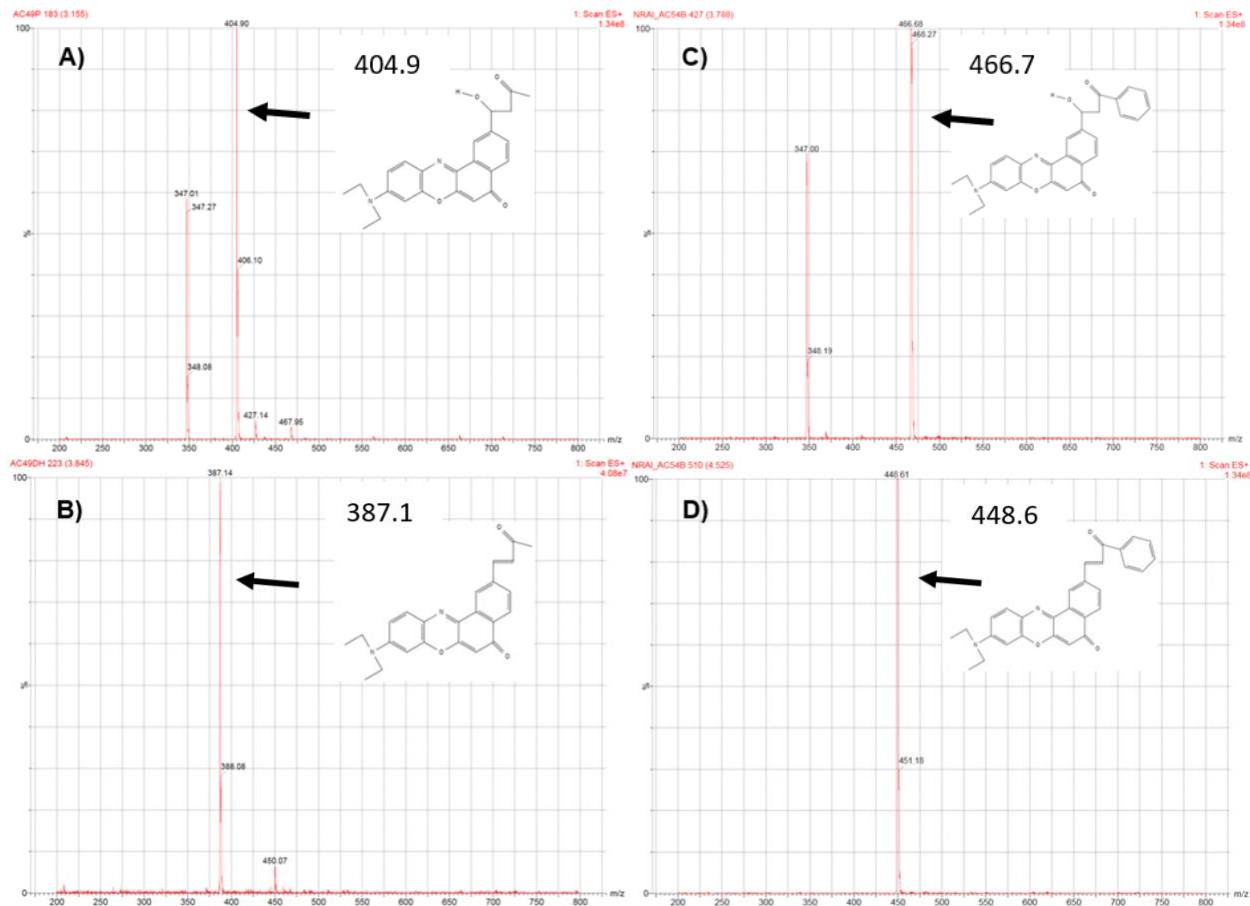


Figure S4. Normalized fluorescence excitation (green) and emission (red) spectra of 1 μ M commercial Nile Red (NR) and 1 μ M (NR-CHO) in DMSO. The absorbance and emission spectra of the newly synthesized derivative of Nile Red are shifted \sim 24 nm and \sim 18 nm respectively to longer wavelengths compared to the commercial Nile Red.

Aldol Reaction Product Mass Spectra

Aldol Reactions Catalyzed by Cs(Mg,Zr)-SiO₂



Aldol Reactions Catalyzed by MgO

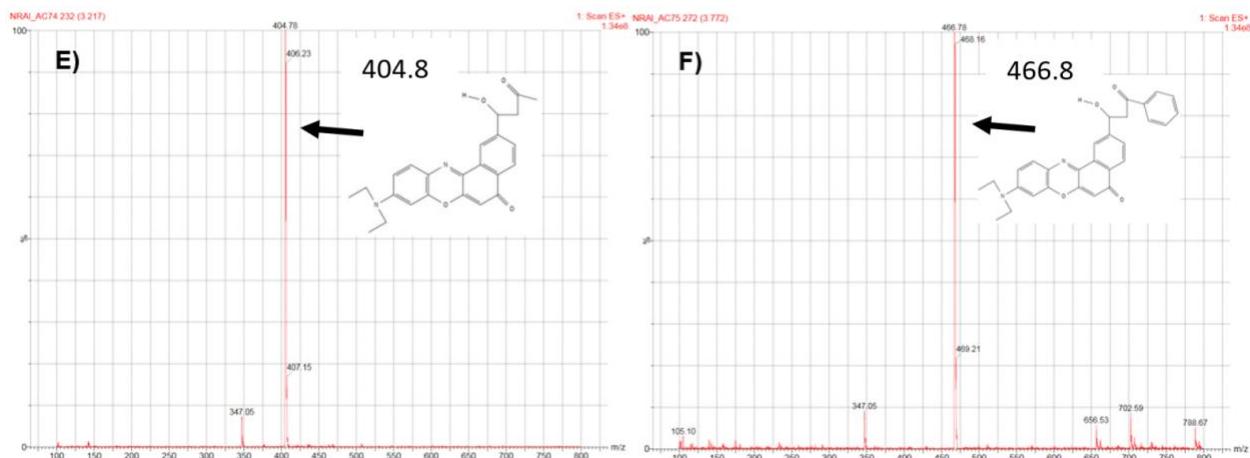


Figure S5 HPLC-MS data from aldol addition and condensation products obtained after 3-day reactions of **(A, B)** NR-CHO with acetone and **(C, D)** NR-CHO with acetophenone using the Cs(Mg,Zr)-SiO₂ catalyst. **(A)** Aldol addition product, 405 amu; **(B)** aldol condensation product, 387 amu; **(C)** aldol addition product, 467 amu; **(D)** aldol condensation product, 449 amu. HPLC-MS of aldol addition products from 3-day reactions of **(E)** NR-CHO with acetone and **(F)** NR-CHO with acetophenone using the MgO catalyst. **(E)** aldol addition product, 405 amu; **(F)** aldol addition product, 467 amu. The molecules were ionized by electrospray ionization.

NR-CHO Stability in Active Heterogeneous Catalysts

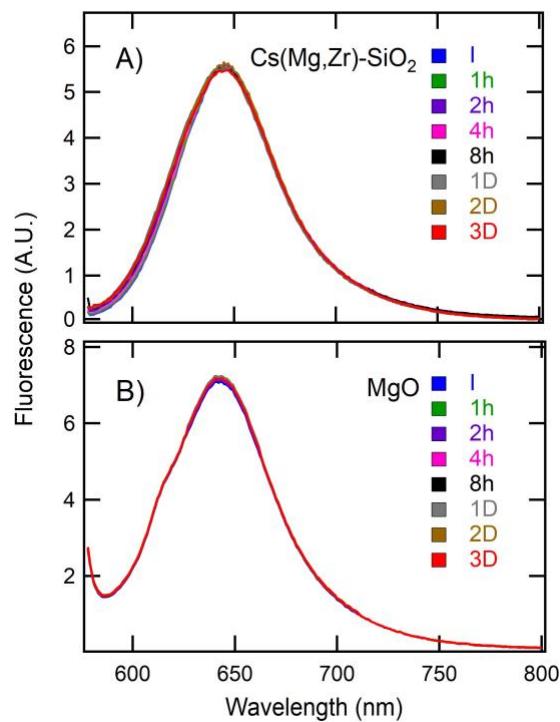


Figure S6 NR-CHO (1 μ M) stability in the presence of active heterogeneous catalysts **A)** Cs(Mg,Zr)-SiO₂, and **B)** MgO. These experiments were run under identical conditions to those described in the main text, with the one exception that no ketone was added to the reaction mixture. Fluorescence was excited at 575 nm in each case. DMSO was employed as the solvent. All reactions were stirred at room temperature.

Spectra of Pure Aldol Products

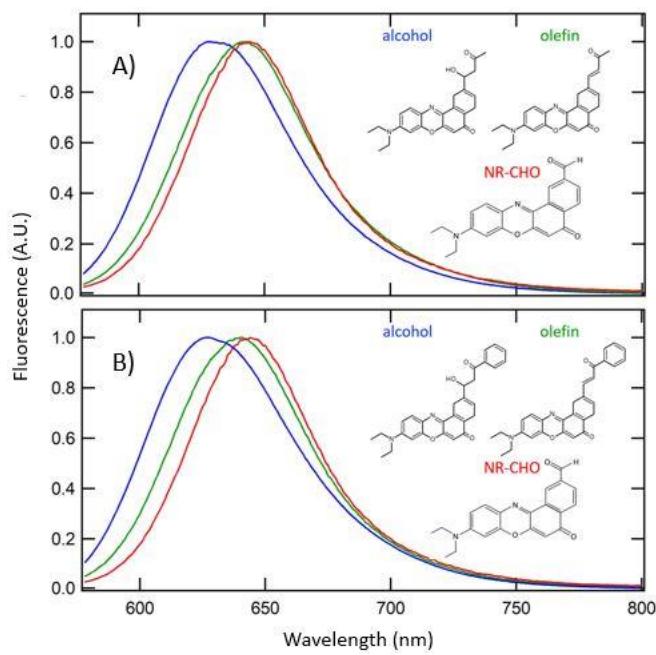


Figure S7 **A)** Normalized fluorescence emission spectra of 1 μ M NR-CHO (red), products from NR-CHO-acetone aldol reaction: alcohol (blue), and olefin (green) in DMSO. **B)** Normalized fluorescence emission spectra of 1 μ M NR-CHO (red), products from NR-CHO-acetophenone aldol reaction: alcohol (blue), and olefin (green) in DMSO. The emission spectra of the alcohol and olefin products in both aldol reactions are shifted \sim 13 nm and \sim 2-4 nm respectively to shorter wavelengths compared to NR-CHO.

Aldol Reaction Kinetics and Product Formation

Based on kinetic data from Figures 6A, D in the main text, it appears the Cs(Mg,Zr)-SiO₂ catalyzed reactions with acetone and acetophenone were nearly complete at \sim 2 h and \sim 12 h, respectively. Analytical HPLC was again used to confirm completeness of the reactions and to further characterize the products formed. These reactions were again run at high NR-CHO concentration (1 mM) and under identical reaction conditions to those described in the main text. Product mixtures obtained from the acetone and acetophenone reactions after arbitrarily selected

times of 8 h and 17 h, respectively, were compared to those obtained after 3 days. The HPLC data showed that the product mixtures obtained from the acetone reaction after 8h and 3 days were very similar. Figures S7A, B show these results. Only a small amount of NR-CHO remained after 8h and that amount was significantly lower after 3 days. Greater conversion of the aldol addition product to the condensation product was also observed after 3 days. The acetophenone reaction was once again found to be much slower. Figures S7C, D show that after 17 h, a significant amount NR-CHO remained in the mixture. After 3 days, more NR-CHO had been converted to the aldol addition product. However, the longer reaction time did not appear to result in higher conversion of aldol addition product to the condensation product. It is uncertain why more condensation product was not found in the reaction mixture after 3 days.

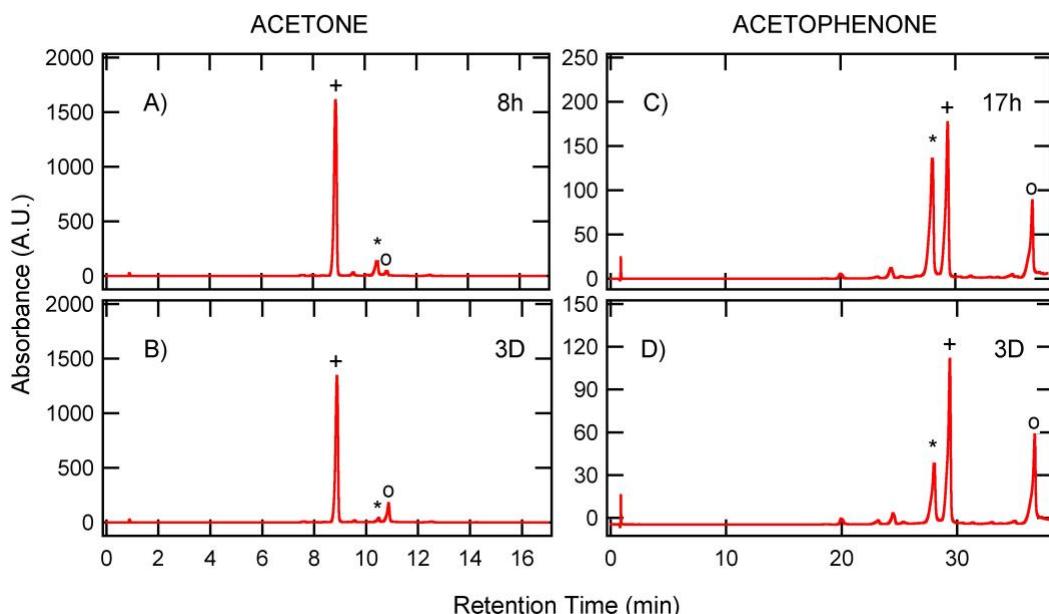


Figure S8. HPLC chromatograms (575 nm absorbance) of aldol products for the NR-CHO (1mM) reaction with acetone at 8h and 3 days (**A, B**), and acetophenone at 17h and 3 days (**C, D**) in the presence of $\text{Cs}(\text{Mg},\text{Zr})\text{-SiO}_2$ at room temperature. The chromatograms show unreacted NR-CHO (*), the aldol addition product (+), and the aldol condensation product (o). Aldol reactions at 8h and 17h had a higher amount of unreacted NR-CHO than 3-day reactions. The peak area ratio of unreacted NR-CHO to aldol condensation product was ~ 4 and ~ 1.5 for acetone and acetophenone 8h and 17h reactions, respectively. While that peak area ratio was \sim

0.2 and \sim 0.8 for acetone and acetophenone 3-day reactions, respectively. Note that aldol reactions with acetone are faster than those with acetophenone, likely due to the greater number of acidic hydrogens on the former. Allowing the NR-CHO-acetone reaction to run for 3 days yielded a higher conversion of aldol addition to aldol condensation products, yielding peak area ratios of \sim 31 and \sim 7 in 8h and 3-day reactions, respectively. However, in the NR-CHO-acetophenone reaction, the peak area ratio of aldol addition to aldol condensation products remained the same (\sim 2) even after 3-days. Note that the peak area ratio of unreacted NR-CHO to aldol addition product was lower after 3-days (i.e., \sim 0.5 compared to \sim 0.8 in the 17h reaction). For **A**, **B**, the mobile phase gradient comprised H₂O and acetonitrile mixtures at 0.800 mL/min flow rate with the following compositions: 0-10 min: 95-40% H₂O; 10-15 min: 40-20% H₂O; 15-17 min: 20% H₂O. For **C**, **D** the mobile phase compositions were: 0-35 min: 95-45% H₂O; 35-36 min: 45-20% H₂O; 36-38 min: 20% H₂O. A Thermo Scientific Hypersil GOLD C18 column (100 X 3 mm², 3 μ m particle size) was employed.

3-Perylenecarboxaldehyde Fluorescence Spectra and Aldol Reaction Results

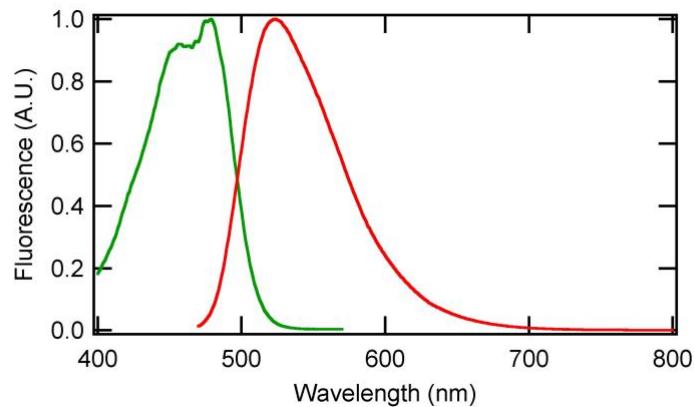


Figure S9. Normalized fluorescence excitation (green) and emission (red) spectra of 1 μ M 3-perylenecarboxaldehyde in DMSO. The excitation spectrum is peaked at 476 nm with a vibronic band appearing at 456 nm. The emission spectrum is centered at 523 nm.

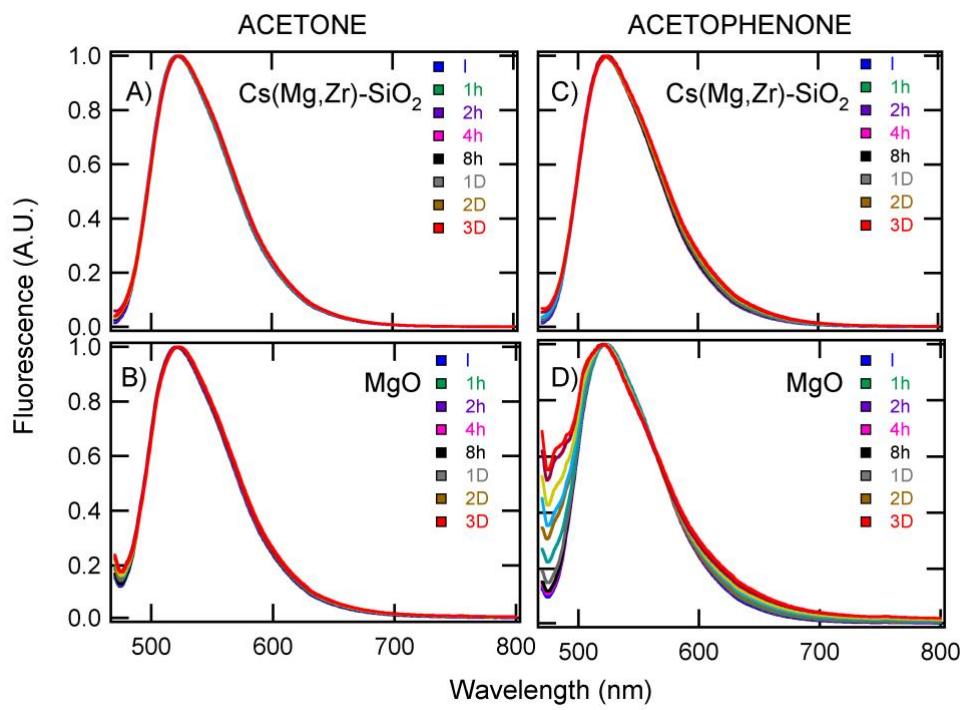


Figure S10. **A), B)** Fluorescence from 3-perylenecarboxaldehyde as a function of time in the presence of the catalysts listed with acetone employed as the ketone. **C), D)** Fluorescence from 3-perylenecarboxaldehyde as a function of time in the presence of the catalysts listed with acetophenone employed as the ketone. The fluorescence was excited at 465 nm in each

experiment. DMSO was employed as the solvent. All reactions were stirred at room temperature. Aldol reactions with acetone yielded nearly identical fluorescence spectra for the two different catalysts. While aldol reactions of the dye with acetophenone and MgO catalyst gave slightly different fluorescence spectra where it appeared that a very small shoulder centered at ~ 480 nm arose after 2 days. In contrast, very distinct fluorescence spectra were observed after only a few hours in aldol reactions with the reactive NR-CHO dye and the same catalysts, Figure 4 in the main text. MS data provided support for the formation of aldol products under the above experimental conditions in reactions employing a higher concentration of 3-perylenecarboxaldehyde (1 mM), data not shown.

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