Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

A silver-promoted solid-phase guanidylation process enables the first total synthesis of Stictamide A

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1. General Information

1.1 Materials

Fmoc-protected amino acids, resin and HCTU were purchased from Glbiochem (Shanghai, China). Further reagents and solvents were purchased from Acros Organics, Alfa Aesar or Sinopharm Chemical Reagent Co. Ltd and were purified when necessary. THF were distilled from sodium/benzophenone ketyl before use. DMF was distilled under reduced pressure from sodium sulfate and stored in flask containing 4 Å molecular sieves. Et_3N and CH_2Cl_2 were distilled from calcium hydride immediately prior to use. All organic extracts were dried over sodium sulfate and concentrated under rotary evaportor. All other commercially obtained reagents and solvents were used directly without further purification. TLC was performed on plates pre-coated with silica gel 60 F_{254} (250 layer thickness). Flash column chromatographic purification of products was finished using forced-flow chromatography on Silica Gel (300-400 mesh). Visualization was accomplished with UV light, and/or phosphomolybdic acid (PMA) solution.

1.2 HPLC

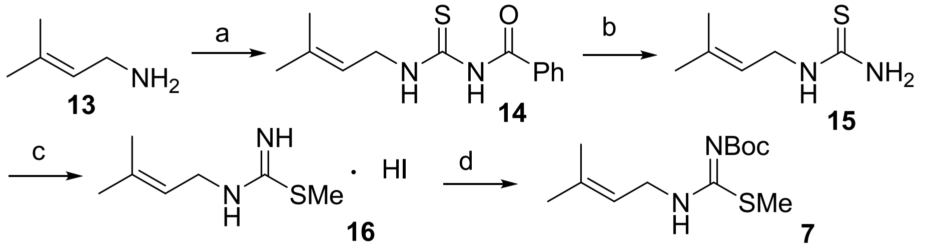
Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using an analytical column (Grace Vydac "Protein & Peptide C18", 250 X 4.6 mM, 5 μm particle size, flow rate 1.0 mL/min, rt). Analytical injections were monitored at 210 nm, 254 nm. Semi preparative HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using a semi preparative column (Grace Vydac "Peptide C18", 250 X 10 mM, 10 μm particle size, flow rate 4 mL/min). Solution A was 0.1% TFA in water, and solution B was 0.1% TFA in MeCN. Gradient A: A linear gradient of 5% to 5% B over 2 min, then a linear gradient of 5% to 85% B over 25 min. Gradient B: A linear gradient of 1% to 1% B over 2 min, then a linear gradient of 1% to 60% B over 25 min.

1.3 Mass spectrometry and NMR

ESI-MS was performed on a Agilent 1200/6340 mass spectrometer in Center of Biomedical Analysis or a Bruker Daltonics Inc. APEX II Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer (Tsinghua University). ^{1}H-NMR and ^{13}C-NMR spectrum were obtained on a Bruker Avance 300 MHz or 600 MHz NMR Spectrometer. The chemical shifts of protons are given on the δ scale, ppm, with tetramethylsilane (TMS) as the internal standard. HR-Q-TOF-MS were measured on an Agilent 6538 UHD Accurate Mass Q-TOF LC/MS mass spectrometer.

2. Chemical synthesis

2.1 Synthesis of building block 7



Reagents and conditions: a) benzoyl chloride, KSCN, acetone, acetonitrile, 2h; b) K_2CO_3, methanol, 6h; c) CH_3I, DMF, 12h; d) Boc_2O, Et_3N, DMAP, DCM.

2.1.1 Synthesis of 1-Benzoyl-3-(3-methylbut-2-enyl)thiourea 14

To a suspension of KSCN (2.1 g, 21.1 mmol) in 20 mL of anhydrous acetone was added slowly benzoyl chloride (2.7 mL, 21.1 mmol). After the mixture was stirred at room temperature for 1 h, the resultant precipitant was filtered off and the filtrate was concentrated to dryness. The residue was dissolved with anhydrous acetonitrile (30 ml) and then a solution of 3-methylbut-2-enylamine (1.8 g, 21.1 mmol) in 10 mL of anhydrous acetonitrile was added. The resultant solution was stirred overnight before the solvent was removed under reduced pressure. The residue was purified by column chromatography eluting with 2/1 petroleum ether/ethyl acetate to give 3.0 g of **14** in 57% yield. ^{1}H-NMR (300 MHz, CD_3Cl_3): δ 10.73 -10.41 (m, 1H), 9.10 (s, 1H), 7.92 -7.71 (m, 2H), 7.59 (t, J = 7.4 Hz, 1H), 7.47 (t, J = 7.6 Hz, 2H), 5.46 -5.19 (m, 1H), 4.39 -4.11 (m, 2H), 1.73 (d, J = 9.7 Hz, 6H).

2.1.2 Synthesis of 3-(3-methylbut-2-enyl)thiourea 15

To a solution of **14** (923 mg, 3.8 mmol) in 20 mL of methanol was added K_2CO_3 (993 mg, 7.2 mmol) and the resulting mixture was stirred at room temperature for 6 h. The solvent was removed under reduced pressure and then DCM (30 ml) was added to dissolve the residue. The resultant solution was washed with water and brine and dried over sodium sulfate. After removal of the solvent, the residue was chromatographed eluting with 30/1DCM/methanol to give 443 mg of **15** in 80% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.06 (s, 1H), 6.44 (s, 2H), 5.15 (s, 1H), 4.02 -3.70 (m, 2H), 1.64 (d, *J* = 9.7 Hz, 6H).

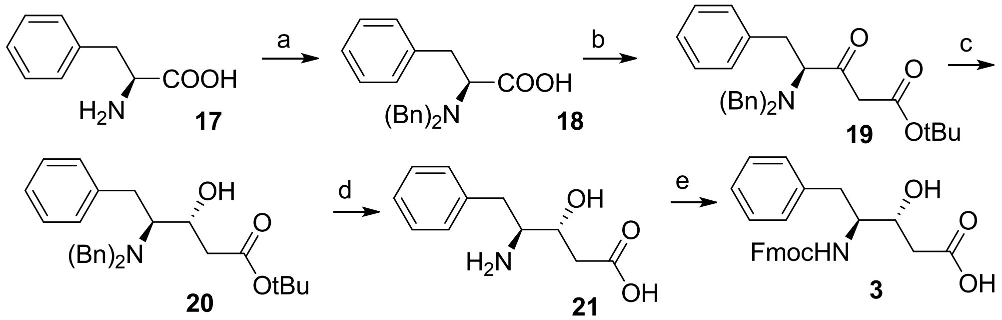
2.1.3 Synthesis of methyl isothiourea hydroiodide salt 16

A solution of **15** (960 mg, 6.4 mmol) and CH_3I (0.96 mL, 13.4 mmol) in anhydrous DMF (20 ml) was stirred at room temperature overnight. The solvent was removed in vacuo to give 965 mg of a crude product of **24** without purification.

2.1.3 Synthesis of 1-(tert-Butoxycarbonyl)-3-(3-methylbut-2-enyl)-2-methylisothiourea 7

To a solution of above crude product, Et_3N (0.6 mL, 4.5 mmol), and catalytic DMAP in 20 mL of anhydrous DCM was added $(Boc)_2O$ (0.52 g, 2.3 mmol). After the mixture was stirred at room temperature for 4 h, it was washed by water and brine. The organic layer was dried over sodium sulfate and concentrated. The residue was purified by column chromatography eluting with 30/1 DCM/methanol to give 745 g of **5** in 85% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.61 (s, 1H), 5.34 – 5.11 (m, 1H), 3.87 (t, *J* = 5.8 Hz, 2H), 2.45 (s, 3H), 1.71 (d, *J* = 8.7 Hz, 3H), 1.67 (s, 3H), 1.47 (d, *J* = 9.6 Hz, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.59, 162.69, 138.00, 119.29, 79.70, 42.32, 28.81, 28.81, 28.81, 26.21, 18.59, 14.21. ESI MS: calcd. for $C_{12}H_{23}N_2O_2S^+$ $[M+H]^+$ m/z, 259.15; found, 259.25.

2.2 Synthesis of building block 3



Reagents and conditions: a) K_2CO_3, benzoyl bromine, ethanol, reflux, 5h; then NaOH, H_2O, dioxane; b) BuLi, DIEA, butyl acetate, CDI, THF, 2h; c) $NaBH_4$, methanol, 12h; d) TFA/DCM; then Pd-C, H_2, methanol; e) Fmoc-Cl, DMAP, DCM, 75%.

2.2.1 Synthesis of *(S)*-2-(dibenzylamino)-3-phenylpropanoic acid 18.

To a 1 L round bottom flask was added L-Phenylalanine (14.2 g, 86.2 mmol), benzyl bromide (35.8 mL, 302.0 mmol), K_2CO_3 (53.6 g, 388.3 mmol), and ethanol (500 mL). The mixture was heated at 80 °C under a reflux condenser for 5 hours. The mixture was filtered through a coarse scintered glass funnel washing with ethyl acetate, and the filtrate was evaporated. The residue was taken up in DCM (300 mL) and washed with brine (200 mL), dried over sodium sulfate, and evaporated to a yellow oil. The crude product was stirred in 6:1:3 (dioxane 360 mL: methanol 60 mL: 2 N NaOH 120 mL, 600 mL) for 5 hours. The mixture was evaporated to an aqueous solution and ether (300 mL) was added. The aqueous layer was extracted again with ether (100 mL), the combined organic layer was washed with brine (200 mL), dried over sodium sulfate, and evaporated to a yellow oil. The residue was purified by column chromatography eluting with 10/1 petroleum ether/ethyl acetate to give 25.5 g of **18** in 86% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.98 (s, 1H), 7.49 -7.27 (m, 15H), 4.51 (d, J = 15.0 Hz, 2H), 4.23 (dd, J = 8.3, 6.4 Hz, 1H), 4.02 (d, J = 13.2 Hz, 2H), 3.71 -3.63 (m, 1H), 3.30 (dd, J = 15.0, 8.5 Hz, 1H). ^{13}C NMR (75-MHz, $CDCl_3$): δ 169.91, 135.35, 132.80, 132.34, 131.44, 130.57, 130.29, 130.14, 129.93, 129.85, 129.71, 129.51, 129.33, 129.24, 129.13, 128.87, 128.52, 128.34, 127.74, 64.92, 55.75, 55.63, 32.59. ESI MS: calcd. for $C_{23}H_{24}NO_2^+$ $[M+H]^+$ m/z, 346.18; found, 346.57.

2.2.2 Synthesis of *(S)*-tert-butyl 4-(dibenzylamino)-3-oxo-5-phenylpentanoate 19.

To a stirred solution of N,N-dibenzylamino acid **18** (1.48 g, 4.30 mmol) in THF (20 mL) was added CDI (767 mg, 4.70 mmol) at room temperature under a N_2 atmosphere. The resulting solution was stirred at room temperture for 1 h. Meanwhile, a solution of lithium (tertbutoxycarbonyl)methanide was made from BuLi (2.50 M, 3.60 mL, 9.00 mmol), DIEA (1.30 mL, 9.00 mmol), and tert-butyl acetate (1.22 mL, 9.00 mmol). The pale yellow solution of the lithium enloate was added dropwise to the imidazole solution at -78 °C under a N_2 atmosphere. The resulting mixture was stirred at-78 °C for 2.5 h, quenched with 1 N HCl (50 mL), and extracted with ethyl acetate (3*50 mL). The combined organic extracts were washed with brine (200 mL), dried over sodium sulfate and concentrated to dryness. The residue was purified by column chromatography eluting with 40/1 petroleum ether/ethyl acetate to give 1.4 g of **19** in 76% yield. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.39 -7.17 (m, 15H), 3.87 (d, J = 13.4 Hz, 2H), 3.66 -3.54 (m, 4H), 3.43 (d, J = 15.5 Hz, 1H), 3.27 -3.19 (m, 1H), 2.99 (dd, J = 13.5, 3.6 Hz, 1H), 1.27 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 203.13, 167.00, 139.89, 139.36, 139.36, 130.16, 130.16, 129.65, 129.65, 129.65, 129.08, 129.08, 129.08, 128.99, 128.99, 127.99, 126.64, 82.10, 68.89, 55.20, 55.20, 48.39, 29.10, 28.36, 28.36, 28.36.

ESI MS: calcd. for $C_{29}H_{34}NO_3^+$ $[M+H]^+$ m/z, 444.25; found, 444.46.

2.2.3 *(3R,4S)*-tert-butyl-4-(dibenzylamino)-3-hydroxy-5-phenylpentanoate 20.

The compound **19** (350 mg, 0.79 mmol) was dissolved in methanol (15 mL). The resulting solution was then cooled to -22 °C and treated with $NaBH_4$ (61 mg, 1.6 mmol). The reaction was monitored by TLC. After 3.5 h, the solution was quenched with H_2O (60 mL) at pH 5-6 (adjusted by 1 N HCl) and extracted with ether (3*100 mL). The combined organic layer was washed by brine (100 mL), dried over sodium sulfate and concentrated to dryness. The residue was purified by column chromatography eluting with 30/1 petroleum ether/ethyl acetate to give 310 mg of **20** in 88% yield. $[\alpha]^{20}_D = 13.8$ (c 1.37, $CHCl_3$); ^1H-NMR (300 MHz, $CDCl_3$): δ 7.36 -7.20 (m, 15H), 4.11 -4.07 (m, 3H), 4.00 -3.93 (m, 1H), 3.47 (d, $J = 13.4$ Hz, 2H), 3.16 (dd, $J = 13.3, 4.6$ Hz, 1H), 2.91 -2.78 (m, 2H), 2.46 -2.37 (m, 1H), 2.10, (dd, $J = 16.1, 2.4$ Hz, 1H), 1.41 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.99, 140.84, 139.97, 139.97, 129.87, 129.87, 129.58, 129.58, 129.58, 129.16, 129.16, 128.99, 128.99, 128.99, 127.70, 126.73, 81.43, 68.63, 63.63, 55.13, 55.13, 41.30, 31.48, 28.67, 28.67, 28.67. ESI MS: calcd. for $C_{29}H_{36}NO_3^+$ $[M+H]^+$ m/z, 446.27; found, 446.45.

2.2.4 Synthesis of *(3R,4S)*-4-amino-3-hydroxy-5-phenylpentanoic acid 21.

The compound **20** (310 mg, 0.7 mmol) was dissolved in TFA/DCM (1:1) (10 mL) and stirred at room temperature for 2h. The solution was co-evaporated with toluene (10 * 3 ml) to dryness under reduced pressure. Then the residue was dissolved in anhydrous methanol (10 ml) and was stirred for 8h in the presence of a catalytic amount of Pd-C (10%) under hydrogen at room temperature. The catalyst Pd-C was removed by filtration, then the resulting filtrate was concentrated under reduced pressure to give 146 mg of **21** as a colorless oil in 99% yield. ^1H-NMR (300 MHz, CD_3OD): δ 7.40 -7.29 (m, 5H), 4.09 -4.04 (m, 1H), 3.66 (s, 1H), 3.56 -3.49 (m, 1H), 3.35 (s, 1H), 3.12 -2.89 (m, 2H), 2.64 -2.58 (m, 2H), 2.04 (s, 1H). ^{13}C-NMR (75 MHz, CD_3OD): δ 173.55, 136.19, 129.63, 129.63, 129.24, 129.24, 127.62, 65.89, 56.82, 39.16, 36.52. ESI MS: calcd. for $C_{11}H_{14}NO_3^-$ $[M-H]^-$ m/z, 208.10; found, 208.29.

2.2.5 Synthesis of *(3R,4S)*-4-Fmoc-amino-3-hydroxy-5-phenylpentanoic acid 3.

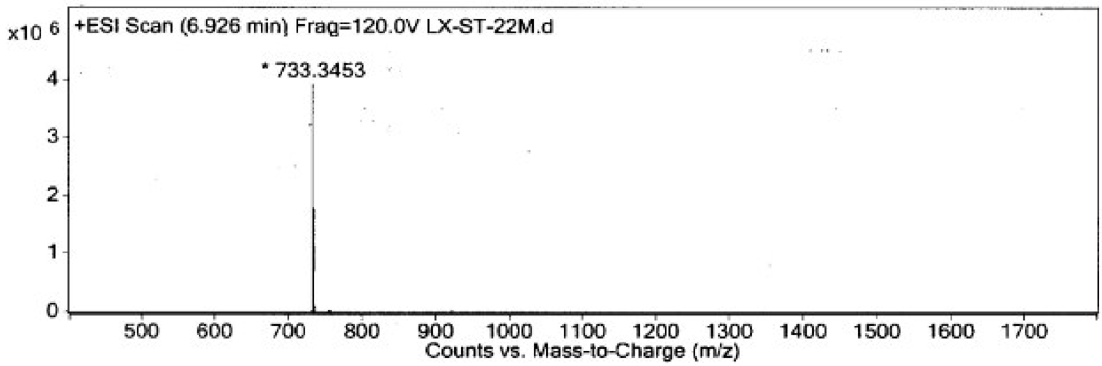
To a stirred solution of the compound **21** (418 mg, 2.0 mmol) in THF (10 mL) and water (25 ml) was added Na_2CO_3 (445 mg, 4.2 mmol) at room temperature. The resulting solution was stirred at room temperture for 1 h. Then, a solution of Fmoc-Cl (541 mg, 2.14 mmol) in THF (10 ml) was was added dropwise to the above solution at 0 °C. The resulting mixture was stirred for 1.5 h, diluted with DCM (100 ml) and water (50 ml) and adjusted to pH 2 with 1N HCl. The resulting solution was extracted with DCM (3*50 mL). The combined organic extracts were washed with brine (200 mL), dried over sodium sulfate and concentrated to dryness. The residue was purified by column chromatography eluting with 1/1 petroleum ether/ethyl acetate to give 630 mg of **3** in 73% yield. ^{1}H-NMR (300 MHz, CD_3OD): δ 7.79 (d, J = 7.4 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.40 -7.14 (m, 9H), 5.49 (s, 1H), 4.30 -4.21 (m, 2H), 4.21 -4.06 (m, 2H), 3.88 -3.84 (m, 1H), 2.96 -2.71 (m, 2H), 2.46 -2.41 (m, 2H). ^{13}C-NMR (75 MHz, CD_3OD): δ 157.91, 144.49, 144.24, 141.69, 141.66, 139.14, 129.54, 129.54, 128.57, 128.57, 128.57, 127.99, 127.99, 127.39, 127.39, 126.51, 125.47, 125.34, 120.08, 120.08, 69.38, 66.93, 57.28, 57.18, 39.13, 37.68. ESI MS: calcd. for $C_{26}H_{26}NO_5^+$ $[M+H]^+$ m/z, 432.18; found, 432.38.

2.3 Synthesis of Stictamide A

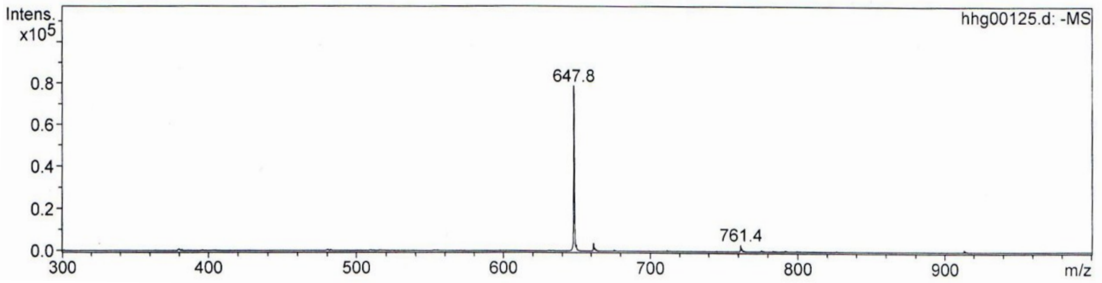
After a mixture of 2-Cl-trityl-Cl resin (400.0 mg, active Cl, 0.20 mmol), Fmoc-Tyr(OtBu)-OH (468.0 mg, 1.0 mmol) and DIEA (365.0 μL) in DMF (5 mL) was shaken on a vortex mixer at rt overnight, the resin was filtered off and washed several times with methanol, DCM and DMF. And then the Fmoc group is deprotected with 20% piperidine in DMF (15 min) to afford the Tyr-linked resin **8** which was then used for the construction of the title peptide. Then, **8** were shaken in DMF (10 ml) with 5 eq. of fragment **3**, 4.5 eq. of HCTU and 10 eq. of DIEA at rt for 4h. The Fmoc group is deprotected with 20% piperidine in DMF (15 min) to give the resin **9**. The Fmoc-D-Orn(Aloc)-OH (**6**) and fragment **5** was then installed to the resin and deprotected as before to afford the resin **11**. The removal of allyloxycarbonyl group on the resin **11** was performed in the presence of $Pd[P(Ph)_3]_4$ (2 eq.) and Phenylsilane (5 eq.) in DCM (5 ml) to afford the compound **12**. Then, a solution of compound **7** (3 eq.), Et_3N (10 eq.) and $AgNO_3$ (3 eq.) in 5 mL of anhydrous DMF was added into the resin **12**. After the mixture was shaken for 8 h in the dark, the resultant mixture was filtered and the resin was washed thoroughly by DMF, methanol, and DCM to compound **1a**. Then, the peptide-loaded resin was treated with a mixture of Water/TFA (5:95, 10 mL) for 2 h at rt and the tBu group was removed at the same time under this condition. The resin was filtered off and washed with TFA. The washings were combined and condensed in vacuum to give crude **1**.

The crude product **1** was dissolved in water and purified by HPLC (conditions: Grace Vydac “Peptide C18”, 250 X 10 mM, 10 μm particle size, suitable ratio of acetonitrile-0.1%TFA in water-0.1%TFA, 4 mL/min) to give the pure **1**. ^{1}H NMR (600 MHz, CD_3OD): *δ* 7.24 -7.19 (m, 5H), 7.14 -7.12 (m, 1H), 7.07 -6.99 (m, 5H), 6.70 (dd, *J* = 10.1, 8.6 Hz, 5H), 5.35 -5.34 (m, 1H), 5.26 -5.23 (m, 1H), 4.49 (dd, *J* = 8.3, 4.4 Hz, 1H), 4.23 -4.21 (m, 1H), 4.16 -4.13 (m, 1H), 4.10 -4.07 (m, 1H), 3.78 (d, *J* = 6.5Hz, 2H), 3.16 -3.03 (m, 3H), 2.98 - 2.82 (m, 4H), 2.54 (t, *J* = 7.5 Hz, 2H), 2.43 -2.36 (m, 1H), 2.33 -2.18 (m, 4H), 1.86 (t, *J* = 7.8 Hz, 2H), 1.76 (s, 3H), 1.70 (s, 3H), 1.62 -1.59 (m, 2H), 1.46 -1.43 (m, 2H). ^{13}C NMR (150 MHz, CD_3OD): *δ* 175.13, 175.04, 172.93, 171.89, 155.18, 154.62, 154.62, 138.37, 137.92, 132.77, 130.15, 129.57, 129.16, 129.01, 128.72, 128.08, 126.04, 117.85, 114.90, 114.90, 114.85, 69.29, 54.92, 54.92, 53.16, 40.61, 40.26, 39.11, 36.64, 36.32, 34.90, 33.92, 27.50, 24.49, 24.49, 16.80. HR-Q-TOF-MS: calcd. for $C_{41}H_{55}N_6O_8^+$ $[M+H]^+$ m/z, 759.4076; found, 759.4080.

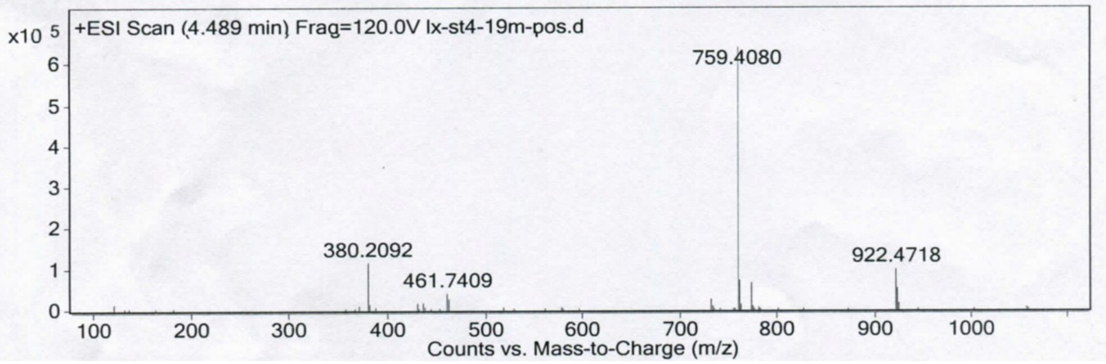
HR-Q-TOF-MS for key immediate **11** (the tBu group is removed when **11** was cut from the resin)



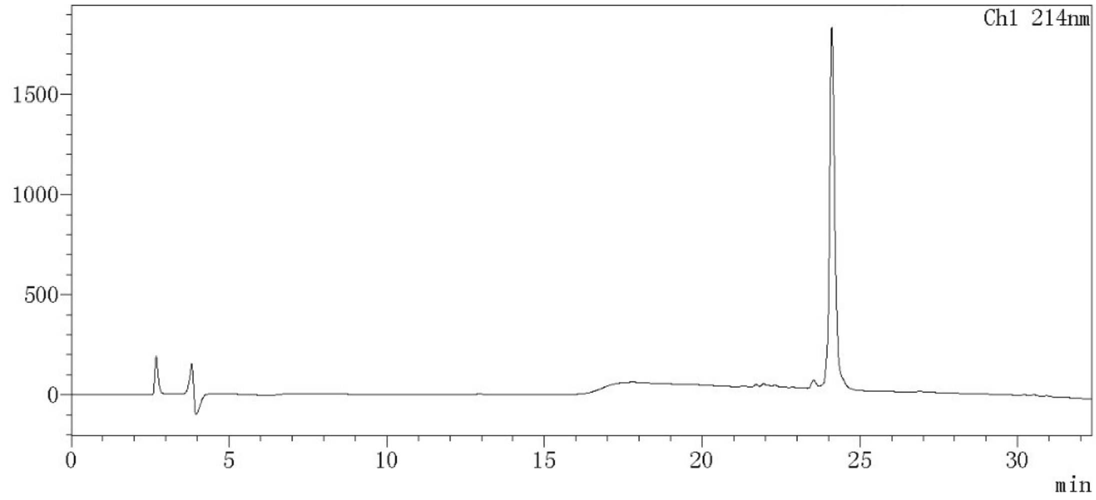
ESI-MS for key immediate **12** (the tBu group is removed when **12** was cut from the resin)



HR-Q-TOF-MS for the compound **1**

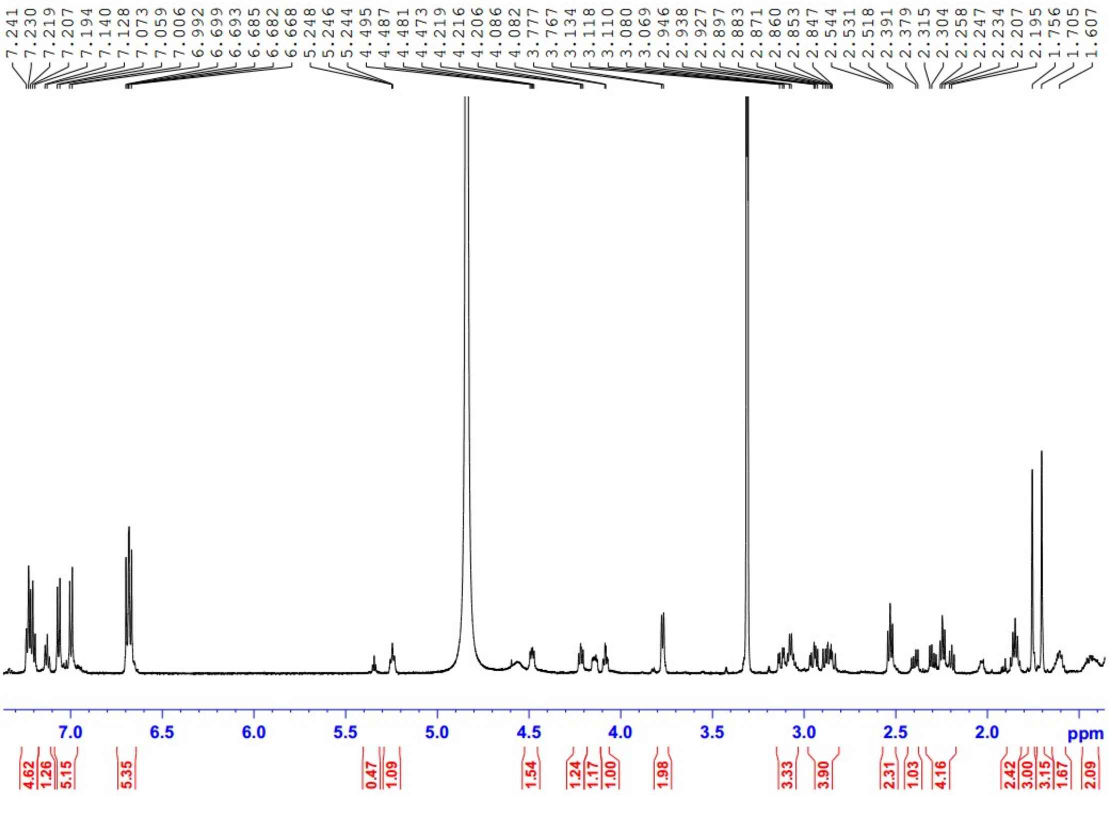


3. Figures and NMR spectrum

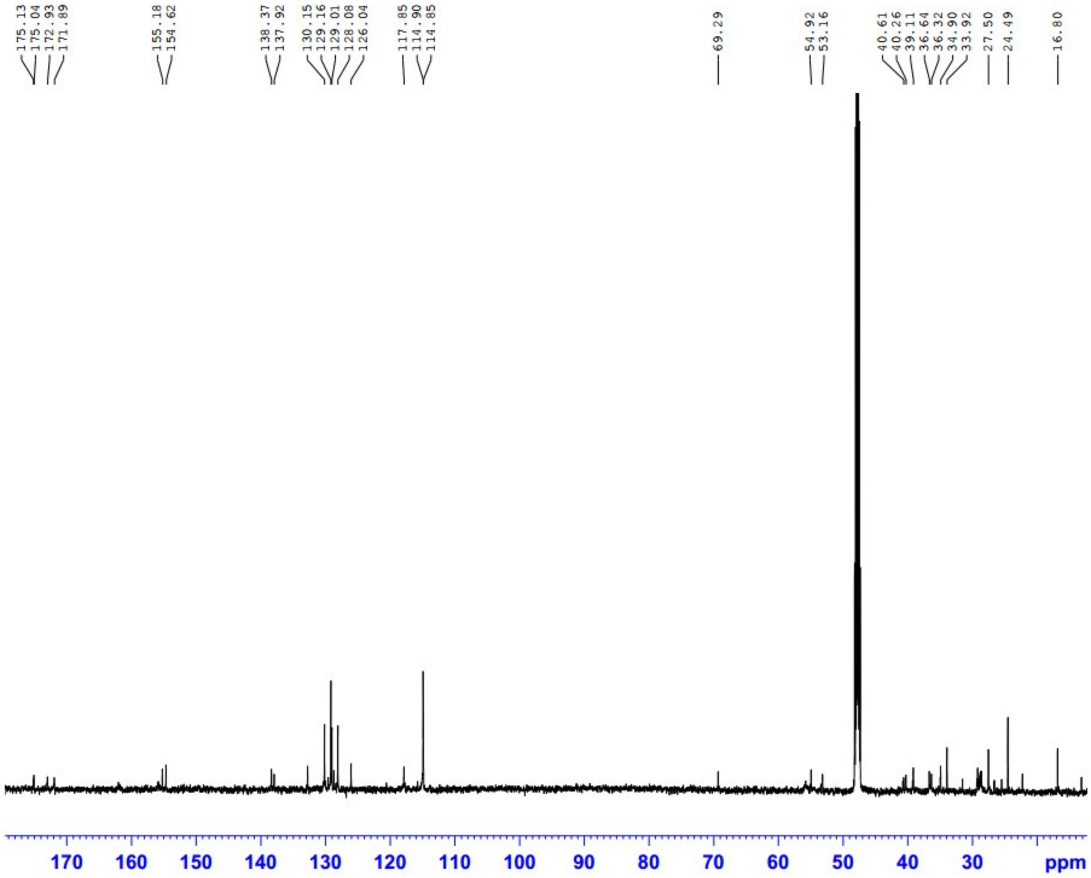


Analytical HPLC trace of compound **1**

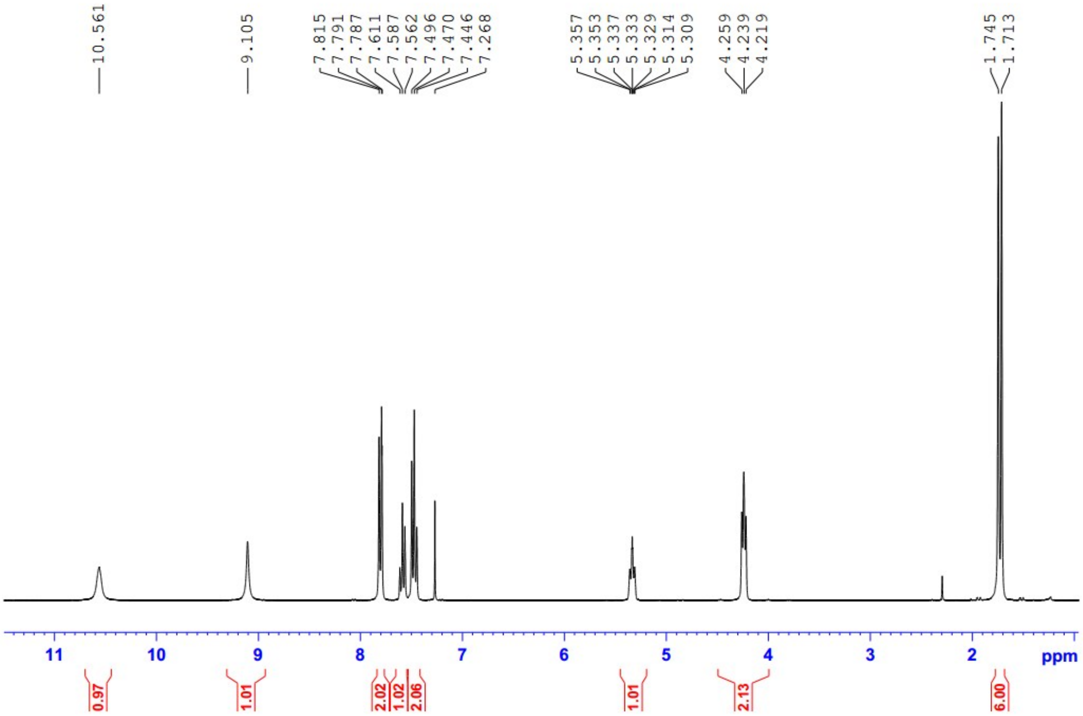
^{1}H-NMR of compound **1** (CD_3OD)



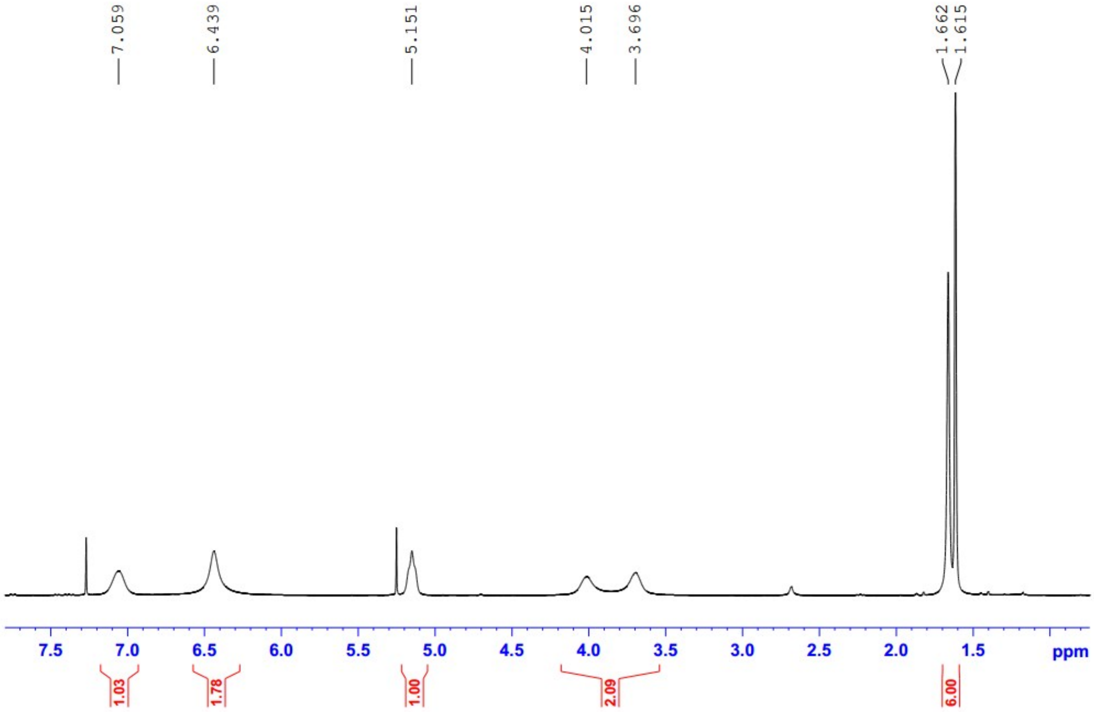
^{13}C-NMR of compound **1** (CD_3OD)



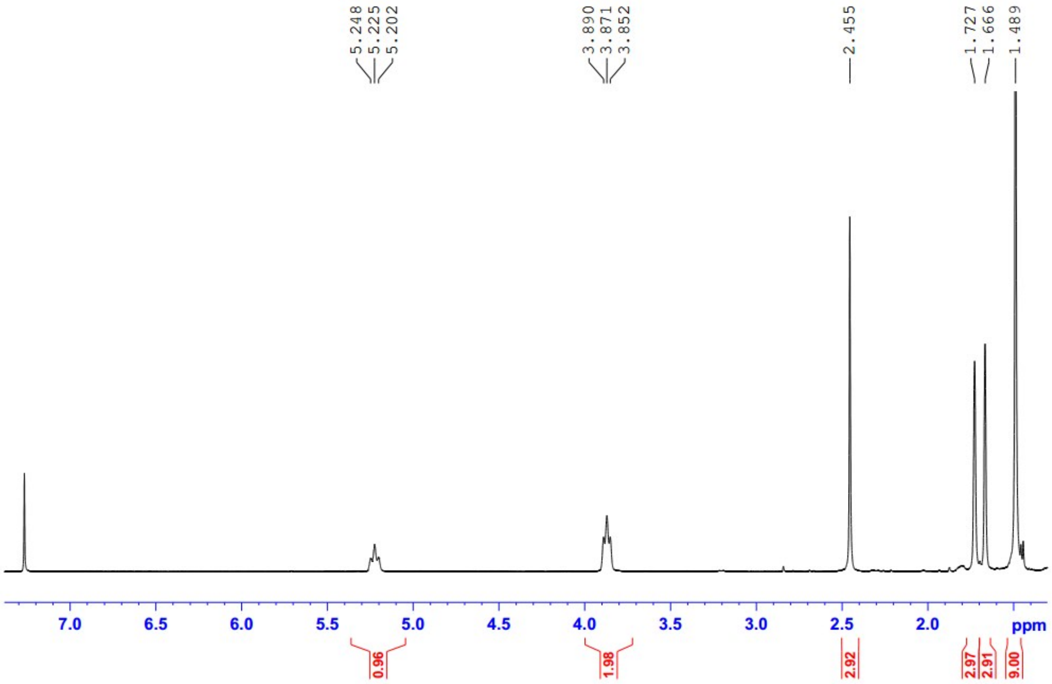
^{1}H-NMR of compound **14** ($CDCl_3$)



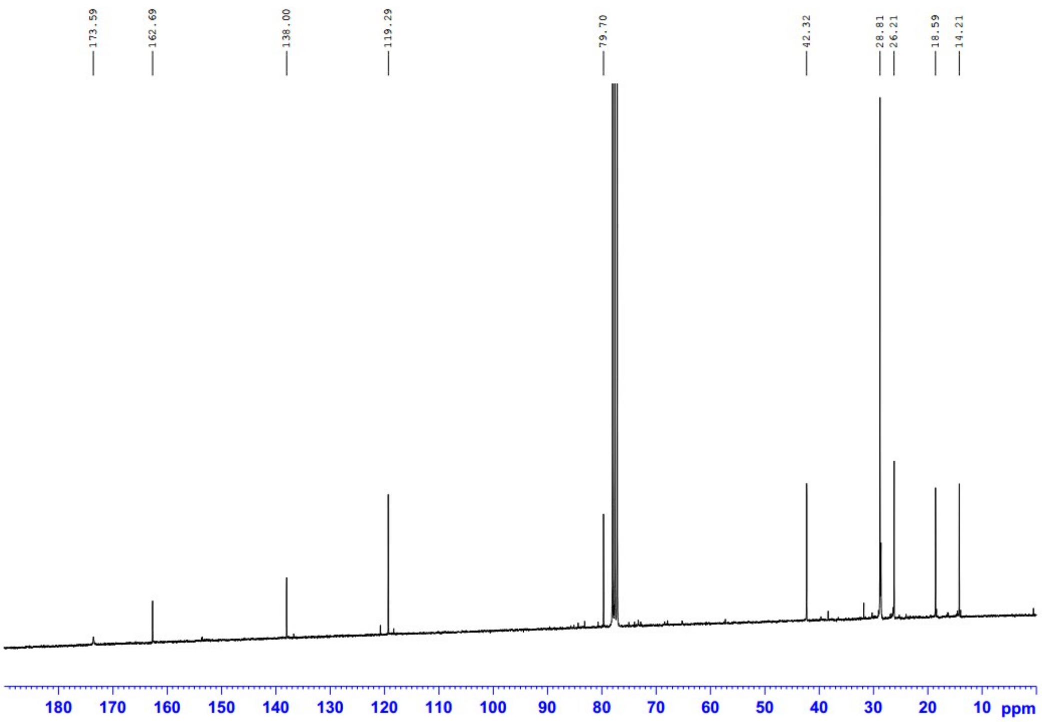
^{1}H-NMR of compound **15** ($CDCl_3$)



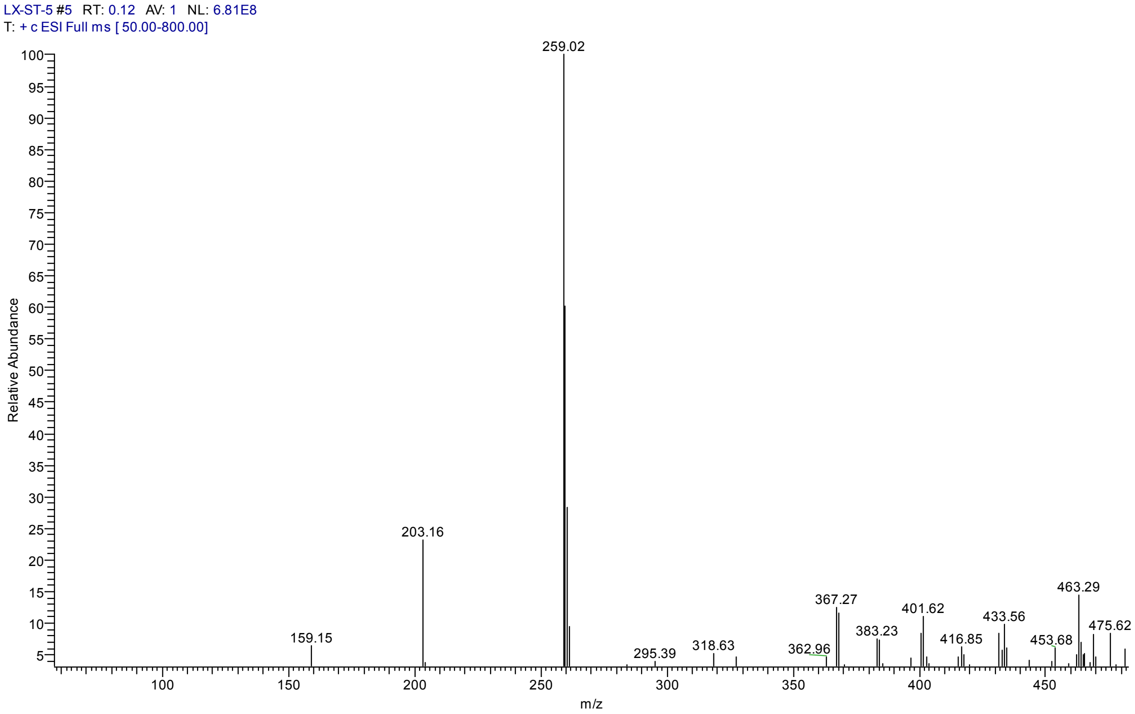
^{1}H-NMR of compound **7** ($CDCl_3$)



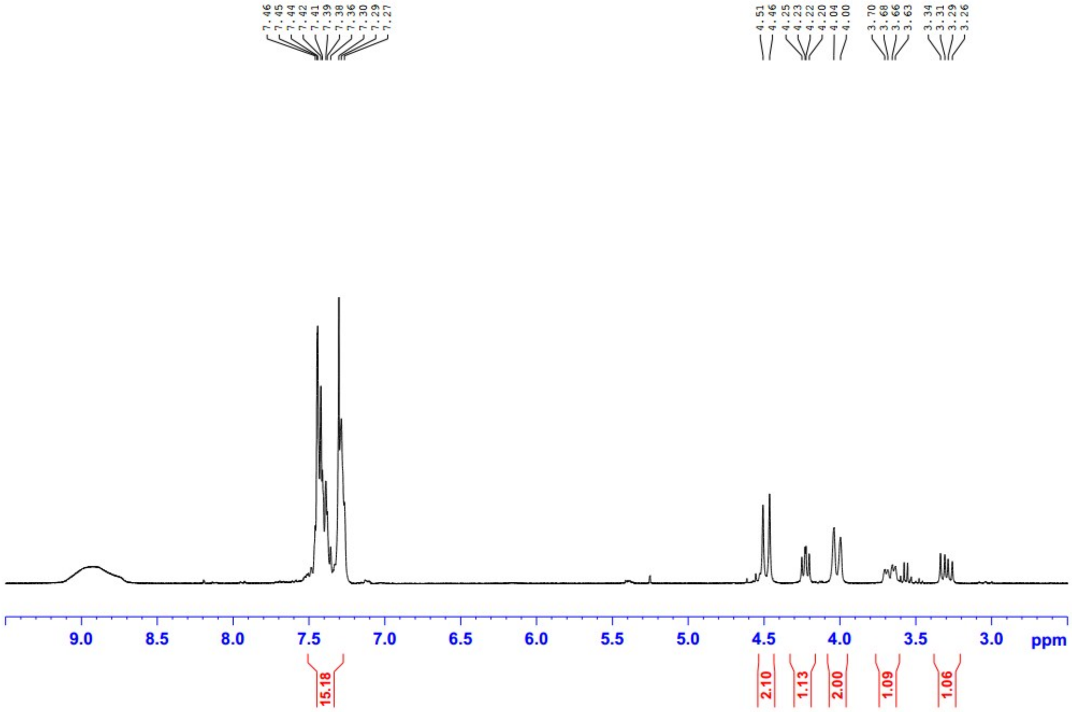
^{13}C-NMR of compound **7** ($CDCl_3$)



ESI MS of compound **7**



^{1}H-NMR of compound **18** ($CDCl_3$)



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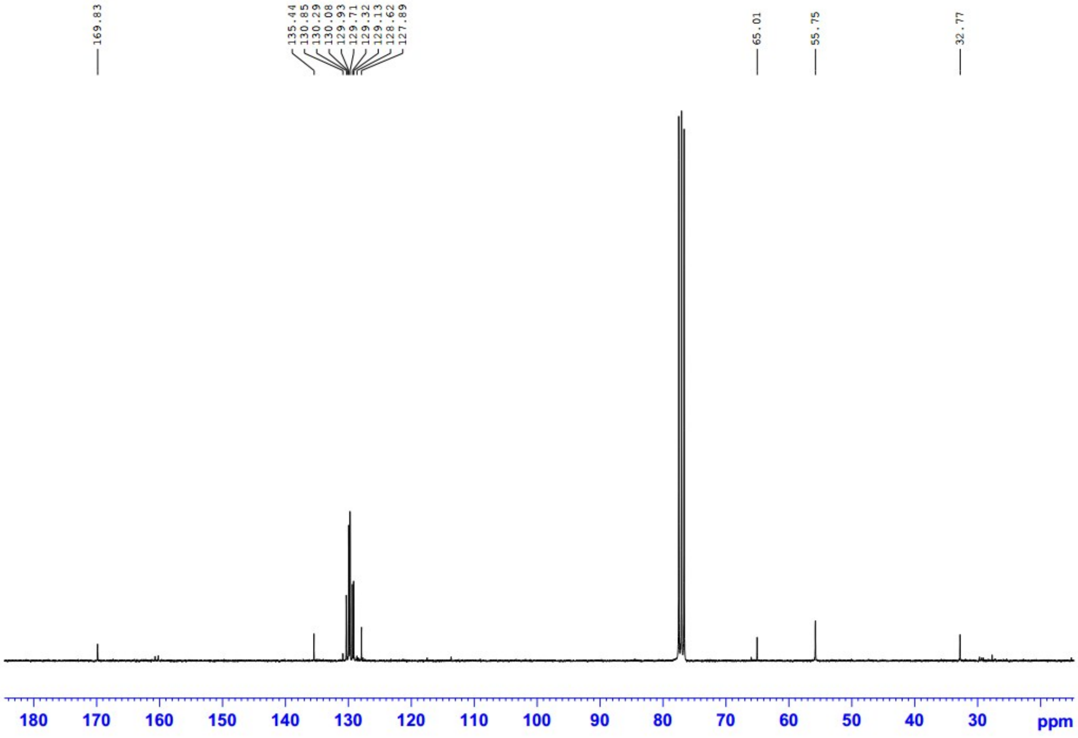
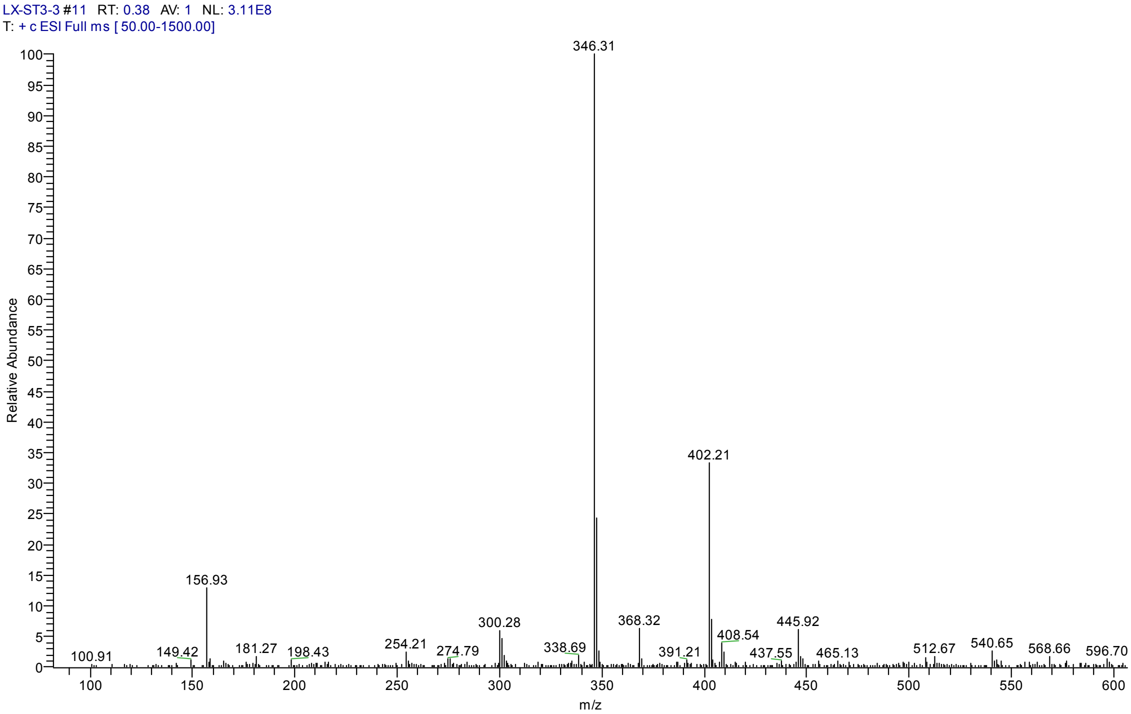
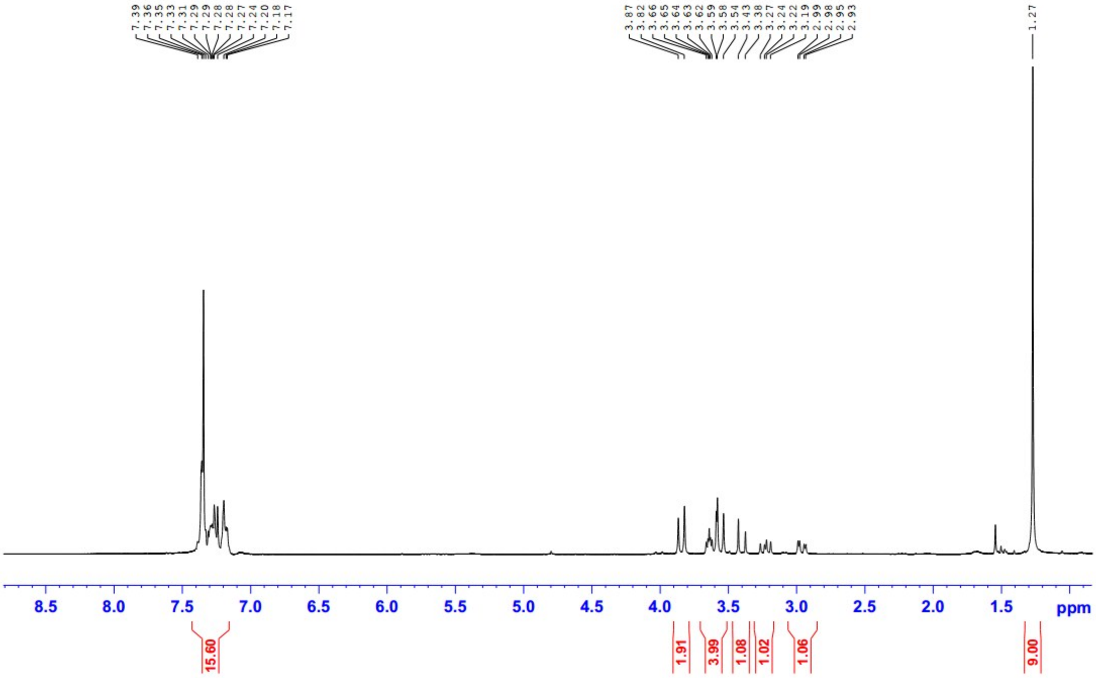


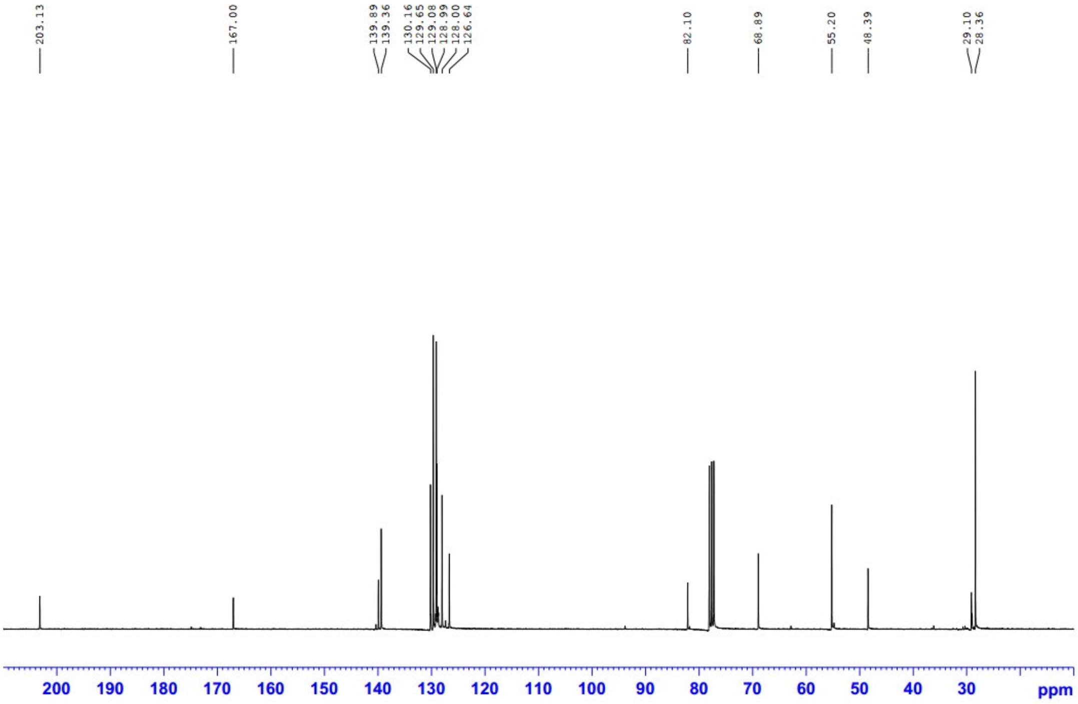
ESI MS of compound **18**



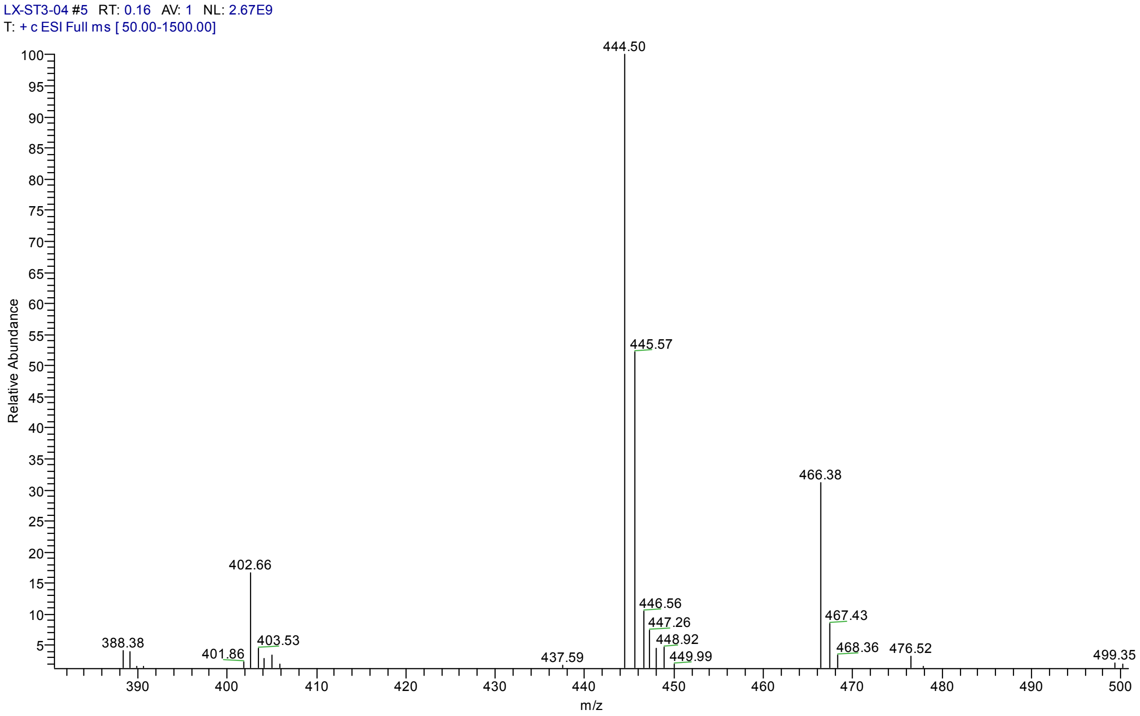
^{1}H-NMR of compound **19** ($CDCl_3$)



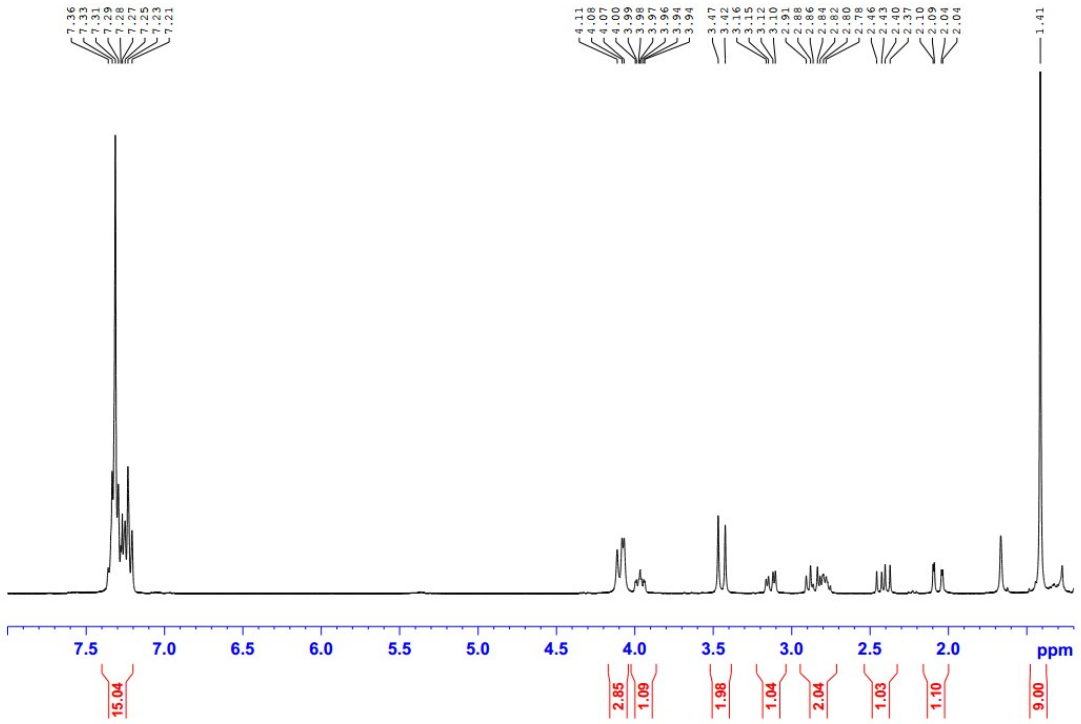
^{13}C-NMR of compound **19** ($CDCl_3$)



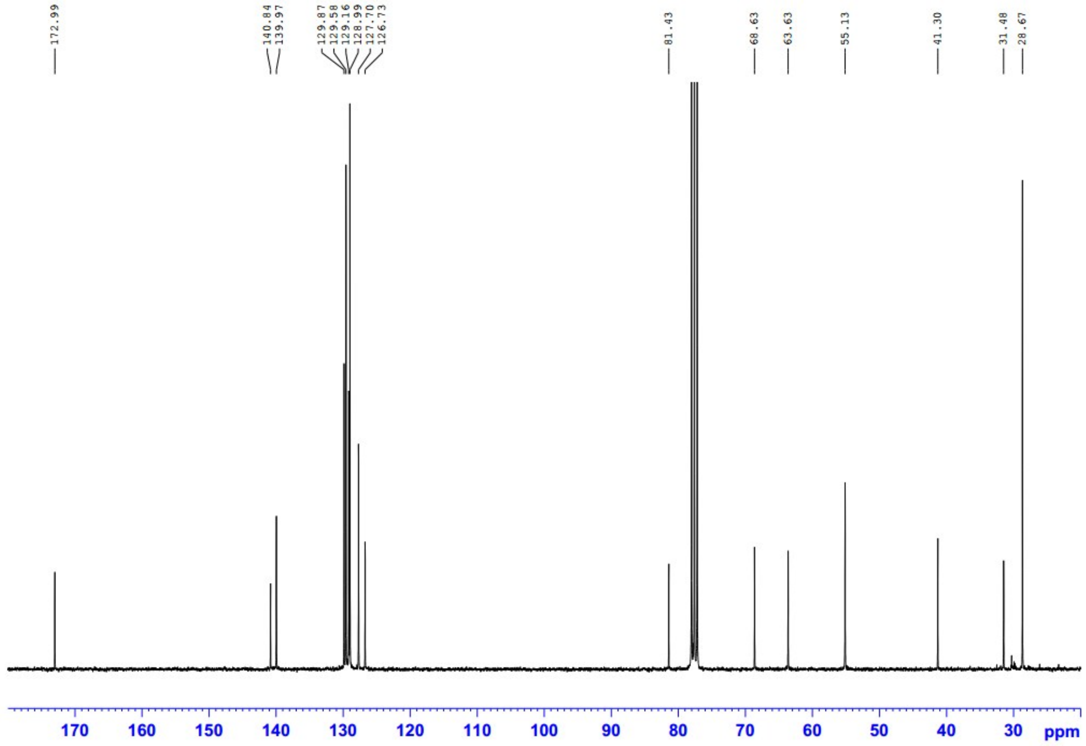
ESI MS of compound **19**



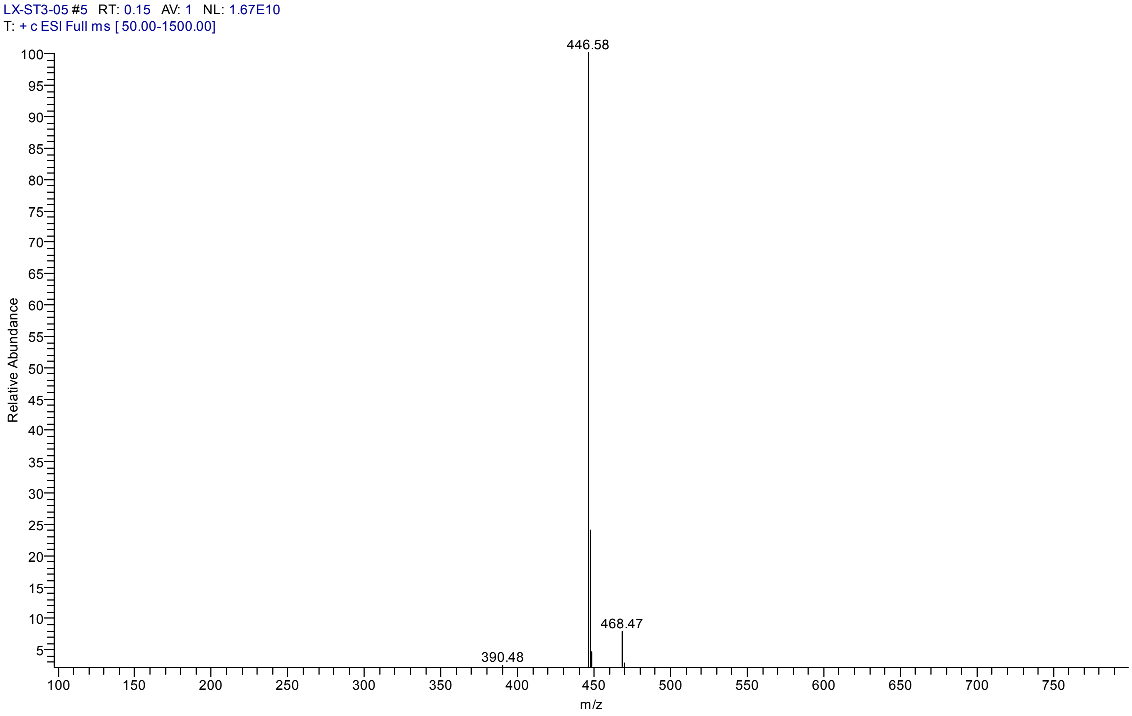
^{1}H-NMR of compound **20** (CDCl$_3$)



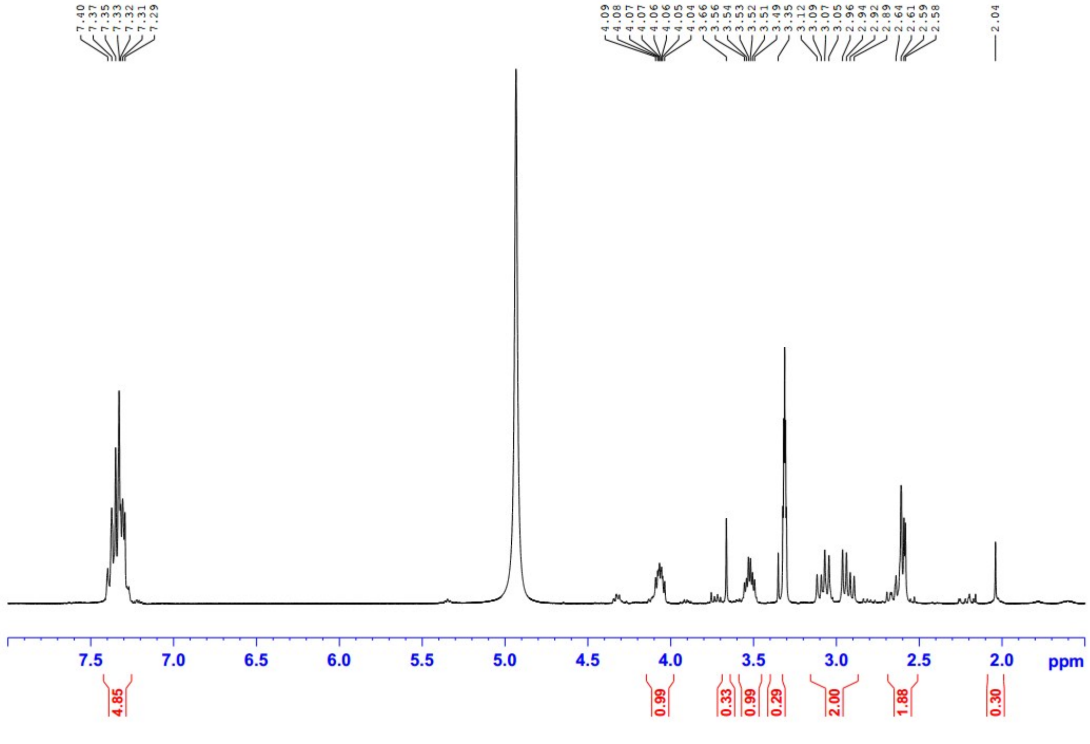
^{13}C-NMR of compound **20** (CDCl$_3$)



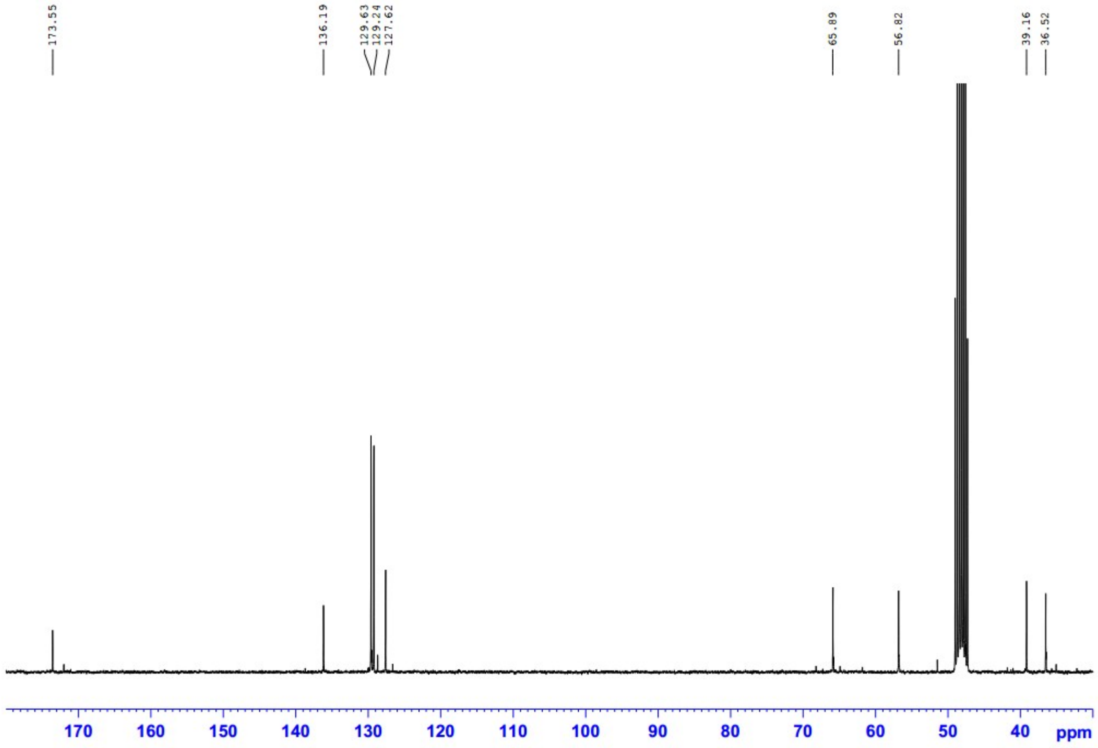
ESI MS of compound **20**



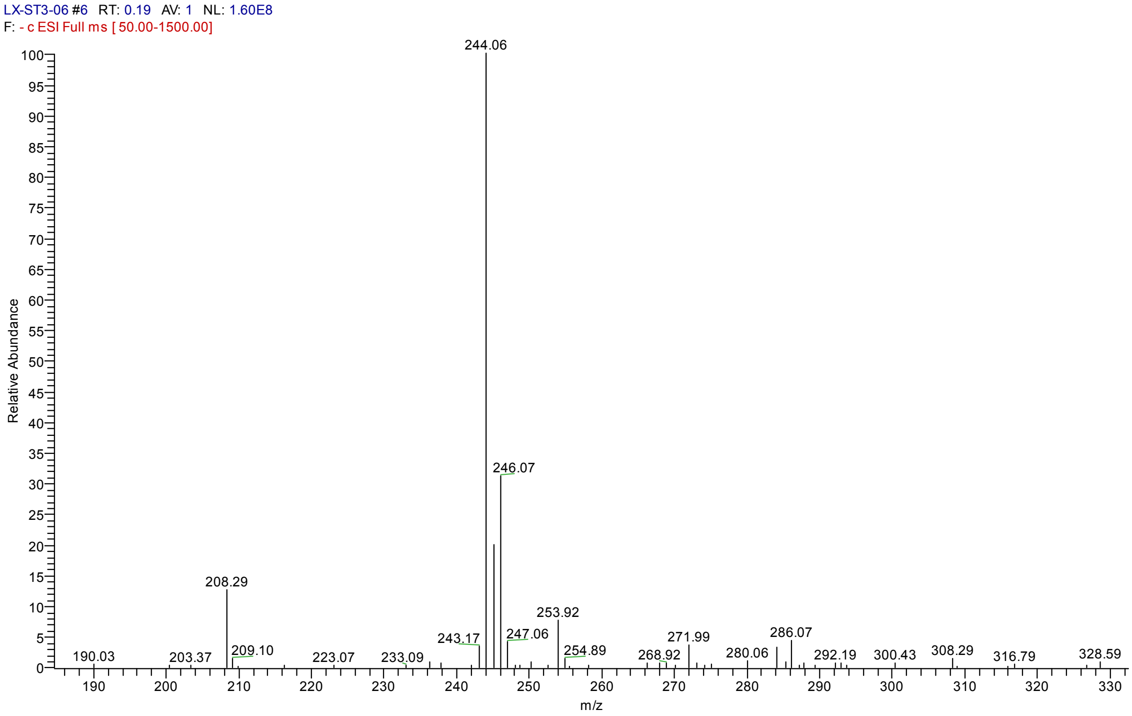
^{1}H-NMR of compound **21** (CD_3OD)



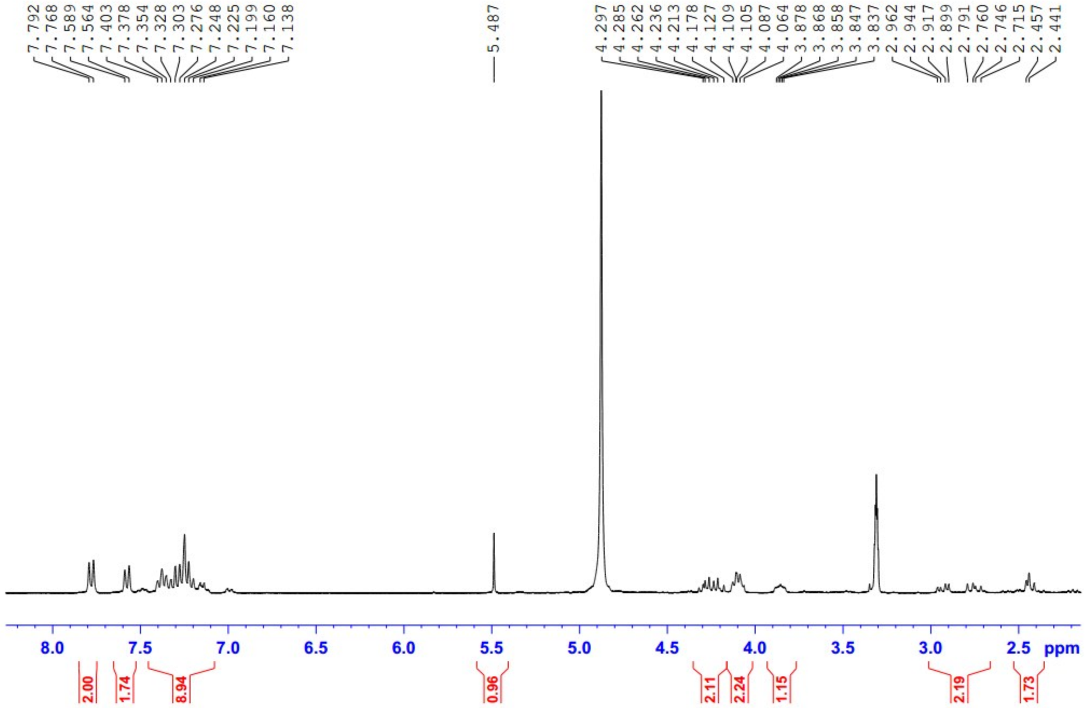
^{13}C-NMR of compound **21** (CD_3OD)



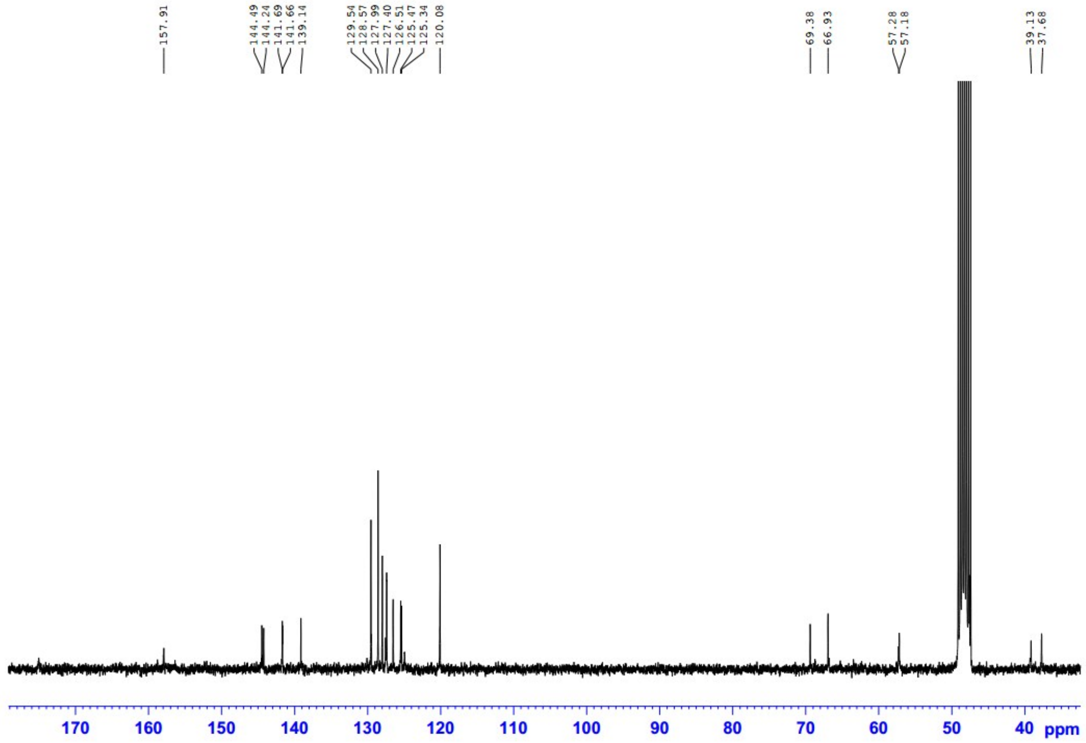
ESI MS of compound **21**



^{1}H-NMR of compound **3** (CD_3OD)



^{13}C-NMR of compound **3** (CD_3OD)



ESI MS of compound **3**