

**Synthesis and biological evaluation of natural *Lachnophyllum* methyl ester,
Lachnophyllum lactone and synthetic analogues thereof**

Supporting information

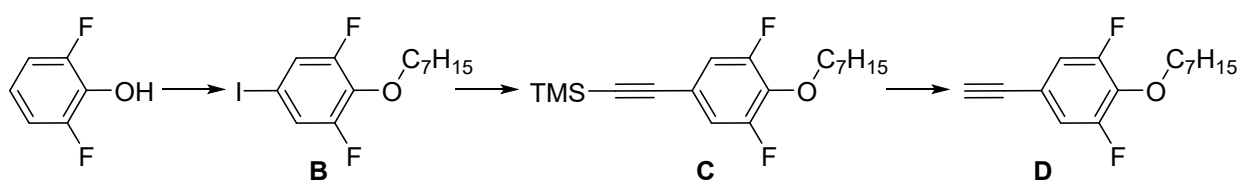
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Chemical synthesis

All reagents were obtained from commercial suppliers and used without any further purification. If not specified, reactions were run under nitrogen atmosphere in oven-dried glassware. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. Toluene, dichloromethane (DCM), tetrahydrofuran (THF), dimethylformamide (DMF) and diethyl ether (Et₂O) were obtained by filtration through a drying column on a filtration system. Thin-layer chromatography (TLC) analyses were performed on precoated, aluminum-backed silica gel (Merck 60 F254). Visualization of the developed chromatogram was performed by UV light (254 nm) and using 10% phosphomolybdic acid in EtOH, or aqueous potassium permanganate (KMnO₄) stain. Flash chromatography columns were performed using flash silica gel (SDS 35-70 μm). Nuclear magnetic resonance spectra were recorded on a Bruker Advance 300 or 400 or 500 MHz spectrometer. Chemical shifts for ¹H NMR spectra are given in parts per million (ppm) with the residual solvent resonance as the reference CHCl₃ (δ = 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet and br = broad), coupling constant in Hz and integration. Chemical shifts for ¹³C NMR spectra are given in ppm using the central peak of CDCl₃ (δ = 77.16 ppm) as the reference. All ¹³C NMR spectra were obtained with complete proton decoupling. Infrared analyses were run on a Thermo-Nicolet Diamond ATR (4 cm⁻¹ of resolution, 16 scans) equipped with a DTGS detector and are reported in reciprocal centimeters (cm⁻¹). High-resolution mass spectrometry (HRMS) was performed on a Thermo-Finnigan MAT 95 XL instrument. Mass spectrometry *m/z* values are given in Dalton units.

Synthesis of 5-ethynyl-1,3-difluoro-2-(heptyloxy)benzene (D):



1,3-Difluoro-2-(heptyloxy)-5-iodobenzene (B)

At 0 °C, NaOH (307 mg, 7.68 mmol, 2 eq.) in H₂O (19 mL) was added dropwise to the reacting mixture containing 2,6-difluorophenol (500 mg, 3.84 mmol, 1 eq.), KI (957 mg, 5.76 mmol, 1.5 eq.), I₂ (1.4 g, 5.76 mmol, 1.5 eq.) in water (19 mL). The resulting solution was stirred at rt for 4 h. After completion of the reaction, sat. NH₄Cl aqueous solution and sat. Na₂S₂O₃ aqueous solution were added to quench the reaction. The resulting solution was extracted with diethyl ether. Combined organic layers were washed with sat. Aqueous NaCl solution and dried over MgSO₄, concentrated under reduced pressure and used without further purification into the next step. Cs₂CO₃ (1.26 g, 3.87 mmol, 1 eq.) was added to a solution of the iodophenol in DMF (10 mL). After stirring at rt for 30 min, TBAI (59 mg, 5 mol%) and heptylbromide (608 μL, 3.87 mmol, 1 eq.) were added. The resulting mixture was stirred at 80 °C for 4 h. After completion of the reaction, water was added to quench the reaction. The resulting solution was extracted with diethyl ether. Combined organic layers were washed with sat. NaCl aqueous solution and dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel in pentane. The iodobenzene **B** (816 mg, 71% yield) was isolated as a pale-yellow oil. ¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.31-7.21 (m, 2H), 4.13 (t, *J* = 6.6 Hz, 2H), 1.84-1.68 (m, 2H), 1.52-1.25 (m, 8H), 0.96-0.87 (m, 3H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm) 156.0 (dd, *J* = 253.2, 6.2 Hz), 136.2 (m), 122.0-121.4 (m), 82.1 (t, *J* = 9.7 Hz), 74.9 (t, *J* = 3.0 Hz), 31.7, 29.9, 28.9, 25.5, 22.6, 14.1. ¹⁹F {¹H} NMR (282 MHz, CDCl₃) δ (ppm) -126.3. HRMS (DCI-CH₄): calcd for C₁₃H₁₇OF₂I [M]⁺: 354.0292 *m/z*, found: 354.0302 *m/z*.

((3,5-Difluoro-4-(heptyloxy)phenyl)ethynyl)trimethylsilane (C)

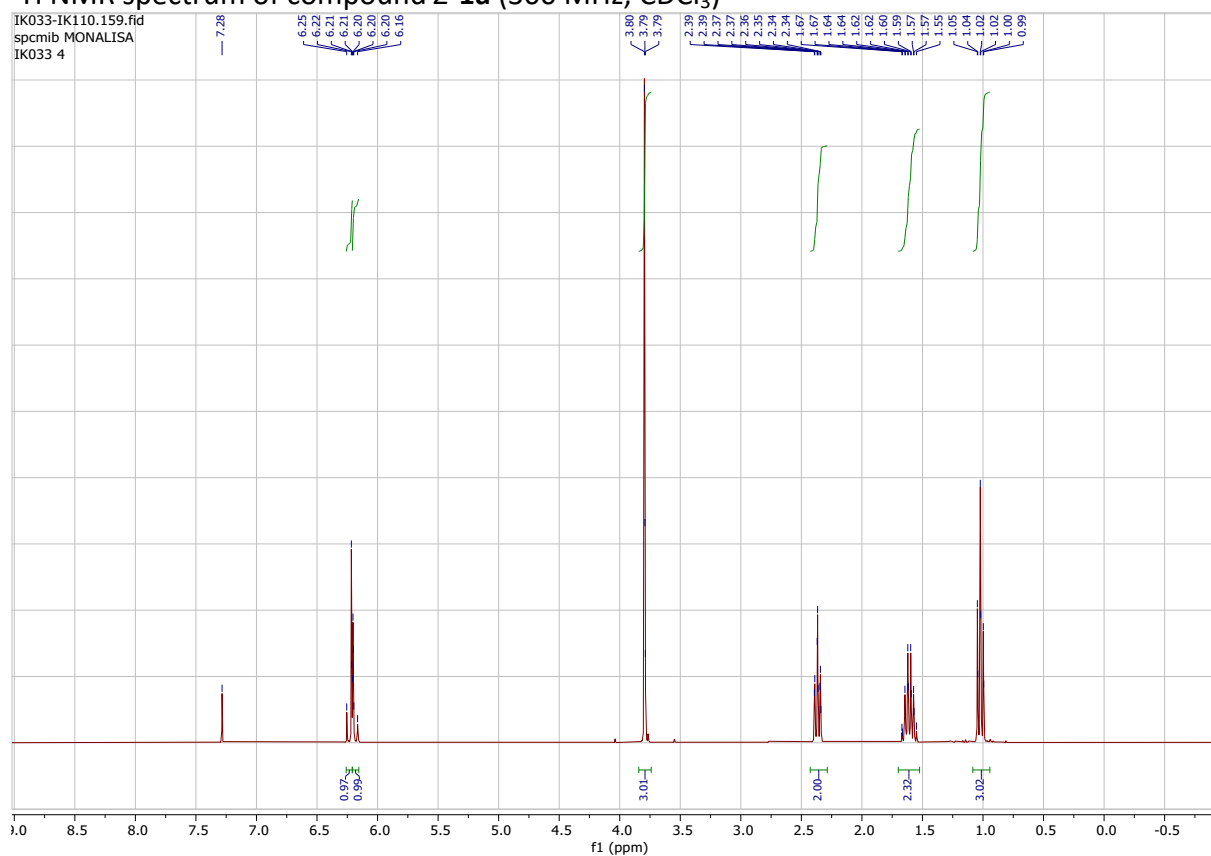
Anhydrous THF was degassed with nitrogen over 30 min before using. To a solution of aryl iodide **B** (400 mg, 1.13 mmol, 1 eq.) in anhydrous degassed THF (0.5M) in a two-necked flask under nitrogen, PdCl₂(PPh₃)₂ (31.7 mg, 0.045 mmol, 0.04 eq.), CuI (8.6 mg, 0.045 mmol 0.04 eq.) and diisopropylamine (112 μL, 0.80 mmol, 0.7 eq.) were added at 0 °C, followed immediately by trimethylsilyl acetylene (114 μL, 0.80 mmol, 0.7 eq.). The mixture was stirred over 2 h and quenched with sat. NH₄Cl aqueous solution. Aqueous layer was extracted with diethyl ether and combined organic layers were dried with MgSO₄ and concentrated under reduced pressure to give the silylated product **C** as yellow oil (301 mg, 82% yield). ¹H NMR (300 MHz, CDCl₃) δ (ppm) 7.09-6.93 (m, 2H), 4.16 (t, *J* = 6.6 Hz, 2H), 1.77 (q, *J* = 14.2, 6.8 Hz, 2H), 1.51-1.41 (m, 2H), 1.39-1.26 (m, 6H), 0.91 (t, *J* = 35.1 Hz, 3H), 0.26 (s, 9H).

5-Ethynyl-1,3-difluoro-2-(heptyloxy)benzene (D)

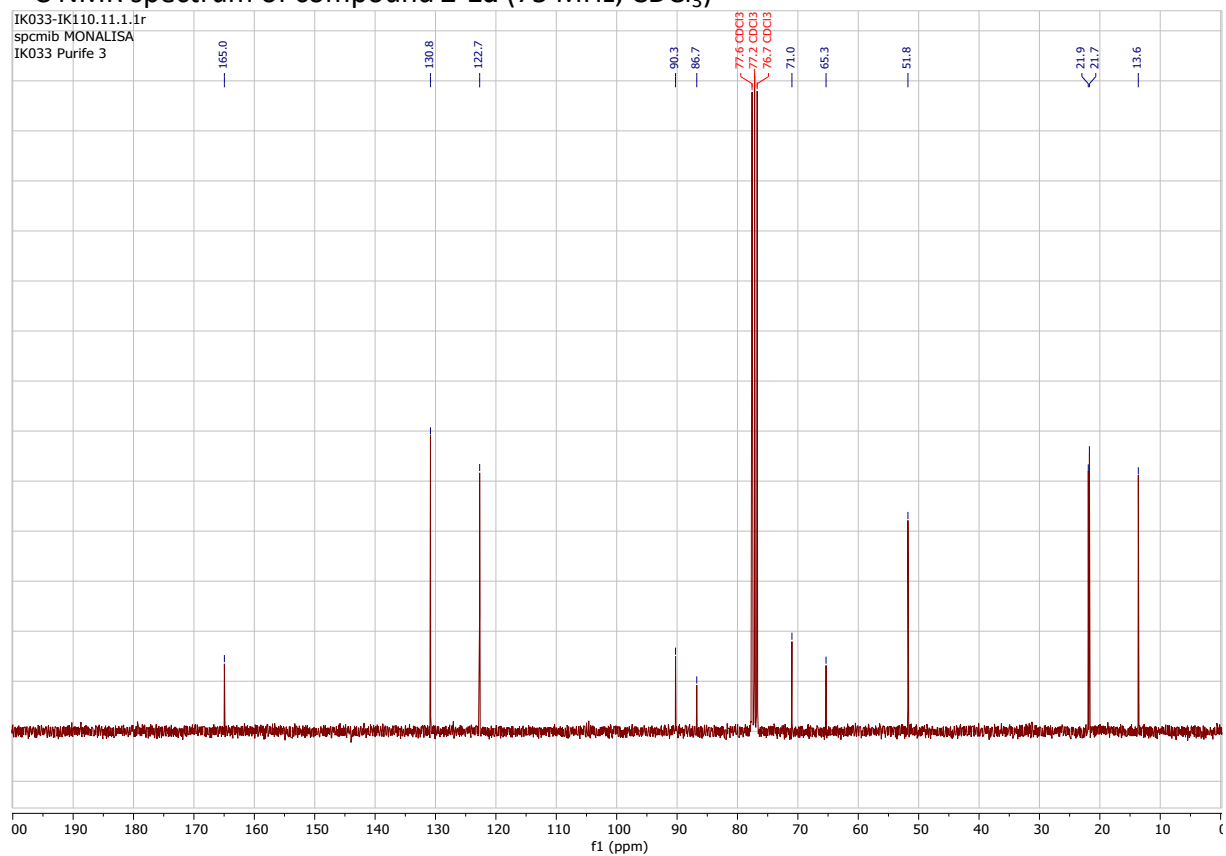
To a stirred solution of silylated alkyne **C** (300 mg, 0.924 mmol, 1 eq.) in methanol (0.06 M), was added K_2CO_3 (38.3 mg, 0.28 mmol, 0.3 eq) and the reaction mixture was warm at rt for 2h. The mixture was quenched with dichloromethane and water. The organic layer was extracted with dichloromethane (3x), dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel with 100% pentane to give alkyne aryl **D** (132 mg, 56% yield) 1H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.09-6.97 (m, 2H), 4.17 (tt, $J = 6.6, 1.0$ Hz, 2H), 3.09 (s, 1H), 1.77 (q, 2H), 1.54-1.41 (m, 2H), 1.41-1.24 (m, 6H), 0.91 (t, $J = 35.1$ Hz, 3H), ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) 155.6 (dd, $J = 248.6, 6.8$ Hz), 137.2 (t, $J = 14.1$ Hz), 116.5-115.77 (m, 3C), 81.5 (t, $J = 3.5$ Hz), 78.1, 74.9 (t, $J = 3.2$ Hz), 32.0, 30.1, 29.1, 25.7, 22.7, 14.2. ^{19}F $\{^1H\}$ NMR (282 MHz, $CDCl_3$) δ (ppm) -127.7. MS (DCI- NH_3): $C_{15}H_{22}F_2NO$ $[M+NH_4]^+$: 270.2 m/z .

NMR spectra

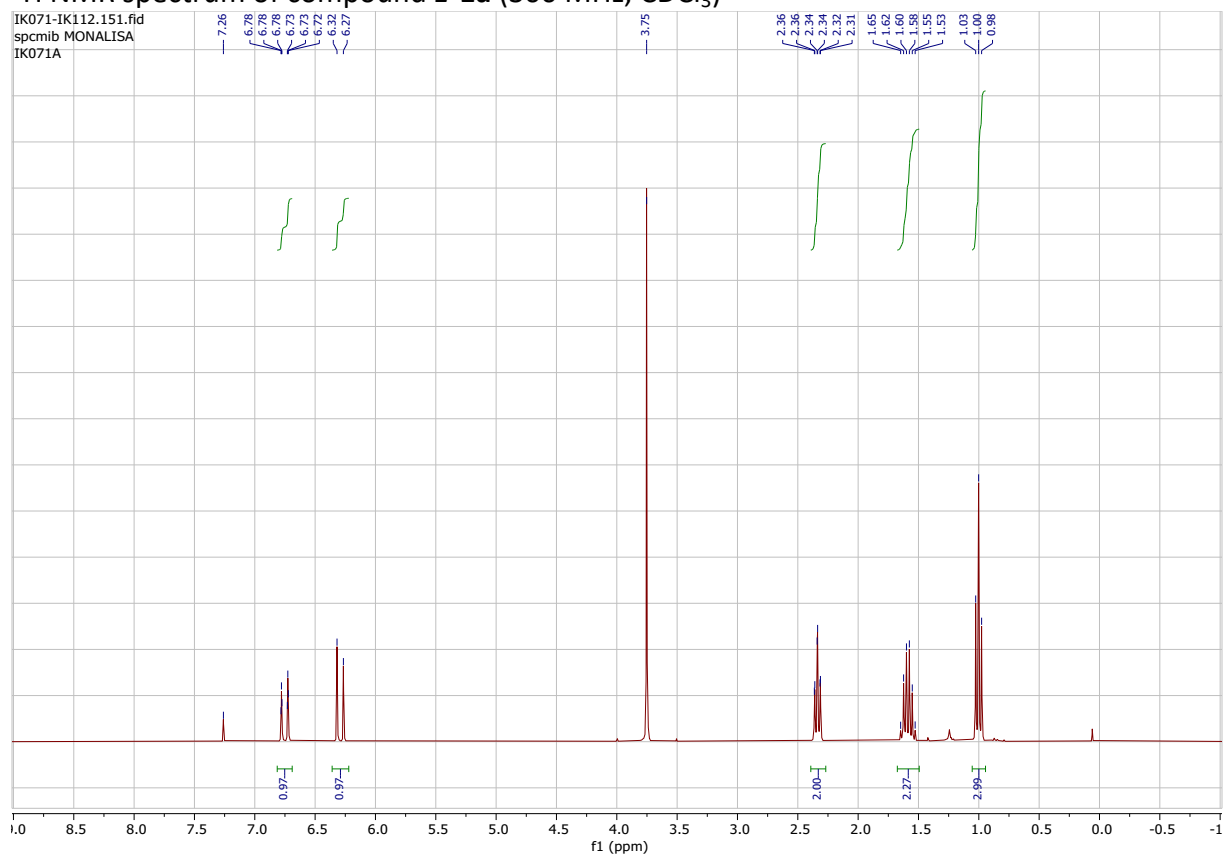
^1H NMR spectrum of compound Z-1a (300 MHz, CDCl_3)



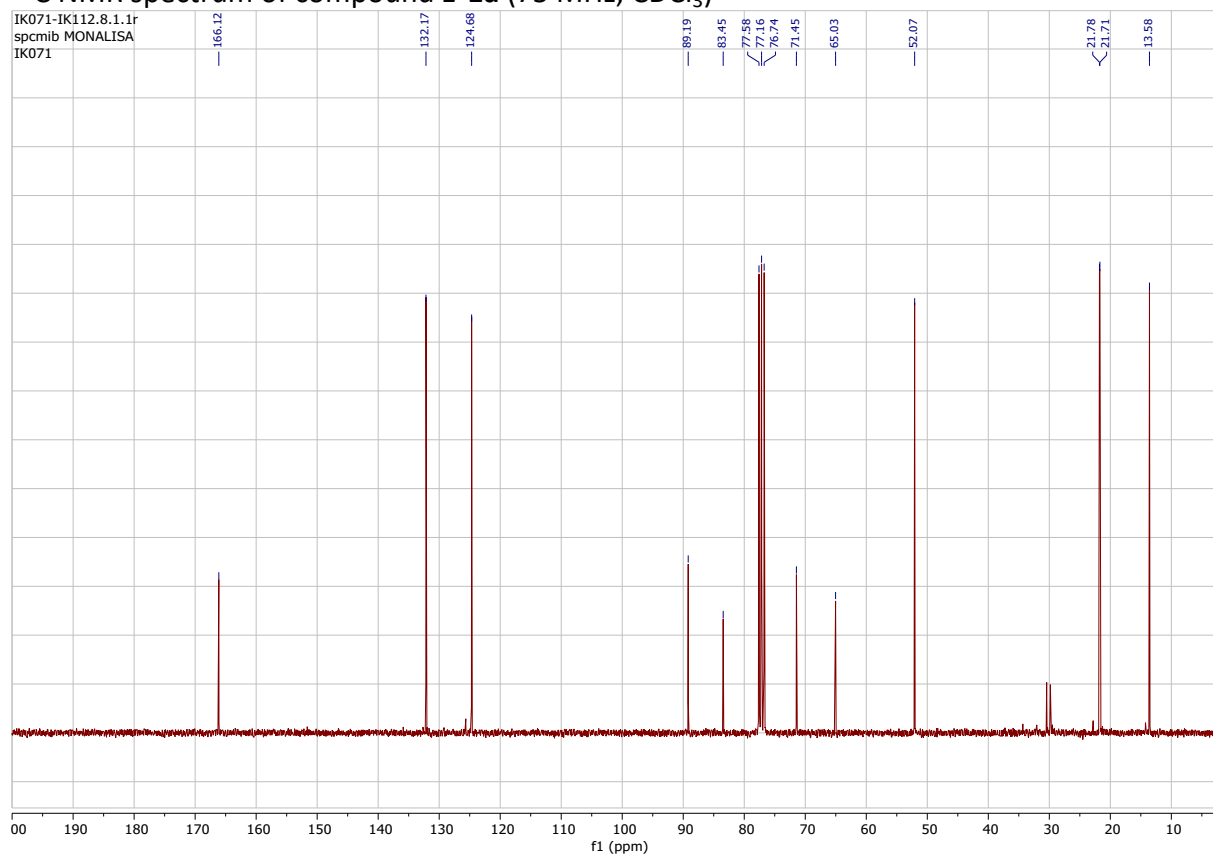
^{13}C NMR spectrum of compound Z-1a (75 MHz, CDCl_3)



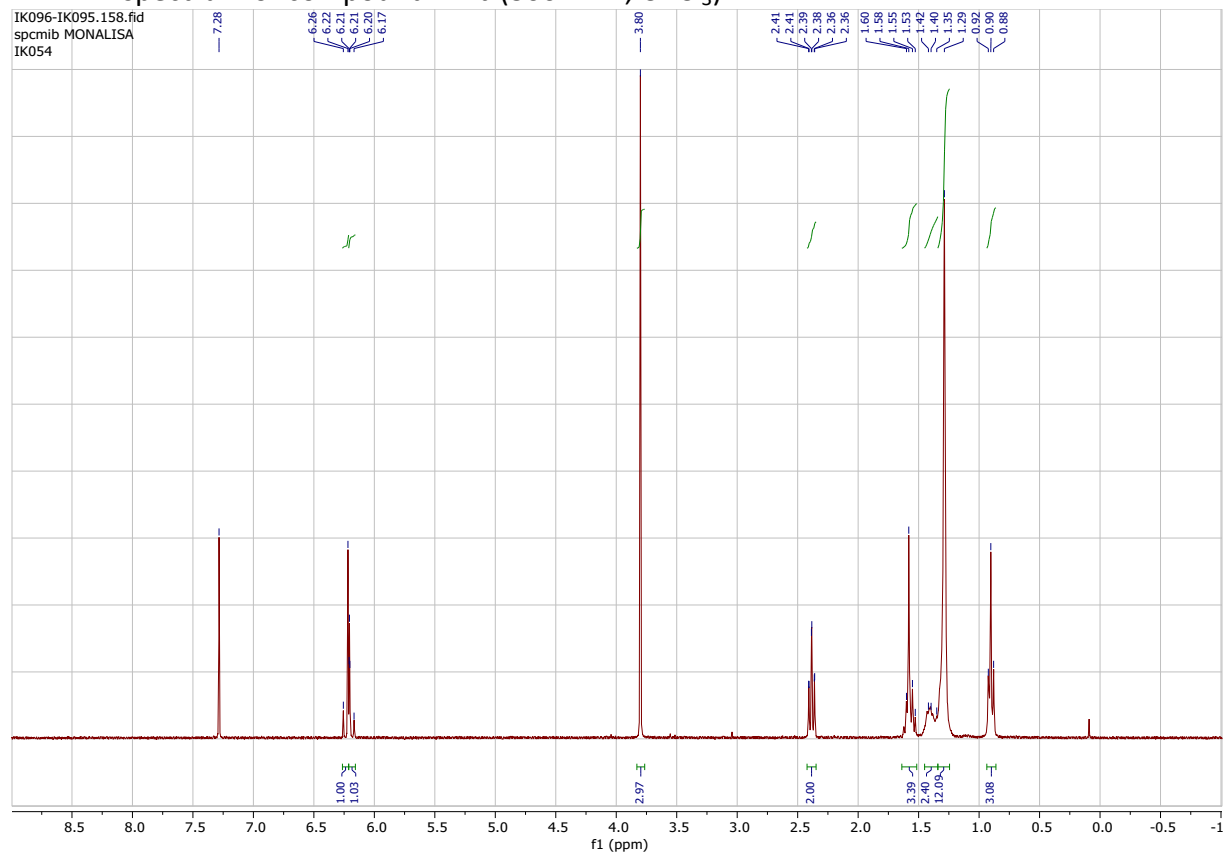
¹H NMR spectrum of compound *E-1a* (300 MHz, CDCl₃)



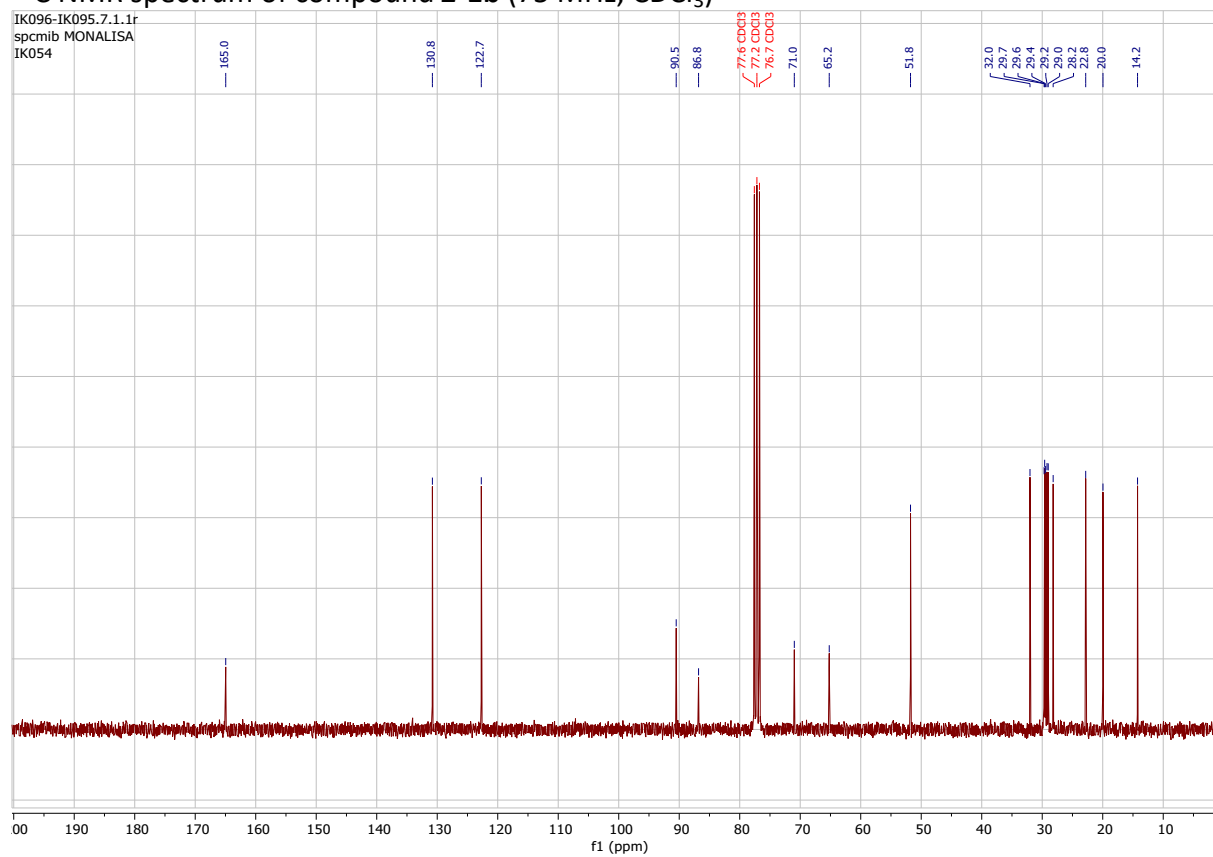
¹³C NMR spectrum of compound *E-1a* (75 MHz, CDCl₃)



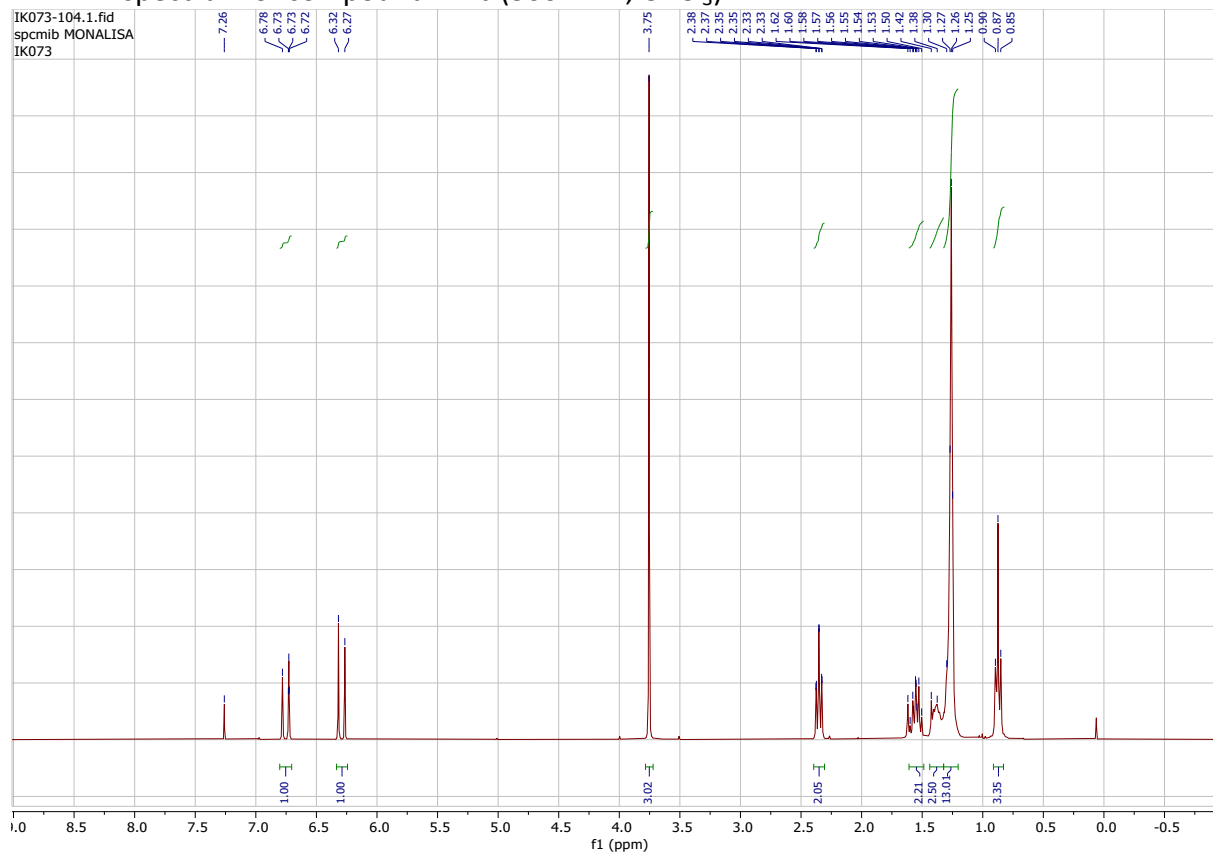
¹H NMR spectrum of compound Z-1b (300 MHz, CDCl₃)



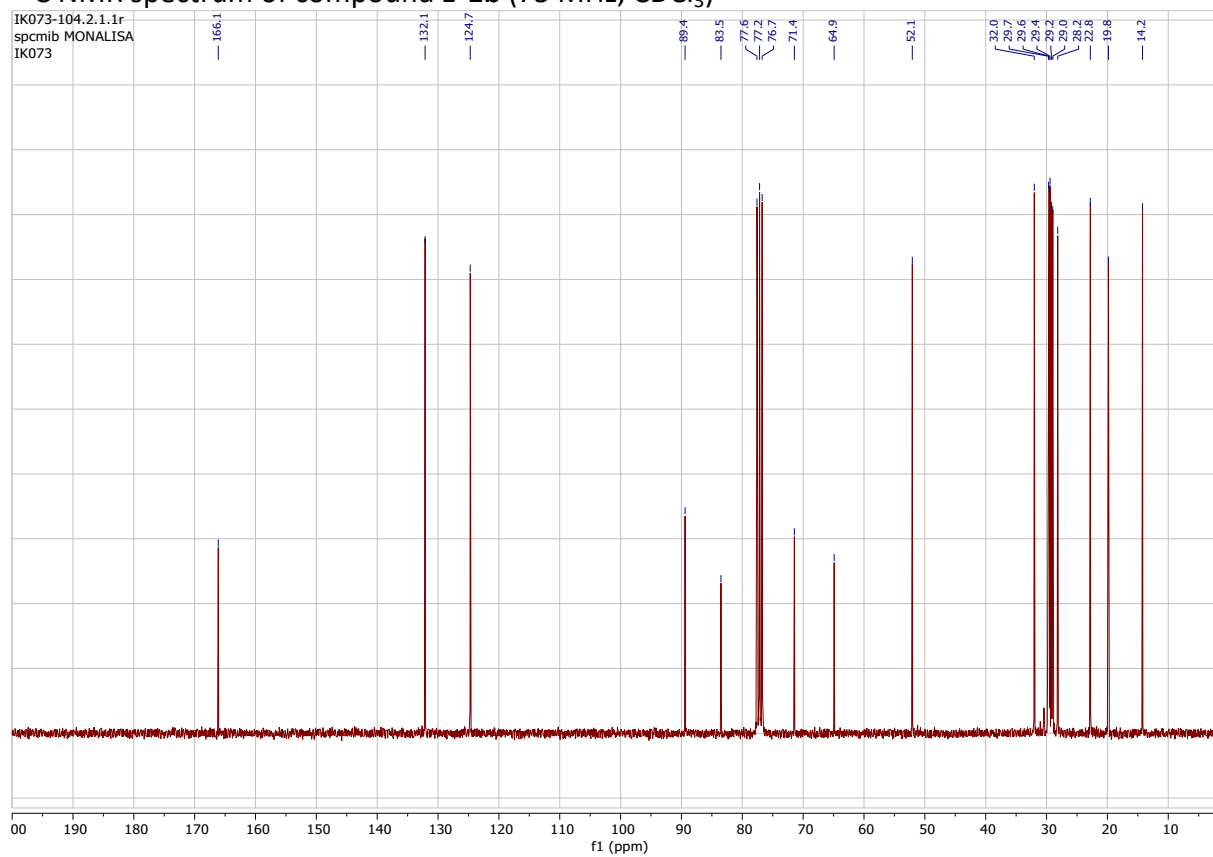
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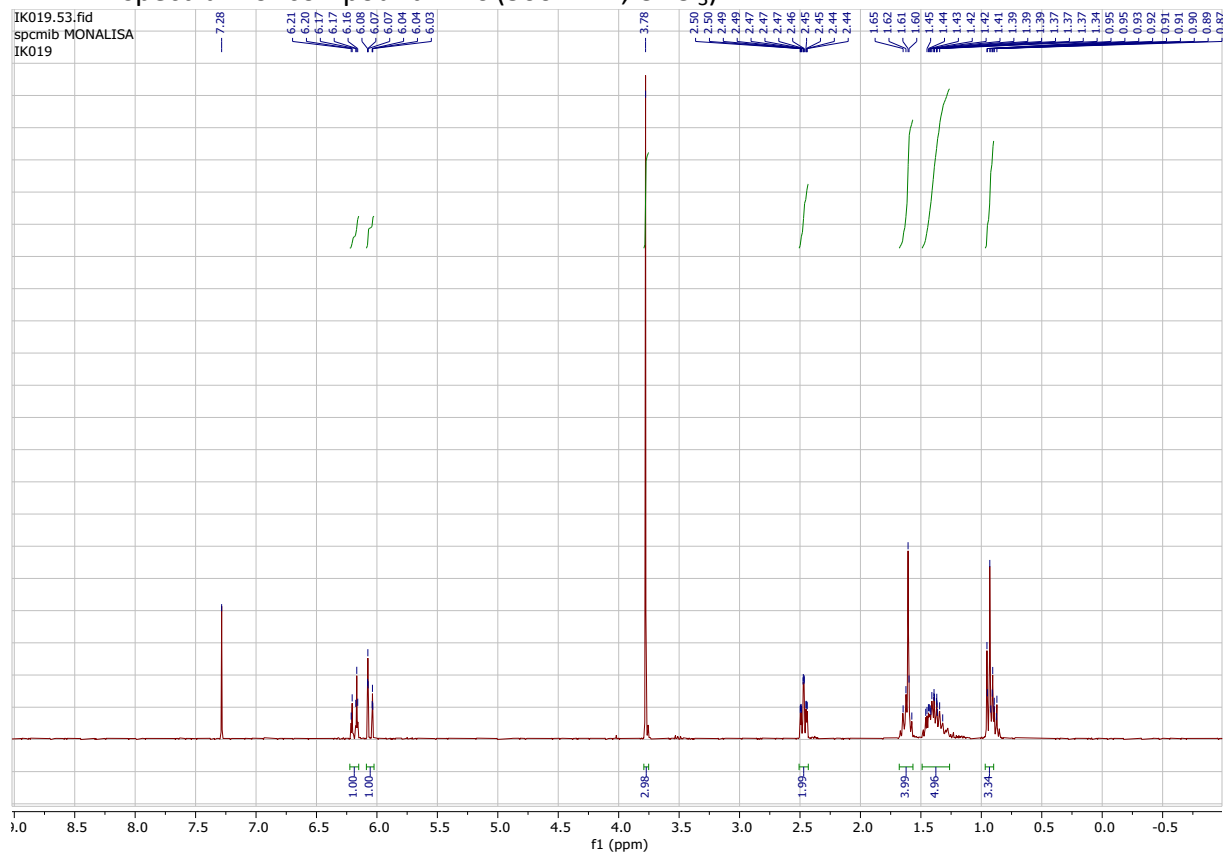
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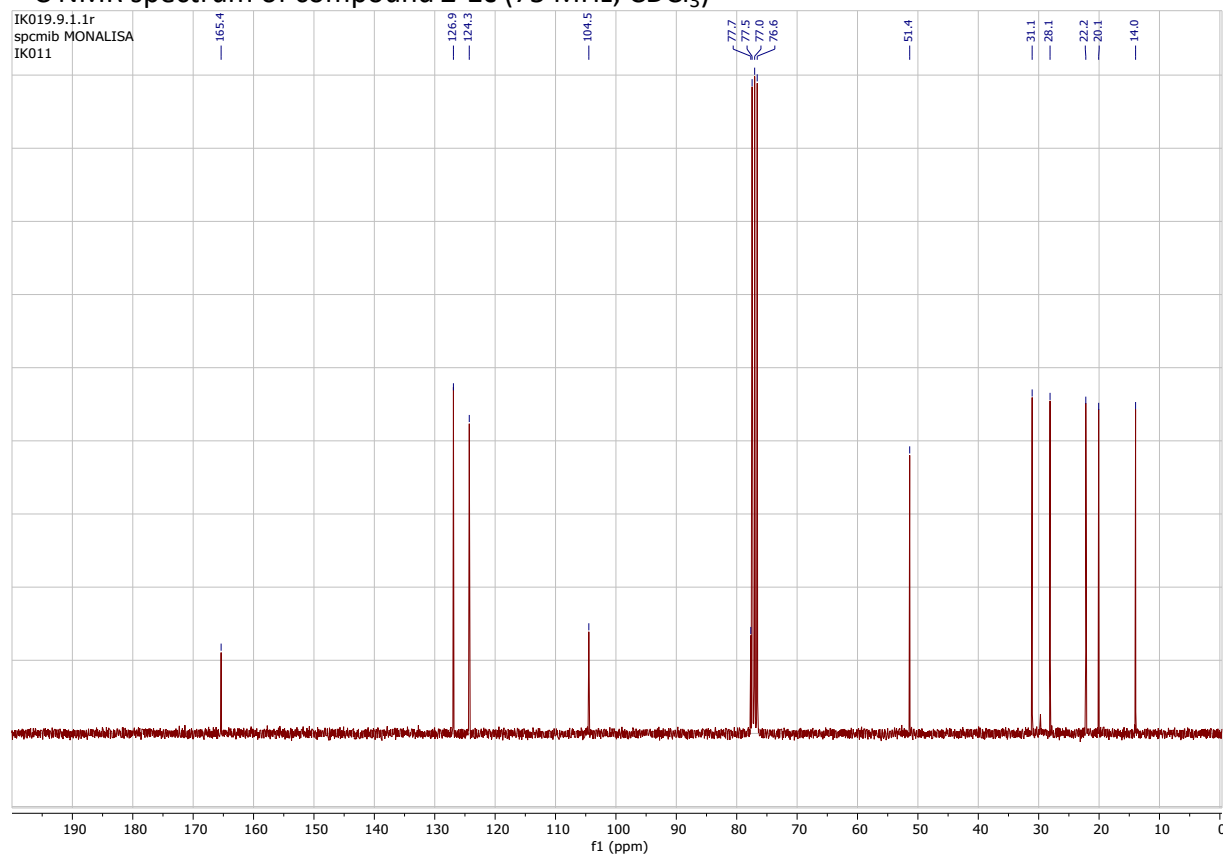
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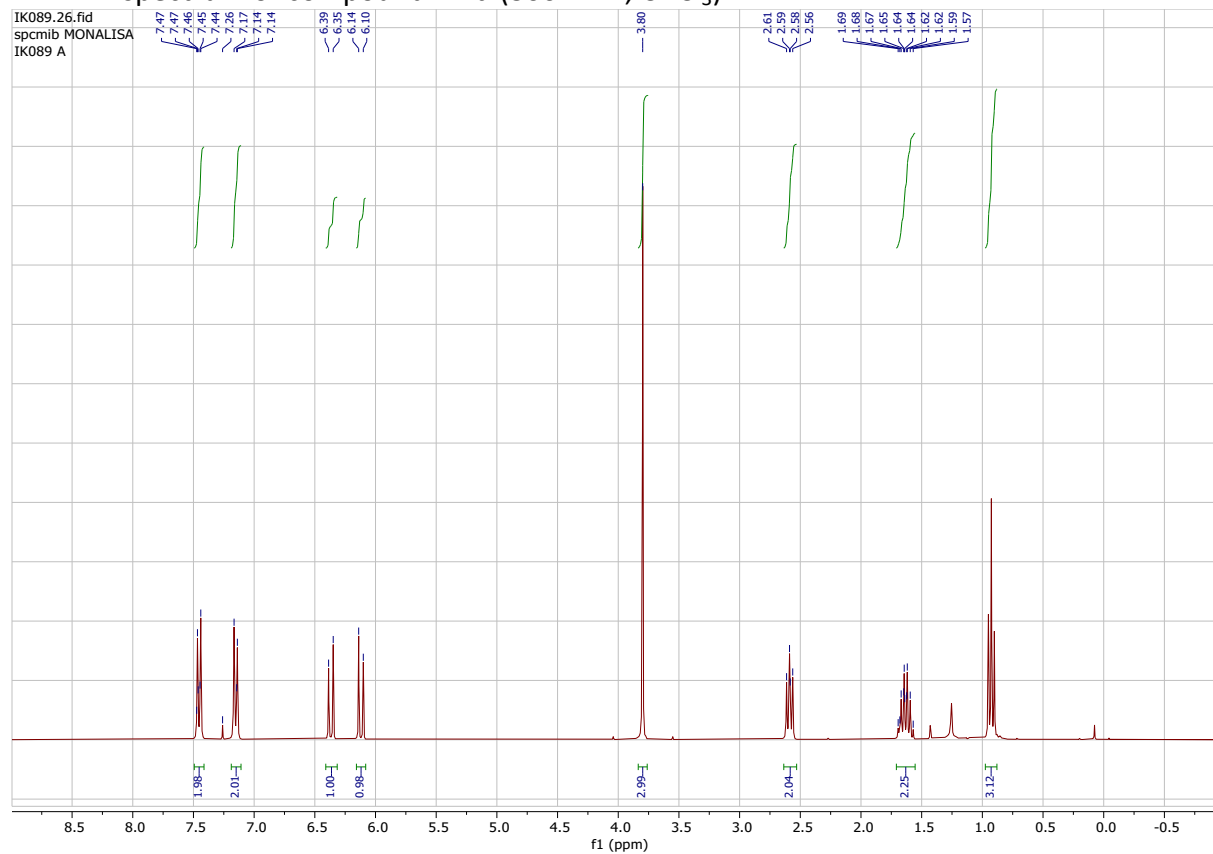
¹H NMR spectrum of compound Z-1c (300 MHz, CDCl₃)



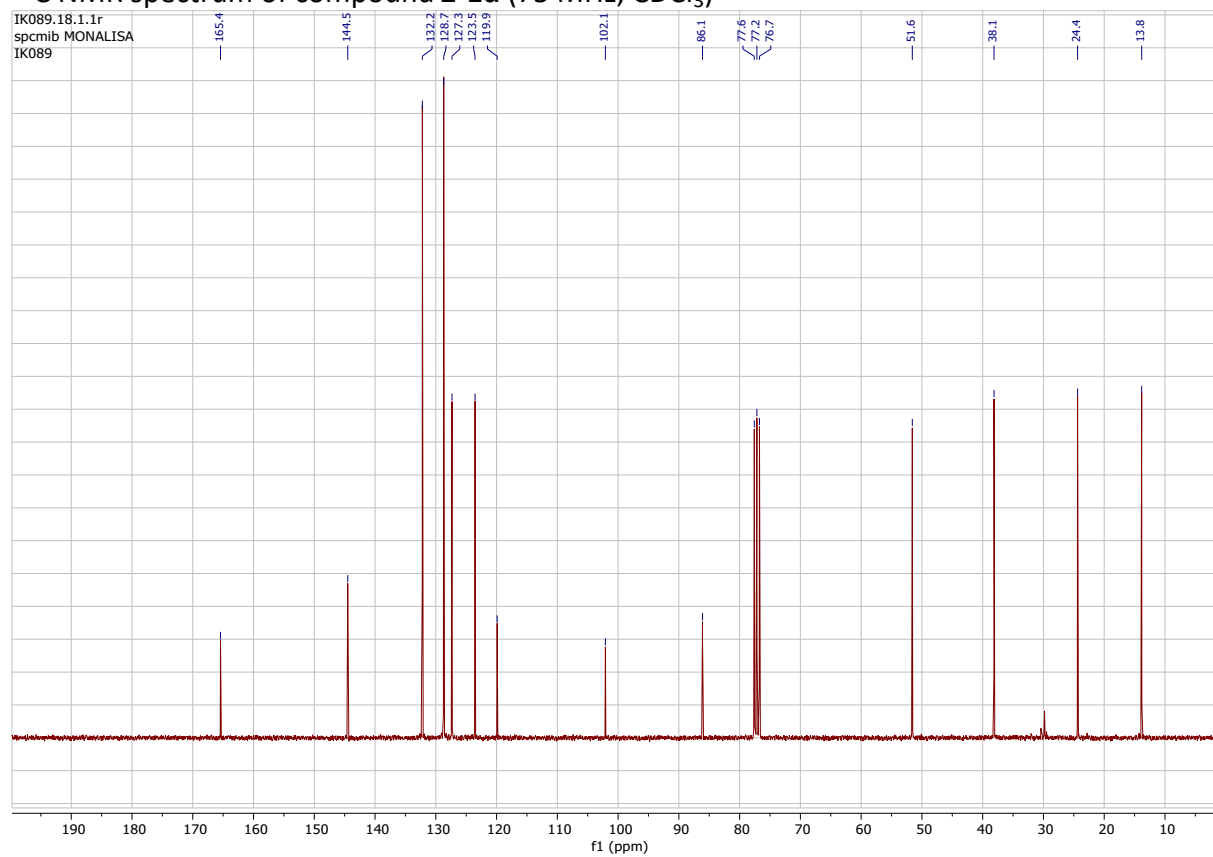
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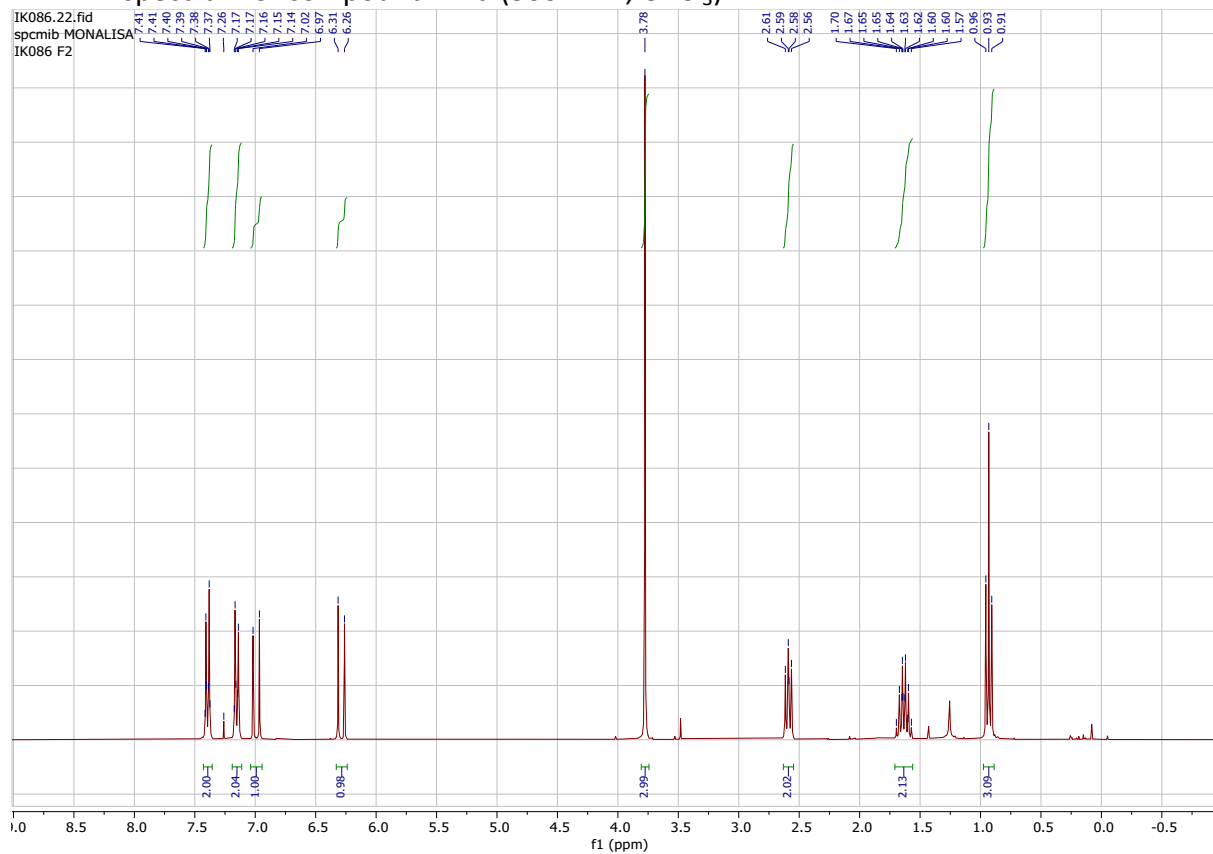
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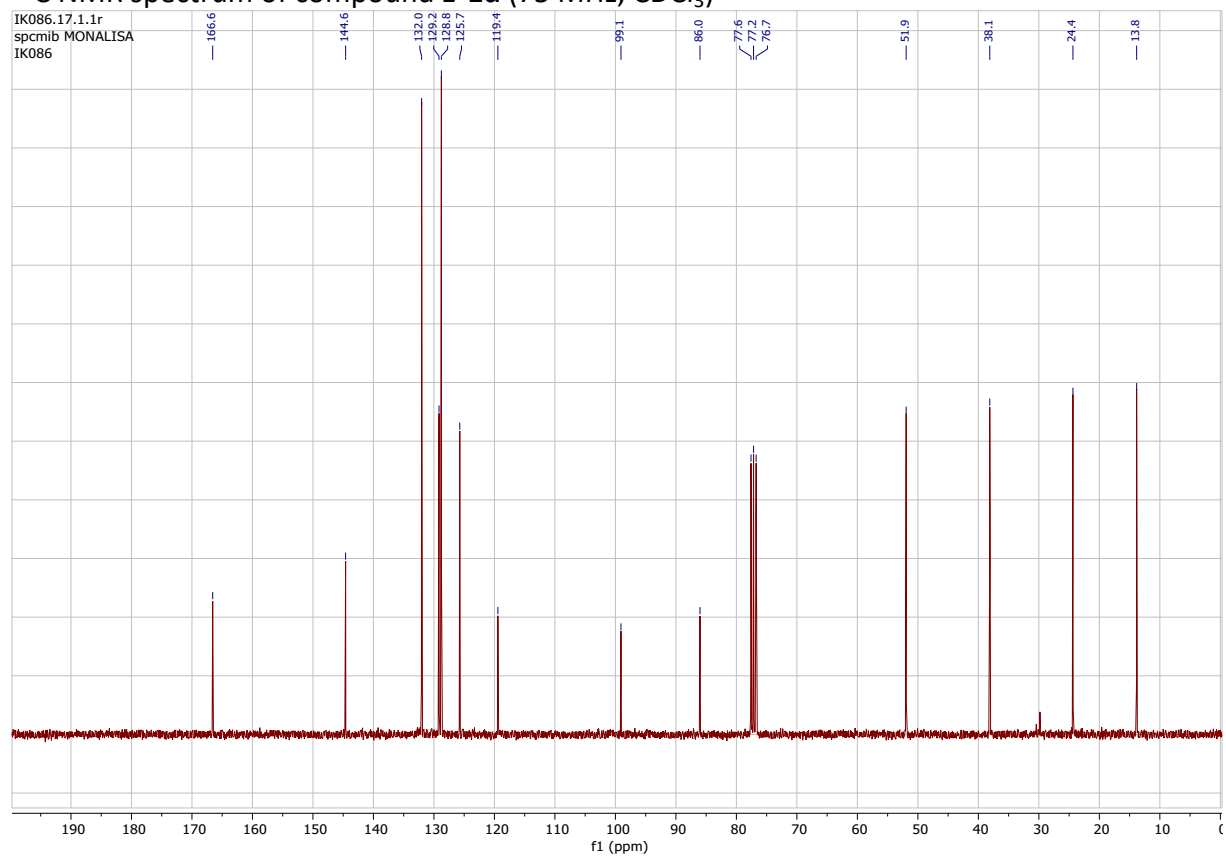
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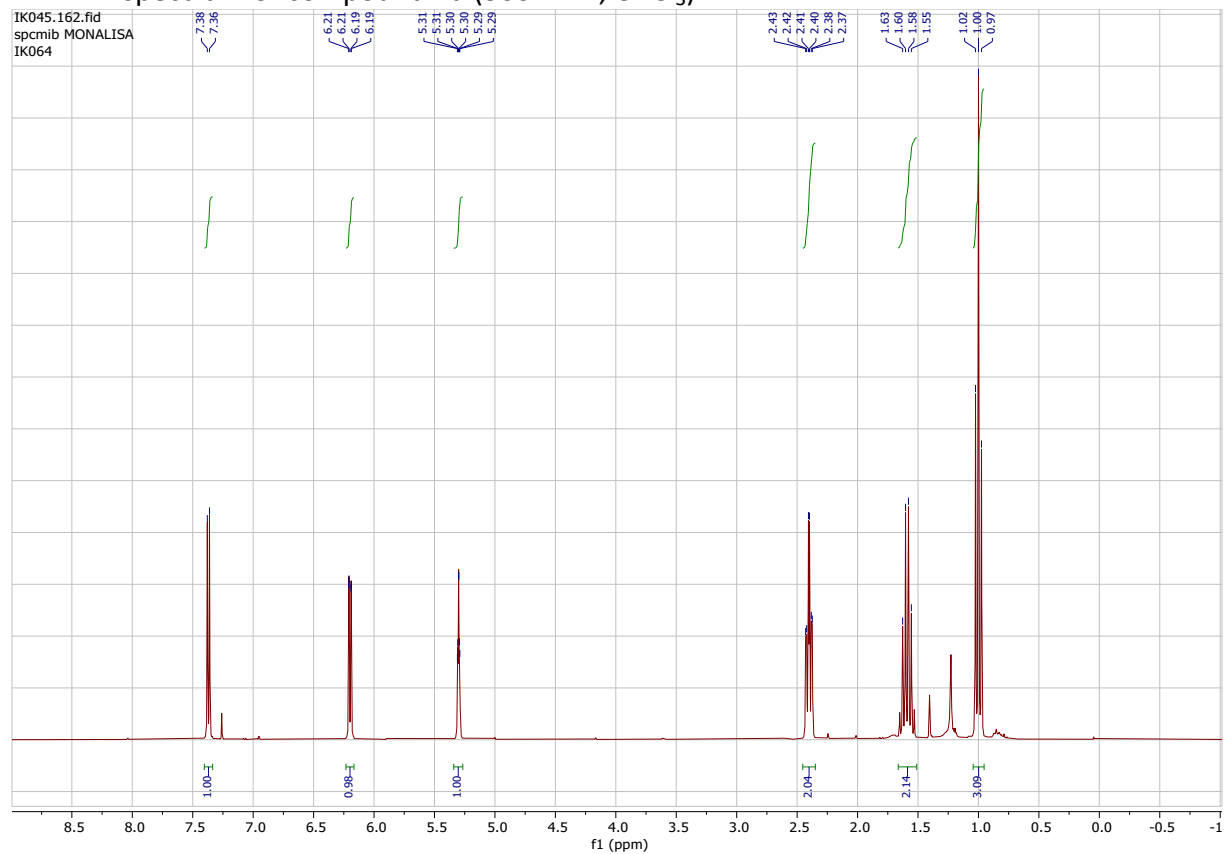
¹H NMR spectrum of compound E-1d (300 MHz, CDCl₃)



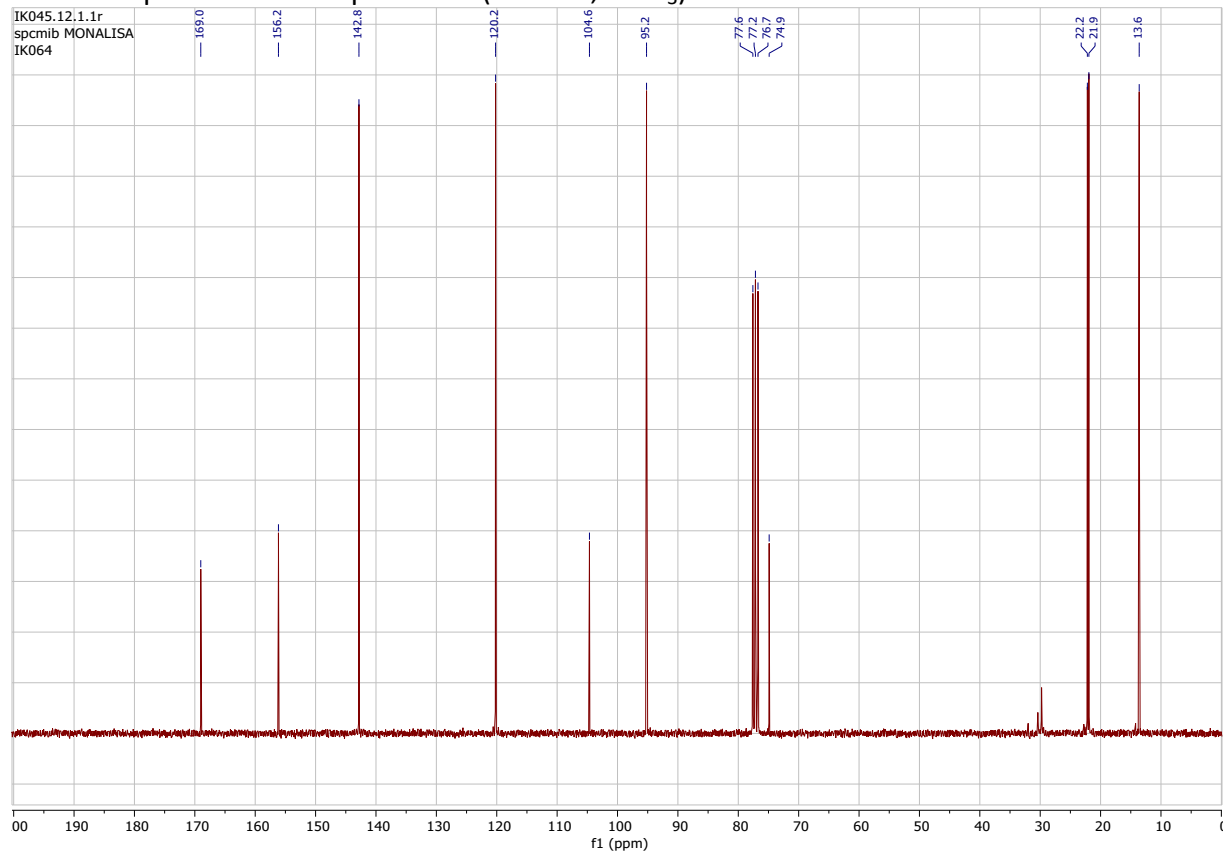
¹³C NMR spectrum of compound E-1d (75 MHz, CDCl₃)



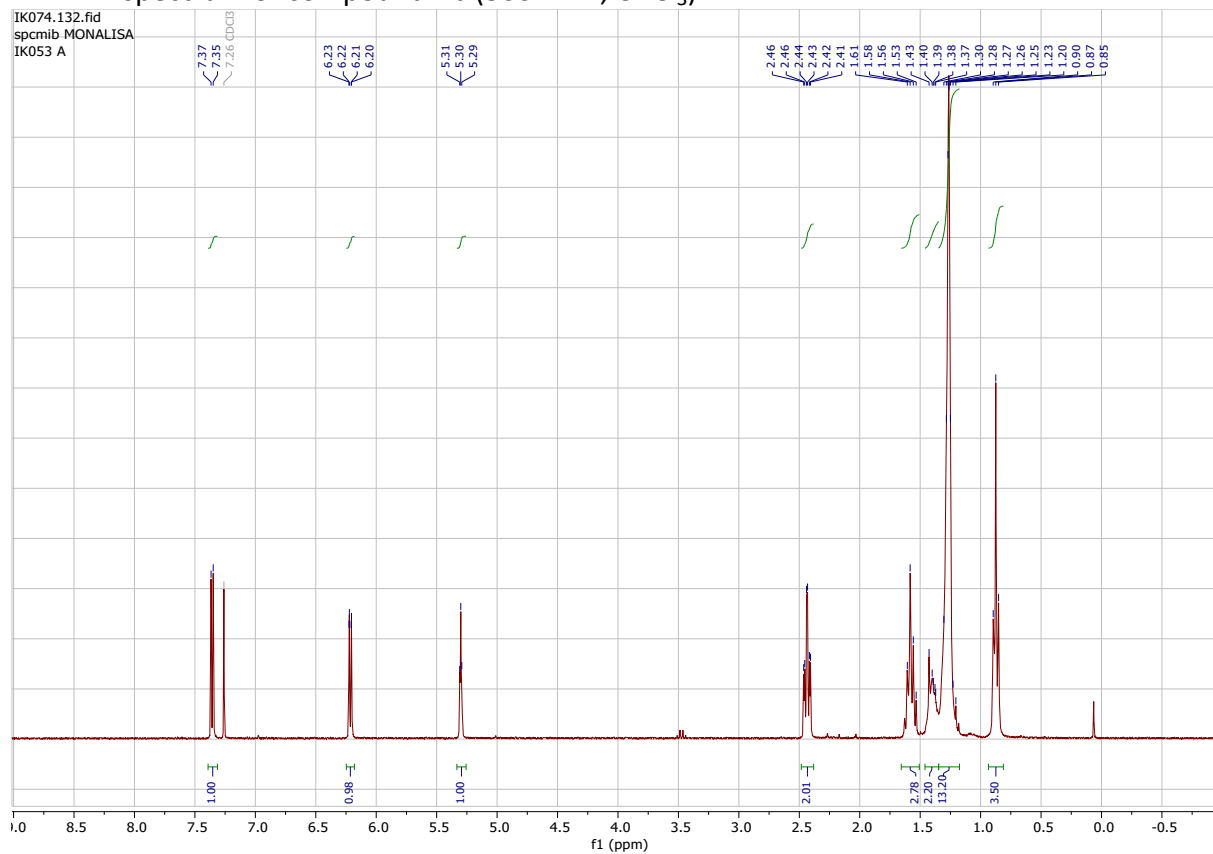
¹H NMR spectrum of compound **4a** (300 MHz, CDCl₃)



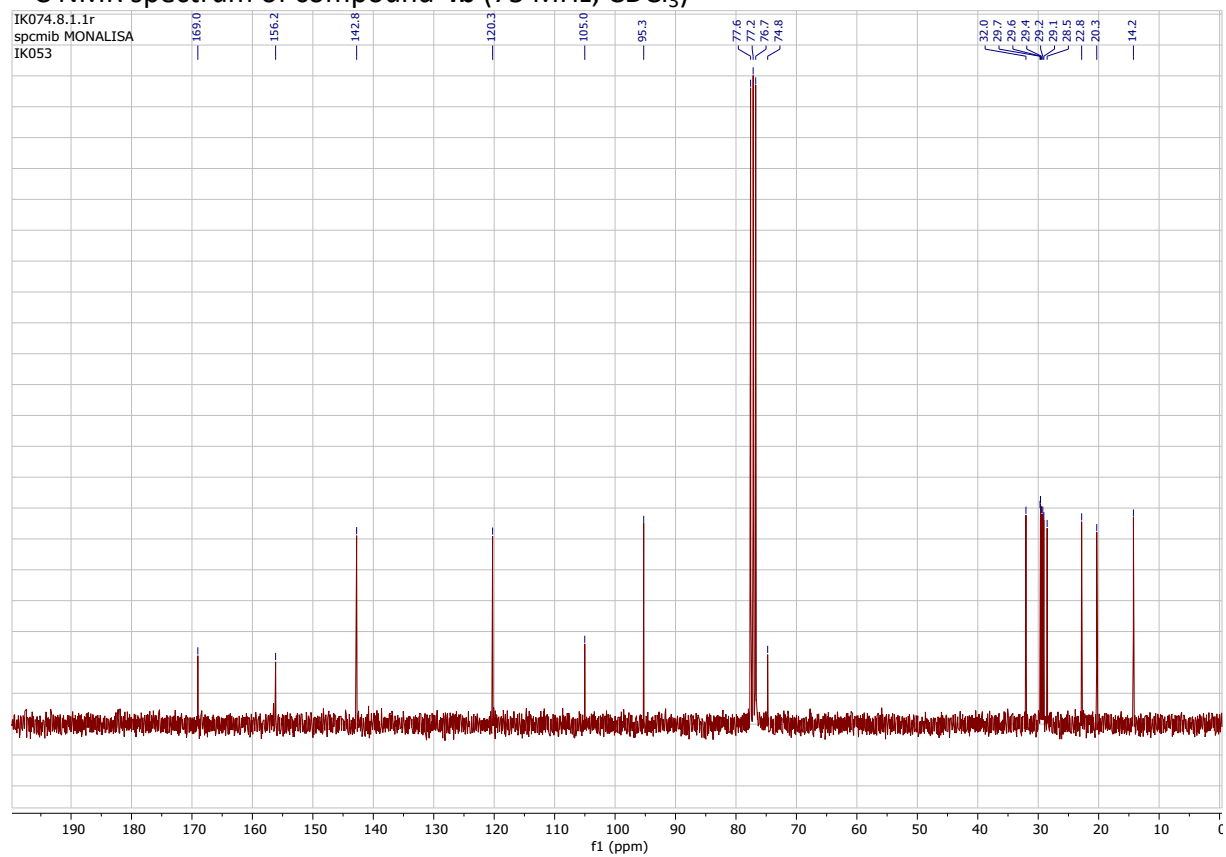
¹³C NMR spectrum of compound **4a** (75 MHz, CDCl₃)



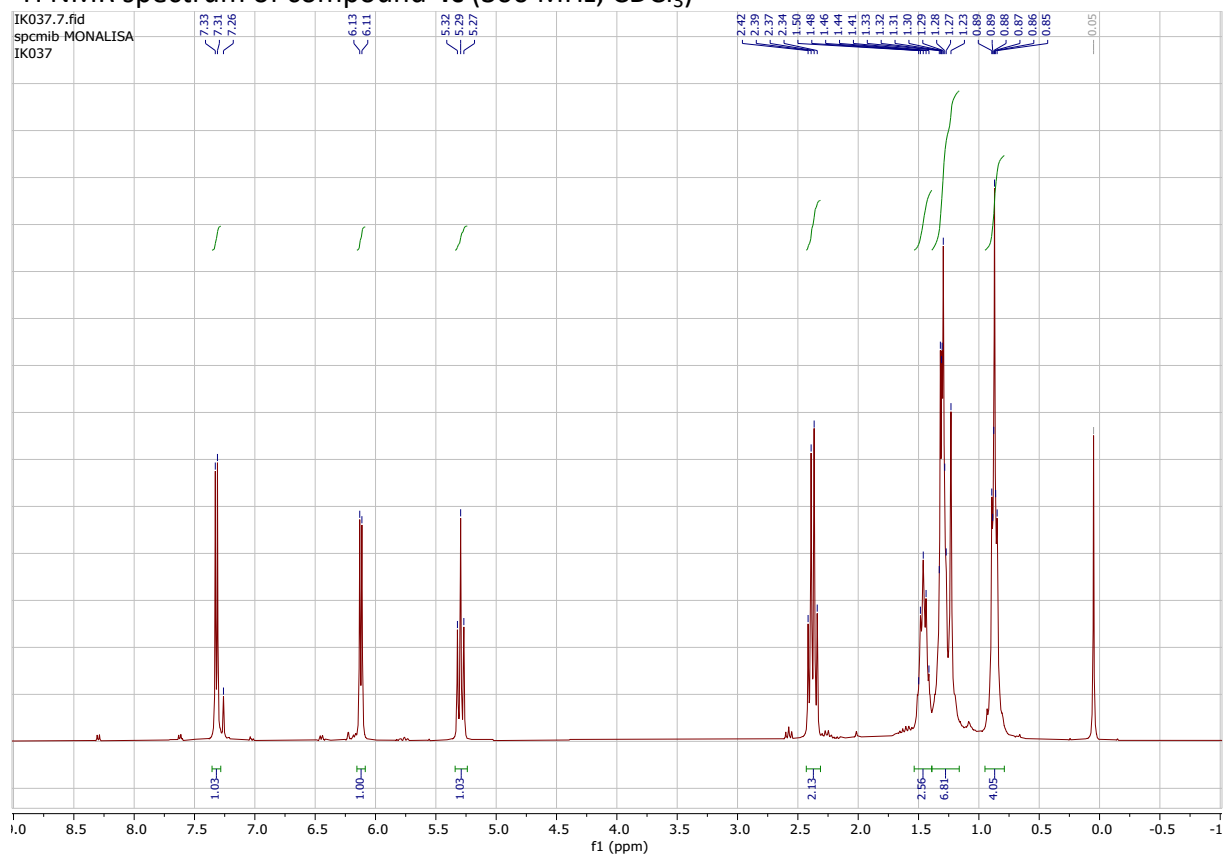
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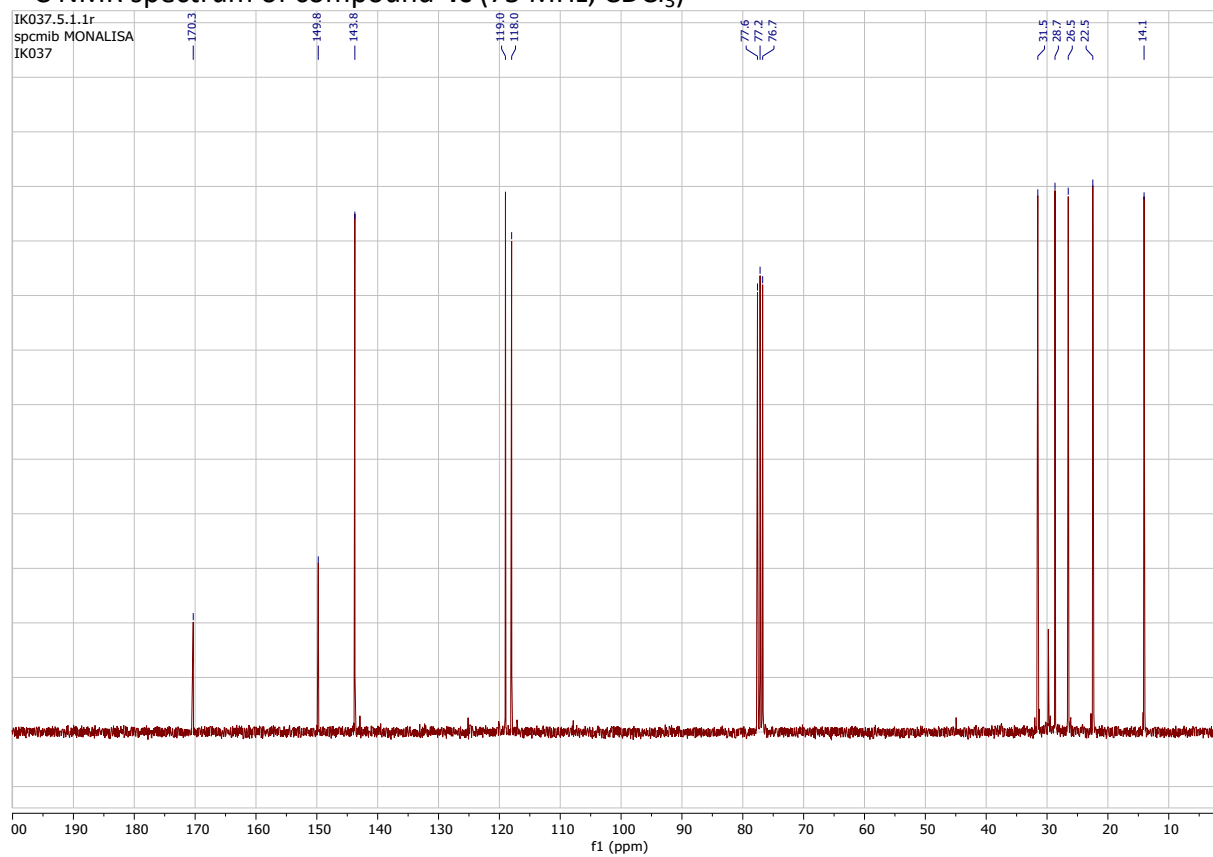
¹³C NMR spectrum of compound **4b** (75 MHz, CDCl₃)



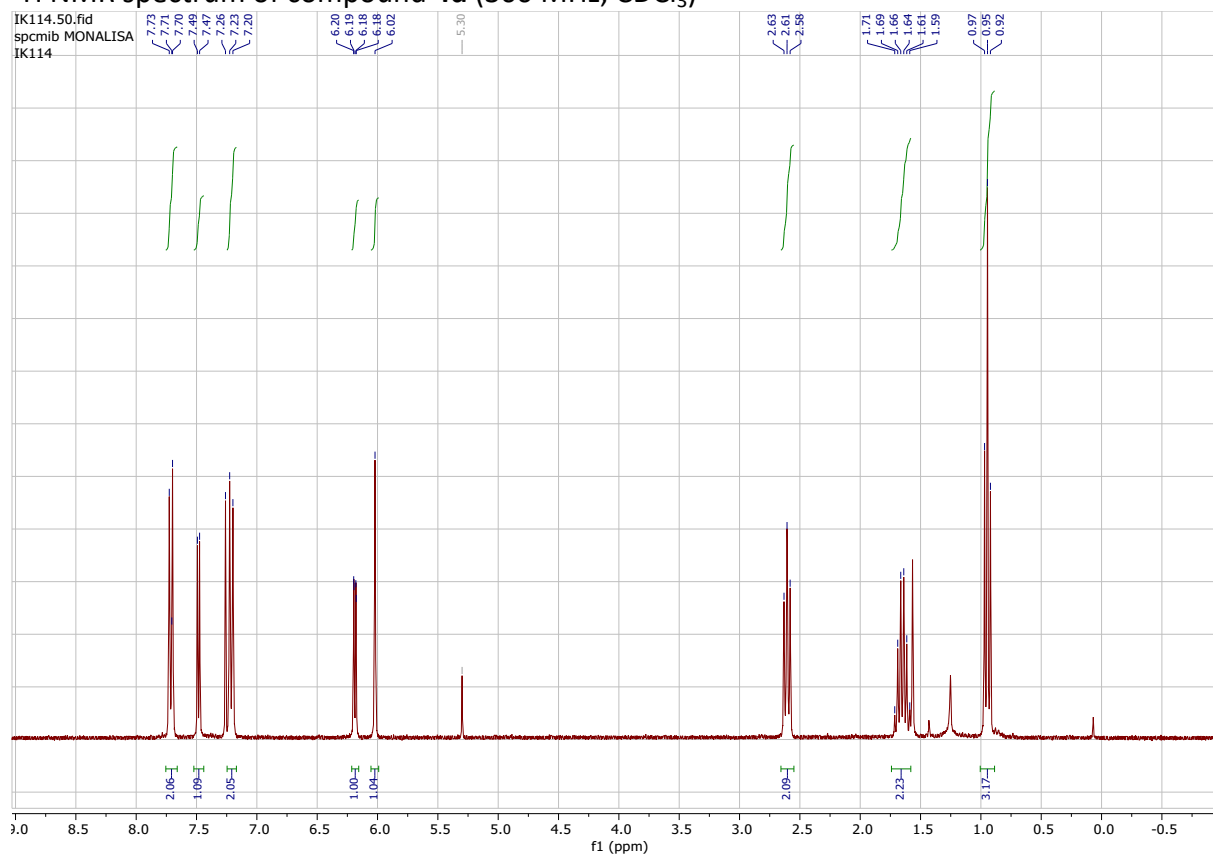
¹H NMR spectrum of compound **4c** (300 MHz, CDCl₃)



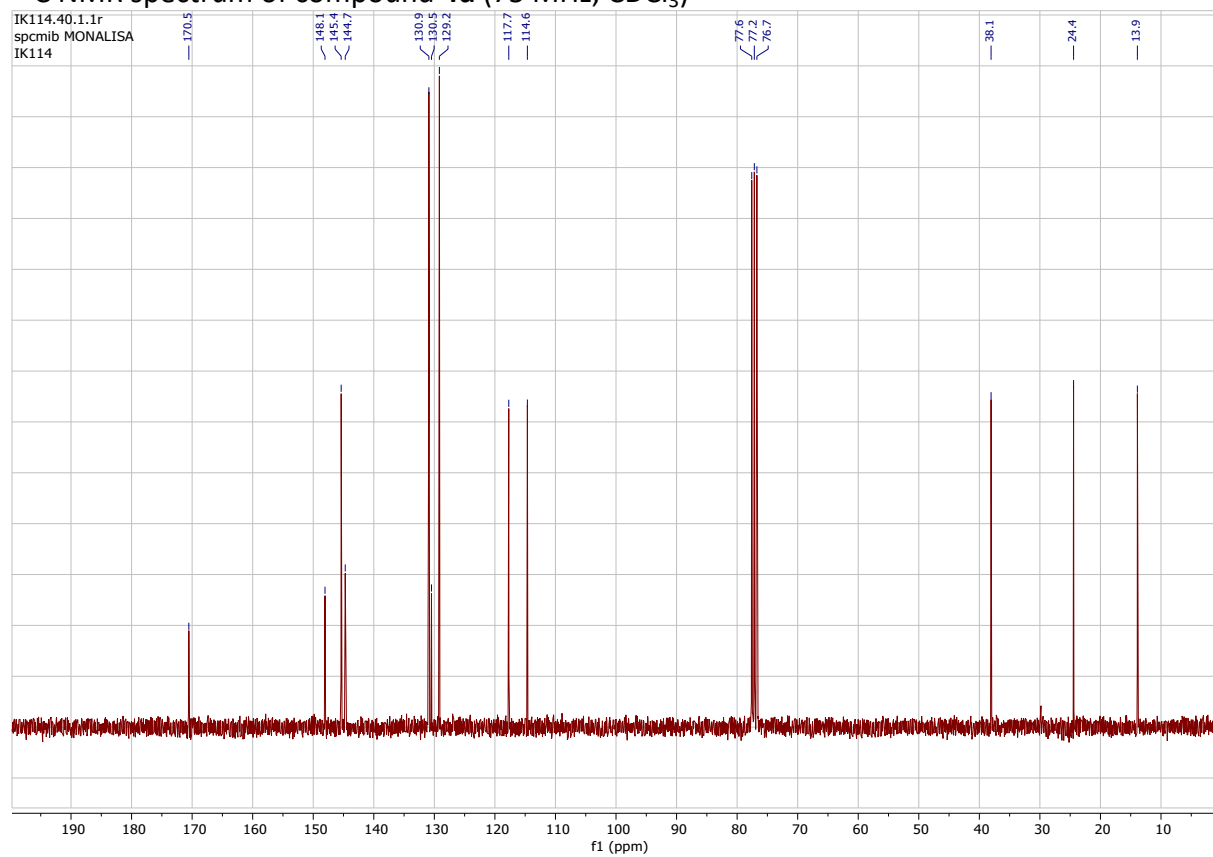
¹³C NMR spectrum of compound **4c** (75 MHz, CDCl₃)



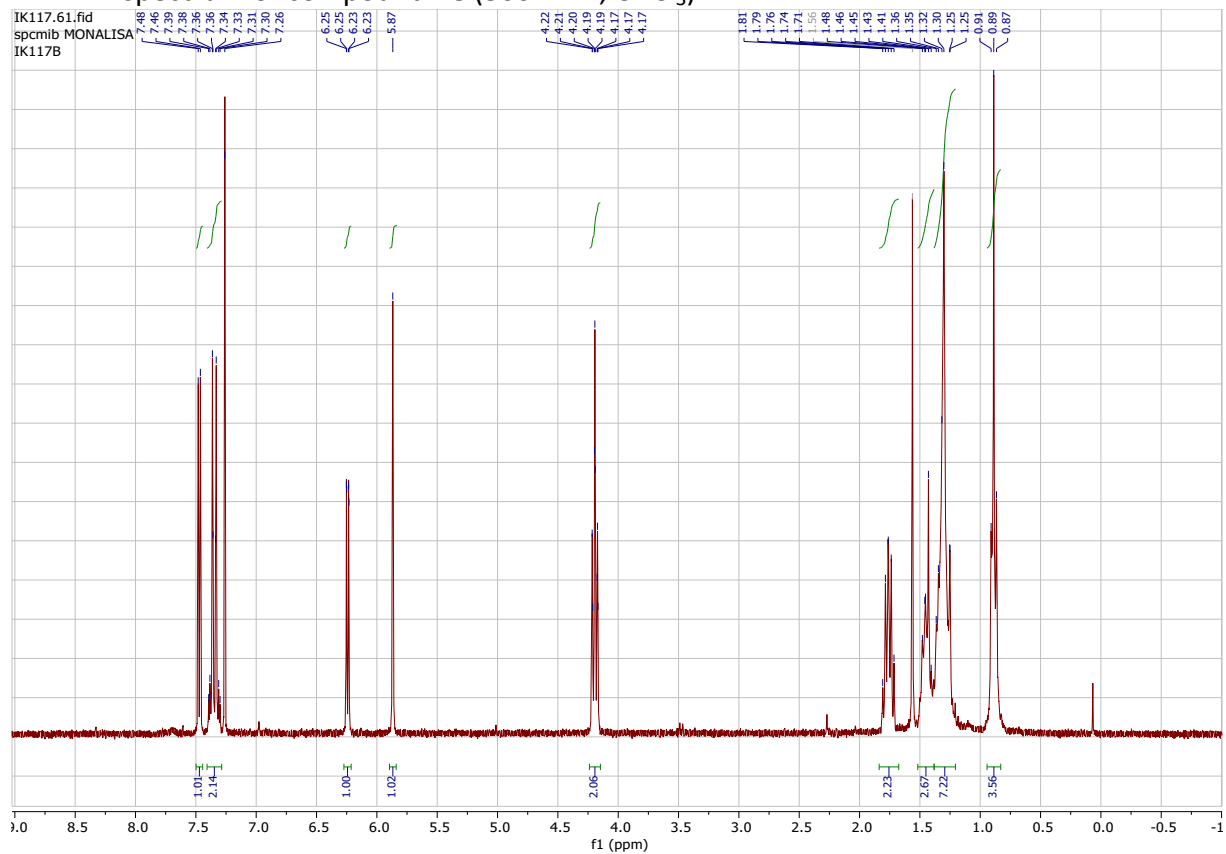
¹H NMR spectrum of compound **4d** (300 MHz, CDCl₃)



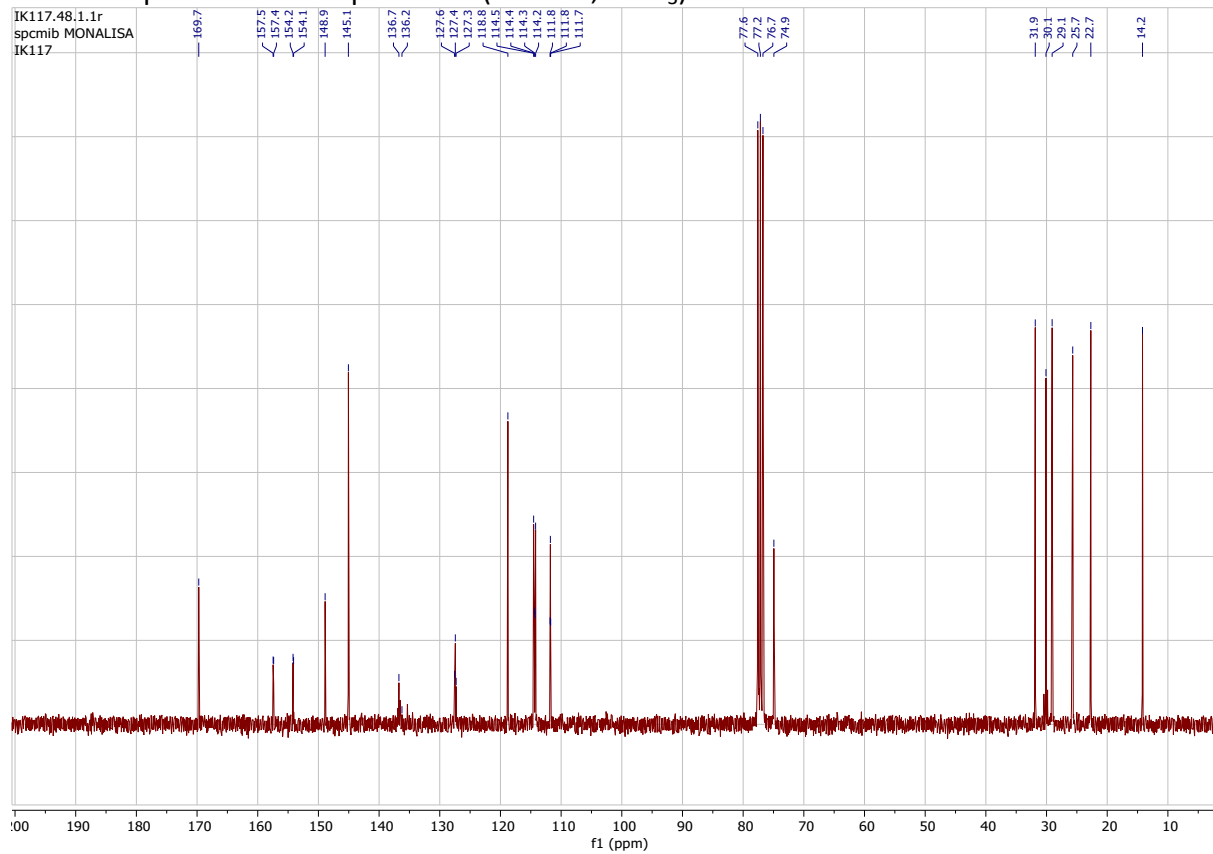
¹³C NMR spectrum of compound **4d** (75 MHz, CDCl₃)



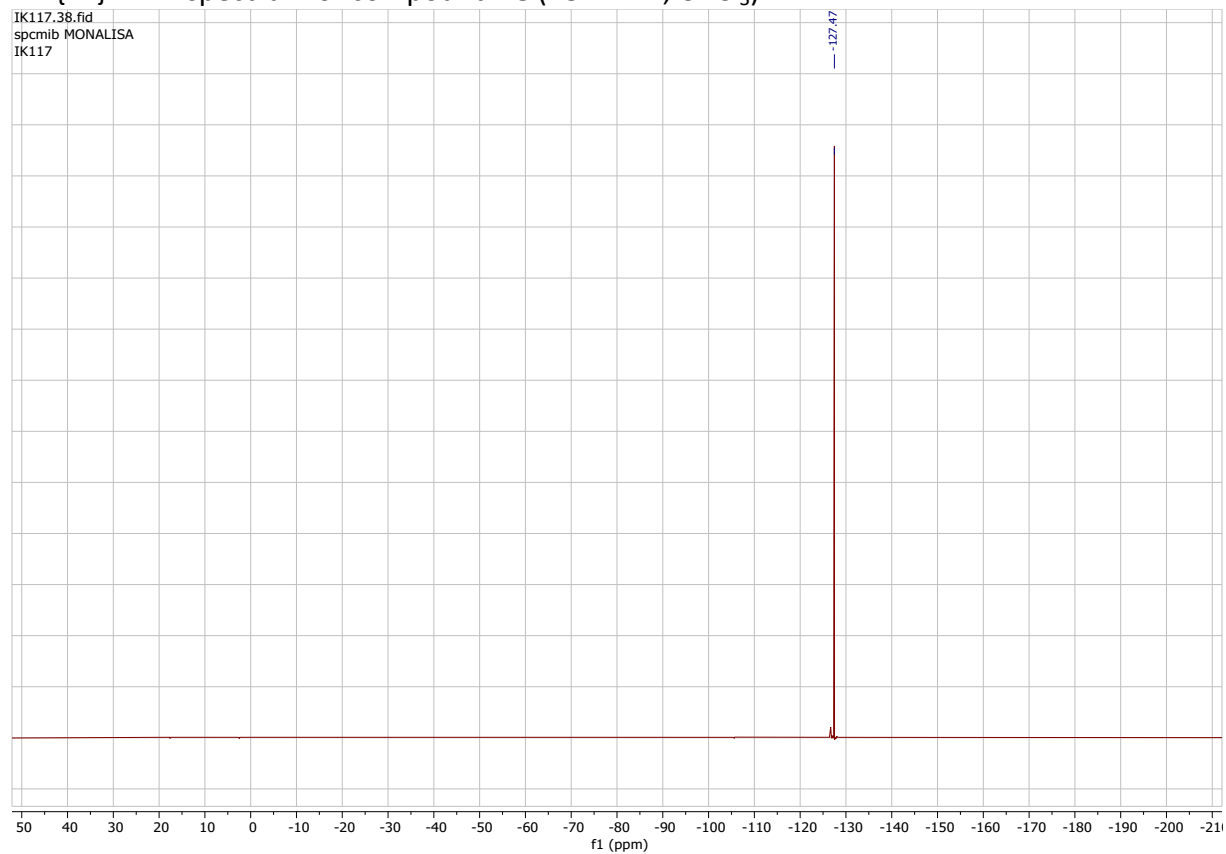
¹H NMR spectrum of compound **4e** (300 MHz, CDCl₃)



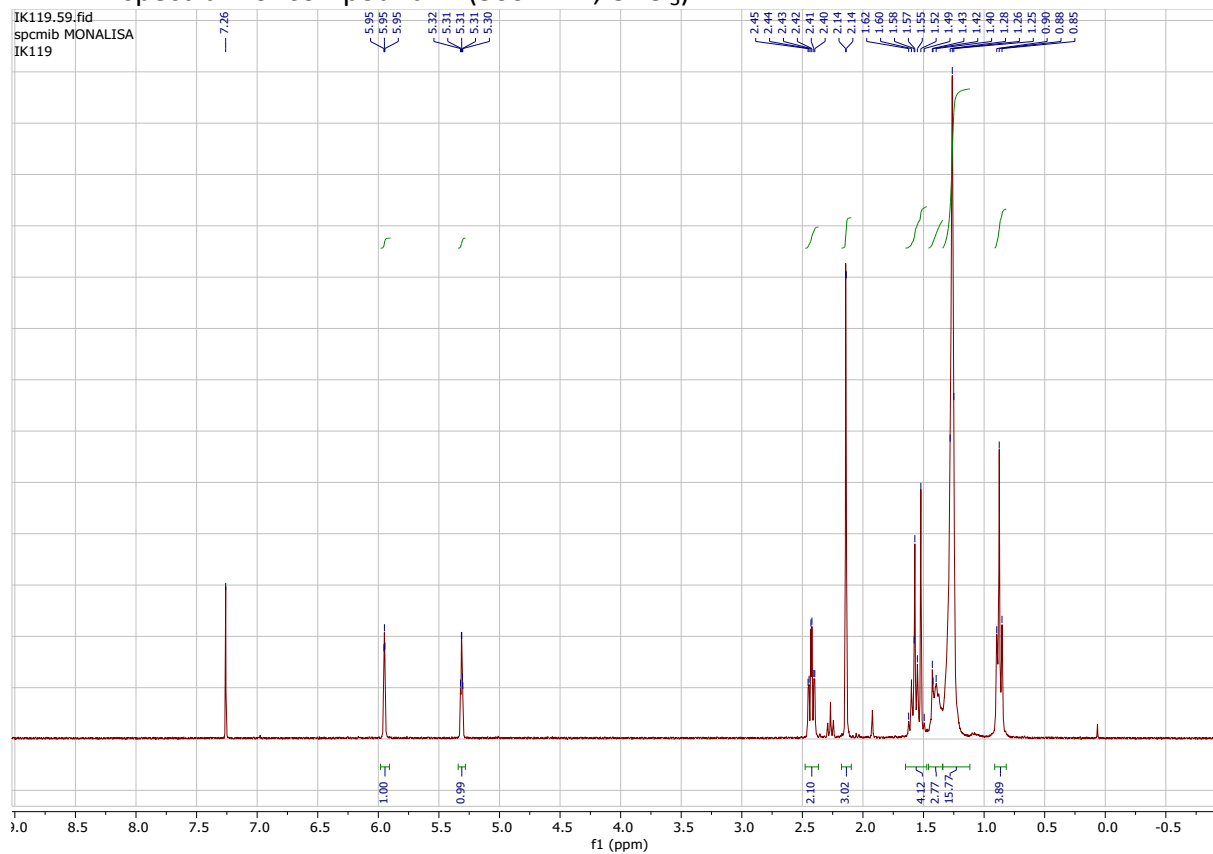
¹³C NMR spectrum of compound **4e** (75 MHz, CDCl₃)



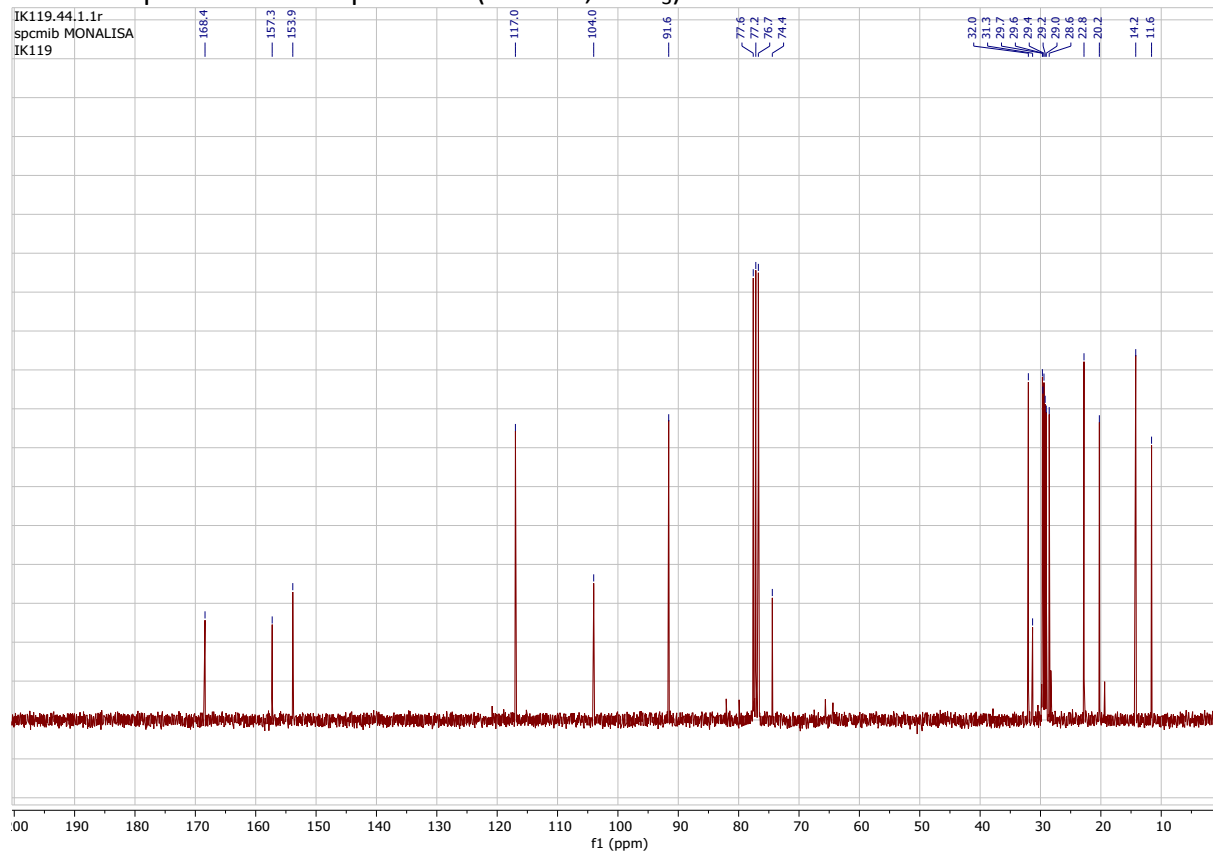
^{19}F $\{^1\text{H}\}$ NMR spectrum of compound **4e** (282 MHz, CDCl_3)



^1H NMR spectrum of compound **4f** (300 MHz, CDCl_3)

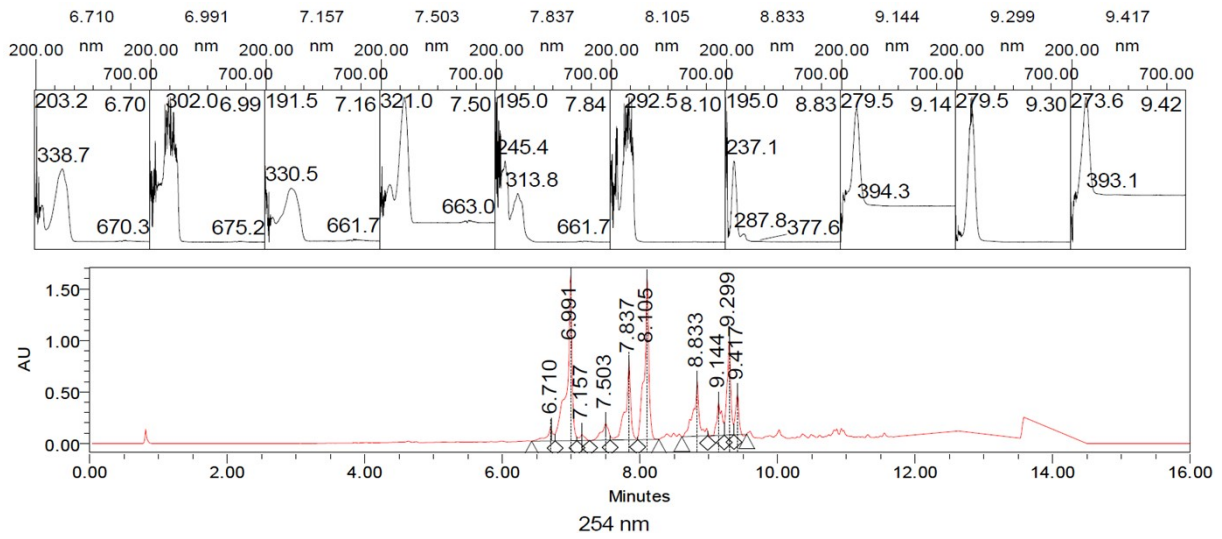


^{13}C NMR spectrum of compound **4f** (75 MHz, CDCl_3)

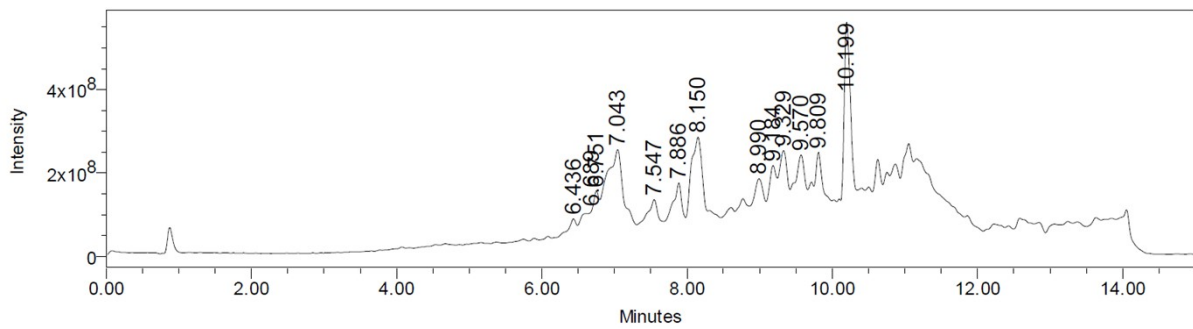


UHPLC analyses of extracts

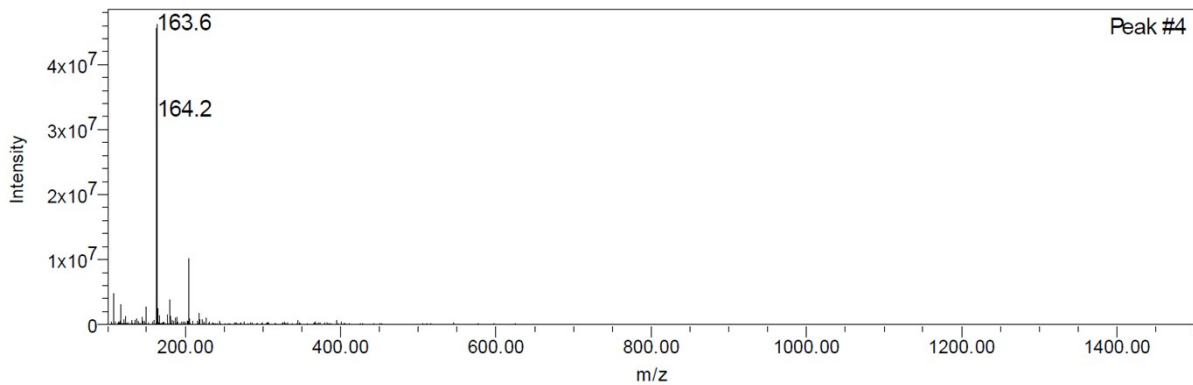
Hexanoic extract obtained according Adande *et al.*¹



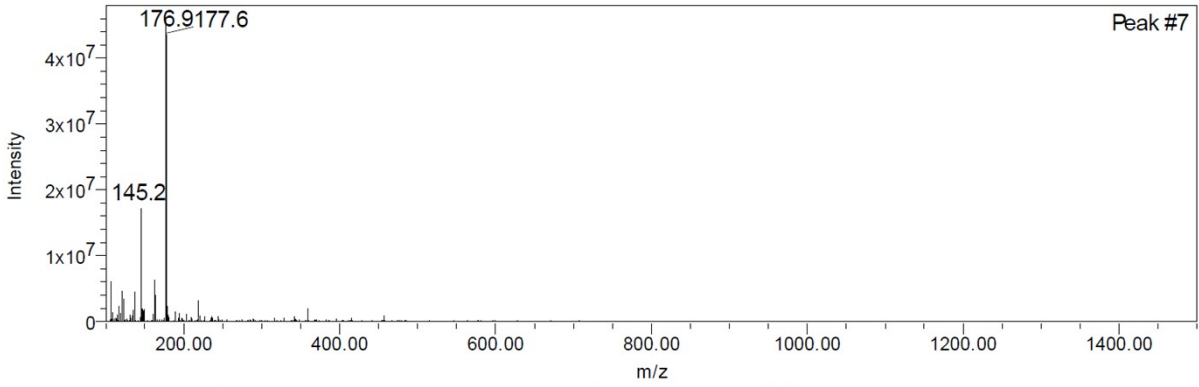
UV detection at 254 nm



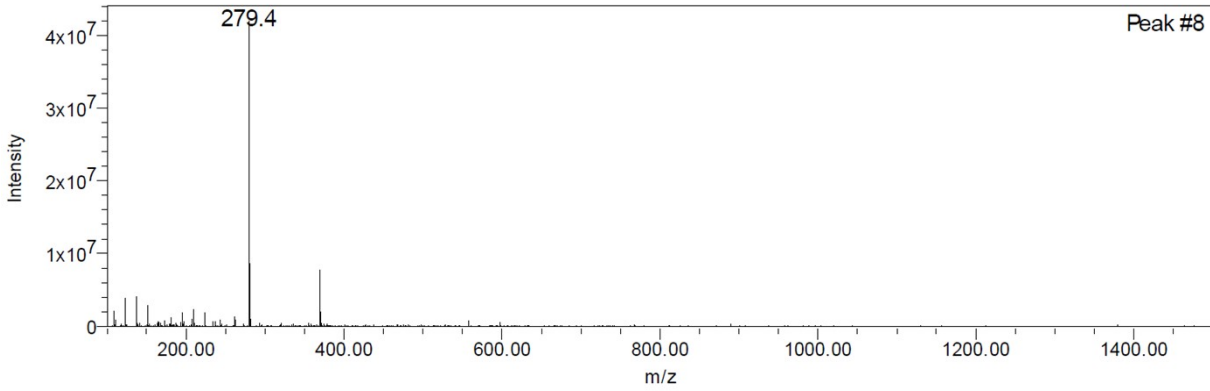
Total-ion current in ESI(+)



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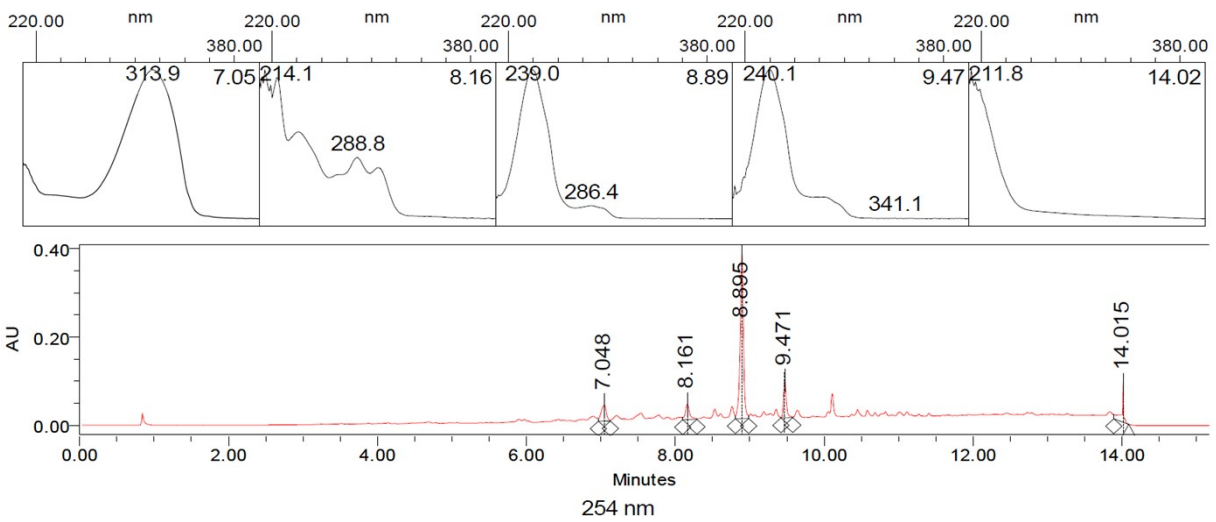


SampleName: Hex A Togo Date Acquired: 6/20/2023 12:42:44 PM CET Retention Time 8.150

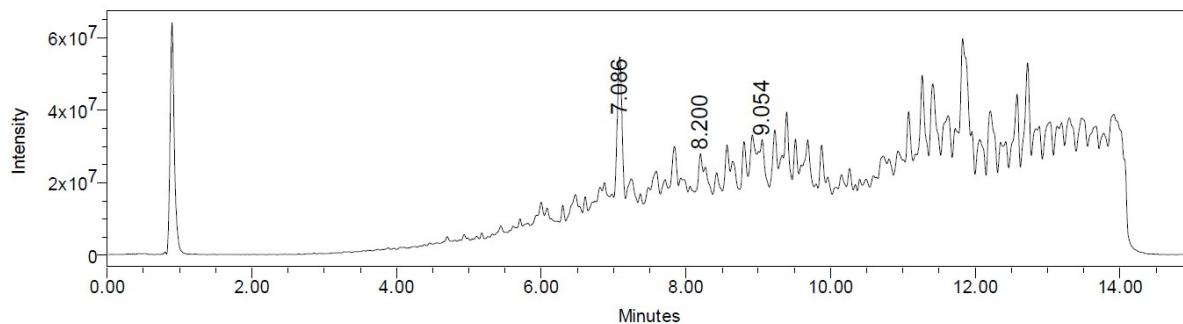


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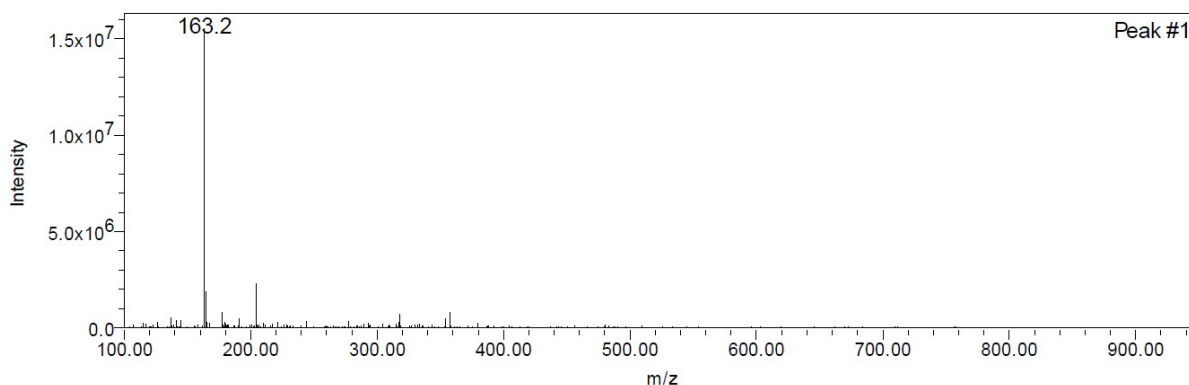
ASE MTBE extract



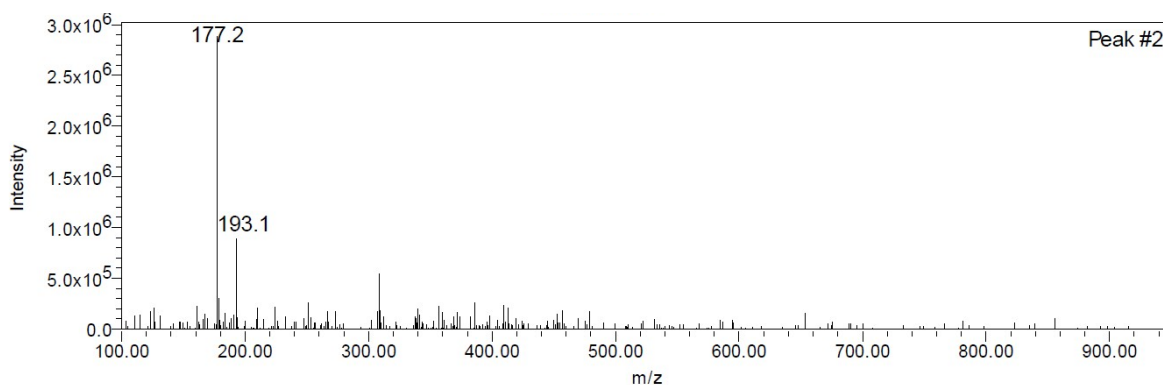
UV detection at 254 nm



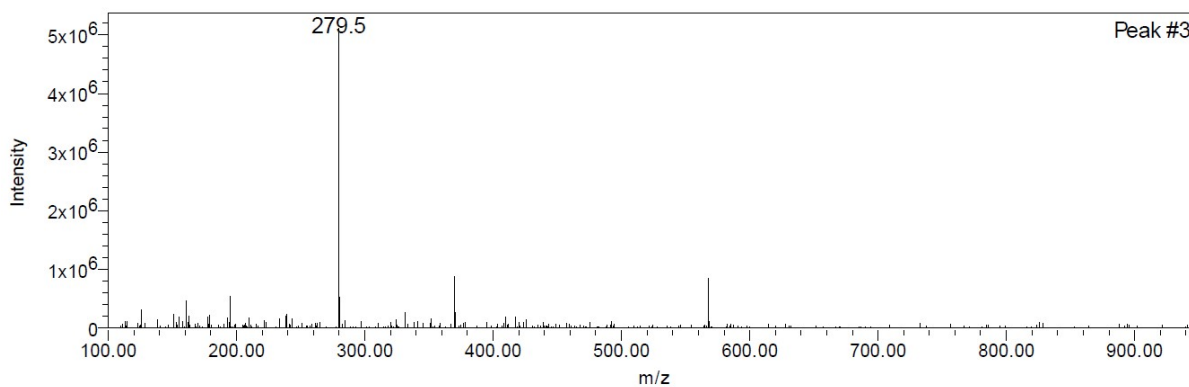
Total-ion current in ESI(+)



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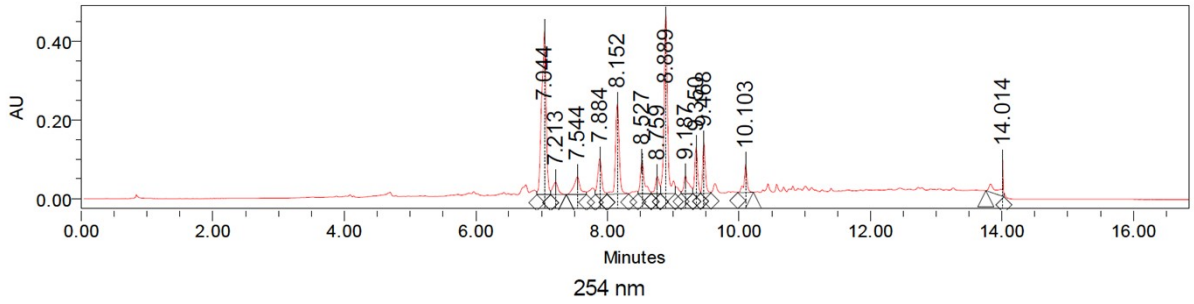
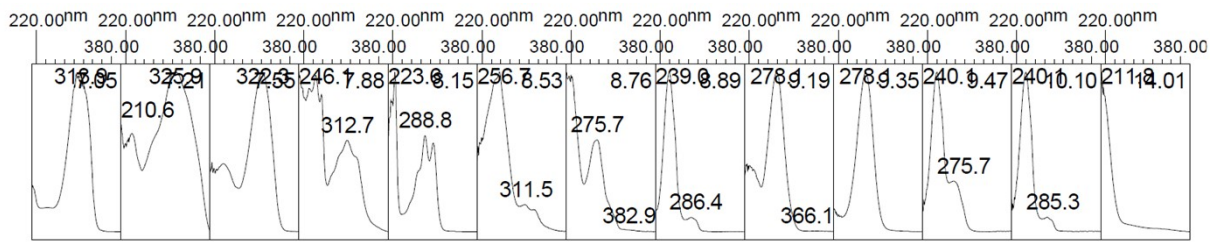


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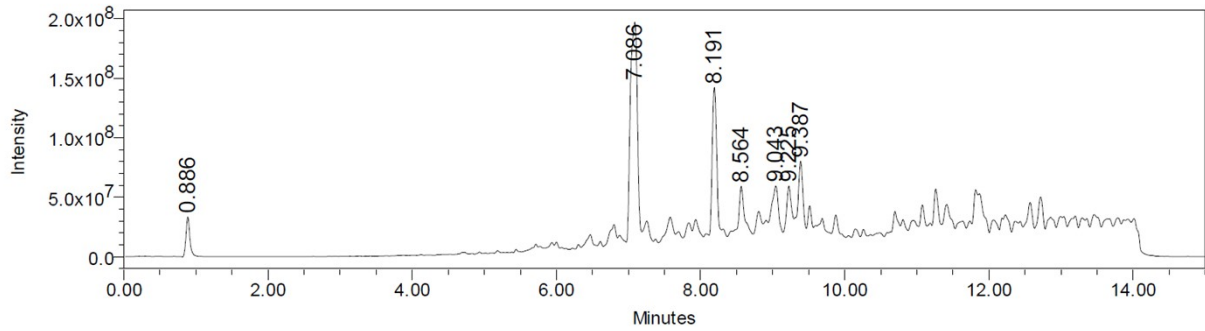


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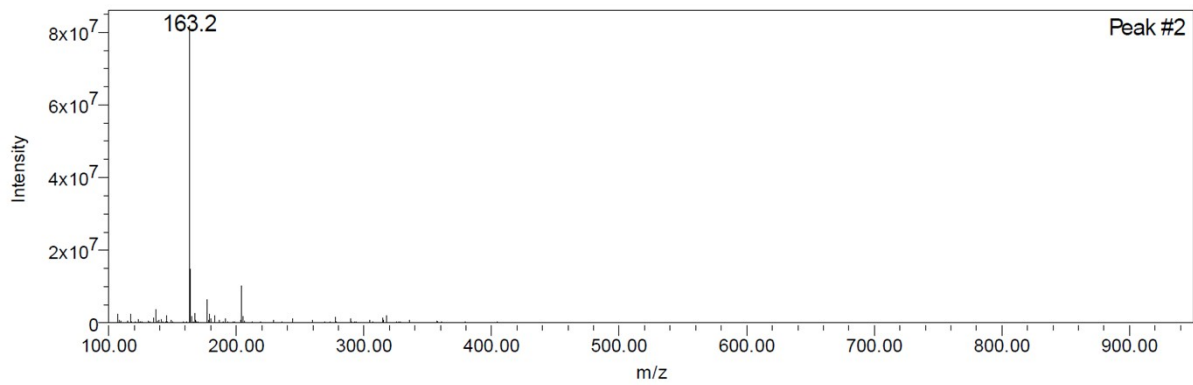
ASE Heptane extract



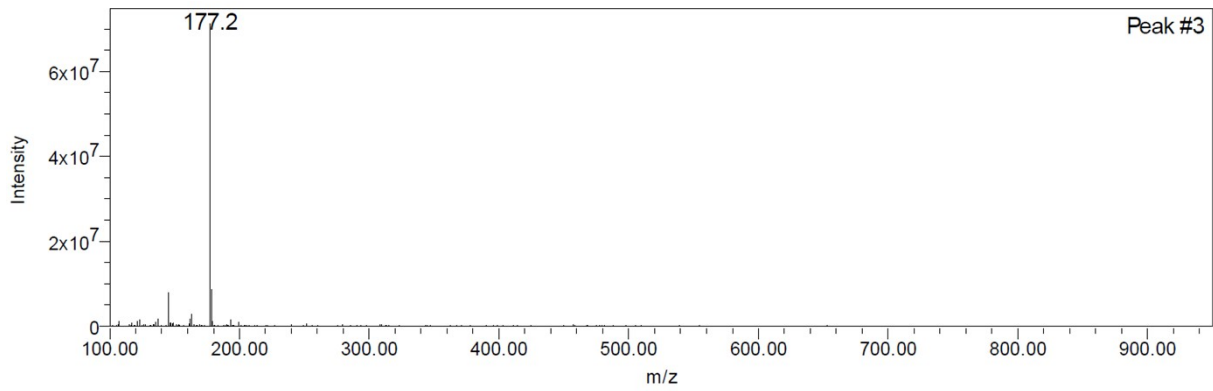
UV detection at 254 nm



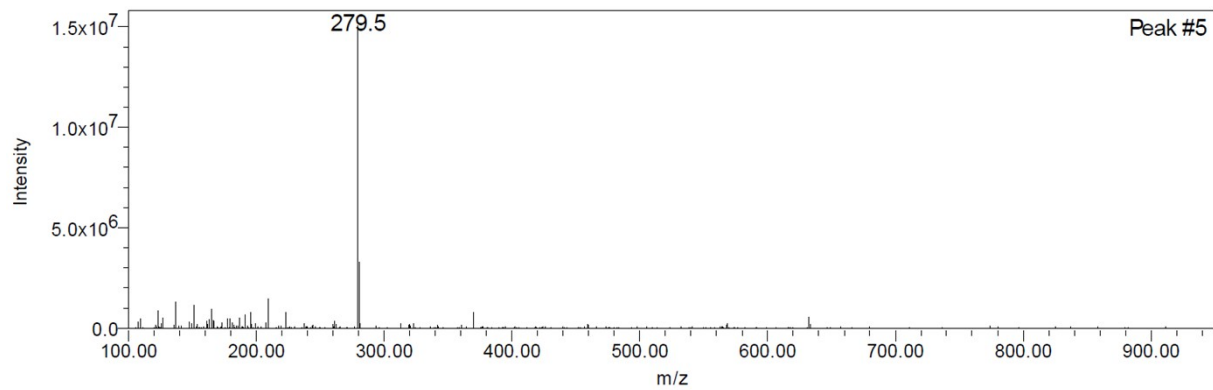
Total-ion current in ESI(+)



SampleName: ASE Heptane 1 Date Acquired: 10/18/2023 8:37:40 AM CET Retention Time 7.086

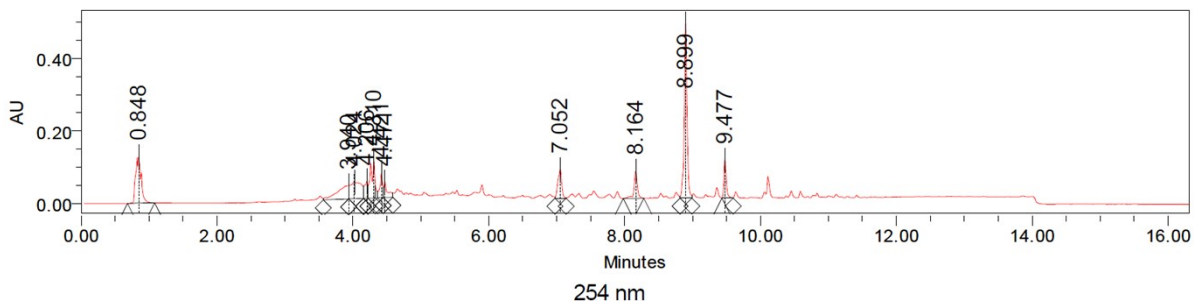
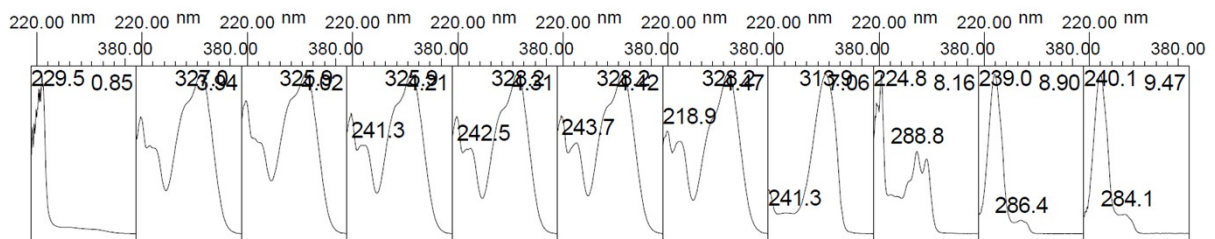


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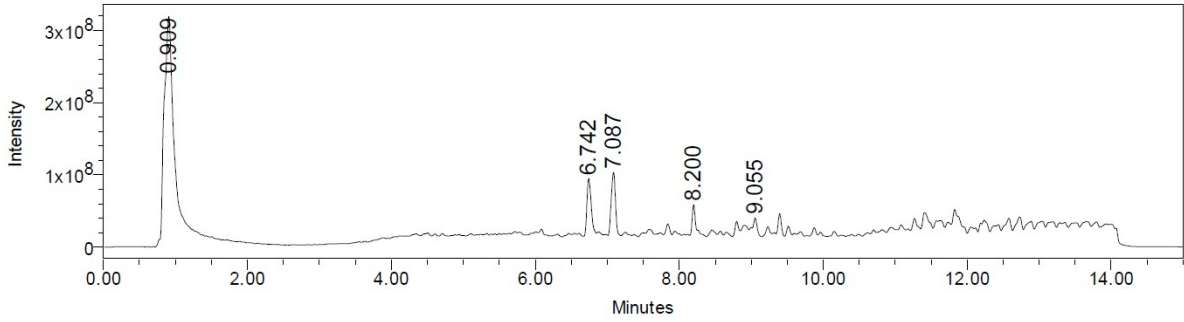


SampleName: ASE Heptane 1 Date Acquired: 10/18/2023 8:37:40 AM CET Retention Time 9.043

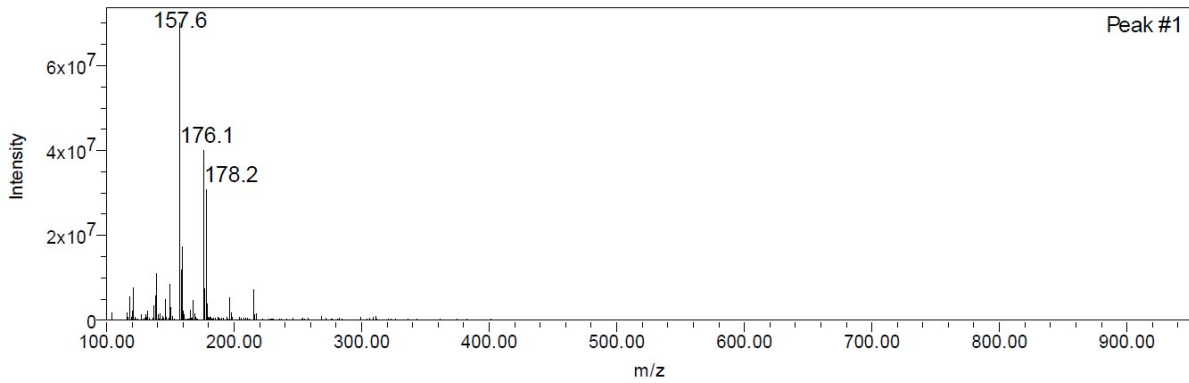
ASE Ethanol extract



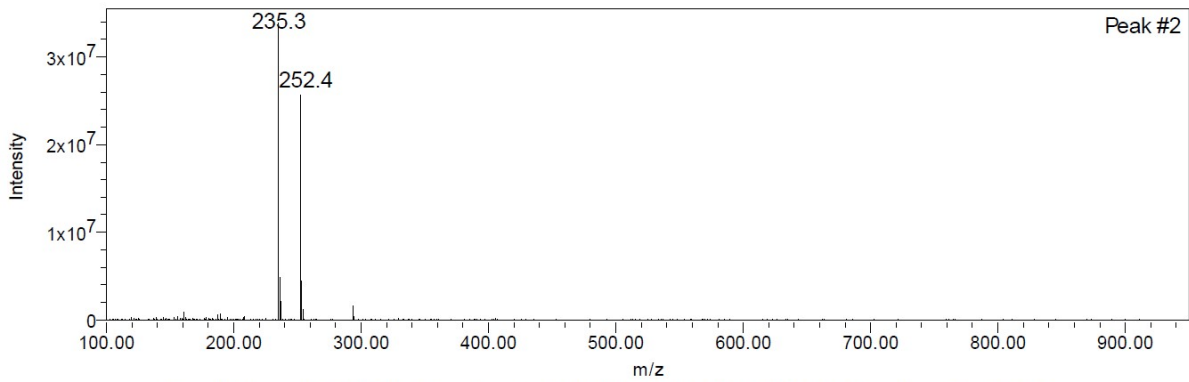
UV detection at 254 nm



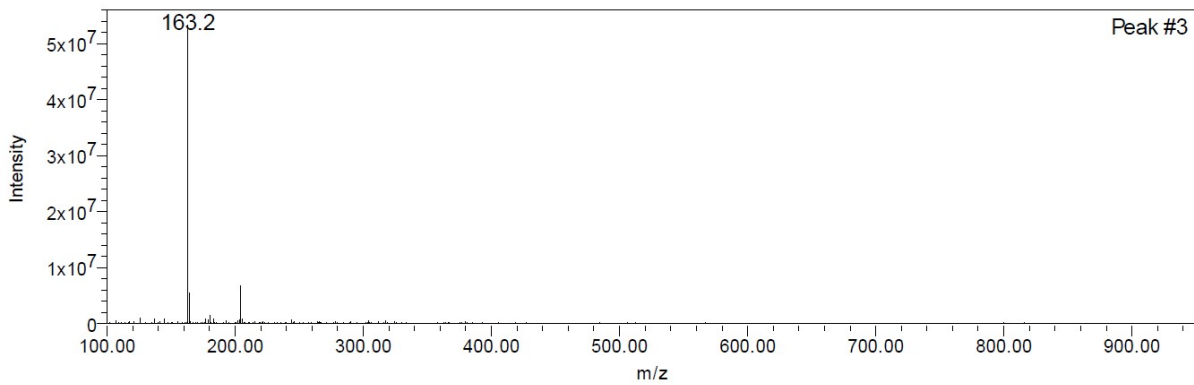
Total-ion current in ESI(+)



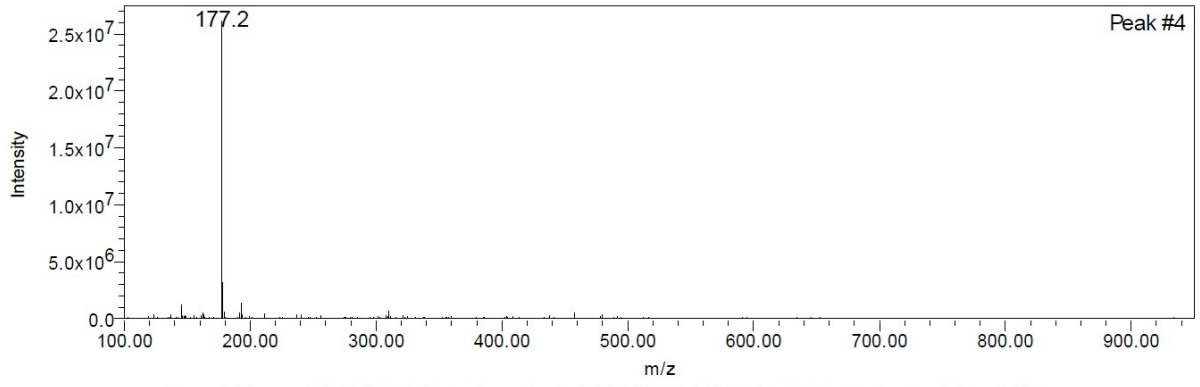
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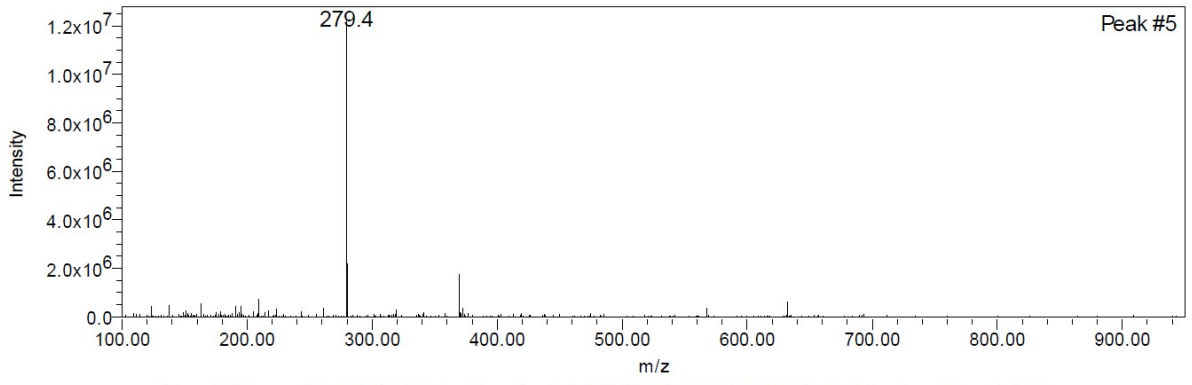
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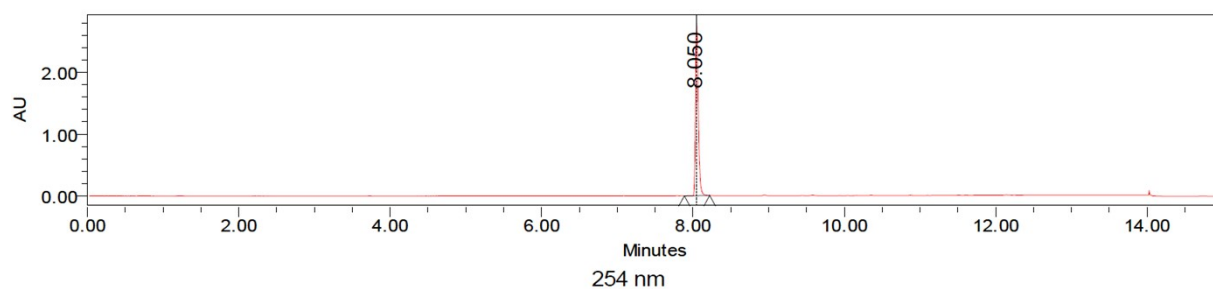
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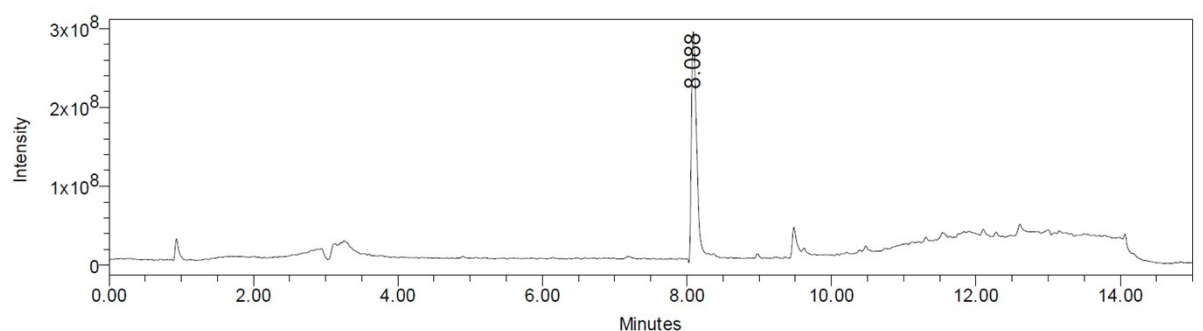
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UHPLC analyses of synthetic samples of naturally occurring compounds

(2Z)-Lachnophyllum methyl ester (Z-1a)

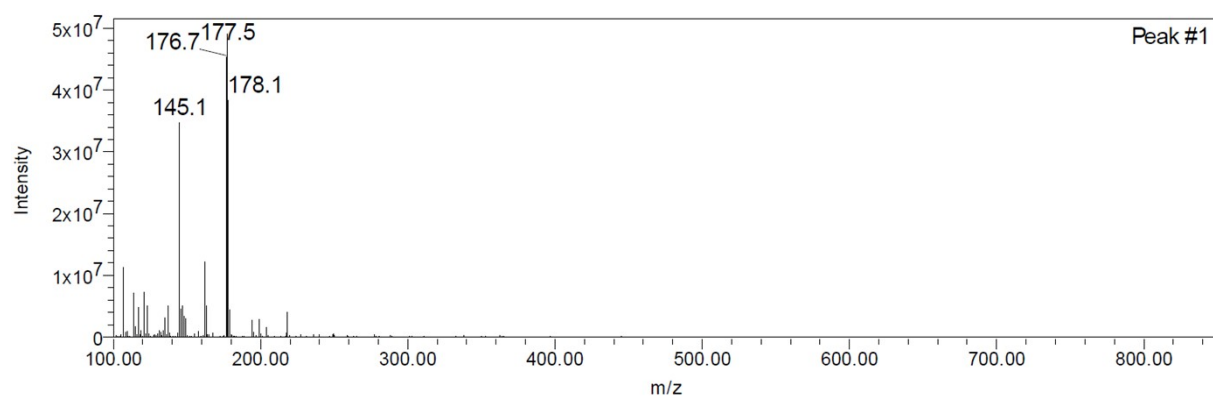


UV detection at 254 nm



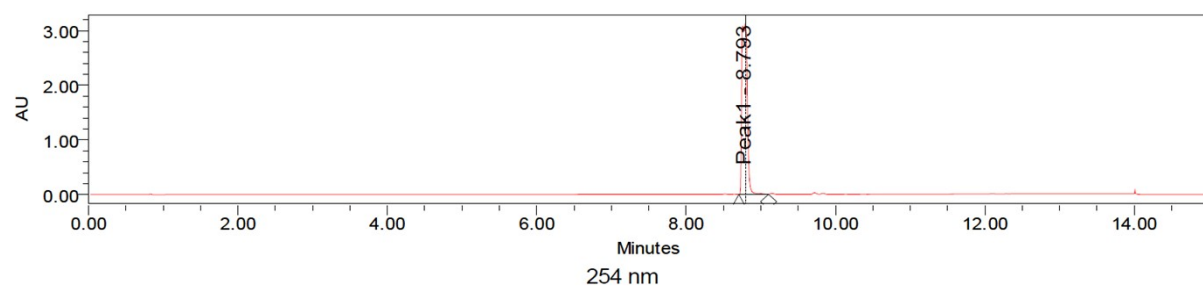
SampleName: IK110; Vial: 1:A,2; Injection: 1; Channel: SQ 1: MS Scan; Date Acquired: 7/17/2024 10:28:27 AM

Total-ion current in ESI(+)

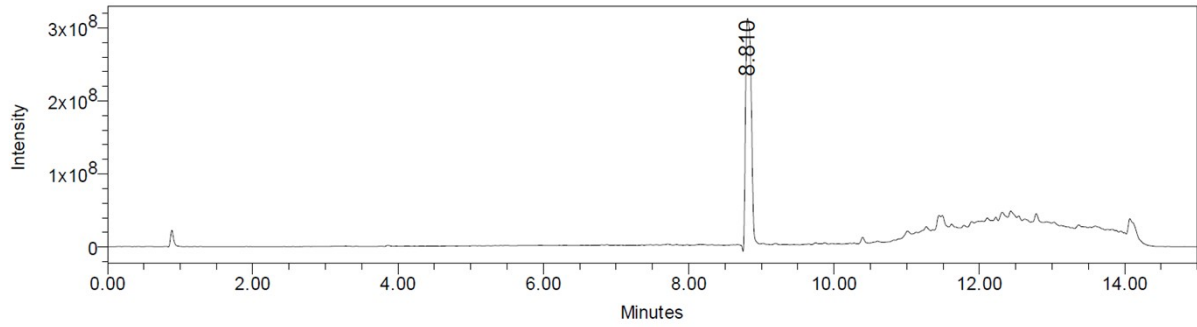


SampleName: IK110 Date Acquired: 7/17/2024 10:28:27 AM CET Retention Time 8.088

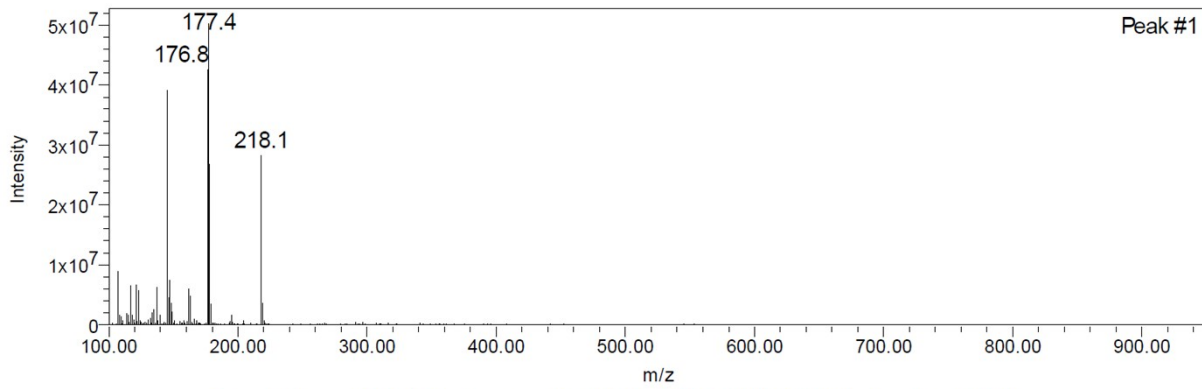
(2E)-Lachnophyllum methyl ester (E-1a)



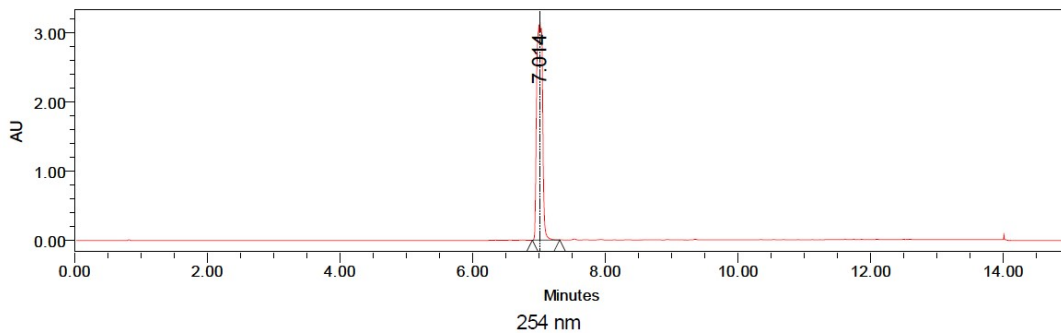
UV detection at 254 nm



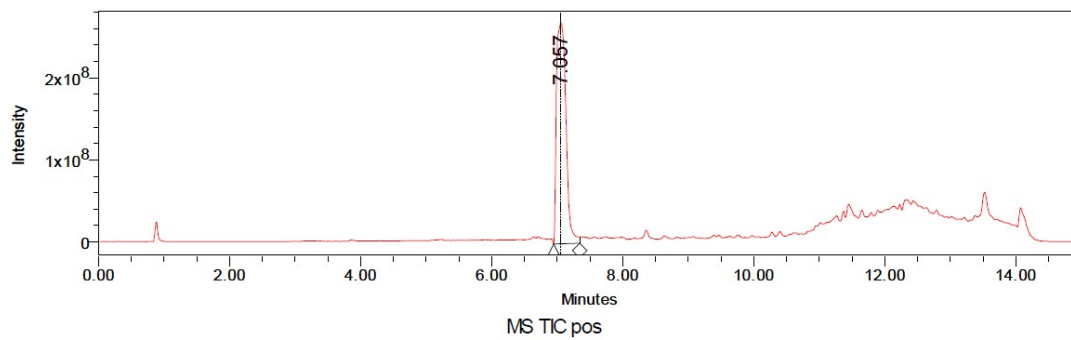
Total-ion current in ESI(+)



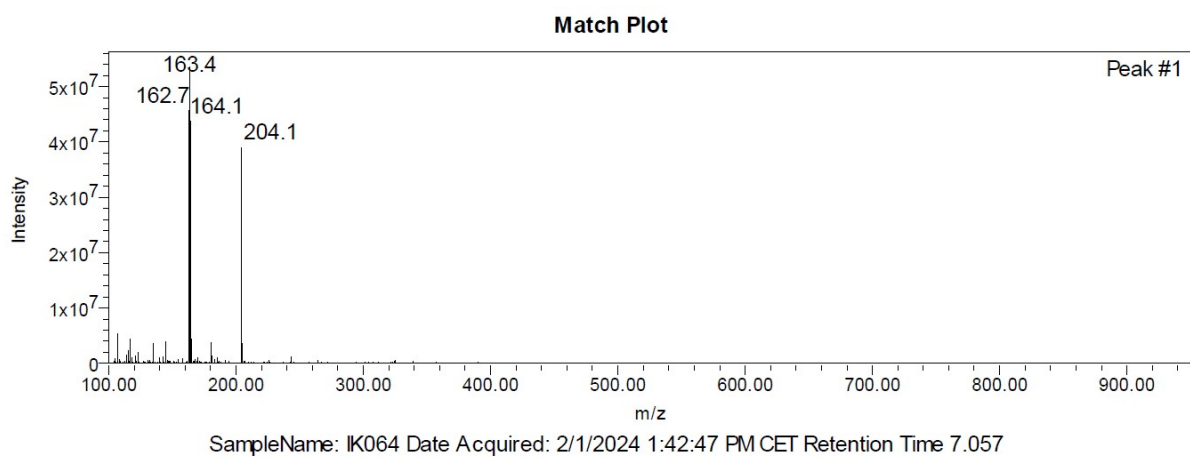
(4Z)-Lachnophyllum lactone (4a)



UV detection at 254 nm



Total-ion current in ESI(+)



1. K. Adande, K. Elo, O. Simalou, M.-F. Bakaï and P. Caboni, *Am. J. Anal. Chem.*, 2023, **14**, 95-120.