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Photo-induced synthesis and antitumor activity of marine Zygosporamide analogs with isoindolinone fragment

Lishuang Zhao,[a] Yuanhao Deng[a], Yuxin Ji[a], Fujing Guan*[b], Yujun Bao*[c], Xuewei Li *[a] and Liguo Wei*[a]

[a] College of Environmental and Chemical Engineering, Heilongjiang University of Science and Technology, Harbin, 150025, China. Email: hsdzls@163.com (Z.L.); xwli2020@163.com (L.X.).

[b] Harbin Center for Disease Control and Prevention, Harbin 150036, China.

[c] College of Life Science and Technology, Harbin Normal University, Harbin, 150025, China

Contents

Experimental

General

Boc-L-leucine (13139-15-6), Boc-D-leucine (16937-99-8), Boc-L-phenylalanine (13734-34-4), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinline (EEDQ, 16357-59-8), *N*-[(trimethylsilyl)methyl]benzylamine (BnTMS, 53215-95-5), phthalylglycyl chloride (6780-38-7) and trifluoroacetic acid (TFA, 76-05-1) were purchased from Energy Chemical. Dichloromethane, methanol, ethyl acetate, petroleum ether, 1,4-dioxane were analytical reagent. Dulbecco's modied eagle medium (DMEM), penicillin, fetal bovine serum (FBS), and streptomycin were purchased from Beijing Dingguo Biotechnology Co. Phosphatebuffered saline (PBS) purchased from Invitrogen (10010) was used as a balanced salt solution in cell culture. All the solvents were distilled and puried by standard procedures. All the above chemicals reagents were used without further purification. ^{1}H and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, on an AMX400 spectrometer (Bruker, Bremen, Germany) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a JEOL JMS-700 spectrometer using the fast atom bombardment (FAB) or electron impact (EI) mode. A 450 W Hanovia medium-pressure mercury lamp surrounded by a Pyrex glass filter (λ > 290 nm) was used for electronic excitation.

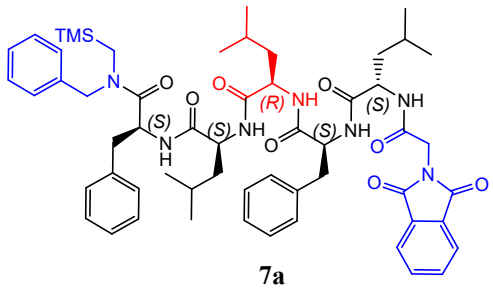
Preparation of trimethylsilylbenzylamido peptides

The Boc-L-phenylalanine (2.65 g, 10 mmol) and *N*-[(Trimethylsilyl) methyl]benzylamine (1.93 g, 10 mmol) was dissolved in 30 mL of anhydrous dichloromethane, EEDQ (2.96 g, 12 mmol in 10 mL of dichloromethane) was added dropwise with stirring at room temperature. After the reaction, the reaction solution was washed twice with 20 mL of water, the organic layer was dried over anhydrous sodium sulfate and concentrated, the residue was purified by silica gel column chromatography (mobile phase V_{EA}/V_{PE} = 1:3) to obtain pure *N*-Boc-Phe-Si$(CH_3)_3$ (white solid). The *N*-Boc-Phe-Si$(CH_3)_3$ was dissolved in 20 mL of anhydrous dichloromethane and added dropwise 10 mL of trifluoroacetic acid, then stirred for 2 h. After removal of trifluoroacetic acid and dichloromethane was concentrated, the residue was dispersed in 15 mL of dichloromethane, washed twice with 15 mL of water, dried over anhydrous sodium sulfate and concentrated to give a chemically pure Phe-Si$(CH_3)_3$ 2.89 g (85%, white solid). The Phe-Si$(CH_3)_3$ (2.00 g, 7 mmol) and Boc-L- leucine (1.70 g, 7 mmol) was dissolved in 25 mL of anhydrous dichloromethane. EEDQ (1.74 g, 8.4 mmol in 8 ml of dichloromethane) was added dropwise with stirring at room temperature for 20 h. After completion of the reaction, the reaction solution was washed twice with 20 mL of water, the organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was dissolved in 20 mL of anhydrous dichloromethane, added dropwise 10 mL of trifluoroacetic acid and stirred for 2 h. After removal of trifluoroacetic acid, the residue was dispersed in 15 mL of dichloromethane, washed twice with 15 mL of water, dried over anhydrous sodium sulfate and concentrated to give Leu-Phe-Si$(CH_3)_3$ 2.31 g (87%, yellow solid). The same method was used for synthesis of Leu(D)-Leu(L)-Phe(L)-Si$(CH_3)_3$, Leu(L)-Leu(D)-Phe(L)-Si$(CH_3)_3$, Phe(L)-Leu(D)-Leu(L)-Phe(L)-Si$(CH_3)_3$, Phe(L)-Leu(L)-Leu(D)-Phe(L)-Si$(CH_3)_3$, Leu(L)-Phe(L)-Leu(D)-Leu(L)-Phe(L)-Si$(CH_3)_3$ and Leu(L)-Phe(L)-Leu(L)-Leu(D)-Phe(L)-Si$(CH_3)_3$.

Preparation of *N*-Phthalimido-Gly-Leu(L)-Phe(L)-Leu(D)-Leu(L)-Phe(L)-Si$(CH_3)_3$ and *N*-Phthalimido-Gly-Leu(L)-Phe(L)-Leu(L)-Leu(D)-Phe(L)-Si$(CH_3)_3$

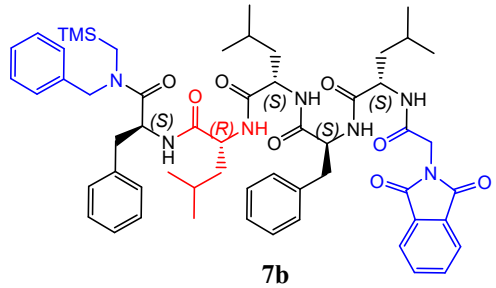
Leu(L)-Phe(L)-Leu(D)-Leu(L)-Phe(L)-Si$(CH_3)_3$ (1.5 g, 10 mmoL) and triethylamine (5 mL) were dissolved in anhydrous dichloromethane, then phthalimide acetyl chloride (0.48 g, 12 mmoL, in 3 mL of 1,4-dioxane) was added dropwise. After stirring at room temperature for 6 h, the reaction solution washed twice with 16 mL of water. The organic layer

was dried over anhydrous sodium sulphate, then concentrated and purified by silica gel column chromatography (mobile phase V_{EA}/V_{PE} = 3:1) to obtain a white solid. The similar method was used for synthesis of *N*-Phthalimido-Gly-Leu(L)-Phe(L)-Leu(D)-Leu(L)-Phe(L)-Si(CH_3)$_3$ (**7a**) and *N*-Phthalimido-Gly-Leu(L)-Phe(L)-Leu(L)-Leu(D)-Phe(L)-Si(CH_3)$_3$ (**7b**).



7a

N*-Phthalimido-Gly-Leu(L)-Phe(L)-Leu(D)-Leu(L)-Phe(L)-Si(CH_3)$_3$** (**7a**) white solid (yield 82%). ^{1}H-NMR($CDCl_3$, 400 MHz) ***δ : -0.18~0.18(m, 9H, $SiMe_3$), 0.81~1.01(m, 18H, CH_3), 1.22~1.42(m, 3H, **CH**(CH_3)$_2$), 1.49~1.81(m, 6H, CH**CH$_2$**CH), 2.80~3.01(m, 2H, CH**CH$_2$**Ph), 3.05~3.31(m, 2H, CH**CH$_2$**Ph), 3.53~3.90(m, 2H, N**CH$_2$**Ph), 3.92~4.19(m, H, NH**CH**CO), 4.20~4.58(m, 4H, N**CH$_2$**CO and N**CH$_2$**C(OH)), 4.60~4.75(m, 2H, NH**CH**CO and NH**CH**CO), 4.78~4.98(m, 1H, NH**CH**CO), 5.08~5.38(m, 1H, NH**CH**CO), 6.78~7.45(m, 15H, ArH), 7.63~7.75(m, 2H, ArH), 7.80~7.99(m, 2H, ArH); ^{13}C-NMR($CDCl_3$, 400 MHz) ***δ*** : 0.0, 8.5, 11.4, 15.4, 23.0, 23..9, 24.2, 25.8 25.9, 26.0, 30.9, 35.9, 36.4, 37.7, 38.3, 39.8, 45.0, 46.8, 53.4, 54.6, 55.3, 64.0, 68.3, 123.3, 125.3, 127.2, 128.0, 129.5, 129.7, 129.9, 130.1, 130.3, 130.7, 130.8, 134.1, 160.2, 162.3, 164.8, 164.9, 165.3, 165.5, 169.0, 171.7. HR-MS (ESI) m/z calcd for $C_{57}H_{75}N_7O_8Si^+$ ($M+Na^+$) 1036.53386, found 1036.53296.

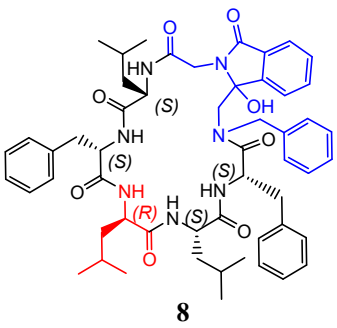


7b

N*-Phthalimido-Gly-Leu(L)-Phe(L)-Leu(L)-Leu(D)-Phe(L)-Si(CH_3)$_3$** (**7b**) white solid (yield 79%). ^{1}H-NMR($CDCl_3$, 400 MHz) ***δ : -0.18~0.19(m, 9H, $SiMe_3$), 0.81~1.01(m, 18H, CH_3), 1.21~1.59(m, 6H, CH**CH$_2$**CH), 1.63~1.75(m, 3H, **CH**(CH_3)$_2$), 2.20~3.05(m, 4H, **CH$_2$**$SiMe_3$ and CH**CH$_2$**Ph), 3.10~3.47(m, 2H, CH**CH$_2$**Ph), 3.63~4.00(m, 1H, NH**CH**CO), 4.13~4.26(m, 2H, N**CH$_2$**Ph), 4.27~4.53(m, 3H, N**CH$_2$**CO and NH**CH**CO), 4.57~4.68(m, 1H, NH**CH**CO), 4.76~5.01(m, 1H, NH**CH**CO), 5.09~5.20(m, 1H, NH**CH**CO), 6.81~7.30(m, 15H, ArH), 7.65~7.75(m, 2H, ArH), 7.76~7.87(m, 2H, ArH); ^{13}C-NMR($CDCl_3$, 400 MHz) ***δ*** : 0.0, 9.7, 9.9, 17.4, 17.8, 18.7, 20.1, 20.2, 22.7, 23.7, 24.1, 24.5, 25.8, 25.9, 26.0, 36.8, 40.9, 41.4, 45.2, 48.5, 52.7, 52.8, 54.6, 120.2, 124.8, 126.7, 127.9, 129.7, 130.1, 131.8, 133.3, 135.4, 137.4, 137.9, 164.8, 166.2, 166.7, 166.9, 168.8, 170.3, 171.6, 172.0. HR-MS (ESI) m/z calcd for $C_{57}H_{75}N_7O_8SiNa^+$ $(M+Na)^+$ 1036.53386, found 1036.53308.

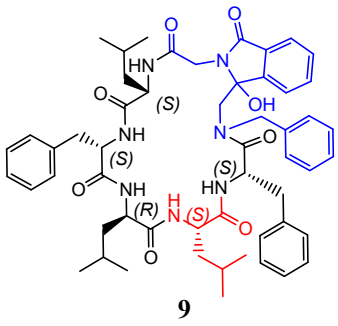
Irradiation of 7a and 7b to obtain cyclic peptide 8 and 9

Nitrogen purged solutions of the substrates in the indicated solvents were irradiated by using Pyrex glass filtered light in an water cooled immersion reactor for time periods required. Concentration of the photoproducts were followed by column chromatography to yield the pure products listed below. In brief, 0.5 g of 7**a** or **7b** in 200 mL of anhydrous methanol were placed in a reactor, then ventilated nitrogen flow for 40 min. Upon maintaining the ventilation of nitrogen, the solutions were irradiated by ultraviolet light (Pyrex tube filtered-light λ > 290 nm).



8

3-Hydroxy-isoindolinone-cyclo-Gly-Leu(L)-Phe(L)-Leu(D)-Leu(L)-Phe(L) (**8**) white solid (yield 44%). ^{1}H-NMR($CDCl_3$, 400 MHz) ***δ*** : 0.75~1.03(m, 18H, CH_3), 1.35~1.45(m, 3H, **CH**(CH_3)$_2$), 1.46~1.75(m, 6H, CH**CH$_2$**CH), 2.87~3.01(m, 2H, CH**CH$_2$**Ph), 3.02~3.35(m, 2H, CH**CH$_2$**Ph), 3.61~3.80(m, 2H, N**CH$_2$**Ph), 3.83~4.34(m, 2H, N**CH$_2$**CO), 4.35~4.51(m, 2H, N**CH$_2$**C(OH)), 4.53~4.77(m, 2H, NH**CH**CO and NH**CH**CO), 4.78~5.18(m, 1H, NH**CH**CO), 5.25~5.40(m, 2H, NH**CH**CO and NH**CH**CO), 6.75~8.01(m, 19H, ArH); ^{13}C-NMR($CDCl_3$, 400 MHz) ***δ*** : 15.8, 16.7, 24.0, 24.2, 25.8, 26.0, 26.1, 28.8, 30.9, 38.5, 41.4, 45.0, 47.5, 48.5, 48.7, 49.1, 52.0, 52.8, 54.5, 92.9, 124.9, 128.2, 129.1, 129.7, 129.9, 130.0, 130.6, 133.3, 135.5, 137.1, 166.7, 167.8, 168.6, 168.9, 170.2, 171.7, 172.0. HR-MS (ESI) m/z calcd for $C_{54}H_{67}N_7O_8Na^+$ $(M+Na)^+$ 964.49433, found 964.49402.



9

3-Hydroxy-isoindolinone-cyclo-Gly-Leu(L)-Phe(L)-Leu(L)-Leu(D)-Phe(L) (**9**) white solid (yield 49%).^{1}H-NMR($CDCl_3$, 400 MHz) ***δ*** : 0.73~1.02(m, 18H, CH_3), 1.41~1.50(m, 3H, **CH**(CH_3)$_2$), 1.52~1.75(m, 6H, CH**CH$_2$**CH), 2.75~3.03(m, 2H, CH**CH$_2$**Ph), 3.05~3.35(m, 2H, CH**CH$_2$**Ph), 3.81~4.20(m, 2H, N**CH$_2$**Ph), 4.21~4.40(m, 2H, N**CH$_2$**CO), 4.42~4.58(m, 2H, N**CH$_2$**C(OH)), 4.60~4.75(m, 2H, NH**CH**CO and NH**CH**CO), 4.76~5.03(m, 1H, NH**CH**CO), 5.04~5.41(m, 1H, NH**CH**CO), 5.42~5.51(m, 1H, NH**CH**CO), 6.89~7.81(m, 19H, ArH); ^{13}C-NMR($CDCl_3$, 400 MHz) ***δ*** : 7.7, 8.7, 12.1, 12.7, 21.2, 22.6, 23.2, 25.0, 29.7, 33.1, 34.9, 39.4, 44.0, 44.3, 45.7, 51.4, 52.7, 54.1, 56.7, 58.2, 62.8, 67.0, 90.1, 125.9, 126.5, 126.9, 128.3, 129.0, 131.6, 133.4, 133.7, 136.9, 137.0, 140.1, 142.2, 164.3, 164.4, 164.5, 167.4, 169.1, 169.6, 173.0. HR-MS (ESI) m/z calcd for $C_{54}H_{67}N_7O_8Na^+$ $(M+Na)^+$ 964.49433, found 964.49451.

HPLC of compound 8 and 9

Shiseido Capcell PAK C18 (150×4.0 mm, 5 μm) was used as the column at 30 ℃ , and the mobile phase flow rate was 1 mL/min. During the analytical run, the elution was carried out using mobile phases A (Ultrapure water) and B (acetonitrile), the percentage of mobile phases B was 55%, while the detection wavelength was 192 nm. From HPLC analysis, the retention time of compound **8** is 12.5 min, and the purity of compound **8** is approximately 99.3%. The retention time of compound **9** is 11.5 min, and the purity of compound **9** is approximately 98.2%.

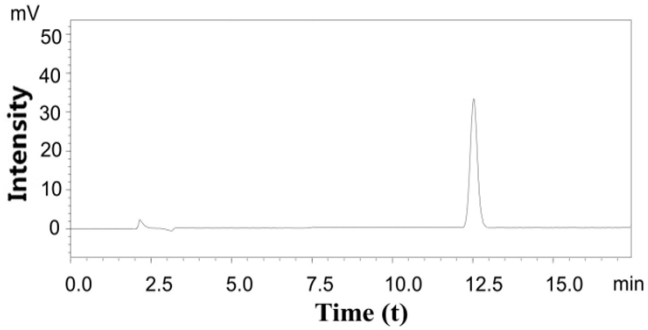


Figure S1. HPLC spectrum of compound **8**.

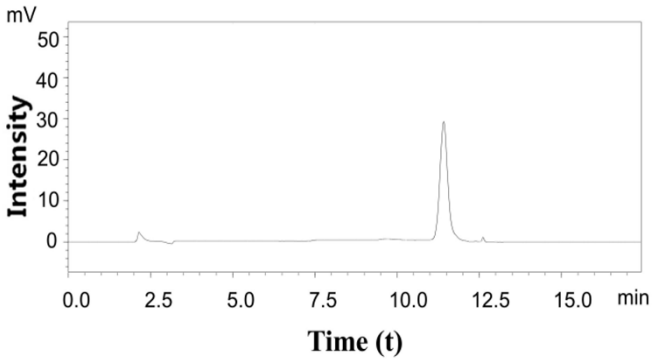


Figure S2. HPLC spectrum of compound **9**.

Cell culture

HepG-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10 % fetal bovine serum (FBS), penicillin G (100 U/mL), and streptomycin (100 g/mL). Cells were cultured at 37 °C in a humidified incubator with 5 % CO_2. Cells were grown on plates and were subcultured after 0.25% trypsin treatment. The experiments were performed when the ratio of cell fusion reached 80%.

MTT assay for cell viability

The activity test were grouped into two groups: cyclic peptide groups and blank group without peptides. Cells were seeded in a 96-well plate at an initial density of 4×10^3 cells per well in DMEM complete medium and incubated at 37 °C in 5% CO_2 for 24 h. Then, they were treated with various concentrations of samples. Each dosage was replicated in six wells. After 48 h incubation, MTT dyes (100 μL, 0.5 mg/mL) were added to the wells, and incubated for 4 h. The MTT solutions were then removed and 150 μL of DMSO was added to dissove the formazan crystals generated. Then microplate reader was used to detect the absorbance of each well at 490 nm. Cell viability (%) was calculated by the following formula:

$$\text{Cell viability (\%)} = A_{490\ (\text{sample})}/A_{490\ (\text{control})} \times 100\%$$

where $A_{490\ (\text{sample})}$ represents A values of the wells treated with various concentrations of samples, and $A_{490\ (\text{control})}$ represents those of the wells treated with DMEM+10% FBS, without any samples. Statistical analyses were performed using the SPSS statistical software version (SPSS Inc., Chicago, IL, USA).

Cell morphological changes of HepG-2 Cells after treatment of sample

After being cultured with compound **8** for 0 h, 12 h, 24 h, 48 h, respectively, the cell phenotype in bright field were analyzed by Leica DM IL LED Fluorescence inverted microscope (FIM). HepG-2 cell lines were incubated on 6 well plates and incubated 24 h. The compound **8** (1 mL, 15 μg/mL)) was added to each well and then incubated for additional 6 h. Then nucleus's morphological variation was immediately observed under FIM.

Molecular docking simulations

Component target prediction: Smiles of compounds were obtained from ChemDRWAw and then uploaded to the Swiss Target Prediction database for target prediction. **Prediction of target of liver cancer**: In the Genecards database, disease targets were searched with the keyword "Liver cancer". **Intersection target acquisition**: Compound **8**, compound **9** and disease targets were intersected to obtain 41 common targets, and Veny plot was drawn. **PPI network mapping:** 41 common targets were imported into the String database to

draw the protein interaction network, and then imported into Cytoscape software to draw the network map and analyze the network topology parameters.

The PPI network diagram was constructed with the help of String database and Cytoscape, as shown in Figure 4. Graph nodes involved a total of 70, the number of edges for 423, one of the nodes represent a protein, and represents the protein role relationship between each other, and the node's size, color said the size of the degree of value, the greater the node corresponding degree of value, the greater the change from sky blue to green, in green orange corresponding threshold, from large to small, edge thickness reaction connection score, the more coarse, the higher the score, The median degree value of 2 times 20 was used as the screening condition, and three targets were obtained as key targets, which were EGFR, MMP9 and SRC. The structure of the compound was drawn in Chemdraw, and then imported into Chemdraw 3D. The MM2 module was used to minimize the energy, and the advantage idea of the lowest energy was obtained and saved as mol2 file. Protein structures were obtained by searching EGFR from Uniprot database.

Docking experiments were performed by Autodock vina 1.1.2. The crystal structure of EGFR protein (**Fig.S3**) was obtained from the Uniprot Protein Data Ban (UPDB). The water of target protein and the original ligand were separated with Mgtools 1.5.6, and then the MDM2 molecule was hydrogenated and charged with ADT. Molecular docking and the complex with the lowest energy was visualized between EGFR and cyclic peptides molecule by PYMOL and discovery studio. The following **table S1** describes the interconnection parameters.

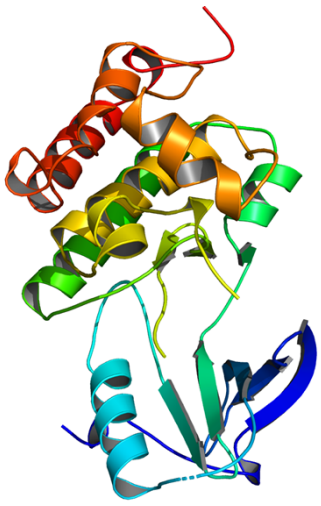
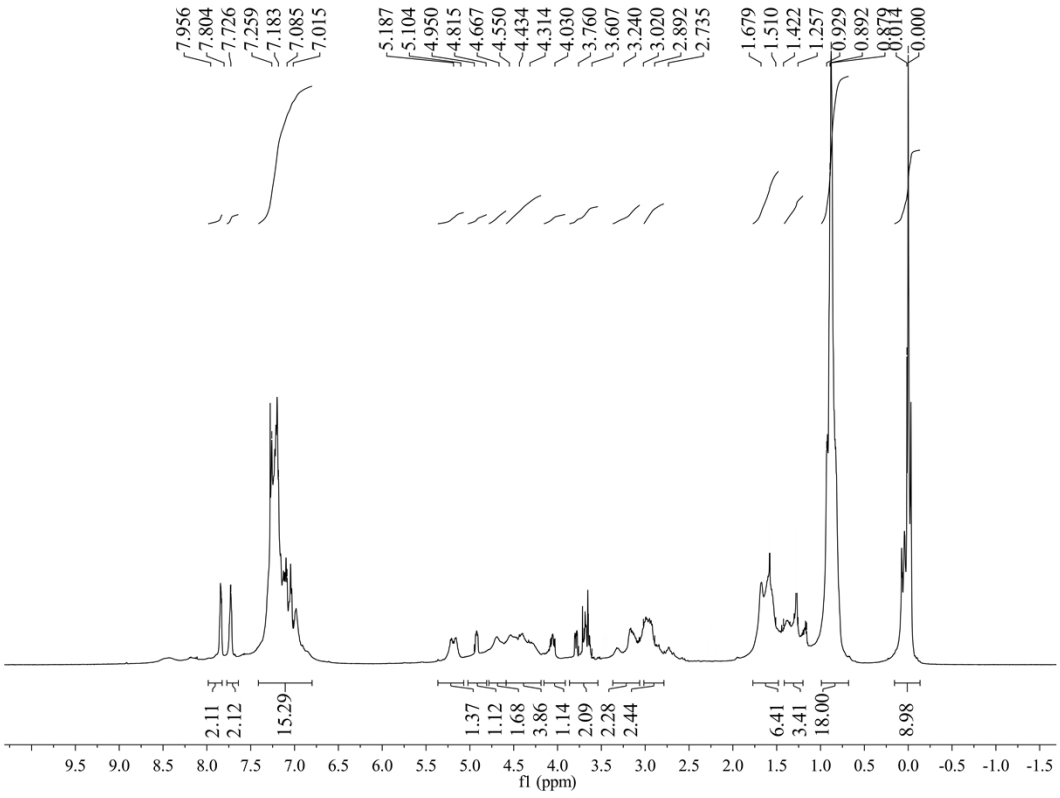


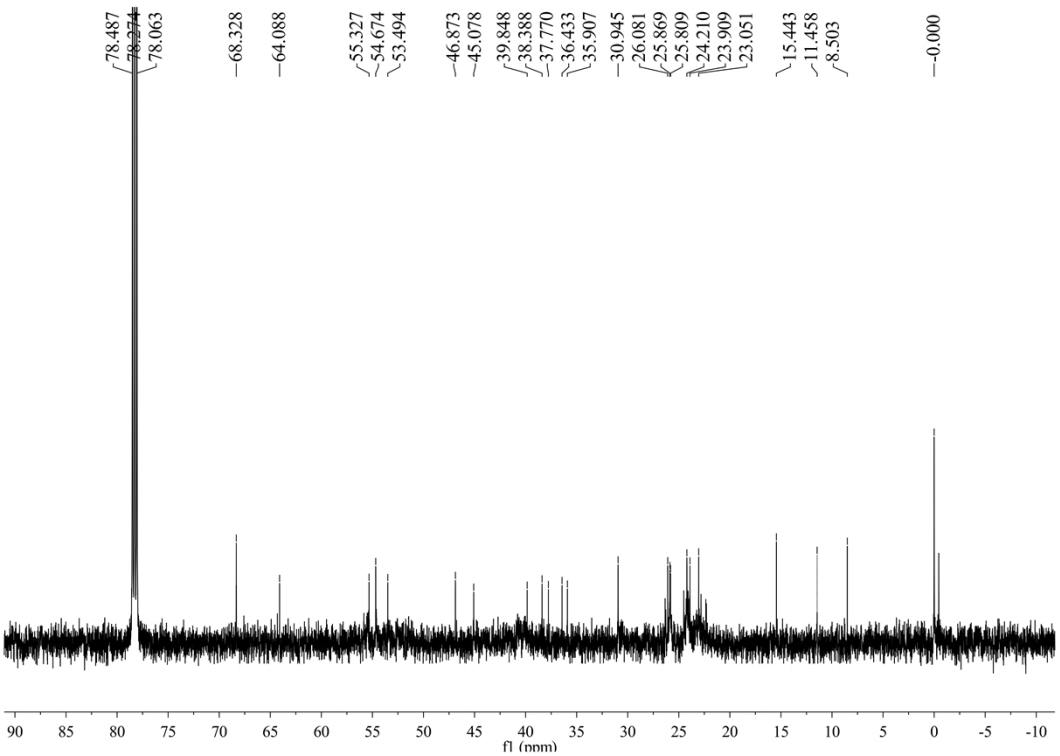
Figure S3. The crystal structure of EGFR protein.

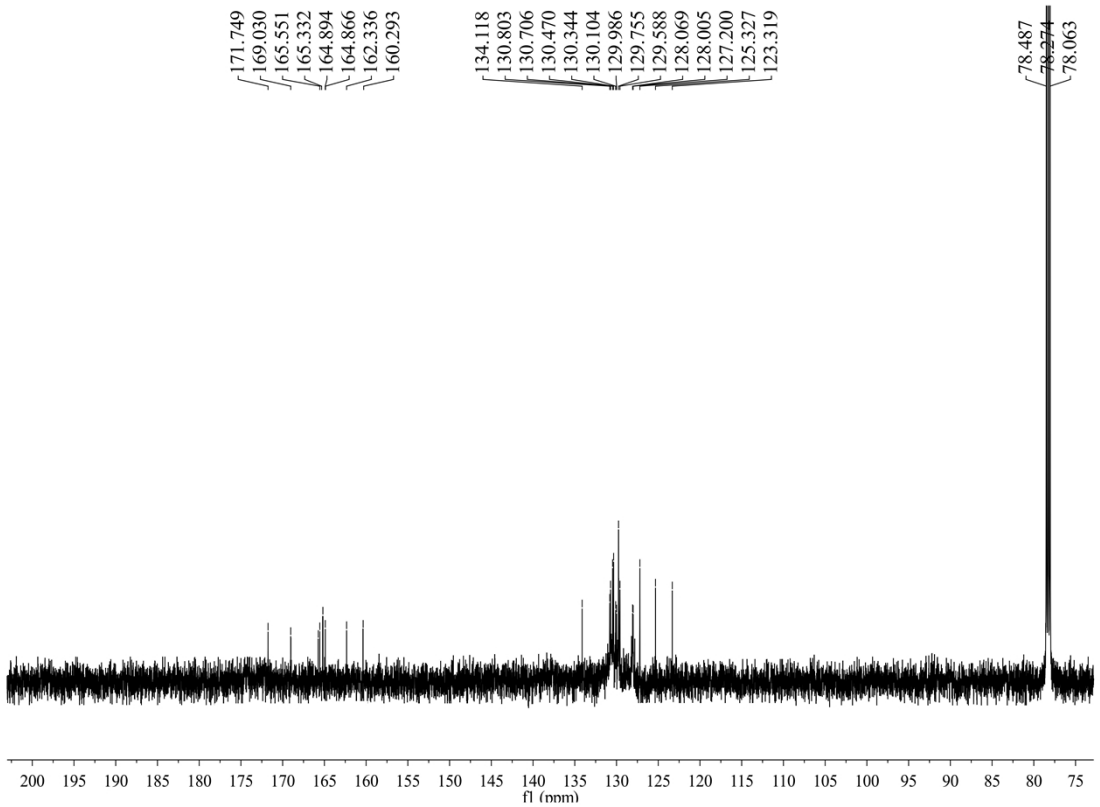
Table S1. Setting of docking parameters between cyclic peptides and EGFR protein

Parameters	Value
center_x	-45.55
center_y	8.948
center_z	-1.498
size_x	30.0
size_y	30.0
size_z	30.0
exhaustiveness	8
num_modes	9
energy_range	3

1. ^{1}H, ^{13}C-NMR and HRMS of **7a**.



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165.332
164.894
164.866
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134.118
130.803
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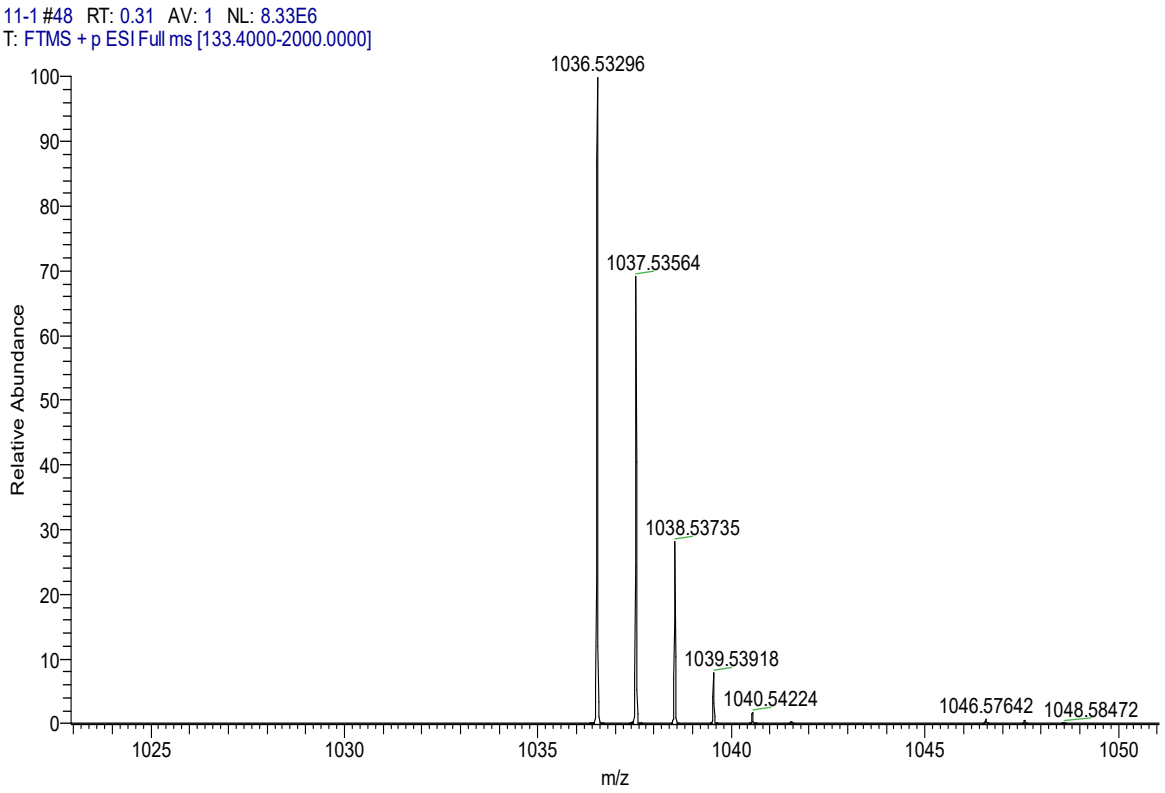
130.344
130.104
129.986
129.755
129.588
128.069
128.005
127.200
125.327
123.319
78.487
78.274
78.063
200 195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75
f1 (ppm)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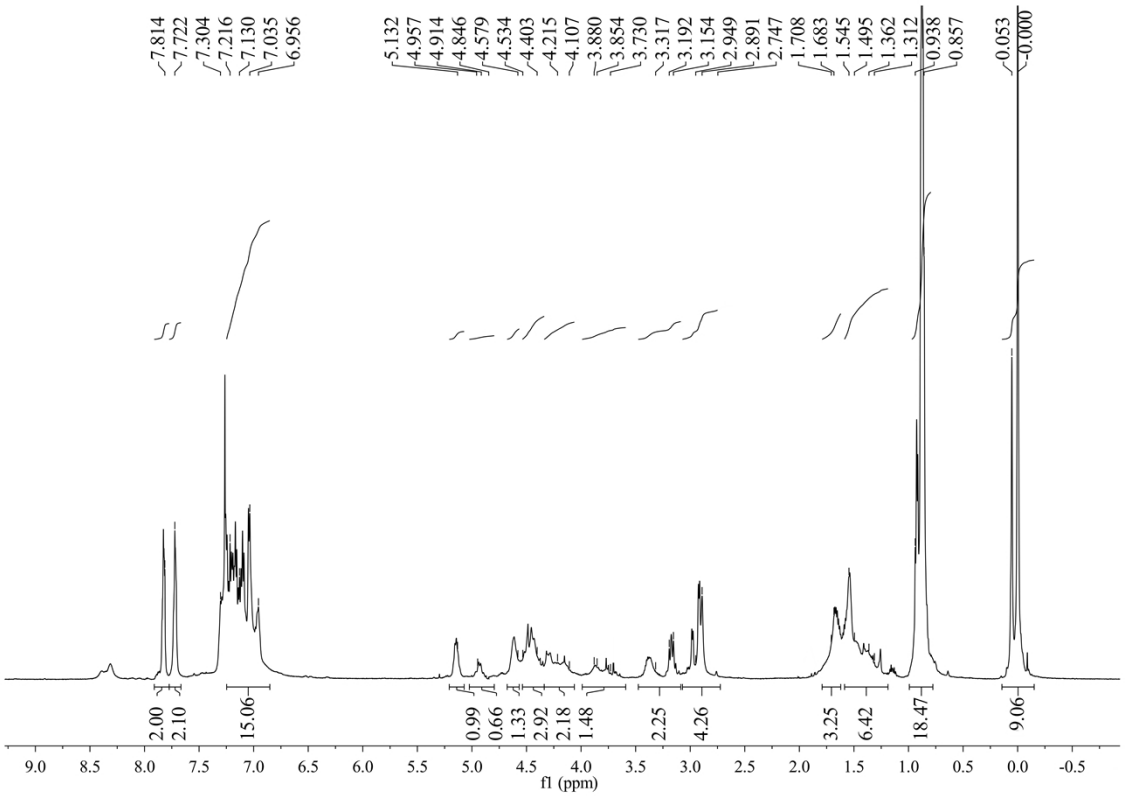
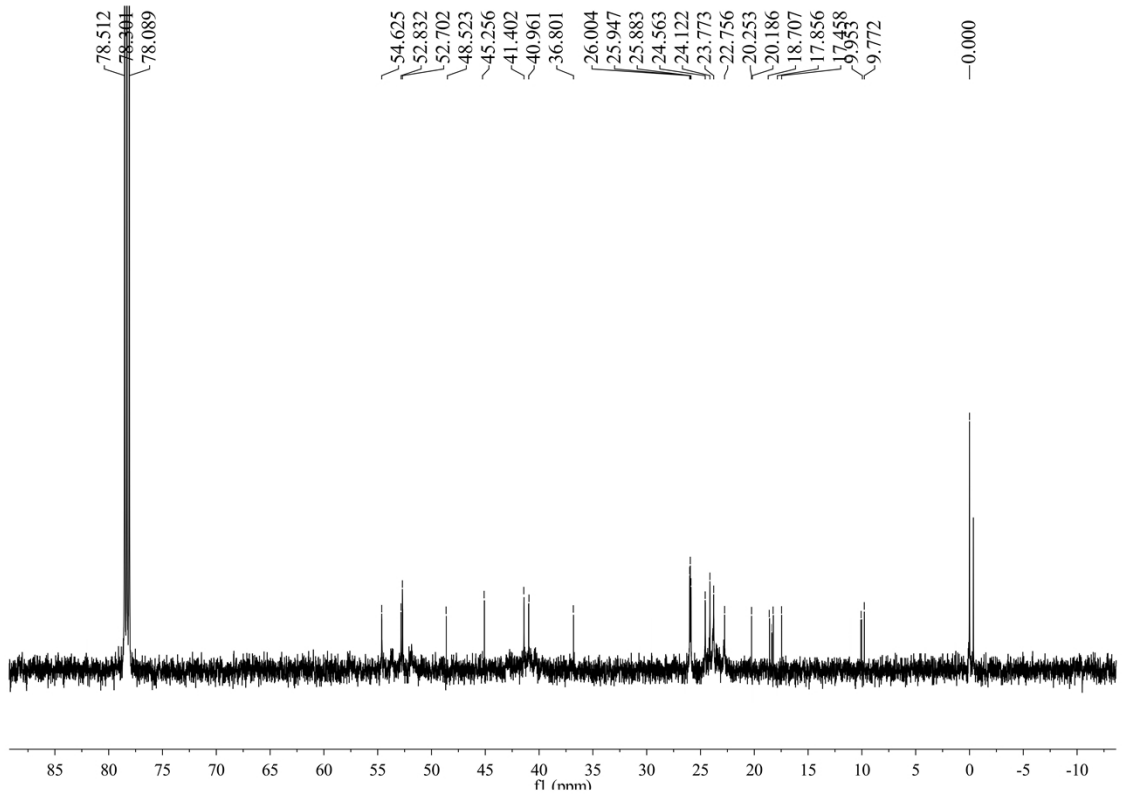
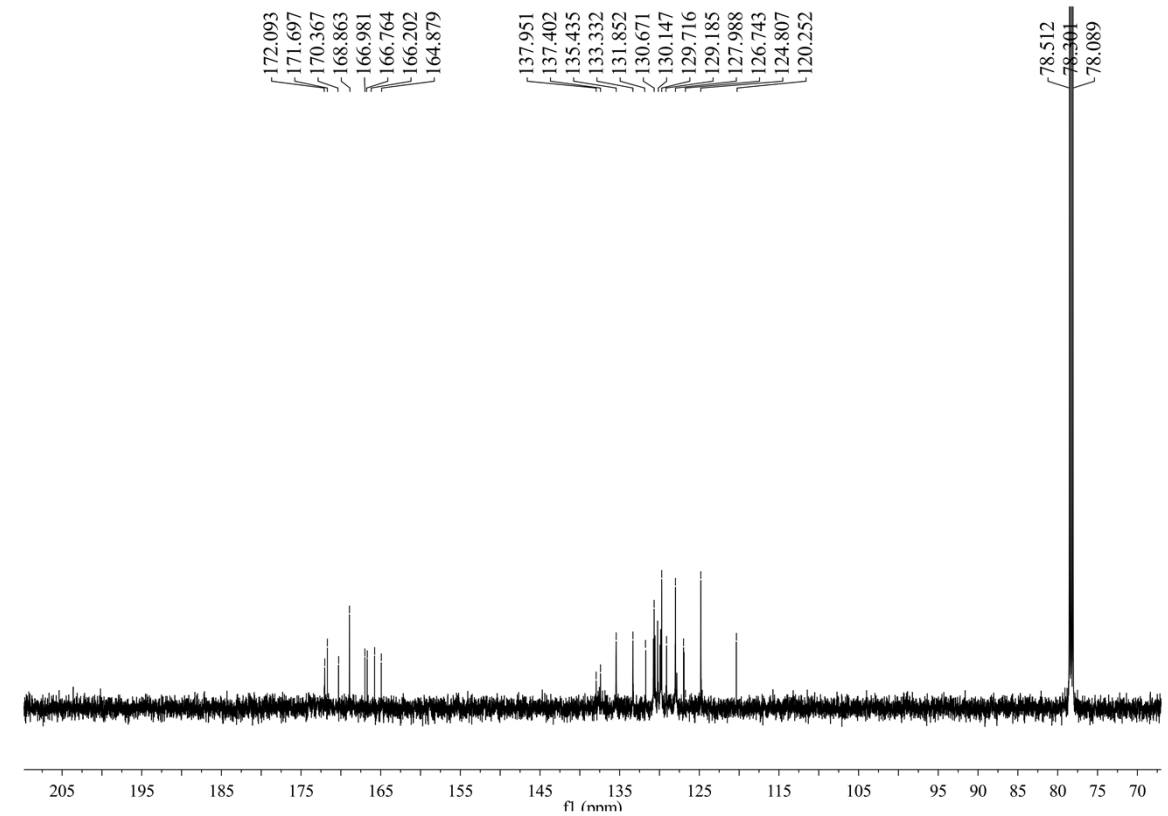
1038.53735
1039.53918
1040.54224
1046.57642
1048.58472
Relative Abundance
m/z
100
90
80
70
60
50
40
30
20
10
0
1025
1030
1035
1040
1045
1050

2. ^{1}H, ^{13}C-NMR and HRMS of **7b.**



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168.863
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166.764
166.202
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135.435
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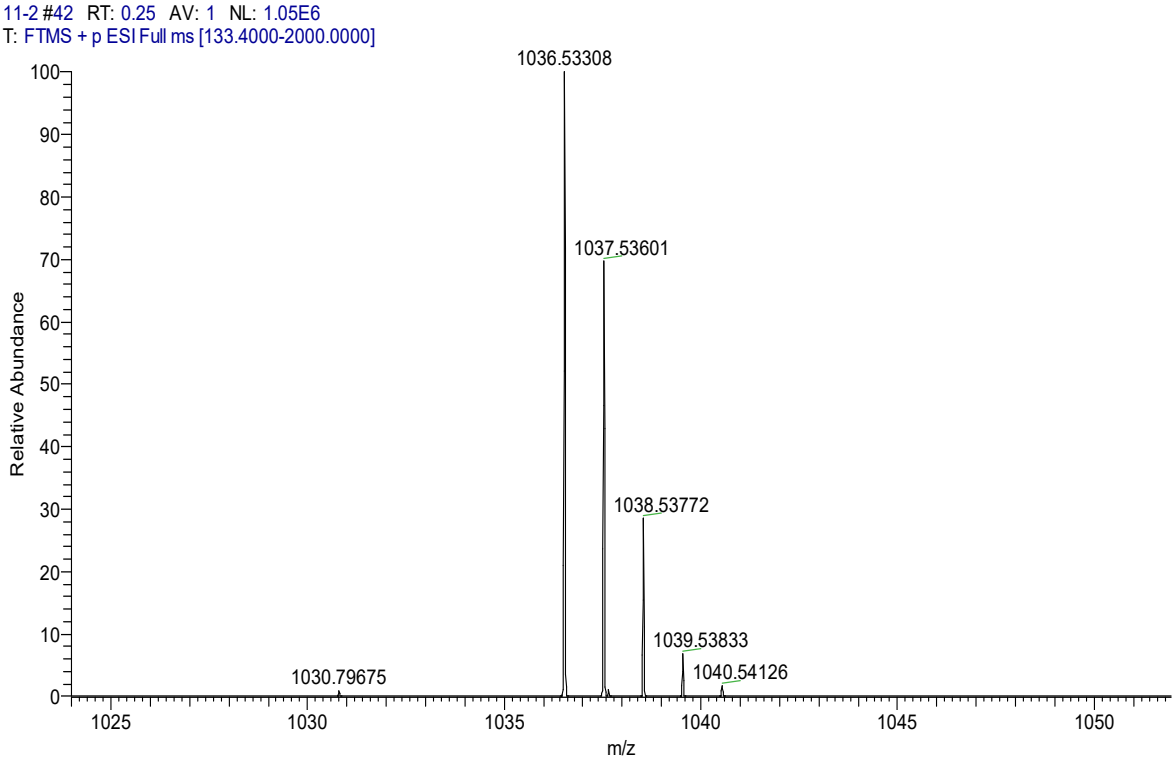
129.716
129.185
127.988
126.743
124.807
120.252
78.512
78.301
78.089
205
195
185
175
165
155
145
135
125
115
105
95
90
85
80
75
70
f1 (ppm)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1037.53601
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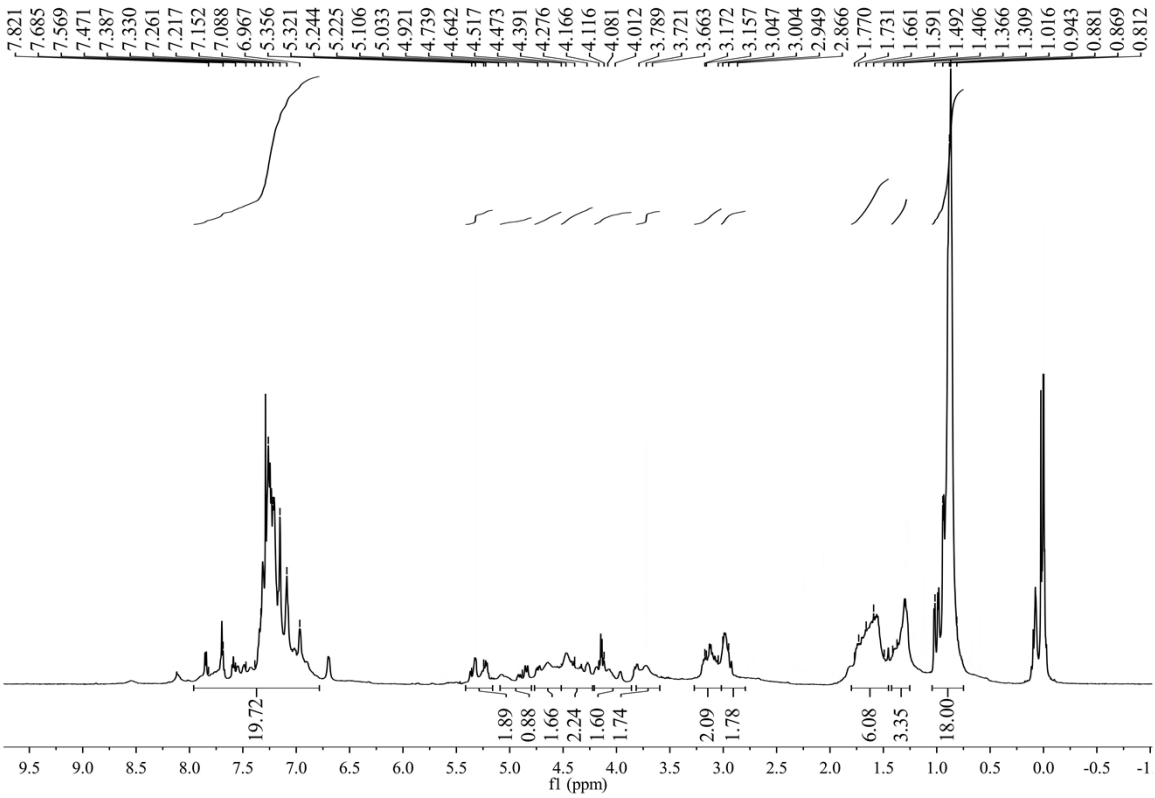
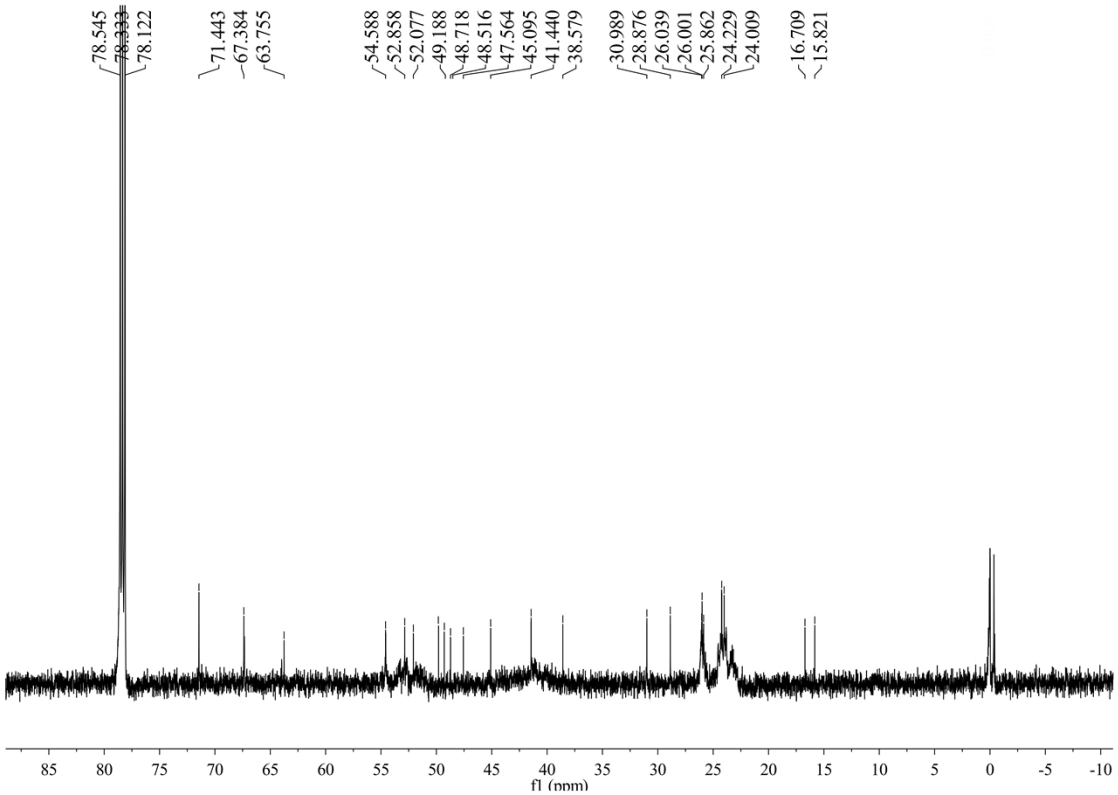
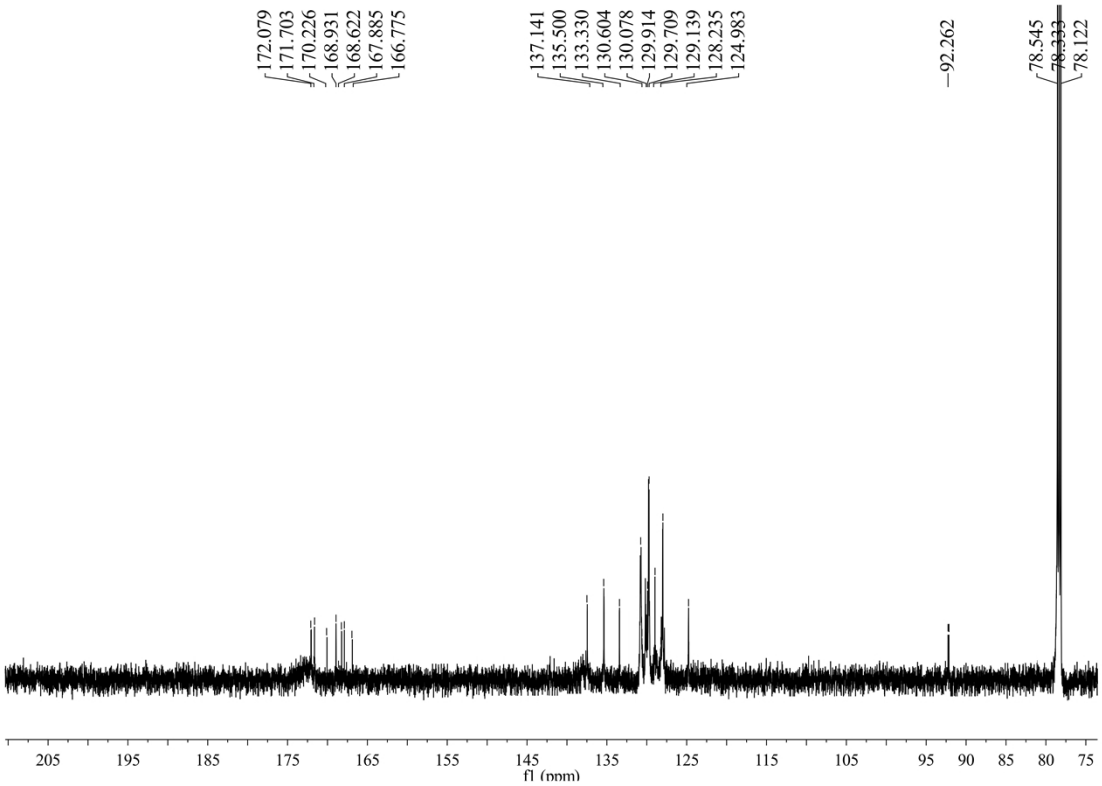
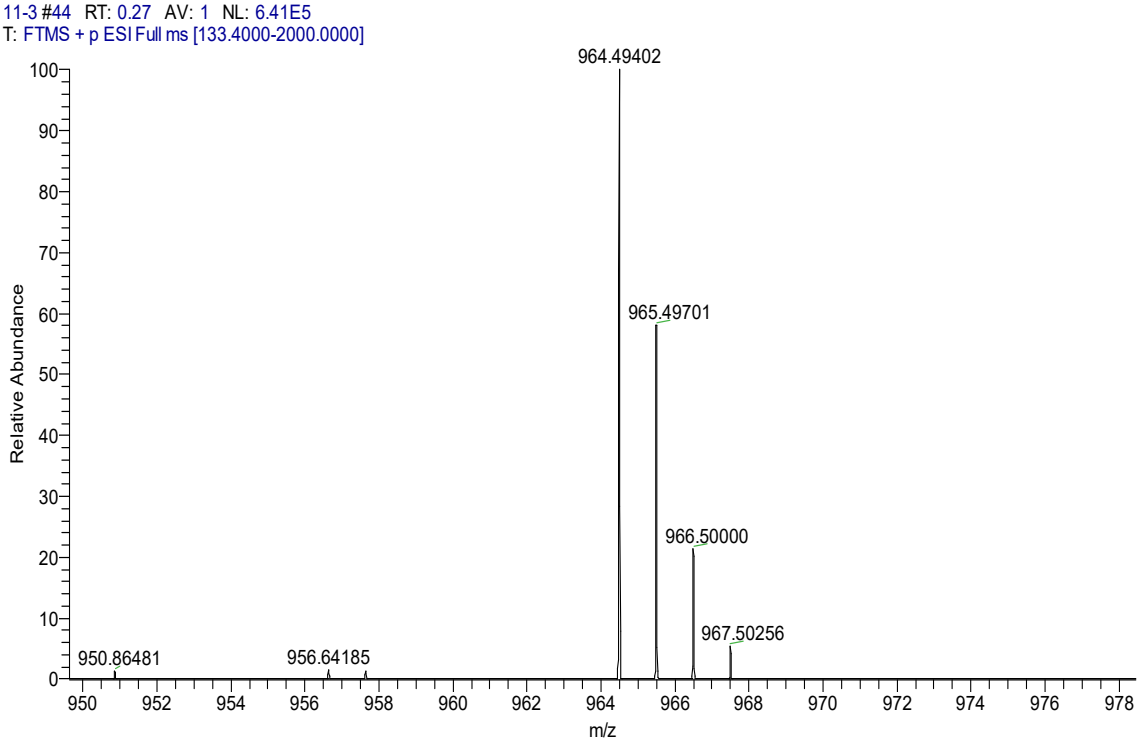
1039.53833
1040.54126
1030.79675
Relative Abundance
m/z
100
90
80
70
60
50
40
30
20
10
0
1025
1030
1035
1040
1045
1050

3. ^{1}H, ^{13}C-NMR and HRMS of **8**.

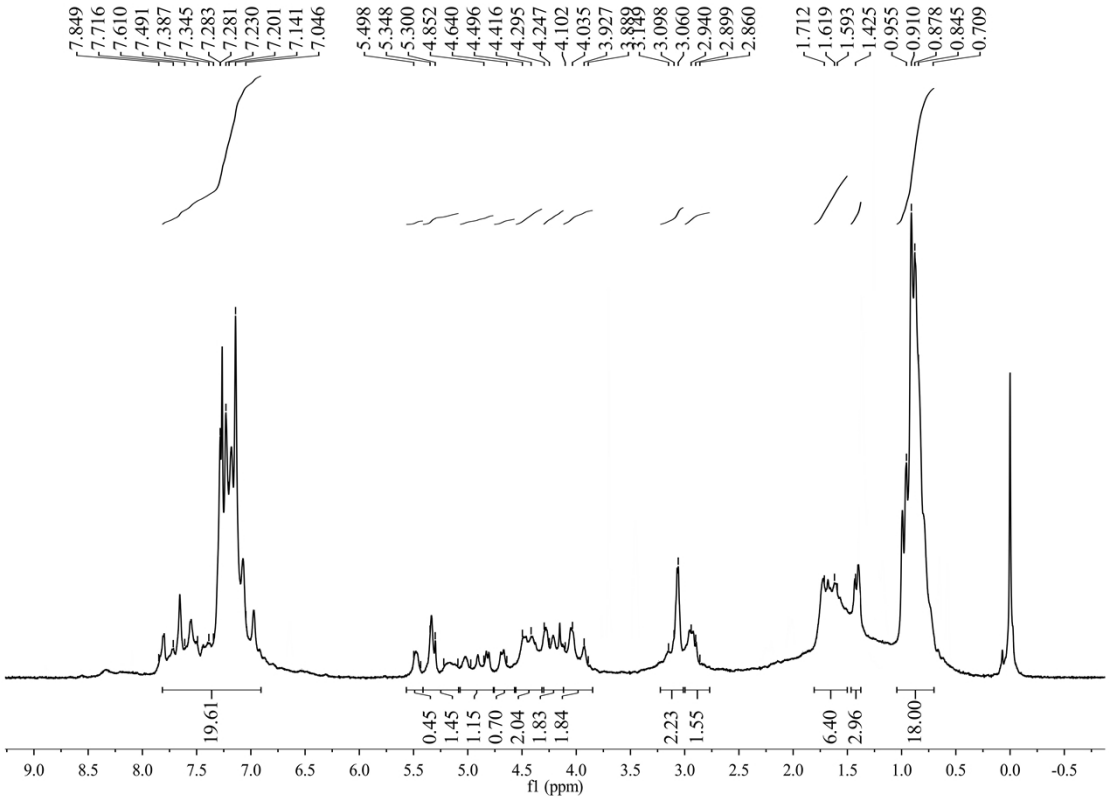


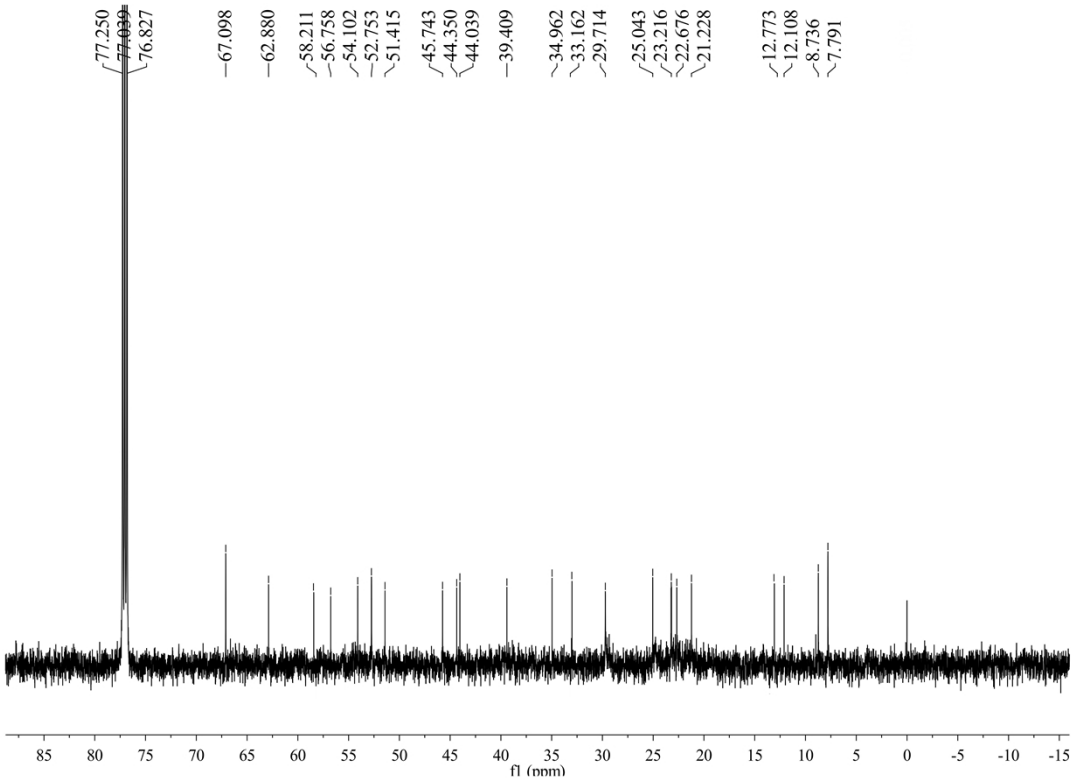


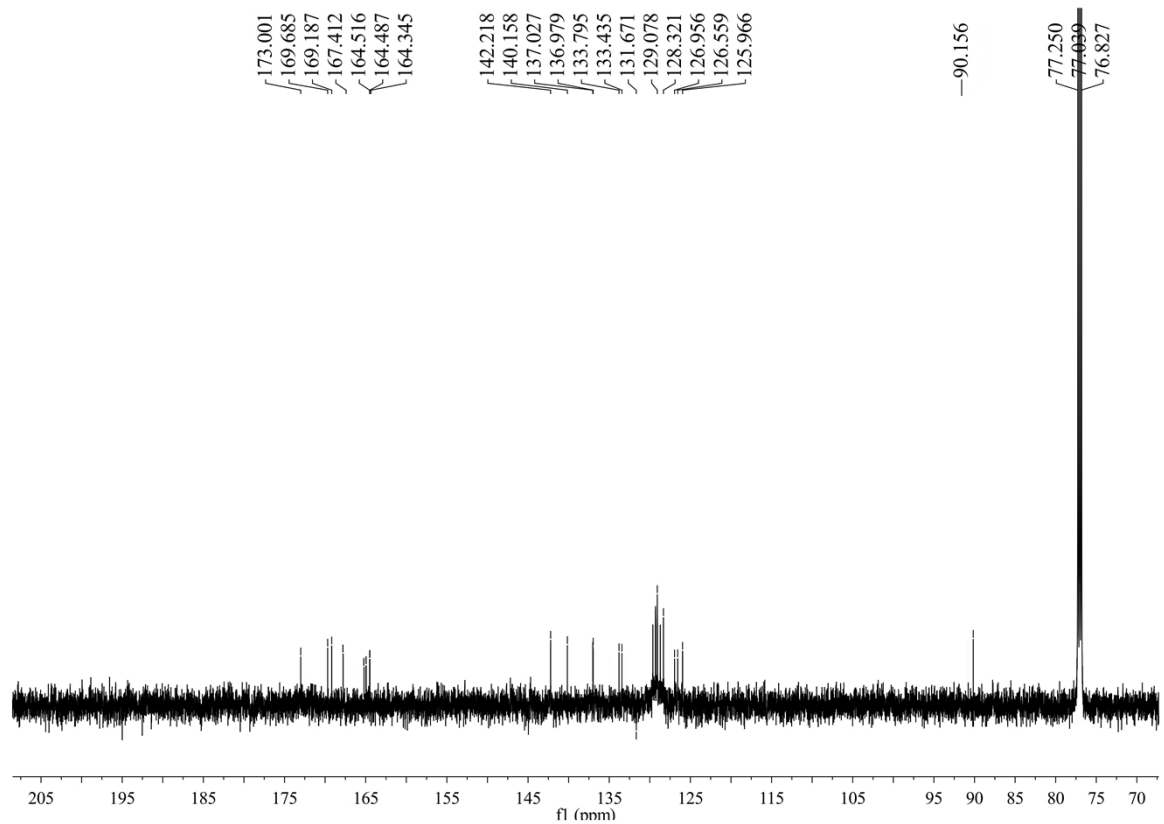

172.079
171.703
170.226
168.931
168.622
167.885
166.775
137.141
135.500
133.330
130.604
130.078
129.914
129.709
129.139
128.235
124.983
92.262
78.545
78.333
78.122
205
195
185
175
165
155
145
135
125
115
105
95
90
85
80
75
f1 (ppm)

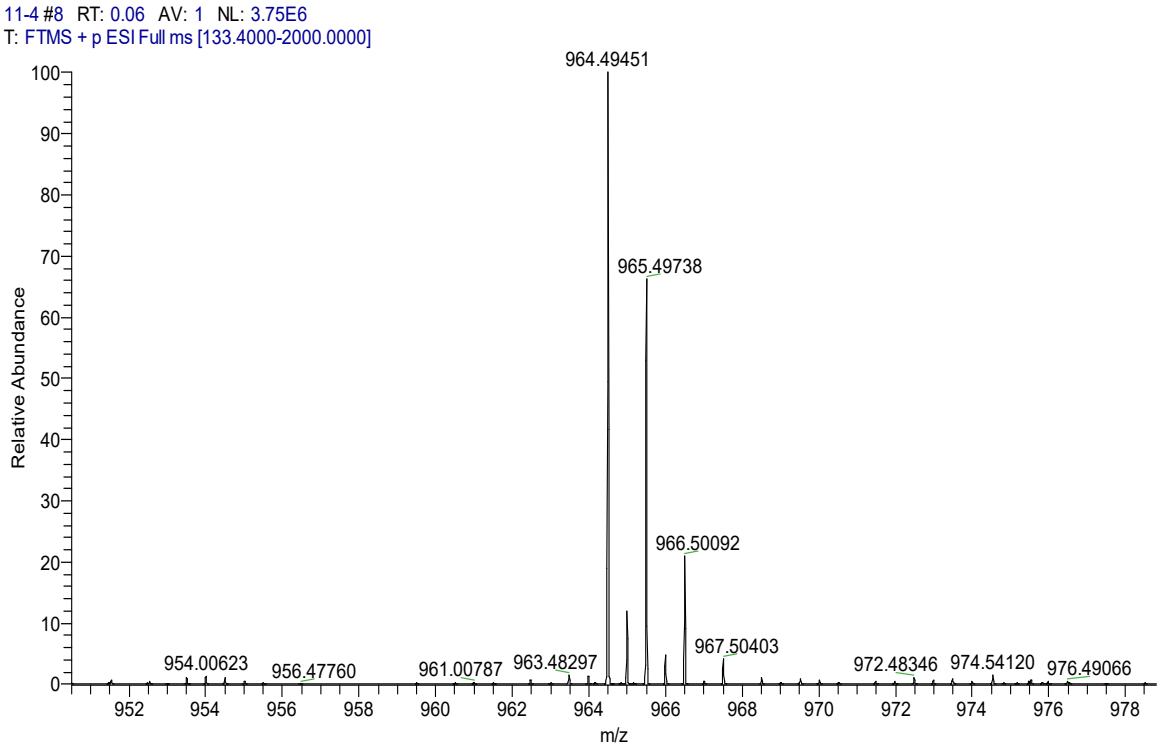

11-3 #44 RT: 0.27 AV: 1 NL: 6.41E5
T: FTMS + p ESI Full ms [133.4000-2000.0000]
964.49402
965.49701
966.50000
967.50256
950.86481
956.64185
Relative Abundance
m/z
100
90
80
70
60
50
40
30
20
10
0
950
952
954
956
958
960
962
964
966
968
970
972
974
976
978

4. ^{1}H, ^{13}C-NMR and HRMS of **9.**






173.001
169.685
169.187
167.412
164.516
164.487
164.345
142.218
140.158
137.027
136.979
133.795
133.435
131.671
129.078
128.321
126.956
126.559
125.966
90.156
77.250
77.039
76.827
205
195
185
175
165
155
145
135
125
115
105
95
90
85
80
75
70
f1 (ppm)


11-4 #8 RT: 0.06 AV: 1 NL: 3.75E6
T: FTMS + p ESI Full ms [133.4000-2000.0000]
964.49451
965.49738
966.50092
967.50403
954.00623
956.47760
961.00787
963.48297
972.48346
974.54120
976.49066
Relative Abundance
m/z