Semisynthesis, anti-oomycete and anti-fungal activities of ursolic acid ester derivatives

Lina Zhu[a], Yuee Tian*[a], Tiewei Wang[c], Xiaobo Huang[a], Lin Zhou[b], Shengming Liu[a], Genqiang Chen*[a], Zhiping Che*[a]

[a] *Laboratory of Pesticidal Design & Synthesis, Department of Plant Protection, College of Horticultrue and Plant Protection, Henan University of Science and Technology, Luoyang 471023, China*

[b] *College of Plant Protection, Henan Agricultural University, Zhengzhou 450002, China*

[c] *ShanDong New Power Biology Science &Technology Co., Ltd., Jinan 250101, China*

** Corresponding author at: Laboratory of Pesticidal Design & Synthesis, Department of Plant Protection, College of Horticultrue and Plant Protection, Henan University of Science and Technology, Luoyang 471023, Henan Province, PR China.*

E-mail address: tianyuee1985@163.com (Y.E. Tian); genqiangchen@126.com (G.Q. Chen); zhipingche@163.com (Z.P. Che).

Abstract: Using ursolic acid (UA) as the lead compound, thirteen UA ester derivatives (**3** and **7a-l**) were synthesized by modifying their C-3 and C-28 positions, respectively, and their structures were well characterized by ^{1}H NMR, ^{13}C NMR, HRMS and melting points. Furthermore, we evaluated the anti-oomycete and anti-fungal activities of these compounds against *Phytophthora capsici* and *Fusarium graminearum* in vitro. The results showed that compound **7h** exhibited prominent anti-oomycete and anti-fungal activities, and the median effective concentration (EC_{50}) values of **7h** against *P. capsici* and *F. graminearum* were 70.49 and 113.21 mg/L, respectively. This study suggested that the anti-oomycete and anti-fungal activities of esters synthesized by introducing acyloxy group at C-3 position of UA was more conspicuous than that of esters synthesized by introducing benzyloxy group at C-28 position. This result will pave the way for further modification of UA to develop potential new fungicides.

Keywords: Natural product, ursolic acid, esterification, anti-oomycete activity, anti-fungal activity

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1.0 Chemistry

Ursolic Acid (**1,** UA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), 4-dimethylaminopyridine (DMAP), phosphorustribromide (PBr_3), arylsulfonyl chloride (R^1SO_2Cl), carboxylic acid (R^2COOH), and triethylamine (Et_3N) were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Triphenylphosphine (PPh_3), phenyl tribromomethyl sulfone (Br_3CSO_2Ph), benzyl alcohol ($C_6H_5CH_2OH$), and Phenol (C_6H_5OH), were ordered from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was purchased from Bide Biochemical Co., Ltd. Anhydrous methanol, ethyl acetate, petroleum ether and dichloromethane were analytical grade obtained from Beichen Fangzheng Reagent Factory (Tianjin, China). *N*, *N*-Dimethylformamide (DMF) was analytical grade obtained from Damao Chemical Reagent Factory (Tianjin, China). Toluene was analytical grade obtained from Haohua Chemical Reagent Factory (Luoyang, China). Thin-layer chromatography (TLC) was performed with a silica gel plate using silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Column chromatography (CC) was performed with silica gel 200-300 mesh (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Melting points were taken on a X-6 microscopic melting point apparatus (Beijing Tech instrument Co., Ltd., Beijing, China) and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were carried out with a Bruker Avance III 400 MHz instrument (Bruker Daltonik, Bremen, Germany) in deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as the internal standard. Electrospray ion trap mass spectrometry (ESI-TRAP-MS) was carried out with a Bruker ESI-TRAP Esquire 6000 plus mass spectrometry instrument (Bruker, Germany).

2.0 Tables

Table S1. Comparison of yields of compounds **7a-l** synthesized by two methods.

Compounds	Method 1 (%)	Method 2 (%)
7a	45	52
7b	46	54
7c	51	60
7d	49	59
7e	39	48
7f	40	63
7g	35	40
7h	38	75
7i	34	45
7j	32	47
7k	30	45
7l	20	46

Table S2. Anti-oomycete activity of **1**, **3** and **7a-l** at 50 and 100 mg/L concentration against *P. capsici in vitro.*

Compounds	Concentration（mg/L）	Inhibition rate (%) [a]
1	50 / 100	25.52 ±0.47 / 36.26 ±0.47
3	50 / 100	17.19 ±0.82 / 26.37 ±1.25
7a	50 / 100	38.02 ±0.94 / 46.46 ±1.25
7b	50 / 100	34.90 ±0.47 / 41.21 ±1.25
7c	50 / 100	32.29 ±0.94 / 50.00 ±0.94
7d	50 / 100	38.54 ±0.94 / 47.25 ±3.27
7e	50 / 100	33.33 ±0.47 / 42.31 ±0.82
7f	50 / 100	35.94 ±0.82 / 45.71 ±0.94
7g	50 / 100	39.06 ±1.41 / 50.55 ±2.16
7h	50 / 100	32.29 ±1.89 / 55.49 ±0.82
7i	50 / 100	39.88 ±0.94 / 49.25 ±0.47
7j	50 / 100	27.60 ±1.41 / 53.05 ±1.25
7k	50 / 100	33.85 ±1.70 / 46.90 ±0.82
7l	50 / 100	26.96 ±1.25 / 50.80 ±1.70
Metalaxyl [b]	50 / 100	83.12 ±0.47 / 86.97 ±0.94

[a] Values are means ±S.D. of three replicates.

[b] Metalaxyl was used as a positive control.

Table S3. Anti-oomycete activity of **7c**, **7d**, **7g**, **7h**, **7j** and **7k** at different concentration gradients against *P. capsici in vitro* [b].

Compounds	EC_{50} (mg/L)	Toxicity regression equation	Correlation coefficient	Confidence interval 95% (mg/L)
7c	99.95	y = 3.2397 + 0.8802x	0.9429	73.48-135.96
7d	107.66	y = 3.5487 + 0.7142x	0.9924	96.20-120.50
7g	82.85	y = 3.0427 + 1.0203x	0.9683	67.35-101.90
7h	70.49	y = 3.6212 + 0.7461x	0.9869	61.99-80.14
7j	74.73	y = 3.4853 + 0.8085x	0.9929	68.01-82.11
7k	105.62	y = 3.3255 + 0.8274x	0.9229	72.54-153.77
Metalaxyl [a]	4.49	y = 4.4601 + 0.8270x	0.9791	2.55-7.90

[a] Metalaxyl was used as a positive control.

[b] Regression analysis by IBM SPSS Statistics 22.0, $p<0.05$.

Table S4. Anti-fungal activity of **1**, **3** and **7a-l** at 100 and 200 mg/L concentration against *F. graminearum in vitro.*

Compounds	Concentration（mg/L）	Inhibition rate (%) [a]
1	100 / 200	10.83 ±0.94 / 25.96 ±1.25
3	100 / 200	16.87 ±0.47 / 26.96 ±0.94
7a	100 / 200	23.50 ±0.47 / 33.67 ±1.70
7b	100 / 200	24.04 ±6.24 / 28.06 ±0.82
7c	100 / 200	20.22 ±1.70 / 31.63 ±0.47
7d	100 / 200	17.21 ±1.25 / 35.20 ±0.47
7e	100 / 200	22.40 ±0.47 / 32.14 ±0.94
7f	100 / 200	21.31 ±0.00 / 33.67 ±1.70
7g	100 / 200	27.32 ±0.47 / 39.29 ±1.70
7h	100 / 200	45.90 ±0.94 / 60.71 ±0.47
7i	100 / 200	20.22 ±0.47 / 30.61 ±0.94
7j	100 / 200	20.77 ±1.25 / 26.00 ±0.82
7k	100 / 200	18.58 ±2.05 / 35.20 ±0.94
7l	100 / 200	26.23 ±0.82 / 27.04 ±1.25
Triadimefon [b]	100 / 200	78.97 ±1.25 / 84.62 ±0.41

[a] Values are means ±S.D. of three replicates.

[b] Triadimefon was used as a positive control.

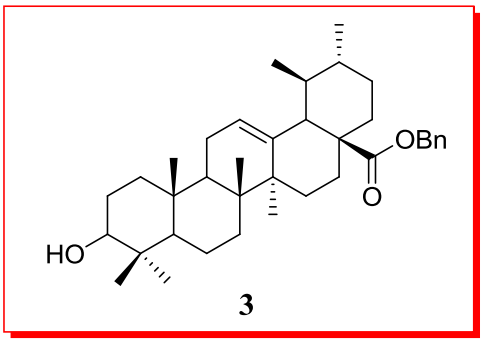
Table S5. Anti-fungal activity of **7h** at different concentration gradients against *F. graminearum in vitro* [b].

Compound	EC_{50} (mg/L)	Toxicity regression equation	Correlation coefficient	Confidence interval 95% (mg/L)
7h	113.21	y = 2.9392 + 1.0034x	0.9715	89.98-142.43
Triadimefon [a]	6.29	y = 4.5307 + 0.5875x	0.9734	3.68-10.74

[a] Triadimefon was used as a positive control.

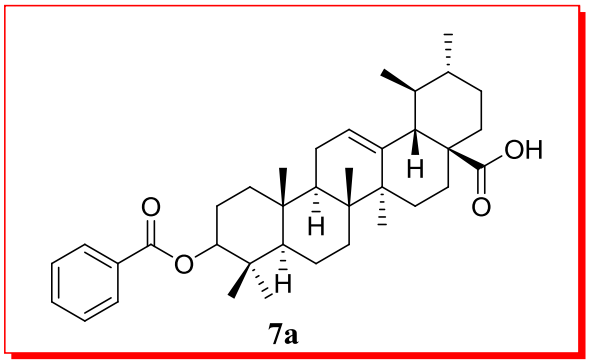
[b] Regression analysis by IBM SPSS Statistics 22.0, $p<0.05$.

3.0 Structural characterization of compounds 3 and 7a-l



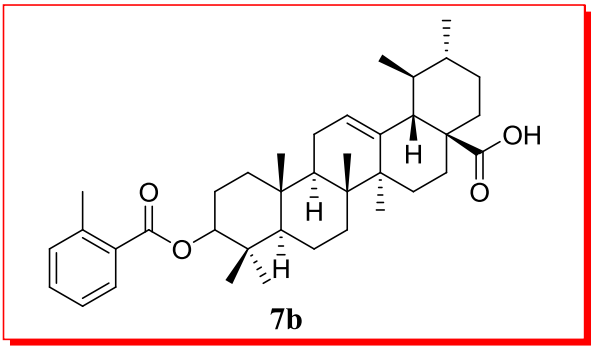
3

Data for **3**: Yield = 85%, White solid, m.p. 193-194 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28-7.37 (m, 5H), 5.24 (t, J = 3.6 Hz, 1H), 5.12 (d, J = 12.8 Hz, 1H), 4.99 (d, J = 12.8 Hz, 1H), 3.23 (dd, J = 11.2 Hz, 4.8 Hz, 1H), 2.24-2.28 (m, 1H), 1.96-2.04 (m, 1H), 1.77-1.94 (m, 3H), 1.66-1.73 (m, 2H), 1.54-1.65 (m, 4H), 1.42-1.53 (m, 4H), 1.25-1.38 (m, 6H), 0.72 (dd, J = 11.6 Hz, 2.0 Hz, 1H), 1.07, 0.98, 0.93, 0.89, 0.85, 0.77, 0.64 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 177.28, 138.09, 136.37, 128.38, 128.13, 127.92, 125.69, 79.03, 65.97, 55.21, 52.88, 48.12, 47.55, 42.03, 39.52, 39.09, 38.83, 38.74, 38.62, 36.95, 36.63, 33.03, 30.66, 28.13, 27.96, 27.23, 24.25, 23.56, 23.26, 21.17, 18.30, 17.01, 16.99, 15.62, 15.44. HRMS (ESI): Calcd for $C_{37}H_{55}O_3^+$, 547.4146; found, 547.4150.



7a

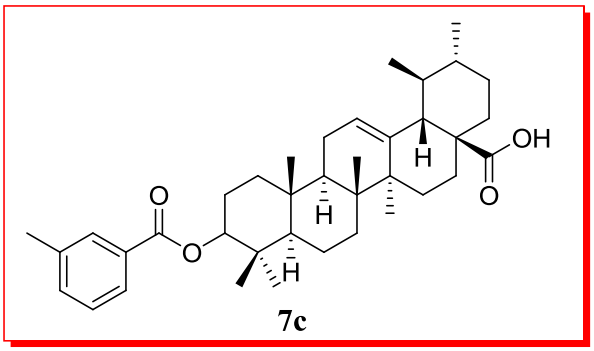
Data for **7a**: Yield = 52%, White solid, m.p. 211-212 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.04 (d, J = 1.2 Hz, 1H), 8.02 (d, J = 1.6 Hz, 1H), 7.61-7.66 (m, 1H), 7.50 (t, J = 7.6 Hz, 2H), 5.38 (t, J = 3.6 Hz, 1H), 3.24 (q, J = 5.2 Hz, 1H), 2.29-2.32 (m, 1H), 2.09-2.17 (m, 1H), 1.94-2.02 (m, 3H), 1.78-1.89 (m, 3H), 1.49-1.68 (m, 9H)，1.31-1.44 (m, 5H), 1.17-1.22 (m, 1H), 1.13, 0.99, 0.97, 0.92, 0.90, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.52, 162.55, 137.60, 134.17, 130.38, 129.39, 128.70, 126.40, 79.03, 55.25, 52.85, 49.95, 47.59, 42.26, 39.65, 39.16, 38.81, 38.75, 38.70, 36.97, 35.80, 33.13, 30.54, 28.14, 28.04, 27.23, 24.43, 23.47, 23.38, 21.10, 18.29, 17.28, 16.94, 15.62, 15.52. HRMS (ESI): Calcd for $C_{37}H_{53}O_4^+$, 561.3938; found, 561.3941.



7b

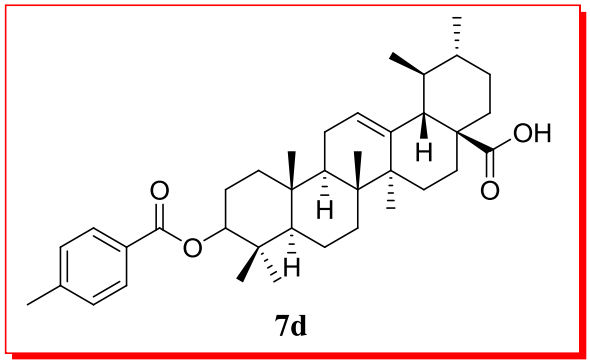
Data for **7b**: Yield = 54%, White solid, m.p. 147-148 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (dd, J = 7.6 Hz, 1.2 Hz, 1H), 7.45-7.49 (m, 1H), 7.25-7.30 (m, 2H), 5.37 (t, J = 4.0 Hz, 1H), 3.24 (q, J = 5.2 Hz, 1H), 2.65 (s, 3H), 2.31 (dd, J = 11.2 Hz, 2.0 Hz, 1H), 2.08-2.15 (m, 1H), 1.97 (dd, J = 9.6 Hz, 3.2 Hz, 2H), 1.76-1.87 (m, 3H), 1.48-1.68 (m, 10H), 1.33-1.43 (m, 5H)，1.16-1.21 (m, 1H), 1.12, 0.99, 0.97, 0.93, 0.89, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 173.00, 162.79, 142.53, 137.61, 133.34, 132.12, 131.24, 128.10, 126.38, 125.86, 79.04, 55.26, 52.82, 49.74, 47.60, 42.27,

39.67, 39.18, 38.80, 38.76, 38.71, 36.97, 35.76, 33.17, 30.56, 28.15, 28.02, 27.24, 24.40, 23.44, 23.38, 21.97, 21.11, 18.31, 17.36, 16.94, 15.63,15.53. HRMS (ESI): Calcd for $C_{38}H_{55}O_4^+$, 575.4095; found, 575.4096.



7c

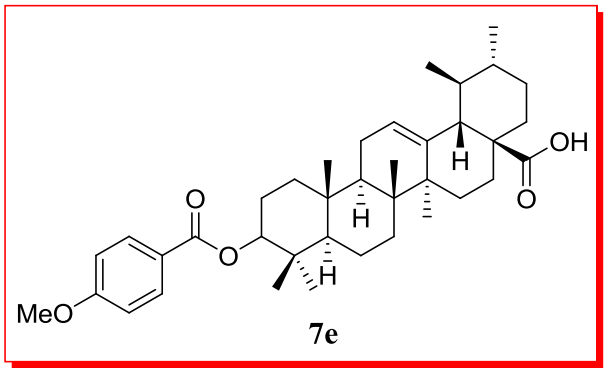
Data for **7c**: Yield = 60%, White solid, m.p. 197-198 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.81-7.84 (m, 2H), 7.42-7.44 (m, 1H), 7.38 (t, J = 7.6 Hz, 1H), 5.39 (t, J = 3.6 Hz, 1H), 3.24 (q, J = 4.8 Hz, 1H), 2.42 (s, 3H), 2.29-2.32 (m, 1H), 2.09-2.16 (m, 1H), 1.95-2.02 (m, 3H), 1.78-1.88 (m, 3H), 1.50-1.68 (m, 8H)，1.31-1.45 (m, 5H), 1.26 (d, J = 2.0 Hz, 1H), 1.16-1.22 (m, 1H), 1.13, 0.99, 0.97, 0.92, 0.89, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.64, 162.77, 138.56, 137.75, 134.98, 130.95, 129.32, 128.57, 127.55, 126.31, 79.03, 55.26, 52.83, 49.93, 47.59, 42.26, 39.65, 39.17, 38.81, 38.76, 38.69, 36.98, 35.77, 33.12, 30.54, 28.15, 28.01, 27.23, 24.41, 23.49, 23.38, 21.31, 21.11, 18.30, 17.30, 16.95, 15.63, 15.51. HRMS (ESI): Calcd for $C_{38}H_{55}O_4^+$, 575.4095; found, 575.4099.



7d

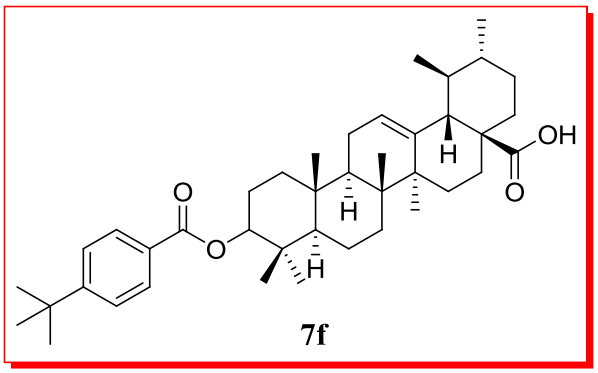
Data for **7d**: Yield = 59%, Yellow solid, m.p. 213-214 ^oC. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.92 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.0 Hz, 2H), 5.37 (t, J = 4.0 Hz, 1H), 3.24 (q, J = 5.2 Hz, 1H), 2.43 (s, 3H), 2.32 (dd, J = 12.0 Hz, 2.0 Hz, 1H), 2.13 (dd, J = 13.6 Hz, 4.0 Hz, 1H), 1.91-2.02 (m, 4H), 1.77-1.90 (m, 4H), 1.64-1.68 (m, 2H), 1.48-1.57

(m, 5H)，1.31-1.41 (m, 6H), 1.12, 0.99, 0.97, 0.92, 0.90, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.66, 162.60, 145.23, 137.66, 130.47, 129.43, 126.65, 126.35, 79.03, 55.26, 52.84, 49.89, 47.60, 42.26, 39.65, 39.17, 38.82, 38.75, 38.70, 36.97, 35.82, 33.14, 30.55, 28.14, 28.03, 27.23, 24.42, 23.47, 23.38, 21.81, 21.11, 18.30, 17.29, 16.94, 15.62, 15.52. HRMS (ESI): Calcd for $C_{38}H_{55}O_4^+$, 575.4095; found, 575.4095.



7e

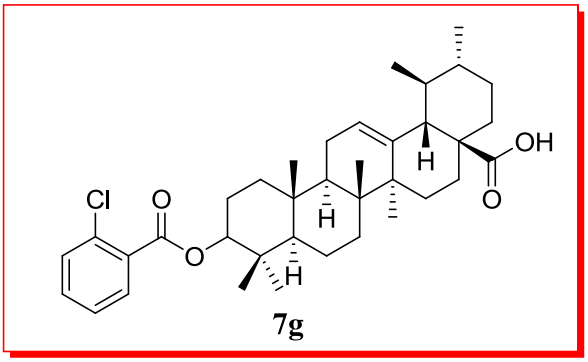
Data for **7e**: Yield = 48%, White solid, m.p. 161-162 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96-8.00 (m, 2H), 6.92-6.96 (m, 2H), 5.37 (t, J = 3.6 Hz, 1H), 3.88 (s, 3H), 3.24 (q, J = 5.2 Hz, 1H), 2.28-2.31 (m, 1H), 2.08-2.16 (m, 1H), 1.94-2.04 (m, 3H), 1.77-1.89 (m, 3H), 1.54-1.68 (m, 9H), 1.31-1.43 (m, 4H), 1.16-1.21 (m, 1H), 1.12, 0.99, 0.97, 0.92, 0.89, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.77, 164.39, 162.27, 137.71, 132.68, 126.31, 121.67, 114.01, 79.03, 55.57, 55.26, 52.84, 49.83, 47.60, 42.26, 39.65, 39.17, 38.82, 38.75, 38.70, 36.97, 35.86, 33.14, 30.56, 28.14, 28.03, 27.24, 24.42, 23.47, 23.39, 21.11, 18.30, 17.29, 16.95, 15.62, 15.52. HRMS (ESI): Calcd for $C_{38}H_{55}O_5^+$, 591.4044; found, 591.4046.



7f

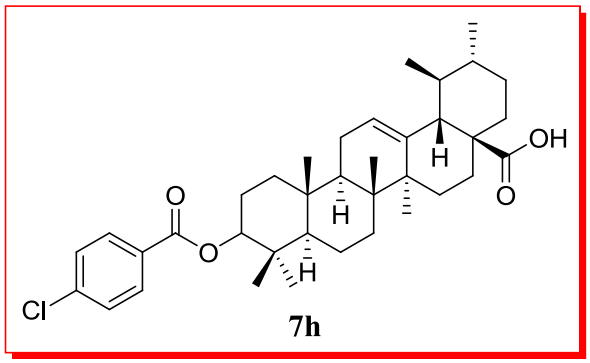
Data for **7f**: Yield = 63%, White solid, m.p. 181-182 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$)

δ: 7.97 (dd, *J* = 6.4 Hz, 1.6 Hz, 2H), 7.50 (dd, *J* = 6.4 Hz, 2.0 Hz, 2H), 5.38 (t, *J* = 3.6 Hz, 1H), 3.24 (q, *J* = 5.2 Hz, 1H), 2.28-2.32 (m, 1H), 2.08-2.16 (m, 1H), 1.94-2.04 (m, 3H), 1.77-1.87 (m, 3H), 1.50-1.68 (m, 9H), 1.36-1.42 (m, 3H), 1.34 (s, 9H), 1.33 (t, *J* = 6.0 Hz, 1H), 1.16-1.25 (m, 2H), 1.12, 0.99, 0.97, 0.93, 0.89, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.44, 162.33, 157.95, 137.44, 130.16, 126.40, 126.18, 125.54, 78.85, 55.08, 52.65, 49.70, 47.42, 42.07, 39.46, 38.98, 38.64, 38.57, 38.52, 36.79, 35.64, 35.08, 32.95, 30.86, 30.37, 27.96, 27.84, 27.05, 24.24, 23.29, 23.21, 20.92, 18.12, 17.11, 16.76, 15.44, 15.33. HRMS (ESI): Calcd for $C_{41}H_{61}O_4^+$, 617.4564; found, 617.4565.



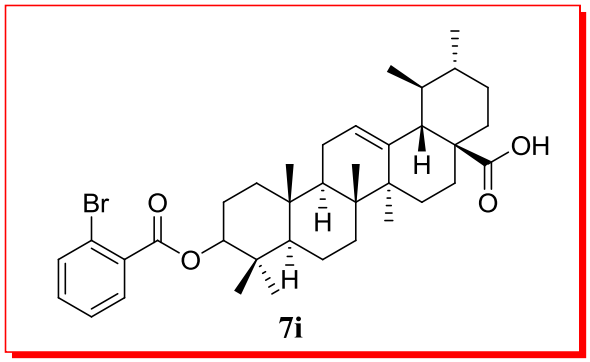
7g

Data for **7g**: Yield = 40%, White solid, m.p. 181-182 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 7.85-7.87 (m, 1H), 7.47-7.49 (m, 2H), 7.33-7.37 (m, 1H), 5.34 (t, *J* = 3.6 Hz, 1H), 3.24 (q, *J* = 5.2 Hz, 1H), 2.29 (d, *J* = 11.2 Hz, 1H), 2.11 (dd, *J* = 13.6 Hz, 4.4 Hz, 1H), 1.90-1.99 (m, 5H), 1.75-1.86 (m, 4H), 1.60-1.68 (m, 5H), 1.56 (d, *J* = 4.0 Hz, 1H), 1.32-1.39 (m, 5H)，1.16-1.19 (m, 1H), 1.11, 0.99, 0.96, 0.93, 0.89, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.27, 161.26, 137.35, 134.69, 133.64, 132.22, 131.49, 128.91, 126.71, 126.49, 79.04, 55.26, 52.76, 49.80, 47.60, 42.25, 39.66, 39.17, 38.75, 38.71, 36.96, 35.50, 33.16, 30.51, 28.14, 28.05, 27.23, 24.34, 23.38, 21.09, 18.30, 17.33, 16.92, 15.63, 15.54. HRMS (ESI): Calcd for $C_{37}H_{52}ClO_4^+$, 595.3549; found, 595.3553.



7h

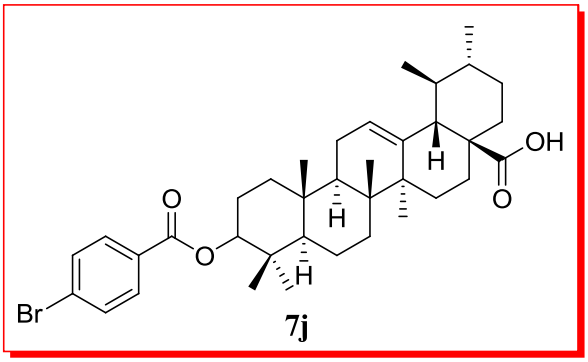
Data for **7h**: Yield = 75%, White solid, m.p. 220-221 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 8.4 Hz, 2H), 5.37 (t, J = 3.6 Hz, 1H), 3.24 (q, J = 5.2 Hz, 1H), 2.29 (d, J = 11.2 Hz, 1H), 2.09-2.17 (m, 1H), 1.77-1.98 (m, 7H), 1.48-1.67 (m, 8H)，1.18-1.42 (m, 6H), 1.13, 0.99, 0.96, 0.92, 0.89, 0.86, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.25, 161.74, 140.82, 137.63, 131.67, 129.13, 127.84, 126.41, 79.02, 55.25, 52.88, 50.02, 47.56, 42.27, 39.63, 39.15, 38.80, 38.75, 38.69, 36.96, 35.77, 33.12, 30.50, 28.14, 28.03, 27.22, 24.44, 23.46, 23.38, 21.08, 18.28, 17.28, 16.93, 15.62, 15.53. HRMS (ESI): Calcd for $C_{37}H_{52}ClO_4^+$, 595.3549; found, 595.3551.



7i

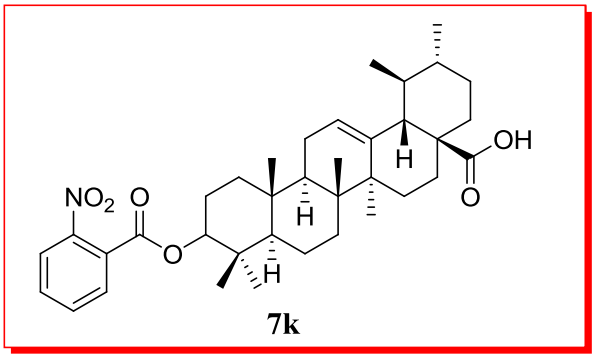
Data for **7i**: Yield = 45%, White solid, m.p. 224-225 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.80-7.83 (m, 1H), 7.68-7.72 (m, 1H), 7.37-7.42 (m, 2H), 5.34 (t, J = 3.6 Hz, 1H), 3.24 (q, J = 5.2 Hz, 1H), 2.29 (dd, J = 11.2 Hz, 2.0 Hz, 1H), 2.06-2.14 (m, 1H), 1.92-1.99 (m, 3H), 1.86 (dd, J = 5.2 Hz, 3.6 Hz, 1H), 1.76-1.82 (m, 2H)，1.59-1.68 (m, 4H), 1.48-1.58 (m, 5H), 1.25-1.42 (m, 5H), 1.15-1.20 (m, 1H), 1.11, 0.99, 0.96, 0.93, 0.88, 0.87, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.26, 161.67, 137.37, 134.83, 133.56, 132.08, 130.92, 127.26, 126.49, 122.56, 79.05, 55.25, 52.78, 49.83, 47.59, 42.26, 39.66, 39.17, 38.75, 38.71, 36.97, 35.52, 33.17, 30.51, 28.14, 28.06,

27.23, 24.34, 23.38, 21.08, 18.30, 17.36, 16.92, 15.63, 15.54. HRMS (ESI): Calcd for $C_{37}H_{52}BrO_4^+$, 639.3043; found, 639.3048.



7j

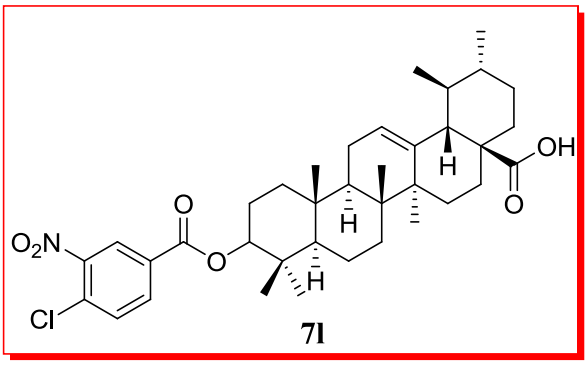
Data for **7j**: Yield = 47%, White solid, m.p. 175-176 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.86-7.89 (m, 2H), 7.61-7.64 (m, 2H), 5.37 (t, J = 4.0 Hz, 1H), 3.24 (q, J = 4.8 Hz, 1H), 2.29 (d, J = 11.2 Hz, 1H), 2.09-2.17 (m, 1H), 1.97 (dd, J = 9.2 Hz, 4.0 Hz, 3H), 1.76-1.86 (m, 3H)，1.48-1.68 (m, 9H), 1.32-1.42 (m, 4H), 1.17-1.26 (m, 2H), 1.12, 0.99, 0.97, 0.92, 0.89, 0.85, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 172.22, 161.90, 137.62, 132.14, 131.74, 129.58, 128.30, 126.41, 79.02, 55.24, 52.88, 50.03, 47.56, 42.27, 39.63, 39.15, 38.80, 38.75, 38.69, 36.96, 35.76, 33.12, 30.49, 28.14, 28.02, 27.22, 24.44, 23.46, 23.38, 21.07, 18.28, 17.28, 16.93, 15.62, 15.53. HRMS (ESI): Calcd for $C_{37}H_{52}BrO_4^+$, 639.3043; found, 639.3044.



7k

Data for **7k**: Yield = 45%, White solid, m.p. 232-233 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (dd, J = 7.2 Hz, 1.6 Hz, 1H), 7.80 (dd, J = 7.6 Hz, 2.0 Hz, 1H), 7.66-7.74 (m, 2H), 5.27 (t, J = 4.0 Hz, 1H), 3.24 (q, J = 4.8 Hz, 1H), 2.18 (dd, J = 11.6 Hz, 2.0 Hz, 2H), 2.06 (dd, J = 13.6 Hz, 4.4 Hz, 1H), 1.87-1.91 (m, 2H), 1.72-1.83 (m, 3H), 1.58-1.68 (m, 5H), 1.45-1.54 (m, 5H)，1.30-1.38 (m, 4H), 1.12-1.17 (m, 1H), 1.08, 0.98, 0.94, 0.94, 0.92, 0.85, 0.79, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$):

171.34, 161.29, 147.79, 137.08, 133.21, 132.41, 130.43, 127.05, 126.52, 124.04, 79.10, 55.23, 52.65, 49.99, 47.56, 42.19, 39.58, 39.12, 38.74, 38.69, 38.62, 36.95, 35.32, 33.09, 30.44, 28.13, 28.01, 27.19, 24.21, 23.34, 23.32, 21.05, 18.29, 17.08, 16.85, 15.63, 15.53. HRMS (ESI): Calcd for $C_{37}H_{52}NO_6^+$, 606.3789; found, 606.3792.

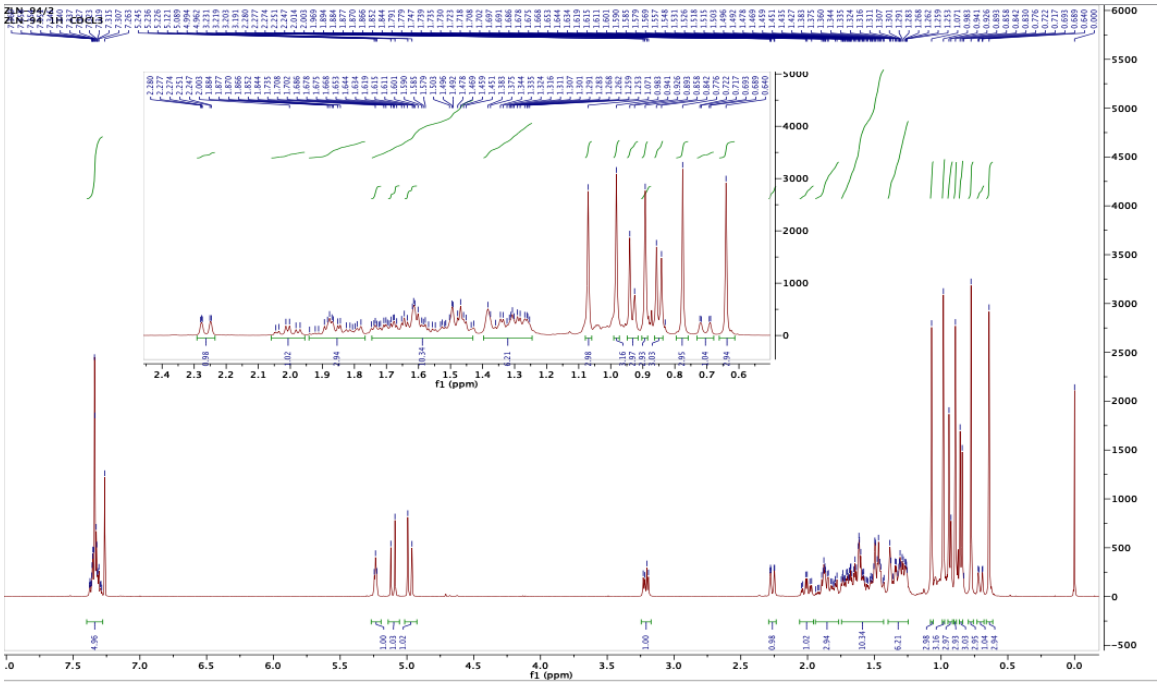


7l

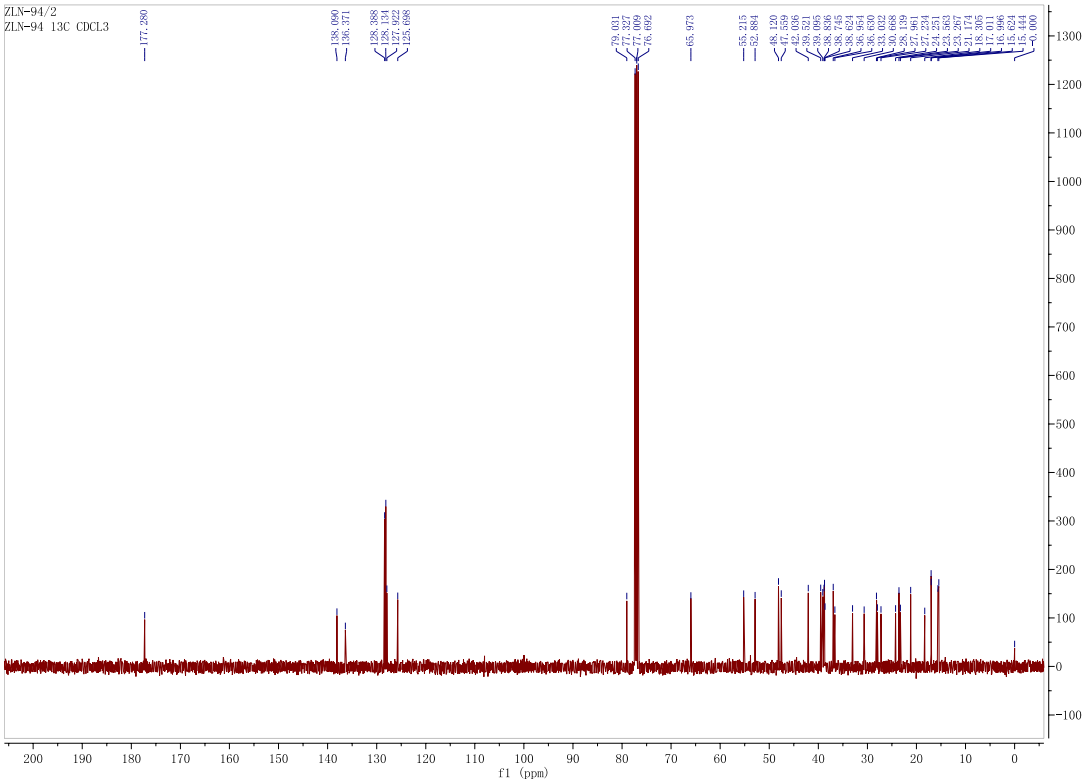
Data for **7l**: Yield = 46%, Yellow solid, m.p. 225-226 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.46 (d, J = 2.0 Hz, 1H), 8.18 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 1H), 5.39 (t, J = 3.6 Hz, 1H), 3.24 (q, J = 5.2 Hz, 1H), 2.24-2.27 (m, 1H), 2.11-2.19 (m, 1H), 1.99 (dd, J = 9.2 Hz, 3.6 Hz, 2H), 1.93 (dd, J = 14.4 Hz, 4.8 Hz, 1H), 1.82-1.85 (m, 2H)，1.75-1.80 (m, 1H), 1.56-1.69 (m, 9H), 1.31-1.44 (m, 5H), 1.19-1.24 (m, 1H), 1.13, 0.99, 0.97, 0.93, 0.89, 0.84, 0.78 (s, each 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 171.57, 159.97, 148.05, 137.74, 134.16, 133.08, 132.69, 129.35, 127.04, 126.59, 79.05, 55.22, 52.92, 50.32, 47.53, 42.28, 39.62, 39.13, 38.78, 38.74, 38.63, 36.97, 35.65, 33.02, 30.39, 28.14, 27.94, 27.20, 24.42, 23.52, 23.31, 21.04, 18.27, 17.27, 16.91, 15.61, 15.43. HRMS (ESI): Calcd for $C_{37}H_{51}ClNO_6^+$, 640.3399; found, 640.3403.

4.0 Copies of ^{1}H NMR and ^{13}C NMR spectra of compounds 3 and 7a-l

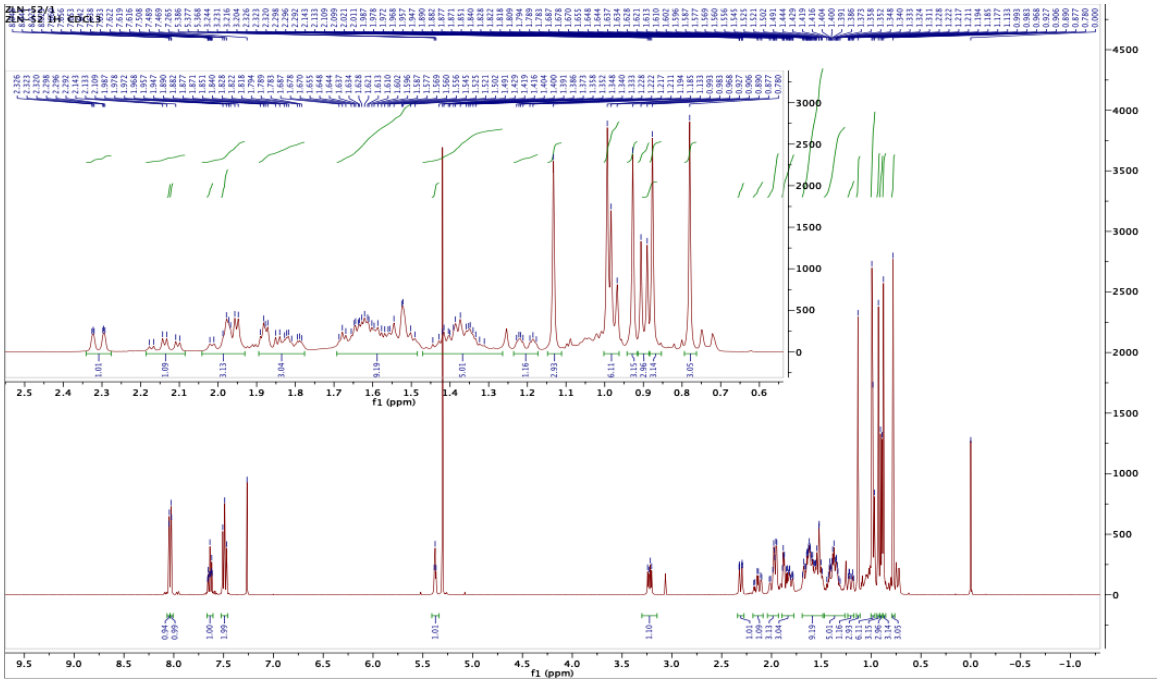
3 ^{1}H NMR



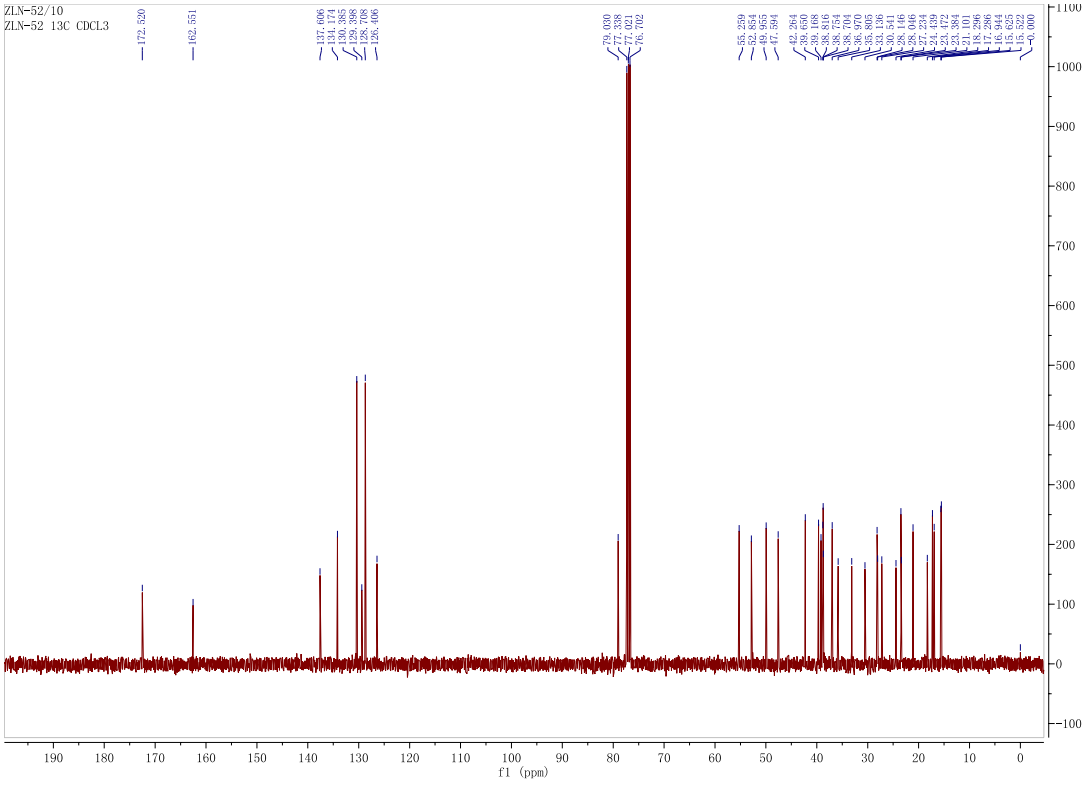
3 ^{13}C NMR



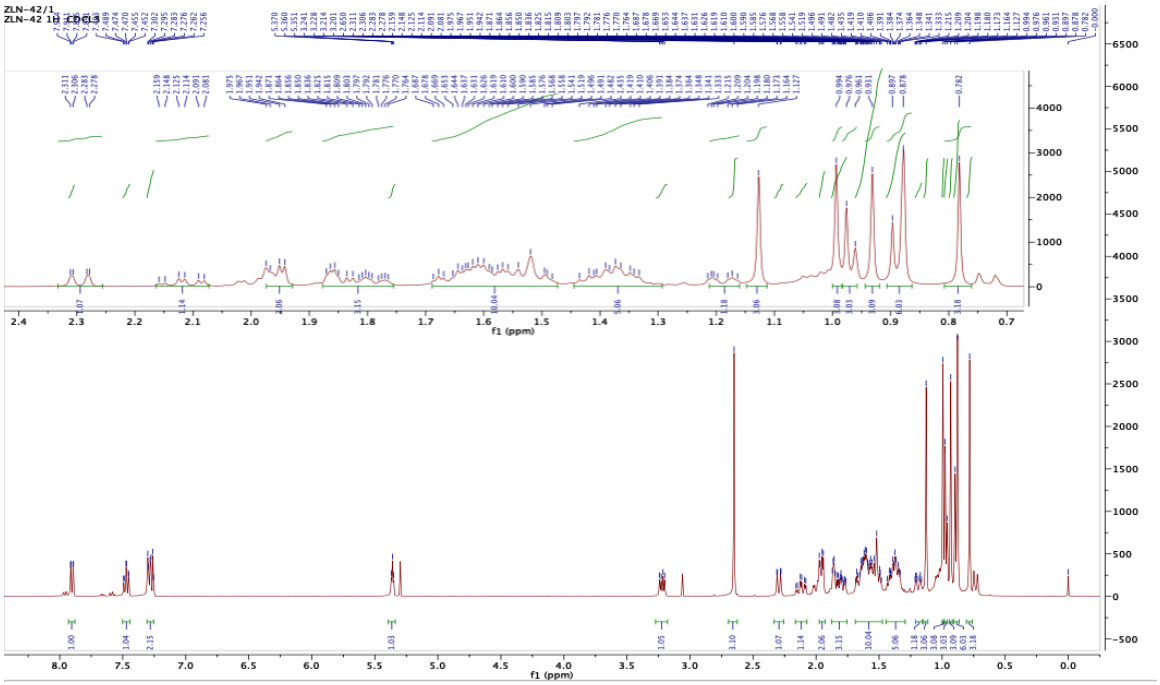
7a ^{1}H NMR



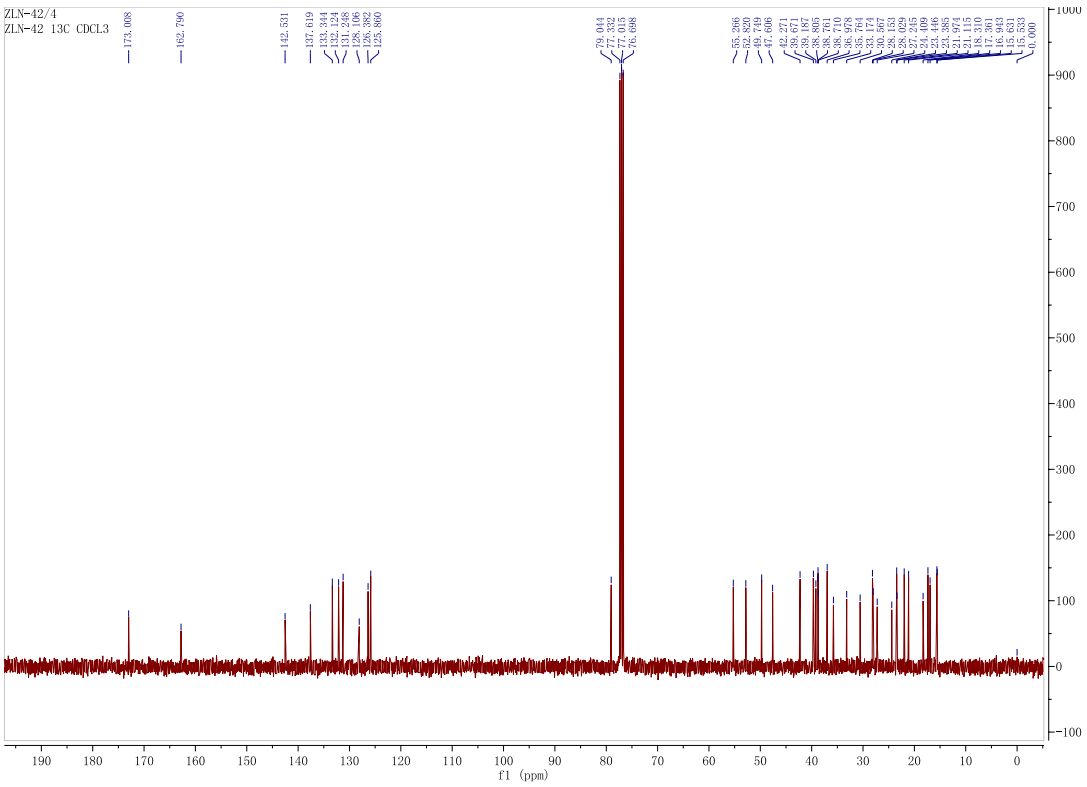
7a ^{13}C NMR



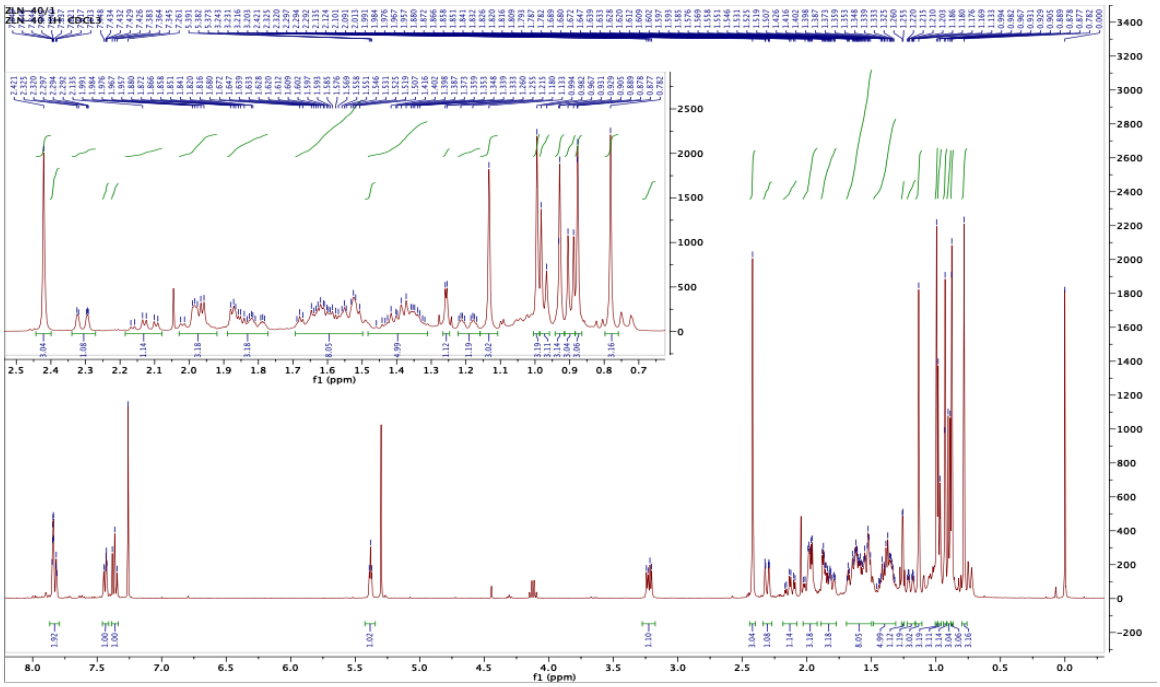
7b ^{1}H NMR



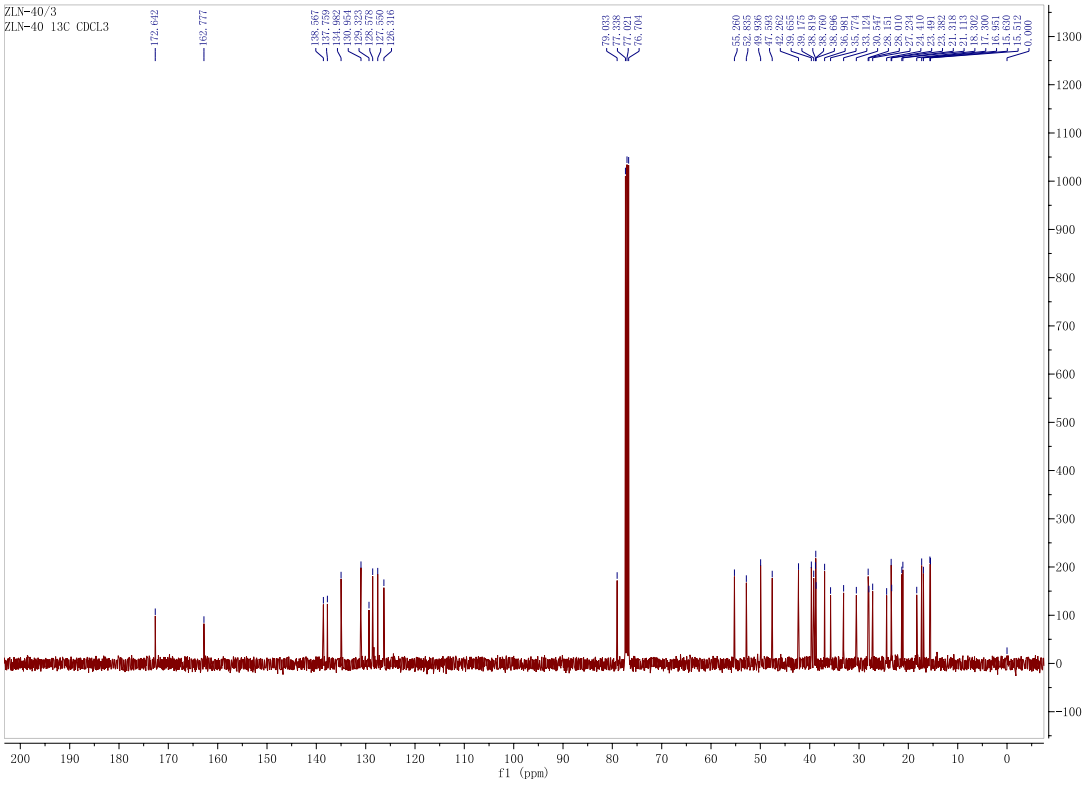
7b ^{13}C NMR



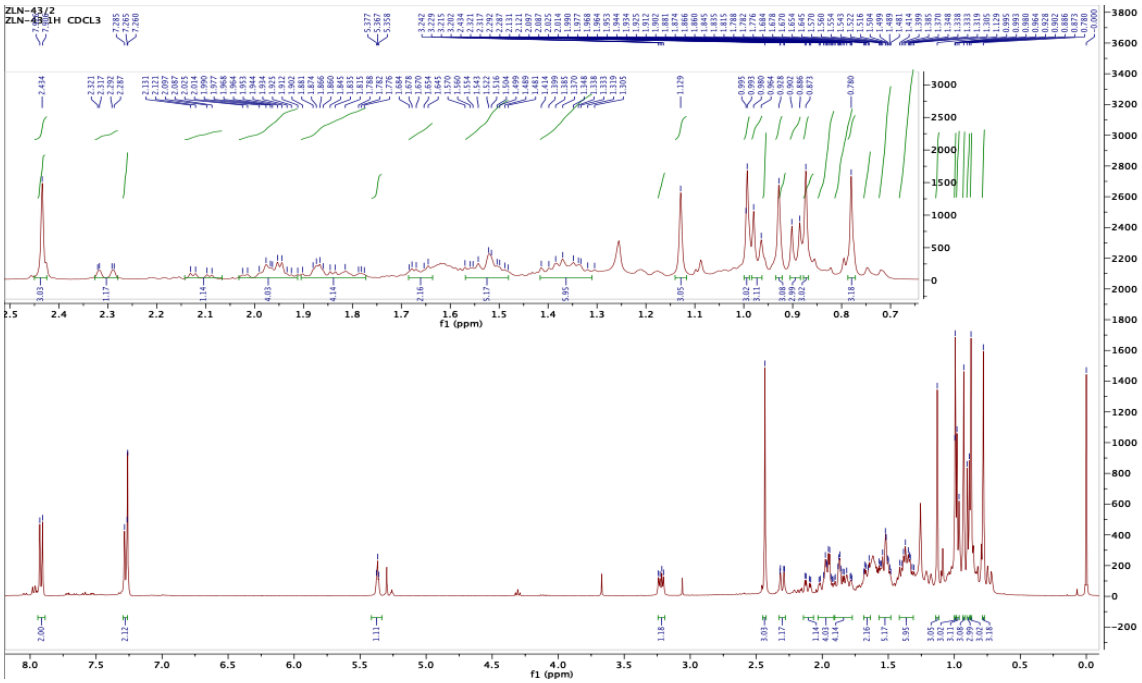
7c ^{1}H NMR



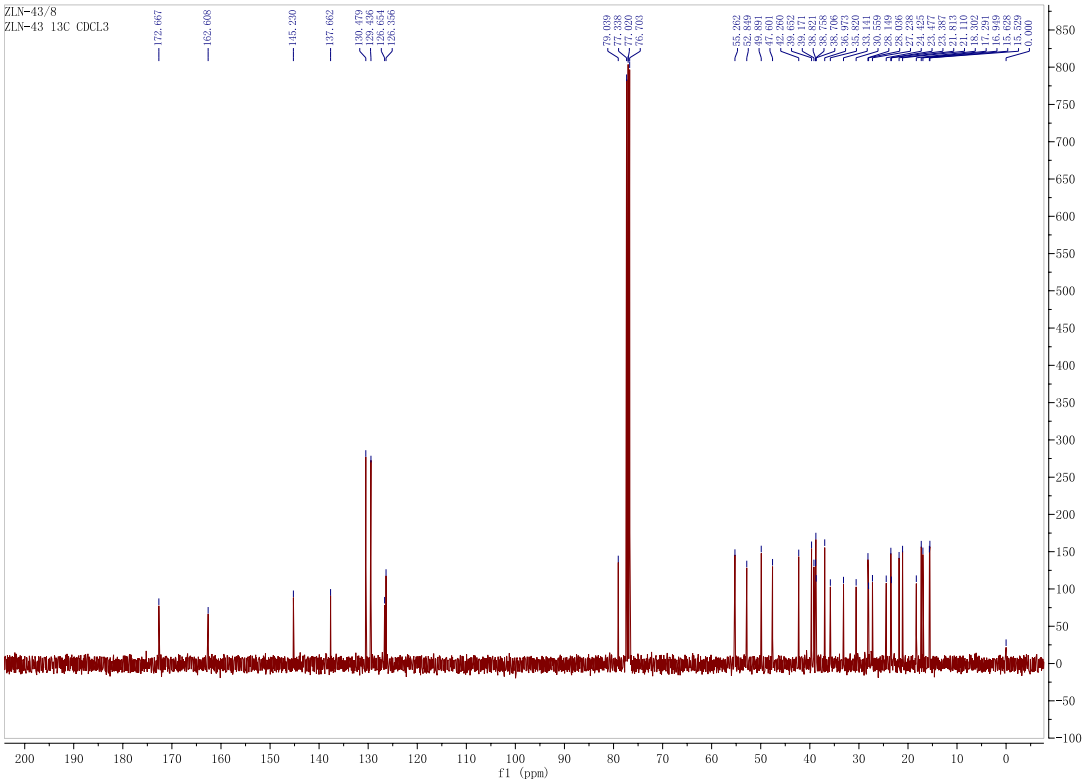
7c ^{13}C NMR



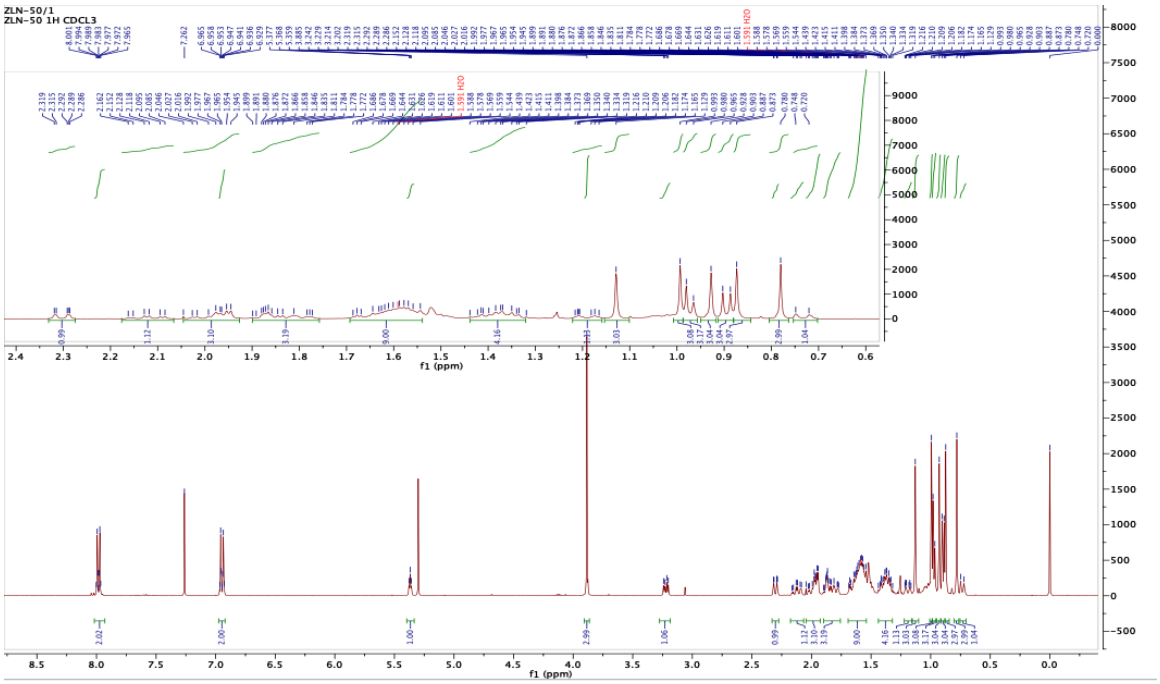
7d ^{1}H NMR



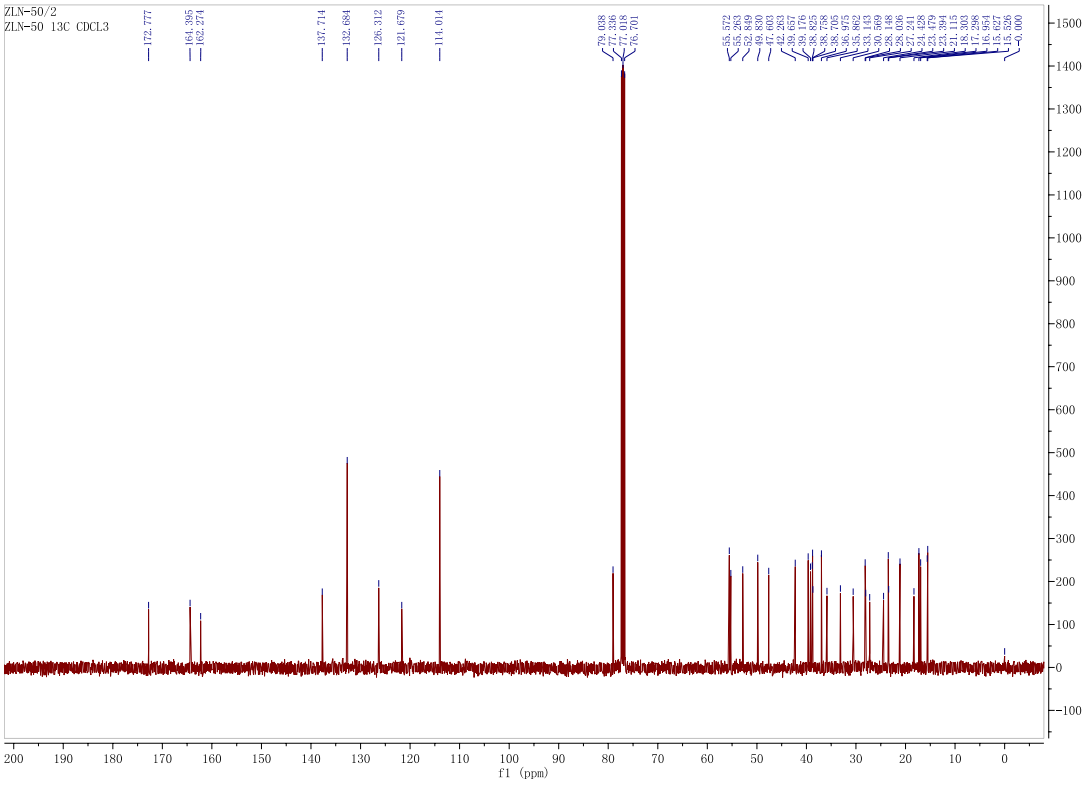
7d ^{13}C NMR



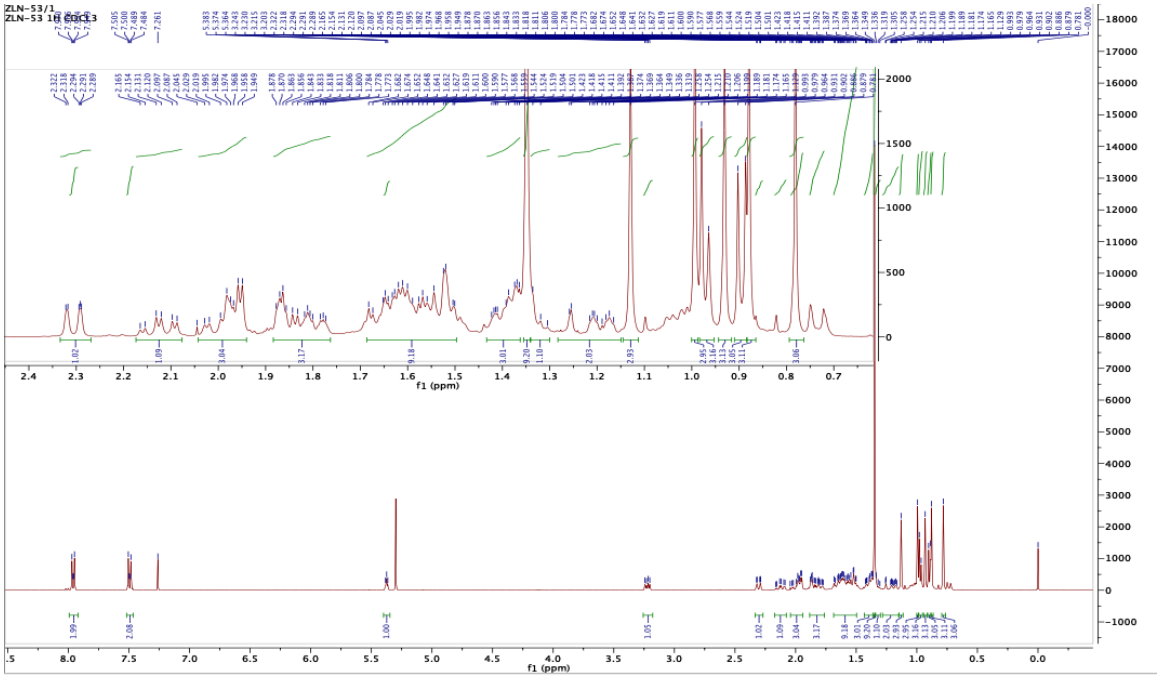
7e ^{1}H NMR



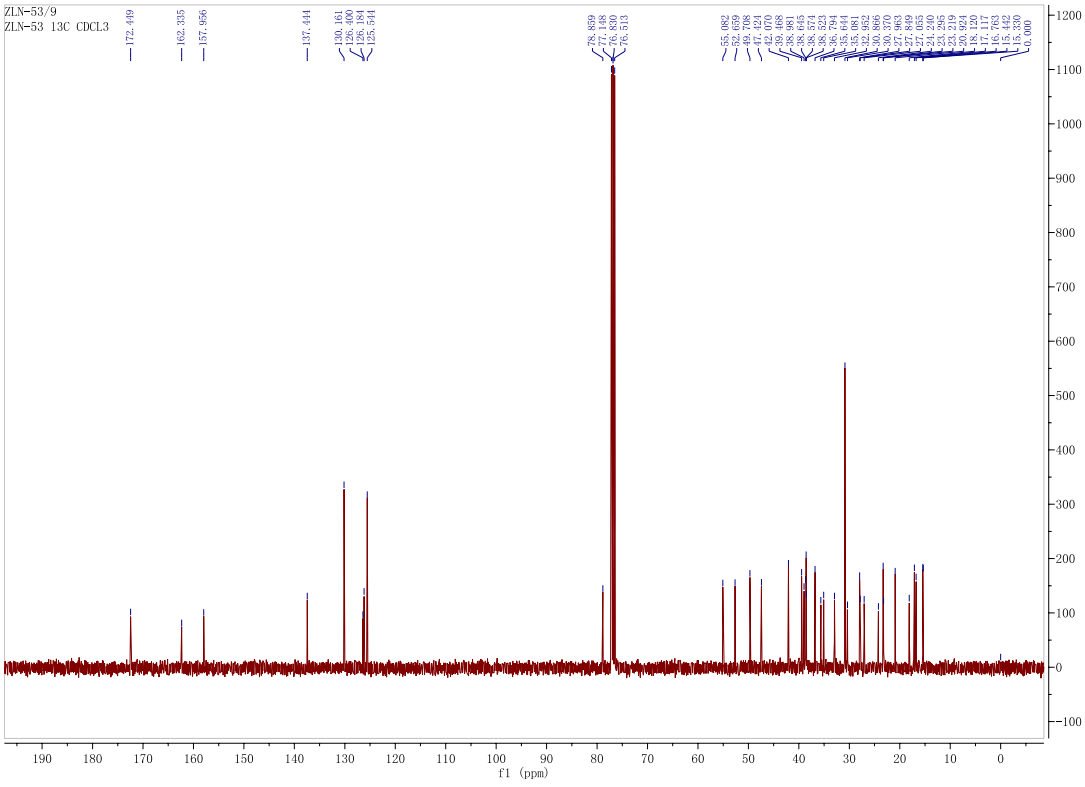
7e ^{13}C NMR



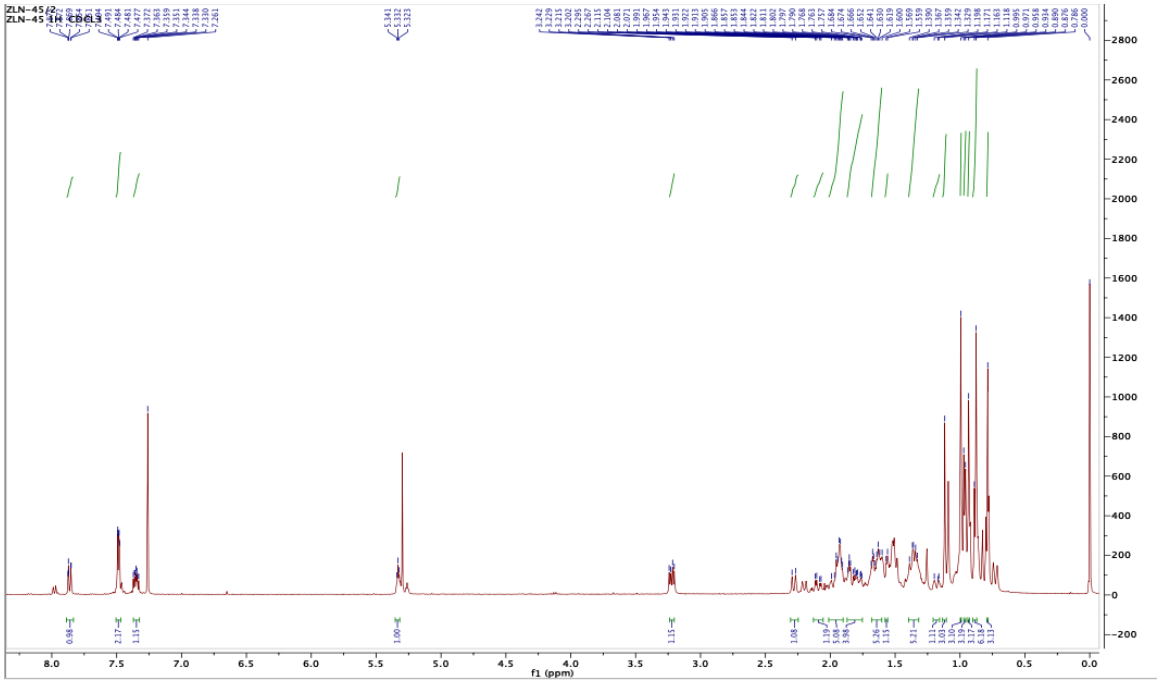
7f ^{1}H NMR



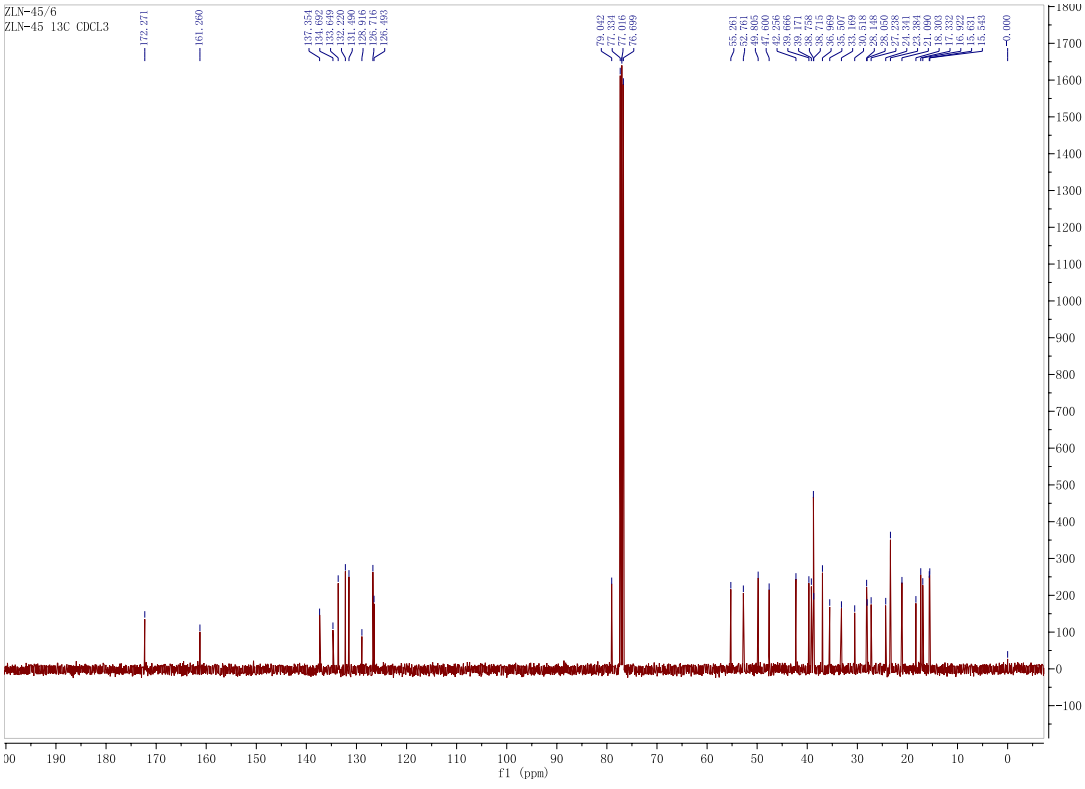
7f ^{13}C NMR



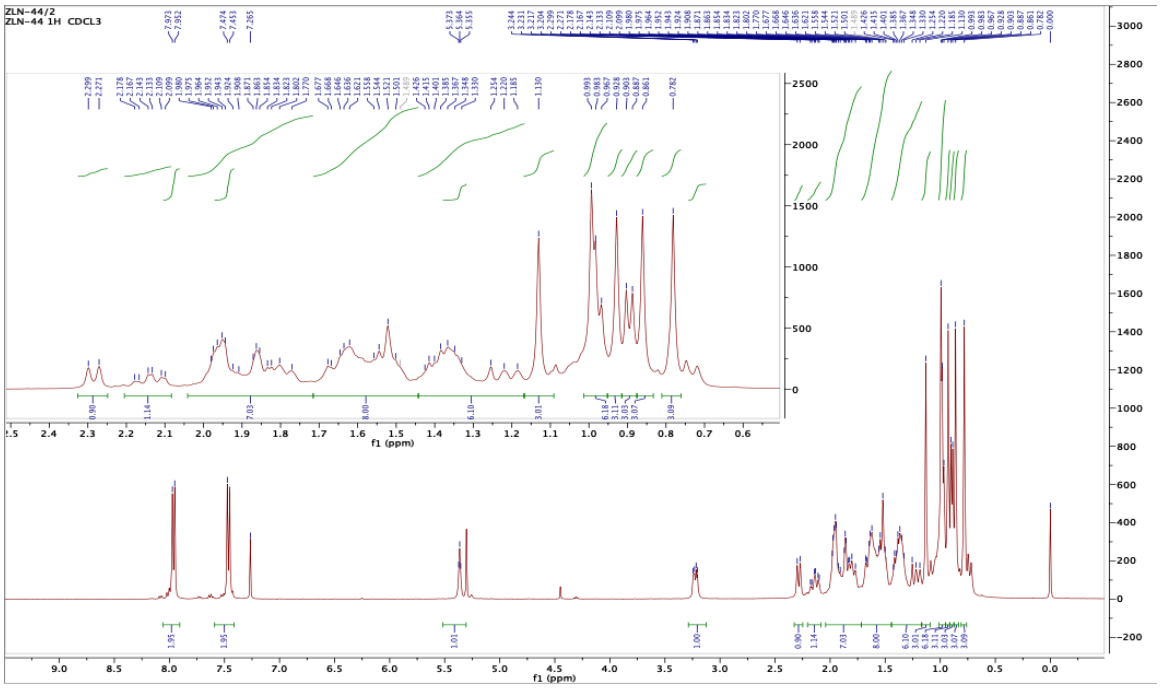
7g ^{1}H NMR



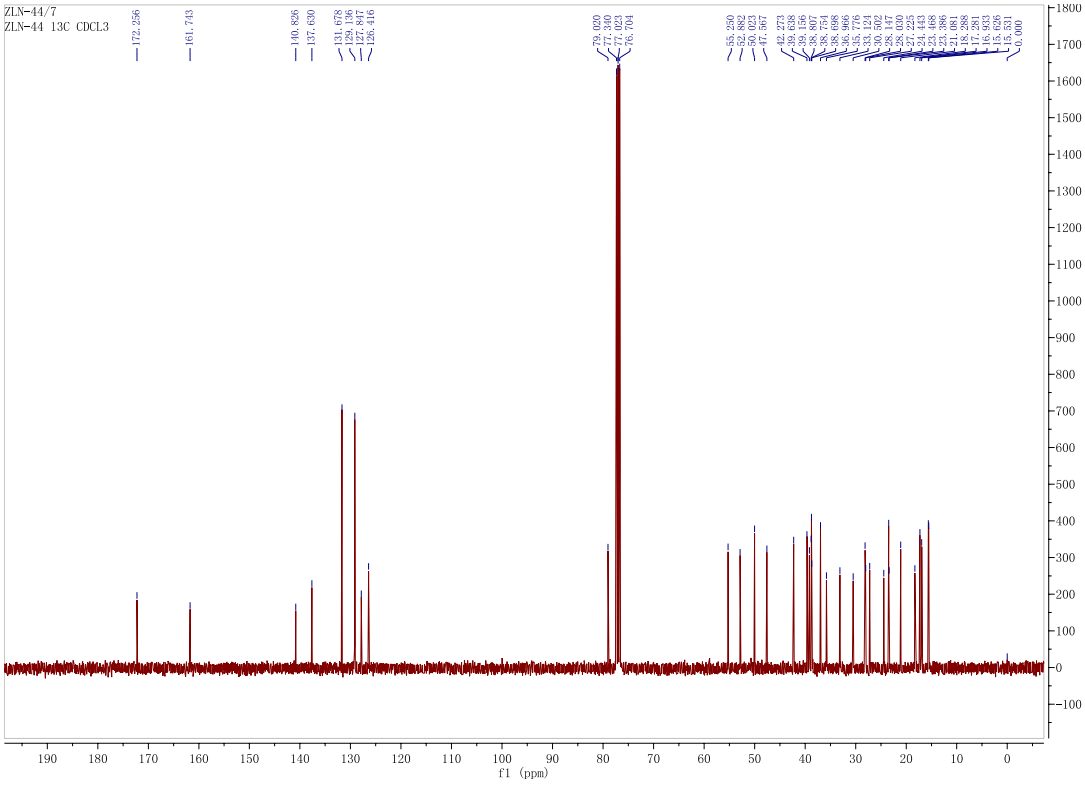
7g ^{13}C NMR



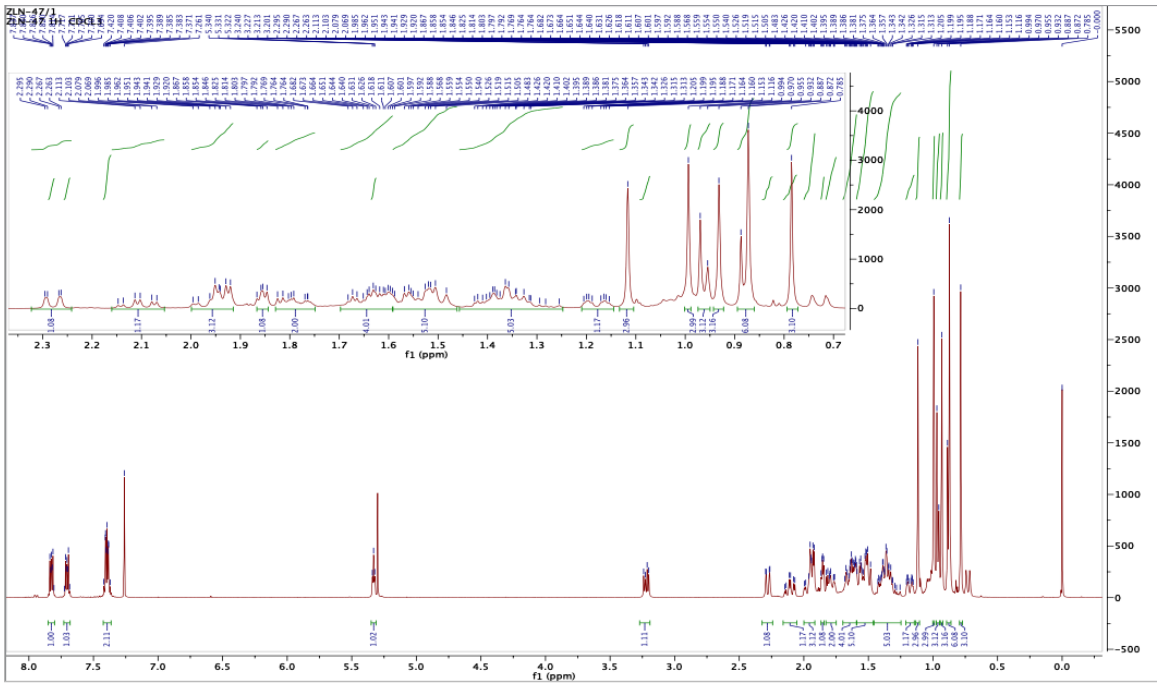
7h ^{1}H NMR



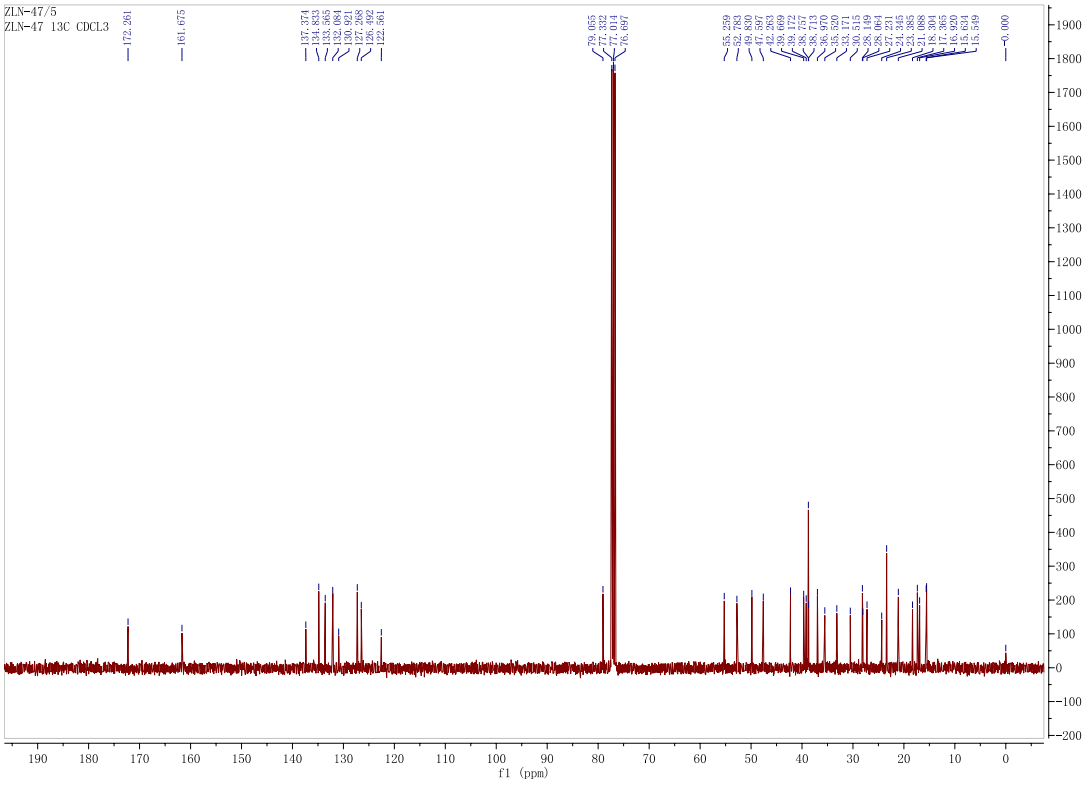
7h ^{13}C NMR



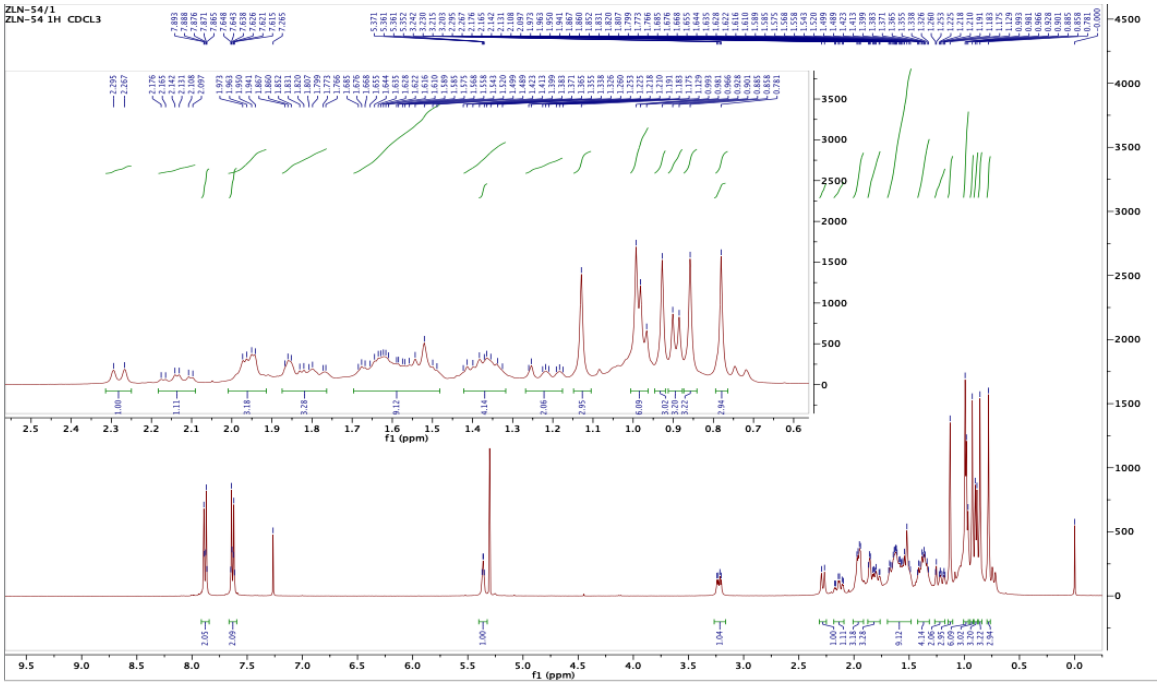
7i ^{1}H NMR



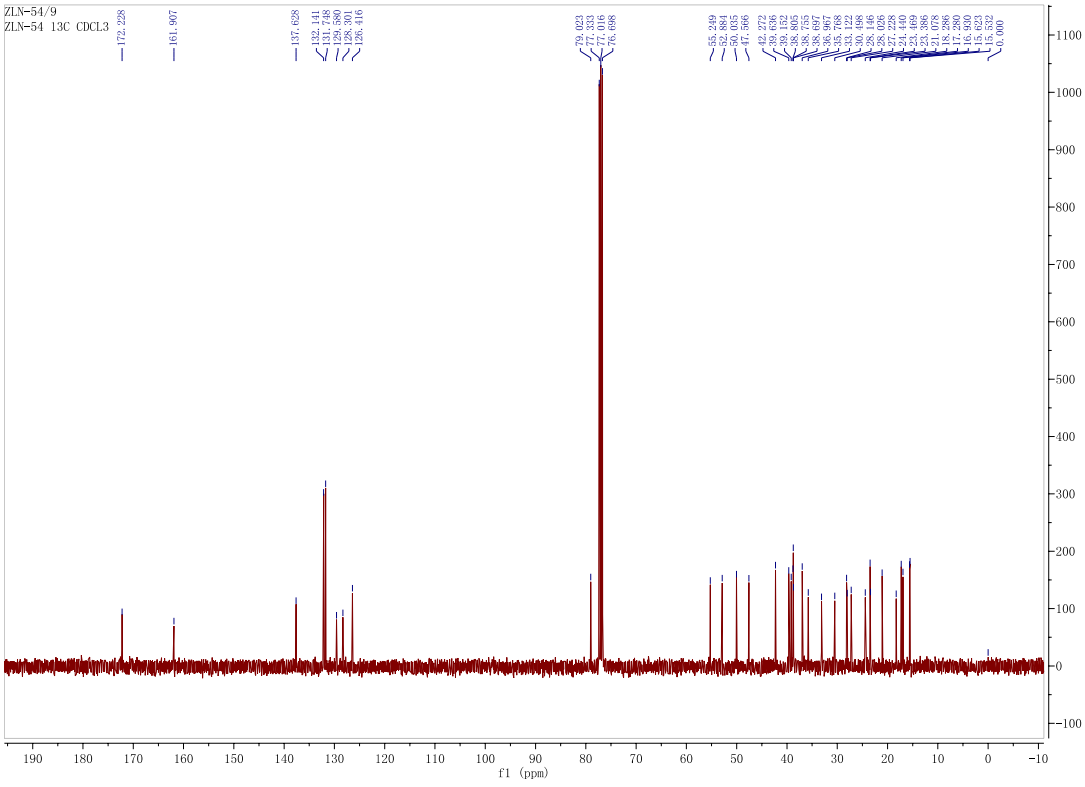
7i ^{13}C NMR



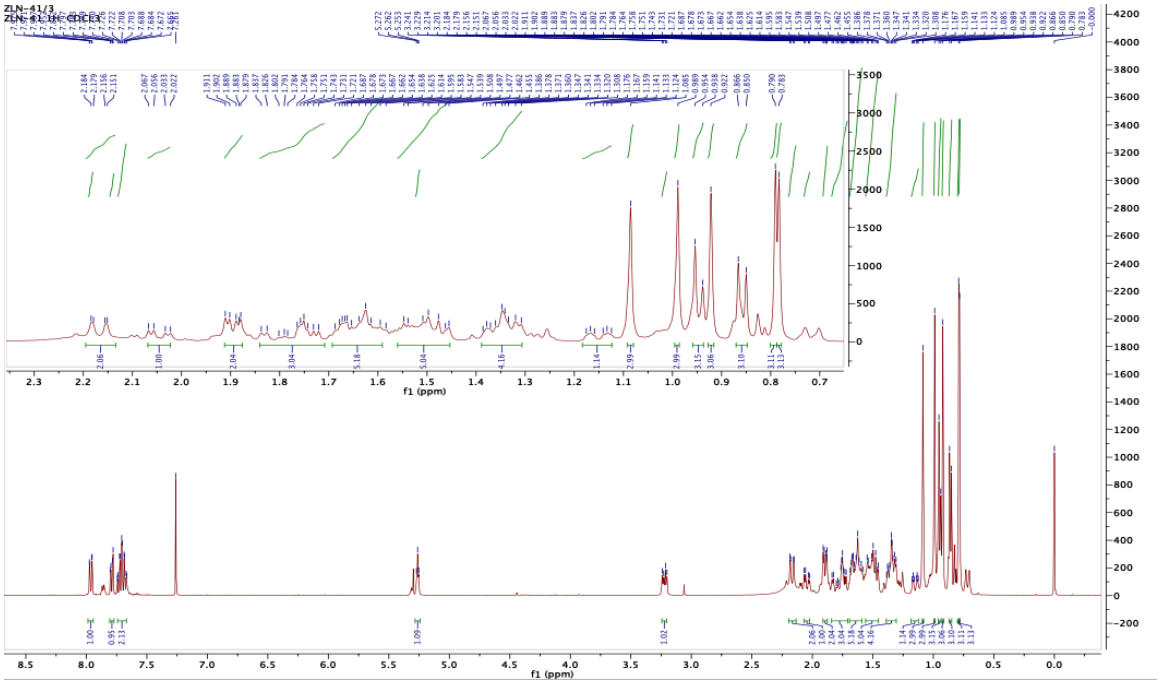
7j ^{1}H NMR



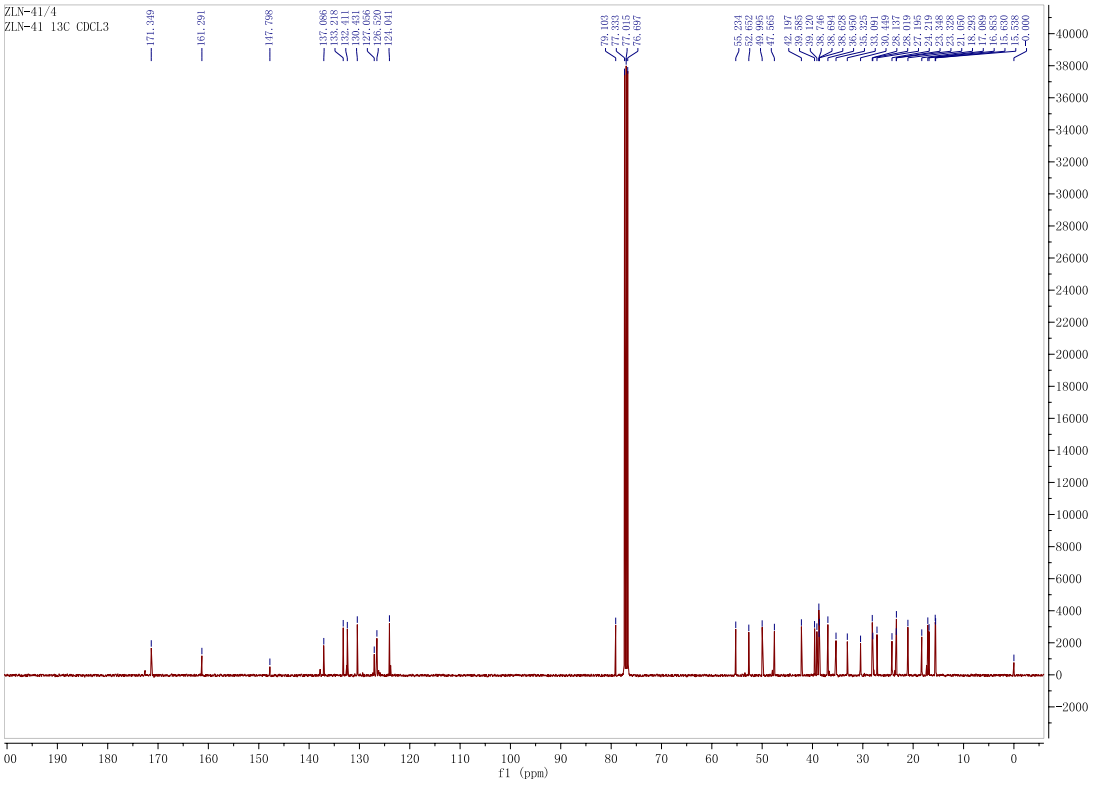
7j ^{13}C NMR



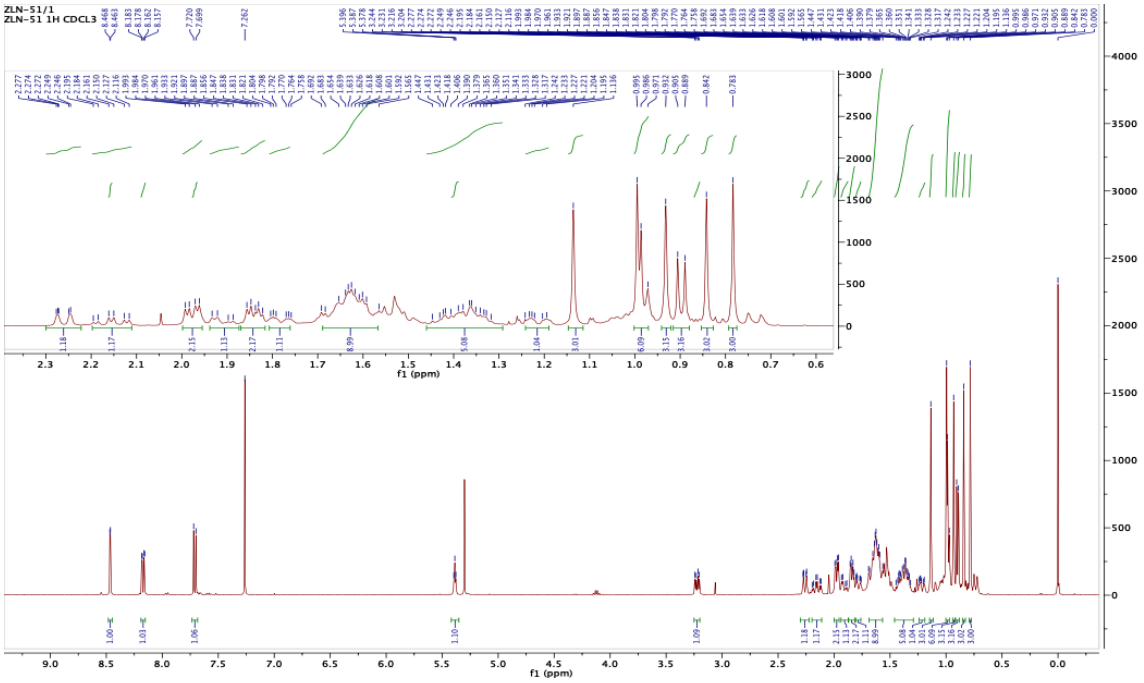
7k ^{1}H NMR



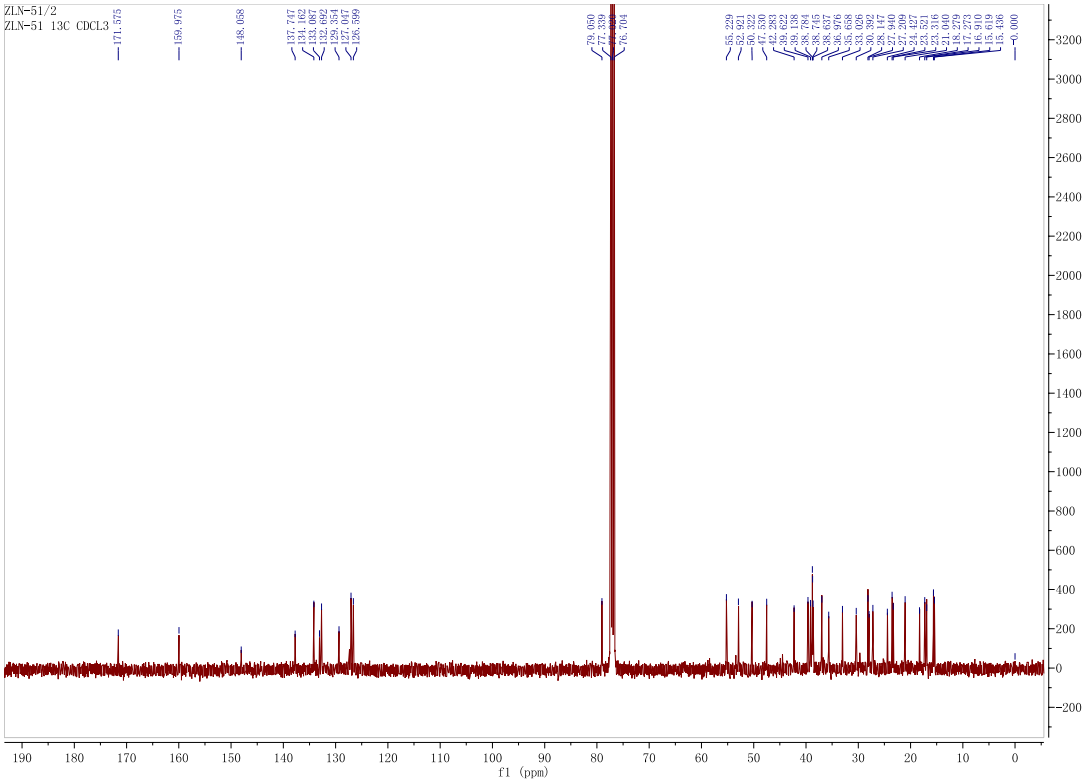
7k ^{13}C NMR



7l ^{1}H NMR



7l ^{13}C NMR



5.0 Copies of HRMS spectra of compounds 3 and 7a-l

3 HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1231

Sample Serial Number: ZLN-94

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:
Target m/z = 547.4146 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9

Additional Search Restrictions:
DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:
Number of Hits = 4

m/z	Delta m/z	DBE	Formula
547.41506	0.00199	37.0	$C_{37}H_{55}O_3^{+1}$
547.41592	-0.00042	22.0	$C_{38}H_{29}NO_3^{+1}$
547.41800	0.00140	17.5	$C_{32}H_{41}N_3O_3S^{+1}$
547.41692	-0.00020	41.5	$C_{33}H_{27}N_2O_4S^{+1}$

7a HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1232

Sample Serial Number: ZLN-52

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:
Target m/z = 561.3938 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9

Additional Search Restrictions:
DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:
Number of Hits = 3

m/z	Delta m/z	DBE	Formula
561.39766	0.00010	27.0	$C_{32}H_{34}ClN_2O_5^{+1}$
561.39411	-0.00123	31.0	$C_{37}H_{53}O_4^{+1}$
561.39432	0.00036	18.5	$C_{41}H_{21}OS^{+1}$

7b HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1233

Sample Serial Number: ZLN-42

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:

Target m/z = 575.4095 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:

Number of Hits = 4

m/z	Delta m/z	DBE	Formula
575.40961	0.00112	37.0	$C_{38}H_{55}O_4^{+1}$
575.40632	-0.00128	22.0	$C_{37}H_{69}NO_3^{+1}$
575.40914	0.00140	17.5	$C_{32}H_{37}N_3O_5S^{+1}$
575.40711	-0.00156	41.5	$C_{33}H_{37}NO_4S_2^{+1}$

7c HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1234

Sample Serial Number: ZLN-40

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:
Target m/z = 575.4095 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9

Additional Search Restrictions:
DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:
Number of Hits = 5

m/z	Delta m/z	DBE	Formula
575.40996	-0.00056	26.0	$C_{38}H_{55}O_4^{+1}$
575.40850	0.00070	37.5	$C_{33}H_{67}ClFN_3O^{+1}$
575.40004	-0.00084	45.5	$C_{32}H_{37}N_3O_5S^{+1}$
575.40823	0.00097	18.0	$C_{29}H_{61}N_5O_6^{+1}$
575.40598	-0.00171	22.5	$C_{37}H_{41}N_3O_3^{+1}$

7d HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1235

Sample Serial Number: ZLN-43

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:
Target m/z = 575.4095 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9

Additional Search Restrictions:
DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:
Number of Hits = 3

m/z	Delta m/z	DBE	Formula
575.40950	0.00050	37.5	$C_{38}H_{55}O_4^{+1}$
575.40598	-0.00076	26.0	$C_{37}H_{69}NO_3^{+1}$
575.40823	0.00077	18.0	$C_{41}H_{55}N_2^{+1}$

7e HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1236

Sample Serial Number: ZLN-50

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:

Target m/z = 591.4044 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:

Number of Hits = 3

m/z	Delta m/z	DBE	Formula
591.40466	0.00127	23.0	$C_{38}H_{55}O_5^{+1}$
591.40721	-0.00044	17.0	$C_{35}H_{63}N_2O_5^{+1}$
591.40633	0.00032	14.5	$C_{33}H_{45}N_5O_5^{+1}$

7f HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1237

Sample Serial Number: ZLN-53

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:
Target m/z = 617.4564 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9

Additional Search Restrictions:
DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:
Number of Hits = 3

m/z	Delta m/z	DBE	Formula
617.45651	0.00126	42.5	$C_{41}H_{61}O_4^{+1}$
617.45693	-0.00053	34.0	$C_{37}H_{65}N_2O_5^{+1}$
617.45754	0.00039	22.5	$C_{44}H_{47}N_3^{+1}$

7g HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1238

Sample Serial Number: ZLN-45

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:

Target m/z = 595.3549 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9
Cl	34.968853	0	2

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:

Number of Hits = 4

m/z	Delta m/z	DBE	Formula
595.35535	0.00086	37.5	$C_{37}H_{52}ClO_4^{+1}$
595.35415	0.00114	18.0	$C_{36}H_{41}N_3O_5^{+1}$
595.35080	-0.00154	22.5	$C_{39}H_{37}N_3O_3^{+1}$
595.35113	-0.00182	42.0	$C_{37}H_{45}N_3O_4^{+1}$

7h HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1239

Sample Serial Number: ZLN-44

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:

Target m/z = 595.3549 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9
Cl	34.968853	0	2

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:

Number of Hits = 4

m/z	Delta m/z	DBE	Formula
595.35444	0.00056	37.5	$C_{35}H_{38}ClN_5S^{+1}$
595.35512	0.00084	18.0	$C_{37}H_{52}ClO_4^{+1}$
595.35484	-0.00184	22.5	$C_{36}H_{29}Cl_2O_4^{+1}$
595.35084	-0.00212	42.0	$C_{39}H_{37}N_3O_3^{+1}$

7i HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1240

Sample Serial Number: ZLN-47

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:
Target m/z = 639.3043 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9
Br	78.918338	0	1

Additional Search Restrictions:
DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:
Number of Hits = 5

m/z	Delta m/z	DBE	Formula
639.30486	0.00075	37.5	$C_{37}H_{52}BrO_4^{+1}$
639.30398	0.00102	18.0	$C_{44}H_{53}N_3O^{+1}$
639.30067	-0.00107	26.5	$C_{41}H_{41}N_3O_2S^{+1}$
639.30117	-0.00125	47.0	$C_{36}H_{41}N_5O_6^{+1}$
639.30133	-0.00126	22.5	$C_{38}H_{40}F_3N_5O^{+1}$

7g HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1241

Sample Serial Number: ZLN-54

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:

Target m/z = 639.3043 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
O	15.994915	0	9
Br	78.918338	0	1

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:

Number of Hits = 5

m/z	Delta m/z	DBE	Formula
639.30444	-0.00028	18.0	$C_{37}H_{52}BrO_4^{+1}$
639.30385	-0.00055	37.5	$C_{38}H_{33}N_5O_5^{+1}$
639.30377	-0.00247	26.5	$C_{44}H_{37}N_3O^{+1}$
639.30125	-0.00275	46.0	$C_{36}H_{41}N_5O_6^{+1}$
639.30518	0.00292	31.5	$C_{36}H_{39}N_4O_5S^{+1}$

7k HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1242

Sample Serial Number: ZLN-41

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:

Target m/z = 606.3789 ± 0.003
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
N	14.003074	0	1
O	15.994915	0	11

Additional Search Restrictions:

DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:

Number of Hits = 3

m/z	Delta m/z	DBE	Formula
606.37923	0.00157	36.5	$C_{37}H_{52}NO_6^{+1}$
606.37818	0.00212	38.5	$C_{35}H_{42}O_5S_2^{+1}$
606.37490	0.00240	19.0	$C_{36}H_{30}O_9^{+1}$

7l HRMS

Shanghai Mass Spectrometry Center
Shanghai Institute of Organic Chemistry
Chinese Academic of Sciences
High Resolution MS DATA REPORT

Instrument: IonSpec 4.7 Tesla FTMS

Card Serial Number: WI11 1243

Sample Serial Number: ZLN-51

Date: 2022/12/20

Operation Mode: MALDI/DHB

Elemental Composition Search Report:

Target Mass:
Target m/z = 640.3399 ± 0.0025
Charge = +1

Possible Elements:

Element:	Exact Mass:	Min:	Max:
C	12.000000	0	100
H	1.007825	0	100
N	14.003074	0	1
O	15.994915	0	11
Cl	34.968853	0	2

Additional Search Restrictions:
DBE Limit Mode = Both Integer and Half-Integer
Minimum DBE = 0

Search Results:
Number of Hits = 4

m/z	Delta m/z	DBE	Formula
640.34036	0.00034	47.5	$C_{37}H_{51}ClNO_6^{+1}$
640.34487	0.00043	36.5	$C_{44}H_{26}Cl_2O^{+1}$
640.34575	-0.00045	31.5	$C_{38}H_{24}O_{10}^{+1}$
640.34623	-0.00093	27.5	$C_{35}H_{27}ClNO_9^{+1}$

6.0 Bioassay Method

The inhibitory activities of one 28-benzyloxy UA derivatives (**3**) and twelve 3-acyloxy UA derivatives (**7a-l**) against *P. capsici* and *F. graminearum* were screened *in vitro*. Potato dextrose agar (PDA) medium was prepared in the flasks and sterilized. The target compounds **3** and **7a-l** were dissolved with dimethyl sulfoxide (DMSO, 1 mL) before mixing with PDA, and Tween 80 (0.01 mL) was added to increase water solubility. The concentration ranges for the assays were defined in the preliminary experiments. DMSO was mixed with PDA as a blank control, while two different commercial agricultural fungicides (Metalaxyl and Triadimefon) were used as positive controls. The inhibitory activities of the two tested strains at 50 and 100 mg/L or 100 and 200 mg/L concentrations were determined respectively, and some compounds with better activity were selected to determine the value of the median effective concentration (EC_{50}). The final concentration of EC_{50} in the medium was determined as 25, 50, 75, 100, 200 mg/L. The medium was poured into the sterilized petri dishes, and the mycelial plugs (7-mm diameter) were cut from the growth edge of a 4-day-old colony and then placed on the PDA plates, and incubated in darkness at 25 ℃, respectively. Mycelial growth rate (mm / 4 days) was measured on PDA medium, each compound was evaluated three plates, and the average colony diameter was calculated. The radial growths of colonies were measured and the data were analyzed statistically. Mean colony diameter (minus the diameter of the plug) for each treatment was measured and expressed as a percentage of growth inhibition. The EC_{50} of **7c, 7d, 7g, 7h, 7j, 7k** and metalaxyl for *P. capsici* and **7h** and triadimefon for *F.*

graminearum isolate was calculated by linear regression of relative percentage of growth inhibition against log-transformed samples concentration.