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Caged Proline in Photoinitiated Organocatalysis

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Abstract

Organocatalysis is an emerging field in which small metal-free organic structures catalyze a diversity of reactions with remarkable stereoselectivity. The ability to selectively switch on such pathways upon demand has proven to be a valuable tool in biological systems. Light as a trigger provides the ultimate spatial and temporal control of activation. However, there have been limited examples of photo triggered catalytic systems. Herein, we describe the synthesis and application of a caged proline system that can initiate organocatalysis upon irradiation. The caged proline was generated using the highly efficient 4-carboxy-5,7-dinitroindolyl (CDNI) photocleavable protecting group in a 4-step synthesis. Advantages of this system include water solubility, biocompatibility, high quantum yield for catalyst release, and responsiveness to two-photon excitation. We showed the light triggered catalysis of a crossed aldol, a Mannich, and a self-aldol condensation reaction. We also demonstrated light initiated catalysis leading to the formation of a biocide *in situ*, which resulted in the growth inhibition of *E. coli*, with as little as 3 minutes of irradiation. This technique can be broadly applied to other systems by which the formation of active forms of drugs can be catalytically assembled remotely via two-photon irradiation.

Graphical Abstract

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Author Contributions

C.G. and N.N. conceived this work, and C.G. developed and performed the synthesis of all compounds reported; S.Y.R. and C.B. participated in the synthetic protocols and purifications. A.K.S. conducted HPLC purifications of final products. C.G. conducted all UV, NMR, and kinetic measurements. S.Y., C.B., and E.G. performed the bioorthogonal study in *E. coli*. N.N. directed the project, and C.G. and N.N. wrote the manuscript. All authors contributed to and approved the final manuscript.

Notes

The authors declare no competing financial interest.

Keywords

organocatalysis; caged proline; photoinitiated; CDNI-Pro; cages; photoprotecting groups; proline

INTRODUCTION

Catalysts that can be activated upon demand are useful tools for multiple applications, especially in recent years alongside the growth of the field of bioorthogonal chemistry.^{1,2} There are multiple types of triggers that include: physical, chemical, biological, as well as mechanical forces.^{3–6} However, triggering pathways using light has shown numerous advantages primarily due to the ability to precisely control the location and time of activation, generally referred to as spatio-temporal control.⁷ Organocatalysts have emerged as biomimetic catalysts displaying advantages of being both greener as well as biocompatible^{8–15} The simple amino acid, proline, has been shown by several groups to be among the simplest and most efficient organocatalyst.^{16–30}

The ability to externally control cellular systems is of extreme value. It can provide ample opportunities for not only learning complex biological pathways but also enabling their manipulation. Optical methods have dramatically enhanced biological research to enable precise spatial and temporal control of specific processes.^{31–33} From the ability to release small molecules upon demand across synaptic junctions to the field of optogenetics,^{34–36} light triggered processes continue to emerge as crucial techniques to study biological pathways^{37–39} and localize treatment of diseases.^{40,41} Molecules that are photo-released upon demand are generally referred to as caged molecules or caged agonists.^{42,43} Another term for the cage is PPG (Photocleavable Protecting Group).^{44–47}

Herein, we report an efficient light initiated catalytic system that can catalyze reactions upon irradiation to generate *in situ* products. We synthesized a protected form of L-proline (proline or Pro) that we refer to as caged proline, and we report the kinetics of its release using light (Figure 1). Proline has two functional groups: a secondary amine and a carboxylic acid group. We have shown that both functional groups play a critical role in catalysis, and here we report the caging of the carboxy end of proline using a 4-step synthesis of 4-carboxymethoxy-5,7-dinitroindolyl proline, **1** (CDNI-Pro). Among photocleavable cages, CDNI has a notably high quantum yield, $\phi \approx 0.5$, and several labs, including ours, have also shown its ability to respond to two-photon excitation at 720 nm.^{46,48} The two photon uncaging cross section for CDNI caged molecules is 0.06 GM at 720 nm wavelength.³⁷ Using CDNI-Pro, we demonstrated light initiated catalysis of a cross aldol reaction, a Mannich reaction, and a self-aldol condensation reaction that generates an antibacterial agent. Furthermore, we showed a biological application by which light initiated catalysis leads to the formation of a biocide that effectively inhibited further growth in *Escherichia coli*.

RESULTS AND DISCUSSION

Catalytic Importance of Proline's Functional Groups

Since proline has two functional groups: the carboxylic acid and the secondary amine, we hypothesized that protecting either of its functional groups would inhibit its catalytic activity. However, there are published mechanisms that demonstrate different mechanistic pathways in proline catalysis,^{28,49} and these pathways vary in the role of these two functional groups. Hence, we initially verified that adding the protection to either group would eliminate catalytic activity in the asymmetric aldol reaction. Using commercially available *N*-Boc-Pro and the methyl-L-prolinate (synthesized according to standard procedures),⁵⁰ we demonstrated that both functional units are critical toward proline's catalytic potential in the asymmetric aldol reaction.^{51,52} Other experimental as well as computational mechanistic studies also showed the role of the two functional groups and the ratio of the Mannich route to the aldol condensation *vs.* the aldol type product.⁵³

The classic cross aldol reaction between acetone (**2**) and 4-nitrobenzaldehyde (**3**) was conducted in the presence of three versions of proline, displayed as three entries in Table S1: 1. Pro, 2. *C*-protected-Pro, and 3. *N*-protected-Pro. Within the span of 4 h of reaction time, we observed the formation of aldol product **4** only in the presence of native proline, but not in the presence of the other two protected versions. This highlights the need for both functional groups to be present to catalyze this reaction. The lack of reactivity may, in part, be due to the bulk of the carboxy protection since pyrrolidine (i.e. proline lacking the carboxy unit) has been shown to be an active catalyst in other Schiff base promoted reactions such as the Stork enamine synthesis.^{53,54} Some reactivity of the pyrrolidine unit has also been observed after 24 h reaction time.⁵³ Table S1 summarizes the control experiments conducted to highlight the critical role of both the secondary amine as well as the carboxylic acid unit in these types of aldol reactions.

Synthesis of CDNI-Pro

Since protection of either functional group eliminates proline's catalytic potential, we sought to protect the carboxylic end of proline with a photocleavable protecting group (PPG) to generate CDNI-Pro (**1**). Although we have shown that CDNI could also be used to protect the amine functionality, our quantum yields of uncaging were far superior when CDNI was attached to the carboxy moiety.⁴⁸ A recent report discussed the protection of the secondary amine with NVOC,⁵⁵ however, here we resorted to the CDNI cage possessing the much higher quantum yield, and which can also respond to two-photon excitation.

Proline was coupled to the CDNI cage to form the amide bond in CDNI-Pro. The protected carboxylic acid functionality rendered this caged proline catalytically inactive. The CDNI cage is photocleavable under UV radiation of 350 nm wavelength,^{46,56,57} or with two-photon fast pulse laser at 720 nm.^{46,48,58,59} Thus irradiation leads to the uncaging of CDNI-Pro and the release of the carboxylic acid group, leading to the active catalyst, proline (Figure 1). This process provided a method to trigger the activation of proline, using an external source, where the precision of its spatial and temporal control adds extreme value in its broader applications. This concept could be expanded to assemble or cross-link drugs *in*

situ via photoactivation. Furthermore, other catalysts can be conjugated to CDNI to enable their external activation for specific pathways.

Our synthesis of CDNI-Pro (scheme 1) relied on an improved synthetic procedure for CDNI-Glu that we recently reported.⁴⁸ Starting with 4-hydroxyindole, *tert*-butyl 2-bromoacetate was used to form **6** in 80% yield, under mild basic conditions of K₂CO₃. A subsequent reduction of the pyrrole ring of the indole with NaBH₃CN broke down its aromaticity. This frees the lone pair of electrons on the pyrrolidine moiety making this secondary amine more reactive. The Boc protected proline, **8**, was linked to compound **7** via EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) based coupling reaction to form **9**. The *tert*-butyl and Boc protection were removed with TFA (trifluoroacetic acid) and followed by nitration using NO₂BF₄.^{48,60–62} The final product CDNI-Pro, **1**, was purified by HPLC using a reverse phase C18 column. The nitration step and all subsequent steps were conducted in the dark under dim red light. The four steps to prepare CDNI-Pro are relatively safe and the only necessary precaution is to avoid moisture in the final stages, as it could lead to the formation of caustic nitric acid. Furthermore, TFA must be handled with care and ample ventilation due its volatility and toxic nature.

Photoinitiated Catalysis

The purified photo responsive catalyst, CDNI-Pro, was introduced to three different reactions: aldol addition, Mannich reaction, and a self-aldol condensation reaction. All three reactions were conducted in pairs: 1) a control reaction in the dark, as well as, 2) the identical condition followed by irradiation (Figure 2). The reactions were irradiated with UV light at a wavelength of 350 nm to uncage CDNI-Pro. A photochemical reactor with an intensity of 60.97 Wm⁻² was used as the light source. In the cross aldol reaction, acetone (**2**) was reacted with 4-nitrobenzaldehyde (**3**) in the presence of CDNI-Pro at rt for 24 h.⁵¹ The control reaction that lacked light as a trigger did not yield any product, while the irradiated reaction gave the aldol addition product, 4-hydroxy-4-(4-nitrophenyl)butan-2-one, **4**, in 65% yield. Similarly, a Mannich reaction²⁷ was performed with acetone (**2**), 4-nitrobenzaldehyde (**3**) and 4-methoxyaniline (**10**). The irradiated reaction produced the expected product, **11**, in 45% yield, while the analogous one in the dark did not yield any product in the span of 4 h. The self-aldol condensation reaction was conducted using butyraldehyde (**12**) as the only substrate in the presence of the caged catalyst. Irradiation resulted in the catalysis of the self-aldol condensation reaction of butyraldehyde affording **13** in 47% yield, while the control setup did not lead to any product.⁵⁷

It is interesting to note that the cross aldol and the self-aldol reactions formed different types of products, namely, the aldol adduct and the enal product of the aldol condensation, respectively. It is likely that the condensation product is driven by the formation of a Mannich type reaction, in which the enamine adds to the iminium, resulting from the condensation of proline with butyraldehyde, leading to a protonated β -ammonium intermediate, which eliminates far easier than the corresponding β -hydroxy in the cross aldol reaction. In the latter reaction, the 4-nitrobenzaldehyde is substantially more electrophilic than butyraldehyde, and thus may react with the proline-generated enamine without the need of forming the electrophilic iminium species formed in the self-aldol of butyraldehyde. The

distribution of the two types of products resulting from proline catalysis has been elegantly described by Gschwind,⁴⁹ Bargeman,⁵³ and co-workers.

Spectroscopic Studies

When CDNI-Pro is irradiated with UV light, the *ortho* nitro aromatic system is predicted to get excited to a singlet state which can intersystem cross to a triplet state leading to the cleavage of the amide bond thus releasing proline.^{44,63,64} A rapid method to follow the photochemical progress of catalyst release can generally be accomplished via UV measurements. The shift in UV absorbance during photolysis was studied and is displayed in Figure S4, and described in SI. CDNI-Pro has a λ_{max} of 330 nm and a calculated molar attenuation coefficient of 6400 Lmol⁻¹cm⁻¹.

Although UV is generally the method of choice for following reactions involving conjugated systems, the lack of a clear isosbestic point precludes precise kinetic calculations. The starting CDNI cage carries two nitro groups at positions C-5 and C-7, and upon irradiation, the nitro group at C-7 is converted to the less electron withdrawing nitroso group (Figure 1). Therefore, since the starting cage and the byproduct of uncaging both harbor the additional nitro group at C-5, the UV properties of the starting cage and the spent cage product are not sufficiently distinct to result in a tangible shift in absorption of reactant vs. product. We have previously observed this phenomenon with CDNI-Glu but not with the mononitrated cage MNI-Glu which bears only one nitro group at C-7, and subsequently gets converted to the nitroso in the spent cage.⁵⁶

Kinetics of Catalyst Release by NMR

Since UV-vis spectra were not sufficiently distinct to enable kinetic assessment of catalyst release, we monitored the release of proline via ¹H NMR spectroscopy (Figure 3). This enabled a more accurate determination of the concentration of species throughout photolysis. A specific amount of CDNI-Pro and maleic acid were dissolved in 0.5 mL of D₂O and transferred in to an NMR tube. The maleic acid was used as an internal integration reference, and all the peak areas were calculated relative to the area of the maleic acid peak at 6.39 ppm. This experiment was conducted in the dark with the assistance of dim red light. The NMR tube was irradiated in the photochemical reactor at a wavelength of 350 nm, with an intensity of 60.97 Wm⁻², for 30 s intervals. After each irradiation, ¹H NMR spectra were acquired. One of the main advantages of using NMR spectroscopy is the fact that the aromatic proton of the nitroindoliny system is a singlet and therefore simple to quantify with reliable accuracy. During the photoinitiated release of proline, the aromatic hydrogen of CDNI's benzene ring, shifts upfield from 8.59 ppm (a) to 7.94 ppm (b). We monitored this peak shift and its peak area diminution with respect to the maleic acid peak (internal standard). As a result of photolysis, the indoliny ring of CDNI gets oxidized to indole, thus forming a double bond in the five membered ring which leads to the formation of two new aromatic peaks, c at 7.20 ppm and d at 6.74 ppm, that gradually became visible with increased photolysis time across sequential 30s intervals of irradiation. The rate of the photocleavage reaction for CDNI-Pro was thus determined using this experiment.

To obtain the photolysis rate of CDNI-Pro, the decreasing area of aromatic hydrogen, H_a , was used to calculate the concentration of the starting cage. The mean of the results of duplicate photolysis experiments were used to calculate the rate constant. The photolysis of CDNI-Pro follows a first order rate law with a rate constant of $7.23 \times 10^{-3} \text{ s}^{-1}$. The quantum yield, (ϕ), of CDNI-Pro was calculated to be 0.52, similar to previously published CDNI based cages (see SI for details).^{37,44} The photo cross-section, ($\epsilon\phi$), of CDNI-Pro was calculated to be $3328 \text{ Lmol}^{-1}\text{cm}^{-1}$.

Biological Application: *In Situ* Catalysis of the Formation of an Antibacterial

The photo activatable catalyst, CDNI-Pro, was also shown to be a valuable tool as a bioorthogonal system under physiological conditions. The self-aldol condensation reaction of **12**, photoinitiated with CDNI-Pro, was used for the *in situ* generation of a toxic product, (*E*)-2-ethylhex-2-enal (**13**), and then its effects on bacteria growth were measured.^{65–67}

In this assay (summarized in Figure 4), *E. coli* bacteria (DH5 α) were transferred to LB media (Lysogenic broth) containing CDNI-Pro and butyraldehyde under dark conditions. Then, the mixture was exposed to UV irradiation for 3 min and allowed to stir for another 2 h. Finally, the bacteria were plated onto LB-agar, incubated for 24 h, and the bacterial colonies were counted. Five control reactions were done to verify the significance of each parameter, Table 1. The results showed that there were large amounts of *E. coli* growing on all the plates, except for entry 5. Since nitrosoarenes are known to induce cellular toxicities, we included a control experiment to photorelease proline and the spent nitrosoarene cage, and we observed that these cleaved products did not affect bacterial growth (entry 3, Table 1). Hence, the coexistence of all three components: CDNI-Pro, butyraldehyde, and UV irradiation is critical to the formation of **13**, which inhibits bacterial growth.

As summarized in Table 1, CDNI-Pro was innocuous to *E. coli*, highlighting its compatibility as a bioorthogonal tool. Proline was released only after irradiation, which resulted in the catalysis of the self-aldol condensation of butyraldehyde (**12**) forming the antibacterial **13**. We further optimized the assay conditions by performing a series of experiments with variations in the concentration of CDNI-Pro.

The assay was conducted according to the diagram shown in Figure 4. The steps included: (a) the average colony count of *E. coli* was measured as 8.70×10^4 in 10 mL of LB media, (b) the butyraldehyde, CDNI-Pro and *E. coli* were transferred in to the sterilized Eppendorf tubes in dark, as per the ratios shown in Table 2. Fifteen sterilized Eppendorf tubes were prepared in triplicate trials as outlined by entries 1–5 (Table 2).

Subsequent steps included (Figure 4): (c) 350 nm UV irradiation of each trial for 3 min; the reactor was cooled down to rt between each irradiation, (d) trials were incubated in the dark for 2 h with gentle shaking. For the final dilution, 5 μL from each irradiated tube were transferred to a new tube with 1 mL of LB media, (e) then, 300 μL from each tube was transferred to the sterilized LB plates and plated with sterile glass beads, (f) samples were incubated at 37 °C for 24 h, and colonies were counted for each plate and averaged.

We observed a drastic reduction in *E. coli* growth when the CDNI-Pro concentration was 25% of butyraldehyde. The colony count was reduced from 3.54×10^9 CFU/mL (colony forming unit per mL) to 3.45×10^7 CFU/mL. With increased CDNI-Pro concentration, *E. coli* growth was further inhibited. Ultimately, there were undetectable levels of *E. coli* when the CDNI-Pro concentration was at stoichiometric amounts relative to the substrate, Figure 5.

As a control, an experiment was performed with the same conditions as per entry 5 of Table 2, but without *E. coli*. After following each step, the product was extracted using dichloromethane from the LB media and confirmed by ^1H NMR spectroscopy and DART (Direct Analysis in Real Time) mass spectrometry to be aldol product, (*E*)-2-ethylhex-2-enal (**13**), identical to the one obtained from the reaction presented in Figure 2.

CONCLUSION

In summary, we have demonstrated the photoinitiation of proline catalysis using light. This spatiotemporal control of organocatalysis enhances its applicability in noninvasive treatments, where CDNI-Pro and similarly inspired structures could be used as bioorthogonal tools. We used a bioassay to show how light can be applied as an external trigger to release an active catalyst that assembled a biocide in cells within 3 minutes. This methodology could broaden the field of *in situ* drug synthesis for varying applications including but not limited to cancer therapeutics.^{68–72} Furthermore, CDNI-Pro could be used for applications that involve other roles for proline beyond aldol catalysis.^{73,74} Future work aims to expand the irradiation wavelengths to enable catalyst activation with visible to near IR regions for a more functionally versatile caged catalyst.

EXPERIMENTAL SECTION

General Experimental Methods

Unless otherwise mentioned all reagents were purchased in highly pure form from Fisher or Sigma-Aldrich. The solvents used in the experiments were purchased from VWR or Fisher. When required anhydrous methylene chloride (CH_2Cl_2) was obtained from a solvent purification system, which uses an activated alumina column ((MBRAUN, MB-SPS). Reaction vessels were flame dried (alternative vacuum and N_2 flow three times) using a Schlenk line. The reactions were done under Ar or N_2 conditions. The progress of reactions was monitored by TLC, carried out on EMD Millipore TLC Silica Gel 60 F254 plates. Short/long wavelength UV light or stains (*p*-anisaldehyde), followed by heating, were used to detect the progress of appropriate spots in TLC. Column chromatography (using 60 Å silica gel from Fisher) was conducted to purify the products from steps **a** to **c** (scheme 1). The product from step **d**, compound (**1**), was purified using HPLC, Waters 2695 separation module with Waters 996 photodiode array detector with an XSELECT C18 OBD prep column (10 mm $\times$ 25 mm). The purified product was lyophilized via LABCONCO Freezone 4.5. From step **d** onwards, all characterizations and experiments were conducted in the dark with the aid of a low intensity red lamp. The purified products were stored at -20°C temperature and compound (**1**) was covered with an aluminum foil to avoid exposure to light. The products were characterized by NMR spectroscopy (Bruker 400 MHz NMR), UV/Vis spectroscopy (Agilent 8453 diode array UV–vis), high-resolution mass

spectroscopy (JEOL DART-AccuTOF mass spectrometer) and low-resolution LCMS (Agilent LC-MS with ES+ and 6120 quadrupole). The HRMS for compounds **1**, **11** and **13** was conducted at University of Florida-Mass Spectrometry Research and Education Center using Agilent 6220 Time-of-Flight spectrometer (funding from NIH S10 OD021758–01A1). Solvents for NMR experiments were purchased from Cambridge Isotopes and Acros (Fisher). Chemical shifts were calculated relative to the solvent residual peaks ($\text{CDCl}_3 = 7.26$ ppm, $\text{D}_2\text{O} = 4.79$ ppm). The following abbreviations were used to describe NMR peak multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. For uncaging of CDNI-Pro, Rayonet photochemical reactor (RPR 100), with 16 UV lamps (wavelength of 350 nm), was used. The photo intensity of the reactor was calculated as 60.97 Wm^{-2} . The instrument was equipped with a fan to regulate the temperature inside. After each irradiation (3–5 min), the instrument was cooled down to room temperature. For the biological experiment *E. coli* DH5 α strain was used.

tert-butyl (S)-2-(4-(2-(tert-butoxy)-2-oxoethoxy)indoline-1-carbonyl)pyrrolidine-1-carboxylate (9). Up to compound **7**, the syntheses for steps **a** and **b** were done per our previously published procedure.⁴⁸ Then a 50 mL round bottomed flask was charged with compound **7** (250.00 mg, 1.07 mmol), EDC (155.26 mg, 1.00 mmol), DMAP (61.13 mg, 0.50 mmol), and 10 mL of dry CH_2Cl_2 . The reaction mixture was allowed to stir for 15 min until complete solubility of all components. Subsequently, *N*-(*tert*-Butoxycarbonyl)-L-proline (*N*-Boc-proline, **8**) (215.30 mg, 1.00 mmol) dissolved in 5 mL of dry CH_2Cl_2 , was slowly added to the reaction mixture. The reaction was allowed to stir for 18 h at rt under Ar. After completion, the organic layer was extracted with CH_2Cl_2 , neutralized with NaHCO_3 , washed with brine, and dried over anhydrous Na_2SO_4 . The crude product was purified by column chromatography (2:1 hexane/ethyl acetate) to isolate (1-*tert*-butoxypyrrolidin-2-yl)-*tert*-butyl 2-(indolin-4-yloxy) acetate, compound **9**, in 62% yield as a brown oil, (230.00 mg, 0.66 mmol). ^1H NMR (400 MHz, CDCl_3) δ 7.90 – 7.86 (m, 1H), 7.16 – 7.08 (m, 1H), 6.43 (t, $J = 8.8$ Hz, 1H), 4.60 – 4.58 (m, 1H), 4.54 (s, 1H), 4.52 (s, 1H), 4.24–4.17 (m, 1H), 4.10–4.04 (m, 1H), 3.70–3.60 (m, 1H), 3.54–3.43 (m, 1H), 3.22 (t, $J = 8.4$, 2H), 2.29–2.07 (m, 2H), 2.05–1.83 (m, 2H), 1.47 (s, 9H), 1.45 – 1.33 (m, 9H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 172.0 – 170.4 (m), 167.9, 155.1 – 153.0 (m), 144.7, 128.9, 118.9, 111.0, 106.9, 82.3, 79.7, 65.7, 60.4, 58.7, 48.1, 46.8, 30.4, 29.5, 28.9, 25.3, 24.0, 21.0, 14.1. HRMS (DART-AccuTOF) m/z $[\text{M}+\text{H}]^+$ calcd for (without Boc protection) $\text{C}_{19}\text{H}_{27}\text{N}_2\text{O}_4^+$ 347.1966, found 347.1994.

(S)-2-((5,7-dinitro-1-prolylindolin-4-yl)oxy)acetic acid (1). A 25 mL round bottomed flask was charged with compound **9** (125.0 mg, 0.20 mmol) and TFA (2 mL) and allowed to stir for 1 h at rt. A TLC was taken (3:2 hexane/ethyl acetate) to determine the completion of the reaction. After complete deprotection, the TLC spot for the product will remain on the baseline. Subsequently, a nitrogen stream was applied to remove the TFA. To the same flask, 2 mL of fresh TFA was added and cooled to 0 °C. Then, nitronium tetrafluoroborate (NO_2BF_4) (106.0 mg, 0.80 mmol) was added under a N_2 flow and stirred in dark for 1.5 h. Finally, TFA was removed via a nitrogen stream followed by vacuum. The product was then purified using reverse phase HPLC via isocratic elution at 2 mL min^{-1} using a solvent system of 60% water and 40 % acetonitrile acidified with 0.1% TFA, $t_R = 9$ min, (Figure S1

in SI). Finally, product was lyophilized to dryness, generating compound **1** in 24% yield as a light-yellow powder (18.25 mg, 0.05 mmol). ^1H NMR (400 MHz, D_2O) δ 8.58 (s, 1H), 4.96 (td, J = 6.8 Hz, 2.4 Hz, 1H), 4.77 (s, 2H), 4.52 (td, J = 10.4 Hz, 4 Hz, 1H), 4.35 (q, J = 10 Hz, 1H), 3.52–3.48 (m, 1H), 3.46 (t, J = 5.2 Hz, 1H), 3.44–3.38 (m, 1H), 2.64–2.56 (m, 1H), 2.27–2.20 (m, 2H), 2.18–2.08 (m, 2H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, D_2O) δ 172.0, 168.0, 151.2, 139.2, 138.4, 135.0, 132.3, 123.0, 69.3, 60.0, 50.7, 46.2, 28.1, 26.4, 23.7. LC–MS (ESI) 381.1 $[\text{M} + \text{H}]^+$; HRMS (ESI-TOF) m/z $[\text{M} + \text{H}]^+$ calcd: $\text{C}_{15}\text{H}_{17}\text{N}_4\text{O}_8^+$ 381.1041, found 381.1054 (MS spectrum can be found in SI).

Photoinitiated organocatalytic reactions

Cross aldol reaction to form 4-hydroxy-4-(4-nitrophenyl)butan-2-one (4). The photoactivatable catalyst **1** (7.6 mg, 0.02 mmol) was stirred in 5 mL of DMSO:acetone (4:1) with 4-nitrobenzaldehyde (15.12 mg, 0.1 mmol) in a 25 mL round-bottomed flask, in the dark at rt. After 24 h, no reaction was observed. Then, the reaction mixture was irradiated in the Rayonet photochemical reactor at 350 nm for a total irradiation time of 5 min. Afterwards, the mixture was stirred for 4 h at rt. The organic layer was extracted with ethyl acetate and dried over anhydrous Na_2SO_4 , filtered, and concentrated under vacuum. The crude product was purified through column chromatography (1:1 hexane/ethyl acetate) to give compound **4** in 65% yield, as a brown oil (13.60 mg, 0.065 mmol). ^1H NMR (400 MHz, CDCl_3) δ 8.22 (d, J = 7.9 Hz, 2H), 7.54 (d, J = 8.1 Hz, 2H), 5.34–5.20 (m, 1H), 3.56 (s, 1H), 2.85 (d, J = 7.5 Hz, 2H), 2.20 (d, J = 21.5 Hz, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 208.3, 150.4, 147.4, 126.6, 123.5, 68.7, 51.8, 30.6. HRMS (DART-AccuTOF) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{10}\text{H}_{12}\text{NO}_4^+$ 210.0761, found 210.0778.

Mannich reaction to form 4-((4-methoxyphenyl)amino)-4-(4-nitrophenyl)butan-2-one (11). The photoactivatable catalyst **1** (76 mg, 0.2 mmol), was stirred in 8 mL of DMSO, *p*-anisidine (135 mg, 1.1 mmol), 4-nitrobenzaldehyde (151.12 mg, 1.0 mmol) and 2 mL of acetone in a 25 mL round-bottomed flask in the dark at rt. After 24 h, no product could be observed. Subsequently, the reaction mixture was irradiated in the Rayonet photochemical reactor at 350 nm for a total irradiation time of 5 min and stirred at rt for 24 h. The organic layer was extracted with ethyl acetate, dried over anhydrous Na_2SO_4 , filtered, and then concentrated under vacuum. The crude product was purified through column chromatography (1:1 hexane/ethyl acetate) to give compound **11** in 40% yield, as dark brown oil (141.45 mg, 0.45 mmol). ^1H NMR (400 MHz, CDCl_3) δ 8.17 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 6.69 (d, J = 8.9 Hz, 2H), 6.46 (d, J = 8.9 Hz, 2H), 4.85 (t, J = 7.5 Hz, 1H), 4.28 (s, 1H), 3.69 (s, 3H), 2.95 (d, J = 6.6 Hz, 2H), 2.15 (s, 3H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 206.0, 153.0, 150.7, 140.2, 140.0, 129.1, 127.4, 124.2, 122.7, 116.44, 115.4, 114.8, 114.6, 55.9, 54.6, 50.8, 30.7. HRMS (ESI-TOF) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_4^+$ 315.1339, found 315.1346.

Self-aldol condensation to form (E)-2-ethylhex-2-enal (13). CDNI-Pro (**1**) (840 mg, 2.2 mmol) and butyraldehyde (1 mL, 11.10 mmol) were mixed in 5 mL of methanol in a 25 mL round-bottomed flask at rt for 24 h. No reaction product could be observed under dark conditions. Then mixture was irradiated in the Rayonet photochemical reactor at 350 nm for a total irradiation time of 5 min. Subsequently, the reaction mixture was stirred at rt for 4 h.

The solvent was subsequently removed under vacuum. The crude product was purified through column chromatography (4:1 hexane/ethyl acetate) to give compound **13** in 47% yield, as a brown oil (658.76 mg, 5.22 mmol). ^1H NMR (400 MHz, CDCl_3) δ 9.3 (s, J, 1H), 6.34 (t, J = 7.4 Hz, 1H), 2.25 (q, J = 7.4 Hz, 2H), 2.19 (q, J = 7.5 Hz, 2H), 1.50–1.45 (m, 2H), 0.90–0.85 (m, 1H). $^{13}\text{C}\{^1\text{H}\}$ NMR (101 MHz, CDCl_3) δ 195.0, 154.5, 145.1, 30.7, 22.1, 17.2, 13.7, 13.2. HRMS (ESI-TOF) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_8\text{H}_{15}\text{O}^+$ 127.1117, found 127.1121.

Generation of in-situ biocide (*E*)-2-ethylhex-2-enal to inhibit *E. coli* growth via photo initiated self-aldol condensation

Preparation of minimal media with *E. coli*—Initially, 1 L of LB (Lysogeny broth) agar media was sterilized and poured to 40 plates, which were stored at 0 °C. Two tubes of sterilized LB agar liquid media (30 mL for each) were prepared. One of the two tubes were referred as the “Blank” and a minimum amount of –150 °C stored DH5 α *E. coli* was transferred to the other tube. The two tubes were incubated at 30 °C with gentle shaking for 24 h. Then, 30 μL of liquid from the tube with *E. coli* was transferred to the “Blank” and the new solution was incubated at 30 °C with gentle shaking for 2 hours after which, OD (optical density) value was measured, and dilutions were performed based on the colonies number. The average colony count was calculated as 4.35×10^4 .

Measuring *E. coli* growth with varying CDNI-Pro concentration—Five trials were conducted varying the CDNI-Pro concentration. Fifteen sterilized Eppendorf tubes were prepared and grouped as triplicate trials. The solution for each entry was prepared according to the details in Table S3 in SI. Stock solutions of 0.79 M CDNI-Pro (2.00 mg in 300 μL LB solution), 0.082 M butyraldehyde (12.96 μL in 1 mL LB solution) and *E. coli* minimal media (with 4.35×10^4 colony count), were used to prepare each trial sample. Samples were prepared in dark with an aid of a red dim light. All the sample tubes were treated with UV light of 350 nm in the photoreactor for 3 min. The photo reactor was cooled to rt before each irradiation. Then, all the tubes were incubated in the dark for 2 h with gentle shaking. New sets of 15 sterilized Eppendorf tubes were prepared with 1 mL of sterilized LB liquid media. Then, 5 μL from each trial tube was transferred to the new tube for dilution. Finally, 300 μL from each reaction mixture was transferred to a prepared LB plate, and 15 plates were incubated at 37 °C for 24 h. Colonies were counted for each plate, and CFU values were calculated.

ASSOCIATED CONTENT

The supporting Information is available free of charge on the ACS Publication website at: <http://pubs.acs.org>.

NMR spectra of CDNI-Pro (**1**) and all intermediates leading up to it; Kinetic data; LCMS data, and Direct Analysis in Real Time (DART)-MS data, ESI HRMS for **1**.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Photoinitiated Organocatalyst

Inactive catalyst

Active catalyst

**Caged Proline
1
CDNI-Pro**

**L-Proline
Pro**

spent cage

Figure 1.

Photoinitiated release of catalytically active proline from CDNI-Pro under UV light.

Reaction		Caged <i>CDNI-Pro</i> In dark/ 24 h	Uncaged <i>CDNI-Pro</i> UV 350 nm (5 min)
cross aldol reaction			
<p>2 + 3 $\xrightarrow[\text{DMSO, 25 } ^\circ\text{C}]{20\% \text{ } CDNI-Pro}$</p>	0%	<p>4 65%</p>	
Mannich reaction			
<p>2 + 3 + 10 $\xrightarrow[\text{DMSO, 25 } ^\circ\text{C}]{20\% \text{ } CDNI-Pro}$</p>	0%	<p>11 45%</p>	
self-aldol condensation			
<p>12 $\xrightarrow[\text{DMSO, 25 } ^\circ\text{C}]{20\% \text{ } CDNI-Pro}$</p>	0%	<p>13 47%</p>	

Figure 2.

Reactions catalyzed by CDNI-Pro in the dark and upon irradiation: Cross aldol reaction between $(\text{CH}_3)_2\text{CO}$ (**2**) and 4-nitrobenzaldehyde (**3**); Mannich reaction between $(\text{CH}_3)_2\text{CO}$ (**2**), 4-nitrobenzaldehyde (**3**) and 4-methoxyaniline (**10**); self-aldol condensation reaction of butyraldehyde (**12**).

Figure 3. ^1H NMR (400 MHz, D_2O) of selected spectra for progressive photolysis of CDNI-Pro, concentration of 0.94 mM, with a wavelength of 350 nm at 60 s intervals, rt.

Figure 4.
Graphical illustration of biological assay.

Figure 5.
Plot of *E. coli* colony count (CFU/mL) with respect to CDNI-Pro percentage.

Scheme 1. Synthetic pathway for CDNI-Pro^a

^aExperimental conditions: a) K_2CO_3 , dry $(CH_3)_2CO$, rt, 18 h, 80%; b) $NaBH_3CN$, dry CH_3COOH , rt, 2 h, 97%; c) *N*-(tert-butoxycarbonyl)-L-proline (**8**), EDC, DMAP, dry CH_2Cl_2 , rt, 18 h, 62%; d) (i) TFA, rt, 1 h, quantitative; (ii) TFA, NO_2BF_4 , 0 °C, 1 h, 24%.

Table 1.

Bioassay Studies:

including all the control experiments revealing the need for all three components of caged proline, substrate, and light to catalyze the formation of **13**, which leads to the inhibition of bacterial growth^a

entry	CDNI-Pro (2.63×10^{-2} M)	Butyraldehyde (2.63×10^{-2} M)	Irradiation (350 nm)	<i>E. coli</i> growth
1	×	×	×	Yes
2	×	✓	✓	Yes
3	✓	×	✓	Yes
4	✓	✓	×	Yes
5	✓	✓	✓	No

^aExperimental conditions: CDNI-Pro (1 eq), butyraldehyde (1 eq) was dissolved in LB media with *E. coli* at pH 7 in RT. Each parameter was either × = missing or ✓ = present in the reaction media. Entries 2, 3 and 5 were irradiated for 3 min in the photochemical reactor at 350 nm with an intensity of 60.97 Wm^{-2} .

Table 2.

a

E. coli. growth relative to CDNI-Pro concentration^a

The reaction scheme shows a green *E. coli* cell reacting with butyraldehyde (SMILES: CCCC=O) under UV 350 nm (3 min) in the presence of CDNI-Pro and LB Media. The products are a branched aldehyde (SMILES: CCCC(=O)C(C)C) and a dead, red *E. coli* cell.

	12		13	
entry	CDNI-Pro (μL)	Butyraldehyde (μL)	LB (μL)	<i>E. coli</i> (μL)
1	0	5	45	50
2	40	5	5	50
3	30	5	15	50
4	20	5	25	50
5	10	5	35	50

^aExperimental conditions: Samples were prepared with LB media, [CDNI-Pro] = 0.02 M stock solution; [butyraldehyde] = 0.14 M stock solution; *E. coli* = 8.70×10^3 CFU mL⁻¹.

Samples were prepared in sterile conditions in the dark, at rt.