Electronic Supplementary information for:

Selective activation of organocatalysts by specific signals

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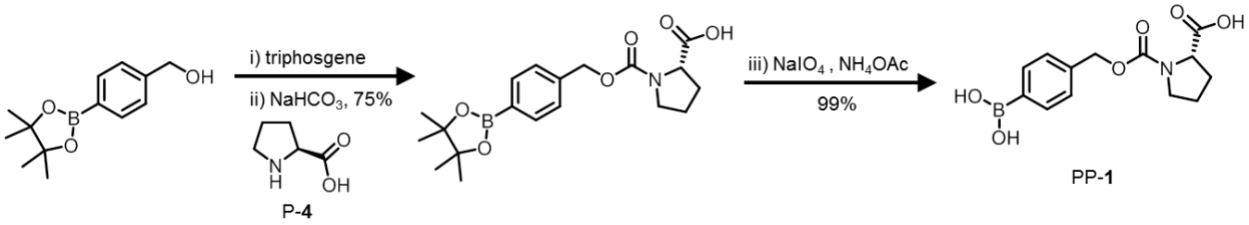
1. General methods

Starting materials were commercially available and used as received unless stated otherwise. **TLC** was performed on Merck Silica Gel 60 F254 TLC plates with a fluorescent indicator with a 254 nm excitation wavelength. Compounds were visualized under UV light at 254 nm. **NMR** spectra were recorded on a Bruker Avance-400 spectrometer (399.90 MHz for ^{1}H and 100.56 MHz for ^{13}C) at 298 K using residual protonated solvent signals as internal standard (^{1}H: $\delta(CHCl_3)$ = 7.26 ppm, $\delta((CH_3)_2SO)$ = 2.50 ppm, $\delta(CH_3OH)$ = 3.31 ppm, $\delta(D_2O)$ = 4.79 ppm, and ^{13}C: $\delta(CHCl_3)$ = 77.16 ppm, $\delta((CH_3)_2SO)$ = 39.52 ppm, $\delta(CH_3OH)$ = 49.00 ppm, D_2O was referenced to internal dioxane, 67.19 ppm. NMR spectra were processed by Mnova NMR software (mestrelab research). **HPLC-MS** analysis was performed on a Shimadzu Liquid Chromatograph Mass Spectrometer, LCMS-2010, LC-8A pump with a diode array detector SPD-M20. The column used was the Xbridge Shield RP 18.5µm (4.6x150mm). **UV-Vis** spectroscopic measurements were performed on an Analytik Jena Specord 250 spectrophotometer.

2. Synthesis of pro-prolines

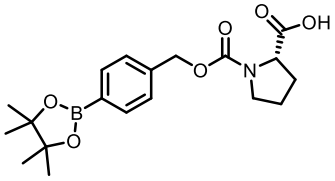
2.1 Synthesis of PP-1:

PP-**1** was obtained by reaction between the boronate ester chloroformate derivative and L-proline and subsequent removal of the pinacol ester (Scheme S1).



Scheme S1: Synthetic pathway for the preparation of PP-**1**.

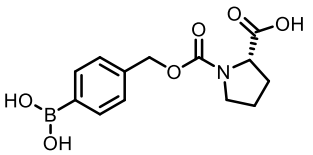
1-(((4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)oxy)carbonyl)pyrrolidine-2-carboxylic acid (boronate-ester derivative):



4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzylchloroformate was synthesized adopting the literature procedure.[1] To a stirred solution of L-proline (0.48 g, 4.2 mmol) and $NaHCO_3$ (0.88 g, 10.5 mmol) in water (10.0 mL) was added 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzylchloroformate (1.03 g, 1.0 mmol) dropwise at 0 °C. The mixture was allowed to reach ambient temperature and was stirred overnight. After that, the reaction mixture was quenched with 1M HCl solution and diluted with ethyl acetate. The organic layer was washed with water, brine and dried over $MgSO_4$. Concentration under reduced pressure

provided the desired compound as a sticky solid (0.98 g, 75 %). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.74–7.67 (m, 2H, Ar-*H*), 7.30-7.22 (m, 2H, Ar-*H*), 5.15-5.04 (m, 2H, Ar-C*H*$_2$), 5.21 (s, 2H, -C*H*$_2$-), 4.36-4.29 (m, 1H, -C*H*(CO_2H)), 3.55-3.36 (m, 2H, -CO_2NCH_2-), 2.20-1.98 (m, 2H, -C*H*$_2$-), 1.90-1.77 (m, 2H, -CH_2-), 1.26-1.24 (d, 12H, -C*H*$_3$). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 176.3 & 175.9 (-*C*O_2H-), 155.1 & 154.3 (-*C*O_2-), 139.3 & 139.2 (C_{Ar}), 134.7 & 134.6 (C_{Ar}), 126.8 (C_{Ar}), 126.4 (C_{Ar}), 83.6 & 83.5 (-*C*Me_2-), 67.0 & 66.8 (Ar-*C*H_2-), 58.9 & 58.4 (-*C*H(CO_2H)), 46.7 & 46.3 (-CO_2NCH_2-), 30.6 & 29.5 (-*C*H_2-), 24.6 (-*C*H_3), 24.0 & 23.2 (-*C*H_2-). Two signals are due to the presence of two rotational isomers, which is well known for carbamate rotamers.[2] **MS** (ESI neg) m/z: 374.2 [$(M-H)^+$] (expected m/z = 374.19).

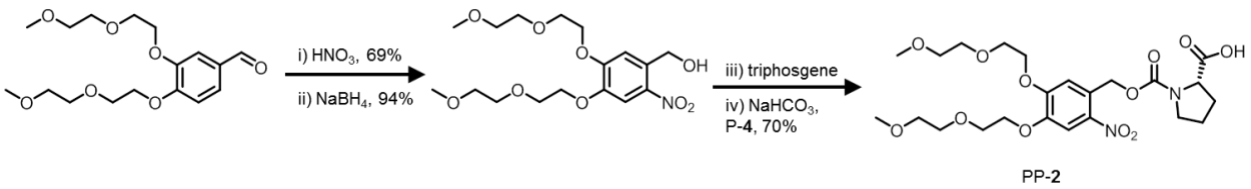
PP-1:



Boronate-ester derivative (0.61 g, 1.6 mmol) was dissolved in acetone (10.0 mL). To that, $NaIO_4$ (2.10 g, 9.8 mmol) and NH_4OAc (0.76 g, 9.8 mmol) in water (20.0 mL) were added and the mixture was stirred at room temperature. After completion of the reaction, it was acidified with 1M HCl and concentrated under reduced pressure. The crude product was diluted with ethyl acetate and the mixture was washed with water, brine and dried over $MgSO_4$. Concentration under reduced pressure provided the desired compound as a yellow-brown solid (0.48 g, 99 %). **^{1}H NMR** (400 MHz, DMSO-D_6): δ = 7.67 (bs, 2H, Ar-*H*), 7.35-7.29 (m, 2H, Ar-*H*), 5.13-5.08 (m, 2H, ArC*H*$_2$), 4.36-4.30 (m, 1H, -C*H*-CO_2H), 3.58-3.44 (m, 2H, -CO_2NCH_2-), 2.34-2.23 & 2.07-2.00 (m, 2H, -C*H*$_2$-), 1.96-1.88 (m, 2H, -C*H*$_2$-). **^{13}C NMR** (100 MHz, CD_3OD): δ = 174.0 & 173.7 (-*C*O_2H), 154.0 & 153.7 (-*C*O_2N-), 139.0 & 138.7 (C_{Ar}), 134.2 & 134.1 (C_{Ar}), 126.4 (C_{Ar}), 126.0 (C_{Ar}), 67.0 & 66.8 (Ar-*C*H_2-), 58.9 & 58.4 (-*C*H(CO_2H)), 46.8 & 46.2 (-CO_2NCH_2-), 30.5 & 29.4 (-*C*H_2-), 23.9 & 23.0 (-*C*H_2-). Two signals are due to the presence of two rotational isomers, which is well known for carbamate rotamers.[2] **MS** (ESI Neg.) m/z: 292.0 [$(M-H)^+$] (expected m/z = 292.11).

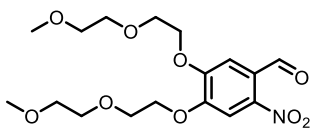
2.2 Synthesis of PP-2:

PP-**2** was synthesized (Scheme S2) by the reaction of L-proline and the corresponding chloroformate derivative which was obtained from 4,5-bis(2-(2-methoxyethoxy)ethoxy)-2-nitrophenyl methanol by the treatment with phosgene. 4,5-Bis(2-(2-methoxyethoxy)ethoxy)-2-nitrophenyl methanol was obtained from 3,4-bis(2-(2-methoxyethoxy)ethoxy)benzaldehyde[3] via nitration with HNO_3, followed by $NaBH_4$ reduction of the aldehyde group to an alcohol functional group.



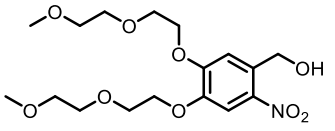
Scheme S2: Synthetic pathway for the preparation of PP-**2**.

4,5-Bis(2-(2-methoxyethoxy)ethoxy)-2-nitrobenzaldehyde:



3,4-Bis(2-(2-methoxyethoxy)ethoxy)benzaldehyde (1.03 g, 3.0 mmol) was added while stirring to a chilled nitric acid solution (70 %, 3.0 mL) at 0 °C. The mixture was then allowed to reach ambient temperature. After completion of the reaction, it was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, brine and dried over $MgSO_4$. Concentration under reduced pressure provided the desired compound as a yellow oil (0.80 g, 69 %). **^{1}H NMR** (400 MHz, DMSO-D_6): δ = 10.41 (s, 1H, -C*H*O), 7.68 (s, 1H, Ar-*H*), 7.45 (s, 1H, Ar-*H*), 4.33-4.29 (m, 4H, ArOC*H*$_2$CH$_2$-), 3.93-3.90 (m, 4H, ArOCH$_2$C*H*$_2$-), 3.73-3.71 (m, 4H, -OC*H*$_2$CH$_2$OCH$_3$), 3.56-3.54 (m, 4H, -OCH$_2$C*H*$_2$OCH$_3$), 3.37 (s, 6H, -OC*H*$_3$). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 187.6 (-*C*HO), 152.9 (2x*C*$_{Ar}$), 152.1 (*C*$_{Ar}$), 125.6 (*C*$_{Ar}$), 111.4 (*C*$_{Ar}$), 109.2 (*C*$_{Ar}$), 71.9 (ArO*C*H$_2$CH$_2$O-, for 2 peaks), 71.0 (ArOCH$_2$*C*H$_2$O-), 70.9 (ArOCH$_2$*C*H$_2$O-), 69.5 (-O*C*H$_2$CH$_2$OMe), 69.4 (-OCH$_2$CH$_2$OMe), 69.3 (-OCH$_2$*C*H$_2$OMe), 69.2 (-OCH$_2$*C*H$_2$OMe), 59.2 (-O*C*H$_3$, for 2 peaks).

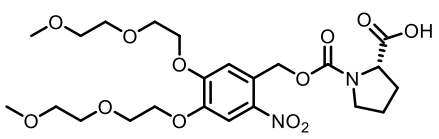
4,5-Bis(2-(2-methoxyethoxy)ethoxy)-2-nitrophenyl)methanol:



$NaBH_4$ (0.03 g, 0.70 mmol) was added slowly to a solution of 4,5-bis(2-(2-methoxyethoxy)ethoxy)-2-nitrobenzaldehyde (0.54 g, 1.4 mmol) in methanol (8.0 mL). The mixture was stirred at room temperature for 1 h. After completion of the reaction, the mixture was concentrated under reduced pressure, and the residue was partitioned between ethyl acetate and water. After extraction the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The obtained crude compound was purified by flash column chromatography (silica gel, $CHCl_3$/MeOH 9/1) to obtain the desired compound as a pale yellow oil (0.51 g, 94 %). **^{1}H NMR** (400 MHz, $CDCl_3$): δ = 7.57 (s, 1H, Ar-*H*), 7.19 (s, 1H, Ar-*H*), 4.84 (s, 2H, ArC*H*$_2$), 4.17-4.08 (m, 4H, Ar-OC*H*$_2$CH$_2$-), 3.80-3.78 (t, 4H, Ar-OCH$_2$C*H*$_2$-), 3.65-3.62 (m, 4H, -OC*H*$_2$CH$_2$-OMe), 3.49-3.46 (m, 4H, -OCH$_2$C*H*$_2$-OMe), 3.29 & 3.28 (s, 6H, -OC*H*$_3$). **^{13}C NMR** (100 MHz, $CDCl_3$): δ = 153.4 (*C*$_{Ar}$), 146.6 (*C*$_{Ar}$), 138.7 (*C*$_{Ar}$), 133.5 (*C*$_{Ar}$), 111.4 (*C*$_{Ar}$), 110.1 (*C*$_{Ar}$), 71.6 (ArO*C*H$_2$CH$_2$O-, for 2 peaks), 70.5 (ArOCH$_2$*C*H$_2$O-), 70.4 (ArOCH$_2$*C*H$_2$O-),

69.2 (-O*C*H_2CH$_2$OMe, for 2 peaks), 68.9 (-OCH$_2$*C*H_2OMe), 68.5 (-OCH$_2$*C*H_2OMe), 61.6 (Ar*C*H_2-), 58.7 (-O*C*H_3), 58.6 (-O*C*H_3).

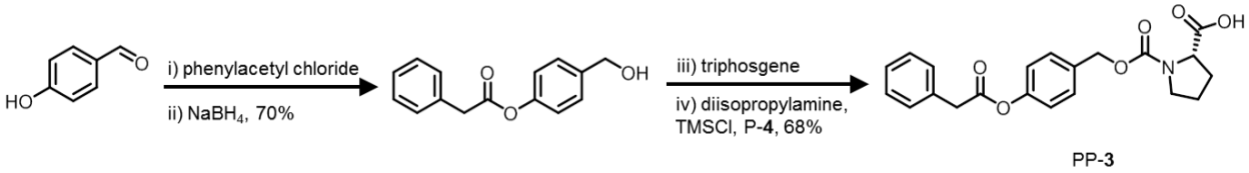
PP-2:



K_2CO_3 (2.49 g, 18.0 mmol) was oven dried in a round-bottom flask. The flask was cooled in an ice bath and triphosgene (1.19 g, 4.0 mM) in toluene (10.0 mL) was added. After stirring for 30 minutes at 0 °C, 4,5-bis(2-(2-methoxyethoxy)ethoxy)-2-nitrophenyl)methanol (0.78 g, 2.0 mmol) in toluene (10.0 mL) was added and stirred at ambient temperature. After completion of the reaction, it was worked-up by diluting with $CHCl_3$, filtering and concentrating under reduced pressure. The obtained chloroformate derivative was used without further purification. To a stirred solution of L-proline (0.25 g, 2.2 mM) and $NaHCO_3$ (0.23 g, 2.8 mM) in water (10.0 mL), the chloroformate derivative was added dropwise and the reaction mixture was stirred overnight at room temperature. After that, it was quenched with 1M HCl (pH 2) and then it was extracted with ethyl acetate. The organic layer was washed with water, brine and dried over $MgSO_4$. Concentration under reduced pressure provided the desired compound as a light brown oil (0.74 g, 70 %). **^{1}H NMR** (400 MHz, CD_3OD): δ = 7.80 (s, 1H, Ar-*H*), 7.14 (s, 1H, Ar-*H*), 5.60-5.30 (m, 2H, ArC*H*$_2$), 4.48-4.26 (m, 3H, -C*H*CO_2H & ArOC*H*$_2$-), 4.24-4.21 (m, 2H, ArOC*H*$_2$-), 3.89-3.83 (m, 4H, ArOCH$_2$C*H*$_2$-), 3.72-3.53 (m, 10H, -OC$_2$*H*$_4$-; 8 protons & -CO_2NC*H*$_2$-), 3.34 (bs, 6H, -OC*H*$_3$), 2.38-2.06 (m, 2H, -C*H*$_2$-), 2.01-1.91 (m, 2H, -C*H*$_2$-). **MS** (ESI Neg.) m/z: 529.3 [(M-H)$^+$] (expected 529.21).

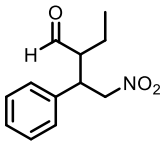
2.3 Synthesis of PP-3

PP-**3** is a known compound and was synthesized following a reported procedure (Scheme S3).[4] The analytical data were in accordance with the literature. A short description: 4-(phenylacetoxy)benzyloxycarbonyl chloride was obtained via acylation of 4-hydroxybenzaldehyde, followed by reduction of the aldehyde group to give the benzyl alcohol, and conversion to the chloroformate. Reaction between the chloroformate and proline gave PP-**3**.



Scheme S3: Synthetic pathway for the preparation of **PP-3**.

2.4 Synthesis of Michael product 10



Trans-β-nitrostyrene **8** (200 mg, 1.34 mmol) and *L*-proline (34.5 mg, 0.30 mmol) were dissolved in methanol (2 mL). Butanal **9** (181 µL, 0.145 g, 2.01 mmol) was added and the reaction was stirred overnight until NMR confirmed complete conversion. The reaction mixture was concentrated under reduced pressure and purified using column chromatography (eluent 5% ethyl acetate in petroleum ether) to yield the product as a colourless oil (0.134 g, 0.60 mmol, 45%). The double peaks in the NMR spectrum are due to the presence of two different diastereoisomers in a 1:3 ratio. **^{1}H NMR** (400 MHz, methanol-d4) δ 9.69 (s, 1H), 7.28 (m, 5H), 4.94 (d, 1H, *J* = 5.4), 4.82 (d, 1H, *J* = 4.9), 3.83 (m, 1H), 2.73 (m, 1H), 1.45 (m, 2H), 0.78 (m, 3H). **^{13}C NMR** (100.5 MHz, methanol-d4) δ 205.1, 139.0, 130.0, 79.7, 56.1, 44.0, 21.0, 10.8. **MS** (ESI Neg.) m/z: 220.1 [$(M-H)^-$] (expected m/z = 220.11).

3. Release of catalyst by the signal:

3.1 H_2O_2 triggered proline release:

H_2O_2 (1.0 mL, 30 % aq. H_2O_2 solution, excess) was added to a solution of PP-**1** (0.05 g) in MeOH (2.0 mL). The mixture was stirred for 10 minutes. After completion of the reaction (monitored via TLC analysis), the mixture was concentrated and diluted with ethyl acetate. The organic layer was washed with sodium sulfite, brine and dried over $MgSO_4$. After filtration, the obtained solution was concentrated under reduced pressure and the residue was analyzed by ^{1}H NMR spectroscopy and mass spectroscopy.

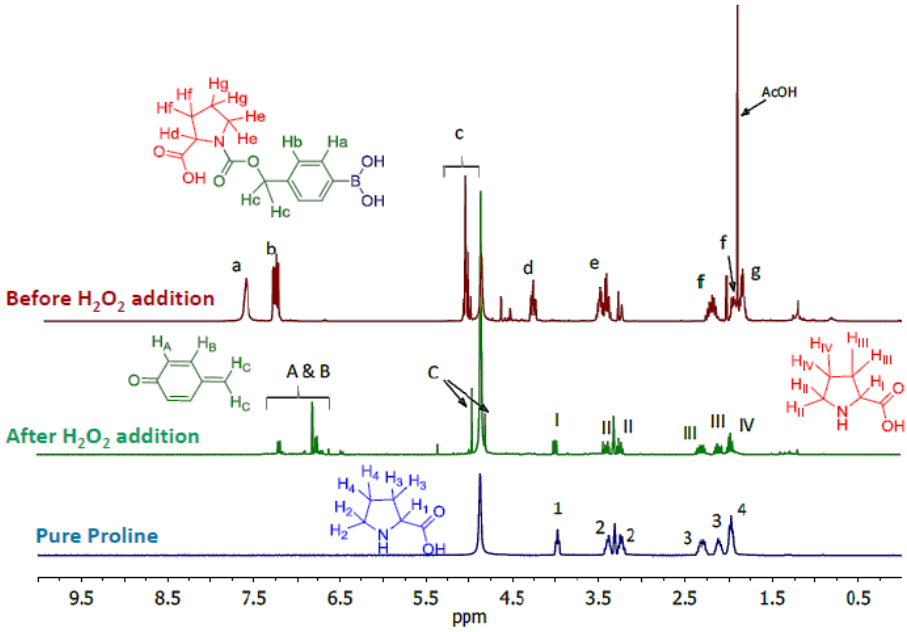


Figure S1: ^{1}H NMR analysis of chemical (H_2O_2) trigger proline release from PP-**1** in CD_3OD at 25 °C: PP-**1** before (top in red) and after H_2O_2 treatment (middle in green). The released proline has comparable spectrum as pure proline (bottom in blue).

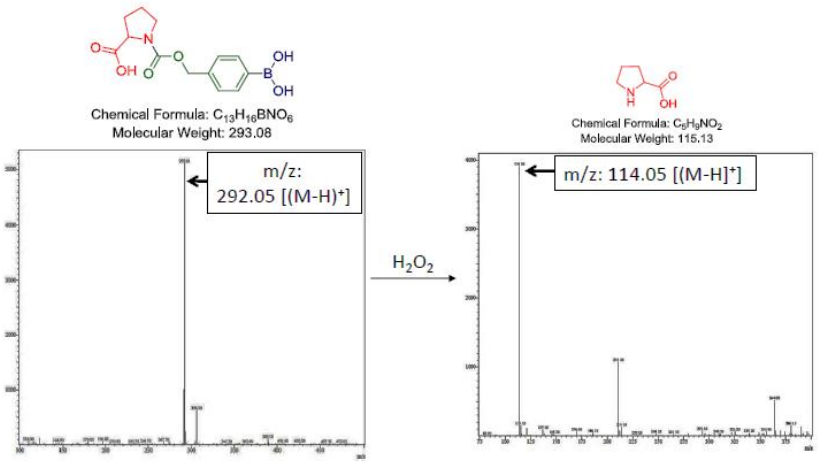


Figure S2: Mass spectrometry analysis of chemical (H_2O_2) triggered proline release from PP-**1**: before (left) and after (right) the treatment of H_2O_2.

3.2 Light-triggered proline release

Light activation method:

Light signal experiment was performed using an optical fiber illuminator with a Nikon Intensilight C-HGFI lamp (lamp-ultrahigh pressure 130W mercury lamp, with 100 % light intensity). In a typical experiment, a vial containing only a solution of PP-**2** in CD_3OD or a mixture of PP-**2** and precursors for aldol reaction in buffer solution was placed directly in front of the optical fiber aperture and illuminated with light for 30 minutes.

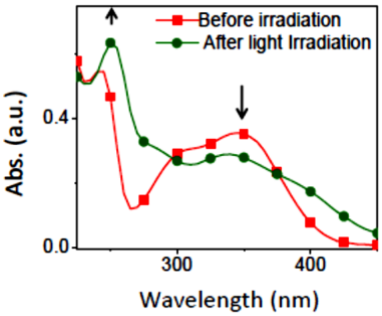


Figure S3: UV-Vis absorption spectra of PP-**2** before (red) and after (green) light irradiation.

Light-triggered proline release:

A vial with a solution of PP-**2** (0.05 g) in MeOH (2.0 mL) was placed directly in front of the optical fiber aperture and illuminated with light for 30 minutes. The mixture was then concentrated and the residue was analyzed by ^{1}H NMR spectroscopy and mass spectroscopy.

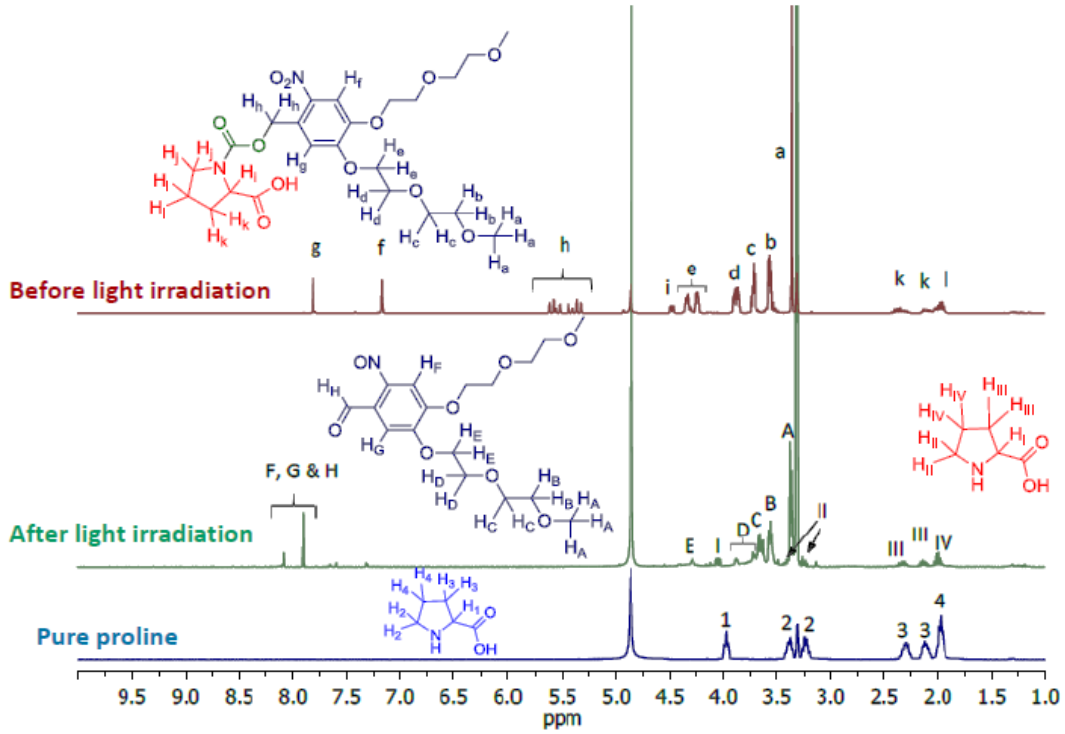


Figure S4: ^{1}H NMR analysis of light triggered proline release from PP-**2** in CD_3OD at 25 °C, before (top in red) and after light irradiation (middle in green). The released proline has a comparable spectrum to pure proline (bottom in blue).

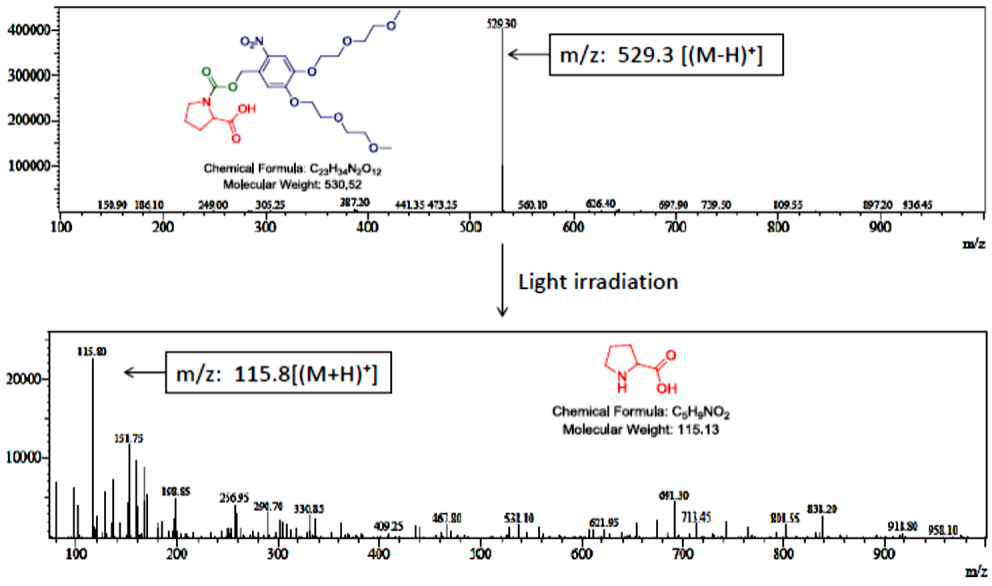


Figure S5: Mass spectrometry analysis of proline release from PP-**2**: before (top) and after (bottom) light irradiation.

3.3 Enzyme triggered proline release

Penicillin Acylase (5.5 mg, 98 U, Penicillin Acylase from *Escherichia coli*, Sigma Aldrich) in sodium phosphate buffer (100 mM, pH 7.4, 100 µL) was added to a solution of PP-**3** (0.5 mg) in acetone (10 µL) and the mixture was stirred for 10 minute at room temperature. After that, it was concentrated under reduced pressure and diluted with CH_3OH, followed by filtration. After concentration under reduced pressure, the obtained residue was analyzed by ^1H NMR and mass spectrometry.

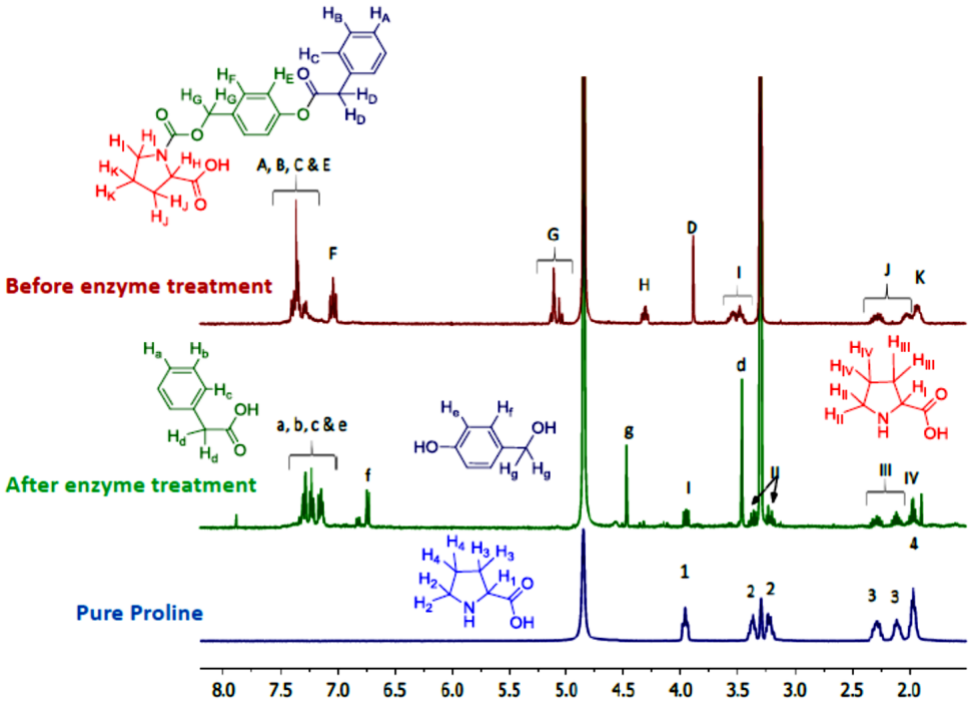


Figure S6: ^1H NMR analysis of enzyme (Peniciline Acylase) triggered proline release from PP-**3** in CD_3OD at 25 °C: PP-**3** before (top in red) and after enzyme treatment (middle in green). The released proline has a comparable spectrum to pure proline (bottom in blue).

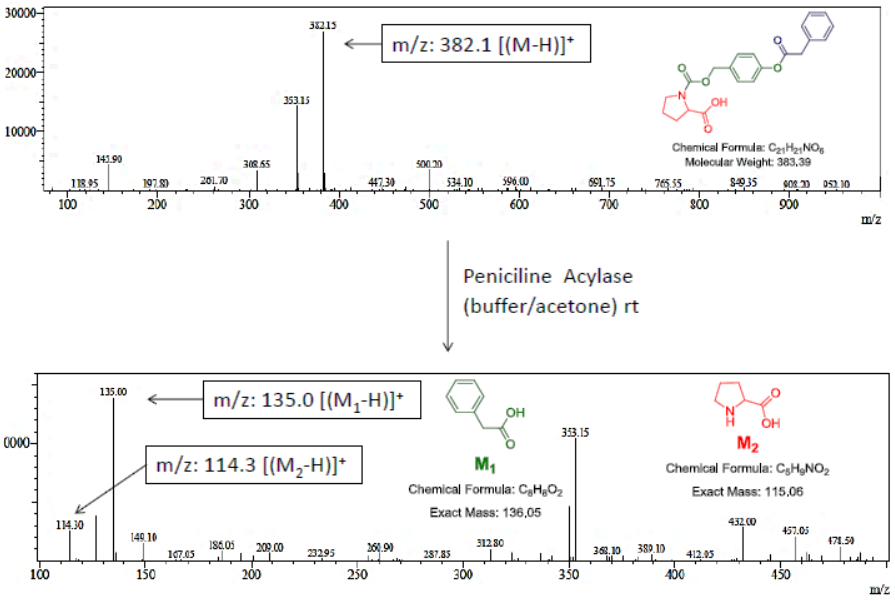
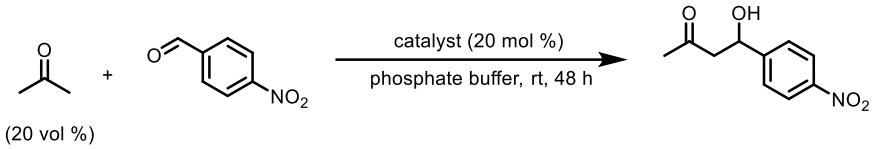


Figure S7: Mass spectrometry analysis of enzyme (Penicilin Acylase) triggered proline release from PP-**3**: before (top) and after (bottom) enzyme treatment.

4. Procedure for the aldol reaction



Scheme S4: General reaction scheme for direct aldol reaction**.**

4.1 General procedure to follow the aldol reactions

In a typical experiment, 4-nitrobenzyldehyde **6** (10.0 mmol) was added to the mixture of aqueous solution (480 µL) and acetone **5** (120 µL, 20 vol %) of a total volume of 600 µL. The aqueous solution consist of 430 µL of 100 mM sodium phosphate buffer of pH 7.4 and 50 µL D_2O. Sodium dodecyl sulfate (SDS, 0.1 equivalents) was added as an additive following the reported procedure.[5] The pro-prolines and signals were added to the mixture and in the mixture was transferred to an NMR tube. Conversion of the aldol reaction was determined by ^{1}H NMR analysis. We did not investigate the enantioselectivity of the reaction. The direct aldol reaction was compared using proline (0.14 mg, 2.0 mmol, 20 mol %) as catalyst.

4.2 H_2O_2 triggered aldol reaction:

In an NMR tube, 4-nitrobenzaldehyde **6** (0.9 mg, 10.0 mmol) was added to the mixture of sodium phosphate buffer of pH 7.4 (100 mM, 430 µL), D_2O (50 µL), acetone **5** (120 µL, 20 vol

%), SDS (0.2 mg, 1.0 mM) and PP-**1** (0.4 mg, 20 mol %). To this mixture H_2O_2 was added and reaction was followed via analyzing ^{1}H NMR spectra. For the control reactions, PP-**1** (0.4 mg, for non-activated catalysis), PP-**1** (0.4 mg) & light irradiation for 30 minutes (a wrong trigger) or nothing (for background reaction) was used replacing PP-**1** and H_2O_2. A controlled chemical triggered direct aldol reaction was performed when PP-**1** (0.4 mg) was used in the reaction mixture and H_2O_2 (1 eq.) was added after 22 h.

Table S1: H_2O_2 triggered proline release from PP-**1** and direct aldol reaction between 4-nitrobenzaldehyde **6** and acetone **5** (pH 7.4, room temperature, 48 h)

Entry	trigger	Compound used for catalysis[a]	Yield (%) of aldol product[b]
1	-	-	65
2	-	PP-**1**	65
3	H_2O_2[C]	PP-**1**	93
4	-	P-**4**	99
5	Light	PP-**1**	67

[a]0.1 equivalent SDS used as an additive, [b]conversion as determined by ^{1}H NMR, [C]1 equivalent H_2O_2 with respect to PP-**1**.

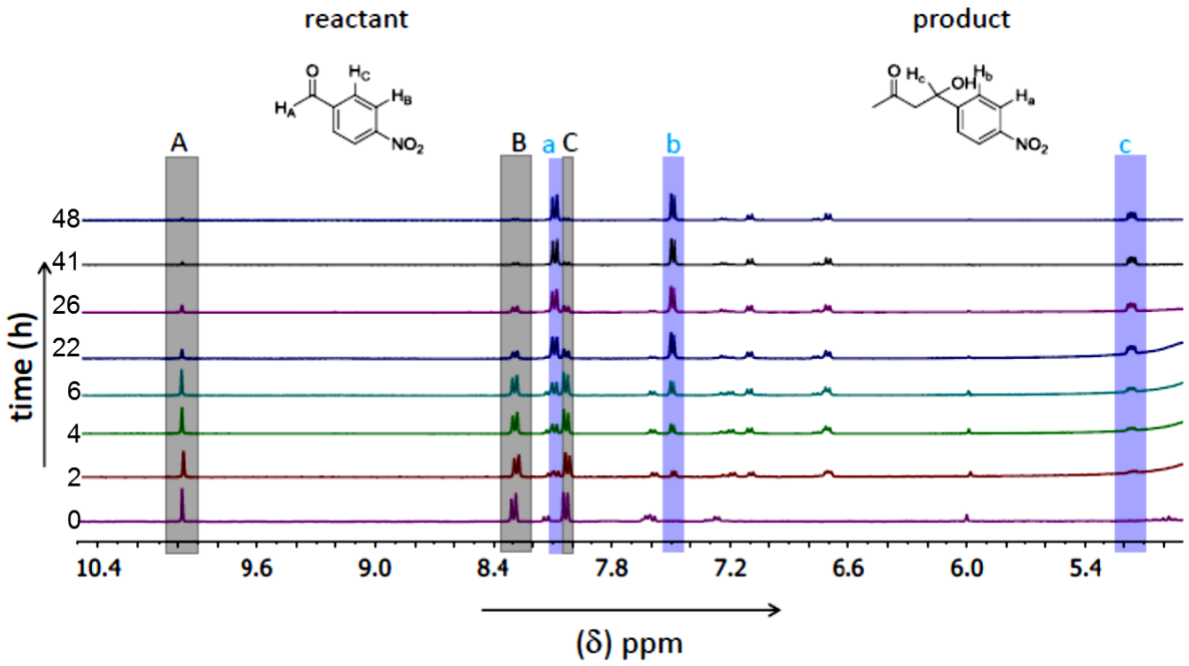
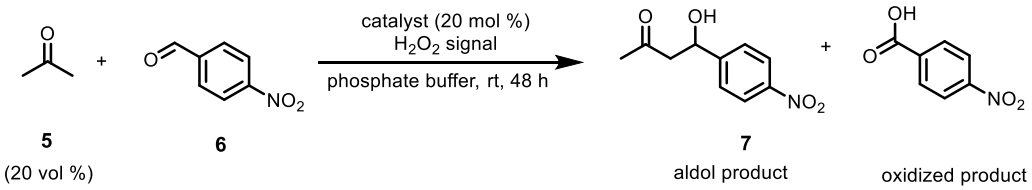


Figure S8: ^{1}H NMR (partial) spectral analysis of H_2O_2 (1 eq.) triggered direct aldol reaction using PP-**1** in phosphate buffer (100 mM, pH 7.4) at room temperature at different time intervals.



Scheme S5: Aldol formation and the oxidation of 4-nitrobenzaldehyde **6**.

Table S2: H_2O_2 trigger direct aldol reaction between 4-nitrobenzaldehyde and acetone at room temperature using PP-**1.**

Entry	Equivalence of H_2O_2 with respect to PP-**1**[a]	Yield (%) of aldol product[b]	Yield (%) of oxidized product
1	0	65	0
2	1	93	<1.0
3	2	93	1.5
4	4	92	3.5
5	6	90	5.0
6	40	75	20.0

[a]0.1 equivalent SDS used as an additive, [b]conversion as determined by ^{1}H NMR.

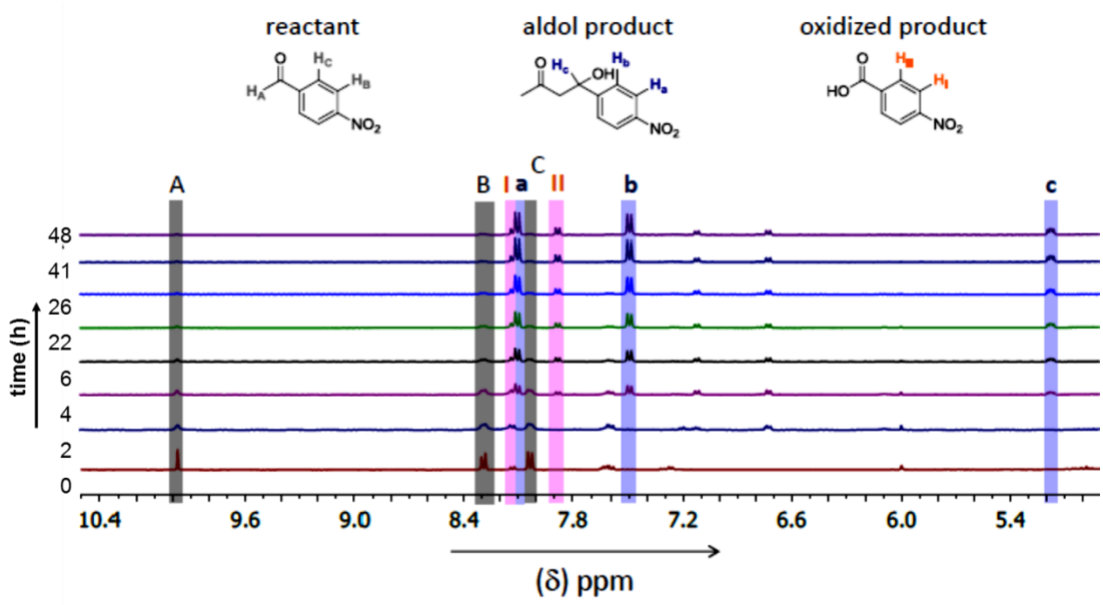


Figure S9: Partial ^{1}H NMR spectra of H_2O_2 (40 eq.) triggered direct aldol reaction using PP-**1** in phosphate buffer (100 mM, pH 7.4) at room temperature at different time intervals.

4.3 Light triggered aldol reaction

In a vial, 4-nitrobenzaldehyde **6** (0.9 mg, 10.0 mmol) was added to the mixture of sodium phosphate buffer of pH 7.4 (100 mM, 430 µL), D_2O (50 µL), acetone **5** (120 µL, 20 vol %), SDS

(0.2 mg, 1.0 mmol) and PP-**2** (0.6 mg, 20 mol %). The mixture was then placed directly in front of the optical fiber aperture and illuminated with light. After that the mixture was transferred to an NMR tube and the aldol reaction was followed via analyzing ^{1}H NMR spectra. For control reactions, PP-**2** (0.6 mg, 20 mol %, for inactivated catalysis), PP-**2** (0.6 mg) & H_2O_2 (a wrong trigger) or nothing (for background reaction) was added replacing PP-**2** and light illumination. A controlled light triggered aldol reaction was performed when PP-**2** was added to the reaction mixture and after 22 h light was irradiated and aldol reaction was monitored via ^{1}H NMR spectroscopy.

Table S3: Direct aldol reaction between 4-nitrobenzaldehyde **6** and acetone **5** at room temperature after (pH 7.4, 48 h, room temperature)

Entry	Compound used for catalysis[a]	Trigger	Yield (%) of aldol product[b]
1	-	-	65
2	PP-**2**	-	65
3	PP-**2**	Light	93
4	P-**4**	-	99
5	PP-**2**	H_2O_2	67

[a]0.1 equivalent SDS used as an additive, [b]conversion as determined by ^{1}H NMR.

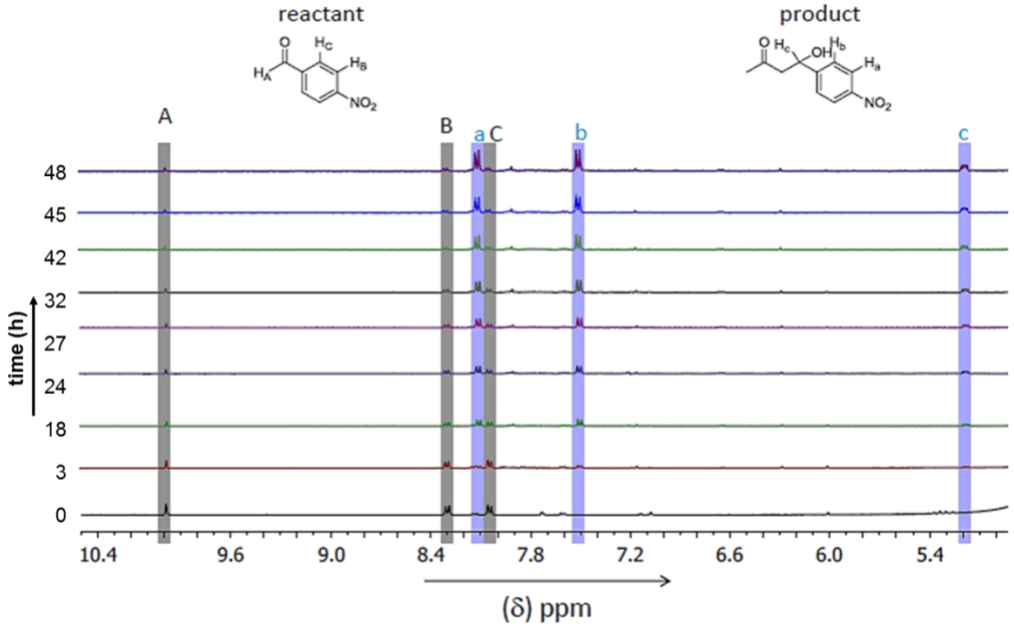


Figure S10: ^{1}H NMR (partial) spectral analysis of light triggered direct aldol reaction using PP-**2** in phosphate buffer (100 mM, pH 7.4) at room temperature at different time intervals.

4.4 Enzyme triggered aldol reaction

In an NMR tube, 4-nitrobenzaldehyde **6** (0.9 mg, 10.0 mmol) was added to the mixture of phosphate buffer of pH 7.4 (100 mM, 330 µL), D_2O (50 µL), acetone **5** (120 µL, 20 vol %), SDS (0.2 mg, 1.0 mmol) and PP-**3** (0.5 mg, 20 mol %). To this mixture Penicillin Acylase (5.5 mg, 98 U, Penicillin Acylase from *Escherichia coli*, Sigma Aldrich) in phosphate buffer (100 µL) was added and reaction was followed via analyzing ^{1}H NMR spectra. For control reaction, PP-**3** (0.5 mg, 20 mol %, for inactivated catalysis) or PP-**3** (0.5 mg) & light irradiation for 30 minutes (a wrong trigger) was employed. A controlled enzyme triggered aldol reaction was performed when PP-**3** (0.5 mg) was used and after 22h Penicillin Acylase enzyme (5.5 mg) was added to the reaction mixture.

Table S4: Direct aldol reaction between 4-nitrobenzaldehyde and acetone at room temperature (pH 7.4, 48 h, room temperature)

Entry	Compound used for catalysis[a]	Trigger	Yield (%) of aldol product[b]
1	None (background)	-	65
2	PP-**3**	-	67
3	PP-**3**	Penicillin Acylase	97
4	P-**4**	-	99
5	PP-**3**	Light	66

[a]0.1 equivalent SDS used as an additive, [b]conversion as determined by ^{1}H NMR.

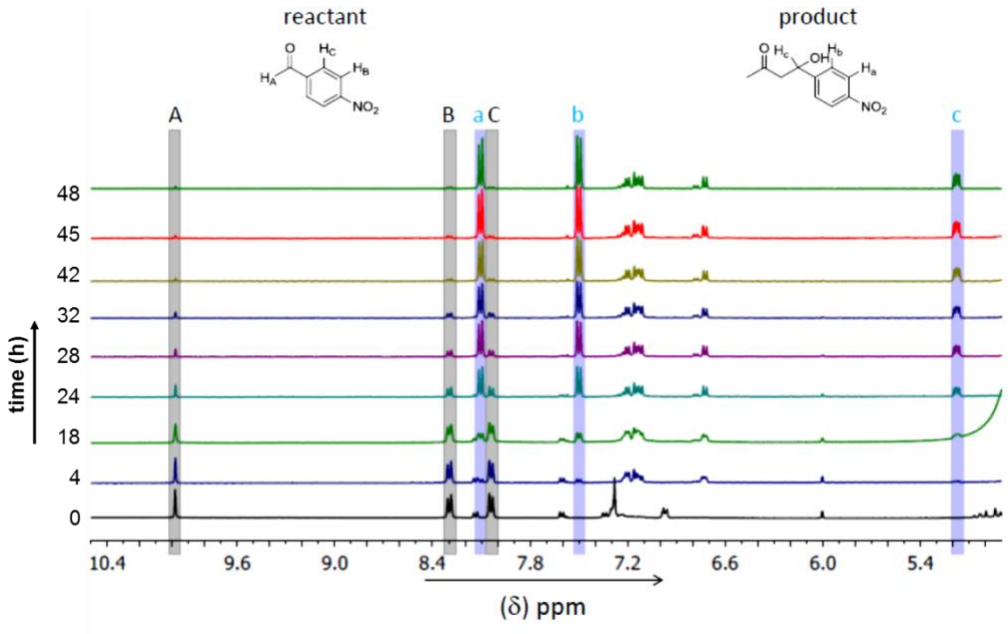
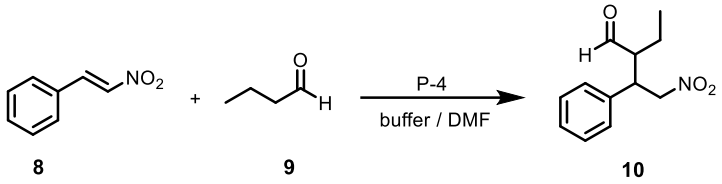


Figure S11: ^{1}H NMR (partial) spectral analysis of enzyme triggered direct aldol reaction using PP-**3** in phosphate buffer (100 mM, pH 7.4) at room temperature at different time intervals.

5. Michael reaction



8 **9** **10**

Scheme S6: Michael reaction between *trans*-β-nitrostyrene **8** and butanal **9**.

H_2O_2 triggered Michael reaction:

The Michael reaction was performed in 20% DMF-d7 in a sodium phosphate buffer (10 mM, pH 8.0), containing *trans*-β-nitrostyrene **8** (10 mM), butanal **9** (100 mM), PP-**1** (10 mM) or P-**4** (10 mM), or H_2O_2 (100 mM). The NMR tube contained a total solvent volume of 0.6 mL. The stock solutions were added as follows: *trans*-β-nitrostyrene **8** solution (100 mM in DMF-d7), DMF-d7, PP-**1** (100 mM in DMF-d7), P-**4** (100 mM in buffer), buffer. H_2O_2 (6.1 µL, 30%) and butanal **9** (5.4 µL, 99%) were added at the last possible moment before the NMR measurement started.

Table S5: H_2O_2 triggered proline release from PP-**1** and Michael reaction between *trans*-β-nitrostyrene **8** and butanal **9** (20% DMF-d7 in 10 mM sodium phosphate buffer pH 8.0).

Catalyst system	***Yield (%) of Michael product after 8 h of reaction***
None	0
PP-**1**	0
H_2O_2	0
P-**4**	100
PP-**1** + H_2O_2	89
P-**4** + H_2O_2	72

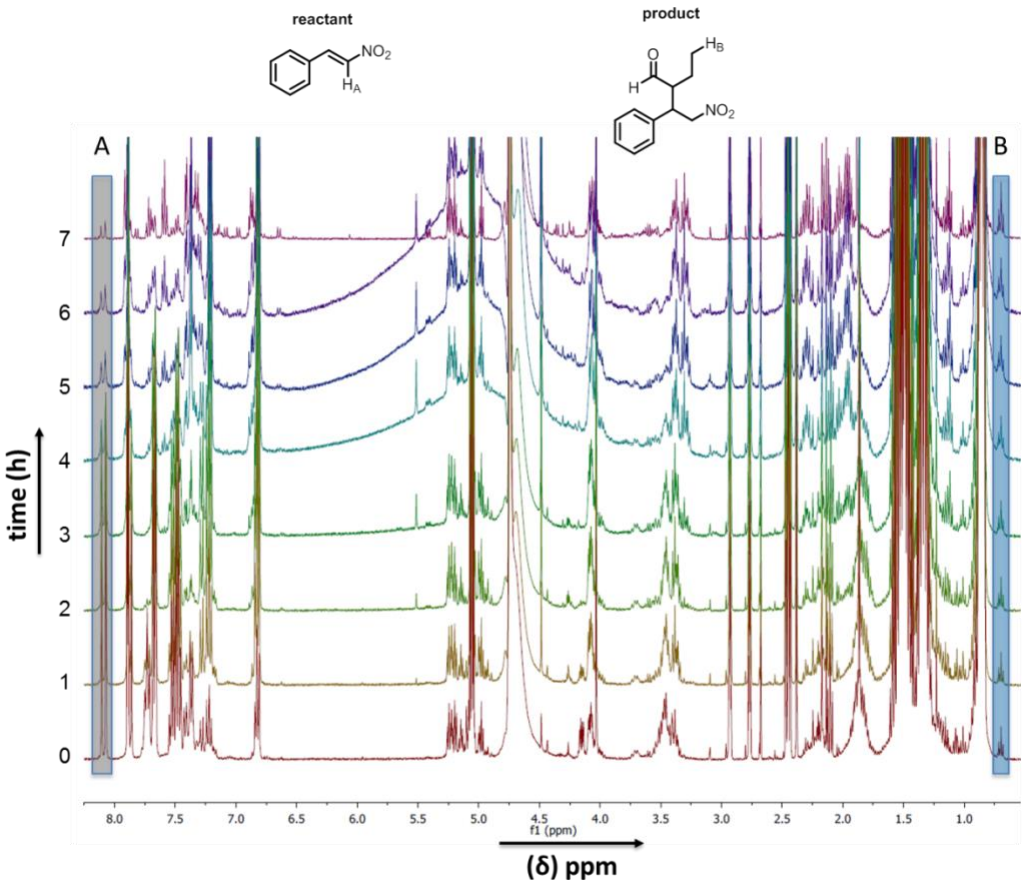
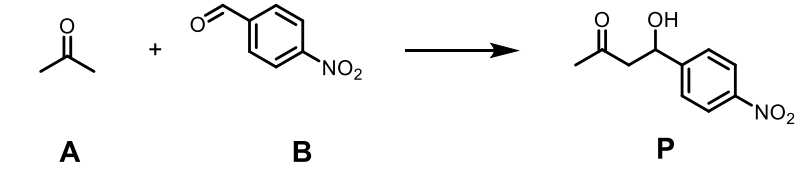


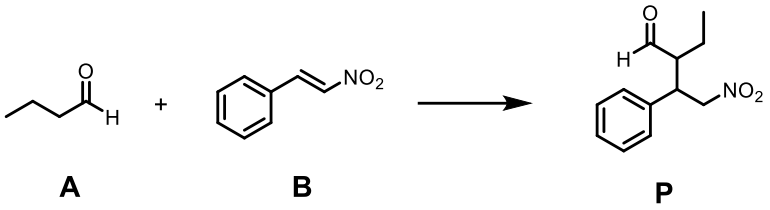
Figure S12: ^{1}H NMR (partial) spectral analysis of H_2O_2 (10 eq.) triggered Michael reaction between *trans*-β-nitrostyrene **8** and butanal **9** using PP-**1** (20% DMF-d7 in 10 mM sodium phosphate buffer pH 8.0) at 25 °C. At the position of peak B, a small, unchanging peak is visible from t = 0. The integral of this background peak is subtracted from all integrals of peak B in the kinetic analysis.

6. Reaction kinetics

6.1 General procedure

The aldol reaction and the Michael reaction are both second order reactions. The reactions were performed at pseudo-first order conditions with one of the reagents in excess. Concentrations used in the aldol reaction: 2.72 M acetone, 0.01 M 4-nitrobenzaldehyde. Concentrations used in the Michael reaction: 100 mM butanal, 10 mM *trans*-β-nitrostyrene.





Start, t = 0	$[A]_0$	$[B]_0$	0
At t = t	$[A]_t = [A]_0 - [P]_t$	$[B]_t = [B]_0 - [P]_t$	$[P]_t$

Here, [A] is the concentration of the reagent used in excess: acetone in the aldol reaction and the concentration of butanal in the Michael reaction. [B] is the concentration of 4-nitrobenzaldehyde in the aldol reaction and *trans*-β-nitrostyrene in the Michael reaction. [P] is the aldol product in the aldol reaction and the Michael product in the Michael reaction.

The 2nd order reaction rate equation (assuming 1st order in both A and B) can be written as:

$Rate = -k[A]_t[B]_t$

For $[A]_0 \neq [B]_0$, the integrated second order rate law can be written as:

$\ln([A]_t/[A]_0) - \ln([B]_t/[B]_0) = k([A]_0-[B]_0)t$

Because a large excess of A (acetone and butanal) was used in the reaction, it can be assumed that:

$[A]_0 >>]B]_0$, $[A]_t/[A]_0 \approx 1$ and $[A]_0-[B]_0 \approx [A]_0$

The rate law can be written as:

$[B]_t/[B]_0 = \exp(-k[A]_0t)$

Rearranging $[B]_t = [B]_0 - [P]_t$ to $[B]_0 = [B]_t + [P]_t$ and replacing $[B]_0$ at the rate law, it can be rewritten as:

$[B]_t/([B]_t + [P]_t) = \exp(-k[A]_0t)$

This is rearranged to:

$$\ln\left\{1-\frac{[P]_t}{([B]_t + [P]_t)}\right\} = -k[A]_0t$$

where, $[B]_t$ is the concentration of *trans*-β-nitrostyrene in the Michael reaction and 4-nitrobenzaldehyde in the aldol reaction. $[P]_t$ is the concentration of the Michael product and the aldol product at every specified time obtained from ^{1}H NMR spectral analysis. $[A]_0$ is the initial concentration of acetone in the aldol reaction and the initial concentration of butanal in the Michael reaction. $[B]_0$ is the initial concentration of 4-nitrobenzaldehyde in the aldol reaction and the initial concentration *trans*-β-nitrostyrene in the Michael reaction. A line was obtained via plotting $\ln\left\{1-\frac{[P]_t}{([B]_t + [P]_t)}\right\}$ vs t and it was fitted to the y = mx line in Origin to obtain the rate constants of the pseudo-first order reactions.

6.2 Reaction kinetics of the aldol reaction

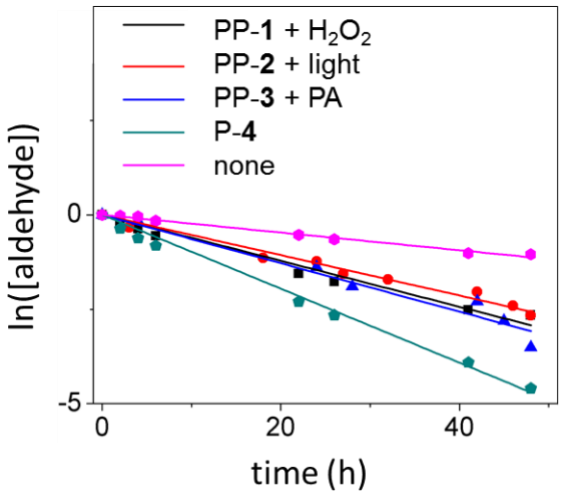


Figure S13: Signal triggered proline release and subsequent catalysis of an aldol reaction between 4-nitrobenzaldehyde **6** and acetone **5** in aqueous buffer media (100 mM, pH 7.4), where $[\text{aldehyde}] = \ln\left\{1-\frac{[P]_t}{([B]_t + [P]_t)}\right\}$. Signal triggered aldol reaction was compared with proline catalysed aldol reaction and background reaction.

For the delayed activation procedure, aldol product was formed due to the combination of uncatalyzed (background) reaction and proline catalyzed reaction (after signal addition). Therefore two lines were obtained via plotting $\ln\left\{1-\frac{[P]_t}{([B]_t + [P]_t)}\right\}$ vs t and it was fitted to the

equation if x<22h, y = m_1x, else y = m_2x in Origin to obtain the rate constants for delayed activation procedure.

Table S6: Direct aldol reaction between 4-nitrobenzaldehyde and acetone (pH 7.4, 48 h, room temperature) using pro-prolines, whereas they were activated with the signal after 22 h (delayed activation) with calculated second order rate constant.

Pro-proline+ signal	Rate constant ($M^{-1}s^{-1}$) before signal added	Rate constant ($M^{-1}s^{-1}$) after signal added
PP-**1** + H_2O_2	$2.76x10^{-6}$	$4.53\ x10^{-6}$
PP-**2** + Light	$2.83x10^{-6}$	$4.17\ x10^{-6}$
PP-**3** + *Penicillin Acylase*	$2.75\ x10^{-6}$	$3.78\ x10^{-6}$

Rate constant before the signal was employed corresponds to the background reaction and after the signal was employed, the reaction was catalyzed by released proline. Slower reaction rates for the delayed activation procedure compared to when the signal is used at the beginning is due to lower concentrations of the reagents after 22 h.

6.3 Reaction kinetics of the Michael reaction

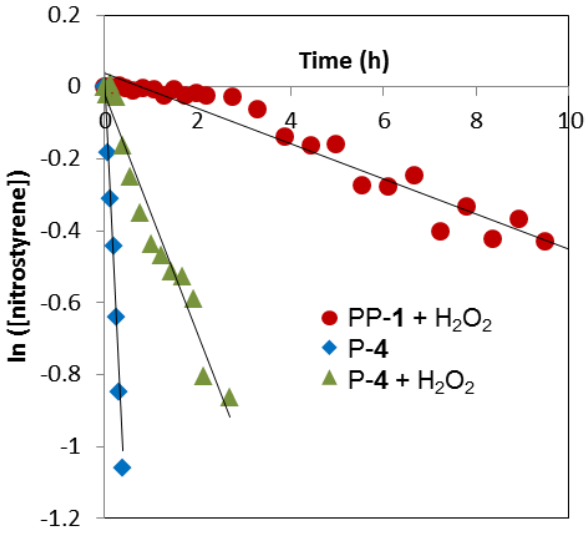


Figure S14: H_2O_2 triggered proline release and subsequent catalysis of Michael reaction between trans-β-nitrostyrene **8** and butanal **9** (20% DMF-d7 in 10 mM sodium phosphate buffer pH 8.0) at 25 °C, where $[nitrostyrene] = \ln\left\{1-\frac{[P]_t}{([B]_t + [P]_t)}\right\}$.

7. NMR spectra

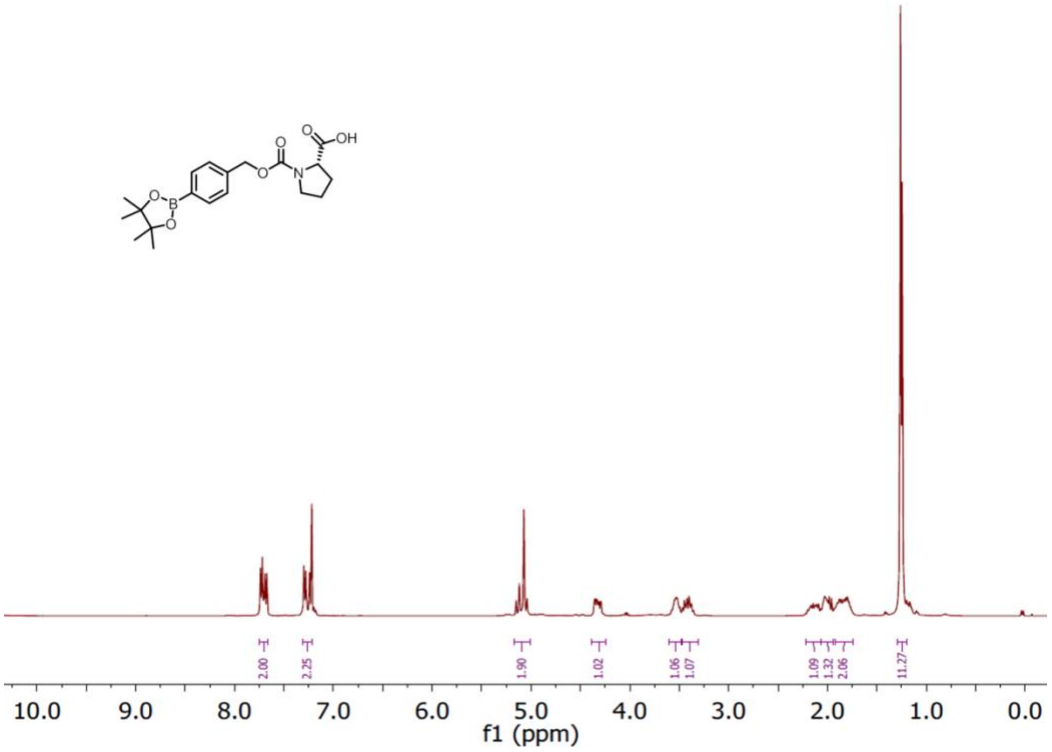


Figure **S15**: ^{1}H NMR spectrum of boronate-ester derivative in $CDCl_3$ at 25 °C.

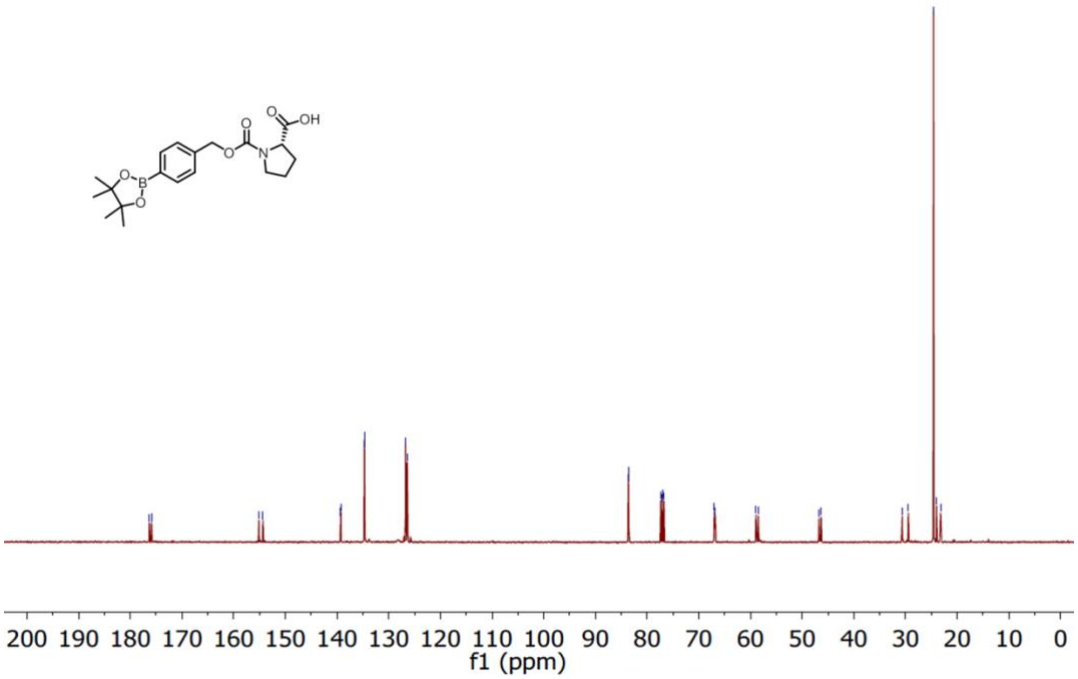


Figure **S16**: ^{13}C NMR spectrum of boronate-ester derivative in $CDCl_3$ at 25 °C.

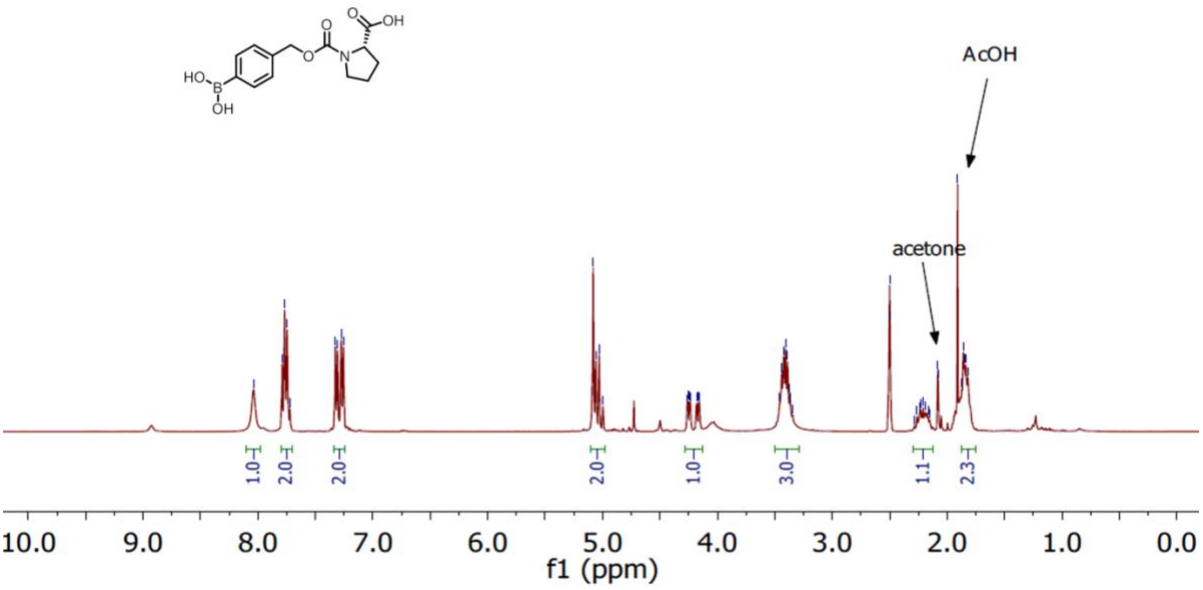


Figure **S17**: ^{1}H NMR spectrum of PP-**1** in DMSO-d6 at 25 °C.

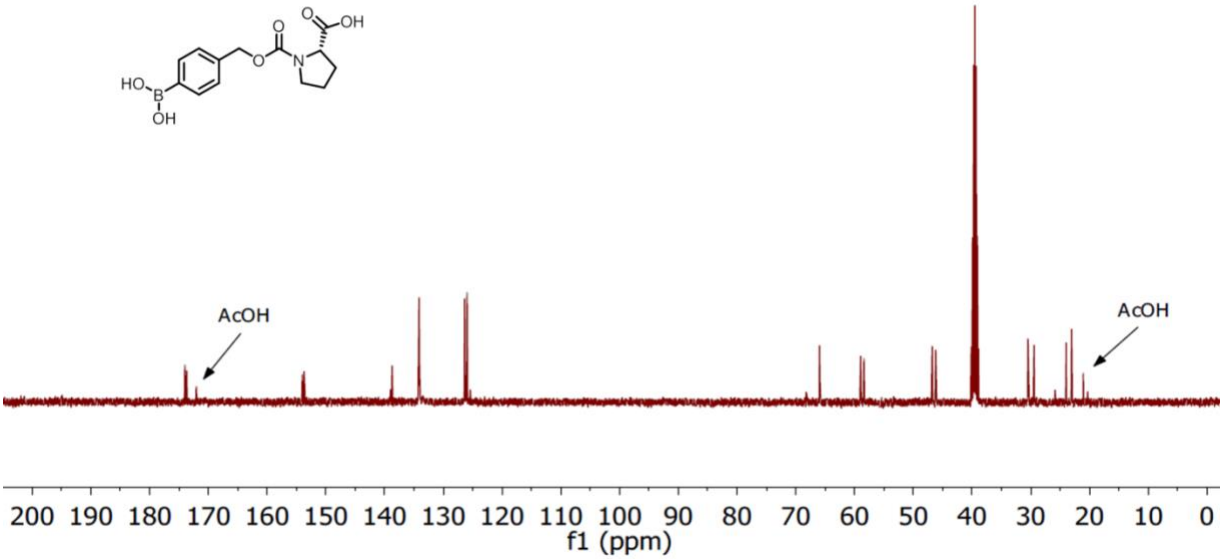


Figure **S18**: ^{13}C NMR spectrum of PP-**1** in DMSO-D_6 at 25 °C.

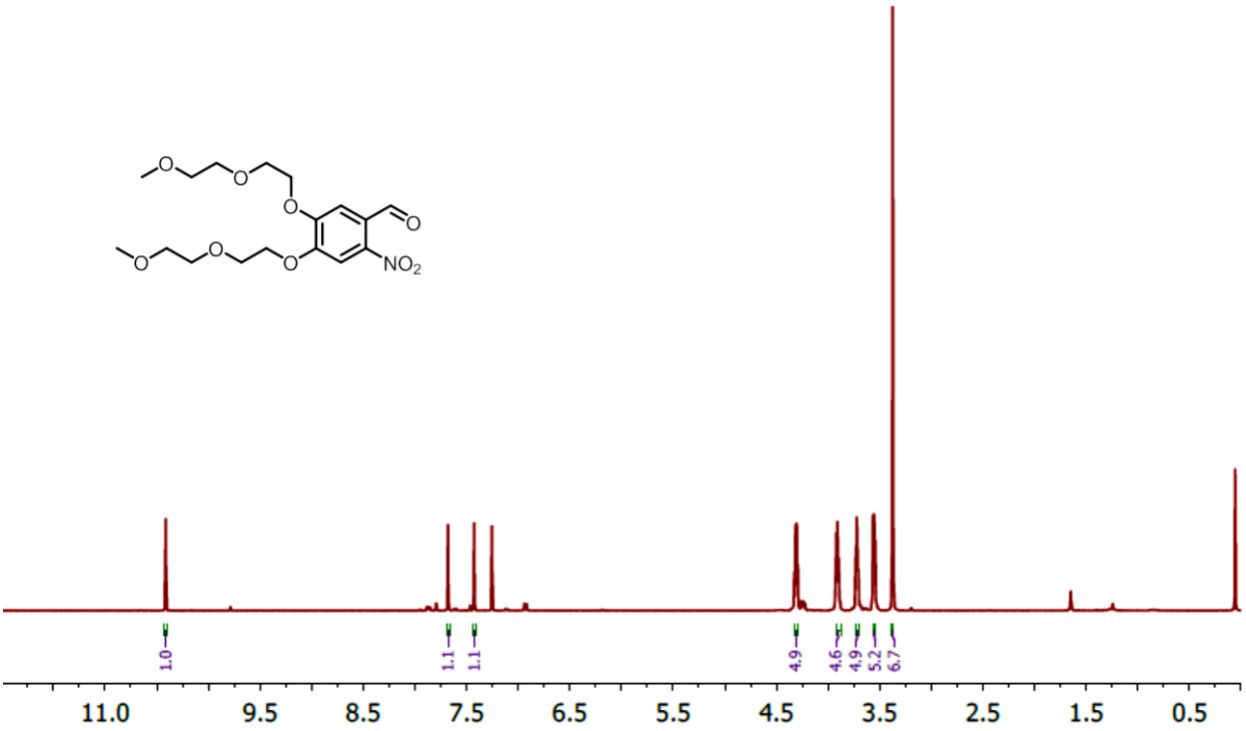


Figure **S19**: ^{1}H NMR spectrum of 4,5-bis(2-(2-methoxyethoxy)ethoxy)-2-nitrobenzaldehyde in $CDCl_3$ at 25 °C.

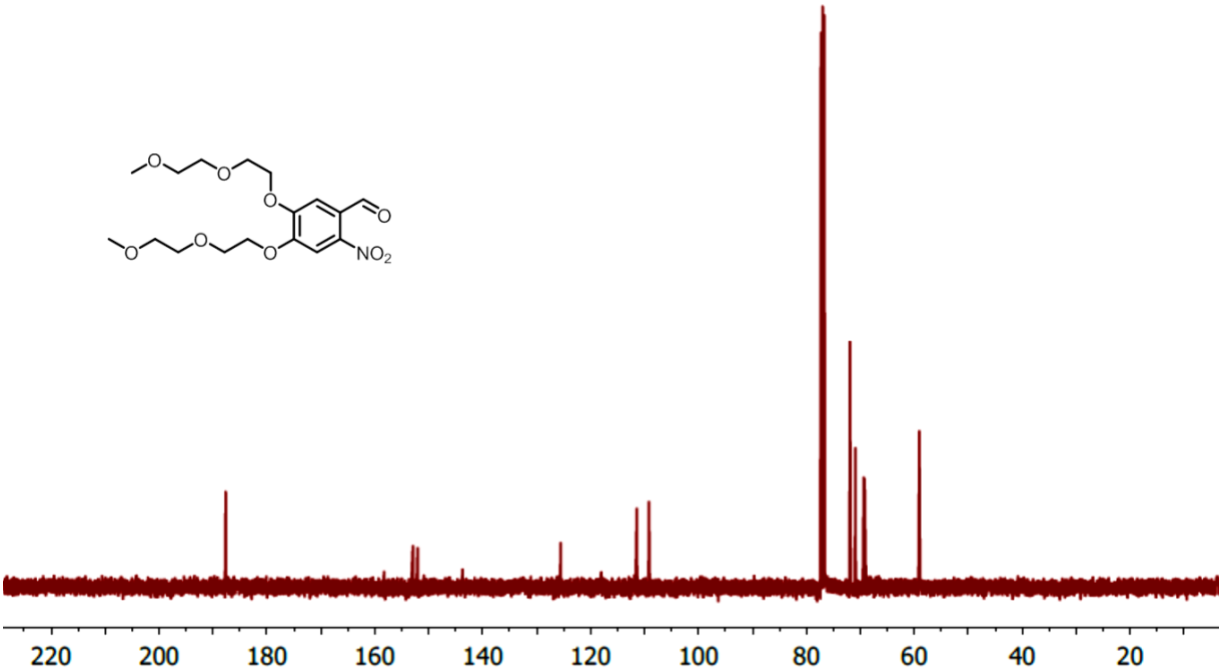


Figure **S20**: ^{13}C NMR spectrum of 4,5-bis(2-(2-methoxyethoxy)ethoxy)-2-nitrobenzaldehyde in CDCl3 at 25 °C.

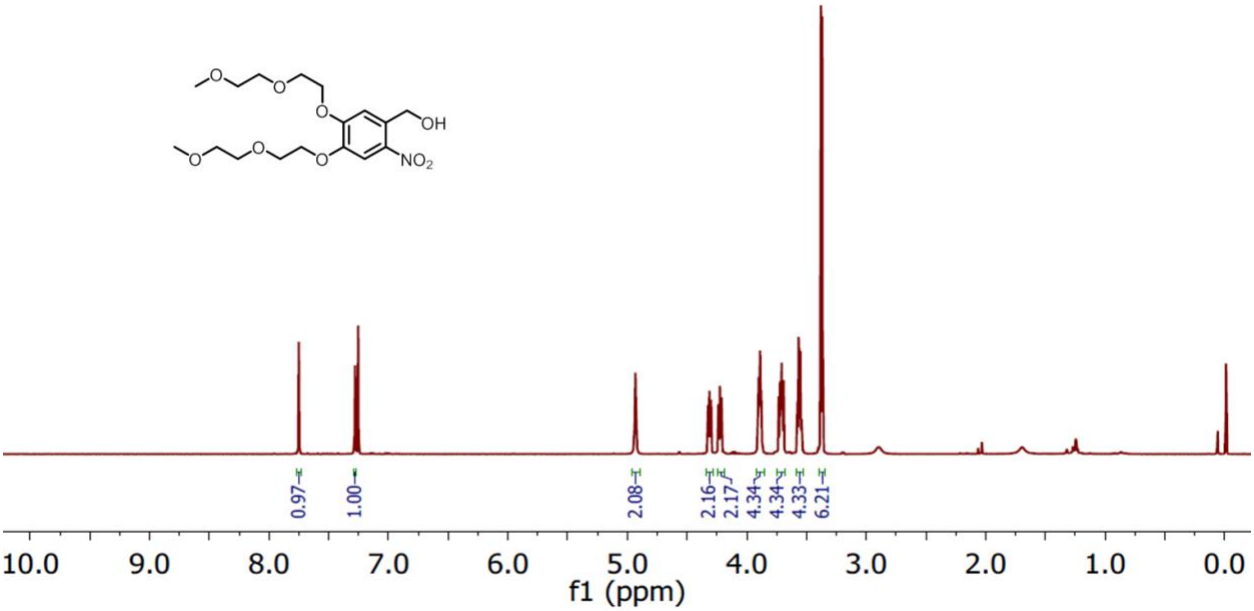


Figure **S21**: ^{1}H NMR spectrum of 4,5-bis(2-(2-methoxyethoxy)ethoxy)-2-nitrophenyl)methanol in $CDCl_3$ at 25 °C.

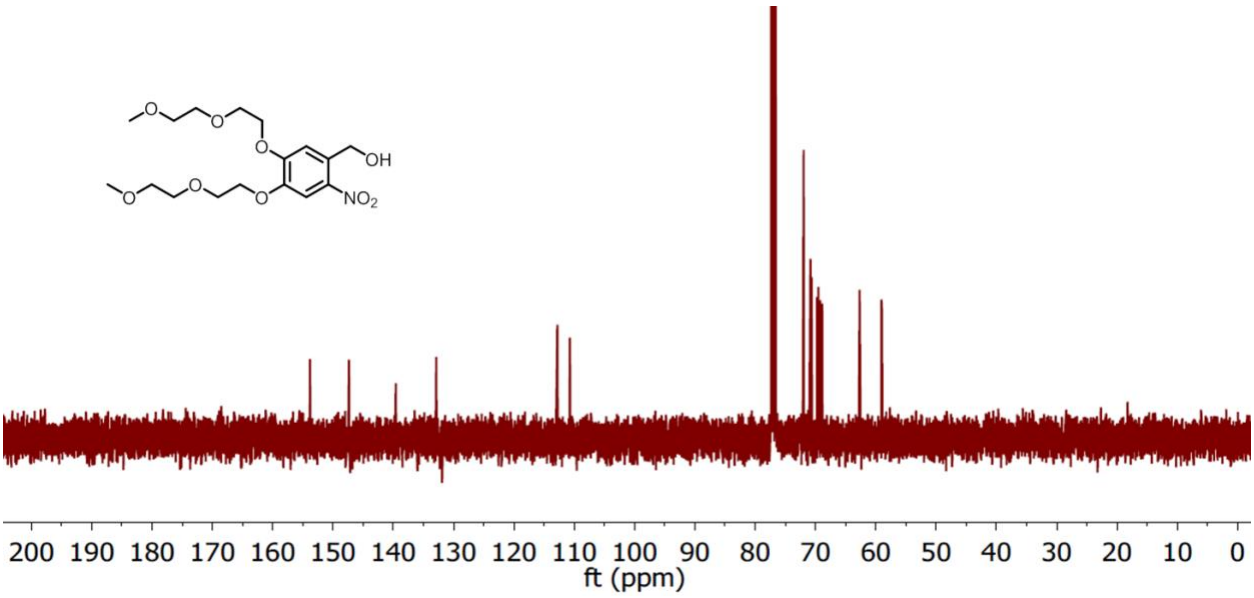


Figure **S22**: ^{13}C NMR spectrum of 4,5-bis(2-(2-methoxyethoxy)ethoxy)-2-nitrophenyl)methanol in $CDCl_3$ at 25 °C.

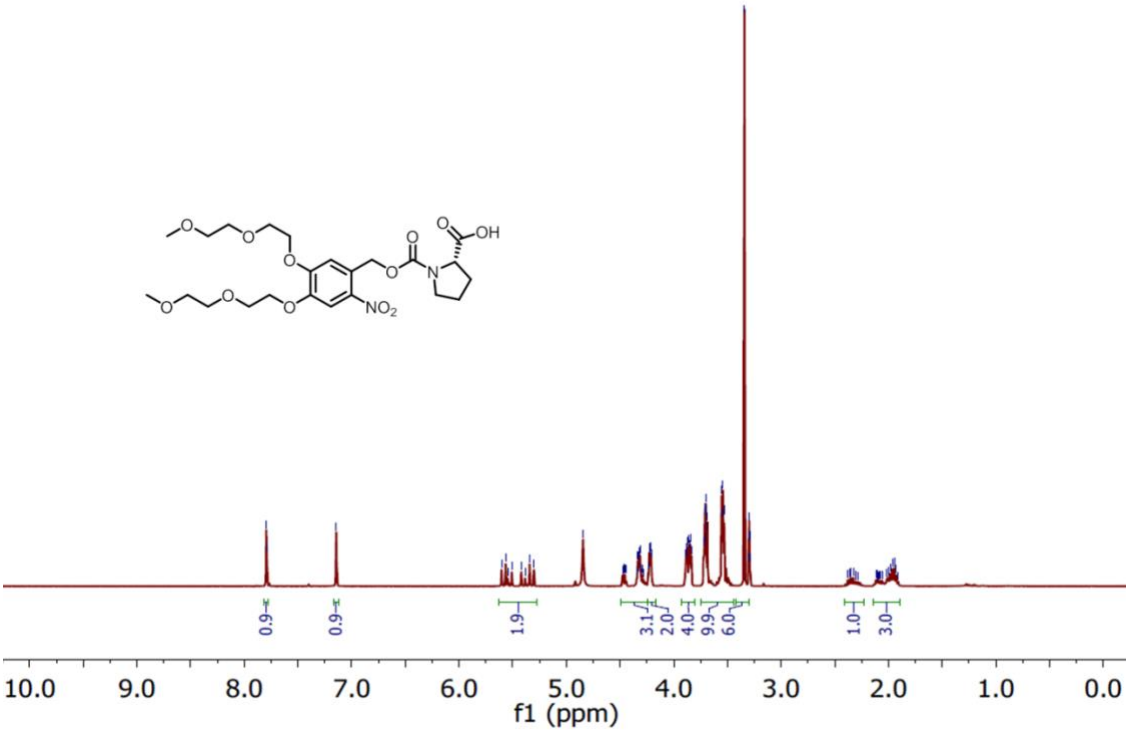


Figure **S23**: ^{1}H NMR spectrum of PP-**2** in CD_3OD at 25 °C.

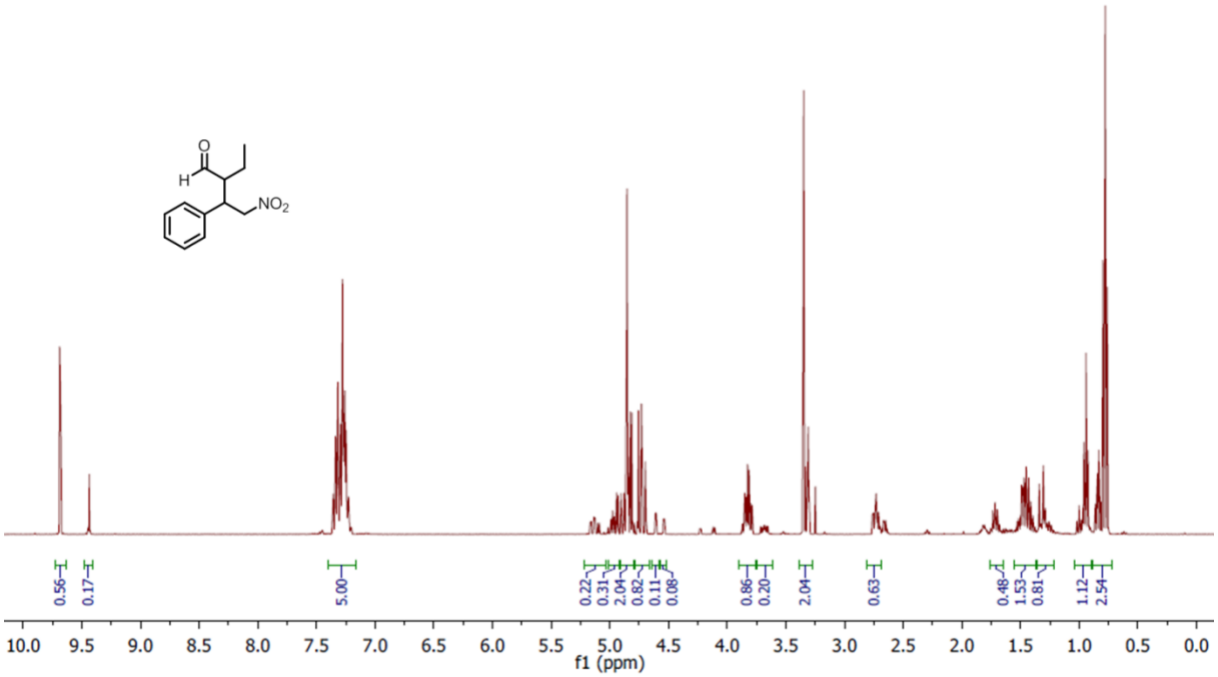


Figure S24: ^{1}H NMR spectrum of the Michael product **10** in CD_3OD.

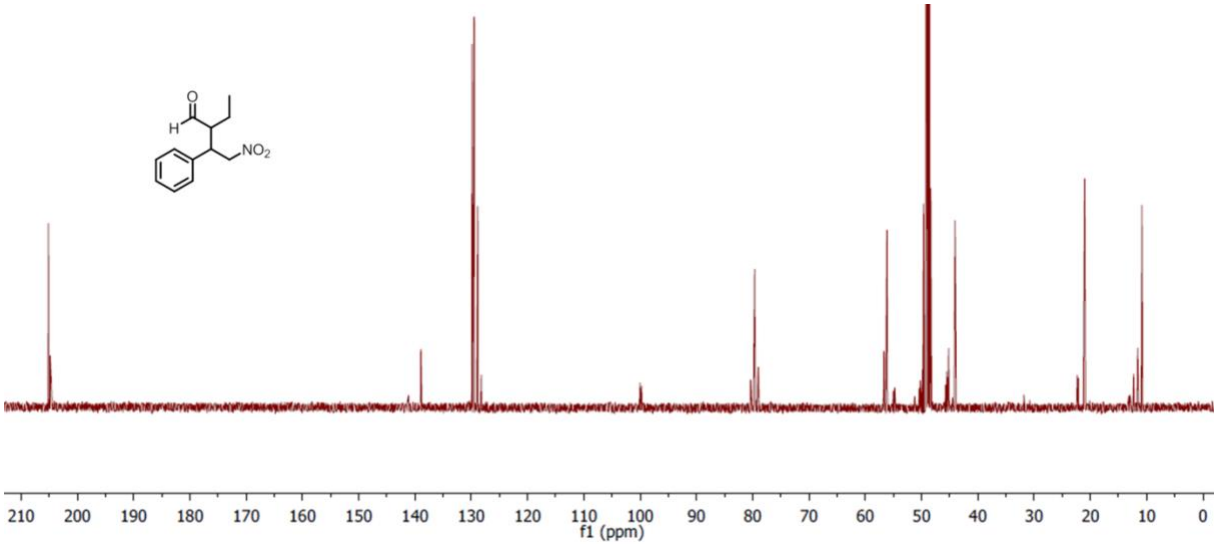


Figure S25: ^{13}C NMR spectrum of the Michael product **10** in CD_3OD.

8. References

(1) Chung, C., Srikun, D., Lim, C. S., Chang, C. J., Cho, B. R. *Chem. Commun*., **2011,** *47*, 9618.

(2) For the examples of carbamate rotamers, see: i) Takeuchi, Y., Yamada, A., Suzuki, T., Koizumi, T. *Tetrahedron*, **1996,** *52*, 225-232. ii) Aitken, R. A., Karodia, N., Massil, T., Young, R. J. *J. Chem. Soc., Perkin Trans*. **2002,** *1*, 533–541.

(3) Poolman, J. M., Boekhoven, J., Besselink, A., Olive, A. G. L., van Esch, J. H., Eelkema, R. *Nat. Protocols* **2014,** *9*, 977.

(4) Machauer, R., Waldmann, H. *Chem. Eur. J.*, **2001,** *7*, 2940.

(5) Cordova, A., Notz, W., Barbas III, C. F. *Chem. Commun.,* **2002,** 3024.