Supplementary Material

Optimization of Substituted Imidazobenzodiazpines as Novel Asthma Treatments

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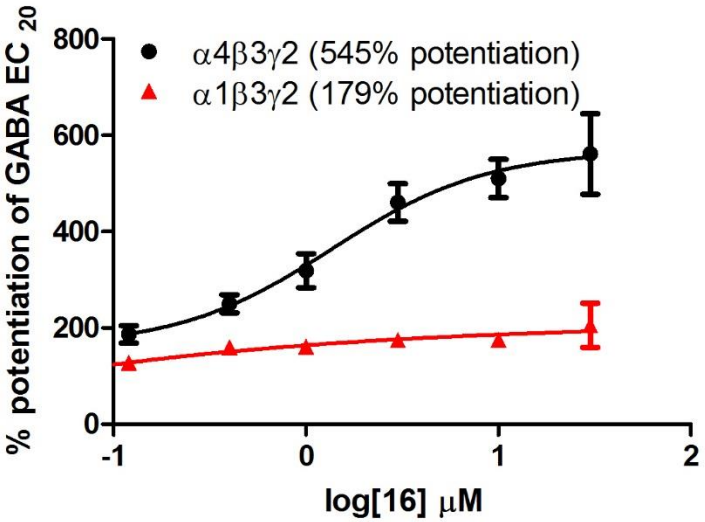
Patch clamp assay: HEK293T stably expressing α1β3γ2 $GABA_AR$ or α4β3γ2 were maintained RPMI 1640 medium with L-glutamine supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin. Automated patch-clamp studies were conducted as described previously.[1] Briefly, the IonFlux plate layout consists of units of 12 wells: two wells contain intracellular solution (ICS containing 140 mM CsCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM EGTA, 10 mM HEPES, pH 7.2 with CsOH), one contains cells diluted in extracellular solution (ECS containing 140 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 10 mM D-glucose monohydrate, and 10 mM HEPES, pH 7.4 with NaOH), eight contain different concentration of **16** in the presence of GABA at 0.1% DMSO. Well 1 is for waste collection. Cells are captured from suspension by

applying suction to microscopic channels in ensemble recording arrays. Once the array is fully occupied, the applied suction breaks the membranes of captured cells, which establishes whole cell voltage clamp. For compound applications, pressure is applied to the appropriate compound wells, which introduces the compound into the extracellular solution rapidly flowing over the cells. For recording $GABA_AR$ induced currents, cell arrays were voltage clamped at a hyperpolarizing holding potential of −80 mV. Prior to use on the automated patch clamp, cells were centrifuged at 380g for 5 minutes and resuspended gently in ECS. This was repeated two more times before the cells were dispensed into the plate. All compound application were carried out for 3 seconds followed by a 5 second washout.



Microsomal stability assay procedure

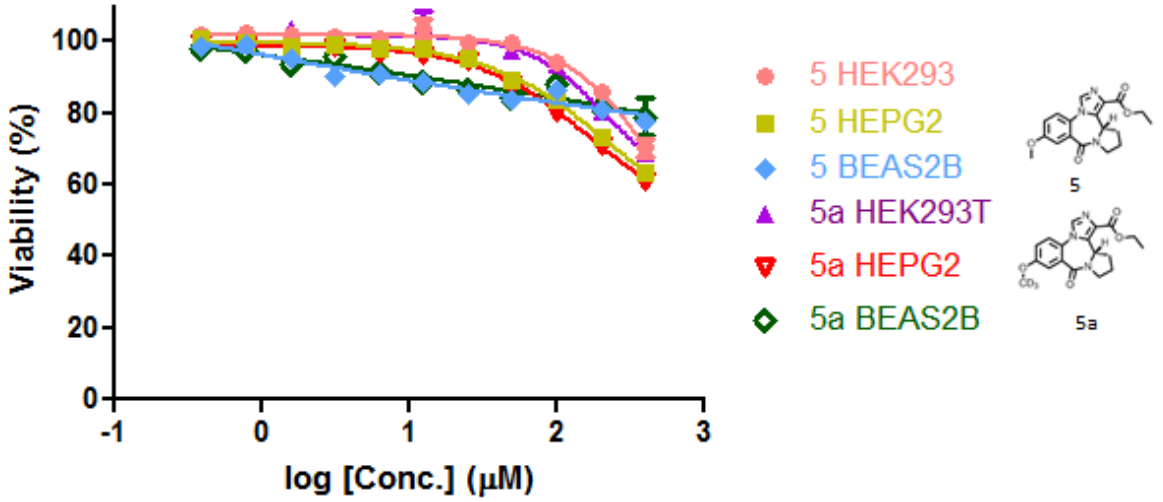
4 µL of 1 mM test compound at a final concentration of 10 µM in DMSO were preincubated at 37°C for 5 minutes on a digital heating shaking dry bath (Fischer scientific, Pittsburgh, PA) in a mixture containing 282 µL of water, 80 µL of phosphate buffer (0.5 M, pH 7.4) 20 µL of NADPH Regenerating System Solution A (BD Bioscience, San Jose, CA) and 4 µL of NADPH Regenerating System Solution B (BD Bioscience, San Jose, CA) in a total volume of 391.2 µL. Following preincubation, the reaction was initiated by addition of 8.8 µL of either human liver microsomes (BD Gentest, San Jose, CA) or mouse liver microsomes (Life technologies, Rockford, IL) at a protein concentration of 0.5 mg/mL. Aliquots of 50 µL were taken at time intervals of 0 (without microsomes), 10, 20, 30, 40, 50 and 60 minutes. Each aliquot was added to 100 µL of cold acetonitrile solution containing 1 µM of verapamil HCl as internal standard. This was followed by sonication for 10 seconds and centrifugation at 10,000 rpm for 5 minutes. 100 µL of the supernatant was transferred into Spin-X HPLC filter tubes (Corning Incorporated, NY) and centrifuged at 13,000 rpm for 5 minutes. The filtrate was diluted 100 fold and subsequently analyzed by LC-MS/MS with Shimadzu LCMS 8040, (Shimadzu Scientific Instruments, Columbia, MD). The ratio of the peak areas of the internal standard and test compound was calculated for every time point and the natural log of the ratio were plotted against time to

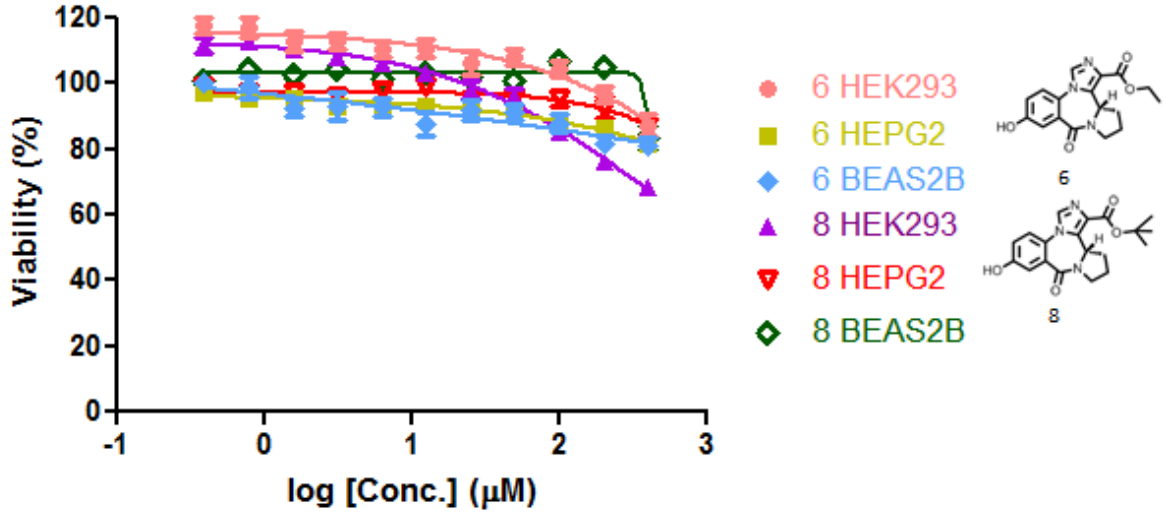
determine the linear slope (k). The metabolic rate ($k*C_0/C$), half-life (0.693/k), and internal clearance (V*k) were calculated, where k is the slope, C_0 is the initial concentration of test compound, C is the concentration of microsomes, and V is the volume of incubation in µL per microsomal protein in mg. All experiments were repeated three times in duplicates.

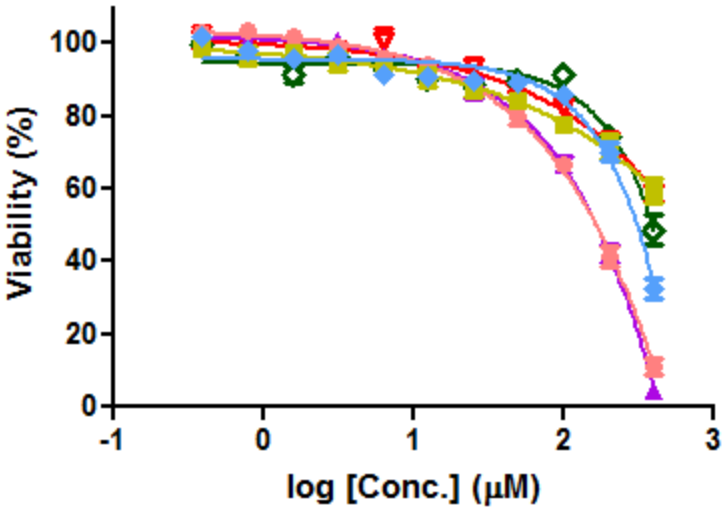
Entry	Compound	Microsomal stability (human) Half-life (min)	Microsomal stability (human) % left after 1 hour	Microsomal stability (mouse) Half-life (min)	Microsomal stability (mouse) % left after 1 hour
1	**5**	-	99.1 ± 0.1	318.3 ± 66	85.4 ± 0.3
2	**5a**	841± 133	92.8 ± 0.3	351 ± 96	85.5 ± 0.3
3	**7**	713 ± 171	92.1 ± 1.0	24.1 ± 0.8	16.2 ± 0.2
4	**7a**	1014 ± 241	91.9 ± 0.4	25.7 ± 1.1	17.6 ± 0.2
5	**6**	659 ± 178	91.74 ± 0.2	540 ± 83	91.05 ± 0.14
6	**8**	579 ± 105	90.55 ± 0.15	57.6 ± 2.8	46.6 ± 0.3
7	**9**	80.1 ± 10.1	56.1 ± 0.5	73.4 ± 6.5	52.9 ± 0.5
8	**9a**	336 ± 70	84.6 ± 0.3	282.6 ± 28.5	84.6 ± 0.16
9	**10**	666 ± 200	89.74± 0.22	128.85 ± 8	70.32 ± 0.2
10	**10a**	979 ± 406	92.9± 0.2	285 ± 34	85.88 ± 0.2
11	**11**	169 ± 13	77.8± 0.2	19.5± 1.0	13.38 ± 0.9
12	**11a**	201 ± 18	78.7± 0.2	26.9± 1.0	22.36 ± 0.25
13	**12**	Insoluble	For	Rotarod	Studies
14	**12a**	204 ± 25	77.05 ± 0.25	109.09 ± 9.83	62.48 ± 0.30
15	**13**	Insoluble	For	Rotarod	Studies
16	**13a**	Insoluble	For	Rotarod	studies
17	**14**	Insoluble	For	Rotarod	Studies
18	**14a**	Insoluble	For	Rotarod	Studies
19	**15**	976 ± 466	93.8± 0.3	64.7 ± 2.3	54.3 ± 0.2
20	**15a**	1433 ± 845	94.9 ± 0.2	77.4 ± 5.7	56.8 ± 0.3
21	**16**	705 ± 281	90.7 ± 0.3	63.3 ± 3.4	50.1 ± 0.2
22	**16a**	799.3 ± 217	93.5± 0.2	1482 ± 910	94.8 ± 0.2
23	**17**	732.6 ± 281	92.4± 0.2	283 ± 59	82.5 ± 0.3
24	**17a**	1280 ± 987	96.3± 0.3	273 ± 34	83.1 ± 0.2
25	**18**	194 ± 19	79.9± 0.2	624 ± 184	91.3 ± 0.2
26	**18a**	429 ± 73	88.1± 0.2	1290 ± 834	94.1 ± 0.2
27	**19**	788 ± 114	92.8 ± 0.5	13.3 ± 0.5	4.2 ± 0.2
28	**19a**	1799 ± 1077	95.6± 0.2	17.7 ± 1.2	9.4 ± 0.4

Cytotoxicity assay. Human liver hepatocellular carcinoma (HEPG2), human embryonic kidney 293T (HEK293T) and human bronchial epithelial (BEAS 2B) cell lines were purchased (ATCC) and cultured in 75 cm^2 flasks (CellStar). Cells were grown in DMEM/High Glucose (Hyclone, #SH3024301) media to which non-essential amino acids (Hyclone, #SH30238.01), 10 mM HEPES (Hyclone, #SH302237.01), 5 x 10^6 units of penicillin and streptomycin (Hyclone, #SV30010), and 10% of heat inactivated fetal bovine serum (Gibco, #10082147) were added. Cells were harvested using 0.05% Trypsin (Hyclone, #SH3023601), washed with PBS, and dispensed into sterile white, optical bottom 384-well plates (NUNC, #142762). After three hours, small molecule solutions

were transferred with a Tecan Freedom EVO liquid handling system equipped with a 100 nL pin tool (V&P Scientific). The controls were 3-dibutylamino-1-(4-hexyl-phenyl)-propan-1-one (25 mM in DMSO, positive control) and DMSO (negative control). The cells were incubated for 48 hours followed by the addition of CellTiter-Glo™, a luminescence-based cell viability assay (Promega, Madison, WI). All luminescence readings were performed on a Tecan Infinite M1000 plate reader. The assay was carried out in quadruplet with three independent runs. The data was normalized to the controls and analyzed by nonlinear regression (GraphPad Prism).







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7 HEPG2
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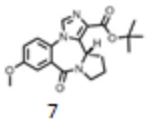
7a HEK293
7a HEPG2
7a BEAS2B



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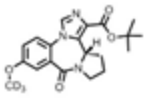

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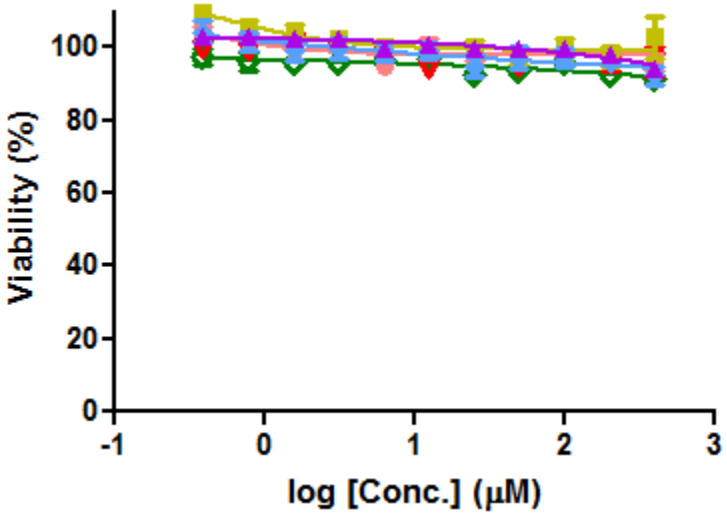

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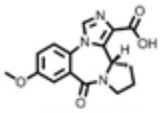

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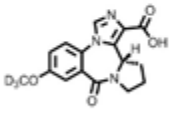

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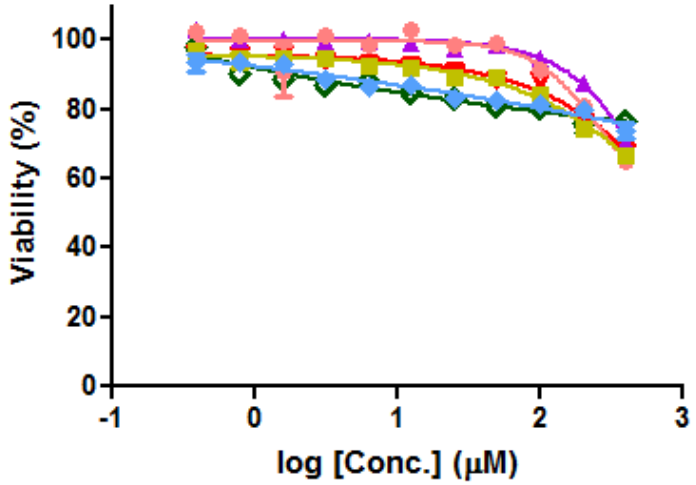

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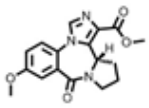

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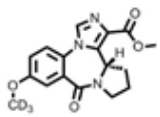

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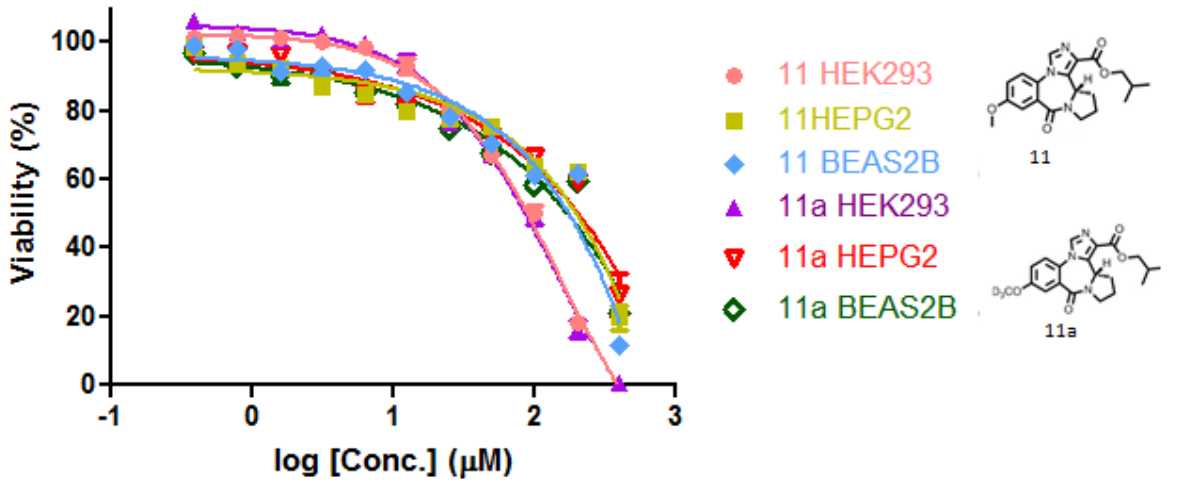
Viability (%)
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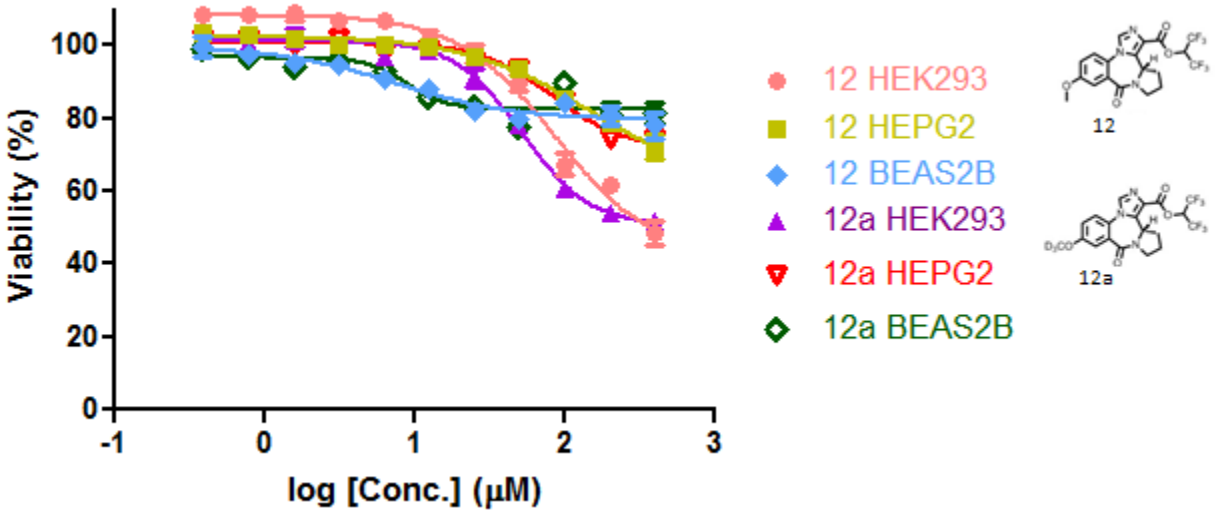
Viability (%)
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12a HEK293
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12a BEAS2B
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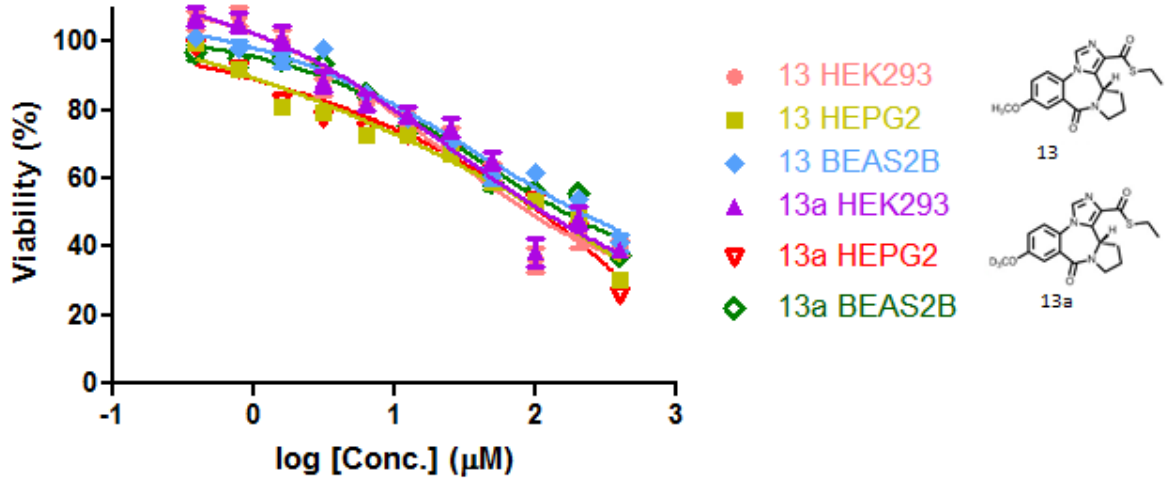
12a


Viability (%)
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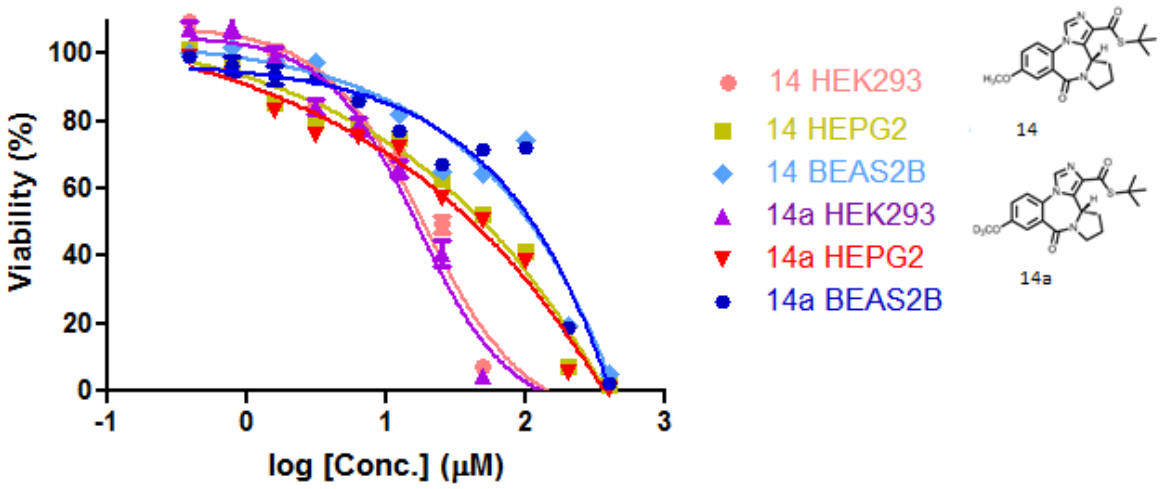
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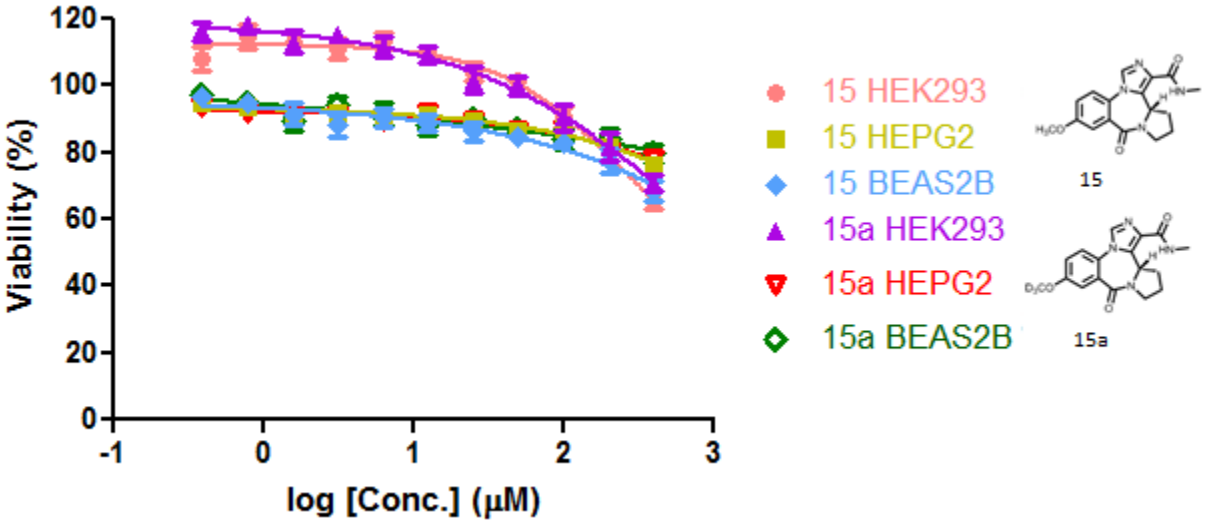
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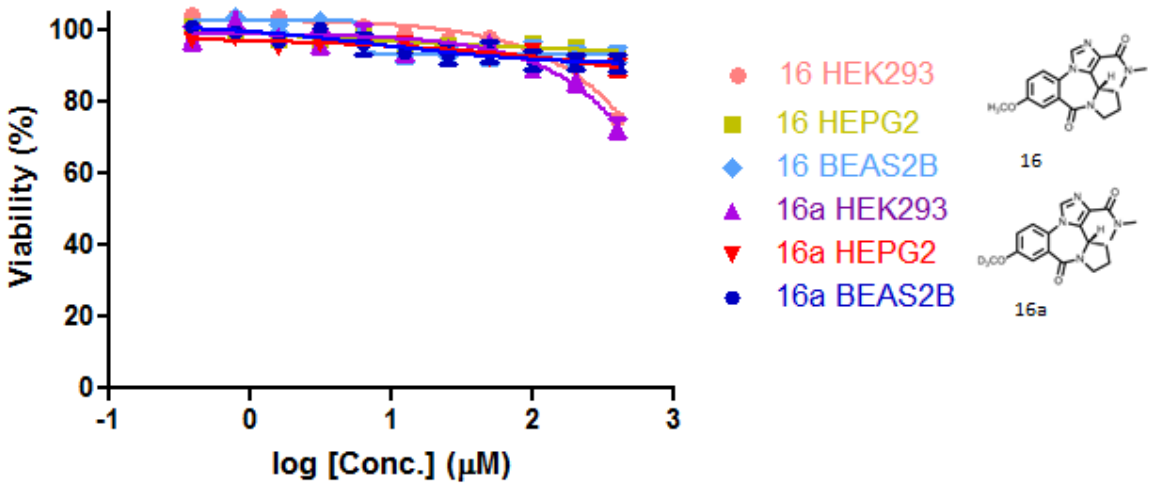
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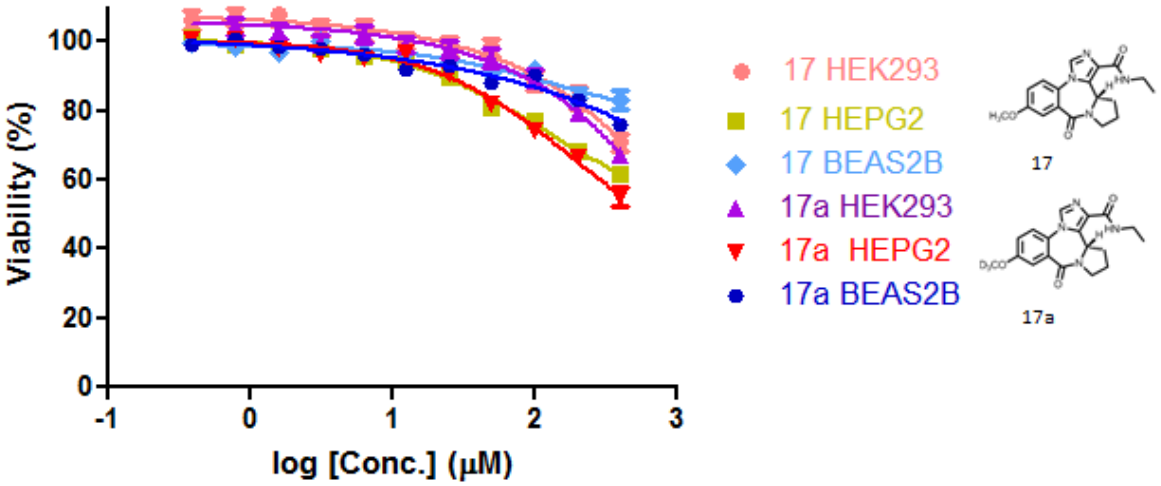
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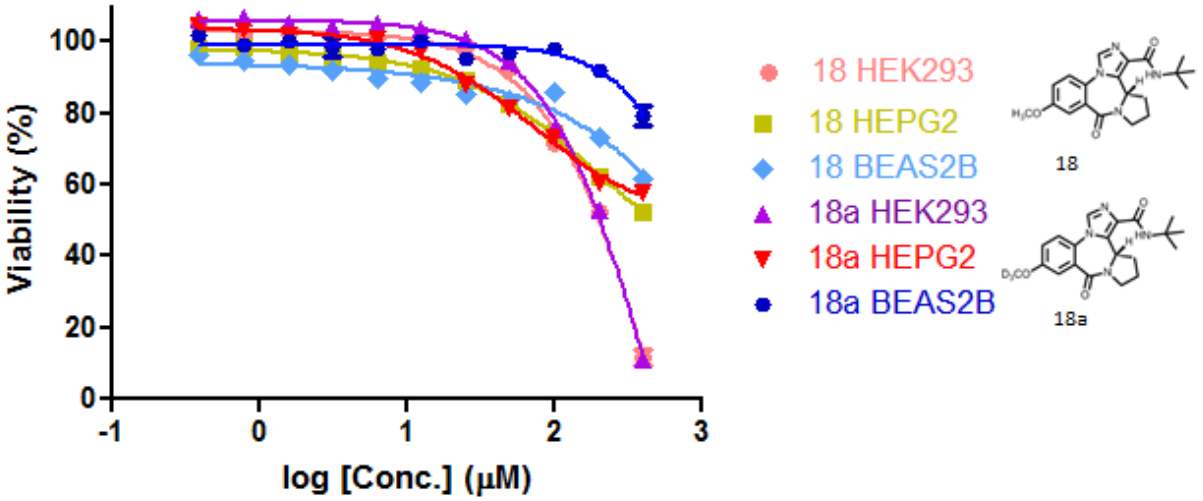
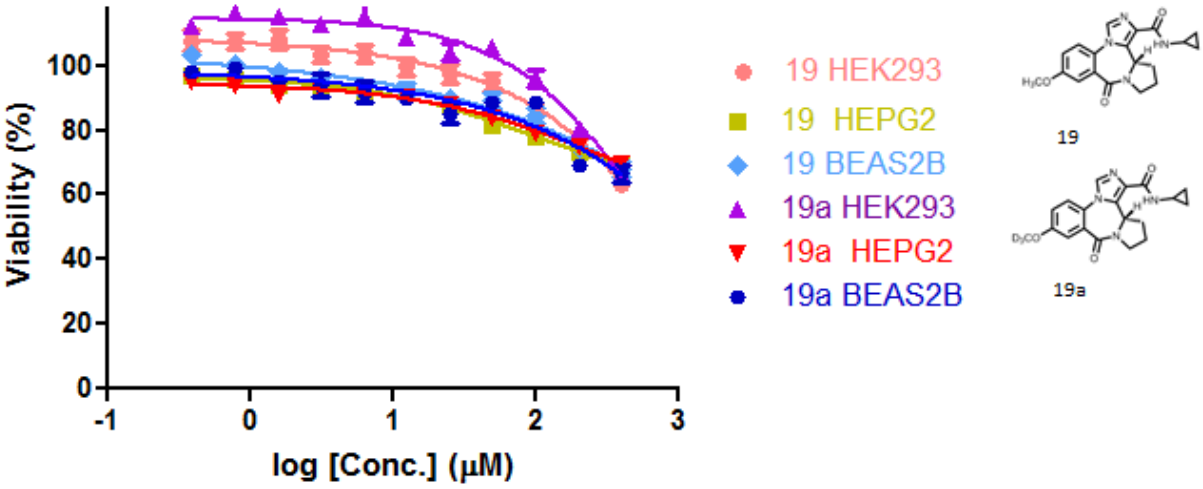




Pharmacokinetic study: Blood samples were thawed on ice, vortexed for 10 seconds, and a 100 µL aliquot was taken and added to 400 µL cold acetonitrile containing [20 nM] internal standard 1 (Hz166). Samples were vortexed for 30 seconds and centrifuged at 13,000 RPM for 3 minutes. The supernatant layer was then transferred to clean tubes and evaporated overnight. The residue was reconstituted with 300 µL of mobile phase and spin-filtered through 0.22 µm nylon centrifugal filter units (Costar). After reconstitution, verapamil (internal standard 2) was added and 5 µL of the sample was injected to the LC–MS/MS.

Brain and lung tissue samples were stored in liquid nitrogen prior to homogenization and extraction. Whole organs were thawed over ice, weighed, and homogenized directly into 500 µL ACN containing internal standard 1 (HZ166) using a Benchmark Scientific BeadBug Homogenizer with three 3.2mm stainless steel beads. Samples were homogenized for 30 seconds, centrifuged for 3 minutes at 13,000 RPM. This process was repeated for a total of three extractions.

The supernatants were combined and prepared in the same manner as the blood samples for LC-MS/MS analysis.

High performance liquid chromatography (HPLC) was performed with Shimadzu Nexera X2 LC30AD series pumps (Shimadzu, Kyoto, Japan) that include a 20A5R degassing unit, SIL 30AC autosampler and a 20A column oven. Analytes were separated by a Restek Ultra Biphenyl II column (2.1 mm × 50 mm, 5 um particle size, Restek, California, US) under gradient elution at a flow rate of 0.6 mL/min. The mobile phase was acetonitrile and water (both containing 0.1% formic acid). Time program: 10% B → 99% B (3 min), hold at 99% B (3.75 min), return to 10% B (4 min), hold (4.5 min). Column Temperature: 50°C.

Analytes were monitored under positive mode by Shimadzu 8040 triple quadrupole mass analyzer (Shimadzu, Kyoto, Japan) electrospray (ESI) and atmospheric pressure ionization (APCI) run in dual (DUIS) mode. The following transitions are monitored in multiple reaction monitoring (MRM) mode. Ion transition pairs for **17** are 340.85 > 296.00, 340.85 > 277.95, 340.85 > 268.10 and 340.85 > 227.15. Transition pairs for HZ-166 are m/z 356.90 > m/z 311.15, m/z 356.90 > m/z 283.15, and m/z 356.90 > m/z 282.15. Transition pairs for verapamil (internal standard) are 454.70 > 165.15, 454.70 > 150.20, and 454.70 > 303.30. Collision energy is optimized for each transition to obtain optimal sensitivity. The mass spectrometer was operated with the heat block temperature of 400°C, drying gas flow of 15 L/min, desolvation line temperature of 250°C, nebulizing gas flow of 1.5 L/min, and both needle and interface voltages of 4.5 kV. The response acquisition was performed using LabSolutions software.

Sample preparation of calibration standards and quality control for LC–MS/MS: HZ-166 was chosen as an internal standard (I.S.) because it has similar chemical structure as that of **17** and was therefore used to account for sample dilution, evaporation, and matrix effects. Verapamil was also used as a second standard to monitor instrumental variations. Stock solutions of all were prepared at concentration of 2 mg/mL separately in ACN and stored in a −20 ◦C freezer, with the exception of the acid which was prepared in 80:20 ACN:water. Intermediate working solutions of each were prepared by serial dilution with mobile phase (80:20, ACN:water with 0.1% formic acid). Calibration curve cocktails were prepared at concentrations of 1, 5, 10, 15, 25, 50, 75, 100, and 150 nM.

The intra-run/within-run validation was performed on concentrations of 10, 25 and 75 nM with three replicates for each concentration. For separate validations, separate standard curves were freshly prepared. The standard curves were fitted by a linear regression and the validation samples were calculated back by the calibration curve of that day. The mean and the coefficient of variance (CV) were calculated accordingly. Accuracy was calculated by comparing calculated concentrations to corresponding nominal.

Pharmacokinetic parameters were calculated with PK solutions software 2.0 and fitted to the following equation: $c = A \bullet e^{-at} + B \bullet e^{-bt}$. Due to the rapid absorption two phases could be identified as distribution/absorption phase and elimination phase.

Compound characterization

*(S)-tert-Butyl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxylate (**7a**):* The *tert*-butyl ester **7a** was prepared from ethyl ester **5a** by following the same procedure employed for preparation of *tert*-Butyl ester **7** in 67% yield: M.p = 114-115 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.65 (s, 9H), 2.15-2.34 (m, 3H), 3.50-3.63 (m, 2H), 3.77-3.84 (m, 1H), 4.75 (d, 1H, *J* = 7.2 Hz), 7.15 (dd, 1H, *J* = 8.8 Hz, 2.8 Hz), 7.27-7.32 (m, 1H), 7.59 (d, 1H, *J* = 2.8 Hz), 7.79 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 28.2, 28.4, 46.7, 53.5, 81.8, 114.4, 119.8, 124.6, 126.3, 129.3, 130.6, 135.7, 136.5, 159.3, 162.6, 163.8; HRMS (ESI) $(M+H)^+$, calcd. for $C_{20}H_{21}{}^2H_3N_3O_4$ 373.1950; Found 373.1951.

The $\underline{^{13}C}$-D signal was not observed due to *due to long relaxation time, reduced NOE effect and spin-spin coupling for most* deuterium analogs.

*(S)-tert-Butyl-7-hydroxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxylate (**8**): The tert*-butyl ester **8** was prepared from phenolic ethyl ester **6** following the same procedure employed for preparation of *tert*-Butyl ester **7** in 65% yield: M.p = 174-175 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.63 (s, 9H), 2.09-2.34 (m, 3H), 3.50-3.62 (m, 2H), 3.77-3.85 (m, 1H), 4.79 (d, 1H, *J* = 7.1 Hz), 7.10 (dd, 1H, *J* = 8.6 Hz, 2.2 Hz), 7.23-7.28 (m, 1H), 7.77 (bs, 1H), 7.85 (s, 1H), 9.75 (bs, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 28.2, 28.3, 46.9, 53.7, 82.0, 117.4, 120.7, 124.9, 125.1, 129.1, 129.6, 135.8, 136.2, 157.7, 162.3, 164.6; HRMS (ESI) $(M+H)^+$, calcd. for $C_{19}H_{22}N_3O_4$ 356.1605; Found 356.1615.

*(S)-11,12,13,13a-Tetrahydro-7-($^2H_3$)-methoxy-9-oxo-9H-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodia-zepine-1-carboxylic acid (**9a**):* The acid **9a** was prepared from ester **5a** following the procedure employed for preparation of acid **9** in 82% yield: M.p = 210-212 ˚C; $[\alpha]_D^{25}$= +8.00 (c 0.25%, in CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17-2.34 (m, 3H), 3.48-3.83 (m, 3H), 4.72 (d, 1H, *J* = 7.1 Hz), 7.17 (dd, 1H, *J* = 8.7 Hz, 2.3 Hz), 7.31-7.35 (m, 1H), 7.60 (d, 1H, *J* = 2.3 Hz), 7.82 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 28.5, 46.7, 53.2, 114.7, 119.8, 124.5, 125.7, 126.7, 130.8, 134.8, 137.5, 159.7, 161.5, 163.6; HRMS (ESI) $(M+H)^+$, calcd. for $C_{16}H_{13}{}^2H_3N_3O_4$ 317.1324; Found 317.1328. This material was employed directly in the next step.

*(S)-Methyl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo-[1,2-a][1,4]diazepine-1-carboxylate (**10a**):* The methyl ester **10a** was prepared from acid **5a** following the procedure employed for preparation of methyl ester **10** in 97% yield: M.p = 182-183 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.17-2.37 (m, 3H), 3.50-3.63 (m, 2H), 3.75-3.83 (m, 1H), 3.95 (s, 3H), 4.76 (d, 1H, *J* = 7.0 Hz), 7.17 (dd, 1H, *J* = 8.8 Hz, 2.9 Hz), 7.34 (d, 1H, *J*= 8.8 Hz), 7.60 (d, 1H, J = 2.9 Hz), 7.82 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 28.4, 46.6, 52.2, 53.5, 114.5, 119.8, 124.6, 126.0, 127.3, 130.6, 135.9, 137.9, 159.4, 163.2, 163.7. HRMS (ESI) $(M+H)^+$, calcd. for $C_{17}H_{15}{}^2H_3N_3O_4$ 331.1480; Found 331.1486.

*(S)-Isobutyl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxylate (**11a**):* The isobutyl ester **11a** was prepared from acid **9a** following the general procedure with dry isobutanol as the nucleophile. The crude residue was

purified by flash column chromatography [silica gel, EtOAc/hexane (7:3)] to yield pure isobutyl ester **11a** as a solid in 61% yield: M.p = 125-127 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.03 (d, 6H, J = 6.7 Hz), 2.12-2.31 (m, 4H), 3.51-3.62 (m, 2H), 3.76-3.82 (m, 1H), 4.14 (d, 2H, J = 6.9 Hz), 4.76 (d, 1H, J = 6.9 Hz), 7.16 (dd, 1H, J= 8.8 Hz, 2.9 Hz), 7.32 (d, 1H, J = 8.8 Hz), 7.59 (d, 1H, J = 2.9 Hz), 7.81 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.1, 24.2, 27.6, 28.2, 46.4, 53.3, 71.0, 114.3, 119.5, 124.5, 125.9, 127.5, 130.4, 135.7, 137.4, 159.2, 162.8, 163.5. HRMS (ESI) $(M+H)^+$, calcd. for $C_{20}H_{21}{}^2H_3N_3O_4$ 373.1950; Found 373.1955.

*(S)-1,1,1,3,3,3-Hexafluoropropan-2-yl-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]-imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxylate (**12**):* The hexafluoro isopropyl ester **12** was prepared from acid **9** following the general procedure with dry 1,1,1,3,3,3-hexafluoropropan-2-ol as the nucleophile. The crude residue was purified by flash column chromatography [silica gel, EtOAc/hexane (1:1)] to yield pure ester **12** as a solid in 95% yield: M.p = 204-205 ˚C; $[\alpha]_D^{25}$= +16.67 (c 0.3%, in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16-2.29 (m, 3H), 3.38-3.40 (m, 1H), 3.52-3.62 (m, 1H), 3.79-3.85 (m, 1H), 3.92 (s, 3H), 4.77 (d, 1H, *J* = 7.3 Hz), 5.98-6.08 (m, 1H), 7.18 (dd, 1H, *J* = 8.8 Hz, 2.8 Hz), 7.33 (d, 1H, *J* = 8.8 Hz), 7.61 (d, 1H, *J* = 2.8 Hz), 7.87 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 28.3, 46.6, 53.4, 55.9, 66.7 (sep, *J* = 34.5 Hz), 114.7, 119.9, 120.6 (q, *J* = 279 Hz), 124.3, 124.6, 125.6, 130.6, 136.8, 140.6, 159.4, 159.7, 163.6; HRMS (ESI) $(M+Na)^+$, calcd. for $C_{19}H_{15}F_6N_3O_4Na$ 486.0864; Found 486.0875.

*(S)-1,1,1,3,3,3-Hexafluoropropan-2-yl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxylate (**12a**):* The hexafluoro isopropyl ester **12a** was prepared from acid **9a** following the general procedure with dry 1,1,1,3,3,3-hexafluoropropan-2-ol as the nucleophile. The crude residue was purified by column chromatography [silica gel, EtOAc/hexane (7:3)] to yield pure ester **12a** as a solid in 97% yield: M.p = 204-206 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15-2.34 (m, 3H), 3.38-3.42 (m, 1H), 3.53-3.63 (m, 1H), 3.80-3.86 (m, 1H), 4.79 (d, 1H, *J* = 7.3 Hz), 5.98-6.10 (sep, 1H, *J* = 6.1 Hz), 7.19 (dd, 1H, *J* = 8.8 Hz, 2.9 Hz), 7.34 (d, 1H, *J* = 8.8 Hz), 7.62 (d, 1H, *J* = 2.9 Hz), 7.89 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 28.3, 46.6, 53.4, 66.7 (sep, *J* = 34.5 Hz), 114.6, 119.9, 120.6 (q, *J* = = 279 Hz), 124.3, 124.6, 125.6, 130.6, 136.8, 140.6, 159.5, 159.7, 163.6; HRMS (ESI) $(M+Na)^+$, calcd. for $C_{19}H_{13}{}^2H_3F_6N_3O_4$ 467.1228; Found 467.1230.

*(S)-S-Ethyl-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carbothioate (**13**):* The thio ethyl ester **13** was prepared from acid **9** following the general procedure with dry ethanethiol as the nucleophile. The crude residue was purified by flash column chromatography [silica gel, EtOAc/hexane (8:2)] to yield pure thio ester **13** as a solid in 70% yield: M.p = 228-230 ˚C; $[\alpha]_D^{25}$= -14.29 (c 0.28%, in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, 3H, *J* = 7.4 Hz), 2.14-2.25 (m, 3H), 3.00 (q, 2H, *J* = 7.4 Hz), 3.42-3.60 (m, 2H), 3.74-3.82 (m, 1H), 3.91 (s, 3H), 4.71 (d, 1H, *J* = 7.2 Hz), 7.15 (dd, 1H, *J* = 8.8 Hz, 2.8 Hz), 7.31 (d, 1H, *J* = 8.8 Hz), 7.59 (d, 1H, *J* = 2.8 Hz), 7.79 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 23.1, 24.4, 28.6, 46.5, 53.3, 55.9, 114.5, 119.8, 124.5, 125.9, 130.6, 132.5, 134.6, 135.5, 159.5, 163.6, 188.1. HRMS (ESI) $(M+Na)^+$, calcd. for $C_{18}H_{19}N_3O_3SNa$ 380.1045; Found 380.1047.

*(S)-S-Ethyl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carbothioate (**13a**):* The thio ethyl ester **13a** was prepared from **9a** following the general procedure with dry ethanethiol as the nucleophile. The crude residue was purified by flash column chromatography [silica gel, EtOAc/hexane (8:2)] to yield pure thio ester **13a** as a solid in 80% yield: M.p = 229-231 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (t, 3H, *J* = 7.4 Hz), 2.18-2.26 (m, 3H), 3.02 (q, 2H, *J* = 7.4 Hz), 3.44-3.61 (m, 2H), 3.76-3.83 (m, 1H), 4.73 (d, 1H, *J* = 6.9 Hz), 7.17 (dd, 1H, *J* = 8.8 Hz, 2.9 Hz), 7.33 (d, 1H, J = 8.8 Hz), 7.60 (d, 1H, *J* = 2.9 Hz), 7.81 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.6, 23.1, 24.4, 28.6, 46.5, 53.4, 114.6, 119.8, 124.5, 125.9, 130.7, 133.6, 134.6, 135.5, 159.5, 163.6, 188.1. HRMS (ESI) $(M+H)^+$, calcd. for $C_{18}H_{17}{}^2H_3N_3O_3S$ 361.1408; Found 361.1405.

*(S)-S-tert-Butyl-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo-[1,2-a][1,4]diazepine-1-carbothioate (**14**):* The thio *tert*-butyl ester **14** was prepared from acid **9** following the general procedure with dry *tert*-butyl mercaptan as the nucleophile. The crude residue was purified by flash column chromatography [silica gel, EtOAc/hexane (8:2)] to yield pure thio ester **14** as a solid in 82% yield: M.p = 130-132 ˚C; $[\alpha]_D{}^{27}$= -23.54 (c 0.17% in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.57 (s, 9H), 2.18-2.20 (m, 3H), 3.41-3.59 (m, 2H), 3.74-3.80 (m, 1H), 3.90 (s, 3H), 4.69 (d, 1H, *J* = 7.2 Hz), 7.14 (dd, 1H, *J* = 8.9 Hz, 3.0 Hz), 7.29 (d, 1H, *J* = 8.9 Hz), 7.58 (d, 1H, *J* = 3.0 Hz), 7.74 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.3, 28.5, 29.7, 46.4, 47.1, 53.3, 55.8, 114.4, 119.6, 124.5, 125.9, 130.5, 134.2, 135.1, 159.3, 163.5, 188.7 ; HRMS (ESI) $(M+H)^+$, calcd. for $C_{20}H_{24}N_3O_3S$ 386.1533; Found 386.1532.

*(S)-S-tert-Butyl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carbothioate (**14a**):* The thio *tert*-butyl ester **a14a** was prepared from **9a** following the general procedure with dry *tert*-butyl mercaptan as the nucleophile. The crude residue was purified by flash column chromatography [silica gel, EtOAc/hexane (1:1)] to yield pure thio ester **14a** as a solid in 89% yield: M.p = 129-131 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.11-2.20 (m, 3H), 3.31-3.53 (m, 2H), 3.69-3.75 (m, 1H), 4.67 (d, 1H, *J* = 5.9 Hz), 7.08-7.13 (m, 1H), 7.26-7.30 (m, 1H), 7.52 (d, 1H, *J* = 2.0 Hz), 7.72 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 28.6, 29.8, 46.5, 47.1, 53.4, 114.5, 119.7, 124.6, 126.0, 130.6, 134.3, 134.4, 135.2, 159.4, 163.6, 188.8 ; HRMS (ESI) $(M+H)^+$, calcd. for $C_{20}H_{21}{}^2H_3N_3O_3S$ 389.1721; Found 389.1725.

*(S)-7-Methoxy-N-methyl-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**15**):* The *N*-methyl amide **15** was prepared from acid **9** following the general procedure with a solution of methylamine (33 wt % in absolute ethanol, 3 mL) as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, 1% MeOH in CH_2Cl_2] to yield pure amide **15** as a solid in 75% yield: M.p = 180-182 ˚C; $[\alpha]_D{}^{25}$= +3.70 (c 0.5%, in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.12-2.25 (m, 2H), 2.30-2.41 (m, 1H), 2.98 (d, 1H, *J* = 4.6 Hz), 3.50-3.62 (m, 1H), 3.73-3.89 (m, 2H), 3.91 (s, 3H), 4.73 (d, 1H, *J* = 7.8 Hz), 7.16 (dd, 1H, *J* = 8.6 Hz, 2.3 Hz), 7.32 (d, 1H, J = 8.6 Hz), 7.59 (d, 1H, J = 2.3 Hz), 7.72 (brs, 1H), 7.91 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.5, 26.2, 28.7, 46.8, 53.5, 55.9, 114.7,

119.8, 124.7, 125.6, 128.5, 130.9, 134.2, 135.6, 159.8, 161.3, 163.5. HRMS (ESI) $(M+Na)^+$, calcd. for $C_{17}H_{18}N_4O_3Na$ 349.1277; Found 349.1300.

*(S)-7-($^2H_3$)-Methoxy-N-methyl-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**15a**):* The *N*-methyl amide **15a** was prepared from acid **9a** following the general procedure with a solution of methylamine (33 wt % in absolute ethanol, 3 mL) as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, 1% MeOH in CH_2Cl_2] to yield pure amide **15a** as a solid in 92 % yield: M.p = 180-182 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.12-2.25 (m, 2H), 2.30-2.42 (m, 1H), 2.97 (d, 1H, *J* = 5.0 Hz), 3.50-3.60 (m, 1H), 3.74-3.91 (m, 2H), 4.73 (d, 1H, *J* = 7.6 Hz), 7.14 (dd, 1H, *J* = 8.8 Hz, 2.9 Hz), 7.29 (d, 1H, J = 8.8 Hz), 7.48-7.54 (bm, 1H), 7.58 (d, 1H, J = 2.9 Hz), 7.70 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.5, 25.8, 28.6, 46.5, 53.4, 114.3, 119.4, 124.4, 126.2, 130.2, 130.6, 134.5, 134.9, 159.1, 162.8, 163.7. HRMS (ESI) $(M+H)^+$, calcd. for $C_{17}H_{16}{}^2H_3N_4O_3$ 330.1640; Found 330.1637.

*(S)-7-Methoxy-N,N-dimethyl-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**16**):* The *N, N*-dimethyl amide **16** was prepared from acid **9** following the general procedure with a solution of *N,N*-dimethylamine (11% in ethanol, 5 mL) as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, 5% MeOH in CH_2Cl_2] to yield pure dimethyl amide **16** as a solid in 70% yield: M.p = 186-188 ˚C; $[\alpha]_D{}^{25}$= +52.00 (c 0.25%, in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01-2.08 (m, 2H), 2.25-2.38 (m, 1H), 2.89-2.94 (m, 1H), 3.08 (s, 3H), 3.14 (s, 3H), 3.63-3.82 (m, 2H), 3.91 (s, 3H), 4.73 (dd, 1H, *J* = 8.4, 3.0 Hz), 7.15 (dd, 1H, *J* = 8.9 Hz, 2.9 Hz), 7.34 (d, 1H, *J* = 8.9 Hz), 7.57 (d, 1H, *J* = 2.9 Hz), 7.97 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.9, 27.8, 35.3, 39.1, 47.2, 52.3, 55.8, 114.9, 119.7, 124.2, 126.1, 130.1, 131.0, 132.0, 134.8, 159.1, 164.2, 165.7. HRMS (ESI) $(M+Na)^+$, calcd. for $C_{18}H_{20}N_4O_3Na$ 363.1433; Found 363.1410.

*(S)-7-($^2H_3$)-Methoxy-N,N-dimethyl-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]-pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**16a**):* The *N, N*-dimethyl amide **16a** was prepared from acid **9a** following the general procedure with a solution of *N,N*-dimethylamine (11% in ethanol, 5 mL) as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, 5% MeOH in CH_2Cl_2] to yield pure amide **16a** as a solid in 88% yield: M.p = 185-186 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01-2.10 (m, 2H), 2.24-2.37 (m, 1H), 2.89-2.98 (m, 1H), 3.07 (s, 3H), 3.14 (s, 3H), 3.63-3.82 (m, 2H), 4.72 (dd, 1H, *J* = 8.3 Hz, 2.9 Hz), 7.13 (dd, 1H, *J* = 8.7 Hz, 2.9 Hz), 7.29 (d, 1H, *J* = 8.7 Hz), 7.56 (d, 1H, *J* = 2.9 Hz), 7.77 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.9, 27.9, 35.3, 39.1, 47.2, 52.3, 114.9, 119.7, 124.2, 126.3, 130.1, 131.4, 132.0, 134.9, 159.0, 164.3, 166.1. HRMS (ESI) $(M+H)^+$, calcd. for $C_{18}H_{18}{}^2H_3N_4O_3$ 344.1796; Found 344.1798.

*(S)-N-Ethyl-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**17**):* The *N*-ethyl amide **17** was prepared from acid **9** following the general procedure with a solution of ethylamine (2.0 M in THF, 1 mL) as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, EtOAc/hexane

(8:2)] to yield pure ethyl amide **17** as a solid in 75% yield: M.p = 175-177 ˚C; $[\alpha]_D^{27}$=+8.33 (c 0.12%, in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H, *J* = 7.3 Hz), 2.13-2.41 (m, 3H), 3.40-3.59 (m, 3H), 3.74-3.87 (m, 2H), 3.90 (s, 3H), 4.72 (d, 1H, *J* = 7.8 Hz), 7.14 (dd, 1H, *J* = 8.7 Hz, 3.0 Hz), 7.28 (d, 1H, *J* = 8.7 Hz), 7.48-7.50 (bm, 1H), 7.58 (d, 1H, *J* = 2.7 Hz), 7.69 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.9, 24.6, 28.8, 34.0, 46.7, 53.6, 55.8, 114.4, 119.6, 124.5, 126.3, 130.3, 130.7, 134.5, 135.2, 159.3, 162.0, 163.8; HRMS (ESI) $(M+H)^+$, calcd. for $C_{18}H_{21}N_4O_3$ 341.1608; Found 341.1601.

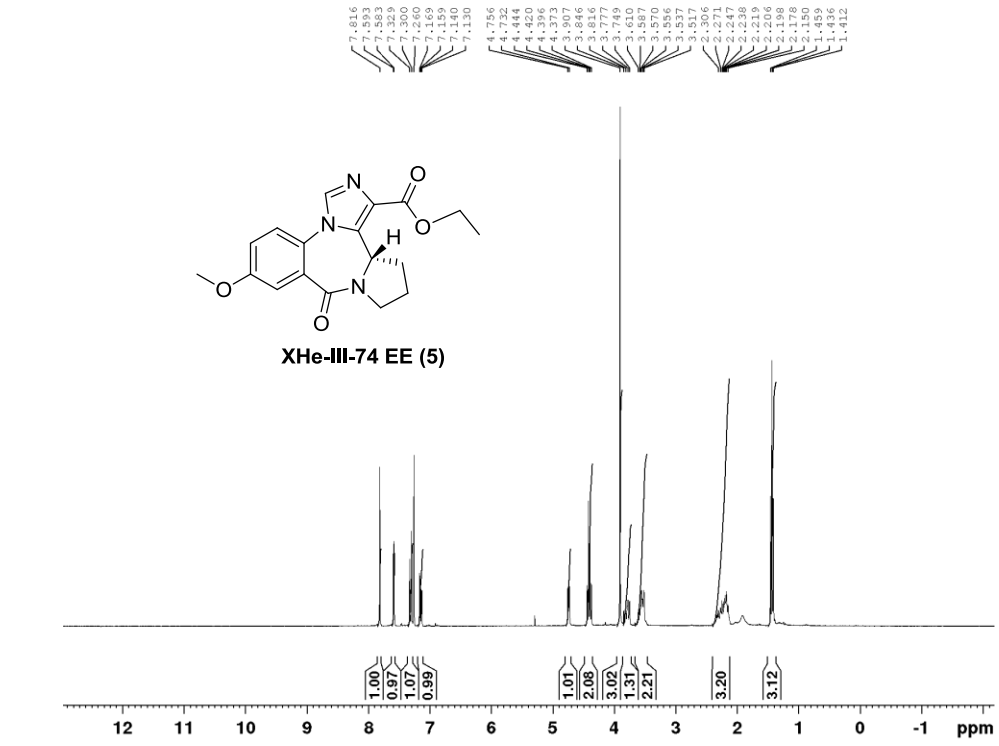
*(S)-N-Ethyl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**17a**):* The *N*-ethyl amide **17a** was prepared from **9a** following the general procedure with a solution of ethylamine (2.0 M in THF, 1 mL) as the nucleophile. The crude residue was purified by flash column chromatography [Neutral alumina, EtOAc/hexane (8:2)] to yield pure amide **17a** as a solid in 96 % yield: M.p = 176-177 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, 3H, *J* = 7.3 Hz), 2.12-2.24 (m, 2H), 2.30-2.42 (m, 1H), 3.40-3.60 (m, 3H), 3.74-3.91 (m, 2H), 4.72 (d, 1H, *J* = 7.7 Hz), 7.14 (dd, 1H, *J* = 8.8 Hz, 2.9 Hz), 7.29 (d, 1H, J = 8.8 Hz), 7.47-7.53 (bm, 1H), 7.58 (d, 1H, *J* = 2.9 Hz), 7.70 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.9, 24.6, 28.8, 34.0, 46.7, 53.6, 114.4, 119.6, 124.5, 126.4, 130.4, 130.7, 134.6, 135.2, 159.3, 162.1, 163.8; HRMS (ESI) $(M+H)^+$, calcd. for $C_{18}H_{18}{}^2H_3N_4O_3$ 344.1796; Found 344.1797.

*(S)-N-(tert-Butyl)-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**18**):* The *N-tert*-butyl amide **18** was prepared from acid **9** following the general procedure with *tert*-butyl amine as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, EtOAc/hexane (8:2)] to yield pure amide **18** as a solid in 80 % yield: M.p = 152-154 ˚C; $[\alpha]_D^{27}$= +170.00 (c 0.10%, in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 9H), 2.11-2.37 (m, 3H), 3.50-3.60 (m, 1H), 3.74-3.86 (m, 2H), 3.91 (s, 3H), 4.71 (d, 1H, *J* = 7.8 Hz), 7.14 (dd, 1H, *J* = 8.7 Hz, 3.0 Hz), 7.26-7.29 (m, 1H), 7.45 (bs, 1H), 7.58 (d, 1H, *J* = 3.0 Hz), 7.77 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.5, 28.8, 46.7, 50.9, 53.6, 55.8, 114.4, 119.6, 124.6, 126.3, 130.7, 131.2, 134.3, 134.9, 159.2, 161.5, 163.8; HRMS (ESI) $(M+H)^+$, calcd. for $C_{20}H_{25}N_4O_3$ 369.1921; Found 369.1920.

*(S)-N-(tert-Butyl)-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]-pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**18a**):* The *N-tert*-butyl amide **18a** was prepared from acid **9a** following the general procedure with *tert*-butyl amine as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, EtOAc/hexane (8:2)] to yield pure amide **18a** as a solid in 85% yield: M.p = 153-155 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 9H), 2.12-2.39 (m, 3H), 3.51-3.61 (m, 1H), 3.75-3.94 (m, 2H), 4.73 (d, 1H, *J* = 7.9 Hz), 7.14 (dd, 1H, *J* = 8.8 Hz, 2.9 Hz), 7.27-7.30 (m, 1H), 7.39 (bs, 1H), 7.59 (d, 1H, *J* = 2.9 Hz), 7.68 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.6, 28.9, 46.7, 50.9, 53.7, 114.4, 119.7, 124.5, 126.5, 130.7, 131.4, 134.3, 134.8, 159.3, 161.7, 163.8; HRMS (ESI) $(M+H)^+$, calcd. for $C_{20}H_{21}{}^2H_3N_4O_3$ 372.2108; Found 372.2109.

*(S)-N-Cyclopropyl-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**19**):* The *N*-cyclopropyl amide **19** was prepared from acid **9** following the general procedure with dry cyclopropylamine as the nucleophile. The crude residue was purified by flash column chromatography [neutral alumina, EtOAc/hexane (1:1)] to yield pure amide **19** as a solid in 82% yield: M.p = 189-190 ˚C; $[\alpha]_D^{25}$= -6.67 (c 0.3%, in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.54-0.59 (m, 2H), 0.76-0.82 (m, 2H), 2.06-2.18 (m, 2H), 2.25-2.36 (m, 1H), 2.75-2.81 (m, 1H), 3.43-3.53 (m, 1H), 3.68-3.81 (m, 2H), 3.84 (s, 3H), 4.65 (d, 1H, *J* = 8.1 Hz), 7.07 (dd, 1H, *J* = 8.7 Hz, 2.8 Hz), 7.19-7.22 (m, 1H), 7.50 (bs, 1H), 7.52 (d, 1H, *J* = 2.8 Hz), 7.61 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.5, 6.6, 22.3, 24.6, 28.8, 46.6, 53.6, 55.8, 114.4, 119.6, 124.4, 126.3, 130.0, 130.7, 134.5, 135.3, 159.3, 163.7, 163.8; HRMS (ESI) $(M+Na)^+$, calcd. for $C_{19}H_{20}N_4O_3Na$ 375.1433; Found 375.1440.

*(S)-N-Cyclopropyl-7-($^2H_3$)-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-benzo[e]imidazo[5,1-c]-pyrrolo[1,2-a][1,4]diazepine-1-carboxamide (**19a**):* The *N*-cyclopropyl amide **19a** was prepared from acid **9a** following the general procedure with dry cyclopropylamine as the nucleophile. The crude residue was purified by column chromatography [neutral alumina, EtOAc/hexane (1:1)] to yield pure amide **19a** as a solid in 86% yield: M.p = 189-190 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.62-0.67 (m, 2H), 0.84-0.90 (m, 2H), 2.12-2.26 (m, 2H), 2.33-2.46 (m, 1H), 2.83-2.89 (m, 1H), 3.51-3.60 (m, 1H), 3.75-3.93 (m, 2H), 4.73 (d, 1H, *J* = 8.2 Hz), 7.15 (dd, 1H, J = 8.8 Hz, 2.9 Hz), 7.27-7.31 (m, 1H), 7.57-7.60 (m, 2H), 7.69 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.5, 6.6, 22.3, 24.7, 28.8, 46.7, 53.6, 114.4, 119.7, 124.5, 126.3, 130.1, 130.8, 134.6, 135.3, 159.3, 163.7, 163.8; HRMS (ESI) $(M+H)^+$, calcd. for $C_{19}H_{18}{}^2H_3N_4O_3$ 356.1796; Found 356.1796.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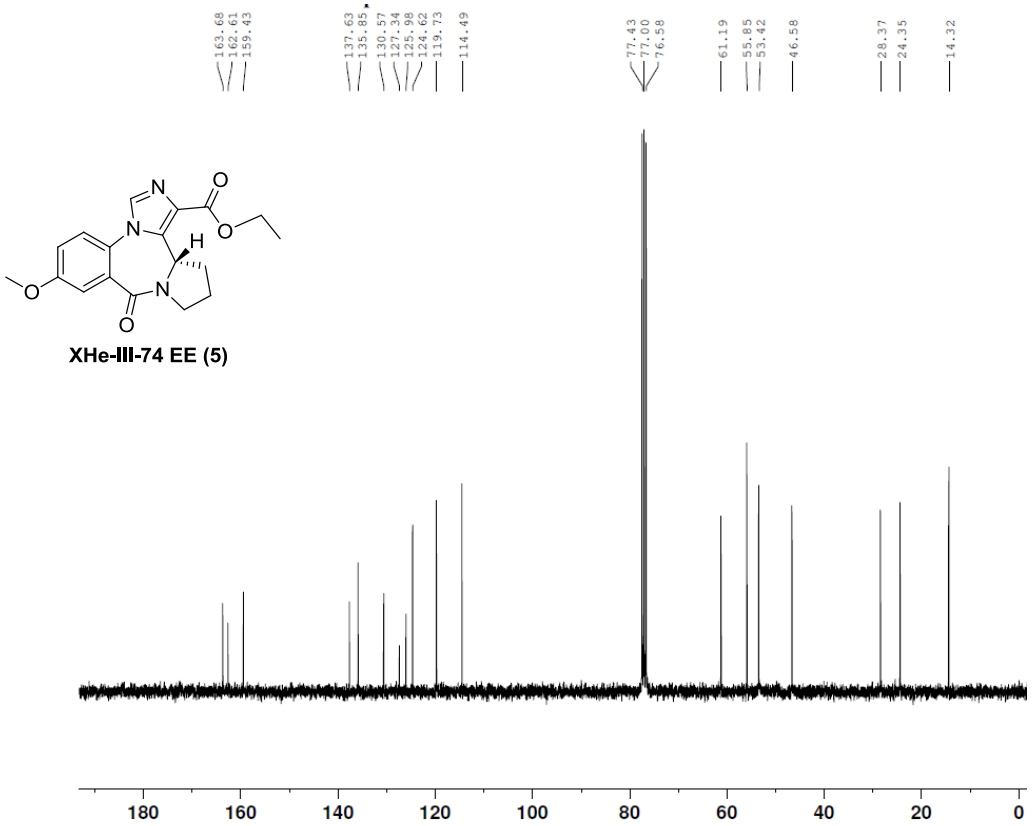
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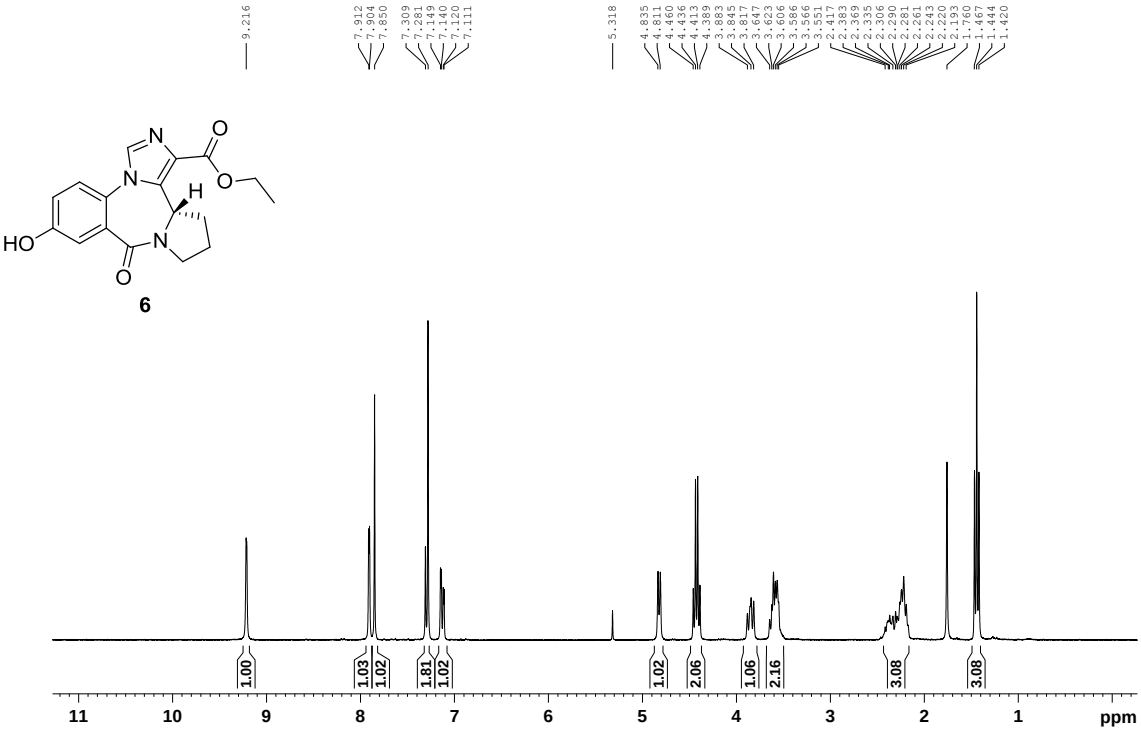
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3.647
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3.586
3.566
3.551
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2.306
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2.243
2.220
2.193
1.760
1.467
1.444
1.420
HO
N
O
H
6
1.00
1.03
1.02
1.81
1.02
1.02
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7
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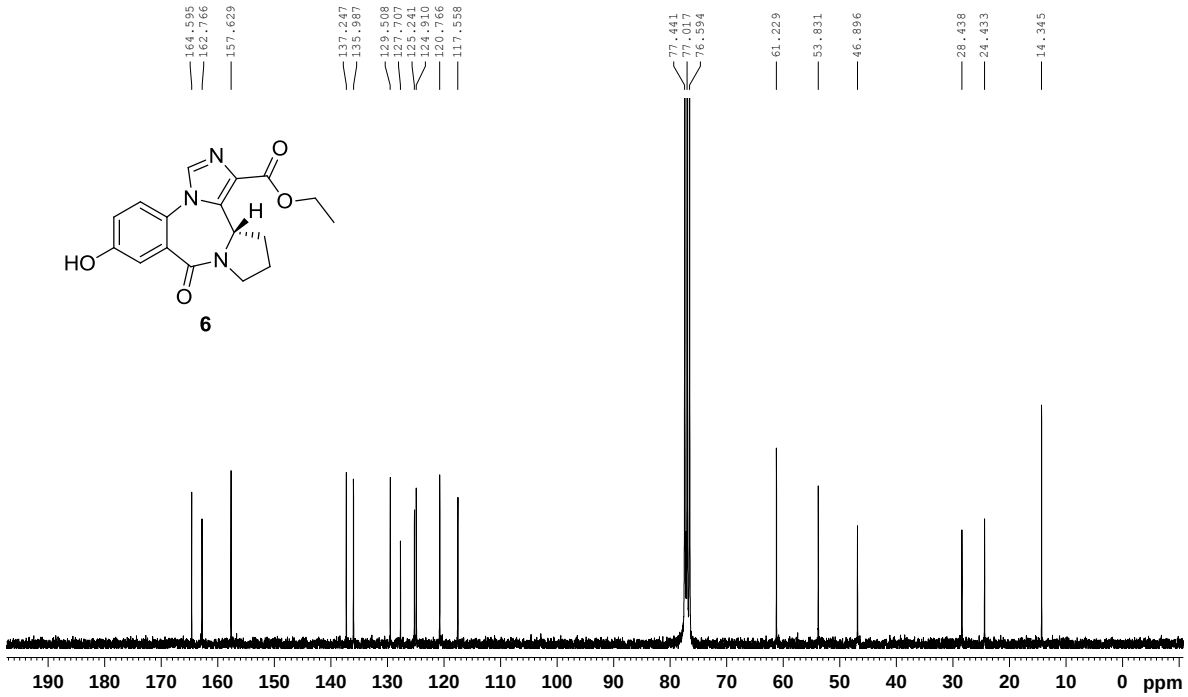
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76.594
61.229
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46.896
28.438
24.433
14.345
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O
H
6
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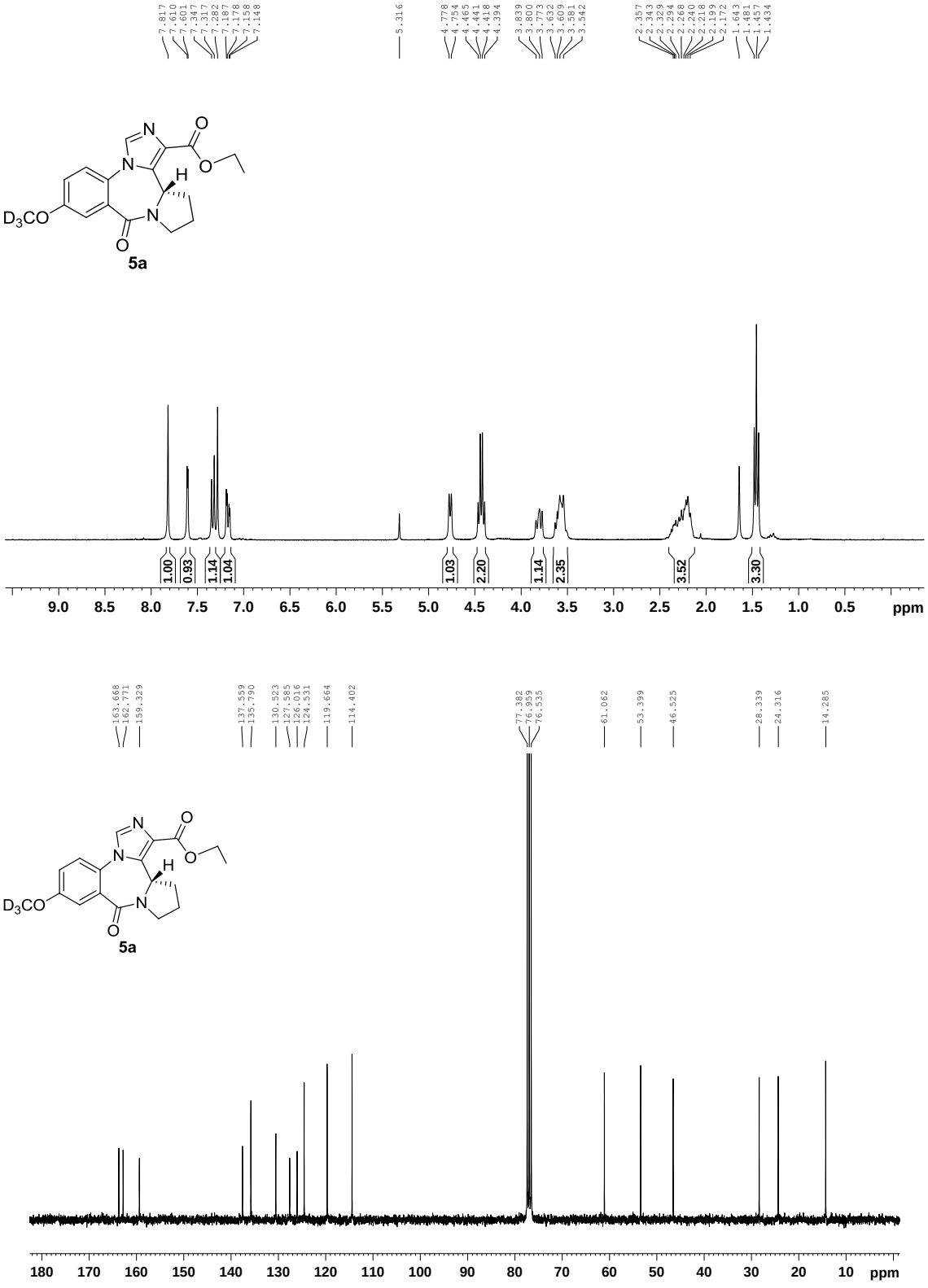
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H
D3CO
N
O
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1.14
1.04
1.03
2.20
1.14
2.35
3.52
3.30
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
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159.329
137.559
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124.531
119.664
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28.339
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O
O
N
H
D3CO
N
O
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30
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10
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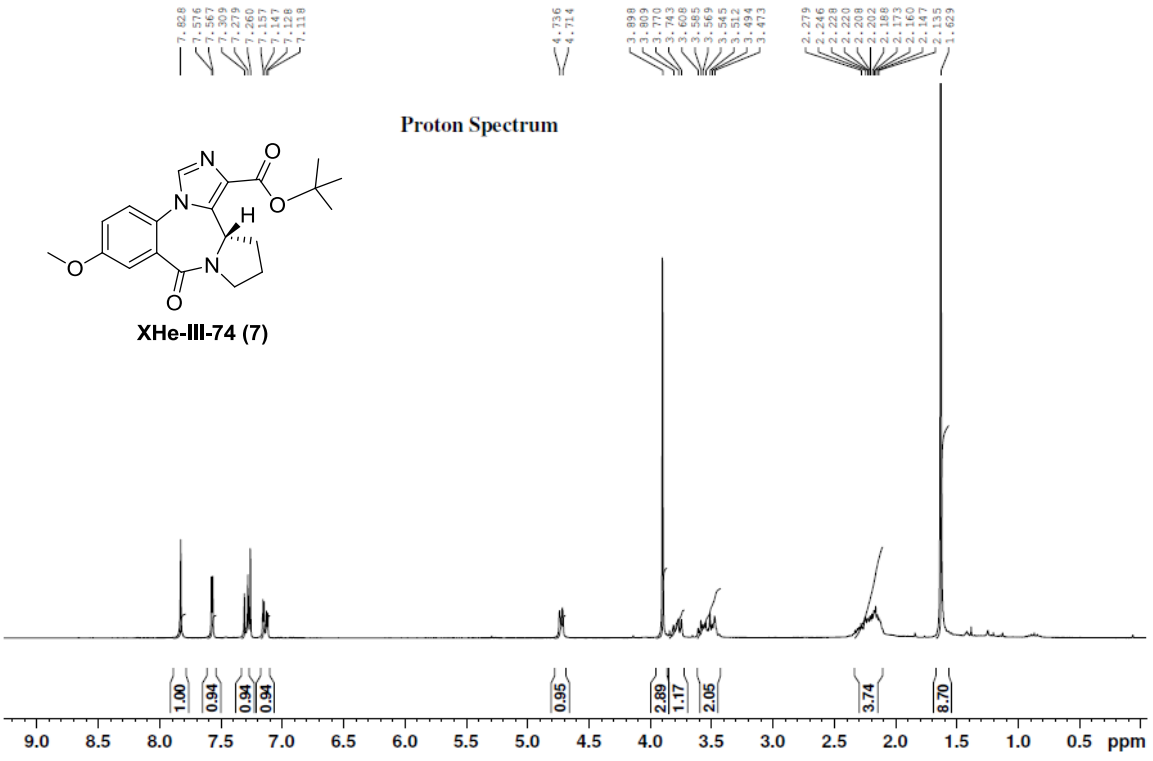
Proton Spectrum
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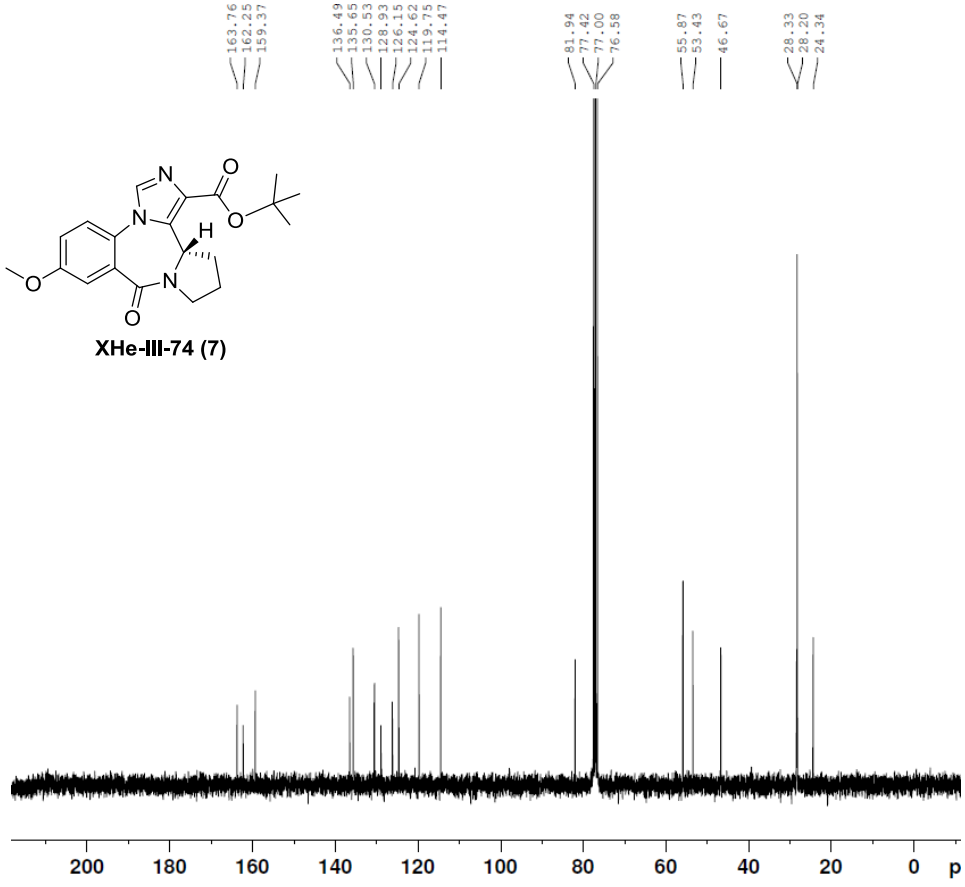
ppm


XHe-III-74 (7)
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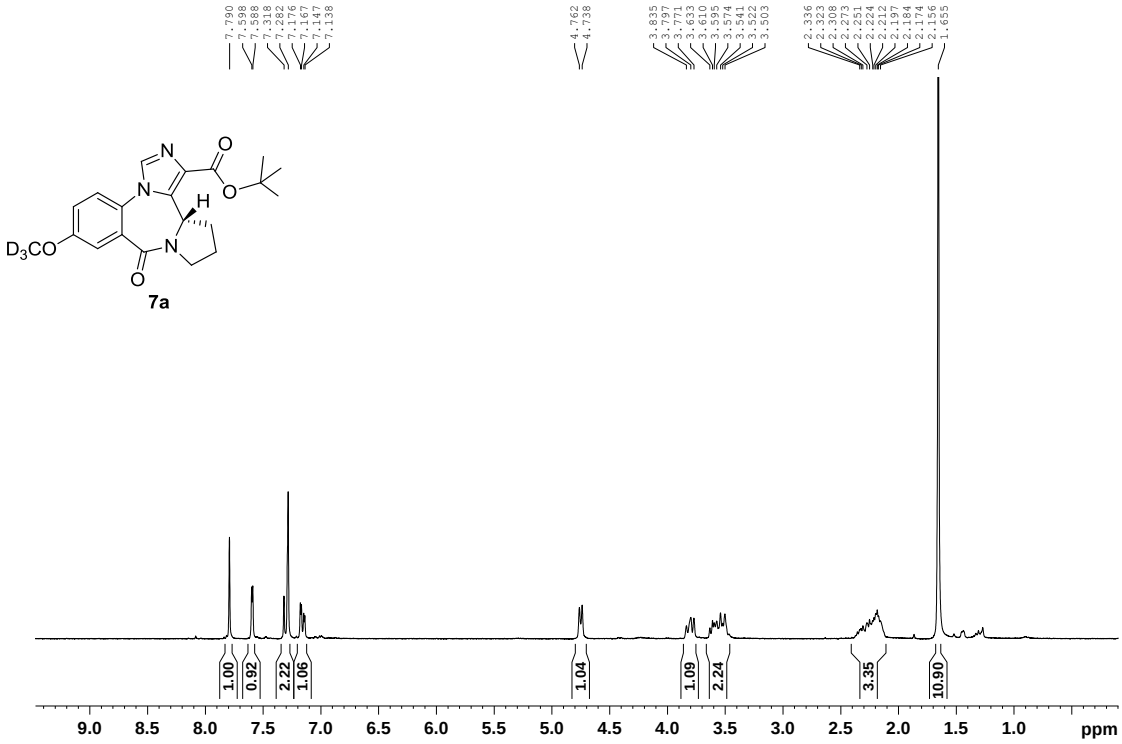
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3.797
3.771
3.633
3.610
3.595
3.574
3.541
3.522
3.503
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2.323
2.308
2.273
2.251
2.224
2.212
2.197
2.184
2.174
2.156
1.655
1.00
0.92
2.22
1.06
1.04
1.09
2.24
3.35
10.90
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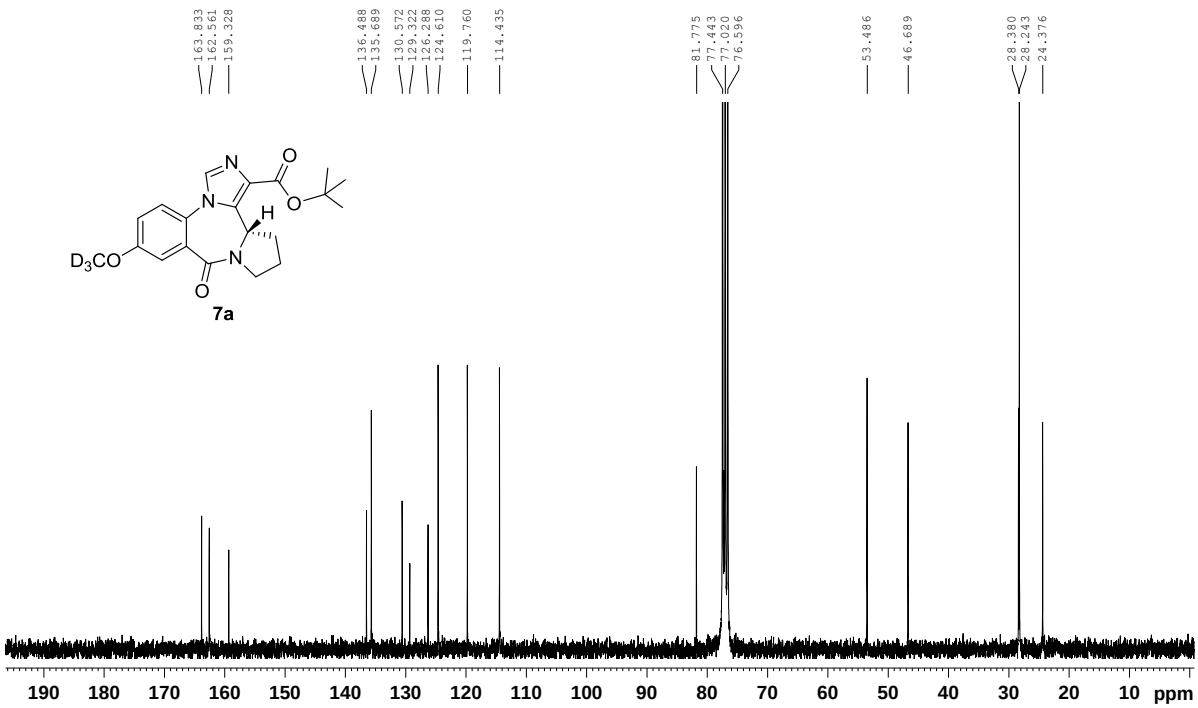
ppm

7a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77.443
77.020
76.596
53.486
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24.376
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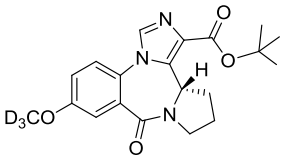
ppm

7a

^{2}H-NMR



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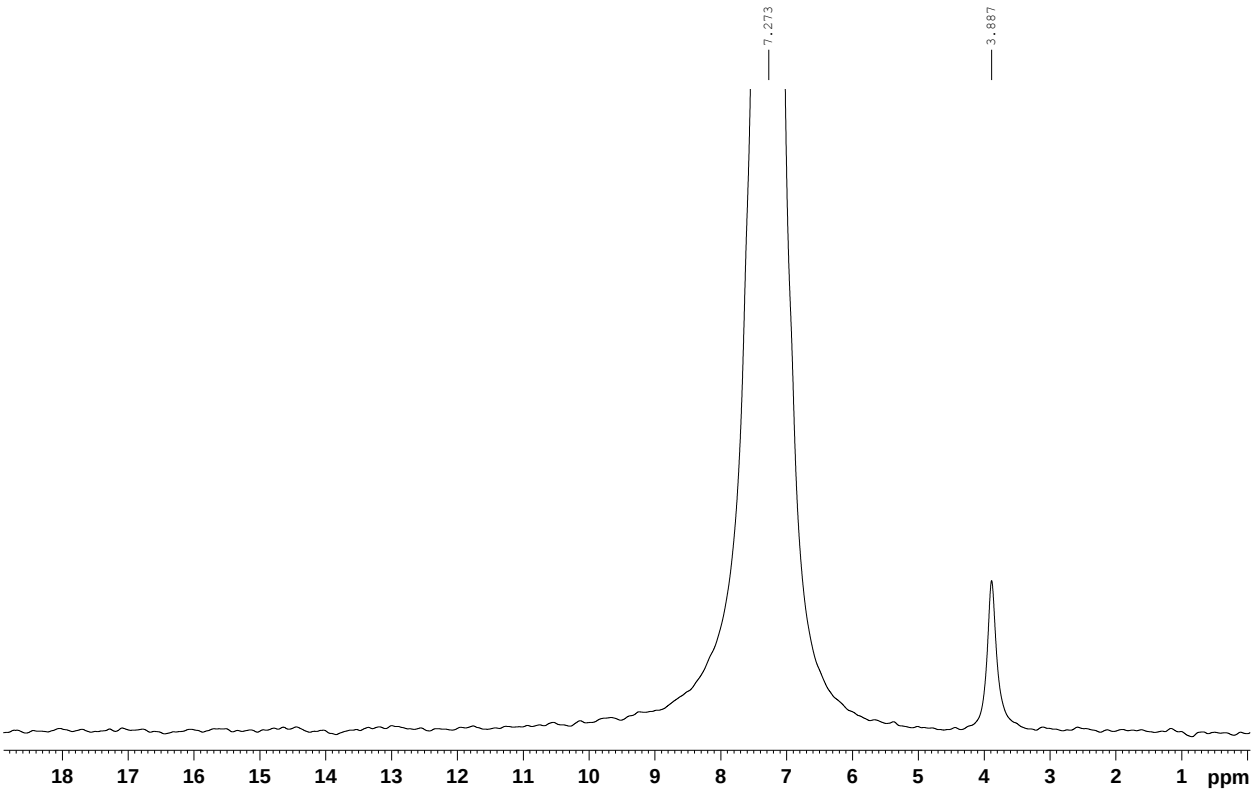

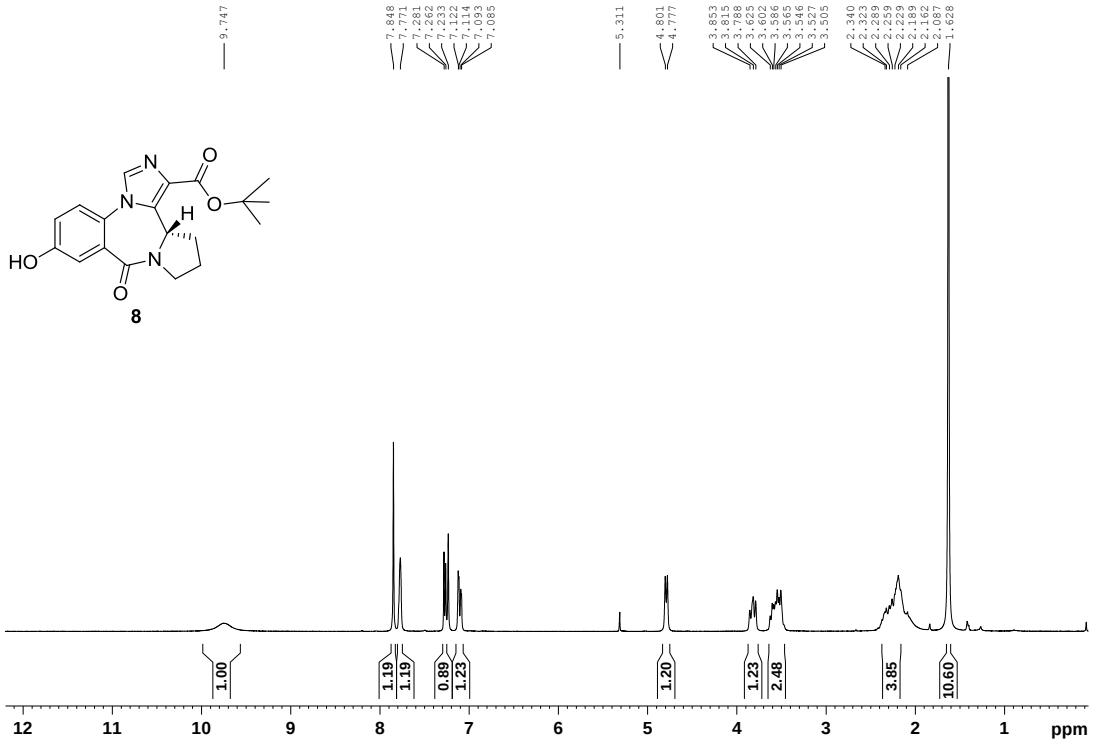
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4.777
3.853
3.715
3.688
3.625
3.602
3.586
3.565
3.546
3.527
3.505
2.340
2.323
2.289
2.259
2.229
2.189
2.162
2.087
1.628
1.00
1.19
1.19
0.89
1.23
1.20
1.23
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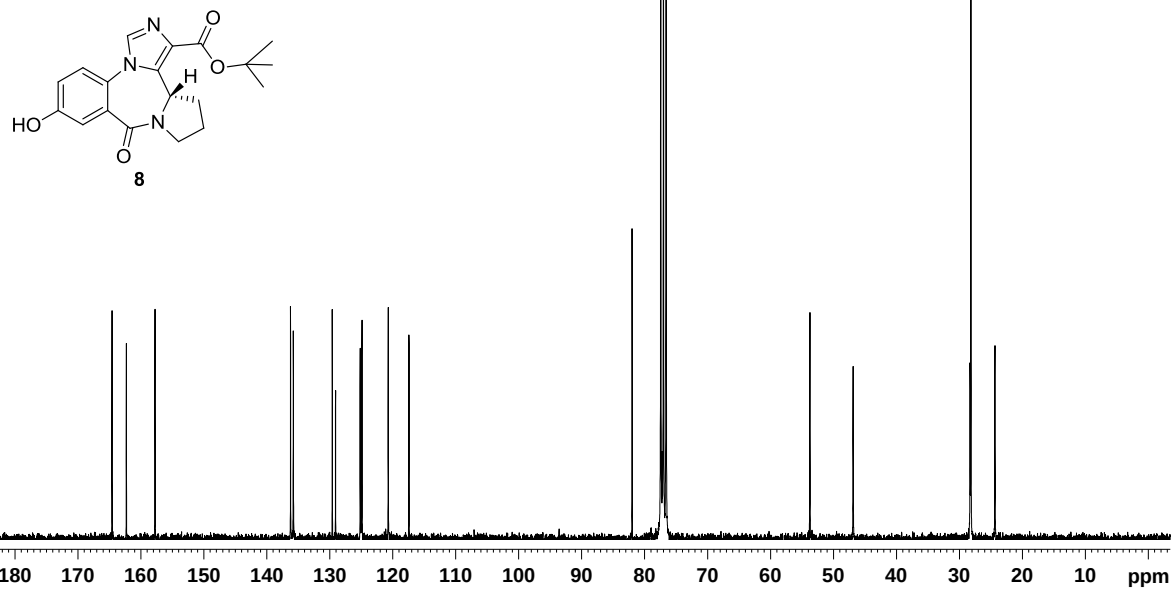
3.85
10.60
12
11
10
9
8
7
6
5
4
3
2
1
ppm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77.029
76.605
53.755
46.898
28.328
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24.376
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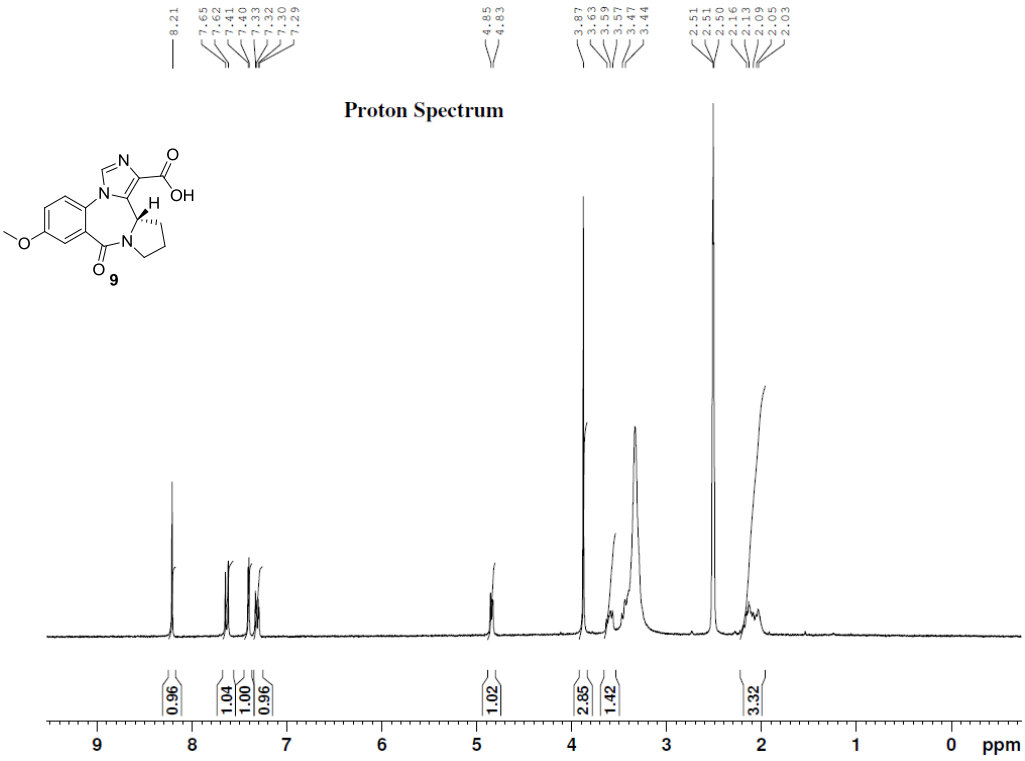
ppm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2.50
2.16
2.13
2.09
2.05
2.03
Proton Spectrum
N
O
OH
N
H
O
N
O
9
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1.04
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6
5
4
3
2
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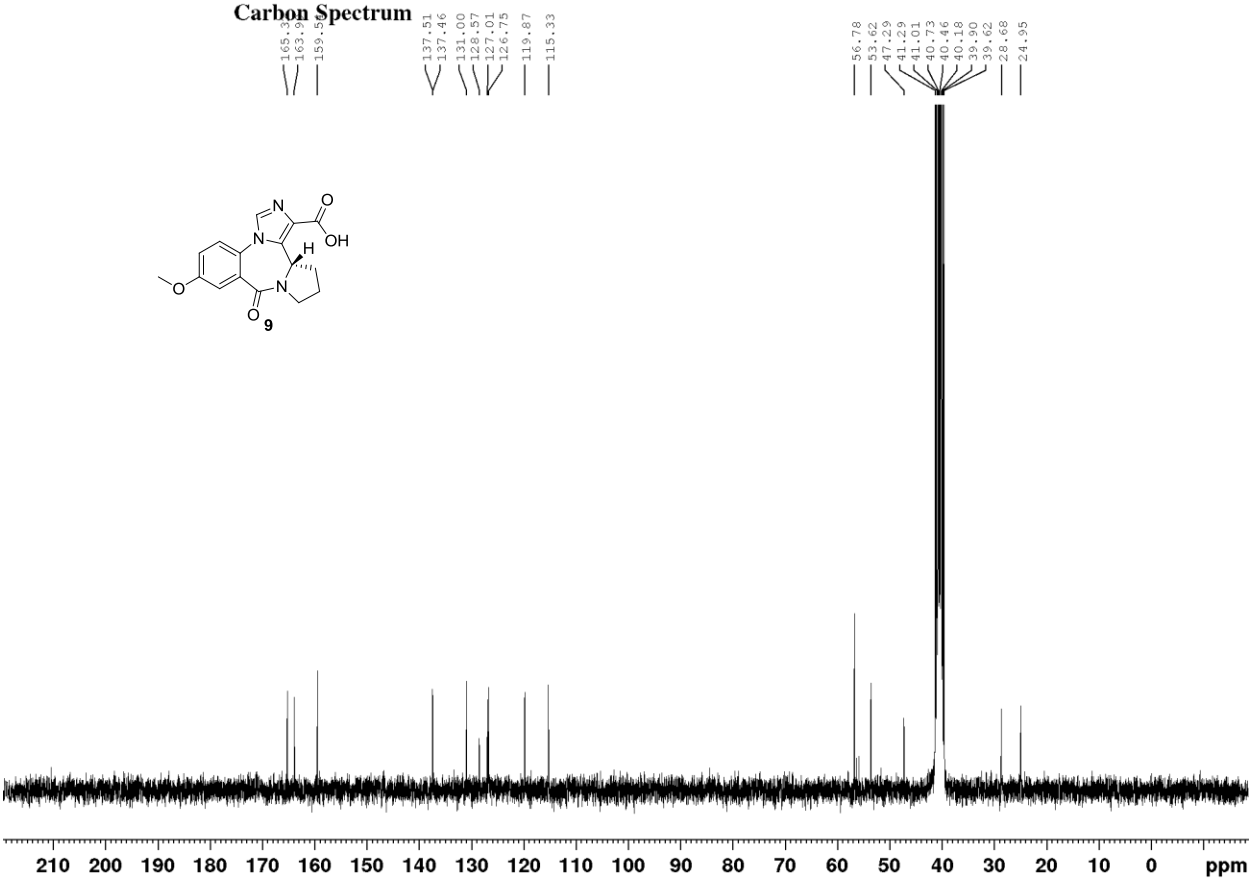
0
ppm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53.62
47.29
41.29
41.01
40.73
40.46
40.18
39.90
39.62
28.68
24.95
N
O
OH
N
H
O
N
O
9
210
200
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130
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70
60
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10
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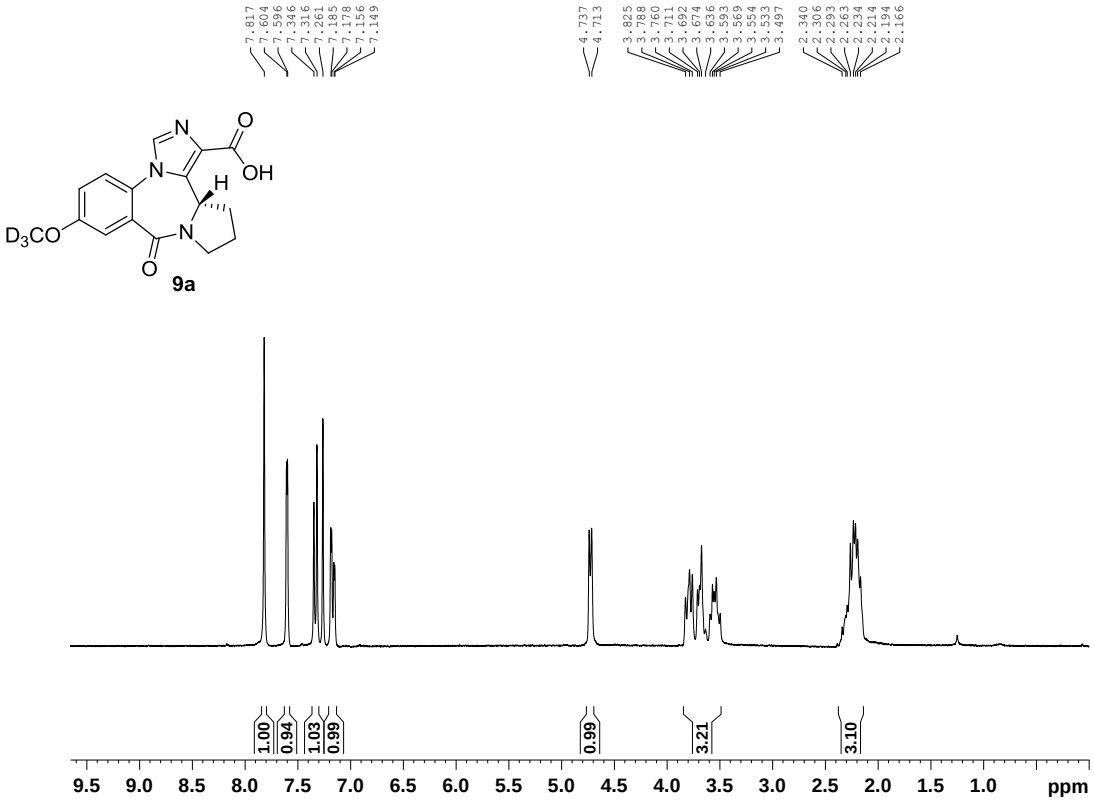
ppm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3.788
3.760
3.711
3.692
3.674
3.636
3.593
3.569
3.554
3.533
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2.340
2.306
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D3CO
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O
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1.03
0.99
0.99
3.21
3.10
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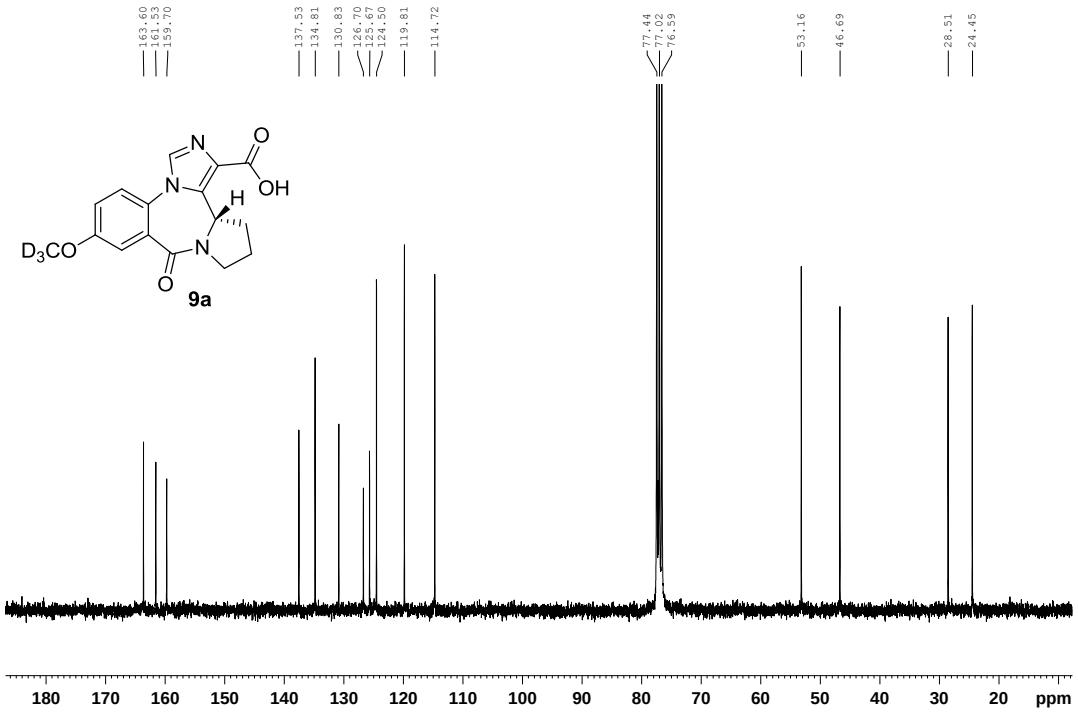
ppm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126.70
125.67
124.50
119.81
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53.16
46.69
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OH
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O
9a
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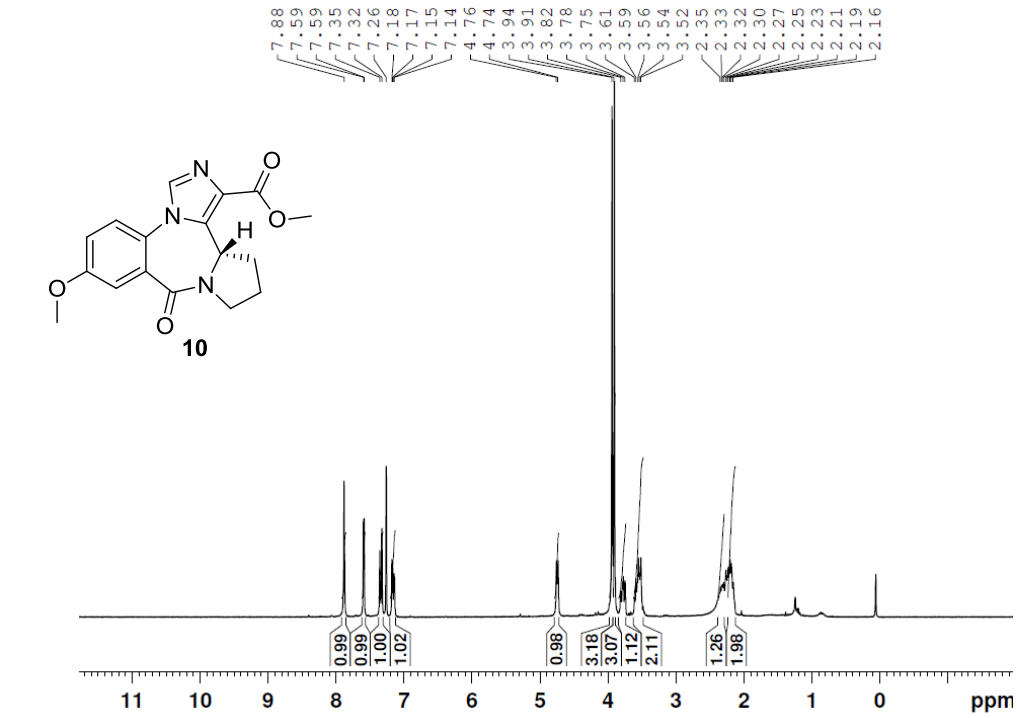
ppm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4.74
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3.91
3.82
3.78
3.75
3.61
3.59
3.56
3.54
3.52
2.35
2.33
2.32
2.30
2.27
2.25
2.23
2.21
2.19
2.16
0.99
0.99
1.00
1.02
0.98
3.18
3.07
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1.26
1.98
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8
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6
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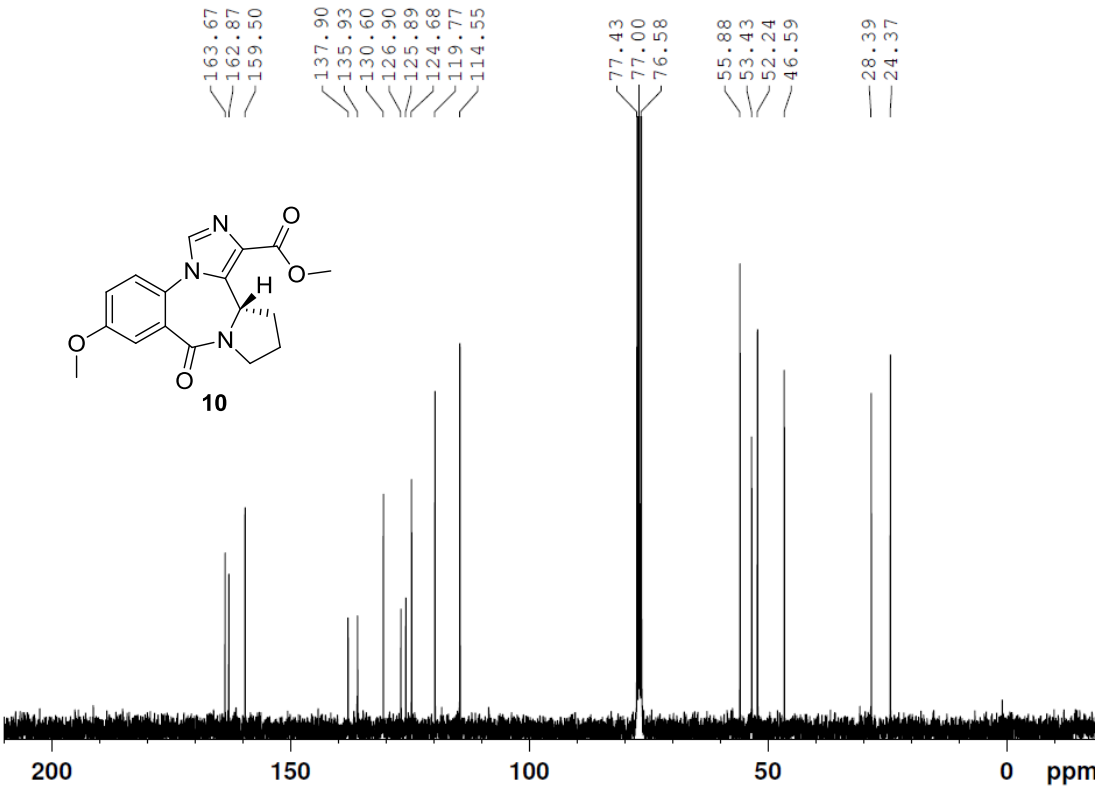
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125.89
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114.55
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55.88
53.43
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24.37
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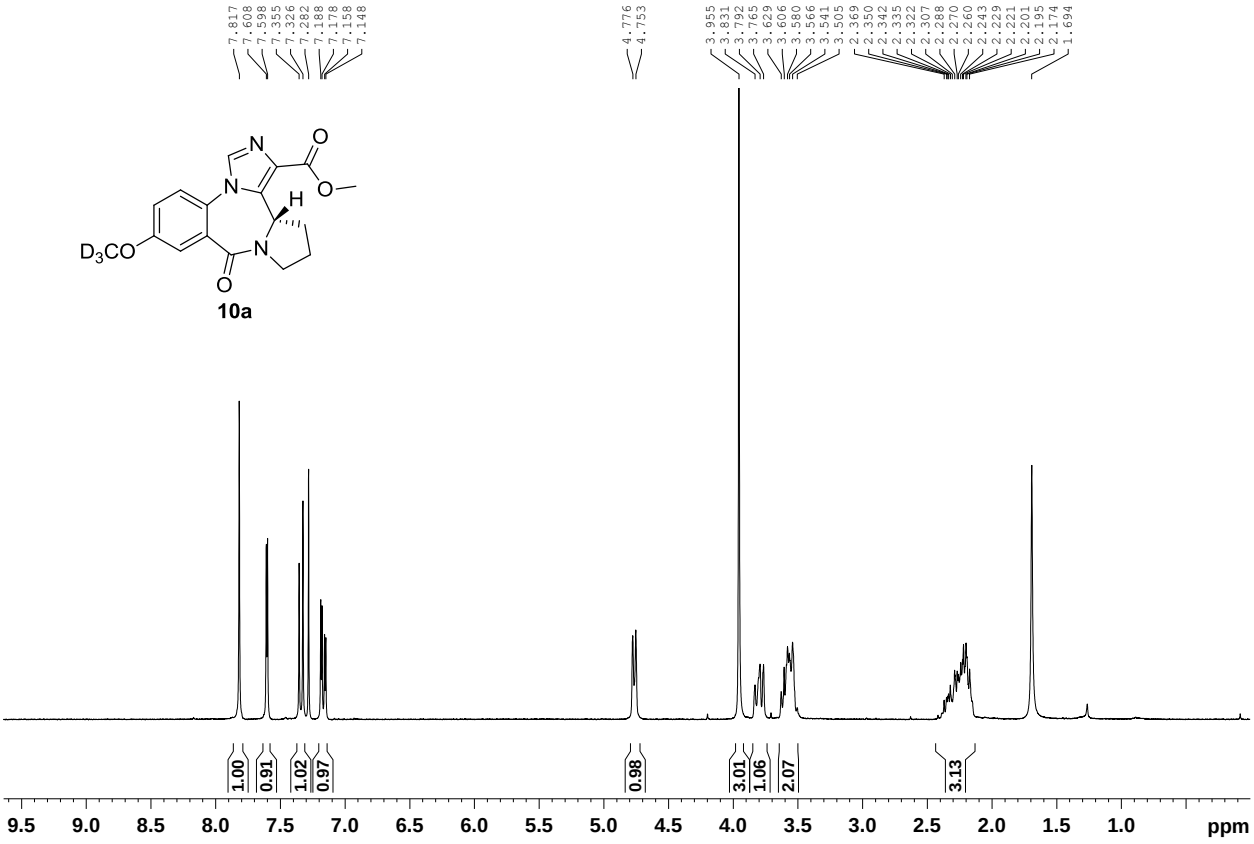
50
0
ppm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7.355
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7.178
7.158
7.148
4.776
4.753
3.955
3.831
3.792
3.765
3.629
3.606
3.580
3.566
3.541
3.505
2.369
2.350
2.342
2.335
2.322
2.307
2.288
2.270
2.260
2.243
2.229
2.221
2.201
2.195
2.174
1.694
N
O
O
N
H
D3CO
N
O
10a
1.00
0.91
1.02
0.97
0.98
3.01
1.06
2.07
3.13
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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5.0
4.5
4.0
3.5
3.0
2.5
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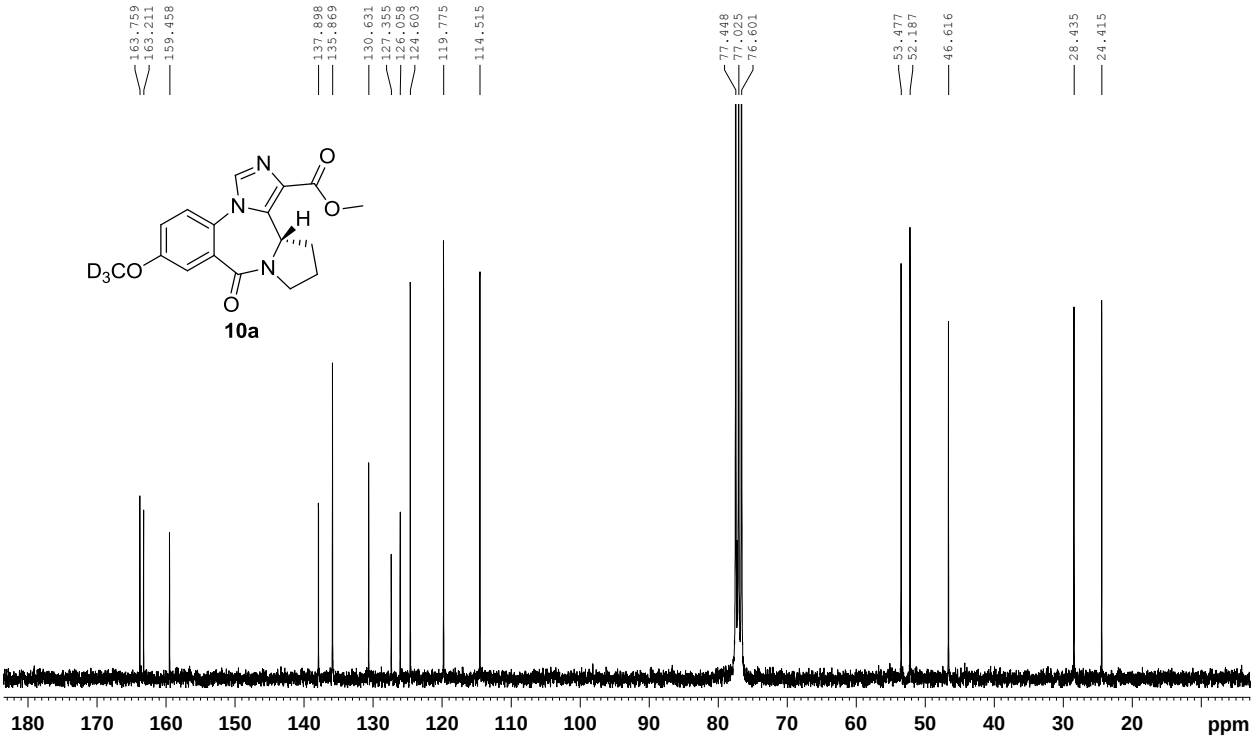
1.5
1.0
ppm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126.058
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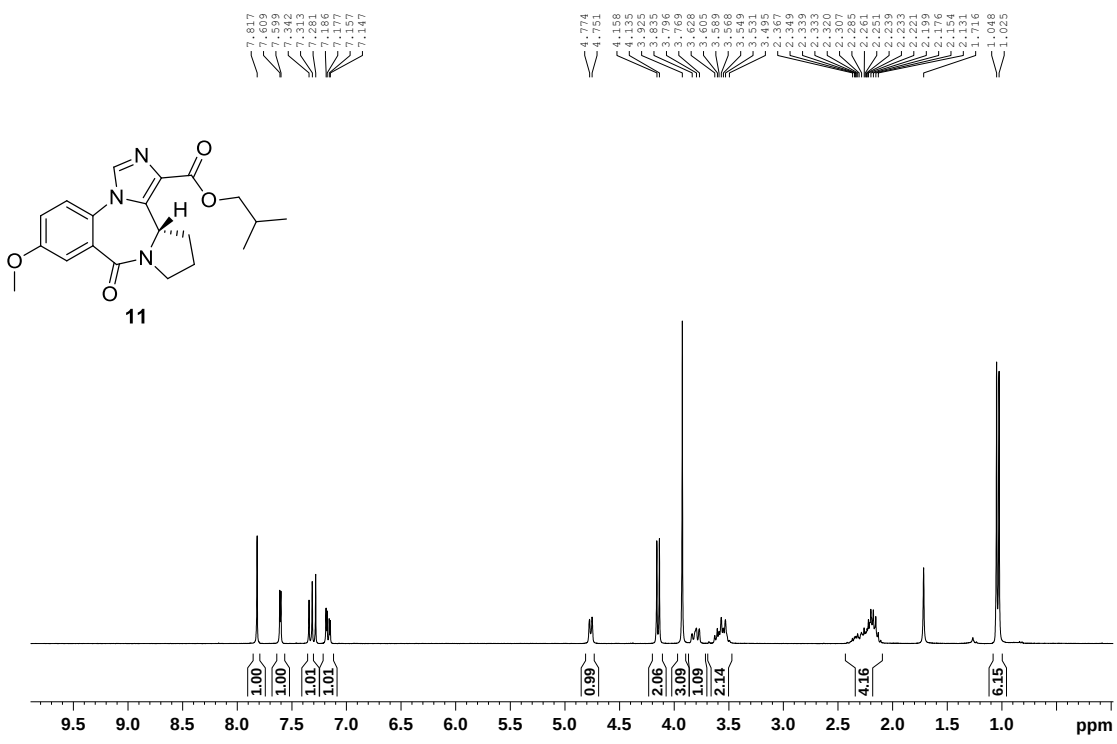
119.775
114.515
77.448
77.025
76.601
53.477
52.187
46.616
28.435
24.415
N
O
O
N
H
D3CO
N
O
10a
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm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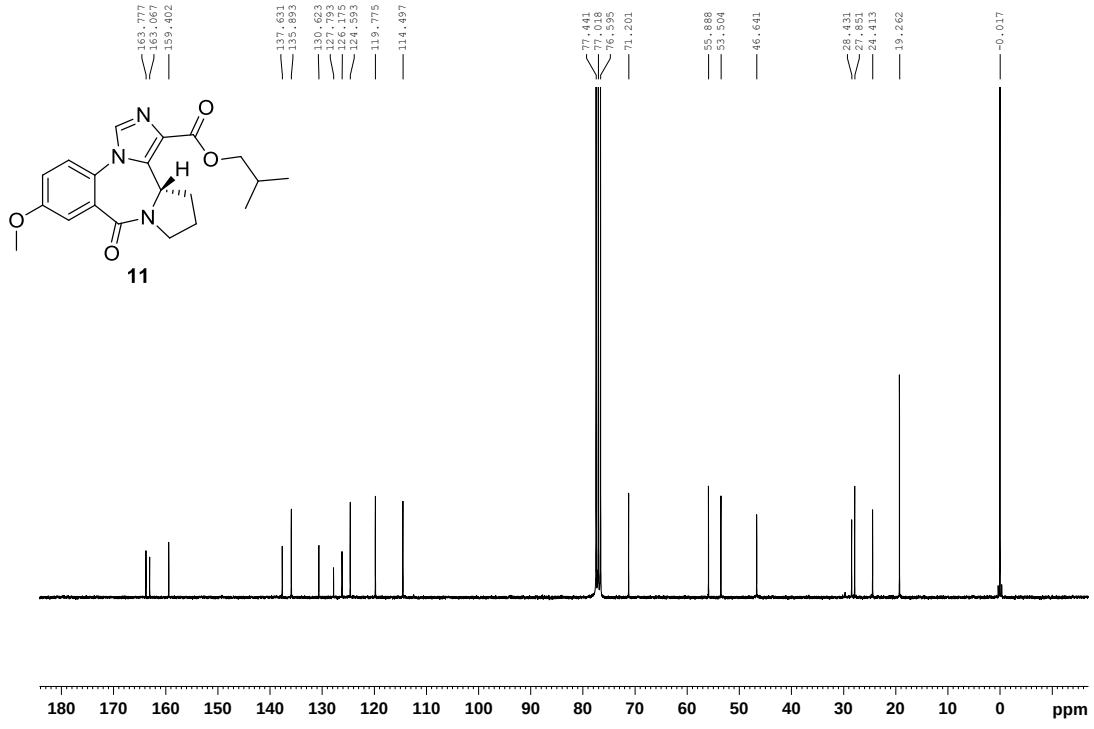
7.281
7.186
7.177
7.157
7.147
4.774
4.751
4.158
4.135
3.925
3.835
3.796
3.768
3.628
3.605
3.589
3.568
3.549
3.331
3.495
3.467
2.349
2.339
2.333
2.320
2.307
2.285
2.261
2.251
2.238
2.233
2.221
2.199
2.176
2.154
2.131
1.716
1.048
1.025
11
1.00
1.00
1.01
1.01
0.99
2.06
3.09
1.09
2.14
4.16
6.15
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
ppm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126.175
124.593
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114.497
77.441
77.018
76.595
71.201
55.888
53.504
46.641
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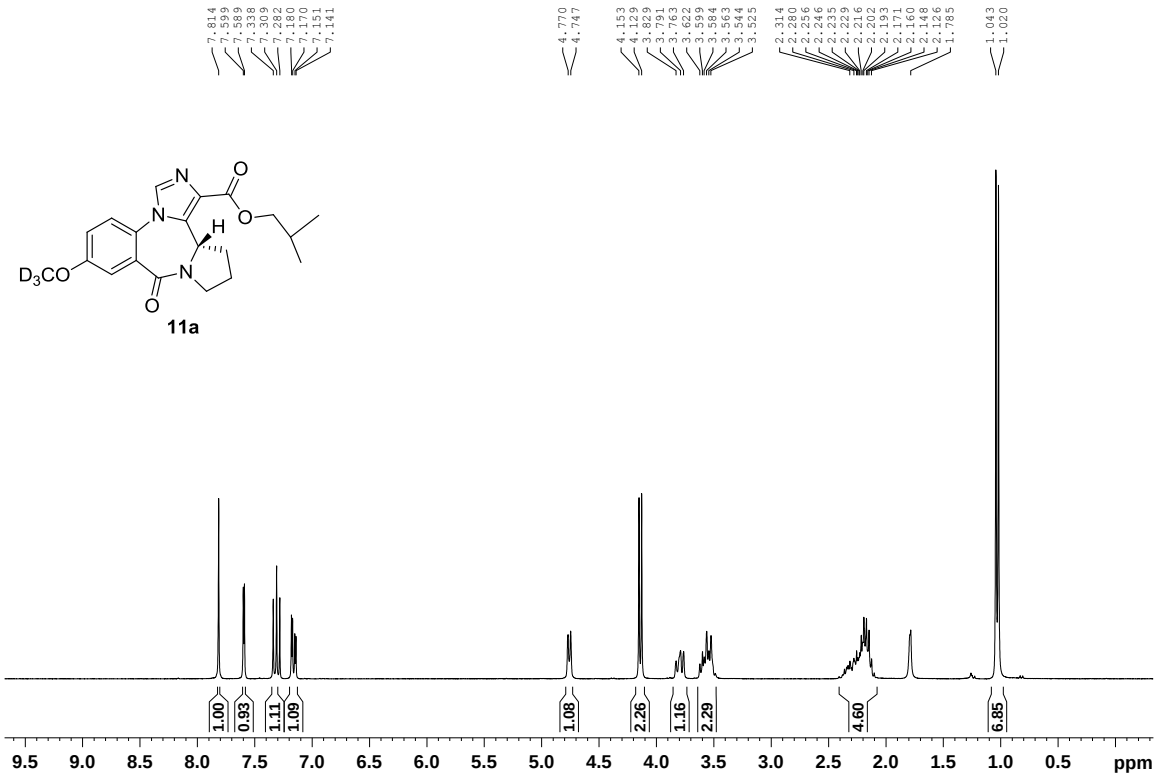
27.851
24.413
19.262
-0.017
11
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm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7.589
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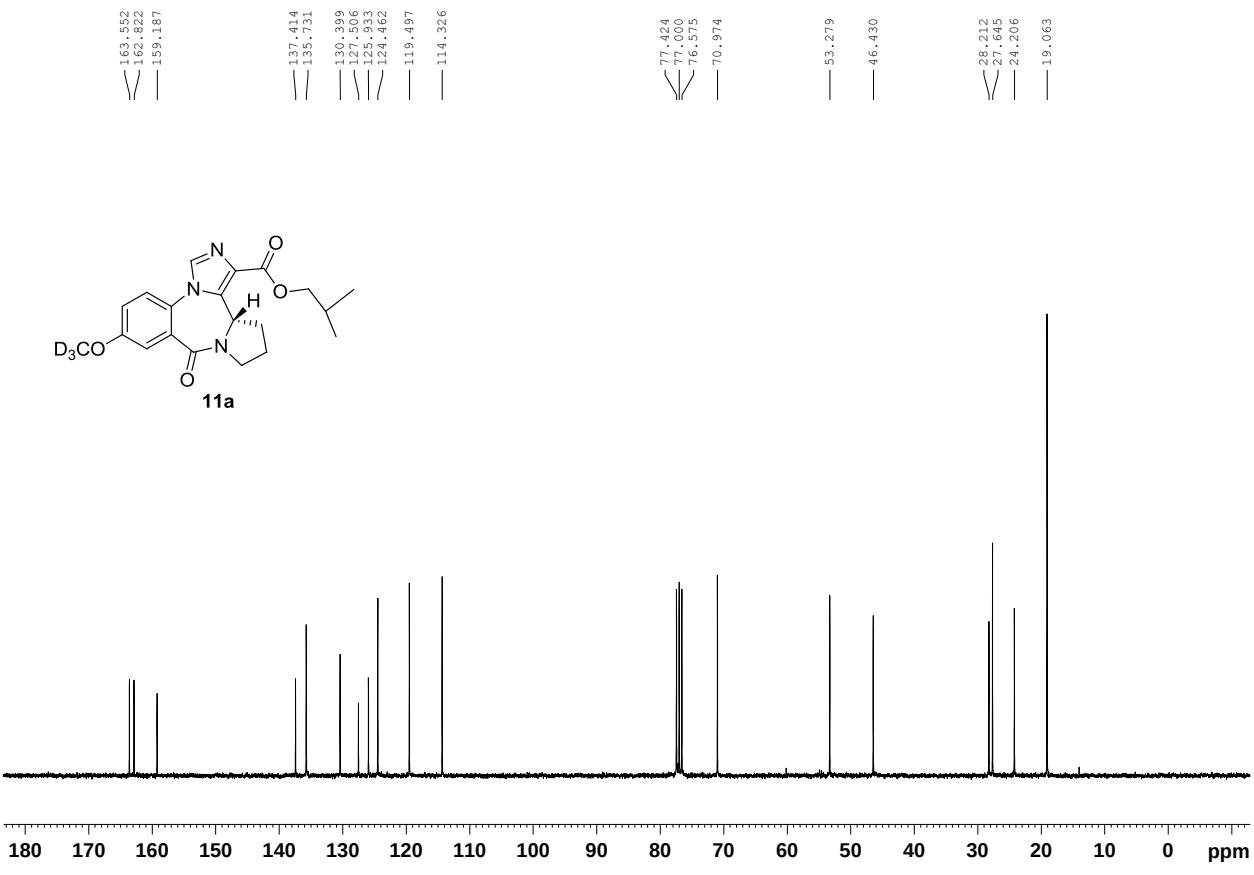
7.309
7.282
7.180
7.170
7.151
7.141
4.770
4.747
4.153
4.129
3.829
3.791
3.783
3.622
3.599
3.584
3.563
3.544
3.525
2.314
2.280
2.256
2.246
2.235
2.229
2.216
2.202
2.193
2.171
2.160
2.148
2.126
1.785
1.043
1.020
D3CO
11a
1.00
0.93
1.11
1.09
1.08
2.26
1.16
2.29
4.60
6.85
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm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159.187
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77.424
77.000
76.575
70.974
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46.430
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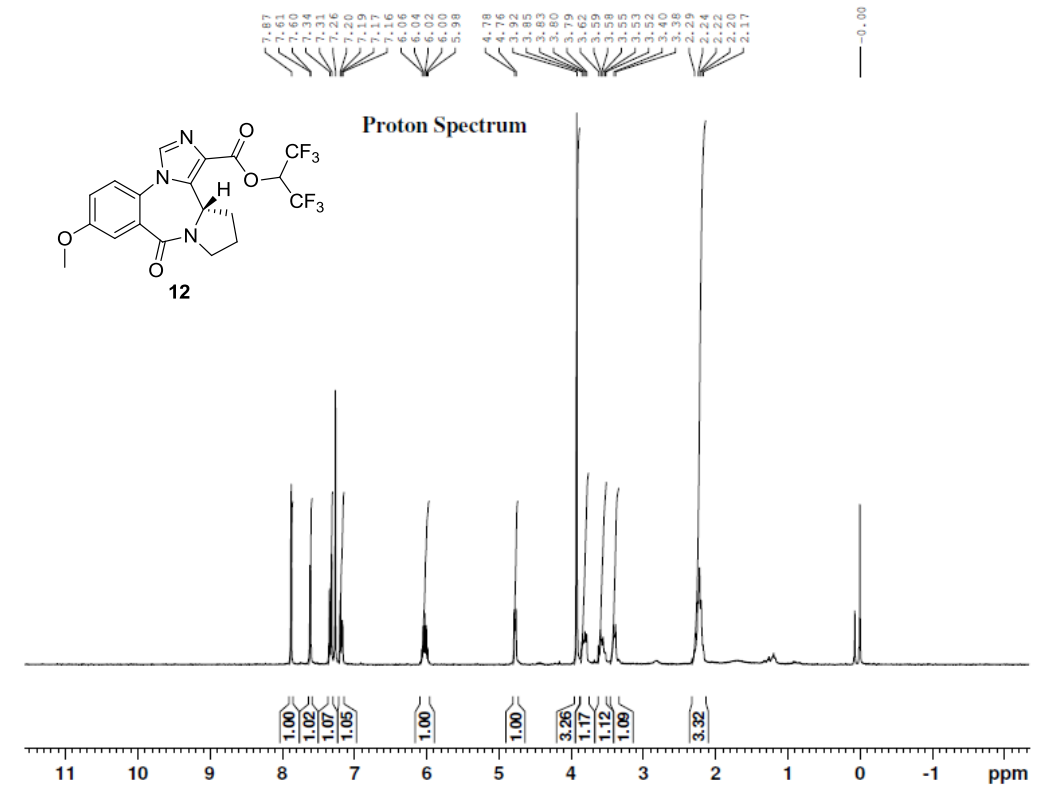
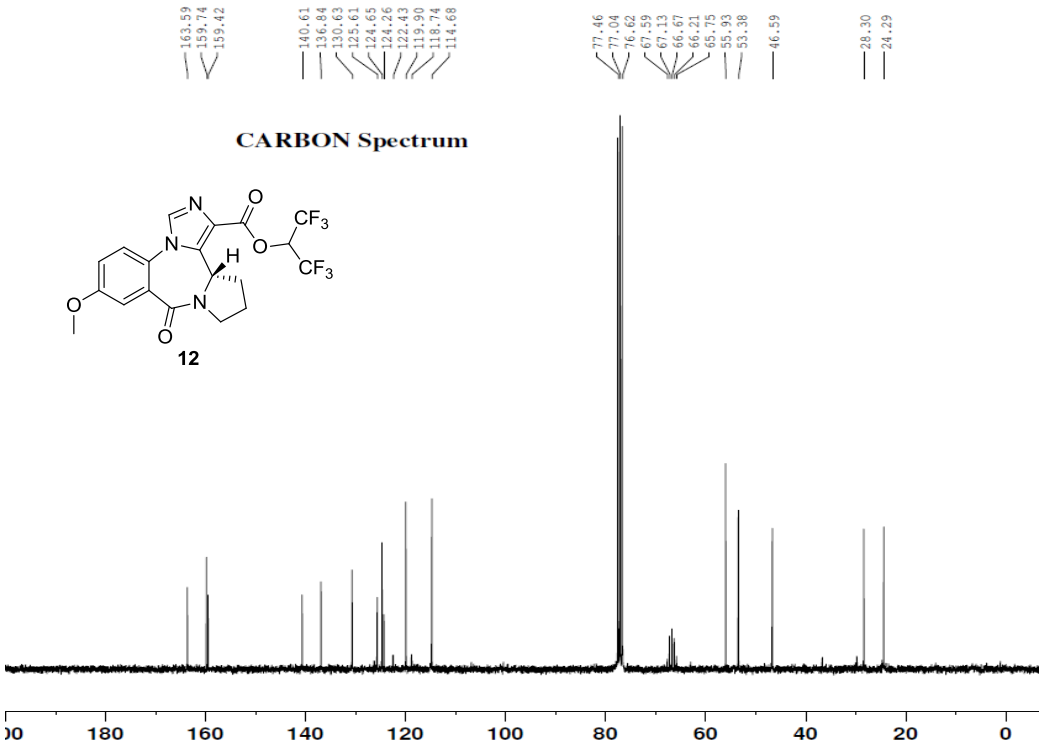
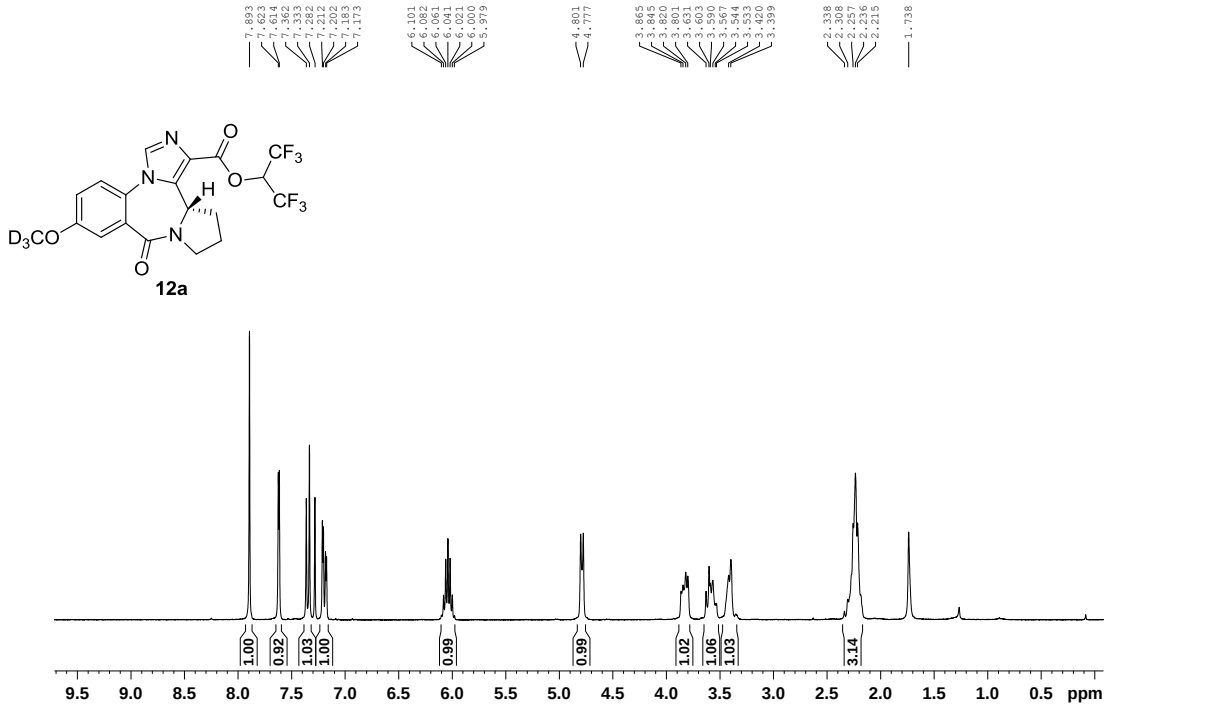
27.645
24.206
19.063
D3CO
11a
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


Proton Spectrum
12
ppm


CARBON Spectrum
12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7.623
7.614
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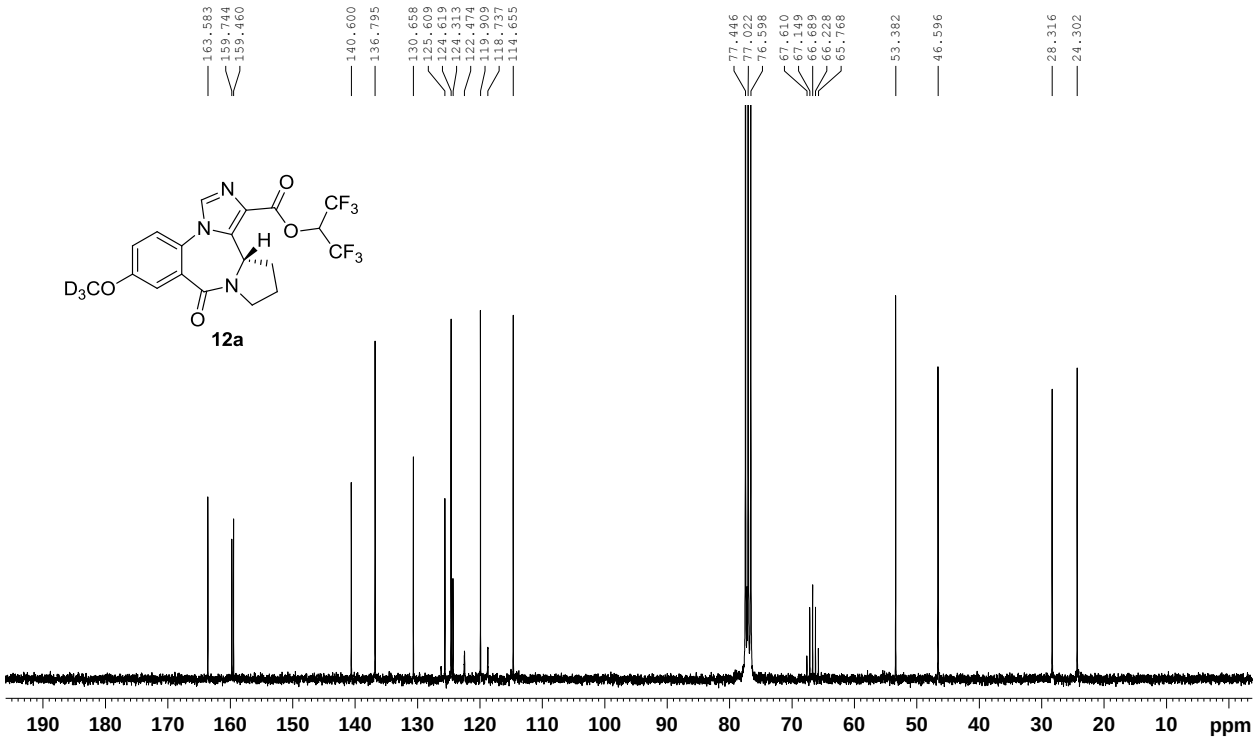
7.353
7.282
7.212
7.202
7.183
7.173
6.101
6.082
6.061
6.041
6.021
6.000
5.979
4.801
4.777
3.865
3.845
3.820
3.601
3.581
3.603
3.590
3.567
3.544
3.533
3.420
3.399
2.338
2.308
2.257
2.236
2.215
1.738
D3CO
CF3
CF3
12a
1.00
0.92
1.03
1.00
0.99
0.99
1.02
1.06
1.03
3.14
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm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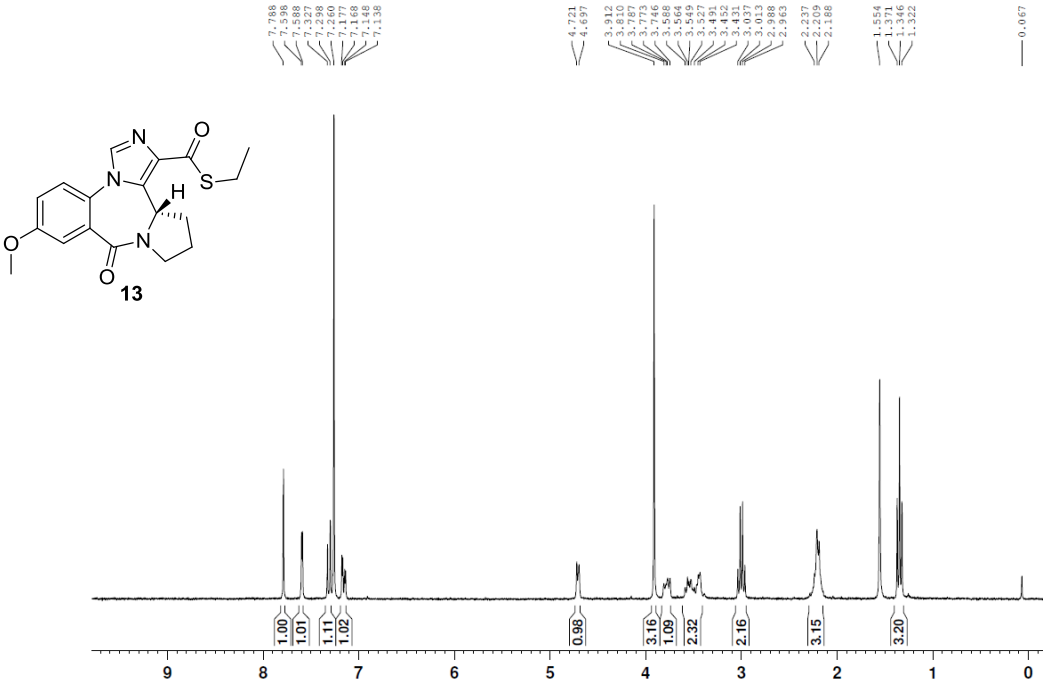
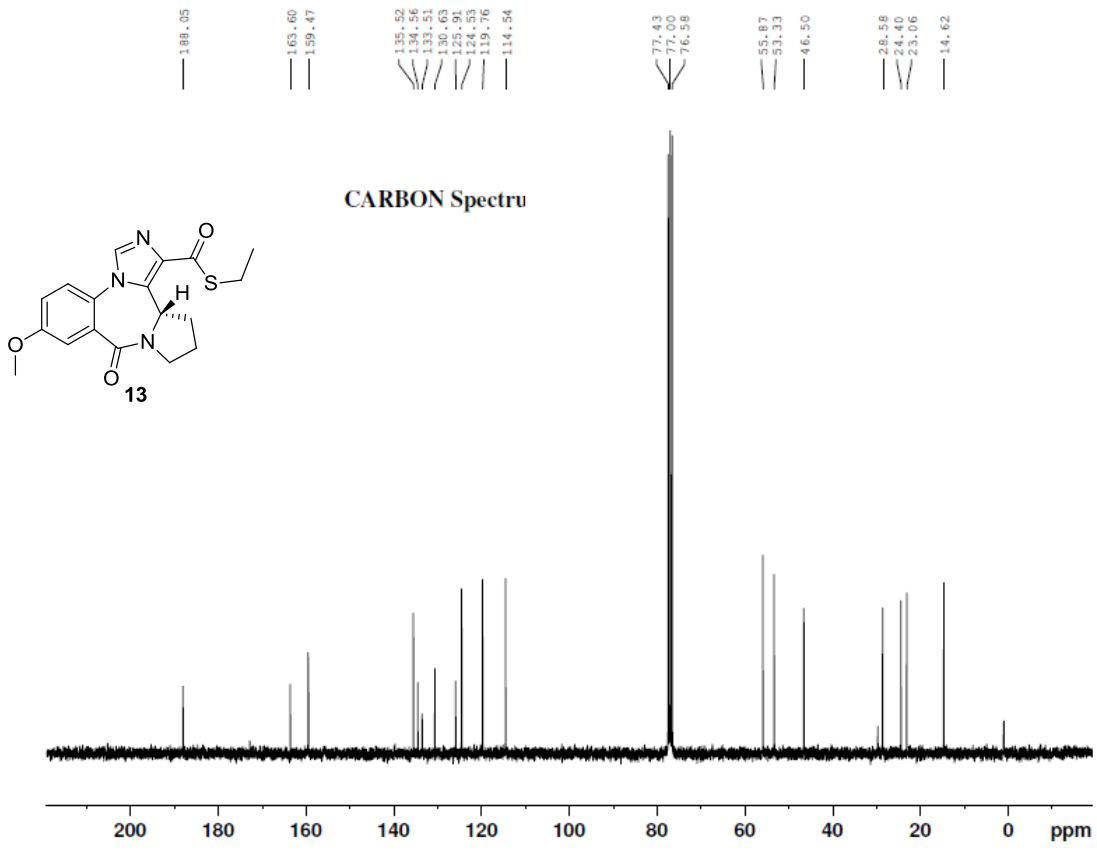
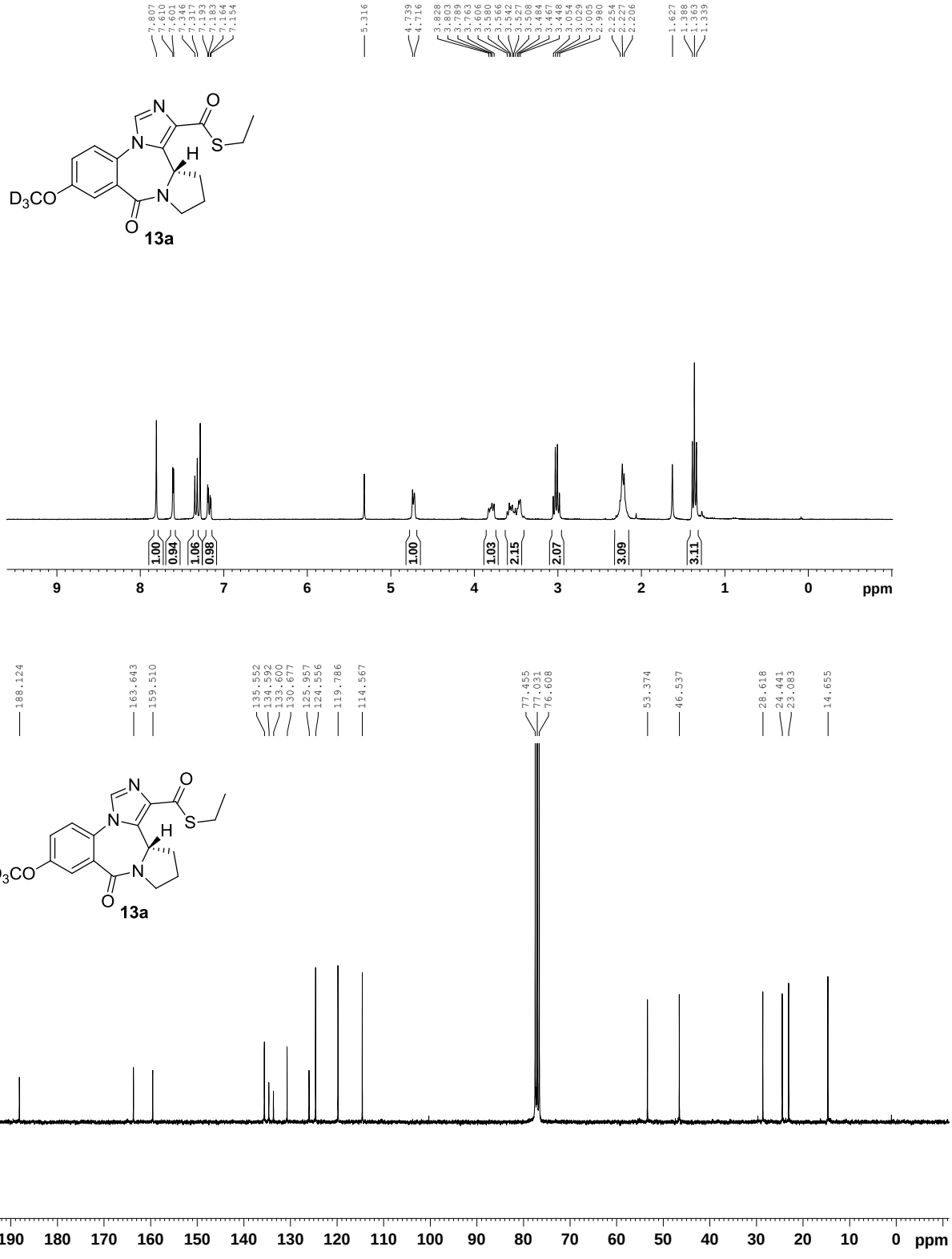
125.609
124.619
124.313
122.474
119.909
118.737
114.655
77.446
77.022
76.598
67.610
67.149
66.689
66.228
65.768
53.382
46.596
28.316
24.302
D3CO
CF3
CF3
12a
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


13


CARBON Spectru
13
ppm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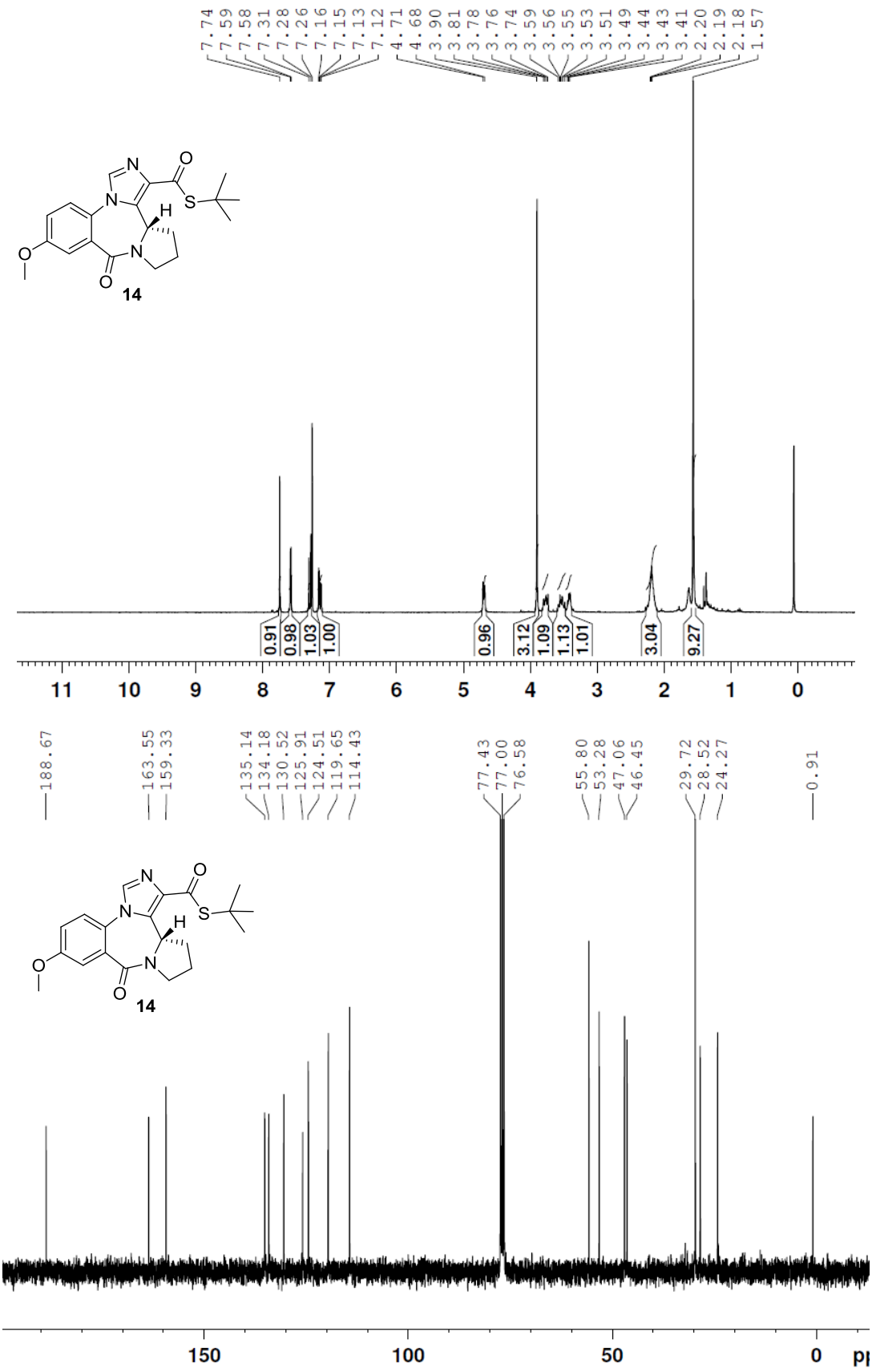
7.164
7.154
5.316
4.739
4.716
3.828
3.803
3.783
3.763
3.606
3.580
3.566
3.542
3.527
3.508
3.484
3.467
3.448
3.054
3.029
3.005
2.980
2.254
2.227
2.206
1.627
1.388
1.363
1.339
N
O
S
H
N
N
D3CO
O
13a
1.00
0.94
1.06
0.98
1.00
1.03
2.15
2.07
3.09
3.11
9
8
7
6
5
4
3
2
1
0
ppm
188.124
163.643
159.510
135.552
134.592
133.600
130.677
125.957
124.556
119.786
114.567
77.455
77.031
76.608
53.374
46.537
28.618
24.441
23.083
14.655
N
O
S
H
N
N
D3CO
O
13a
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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7.31
7.28
7.26
7.16
7.15
7.13
7.12
4.71
4.68
3.90
3.81
3.78
3.76
3.74
3.59
3.56
3.55
3.53
3.51
3.49
3.44
3.43
3.41
2.20
2.19
2.18
1.57
14
0.91
0.98
1.03
1.00
0.96
3.12
1.09
1.13
1.01
3.04
9.27
11
10
9
8
7
6
5
4
3
2
1
0
188.67
163.55
159.33
135.14
134.18
130.52
125.91
124.51
119.65
114.43
77.43
77.00
76.58
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47.06
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0.91
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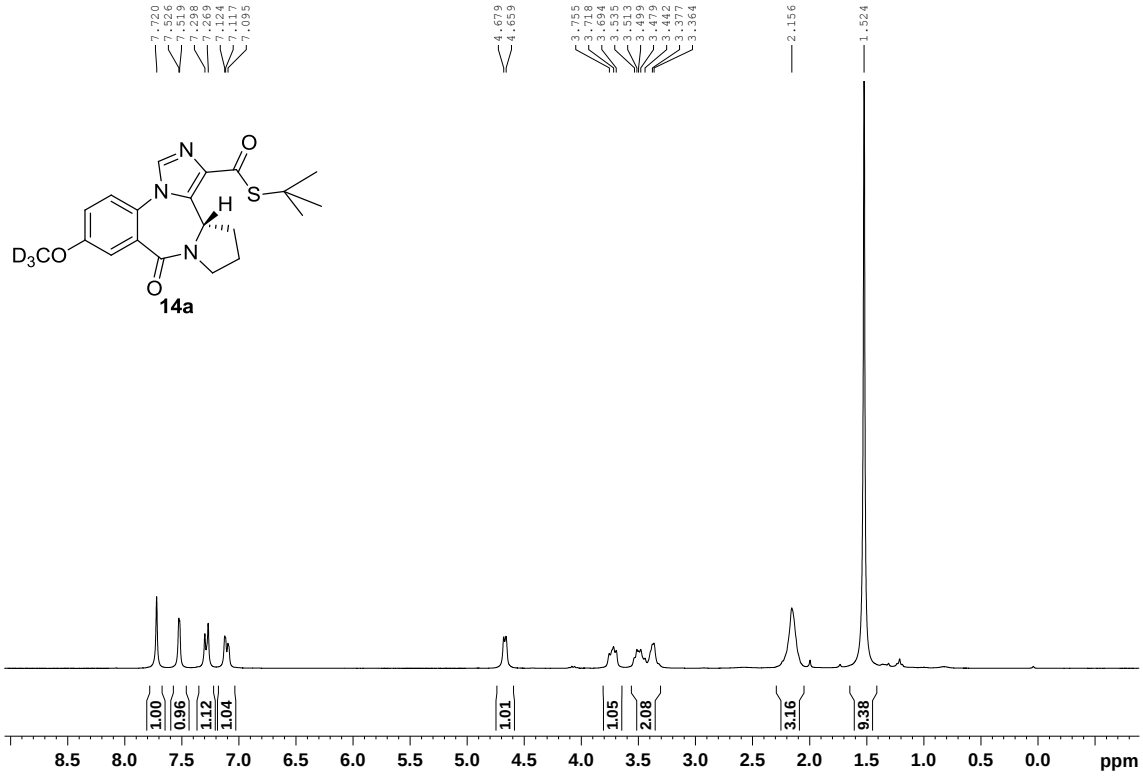
150
100
50
0
pp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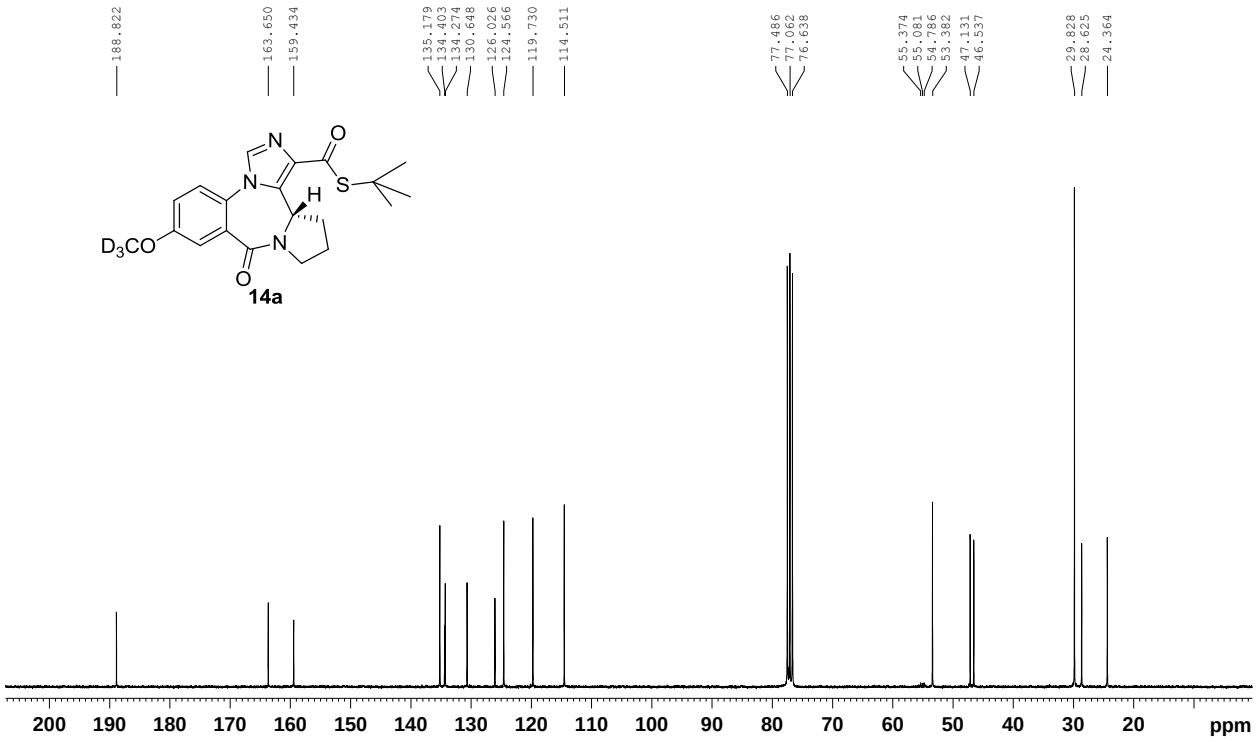
7.526
7.519
7.298
7.269
7.124
7.117
7.095
4.679
4.659
3.755
3.718
3.694
3.535
3.513
3.499
3.479
3.442
3.377
3.364
2.156
1.524
D3CO
14a
1.00
0.96
1.12
1.04
1.01
1.05
2.08
3.16
9.38
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm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135.179
134.403
134.274
130.648
126.026
124.566
119.730
114.511
77.486
77.062
76.638
55.374
55.081
54.786
53.382
47.131
46.537
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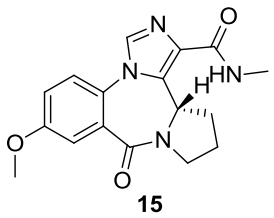
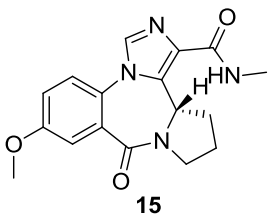
28.625
24.364
D3CO
14a
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
ppm

Proton Spectrum

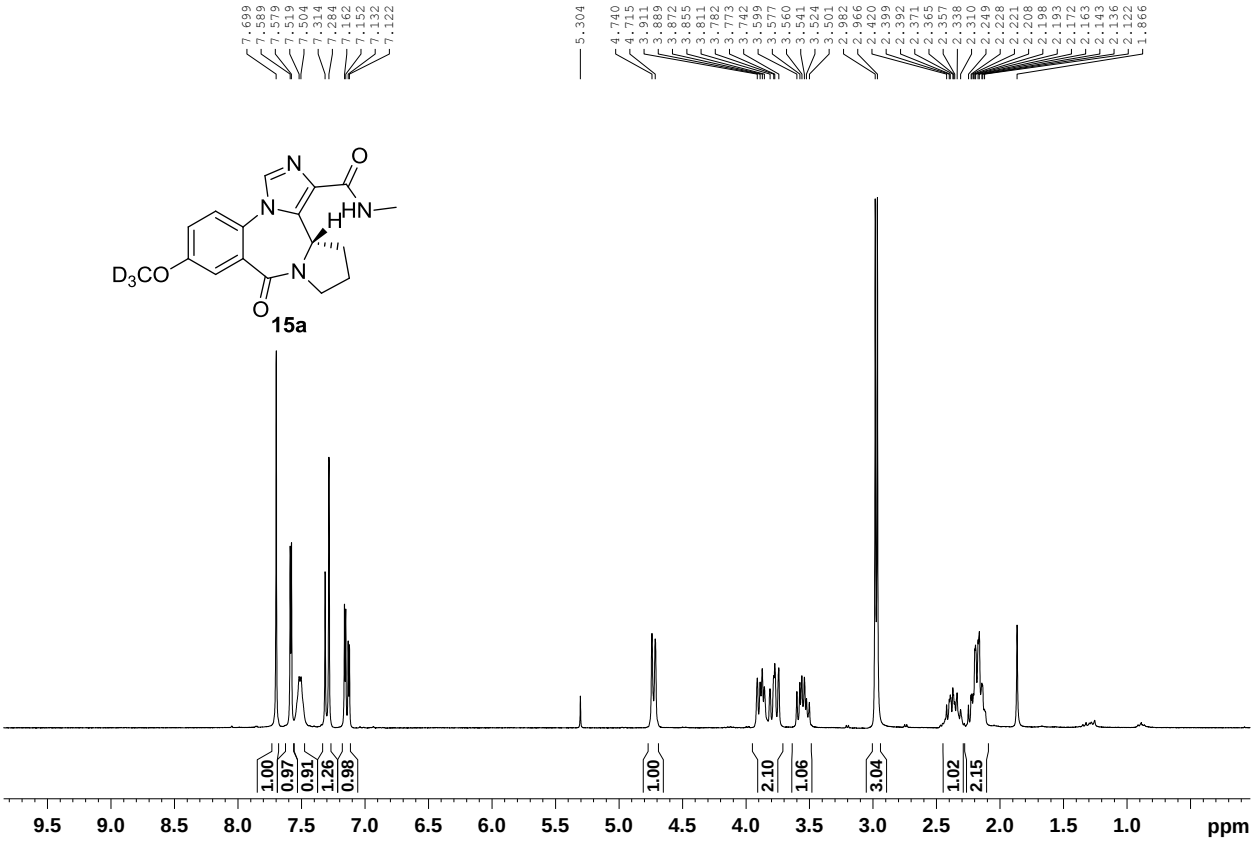


15

Carbon Spectrum



15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7.314
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7.162
7.152
7.132
7.122
5.304
4.740
4.715
3.911
3.889
3.872
3.855
3.811
3.782
3.773
3.742
3.599
3.577
3.560
3.541
3.524
3.501
2.982
2.966
2.420
2.399
2.392
2.371
2.365
2.357
2.338
2.310
2.249
2.228
2.221
2.208
2.198
2.193
2.172
2.163
2.143
2.136
2.122
1.866
D3CO
HN
H
O
N
15a
1.00
0.97
0.91
1.26
0.98
1.00
2.10
1.06
3.04
1.02
2.15
9.5
9.0
8.5
8.0
7.5
7.0
6.5
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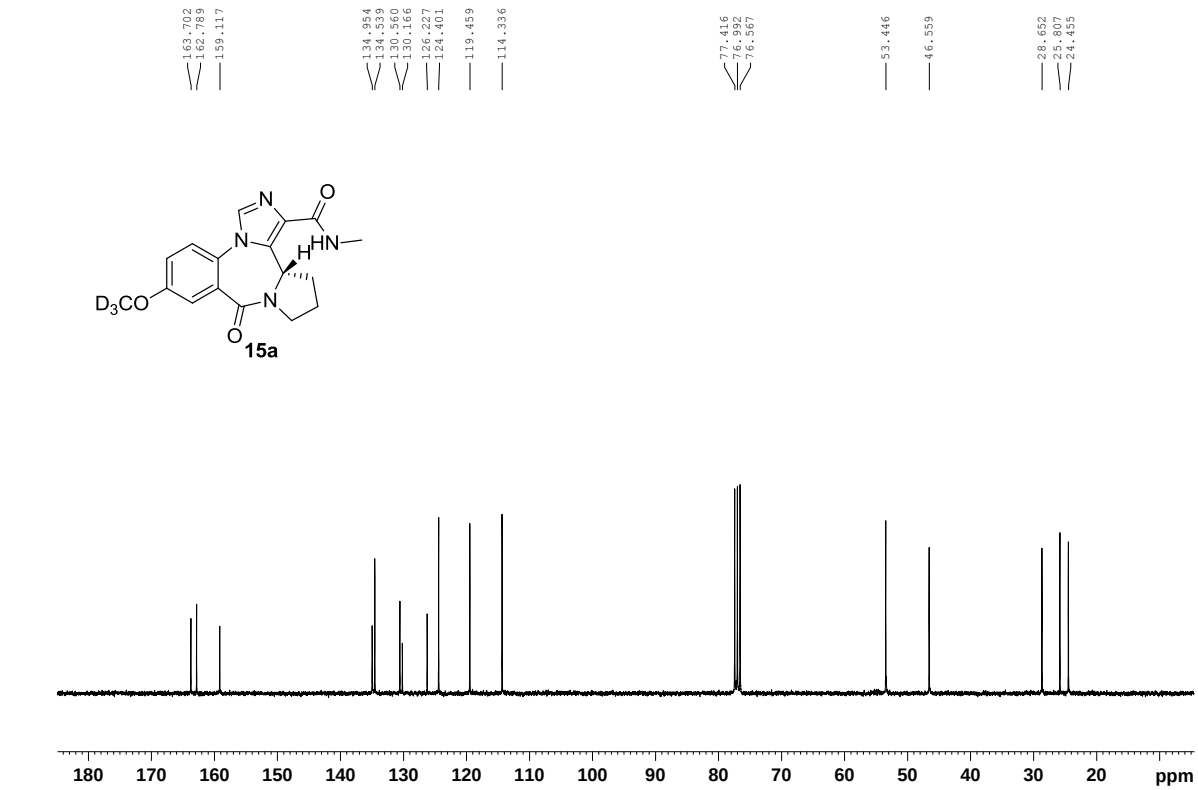
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm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126.227
124.401
119.459
114.336
77.416
76.992
76.567
53.446
46.559
28.652
25.807
24.455
D3CO
HN
H
O
N
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160
150
140
130
120
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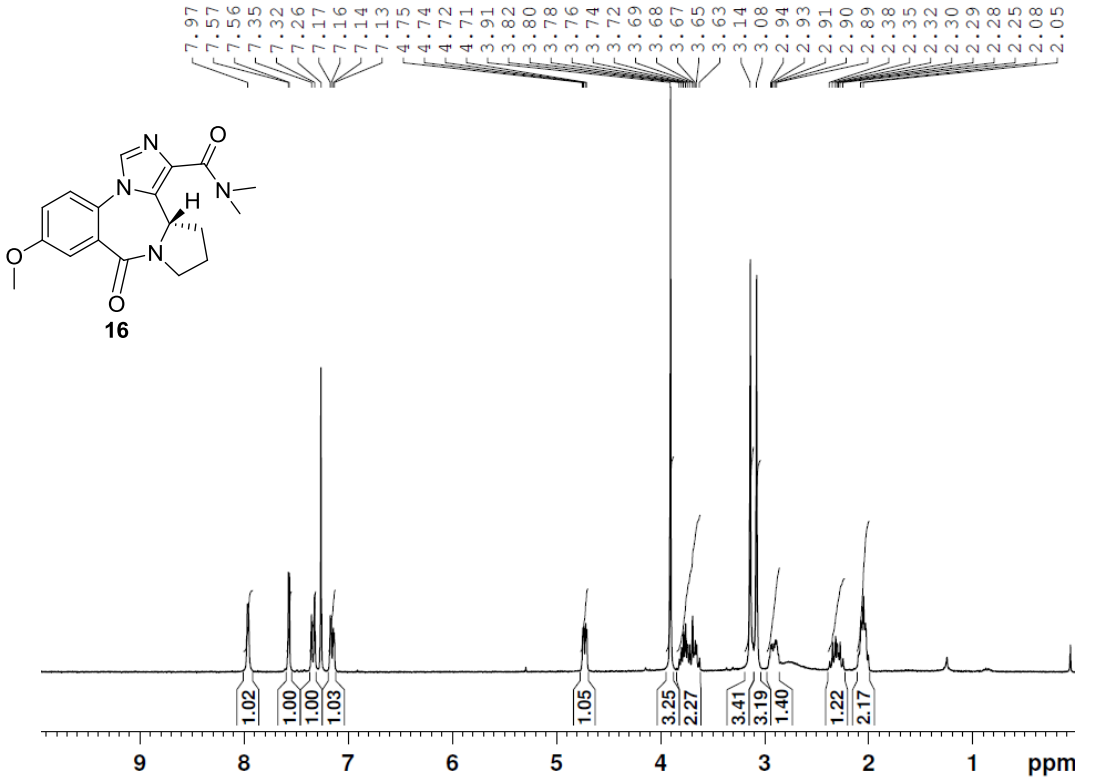
100
90
80
70
60
50
40
30
20
ppm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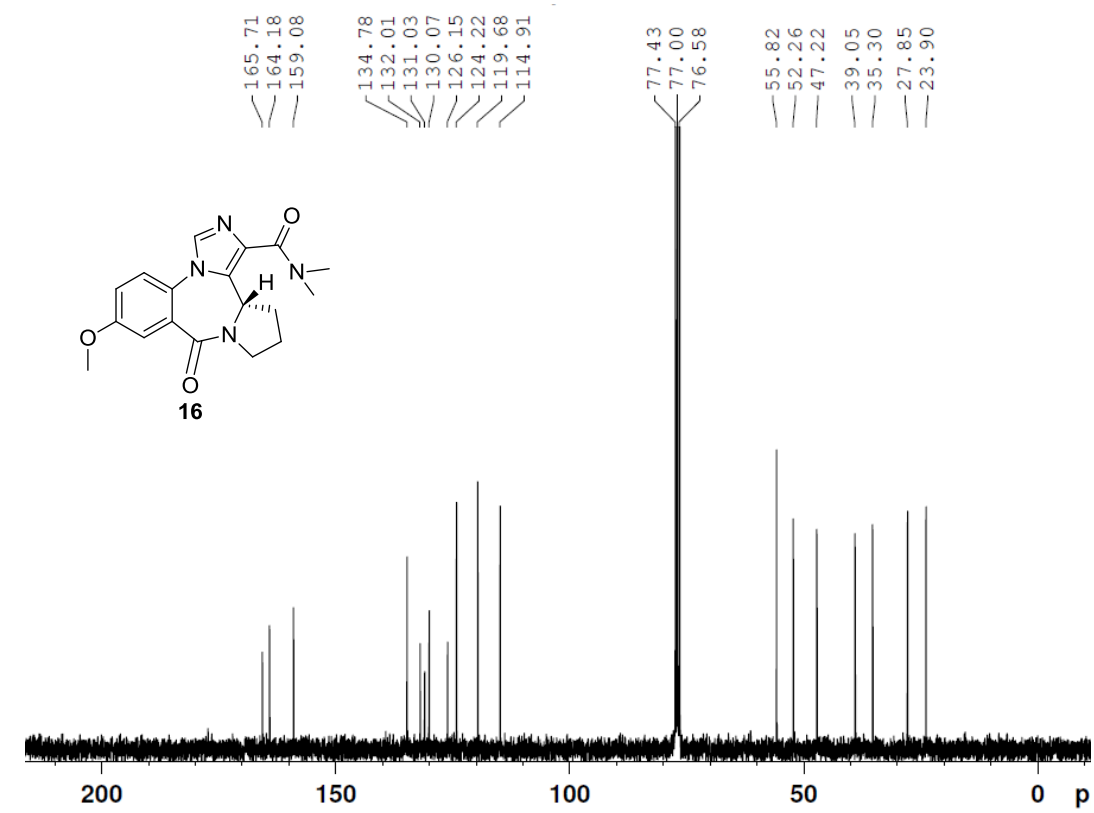
7.16
7.14
7.13
4.75
4.74
4.72
4.71
3.91
3.82
3.80
3.78
3.76
3.74
3.72
3.69
3.68
3.67
3.65
3.63
3.14
3.08
2.94
2.93
2.91
2.90
2.89
2.38
2.35
2.32
2.30
2.29
2.28
2.25
2.08
2.05
16
1.02
1.00
1.00
1.03
1.05
3.25
2.27
3.41
3.19
1.40
1.22
2.17
9
8
7
6
5
4
3
2
1
ppm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159.08
134.78
132.01
131.03
130.07
126.15
124.22
119.68
114.91
77.43
77.00
76.58
55.82
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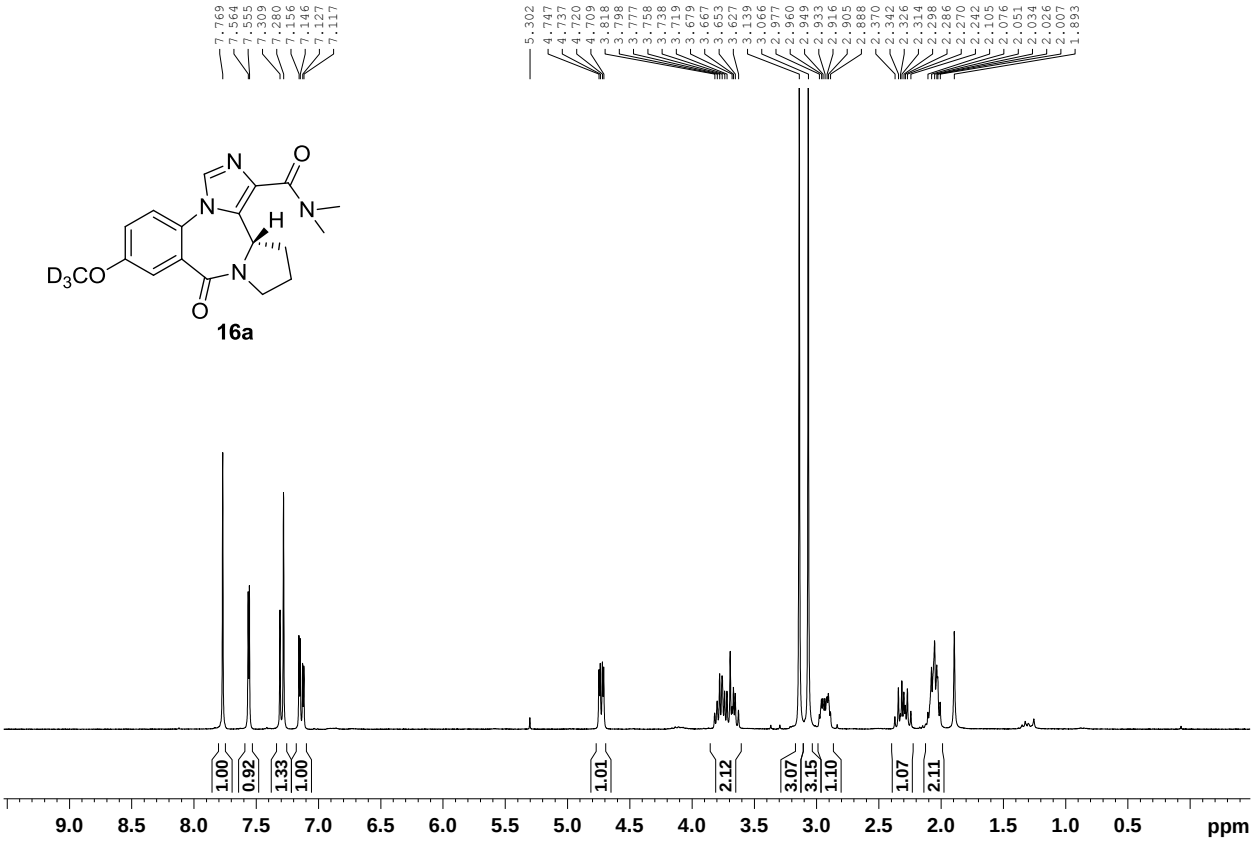
47.22
39.05
35.30
27.85
23.90
16
200
150
100
50
0
p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7.117
5.302
4.747
4.737
4.720
4.709
3.818
3.798
3.777
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3.679
3.667
3.653
3.627
3.139
3.066
2.977
2.960
2.949
2.933
2.916
2.905
2.888
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2.342
2.326
2.314
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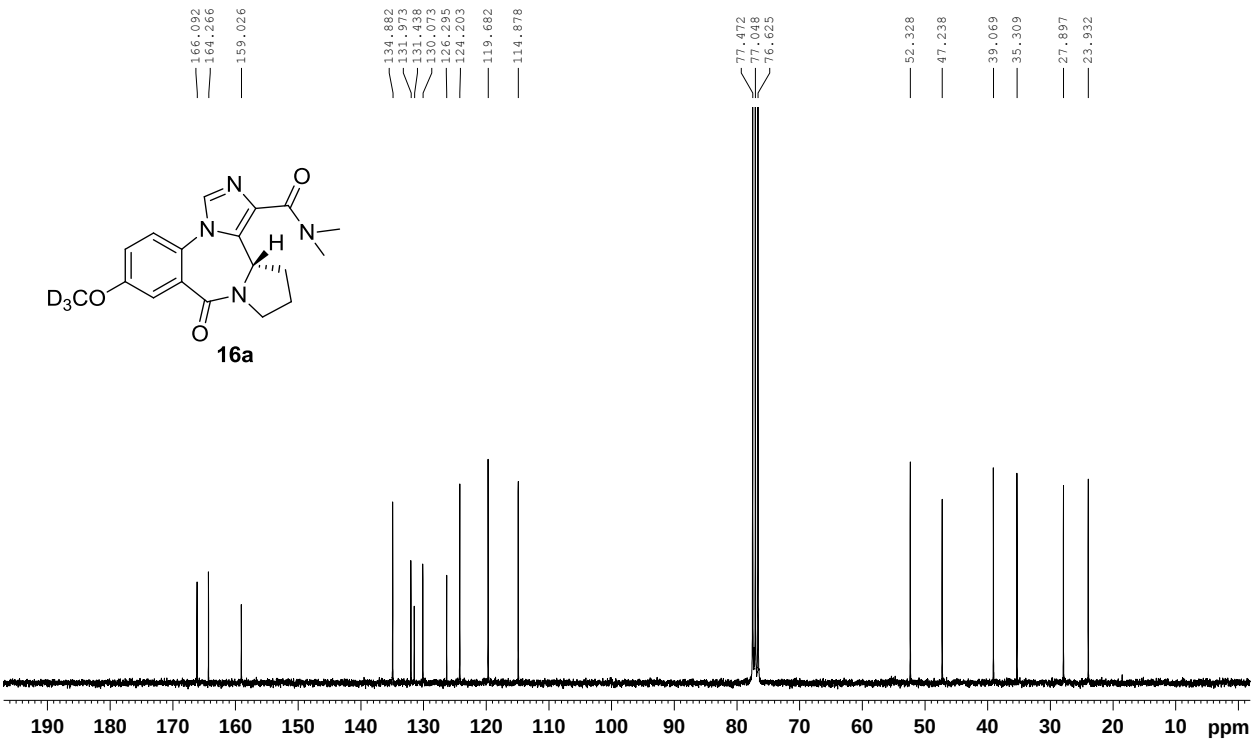
2.286
2.270
2.242
2.105
2.076
2.051
2.034
2.026
2.007
1.893
D3CO
16a
1.00
0.92
1.33
1.00
1.01
2.12
3.07
3.15
1.10
1.07
2.11
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm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76.625
52.328
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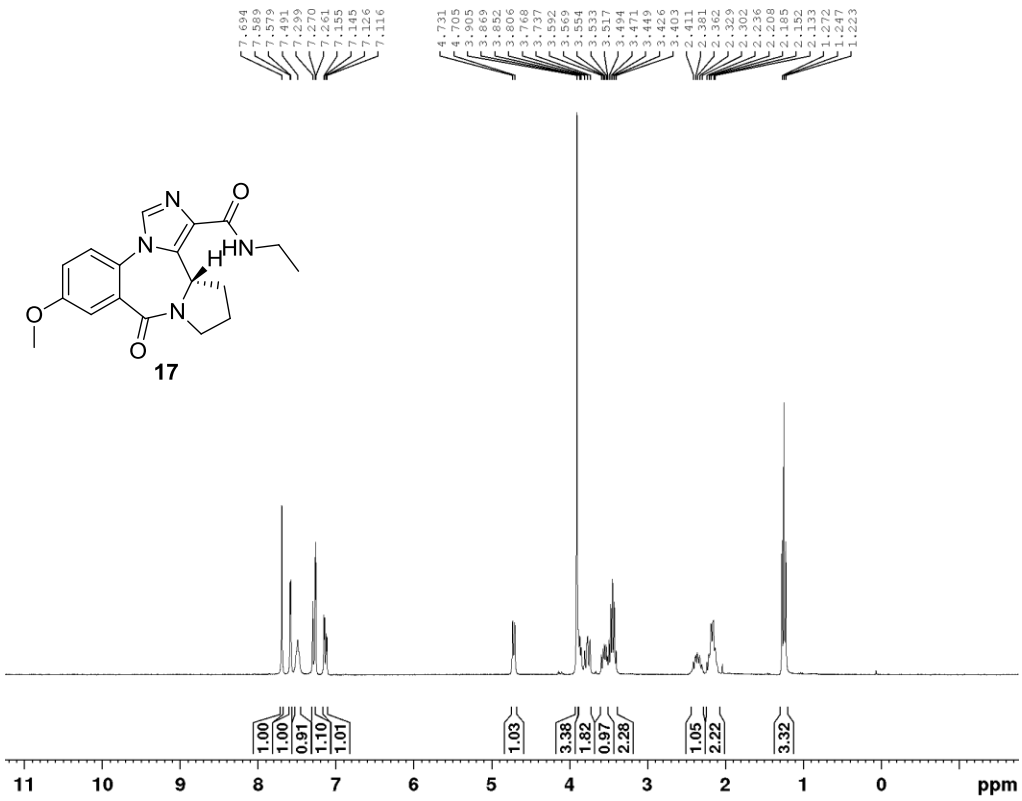
27.897
23.932
D3CO
16a
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
ppm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4.705
3.905
3.869
3.852
3.806
3.768
3.737
3.592
3.569
3.554
3.533
3.517
3.494
3.471
3.449
3.426
3.403
2.411
2.381
2.362
2.329
2.302
2.236
2.208
2.185
2.152
2.133
1.272
1.247
1.223
1.00
1.00
0.91
1.10
1.01
1.03
3.38
1.82
0.97
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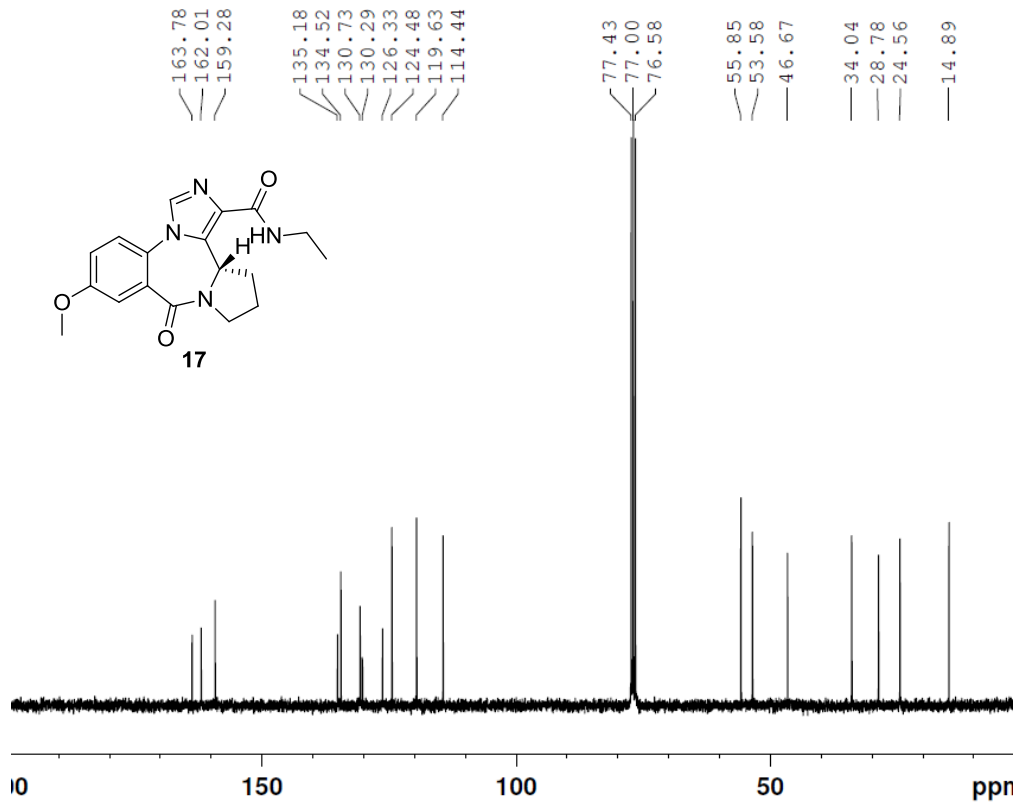
3.32
11
10
9
8
7
6
5
4
3
2
1
0
ppm
17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53.58
46.67
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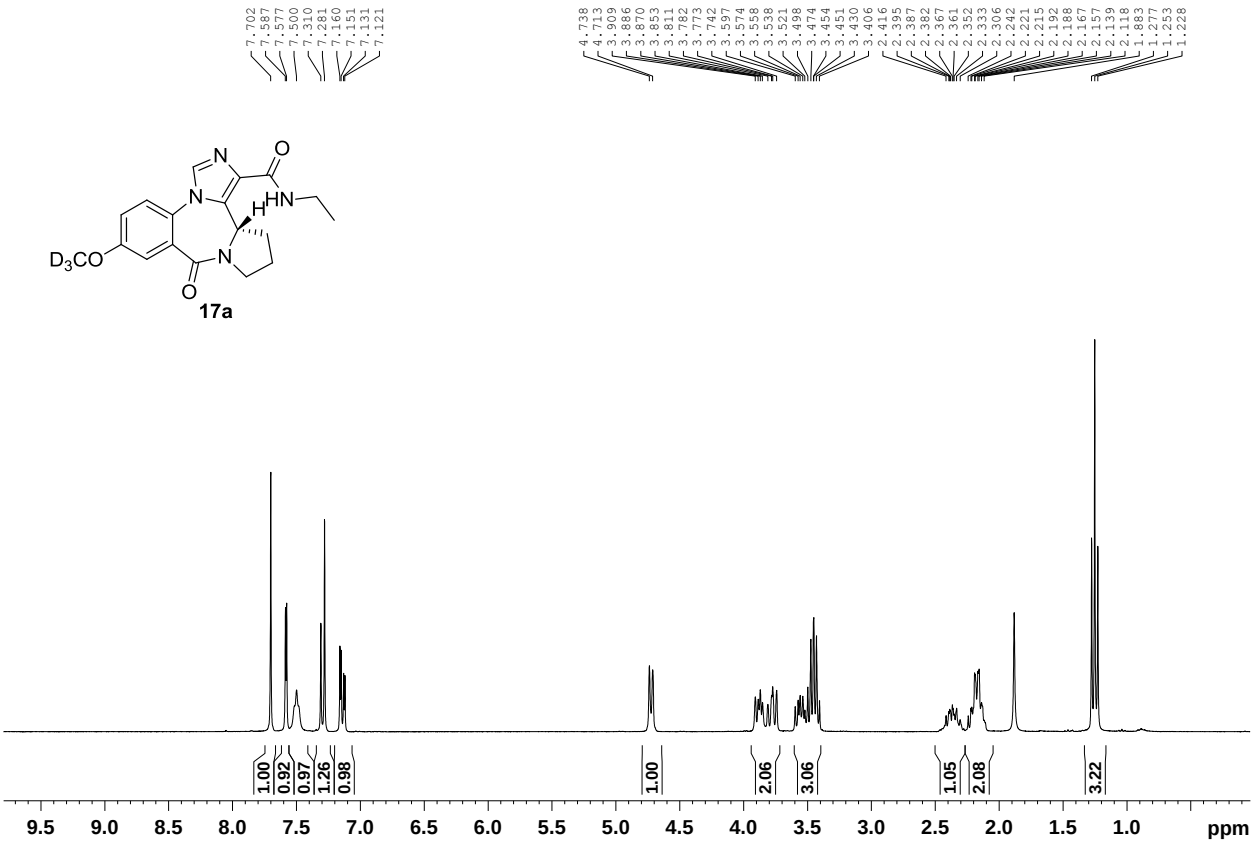
150
100
50
ppm
17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7.577
7.500
7.310
7.281
7.160
7.151
7.131
7.121
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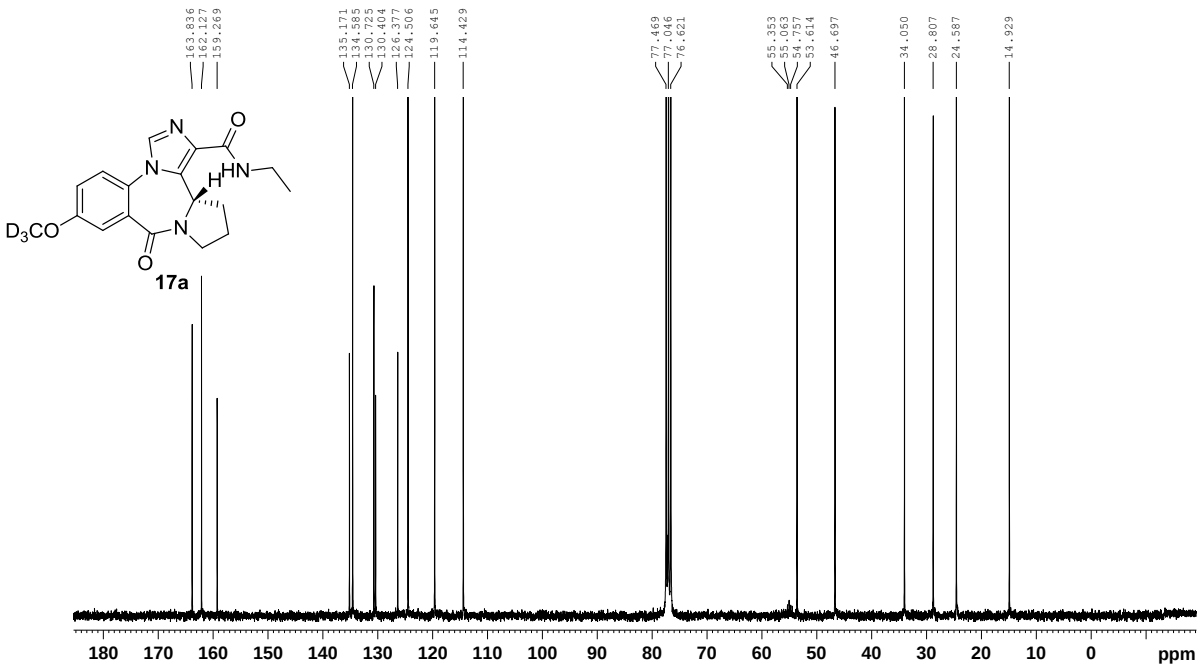
4.713
3.909
3.886
3.870
3.853
3.811
3.782
3.773
3.742
3.597
3.574
3.558
3.538
3.521
3.498
3.474
3.454
3.451
3.430
3.406
2.416
2.395
2.387
2.382
2.367
2.361
2.352
2.333
2.306
2.242
2.221
2.215
2.192
2.188
2.167
2.157
2.139
2.118
1.883
1.277
1.253
1.228
O
N
N
H
HN
D3CO
N
O
17a
1.00
0.92
0.97
1.26
0.98
1.00
2.06
3.06
1.05
2.08
3.22
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm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77.046
76.621
55.353
55.063
54.757
53.614
46.697
34.050
28.807
24.587
14.929
O
N
N
H
HN
D3CO
N
O
17a
180
170
160
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140
130
120
110
100
90
80
70
60
50
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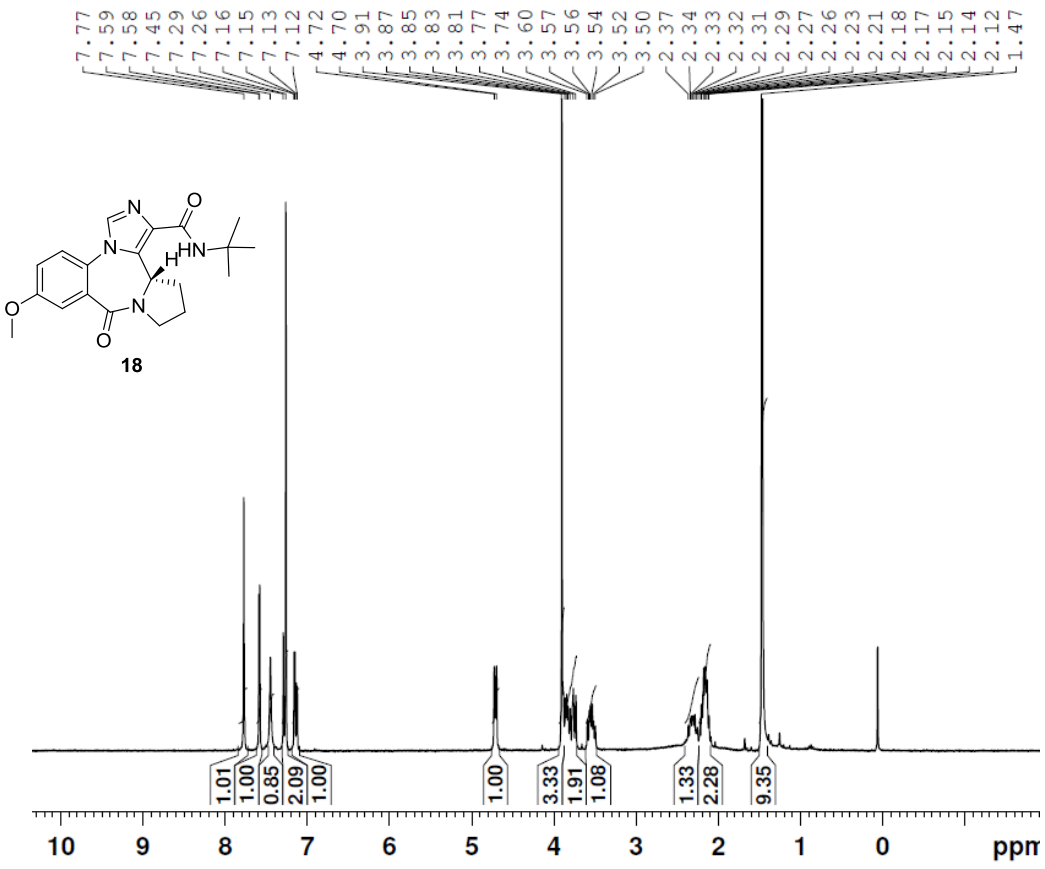
30
20
10
0
ppm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7.29
7.26
7.16
7.15
7.13
7.12
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4.70
3.91
3.87
3.85
3.83
3.81
3.77
3.74
3.60
3.57
3.56
3.54
3.52
3.50
2.37
2.34
2.33
2.32
2.31
2.29
2.27
2.26
2.23
2.21
2.18
2.17
2.15
2.14
2.12
1.47
1.01
1.00
0.85
2.09
1.00
1.00
3.33
1.91
1.08
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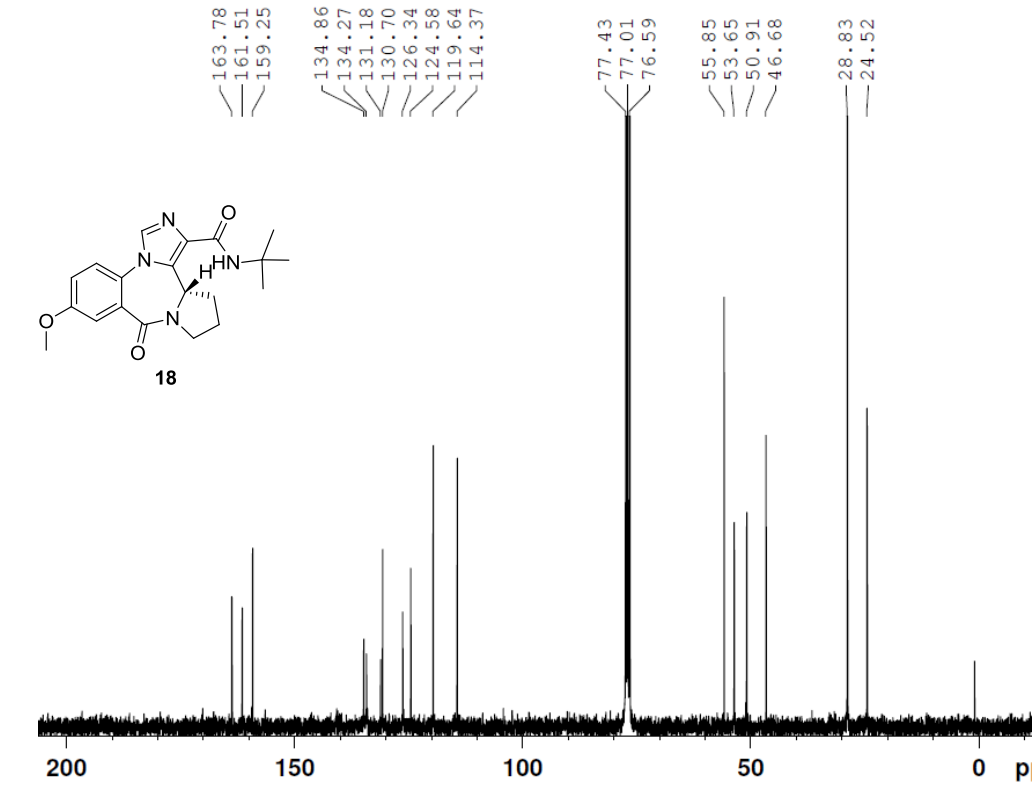
2.28
9.35
10
9
8
7
6
5
4
3
2
1
0
ppm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126.34
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119.64
114.37
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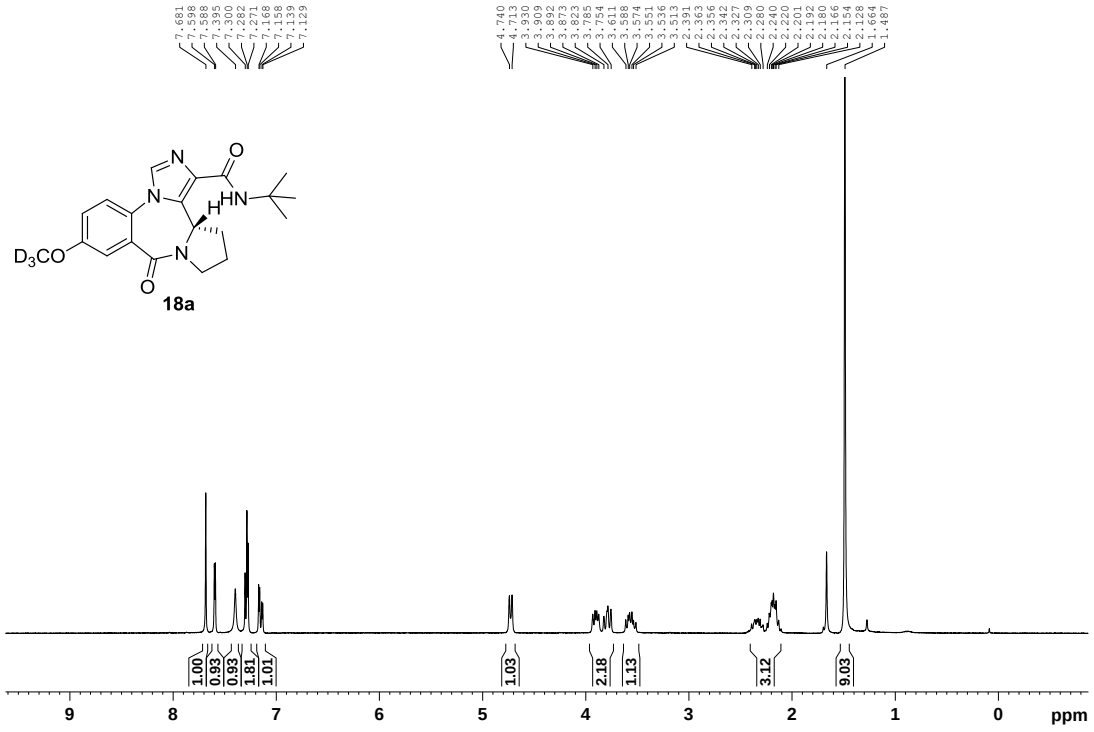
55.85
53.65
50.91
46.68
28.83
24.52
200
150
100
50
0
p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7.139
7.129
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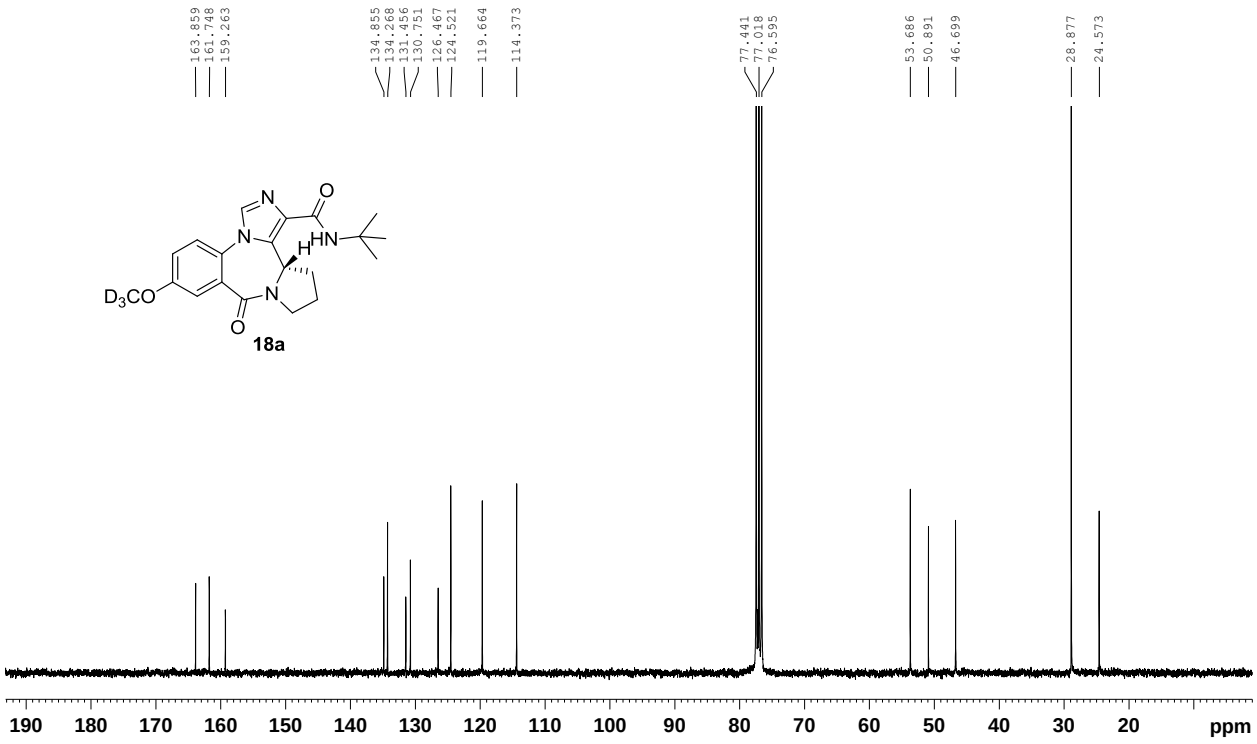
4.713
3.930
3.909
3.892
3.873
3.823
3.785
3.754
3.711
3.588
3.574
3.551
3.536
3.513
2.391
2.363
2.355
2.342
2.327
2.309
2.280
2.240
2.220
2.201
2.192
2.180
2.166
2.154
2.128
1.664
1.487
1.00
0.93
0.93
1.81
1.01
1.03
2.18
1.13
3.12
9.03
9
8
7
6
5
4
3
2
1
0
ppm
18a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130.751
126.467
124.521
119.664
114.373
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77.018
76.595
53.686
50.891
46.699
28.877
24.573
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170
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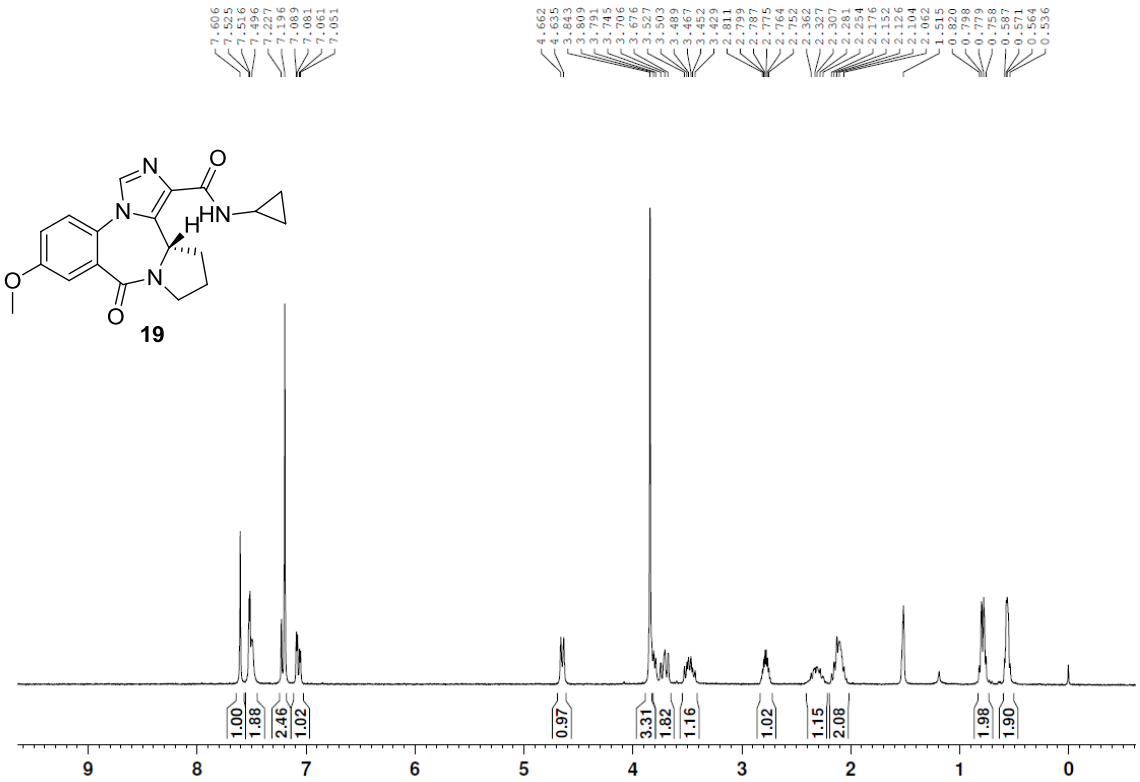
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm
18a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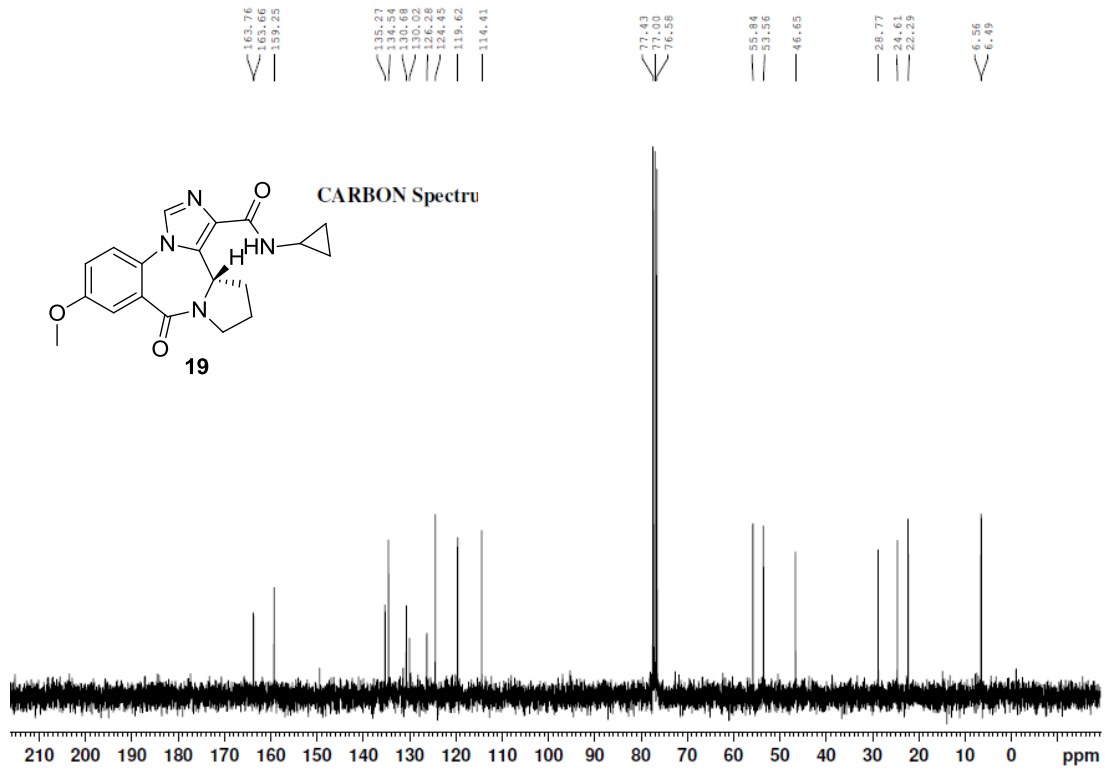
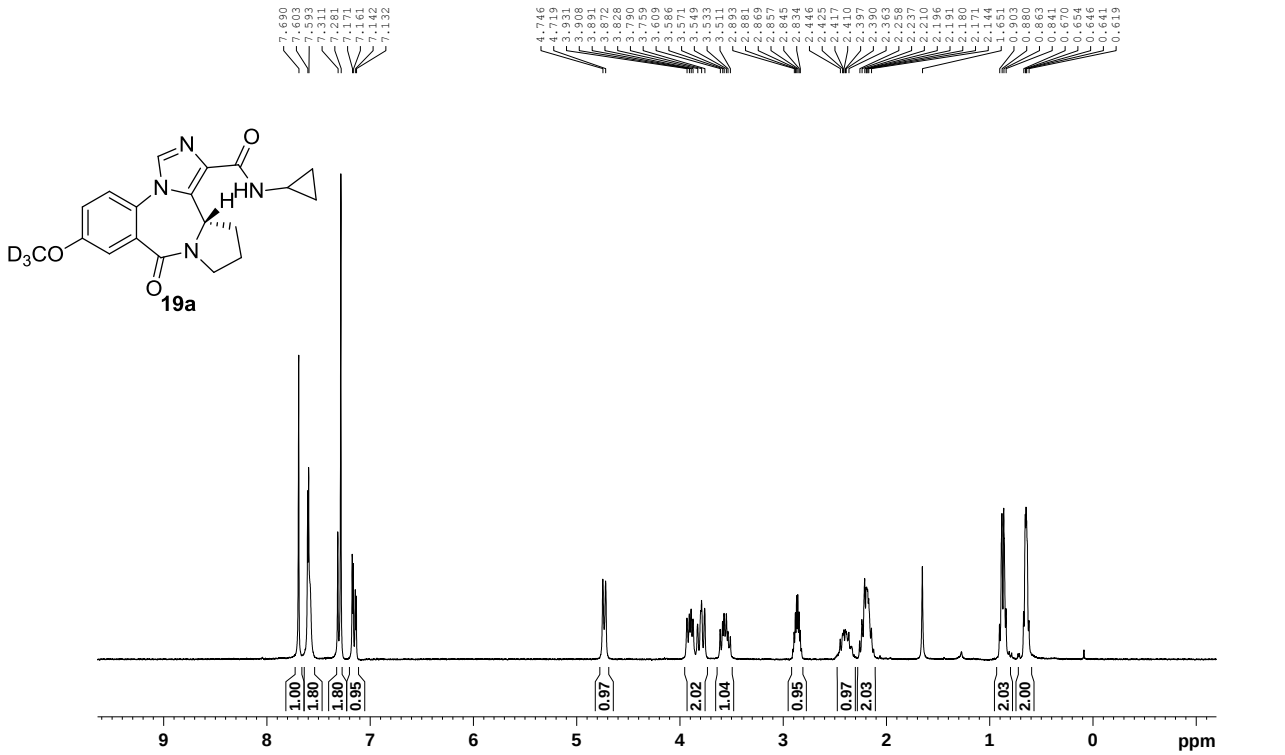
1.82
1.16
1.02
1.15
2.08
1.98
1.90


CARBON Spectru
19
ppm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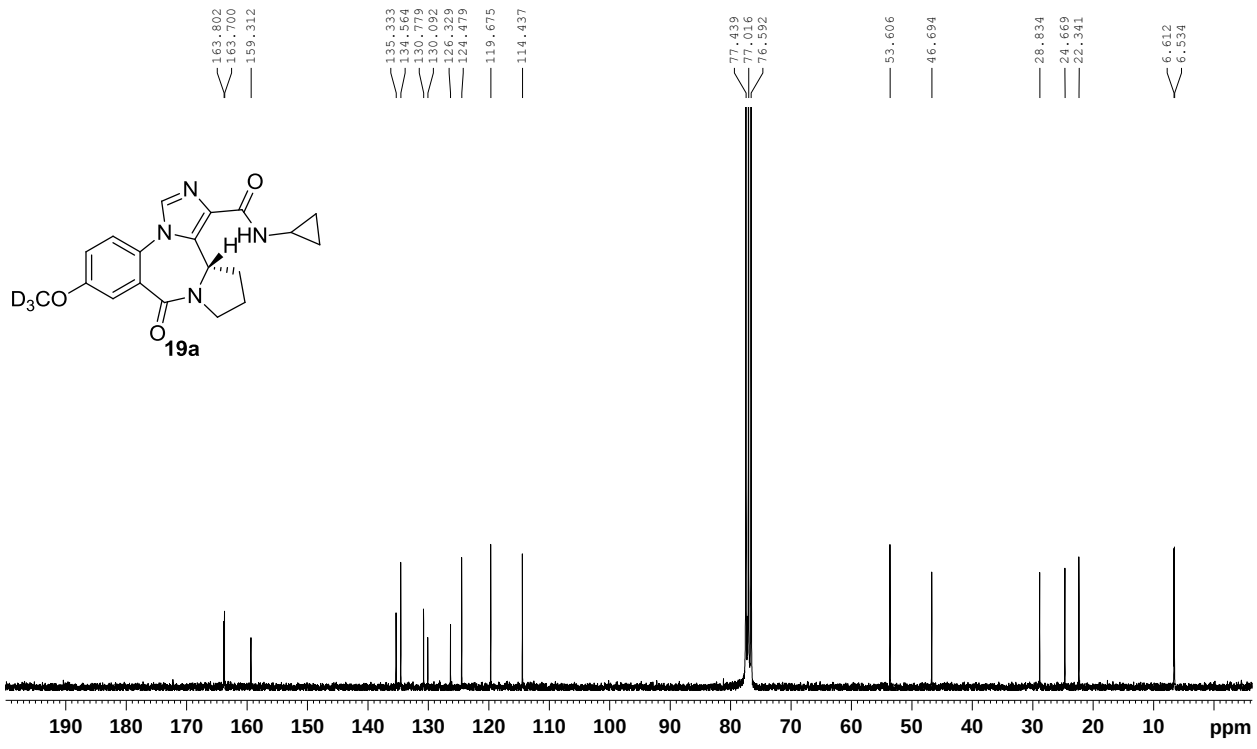
4.719
3.931
3.908
3.891
3.872
3.828
3.790
3.759
3.609
3.586
3.571
3.549
3.533
3.511
2.893
2.881
2.869
2.857
2.845
2.834
2.446
2.425
2.417
2.410
2.397
2.390
2.363
2.258
2.237
2.210
2.196
2.191
2.180
2.171
2.144
1.651
0.903
0.880
0.863
0.841
0.670
0.654
0.646
0.641
0.619
N
O
HN
H
N
N
D3CO
O
19a
1.00
1.80
1.80
0.95
0.97
2.02
1.04
0.95
0.97
2.03
2.03
2.00
9
8
7
6
5
4
3
2
1
0
ppm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119.675
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77.439
77.016
76.592
53.606
46.694
28.834
24.669
22.341
6.612
6.534
N
O
HN
H
N
N
D3CO
O
19a
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

[1] G.S. Forkuo, M.L. Guthrie, N.Y. Yuan, A.N. Nieman, R. Kodali, R. Jahan, M.R. Stephen, G.T. Yocum, M. Treven, M.M. Poe, G. Li, O.B. Yu, B.D. Hartzler, N.M. Zahn, M. Ernst, C.W. Emala, D.C. Stafford, J.M. Cook, L.A. Arnold, Development of GABAA Receptor Subtype-Selective Imidazobenzodiazepines as Novel Asthma Treatments, Mol Pharm, 13 (2016) 2026-2038.