Biotransformation of neuro-inflammation inhibitor Kellerin by *Angelica sinensis* (Oliv.) Diels callus

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Spectroscopic data of known compounds

1. (3′*S*, 5′*S*, 8′*R*, 9′*S*, 10′*R*)-deacetylkellerin (**5**)

White crystal (actone). MS $[M + Na]^+$ *m/z* 423.4 ($C_{24}H_{32}NaO_5$), ^1H NMR (400 MHz, CD_3OD): δ (ppm) 7.88 (1H, d, *J* = 9.5 Hz, H-4), 7.52 (1H, d, *J* = 8.4 Hz, H-5), 6.95 (2H, m, H-6, 8), 6.23 (1H, d, *J* = 9.5 Hz, H-3), 4.32 (1H, dd, *J* = 10.4, 3.2 Hz, H-11'a), 4.05 (1H, dd, *J* = 10.4, 3.1 Hz, H-11'b), 3.64 (1H, t, *J* = 2.6 Hz, H-3'), 1.37 (3H, s, H-15'), 1.27 (3H, s, H-12'), 0.97 (3H, s, H-13'), 0.88 (3H, s, H-14').

2. ferukrin (**6**)

White crystal (actone). MS $[M + Na]^+$ *m/z* 423.4 ($C_{24}H_{32}NaO_5$), ^1H NMR (400 MHz, CD_3OD): δ (ppm) 7.89 (1H, d, *J* = 9.6 Hz, H-4), 7.54 (1H, br.d, *J* = 8.4 Hz, H-5), 6.90 (2H, m, H-6, 8), 6.25 (1H, d, *J* = 9.6 Hz, H-3), 4.19 (1H, dd, *J* = 10.4, 3.2 Hz, H-11'a), 4.18 (1H, dd, *J* = 10.4, 3.2 Hz, H-11'b), 3.09 (1H, dd, *J* = 11.2, 4.8 Hz, H-3'), 1.34 (3H, s, H-15'), 1.27 (3H, s, H-12'), 1.03 (3H, s, H-13'), 0.82 (3H, s, H-14').

3. gummosin (**7**)

White crystal (actone). MS $[M + Na]^+$ *m/z* 405.4 ($C_{24}H_{30}NaO_4$), ^1H NMR (400 MHz, CD_3OD): δ (ppm) 7.88 (1H, d, *J* = 9.6 Hz, H-4), 7.51 (1H, d, *J* = 8.8 Hz, H-5), 6.96 (1H, d, *J* = 2.4 Hz, H-8), 6.90 (1H, d, *J* = 8.8, 2.4 Hz, H-6), 6.23 (1H, d, *J* = 9.6 Hz, H-3), 4.80 (1H, br. s, H-12'a), 4.70 (1H, br. s, H-12'b), 4.45 (1H, dd, *J* = 10.0, 5.4 Hz, H-11'a), 4.13 (1H, dd, *J* = 10.0, 6.9 Hz, H-11'b), 3.41 (1H, br. s, H-3'), 1.78-1.48 (9H, m, H-1', 2', 5', 6', 7') 1.04 (3H, s, H-15') 0.99 (3H, s, H-13'), 0.87 (3H, s, H-14').

4. 7-hydroxycoumarin (**8**)

Light yellow needle crystal (CH_2Cl_2-CH_3OH). MS $[M + Na]^+$ *m/z* 185.1 ($C_9H_6NaO_3$), $[M - H]^-$ *m/z* 160.9 ($C_9H_5O_3$), ^1H NMR (400 MHz, CD_3OD): δ (ppm) 7.84 (1H, d, *J* = 9.2 Hz, H-4), 7.44 (1H, d, *J* = 8.4 Hz, H-5), 6.78 (1H, dd, *J* = 2.4, 8.4 Hz, H-6), 6.69 (1H, d, *J* = 2.4 Hz, H-8), 6.17 (1H, d, *J* = 9.2 Hz, H-3).

5. ferulic acid (**9**)

White crystal (CH_2Cl_2-CH_3OH). MS $[M + Na]^+$ *m/z* 217.3 ($C_{10}H_{10}NaO_4$); $[M - H]^-$ *m/z* 192.9 ($C_{10}H_9O_4$), ^1H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.50 (1H, d, *J* = 15.9 Hz, H-7), 7.28 (1H, d, *J* = 1.8 Hz, H-2), 7.08 (1H, dd, *J* = 8.0, 1.8 Hz, H-6), 6.79 (1H, d, *J* = 8.0 Hz, H-5), 6.37 (1H, d, *J* = 15.9 Hz, H-8), 3.81 (3H, s, -OCH_3).

6. bis (2-ethylhexyl) phthalate (**10**)

Colorless oil (CH_3OH). MS $[M + Na]^+$ *m/z* 413.5 ($C_{24}H_{38}NaO_4$), ^1H NMR (400 MHz, CD_3OD): δ (ppm) 7.71 (2H, m, H-3,6), 7.62 (2H, m, H-4,5), 4.22 (4H, dd, *J* = 2.8, 5.5 Hz, H-1',1"), 1.43-1.29 (8H, m), 0.94 (6H, t, *J* = 7.5 Hz, H-2', 2"), 0.90 (6H, t, *J* = 7.8 Hz, H-6', 6"); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 169.5 (C=O), 133.7 (C-1, 2), 132.6 (C-4, 5), 130.0 (C-3, 6), 69.3 (C-1', 1"), 40.3 (C-2', 2"), 31.8 (C-3', 3"), 30.3 (C-5', 5"), 25.1 (C-4', 4"), 14.6 (CH_3-2', 2"), 11.6 (CH_3-6', 6").

7. 3-heptyl-3-hydroxy-2,4 (1*H*, 3*H*)-quinolinediones (**11**)

Yellow solid (CH_3OH). MS $[M + Na]^+$ *m/z* 298.3 ($C_{16}H_{21}NaNO_3$), ^1H NMR (600 MHz, DMSO-d_6): δ (ppm) 10.76 (s, 1H), 7.70 (1H, dd, *J* = 1.2, 7.2 Hz, H-5), 7.58 (1H, td, *J* = 1.2, 7.2 Hz, H-6), 7.10 (1H, td, *J* = 1.2, 7.2 Hz, H-7), 7.05 (1H, d, *J* = 7.2 Hz, H-8), 5.66 (1H, s, NH), 1.66 (2H, m, H-1'), 1.23-1.36 (10H, m), 0.80 (3H, t, *J* = 7.0 Hz, H-6').

8. 7-methoxy-1-methyl-9*H*-pyrido[3,4-*b*]indole (**12**)

Yellow solid (CH_3OH). MS $[M + H]^+$ *m/z* 213.3 ($C_{13}H_{13}N_2O$), ^1H NMR (600 MHz, CD_3OD): δ (ppm) 8.01 (1H, d, *J* = 5.4 Hz, H-3), 7.99 (1H, d, *J* = 8.7 Hz, H-5), 7.80 (1H, d, *J* = 5.4 Hz, H-4), 7.05 (1H, d, *J* = 2.1 Hz, H-8), 6.86 (1H, dd, *J* = 2.1, 8.7 Hz, H-6), 3.91 (3H, s, 7-OCH_3), 2.77 (3H, s); ^{13}C NMR (150 MHz, methanol-d_4): δ (ppm) 162.7 (C-7), 144.5 (C-1), 142.4 (C-13), 138.1 (C-3), 130.8 (C-13), 130.3 (C-11), 123.7 (C-5), 116.6 (C-12), 113.5 (C-4), 111.2 (C-6), 95.6 (C-8), 56.1 (CH_3O), 19.6

(CH_3).

9. methyl butyrate (**13**)

Yellow solid (CH_3OH). MS $[M - H]^-$ *m/z* 101.0 ($C_5H_9O_2$), ^1H NMR (400 MHz, CD_3OD): δ (ppm) 3.65 (3H, s, OCH_3), 2.32 (2H, t, J = 7.2 Hz, H-2), 1.60 (2H, m, H-3), 0.92 (3H, t, J = 6.4 Hz, H-4).

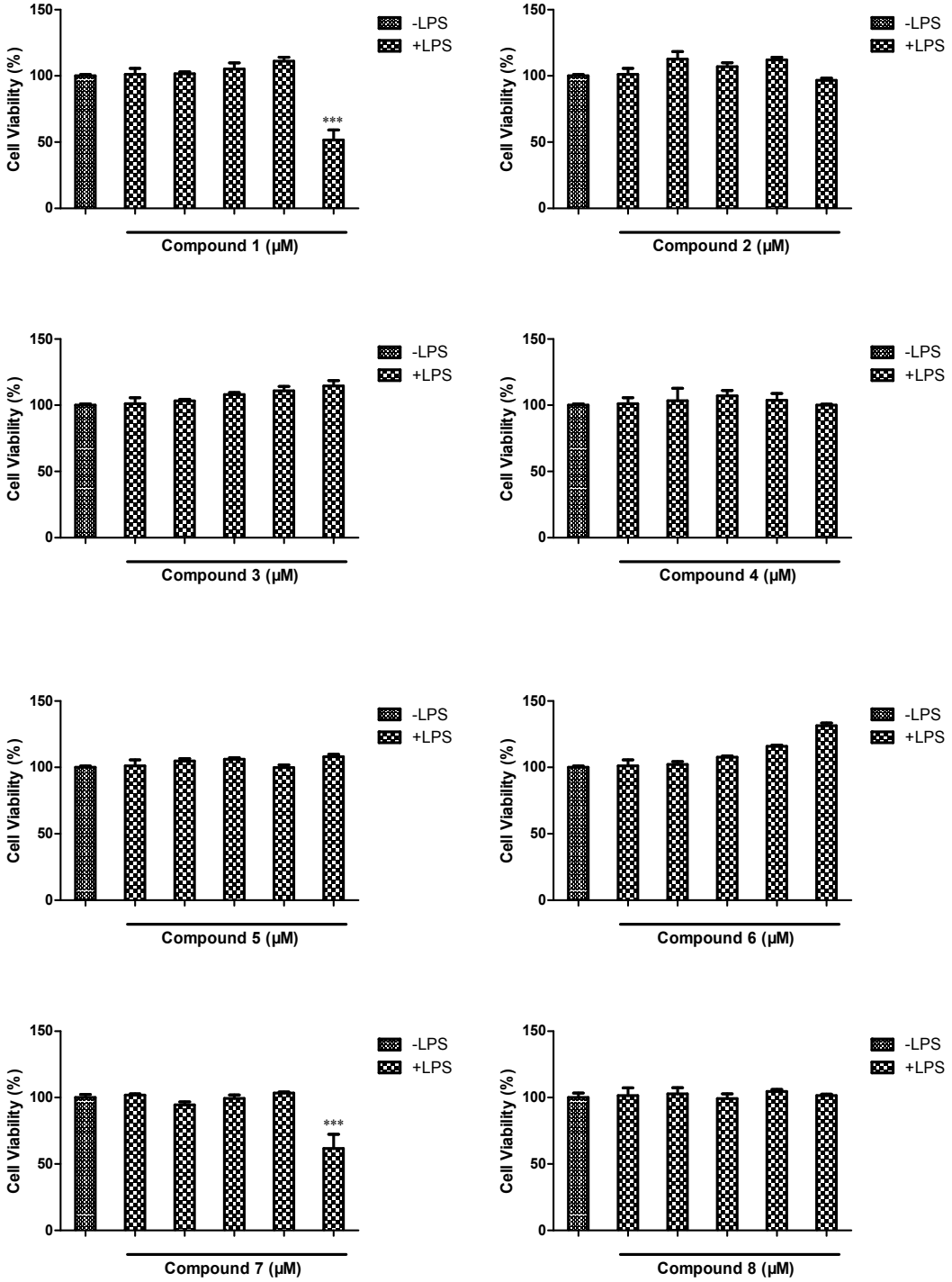
10. (*Z*)-3-methyl-4-oxobut-2-enoic acid (**14**)

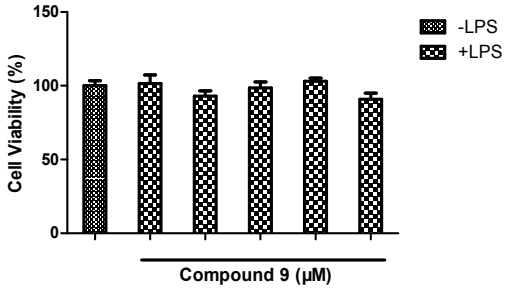
White needles (CH_3OH). MS $[M + Na]^+$ *m/z* 137.0 ($C_5H_6NaO_3$); $[M - H]^-$ *m/z* 112.9 ($C_5H_5NO_3$), ^1H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.98 (1H, s, COOH), 10.72 (1H, s, CHO), 7.26 (1H, s, H-2), 1.72 (3H, s, H-3); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 174.6 (CHO), 161.1 (COOH), 147.4 (C-2), 117.3 (C-3), 21.5 (CH_3).

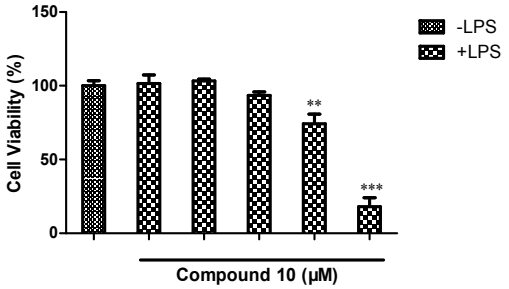
11. Substrate: (3′*S*, 5′*S*, 8′*R*, 9′*S*, 10′*R*)-kellerin

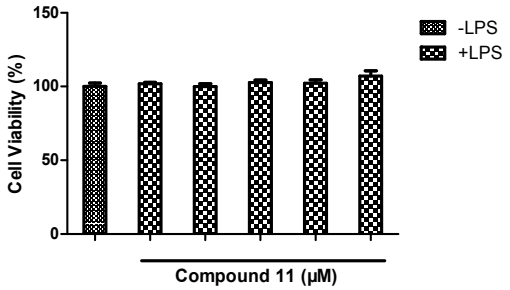
Yellow oil (CH_3OH). $[M + Na]^+$ *m/z* 465.4 ($C_{26}H_{34}NaO_5$), ^1H-NMR (400 MHz, CD_3OD): δ (ppm) 7.89 (1H, d, J = 9.5 Hz, H-4), 7.35 (1H, d, J = 8.5 Hz, H-5), 6.83 (1H, dd, J = 8.5, 2.4 Hz, H-6), 6.80 (1H, d, J = 2.2 Hz, H-8), 6.22 (1H, d, J = 9.5 Hz, H-3), 5.27 (1H, s, -OH), 4.60 (1H, br. s, H-3'), 4.14 (2H, d, J = 2.6 Hz, H-11'), 1.75 (3H, s, $-COCH_3$), 1.73 (1H, m, H-5'), 1.50 (1H, m, H-9'), 1.33 (3H, s, H-15'), 1.29 (3H, s, H-12'), 0.90 (3H, s, H-14'), 0.87 (3H, s, H-13').

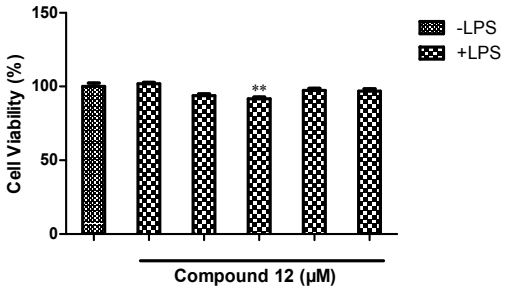
Fig. 1S BV2 cell viability assay of transformed products and substrate on LPS-induced NO production in BV2 microglial cells (Each bar represents the means ± SE of three independent experiments. Significance: **P*<0.05, ***P*<0.01, ****P*<0.001 compared to LPS groups. $^{###}P$<0.001 compared to control groups. LPS: lipopolysaccharide).

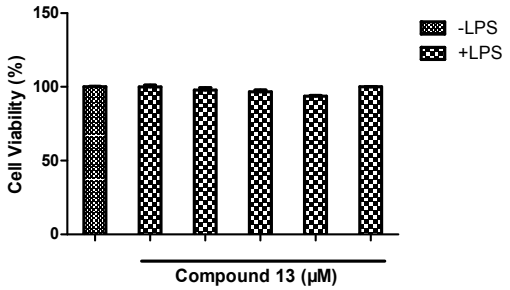


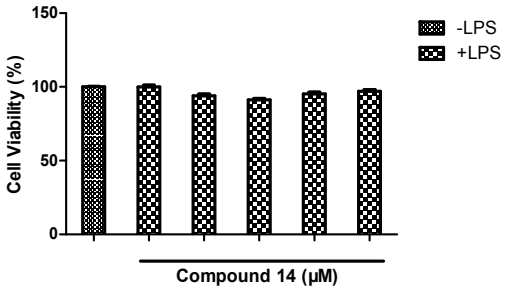

Cell Viability (%)
150
100
50
0
-LPS
+LPS
Compound 9 (μM)

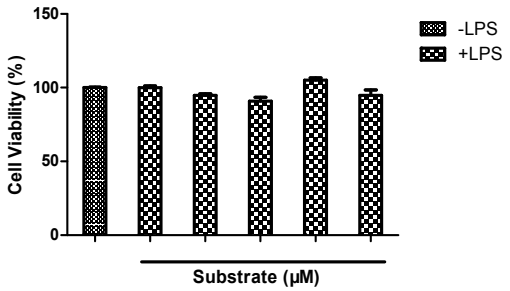

Cell Viability (%)
150
100
50
0
**
***
-LPS
+LPS
Compound 10 (μM)

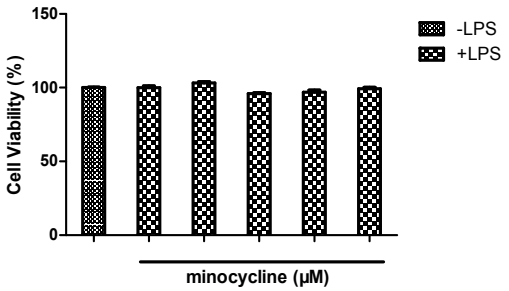

Cell Viability (%)
150
100
50
0
-LPS
+LPS
Compound 11 (μM)


Cell Viability (%)
150
100
50
0
**
-LPS
+LPS
Compound 12 (μM)

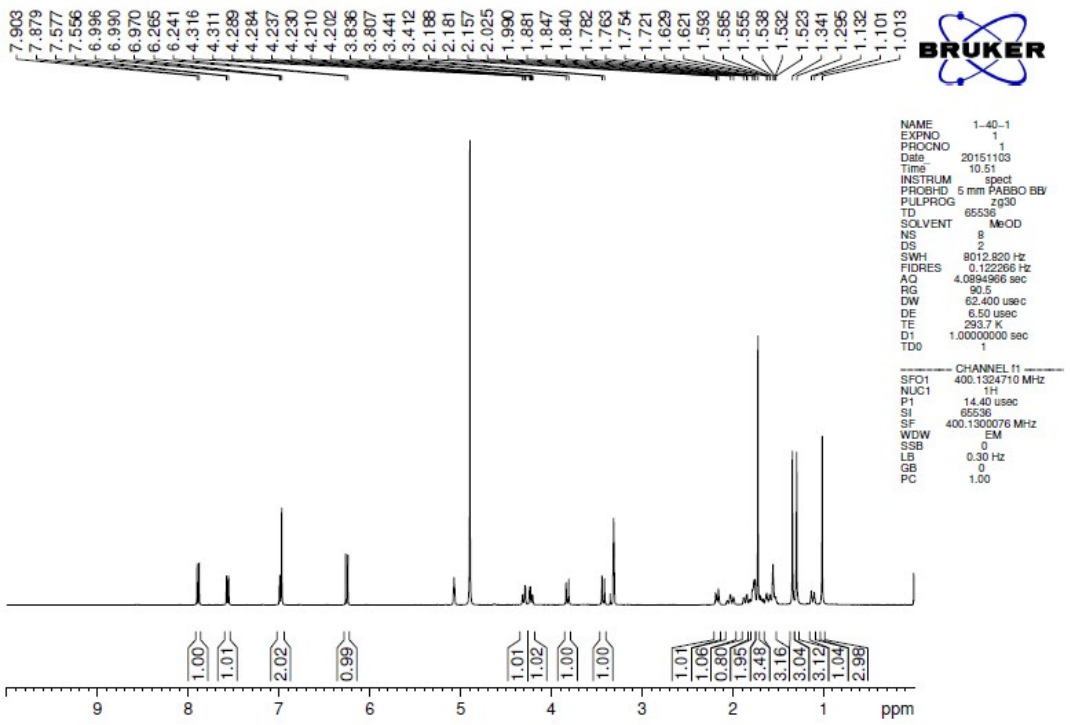

Cell Viability (%)
150
100
50
0
-LPS
+LPS
Compound 13 (μM)


Cell Viability (%)
150
100
50
0
-LPS
+LPS
Compound 14 (μM)

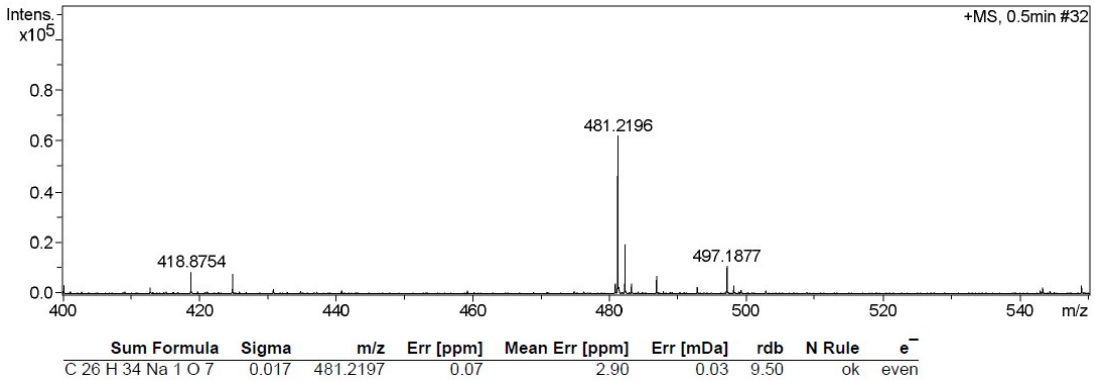

Cell Viability (%)
150
100
50
0
-LPS
+LPS
Substrate (μM)


Cell Viability (%)
150
100
50
0
-LPS
+LPS
minocycline (μM)

[1]H NMR and MS spectra of all products

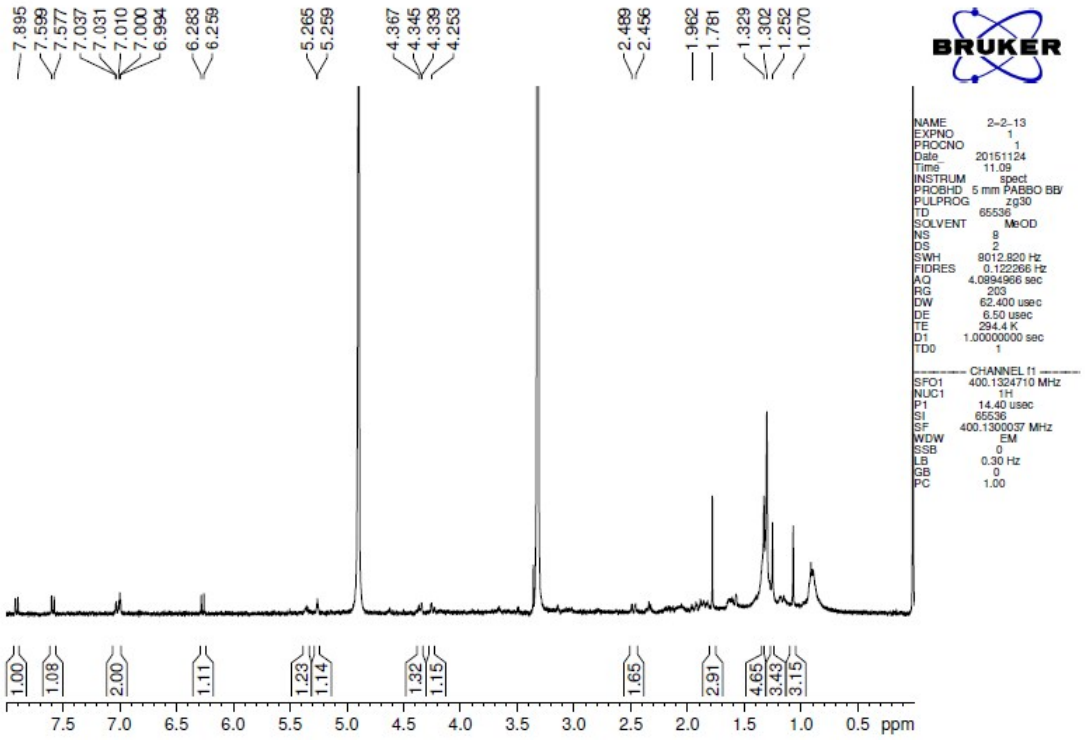


[1]H-NMR spectrum of compound **1**

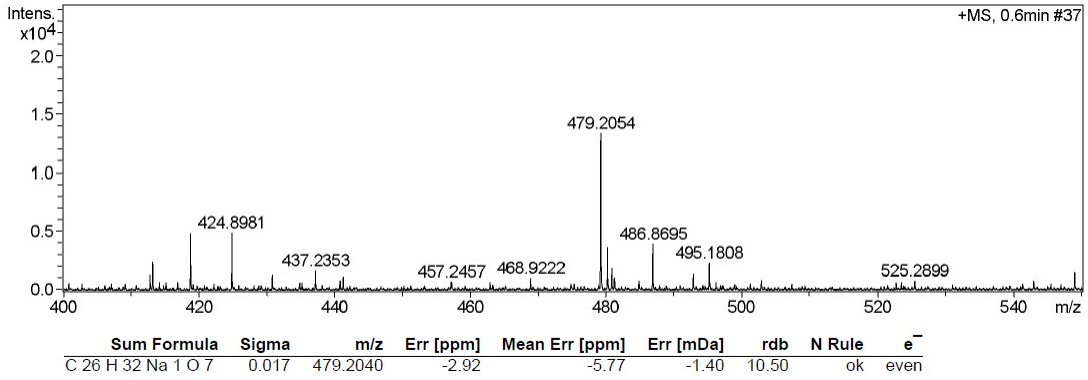


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 26 H 34 Na 1 O 7	0.017	481.2197	0.07	2.90	0.03	9.50	ok	even

HR-ESI-MS spectrum of compound **1**

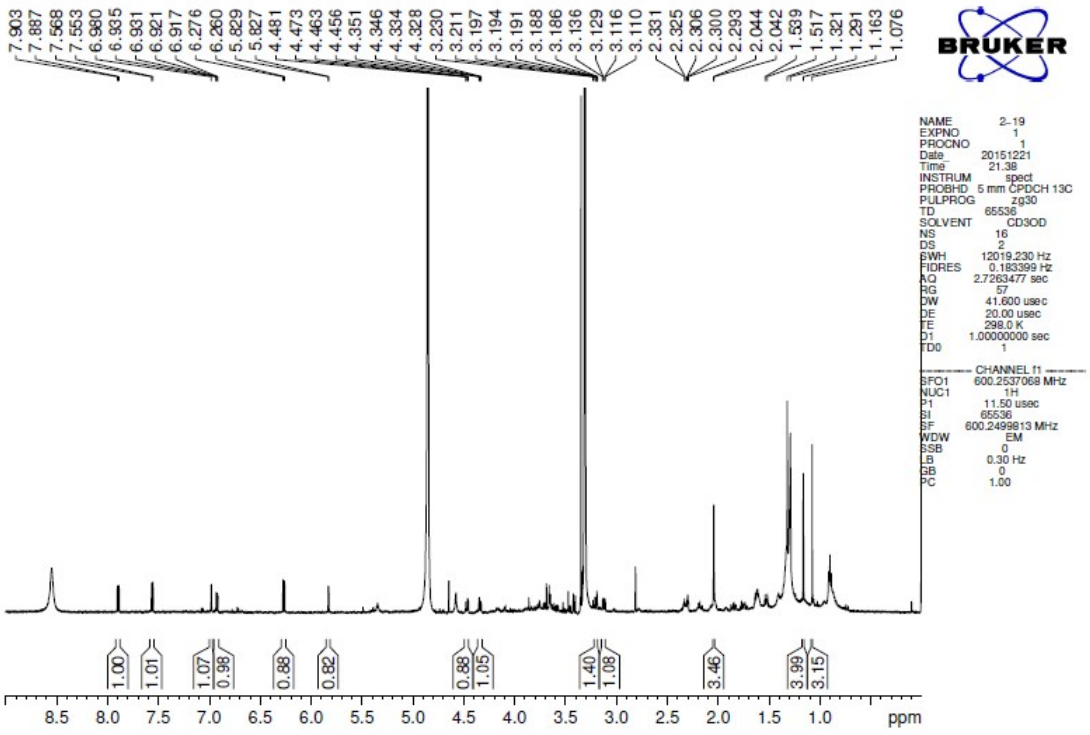


^{1}H-NMR spectrum of compound **2**

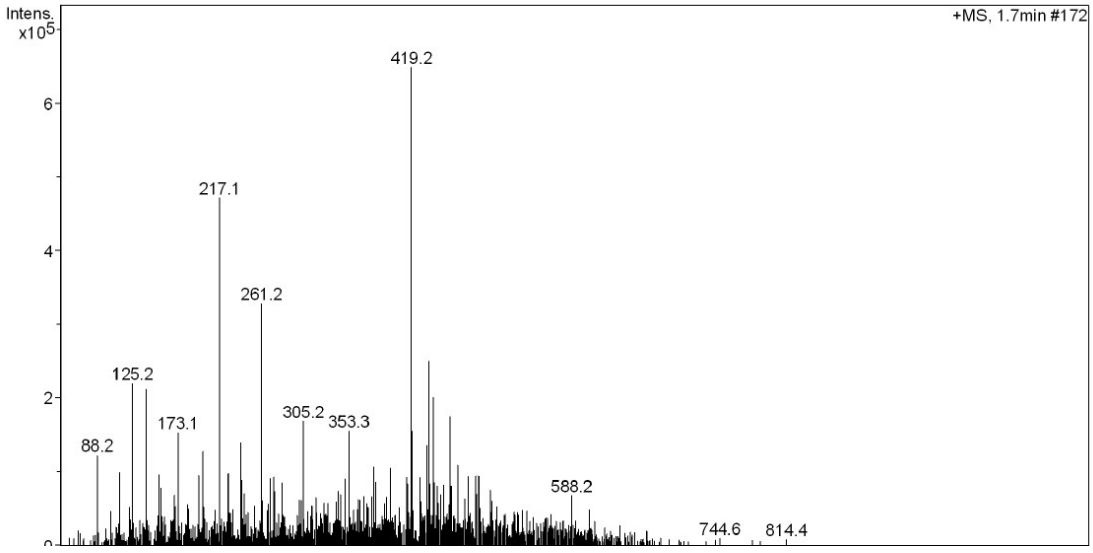


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 26 H 32 Na 1 O 7	0.017	479.2040	-2.92	-5.77	-1.40	10.50	ok	even

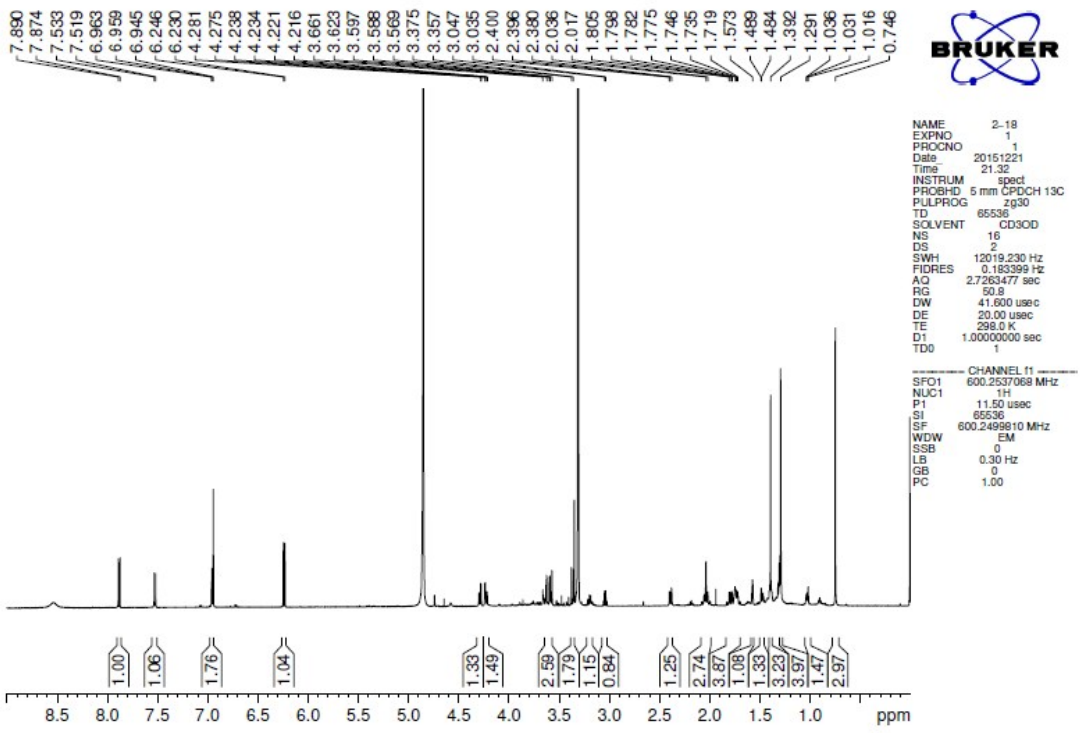
HR-ESI-MS spectrum of compound **2**



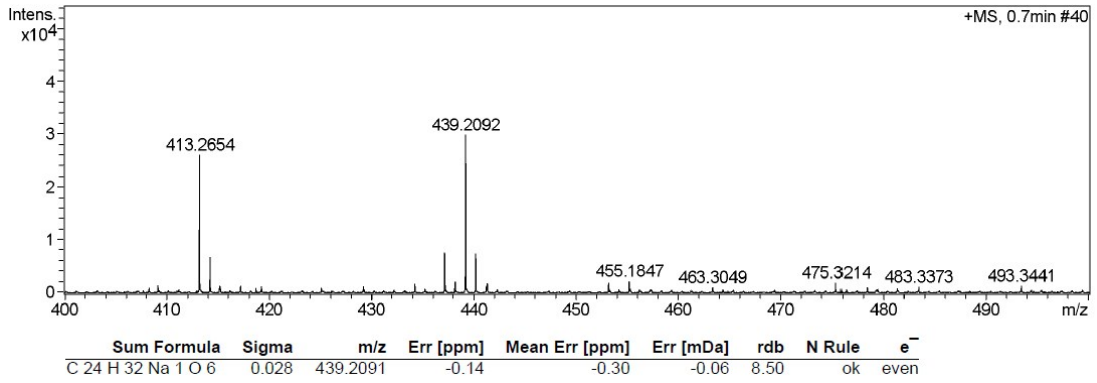
^{1}H-NMR spectrum of compound **3**



MS spectrum of compound **3**

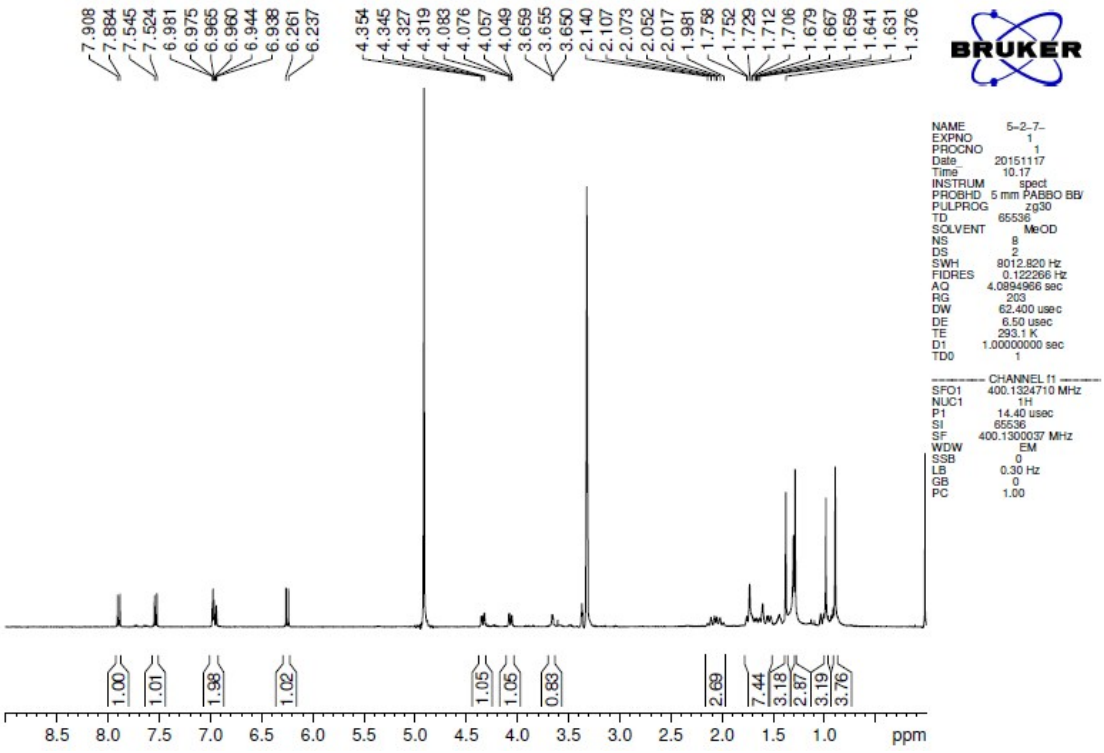


^{1}H-NMR spectrum of compound **4**

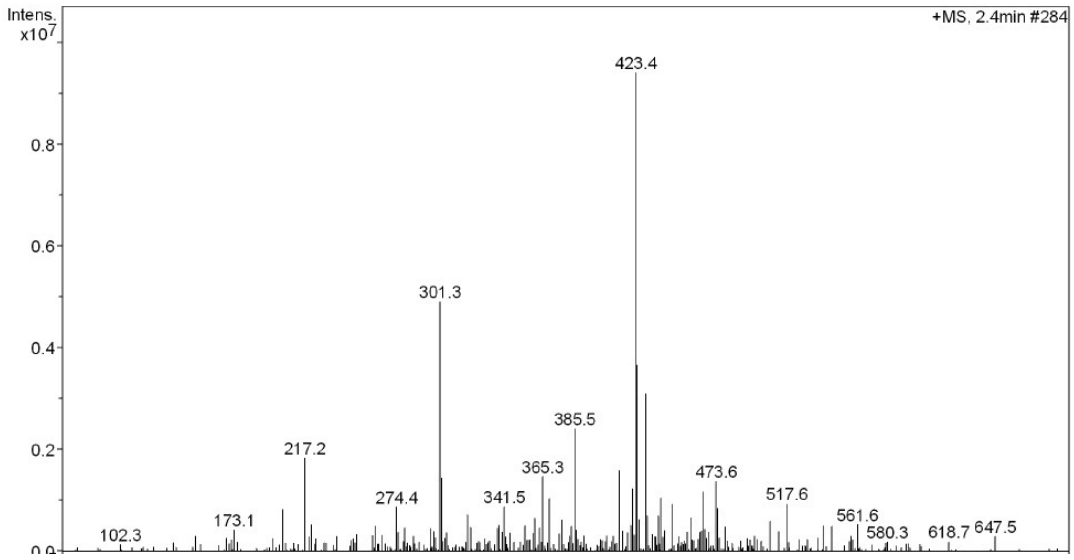


Sum Formula	Sigma	m/z	Err [ppm]	Mean Err [ppm]	Err [mDa]	rdb	N Rule	e⁻
C 24 H 32 Na 1 O 6	0.028	439.2091	-0.14	-0.30	-0.06	8.50	ok	even

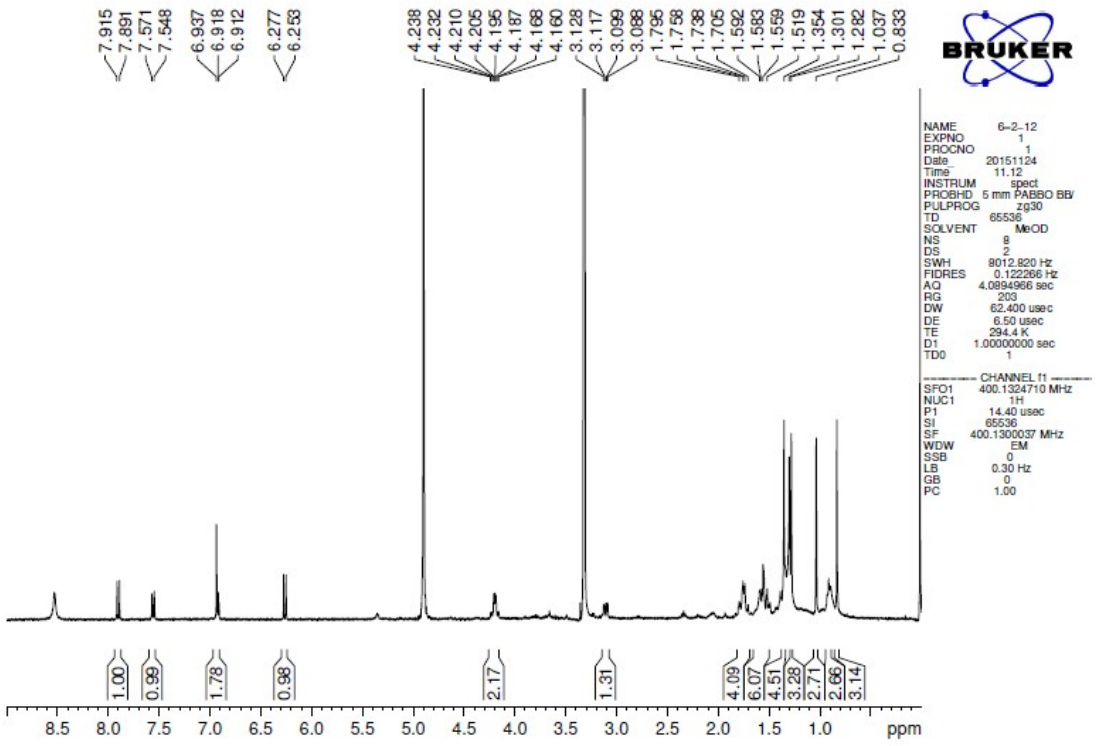
HR-ESI-MS spectrum of compound **4**



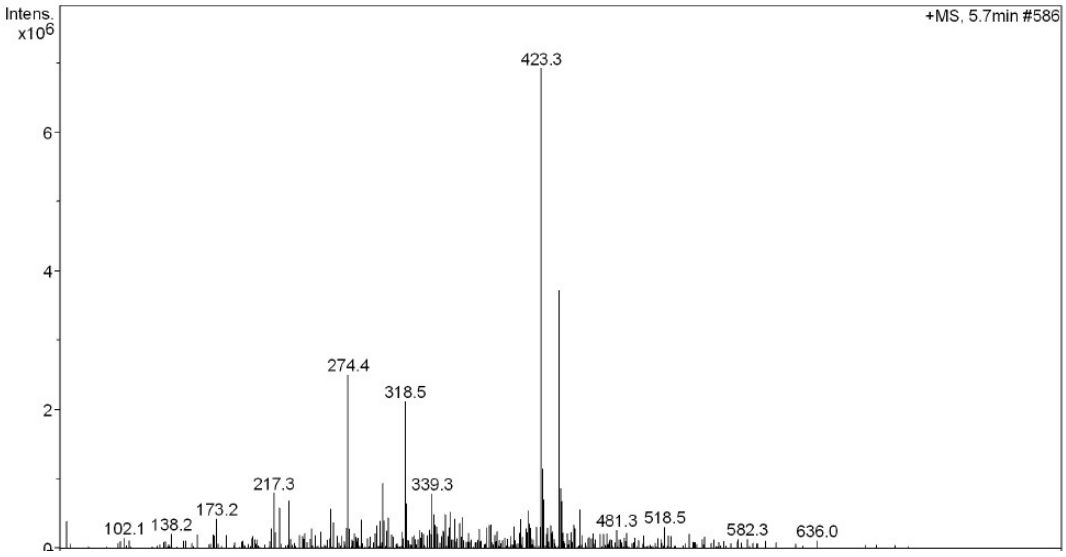
^{1}H-NMR spectrum of compound **5**



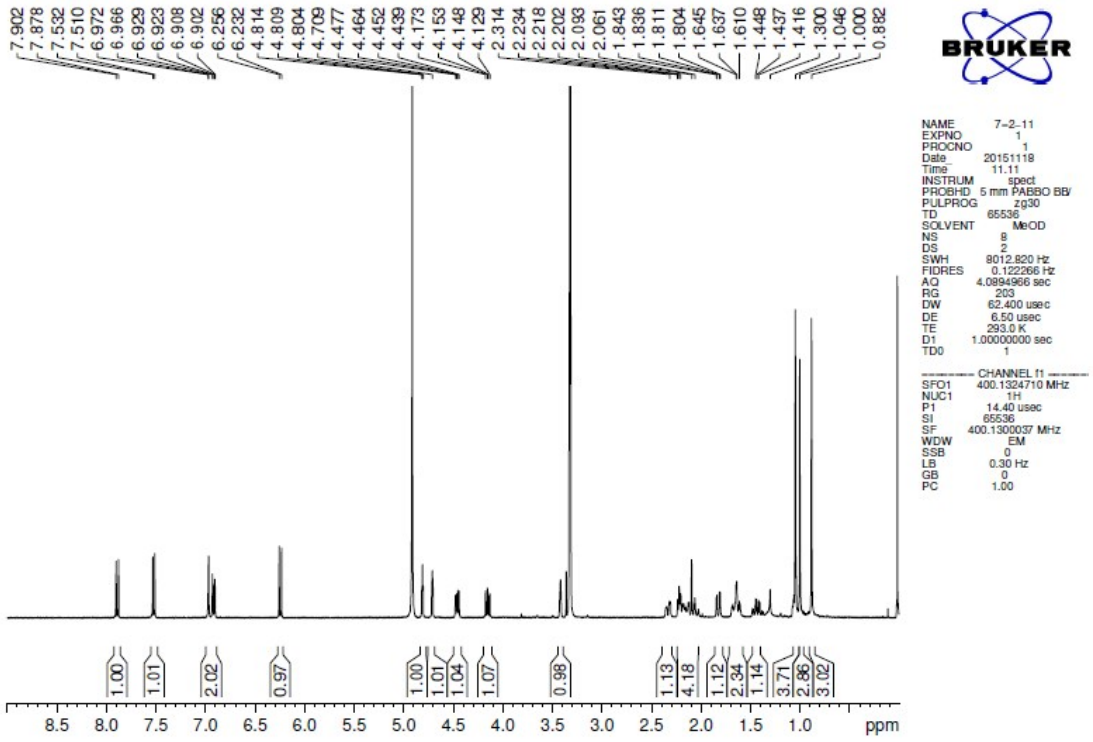
MS spectrum of compound **5**



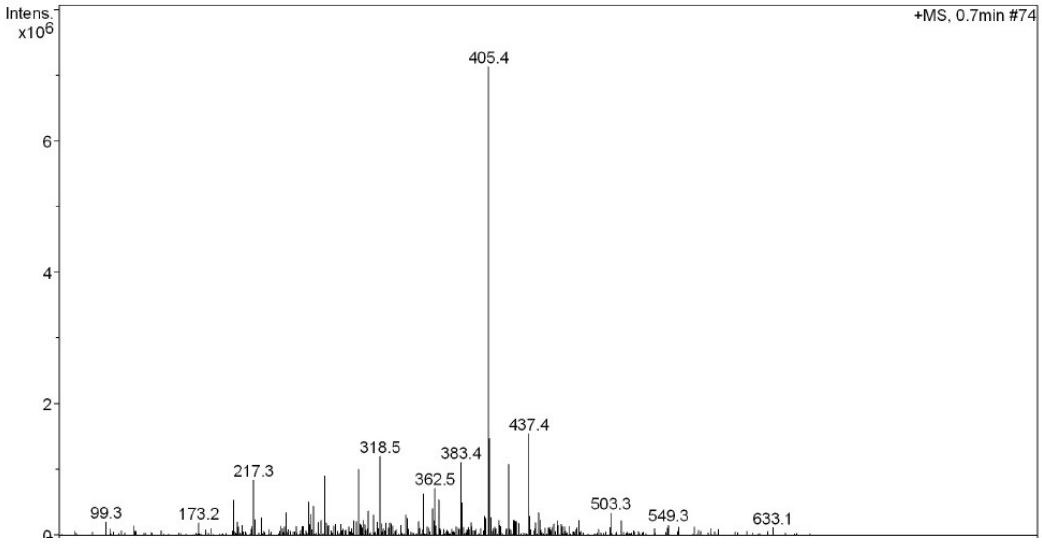
^{1}H-NMR spectrum of compound **6**



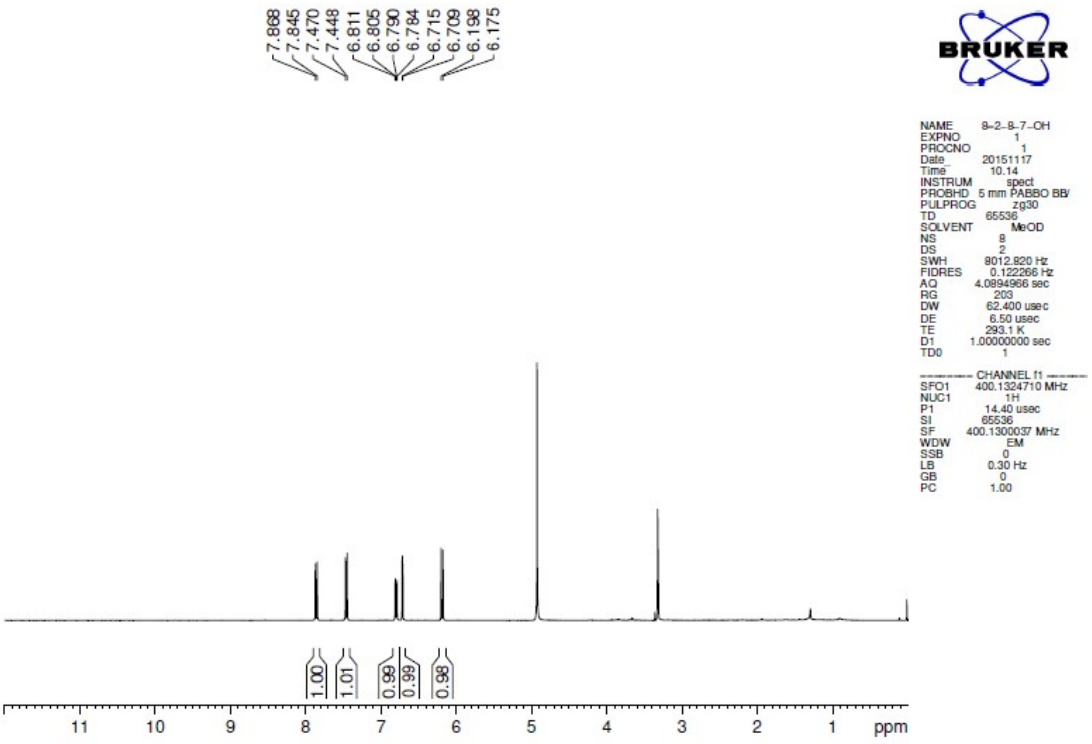
MS spectrum of compound **6**



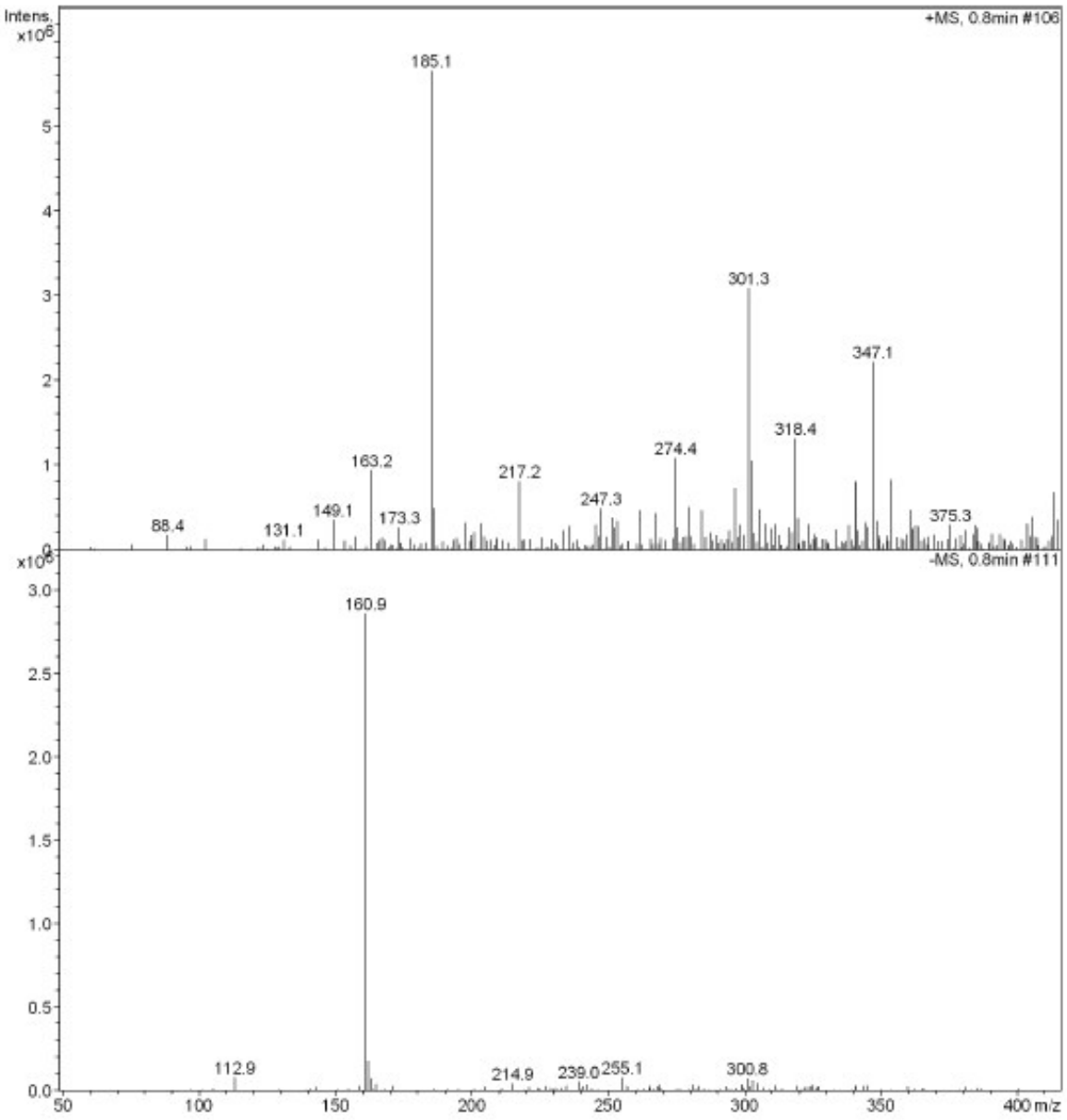
^{1}H-NMR spectrum of compound **7**



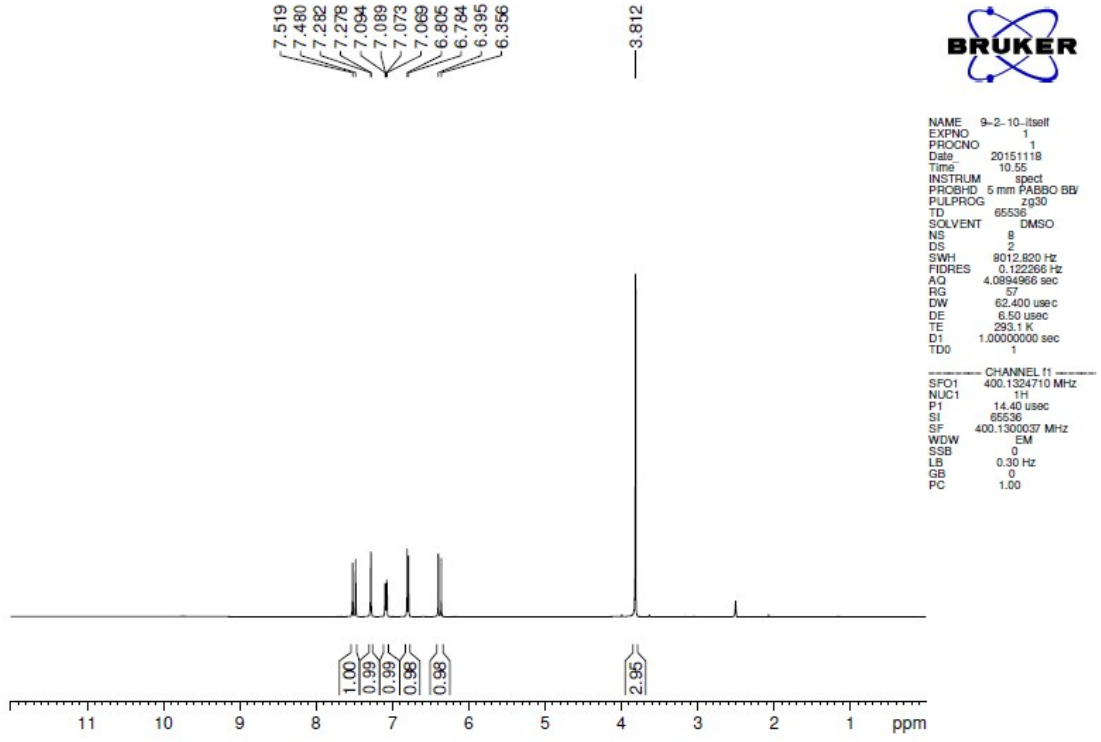
MS spectrum of compound **7**



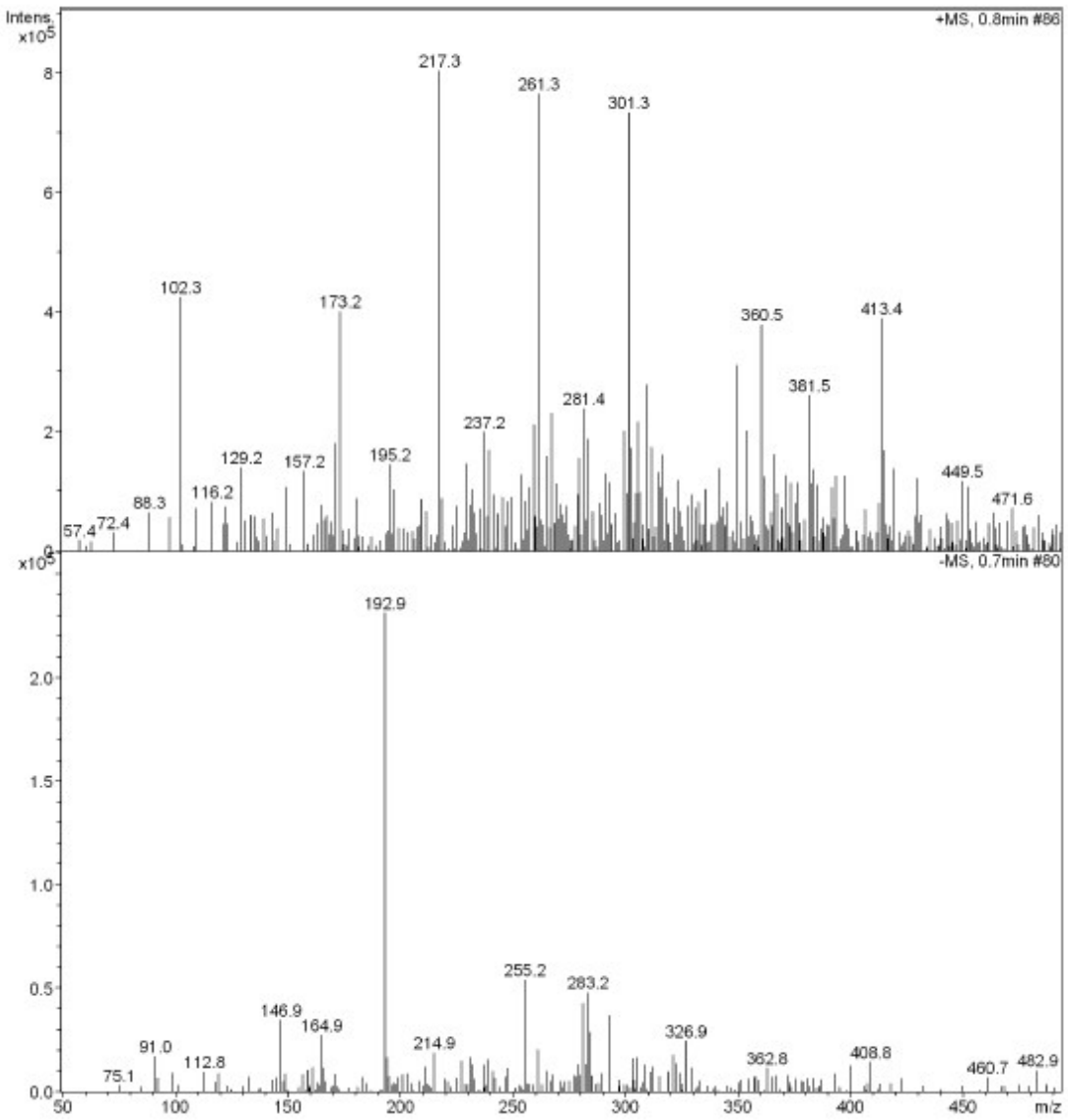
^{1}H-NMR spectrum of compound **8**



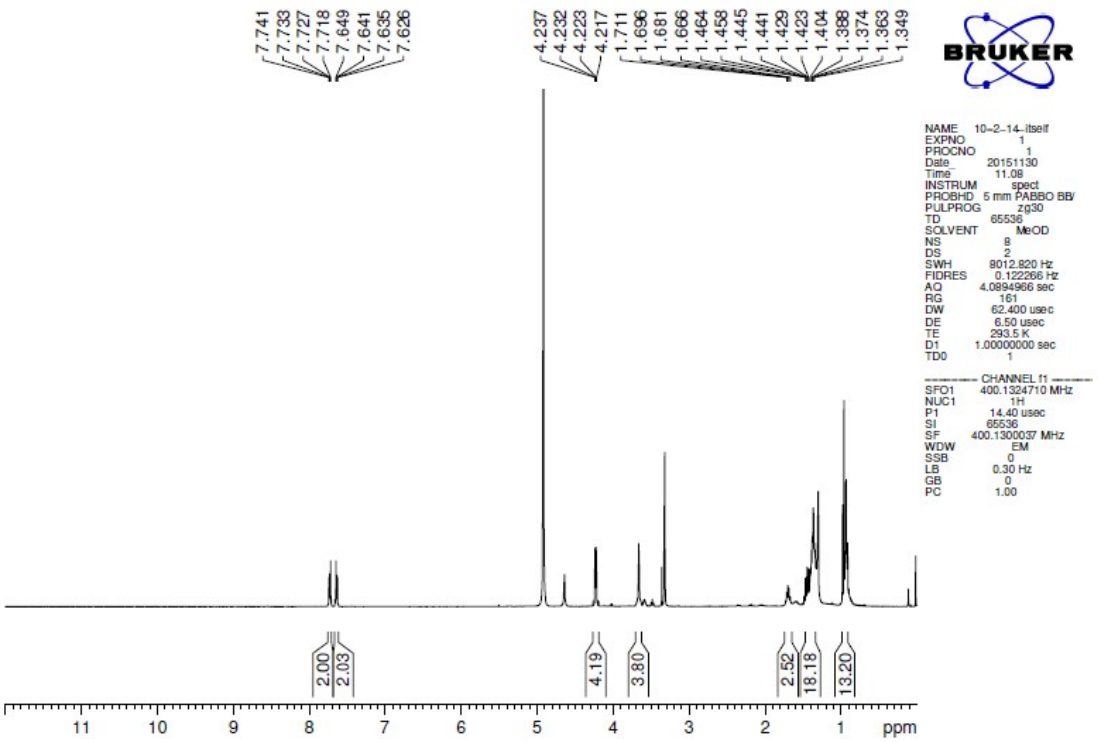
MS spectrum of compound **8**



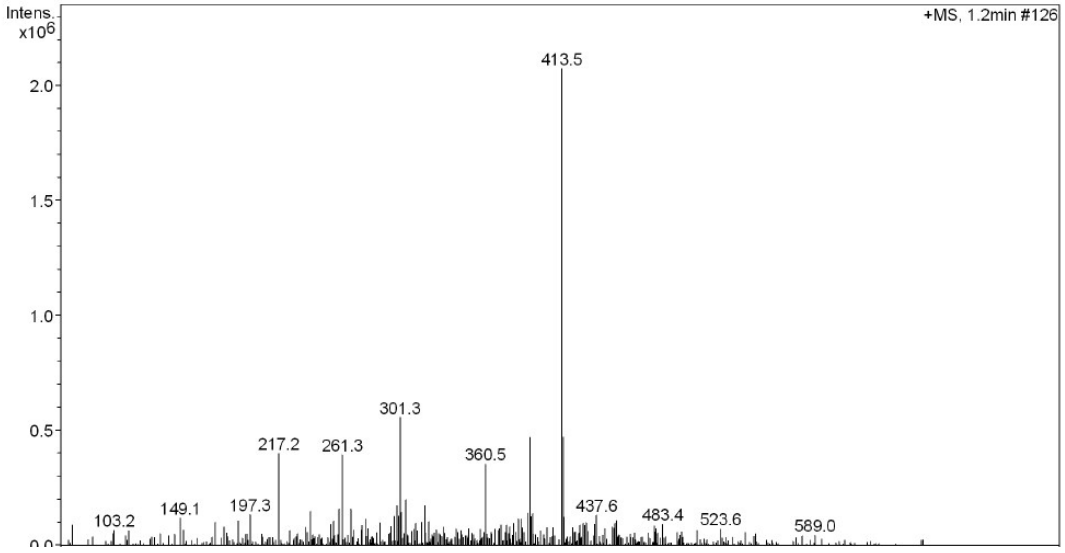
^{1}H-NMR spectrum of compound **9**



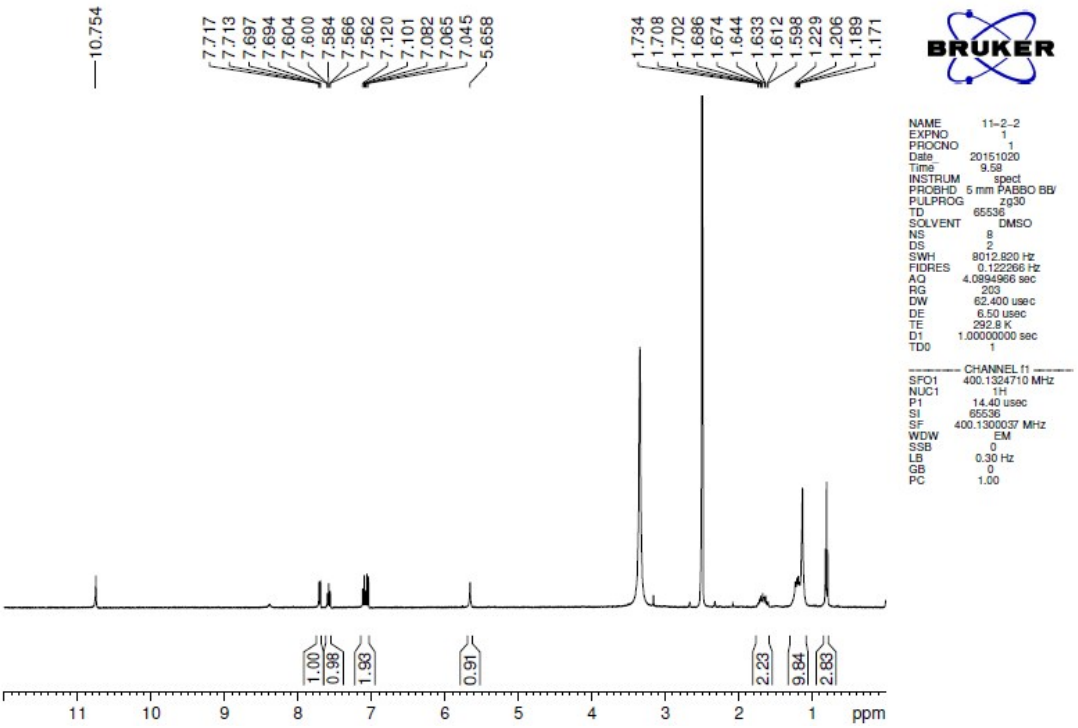
MS spectrum of compound **9**



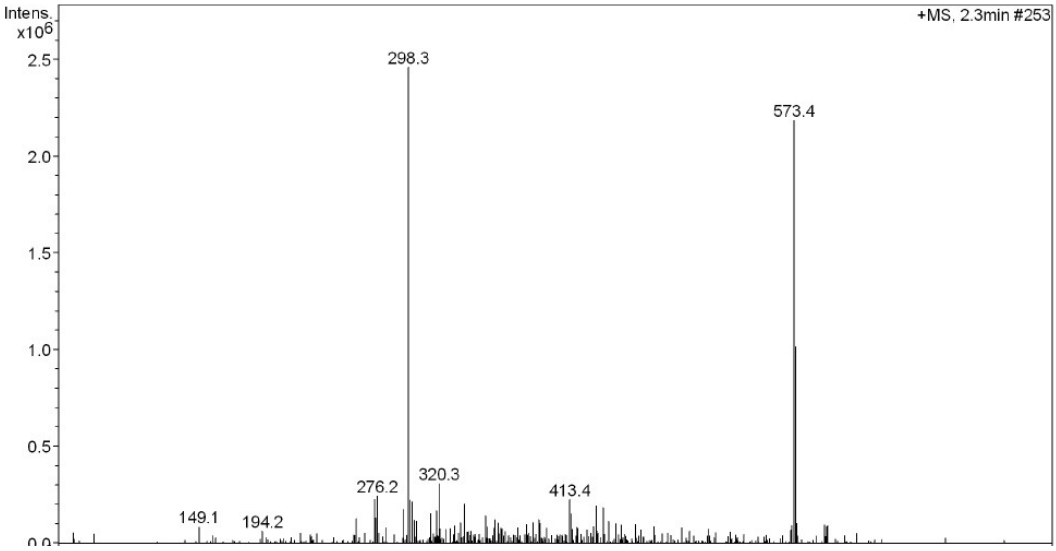
^{1}H-NMR spectrum of compound **10**



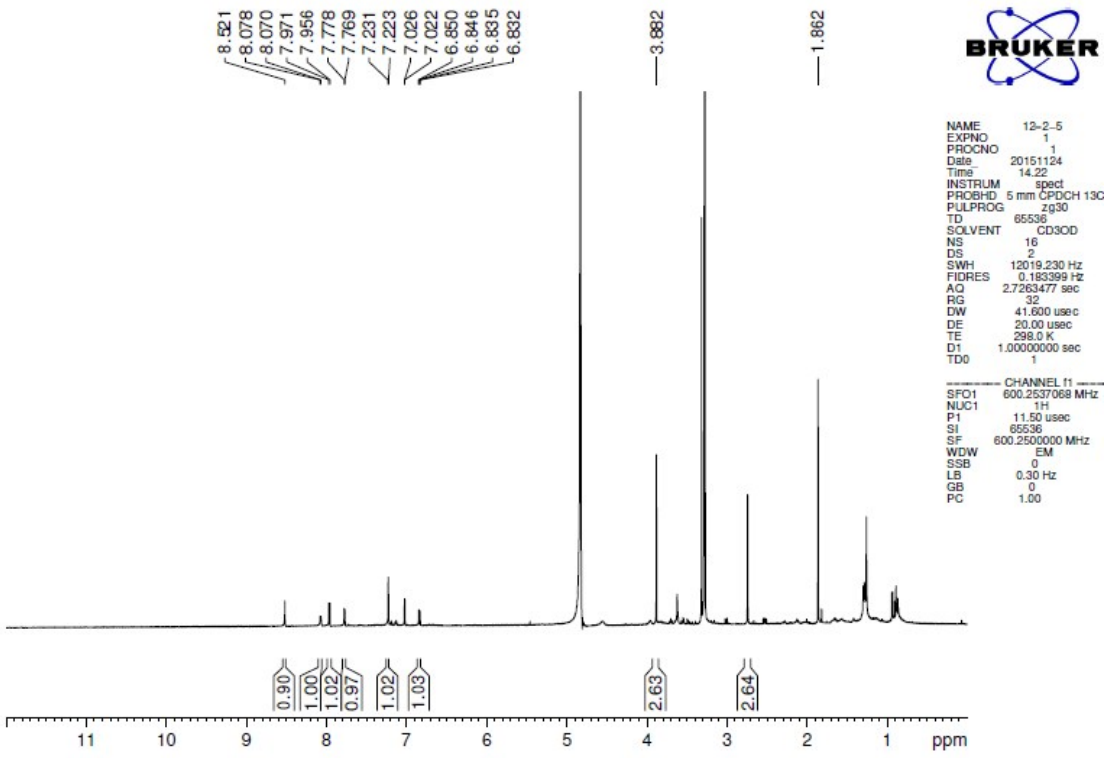
MS spectrum of compound **10**



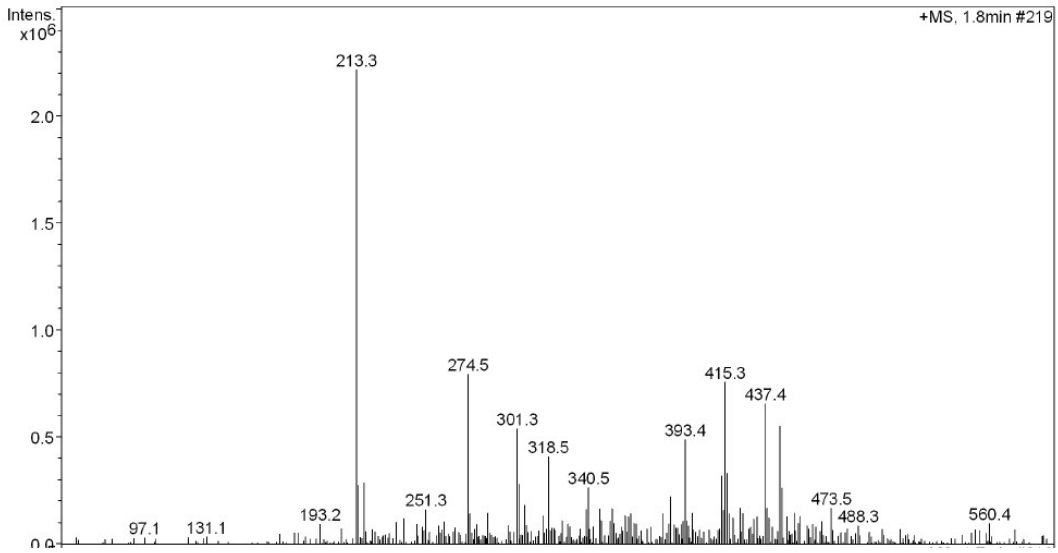
^{1}H-NMR spectrum of compound **11**



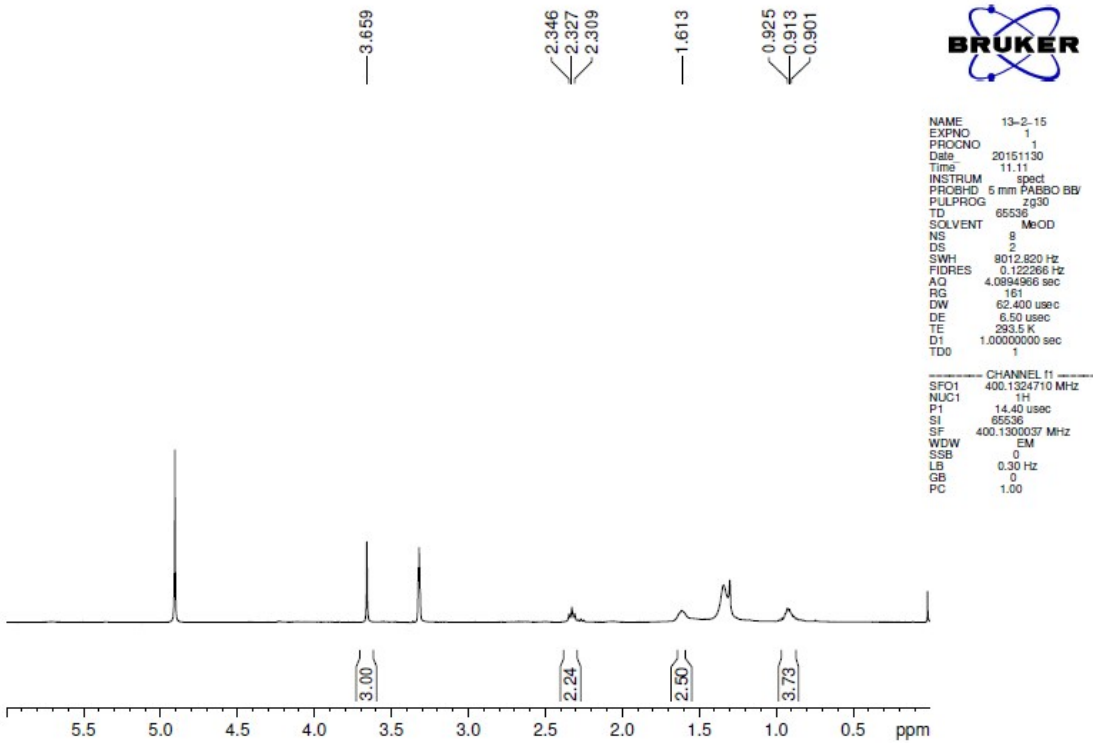
MS spectrum of compound **11**



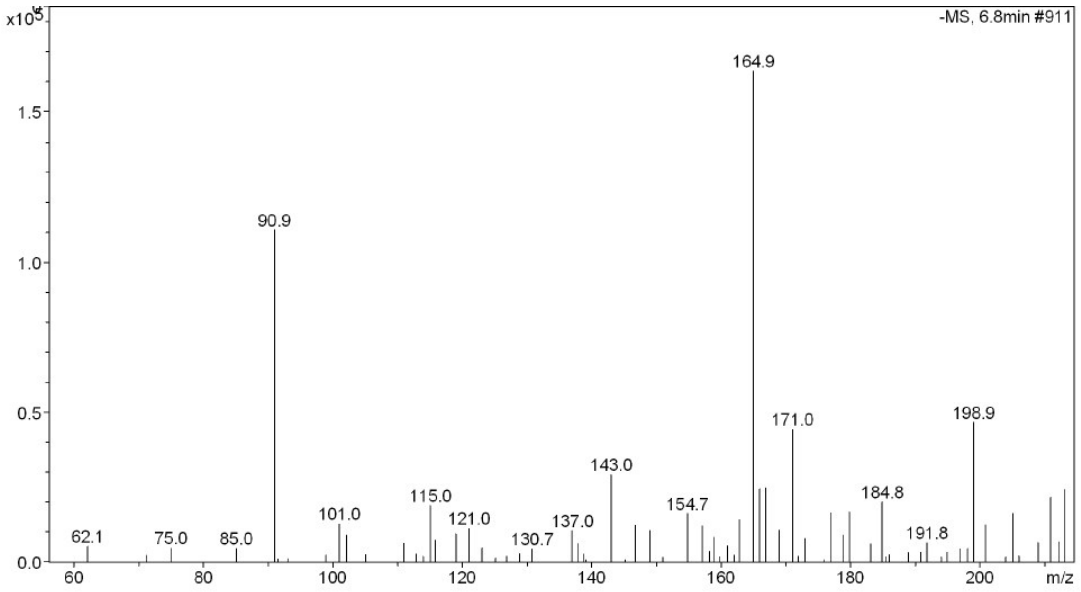
^{1}H-NMR spectrum of compound **12**



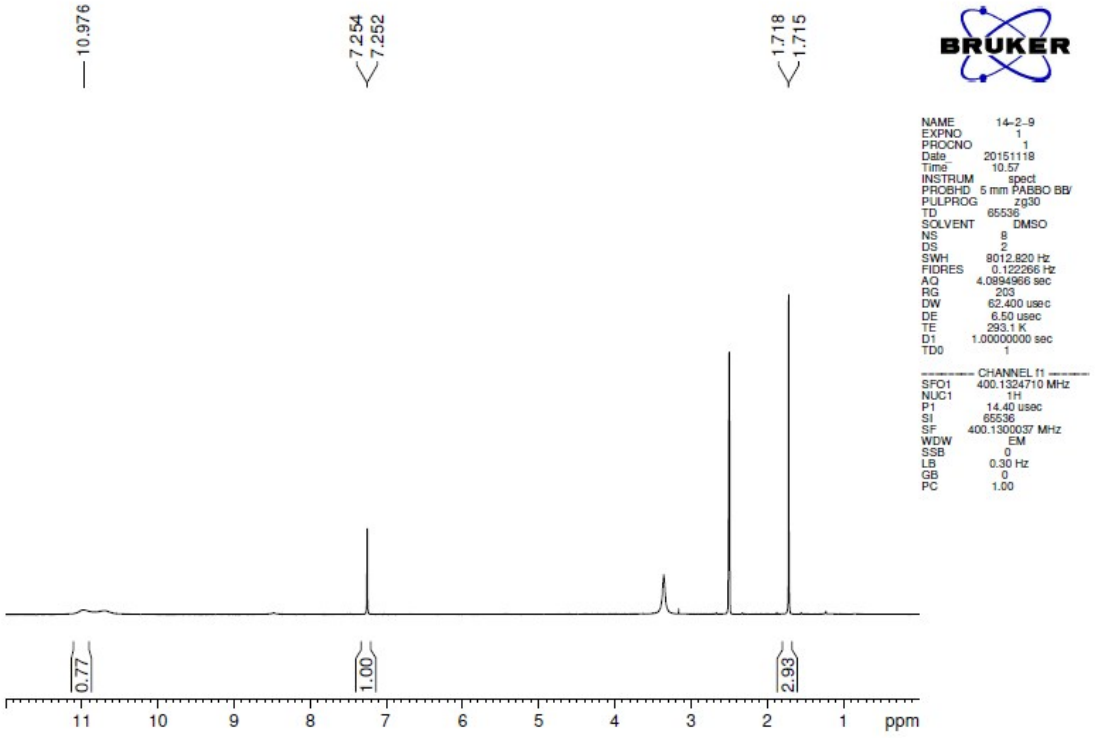
MS spectrum of compound **12**



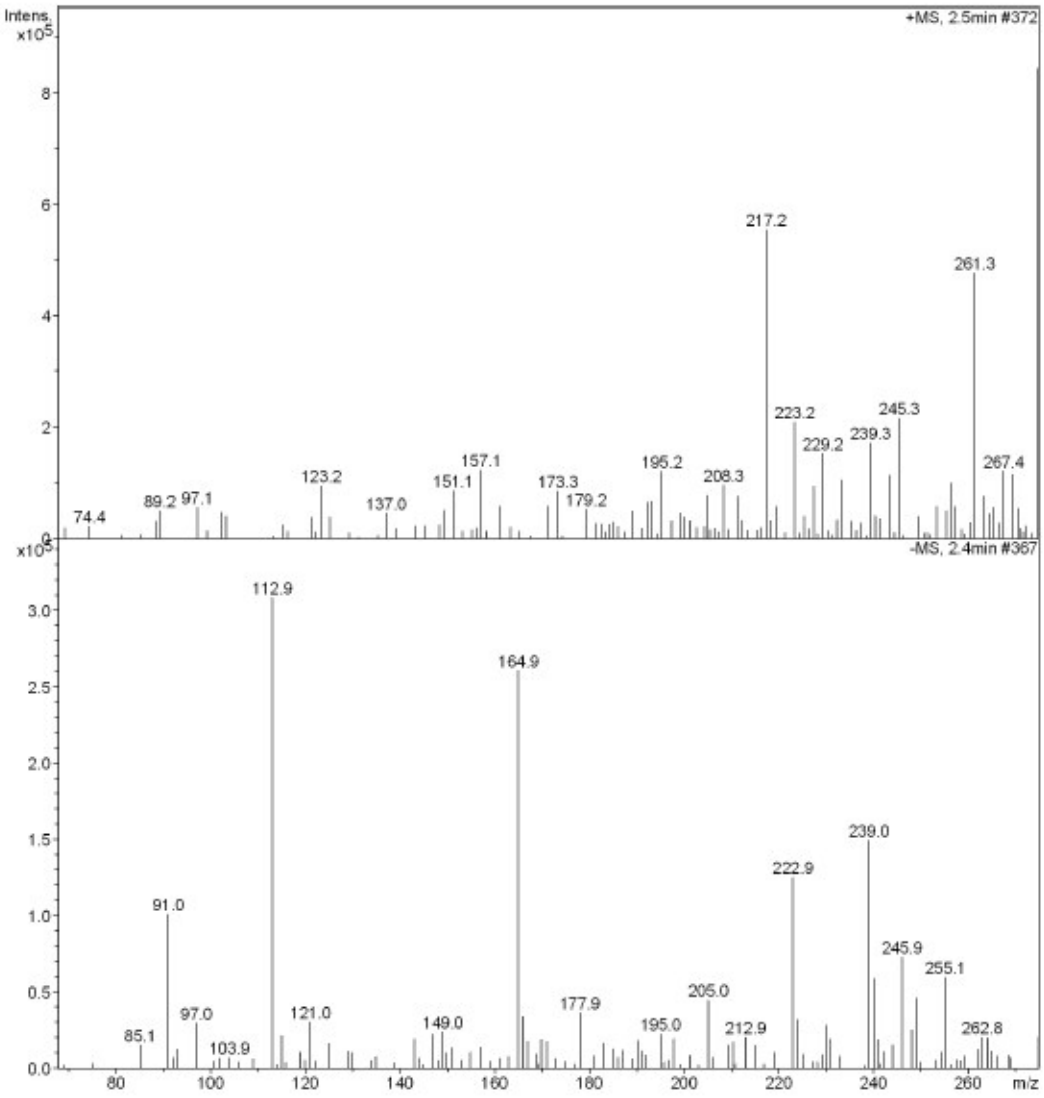
^{1}H-NMR spectrum of compound **13**



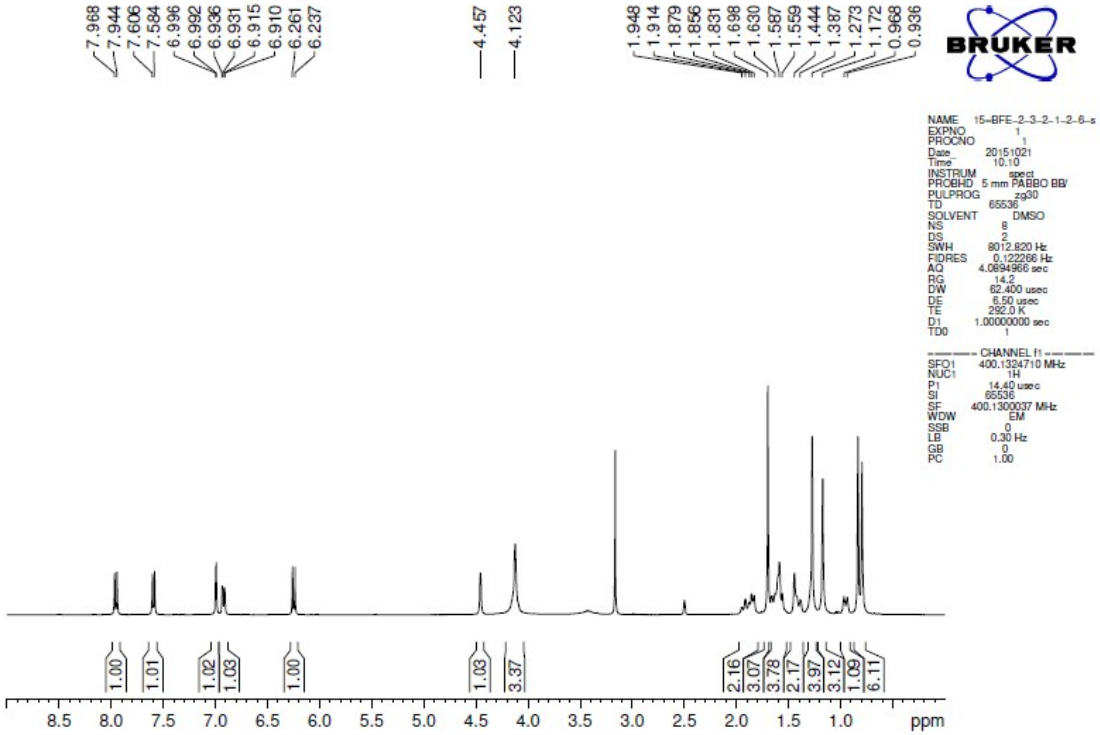
MS spectrum of compound **13**



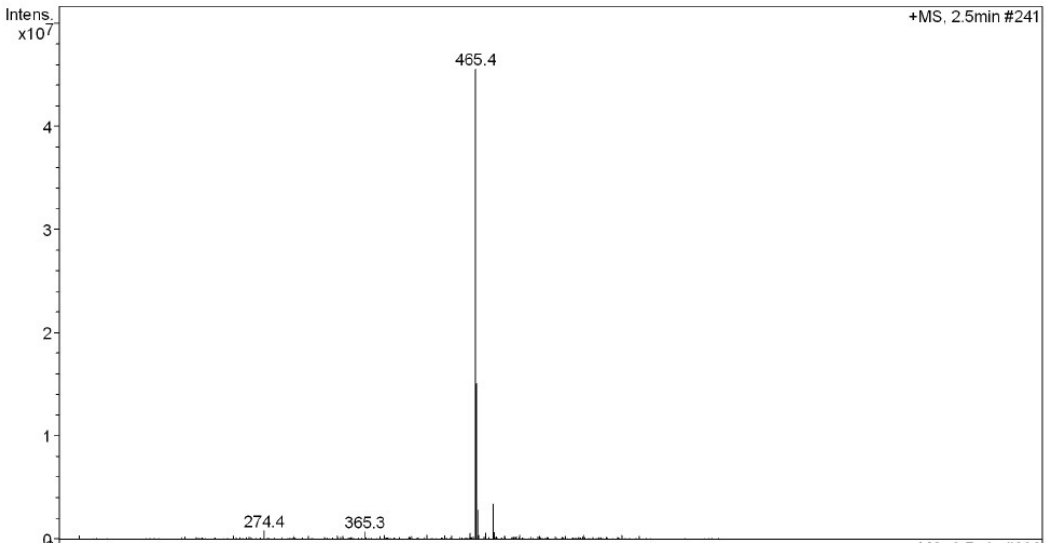
^{1}H-NMR spectrum of compound **14**



MS spectrum of compound **14**



^{1}H-NMR spectrum of substrate



MS spectrum of substrate