Supporting Information

Chemoenzymatic Asymmetric Synthesis of Pregabalin-Precursors via Asymmetric Bioreduction of β-Cyano-Acrylate Esters Using Ene-Reductases

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Cloning, his-tagging, cultivation and purification of EBP1 from *Candida albicans*

AA-Sequence:

MTIESTNSFVVPSDTKLIDVTPLGSTKLFQPIKVGNNVLPQRIAYVPTTRFRASKDHIPS
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LA

EBP1 DNA-Sequence

>gi|68483570:c70174-68951 *Candida albicans* SC5314 chromosome 6 Ctg19-10035, whole genome shotgun sequence:

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CATAG

Cloning and His-tagging

The design of the primers for adding a HIS-tag on the C-terminus of EBP1 using a pET22-b(+) vector was done using OligoCalc (www.basic.northwestern.edu/biotools/oligocalc.html). As template DNA for the PCR a plasmid preparation of EBP1 was done using the kit of Qiagen. For the PCR 5 x Phusion HF buffer (10 µL), dNTPs (1 µL, 10 mM), forward primer (2.5 µL, 10 µmol) and reverse primer (2.5 µmol), Phusion DNA Polymerase (Hot Start, 0.5 µL), template DNA (1 pg – 10 ng plasmid DNA; 1 µL) and H_2O (32.5 µL) were used. The PCR program started with initial denaturation for 30 sec at 98 °C followed by 35 cycles of denaturation at 98 °C for 10 sec, annealing at 60.9 °C for 25 sec and extension at 72 °C for 20 sec. The program ended with the final elongation at 72 °C for 7 min and 4 °C hold. The PCR product was purified via agarose gel before the restriction with NdeI and XhoI for 1.5 hours at 37 °C. For the restriction PCR product (0.4 µg, 20 µL), XhoI (Fermentas Fast Digest; 2 µL), NdeI (Fermentas Fast Digest; 2 µL), 10 x Fermentas Fast Digest Green Buffer (4 µL) and H_2O (32 µL) were used. For the restriction pET22-b(+) vector (2.86 µg, 40 µL), XhoI (4 µL),

NdeI (2 µL), 10 x 0 buffer (6 µL) and H_2O (8 µL) were used for 3 hours at 37 °C. The size of the vector was 5500 base pairs and the size of the plasmid-insert 1100 base pairs. After purification of the restriction the ligation of the insert into the vector was performed using the insert (36.4 ng/µL; 1.7 µL), 10 x ligation buffer (2 µL), vector (38.7 ng/µL; 2.6 µL), H_2O (12.7 µL) and ligase (1 µL). After 1 hour of ligation at 22 °C the inactivation followed at 65 °C for 10 min. Then the ligated vector was re-cooled on ice. The transformation was done into 50 µL Rosetta™ 2 (DE3) pLysS competent cells (Novagen) using 5 µL of ligation product. The mixture was kept on ice for 30 min before heat-shocking for 30 sec on 42 °C and re-cooling on ice for 2 min afterwards. Then 250 µL SOC-media were added and incubated at 37 °C, 300 rpm for 1 hour. Colonies were cultivated on agar-plates containing ampicillin and chloramphenicol overnight at 37 °C. Forward NdeI: GCC GCA GGC cat atg ACT ATT GAA TCA ACT AAT TCA TTT GTT GTC; melting point: 56.79 °C; reverse XhoI: GTG GTG ctc gag TGC CAA TGG TTT ACC AAT GAC TTG C; melting point: 56.66 °C

Cultivation and Purification

The overnight culture was cultivated for 14-16 hours in LB-Media at 37 °C and 120 rpm. ampicillin (100 mg/L) and chloramphenicol (20 mg/L) were added. The inoculation was done using glycerol stock solution of EBP1 (20 µL). The main culture of EBP1 was done in an auto-induction media. For the media (total 670 mL) separately autoclaved solutions of 8 x ZY (276 mL), 50 x Lac (13.4 mL), NPSC (33.5 mL), trace element mix (1-3 mL), $MgSO_4$ (1 M, 1.34 mL), H_2O (306 mL) and sodium succinate (33.5 mL, 0.5 M), were combined. The cultivation was at 20 °C and 120 rpm for 3 days in a 2L shaking-flask containing 500 mL of media. For inoculation of 500 mL media 15 mL of ONC were used. After 3 days the cells were collected by centrifuging at 8000 rpm and 4 °C for 20 min. The wet cells were frozen at -20 °C for at least one night. For purification, thawed cells (15 g) were suspended in lysis-buffer (10 mM imidazole, 50 mM NaH_2PO_4, 300 mM NaCl, 25 mL) adding traces of FMN.

The cell disruption was done by ultrasonication using 35 % amplitude for 5 min (1 sec on, 3 sec off) while cooling with ice-water. Afterwards cells were centrifuged twice for 20 min at 4 °C and 1800 rpm. The clear supernatant was used for Ni-affinity chromatography purification using a 5 mL His trap HP column from GE Healthcare. The column first was equilibrated with lysis-buffer before applying the cell-free extract (flow: 1 mL/min). Then the column was washed with wash-buffer (20 mM imidazole, 50 mM NaH_2PO_4, 300 mM NaCl) until the eluting buffer stayed clear. Then the protein was eluted (1 mL per fraction) using elution-buffer (150 mM imidazole, 50 mM NaH_2PO_4, 300 mM NaCl). All active fractions were combined and dialysed overnight in (50 mM Tris-HCl buffer pH 7.5) before concentration and storage at -20 °C.

Buffers and Solutions:

ZY: 40 g/L yeast extract, 80 g/L trypton; NPSC: 1 M NH_4Cl, 0.1 M Na_2SO_4, 0.5 M KH_2PO_4, 0.5 M Na_2HPO_4; 50 x Lac: 25 % glycerol, 2.5 % glucose, 10 % lactose; succinate: 0.5 M disodium succinate; trace elements: 50 mM $FeCl_3$, 20 mM $CaCl_2$, 10 mM $MnCl_2$, 10 mM $ZnSO_4$, 2 mM $CoCl_2$, 2 mM $CuCl_2$, 2 mM Na_2MoO_4, 2 mM H_3BO_3, 2mM Na_2SeO_3, 2 mM $NiCl_2$, diluted with 60 mM HCl conc. to 1:20.

Analytical methods:

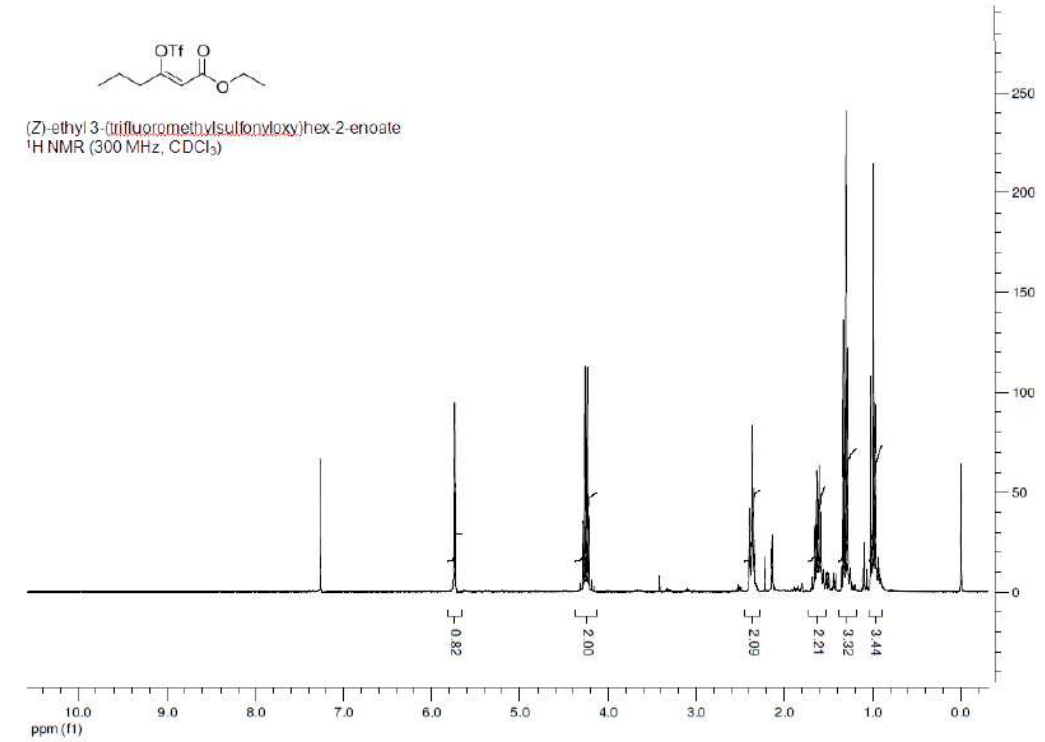
Chiral and achiral GC analyses were carried out using a Hydrodex-β-TBDAc capillary column (250 m x 0.25 mm id).

Table S1. GC-Retention times for substrates **1a-7a** and products (*R*)- and (*S*)-**1b-7b**.

Compound	Method	(*E*)-Isomer	(*Z*)-Isomer	(*R*)-Product	(*S*)-Product
1	A	3.71	5.78	6.37	5.59
2	A	4.00	6.59	6.22	5.88
3	B	30.13	52.87	41.49	41.27
4	C	5.09	9.58	8.36	8.58
5	C	3.96	7.69	6.82	6.99
6	B	-	41.03	39.59	38.88
7	C	7.32	-	11.73	11.85

Methods: A: 140 °C hold 10 min, 10 °C min^{-1} to 180 °C; B: 80 °C, 1 °C min^{-1} to 120 °C, 10 °C min^{-1} to 180 °C; C: 140 °C hold 10 min, 10 °C min^{-1} to 160 °C, hold 10 min.

NMR:


OTf O
O
(Z)-ethyl 3-(trifluoromethylsulfonyloxy)hex-2-enoate
¹H NMR (300 MHz, CDCl₃)
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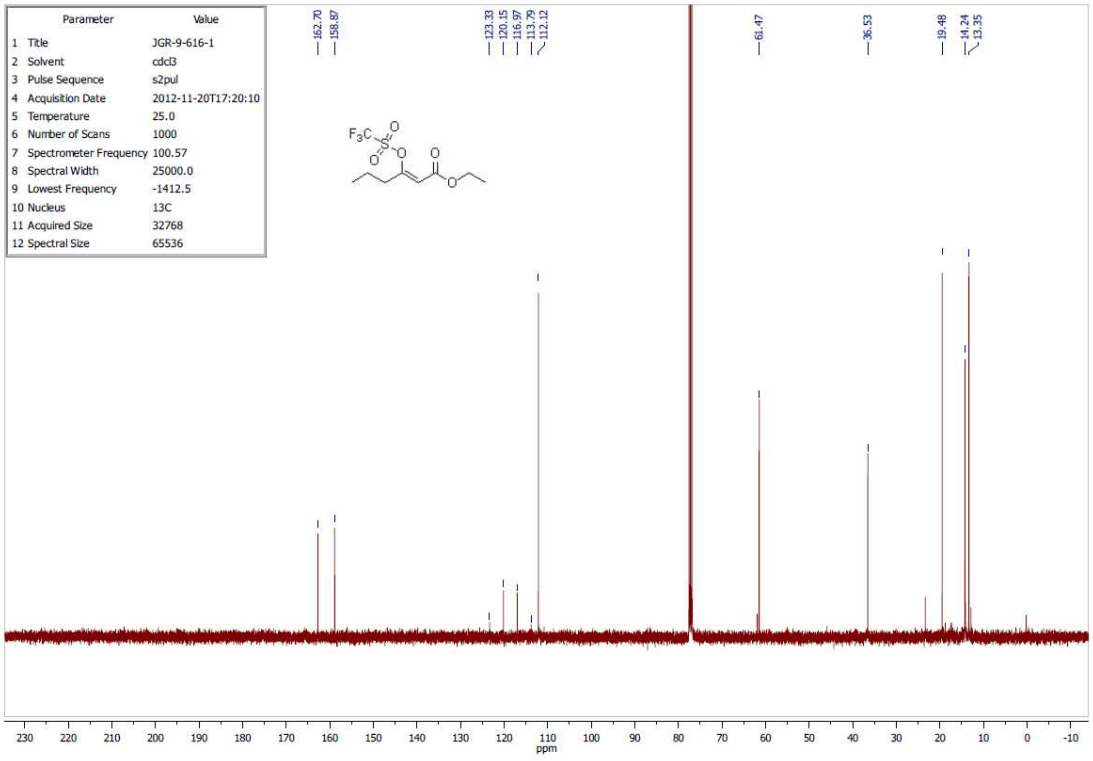
2.00
2.09
2.21
3.32
3.44
ppm (f1)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5 Temperature 25.0
6 Number of Scans 1000
7 Spectrometer Frequency 100.57
8 Spectral Width 25000.0
9 Lowest Frequency -1412.5
10 Nucleus 13C
11 Acquired Size 32768
12 Spectral Size 65536
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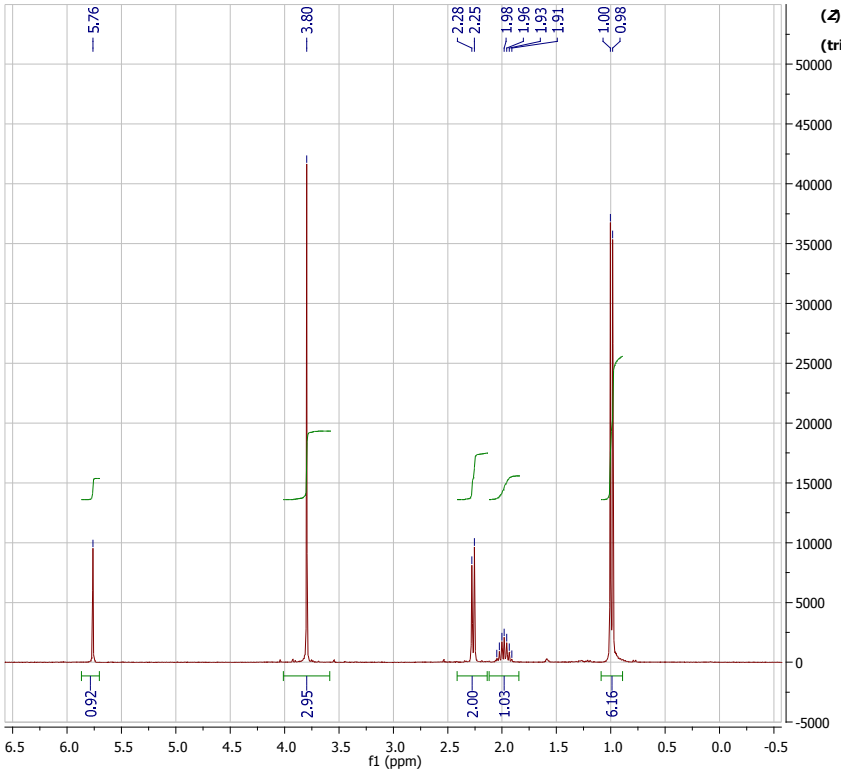
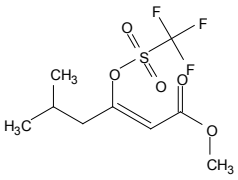
14.24
13.35
F3C
ppm



(*Z*)-Methyl 5-methyl-3-(trifluoromethylsulfonyloxy)hex-2-enoate.



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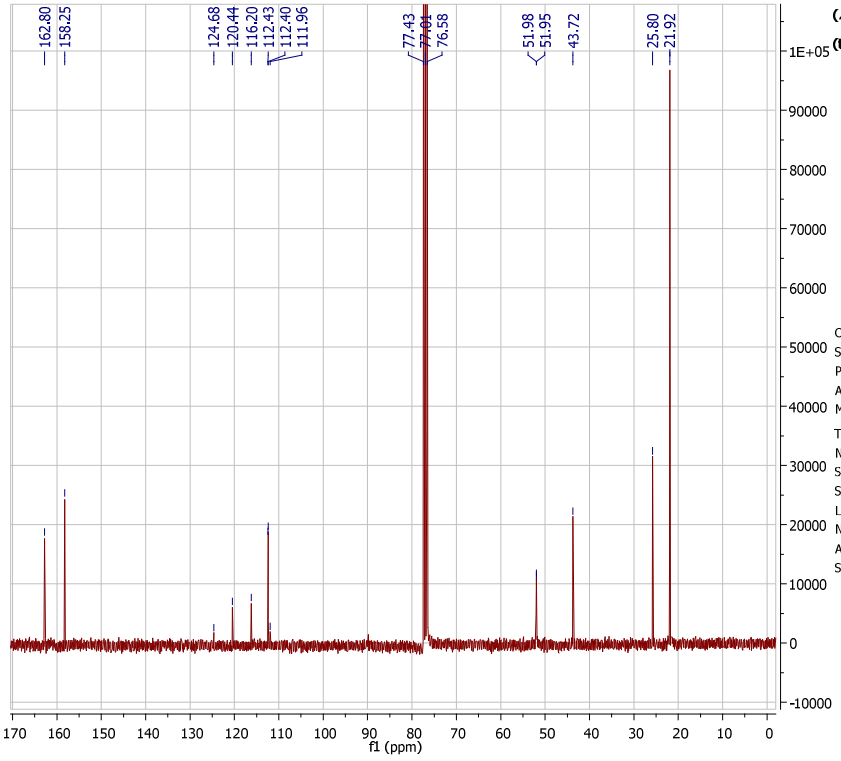
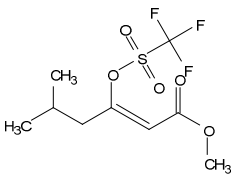
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



(*Z*)-Methyl 5-methyl-3-(trifluoromethylsulfonyloxy)hex-2-enoate.



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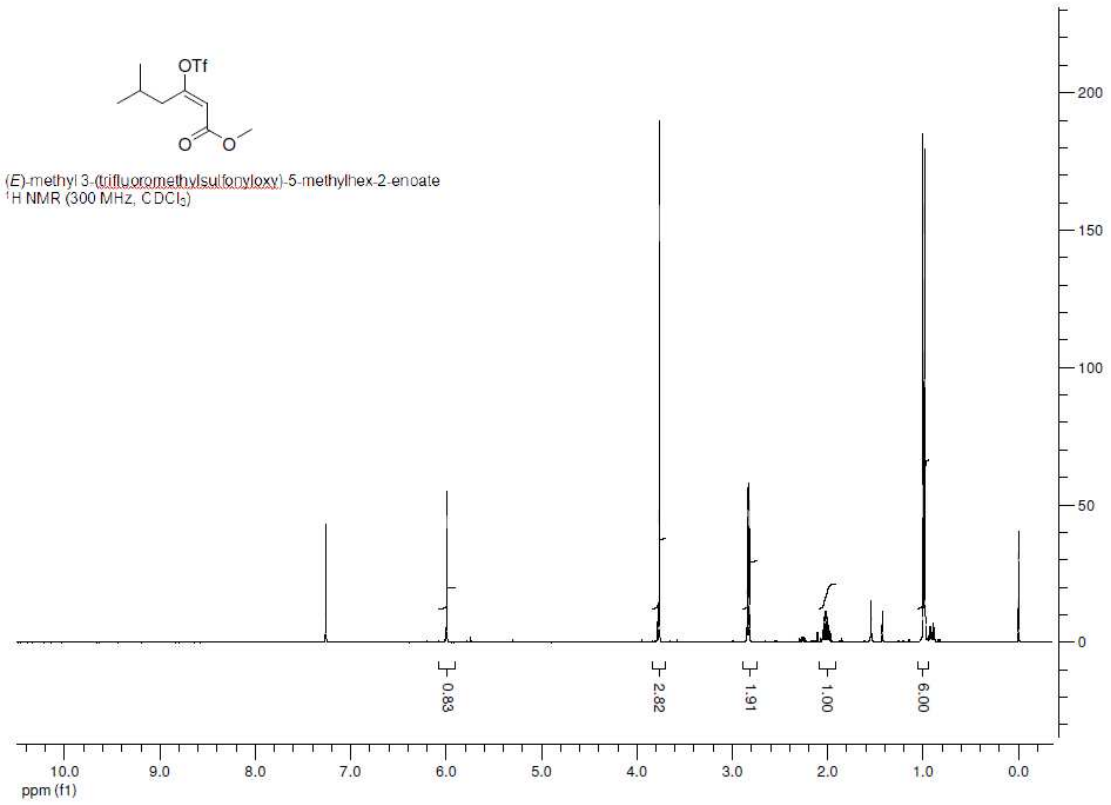
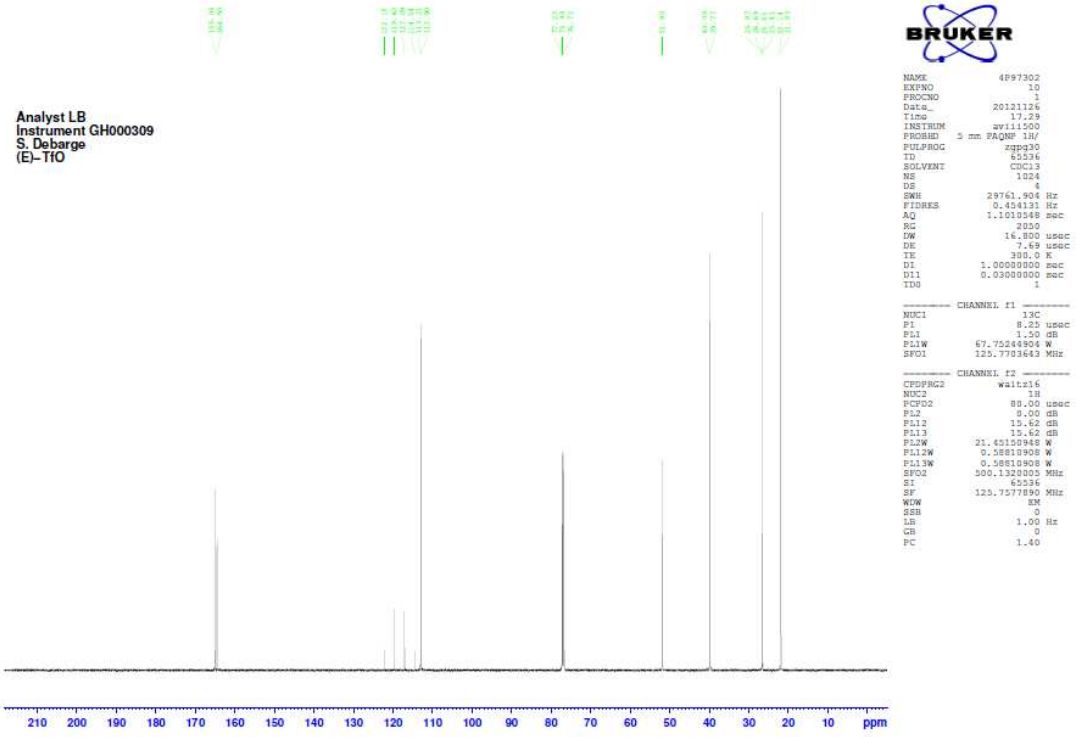
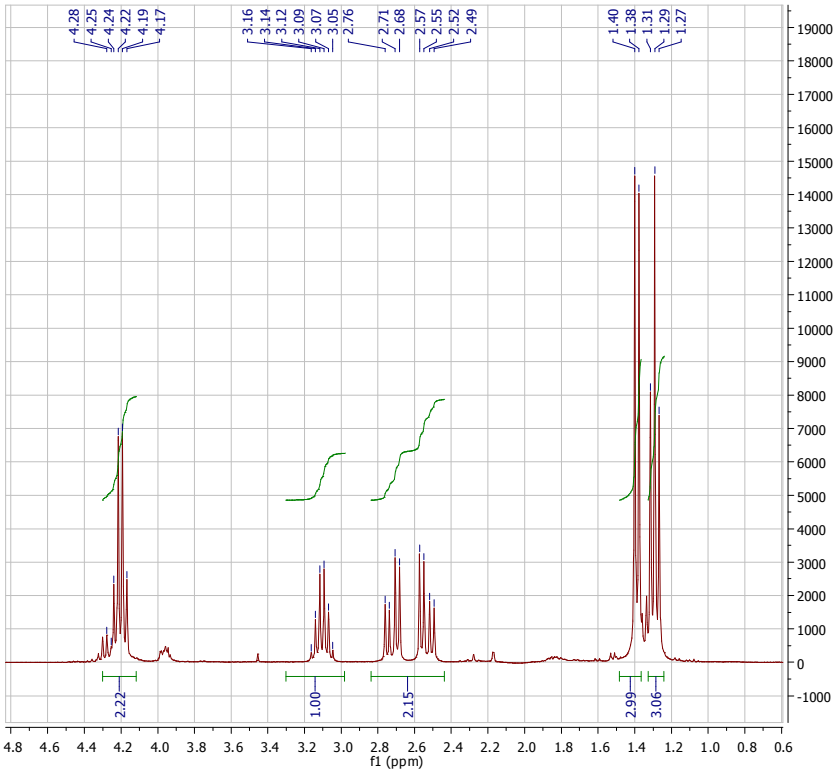
Nucleus	13C
Acquired Size	18027
Spectral Size	65536

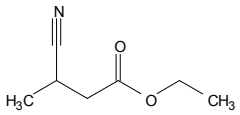


(E)-Methyl (trifluoromethylsulfonyloxy)-5-methylhex-2-enoate





rac-**1b**



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Modification Date	
Temperature	296.1
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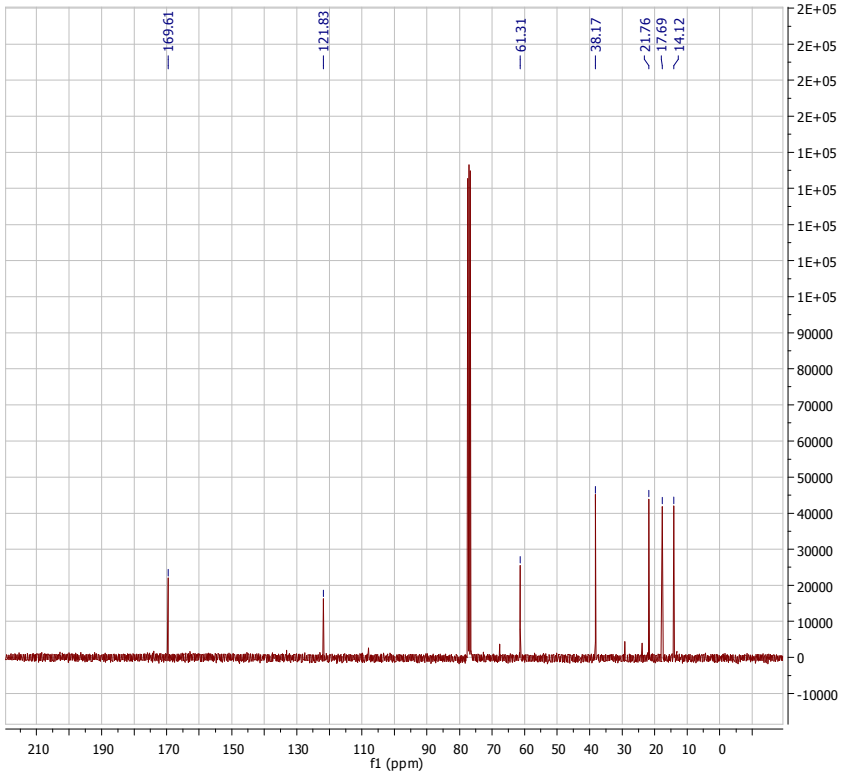
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



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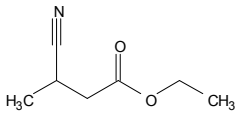

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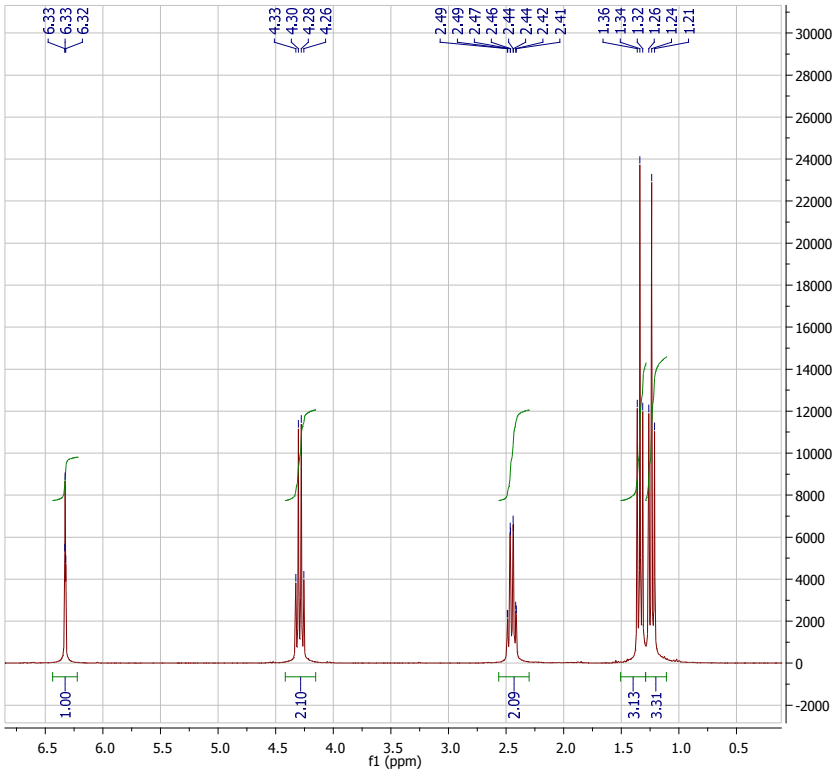
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



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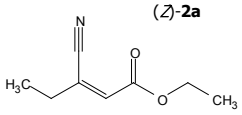

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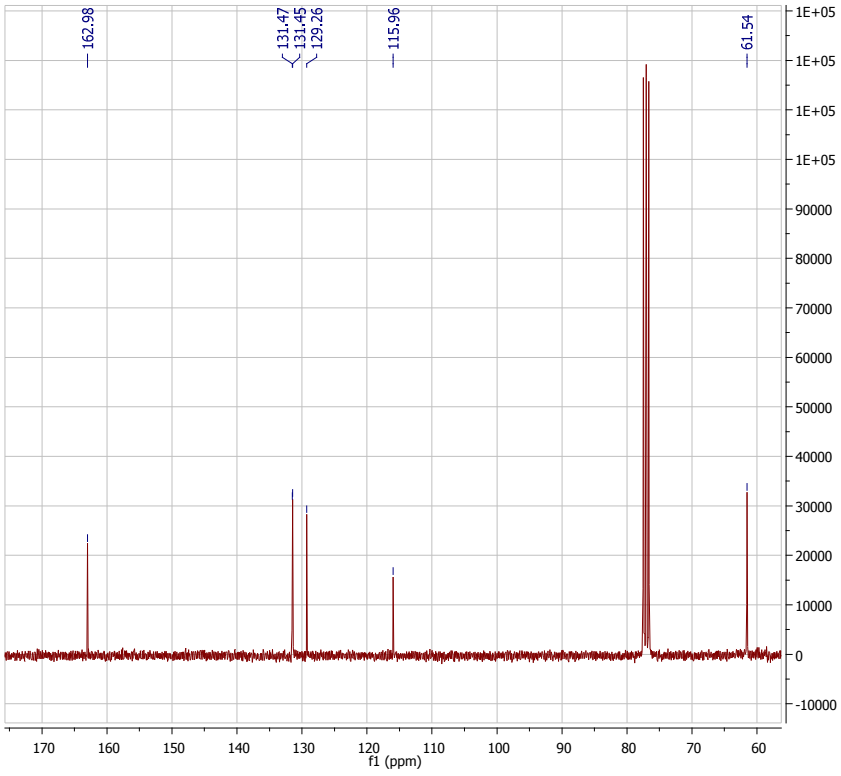
Spectral Size	65536



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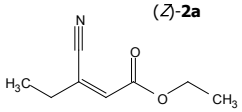

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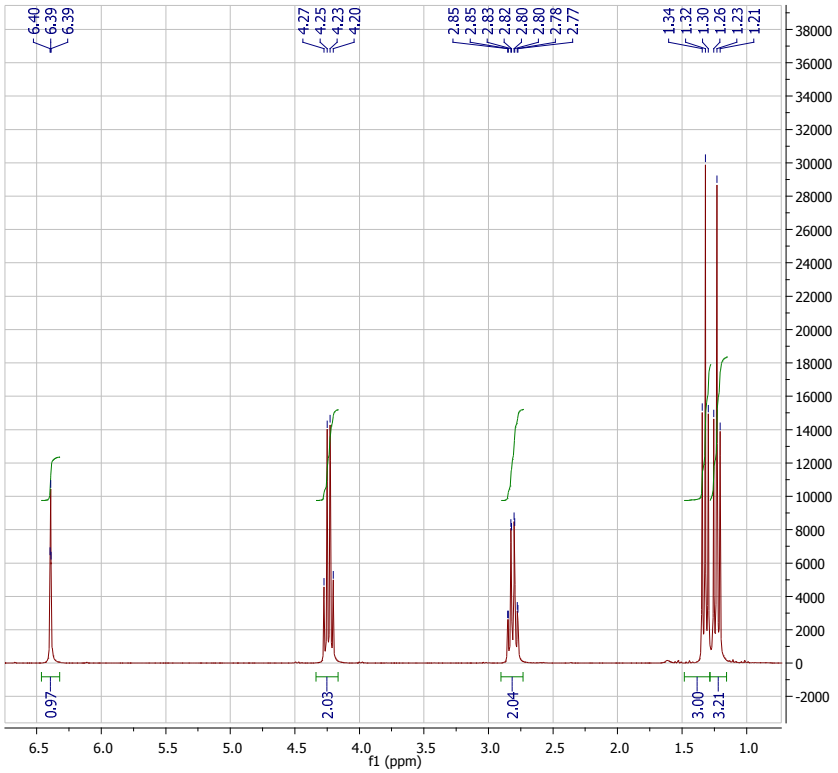

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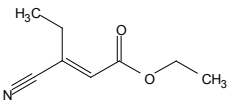

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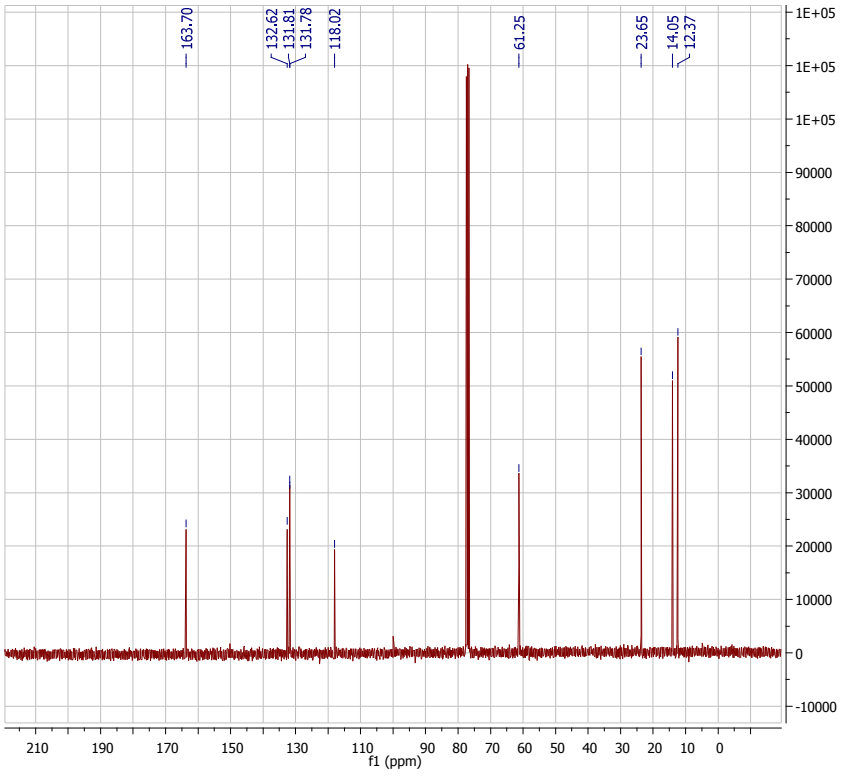

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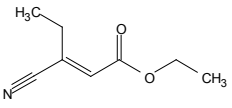

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Nucleus	13C
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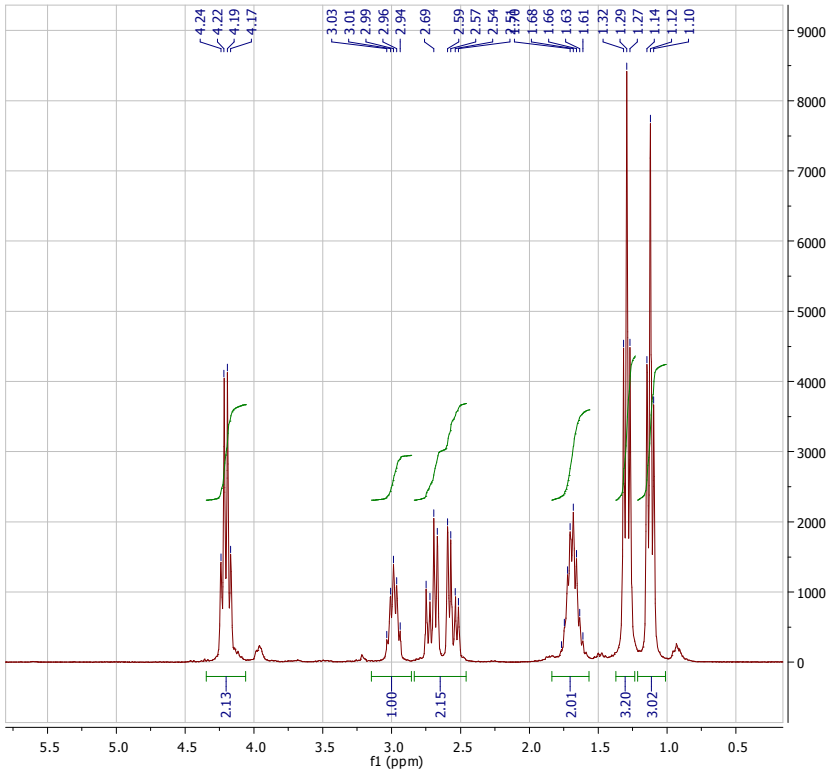
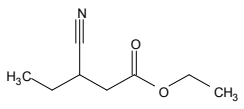
Spectral Size	65536



rac-**2b**



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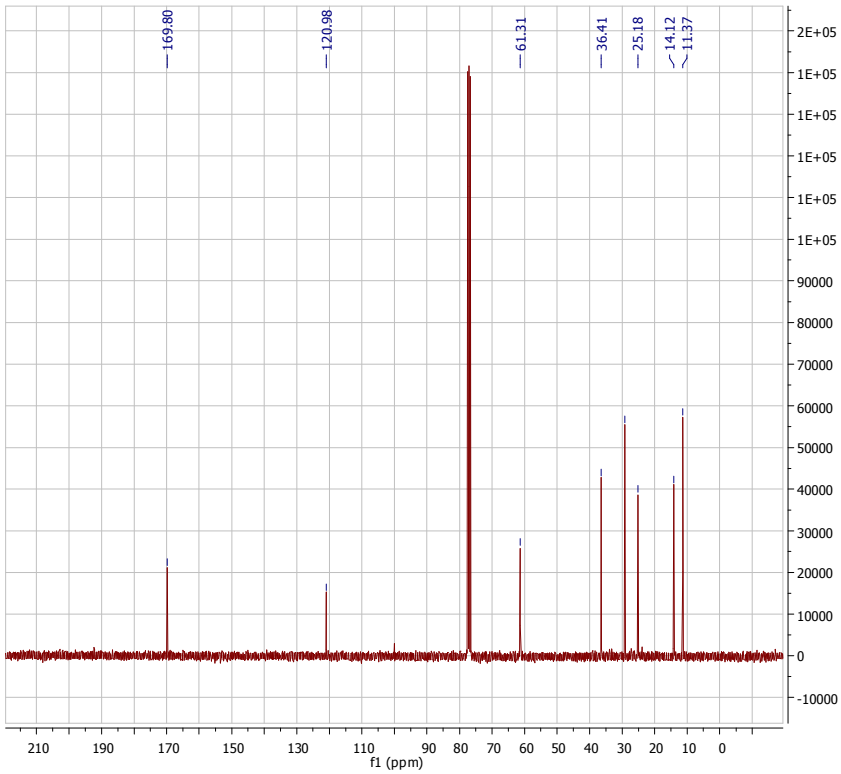
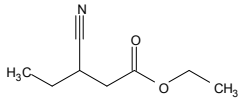
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



rac-**2b**



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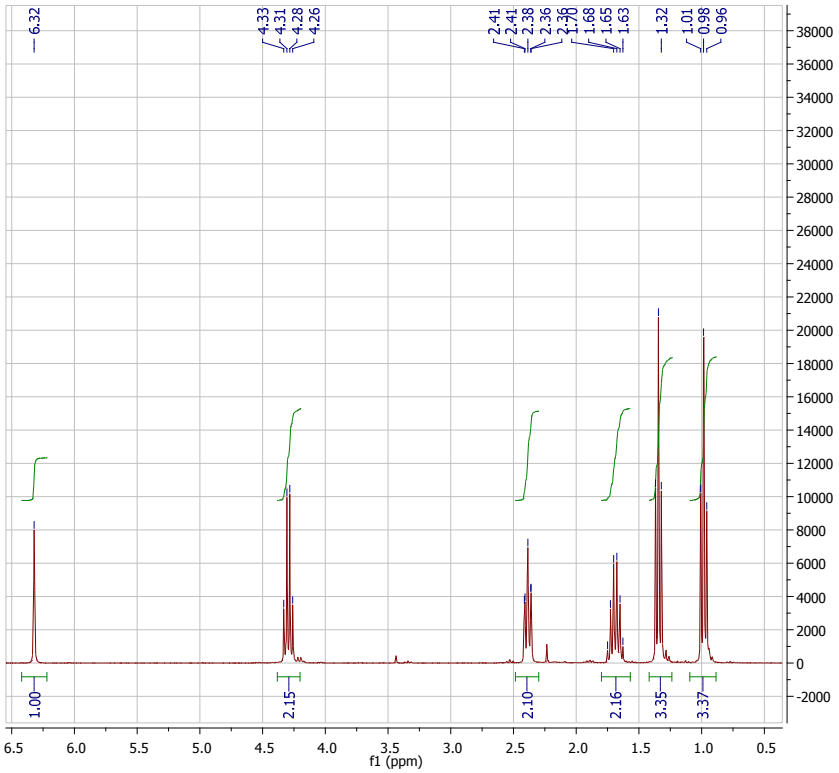

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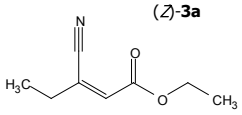

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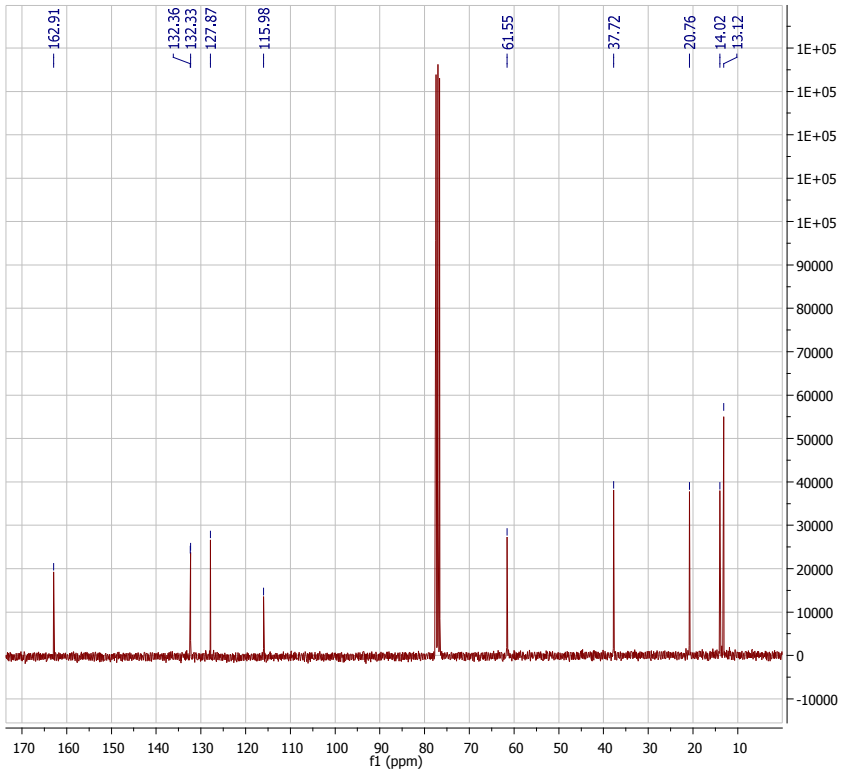
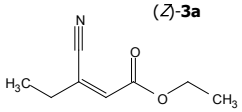
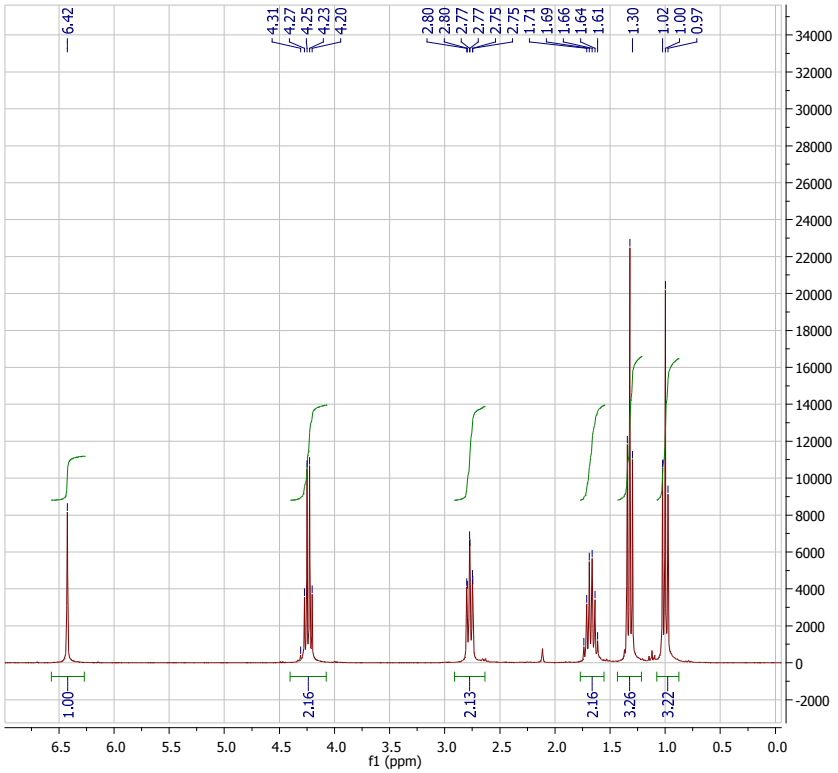
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



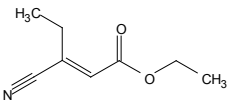
(*Z*)-**3a**



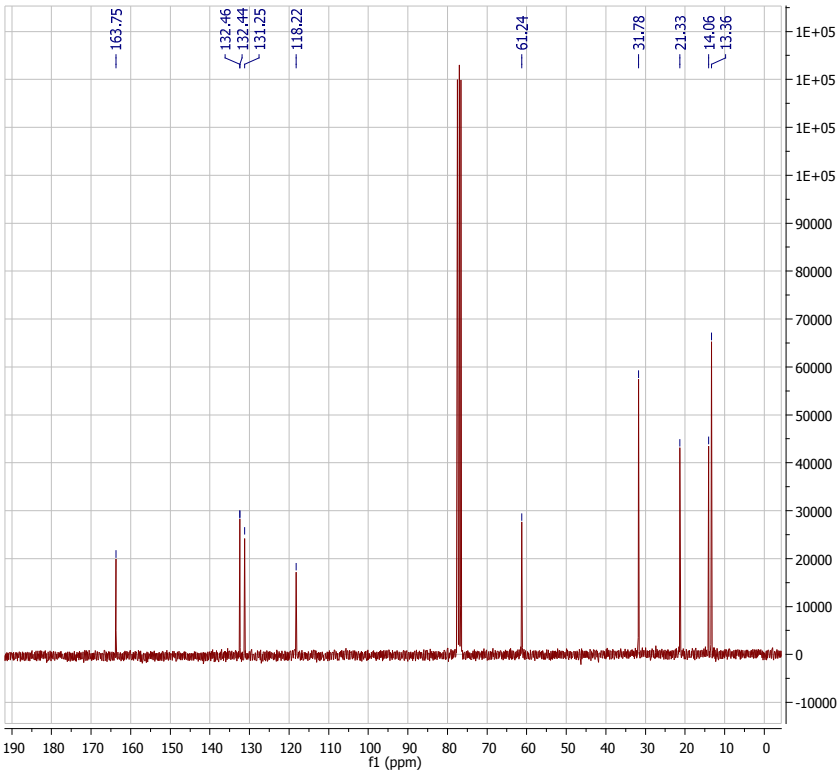
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2012-11-16T18:20:25
Modification Date	
Temperature	296.8
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



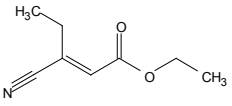
(*E*)-**3a**



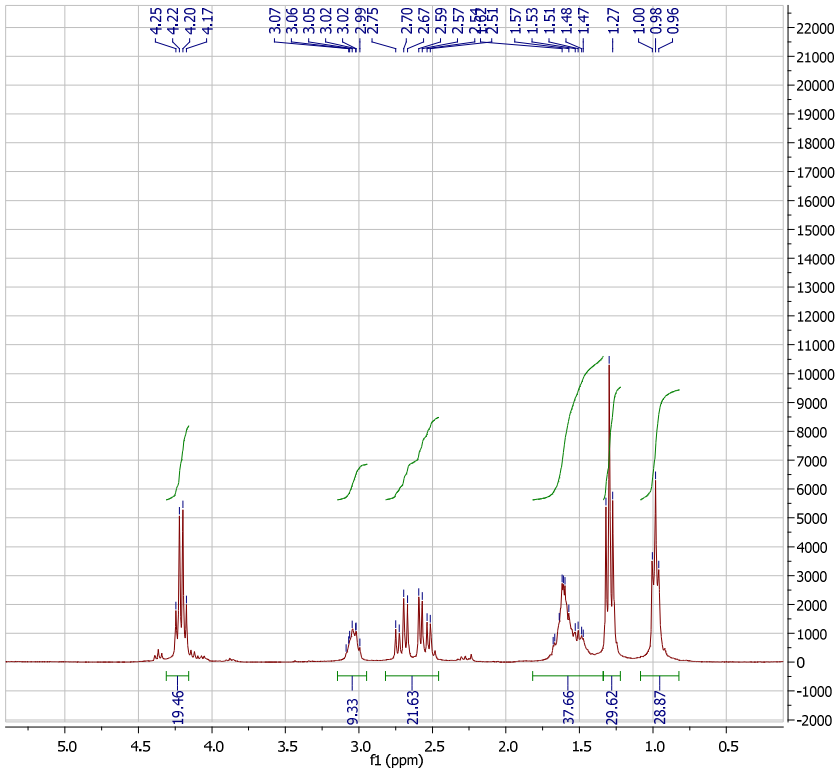
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2012-11-16T15:30:09
Modification Date	
Temperature	296.7
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



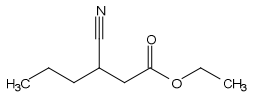
(*E*)-**3a**



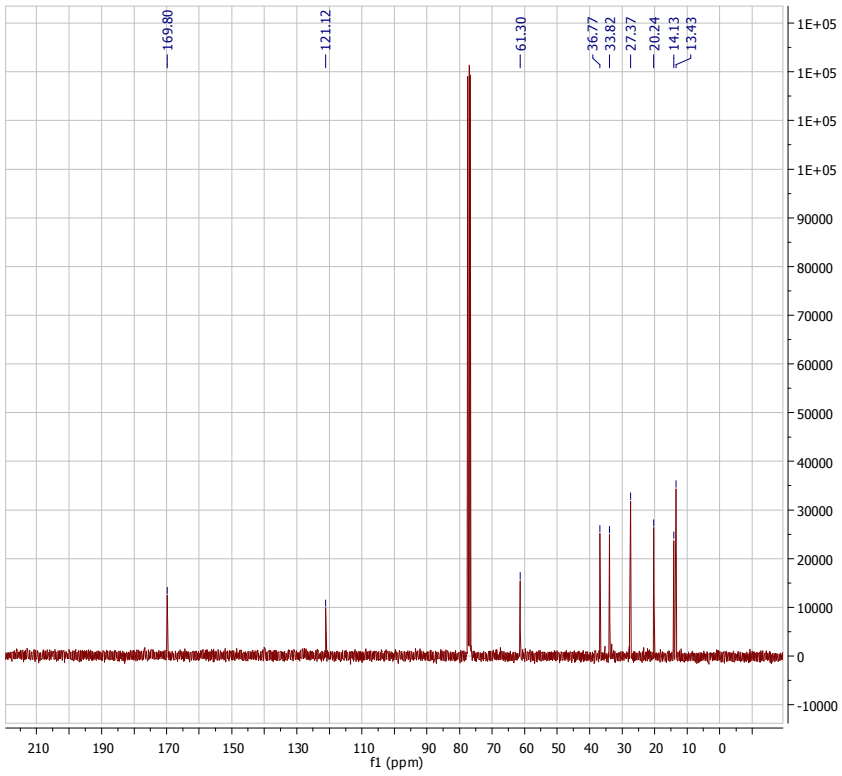
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2012-11-16T16:23:27
Modification Date	
Temperature	297.2
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



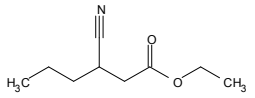
rac-**3b**



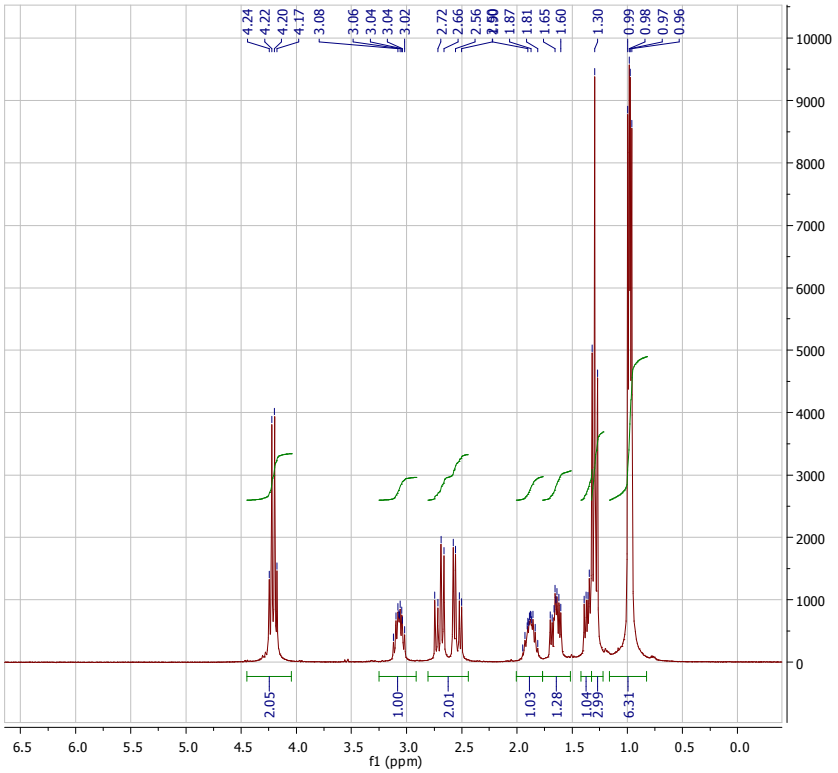
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCB
Pulse Sequence	zg30
Acquisition Date	2011-11-15T19:53:19
Modification Date	
Temperature	296.6
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



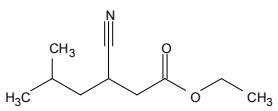
rac-**3b**



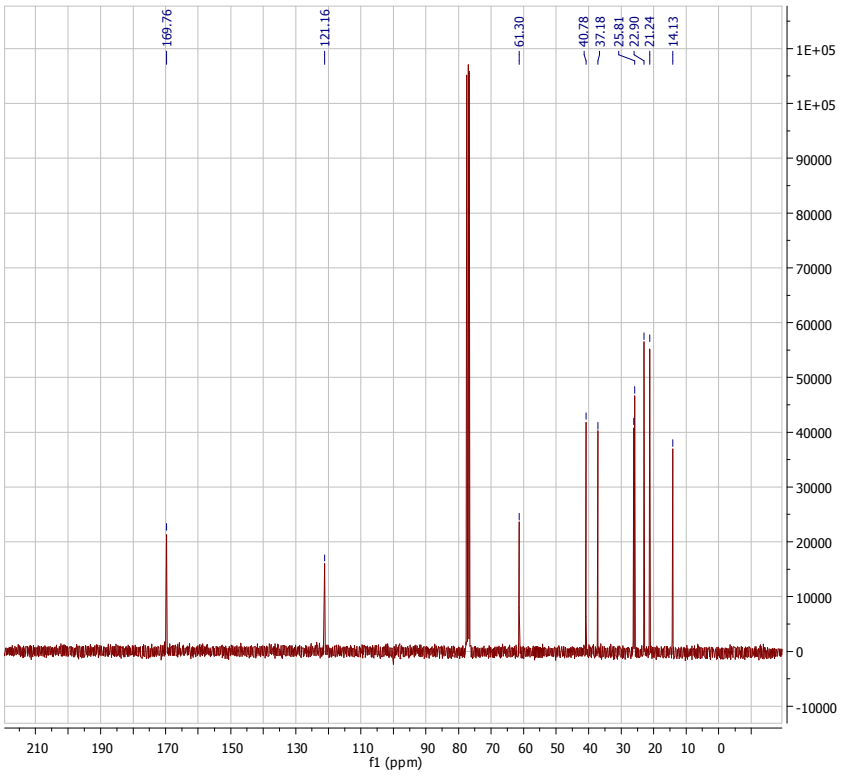
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-11-15T20:46:37
Modification Date	
Temperature	297.2
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



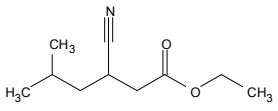
rac-**4b**



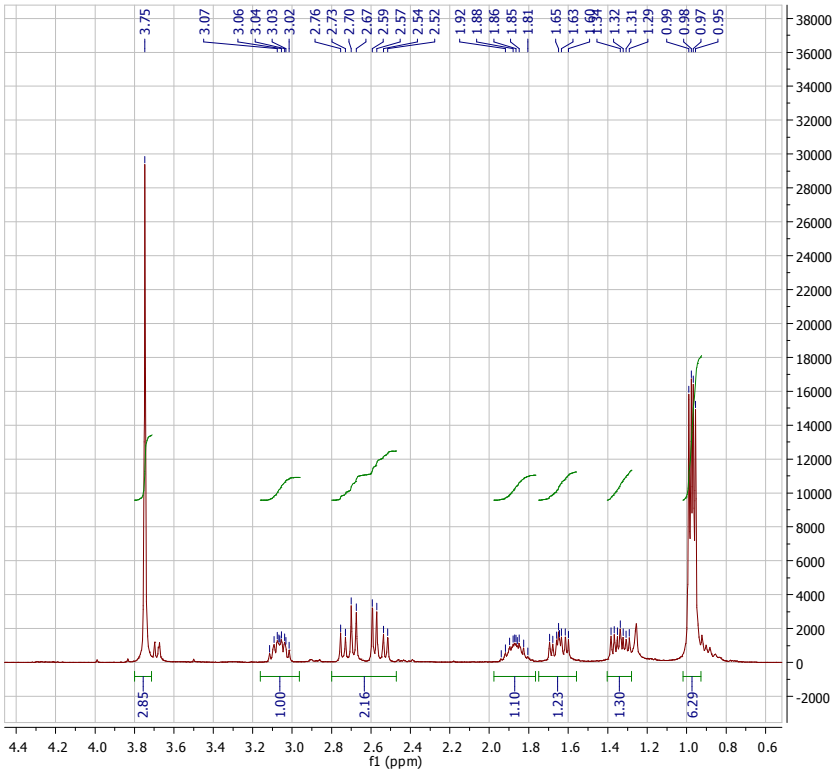
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2011-11-15T18:55:39
Modification Date	
Temperature	296.6
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



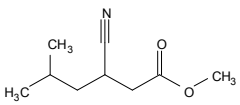
rac-**4b**



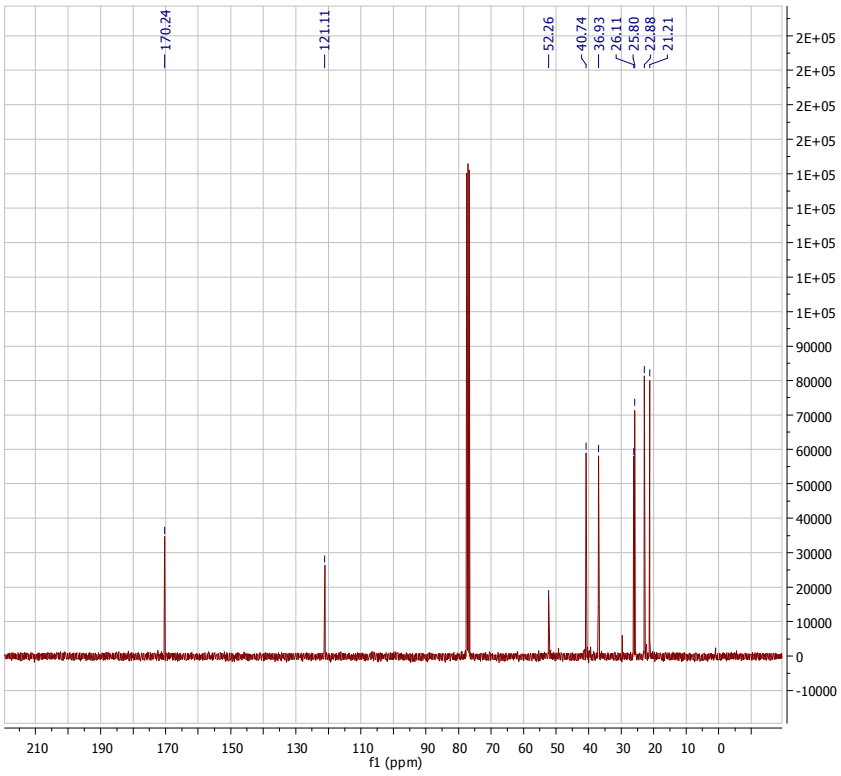
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-11-15T19:48:57
Modification Date	
Temperature	296.9
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



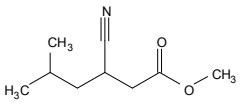
rac-**5b**



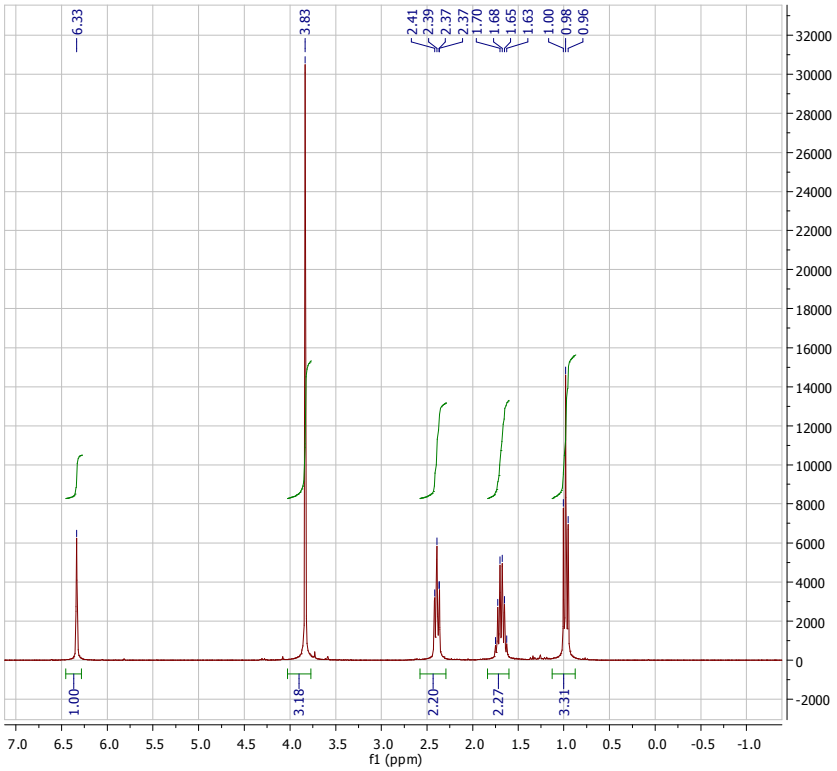
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2011-11-23T08:58:47
Modification Date	
Temperature	295.8
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



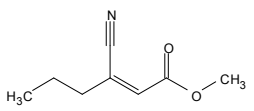
rac-**5b**



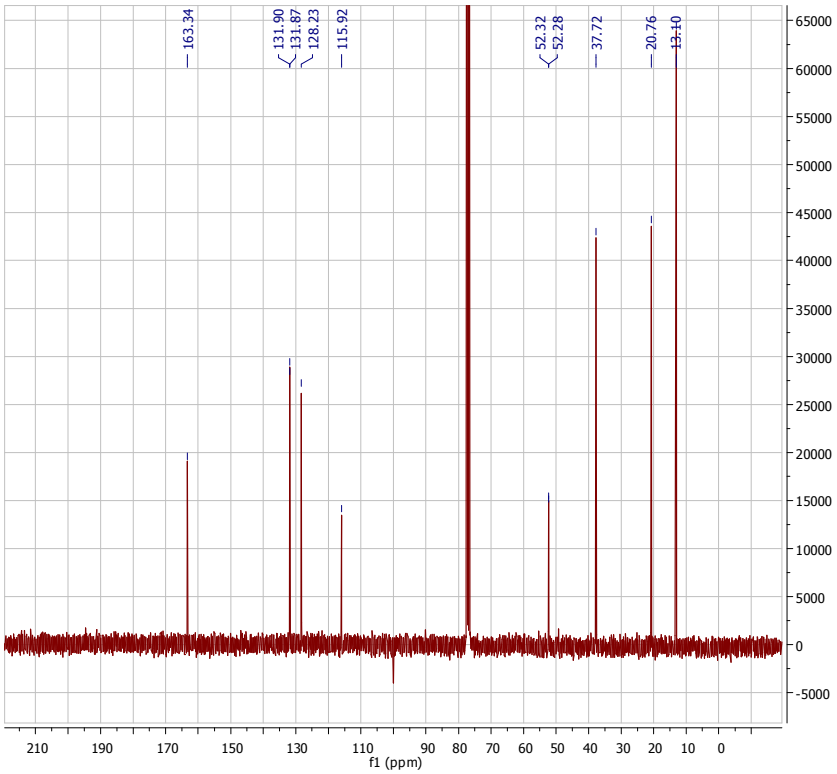
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-11-23T09:52:06
Modification Date	
Temperature	296.1
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



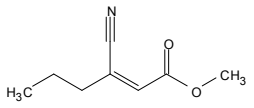
(*Z*)-**6a**



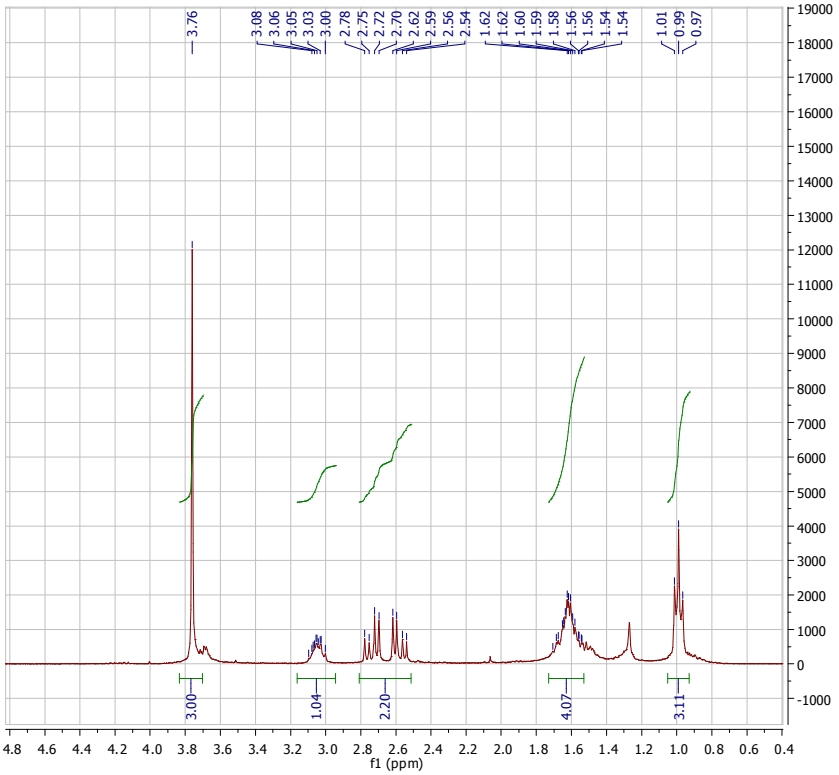
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2011-12-13T16:13:01
Modification Date	
Temperature	296.0
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536



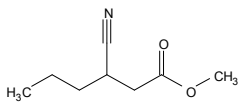
(*Z*)-**6a**



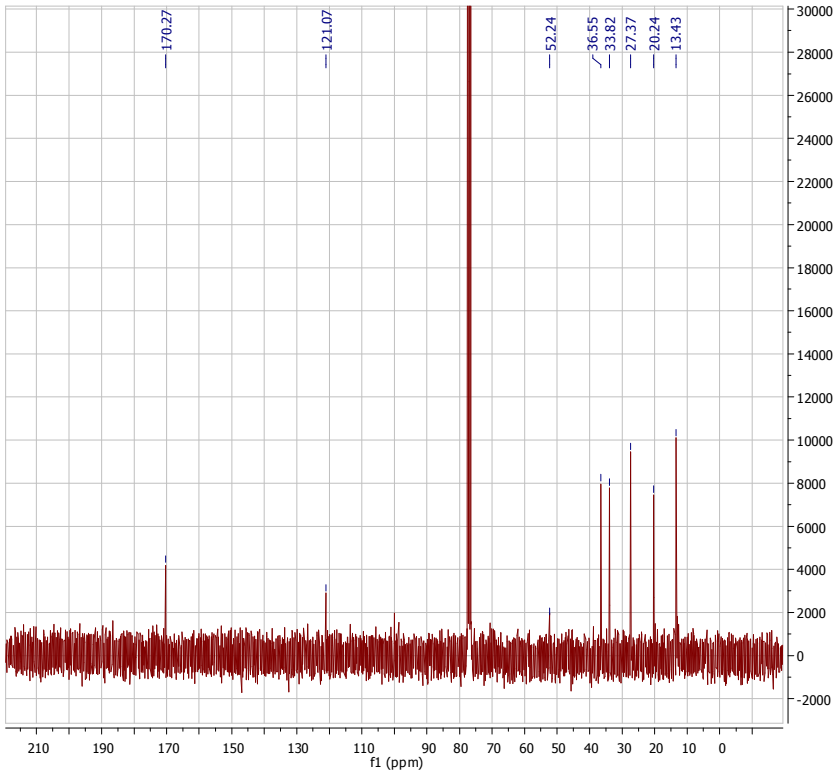
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-12-13T17:06:22
Modification Date	
Temperature	296.5
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



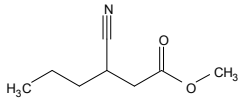
rac-**6b**



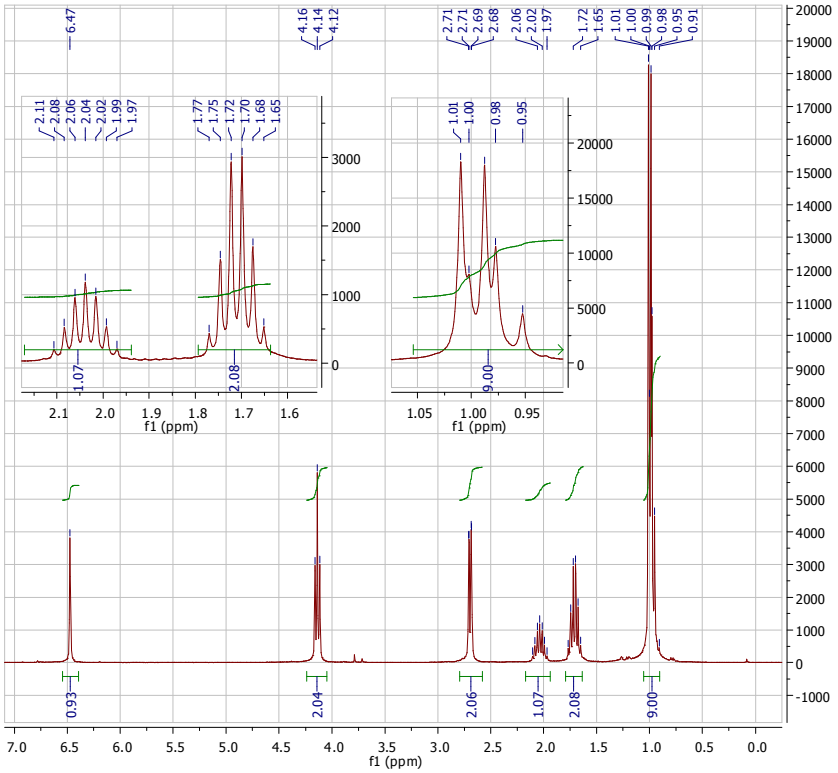
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2011-12-06T14:52:06
Modification Date	
Temperature	295.9
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536

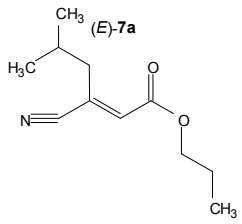


rac-**6b**

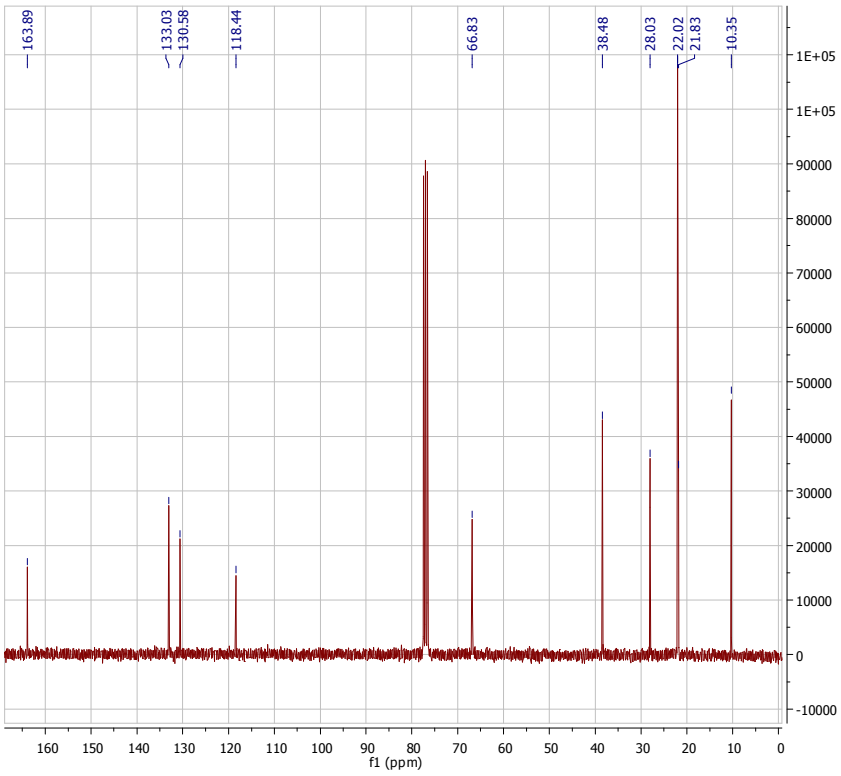


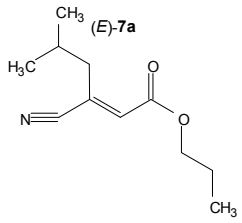
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-12-06T15:45:24
Modification Date	
Temperature	296.5
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



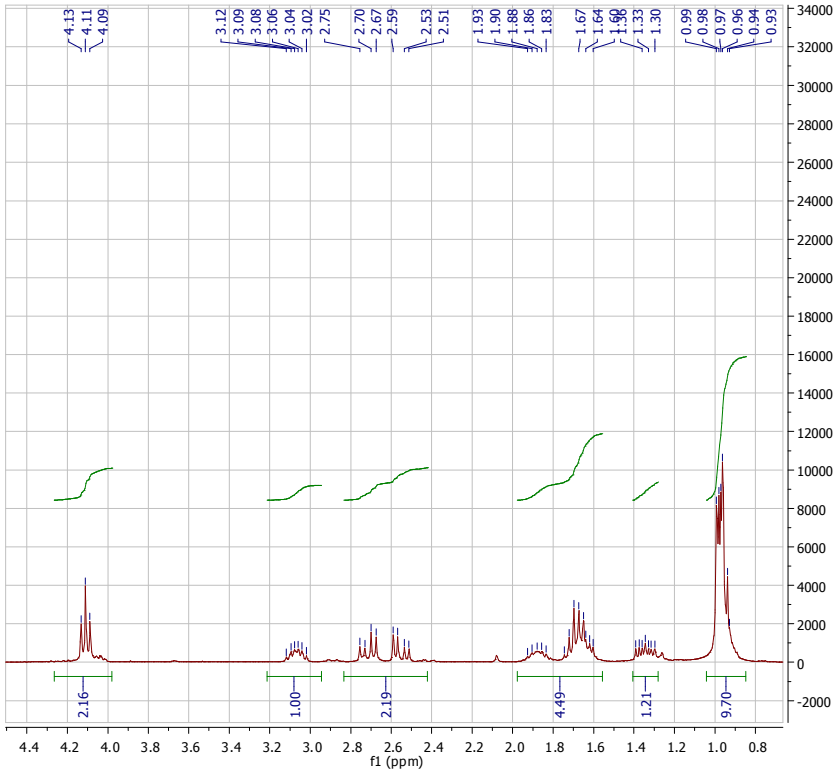


Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2011-12-05T18:48:03
Modification Date	
Temperature	296.3
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536

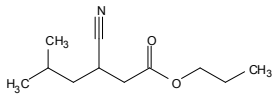




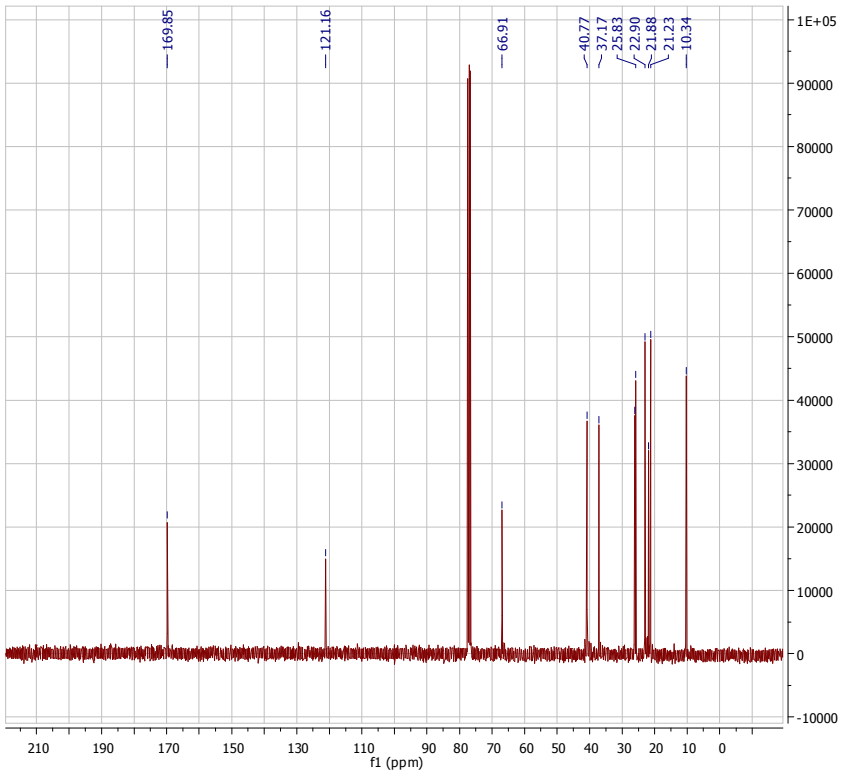
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-12-05T19:41:21
Modification Date	
Temperature	296.7
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536



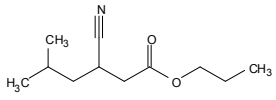
rac-**7b**



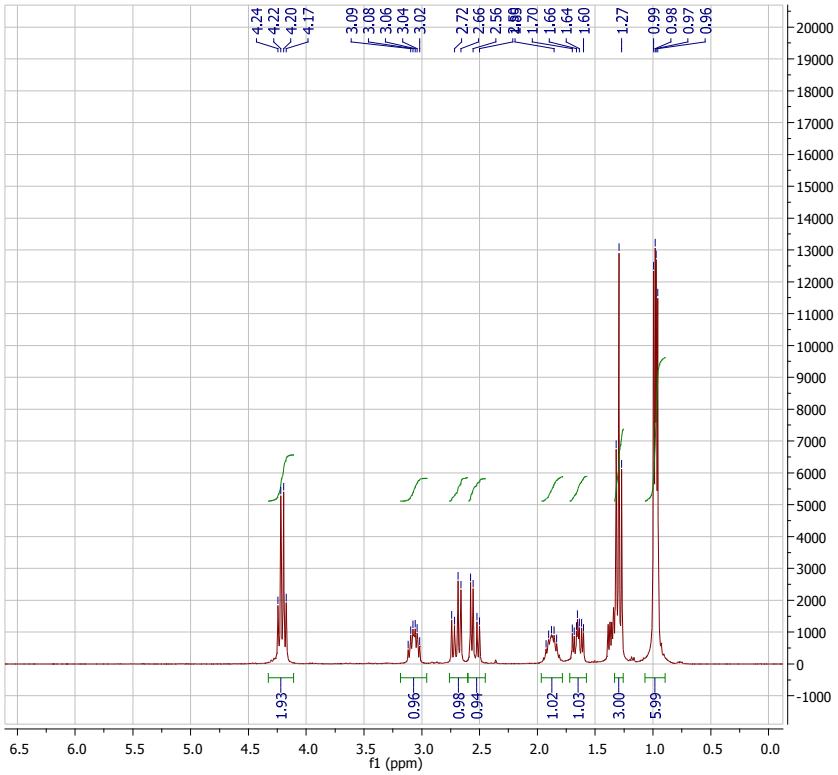
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2011-12-05T15:52:40
Modification Date	
Temperature	296.5
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536

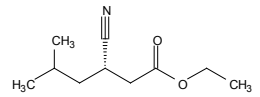


rac-**7b**



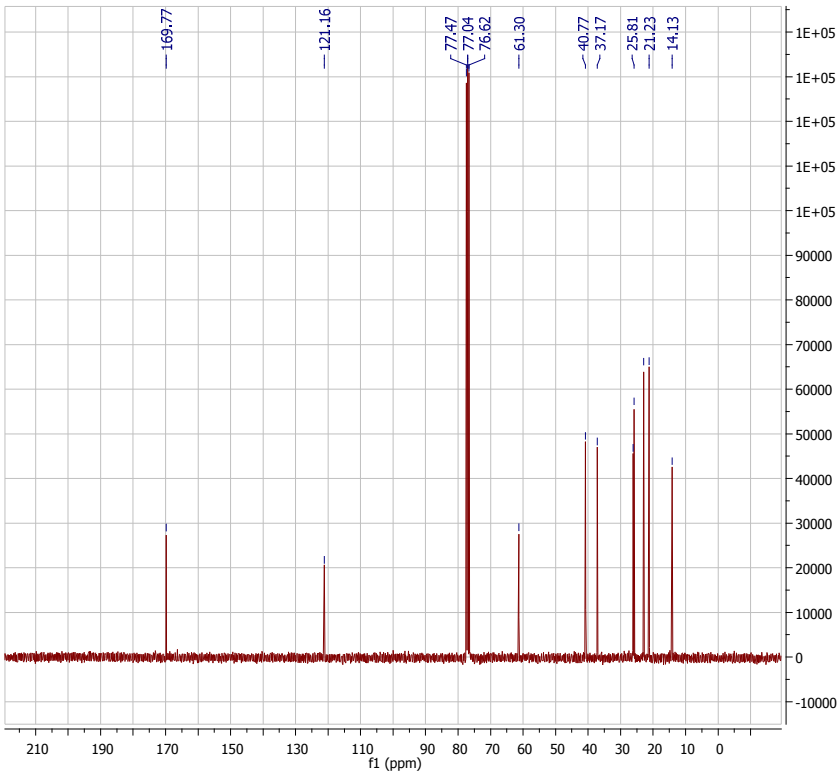
Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-12-05T16:46:15
Modification Date	
Temperature	296.6
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536

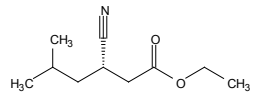




(*S*)-**4b**

Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zg30
Acquisition Date	2011-11-23T08:00:43
Modification Date	
Temperature	296.3
Number of Scans	16
Spectrometer Frequency	300.13
Spectral Width	6188.1
Lowest Frequency	-1240.6
Nucleus	1H
Acquired Size	24752
Spectral Size	65536





(*S*)-**4b**

Parameter	Value
Origin	Bruker BioSpin GmbH
Solvent	CDCl3
Pulse Sequence	zgpg30
Acquisition Date	2011-11-23T08:54:00
Modification Date	
Temperature	296.6
Number of Scans	1024
Spectrometer Frequency	75.47
Spectral Width	18028.8
Lowest Frequency	-1468.1
Nucleus	13C
Acquired Size	18027
Spectral Size	65536