Supplementary Material for:

The paths of syntheses, chemical characteristics and stability tests for selected synthetic cannabinoids: 5F-PB-22, NM-2201, UR-144, and AB-CHMINACA

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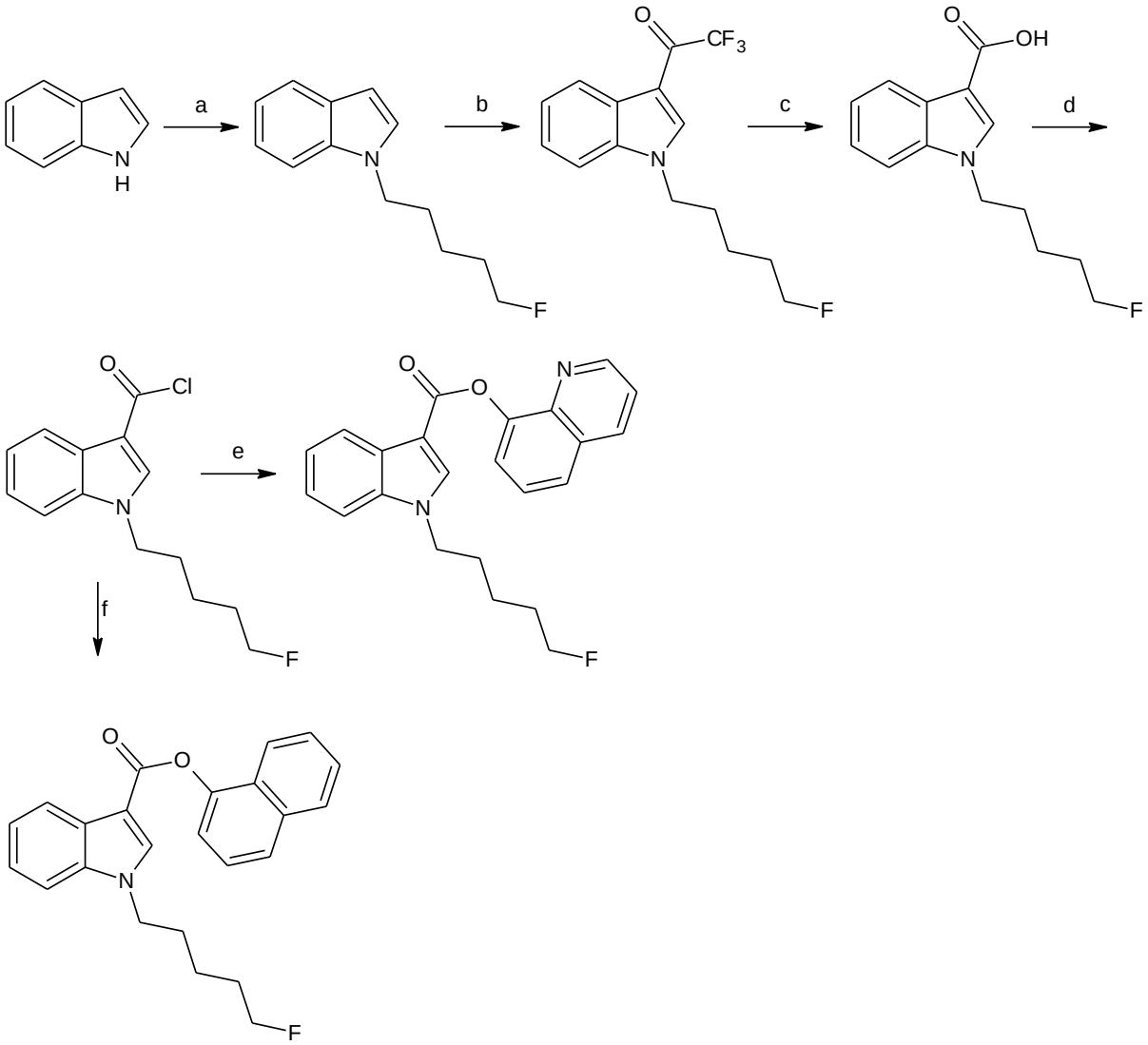
Synthesis

Synthesis of 5F-PB-22 and NM-2201



a) 1-bromo-5-fluoropentane, 30% potassium hydride, dimethylformamide; b) trifluoroacetic anhydride, dimethylformamide; c) potassium hydroxide, methanol, toluene; d) oxalyl chloride, dimethylformamide, methylene chloride; e) 8-hydroxyquinoline, triethylamine, methylene chloride; f) 1-naphthol, triethylamine, methylene chloride

1-(5-fluoropentyl)-1*H*-indole

To a cooled (0°C) solution of 1*H*-indole (25 mM, 2.93 g) in dimethylformamide (65 mL) potassium hydride (30% dispersion in mineral oil, 100 mM, 4.01 g) was slowly added. The mixture was stirred for 30 min at room temperature and then cooled to 0°C. Then 1-bromo-5-fluoropentane (26.2 mM, 3.26 mL) was added. After the addition was complete, the ice bath was removed and the mixture was stirred for 2 h at room temperature. The crude product was used for the next step without isolation. R_f [ethyl acetate:hexane 1:9] = 0.5

1-(5-fluoropentyl)-1*H*-3-trifluoroacetylindole

To a cooled (0°C) solution of 1-(5-fluoropentyl)-1*H*-indole in dimethylformamide, trifluoroacetic anhydride (62.5 mM, 8.69 mL) was added dropwise. The mixture was stirred for 1 h at room temperature. The turbidity was removed by filtration, and the filtrate was poured into water and the obtained precipitate was filtered off, washed with hexane and dried to give a 1-(5-fluoropentyl)-1*H*-3-trifluoroacetylindole (19.3 mM, 5.82 g, yield = 77.3%) as a pink solid. R_f [ethyl acetate:hexane 1:9] = 0.15

1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid

To a refluxed solution of potassium hydroxide (63.53 mM, 3.56 g) in methanol (20 mL), a solution of 1-(5-fluoropentyl)-1*H*-3-trifluoroacetylindole (19.25 mM, 5.80 g) in toluene (5 mL) was added dropwise, and the mixture refluxed for 2 h. Then the mixture was cooled and diluted with water. Layers were separated, and the organic layer was washed with 1 N aq. sodium hydroxide. The combined aqueous layers was acidified to pH~1 and extracted with ethyl acetate (three times, 150 mL each). The combined organic phases were dried using anhydrous magnesium sulfate and subsequently concentrated under vacuum. The crude material was recrystallized from ethyl acetate and hexane to give 1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid (16.7 mM, 4.18 g, yield = 87.1%) as a pink solid. R_f [ethyl acetate:hexane 1:1] = 0.43

1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid chloride

To a cooled (0°C) solution of 1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid (16.65 mM, 4.15 g) in methylene chloride (35 mL), oxalyl chloride (33 mM, 2.89 mL) and a one drop of dimethylformamide were added. The mixture was stirred for 1 h at room temperature, concentrated under vacuum and used for the next step without purification. R_f [ethyl acetate:hexane 1:1] = 0.85

5F-PB-22

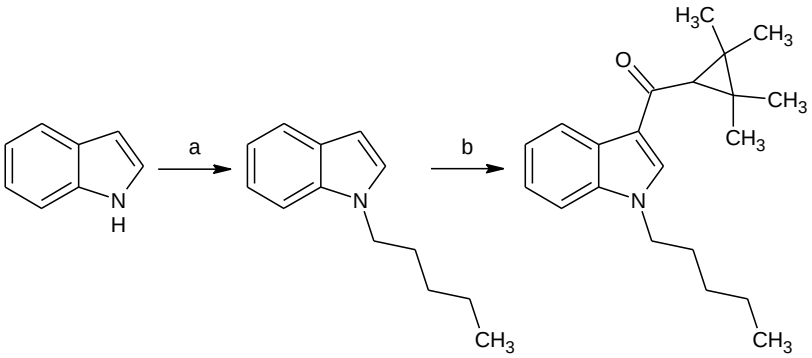
To a cooled (0°C) solution of 1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid chloride (16.65 mM) and triethylamine (58.5 mM, 8.13 mL) in methylene chloride (80 mL), a solution of 8-hydroxyquinoline (20 mM, 2.9 g) in methylene chloride (80 mL) was added dropwise. The mixture was stirred for 20 h at room temperature. The solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (250 mL) and washed with water (three times, 25 mL each). The organic layer was dried with magnesium sulfate and concentrated under vacuum. The crude material was purified using flash chromatography (0-100% ethyl

acetate in hexane, 55 min), and recrystallized from ethyl acetate and hexane to give 5F-PB-22 (13.48 mM, 5.27 g, yield = 84.1%) as a white solid. R_f [chloroform:methanol:acetic acid 95:5:1] = 0.54; m.p. = 117.7°C

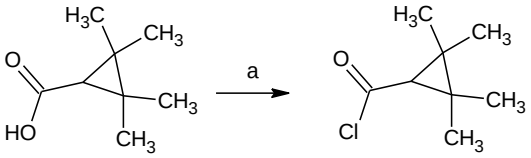
NM-2201

To a cooled (0°C) solution of 1-(5-fluoropentyl)-1*H*-indole-3-carboxylic acid chloride (11.83 mM) and triethylamine (41.4 mM, 5.8 mL) in methylene chloride (65 mL), a 1-naphthol (14.2 mM, 2.05 g) was added. The mixture was stirred for 20 h at room temperature. The solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (250 mL) and washed with 5% aq. sodium hydrogen carbonate (three times, 25 mL each), a brine (25 mL) and water (25 mL). The organic layer was dried using anhydrous magnesium sulfate and subsequently concentrated under vacuum. The crude material was purified using flash chromatography (0-30% ethyl acetate in hexane, 35 min), and recrystallized from ethyl acetate and hexane to give NM-2201 (9.58 mM, 3.58 g, yield = 81%) as a white solid. R_f [ethyl acetate:hexane 1:3] = 0.36; m.p. = 83.8°C

Synthesis of UR-144



a) 1-bromopentane, 30% potassium hydride, dimethylformamide; b) 2,2,3,3-tetramethylcyclopropanecarboxylic acid chloride, 1 N solution of ethylmagnesium bromide in tetrahydrofuran, 1 N solution of zinc chloride in diethyl ether, methylene chloride



a) oxalyl chloride, dimethylformamide, methylene chloride

1-pentyl-1*H*-indole

To a cooled (0°C) solution of 1*H*-indole (54 mM, 6.33 g) in dimethylformamide (100 mL) potassium hydride (30% dispersion in mineral oil, 200 mM, 8.02 g) was slowly added. The mixture was stirred for 1 h at room temperature and then cooled to 0°C. Then 1-bromopentane (65 mM, 8.08 mL) was added. After the addition was complete, the ice bath was removed and the mixture was stirred for 24 h at room temperature. The mixture was transferred to a separating funnel, the oil phase discarded, and the residue evaporated under reduced pressure. Next, the residue was diluted with ethyl acetate (250 mL) and washed with water (three times, 25 mL each). The organic layer was dried with magnesium sulfate and concentrated under vacuum. The crude material was purified using flash chromatography (0-100% ethyl acetate in hexane, 10 min) to give 1-pentyl-1*H*-indole (51.84 mM, 9.76 g, yield = 96%) as an yellow oil. R_f [ethyl acetate:hexane 1:9] = 0.63

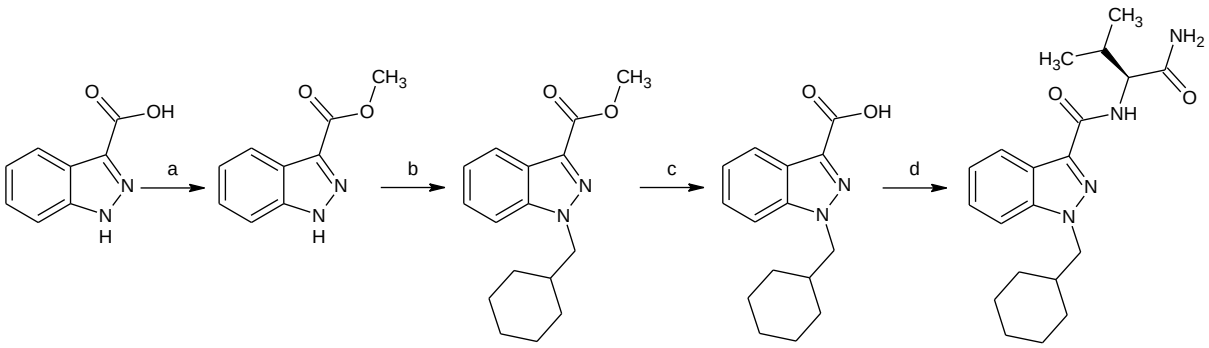
2,2,3,3-tetramethylcyclopropanecarboxylic acid chloride

To a cooled (0°C) solution of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (42 mM, 5.97 g) in methylene chloride (150 mL), oxalyl chloride (84 mM, 7.33 mL) and a one drop of DMF were added. The mixture was stirred for 1 h at room temperature, concentrated under vacuum and used for the next step without purification.

UR-144

To a stirred solution of 1-pentyl-1*H*-indole (75 mM, 14.06 g) in methylene chloride (30 mL), a 1 N solution of ethylmagnesium bromide in tetrahydrofuran (105 mM, 13.85 mL) was added. After 15 min a 1 N solution of zinc chloride in diethyl ether (105 mM, 17.14 mL) was added and the mixture stirred for 30 min. Then 2,2,3,3-tetramethylcyclopropanecarboxylic acid chloride (75 mM) in methylene chloride (30 mL) was added and the mixture stirred for 10 min. Next, a 1 N aq. hydrochloric acid (20 mL) were added, and transferred to a separating funnel. The aqueous phase was extracted with methylene chloride (three times, 15 mL each). The combined organic phases were washed with 1 N aq. sodium hydroxide (three times, 10 mL each), dried with magnesium sulfate and concentrated under vacuum. The crude material was purified using flash chromatography (0-5% ethyl acetate in hexane, 60 min), and recrystallized from pentane to give UR-144 (38.25 mM, 11.82 g, yield = 51%) as a white solid. R_f [ethyl acetate:hexane 1:9] = 0.35; m.p. = 69°C

Synthesis of AB-CHMINACA



a) concentrated sulfuric acid, methanol; b) (bromomethyl)cyclohexane, cesium carbonate, acetonitrile; c) 1 N aq. sodium hydroxide, methanol; d) L-valinamide hydrochloride, HATU, *N*,*N*-diisopropylethylamine, dimethylformamide

Methyl 1*H*-indazole-3-carboxylate

To a stirred solution of 1*H*-indazole-3-carboxylic acid (50 mM, 8.11 g) in methanol (160 mL), concentrated sulfuric acid (4 mL) was slowly added, and the mixture stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (250 mL) and washed with 1 N aq. sodium hydrogen sulfate (three times, 25 mL each), 5% aq. sodium hydrogen carbonate (three times, 25 mL each), brine (25 mL) and water (25 mL). The organic layer was dried using anhydrous magnesium sulfate, concentrated under vacuum to give methyl 1*H*-indazole-3-carboxylate (39 mM, 6.88 g, yield = 78%) as a yellow solid and used for the next step without further purification. R_f [chloroform:methanol 9:1] = 0.6; R_f [ethyl acetate:hexane 1:3] = 0.13

Methyl 1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylate

To a cooled (0 °C) solution of methyl 1*H*-indazole-3-carboxylate (82 mM, 14.45 g) in acetonitrile (150 mL) cesium carbonate (142 mM, 46.5 g) was added, and the mixture stirred at room temperature for 30 min. The cooled (0 °C) mixture was treated with (bromomethyl)cyclohexane (180 mM, 25 mL), and the mixture stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (500 mL) and washed with 1 N aq. sodium hydrogen sulfate (three times, 50 mL each), 5% aq. sodium hydrogen carbonate (three times, 50 mL each), brine (50 mL) and water (50 mL). The organic layer was dried with magnesium sulfate and concentrated under vacuum. The crude material was purified using flash chromatography (0-30% ethyl acetate in hexane, 40 min) to give methyl 1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylate (24.4 mM, 6.66 g, yield = 30%) as an yellow oil and methyl 2-(cyclohexylmethyl)-1*H*-indazole-3-

carboxylate (9.8 mM, 2.83 g, yield = 12%) as an yellow oil. R_f [ethyl acetate:hexane 1:3] = 0.48; R_f [ethyl acetate:hexane 1:3] = 0.65

1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylic acid

To a solution of methyl 1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylate (24.4 mM, 6.66 g) in methanol (100 mL), a solution of 1 N aq. sodium hydroxide (40 mL) was added, and the solution stirred at room temperature for 4 h. The solvent was evaporated under reduced pressure. The residue was washed with diethyl ether (three times, 5 mL each) and the aqueous phase was asjusted to pH~2 using 1 N aq. hydrochloride acid solution, and extracted with ethyl acetate (three times, 50 mL each). The combined organic phases were washed with brine (15 mL) and water (15 mL), dried with magnesium sulfate and concentrated under vacuum to give 1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylic acid (23.2 mM, 6.0 g, yield = 95%) as a yellow solid and used for the next step without further purification. R_f [ethyl acetate:hexane 1:3] = 0.09

AB-CHMINACA

To a stirred solution of 1-(cyclohexylmethyl)-1*H*-indazole-3-carboxylic acid (12.4 mM, 3.18 g) in dimethylformamide (5 mL), HATU (12.4 mM, 4.71 g), and *N*,*N*-diisopropylethylamine (37.2 mM, 6.5 mL) were added. After stirring the mixture for 10 min, L-valinamide hydrochloride (12.4 mM, 1.90 g) was added. Then the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (150 mL) and washed with 1 N aq. sodium hydrogen sulfate (three times, 5 mL each), 5% aq. sodium hydrogen carbonate (three times, 5 mL each), brine (5 mL) and water (5 mL), dried with magnesium sulfate and concentrated under vacuum. The crude material was purified using flash chromatography (0-100% ethyl acetate in hexane, 60 min) to give AB-CHMINACA (12.4 mM, 4.42 g, yield = 100%) as a white solid. R_f [ethyl acetate:hexane 1:1] = 0.40; m.p. 87.2°C

HPLC UV-VIS analysis

Methods development consisted of selecting the appropriate wave lengths and choosing of stationary and mobile phases. The spectrum of standard solutions for each compound was recorded separately on UV spectrophotometer to choose maximum absorbance. An optimization of mobile phase was performed based on asymmetry factor and peak area obtained. The validation was performed to evaluate the method in terms of specificity, linearity, precision, accuracy. Purity of all synthesized compounds were determined using validated HPLC methods.

*System suitability.*The system suitability was performed to determine the accuracy and precision of the system, by making six replicate injection of a standard solutions and analyzing each solute for their peak area, retention time, theoretical plate and peak tailing factor. The system suitability requirements for 10 µg/ml of standards was a %RSD for peak area <1, retention time <0.5, a peak tailing factor <1.5. All validated methods have met these requirements.

A blank sample consists only of the solvent was always the first sample injected in the HPLC instrument to check for potential sources of contamination due to the chromatographic system. Injector carry-over test was performed and no carry-over was observed.

Specificity. The specificity of the method was determined by analysis of blank, standard and sample. The identity of the synthesized compounds was confirmed by comparison of retention times of standard and sample. No interferences were observed in retention time of substances of interest.

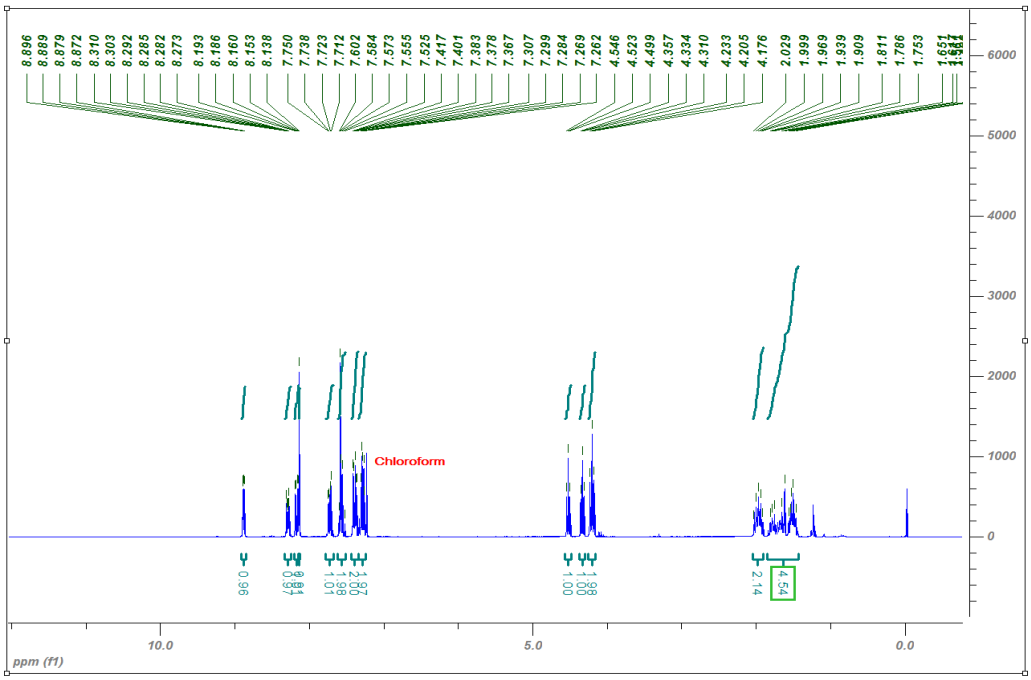
Linearity and Calibration Curve. Working ranges were for 5F-PB-22 (0.1-204 µg/ml), AB-CHMINACA (0.5-212 µg/ml), NM-2201 (0.1-2018 µg/ml), UR-144 (0.1-200 µg/ml). The calibration curves were studied with three concentration points of two injections for each calibration standard. Samples for the calibration curves were injected into liquid chromatographic system, chromatograms were obtained and peak area was determined for each concentrations of standards. In all cases, the correlation coefficients of linear functions were ≥0.999.

Precision. To study the intraday precision, six replicate of each standard solutions were injected. The relative standard deviation (%RSD) was calculated and for each standard the value was within the acceptable criteria of not more than 2.0.

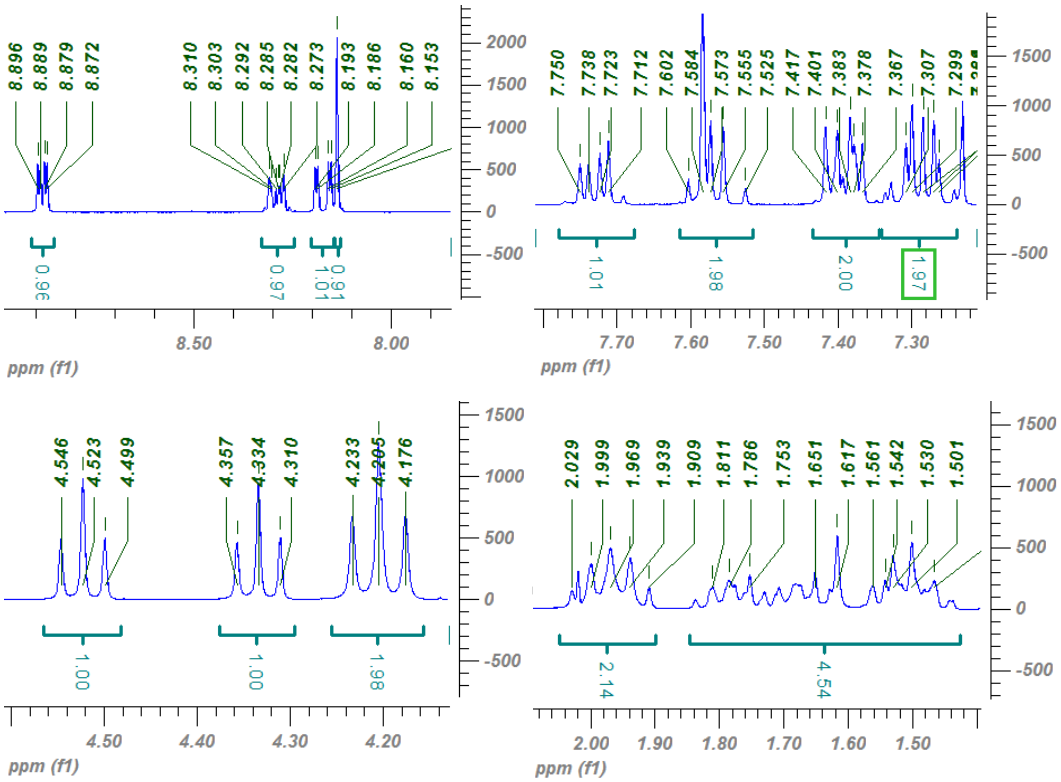
Accuracy. The accuracy of the methods were assessed using nine determinations (three concentration levels including 50%, 100% and 150% in three replicates each) over of covering the specified range. Accuracy was reported as the difference between the mean and the true value. All the measurements met acceptance criteria which was relative error smaller than 2%.

5F-PB-22

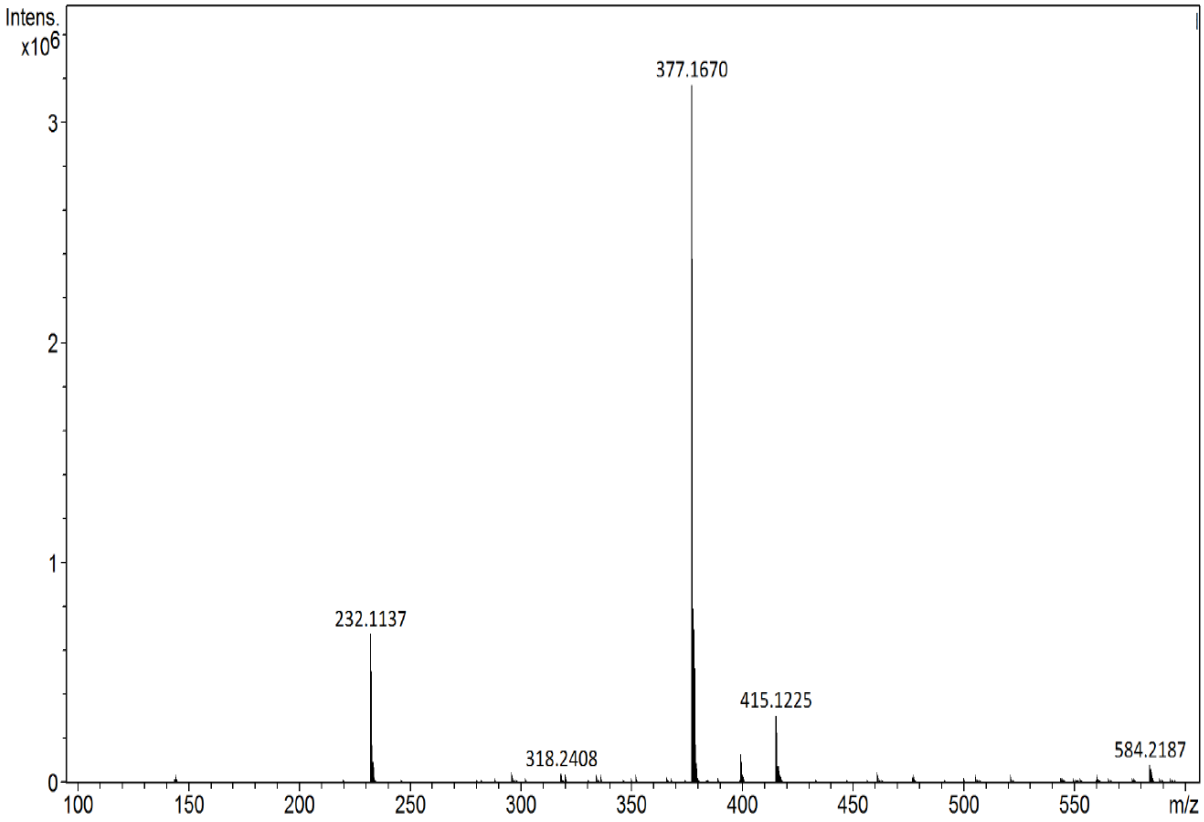
^{1}H NMR Spectrum of 5F-PB-22



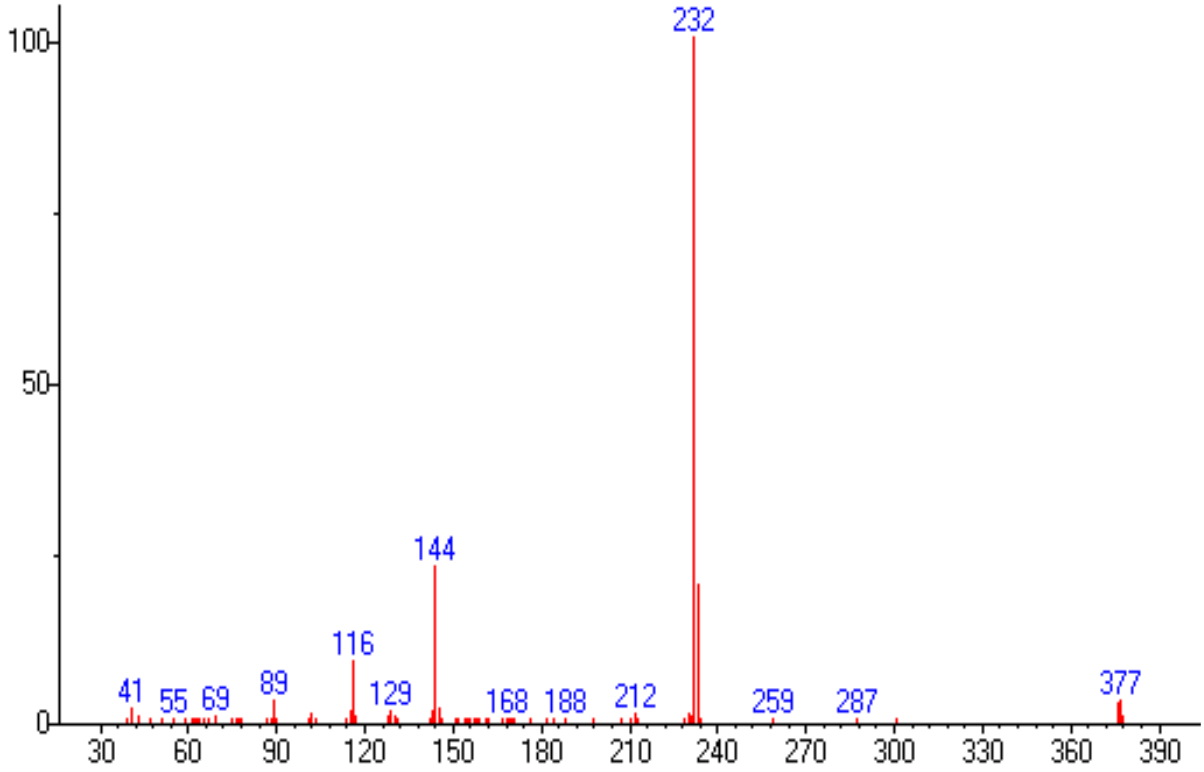
^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) = 1.45-1.59 (2H, CH_2, m); 1.64-1.87 (2H, CH_2, m); 1.99 (2H, CH_2, qui, J = 7.5 Hz); 4.23 (2H, CH_2-N, t, J = 7.1 Hz); 4.46 (2H, CH_2-F, dt, J = 47.3 Hz, J = 5.8 Hz); 7.29-7.44 (4H, CH_{arom}, m); 7.59 (1H, CH_{arom}, dd, J = 11.9 Hz, J = 7.4 Hz); 7.61 (1H, CH_{arom}, s); 7.76 (1H, CH_{arom}, dd, J = 8.0 Hz, J = 2.9 Hz); 8.16 (1H, CH_{arom}, s); 8.20 (1H, CH_{arom}, dd, J = 8.4 Hz, J = 1.6 Hz); 8.28-8.35 (1H, CH_{arom}, m); 8.91 (1H, CH_{arom}, dd, J = 4.2 Hz, J = 1.7 Hz)



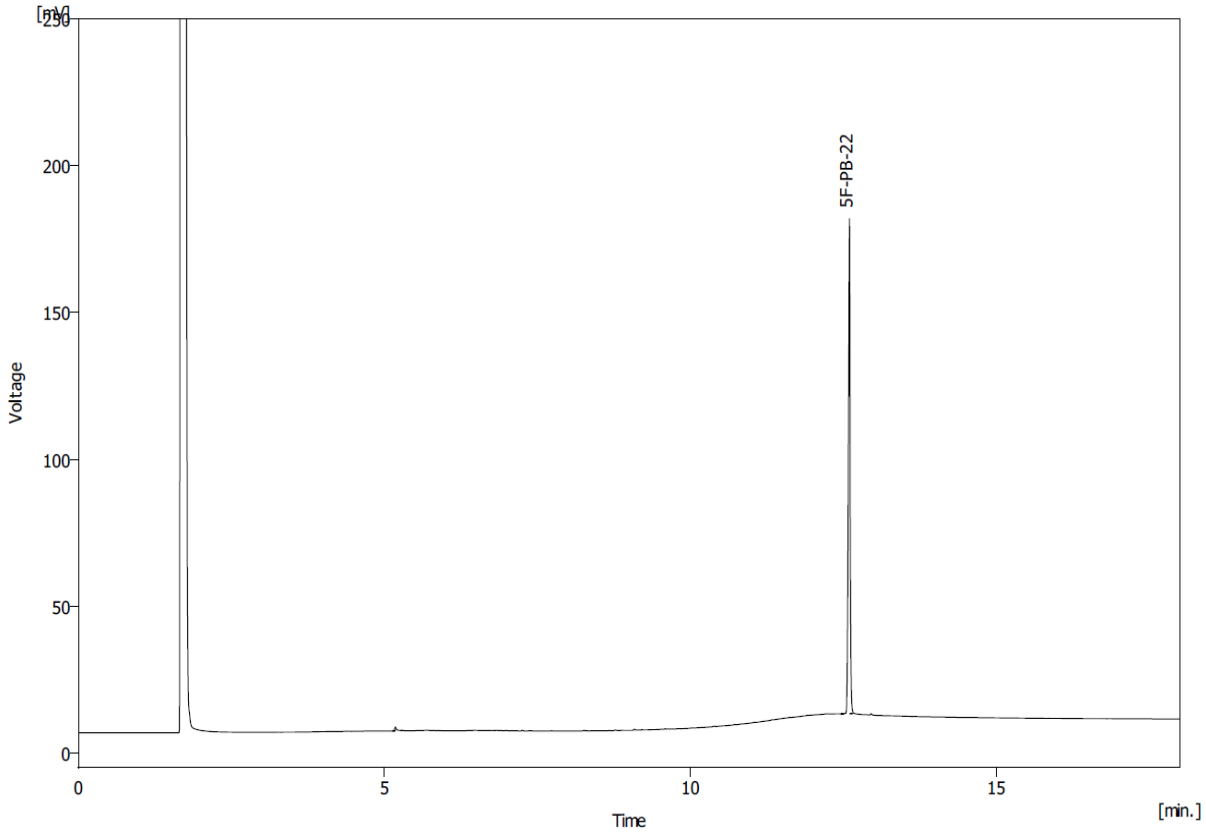
ESI-QTOF-MS spectrum of 5F-PB-22



EI spectrum (GC–MS) of 5F-PB-22



GC-FID chromatogram of 5F-PB-22, t = 0

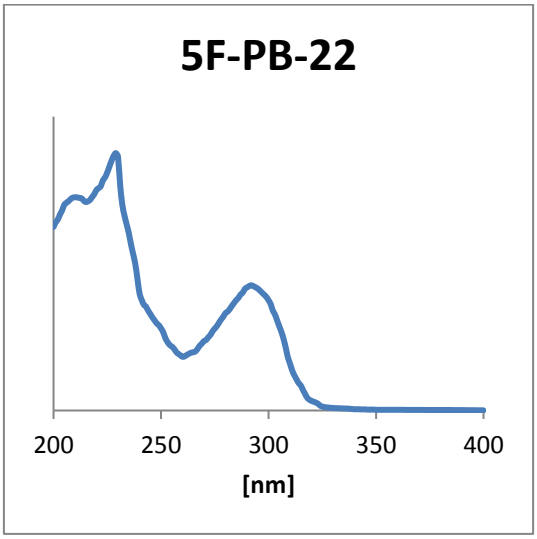


Relative content of 5F-PB-22 by GC-FID method

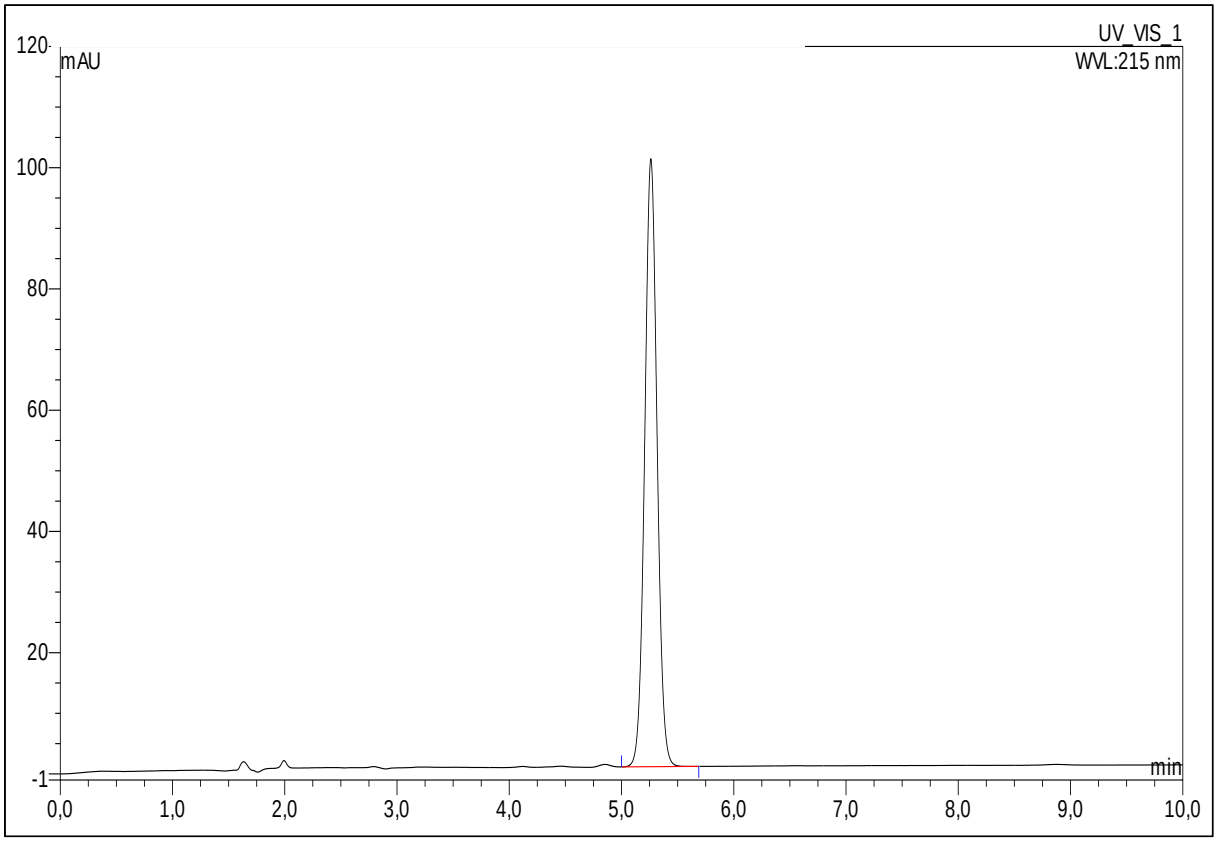
		Conditions			
		-20°C	4°C	RT	40°C
Time [month]	0	99.2%	-	-	-
	1	99.2%	99.5%	99.3%	99.3%
	2	98.1%	98.7%	98.3%	98.3%
	3*	99.4%	99.6%	99.4%	99.4%

*compound in lower concentration than in 2nd month

Spectrum UV of 5F-PB-22

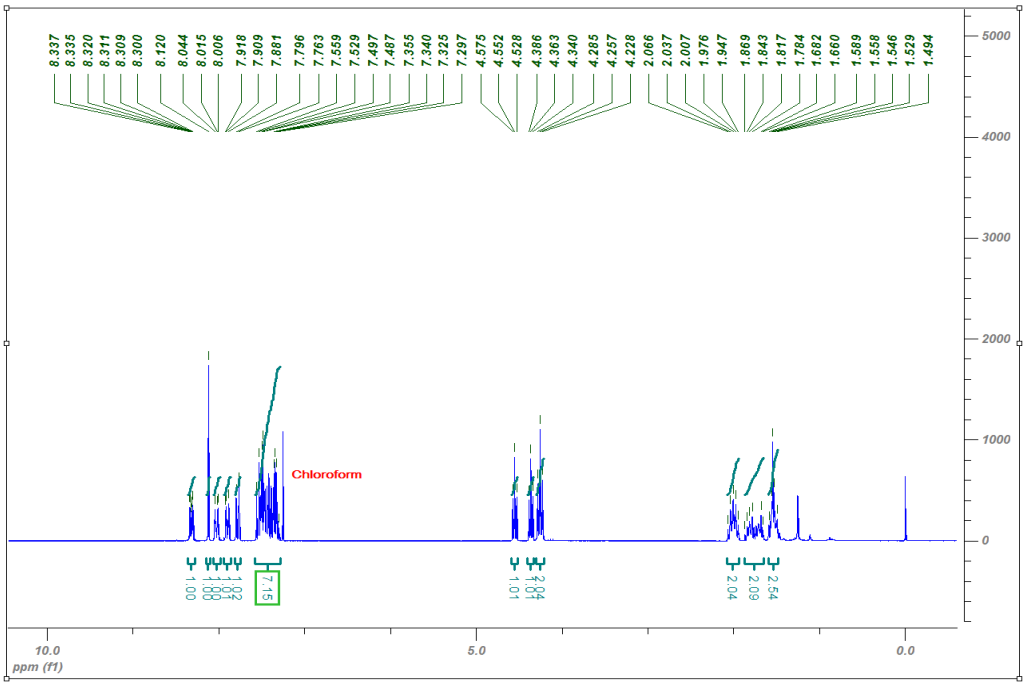


HPLC chromatogram of 5F-PB-22, c = 10 µg/mL

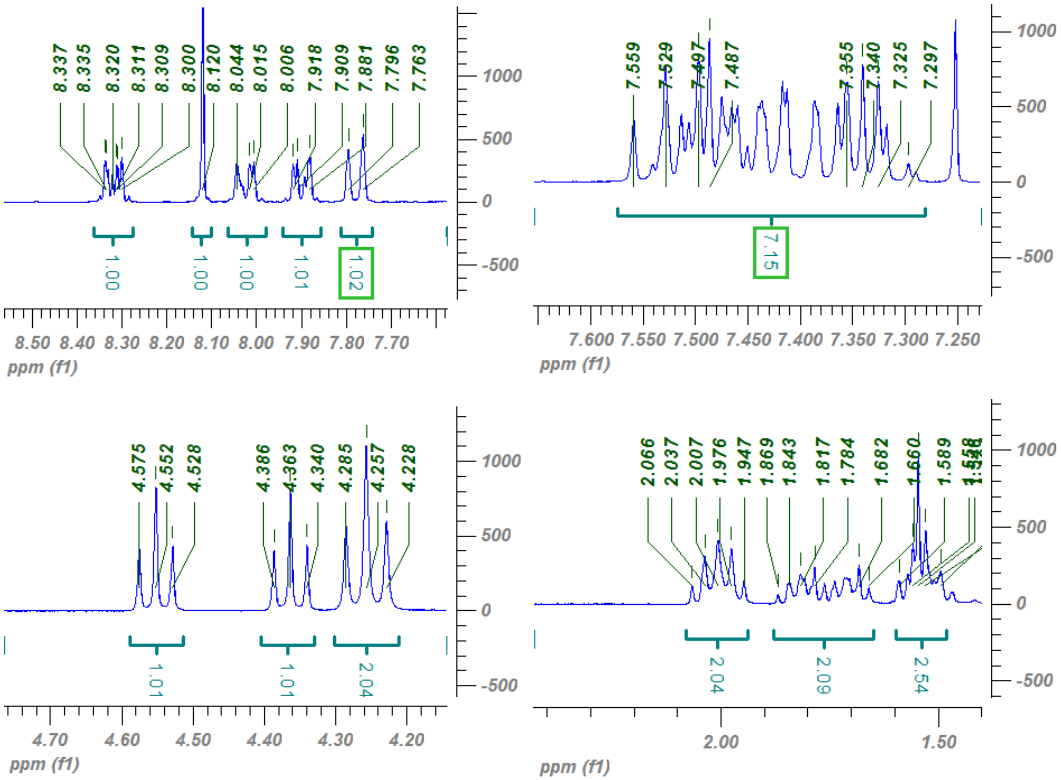


NM-2201

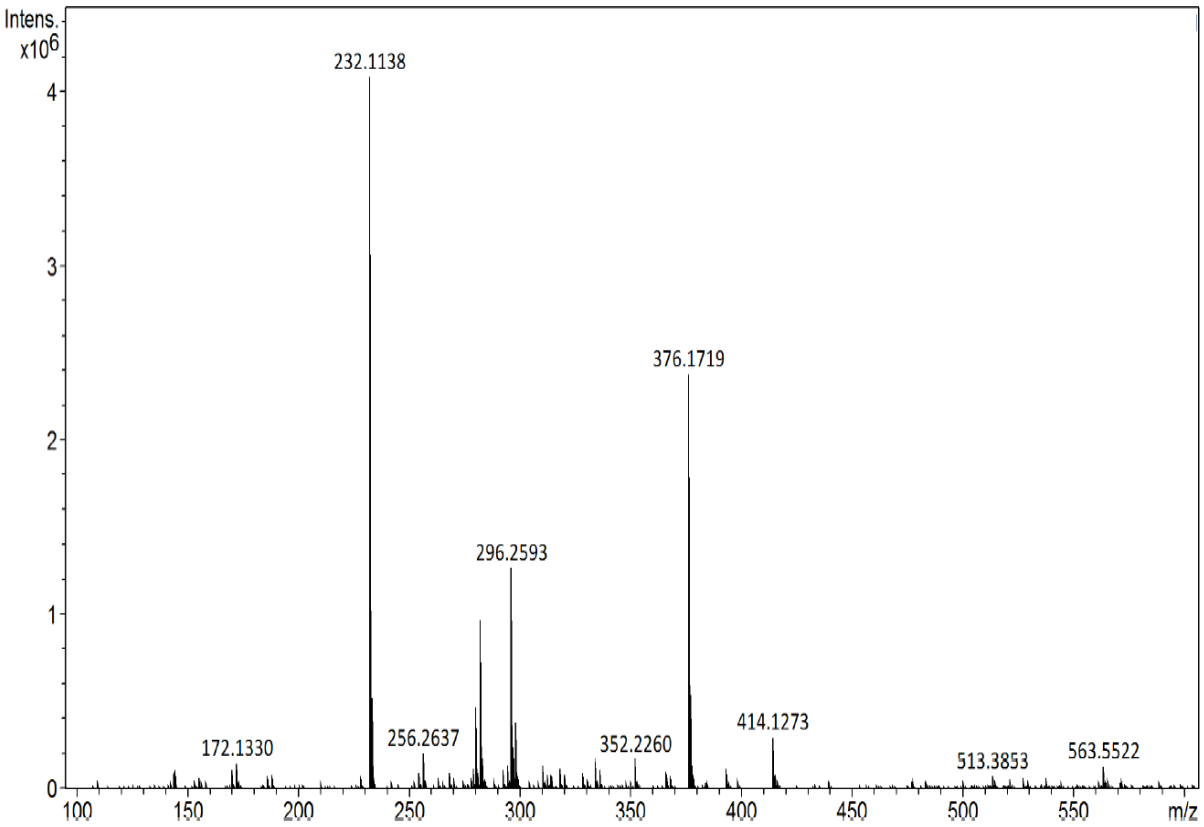
^{1}H NMR Spectrum of NM-2201



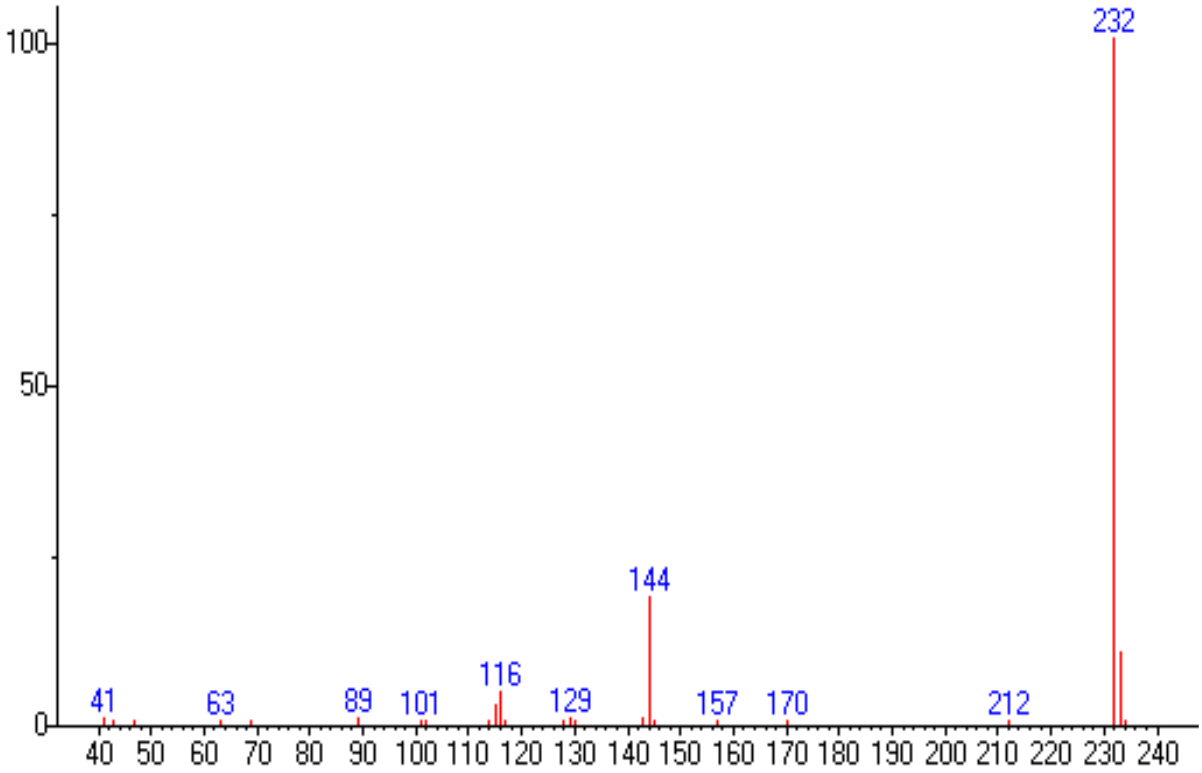
^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) = 1.49-1.60 (2H, CH_2, m); 1.66-1.86 (2H, CH_2, m); 2.01 (2H, CH_2, qui, J = 7.5 Hz); 4.26 (2H, CH_2-N, t, J = 7.1 Hz); 4.46 (2H, CH_2-F, dt, J = 47.1 Hz, J = 5.8 Hz); 7.30-7.56 (7H, 7 x CH_{arom}, m); 7.78 (1H, CH_{arom}, d, J = 8.2 Hz); 7.86-7.93 (1H, CH_{arom}, m); 7.88-8.05 (1H, CH_{arom}, m); 8.12 (1H, CH_{arom}, s); 8.28-8.35 (1H, CH_{arom}, m)



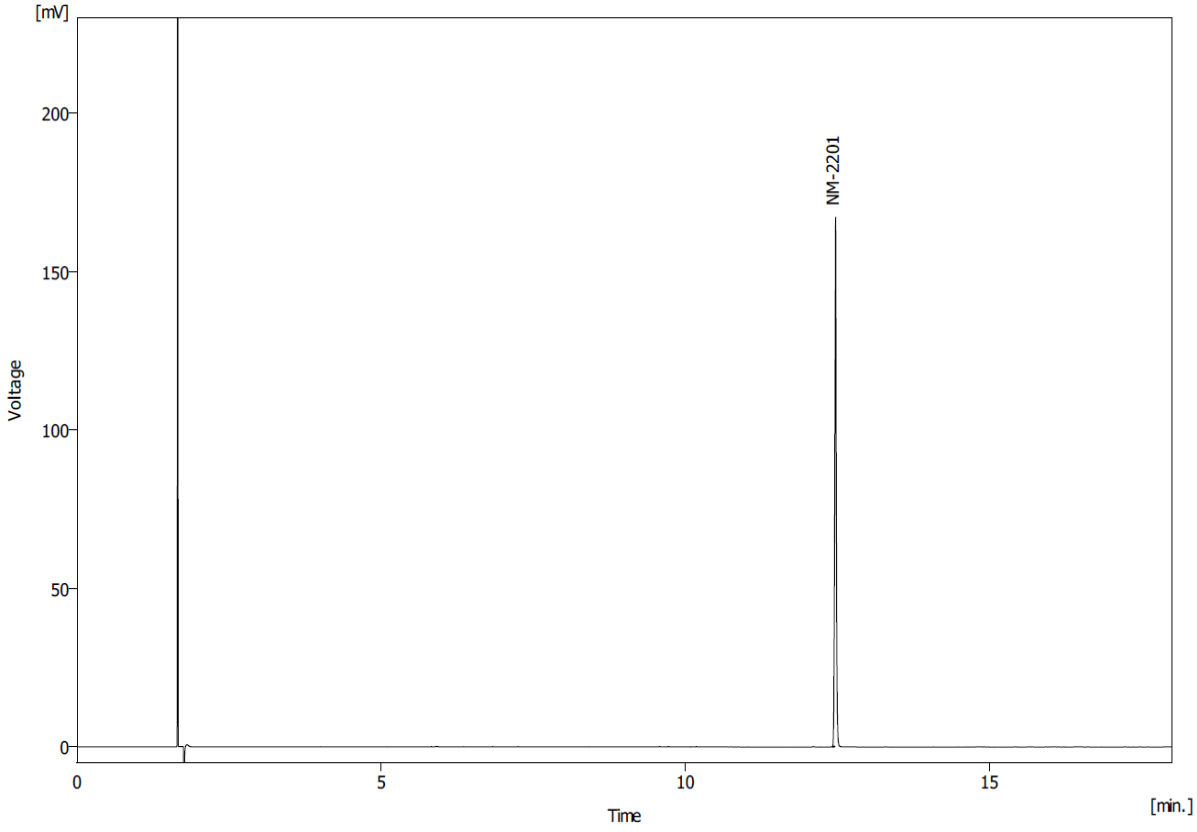
ESI-QTOF-MS spectrum of NM-2201



EI spectrum (GC–MS) of NM-2201



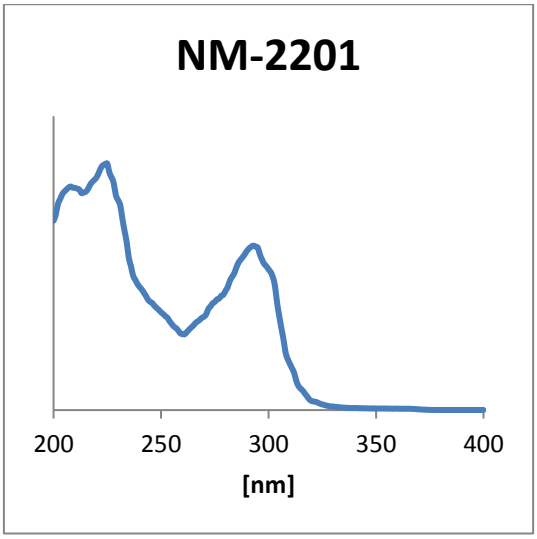
GC-FID chromatogram of NM-2201, t = 0



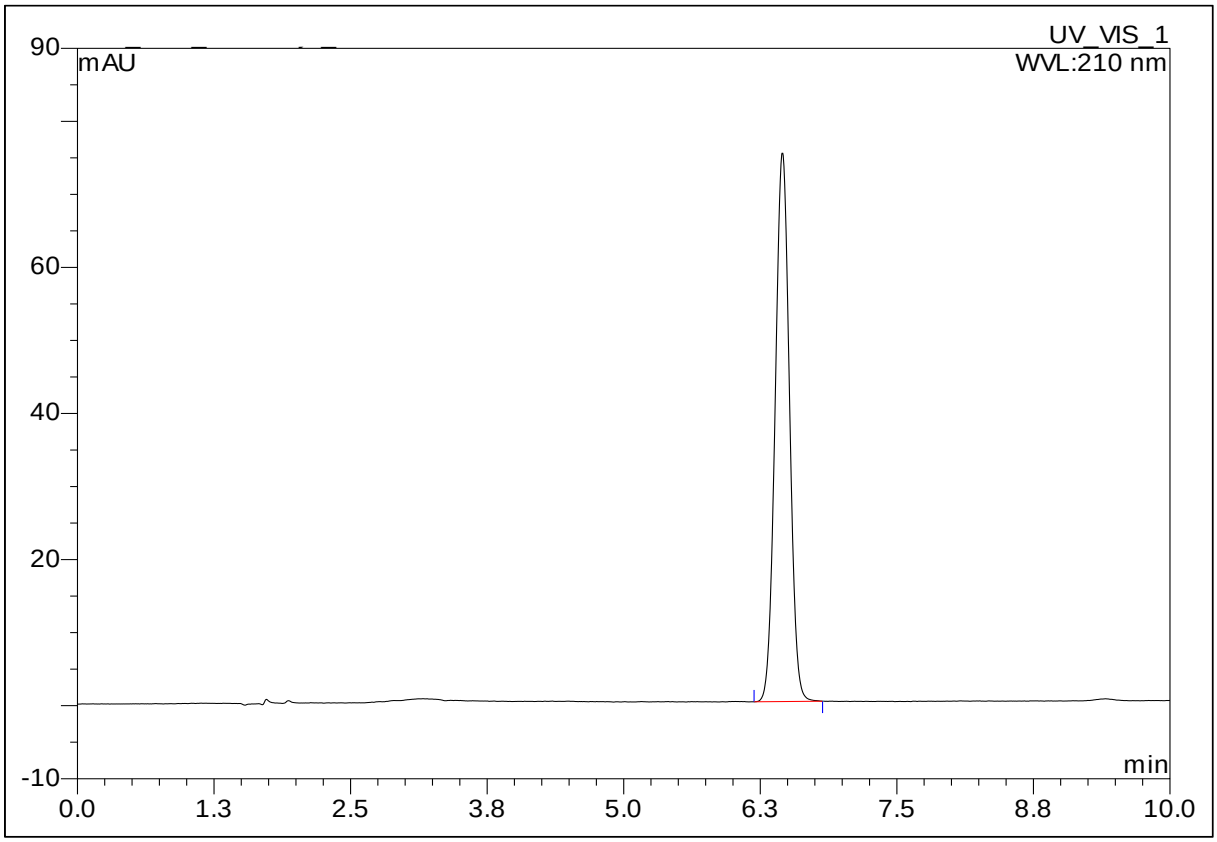
Relative content of NM-2201 by GC-FID method

		Conditions			
		-20°C	4°C	RT	40°C
Time [month]	0	100%	-	-	-
	1	100%	100%	100%	100%
	2	100%	100%	99.9%	99.8%
	3	98.7%	98.5%	98.6%	98.9%

Spectrum UV of NM-2201

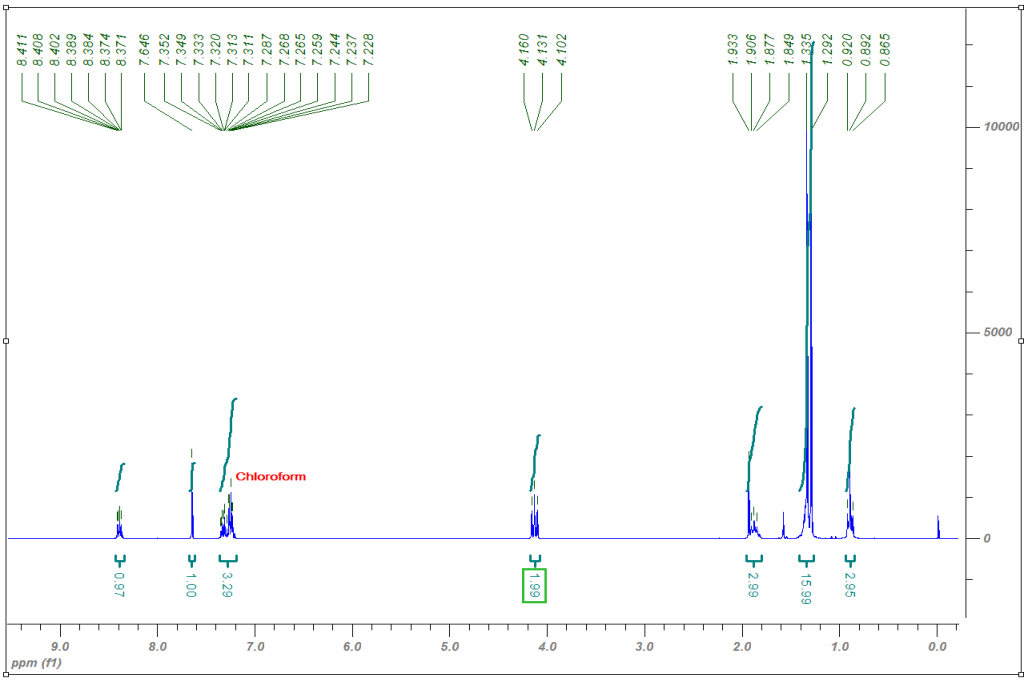


HPLC chromatogram of NM-2201, c = 10 µg/mL

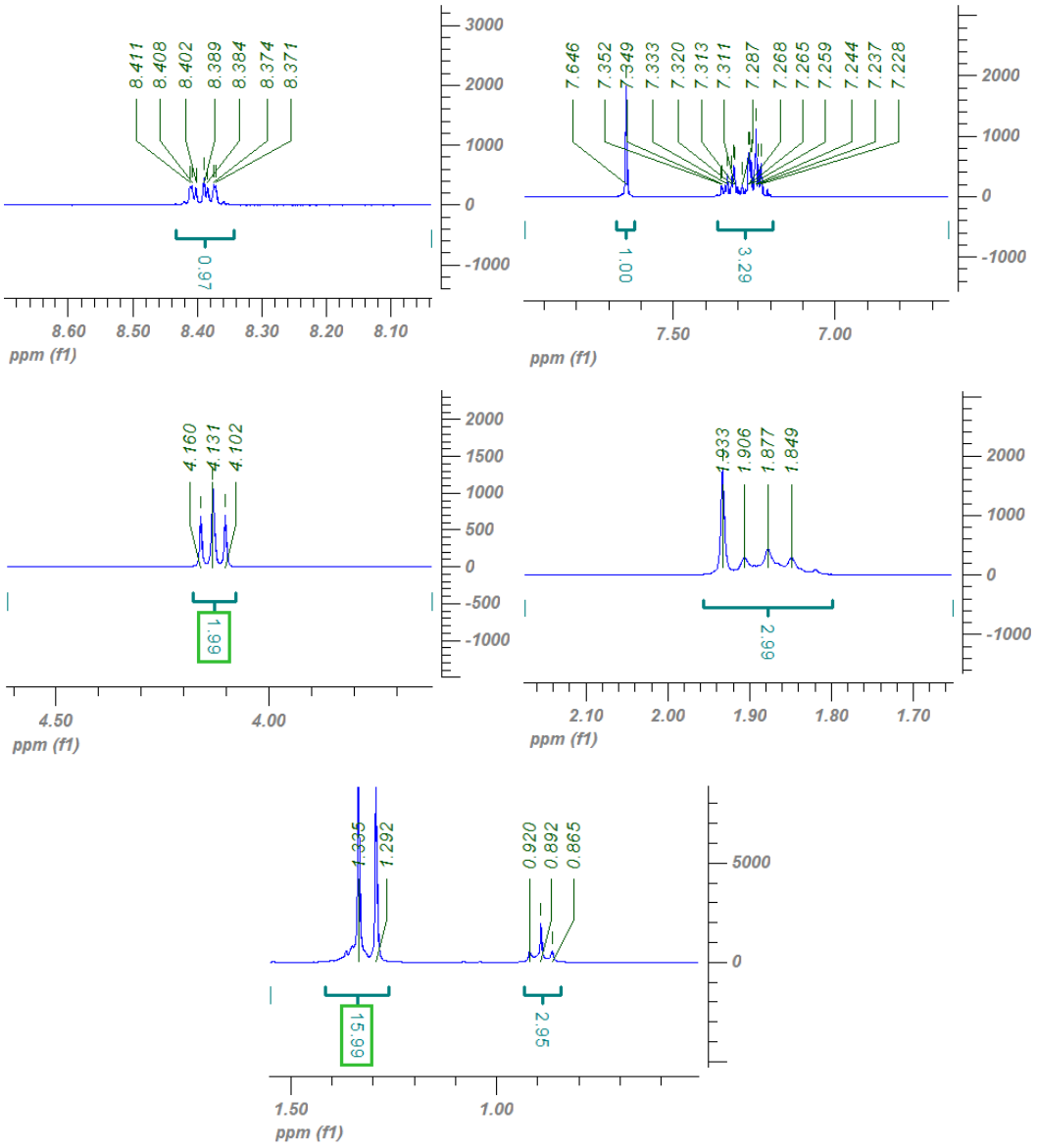


UR-144

^{1}H NMR Spectrum of UR-144



^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) = 0.90 (3H, CH_3 (pentyl), t, J = 6.25 Hz); 1.25-1.43 (4H, 2 x CH_2 (N-$(CH_2)_2$-$(CH_2)_2$-CH_3), m); 1.30 (6H, 2 x CH_3 (tetramethylcyclopropyl), s); 1.35 (6H, 2 x CH_3 (tetramethylcyclopropyl), s); 1.89 (2H, CH_2 (N-CH_2-CH_2), qui, J = 7.5 Hz); 1.95 (1H, CH (tetramethylcyclopropyl), s); 4.14 (2H, CH_2 (N-CH_2), t, J = 7.5 Hz); 7.21-7.38 (3H, 3 x CH_{arom}, m); 7.66 (1H, CH_{arom}, s); 8.37-8.45 (1H, CH_{arom}, m)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8.374
8.371
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8.50
8.40
8.30
8.20
8.10
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7.349
7.333
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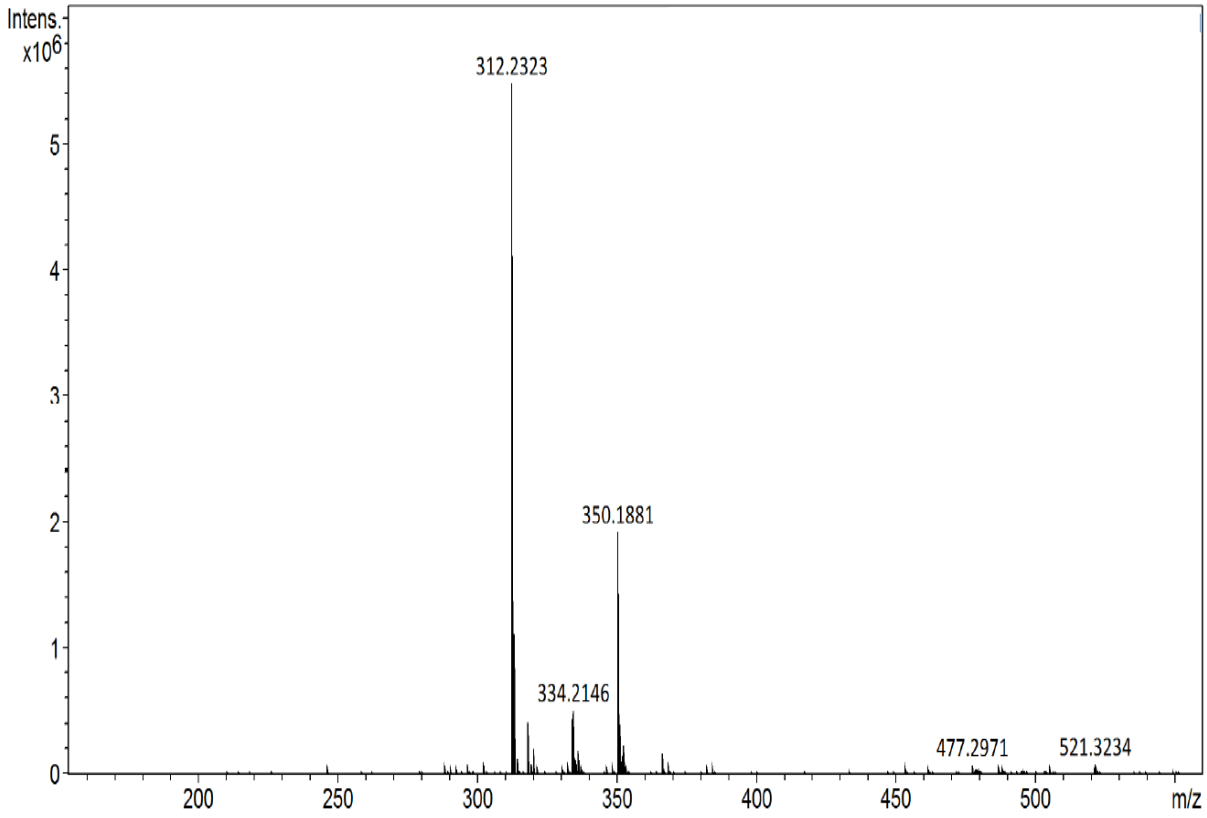
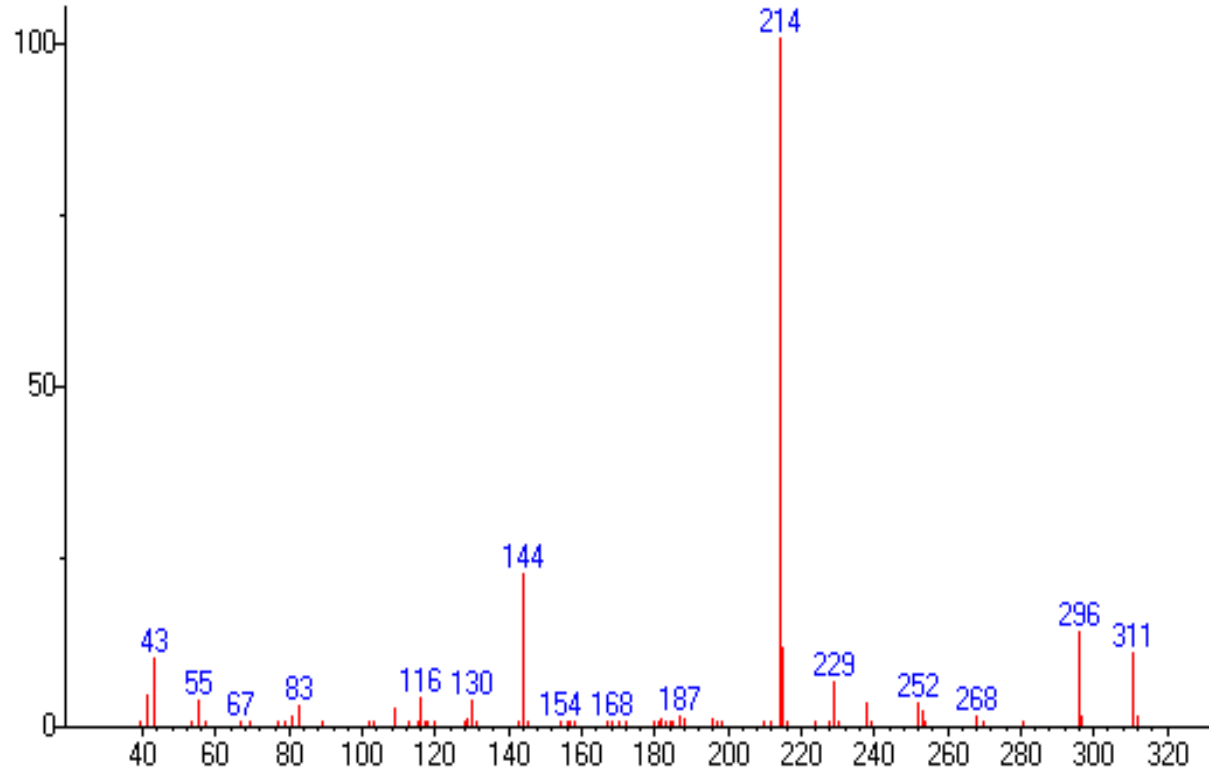
1.877
1.849
2.99
2.10
2.00
1.90
1.80
1.70
ppm (f1)
1.292
0.920
0.892
0.865
15.99
2.95
1.50
1.00
ppm (f1)

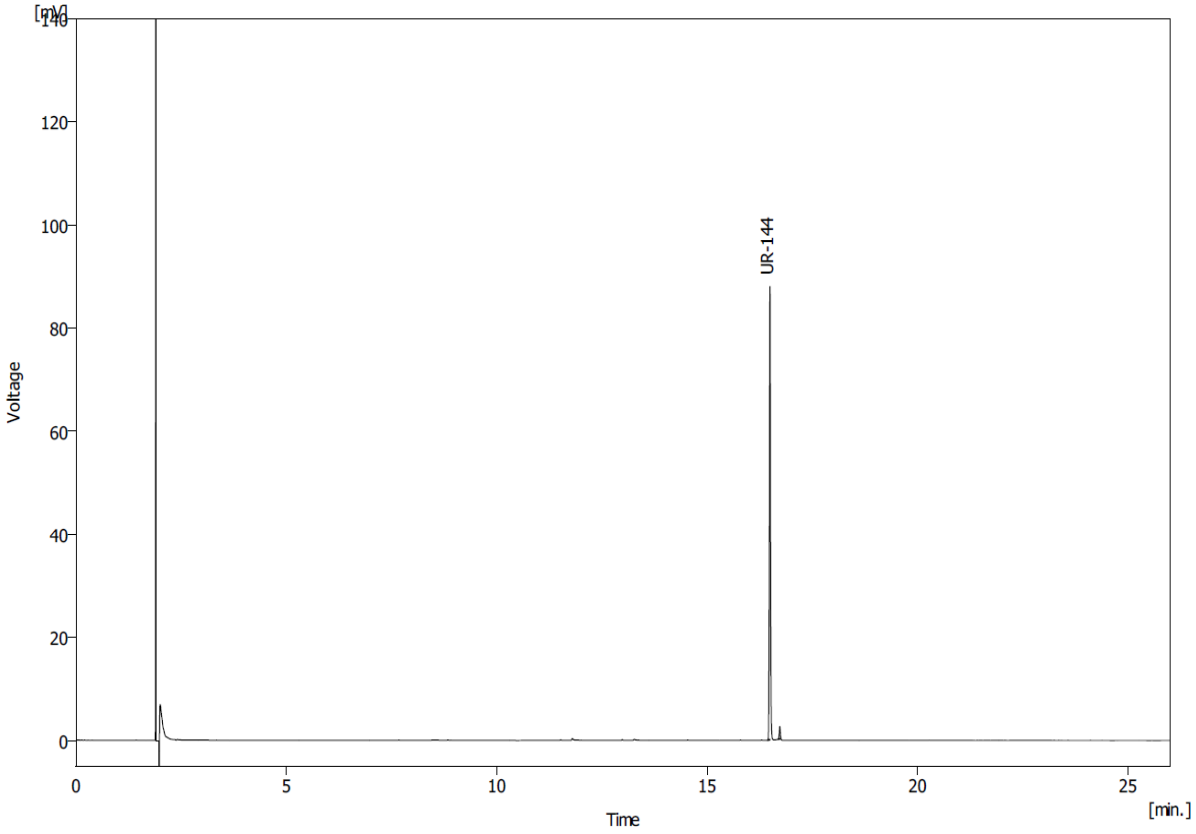
ESI-QTOF-MS spectrum of UR-144



EI spectrum (GC–MS) of UR-144



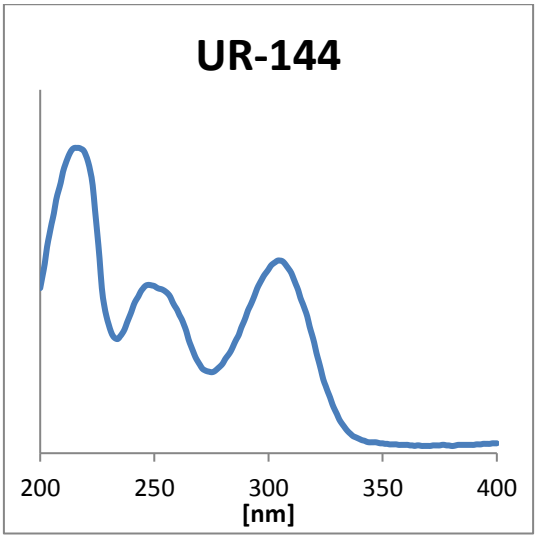
GC-FID chromatogram of UR-144, t = 0



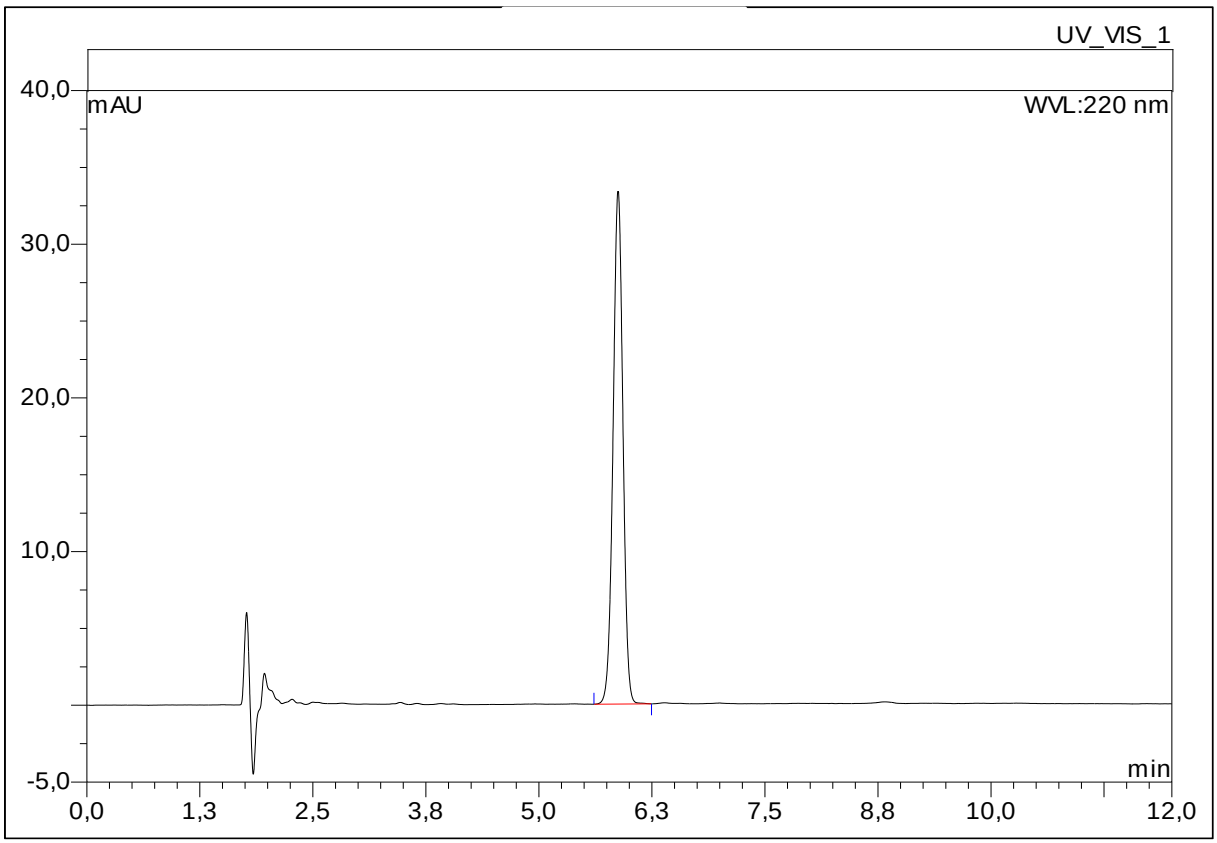
Relative content of UR-144 by GC-FID method

		Conditions			
		-20°C	4°C	RT	40°C
Time [month]	0	97.5%	-	-	-
	1	96.9%	96.8%	96.8%	96.8%
	2	96.6%	96.7%	96.6%	96.7%
	3	96.4%	96.3%	96.2%	96.3%

Spectrum UV of UR-144

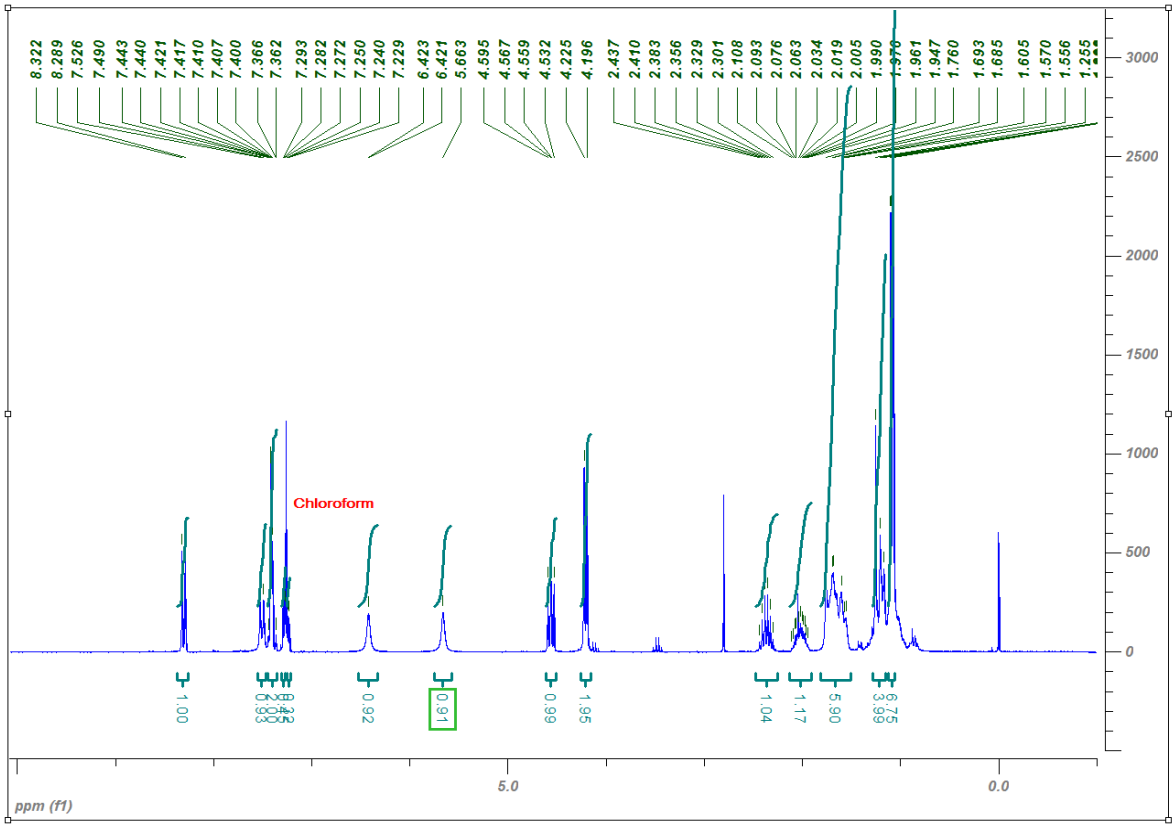


HPLC chromatogram of UR-144, c = 10 µg/mL

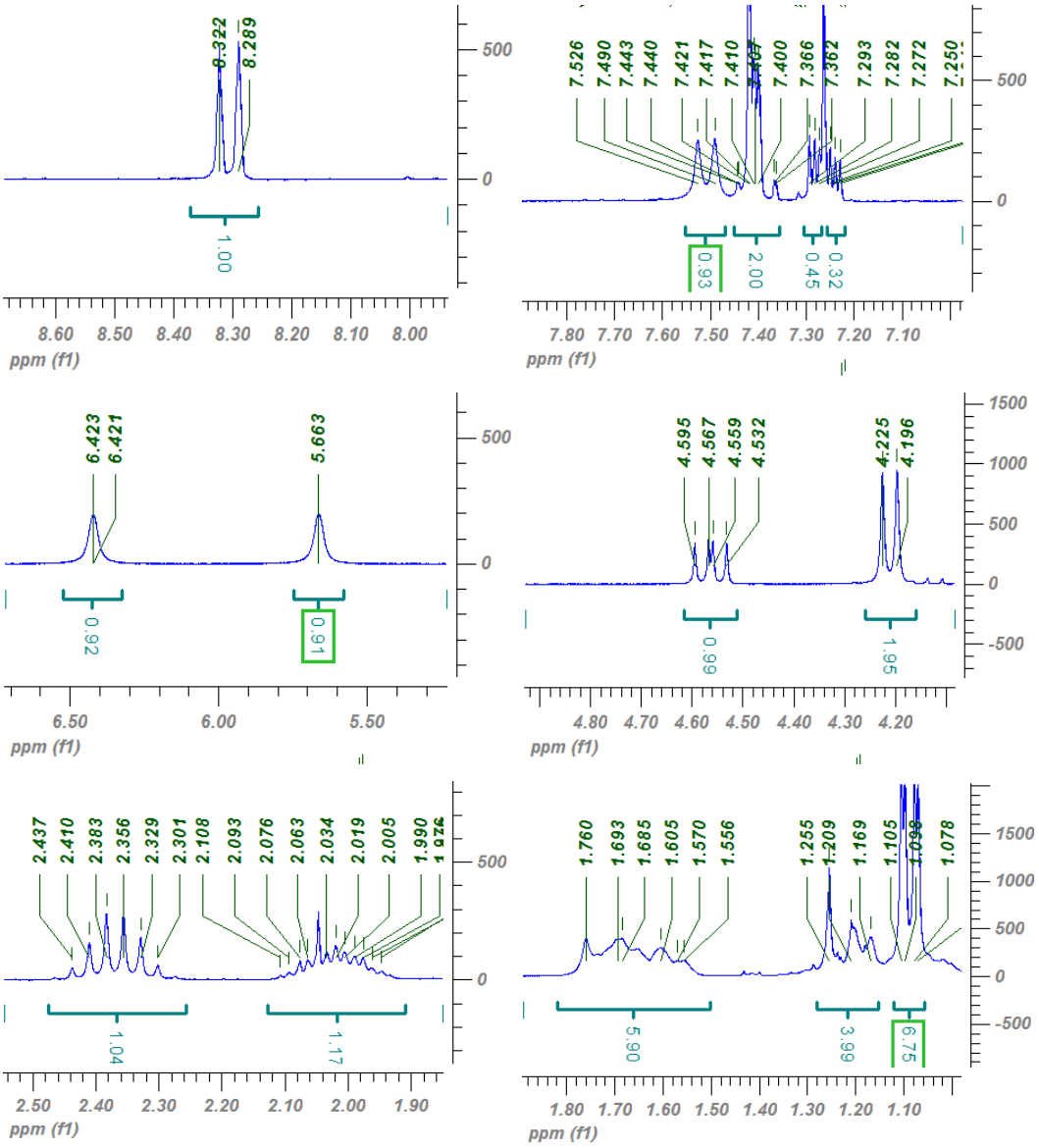


AB-CHMINACA

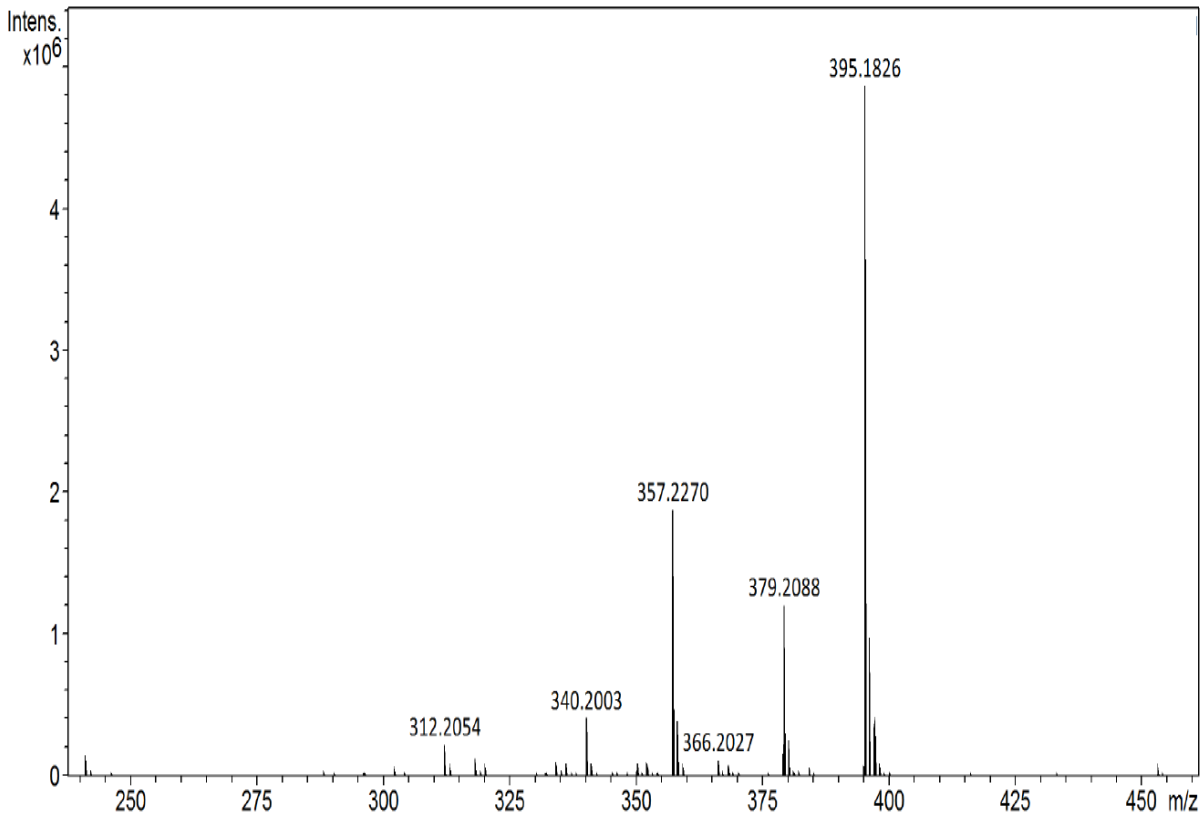
^{1}H NMR Spectrum of AB-CHMINACA



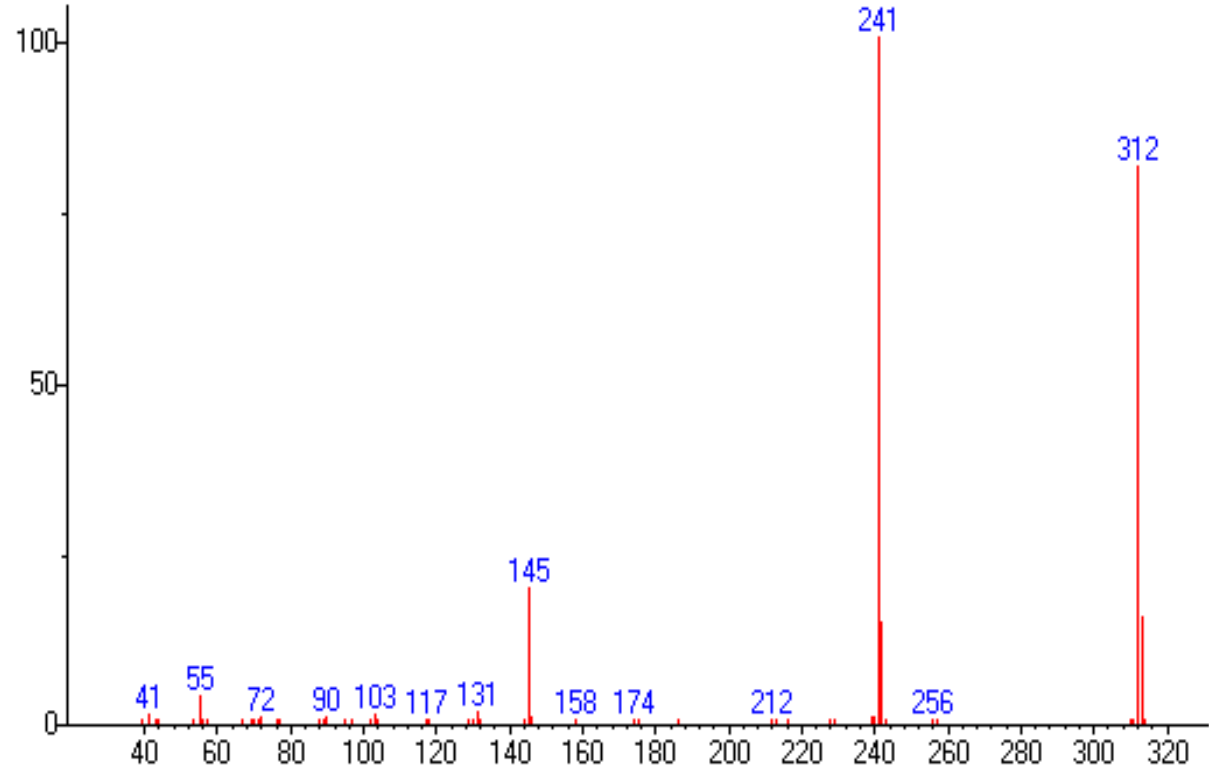
^{1}H NMR (250 MHz, $CDCl_3$), δ (ppm) = 1.07 (3H, CH_3, d, J = 1.80 Hz); 1.10 (3H, CH_3, d, J = 1.83 Hz); 1.13 - 1.28 (4H, CH_2, m); 1.56 - 1.76 (6H, CH_2, m); 2.02 (1H, CH (cyclo), m,); 2.37 (1H, CH (Val), sxt, J = 6.76 Hz); 4.21 (2H, CH_2, d, J = 7.1 Hz); 4.56 (1H, CH, dd, J = 8.90 Hz, J = 6.85 Hz); 5.66 (1H, NH_2, bs) 6.42 (1H, NH_2, bs); 7.22-7.30 (1H, CH_{arom}, m); 7.36-7.45 (2H, CH_{arom}, m); 7.51 (1H, NH, d, J = 8.75 Hz); 8.31 (1H, CH_{arom}, d, J = 8.00 Hz)


8.322
8.289
1.00
8.60 8.50 8.40 8.30 8.20 8.10 8.00
ppm (f1)
7.526
7.490
7.443
7.440
7.421
7.417
7.410
7.400
7.366
7.362
7.293
7.282
7.272
7.250
0.93
2.00
0.45
0.32
7.80 7.70 7.60 7.50 7.40 7.30 7.20 7.10
ppm (f1)
6.423
6.421
5.663
0.92
0.91
6.50 6.00 5.50
ppm (f1)
4.595
4.567
4.559
4.532
4.225
4.196
0.99
1.95
4.80 4.70 4.60 4.50 4.40 4.30 4.20
ppm (f1)
2.437
2.410
2.383
2.356
2.329
2.301
2.108
2.093
2.076
2.063
2.034
2.019
2.005
1.990
1.04
1.17
2.50 2.40 2.30 2.20 2.10 2.00 1.90
ppm (f1)
1.760
1.693
1.685
1.605
1.570
1.556
1.255
1.209
1.169
1.105
1.078
5.90
3.99
6.75
1.80 1.70 1.60 1.50 1.40 1.30 1.20 1.10
ppm (f1)

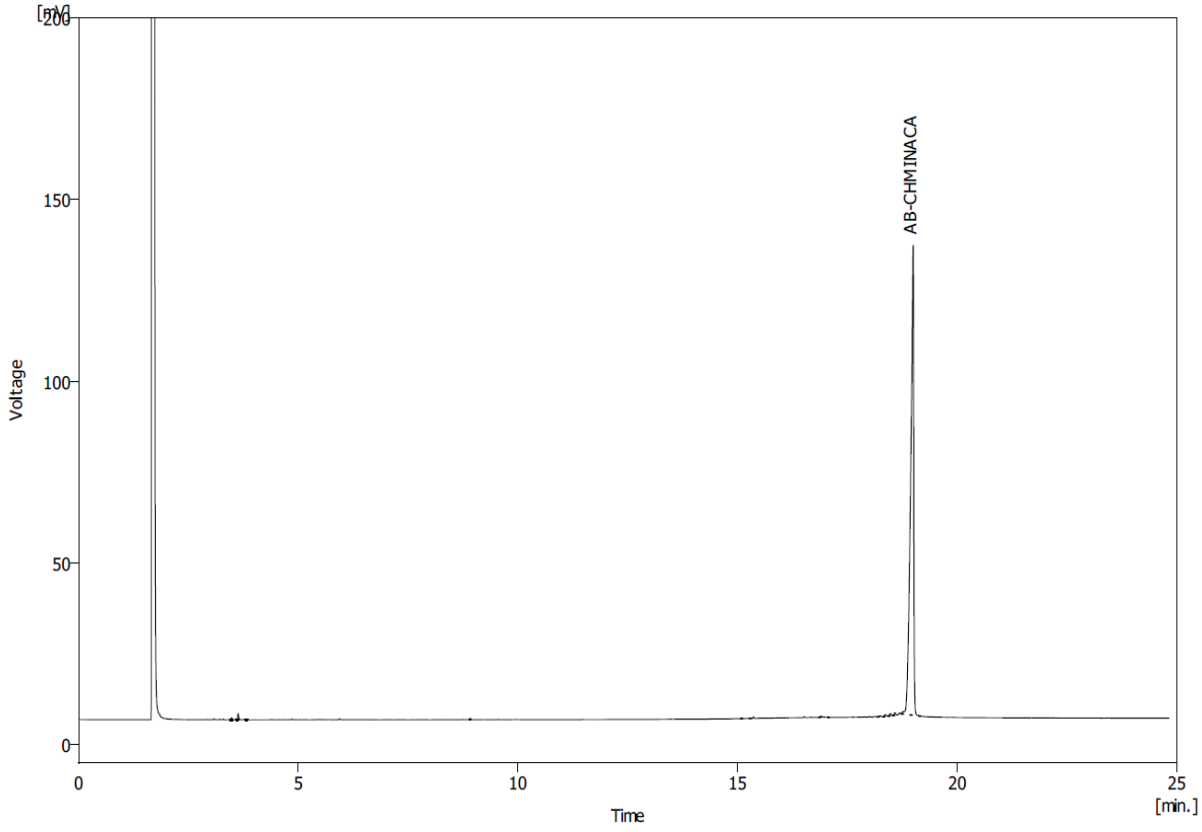
ESI-QTOF-MS spectrum of AB-CHMINACA



EI spectrum (GC–MS) of AB-CHMINACA



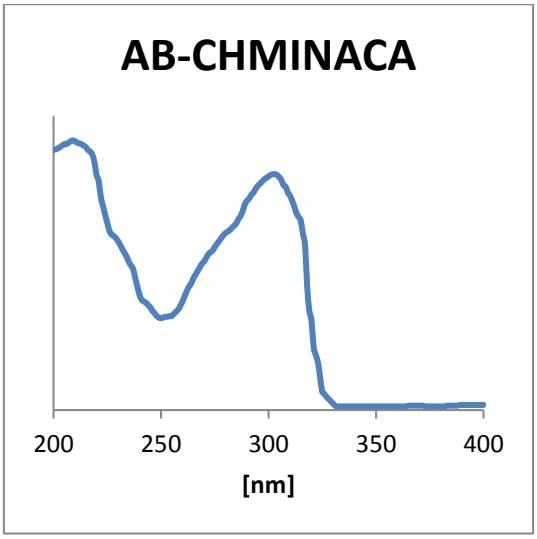
GC-FID chromatogram of AB-CHMINACA



Relative content of AB-CHMINACA by GC-FID method

		Conditions			
		-20°C	4°C	RT	40°C
Time [month]	0	98.2%	-	-	-
	1	97.9%	97.2%	97.3%	97.5%
	2	95.6%	95.6%	96.2%	95.2%
	3	93.5%	93.1%	93.3%	91.6%

Spectrum UV of AB-CHMINACA



HPLC chromatogram of AB-CHMINACA, c = 10.1 µg/mL