Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Stereoselective synthesis of allele-specific BET inhibitors

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Supporting Information

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Supplementary Figures

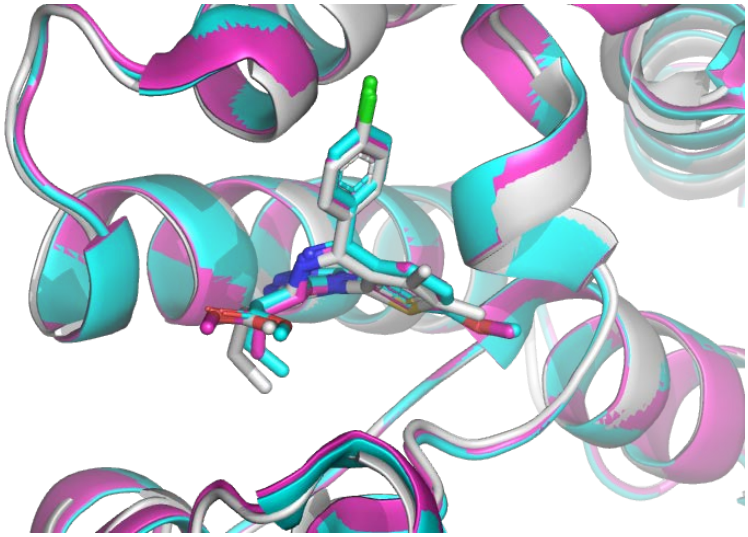


Figure S1. Co-crystal structure of Brd2(2)^{L383V} (grey cartoon representation) in complex with ET-JQ1-OMe (stick representation, grey carbons) superimposed with co-crystal structures of Brd2(2)^{L383V} (magenta (PDB code 5O3C)[1] and cyan (PDB code 5O3D)[1] cartoon representations) in complex with 9-ME-1 (stick representation, magenta carbons (5O3C)) and 9-ET-1 (stick representation, cyan carbons (5O3D)).

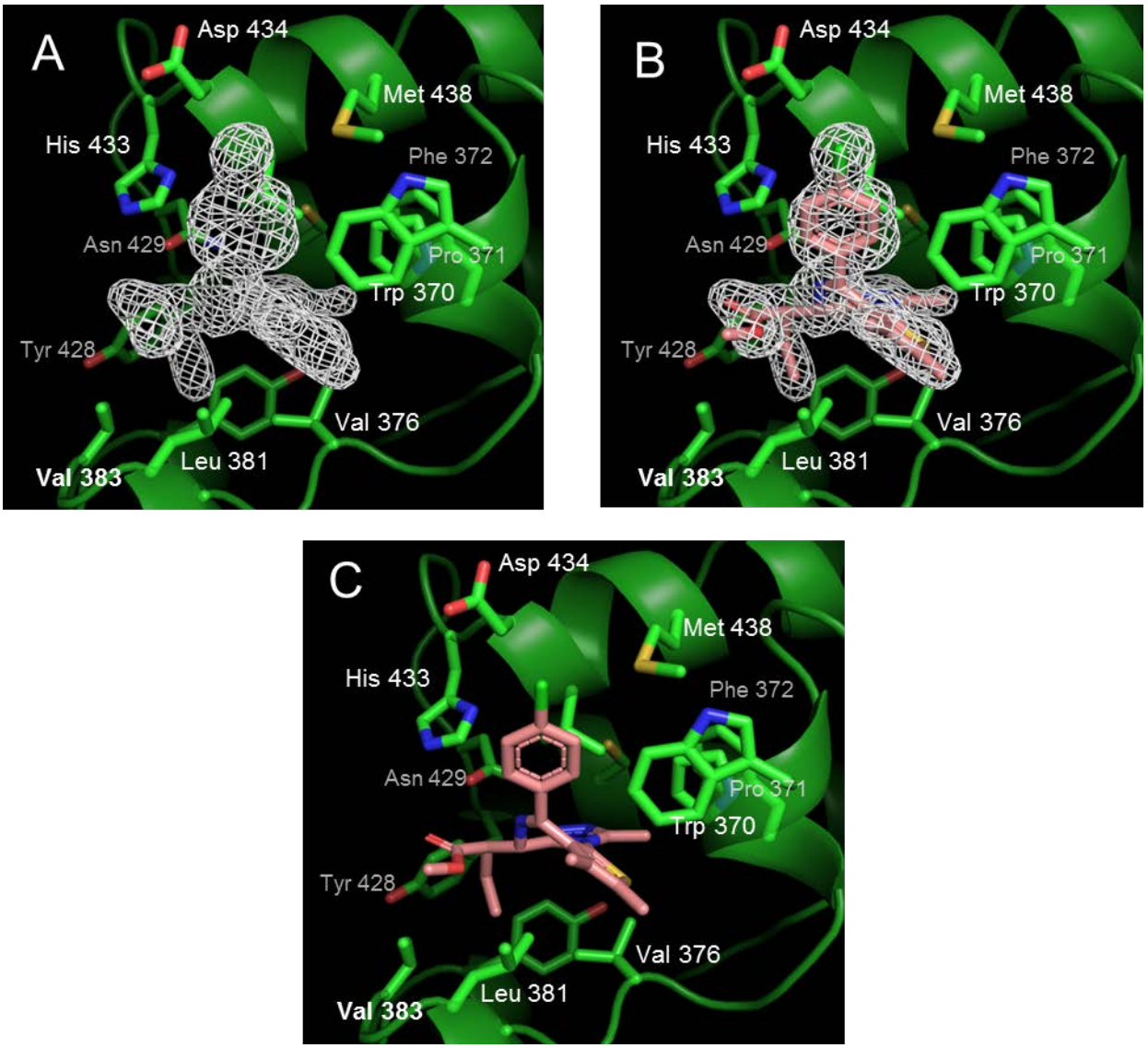


Figure S2. X-ray structure of Brd2(2)^{L383V} (green) co-crystallised with ET-JQ1-OMe (pink carbons). (A) Fo-Fc omit map (white mesh, contour: 3σ); (B) Fo-Fc omit map with ET-JQ1-OMe (pink carbons); (C) ET-JQ1-OMe (pink carbons) with no omit map.

ITC Data

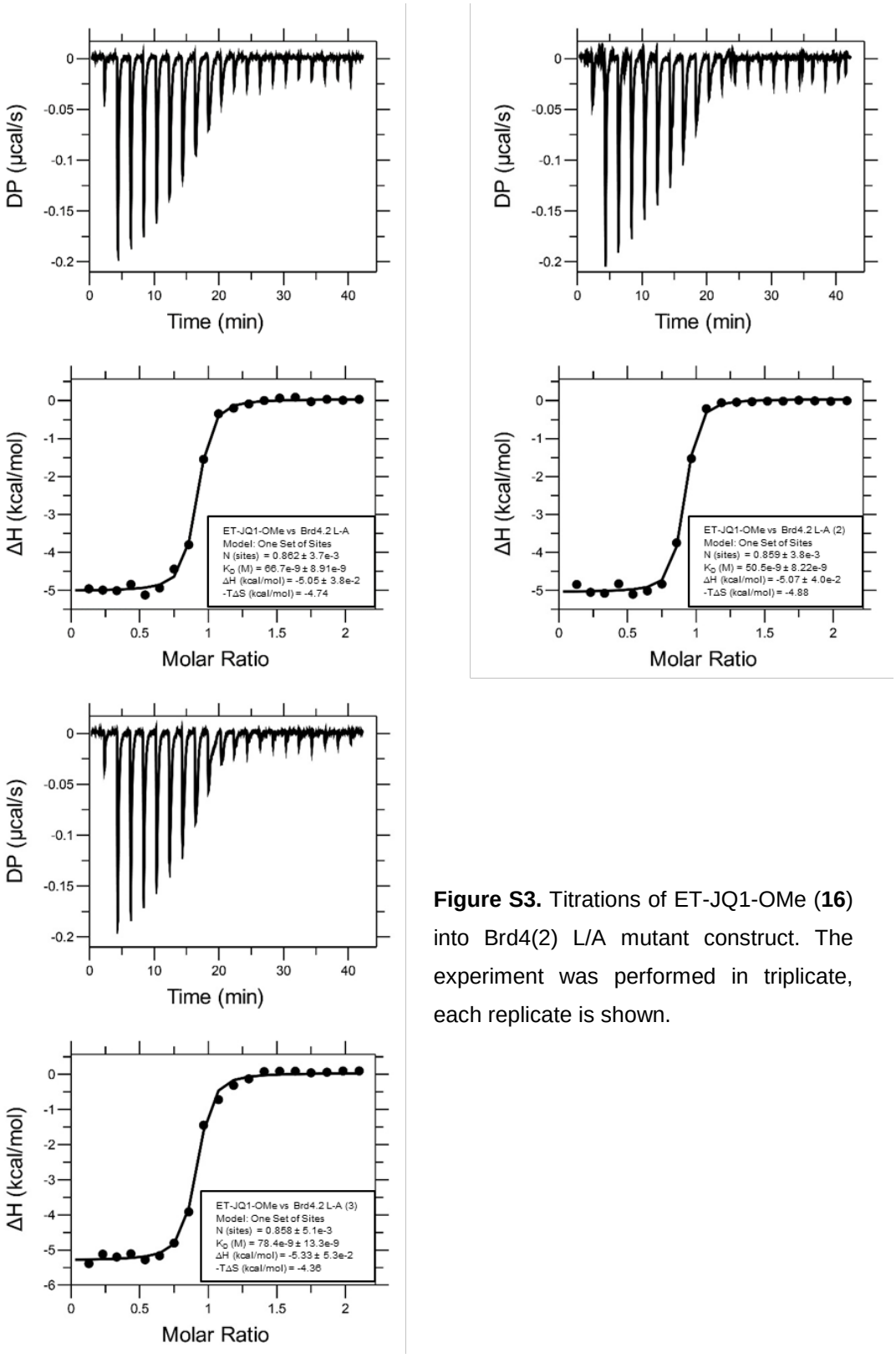


Figure S3. Titrations of ET-JQ1-OMe (**16**) into Brd4(2) L/A mutant construct. The experiment was performed in triplicate, each replicate is shown.

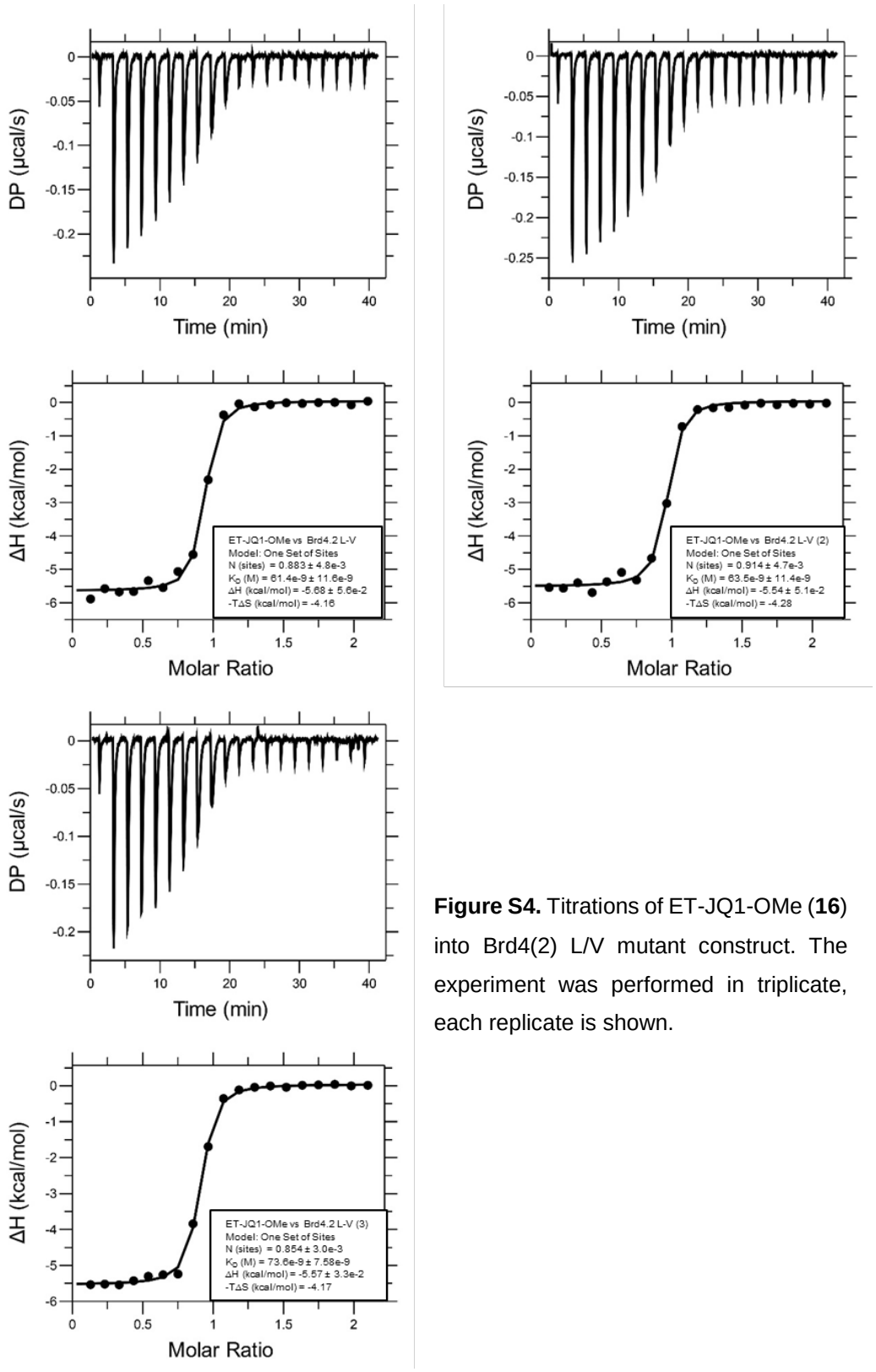


Figure S4. Titrations of ET-JQ1-OMe (**16**) into Brd4(2) L/V mutant construct. The experiment was performed in triplicate, each replicate is shown.

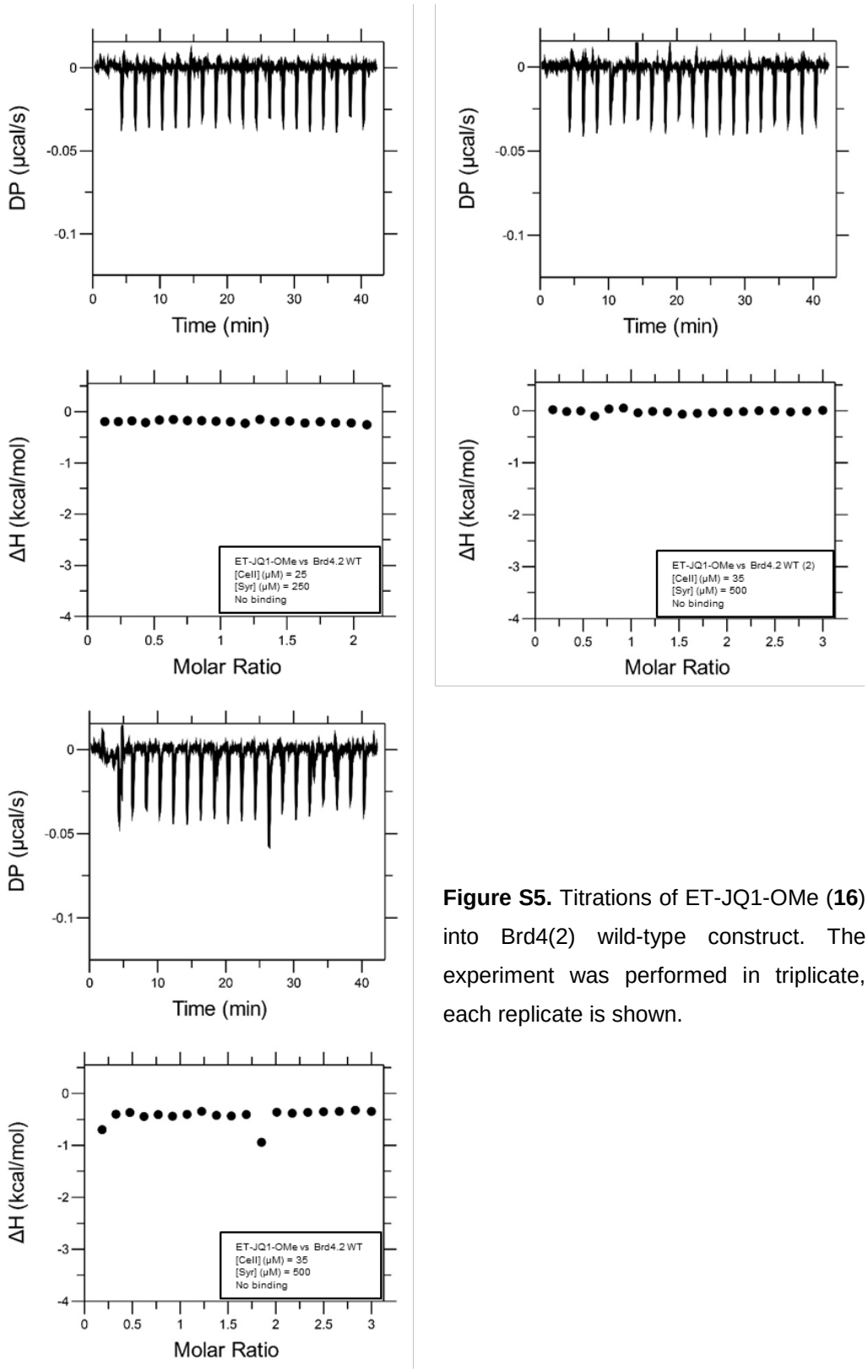
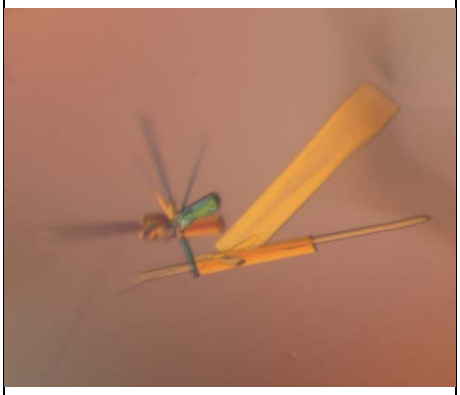


Figure S5. Titrations of ET-JQ1-OMe (**16**) into Brd4(2) wild-type construct. The experiment was performed in triplicate, each replicate is shown.

Table S1. X-ray data collection and refinement statistics.

Compound	**ET-JQ1-OMe (16)**
PDB code	6YTM
Resolution range	39.95 - 1.56 (1.616 - 1.56)
Space group	P 21 21 21
Unit cell	34.05 50.535 130.486 90 90 90
Total reflections	402700 (33778)
Unique reflections	32590 (3130)
Multiplicity	12.4 (10.7)
Completeness (%)	98.71 (96.45)
Mean *I*/sigma(*I*)	14.08 (2.88)
Wilson B-*f*actor	15.66
R-merge	0.1846 (2.556)
R-meas	0.1926 (2.689)
R-pim	0.0543 (0.8114)
$CC_{1/2}$	0.998 (0.55)
CC*	0.999 (0.843)
Reflections used in refinement	32557 (3129)
Reflections used for R-*f*ree	1589 (147)
R-work	0.1723 (0.2552)
R-*f*ree	0.2034 (0.2988)
CC(work)	0.962 (0.784)
CC(free)	0.943 (0.587)
Number of non-hydrogen atoms	2108
macromolecules	1846
ligands	76
solvent	186
Protein residues	218
RMS(bonds)	0.009

RMS(angles)	0.89
Ramachandran favored (%)	99.07
Ramachandran allowed (%)	0.93
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.51
Clashscore	1.33
Average B-*f*actor	23.40
macromolecules	22.51
ligands	24.84
solvent	31.68
Number of TLS groups	12
	

Biophysical Methods

All protein constructs used in this work were gifted by Dr. Kwok-Ho Chan and were expressed and purified using the same procedures described by Runcie et al..[1]

X-ray Crystallography – Co-crystallisation of **16** with Brd2(2) L/V

Purified Brd2(2) L383V protein at 18 mg/ml (≈1.3 mM) was mixed with an excess amount of **16** (2 mM) to give a 1:1.5 ratio of protein to ligand with a final DMSO concentration of 5%. This was incubated at 4°C for 30 min to allow for complex formation before mixing 1:1 (1 µL:1 µL) with the precipitation solution in sitting-drop vapour diffusion format. A 24-well plate was set up with conditions ranging from 0.1 M Tris pH 7.77 – 9.00 and varying concentrations of pentaerythiol propoxylate (5/4 PO/OH) 45 – 60%. Crystals formed after 16 h and were fully grown after 3-4 days.

Diffraction data was collected at Diamond Light Source beamline I24 using a Pilatus 6M detector at a wavelength of 0.9686 Å. Crystals grown in 0.1 M Tris pH 8.75 with 55% pentaerythiol propoxylate had the best diffraction of highest resolution. Data was taken from the Diamond Light Source autoPROC,[2] auto processing function. The structure was solved by molecular replacement in Phaser,[3] using two copies of search models derived from the coordinates of 9-ET-1 with Brd2(2)^{L383V} (PDB entry 5O3D). The model was iteratively refined using PHENIX,[4] and COOT. Ligand restraints were generated in eLBOW.[5] The structure models have been deposited in the protein data bank (PDB) and data collection and refinement statistics are presented in the supplementary information. All figures were generated using PyMOL 2.3.0.

Isothermal Titration Calorimetry (ITC)

ITC titrations were performed on an ITC200 instrument (MicroCal™, GE Healthcare). Proteins and **16** were dissolved in ITC buffer (20 mM HEPES, 100 mM NaCl, pH 7.5). Protein samples underwent buffer exchange via dialysis using 6-8 kDa mini D-tubes (Millipore). ITC titrations were performed at 25°C and consisted of 20 titrations: 1 initial injection of 0.4 µl over 0.8s, followed by 19 injections of 2 µl over 4s, at 2 min intervals. Data was analysed using MicroCal PEAQ-ITC Analysis Software, using a single site binding model, to determine thermodynamic values such as K_d and enthalpy of binding ΔH.

For Brd4(2) L/A and L/V constructs, 250 µM of **16** was titrated into 25 µM of protein with a final DMSO concentration of 2.5%. This was repeated to provide triplicates. For Brd4(2) wild-type,

500 μM of **16** was titrated into 35 μM of protein with a final DMSO concentration of 2.5%. This was repeated to provide duplicates. A third experiment was performed with 250 μM of **16** titrating into 25 μM of protein with a final DMSO concentration of 2.5% to provide a comparison with data from mutant constructs.

Chemistry – Materials and Methods

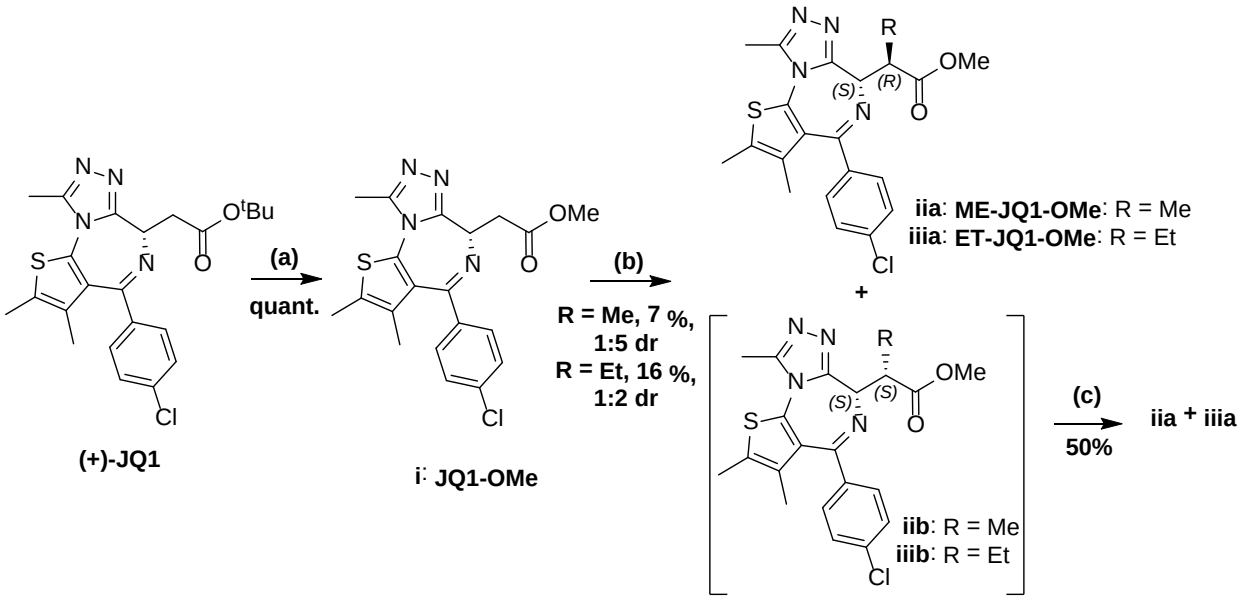
All chemicals, reagents and solvents used, unless stated otherwise, were obtained from commercial sources and used without further purification. Intermediates were purified by flash column chromatography using a Teledyne Isco Combiflash Rf or Rf200i, with Normal Phase RediSep Rf Disposable Columns or with Reverse Phase RediSep Rf Gold C18 Reusable Columns. Final compounds were purified by HPLC (High Performance Liquid Chromatography) using a Gilson Preparative HPLC System equipped with a Waters X-Bridge C18 column (100 mm x 19 mm; 5 μm particle size) using a gradient from 5% to 95% of acetonitrile in water containing 0.1% formic over 10 min at a flow rate of 25 mL/min unless stated otherwise.

Compound characterisation using NMR was performed either on a Bruker 500 Ultrashield or Bruker Ascend 400 spectrometers. The proton (^{1}H) and carbon (^{13}C) reference solvents used were as follows: d_1-Chloroform – $CDCl_3$ ((δH = 7.26 ppm / δC = 77.15 ppm) and d_5-Methanol – CD_3OD (δH = 3.31 ppm / δC = 49.00 ppm). Signal patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br.), or a combination of the listed splitting patterns. NMR spectra for all compounds were processed using Bruker TopSpin 4.0.5.

Reactions were monitored using an Agilent Technologies 1200 series analytical HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS containing an Agilent diode array detector and a Waters XBridge column (50 mm × 2.1 mm, 3.5 μm particle size). Samples were eluted with a 3 min gradient of 5% to 95% acetonitrile: water containing 0.1% formic acid at a flow rate of 0.7 mL/min. High resolution mass spectrometry (HRMS) data was performed on a Bruker microTOF. Chiral HPLC analysis was outsourced at Reach Separations Ltd, BioCity Nottingham, UK.

Chemistry – Experimental

Scheme S1: Alkylation of (+)-JQ1



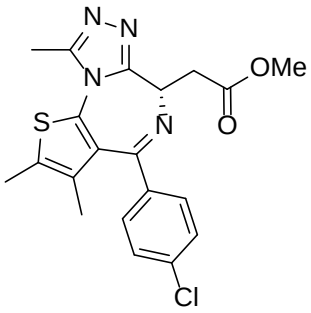
Conditions: (**a**) i) TFA, DCM, r.t., 2 h, ii) $SOCl_2$, MeOH, r.t., 3 h; (**b**) i) KHMDS, THF, −78°C, 1 h, ii) MeI/EtI, −78°C to r.t., 16 h, iii) HPLC separation; (**c**) i) NaOMe, MeOH, 120°C M.W., 40 min, ii) HPLC separation.

General procedure for direct alkylation of (+)-JQ1

(+)-JQ1 (300 mg, 0.66 mmol) was dissolved in a 1:1 mixture of DCM (1.8 ml) to TFA (1.8 ml) and stirred at r.t. until complete conversion to the free acid was observed by LC-MS. The reaction was then concentrated *in vacuo* and freeze dried (x3) to remove excess TFA and leave a yellow solid. The solid was then immediately dissolved in MeOH (5 ml/mmol) and $SOCl_2$ (3 eq.) was added and left to stir at r.t. for 3 h. The reaction mixture was then concentrated *in vacuo* to afford JQ1-OMe (**i**) as a yellow solid in quantitative yields. JQ1-OMe (1 eq.) was then dissolved in THF (17.5 ml/mmol) and cooled to -78°C. A solution of 0.5 M KHMDS in toluene (1.4 eq.) was added dropwise to the flask and stirred at -78°C for 1 h. The appropriate alkyl iodide (1.4 eq) was then added and the reaction was left to warm to r.t. over 16 h. LCMS showed a 1:5 (methyl) or 1:2 (ethyl) ratio of diastereomers with the major (*S*,*S*) isomer eluting later on silica. The reaction was then quenched with AcOH and concentrated *in vacuo* and diastereomers were separated by HPLC using a linear gradient of 30% to 70% MeCN in 0.1% formic acid in water over 10 minutes affording diastereomers with total alkylation isolated yields of ≈20%. For epimerisation, major (*S*,*S*) diastereomers, **iib** and **iiib**, (1 eq., 50 µmol) and NaOMe (10 eq.) were dissolved in

anhydrous MeOH (4 ml) and heated to 120°C by microwave irradiation for 40 min. The reaction was acidified with AcOH (1 ml) at 60°C and then concentrated *in vacuo*. Diastereomers were separated by HPLC using a linear gradient of 30 to 70% MeCN in 0.1% formic acid in water over 10 minutes affording (*S*,*S*) and (*S*,*R*) isomers in a 1:1 ratio.

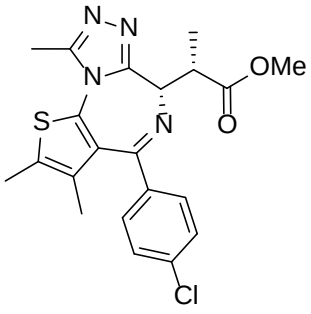
methyl (*S*)-2-(4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)acetate (**JQ1-OMe** (**i**))



^{1}H-NMR (500 MHz, $CDCl_3$): δ, ppm 7.41 (2H, d, *J* = 8.6 Hz), 7.33 (2H, d, *J* = 8.8 Hz), 4.62 (1H, dd, *J* = 6.1, 8.0 Hz), 3.78 (3H, s), 3.69 - 3.58 (2H, m), 2.68 (3H, s), 2.41 (3H, s), 1.70 (3H, s);

LC-MS m/z calc. for $C_{20}H_{20}ClN_4O_2S$ $[M+H]^+$ 415.1, found: 414.9.

methyl (*S*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)propanoate (**iib**)



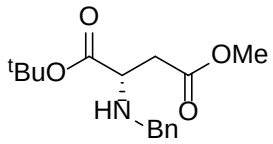
^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.43 (2H, d, *J* = 8.5 Hz), 7.33 (2H, d, *J* = 8.5 Hz), 4.31 (1H, d, *J* = 9.8 Hz), 3.88 (1H, qd, *J* = 7.2, 9.7 Hz), 3.72 (3H, s), 2.64 (3H, s), 2.41 (3H, s), 1.70 (1H, s), 1.62 (3H, d, *J* = 7.1 Hz);

^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 176.1, 163.9, 155.5, 149.6, 136.95, 136.89, 132.7, 130.8, 130.4, 129.9, 129.8, 128.8, 58.5, 52.1, 41.2, 15.4, 14.5, 13.2, 11.9;

LC-MS m/z calc. for $C_{21}H_{22}ClN_4O_2S$ $[M+H]^+$ 429.1, found: 429.0.

Stereoselective Synthesis

1-(*tert*-butyl) 4-methyl benzyl-*L*-*a*spartate (**2**)



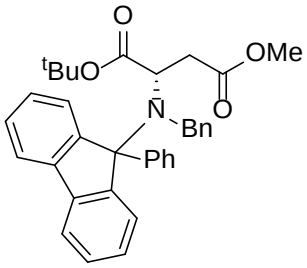
1-(*tert*-butyl) 4-methyl-*L*-*a*spartate hydrogen chloride (4.11 g, 17.1 mmol) was first converted to the free amine by dissolving in saturated $NaHCO_3$ (100 ml) and EtOAc (100 ml). After stirring at r.t. for 10 min, the aqueous layer was extracted with EtOAc (3 x 50 ml). The combined organic layers were dried with anhydrous $MgSO_4$ and concentrated *in vacuo* leaving a colourless oil with a mass of 2.86 g, 14.1 mmol. The free amine was subsequently dissolved in anhydrous DCM (70 ml) which contained a suspension of anhydrous $MgSO_4$ (3.39 g, 28.2 mmol). Freshly distilled benzaldehyde (2.70 g, 25.4 mmol) was then added and the reaction was left to stir at r.t. and monitored by NMR. After 2 h, NMR analysis showed the presence of the imine and excess benzaldehyde. MeOH (30 ml) was added and the reaction was then cooled to 0°C. $NaBH_4$ (1.07 g, 28.2 mmol) was added at once to the flask. After 10 min of stirring, the reaction was allowed to warm to r.t. and monitored by NMR. After 1 h the reaction mixture was filtered into a solution of saturated $NaHCO_3$ (200 ml) and stirred for 10 min. The aqueous phase was extracted with DCM (4 x 50 ml) and the combined organic layers were dried with $MgSO_4$ and concentrated *in vacuo.* The residue was purified by flash column chromatography (120 g silica column) using a linear gradient from 0% to 30% acetone in heptane to afford 1-(*tert*-butyl) 4-methyl benzyl-*L*-*a*spartate (2.72 g, 66%) as a colourless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 7.34 - 7.28 (4H, m), 7.25 - 7.22 (1H, m), 3.88 (1H, d, *J* = 13.0 Hz), 3.72 (1H, d, *J* = 12.8 Hz), 3.68 (3H, s), 3.55 (1H, t, *J* = 6.5 Hz), 2.69 (1H, dd, *J* = 6.2, 15.5 Hz), 2.63 (1H, dd, *J* = 6.9, 15.5 Hz), 2.07 (1H, br. s), 1.47 (9H, s);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 172.9, 171.6, 139.9, 128.5, 128.4, 127.2, 81.8, 57.9, 52.2, 51.9, 38.4, 28.2;

HRMS m/z calc. for $C_{16}H_{24}NO_4$ $[M+H]^+$ 294.1705, found: 294.1708.

1-(*tert*-butyl) 4-methyl *N*-benzyl-*N*-(9-phenyl-9H-*f*luoren-9-yl)-L-*a*spartate (**3**)



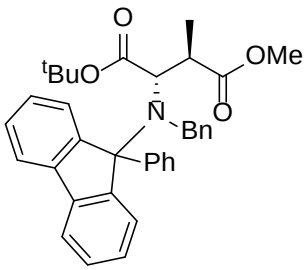
1-(*tert*-butyl) 4-methyl benzyl-*L*-*a*spartate (5.49 g, 18.7 mmol), 9-bromo-9-phenyl-9H-*f*luorene (7.59 g, 22.5 mmol), tribasic potassium phosphate (7.94 g, 37.4 mmol) and lead (II) nitrate (4.95 g, 14.96 mmol) were dissolved in MeCN (180 ml) and left to stir at r.t. TLC analysis confirmed that the reaction had gone to completion after 4 h. The mixture was then filtered through a celite pad and the solid was washed with DCM. The combined filtrate was then concentrated *in vacuo.* The residue was dissolved in diethyl ether (100 ml) and washed with 50 mM citric acid (2 x 100 ml) and then water (100 ml). The organic layer was dried with $MgSO_4$ and concentrated *in vacuo.* The residue was purified by flash column chromatography (crude split between 2 x 120 g silica columns) using a linear gradient from 5% to 15% MTBE in heptane to afford 1-(*tert*-butyl) 4-methyl *N*-benzyl-*N*-(9-phenyl-9H-*f*luoren-9-yl)-*L*-*a*spartate (7.91 g, 79%) as a fluffy white solid.

^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 7.82 (2H, d, *J* = 7.8 Hz), 7.73 (2H, t, *J* = 7.5 Hz), 7.69 (1H, d, *J* = 7.4 Hz), 7.56 (1H, d, *J* = 7.5 Hz), 7.47 (2H, d, *J* = 7.2 Hz), 7.37 (2H, dq, *J* = 1.1, 7.9 Hz), 7.31 - 7.27 (4H, m), 7.25 - 7.17 (3H, m), 4.21 (1H, d, *J* = 13.9 Hz), 3.91 - 3.84 (2H, m), 3.42 (3H, s), 2.54 (1H, dd, *J* = 10.8, 15.7 Hz), 1.94 (1H, dd, *J* = 2.9, 15.8 Hz), 1.14 (9H, s);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 172.1, 171.4, 147.6, 146.5, 143.9, 140.9, 140.4, 139.1, 129.6, 128.74, 128.68, 128.5, 128.2, 127.7, 127.6, 127.4, 127.3, 127.1, 126.7, 120.5, 120.1, 80.8, 79.7, 57.8, 51.9, 51.5, 34.0, 27.8;

HRMS m/z calc. for $C_{35}H_{36}NO_4$ $[M+H]^+$ 534.2644, found: 534.2658.

1-(*tert*-butyl) 4-methyl (2*S*,3*R*)-2-(benzyl(9-phenyl-9H-*f*luoren-9-yl)amino)-3-methylsuccinate (**4a**)



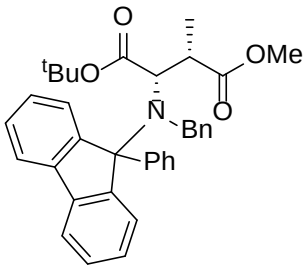
1-(*tert*-butyl) 4-methyl *N*-benzyl-*N*-(9-phenyl-9H-*f*luoren-9-yl)-*L*-*a*spartate (3.42 g, 6.41 mmol) was dissolved in anhydrous THF (50 ml) under an atmosphere of N_2 and cooled to -23°C. A solution of 1.0 M LHMDS in THF (9.62 ml, 9.62 mmol) was added dropwise to the flask and stirred at -23°C for 1 h. The solution turned orange. The reaction was then cooled to -78°C and methyl iodide (0.5 ml, 8.01 mmol) was added dropwise and left to warm to -40°C over 16h. LCMS showed a 1:6 ratio of diastereomers with the major isomer eluting later on silica. After completion, the reaction was quenched with saturated NH_4Cl solution and extracted with MTBE (3 x 50 ml). The combined organic layers were washed with brine (100 ml), dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified and diastereomers separated by flash column chromatography (crude split between 2 x 120 g silica columns) using a linear gradient from 0% to 15% MTBE in heptane to afford 1-(*tert*-butyl) 4-methyl (2*S*,3*R*)-2-(benzyl(9-phenyl-9H-*f*luoren-9-yl)amino)-3-methylsuccinate (2.28 g, 65%) as a fluffy white solid. This also afforded the minor (*S*,*S*) diastereomer (358 mg, 10%) as a fluffy white solid.

^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.94 - 7.88 (1H, m), 7.74 (1H, d, *J* = 7.5 Hz), 7.69 - 7.60 (4H, m), 7.51 (2H, d, *J* = 7.5 Hz), 7.42 (1H, dt, J=0.8, 7.5 Hz), 7.32 - 7.27 (4H, m), 7.25 - 7.14 (5H, m), 4.67 (1H, d, *J* = 14.2 Hz), 4.31 (1H, d, *J* = 14.0 Hz), 3.87 (1H, d, *J* = 10.5 Hz), 3.56 (3H, s), 2.66 (1H, qd, *J* = 7.0, 10.4 Hz), 1.04 (1H, s), 0.73 (3H, d, *J* = 7.1 Hz);

^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 174.8, 170.4, 147.3, 146.0, 145.5, 142.4, 141.9, 139.5, 129.5, 128.7, 128.4, 128.24, 128.20, 127.9, 127.75, 127.68, 127.54, 127.49, 127.1, 126.8, 120.3, 119.4, 80.9, 80.6, 64.2, 51.6, 51.3, 42.2, 27.7, 15.6;

HRMS m/z calc. for $C_{36}H_{38}NO_4$ $[M+H]^+$ 548.2801, found: 548.2800.

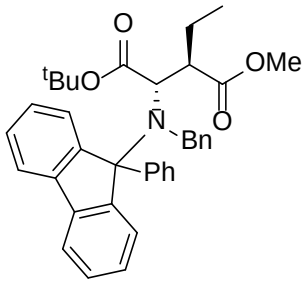
1-(*tert*-butyl) 4-methyl (2*S*,3*S*)-2-(benzyl(9-phenyl-9H-*f*luoren-9-yl)amino)-3-methylsuccinate (**4b**)



^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.77 - 7.71 (3H, m), 7.66 - 7.57 (5H, m), 7.45 (1H, t, *J* = 7.3 Hz), 7.34 - 7.27 (6H, m), 7.25 - 7.19 (3H, m), 4.39 (1H, d, *J* = 13.5 Hz), 4.31 (1H, d, *J* = 13.4 Hz), 3.60 (1H, d, *J* = 11.2 Hz), 3.36 (3H, s), 2.39 (1H, qd, *J* = 7.1, 11.2 Hz), 1.16 (3H, d, *J* = 7.1 Hz), 1.06 (9H, s);

^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 175.6, 170.9, 147.5, 146.7, 145.7, 142.0, 140.4, 129.8, 128.7, 128.5, 128.4, 128.0, 127.8, 127.6, 127.5, 127.3, 127.2, 126.8, 120.6, 120.1, 80.6, 63.9, 52.9, 51.4, 41.9, 27.6, 15.9;

1-(*tert*-butyl) 4-methyl (2*S*,3*R*)-2-(benzyl(9-phenyl-9H-*f*luoren-9-yl)amino)-3-ethylsuccinate (**5a**)

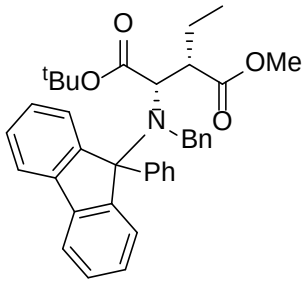


1-(*tert*-butyl) 4-methyl *N*-benzyl-*N*-(9-phenyl-9H-*f*luoren-9-yl)-*L*-*a*spartate (1.2 g, 2.25 mmol) was dissolved in anhydrous THF (6 ml) under an atmosphere of N_2 and cooled to -78°C. A solution of 1.0 M LHMDS in THF (4.38 ml, 4.38 mmol) was added dropwise to the flask and stirred at -78°C for 1 h. Ethyl iodide (1.09 ml, 13.5 mmol) was then added dropwise and the reaction was left to stir at -78°C for 16 h. The reaction was then warmed to between -23 and -30°C and stirred for 24 h until the starting material was consumed. LCMS showed a 1:2 ratio of diastereomers with the major isomer eluting later on silica. After completion, the reaction was quenched with saturated NH_4Cl solution and extracted with MTBE (3 x 20 ml). The combined organic layers were washed with brine (50 ml), dried with $MgSO_4$ and concentrated *in vacuo*. The residue was purified and diastereomers separated by flash column chromatography (40 g silica column) using a linear gradient from 5% to 15% MTBE in heptane to afford 1-(*tert*-butyl) 4-methyl (2*S*,3*R*)-2-(benzyl(9-phenyl-9H-*f*luoren-9-yl)amino)-3-ethylsuccinate (612 mg, 48%) as a fluffy white solid. This also afforded the minor (*S*,*S*) diastereomer (293 mg, 23%) as a fluffy white solid.

^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.93 - 7.89 (1H, m), 7.75 (1H, d, *J* = 7.6 Hz), 7.66 - 7.59 (4H, m), 7.53 (2H, d, *J* = 7.6 Hz), 7.45 (1H, t, *J* = 7.5 Hz), 7.33 - 7.14 (9H, m), 4.65 (1H, d, *J* = 13.5 Hz), 4.32 (1H, d, *J* = 13.9 Hz), 3.89 (1H, d, *J* = 11.4 Hz), 3.57 (3H, s), 2.57 (1H, ddd, *J* = 3.9, 9.0, 11.0 Hz), 1.20 - 1.11 (1H, m), 1.10 - 1.02 (1H, m), 1.02 (9H, s), 0.51 (3H, t, *J* = 7.4 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 173.8, 170.4, 147.3, 145.8, 145.7, 142.6, 141.5, 139.4, 130.0, 128.7, 128.4, 128.2, 127.8, 127.6, 127.5, 127.1, 126.9, 120.4, 119.3, 80.8, 80.7, 62.6, 51.6, 51.1, 48.8, 27.6, 23.0, 10.6;

1-(*tert*-butyl) 4-methyl (2*S*,3*S*)-2-(benzyl(9-phenyl-9H-*f*luoren-9-yl)amino)-3-ethylsuccinate (**5b**)



^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.77 - 7.69 (3H, m), 7.65 - 7.61 (4H, m), 7.58 (1H, d, *J* = 7.9 Hz), 7.45 (1H, t, *J* = 7.3 Hz), 7.35 - 7.27 (6H, m), 7.26 - 7.19 (3H, m), 4.45 (1H, d, *J* = 13.3 Hz), 4.38 (1H, d, *J* = 13.5 Hz), 3.60 (1H, d, *J* = 11.1 Hz), 3.36 (3H, s), 2.29 - 2.16 (2H, m), 1.42 - 1.32 (1H, m), 0.44 (3H, t, *J* = 7.4 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 174.9, 170.6, 147.5, 146.4, 145.6, 142.1, 140.7, 139.9, 129.7, 128.7, 128.4, 128.1, 127.8, 127.6, 127.5, 127.25, 127.17, 126.5, 120.5, 120.1, 80.5, 63.2, 52.2, 51.2, 49.6, 27.6, 23.1, 11.5;

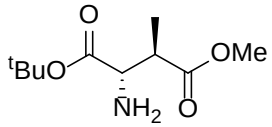
HRMS m/z calc. for $C_{37}H_{40}NO_4$ $[M+H]^+$ 562.2957, found: 562.2972.

General procedure for amino acid deprotection

Compounds **4a**, **5a**, **5b** were dissolved in acetic acid (6 L/mol) (MeOH was used as a co-solvent for the (*S*,*S*) diastereomer, **5b**) and a catalytic amount of Pd/C (15% wt/wt) was added. The reaction was left to stir under an atmosphere of hydrogen until no starting material was remaining by LC-MS analysis. The reaction was then filtered through PTFE syringe filters. The crude was *in vacuo* and the residue was partitioned between Et_2O and 1.0 M H_3PO_4 solution. The organic phase was extracted with 1.0 M H_3PO_4 solution (x 2) and the combined aqueous fractions were adjusted to pH 9 using solid K_2CO_3. The basified solution was extracted with EtOAc (x 5) and the combined organic fractions were dried with $MgSO_4$ and concentrated *in vacuo* to afford free amines, **6**, **7** and **7*** in quantitative yields as colourless oils.

The resulting free amines were dissolved in a 1:1 mixture of DCM to TFA (1 ml/100 mg) and stirred for 1 h. The reaction was monitored by NMR to see the disappearance of the *tert*-butyl group. After completion the reaction mixture was concentrated *in vacuo*. The resulting TFA salt was then dissolved in 2.0 M HCl and freeze dried to convert the TFA salt to the HCl salt. This process was repeated 2-3 times to afford the amino acids **8**, **9** and **9*** as HCl salts in quantitative yields.

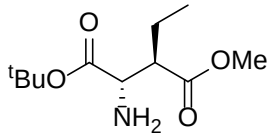
1-(*tert*-butyl) 4-methyl (2*S*,3*R*)-2-*a*mino-3-methylsuccinate (**6**)



Yield: 561 mg (quant.); ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 3.69 (3H, s), 3.55 (1H, d, *J* = 5.4 Hz), 2.89 (1H, dq, *J* = 5.5, 7.1 Hz), 1.62 (2H, s), 1.46 (9H, s), 1.21 (3H, d, *J* = 7.2 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 174.4, 173.3, 81.7, 57.7, 51.8, 44.0, 28.1, 13.4;

1-(*tert*-butyl) 4-methyl (2*S*,3*R*)-2-*a*mino-3-ethylsuccinate (**7**)

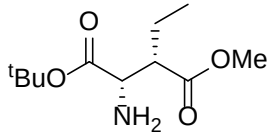


Yield: 451 mg (quant.); ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 3.69 (3H, s), 3.50 (1H, d, *J* = 6.0 Hz), 2.70 (1H, td, *J* = 5.9, 8.9 Hz), 1.82 - 1.72 (1H, m), 1.65 - 1.55 (3H, m), 1.47 (9H, s), 0.96 (3H, t, *J* = 7.4 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 174.1, 173.7, 81.6, 56.5, 51.6, 51.1, 28.1, 22.2, 12.2;

HRMS m/z calc. for $C_{11}H_{22}NO_4$ $[M+H]^+$ 232.1549, found: 232.1552.

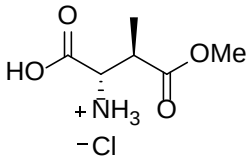
1-(*tert*-butyl) 4-methyl (2*S*,3*S*)-2-*a*mino-3-ethylsuccinate (**7***)



Yield: 150 mg (quant.); ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 3.71 (3H, s), 3.67 (1H, d, *J* = 5.6 Hz), 2.67 - 2.62 (1H, m), 1.84 - 1.74 (1H, m), 1.62 - 1.54 (1H, m), 1.53 (2H, s), 1.47 (9H, s), 0.95 (3H, t, *J* = 7.3 Hz);

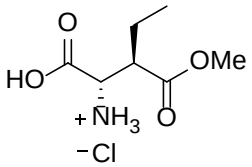
^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 174.3, 173.3, 81.7, 56.4, 51.8, 51.0, 28.1, 20.8, 12.3;

(2*S*,3*R*)-2-*a*mino-4-methoxy-3-methyl-4-oxobutanoic acid hydrogen chloride salt (**8**)



Yield: 607 mg (quant.); ^{1}H-NMR (500 MHz, MeOD) δ, ppm: 4.31 (1H, d, *J* = 4.2 Hz), 3.76 (3H, s), 3.31 - 3.25 (1H, m), 1.34 (3H, d, *J* = 7.4 Hz);

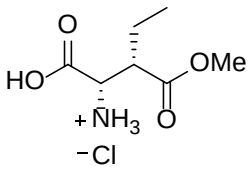
(2*S*,3*R*)-2-*a*mino-3-(methoxycarbonyl)pentanoic acid hydrogen chloride salt (**9**)



Yield: 408 mg (quant.); ^{1}H-NMR (500 MHz, MeOD) δ, ppm: 4.23 (1H, d, *J* = 4.6 Hz), 3.76 (3H, s), 3.11 - 3.06 (1H, m), 1.94 - 1.82 (1H, m), 1.75 - 1.64 (1H, m), 1.06 (3H, t, *J* = 7.4 Hz);

^{13}C-NMR (500 MHz, MeOD) δ, ppm: 173.5, 170.2, 54.4, 53.0, 47.7, 22.2, 12.3;

(2*S*,3*S*)-2-*a*mino-3-(methoxycarbonyl)pentanoic acid hydrogen chloride salt (**9***)



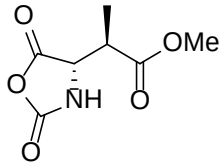
Yield: 128 mg (quant.) ^{1}H-NMR (400 MHz, MeOD) δ, ppm: 4.28 (1H, d, *J* = 4.3 Hz), 3.77 (3H, s), 3.03 - 2.95 (1H, m), 1.97 - 1.82 (1H, m), 1.79 - 1.66 (1H, m), 1.04 (3H, t, *J* = 7.3 Hz);

^{13}C-NMR (400 MHz, MeOD) δ, ppm: 173.7, 170.1, 54.5, 53.0, 48.3, 22.7, 12.4;

General procedure for the synthesis of *N*-carboxyanhydrides (**10**, **11** and **11***)

Amino acids **8**, **9** and **9*** (1.0 eq.) were dissolved in anhydrous THF (3 L/mol). Triphosgene (0.67 eq.) was then added and the flask was flushed with N_2 and left to stir for 16 h in a closed vial. The reaction was monitored by ^{1}H-NMR. The flask was then concentrated *in vacuo* to afford *N*-carboxyanhydrides **10**, **11** and **11*** in quantitative yields as pale brown oils.

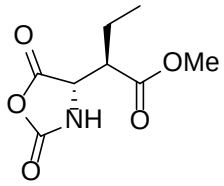
methyl (*R*)-2-((*S*)-2,5-dioxooxazolidin-4-yl)propanoate (**10**)



Yield: 575 mg (quant.); ^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 6.10 (1H, br. s), 4.36 (1H, d, *J* = 7.0 Hz), 3.77 (3H, s), 3.04 (1H, dq, *J* = 7.2, 7.2 Hz), 1.44 (3H, d, *J* = 7.4 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 172.5, 167.9, 151.8, 59.1, 52.9, 42.0, 13.6;

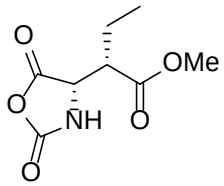
methyl (*R*)-2-((*S*)-2,5-dioxooxazolidin-4-yl)butanoate (**11**)



Yield: 386 mg (quant.); ^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 6.12 (1H, br. s), 4.45 (1H, d, *J* = 6.7 Hz), 3.76 (3H, s), 2.94 (1H, dt, *J* = 6.5, 6.6 Hz), 2.09 - 1.97 (1H, m), 1.91 - 1.80 (1H, m), 1.03 (3H, t, *J* = 7.5 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 171.9, 168.3, 152.0, 57.1, 52.8, 48.4, 21.5, 11.1;

methyl (*S*)-2-((*S*)-2,5-dioxooxazolidin-4-yl)butanoate (**11***)



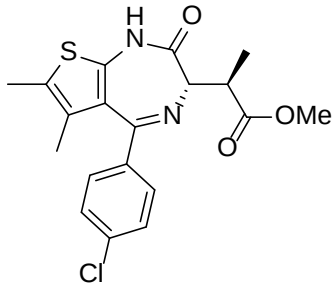
Yield: 121 mg (quant.) ^{1}H-NMR (400 MHz, MeOD) δ, ppm: 4.59 (1H, d, *J* = 4.7 Hz), 3.73 (3H, s), 2.87 - 2.81 (1H, m), 1.93 - 1.69 (2H, m), 1.02 (3H, t, *J* = 7.5 Hz);

^{13}C-NMR (400 MHz, MeOD) δ, ppm: 173.9, 170.5, 154.2, 58.8, 52.8, 50.1, 22.4, 12.2;

General procedure for thienodiazepines (**13**, **14** and **14***)

(2-amino-4,5-dimethylthiophen-3-yl)(4-chlorophenyl)methanone (1 eq.) was suspended in toluene (830 mL/mol). 4 Å molecular sieves and TFA (2 eq.) were then added and stirred at r.t. for 5 min. *N*-carboxyanhydrides, **10**, **11** and **11***, (1.2 eq.) were dissolved in toluene (210 mL/mol) (DCM for compound **10**) and subsequently added to the flask which was then heated at 60°C for 1 h. The conversion of the amino ketone was monitored by LC-MS. TEA (2.5 eq.) was then added and the reaction was heated to 80°C and stirred for 2 h. The mixture was then cooled to r.t. and concentrated *in vacuo*. The residue was purified by flash column chromatography (12 g silica column) using a linear gradient from 0% to 40% EtOAc in heptane to afford **13**, **14** and **14*** in 29 – 51% yields.

methyl (*R*)-2-((*S*)-5-(4-chlorophenyl)-6,7-dimethyl-2-oxo-2,3-dihydro-1H-thieno[2,3-e][1,4]diazepin-3-yl)propanoate (**13**)

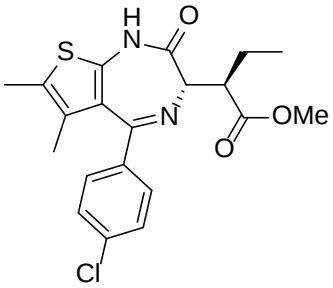


Yield: 228 mg (44%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 9.64 (1H, s), 7.33 (2H, d, *J* = 8.7 Hz), 7.29 (2H, d, *J* = 8.9 Hz), 3.86 (1H, d, *J* = 10.1 Hz), 3.78 (3H, s), 3.71 (1H, qd, *J* = 6.8, 10.2 Hz), 2.28 (3H, s), 1.58 (3H, s), 1.38 (3H, d, *J* = 6.9 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 176.0, 168.0, 164.7, 141.1, 136.9, 136.5, 130.3, 129.8, 128.7, 127.9, 126.9, 67.1, 51.7, 42.0, 15.1, 14.5, 13.0;

LC-MS m/z calc. for $C_{19}H_{20}ClN_2O_3S$ $[M+H]^+$ 391.1, found: 391.1.

methyl (*R*)-2-((*S*)-5-(4-chlorophenyl)-6,7-dimethyl-2-oxo-2,3-dihydro-1H-thieno[2,3-e][1,4]diazepin-3-yl)butanoate (**14**)

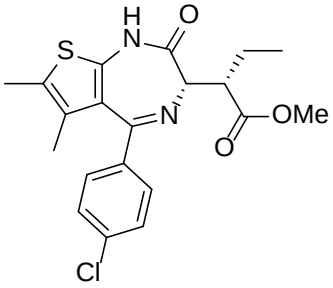


Yield: 119 mg (51%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 9.64 (1H, s), 7.31 (2H, d, *J* = 8.6 Hz), 7.28 (2H, d, *J* = 8.6 Hz), 3.83 (1H, d, *J* = 10.4 Hz), 3.80 (3H, s), 3.64 (1H, dt, *J* = 3.7, 10.6 Hz), 2.27 (3H, s), 1.99 (3H, s), 1.95 - 1.85 (1H, m), 1.64 - 1.54 (4H, m), 1.02 (3H, t, *J* = 7.5 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 175.5, 167.7, 164.8, 141.0, 136.8, 136.5, 130.3, 129.9, 128.7, 127.9, 126.9, 66.4, 51.6, 49.5, 23.5, 14.6, 13.0, 12.0;

LC-MS m/z calc. for $C_{20}H_{22}ClN_2O_3S$ $[M+H]^+$ 405.1, found: 405.1.

methyl (*S*)-2-((*S*)-5-(4-chlorophenyl)-6,7-dimethyl-2-oxo-2,3-dihydro-1H-thieno[2,3-e][1,4]diazepin-3-yl)butanoate (**14***)



Yield: 35 mg (29%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 9.55 (1H, s), 7.42 (2H, d, *J* = 8.6 Hz), 7.33 (2H, d, *J* = 8.2 Hz), 3.99 (1H, d, *J* = 11.1 Hz), 3.77 (3H, s), 3.47 (1H, ddd, *J* = 4.0, 8.9, 10.7 Hz), 2.27 (3H, s), 2.18 - 2.06 (1H, m), 1.81 - 1.68 (1H, m), 1.59 (3H, s), 0.96 (3H, t, *J* = 7.5 Hz);

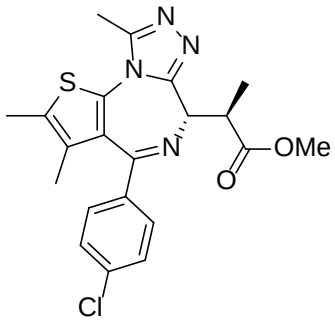
^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 175.7, 169.5, 165.3, 141.6, 137.2, 136.5, 130.3, 129.6, 128.7, 127.5, 126.7, 65.0, 51.7, 47.3, 22.6, 14.5, 12.9, 11.0;

LC-MS m/z calc. for $C_{20}H_{20}ClN_2O_3S$ $[M+H]^+$ 405.1, found: 405.1.

General procedure for triazolothienodiazepines (**15**, **16** and **16***)

Thienodiazepines **13**, **14** and **14***, were dissolved in THF (5 L/mol) and cooled to -78°C. 1.0 M KO^{t}Bu in THF (1.5 eq.) was added dropwise and stirred at -78°C for 1 h. Diethyl chlorophosphate (1.5 eq.) was added dropwise and left to warm to -10°C over 2 h. Conversion to the phosphorylimidate intermediate was monitored by LC-MS. Acetyl hydrazine (2 eq.) was then added and left to stir at r.t. for 1 h. *n*-Butanol (7.6 L/mol) was added and the reaction was heated to 90°C and stirred for 2 h. The mixture was then cooled to r.t. and concentrated *in vacuo*. The residue was purified by reverse phase flash column chromatography (50 g C18 gold column) using a linear gradient from 30% to 75% MeCN in 0.1% formic acid in water to afford **15**, **16** and **16*** in 12 – 39% yields as pale yellow sticky solids.

methyl (*R*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)propanoate (**ME-JQ1-OMe** (**15**))

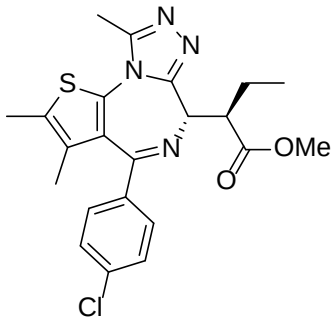


Yield: 98 mg (39%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.32 (2H, d, *J* = 8.3 Hz), 7.28 (2H, d, *J* = 8.3 Hz), 4.23 (1H, d, *J* = 10.7 Hz), 4.07 - 3.98 (1H, m), 3.80 (3H, s), 2.65 (3H, s), 2.39 (3H, s), 1.66 (3H, s), 1.48 (3H, d, *J* = 7.0 Hz);

^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 175.8, 163.2, 154.3, 149.8, 136.8, 136.5, 131.9, 131.1, 130.9, 130.7, 129.9, 128.7, 60.2, 51.8, 42.5, 15.3, 14.4, 13.1, 11.7;

LC-MS m/z calc. for $C_{21}H_{22}ClN_4O_2S$ $[M+H]^+$ 429.1, found: 429.1.

methyl (*R*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)butanoate (**ET-JQ1-OMe** (**16**))



Enantiomeric excess of 99.0% (determined with chiral SFC).

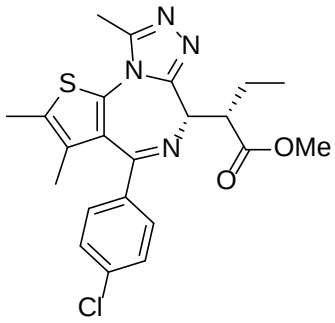
Column Details	Amy SA (4.6mm x 250mm, 5um)
Column Temperature	40°C
Flow Rate	4 mL/min
Isocratic Conditions	25:75 MeOH:CO_2 (0.2% v/v NH_3)

Yield: 45 mg (35%); ^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.31 (2H, d, *J* = 8.9 Hz), 7.28 (2H, d, *J* = 8.6 Hz), 4.22 (1H, d, *J* = 10.9 Hz), 3.97 (1H, ddd, *J* = 10.7, 10.7, 3.6 Hz), 3.82 (3H, s), 2.64 (3H, s), 2.39 (3H, s), 2.22 - 2.11 (1H, m), 1.72 - 1.60 (4H, m), 1.00 (3H, t, *J* = 7.4 Hz);

^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 175.4, 163.2, 154.5, 149.8, 136.8, 136.6, 132.2, 131.0, 130.9, 130.5, 129.9, 128.7, 59.5, 51.6, 49.7, 23.3, 14.5, 13.2, 11.9, 11.7;

HRMS m/z calc. for $C_{22}H_{24}ClN_4O_2S$ $[M+H]^+$ 443.1308, found: 443.1303.

methyl (*S*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)butanoate (**16***)

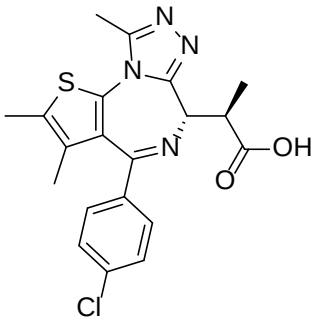


Yield: 4.5 mg (12%); ^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 7.43 (2H, d, *J* = 8.6 Hz), 7.34 (2H, d, *J* = 8.7 Hz), 4.31 (1H, d, *J* = 10.9 Hz), 3.84 (1H, ddd, *J* = 3.8, 9.3, 10.9 Hz), 3.73 (3H, s), 2.64 (3H, s), 2.42 (3H, s), 2.38 - 2.27 (1H, m), 1.93 - 1.81 (1H, m), 1.70 (3H, s), 1.06 (3H, t, *J* = 7.5 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 175.5, 163.9, 155.5, 149.6, 136.95, 136.91, 132.7, 130.81, 130.75, 130.3, 129.9, 128.8, 57.6, 51.9, 47.6, 23.4, 14.5, 13.2, 12.0, 11.2;

HRMS m/z calc. for $C_{22}H_{24}ClN_4O_2S$ $[M+H]^+$ 443.1308, found: 443.1305.

(R)-2-((S)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)propanoic acid (**ME-JQ1-OH** (**17**))



Methyl (*R*)-2-((*S*)-6-(4-chlorophenyl)-9-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)propanoate (133 mg, 0.31 mmol) was dissolved in THF (4.59 ml). LiOH (15 mg, 0.62 mmol) was subsequently dissolved in water (1.15 ml) and added to the flask. The flask was heated to 30°C and stirred for 48 h. The conversion of the ester to the acid was monitored by LC-MS. Water (0.25 ml) was added at regular intervals (every 12 h) to assist with the conversion. After 100% conversion, the solution was neutralised with 2.0 M HCl solution and freeze dried. Slight epimerisation of the carbon adjacent to the carbonyl had occurred so the residue was purified by HPLC using a linear gradient of 35 to 75% MeCN in 0.1% formic acid in water over 10 minutes to afford (*R*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)propanoic acid (isolated yield of 77 mg, 61%) as a white solid with an enantiomeric excess of 98.7% (determined with chiral SFC).

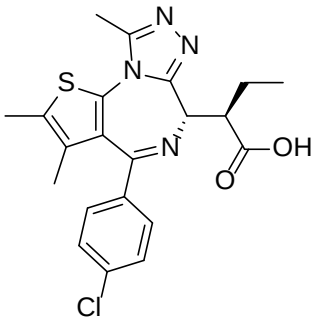
Column Details	Chiralpak IG (4.6mm x 250mm, 5um)
Column Temperature	40°C
Flow Rate	4 mL/min
Isocratic Conditions	45:55 MeOH:CO_2 (0.2% v/v NH_3)

^{1}H-NMR (500 MHz, $CDCl_3$) δ, ppm: 7.39 (2H, d, *J* = 8.2 Hz), 7.28 (2H, d, *J* = 8.1 Hz), 4.22 (1H, d, *J* = 8.0 Hz), 3.93 (1H, dq, *J* = 7.2, 7.2 Hz), 2.68 (3H, s), 2.41 (3H, s), 1.69 (3H, s), 1.54 (3H, d, *J* = 6.9 Hz);

^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 177.1, 164.0, 154.7, 150.0, 137.2, 136.2, 132.1, 131.4, 131.1, 130.6, 130.1, 128.8, 59.6, 41.9, 15.7, 14.6, 13.2, 11.8;

HRMS m/z calc. for $C_{20}H_{20}ClN_4O_2S$ $[M+H]^+$ 415.0995, found: 415.1009.

(*R*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)butanoic acid (**ET-JQ1-OH** (**18**))



Methyl (*R*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)butanoate (60 mg, 0.14 mmol) was dissolved in THF (2.0 ml). LiOH (8 mg, 0.34 mmol) was subsequently dissolved in water (0.5 ml) and added to the flask. The flask was heated to 45°C and stirred for 6 days. The conversion of the ester to the acid was monitored by LC-MS. Water (0.25 ml) and 0.65 M LiOH solution (0.25 ml) was added at regular intervals (every 12 h) to assist with the conversion. After 100% conversion, the solution was neutralised with 2.0 M HCl solution and freeze dried. Slight epimerisation of the carbon adjacent to the carbonyl had occurred so the residue was purified by HPLC using a linear gradient of 35 to 75% MeCN in 0.1% formic acid in water over 10 minutes to afford (*R*)-2-((*S*)-4-(4-chlorophenyl)-2,3,9-trimethyl-6H-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepin-6-yl)butanoic acid (isolated yield of 48 mg, 83%) as a white solid with an enantiomeric excess of 99.0% (determined with chiral SFC).

Column Details	Amy SA (4.6mm x 250mm, 5um)
Column Temperature	40°C
Flow Rate	4 mL/min
Isocratic Conditions	35:65 EtOH:CO_2 (0.2% v/v NH_3)

^{1}H-NMR (400 MHz, $CDCl_3$) δ, ppm: 7.38 (2H, d, *J* = 8.5 Hz), 7.26 (2H, d, *J* = 8.6 Hz), 4.25 (1H, d, *J* = 8.2 Hz), 3.81 (1H, ddd, *J* = 4.6, 4.6, 13.1 Hz), 2.67 (3H, s), 2.40 (3H, s), 2.12 - 2.00 (1H, m), 1.93 - 1.80 (1H, m), 1.68 (3H, s), 1.07 (3H, t, *J* = 7.4 Hz);

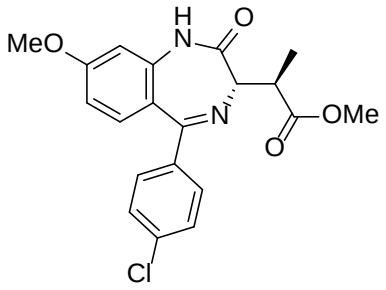
^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 176.6, 164.1, 154.7, 150.0, 137.1, 136.2, 132.2, 131.4, 131.1, 130.4, 130.1, 128.8, 58.5, 49.0, 23.5, 14.5, 13.2, 11.77, 11.75;

HRMS m/z calc. for $C_{21}H_{22}ClN_4O_2S$ [M+H]+ 429.1152, found: 429.1137.

General procedure for benzodiazepines (**20** and **21**)

(2-*a*mino-4-methoxyphenyl)(4-chlorophenyl)methanone (1 eq.) was suspended in toluene (830 mL/mol). 4 Å molecular sieves and TFA (2 eq.) were then added and stirred at r.t. for 5 min. *N*-carboxyanhydrides, **10** and **11** (1.2 eq.) were dissolved in toluene (210 mL/mol) (DCM for compound **10**) and subsequently added to the flask which was then heated at 60°C for 2 h. The conversion of the amino ketone was monitored by LC-MS. TEA (2.5 eq) was then added and the reaction was heated to 80°C and stirred for 2 h. The mixture was then cooled to r.t. and concentrated *in vacuo*. The residue was purified by HPLC using a linear gradient of 35 to 75% MeCN in 0.1% formic acid in water over 10 minutes to afford **20** and **21** with isolated yields of 30 and 50% yields respectively.

methyl (*R*)-2-((*S*)-5-(4-chlorophenyl)-8-methoxy-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)propanoate (**20**)

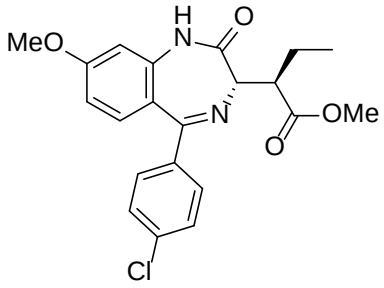


Yield: 14 mg (30%); ^{1}H-NMR (500 MHz, $CDCl_3$): δ, ppm: 9.09 (1H, s), 7.36 (2H, d, *J* = 8.4 Hz), 7.30 (2H, d, *J* = 8.3 Hz), 7.20 (1H, d, *J* = 8.8 Hz), 6.72 (1H, dd, *J* = 2.1, 8.8 Hz), 6.63 (1H, d, *J* = 2.2 Hz), 3.88 (3H, s), 3.81 - 3.77 (4H, m), 3.71 (1H, qd, *J* = 6.7, 10.2 Hz), 1.39 (3H, d, *J* = 6.7 Hz);

^{13}C-NMR (500 MHz, $CDCl_3$) δ, ppm: 176.0, 169.6, 167.9, 162.4, 139.9, 137.8, 136.6, 132.9, 131.3, 128.5, 120.3, 111.0, 105.4, 66.3, 55.8, 51.8, 42.1, 15.0;

LC-MS m/z calc. for $C_{20}H_{20}ClN_2O_4$ $[M+H]^+$ 387.1, found: 387.0.

methyl (*R*)-2-((*S*)-5-(4-chlorophenyl)-8-methoxy-2-oxo-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl)butanoate (**21**)



Yield: 14.8 mg (50%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ, ppm: 8.50 (1H, s), 7.36 (2H, d, *J* = 8.6 Hz), 7.31 (2H, d, *J* = 8.6 Hz), 7.21 (1H, d, *J* = 8.8 Hz), 6.73 (1H, dd, *J* = 2.5, 8.8 Hz), 6.60 (1H, d, *J* = 2.4 Hz), 3.88 (3H, s), 3.81 (3H, s), 3.76 (1H, d, *J* = 10.6 Hz), 3.64 (1H, dt, *J* = 3.6, 10.5 Hz), 1.97 - 1.86 (1H, m), 1.63 - 1.51 (1H, m);

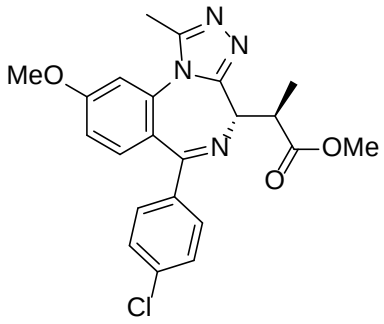
^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 175.5, 169.2, 167.9, 162.4, 139.7, 137.8, 136.7, 133.0, 131.3, 128.5, 120.3, 111.0, 105.3, 65.7, 55.8, 51.6, 49.6, 23.5, 12.0;

LC-MS m/z calc. for $C_{21}H_{22}ClN_2O_4$ $[M+H]^+$ 401.1, found: 401.1.

General procedure for triazolobenzodiazepines (**22** and **23**)

Benzodiazepines **20** and **21**, were dissolved in THF (5 L/mol) and cooled to -78°C. 1.0 M KO^{t}Bu in THF (1.5 eq.) was added dropwise and stirred at -78°C for 1 h. Diethyl chlorophosphate (3 eq.) was added dropwise and left to warm to -10°C over 2 h. Conversion to the phosphorylimidate intermediate was monitored by LC-MS. Acetyl hydrazine (3 eq.) was then added and left to stir at r.t. for 1 h. *n*-Butanol (7.6 L/mol) was added and the reaction was heated to 90°C and stirred for 2 h. The mixture was then cooled to r.t. and concentrated *in vacuo*. The residue was purified by HPLC using a linear gradient of 5 to 95% MeCN in 0.1% formic acid in water over 15 minutes to afford **22** and **23** in isolated yields of 11% and 8% respectively, with enantiomeric excess of 99.0% (determined with chiral SFC).

methyl (*R*)-2-((*S*)-6-(4-chlorophenyl)-9-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)propanoate (**9-ME-1** (**22**))



Enantiomeric excess of 99.0% (determined with chiral SFC).

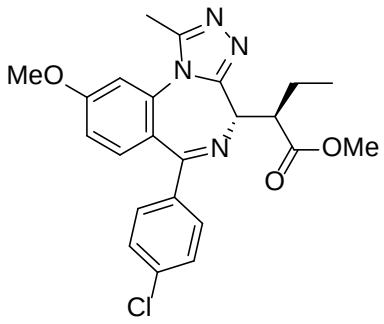
Column Details	Lux C1 (4.6mm x 250mm, 5um)
Column Temperature	40°C
Flow Rate	4 mL/min
Isocratic Conditions	30:70 EtOH:CO_2 (0.2% v/v NH_3)

Yield: 1.7 mg (11%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ, ppm: 7.39 (2H, d, *J* = 8.5 Hz), 7.36 (1H, d, *J* = 8.8 Hz), 7.31 (2H, d, *J* = 8.7 Hz), 6.98 (1H, dd, *J* = 2.5, 8.7 Hz), 6.94 (1H, d, *J* = 2.4 Hz), 4.24 (1H, d, *J* = 10.7 Hz), 4.07 (1H, qd, *J* = 6.9, 10.7 Hz), 3.95 (3H, s), 3.82 (3H, s), 2.64 (3H, s), 1.49 (3H, d, *J* = 6.9 Hz);

^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 176.2, 165.8, 161.8, 155.2, 150.3, 137.6, 136.9, 135.0, 133.5, 131.0, 128.6, 121.6, 112.9, 109.6, 59.8, 56.1, 51.9, 42.6, 15.4, 12.5;

LC-MS m/z calc. for $C_{22}H_{22}ClN_4O_3$ $[M+H]^+$ 425.1, found: 425.0.

Methyl (*R*)-2-((*S*)-6-(4-chlorophenyl)-9-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-*a*][1,4]diazepin-4-yl)butanoate (**9-ET** (**23**))



Enantiomeric excess of 99.1% (determined with chiral SFC).

Column Details	Lux C1 (4.6mm x 250mm, 5um)
Column Temperature	40°C
Flow Rate	4 mL/min
Isocratic Conditions	25:75 EtOH:CO_2 (0.2% v/v NH_3)

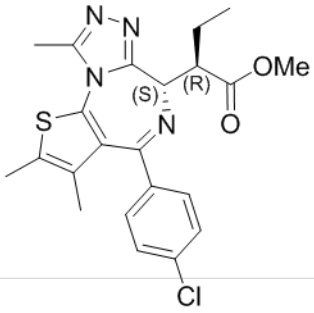
Yield: 1.6 mg (8%); ^{1}H-NMR (400 MHz, $CDCl_3$): δ, ppm: 7.39 - 7.33 (3H, m), 7.30 (2H, d, *J* = 8.7 Hz), 6.98 (1H, dd, *J* = 2.5, 8.8 Hz), 6.93 (1H, d, *J* = 2.5 Hz), 4.23 (1H, d, *J* = 11.0 Hz), 3.99 (1H, dt, *J* = 3.7, 10.8 Hz), 3.95 (3H, s), 3.84 (3H, s), 2.63 (3H, s), 2.24 - 2.12 (1H, m), 1.70 - 1.60 (1H, m), 1.01 (3H, t, *J* = 7.4 Hz);

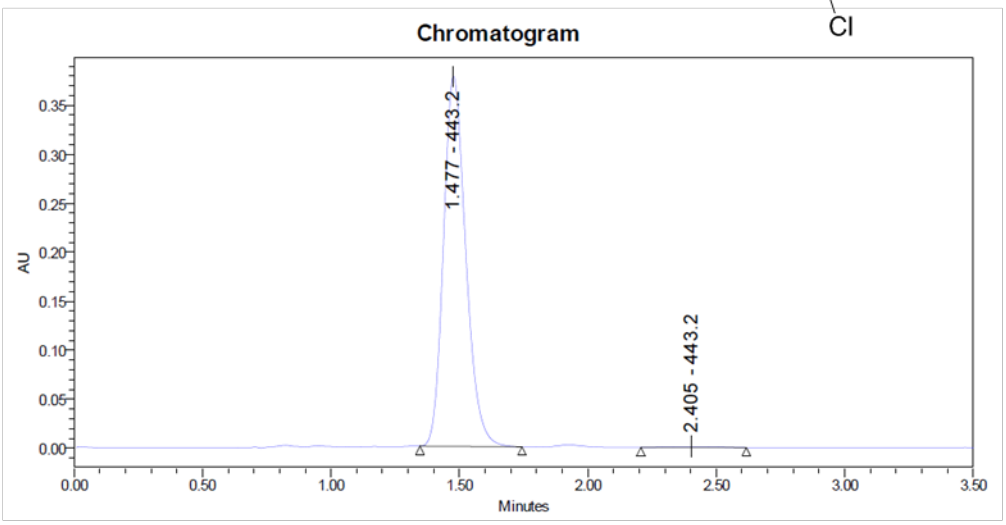
^{13}C-NMR (400 MHz, $CDCl_3$) δ, ppm: 175.7, 165.9, 161.7, 155.2, 150.4, 137.5, 136.9, 134.9, 133.6, 131.0, 128.5, 121.5, 112.8, 109.6, 58.9, 56.1, 51.7, 49.8, 23.3, 12.5, 11.7;

LC-MS m/z calc. for $C_{23}H_{24}ClN_4O_3$ $[M+H]^+$ 439.2, found: 439.0.

Chiral HPLC Chromatograms

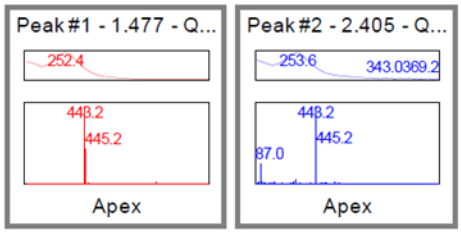
ET-JQ1-OMe (**16**)

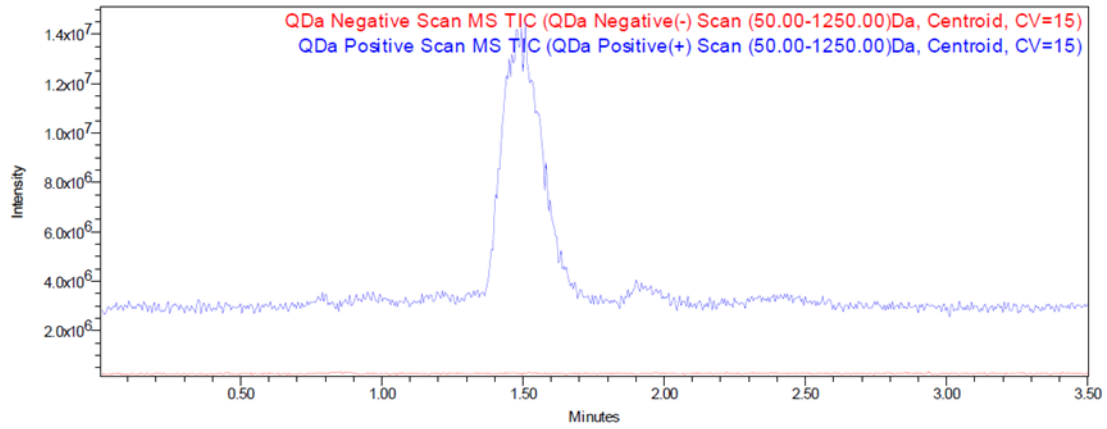




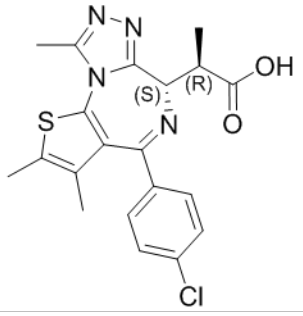
Peak Results

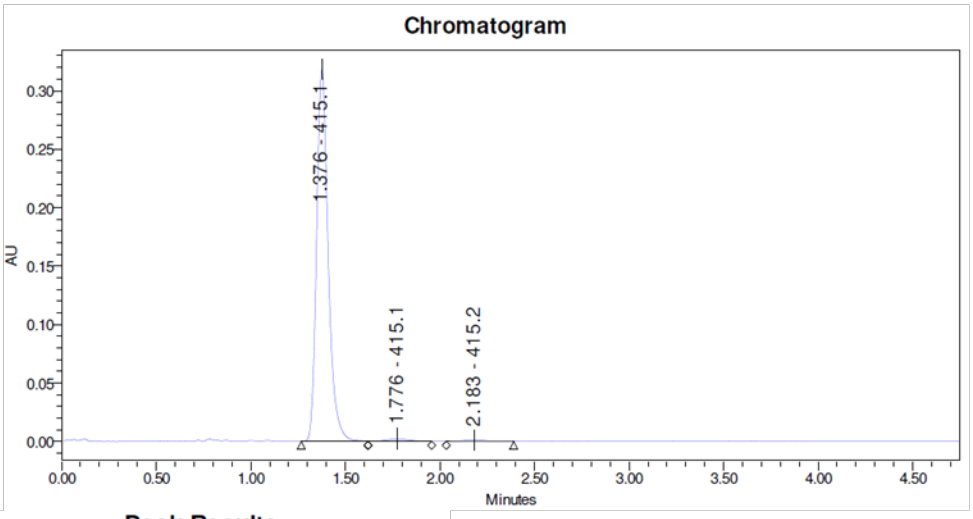
	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.48	2275544	99.5	0.09195
2	2.40	11025	0.5	0.19363





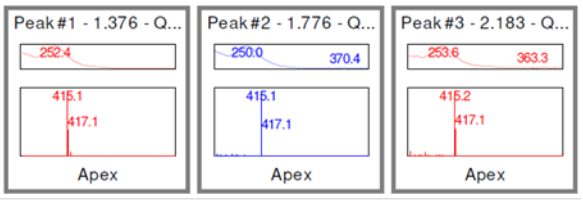
Me-JQ1-OH (**17**)

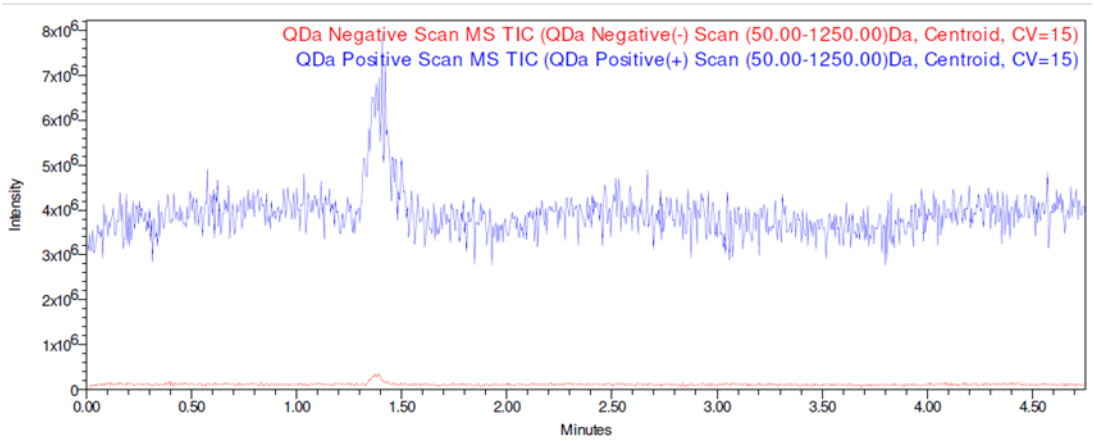




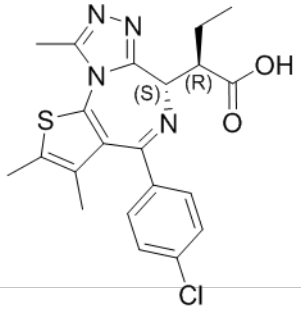
Peak Results

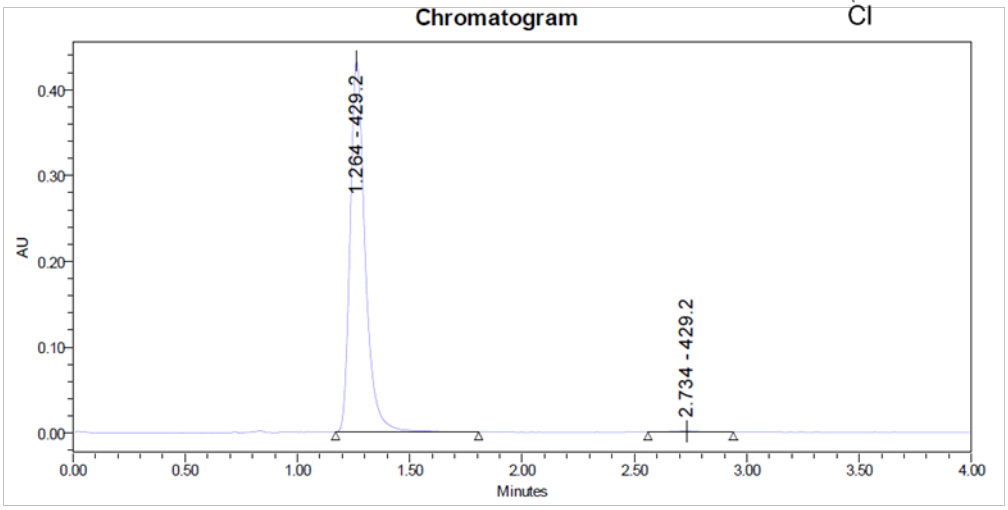
	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.38	1351725	98.0	0.06316
2	1.78	18099	1.3	0.11841
3	2.18	9703	0.7	0.13129





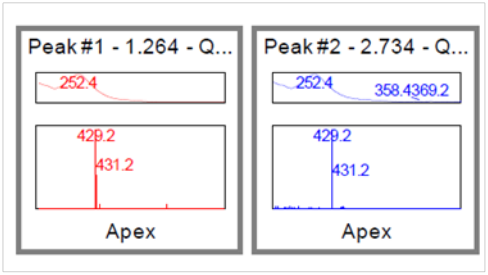
ET-JQ1-OH (**18**)

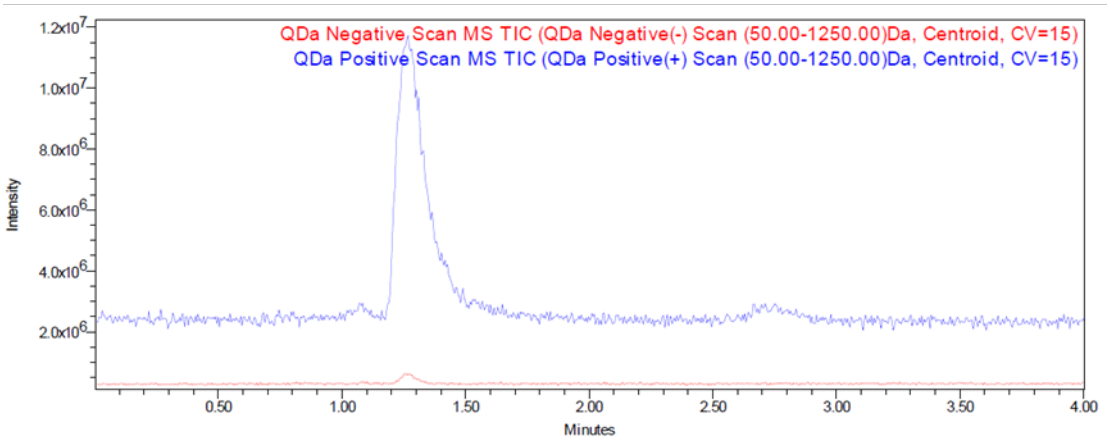




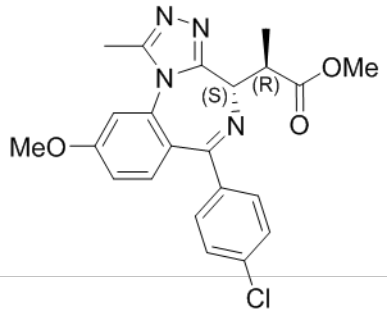
Peak Results

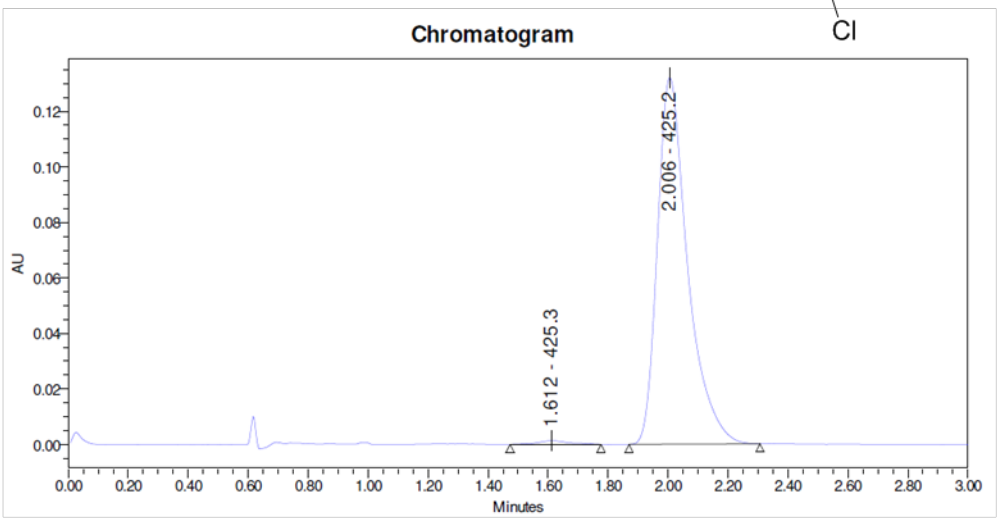
	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.26	1996022	99.5	0.06743
2	2.73	11033	0.5	0.17091





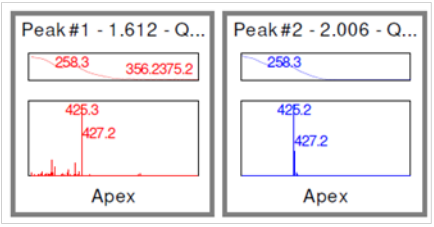
9-ME-1 (**22**)

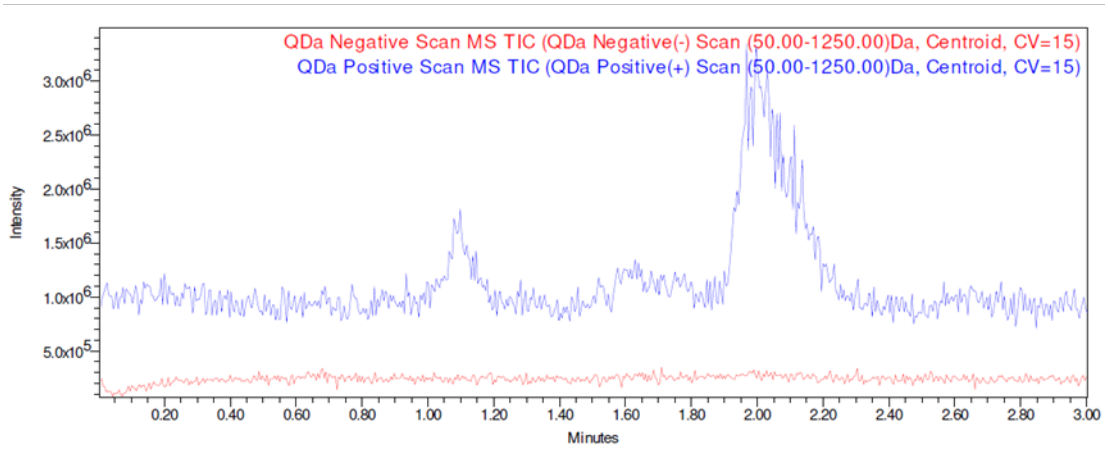




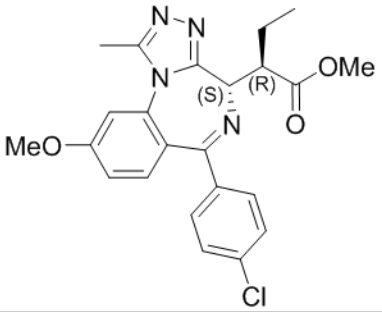
Peak Results

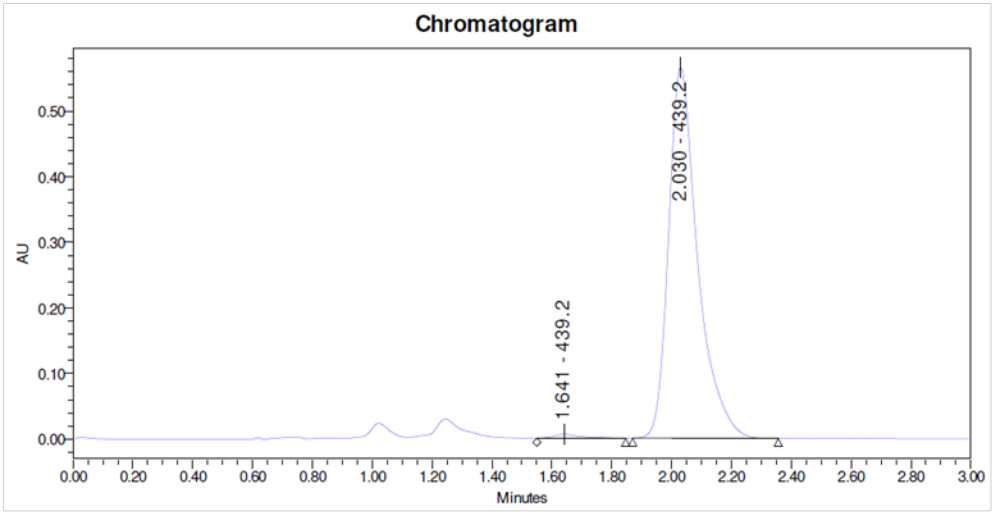
	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.61	9770	1.0	0.10312
2	2.01	925436	99.0	0.10238





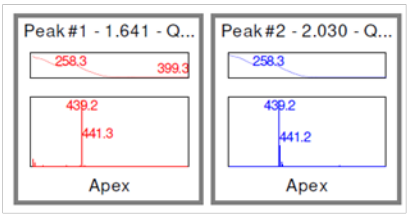
9-ET-1 (**23**)

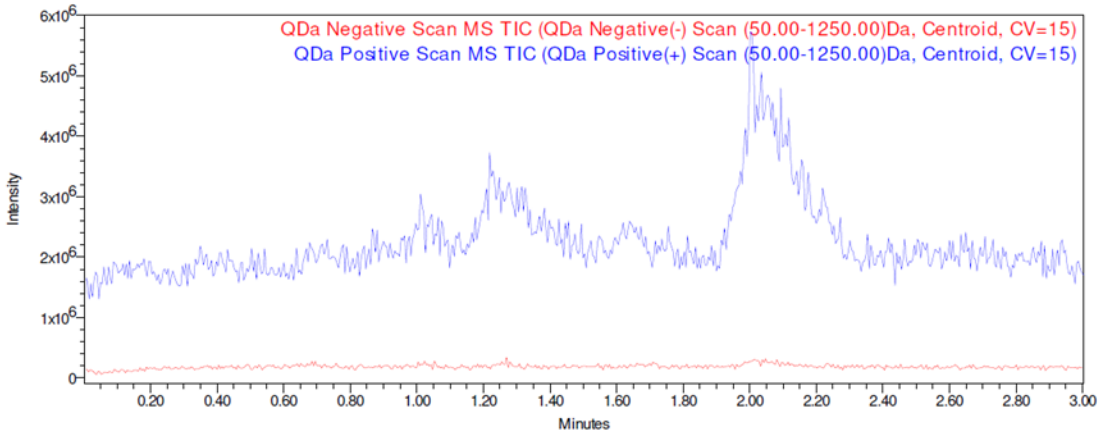




Peak Results

	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.64	36447	0.9	0.08902
2	2.03	3824661	99.1	0.09786





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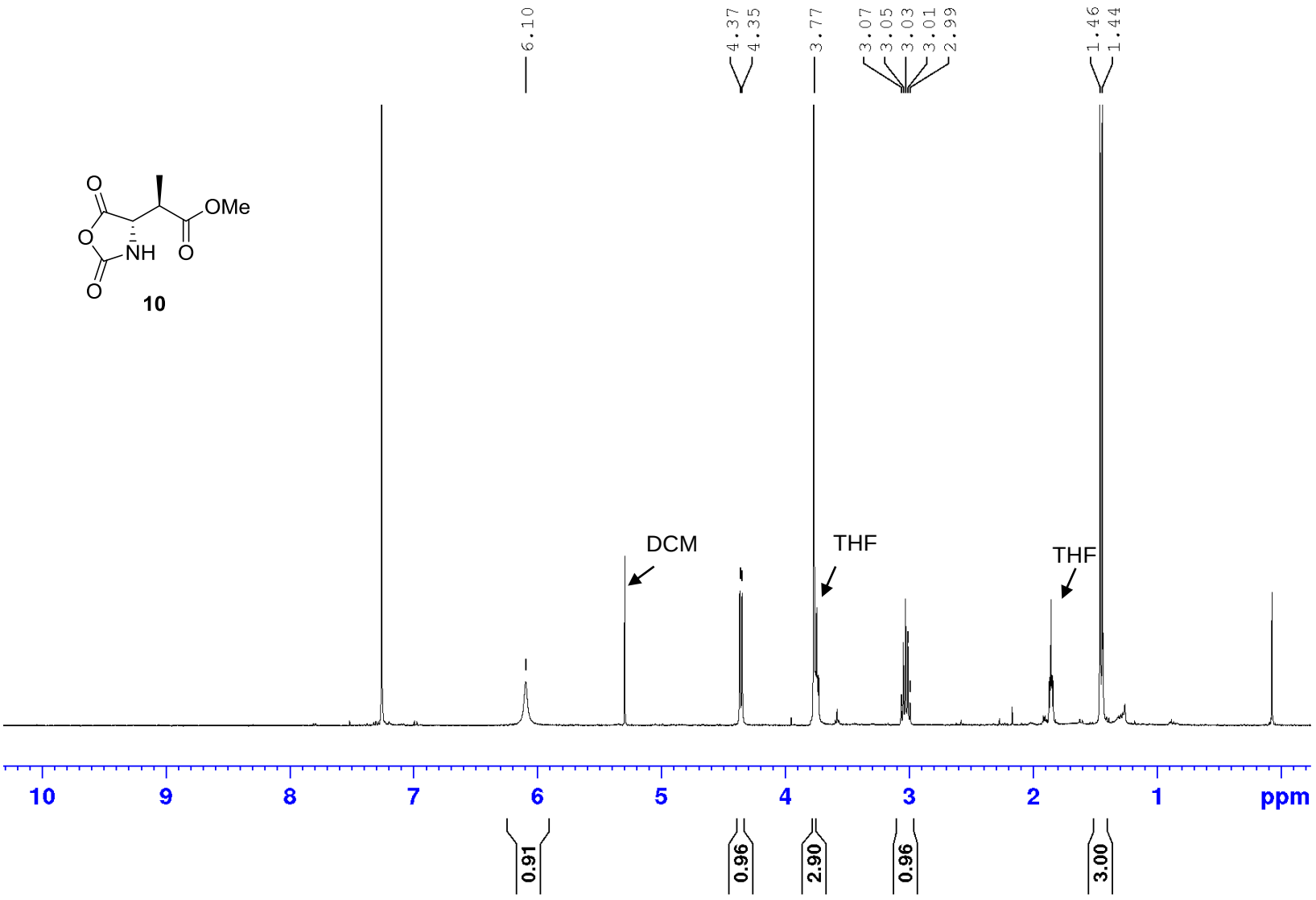
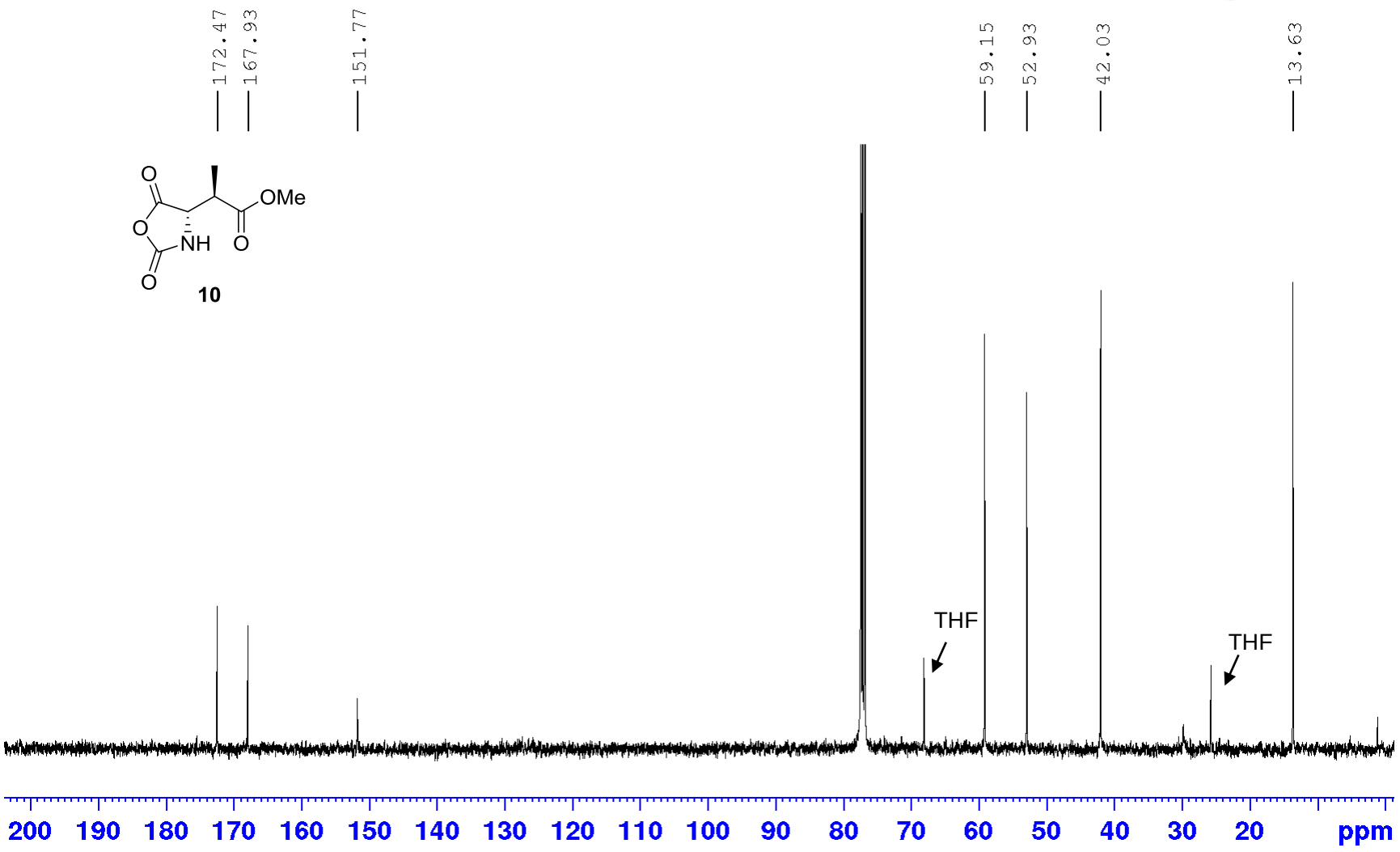
^{1}H-NMR, $CDCl_3$

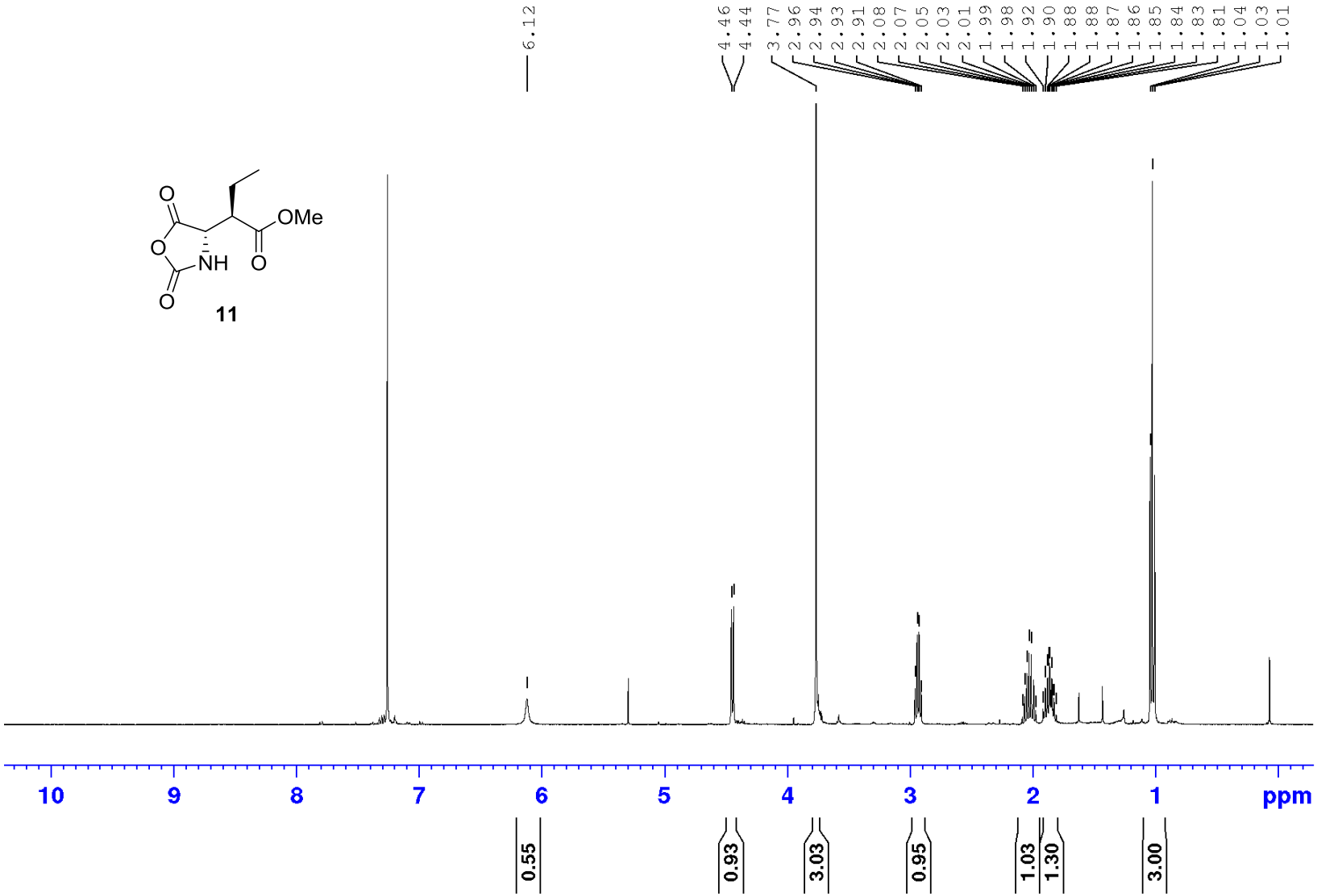


10

^{13}C-NMR, $CDCl_3$



^{1}H-NMR, $CDCl_3$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2.94
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2.91
2.08
2.07
2.05
2.03
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1.99
1.98
1.92
1.90
1.88
1.88
1.87
1.86
1.85
1.84
1.83
1.81
1.04
1.03
1.01
O
OMe
O
NH
O
O
11
10
9
8
7
6
5
4
3
2
1
ppm
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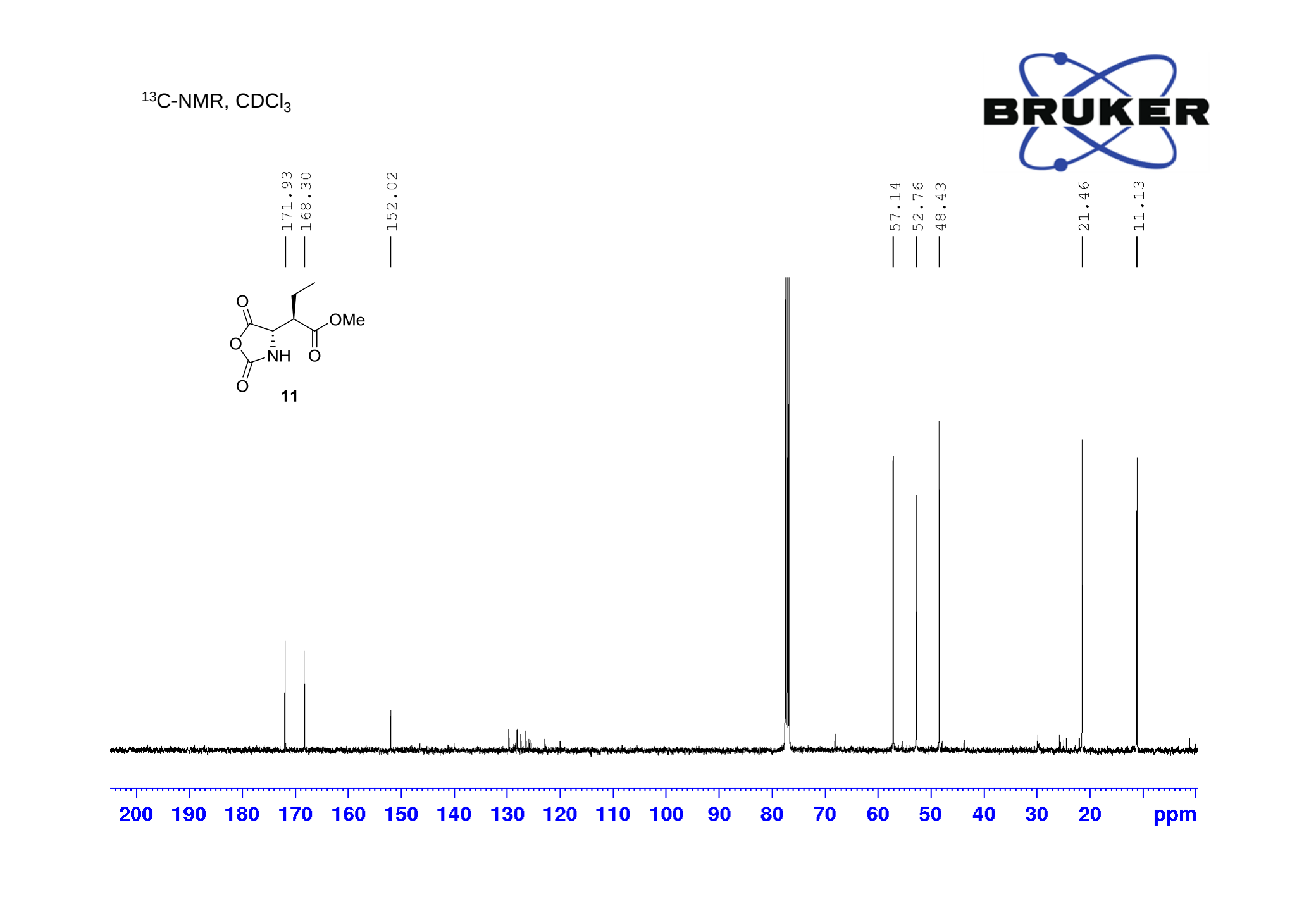
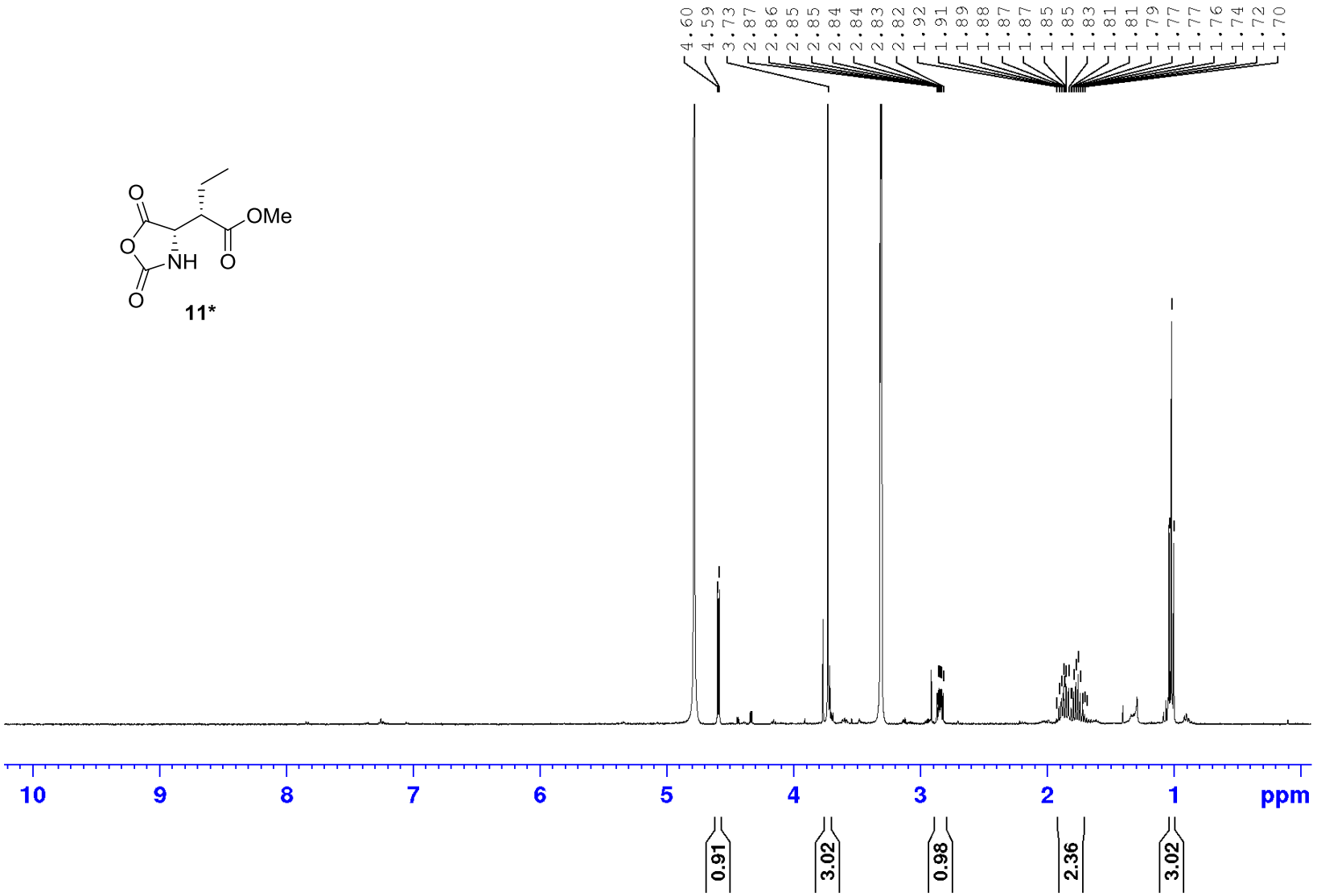
0.93
3.03
0.95
1.03
1.30
3.00

^{13}C-NMR, $CDCl_3$



^{1}H-NMR, MeOD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2.86
2.85
2.85
2.84
2.84
2.83
2.82
1.92
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1.89
1.88
1.87
1.87
1.85
1.85
1.83
1.81
1.81
1.79
1.77
1.77
1.76
1.74
1.72
1.70
O
O
NH
O
O
OMe
11*
10
9
8
7
6
5
4
3
2
1
ppm
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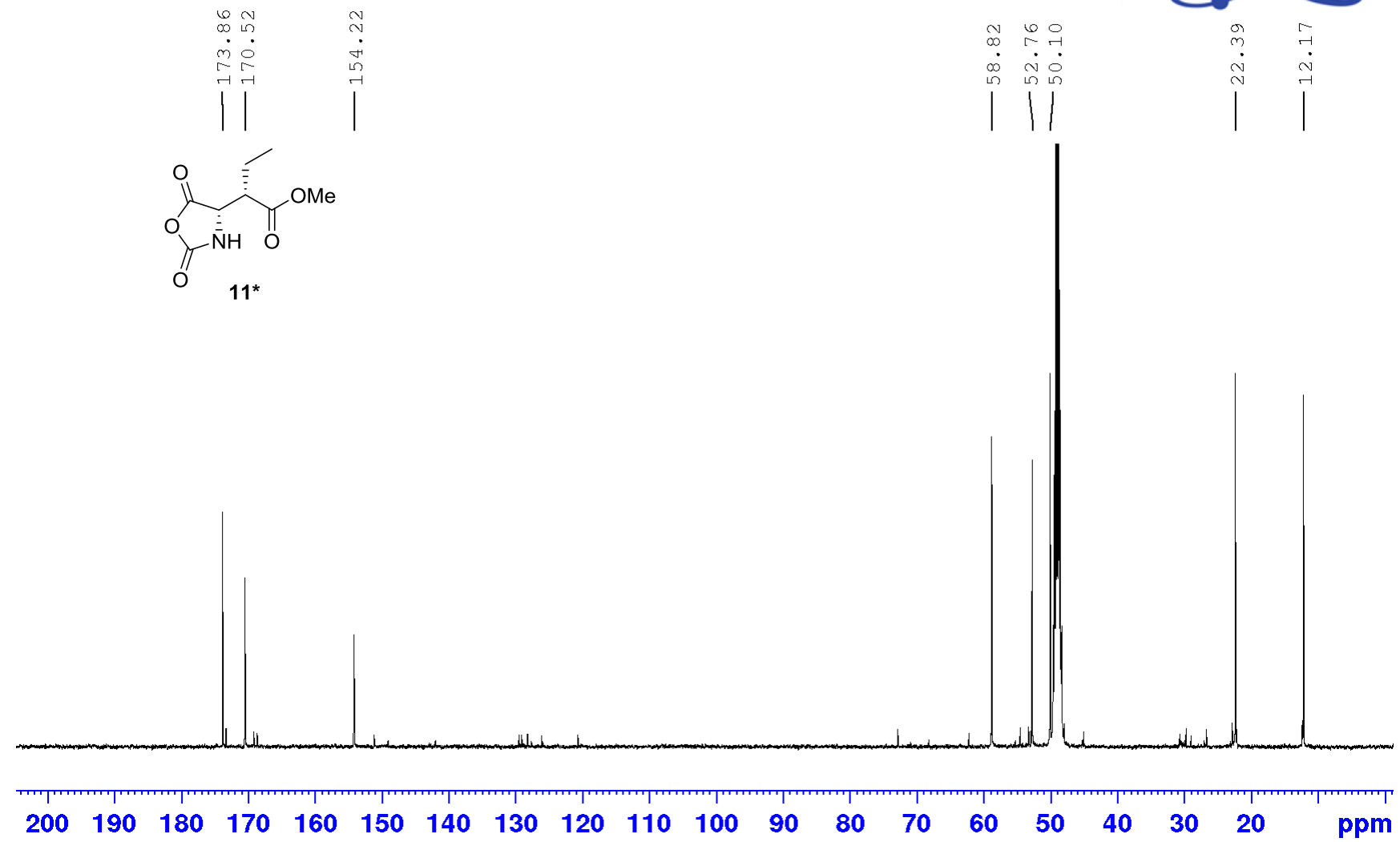
0.98
2.36
3.02

^{13}C-NMR, MeOD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58.82
52.76
50.10
22.39
12.17
O
O
NH
O
O
OMe
O
11*
200
190
180
170
160
150
140
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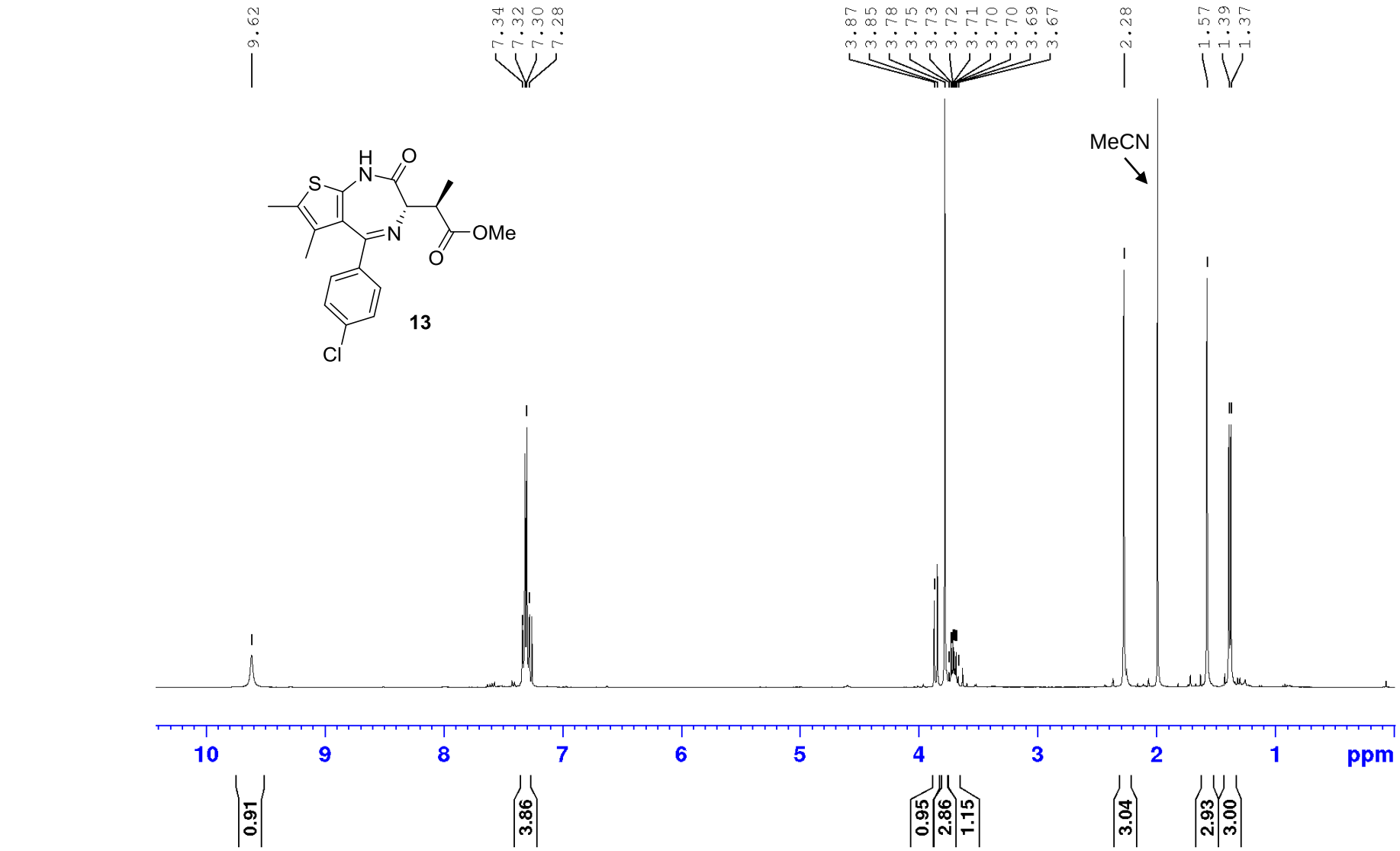
120
110
100
90
80
70
60
50
40
30
20
ppm

^{1}H-NMR, $CDCl_3$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3.70
3.69
3.67
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1.39
1.37
MeCN
H
N
O
S
N
OMe
O
Cl
13
10
9
8
7
6
5
4
3
2
1
ppm
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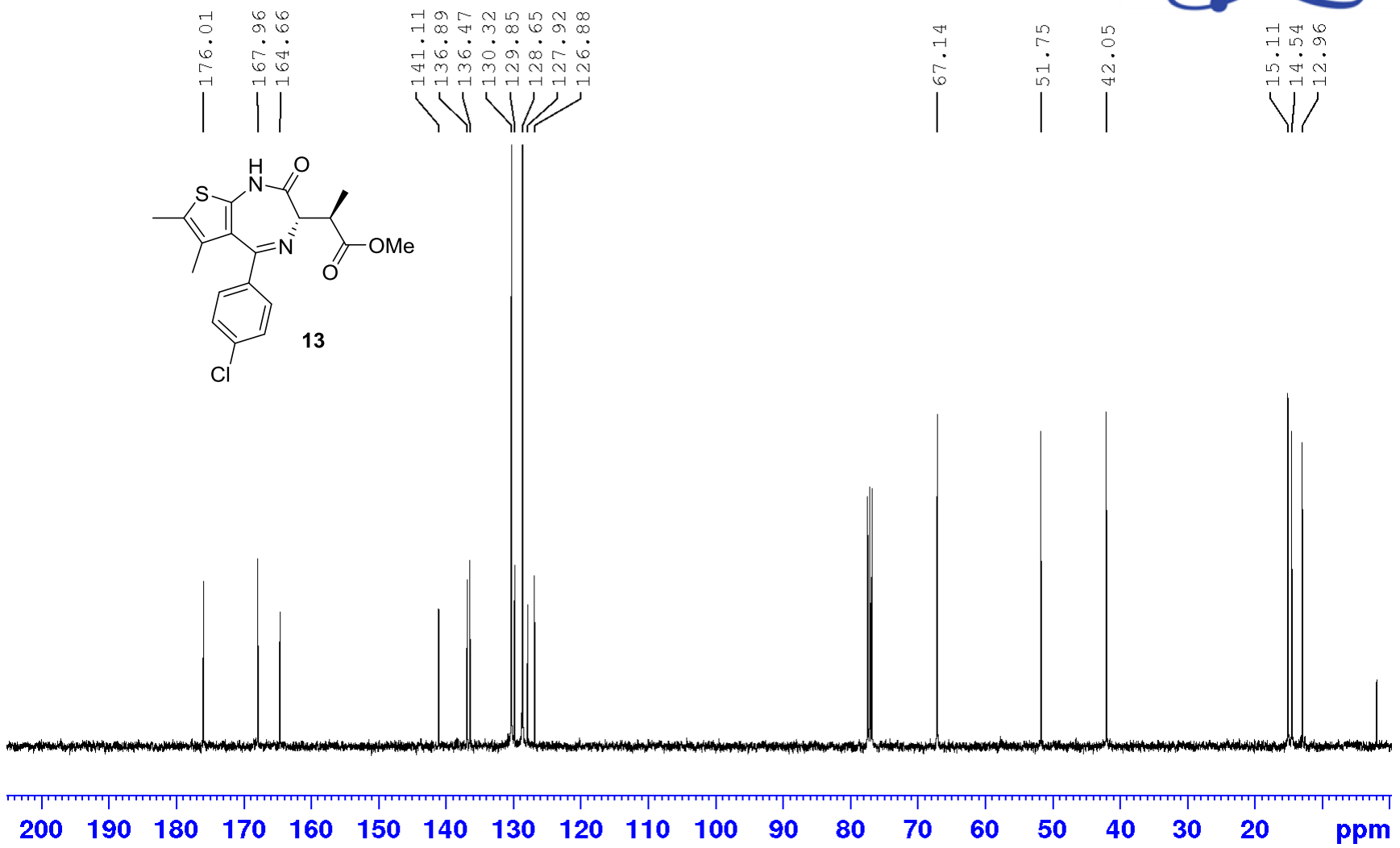
2.93
3.00

^{13}C-NMR, $CDCl_3$



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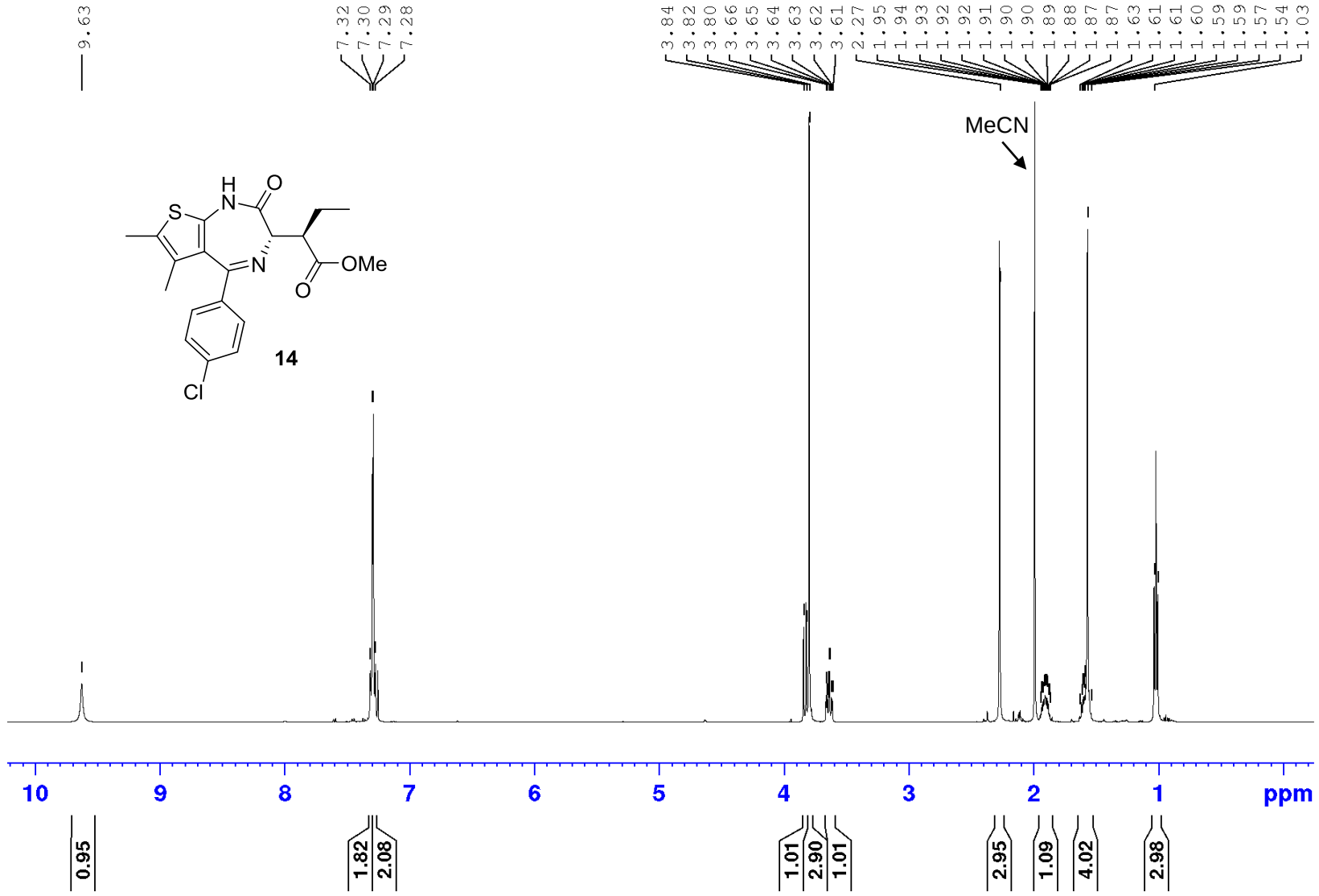
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3.80
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3.64
3.63
3.62
3.61
2.27
1.95
1.94
1.93
1.92
1.92
1.91
1.90
1.90
1.89
1.88
1.87
1.87
1.63
1.61
1.61
1.60
1.59
1.59
1.57
1.54
1.03
MeCN
H
N
O
S
N
OMe
O
Cl
14
10
9
8
7
6
5
4
3
2
1
ppm
0.95
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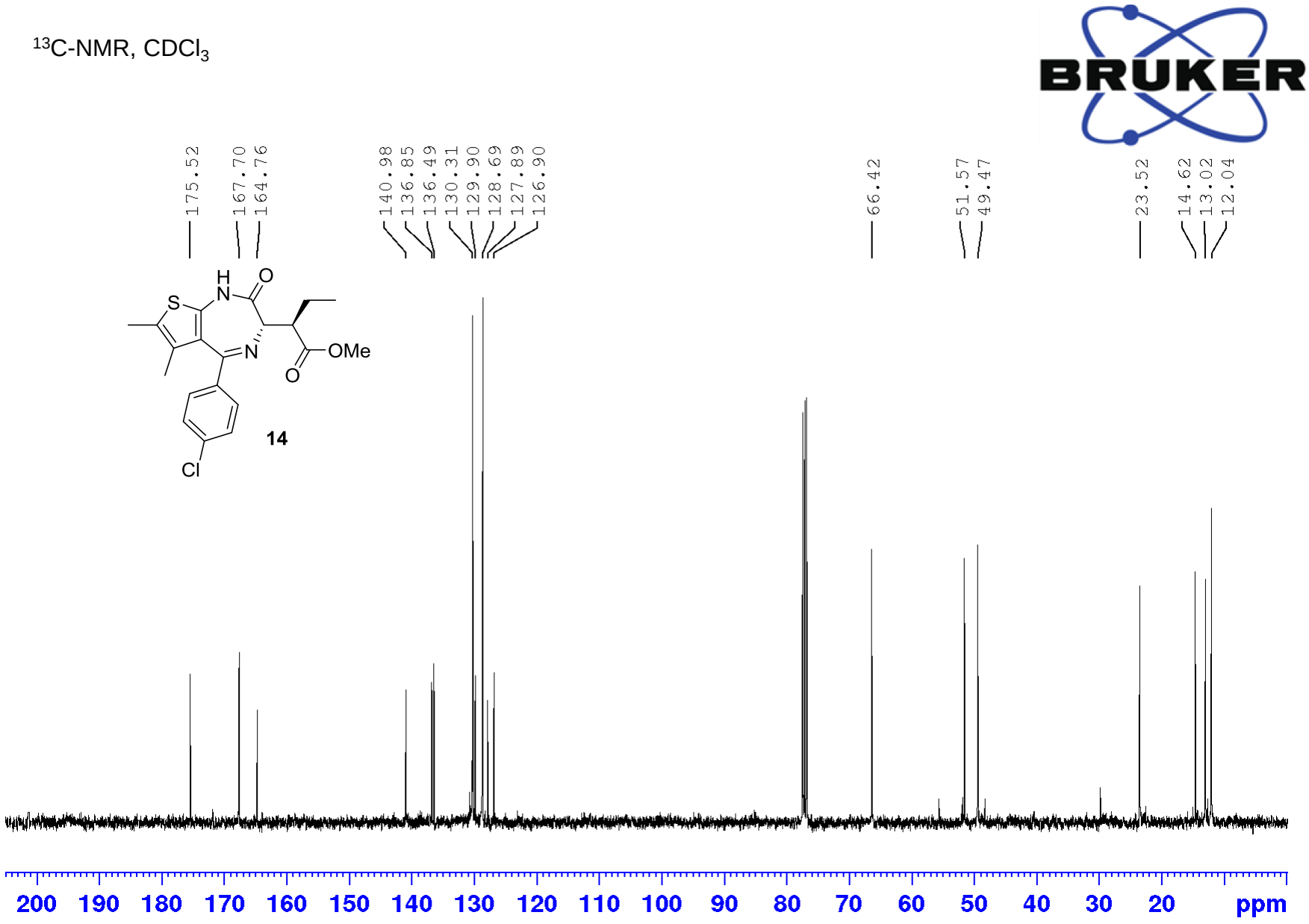
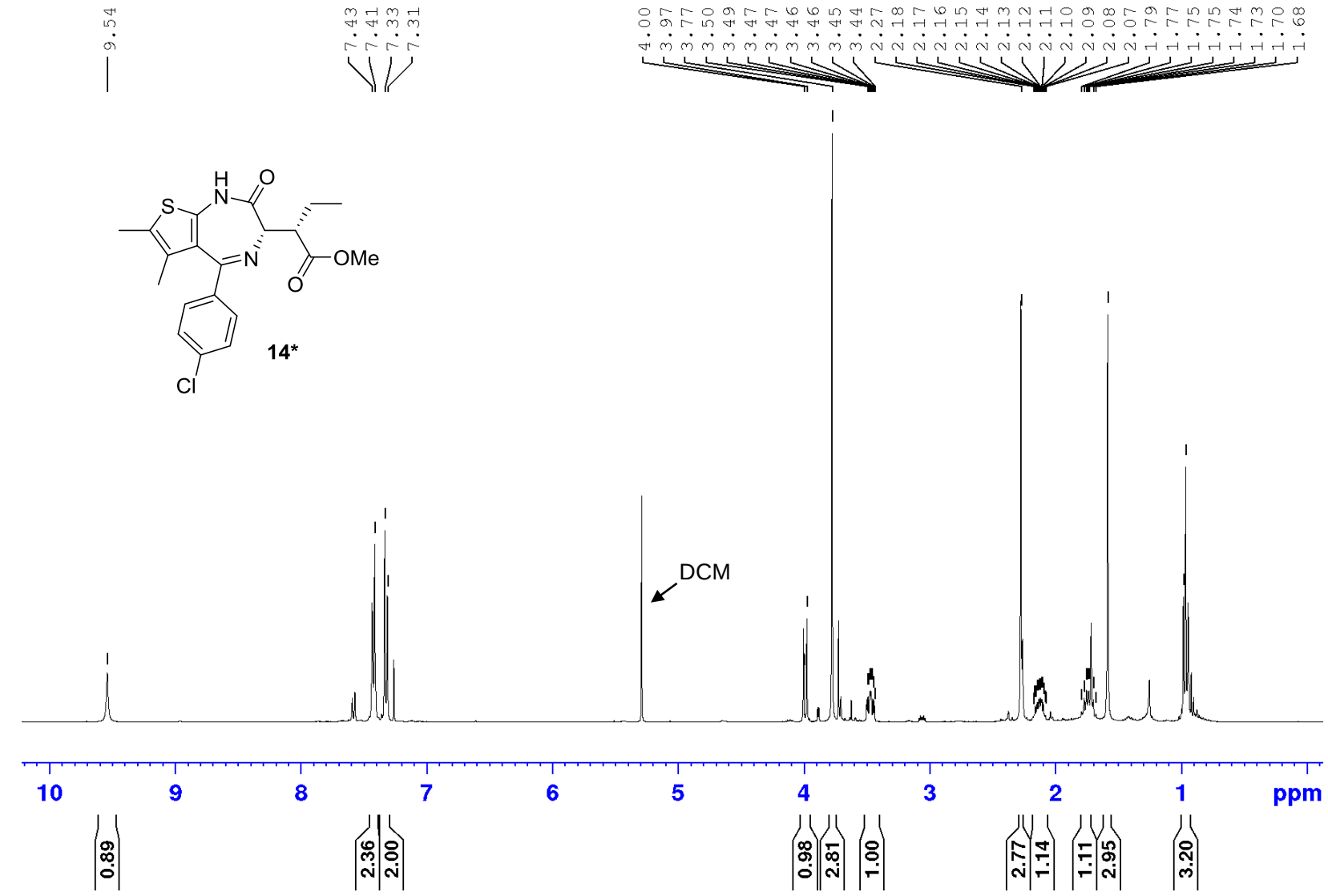
2.90
1.01
2.95
1.09
4.02
2.98

^{13}C-NMR, $CDCl_3$



^{1}H-NMR, $CDCl_3$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3.46
3.46
3.45
3.44
2.27
2.18
2.17
2.16
2.15
2.14
2.13
2.12
2.11
2.10
2.09
2.08
2.07
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1.77
1.75
1.75
1.74
1.73
1.70
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O
S
N
OMe
O
Cl
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DCM
10
9
8
7
6
5
4
3
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1
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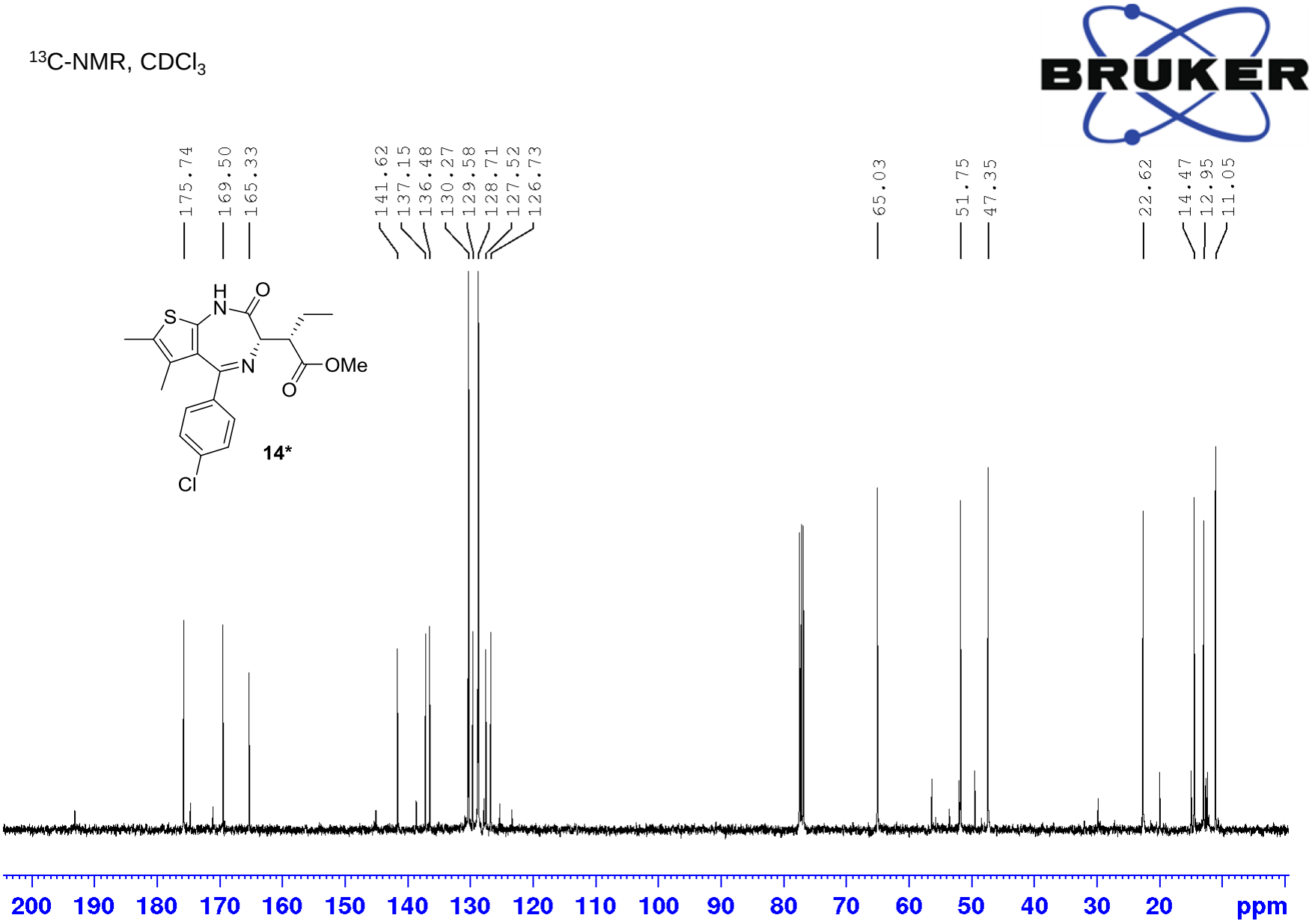
3.20

^{13}C-NMR, $CDCl_3$



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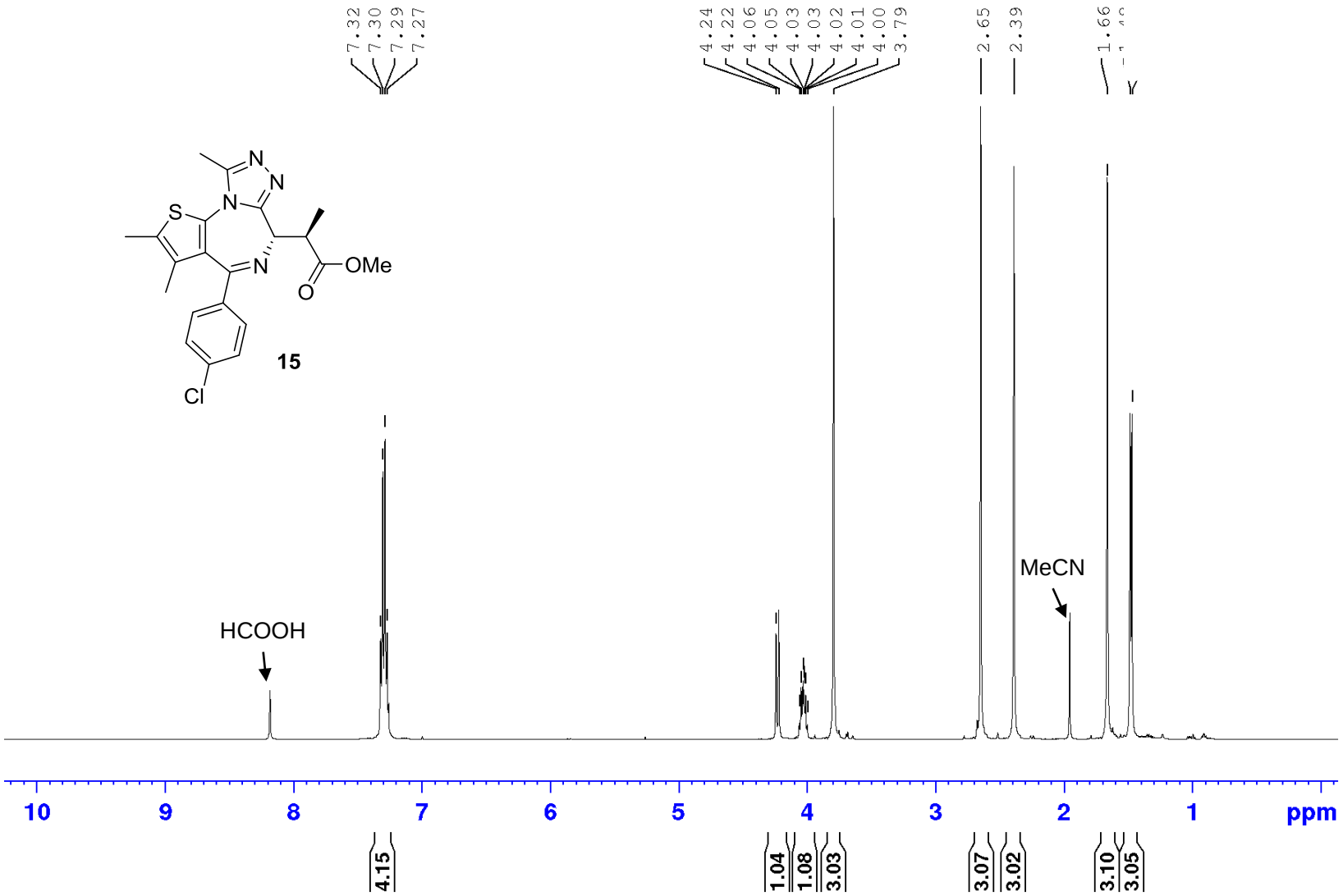
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4.05
4.03
4.03
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4.01
4.00
3.79
2.65
2.39
1.66
OMe
O
15
Cl
HCOOH
MeCN
10
9
8
7
6
5
4
3
2
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ppm
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1.08
3.03
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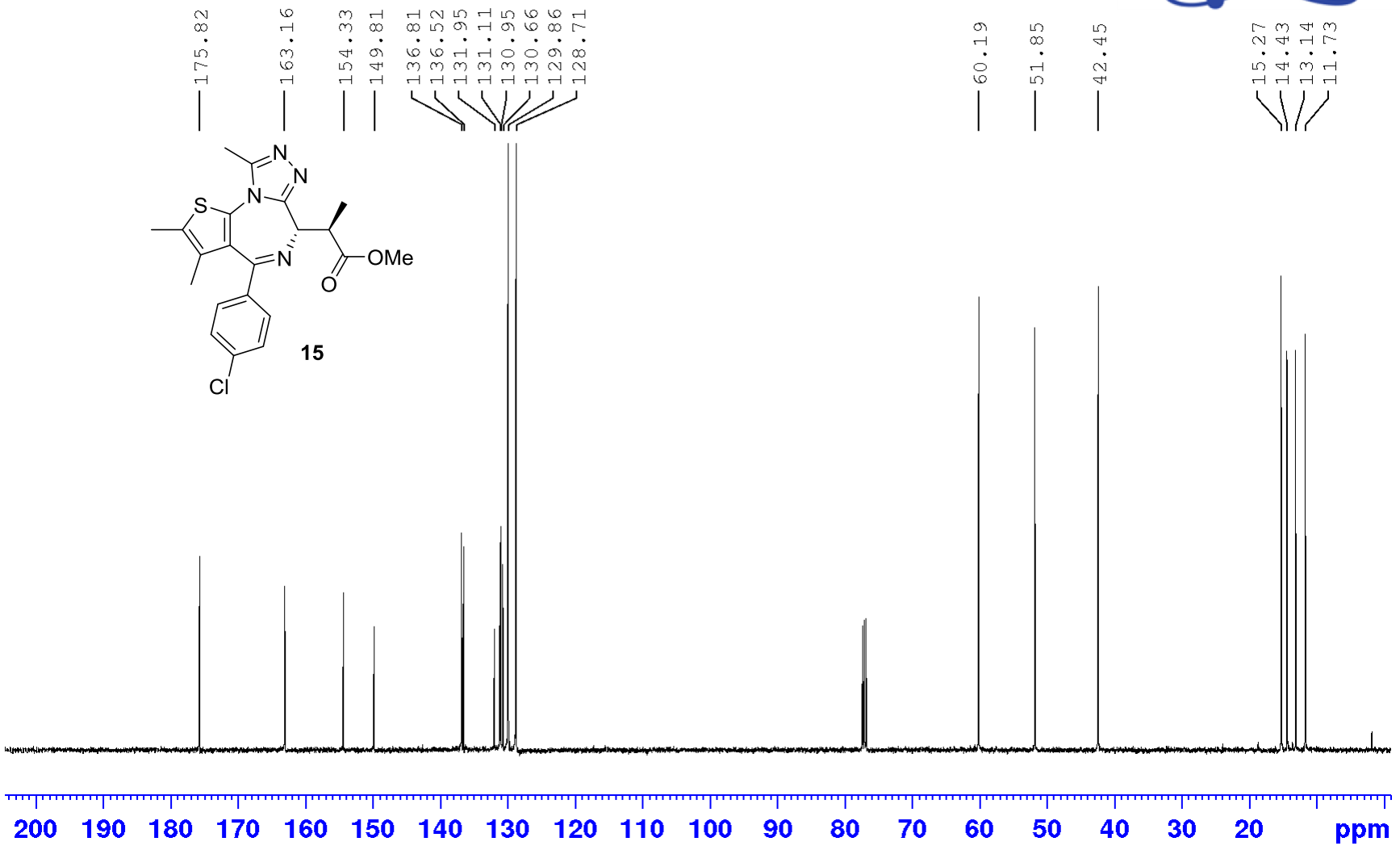
3.10
3.05

^{13}C-NMR, $CDCl_3$



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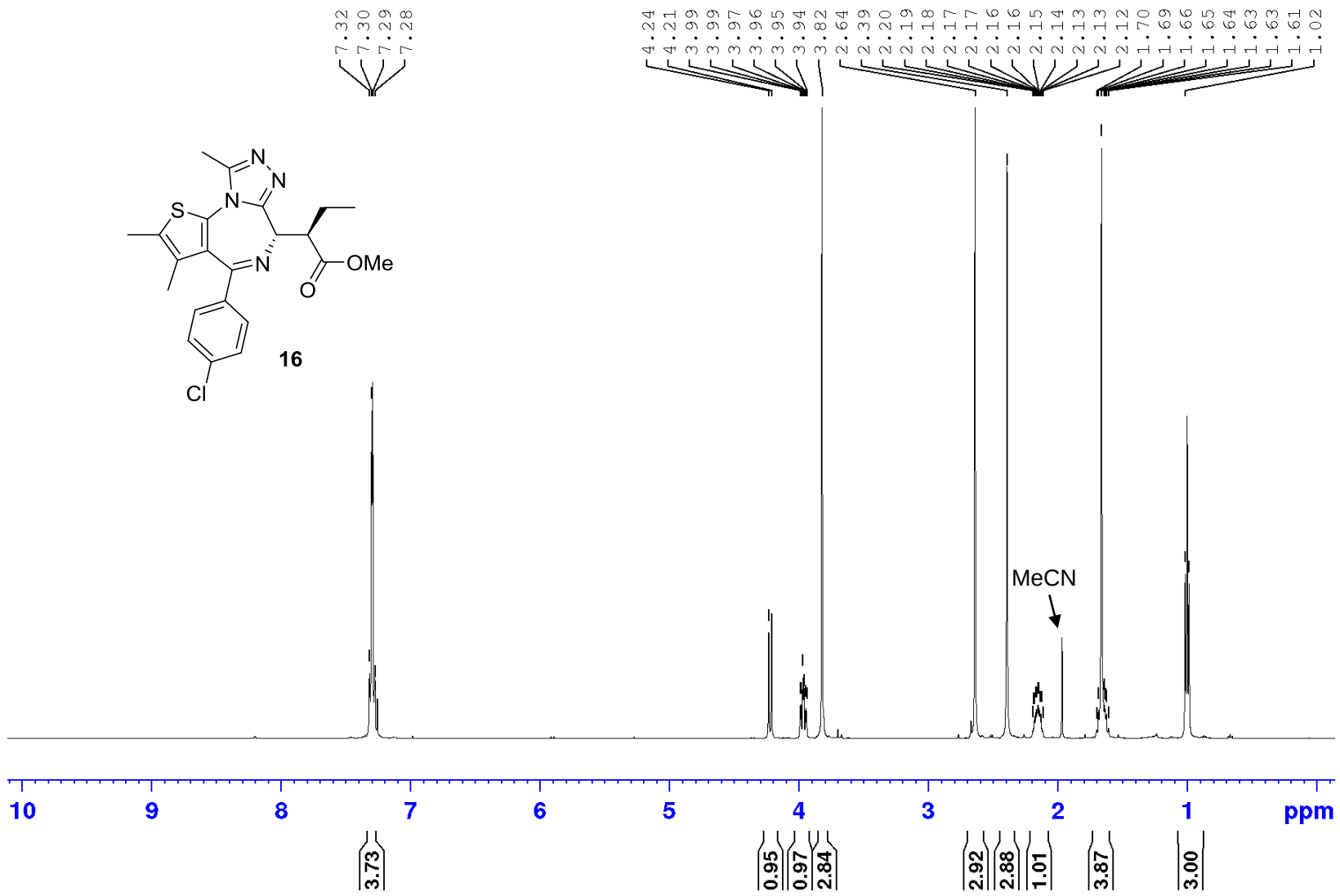
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3.82
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2.18
2.17
2.17
2.16
2.16
2.15
2.14
2.13
2.13
2.12
1.70
1.69
1.66
1.65
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1.63
1.63
1.61
1.02
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N
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S
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OMe
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Cl
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7
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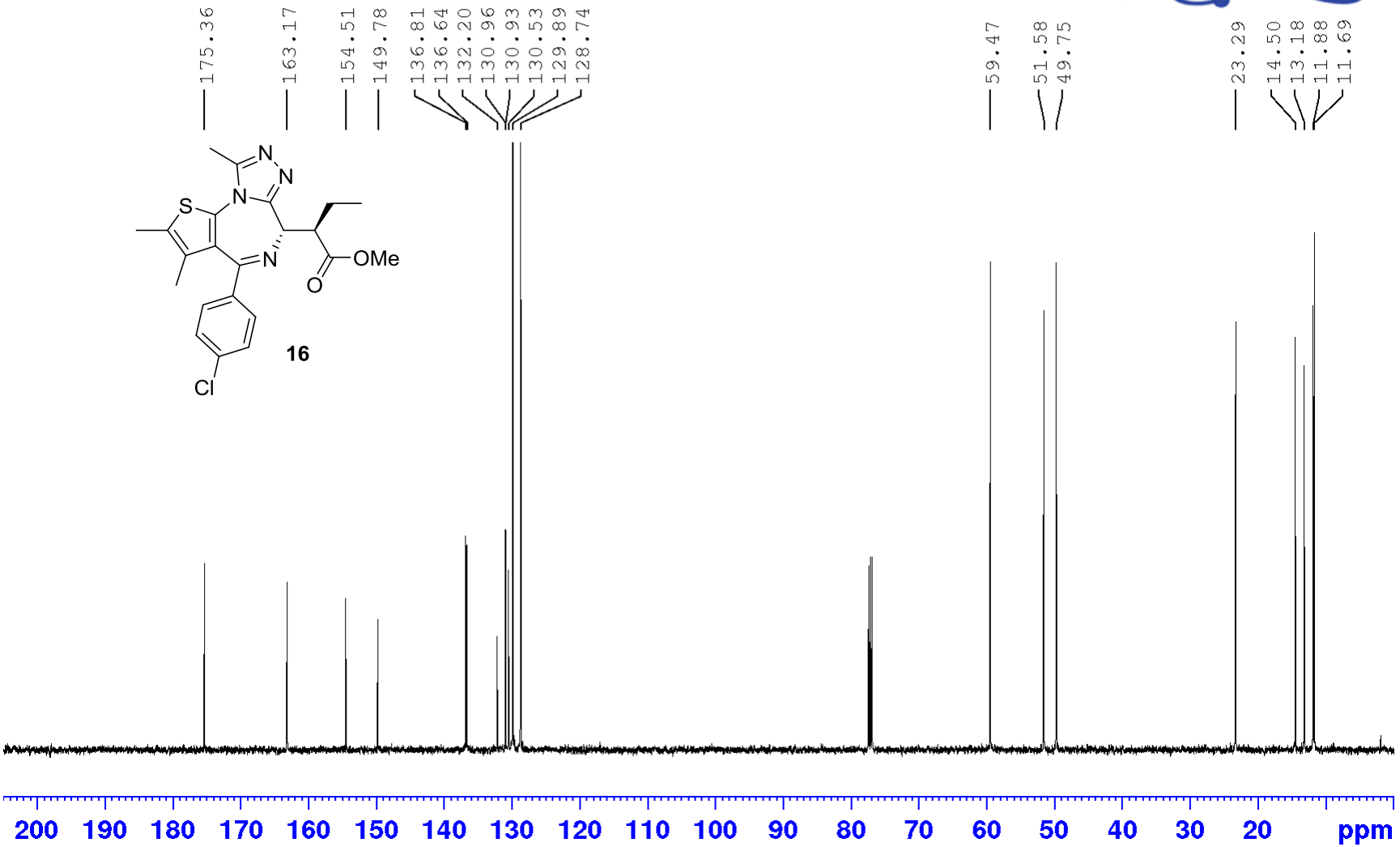

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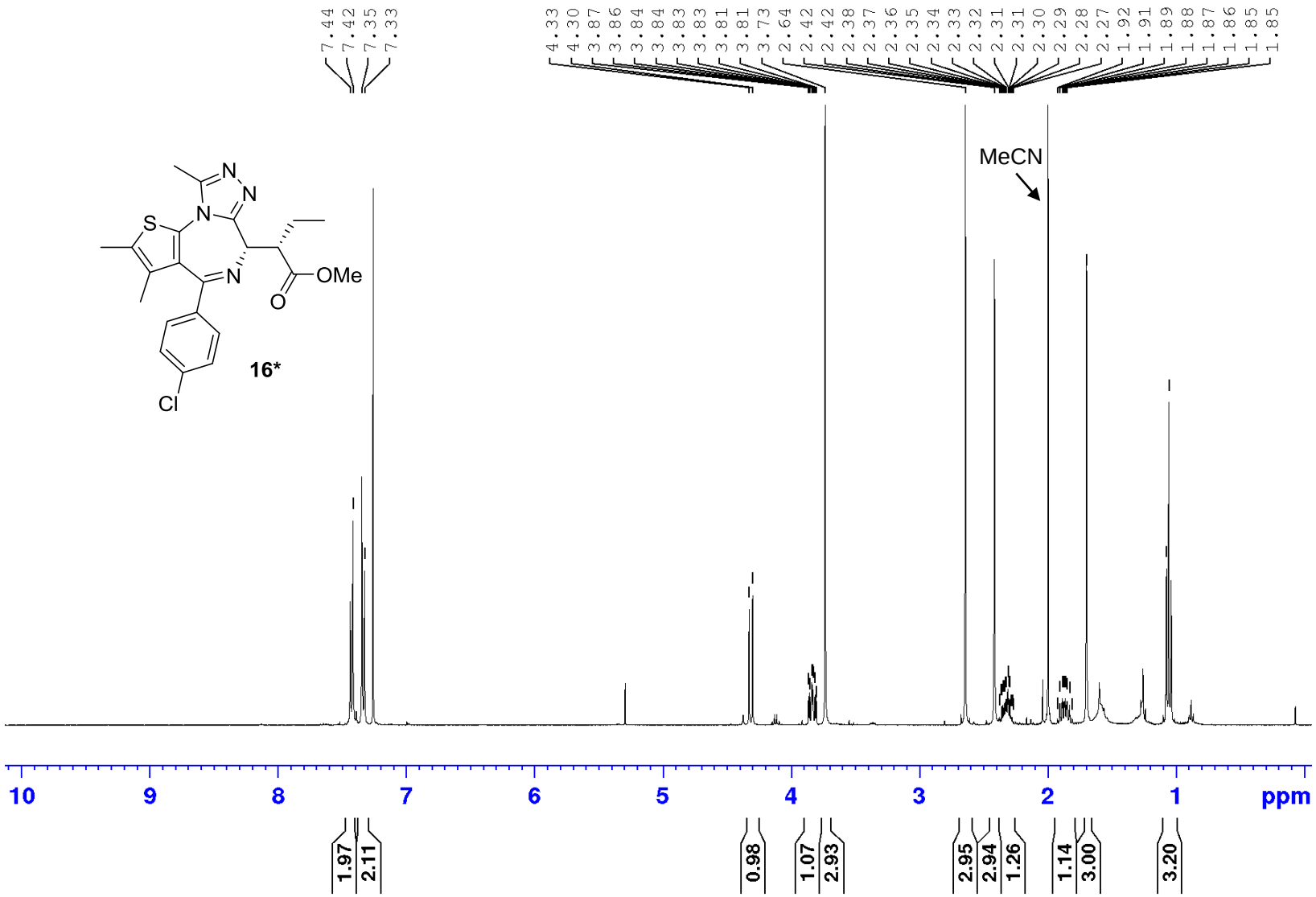
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3.81
3.81
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2.64
2.42
2.42
2.38
2.37
2.36
2.35
2.34
2.33
2.32
2.31
2.31
2.30
2.29
2.28
2.27
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1.91
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1.88
1.87
1.86
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1.85
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OMe
O
Cl
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7
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ppm
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3.00
3.20

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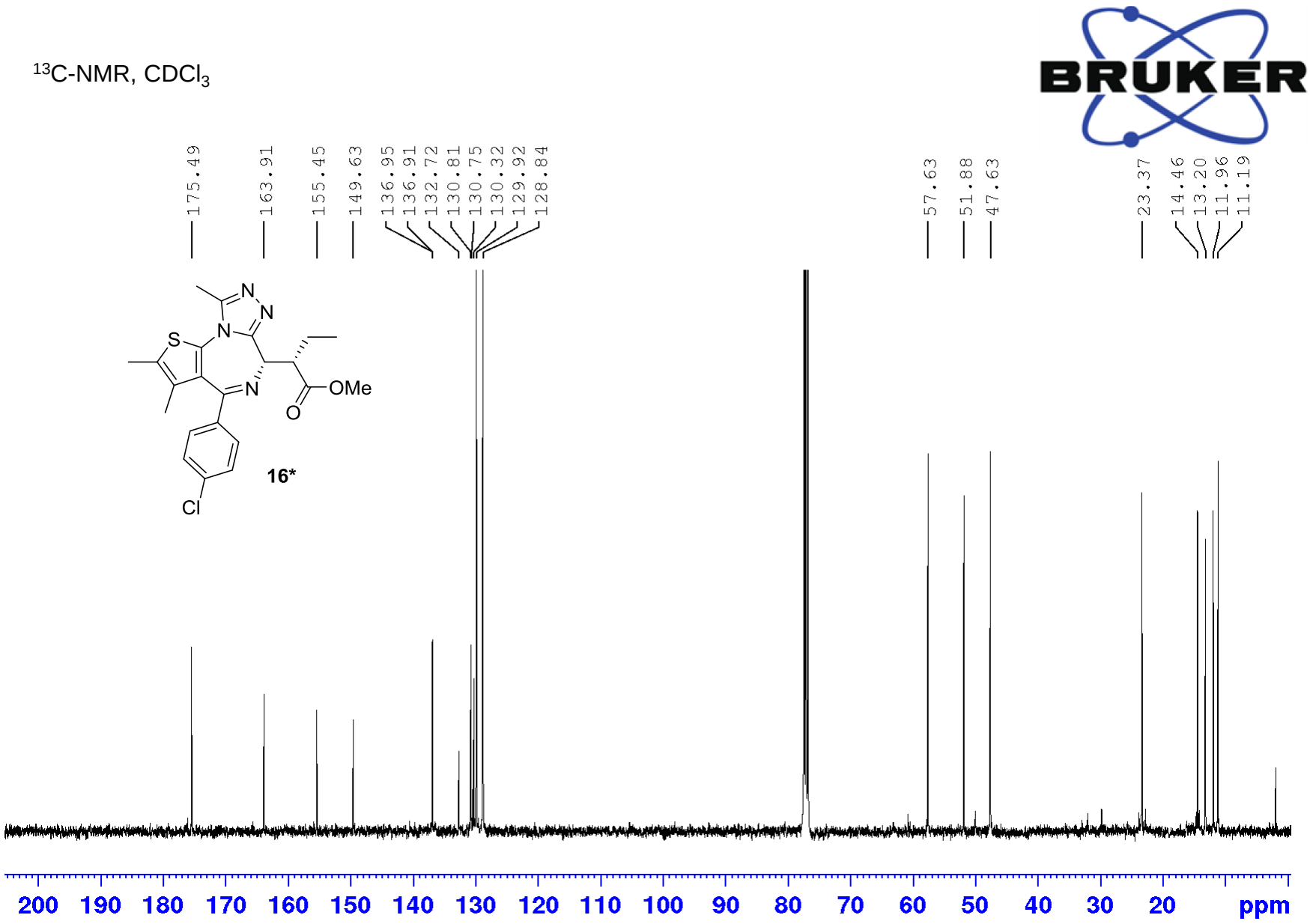

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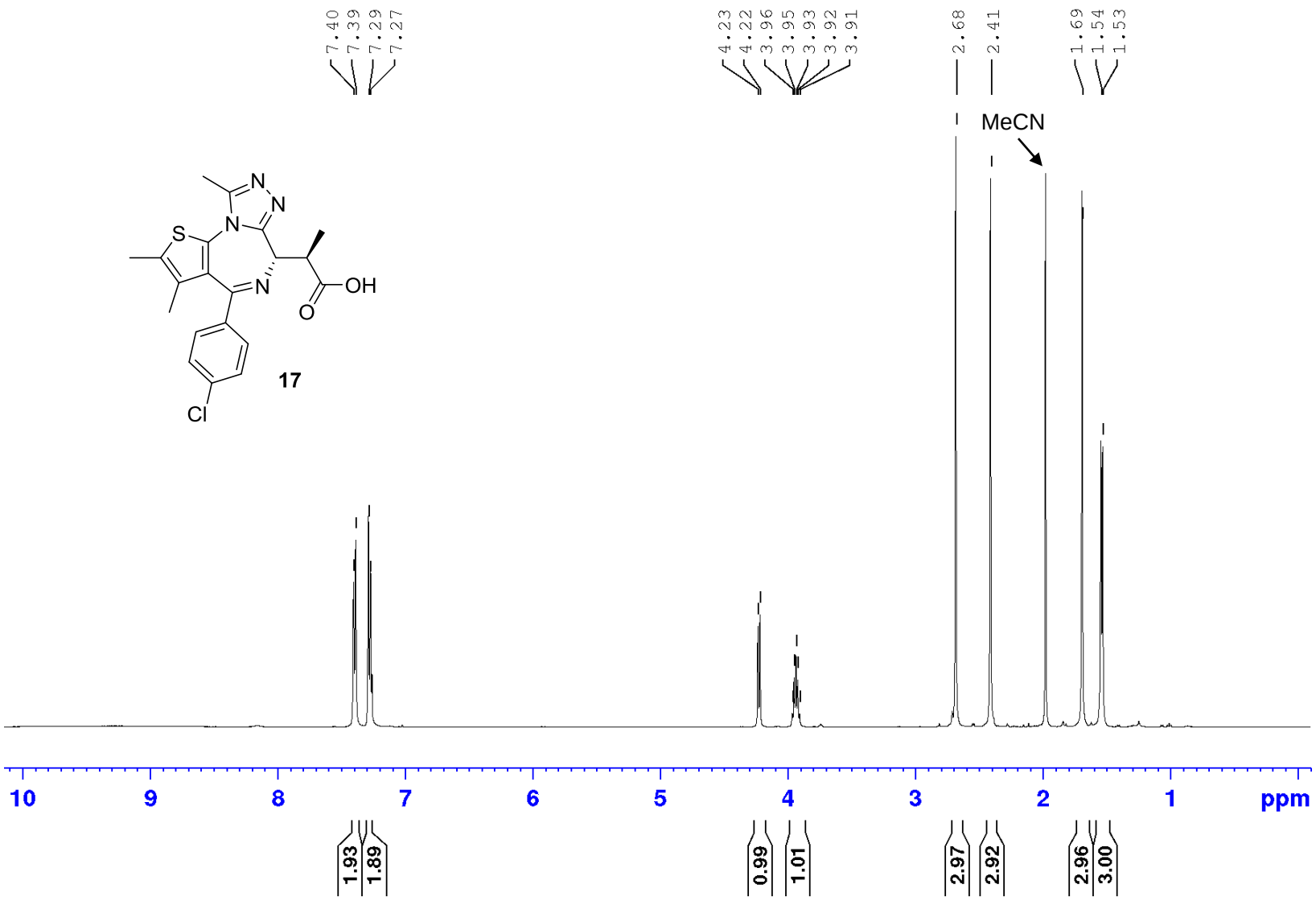
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3.95
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1.54
1.53
MeCN
N
N
N
S
N
OH
O
Cl
17
10
9
8
7
6
5
4
3
2
1
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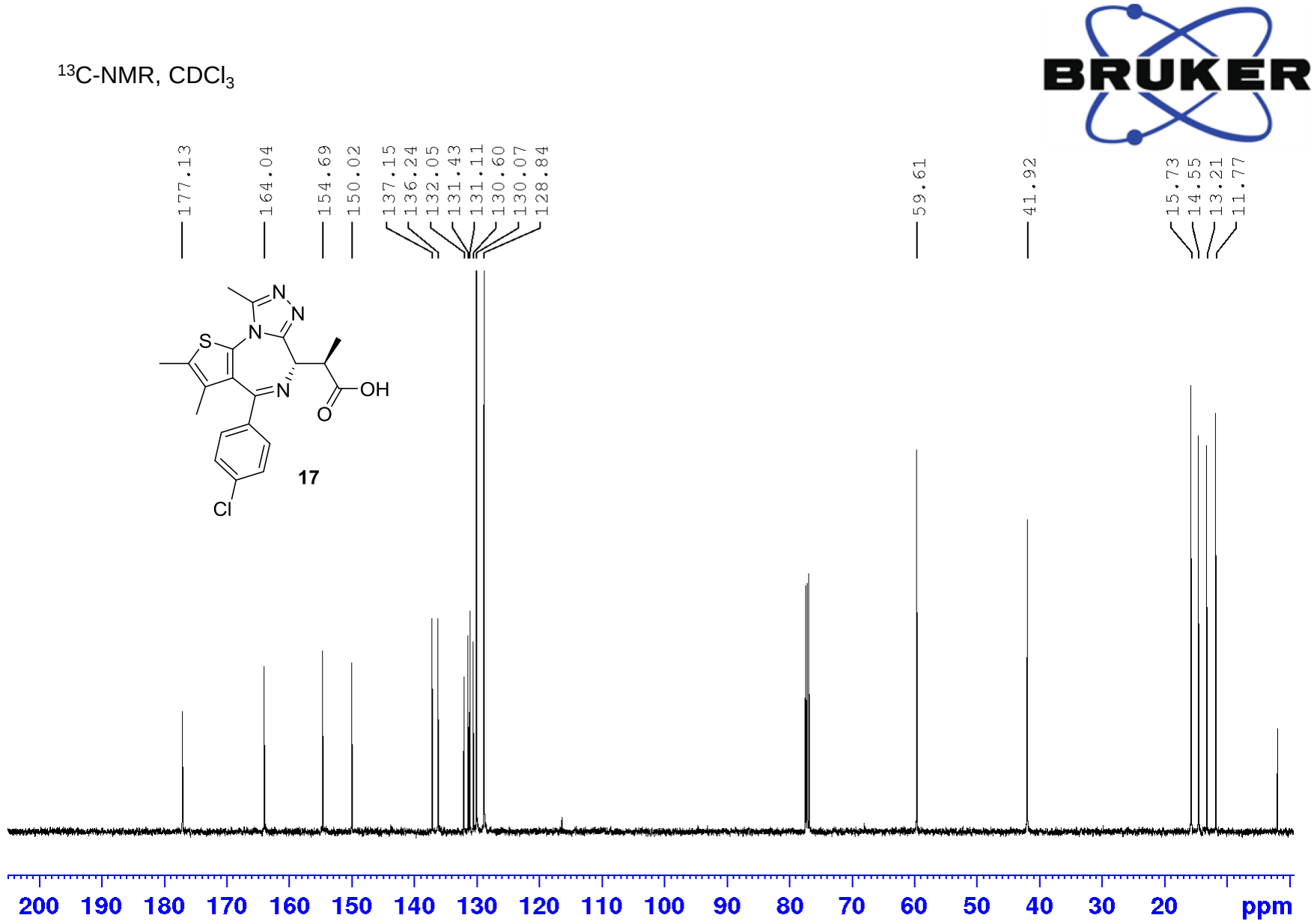
2.92
2.96
3.00

^{13}C-NMR, $CDCl_3$



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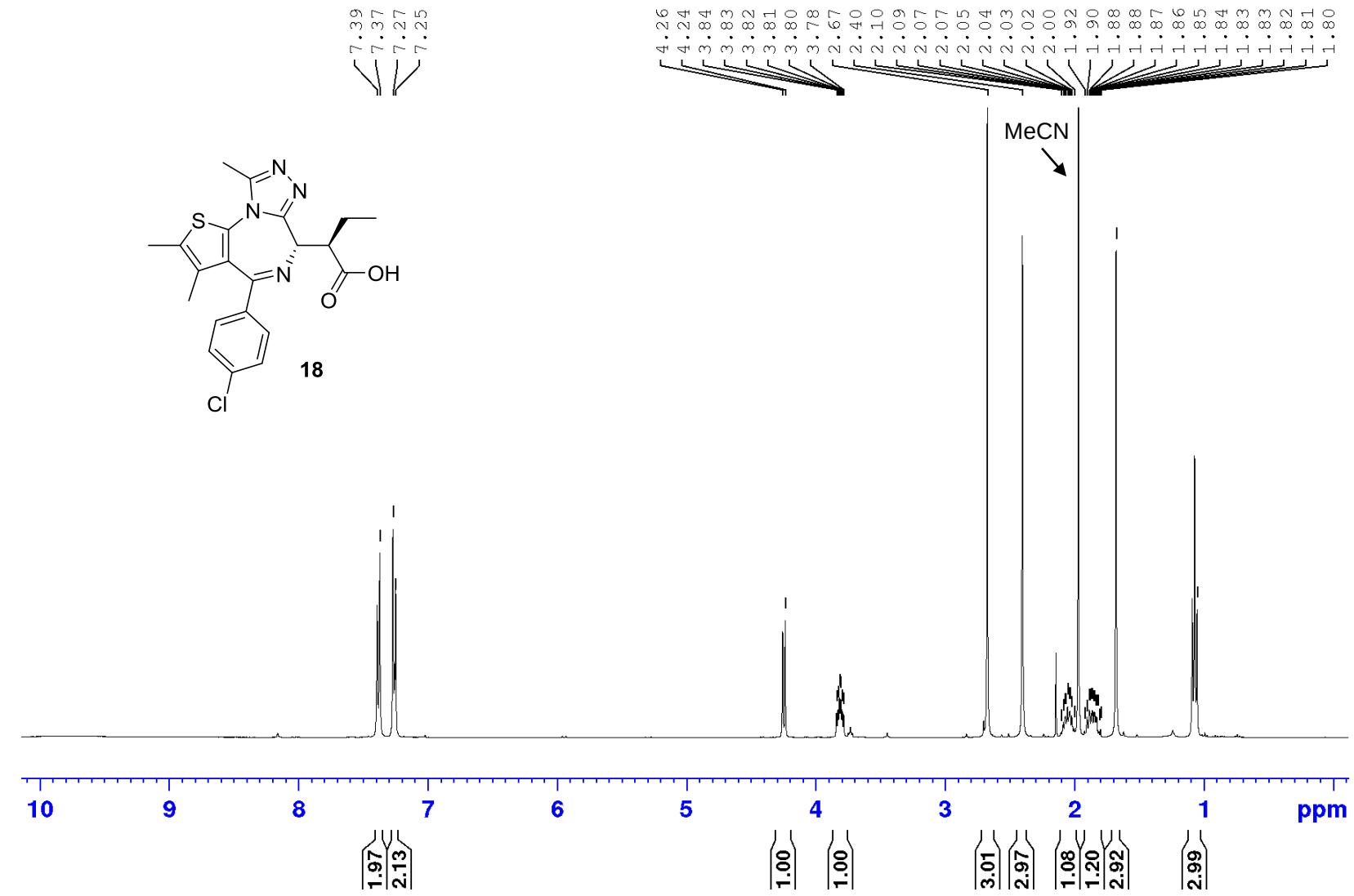
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2.10
2.09
2.07
2.07
2.05
2.04
2.03
2.02
2.00
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1.90
1.88
1.88
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1.85
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1.83
1.82
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7
6
5
4
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2
1
ppm
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2.92
2.99

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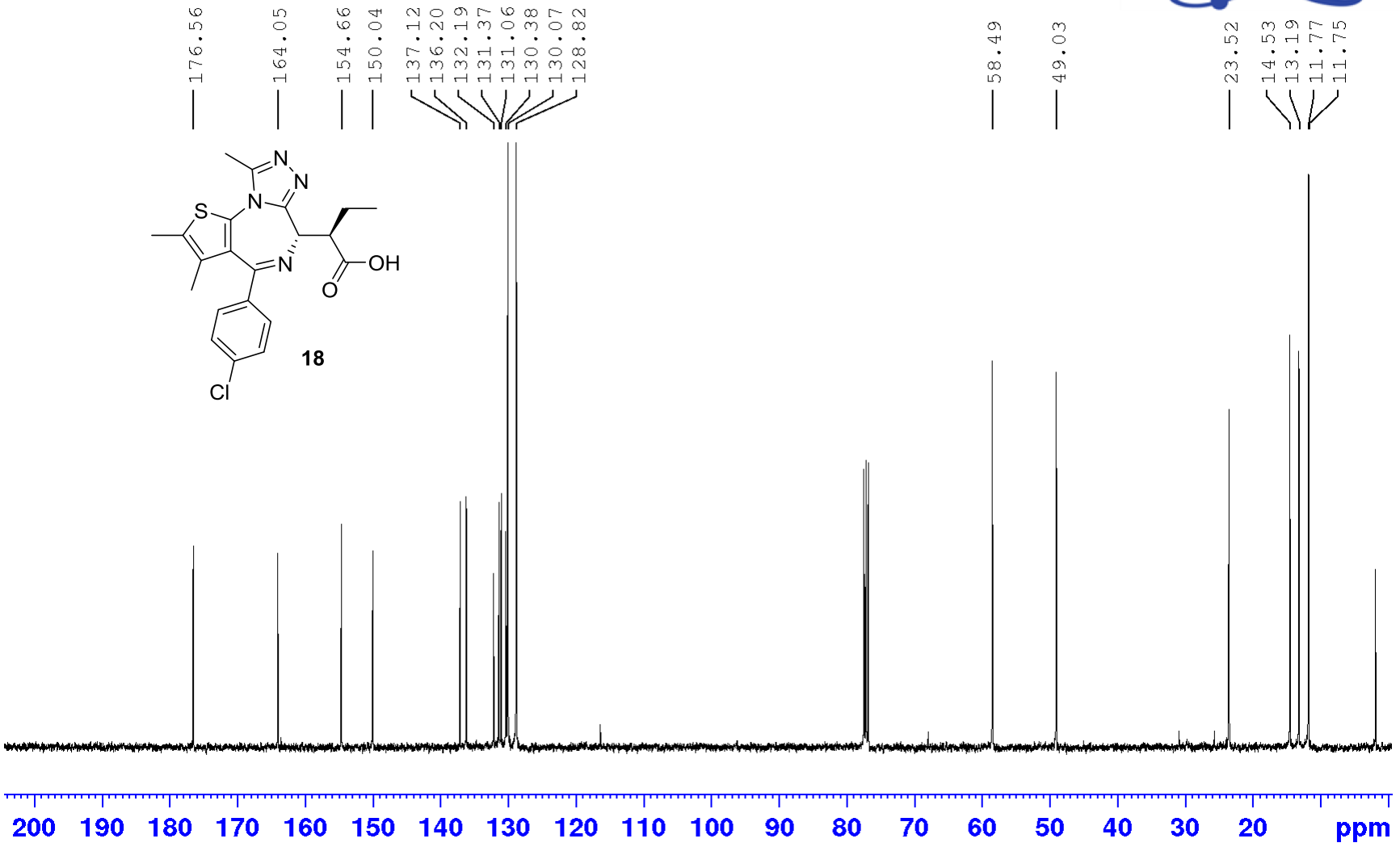
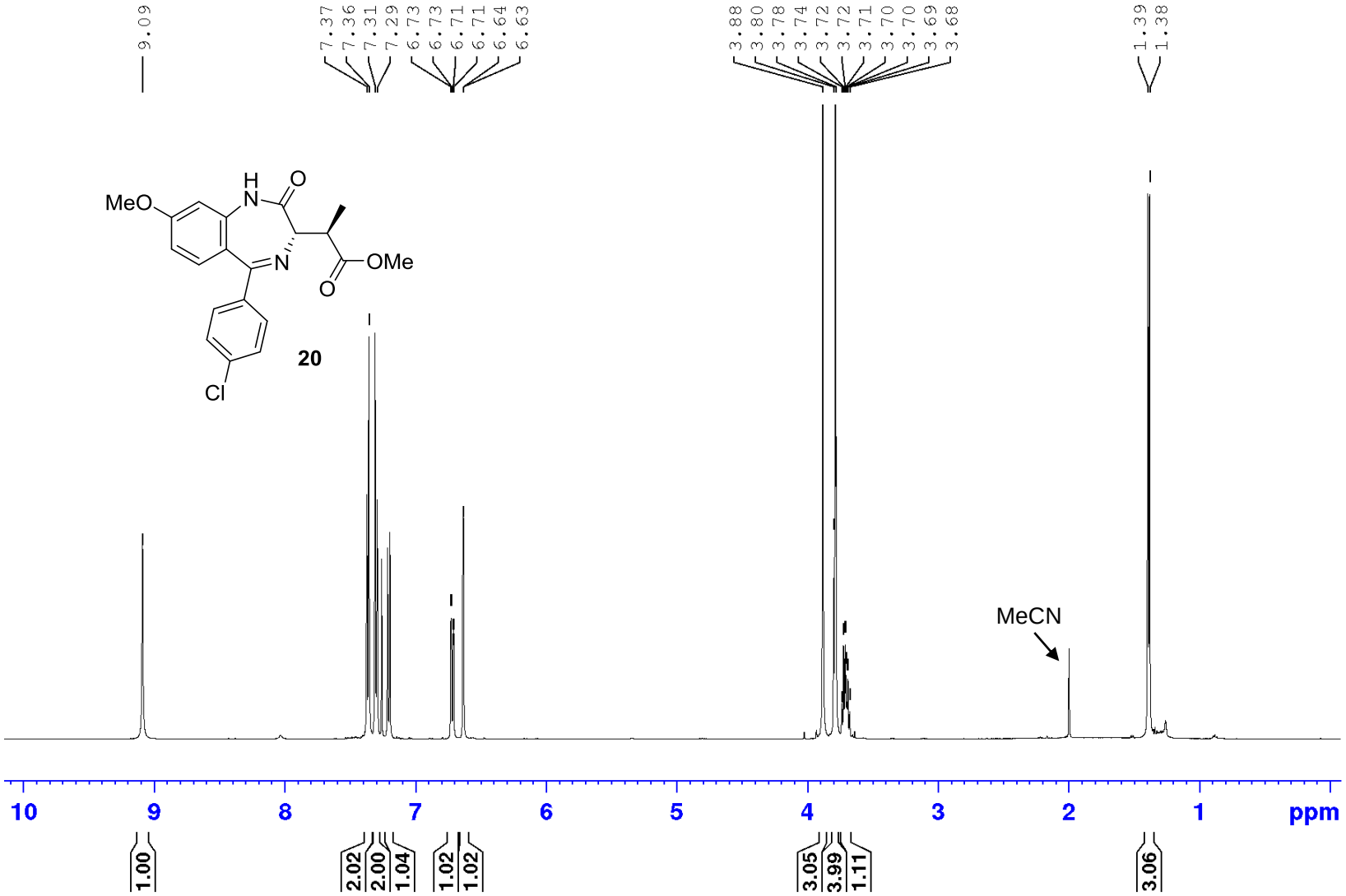


^{1}H-NMR, $CDCl_3$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6.63
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3.80
3.78
3.74
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3.72
3.71
3.70
3.70
3.69
3.68
1.39
1.38
MeO
H
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O
N
OMe
O
Cl
20
MeCN
10
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8
7
6
5
4
3
2
1
ppm
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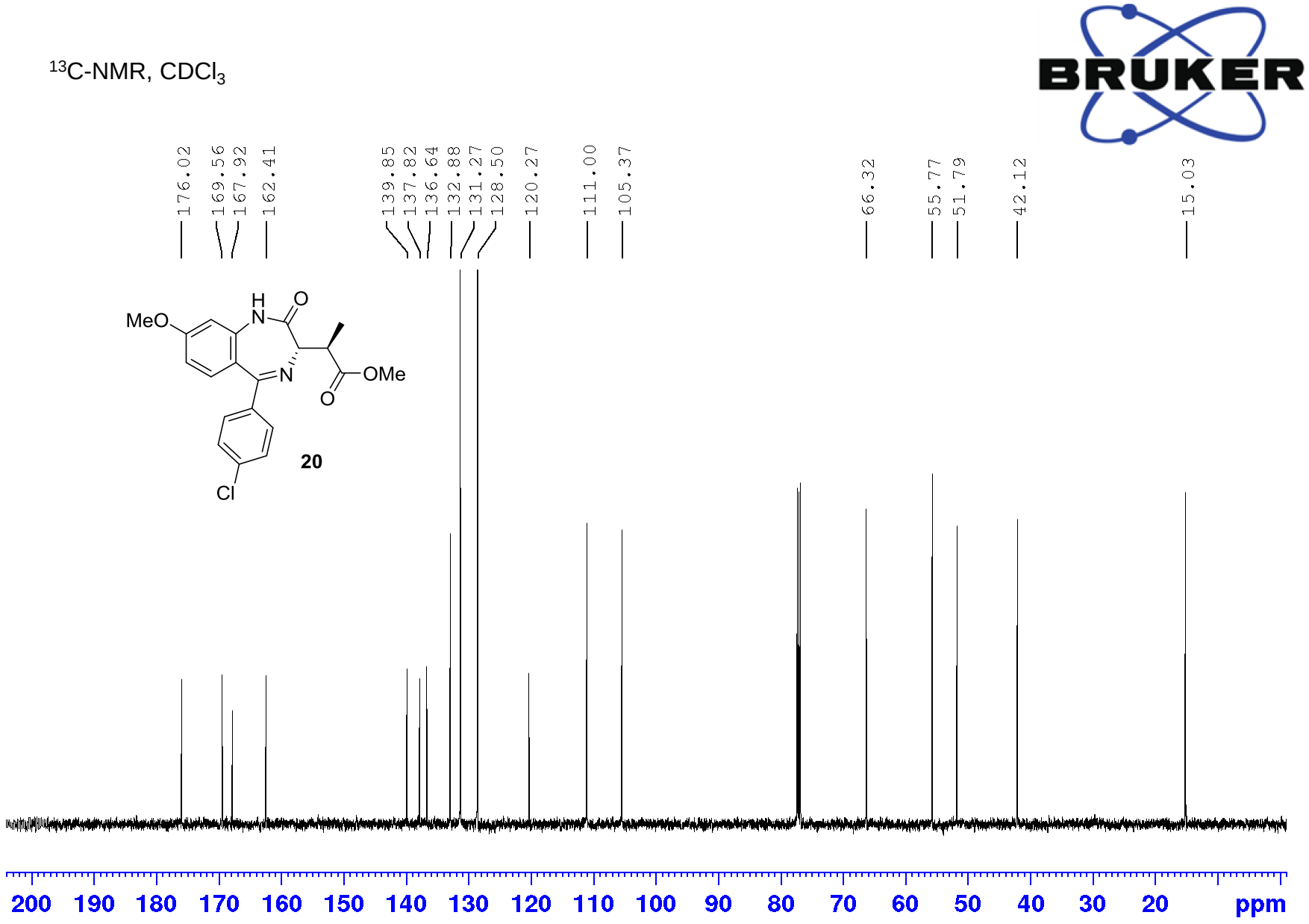
1.11
3.06

^{13}C-NMR, $CDCl_3$



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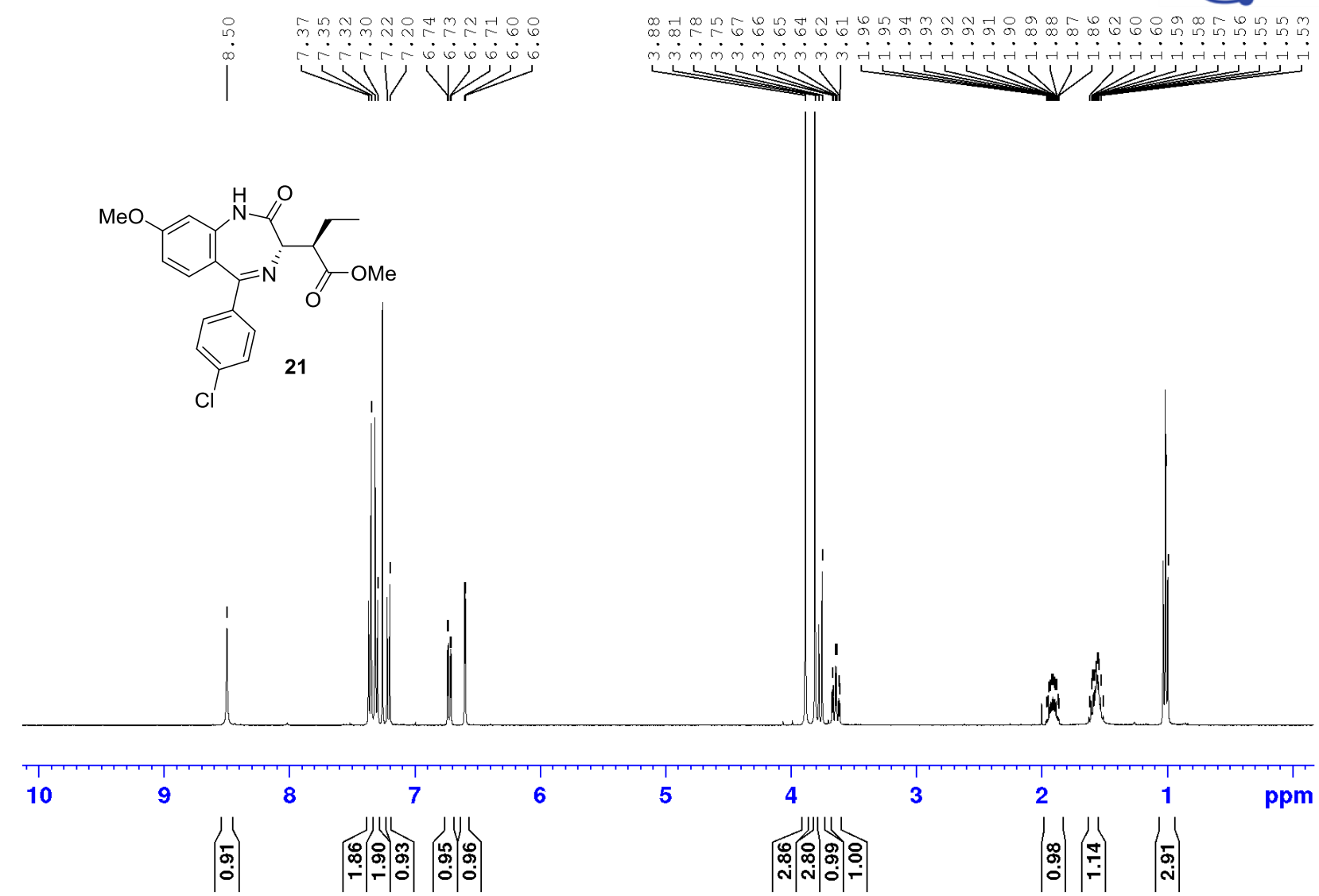
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6.72
6.71
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6.60
3.88
3.81
3.78
3.75
3.67
3.66
3.65
3.64
3.62
3.61
1.96
1.95
1.94
1.93
1.92
1.92
1.91
1.90
1.89
1.88
1.87
1.86
1.62
1.60
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1.59
1.58
1.57
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1.55
1.53
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H
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O
N
OMe
O
Cl
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10
9
8
7
6
5
4
3
2
1
ppm
0.91
1.86
1.90
0.93
0.95
0.96
2.86
2.80
0.99
1.00
0.98
1.14
2.91

^{13}C-NMR, $CDCl_3$