A short, chemoenzymatic route to chiral β-aryl-γ-amino acids using reductases from anaerobic bacteria.

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GENERAL INFORMATION.

CHEMISTRY.

NMR spectra were recorded on 300 MHz or 400 MHz spectrometers and referenced to the solvent, unless stated otherwise. The chemical shifts are reported in ppm and the coupling constants (*J*) are given in hertz (Hz). GC-MS analysis was performed using a RESTEK Rtx®-5MS column (30 m, 0.25 mm ID, 0.25 μm) or a DB-Wax column (30 m, 0.32 mm, 0.25 μm) and a MS unit equipped with ion trap. Melting points were determined using electrothermal capillary apparatus and are uncorrected. IR spectra were recorded neat on NaCl plates. $[\alpha]_D$ values are given in $10^{-1}\cdot deg\cdot cm^2\cdot g^{-1}$. HPLC analysis was performed using an instrument equipped with a UV detector. *Ee* was determined by HPLC using Chiralcel OD or Chiralcel OJ columns (ø 4.6 mm × 250 mm), as described for each compound in the experimental section. UV-visible data were recorded with a diode array spectrophotometer. $[\alpha]_D$ All reactions were monitored by GC or by TLC on standard silica gel plates. All chemicals were obtained from commercial sources and the solvents were of analytical grade.

GROWTH OF MICRORGANISMS AND PREPARATION OF CRUDE EXTRACTS.

Clostridium sporogenes DSM 795, *Acetobacterium woodii* DSM 1030 and *Ruminococcus productus* DSM 3507 strains were obtained from DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. All bacterial strains were stored and cultured at small scale (20 mL) at 30 ºC in an anaerobic cabinet operating under 10% H_2, 10% CO_2 and 80% N_2. To produce cell-free extracts, *R. productus* and *A. woodii* were grown in a 3 L fermentor with stirring at 200 rpm and sparged (1.5 mL min^{-1}) with N_2 (80%) and CO_2 (20%). *C. sporogenes* was grown in 2 L Duran bottles in the anaerobic cabinet at 30 ºC with stirring at 200 rpm. Anaerobic methods for preparation of media, buffers, solvents, growth of cultures and centrifugation were as described previously.[1-3]

Clostridium sporogenes: Stock cultures were maintained on anaerobic cooked meat medium agar consisting of cooked meat powder (5 g/L), agar (20 g/L), KH_2PO_4 (5 g/L) and L-cysteine·HCl (5 g/L). Oxygen-free media for the large scale growth of bacterial cultures were prepared by autoclaving peptone (20 g/L) and yeast extract (5 g/L) in deionized water prior to cooling under nitrogen gas and moving to the anaerobic cabinet. The following sterile anaerobic solutions were then added at the stated volume per L of medium: salt solution (131 g/L K_2HPO_4; 34 g/L KH_2PO_4; 0.021 g/L $NaSeO_3\cdot 5H_2O$; 20 mL); sodium thioglycolate (10 mL, 30 g/L); trace element solution (10 mL; 3.3 g/L $MgCl_2\cdot 6\,H_20$, 4 g/L $CaCl_2\cdot 2H_20$, 2.9 g/L $FeSO_4$, 1 g/L $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$). Anaerobic, filter sterilized solutions of phenylalanine (50 mL/L; 363 mM) and vitamins (10 mL/L; riboflavin, 0.02 g/L; p-amino-benzoic acid, 0.08 g/L; d-biotin, 0.004 g/L) were added immediately prior to inoculation from stock cultures. Total protein concentration of crude extracts was determined to be 36 mg/mL.

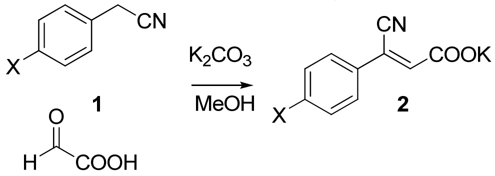
Ruminococcus productus: stock cultures were maintained on anaerobic cooked meat medium plates consisting of cooked meat powder (1 g/L), Agar (20 g/L), KH_2PO_4 (1 g/L), caffeate (180 mg/L), L-cysteine·HCl (250 mg/L) and the pH was adjusted to 7.0 with HCl.[3] Basal medium pH 7 contained: KH_2PO_4 (25 mg/L), K_2HPO_4 (400 mg/L), NH_4Cl (3 g/L), and yeast extract (2 g/L). After sterilization, the following sterile, anaerobic solutions were added to give a final volume of 1000 mL: $MgSO_4\cdot 7H_2O$ (0.02 g/L, 20 mL), $NaHCO_3$ (0.1 g/L, 20 mL in water degassed with oxygen-free CO_2), fructose (0.254 g/L, 10 mL), caffeate (18 mg/L, 10 mL), selenite-tungstate solution (H_2SeO_4 250 mg/L, 2 mL; $Na_2WO_4\cdot 2H_2O$ 50 mg/L, 2 mL), cysteine HCl (250 mg/L, 2 mL), Na_2S (250 mg/L, 2 mL), trace elements (10 mL but with 0.024 g/L $NiCl_2\cdot 6H_2O$ and 0.01 g/L, NaOH and without $MgSO_4\cdot 7H_2O$) and vitamins (10 mL[4], but with only 0.01 mg/L cyanocobalamin). Total protein concentration of crude extracts was determined to be 5.8 mg/mL.

Acetobacterium woodii: stock cultures were maintained on anaerobic agar plates of Balch's medium[5] consisting of NH_4Cl (1 g/L), $MgSO_4$ (0.1 g/L), fructose (3 g/L), agar (20 g/L), $NaHCO_3$ (2 g/L dissolved in water degassed with oxygen-free CO_2), yeast extract (2 g /L), KH_2PO_4 (0.4 g/L), K_2HPO_4 (0.4 g /L) and L-cysteine·HCl (0.5 g/L). The pH of the medium was adjusted to 7.0 before sterilization. The liquid medium was prepared in the same way except that agar was omitted and the medium was supplemented with a vitamins solution[4], and a selenite tungstate solution exactly as described above. Total protein concentration of crude extracts was determined to be 10.7 mg/mL.

Cell harvesting and crude extract preparation: Whole cells were grown overnight to an $OD_{660} = 4.0$ and harvested by centrifugation at 13000 x g in 250 mL centrifuge pots using the anaerobic methods described previously.[1] Cell pellets were washed with anaerobic 50 mM potassium phosphate buffer pH 7.0 and the suspension centrifuged again. The wash step was repeated twice to remove all traces of growth medium. Cells were finally resuspended anaerobically with anaerobic 100 mM phosphate buffer, pH 7.0, containing dithiothreitol (0.1 g/L), 10 μM FAD and a sufficient quantity of a protease inhibitor cocktail (Complete, EDTA-free protease inhibitor cocktail tablets) at the approximate ratio of 2.0 mL of buffer to 1g wet weight cells. Cells were lysed by passage through a French Pressure cell operating at a pressure of 1380 bar and the extract was collected under N_2 and used in biotransformations. Protein concentrations were estimated with the Folin phenol reagent using bovine serum albumin as a standard.

Synthesis of cyano-propanoic acid potassium salts 2

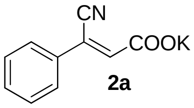
General procedure 1 (according to Dean *et al.*)[6]



To the solution of the nitrile **1** (20.00 mmol) and glyoxalic acid monohydrate (19.95 mmol, 1.84 g) in MeOH (100 mL), K_2CO_3 (30.00 mmol, 4.20 g) was added. The mixture was heated under reflux for 2-16 h, until TLC showed the complete consumption of the starting material. The precipitate was filtered off, washed with dichoromethane and recrystallized from water to give product **2** as white needles.

Characterization data for salts 2

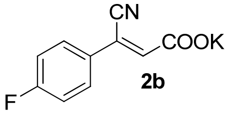
(*Z*)-3-Cyano-3-(phenyl)-propenoic acid potassium salt (*Z*)-2a [6]



2a

The product was prepared according to General procedure 1 as white crystals (57% yield): mp >250 °C (water); ^{1}H NMR (D_2O, 400 MHz) δ 7.0 (s, 1H), 7.35-7.37 (m, 3H), 7.54-7.57 (m, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 117.0, 118.7, 126.4, 129.1, 130.3, 132.1, 139.7, 171.3; HR-MS (ESI(−)) *m/z* calcd for $[M-K]^-$ $C_{10}H_6NO_2$: 172.0399; Found. 172.0406; IR (neat) ν_{max}/cm^{-1} 1397, 1584, 1613, 2220. All spectral data were in accordance with those reported in the literature.[6]

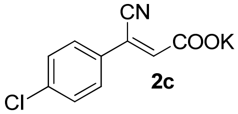
(*Z*)-3-Cyano-3-(4-fluoro-phenyl)-propenoic acid potassium salt ((*Z*)-2b)[7]



2b

The product was prepared according to General procedure 1 as white crystals (65% yield): mp >250 °C (water); ^{1}H NMR (D_2O, 400 MHz) δ 7.94 (s, 1H), 7.08 (t, J = 9.0 Hz, 2H), 7.56 (dd, J_{HF} = 5.2 Hz, J = 9.0 Hz, 2H); HR-MS (ESI(−)) *m/z* calcd for $[M-K]^-$ $C_{10}H_5FNO_2$: 190.0304; Found. 190.0305; IR (neat) ν_{max}/cm^{-1} 830, 1389, 1578, 1612, 2365.

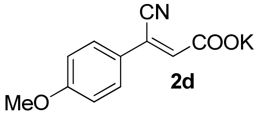
(*Z*)-3-(4-Chloro-phenyl)-3-cyano-propenoic acid potassium salt ((*Z*)-2c)[6]



2c

The product was prepared according to General procedure 1 as white crystals (73% yield): mp >250 °C (water); ^{1}H NMR (D_2O, 400 MHz) δ 7.00 (s, 1H), 7.34 (d, J = 8.9 Hz, 2H), 7.50 (d, J = 8.9 Hz, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 109.1, 117.8, 127.8, 129.1, 130.7, 135.7, 140.0, 171.1; HR-MS (ESI(−)) *m/z* calcd for $[M-K]^-$ $C_{10}H_5ClNO_2$: 206.0009; Found. 206.0003; IR (neat) ν_{max}/cm^{-1} 828, 1208, 1736, 2400. All spectral data were in accordance with those reported in the literature.[6]

(*Z*)-3-Cyano-3-(4-methoxy-phenyl)-propenoic acid potassium salt ((*Z*)-2d)[8]



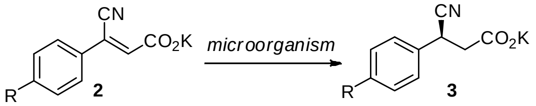
2d

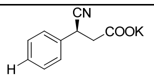
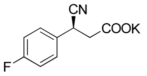
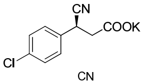
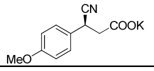
The product was prepared according to General procedure 1 as white crystals (32% yield): mp >250 °C (water); ^{1}H NMR (D_2O, 400 MHz) δ 3.71 (s, 3H), 6.84 (s, 1H), 6.89 (d, J = 9.0 Hz, 2H), 7.48 (d, J = 9.0 Hz, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 55.4, 114.5, 117.1, 118.6, 124.8, 128.0, 137.2, 160.6, 171.5; HR-MS (ESI(−)) *m/z* calcd for $[M{-}K]^-$ $C_{10}H_8NO_3$: 202.0504; Found. 202.0501; IR (neat) ν_{max}/cm^{-1} 817, 1035, 1249, 1575, 1610, 2390. All spectral data were in accordance with those reported in the literature.[8]

Typical experimental procedure for analytical assays for bioreduction of propenoates 2

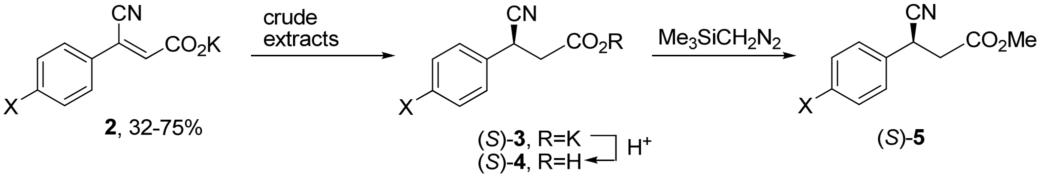
Salt **2** (0.09 mmol); NADH (0.10 mmol, 76 mg); crude extracts of *C. sporogenes, R. productus* and *A. woodii* (1 mL, crude extracts of *C. sporogenes* - 36 mg of protein/mL; *Acetobacterium woodii* - 10.7 mg of protein /mL and *Ruminococcus productus* - 5.8 mg of protein /mL), phosphate buffer (20 mL, 50 mM, pH 7.0) were incubated under H_2 atmosphere at 30 °C for 3 days. The reactions were monitored by GC (conversion) and HPLC (*ee*, Chiralcel OD), after acidification and derivatization of the product and unreacted substrate to their respective methyl esters **5**, using a (trimethylsilyl)diazomethane solution (2M in hexanes).

Table S1. Reduction of 3-nitrilo-3-aryl-propanoic acid salts (**2a-d**) by crude extracts from *C. sporogenes*, *A. woodii* and *R. productus* after 3 and 6 days.



		C. sporogenes			*A. woodii*			*R. productus*		
Substrate	Product	Conf	Conv. (*ee*)		Conf	Conv. (*ee*)		Conf	Conv.(*ee*)	
			3 days	6 days		3 days	6 days		3 days	6 days
2a	**3a** 	*S*	>99 (93%)	>99 (88%)	*S*	>99 (92%)	>99 (84%)	*S*	>99 (94%)	>99 (92%)
2b	**3b** 	*S*	>99 (98%)	>99 (96%)	*S*	>99 (57%)	>99 (49%)	*S*	>99 (95%)	>99 (93%)
2c	**3c** 	*S*	95 (96%)	98 (92%)	*S*	95 (89%)	98 (81%)	*S*	94 (91%)	98 (91%)
2d	**3d** 	*S*	94 (≥99%)	98 (≥99%)	*S*	95 (≥99%)	98 (≥99%)	*S*	93 (≥99%)	98 (≥99%)

General procedure 2 Preparative scale reduction of compounds 2 by crude extracts of *C. sporogenes*.

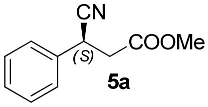


To the solution of the cyano-propenoic acid potassium salt (**2**, 0.70 mmol) and NADH (0.77 mmol, 588 mg) in potassium buffer (142 mL, 50 mM, pH 7.0), crude extracts of *C. sporogenes* (8 mL, 36 mg of protein/mL) was added. The flasks were flushed with hydrogen and shaken at 200 rpm at 30 ºC. Aliquots (2.0 mL) were taken every 8 h to monitor the reaction progress. The aliquots were acidified with 5 M HCl (0.1 mL) and extracted with Et_2O (2.5 mL) containing an internal standard (*t*-Bu-benzene, 5 μL/mL). To the ethereal phase (0.5 mL), MeOH (0.1 mL) was added, followed by a few drops of (trimethylsilyl)diazomethane solution (2.0 M in diethyl ether), until the mixture had a persistent yellow color. Acetic acid (15 μL) was added after 30 min and the sample was analyzed by GC-MS.

Upon the completion of the biotransformation, as indicated by GC, the reaction mixture was acidified with 1 M HCl (15 mL) to pH 1, extracted with Et_2O (2 × 150 mL) and $CHCl_3$ (1 × 150 mL). The combined organic layers were dried ($MgSO_4$) and evaporated to dryness to give crude acid (*S*)-**4** that was used without further purification. The product **4** was dissolved in Et_2O (5 mL) and MeOH (5 mL) on cooled to 0 °C. (Trimethylsilyl)diazomethane solution (2.0 M in diethyl ether) was added drop wise until gas stopped evolving and the mixture maintained a persistent yellow color. The mixture was allowed to warm up to rt and it was stirred for 1.5 h, then cooled again and a few drops of acetic acid were added until gas stopped evolving. The solvent was evaporated in *vacuo* and the product (*S*)-**5** was purified by flash chromatography (Hexane:AcOEt, 9→1 to 8→2). The yields and conversions are reported for the overall two step bioreduction-esterification sequence. The optical purity of the products **5** was determined by chiral HPLC, using Chiralcel OJ or OD columns and the details are given for each compound.

Characterization data for the optically active methyl 3-aryl-3-cyano-propanoates (*S*)-5

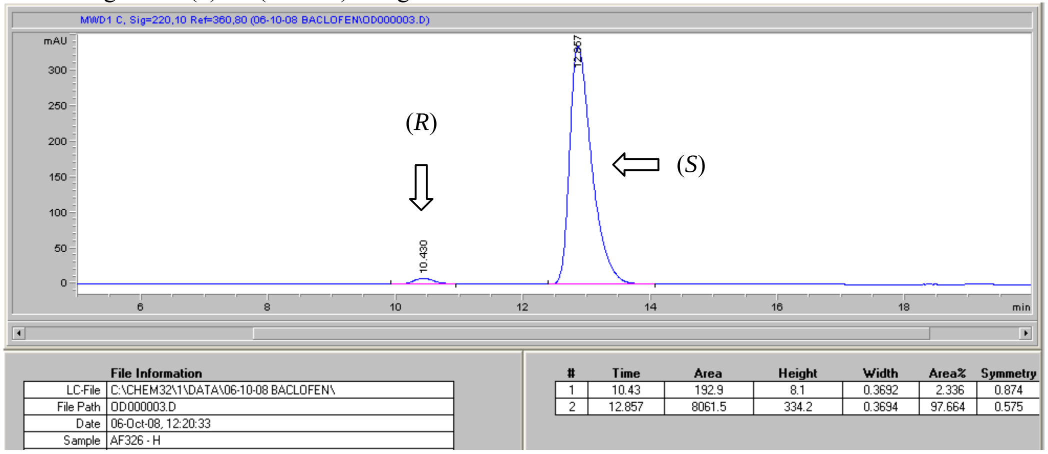
(*S*)-3-Cyano-3-(phenyl)-propanoic acid methyl ester ((*S*)-5a)



5a

The product was prepared according to General procedure 2 and purified by chromatography (hexane/AcOEt, 85:15) to give (*S*)-**5a** as transparent oil that solidified (82% total yield from **2c**): mp 29-30 °C (hexane/AcOEt); ^{1}H NMR (CDCl$_3$, 400 MHz) δ 2.86 (dd, *J* = 6.7 Hz, *J* = 16.6 Hz, 1H), 3.03 (dd, *J* = 8.3 Hz, *J* = 16.6 Hz, 1H), 3.73 (s, 3H), 4.31 (dd, *J* = 6.7 Hz, *J* = 8.3 Hz, 1H), 7.35-7.41 (m, 5H); ^{13}C NMR (CDCl$_3$, 100 MHz) δ 33.1, 39.8, 52.3, 119.8, 127.2, 128.5, 129.2, 134.3, 169.6; $[\alpha]_D = -15.3$ (*c* 1.15, MeOH, (*S*)-enantiomer, 29 °C, 94% *ee*), Chiralcel OD: hexane/*i*-PrOH, 9:1, t_R = 10.7 min, t_S = 13.7 min; MS (EI) *m/z* 189 (60%, M$^+$), 129 (100%); IR (neat) ν_{max}/cm^{-1} 1210, 1737, 2236; Anal. calcd for C$_{11}$H$_{11}$NO$_2$: C, 69.83; H, 5.86; N, 7.40; Found: C, 69.94; H, 5.95; N, 7.35. The corresponding racemic compound was synthesized according to General procedure 5 and obtained as a white solid (81% yield): mp 58 °C (hexane/AcOEt).

Chromatogram of (*S*)-**5a** (95% *ee*) using Chiralcel OD column



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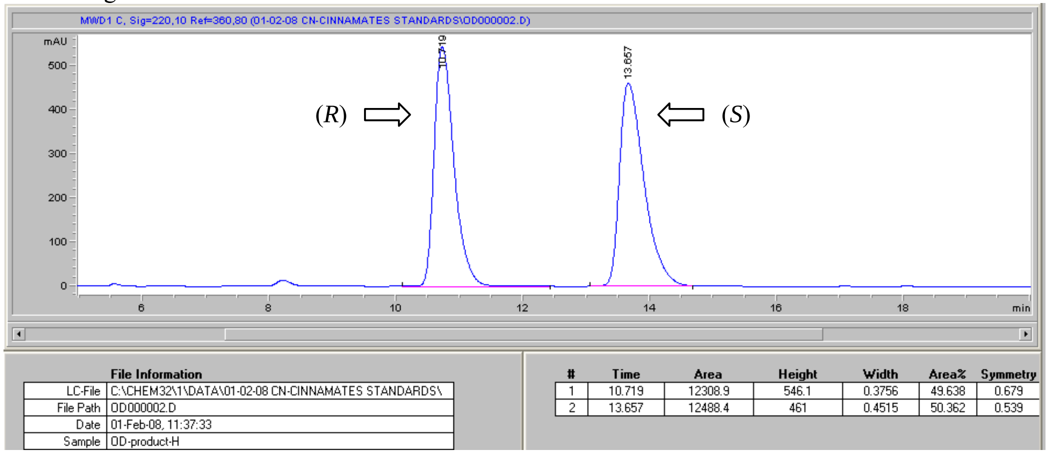
Chromatogram of *rac*-**5a** on Chiralcel OD



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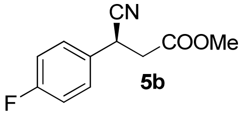
2	13.657	12488.4	461	0.4515	50.362	0.539

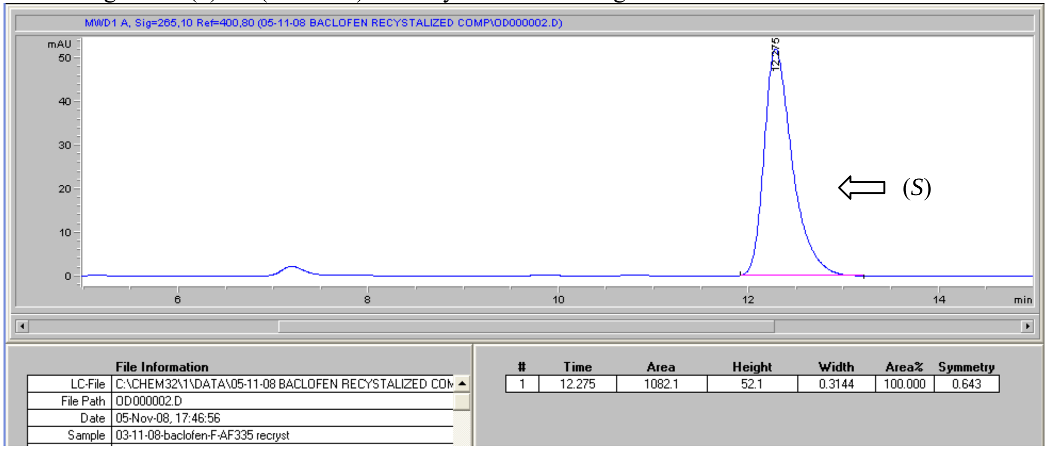
(*S*)-3-Cyano-3-(4-fluoro-phenyl)-propanoic acid methyl ester ((*S*)-5b)



5b

The product was prepared according to General procedure 2 and purified by chromatography (hexane/AcOEt, 85:15) to give (*S*)-**5b** as white solid. The product was recrystallized to give white needles (80% total yield from **2c**): mp 59-60 °C (hexane/ Et_2O); (≥99% *ee*); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.76 (dd, J = 7.2 Hz, J = 16.8 Hz, 2H), 2.94 (dd, J = 8.0 Hz, J = 16.8 Hz, 2H), 3.64 (s, 3H), 4.22 (t, J = 7.6 Hz, 1H), 6.98-7.03 (m, 2H), 7.26-7.30 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 32.3, 39.7, 52.3, 116.4 (d, J = 22.1 Hz), 119.7, 129.2 (d, J = 8.3 Hz), 130.2 (d, J = 3.1 Hz), 162.7 (d, J = 248.0 Hz), 169.4; $[\alpha]_D = -9.3$ (*c* 1.48, MeOH, (*S*)-enantiomer, 20 °C, 91% *ee*), Chiralcel OD: hexane/*i*-PrOH, 9:1, t_R = 11.1 min, t_S = 12.9 min; MS (EI) *m/z* 207 (65%, M$^+$), 147 (100%), 134 (100%); IR (neat) ν_{max}/cm^{-1} 1227, 1510, 1736, 2246; Anal. calcd for $C_{11}H_{10}NO_2F$:C, 63.76; H, 4.86; N, 6.76; Found: C, 63.85; H, 4.67; N, 6.52. The corresponding racemic compound was synthesized according to General procedure 5 in 66% yield as white solid: mp 56-57 °C (hexane/ Et_2O).

Chromatogram of (*S*)-**5b** (>99% *ee*) after crystallization using Chiralcel OD column



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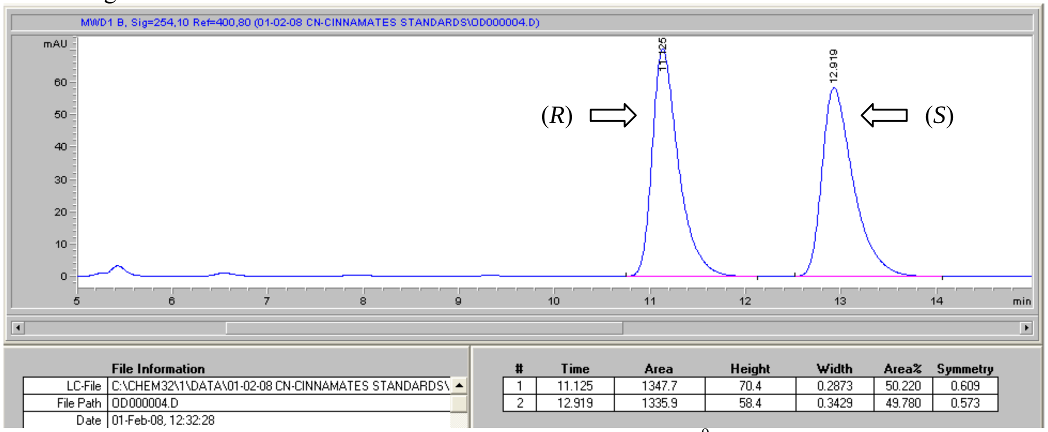
Chromatogram of *rac*-**5b** on Chiralcel OD



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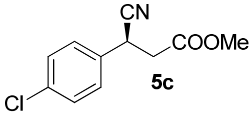
2	12.919	1335.9	58.4	0.3429	49.780	0.573

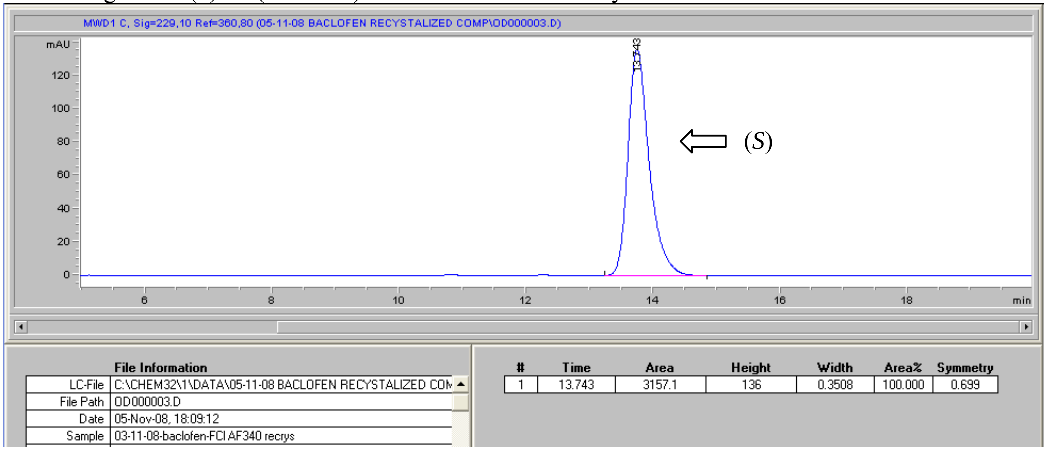
3-(4-Chloro-phenyl)-3-cyano-propanoic acid methyl ester ((*S*)-5c)[9]



5c

The product was prepared according to General procedure 2 and purified by chromatography (hexane/AcOEt, 85:15) to give (*S*)-**5c** as white solid (95% *ee*, 83% total yield from **2c**). Single recystallization gave pure enantiomer >99% *ee* as white needle crystals: mp 53°C (hexane/ Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.85 (dd, J = 7.0 Hz, J = 16.7 Hz, 1H), 3.03 (dd, J = 7.8 Hz, J = 16.7 Hz, 1H), 3.73 (s, 3H), 4.31 (dd, J = 7.0 Hz, J = 7.8 Hz, 1H), 7.33 (d, J = 8.0 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 32.6, 39.6, 52.5 119.5, 128.8, 129.5, 132.9, 134.7, 169.4; $[\alpha]_D$ = – 7.49 (*c* 2.15, MeOH, (*S*)-enantiomer, 29 °C, 91% *ee*), lit. $[\alpha]_D$ = +8.7 (*c* 1.1, MeOH, (*R*)-enantiomer),[9] Chiralcel OD: hexane/*i*-PrOH, 9:1, t_R = 12.8 min, t_S = 14.4 min; MS (EI) *m/z* 225 (22%, M^+), 223 (66%, M^+), 163 (100%); IR (neat) ν_{max}/cm^{-1} 827, 1251, 1294, 1512, 1601. The respective racemic compound was synthesized according to General procedure 5 in 92% yield and obtained as white crystals: mp 67-68 °C (hexane/ Et_2O). Anal. calcd for $C_{11}H_{10}ClNO_2$: C, 59.07; H, 4.51; N, 6.26; Anal. Found: C, 58.75; H, 4.32; N, 6.20. All spectral data were in accordance with those reported in literature.[9]

Chromatogram of (*S*)-**5c** (>99% *ee*) on Chiralcel OD after crystallization



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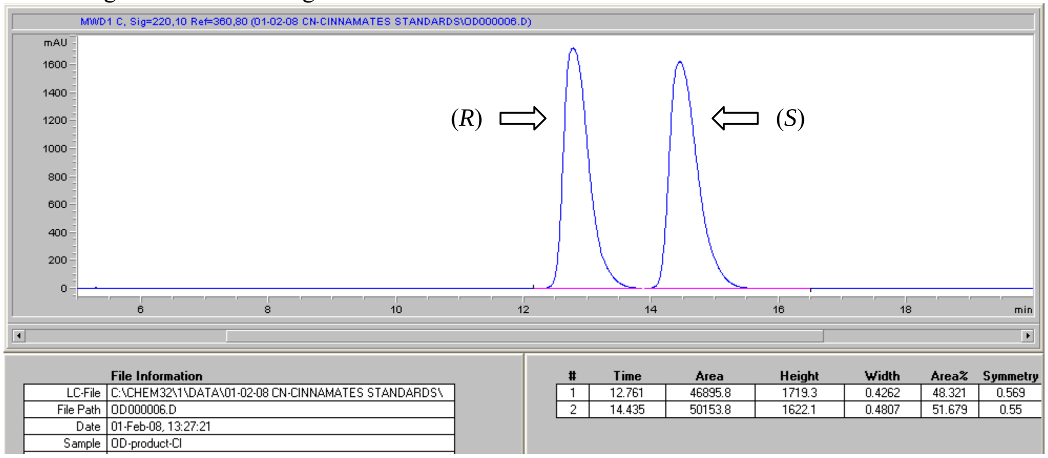
File Path	OD000003.D
Date	05-Nov-08, 18:09:12
Sample	03-11-08-baclofen-FCI AF340 recrys

#	Time	Area	Height	Width	Area%	Symmetry
1	13.743	3157.1	136	0.3508	100.000	0.699

Chromatogram of *rac*-**5c** using Chiralcel OD column



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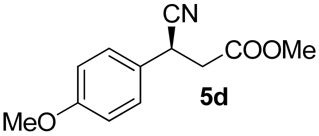
File Path	OD000006.D
Date	01-Feb-08, 13:27:21
Sample	OD-product-Cl

#	Time	Area	Height	Width	Area%	Symmetry
1	12.761	46895.8	1719.3	0.4262	48.321	0.569
2	14.435	50153.8	1622.1	0.4807	51.679	0.55

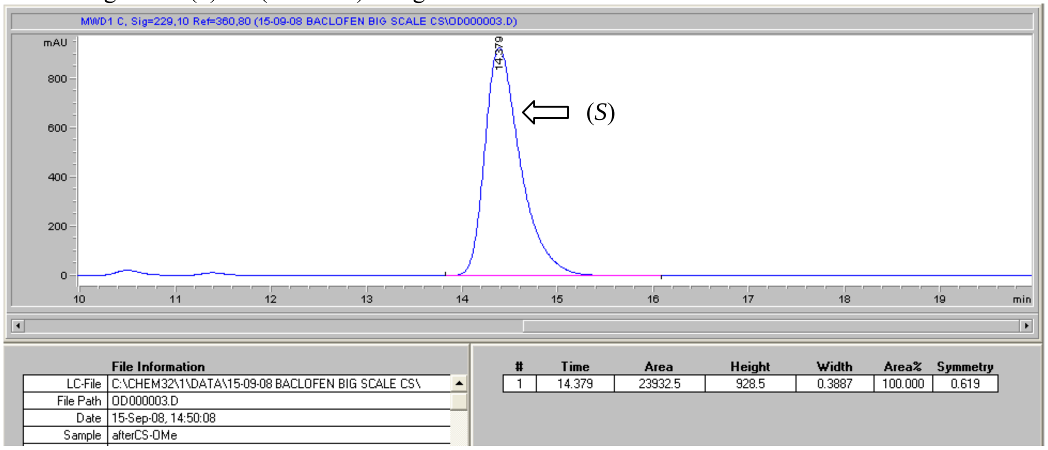
3-Cyano-3-(4-methoxy-phenyl)-propanoic acid methyl ester ((*S*)-5d)



5d

The product was prepared according to General procedure 2 and purified by chromatography (hexane/AcOEt, 8:2) to give (*S*)-**5d** as a transparent oil (77% yield total yield from **2d**): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.82 (dd, *J* = 6.9 Hz, *J* = 16.6 Hz, 2H), 3.00 (dd, *J* = 8.0 Hz, *J* = 16.6 Hz, 2H), 3.71 (s, 3H), 3.81 (s, 3H), 4.31 (dd, *J* = 6.9 Hz, *J* = 8.0 Hz, 1H), 6.90 (d, *J* = 8.8 Hz, 2H), 7.27 (d, *J* = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.9, 39.8, 52.2, 55.3, 114.5, 120.1, 126.2, 128.4, 159.6, 169.6; $[\alpha]_D$ = - 4.9 (*c* 1.45, MeOH, (*S*)-enantiomer, 29 °C, ≥99% *ee*), Chiralcel OD: hexane/*i*-PrOH, 9:1, t_R = 13.5 min, t_S = 14.6 min; MS (EI) *m/z* 219 (55%, M^+), 159 (100%); IR (neat) ν_{max}/cm^{-1} 1253, 1514, 1740, 2244; Anal. calcd for $C_{12}H_{13}ClNO_3$: C, 65.74; H, 5.98; N, 6.39; Found: C, 65.81; H, 6.17; N, 6.16. A respective racemic compound was synthesized according to General procedure 5 in 96% yield as transparent oil. Anal. Found: C, 65.40; H, 6.11; N, 6.21.

Chromatogram of (*S*)-**5d** (>99% *ee*) using Chiralcel OD column



File Information

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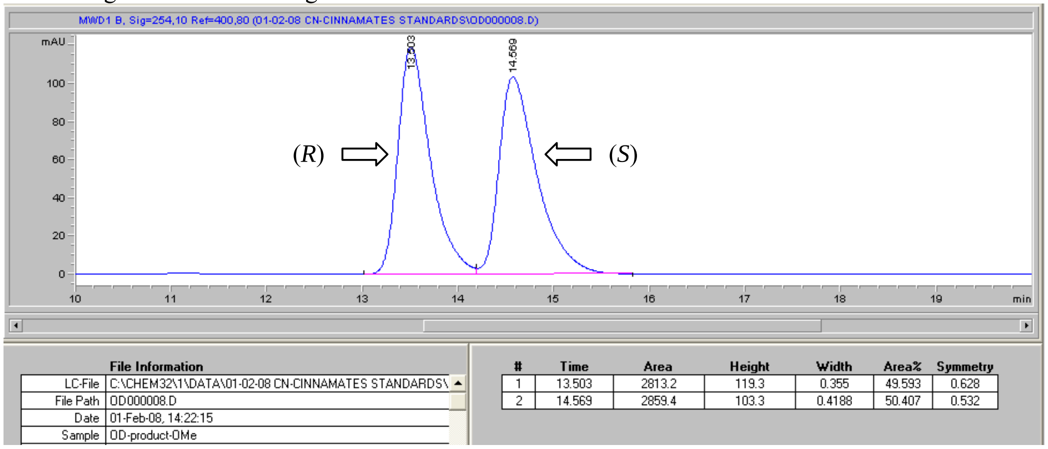
File Path	OD000003.D
Date	15-Sep-08, 14:50:08
Sample	afterCS-OMe

#	Time	Area	Height	Width	Area%	Symmetry
1	14.379	23932.5	928.5	0.3887	100.000	0.619

Chromatogram of *rac*-**5d** using Chiralcel OD column

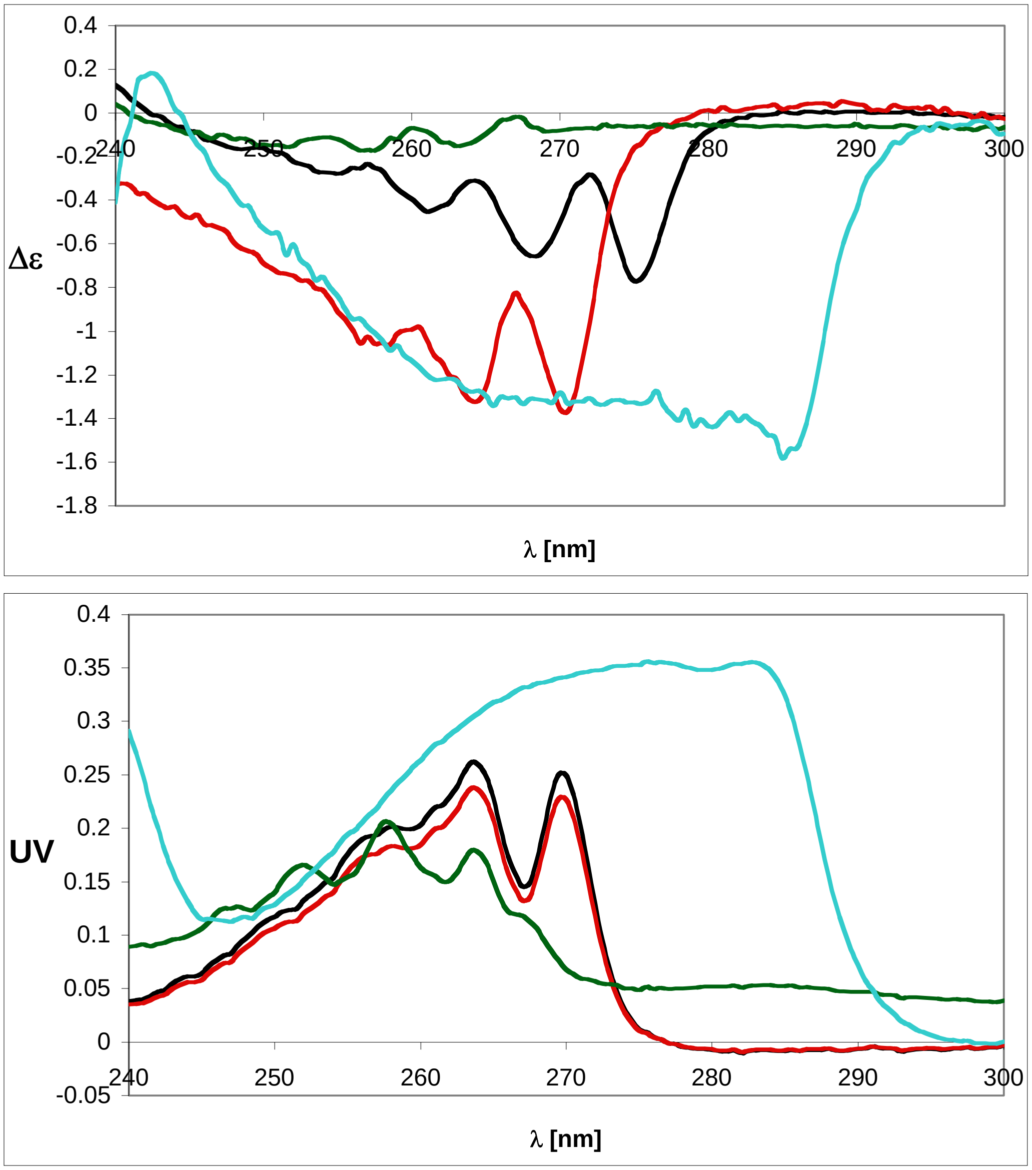


File Information

LC-File	C:\CHEM32\1\DATA\01-02-08 CN-CINNAMATES STANDARDS\
File Path	OD000008.D
Date	01-Feb-08, 14:22:15
Sample	OD-product-OMe

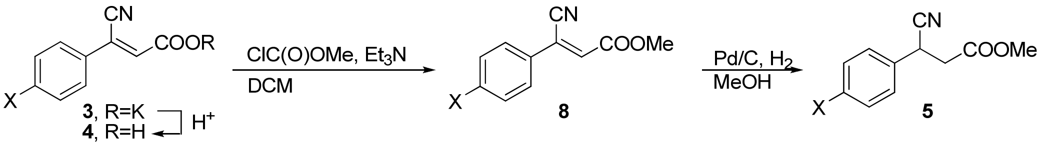
#	Time	Area	Height	Width	Area%	Symmetry
1	13.503	2813.2	119.3	0.355	49.593	0.628
2	14.569	2859.4	103.3	0.4188	50.407	0.532

Figure 1 CD and UV spectra of compounds.[a]



[a] (*S*)-**5a** (—), (*S*)-**5b** (—), (*S*)-**5c** (—) and (*S*)-**5d** (—). Spectra were recorded in MeCN at rt for the following concentrations: (*S*)-**5a** 0.00515 mM, (*S*)-**5b** 0.00241 mM, (*S*)-**5c** 0.00567 mM and (*S*)-**5d** 0.00178. Negative Cotton effect corresponds to aryl chromophore.

Synthesis of racemic propanoates 5



General procedure 3: Synthesis of 3-aryl-3-cyano-propenoic acids 4

To the suspension of cyano-propenoic acid potassium salt **2** in water, 1 M HCl was added to pH 1 and the mixture was stirred for 20 min at rt. The precipitate was filtered off and redissolved in hot AcOEt and filtered again. The filtrate was concentrated in *vacuo* and recrystallized from AcOEt/hexane to give pure cyano-propenoic acid **4**.

General procedure 4: Synthesis of methyl 3-aryl-3-cyano propenoates 8

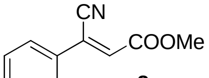
To the solution of cyano-propenoic acid **4** (1.20 mmol) and DMAP (cat) in dry dichloromethane (10 mL) under argon, Et_3N (1.30 mmol, 130 mg, 180 μL) was added drop wise at 0 °C, followed after 15 min by methyl chloroformate (1.30 mmol, 123 mg, 100 μL). The mixture was allowed to warm up to rt and MeOH (100 μL) was added and the stirring was continued for 2 h. The solution was washed with brine and dried ($MgSO_4$). Evaporation of the solvent gave product **8** that was purified by flash chromatography (hexane/Et_2O or hexane/AcOEt, as described for each compound).

General procedure 5: Synthesis of racemic methyl 3-aryl-3-cyano-cyano-propanoates 5

Hydrogen was purged through a suspension of Pd/C (5%, 20 mg) and the compound **8** (0.5 mmol) in MeOH (10 mL) overnight. The catalyst was filtered off through a Celite plug, the solution was evaporated to give crude product that was purified by flash chromatography (hexane/Et_2O or hexane/AcOEt, as described for each compound). Yields and characterization data are given next to the characterisation data for the respective optically active compounds **5**.

Characterization data for methyl propenoates 8

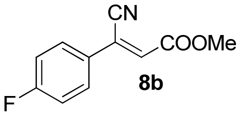
(*Z*)-3-Cyano-3-(phenyl)-propenoic acid methyl ester ((*Z*)-8a)[10, 11]



8a

The product was prepared according to (General procedure 4) and purified by chromatography (hexane/Et_2O, 9:1) to give white crystals (40% yield); mp 83-84 °C (hexane/ Et_2O; lit 78-81 C[11]); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.91 (s, 3H), 6.89 (s, 2H), 7.42-7.51 (m, 3H), 7.71-7.74 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.5, 114.9, 126.3, 127.0, 128.8, 129.2, 131.6, 131.7, 163.6; MS (EI) *m/z* 187 (100%, M; IR (neat) ν_{max}/cm^{-1} 1198, 1345, 1717, 2400; UV-VIS: 297 nm, 227 nm; Anal. calcd for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48; Found: C, 70.60; H, 4.82; N, 7.37. All spectral data were in accordance with those reported in the literature.[10, 11]

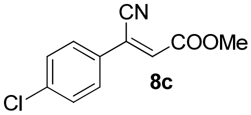
(*Z*)-3-Cyano-3-(4-fluoro-phenyl)-propenoic acid methyl ester ((*Z*)-8b)



8b

The product was prepared according to (General procedure 4) and purified by chromatography (hexane/Et_2O, 9:1) to give white crystals (46% yield): mp 86 ºC (hexane/ Et_2O); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.90 (s, 3H), 6.83 (s, 2H), 7.15-7.19 (m, 2H), 7.71-7.74 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.6, 114.9, 116.3, 116.7, 125.1, 128.0, 128.6, 129.2, 129.3, 163.6, 165.9; MS (EI) *m/z* 205 (100%, M^+), 174 (100%); IR (neat) ν_{max}/cm^{-1} 837, 1196, 1342, 1590, 1720, 2345; UV-VIS: 294 nm, 226 nm; Anal. calcd for $C_{11}H_8FNO_2$: C, 64.39; H, 3.93; N, 6.83; Found: C, 64.14; H, 3.81; N, 6.76.

(*Z*)-3-(4-Chloro-phenyl)-3-cyano-propenoic acid methyl ester ((*Z*)-8c)[11]

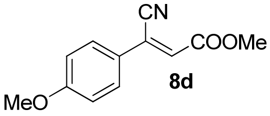


8c

The product was prepared according to (General procedure 4) and purified by chromatography (hexane/Et_2O, 9:1) to give white crystals (84% yield): mp 104-105 °C (hexane/ Et_2O; lit. 102.5-

103.5 °C)[11]; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.88 (s, 3H), 6.86 (s, 2H), 7.44 (d, *J* = 8.3 Hz, 2H), 7.64 (d, *J* = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.6, 114.7, 125.2, 128.6, 129.2, 129.6, 130.2, 138.0, 163.5; MS (EI) *m/z* 223 (20%, M^+), 221 (50%, M^+), 190(100%); IR (neat) ν_{max}/cm^{-1} 1095, 1175, 1198, 1339, 1731, 2246; UV-VIS: 297 nm, 229 nm; Anal. calcd for $C_{11}H_8ClNO_2$: C, 59.61; H, 3.64; N, 6.32; Found: C, 59.67; H, 3.57; N, 6.29. All spectral data were in accordance with those reported in the literature.[11]

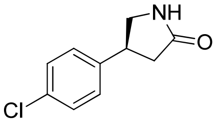
(*Z*)-3-Cyano-3-(4-methoxy-phenyl)-propenoic acid methyl ester ((*Z*)-8d)[12]



8d

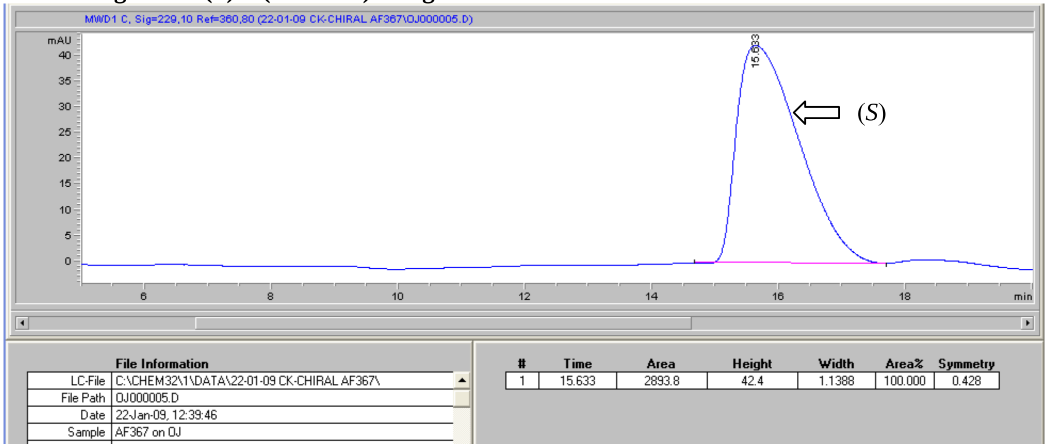
The product was prepared according to (General procedure 4) and purified by chromatography (hexane/Et_2O, 9:1) to give white crystals (67% yield): mp 74-76 °C (hexane/ Et_2O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.88 (s, 3H), 3.89 (s, 3H), 6.78 (s, 1H), 6.97 (d, *J* = 9.1 Hz, 2H), 7.69 (d, *J* = 9.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.4, 55.5, 114.7, 115.2, 124.2, 125.8, 125.9, 128.7, 162.4, 164.0; IR (neat) ν_{max}/cm^{-1} 828, 1190, 1298, 1713, 2354; UV-VIS: 328 nm, 227 nm; Anal. calcd for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45; Found: C, 66.31; H, 5.06; N, 6.38.

Synthesis of (+)-4-(4-chloro-phenyl)-pyrrolidin-2-one 6 and (*S*)-6[9]



γ-Lactam (*S*)-**6** was synthesized from nitrile (*S*)-**5c** (≥99% *ee*) according to the published procedure[9] and purified by chromatography (AcOEt/EtOH, 9:1) to give white solid (70% yield): mp 108-111 °C (EtOH, lit. 111 °C[9]); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.45 (dd, *J* = 8.8 Hz, *J* = 17.0 Hz, 1H), 2.75 (dd, *J* = 9.1 Hz, *J* = 17.0 Hz, 1H), 3.35-3.40 (m, 1H), 3.64-3.69 (m, 1H), 3.75-3.79 (m, 1H), 6.06 (bs, 1H), 7.20 (d, *J* = 6.5 Hz, 2H), 7.32 (d, *J* = 6.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.9, 39.7, 49.4, 126.8, 128.2, 129.0, 132.9, 140.7, 177.5; $[\alpha]_D$ = + 31.7 (*c* 0.75, EtOH, (*S*)-enantiomer, 18 °C), lit. – 39 (c 1.00, EtOH, (*R*)-enantiomer[9]); Chiralcel OJ: hexane/*i*-PrOH, 9:1, t_R = 14.3 min, t_S = 15.6 min; Chiralcel OD: hexane/*i*-PrOH, 9:1, t_R = 21.5 min, t_S = 22.5 min. The respective racemic compound was synthesized in the same manner in 78% yield and obtained as a white solid: mp 109-111 °C (EtOH, lit. 111[9, 13, 14]). All spectral data were in accordance with those reported in literature.[9]

Chromatogram of (*S*)-6 (≥99% *ee*) using Chiralcel OJ column



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Sample	AF367 on OJ

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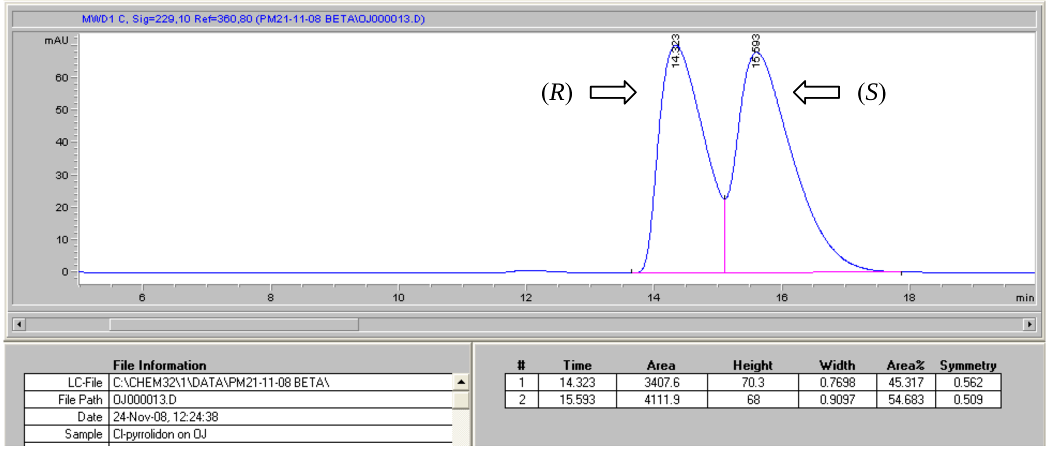
1	15.633	2893.8	42.4	1.1388	100.000	0.428

Chromatogram of *rac*-**6** using Chiralcel OJ column



File Information

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Sample	Cl-pyrrolidon on OJ

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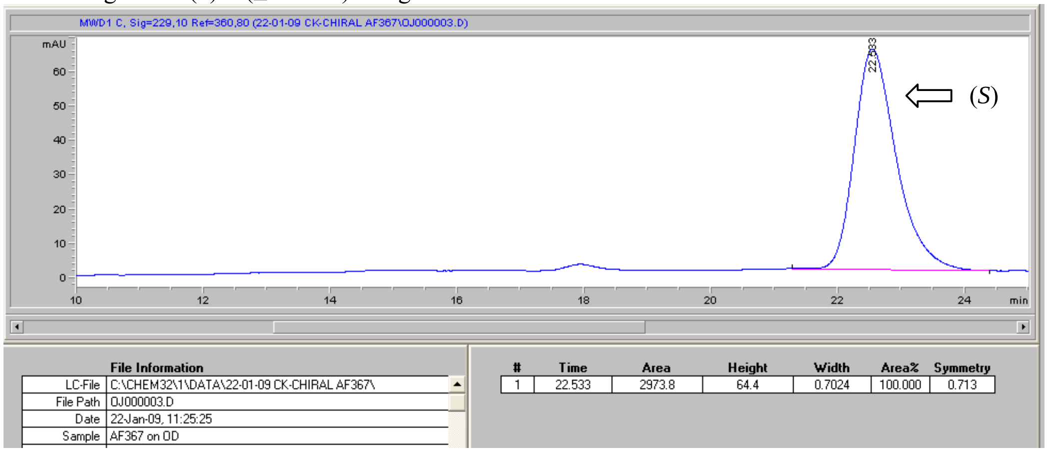
1	14.323	3407.6	70.3	0.7698	45.317	0.562
2	15.593	4111.9	68	0.9097	54.683	0.509

Chromatogram of (*S*)-**6** (≥99% *ee*) using Chiralcel OD column



File Information

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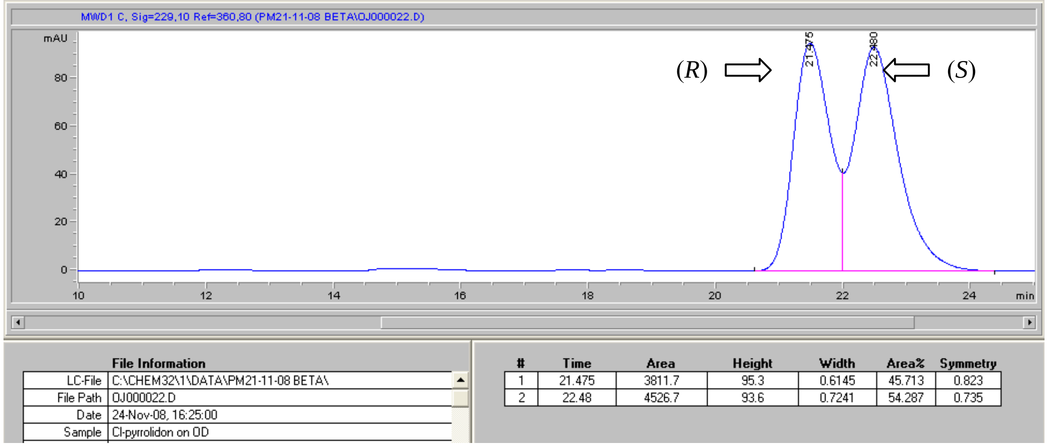
File Path	OJ000003.D
Date	22-Jan-09, 11:25:25
Sample	AF367 on OD

#	Time	Area	Height	Width	Area%	Symmetry
1	22.533	2973.8	64.4	0.7024	100.000	0.713

Chromatogram of *rac*-**6** using Chiralcel OD column

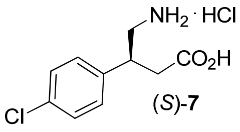


File Information

LC-File	C:\CHEM32\1\DATA\PM21-11-08 BETA\
File Path	OJ000022.D
Date	24-Nov-08, 16:25:00
Sample	Cl-pyrrolidon on OD

#	Time	Area	Height	Width	Area%	Symmetry
1	21.475	3811.7	95.3	0.6145	45.713	0.823
2	22.48	4526.7	93.6	0.7241	54.287	0.735

Synthesis of (*S*)-4-amino-3-(4-chloro-phenyl)-butanoic acid hydrochloride [(*S*)-baclofen] 7.

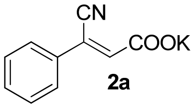


(*S*)-**7**

(*S*)-Baclofen (**7**) was synthesized in 82% yield from *γ*-lactam (*S*)-**6** (≥99% *ee*) according to the published procedure[9] and obtained as a white solid after trituration in *iso*-propanol; ^{1}H NMR (CD_3OD, 400 MHz) δ 2.57 (dd, *J* = 7.6 Hz, *J* = 16.4 Hz, 1H), 2.70 (dd, *J* = 6.8 Hz, *J* = 16.4 Hz, 1H), 3.08 (dd, *J* = 9.5 Hz, *J* = 12.7 Hz, 1H), 3.15-3.31 (m, 2H), 7.23-7.31 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 39.3, 41.2, 44.9, 130.3, 130.6, 134.8, 139.5, 174.4; Anal. calcd for $C_{10}H_{13}Cl_2NO_2$: C, 48.02; H, 5.24; N, 5.60; Found: C, 48.22; H, 4.97; N, 5.28. All spectral data were in accordance with those reported in literature.[9] The respective racemic compound was synthesized in the same manner in 80% yield and obtained as white solid: mp 183-184 °C (lit. 194 °C[9]).

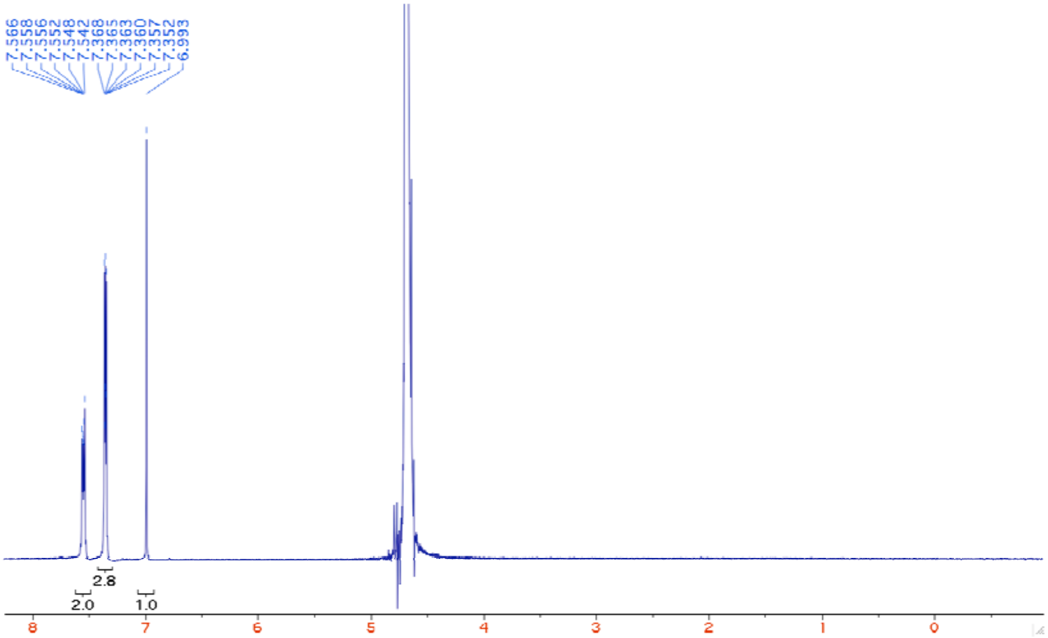
NMR spectra of unknown compounds

(Z)-3-Cyano-3-(phenyl)-propenoic acid potassium salt ((Z)-*2a*)

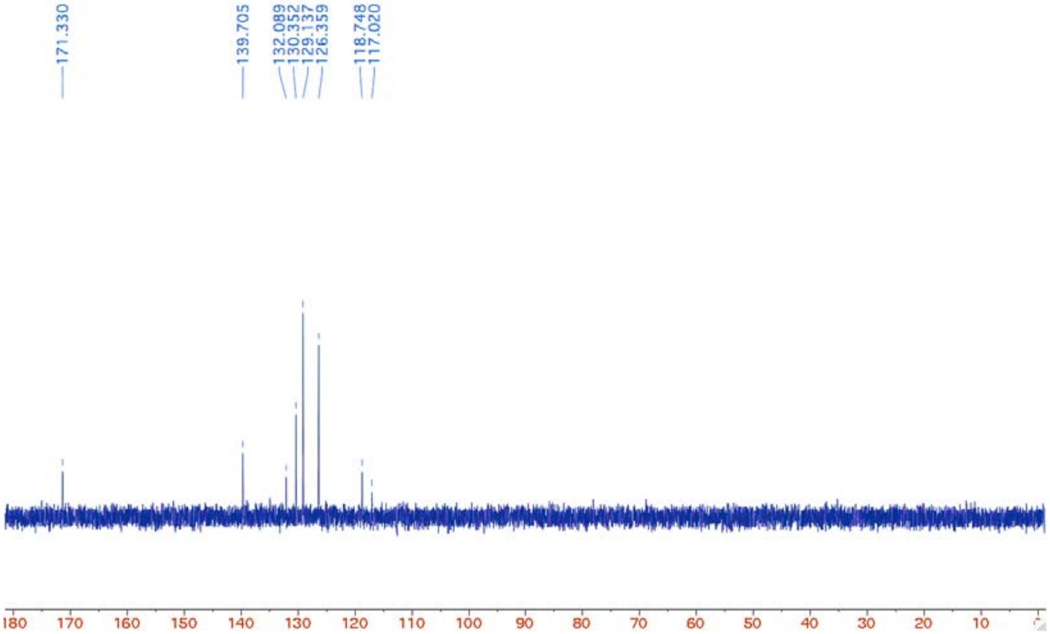


2a

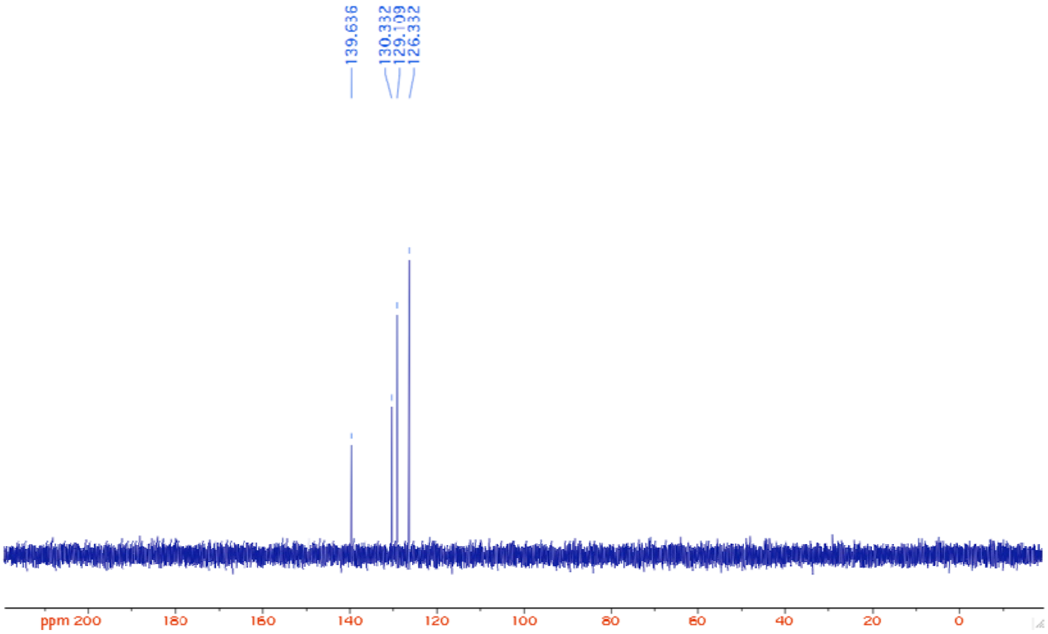
^{1}H NMR



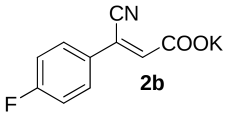
^{13}C NMR



^{13}C NMR – DEPT 135

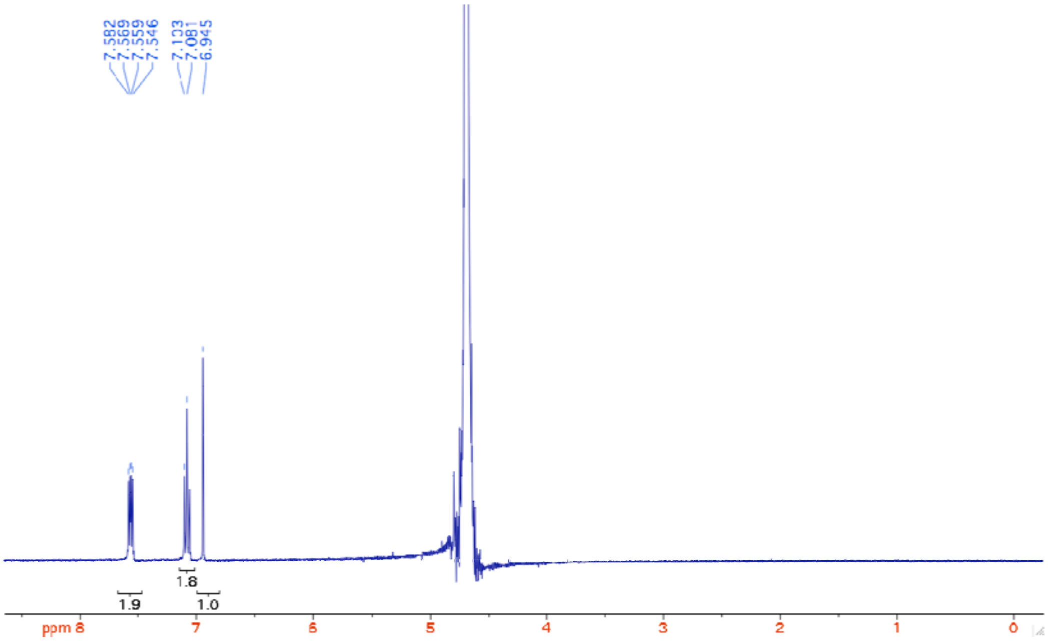


(Z)-3-Cyano-3-(4-fluoro-phenyl)-propenoic acid potassium salt ((Z)-2b)

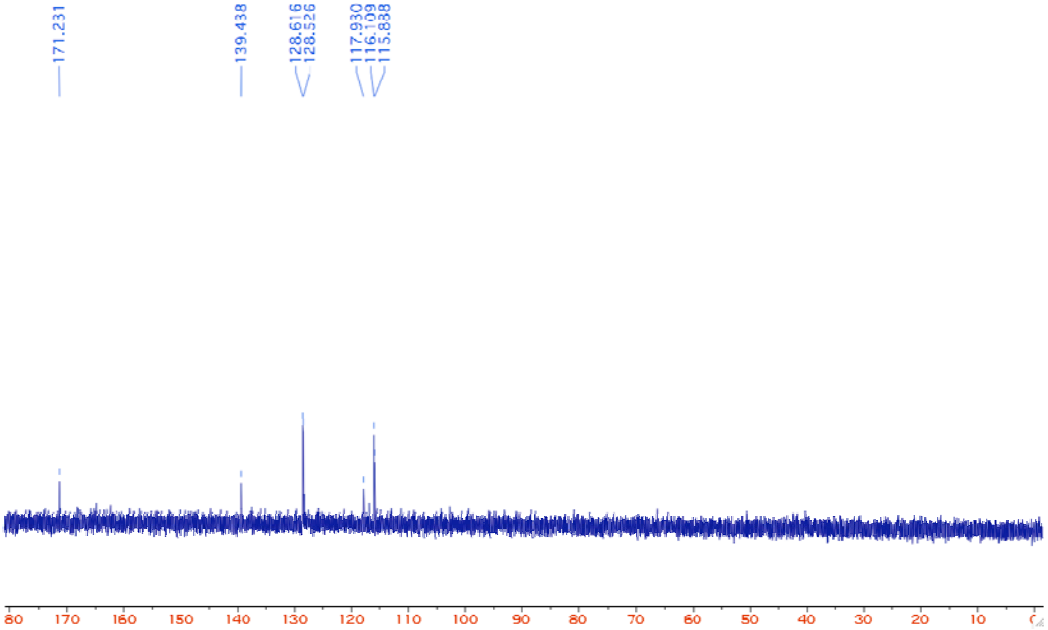


2b

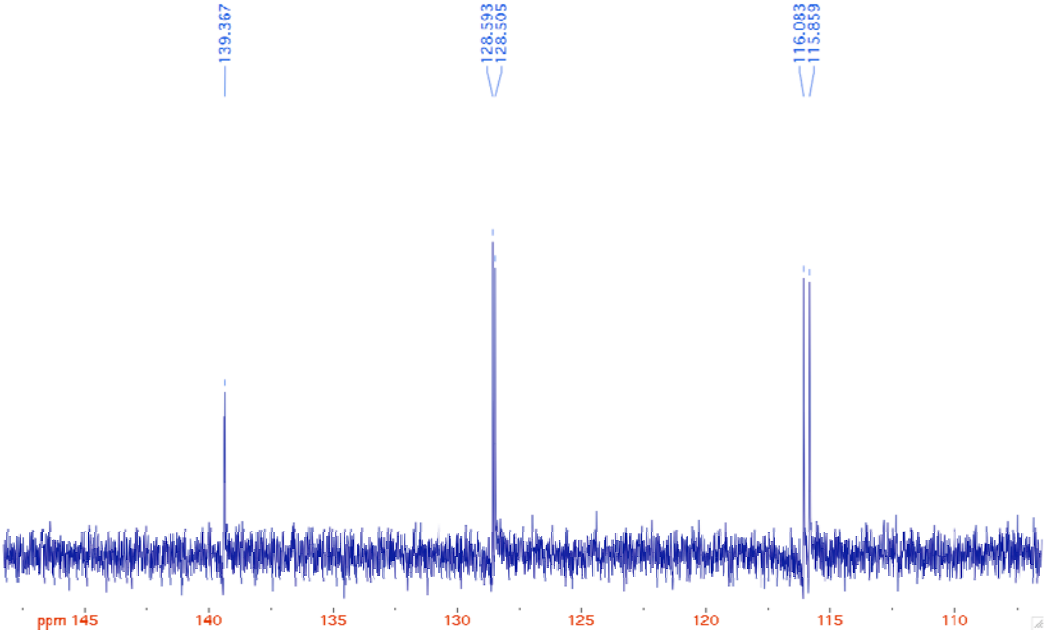
^{1}H NMR



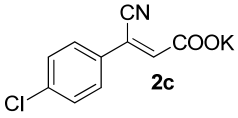
^{13}C NMR



^{13}C NMR – DEPT 135

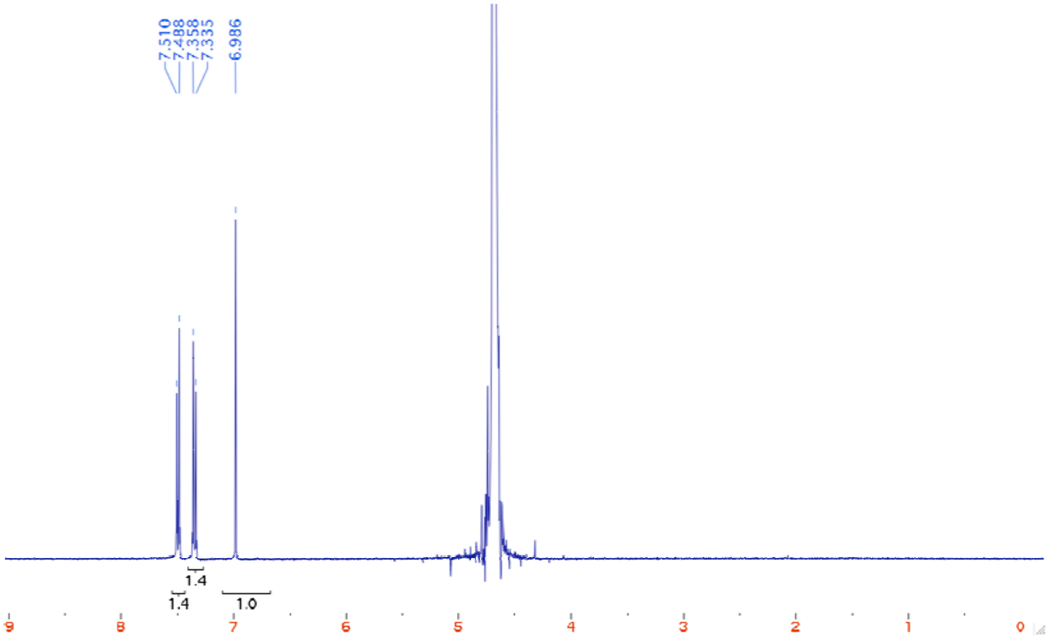


(Z)-3-(4-Chloro-phenyl)-3-Cyano-propenoic acid potassium salt ((Z)-2c)



2c

^{1}H NMR



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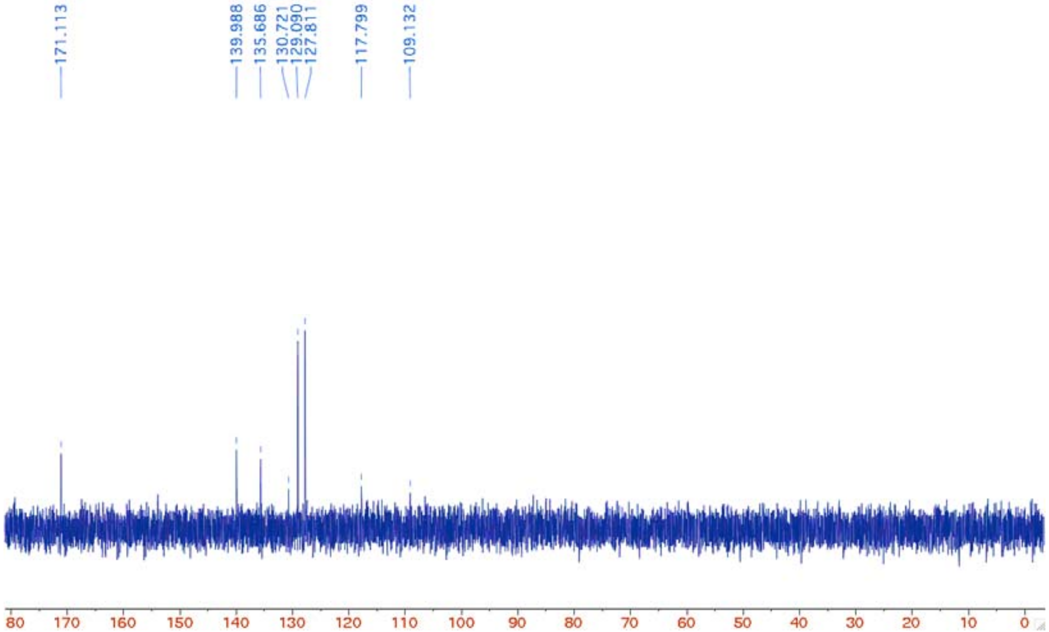
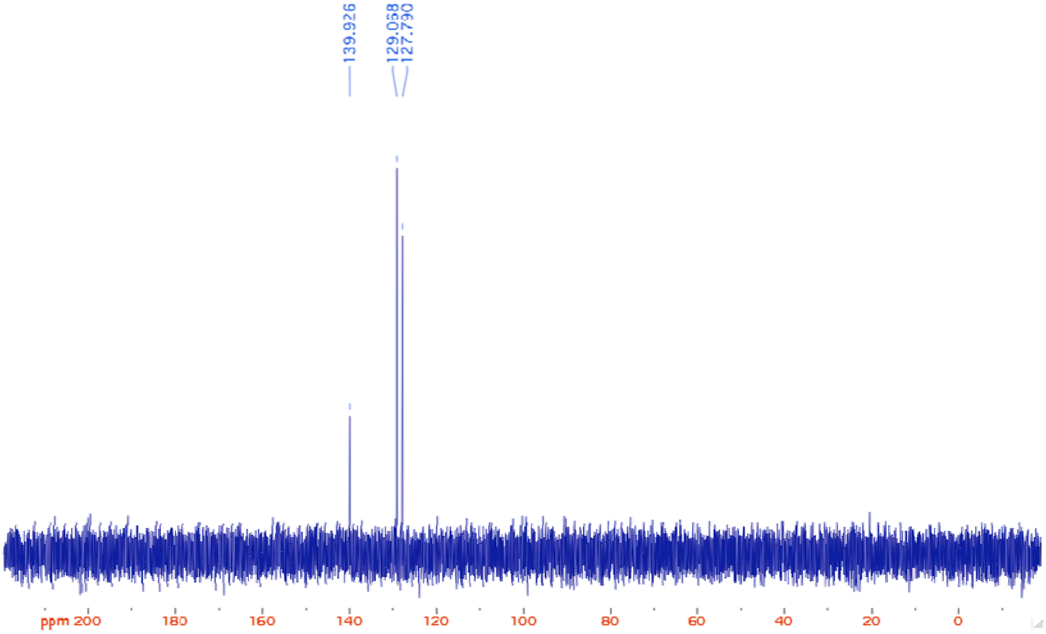
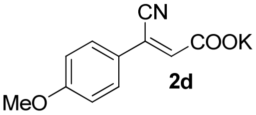


^{13}C NMR – DEPT 135

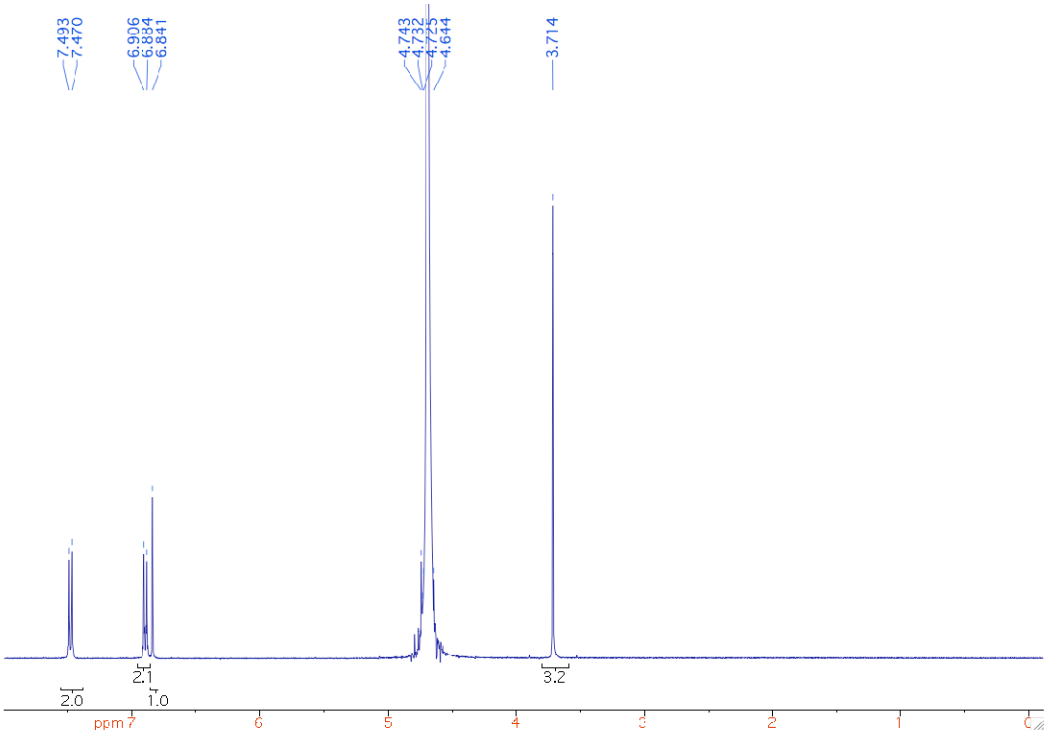


(Z)-3-Cyano-3-(4-methoxy-phenyl)-propenoic acid potassium salt ((Z)-2d)



2d

^{1}H NMR



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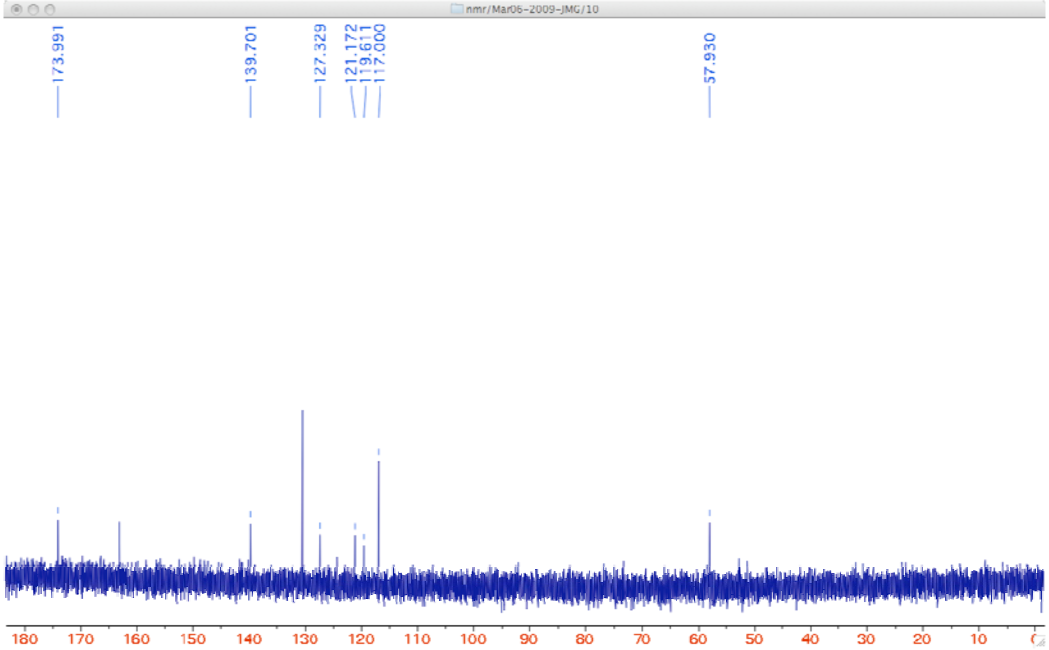
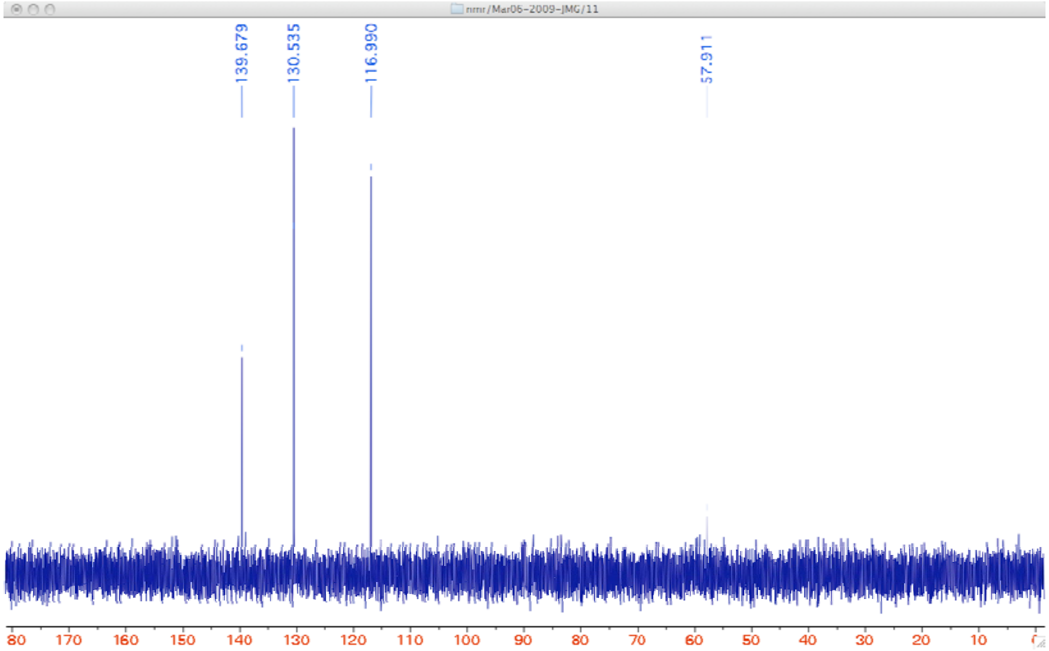
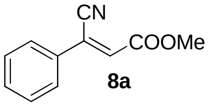


^{13}C NMR – DEPT 135

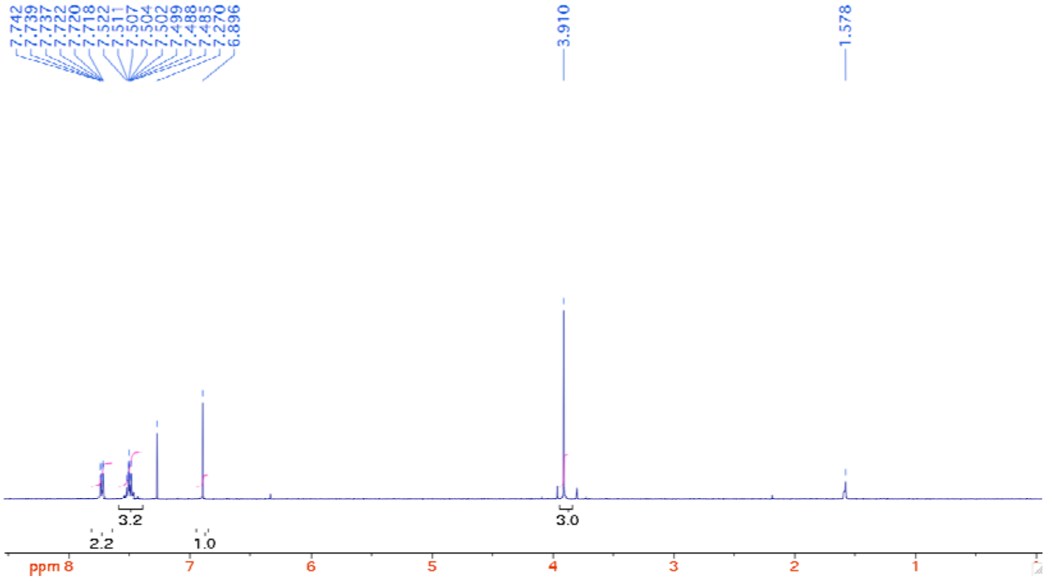


(Z)-3-Cyano-3-(phenyl)-propenoic acid methyl ester ((Z)-8a)



8a

^{1}H NMR



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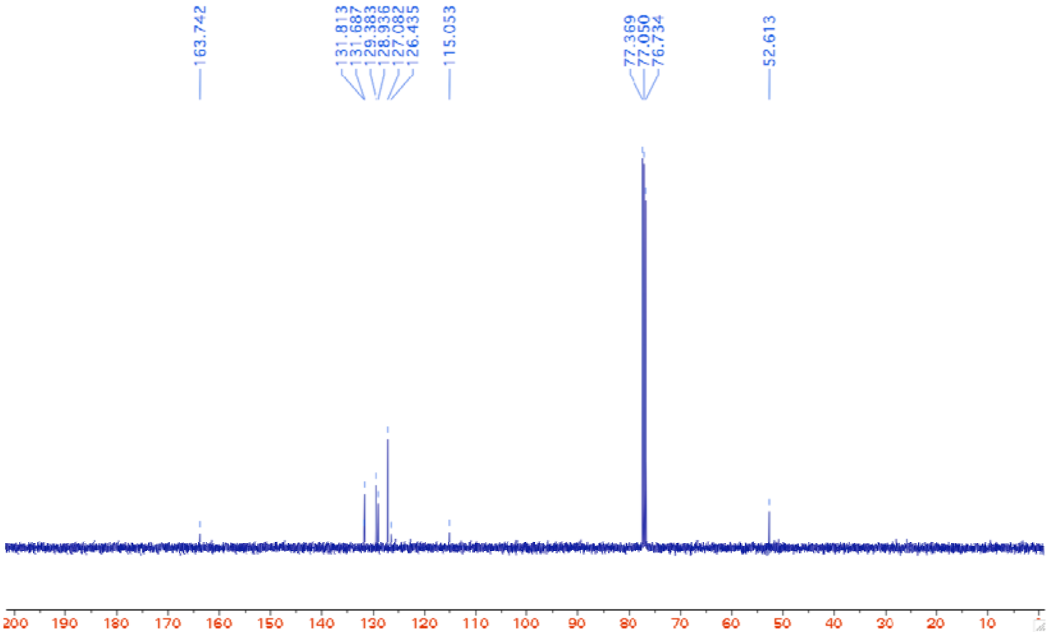
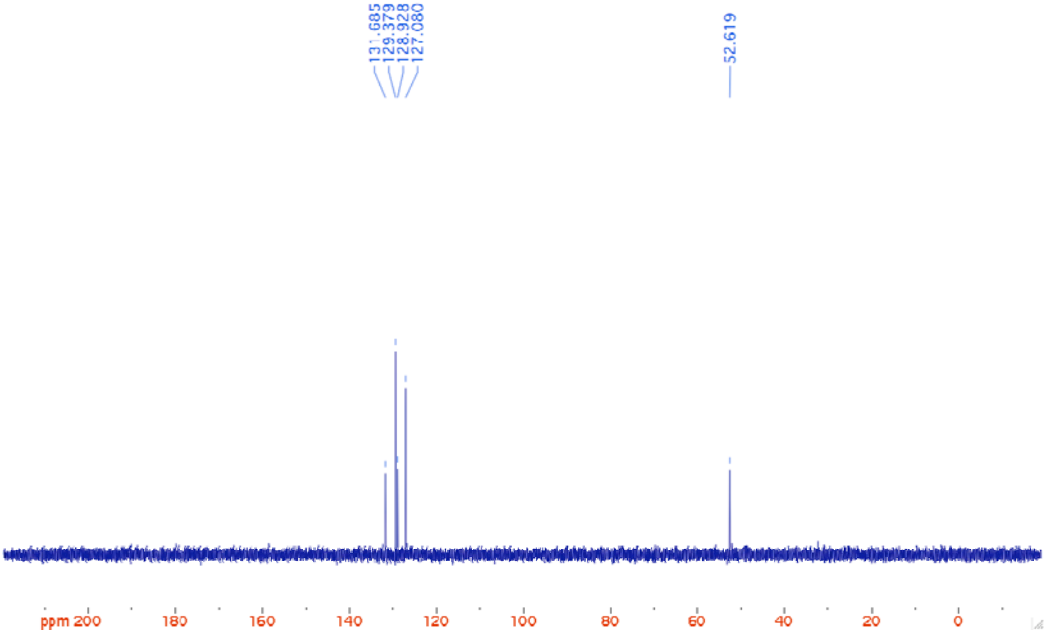
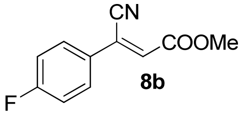


^{13}C NMR – DEPT 135

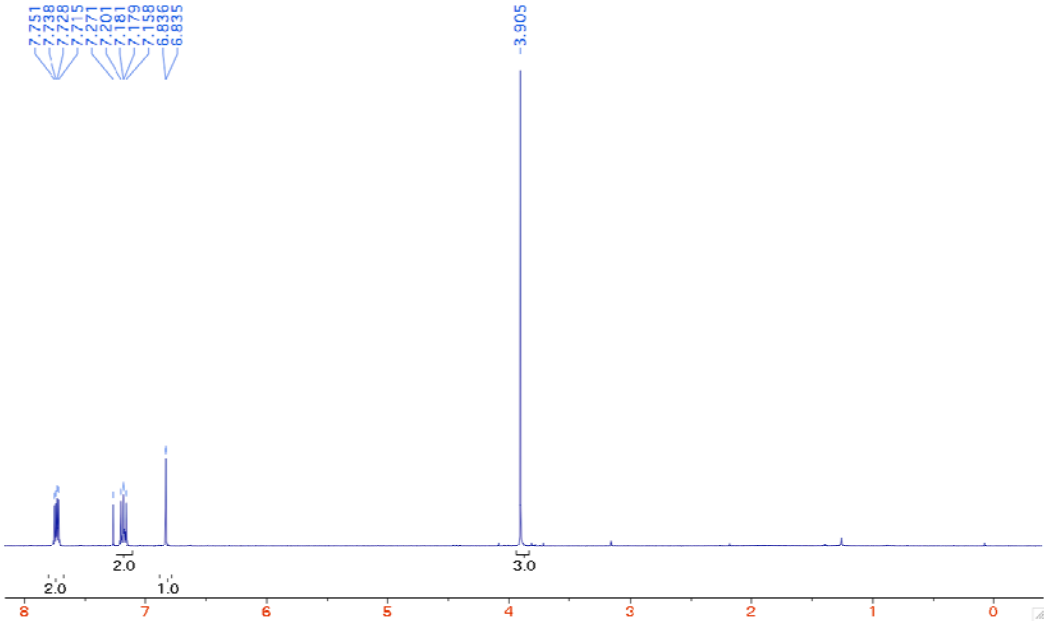


(Z)-3-Cyano-3-(4-fluoro-phenyl)-propenoic acid methyl ester ((Z)-8b)



8b

^{1}H NMR



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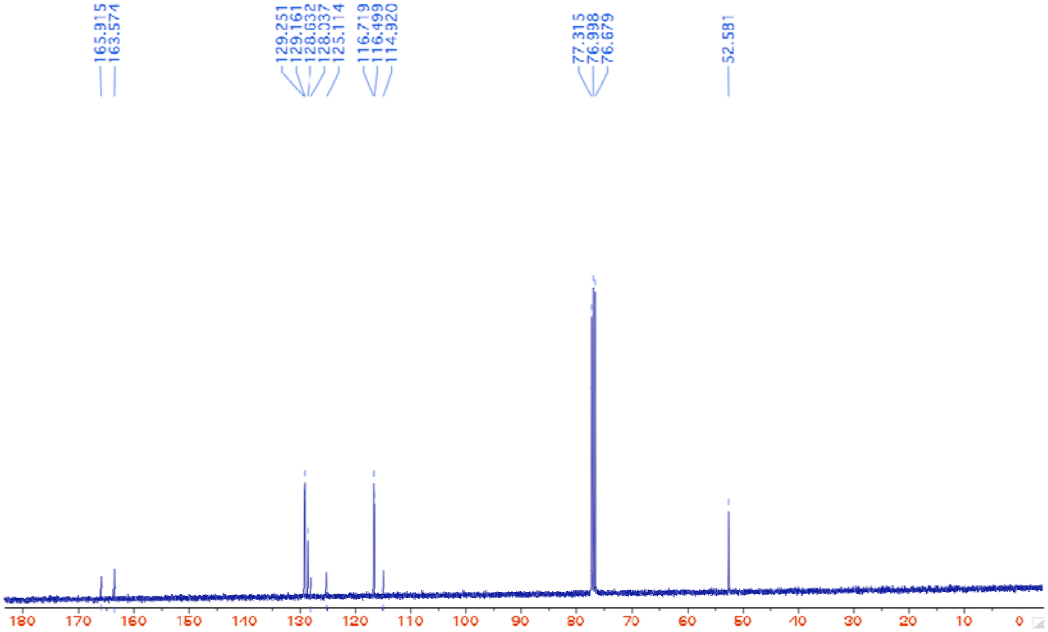
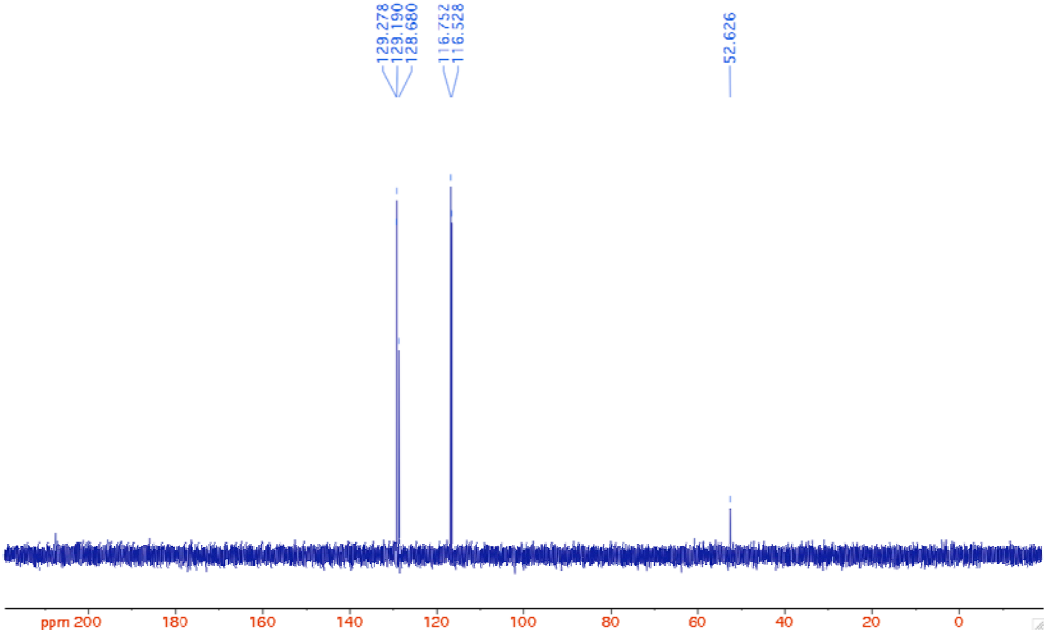
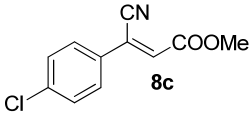


^{13}C NMR – DEPT 135

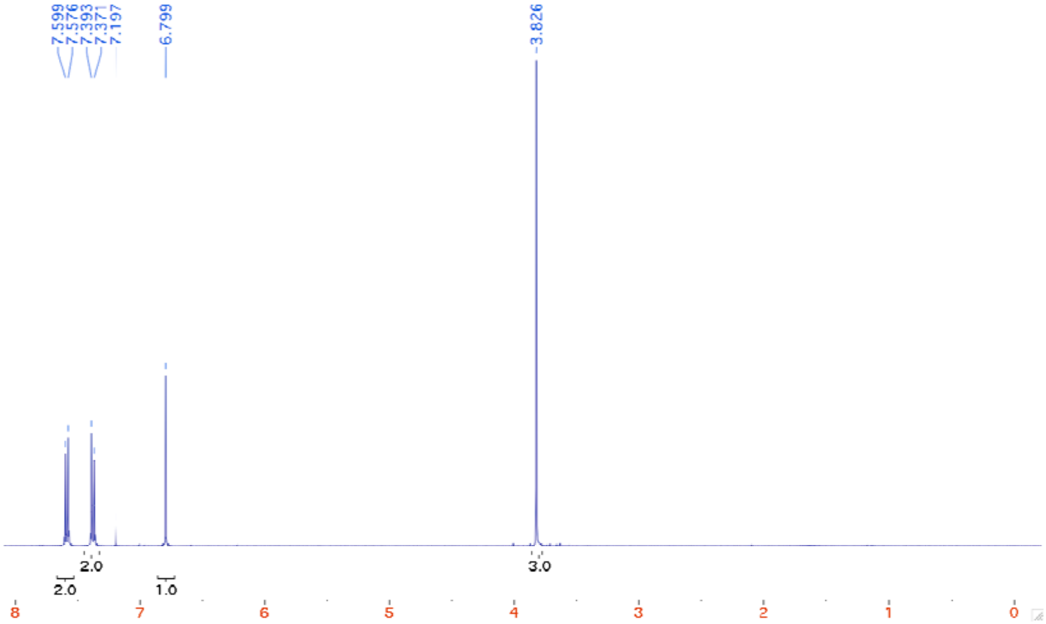


(Z)-3-(4-Chloro-phenyl)-3-cyano propenoic acid methyl ester ((Z)-8c)



8c

^{1}H NMR



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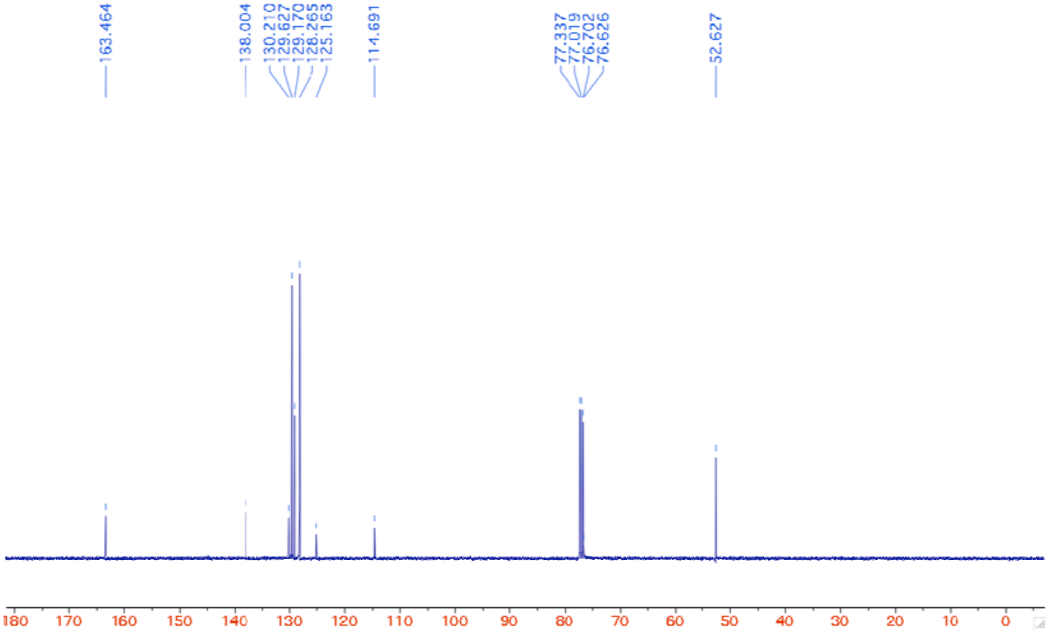

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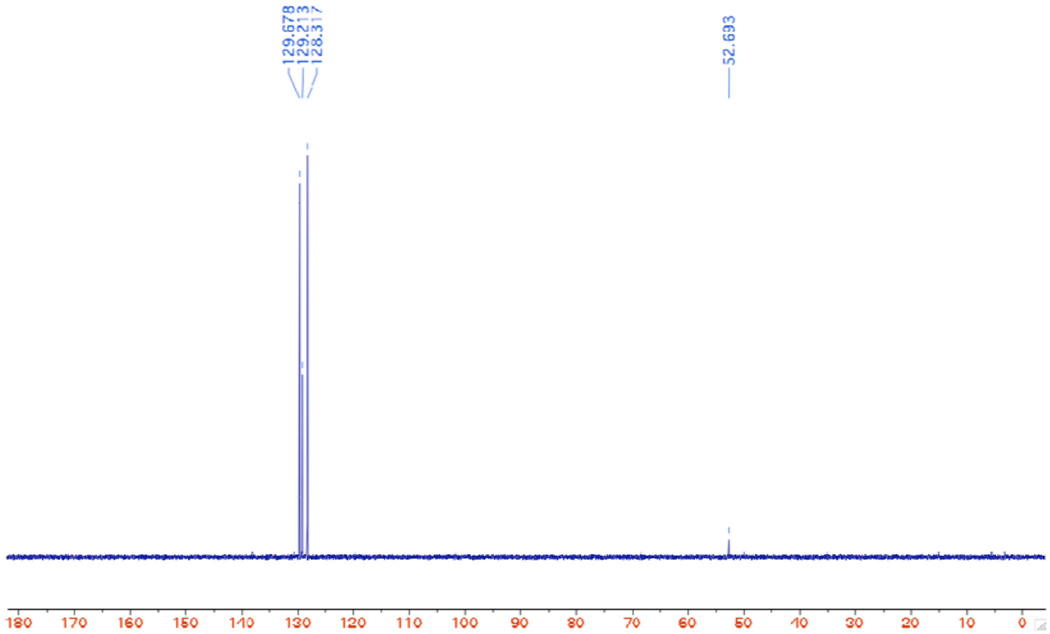
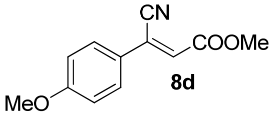
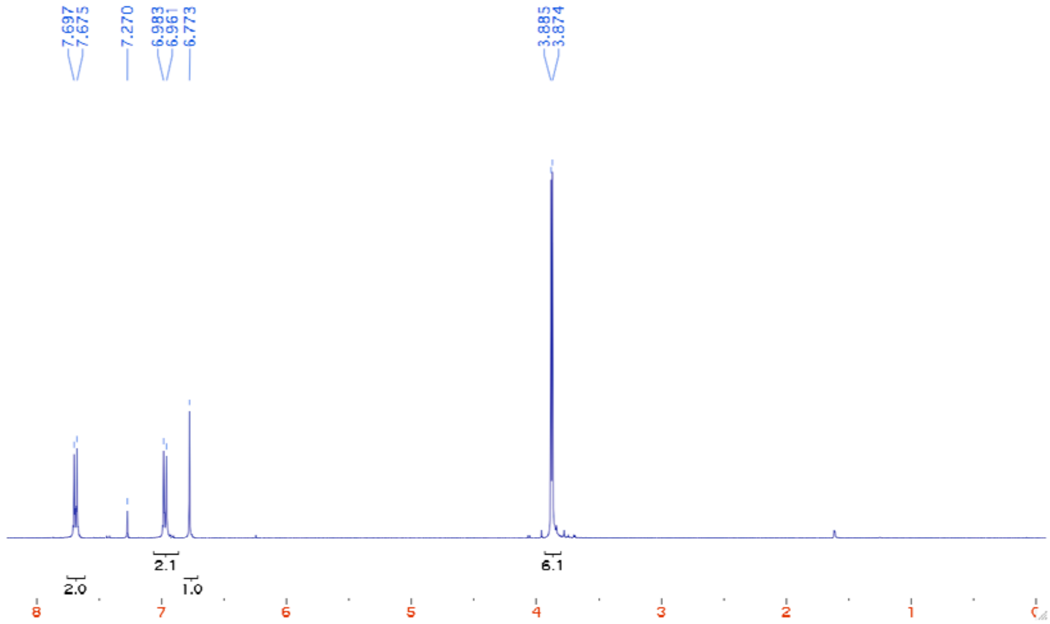


(Z)-3-Cyano-3-(4-methoxy-phenyl)-propenoic acid methyl ester ((Z)-8d)



8d

^{1}H NMR



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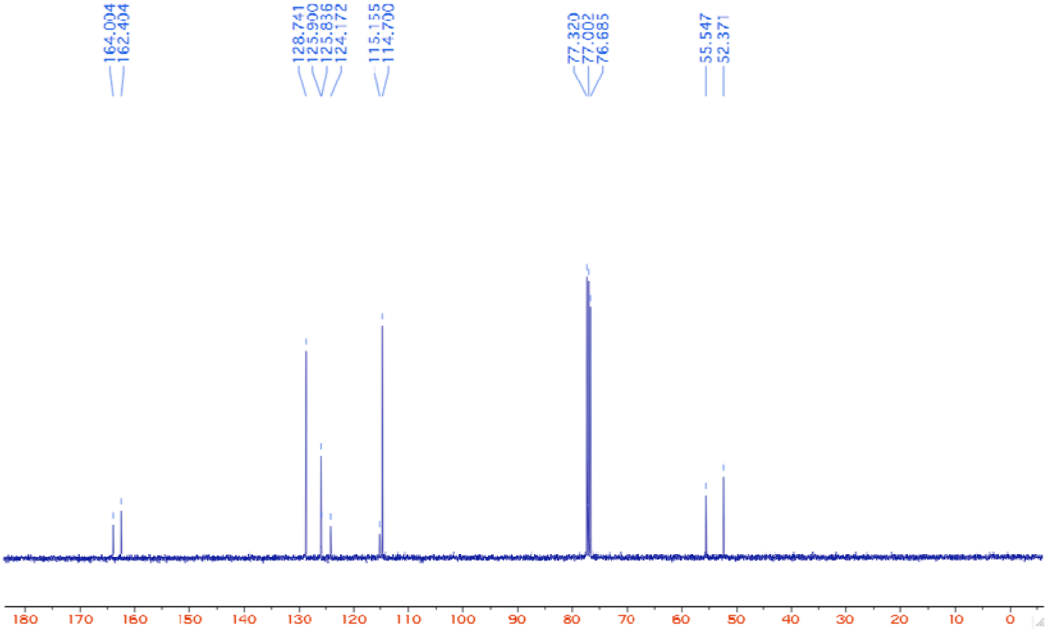
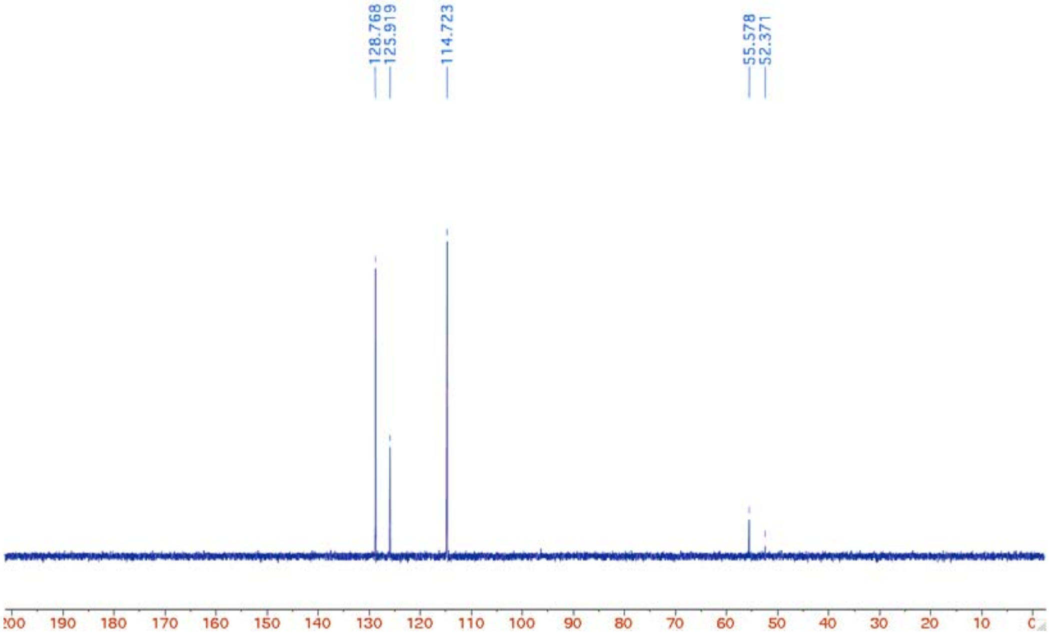
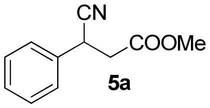


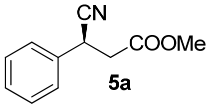
^{13}C NMR – DEPT 135



3-Cyano-3-(phenyl)-propanoic acid methyl ester (5a)

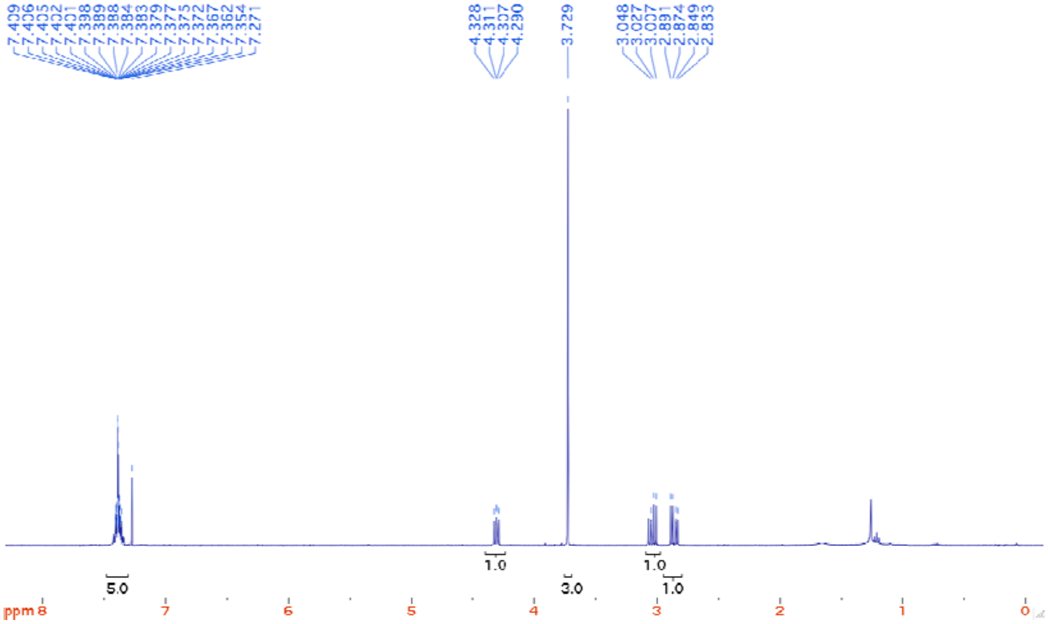


5a



5a

^{1}H NMR



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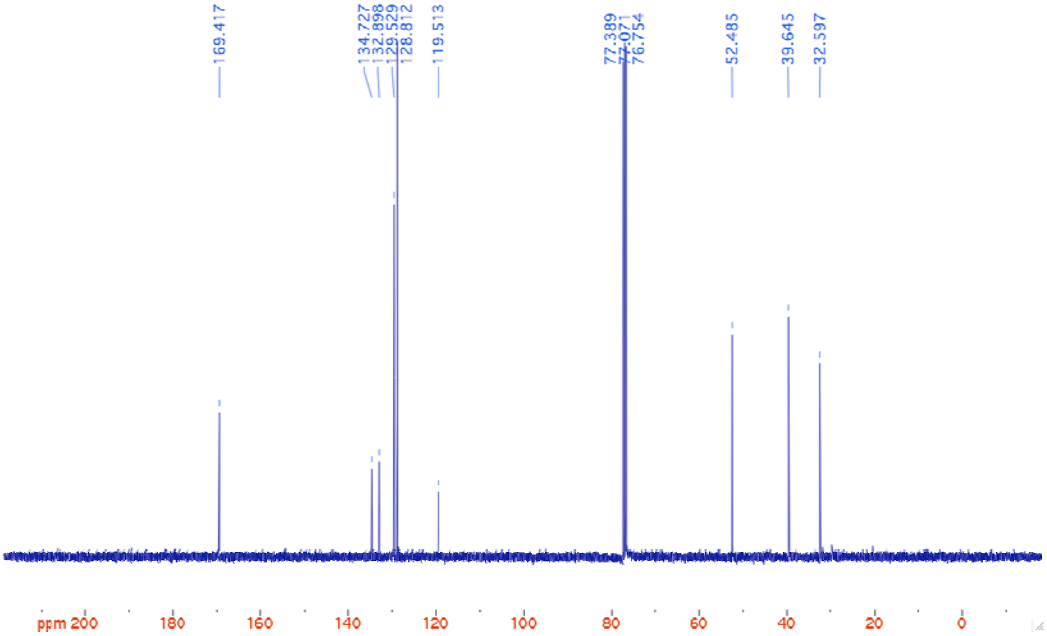
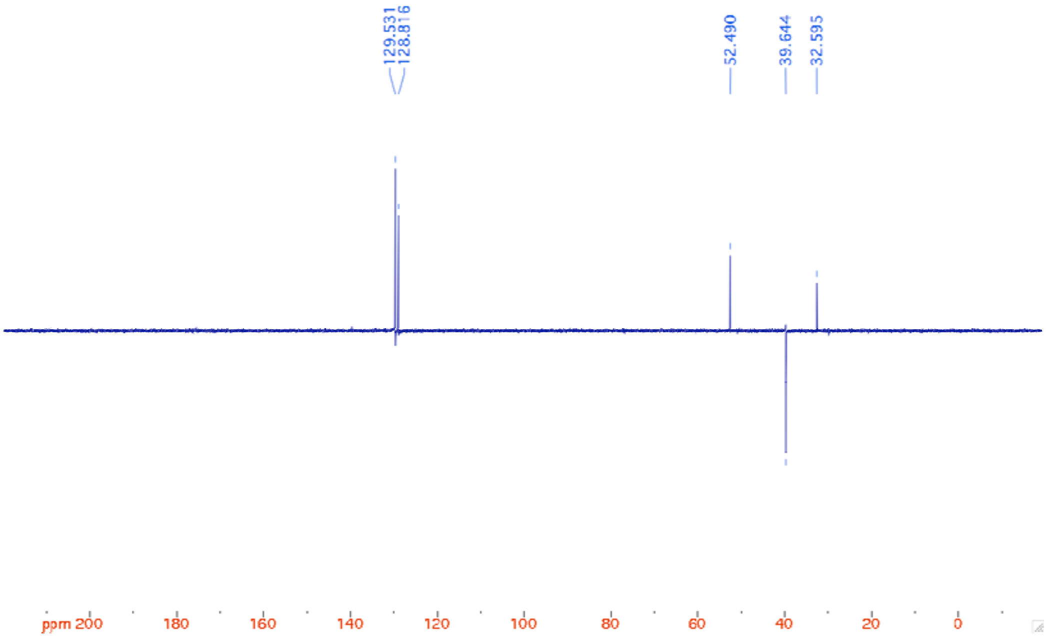
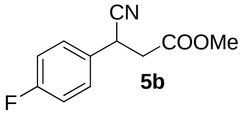


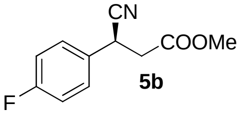
^{13}C NMR – DEPT 135



3-Cyano-3-(4-fluoro-phenyl)-propanoic acid methyl ester (5b)



5b



5b

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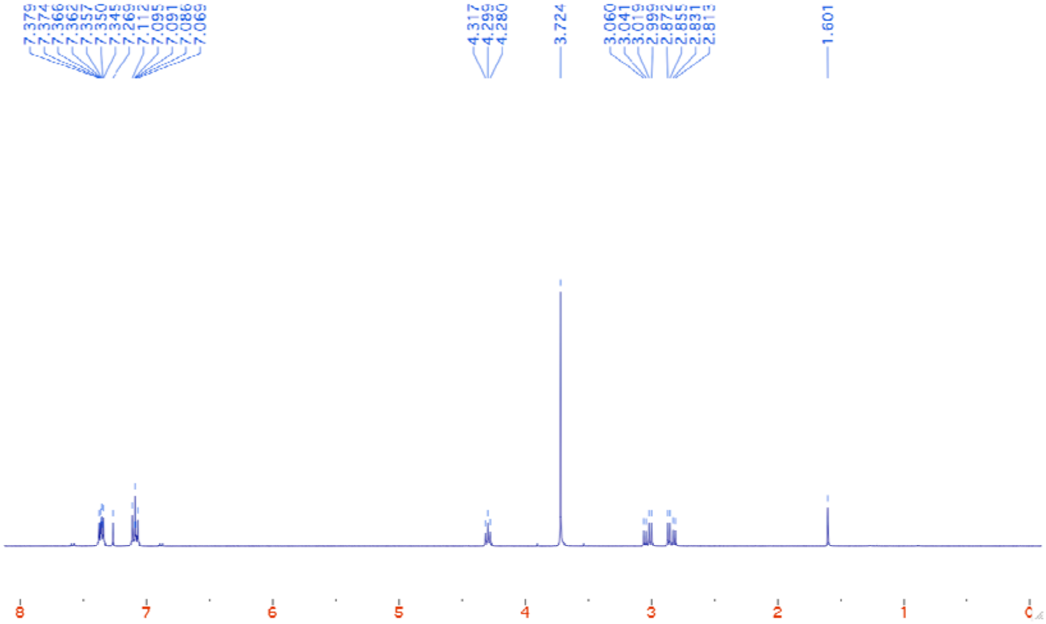

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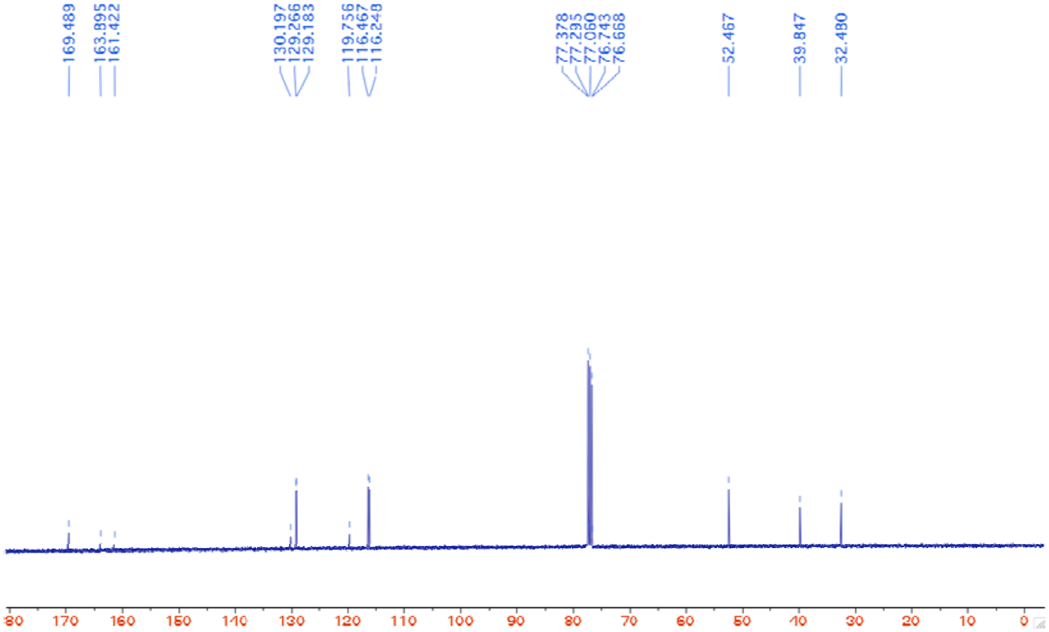

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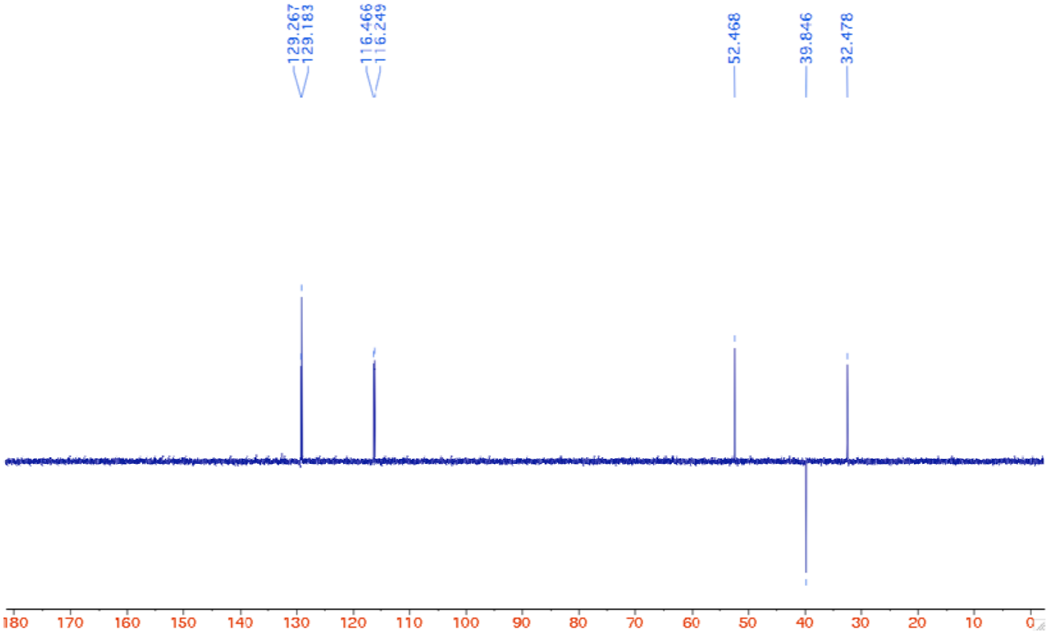
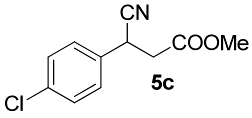


3-(4-Chloro-phenyl)-3-cyano propanoic acid methyl ester (5c)



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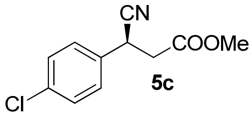

5c

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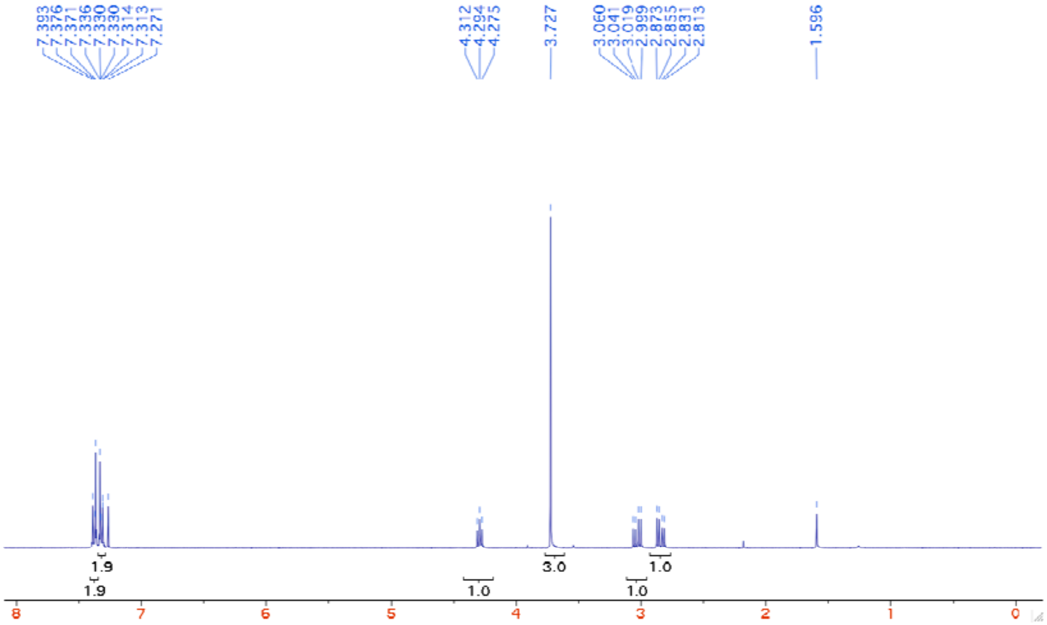

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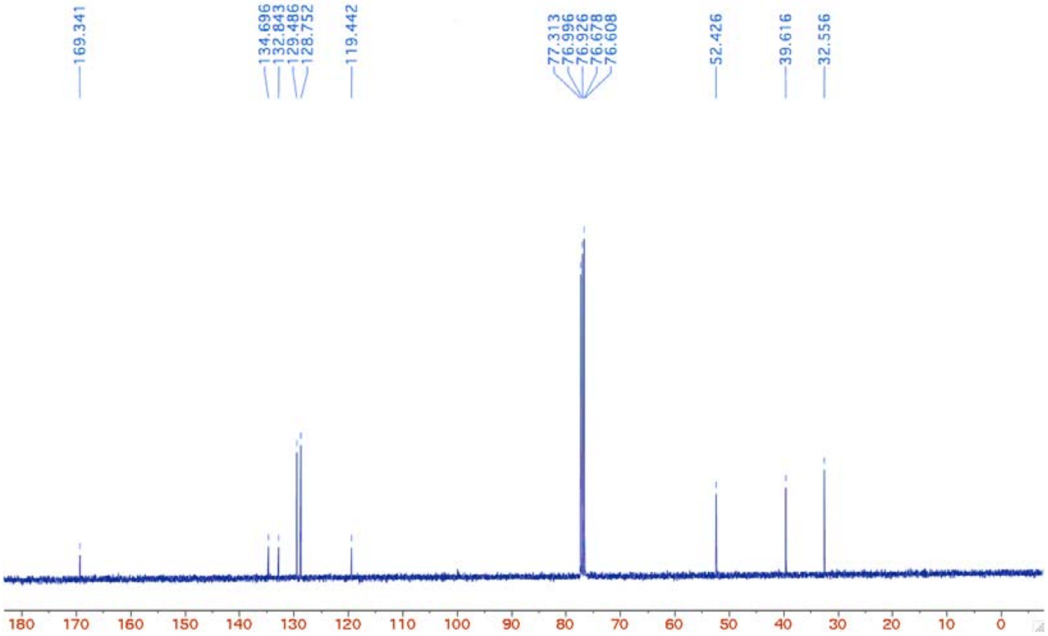
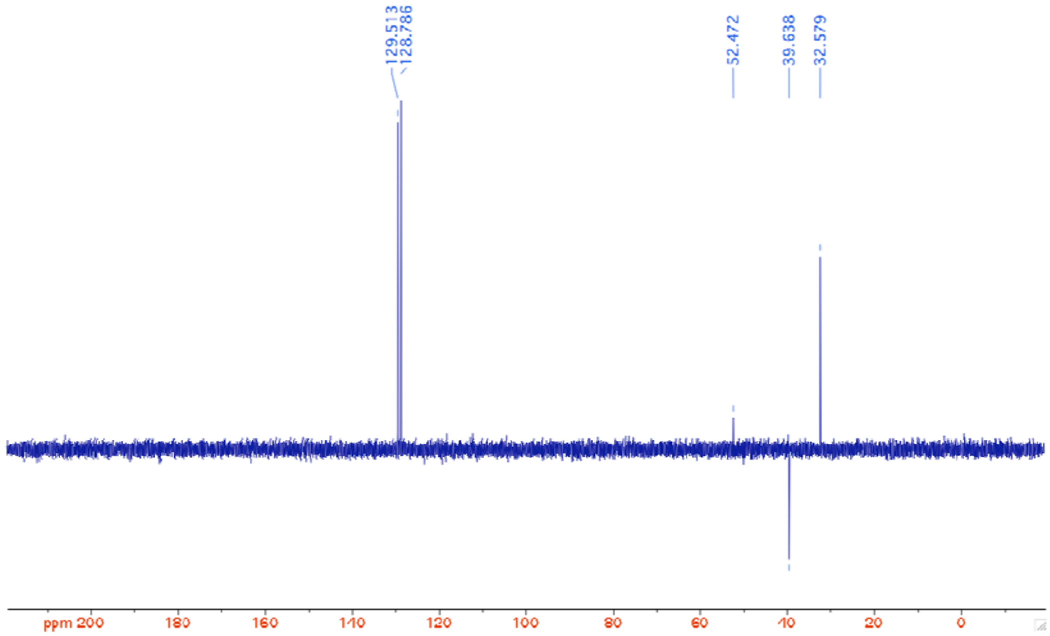
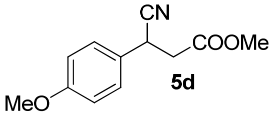


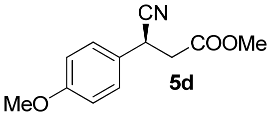
^{13}C NMR – DEPT 135



***3-Cyano-3-(4-methoxy-phenyl)-propanoic acid methyl ester** (5d)*

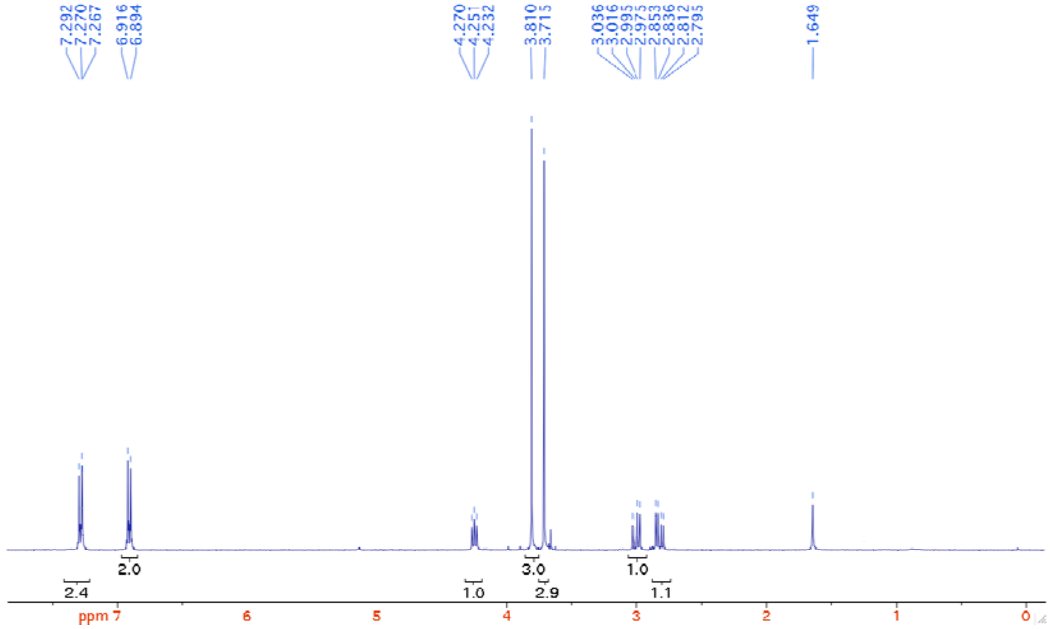


5d



5d

^{1}H NMR



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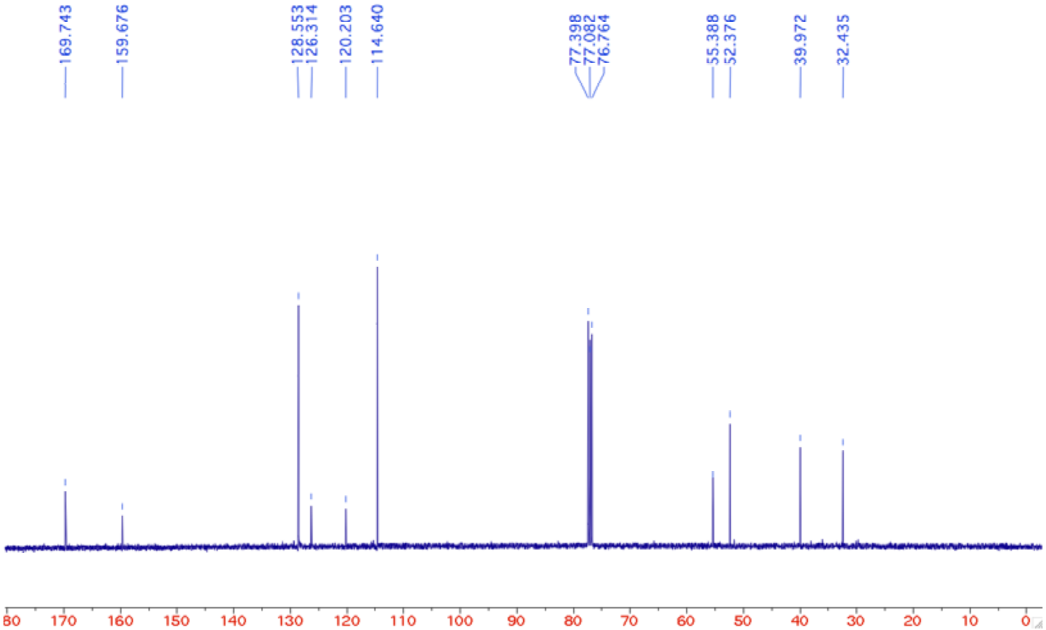
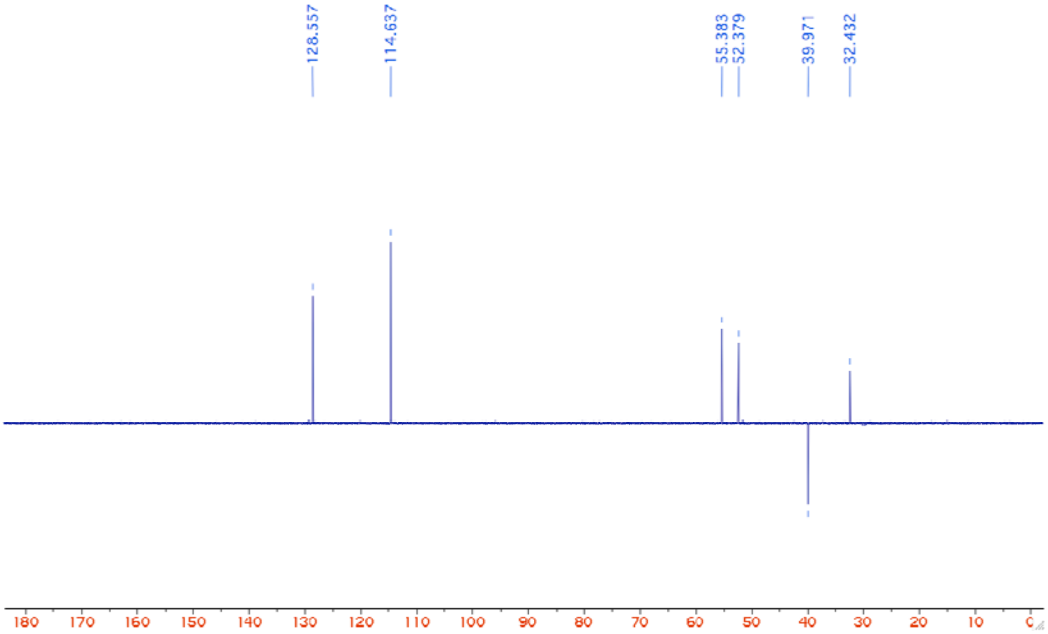


^{13}C NMR – DEPT 135



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