SUPPORTING INFORMATION

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Title: Stereochemical Control of Enzymatic Carbon–Carbon Bond-Forming Michael-Type Additions by “Substrate Engineering”
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1. General information

Materials

All the chemicals including nitrostyrene derivatives 2a-n were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). The sources for buffers, solvents, and components of Luria-Bertani (LB) medium are reported elsewhere.[1] Synthetic 4-OT peptide with high purity (92% purity) was purchased from GenScript USA Inc. (Piscataway, NY) and folded into the active homohexamer as described before.[2]

General methods

Techniques for transformation and other molecular biology manipulations were based on methods described elsewhere.[3] Proteins were analyzed by polyacrylamide gel electrophoresis (PAGE) using sodium dodecyl sulfate (SDS) gels containing 10% polyacrylamide. The gels were stained with InstantBlue™ from Expeadeon (Harston, UK). Protein concentrations were determined using the method of Waddell.[4] Enzymatic assays were monitored using a V-660 spectrophotometer purchased from Jasco (IJsselein, The Netherlands). NMR spectra were recorded on a Bruker DRX-500 (500 MHz) spectrometer. Data for 1H NMR are reported as chemical shift, multiplicity (s = singlet, b = broad, d = doublet, dd = double doublet, ddd = double double doublet, dddd = double double double doublet, t = triplet, m = multiplet), coupling constant (Hz), integration, and assignment. Chemical shifts for protons are reported in parts per million scale ( scale) and are referenced to CHCl3 ( = 7.26). Data for 13C NMR are reported as chemical shifts. Reverse phase HPLC was carried out using an in-house analytical HPLC equipped with a Shimadzu LC-10 AT pump and a Shimadzu SPD-M10A diode array detector using a Daicel Chiralpak AD-RH or Chiralpak ID column (reverse phase, Chiral Technologies Europe, Illkirch Cedex, France). Chromatographic data were analyzed using LC solutions provided by Shimadzu. Kinetic parameters were determined using SigmaPlot. Optical rotations were measured in CHCl3 on a Schmidt + Haensch polarimeter (Polartronic MH8) with a 10 cm cell (c given in g/100 mL).

2. Expression and purification of 4-OT

The construction of the expression vector for wild-type 4-OT was reported previously.[5] Wild type 4-OT enzyme was produced in E. coli BL21(DE3) using the pET20b(+) expression system and purified according to the protocol described before.[6]

3. General procedures for 4-OT-catalyzed Michael-type addition reactions

General procedure for analytical scale reactions

The UV-spectroscopic assays to monitor the 4-OT-catalyzed Michael-type addition reactions were performed at 25 °C by following the decrease in absorbance at max of the nitrostyrene derivatives 2b-l in course of time which corresponds to the depletion of 2b-l (see Table S1 for
specific $\lambda_{\text{max}}$ values of 2b-l). A fresh stock solution of acetaldehyde (1) was prepared in 20 mM NaH$_2$PO$_4$ buffer (pH 5.5 or 7.3), while separate stock solutions of nitrostyrene derivatives 2b-l were prepared in absolute ethanol or DMSO (see Table S1). An aliquot of enzyme (4-OT, 36 $\mu$M) and acetaldehyde (1, 100 mM) were incubated in 20 mM NaH$_2$PO$_4$ buffer in a 1 mm cuvette, after which the assay was initiated by the addition of one of the nitrostyrene derivatives (2b-l, 2 mM). The enzyme-catalyzed reactions between acetaldehyde and nitrostyrene derivatives 2c and 2h-i were performed at pH 7.3, while the reactions with nitrostyrene derivatives 2b, d-f and 2j-l were performed at pH 5.5 to reduce non-enzymatic background reactions. The final concentrations of nitrostyrene derivatives (2b-l) and acetaldehyde (1), the final percentage (v/v) of co-solvent, the pH of the buffer, and the total reaction time ($t$) are summarized in Table S1 for each reaction. The reactions were monitored in course of time by recording absorbance spectra from 200 to 400 or 200 to 500 nm, and the spectra were analyzed using the software provided with the UV-VIS spectrophotometer (Jasco).

Table S1. Conditions of analytical scale, 4-OT-catalyzed Michael-type additions of acetaldehyde 1 to nitrostyrene derivatives 2b-l.

| entry | nitrostyrene derivative | $\lambda_{\text{max}}$ (nm) | $\varepsilon_{\text{max}}$ (mM$^{-1}$ cm$^{-1}$) | co-solvent (v/v) | buffer pH | Final concentration (mM) | $t$ (h) |
|-------|-------------------------|-----------------------------|----------------------------------|-----------------|-----------|-------------------------|--------|
| 1     | 2b                      | 316                         | 9.8                              | DMSO 40%        | 5.5       | 100                     | 2      | 2.0                     |
| 2     | 2c                      | 318                         | 12.7                             | EtOH 10%        | 7.3       | 100                     | 2      | 0.5                     |
| 3     | 2d                      | 365                         | 18.1                             | DMSO 40%        | 5.5       | 100                     | 2      | 1.0                     |
| 4     | 2e                      | 362                         | 11.0                             | DMSO 40%        | 5.5       | 100                     | 2      | 0.5                     |
| 5     | 2f                      | 312                         | 8.6                              | DMSO 40%        | 5.5       | 100                     | 2      | 1.5                     |
| 6     | 2h                      | 317                         | 11.1                             | EtOH 25%        | 7.3       | 100                     | 2      | 1.3                     |
| 7     | 2i                      | 312                         | 12.5                             | EtOH 25%        | 7.3       | 100                     | 2      | 2.7                     |
| 8     | 2j                      | 320                         | 16.8                             | DMSO 40%        | 5.5       | 100                     | 2      | 1.3                     |
| 9     | 2k                      | 267                         | 13.3                             | DMSO 40%        | 5.5       | 100                     | 2      | 0.5                     |
| 10    | 2l                      | 289                         | 15.7                             | DMSO 40%        | 5.5       | 100                     | 2      | 1.5                     |

The following control experiments were conducted for each Michael-type addition reaction: 1) reactions were carried out using identical conditions as listed in Table S1 but in the absence of 4-OT (blank experiments); and 2) reactions were carried out using identical conditions as listed in Table S1 but using folded synthetic 4-OT (22-36 $\mu$M) instead of recombinant 4-OT. During all blank reactions (without enzyme), no significant decreases of absorbance at $\lambda_{\text{max}}$ values of 2b-l were observed. Control reactions with synthetic 4-OT (syn-4-OT) indeed demonstrated that 4-OT is responsible for catalysis (rather than a contaminating protein copurified from the expression host). In general, folded synthetic 4-OT does show somewhat lower specific activity than recombinant 4-OT. UV-spectra monitoring the progress of each analytical scale reaction are visualized in Figures S1-S10.
Figure S1. UV spectra showing the depletion of trans-2-hydroxy-β-nitrostyrene (2h, 2 mM) incubated in 20 mM NaH₂PO₄ buffer/40% DMSO (v/v) at pH 5.5 with A) acetaldehyde (1, 100 mM); B) acetaldehyde (1, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (1, 100 mM) and synthetic 4-OT (36 µM, 1.8 mol %).

Figure S2. UV spectra showing the depletion of trans-3-hydroxy-β-nitrostyrene (2c, 2 mM) incubated in 20 mM NaH₂PO₄ buffer/10% EtOH (v/v) at pH 7.3 with A) acetaldehyde (1, 100 mM); B) acetaldehyde (1, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (1, 100 mM) and synthetic 4-OT (22 µM, 1.1 mol %).
Figure S3. UV spectra showing the depletion of trans-4-methoxy-β-nitrostyrene (2d, 2 mM) incubated in 20 mM NaH$_2$PO$_4$ buffer/40% DMSO (v/v) at pH 5.5 with A) acetaldehyde (I, 100 mM); B) acetaldehyde (I, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (I, 100 mM) and synthetic 4-OT (36 µM, 1.8 mol %).

Figure S4. UV spectra showing the depletion of trans-2-methoxy-β-nitrostyrene (2e, 2 mM) incubated in 20 mM NaH$_2$PO$_4$ buffer/40% DMSO (v/v) at pH 5.5 with A) acetaldehyde (I, 100 mM); B) acetaldehyde (I, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (I, 100 mM) and synthetic 4-OT (36 µM, 1.8 mol %).
Figure S5. UV spectra showing the depletion of trans-3-methoxy-β-nitrostyrene (2f, 2 mM) incubated in 20 mM NaH$_2$PO$_4$ buffer/40% DMSO (v/v) at pH 5.5 with A) acetaldehyde (I, 100 mM); B) acetaldehyde (I, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (I, 100 mM) and synthetic 4-OT (36 µM, 1.8 mol %).

Figure S6. UV spectra showing the depletion of trans-2-chloro-β-nitrostyrene (2h, 2 mM) incubated in 20 mM NaH$_2$PO$_4$ buffer/25% EtOH (v/v) at pH 7.3 with A) acetaldehyde (I, 100 mM); B) acetaldehyde (I, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (I, 100 mM) and synthetic 4-OT (22 µM, 1.1 mol %).
Figure S7. UV spectra showing the depletion of trans-3-chloro-β-nitrostyrene (2i, 2 mM) incubated in 20 mM NaH₂PO₄ buffer/25% EtOH (v/v) at pH 7.3 with A) acetaldehyde (1, 100 mM); B) acetaldehyde (1, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (1, 100 mM) and synthetic 4-OT (22 µM, 1.1 mol %).

Figure S8. UV spectra showing the depletion of β,4-dinitrostyrene (2j, 2 mM) incubated in 20 mM NaH₂PO₄ buffer/40% DMSO (v/v) at pH 5.5 with A) acetaldehyde (1, 100 mM); B) acetaldehyde (1, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (1, 100 mM) and synthetic 4-OT (36 µM, 1.8 mol %).
Figure S9. UV spectra showing the depletion of β,2-dinitrostyrene (2k, 2 mM) incubated in 20 mM NaH$_2$PO$_4$ buffer/40% DMSO (v/v) at pH 5.5 with A) acetaldehyde (1, 100 mM); B) acetaldehyde (1, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (1, 100 mM) and synthetic 4-OT (36 µM, 1.8 mol %).

Figure S10. UV spectra showing the depletion of β,3-dinitrostyrene (2l, 2 mM) incubated in 20 mM NaH$_2$PO$_4$ buffer/40% DMSO (v/v) at pH 5.5 with A) acetaldehyde (1, 100 mM); B) acetaldehyde (1, 100 mM) and recombinant 4-OT (36 µM, 1.8 mol %); C) acetaldehyde (1, 100 mM) and synthetic 4-OT (36 µM, 1.8 mol %).
General procedure for preparative scale reactions

A stock solution of nitrostyrene derivative 2b-m (~20 mg, 1.5-2.0 mM) was prepared in 6 mL absolute EtOH or DMSO, respectively. The stock solution was slowly added to buffer (20 mM NaH$_2$PO$_4$, pH 5.5) containing 4-OT (15-20 µM, 1.0 mol %) in a 150 mL round flask. The reaction was initiated by the addition of acetaldehyde 1 (50 eq, 75-100 mM). The total volume of the reaction mixture, the final concentrations of acetaldehyde, nitrostyrene derivative and 4-OT, and the final ratios of buffer/co-solvent are summarized in Table S2. The reaction progress was monitored by UV-spectroscopy at $\lambda_{\text{max}}$, 2b-m and visualized in Figure S11-S14. The conversion and final reaction time of each preparative scale reaction were determined based on the progress curves and are summarized in Table S2. For each preparative scale reaction, a blank reaction was performed under the same conditions but without 4-OT. The progress of the blank reactions was followed by UV-spectroscopy using the same time intervals as used in the 4-OT-catalyzed reactions (see progress curves of blank reactions in Figure S11-S14).

Table S2. Conditions of preparative scale, 4-OT-catalyzed Michael-type additions of acetaldehyde (1) to nitrostyrene derivatives (2b-m) in 20 mM NaH$_2$PO$_4$ buffer (pH 5.5).

| entry | nitrostyrene derivative | final volume (mL) | final concentrations (mol %) | final volume (mL) | final concentrations (mol %) | final volume (mL) | final concentrations (mol %) | co-solvent (v/v) | t (h) | conversion (%) | product |
|-------|------------------------|------------------|-----------------------------|------------------|-----------------------------|------------------|-----------------------------|----------------|------|---------------|--------|
| 1     | 2b                     | 60               | 2.0                         | 100              | 20                          | 1.0              | EtOH 10%                    | 6.1            | 96              | 5       |
| 2     | 2c                     | 60               | 2.0                         | 100              | 20                          | 1.0              | EtOH 10%                    | 1.0            | 99              | 3c      |
| 3     | 2d                     | 60               | 1.9                         | 93               | 20                          | 1.0              | DMSO 40%                    | 3.0            | 99              | 3d      |
| 4     | 2e                     | 60               | 1.9                         | 93               | 20                          | 1.0              | DMSO 40%                    | 1.0            | 99              | 3e      |
| 5     | 2f                     | 60               | 1.9                         | 93               | 20                          | 1.0              | DMSO 40%                    | 0.7            | 92              | 3f      |
| 6     | 2h                     | 63               | 1.8                         | 90               | 18                          | 1.0              | EtOH 25%                    | 2.8            | 98              | 3h      |
| 7     | 2i                     | 60               | 1.8                         | 90               | 18                          | 1.0              | EtOH 25%                    | 3.9            | 93              | 3i      |
| 8     | 2j                     | 60               | 1.8                         | 90               | 18                          | 1.0              | DMSO 40%                    | 3.3            | 83              | 3j      |
| 9     | 2k                     | 60               | 1.8                         | 90               | 18                          | 1.0              | DMSO 40%                    | 3.7            | 48              | 3k      |
| 10    | 2l                     | 60               | 1.8                         | 90               | 18                          | 1.0              | DMSO 40%                    | 4.0            | 84              | 3l      |
| 11    | 2m                     | 60               | 1.5                         | 75               | 15                          | 1.0              | DMSO 40%                    | 1.2            | 98              | 3m      |

After reaching maximal conversion, the enzyme reaction mixtures and the mixtures of the blank reactions using EtOH as a co-solvent were extracted directly with ethyl acetate (3 × 30 mL). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. For enzyme and blank reactions that were performed in buffer containing 40% DMSO (v/v), the mixtures were first diluted with water to reduce the concentration of DMSO in the buffer to 10% (v/v) and then extracted with ethyl acetate (3 × 60 mL). The combined organic layers were then washed with water (3 × 30 mL) to remove the remaining DMSO in ethyl acetate and dried over Na$_2$SO$_4$, and concentrated in vacuo after filtration.

For the blank reactions without enzyme, no Michael-addition (~cyclization) product (3c-m, 5) was observed (as confirmed by $^1$H NMR spectroscopy) as expected. In the case of nitrostyrene derivatives 2j-l, nonenzymatic water addition resulted in the corresponding alcohols as confirmed by $^1$H NMR spectroscopy.
Figure S11. Progress curves of preparative scale 4-OT-catalyzed Michael-type addition of acetaldehyde (1) to A) trans-2-hydroxy-β-nitrostyrene (2b); B) trans-3-hydroxy-β-nitrostyrene (2c) as monitored by UV-spectroscopy.

Figure S12. Progress curves of preparative scale 4-OT-catalyzed Michael-type addition of acetaldehyde (1) to A) trans-4-methoxy-β-nitrostyrene (2d); B) trans-2-methoxy-β-nitrostyrene (2e); C) trans-3-methoxy-β-nitrostyrene (2f) as monitored by UV-spectroscopy.
Figure S13. Progress curves of preparative scale 4-OT-catalyzed Michael-type addition of acetaldehyde (1) to A) trans-2-chloro-β-nitrostyrene (2h); B) trans-3-chloro-β-nitrostyrene (2i) as monitored by UV-spectroscopy.

Figure S14. Progress curves of preparative scale 4-OT-catalyzed Michael-type addition of acetaldehyde (1) to A) β,4-dinitrostyrene (2j); B) β,2-dinitrostyrene (2k); C) β,3-dinitrostyrene (2l); D) trans-3-bromo-β-nitrostyrene (2m) as monitored by UV-spectroscopy.
4. Characterization of products 3c-m and 5

After work-up procedures, products 3c-m and 5 from preparative-scale reactions were characterized by NMR spectroscopy and the results were compared with NMR data reported in the literature. In all cases, the $^1$H NMR (and $^{13}$C NMR spectra) indicated the formation of Michael adducts (see Figure S15-S25 for NMR spectra of products 3c-m and 5). Residues containing products 3c-f, 3h, and 3m showed complete conversion of nitrostyrene derivatives 2c-f, 2h, 2m (Table S2, entry 2-6, 11), while unconverted nitrostyrene derivatives (in the case of 2b, 2i and 2k) or hydration products (in the case of 2j-l) were observed in residues containing products 5, 3i-l (Table S2, entry 1, 7-10). The crude mixtures containing products 3i-l were purified by column chromatography (silica gel, EtOAc/n-heptane) to afford the Michael adducts 3i-l. No formation of the Michael adducts (i.e. 3c-m and 5) was observed by $^1$H NMR spectroscopy in the residues isolated from reaction mixtures of blank experiments.

The enantiomeric excess (ee) of the Michael products 3c-m were determined by their derivatization to alcohols 4c-m, which were analyzed by HPLC with a chiral stationary phase (see details of derivatization in section 5). The diastereomeric ratio (dr) of the Michael-addition-cyclization product 5 was determined by $^1$H NMR and comparison with literature data (see Figure S15 for assignment of the diastereoisomers). The optical rotations of the enzymatically obtained products 3c-m and 5 were measured for absolute configuration determination (see section 7 for details of optical rotation measurements).

4-(Nitromethyl)-chroman-2-ol (5) (Table S2, entry 1)

$$\text{HO}$$
$$\text{O}$$
$$\text{NO}_2$$
$$\text{H}$$

The title compound 5 (24.4 mg, 0.12 mmol, yield: 95%) was obtained from acetaldehyde (1) and trans-2-hydroxy-β-nitrostyrene 2b (21.5 mg, 0.13 mmol) according to the general procedure as a red-brownish oil. The $^1$H NMR spectroscopic data of 5 are in agreement with published data. Diastereomeric ratio (dr) was determined by $^1$H NMR spectroscopy and comparison with literature data. $[\alpha]^{25}_D = -25.0$ (c = 2.2 in CHCl$_3$).

3-(3-Hydroxy-phenyl)-4-nitrobutanal (3c) (Table S2, entry 2)

$$\text{HO}$$
$$\text{O}$$
$$\text{NO}_2$$
$$\text{H}$$

The title compound 3c (23.2 mg, 0.11 mmol, yield: 94%) was obtained from acetaldehyde (1) and trans-3-hydroxy-β-nitrostyrene 2c (20.4 mg, purity: 96%, 0.12 mmol) according to the general procedure as a brownish oil. $^1$H NMR (500 MHz, CDCl$_3$) δ 9.68 (s, 1H), 7.19 (t, $J = 7.9$ Hz, 1H), 6.78 – 6.73 (m, 2H), 6.71 (t, $J = 2.1$ Hz, 1H), 4.64 (dd, $J = 12.6, 7.2$ Hz, 1H), 4.58 (dd, $J = 12.6, 7.6$ Hz, 1H), 4.35 (b, 1H), 4.00 (dddd, $J = 7.6, 7.6, 7.2, 6.8$ Hz, 1H), 2.93 (dd, $J = 18.1, 7.6$ Hz, 1H), 2.88 (dd, $J = 18.1, 6.8$ Hz, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 199.04, 156.51, 139.83, 130.44, 119.25, 115.22, 114.51, 79.30, 46.30, 37.83. Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4c using a Chiralpak ID column (Water/MeCN: 95/5, 25 °C) at 1 mL/min, UV detection at
220 nm: $t_R$ (major) = 8.0 min, (minor) = 9.9 min, $ee = 95\%$ (see HPLC chromatogram in Figure S46); $[\alpha]^{25}_D = -4.3$ (c = 2.1 in CHCl$_3$); MS (EI): $m/z$ 208.1 (M$^+$).

3-(4-Methoxy-phenyl)-4-nitrobutanal (3d) (Table S2, entry 3)

The title compound 3d (21.3 mg, 0.095 mmol, yield: 85\%) was obtained from acetaldehyde (1) and trans-4-methoxy-β-nitrostyrene 2d (20.0 mg, 0.11 mmol) according to the general procedure as a yellowish oil after column chromatography (silica gel, heptane/EtOAc: 4/1~2/1). The $^1$H NMR spectroscopic data of 3d are in agreement with published data.$^{[10,11,12]}$ Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4d using a Chiralpak AD-RH column (Water/MeCN: 85/15, 25 °C) at 1 mL/min, UV detection at 220 nm: $t_R$ (minor) = 29.1 min, (major) = 37.0 min, $ee = 94\%$ (see HPLC chromatogram in Figure S47); $[\alpha]^{25}_D = -16.2$ (c = 2.2 in CHCl$_3$).

3-(2-Methoxy-phenyl)-4-nitrobutanal (3e) (Table S2, entry 4)

The title compound 3e (24.1 mg, 0.11 mmol, yield: 93\%) was obtained from acetaldehyde (1) and trans-2-methoxy-β-nitrostyrene 2e (21.4 mg, purity: 97\%, 0.12 mmol) according to the general procedure as a colorless oil after column chromatography (silica gel, heptane/EtOAc: 4/1~2/1). The $^1$H NMR spectroscopic data of 3e are in agreement with published data.$^{[11,12]}$ Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4e using a Chiralpak AD-RH column (Water/MeCN: 90/10, 25 °C) at 1 mL/min, UV detection at 220 nm: $t_R$ (major) = 36.6 min, (minor) = 43.6 min, $ee = 97\%$ (see HPLC chromatogram in Figure S48); $[\alpha]^{25}_D = +30.4$ (c = 2.4 in CHCl$_3$).

3-(3-Methoxy-phenyl)-4-nitrobutanal (3f) (Table S2, entry 5)

The title compound 3f (11.2 mg, 0.05 mmol, yield: 81\%) was obtained from acetaldehyde (1) and trans-3-methoxy-β-nitrostyrene 2f (11.3 mg, purity: 99\%, 0.062 mmol) according to the general procedure as a yellowish oil after column chromatography (silica gel, heptane/EtOAc: 4/1~2/1). The $^1$H NMR spectroscopic data of 3f are in agreement with published data.$^{[11]}$ Enantiomeric excess was determined by reverse phase HPLC with derivatized 4f using a Chiralpak AD-RH column (Water/MeCN: 85/15, 25 °C) at 1 mL/min, UV detection at 220 nm: $t_R$ (major) = 23.5 min, (minor) = 30.9 min, $ee = 96\%$ (see HPLC chromatogram in Figure S49); $[\alpha]^{25}_D = -4.0$ (c = 0.7 in CHCl$_3$).
3-(2-Chloro-phenyl)-4-nitrobutanal (3h) (Table S2, entry 6)

The title compound 3h (23.3 mg, 0.102 mmol, yield: 94%) was obtained from acetaldehyde (1) and trans-2-chloro-β-nitrostyrene 2h (20.6 mg, purity: 97%, 0.11 mmol) according to the general procedure as colorless oil. The 1H NMR spectroscopic data of 3h are in agreement with published data.\textsuperscript{[12,13]} Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4h using a Chiralpak ID column (Water/MeCN: 95/5, 25 ºC) at 2 mL/min, UV detection at 192 nm: $t_R$ (major) = 122 min, (minor) = 144.5 min, $ee = 82\%$ (see HPLC chromatogram in Figure S50); $[\alpha]^{25}_D = +8.3$ (c = 1.5 in CHCl$_3$).

3-(3-Chloro-phenyl)-4-nitrobutanal (3i) (Table S2, entry 7)

The title compound 3i (22.5 mg, 0.099 mmol, yield: 93%) was obtained from acetaldehyde (1) and trans-3-chloro-β-nitrostyrene 2i (20.1 mg, purity: 97%, 0.11 mmol) according to the general procedure as colorless oil. The 1H NMR spectroscopic data of 3i are in agreement with published data.\textsuperscript{[12,13]} Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4i using a Chiralpak ID column (Water/MeCN: 85/15, 25 ºC) at 1 mL/min, UV detection at 220 nm: $t_R$ (major) = 19.3 min, (minor) = 23.1 min, $ee = 84\%$ (see HPLC chromatogram in Figure S51); $[\alpha]^{25}_D = -2.2$ (c = 2.3 in CHCl$_3$).

3-(4-Nitro-phenyl)-4-nitrobutanal (3j) (Table S2, entry 8)

The title compound 3j (9.4 mg, 0.039 mmol, yield: 37%) was obtained from acetaldehyde (1) and β,4-dinitrostyrene 2j (21.1 mg, purity: 97%, 0.11 mmol) according to the general procedure as yellowish oil after column chromatography (silica gel, heptane/EtOAc: 4/1~2/1). The 1H NMR spectroscopic data of 3j are in agreement with published data.\textsuperscript{[10,11]} Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4j using a Chiralpak ID column (Water/MeCN: 85/15, 25 ºC) at 1 mL/min, UV detection at 220 nm: $t_R$ (minor) = 15.1 min, (major) = 17.1 min, $ee = 87\%$ (see HPLC chromatogram in Figure S52); $[\alpha]^{25}_D = -9.2$ (c = 0.9 in CHCl$_3$).

3-(2-Nitro-phenyl)-4-nitrobutanal (3k) (Table S2, entry 9)

The title compound 3k (7.9 mg, 0.033 mmol, yield: 31%) was obtained from acetaldehyde (1) and β,2-dinitrostyrene 2k (21.2 mg, purity: 97%, 0.11 mmol) according to the general procedure as yellowish oil after column chromatography (silica gel, heptane/EtOAc: 4/1~2/1). The 1H
NMR spectroscopic data of 3k are in agreement with published data.\[11,12\] Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4k using a Chiralpak ID column (Water/MeCN: 95/5, 25 ºC) at 1.5 mL/min, UV detection at 220 nm: \(t_R\) (major) = 23.3 min, (minor) = 28.7 min, \(ee = 94\%\) (see HPLC chromatogram in Figure S53); \([\alpha]^{25}_D = +5.8\ (c = 0.8\) in CHCl\(_3\)).

3-(3-Nitro-phenyl)-4-nitrobutanal (3l) (Table S2, entry 10)

The title compound 3l (4.4 mg, 0.018 mmol, yield: 35\%) was obtained from acetaldehyde (1) and \(\beta,3\)-dinitrostyrene 2l (10.5 mg, purity: 98\%, 0.054 mmol) according to the general procedure as a colorless oil after column chromatography (silica gel, heptane/EtOAc: 4/1~2/1). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 9.75 (s, 1H), 8.18 (dd, \(J = 7.8, 2.0\ Hz, 1H\)), 8.13 (t, \(J = 1.7\ Hz, 1H\)), 7.62 (dt, \(J = 7.8, 1.7\ Hz, 1H\)), 7.55 (t, \(J = 7.8\ Hz, 1H\)), 4.76 (dd, \(J = 12.9, 6.9\ Hz, 1H\)), 4.67 (dd, \(J = 12.9, 7.8\ Hz, 1H\)), 4.23 (ddd, \(J = 7.8, 6.9, 6.8\ Hz, 1H\)). \(13\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 197.86, 148.60, 140.63, 134.16, 130.23, 123.17, 122.28, 78.64, 46.14, 37.35. Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4l using a Chiralpak ID column (Water/MeCN: 95/5, 25 ºC) at 1.5 mL/min, UV detection at 220 nm: \(t_R\) (major) = 34.3 min, (minor) = 42.3 min, \(ee = 95\%\) (see HPLC chromatogram in Figure S54); \([\alpha]^{25}_D = -2.4\ (c = 0.5\) in CHCl\(_3\)); MS (EI): \(m/\zeta = 237.2\ (M^+)\).

3-(3-Bromo-phenyl)-4-nitrobutanal (3m) (Table S2, entry 11)

The title compound 3m (24.0 mg, 0.088 mmol, yield: 96\%) was obtained from acetaldehyde (1) and trans-3-bromo-\(\beta\)-nitrostyrene 2m (20.6 mg, purity: 97\%, 0.09 mmol) according to the general procedure as yellowish oil. \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 9.71 (s, 1H), 7.42 (d, \(J = 7.8\ Hz, 1H\)), 7.38 (s, 1H), 7.22 (t, \(J = 7.8\ Hz, 1H\)), 7.19 (d, \(J = 7.8\ Hz, 1H\)), 4.67 (dd, \(J = 12.8, 6.9\ Hz, 1H\)), 4.60 (dd, \(J = 12.8, 7.8\ Hz, 1H\)), 4.05 (dd, \(J = 7.8, 6.9, 6.8\ Hz, 1H\)), 2.95 (d, \(J = 6.8, 2H\)); \(13\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 198.17, 140.55, 131.31, 130.72, 130.47, 126.18, 123.15, 123.17, 78.90, 46.24, 37.43. Enantiomeric excess was determined by reverse phase HPLC with derivatized alcohol 4m using a Chiralpak ID column (Water/MeCN: 85/15, 25 ºC) at 1 mL/min, UV detection at 220 nm: \(t_R\) (major) = 27.2 min, (minor) = 33.3 min, \(ee = 92\%\) (see HPLC chromatogram in Figure S55); \([\alpha]^{25}_D = -5.0\ (c = 2.3\) in CHCl\(_3\)); MS (EI): \(m/\zeta = 270.3\ (M^+)\).

5. Derivatization of \(\gamma\)-nitroaldehydes 3c-m to \(\gamma\)-nitroalcohols 4c-m

The chemically synthesized aldehydes rac-3c-m as well as enzymatically obtained 3c-m were converted into the corresponding alcohols based on a literature procedure.\[14\]
General procedure: γ-nitroaldehyde 3c-m (~5-10 mg, 0.018-0.045 mmol) was dissolved in 5 mL EtOH and the solution was cooled to 0 °C (ice-brine bath). An aliquot of NaBH₄ (0.009-0.023 mmol, 0.5 eq) from a stock solution (200 mM in EtOH) was added, drop wise. The solution was stirred at 0 °C (ice-brine bath) for 1 h and then slowly warmed up to ambient temperature. The reaction progress was monitored by TLC (EtOAc/n-Heptane: 1:1). After the reaction was completed, the solution was quenched with H₂O (~ 1 mL) and extracted with CH₂Cl₂ (3 × 1 mL). The combined organic layers were washed with brine (1 mL) and dried with Na₂SO₄. The residue was submitted to NMR spectroscopy after the solvent was evaporated. NMR spectroscopic data for 4c-m are given in Figure S37-S45.

3-(3-Hydroxy-phenyl)-4-nitrobutanol (4c)

The title compound 4c (4 mg, 0.019 mmol, yield: 34%) was obtained from 3c (11.6 mg, 0.056 mmol) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 7.21 (t, J = 7.9 Hz, 1H), 6.79 (d, J = 7.9 Hz, 1H), 6.74 (dd, J = 7.9, 1.9 Hz, 1H), 6.71 (d, J = 1.9 Hz, 1H), 4.64 (dd, J = 12.4, 7.2 Hz, 1H), 4.59 (dd, J = 12.4, 8.2 Hz, 1H), 3.70 – 3.61 (m, 2H), 3.55 – 3.48 (m, 1H), 2.01 – 1.84 (m, 2H); ¹³C NMR (126 MHz, CDCl₃) δ 156.03, 140.72, 130.34, 119.80, 114.82, 114.65, 80.51, 59.89, 40.94, 35.53; HRMS (ESI): [M-H]⁻ calculated for C₁₀H₁₂NO₄: 210.07718, found: 210.07754.

3-(4-Methoxy-phenyl)-4-nitrobutanol (4d)

The title compound 4d (5 mg, 0.022 mmol, yield: 50%) was obtained from 3d (10.3 mg, 0.044 mmol) as a colorless oil. The ¹H NMR spectroscopic data of 4d are in agreement with literature data.[¹⁴]

3-(2-Methoxy-phenyl)-4-nitrobutanol (4e)

The title compound 4e (5 mg, 0.020 mmol, yield: 46%) was obtained from 3e (10.3 mg, 0.045 mmol) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 7.26 (ddd, J = 8.3, 8.2, 1.6 Hz, 1H), 7.14 (dd, J = 7.5, 1.6 Hz, 1H), 6.94 (ddd, J = 8.3, 7.5, 0.9 Hz, 1H), 6.90 (dd, J = 8.2, 0.9 Hz, 1H), 4.75 (dd, J = 12.3, 7.7 Hz, 1H), 4.68 (dd, J = 12.3, 7.3 Hz, 1H), 4.01 – 3.98 (m, 1H), 3.86 (s, 3H), 3.63 – 3.58 (m, 1H), 3.48 – 3.43 (m, 1H), 2.01 – 1.96 (m, 2H); ¹³C NMR (126 MHz, CDCl₃) δ
3-(3-Methoxy-phenyl)-4-nitrobutanol (4f)

The title compound 4f (6.0 mg, 0.026 mmol, yield: 21%) was obtained from 3f (11.2 mg, 0.12 mmol) as a colorless oil. The $^1$H NMR spectroscopic data of 4f are in agreement with literature data.\[16\]

3-(2-Chloro-phenyl)-4-nitrobutanol (4h)

The title compound 4h (4.0 mg, 0.017 mmol, yield: 66%) was obtained from 3h (6.0 mg, 0.026 mmol) as a colorless oil. The $^1$H NMR spectroscopic data of 4h are in agreement with literature data.\[15\]

3-(3-Chloro-phenyl)-4-nitrobutanol (4i)

The title compound 4i (2.0 mg, 0.009 mmol, yield: 17%) was obtained from 3i (10.3 mg, 0.051 mmol) as a colorless oil. The $^1$H NMR spectroscopic data of 4i are in agreement with literature data.\[15\]

3-(4-Nitro-phenyl)-4-nitrobutanol (4j)

The title compound 4j (7.0 mg, 0.029 mmol, yield: 74%) was obtained from 3j (9.4 mg, 0.039 mmol) as a yellowish oil. The $^1$H NMR spectroscopic data of 4j are in agreement with literature data.\[16\]

3-(2-Nitro-phenyl)-4-nitrobutanol (4k)

The title compound 4k (3.0 mg, 0.012 mmol, yield: 38%) was obtained from 3k (8.0 mg, 0.033 mmol) as a yellowish oil. $^1$H NMR (500 MHz, CDCl$_3$) δ 7.84 (d, $J = 7.7$ Hz, 1H), 7.62 (ddd, $J = 7.7, 7.7, 1.1$ Hz, 1H), 7.47 – 7.43 (m, 2H), 4.79 – 4.76 (m, 2H), 4.36 – 4.30 (m, 1H), 3.75 – 3.67 (m, 1H), 3.62 – 3.57 (m, 1H), 2.13 – 2.00 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 140.51$^{[17]}$, 133.65, 133.14, 128.58, 128.34, 124.98, 79.43, 59.81, 37.63, 35.23; HRMS (ESI): [M-H]$^-$ calculated for C$_{10}$H$_{12}$N$_2$O$_5$: 239.06735, found: 239.06752.
3-(3-Nitro-phenyl)-4-nitrobutanol (4l)

The title compound 4l (1.0 mg, 0.004 mmol, yield: 28%) was obtained from 3l (3.5 mg, 0.015 mmol) as a yellowish oil. $^1$H NMR (500 MHz, CHCl$_3$) $\delta$ 8.21 – 8.17 (m, 1H), 8.16 (t, $J = 1.8$ Hz, 1H), 7.63 (d, $J = 7.7$ Hz, 1H), 7.57 (t, $J = 7.9$ Hz, 1H), 4.80 (dd, $J = 12.8$, 6.1 Hz, 1H), 4.70 (dd, $J = 12.8$, 9.2 Hz, 1H), 3.98 – 3.91 (m, 1H), 3.78 – 3.68 (m, 1H), 3.55 – 3.50 (m, 1H), 2.13 – 1.93 (m, 2H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 148.66, 141.36, 134.13, 130.06, 122.93, 122.41, 79.86, 59.36, 40.70, 35.36; HRMS (ESI): [M-H]$^-$ calculated for C$_{10}$H$_{12}$N$_2$O$_5$: 239.06735, found: 239.06755.

3-(3-Bromo-phenyl)-4-nitrobutanol (4m)

The title compound 4m (3.0 mg, 0.011 mmol, yield: 26%) was obtained from 3m (11.5 mg, 0.042 mmol) as a colorless oil. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 (ddd, $J = 7.9$, 1.5, 1.5 Hz, 1H), 7.38 (dd, $J = 1.5$, 1.5 Hz, 1H), 7.22 (dd, $J = 7.9$, 7.7 Hz, 1H), 7.17 (ddd, $J = 7.7$, 1.5, 1.5 Hz, 1H), 4.67 (dd, $J = 12.6$, 6.9 Hz, 1H), 4.60 (dd, $J = 12.6$, 8.6 Hz, 1H), 3.74 – 3.69 (m, 1H), 3.68 – 3.62 (m, 1H), 3.52 – 3.47 (m, 1H), 2.02 – 1.94 (m, 1H), 1.93 – 1.85 (m, 1H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 141.34, 131.01, 130.66, 130.60, 126.33, 123.09, 80.20, 59.63, 40.69, 35.48; HRMS (ESI): [M-H]$^-$ calculated for C$_{10}$H$_{11}$NO$_3$Br: 271.99278, found: 271.99299.

6. Synthesis of racemic 3c-m and its derivatization to racemic 4c-m for enantiomeric excess determination

Racemic $\gamma$-nitroaldehydes 3c-m (rac-3c-m) were synthesized to serve as reference compounds to determine the enantiopurity of enzymatically obtained Michael adducts 3c-m. The racemic 3d, 3h-i were synthesized according to a previously reported protocol. In separate experiments, nitrostyrene derivatives (2d, 2h-i), piperidine (0.2 eq) and acetaldehyde 1 (10 eq) were dissolved in MeOH (3 mL). The solution was stirred at 0 °C for 24 – 48 h. The racemic 3c, 3e-f, 3j-m were synthesized according to a literature protocol. Nitrostyrene derivative (2c, 2e-f, 2j-m) was dissolved in 1,4-dioxane (0.3 mL). (R/S)-diphenyltrimethylsiloxymethylpyrrolidine (0.1 eq) was added and the reaction mixture was cooled to 0 °C. Acetaldehyde 1 (10 eq) was cooled to -10 °C and added to the reaction mixture. The solution was slowly warmed up to ambient temperature and stirred for 44 h. The reaction progress was monitored by thin layer chromatography (TLC, silica, EtOAc/n-heptane: 2:1). The crude mixtures were purified by column chromatography (silica gel, EtOAc/n-heptane) to enrich the racemic 3c-m. The $^1$H NMR spectra of the synthesized racemic 3c-m (Figure S27-S34) were in agreement with those of enzymatically prepared 3c-m and published data.

The chemically synthesized aldehydes rac-3c-m were derivatized into racemic alcohols 4c-m (rac-4c-m) for enantiomeric excess determination following the same procedure as described above (see section 5). The NMR spectra of rac-4c-m, obtained from rac-3c-m, were in agreement with those of 4c-m.
7. Absolute configuration determination of major enantiomers of 3c-m and 5

Optical rotations of enzymatically prepared 3c-m and 5 were measured to elucidate the absolute configuration of the major enantiomers. The measured optical rotations of 3c-m, 5 and the reported optical rotation data of these compounds in literature are summarized in Table S3.

Table S3. Absolute configuration determination of major enantiomers of enzymatically obtained 3c-m and 5 by optical rotation.

| Product | R     | Measured optical rotation \[\alpha\] \[^{25}\text{D}\] | Optical rotation and absolute configuration data in literature | ee \(\%\) \[^{[a]}\] | Abs. configuration \[^{[b]}\] |
|---------|-------|--------------------------------------------------|--------------------------------------------------------------|-----------------|-------------------|
| 5       | o-OH  | -25.0 \(c = 2.2, \text{CHCl}_3\)                 | -5.06 \(c = 1.0, \text{CHCl}_3\) (4S)\[^{[9]}\]             | n.d.            | n.d. \[^{[e]}\]   |
| 3c      | m-OH  | -4.3 \(c = 2.1, \text{CHCl}_3\)                 | n.a. \[^{[d]}\]                                               | 95              | S\[^{[f]}\]       |
| 3d      | p-MeO | -16.2 \(c = 2.2, \text{CHCl}_3\)                | -11.2 \(c = 1.0, \text{CHCl}_3\) (S)\[^{[10,11]}\]         | 94              | S.                |
| 3e      | o-MeO | +30.4 \(c = 2.4, \text{CHCl}_3\)                | -3.9 \(c = 1.0, \text{CHCl}_3\) (S)\[^{[11]}\]             | 97              | R.                |
| 3f      | m-MeO | -4.0 \(c = 0.7, \text{CHCl}_3\)                | -5.9 \(c = 2.0, \text{CHCl}_3\) (S)\[^{[11]}\]             | 96              | S.                |
| 3g\[^{[c]}\] | p-Cl | -5.2 \(c = 0.9, \text{CHCl}_3\)               | -24.0 \(c = 0.8, \text{CHCl}_3\) (S)\[^{[11]}\]           | 69              | S.                |
| 3h      | o-Cl  | +8.3 \(c = 1.5, \text{CHCl}_3\)                | n.a.                                                         | 82              | R\[^{[g]}\]       |
| 3i      | m-Cl  | -2.2 \(c = 2.3, \text{CHCl}_3\)                | -10.8 \(c = 0.5, \text{CH}_{2}\text{Cl}_2\) (S)\[^{[20]}\] | 84              | S.                |
| 3j      | p-NO\(_2\) | -9.2 \(c = 0.9, \text{CHCl}_3\)           | -19.0 \(c = 1.9, \text{CHCl}_3\) (S)\[^{[10]}\]           | 87              | S.                |
| 3k      | o-NO\(_2\) | +5.8 \(c = 0.8, \text{CHCl}_3\)         | -1.8 \(c = 0.8, \text{CHCl}_3\) (S)\[^{[11]}\]           | 94              | R.                |
| 3l      | m-NO\(_2\) | -2.4 \(c = 0.5, \text{CHCl}_3\)          | n.a.                                                        | 95              | S\[^{[f]}\]       |
| 3m      | m-Br  | -5.0 \(c = 2.3, \text{CHCl}_3\)               | n.a.                                                        | 92              | S\[^{[f]}\]       |

[a] ee of the major enantiomer of enzymatically obtained 3c-m determined by reverse phase HPLC with the derivatized alcohol 4c-m. [b] Absolute configuration determined by comparison of measured optical rotation data of 3c-m with literature data. [c] Previously reported data.\[^{[18]}\] [d] n.a. = not available (i.e. have not been reported in literature). [e] n.d. = not determined. [f] Absolute configuration assumed by comparing the optical rotation data and HPLC chromatogram with those of products 3i and 3f. [g] Absolute configuration assumed by comparing the optical rotation data with those of 3e and 3k.
For γ-nitroaldehydes obtained from meta- and para-substituted nitrostyrene derivatives (i.e. products $3c$, $3d$, $3f$, $3g$, $3i$, $3j$, $3l$, $3m$), a negative rotation was observed. In the case of products that were obtained from ortho-substituted nitrostyrene derivatives (i.e. products $3e$, $3h$, $3k$), a positive rotation was found. Comparison with the literature data revealed that the major enantiomers of meta- and para-substituted γ-nitroaldehydes ($3d$, $3f$, $3i$, $3j$) have the $(S)$-configuration, while the major enantiomers of the ortho-substituted γ-nitroaldehydes ($3e$, $3h$, $3k$) have the $(R)$-configuration. The negative optical rotation of products $3c$, $3l$, $3m$ could not be compared with literature data since they have not been reported in literature so far. Since $3c$, $3l$-$m$ gave the same negative rotation as $3f$ and $3i$, we assume that the chiral centers of $3c$, $3l$, $3m$ have the identical geometry (i.e. $S$-configuration) as compared to the chiral centers of $3f$ and $3i$. Similarly, we assume that the chiral center of $3h$ has the same geometry (i.e. $R$-configuration) as $3e$ and $3k$ by comparing their optical rotation data.

8. General procedure for kinetic assays

Kinetic assays were performed at 25 °C in DMSO/phosphate buffer (40/60, v/v) by following the decrease in absorbance at 316-360 nm corresponding to the depletion of nitrostyrene derivatives $2a$-$n$. The kinetic assay of the 4-OT-catalyzed reaction between acetaldehyde (1) and trans-β-nitrostyrene (2n) was performed at both pH 7.3 and pH 5.5 for comparison with previously reported data. Kinetic assays with meta- and para-substituted nitrostyrene derivatives (i.e. $2a$, $2c$, $2d$, $2f$-$g$, $2i$-$j$, $2l$-$m$) were performed at pH 7.3, while kinetic assays with ortho-substituted nitrostyrene derivatives (i.e. $2b$, $2e$, $2h$, $2k$) were performed at pH 5.5 due to strong non-enzymatic background reaction (i.e. hydration of nitrostyrene derivatives) at pH 7.3.

Assay procedure: a stock solution of 4-OT (3 mg/ml, monomer concentration) was prepared in 20 mM NaH$_2$PO$_4$ buffer (pH 7.3). A fresh stock solution of acetaldehyde 1 (1 M) was prepared in 20 mM NaH$_2$PO$_4$ buffer (pH 7.3 or 5.5) while stock solutions of nitrostyrene derivatives $2a$-$n$ were prepared in DMSO. An aliquot (15 μl) of the 4-OT stock solution was added to the 1 mm cuvette (total volume: 300 μl) containing DMSO and NaH$_2$PO$_4$ buffer (20mM, pH 7.3 or 5.5). The assays were initiated by the addition of acetaldehyde 1 (30 μl from the stock solution) and nitrostyrene derivatives $2a$-$n$ (15 μl from the stock solution), yielding final concentrations of 22 μM 4-OT, 100 mM acetaldehyde (1), 0.125 to 3 mM nitrostyrene derivatives ($2a$-$n$), and 40% DMSO (v/v). The initial rates (μM/s) were plotted versus the concentrations of nitrostyrene derivatives ($2a$-$n$). SigmaPlot was used to fit the data to Michaelis-Menten kinetics and determine the kinetic parameters.
9. NMR spectra and HPLC chromatograms

_NMR spectra of enzymatically obtained 3c-m and 5_

Figure S15. \(^1\)H NMR spectrum of enzymatically prepared 4-(nitromethyl)-chroman-2-ol (5).
Figure S16. $^1$H NMR and $^{13}$C NMR spectrum of enzymatically prepared 3-((3-hydroxyphenyl)-4-nitrobutanal (3c).
Figure S17. $^1$H NMR spectrum of enzymatically prepared 3-(4-methoxy-phenyl)-4-nitrobutanal (3d).

Figure S18. $^1$H NMR spectrum of enzymatically prepared 3-(2-methoxy-phenyl)-4-nitrobutanal (3e).
Figure S19. $^1$H NMR spectrum of enzymatically prepared 3-(3-methoxy-phenyl)-4-nitrobutanal (3f).

Figure S20. $^1$H NMR spectrum of enzymatically prepared 3-(2-chloro-phenyl)-4-nitrobutanal (3h).
Figure S21. $^1$H NMR spectrum of enzymatically prepared 3-(3-chloro-phenyl)-4-nitrobutanal (3i).

Figure S22. $^1$H NMR spectrum of enzymatically prepared 3-(4-nitro-phenyl)-4-nitrobutanal (3j).
Figure S23. $^1$H NMR spectrum of enzymatically prepared 3-(2-nitro-phenyl)-4-nitrobutanal (3k).
Figure S24. $^1$H NMR and $^{13}$C NMR spectrum of enzymatically prepared 3-(3-nitro-phenyl)-4-nitrobutanal (3l).
Figure S25. $^1$H NMR and $^{13}$C NMR spectrum of enzymatically prepared 3-(3-bromo-phenyl)-4-nitrobutanal (3m).
$^1$H NMR spectra of chemically synthesized racemic 3c-m (rac-3c-m)

Figure S26. $^1$H NMR spectrum of chemically synthesized racemic 3-(3-hydroxy-phenyl)-4-nitrobutanal (rac-3c).

Figure S27. $^1$H NMR spectrum of chemically synthesized racemic 3-(4-methoxy-phenyl)-4-nitrobutanal (rac-3d).
Figure S28. $^1$H NMR spectrum of chemically synthesized racemic 3-(2-methoxy-phenyl)-4-nitrobutanal ($rac\text{-}3e$).

Figure S29. $^1$H NMR spectrum of chemically synthesized racemic 3-(3-methoxy-phenyl)-4-nitrobutanal ($rac\text{-}3f$).
Figure S30. $^1$H NMR spectrum of chemically synthesized racemic 3-(2-chloro-phenyl)-4-nitrobutanal ($rac$-$3h$).

Figure S31. $^1$H NMR spectrum of chemically synthesized racemic 3-(3-chloro-phenyl)-4-nitrobutanal ($rac$-$3i$).
Figure S32. $^1$H NMR spectrum of chemically synthesized racemic 3-(4-nitro-phenyl)-4-nitrobutanal (rac-3j).

Figure S33. $^1$H NMR spectrum of chemically synthesized racemic 3-(2-nitro-phenyl)-4-nitrobutanal (rac-3k).
Figure S34. $^1$H NMR spectrum of chemically synthesized racemic 3-(3-nitro-phenyl)-4-nitrobutanal (rac-3l).

Figure S35. $^1$H NMR spectrum of chemically synthesized racemic 3-(3-bromo-phenyl)-4-nitrobutanal (rac-3m).
NMR spectra of alcohols 4c–m derived from enzymatically prepared 3c–m

Figure S36. $^1$H NMR and $^{13}$C NMR spectrum of 3-(3-hydroxy-phenyl)-4-nitrobutanol (4c) derived from enzymatically obtained 3c.
Figure S37. $^1$H NMR spectrum of 3-(3-methoxy-phenyl)-4-nitrobutanol (4d) derived from enzymatically obtained 3d.
Figure S38. $^1$H NMR and $^{13}$C NMR spectrum of 3-(2-methoxy-phenyl)-4-nitrobutanol (4e) derived from enzymatically obtained 3e.
Figure S39. $^1$H NMR spectrum of 3-(3-methoxy-phenyl)-4-nitrobutanol (4f) derived from enzymatically obtained 3f.

Figure S40. $^1$H NMR spectrum of 3-(2-chloro-phenyl)-4-nitrobutanol (4h) derived from enzymatically obtained 3h.
Figure S41. $^1$H NMR spectrum of 3-(3-chloro-phenyl)-4-nitrobutanol ($4i$) derived from enzymatically obtained $3i$.

Figure S42. $^1$H NMR spectrum of 3-(4-nitro-phenyl)-4-nitrobutanol ($4j$) derived from enzymatically obtained $3j$. 
Figure S43. $^1$H NMR and $^{13}$C NMR spectrum of 3-(2-nitro-phenyl)-4-nitrobutanol (4k) derived from enzymatically obtained 3k.
Figure S44. $^1$H NMR and $^{13}$C NMR spectrum of 3-(3-nitro-phenyl)-4-nitrobutanol (4l) derived from enzymatically obtained 3l.
Figure S45. $^1$H NMR and $^{13}$C NMR spectrum of 3-(3-bromo-phenyl)-4-nitrobutanol (4m) derived from enzymatically obtained 3m.
**Figure S46.** HPLC chromatograms of A) racemic 4c (rac-4c) derived from chemically synthesized rac-3c; B) 4c derived from enzymatically obtained 3c; C) mixture of rac-4c and 4c in molar ratio of 1:2.
Figure S47. HPLC chromatograms of A) racemic 4d (rac-4d) derived from chemically synthesized rac-3d; B) 4d derived from enzymatically obtained 3d; C) mixture of rac-4d and 4d in molar ratio of 1:1.
Figure S48. HPLC chromatograms of A) racemic 4e (rac-4e) derived from chemically synthesized rac-3e; B) 4e derived from enzymatically obtained 3e; C) mixture of rac-4e and 4e in molar ratio of 1:1.
Figure S49. HPLC chromatograms of A) racemic 4f (rac-4f) derived from chemically synthesized rac-3f; B) 4f derived from enzymatically obtained 3f; C) mixture of rac-4f and 4f in molar ratio of 1:1.
Figure S50. HPLC chromatograms of A) racemic 4h (rac-4h) derived from chemically synthesized rac-3h; B) 4h derived from enzymatically obtained 3h; C) mixture of rac-4h and 4h in molar ratio of 1:1.
Figure S51. HPLC chromatograms of A) racemic 4i (rac-4i) derived from chemically synthesized rac-3i; B) 4i derived from enzymatically obtained 3i; C) mixture of rac-4i and 4i in molar ratio of 1:1.
Figure S52. HPLC chromatograms of A) racemic 4j (rac-4j) derived from chemically synthesized rac-3j; B) 4j derived from enzymatically obtained 3j; C) mixture of rac-4j and 4j in molar ratio of 1:1.
Figure S53. HPLC chromatograms of A) racemic 4k (rac-4k) derived from chemically synthesized rac-3k; B) 4k derived from enzymatically obtained 3k; C) mixture of rac-4k and 4k in molar ratio of 1:1.
Figure S54. HPLC chromatograms of A) racemic 4l (rac-4l) derived from chemically synthesized rac-3l; B) 4l derived from enzymatically obtained 3l; C) mixture of rac-4l and 4l in molar ratio of 1:1.
**Figure S55.** HPLC chromatograms of A) racemic 4m (*rac-4m*) derived from chemically synthesized *rac-3m*; B) 4m derived from enzymatically obtained 3m; C) mixture of *rac-4m* and 4m in molar ratio of 1:1.
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