Electronic Supplementary Information (ESI)

Biocatalytic Access to Nonracemic γ-Oxo Esters *via* Stereoselective Reduction Using Ene-Reductases

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1. General information

Unless stated otherwise, all solvents and commercially available reagents were used as purchased. Cyclohexane was distilled prior to use. Anhydrous toluene, dichloromethane and tetrahydrofuran were distilled over sodium, calcium hydride and sodium/potassium alloy, respectively, and stored under nitrogen on 4 Å molecular sieves.

Melting points were recorded on a Büchi M-565 melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 300 MHz NMR (75.47 MHz for ^{13}C) with chemical shifts reported relative to TMS (δ = 0.00 ppm) or a Bruker Avance 500 (125.78 MHz for ^{13}C) or Bruker Avance 400 (100.62 MHz for ^{13}C) using the residual solvent as internal standard (^{1}H: δ 7.26 ppm, $^{13}C\{^{1}H\}$: δ 77.16 ppm for $CDCl_3$, ^{1}H: δ 2.50 ppm, $^{13}C\{^{1}H\}$: δ 39.52 ppm for DMSO-d6). Chemical shifts (δ) are given in ppm and coupling constants (*J*) are quoted in Hertz (Hz). Multiplicities are described as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sex (sextet), sep (septet), br (broad singlet) and m (multiplet) or combinations thereof. Infrared (IR) spectra were recorded neat using a Shimadzu FTIR-8400s spectrophotometer and wavelengths are reported in cm^{-1}. Electrospray Ionization (ESI) high-resolution mass spectrometry (HRMS) was carried out using a Bruker microTOF-Q instrument in positive ion mode (capillary potential of 4500 V). Flash chromatography was performed on Silicycle Silia-P Flash Silica Gel (particle size 40-63 µm, pore diameter 60 Å) using the indicated eluent. Thin Layer Chromatography (TLC) was performed using TLC plates from Merck (Kieselgel 60 F_{254} neutral, on aluminium sheets with fluorescence indicator).

2. Access to enzymes

2.1. List of protein accession numbers (Uniprot)

Enzyme	Protein accession number
OYE1	**Q02899**
OYE2	**Q03558**
OYE3	**P41816**
NCR	**Q5NLA1**
YqjM	**P54550**
OPR1	**Q9XG54**
OPR3	**Q9FEW9**
XenA	**Q88NF7**
EBP1	**P43084**

2.2. Preparation of biocatalysts: typical procedure

Synthetic genes and *E. coli* BL21 DE3 cells were obtained from Invitrogen. NEB5α cells were obtained from New England Biolabs. Restriction enzymes, buffers and ligases were obtained from Thermo Fisher Scientific. For DNA extraction, the QIAGEN QIAquick gel extraction kit was used. Plasmid preparation was

performed with the QIAGEN QIAprep Spin Miniprep Kit. For enzyme purification, a HisTrap FF column (GE Healthcare, 5 mL column volume) was employed. Desalination was achieved by passing the purified enzyme through a PD-10 column (GE Healthcare).

2.2.1 Cloning

The synthetic genes (plasmid) were cloned into a pET28a(+)-vector, followed by transformation into NEB5α cells. Six overnight cultures (ONCs) were prepared and the plasmid was isolated the following day (QIAgen). Sequencing showed successful incorporation of the proper gene into the vector. Agarose-gel confirmed the insertion of the desired DNA-fragment.

2.2.2 Transformation of plasmids

To 100 µL of cell suspension (*E. coli* BL21 DE3) in 1.5 mL Eppendorf tubes, 10 µL of plasmid were added and incubated on ice for 30 min. The samples were then placed in a thermoshaker at 42 °C for 10 s. Afterwards, 250 µL of LB-medium were added and the cells were incubated in the thermomixer for 1 h at 37 °C and 300 rpm. 100 µL of the resulting product were plated on kanamycin-containing agar plates.

2.2.3 Overexpression

Colonies obtained from the transformation step were used to prepare overnight cultures (10 mL LB-medium, 50 µg/mL kanamycin). They were placed in the shaker overnight (37 °C, 120 rpm) and used on the next day to inoculate the main cultures (330 mL LB-medium per shaking flask; 3.3 mL of the ONCs, 50 µg/mL kanamycin). The main cultures were placed in the shaker at 37 °C and 140 rpm until an OD_{600} of 0.6 was reached. Then, IPTG (0.2 mM) was used for induction. The cultures were again placed in the shaker overnight at 37 °C and 140 rpm. On the next day, the cells were harvested by centrifugation (4000 rpm, 20 min, 4 °C) and the supernatant was discarded. The pellet was resuspended with cold 0.9% NaCl solution and centrifuged again (4000 rpm, 20 min, 4°C). The supernatant was discarded and the pellets were stored (-20 °C freezer) until purification was performed.

2.2.4 Enzyme purification

The pellets obtained from the overexpression were all treated as follows. Lysis buffer (Tris-HCl buffer, 50 mM, pH 7.5) was added to resuspend the pellet. The cells were then ultrasonicated (40% amplitude, 1 s pulse on, 4 s pulse off, total pulse time: 5 min), centrifuged (14000 rpm, 30 min, 4 °C). The supernatant was filtered through a 0.45 µM syringe filter and transferred into a 50 mL Sarstedt tube. Purification was performed by Ni-affinity chromatography. Wash buffer: TrisHCl (50 mM, 25 mM imidazole and 300 mM

NaCl), elution buffer: TrisHCl (50 mM, 250 mM imidazole, 300 mM NaCl). Following buffer exchange (lysis buffer), the enzyme concentration was determined by Bradford-assay. The enzymes were divided into aliquots of 800 µL and stored at -20 °C.

3. General synthetic procedures

3.1. Synthesis of substrates

Note: some substrates were found to be volatile and/or unstable over time (polymerization).

Procedure A. Wittig-Horner reaction with diketones[1]

To a solution of (alkoxycarbonylmethyl)triphenylphosphonium bromide (20 mmol, 1 equiv) in water (200 mL) was added NaOH (22 mmol, 1.1 equiv) precipitating the phosphorane as white solid. The suspension was extracted twice with CH_2Cl_2, dried over Na_2SO_4 and concentrated *in vacuo*. The resulting white powder was redissolved in CH_2Cl_2 (100 mL) and the diketone (20 mmol, 1 equiv) was added. The mixture was stirred at room temperature for 1-5 days, concentrated *in vacuo* and subjected to purification by column chromatography on silica gel.

Procedure B. Isomerization of exo- to endocylic alkenes[2]

To a solution of substrate (3 mmol, 1 equiv) in cyclohexane (30 mL) was added DBU (0.1-0.4 equiv). The solution was refluxed for 3-24 hours (until TLC indicated full consumption of starting material) and then cooled to room temperature. The product was washed with saturated NH_4Cl solution, dried over Na_2SO_4 and concentrated *in vacuo* yielding a yellow oil after purification by column chromatography on silica gel.

Procedure C. γ-Oxidation of α,β-unsaturated esters[3]

To a solution of α,β-unsaturated ester (4 mmol, 1 equiv) in CH_2Cl_2 (7 mL) cooled on an ice bath was added dropwise over 30 minutes a CrO_3 solution (11 mmol, 2.75 equiv) in acetic acid (2.6 mL) and acetic anhydride (1.25 mL). The solution stirred at room temperature for 90 minutes and then cautiously neutralized with concentrated KOH solution. The product was extracted with CH_2Cl_2, washed with water and concentrated *in vacuo* yielding a colorless oil after purification by column chromatography on silica gel.

3.2. Synthesis of product standards

Procedure D. Hydrogenation of activated alkenes

To a solution of alkene (1 mmol) in ethyl acetate (10 mL) was added 10 w% Pd/C (25% w/w to substrate). The mixture was stirred under atmospheric pressure of H_2 (balloon) at room temperature for 1-2 days. The mixture was then filtered over Celite and concentrated *in vacuo* yielding the product. If necessary, the product was purified by column chromatography on silica gel.

Procedure E. Basic hydrolysis of esters

To a solution of ester (5 mmol, 1 equiv) in a water/methanol mixture (10:1, 5 mL) was added NaOH (10 mmol, 2 equiv). The solution was stirred at room temperature for 2-24 hours before acidifying to pH = 1 with 6 M HCl. The mixture was extracted with ethyl acetate twice, dried over Na_2SO_4 and concentrated *in vacuo* to give the pure product.

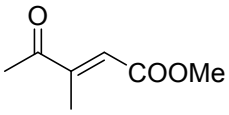
Procedure F. Esterification of carboxylic acids

To a solution of carboxylic acid (1 mmol) in ethanol or methanol (2 mL) was added a solution of thionyl chloride (1 mmol) in toluene (0.1 mL) dropwise over 30 minutes at 0-5 °C. The solution was then stirred at room temperature for 5 hours and concentrated *in vacuo*. The crude product was taken up in CH_2Cl_2, washed twice with saturated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated *in vacuo* to give the ethyl or methyl ester, respectively.

Ethyl esters were generally prepared by hydrogenation of corresponding alkenes on Pd/C (Procedure D). Methyl esters were generally prepared by esterification from corresponding carboxylic acids (Procedure F). Carboxylic acids were generally prepared by saponification of the ethyl esters (Procedure E). Racemic **9b** was purchased from Sigma Aldrich. All product standards are known compounds: **1b**,[4] **2b**,[5] **3b**,[8] **4b**,[9] **5b**,[6] **6b**,[7] **7b**,[10] **8b**,[11] **9b**,[12] **10b**,[13] **11b**,[8] **12b**,[11] **13b**.[14]

4. Characterization of substrates

Methyl 3-methyl-4-oxopent-2-enoate (*E*)- and (*Z*)-1a[15]



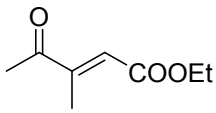
Prepared according to Procedure A from diacetyl (878 µL, 10 mmol) and methoxycarbonylmethyltriphenylphosphonium bromide (4.17 g, 10 mmol). Purification: column chromatography on silica gel (cyclohexane/ethyl acetate, 19/1); diastereoisomers could be separated [R_f = 0.22 (*E*) and R_f = 0.14 (*Z*)] and were isolated as colorless oils. Yield: 0.457 g, 0.36 mmol, 36% of combined (*E*) and (*Z*) isomers, 2.5:1 ratio. Note: The *Z* isomer isomerizes slowly to the *E*.

(*E*)-**1a***:* ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.12 (s, 3H), 2.31 (s, 3H), 3.70 (s, 3H), 6.51 (s, 1H) ppm; $^{13}C\{^1H\}$-NMR ($CDCl_3$, 125 MHz) δ 13.1 (CH_3), 26.2 (CH_3), 51.8 (CH_3), 126.0 (CH), 150.7 (C), 166.6 (C), 199.8 (C) ppm; IR (neat): vmax (cm^{-1}) =1955 (w), 1724 (s), 1682 (s), 1437 (m), 1364 (m), 1246 (s), 1196 (s), 1180 (s), 1105 (m), 1040 (m), 876 (m);

(*Z*)-**1a***:* ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.96 (s, 3H), 2.32 (s, 3H), 3.66 (s, 3H), 5.66 (s, 1H) ppm; $^{13}C\{^1H\}$-NMR ($CDCl_3$, 125 MHz) δ 20.3 (CH_3), 28.6 (CH_3), 51.8 (CH_3), 116.6 (CH), 157.7 (C), 165.7 (C), 206.3 (C) ppm; IR (neat): vmax (cm^{-1}) = 2953 (w), 1705 (s), 1643 (m), 1437 (m), 1362 (m), 1348 (m), 1248 (s), 1161 (s), 1011 (m), 858 (m);

HRMS (ESI): *m/z* calculated for $C_7H_{10}NaO_3$ $[M+Na]^+$ 165.0523, found 165.0522.

Ethyl 3-methyl-4-oxopent-2-enoate (*E*)- and (*Z*)-2a[16]



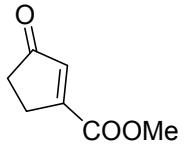
Prepared according to Procedure A from diacetyl (1.78 mL, 20 mmol) and ethoxycarbonylmethyltriphenylphosphonium bromide (8.59 g, 20 mmol). Purification: column chromatography on silica gel (cyclohexane/ethyl acetate, 15/1); diastereoisomers could be separated [R_f = 0.22 (*E*) and R_f = 0.14 (*Z*)] and were isolated as colorless oils. Yield: 2.41 g, 15.6 mmol, 78% of combined (*E*) and (*Z*) isomers, 2.5:1 ratio. Note: The *Z* isomer isomerizes slowly to the *E*.

(*E*)-**2a**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.31 (t, J = 7.0 Hz, 3H), 2.19 (s, 3H), 2.37 (s, 3H), 4.23 (q, J = 7.0 Hz, 2H), 6.56 (s, 1H); $^{13}C\{^1H\}$-NMR ($CDCl_3$, 125 MHz) δ 13.2 (CH_3), 14.3 (CH_3), 26.3 (CH_3), 60.9 (CH_2), 126.7 (CH), 150.5 (C), 166.3 (C), 200.0 (C) ppm; IR (neat): vmax (cm^{-1}) = 2981 (w), 1720 (s), 1686 (s), 1366 (m), 1246 (s), 1188 (s), 1103 (s), 1045 (m), 633 (m), 536 (s), 498 (s);

(*Z*)-**2a**: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.25 (t, J = 7.0 Hz, 3H), 1.97 (s, 3H), 2.34 (s, 3H), 4.14 (q, J = 7.0 Hz, 2H), 5.67 (s, 1H) ppm; $^{13}C\{^1H\}$-NMR ($CDCl_3$, 125 MHz) δ 14.3 (CH_3), 20.4 (CH_3), 28.8 (CH_3), 60.8 (CH_2), 117.1 (CH), 157.4 (C), 166.3 (C), 200.0 (C) ppm; IR (neat): vmax (cm^{-1}) = 2982 (w), 1720 (s), 1686 (s), 1366 (m), 1246 (s), 1188 (s), 1045 (m), 633 (m), 536 (s), 498 (s);

HRMS (ESI): *m/z* calculated for $C_8H_{12}NaO_3$ $[M+Na]^+$ 179.0679, found 179.0684.

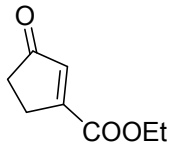
Methyl 3-oxocyclopent-1-ene-1-carboxylate 3a[20]



Prepared according to procedure C from methyl cyclopent-1-ene-1-carboxylate (0.49 mL, 4 mmol). Purification by column chromatography on silica gel (pentane/diethyl ether, 2/1). Isolated as a pale yellow solid. Yield: 230 mg, 1.6 mmol, 41% (contains traces of AcOH and Ac_2O).

m.p.: 35.0-38.5 °C. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.42-2.47 (m, 2H), 2.74-2.79 (m, 2H), 3.78 (s, 3H), 6.65 (s, 1H) ppm; $^{13}C\{^1H\}$-NMR ($CDCl_3$, 125 MHz) δ 27.4 (CH_2), 35.5 (CH_2), 52.5 (CH_3), 138.0 (CH), 163.9 (C), 164.7 (C), 209.2 (C) ppm; IR (neat): vmax (cm^{-1}) = 2959 (w), 1709 (s), 1431 (m), 1219 (s), 1161 (s), 991 (m), 737 (m), 435 (m); HRMS (ESI): *m/z* calculated for $C_7H_8NaO_3$ $[M+Na]^+$ 163.0371, found 163.0364.

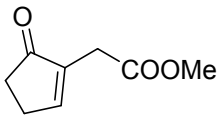
Ethyl 3-oxocyclopent-1-ene-1-carboxylate 4a[21]



Prepared according to procedure C from ethyl cyclopent-1-ene-1-carboxylate (530 mg, 3.8 mmol). Purification by column chromatography on silica gel (pentane/diethyl ether, 1/2). Isolated as a pale yellow oil. Yield: 153 mg, 1 mmol, 26%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.33 (t, J = 7.0 Hz, 3H), 2.50-2.55 (m, 2H), 2.82-2.87 (m, 2H), 4.30 (q, J = 7.0 Hz, 2H), 6.73 (t, J = 2.0 Hz, 1H) ppm; $^{13}C\{^1H\}$-NMR ($CDCl_3$, 125 MHz) δ 14.3 (CH_3), 27.5 (CH_2), 35.7 (CH_2), 61.8 (CH_2), 138.2 (CH), 164.5 (C), 209.4 (C) ppm; IR (neat): vmax (cm^{-1}) = 2982 (w), 1713 (s), 1613 (w), 1213 (s), 1161 (s), 1070 (m), 1009 (m), 741 (m), 631 (m); HRMS (ESI): *m/z* calculated for $C_8H_{12}O_3$ $[M+H]^+$ 155.0704, found 155.0703.

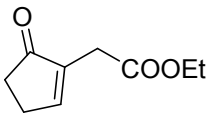
Methyl 2-(5-oxocyclopent-1-en-1-yl)acetate 5a[17]



To a solution of *exo*-**6a** (1.30 g, 7.75 mmol, 1 equiv) in methanol (16 mL) was added 0.5 mL 37% HCl. The mixture was refluxed for 18 hours and then cooled to room temperature. The mixture was neutralized by adding solid $NaHCO_3$, the salts were filtered off and the solution was concentrated *in vacuo* to give the product as a yellow oil (696 mg, 4.5 mmol, 58%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.40-2.46 (m, 2H), 2.62-2.67 (m, 2H), 3.24 (s, 2H), 3.70 (s, 3H), 7.60 (quint, J = 1.0 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 27.0 (CH_2), 30.2 (CH_2), 34.1 (CH_2), 52.2 (CH_3), 139.0 (C), 160.9 (CH), 171.1 (C), 208.6 (C) ppm; IR (neat): vmax (cm^{-1}) = 2953 (w), 1734 (s), 1697 (s), 1437 (m), 1258 (m), 1163 (m), 1001 (m), 791 (w), 633 (w), 492 (m); HRMS (ESI): *m/z* calculated for $C_8H_{10}NaO_3$ $[M+Na]^+$ 177.0523, found 177.0522.

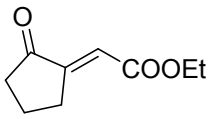
Ethyl 2-(5-oxocyclopent-1-en-1-yl)acetate *endo*-6a[18]



To a solution of *exo*-**6a** (317 mg, 1.88 mmol, 1 equiv) in ethanol (4 mL) was added 0.125 mL of 37% HCl. The mixture was refluxed for 17 hours and then cooled to room temperature. The mixture was neutralized by adding solid $NaHCO_3$, the salts were filtered off and the solution was concentrated *in vacuo* to give the product as a dark brown oil (298 mg, 1.76 mmol, 94%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.23 (t, J = 7.0 Hz, 3H), 2.40-2.48 (m, 2H), 2.62-2.68 (m, 2H), 3.22 (s, 2H), 4.16 (q, J = 7.0 Hz, 2H), 7.60 (quint, J = 1.5 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.2 (CH_3), 26.9 (CH_2), 30.4 (CH_2), 34.0 (CH_2), 61.0 (CH_2), 139.1 (C), 160.8 (CH), 170.6 (C), 208.6 (C) ppm; IR (neat): vmax (cm^{-1}) = 2982 (w), 2928 (w), 1732 (s), 1697 (s), 1258 (m), 1159 (m), 1026 (m), 1026 (m), 789 (w), 498 (w); HRMS (ESI): *m/z* calculated for $C_9H_{13}O_3$ $[M+H]^+$ 169.0860, found 169.0859.

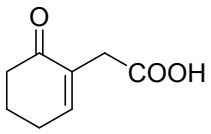
Ethyl (*E*)-2-(2-oxocyclopentylidene)acetate *exo*-6a[19]



To a solution of 1-morpholinocyclopentene (1.7 mL, 10 mmol, 1 equiv) in cyclohexane (10 mL) was added ethyl glyoxylate (50% in toluene, 2 mL, 10 mmol, 1 equiv). The mixture was refluxed using a Dean-Stark apparatus for 23 hours. After cooling to room temperature 6 M HCl (6 mL) was added and the mixture was stirred for 5 hours at room temperature. The product was extracted with toluene (two times), dried over Na_2SO_4 and concentrated *in vacuo*. The product was isolated by column chromatography on silica gel (cyclohexane/ethyl acetate, 13/1, R_f = 0.24) as a brown oil (574 mg, 3.4 mmol, 34%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.28 (t, J = 7.5 Hz, 3H), 2.00 (quint, J = 7.5 Hz, 2H), 2.39 (t, J = 7.5 Hz, 2H), 3.08 (td, J = 7.5, 3.0 Hz, 2H), 4.21 (q, J = 7.5 Hz, 2H), 6.48 (t, J = 3.0 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.3 (CH_3), 19.7 (CH_2), 29.5 (CH_2), 38.0 (CH_2), 60.9 (CH_2), 119.8 (CH), 150.9 (C), 166.5 (C), 207.5 (C) ppm; IR (neat): vmax (cm^{-1}) = 2380 (w), 1709 (s), 1369 (m), 1346 (m), 1223 (m), 1202 (s), 1173 (m), 1022 (m), 525 (m); HRMS (ESI): *m/z* calculated for $C_9H_{13}O_3$ $[M+H]^+$ 169.0860, found 169.0832.

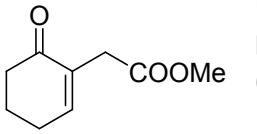
2-(6-Oxocyclohex-1-en-1-yl)acetic acid 7a[22]



Prepared according to Procedure E from *endo*-**9a** (60 mg, 0.33 mmol). Isolated as an off-white amorphous solid used without further purification. Yield: 45 mg, 0.3 mmol, 90%).

^{1}H-NMR ($CDCl_3$-DMSO-d6, 500 MHz) δ 1.64 (quint, J = 6.5 Hz, 2H), 1.95-2.10 (m, 4H), 2.73 (s, 2H), 6.51 (t, J = 4.5 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$/DMSO-d6, 125 MHz) δ 22.1 (CH_2), 25.2 (CH_2), 34.7 (CH_2), 37.2 (CH_2), 132.9 (C), 147.6 (CH), 172.3 (C), 197.5 (C) ppm; IR (neat): vmax (cm^{-1}) = 2933 (w), 1715 (s), 1695 (s), 1231 (m), 1180 (s); HRMS (ESI): *m/z* calculated for $C_8H_{10}NaO_3$ $[M+Na]^+$ 177.0522, found 177.0529.

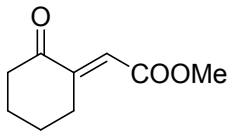
Methyl 2-(6-oxocyclohex-1-en-1-yl)acetate *endo*-**8a**[22]



Prepared according to Procedure B from *exo*-**8a** (587 mg, 3 mmol) and DBU (179 µL, 1.2 mmol). Purification: column chromatography on silica gel (cyclohexane/ethyl acetate, 9/1, R_f = 0.15). Isolated as a yellow oil. Yield: 297 mg, 1.77 mmol, 59%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.03 (quint, J = 7.0 Hz, 2H), 2.41 (q, J = 4.5 Hz, 2H), 2.47 (t, J = 7.0 Hz, 2H), 3.20 (s, 2H), 3.67 (s, 3H), 6.88 (t, J = 4.0 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 23.0 (CH_2), 26.2 (CH_2), 35.3 (CH_2), 38.1 (CH_2), 52.1 (CH_3), 133.6 (C), 148.6 (CH), 172.0 (C), 198.5 (C) ppm; IR (neat): vmax (cm^{-1}) = 2951 (w), 1736 (s), 1670 (s), 1435 (m), 1339 (m), 1258 (m), 1169 (s), 987 (w), 601 (m), 498 (m); HRMS (ESI): *m/z* calculated for $C_9H_{12}NaO_3$ $[M+Na]^+$ 191.0679, found 191.0679.

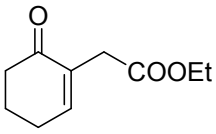
Methyl (*E*)-2-(2-oxocyclohexylidene)acetate *exo*-**8a**[23]



Prepared according to Procedure A from 1,2-cyclohexanedione (1.12 g, 10 mmol) and methoxycarbonylmethyltriphenylphosphonium bromide (4.16 g, 10 mmol). Purification: column chromatography on silica gel (cyclohexane/ethyl acetate, 19/1, R_f = 0.17). Isolated as a yellow oil. Yield: 0.91 g, 5.4 mmol, 54%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.68-1.77 (m, 2H), 1.80-1.91 (m, 2H), 2.47 (t, J = 6.5 Hz, 2H), 2.98-3.06 (m, 2H), 3.68 (s, 3H), 6.39 (t, J = 2.0 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 23.4 (two CH_2s), 28.8 (CH_2), 41.0 (CH_2), 51.6 (CH_3), 121.6 (CH), 151.7 (C), 166.5 (C), 201.1 (C) ppm; IR (neat): vmax (cm^{-1}) = 2949 (w), 1718 (s), 1693 (s), 1435 (m), 1362 (m), 1256 (m), 1196 (s), 1175 (s), 1138 (s), 1015 (m), 816 (m), 631 (m), 527 (m); HRMS (ESI): *m/z* calculated for $C_9H_{13}O_3$ $[M+H]^+$ 169.0860, found 169.0859.

Ethyl 2-(6-oxocyclohex-1-en-1-yl)acetate *endo*-**9a**[2]

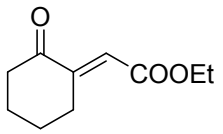


Prepared according to Procedure B from *exo*-**9a** (400 mg, 2.2 mmol) and DBU (132 µL, 0.4 mmol) and used without further purification. Isolated as a brown oil. Yield: 311 mg, 1.71 mmol, 78%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.24 (t, J = 7.0 Hz, 3H), 2.03 (quint, J = 6.5 Hz, 2H), 2.38-2.44 (m, 2H), 2.46 (t, J = 7.0 Hz, 2H), 3.18 (d, J = 1 Hz, 2H), 4.13 (q, J = 7.0 Hz, 2H), 6.87 (t, J = 3.5 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.3 (CH_3), 23.1 (CH_2), 26.2 (CH_2), 35.5 (CH_2), 38.1 (CH_2), 60.9 (CH_2), 133.7 (C), 148.4

(CH), 171.6 (C), 198.4 (C) ppm; IR (neat): vmax (cm^{-1}) = 2935 (w), 1732 (s), 1670 (s), 1369 (m), 1331 (m), 1258 (m), 1173 (s), 1030 (m), 625 (m), 463 (m); HRMS (ESI): *m/z* calculated for $C_{10}H_{14}NaO_3$ $[M+Na]^+$ 205.0835, found 205.0846.

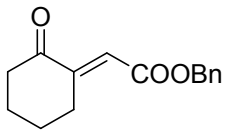
Ethyl (*E*)-2-(2-oxocyclohexylidene)acetate *exo*-**9a**[1]



Prepared according to Procedure A from 1,2-cyclohexanedione (113 mg, 1 mmol) and ethoxycarbonylmethyltriphenylphosphonium bromide (430 mg, 1 mmol). Purification: column chromatography on silica gel (cyclohexane/ethyl acetate, 19/1, R_f = 0.17). Isolated as a colorless oil. Yield: 90 mg, 0.55 mmol, 55%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.28 (t, J = 7.0 Hz, 3H), 1.7-1.82 (m, 2H), 1.87-1.95 (m, 2H), 2.52 (t, J = 7.0 Hz, 2H), 3.08 (td, J = 7.0 Hz, J = 2.0 Hz, 2H), 4.19 (q, J = 7.0 Hz, 2H), 6.46 (t, J = 2.0 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.3 (CH_3), 23.5 (CH_2), 23.6 (CH_2), 28.9 (CH_2), 41.1 (CH_2), 60.6 (CH_2), 122.3 (CH), 151.4 (C), 166.2 (C), 201.4 (C) ppm; IR (neat): vmax (cm^{-1}) = 2939 (w), 1717 (s), 1369 (m), 1184 (s), 633 (m), 532 (s), 498 (s); HRMS (ESI): *m/z* calculated for $C_{10}H_{14}NaO_3$ $[M+Na]^+$ 205.0835, found 205.0846.

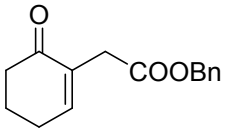
Benzyl (*E*)-2-(2-oxocyclohexylidene)acetate *exo*-**10a**



Prepared according to Procedure A from 1,2-cyclohexanedione (560 mg, 5 mmol) and benzoxycarbonylmethyltriphenylphosphonium bromide (2.95 g, 6 mmol). Purification: column chromatography on silica gel (cyclohexane/ethyl acetate, 20/1, R_f = 0.12). Isolated as a yellow oil. Yield: 950 mg, 3.9 mmol, 78%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.78 (quint, J = 5.0 Hz, 2H), 1.90 (quint, J = 6.0 Hz, 2H), 2.52 (t, J = 7.0 Hz, 2H), 3.10 (td, J = 7.0 Hz, J = 2.0 Hz, 2H), 5.18 (s, 2H), 6.52 (t, J = 2.0 Hz, 1H), 7.28-7.38 (m, 5H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 23.4 (two CH_2s), 28.9 (CH_2), 41.0 (CH_2), 66.3 (CH_2), 121.7 (CH), 128.2 (CH), 128.3 (CH), 128.6 (CH), 135.7 (C), 152.0 (C), 165.8 (C), 201.1 (C) ppm; IR (neat): vmax (cm^{-1}) = 2900 (w), 1716 (s), 1696 (s), 1685 (s), 1231 (m), 1168 (s), 737 (m), 696 (s); HRMS (ESI): *m/z* calculated for $C_{15}H_{16}NaO_3$ $[M+Na]^+$ 267.0992, found 267.0985.

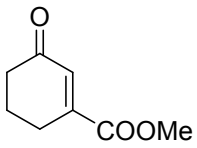
Benzyl 2-(6-oxocyclohex-1-en-1-yl)acetate *endo*-**10a**



Prepared according to Procedure B from *exo*-**10a** (500 mg, 2 mmol) and DBU (60 µL, 0.4 mmol). Purification by column chromatography on silica gel (cyclohexane/ethyl acetate, 8/1, R_f = 0.25). Isolated as a yellow oil. Yield: 400 mg, 1.6 mmol, 80%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.02 (quint, J = 7.0 Hz, 2H), 2.39 (q, J = 5.0 Hz, 2H), 2.46 (t, J = 5.0 Hz, 2H), 3.24 (s, 2H), 5.11 (s, 2H), 6.86 (t, J = 4.0 Hz, 1H), 7.28-7.38 (m, 5H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 23.0 (CH_2), 26.1 (CH_2), 35.5 (CH_2), 38.0 (CH_2), 66.6 (CH_2), 128.2 (CH), 128.3 (CH), 128.6 (CH), 133.5 (C), 136.0 (C), 148.6 (CH), 171.3 (C), 198.3 (C) ppm; IR (neat): vmax (cm^{-1}) = 2940 (w), 1743 (s), 1730 (s), 1684 (s), 1231 (m), 1163 (s), 745 (m), 698 (m); HRMS (ESI): *m/z* calculated for $C_{15}H_{16}NaO_3$ $[M+Na]^+$ 267.0992, found 267.0982.

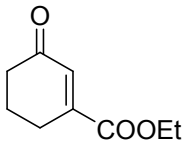
Methyl 3-oxocyclohex-1-ene-1-carboxylate 11a[3]



Prepared according to Procedure C from methyl cyclohex-1-ene-1-carboxylate (1 g, 7.1 mmol). Purification by column chromatography on silica gel (cyclohexane/ethyl acetate, 4/1, R_f = 0.26). Isolated as a yellow oil. Yield: 600 mg, 3.9 mmol, 55%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.97 (quint, J = 7.0 Hz, 2H), 2.34 (t, J = 7.0 Hz, 2H), 2.45-2.53 (m, 2H), 3.73 (s, 3H), 6.61 (s, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 22.0 (CH_2), 24.7 (CH_2), 37.6 (CH_2), 52.5 (CH_3), 132.9 (CH), 148.7 (C), 166.8 (C), 199.9 (C) ppm; IR (neat): νmax (cm^{-1}) = 2953 (w), 1721 (s), 1682 (s), 1435 (m), 1250 (s), 1225 (s), 1076 (m), 1043 (m), 743 (m), 727 (m); HRMS (ESI): *m/z* calculated for $C_{18}H_{10}NaO_3$ $[M+Na]^+$ 177.0521, found 177.0522.

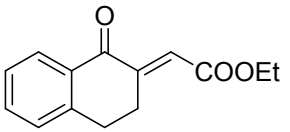
Ethyl 3-oxocyclohex-1-ene-1-carboxylate 12a[24]



Prepared according to Procedure C from ethyl cyclohex-1-ene-1-carboxylate (325 mg, 2.1 mmol). Purification by column chromatography on silica gel (cyclohexane/ethyl acetate, 9/1, R_f = 0.19). Isolated as a pale yellow oil. Yield: 184 mg, 1.1 mmol, 52%.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.26 (t, J = 7.0 Hz, 3H), 2.00 (quint, J = 7.0 Hz, 2H), 2.37 (t, J = 7.0 Hz, 2H), 2.51 (td, J = 7.0 Hz, J = 1.0 Hz, 2H), 4.20 (q, J = 7.0 Hz, 2H), 6.65 (t, J = 1.0 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.1 (CH_3), 22.1 (CH_2), 24.8 (CH_2), 37.7 (CH_2), 61.6 (CH_2), 132.8 (CH), 149.2 (C), 166.4 (C), 200.1 (C) ppm; IR (neat): νmax (cm^{-1}) = 2955 (w), 1717 (s), 1682 (s), 1250 (s), 1223 (s), 1076 (m), 1042 (m), 964 (m), 741 (m), 633 (m), 498 (s); HRMS (ESI): *m/z* calculated for $C_9H_{13}O_3$ $[M+H]^+$ 169.0865, found 169.0845.

Ethyl (*E*)-2-(1-oxo-3,4-dihydronaphthalen-2(1*H*)-ylidene)acetate 14a

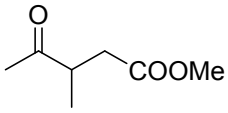


To a solution of *α*-tetralone (1.35 mL, 10 mmol) in toluene (50 mL) were added $MgSO_4$ (8 g), ethyl glyoxylate (50% solution in toluene, 2 mL, 10 mmol) and *p*-toluenesulfonic acid monohydrate (190 mg, 1 mmol). The mixture was refluxed for 15 hours. After cooling, $MgSO_4$ was filtered off. The solution was diluted with ethyl acetate and washed with water and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The product was isolated by column chromatography on silica gel (cyclohexane/ethyl acetate, 13/1) as a purple solid (1.77g, 7.7 mmol, 77%). m.p.: 104-107.5 °C.

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.34 (t, J = 7.5 Hz, 3H), 3.03 (t, J = 6.5 Hz, 2H), 3.44 (td, J = 6.5 Hz, J = 1.5 Hz, 2H), 4.26 (q, J = 7.5 Hz, 2H), 6.90 (t, J = 1.5 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H), 7.37 (t, J = 7.5 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 8.10 (t, J = 7.5 Hz, 1H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.4 (CH_3), 27.2 (CH_2), 28.7 (CH_2), 60.8 (CH_2), 123.7 (CH), 127.4 (CH), 128.6 (CH), 128.6 (CH), 132.7 (C), 134.1 (CH), 144.3 (C), 149.7 (C), 166.4 (C), 187.1 (C) ppm; IR (neat): νmax (cm^{-1}) = 2974 (w), 1705 (s), 1666 (m), 1593 (m), 1292 (s), 1242 (s), 1161(s), 903 (m), 744 (s); HRMS (ESI): *m/z* calculated for $C_{14}H_{14}NaO_3$ $[M+Na]^+$ 239.0679, found 239.0682.

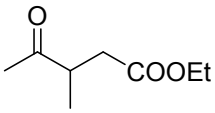
5. Characterization of products

Methyl 3-methyl-4-oxopentanoate *rac*-**1b**[4]



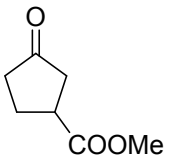
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.12 (d, J = 7.0 Hz, 3H), 1.21 (m, 3H), 2.20 (s, 3H), 2.27 (dd, J = 17.0 Hz, J = 5.0 Hz, 1H), 2.74 (dd, J = 17.0 Hz, J = 8.5 Hz, 1H), 2.93-3.03 (m, 1H), 3.63 (s, 3H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 16.6 (CH_3), 28.5 (CH_3), 36.7 (CH_2), 42.8 (CH), 51.8 (CH_3), 172.9 (C), 210.9 (C) ppm.

Ethyl 3-methyl-4-oxopentanoate *rac*-**2b**[5]



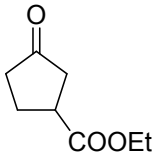
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.11 (d, J = 7.0 Hz, 3H), 1.21 (t, J = 7.0 Hz, 3H), 2.18 (s, 3H), 2.25 (dd, J = 17.0 Hz, J = 5.0 Hz, 1H), 2.71 (dd, J = 17.0 Hz, J = 8.5 Hz, 1H), 2.92-3.02 (m, 1H), 4.07 (q, J = 7.0 Hz, 2H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.2 (CH_3), 16.6 (CH_3), 28.5 (CH_3), 37.0 (CH_2), 42.8 (CH), 60.5 (CH_2), 172.4 (C), 210.9 (C) ppm.

Methyl 3-oxocyclopentane-1-carboxylate *rac*-**3b**[8]



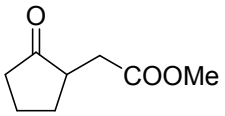
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.13-2.51 (m, 6H), 3.13 (quint, J = 8.0 Hz, 1H), 3.73 (s, 3H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 26.7 (CH_2), 37.6 (CH_2), 40.9 (CH_2), 41.3 (CH), 52.3 (CH_3), 174.9 (C), 216.7 (C) ppm.

Ethyl 3-oxocyclopentane-1-carboxylate *rac*-**4b**[9]



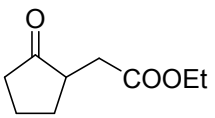
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.27 (t, J = 7.0 Hz, 3H), 2.12-2.50 (m, 6H), 3.11 (quint, J = 8.0 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.3 (CH_3), 26.7 (CH_2), 37.6 (CH_2), 41.1 (CH), 41.3 (CH_2), 61.2 (CH_2), 174.4 (C), 216.9 (C) ppm.

Methyl 2-(2-oxocyclopentyl)acetate *rac*-**5b**[6]



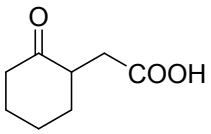
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.55-1.65 (m, 1H), 1.75-1.87 (m, 1H), 2.02-2.10 (m, 1H), 2.11-2.23 (m, 1H), 2.29-2.44 (m, 4H), 2.68-2.80 (m, 1H), 3.68 (s, 3H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 20.7 (CH_2), 29.5 (CH_2), 33.9 (CH_2), 37.6 (CH_2), 45.8 (CH), 51.9 (CH_3), 172.8 (C) ppm. Note: the cyclopentanone C=O appears at δ >217 ppm.

Ethyl 2-(2-oxocyclopentyl)acetate *rac*-**6b**[7]



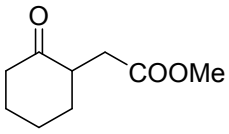
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.21 (t, J = 7.0 Hz, 3H), 1.53-1.64 (m, 1H), 1.70-1.83 (m, 1H), 1.96-2.06 (m, 1H), 2.08-2.18 (m, 1H), 2.20-2.44 (m, 4H), 2.63-2.71 (m, 1H), 4.08 (q, J = 7.0 Hz, 2H) ppm; ^{13}C{^{1}H}-NMR ($CDCl_3$, 125 MHz) δ 14.2 (CH_3), 20.7 (CH_2), 29.3 (CH_2), 34.0 (CH_2), 37.5 (CH_2), 45.6 (CH), 60.7 (CH_2), 172.1 (C) ppm. Note: the cyclopentanone C=O appears at δ >217 ppm.

2-(2-Oxocyclohexyl)acetic acid *rac*-**7b**[10]



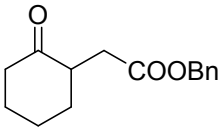
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.41 (qd, J = 13.5 Hz, 2.0 Hz, 1H), 1.55-1.80 (m, 2H), 1.87 (d, J = 13.5 Hz, 1H), 2.03-2.25 (m, 3H), 2.30-2.48 (m, 2H), 2.74-2.88 (m, 2H), 11.31 (br, 1H) ppm; $^{13}C\{^{1}H\}$-NMR ($CDCl_3$, 125 MHz) δ 25.3 (CH_2), 27.9 (CH_2), 33.9 (CH_2), 34.4 (CH_2), 41.9 (CH_2), 47.0 (CH), 178.7 (C), 211.3 (C) ppm.

Methyl 2-(2-oxocyclohexyl)acetate *rac*-**8b**[11]



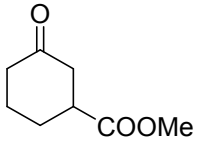
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.36 (qd, J = 13.5 Hz, 2.0 Hz, 1H), 1.51-1.73 (m, 2H), 1.82 (d, J = 13.5 Hz, 1H), 2.03-2.14 (m, 3H), 2.25-2.38 (m, 2H), 2.71 (dd, J = 16.5 Hz, J = 7.5 Hz, 1H), 2.81 (sex, J = 7.0, 1H), 3.60 (s, 3H) ppm; $^{13}C\{^{1}H\}$-NMR ($CDCl_3$, 125 MHz) δ 25.1 (CH_2), 27.8 (CH_2), 33.9 (CH_2), 34.2 (CH_2), 41.8 (CH_2), 47.0 (CH), 51.6 (CH_3), 173.0 (C), 211.0 (C) ppm.

Benzyl 2-(2-oxocyclohexyl)acetate *rac*-**10b**[13]



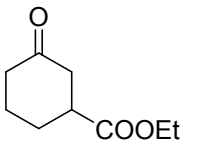
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.40 (qd, J = 13.5, 2.0 Hz, 1H), 1.55-1.80 (m, 2H), 1.80-1.93 (m, 1H), 2.03-2.26 (m, 3H), 2.28-2.48 (m, 2H), 2.74-2.95 (m, 2H), 5.11 (s, 2H), 7.26-7.40 (m, 5H) ppm; $^{13}C\{^{1}H\}$-NMR ($CDCl_3$, 125 MHz) δ 25.3 (CH_2), 27.9 (CH_2), 34.0 (CH_2), 34.4 (CH_2), 41.9 (CH_2), 47.1 (CH), 66.3 (CH_2), 128.1 (CH), 128.2 (CH), 128.5 (CH), 136.0 (C), 172.6 (C), 211.0 (C) ppm.

Methyl 3-oxocyclohexane-1-carboxylate *rac*-**11b**[8]



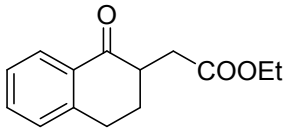
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.67-1.78 (m, 1H), 1.85 (qd, J = 12.0 Hz, 3.5 Hz, 1H), 2.01-2.16 (m, 2H), 2.27-2.42 (m, 2H), 2.55 (d, J = 8.0 Hz, 2H), 2.75-2.86 (m, 1H), 3.71 (s, 3H) ppm; $^{13}C\{^{1}H\}$-NMR ($CDCl_3$, 125 MHz) δ 24.6 (CH_2), 27.9 (CH_2), 41.0 (CH_2), 43.1 (CH), 43.2 (CH_2), 52.3 (CH_3), 174.3 (C), 209.4 (C) ppm.

Ethyl 3-oxocyclohexane-1-carboxylate *rac*-**12b**[11]



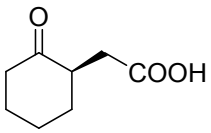
^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.26 (t, J = 7.0 Hz, 3H), 1.67-1.75 (m, 1H), 1.76-1.88 (m, 1H), 2.02-2.15 (m, 2H), 2.25-2.40 (m, 2H), 2.54 (d, J = 8.0 Hz, 2H), 2.74-2.82 (m, 1H), 4.15 (q, J = 7.0 Hz, 2H) ppm; $^{13}C\{^{1}H\}$-NMR ($CDCl_3$, 125 MHz) δ 14.3 (CH_3), 24.6 (CH_2), 27.9 (CH_2), 41.1 (CH_2), 43.3 (CH_2), 43.4 (CH), 61.0 (CH_2), 173.9 (C), 209.5 (C) ppm.

Ethyl 2-(1-oxo-1,2,3,4-tetrahydronaphthalen-2-yl)acetate *rac*-**14b**[14]



^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.28 (t, J = 7.5 Hz, 3H), 1.97 (dq, J = 13.0 Hz, J = 4.0 Hz, 1H), 2.25 (dd, J = 9.0 Hz, J = 4.0 Hz, 1H), 2.42 (dd, J = 6.5 Hz, J = 15.5 Hz, 1H), 2.96-3.13 (m, 4H), 4.19 (q, J = 7.5 Hz, 2H), 7.24 (d, J = 7.5 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.47 (t, J = 7.5 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H) ppm; $^{13}C\{^{1}H\}$-NMR ($CDCl_3$, 125 MHz) δ 14.4 (CH_3), 29.4 (CH_2), 35.3 (CH_2), 44.9 (CH), 60.7 (CH_2), 126.8 (CH), 127.6 (CH), 128.9 (CH), 132.3 (C), 133.5 (CH), 144.1 (C), 172.7 (C), 198.6 (C) ppm.

(*R*)-2-(2-Oxocyclohexyl)acetic acid (*R*)-7b



To a solution of **10b** obtained from scale up of thew bioreduction of *endo*-**10a** with NCR (90 mg, 0.36 mmol) in ethyl acetate (1.5 mL) was added 10 w% Pd/C (45 mg, 50% w/w). The mixture was stirred under atmospheric pressure of H_2 (balloon) at room temperature for 21 h. The mixture was then filtered over Celite (the filter thoroughly washed with ethyl acetate) and concentrated *in vacuo* yielding the product of sufficient purity.

Isolated as an off-white solid. Yield: 49 mg, 0.31 mmol, 70%. $[\alpha]_D^{20}$ = +36.7 (c = 1.8, $CHCl_3$). NMR as described above.

The *ee* (85% for (*R*)-**7b**) was determined by chiral GC upon derivatization to **8b** with $TMSCHN_2$ in MeOH-$CHCl_3$. The compound most likely epimerizes slowly.

6. Interpretation of isotopic labeling experiments

Reduction of 2a

In the ^{1}H-NMR spectrum of product **2b** obtained in non-deuterated medium, the proton at Cα of the ketone carbonyl gives a typical signal at 2.92-3.02 ppm (multiplet), while the methyl group attached to that Cα gives an expected doublet at 1.11 pm. The two protons on Cβ (i.e. Cα of the ester moiety) give two distinguishable signals (doublet of doublets) at 2.25 and 2.71 ppm, respectively.

Upon reduction of (*Z*)-**2a** by NCR in D_2O yielding (*R*)-**2b**, the multiplet at 2.92-3.02 ppm disappears while the doublet at 1.11 pm is replaced by a singlet, which is a strong evidence that the proton at Cα has been replaced by deuterium. At the same time, the dd signals of both protons on Cβ are now doublets, confirming insertion of D at Cα of the ketone carbonyl, which is the binding group in NCR.

Bioreduction of (*E*)-**2a** by NCR at the expense of NADD forming (*S*)-**2b** resulted in a complex ^{1}H-NMR spectrum, despite full conversion and purification of the product. Importantly, the doublet at 1.11 pm corresponding to the methyl group on Cα is still present, indicating the presence of a proton on that carbon. The corresponding signal of that proton clearly appears as a quintuplet, indicating that the neighboring carbon Cβ now bears a single proton. Additionally, the dd signal at 2.25 ppm corresponding to one of the protons on Cβ has disappeared. All this indicates that D has been incorporated on Cβ to the ketone carbonyl, which remains the binding group of (*E*)-**2a** in NCR. The ^{13}C NMR spectrum supports this conclusion as it shows a splitting of the Cβ signal (37.0 ppm), a typical sign of a deuterium being present on a carbon atom.

Reduction of 3a

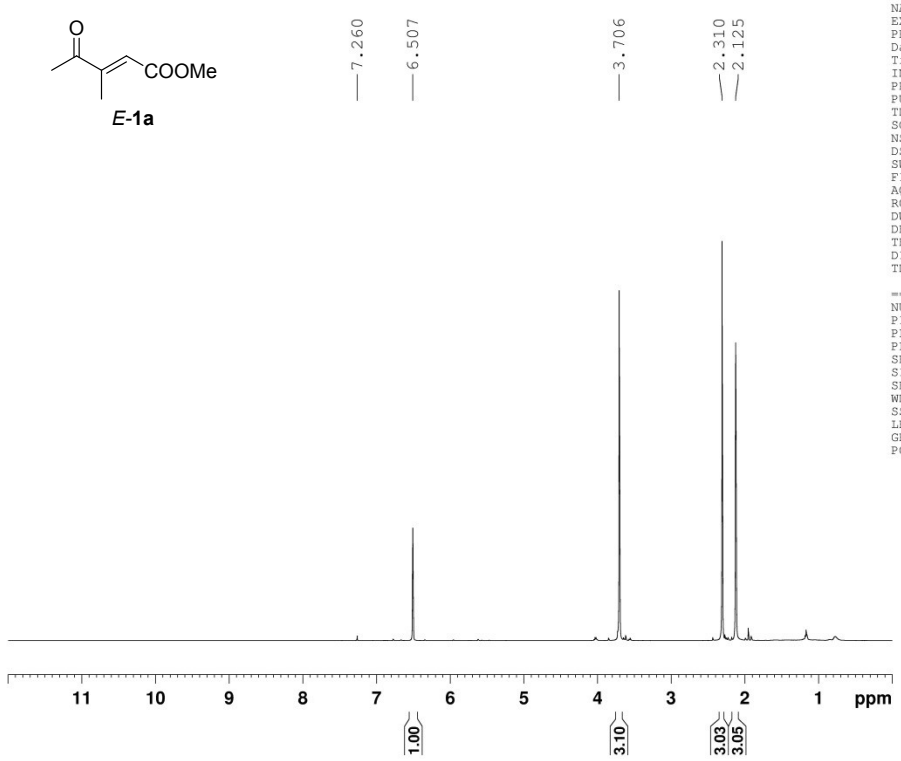
For product **3b**, the ^{1}H-NMR spectrum of a non-deuterated sample displays a typical signal for the proton on the most substituted carbon (Cβ to the ketone carbonyl) as a multiplet at 3.07-3.18 ppm. Upon reduction of **3a** at the expense of NADD by OYE2 to (*S*)-**3b** and by XenA to (*R*)-**3b**, a major decrease in the abundance of that signal is observed, without change of the shape, indicating that this position has been

deuterated, and possibly that exchange with the solvent (H_2O) occurred (incorporation of D at Cα would have modified the shape of that signal). Furthermore, in ^{13}C NMR, splitting of the Cβ signal at position 40.9 ppm indicates incorporation of deuterium at that position. In both cases, the ketone carbonyl is activating. The HSQC-spectrum also clearly shows disappearance of the coupling between the Cβ (40.9 ppm) and the corresponding H (replaced by D).

Reduction of 5a

(*R*)-**5b** was obtained from reduction of **5a** by NCR in D_2O. Analysis of the resulting ^1H-NMR spectrum indicates a change in the shape and integral of signals at 2.71-2.76 ppm and 2.28-2.55 ppm. However, overlap with the signal of the proton on Cα to the ketone carbonyl, which gives a multiplet under non-deuterating conditions, does not allow integration of the signals, which were significantly altered. This indicates (partial) incorporation of D at Cα and thus suggests that the ketone carbonyl is the binding group. In addition, the carbon signal in ^{13}C NMR of Cα at 45.7 ppm shows a splitting and reduced intensity, indicating incorporation of a deuterium atom. Finally, the HSQC-spectrum clearly shows no coupling of the Cα (45 ppm) to any hydrogen (the signal at {2.4,45} ppm has disappeared). The ketone carbonyl is the sole binding group.

7. NMR spectra

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PROCNO                  1
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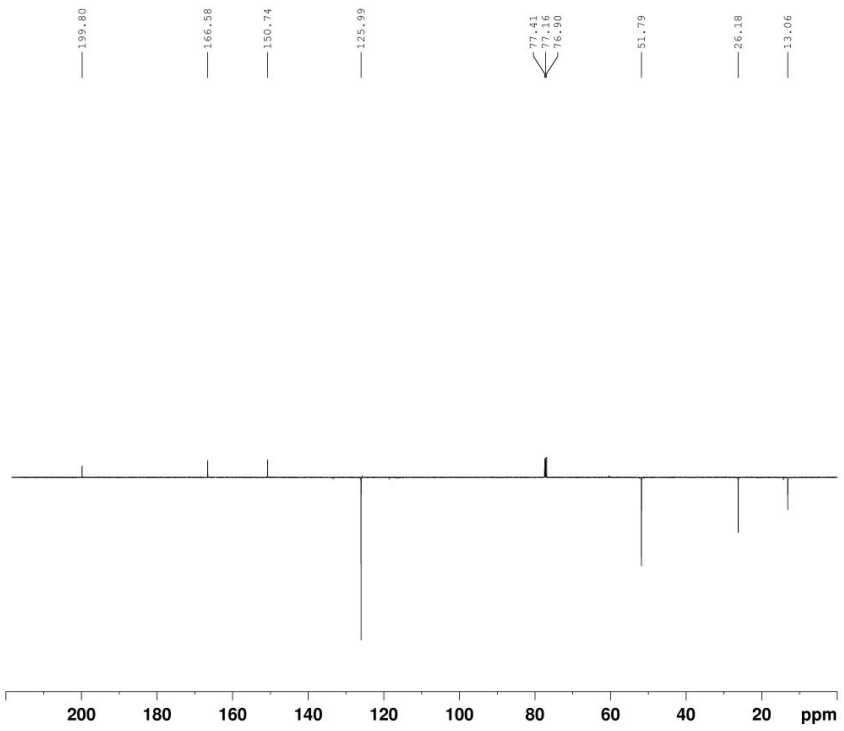
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH             10330.578 Hz
FIDRES          0.157632 Hz
AQ              3.1719923 sec
RG                      9
DW                 48.400 usec
DE                  6.50 usec
TE                 293.2 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                   6.70 usec
PL1                  4.00 dB
PL1W          8.72000027 W
SFO1         500.2330891 MHz
SI                  32768
SF           500.2300101 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

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NAME        DNT_223E_pure
EXPNO                   2
PROCNO                  1
Date_            20140222
Time                12.12
INSTRUM             spect
PROBHD    5 mm CPTCI 1H-
PULPROG              jmod
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH             29761.904 Hz
FIDRES          0.454131 Hz
AQ              1.1010548 sec
RG                   2050
DW                 16.800 usec
DE                  6.50 usec
TE                 293.2 K
CNST2         145.0000000
CNST11          1.0000000
D1              2.00000000 sec
D20             0.00689655 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                  11.20 usec
P2                  22.40 usec
PL1                 -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2               80.00 usec
PL2                  4.00 dB
PL12                25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2         500.2320009 MHz
SI                  32768
SF           125.7829265 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
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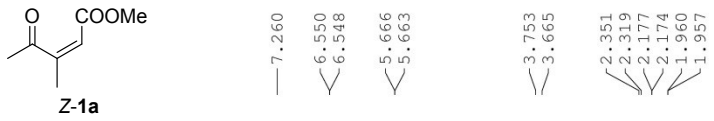

Z-**1a**

Contains 7% of the *E*-isomer

```
NAME        DNT_223Z_pure
EXPNO               4
PROCNO              1
Date_        20140224
Time            20.42
INSTRUM         spect
PROBHD   5 mm CPTCI 1H-
PULPROG          zg30
TD              65536
SOLVENT         CDCl3
NS                 16
DS                  2
SWH         10330.578 Hz
FIDRES       0.157632 Hz
AQ           3.1719923 sec
RG                 11.3
DW              48.400 usec
DE               6.50 usec
TE              293.1 K
D1           1.00000000 sec
TD0                 1

======== CHANNEL f1 ========
NUC1               1H
P1              6.70 usec
PL1             4.00 dB
PL1W       8.72000027 W
SFO1      500.2330891 MHz
SI             32768
SF        500.2300105 MHz
WDW               EM
SSB                0
LB              0.30 Hz
GB                 0
PC              1.00
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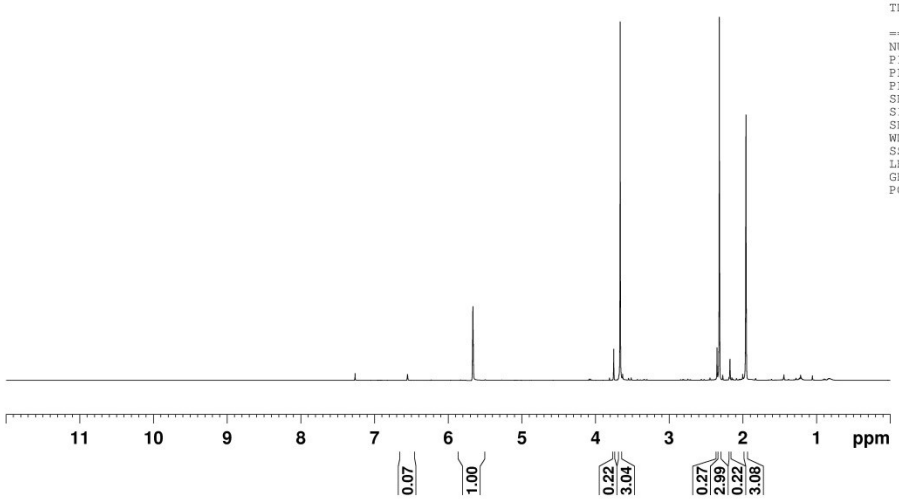


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NAME        DNT_223Z_pure
EXPNO               2
PROCNO              1
Date_        20140222
Time            12.35
INSTRUM         spect
PROBHD   5 mm CPTCI 1H-
PULPROG          jmod
TD              65536
SOLVENT         CDCl3
NS                256
DS                  4
SWH         29761.904 Hz
FIDRES       0.454131 Hz
AQ           1.1010548 sec
RG                2050
DW              16.800 usec
DE               6.50 usec
TE              293.2 K
CNST2       145.0000000
CNST11        1.0000000
D1           2.00000000 sec
D20          0.00689655 sec
TD0                 1

======== CHANNEL f1 ========
NUC1               13C
P1             11.20 usec
P2             22.40 usec
PL1             -2.00 dB
PL1W       88.77790070 W
SFO1      125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2               1H
PCPD2           80.00 usec
PL2              4.00 dB
PL12            25.28 dB
PL2W        8.72000027 W
PL12W       0.06494062 W
SFO2      500.2320009 MHz
SI             32768
SF        125.7829244 MHz
WDW               EM
SSB                0
LB              1.00 Hz
GB                 0
PC              1.40
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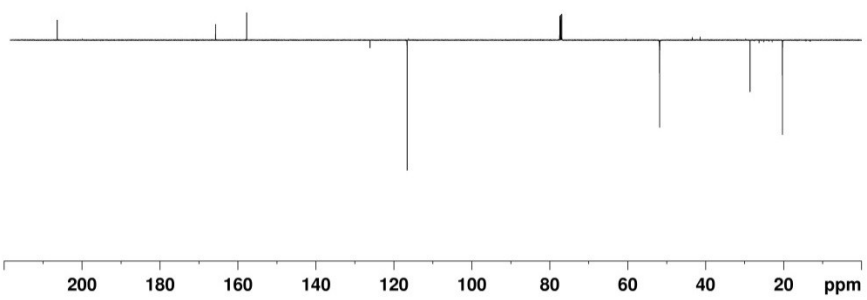

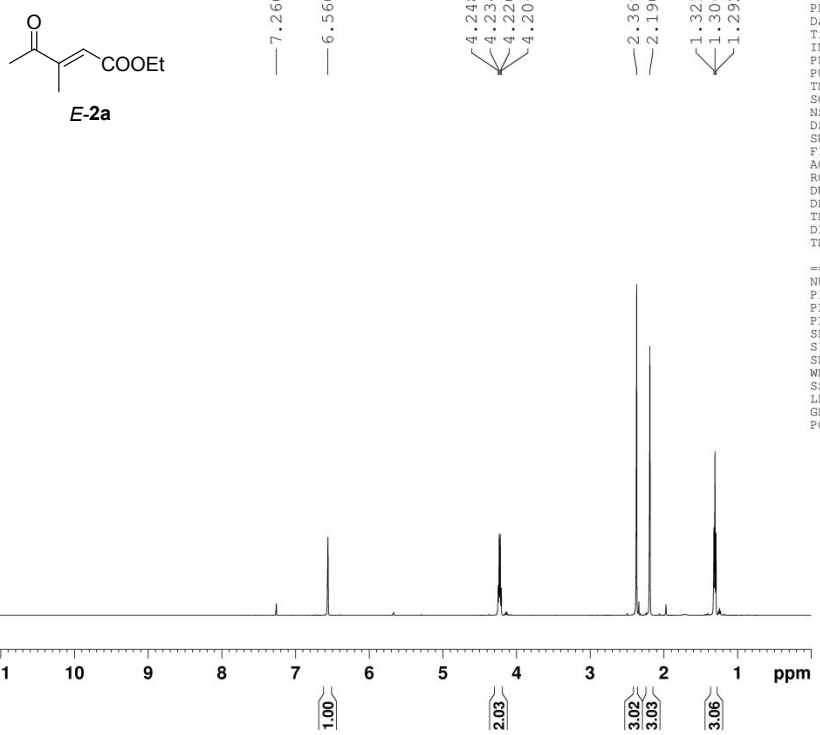


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NAME          DNT_188_pure
EXPNO                    1
PROCNO                   1
Date_             20140203
Time                19.00
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG                zg30
TD                    65536
SOLVENT             CDCl3
NS                       4
DS                       2
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719923 sec
RG                    14.2
DW                  48.400 usec
DE                   6.50 usec
TE                  293.2 K
D1                1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                    6.70 usec
PL1                   4.00 dB
PL1W           8.72000027 W
SFO1          500.2330891 MHz
SI                    32768
SF            500.2300110 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
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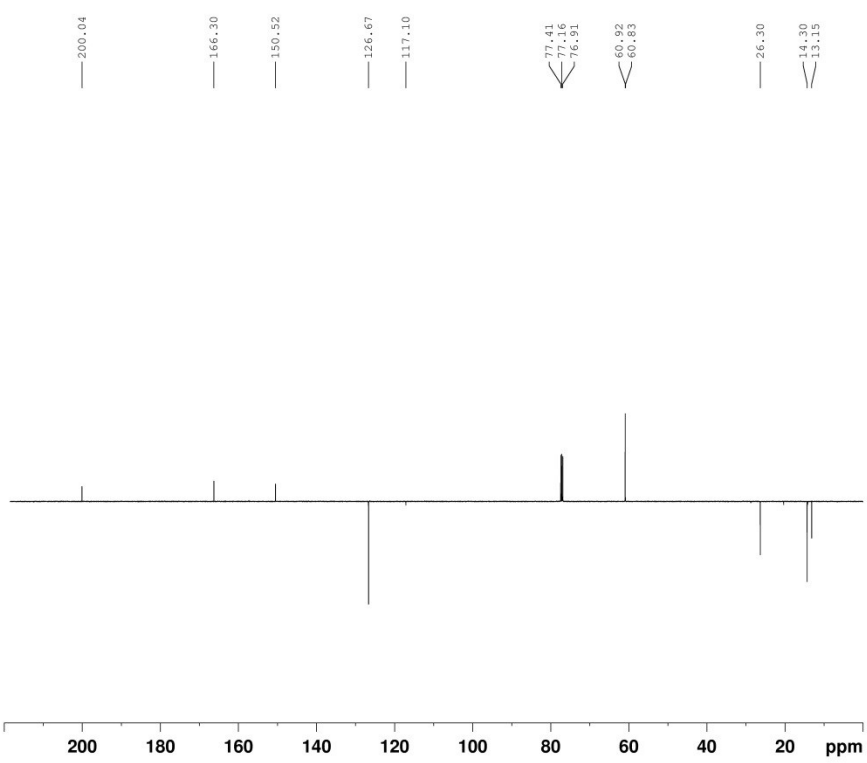


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NAME          DNT_188_pure
EXPNO                    2
PROCNO                   1
Date_             20140203
Time                19.15
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG               jmod
TD                    65536
SOLVENT             CDCl3
NS                     256
DS                       4
SWH              29761.904 Hz
FIDRES            0.454131 Hz
AQ                1.1010548 sec
RG                    2050
DW                  16.800 usec
DE                   6.50 usec
TE                  293.2 K
CNST2           145.0000000
CNST11            1.0000000
D1                2.00000000 sec
D20               0.00689655 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                   11.20 usec
P2                   22.40 usec
PL1                  -2.00 dB
PL1W           88.77790070 W
SFO1          125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                    1H
PCPD2                80.00 usec
PL2                   4.00 dB
PL12                 25.28 dB
PL2W           8.72000027 W
PL12W          0.06494062 W
SFO2          500.2320009 MHz
SI                    32768
SF            125.7829203 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
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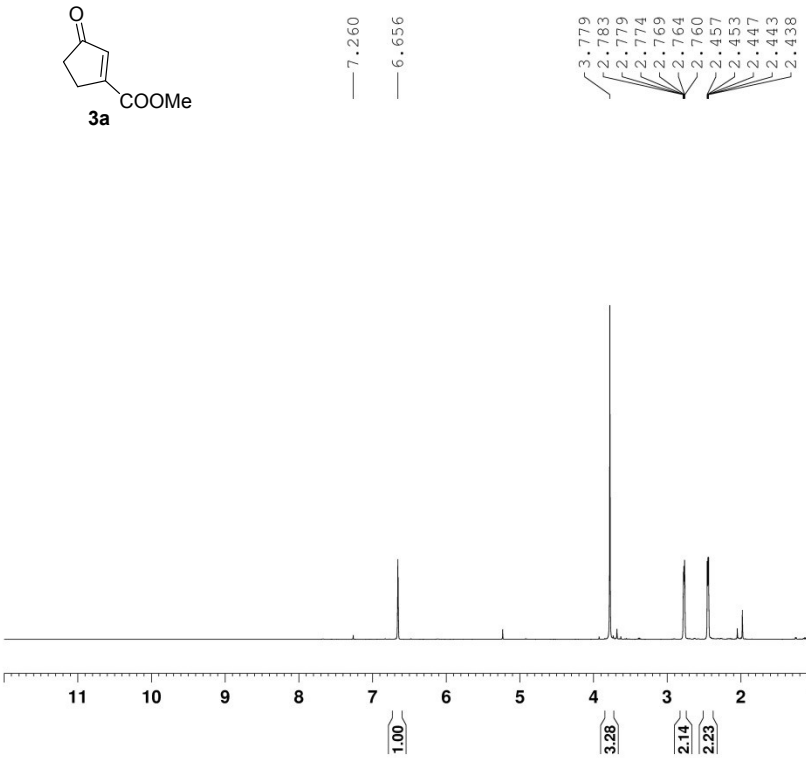


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EXPNO                   2
PROCNO                  1
Date_            20140127
Time                15.10
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH            10330.578 Hz
FIDRES          0.157632 Hz
AQ             3.1719923 sec
RG                   11.3
DW                 48.400 usec
DE                  6.50 usec
TE                 293.1 K
D1            1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                   6.70 usec
PL1                  4.00 dB
PL1W          8.72000027 W
SFO1         500.2330891 MHz
SI                  32768
SF           500.2300102 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
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PC                   1.00
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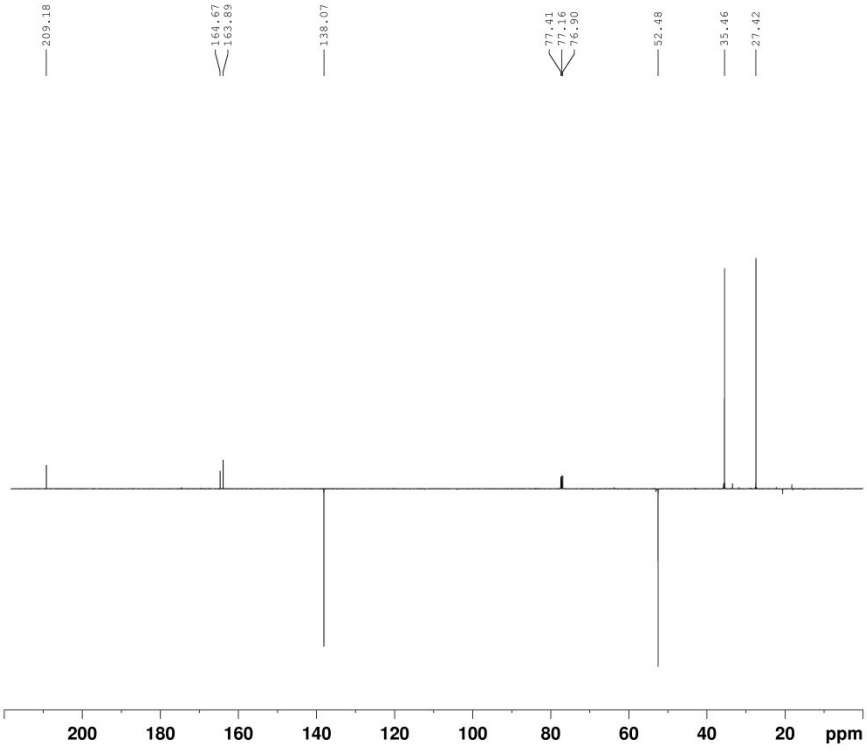


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PROCNO                  1
Date_            20140127
Time                15.24
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG             jmod
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH            29761.904 Hz
FIDRES          0.454131 Hz
AQ             1.1010548 sec
RG                   2050
DW                 16.800 usec
DE                  6.50 usec
TE                 293.2 K
CNST2         145.0000000
CNST11          1.0000000
D1            2.00000000 sec
D20           0.00689655 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                  11.20 usec
P2                  22.40 usec
PL1                 -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2               80.00 usec
PL2                  4.00 dB
PL12                25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2         500.2320009 MHz
SI                  32768
SF           125.7829363 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
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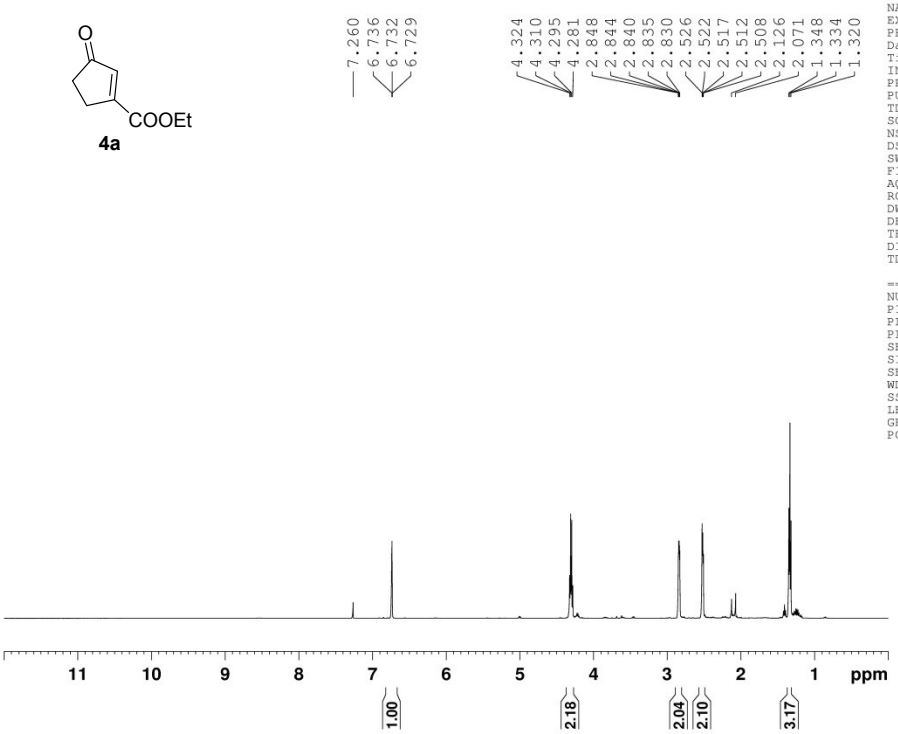


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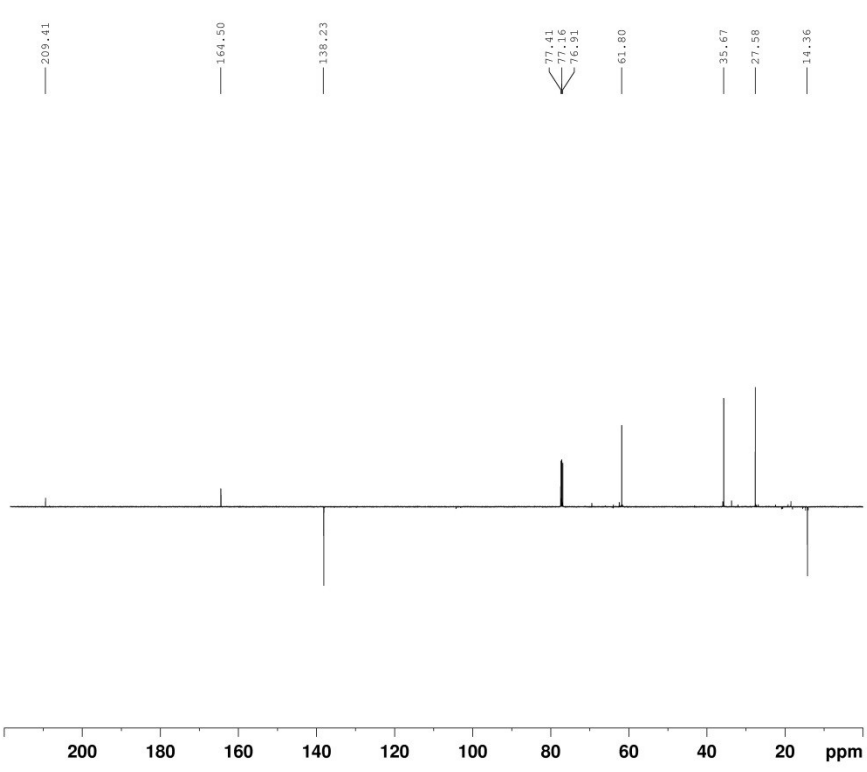
EXPNO                    1
PROCNO                   1
Date_             20140303
Time                 13.42
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                       3
DS                       2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                    14.2
DW                48.400 usec
DE                 6.50 usec
TE                293.2 K
D1            1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   6.70 usec
PL1                  4.00 dB
PL1W          8.72000027 W
SFO1        500.2330891 MHz
SI                   32768
SF          500.2300107 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

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PROCNO                   1
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Time                 13.57
INSTRUM              spect
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PULPROG              jmod
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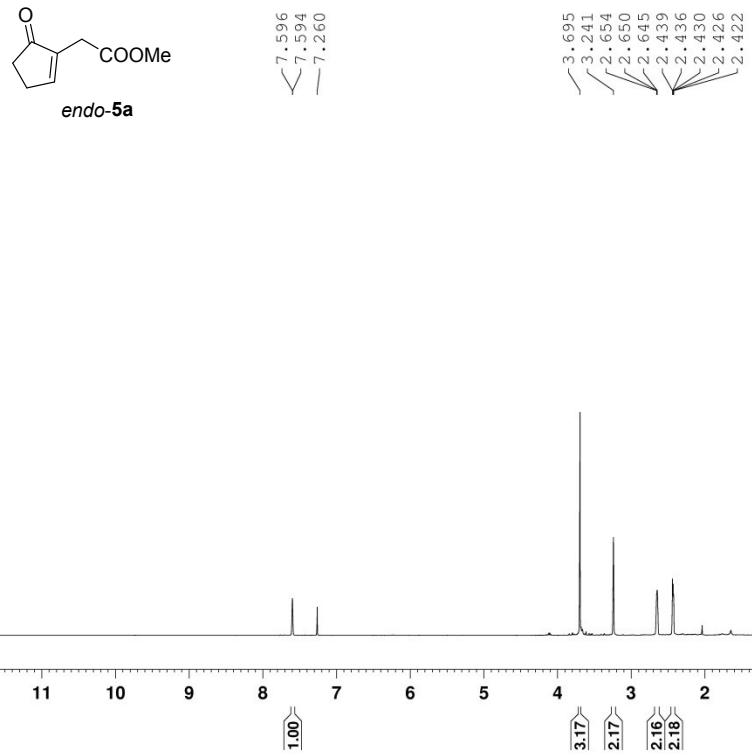
SOLVENT             CDCl3
NS                     256
DS                       4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ             1.1010548 sec
RG                    2050
DW                16.800 usec
DE                 6.50 usec
TE                293.2 K
CNST2       145.0000000
CNST11        1.0000000
D1            2.00000000 sec
D20           0.00689655 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                  11.20 usec
P2                  22.40 usec
PL1                 -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2               80.00 usec
PL2                  4.00 dB
PL12                25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2        500.2320009 MHz
SI                   32768
SF          125.7829220 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

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PROCNO                   1
Date_             20140304
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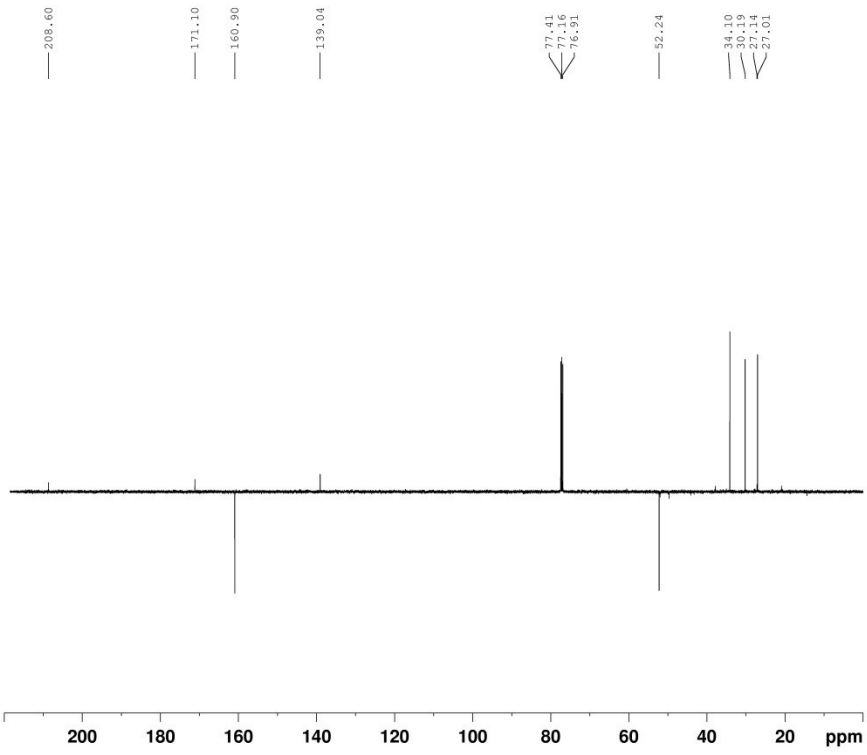
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                     16
DW                48.400 usec
DE                 6.50 usec
TE                293.3 K
D1            1.00000000 sec
TD0                     1

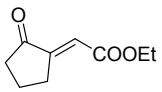
======== CHANNEL f1 ========
NUC1                   1H
P1                  6.70 usec
PL1                 4.00 dB
PL1W          8.72000027 W
SFO1         500.2330891 MHz
SI                  32768
SF           500.2300112 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```




```
NAME          DNT_233_pure
EXPNO                    3
PROCNO                   1
Date_             20140304
Time                 21.59
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG              jmod
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ            1.1010548 sec
RG                   2050
DW                16.800 usec
DE                 6.50 usec
TE                293.3 K
CNST2        145.0000000
CNST11         1.0000000
D1             2.00000000 sec
D20            0.00689655 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                 11.20 usec
P2                 22.40 usec
PL1                -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

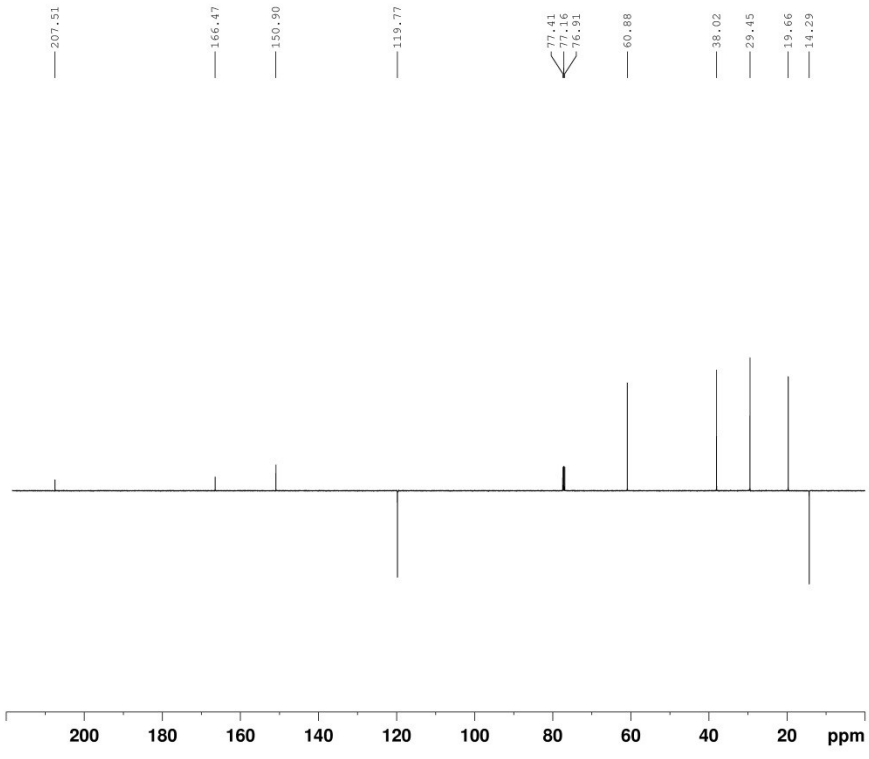
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                 4.00 dB
PL12               25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2         500.2320009 MHz
SI                  32768
SF           125.7829192 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```



exo-**6a**


```
NAME          DNT_213_pure
EXPNO                    2
PROCNO                   1
Date_             20140211
Time                 1.16
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDC13
NS                      8
DS                      2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ           3.1719923 sec
RG                     18
DW                48.400 usec
DE                 6.50 usec
TE                293.2 K
D1           1.00000000 sec
TD0                     1

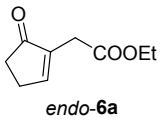
======== CHANNEL f1 ========
NUC1                   1H
P1                  6.70 usec
PL1                 4.00 dB
PL1W         8.72000027 W
SFO1        500.2330891 MHz
SI                  32768
SF          500.2300107 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                 1.00
```



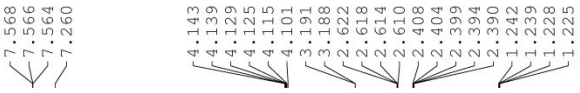

```
NAME          DNT_213_pure
EXPNO                    1
PROCNO                   1
Date_             20140211
Time                 1.15
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG              jmod
TD                  65536
SOLVENT             CDC13
NS                    256
DS                      4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ           1.1010548 sec
RG                   2050
DW                16.800 usec
DE                 6.50 usec
TE                293.2 K
CNST2        145.0000000
CNST11         1.0000000
D1           2.00000000 sec
D20          0.00689655 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                 11.20 usec
P2                 22.40 usec
PL1                -2.00 dB
PL1W        88.77790070 W
SFO1       125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                 4.00 dB
PL12               25.28 dB
PL2W         8.72000027 W
PL12W        0.06494062 W
SFO2        500.2320009 MHz
SI                  32768
SF          125.7829220 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                 1.40
```

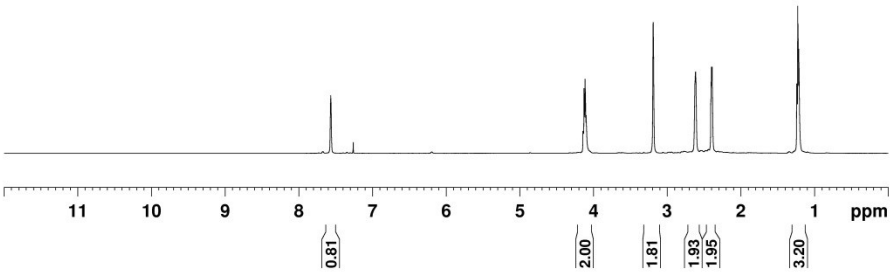


endo-**6a**




```
NAME        DNT_218_pure
EXPNO                  1
PROCNO                 1
Date_           20140212
Time                4.00
INSTRUM            spect
PROBHD   5 mm CPTCI 1H-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     3
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                  12.7
DW                48.400 usec
DE                 6.50 usec
TE                293.3 K
D1            1.00000000 sec
TD0                    1

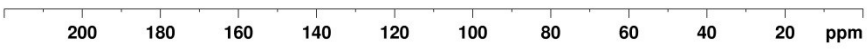
======== CHANNEL f1 ========
NUC1                  1H
P1                 6.70 usec
PL1                4.00 dB
PL1W         8.72000027 W
SFO1        500.2330891 MHz
SI                 32768
SF          500.2300107 MHz
WDW                   EM
SSB                    0
LB                 0.30 Hz
GB                     0
PC                 1.00
```

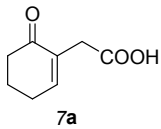



```
NAME        DNT_218_pure
EXPNO                  2
PROCNO                 1
Date_           20140212
Time                4.15
INSTRUM            spect
PROBHD   5 mm CPTCI 1H-
PULPROG             jmod
TD                 65536
SOLVENT            CDCl3
NS                   256
DS                     4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ            1.1010548 sec
RG                  2050
DW                16.800 usec
DE                 6.50 usec
TE                293.1 K
CNST2         145.0000000
CNST11          1.0000000
D1            2.00000000 sec
D20           0.00689655 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                11.20 usec
P2                22.40 usec
PL1               -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             80.00 usec
PL2                4.00 dB
PL12              25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2        500.2320009 MHz
SI                 32768
SF          125.7829245 MHz
WDW                   EM
SSB                    0
LB                 1.00 Hz
GB                     0
PC                 1.40
```

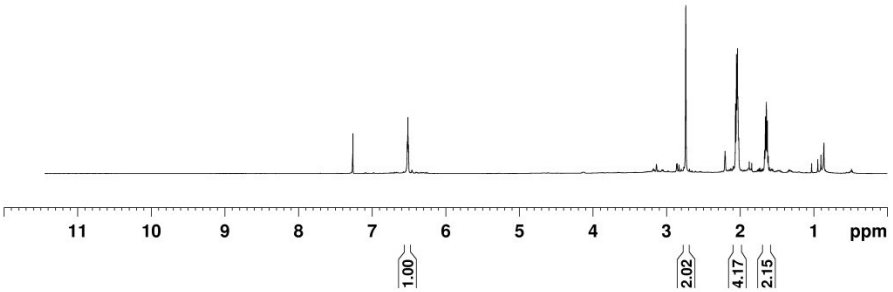




7a


```
NAME          RCC_977_pure
EXPNO                    4
PROCNO                   1
Date_             20151002
Time                 11.00
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDC13
NS                     16
DS                      2
SWH             10330.578 Hz
FIDRES          0.157632 Hz
AQ              3.1719923 sec
RG                   14.2
DW                 48.400 usec
DE                  6.50 usec
TE                 298.2 K
D1              1.00000000 sec
TD0                     1

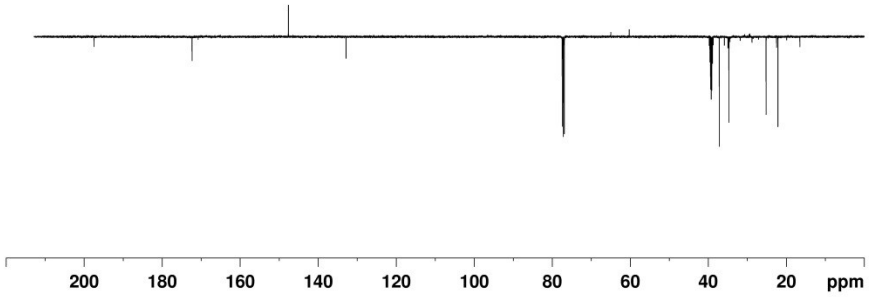
======== CHANNEL f1 ========
NUC1                   1H
P1                   6.70 usec
PL1                  4.00 dB
PL1W          8.72000027 W
SFO1        500.2330891 MHz
SI                  32768
SF          500.2325294 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

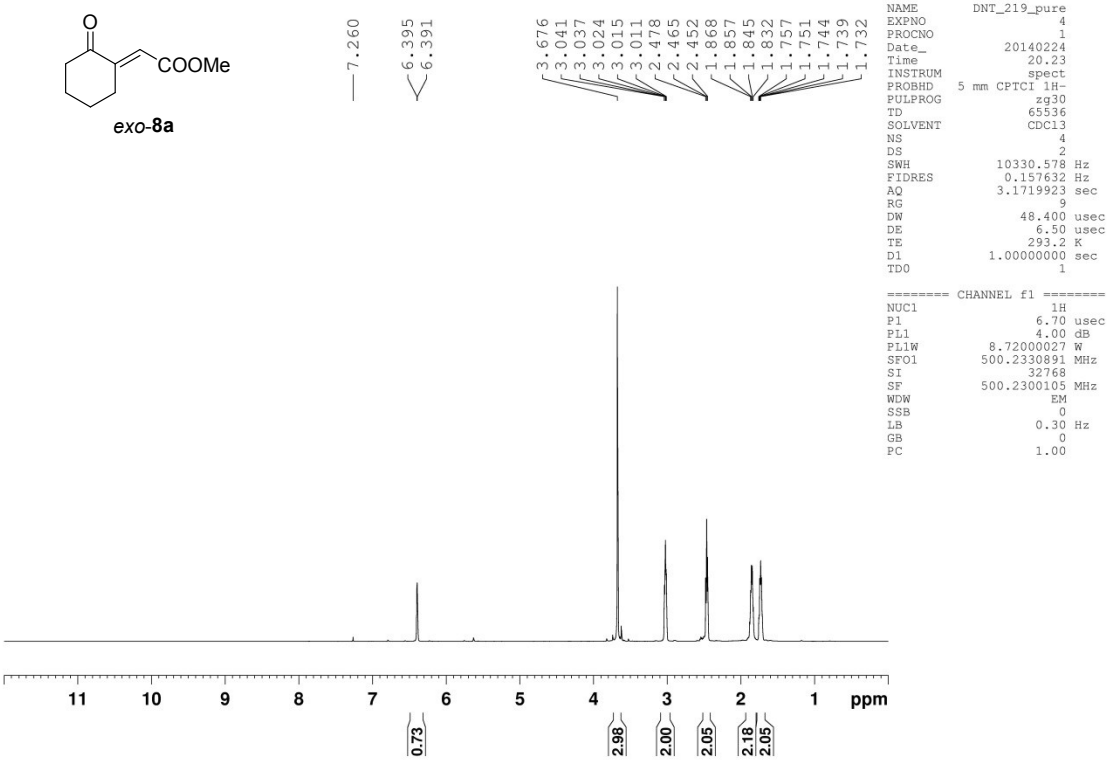


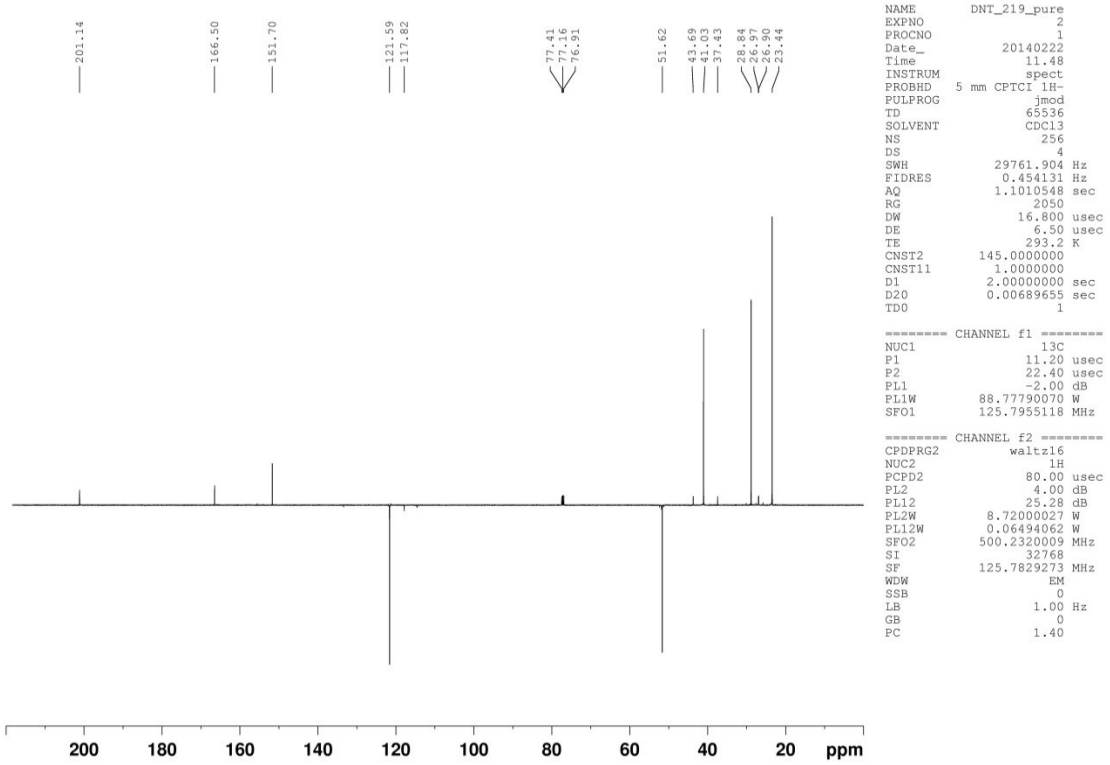

```
NAME          RCC_977_pure
EXPNO                    5
PROCNO                   1
Date_             20151002
Time                 11.14
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG              jmod
TD                  65536
SOLVENT             CDC13
NS                    256
DS                      4
SWH             29761.904 Hz
FIDRES          0.454131 Hz
AQ              1.1010548 sec
RG                   2050
DW                 16.800 usec
DE                  6.50 usec
TE                 298.2 K
CNST2          145.0000000
CNST11           1.0000000
D1               2.00000000 sec
D20              0.00689655 sec
TD0                     1

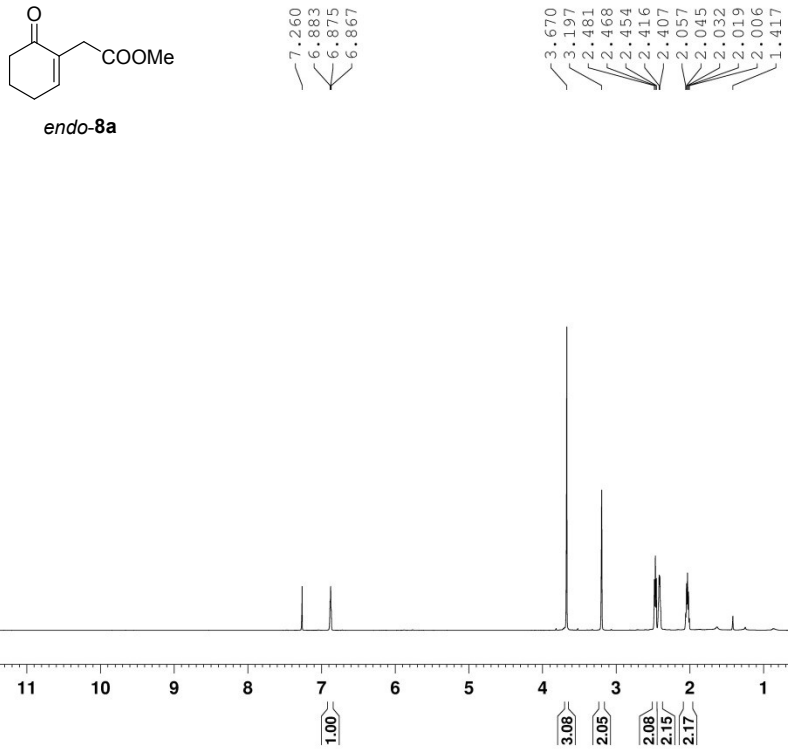
======== CHANNEL f1 ========
NUC1                  13C
P1                 11.20 usec
P2                 22.40 usec
PL1                -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                 4.00 dB
PL12               25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2        500.2320009 MHz
SI                  32768
SF          125.7836322 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```




O
COOMe
exo-8a
7.260
6.395
6.391
3.676
3.041
3.037
3.024
3.015
3.011
2.478
2.465
2.452
1.868
1.857
1.845
1.832
1.757
1.751
1.744
1.739
1.732
NAME DNT_219_pure
EXPNO 4
PROCNO 1
Date_ 20140224
Time 20.23
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 4
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719923 sec
RG 9
DW 48.400 usec
DE 6.50 usec
TE 293.2 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 6.70 usec
PL1 4.00 dB
PL1W 8.72000027 W
SFO1 500.2330891 MHz
SI 32768
SF 500.2300105 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11 10 9 8 7 6 5 4 3 2 1 ppm
0.73 2.98 2.00 2.05 2.18 2.05

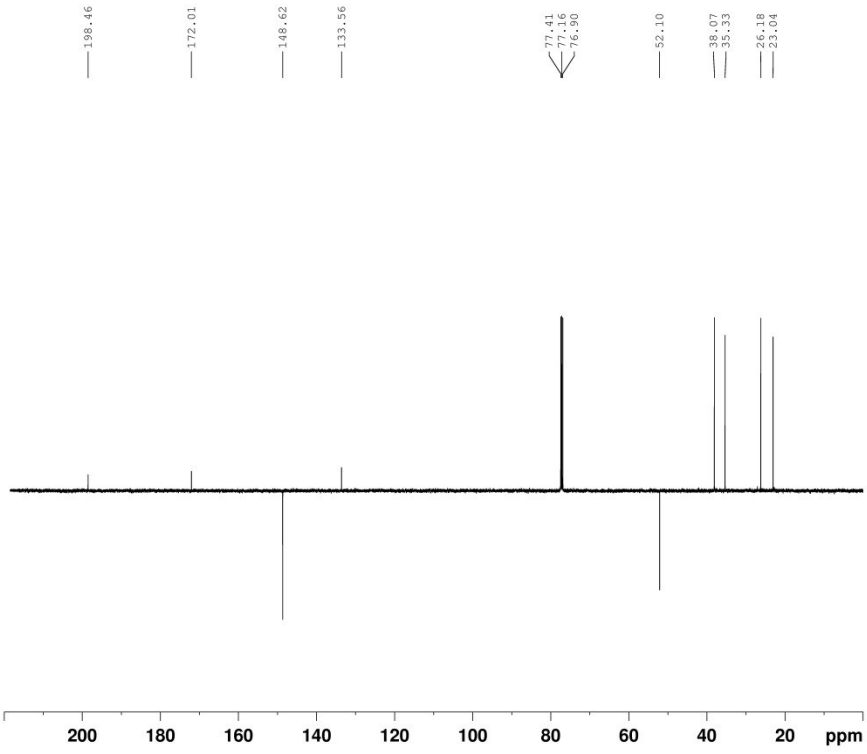

201.14
166.50
151.70
121.59
117.82
77.41
77.16
76.91
51.62
43.69
41.03
37.43
28.84
26.97
26.90
23.44
NAME DNT_219_pure
EXPNO 2
PROCNO 1
Date_ 20140222
Time 11.48
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG jmod
TD 65536
SOLVENT CDCl3
NS 256
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 2050
DW 16.800 usec
DE 6.50 usec
TE 293.2 K
CNST2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
D20 0.00689655 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 11.20 usec
P2 22.40 usec
PL1 -2.00 dB
PL1W 88.77790070 W
SFO1 125.7955118 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 4.00 dB
PL12 25.28 dB
PL2W 8.72000027 W
PL12W 0.06494062 W
SFO2 500.2320009 MHz
SI 32768
SF 125.7829273 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 ppm



endo-**8a**

```
NAME          DNT_228_pure
EXPNO                    1
PROCNO                   1
Date_             20140304
Time                 21.21
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ           3.1719923 sec
RG                     16
DW               48.400 usec
DE                6.50 usec
TE                293.2 K
D1           1.00000000 sec
TD0                     1

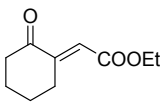
======== CHANNEL f1 ========
NUC1                   1H
P1                   6.70 usec
PL1                  4.00 dB
PL1W         8.72000027 W
SFO1        500.2330891 MHz
SI                  32768
SF          500.2300112 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



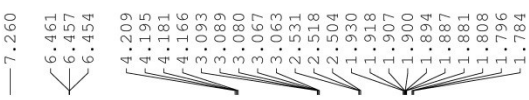

```
NAME          DNT_228_pure
EXPNO                    3
PROCNO                   1
Date_             20140304
Time                 21.35
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG              jmod
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ           1.1010548 sec
RG                   2050
DW               16.800 usec
DE                6.50 usec
TE                293.2 K
CNST2        145.0000000
CNST11         1.0000000
D1           2.00000000 sec
D20          0.00689655 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                  11.20 usec
P2                  22.40 usec
PL1                 -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2               80.00 usec
PL2                  4.00 dB
PL12                25.28 dB
PL2W         8.72000027 W
PL12W        0.06494062 W
SFO2        500.2320009 MHz
SI                  32768
SF          125.7829194 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

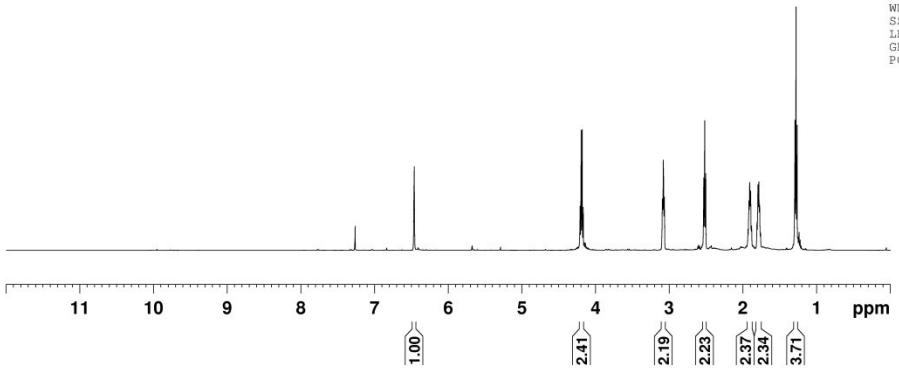


exo-**9a**




```
NAME            DNT_176
EXPNO                 1
PROCNO                1
Date_          20131214
Time               7.41
INSTRUM           spect
PROBHD   5 mm CPTCI 1H-
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ           3.1719923 sec
RG                   16
DW              48.400 usec
DE               6.50 usec
TE               298.2 K
D1          1.00000000 sec
TD0                   1

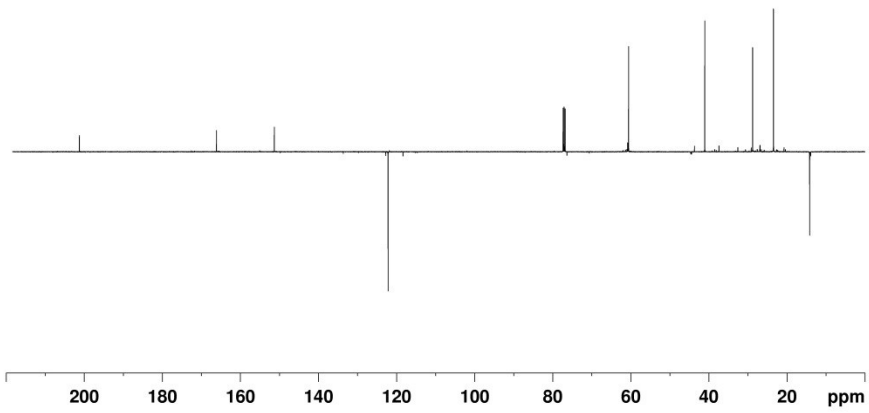
======== CHANNEL f1 ========
NUC1                 1H
P1                6.70 usec
PL1               4.00 dB
PL1W        8.72000027 W
SFO1       500.2330891 MHz
SI                32768
SF         500.2300106 MHz
WDW                  EM
SSB                   0
LB                0.30 Hz
GB                    0
PC                1.00
```

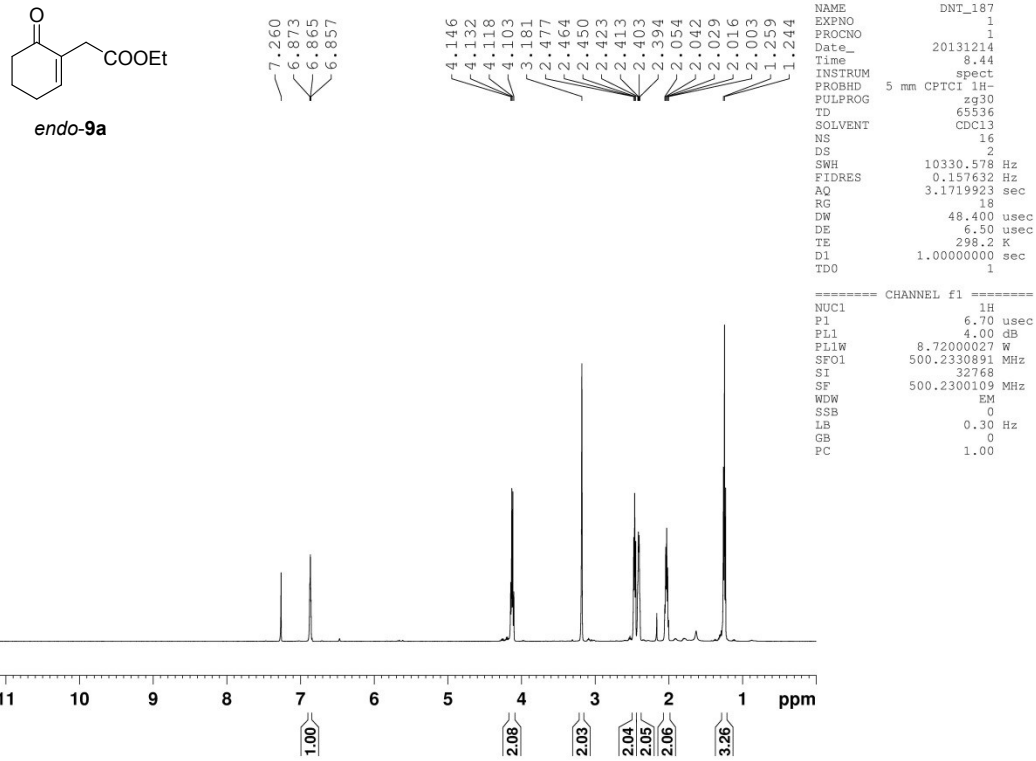


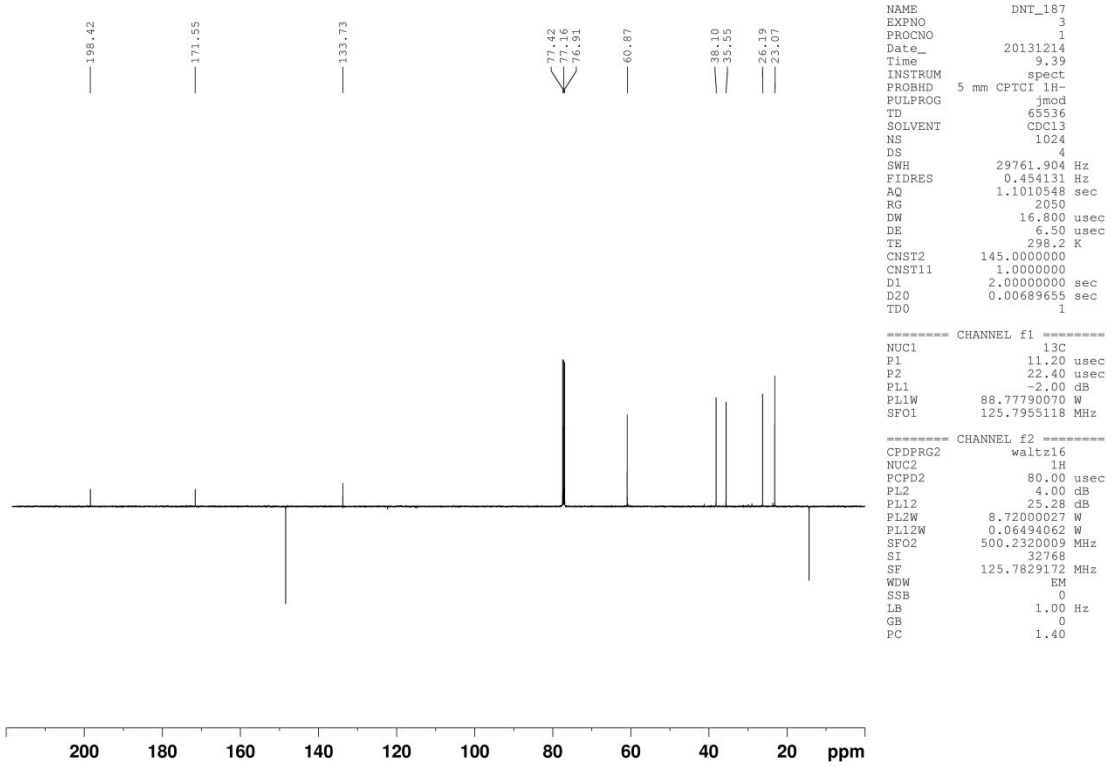

```
NAME            DNT_176
EXPNO                 2
PROCNO                1
Date_          20131214
Time               8.35
INSTRUM           spect
PROBHD   5 mm CPTCI 1H-
PULPROG           jmod
TD                65536
SOLVENT           CDCl3
NS                 1024
DS                    4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ           1.1010548 sec
RG                 2050
DW              16.800 usec
DE               6.50 usec
TE               298.1 K
CNST2       145.0000000
CNST11        1.0000000
D1          2.00000000 sec
D20         0.00689655 sec
TD0                   1

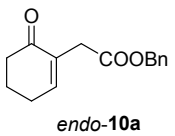
======== CHANNEL f1 ========
NUC1                13C
P1               11.20 usec
P2               22.40 usec
PL1              -2.00 dB
PL1W        88.77790070 W
SFO1       125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                 1H
PCPD2            80.00 usec
PL2               4.00 dB
PL12             25.28 dB
PL2W         8.72000027 W
PL12W        0.06494062 W
SFO2       500.2320009 MHz
SI                32768
SF         125.7829340 MHz
WDW                  EM
SSB                   0
LB                1.00 Hz
GB                    0
PC                1.40
```

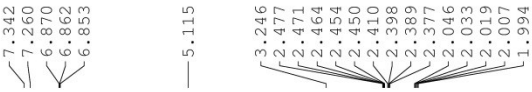



O
COOEt
endo-9a
7.260
6.873
6.865
6.857
4.146
4.132
4.118
4.103
3.181
2.477
2.464
2.450
2.423
2.413
2.403
2.394
2.054
2.042
2.029
2.016
2.003
1.259
1.244
NAME DNT_187
EXPNO 1
PROCNO 1
Date_ 20131214
Time 8.44
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG zg30
TD 65536
SOLVENT CDC13
NS 16
DS 2
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719923 sec
RG 18
DW 48.400 usec
DE 6.50 usec
TE 298.2 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 6.70 usec
PL1 4.00 dB
PL1W 8.72000027 W
SFO1 500.2330891 MHz
SI 32768
SF 500.2300109 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11 10 9 8 7 6 5 4 3 2 1 ppm
1.00 2.08 2.03 2.04 2.05 2.06 3.26


198.42
171.55
133.73
77.42
77.16
76.91
60.87
38.10
35.55
26.19
23.07
NAME DNT_187
EXPNO 3
PROCNO 1
Date_ 20131214
Time 9.39
INSTRUM spect
PROBHD 5 mm CPTCI 1H-
PULPROG jmod
TD 65536
SOLVENT CDC13
NS 1024
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010548 sec
RG 2050
DW 16.800 usec
DE 6.50 usec
TE 298.2 K
CNST2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
D20 0.00689655 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 11.20 usec
P2 22.40 usec
PL1 -2.00 dB
PL1W 88.77790070 W
SFO1 125.7955118 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 4.00 dB
PL12 25.28 dB
PL2W 8.72000027 W
PL12W 0.06494062 W
SFO2 500.2320009 MHz
SI 32768
SF 125.7829172 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 ppm

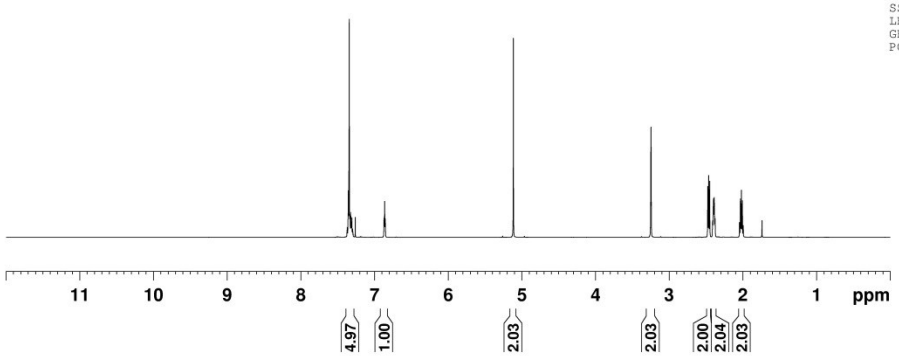


endo-**10a**




```
NAME            RCC_Benz
EXPNO                  1
PROCNO                 1
Date_           20150223
Time               17.13
INSTRUM            spect
PROBHD   5 mm CPTCI 1H-
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719923 sec
RG                    16
DW               48.400 usec
DE                6.50 usec
TE               293.2 K
D1            1.00000000 sec
TD0                    1

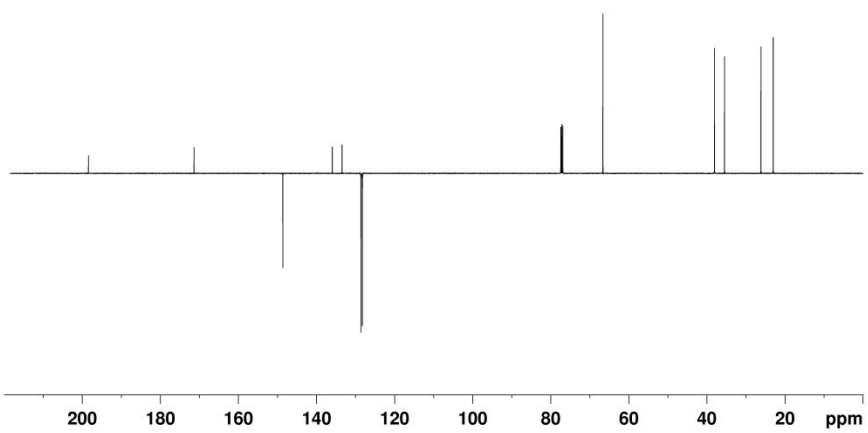
======== CHANNEL f1 ========
NUC1                  1H
P1                 6.70 usec
PL1                4.00 dB
PL1W         8.72000027 W
SFO1        500.2330891 MHz
SI                 32768
SF          500.2300118 MHz
WDW                   EM
SSB                    0
LB                0.30 Hz
GB                     0
PC                1.00
```

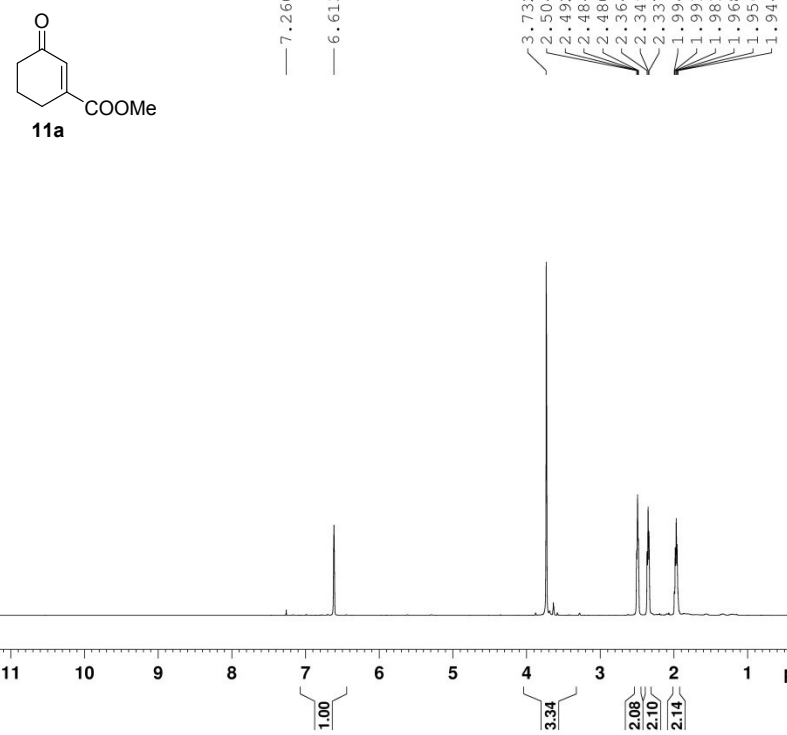



```
NAME            RCC_Benz
EXPNO                  2
PROCNO                 1
Date_           20150223
Time               17.28
INSTRUM            spect
PROBHD   5 mm CPTCI 1H-
PULPROG             jmod
TD                 65536
SOLVENT            CDCl3
NS                   256
DS                     4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ            1.1010548 sec
RG                  2050
DW               16.800 usec
DE                6.50 usec
TE               293.2 K
CNST2        145.0000000
CNST11         1.0000000
D1             2.00000000 sec
D20            0.00689655 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  13C
P1                11.20 usec
P2                22.40 usec
PL1               -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

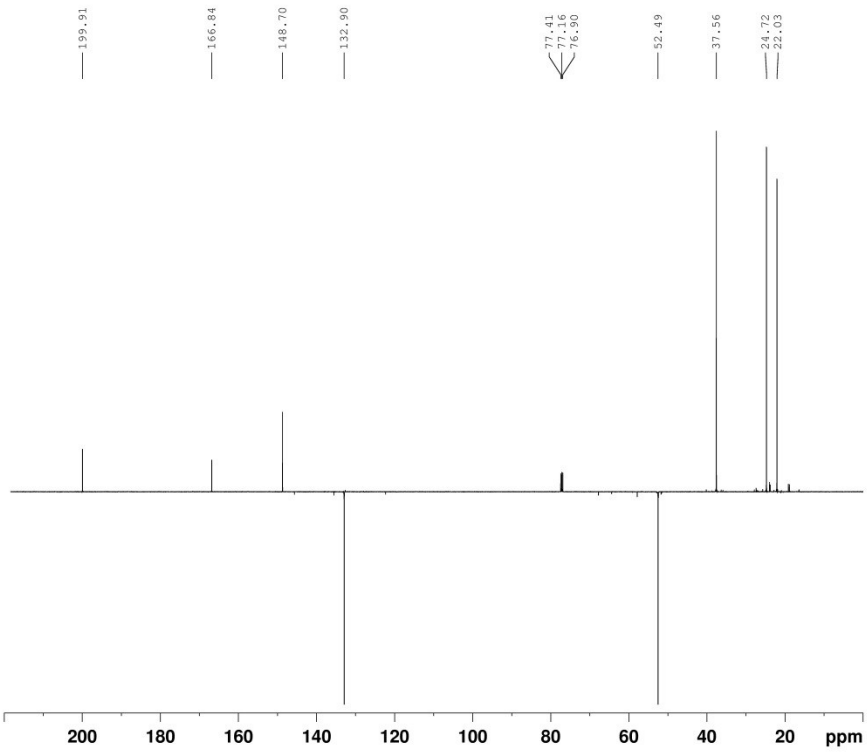
======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2              80.00 usec
PL2                4.00 dB
PL12              25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2        500.2320009 MHz
SI                 32768
SF          125.7829258 MHz
WDW                   EM
SSB                    0
LB                1.00 Hz
GB                     0
PC                1.40
```






```
NAME          RCC_547_col
EXPNO                   1
PROCNO                  1
Date_            20140207
Time                18.40
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDC13
NS                      5
DS                      2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                      9
DW                 48.400 usec
DE                  6.50 usec
TE                 293.2 K
D1             1.00000000 sec
TD0                     1

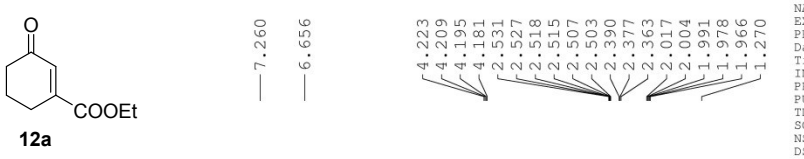
======== CHANNEL f1 ========
NUC1                   1H
P1                  6.70 usec
PL1                 4.00 dB
PL1W          8.72000027 W
SFO1         500.2330891 MHz
SI                  32768
SF           500.2300104 MHz
WDW                    EM
SSB                     0
LB                  0.30 Hz
GB                      0
PC                  1.00
```




```
NAME          RCC_547_col
EXPNO                   2
PROCNO                  1
Date_            20140207
Time                18.55
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG             jmod
TD                  65536
SOLVENT             CDC13
NS                    256
DS                      4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ             1.1010548 sec
RG                   2050
DW                 16.800 usec
DE                  6.50 usec
TE                 293.2 K
CNST2        145.0000000
CNST11         1.0000000
D1             2.00000000 sec
D20            0.00689655 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   13C
P1                 11.20 usec
P2                 22.40 usec
PL1                -2.00 dB
PL1W         88.77790070 W
SFO1        125.7955118 MHz

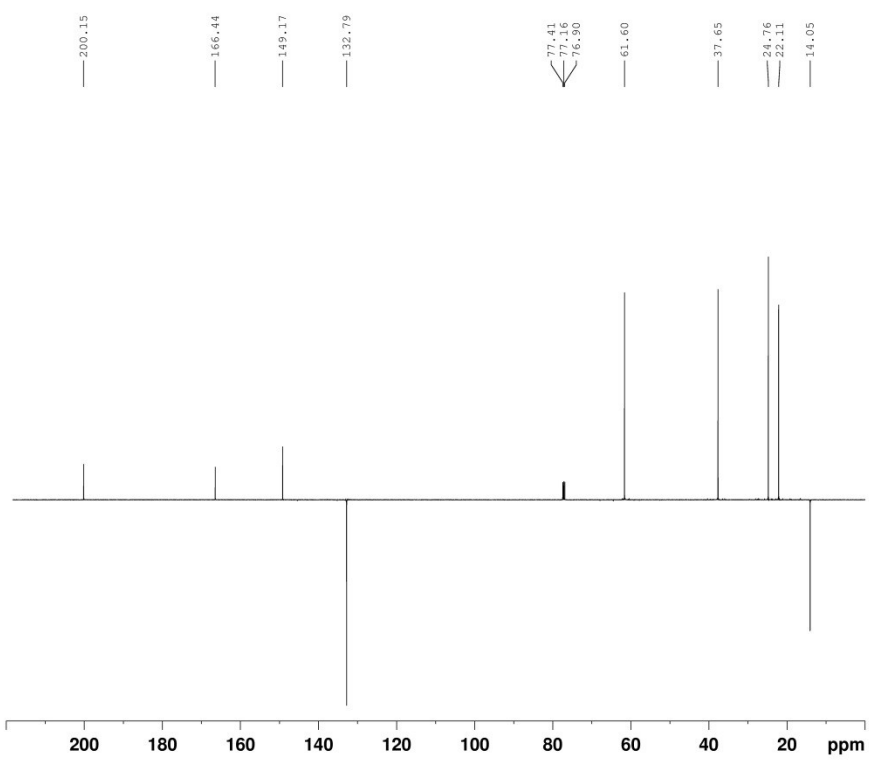
======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                 4.00 dB
PL12               25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2         500.2320009 MHz
SI                  32768
SF           125.7829377 MHz
WDW                    EM
SSB                     0
LB                  1.00 Hz
GB                      0
PC                  1.40
```



12a


```
NAME          RCC_543_co1
EXPNO                   1
PROCNO                  1
Date_            20140127
Time                15.32
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      3
DS                      2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                     10
DW                48.400 usec
DE                 6.50 usec
TE                293.2 K
D1             1.00000000 sec
TD0                     1

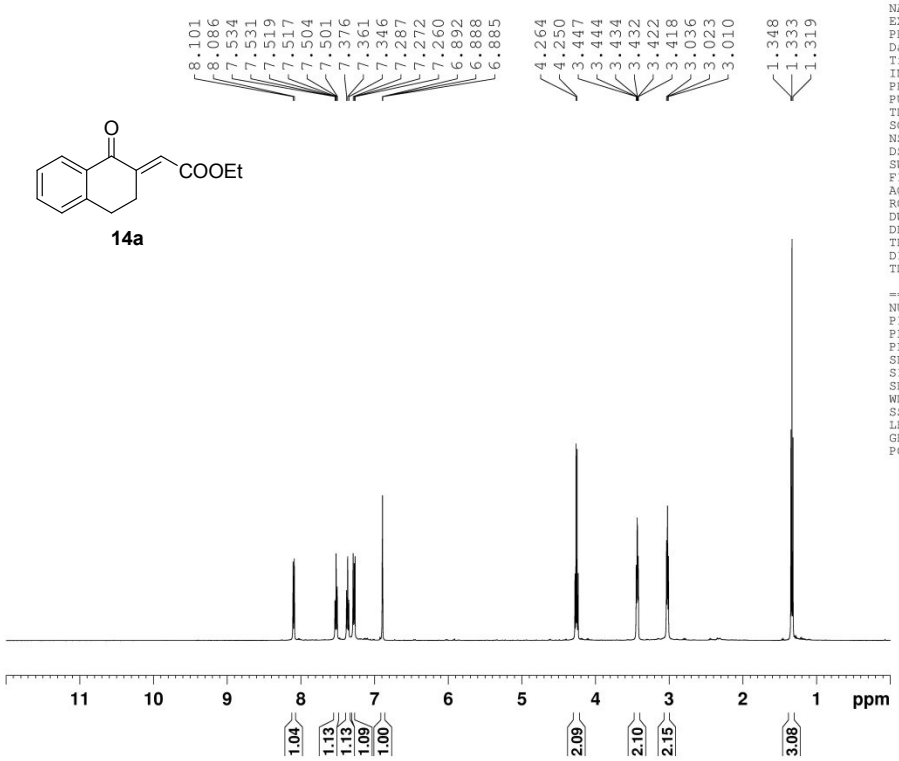
======== CHANNEL f1 ========
NUC1                   1H
P1                   6.70 usec
PL1                  4.00 dB
PL1W          8.72000027 W
SFO1        500.2330891 MHz
SI                  32768
SF          500.2300103 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```




```
NAME          RCC_543_co1
EXPNO                   2
PROCNO                  1
Date_            20140127
Time                15.47
INSTRUM             spect
PROBHD   5 mm CPTCI 1H-
PULPROG             jmod
TD                  65536
SOLVENT             CDCl3
NS                    256
DS                      4
SWH           29761.904 Hz
FIDRES         0.454131 Hz
AQ             1.1010548 sec
RG                   2050
DW                16.800 usec
DE                 6.50 usec
TE                293.2 K
CNST2         145.0000000
CNST11          1.0000000
D1              2.00000000 sec
D20             0.00689655 sec
TD0                     1

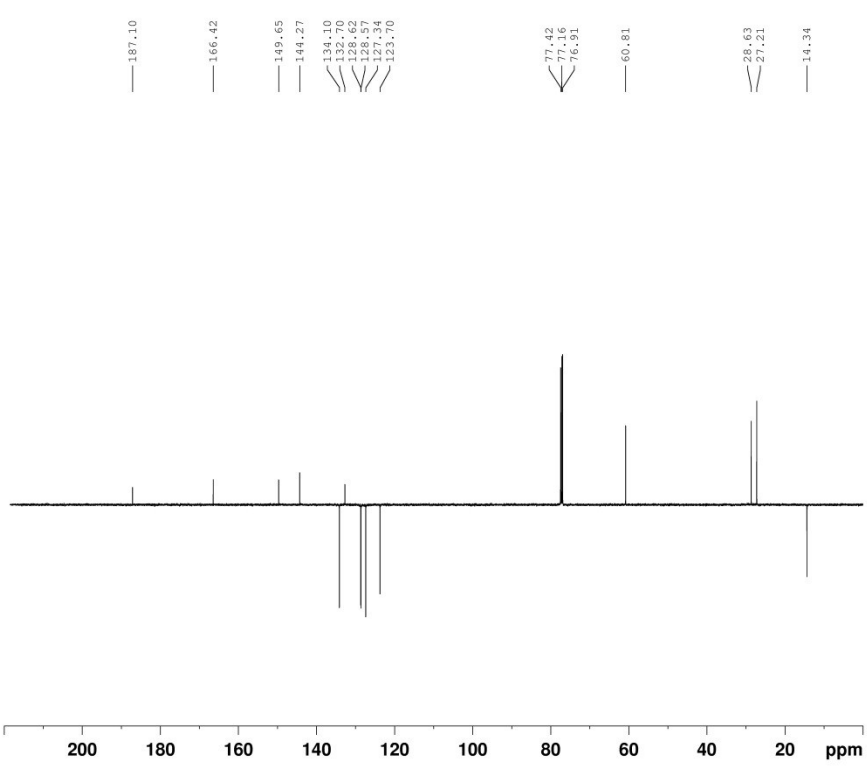
======== CHANNEL f1 ========
NUC1                   13C
P1                  11.20 usec
P2                  22.40 usec
PL1                 -2.00 dB
PL1W          88.77790070 W
SFO1        125.7955118 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                   1H
PCPD2               80.00 usec
PL2                  4.00 dB
PL12                25.28 dB
PL2W          8.72000027 W
PL12W         0.06494062 W
SFO2        500.2320009 MHz
SI                  32768
SF          125.7829323 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```




```
NAME          DNT_148_pure
EXPNO                    4
PROCNO                   1
Date_             20150429
Time                 11.35
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                       2
DS                       2
SWH             10330.578 Hz
FIDRES          0.157632 Hz
AQ              3.1719923 sec
RG                      18
DW                 48.400 usec
DE                  6.50 usec
TE                 300.1 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   6.70 usec
PL1                  4.00 dB
PL1W           8.72000027 W
SFO1          500.2330891 MHz
SI                   32768
SF            500.2300113 MHz
WDW                     EM
SSB                      0
LB                  0.30 Hz
GB                       0
PC                    1.00
```



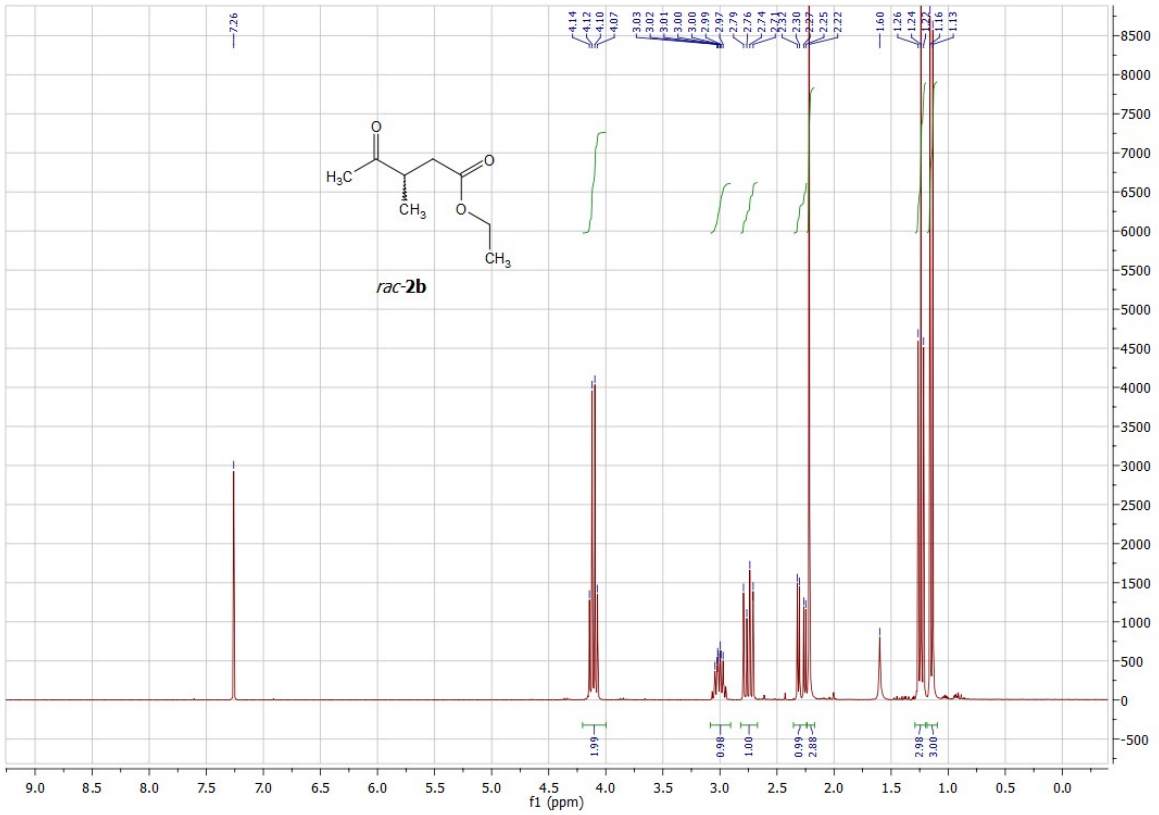

```
NAME          DNT_148_pure
EXPNO                    5
PROCNO                   1
Date_             20150429
Time                 11.42
INSTRUM              spect
PROBHD   5 mm CPTCI 1H-
PULPROG              jmod
TD                   65536
SOLVENT              CDCl3
NS                     128
DS                       4
SWH             29761.904 Hz
FIDRES          0.454131 Hz
AQ              1.1010548 sec
RG                    2050
DW                 16.800 usec
DE                  6.50 usec
TE                 300.0 K
CNST2          145.0000000
CNST11           1.0000000
D1               2.00000000 sec
D20              0.00689655 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                   13C
P1                  11.20 usec
P2                  22.40 usec
PL1                 -2.00 dB
PL1W           88.77790070 W
SFO1          125.7955118 MHz

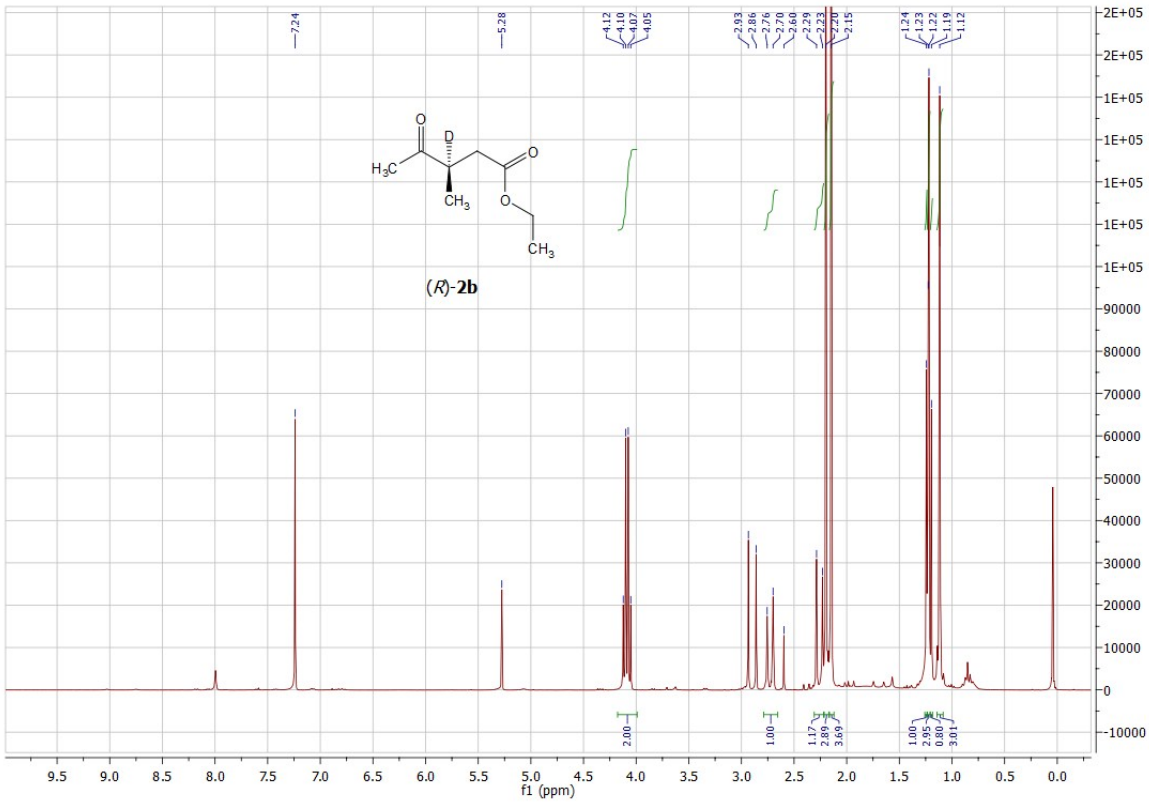
======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2               80.00 usec
PL2                  4.00 dB
PL12                25.28 dB
PL2W            8.72000027 W
PL12W           0.06494062 W
SFO2          500.2320009 MHz
SI                   32768
SF            125.7829178 MHz
WDW                     EM
SSB                      0
LB                  1.00 Hz
GB                       0
PC                    1.40
```

8. NMR spectra from isotopic labeling

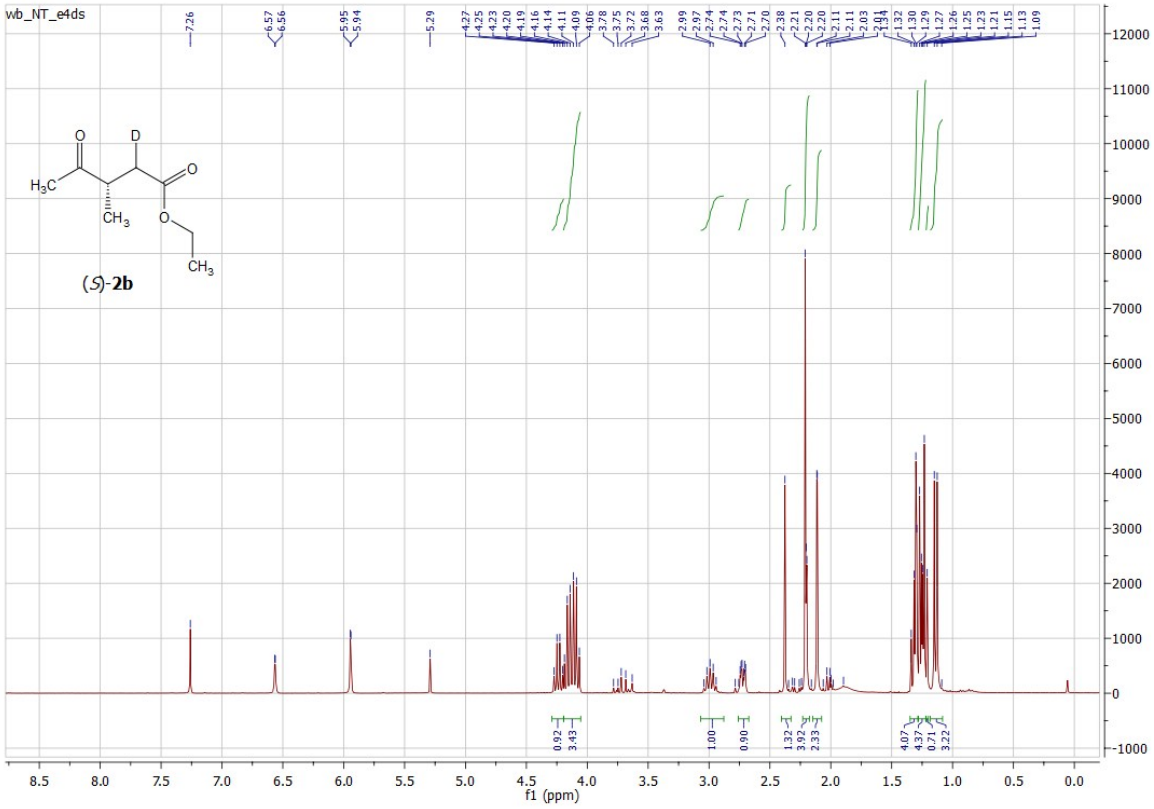
rac-**2b**



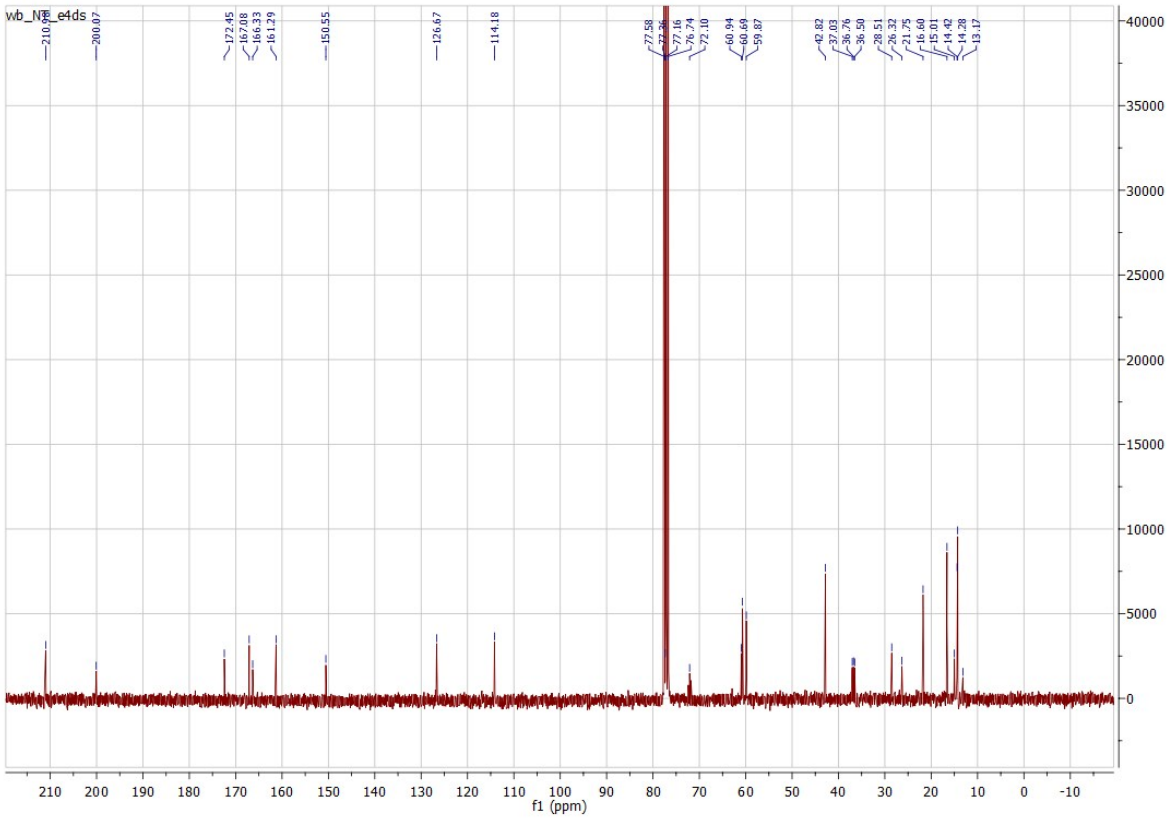
(*R*)-**2b**



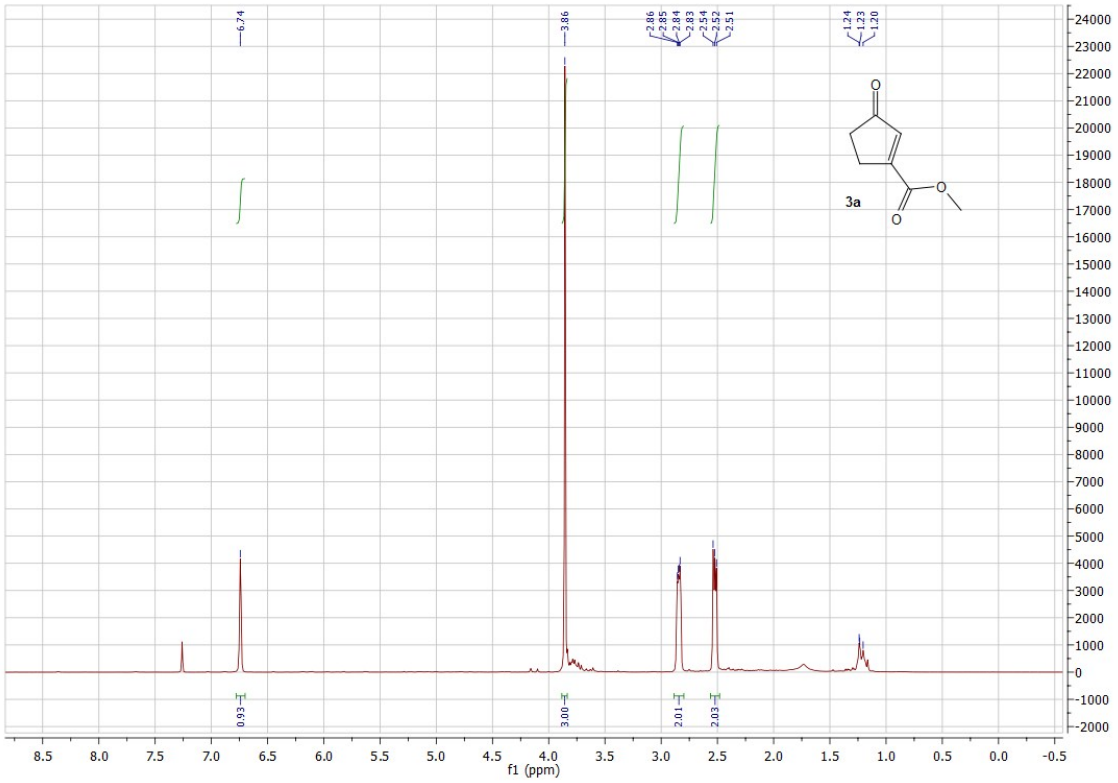
(*S*)-**2b**



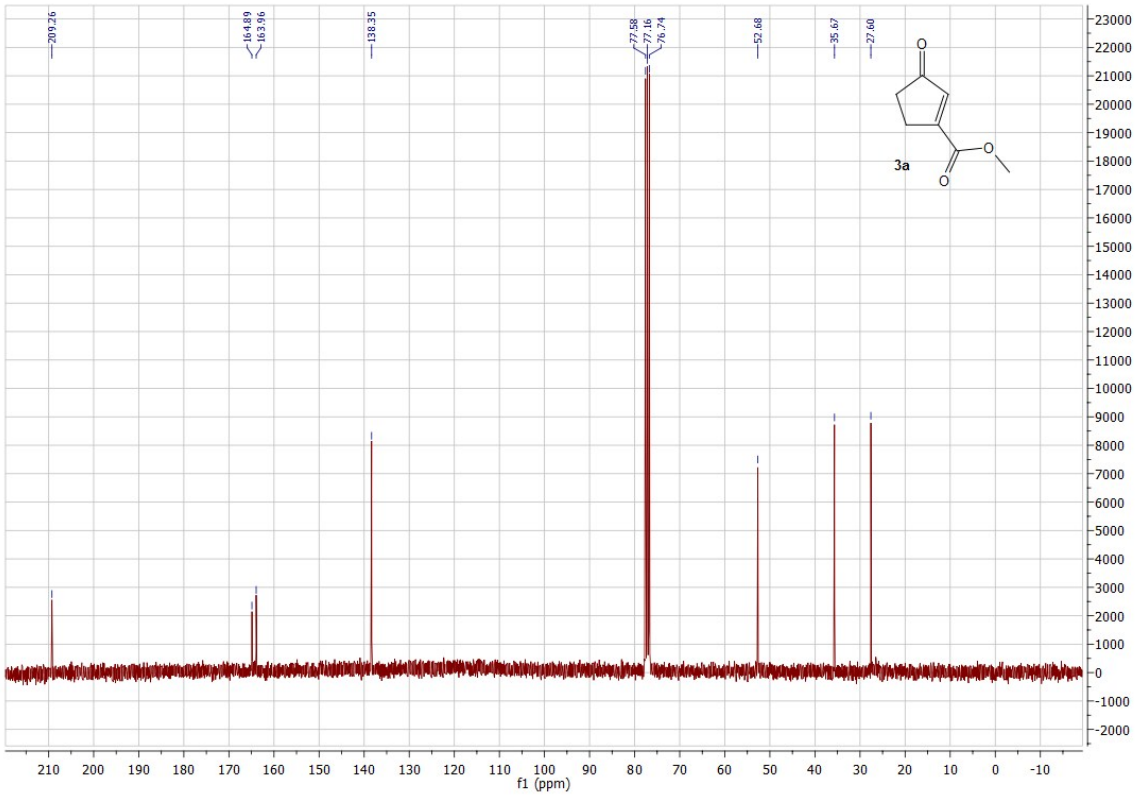
(*S*)-**2b** ^{13}C



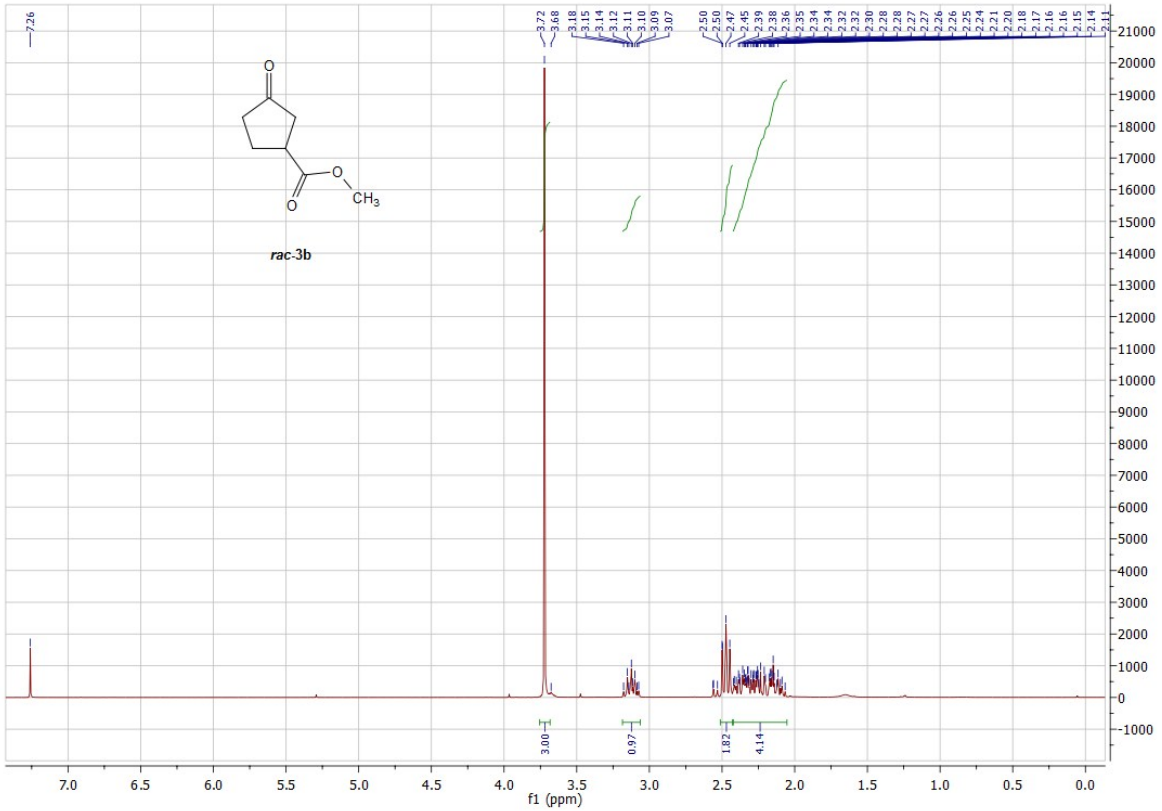
3a


6.74
3.86
2.86
2.85
2.84
2.83
2.54
2.52
2.51
1.24
1.23
1.20
3a
0.93
3.00
2.01
2.03
f1 (ppm)

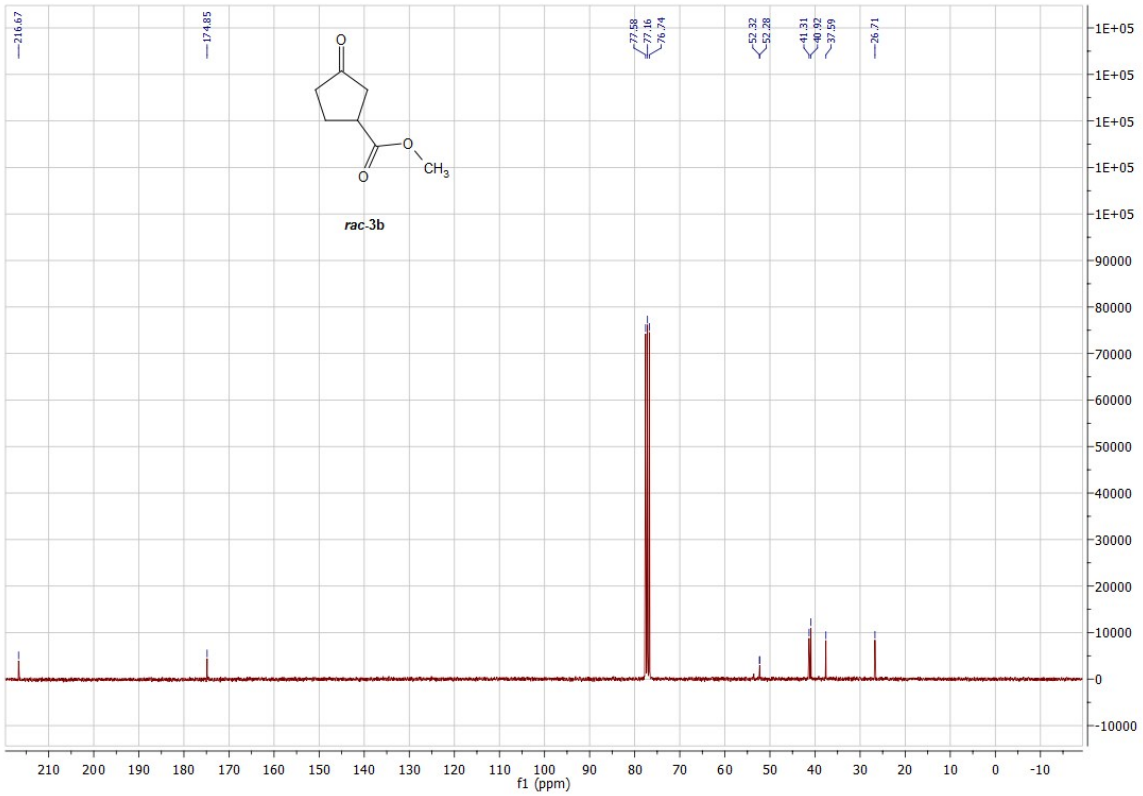
3a ^{13}C


209.26
164.89
163.96
138.35
77.58
77.16
76.74
52.68
35.67
27.60
3a
f1 (ppm)

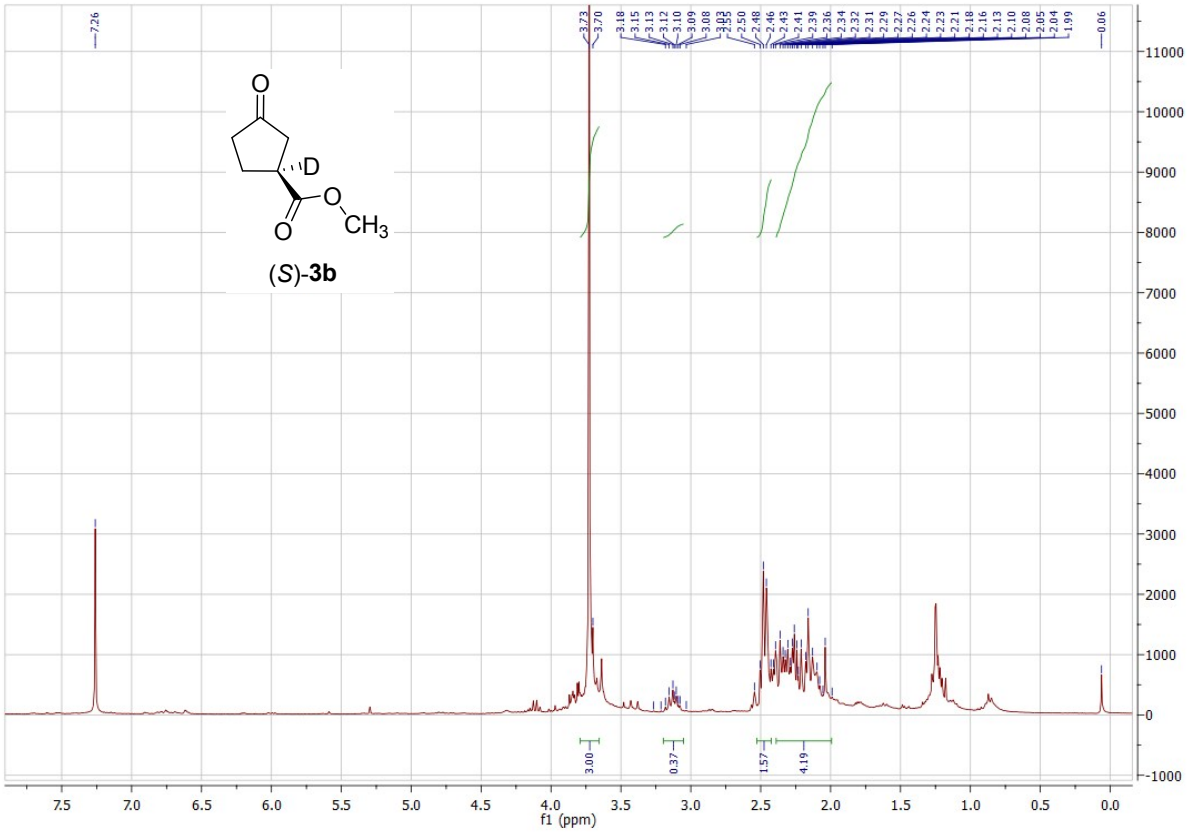
rac-**3b**



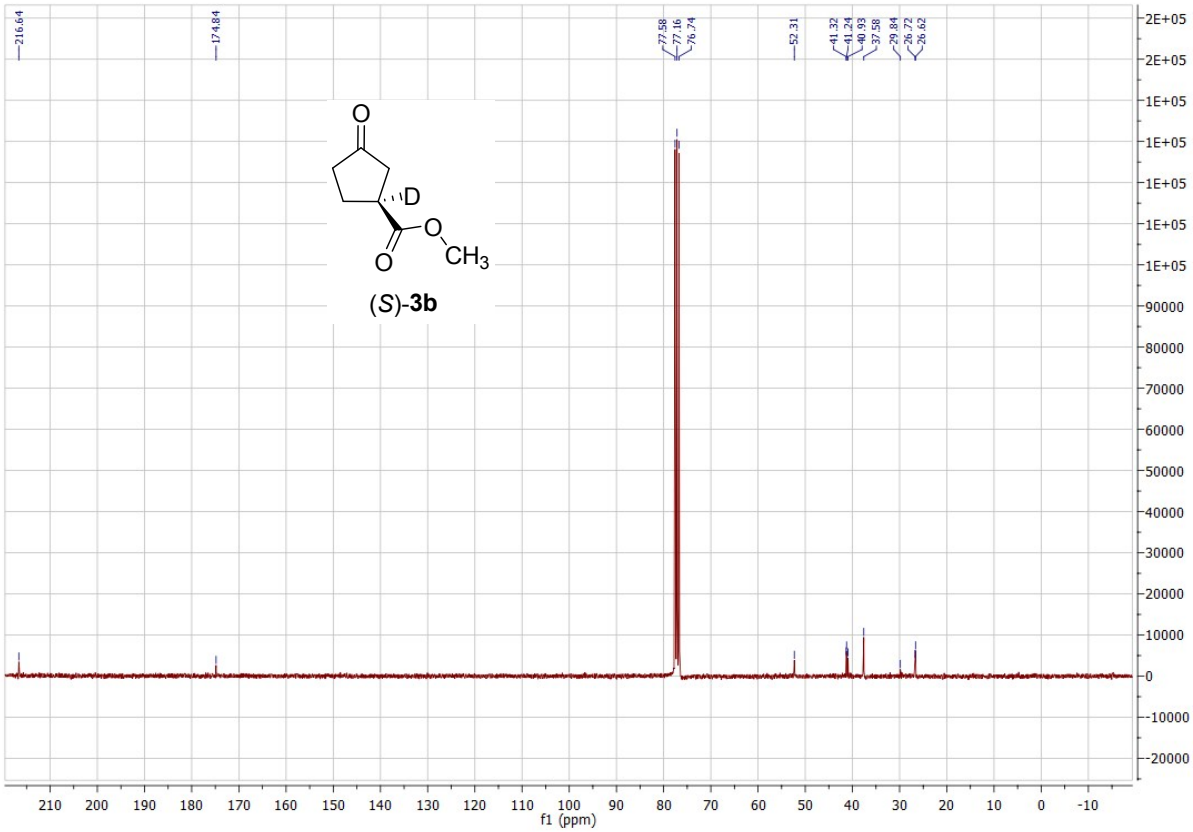
rac-**3b** ^{13}C



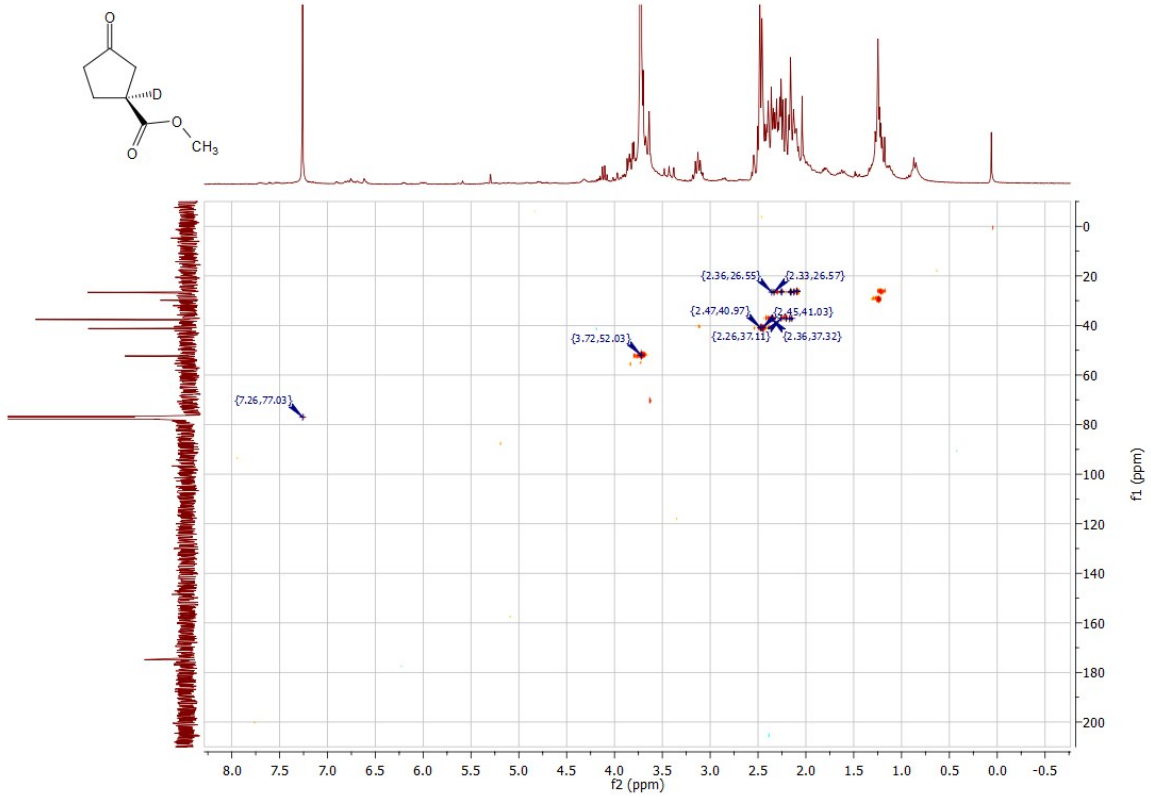
(*S*)-**3b** from OYE2



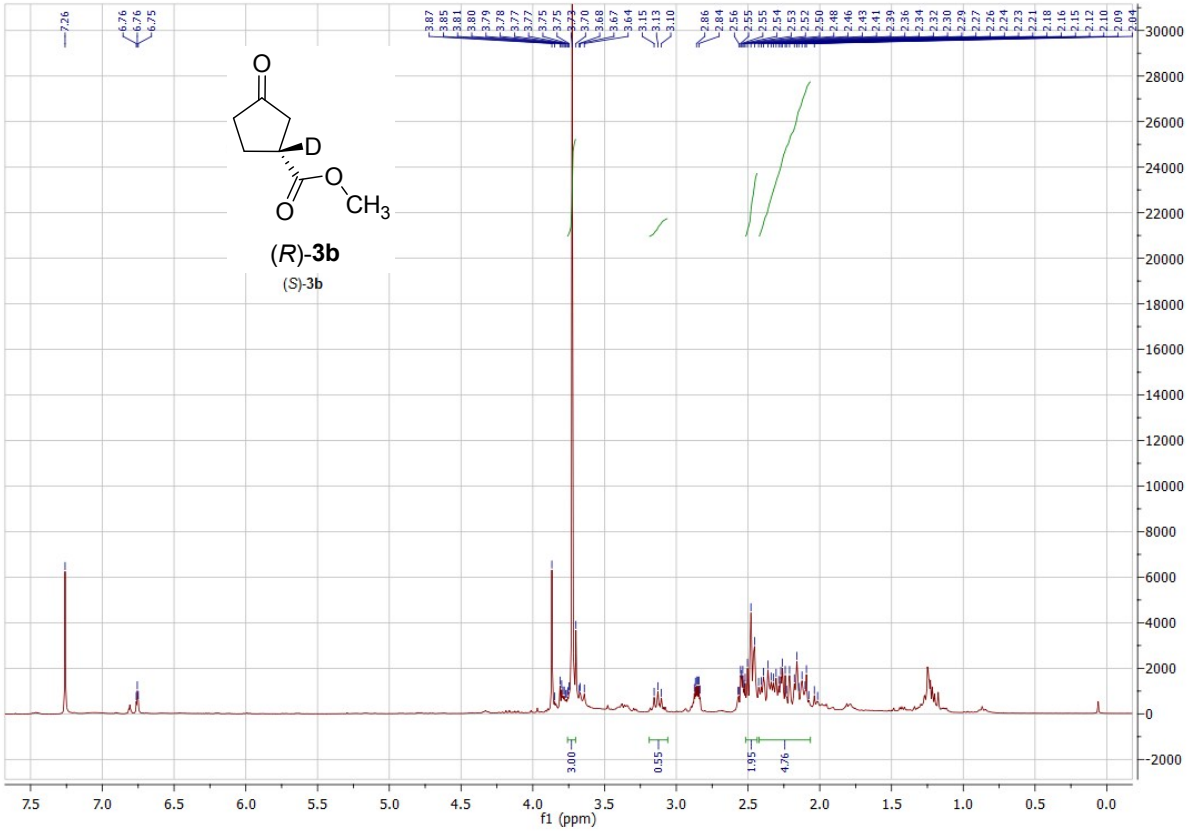
(*S*)-**3b** from OYE2 ^{13}C



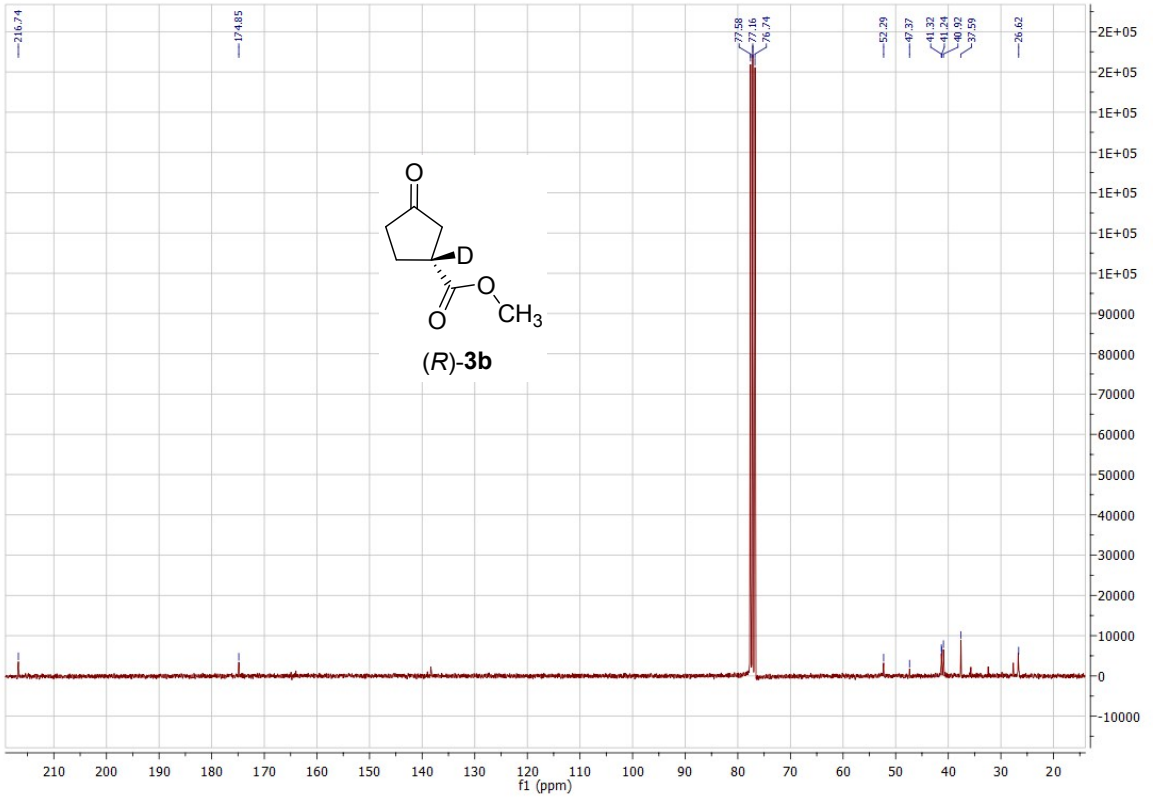
(*S*)-**3b** from OYE2 HSQC (signals at around 1.2 ppm are impurities already present in the substrate)



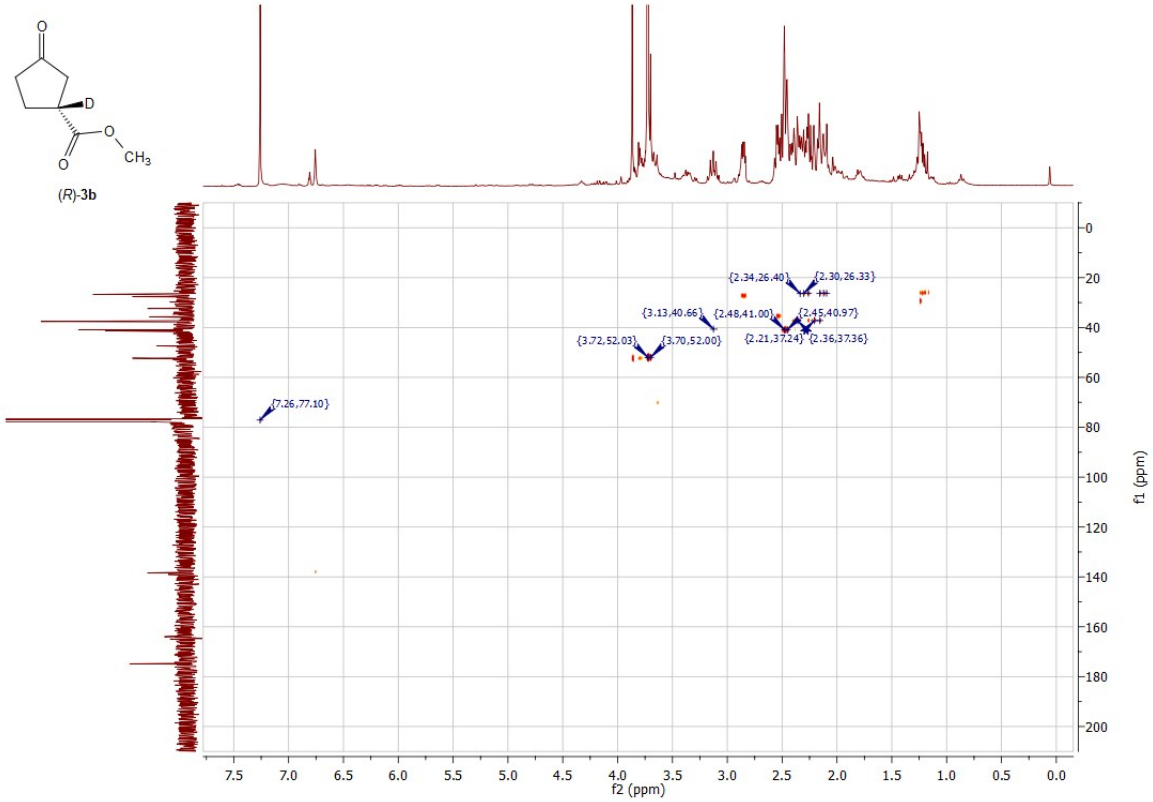
(*R*)-**3b** from XenA



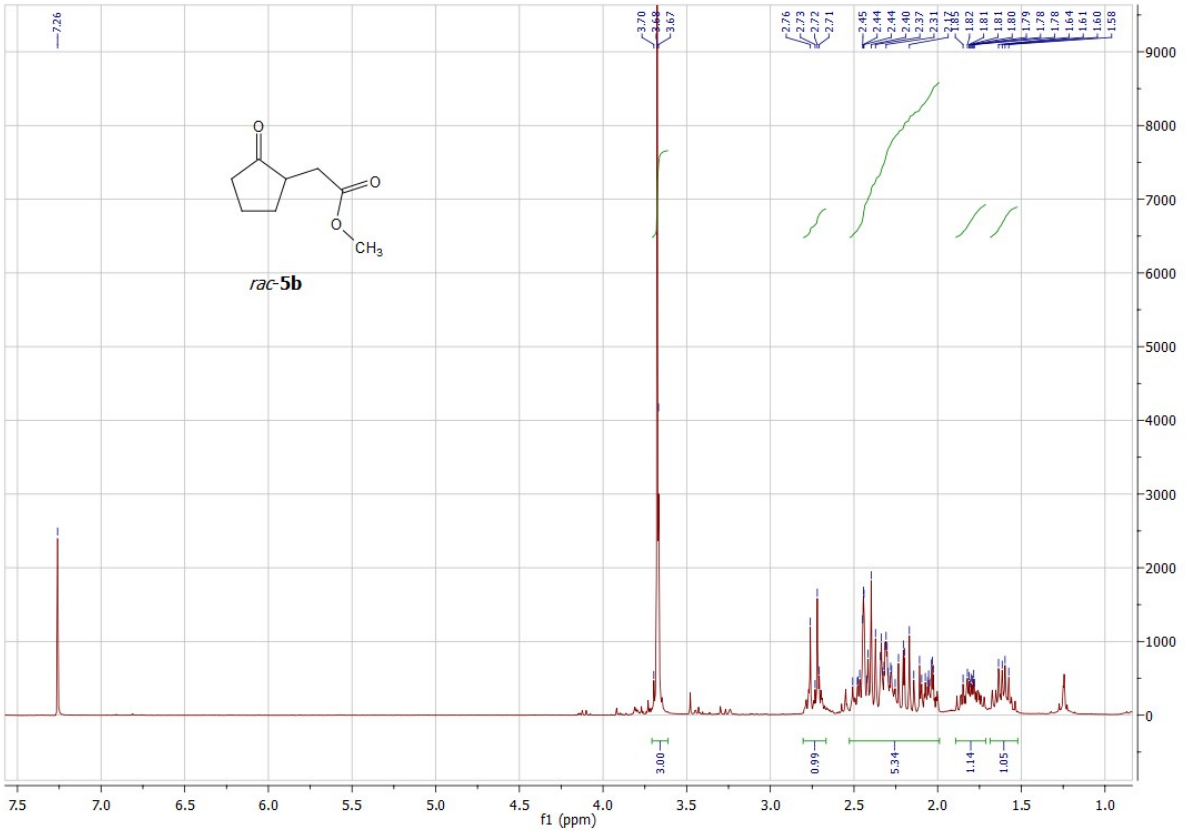
(*R*)-**3b** from XenA ^{13}C



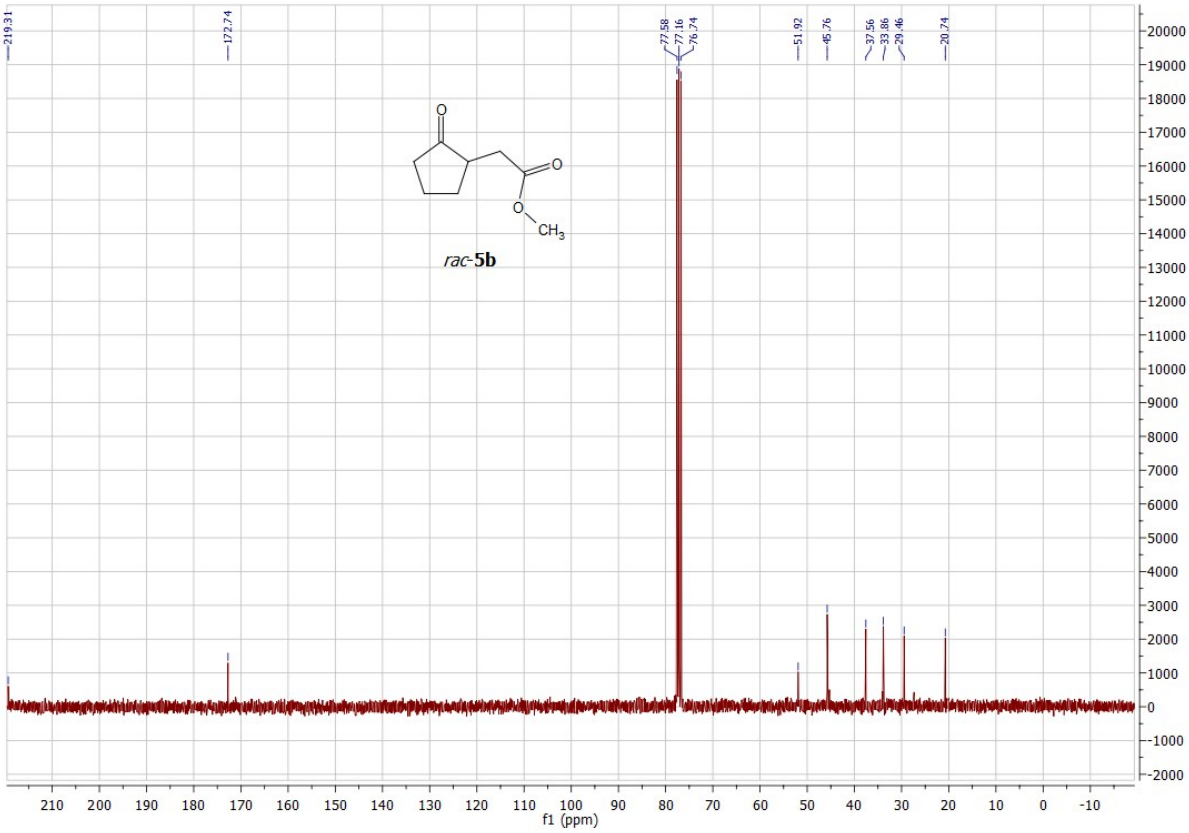
(*R*)-**3b** from XenA HSQC (signals at around 1.2 ppm are impurities already present in the substrate)



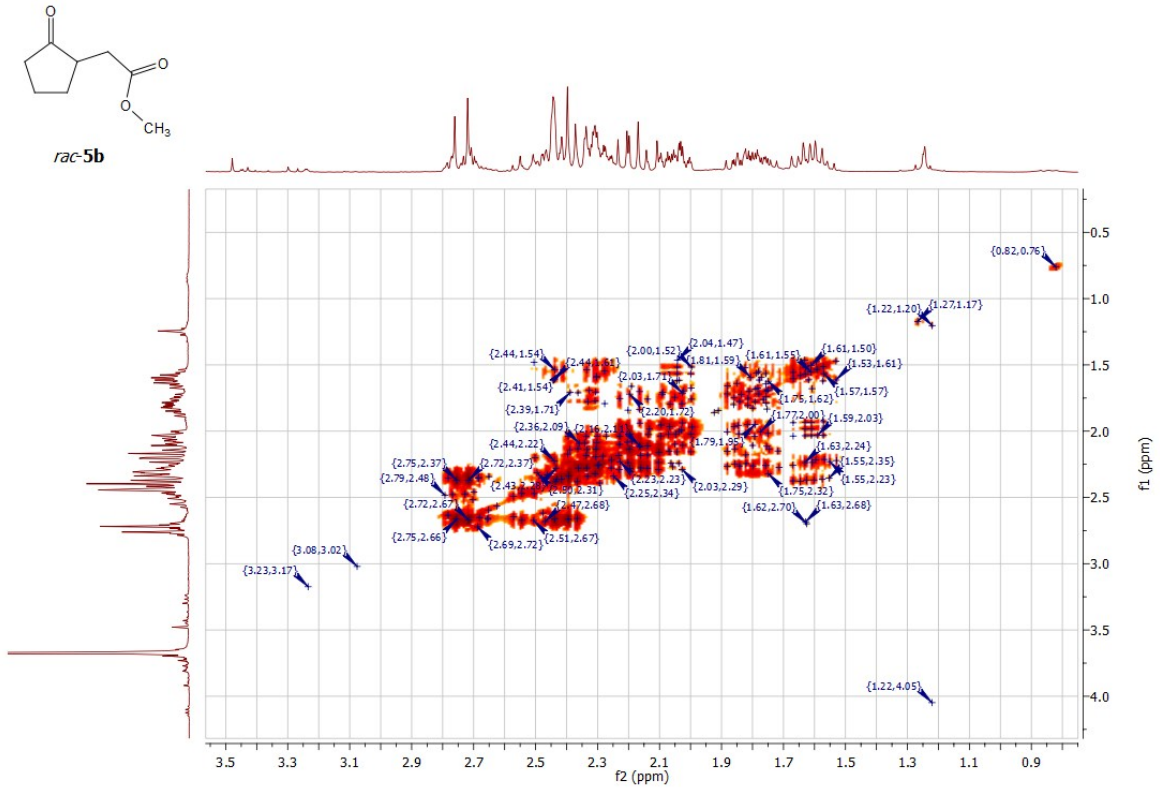
rac-**5b**



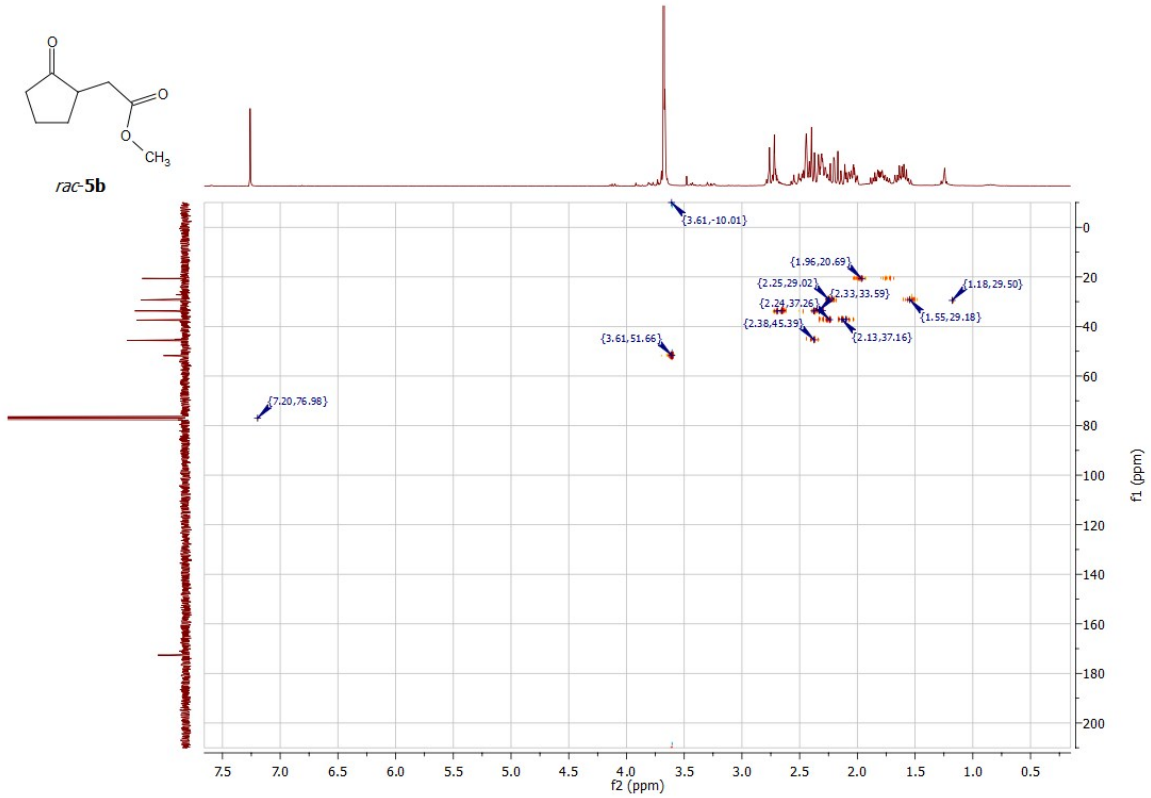
rac-**5b** ^{13}C



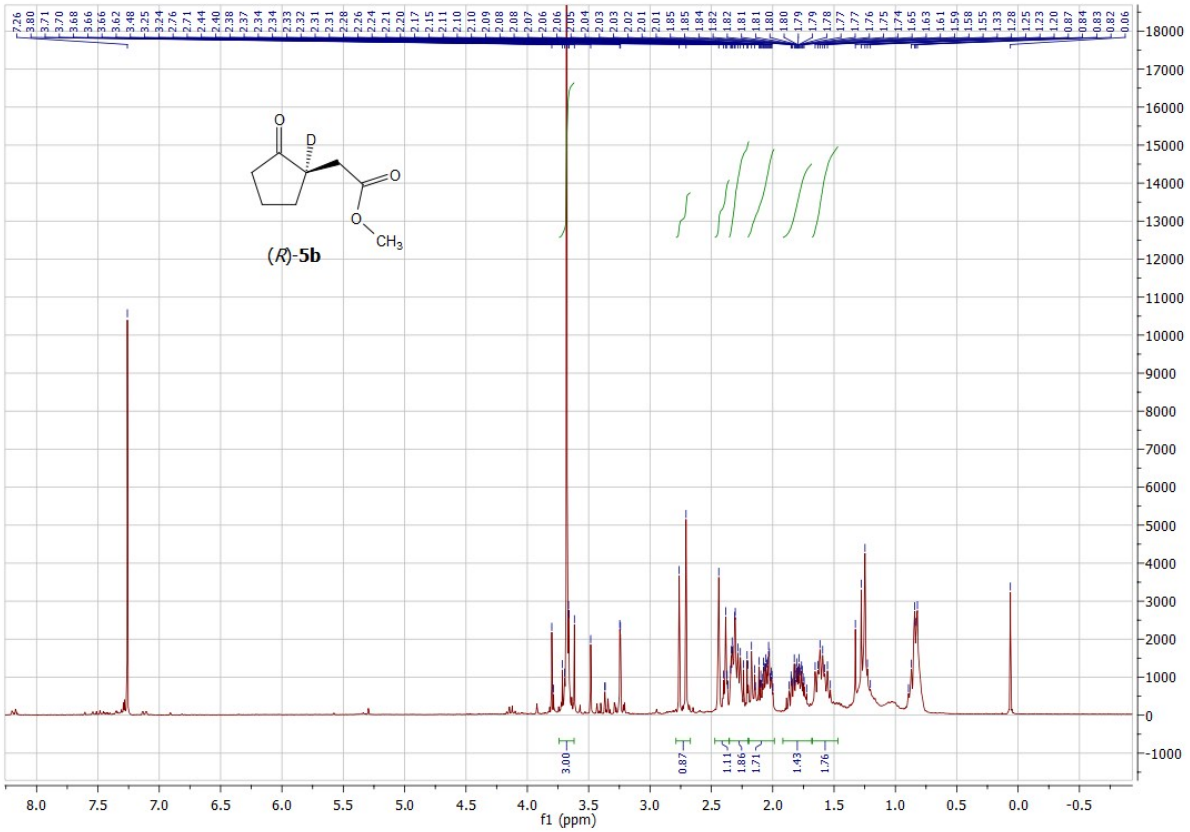
rac-**5b** COSY



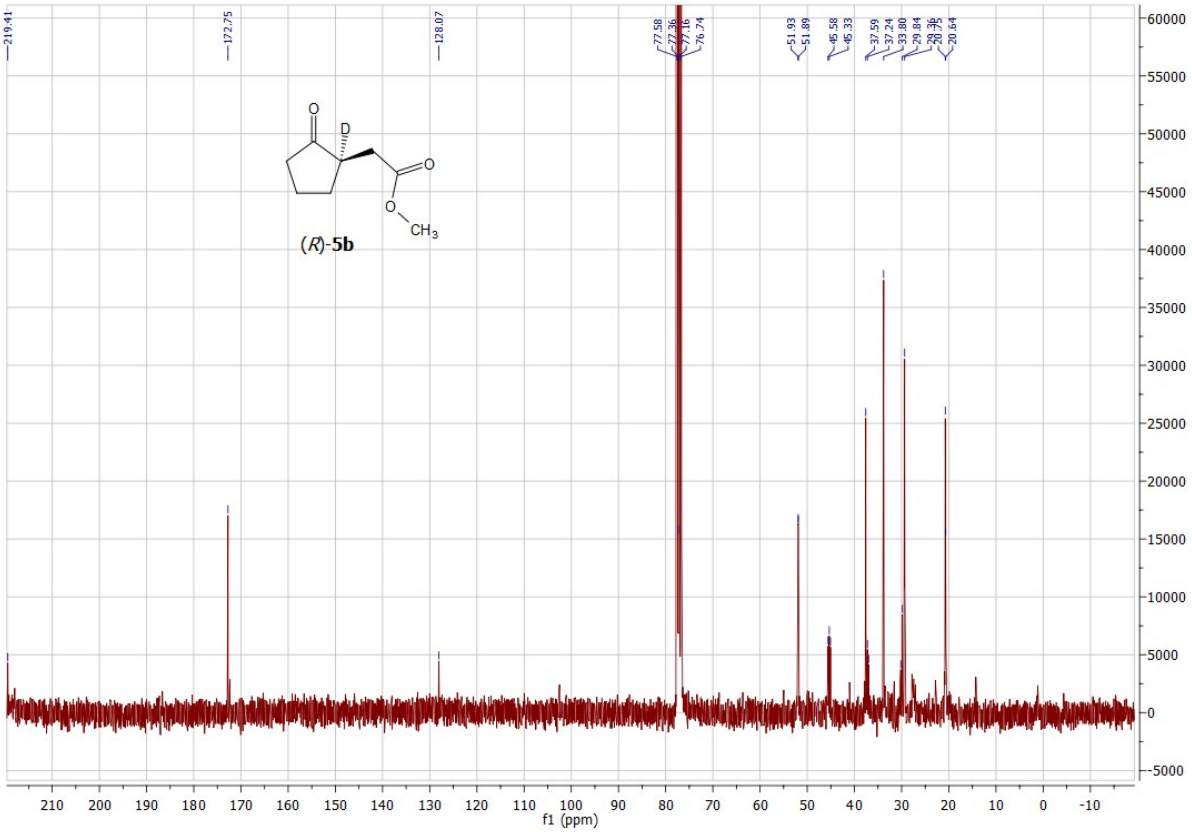
rac-**5b** HSQC



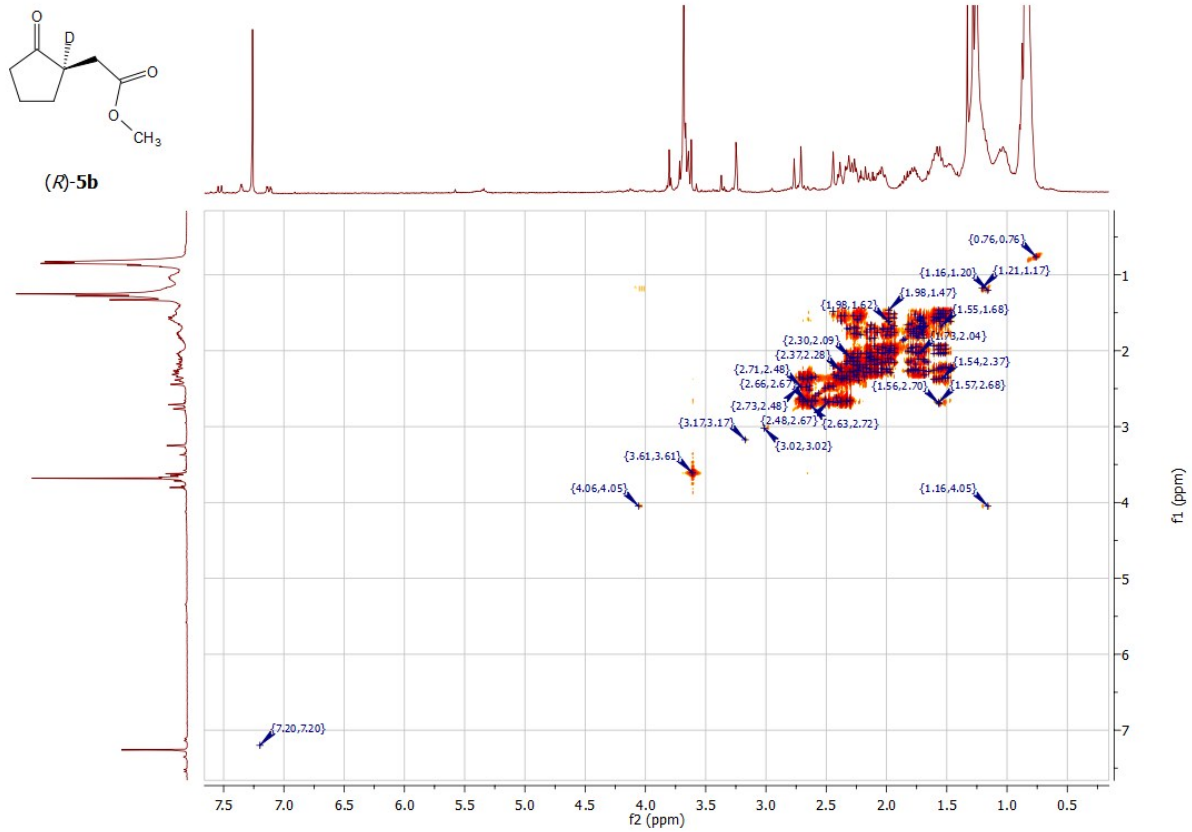
(*R*)-**5b** from NCR



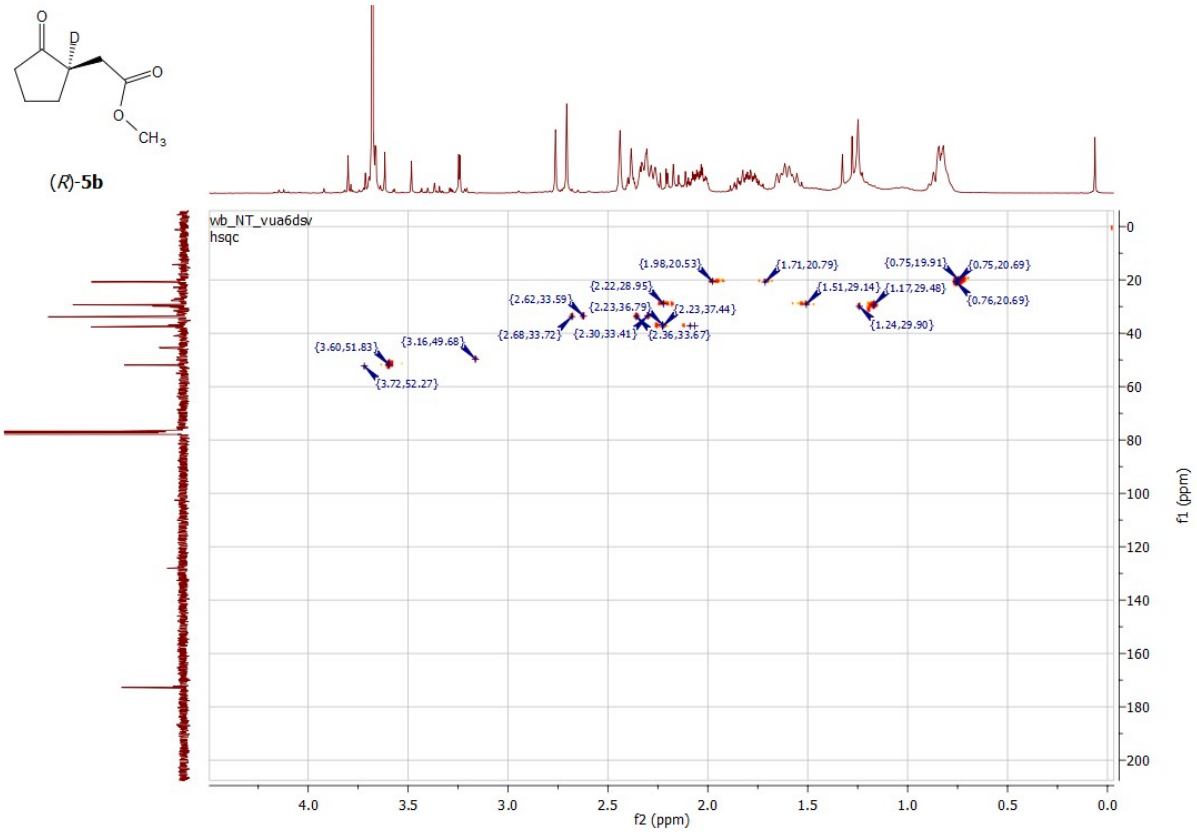
(*R*)-**5b** from NCR ^{13}C



(*R*)-**5b** from NCR COSY



(*R*)-**5b** from NCR HSQC



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