Supplementary table 1. Fatty acid composition of total lipids in the CC1010 cells grown with different concentrations of CO_2. Experiments were performed in duplicate or quadruplicate. Measurements were performed in duplicate in each experiment. Each value is a mean ± standard error of the results obtained from four or eight measurements.

Fatty acid	Aeration					
	MBM			MBM-noN		
	Air	1% CO_2	5% CO_2	Air	1% CO_2	5% CO_2
14:0	0.5±0.1	0.5±0.0	0.5±0.0	0.4±0.0	0.7±0.0	0.5±0.0
16:0	21.0±0.2	22.6±0.4	19.0±0.4	29.0±0.4	35.8±0.4	30.7±0.8
16:1(7) and 16:1(3t)	2.9±0.3	3.4±0.3	5.2±0.3	2.2±0.4	1.1±0.1	2.3±0.3
16:1(9)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
16:1(11)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
16:2(7,10)	3.7±0.6	2.7±0.4	4.1±0.3	3.7±0.1	1.5±0.0	2.1±0.4
16:3(4,7,10)	1.7±0.1	1.9±0.0	2.3±0.0	1.1±0.1	1.0±0.0	1.5±0.1
16:3(7,10,13)	4.6±0.2	3.8±0.1	3.8±0.1	3.8±0.3	2.3±0.0	2.5±0.1
16:4(4,7,10,13)	12.8±0.9	13.6±0.7	12.5±0.4	5.2±0.1	6.8±0.3	8.2±0.5
17:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
18:0	2.7±0.2	2.7±0.1	2.1±0.1	2.8±0.0	3.0±0.1	3.4±0.3
18:1(9)	1.6±0.3	3.6±0.6	7.8±0.4	8.8±1.2	5.8±0.2	3.7±0.5
18:1(11)	3.7±0.3	2.9±0.0	2.7±0.1	5.7±0.1	5.4±0.0	5.1±0.2
18:2(9,12)	12.4±1.3	11.4±0.8	12.7±0.6	15.2±0.5	11.6±0.7	10.1±0.9
18:3(5,9,12)	7.5±0.4	8.1±0.9	6.8±0.2	6.8±0.4	10.0±0.3	12.0±0.6
18:3(9,12,15)	22.2±1.2	20.7±1.1	18.3±0.5	13.8±0.2	13.4±0.2	15.3±0.6
18:4(5,9,12,15) and (6,9,12,15)	2.8±0.1	2.1±0.0	2.1±0.1	1.6±0.0	1.7±0.2	2.4±0.0

Supplementary table 2. Fatty acid composition of TAG in the CC1010 cells grown with different concentrations of CO_2. Experiments were performed in duplicate or quadruplicate. Measurements were performed in duplicate in each experiment. Each value is a mean ± standard error of the results obtained from four or eight measurements.

Fatty acid	Aeration					
	MBM			MBM-noN		
	Air	1% CO_2	5% CO_2	Air	1% CO_2	5% CO_2
14:0	9.7±3.1	11.0±3.0	1.8±0.3	0.5±0.0	0.9±0.1	0.9±0.1
16:0	42.0±2.4	48.7±1.1	12.5±1.9	31.1±1.0	38.8±0.3	35.3±1.8
16:1(7) and 16:1(3t)	2.5±0.9	3.5±0.6	2.6±0.4	2.2±0.6	1.2±0.1	2.6±0.2
16:1(9)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
16:1(11)	0.3±0.3	0.0±0.0	0.1±0.1	0.0±0.0	0.0±0.0	0.0±0.0
16:2(7,10)	1.9±0.8	3.8±0.4	3.7±0.1	3.8±0.1	1.7±0.1	1.9±0.3
16:3(4,7,10)	0.2±0.1	0.2±0.2	1.4±0.1	0.7±0.1	0.4±0.0	0.6±0.1
16:3(7,10,13)	1.1±0.6	0.5±0.3	4.0±0.2	3.4±0.3	2.2±0.1	2.6±0.3
16:4(4,7,10,13)	2.4±0.8	1.7±0.1	16.1±0.9	4.1±0.0	5.2±0.3	7.6±0.4
17:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
18:0	17.3±2.6	9.5±1.4	2.9±0.5	3.1±0.1	2.5±0.1	2.5±0.1
18:1(9)	4.9±0.8	7.0±0.1	6.1±0.1	12.6±1.0	9.3±0.3	5.9±0.7
18:1(11)	2.6±0.8	2.5±0.3	2.0±0.1	6.3±0.1	5.5±0.1	4.9±0.2
18:2(9,12)	6.8±2.4	4.1±1.1	10.8±0.2	14.2±0.2	13.0±0.9	10.7±0.5
18:3(5,9,12)	2.8±1.0	3.7±1.3	12.0±0.3	6.0±0.4	8.6±0.1	10.6±0.6
18:3(9,12,15)	3.7±1.6	2.5±0.7	17.1±0.5	10.5±0.1	9.0±0.1	11.2±0.5
18:4(5,9,12,15) and (6,9,12,15)	1.6±0.5	1.3±0.4	6.9±0.6	1.6±0.1	1.6±0.1	2.9±0.1

Supplementary document

Critical identification of fatty acids in Chlamydomonas by GC-MS

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Contents

Materials and Methods

Algal strain

The green alga *Chlamydomonas reinhardtii* strain CC1010 was obtained from the Chlamydomonas Resource Center (St. Paul, MN).

Growth

The algal cells were grown at 25°C in flat culture flasks (capacity: 500 ml with air space) filled with 500 ml of modified Bristol's medium (MBM: 1) with continuous aeration by 1% CO_2 in air. In some experiments, aeration was provided by either 5% CO_2 in air or ordinary air. Light (45 μmol m^{-2} s^{-1}) was provided by a bank of white fluorescent tubes (model FL20S FR P, Panasonic, Osaka, Japan; color temperature at 6700 K). MBM contained, per liter, 250 mg KNO_3, 7.5 mg $MgSO_4$ $7H_2O$, 25 mg NaCl, 7.5 mg K_2HPO_4, 175 mg KH_2PO_4, 13 mg $Ca(NO_3)$ $4H_2O$, 2 ml Fe stock solution and 1 mL A5 solution (pH was adjusted to 6.5 with 1N NaOH). The Fe stock solution contained 0.5 g L^{-1} $FeSO_4$ $7H_2O$, whereas the A5 solution contained, per liter, 2.8 g H_3BO_3, 2.5 g $MnSO_4$ $7H_2O$, 0.2 g $ZnSO_4$ $7H_2O$, 0.07 g $CuSO_4$ $5H_2O$ and 0.02 g Na_2MoO_4 $2H_2O$. KNO_3 was not added in the nitrogen-deplete medium, which we designated as MBM-noN. It included 10 mg L^{-1} $CaCl_2$ $2H_2O$ instead of $Ca(NO_3)$ $4H_2O$.

Extraction of lipids

The algal cells were harvested by centrifugation (3,000 × *g*, 10 min, at 4°C) at a density of 2×10^6 - 5×10^6 cells ml^{-1} (about 40 ml). Total lipids were extracted by the method of Bligh and Dyer (1959) (2). The chloroform phase was evaporated under vacuum. The lipids were dissolved in 0.4 ml of chloroform-methanol (2:1, v/v), and stored at -20°C until use.

Separation of lipids

Lipid classes were separated by two-dimensional thin-layer chromatography (2D-TLC) according to Sato and Furuya (1983) (3). Lipid solution (180 μl) was spotted on a silica gel plate (20 cm × 20 cm, TLC Silica gel 60 plate, Catalog Number 5721, Merck, Darmstadt,

Germany) at a position 25 mm × 25 mm from one corner. The first dimension was developed with acetone-benzene-methanol-water (8:3:2:1, by volume) to the top of the plate. After drying for 30 min, the second dimension was developed with chloroform-acetone-methanol-acetic acid-water (10:4:2:3:1, by volume) until the front reached the height of 12.5 cm. After drying again, the plate was further developed in the second dimension with *n*-hexane-diethyl ether-acetic acid (80:30:1, by volume) to the top of the plate. After drying, the plate was sprayed with 0.01% primuline in 80% aqueous acetone. Lipid spots were detected under UV light at 365 nm.

Preparation of fatty acid methyl esters

Each lipid class was scraped off the plate with a razor blade, and transferred in a Pyrex glass tube with a screw cap. Then, 2 ml of 2.5% HCl in anhydrous methanol (Kanto Kagaku, Ltd., Tokyo, Japan) was added. The tube was placed in a heating block at 85°C for 2.5 h. After cooling, fatty acid methyl esters (FAME) were extracted four times with 2 ml *n*-hexane. For the final extraction, 1 ml water was added to achieve complete extraction of FAME into the *n*-hexane phase. The solvent was removed under vacuum, and the resultant FAME was dissolved in a small volume of *n*-hexane, and stored at -20°C until analysis. Pentadecanoic acid (15:0) was added before the methanolysis as an internal standard for quantification of fatty acid methyl esters by gas chromatography (GC).

Preparation of fatty acid pyrrolidides

An aliquot of FAME solution was evaporated to dryness, and then dissolved in 20 µl of pyrrolidine. After addition of 2 µl of acetic acid, the mixture was placed in a heating block at 100°C for 30 min. The reaction mixture was directly analyzed by GC (4).

Preparation of trimethylsililated hydroxy derivatives

An aliquot of FAME solution was evaporated to dryness and then dissolved in 500 µl of dioxane-pyridine (8:1, by volume). After adding of 50 µl of 5% osmium tetroxide in dioxane, the mixture was kept at ambient temperature for 30 min with continuous stirring. Then 1.25 ml of methanol and 4.25 ml of 20% aqueous sodium sulfite were added to the mixture. After

standing for 1 h, the mixture was centrifuged at 750 × *g* for 20 min at 15°C. The supernatant was clarified by filtration through a glass-fiber paper (type GF/F, Whatman International Ltd., Maidstone, Kent, UK), and then evaporated under vacuum. The residue (perhydroxylated FAME) was completely dried in a vacuum desiccator for 15 min. The final residue was extracted with diethyl ether and then with methanol. Each of the extracts was dried under vacuum. Trimethylsilylation was performed by adding 20 µl of the reagent TMS-BA (Tokyo Kasei, Ltd., Tokyo, Japan) and the mixture was kept at 80°C for 10 min (5-7).

Gas chromatography

FAME was analyzed by a gas chromatograph (model GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a capillary column (ULBON HR-SS-10, 50 m in length, 0.25 mm in internal diameter, Shinwa Chemical Co., Kyoto, Japan). The temperature of the column was kept at 180°C for 5 min, and then elevated to 230°C at a rate of 3°C min^{-1}. The flow rate of the carrier gas (nitrogen) was 1 ml min^{-1}. Peak areas on the gas chromatogram were used to calculate the relative molar amounts of fatty acids. Absolute amounts were calculated using the internal standard 15:0. The amounts of lipid classes were determined based on the amounts of fatty acids.

Gas chromatography-mass spectrometry (GC-MS)

FAME, fatty acid pyrrolidides, and trimethylated derivatives of hydroxyl fatty acids were analyzed by a gas chromatograph-mass spectrometer (model GCMS-QP2010 Ultra, Shimadzu). High-grade pure helium (He) was used as the carrier gas. The Ionization voltage was 70 eV, and ionization temperature was 200°C. Mass spectra were scanned every 0.2 s.

For the analysis of FAME, a BPX70 column (60 m in length, 0.22 mm in internal diameter, SGE Analytical Science, Victoria, Australia) was used. The column temperature was elevated from 170°C to 250°C at a rate of 3°C min^{-1}, and then kept at 250°C for 5 min. The flow rate of helium carrier gas was 0.85 ml min^{-1}.

Fatty acid pyrrolidides were also analyzed with the BPX70 column. The column temperature was first kept at 200°C for 1 min, elevated to 250°C at a rate of 10°C min^{-1}, kept at 250°C for 25 min, elevated to 260°C at a rate of 2°C min^{-1}, and finally kept at 260°C for 5

min. The flow rate ofHe carrier was 0.84 ml min^{-1}.

Trimethylsilylated derivatives of hydroxylated fatty acids were analyzed with a non-polar column, Rtx-5MS (30 m in length, 0.25 mm in internal diameter, RESTEK, Bellefonte, PA). The column temperature was initially kept at 150°C for 1 min, elevated to 300°C at a rate of 7°C min^{-1}, kept at 300°C for 10 min, elevated to 330°C at a rate of 7°C min^{-1}, and finally kept at 330°C for 5 min. The flow rate of He gas was 1.40 ml min^{-1}.

We present representative spectra. It was sometimes necessary to show spectra obtained with different classes of lipids to show better spectra of overlapping isomers. By combining the three different methods, we were able to identify various different isomers of fatty acids. We also detected various fatty acids with odd chain legths, which have been neglected in the past studies. The information on such atypical fatty acids will shed new light on the mechanism of synthesis of fatty acids in *Chlamydomonas* and other algae. Note that 14:0 and 15:0 are not normally detected in *Chlamydomonas*, but they are useful as internal standards.

References

1. **Watanabe A.** 1960. List of algal strains in collection at the Institute of Applied Microbiology, University of Tokyo. J. Gen. Appl. Microbiol. **6:**283-292.
2. **Bligh EG, Dyer WJ.** 1959. A rapid method of total lipid extraction and purification. Can. J. of Biochem. and Physiol. **37:**911-917.
3. **Sato N, Furuya M.** 1983. Isolation and identification of diacylglyceryl-*O*-4'-(*N,N,N*-trimethyl)-homoserine from the fern Adiantum capillusveneris L. Plant Cell Physiol. **24:**1113-1120.
4. **Andersson B, Holman R.** 1974. Pyrrolidides for mass spectrometric determination of the position of the double bond in monounsaturated fatty acids. Lipids. **9:**185-190.
5. **Capella P, Zorzut C.** 1968. Determination of double bond position in monounsaturated fatty acids esters by mass spectrometry of their trimethylsilyloxy derivatives. Anal.

Chem. **40:**1485-1463.

6. **Janssen G, Parmentier G.** 1978. Determination of double bond positions in fatty acids with conjugated double bonds. Biomed. Mass Spectrom. **5:**439-443.
7. **Janssen G, Parmentier G, Verhulst A, Eyssen H.** 1985. Location of the double bond positions in microbial isomerization and hydrogenation products of α– and γ–linolenic acids. Biomed. Mass Spectrom. **3:**134-138.

Table1. Retention times of fatty acid methylesters, pyrrolidide derivatives, and trimethylsilyl derivatives on gas chromatography.

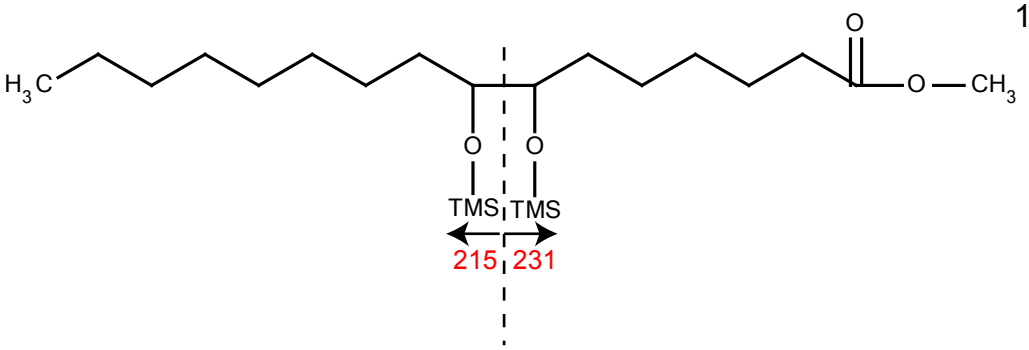
Fatty acid	Retention time (min)			
	Fatty acid methylester		Pyrrolidide derivative	Trimethylsilyl derivative
	SS10	BPX70	BPX70	Rtx5MS
14:0	5.101	7.145	14.425	
15:0	5.683	8.000	16.070	
16:0	6.434	9.085	18.035	
16:1(7)	6.733	9.485	19.035	13.550 13.740
16:1(3t)	6.698	9.480	20.405	14.635
16:1(9)	6.808	9.595	19.535	13.830
16:1(11)	6.949	9.675	19.950	14.145
16:2(7,10)	7.320	10.275	20.935	16.670
16:3(4,7,10)	7.597	10.615	22.460	19.540
16:3(7,10,13)	8.113	11.345	23.660	20.220
16:4(4,7,10,13)	8.407	11.745	24.125	23.420
17:0	7.264	10.210	20.335	
17:1(8) 17:1(9)	7.681	10.800	21.850	14.435 14.865
17:1(10) 17:1(11)	7.681	10.800	21.925	14.545 15.060
18:0	8.273	11.545	23.120	
18:1(9)	8.735	12.145	24.865	15.910
18:1(11) 18:1(13)	8.829 8.976	12.265 12.450	25.380	16.025 16.340
18:2(9,12)	9.553	13.165	28.255	18.605
18:3(5,9,12)	9.956	13.630	28.820	21.250
18:3(9,12,15)	10.575	14.365	32.240	21.835 21.930
18:4(5,9,12,15) 18:4(6,9,12,15)	10.994	14.850	32.255 32.300	24.870 24.815
19:0	9.390	12.985	26.090	
19:1(10) 19:1(13)	9.774	13.690	28.975	16.555 16.480
20:1(11)	11.237	15.210	25.380	17.930
22:1(13)		18.515	40.590	19.865

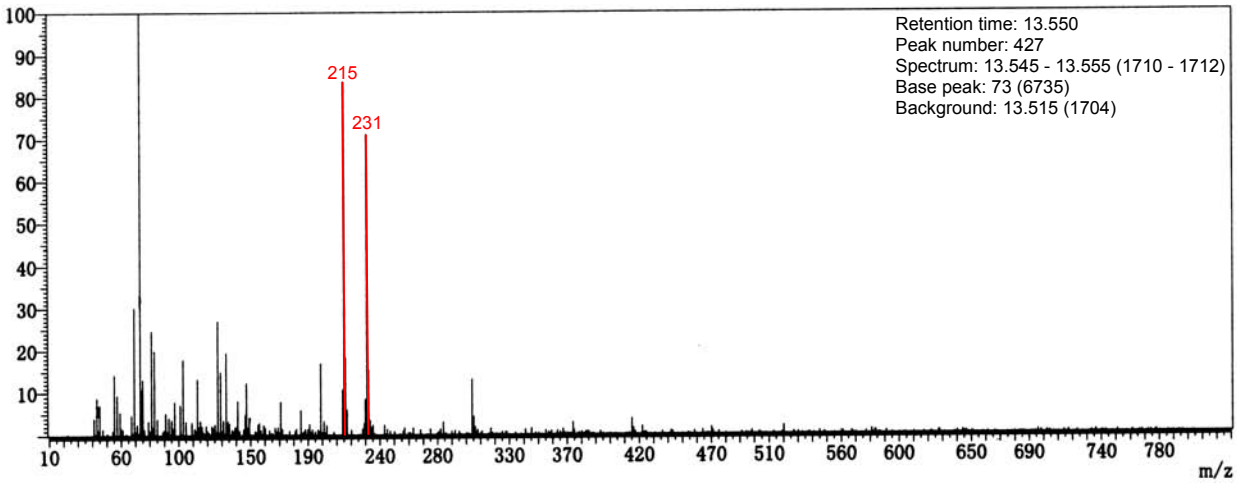
Table2. Molecular weights of fatty acid methylesters, pyrrolidide derivatives, and trimethylsilyl derivatives.

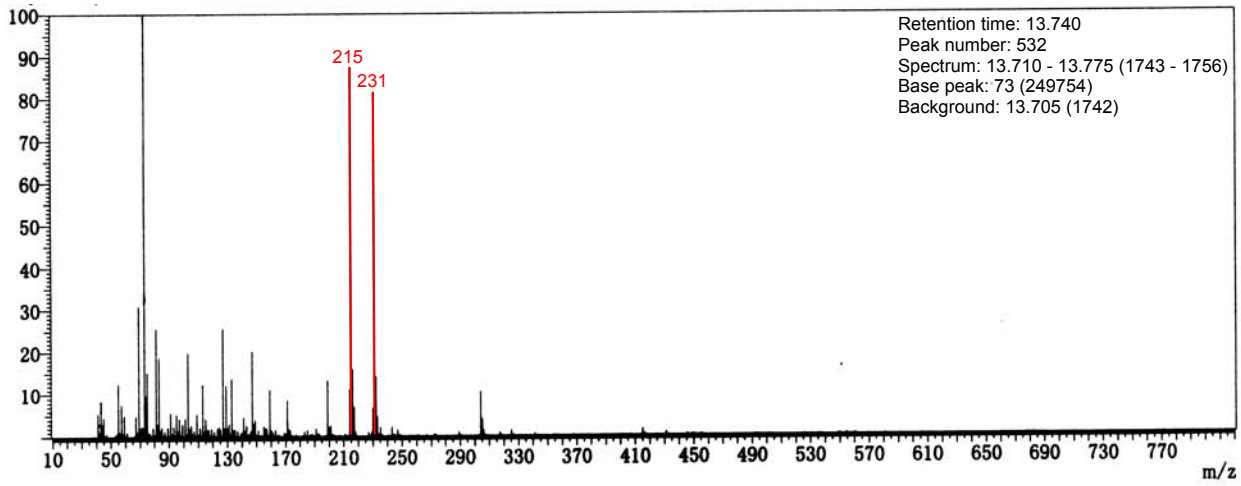
Fatty acid	Molecular weight		
	Fatty acid methylester	Pyrrolidide derivative	Trimethylsilyl derivative
14:0	242	281	242
15:0	256	295	256
16:0	270	309	270
16:1(7)	268	307	446
16:1(3t)	268	307	446
16:1(9)	268	307	446
16:1(11)	268	307	446
16:2(7,10)	266	305	622
16:3(4,7,10)	264	303	798
16:3(7,10,13)	264	303	798
16:4(4,7,10,13)	262	301	974
17:0	284	323	284
17:1(8)	282	321	460
17:1(9)	282	321	460
17:1(10)	282	321	460
17:1(11)	282	321	460
18:0	298	337	298
18:1(9)	296	335	474
18:1(11)	296	335	474
18:1(13)	296	335	474
18:2(9,12)	294	333	650
18:3(5,9,12)	292	331	826
18:3(9,12,15)	292	331	826
18:4(5,9,12,15)	290	329	1002
18:4(6,9,12,15)	290	329	1002
19:0	312	351	312
19:1(10)	310	349	488
19:1(13)	310	349	488
20:1(11)	324	363	502
22:1(13)	352	391	530

Trimethylsilyl derivative

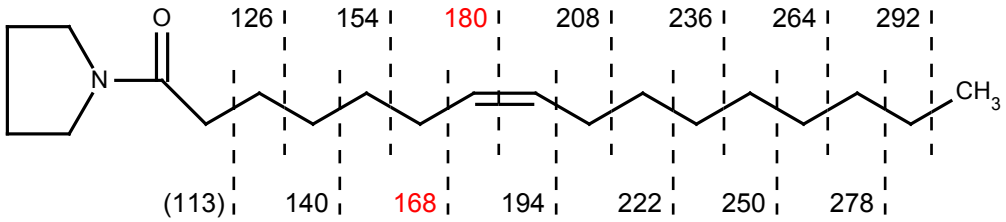
16:1 (7)

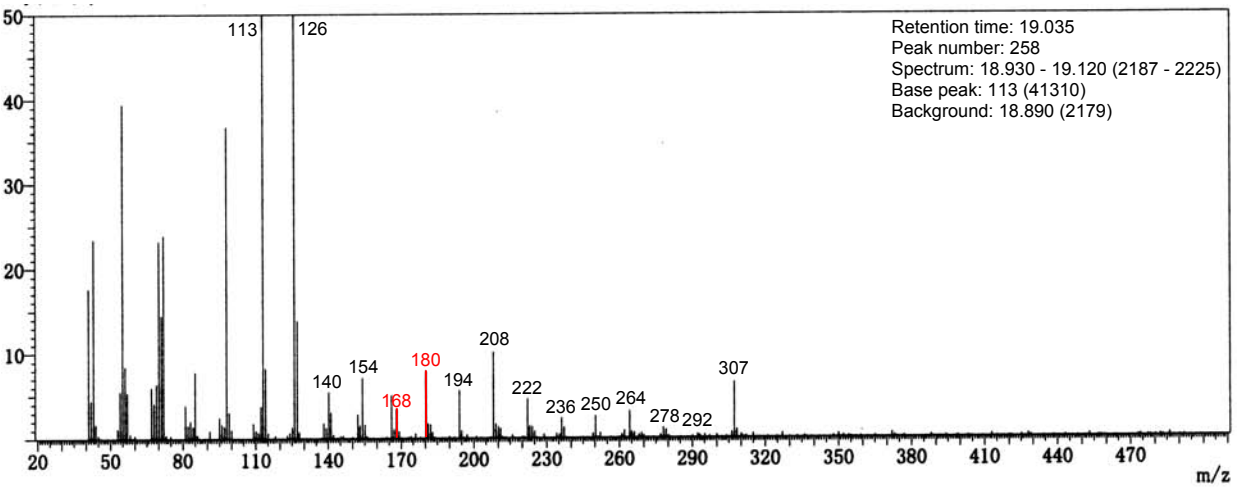






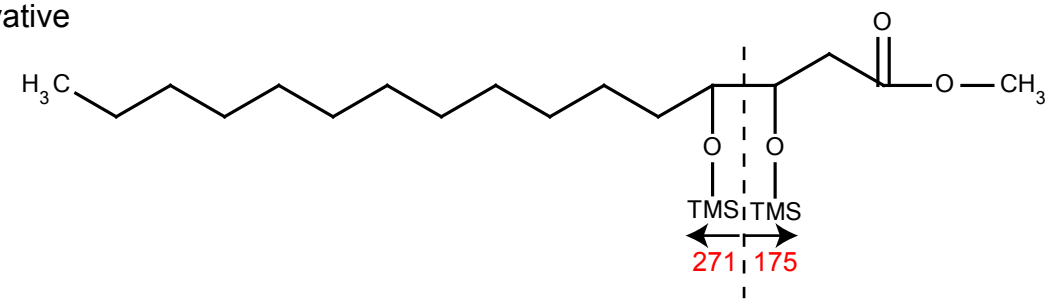
Pyrrolidide derivative

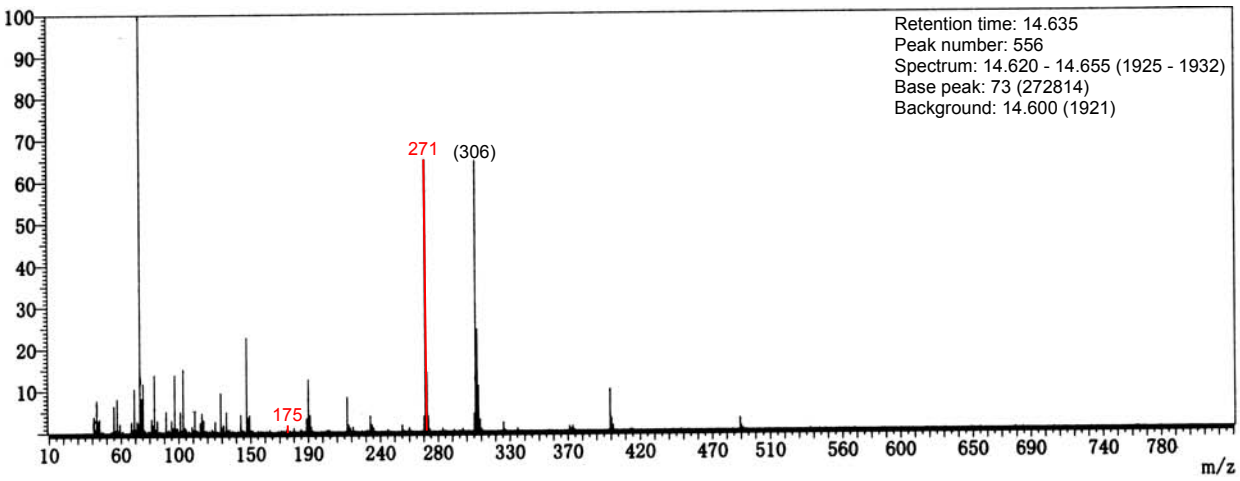




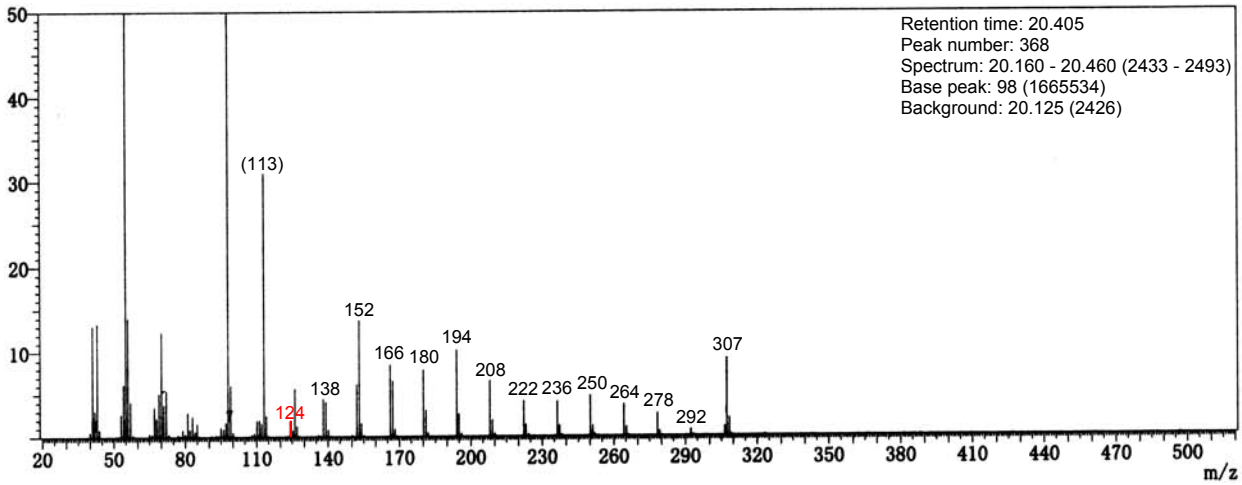
Trimethylsilyl derivative

16:1 (3t)



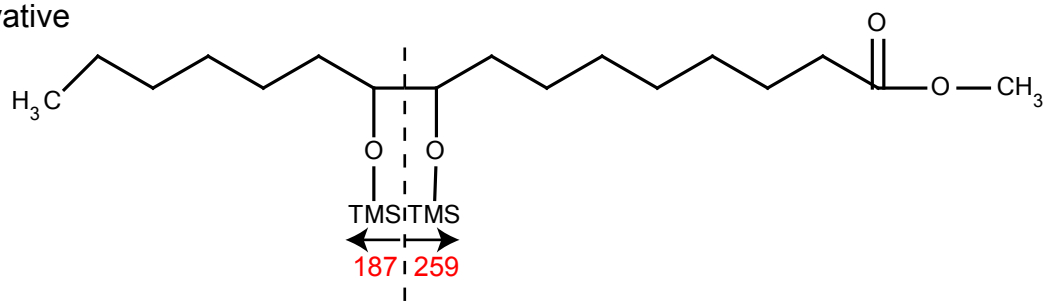


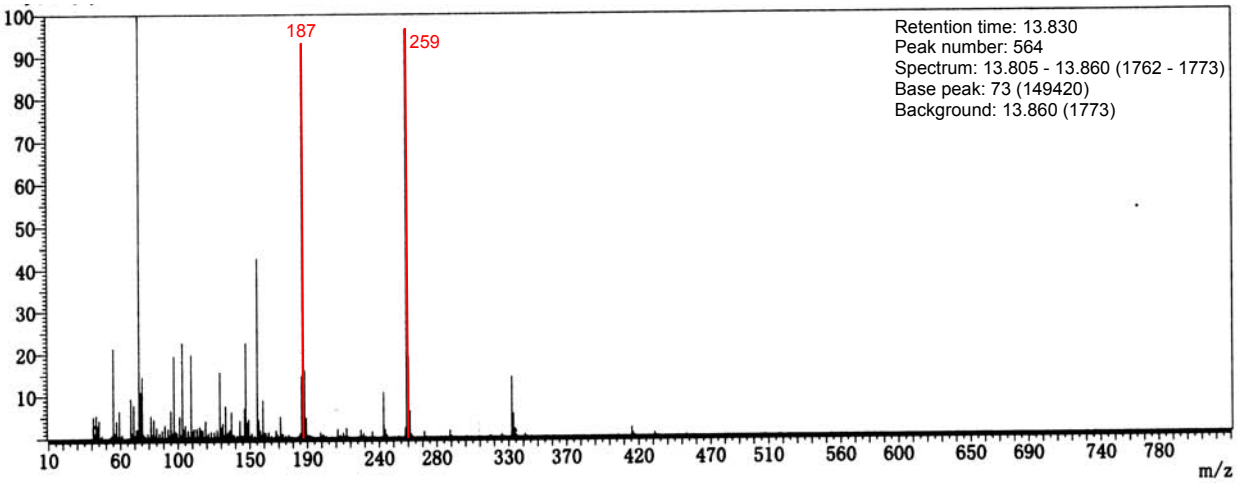
Pyrrolidide derivative



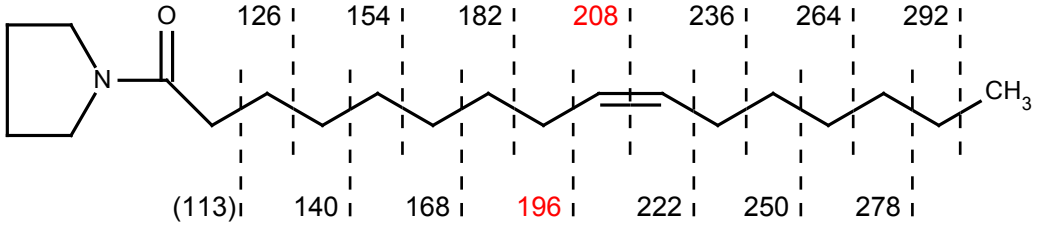
Trimethylsilyl derivative

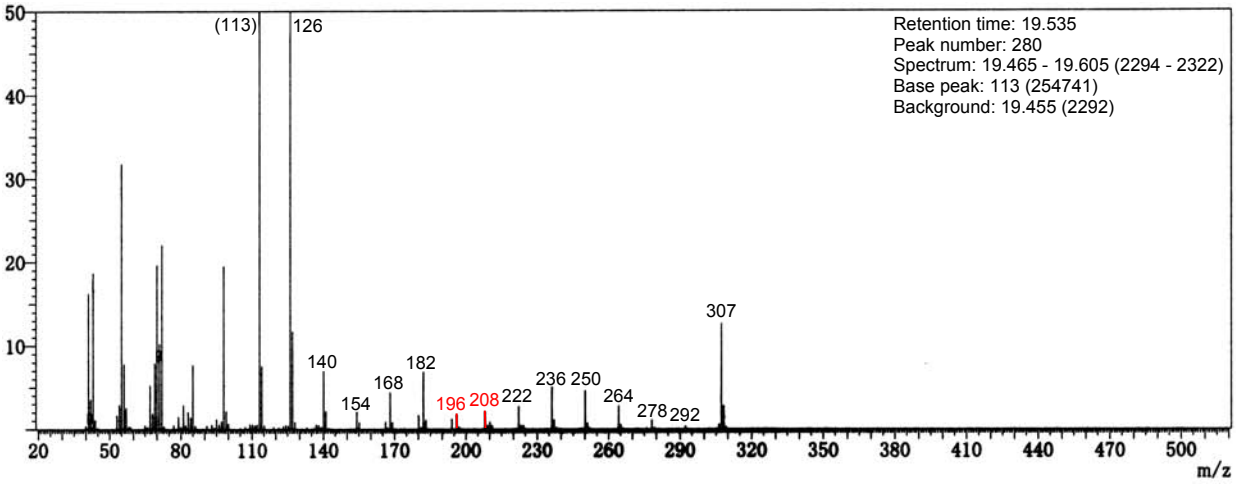
16:1 (9)





Pyrrolidide derivative





Trimethylsilyl derivative

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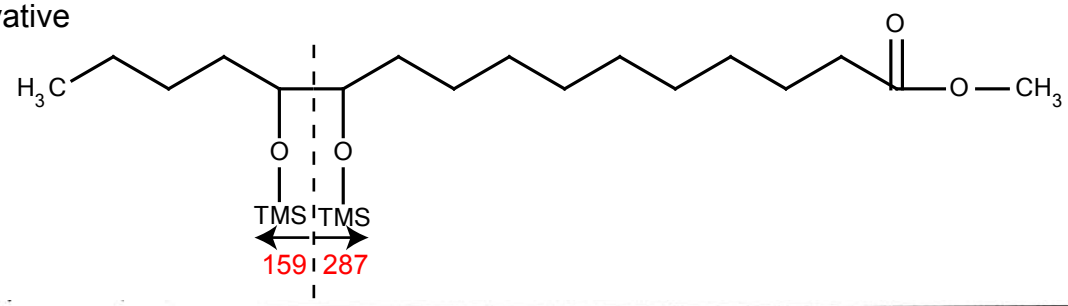
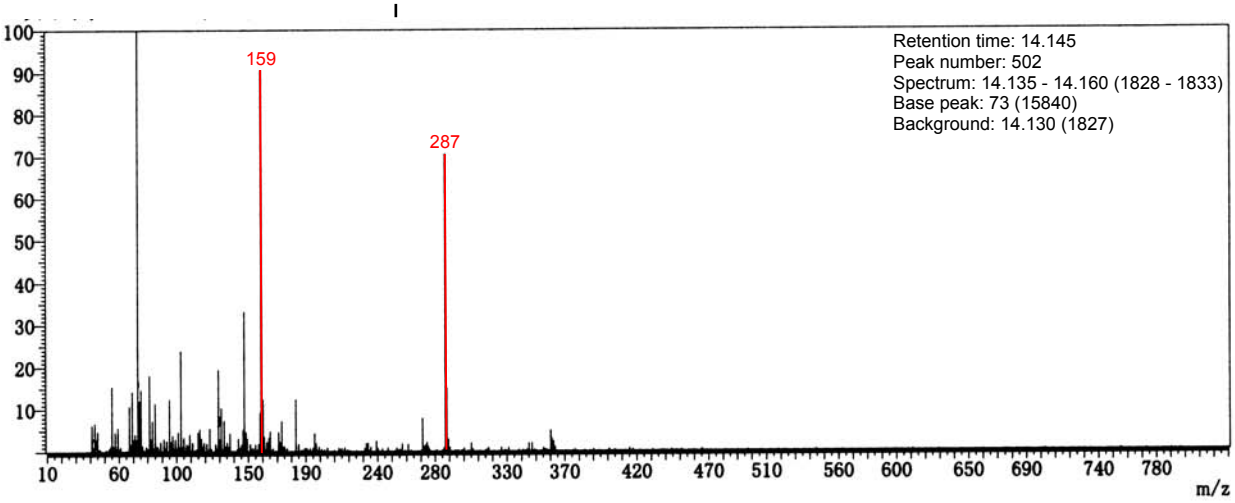
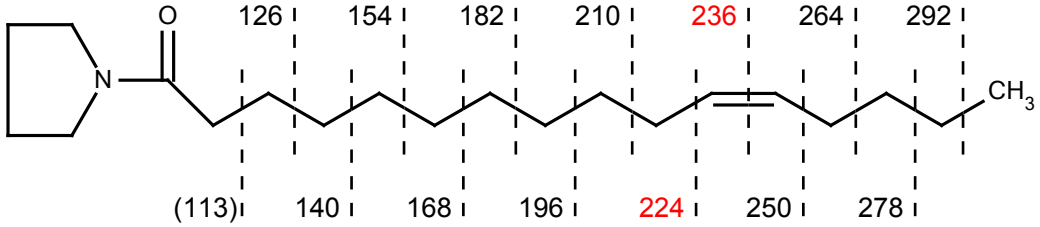
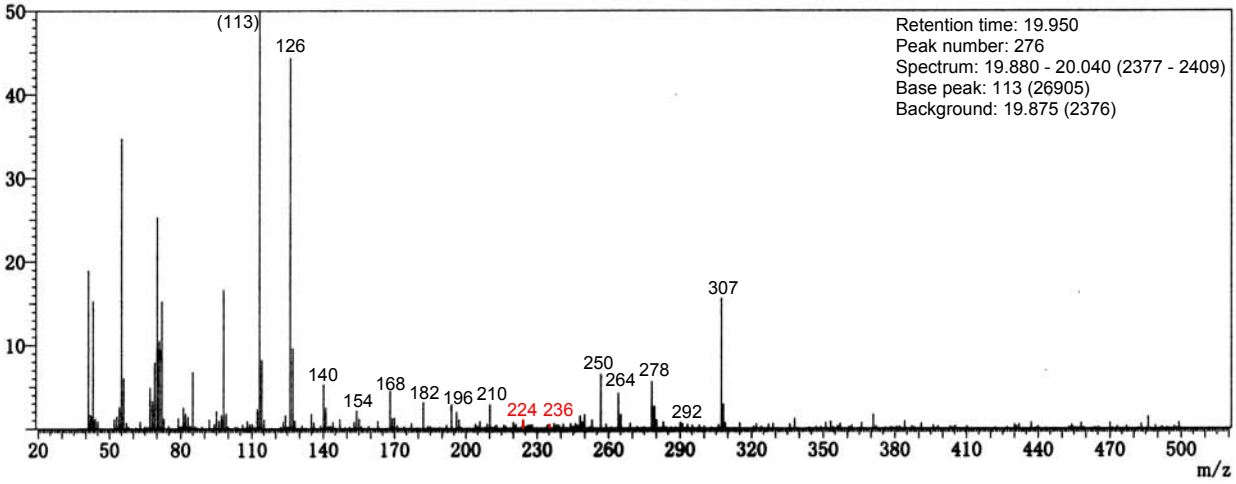




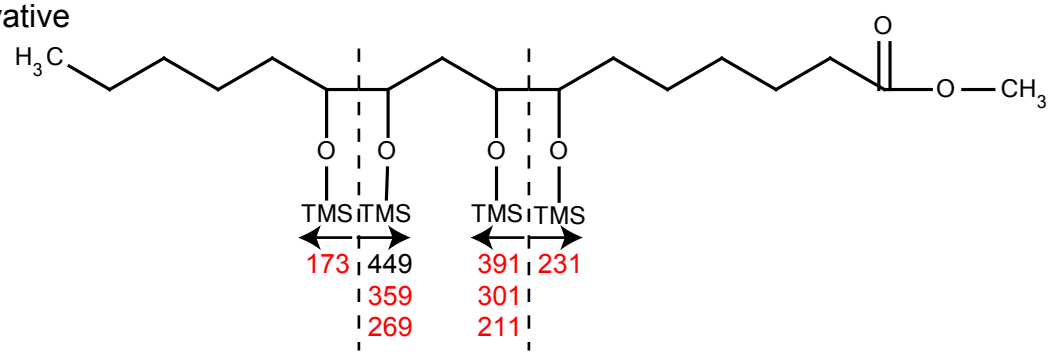
Pyrrolidide derivative

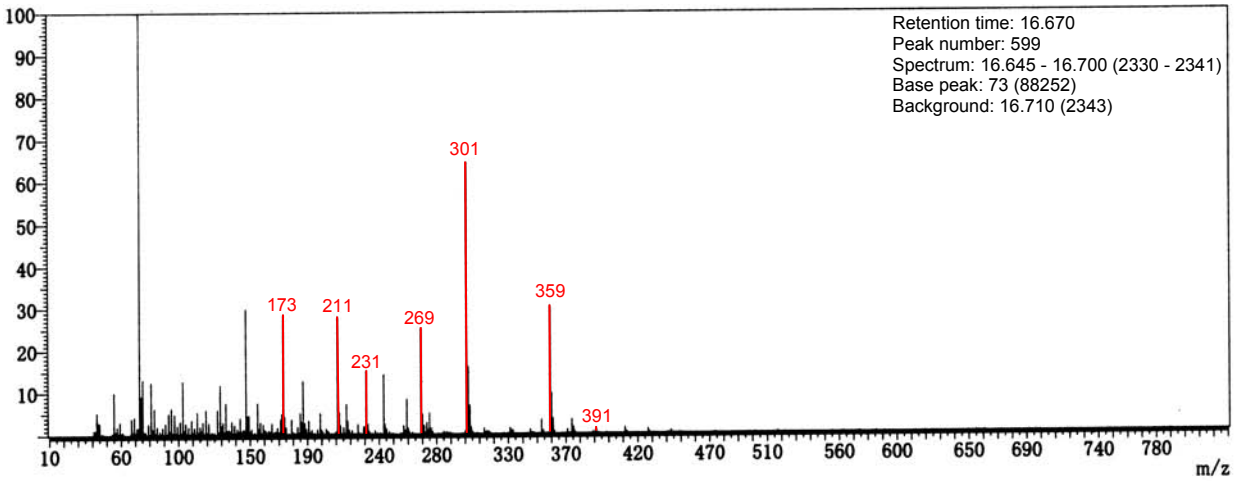




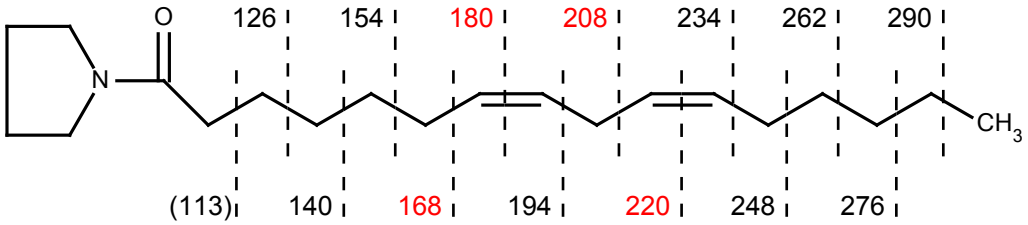
Trimethylsilyl derivative

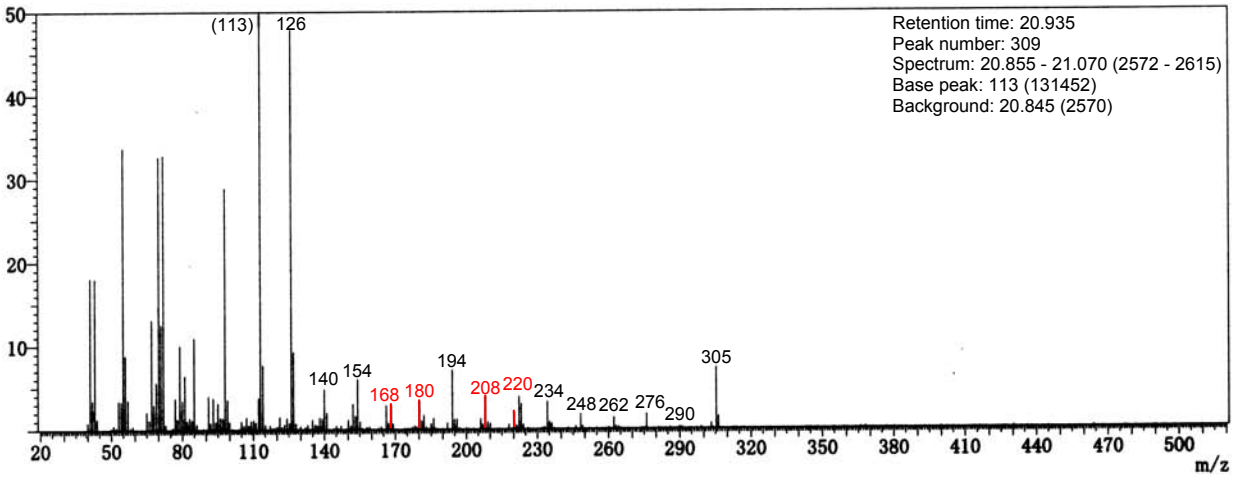
16:2 (7,10)





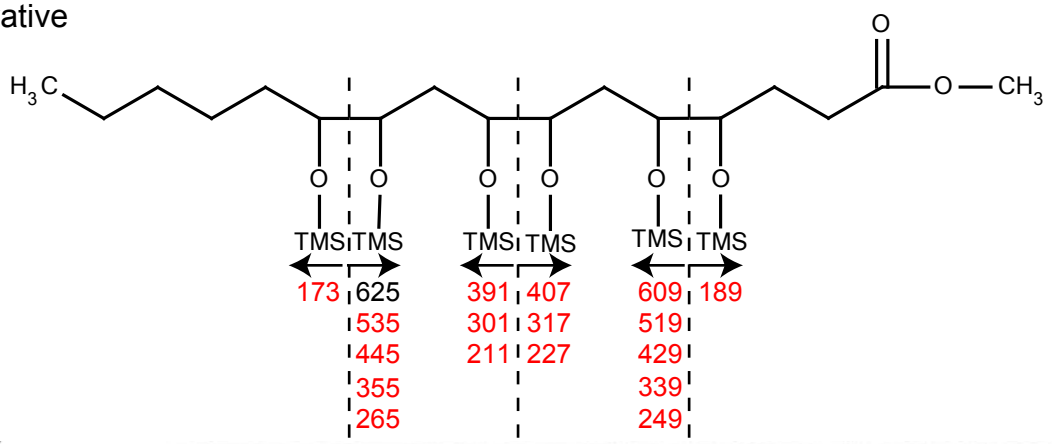
Pyrrolidide derivative

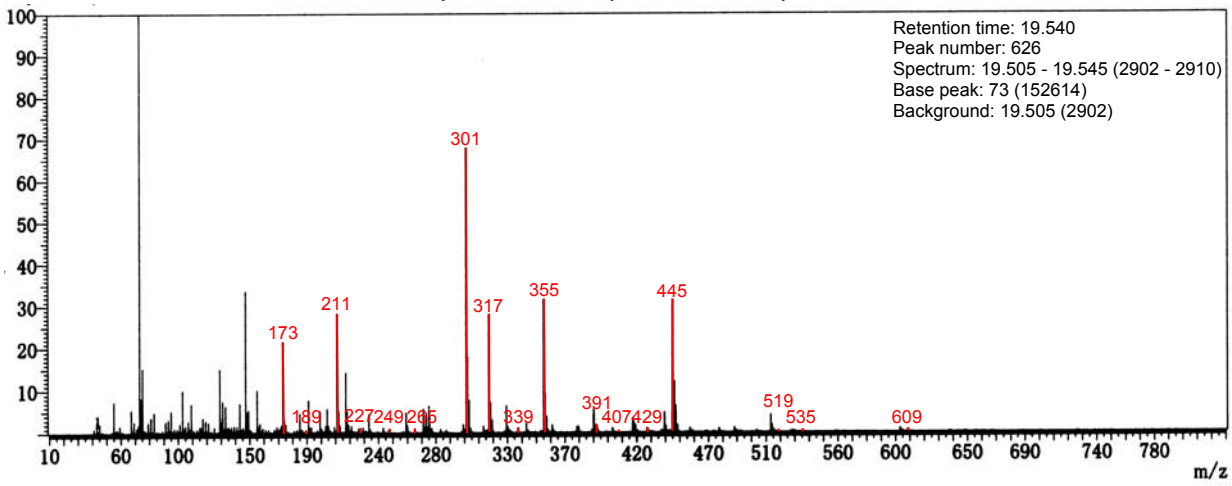




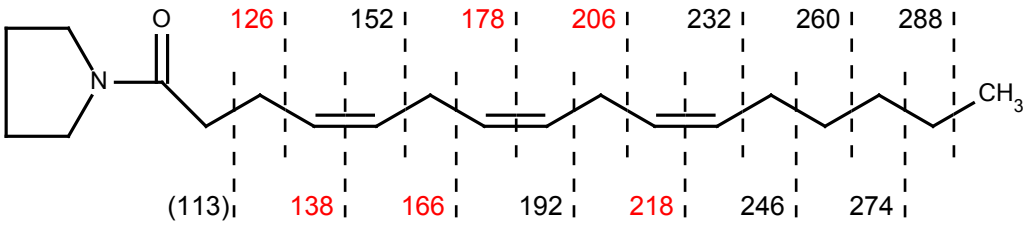
Trimethylsilyl derivative

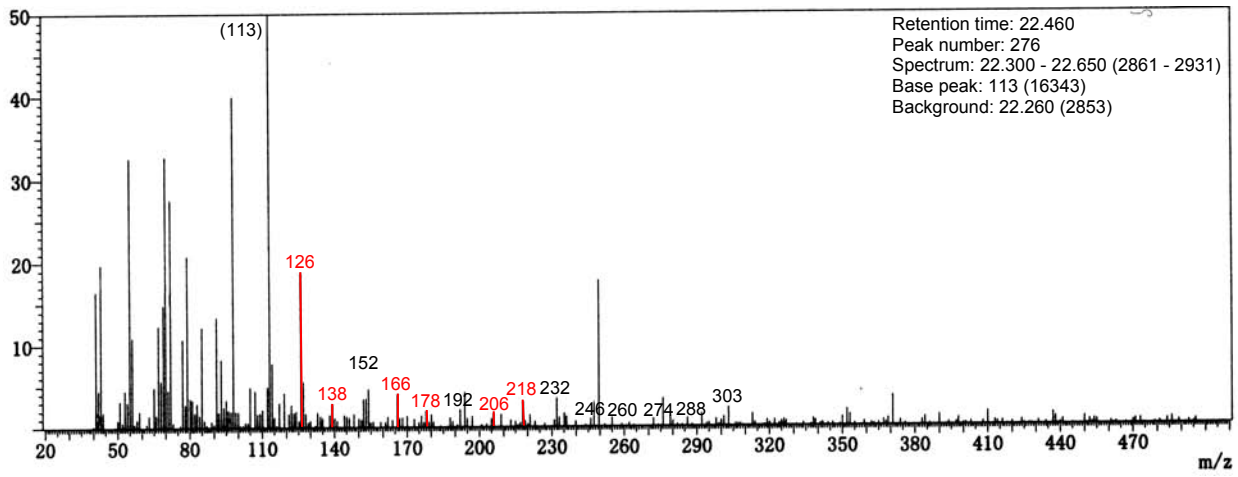
16:3 (4,7,10)





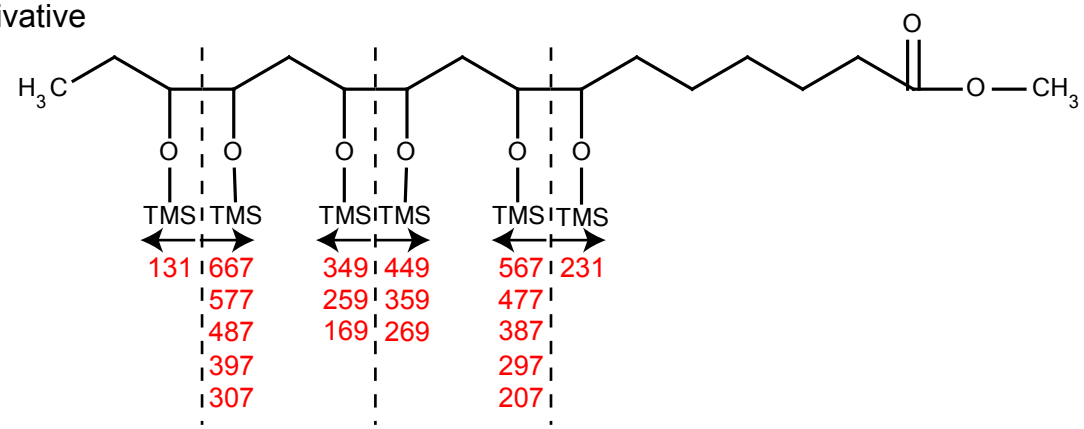
Pyrrolidide derivative

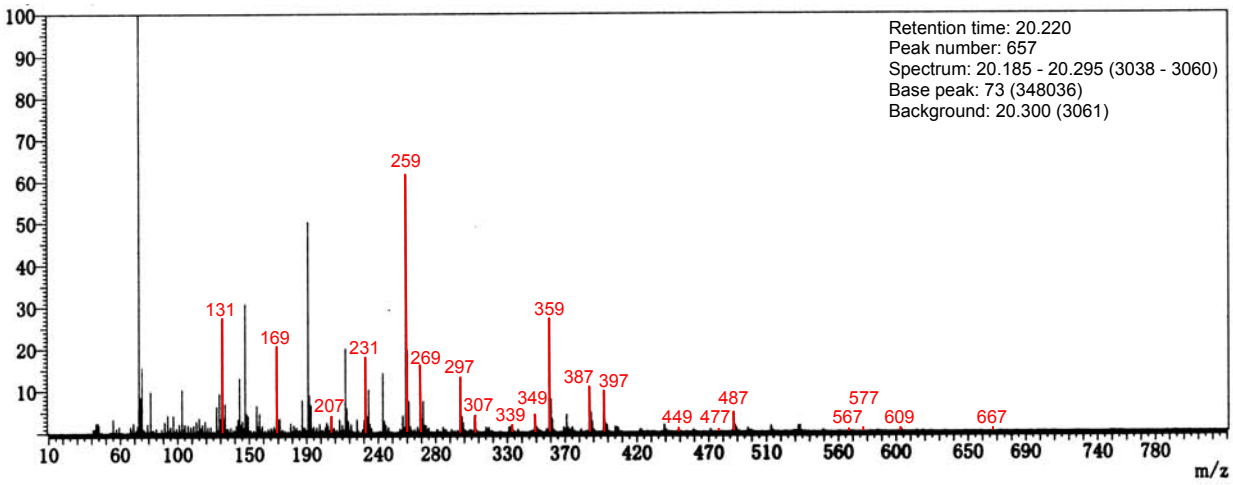




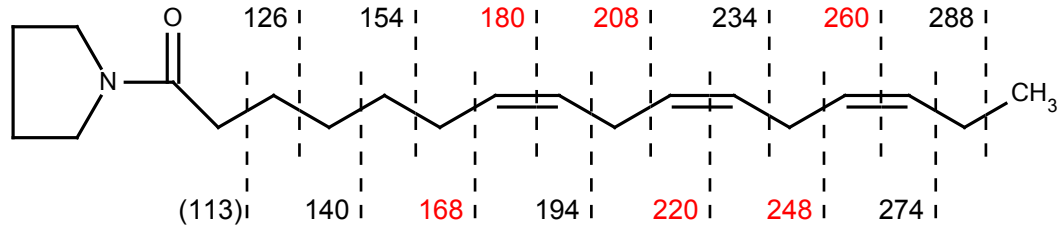
Trimethylsilyl derivative

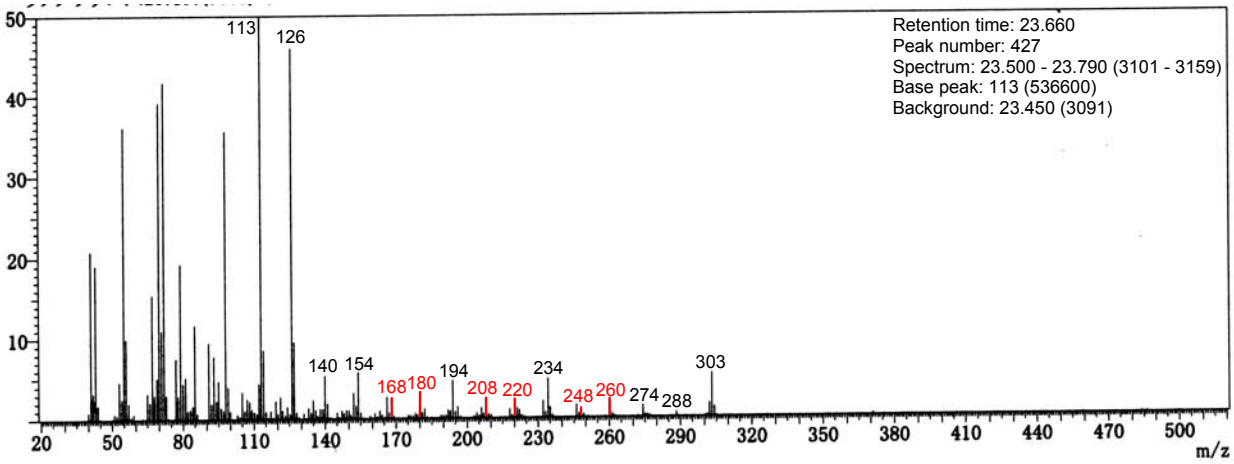
16:3 (7,10,13)





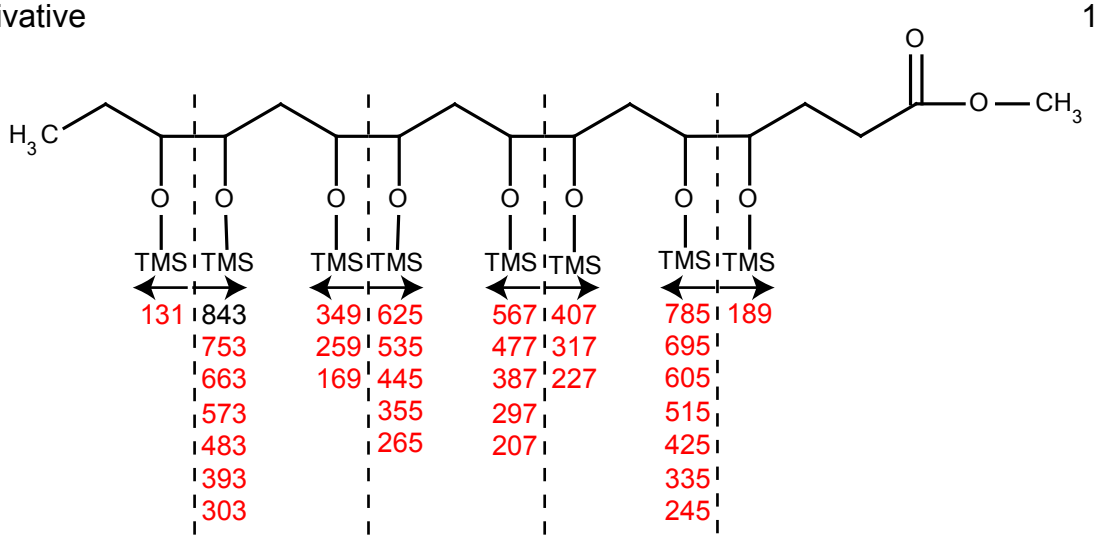
Pyrrolidide derivative

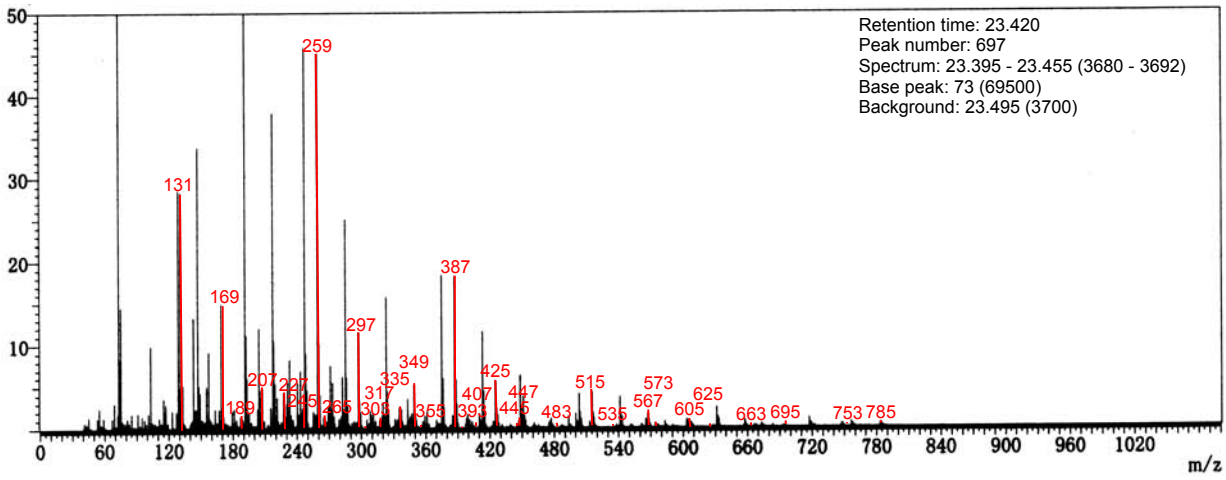




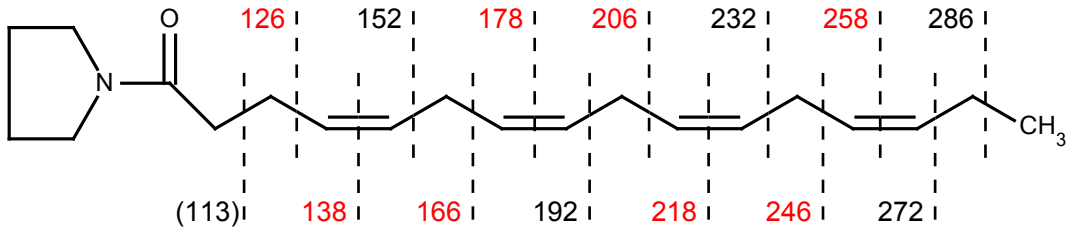
Trimethylsilyl derivative

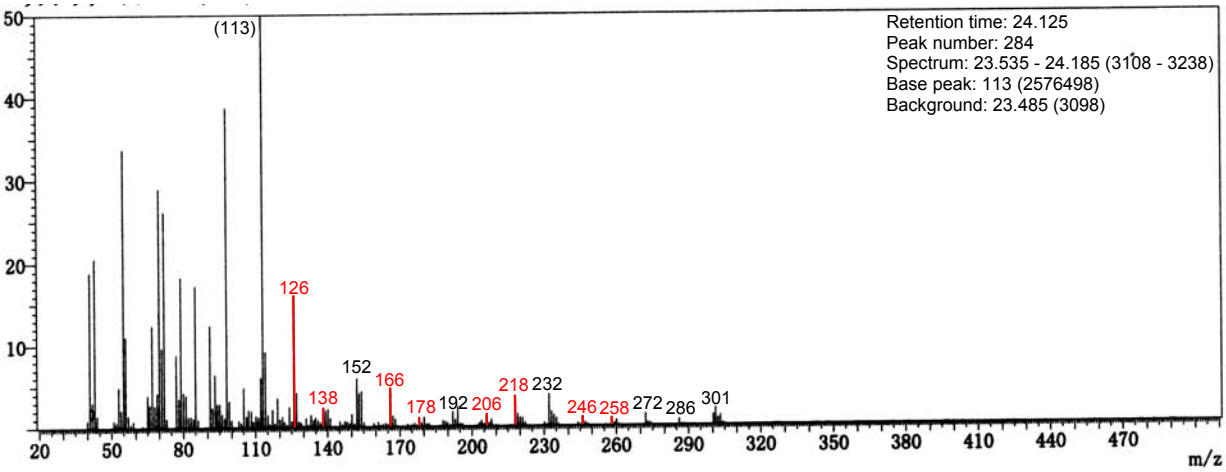
16:4 (4,7,10,13)



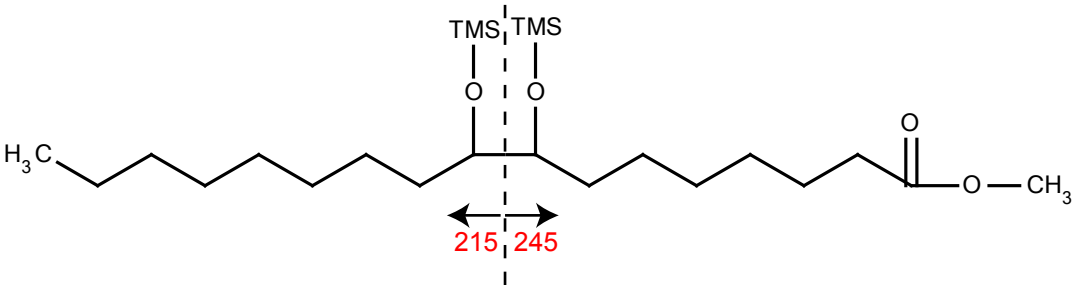


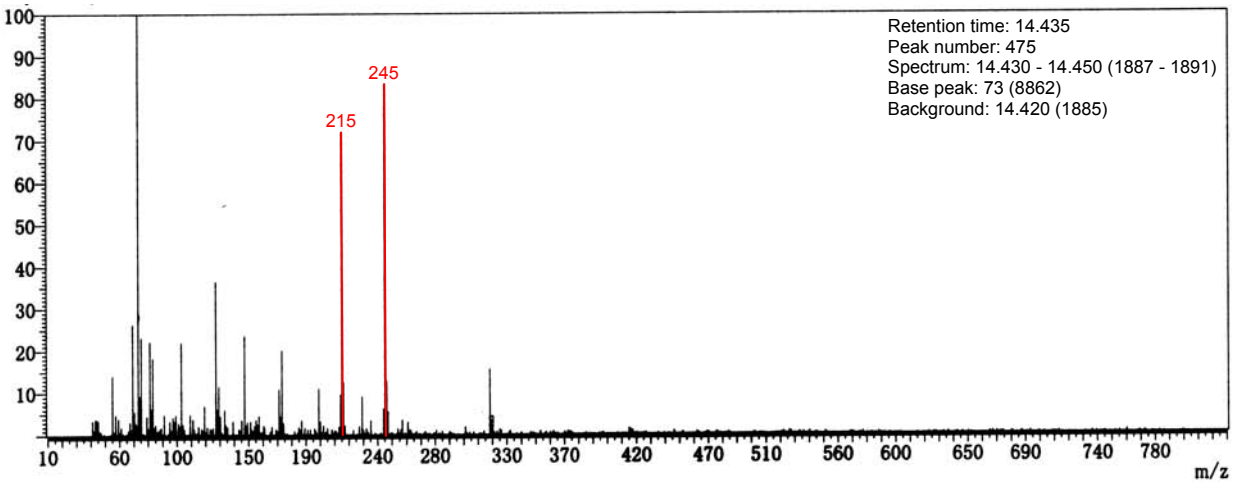
Pyrrolidide derivative



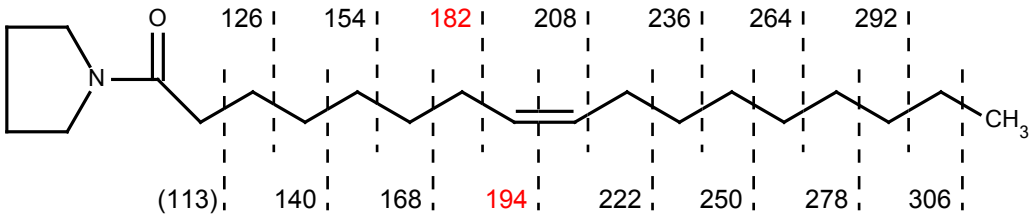


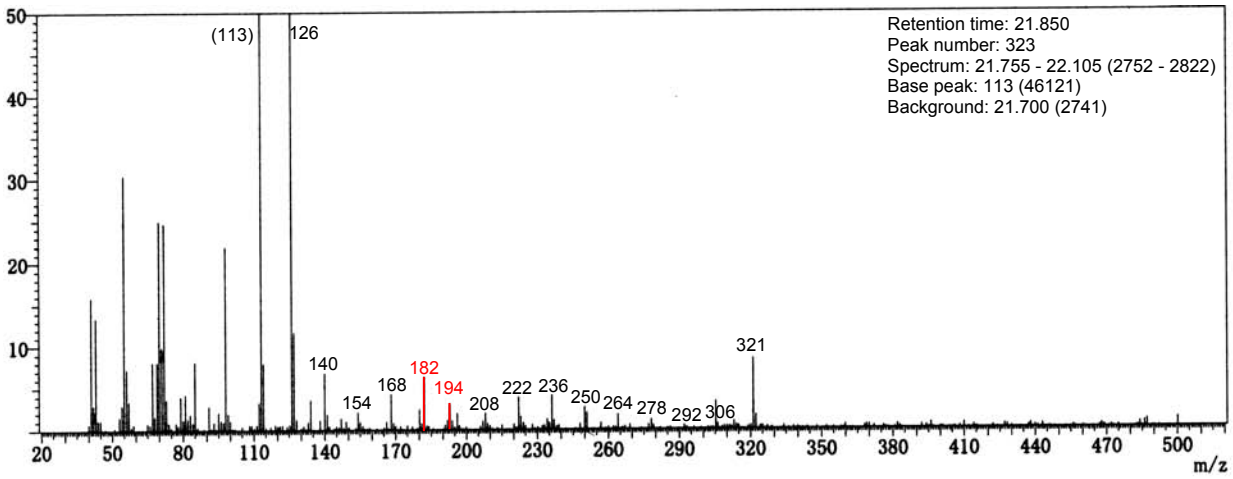
Trimethylsilyl derivative





Pyrrolidide derivative

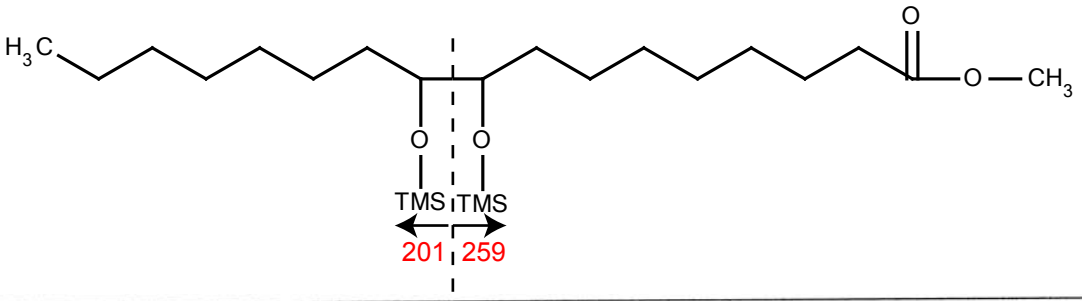


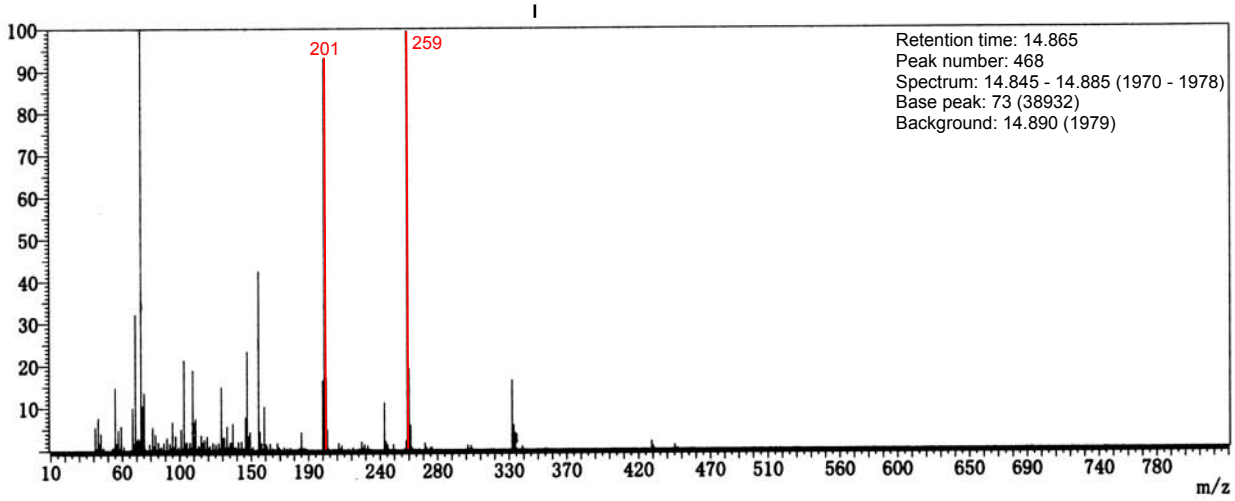


In the pyrroridide derivatives of 17:1(8) and 17:1(9), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.

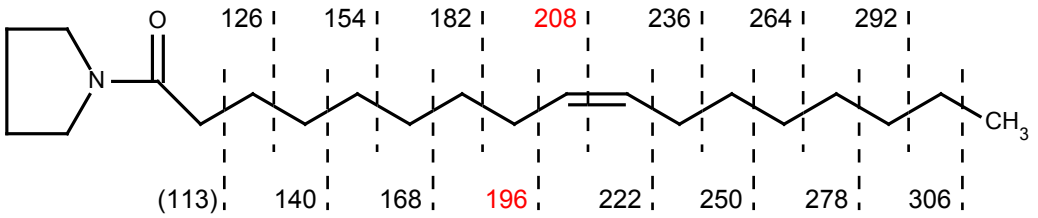
Trimethylsilyl derivative

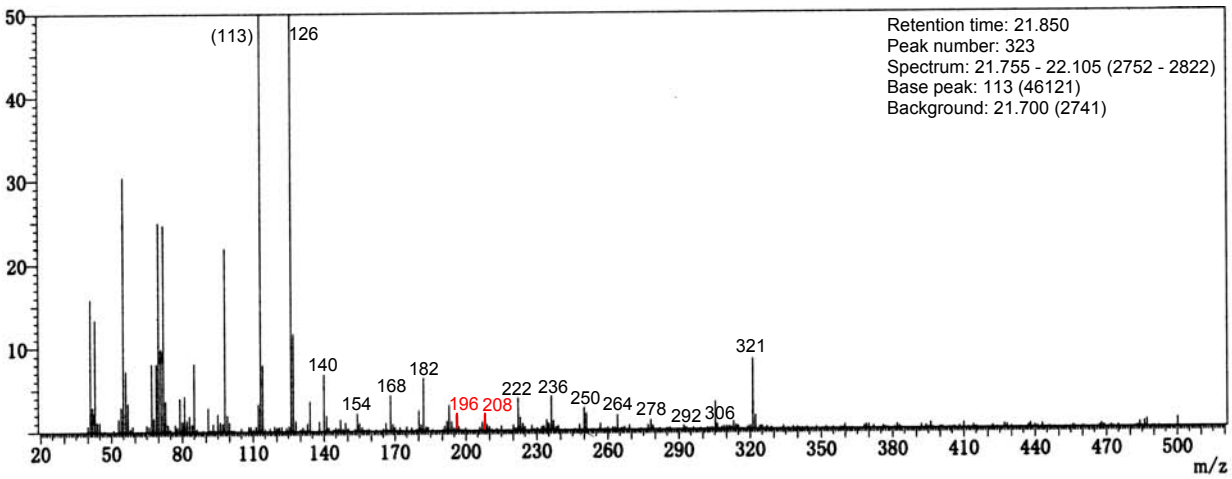
17:1 (9)





Pyrrolidide derivative

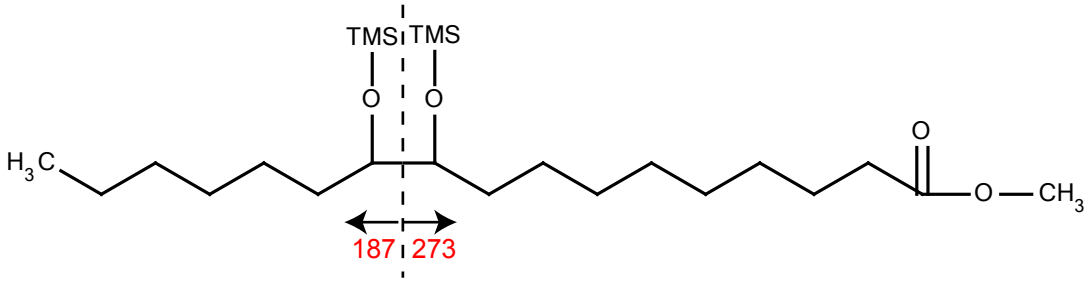


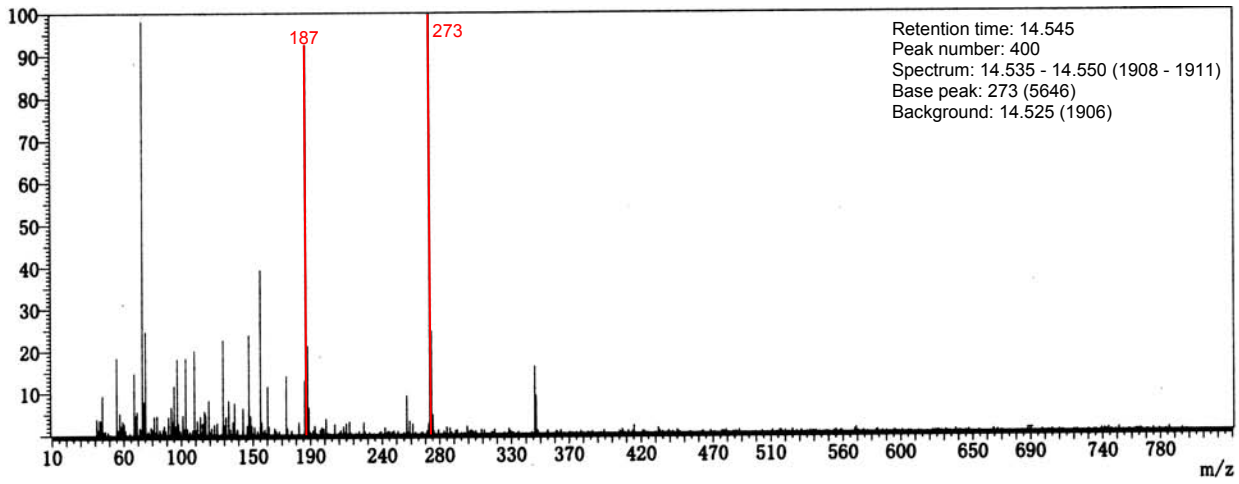


In the pyrroridide derivatives of 17:1(8) and 17:1(9), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.

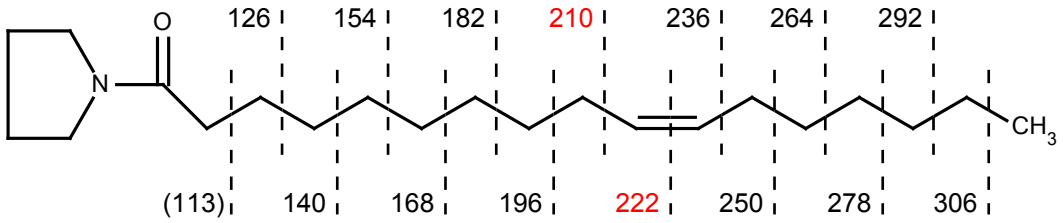
Trimethylsilyl derivative

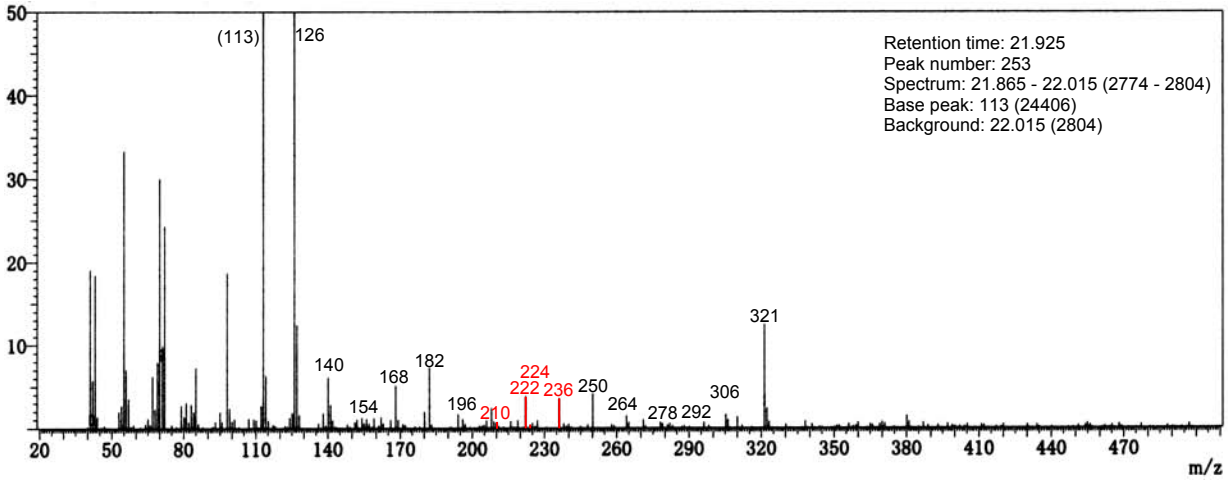
17:1 (10)





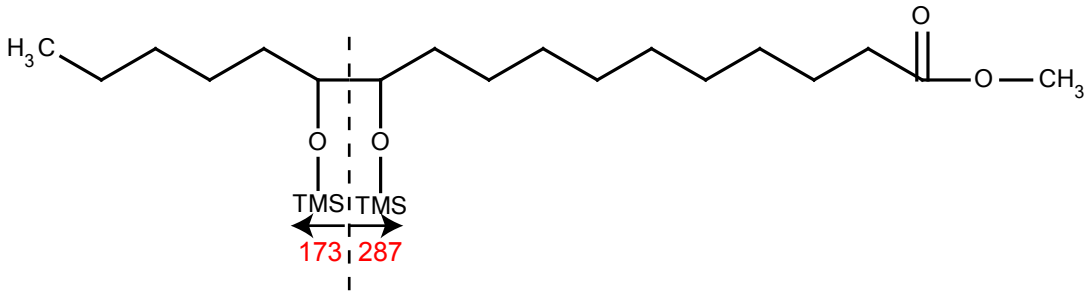
Pyrrolidide derivative

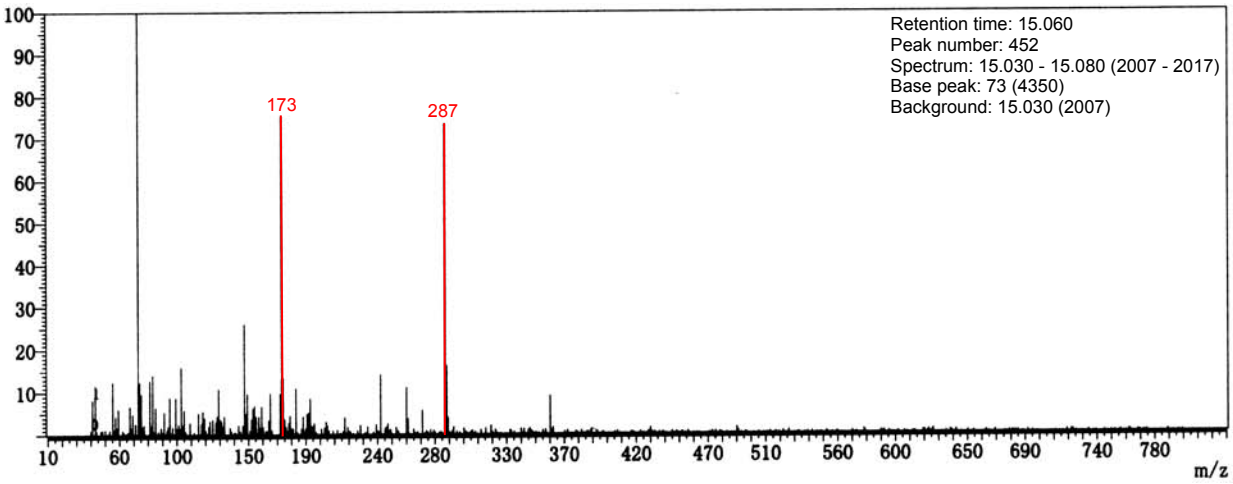




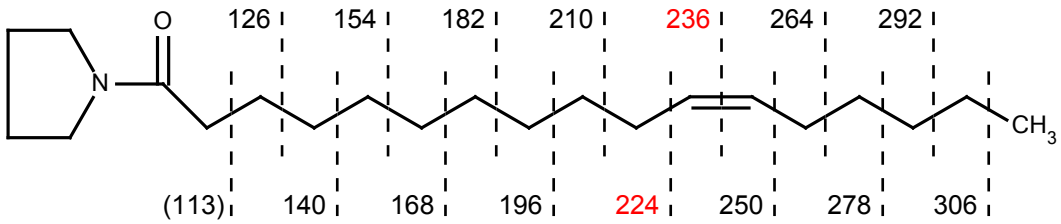
In the pyrroridide derivatives of 17:1(10) and 17:1(11), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.

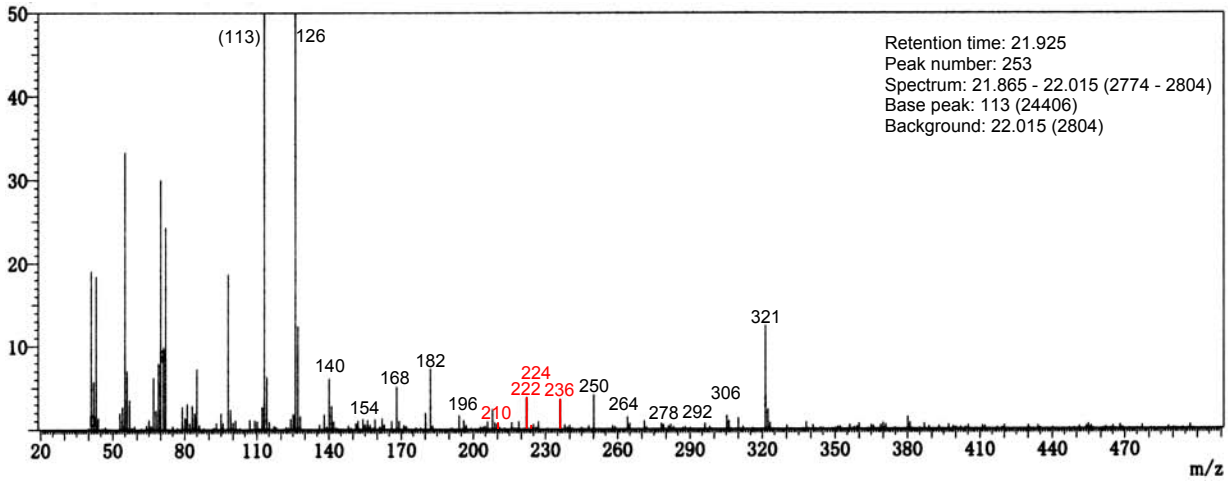
Trimethylsilyl derivative





Pyrrolidide derivative

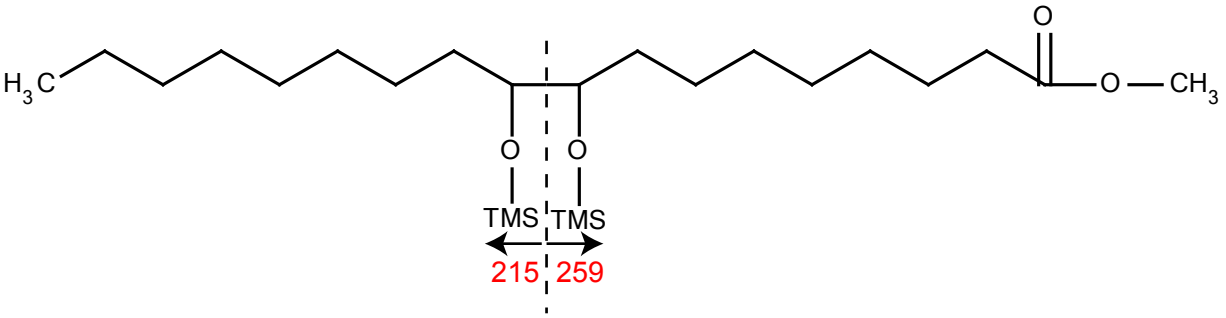


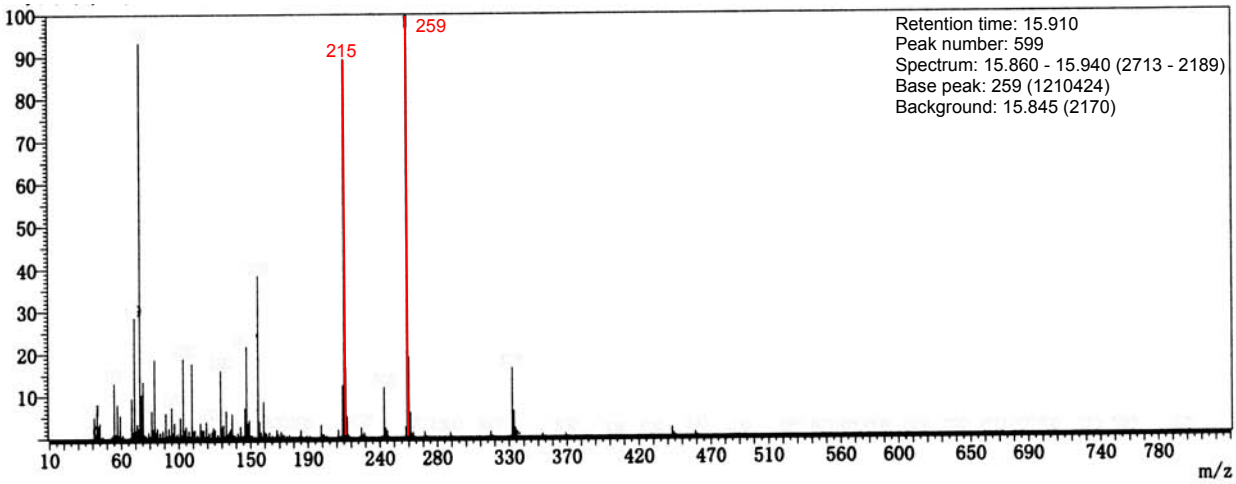


In the pyrroridide derivatives of 17:1(10) and 17:1(11), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.

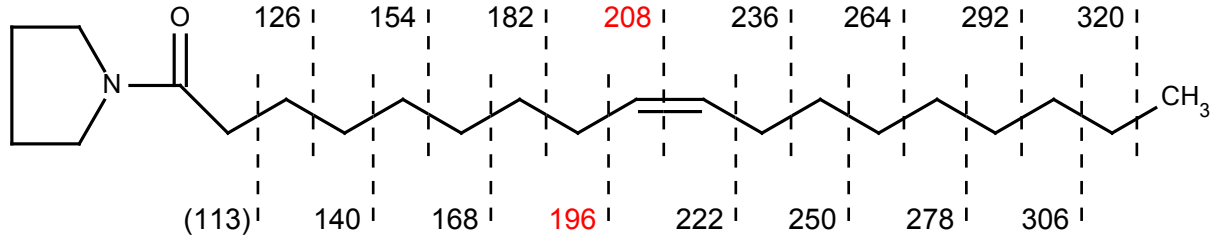
Trimethylsilyl derivative

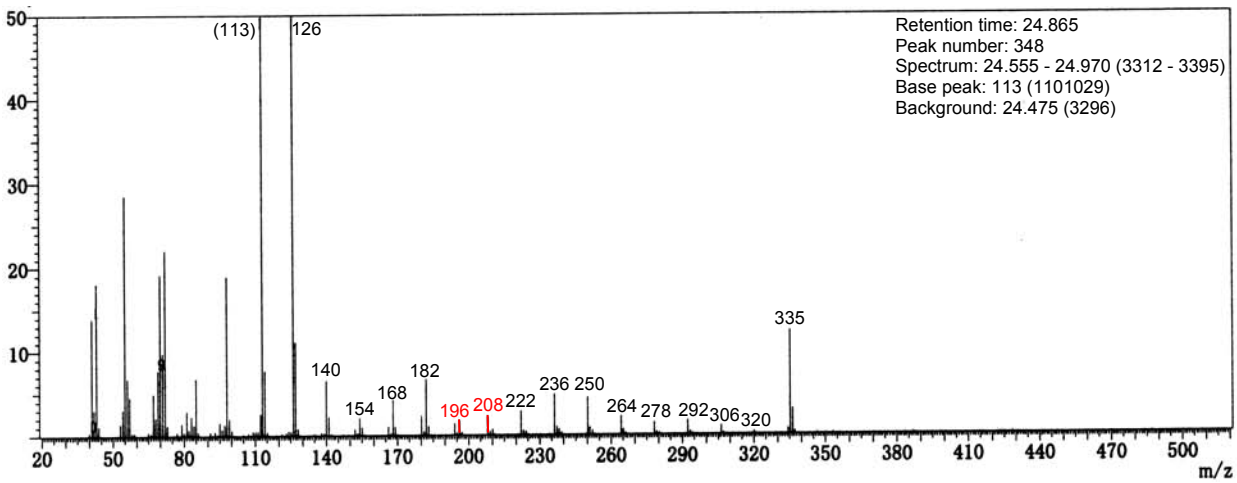
18:1 (9)

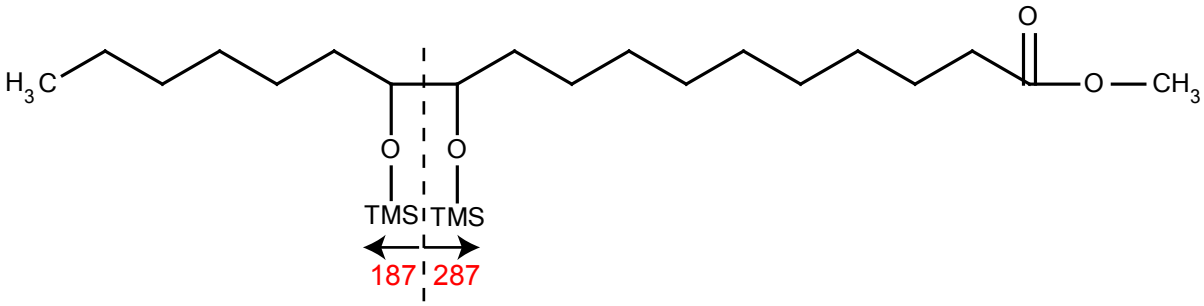


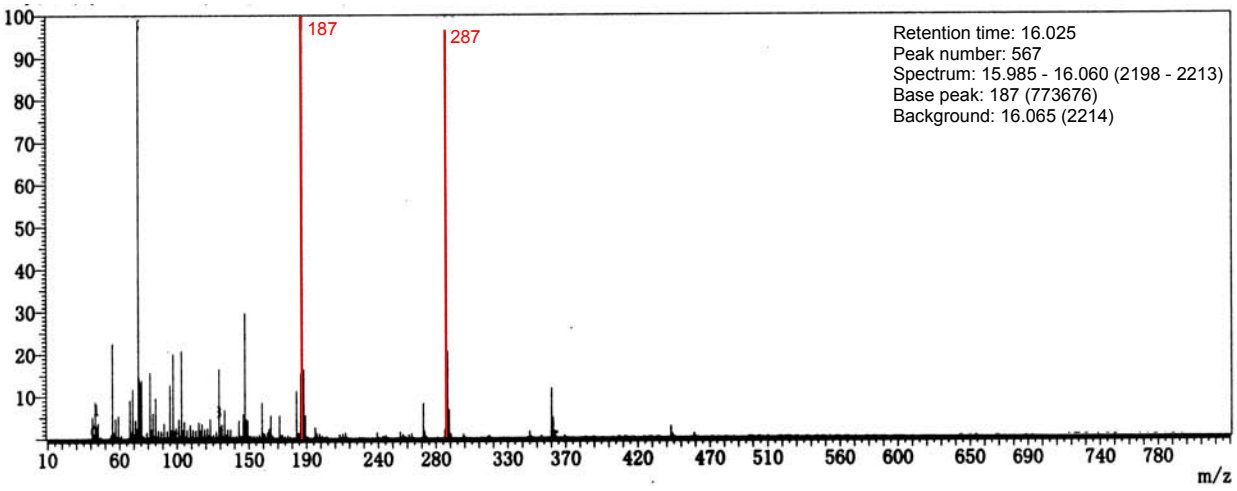


Pyrrolidide derivative

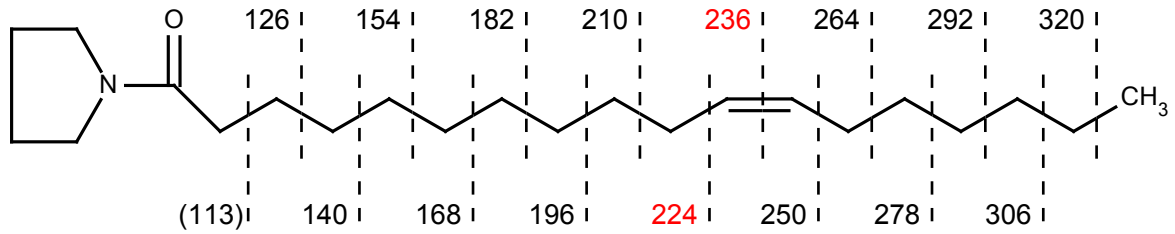


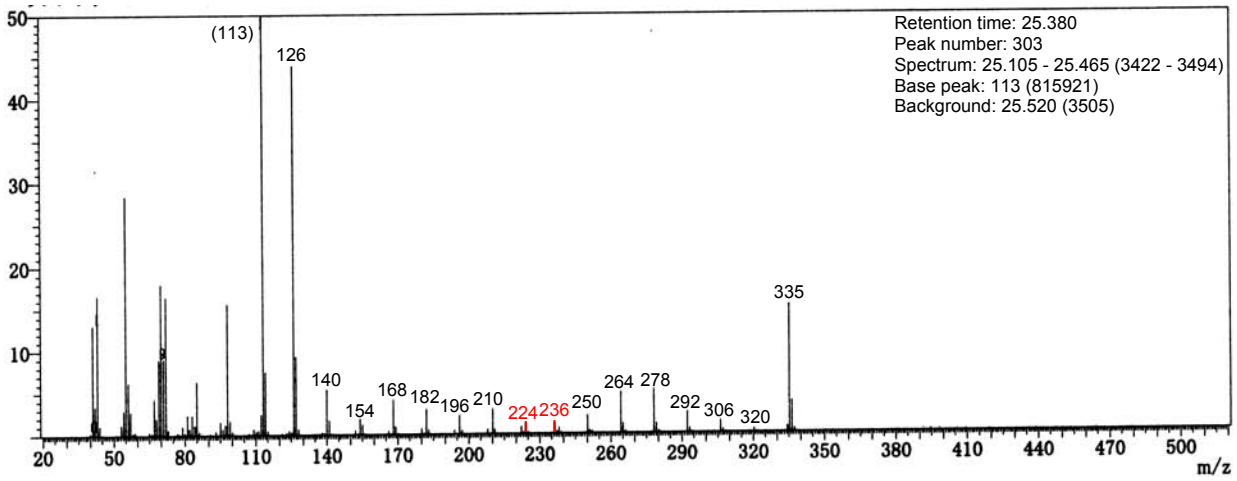






Pyrrolidide derivative

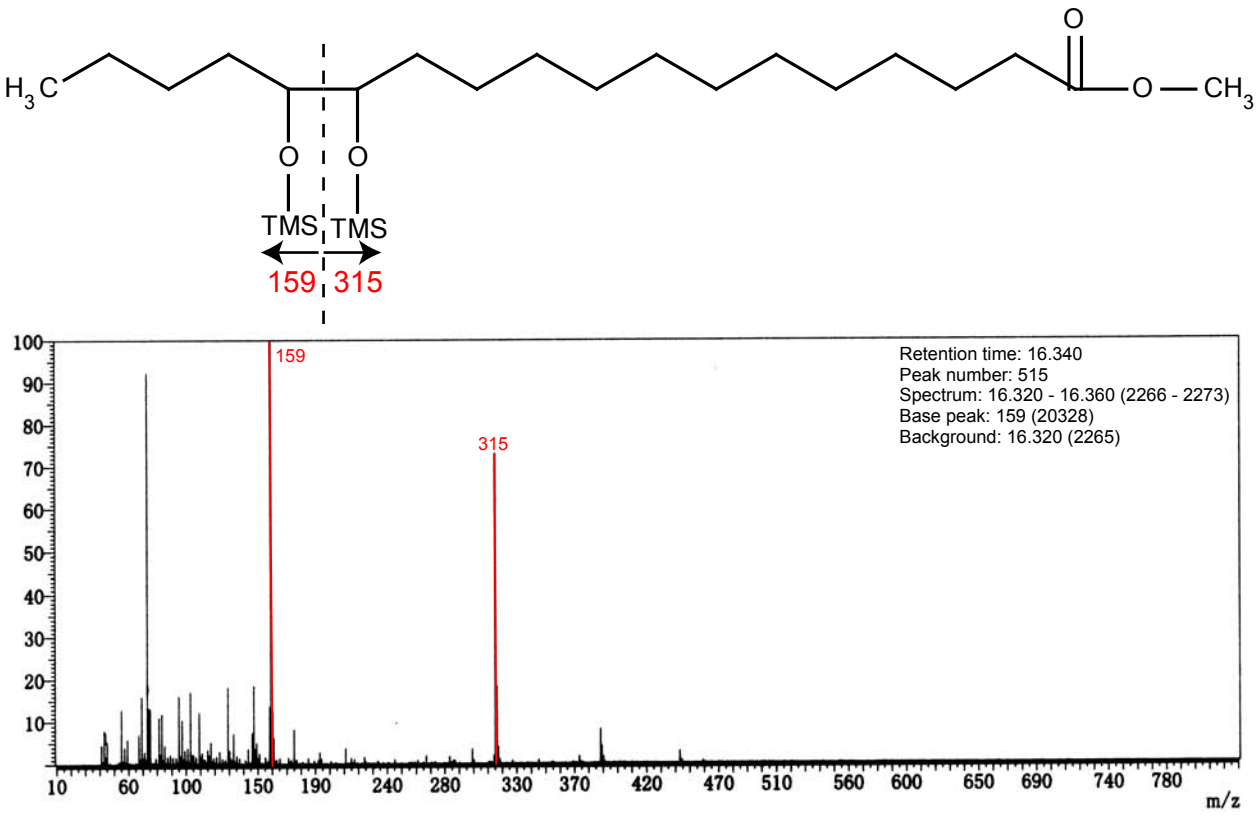




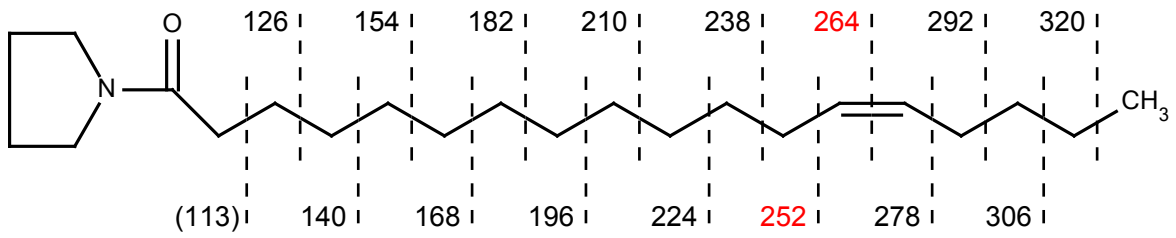
In the pyrroridide derivatives of 18:1(11) and 18:1(13), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.

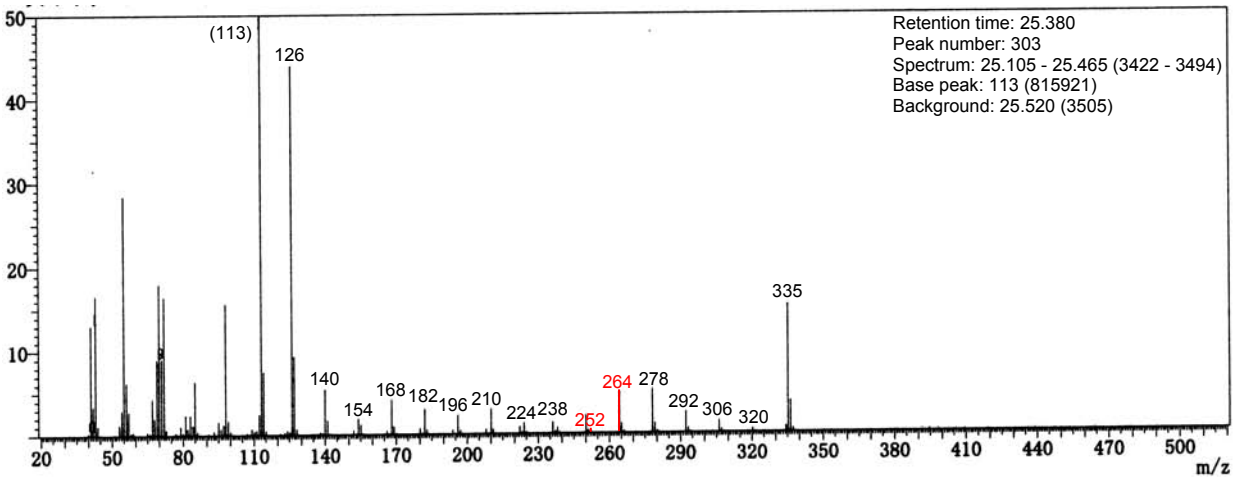
Trimethylsilyl derivative

18:1 (13)



Pyrrolidide derivative

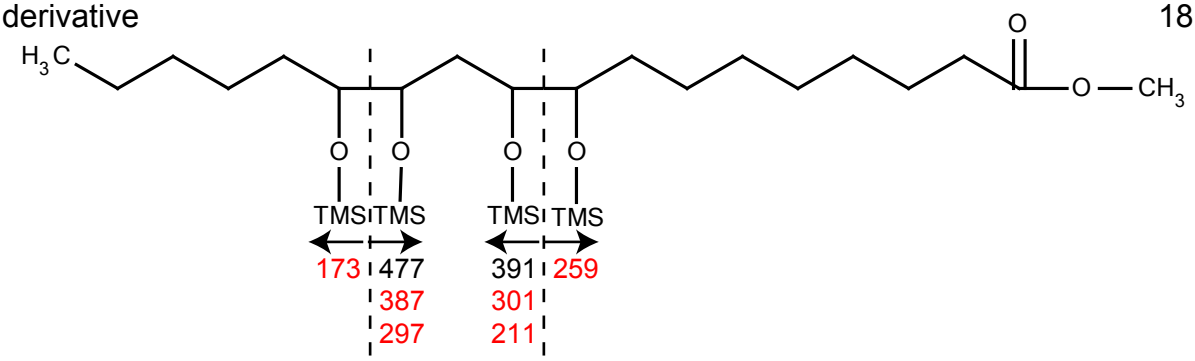


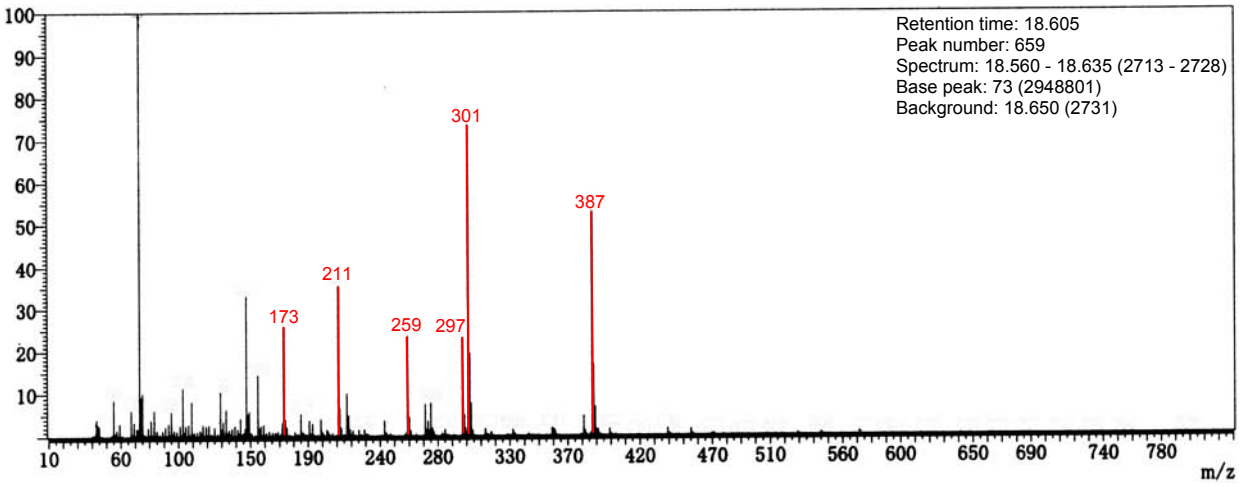


In the pyrroridide derivatives of 18:1(11) and 18:1(13), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.

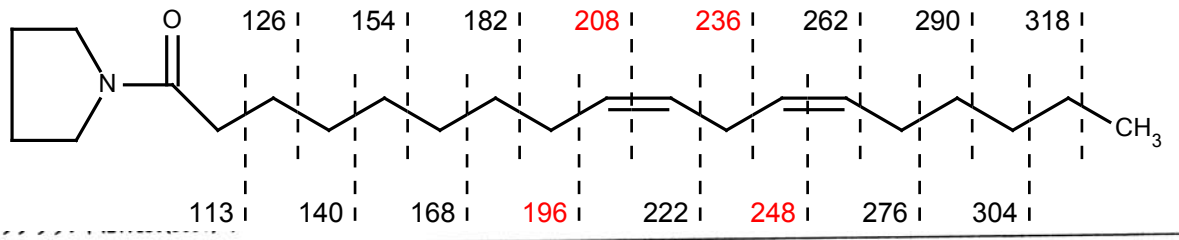
Trimethylsilyl derivative

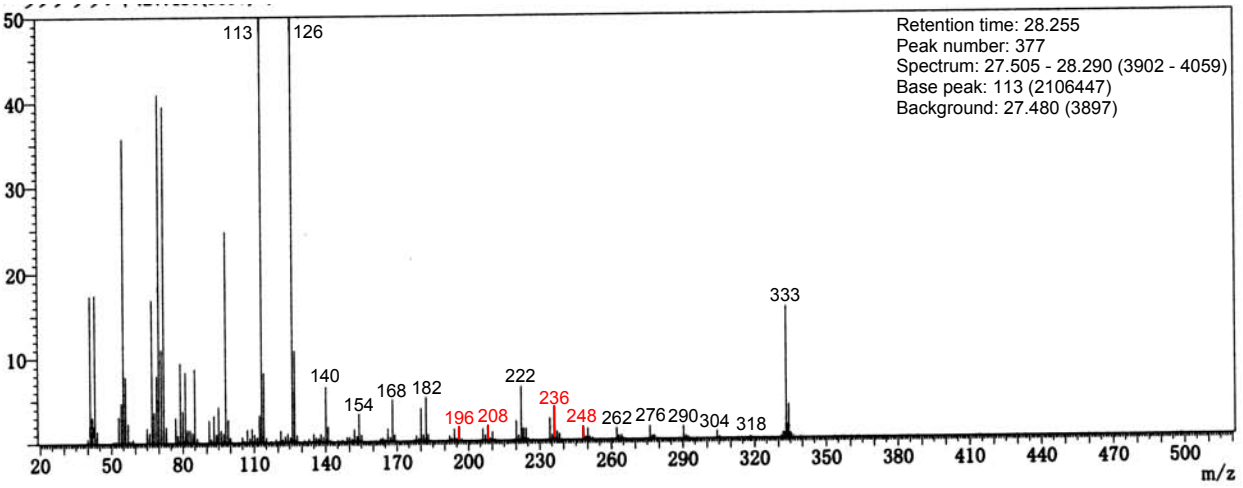
18:2 (9.12)





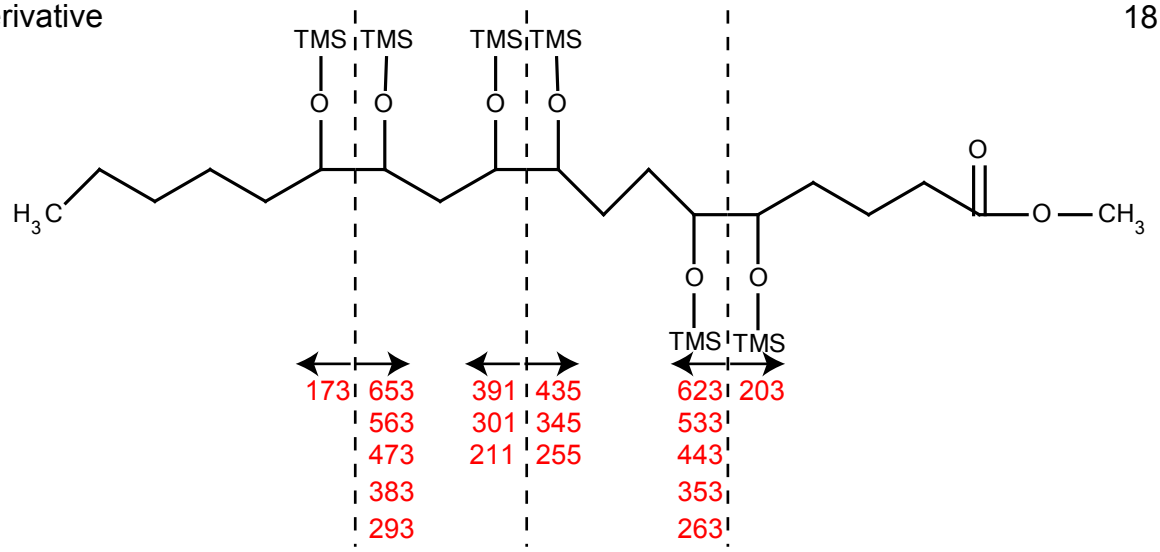
Pyrrolidide derivative

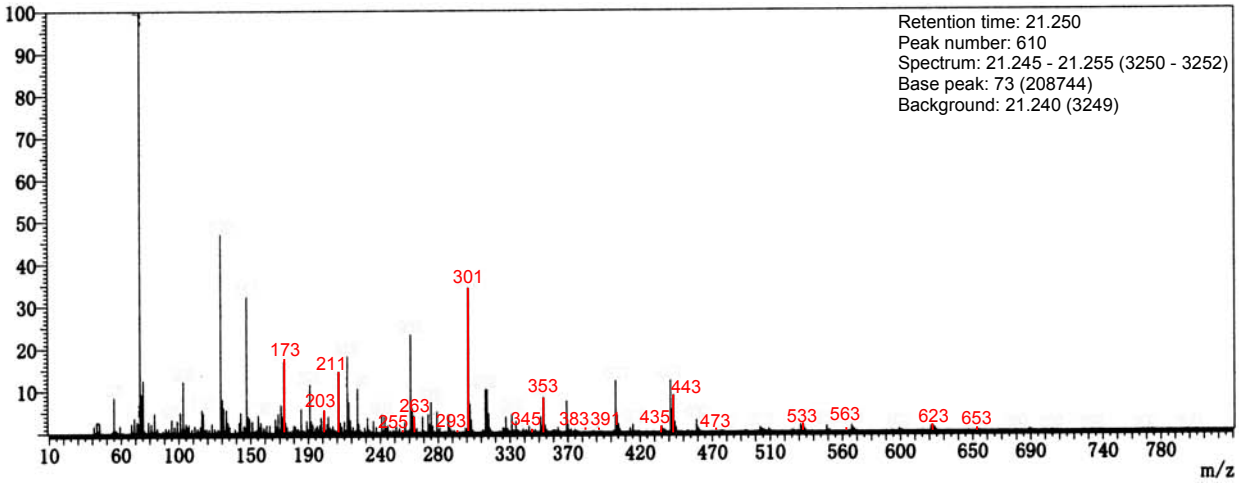




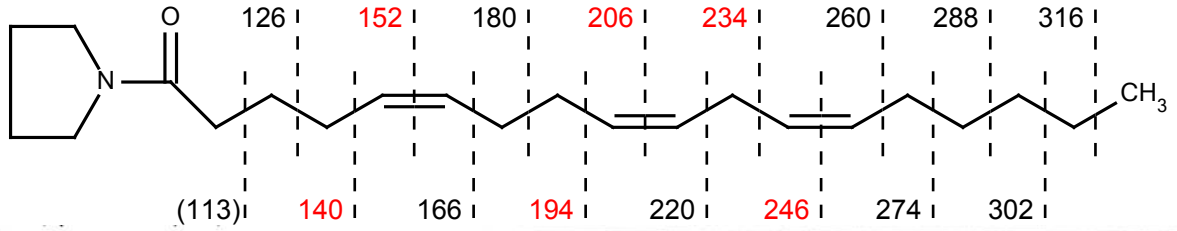
Trimethylsilyl derivative

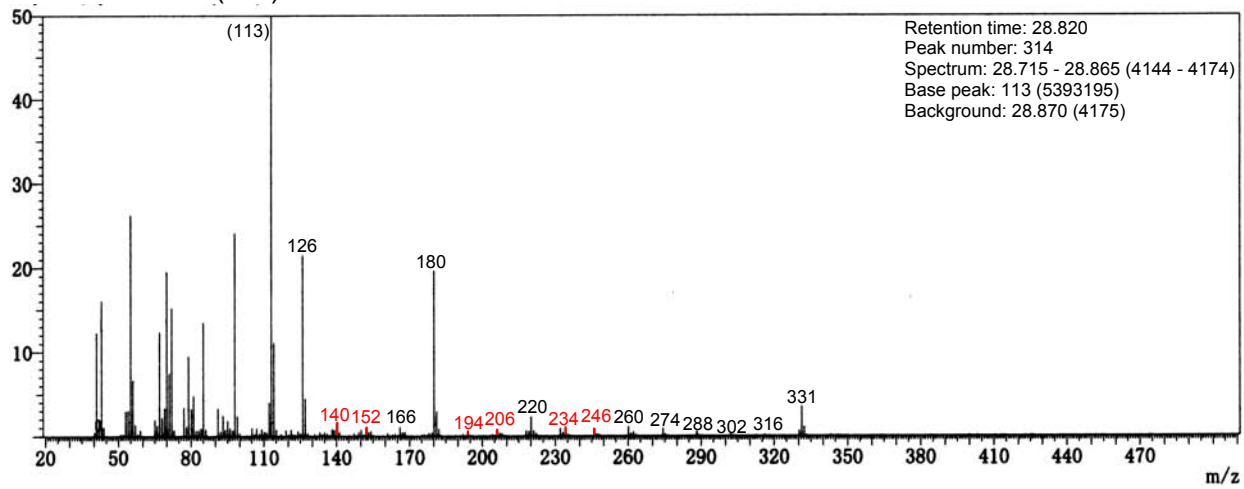
18:3 (5,9,12)





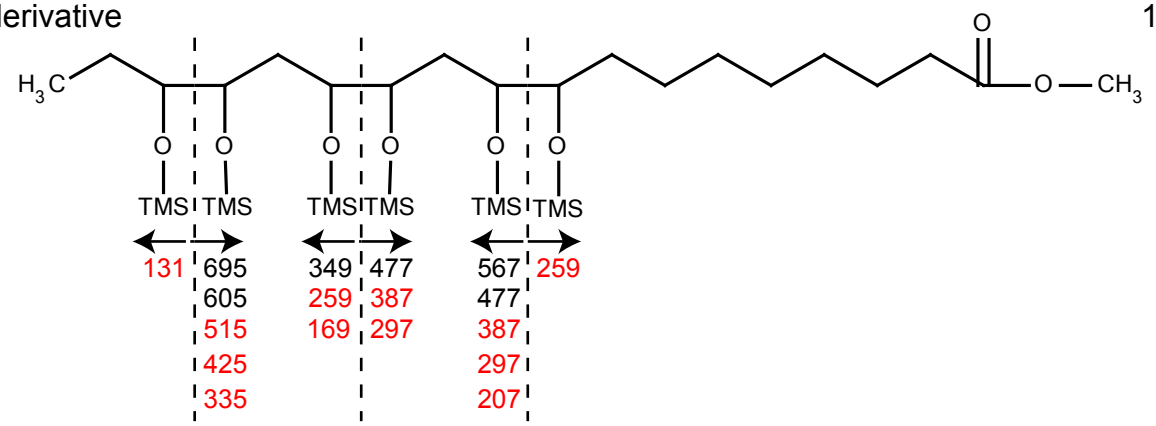
Pyrrolidide derivative

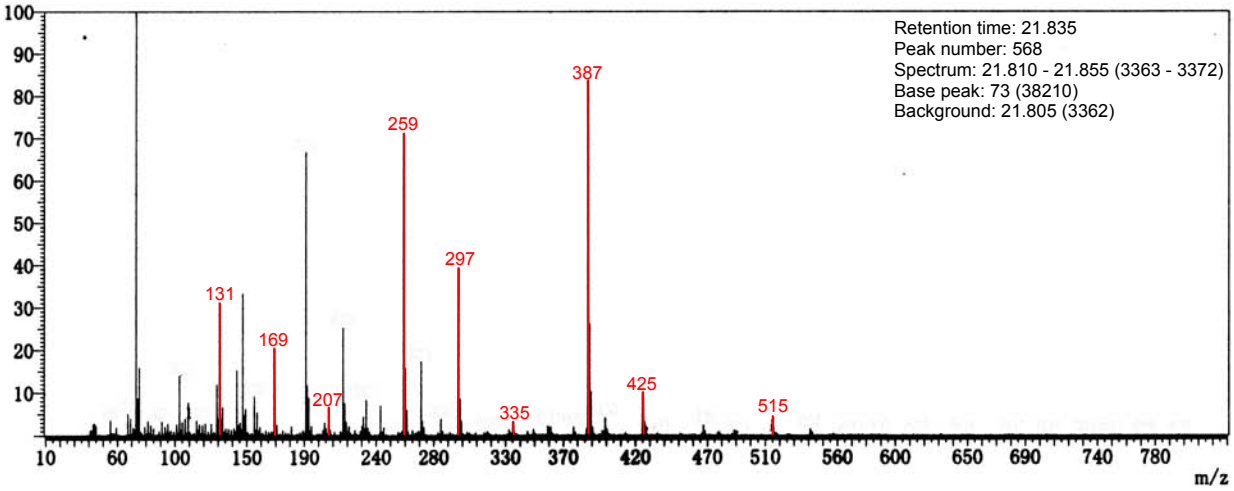


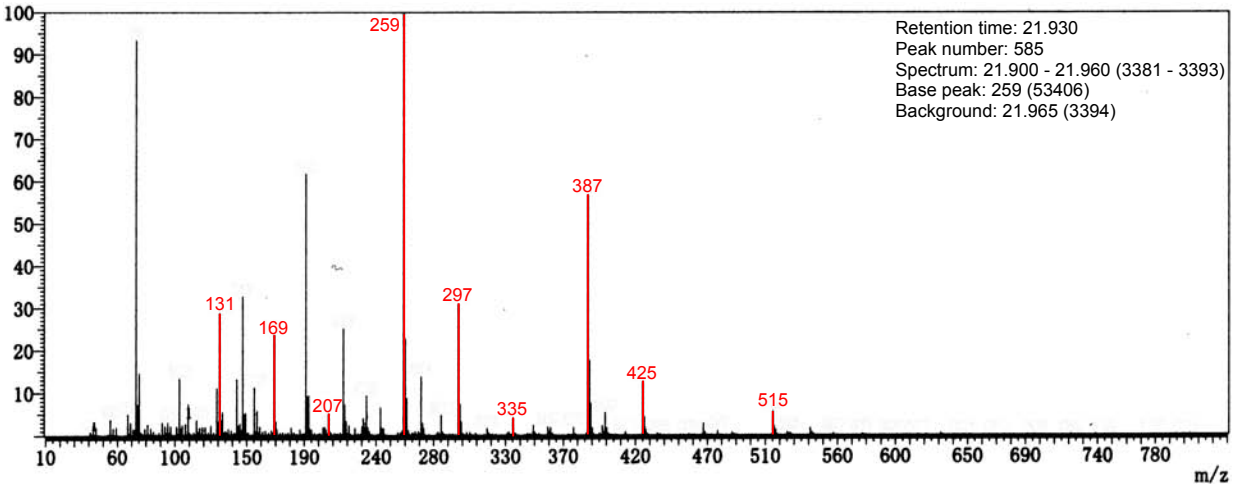


Trimethylsilyl derivative

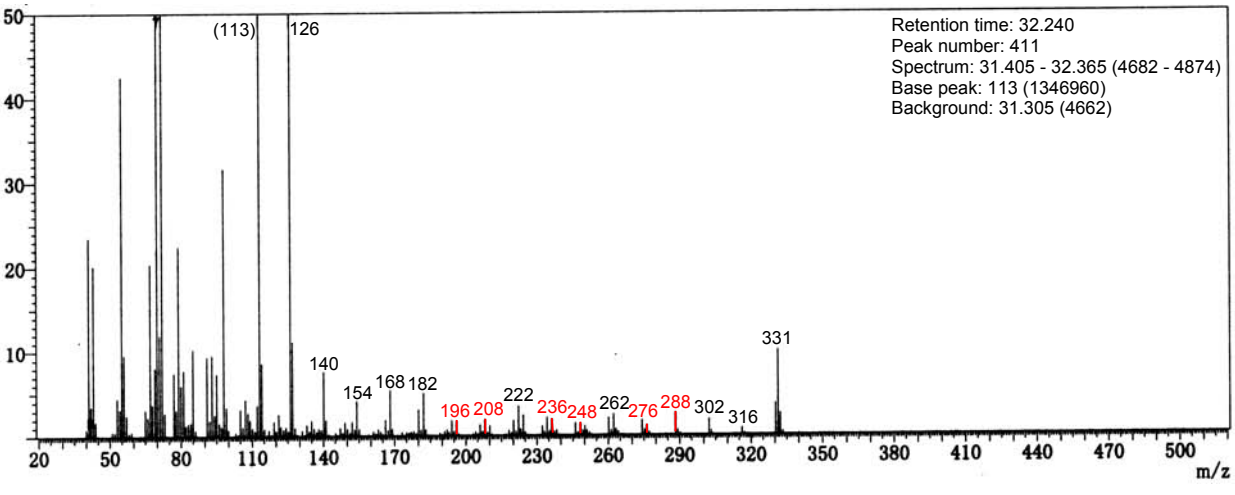
18:3 (9.12,15)





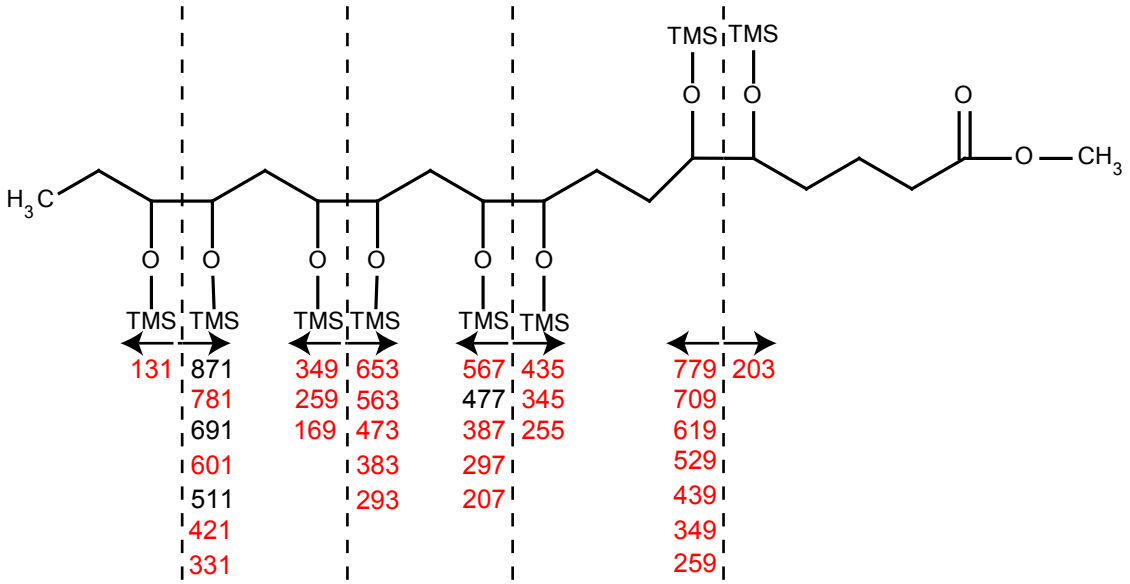


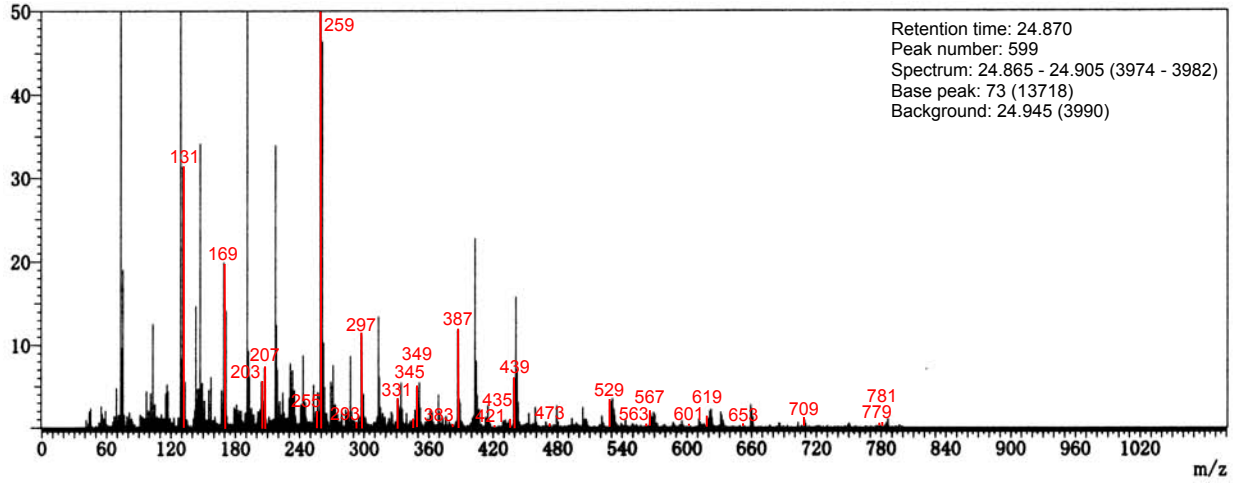
Pyrrolidide derivative



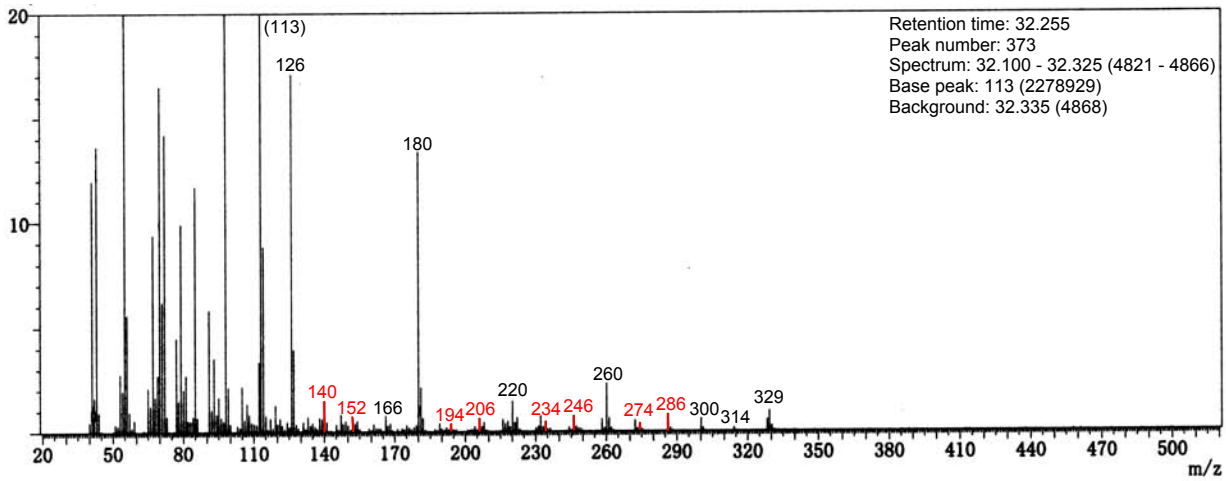
Trimethylsilyl derivative

18:4 (5,9,12,15)





Pyrrolidide derivative

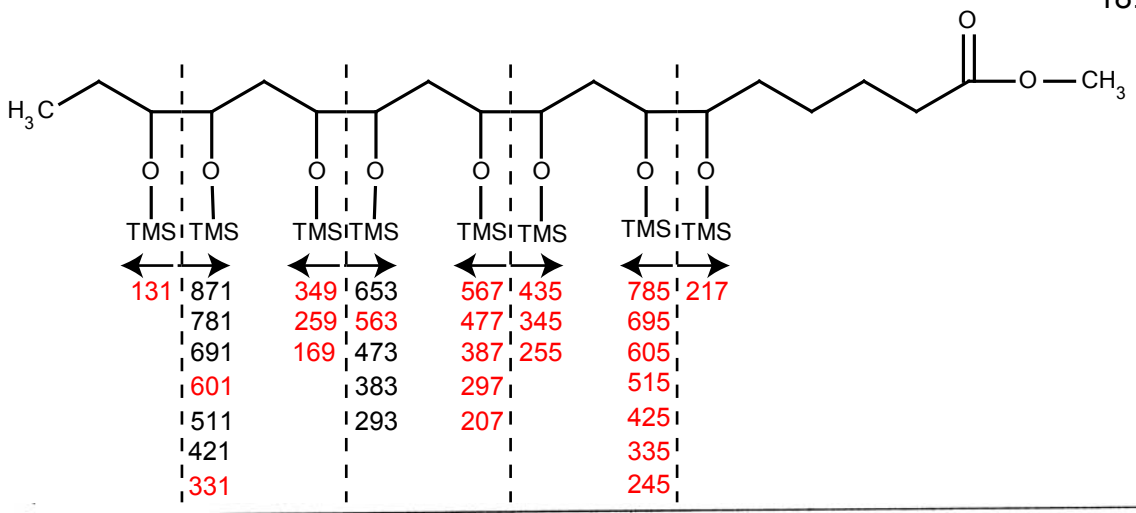


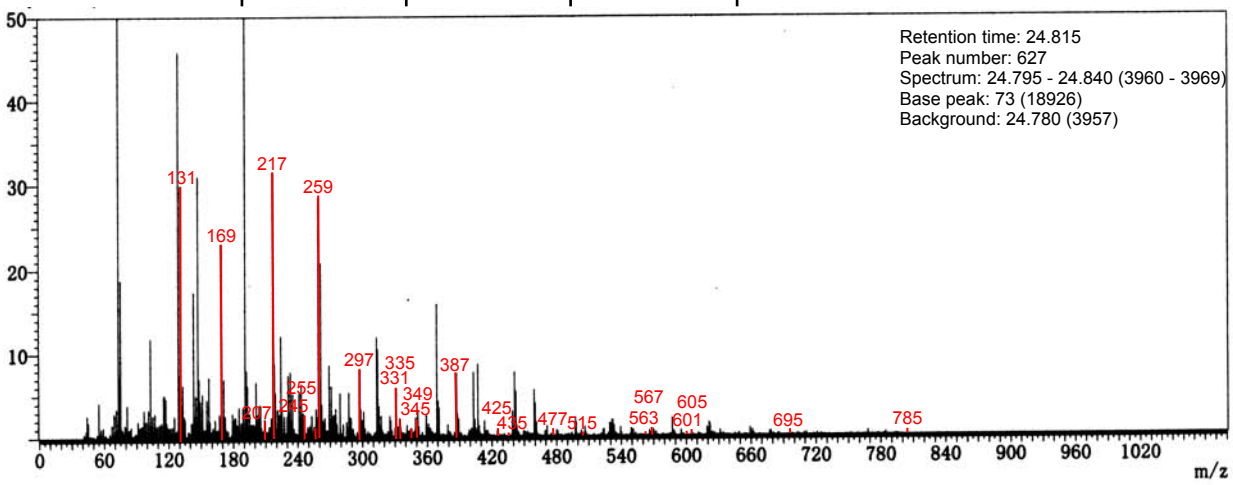
In the trimethylsilyl derivative of 18:4(5,9,12,15) and 18:4(6,9,12,15), the mass spectra of each derivative were obtained from the same peak of gas chromatogram.

In the pyrrolidide derivative of 18:4(5,9,12,15) and 18:4(6,9,12,15), the mass spectra of each derivative were obtained from the same peak of gas chromatogram.

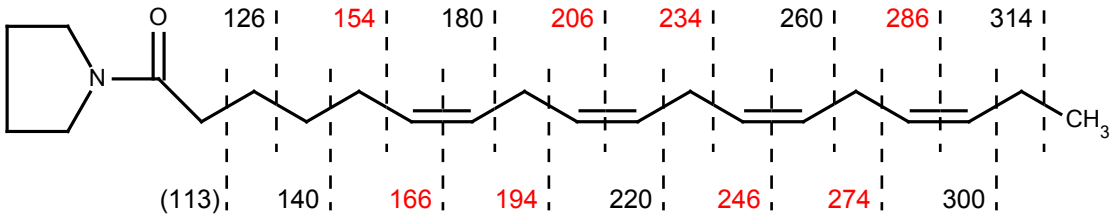
Trimethylsilyl derivative

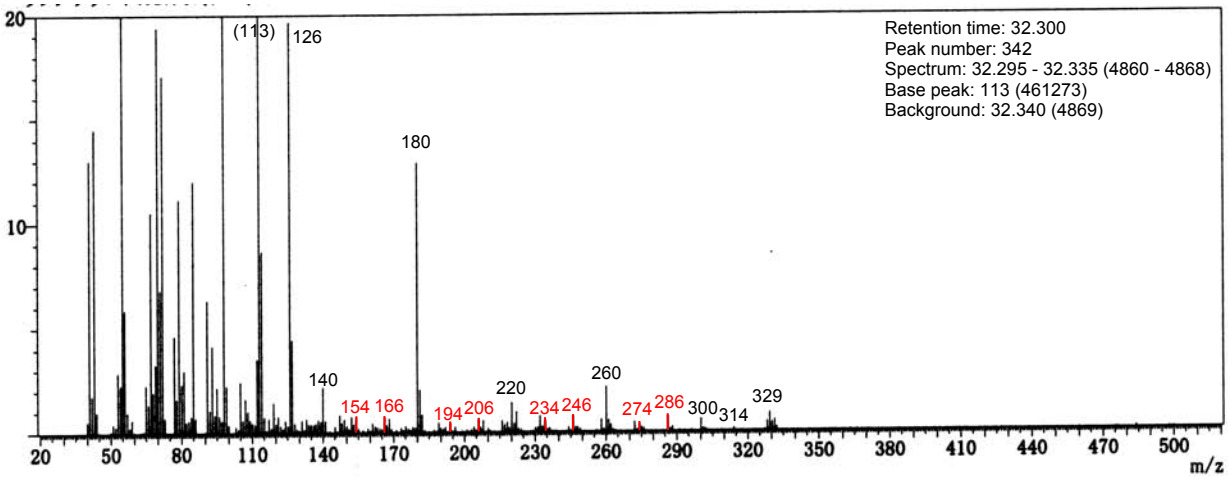
18:4 (6,9,12,15)





Pyrrolidide derivative



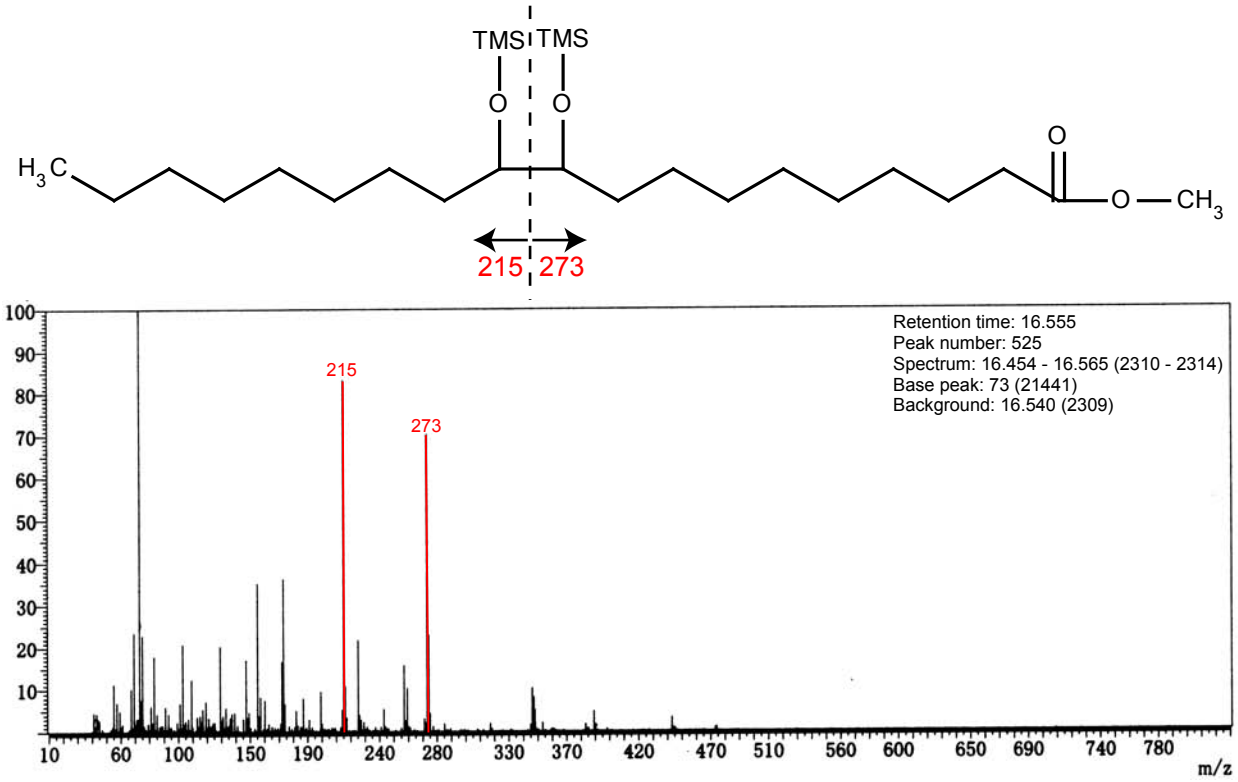


In the trimethylsilyl derivative of 18:4(5,9,12,15) and 18:4(6,9,12,15), the mass spectra of each derivative were obtained from the same peak of gas chromatogram.

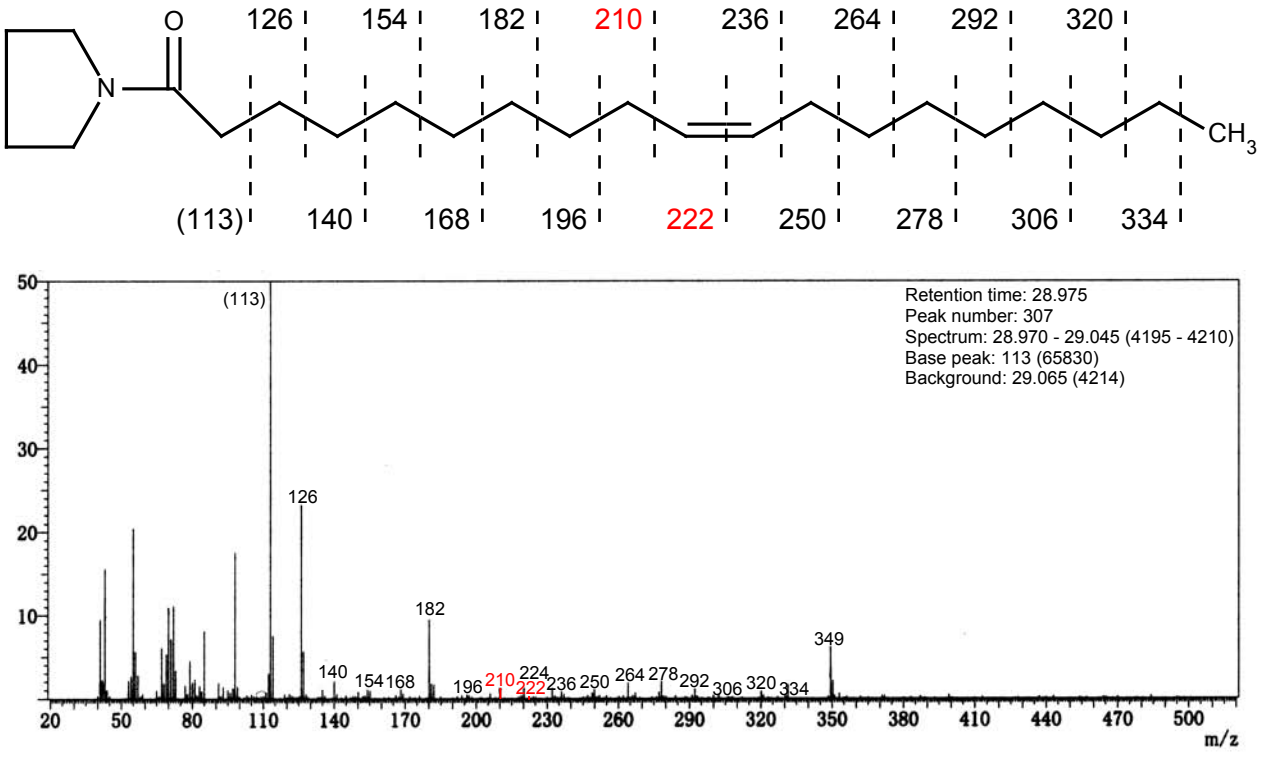
In the pyrrolidide derivative of 18:4(5,9,12,15) and 18:4(6,9,12,15), the mass spectra of each derivative were obtained from the same peak of gas chromatogram.

Trimethylsilyl derivative

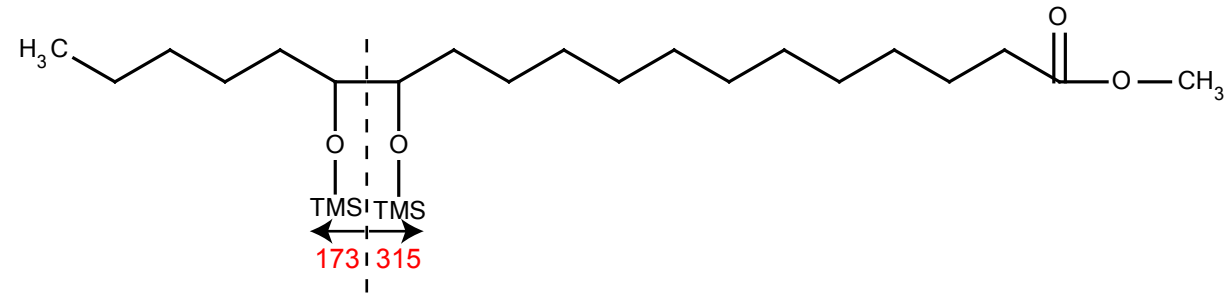
19:1 (10)

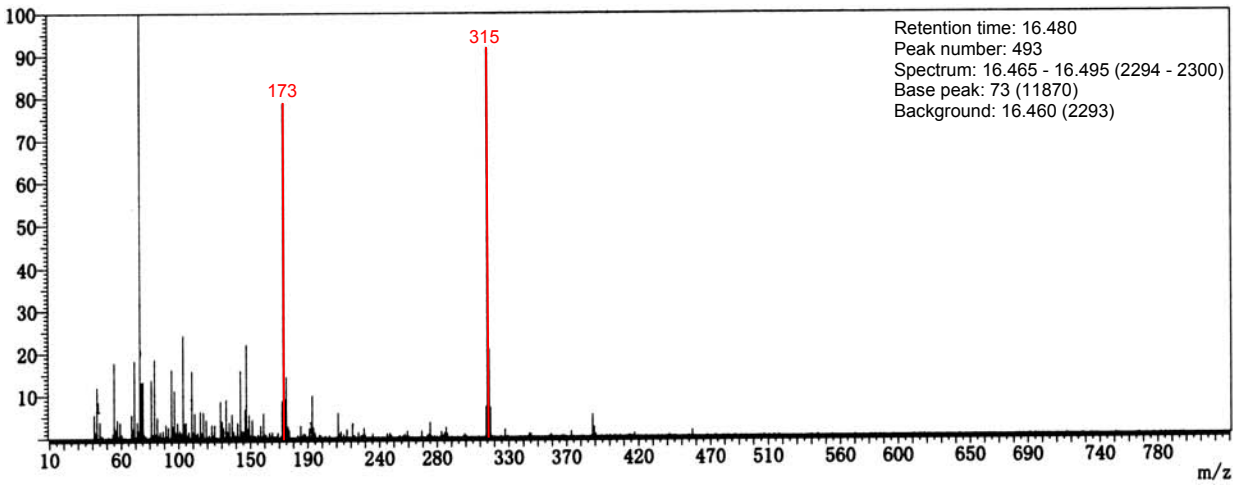


Pyrrolidide derivative

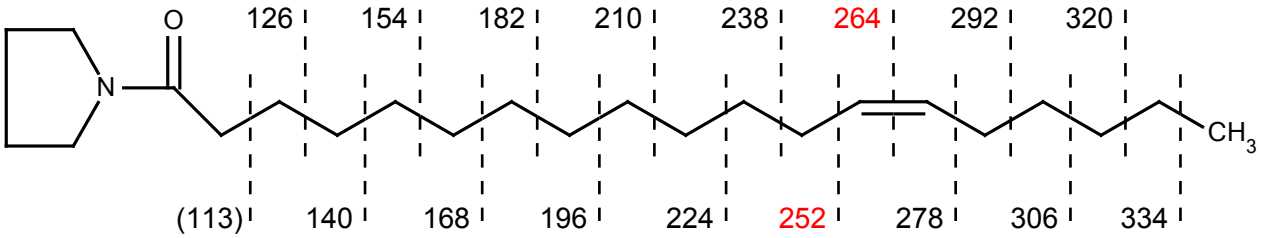


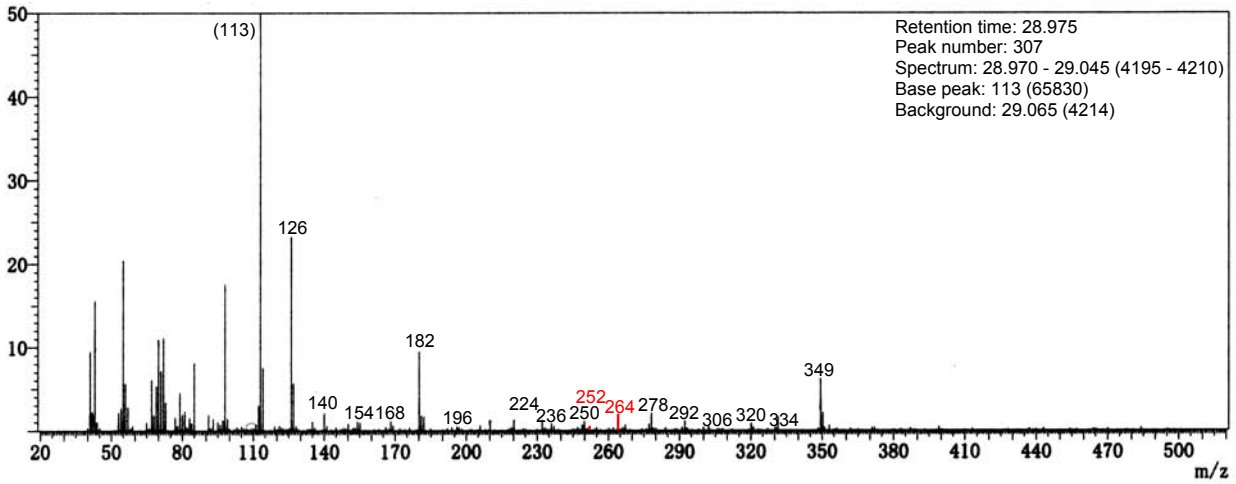
In the pyrroridide derivatives of 19:1(10) and 19:1(13), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.



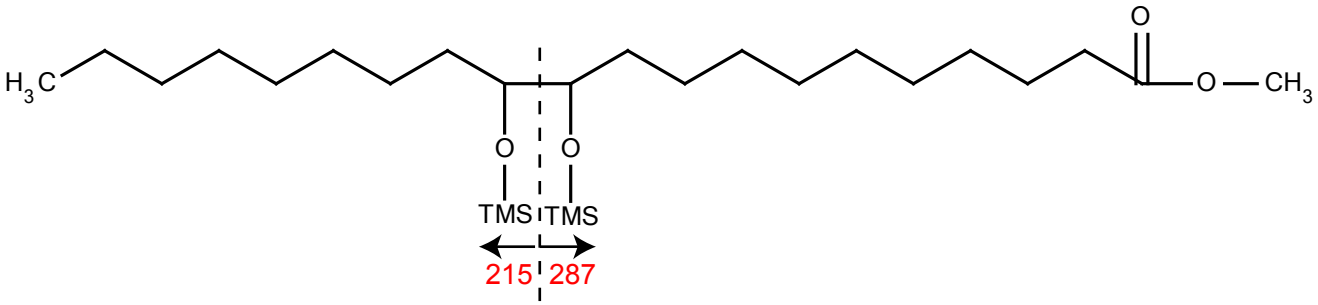


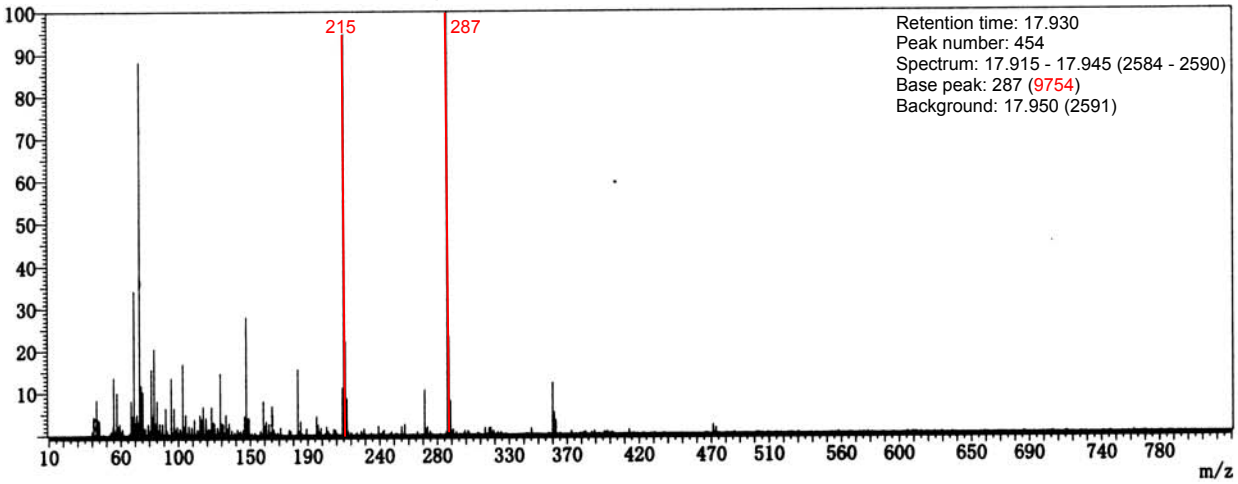
Pyrrolidide derivative



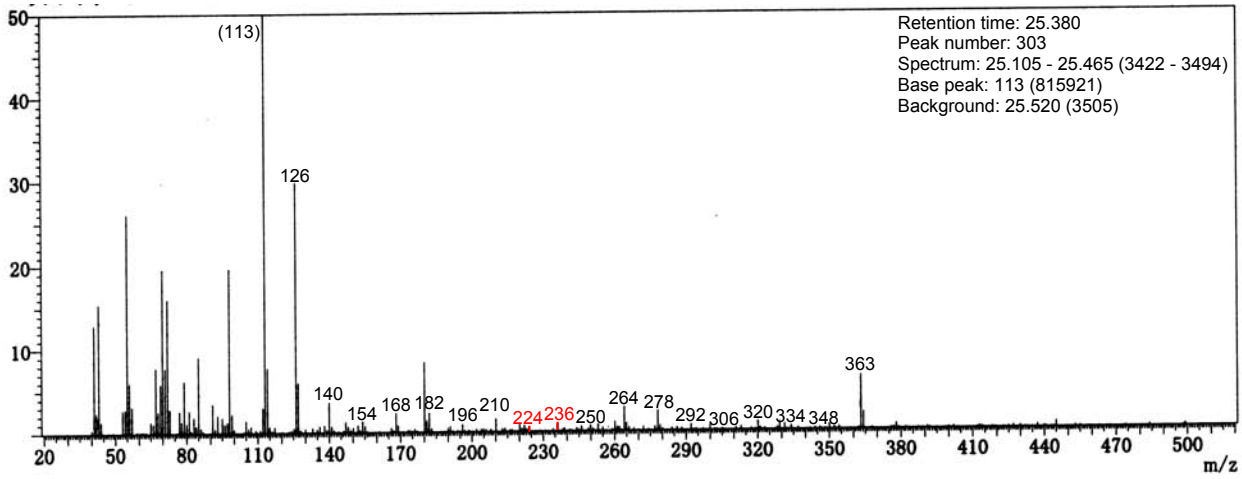


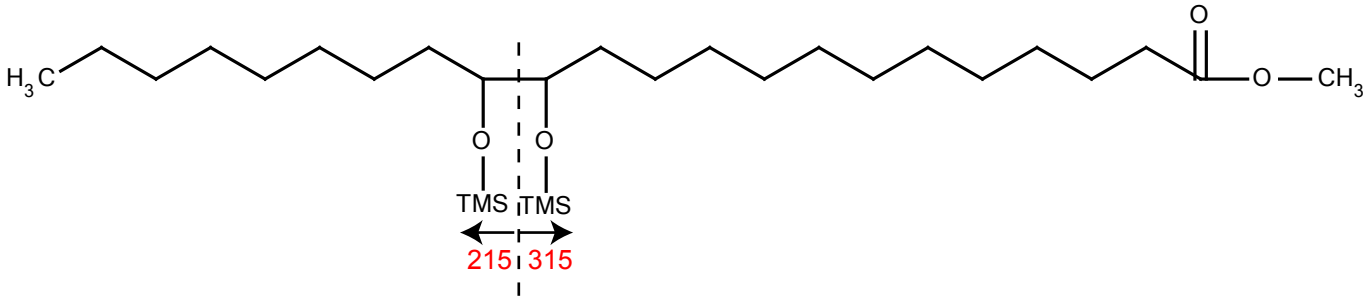
In the pyrroridide derivatives of 19:1(10) and 19:1(13), the mass spectrum of these pyrrolidide derivatives was obtained from the same peak of gas chromatogram.

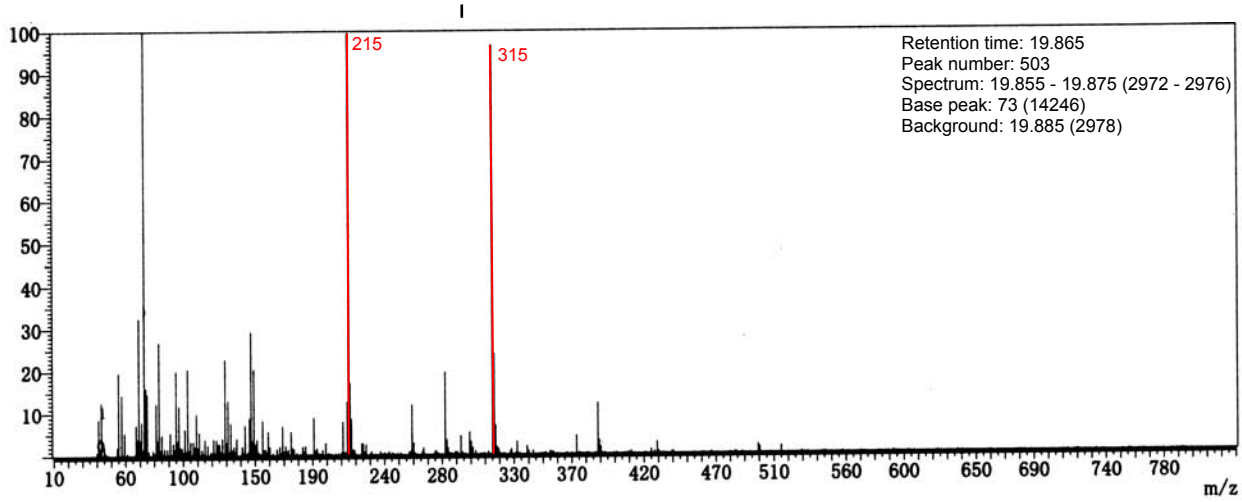




Pyrrolidide derivative







Pyrrolidide derivative