Design and Synthesis of Naturally-Inspired SARS-CoV-2 Inhibitors

Haitham Hassan,*[a] Jeanne Chiavaralli,[b,#] Afnan Hassan,[c,d,#] Loay Bedda,[c,d,#], Tim Krischuns,[e] Kuang-Yu Chen,[e] Alice Shi Ming Li,[f] Adrien Delpal,[g] Etienne Decroly,[g] Masoud Vedadi,[f,h] Nadia Naffakh,[e] Fabrice Agou,[b] Sergio Mallart,[a] Reem K. Arafa,*[c,d] and Paola B. Arimondo [a]*

[a] Institut Pasteur, Université Paris Cité, CNRS UMR n°3523 Chem4Life, Epigenetic Chemical Biology, Department of Structural Biology and Chemistry, F-75015 Paris, France.
[b] Institut Pasteur, Université Paris Cité, CNRS UMR n°3523 Chem4Life, Chemogenomic and Biological Screening platform, Center for Technological Resources and Research (C2RT), F-75015 Paris, France.
[c] Drug Design and Discovery Lab, Zewail City of Science and Technology, 12578 Cairo, Egypt.
[d] Biomedical Sciences Program, University of Science and Technology, Zewail City of Science and Technology, 12578 Cairo, Egypt.
[e] Institut Pasteur, Université Paris Cité, CNRS UMR3569, Unité Biologie des ARN et Virus Influenza, F-75015 Paris, France.
[f] Department of Pharmacology and Toxicology, University of Toronto, Canada.
[g] CNRS - UMR7257 -AFMB - Aix-Marseille Université, Marseille, France.
[h] QBI COVID-19 Research Group (QCRG), San Francisco, CA, USA.

Chemical Syntheses

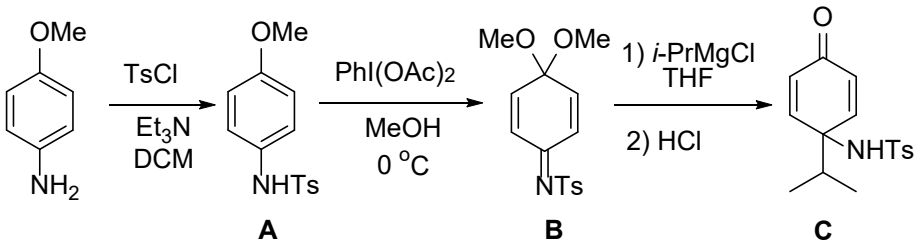
General information

All reactions were performed under an atmosphere of argon unless otherwise stated. Water-sensitive reactions were performed in oven-dried glassware, cooled under argon before use. Solvents were removed in vacuo using a Büchi rotary evaporator and a Vacuubrand® PC2001 Vario diaphragm pump. A Genevac EZ-2 Elite centrifugal evaporator was used for the removal of MeOH–H_2O. Anhydrous tetrahydrofuran (THF), dichloromethane (DCM), toluene, acetonitrile, *N*,*N*-dimethylacetamide (DMA), *N*,*N*-dimethylformamide (DMF) and 1,4-dioxane were obtained in SureSeal® bottles from Sigma-Aldrich (Merck-France). All other solvents used were of chromatography or analytical grade. Commercially available starting materials were obtained from Fluka-Sigma-Aldrich (Merck-France) or Thermo Fisher Scientific (Acros Chemicals and Alfa-Aesar) and were used without purification unless stated. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogeneous materials, unless otherwise stated.

^{1}H-NMR and ^{13}C-NMR were recorded on an Agilent DirectDrive 500 spectrometer (Agilent Technologies, Santa Clara) with a proton resonating frequency of 499.8 MHz. Spectra were recorded using VnmrJ 4.2A (Agilent Technologies). The chemical shifts (δ) and coupling constants (J) are expressed in ppm and Hz respectively. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, quint = quintuplet, hex = hexuplet. High-resolution mass spectra (HRMS) were recorded with a Waters Q-TOF 2 spectrometer in the electrospray ionization (ESI) mode. Analytical thin layer chromatography was performed using silica gel 60 F254 pre-coated plates (Merck) with visualization by ultraviolet light and potassium permanganate stain. Flash chromatography was

performed on a PuriFlash® (Advion Interchim) automated column chromatography using prepacked PuriFlash® Slica gel columns (60Å, 15/35 μm).

Synthesis of the precursor: N-(1-isopropyl-4-oxocyclohexa-2,5-dien-1-yl)-4-methylbenzene sulfonamide (Intermediate C)



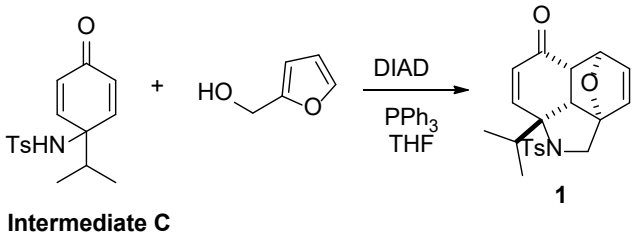
To a solution of *p*-Anisidine (5.0 g, 40.6 mmol) in dichloromethane was added Et_3N (5.65 mL, 40.6 mmol, 1.0 equiv.). The resulting solution was cooled to 4°C and tosyl chloride (8.5 g, 44.6 mmol, 1.1 equiv.) was added as a solid by portions. The reaction mixture was allowed to warm to room temperature (RT) and stirred overnight. Then, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL). The solution was allowed to stir with saturated sodium carbonate solution (100 mL) for one hour. The phases were separated and the organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product (11.2 g) was used without further purification in the following step.

To a solution of **A** (3.5 g, 12.61 mmol, 1.0 equiv.), in MeOH (63 mL), $PhI(OAc)_2$ (4.06 g, 12.61 mmol, 1.0 equiv.) was added as a solid by portions at 0°C under nitrogen atmosphere. The resulting mixture was stirred for 3 hours at 0°C. Then, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL). An aqueous solution of saturated $NaHCO_3$ was carefully added to quench the reaction. The phases were separated, the aqueous layer was extracted with 2x 50 mL AcOEt. The combined organic phases were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product which was purified by flash chromatography (eluent: Cyclohexane / EtOAc = 8 / 1~4 / 1) to give **intermediate B** as a white solid (2.1 g, 54%). The spectral data are in agreement with the reported synthesis. [1]

To a solution of **intermediate B** (2.0 g, 6.5 mmol, 1.0 equiv.) in anhydrous THF (20.0 mL), *i*-PrMgCl (2M) (4.22 mL, 8.45 mmol, 1.3 equiv.) was carefully added dropwise at -78 °C under argon atmosphere. The reaction was allowed to warm up gradually to RT and stirred overnight. Then, the reaction was quenched by HCl (2.0 M aq). The reaction mixture was extracted 3 times with EtOAc and the combined organic layers were washed with brine and dried over Na_2SO_4. Then, the solution was concentrated under vacuum to yield the crude product, which was purified by a flash chromatography (eluent: Cyclohexane/EtOAc = 8/1 ~ 6/1) to furnish **intermediate C** as white solid (830 mg, 42%). The spectral data are in agreement with the reported synthesis. [1]

[1] Cao, B.; Wei, Y.; Shi, M., *Org. Biomol. Chem.*, **2019**, *17*, 3737–3740.

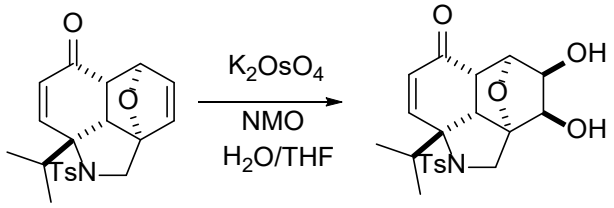
Synthesis of 8a-isopropyl-1-tosyl-2a1,5,5a,8a-tetrahydro-1*H*-2a,5-epoxybenzo[cd]indol-6(2H)-one (1)



Intermediate C **1**

To a solution of **intermediate C** (830 mg, 2.71 mmol, 1.0 equiv.), 2-furanylmethanol (400 mg, 4.07 mmol, 1.5 equiv.), triphenylphosphane (852.9 mg, 3.25 mmol, 1.2 equiv.) and 4 Å molecular sieves (1800 mg) in dry THF (27.30 mL), DIAD (603.93 mg, 2.98 mmol, 1.1 equiv.) was added dropwise at 0 °C. The resulting mixture was allowed to warm to RT and was stirred for 12 hours. Then, the resulting solution was concentrated under reduced pressure and the residue was purified by flash chromatography (eluent: Cyclohexane / EtOAc = 8 / 1) to give the desired product **1** as a white solid (630 mg, 60%). The spectral data are in agreement with the reported synthesis.[1]

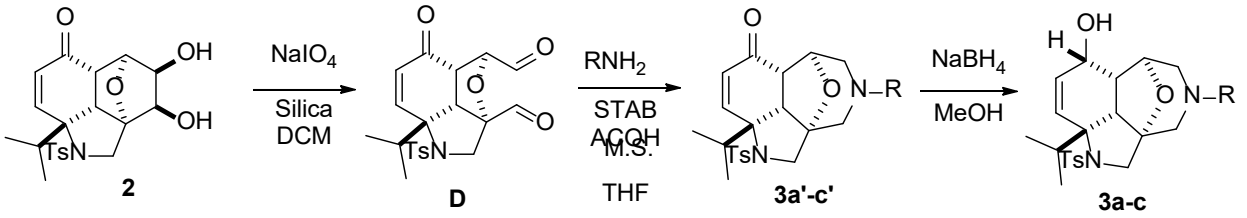
Synthesis of 3,4-dihydroxy-8a-isopropyl-1-tosyl-1,2,2a1,3,4,5,5a,8a-octahydro-6*H*-2a,5-epoxybenzo[cd]indol-6-one (2)



1 **2**

To a solution of the crude product of **1** (1600 mg, 4.15 mmol, 1.0 equiv.) in THF (80 mL) and water (14.4 mL) were added N-methyl-morpholine N-oxide (NMO) (583.50 mg, 4.98 mmol, 1.2 equiv.) and $K_2OsO_4.2H_2O$ (12.2 mg, 0.033 mmol, 0.008 equiv.). The solution was stirred at RT for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was diluted by a solution of $NaHSO_3$ 15% (15 ml) and the mixture was extracted three times with Et_2O. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified through column chromatography eluting with 100% EtOAc to give the titled compound **2** as white solid (550 mg, 27% over two steps). HRMS (ESI): $[M+H]^+$ $C_{21}H_{26}NO_6S$: calcd. 420.1475 found 420.1479. ^1H NMR (500 MHz, CD_3OD): δ (ppm) 7.74-7.72 (m, 2H), 7.35-7.33 (m, 2H), 7.31-7.29 (dd, 1H, *J* = 1.0, 11.0 Hz), 6.09 (d, 1H, *J* = 10.5 Hz), 4.16 (s, 1H), 4.10 (d, 1H, *J* = 6.0 Hz), 3.98 (d, 1H, *J* = 6.5 Hz), 3.71 (d, 1H, *J* = 12.0 Hz), 3.63 (d, 1H, *J* = 12.0 Hz), 2.78 (d, 1H, *J* = 9.0 Hz), 2.68-2.63 (m, 2H), 2.40 (s, 1H), 1.10 (d, 3H, *J* = 6.5 Hz), 0.78 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, CD_3OD): δ (ppm) 199.4, 152.8, 146.2, 140.4, 131.9, 131.2, 129.3, 97.2, 90.8, 76.8, 74.8, 72.7, 52.5, 52.0, 47.6, 38.9, 22.6, 19.1, 17.6.

Ring Expansion



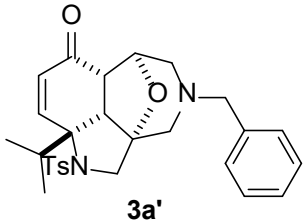
To a vigorously stirred solution of silica (column grade) (200 mg) in DCM (20 ml), a solution of $NaIO_4$ in H_2O (0.68 ml of a 0.65M, 1.4 equiv.) was added dropwise until a formation of a flaky suspension was observed. Solution of the diol **2** (400 mg, 0.95 mmol. 1.0 equiv.) in DCM (11 mL) was then added and the mixture was monitored by TLC and kept under stirring until complete disappearance of the starting material (typically 16 h). The solution was then filtered through a sintered glass funnel packed with Na_2SO_4 and concentrated under reduced pressure. The crude product was used directly without further purifications in the next step.

General Procedure (A): Reductive amination:
To stirred solution of the crude bis-aldehyde (intermediate **D**) in THF or DCM was added primary amine (1.2 equiv.), sodium tri-acetoxy borohydride (STAB) (3.0 equiv.), AcOH (0.1 equiv.), and 4 Å M.S. The reaction stirred at RT for 2h. Then the mixture was filtered through pad of Celite® and the filtrate was diluted with EtOAc and saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted for three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: Hexane / EtOAc = 6 / 1).

General Procedure (B): Reduction of ketone to alcohol:
To a solution of ketone (3a'-c') in MeOH, $NaBH_4$ (1.1 equiv.) was carefully added in one portion at 0 °C. The resulting mixture was allowed to warm to RT and stirred for 30 min. The reaction was quenched by a few drops of water. The solvent was removed under reduced pressure then the resulting mixture was diluted by EtOAc and saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted for three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by column chromatography (eluent: Cyclohexane / EtOAc = 4 / 1).

General Procedure (C): Reaction of Alcohol with aryl or alkyl chlorides (RCl).
To a suspension of NaH (60 % dispersion in mineral oil) (1.5 equiv.) in dry THF was added the crude alcohol (1.0 equiv.) in dry THF at 0 ºC. The mixture was stirred for 5 min at this temperature, and then RCl (1.5 equiv.) was added to the reaction mixture. The reaction was stirred at RT overnight. Then, the reaction was quenched by adding a few drops of H_2O at 0 ºC. The volatiles were removed under reduced pressure and the residue was diluted with EtOAc and saturated solution of $NaHCO_3$.The organic layer was separated and the aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography.

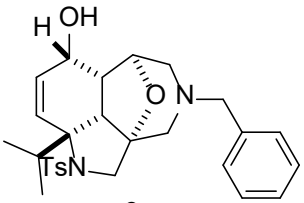
4-benzyl-9a-isopropyl-1-tosyl-1,2a1,3,4,5,6,6a,9a-octahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7(2H)-one (3a')



3a'

The titled compound was synthesized according to general procedure **(A)** from bis-aldehyde **D** (396 mg, 0.95 mmol. 1.0 equiv.) in THF (22 mL), benzylamine (122.15 mg, 1.14 mmol), STAB (604.02 mg, 2.85 mmol), AcOH (5.70 mg, 0.095 mmol), and 4 Å M.S. (200 mg). The crude product was purified by column chromatography eluting with cyclohexane/EtOAc 80/20, to give the titled compound **3a'** as white solid (341 mg, 73%). HRMS (ESI): $[M+H]^+$ $C_{28}H_{33}N_2O_4S$: calcd. 493.2156 found 493.2142. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.68-7.66 (m, 2H), 7.36-7.7.34 (m, 3H), 7.28-7.26 (m, 3H), 7.25-7.723 (m, 2H), 6.11 (d, 1H, *J* = 11.0 Hz), 7.38 (s, 1H), 3.59-3.52 (m, 3H), 3.31-3.21 (m, 2H), 3.07 (d, 1H, *J* = 9.5 Hz), 2.76-2.73 (m, 2H), 2.63 (d, 1H, *J* = 10.5 Hz), 2.38 (s, 3H), 2.35-2.30 (m, 2H), 1.07 (d, 3H, *J* = 7.0 Hz), 0.86 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 200.8, 152.9, 146.0, 140.4, 132.3, 131.3, 131.2, 131.1, 130.1, 129.6, 91.7, 85.4, 72.9, 64.3, 59.9, 59.2, 57.2, 56.1, 51.6, 39.5, 32.3, 24.1, 20.6, 19.1.

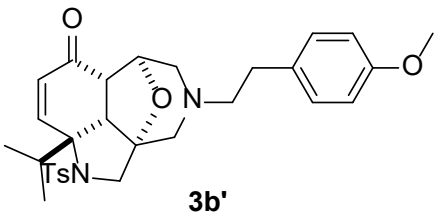
4-benzyl-9a-isopropyl-1-tosyl-1,2,2a1,3,4,5,6,6a,7,9a-decahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7-ol (3a)



3a

The titled compound was synthesized according to general procedure **(B)** from **3a'** (20 mg, 0.051 mmol, 1.0 equiv.) in MeOH (3.0 mL), $NaBH_4$ (1.6 mg, 0.042 mmol, 1.1 equiv.). The crude product was purified by column chromatography (Cyclohexane/EtOAc 70/30) to give the titled compound **3a** as white solid (18 mg, 70%). HRMS (ESI): $[M+H]^+$ $C_{28}H_{35}N_2O_4S$: calcd. 495.2312 found 495.2314. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.74-7.72 (m, 2H), 7.34-7.28 (m, 5H), 7.24-7.22 (m, 2H), 6.32 (dd, 1H, J = 2.5, 10.5 Hz), 5.77-5.74 (m, 1H), 4.63 (s, 1H), 4.37-4.35 (dt, 1H, *J* = 7.5, 2.5 Hz), 3.65 (s, br, 1H), 3.53-3.51 (m, 1H), 3.47 (d, 1H, *J* = 11.0 Hz), 3.19 (d, 1H, J = 11.5 Hz), 3.05-3.04 (m, 1H), 2.99-2.98 (m, 1H), 2.79 (s dr, 1H), 2.71-2.68 (m, 1H), 2.60 (s br, 1H), 2.73 (m, 5H), 1.04 (d, 3H, *J* = 7.0 Hz), 0.80 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 152.3, 145.4, 140.9, 134.7, 132.3, 132.1, 131.7, 131.1, 130.2, 129.6, 90.2, 79.2, 75.6, 67.3, 64.6, 59.7, 57.5, 56.0, 50.9, 48.7, 40.0, 24.1, 20.6, 19.1.

9a-isopropyl-4-(4-methoxyphenethyl)-1-tosyl-1,2a1,3,4,5,6,6a,9a-octahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7(2H)-one (3b').



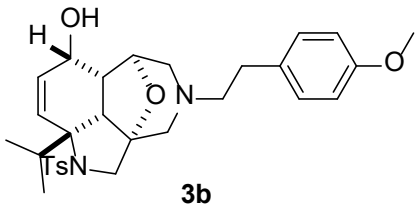
3b'

The titled compound was synthesized according to general procedure **(A)** from the crude bis-aldehyde **D** (47 mg, 0.11 mmol) in DCM (5.0 mL), 4-Methoxyphenethylamine (20.4 mg, 0.13 mmol), STAB (69.9 mg, 0.33 mmol) and 4 Å M.S. (100 mg). The crude product was purified by column chromatography eluting (Cyclohexane/ EtOAc 70/30) to give the titled compound **3b'** as white solid (46 mg, 77%).

HRMS (ESI): $[M+H]^+$ $C_{30}H_{37}N_2O_5S$: calcd. 537.2418 found 537.2403. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.67 (s, 1H), 7.65 (s, 1H), 7.31 (d, 1H, *J* = 10.5 Hz), 7.25-7.23 (m, 2H), 7.10 (s,

1H), 7.09 (s, 1H), 6.82-6.81 (m, 2H), 6.09 (d, 1H, *J* = 10.5 Hz), 4.36 (s, 1H), 3.77 (s, 3H), 3.51 (d, 1H, *J* = 11.5 Hz), 3.26 (d, 1H, *J* = 11 Hz), 2.95 (d, 1H, *J* = 9.5 Hz), 2.88 (d, 1H, *J* = 9.5 Hz), 2.77 (d, 1H, *J* = 11 Hz), 2.75-2.60 (m, 6H), 2.85 (s, 3H), 2.37-2.36 (m, 1H), 2.30 (d, 1H, *J* = 11.0 Hz), 1.05 (d, 3H, *J* = 6.5 Hz), 0.75 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 200.9, 160.6, 152.9, 145.9, 140.3, 134.5, 132.2, 132.0, 131.1, 129.5, 116.4, 91.6, 85.3, 72.8, 60.7, 59.7, 59.3, 57.8, 57.2, 55.9, 51.3, 39.5, 34.7, 24.1, 20.3, 19.0.

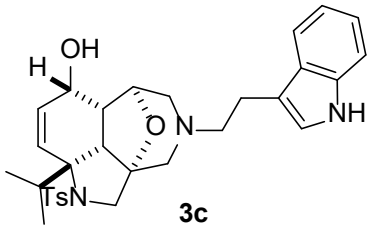
9a-isopropyl-4-(4-methoxyphenethyl)-1-tosyl-1,2,2a1,3,4,5,6,6a,7,9a-decahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7-ol (3b).



3b

The titled compound was synthesized according to general procedure **(A)** from the crude bis-aldehyde **D** (47 mg, 0.11 mmol) in DCM (5.0 mL), 4-Methoxyphenethylamine (20.4 mg, 0.13 mmol), STAB (69.9 mg, 0.33 mmol) and 4 Å M.S. (100 mg). Then, the crude product was dissolved in MeOH (3.0 mL) and $NaBH_4$ (3.2 mg, 0.085 mmol) was added and the synthesis was continued following general procedure **(B)**. The crude product was purified by column chromatography eluting with cyclohexane/ EtOAc 70/30, to give **3b** as white solid (46 mg, 77%). HRMS (ESI): $[M+H]^+$ $C_{30}H_{39}N_2O_5S$: calcd. 539.2574 found 539.2575. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.75-7.73 (m, 2H), 7.24-7.23 (m, 2H), 7.10-7.08 (m, 2H), 6.83-6.80 (m, 2H), 6.30 (dd, 1H, *J* = 2.5, 10.5 Hz), 5.75 (m, 1H), 4.62 (s, 1H), 4.33-4.32 (dt, 1H, *J* = 7.5, 2.5 Hz), 3.77 (s, 3H), 3.47-3.44 (d, 1H, *J* = 11.5 Hz), 3.21-3.19 (d, 1H, *J* = 11.5 Hz), 2.93-2.90 (m, 1H), 2.83-2.82 (m, 1H), 2.71-2.58 (m, 7H), 2.36 (s, 3H), 2.34-2.32 (m, 1H), 2.28-2.26 (m, 1H), 1.06 (d, 3H, *J* = 6.5 Hz), 0.76 (d, 3H, *J* = 7.0 Hz).^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 160.5, 145.3, 140.9, 134.7, 132.5, 132.16, 132.14, 131.1, 129.6, 116.3, 90.3, 75.6, 67.4, 61.7, 60.1, 59.2, 57.8, 57.6, 56.0, 51.0, 48.7, 40.1, 34.9, 24.1, 20.5, 19.1.

4-(2-(1H-indol-3-yl)ethyl)-9a-isopropyl-1-tosyl-1,2,2a1,3,4,5,6,6a,7,9a-decahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7-ol (3c).

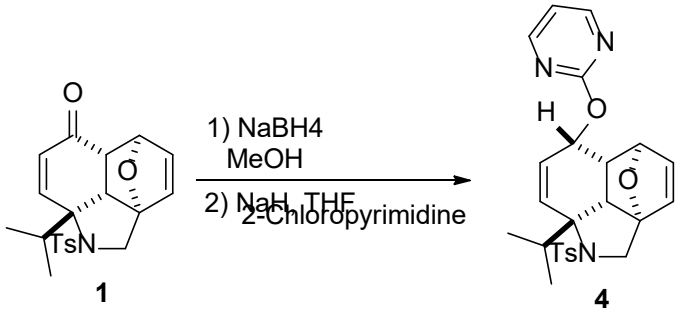


3c

The titled compound was synthesized according to general procedure **(A)** from the crude bis-aldehyde **D** (30 mg, 0.077 mmol) in DCM (5.0 mL), Tryptamine (13.8 mg, 0.086 mmol), STAB (45.14 mg, 0.21 mmol) and 4 Å M.S. (100 mg). Then, the crude product was dissolved in MeOH (3.0 mL) and $NaBH_4$ (3.2 mg, 0.085 mmol) was added and the synthesis was continued following general procedure **(B)**. The crude product was purified by column chromatography eluting with cyclohexane/ EtOAc 70/30 to give **3c** as yellow solid (18 mg, 47%). HRMS (ESI): $[M+H]^+$ $C_{31}H_{38}N_3O_4S$: calcd. 548.2578 found 548.2575. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.03 (s, br, 1H), 7.77-7.48 (m, 2H), 7.59 (d, 1H, *J* = 8.0 Hz), 7.35-7.33 (m, 1H), 7.25-7.23 (m, 2H), 7.20-7.16 (m, 1H), 7.12-7.09 (m, 1H), 7.01 (d, 1H, *J* = 2.0 Hz), 6.33 (dd, 1H, *J* = 2.5, 10.5 Hz), 5.77-5.74 (m, 1H), 4.64 (s, 1H), 4.33 (m, 1H), 3.48 (d, 1H, *J* = 11.5 Hz), 3.22 (d, 1H, *J* = 11.5 Hz), 2.94-2.89 (m, 4H), 2.75-2.67 (m, 5H), 2.37 (s, 3H), 2.35-2.32 (m, 2H), 1.07 (d, 3H, *J* = 7.0 Hz), 0.78 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 145.4, 140.9,

138.8, 134.7, 132.4, 132.1, 130.1, 129.6, 129.3, 124.6, 124.0, 121.8, 121.3, 113.8, 90.3, 75.7, 67.5, 60.7, 60.1, 59.4, 57.6, 56.0, 51.0, 48.7, 40.0, 25.3, 24.1, 20.6, 19.1.

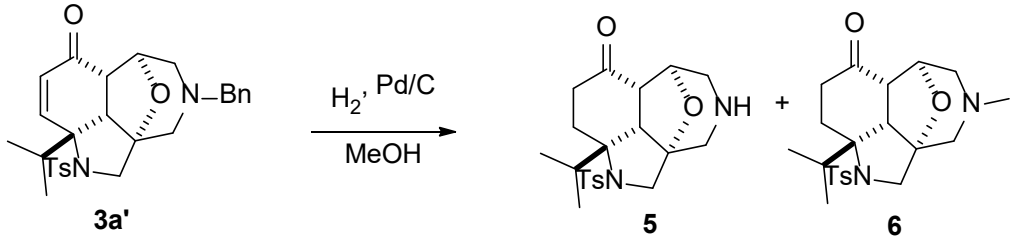
8a-isopropyl-6-(pyrimidin-2-yloxy)-1-tosyl-2,2a1,5,5a,6,8a-hexa hydro-1H-2a,5-epoxybenzo[cd]indole (4).



1 4

The tilted compound was synthesized firstly according to general procedure **(B)** from **1** (50 mg, 0.12 mmol) in MeOH (10.0 mL), $NaBH_4$ (5.0 mg, 0.13 mmol, 1.1 equiv.). The crude product was used directly in the next step. Then, following the general procedure **(C)**, NaH (60 % dispersion in mineral oil) (1.3 mg, 0.050 mmol, 1.5 equiv.) in dry THF (1 mL), the crude alcohol (13 mg, 0.033 mmol, 1.0 equiv.) in THF (3.0 mL) and 2-ChloroPyrimidine (5.7 mg, 0.050 mmol, 1.5 equiv.). The crude product was purified by column chromatography eluting with Cyclohexane/EtOAc 50/50, to give the titled compound **4** as faint yellow solid (10 mg, 65%). HRMS (ESI): $[M+H]^+$ $C_{25}H_{28}N_3O_4S$: calcd. 466.1795 found 466.1792. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.54 (d, 2H, *J* = 5.0 Hz), 7.78-7.77 (m, 2H), 7.25-7.23 (m, 2H), 6.97 (t, 1H, *J* = 5.0 Hz), 6.50 (dd, 1H, *J* = 2.0, 6.0 Hz), 6.41-6.38 (m, 1H), 6.35 (d, 1H, *J* = 15.0 Hz), 5.89-5.87 (m, 1H), 5.83-5.81 (m, 1H), 5.37 (d, 1H, *J* = 1.5 Hz), 3.80-3.74 (m, 2H), 2.85-2.77 (m, 1H), 2.59-2.56 (td, 1H, *J* = 8.5, 1.0 Hz), 2.41 (d, 1H, *J* = 8.5 Hz), 2.37 (s, 3H), 1.18 (d, 3H, *J* = 6.5 Hz), 0.88 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 167.1, 162.1, 145.4, 142.8, 140.9, 136.6, 134.5, 132.2, 129.6, 129.1, 117.9, 96.2, 82.8, 75.9, 74.1, 53.1, 46.6, 43.9, 39.8, 24.1, 20.9, 18.9.

2a-isopropyl-2-tosyldecahydro-6,9a-epoxyazepino[3,4,5-*cd*]indol-5(1*H*)-one (5) and 2a-isopropyl-8-methyl-2-tosyldecahydro-6,9a-epoxyazepino[3,4,5-*cd*]indol-5(1*H*)-one (6).



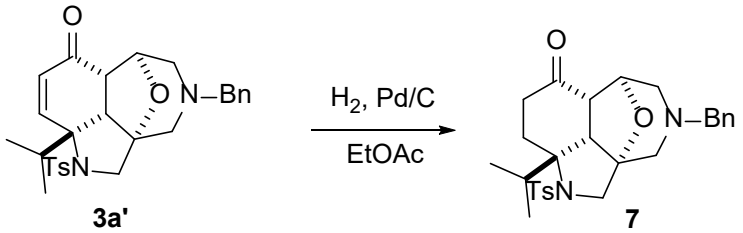
3a' 5 6

In 25 ml round bottom flask charged with **3a'** (35 mg, 0.071 mmol) and 10% Pd/C (10 mg, 0.01 mmol, 0.14 equiv.) and MeOH (1.0 mL) was evacuated and backfilled with H_2 for three times and stirred at 25 °C for 24 h under H_2 (1 bar). The mixture was filtered through a pad of celite® and the resulting filtrate was concentrated under vacuum and the residue was purified by a silica gel column chromatography (EtOAc 100 %) to give the **5** as a white solid (16 mg, 57%) and **6** (4.0 mg, 13%).

Compound 5: **2a-isopropyl-2-tosyldecahydro-6,9a-epoxyazepino[3,4,5-*cd*]indol-5(1*H*)-one**
HRMS (ESI): $[M+H]^+$ $C_{21}H_{29}N_2O_4S$: calcd. 405.1843 found 405.1845. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.72-7.69 (m, 2H), 7.28-7.26 (m, 2H), 4.16 (s, 1H), 3.64 (d, 1H, *J* = 11.5 Hz), 3.39 (d, 1H, *J* = 11.5 Hz), 3.24 (d, 1H, *J* = 9.5 Hz), 2.98-2.94 (m, 2H), 2.87-2.81 (m, 2H), 2.74-2.66 (m, 3H), 2.40 (s, 3H), 2.05-1.89 (m, 4H), 1.04 (d, 3H, *J* = 7.0 Hz), 0.97 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 215.7, 145.9, 141.1, 132.2, 129.2, 91.5, 86.0, 77.2, 58.9, 58.8, 54.0, 52.8, 51.5, 40.8, 39.2, 32.0, 24.1, 21.7, 21.0.

Compound 6: **2a-isopropyl-8-methyl-2-tosyldecahydro-6,9a-epoxyazepino[3,4,5-*cd*]indol-5(1*H*)-one**
HRMS (ESI): $[M+H]^+$ $C_{22}H_{31}N_2O_4S$: calcd. 419.1999 found 419.2000. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.71(d, 2H, *J* = 8.0 Hz), 7.27 (d, 2H, *J*= 8.0 Hz), 4.17 (s, 1H), 3.67 (d, 1H, *J* = 11.5 Hz), 3.41 (d, 1H, *J* = 11.5 Hz), 3.18 (d, 1H, *J* = 9 Hz), 2.84-2.78 (m, 2H), 2.69-2.61 (m, 3H), 2.40 (s, 3H), 2.25 (m, 4H), 2.07-1.88 (m, 4H), 1.05 (d, 3H, *J* = 6.5 Hz), 0.96 (d, 3H, *J* = 6.0 Hz).

8-benzyl-2a-isopropyl-2-tosyldecahydro-6,9a-epoxyazepino[3,4,5-cd]indol-5(1H)-one (7)



3a' **7**

In 25 ml round bottom flask charged with **3a'** (35 mg, 0.071 mmol) and Pd/C (10 mg, 0.01 mmol, 0.14 equiv.) and AcOEt (1.0 mL) was evacuated and backfilled with H_2 for three times and stirred at 25 °C for 24 h under H_2 (1 bar). The mixture was filtered through a pad of celite® and the resulting filtrate was concentrated under vacuum and the residue was purified by a silica gel column chromatography (EtOAc 100 %) to give the **7** as a white solid (5 mg, 14%). HRMS (ESI): $[M+H]^+$ $C_{28}H_{35}N_2O_4S$: calcd. 495.2312 found 495.2316. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.70-7.69 (d, 2H, *J* = 8.5 Hz), 7.32-7.24 (m, 7H), 4.18 (s, 1H), 3.66 (d, 1H, *J* = 11.5 Hz), 3.53 (s, 2H), 3.36 (d, 1H, *J* = 11.5 Hz), 3.25 (d, 1H, *J* = 9.5 Hz), 2.85-2.80 (m, 2H), 2.67-2.60 (m, 3H), 2.39 (s, 3H), 2.32-2.27 (m, 2H), 2.04-1.91 (m, 3H), 0.98 (d, 3H, *J* = 6.5 Hz), 0.95 (d, 3H, *i* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 215.8, 145.8, 141.1, 140.0, 132.2, 131.1, 131.0, 130.0, 129.2, 91.1, 85.3, 77.0, 64.1, 59.7, 59.2, 58.7, 58.6, 54.5, 40.7, 39.3, 31.9, 24.1, 21.8, 20.9.

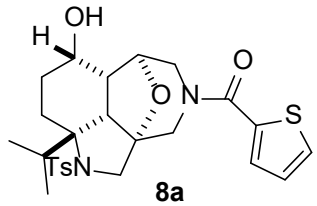
General Procedure (D):
In 10 ml round bottom flask charged with **5** (1.0 equiv.), acid chloride (1.1 equiv.) in DCM was added Et_3N (1.1 equiv.). The reaction mixture was stirred at RT for 2 h. Then, the reaction mixture was diluted with saturated solution of $NaHCO_3$ and the organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was used directly in the next step. To a solution of the ketone from the previous step in MeOH, $NaBH_4$ (1.1 equiv.) was carefully added in one portion at 0°C. The resulting mixture was allowed to warm to RT and was stirred for 30 min. The reaction was quenched by a few drops of water. After that the solvent was removed under reduced pressure and the resulting mixture was diluted by EtOAc and saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted three times with

EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by column chromatography.

General Procedure (E):

In 10 ml round bottom flask charged with **5** (1.0 equiv.), carboxylic acid (1.1 equiv.), TBTU (1.5 equiv.) and DCM (1.0 mL) was added DIPEA (2.5 equiv.). The reaction mixture was stirred at RT for 2 h. The reaction mixture was diluted with saturated solution of $NaHCO_3$ and the organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was used directly in the next step. To a solution of the ketone from pervious step in MeOH, $NaBH_4$ (1.1 equiv.) was carefully added in one portion at 0°C. The resulting mixture was allowed to warm to RT and was stirred for 30 min. The reaction was quenched by a few drops of water. After that the solvent was removed under reduced pressure and the resulting mixture was diluted by EtOAc and saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted for three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by column chromatography.

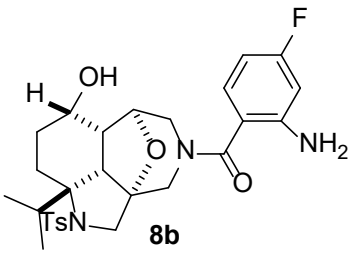
7-hydroxy-9a-isopropyl-1-tosyloctahydro-2a,6-epoxyazepino[3,4,5-cd]indol-4(2H,2a1H,3H)-yl)(thiophen-2-yl)methanone (8a).



8a

The titled compound was synthesized according to general procedure **(E)** from **5** (15 mg, 0.037 mmol) in DCM (1.0 mL), 2-thiophenecarboxylic acid (5.1 mg, 0.040 mmol), TBTU (17.8 mg, 0.055 mmol) and DIPEA (16µL, 0.092 mmol).Then, the next step (reduction of the ketone), the crude product was dissolved in MeOH (1.0 mL) and $NaBH_4$ (1.5 mg, 0.040 mmol). The crude product was purified by column chromatography eluting by (Cyclohexane/EtOAc 70/30) to give **8a** as yellow solid (3.0 mg, 15%). HRMS (ESI): $[M+H]^+$ $C_{26}H_{33}N_2O_5S_2$: calcd. 517.1825 found 517.1832. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.73-7.72 (m, 2H), 7.49-7.47 (m, 1H), 7.30-7.29 (m, 1H), 7.27 (m, 1H), 7.25 (m,1H), 7.07-7.05 (m, 1H), 4.78 (br s, 1H), 3.78-3.74 (m, 1H), 3.67-3.65 (m, 1H), 3.38-3.35 (m, 2H), 2.68-2.67 (m, 1H), 2.65-2.63 (m,1H), 2.58-2.51 (m, 1H), 2.49-2.45 (m,1H), 2.41 (s, 3H), 1.66-1.61 (m, 3H), 1.39-1.33 (m, 2H), 1.05-0.89 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 167.4, 145.8, 145.2, 142.0, 132.1, 131.9, 131.9, 129.6, 129.0, 90.3, 71.8, 58.1, 56.0, 52.1, 50.6, 41.8, 41.2, 33.5, 32.6, 32.3, 29.6, 24.1, 21.4, 20.4.

(2-amino-4-fluorophenyl)(7-hydroxy-9a-isopropyl-1-tosyloctahydro-2a,6-epoxyazepino[3,4,5-cd]indol-4(2H,2a1H,3H)-yl)methanone (8b).

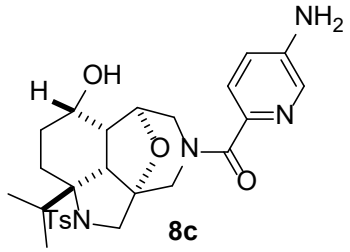


8b

The titled compound was synthesized according to general procedure **(E)** from the **5** (15 mg, 0.037 mmol) in DCM (1.0 mL), 2-amino-4-fluorobenzoic acid (6.2 mg, 0.040 mmol), TBTU (17.8 mg, 0.055 mmol) and DIPEA (16µL, 0.092 mmol).Then, The next step (reduction of the ketone), the crude product was dissolved in MeOH (1.0 mL) and $NaBH_4$ (1.5 mg, 0.040 mmol). The crude product was purified by column chromatography eluting by (Cyclohexane/EtOAc 50/50) to give **8b** as white solid (15 mg, 75%). HRMS (ESI): $[M+H]^+$ $C_{28}H_{35}FN_3O_5S$: calcd. 544.2276 found 544.2277. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.70 (m,

2H), 7.26 (s, 1H), 7.25 (s, 1H), 6.99-6.95 (m, 1H), 6.44-6.52 (m, 2H), 4.73 (br s, 1H), 3.76-3.71 (m, 1H), 3.62-3.59 (m, 1H), 3.33-3.16 (m, 4H), 2.80-2.71 (m, 1H), 2.65-2.61 (m, 1H), 2.57-2.52 (q, 1H, J = 6.5 Hz),2.43-2.43 (m, 1H) 2.39 (s, 3H), 1.66-1.59 (dt, 1H, J = 3.5, 12.5 Hz), 1.40-1.32 (m, 1H), 1.25-1.20 (m, 2H), 0.94-0.89 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.1, 168.0, 166.0, 145.5, 141.8, 132.1, 129.3, 129.0, 117.6, 107.6, 107.4, 106.2, 106.0, 90.5,78.6, 76.7, 71.7, 57.9, 53.5, 51.8, 50.4, 41.8, 32.6, 29.6, 24.1, 21.3, 20.3.

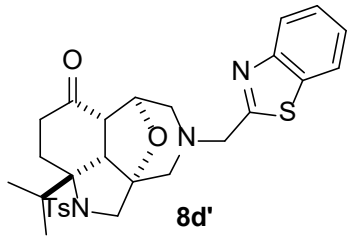
5-aminopyridin-2-yl(7-hydroxy-9a-isopropyl-1-tosyloctahydro-2a,6-epoxyazepino[3,4,5-cd]indol-4(2H,2a1H,3H)-yl)methanone (8c).



8c

The titled compound was synthesized according to general procedure **(E)** from the **5** (15 mg, 0.037 mmol) in DCM (1.0 mL), 5-aminopyridine-2-carboxylic acid (5.5 mg, 0.040 mmol), TBTU (17.8 mg, 0.055 mmol) and DIPEA (16µL, 0.092 mmol).Then, The next step (reduction of the ketone), the crude product was dissolved in MeOH (1.0 mL) and $NaBH_4$ (1.5 mg, 0.040 mmol). The crude product was purified by column chromatography eluting by (EtOAc 100%) to give **8c** as yellow solid (16.0 mg, 82%). HRMS (ESI): $[M+H]^+$ $C_{27}H_{35}N_4O_5S$: calcd. 527.2323 found 527.2325. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.96-7.94 (m, 1H), 7.73-7.70 (m, 2H), 7.56-7.54 (m, 1H) , 7.25-7.24 (m, 2H), 7.01-6.99 (dd, 1H, J = 2.5, 11 Hz), 4.84 (s, 1H), 4.45-4.43 (m, 1H), 4.23-4.20 (m, 1H), 3.72-3.66 (m, 1H), 3.62-3.58 (m, 1H), 3.44-3.54 (m, 2H), 3.28-3.26 (m, 1H), 3.22-3.16 (m, 1H), 3.09-3.00 (m, 2H), 2.78-2.53 (m, 4H), 2.38 (s, 3H), 1.64-1.59 (m 1H), 1.38-1.031 (m, 1H), 1.14-1.05 (m, 3H), 0.97-0.87 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 171.3, 146.5, 145.4, 145.0, 142.1, 137.1, 132.1, 129.0, 128.5, 124.0, 90.6, 78.8, 77.0, 71.8, 58.1, 54.3, 51.4, 51.0, 50.4, 42.1, 32.8, 29.3, 24.1, 21.4, 20.1.

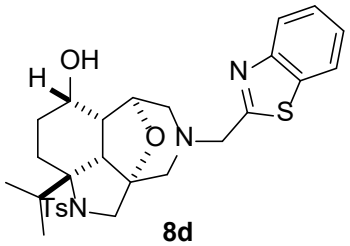
4-(benzo[d]thiazol-2-ylmethyl)-9a-isopropyl-1-tosyldecahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7(2H)-one (8d').



8d'

The titled compound was synthesized according to general procedure **(A)** from **5** (15.0 mg, 0.037 mmol. 1.0 equiv.) in THF (3.0 mL), benzothiazole-2-carboxaldehyde (7.2 mg, 0.044 mmol, 1.2 equiv.), STAB (23.5 mg, 0.11 mmol, 3.0 equiv.) and 4 Å M.S. (100 mg). The crude product was purified by column chromatography eluting by (Hex/EtOAc 70/30) to give **8d'** as white solid (15 mg, 73%). HRMS (ESI): $[M+H]^+$ $C_{29}H_{34}N_3O_4S_2$: calcd. 552.1985 found 552.1994. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.96-7.94 (m, 1H), 7.87-7.88 (m, 1H), 7.71-7.69 (m, 2H), 7.48-7.45 (m, 1H), 7.40-7.36 (m, 1H), 7.28-7.26 (m, 2H), 4.24 (s, 1H), 4.00 (s, 2H), 3.70 (d, 1H, J = 11.5 Hz), 3.44-3.39 (dd, 2H, J = 9.5 ,18.0 Hz), 2.95 (d, 1H, J = 9.5 Hz), 2.89-2.82 (m, 3H), 2.68 (q, 1H, J = 6.5 Hz), 261-2.67 (m, 2H), 2.39 (s, 3H), 2.06-1.96 (m, 3H), 1.06 (d, 3H, J = 6.5 Hz), 1.00 (d, 3H, J = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 215,4, 173.6, 155.8, 145.9, 141.1, 137.5, 132.3, 129.2, 128.8, 127.9, 125.6, 124.3, 91.1, 58.1, 77.0, 61.6, 59.9, 59.0, 58.7, 58.6, 54.4, 40.9, 39.3, 31.8, 24.1, 21.8, 21.1.

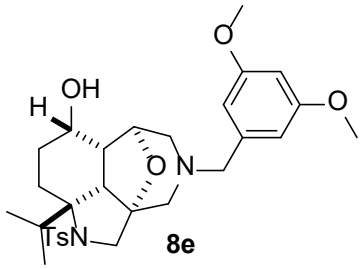
4-(benzo[d]thiazol-2-ylmethyl)-9a-isopropyl-1-tosyldodecahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7-ol (8d).



8d

The titled compound was synthesized according to general procedure (**B**) from **8d'** (13 mg, 0.023 mmol, 1.0 equiv.) in MeOH (1.0 mL), $NaBH_4$ (1.06 mg, 0.028 mmol, 1.2 equiv.). The crude product was purified by column chromatography on silica gel (Cyclohexane/EtOAc 50/50) to give the titled compound **8d** as yellow solid (9.0 mg, 70%).HRMS (ESI): $[M+H]^+$ $C_{29}H_{36}N_3O_4S_2$: calcd. 554.2142 found 554.2148. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.96 (d, 1H, *J* = 3.0 Hz), 7.88-7.87 (m, 1H), 7.73 (s, 1H), 7.72 (s, 1H), 7.48-7.45 (m, 1H), 7.39-7.36 (m, 1H), 7.25-7.24 (m, 2H), 4.72 (s, 1H), 3.99 (br s, 1H), 3.85-3.81 (m, 1H), 3.61 (d, 1H, *J* = 11.5 Hz), 3.27 (d, 1H, *J* = 11.5 Hz), 3.06 (m, 1H), 2.83 (m, 2H), 2.73-2.57 (m, 5H), 2.38 (s, 3H), 1.73-1.60 (s, 3H), 1.48-1.41 (m, 1H), 1.03-1.01 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 173.7, 155.8, 145.3, 142.1, 137.7, 132.1, 129.0, 128.7, 127.7, 125.5, 124.3, 90.5, 78.9, 76.6, 72.2, 63.0, 58.5, 52.5, 51.2, 42.1, 32.8, 32.3, 30.0, 24.1, 23.6, 21.5, 20.5.

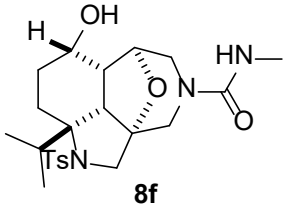
4-(3,5-dimethoxybenzyl)-9a-isopropyl-1-tosyldodecahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7-ol (8e).



8e

The titled compound was synthesized according to general procedure **(A)** from **5** (12.0 mg, 0.029 mmol. 1.0 equiv.) in THF (3.0 mL), 3,5-Dimethoxybenzaldehyde (5.7 mg, 0.035 mmol, 1.2 equiv.), STAB (18.4 mg, 0.087 mmol, 3.0 equiv.) and 4 Å M.S. (100 mg). Then, The next step (reduction of the ketone), the crude product was dissolved in MeOH (1.0 mL) and $NaBH_4$ (1.3 mg, 0.034 mmol). The crude product was purified by column chromatography eluting by (Cyclohexane/EtOAc 70/30 to give **8e** as white solid (14 mg, 85%). HRMS (ESI): $[M+H]^+$ $C_{30}H_{41}N_2O_6S$: calcd. 557.2680 found 557.2684. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.73-7.72 (m,2H), 7.25 (s, 1H), 7.23 (m, 1H), 6.74 (m, 2H), 6.35 (m, 1H), 4.65 (s, 1H), 3.78 (s, 6H), 3.57 (d, 1H, *J* =11.5 Hz), 3.49-3.42 (m, 2H), 3.25 (d, 1H, *J* = 11.5 Hz), 2.91 (d, 1H, *J* = 9.5 Hz), 2.70-2.53 (m, 5H), 2.38 (s, 3H), 2.30-2.23 (m, 2H), 1.71-1.65 (m, 2H), 1.47-1.144 (m, 1H), 1.27-1.21 (m, 1H), 1.00 (d, 3H, *J* = 7.0 Hz), 0.95 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 163.4, 145.3, 143.1, 142.1, 132.1, 129.0, 109.0, 101.6, 90.6, 79.1, 76.5, 72.2, 64.6, 59.9, 59.4, 58.6, 57.9, 52.5, 51.4, 42.0, 32.8, 30.0, 24.1, 21.4, 20.5.

7-hydroxy-9a-isopropyl-N-methyl-1-tosyldecahydro-2a,6-epoxyazepino[3,4,5-cd]indole-4(2H)-carboxamide (8f).

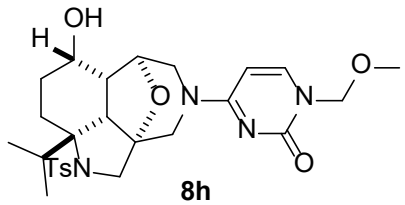


8f

To s solution of **5** (15 mg, 0.037 mmol), *N*-methyl-1*H*-imidazole-1-carboxamide (5.5 mg, 0.040 mmol, 1.1 equiv.) in DCM (1.0 mL) was added Et_3N (6.8 µL, 0.092 mmol, 2.5 equiv.). The reaction was stirred at RT for 2 h. Then, the reaction mixture was diluted with saturated solution of $NaHCO_3$ and the organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was used directly

in the next step. To a solution of the ketone from pervious step in MeOH, $NaBH_4$ (1.5 mg, 0.040 mmol, 1.5 equiv.) was carefully added in one portion at 0 °C. The resulting mixture was allowed to warm to RT and was stirred for 30 min. The reaction was quenched by drop of water. After that the solvent was removed under reduced pressure and the resulting mixture was diluted by EtOAc and saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted for three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography eluting by (EtOAc 100%) to give **8f** as white solid (17 mg, quantitative yield). HRMS (ESI): $[M+H]^+$ $C_{23}H_{34}N_3O_5S$: calcd. 464.2214 found 464.2218. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.80 (br s, 1H), 7.74 (s, 1H), 7.72 (s, 1H), 7.27-7.25 (m, 2H), 7.12 (s br, 1H), 4.78 (s, 1H), 4.57-5.56 (m, 1H), 3.77-3.72 (m, 1H), 3.66 (d, 2H, *J* = 11.5 Hz), 3.37-3.33 (m, 2H), 3.09-3.06 (m, 1H), 2.98-2.95 (m, 1H), 2.81-2.80 (d, 3H, *J* = 4.5 Hz), 2.70-2.68 (m, 1H), 2.67-2.63 (m, 1H), 2.55-2.50 (m, 2H), 2.39 (s, 3H), 1.67-1.60 (m, 1H), 1.41-1.33 (dq, 1H, *J* = 2.5, 12 Hz), 1.04 (d, 3H, *J* = 7.0 Hz), 0.90 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 161.8, 145.5, 142.0, 132.1, 129.3, 129.0, 89.7, 77.7, 76.7, 71.9, 58.4, 52.3, 51.0, 50.9, 50.7, 41.8, 32.8, 30.2, 29.4, 24.1, 21.4, 20.5.

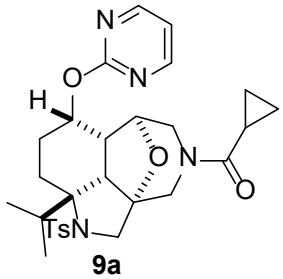
4-(7-hydroxy-9a-isopropyl-1-tosyloctahydro-2a,6-epoxyazepino[3,4,5-cd]indol-4(2H,2a1H,3H)-yl)-1-(methoxymethyl)pyrimidin-2(1H)-one (8h).



8h

To s solution of **5** (15 mg, 0.037 mmol) and 1-(methoxymethyl)-4-(1H-1,2,4-triazol-1-yl)pyrimidin-2(1*H*)-one (9.1 mg, 0.044 mmol, 1.2 equiv.) in MeCN (1.0 mL), was added Et_3N (6.8 μL, 0.092 mmol, 2.5 equiv.). The reaction was stirred at 70 °C for 2 days. Then, the reaction mixture was cooled to RT and diluted with saturated solution of $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was used directly in the next step. To a solution of the ketone from pervious step in MeOH, $NaBH_4$ (1.5 mg, 0.040 mmol, 1.5 equiv.) was carefully added in one portion at 0 °C. The resulting mixture was allowed to warm to RT and was stirred for 30 min. The reaction was quenched by drop of water. After that the solvent was removed under reduced pressure and the resulting mixture was diluted by EtOAc and saturated solution of $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted for three times with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography eluting by (EtOAc 100%) to give **8h** as white solid (6.0 mg, 30%). HRMS (ESI): $[M+H]^+$ $C_{27}H_{37}N_4O_6S$: calcd. 545.2428 found 545.2435. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.74-7.69 (m, 2H), 7.46-7.44 (m, 1H), 7.27-724 (m, 2H), 5.88-5.83 (m, 1H), 5.19-5.16 (m, 1H), 4.82-4.77 (, 2H), 3.75-3.65 (m, 2H), 3.40 (s, 3H), 3.39-3.35 (m, 1H), 3.33-3.27 (m, 3H), 2.66-2.51 (m, 3H), 2.38 (s, 3H), 2.03-1.92 (m, 2H), 1.65-.59 (m, 3H), 1.05-1.02 (m, 3H), 0.90-0.86 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 147.1, 145.6, 142.0, 132.2, 132.1, 129.08, 129.02, 94.3, 83.4, 81.6, 80.9, 77.2, 59.7, 58.1, 56.7, 52.4, 50.8, 46.6, 43.2, 41.9, 33.5, 29.6, 24.7, 24.1, 21.4.

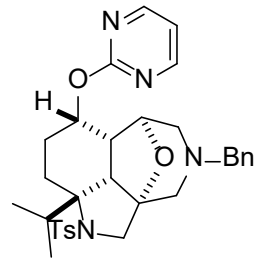
cyclopropyl(9a-isopropyl-7-(pyrimidin-2-yloxy)-1-tosyloctahydro-2a,6-epoxyazepino[3,4,5-cd]indol-4(2H,2a1H,3H)-yl)methanone (9a)



9a

The titled compound was synthesized firstly, according to general procedure (**D**) from the **5** (15 mg, 0.037 mmol) in DCM (1.0 mL), cyclopropanecarbonyl chloride (3.6 μL, 0.040 mmol) and Et_3N (5.5μL, 0.040 mmol).Then, the next step (reduction of the ketone), the crude product from previous step was dissolved in MeOH (1.0 mL) and $NaBH_4$ (1.5 mg, 0.040 mmol) to give **8g**. Then, the next step (reaction of alcohol with RCl), following to the general procedure (**C**) crude product from the previous step, NaH (60 % dispersion in mineral oil) (1.48 mg, 0.055 mmol, 1.5 equiv.) in dry THF (1 mL) and 2-chloropyrimidine (6.3 mg, 0.055 mmol, 1.5 equiv.). The crude product was purified by column chromatography on silica gel eluting by (EtOAc 100%) to give the titled compound **9a** as faint yellow solid (16 mg, 78%). HRMS (ESI): $[M+H]^+$ $C_{29}H_{37}N_4O_5S$: calcd. 553.2484 found 553.2484. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.48-8.45 (m, 2H), 7.74-7.72 (m, 2H), 7.28-7.23 (m, 2H), 6.94-6.90 (m, 1H), 4.97-4.95 (m, 1H), 4.92-4.87 (m, 1H), 4.27 (d, 1H, *J* = 13 Hz), 4.03-3.87 (m, 1H), 3.79-3.74 (m, 1H), 3.67 (d, 1H, *J* = 12.5 Hz), 3.46 (d, 1H, *J* = 11.5 Hz), 3.41-3.37 (m,1H), 2.87-2.54 (m, 5H), 2.38 (s, 3H), 1.77-1.66 (m, 3H), 1.57-1.49 (m, 2H), 1.46-1.38 (m, 1H), 1.11-1.07 (m, 3H), 1.00-0.94 (m, 4H), 0.74-0.73 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 176.5, 166.9, 162.0, 145.4, 142.3, 132.1, 129.0, 117.8, 90.1, 78.5, 77.4, 76.7, 58.4, 56.0, 52.8, 49.4, 48.6, 41.8, 32.5, 25.4, 24.1, 21.5, 20.7, 20.4, 13.9, 10.3.

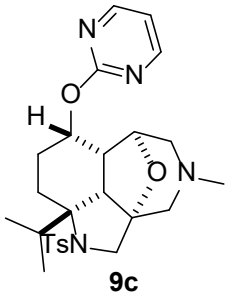
4-benzyl-9a-isopropyl-7-(pyrimidin-2-yloxy)-1-tosyldodecahydro-2a,6-epoxyazepino[3,4,5-cd]indolemethanone (9b).



9b

The titled compound was synthesized firstly, according to general procedure (**B**) from ketone **7** (9 mg, 0.018 mmol) in MeOH (0.5 mL) and $NaBH_4$ (0.75 mg, 0.020 mmol). Then, the next step (reaction of alcohol with RCl), following to the general procedure (**C**) crude product from the previous step, NaH (60 % dispersion in mineral oil) (0.72 mg, 0.027 mmol, 1.5 equiv.) in dry THF (0.5 mL) and 2-chloropyrimidine (3.1 mg, 0.027 mmol, 1.5 equiv.). The crude product was purified by column chromatography on silica gel eluting by (Cyclohexane/EtOAc 80/20) to give the titled compound **9b** as faint yellow solid (7.0 mg, 86%). HRMS (ESI): $[M+H]^+$ $C_{32}H_{39}N_4O_4S$: calcd. 575.2687 found 575.2695. ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.45 (s, 1H), 8.44 (s, 1H), 7.73-7.72 (m, 2H), 7.29-7.27 (m, 3H), 7.24-7.21 (m, 3H), 6.87 (t, 1H, *J* = 5.0 Hz), 4.99-4.95 (m, 1H), 4.78 (br s, 1H), 3.70 (d, 1H, *J* = 12 Hz), 3.54 (d, 1H, *J* =13.0 Hz), 3.47 (d, 1H, *J* =13.0 Hz), 3.30 (d, 1H, *J* = 13.0Hz), 3.05-3.02 (m, 1H), 2.99-2.97 (m, 1H), 2.72 (dt, 1H, *J* = 16.0, 3.0 Hz), 2.63 (d, 1H, *J* = 10.5 Hz), 2.59-2.57 (m, 1H), 2.41-2.38 (m, 1H), 2.37 (s, 3H), 2.28 (d, 1H, *J* = 11 Hz), 2.24-2.22 (m, 1H), 1.78-1.71 (m, 1H), 1.52-1.42 (m, 3H), 1.06-1.03 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 167.0, 161.9, 145.2, 142.4, 140.5, 132.0, 131.2, 130.9, 129.8, 128.9, 117.5, 90.6, 78.4, 76.7, 64.4, 59.8, 59.3, 58.9, 53.4, 49.0, 41.7, 32.5, 32.3, 25.8, 24.1, 21.7, 20.5.

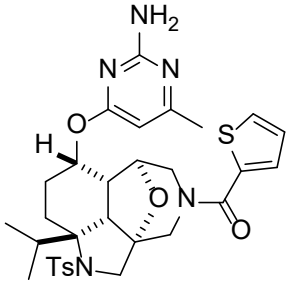
9a-isopropyl-4-methyl-7-(pyrimidin-2-yloxy)-1-tosyldodecahydro-2a,6-epoxyazepino[3,4,5-cd]indole (9c).



9c

The titled compound was synthesized firstly, according to general procedure (**B**) from the **6** (4 mg, 0.0095 mmol) in MeOH (0.25 mL) and $NaBH_4$ (0.39 mg, 0.010 mmol). Then, the next step (reaction of alcohol with RCl), following to the general procedure (**C**) crude product from the previous step, NaH (60 % dispersion in mineral oil) (0.33 mg, 0.014 mmol, 1.5 equiv.) in dry THF (0.25 mL) and 2-chloropyrimidine (1.6 mg, 0.014 mmol, 1.5 equiv.). The crude product was purified by column chromatography on silica gel eluting by (Cyclohexane/EtOAc 80/20) to give the titled compound **9c** as faint yellow solid (4.0 mg, 84%). HRMS (ESI): $[M+H]^+$ $C_{26}H_{35}N_4O_4S$: calcd. 499.2374 found 499.2373. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.47 (s, 1H), 8.46 (s, 1H), 7.75-7.73 (m, 2H), 7.24-7.22 (m, 2H), 6.89 (t, 1H, J = 4.5 Hz), 4.98-4.94 (m, 1H), 4.78 (br s, 1H), 6.70 (d, 1H, J = 12 Hz), 3.34 (d, 1H, J = 11.5 Hz), 3.03-3.00 (m, 1H), 2.93-2.90 (m, 1H), 2.71 (dt, 1H, J = 16.0, 3.0 Hz), 2.63-2.56 (m, 2H), 2.40-2.39 (m, 1H), 2.38 (s, 3H), 2.23 (s, 3H), 2.20-2.16 (m, 2H), 1.77-1.71 (m, 1H), 1.50-1.44 (m, 2H), 1.10 (d, 3H, J = 7.0 Hz), 1.03 (d, 3H, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 167.1, 161.9, 145.3, 142.4, 132.1, 128.9, 117.6, 90.3, 78.4, 76.8, 61.8, 61.6, 58.9, 53.3, 48.8, 48.0, 41.8, 32.6, 32.3, 25.7, 24.1, 21.5, 20.5.

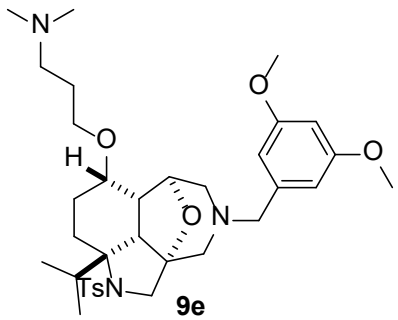
7-((2-amino-6-methylpyrimidin-4-yl)oxy)-9a-isopropyl-1-tosyloctahydro-2a,6-epoxyazepino[3,4,5-cd]indol-4(2H,2a1H,3H)-yl)(thiophen-2-yl)methanone (9d).



9d

The titled compound was synthesized following to the general procedure (**C**) from **8a** (3.0 mg, 0.0058 mmol), NaH (60 % dispersion in mineral oil) (0.33 mg, 0.0087 mmol, 1.5 equiv.) in dry THF (0.25 mL) and 2-amino-4-chloro-6-methylpyrimidine (1.24 mg, 0.0087 mmol, 1.5 equiv.). The crude product was purified by column chromatography on silica gel eluting by (Cyclohexane/EtOAc 50/50) to give the titled compound **9d** as faint yellow solid (3.0 mg, 82%). HRMS (ESI): $[M+H]^+$ $C_{31}H_{38}N_5O_5S_2$: calcd. 624.2309 found 624.2314. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.74-7.77 (m, 3H), 7.48-7.47 (m, 1H), 7.27-7.25 (m, 2H), 7.06-7.03 (m, 1H), 5.87 (s, 1H), 4.91-4.87 (m, 1H), 4.69-4.64 (m, 1H), 3.71-3.68 (m, 1H), 3.41-3.39 (m, 1H), 3.35-3.31 (m, 1H), 3.14-3.05 (m, 1H), 2.92-2.89 (m, 1H), 2.79-2.75 (m, 2H), 2.64-2.55 (m, 1H), 2.47-2.44 (m, 1H), 2.39 (s, 3H), 2.39-2.38 (m, 1H), 2.34-2.32 (m, 1H), 2.27 (s, 3H), 1.73-1.67 (m, 1H), 1.50-1.35 (m, 3H), 1.06-0.99 (m, 3H), 0.98-0.96 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 172.6, 167.5, 145.5, 141.9, 138.7, 132.1, 132.0, 131.9, 130.2, 129.7, 129.0, 100.3, 90.4, 78.9, 76.9, 59.9, 58.2, 56.0, 52.6, 51.0, 48.3, 41.6, 32.33, 32.31, 25.9, 24.1,22.1, 21.6, 20.5.

3-(4-(3,5-dimethoxybenzyl)-9a-isopropyl-1-tosyldodecahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7-yl)oxy)-N,N-dimethylpropan-1-amine (9e).

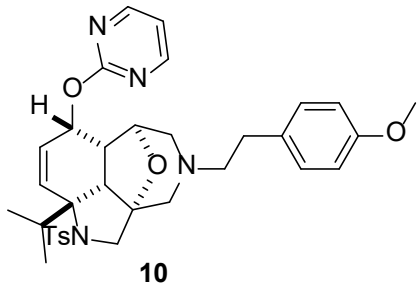


9e

The titled compound was synthesized following to the general procedure (**C**) from **8e** (9.0 mg, 0.016 mmol), NaH (60 % dispersion in mineral oil) (0.64 mg, 0.024 mmol, 1.5 equiv.) in dry THF (0.5 mL) and dimethylaminopropylchloride HCl (3.7 mg, 0.024 mmol, 1.5 equiv.) and KI (0.26 mg, 0.0016 mmol, 0.1 equiv.). The crude product was purified by column chromatography on silica gel eluting by (Cyclohexane/EtOAc 50/50) to give the titled compound **9e** as faint yellow solid (5.0 mg, 49%). HRMS (ESI): $[M+H]^+$ $C_{35}H_{52}N_3O_6S$: calcd. 642.3571 found 642.3553. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.71-7.70 (m, 2H), 7.24-7.22 (m, 2H), 6.47 (m, 2H), 6.35 (t, 1H, *J* = 2 Hz), 4.56 (s, 1H), 3.78 (s, 6H), 3.57 (d, 1H, *J* = 11.5 Hz), 3.49-3.36 (m, 4H), 3.28-3.24 (m, 1H), 3.24-3.21 (m, 1H), 2.89-2.87 (m, 1H), 2.81-2.77 (m, 1H), 2.68-2.50 (m, 6H), 2.37 (s, 3H), 2.34 (s, 6H), 2.30-2.78 (s, 2H), 2.23-2.20 (d, 1H, *J* = 10.5 Hz), 1.79-1.74 (m, 2H), 1.59-1.53 (m, 1H), 1.34-1.27 (m, 2H), 0.99 (d, 3H, *J* = 7.0 Hz), 0.95-0.93 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 163.5, 145.2, 143.1, 142.2, 132.1, 129.0, 108.9, 101.7, 90.6, 80.6, 79.1, 77.3, 68.6, 64.6, 59.7, 59.0, 58.7, 57.9, 52.9, 49.4, 47.2, 41.5, 34.5, 33.0, 32.3, 32.0, 26.2, 25.3, 24.1, 21.5, 20.5.

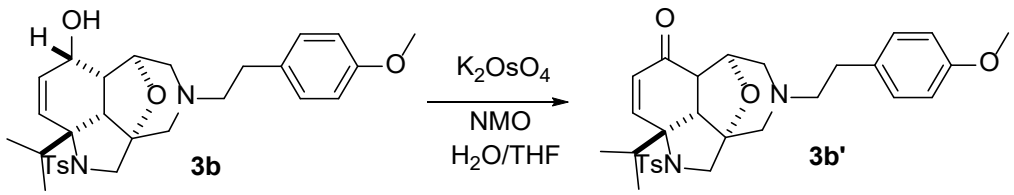
9a-isopropyl-4-(4-methoxyphenethyl)-7-(pyrimidin-2-yloxy)-1-tosyl-1,2,2a1,3,4,5,6,6a,7,9a-decahydro-2a,6-epoxyazepino[3,4,5-cd]indole (10).

The titled compound was synthesized following to the general procedure (**C**) from **3b** (30.0 mg, 0.055 mmol), NaH (60 % dispersion in mineral oil) (2.2 mg, 0.083 mmol, 1.5 equiv.) in dry THF (0.5 mL) and 2-chloropyrimidine (9.56 mg, 0.083 mmol, 1.5 equiv.). The crude product was purified by column chromatography on silica gel eluting by (Cyclohexane/EtOAc 70/30) to give the titled compound **10** as faint yellow solid (30.0 mg, 88%). HRMS (ESI): $[M+H]^+$ $C_{34}H_{41}N_4O_5S$: calcd. 617.2792 found 617.2779. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.52 (s, 1H), 8.51 (s, 1H), 7.78 (s, 1H), 7.76 (s, 1H), 7.25-7.23 (m, 2H), 7.07-7.06 (m, 2H), 6.95 (t, 1H, *J* = 4.5 Hz), 6.82-6.80 (m, 2H), 6.42 (dd, 1H, *J* = 2.5 ,13.5 Hz), 5.88 (dt, 1H, *J* = 11.0 , 1.0 Hz), 5.61 (d, 1H, *J* = 7.5 Hz), 4.78 (br s, 1H), 3.77 (s, 3H), 3.50 (d, 1H, *J* = 11.5 Hz), 3.36 (m, 1H), 3.22 (d, 1H, *J* = 11.5 Hz), 2.90 (m, 1H), 2.78-2.73 (m, 1H), 2.65 (m, 3H), 2.55 (m, 2H), 2.44 (m, 1H), 2.37 (s, 3H), 2.30 (m, 2H), 1.12 (d, 3H, *J* = 7.0 Hz), 0.90 (d, 3H, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 167.1, 162.2, 162.0, 145.3, 141.0, 135.3, 134.7, 132.1, 129.6, 129.0, 122.3, 117.9, 116.3, 90.3, 75.9, 73.6, 61.7, 60.0, 59.0, 57.8, 57.5, 50.9, 45.9, 39.9, 34.8, 32.3, 24.1, 20.1, 20.6, 19.0.

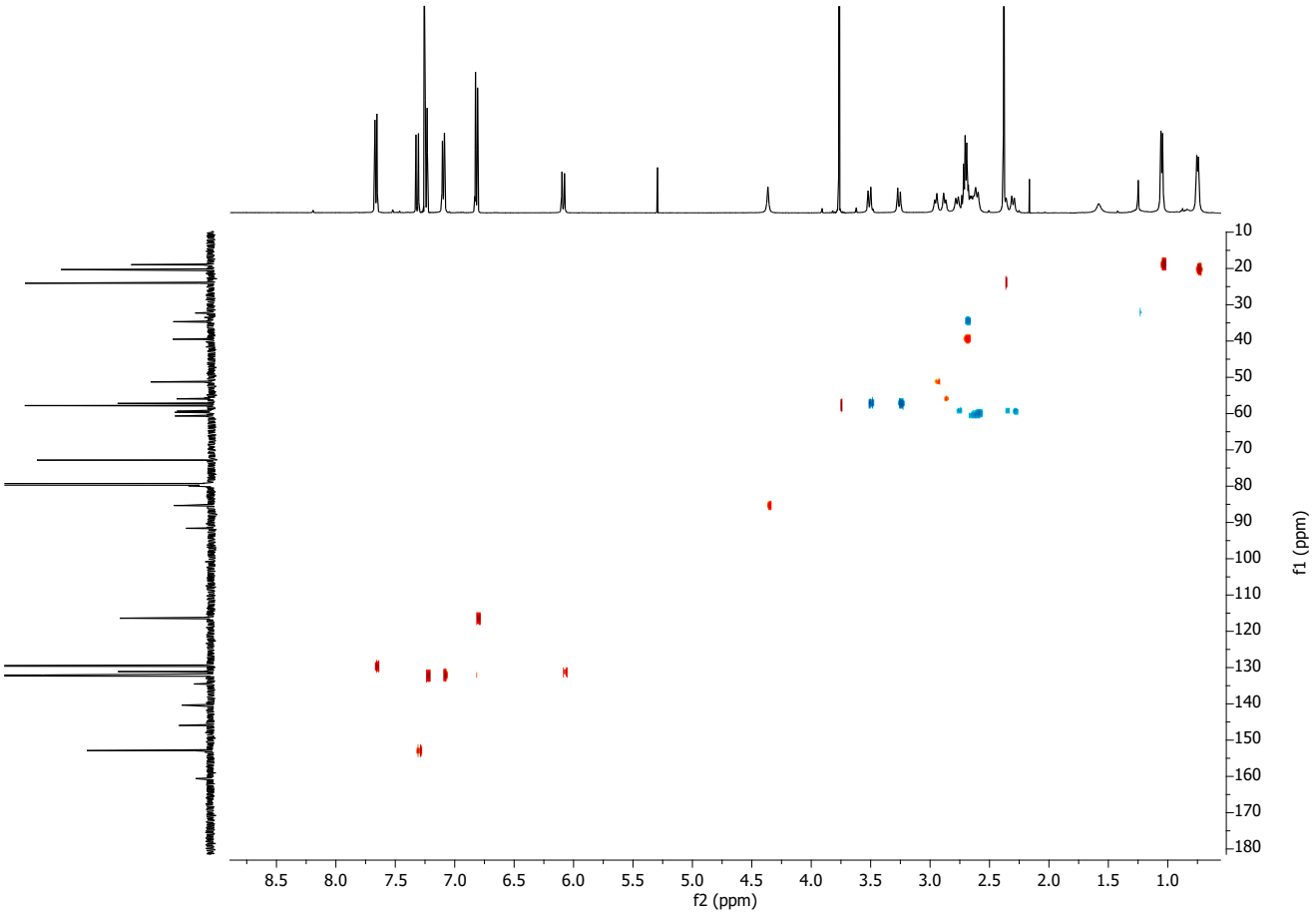


10

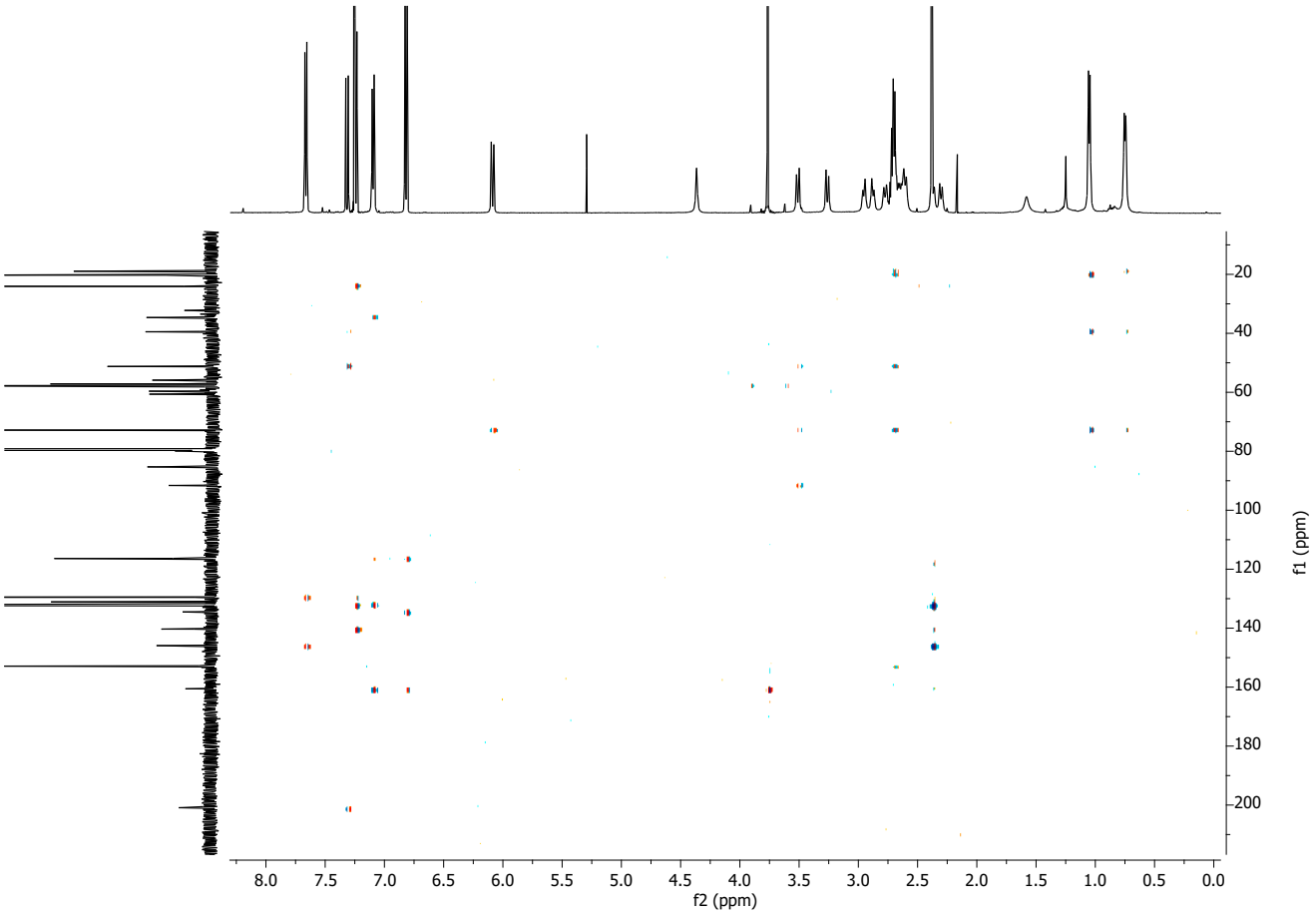
9a-isopropyl-4-(4-methoxyphenethyl)-1-tosyl-1,2a1,3,4,5,6,6a,9a-octahydro-2a,6-epoxyazepino[3,4,5-cd]indol-7(2H)-one (3b').



To a solution of **3b** (15 mg, 0.027 mmol, 1.0 equiv.) in THF (0.52 mL) and water (0.093 mL) were added NMO (3.9 mg, 0.033 mmol, 1.2 equiv.) and $K_2OsO_4.2H_2O$ (0.079 mg, 0.216 µmol, 0.008 equiv.). The solution was stirred at RT for 16 h. The reaction mixture was concentrated under reduced pressure and the residue was diluted by a solution of 15% of $NaHSO_3$ (15 ml) and the mixture was extracted three times with Et_2O. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified through column chromatography eluting (Cyclohexane/EtOAc 90/10) to give the titled compound **3b'** as white solid (11.0 mg, 76%). HRMS (ESI): $[M+H]^+$ $C_{30}H_{37}N_2O_5S$: calcd. 537.2418 found 537.2403. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.67 (s, 1H), 7.65 (s, 1H), 7.31 (d, 1H, *J* = 10.5 Hz), 7.25-7.23 (m, 2H), 7.10 (s, 1H), 7.09 (s, 1H), 6.82-6.81 (m, 2H), 6.09 (d, 1H, *J* = 10.5 Hz), 4.36 (s, 1H), 3.77 (s, 3H), 3.51 (d, 1H, *J* = 11.5 Hz), 3.26 (d, 1H, *J* = 11 Hz), 2.95 (d, 1H, *J* = 9.5 Hz), 2.88 (d, 1H, *J* = 9.5 Hz), 2.77 (d, 1H, *J* = 11 Hz), 2.75-2.60 (m, 6H), 2.85 (s, 3H), 2.37-2.36 (m, 1H), 2.30 (d, 1H, *J* = 11.0 Hz), 1.05 (d, 3H, *J* = 6.5 Hz), 0.75 (d, 3H, *J* = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 200.9, 160.6, 152.9, 145.9, 140.3, 134.5, 132.2, 132.0, 131.1, 129.5, 116.4, 91.6, 85.3, 72.8, 60.7, 59.7, 59.3, 57.8, 57.2, 55.9, 51.3, 39.5, 34.7, 24.1, 20.3, 19.0.



HSQC spectrum for compound 3b'



HMBC spectrum for compound 3b'

Viral assay and Cytotoxicity

The lung cancer A549 cell line (adenocarcinomic human alveolar basal epithelial cells, source ATTC, reference CCL-158) stably transfected with a lentiviral construct bearing the human ACE2 receptor were kindly provided by Olivier Schwartz (Institut Pasteur). A549-ACE2 were cultured in DMEM with 10% FBS, 1% penicillin/streptomycin and 10 µg/ml blasticidin. The SARS-CoV-2 isolate BetaCoV/France/IDF0372/2020 (European Beta) and hCoV-19/France/PDL-IPP46934/2021 (B.1.1.529 (BA1)) were supplied by the National Reference Centre for Respiratory Viruses hosted by Institut Pasteur (Paris, France) and headed by Dr. Sylvie van der Werf. Viral stocks were prepared in Vero E6 cells in DMEM 2% FBS All experiments involving live SARS-CoV-2 were performed in compliance with Institut Pasteur Paris's guidelines for Biosafety Level 3 (BSL-3) containment procedures in approved laboratories. All experiments were performed in at least three biologically independent samples.

A549-ACE2 cells were seeded in 384-well plates (Greiner Bio-One, $2x10^4$ cells/well). The following day, compounds were added at indicated concentrations 2 h prior infection. DMSO only (0.5%) as negative control and 10 µM remdesivir (Selleckchem) as positive control were added in each plate. After the pre-incubation period, the drugs were removed and replaced with virus inoculum (MOI of 0,1 PFU/cell) for 1 h at 37°C. Following the adsorption period, the inoculum was removed and replaced with 2% FBS/DMEM containing the individual drugs. After 72 h of incubation, the supernatant was recovered and the measurement of viral replication was carried out by quantitative RT-PCR using previously described [2] SARS-CoV-2 specific primers targeting the N gene. RT-qPCR was performed using the Luna Universal One-Step RT-qPCR Kit (NEB) in an Applied Biosystems QuantStudio 7 thermocycler. The quantity of viral genomes is expressed as Ct and was normalized against the Ct values of the negative and positive controls.

In parallel, cytotoxicity was assessed using the CellTiter-Glo luminescent cell viability kit (Promega). 2,000 cells/well of A549-ACE2 were seeded in white with clear bottom 384-well plates. The following day, compounds were added at concentrations indicated. DMSO only (0,5%) and 10 µM camptotecin (Sigma-Aldrich) controls were added in each plate. After 72 h incubation, 10 µl of CellTiter Glo reagent was added in each well and the luminescence was recorded using a luminometer (Berthold Technologies) with 0,5 sec integration time.

Raw data were normalized against appropriate negative (0 %) and positive controls (100 %) and are expressed in % of viral replication inhibition or % of cytotoxicity. Curve fits and IC50/CC50 values were obtained in Prism using the variable Hill slope model.

[2] D. K. W. Chu, Y. Pan, S. M. S. Cheng, K. P. Y. Hui, P. Krishnan, Y. Liu, D. Y. M. Ng, C. K. C. Wan, P. Yang, Q. Wang, M. Peiris and L. L. M. Poon, *Clin Chem,* 2020, 66, 549.

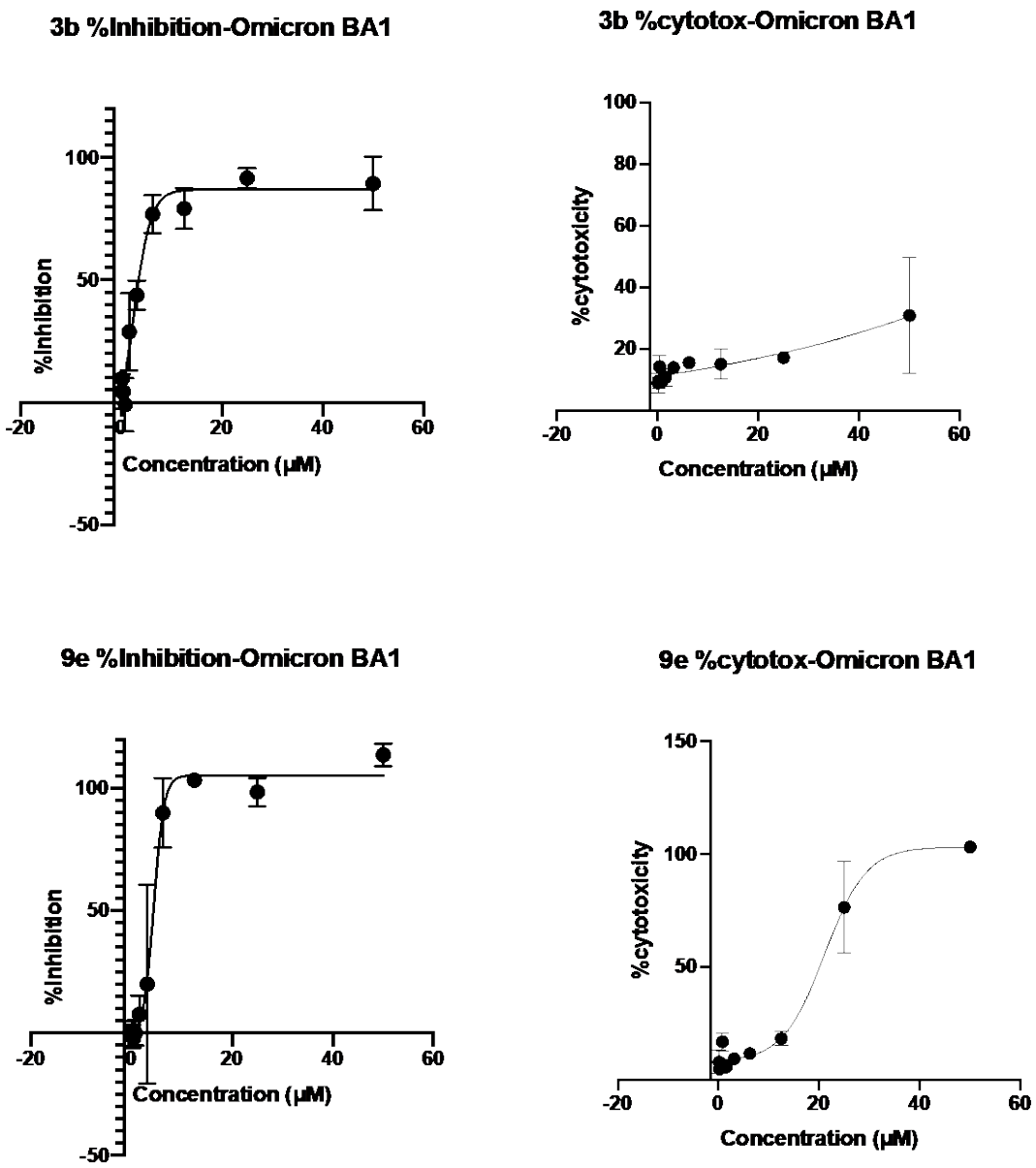


Figure S1. Anti-SARS-CoV-2 variant Omicron BA1 dose response curve (left) and cytotoxicity (right). Top: Compound **3b**. Bottom: Compound **9e**. Error bars of triplicates are shown.

Docking and molecular dynamics:

Preparation of protein structures

To computationally verify the *ex vivo* results of the synthetic compounds on the lung cell-lines infected with SARS-CoV-2 and predict the target protein of the best hits, four different SARS-CoV-2 target proteins were chosen to carry out comprehensive virtual screening, including COVID-19 main protease (M^{pro}) (PDB ID: 6LU7), nucleocapsid phosphoprotein (PDB ID: 6VYO), non-structural protein (nsp10-nsp16 complex) (PDB ID: 6W4H) and RBD of spike protein/ACE2 complex (PDB ID: 6M17), retrieved from Protein Data Bank (http://www.pdb.org) with resolutions of 2.16 Å, 1.70 Å, 1.80 Å, and 2.90 Å, respectively.

Water molecules were removed from the crystal structures and only main-chain amino acids were retained which are essential for binding of the co-crystallized ligand. All the four 3D structures were prepared by using AMBER10:EHT force field for energy minimization; where AMBER parameters are suitable for proteins and nucleic acids (ff10); EHT parameters are suitable for small molecules (Preliminary Release). The protons were added by employing the 3D protonation feature in MOE v.2019.01, Asn, Gln and is flips were allowed during 3D protonation and finally, the complexes were refined to RMS Gradient of 0.1 Kcal/mol/Å.

Re-docking of the co-crystallized ligand and virtual screening

The 25 compounds screening library were docked over the 4 COVID-19 target proteins M^{Pro}, nucleocapsid phosphoprotein, nsp10-nsp16 complex/nsp16 complex and membrane spike glycoprotein employing MOE v.2019.01. Also, the native co-crystallized ligands of M^{pro} and nsp10-nsp16 complex/nsp16 were redocked for the sake of validation and comparison. For docking scoring, triangle matcher placement was chosen; the first rescoring function was set to be London dG while GBVI/WSA dG was chosen to be the second rescoring function and finally it was refined using MMFF94 forcefield retaining 30 docked structures for each compound. To validate the docking performance, Root Mean Square Deviation (RMSD) values between the docked conformation and the reference conformation presented in Å were utilized.[3] Figure S2 shows the binding poses, ligands' alignments and their interactions with the two proteins. Self-docking results showed an RMSD of 0.585 Å for SAM, and 2.009 Å for the peptide-like inhibitor PRD_002214. Since the optimum RMSD for docking validation is conventionally ≤ 2 Å and the results obtained by MOE 2019.01 were within this range, the adopted docking protocol was considered reliable.

[3] S. C. Tuble, J. Anwar and J. D. Gale, *J. Am. Chem. Soc. 2004*, **126**, 396.

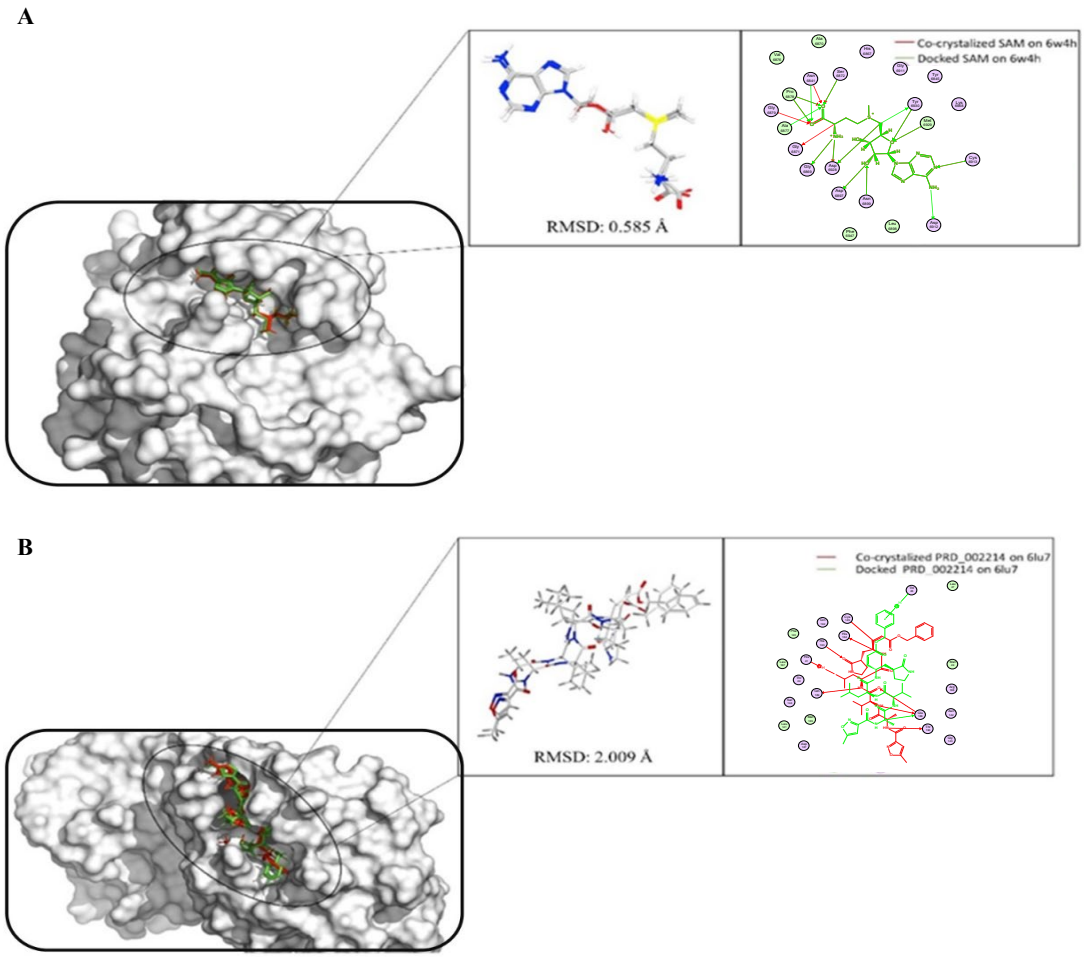


Figure S2. Binding poses alignment, and binding interactions of the co-crystallized and self-docking ligands for: **(A)** SAM with nsp10-nsp16 complex/nsp16 complex (PDB ID: 6W4H) and **(B)** the peptide-like inhibitor PRD_002214 with M^{pro} (PDB ID: 6LU7).

The binding potential of the 25 synthesized compounds was assessed and their potential binding affinity was reported as S-scores (MOE internal scoring function) and compared to those of the co-crystallized ligands, when applicable, for each of the four target proteins (Table S1). The 2D interactions of the molecules compared to the co-crystallized ligands demonstrated that compounds **3b**, **3c**, **8e**, **8d, 8h**, **8d'**, **9a**, **9b**, **9d**, **9e**, **10** and **11** showed the best S-scores for M^{pro} some even better than the co-crystallized ligand. Regarding the SARS-CoV-2 nucleocapsid phosphoprotein, compounds **3b**, **4**, **9d**, **9e** and **10** showed the best binding affinities. Only five compounds demonstrated S-scores of above -6.00 Kcal/mol with the spike protein membrane complex *viz.* **3b**, **8h**, **9d**, **9e** and **10**. Finally, compounds **3b**, **9a**, **9b** and **9e** were the best ligands for nsp10-nsp16 complex. Accordingly, **3b**, **8d**, **8d'**, **8e** and **9e** were the best virtual hits (Table S1), which is in line with the antiviral activity profile of these compounds.

Table S1. Docking S-score (Kcal/mol) for the compounds of the chemical library compared the co-crystallized ligands for each SARS-CoV-2 target proteins.

Compound	Main protease (M^{Pro}) (PDB ID: 6LU7)	Nucleocapsid phosphoprotein (PDB ID: 6VYO)	Non-structural protein (nsp10-nsp16 complex/nsp16 complex) (PDB ID: 6W4H)	Spike protein membrane complex (PDB ID: 6M17)
1	-6.83	-5.37	-6.09	-4.90
2	-6.88	-5.68	-6.44	-4.94
3a'	-7.26	-5.70	-7.05	-5.68
3a	-6.95	-5.70	-6.98	-5.54
3b'	-7.70	-5.34	-7.15	-5.55
3b	-7.52	-6.09	-7.67	-6.17
3c	-7.65	-5.97	-7.21	-5.89
4	-6.87	-6.18	-6.58	-5.32
5	-7.03	-5.60	-6.58	-5.09
6	-7.16	-4.96	-5.98	-5.08
7	-7.36	-5.53	-6.81	-5.77
8a	-7.23	-5.44	-7.09	-5.55
8b	-7.17	-5.69	-7.26	-5.46
8c	-7.10	-5.59	-7.19	-5.57
8d'	-7.49	-5.59	-7.36	-5.49
8d	-7.58	-5.39	-7.35	-5.88
8e	-7.40	-5.94	-6.84	-5.90
8f	-6.94	-5.62	-6.68	-5.49
8h	-7.53	-5.49	-7.50	-6.22
9a	-7.55	-5.81	-8.60	-5.87
9b	-7.57	-5.83	-7.75	-5.98
9c	-7.37	-5.76	-7.06	-5.46
9d	-7.75	-6.56	-7.56	-6.14
9e	-8.74	-6.94	-8.30	-6.55
10	-7.67	-6.17	-7.63	-6.22
co-crystallized ligand	-8.25	N/A	-9.43	N/A

N/A= not applicable

Binding interactions of **3b** and **9e** along with the co-crystallized ligands with M^{Pro} and nsp10-nsp16 complex are shown in Table S2.

Table S2: Comparison of the 2D interactions of the screening library compounds **3b, 9e**, and the co-crystallized ligands

Compound	Main protease (M^{Pro}) (PDB ID: 6LU7)	Non-structural protein (nsp10-nsp16 complex/nsp16 complex) (PDB ID: 6W4H)
3b	6 interactions -4 H-bond donors; one between O11 of the ligand and Leu 141 in the pocket , the second between O31 of the ligand and Cys 145 in the pocket, the third between C32 of the ligand and Met 49 in the pocket and the fourth between O63 of the ligand and Cys 145 in the pocket with distances 3.14 Å, 3.42 Å, 3.61 Å and 3.72 Å, respectively and with energy scores of -0.6 Kcal/mol, -0.8 Kcal/mol, -0.8 Kcal/mol, and -1.6 Kcal/mol, respectively. - 2 Pi-H interactions between the ligand's 6-membered ring and Thr 25 and Thr 26 in the pocket with distances 4.39 Å and 4.22 Å, respectively and with energy scores of -1.1 Kcal/mol, and -2.0 Kcal/mol, respectively.	2 interactions -H-bond acceptor between N53 of the ligand and Tyr 6930 in the pocket with distance 3.64 Å and energy scores of -0.6 Kcal/mol. -Pi-H interaction between the ligand's 6-membered ring and Asp 6873 in the pocket with distance 3.67 Å and energy scores of -0.9 Kcal/mol.

9e	2 interactions - 2 H-bond donors between C62 and O76 of the ligand and Cys 145 in the pocket with distances 4.08 Å and 3.47 Å, respectively and with energy scores of -0.6 Kcal/mol and -0.7 Kcal/mol, respectively.	2 interactions - 2 H-bond acceptors one between O48 of the ligand and Asn 6841 of the receptor, and the second between O77 of the ligand and Asp 6873 in the pocket with distances 3.17 Å and 3.44 Å, respectively and with energy scores of -1.5 Kcal/mol, and -0.6 Kcal/mol, respectively.
Co-crystallized ligand	7 interactions - 4 as H-donor, first between N13 of ligand and Thr 190, a second between N23 and Glu 166, a third between N39 and Gln 189, and a fourth between N5 and His 164. Interaction distances were 2.85 Å, 2.83 Å, 2.93 Å, and 3.07 Å, while the energy scores were -2.6 Kcal/mol, -4.8 Kcal/mol, 3.3 Kcal/mol and -1.8 Kcal/mol, respectively. - 2 as H acceptor, first between O28 and Glu1 66 and the second between O8 and His 164. Interactions distances were 2.98 Å and 2.52 Å, and interactions energy scores were -3.3 and -3.4 Kcal/mol, respectively - One H-pi between CD1 and His 41 with a distance of 4.08 Å and energy of -0.5 Kcal/mol	11 interactions - 6 H-bond donors one between N1 of the ligand and Gly 6869 of the receptor, the second between N1 and Asp 6928, the third CA5 and Gly 6871, the fourth between C5' of the ligand and Asp 6928 in the pocket, the fifth and sixth between O2' and O3' of the ligand and Asp 6897 in the pocket, with distances 2.89 Å, 2.75 Å , 3.08 Å, 3.29 Å, 2.65 Å and 2.72 Å, respectively and with energy scores of -8.0 Kcal/mol, -5.8 Kcal/mol , -0.8 Kcal/mol, -1.5 Kcal/mol, -4.1 Kcal/mol, and -4.0 Kcal/mol, respectively. - 4 H-bond acceptors one between O8 of the ligand and Gly 6879 of the receptor , the second between OXT9 of the ligand and Asn 6841 in the pocket, the third between O4' of the ligand and Tyr 6930 in the pocket, the fourth between N1 of the ligand and Cys 6913 with distances 2.86 Å, 2.74 Å, 3.38 Å, and 3.03 Å, respectively and with energy scores of -5.5 Kcal/mol, -3.0 Kcal/mol, -0.5 Kcal/mol and -4.8 Kcal/mol, respectively - Ionic bond between N1 of the ligand and Asp 6928 in the protein pocket with distance 2.75 Å and energy scores of -6.4 Kcal/mol.

Molecular Dynamics Simulation for Compounds 3b and 9e

Compounds ***3b*** *and* ***9e*** displayed the best binding interactions and free energies for the 4 target proteins among the 25 investigated compounds in addition to the best IC_{50} scores with the best toxicity profiles. Accordingly, they were subjected to 100 ns molecular dynamics (MD) investigation against the 4 target proteins; COVID-19 main protease, nucleocapsid phosphoprotein, membrane glycoprotein and non- structural protein (nsp10-nsp16 complex).

MD simulations were performed using GROMACS 2021 software package where the CHARMM36 forcefield was used for protein topology preparation and the official CHARMM General Force Field server (CGenFF) for ligand topology preparation. The solvation method used here was a dodecahedron box of common simple point charge (SPC) water model where explicit solvent periodic boundary conditions were applied. Charge neutralization using sodium and chloride ions was carried out for the 8 solvated complexes. The systems were subjected to energy minimization to resolve any steric clashes or inappropriate geometry employing the steepest descent method through 5000 steps. System equilibration was also manipulated to ensure a reasonable starting structure using NVT; equilibration under constant number of particles, volume, and temperature (NVT) for 100 ps using a Berendsen thermostat.[4] Then re-equilibration was performed for another 100 ps under constant pressure (Isothermal-isobaric (NPT) ensemble) using the Parrinello-Rahman barostat using a time step of 2 fs for each equilibration round.[5] Finally, MD production phase was carried out for 100 ns using a time step of 2 fs at a constant temperature of 300 K and constant pressure at 1 atm. Simulation results were analyzed using Visual Molecular Dynamics (VMD) software, ver.1.9.3.[6]

[4] V. L. Golo, K. V. Shaĭtan. *Biofizika. 2002,* **47**, 611

[5] R. Kumari, R. Kumar and A. Lynn, *J. of Chem. Inf. Model.*, 2014, **54**, 1951.

[6] W. Humphrey, A. Dalke and K. *J.* Schulten*, Molecular Graphics*, 1996, **14**, 33.

Validation of the RMSD results

To further validate the RMSD results and the complex stability, the radius of gyration (R_g) was calculated for the proteins in each complex. R_g is an excellent parameter to be considered when testing the stability of the protein upon binding to the ligand, as R_g measures the compactness of the protein and describes its folding behaviour throughout the simulation.

For further verification of the complexes' stability, R_g, SASA, total complex H-bonds, and the complexes energies were assessed as displayed in figure S3. Figure S3.A shows a very stable radius of gyration for the 2 complexes with minimal fluctuation less than 0.5 Å. The nsp10-nsp16 complex and M^{pro} complexed with **3b** to exhibit R_g of value ~ 19.3 Å and 22.25 Å respectively. The SASA for the 2 complexes exhibited very low fluctuation (~ 10 nm^2), confirming their stable folding when **3b** is bounded where complex with the main protease (M^{pro}) showed better stability than that with nsp10-nsp16 complex (figure S3.B). The total H-bonds of the complexes (shown in figure S3.C) gave the same results as the complexes bound to **9e** shown in figure S4.C below. Figure S3.D confirms the previously results of the high stability of **3b** bound to M^{pro}, with stable energies of -150 kJ/mol. Furthermore, **3b** bound to nsp10-nsp16 complex showed higher unstable energy where it starts with -150 kJ/mol at the first 40 ns then fluctuates between -70 kJ/mol and 0 kJ/mol throughout the last 60 ns.

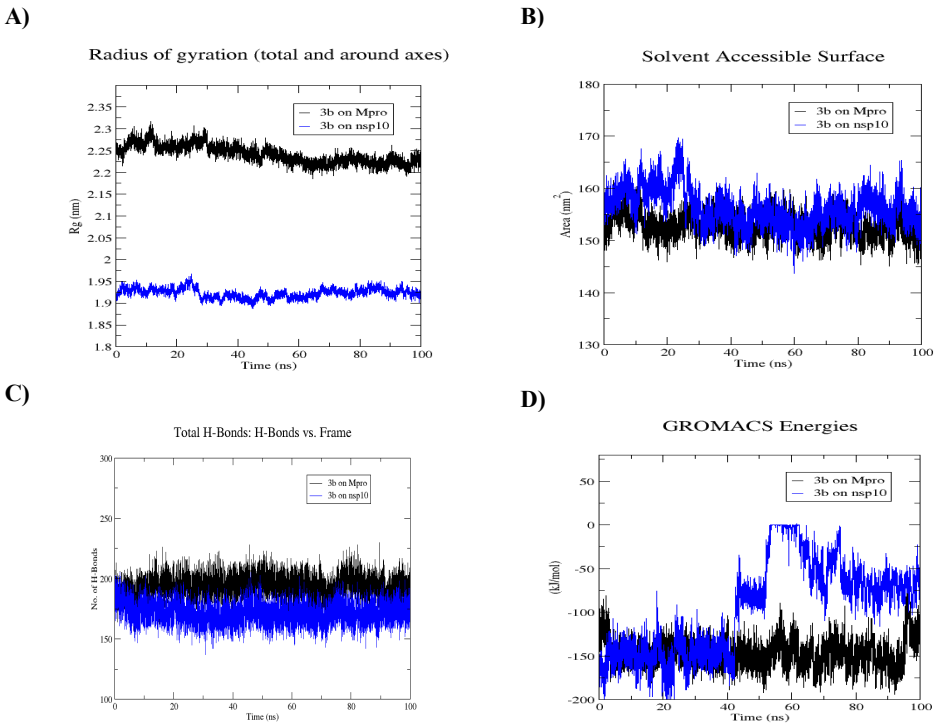


Figure S3. Structural dynamics of compound **3b** bound to M^{pro} (black curve) and nsp10-nsp16 complex (blue curve); Radius of gyration **(A),** SASA values calculated during the 100 ns of MD trajectories **(B)**, total H-bonds formed **(C)**, and Lennard-Jones energies for the 2 complexes **(D)**.

Nsp10-nsp16 complex and M^{pro} were complexed with **9e** to exhibit R_g value of ~ 19.25 Å and 22.5 Å, respectively. Figure S4.A reveals the R_g of the 2 complexes where M^{pro} and nsp10-nsp16 complex demonstrated minor alterations, indicating the most consistent compactness and the overall stability. The solvent-accessible area analysis (SASA) was also explored. SASA, in this case, can give indication of the stability of the protein folding and its conformational changes by

investigating changes throughout the 100 ns run. If the protein suffers from instability, conformational changes will occur, which will lead to changing in the solvent-accessible area. Figure S4.B shows the calculated SASA for the two proteins. Both M^{pro} and nsp10-nsp16 complex showed very good convergence between 145 and 165 nm^2 for both. Figure S4.C reveals the changes in the total intramolecular hydrogen bonds throughout the simulation for the two proteins. This parameter serves as SASA. Different rates of convergence reveal the protein's stability as the number of intramolecular hydrogen bonds change and alterations appear due to conformational changes. It is important to note that the SARS-CoV-2 main protease (M^{pro}) showing stable protein structure does not contradict our previous conclusion of it exhibiting poor complex stability and binding interactions. This is attributed to the fact that both the protein and the ligand converged and showed stability once they broke apart, that is why GROMACS energies were calculated for the two complexes (short-range Lennard-Jones energy). As noticed in figure S3.D, M^{pro} exhibited significant instability when complexed with **9e** unlike nsp10-nsp16 complex, which was noticeably more stable, -80 kJ/mol in agreement with the previous analysis.

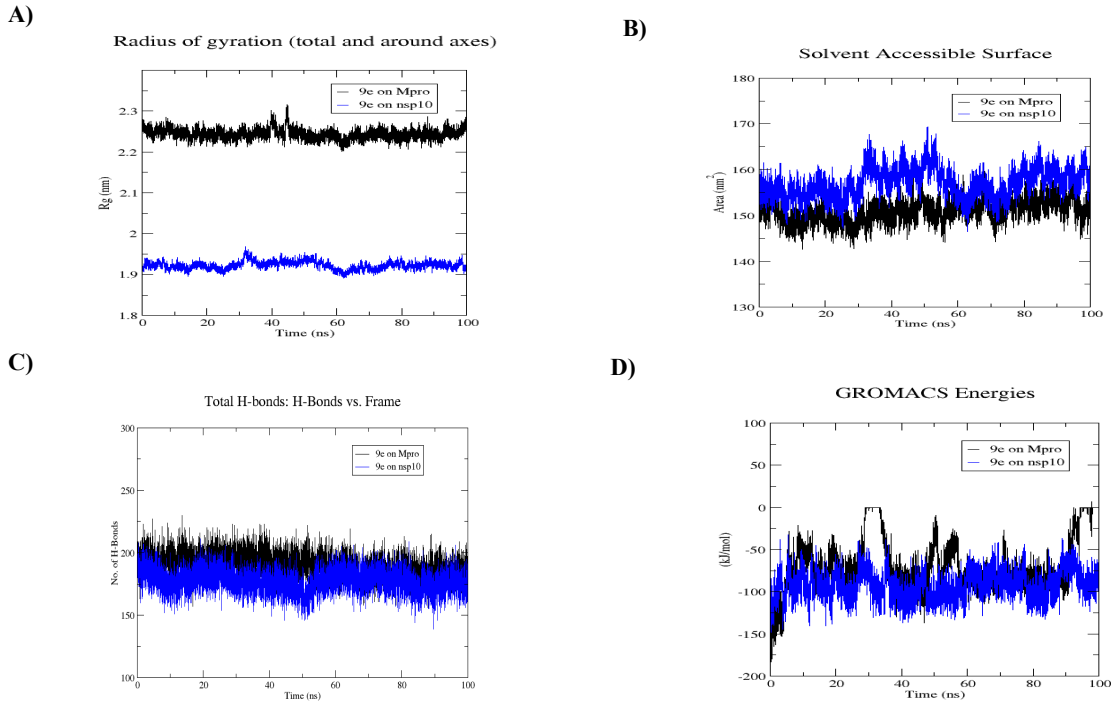


Figure S4. Structural dynamics of compound **9e** bound to M^{pro} (black curve) and nsp10-nsp16 complex (blue curve); Radius of gyration **(A)**, SASA values calculated during the 100 ns of MD trajectories **(B)**, total H-bonds formed **(C),** and Lennard-Jones energies for the two complexes **(D).**

Molecular Dynamics Simulation for Compounds 3b and 9e

Stable binding of **3b** and **9e** should reflect on the stability of the residues within the binding pocket. The rigidity and flexibility of such residues can be investigated through the root mean square fluctuation (RMSF) of the Cα atoms during the simulation. For M^{pro}, figure S5 reveals the RMSF of the residues in proximity of 5 Å from **3b** and **9e**; residues (24:27, 41:42, 49, 140:145, 163:168, 172, 185:186, 188:192); especially Cys145 and His41 that constitute the catalytic dyad. The RMSF of **3b** and M^{pro} complex showed low fluctuations. **9e** exhibited fluctuations (~ 1.3-3.2 Å) in some residues, with higher fluctuations at residues (185:192) than **3b**. In parallel, nsp10-nsp16 complex/nsp16 complex residues (6841, 6844, 6845, 6867:6874, 6911:6714, 6916, 6928:6934,

6945, 6968) are within 5 Å proximity from **3b** and **9e** and they exhibited lower fluctuations (~ 0.4-1.4 Å). It thus can be concluded that **3b** forms more stable complexes with the two investigated targets.

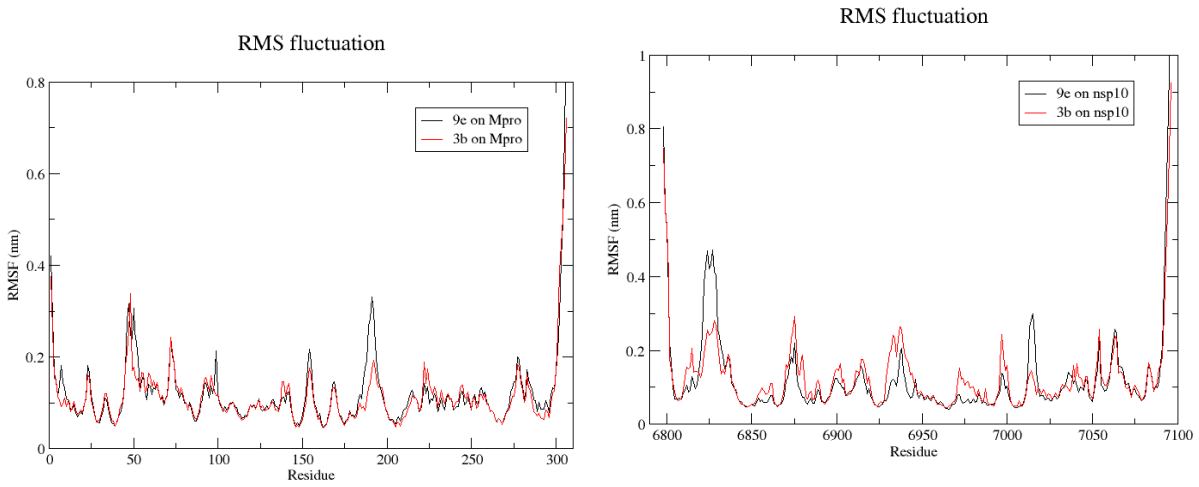


Figure S5. Root Mean Square fluctuation (RMSF) of compounds **9e** (black line) and **3b** (red line) complexed with M^{pro} (left) and nsp10-nsp16 complex/nsp16 complex (right).

Nsp10-nsp16 complex/nsp16 complex residues (6841, 6844, 6845, 6867:6874, 6911:6714, 6916, 6928:6934, 6945, 6968) are within 5 Å proximity from **3b** and **9e** and they exhibited lower fluctuations (~ 0.4-1.4 Å) (Figure **S5** right).

Post MD analysis, Trajectory post-processing

After obtaining the trajectories of the 2 complexes resulting from the MD simulation, the complexes were re-centered and re-wrapped within the unit cells using the trjconv function of GROMACS. Then to check the trajectories stability throughout the 100 ns simulation the radius of gyration and the root-mean-square deviation (RMSD) of the protein backbone referenced to its initial position were generated with 10 ps intervals. In addition, solvent accessible area analysis was carried out along with root mean square fluctuations. The total hydrogen bonds were investigated as a representation for stability. Besides, intermolecular hydrogen bonds between the protein and the ligand was investigated to have a general view about the interaction stability. GROMACS energies were calculated for the two complexes (short-range Lennard-Jones energy) to show an overview of the binding energetics throughout the 100 ns run.

Hydrogen bond analysis was performed to get closer insights into the binding affinity and interactions. Intermolecular hydrogen bonds between the M^{pro} and nsp10-nsp16 complex/nsp16 complex and the two hits **3b** and **9e** were investigated using VMD 1.9.4a51. The distance cut-off for hydrogen bond calculations was set at 3.5 Å and the angle cut-off at 30°. Compound **3b** maintained 2-3 hydrogen bonds with M^{pro} in the first 40 ns, then, it maintained 1-2 hydrogen bonds throughout the rest of the run. The hydrogen bonds were alternating between Gln189, Gln192, Thr190, and Glu166 (figure S6.A). On contrary, **3b** made only 1-2 hydrogen bonds with nsp10-nsp16 complex/nsp16 complex. These bonds were not maintained throughout the whole 100 ns simulation (figure S6.A). For **9e**, it barely maintained one hydrogen bond with M^{pro} protease (figure S6.C). This is consistent with the predicted poor stability of the complex. On contrary, **9e** strongly maintained 3-4 hydrogen bonds with nsp10-nsp16 complex/nsp16 complex (figure S6.D).

Two of these bonds are firmly maintained with Asp6897 and Asp6873, and 1-2 additional bonds were alternating with other residues throughout the simulation. This favours the conclusion that **9e** binds to nsp10-nsp16 complex/nsp16 complex with a higher affinity.

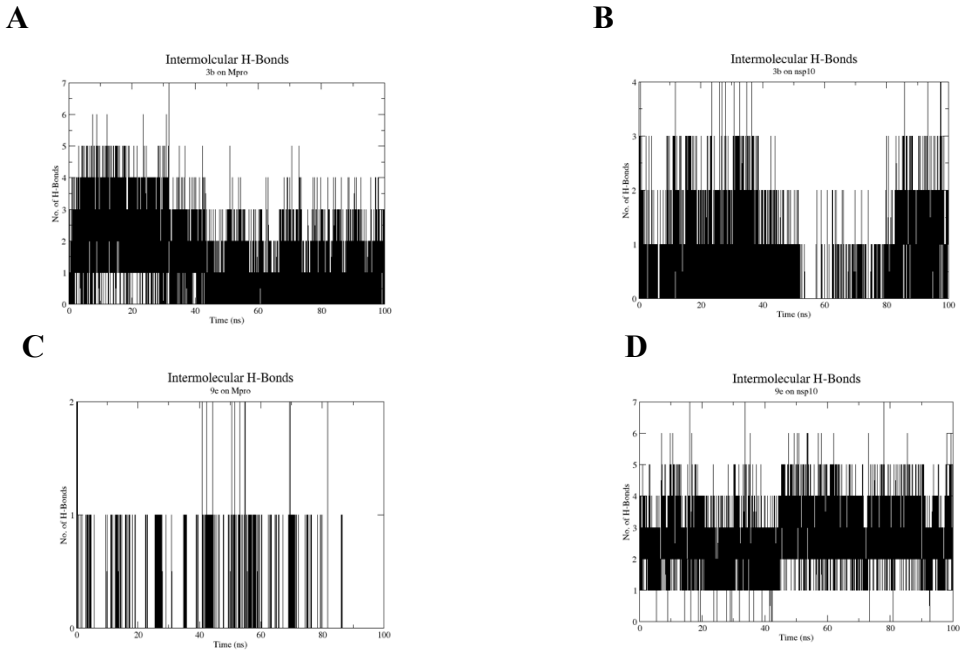


Figure S6. Intermolecular H-bonds of compound **3b** bound to main protease M^{pro} (A) and nsp10-nsp16 complex/nsp16 complex **(B)** and compound **9e** to main protease M^{pro} **(C)** and nsp10-nsp16 complex/nsp16 complex **(D).**

In-silico toxicity prediction

In-silico toxicity profile was predicted for **3b** and **9e** as they demonstrated the best antiviral IC_{50} values in addition to their good binding affinities towards the 4 target SARS-CoV-2 proteins. pkCSM online web tool (http://biosig.unimelb.edu.au/pkcsm/prediction) was used and the results are shown in Table S3. Both compounds showed no Ames toxicity, no hERG I potential inhibition and no skin sensitization. They also exhibited good human maximum tolerated doses. Regarding the oral rat acute toxicity (LD_{50}) they exhibited good results; 2.802 and 3.373 mol/kg, respectively. Similarly, both derivatives had good Oral Rat Chronic Toxicity 1.498 and 1.813 mg/kg_bw/day, respectively.[3]

Table S3: Toxicity prediction results for **3b** and **9e**.

Compound #	AMES toxicity	Max. tolerated dose (human) (log mg/kg/ day)	hERG I inhibitor	Oral Rat Acute Toxicity (LD_{50}) (mol/kg)	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	Skin Sensitization
3b	No	-0.624	No	3.373	1.498	No
9e	No	-0.409	No	2.802	1.813	No

Nsp10-nsp16 complex and nsp14 methyltransferase activity assays

SARS-CoV-2 nsp10-nsp16 complex activity assay was performed as previously described [7] with some modifications. Briefly, compounds were titrated in 100% DMSO from 8 μM to 2 mM before directly added to reaction mixture with 5% final concentration of DMSO. Reaction mixtures were prepared in 50 mM Tris-HCl (pH 7.5), 1.5 mM $MgCl_2$, 1 mM TCEP, 0.01% Triton X-100. Compounds were pre-incubated with 125 nM SARS-CoV-2 nsp10-nsp16 complex (8:1 molar ratio) for 2 hours at 23°C before the addition of 0.8 μM RNA substrate and 2 μM SAM (30% ^{3}H-SAM). Reaction mixtures (10 μL) were incubated for 30 minutes at 23°C, quenched, and the radioactivity signal was read.

SARS-Cov-2 nsp14 activity assay experiments were performed as previously described [8] with some modifications. Briefly, compounds were titrated in 100% DMSO from 8 μM to 2 mM before directly added to reaction with 5% final concentration of DMSO. Reaction mixtures were prepared in 20 mM Tris-HCl (pH 7.5), 0.25 mM $MgCl_2$, 1 mM TCEP, 0.01% Triton X-100. Compounds were pre-incubated with 1.5 nM SARS-Cov-2 nsp14 for 2 hours at 23°C before the addition of 50 nM RNA substrate and 0.25 μM ^{3}H-SAM. Reaction mixtures (30 μL) were incubated for 20 minutes at 23 C, quenched, and the radioactivity signal was read as previously described.

[7] Khalili Yazdi A, Li F, Devkota K, Perveen S, Ghiabi P, Hajian T, Bolotokova A, Vedadi M. A High-Throughput Radioactivity-Based Assay for Screening SARS-CoV-2 nsp10-nsp16 Complex. *SLAS Discov.* 2021 Jul;26(6):757-765. doi: 10.1177/24725552211008863.

[8] Devkota K, Schapira M, Perveen S, Khalili Yazdi A, Li F, Chau I, Ghiabi P, Hajian T, Loppnau P, Bolotokova A, Satchell KJF, Wang K, Li D, Liu J, Smil D, Luo M, Jin J, Fish PV, Brown PJ, Vedadi M. Probing the SAM Binding Site of SARS-CoV-2 Nsp14 In Vitro Using SAM Competitive Inhibitors Guides Developing Selective Bisubstrate Inhibitors. *SLAS Discov*. 2021 Oct;26(9):1200-1211. doi: 10.1177/24725552211026261.

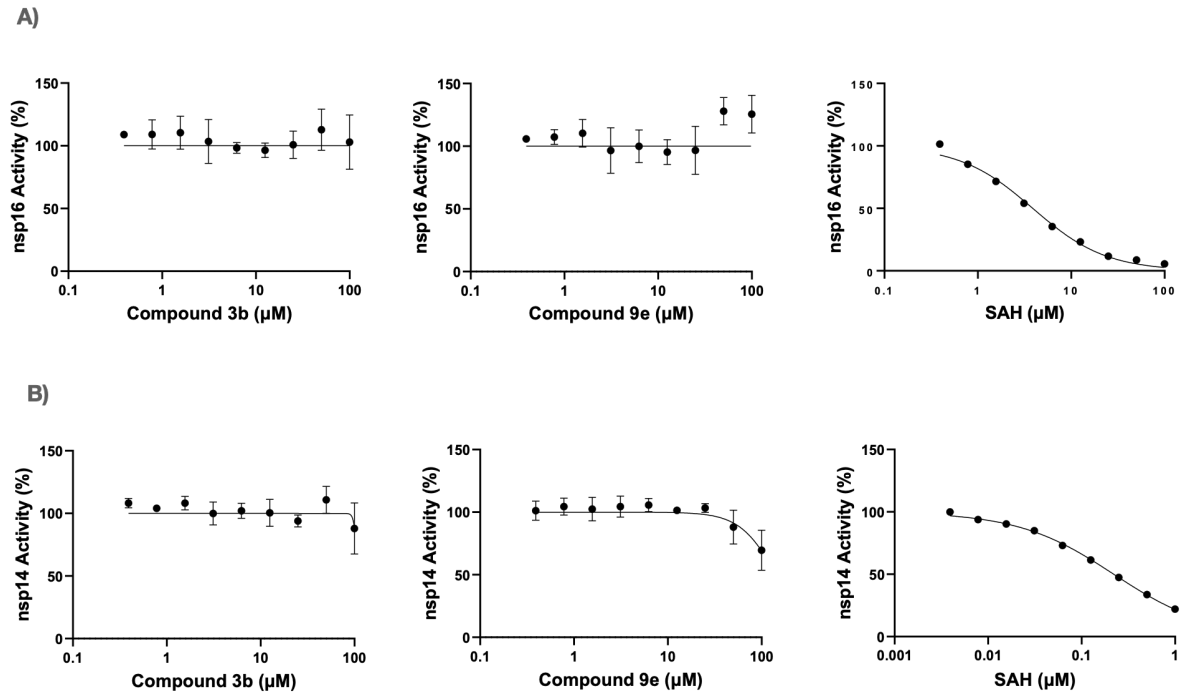


Figure S7. Effect of **3b** and **9e** on methyltransferase activities of nsp10-nsp16 complex and nsp14. Compounds were tested in triplicate against nsp10-nsp16 complex **(A)** and nsp14 **(B)**. SAH was used as a positive control. The bottom and top of the plots were constrained to 0 and 100, respectively.

Viral protease activity cellular assays

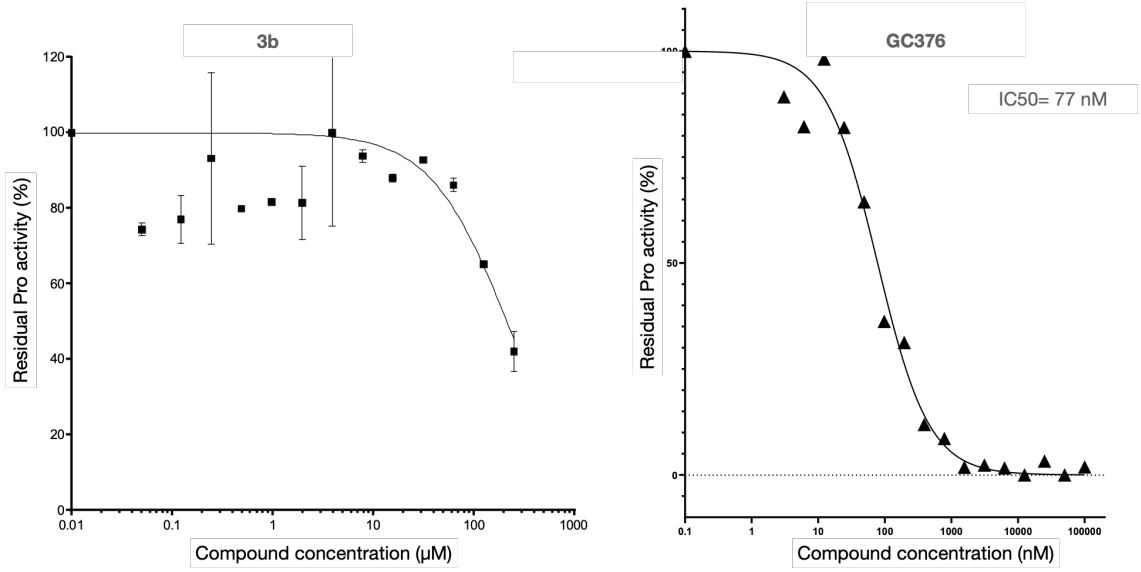
The viral protease activity assay was performed as described.[9] Briefly, ~10^7 HEK-293T cells were transfected in 50 cm2 dishes with 1 µg of the Rev-Nluc-CoV reporter plasmid and 15 µg of nsp4-5-6 expression plasmids encoding the wild-type nsp5 or the catalytically inactive C145A mutant nsp5 protein. Increasing amounts of the tested compounds, corresponding to final concentrations of 0.1 to 50 µM with a constant DMSO concentration of 0.5% were distributed in 384-well white opaque plates using an Echo 555 Liquid Handler (Labcyte). Transfected HEK-293T cells were trypsinized at 6 hours post-transfection (hpt) and distributed in the 384-well plates ($2x10^4$ cells per well in 50 µL). The Nanoluciferase activity was measured at 24 hpt Each 384-well plate included 14 negative control wells (mean DMSO-treated signal = DMSO) and 14 positive-control wells (mean GC376 at 50 µM signal = GC). The compound luciferase signals (compound-treated signal = C) measured in nsp4-5-6 WT expressing cells were normalized as follows: (C - DMSO) / (GC – DMSO) x 100. The luciferase signals measured in nsp4-5-6 C145A expressing cells were normalized as follow: (C / DMSO) x 100.

M^{pro} protein purification and *in vitro* activity assay

[9] D. E. V. Pires, T. L. Blundell and D. B. Ascher, *J. Med. Chem., 2015*, **58**, 4066.

The M^{pro} protein was produced in *E. coli* as previously described.[10] Briefly, the pGEX-6P-1 plasmid was transformed in E coli BL21 DE3 gold, and the nsp5 protein was produced overnight at 17 °C upon IPTG induction (250 µM). Bacterial pellets were resuspended in lysis buffer (50 mM Tris pH 8, 300 mM NaCl, 5 mM MgSO4, 10% Glycerol, 0.1%Triton X-100, supplemented with 0.25 mg/mL Lysosyme, 1 mM PMSF, and 10 µg/mL DNAse I). After 3 cycles of sonication and clarification, the 6xHis-tagged protein was purified by NTA affinity on cobalt beads, and the nsp5 protein was eluted in lysis buffer supplemented with 250 mM imidazole. The nsp5 protein was then concentrated on Vivaspin 20 centrifugal concentrators 10kDa MWCO (GE Healthcare #VS2001), dialysed against the elution buffer in the absence of imidazole, and stored at −80 °C upon addition of 50% glycerol.

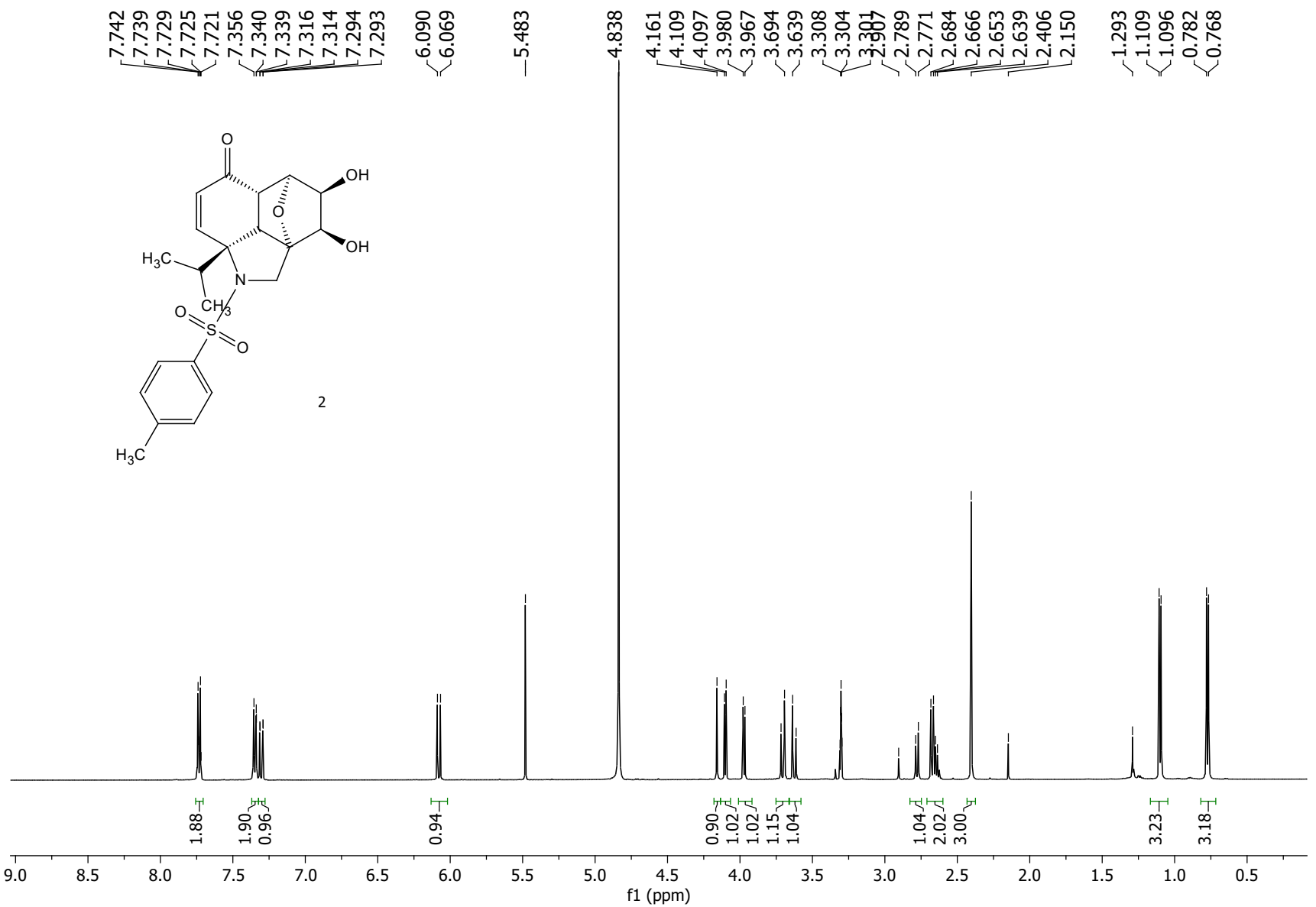
The fluorescence resonance energy transfer (FRET)-based *in vitro* activity assays were performed in black 384-well HiBase non-binding plates (Greiner Bio One #784900) as described in [11]. Briefly, serial dilution of the inhibitors were incubated with purified nsp5 protein (80 nM) and 5 µM of a florescent synthetic peptide (Dabcyl-KTSAVLQ↓SGFRKM-Edans-NH2, purchased from Genscript) in HEPES buffer (20 mM, pH 6.5) containing 120 mM NaCl, 0.4 mM EDTA, 4 mM DTT and 10% glycerol. The DMSO final concentration was adjusted at 0.5%. Cleavage of the synthetic peptide by nsp5 separates the Edans/Dabcyl fluorophore-quencher pair. The time courses of the enzymatic reaction were followed during 40 mn by monitoring the increase of fluorescence emission at 493 nM (excitation 335 nM) using a Tecan Saphir 2 fluorimeter during 40 mn. The enzymatic activities were estimated by taking the slope of the linear part of the reaction curve, and were normalized with respect to the activity measured in the absence of inhibitor. The IC_{50} values were determined by plotting the % of activity as a function of the inhibitor concentration and by fitting the curves with Prism (GraphPad) using the following equation: Y = 100/(1+((X/IC50)^Hill slope).



[10] Zhang L., Lin D., Sun X., Curth U., Drosten C., Sauerhering L., Becker S., Rox K., Hilgenfeld R. Science. 2020;368:409–412.

[11] K. Y. Chen, T. Krischuns, L. O. Varga, E. Harigua-Souiai, S. Paisant, A. Zettor, J. Chiaravalli, A. Delpal, D. Courtney, A. O'Brien, S. C. Baker, E. Decroly, C. Isel, F. Agou, Y. Jacob, A. Blondel and N. Naffakh, *Antiviral Research*, 2022, **201**, 105272.

Figure S8. Activity of compound **3b** (left) on the enzymatic activity of SARS-CoV2 Mpro (50nM) in 20 mM HEPES, 120 mM NaCl, 0.4 mM EDTA, and 4 mM DTT, pH 6.5, on substrate Dabcyl-KTSAVLQ↓SGFRKM-(Edans)-NH_2 compared to reference compound GC376 (right).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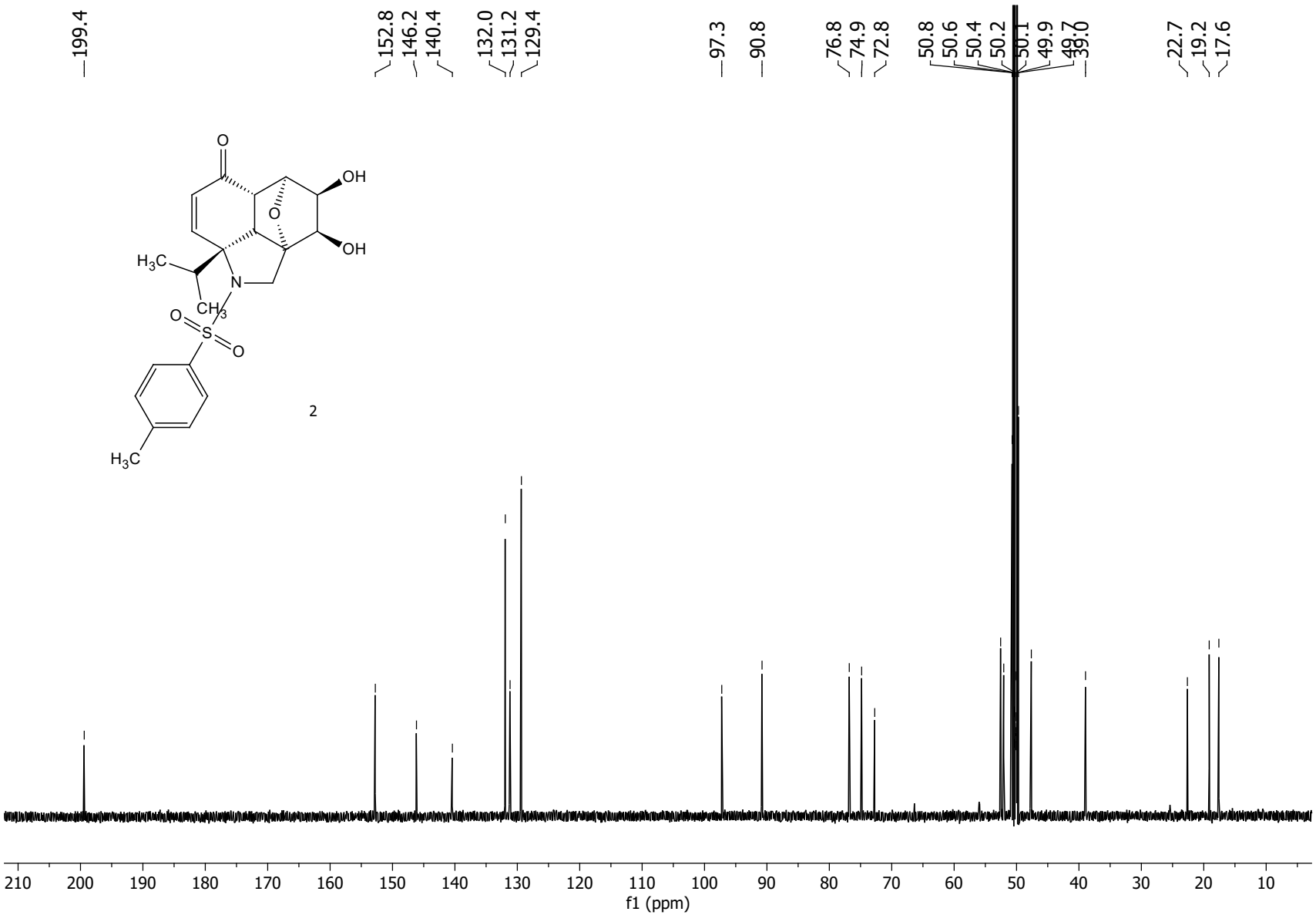
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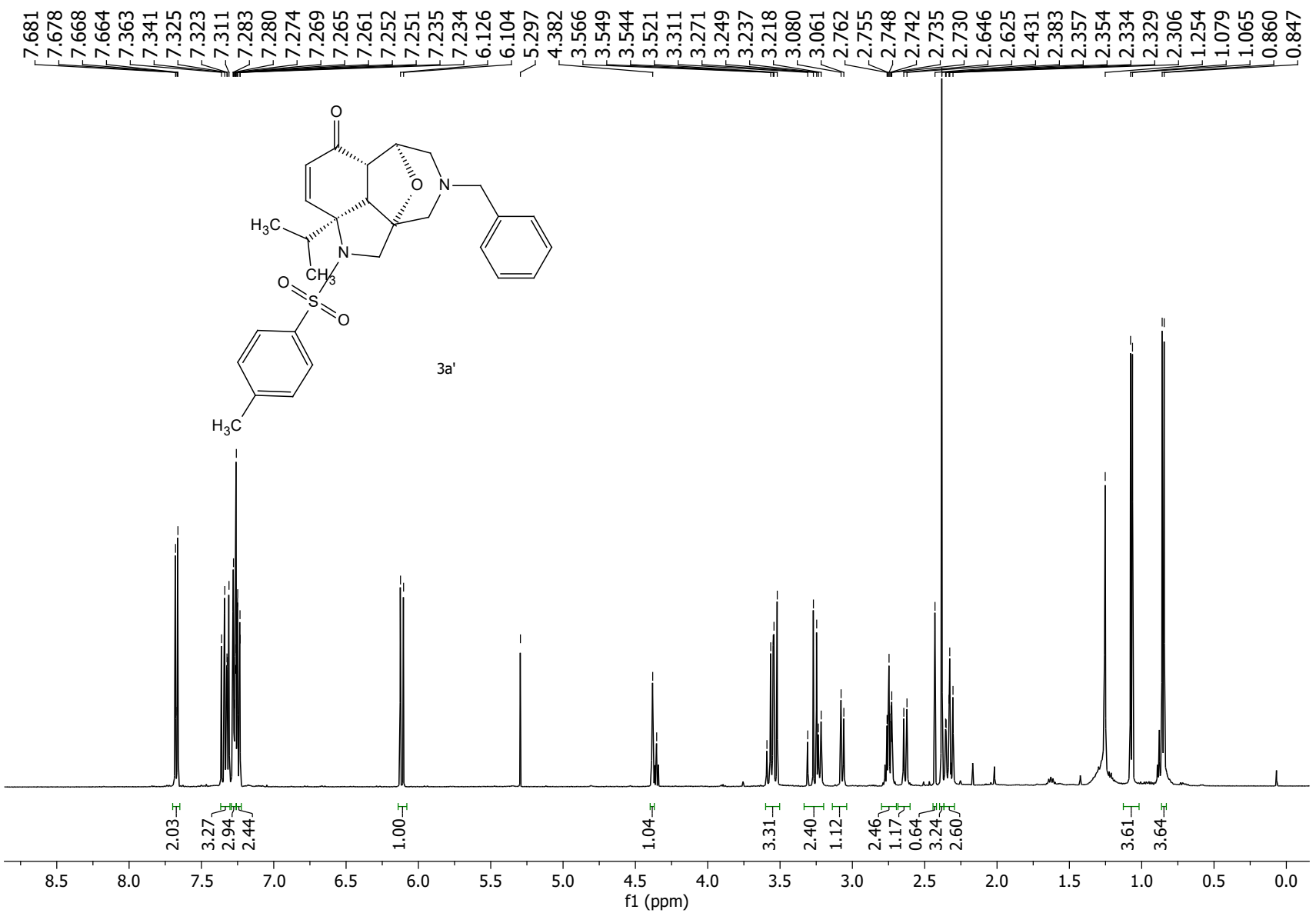
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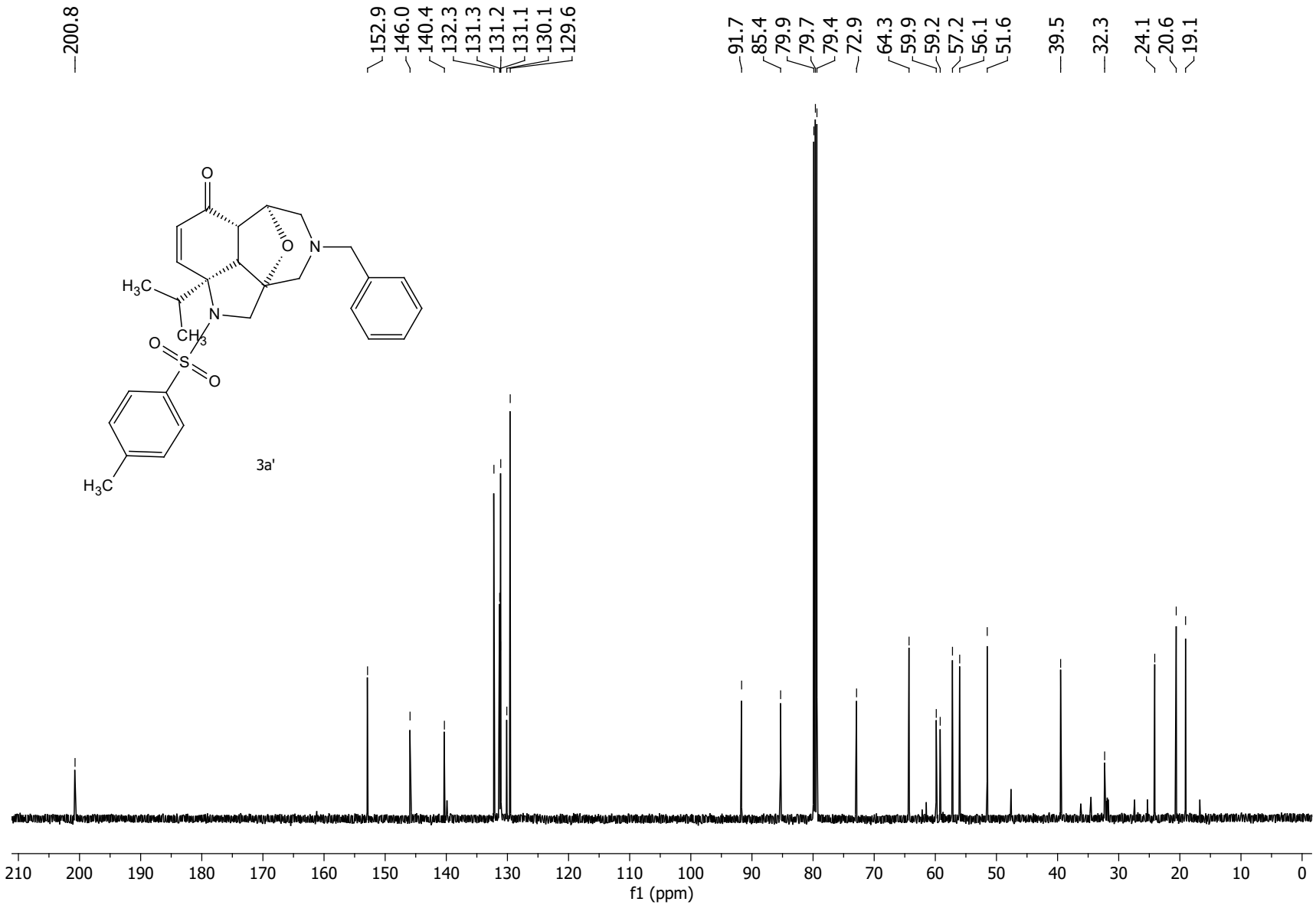
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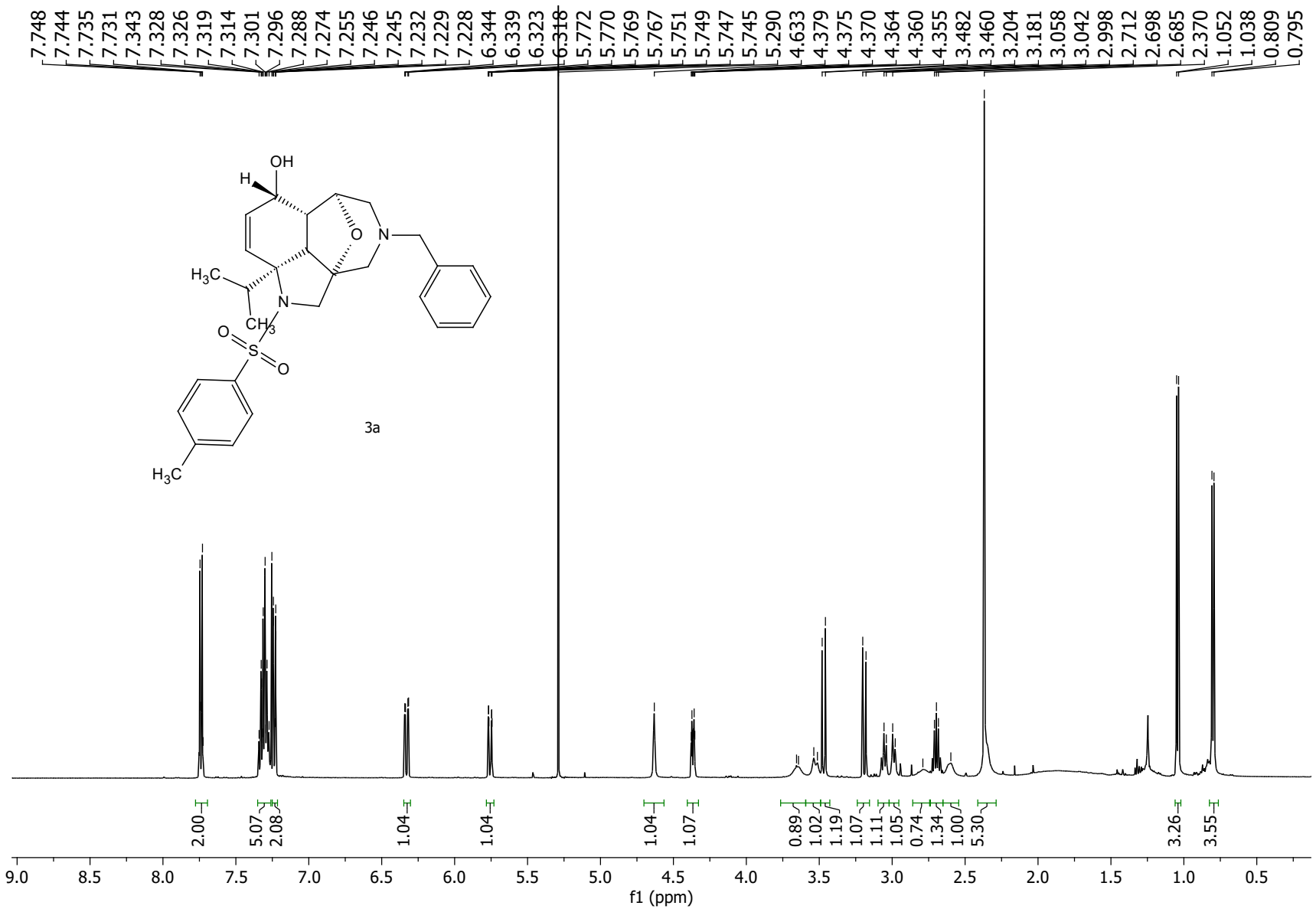
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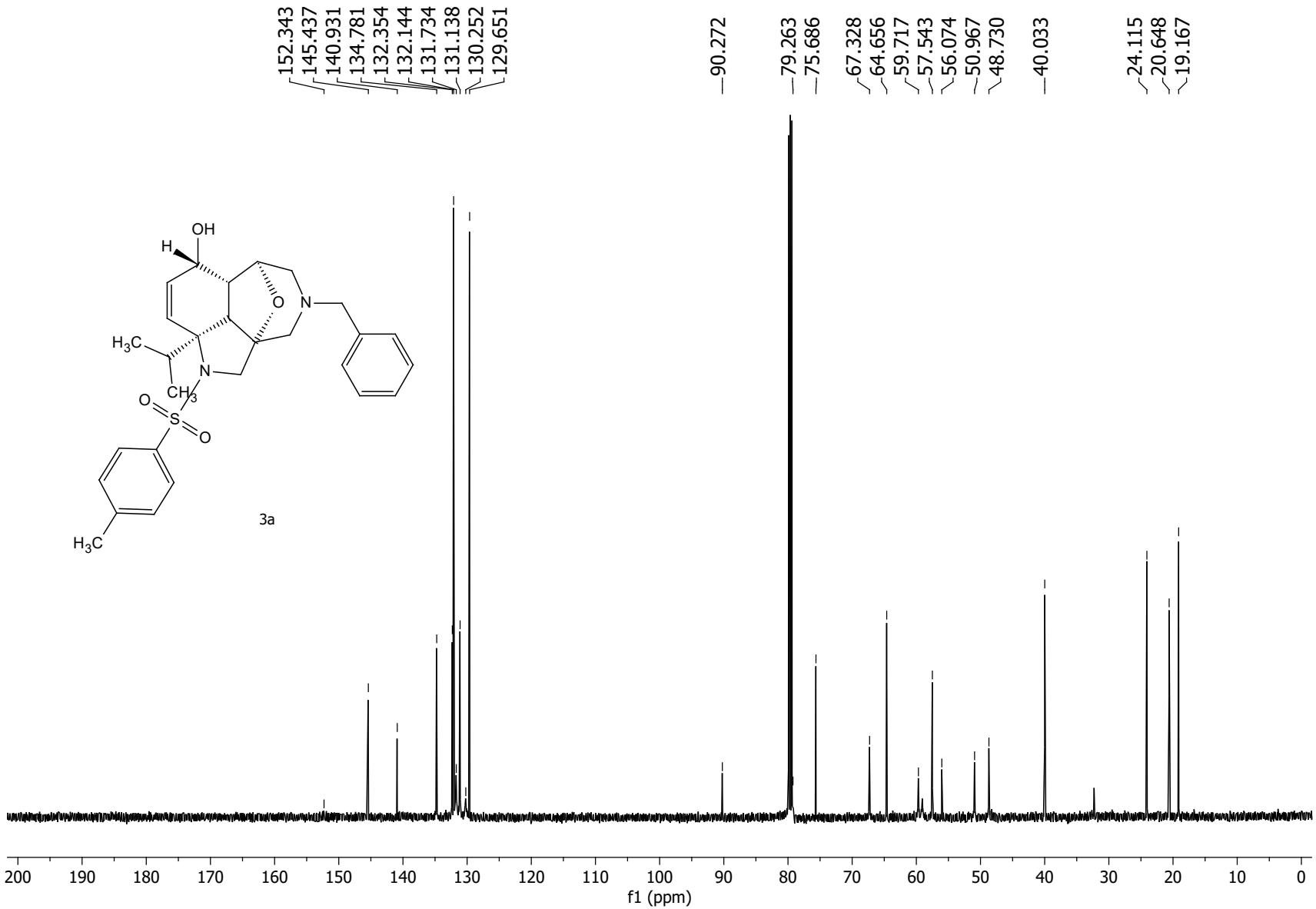
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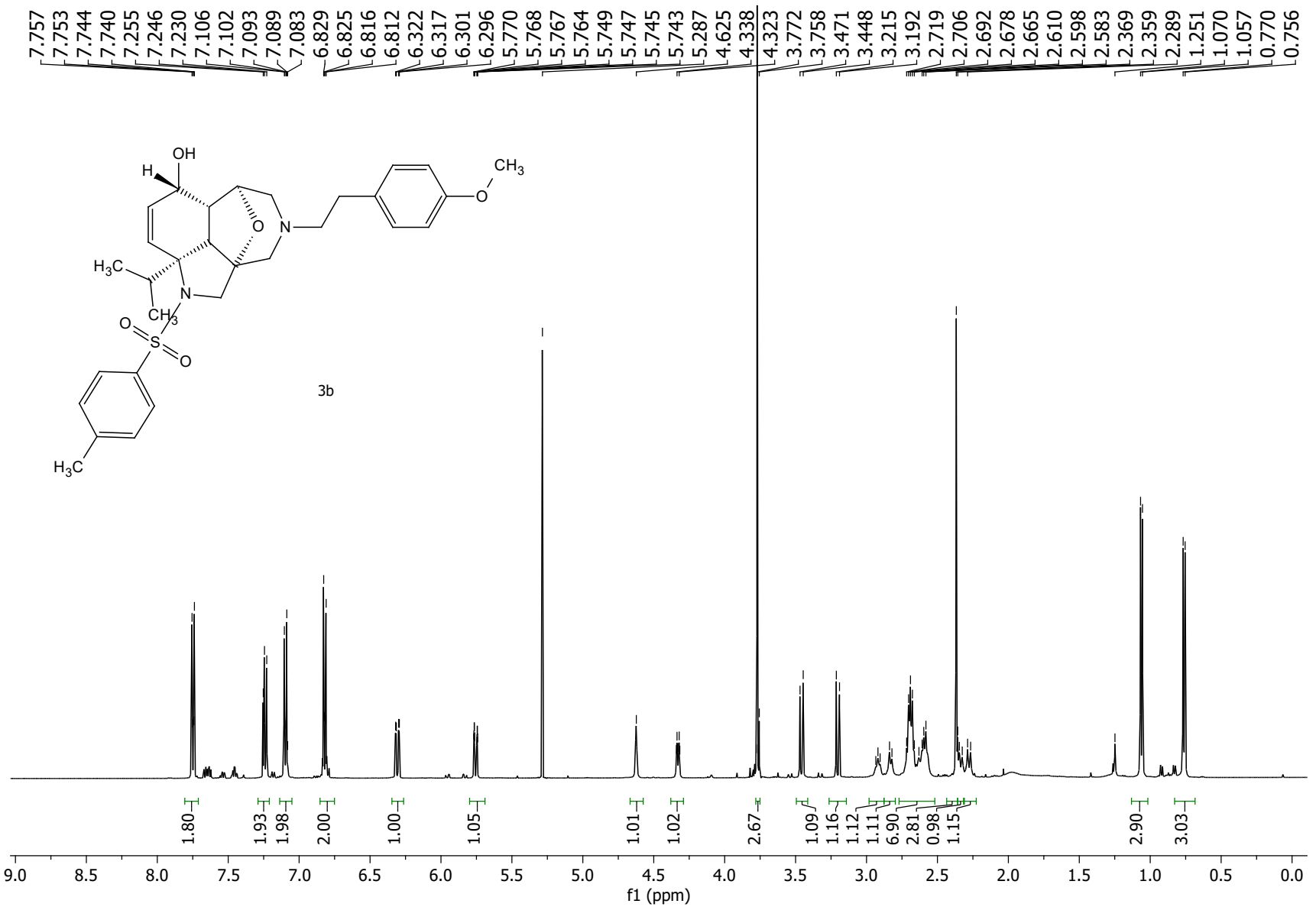
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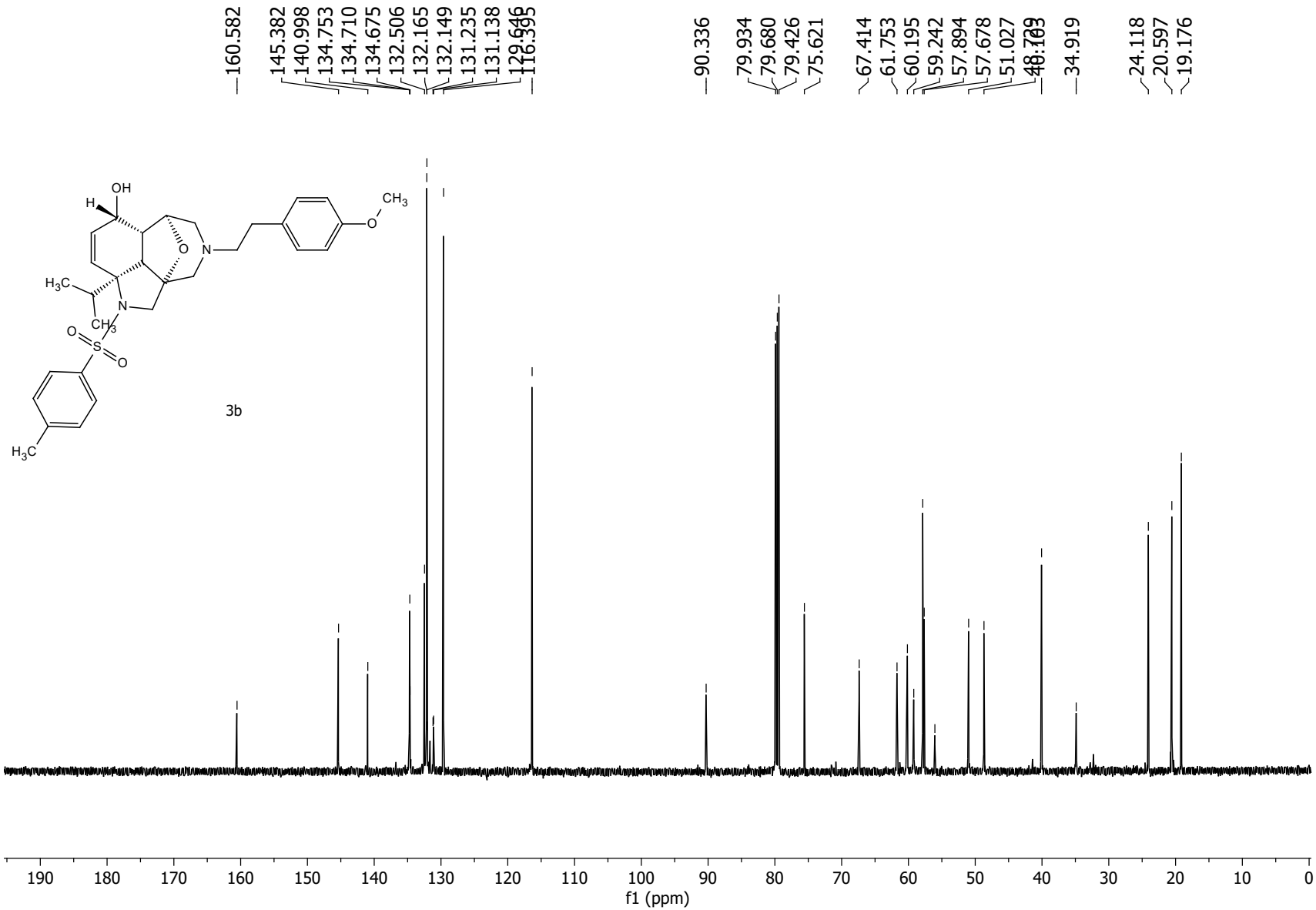
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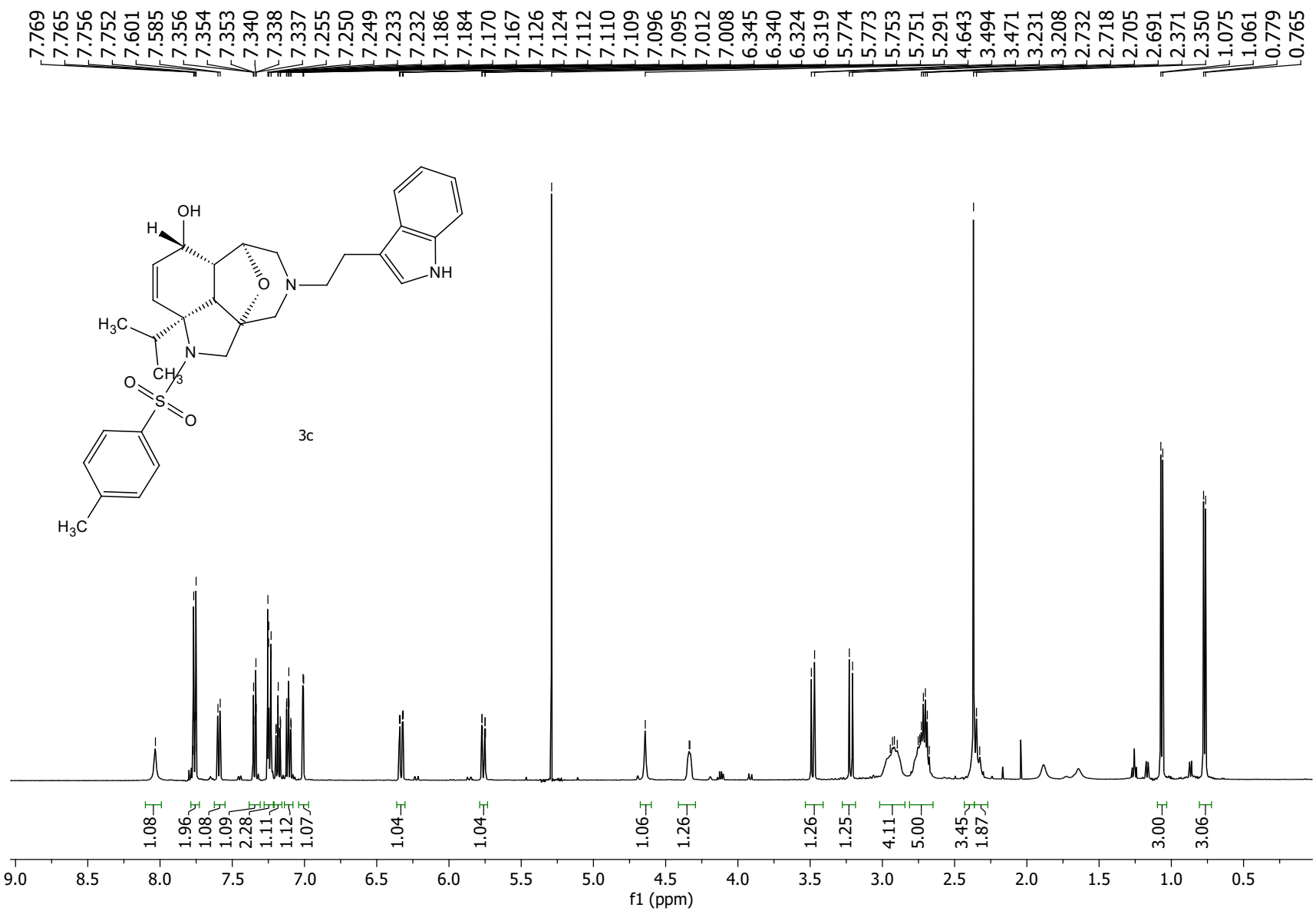
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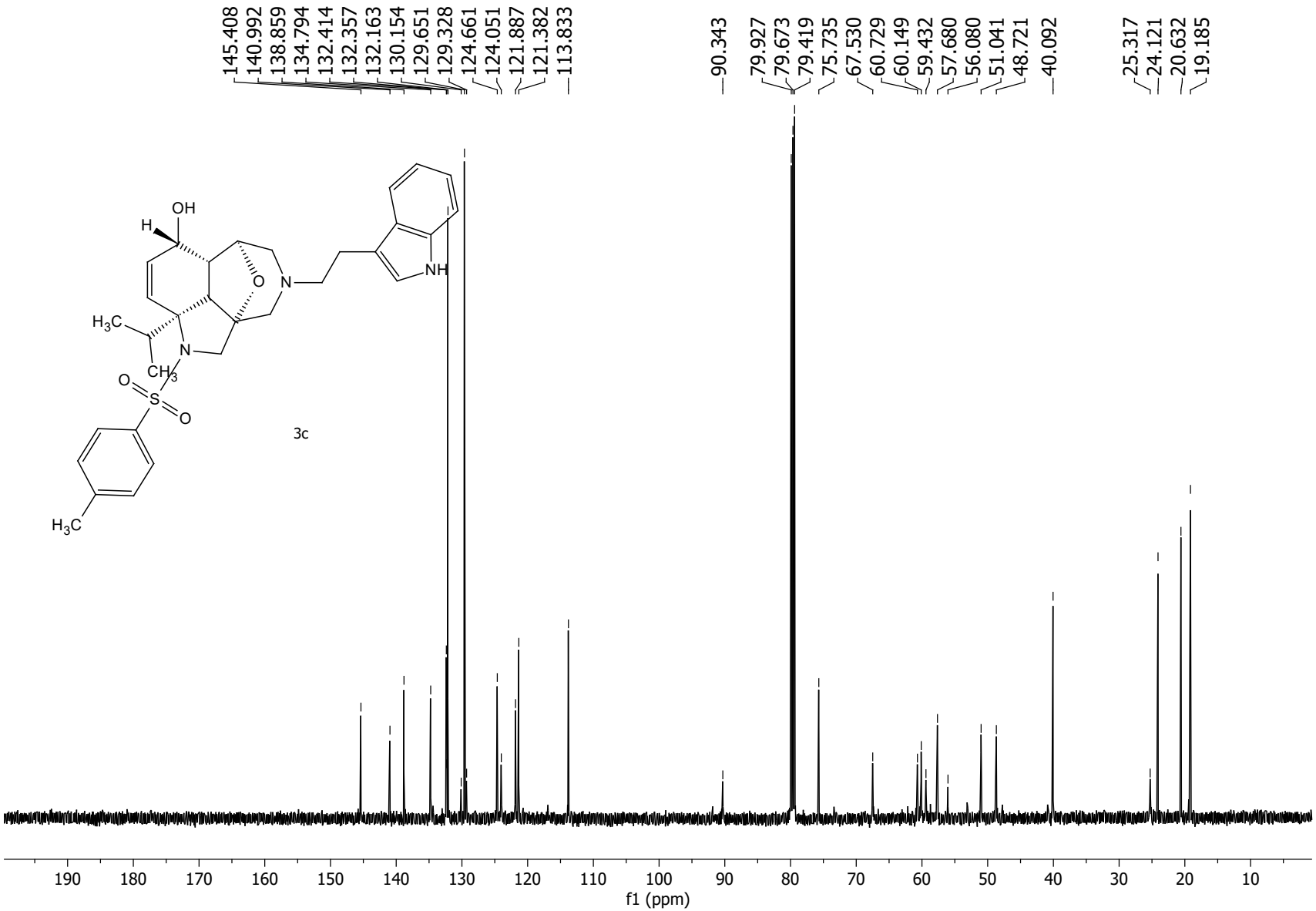
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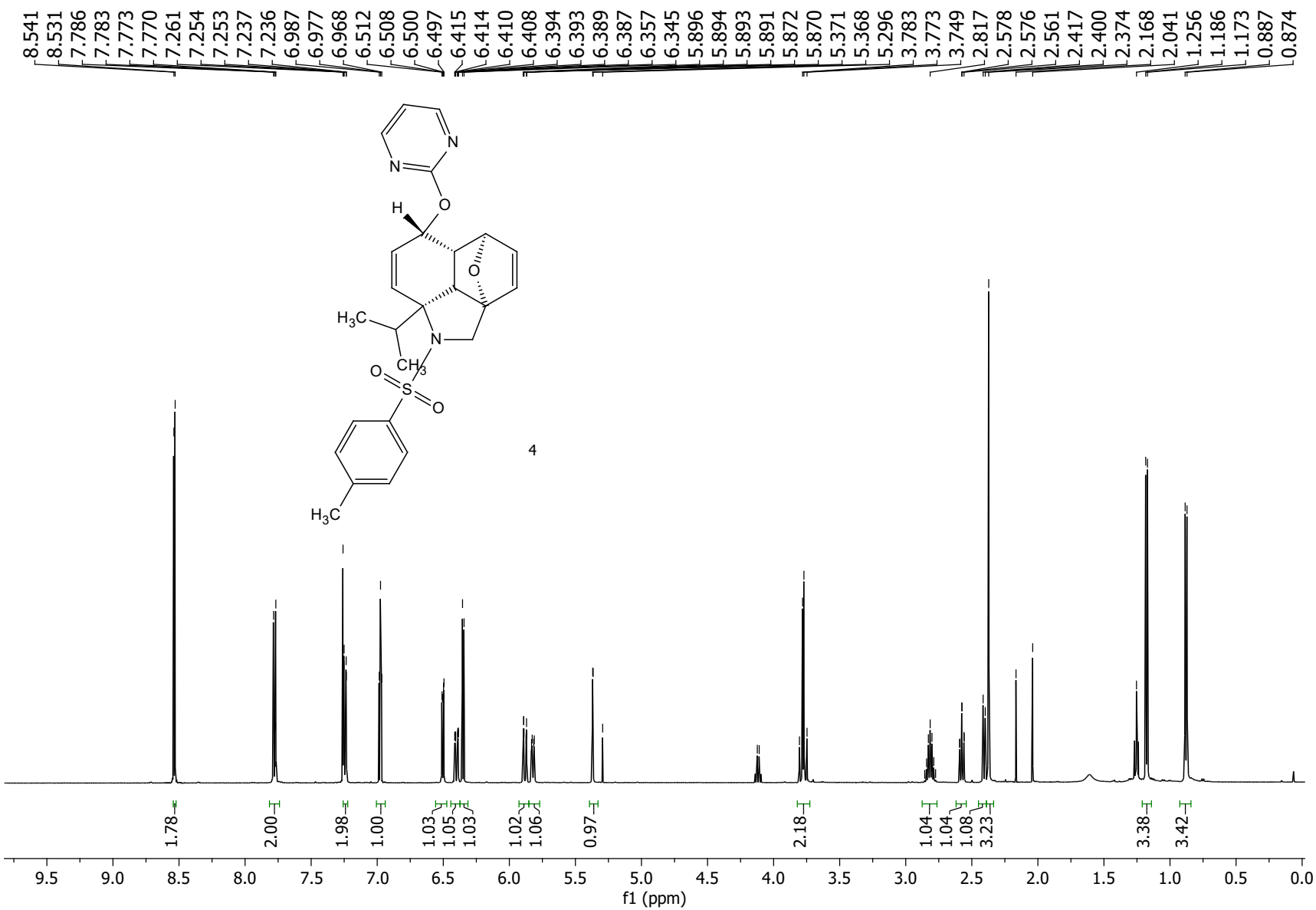
20
10
f1 (ppm)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7.253
7.237
7.236
6.987
6.977
6.968
6.512
6.508
6.500
6.497
6.415
6.414
6.410
6.408
6.394
6.393
6.389
6.387
6.357
6.345
5.896
5.894
5.893
5.891
5.872
5.870
5.371
5.368
5.296
3.783
3.773
3.749
2.817
2.578
2.576
2.561
2.417
2.400
2.374
2.168
2.041
1.256
1.186
1.173
0.887
0.874
4
1.78
2.00
1.98
1.00
1.03
1.05
1.03
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1.04
1.04
1.08
3.23
3.38
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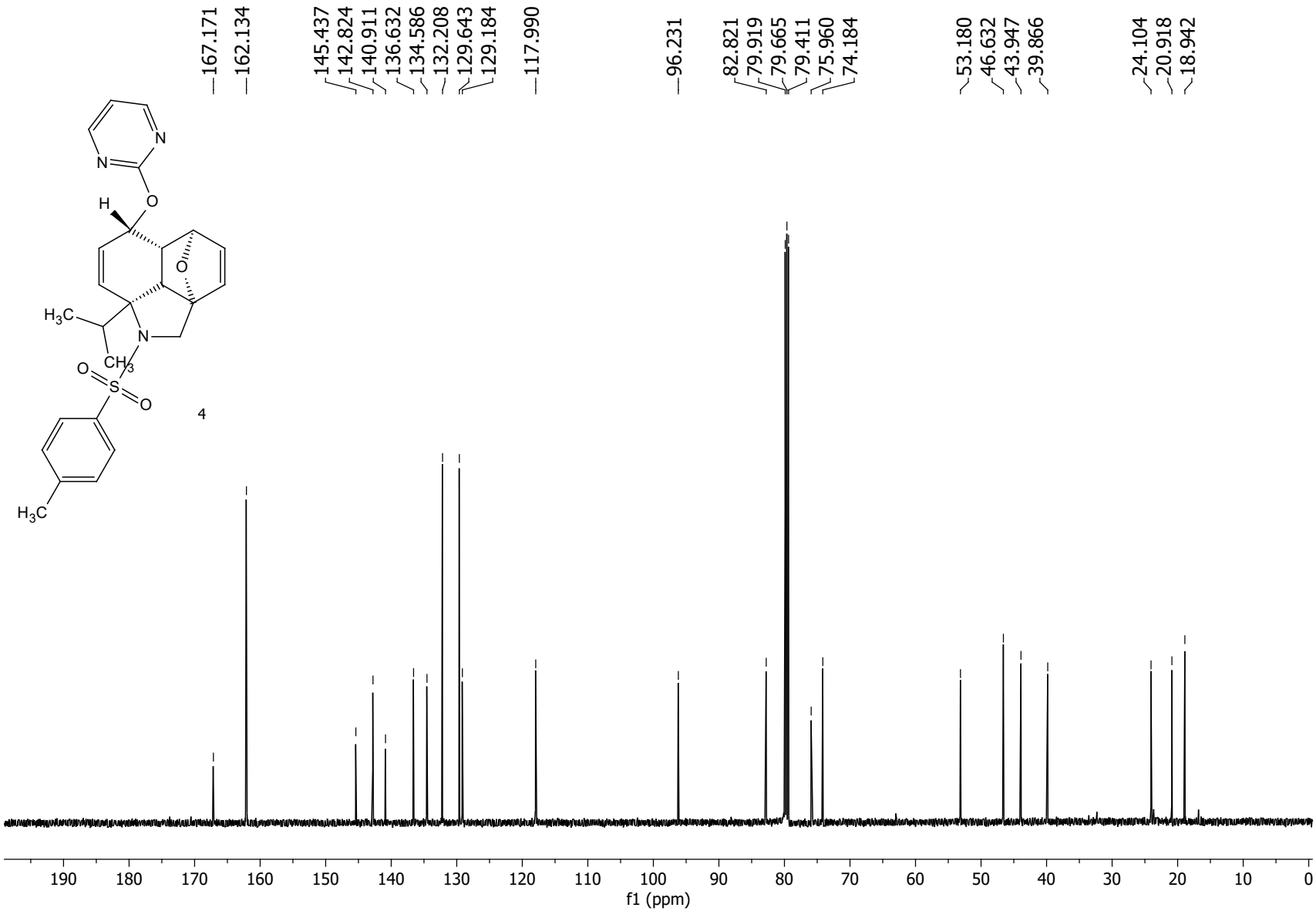
f1 (ppm)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134.586
132.208
129.643
129.184
117.990
96.231
82.821
79.919
79.665
79.411
75.960
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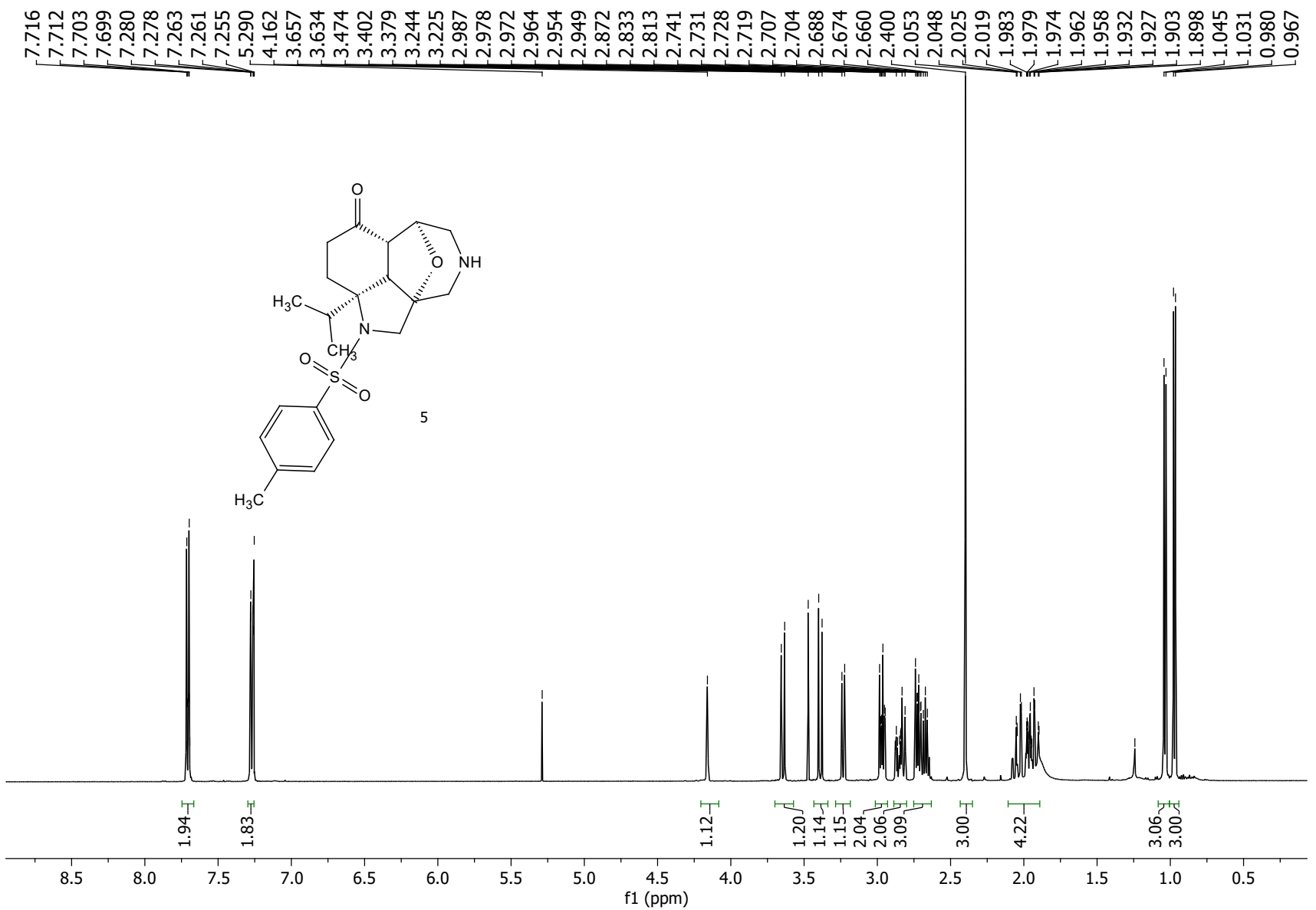
53.180
46.632
43.947
39.866
24.104
20.918
18.942
4
f1 (ppm)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7.263
7.261
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3.634
3.474
3.402
3.379
3.244
3.225
2.987
2.978
2.972
2.964
2.954
2.949
2.872
2.833
2.813
2.741
2.731
2.728
2.719
2.707
2.704
2.688
2.674
2.660
2.400
2.053
2.048
2.025
2.019
1.983
1.979
1.974
1.962
1.958
1.932
1.927
1.903
1.898
1.045
1.031
0.980
0.967
O
O
NH
H3C
N
CH3
O
S
O
H3C
5
1.94
1.83
1.12
1.20
1.14
1.15
2.04
2.06
3.09
3.00
4.22
3.06
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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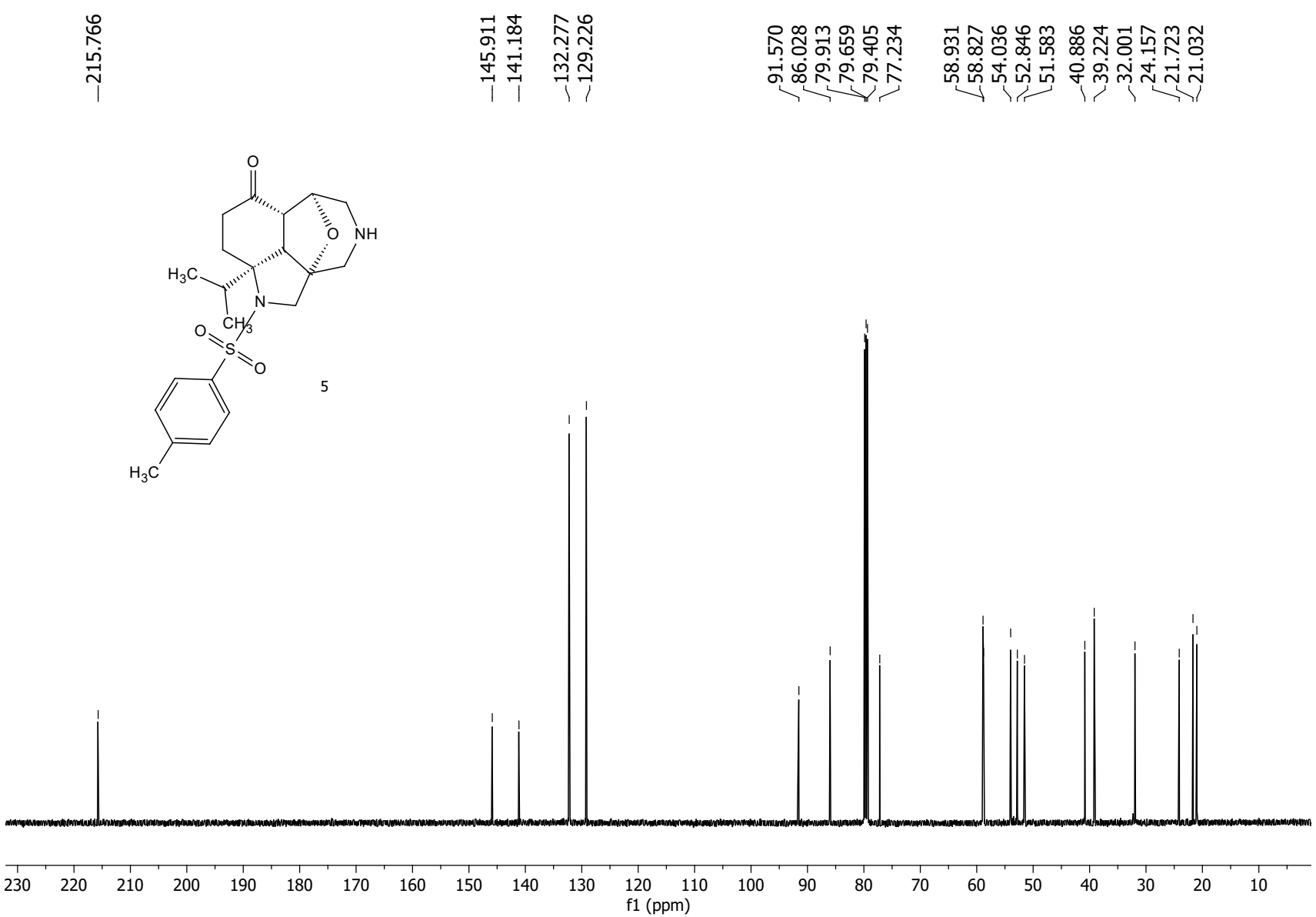
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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79.913
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77.234
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58.827
54.036
52.846
51.583
40.886
39.224
32.001
24.157
21.723
21.032
O
O
NH
H3C
N
CH3
O
S
O
H3C
5
230
220
210
200
190
180
170
160
150
140
130
120
110
100
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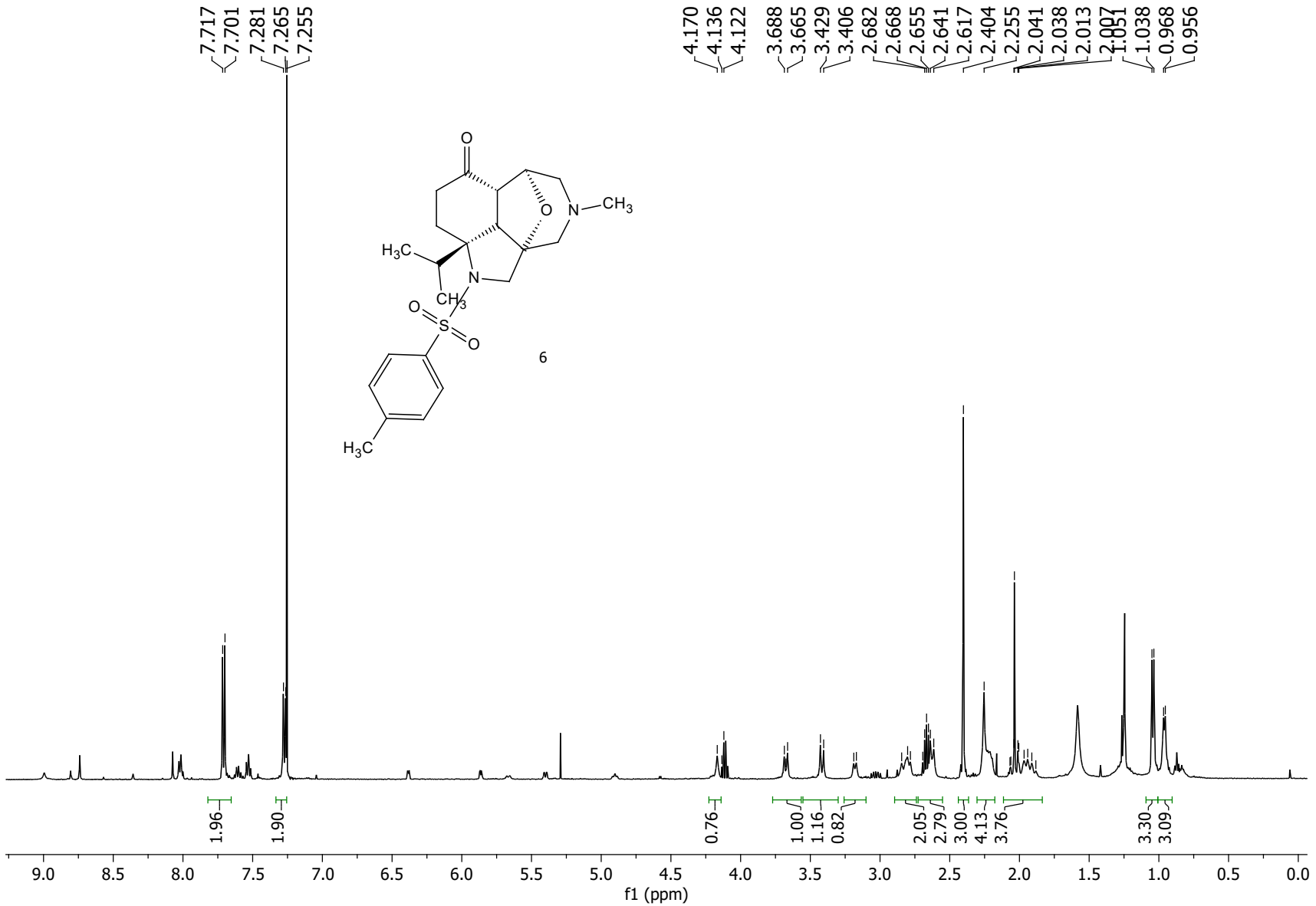
80
70
60
50
40
30
20
10
f1 (ppm)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4.122
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3.429
3.406
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2.668
2.655
2.641
2.617
2.404
2.255
2.041
2.038
2.013
2.007
1.051
1.038
0.968
0.956
O
O
N
CH3
H3C
N
CH3
O
S
O
H3C
6
1.96
1.90
0.76
1.00
1.16
0.82
2.05
2.79
3.00
4.13
3.76
3.30
3.09
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.5
3.0
2.5
2.0
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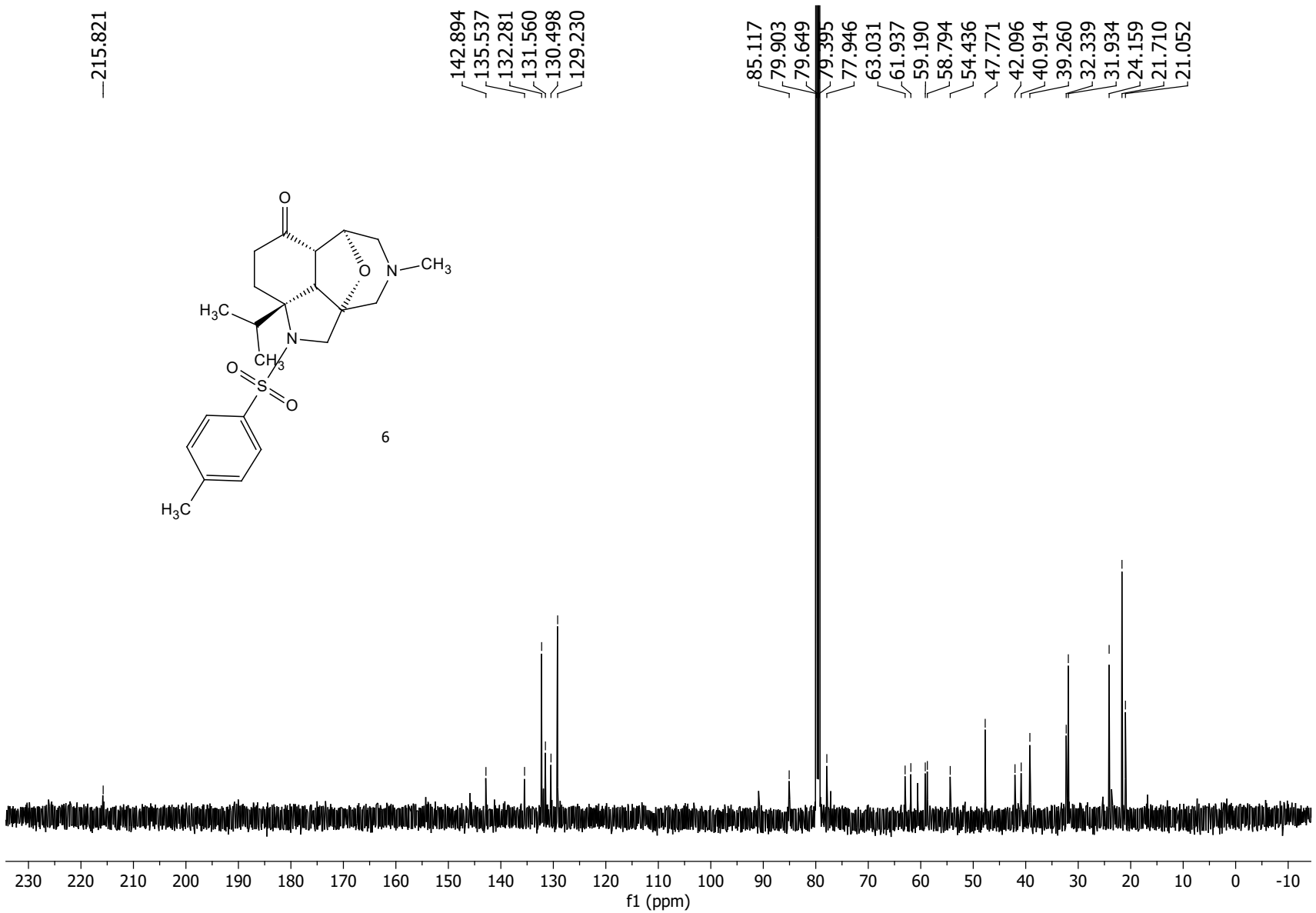
1.0
0.5
0.0
f1 (ppm)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130.498
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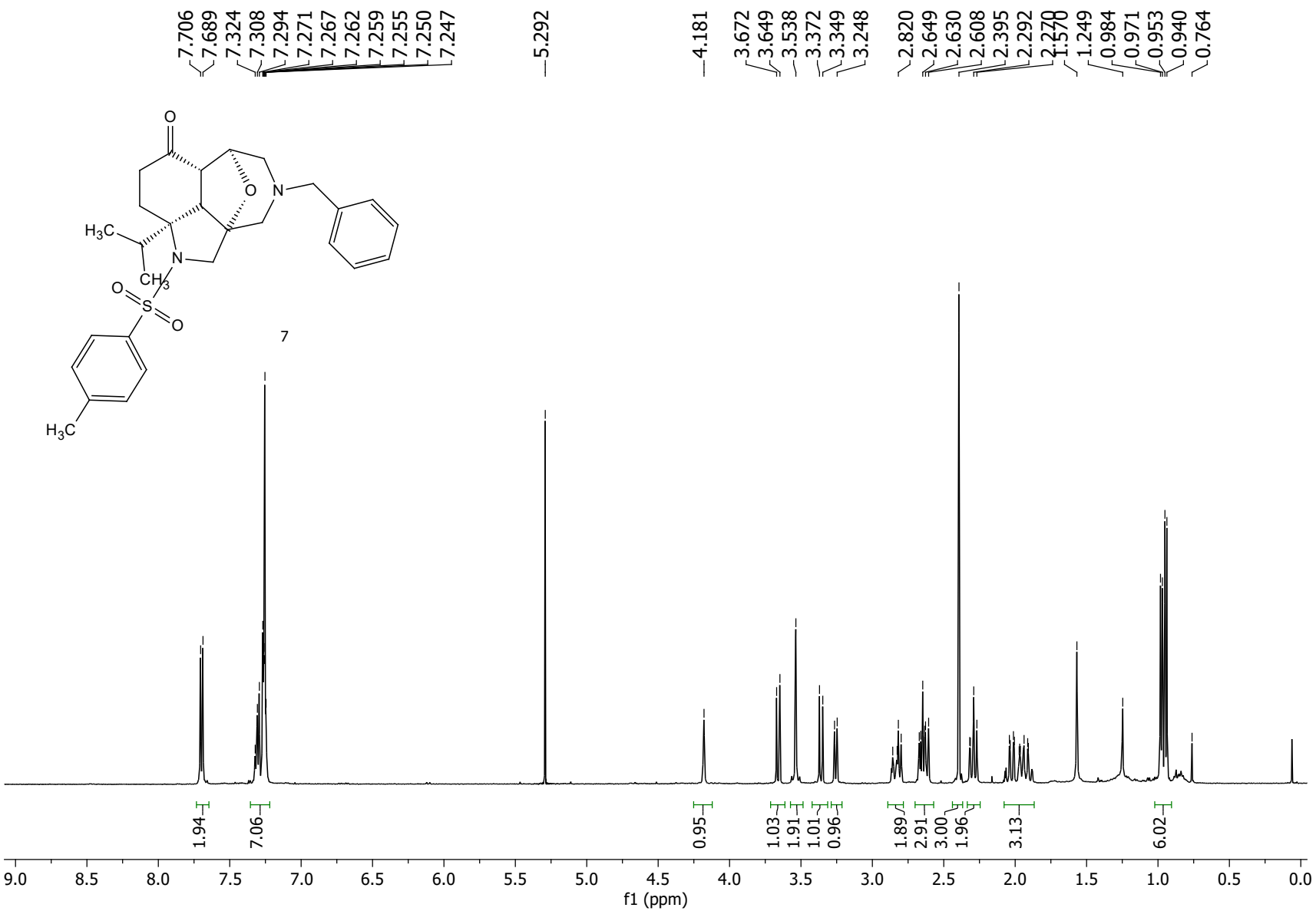
85.117
79.903
79.649
79.395
77.946
63.031
61.937
59.190
58.794
54.436
47.771
42.096
40.914
39.260
32.339
31.934
24.159
21.710
21.052
O
O
N
CH3
H3C
N
CH3
O
S
O
H3C
6
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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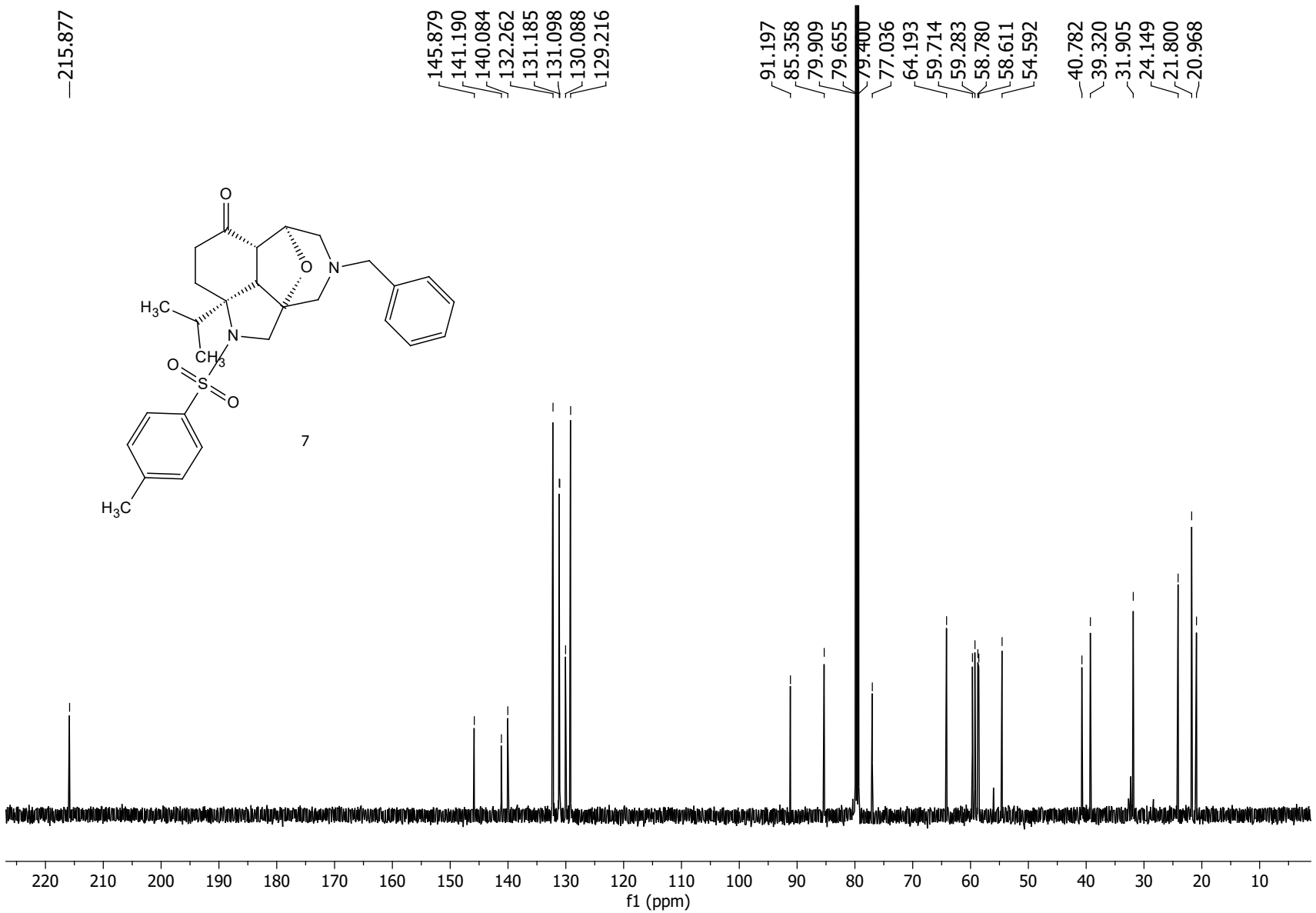
7.324
7.308
7.294
7.271
7.267
7.262
7.259
7.255
7.250
7.247
5.292
4.181
3.672
3.649
3.538
3.372
3.349
3.248
2.820
2.649
2.630
2.608
2.395
2.292
2.270
1.570
1.249
0.984
0.971
0.953
0.940
0.764
O
O
N
N
O
S
O
O
H3C
CH3
H3C
7
1.94
7.06
0.95
1.03
1.91
1.01
0.96
1.89
2.91
3.00
1.96
3.13
6.02
9.0
8.5
8.0
7.5
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
0.0
f1 (ppm)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130.088
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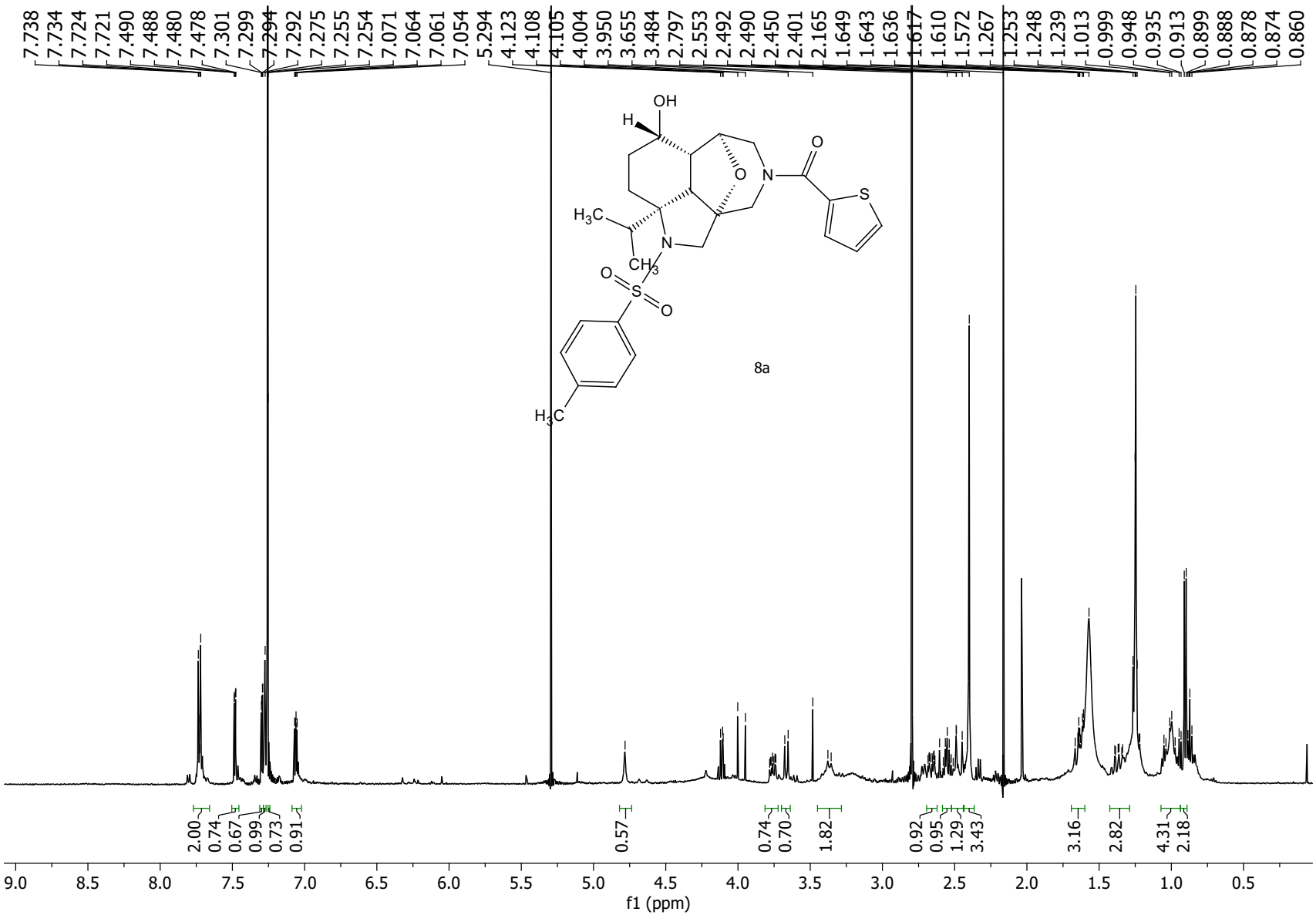
79.655
79.400
77.036
64.193
59.714
59.283
58.780
58.611
54.592
40.782
39.320
31.905
24.149
21.800
20.968
O
O
N
H3C
N
CH3
O
S
O
H3C
7
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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7.299
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7.292
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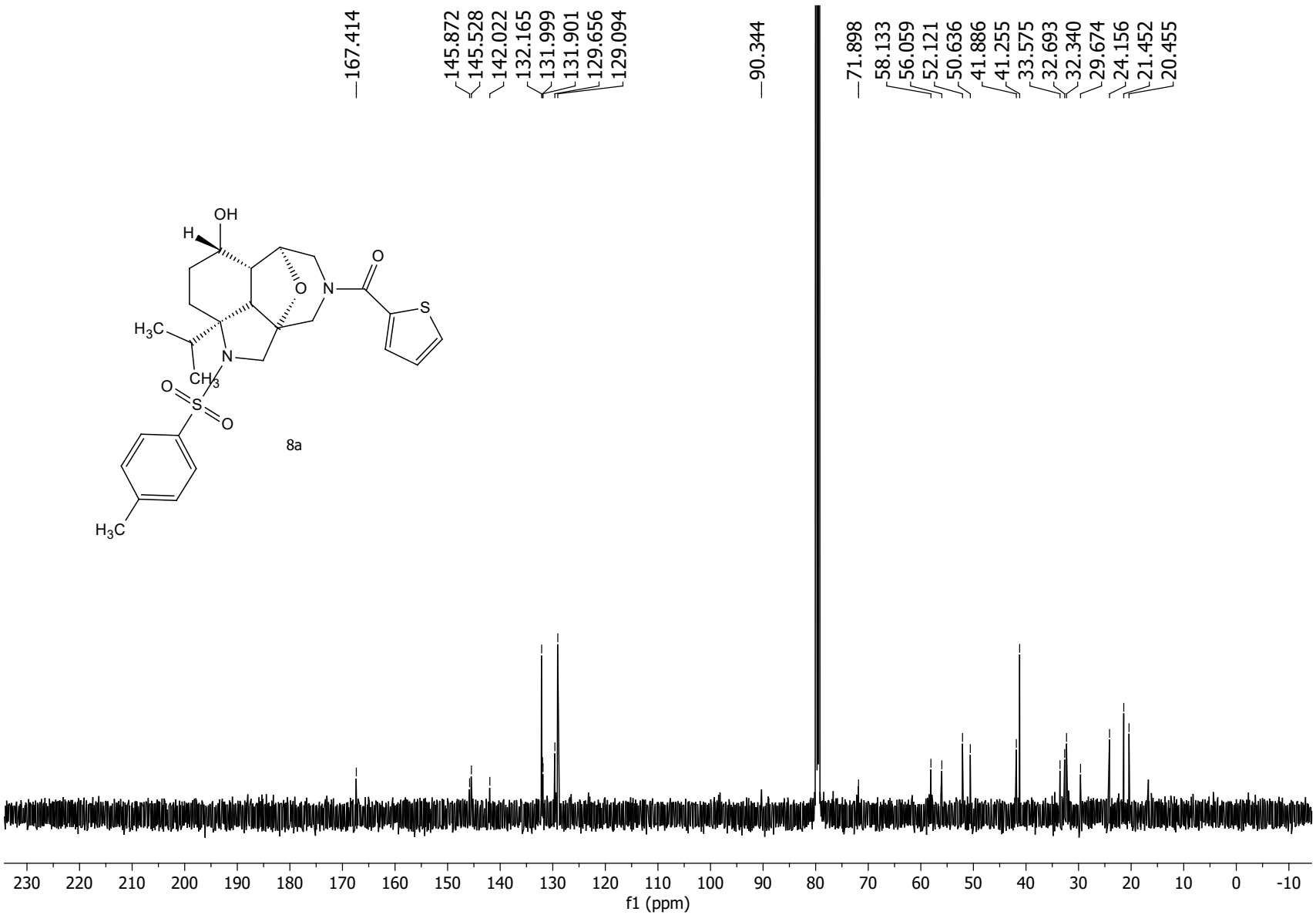
7.255
7.254
7.071
7.064
7.061
7.054
5.294
4.123
4.108
4.105
4.004
3.950
3.655
3.484
2.797
2.553
2.492
2.490
2.450
2.401
2.165
1.649
1.643
1.636
1.617
1.610
1.572
1.267
1.253
1.248
1.239
1.013
0.999
0.948
0.935
0.913
0.899
0.888
0.878
0.874
0.860
OH
H
O
N
O
S
H3C
N
CH3
O
S
O
O
H3C
8a
2.00
0.74
0.67
0.99
0.73
0.91
0.57
0.74
0.70
1.82
0.92
0.95
1.29
3.43
3.16
2.82
4.31
2.18
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
f1 (ppm)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129.656
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90.344
71.898
58.133
56.059
52.121
50.636
41.886
41.255
33.575
32.693
32.340
29.674
24.156
21.452
20.455
OH
H
O
N
S
H3C
N
CH3
O
S
O
H3C
8a
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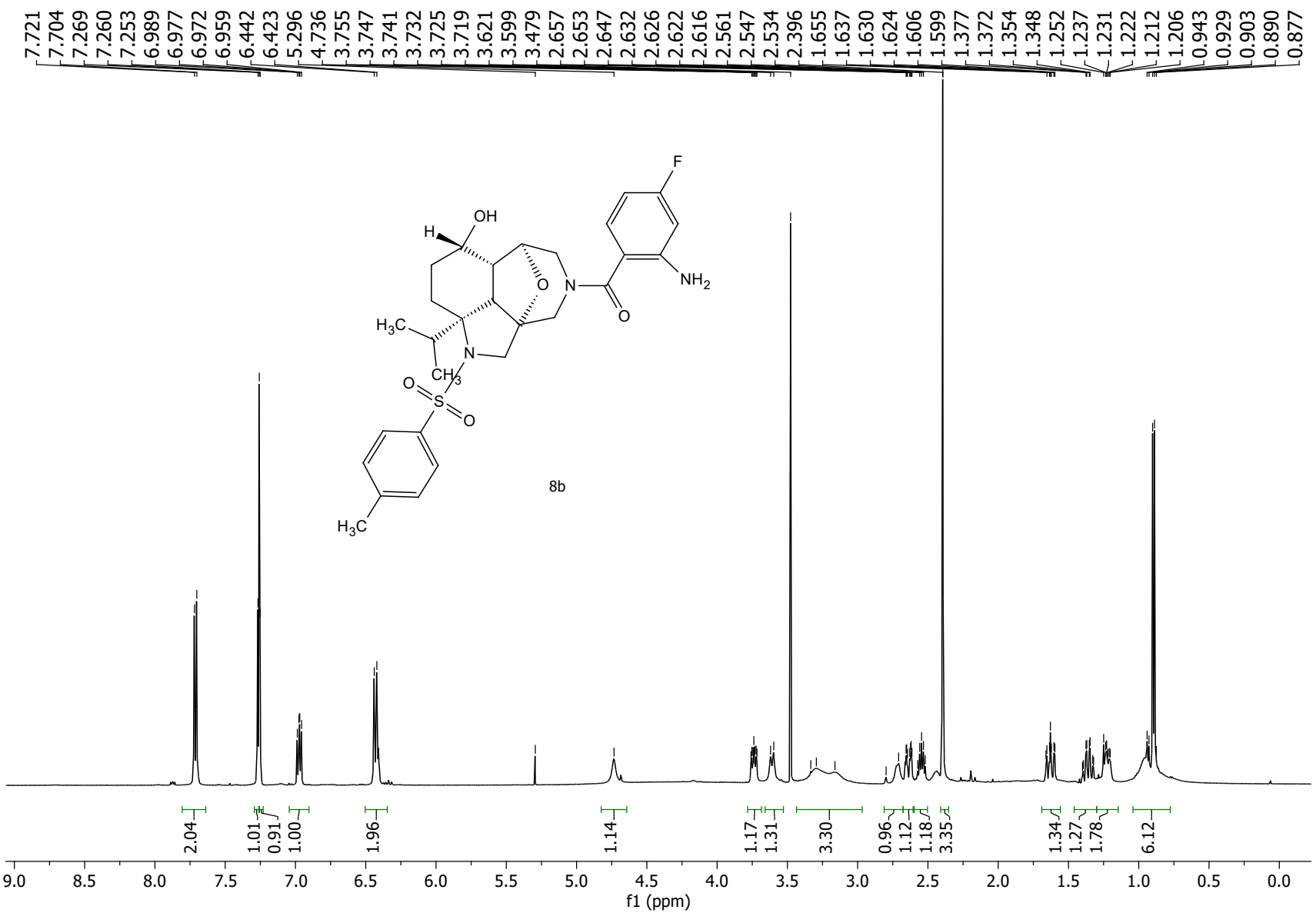
f1 (ppm)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4.736
3.755
3.747
3.741
3.732
3.725
3.719
3.621
3.599
3.479
2.657
2.653
2.647
2.632
2.626
2.622
2.616
2.561
2.547
2.534
2.396
1.655
1.637
1.630
1.624
1.606
1.599
1.377
1.372
1.354
1.348
1.252
1.237
1.231
1.222
1.212
1.206
0.943
0.929
0.903
0.890
0.877
F
OH
H
O
N
NH2
O
H3C
N
CH3
O
S
O
H3C
8b
2.04
1.01
0.91
1.00
1.96
1.14
1.17
1.31
3.30
0.96
1.12
1.18
3.35
1.34
1.27
1.78
6.12
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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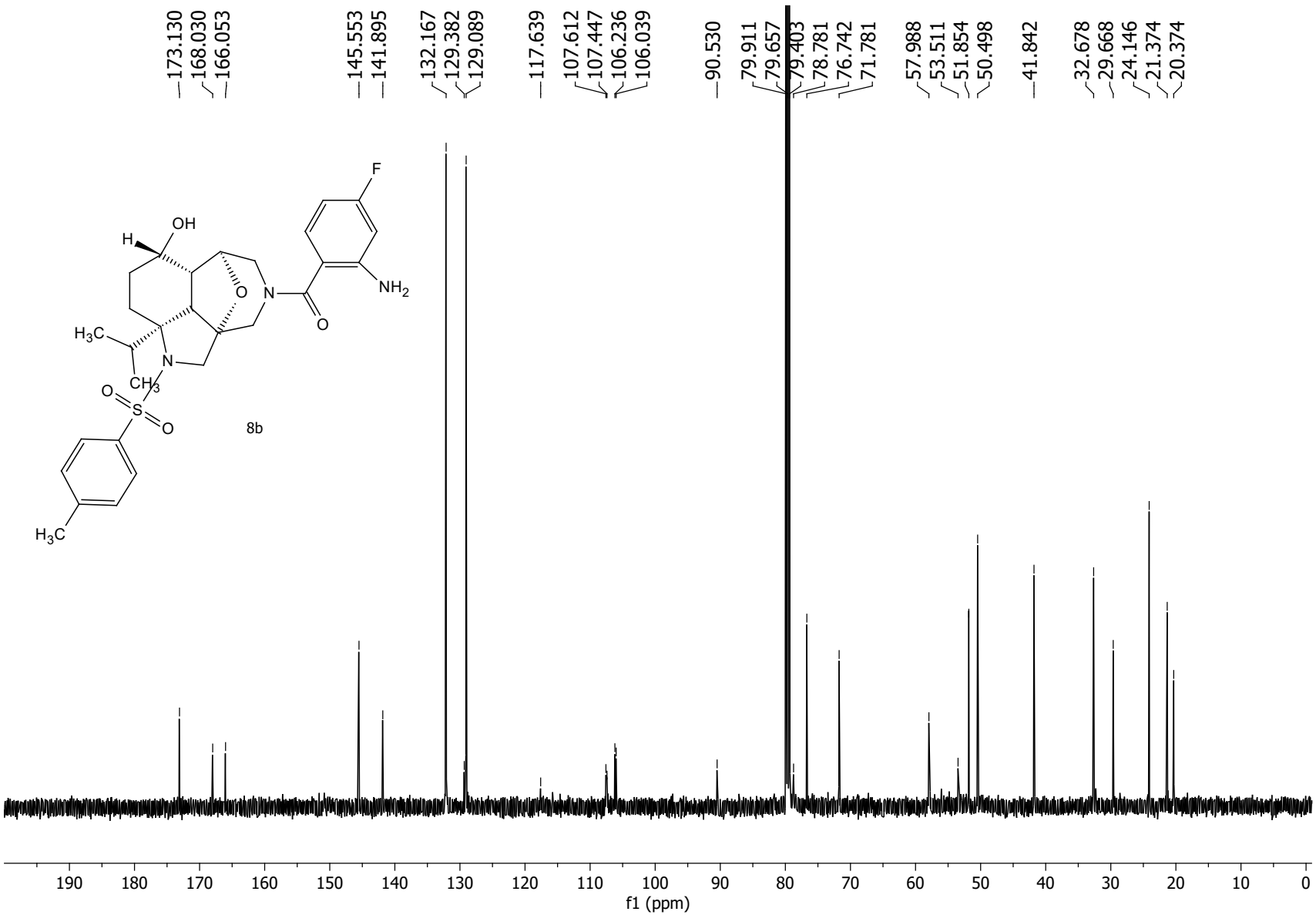
f1 (ppm)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129.089
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106.236
106.039
90.530
79.911
79.657
79.403
78.781
76.742
71.781
57.988
53.511
51.854
50.498
41.842
32.678
29.668
24.146
21.374
20.374
F
OH
H
O
N
NH2
O
H3C
N
CH3
O
S
O
O
H3C
8b
190
180
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140
130
120
110
100
90
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70
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50
40
30
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10
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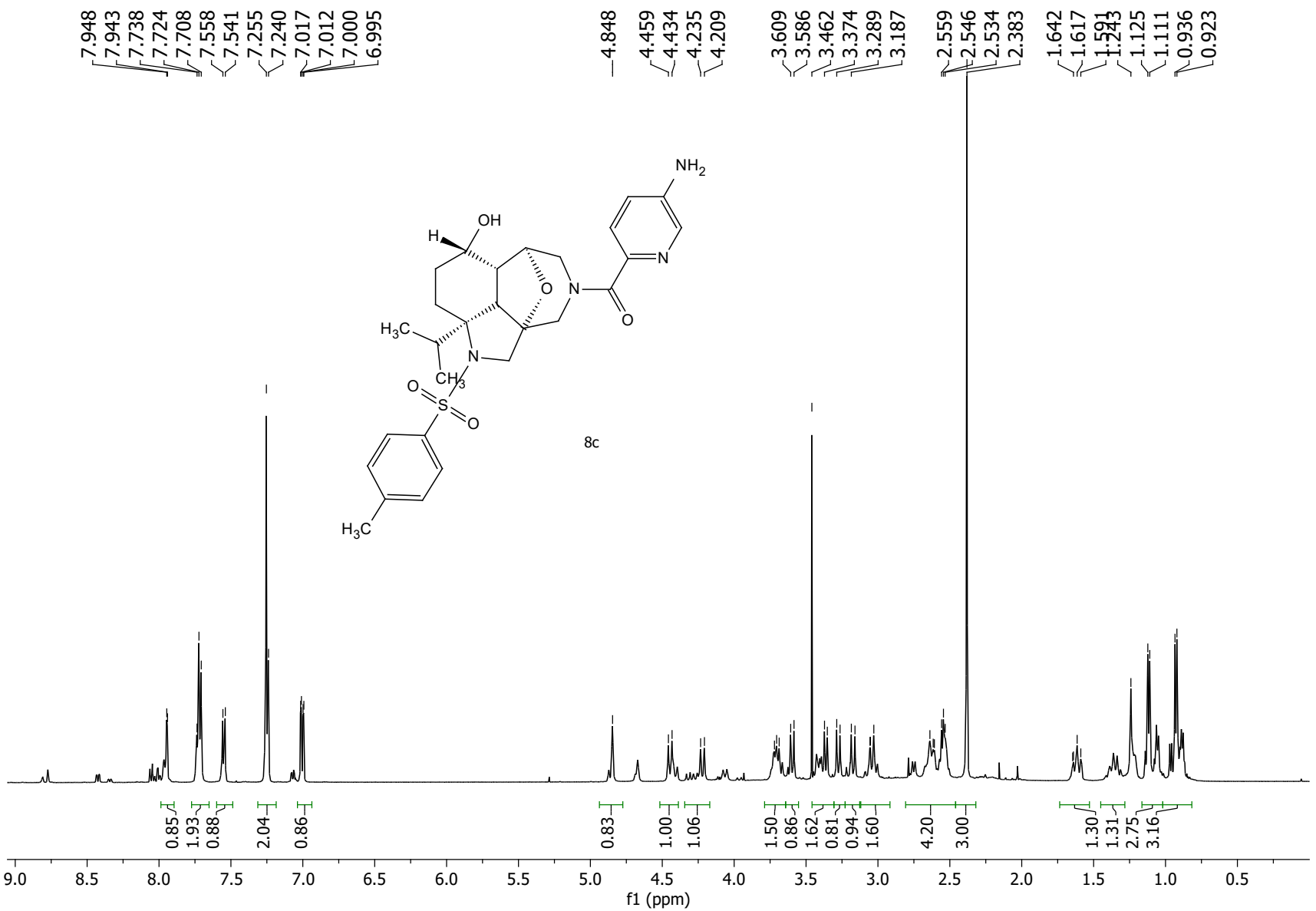
f1 (ppm)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7.255
7.240
7.017
7.012
7.000
6.995
4.848
4.459
4.434
4.235
4.209
3.609
3.586
3.462
3.374
3.289
3.187
2.559
2.546
2.534
2.383
1.642
1.617
1.591
1.243
1.125
1.111
0.936
0.923
NH2
OH
H
N
O
N
O
H3C
N
CH3
O
S
O
H3C
8c
0.85
1.93
0.88
2.04
0.86
0.83
1.00
1.06
1.50
0.86
1.62
0.81
0.94
1.60
4.20
3.00
1.30
1.31
2.75
3.16
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8.5
8.0
7.5
7.0
6.5
6.0
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3.0
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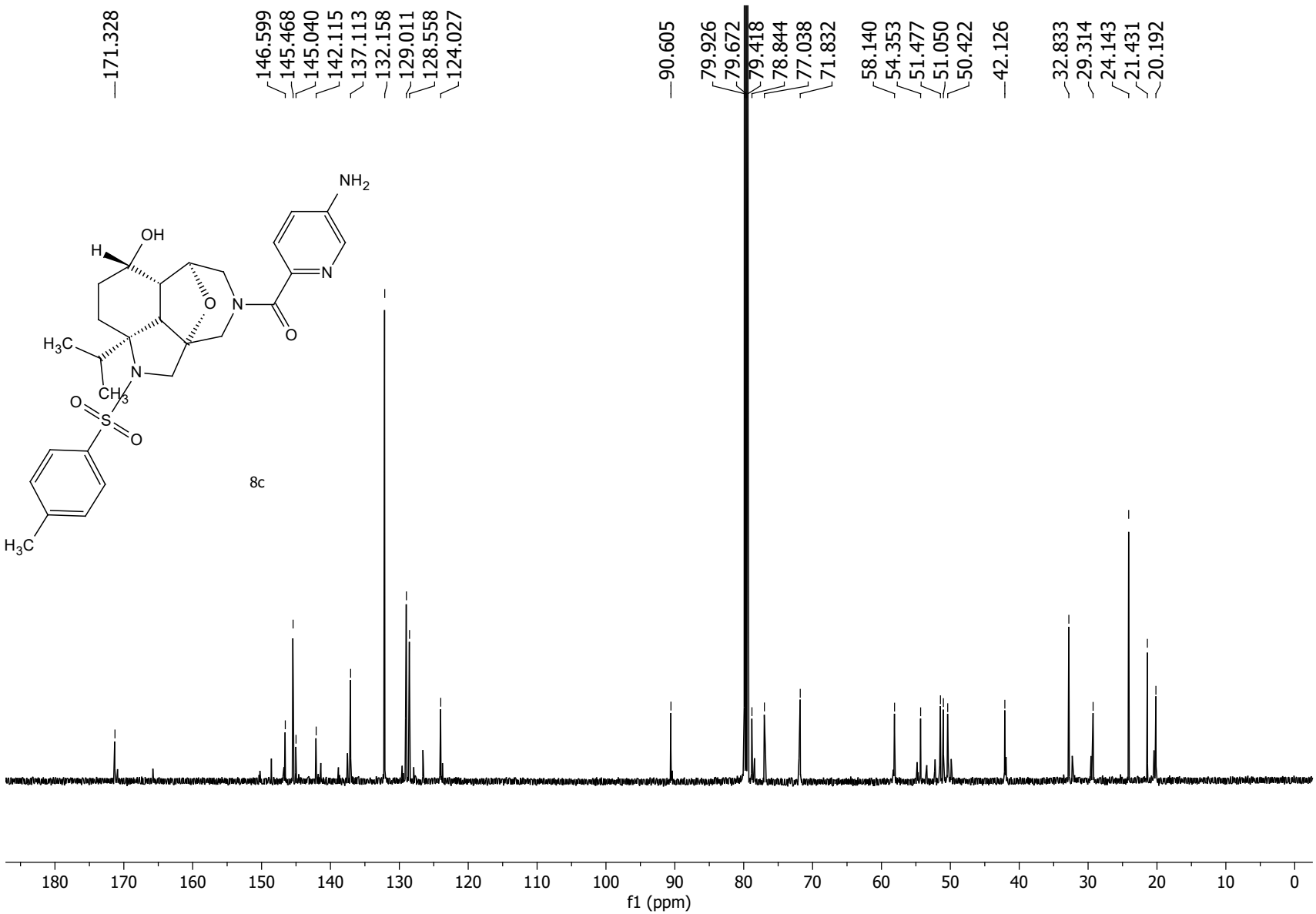
2.0
1.5
1.0
0.5
f1 (ppm)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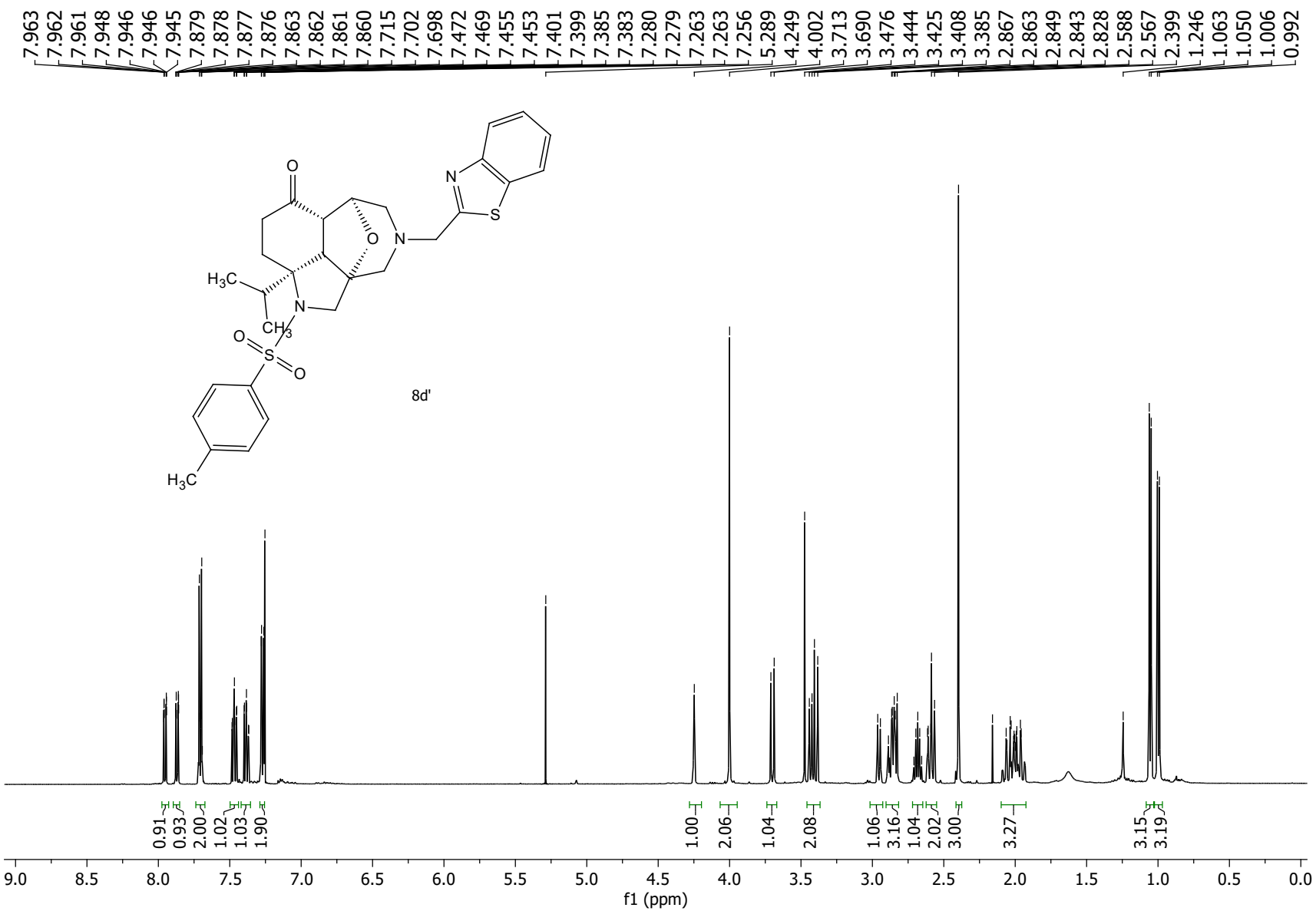
129.011
128.558
124.027
90.605
79.926
79.672
79.418
78.844
77.038
71.832
58.140
54.353
51.477
51.050
50.422
42.126
32.833
29.314
24.143
21.431
20.192
NH2
OH
H
N
O
N
O
H3C
N
CH3
O
S
O
H3C
8c
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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7.863
7.862
7.861
7.860
7.715
7.702
7.698
7.472
7.469
7.455
7.453
7.401
7.399
7.385
7.383
7.280
7.279
7.263
7.263
7.256
5.289
4.249
4.002
3.713
3.690
3.476
3.444
3.425
3.408
3.385
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2.588
2.567
2.399
1.246
1.063
1.050
1.006
0.992
O
N
S
O
N
H3C
CH3
N
O
S
O
H3C
8d'
0.91
0.93
2.00
1.02
1.03
1.90
1.00
2.06
1.04
2.08
1.06
3.16
1.04
2.02
3.00
3.27
3.15
3.19
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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3.5
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2.5
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1.0
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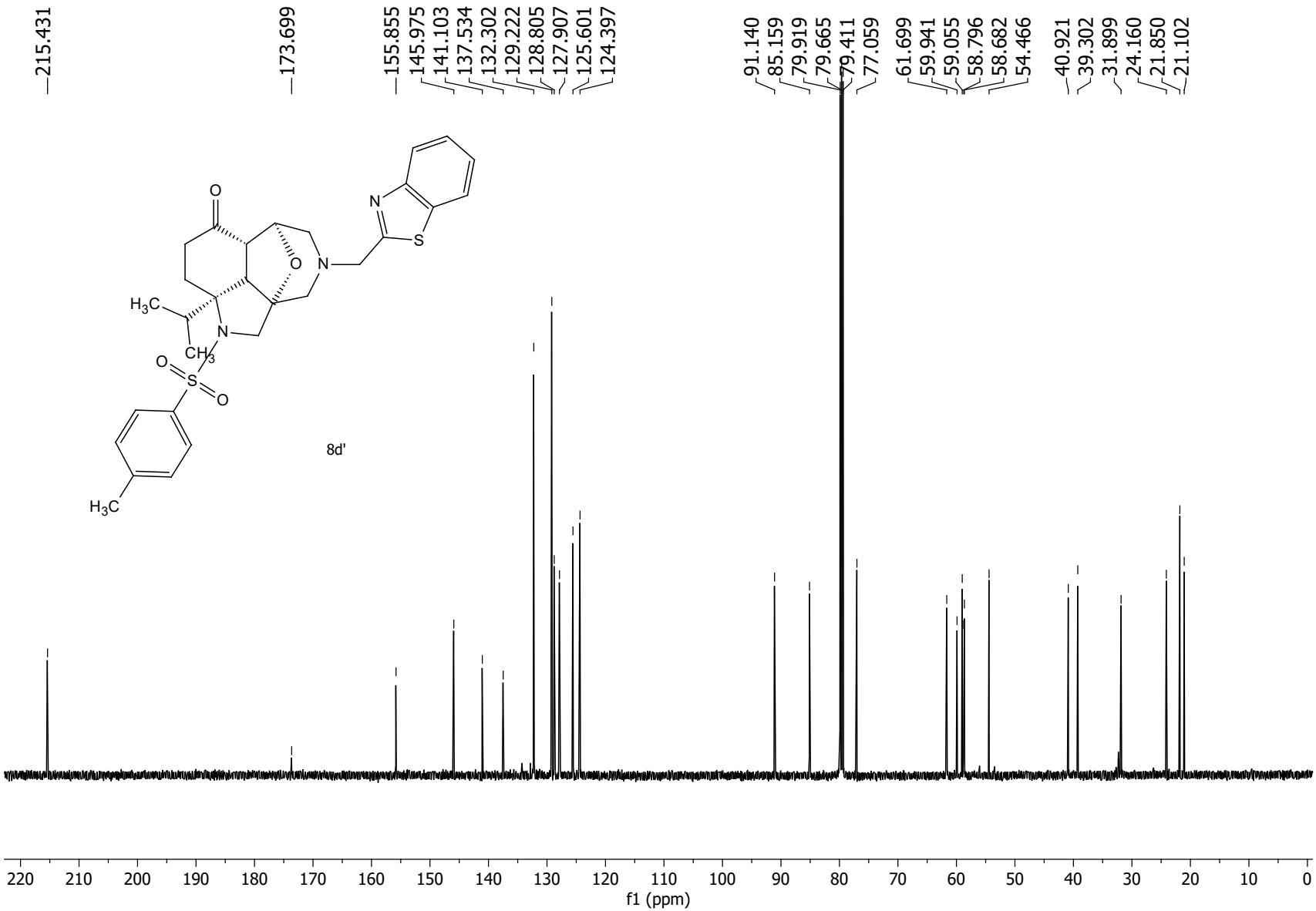
0.0
f1 (ppm)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129.222
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127.907
125.601
124.397
91.140
85.159
79.919
79.665
79.411
77.059
61.699
59.941
59.055
58.796
58.682
54.466
40.921
39.302
31.899
24.160
21.850
21.102
8d'
220
210
200
190
180
170
160
150
140
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120
110
100
90
80
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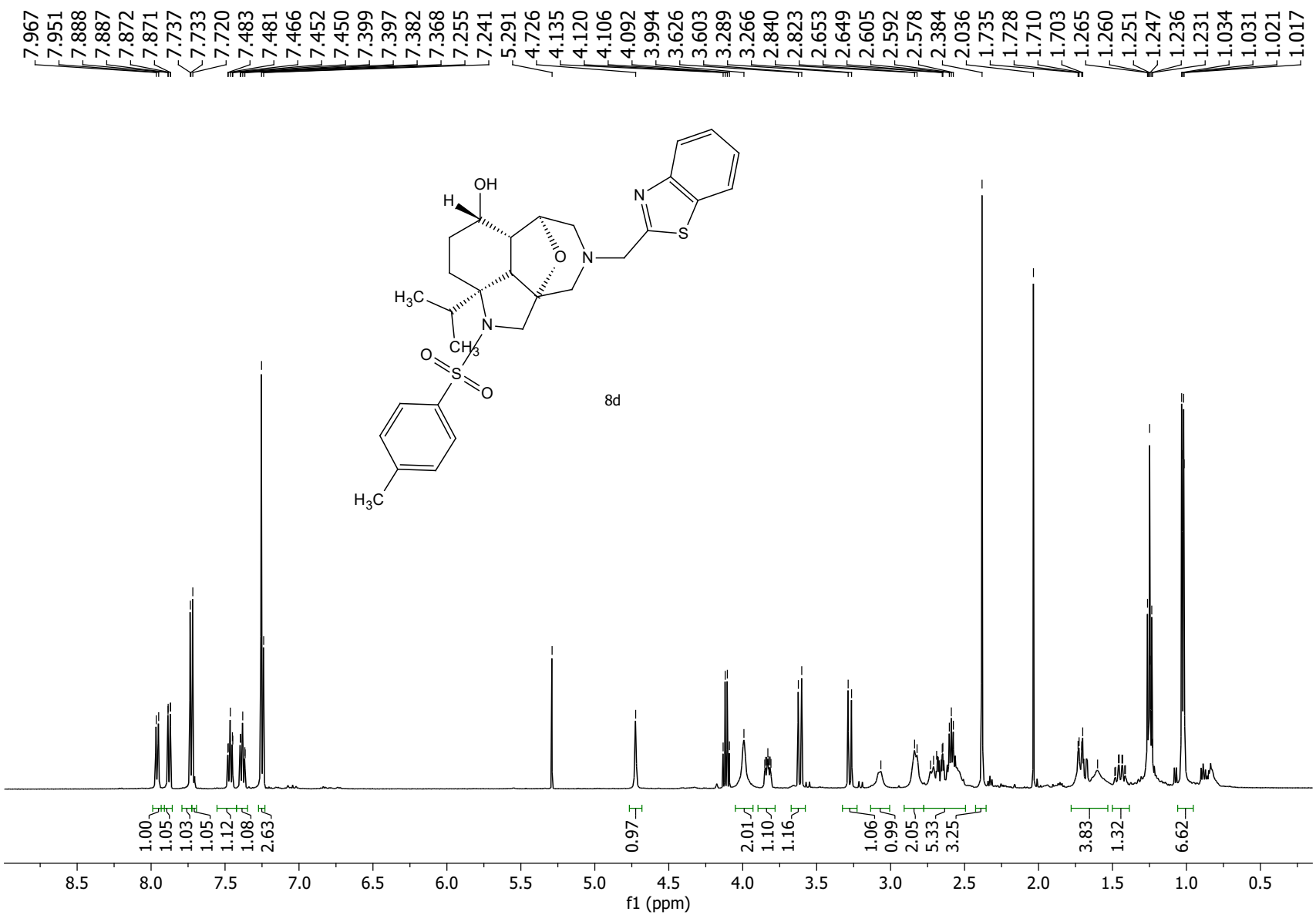
60
50
40
30
20
10
0
f1 (ppm)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7.450
7.399
7.397
7.382
7.368
7.255
7.241
5.291
4.726
4.135
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4.106
4.092
3.994
3.626
3.603
3.289
3.266
2.840
2.823
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2.649
2.605
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2.578
2.384
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1.735
1.728
1.710
1.703
1.265
1.260
1.251
1.247
1.236
1.231
1.034
1.031
1.021
1.017
OH
H
N
S
O
N
H3C
N
CH3
O
S
O
H3C
8d
1.00
1.05
1.03
1.05
1.12
1.08
2.63
0.97
2.01
1.10
1.16
1.06
0.99
2.05
5.33
3.25
3.83
1.32
6.62
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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4.0
3.5
3.0
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1.5
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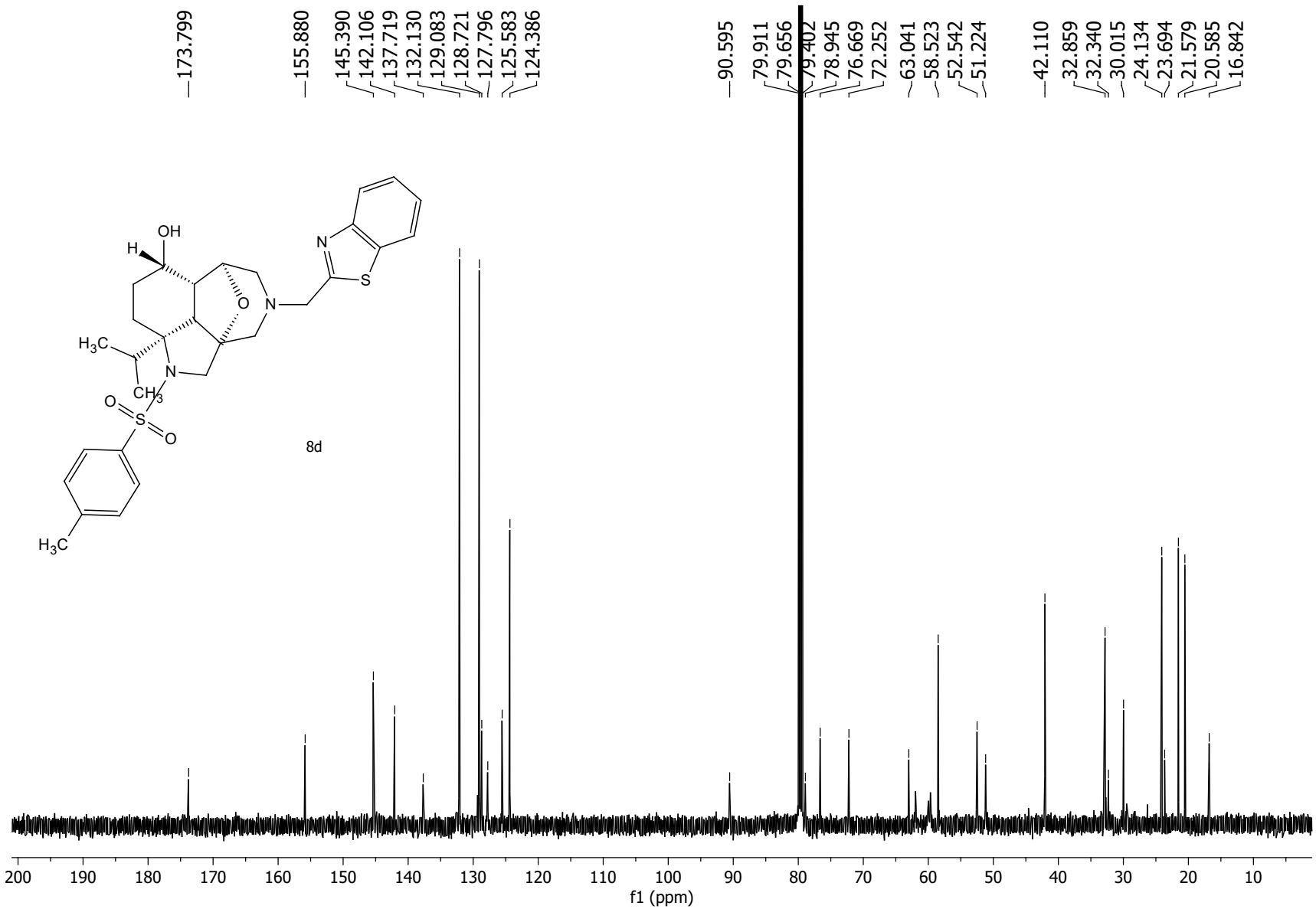
0.5
f1 (ppm)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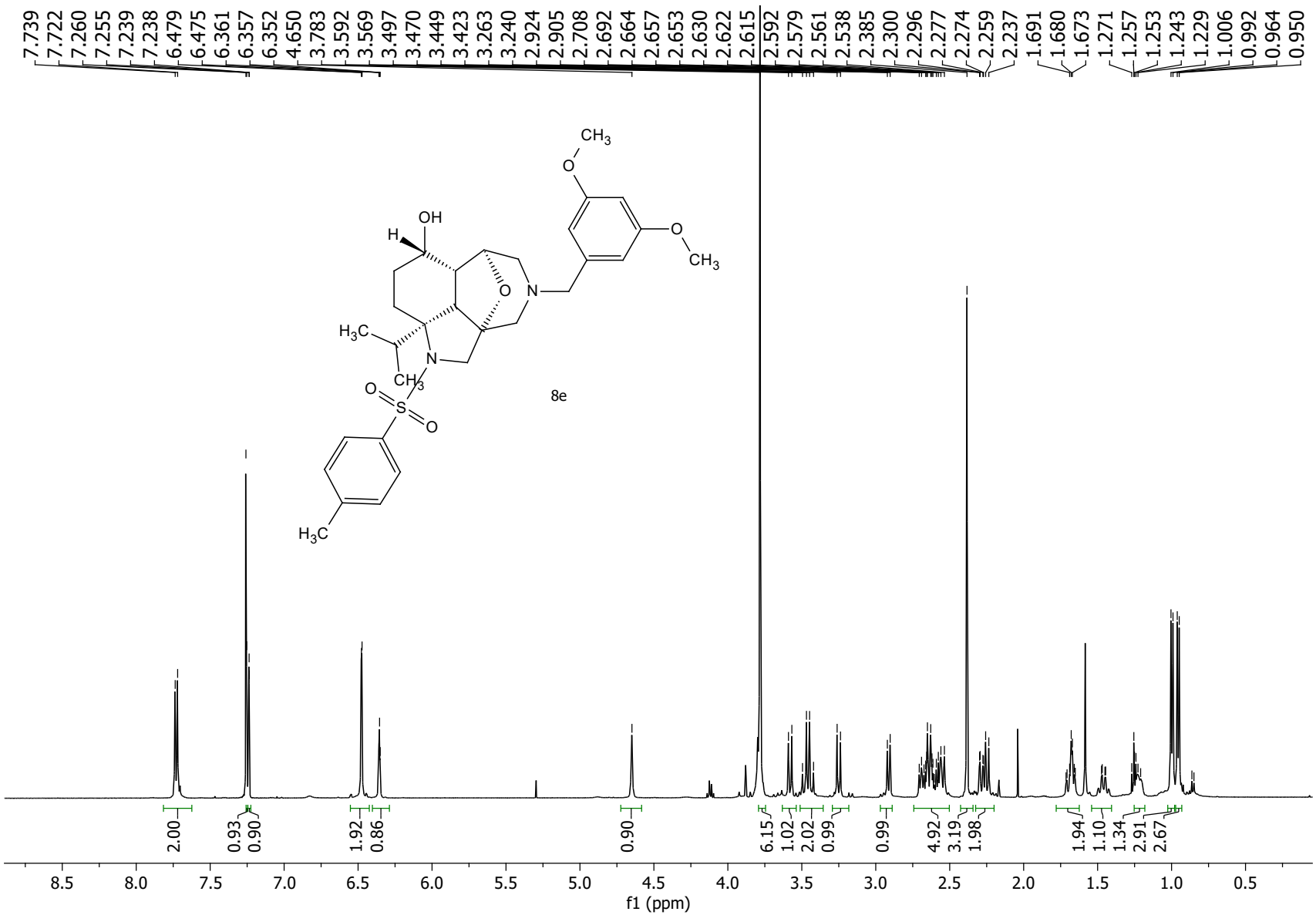
90.595
79.911
79.656
79.402
78.945
76.669
72.252
63.041
58.523
52.542
51.224
42.110
32.859
32.340
30.015
24.134
23.694
21.579
20.585
16.842
OH
H
N
S
O
N
H3C
N
CH3
O
S
O
H3C
8d
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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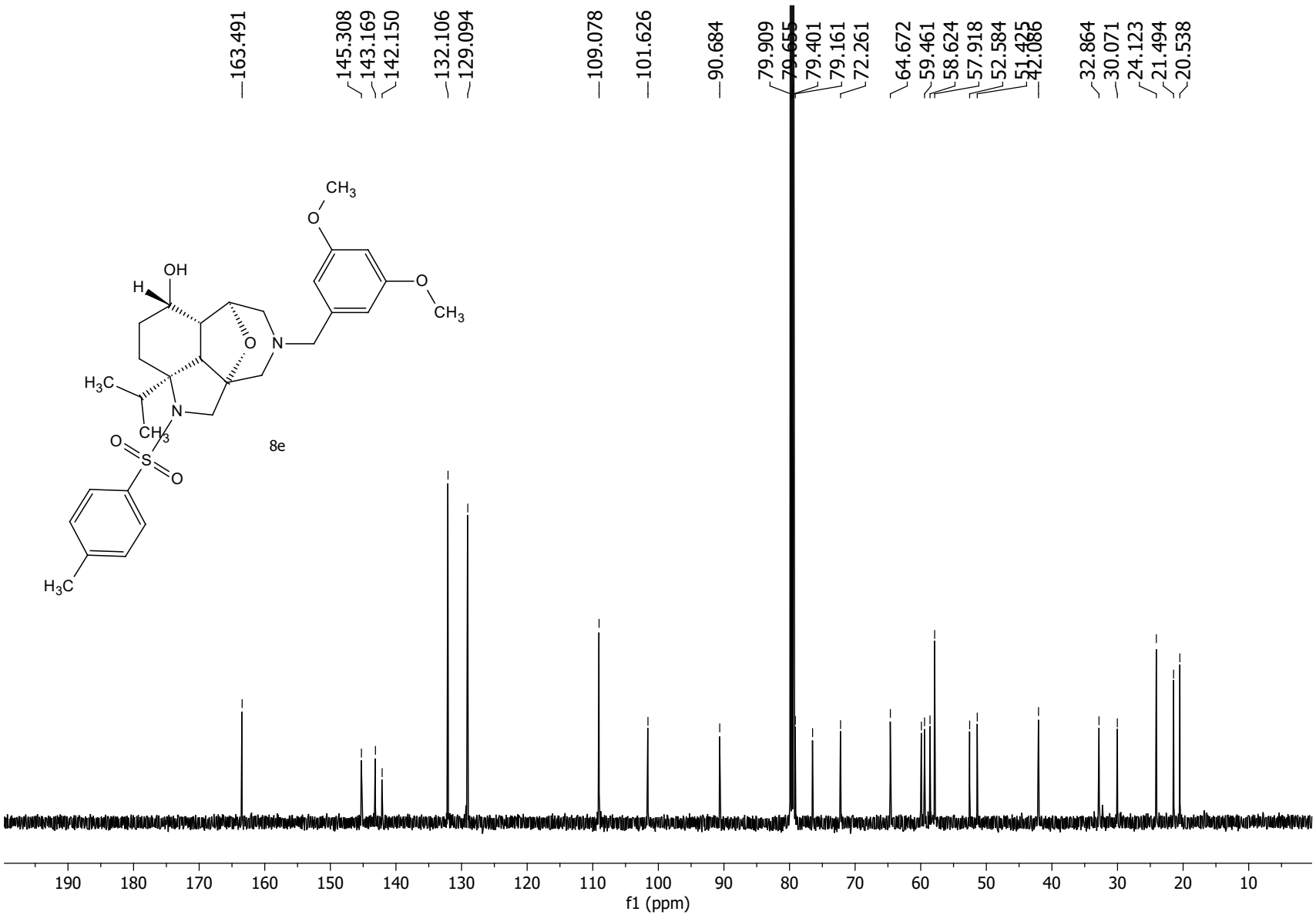
7.238
6.479
6.475
6.361
6.357
6.352
4.650
3.783
3.592
3.569
3.497
3.470
3.449
3.423
3.263
3.240
2.924
2.905
2.708
2.692
2.664
2.657
2.653
2.630
2.622
2.615
2.592
2.579
2.561
2.538
2.385
2.300
2.296
2.277
2.274
2.259
2.237
1.691
1.680
1.673
1.271
1.257
1.253
1.243
1.229
1.006
0.992
0.964
0.950
8e
2.00
0.93
0.90
1.92
0.86
0.90
6.15
1.02
2.02
0.99
0.99
4.92
3.19
1.98
1.94
1.10
1.34
2.91
2.67
f1 (ppm)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101.626
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79.909
79.655
79.401
79.161
72.261
64.672
59.461
58.624
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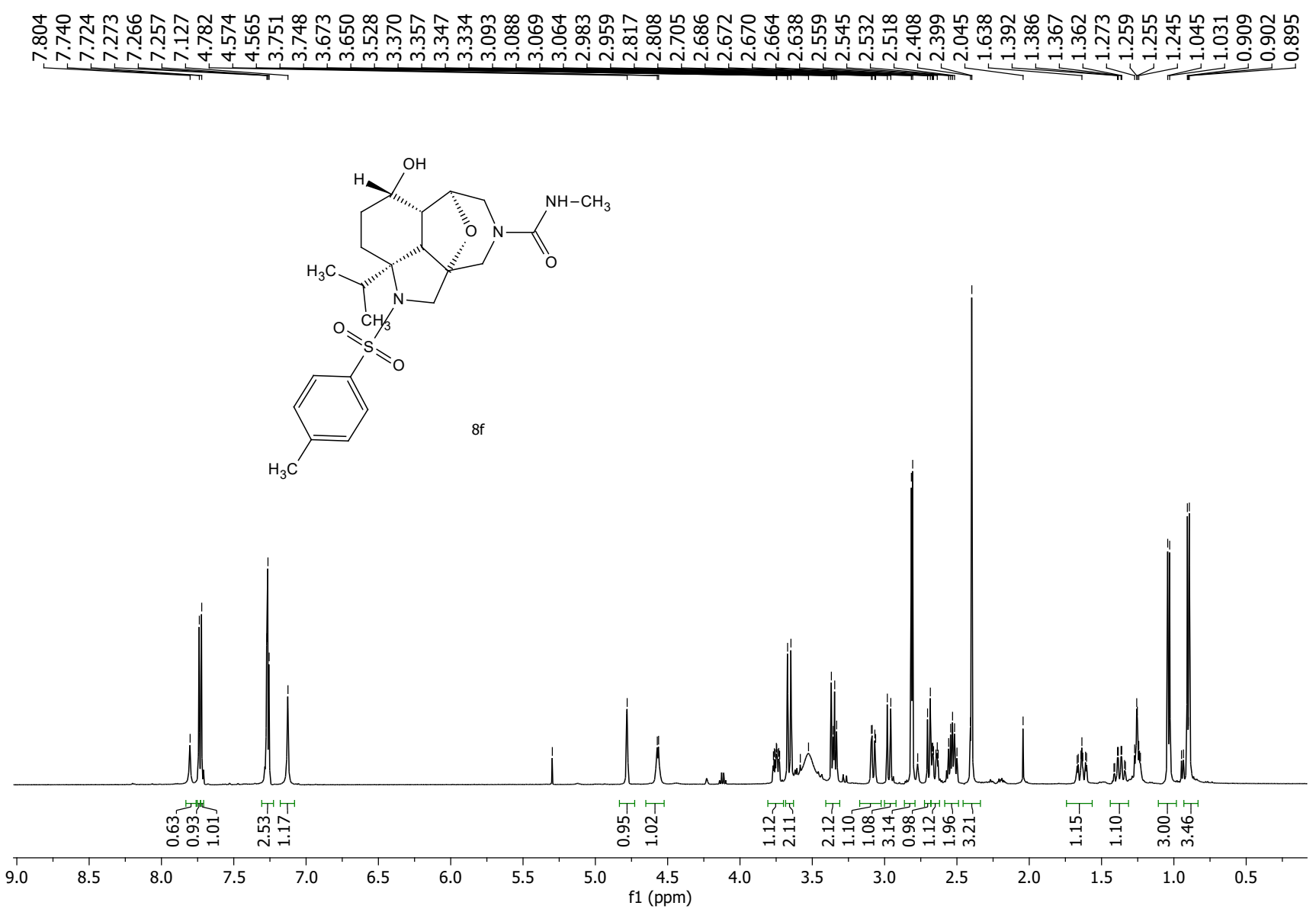
52.584
51.425
42.086
32.864
30.071
24.123
21.494
20.538
8e
f1 (ppm)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4.782
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3.751
3.748
3.673
3.650
3.528
3.370
3.357
3.347
3.334
3.093
3.088
3.069
3.064
2.983
2.959
2.817
2.808
2.705
2.686
2.672
2.670
2.664
2.638
2.559
2.545
2.532
2.518
2.408
2.399
2.045
1.638
1.392
1.386
1.367
1.362
1.273
1.259
1.255
1.245
1.045
1.031
0.909
0.902
0.895
OH
H
NH−CH3
O
N
O
H3C
N
CH3
O
S
O
H3C
8f
0.63
0.93
1.01
2.53
1.17
0.95
1.02
1.12
2.11
2.12
1.10
1.08
3.14
0.98
1.12
1.96
3.21
1.15
1.10
3.00
3.46
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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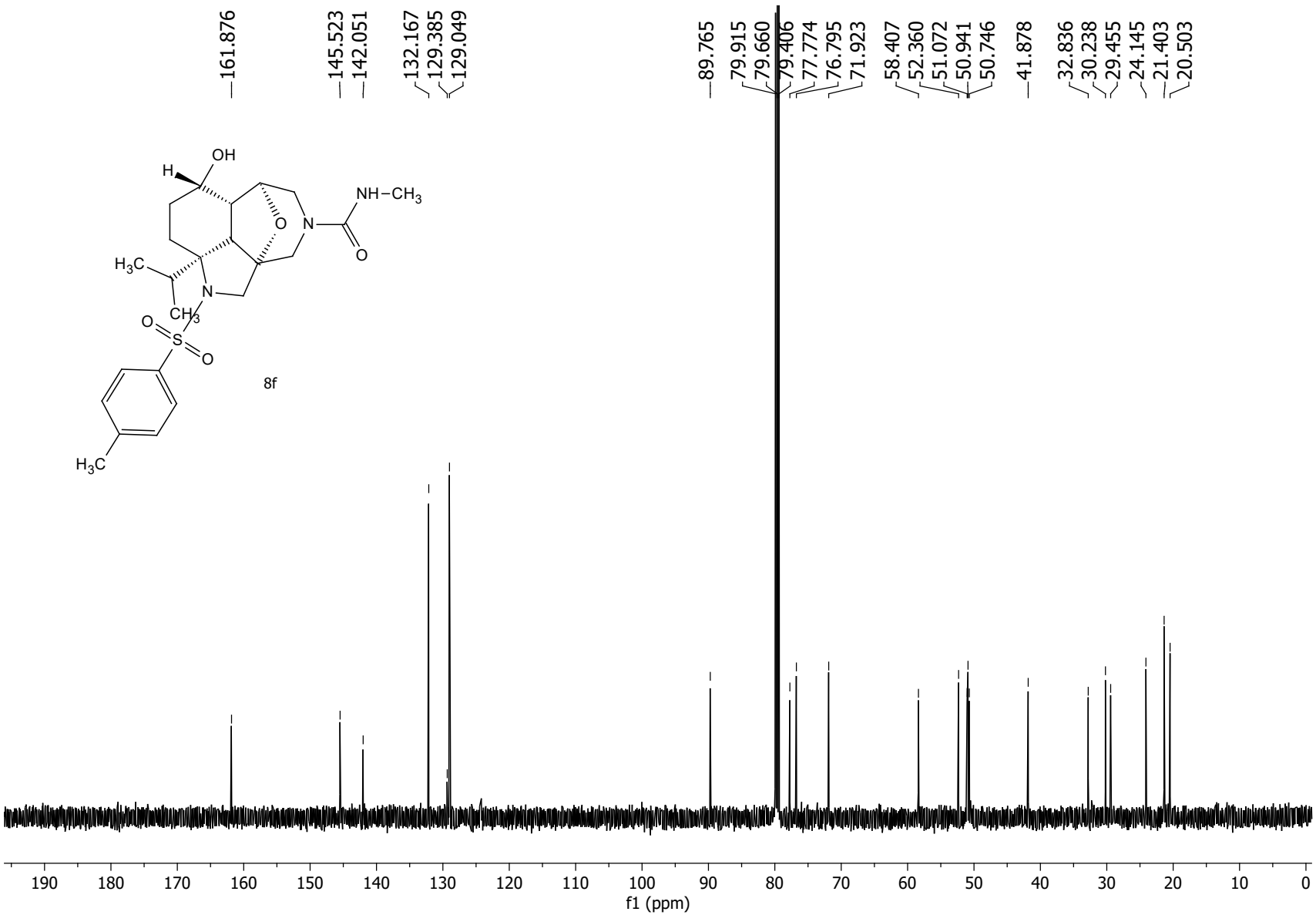
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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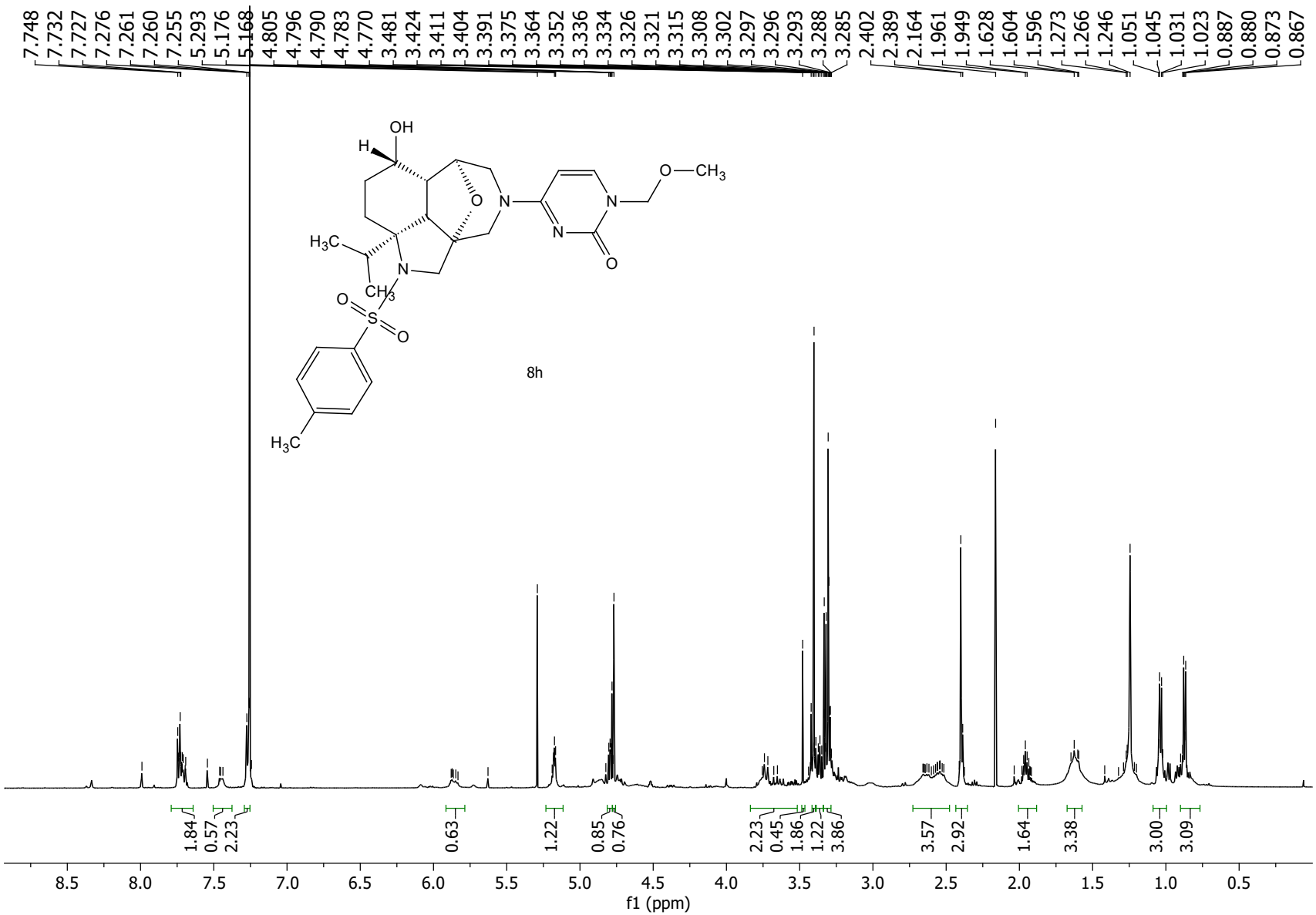
79.915
79.660
79.406
77.774
76.795
71.923
58.407
52.360
51.072
50.941
50.746
41.878
32.836
30.238
29.455
24.145
21.403
20.503
OH
H
NH−CH3
O
N
O
H3C
N
CH3
O
S
O
O
H3C
8f
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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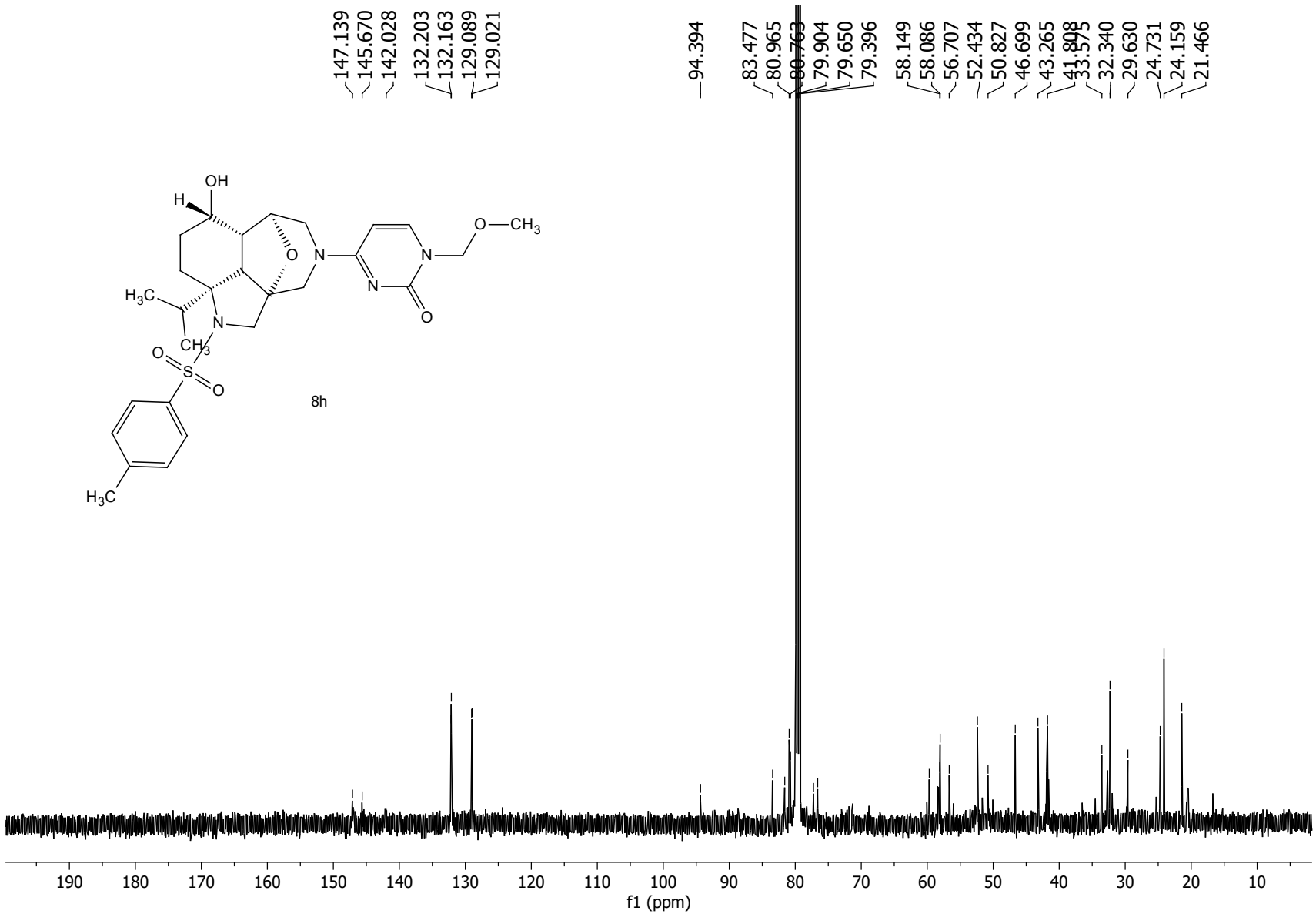
7.727
7.276
7.261
7.260
7.255
5.293
5.176
5.168
4.805
4.796
4.790
4.783
4.770
3.481
3.424
3.411
3.404
3.391
3.375
3.364
3.352
3.336
3.334
3.326
3.321
3.315
3.308
3.302
3.297
3.296
3.293
3.288
3.285
2.402
2.389
2.164
1.961
1.949
1.628
1.604
1.596
1.273
1.266
1.246
1.051
1.045
1.031
1.023
0.887
0.880
0.873
0.867
OH
H
O
N
N
N
O
O—CH3
H3C
CH3
O
S
O
H3C
8h
1.84
0.57
2.23
0.63
1.22
0.85
0.76
2.23
0.45
1.86
1.22
3.86
3.57
2.92
1.64
3.38
3.00
3.09
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
f1 (ppm)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142.028
132.203
132.163
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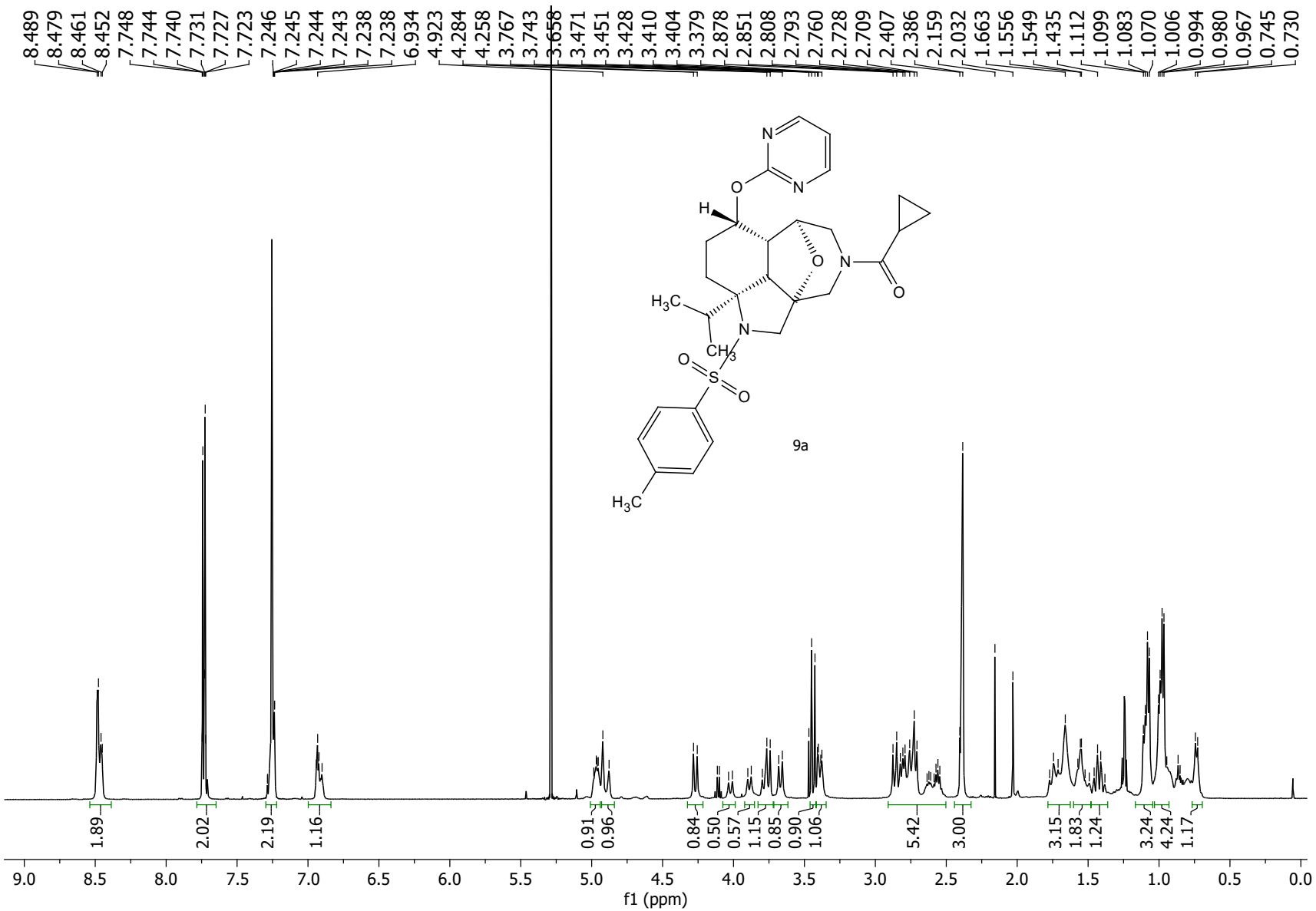
129.021
94.394
83.477
80.965
80.763
79.904
79.650
79.396
58.149
58.086
56.707
52.434
50.827
46.699
43.265
41.808
33.575
32.340
29.630
24.731
24.159
21.466
OH
H
O
N
N
N
O
O—CH3
H3C
CH3
O
S
O
H3C
8h
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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8.452
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7.744
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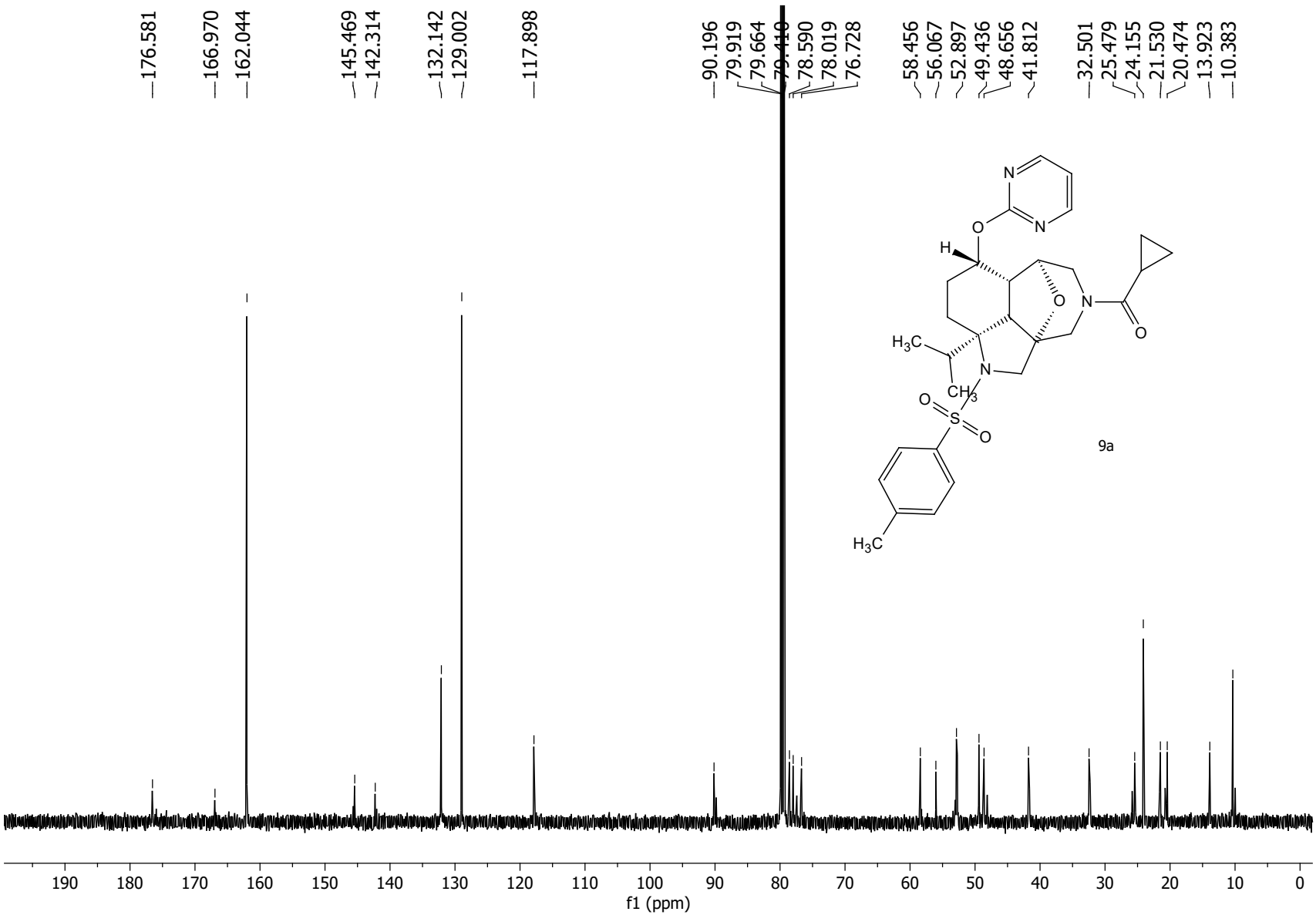
7.723
7.246
7.245
7.244
7.243
7.238
7.238
6.934
4.923
4.284
4.258
3.767
3.743
3.658
3.471
3.451
3.428
3.410
3.404
3.379
2.878
2.851
2.808
2.793
2.760
2.728
2.709
2.407
2.386
2.159
2.032
1.663
1.556
1.549
1.435
1.112
1.099
1.083
1.070
1.006
0.994
0.980
0.967
0.745
0.730
9a
1.89
2.02
2.19
1.16
0.91
0.96
0.84
0.50
0.57
1.15
0.85
0.90
1.06
5.42
3.00
3.15
1.83
1.24
3.24
4.24
1.17
f1 (ppm)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132.142
129.002
117.898
90.196
79.919
79.664
79.410
78.590
78.019
76.728
58.456
56.067
52.897
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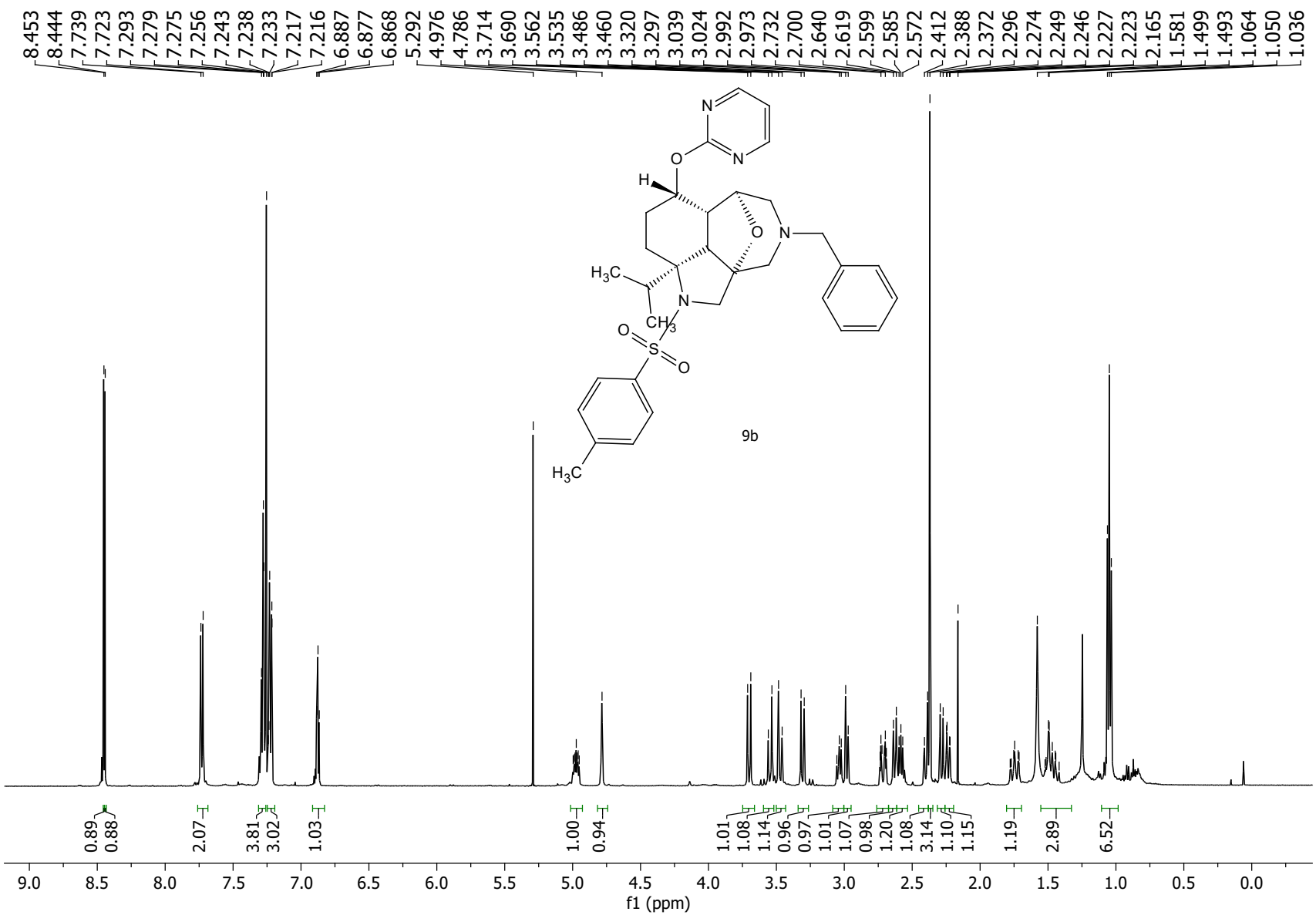
48.656
41.812
32.501
25.479
24.155
21.530
20.474
13.923
10.383
9a
f1 (ppm)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7.256
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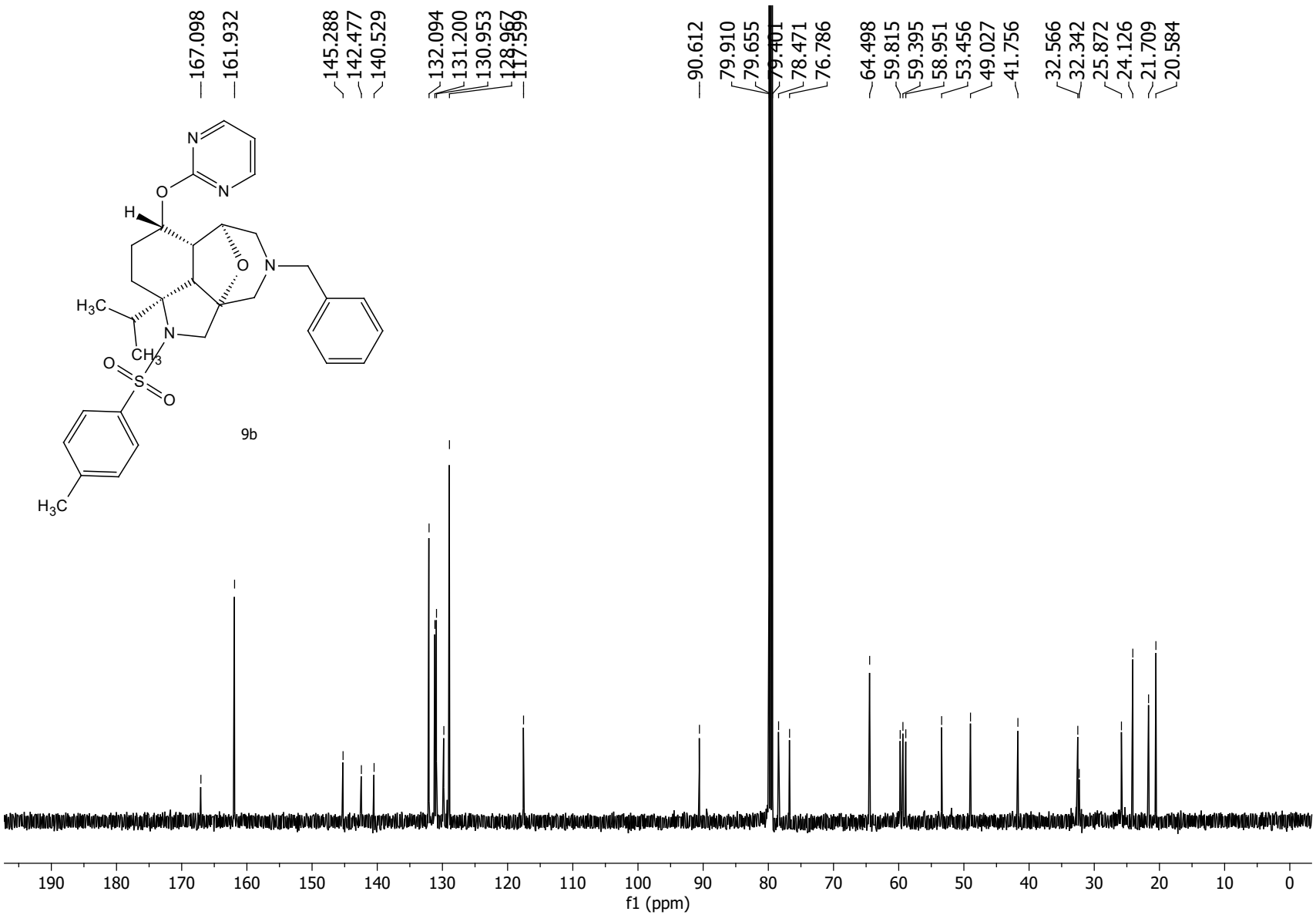
7.238
7.233
7.217
7.216
6.887
6.877
6.868
5.292
4.976
4.786
3.714
3.690
3.562
3.535
3.486
3.460
3.320
3.297
3.039
3.024
2.992
2.973
2.732
2.700
2.640
2.619
2.599
2.585
2.572
2.412
2.388
2.372
2.296
2.274
2.249
2.246
2.227
2.223
2.165
1.581
1.499
1.493
1.064
1.050
1.036
9b
0.89
0.88
2.07
3.81
3.02
1.03
1.00
0.94
1.01
1.08
1.14
0.96
0.97
1.01
1.07
0.98
1.20
1.08
3.14
1.10
1.15
1.19
2.89
6.52
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
0.0
f1 (ppm)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130.953
128.967
117.599
90.612
79.910
79.655
79.401
78.471
76.786
64.498
59.815
59.395
58.951
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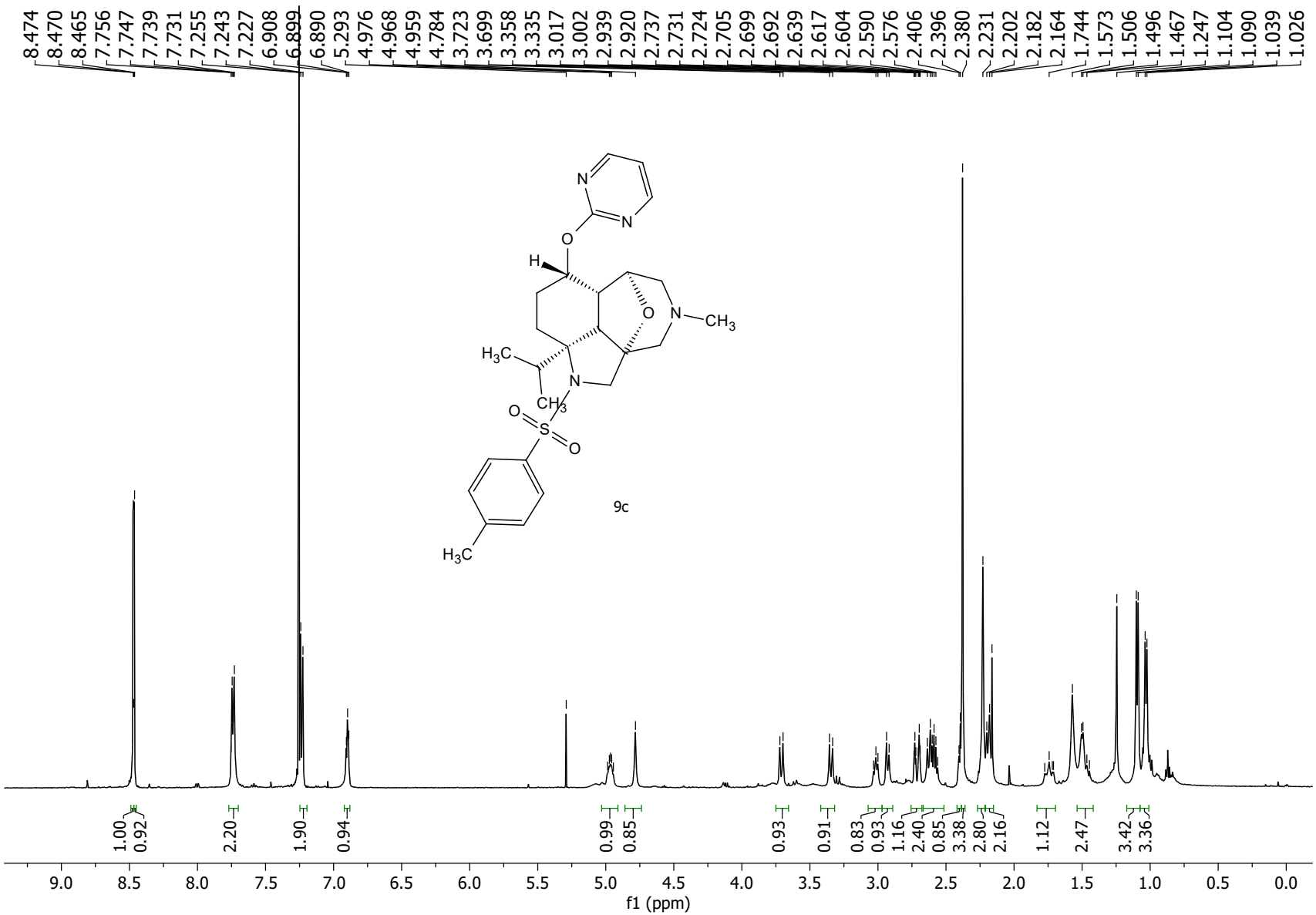
49.027
41.756
32.566
32.342
25.872
24.126
21.709
20.584
9b
f1 (ppm)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7.255
7.243
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6.908
6.899
6.890
5.293
4.976
4.968
4.959
4.784
3.723
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3.358
3.335
3.017
3.002
2.939
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2.731
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2.705
2.699
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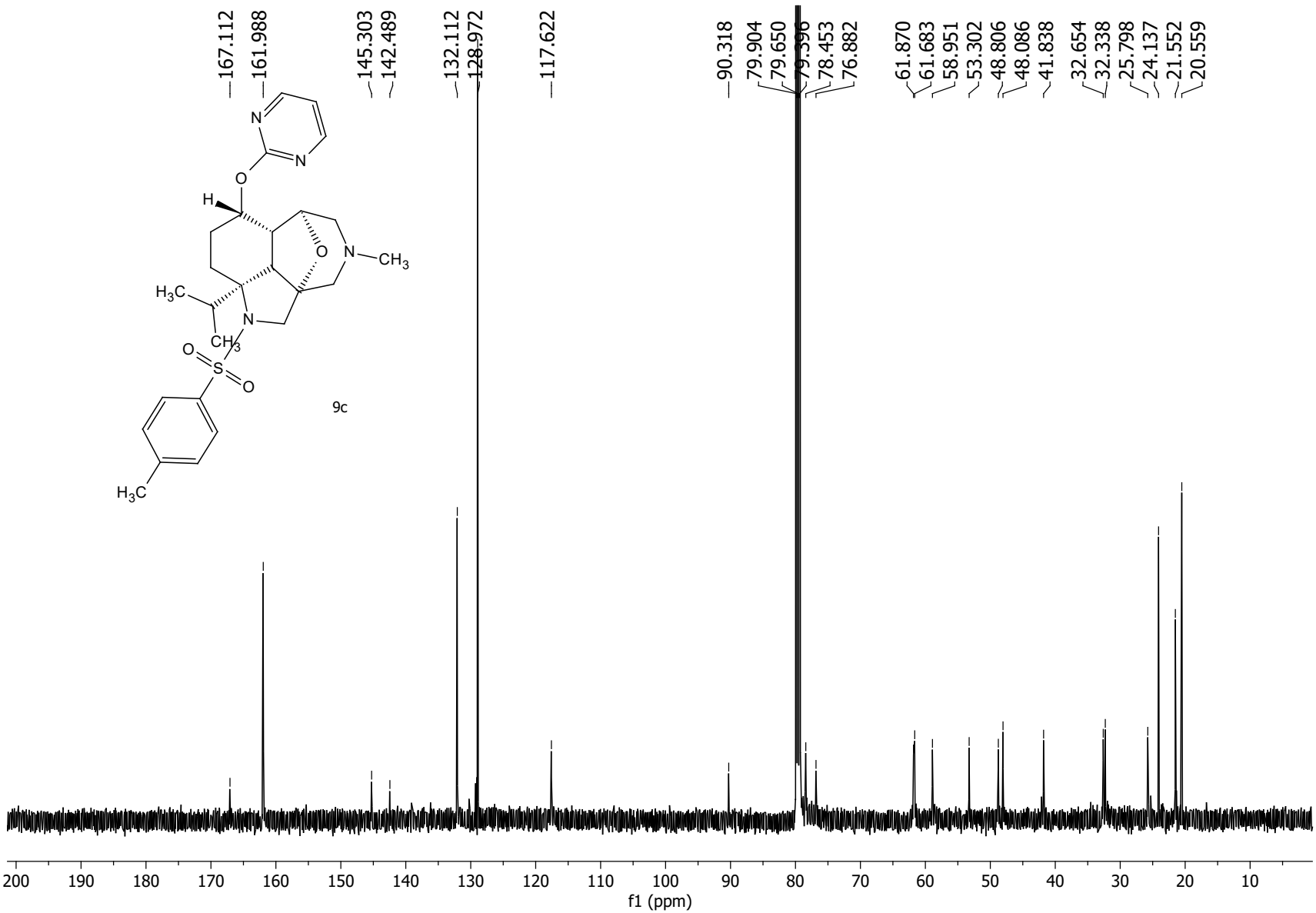
2.639
2.617
2.604
2.590
2.576
2.406
2.396
2.380
2.231
2.202
2.182
2.164
1.744
1.573
1.506
1.496
1.467
1.247
1.104
1.090
1.039
1.026
9c
1.00
0.92
2.20
1.90
0.94
0.99
0.85
0.93
0.91
0.83
0.93
1.16
2.40
0.85
3.38
2.80
2.16
1.12
2.47
3.42
3.36
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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90.318
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79.396
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76.882
61.870
61.683
58.951
53.302
48.806
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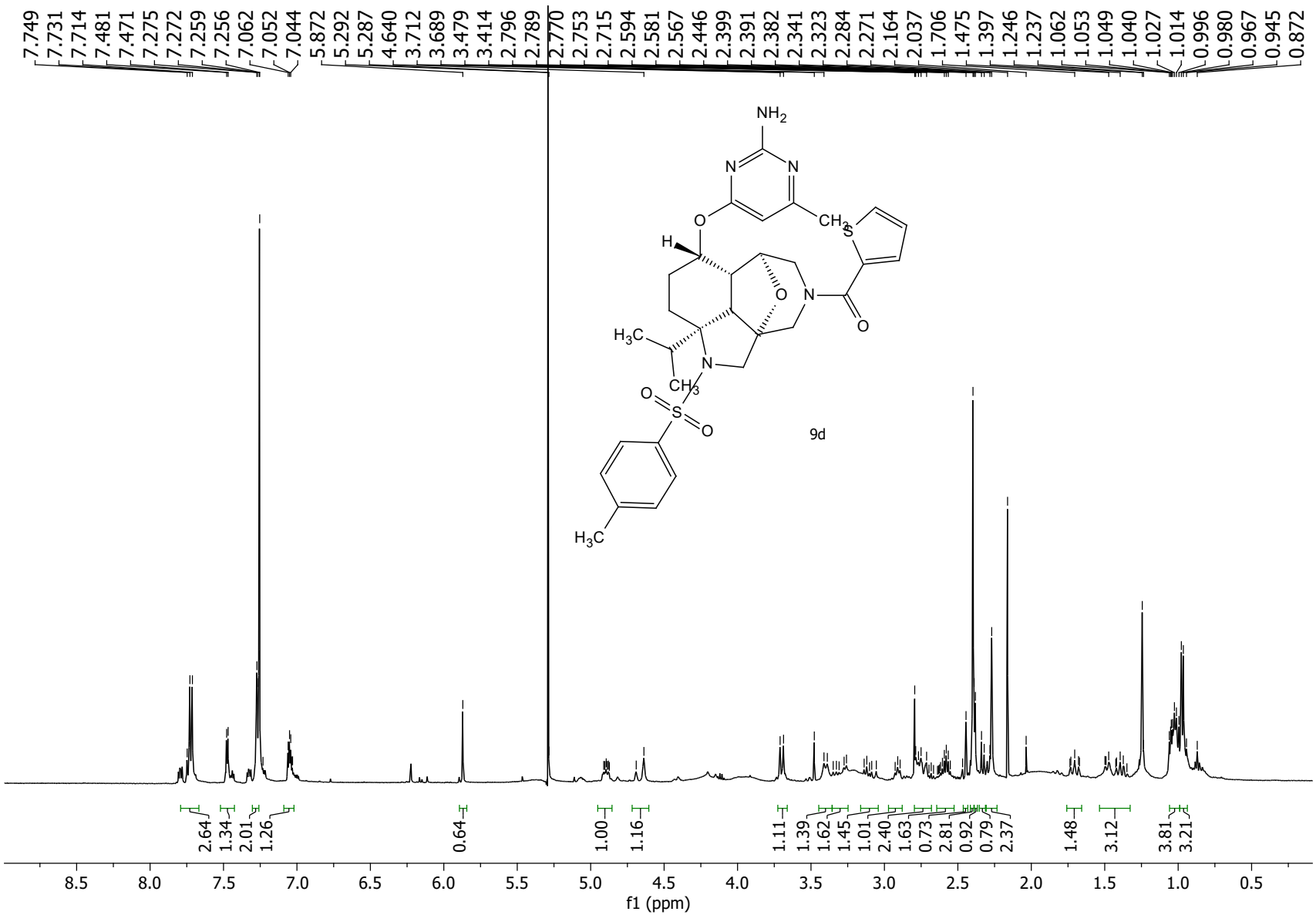
41.838
32.654
32.338
25.798
24.137
21.552
20.559
9c
f1 (ppm)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7.259
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7.062
7.052
7.044
5.872
5.292
5.287
4.640
3.712
3.689
3.479
3.414
2.796
2.789
2.770
2.753
2.715
2.594
2.581
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2.341
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2.284
2.271
2.164
2.037
1.706
1.475
1.397
1.246
1.237
1.062
1.053
1.049
1.040
1.027
1.014
0.996
0.980
0.967
0.945
0.872
NH2
N
N
O
CH3
S
H
O
N
O
H3C
N
CH3
O
S
O
O
H3C
9d
2.64
1.34
2.01
1.26
0.64
1.00
1.16
1.11
1.39
1.62
1.45
1.01
2.40
1.63
0.73
2.81
0.92
0.79
2.37
1.48
3.12
3.81
3.21
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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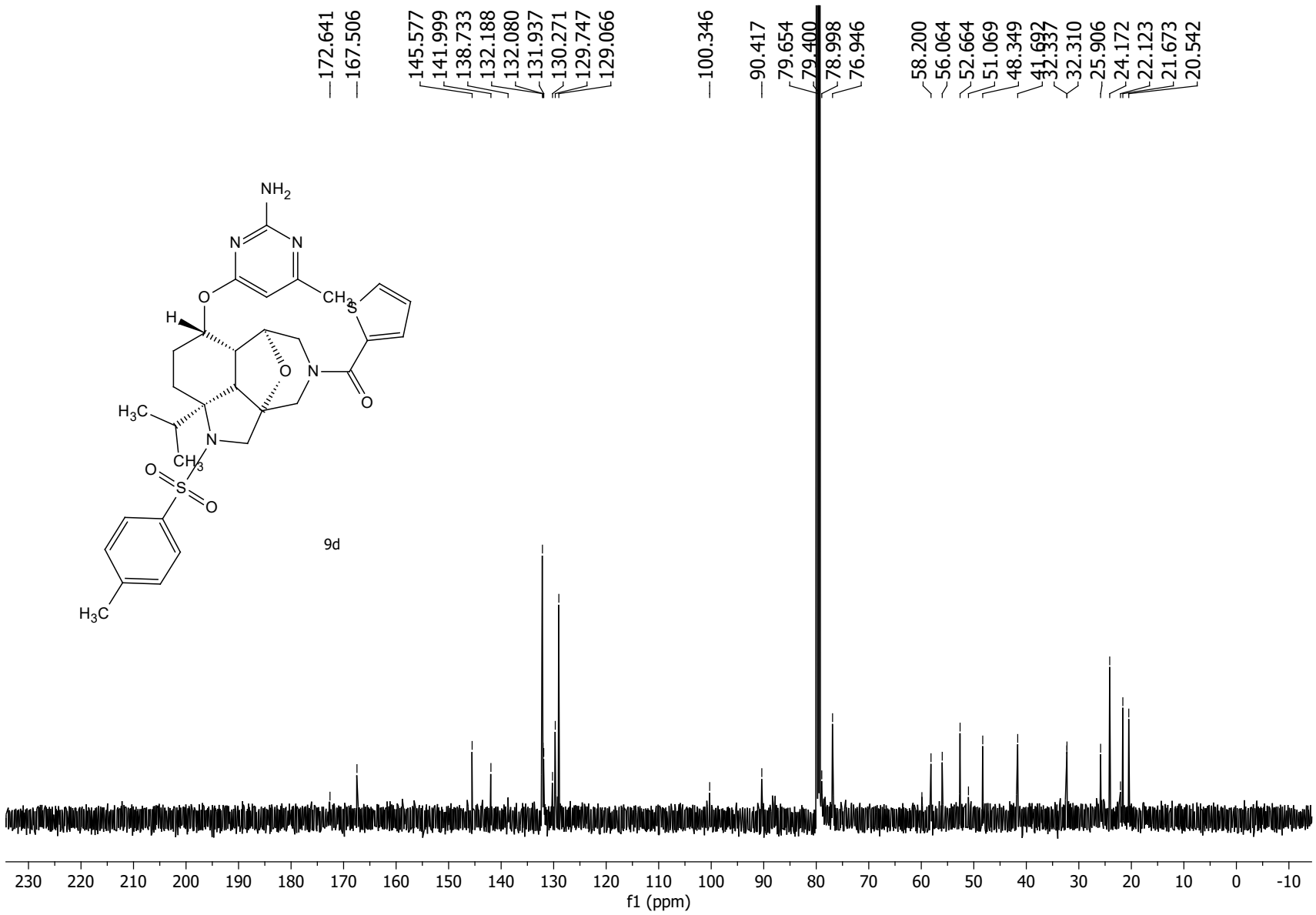
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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131.937
130.271
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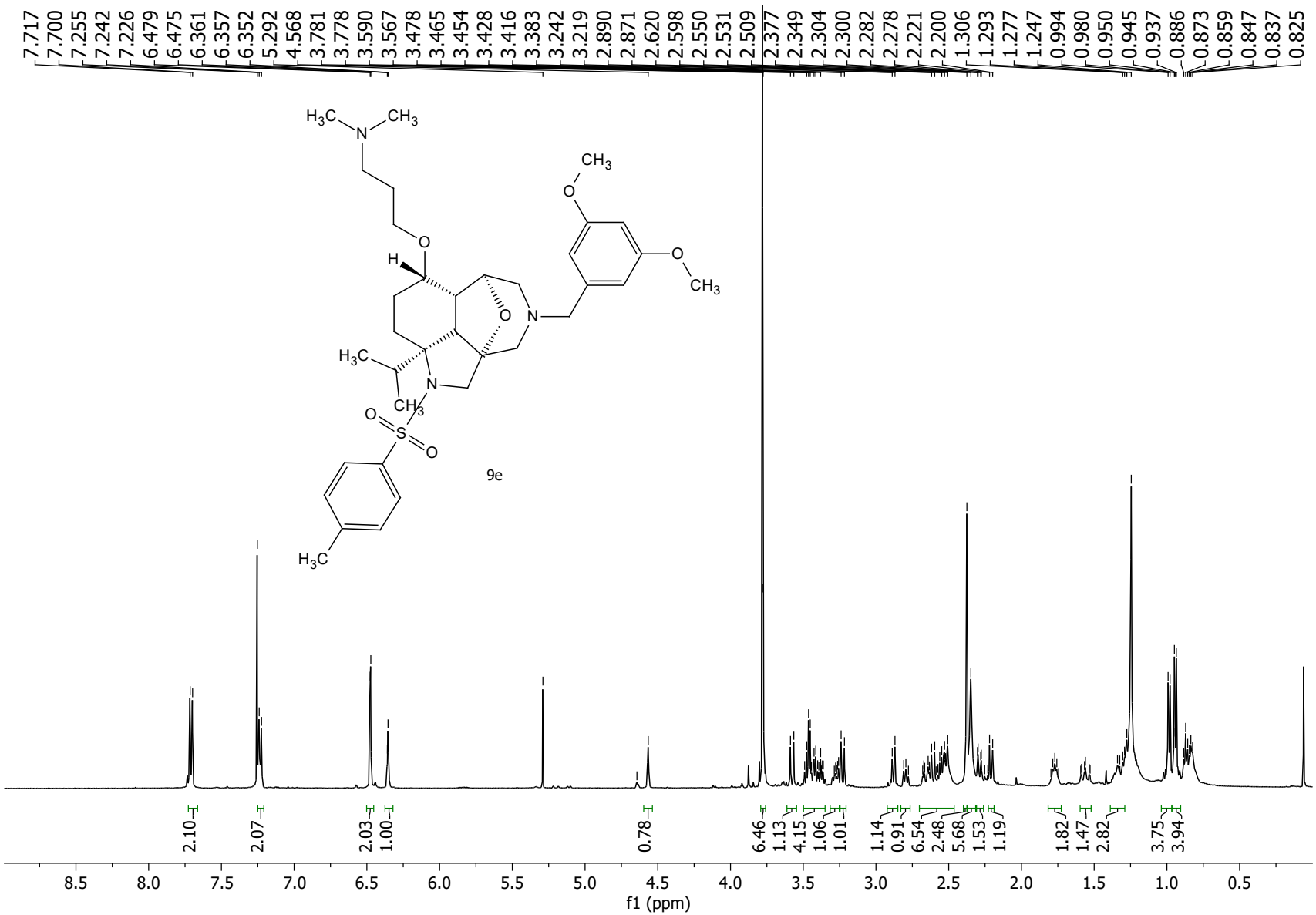
129.066
100.346
90.417
79.654
79.400
78.998
76.946
58.200
56.064
52.664
51.069
48.349
41.692
32.337
32.310
25.906
24.172
22.123
21.673
20.542
NH2
N
N
O
CH3
S
H
O
N
O
H3C
N
CH3
O
S
O
H3C
9d
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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7.700
7.255
7.242
7.226
6.479
6.475
6.361
6.357
6.352
5.292
4.568
3.781
3.778
3.590
3.567
3.478
3.465
3.454
3.428
3.416
3.383
3.242
3.219
2.890
2.871
2.620
2.598
2.550
2.531
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2.377
2.349
2.304
2.300
2.282
2.278
2.221
2.200
1.306
1.293
1.277
1.247
0.994
0.980
0.950
0.945
0.937
0.886
0.873
0.859
0.847
0.837
0.825
9e
2.10
2.07
2.03
1.00
0.78
6.46
1.13
4.15
1.06
1.01
1.14
0.91
6.54
2.48
5.68
1.53
1.19
1.82
1.47
2.82
3.75
3.94
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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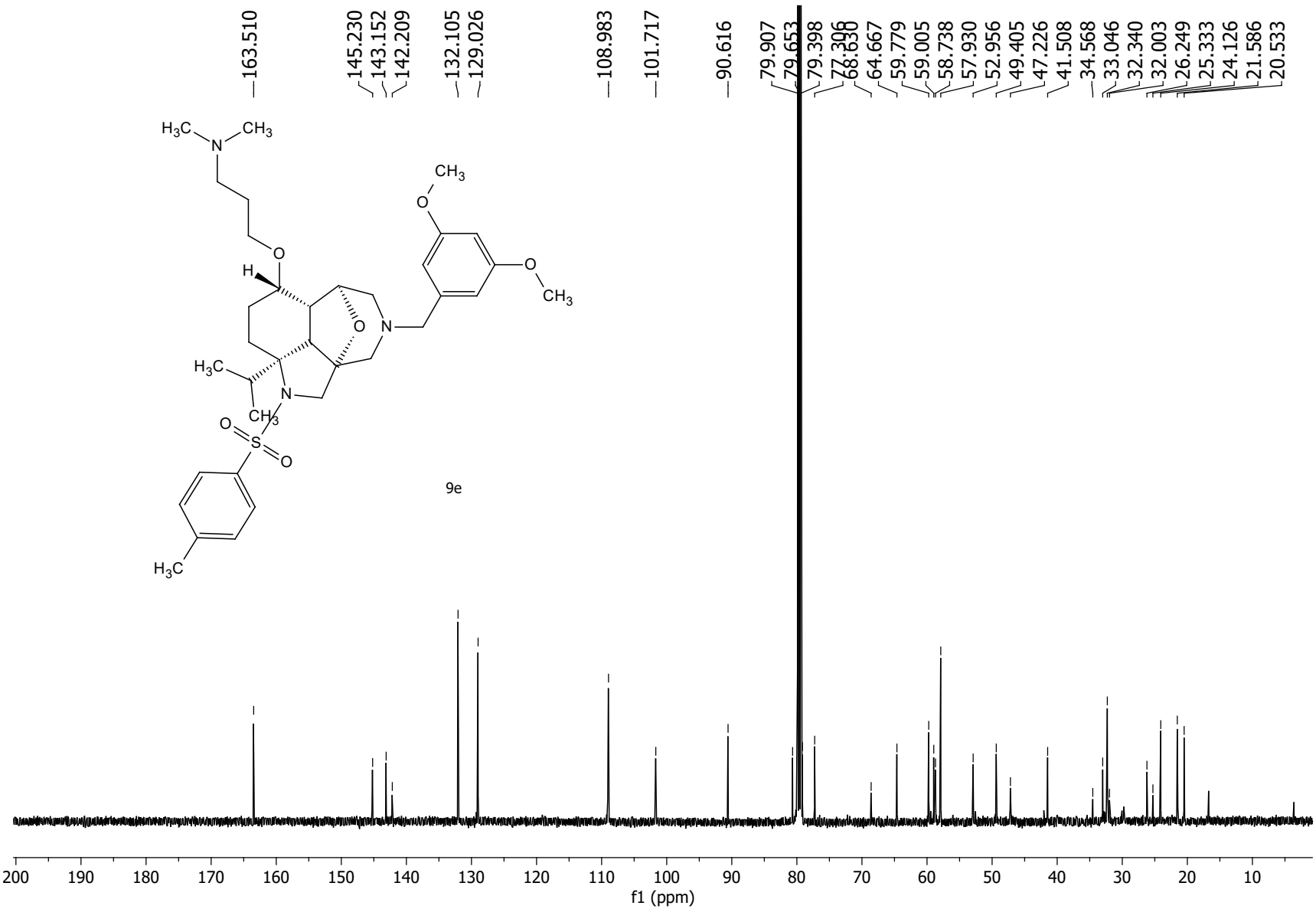
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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143.152
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132.105
129.026
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101.717
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79.907
79.653
79.398
77.306
68.830
64.667
59.779
59.005
58.738
57.930
52.956
49.405
47.226
41.508
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26.249
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20.533
9e
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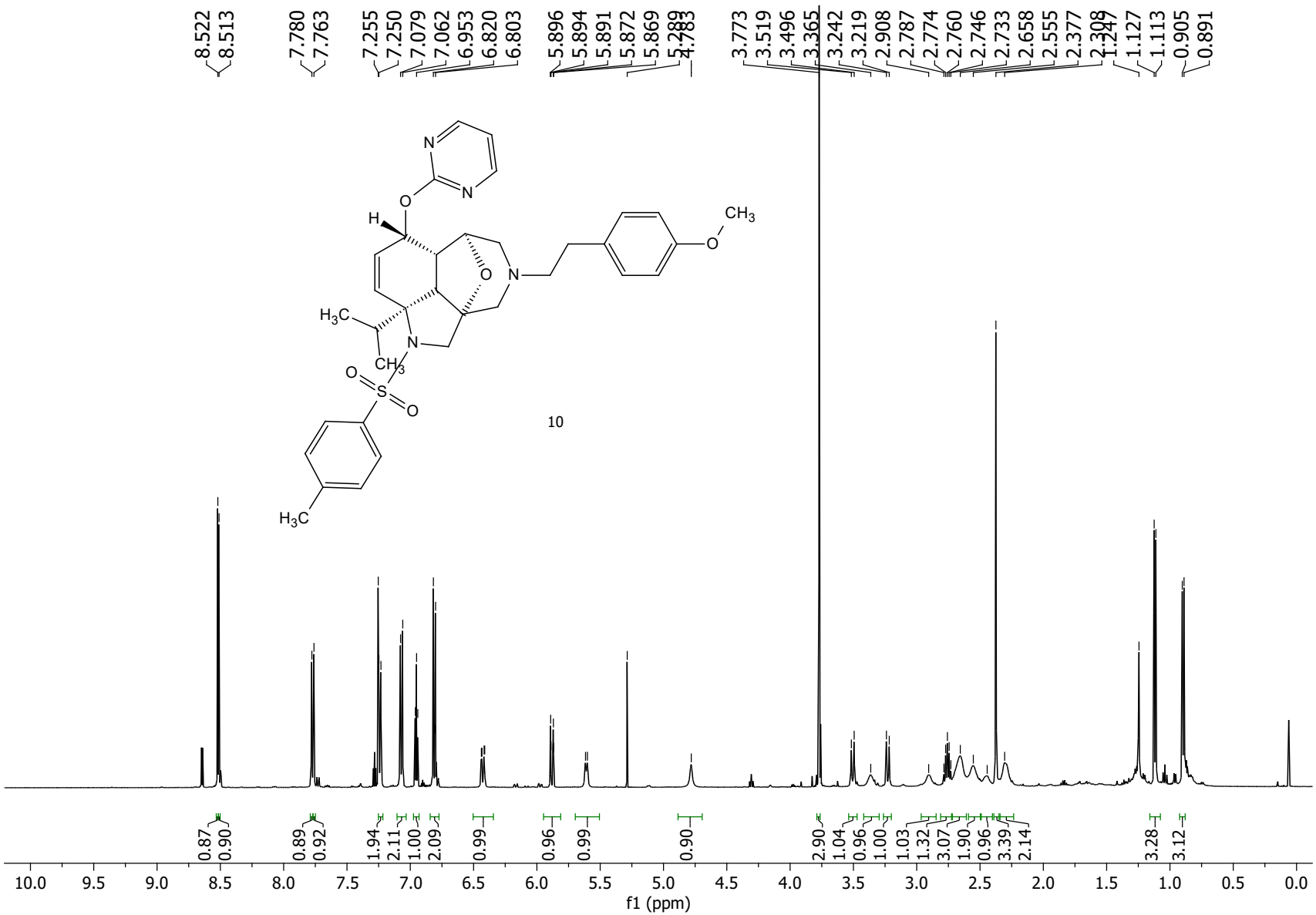
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5.894
5.891
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5.869
4.783
3.773
3.519
3.496
3.365
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3.219
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2.787
2.774
2.760
2.746
2.733
2.658
2.555
2.377
1.247
1.127
1.113
0.905
0.891
10
0.87
0.90
0.89
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1.94
2.11
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2.09
0.99
0.96
0.99
0.90
2.90
1.04
0.96
1.00
1.03
1.32
3.07
1.90
0.96
3.39
2.14
3.28
3.12
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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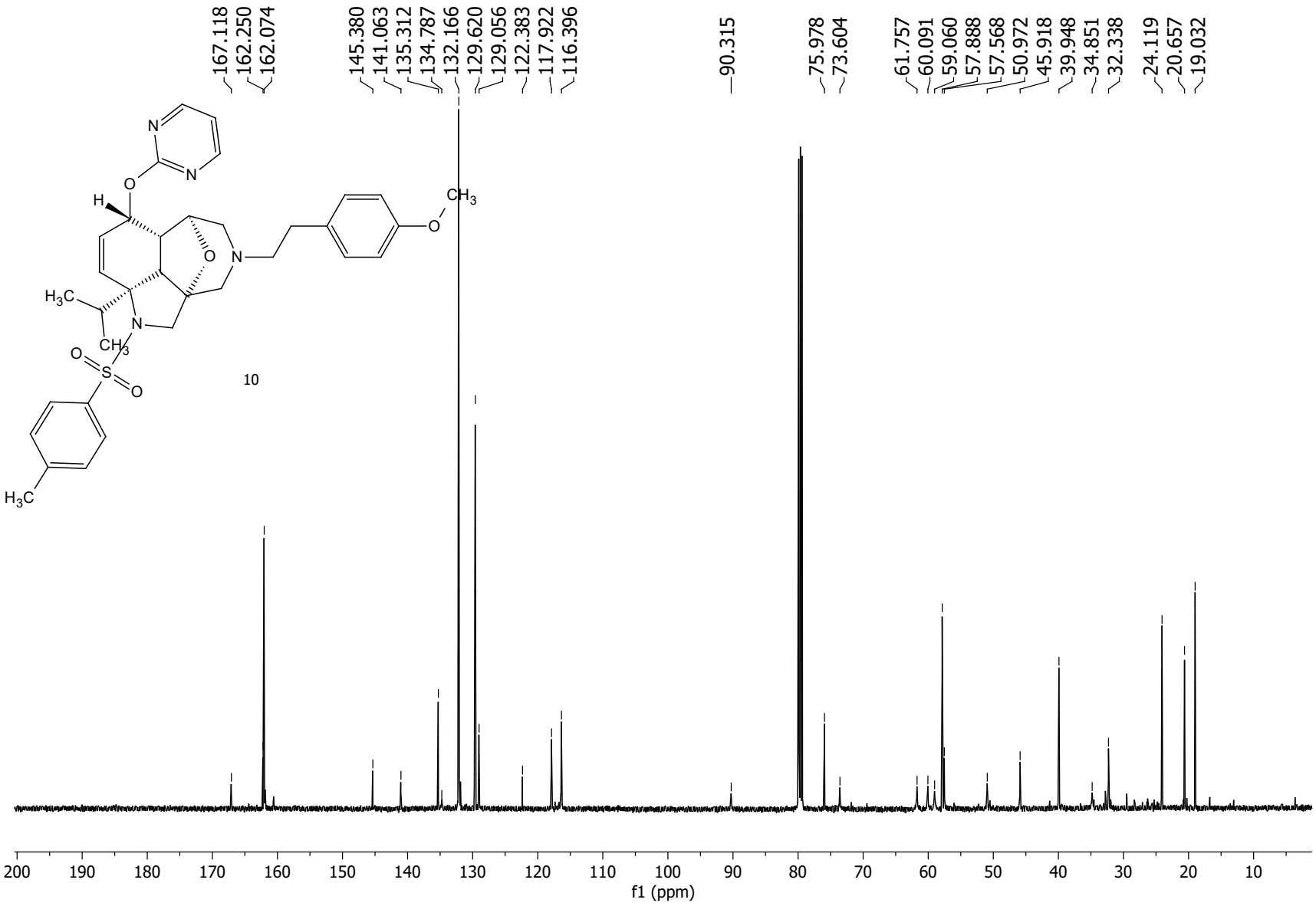
f1 (ppm)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90.315
75.978
73.604
61.757
60.091
59.060
57.888
57.568
50.972
45.918
39.948
34.851
32.338
24.119
20.657
19.032
10
200
190
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120
110
100
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80
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60
50
40
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20
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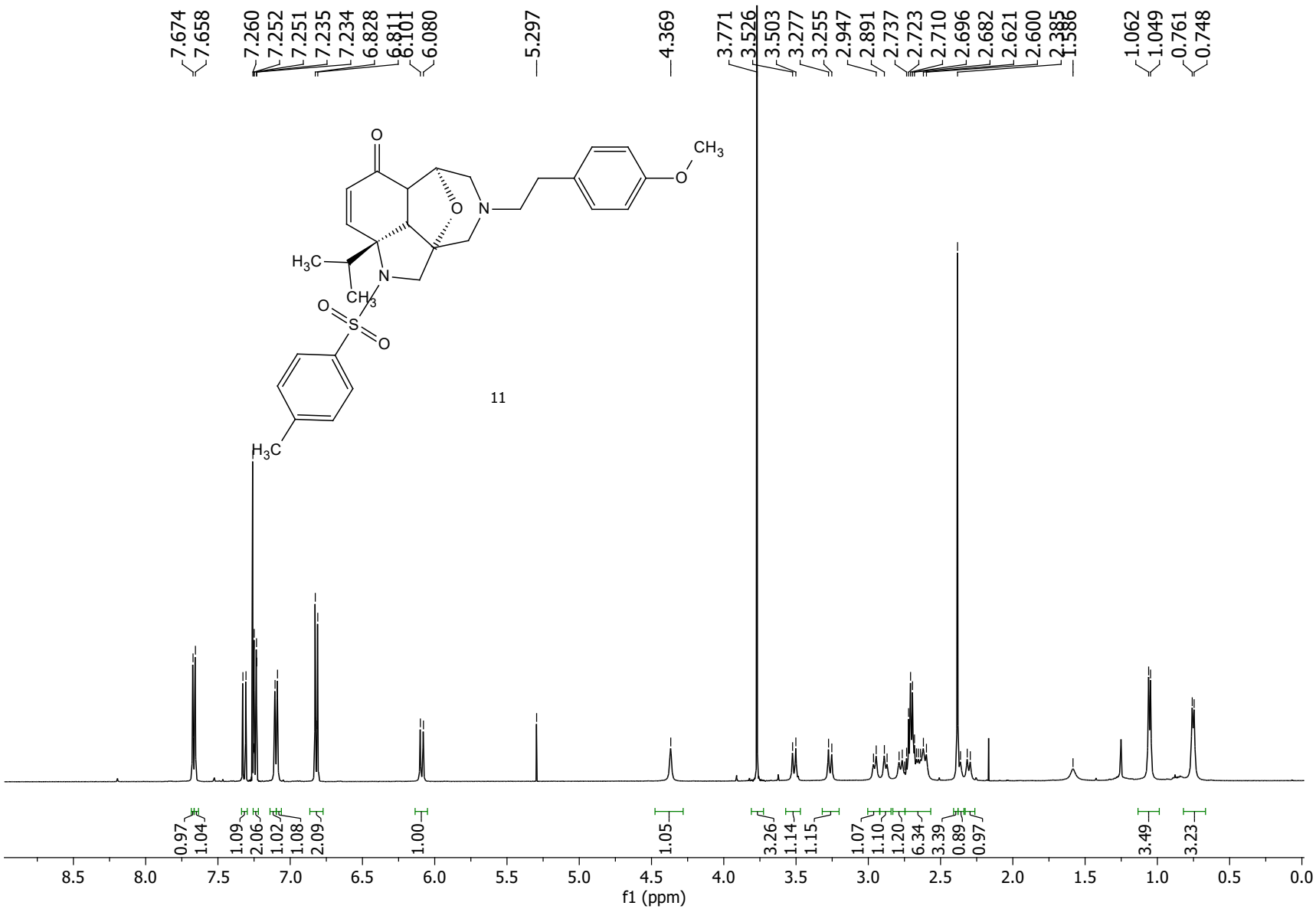
f1 (ppm)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7.252
7.251
7.235
7.234
6.828
6.811
6.101
6.080
5.297
4.369
3.771
3.526
3.503
3.277
3.255
2.947
2.891
2.737
2.723
2.710
2.696
2.682
2.621
2.600
2.385
1.586
1.062
1.049
0.761
0.748
O
CH3
O
O
N
H3C
N
CH3
O
S
O
H3C
11
0.97
1.04
1.09
2.06
1.02
1.08
2.09
1.00
1.05
3.26
1.14
1.15
1.07
1.10
1.20
6.34
3.39
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0.97
3.49
3.23
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7.0
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6.0
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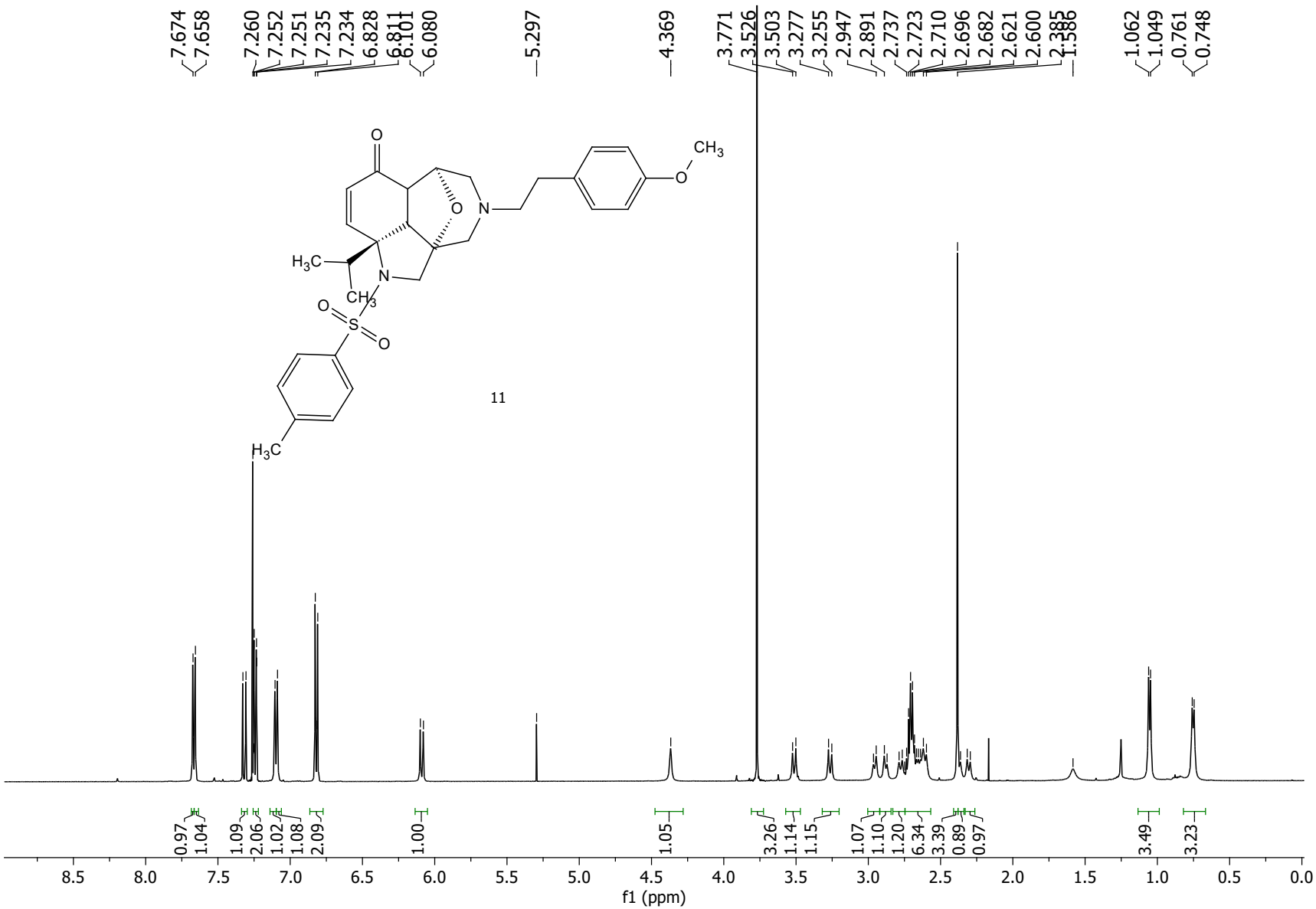
f1 (ppm)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4.369
3.771
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3.255
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2.696
2.682
2.621
2.600
2.385
1.586
1.062
1.049
0.761
0.748
O
CH3
O
O
N
H3C
N
CH3
O
S
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H3C
11
0.97
1.04
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3.26
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3.39
0.89
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2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)