Supporting Information

Enantiocomplementary synthesis of γ-nitroketones using designed and evolved carboligases

Xavier Garrabou, Rebecca Verez, and Donald Hilvert*

Laboratory of Organic Chemistry, ETH Zurich, 8093 Zurich, Switzerland

*e-mail: hilvert@org.chem.ethz.ch

1. Materials and methods

1.1. Materials. Chemicals were purchased from Sigma, Acros, ABCR, Merck, Aldrich or Fluka and were used without further purification. Nitroolefins **1a**, **1b**, and **1c**, and conjugated ketones **2b** and **2c** were purchased from Sigma-Aldrich. Conjugated ketones **2a** and **2d** were prepared in a previous study.[1] Nitroolefin **1d** was prepared using a reported method.[2]

1.2. Assayed retro-aldolase variants. The library of retro-aldolase variants (Table S1) included recently reported mutants (RA95.5-8[3], K83M RA95.5-8[3], RA95.5-8F[4], KN.4[5], T53L/K210H RA95.5-8[1]) and additional variants from our sample collection (S233F RA95.5-8, K210R RA95.5-8, I133L RA95.5-8, T53L RA95.5-8). Enzymes were expressed and purified as described in previous studies,[1] and their identity was confirmed by DNA sequencing of the coding genes and MS analysis of the purified proteins.

Table S1. Mass spectrometry of RA95.5-8 and variants. Calculated masses reflect intracellular cleavage of the *N*-terminal methionine.

Enzyme	*Calculated mass / Da*	*Determined mass / Da*	*Error / Da*
RA95.5-8	29730.2	29729.9	0.3
K83M RA95.5-8	29733.2	29733.8	0.6
RA95.5-8F	29751.2	29751.8	0.6
KN.4	29630.1	29629.7	0.4
T53L/K210H RA95.5-8	29751.2	29750.1	1.1
S233F RA95.5-8	29790.3	29790.8	0.5
K210R RA95.5-8	29758.2	29757.2	1.0
I133L RA95.5-8	29730.2	29729.9	0.3
T53L RA95.5-8	29742.3	29741.8	0.5

1.3. General methods. NMR spectra were recorded on an AVIII 400 (^{1}H 400 MHz, ^{13}C 100 MHz) spectrometer. Optical rotations were measured on JASCO P-1010 operating at the sodium D line with a 100 mm path length cell.

1.4. Chromatographic analysis. A C18 column (Reprosil Gold 120 C18, 3 µm, 100 x 2 mm) was employed for analytical RP-HPLC analysis. The solvent system used was: A: aqueous TFA 0.1% (v/v); and B: acetonitrile 0.08% (v/v) TFA, gradient elution from 5% to 100% B in 5 min, flow rate 0.75 mL min^{-1}, detection 220 nm.

1.5. Chiral analysis. The enantiomeric ratio of the enzymatically prepared compounds (**3a**-**3d**, **3f**) was established by comparison with chemically synthesized racemic mixtures using chiral HPLC or Supercritical Fluid Chromatography (SFC) analysis. Chiral HPLC analysis was performed with a Waters 717plus Autosampler (Waters Corporation) equipped with two Waters 515 HPLC Pumps (Waters Corporation) and a Waters 996 Photodiode Array Detector (Waters Corporation). Separations were performed with a Chiralpak AS (Chiral Technologies Europe) column (150 x 2.1 mm, particles diameter = 10 µm) for **3a**, **3b**, and **3c**, and a Chiralcel OD-H (Chiral Technologies Europe) column (150 x 4.6 mm, particles diameter = 5 µm) was used for **3f**. Analytic conditions for each compound are detailed below. SFC analysis of **3d** was performed on a Jasco liquid chromatography unit, using a Daicel Chiralpak OJH column (4.6 × 250 mm); gradient: 5% isopropanol in CO_2 to 50% isopropanol in CO_2 over 10 min; flow: 3.0 mL min^{-1}; detection: 220 nm.

1.6. Determination of absolute configuration of enzymatically prepared adducts. Literature values for the optical rotation of both enantiomers of **3a**, **3b** and **3c** vary widely in sign and magnitude (see, for example, the values for (*S*)-**3a** reported in references 6-8), possibly due to the presence of chiral impurities that override the low optical signals of these compounds. This may also explain the differences in absolute value obtained in this study for the optical rotations of the enantiomerically enriched products **3a**-**3d** obtained using the two alternative nitro-Michael reactions. Therefore, the absolute configuration of the retro-aldolase adducts was assigned based on the following observations: (a) the elution order in chiral HPLC analysis of the enantiomers of **3a** and **3c** is consistent with reported separations using the same column;[9] (b) the optical rotation obtained for enzymatically prepared (*R*)-**3f** is high and consistent with a reported value (see Page S15);[10] (c) the selectivity obtained for enamine- and iminium-mediated nitro-Michael additions is consistently opposite; (d) and the consistent *Re* facial selectivity observed previously for the addition of nucleophiles to conjugated ketones using T53L/K210H RA95.5-8.[1]

1.7. Analytical scale reactions: addition of nitroolefins and acetone. Analytical scale reactions (400 µL total volume) in 1.5 mL centrifuge tubes were conducted in the dark, incubated in a water bath thermostated at 29 ºC. The nitroolefins **1a-d** (300, 600 or 1200 µM final concentration) were dissolved in acetone (58.7 µL, 2.0 M final concentration), and the enzyme solution (1.2 nmol, 3 µM final concentration) in buffer (341.5 µL, 25 mM HEPES 100mM NaCl, pH = 6.5, 7.0, 7.5 or 8.0) was added. Conversions were measured at 3 h and 24 h reaction time. Reactions were monitored as follows: aliquots (40 µL) were removed, diluted with acetonitrile (160 µL) and analyzed by HPLC under the conditions described on Page S3.

1.8. Analytical scale reactions: addition of conjugated ketones and nitroalkanes. Reactions were conducted in 1.5 mL centrifuge tubes incubated in a water bath thermostated at 29 ºC. The ketone acceptors **2a-d** (0.5 µmol, 5 mM final concentration) and nitromethane, nitroethane or 2-nitropropane (25, 50 75, 150, or 300 mM final concentration) were dissolved in acetonitrile (3.0 µL total volume), and the enzyme solution (0.5-1.0 nmol, 5-10 µM final concentration) in buffer (97 µL, 25 mM HEPES 100 mM NaCl, pH = 7.5) was added. Conversions were determined at 3 h and 24 h reaction time. Reaction monitoring was as follows: aliquots (5 µL) were removed, diluted with methanol (95 µL) and analyzed by HPLC under the conditions described on Page S3.

2. Reaction kinetics

Reaction kinetics were measured using a Perkin Elmer Lambda 35 UV-vis spectrometer equipped with a Peltier system for temperature control. The nitro-Michael additions between **1a** (50-500 μM; higher concentrations were not attainable due to limited solubility) and acetone (2 M) catalyzed by S233F RA95.5-8 and T53L/K210H RA95.5-8 were monitored spectroscopically at 380 nm ($\Delta\varepsilon$ = 17586 M^{-1} cm^{-1}), 400 nm ($\Delta\varepsilon$ = 9413 M^{-1} cm^{-1}), 420 nm ($\Delta\varepsilon$ = 3318 M^{-1} cm^{-1}) or 430 nm ($\Delta\varepsilon$ = 1632 M^{-1} cm^{-1}). Reactions were carried out at 29 °C in aqueous buffer (25 mM HEPES, 100 mM NaCl, pH 6.5) in sealed quartz cuvettes (l = 1.0 cm). Apparent steady-state kinetic parameters were derived by fitting the experimental data to the Michaelis-Menten equation: $v_0/[E] = k_{cat} \cdot [S]/(K_M + [S])$ with the program KaleidaGraph, where v_0 is the initial rate, [E] is the enzyme concentration, and [S] is the concentration of **1a**.

The nitro-Michael additions between **2a** and nitromethane (CH_3NO_2) catalyzed by T53L/K210H RA95.5-8 were monitored spectroscopically at 370 nm ($\Delta\varepsilon$ = 1858.5 M^{-1} cm^{-1}). Reactions were carried out at 29 °C in aqueous buffer (25 mM HEPES, 100 mM NaCl, pH 7.5) in sealed quartz cuvettes (l = 1.0 cm for [**2a**] ≤ 600 μM, l = 0.5 cm for [**2a**] ≥ 700 μM). Acetonitrile at a final concentration of 3.0 % was included as co-solvent to facilitate substrate solubility. Steady-state kinetic parameters were derived by fitting the experimental data to the equation shown below using the R statistics package[11]:

$$v_0/[E] = k_{cat}\cdot[\mathbf{2a}]\cdot[CH_3NO_2]/(K_{M,\mathbf{2a}}\cdot K_{M,\,CH_3NO_2}+K_{M,\mathbf{2a}}\cdot[CH_3NO_2]+K_{M,\,CH_3NO_2}\cdot[\mathbf{2a}]+[\mathbf{2a}]\cdot[CH_3NO_2])$$

The activity of K83M RA95.5-8 was evaluated under the conditions described on Page S4 for analytical scale reactions. Negligible formation of products (< 5 %) was observed by HPLC for either reaction type after 24 h.

3. Kinetic plots

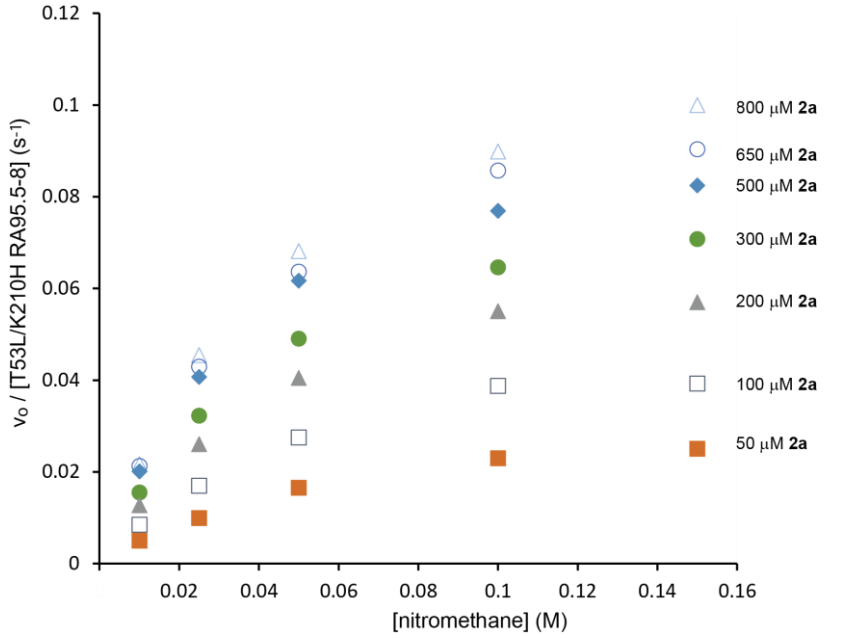


Figure S1. Kinetic characterization of the nitro-Michael addition between **2a** and nitromethane catalyzed by T53L/K210H RA95.5-8.

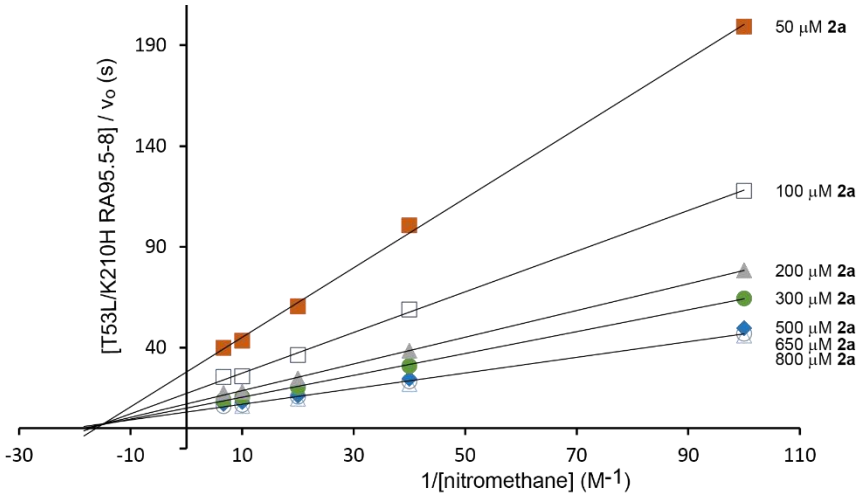


Figure S2. Double-reciprocal plots for the nitro-Michael addition between **2a** and nitromethane catalyzed by T53L/K210H RA95.5-8, showing the dependence of reaction velocity on nitromethane concentration at different fixed concentrations of **2a**.

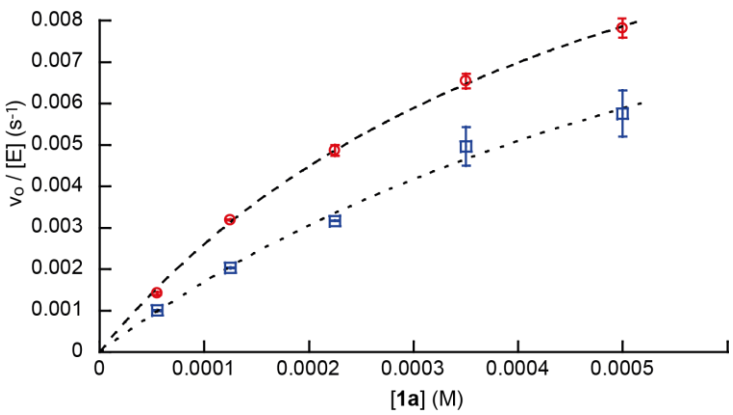
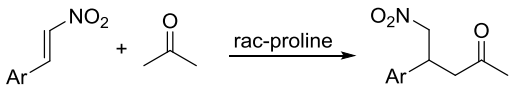
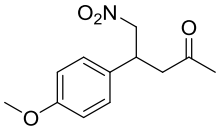


Figure S3. Determination of apparent steady-state parameters for the nitro-Michael addition between **1a** and acetone (2 M) catalyzed by S233F RA95.5-8 (red circles) and T53L/K210H RA95.5-8 (blue squares).

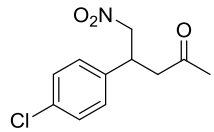
4. Preparation of reference adducts



4.1. Preparation of γ-nitroketones 3a-d. Reference Michael adducts were prepared according to a procedure described by Sakthivel and co-workers with minor modifications.[12] Nitroolefins **1a-d** (1.0 mmol) were dissolved in an acetone/chloroform mixture (1/4, 10 mL), and racemic proline (22.9 mg, 0.2 mmol) was added. The mixture was stirred at 50 ºC in a sealed vial and the conversion was monitored by HPLC. When the maximum conversion was achieved (24 - 96 h), the crude product was transferred to a separatory funnel and diluted in a saturated ammonium chloride solution (30 mL). The aqueous phase was extracted with 3 x AcOEt (30 mL), and the combined organic layer was dried over sodium sulfate and concentrated under vacuum. The crude material was purified by flash chromatography (hexane/AcOEt 3:1) and the purest fractions containing the Michael adducts were concentrated under vacuum, yielding **3a-d** as white solids (10-38% isolated yield).

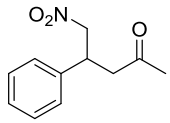


4-(4-Methoxyphenyl)-5-nitropentan-2-one [**(±)-3a**]. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.20 – 7.06 (m, 2H, **Ar**), 7.00 – 6.63 (m, 2H, **Ar**), 4.65 (dd, *J* = 12.2, 6.9 Hz, 1H, C**H**$_2$NO$_2$), 4.55 (dd, *J* = 12.2, 7.7 Hz, 1H, C**H**$_2$NO$_2$), 3.95 (p, *J* = 7.1 Hz, 1H, C**H**), 3.78 (s, 3H, C**H**$_3$O), 2.88 (d, *J* = 7.0 Hz, 2H, C**H**$_2$CO), 2.11 (s, 3H, COC**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 205.66 (**C**O), 159.25, 130.79, 128.57, 114.57 (**Ar**), 79.86 (**C**H$_2$NO$_2$), 55.39 (**C**H$_3$O), 46.42 (**C**H), 38.55 (**C**H$_2$CO), 30.57 (CO**C**H$_3$). **HRMS** (EI, M^+): calc. 237.1001, found 237.0998.

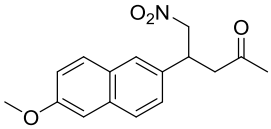


4-(4-Chlorophenyl)-5-nitropentan-2-one [**(±)-3b**]. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.37 – 7.26 (m, 2H, **Ar**), 7.21 – 7.11 (m, 2H, **Ar**), 4.68 (dd, *J* = 12.5, 6.6 Hz, 1H, C**H**$_2$NO$_2$), 4.57 (dd, *J* = 12.5, 7.9 Hz, 1H, C**H**$_2$NO$_2$), 3.99 (dt, *J* = 14.3, 7.0 Hz, 1H, C**H**), 2.89 (dd, *J* = 7.0, 1.5 Hz, 2H, C**H**$_2$CO), 2.13 (s, 3H, COC**H**$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 205.12 (**C**O), 137.47, 133.83, 129.32, 128.92 (**Ar**), 79.28

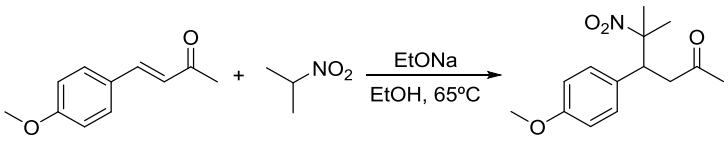
(**C**H_2NO_2), 46.06 (**C**H), 38.50 (**C**H_2CO), 30.46 (CO**C**H_3). **HRMS** (MALDI, MNa^+): calc. 264.0398, found 264.0398.



5-Nitro-4-phenylpentan-2-one [**(±)-3c**]. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.70 – 6.88 (m, 5H, **Ar**), 4.69 (dd, *J* = 12.3, 6.9 Hz, 1H, C**H**$_2NO_2$), 4.60 (dd, *J* = 12.3, 7.7 Hz, 1H, C**H**$_2NO_2$), 4.01 (p, *J* = 7.1 Hz, 1H, C**H**), 2.92 (d, *J* = 7.0 Hz, 2H, C**H$_2$**CO), 2.12 (s, 3H, COC**H$_3$**). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 205.36 (**C**O), 138.81, 129.09, 127.92, 127.38 (**Ar**), 79.46 (**C**H_2NO_2), 46.15 (**C**H), 39.06 (**C**H_2CO), 30.41 (CO**C**H_3). **HRMS** (MALDI, MNa^+): calc. 230.0788, found 230.0787.



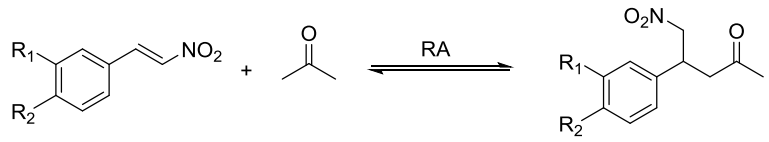
4-(6-Methoxynaphthalen-2-yl)-5-nitropentan-2-one [**(±)-3d**]. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.78 – 7.65 (m, 2H, **Ar**), 7.59 (d, *J* = 1.8 Hz, 1H, **Ar**), 7.33 – 7.27 (m, 1H, **Ar**), 7.15 (dd, *J* = 8.9, 2.5 Hz, 1H, **Ar**), 7.10 (d, *J* = 2.5 Hz, 1H, **Ar**), 4.76 (dd, *J* = 12.3, 6.9 Hz, 1H, C**H**$_2NO_2$), 4.68 (dd, *J* = 12.3, 7.6 Hz, 1H, C**H**$_2NO_2$), 4.14 (p, *J* = 7.1 Hz, 1H, C**H**), 3.91 (s, 3H, C**H$_3$**O), 2.99 (d, *J* = 6.9 Hz, 2H, C**H$_2$**CO), 2.12 (s, 3H, COC**H$_3$**). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 205.57 (**C**O), 158.09, 134.16, 133.89, 129.42, 129.00, 127.92, 126.44, 125.66, 119.54, 105.73 (**Ar**), 79.67 (**C**H_2NO_2), 55.49 (**C**H_3O), 46.39 (**C**H), 39.23 (**C**H_2CO), 30.60 (CO**C**H_3). **HRMS** (EI, M^+): calc. 287.1158, found 287.1166.



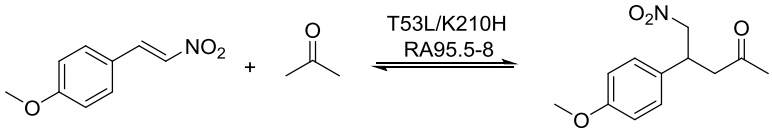
4.2. Preparation of 4-(4-methoxyphenyl)-5-methyl-5-nitrohexan-2-one [(±)-3f]. The acceptor substrate **2a** (0.2 mmol, 35.24 mg) was dissolved in ethanol (4 mL), and 2-nitropropane (2 eq, 0.4 mmol, 36.29 µL) and sodium ethoxide (0.1 eq, 0.02 mmol, 1.36 mg) were added. The mixture was stirred at 65 ºC for 24 h and reaction progress was monitored by HPLC as follows: samples (2 µL) were diluted in acetonitrile (198 µL) and analyzed as described on Page S3. Once the maximum conversion was achieved, the crude product was diluted in a solution of 0.5 M NaH_2PO_4 containing 3 M NaCl (100 mL), transferred to a separatory funnel, and extracted with AcOEt (4 x 30 mL). The organic fractions were combined, dried

with Na_2SO_4, and concentrated under vacuum. The product was purified by flash chromatography (AcOEt / hexane 1:3) and the fractions containing the Michael adduct were concentrated under vacuum, yielding **3f** as a white solid (0.022 mmol, 5.86 mg, 11% isolated yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.15 – 7.06 (m, 2H, **Ar**), 6.87 – 6.78 (m, 2H, **Ar**), 3.86 (dd, J = 10.8, 3.5 Hz, 1H, C**H**), 3.77 (s, 3H, C**H**$_3$O), 3.03 (dd, J = 16.7, 10.8 Hz, 1H, C**H**$_2$CO), 2.67 (dd, J = 16.7, 3.5 Hz, 1H, C**H**$_2$CO), 2.02 (s, 3H, CO**C**H_3), 1.53 [s, 3H, (C**H**$_3$)$_2$$CNO_2$]), 1.47 [s, 3H, (C**H**$_3$)$_2$$CNO_2$]. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 205.45 (**C**O), 159.25, 130.30, 129.51, 114.06, 91.33 (**Ar**), 55.32 (**C**H_3O), 48.34 (**C**H), 44.29 (**C**H_2CO), 30.45 (CO**C**H_3), 25.86 [(**C**H_3)$_2$$CNO_2$], 22.55 [(**C**$H_3$)$_2$$CNO_2$]. **HRMS** (EI, M^+): calc. 265.1314, found 265.1312.

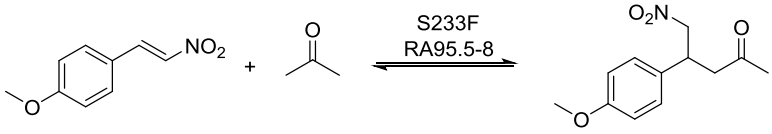
5. Enzymatic addition of acetone to nitrostyrenes 1a-d



In 200 mL Erlenmeyer flasks, acetone solutions (14.7-18.4 mL, 2 M final concentration) containing **1a-d** (60-75 µmol, final concentration 0.6 mM) were prepared. A buffer solution (85.3-106.6 mL, 25 mM HEPES, 100 mM NaCl, pH 6.5) containing enzyme (0.2-0.24 µmol, 2.0 µM final concentration) was added and the mixtures were incubated at 29 ºC and 300 r.p.m. in an orbital shaker. Reaction aliquots (20 µL) diluted in acetonitrile (180 µL) were periodically analyzed by HPLC under the conditions described on Page S3. After 24-72 h, the solutions were saturated with NaCl and extracted with 4 x 30 mL AcOEt. The organic fractions were combined, dried with Na_2SO_4, and concentrated under vacuum. The crude materials were purified by flash chromatography (hexane / AcOEt 3:1). Fractions containing the nitro-Michael adducts were concentrated under vacuum to give **3a-d** as white solids.



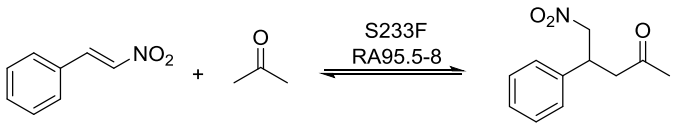
4-(4-Methoxyphenyl)-5-nitropentan-2-one (**3a**) **with T53L/K210H RA95.5-8 as catalyst**. The procedure described above was followed using **1a** in a total reaction volume of 100 mL, T53L/K210H RA95.5-8 as catalyst, and 48 h reaction time, to afford **3a** (36.8 µmol, 8.74 mg, 61% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3a**. The 4*R*/4*S* ratio was 13:87 as determined by chiral HPLC analysis [isocratic hexane/isopropanol 50:7; flow rate 0.57 mL min^{-1}; λ = 223.9 nm; $t_{(S)\text{-}\mathbf{3a}}$ = 11.7 min (major); $t_{(R)\text{-}\mathbf{3a}}$ = 27.9 min (minor)]. $[\alpha]^{25}_D$ = -2.1º (c = 0.73, $CHCl_3$). **HRMS** (EI, M^+): calc. 237.1001, found 237.0996.



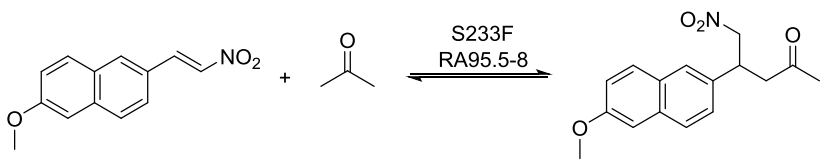
4-(4-Methoxyphenyl)-5-nitropentan-2-one (**3a**) **with S233F RA95.5-8 as catalyst**. The procedure described above was followed using **1a** in a total reaction volume of 100 mL, S233F RA95.5-8 as catalyst, and 48 h reaction time, to afford **3a** (50.70 µmol, 12.03 mg, 84% isolated yield). NMR analysis

was identical to the chemically prepared nitro-Michael adduct **3a**. The 4*R*/4*S* ratio was 14:86 as determined by chiral HPLC analysis [isocratic hexane/isopropanol 50:7; flow rate 0.57 mL min^{-1}; λ = 223.9 nm; $t_{(S)\text{-}\mathbf{3a}}$ = 11.7 min (major); $t_{(R)\text{-}\mathbf{3a}}$ = 27.9 min (minor)]. $[\alpha]^{25}_{D}$ = -0.1º (c = 1.00, $CHCl_3$). **HRMS** (EI, M^+): calc. 237.1001, found 237.1000.

4-(4-Chlorophenyl)-5-nitropentan-2-one (**3b**) **with S233F RA95.5-8 as catalyst**. The procedure described above was followed using **1b** a total reaction volume of 125 mL, S233F RA95.5-8 as catalyst, and 48 h reaction time, to afford **3b** (47.8 µmol, 11.56 mg, 64% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3b**. The 4*R*/4*S* ratio was 12.5:87.5 as determined by chiral HPLC analysis [isocratic hexane/isopropanol 50:7; flow rate 0.57 mL min^{-1}; λ = 219.2 nm; $t_{(S)\text{-}\mathbf{3b}}$ = 7.2 min (major); $t_{(R)\text{-}\mathbf{3b}}$ = 10.3 min (minor)]. $[\alpha]^{25}_{D}$ = 8.5º (c = 0.40, $CHCl_3$). **HRMS** (MALDI, MNa^+): calc. 264.0398, found 264.0398.



5-Nitro-4-phenylpentan-2-one (**3c**) **with S233F RA95.5-8 as catalyst**. The procedure described above was followed using **1c** in a total reaction volume of 100 mL, S233F RA95.5-8 as catalyst, and 48 h reaction time, to afford **3c** (39.0 µmol, 8.08 mg, 65% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3c**. The 4*R*/4*S* ratio was 18:82 as determined by chiral HPLC analysis [isocratic hexane/isopropanol 50:7; flow rate 0.57 mL min^{-1}; λ = 207.5 nm; $t_{(S)\text{-}\mathbf{3c}}$ = 6.2 min (major); $t_{(R)\text{-}\mathbf{3c}}$ = 8.0 min (minor)]. $[\alpha]^{25}_{D}$ = 3.5º (c = 0.44, $CHCl_3$). **HRMS** (MALDI, MNa^+): calc. 230.0788, found 230.0788.

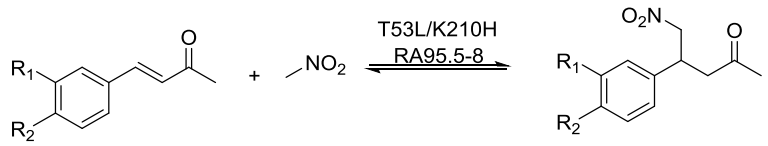


4-(6-Methoxynaphthalen-2-yl)-5-nitropentan-2-one (**3d**) **with S233F RA95.5-8 as catalyst**. The procedure described above was followed using **1d** in a total reaction volume of 125 mL, S233F RA95.5-8 as catalyst, and 72 h reaction time, to afford **3d** (27.5 µmol, 7.91 mg, 37% isolated yield). Conversion may have been limited by the very poor solubility of **1d** in the reaction medium. NMR analysis was

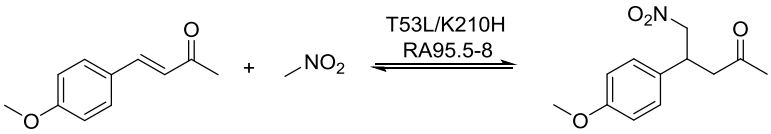
identical to the chemically prepared nitro-Michael adduct **3d**. The 4*R*/4*S* ratio was 44:56 as determined by chiral SFC analysis [$t_{(S)\text{-}\mathbf{3d}}$ = 8.6 min (major); $t_{(R)\text{-}\mathbf{3d}}$ = 9.0 min (minor)]. $[\alpha]^{25}_{D}$ = 3.4º (c = 0.47, $CHCl_3$). **HRMS** (EI, M^+): calc. 287.1158, found 287.1149.

6. Enzymatic addition of nitroolefins to conjugated ketones

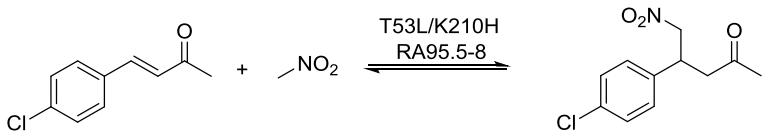
6.1. Enzyme-catalyzed addition of nitromethane to conjugated ketones 2a-d



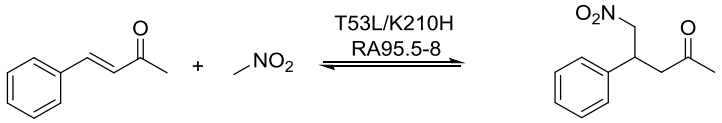
In 200 mL Erlenmeyer flasks, acetonitrile solutions (1.2 % final concentration) containing **2a-d** (final concentration 2-5 mM) and nitromethane (50 mM final concentration) were prepared. A buffer solution (19.4-49.2 mL, 25 mM HEPES, 100 mM NaCl, pH 7.5) containing enzyme (1-10 µM final concentration) was added and the mixtures were incubated at 29 ºC and 300 r.p.m. in an orbital shaker. Reaction aliquots (20 µL) diluted in acetonitrile (180 µL) were periodically analyzed by HPLC under the conditions described on Page S3. After 24-72 h, the solutions were saturated with NaCl and extracted with 4 x 30 mL AcOEt. The organic fractions were combined, dried with Na_2SO_4, and concentrated under vacuum. The crude materials were purified by flash chromatography (hexane / ethyl acetate 3:1). Fractions containing the nitro-Michael adducts were concentrated under vacuum to give **3a-d** as white solids.



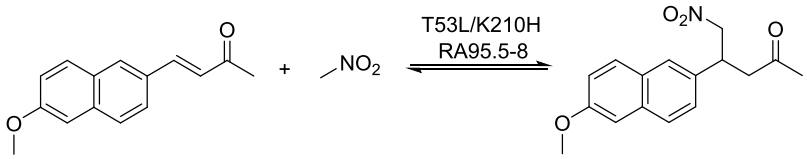
4-(4-Methoxyphenyl)-5-nitropentan-2-one (**3a**). The procedure described above was followed using **2a** (17.62 mg, 100 µmol, 2 mM final concentration) and T53L/K210H RA95.5-8 (1 µM final concentration) in a total reaction volume of 50 mL and 24 h reaction time, to afford **3a** (82.19 µmol, 19.50 mg, 82% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3a**. The 4*R*/4*S* ratio was 96:4 as determined by chiral HPLC analysis [isocratic hexane/isopropanol 50:7; flow rate 0.57 mL min^{-1}; λ = 223.9 nm; $t_{(S)\text{-}\mathbf{3a}}$ = 11.7 min (minor); $t_{(R)\text{-}\mathbf{3a}}$ = 27.9 min (major)]. $[\alpha]^{25}_{D}$ = 0.9º (c = 1.36, $CHCl_3$). **HRMS** (EI, M^+): calc. 237.1001, found 237.1000.



4-(4-Chlorophenyl)-5-nitropentan-2-one (**3b**). The procedure described above was followed using **2b** (18.06 mg, 100 µmol, 5 mM final concentration) and T53L/K210H RA95.5-8 (10 µM final concentration) a total reaction volume of 20 mL and 48 h reaction time, to afford **3b** (69.5 µmol, 16.76 mg, 70 % isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3b**. The 4*R*/4*S* ratio was 94:6 as determined by chiral HPLC analysis (isocratic hexane/isopropanol 50:7; flow rate 0.57 mL min^{-1}; λ = 219.2 nm; $t_{(S)\text{-}\mathbf{3b}}$ = 7.2 min (minor); $t_{(R)\text{-}\mathbf{3b}}$ = 10.3 min (major)]. $[\alpha]^{25}_{D}$ = -1.9º (c = 1.22, $CHCl_3$). **HRMS** (MALDI, MNa^+): calc. calc. 264.0398, found 264.0398.



5-Nitro-4-phenylpentan-2-one (**3c**). The procedure described above was followed using **2c** (14.61 mg, 100 µmol, 2 mM final concentration) and T53L/K210H RA95.5-8 (2 µM final concentration) in a total reaction volume of 50 mL and 48 h reaction time to afford **3c** (65.1 µmol, 13.49 mg, 65% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3c**. The 4*R*/4*S* ratio was 77:23 as determined by chiral HPLC analysis [isocratic hexane/isopropanol 50:7; flow rate 0.57 mL min^{-1}; λ = 207.5 nm; $t_{(S)\text{-}\mathbf{3c}}$ = 6.2 min (minor); $t_{(R)\text{-}\mathbf{3c}}$ = 8.0 min (major)]. $[\alpha]^{25}_{D}$ = -0.5º (c = 1.00, $CHCl_3$). **HRMS** (MALDI, MNa^+): calc. 230.0788, found 230.0787.

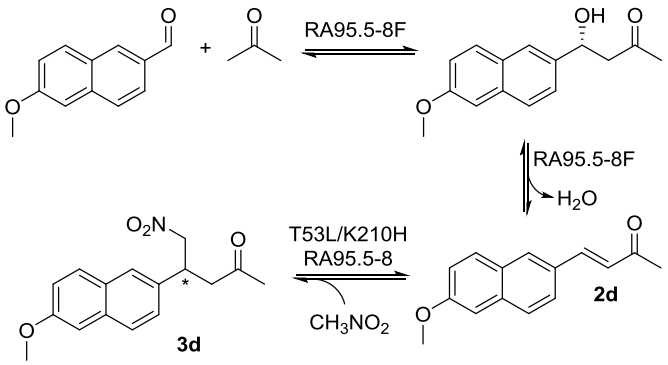


4-(6-Methoxynaphthalen-2-yl)-5-nitropentan-2-one (**3d**). The procedure described above was followed using **2d** (22.62 mg, 100 µmol, 5 mM final concentration) and T53L/K210H RA95.5-8 (10 µM final concentration) in a total reaction volume of 20 mL and 72 h reaction time, to afford **3d** (62.5 µmol, 17.94 mg, 63% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3d**. $[\alpha]^{25}_{D}$ = -5.9º (c = 1.30, $CHCl_3$). The 4*R*/4*S* ratio was 99.3:0.7 as determined by chiral SFC analysis [$t_{(S)\text{-}\mathbf{3d}}$ = 8.6 min (minor); $t_{(R)\text{-}\mathbf{3d}}$ = 9.0 min (major)]. **HRMS** (EI, M^+): calc. 287.1158, found 287.1157.

6.2. Enzyme-catalyzed addition of 2-nitropropane to conjugated ketone 2a

In a 200 mL Erlenmeyer flask, a solution of acetonitrile (764 µL) containing **2a** (27.15 mg, 0.12 mmol, 4 mM final concentration) and 2-nitropropane (136.1 µL, 1.5 mmol, 50 mM final concentration) was prepared. A buffer solution (29.1 mL, 25 mM HEPES, 100 mM NaCl, pH 7.5) containing enzyme (0.24 µmol, 8.0 µM final concentration) was added and the mixture was incubated at 29 ºC and 300 r.p.m. in an orbital shaker. Reaction aliquots (20 µL) diluted in acetonitrile (180 µL) were periodically analyzed by HPLC under the conditions described on page S2. After 72 h, the solution was saturated with NaCl and extracted with 4 x 30 mL AcOEt. The organic fractions were combined, dried with Na_2SO_4, and concentrated under vacuum. The crude materials were purified by flash chromatography (hexane / AcOEt 4:1). Fractions containing the nitro-Michael adduct were concentrated under vacuum to give **3f** as a white solid (97.1 µmol, 30.09 mg, 81% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3f**. $[\alpha]^{25}_D$ = 36.1º (c = 1.61, $CHCl_3$); reported value (95% ee): $[\alpha]_D^{25}$ = +33.7 (c 0.67, $CHCl_3$).[10] The 4*R*/4*S* ratio was 93.5:6.5 as determined by chiral HPLC analysis [isocratic hexane/isopropanol 29:1; flow rate 0.3 mL min^{-1}; λ = 207.5 nm; $t_{(S)\text{-}\mathbf{3f}}$ = 32.6 min (major); $t_{(R)\text{-}\mathbf{3f}}$ = 34.5 min (minor)]. **HRMS** (EI, M^+): calc. 265.1314, found 265.1319.

7. Cascade synthesis of nitro-Michael adduct 3d



In a 200 mL Erlenmeyer flask, an acetone solution (1101.4 µL, 500 mM final concentration) containing 6-methoxy-2-naphthaldehyde (11.73 mg, 60 µmol, 2 mM final concentration) was prepared. A buffer solution (28.8 mL, 25 mM HEPES, 100 mM NaCl, pH 7.5) containing RA95.5-8F (60 nmol, 2.0 µM final concentration) was added and the mixture was incubated at 29 ºC and 300 r.p.m. in an orbital shaker. Reaction aliquots (20 µL) diluted in acetonitrile (180 µL) were periodically analyzed by HPLC under the conditions described on Page S3. After 48 h, almost complete conversion of the aldehyde to **2d** was observed, and nitromethane (80.3 µL, 1.50 mmol, 50 mM final concentration) was added. Formation of **3d** was not observed after 12 h, and the second catalyst T53L/K210H RA95.5-8 (60 nmol, 2.0 µM final concentration) in buffer (28.8 mL, 25 mM HEPES, 100 mM NaCl, pH 7.5) was added. The mixture was stirred for additional 72 h, saturated with NaCl and extracted with 4 x 30 mL AcOEt. The organic fractions were combined, dried with Na_2SO_4, and concentrated under vacuum. The crude materials were purified by flash chromatography (hexane / ethyl acetate 3:1). Fractions containing the nitro-Michael adduct were concentrated under vacuum to give **3d** as a white solid (38.5 µmol, 11.05 mg, 64% isolated yield). NMR analysis was identical to the chemically prepared nitro-Michael adduct **3d**. $[\alpha]^{25}_{D}$ = -3.4º (c = 0.66, $CHCl_3$). The 4*R*/4*S* ratio was 97.7:2.3 as determined by chiral SFC analysis [$t_{(S)\text{-}\mathbf{3d}}$ = 8.6 min (minor); $t_{(R)\text{-}\mathbf{3d}}$ = 9.0 min (major)]. **HRMS** (EI, M^+): calc. 287.1158, found 287.1155.

8. References

1. Garrabou, X.; Beck, T.; Hilvert, D., *Angew. Chem., Int. Ed.* **2015,** *54*, 5609-5612.
2. Reddy, P.; Bandichhor, R., *Tetrahedron Lett.* **2013,** *54*, 3911-3915.
3. Giger, L.; Caner, S.; Obexer, R.; Kast, P.; Baker, D.; *Nat. Chem. Biol*. **2013,** *9*, 494-498.
4. Obexer, R.; Godina, A.; Garrabou, X.; Mittl, P. R. E.; Baker, D.; Griffiths, A. D.; Hilvert, D., *Nat. Chem.* **2017,** *9*, 50-56.
5. Garrabou, X.; Wicky, B. I. M.; Hilvert, D., *J. Am. Chem. Soc.* **2016,** *138*, 6972-6974.
6. Huang, H.; Jacobsen, E. N., *J. Am. Chem. Soc.* **2006,** *128*, 7170-7171.
7. Gu, Q.; Guo, X.-T.; Wu, X.-Y., *Tetrahedron* **2009,** *65*, 5265-5270.
8. Ueda, A.; Umeno, T.; Doi, M.; Akagawa, K.; Kudo, K.; Tanaka, M., *J. Org. Chem.* **2016,** *81*, 6343-6356.
9. Mei, K.; Jin, M.; Zhang, S.; Li, P.; Liu, W.; Chen, X.; Xue, F.; Duan, W.; Wang, W., *Org. Lett.* **2009,** *11*, 2864-2867.
10. Guo, X.-T.; Shen, J.; Sha, F.; Wu, X.-Y., *Synthesis* **2015,** *47*, 2063-2072.
11. R Core Team (**2013**). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
12. Sakthivel, K.; Notz, W.; Bui, T.; Barbas, C. F., *J. Am. Chem. Soc.* **2001,** *123*, 5260-5267.