Supporting Information

Synthesis of γ-nitroaldehydes containing quaternary carbon in α position using 4-oxalocrotonate tautomerase whole-cell biocatalyst †

Jelena Radivojevic,[a,b] Gordana Minovska,[b] Lidija Senerovic,[b] Kevin O'Connor,[c] Predrag Jovanovic,[d] Vladimir Savic,[d] Zorana Tokic-Vujosevic,[d] Jasmina Nikodinovic-Runic,*[b] and Veselin Maslak*[a]†

[a] *Faculty of Chemistry, University of Belgrade, Studentski Trg 12-16, 11000 Belgrade, Serbia. Fax: +381 11 218 4330; Tel: +381 11 333 3655; E-mail: vmaslak@chem.bg.ac.rs*
[b] *Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Vojvode Stepe 444a, P.O. Box 23, 11010 Belgrade, Serbia. Fax: +381 11 397 5808; Tel: +381 11 397 6034; E-mail: jasmina.nikodinovic@gmail.com*
[c] *School of Biomolecular and Biomedical Sciences, Centre for Synthesis and Chemical Biology, University College Dublin, Belfield, Dublin 4, Ireland*
[d] *Department of Organic Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia*

Contents:

1. General information

NMR spectra were recorded on a Varian Gemini 200 (^{1}H NMR at 200 MHz, ^{13}C NMR at 50 MHz, for samples in deuterated chloroform), and on Bruker Avance III 500 (^{1}H NMR at 500 MHz, ^{13}C NMR at 125 MHz). Chemical shifts are expressed in ppm (δ) using tetramethylsilane as internal standard, coupling constants (*J*) are in Hz. The following abbreviations were used for signal multiplicities: s, singlet; d, doublet; qu, quintet; and m, multiplet. NMR data of known compounds are in agreement with literature values.

FTIR spectra were recorded on Perkin-Elmer-FT-IR 1725X spectrophotometer; values are given in cm^{-1}.

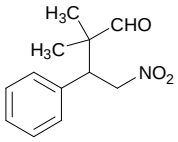
Mass spectra were obtained on Agilent technologies 6210 TOF LC/MS instrument (LC: series 1200). GC/MS analisys were recorded using Agilent technologies 7890A - 5975C inert XL EI CI instrument.

The enantiomeric excess was determined by HPLC (Agilent Technologies, HP110) with CHIRALPAK IA column (Chiral Technologies Europe, Cedex, France) at 215 nm for all samples. General procedure for the synthesis of γ-nitroaldehydes from the corresponding aldehydes catalysed by L-phenylalanine lithium salt was carried out using reported procedures.[1]

[1] M. Yoshida, A. Sato and S. Hara, *Organic & Biomolecular Chemistry*, 2010, **8**, 3031-3036.
A. Sato, M. Yoshida and S. Hara, *Chemical Communications*, 2008, 6242-6244.

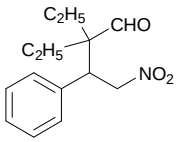
2. Spectral data

*2,2-dimethyl-4-nitro-3-phenylbutanal (**1a**)*



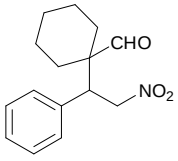
^{1}H NMR (200 MHz, $CDCl_3$) δ 9.53 (s, 1H), 7.40-7.13 (m, 5H), 4.92-4.64 (m, 2H), 3.78 (dd, J_1 =11 Hz, J_2 = 4.4 Hz, 1H), 1.14 (s, 3H), 1.01 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 204.3, 135.3, 129.0, 128.7, 128.1, 76.3, 48.3, 48.2, 21.6, 18.8; HPLC (Chiralpak IA, *i*-Propanol-Heptane= 20/80, flow rate 1 mL min^{-1}, λ=210nm): t_{major}= 2.98 min, t_{minor}= 4.01 min, IR νmax : 2975, 2935, 1725, 1554.

*2,2-diethyl-4-nitro-3-phenylbutanal (**1b**)*



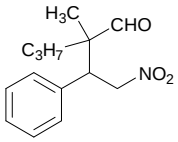
^{1}H NMR (200 MHz, $CDCl_3$) δ 9.58 (s, 1H), 7.38-7.11 (m, 5H), 4.85-4.58 (m, 2H), 3.33 (t, *J* = 7.4 Hz, 1H), 1.80-0.86 (m, 10H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 207.8, 135.2, 129.1, 128.9, 128.8, 128.5, 128.1, 127.4, 48.1, 33.4, 23.5, 22.6, 7.9, 7.5. HRMS (ESI): *m/z* calcd for $C_{14}H_{23}N_2O_3$: 267.1703 $[M+NH_4]^+$; found 267.1693. IR νmax : 2972, 2941, 1718, 1553. HPLC (Chiralpak IA, i-Propanol-Heptane= 30/70, flow rate 1 mL min^{-1}, λ=210nm): t_{major}= 3.87 min, t_{minor}= 4.72 min.

*1-(2-nitro-1-phenylethyl)cyclohexanecarbaldehyde (**1c**)*



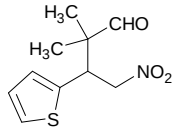
^{1}H NMR (200 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.63-7.02 (m, 5H), 4.90-4.58 (m, 2H), 3.54 (dd, J_1 =10 Hz, J_2 = 5.6 Hz, 1H), 2.39-0.85 (m, 10H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 207.3, 134.8, 129.1, 128.7, 128.1, 76.3, 51.2, 50.4, 30.9, 29.6, 25.0, 22.6, 22.5. IR νmax: 2933, 2856, 1719, 1553. HPLC (Chiralpak IA, i-Propanol-Heptane= 30/70, flow rate 1 mL min^{-1}, λ=210nm): t_{major}= 4.13 min, t_{minor}= 4.89 min.

*2-methyl-2-(2-nitro-1-phenylethyl)pentanal (**1d**)*



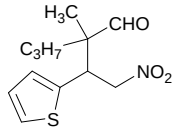
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.54 (major isomer) and 9.52 (minor isomer) (s, 1H), 7.34-7.27 (m, 3H), 7.21-7.16 (m, 2H), 4.85 (minor isomer) and 4.84 (major isomer) (dd, *J* = 11.3, 13.1 and 11.5, 13.0 Hz, 1H), 4.76 (minor isomer) and 4.63 (major isomer) (dd, *J* = 4.3, 13.1 and 3.9, 13.0 Hz, 1H), 3.79 (major isomer) and 3.77 (minor isomer) (dd, *J* = 3.9, 11.4 and 4.3, 11.2 Hz, 1H), 1.63-1.17 (m, 4H), 1.11 (major isomer) and 1.10 (minor isomer) (s, 3H), 0.9 (minor isomer) and 0.84 (major isomer) (t, *J* = 7.2 and 6.9 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.4 (major isomer), 205.0 (minor isomer), 135.3, 129.2, 129.1, 128.7, 128.0, 76.7, 51.7 (major isomer), 51.1 (minor isomer), 49.2 (minor isomer), 47.5 (major isomer), 37.6 (major isomer), 36.5 (minor isomer), 17.3 (minor isomer), 17.0 (major isomer), 15.8, 14.4.

*2,2-dimethyl-4-nitro-3-(thiophen-2-yl)butanal (**4a**)*



^{1}H NMR (200 MHz, $CDCl_3$) δ 9.54 (s, 1H), 7.27-7.22 (m, 1H), 7.00-6.91 (m, 2H), 4.75-4.59 (m, 2H), 4.14 (dd, J_1 =4.9 Hz, J_2 = 10.0 Hz, 1H), 1.21 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 204.0, 137.6, 127.7, 126.0, 124.6, 77.6, 48.3, 44.0, 21.1, 18.9. HPLC (Chiralpak IA, i-Propanol-Heptane= 10/90, flow rate 1 mL min^{-1}, λ=210nm): t_{major}= 8.08 min t_{minor}= 7.82 min.

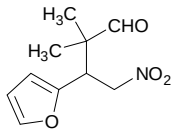
*2-methyl-2-(2-nitro-1-(thiophen-2-yl)ethyl)pentanal (**4d**)*



^{1}H NMR (500 MHz, $CDCl_3$) δ 9.54 (s, 1H), 7.26-7.22 (m, 1H), 6.98-6.89 (m, 2H), 4.78-4.60 (m, 2H), 4.20 (major isomer) and 4.12 (minor isomer) (dd, *J* = 3.8, 11.1 and 4.5, 10.4 Hz, 1H), 1.69-1.22 (m, 4H), 1.20 (major isomer) and 1.17 (minor isomer) (s, 3H), 0.92 (minor isomer) and 0.85 (major isomer) (t, *J* = 7.2 and 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.6 (major isomer) and 204.2 (minor isomer), 137.8, 127.93 (major isomer) and 127.86 (minor isomer), 126.9, 125.58(minor isomer) and 125.46 (major isomer), 78.2, 51.8 (major isomer) and 51.1 (minor isomer), 44.4 (minor isomer) and 42.4 (major isomer), 37.6 (major isomer) and 36.4 (minor isomer), 17.1 (minor isomer) and 17.0 (major isomer), 16.0, 14.5

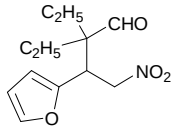
(minor isomer) and 14.3 (major isomer). IR νmax : 2963, 2935, 1723, 1556. HRMS (ESI): *m/z* calcd for $C_{12}H_{21}N_2O_3S$: 273.1267 $[M+NH_4]^+$; found 273.1270. HPLC (Chiralpak IA, i-Propanol-Heptane= 10/90, flow rate 1 mL min^{-1}, λ=210nm): t_{minor}= 6.55 min, t_{major}= 5.95 min

*3-(furan-2-yl)-2,2-dimethyl-4-nitrobutanal (**5a**)*



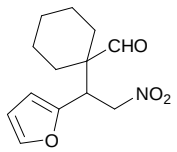
^{1}H NMR (200 MHz, $CDCl_3$) δ 9.53 (s, 1H), 7.38 (d, *J* = 1.6 Hz, 1H), 6.32 (dd, J_1 =1.6 Hz, J_2 = 3.2 Hz, 1H), 6. 20 (d, *J* = 3.2 Hz, 1H), 4.76 (dd, J_1 =10.9 Hz, J_2 = 12.9 Hz, 1H), 4.59 (dd, J_1 =4.1 Hz, J_2 = 12.9 Hz, 1H), 3.92 (dd, J_1 =4.1 Hz, J_2 = 10.9 Hz, 1H), 1.18 (s, 3H), 1.05 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 203.8, 150.1, 143.1, 110.6, 109.9, 74.9, 48.5, 41.8, 21.2, 18.4. HPLC (Chiralpak IA, i-Propanol-Heptane= 10/90, flow rate 1 mL min^{-1}, λ=210nm): t_{major}= 6.63 min, t_{minor}= 7.20 min.

*2,2-diethyl-3-(furan-2-yl)-4-nitrobutanal (**5b**)*



^{1}H NMR (200 MHz, $CDCl_3$) δ 9.61 (s, 1H), 7.36 (dd, J_1 = 0.6 Hz, J_2 = 1.8 Hz 1H), 6.31 (dd, J_1 =1.8 Hz, J_2 = 3.2 Hz, 1H), 6. 21 (dd, J_1 = 0.6 Hz, J_2 = 3.2 Hz, 1H), 4.81-4.62 (m, 2H), 3.88 (dd, J_1 =4.9 Hz, J_2 = 10.0 Hz, 1H), 1.72-1.51 (m, 4H), 0.91 (t, *J* = 7.5 Hz, 3H), 0.89 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$). δ 206.0, 149.4, 142.4, 110.4, 109.8, 75.2, 53.7, 41.0, 23.6, 22.9, 7.9, 7.6. IR νmax : 2971, 2936, 2883, 1719, 1555. HRMS (ESI): *m/z* calcd for $C_{12}H_{21}N_2O_4$: 257.1496 $[M+NH_4]^+$; found 257.1501. HPLC (Chiralpak IA, i-Propanol-Heptane= 10/90, flow rate 1 mL min^{-1}, λ=210nm): t_{major}= 5,58 min, t_{minor}= 5,62 min.

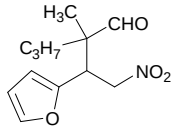
*1-(1-(furan-2-yl)-2-nitroethyl)cyclohexanecarbaldehyde (**5c**)*



^{1}H NMR (200 MHz, $CDCl_3$) δ 9.59 (s, 1H), 7.37 (d, *J* = 1.8 Hz, 1H), 6.31 (dd, J_1 =1.8 Hz, J_2 = 3.2 Hz, 1H), 6. 20 (d, *J* = 3.2 Hz, 1H), 4.76-4.56 (m, 2H), 3.68 (dd, J_1 =4.8 Hz, J_2 = 10.2

Hz, 1H), 2.11-1.16 (m, 10 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 205.9, 149.0, 142.8, 110.4, 109.8, 74.7, 51.0, 44.1, 30.5, 29.5, 24.9, 22.5; HPLC (Chiralpak IA, i-Propanol-Heptane= 10/90, flow rate 1 mL min^{-1}, λ=210nm): t_{major}= 5.79 min, t_{minor}= 6.09 min.

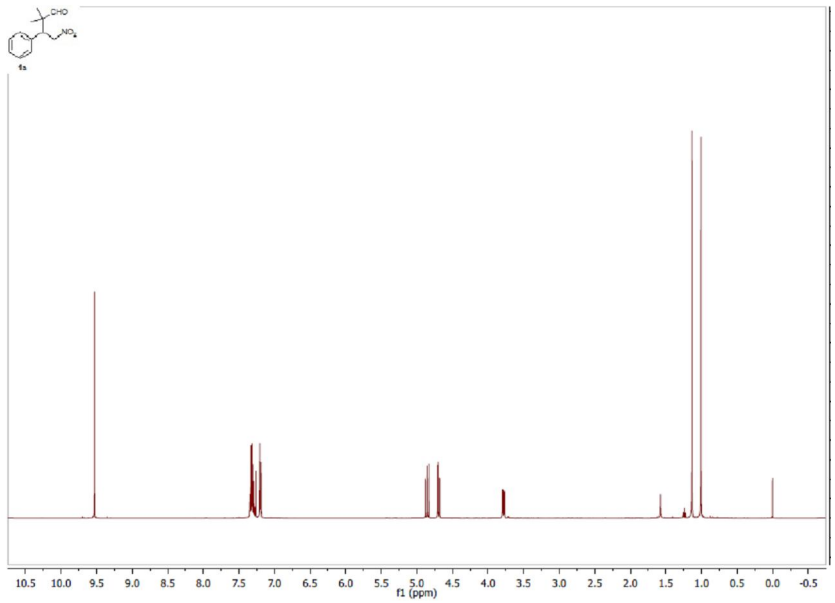
*2-(1-(furan-2-yl)-2-nitroethyl)-2-methylpentanal (**5d**)*

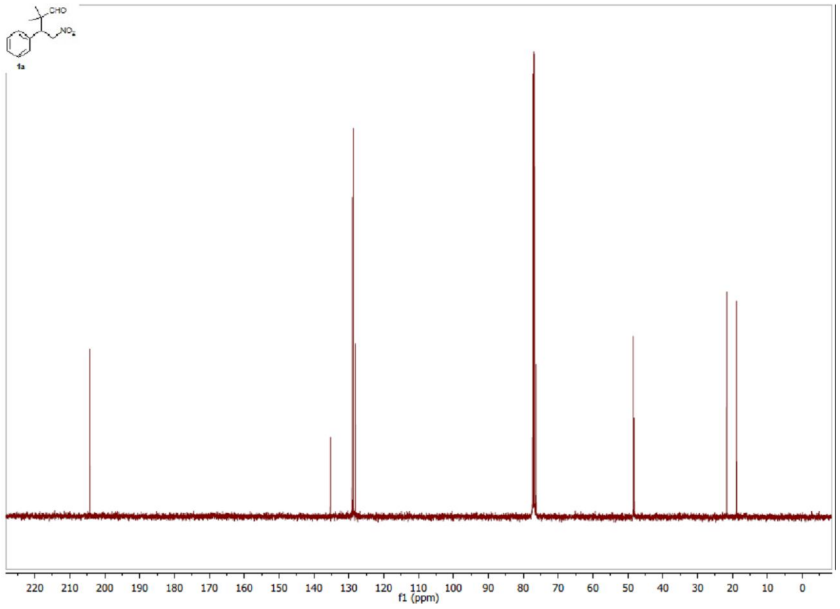


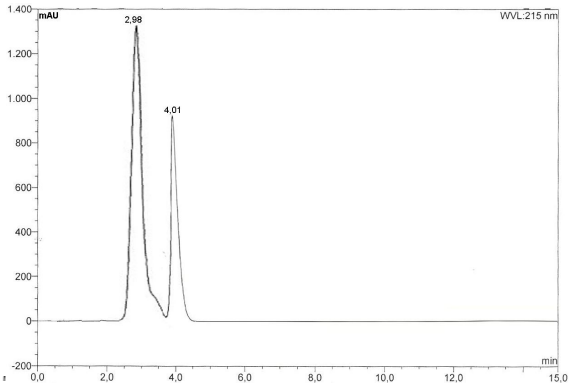
^{1}H NMR (500 MHz, $CDCl_3$) δ 9.55 (minor isomer) and 9.49 (major isomer) (s, 1H), 7.38 (major isomer) and 7.35 (minor isomer) (dd, *J* = 0.6, 1.8 and 0.6, 1.9 Hz, 1H), 6.32 (major isomer) and 6.30 (minor isomer) (dd, *J* = 1.8, 3.2 and 1.9, 3.2 Hz, 1H), 6.22 (major isomer) and 6.20 (minor isomer) (dd, *J* = 0.6, 3.2 and 0.6, 3.2 Hz, 1H), 4.76 (minor isomer) and 4.73 (major isomer) (dd, *J* = 11.1, 13.0 and 11.3, 12.8 Hz, 1H), 4.64 (minor isomer) and 4.55 (major isomer) (dd, *J* = 3.8, 13.0 and 3.6, 12.8 Hz, 1H), 3.99 (major isomer) and 3.90 (minor isomer) (dd, *J* = 3.6, 11.3 and 3.8, 11.1 Hz, 1H), 1.59-1.18 (m, 4H), 1.17 (major isomer) and 1.11 (minor isomer) (s, 3H), 0.91 (minor isomer) and 0.85 (major isomer) (t, *J* = 7.2 and 7.1, 3H) ^{13}C NMR 204.1, 149.7, 142.7, 110.6, 109.2, 75.3 (major isomer) and 74.7 (minor isomer), 51.5 (major isomer) and 51.1 (minor isomer), 42.7 (minor isomer) and 40.5 (major isomer), 37.6 (major isomer) and 36.6 (minor isomer), 17.1 (minor isomer) and 16.9 (major isomer), 16.5, 14.6 (minor isomer) and 14.4 (major isomer). IR νmax : 2964, 2935, 2874, 1723, 1557. HRMS (ESI): *m/z* calcd for $C_{12}H_{21}N_2O_4$: 257.1496 $[M+NH_4]^+$; found 257.1500.

3. *Scanned spectra*

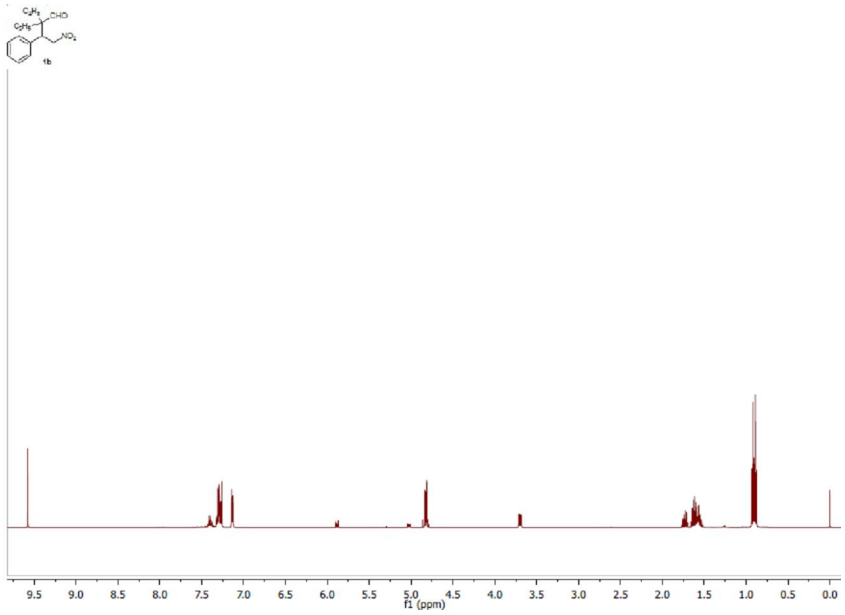
*2,2-dimethyl-4-nitro-3-phenylbutanal (***1a***)*

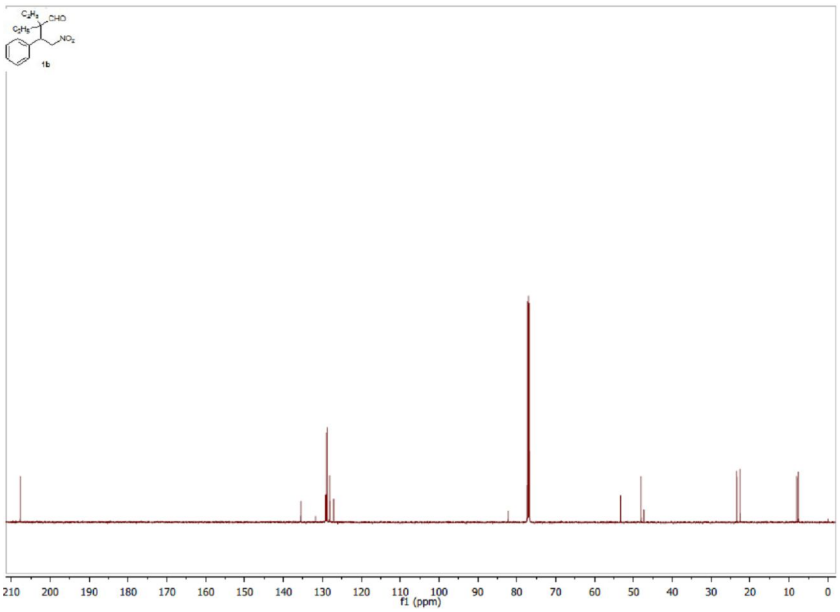


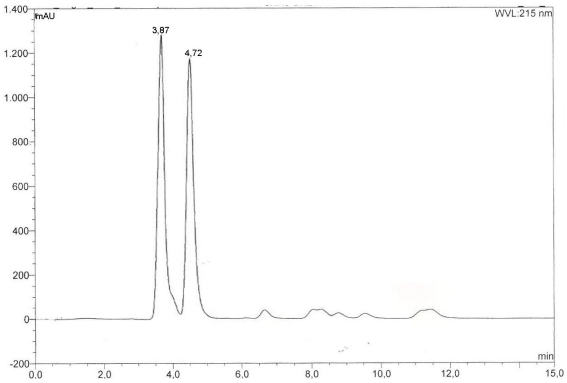




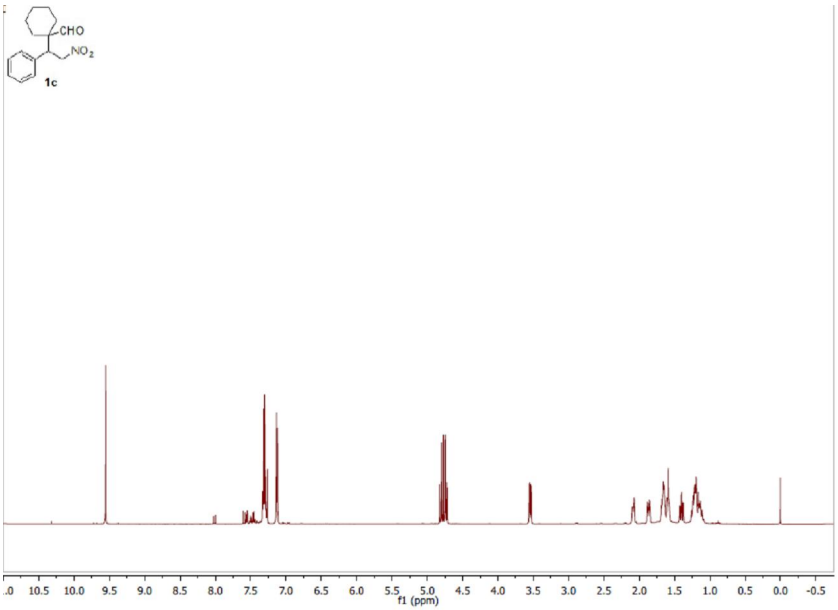
*2,2-diethyl-4-nitro-3-phenylbutanal (***1b***)*

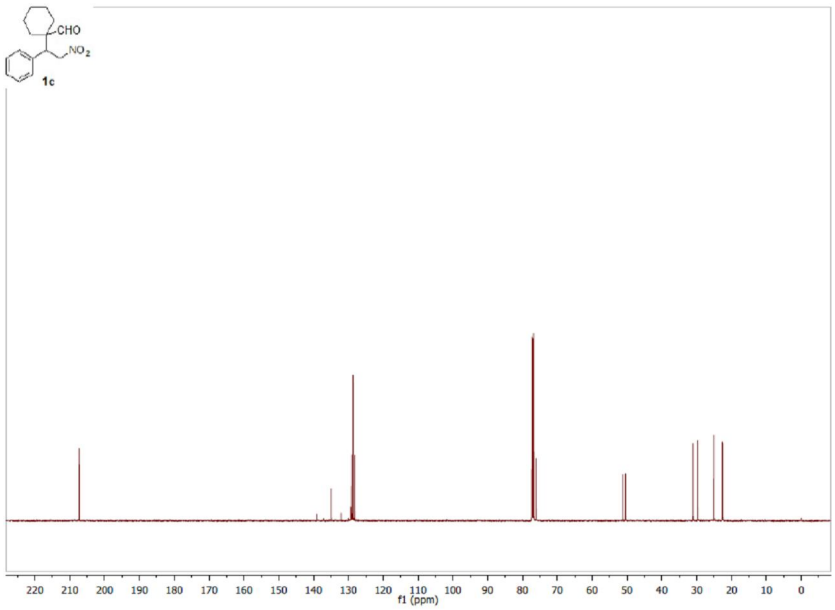


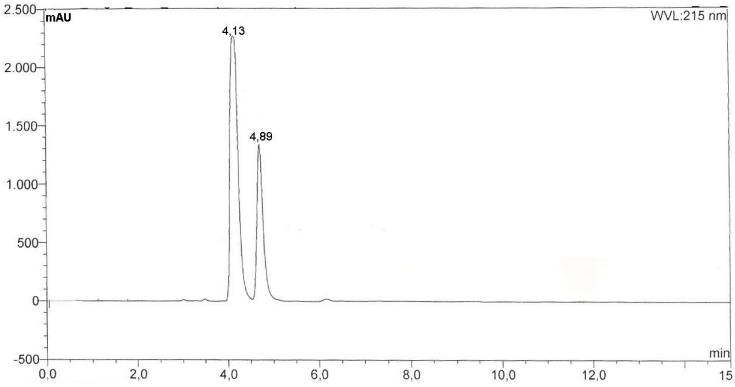




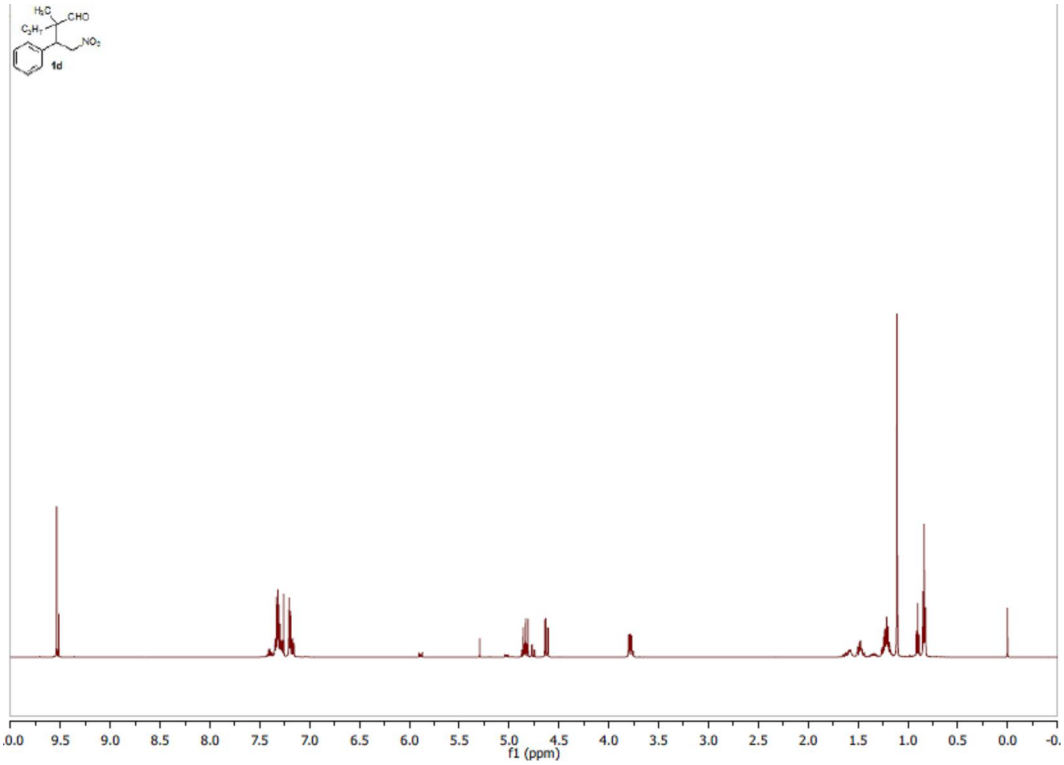
*1-(2-nitro-1-phenylethyl)cyclohexanecarbaldehyde (***1c***)*

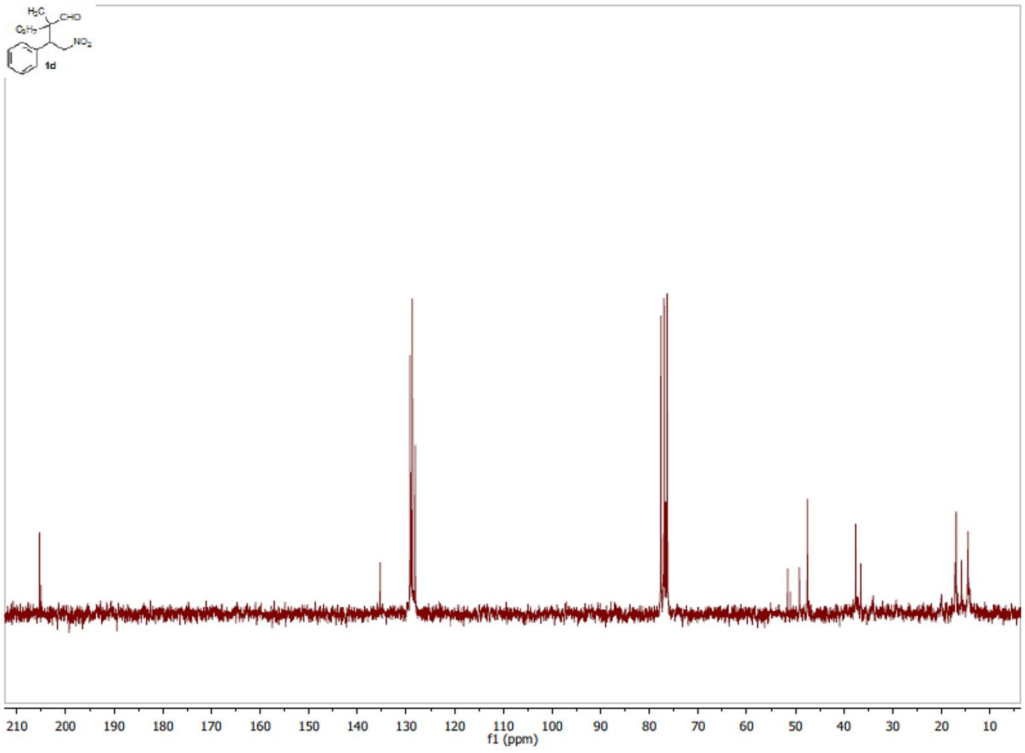




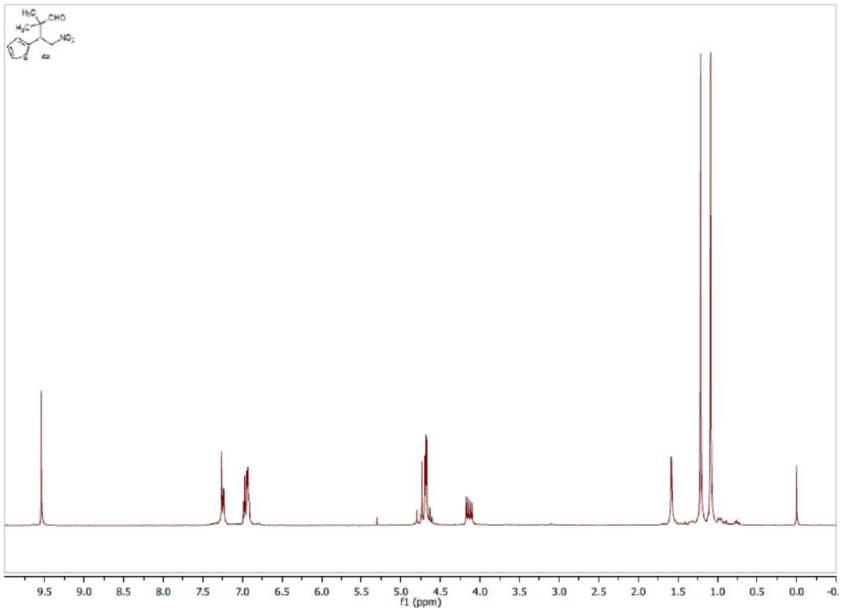


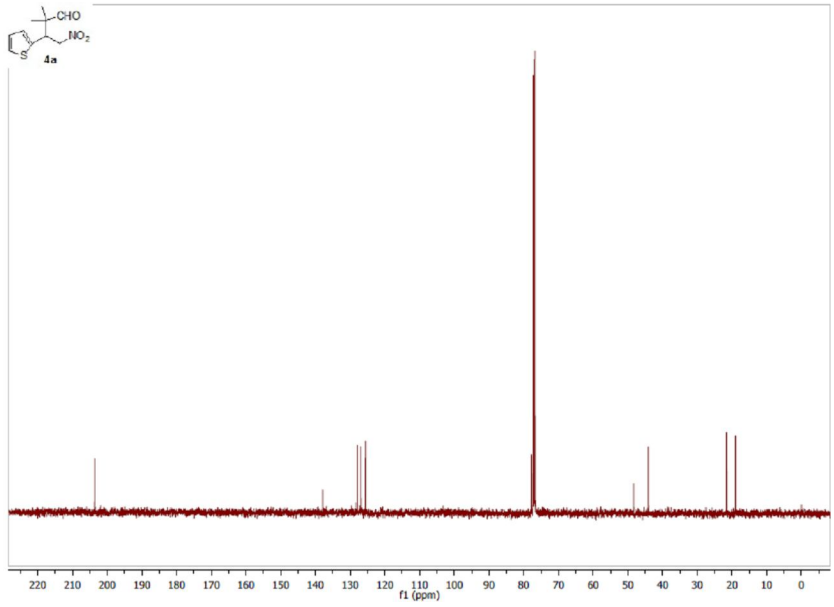
*2-methyl-2-(2-nitro-1-phenylethyl)pentanal (***1d***)*

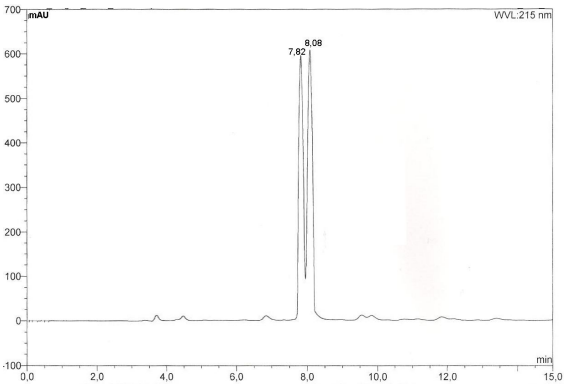




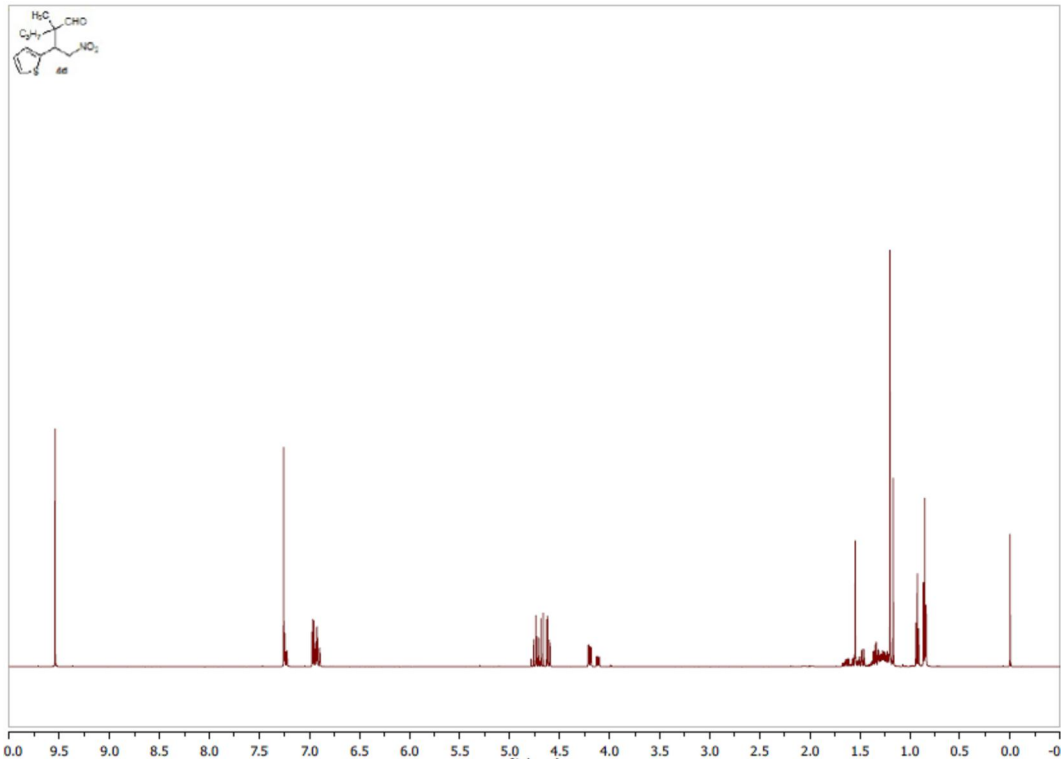
*2,2-dimethyl-4-nitro-3-(thiophen-2-yl)butanal (**4a**)*

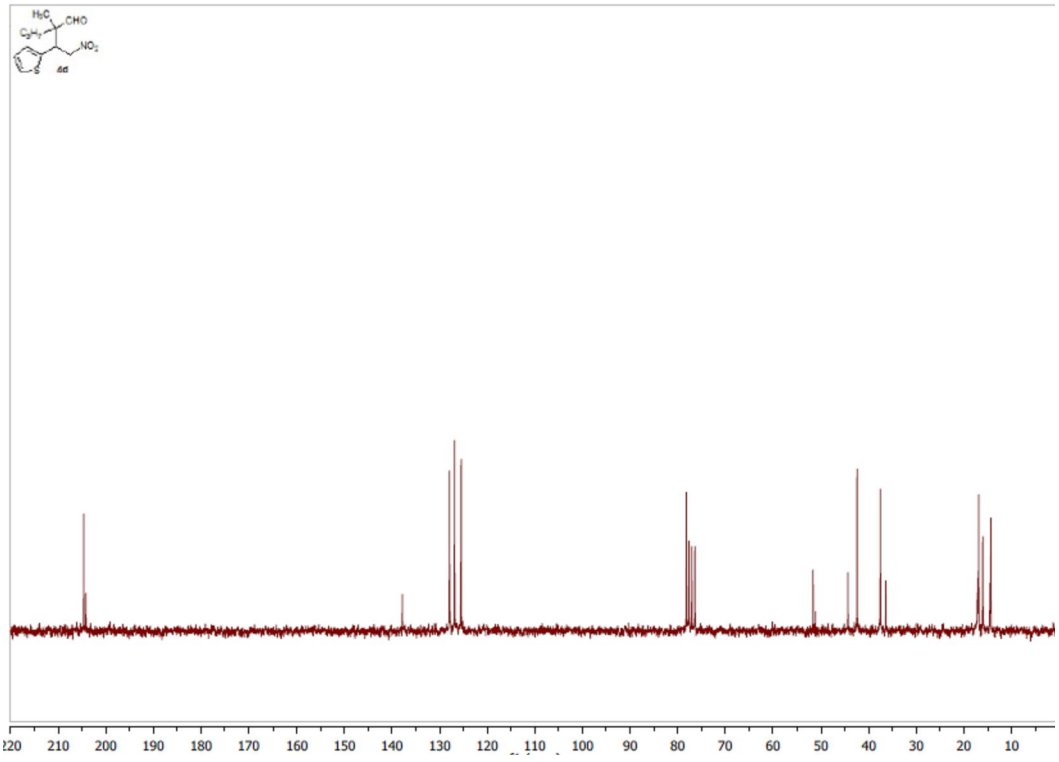




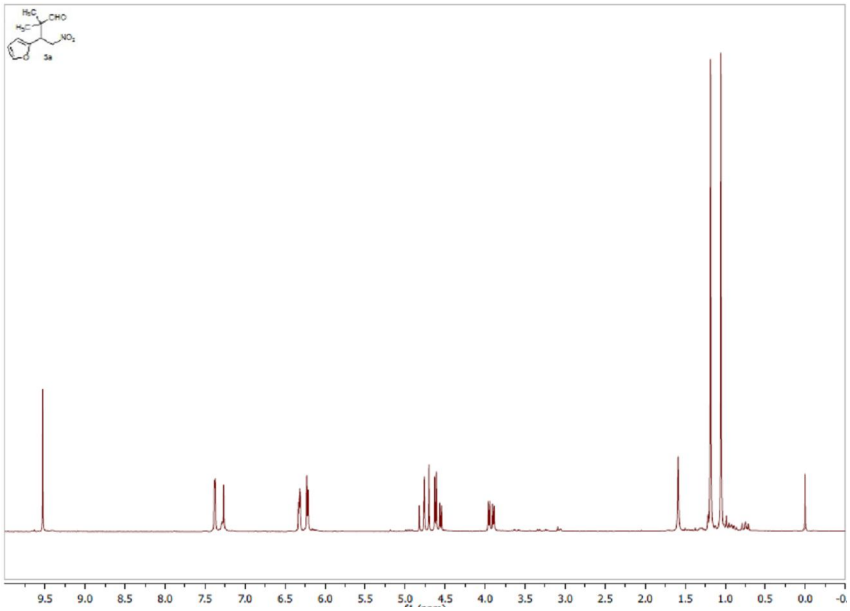


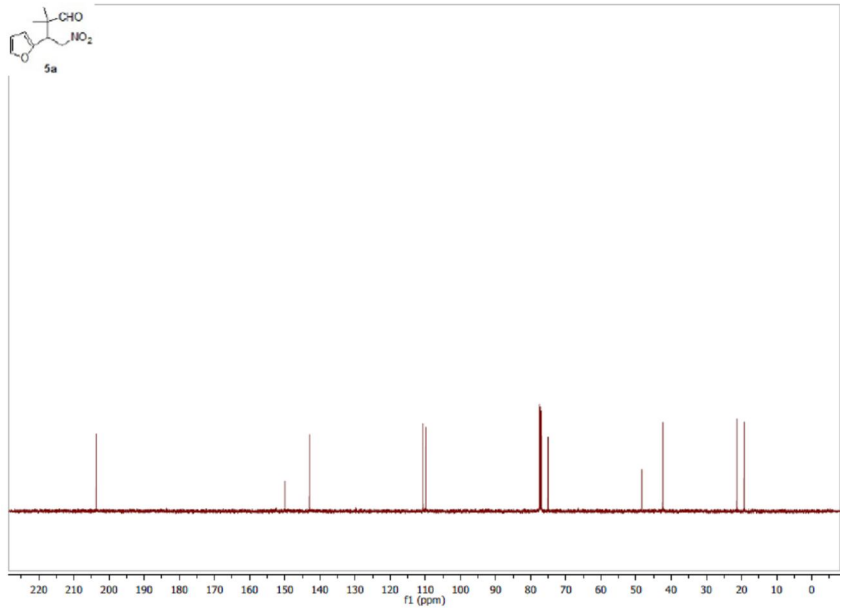
*2-methyl-2-(2-nitro-1-(thiophen-2-yl)ethyl)pentanal (**4d**)*

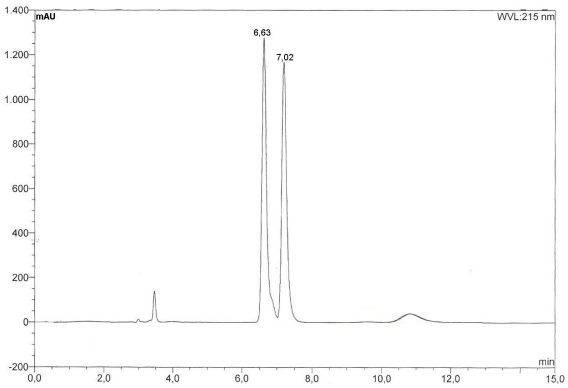




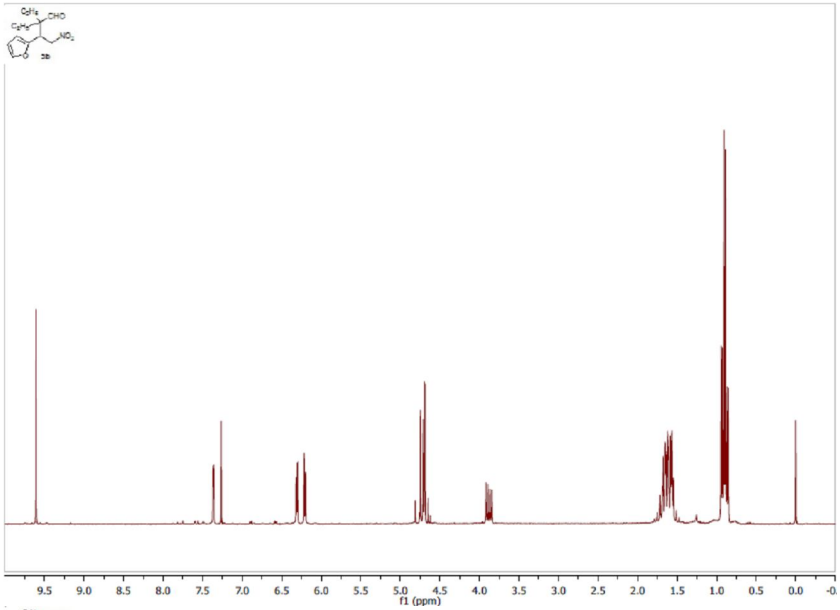
*3-(furan-2-yl)-2,2-dimethyl-4-nitrobutanal (**5a**)*

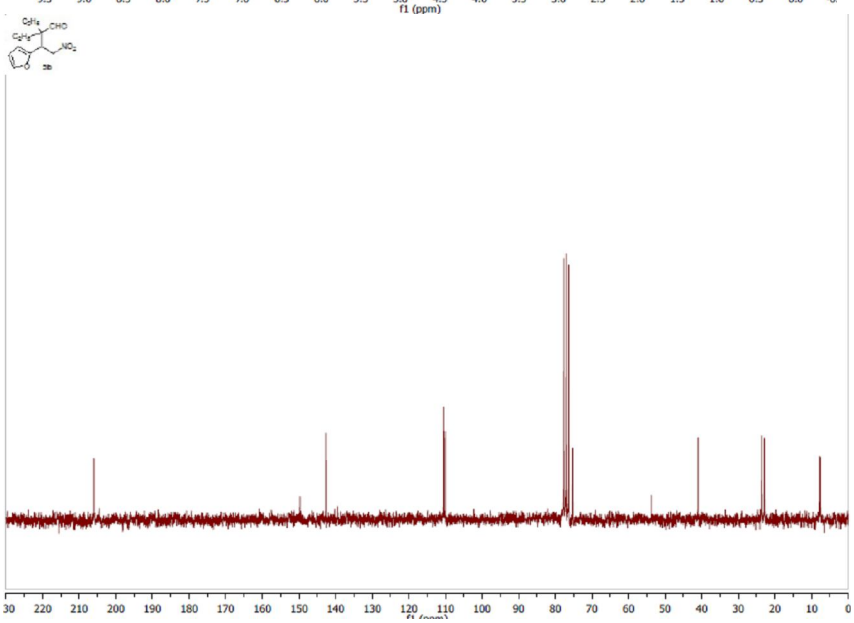


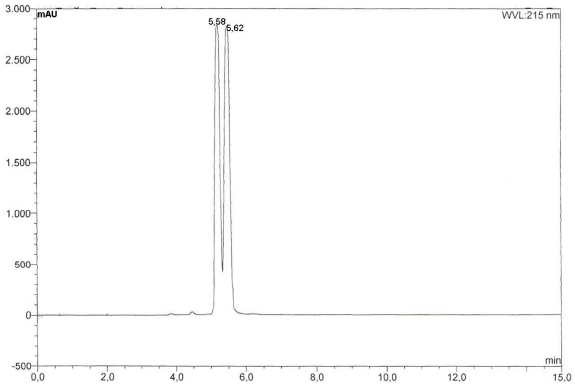




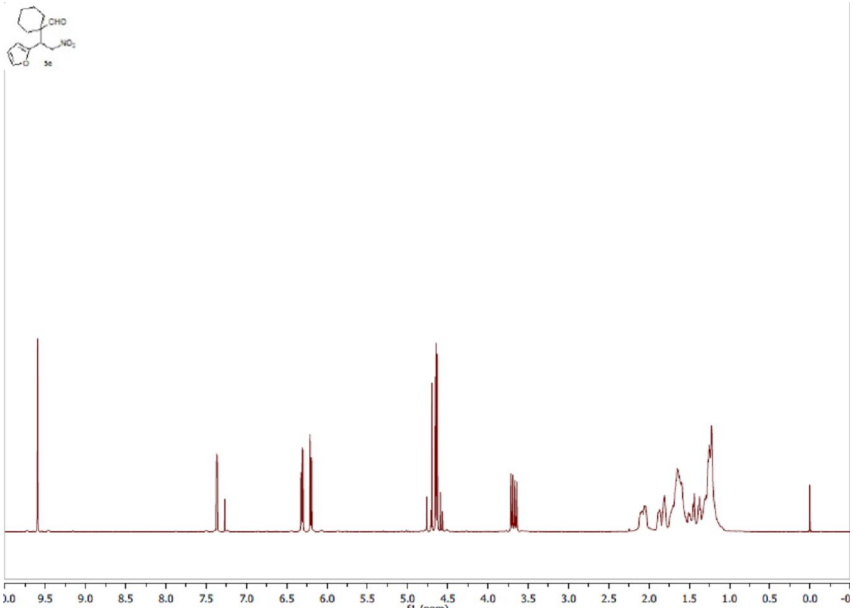
*2,2-diethyl-3-(furan-2-yl)-4-nitrobutanal (5**b**)*

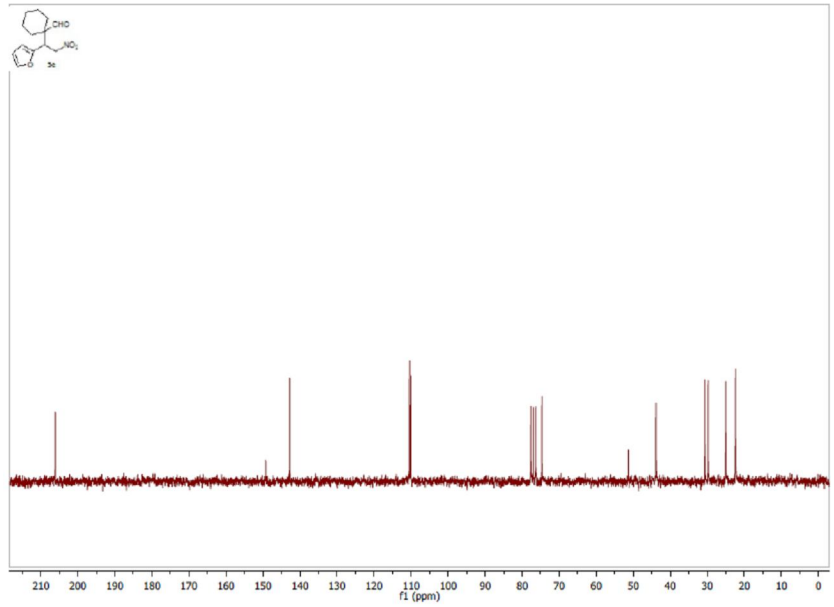


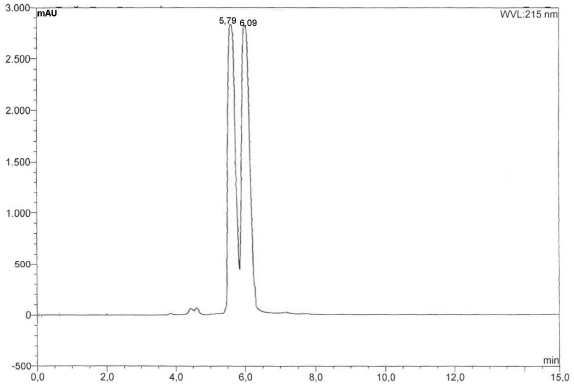




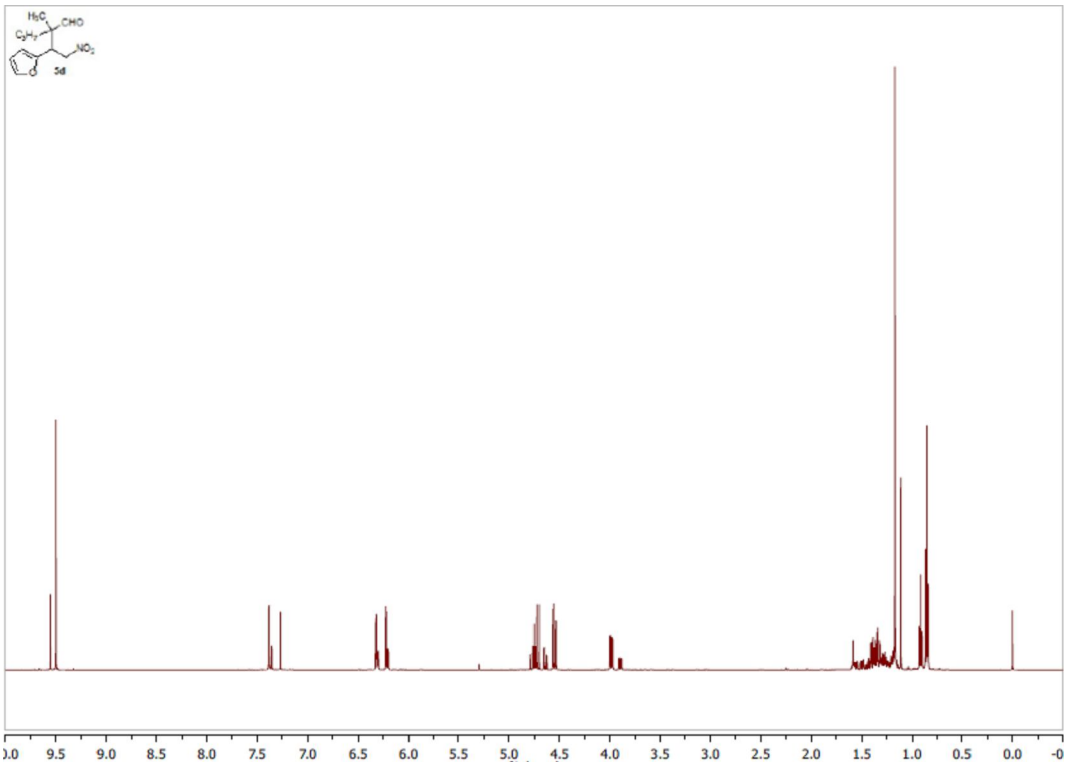
*1-(1-(furan-2-yl)-2-nitroethyl)cyclohexanecarbaldehyde (**5c**)*

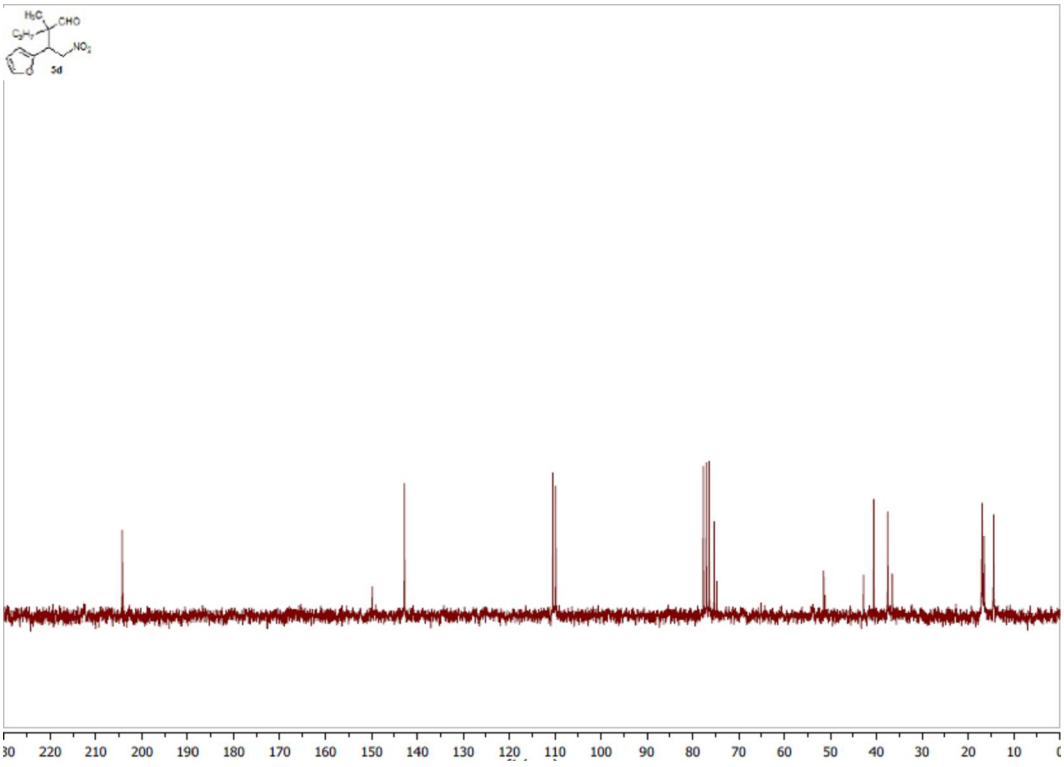






*2-(1-(furan-2-yl)-2-nitroethyl)-2-methylpentanal (**5d**)*





4. Supplementary Table 1.

Supplementary Table 1. Synthesis of **3** (2,2-dimethyl-4-nitro-3-phenylbutanal) through biotransformation of β-nitrostyrene (**1**) and isobutylaldehyde (**a**) by recombinant *E. coli* BL21(4-OT) and cell free extracts of *E. coli* BL21(4-OT).

Entry	Reaction conditions [a]	Reaction time (h)	Yield (%)	*ee* (%)
I	pH 7.2, 28°C	4	60	48
I'		8	57	50
II	pH 5.5, 28°C	4	26	46
II'		12	53	47
III	pH 7.2, 20°C	8	32	48
III'		20	61	46
IV	pH 5.5, 10°C	20	15	48
IV'		24	47	49

[a] β-nitrostyrene (**1**) was added to reaction as stock solution in 2-butanol.