Membrane-permeant, environment-sensitive dyes generate biosensors within living cells

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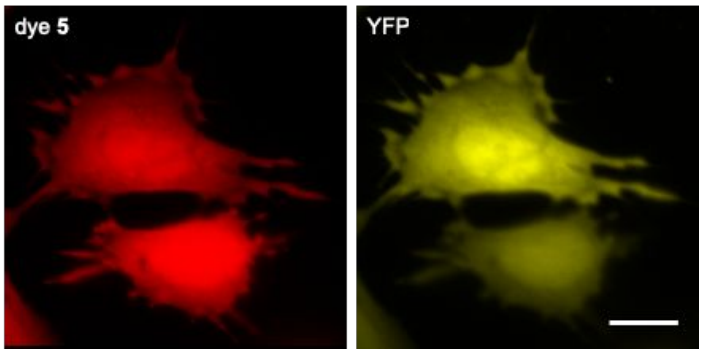


Figure S1. Distribution of the dye **5** relative to YFP, used as a volume indicator. Images were scaled individually to show subcellular distribution, so do not indicate the relative brightness of each cell. Mouse embryo fibroblasts, bar = 20 μm.

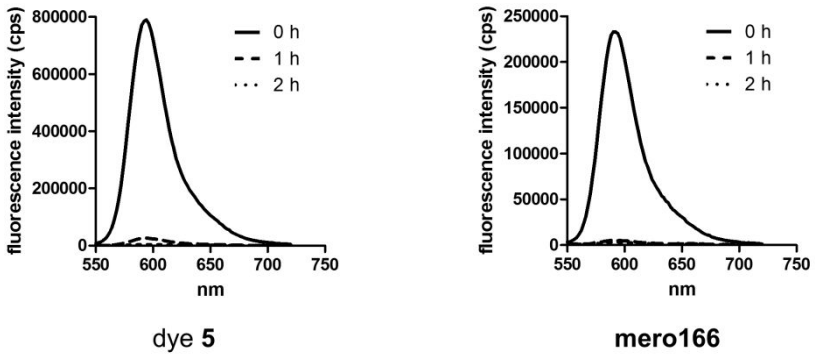


Figure S2. Export of dye **5** and **mero166** over time. HeLa cells were incubated with test dye (5.0 μM) and co-treated with Hoechst 33342 (1.0 μM) to control for cell number variation in 12-well dish. Cells were incubated for 15 min, washed twice with PBS, and trypsinized at 0, 1, and 2 h post-wash. Cells were suspended in PBS for fluorescence measurements.

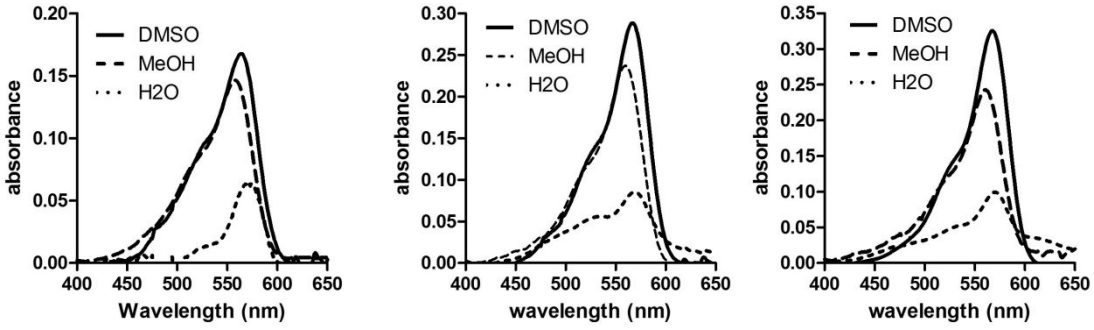


Figure S3. UV-Vis spectra of conjugatable merocyanine dyes bearing acetoxymethylester groups. Spectra obtained in DMSO, MeOH, and water (2.5 μM).

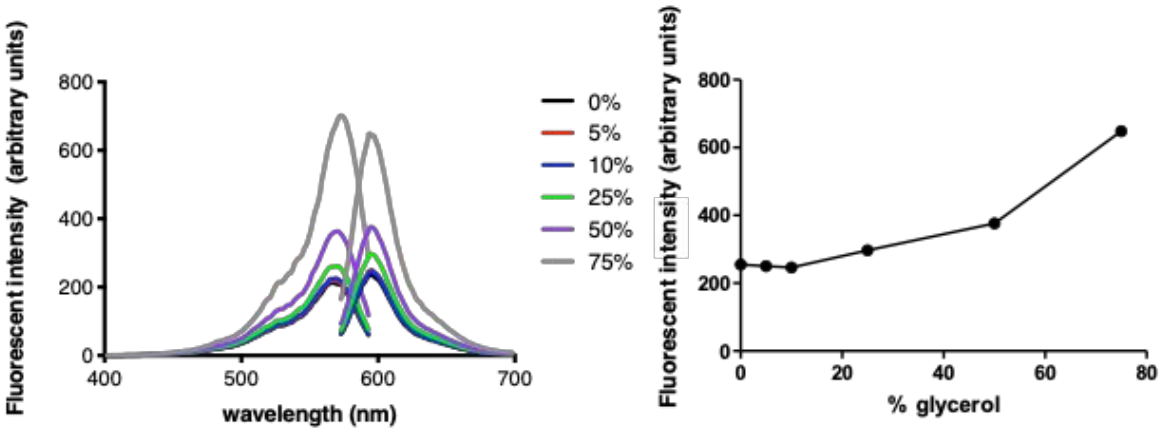


Figure S4. Emission and excitation spectra of **mero166** with increasing viscosity. Stock **mero166** solution was prepared at 10 uM and diluted to 1.0 uM in solutions with increasing percentages of glycerol (0, 5, 10, 25, 50, and 75%).

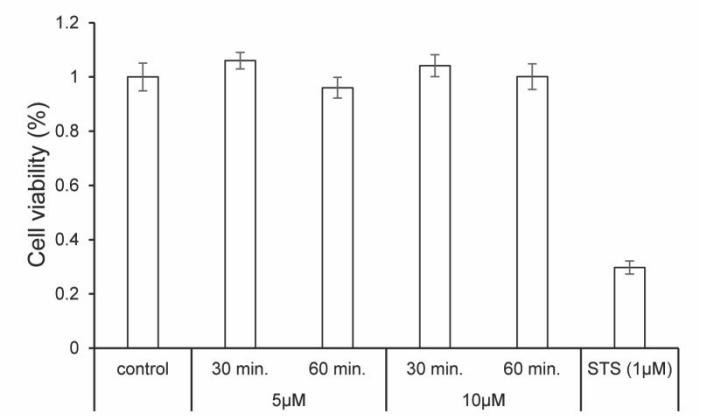


Figure S5. The cytotoxicity of **mero166** was measured by monitoring dehydrogenase activity (CCK-8 assay).[1] HeLa cells were exposed to 5μM and 10μM **mero166** for 30 and 60 minutes. Cells treated with vehicle (DMSO) were used as a negative control and cells treated for 5 hours with 1μM Staurosporine (STS), a common inducer of apoptosis, were used as a positive control. Each treatment had 6 replicates. Data are presented as mean ± standard deviation (SD).

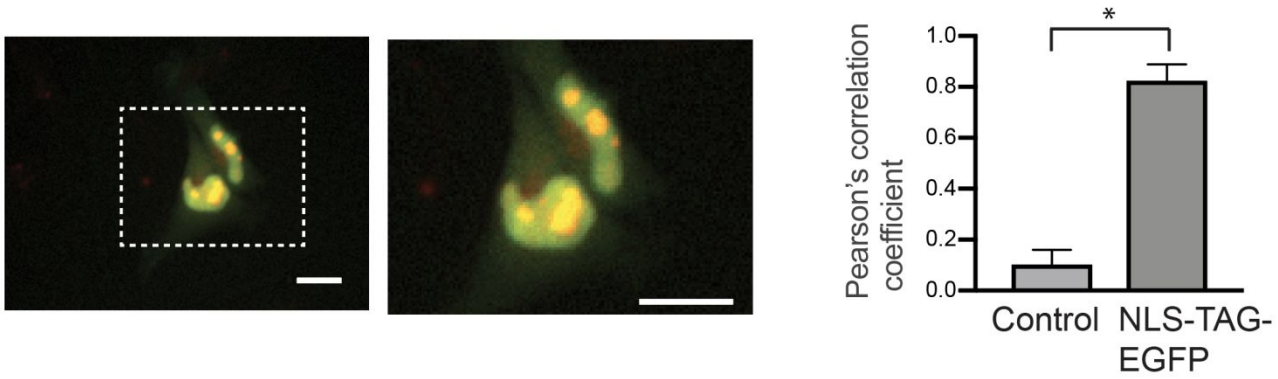


Figure S6. HeLa cells co-transfected with the test construct NLS-TAG-EGFP and a plasmid expressing the tRNA synthetase/tRNA pair. The EGFP and **mero166** images shown in Figure 4 were are shown merged here (green, EGFP; red, mero166). The rectangle in the left image was enlarged on the right. Pearson's correlation coefficient was quantified in non-EGFP expressing (control) and NLS-TAG-EGFP-expressing cells. More than 10 cells were quantified for each condition. NLS, nuclear localization signal. (Scale bars 20 μm, dye excitation filter 545/50, dye emission filter 630/45, EGFP excitation filter 470/40, EGFP emission filter 630/45, * $p < 0.0001$ unpaired student t-test).

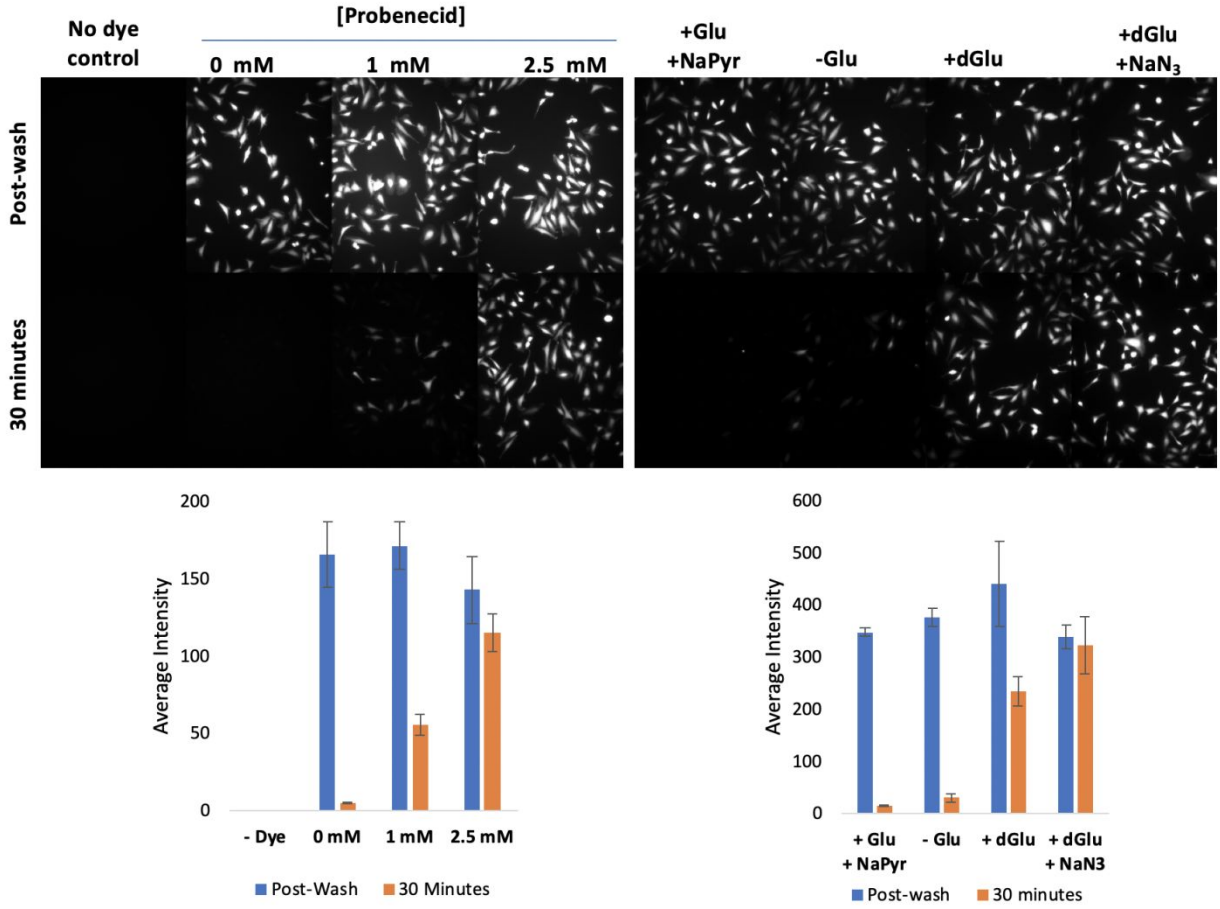


Figure S7. Probing the mechanism of **mero166** export. Probenecid inhibits organic anion transport by targeting multidrug resistance-associated proteins (MRP), which have been shown to actively export the anionic calcium probe calcein.[2-3] Active anion export can also be indirectly inhibited using 2-deoxyglucose (dGlu) and sodium azide (NaN_3), which deplete ATP levels in the cell.[3-4] Glucose (Glu) and sodium pyruvate (NaPyr) sustain ATP production. In the absence of treatments affecting ion transport, washing resulted in rapid loss of dye from the cells. When anion transport was inhibited, cells retained dye. This is consistent with hydrolysis of the acetoxymethyl ester to an anion, and removal of the dye from the cells by active anion transport. This can explain why hydrolyzed acetoxymethyl ester is effectively transported from the cells.

For all steps, the media contained Probenecid or the indicated reagents at the following concentrations: 5 mM Glu, 5 mM dGlu, 1 mM NaPyr, 10 mM NaN_3. Prior to dye treatment, cells were treated with the indicated media for 1 hour to allow sufficient changes in ATP concentration. For all dye treatments, cells were incubated with 5 uM **mero166** in media for 15 minutes, washed with PBS, and then incubated with fresh media for 30 minutes to wash out dye. Cells were imaged under identical exposure conditions at the beginning (post-wash) and end (30 minutes) of the washout period. The bar graph compares the average intensity of the entire field of view (average of three fields, representative images shown).

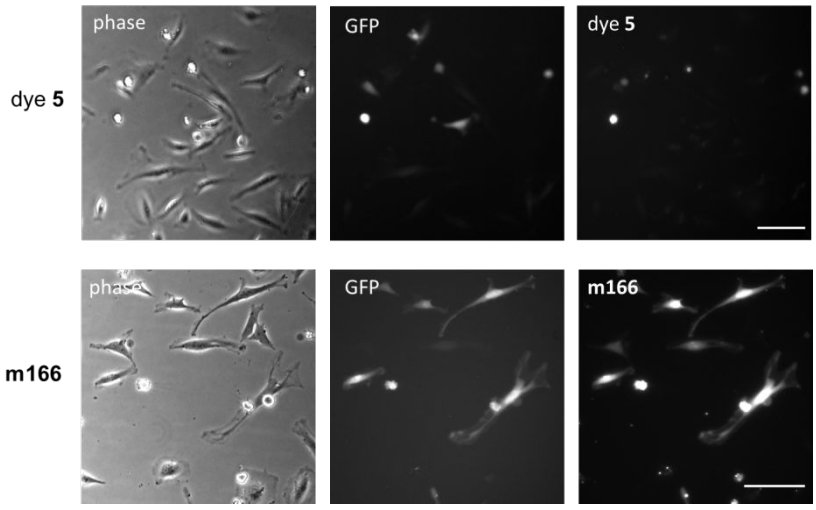


Figure S8. In cell protein labeling is specific to dye with reactive functional group. HeLa cells transfected with CBD-TAG-EGFP for 24 h were incubated with either control dye **5** or **mero166** at 37 °C for 15 min. Dye-containing media was removed, cells were then washed (PBS × 2) and fresh media was added. Cells were allowed to recover for 2 h prior to imaging. (Scale bar 100 □m, dye excitation filter 545/50, dye emission filter 630/45, EGFP excitation filter 470/40, EGFP emission filter 630/45).

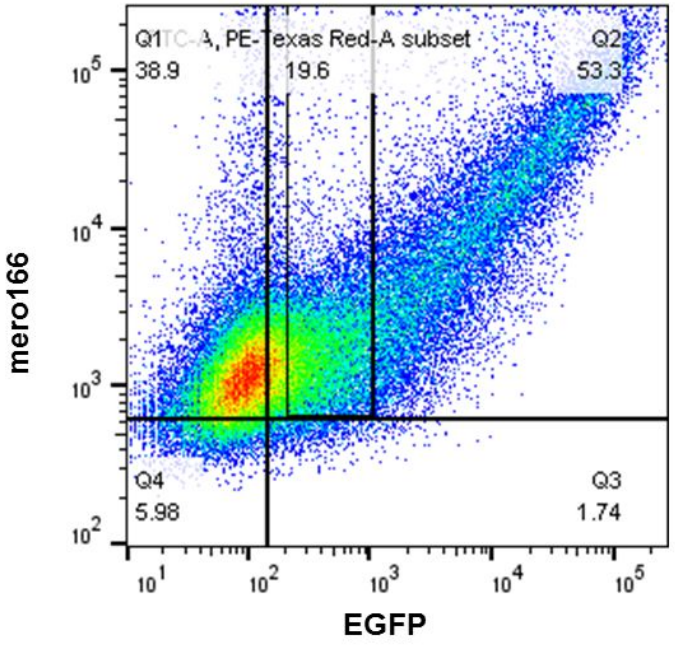


Figure S9. Extent of dye labeling correlates with EGFP expression level. HeLa cells expressing CBD-TAG-EGFP labeled with **mero166** (5 uM, 15 min).

GENERAL MATERIALS AND METHODS

Cloning. Initial studies for unnatural amino acid labeling utilized the construct NLS-TAG-EGFP (donation from Alexander Deiters, University of Pittsburgh). This construct was derived from the EGFP-N1 vector and contained the nucleoplasmin bipartite nuclear localization signal (KRPAATKKAGQAKKKKL) followed by a short linker sequence containing the amber stop codon (TAG), followed by EGFP. For expression of orthogonal tRNA and aminoacyl tRNA synthetase, we modified the vector pAG13-38 (donation from Alexander Deiters, University of Pittsburgh) harboring four copies of tRNA expression cassette (CMV enhancer, U6 promoter and tRNA), and one copy of the CMV-driven acyl tRNA synthetase cassette. The codon optimized *Methanosarcina barkeri* tRNA synthetase was mutated into Y271M, L274G, and C321A for incorporation of bicyclononyne lysine at the amber stop codon by replacing the BsrGI/EcoRI fragment from the original tRNA synthetase with those from a synthetic tRNA synthetase containing those mutations. This plasmid was named pAG13-BCN.

Mammalian expression plasmids for WASP CBD(aa201-314)-F271TAG-EGFP were derived from plasmids described previously.[5] The amber stop codon (TAG) was introduced at F271 of the wild type CBD and the Cdc42-binding defective CBD mutant (H246D, H249D) in pET23a using the Q5 site-directed mutagenesis kit (NEB, Ipswich, MA). The resultant plasmids were used as the templates to amplify CBD-F271TAG-EGFP-His and CBD mutant mCBD-F271TAG-EGFP-His by PCR with primers listed below. These PCR products were ligated into the NcoI/XhoI sites of pTriEx-4 (EMD Millipore, Billerica, MA) using Gibson assembly (NEB). To minimize background GFP signals, two in-frame ATG codons that encode M307 in WASP and M1 in EGFP were mutated into glycine by mutagenesis with primers listed below. We termed these plasmids CBD-F271TAG-EGFP-His v2 and mCBD-F271TAG-EGFP-His v2. Further, to exclude potential electrostatic interaction, the His tag at the end of EGFP was deleted in both CBD and mCBD constructs by Q5 mutagenesis using the primers listed below to give final constructs for labeling studies CBD-F271TAG-EGFP-noHis v2 and mCBD-F271TAG-EGFP-noHis v2

For the optimized mammalian expression vector containing the PylRS/$tRNA_{CUA}$ pair, pU6PylT-MCS, EF1α promoter amplified from PB513B_1 (System Bioscience, Palo Alto, CA) and a synthetic U6PylT cassette (U6-promoter driven PylT expression) containing a U25C mutation[6] were assembled into pEGFP-C1 (Clontech, Mountain View, CA) digested with AgeI and BglII by Gibson assembly (NEB). From pU6PylT-MCS, the U6PylT cassette was excised with SpeI and NheI, and ligated into a unique NheI site in the same plasmid, yielding p2U6PylT-MCS which contains two copies of U6PylT. In a similar way, p4U6PylT-MCS was generated. The tRNA synthetase for incorporation of bicyclononyne was ligated into SalI and BamHI sites of p4U6PylT-MCS, yielding p4U6PylT-aaRS BCN.

All plasmids were verified by sequencing (Genewiz, South Plainfield, NJ) before use.

Primers for amber stop codon mutagenesis:
CCAGATCTGCGGAGTCTGTAGTCCAGGGCAG
CTGCCCTGGACTACAGACTCCGCAGATCTGG

Primers for CBD-4Glycine-EGFP amplification:
ATCAAAGGAGATATACCATGGACATCCAGAACCCTG
ATGGTGATGGTGGTGCTCGAGCTTGTACAG

Primers for ATG mutations:
ATTCGGTGGCGGTGGCACCGGTGTGAGCAAGGGCGAGGAG
TCAAGTGGCTCCTGGCGCCTACCCTCCTGCCGCACAGCCTC

Primers for His tag removal:
TAAGTGATTAACCTCAGGTG
CTTGTACAGCTCGTCCATG

cDNA of tRNA synthetase:
AGCGGCTGTACACCAACGACCGGGAGGACTACCTGGGCAAGCTGGAACGGGACATCACCA
AGTTCTTCGTGGACCGGGGCTTCCTGGAAATCAAGAGCCCCATCCTGATCCCCGCCGAGTA
CGTGGAGCGGATGGGCATCAACAACGACACCGAGCTGTCCAAGCAGATTTTCCGGGTGGA
CAAGAACCTGTGCCTGCGGCCTATGCTGGCCCCCACCCTGATGAACTACGGGCGGAAACTG
GACAGAATCCTGCCTGGCCCCATCAAGATTTTCGAAGTGGGACCCTGCTACCGGAAAGAGA
GCGACGGCAAAGAGCACCTGGAAGAGTTTACAATGGTGAATTTTGCCCAGATGGGCAGCG
GCTGCACCCGGGAGAACCTGGAAGCCCTGATCAAAGAGTTCCTGGATTACCTGGAAATCGA
CTTCGAGATCGTGGGCGACAGCTGCATGGTGTACGGCGACACCCTGGACATCATGCACGGC
GACCTGGAACTGAGCAGCGCCGTGGTGGGACCCGTGTCCCTGGACCGGGAGTGGGGCATC
GACAAGCCCTGGATCGGAGCCGGCTTCGGCCTGGAACGGCTGCTGAAAGTGATGCACGGC
TTCAAGAACATCAAGCGGGCCAGCAGAAGCGAGAGCTACTACAACGGCATCAGCACCAAC
CTGTGATAAGGATCCACTAGTCCAGTGTGGTGGAATTCTGCAGAT

Primers for EF1a promoter:
ACTAGTAAGGATCTGCGATCGCTCCGGT
CGAAGCTTGAGCTCGAGATCTGTAGGCGCCGGTCACAGCTTG

U6PylT cassette:
CCGCCATGCATTAGTTATTAATGCTAGCAAAAACCGCACTTGTCCGGAAACCCCGGGAATC
TAACCCGGCTGAACGGATTTAGAGTCCGTTCGATCTACATGATCAGGTTTCCGGTGTTTCGT
CCTTTCCACAAGATATATAAAGCCAAGAAATCGAAATACTTTCAAGTTACGGTAAGCATAT
GATAGTCCATTTTAAAACATAATTTTAAAACTGCAAACTACCCAAGAAATTATTACTTTCTA
CGTCACGTATTTTGTACTAATATCTTTGTGTTTACAGTCAAATTAATTCTAATTATCTCTCTA
ACAGCCTTGTATCGTATATGCAAATATGAAGGAATCATGGGAAATAGGCCCTCTTCCTGCC
CAACTAGTAAGGATCTGCGATC

Cell Culture. NIH 3T3 mouse embryonic fibroblasts (MEF) and HeLa cells were maintained in 10% CO_2 at 37 °C in Dulbecco's modified Eagle's medium (DMEM, Cellgro) with 10% fetal bovine serum (HyClone, Thermo Scientific) and 2 mM GlutaMax (Gibco, Life Technologies). For imaging, cells were plated on coverslips coated with fibronectin (Sigma-Aldrich) overnight.

Transfection and Dye Labeling. HeLa cells were plated on coverslips that had been coated with 10 μL/mL fibronectin (Sigma-Aldrich) in PBS (1 mL) overnight. Seeding density was 50 K cells/well for 6-well plate (20 K/well 12-well plate). The following day, cells were transfected with the construct containing the protein of interest with a TAG stop codon for unnatural amino acid (UAA) incorporation and the second construct containing the tRNA synthetase/tRNA pair for a specific amino acid (Fugene 6, 1.0 μg DNA/well of 6-well dish, 3:1 μL reagent/μg DNA). UAA containing media was prepared by first dissolving UAA in DMSO (100 mM), then diluting to 1 mL in culture medium and further diluting to reach a final concentration of 0.5 mM. This solution was filtered (0.22 μM Durropore PVDF, Millipore), existing cell media was removed, and freshly prepared UAA solution was added. After 24 h transfection, UAA-containing media was removed, cells were washed with PBS, and fresh UAA-free media was added.

Cells were then were maintained in 10% CO_2 at 37 °C for at least 2 h. Additional media changes were performed after 2 h.

Dye labeling: A stock solution of **mero166** in DMSO was prepared at 10 mM. This stock solution was further diluted in cell culture media to a final concentration of 5.0 μM. Media was removed from the cells and **mero166** media was added. Cells were incubated for at least 15 min at 37 °C. Dye containing media was removed, cells were washed with fresh media and maintained in fresh dye-free media in 10% CO_2 at 37 °C for at least 90 min. Prior to imaging, media was again removed and replaced with fresh media.

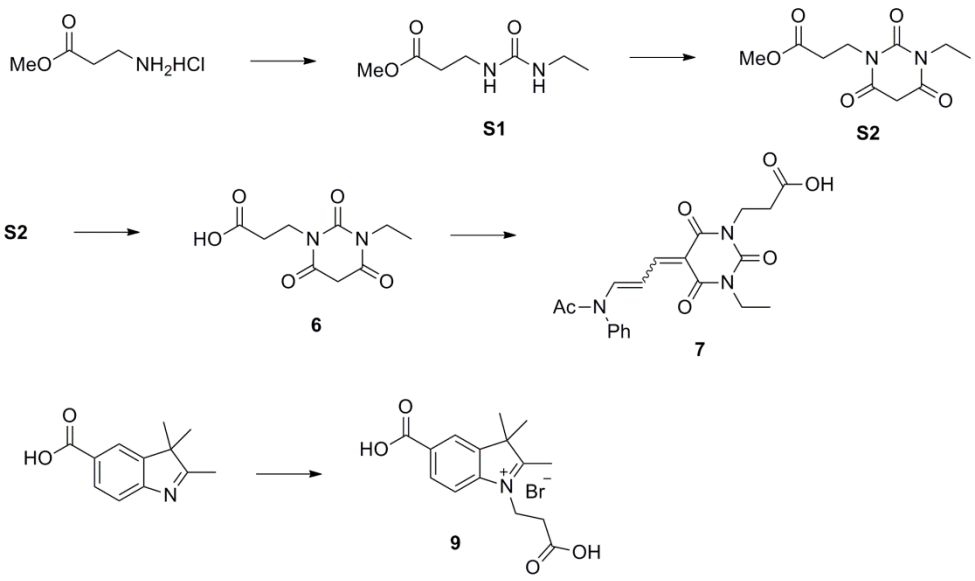
Cell viability/ cytotoxicity assay. Cell viability/ cytotoxicity was measured using the CCK-8 kit from Dojindo Molecular Technologies, Inc. following the manufacturer's instructions. This kit uses tetrazolium salt WST-8, which is reduced by dehydrogenase in cells to a yellow-colored formazan dye. The presence of the dye was used to quantify cell viability / dye cytotoxicity. 5000 cells/well were seeded in a 96-well plate one day prior to the assay, and then exposed to **mero166** for the indicated duration and concentration in a humidified incubator containing 10% CO_2 at 37°C with protection from light. Cells were washed and incubated for another 2 hours with fresh media. After incubation, cells were washed again and 10μl CCK-8 solution were added to each well together with 100ul fresh media. After 1 hour incubation at 37 °C, absorbance (A) at 450nm was measured using a plate reader (FLUOstar Omega, BMG LABTECH GmbH, Germany). The cell survival rate was calculated as: $cell\ survival\ (\%) = \frac{A^{sample} - A^{blank}}{A^{control} - A^{blank}}$.

Image Acquisition. Live cell imaging was carried out on an Olympus IX81 microscope with a UPLFLN 40X oil objective (NA 1.3) and mercury lamp excitation (103W HBO bulb). For EGFP images, we used 470/40 excitation filter, 525/50 emission filter and 480/580 dichroic mirror. For Mero166 and other dye images, we used 545/50 excitation filter, 630/45 emission filter and 480/580 dichroic mirror. Excitation was through a ND 2.0 (1 % T) filter. Images were acquired with a Coolsnap ES2 camera (Photometrics) with a Sony 6.45 x 6.45 μM/pixel chip using 2 x 2 binning. All image acquisition, processing, and analysis was carried out with Metamorph software. Images were shade corrected, background subtracted, and linearly contrast stretched to reveal subcellular features as previously described.[7] To quantify Pearson's correlation coefficient between NLS-TAG-EGFP and **mero166** images, Coloc2 plugin in ImageJ was used. Cell edges were drawn by freehand selection in ImageJ and used as ROI for the quantification. Non EGFP expressing cells were used as a control. More than 10 cells in each condition were quantified.

Chemistry. Bicyclononyne lysine was synthesized as described by Dommerholt et al. and Lang et al.[8-9] β-Alanine methyl ester hydrochloride (Sigma-Aldrich) and malonaldehyde dianilide hydrochloride (TCI America) were purchased and used as received. The following compounds were prepared according to literature procedures: 1,3-diethylbarbituric acid,[10] 1,2,3,3-tetramethylindolium iodide,[11] 1,3-diethyl-5-(3-methoxyallylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione,[12] 2,3,3-trimethyl-5-carboxyindolenine and 5-carboxy-1,2,3,3-tetramethyl-*3H*-indolium iodide,[13] 1-(2-carboxyethyl)-2,3,3-trimethyl-3*H*-indolium bromide,[14] 4,5-dimethoxy-2-nitrobenzyl alcohol,[15] and 2-azidoethylamine.[16] Reaction progress was monitored via thin-layer chromatography (TLC) on EM Science pre-coated glass-backed plates (silica gel 60 Å F_{254}, 0.25 mm thickness). Flash chromatography was carried out with silica gel 60 Å (230 - 400 mesh) and automated chromatography was performed on an Isco Combiflash Companion. Unless otherwise stated, organic extracts were dried over commercially available magnesium sulfate and the solvents were removed by rotary evaporation. ^1H and ^{13}C NMR spectra were referenced to the residual solvent peak (for $CDCl_3$, ^1H δ 7.26 ppm and ^{13}C δ 77.23 ppm; for DMSO-d_6, ^1H δ 2.50 ppm and ^{13}C δ 39.51 ppm). Mass spectra were obtained on a Hewlett-Packard 1100 high-performance liquid

chromatograph equipped with a 1100 mass-selective detector (MS-ESI). UV-visible spectra were obtained with a Hewlett-Packard 8453 diode array spectrophotometer. Emission and excitation spectra were obtained using a Spex Fluorolog 2 spectrofluorometer at 23 °C. All operations with dyes were performed under dim light. Photoreactions were carried out using a Rayonet RMR-600 equipped with 350 nm bulbs. High performance liquid chromatography was performed on a Shimadzu Prominence system with a Vydac 218TP152022 C_{18} column (15-20 μm particle size, 300-Å, 250 X 22 mm) in the water/methanol or acetonitrile/TFA mixture: solvent A (water 95 parts, organic solvent 5 parts, TFA 0.05%), solvent B (water 5 parts, organic solvent 95 parts, TFA 0.05 %). Standard gradient for separation of final products is 10% solvent B, 30 min 90% solvent B, total run time of 45 min.

SYNTHESIS AND ANALYTICAL DATA OF COMPOUNDS



Scheme S1. Synthesis of key intermediates.

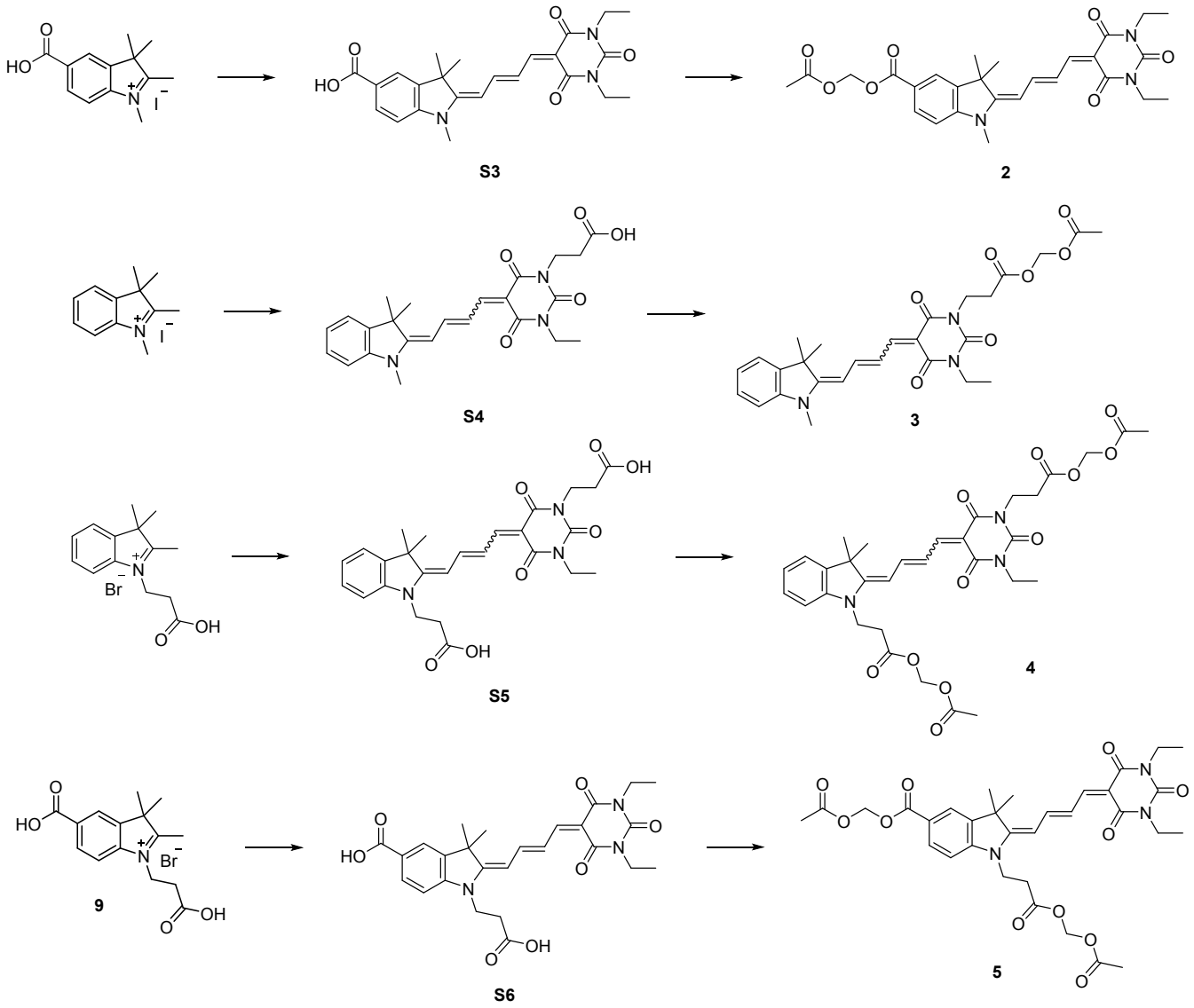
Methyl 3-(3-ethylureido)propanoate (S1). *β*-Alanine methyl ester hydrochloride (34.9 g, 250 mmol) was added to a flame dried flask. The flask was sealed, evacuated, and flushed with argon and CH_2Cl_2 (350 mL) was added via cannula. Triethylamine (38.3 mL, 275 mmol) was added and the reaction was stirred at room temp for 45 min. The flask was cooled in an ice bath and ethyl isocyanate (20.8 mL, 262.5 mmol) was added quickly dropwise. The reaction was allowed to gradually equilibrate to room temp. After stirring for 16 h, the reaction was quenched with half-saturated aq. NH_4Cl (400 mL). The organic layer was separated and the aq. layer was further extracted with CH_2Cl_2 (200 mL x 2). The organic layers were combined, washed with water and brine, dried with $MgSO_4$, filtered, and concentrated. The resulting solid was further dried under high vacuum to give 25.2 g (58%) white solid suitable for use without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (bs, 1H), 4.82 (bs, 1H), 3.66 (s, 3H), 3.44 (q, J = 6.0 Hz, 2H), 3.20 – 3.12 (m, 2H), 2.52 (t, J = 6.0 Hz, 2H), 1.10 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 158.4, 51.9, 36.0, 35.4, 34.9, 15.6. MS-ESI *m/z* 197.0 ($[M + Na]^+$ requires 197.1).

Methyl 3-(3-ethyl-2,4,6-trioxotetrahydropyrimidin-1(2H)-yl)propanoate (S2). Compound **S1** (24.9 g, 143 mmol) and malonic acid (15.6 g, 150 mmol) were suspended in glacial AcOH (200 mL). The mixture was heated to 60 °C and stirred at that temp for 10 min. Ac_2O (53.9 mL, 572 mmol) was then added in bulk and the reaction was heated further to 100 °C and stirred for 1.5 h. The reaction mixture was concentrated and set at room temp open to air. The resulting crystals were re-crystallized from 3:1 hexanes/EtOAc (50 mL). The re-crystallized solid was filtered and washed with ice cold Et_2O (100 mL X 2) to give 23.9 g (69%) white solid suitable for use in the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (t, J = 7.2, 2H), 3.92 (q, J = 7.2 Hz, 2H), 3.67 (s, 3H), 3.65 (s, 2H), 2.62 (t, J = 7.2 Hz, 2H), 1.20 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 164.7, 164.4, 151.2, 52.1, 39.9, 37.9, 37.6, 32.5, 13.4. MS-ESI *m/z* 240.9 ($[M - H]^-$ requires 241.1).

3-(3-Ethyl-2,4,6-trioxotetrahydropyrimidin-1(2H)-yl)propanoic acid (6). Compound **S2** (8.30 g, 34.3 mmol) was diluted in THF (70 mL) and DI (35 mL) was added. $LiOH.H_2O$ (5.75 g, 4.00 eq) was added and the reaction was stirred at room temp for 16 h. The flask was set in an ice bath and the reaction was quenched with 6 N aq. HCl (27 mL) to a pH of ~4. The reaction mixture was concentrated and the resulting precipitate was filtered, washed with DI (2x) and ice chilled Et_2O (2x). The product was dried under high vacuum to give 7.30 g (93%) white solid that required no further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.36 (bs, 1H), 3.93, (t, J = 7.8 Hz, 2H), 3.75 (q, J = 7.2 Hz, 2H), 3.71 (s, 2H), 2.45 (t, J = 7.8 Hz, 2H), 1.08 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO- d_6) δ 172.2, 165.6, 165.4, 151.4, 40.1, 36.7, 36.1, 32.0, 12.9. MS-ESI *m/z* 226.8 ([M - H]$^-$ requires 227.1).

3-(3-ethyl-2,4,6-trioxo-5-((E)-3-(N-phenylacetamido)allylidene)tetrahydropyrimidin-1(2H)-yl)propanoic acid (7). Compound **6** (0.500 g, 2.19 mmol), *N*-(3-(phenylamino)-2-propenylidene)aniline hydrochloride (0.624 g, 2.41 mmol), and sodium acetate (0.198 g, 2.41 mmol) were added together to an oven-dried flask. The flask was sealed, evacuated, and argon flushed and acetic anhydride (2.2 mL) was added. The flask was added to a pre-heated oil bath at 135 °C and stirred for 1 h. The reaction mixture was cooled to room temp and diluted with dichloromethane. The organic layer was washed with saturated sodium bicarbonate, saturated ammonium chloride, and brine, and dried with sodium sulfate. The organic layer was filtered, concentrated, and loaded neat onto a 120 g silica column. The column was eluted with 0 – 75% EtOAc in hexanes over 25 min. Main product fractions were combined and concentrated to give 0.387 g (44%) yellow solid as an approximate 1:1 mixture of *E* and *Z* isomers. Isomer A: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (bs, 1H), 8.67 (d, J = 13.6 Hz, 1H), 8.23 (d, J = 12.4 Hz, 1H), 7.65 – 7.53 (m, 3H), 7.44 – 7.36 (m, 2H), 6.70 (t, J = 13.0 Hz, 1H), 3.86 (t, J = 7.8 Hz, 2H), 3.80 (q, J = 7.2 Hz, 2H), 2.37 (t, J = 7.8 Hz, 2H), 2.09 (s, 3H), 1.08 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 169.9, 161.5, 161.0, 157.2, 150.3, 137.8, 130.3, 129.5, 128.2, 110.5, 109.0, 37.0, 36.2, 35.5, 32.2, 23.1, 13.0. Isomer B: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.28 (bs, 1H), 8.67 (d, J = 13.6 Hz, 1H), 8.22 (d, J = 12.4 Hz, 1H), 7.65 – 7.53 (m, 3H), 7.44 – 7.36 (m, 2H), 6.71 (t, J = 13.0 Hz, 1H), 3.99 (t, J = 7.8 Hz, 2H), 3.68 (q, J = 6.8 Hz, 2H), 2.46 (t, J = 7.8 Hz, 2H), 2.09 (s, 3H), 0.99 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.2, 169.9, 161.4, 161.1, 157.2, 150.3, 137.8, 130.3, 129.6, 128.2, 110.5, 109.0, 37.0, 36.3, 35.5, 32.1, 23.1, 13.1. MS-ESI *m/z* 400.0 ([M + H]$^+$ requires 400.2).

5-Carboxy-1-(2-carboxyethyl)-2,3,3-trimethyl-3*H*-indolium bromide (9). 2,3,3-Trimethyl-5-carboxyindolenine (1.00 g, 4.92 mmol) and bromopropionic acid (0.828 g, 5.41 mmol) were added to a flame-dried 2-necked flask fitted with a condenser and rubber septum. The flask was sealed, evacuated, and argon flushed, and *o*-dichlorobenzene (10 mL) was added. The suspension was heated to 150 °C for 16 h under argon. The reaction mixture was cooled gradually to 0 °C and filtered. The precipitate was washed with chloroform (3 x 20 mL) and dried under high vacuum to give 1.167 g pale red solid that was carried on to the next step as intermediate. MS-ESI *m/z* 355.8 ([M]$^{\cdot-}$ requires 355.0).



Scheme S2. Synthesis of structure permeability screening compound set.

(E)-2-((E)-4-(1,3-diethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)but-2-en-1-ylidene)-1,3,3-trimethylindoline-5-carboxylic acid (S3). 1,3-Diethyl-5-(3-methoxyallylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (0.126 g, 0.500 mmol) was added to a flame dried flask fitted with a condensor. A 1:1 mixture of MeOH and $CHCl_3$ (5 mL) was added and the flask was flushed with argon. 5-Carboxy-1,2,3,3-tetramethyl-*3H*-indolium iodide (0.207 g, 0.600 mmol) was added, followed quickly by NaOAc (0.049 g, 0.600 mmol). The reaction was allowed to stir for 16 h at reflux. Solvent was removed and the residue was redissolved in $CHCl_3$ and loaded onto silica. The silica cake was eluted on a 12 g silica column using 0 – 5% MeOH in CH_2Cl_2. The product was isolated as 0.075 g (34%) purple powdery solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (bs, 1H), 8.21 (d, J = 12.8 Hz, 1H), 8.15 (t, J = 13.2 Hz, 1H), 7.98 (s, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.78 (t, J = 13.2 Hz, 1H), 7.27 (d, J = 8.4 Hz, 1H), 6.14 (d, J = 13.2 Hz, 1H), 3.90 – 3.80 (m, 4H), 3.47 (s, 3H), 1.64 (s, 6H), 1.14 – 1.07 (m, 6H). MS-ESI *m/z* 438.2 ([M + H]$^+$ requires 438.2).

(E)-acetoxymethyl-2-((E)-4-(1,3-diethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)but-2-en-1-ylidene)-1,3,3-trimethylindoline-5-carboxylate (2). Compound **S3** (50.0 mg, 0.114 mmol) was diluted in 2.5 mL anhydrous ACN and the flask was flushed with argon. Diisopropylethylamine (30.0 µL, 0.171 mmol) was added, followed by addition of bromomethylacetate (13.0 µL, 0.137 mmol) and the reaction was stirred at room temperature for 16 h. The reaction was concentrated and the residue was redissolved in a minimum amount of DCM. The crude mixture was eluted on a 12 g silica column with 0 – 50% hexanes in EtOAc. The product was isolated as 52 mg (89%) purple sticky solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 12.8 Hz, 1H), 8.15 (t, J = 13.2 Hz, 1H), 8.00 (d, J= 1.6 Hz, 1H), 7.95 (dd, J = 8.0, 1.6 Hz, 1H), 7.78 (t, J = 12.8 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.14 (d, J = 13.2 Hz, 1H), 5.92 (s, 2H), 3.90 – 3.80 (m, 4H), 3.46 (s, 3H), 2.11 (s, 3H), 1.65 (s, 6H), 1.14 – 1.07 (m, 6H). MS-ESI *m/z* 510.2 ([M + H]$^+$ requires 510.2).

3-((E)-3-ethyl-2,4,6-trioxo-5-((2E,4E)-4-(1,3,3-trimethylindolin-2-ylidene)but-2-en-1-ylidene)tetrahydropyrimidin-1(2H)-yl)propanoic acid (S4). 1,2,3,3-Tetramethylindolium iodide (0.151 g, 0.500 mmol) and compound **7** (0.300 g, 0.750 mmol) were diluted in 1:1 $CHCl_3$/MeOH (5 mL) and NaOAc (0.051 g, 0.625 mmol) was added in bulk. The reaction was stirred at room temp for 16 h and concentrated. The concentrate was prepared as a silica cake and eluted on a 24 g silica column with 0 – 8% MeOH in CH_2Cl_2 over 25 min. Pure product containing fractions were combined and concentrated to give 0.123 g (56%) dark blue solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (bs, 1H), 8.17 – 8.03 (m, 2H), 7.73 (t, J = 13.0 Hz, 0.5H), 7.72 (t, J = 13.0 Hz, 0.5H), 7.51 (d, J = 7.2 Hz, 1H), 7.35 (dt, J = 7.2, 1.2 Hz, 1H), 7.30 – 7.25 (m, 1H), 7.16 (dt, J = 7.2, 1.2 Hz, 1H), 6.16 (d, J = 13.6 Hz, 1H), 4.03 (t, J = 7.6 Hz, 2H), 3.84 (q, J = 6.8 Hz, 2H), 3.51 (s, 3H), 2.47 (t, J = 7.6 Hz, 2H), 1.62 (s, 6H), 1.10 (t, J = 7.0 Hz, 1.5H), 1.09 (t, J = 7.0 Hz, 1.5H). MS-ESI *m/z* 438.1 ([M + H]$^+$ requires 438.2).

Acetoxymethyl 3-((E)-3-ethyl-2,4,6-trioxo-5-((2E,4E)-4-(1,3,3-trimethylindolin-2-ylidene)but-2-en-1-ylidene)tetrahydropyrimidin-1(2H)-yl)propanoate (3). Compound **S4** (33.8 mg, 0.077 mmol) was diluted in 2 mL anhydrous ACN and the flask was flushed with argon. Diisopropylethylamine (20.2 µL, 0.116 mmol) was added, followed by addition of bromomethylacetate (11.4 µL, 0.116 mmol) and the reaction was stirred at room temperature for 16 h. The reaction was concentrated and the residue was redissolved in a minimum amount of CH_2Cl_2. The crude mixture was eluted on a 12 g silica column with 0 – 50% hexanes in EtOAc. The product was isolated as 33.8 mg (93%) dark violet solid. ^{1}H NMR (400 MHz, DMSO-d_6, 50 °C) δ 8.17 – 8.03 (m, 2H), 7.80 – 7.65 (m, 1H), 7.50 (d, J = 7.2 Hz, 1H), 7.35 (dt, J = 7.2, 1.2 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.16 (dt, J = 7.2, 1.2 Hz, 1H), 6.15 (d, J = 14.0 Hz, 1H), 5.66 (s, 2H), 4.09 (t, J = 7.4 Hz, 2H), 3.85 (q, J = 7.2 Hz, 2H), 3.52 (s, 3H), 2.63 (t, J = 7.2 Hz, 2H), 2.07 (s, 3H), 1.63 (s, 6H), 1.11 (t, J = 7.0 Hz, 3H). MS-ESI *m/z* 510.2 ([M + H]$^+$ requires 510.2).

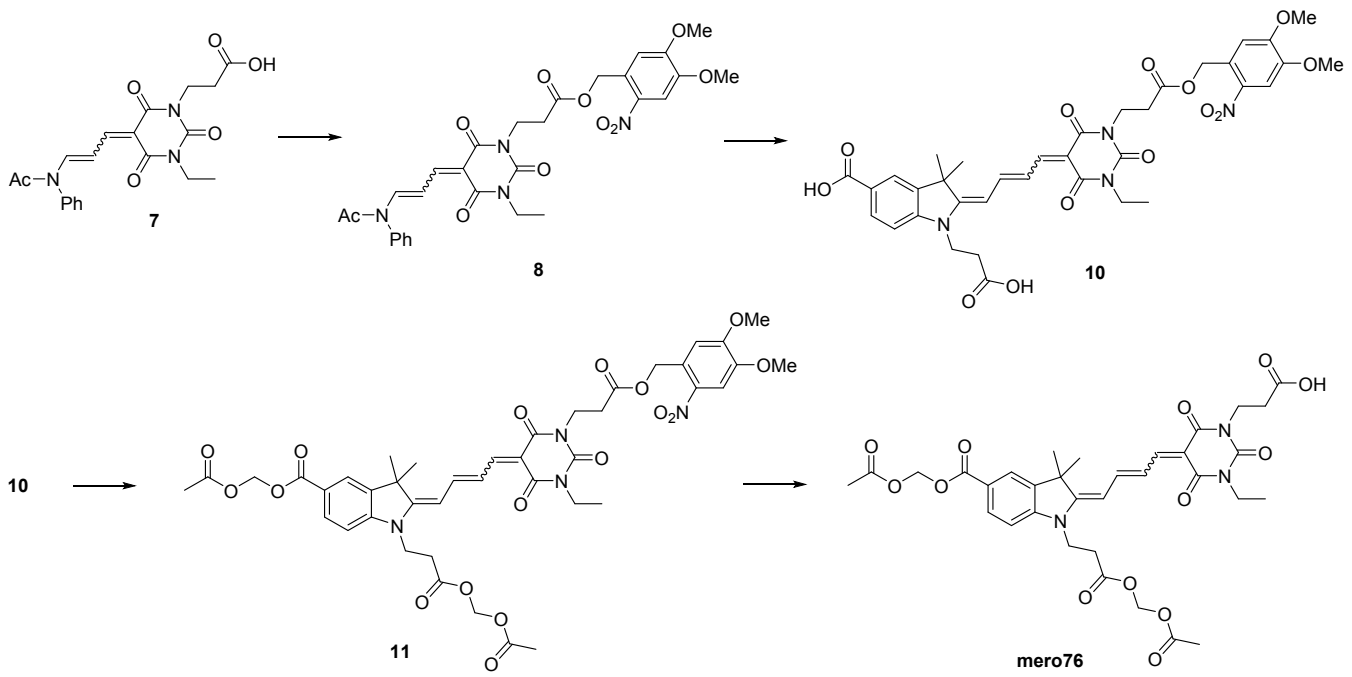
3-((E)-5-((2E,4E)-4-(1-(2-carboxyethyl)-3,3-dimethylindolin-2-ylidene)but-2-en-1-ylidene)-3-ethyl-2,4,6-trioxotetrahydropyrimidin-1(2H)-yl)propanoic acid (S5). 1-(2-Carboxyethyl)-2,3,3-trimethyl-3*H*-indolium bromide (47 mg, 0.150 mmol) compound **7** (90 mg, 0.225 mmol) were diluted in 1:1 $CHCl_3$/MeOH (1.5 mL) and NaOAc (18 mg, 0.225 mmol) was added in bulk. The reaction was stirred at room temp for 16 h and concentrated. The residue was diluted in CH_2Cl_2, prepared as a silica cake, and eluted on a 24 g silica column with 0 – 8% MeOH in CH_2Cl_2 over 25 min. Pure product containing fractions were combined and concentrated to give 27 mg (36%) dark blue solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (bs, 2H), 8.19 – 8.08 (m, 2H), 7.74 (t, J = 13.0 Hz, 0.5H), 7.73 (t, J = 13.0 Hz, 0.5H), 7.50 (d, J = 7.2 Hz, 1H), 7.33 (dt, J = 7.4, 1.2 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.14 (dt, J = 7.2, 1.2 Hz, 1H), 6.23 (d, J = 13.3 Hz, 1H), 4.23 (t, J = 7.2 Hz, 2H), 4.03 (t, J = 7.8 Hz, 2H), 3.84 (q, J = 7.2 Hz, 2H),

2.66 (t, J = 7.2 Hz, 2H), 2.47 (t, J = 7.6 Hz, 2H), 1.62 (s, 6H), 1.10 (t, J = 6.8 Hz, 1.5H), 1.10 (t, J = 6.8 Hz, 1.5H). MS-ESI *m/z* 496.2 ($[M + H]^+$ requires 496.2).

Acetoxymethyl 3-((E)-5-((2E,4E)-4-(1-(3-(acetoxymethoxy)-3-oxopropyl)-3,3-dimethylindolin-2-ylidene)but-2-en-1-ylidene)-3-ethyl-2,4,6-trioxotetrahydropyrimidin-1(2H)-yl)propanoate (4). Compound **S5** (14 mg, 0.028 mmol) was diluted in 1 mL anhydrous ACN and the flask was flushed with argon. Diisopropylethylamine (20 μL, 0.113 mmol) was added, followed after 5 min by addition of bromomethylacetate (11 μL, 0.113 mmol) and the reaction was stirred at room temperature for 16 h. The reaction was concentrated and the residue was redissolved in a minimum amount of CH_2Cl_2. The crude mixture was eluted on a 12 g silica column with 0 – 3% MeOH in CH_2Cl_2. The product was isolated as 16 mg (89%) dark violet solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 – 8.10 (m, 2H), 7.76 (t, J = 13.0 Hz, 0.5H), 7.74 (t, J = 13.0 Hz, 0.5H), 7.50 (d, J = 7.6 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 7.14 (t, J = 7.4 Hz, 1H), 6.23 (d, J = 13.2 Hz, 0.5H), 6.22 (d, J = 13.2 Hz, 0.5H), 5.66 (s, 2H), 5.63 (s, 2H), 4.27 (t, J = 6.8 Hz, 2H), 4.07 (t, J = 6.8 Hz, 2H), 3.84 (q, J = 7.2 Hz, 2H), 2.82 (t, J = 7.2 Hz, 2H), 2.62 (t, J = 7.2 Hz, 2H), 2.08 (s, 3H), 2.02 (s, 3H), 1.62 (s, 6H), 1.10 (t, J = 7.2 Hz, 1.5H), 1.10 (t, J = 6.8 Hz, 1.5H). . MS-ESI *m/z* 662.2 ($[M + Na]^+$ requires 662.2).

(E)-1-(2-carboxyethyl)-2-((E)-4-(1,3-diethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)but-2-en-1-ylidene)-3,3-dimethylindoline-5-carboxylic acid (S6). Compound **9** (0.178 g, 0.500 mmol) and NaOAc (0.051 g, 0.625 mmol) were diluted in 1:1 MeOH/$CHCl_3$ (3.0 mL) and 1,3-diethyl-5-(3-methoxyallylidene)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (0.173 g, 0.650 mmol) was added. The flask was fitted with a condenser and flushed with argon and the reaction was heated to reflux for 16 h. The reaction was concentrated and the residue was diluted in CH_2Cl_2, prepared as a silica cake, and eluted on a 24 g silica column with 0 – 8% MeOH in CH_2Cl_2 over 25 min. Pure product containing fractions were combined and concentrated to give 53 mg (21%) dark blue solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.61 (bs, 2H), 8.22 (d, J = 13.2 Hz, 1H), 8.13 (t, J = 13.4 Hz, 1H), 7.97 (s, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.79 (t, J = 13.0 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 4.20 (t, J = 6.8 Hz, 2H), 3.90 – 3.80 (m, 2H), 2.65 (t, J = 6.8 Hz, 2H), 1.63 (s, 6H), 1.11 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 168.9, 167.0, 162.0, 161.3, 156.4, 155.7, 150.6, 146.1, 140.2, 130.4, 125.0, 123.0, 122.6, 109.3, 101.5, 47.3, 35.9, 35.3, 31.2, 27.4, 13.3. MS-ESI *m/z* 493.9 ($[M - H]^-$ requires 494.2).

(E)-Acetoxymethyl 1-(3-(acetoxymethoxy)-3-oxopropyl)-2-((E)-4-(1,3-diethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)but-2-en-1-ylidene)-3,3-dimethylindoline-5-carboxylate (5). Compound **S6** (36 mg, 0.073 mmol) was diluted in 2.0 mL anhydrous ACN and the flask was flushed with argon. Diisopropylethylamine (51 μL, 0.291 mmol) was added, followed after 5 min by addition of bromomethylacetate (28 μL, 0.291 mmol) and the reaction was stirred at room temperature for 3 h. The reaction was concentrated and the residue was redissolved in a minimum amount of CH_2Cl_2. The crude mixture was eluted on a 12 g silica column with 0 – 3% MeOH in CH_2Cl_2. The product was isolated as 31 mg (67%) dark violet solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (d, J = 12.8 Hz, 1H), 8.14 (t, J = 13.2 Hz, 1H), 7.99 (d, J = 1.6 Hz, 1H), 7.93 (dd, J = 8.4, 1.6 Hz, 1H), 7.81 (t, J = 13.2 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.22 (d, J = 13.2 Hz, 1H), 5.92 (s, 2H), 5.62 (s, 2H), 4.23 (t, J = 6.8 Hz, 2H), 3.90 – 3.80 (m, 4H), 2.81 (t, J = 6.8 Hz, 2H), 2.10 (s, 3H), 2.01 (s, 3H), 1.64 (s, 6H), 1.15 – 1.08 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.6, 169.4, 169.1, 167.8, 164.2, 162.0, 161.3, 156.6, 155.2, 150.5, 147.2, 140.4, 130.9, 123.1, 122.2, 109.4, 105.9, 101.7, 79.6, 79.2, 47.0, 38.4, 35.9, 35.3, 30.8, 30.7, 27.3, 20.5, 20.4, 13.3. MS-ESI *m/z* 640.1 ($[M + H]^+$ requires 640.3).



Scheme S3. Synthesis of **mero76**.

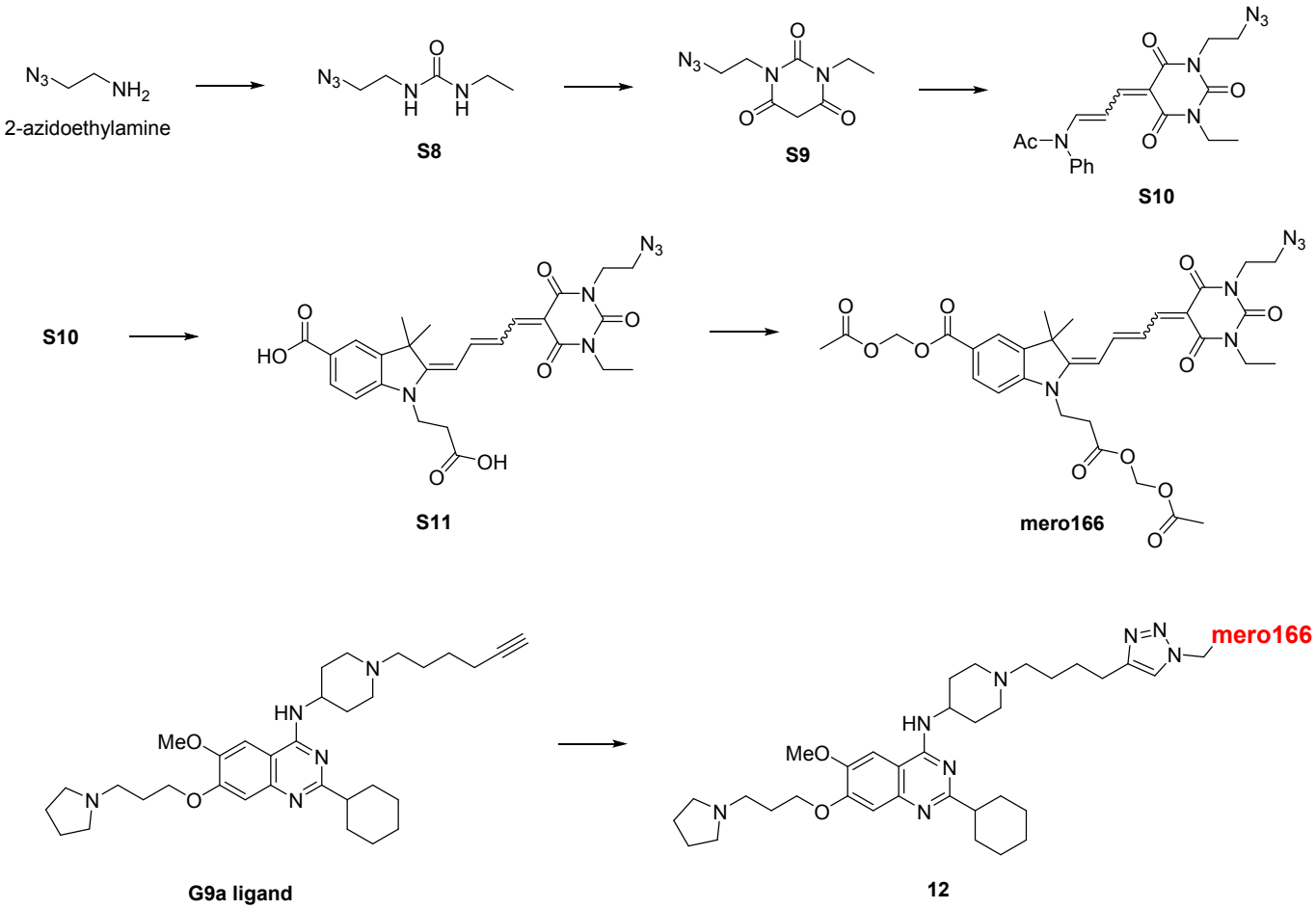
4,5-dimethoxy-2-nitrobenzyl 3-((E)-3-ethyl-2,4,6-trioxo-5-((E)-3-(N-phenylacetamido)allylidene) tetrahydropyrimidin-1(2H)-yl)propanoate (8). Compound **7** (0.399 g, 1.00 mmol), 4,5-dimethoxy-2-nitrobenzyl alcohol (0.213 g, 1.00 mmol), and DMAP (0.012 g, 0.100 mmol) were added to a flame-dried flask. The flask was sealed, evacuated, and argon flushed. Dichloromethane (12.5 mL) was added, followed by *N*,*N*'-dicyclohexylcarbodiimide. The reaction was stirred at room temperature under argon for 16 h. The reaction mixture was then filtered through a short pad of Celite. The filtrate was concentrated and filtered through a 0.45 μM nylon filter. This filtrate was loaded onto a 40 g silica column and the column was eluted with 0 – 50% EtOAc in hexanes. The product was isolated as 0.538 g (82%) dark orange foamy solid. Mixture of *E/Z* isomers (1:1 ratio). Isomer A: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 13.6 Hz, 1H), 8.16 (d, J = 12.8 Hz, 1H), 7.71 (s, 1H), 7.64 – 7.49 (m, 3H), 7.25 – 7.19 (m, 2H), 7.01 (s, 1H), 6.80 (t, J = 12.6 Hz, 1H), 5.45 (s, 2H), 4.17 (t, J = 7.4 Hz, 2H), 3.98 – 3.91 (m, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 2.67 (t, J = 7.4 Hz, 2H), 2.01 (s, 3H), 1.18 (t, J = 7.0 Hz, 3H). Isomer B: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 13.6 Hz, 1H), 8.13 (d, J = 12.8 Hz, 1H), 7.70 (s, 1H), 7.64 – 7.49 (m, 3H), 7.25 – 7.19 (m, 2H), 7.05 (s, 1H), 6.83 (t, J = 12.6 Hz, 1H), 5.50 (s, 2H), 4.28 (t, J = 7.4 Hz, 2H), 3.98 – 3.91 (m, 2H), 3.99 (s, 3H), 3.95 (s, 3H), 2.76 (t, J = 7.4 Hz, 2H), 2.01 (s, 3H), 1.10 (t, J = 7.0 Hz, 3H). MS-ESI m/z 595.0 ($[M + H]^+$ requires 595.2).

(E)-1-(2-carboxyethyl)-2-((2E,4E)-4-(1-(3-((4,5-dimethoxy-2-nitrobenzyl)oxy)-3-oxopropyl)-3-ethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)but-2-en-1-ylidene)-3,3-dimethylindoline-5-carboxylic acid (10). Compound **8** (1.784 g, 3.00 mmol), compound **9** (1.603 g, 4.50 mmol), and NaOAc (0.369 g, 4.50 mmol) were added to a flame dried flask and diluted in 1:1 MeOH/$CHCl_3$ (30 mL). The reaction was heated to reflux and stirred under argon for 16 h. The reaction mixture was concentrated with 10 g silica and the resulting cake was eluted on an 80 g silica column with 0 – 5% MeOH in CH_2Cl_2. The main product was isolated as 0.385 g (17%) dark amber foamy solid. ^{1}H NMR (400 MHz, DMSO-

d_6) δ 12.60 (bs, 2H), 8.22 – 8.07 (m, 2H), 7.97 (d, J = 1.6 Hz, 1H), 7.91 (dd, J = 8.4 Hz, 1.6 Hz, 1H), 7.81 – 7.69 (m, 1H), 7.68 (s, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 3.6 Hz, 1H), 6.28 – 6.16 (m, 1H), 5.37 (s, 2H), 4.21 (t, J = 6.6 Hz, 2H), 4.11 (dt, J = 7.0, 2.6 Hz, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.85 – 3.77 (m, 2H), 2.72 – 2.61 (m, 4H), 1.63 (s, 6H), 1.08 (t, J = 7.0 Hz, 3H). MS-ESI *m/z* 735.1 ($[M + H]^+$ requires 735.3).

(E)-acetoxymethyl 1-(3-(acetoxymethoxy)-3-oxopropyl)-2-((2E,4E)-4-(1-(3-((4,5-dimethoxy-2-nitrobenzyl)oxy)-3-oxopropyl)-3-ethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)but-2-en-1-ylidene)-3,3-dimethylindoline-5-carboxylate (11). Compound **10** (0.270 g, 0.367 mmol) was diluted in ACN (10 mL) and diisopropylethylamine (257 μL, 1.47 mmol) was added, followed by addition of bromomethyl acetate (144 μL, 1.47 mmol). The reaction was allowed to stir under argon for 16 h. Solvent was removed and the residue was redissolved in CH_2Cl_2 and prepared as a silica cake. Sample was eluted on a 24 g silica column with 0 – 75% EtOAc in hexanes over 25 min. Main product isolated as 0.163 g (51%) dark blue solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.02 (m, 2H), 7.92 (d, J = 1.5 Hz, 1H), 7.88 – 7.74 (m, 2H), 7.71 (d, J = 4.3 Hz, 1H), 7.08 (d, J = 8.6 Hz, 1H), 6.99 – 6.93 (m, 1H), 5.99 (s, 2H), 5.91 – 5.84 (m, 1H), 5.72 (d, J = 2.8 Hz, 2H), 5.54 (d, J = 3.6 Hz, 2H), 4.37 – 4.28 (m, 2H), 4.13 (q, J = 7.0 Hz, 2H), 4.02 (d, J = 4.4 Hz, 3H), 4.00 – 3.95 (m, 2H) 3.96 (d, J = 1.9 Hz, 3H), 2.84 – 2.76 (m, 4H), 2.14 (s, 3H), 2.09 (d, J = 4.7 Hz, 3H), 1.66 (s, 3H), 1.65 (s, 3H) 1.22 (t, J = 7.0 Hz, 3H). MS-ESI *m/z* 879.0 ($[M + H]^+$ requires 879.3).

Mero76. Compound **11** (132 mg, 0.150 mmol) was diluted in degassed ACN (75 mL). The flask was sealed, evacuated, and argon flushed. The reaction was irradiated at 365 nm for 24 h. The reaction mixture was concentrated and the residue was loaded in a minimum amount of CH_2Cl_2 onto a 12 g silica column and eluted with 0 – 5% MeOH in CH_2Cl_2. Isolated 88 mg (86%) desired product as a dark blue solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.36 (bs, 1H), 8.25 (d, J = 13.0 Hz, 0.5H), 8.25 (d, J = 13.0 Hz, 0.5H), 8.16 (t, J = 13.0 Hz, 1H), 7.99 (d, J = 1.0 Hz, 1H), 7.93 (dd, J = 8.5, 1.5 Hz, 1H), 7.81 (t, J = 13.0 Hz, 0.5H), 7.80 (t, J = 13.0 Hz, 0.5H), 7.29 (d, J = 8.5 Hz, 1H), 6.24 (d, J = 13.0 Hz, 1H), 5.92 (s, 2H), 5.62 (s, 2H), 4.23 (t, J = 6.8 Hz, 2H), 4.02 (t, J = 7.5 Hz, 2H), 3.88 – 3.80 (m, 2H), 2.81 (t, J = 7.0 Hz, 2H), 2.49 – 2.45 (m, 2H), 2.10 (s, 3H), 2.01 (s, 3H), 1.63 (s, 6H), 1.10 (t, J = 7.0 Hz, 3H). HRMS *m/z* 684.2408 ($[M + H]^+$ requires 684.2405).



Scheme S4. Synthesis of **mero166** and G9a ligand – **mero166** conjugate (**12**).

1-(2-Azidoethyl)-3-ethylurea (S8). 2-Azidoethylamine (2.15 g, 25.0 mmol) was diluted in THF (25 mL) in an oven dried flask under argon and the solution was cooled in a cold water bath. Ethylisocyanate (1.98 mL, 25.0 mmol) was added dropwise and the reaction was stirred for 16 h. The reaction was concentrated and all residual solvents were removed under high vacuum to give 3.02 g (77%) off-white solid suitable for use without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.53 (bs, 1H), 5.26 (bs, 1H), 3.42 – 3.31 (m, 4H), 3.22 – 3.14 (m, 2H), 1.11 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.8, 52.0, 39.9, 35.3, 15.6. MS-ESI *m/z* 158.0 ($[M + H]^+$ requires 158.1).

1-(2-Azidoethyl)-3-ethylpyrimidine-2,4,6(1H,3H,5H)-trione (S9). Compound **S8** (1.57 g, 10.0 mmol) and malonic acid (1.09 g, 10.5 mmol) were added to a flame dried flask and the flask was sealed, evacuated, and argon flushed. AcOH (14 mL) was added and the flask was heated to 60 °C for 10 min. Acetic anhydride (3.8 mL, 40.0 mmol) was added and the reaction was further heated to 95 °C and stirred for 1 h. The reaction was then concentrated and the residue was loaded in a minimum amount of CH_2Cl_2 onto a silica column and eluted with 0 – 2% MeOH in CH_2Cl_2. Main product containing fractions were combined and concentrated to give a pale amber oil that became a waxy solid on cooling. Isolated 1.86 g

(83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (t, *J* = 6.0 Hz, 2H), 3.95 (q, *J* = 7.2 Hz, 2H), 3.70 (s, 2H), 3.52 (t, *J* = 6.0 Hz, 2H), 1.22 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 164.2, 151.3, 48.8, 40.5, 39.8, 37.7, 13.4. MS-ESI *m/z* 223.9 ($[M - H]^-$ requires 224.1).

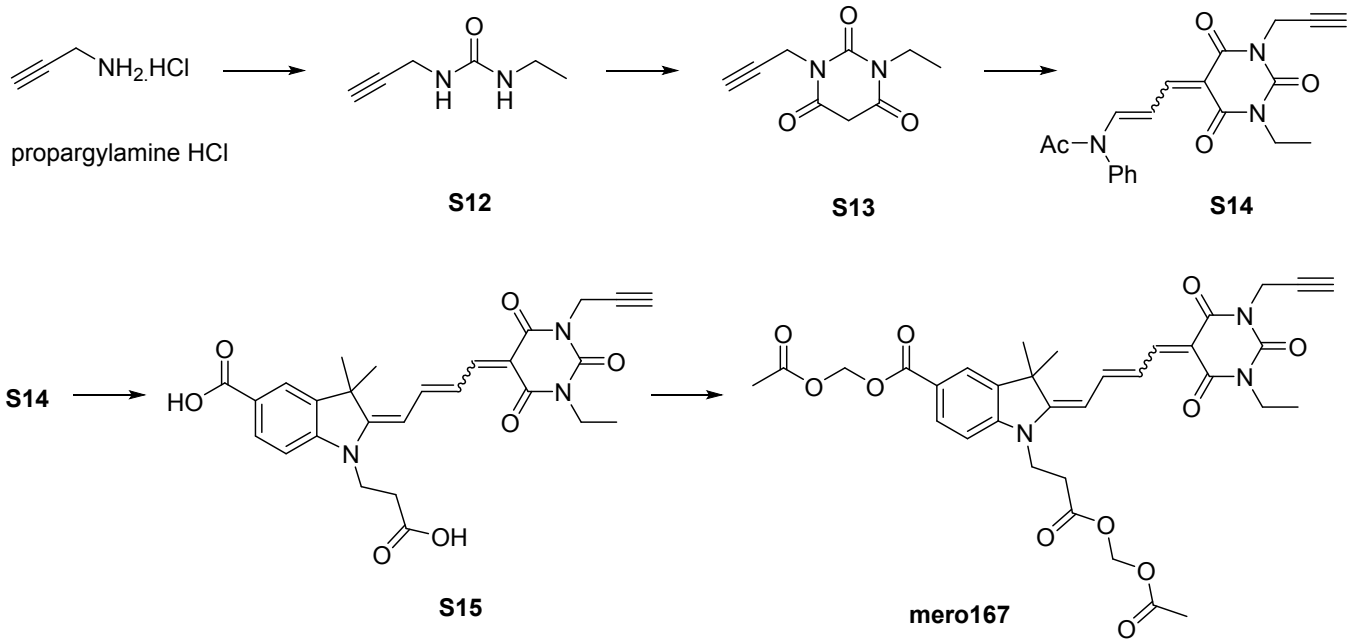
***N*-((1E,3E)-3-(1-(2-Azidoethyl)-3-ethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)prop-1-en-1-yl)-*N*-phenylacetamide (S10)**. Compound **S9** (1.126 g, 5.00 mmol), malonaldehyde dianilide hydrochloride (1.423 g, 5.50 mmol), and sodium acetate (0.451 g, 5.50 mmol) were diluted in acetic anhydride (6.0 mL). The reaction mixture was added to a pre-heated oil bath at 100 °C and stirred for 1 h. The reaction was cooled and added in portions to saturated aq. $NaHCO_3$ (50 mL). The aq. layer was extracted with EtOAc (3 X 50 mL). The organic layers were combined, washed with brine, dried with $MgSO_4$, filtered, and concentrated. The residue was diluted in CH_2Cl_2 and concentrated with Celite. The Celite cake was eluted on an 80 g silica column with 0 – 50% EtOAc in hexanes over 25 min. The product was isolated as 0.479 g (24%) yellow solid as an approximate 1:1 mixture of *E* and *Z* isomers. Isomer A: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 13.6 Hz, 1H), 8.19 (d, *J* = 12.8 Hz, 1H), 7.64 – 7.54 (m, 3H), 7.26 – 7.22 (m, 2H), 6.86 (t, *J* = 13.2 Hz, 1H), 4.17 (t, *J* = 6.2 Hz, 2H), 3.87 (q, *J* = 7.2 Hz, 2H), 3.52 (t, *J* = 6.0 Hz, 2H), 2.01 (s, 3H), 1.13 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 162.1, 161.4, 158.8, 151.1, 150.6, 138.0, 131.0 (2C), 130.4, 128.1 (2C), 111.6, 110.5, 49.0, 40.5, 36.6, 23.6, 13.5. Isomer B: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 13.6 Hz, 1H), 8.19 (d, *J* = 12.4 Hz, 1H), 7.64 – 7.54 (m, 3H), 7.26 – 7.22 (m, 2H), 6.83 (t, *J* = 13.0 Hz, 1H), 4.04 (t, *J* = 6.4 Hz, 2H), 3.98 (q, *J* = 7.2 Hz, 2H), 3.43 (t, *J* = 6.2 Hz, 2H), 2.01 (s, 3H), 1.21 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 162.5, 161.7, 158.8, 151.1, 150.5, 138.0, 131.0 (2C), 130.4, 128.1 (2C), 111.6, 110.6, 48.9, 39.7, 37.4, 23.7, 13.5. MS-ESI *m/z* 418.4 ($[M + Na]^+$ requires 419.1).

(E)-2-((2E,4E)-4-(1-(2-Azidoethyl)-3-ethyl-2,4,6-trioxotetrahydropyrimidin-5(2H)-ylidene)but-2-en-1-ylidene)-1-(2-carboxyethyl)-3,3-dimethylindoline-5-carboxylic acid (S11). Compound **S10** (0.458 g, 1.155 mmol), sodium acetate (0.142 g, 1.733 mmol), and compound **9** (0.617 g, 1.733 mmol) were diluted in 1:1 MeOH/$CHCl_3$ (7.5 mL). The reaction flask was topped with a condenser with argon inlet and heated to reflux for 16 h. The reaction was concentrated and re-diluted in EtOAc and 1 M aq. NaOH (25 mL) was added. The aq. layer was washed with EtOAc (3 X 25 mL), cooled in an ice bath, and acidified with 2 M HCl. The aq. layer was then extracted with CH_2Cl_2. The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated. The residue was prepared as a Celite cake and eluted on a 40 g silica column with 0 – 5% MeOH in CH_2Cl_2. Main product isolated as 0.134 g (22%) dark blue solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (bs, 2H), 8.25 (d, *J* = 13.2 Hz, 1H), 8.18 (t, *J* = 13.2 Hz, 1H), 7.98 (s, 1H), 7.92 (d, *J* = 8.8 Hz, 1H), 7.85 – 7.74 (m, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 6.31 – 6.21 (m, 1H), 4.30 – 4.15 (m, 2H), 4.10 – 4.00 (m, 2H), 3.92 – 3.78 (m, 2H), 3.49 (t, *J* = 5.8 Hz, 2H), 2.66 (t, *J* = 6.8 Hz, 2H), 1.64 (s, 6H), 1.11 (t, *J* = 6.8 Hz, 3H). MS-ESI *m/z* 537.0 ($[M + H]^+$ requires 537.2).

Mero166. Compound **S11** (56 mg, 0.094 mmol) was diluted in ACN (3.0 mL) and bromomethyl acetate (37 μL, 0.38 mmol) was added, followed by addition of *N,N*-diisopropylethylamine (66 μL, 0.38 mmol). The flask was capped and fitted with an argon inlet line and the reaction was stirred at room temp. for 16 h. The reaction mixture was then concentrated and re-diluted in CH_2Cl_2 and prepared as a Celite cake. The sample was eluted on a 12 g silica column with 0 – 50% EtOAc in hexanes over 25 min.

Isolated 37 mg (52%) dark blue solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (d, *J* = 13.0 Hz, 1H), 8.19 (t, *J* = 13.0 Hz, 0.5H), 8.18 (t, *J* = 13.0 Hz, 0.5H), 8.00 (d, *J* = 1.5 Hz, 1H), 7.94 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.82 (t, *J* = 13.0 Hz, 0.5H), 7.80 (t, *J* = 13.0 Hz, 0.5H), 7.31 (d, *J* = 8.5 Hz, 1H), 6.27 (d, *J* = 13.0 Hz, 0.5H), 6.26 (d, *J* = 13.0 Hz, 0.5H), 5.92 (s, 2H), 5.62 (s, 2H), 4.24 (t, *J* = 6.8 Hz, 2H), 4.10 – 4.00 (m, 2H), 3.91 – 3.80 (m, 2H), 3.49 (t, *J* = 6.3 Hz, 2H), 2.81 (t, *J* = 6.8 Hz, 2H), 2.10 (s, 3H), 2.01 (s, 3H), 1.64 (s, 6H), 1.11 (t, *J* = 7.0 Hz, 1.5H), 1.11 (t, *J* = 7.0 Hz, 1.5H). HRMS *m/z* 681.2539 ($[M + H]^+$ requires 681.2520).

G9a ligand – mero166 conjugate (12). **Mero166** (5.1 mg, 7.5 μmol) and **G9a ligand** (6.2 mg, 11 μmol) were diluted in 500 μL CH_2Cl_2. A 15 mM stock solution of tetrakis(acetonitrile)copper(I) hexafluorophosphate was prepared in CH_2Cl_2 and 100 μL was added to the reaction mixture dropwise. After 24 h, the reaction was concentrated and submitted to preparative LC. The product was isolated after lyophilization as 6.8 mg (74%) dark blue solid. MS-ESI *m/z* 1228.2 ($[M + H]^+$ requires 1228.6).



Scheme S5. Synthesis route to **mero167**.

1-ethyl-3-(prop-2-yn-1-yl)urea (S12). Propargylamine hydrochloride (0.976 g, 10.92 mmol) was diluted in THF (10 mL) in an oven dried flask under argon. Triethylamine (1.6 mL, 11.47 mmol) was added dropwise and the solution was cooled in a cold water bath. Ethylisocyanate (0.86 mL, 10.92 mmol) was then added dropwise and the reaction was stirred for 16 h. The solution was combined with half saturated aq. NH_4Cl (15 mL) and diluted with CH_2Cl_2 (15 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 15 mL). Organic layers were combined, washed with brine, dried with $MgSO_4$, filtered, concentrated, and dried under vacuum. Isolated 0.761 g (57%) white solid that required no further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38 (t, J = 5.9 Hz, 1H), 5.19 (t, J = 5.9 Hz, 1H), 3.97 (dd, J = 5.7 Hz, 2H), 3.21 (dq, J = 7.3 Hz, 2H), 2.19 (t, J = 2.7 Hz, 1H), 1.12 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.4, 81.2, 71.0, 35.4, 30.2, 15.6. MS-ESI *m/z* 127.10 ($[M + H]^+$ requires 127.09).

1-ethyl-3-(prop-2-yn-1-yl)pyrimidine-2,4,6(1H,3H,5H)-trione (S13). Compound **S12** (0.708 g, 5.61 mmol) and malonic acid (0.61 g, 5.89 mmol) were added to a flame dried flask and the flask was sealed, evacuated, and argon flushed. Acetic acid (8.0 mL) was added and the flask was heated to 60 °C for 10 min. Acetic anhydride (2.12 mL, 22.4 mmol) was added and the reaction was further heated to 95 °C and stirred for 1 h. The reaction mixture was then concentrated and the residue was loaded in a minimum amount of CH_2Cl_2 onto a 24 g silica column and eluted with 0 – 2% MeOH in CH_2Cl_2. Main product containing fractions were combined and concentrated to give 0.852 g (78%) amber oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.64 (d, J = 2.7, 2H), 3.96 (q, J = 7.0, 2H), 3.70 (s, 2H), 2.22 (t, J = 2.6 Hz, 1H), 1.22 (t, J = 7.0, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 163.9, 150.6, 77.6, 71.8, 39.8, 37.7, 31.2, 13.4. MS-ESI *m/z* 195.17 ($[M + H]^+$ requires 195.08).

***N*-((1*E*,3*E*)-3-(1-ethyl-2,4,6-trioxo-3-(prop-2-yn-1-yl)tetrahydropyrimidin-5(2*H*)-ylidene)prop-1-en-1-yl)-*N*-phenylacetamide (S14).** Compound **S13** (0.84 g, 4.31 mmol), malonaldehyde dianilide hydrochloride (1.41 g, 6.46 mmol), and sodium acetate (0.40 g, 6.46 mmol) were diluted in acetic anhydride (7.0 mL). The reaction mixture was added to a pre-heated oil bath at 100 °C and stirred for 1 h. The reaction was cooled and added in portions to saturated aq. $NaHCO_3$ (50 mL). The aq. layer was extracted with EtOAc (3 X 50 mL). The organic layers were combined, washed with brine, dried with $NaSO_4$, filtered, and concentrated. The residue was diluted in CH_2Cl_2 and concentrated with Celite. The Celite cake was eluted on an 80 g silica column with 0 – 50% EtOAc in hexanes over 30 min. The product was isolated as 0.928 g (59%) yellow solid as an approximate 1:1 mixture of *E* and *Z* isomers. Isomer A: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 13.6 Hz, 1H) 8.20 (d, *J* = 12.4 Hz, 1H), 7.65 – 7.53 (m, 3H), 7.26 – 7.20 (m, 2H), 6.86 (dd, *J* = 12.6 Hz, 1H), 4.58 (d, *J* = 2.8 Hz, 2H), 3.99 (q, *J* = 7.2 Hz, 2H), 2.18 (t, *J* = 2.4 Hz, 1H), 2.00 (s, 3H), 1.21 (t, *J* = 7.2 Hz, 3H), Isomer B: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (d, *J* = 14.0 Hz, 1H), 8.20 (d, *J* = 12.4 Hz, 1H), 7.65 – 7.53 (m, 3H), 7.26 – 7.20 (m, 2H), 6.85 (dd, *J* = 12.4 Hz, 1H), 4.69 (d, *J* = 2.8 Hz, 2H), 3.87 (q, *J* = 7.2 Hz, 2H), 2.14 (t, *J* = 2.6 Hz, 1H), 2.01 (s, 3H), 1.13 (t, *J* = 7.0 Hz, 3H). MS-ESI *m/z* 388.26 ([M + Na]$^+$ requires 388.13).

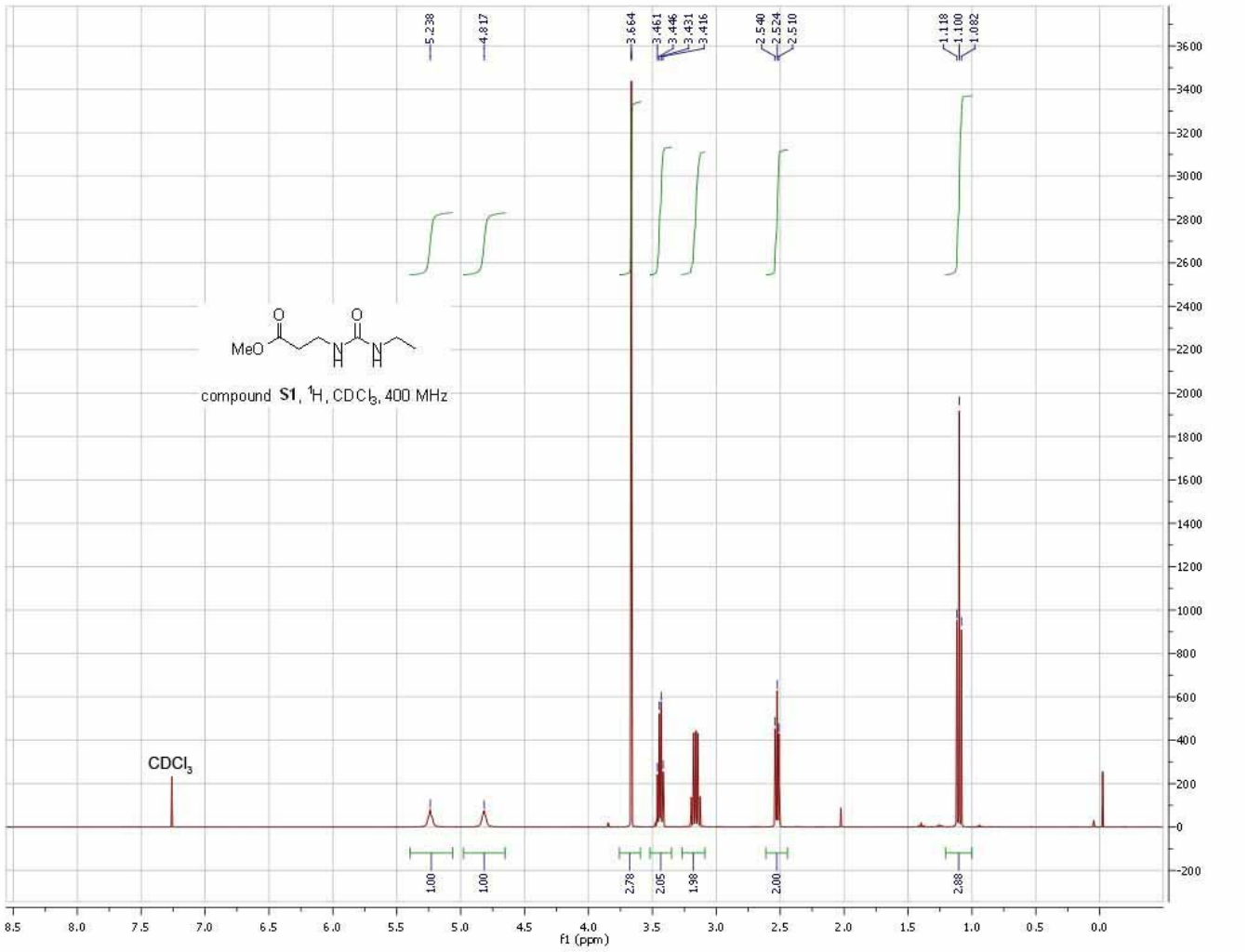
(*E*)-1-(2-carboxyethyl)-2-((2*E*,4*E*)-4-(1-ethyl-2,4,6-trioxo-3-(prop-2-yn-1-yl)tetrahydropyrimidin-5(2*H*)-ylidene)but-2-en-1-ylidene)-3,3-dimethylindoline-5-carboxylic acid (S15). Compound **S14** (0.868 g, 2.38 mmol), sodium acetate (0.293 g, 3.57 mmol), and compound **9** (1.272 g, 3.57 mmol) were diluted in 1:1 MeOH/$CHCl_3$ (15 mL). The reaction flask was topped with a condenser with argon inlet and heated to reflux for 16 h. The reaction was concentrated and re-diluted in EtOAc and minimal amount of MeOH. The residue was prepared as a Celite cake and eluted on a 40 g silica column with 0 – 5% MeOH in CH_2Cl_2 over 36 min. Main product isolated as 0.352 g (29%) dark blue solid. δ ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (bs, 2H), 8.29 – 8.14 (m, 2H), 7.99 (d, *J* = 1.6 Hz, 1H), 7.92 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.83 – 7.73 (m, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 6.27 (d, *J* = 13.2 Hz, 1H), 4.55 (s, 2H), 4.23 (t, *J* = 6.8 Hz 2H), 3.90 – 3.80 (m, 2H), 3.11 – 3.08 (m, 1H), 2.66 (t, *J* = 7.1 Hz, 2H), 1.64 (s, 6H), 1.12 – 1.10 (m, 3H). MS-ESI *m/z* 528.38 ([M + Na]$^+$ requires 528.17).

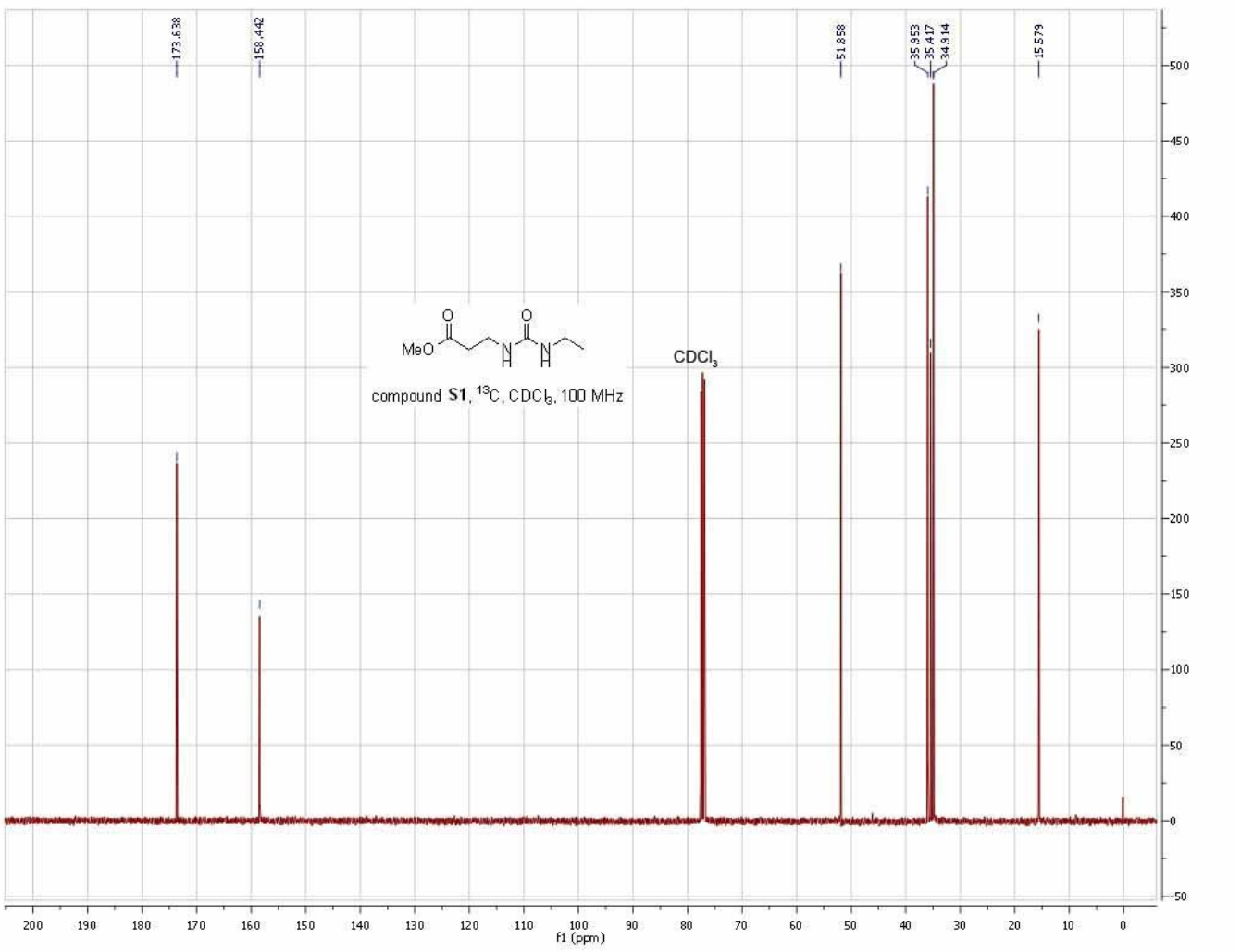
Acetoxymethyl (*E*)-1-(3-(acetoxymethoxy)-3-oxopropyl)-2-((2*E*,4*E*)-4-(1-ethyl-2,4,6-trioxo-3-(prop-2-yn-1-yl)tetrahydropyrimidin-5(2*H*)-ylidene)but-2-en-1-ylidine)-3,3-dimethylindoline-5-carboxylate (mero167). Compound **S15** (0.323 g, 0.638 mmol) was diluted in ACN (35 mL) and bromomethyl acetate (0.27 mL, 2.55 mmol) was added, followed by addition of *N,N*-diisopropylethylamine (0.49 mL, 2.55 mmol). The flask was capped and fitted with an argon inlet line and the reaction was stirred at room temperature for 16 h. The reaction mixture was then concentrated, re-diluted in CH_2Cl_2, and prepared as a Celite cake. The sample was eluted on a 24 g silica column with 0 – 50% EtOAc in hexanes over 25 min. Isolated 0.292 g (71%) dark blue solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (d, *J* = 13.0 Hz, 0.5H), 8.27 (d, *J* = 13.0 Hz, 0.5H), 8.21 (t, *J* = 13.0 Hz, 1H), 8.01 (d, *J* = 1.0 Hz, 1H), 7.94 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.82 (t, *J* = 13.3 Hz, 0.5H), 7.80 (t, *J* = 13.0 Hz, 0.5H), 7.32 (d, *J* = 8.5 Hz, 1H), 6.28 (d, *J* = 13.0 Hz, 1H), 5.92 (s, 2H), 5.62 (s, 2H), 4.55 (t, *J* = 2.5 Hz, 2H), 4.25 (t, *J* = 6.8 Hz, 2H), 3.90 – 3.80 (m, 2H), 3.14 – 3.10 (m, 1H), 2.82 (t, *J* = 7.0 Hz, 2H), 2.10 (s, 3H), 2.01 (s,

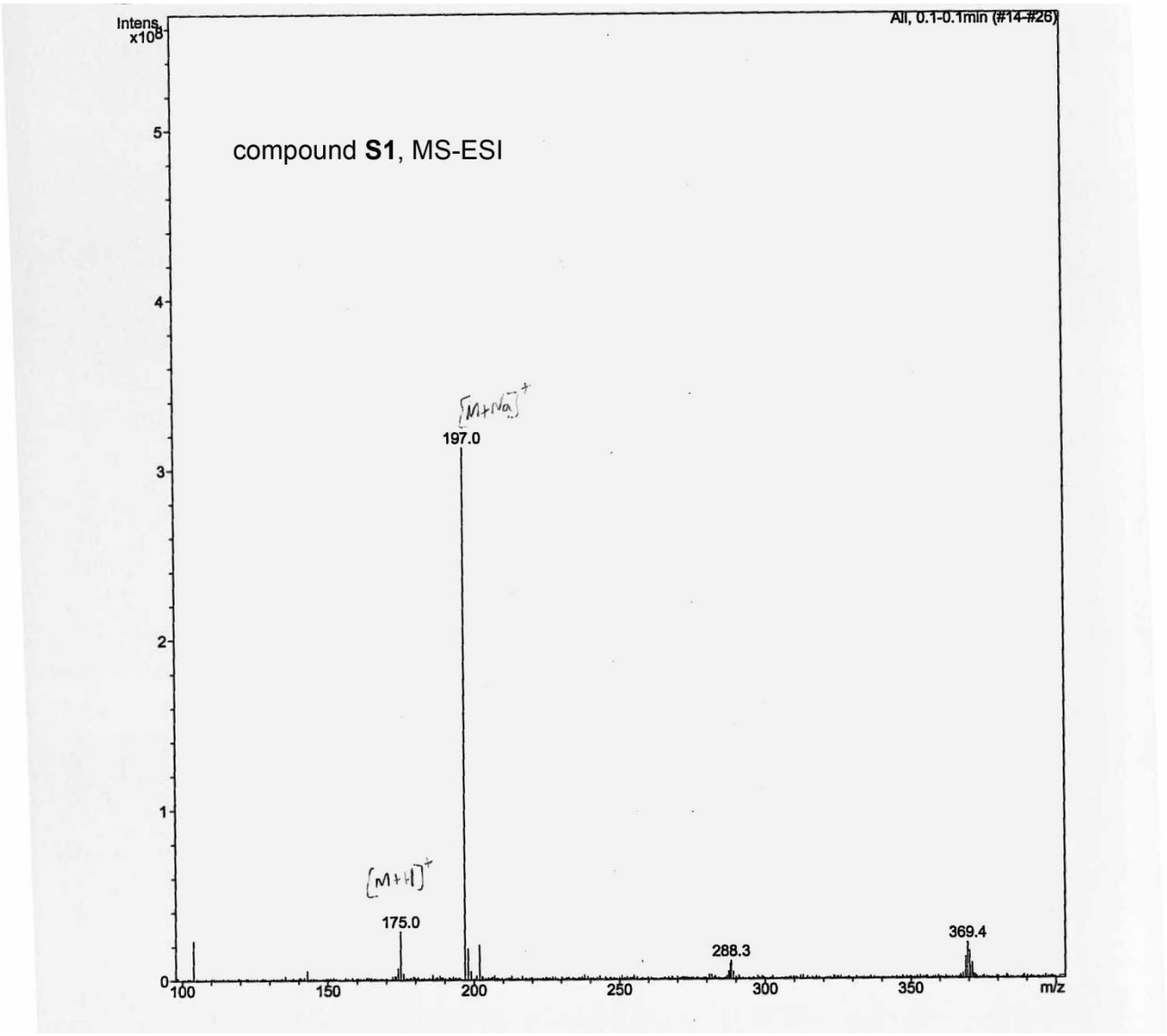
3H), 1.64 (s, 6H), 1.12 (t, J = 7.0 Hz, 1.5H), 1.11 (t, J = 7.0 Hz, 1.5H). MS-ESI *m/z* 672.40 ($[M + Na]^+$ requires 672.22).

SPECTRA OF COMPOUNDS

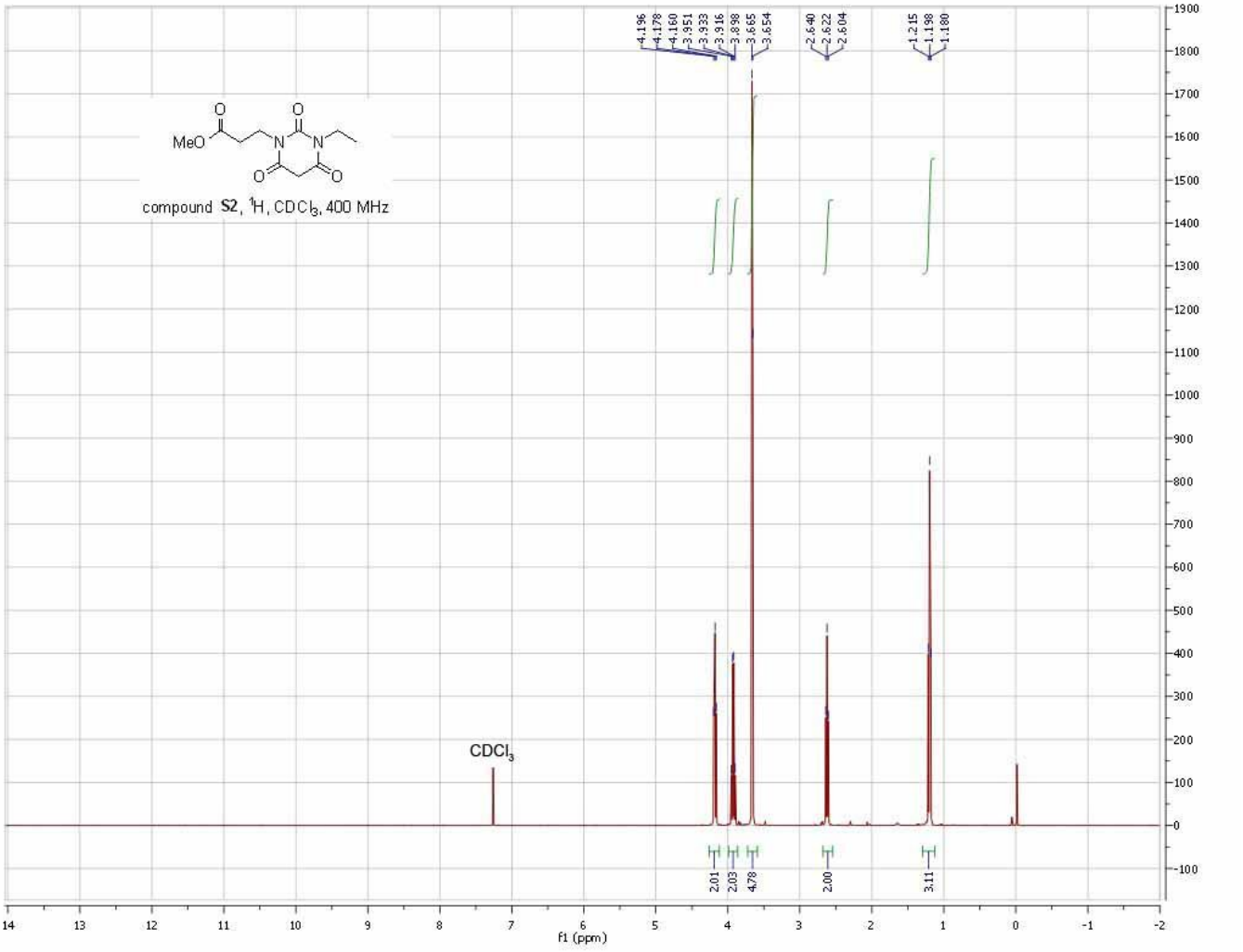
Compound **S1**.




173.638
158.442
51.858
35.953
35.417
34.914
15.579
CDCl3
MeO
compound S1, 13C, CDCl3, 100 MHz
f1 (ppm)


compound **S1**, MS-ESI
Intens.
x10^8
All, 0.1-0.1min (#14-#26)
5
4
3
2
1
0
[M+Na]+
197.0
[M+H]+
175.0
288.3
369.4
100
150
200
250
300
350
m/z

Compound **S2**.


compound S2, ^{1}H, $CDCl_3$, 400 MHz
$CDCl_3$
f1 (ppm)
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4.178
4.160
3.951
3.933
3.916
3.838
3.665
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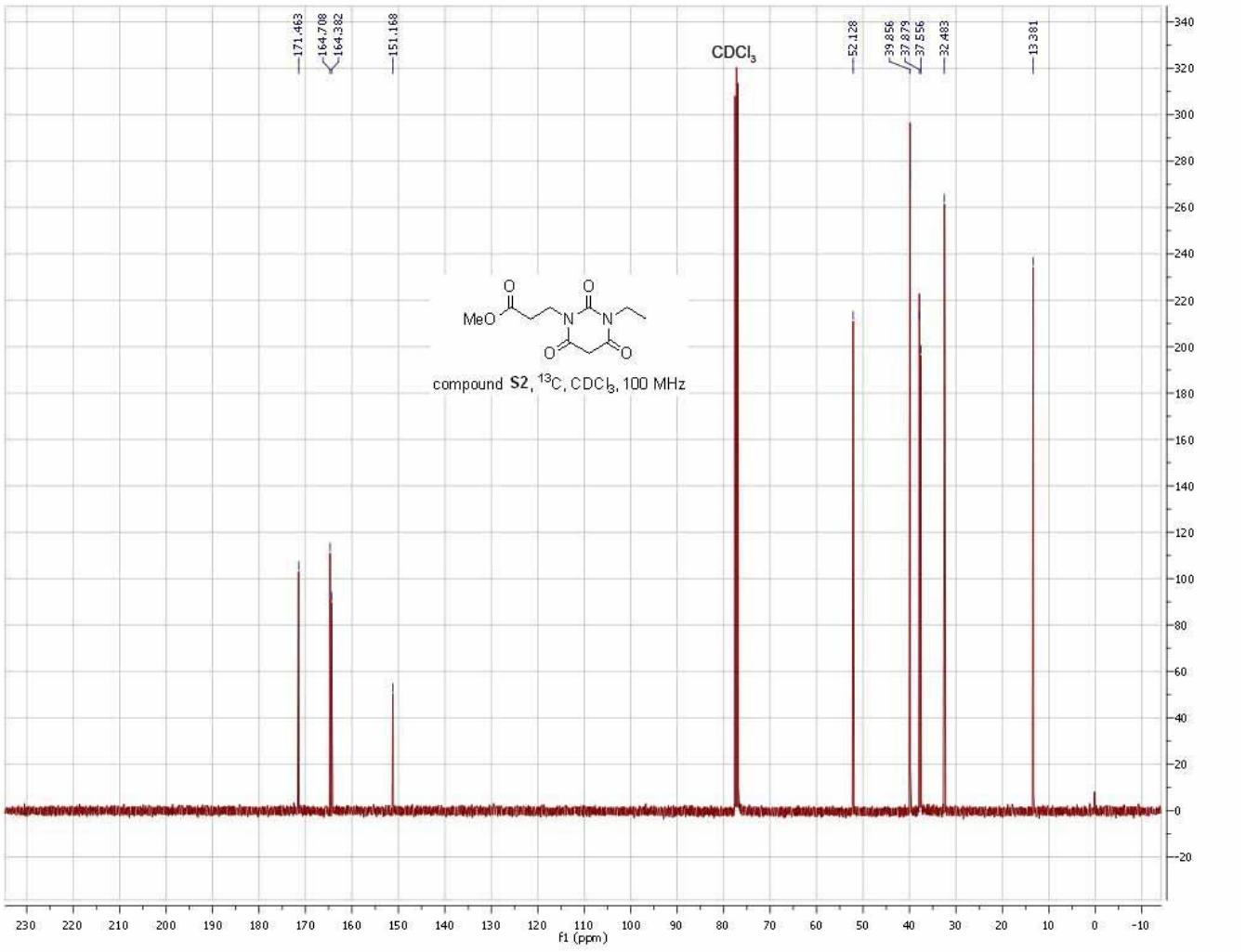
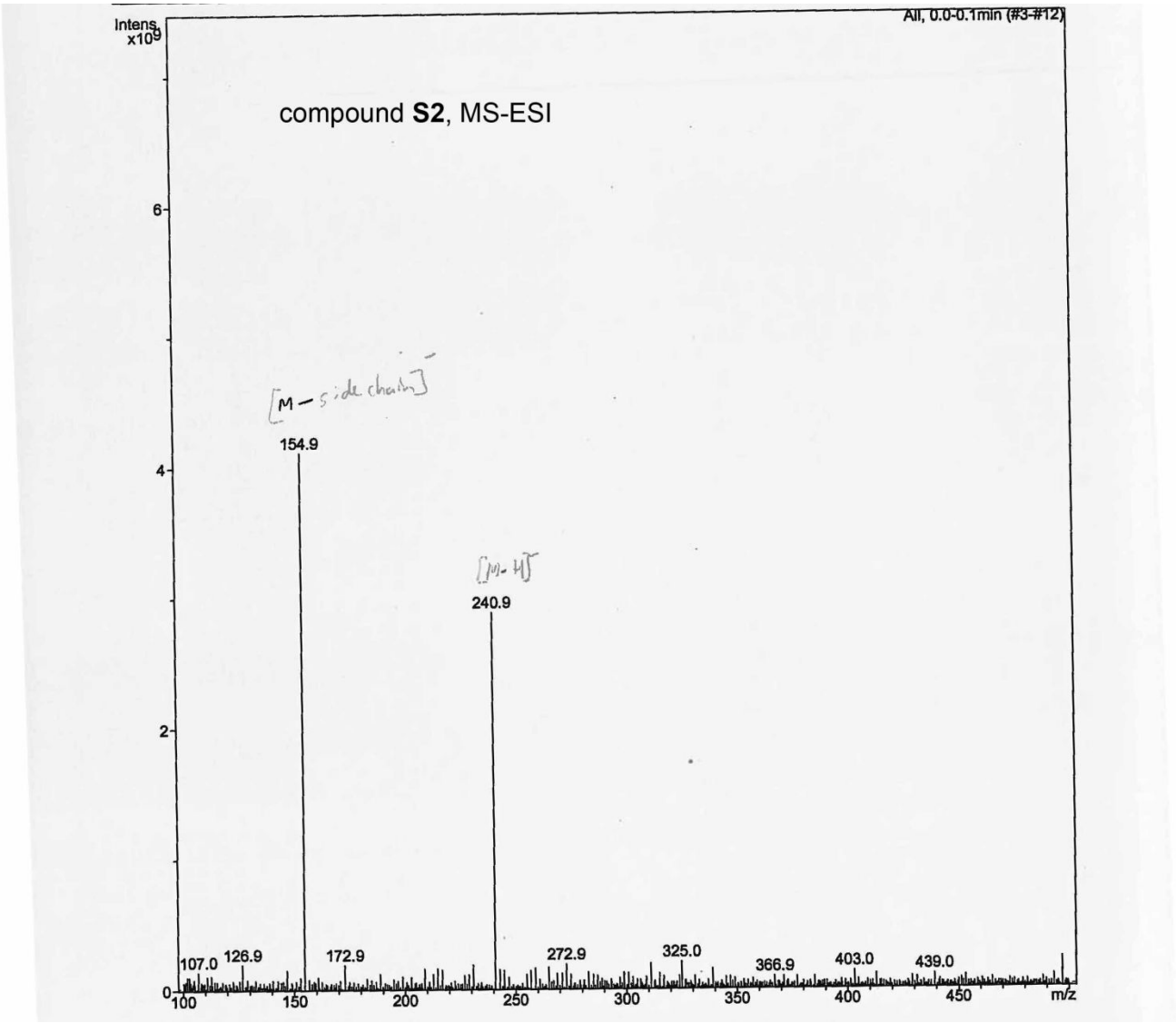
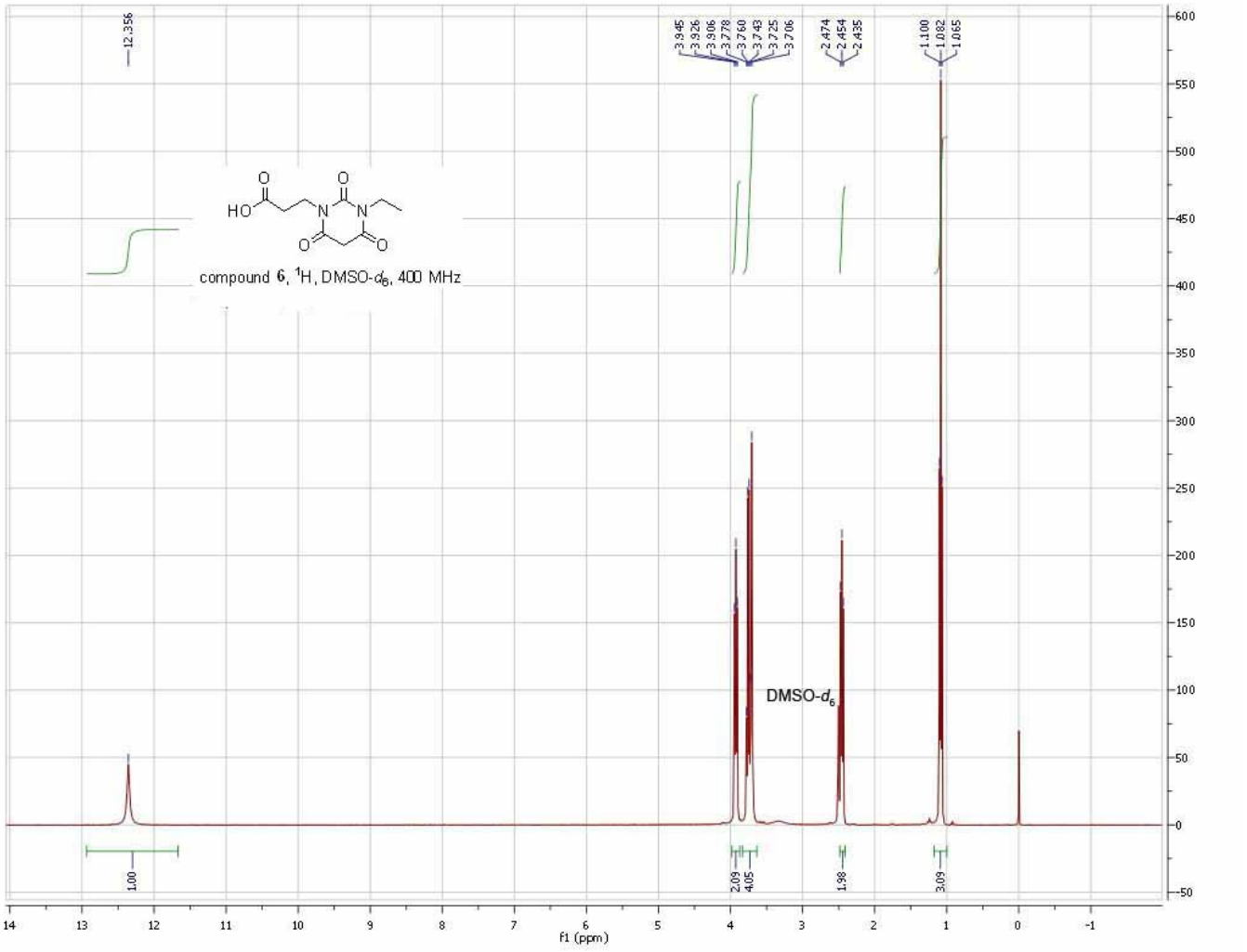
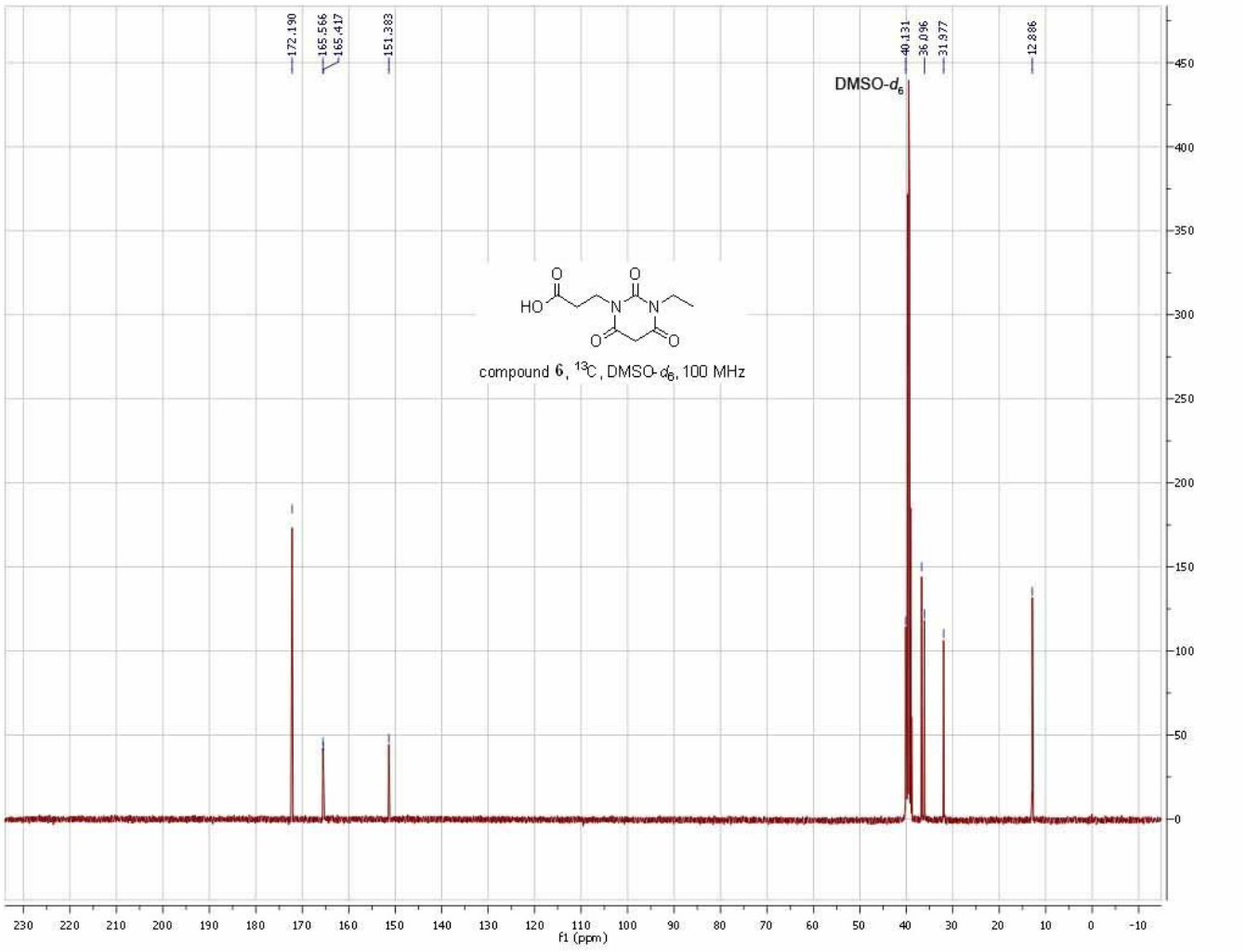
1.180
2.01
2.03
4.78
2.00
3.11

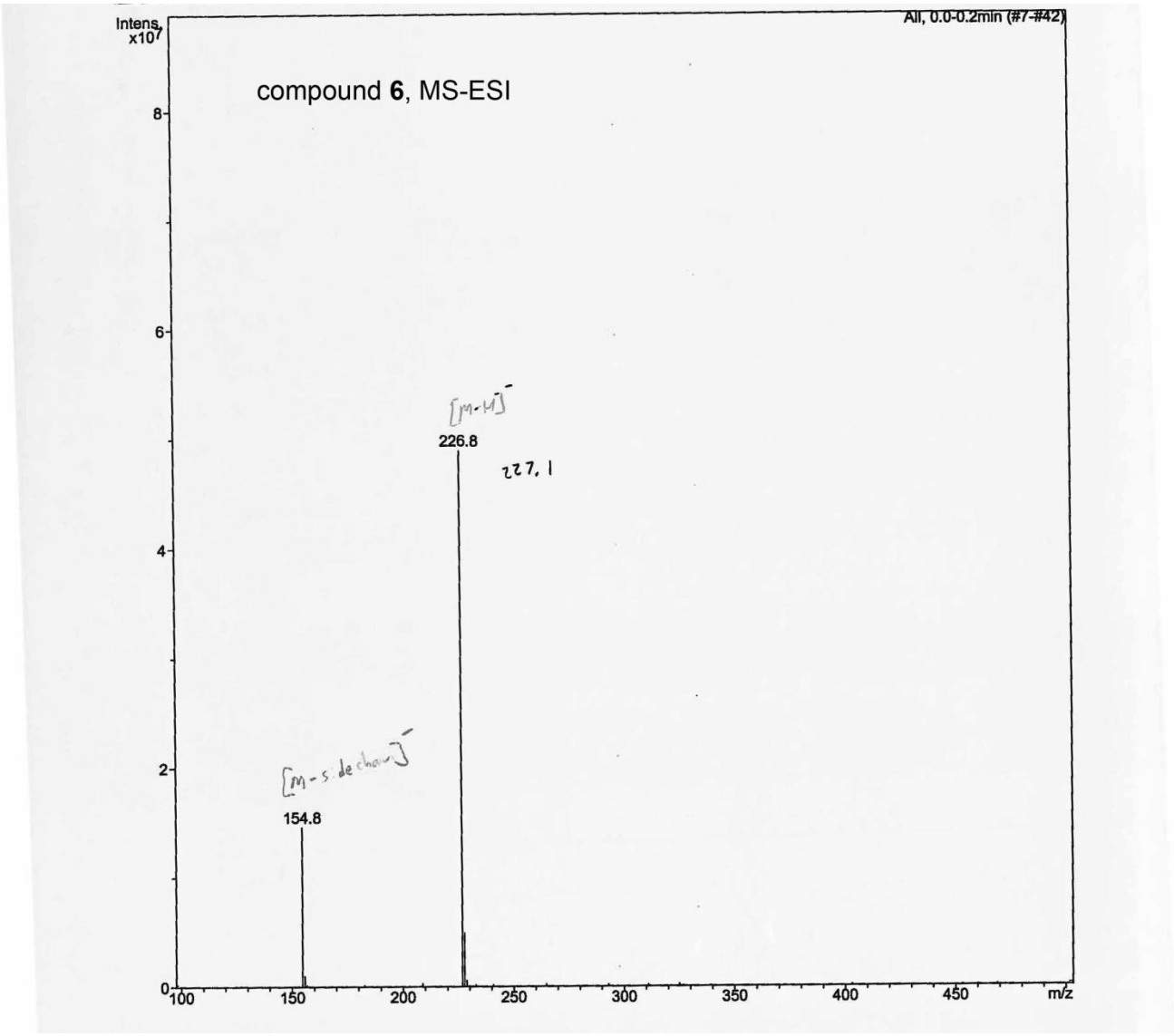

171.463
164.708
164.382
151.168
CDCl3
52.128
39.856
37.879
37.556
32.483
13.381
MeO
compound S2, 13C, CDCl3, 100 MHz
f1 (ppm)


compound **S2**, MS-ESI
Intens
x10^9
All, 0.0-0.1min (#3-#12)
6
4
2
0
[M − side chain]⁻
154.9
[M-H]⁻
240.9
107.0
126.9
172.9
272.9
325.0
366.9
403.0
439.0
100
150
200
250
300
350
400
450
m/z

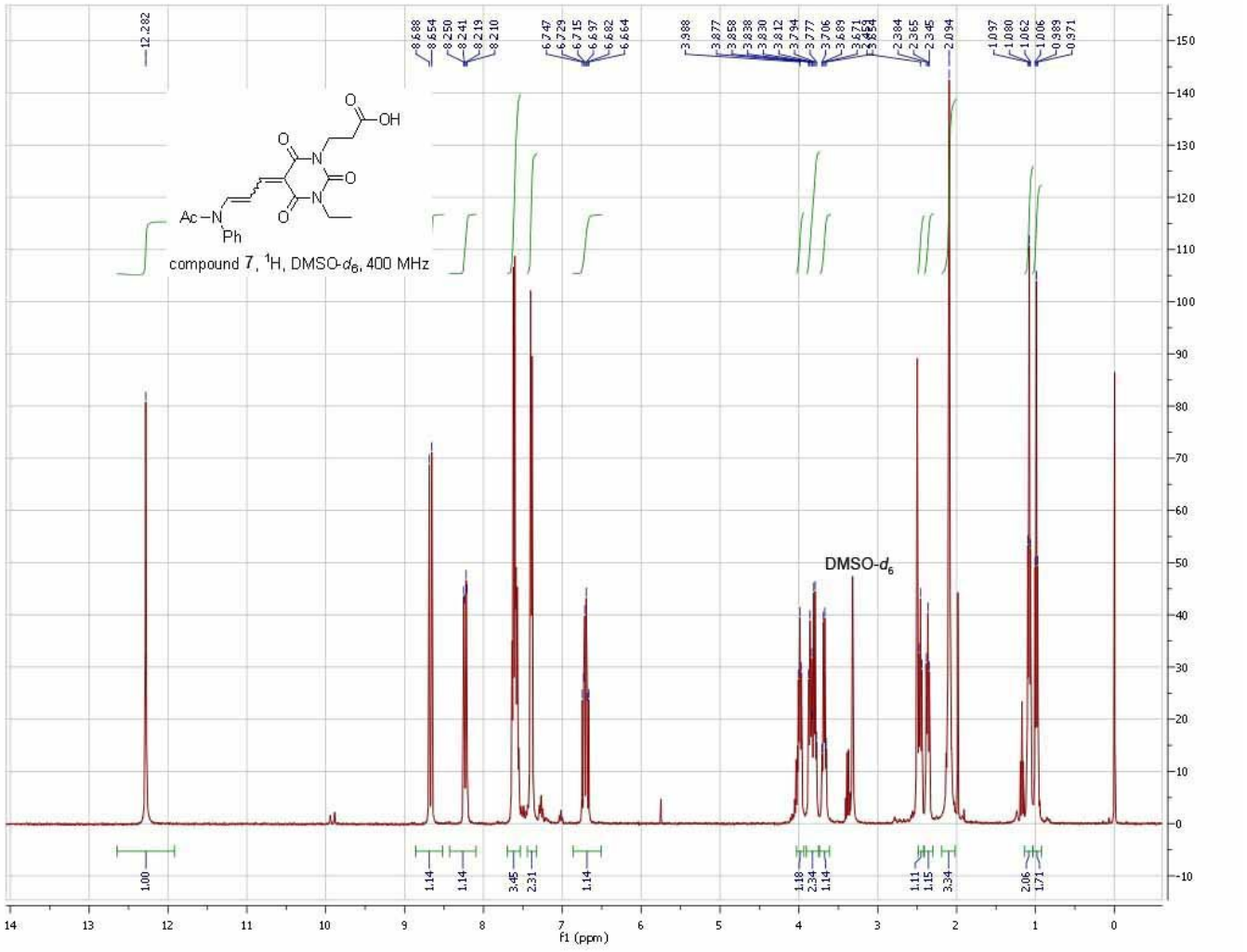
Compound **6**.

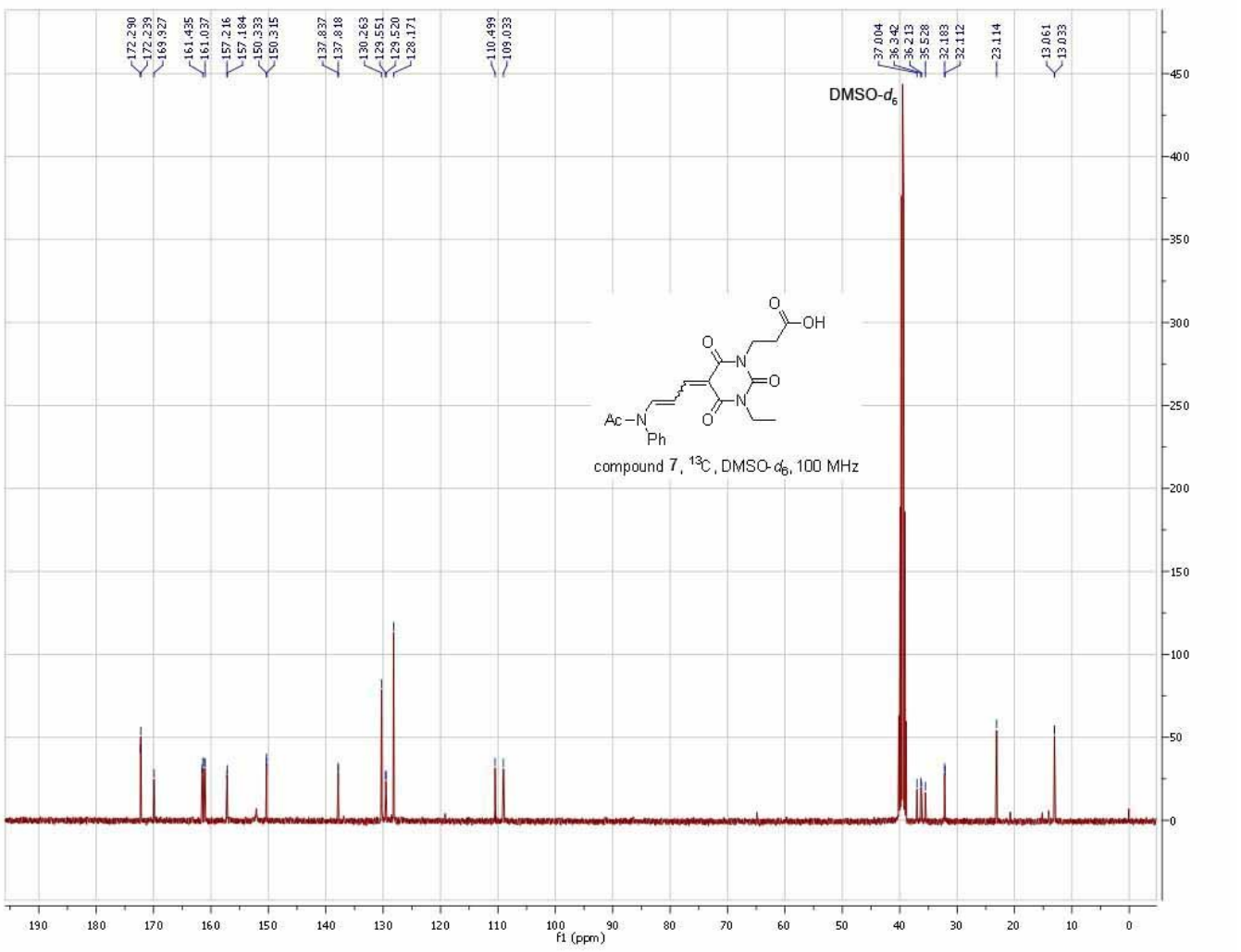

12.356
3.945
3.926
3.906
3.778
3.760
3.743
3.725
3.706
2.474
2.454
2.435
1.100
1.082
1.065
compound 6, 1H, DMSO-d6, 400 MHz
DMSO-d6
1.00
2.09
4.05
1.98
3.09
f1 (ppm)

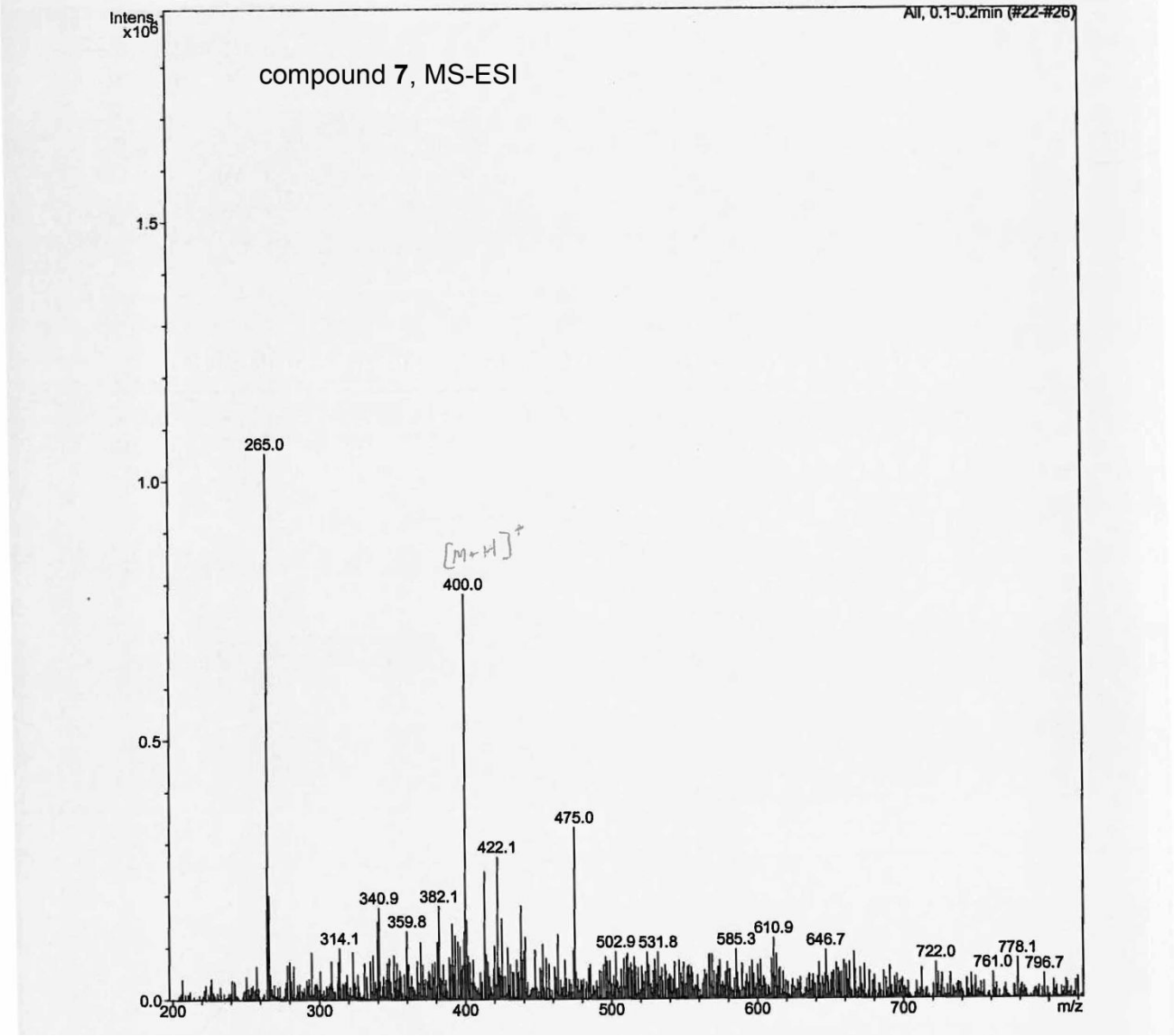

172.190
165.566
165.417
151.383
40.131
39.096
31.977
12.886
DMSO-d_6
HO
O
O
N
N
O
O
compound 6, ^{13}C, DMSO-d_6, 100 MHz
f1 (ppm)


compound **6**, MS-ESI
Intens.
x10⁷
All, 0.0-0.2min (#7-#42)
8
6
4
2
0
226.8
227.1
154.8
100
150
200
250
300
350
400
450
m/z

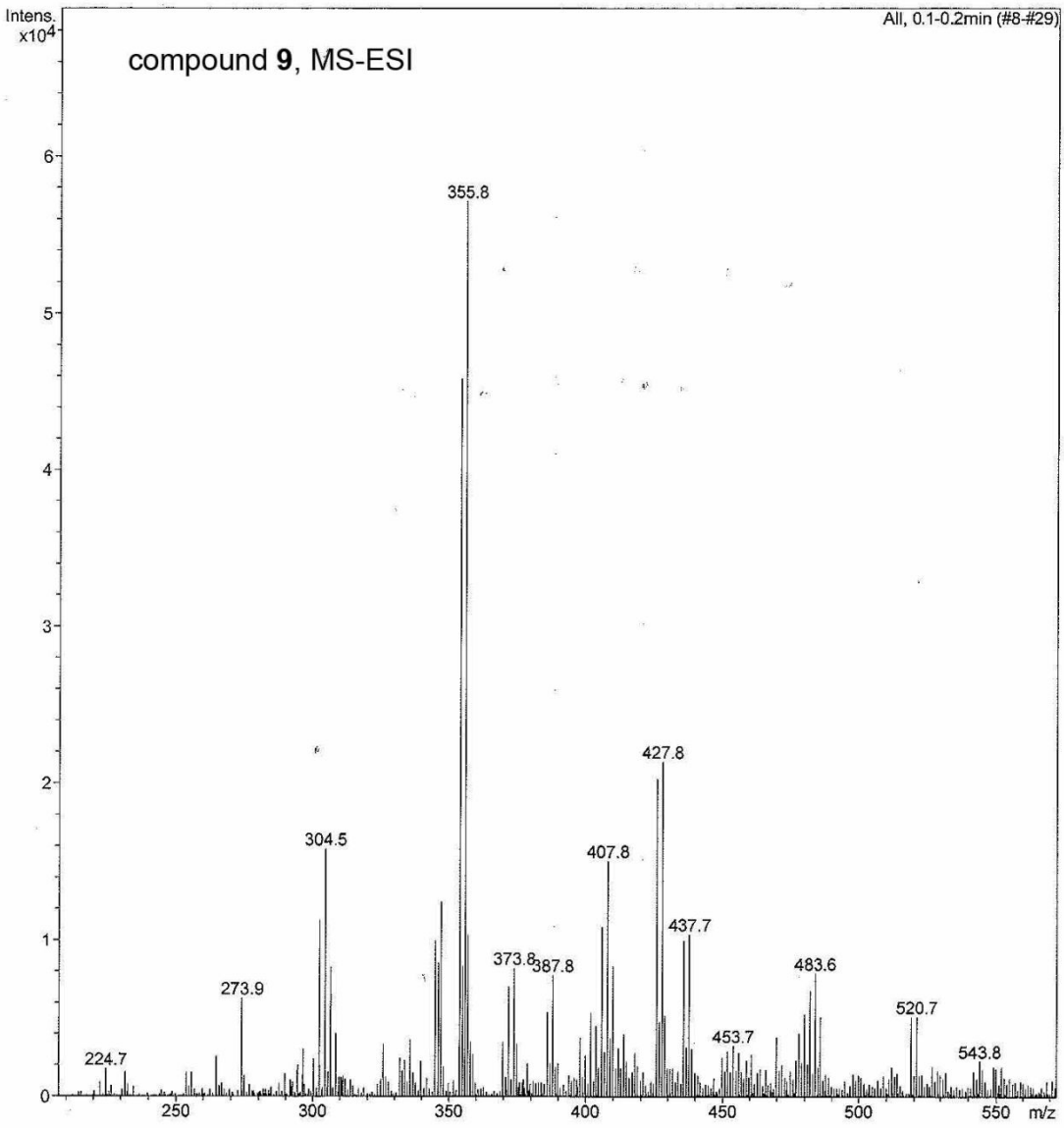
Compound **7**.



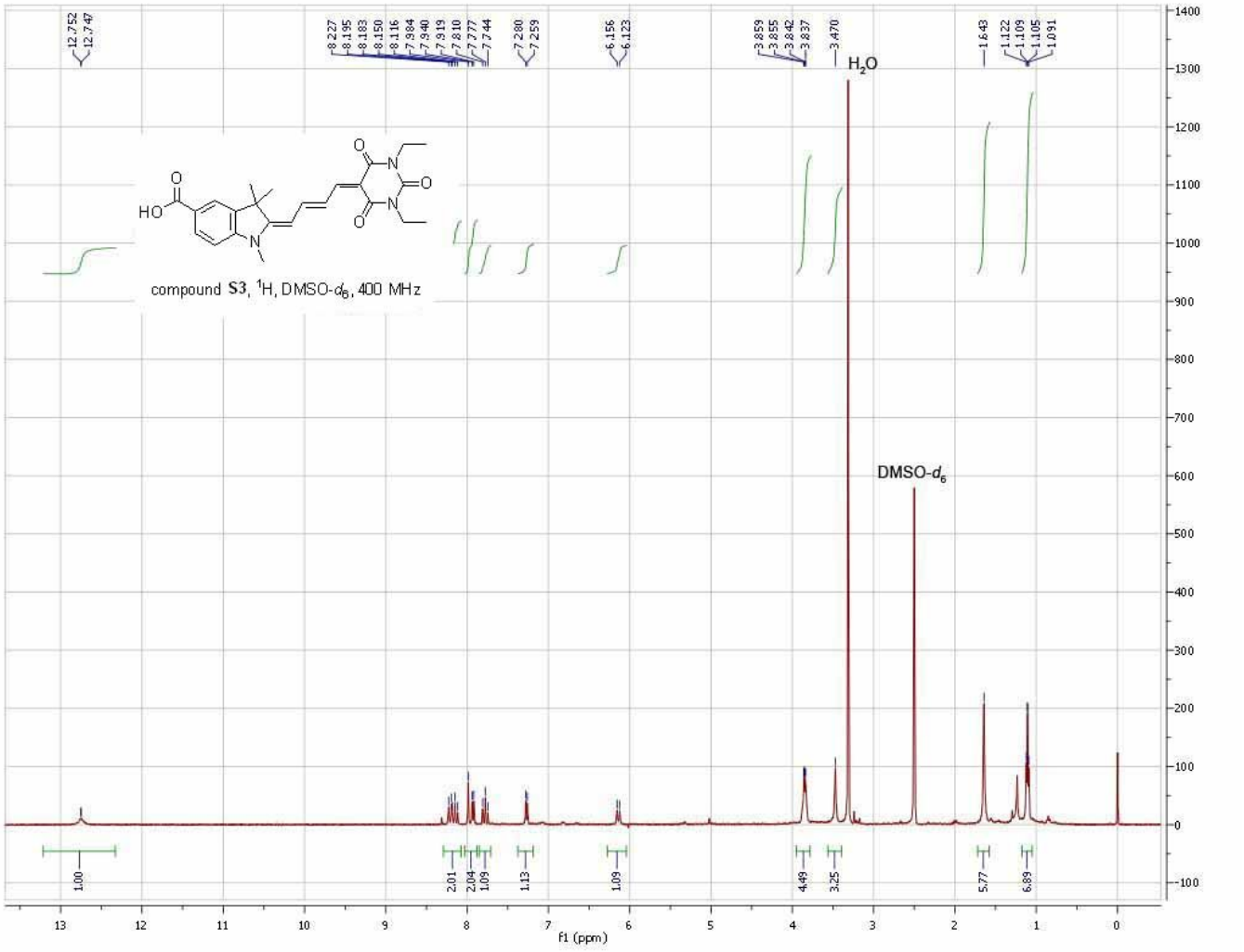

172.290
172.239
169.927
161.435
161.037
157.216
157.184
150.333
150.315
137.837
137.818
130.263
129.551
129.520
128.171
110.499
109.033
37.004
36.342
36.213
35.528
32.183
32.112
23.114
13.061
13.033
DMSO-d_6
compound 7, ^{13}C, DMSO-d_6, 100 MHz
f1 (ppm)

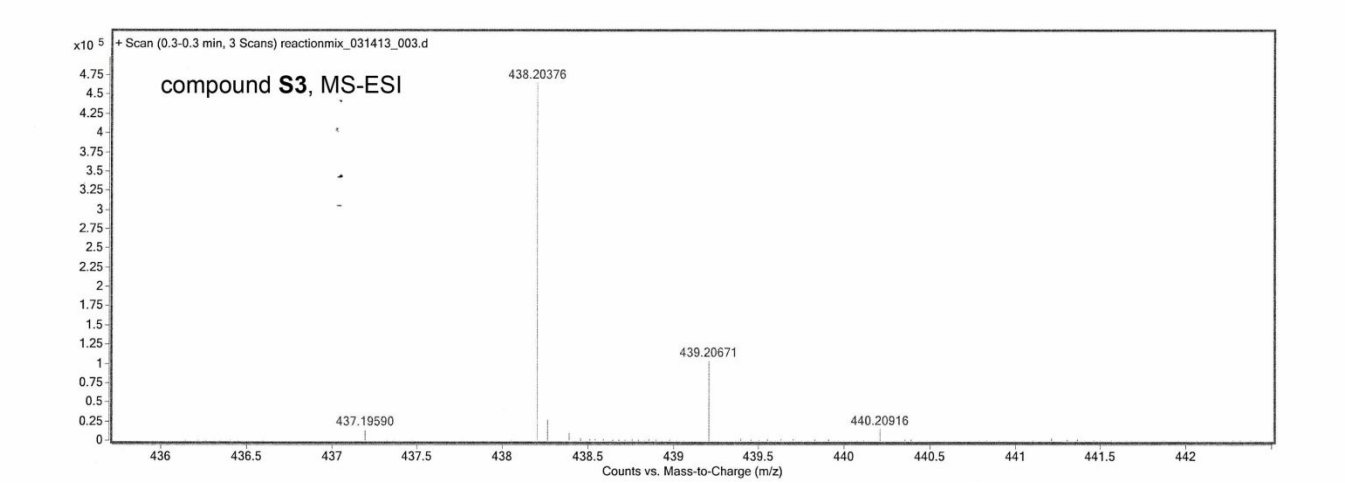

compound 7, MS-ESI
Intens.
x10^6
All, 0.1-0.2min (#22-#26)
1.5
1.0
0.5
0.0
265.0
[M+H]+
400.0
475.0
422.1
340.9
382.1
359.8
314.1
502.9
531.8
585.3
610.9
646.7
722.0
761.0
778.1
796.7
200
300
400
500
600
700
m/z

Compound **9**.

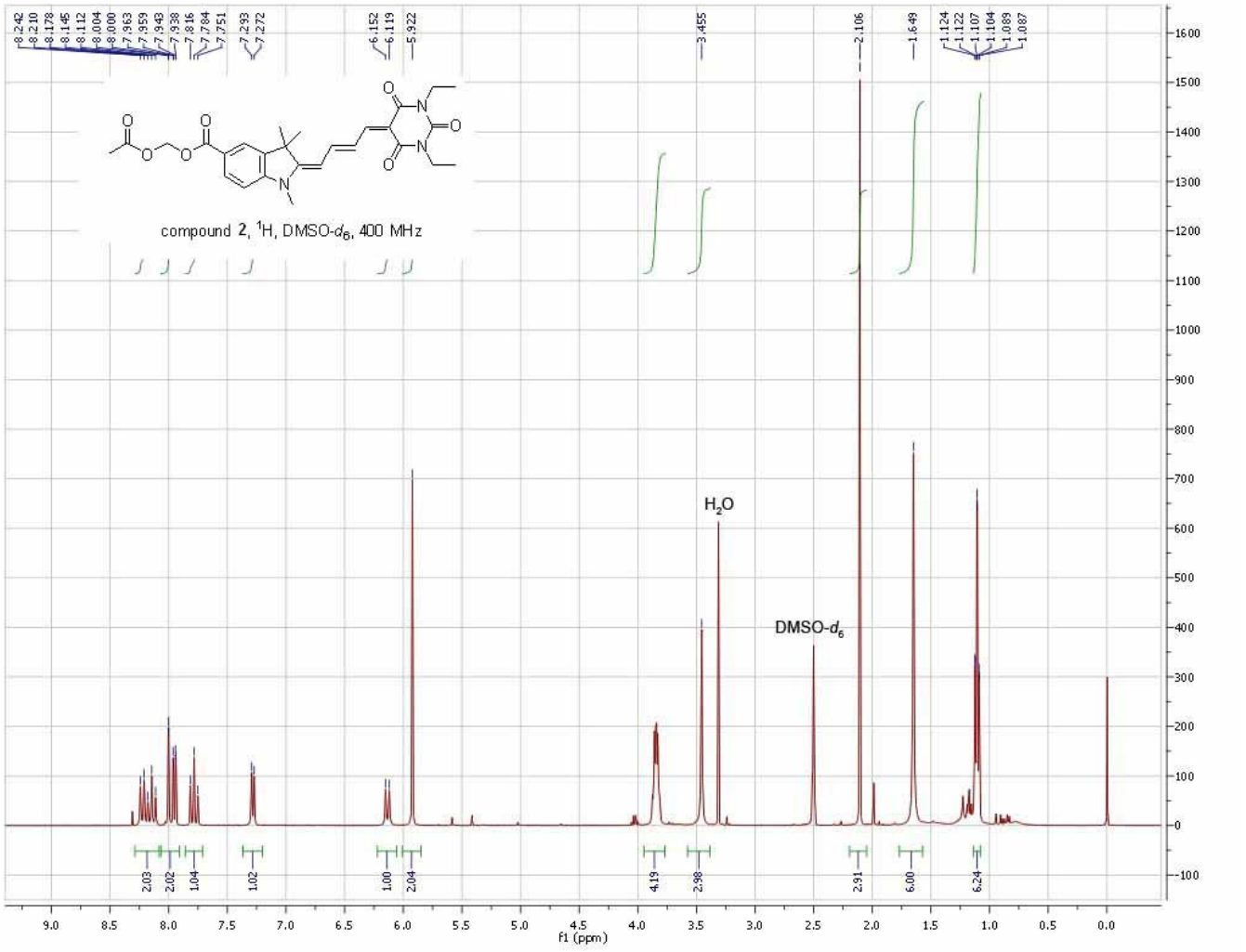

Intens.
x10^4
All, 0.1-0.2min (#8-#29)
compound 9, MS-ESI
355.8
427.8
304.5
407.8
437.7
373.8
387.8
483.6
273.9
520.7
453.7
224.7
543.8
m/z

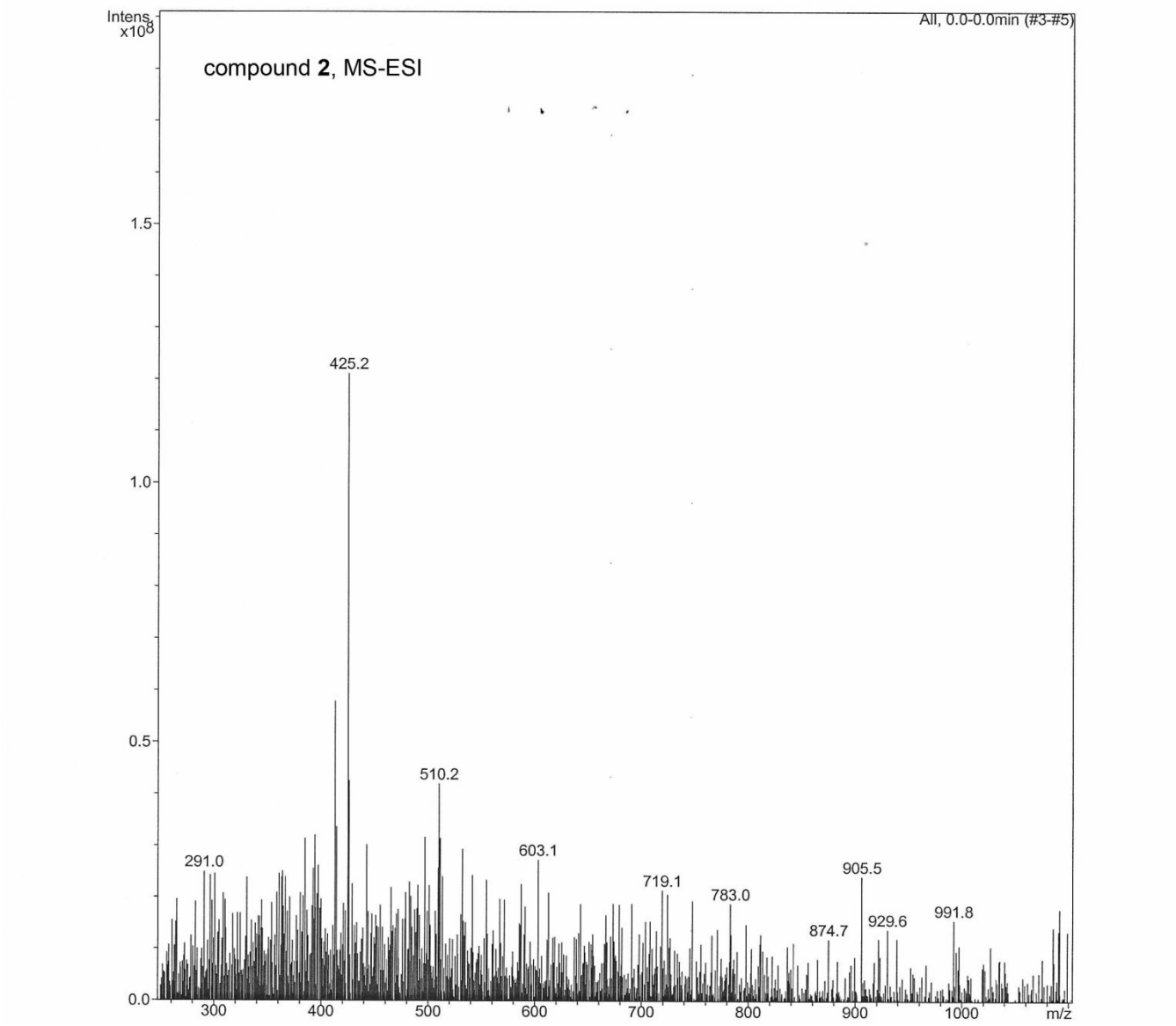
Compound **S3**.



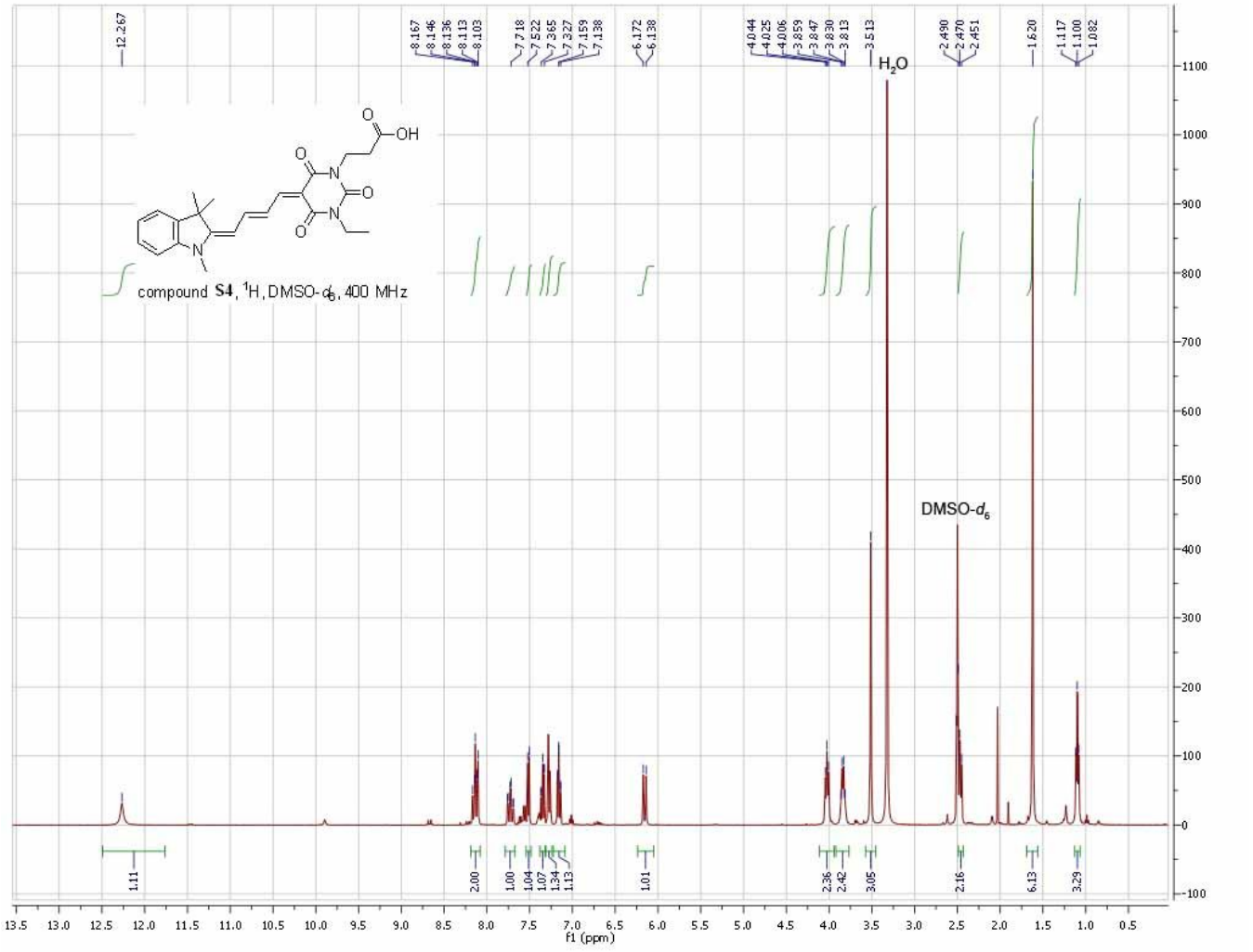

x10 5
+ Scan (0.3-0.3 min, 3 Scans) reactionmix_031413_003.d
compound **S3**, MS-ESI
438.20376
439.20671
437.19590
440.20916
4.75
4.5
4.25
4
3.75
3.5
3.25
3
2.75
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
436
436.5
437
437.5
438
438.5
439
439.5
440
440.5
441
441.5
442
Counts vs. Mass-to-Charge (m/z)

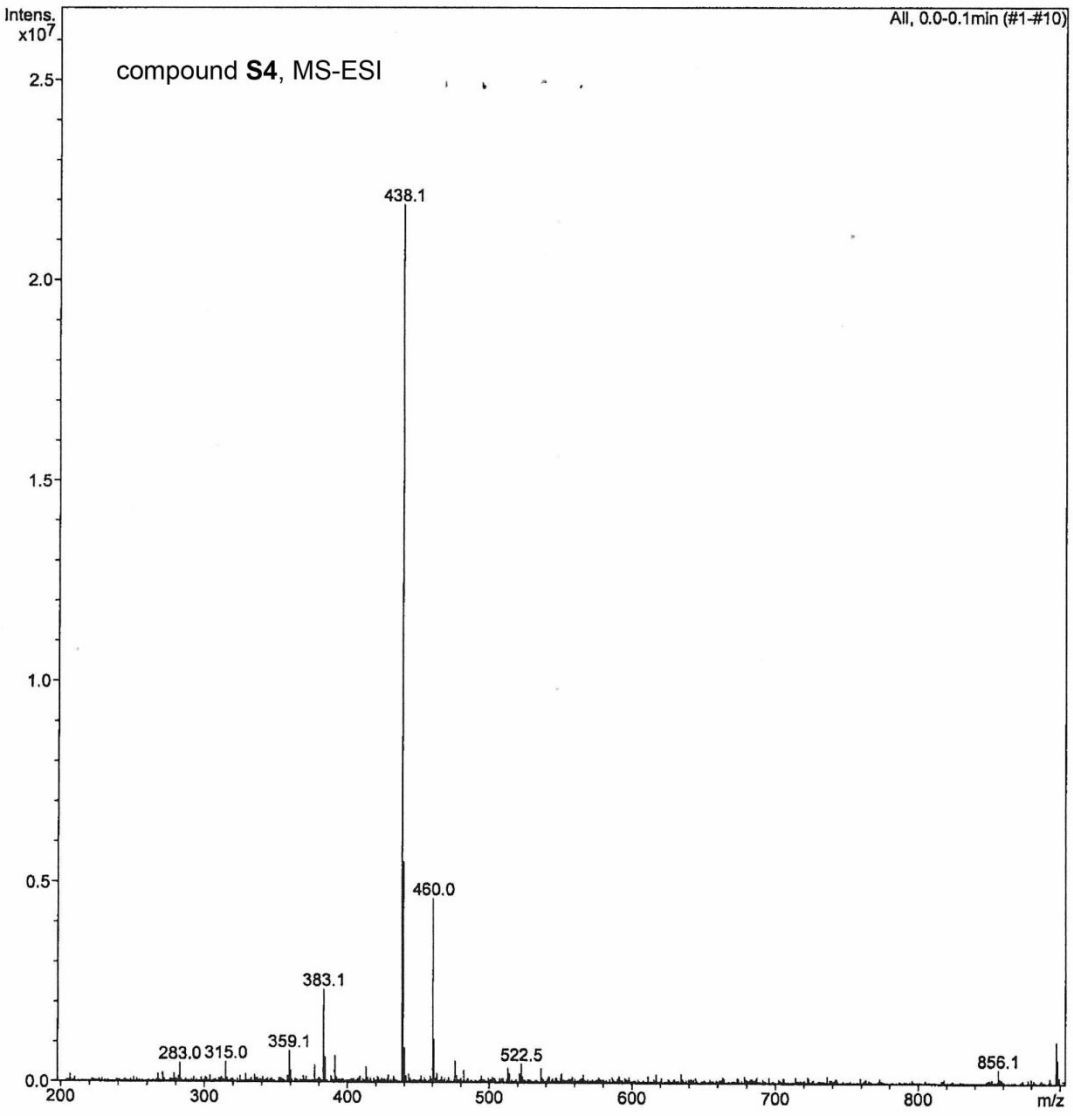
Compound **2**.



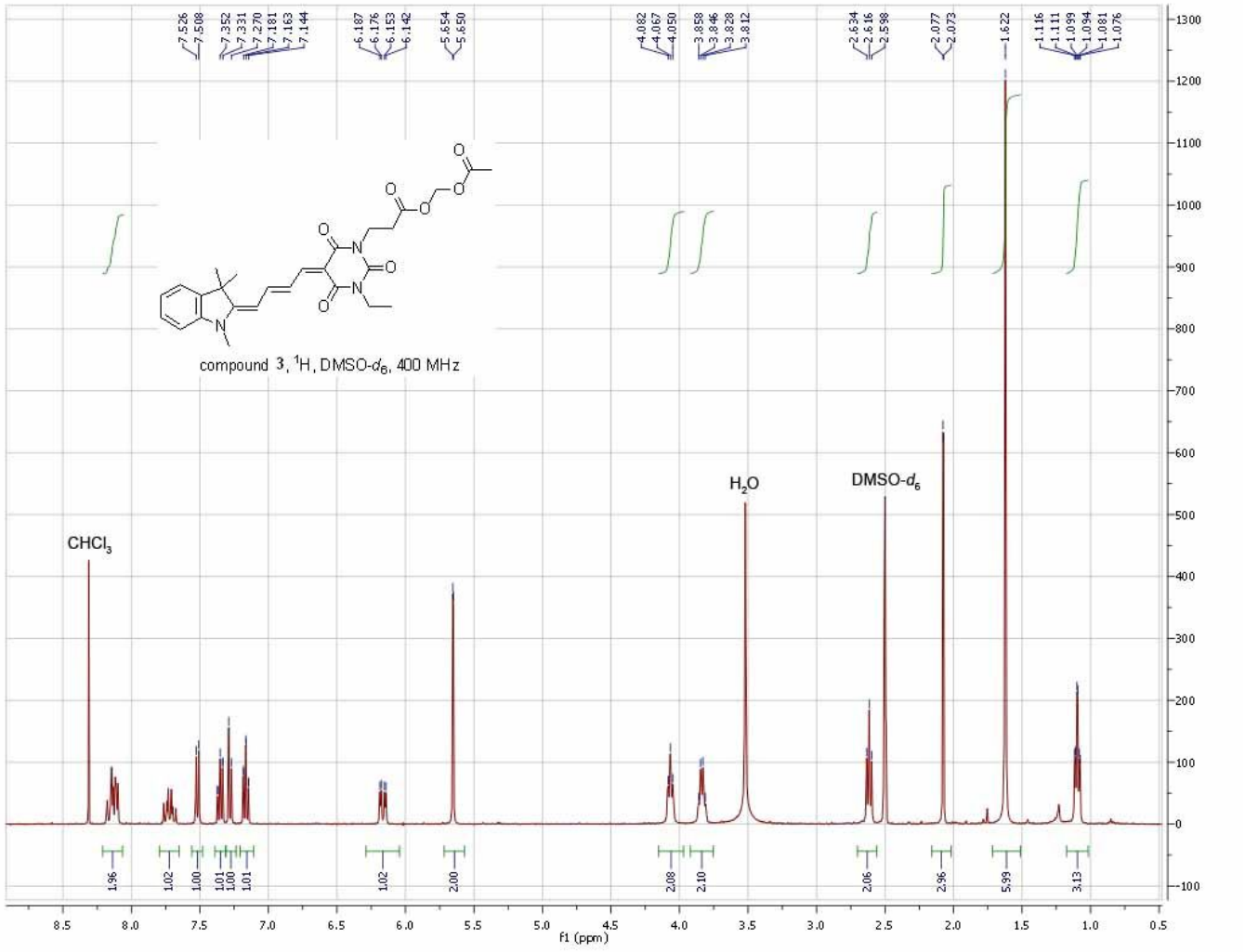

Intens.
x10^8
All, 0.0-0.0min (#3-#5)
compound **2**, MS-ESI
1.5
1.0
0.5
0.0
425.2
510.2
291.0
603.1
719.1
783.0
905.5
929.6
874.7
991.8
300
400
500
600
700
800
900
1000
m/z

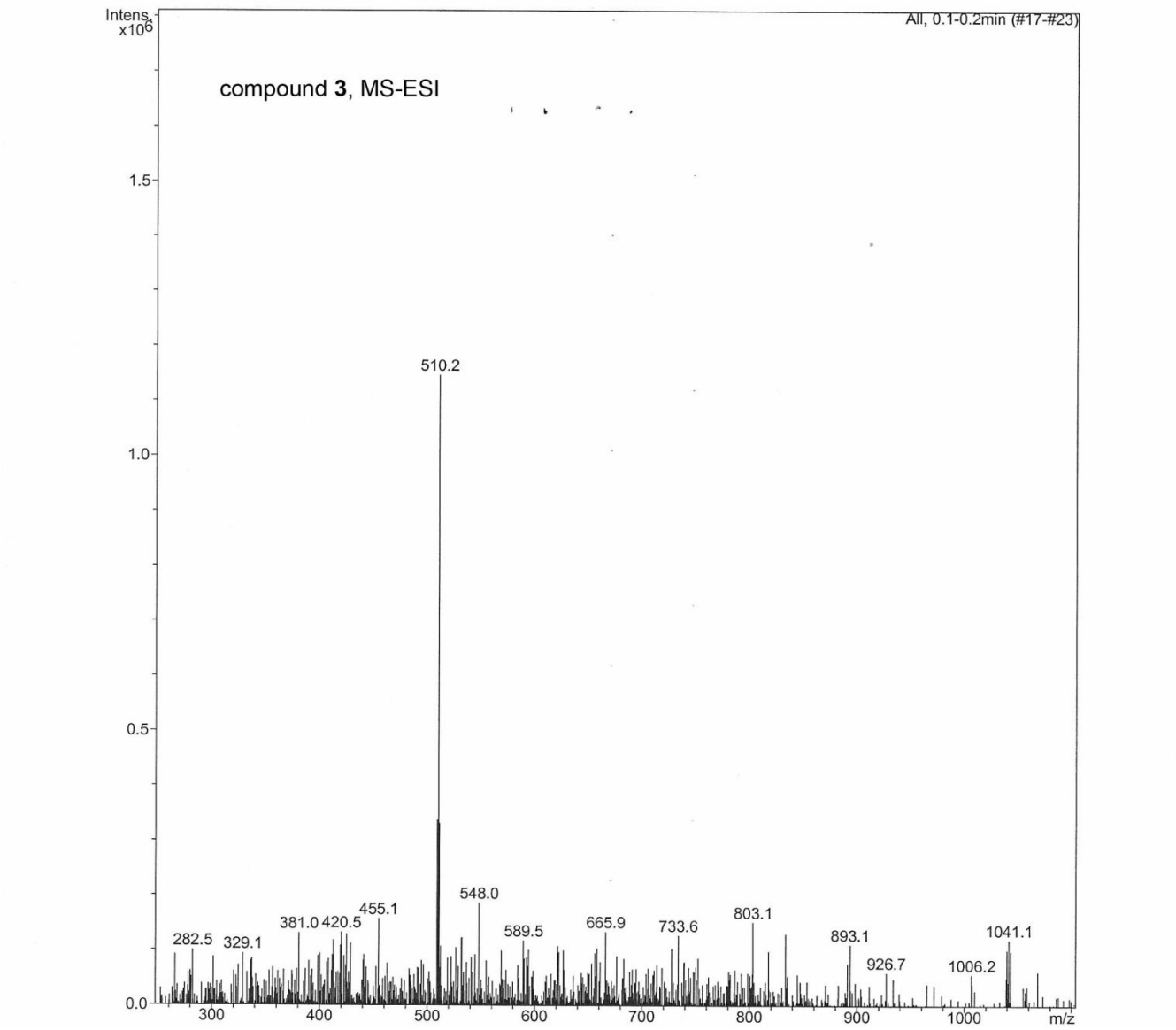
Compound **S4**.



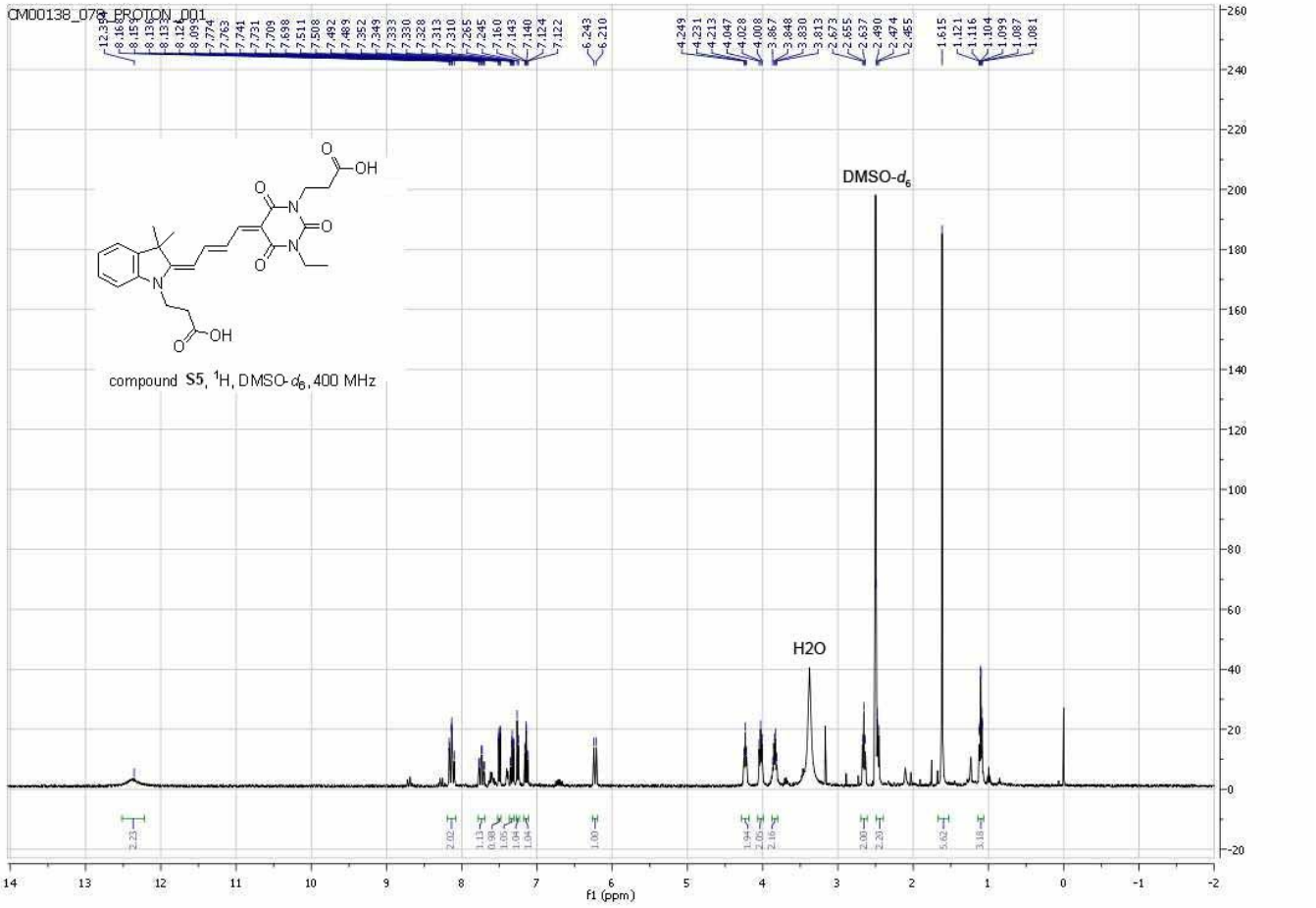

Intens.
x10^7
All, 0.0-0.1min (#1-#10)
compound **S4**, MS-ESI
2.5
2.0
1.5
1.0
0.5
0.0
438.1
460.0
383.1
359.1
283.0
315.0
522.5
856.1
200
300
400
500
600
700
800
m/z

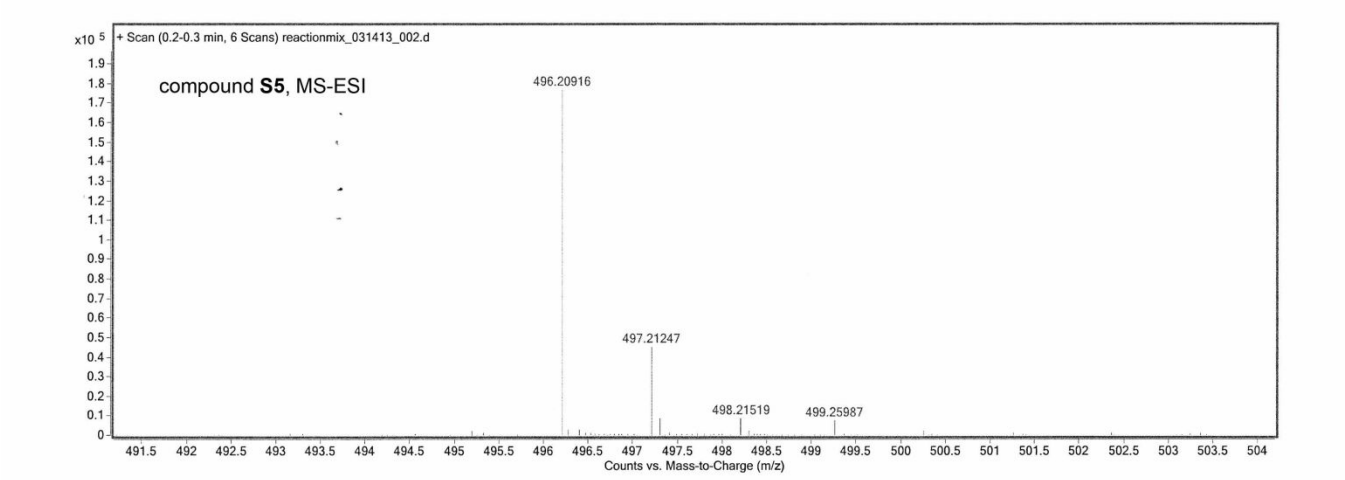
Compound **3**.



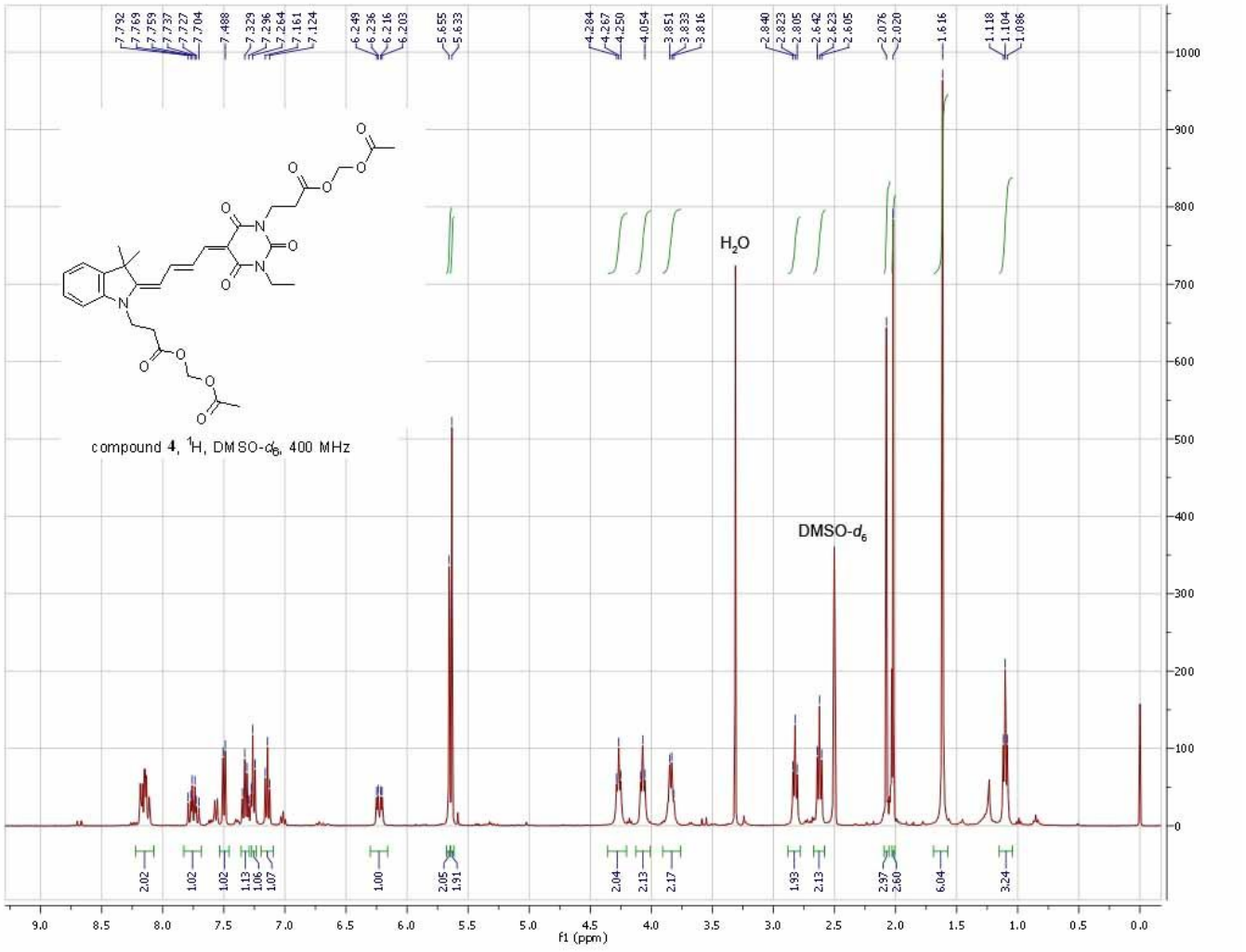

Intens.
x10^6
All, 0.1-0.2min (#17-#23)
compound **3**, MS-ESI
1.5
1.0
0.5
0.0
510.2
548.0
455.1
381.0
420.5
282.5
329.1
589.5
665.9
733.6
803.1
893.1
926.7
1006.2
1041.1
300
400
500
600
700
800
900
1000
m/z

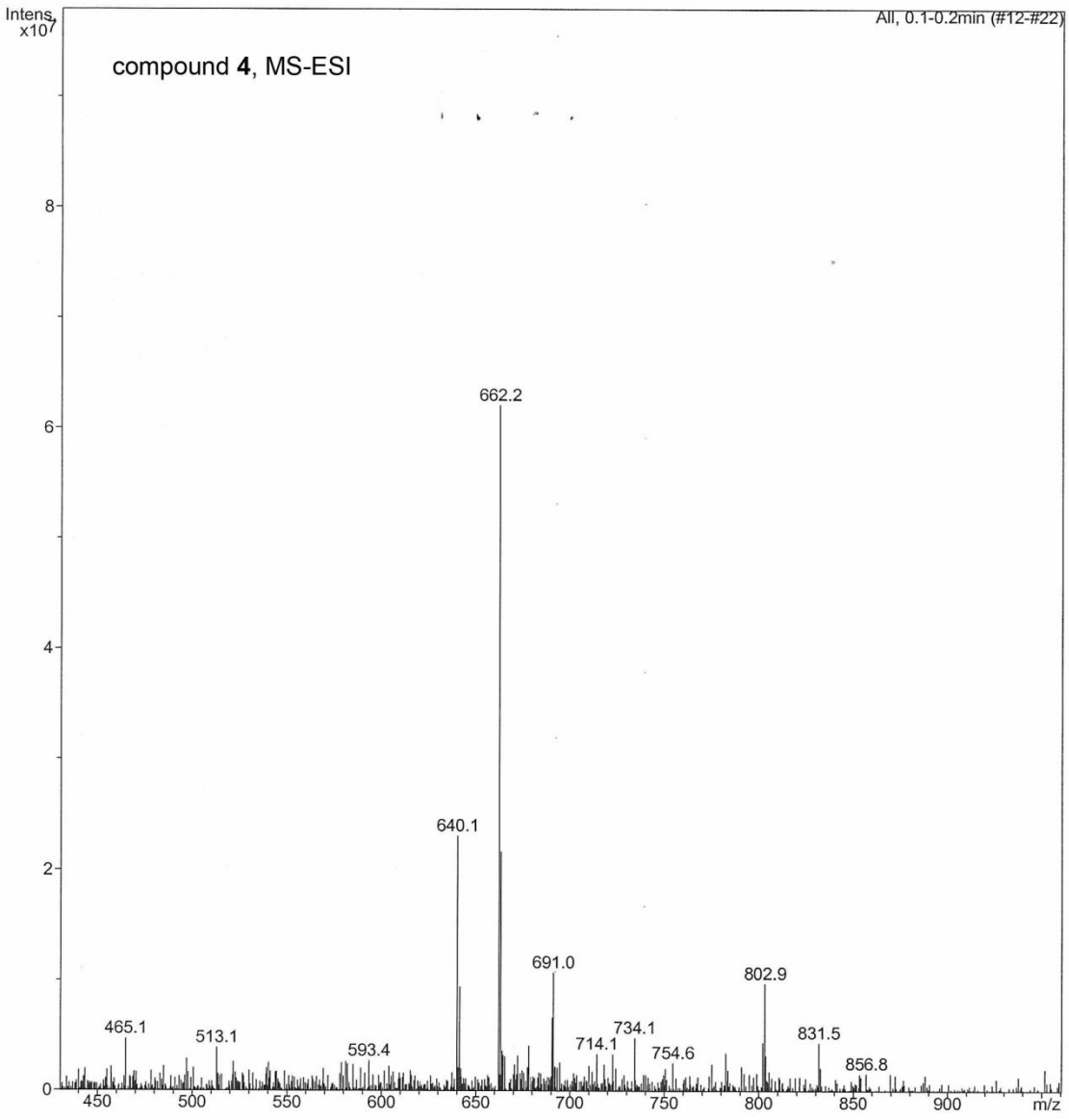
Compound **S5**.


CM00138_078_PROTON_001
DMSO-d_6
H2O
compound S5, ^{1}H, DMSO-d_6, 400 MHz
f1 (ppm)

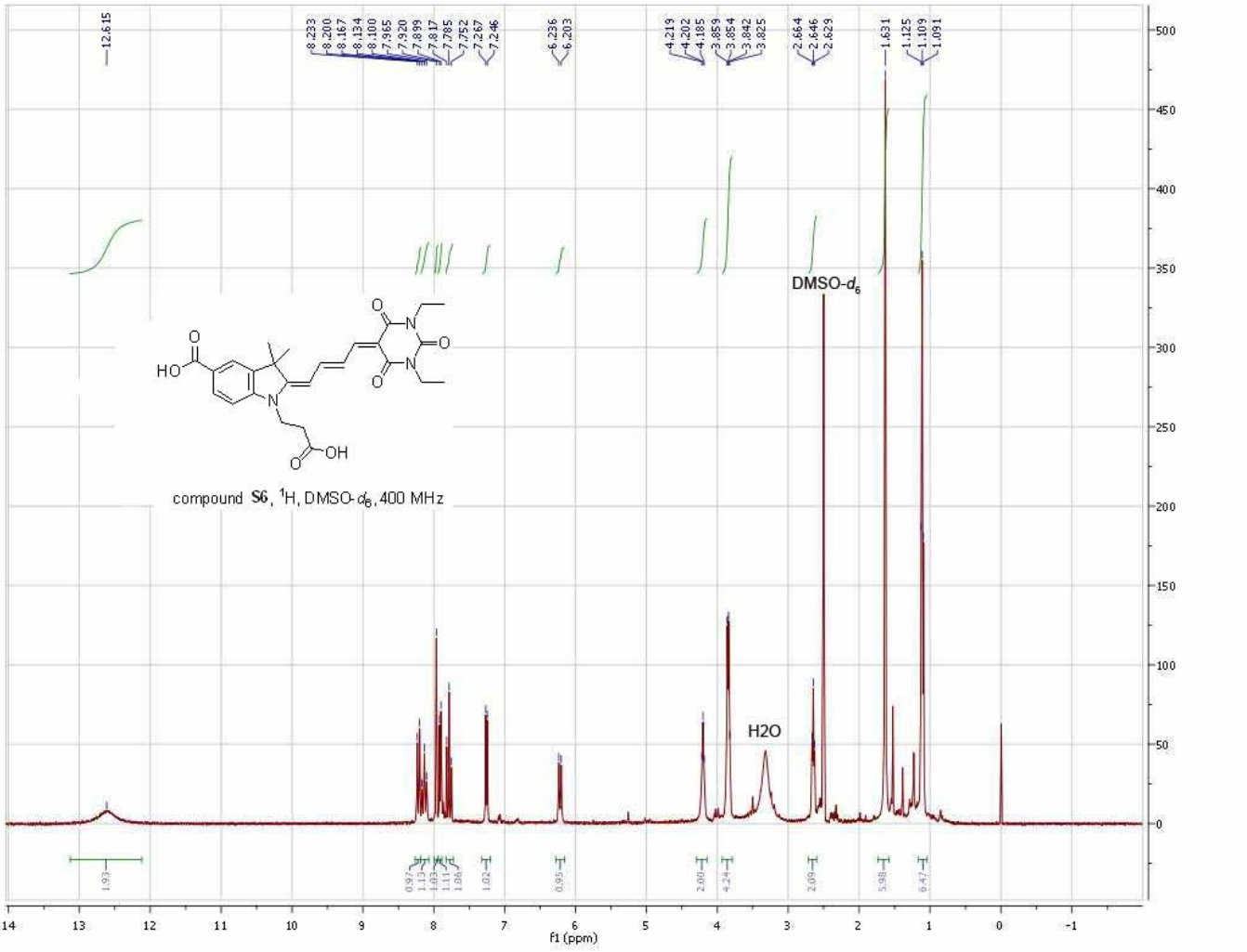

+ Scan (0.2-0.3 min, 6 Scans) reactionmix_031413_002.d
x10 5
compound **S5**, MS-ESI
496.20916
497.21247
498.21519
499.25987
Counts vs. Mass-to-Charge (m/z)

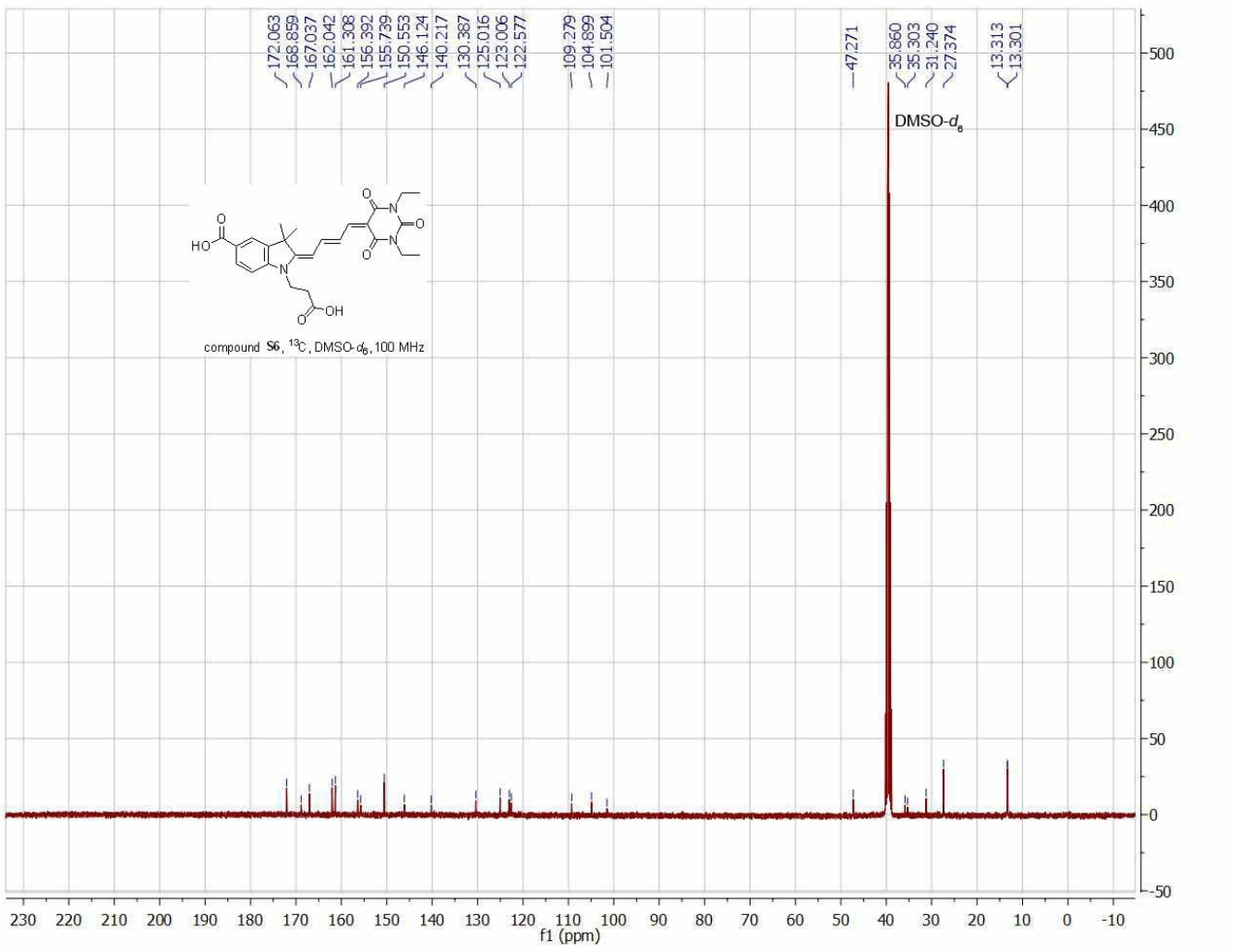
Compound **4**.

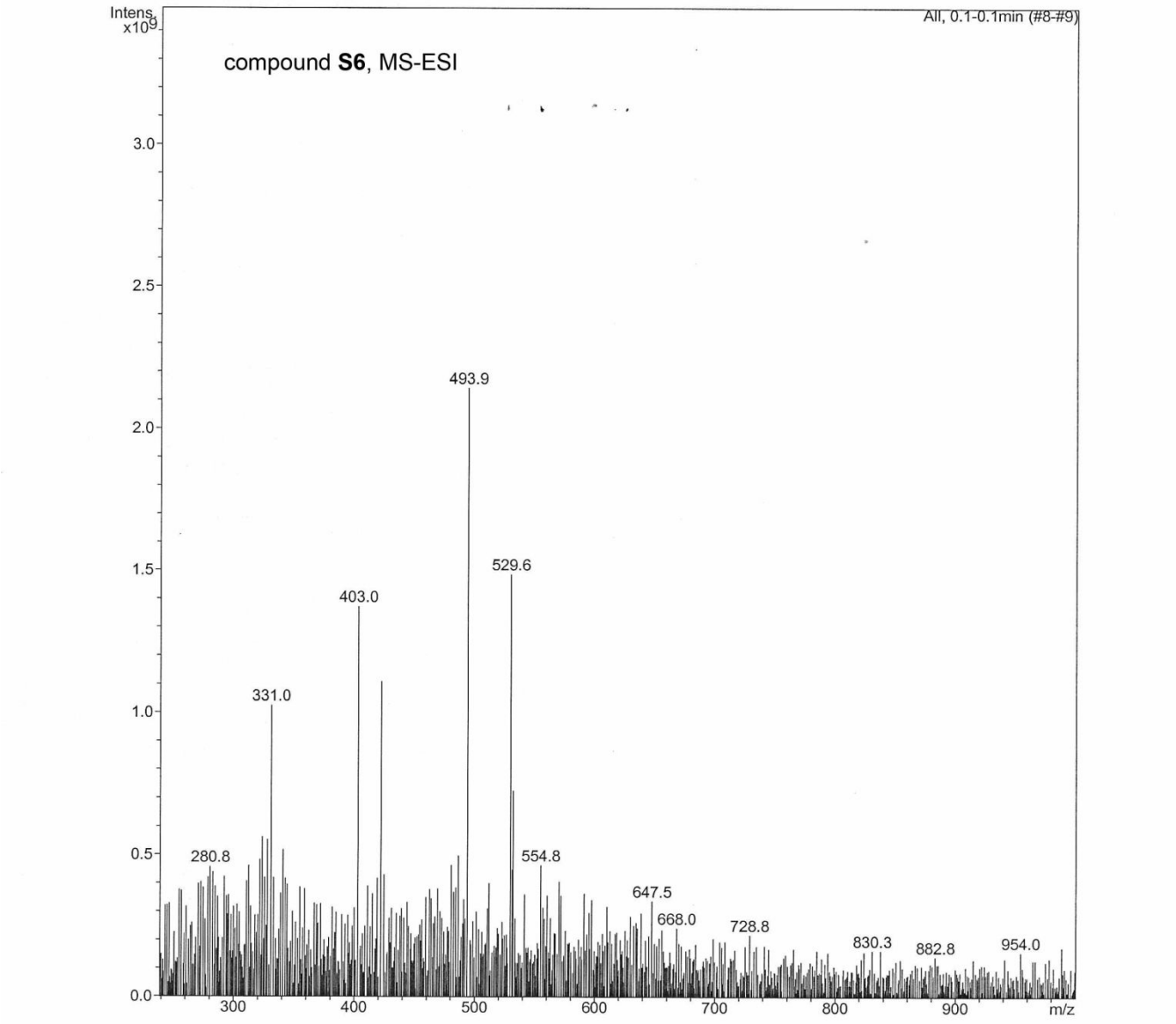



Intens.
x10⁷
All, 0.1-0.2min (#12-#22)
compound **4**, MS-ESI
8
6
4
2
0
662.2
640.1
691.0
802.9
465.1
513.1
593.4
714.1
734.1
754.6
831.5
856.8
450
500
550
600
650
700
750
800
850
900
m/z

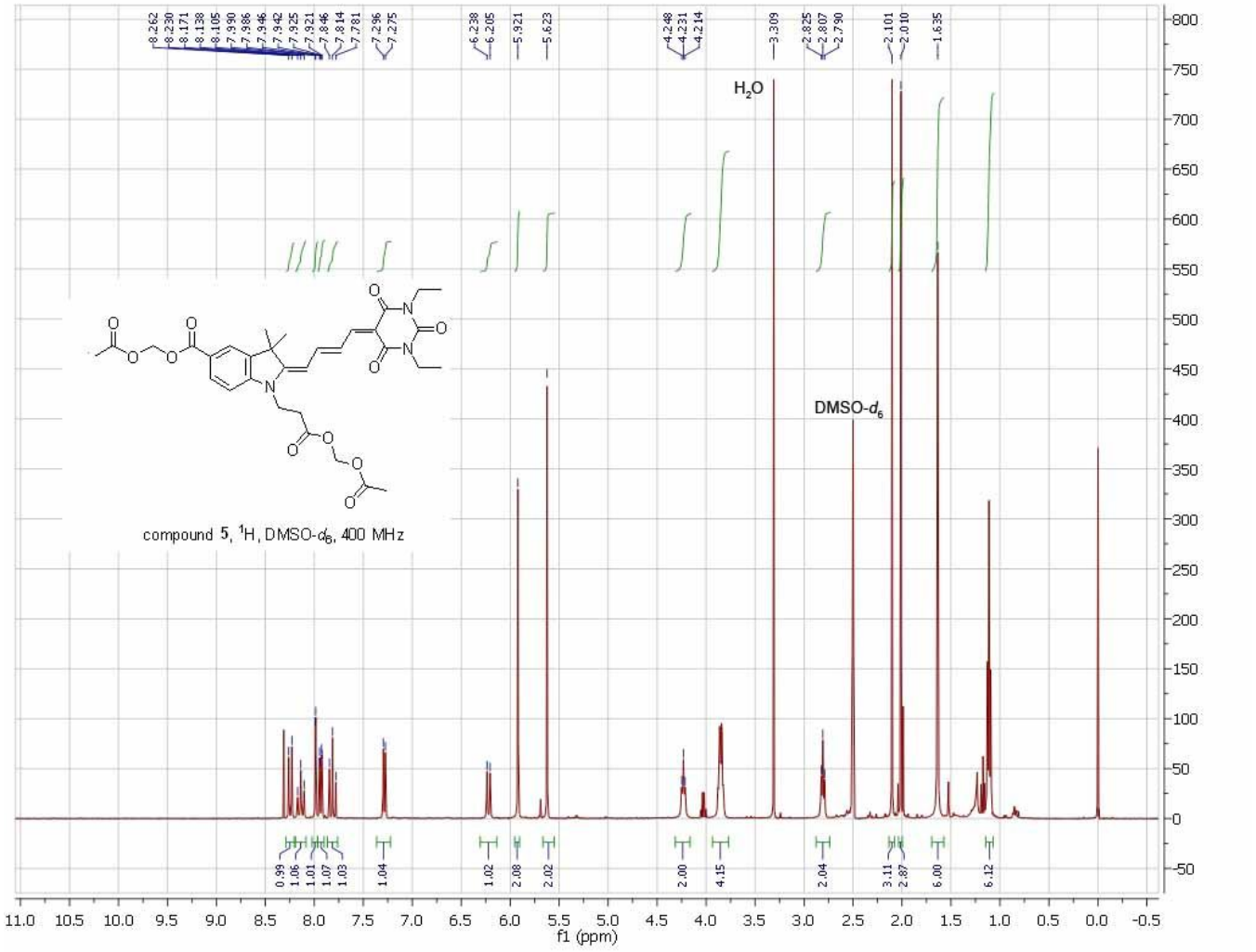
Compound **S6**.

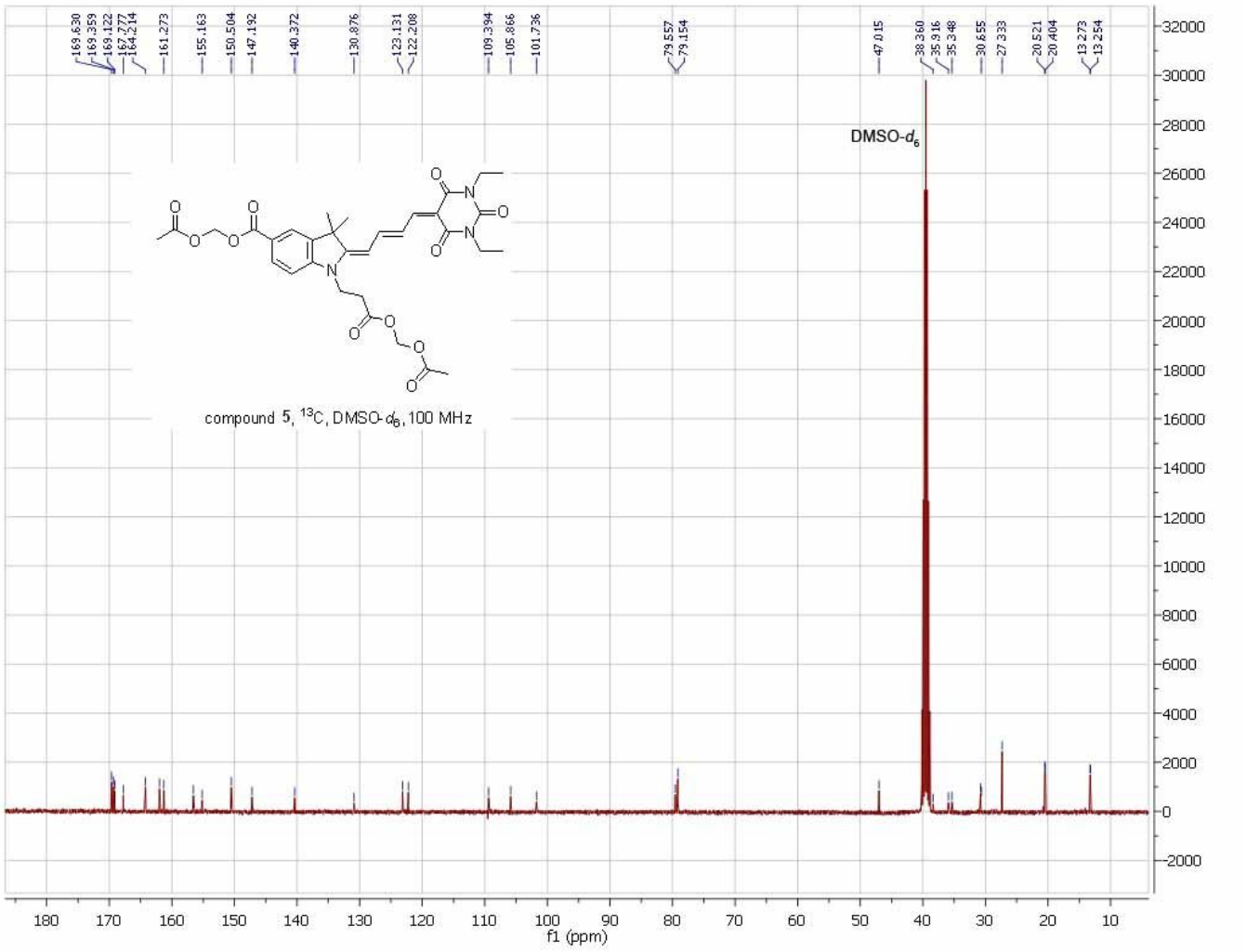


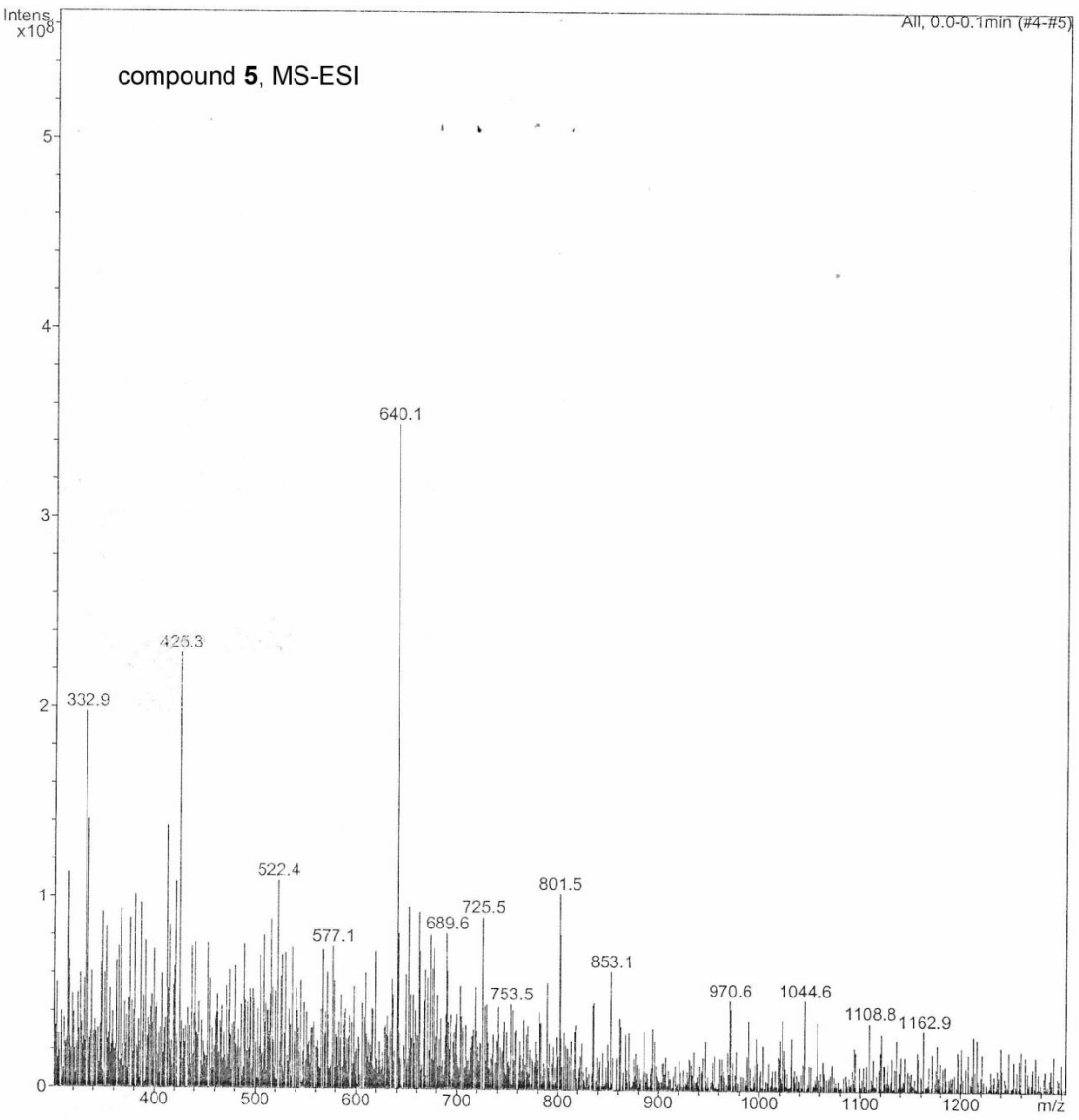

172.063
168.859
167.037
162.042
161.308
156.392
155.739
150.553
146.124
140.217
130.387
125.016
123.006
122.577
109.279
104.899
101.504
47.271
35.860
35.303
31.240
27.374
13.313
13.301
DMSO-d_6
compound S6, ^{13}C, DMSO-d_6, 100 MHz
f1 (ppm)


Intens.
x10^9
All, 0.1-0.1min (#8-#9)
compound **S6**, MS-ESI
3.0
2.5
2.0
1.5
1.0
0.5
0.0
493.9
529.6
403.0
331.0
280.8
554.8
647.5
668.0
728.8
830.3
882.8
954.0
300
400
500
600
700
800
900
m/z

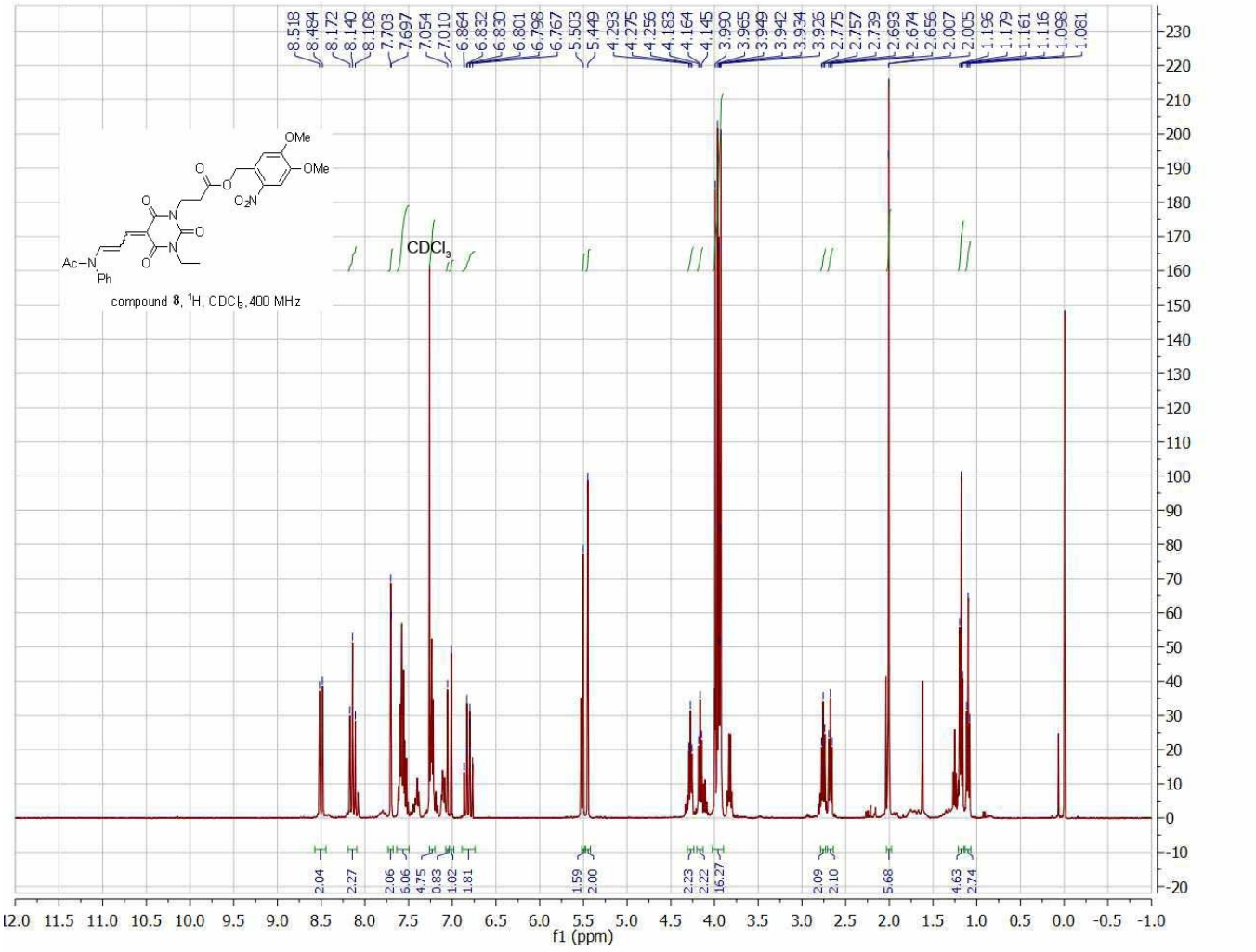
Compound **5**.

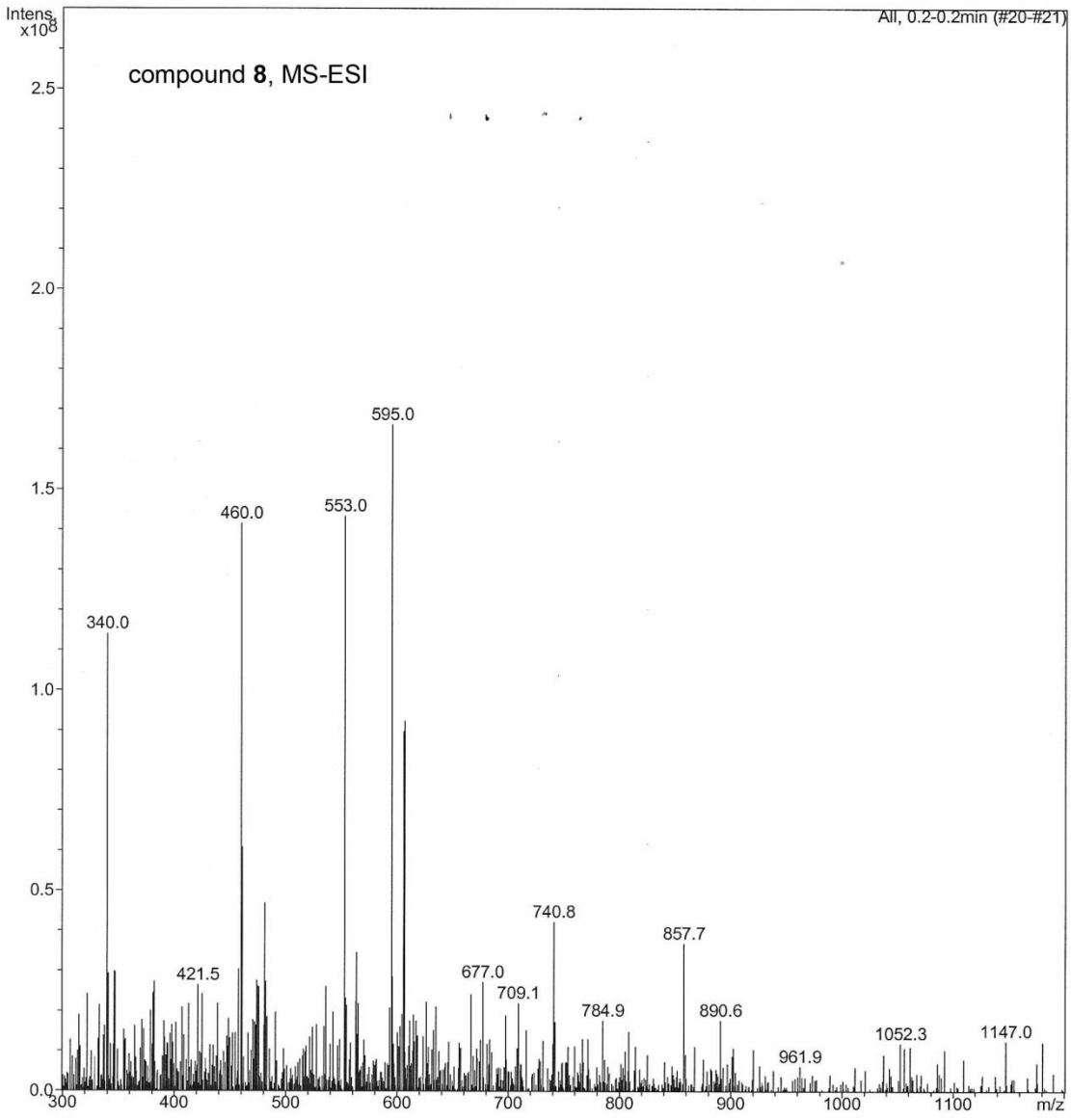



169.630
169.359
169.122
167.277
164.214
161.273
155.163
150.504
147.192
140.372
130.876
123.131
122.208
109.394
105.866
101.736
79.557
79.154
47.015
38.360
35.916
35.348
30.655
27.333
20.521
20.404
13.273
13.254
DMSO-d_6
compound 5, ^{13}C, DMSO-d_6, 100 MHz
f1 (ppm)

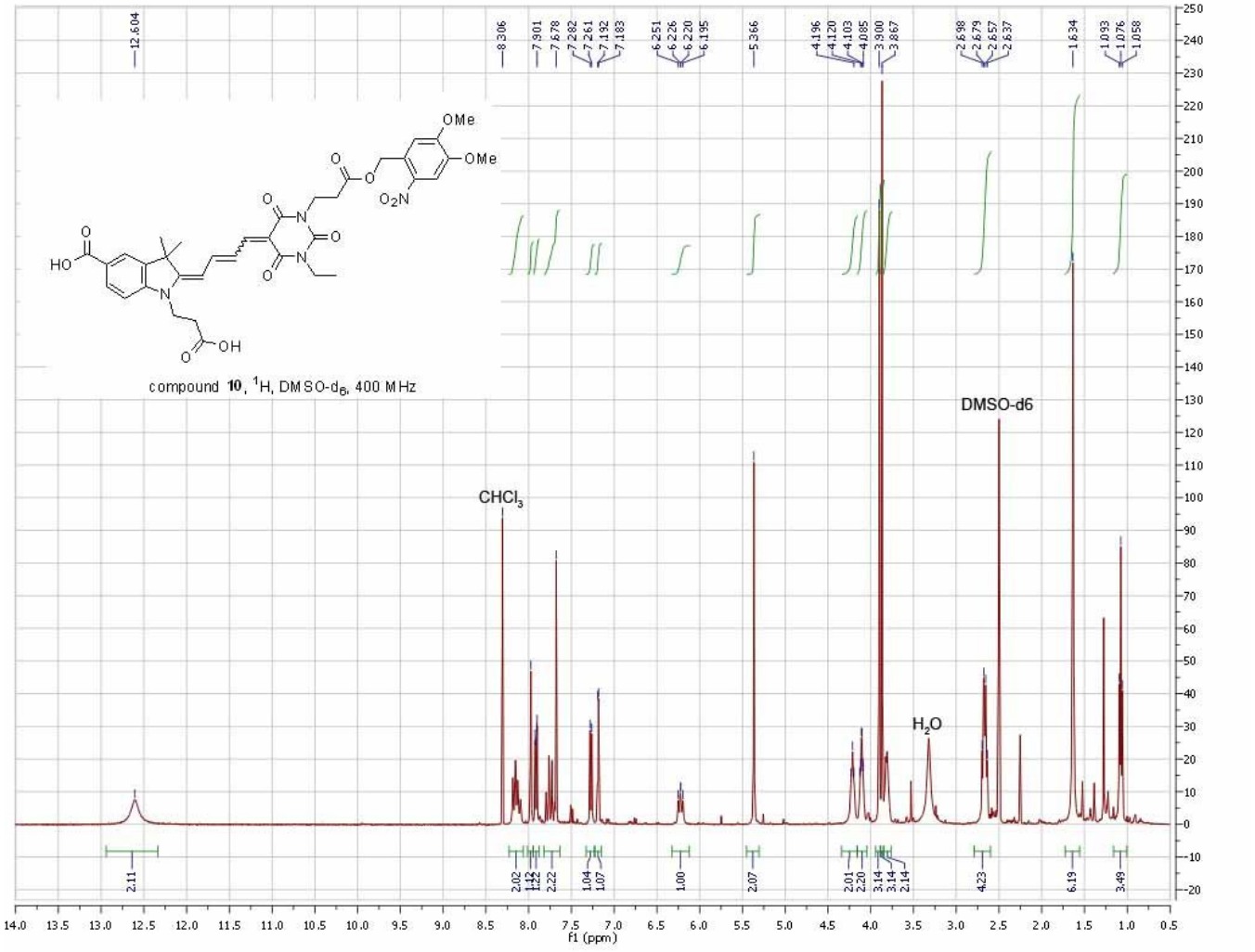

Intens.
x10⁸
All, 0.0-0.1min (#4-#5)
compound **5**, MS-ESI
5
4
3
2
1
0
640.1
425.3
332.9
522.4
577.1
689.6
725.5
801.5
753.5
853.1
970.6
1044.6
1108.8
1162.9
400
500
600
700
800
900
1000
1100
1200
m/z

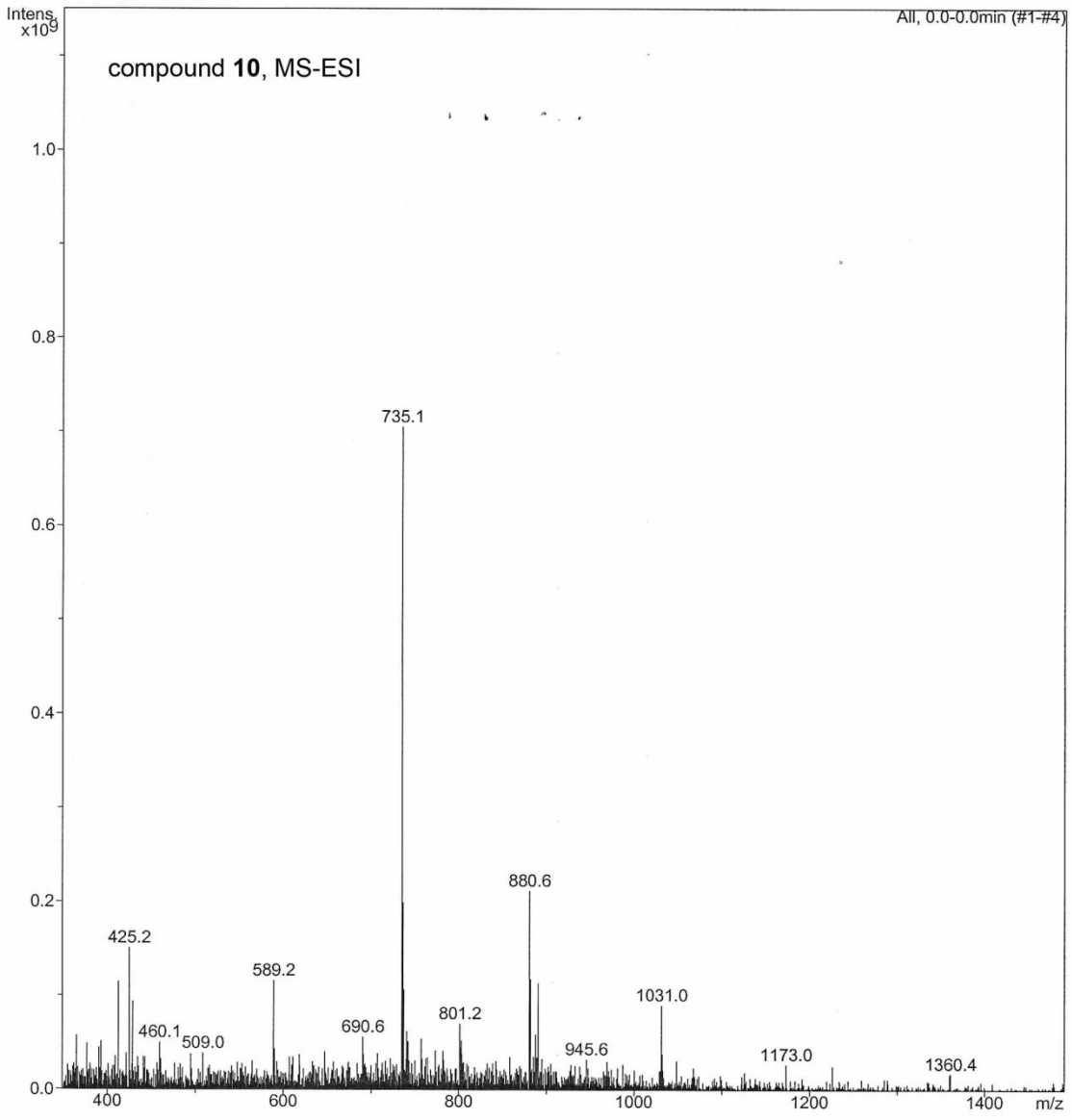
Compound **8**.



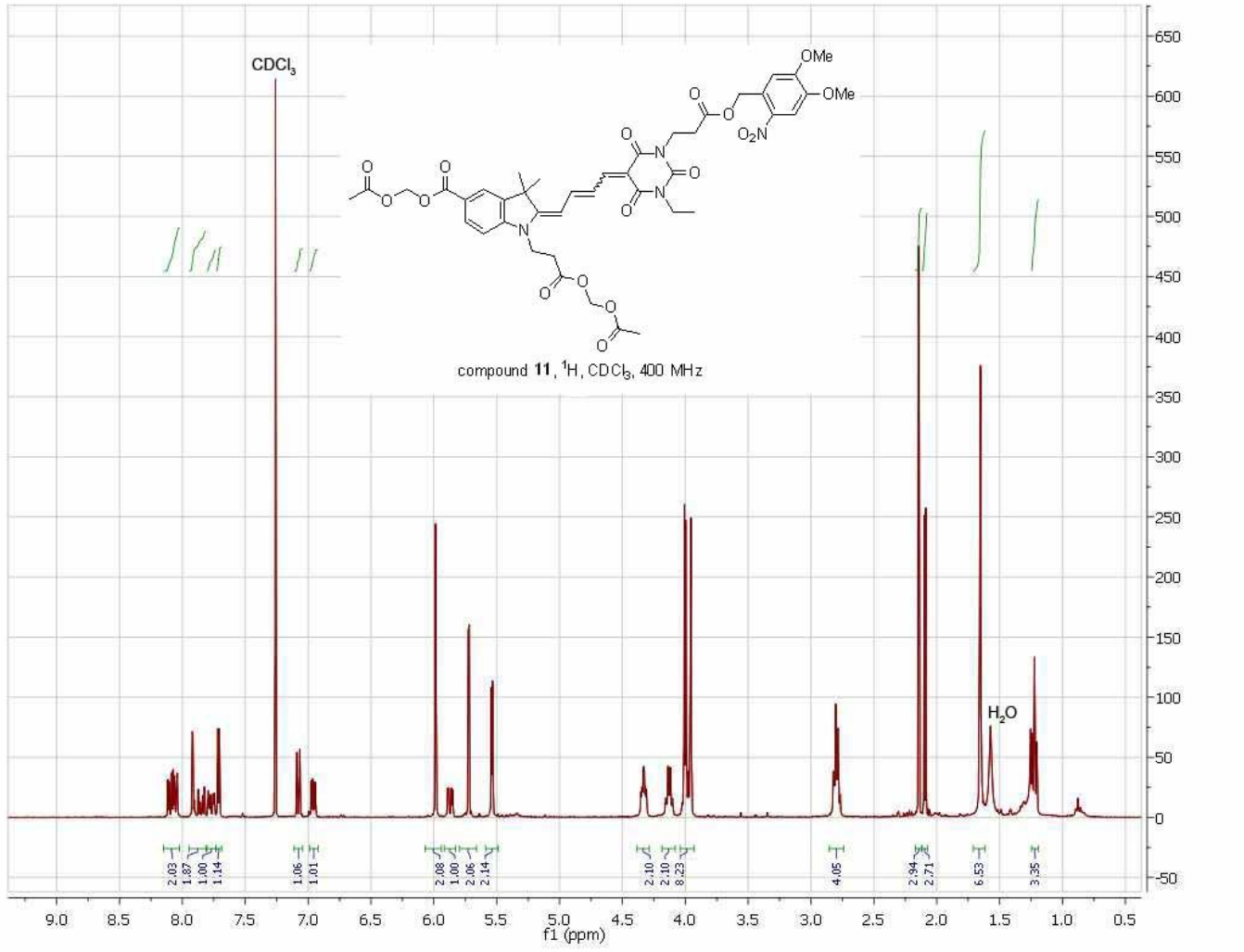

compound **8**, MS-ESI
Intens.
x10⁸
All, 0.2-0.2min (#20-#21)
2.5
2.0
1.5
1.0
0.5
0.0
595.0
460.0
553.0
340.0
421.5
740.8
857.7
677.0
709.1
784.9
890.6
1052.3
1147.0
961.9
300
400
500
600
700
800
900
1000
1100
m/z

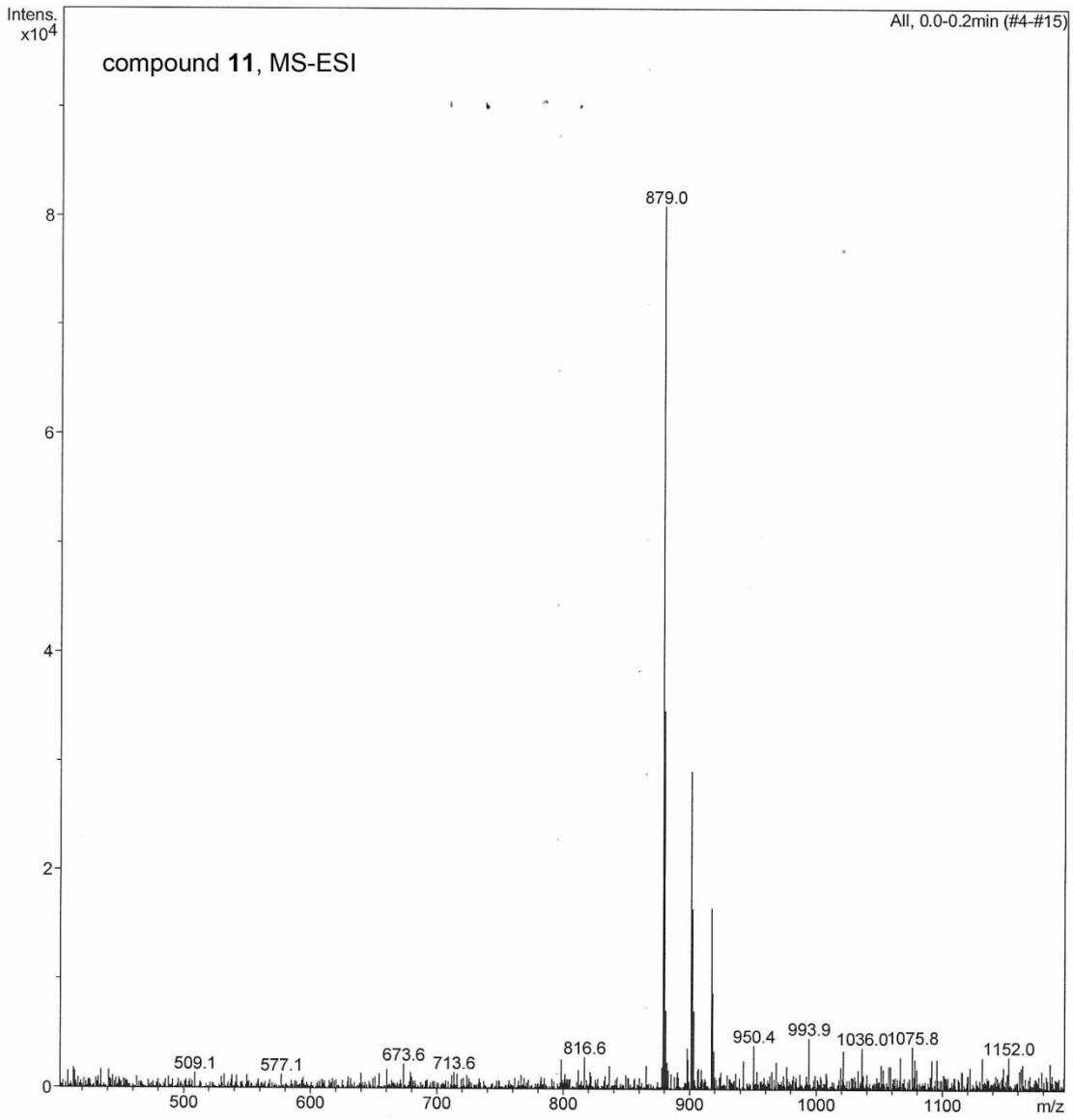
Compound **10**.



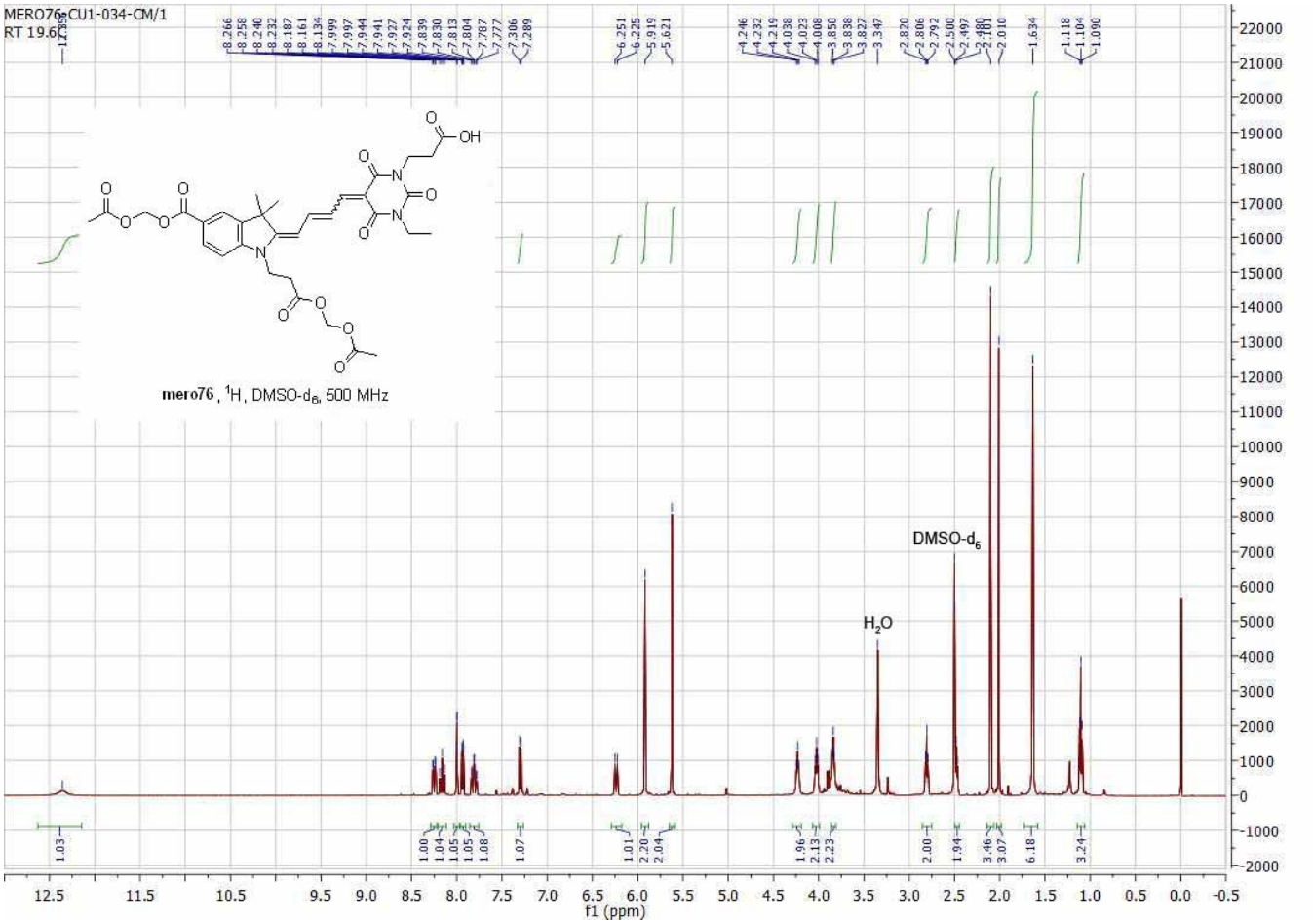

Intens.
x10^9
All, 0.0-0.0min (#1-#4)
compound **10**, MS-ESI
1.0
0.8
0.6
0.4
0.2
0.0
735.1
880.6
425.2
589.2
1031.0
801.2
460.1
509.0
690.6
945.6
1173.0
1360.4
400
600
800
1000
1200
1400
m/z

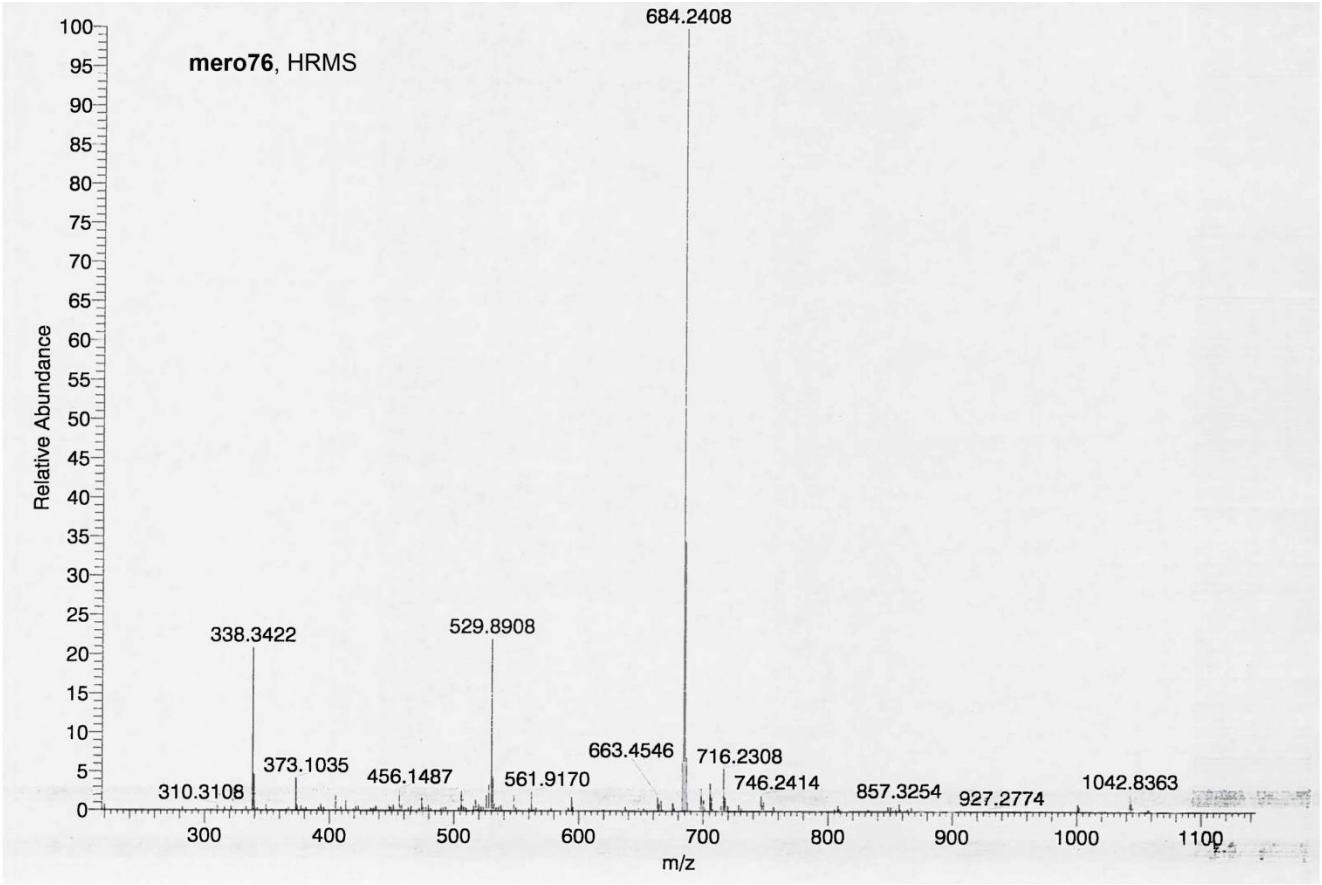
Compound **11**.




Intens.
x10^4
All, 0.0-0.2min (#4-#15)
compound **11**, MS-ESI
879.0
509.1
577.1
673.6
713.6
816.6
950.4
993.9
1036.0
1075.8
1152.0
m/z

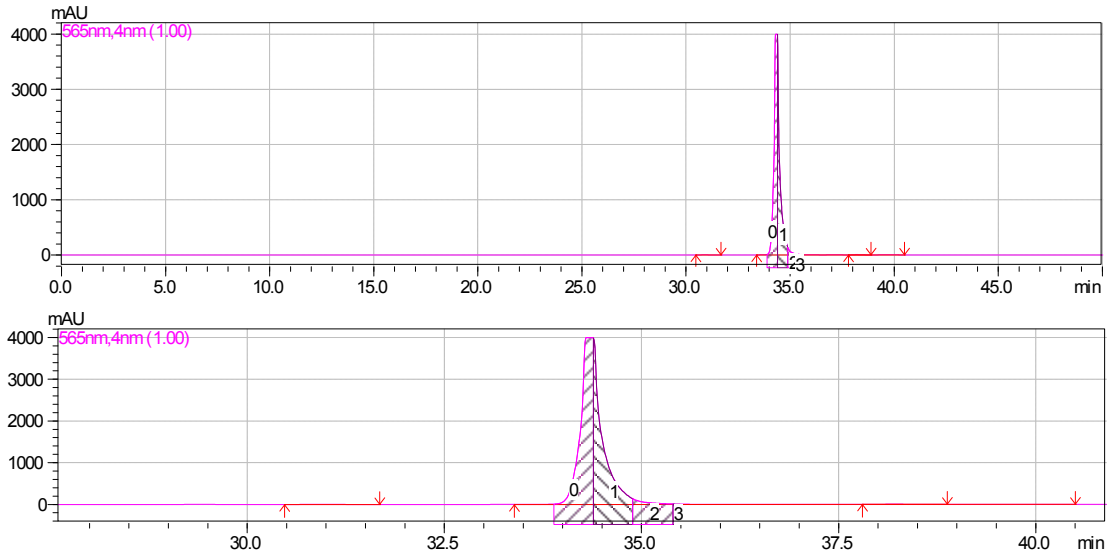
Compound **mero76**.



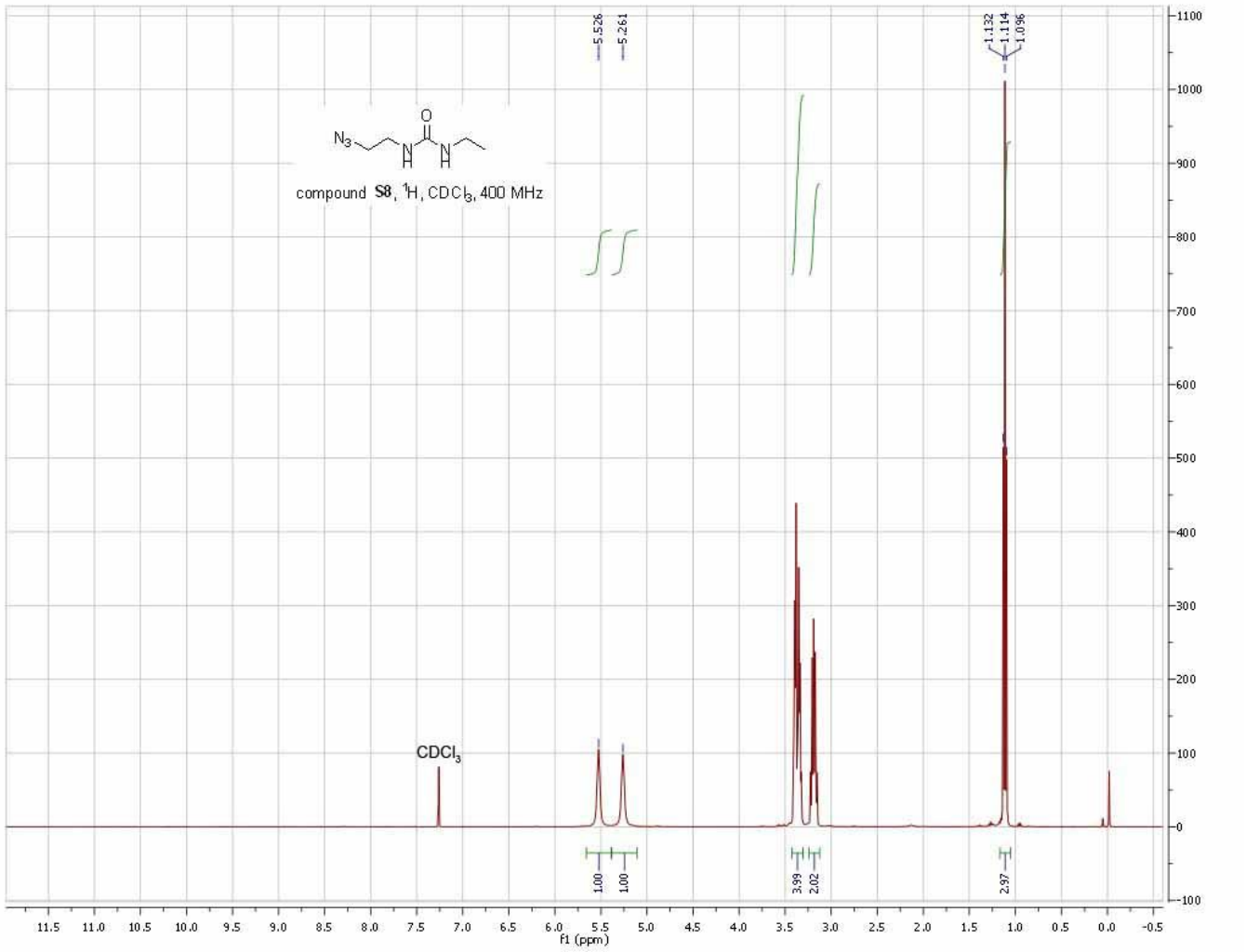

mero76, HRMS
684.2408
338.3422
529.8908
663.4546
716.2308
373.1035
456.1487
561.9170
746.2414
310.3108
857.3254
927.2774
1042.8363
Relative Abundance
m/z
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
300
400
500
600
700
800
900
1000
1100

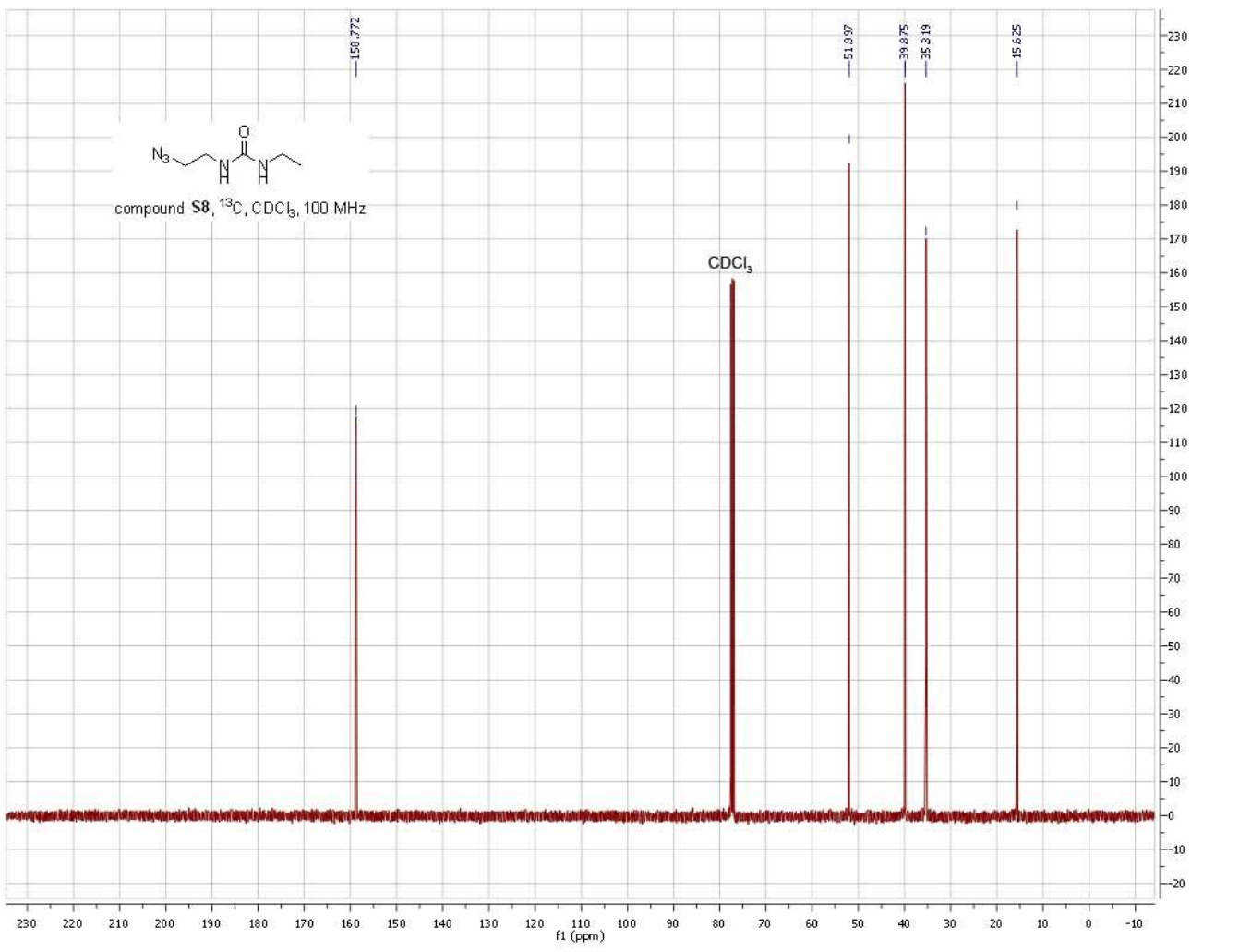
Mero76, HPLC data:

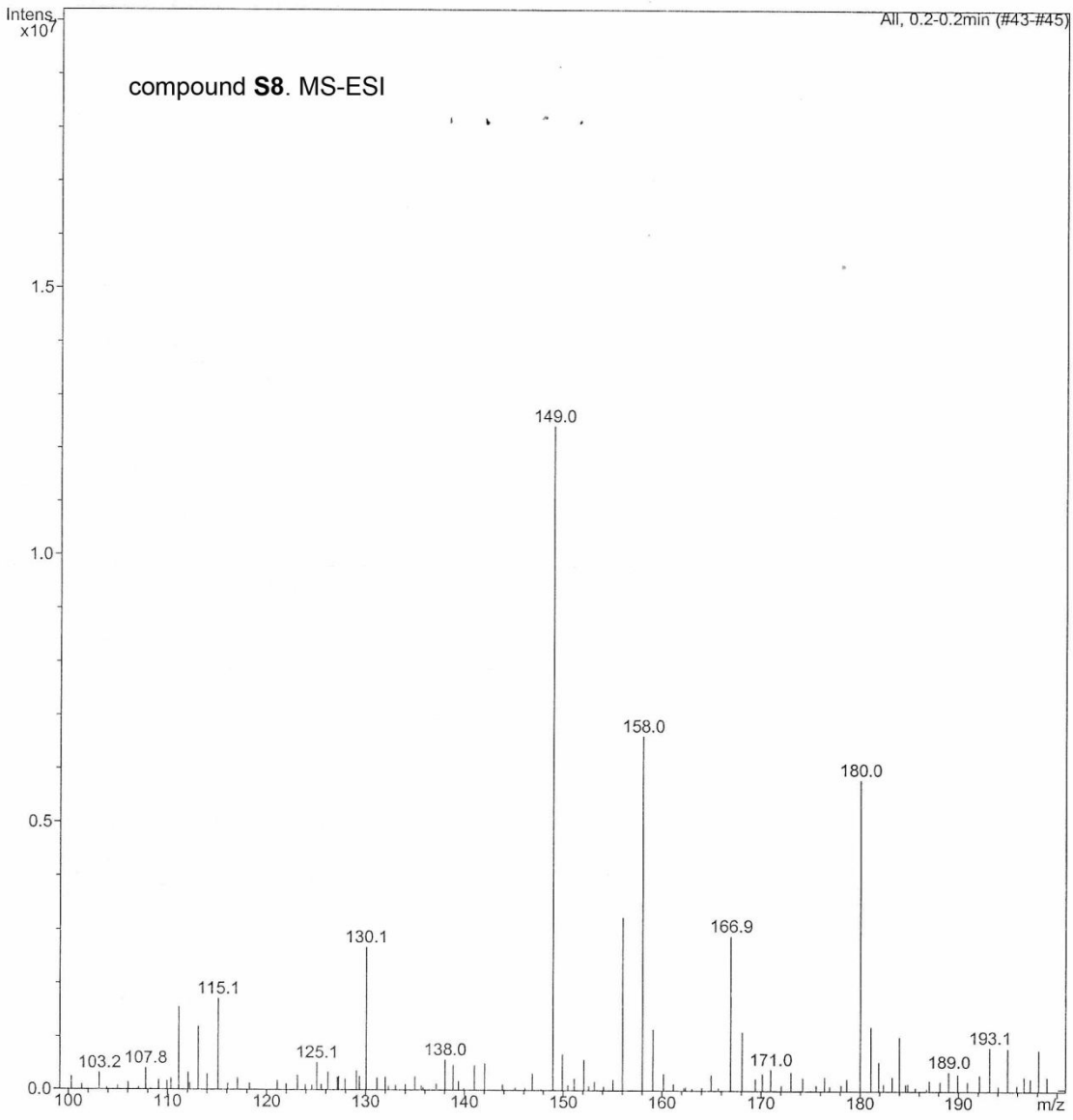
Peak#	Ret. Time	Area	Height	Peak Start	Peak End	Area%
1	30.880	137617	6106	30.475	31.680	0.1792
2	34.379	76520839	3999815	33.387	40.501	99.6338
3	38.191	143638	6156	37.803	38.880	0.1870



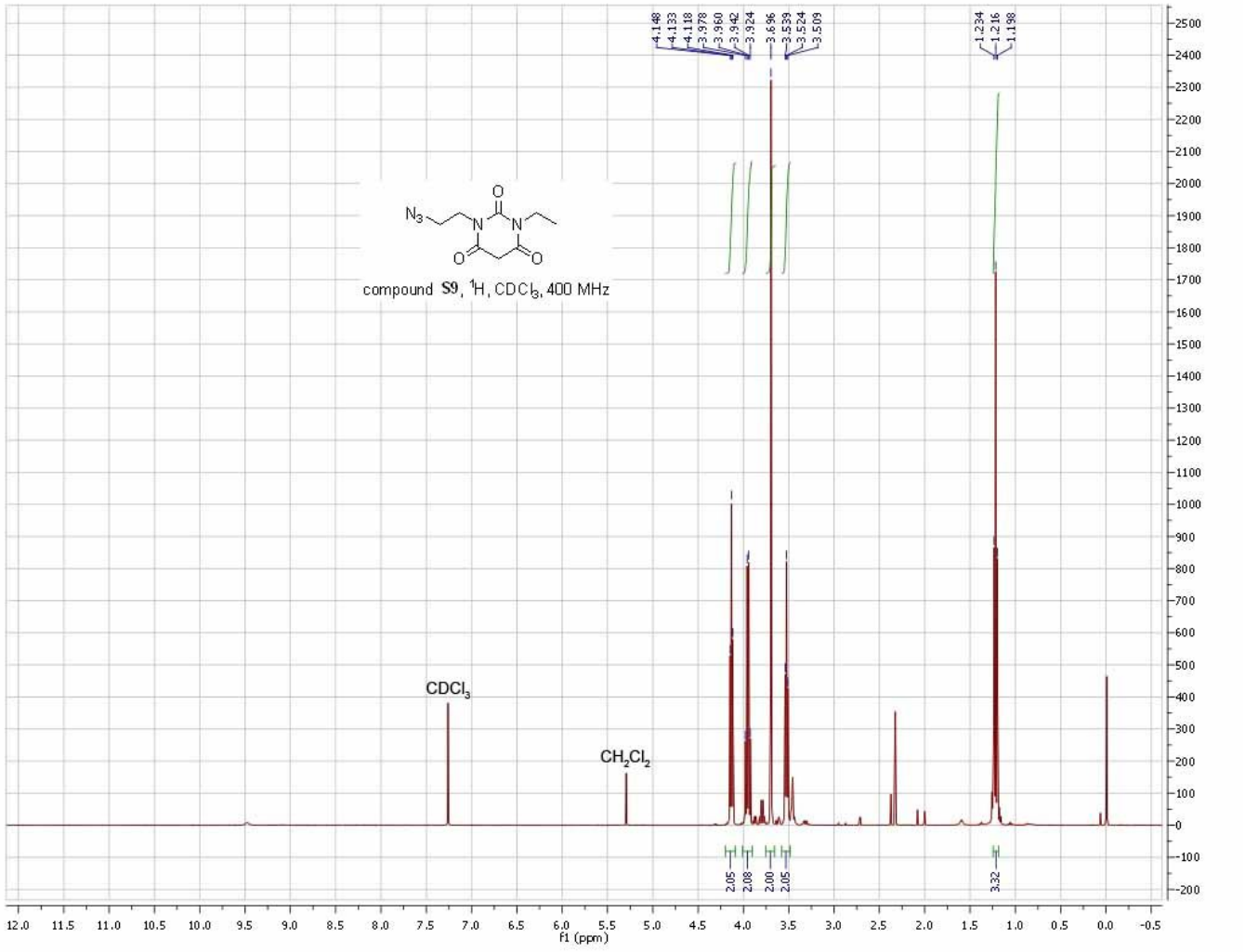
Compound **S8**.

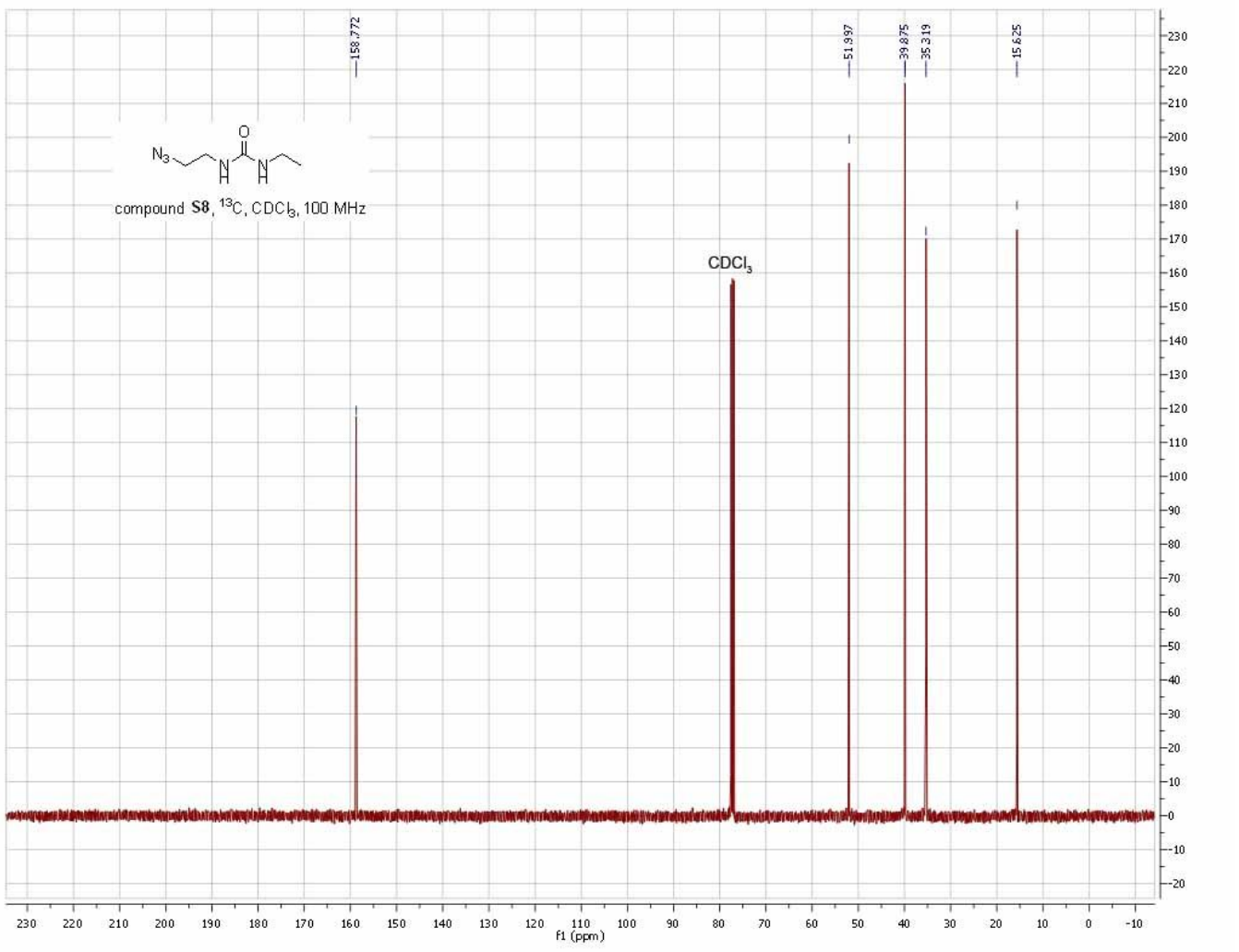

compound S8, ^{1}H, $CDCl_3$, 400 MHz
$CDCl_3$
5.526
5.261
1.132
1.114
1.096
1.00
1.00
3.99
2.02
2.97
f1 (ppm)

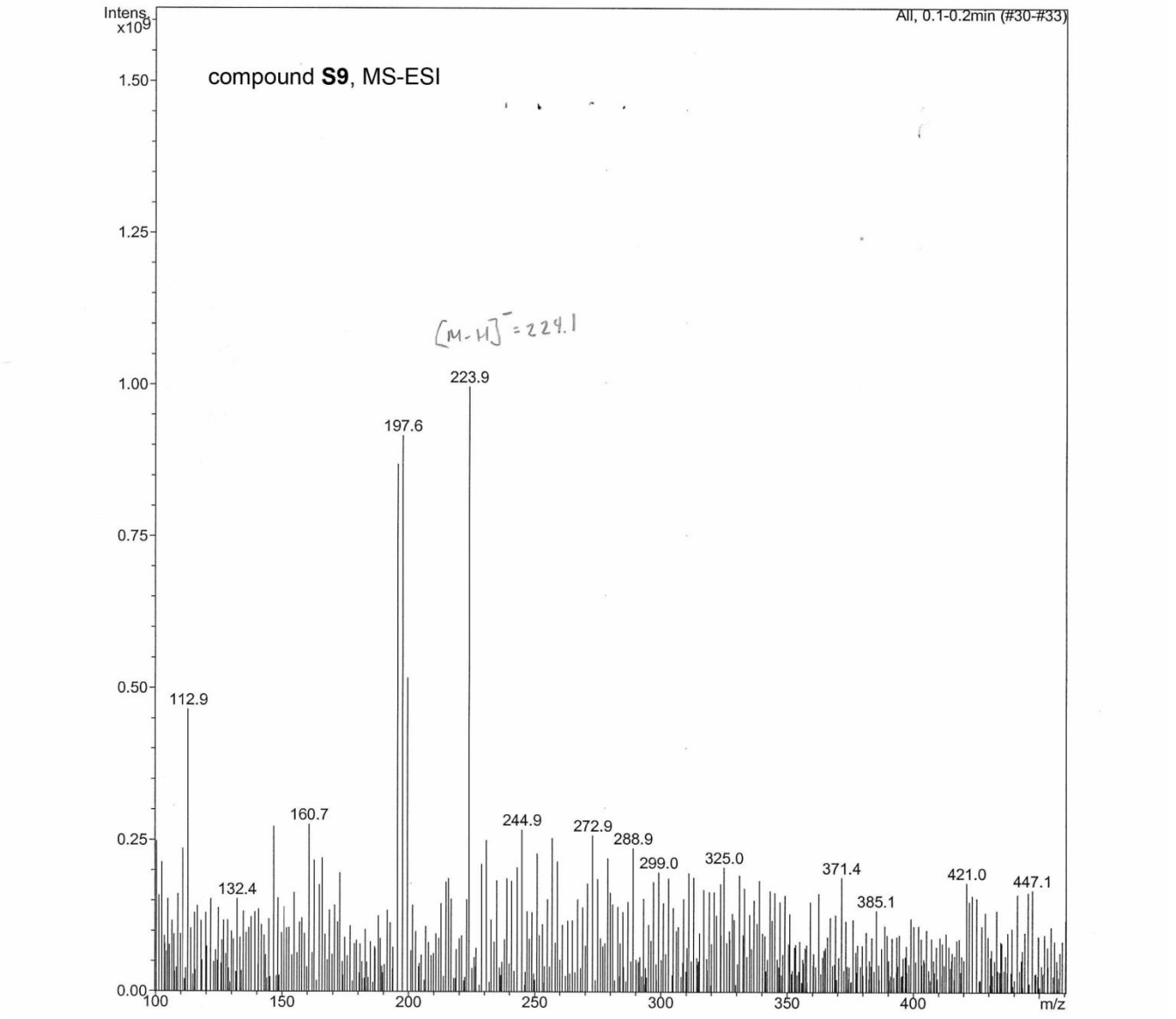

158.772
51.997
39.875
35.319
15.625
CDCl3
compound S8, 13C, CDCl3, 100 MHz
f1 (ppm)


compound **S8**. MS-ESI
Intens.
x10⁷
All, 0.2-0.2min (#43-#45)
1.5
1.0
0.5
0.0
149.0
158.0
180.0
166.9
130.1
115.1
103.2
107.8
125.1
138.0
171.0
189.0
193.1
100
110
120
130
140
150
160
170
180
190
m/z

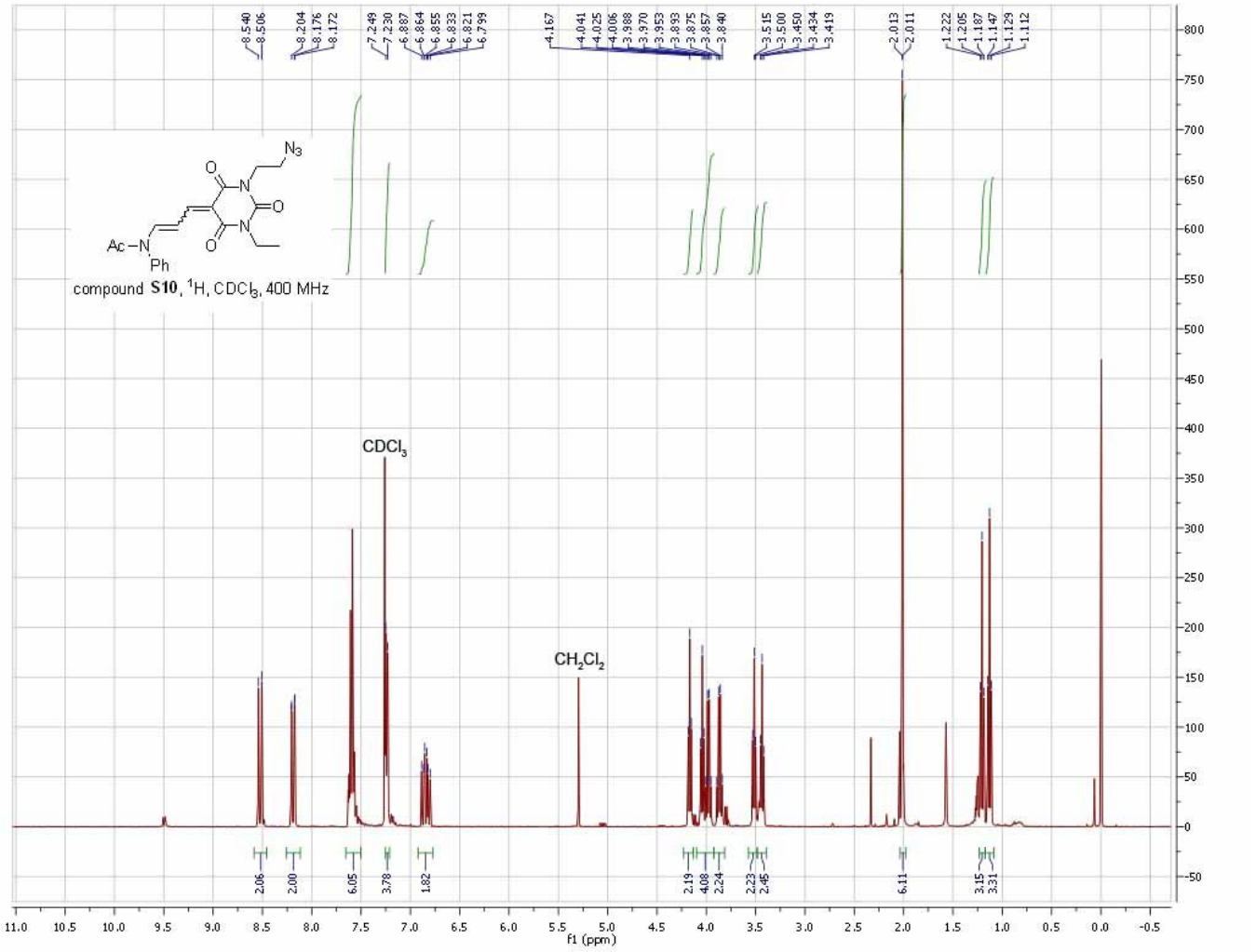
Compound **S9**.

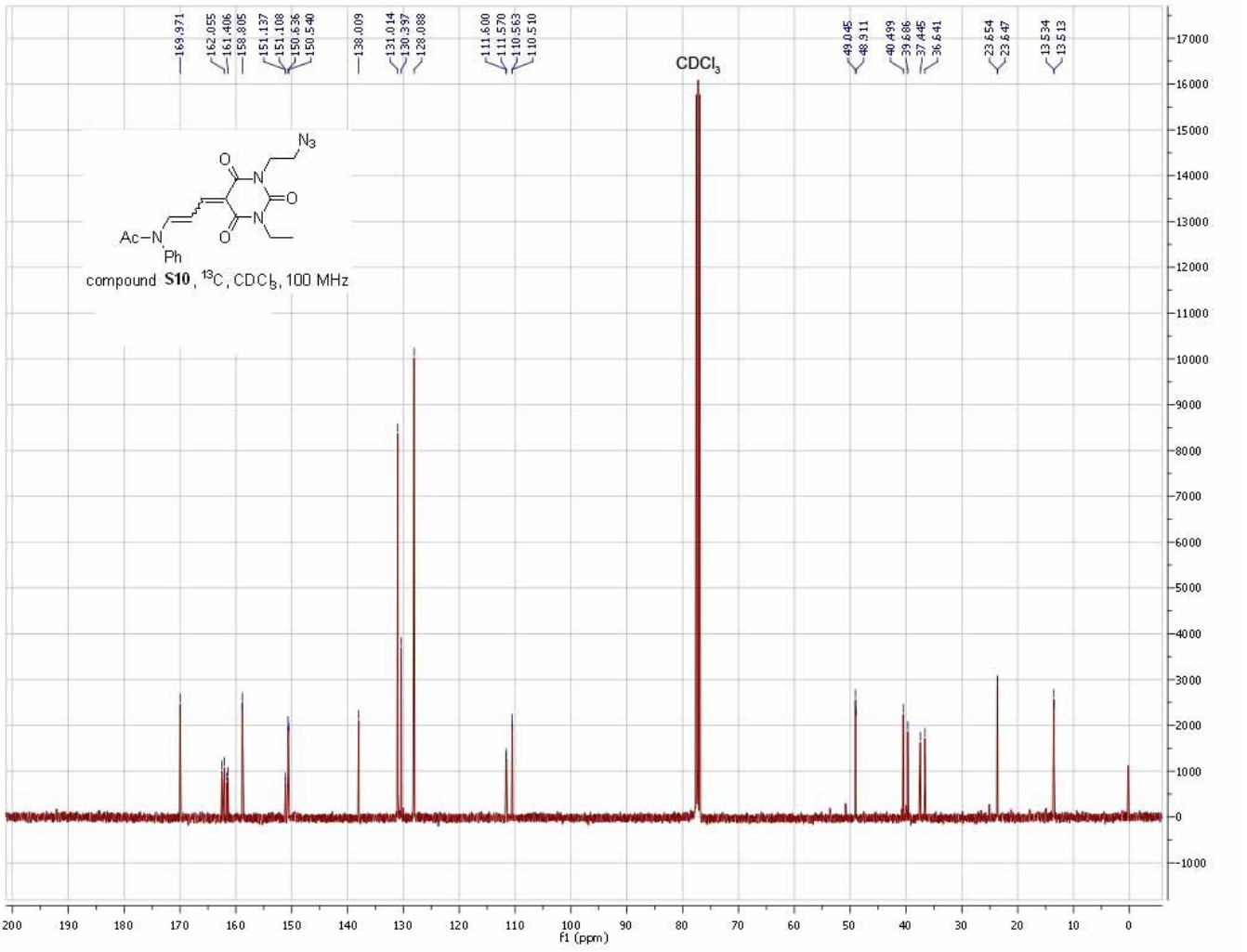


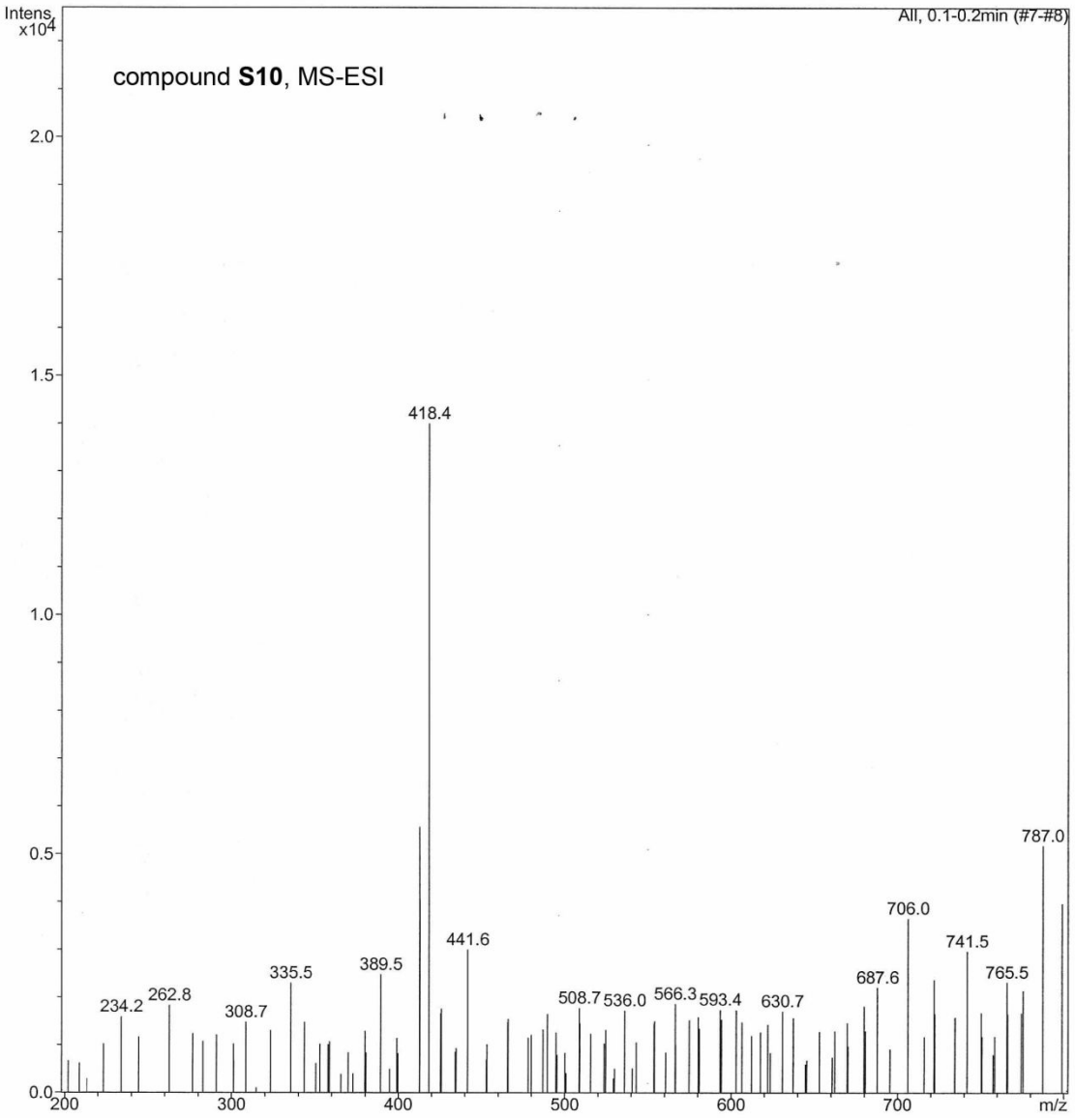

158.772
51.997
39.875
35.319
15.625
CDCl3
compound S8, 13C, CDCl3, 100 MHz
f1 (ppm)


Intens.
x10^9
All, 0.1-0.2min (#30-#33)
compound **S9**, MS-ESI
[M-H]⁻ = 224.1
1.50
1.25
1.00
0.75
0.50
0.25
0.00
223.9
197.6
112.9
160.7
244.9
272.9
288.9
299.0
325.0
371.4
385.1
421.0
447.1
132.4
100
150
200
250
300
350
400
m/z

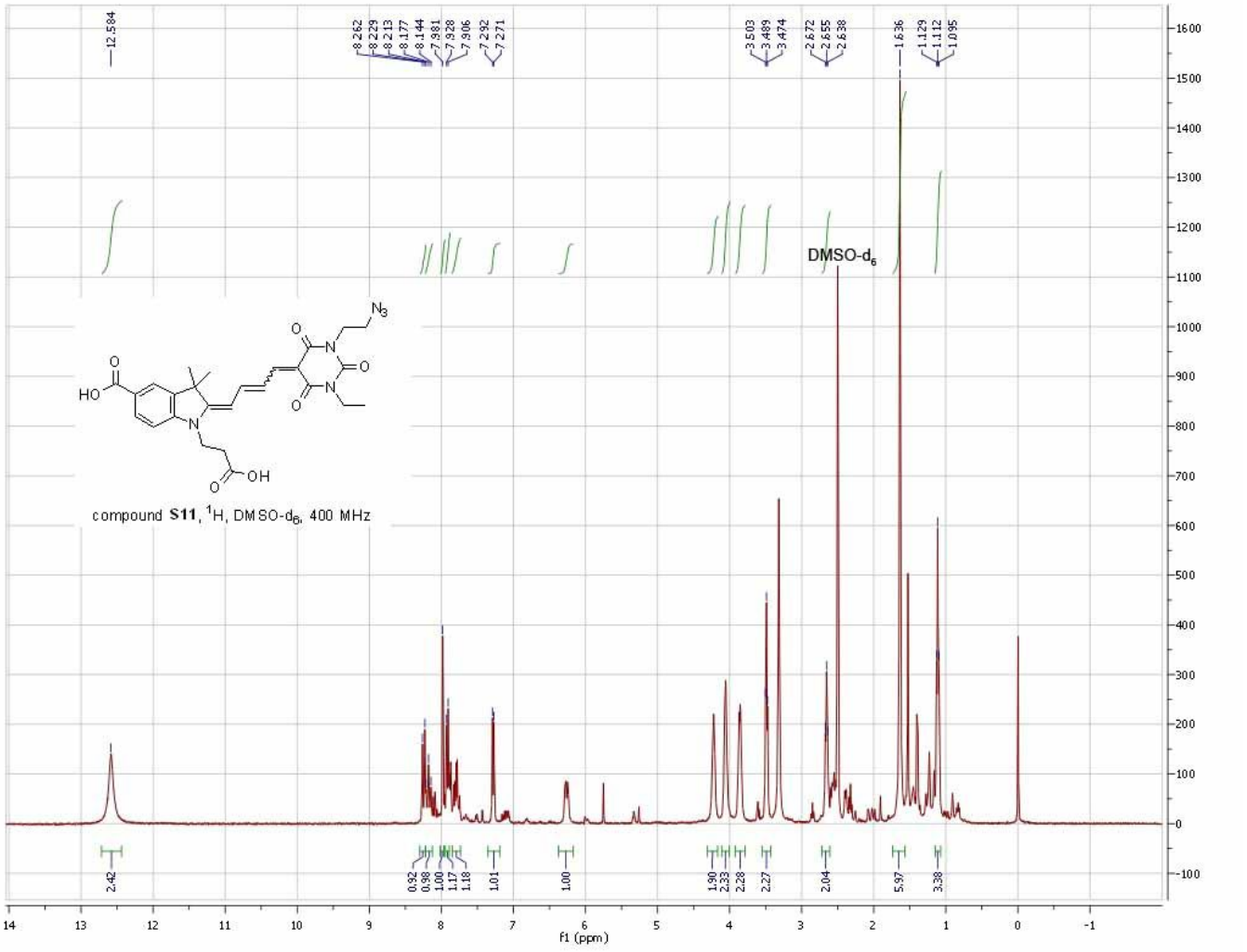
Compound **S10**.

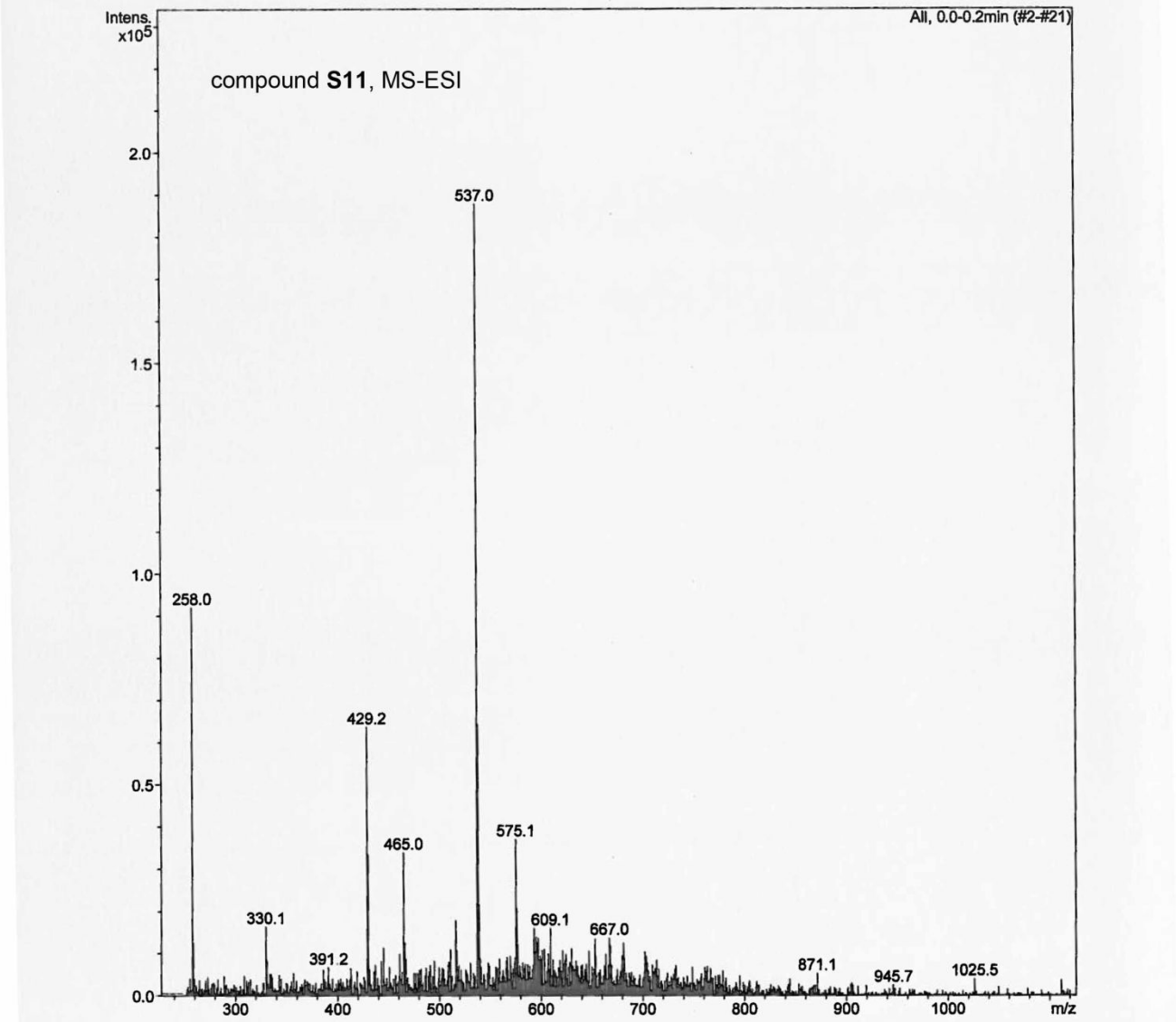



169.971
162.055
161.406
158.805
151.137
151.108
150.636
150.540
138.009
131.014
130.397
128.088
111.600
111.570
110.563
110.510
CDCl3
49.045
48.911
40.499
39.686
37.445
36.641
23.654
23.647
13.534
13.513
N3
O
N
O
N
O
Ac–N
Ph
compound S10, 13C, CDCl3, 100 MHz
f1 (ppm)

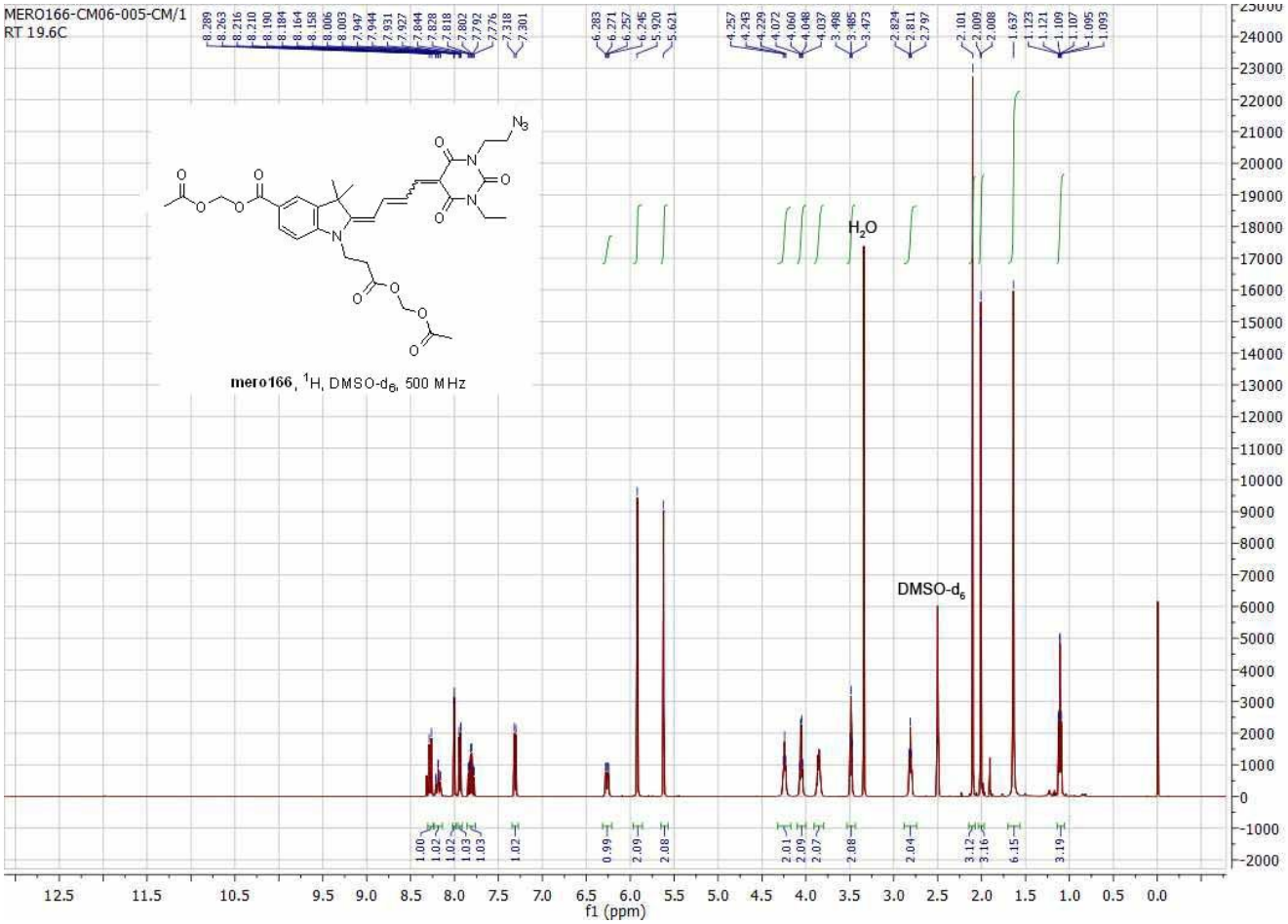

Intens.
x10^4
All, 0.1-0.2min (#7-#8)
compound **S10**, MS-ESI
2.0
1.5
1.0
0.5
0.0
418.4
787.0
706.0
441.6
741.5
389.5
335.5
765.5
687.6
262.8
234.2
308.7
508.7
536.0
566.3
593.4
630.7
200
300
400
500
600
700
m/z

Compound **S11**.



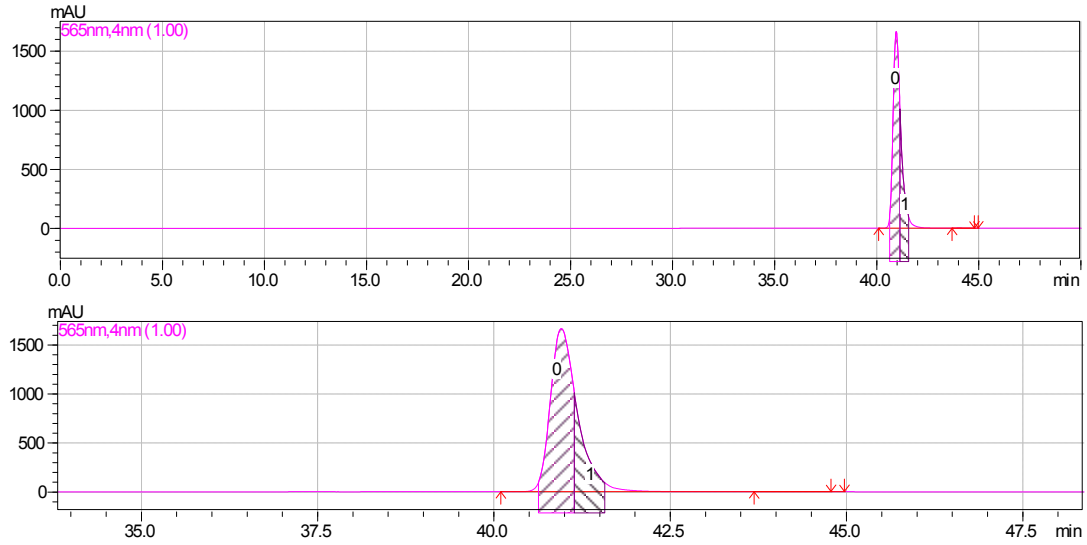

Intens.
x10^5
All, 0.0-0.2min (#2-#21)
compound **S11**, MS-ESI
2.0
1.5
1.0
0.5
0.0
537.0
258.0
429.2
465.0
575.1
330.1
609.1
667.0
391.2
871.1
945.7
1025.5
300
400
500
600
700
800
900
1000
m/z

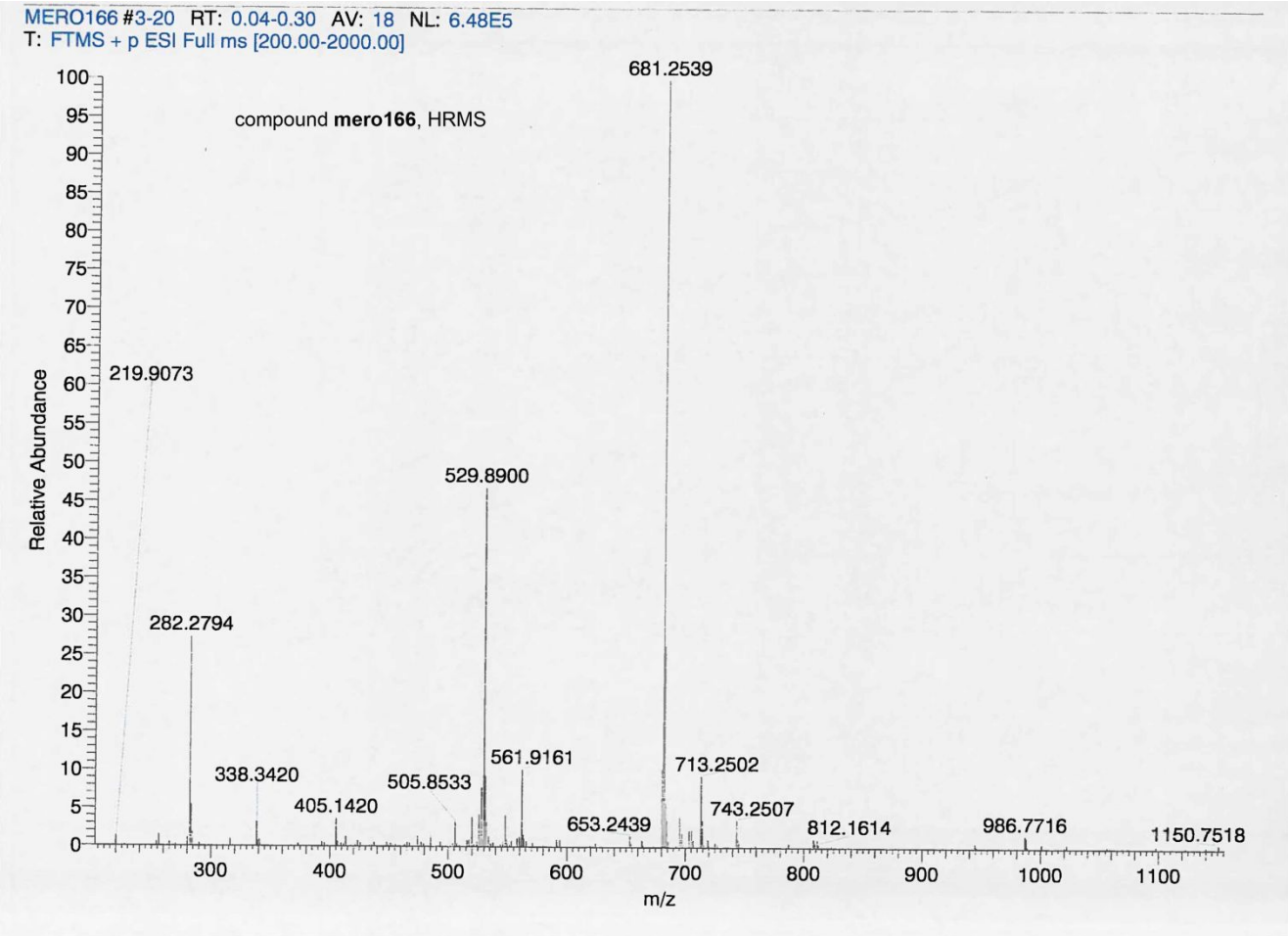
Compound **mero166**.



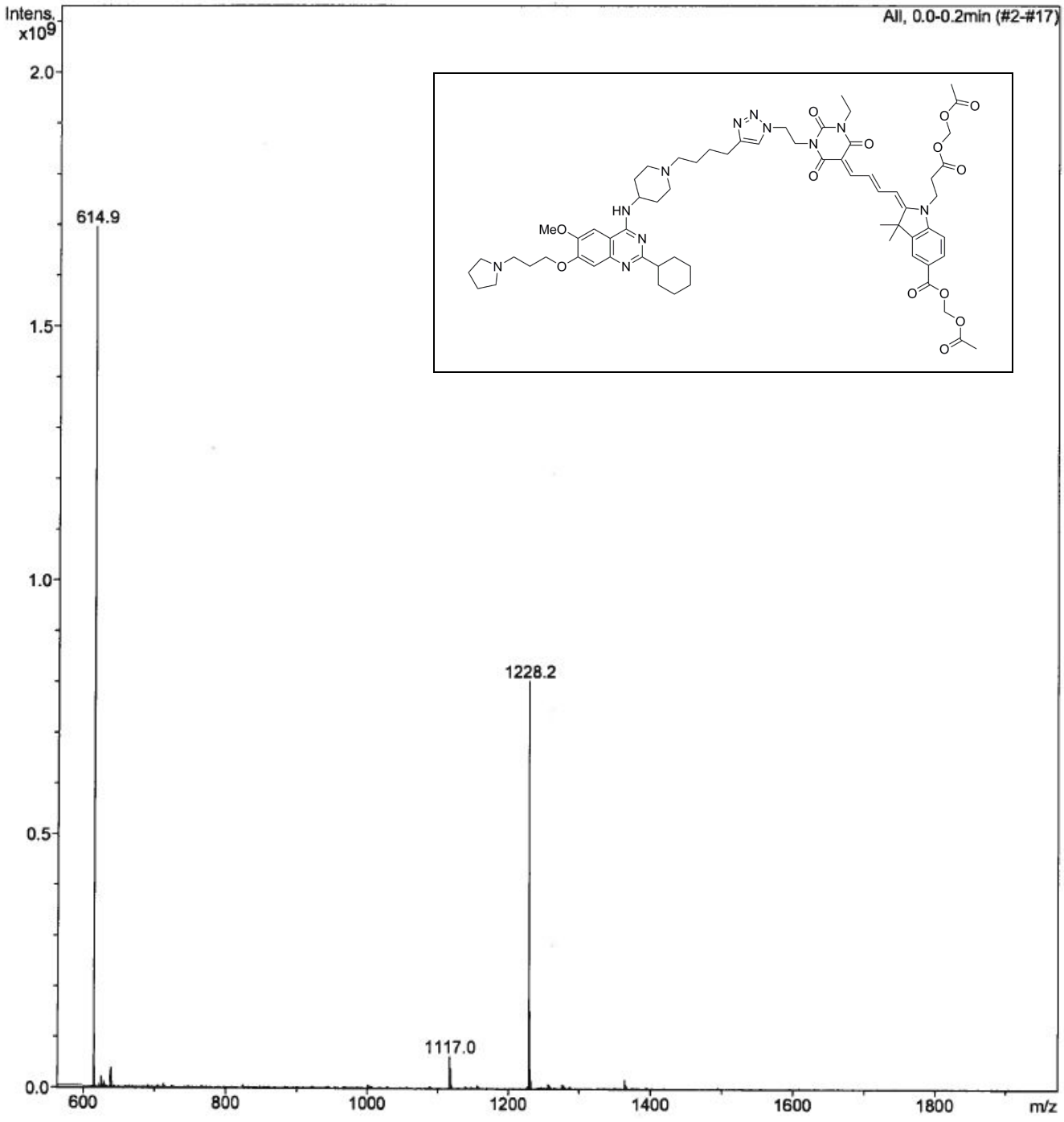
Mero166, HPLC data:

Peak#	Ret. Time	Area	Height	Peak Start	Peak End	Area%
1	40.950	44804980	1665138	40.096	44.971	99.9349
2	44.055	29202	1189	43.691	44.779	0.0651

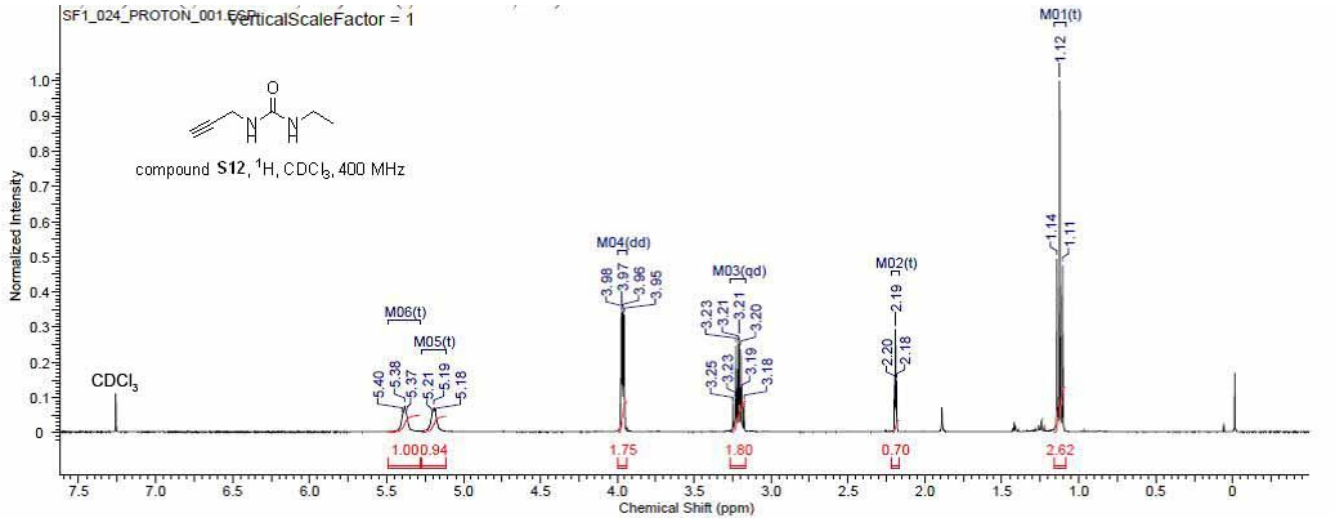


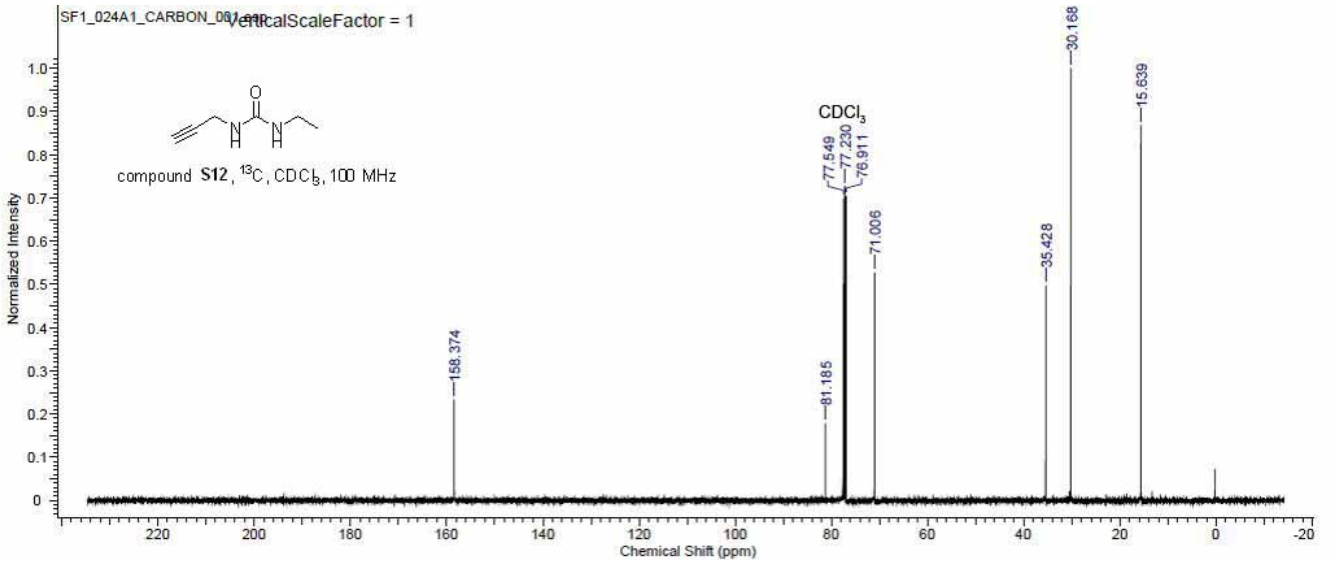

MERO166 #3-20 RT: 0.04-0.30 AV: 18 NL: 6.48E5
T: FTMS + p ESI Full ms [200.00-2000.00]
compound mero166, HRMS
Relative Abundance
m/z
681.2539
219.9073
529.8900
282.2794
561.9161
338.3420
505.8533
713.2502
405.1420
743.2507
653.2439
812.1614
986.7716
1150.7518

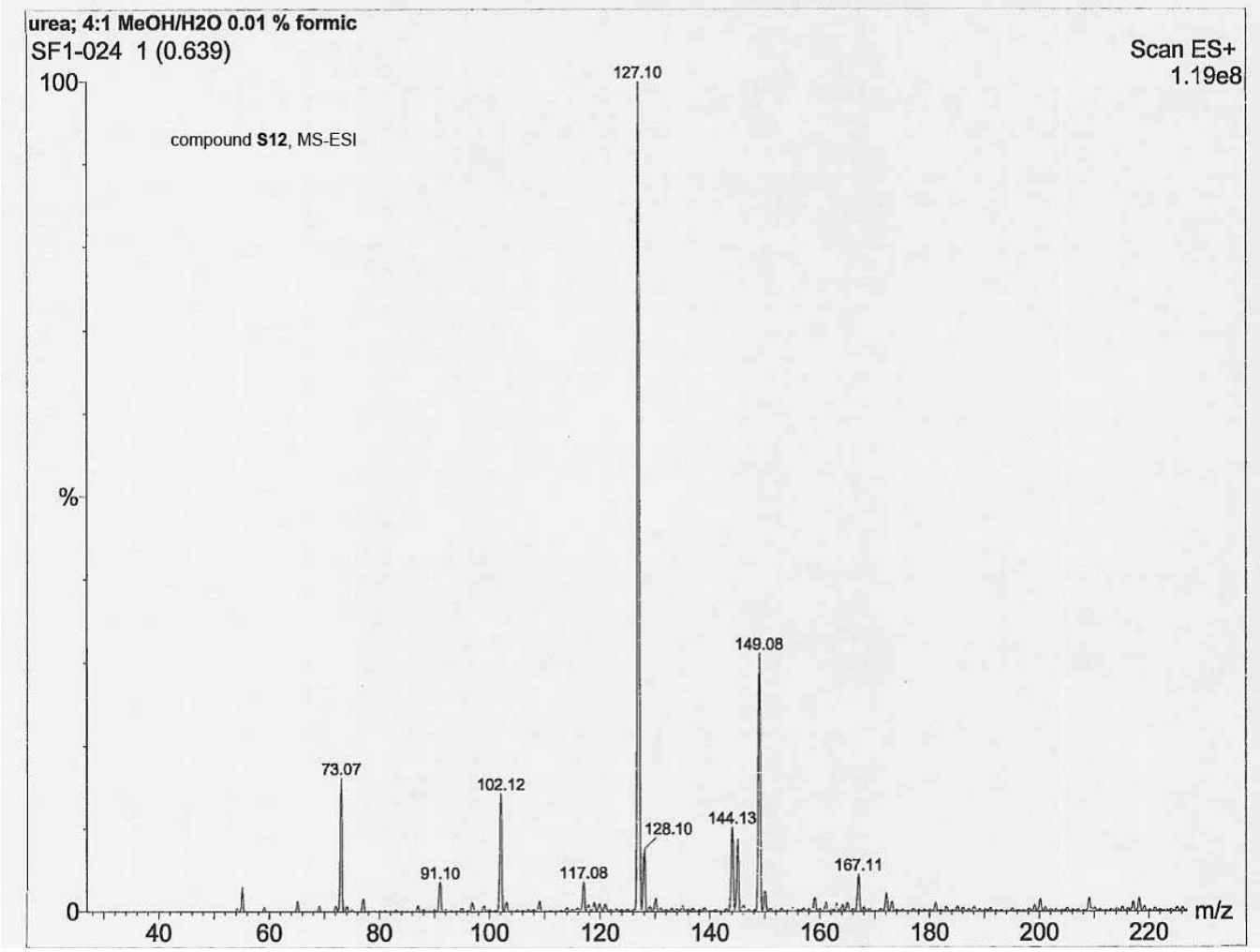
Compound **12** (G9a ligand – mero166 conjugate)



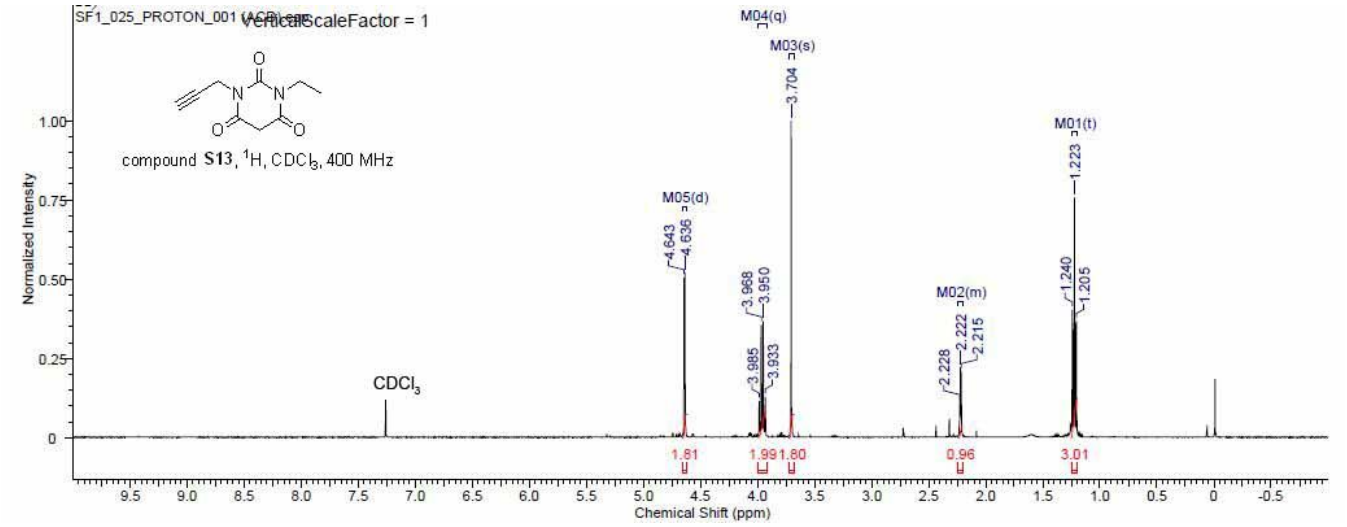
Compound **S12**

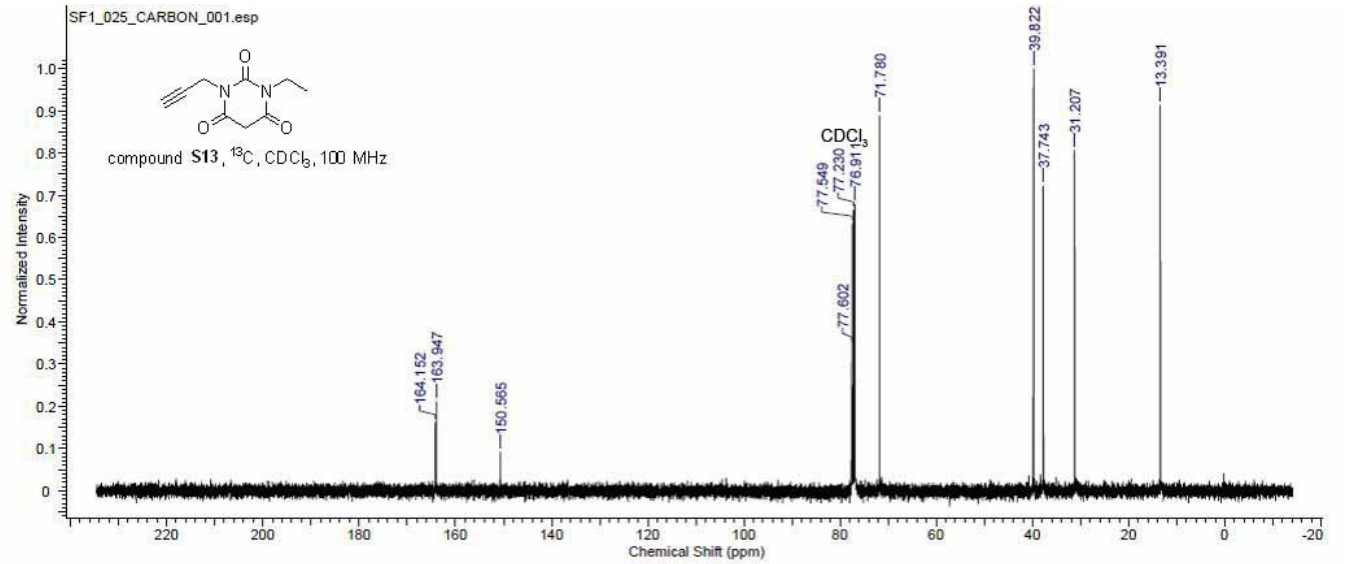


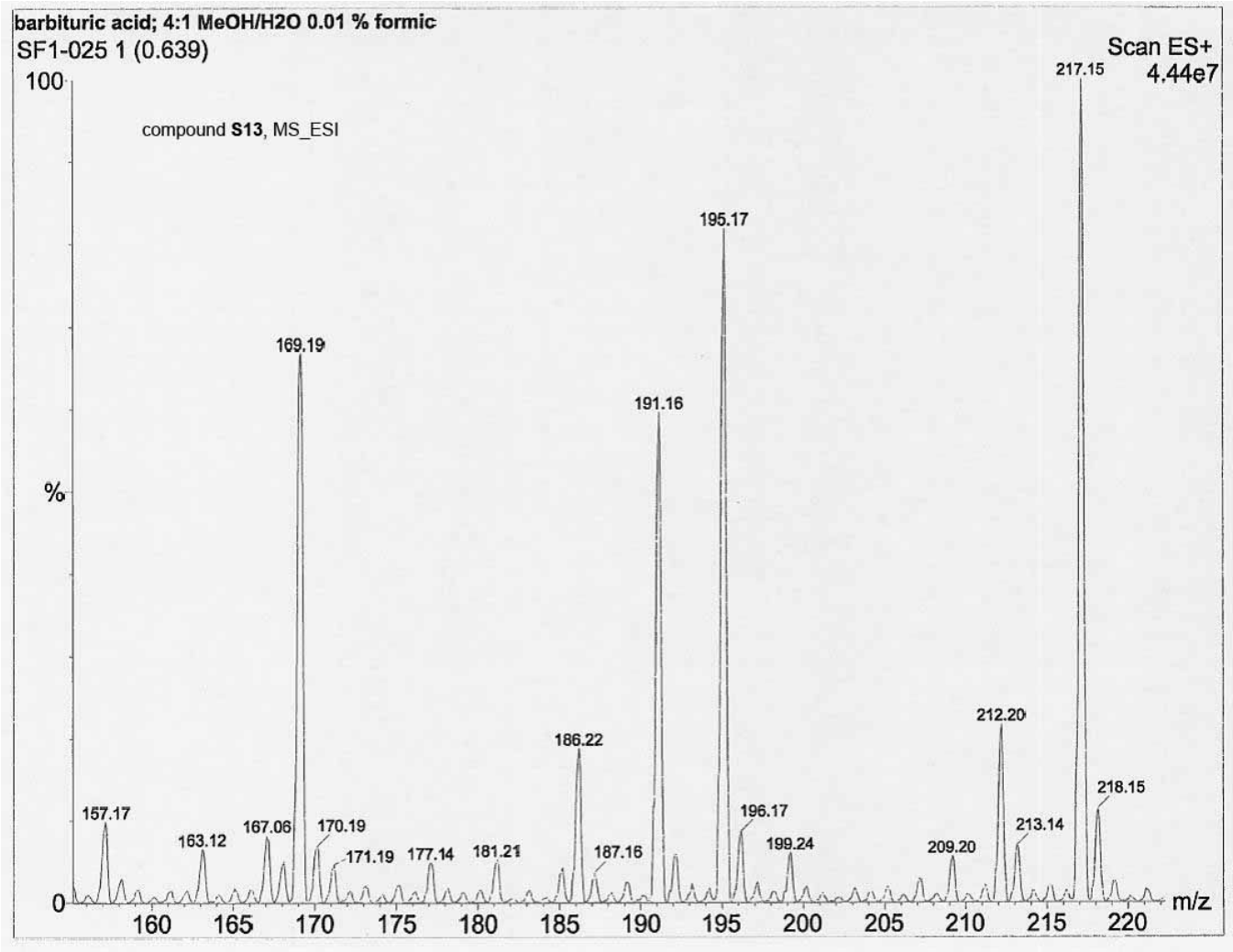



urea; 4:1 MeOH/H2O 0.01 % formic
SF1-024 1 (0.639)
Scan ES+
1.19e8
100
%
0
compound S12, MS-ESI
127.10
149.08
73.07
102.12
144.13
128.10
91.10
117.08
167.11
40
60
80
100
120
140
160
180
200
220
m/z

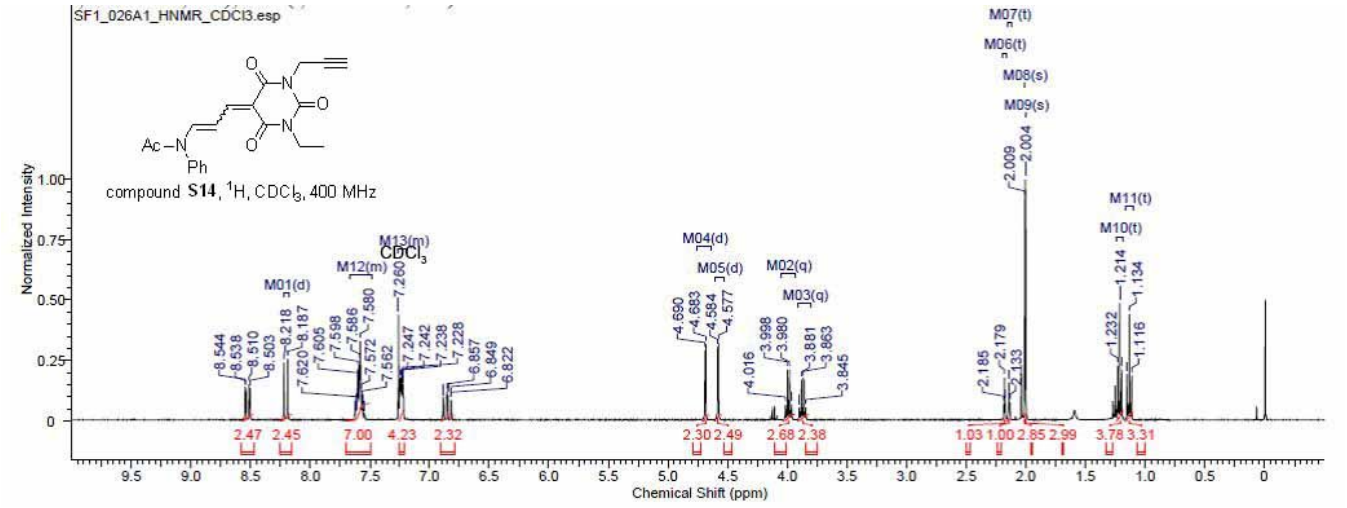
Compound **S13**

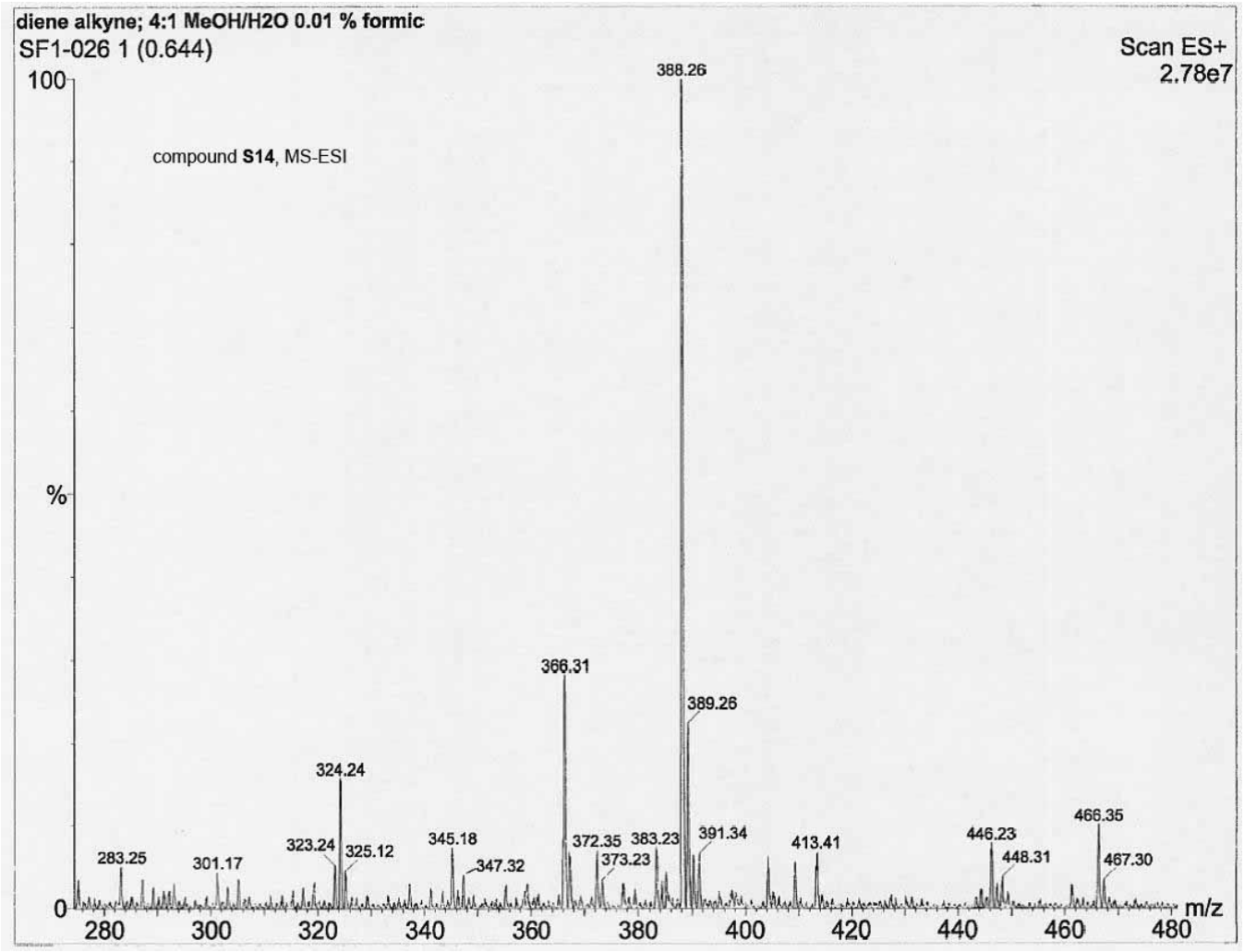




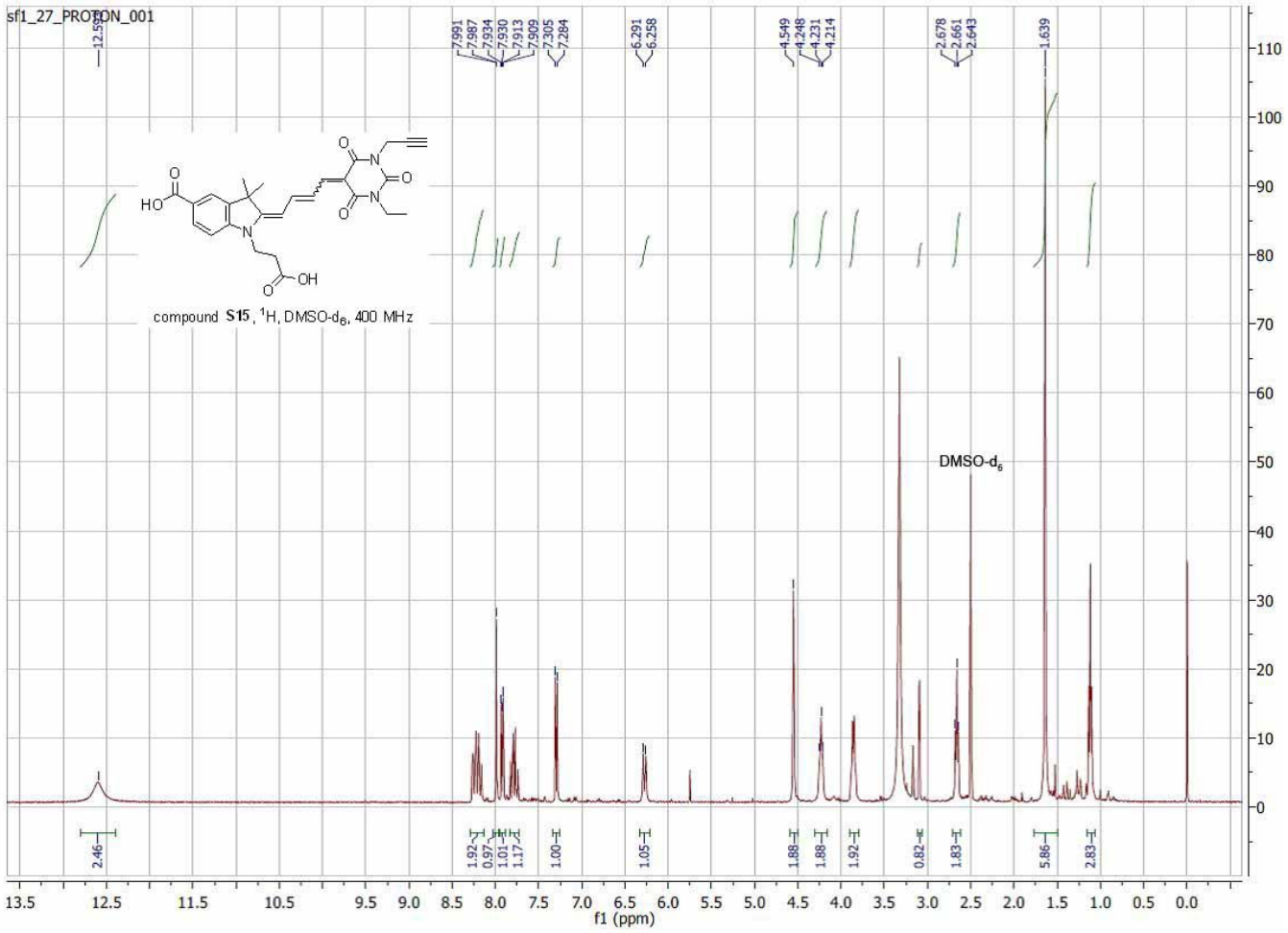

barbituric acid; 4:1 MeOH/H2O 0.01 % formic
SF1-025 1 (0.639)
Scan ES+
4.44e7
100
%
0
compound S13, MS_ESI
217.15
195.17
169.19
191.16
212.20
186.22
218.15
157.17
196.17
213.14
163.12
167.06
170.19
199.24
209.20
171.19
177.14
181.21
187.16
m/z
160
165
170
175
180
185
190
195
200
205
210
215
220

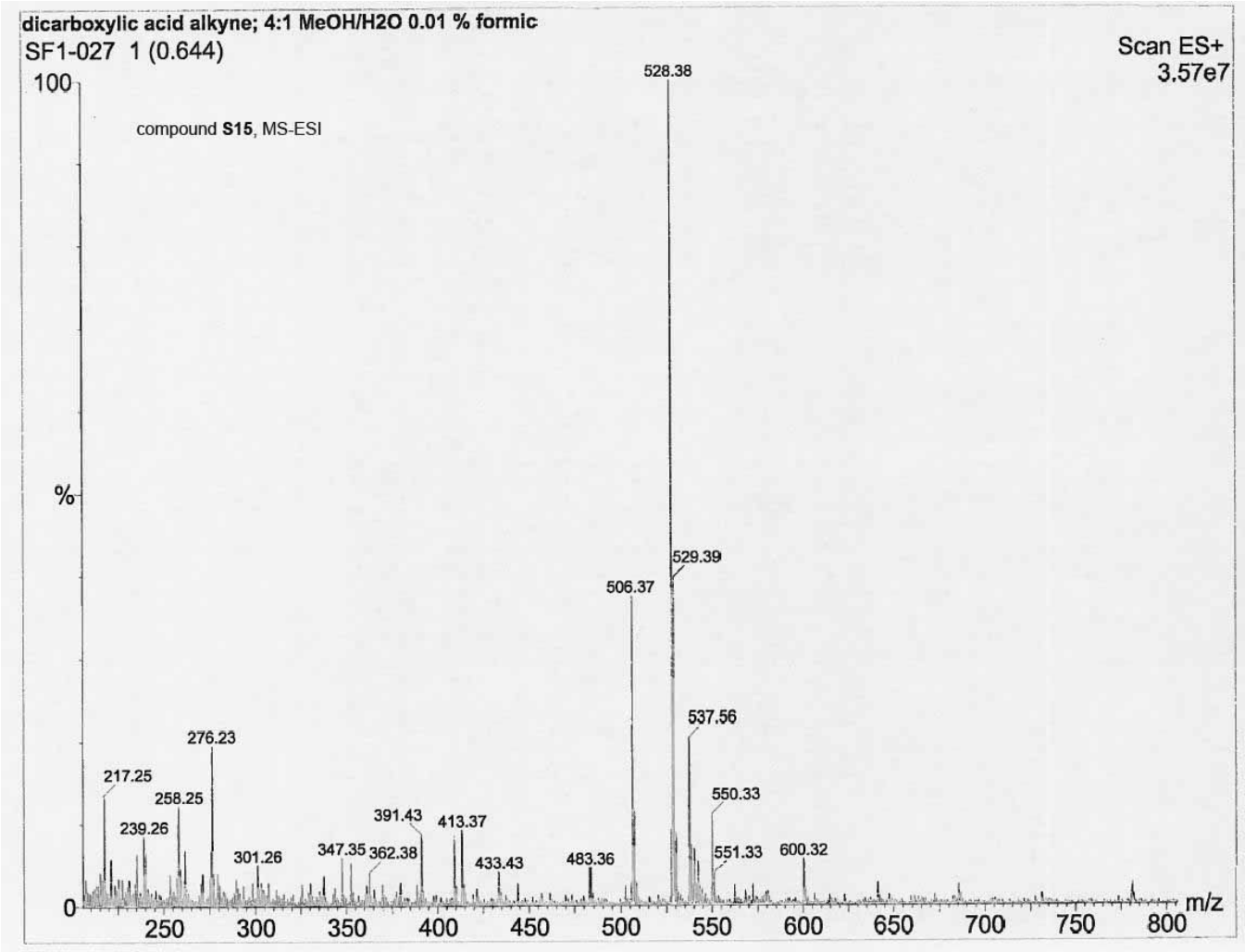
Compound **S14**



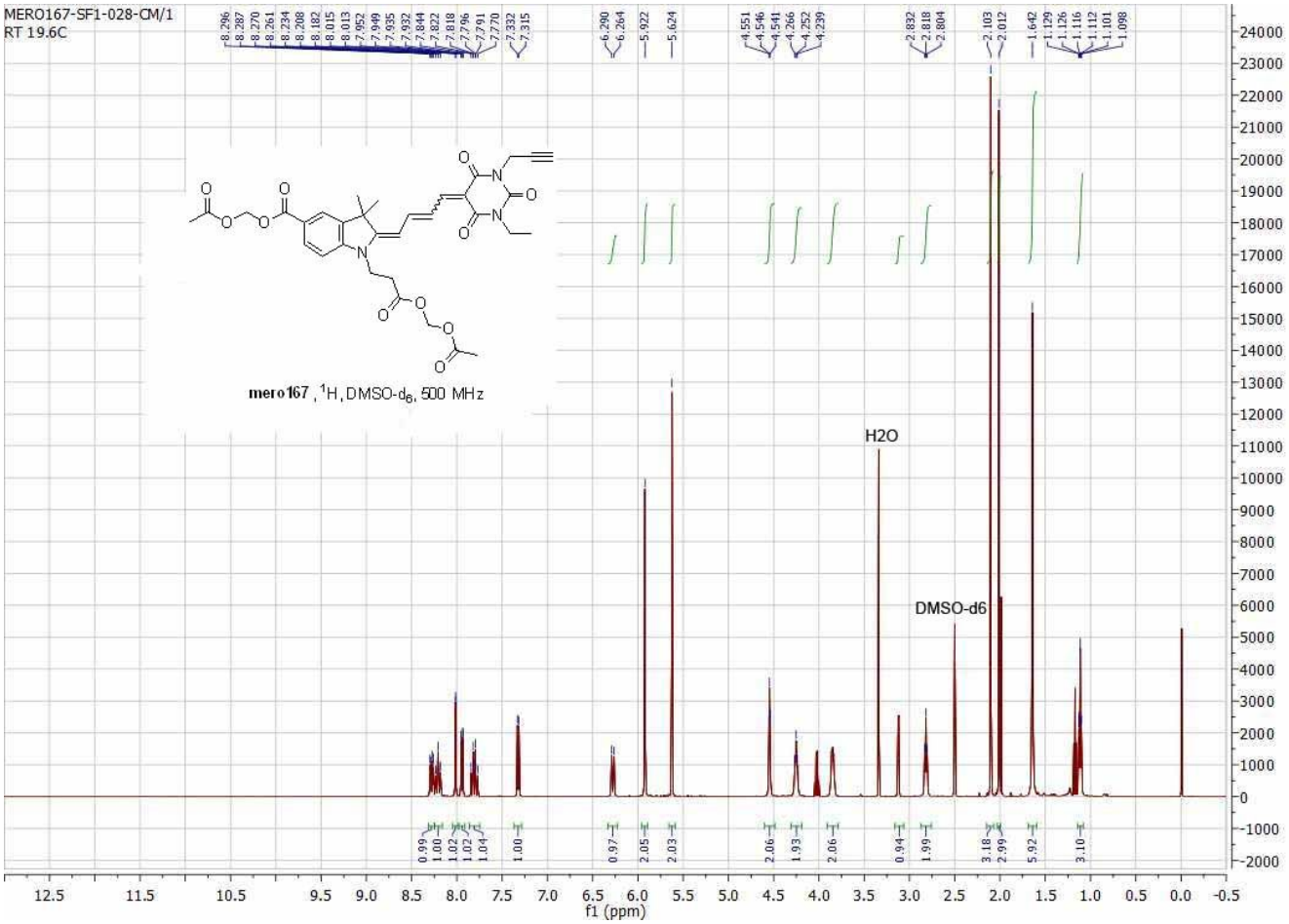


Compound **S15**



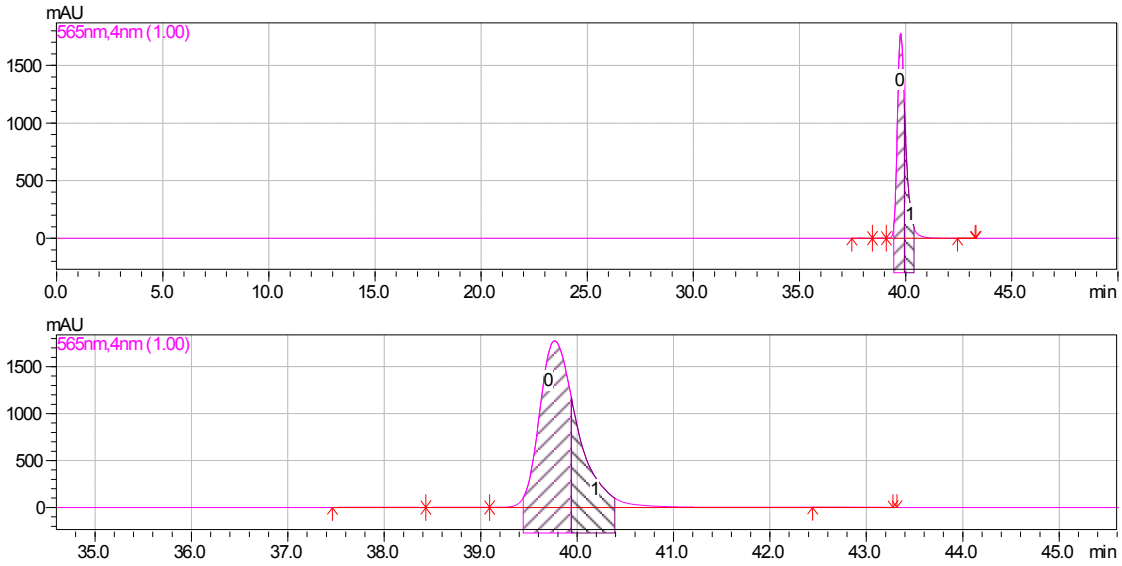

dicarboxylic acid alkyne; 4:1 MeOH/H2O 0.01 % formic
SF1-027 1 (0.644)
Scan ES+
3.57e7
100
%
0
compound S15, MS-ESI
528.38
529.39
506.37
537.56
276.23
217.25
258.25
239.26
550.33
391.43
413.37
551.33
600.32
301.26
347.35
362.38
433.43
483.36
250
300
350
400
450
500
550
600
650
700
750
800
m/z

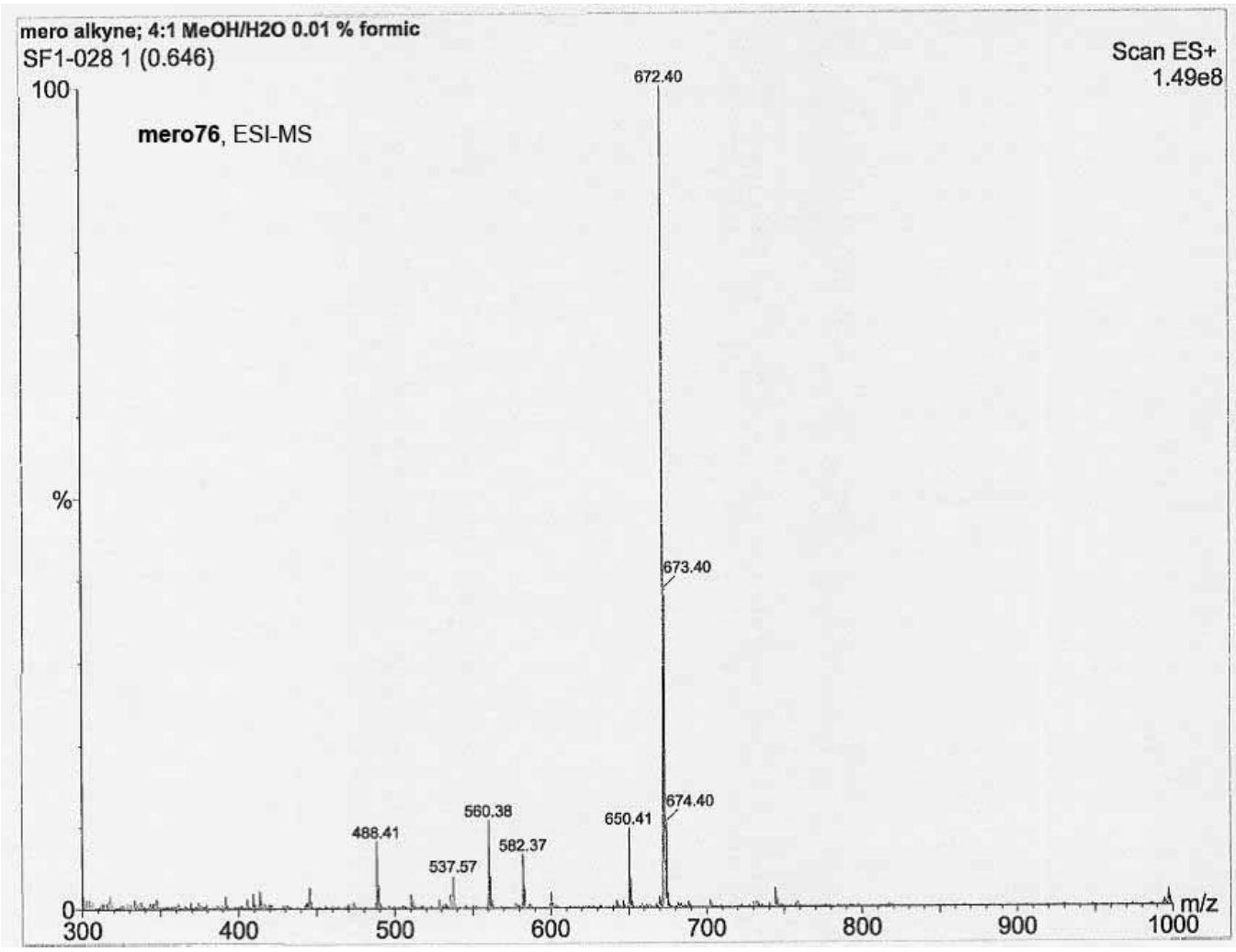
Compound **mero167**



Mero167, HPLC data:

Peak#	Ret. Time	Area	Height	Peak Start	Peak End	Area%
1	37.832	27841	1168	37.461	38.432	0.0571
2	38.834	25957	1119	38.432	39.093	0.0532
3	39.762	48683068	1776210	39.093	43.317	99.8617
4	42.683	13647	554	42.443	43.275	0.0280




mero alkyne; 4:1 MeOH/H2O 0.01 % formic
SF1-028 1 (0.646)
Scan ES+
1.49e8
100
mero76, ESI-MS
672.40
673.40
674.40
650.41
560.38
582.37
488.41
537.57
%
0
m/z
300
400
500
600
700
800
900
1000

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