

Electronic Supplementary Information

New Potent $\alpha_v\beta_3$ Integrin Ligands Based on Azabicycloalkane Scaffolds

M. Pilkington-Miksa, E.M.V. Araldi, D. Arosio, L. Belvisi, M. Civera, and L. Manzoni*

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Chemistry

General. ^1H and ^{13}C NMR spectra were recorded at 300 K on a Bruker AVANCE-400 or a Bruker AVANCE-600 MHz spectrometer.

HPLC-Mass spectra were obtained with Agilent 1100 analytical HPLC equipped with diode array detector and Bruker ion-trap Esquire 3000 plus with ESI. HPLC analysis was performed using the following methods:

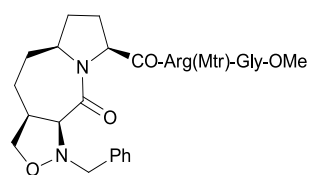
Method A. Column: Waters Atlantis 3 μm 50x4.6 mm; Phase A: H_2O + 0.05% TFA, Phase B: MeCN + 0.05% TFA; Flow: 1 mL/min, Gradient: from 10% B to 90% B in 6 min, washing at 100% B for 1 min, equilibration at 10% B in the next 3 min.

Method B. Column: Supelco Ascentis-Express 2.7 μm 50x4.6 mm; Phase A: H_2O + 0.05% TFA, Phase B: MeCN + 0.05% TFA; Flow: 1 mL/min, Gradient: from 5% B to 95% B in 6 min, washing at 100% B for 1 min, equilibration at 5% B in the next 3 min.

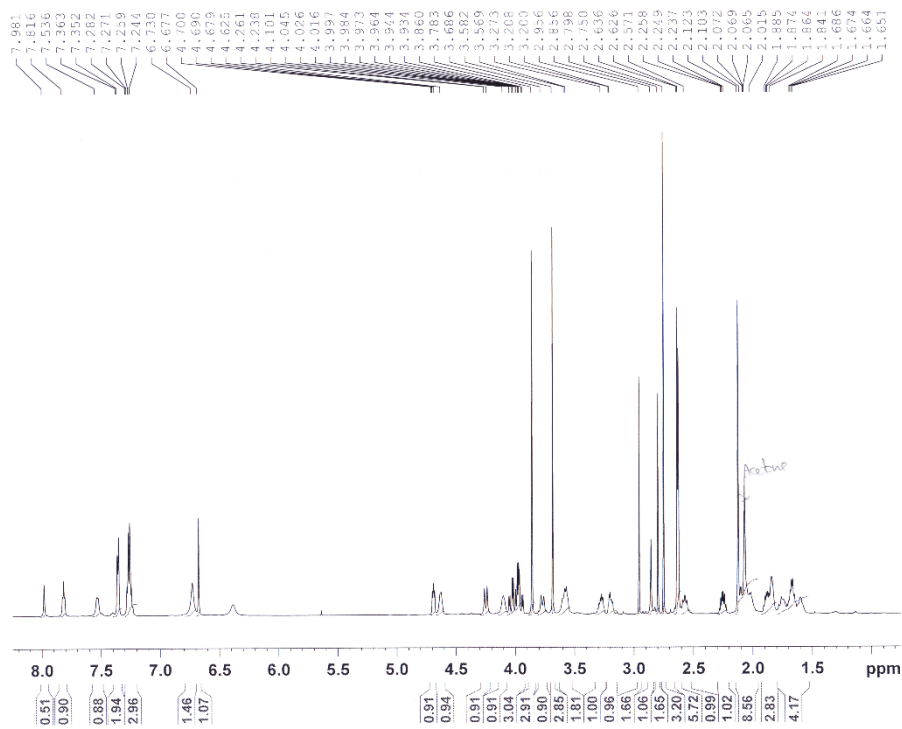
Method C. Column: Waters Atlantis 3 μm 50x4.6 mm; Phase A: H_2O + 0.05% TFA, Phase B: MeCN + 0.05% TFA; Flow: 1 mL/min, Gradient: from 0% B to 30% B in 6 min, from 30% B to 90% B in 1 min, washing at 100% B for 1 min, equilibration at 0% B in the next 3 min.

Method D. Column: Supelco RP-amide 5 μm 6x150 mm; Phase A: H_2O + 0.1% TFA, Phase B: MeCN + 0.1% TFA; Flow: 1 mL/min, Gradient: from 0% B to 30% B in 20 min.

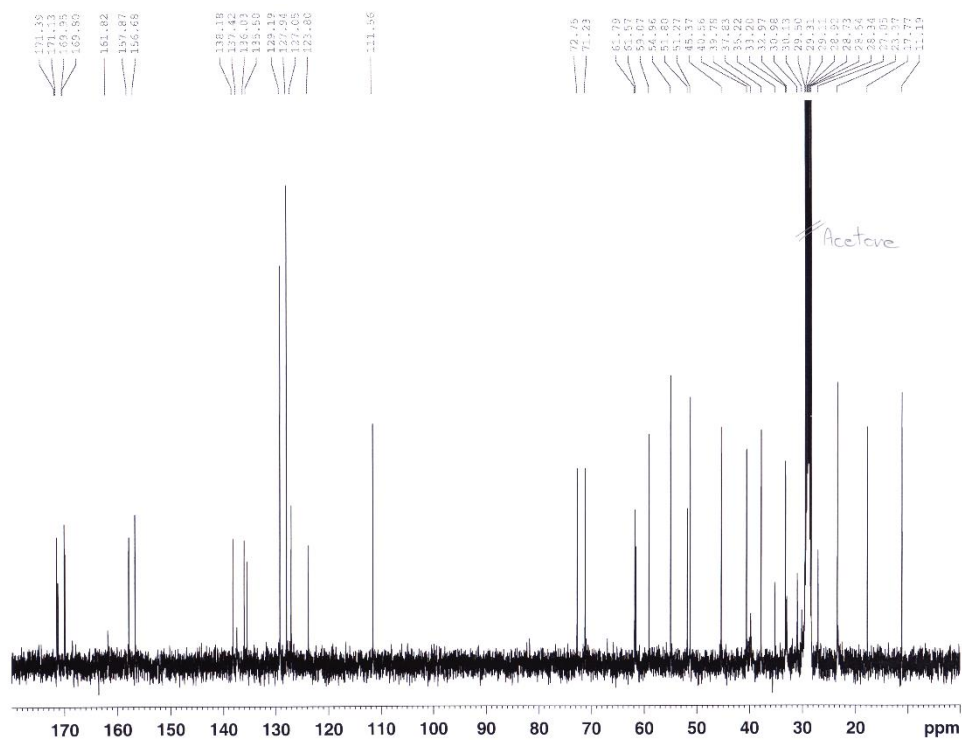
Compound 7.



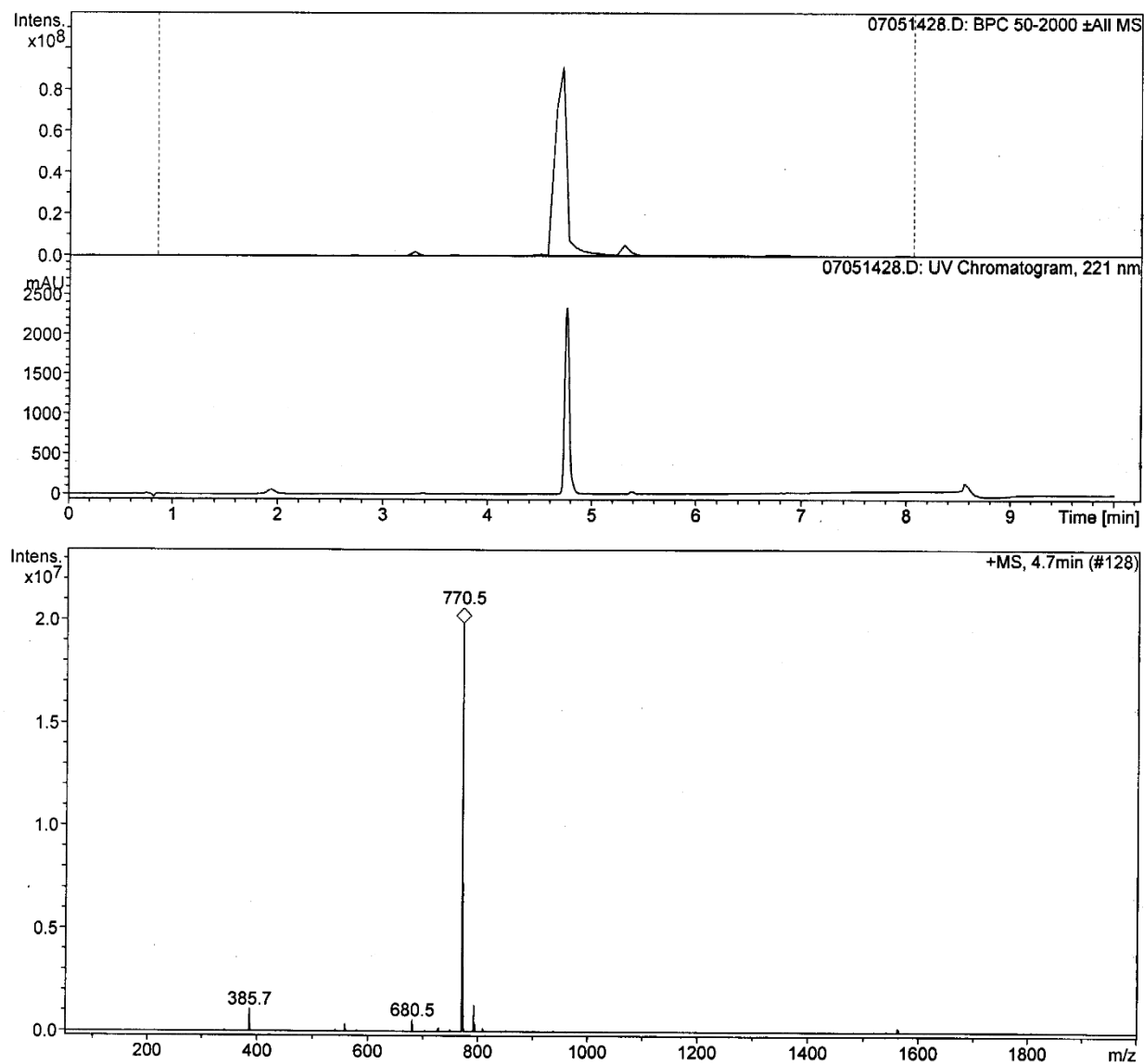
^1H NMR (400 MHz, *Acetone-d*₆)



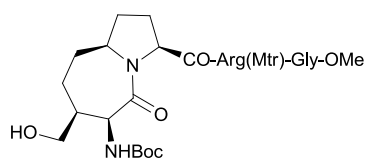
^{13}C NMR (100.6 MHz, *Acetone-d*₆)



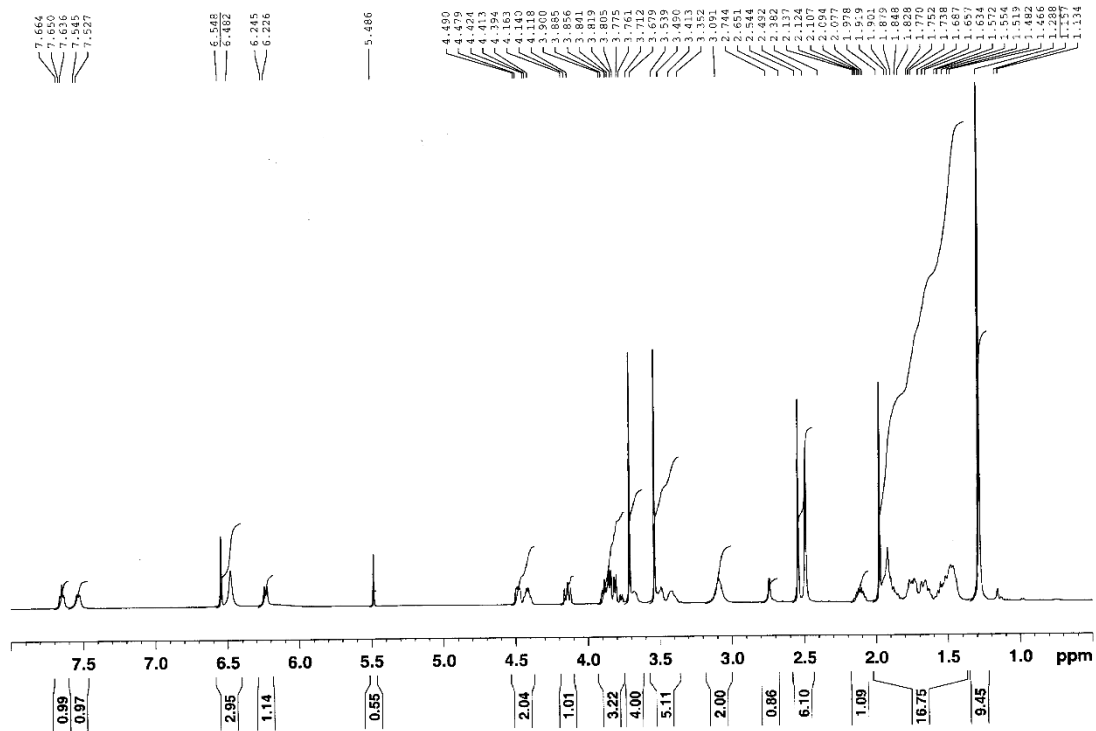
HPLC-MS (analytical method A)



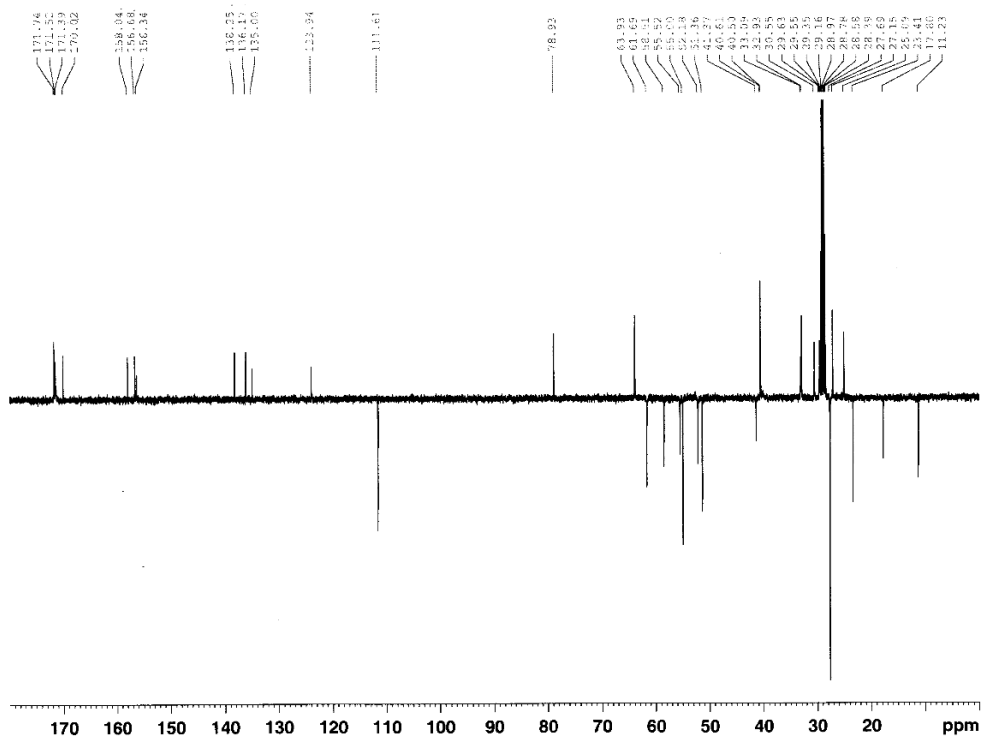
Compound 9.



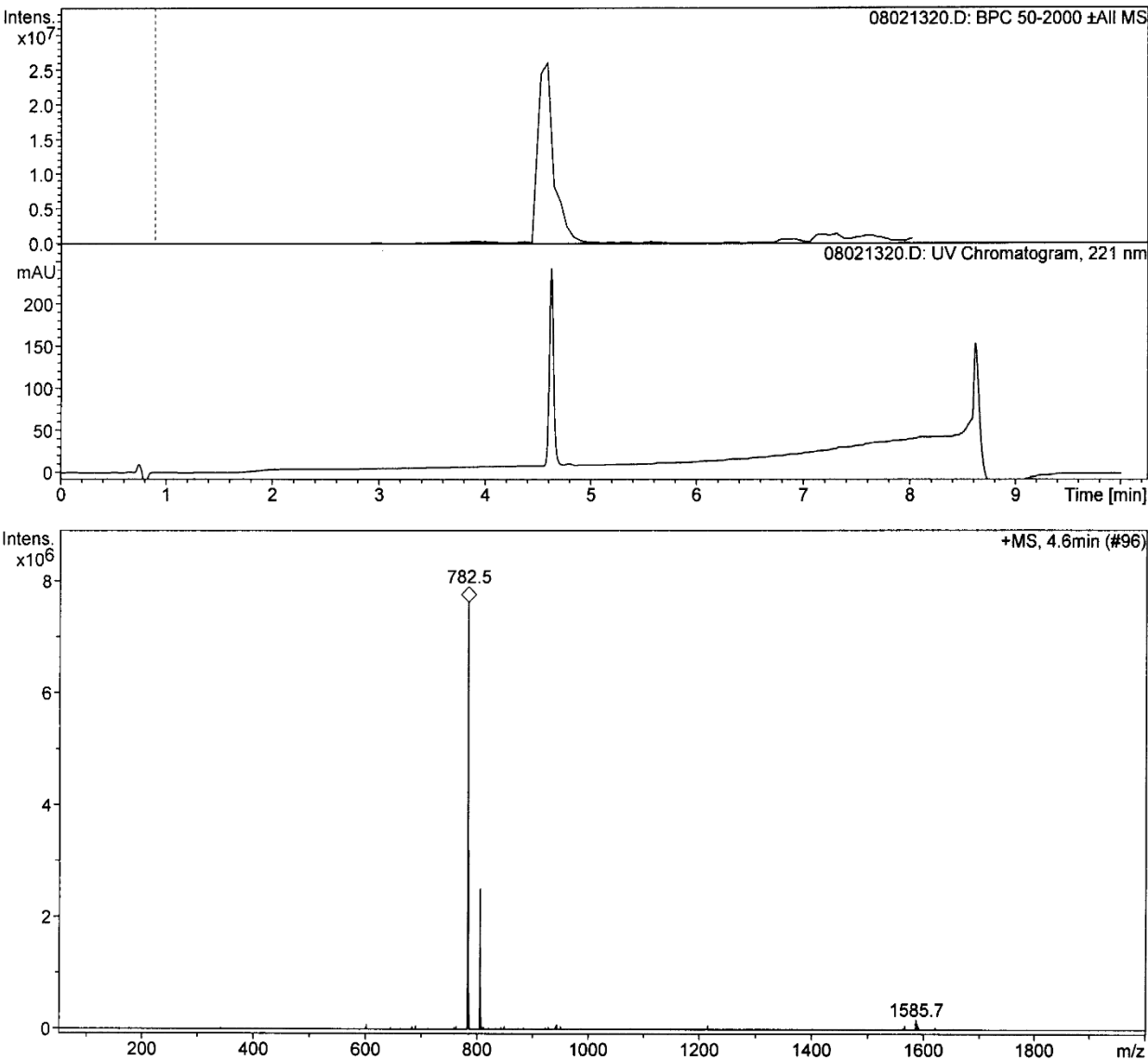
¹H NMR (400 MHz, Acetone-*d*₆)



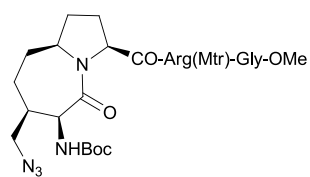
¹³C NMR (100.6 MHz, Acetone-*d*₆)



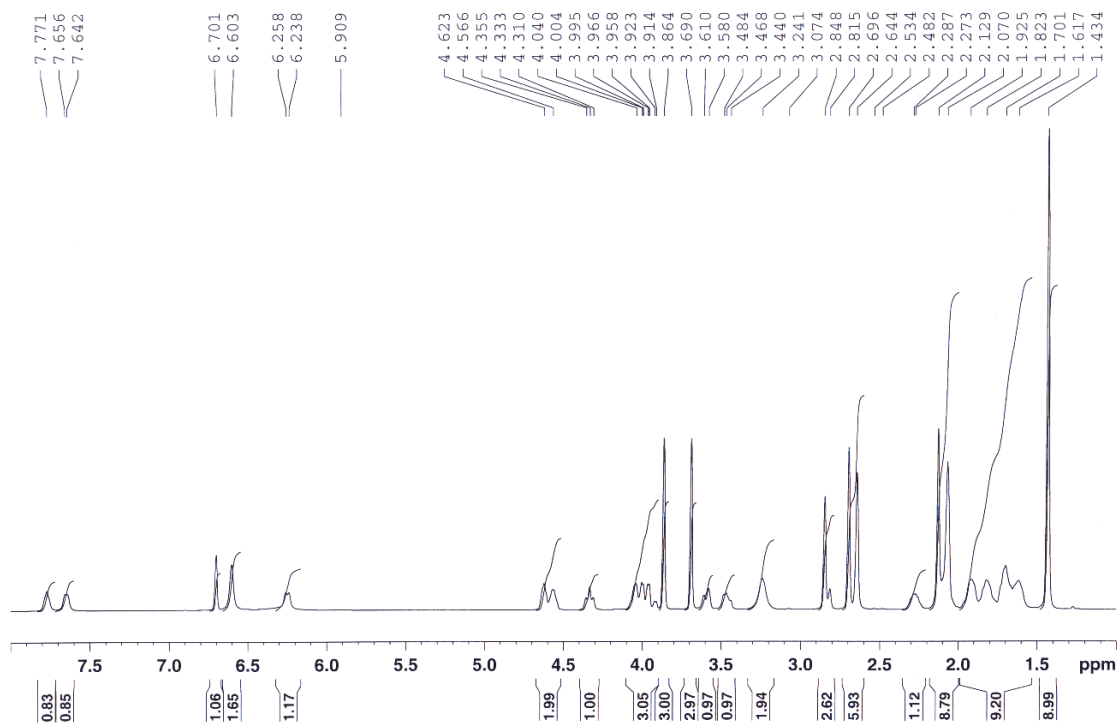
HPLC-MS (analytical method A)



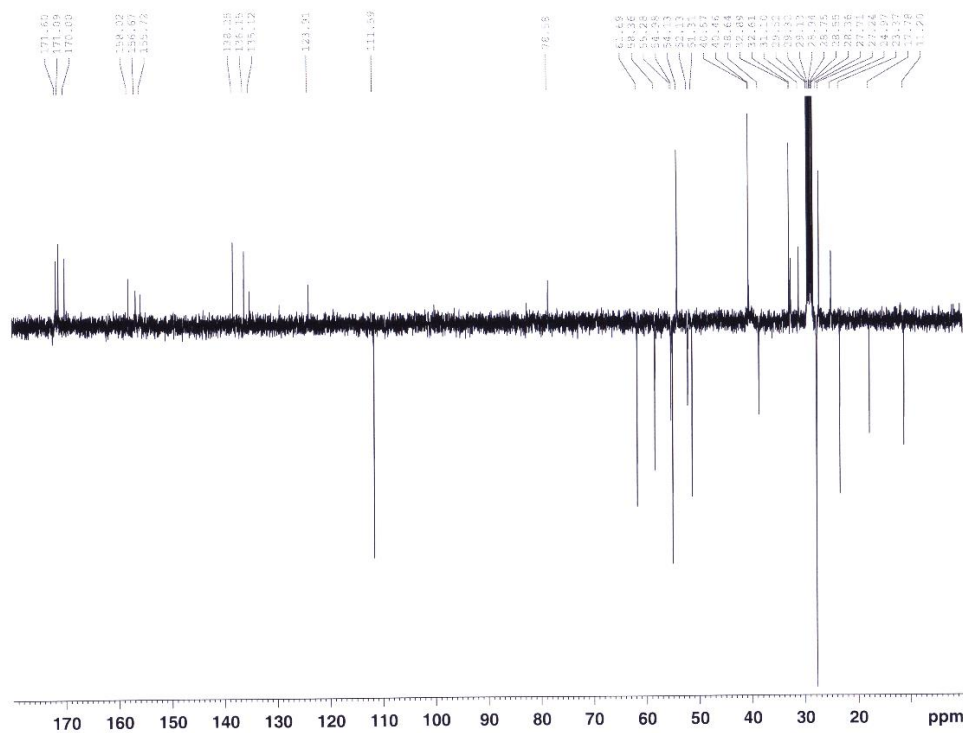
Compound 10



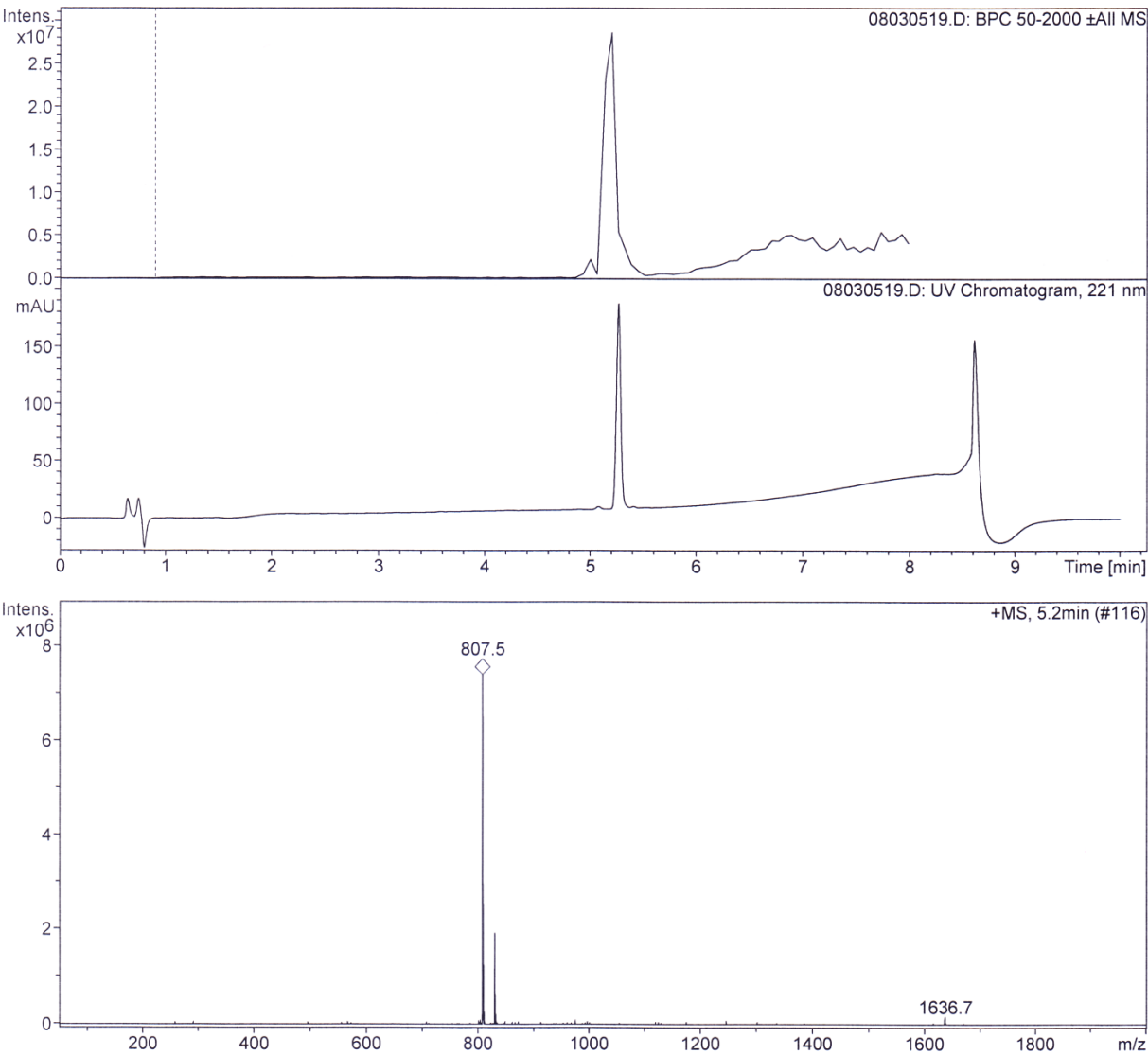
¹H NMR (400 MHz, Acetone-d₆)



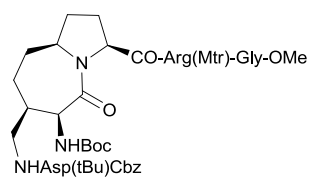
¹³C NMR (100.6 MHz, Acetone-d₆)



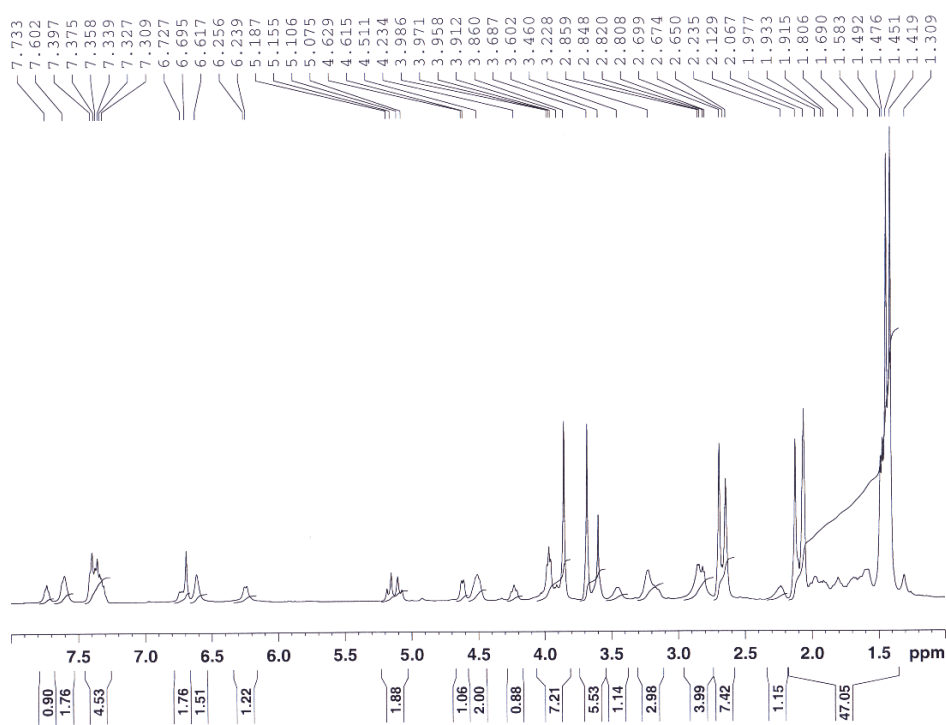
HPLC-MS (analytical method A)



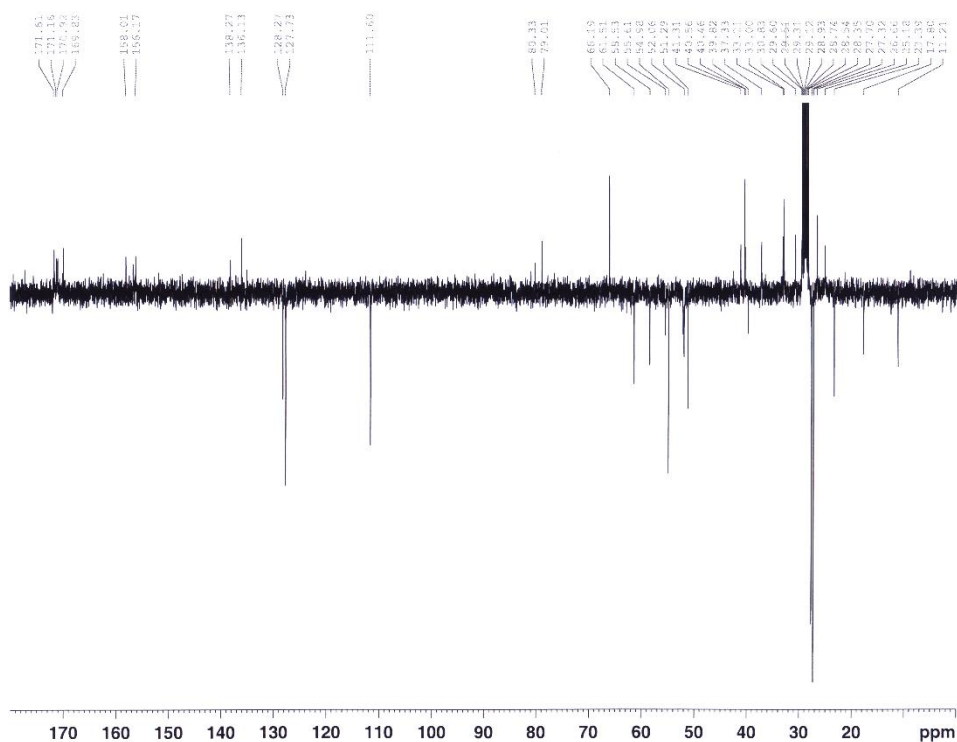
Compound 11



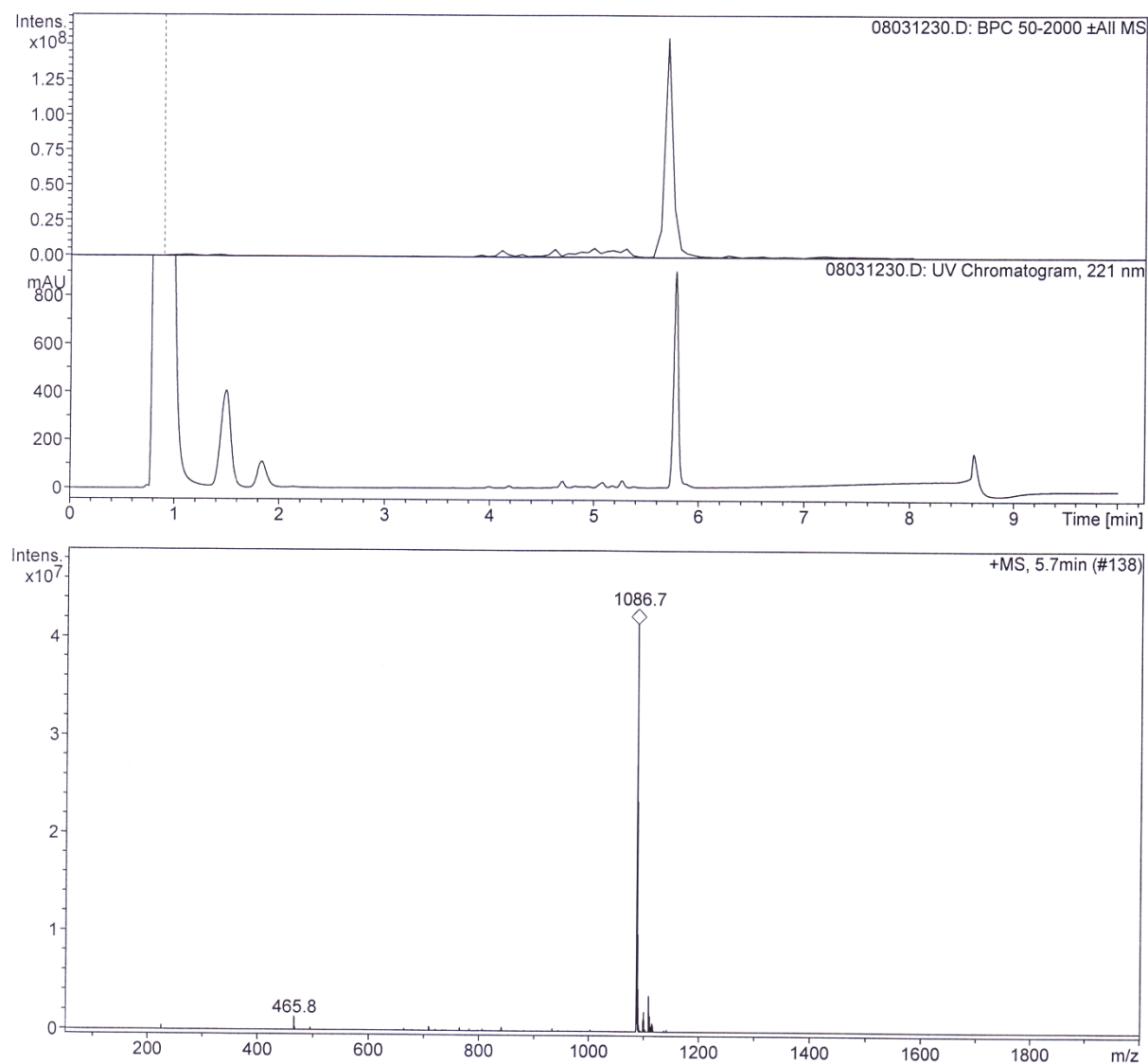
¹H NMR (400 MHz, Acetone-d₆)



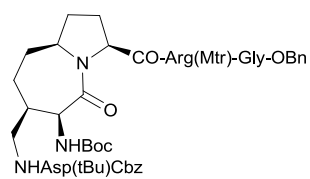
¹³C NMR (100.6 MHz, Acetone-d₆)



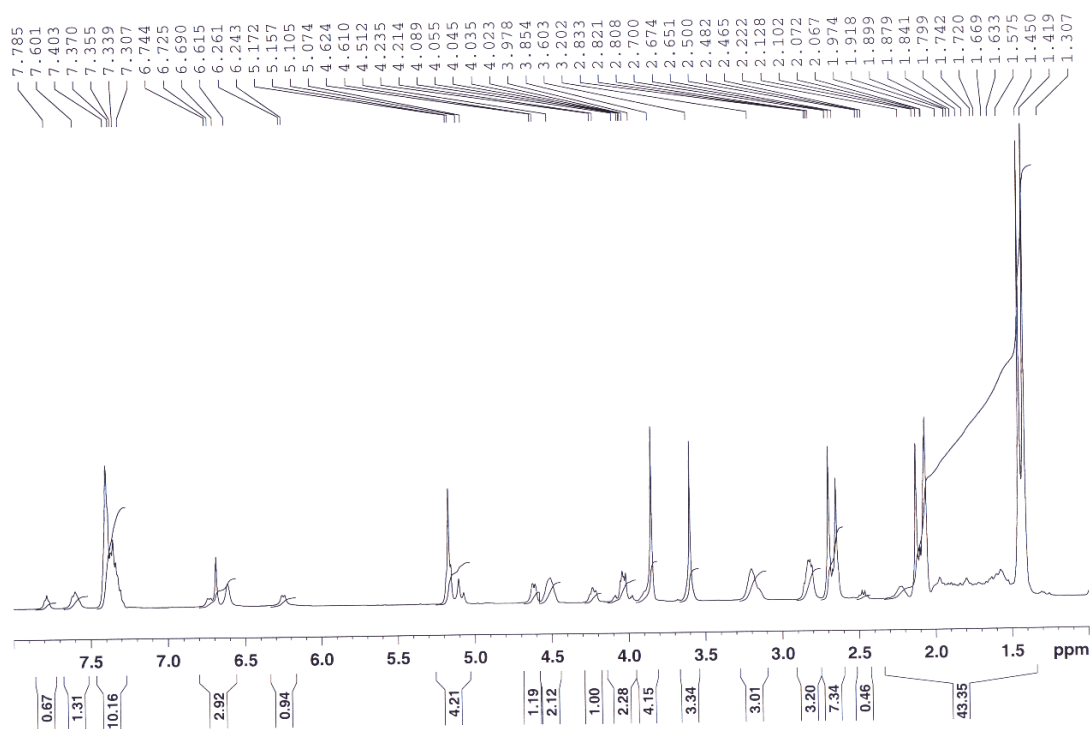
HPLC-MS (analytical method A)



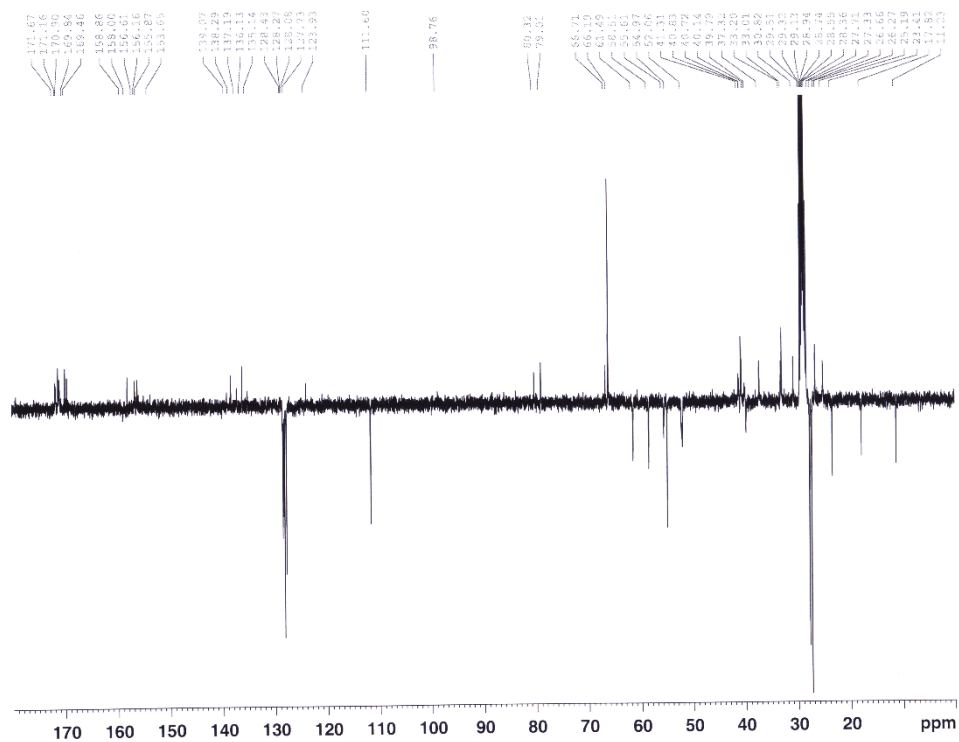
Compound 12



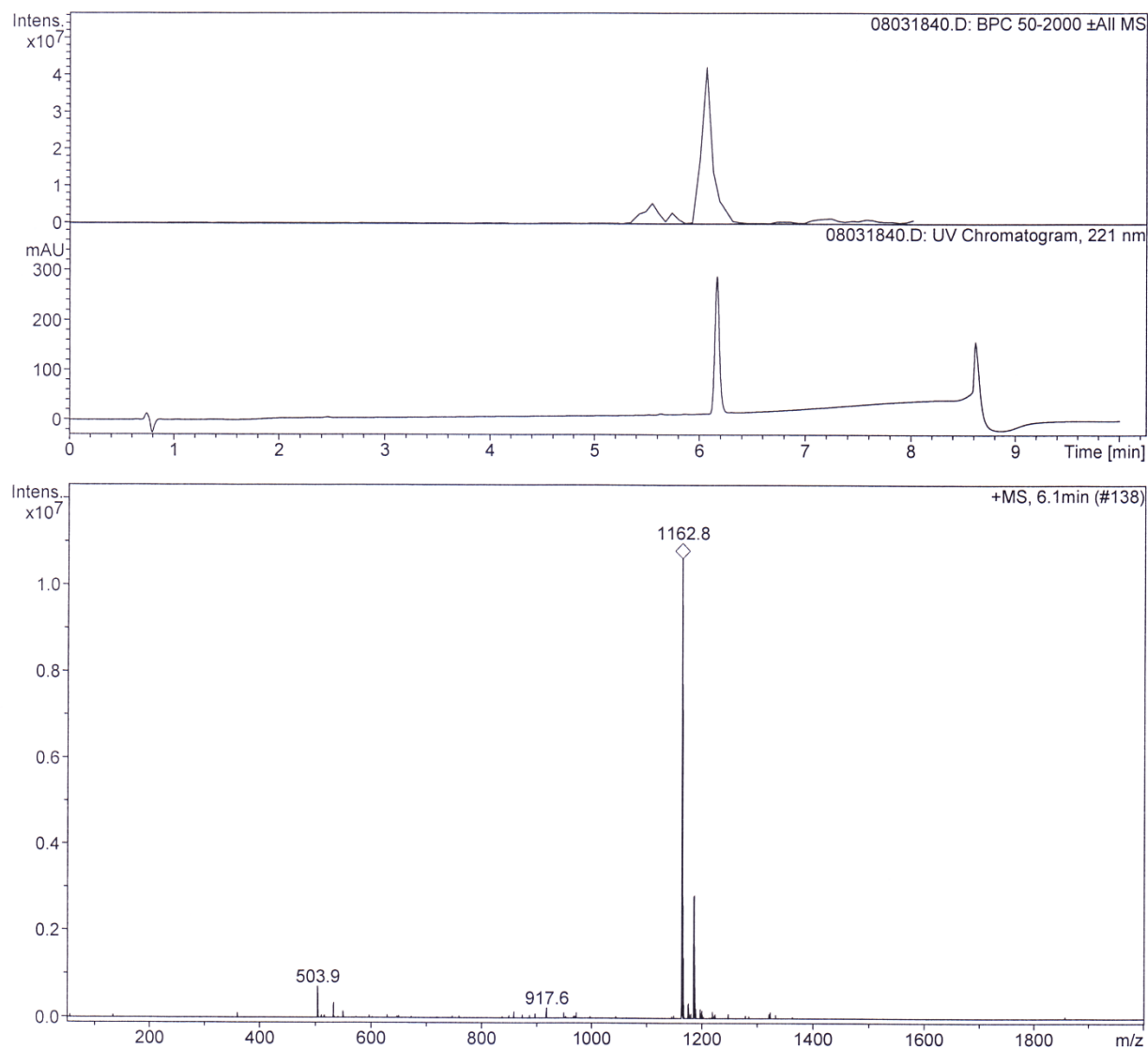
^1H NMR (400 MHz, *Acetone-d*₆)



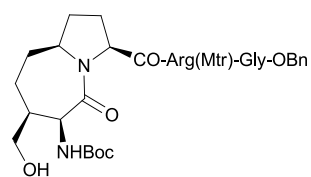
^{13}C NMR (100.6 MHz, *Acetone-d*₆)



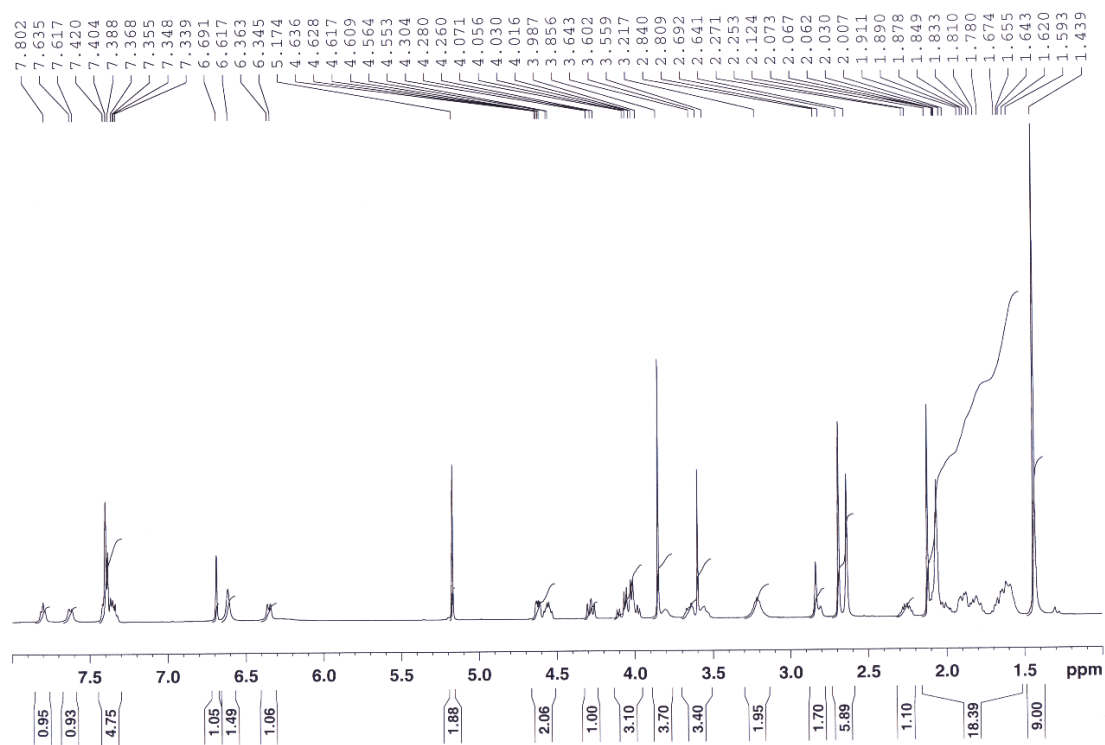
HPLC-MS (analytical method A)



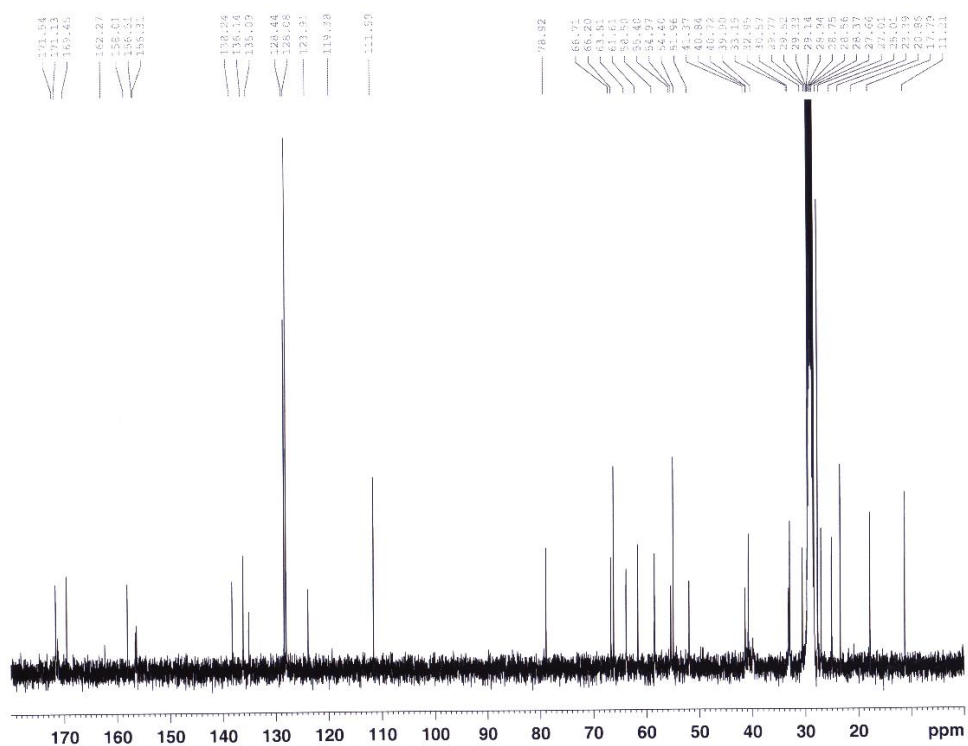
Compound 13



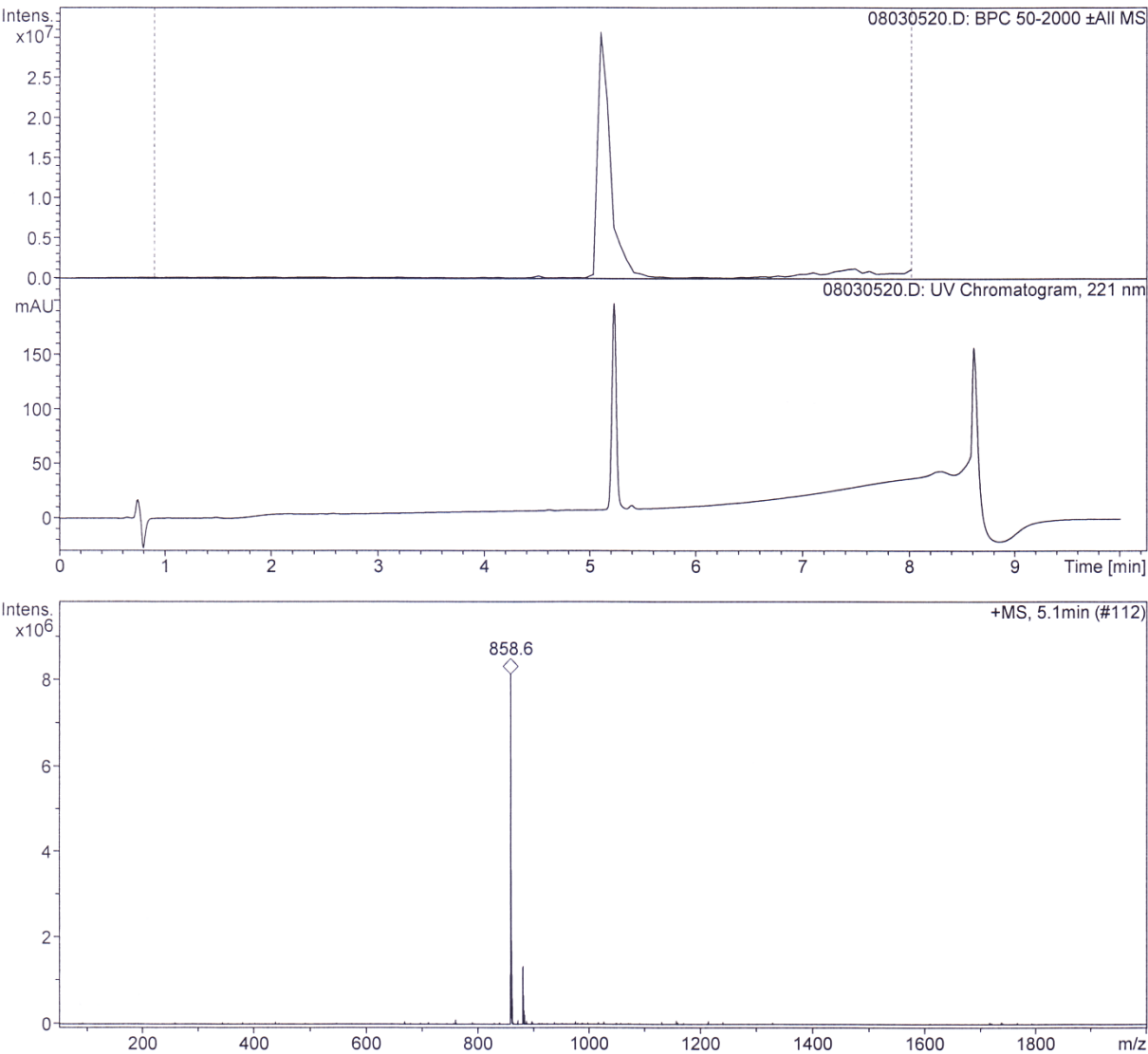
¹H NMR (400 MHz, Acetone-*d*₆)



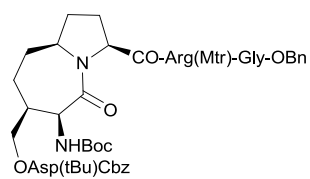
¹³C NMR (100.6 MHz, Acetone-*d*₆)



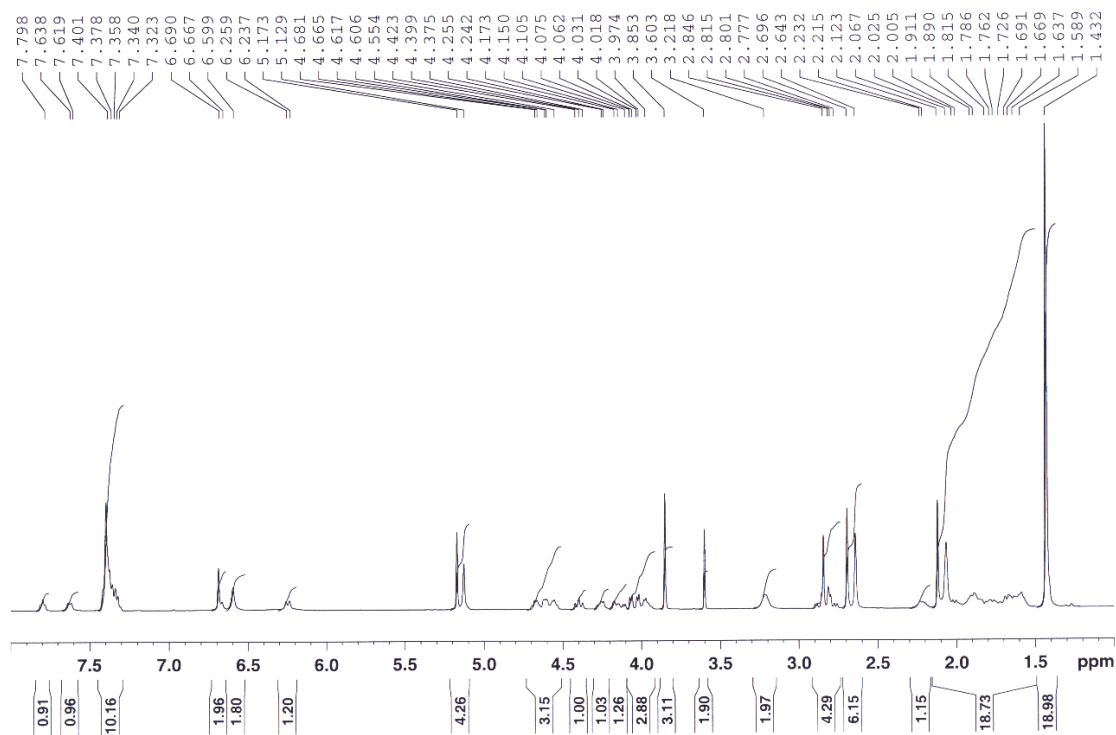
HPLC-MS (analytical method A)



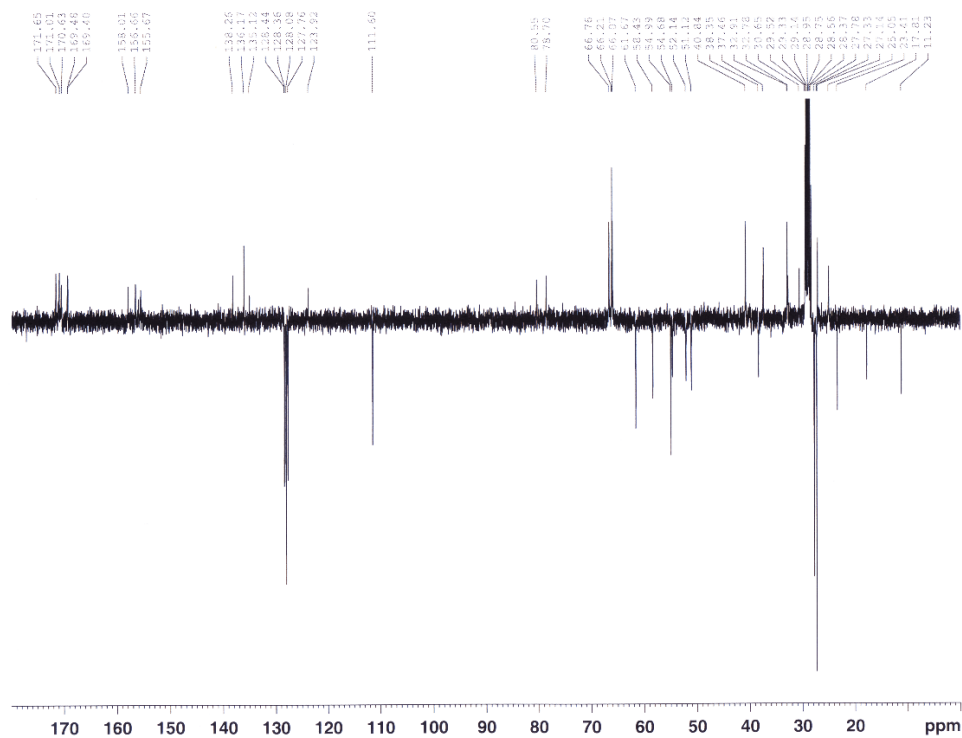
Compound 14



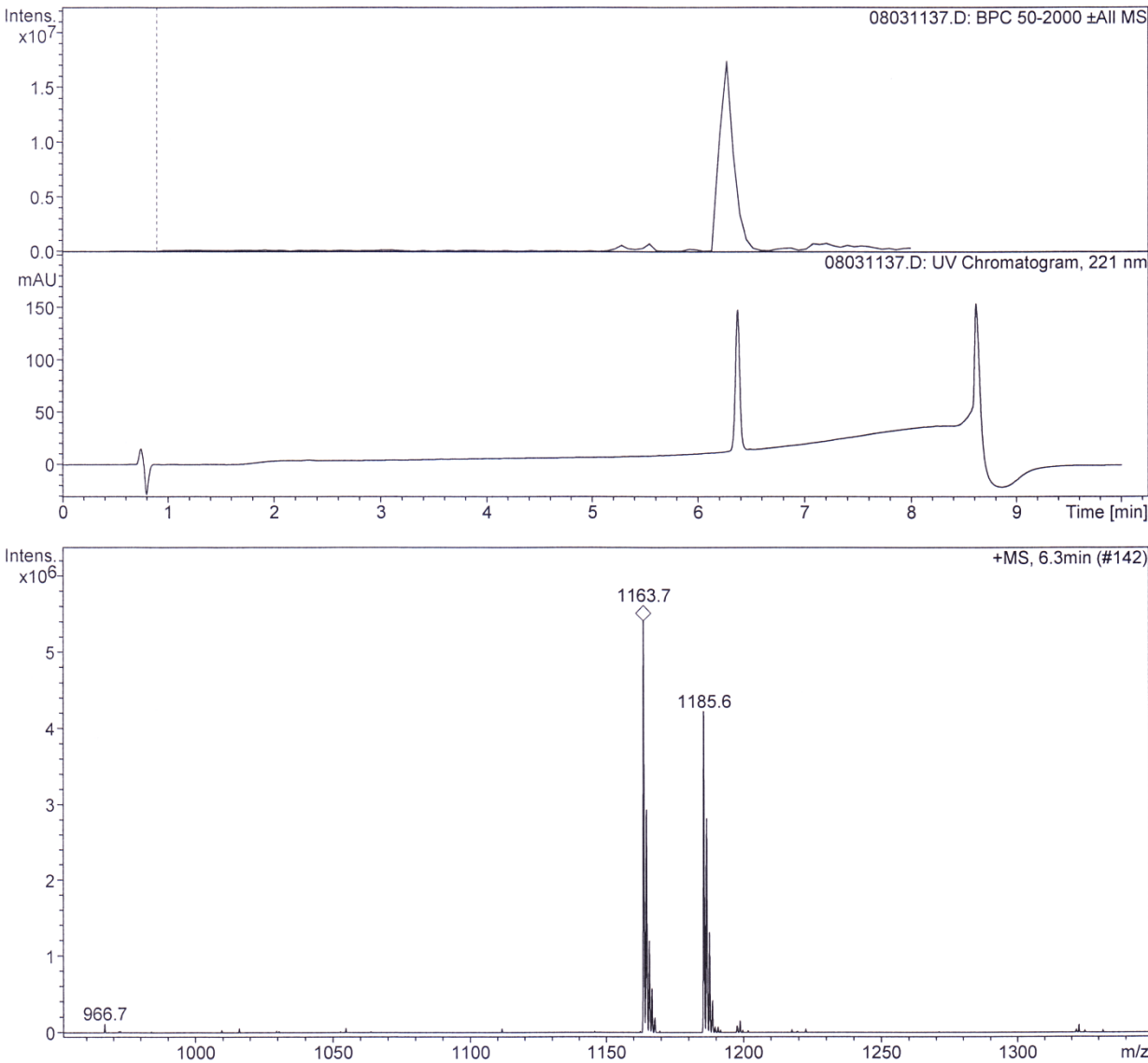
¹H NMR (400 MHz, Acetone-d₆)



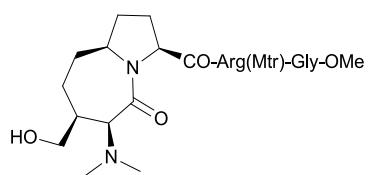
¹³C NMR (100.6 MHz, Acetone-d₆)



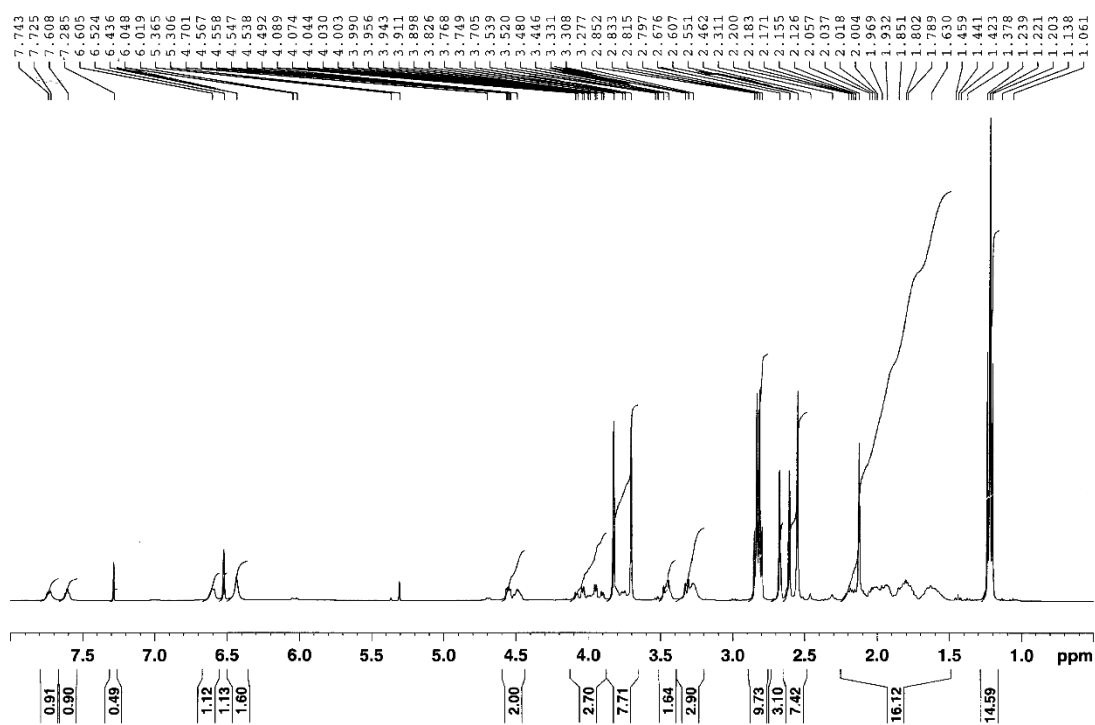
HPLC-MS (analytical method A)



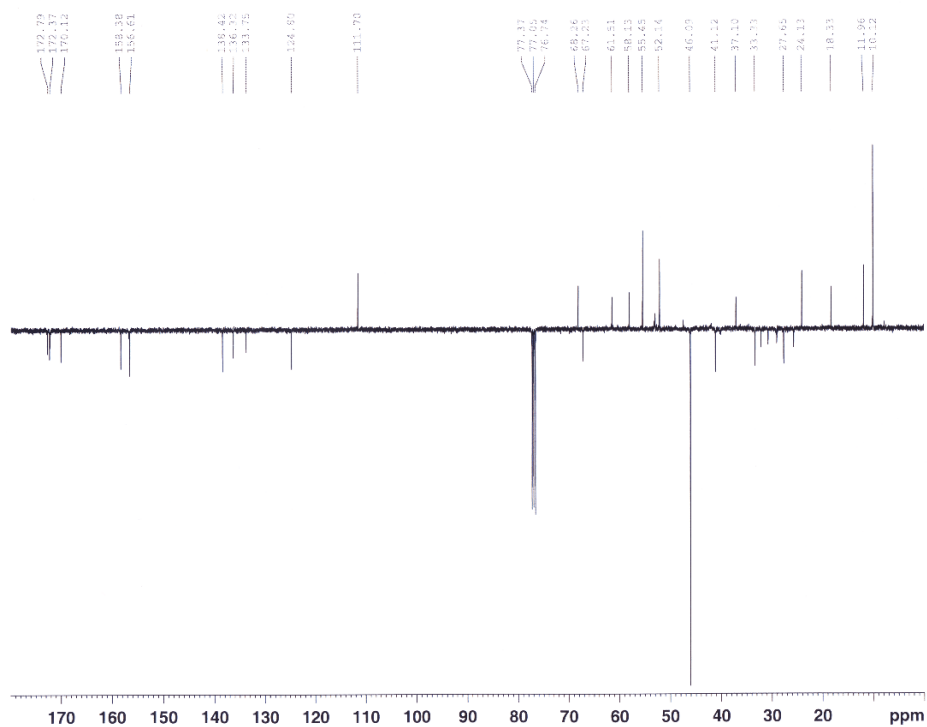
Compound 15



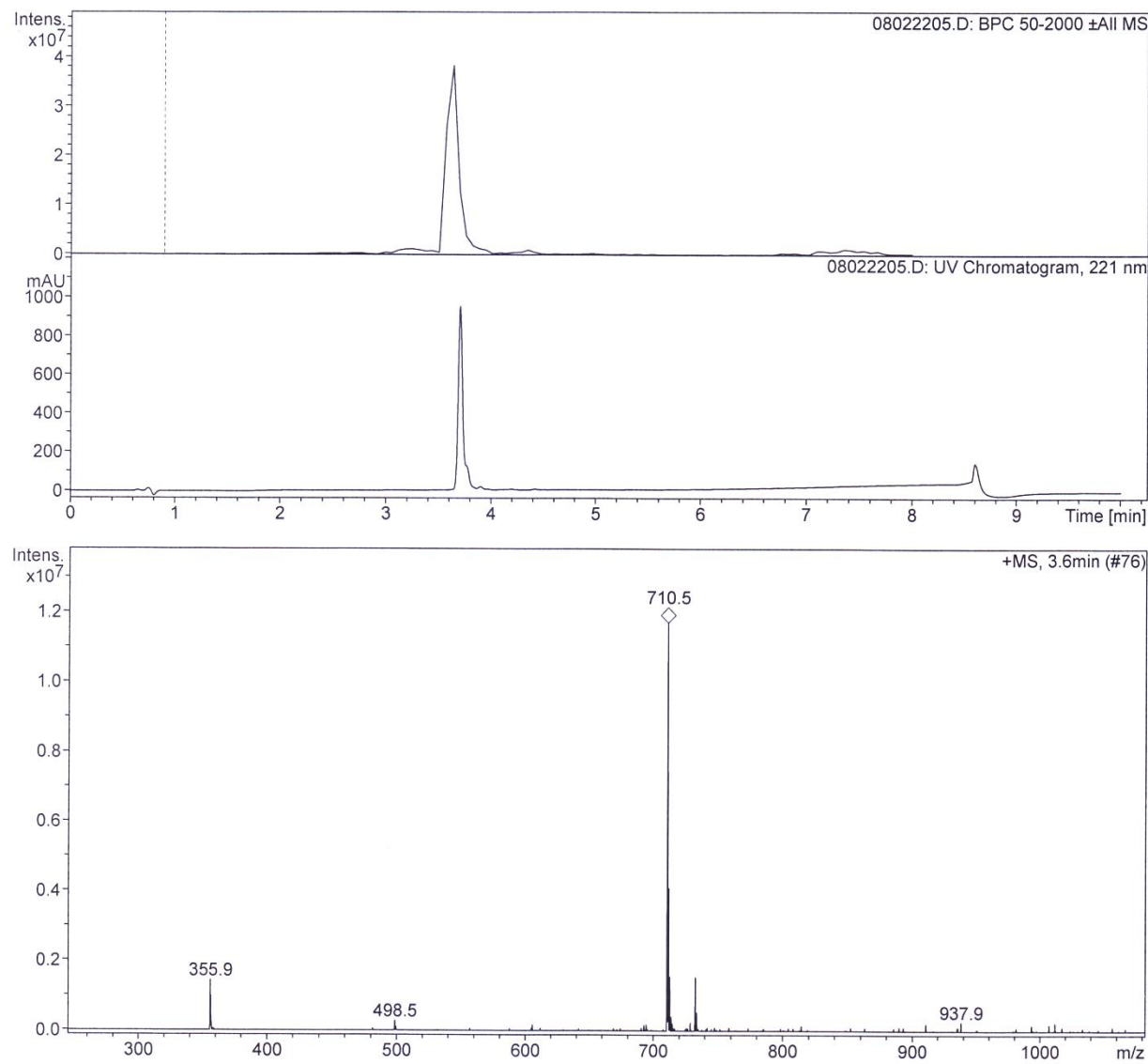
^1H NMR (400 MHz, Acetone- d_6)



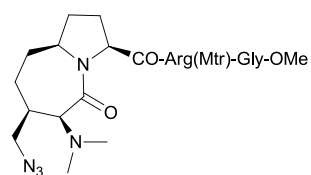
^{13}C NMR (100.6 MHz, Acetone- d_6)



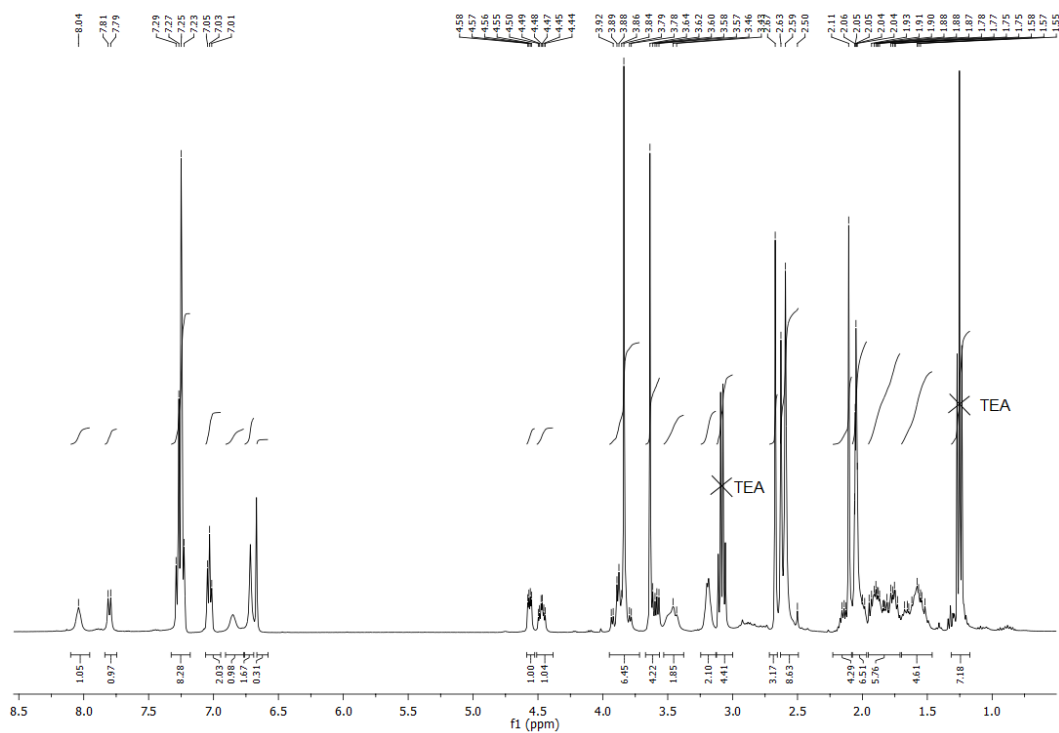
HPLC-MS (analytical method A)



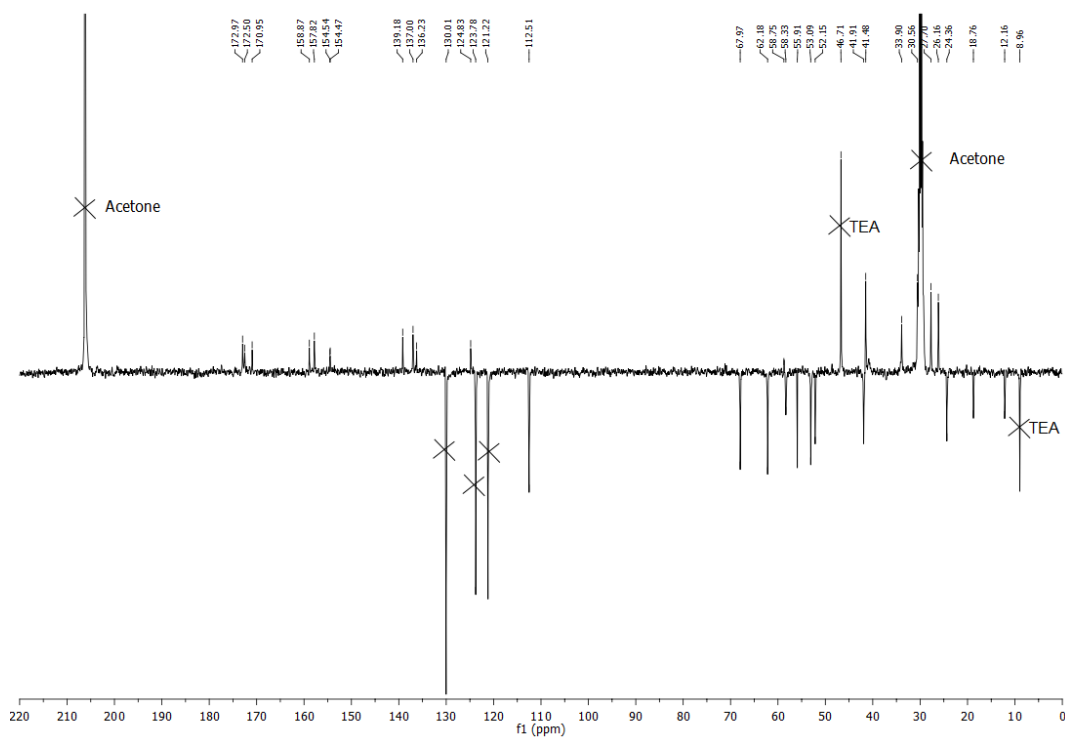
Compound 16



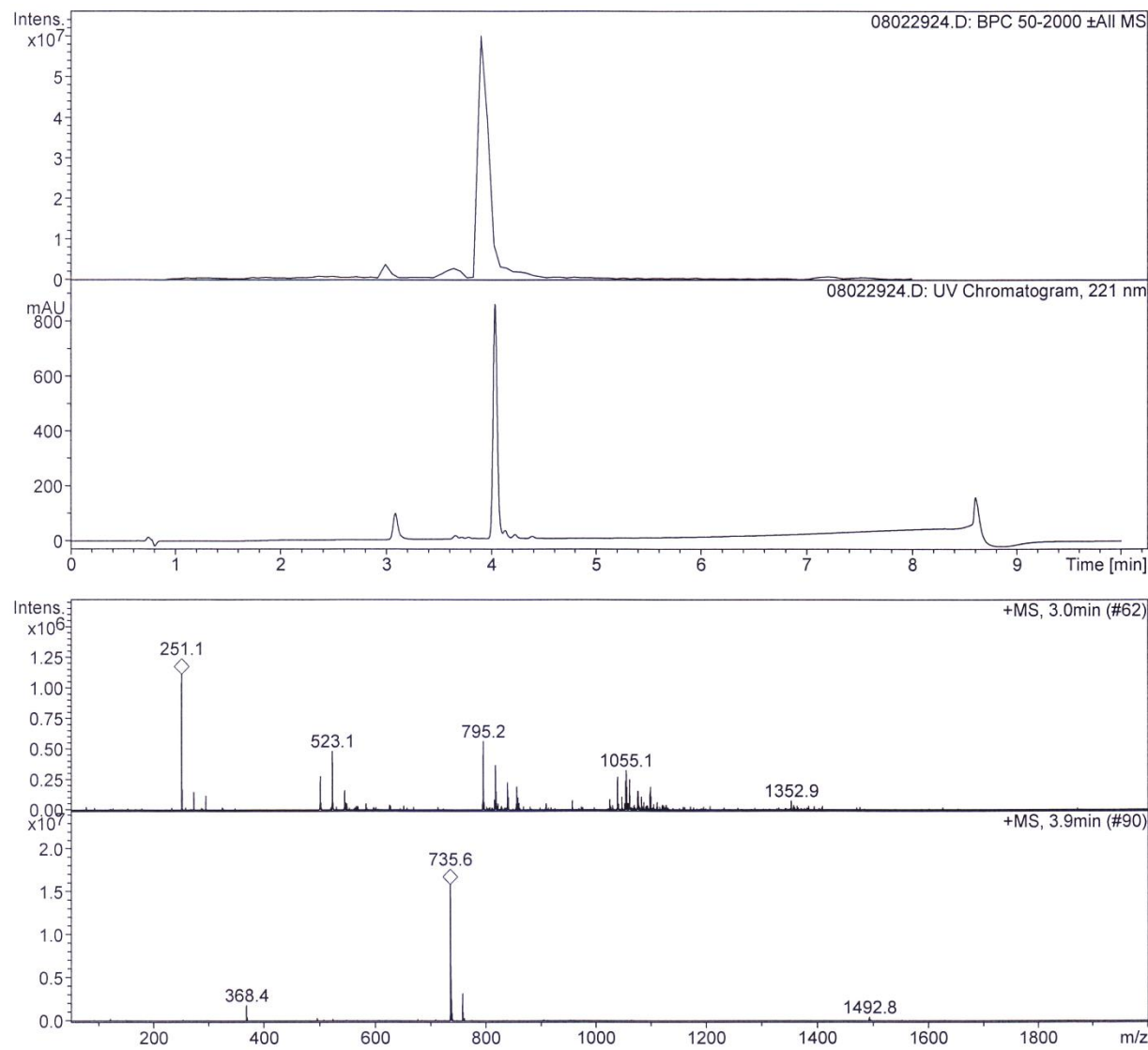
¹H NMR (400 MHz, Acetone-d₆)



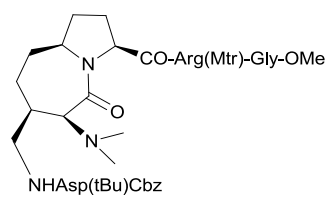
¹³C NMR (100.6 MHz, Acetone-d₆)



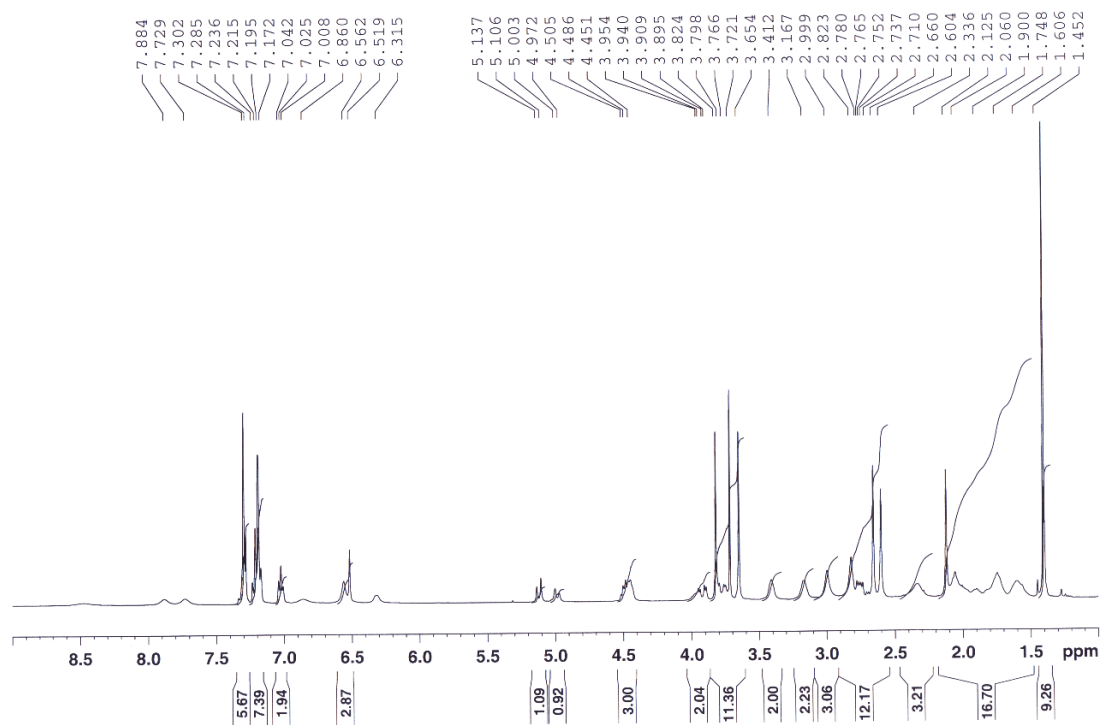
HPLC-MS (analytical method A)



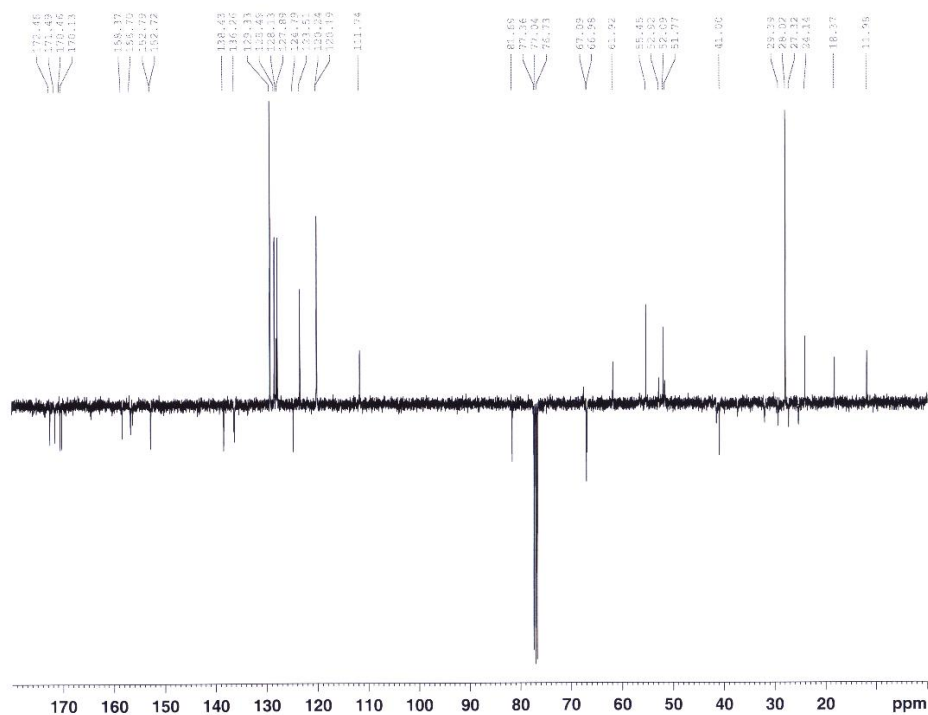
Compound 17



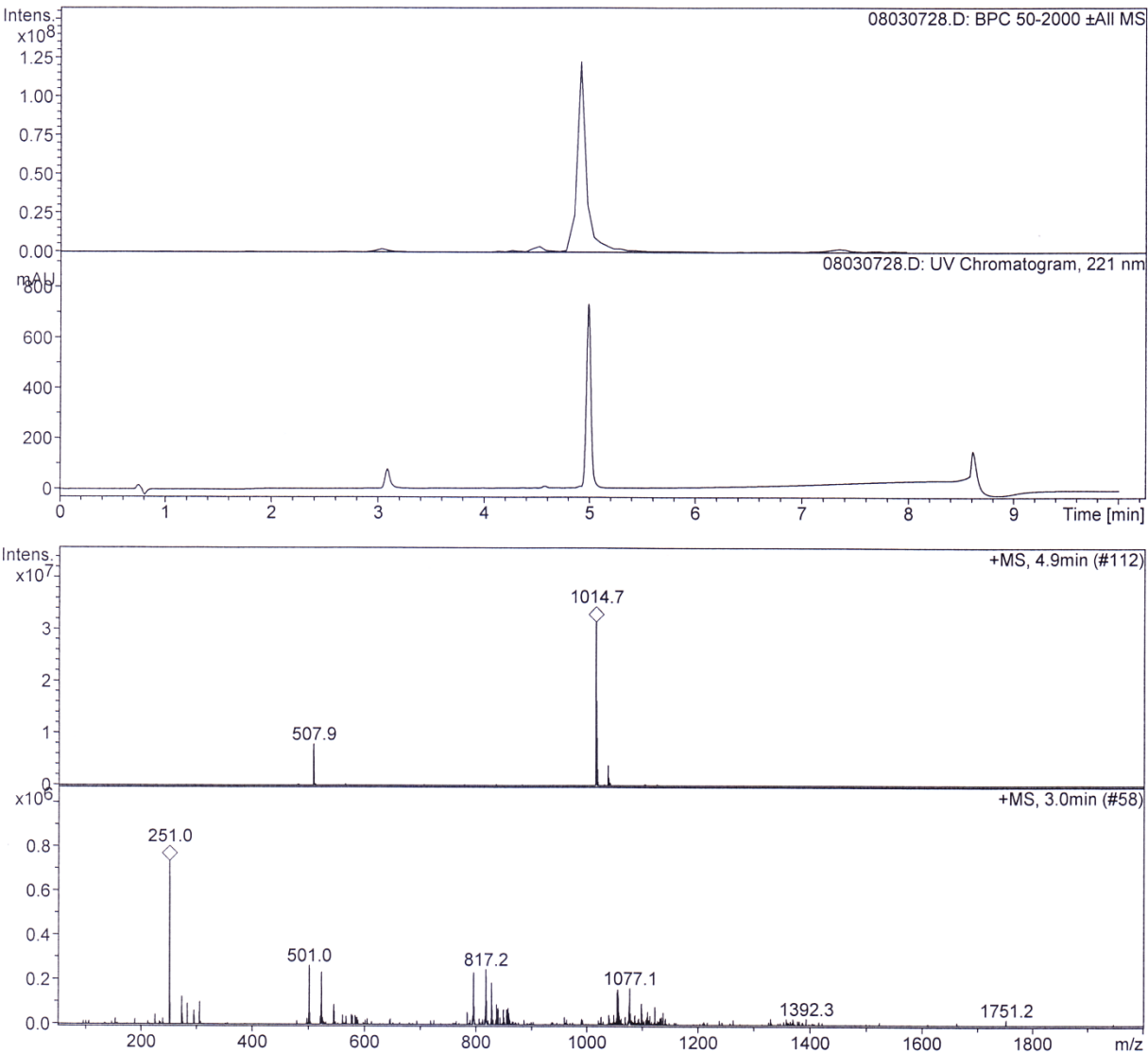
^1H NMR (400 MHz, CDCl_3)



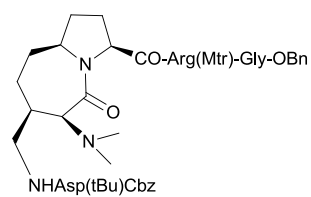
^{13}C NMR (100.6 MHz, CDCl_3)



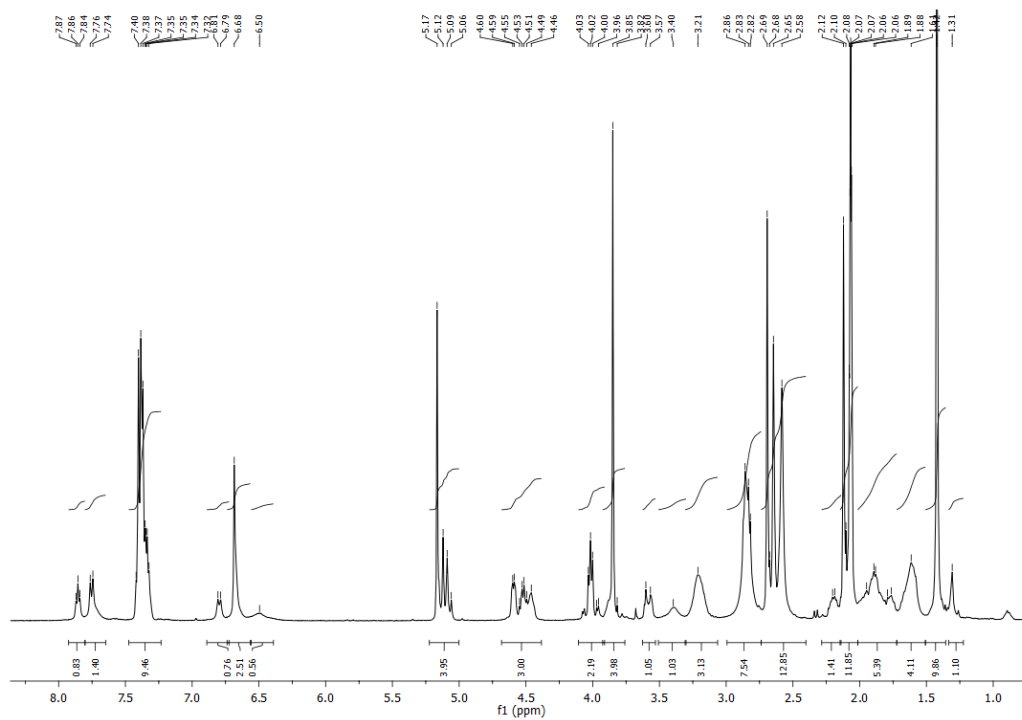
HPLC-MS (analytical method A)



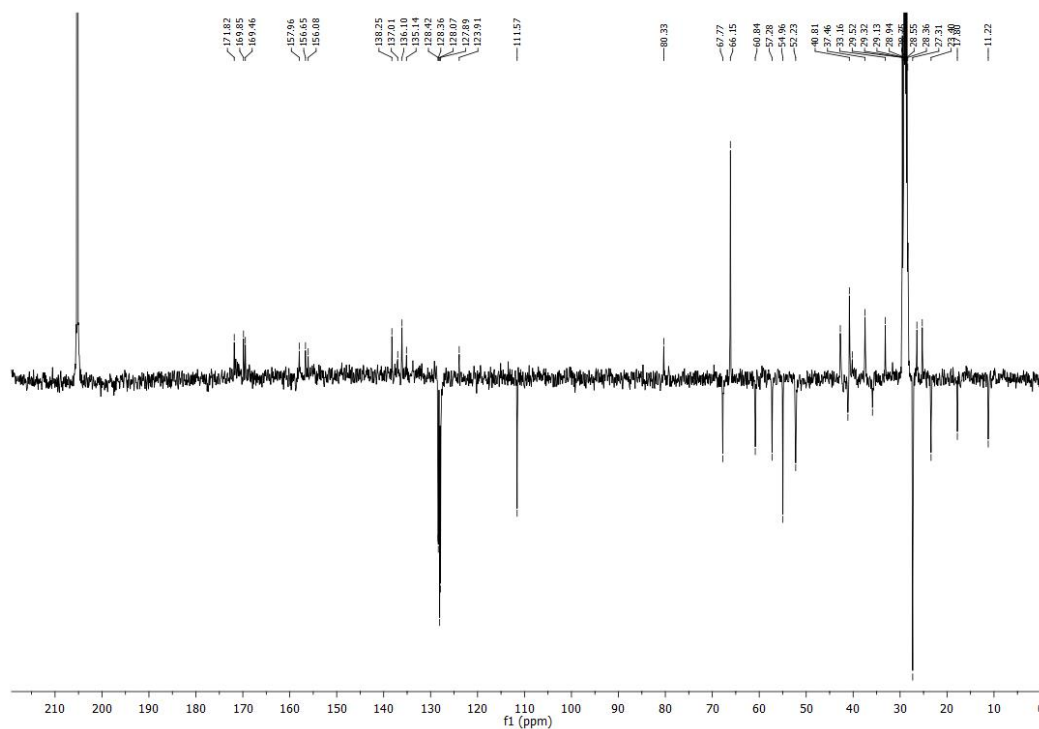
Compound 18



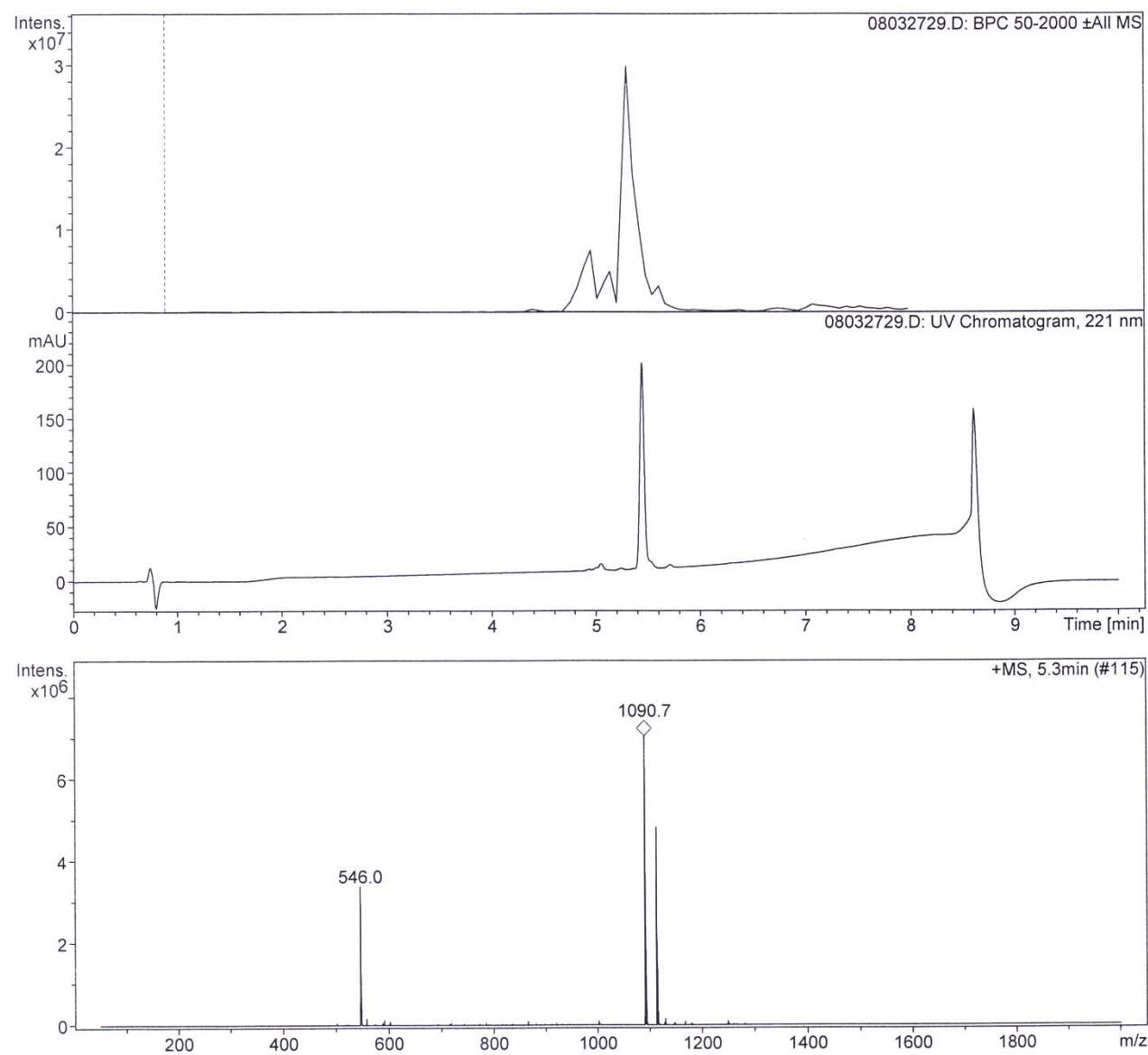
^1H NMR (400 MHz, *Acetone-d*₆)



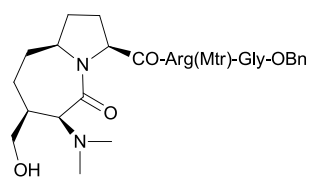
^{13}C NMR (100.6 MHz, *Acetone-d*₆)



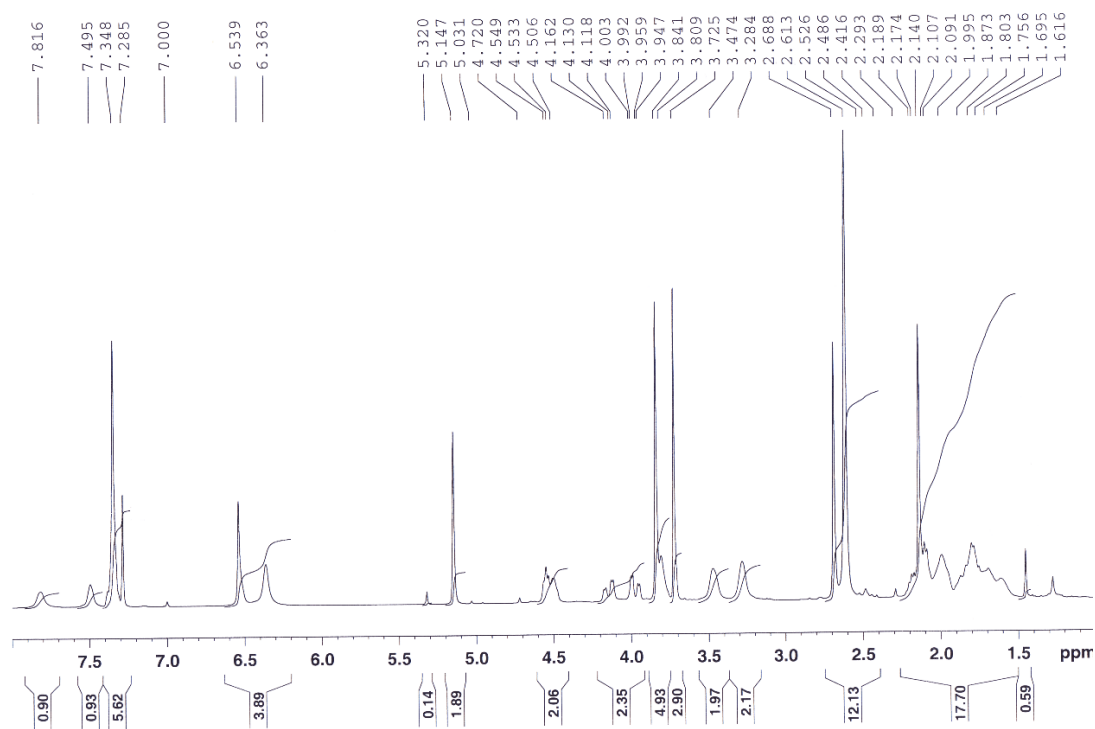
HPLC-MS (analytical method A)



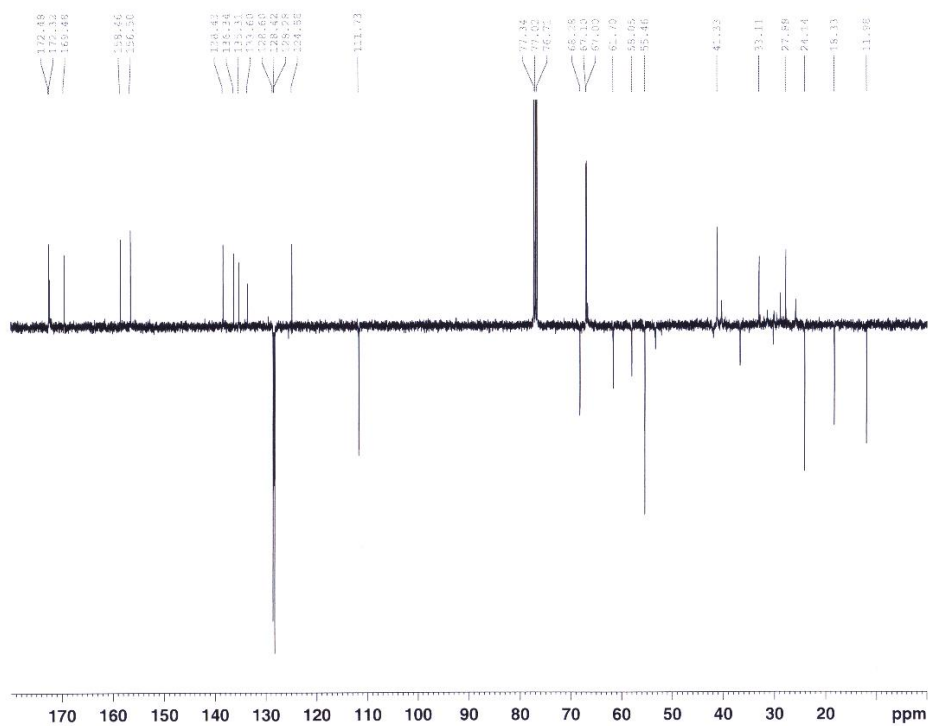
Compound 19



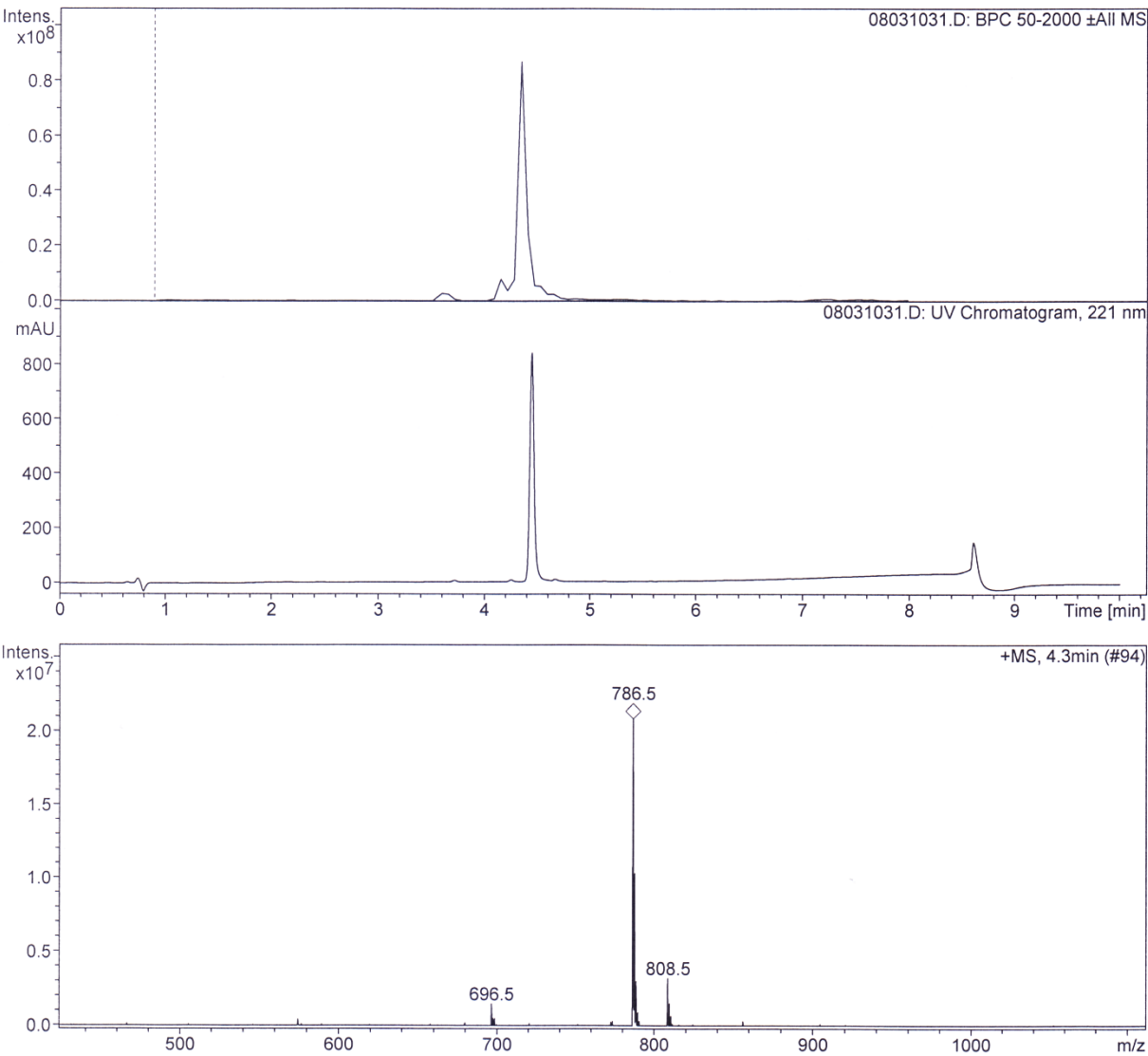
¹H NMR (400 MHz, CDCl₃)



¹³C NMR (100.6 MHz, CDCl₃)



HPLC-MS (analytical method A)

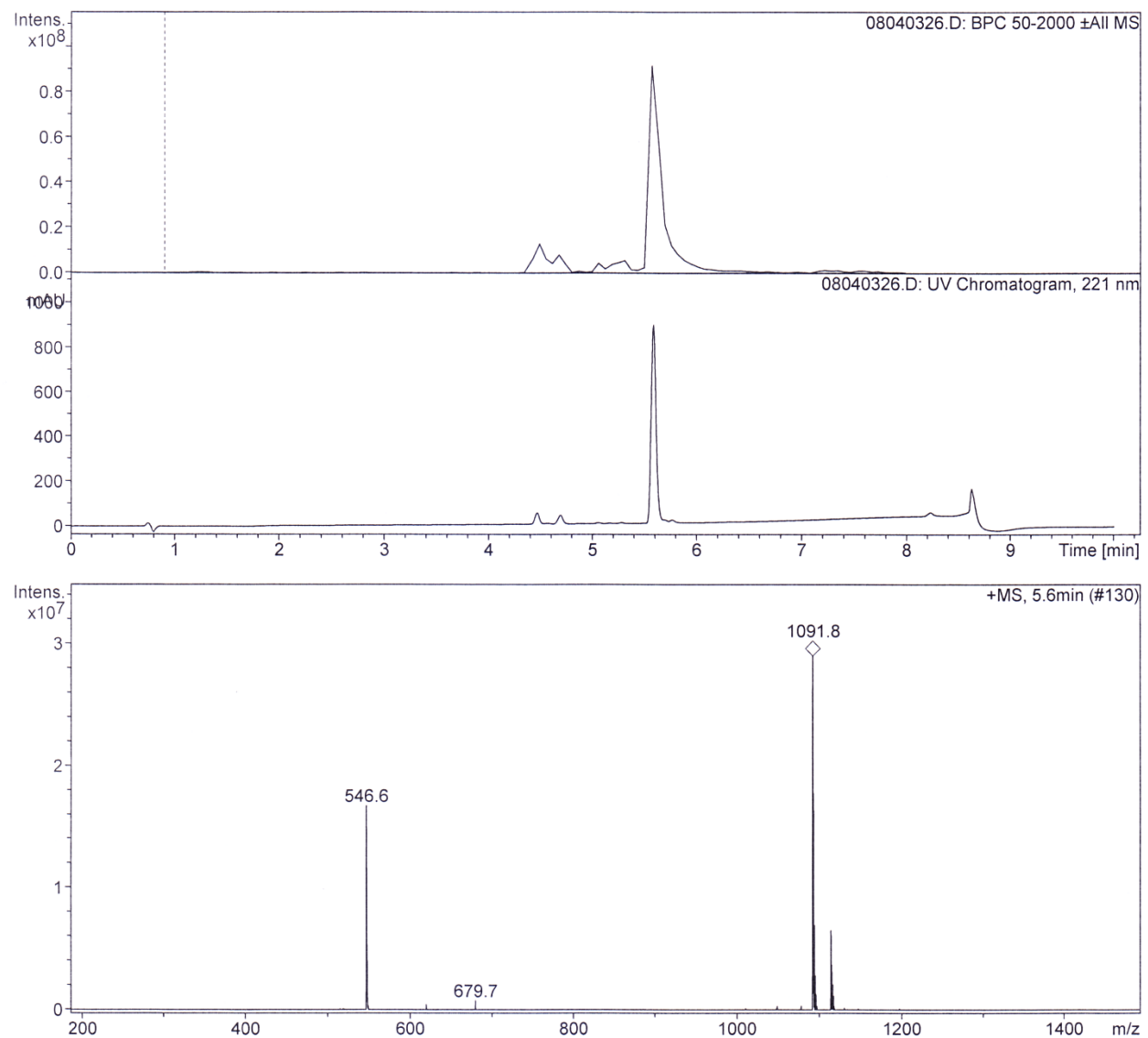


¹H NMR spectrum of compound 10 in CDCl₃. The spectrum shows peaks from 1.4 to 7.8 ppm. Integration values are provided below the baseline: 12.94, 3.78, 0.88, 4.51, 3.00, 0.90, 2.01, 1.05, 3.09, 2.29, 2.85, 0.56, 2.80, 3.24, 3.36, 6.22, 9.49, 3.17, 4.09, 14.81. Chemical shifts are labeled above the peaks: 7.754, 7.369, 7.352, 7.287, 6.538, 6.463, 6.379, 5.965, 5.810, 5.153, 5.136, 5.090, 4.606, 4.527, 4.475, 4.387, 4.252, 4.119, 4.102, 4.014, 3.984, 3.839, 3.728, 3.668, 3.275, 3.082, 2.936, 2.909, 2.788, 2.761, 2.694, 2.619, 2.499, 2.140, 2.062, 1.790, 1.666, 1.593, 1.488, 1.432.

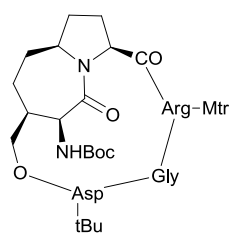
13C NMR spectrum of compound 1. The x-axis represents chemical shift in ppm, ranging from 170 to 20. The spectrum shows several sharp peaks in the aromatic region (120-140 ppm), a cluster of peaks between 40 and 60 ppm, and a very tall, sharp peak at approximately 77 ppm, which is the solvent peak for CDCl₃. A list of chemical shift values is provided at the top of the spectrum.

| Chemical Shift (ppm) |
|----------------------|
| 159.25 |
| 157.28 |
| 151.76 |
| 149.27 |
| 145.07 |
| 138.41 |
| 136.33 |
| 132.40 |
| 131.33 |
| 130.30 |
| 129.17 |
| 128.77 |
| 128.57 |
| 128.44 |
| 127.33 |
| 126.10 |
| 125.90 |
| 124.87 |
| 11.70 |
| 77.00 |
| 76.82 |
| 76.64 |
| 76.46 |
| 76.28 |
| 76.10 |
| 75.92 |
| 75.74 |
| 75.56 |
| 75.38 |
| 75.20 |
| 75.02 |
| 74.84 |
| 74.66 |
| 74.48 |
| 74.30 |
| 74.12 |
| 73.94 |
| 73.76 |
| 73.58 |
| 73.40 |
| 73.22 |
| 73.04 |
| 72.86 |
| 72.68 |
| 72.50 |
| 72.32 |
| 72.14 |
| 71.96 |
| 71.78 |
| 71.60 |
| 71.42 |
| 71.24 |
| 71.06 |
| 70.88 |
| 70.70 |
| 70.52 |
| 70.34 |
| 70.16 |
| 69.98 |
| 69.80 |
| 69.62 |
| 69.44 |
| 69.26 |
| 69.08 |
| 68.90 |
| 68.72 |
| 68.54 |
| 68.36 |
| 68.18 |
| 68.00 |
| 67.82 |
| 67.64 |
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| 65.66 |
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| 65.30 |
| 65.12 |
| 64.94 |
| 64.76 |
| 64.58 |
| 64.40 |
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| 55.22 |
| 55.04 |
| 54.86 |
| 54.68 |
| 54.50 |
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| 46.58 |
| 46.40 |
| 46.22 |
| 46.04 |
| 45.86 |
| 45.68 |
| 45.50 |
| 45.32 |
| 45.14 |
| 44.96 |
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| 42.26 |
| 42.08 |
| 41.90 |
| 41.72 |
| 41.54 |
| 41.36 |
| 41.18 |
| 41.00 |
| 40.82 |
| 40.64 |
| 40.46 |
| 40.28 |
| 40.10 |
| 39.92 |
| 39.74 |
| 39.56 |
| 39.38 |
| 39.20 |
| 39.02 |
| 38.84 |
| 38.66 |
| 38.48 |
| 38.30 |
| 38.12 |
| 37.94 |
| 37.76 |
| 37.58 |
| 37.40 |
| 37.22 |
| 37.04 |
| 36.86 |
| 36.68 |
| 36.50 |
| 36.32 |
| 36.14 |
| 35.96 |
| 35.78 |
| 35.60 |
| 35.42 |
| 35.24 |
| 35.06 |
| 34.88 |
| 34.70</ |

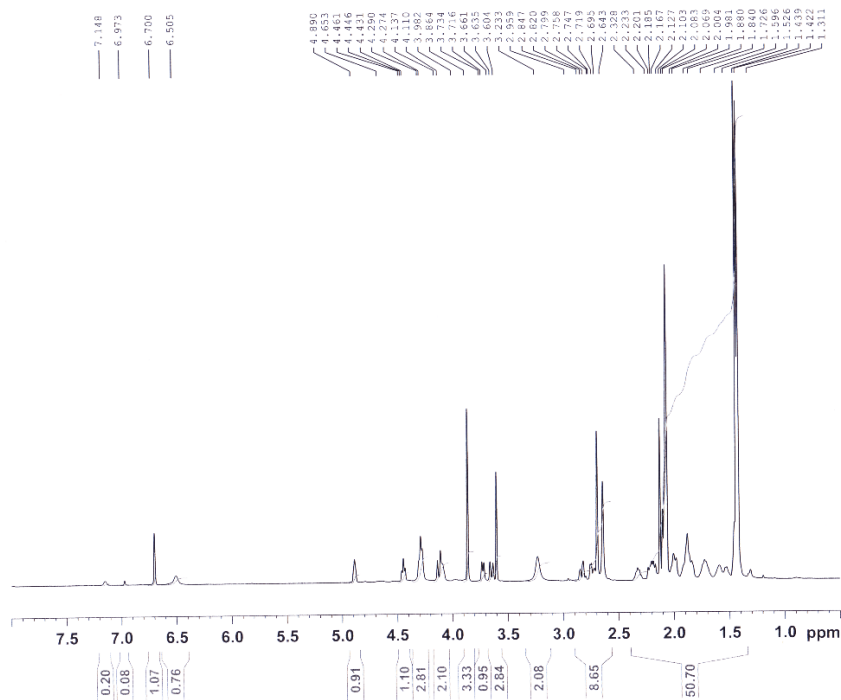
HPLC-MS (analytical method A)



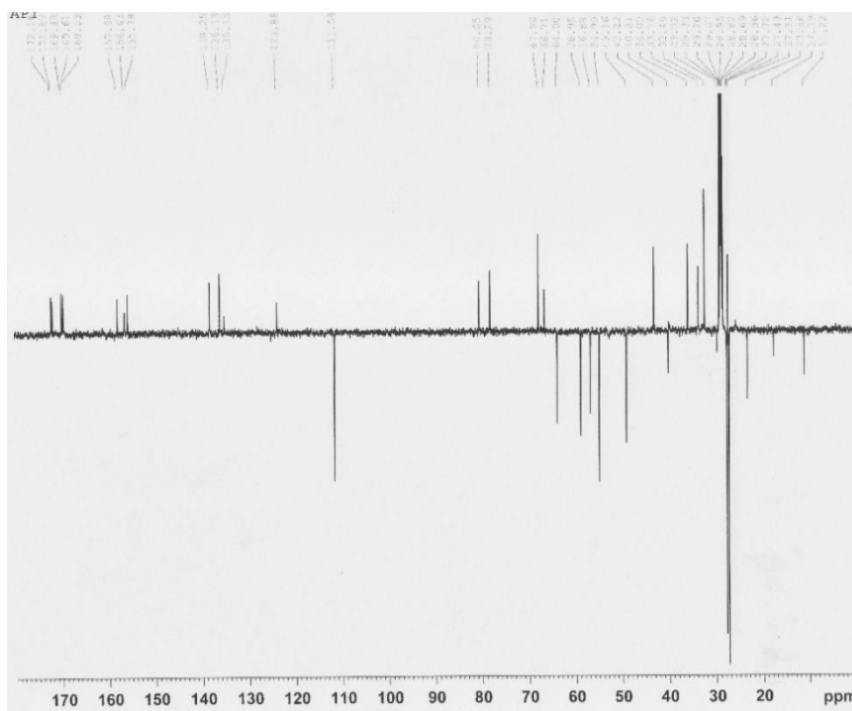
Compound 21



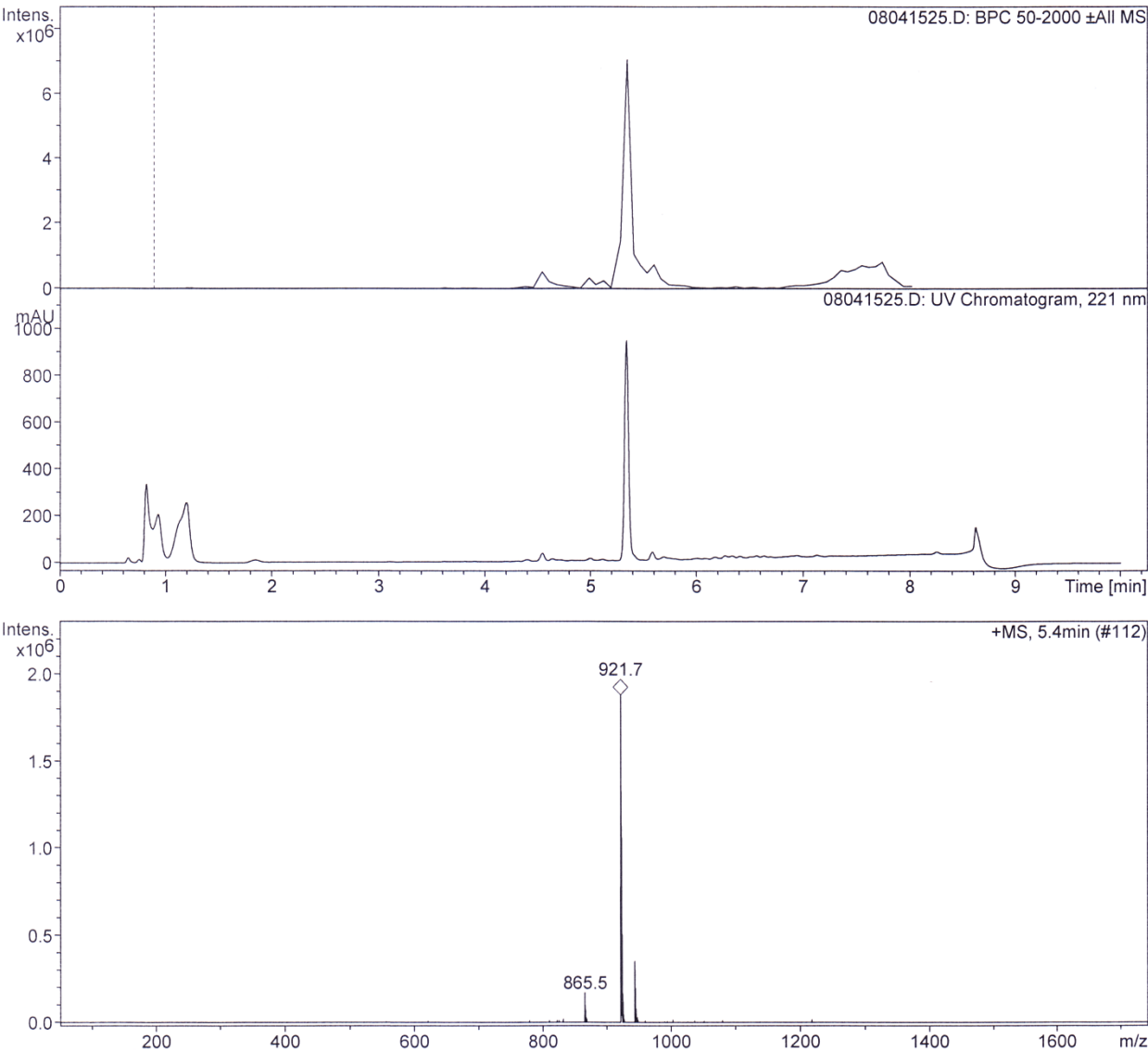
^1H NMR (400 MHz, *Acetone-d*₆)



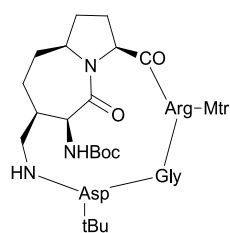
^{13}C NMR (100.6 MHz, *Acetone-d*₆)



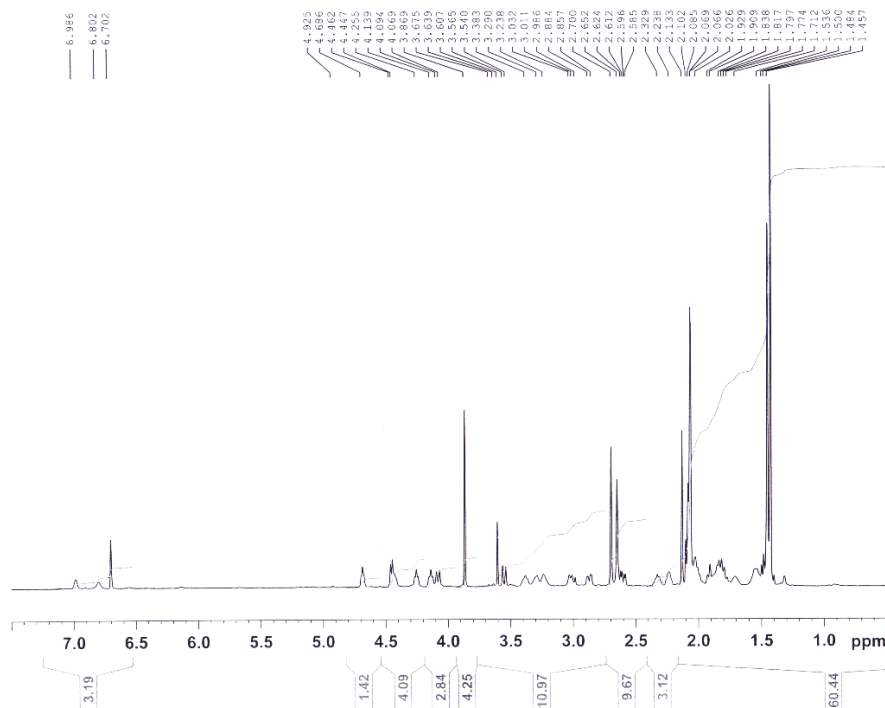
HPLC-MS (analytical method A)



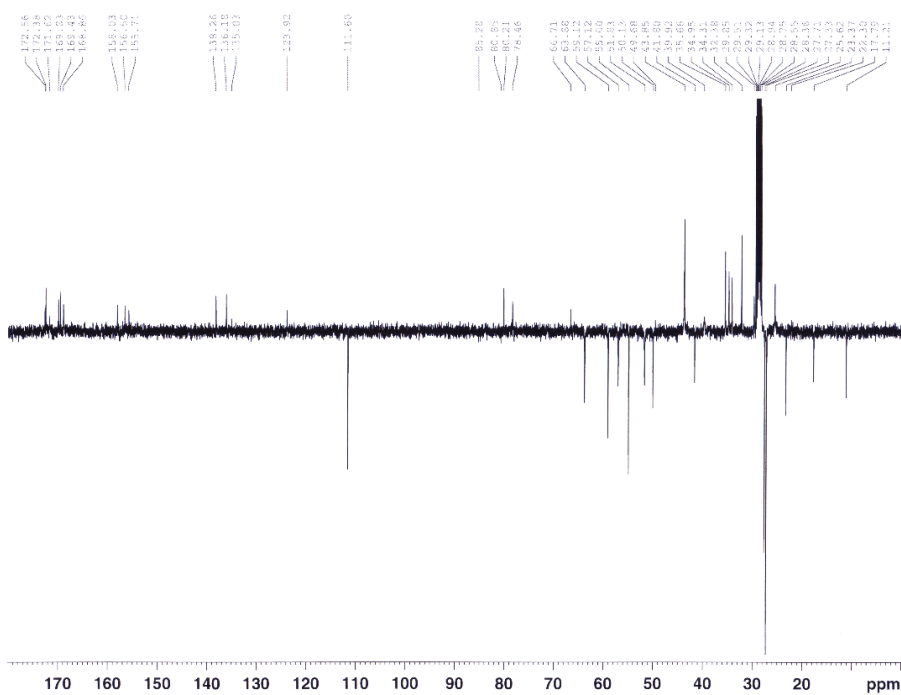
Compound 22



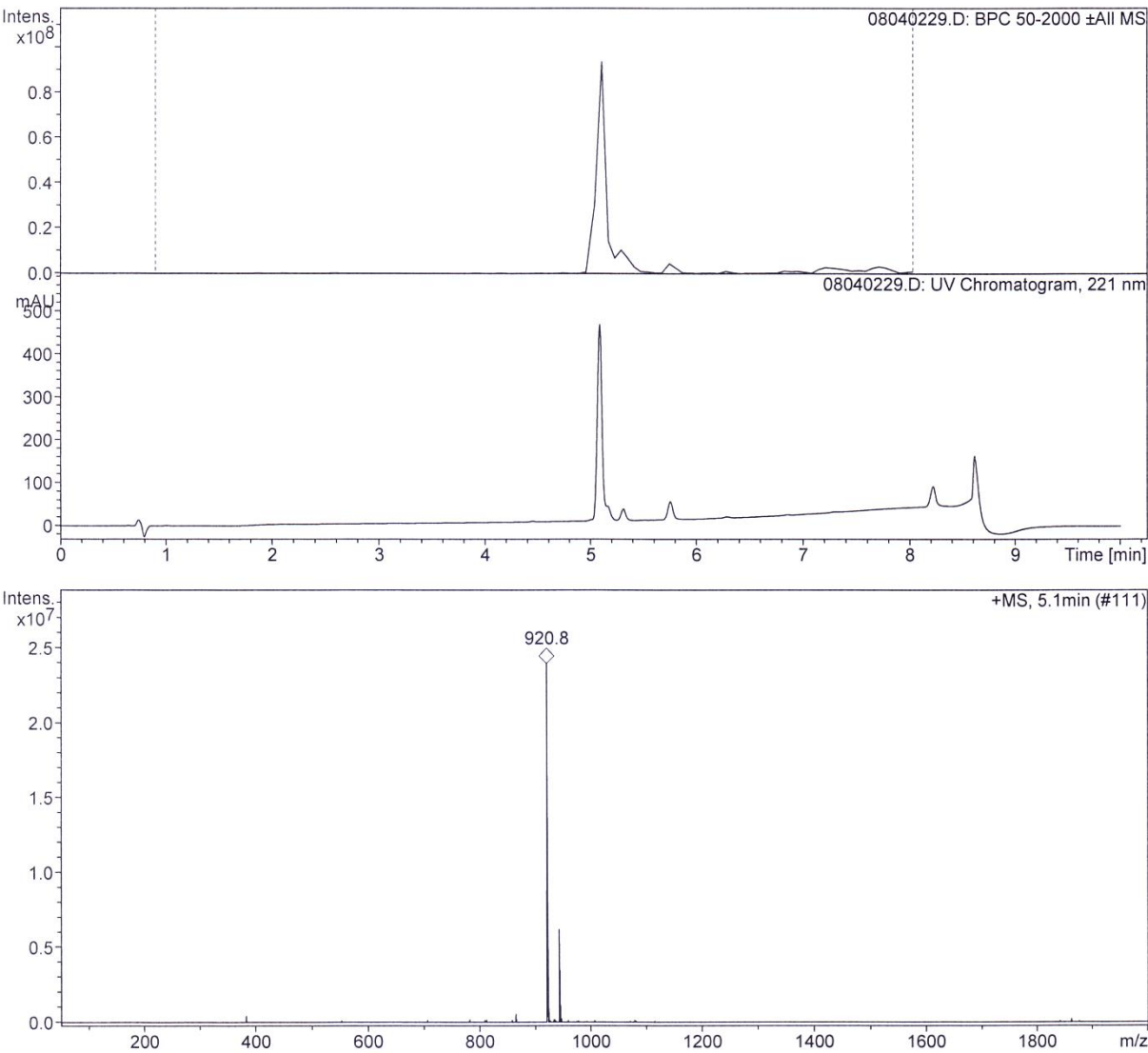
^1H NMR (400 MHz, *Acetone-d*₆)



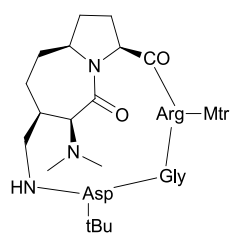
^{13}C NMR (100.6 MHz, *Acetone-d*₆)



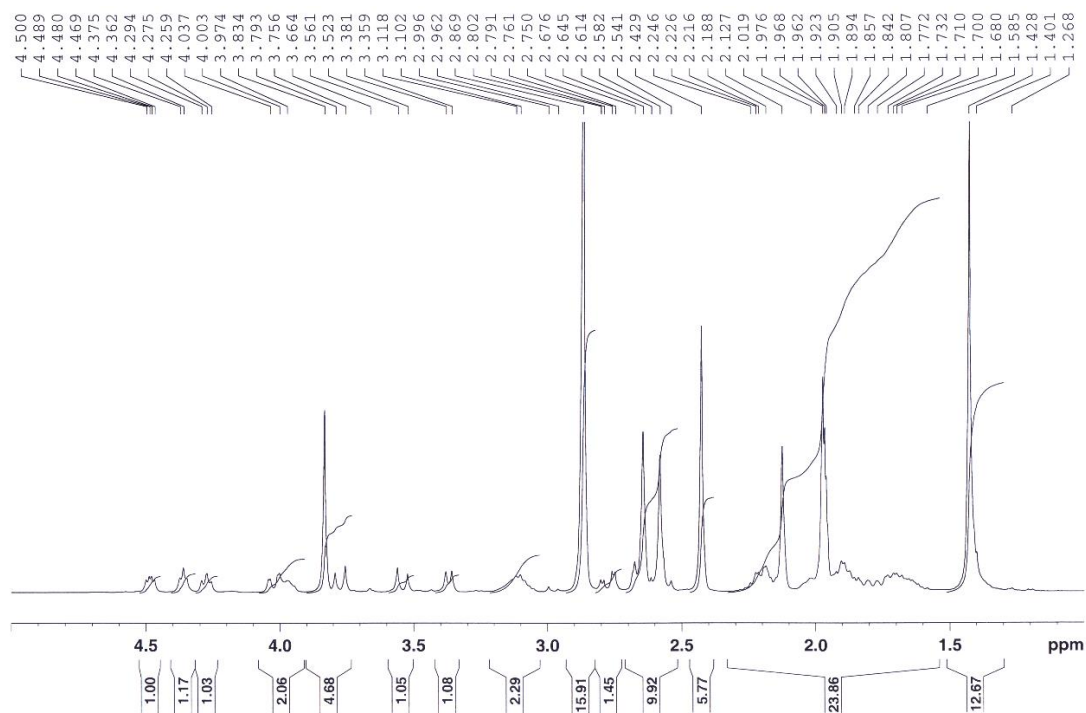
HPLC-MS (analytical method A)



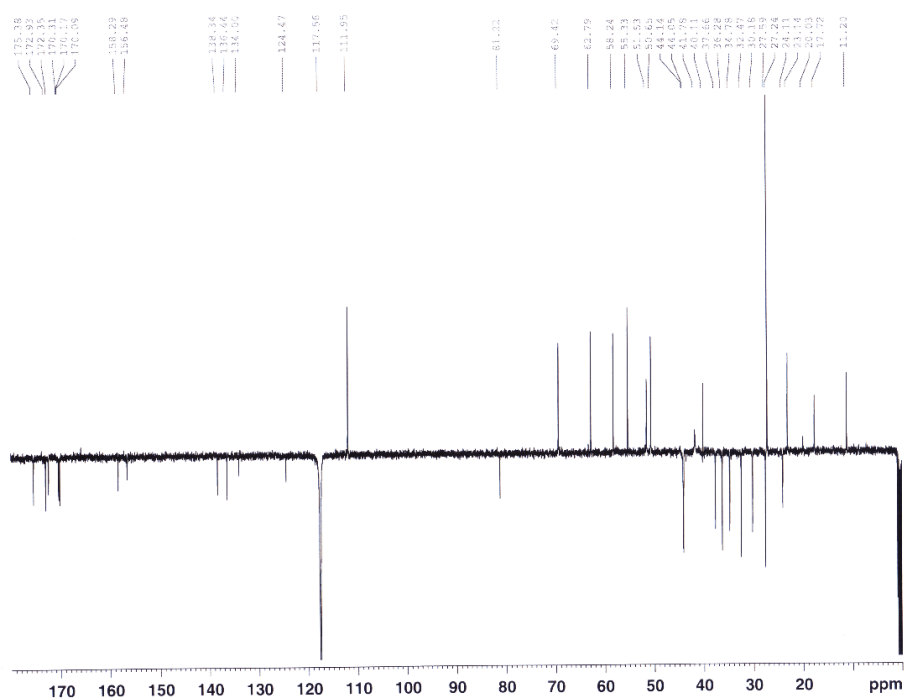
Compound 23



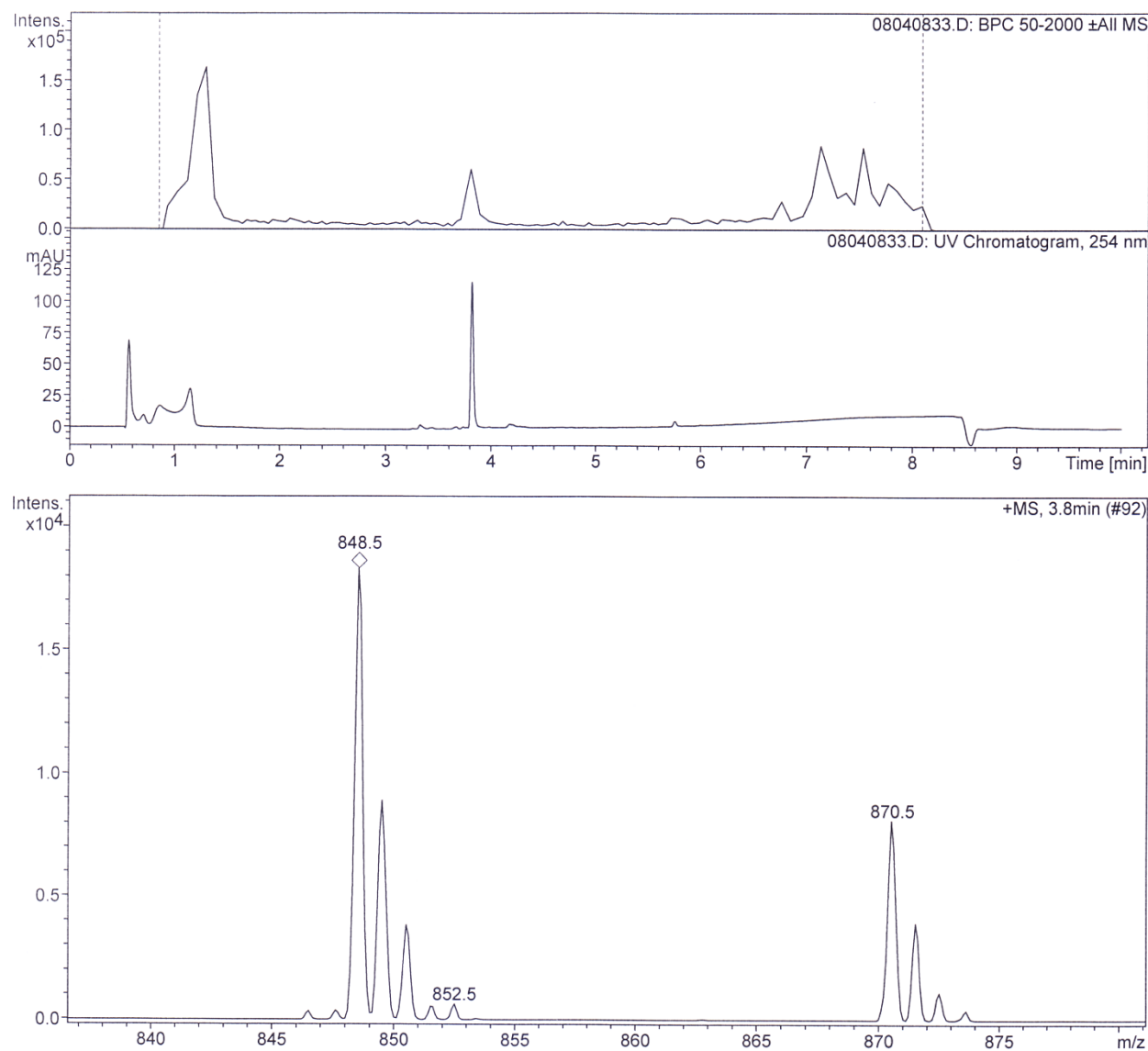
^1H NMR (400 MHz, $\text{CD}_3\text{CN} + \text{D}_2\text{O}$)



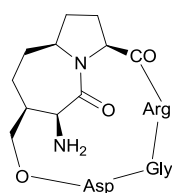
^{13}C NMR (100.6 MHz, $\text{CD}_3\text{CN} + \text{D}_2\text{O}$)



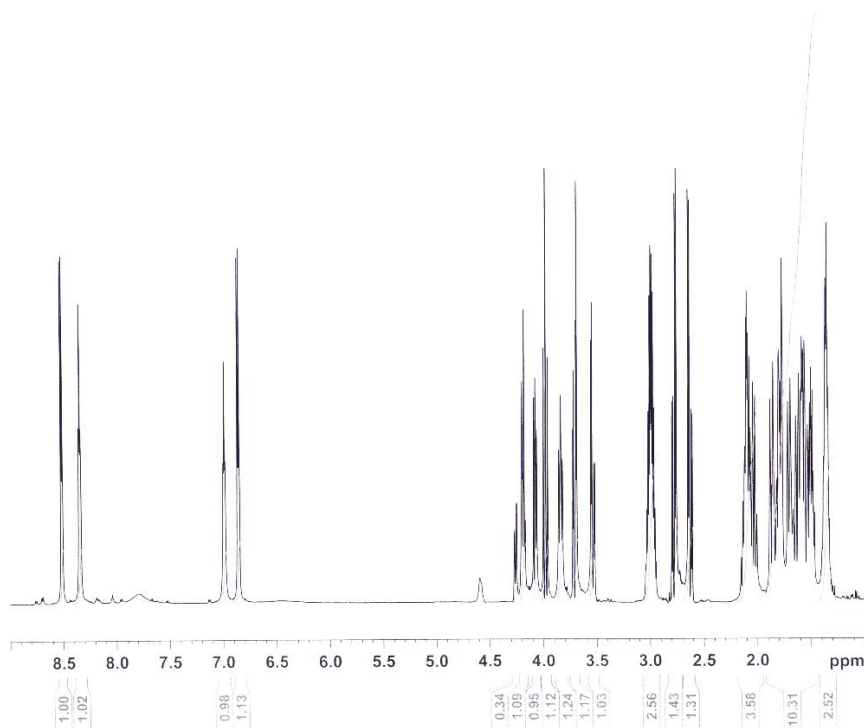
HPLC-MS (analytical method B)



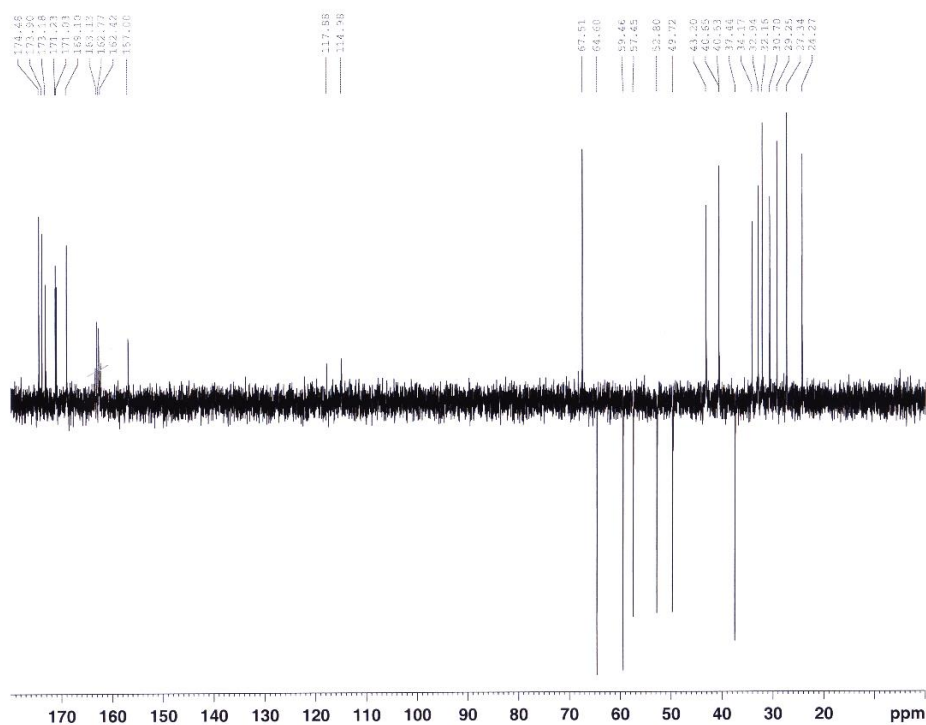
Compound 2



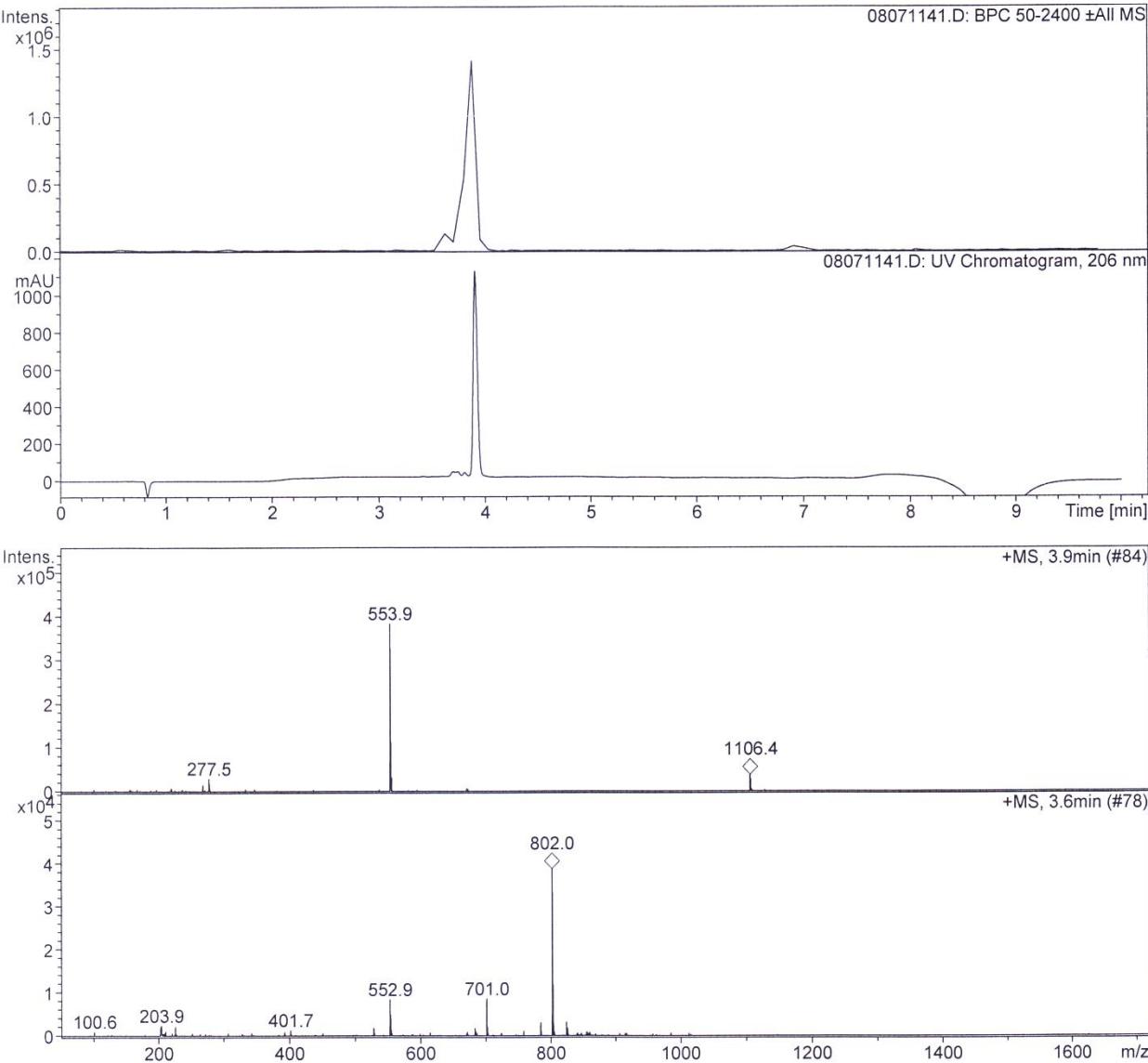
^1H NMR (600 MHz, $\text{D}_2\text{O} + \text{H}_2\text{O}$)



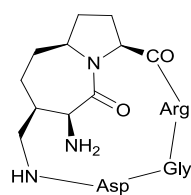
^{13}C NMR (100.6 MHz, $\text{D}_2\text{O} + \text{H}_2\text{O}$)



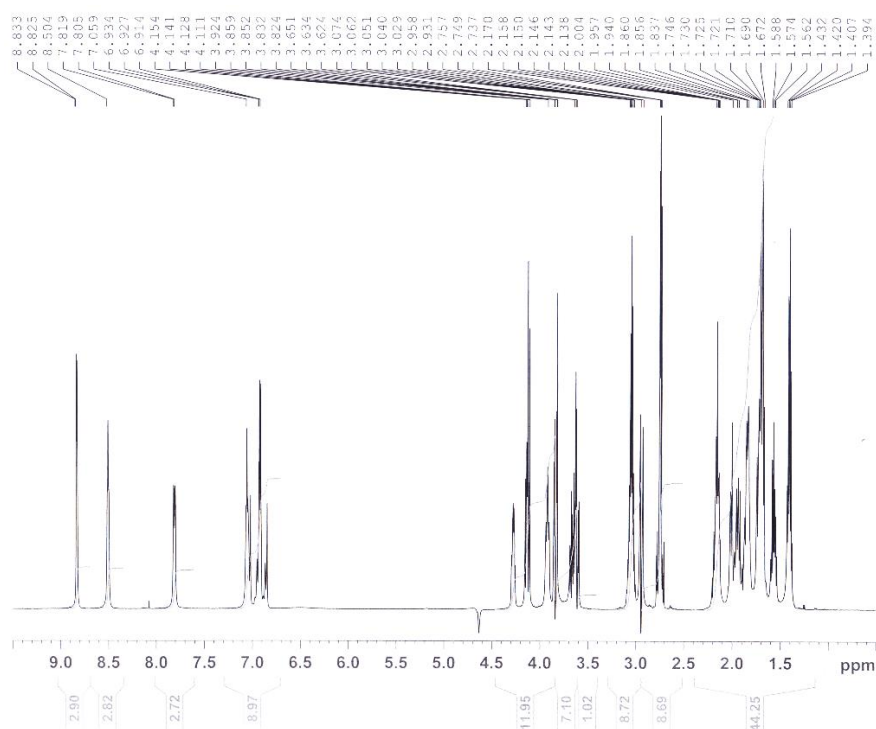
HPLC-MS (analytical method C)



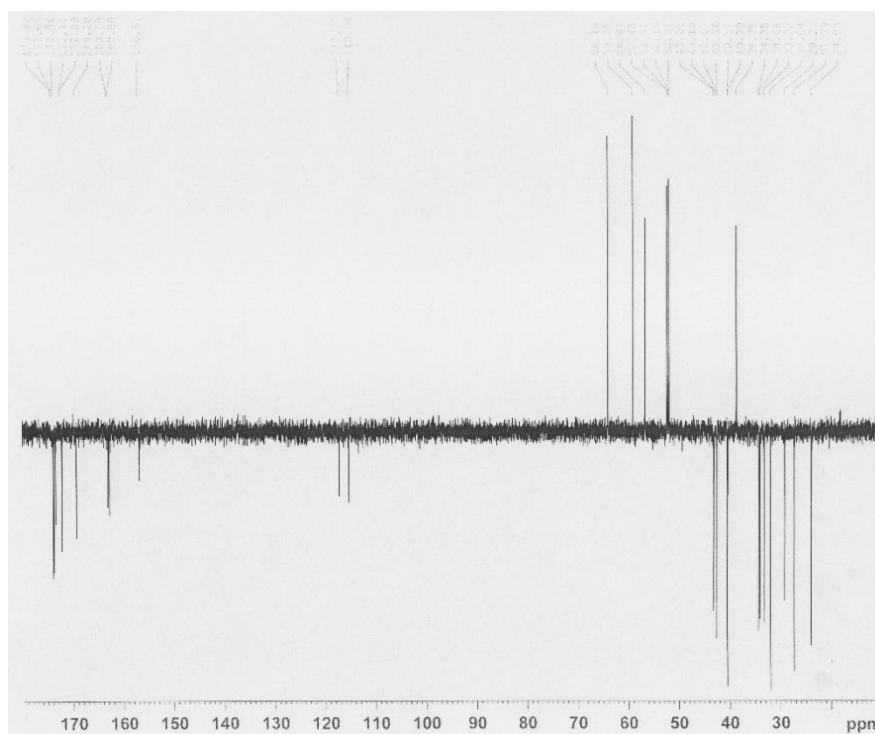
Compound 3



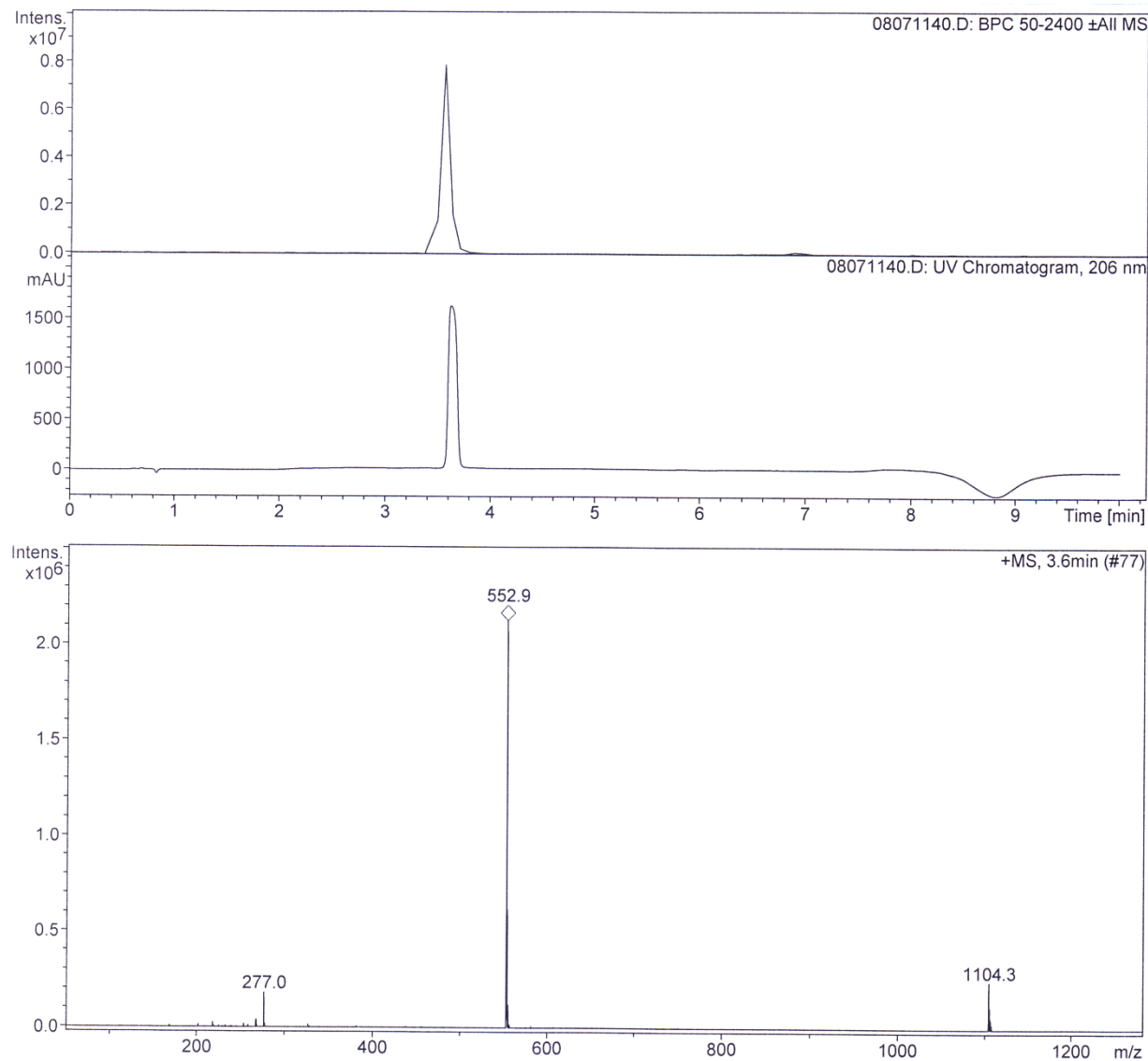
^1H NMR (600 MHz, $\text{D}_2\text{O} + \text{H}_2\text{O}$)



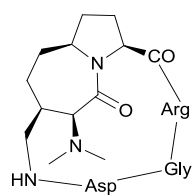
^{13}C NMR (150.95 MHz, $\text{D}_2\text{O} + \text{H}_2\text{O}$)



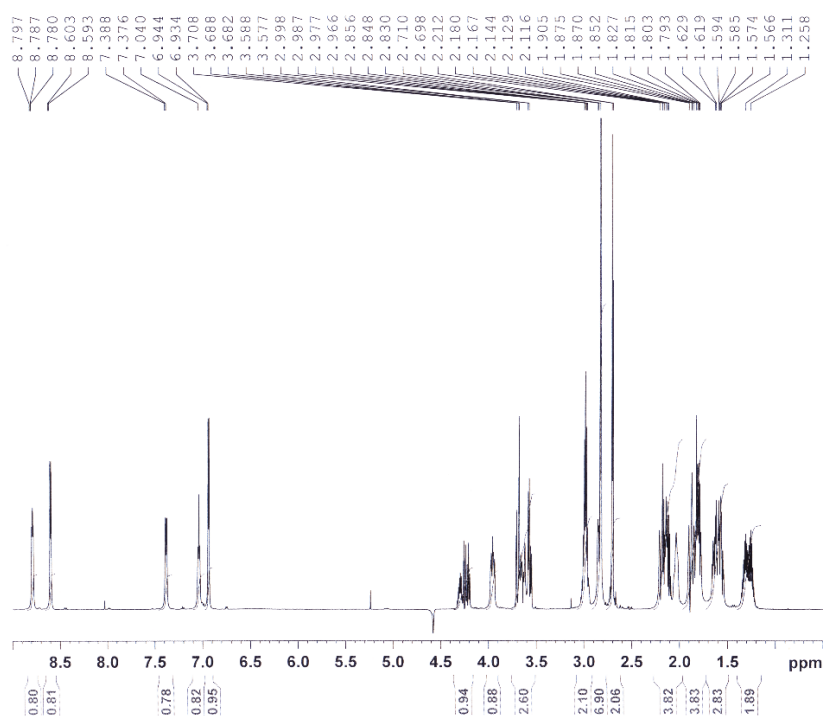
HPLC-MS (analytical method C)



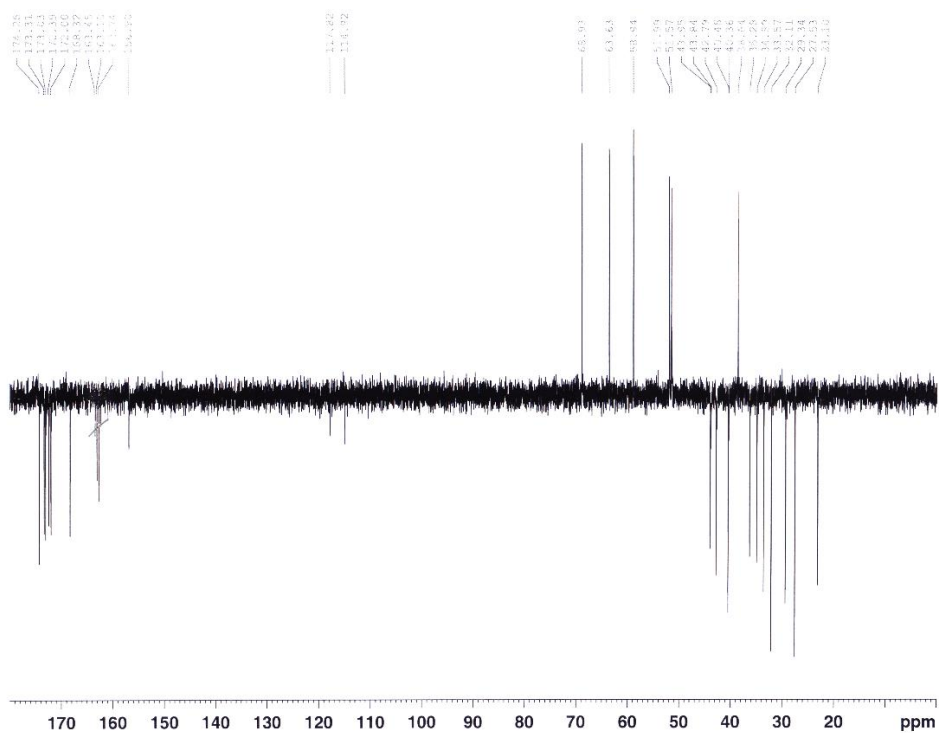
Compound 5



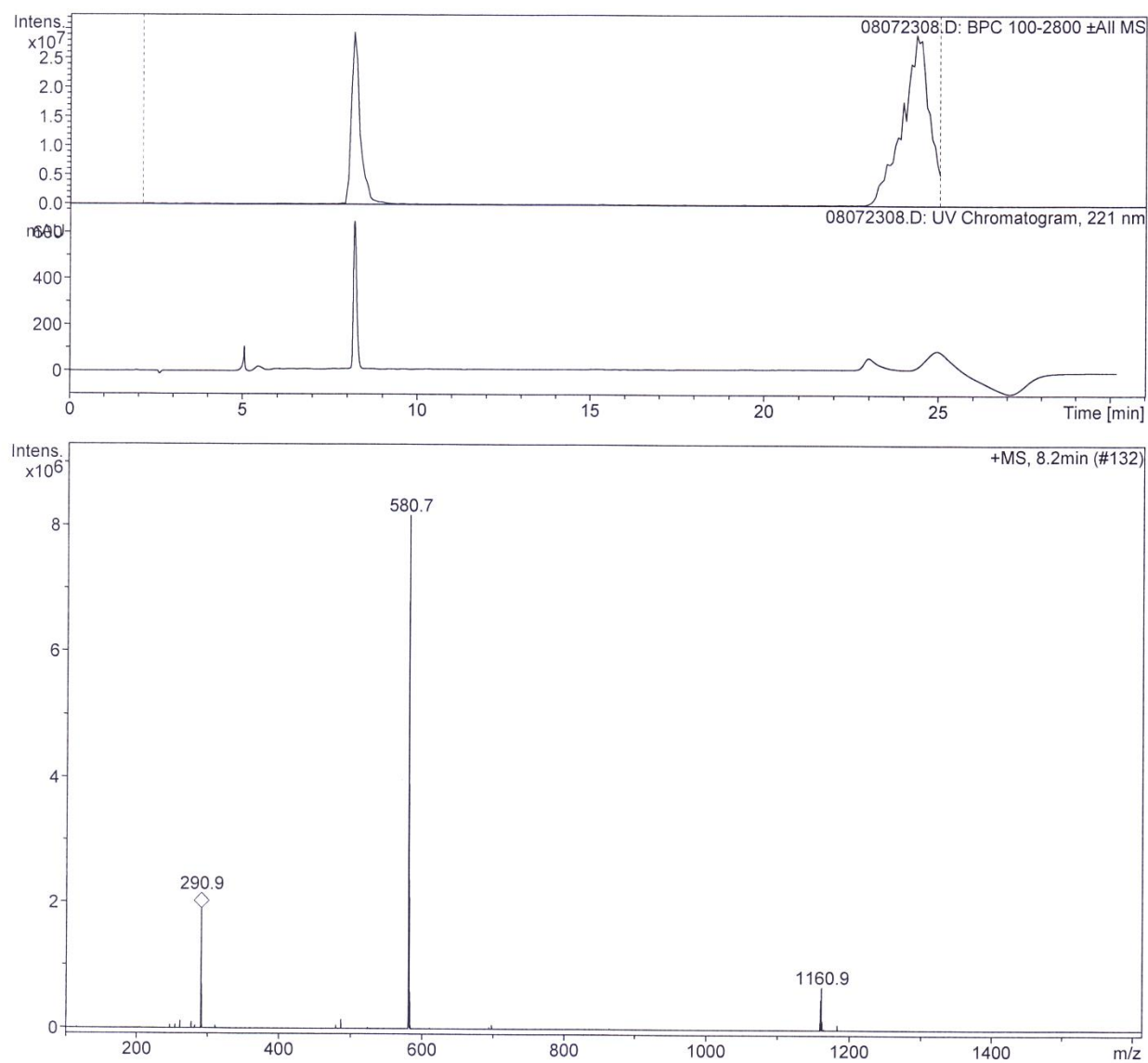
^1H NMR (600 MHz, $\text{D}_2\text{O} + \text{H}_2\text{O}$)



^{13}C NMR (150.95 MHz, $\text{D}_2\text{O} + \text{H}_2\text{O}$)



HPLC-MS (analytical method D)



Biology

Wound healing assay

Round bottomed 24-well plates were coated with vitronectin (Duotech) or fibronectin (Sigma) at 10 $\mu\text{g/mL}$ in PBS, overnight at 37 °C. 1.2×10^4 cells/ cm^2 /well were harvested by trypsinization, seeded and allowed to adhere until a nearly confluent state. Adherent cells were then scratched with a p200 pipette tip and washed three times with culture medium. The scratch was immediately photographed with a Zeiss Axio Observer A1 Inverted Microscope, equipped with a Zeiss AxioCam MRm. Subsequently, cells were incubated for 24 hours (HUVECs) or 48h (T98G) with the IC_{20} of test compound, as graphically extrapolated by the reference IC_{50} values determined by means of adhesion assay. Test compound was diluted in culture medium supplemented with 2% v/v FBS. Control cells were maintained in culture medium supplemented with 2% v/v FBS. After treatment, cells were washed once in culture medium and the scratch was photographed as previously described. This allowed to compare the width of the scratches before and after the treatment. Each experiment was done in duplicate. The best representative images were chosen.

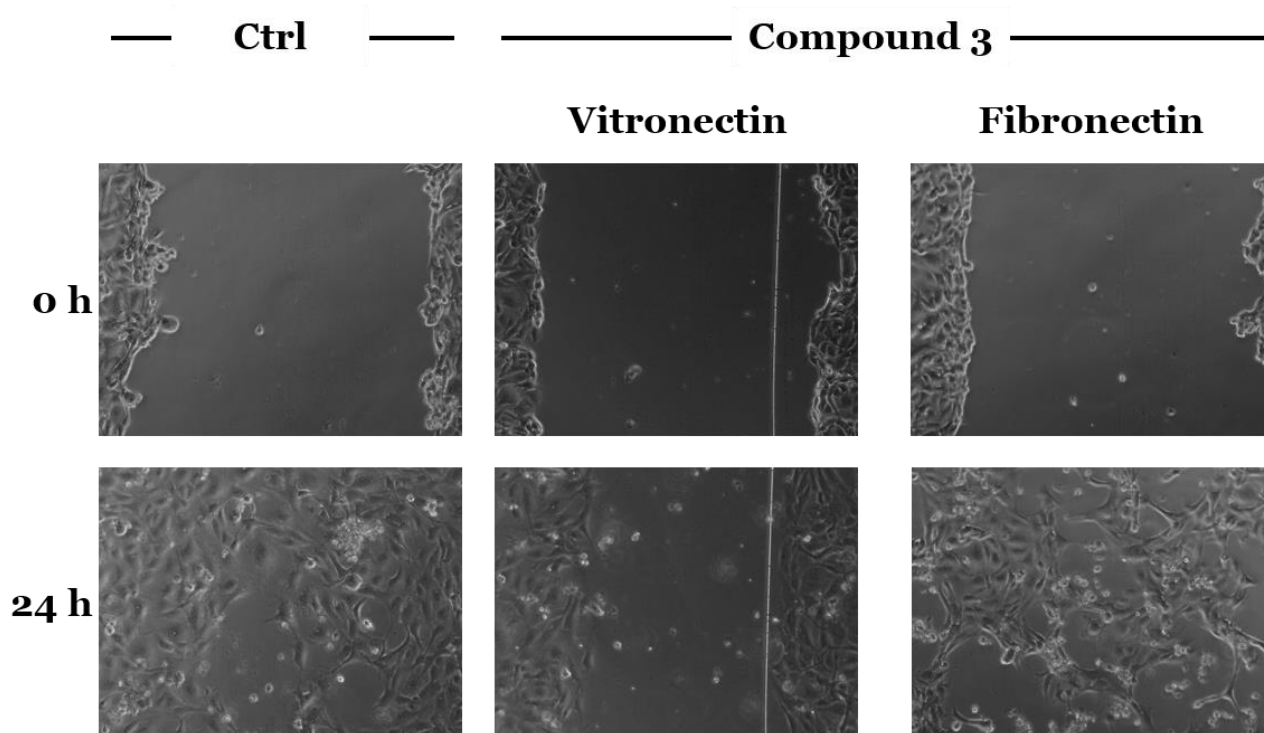


Figure S1. Wound healing assay on HUVECs. The IC_{20} of compound **3** was incubated on HUVECs plated on vitronectin or fibronectin for 24 hours, in order to determine its anti-migratory activity. Original magnification: 10x.

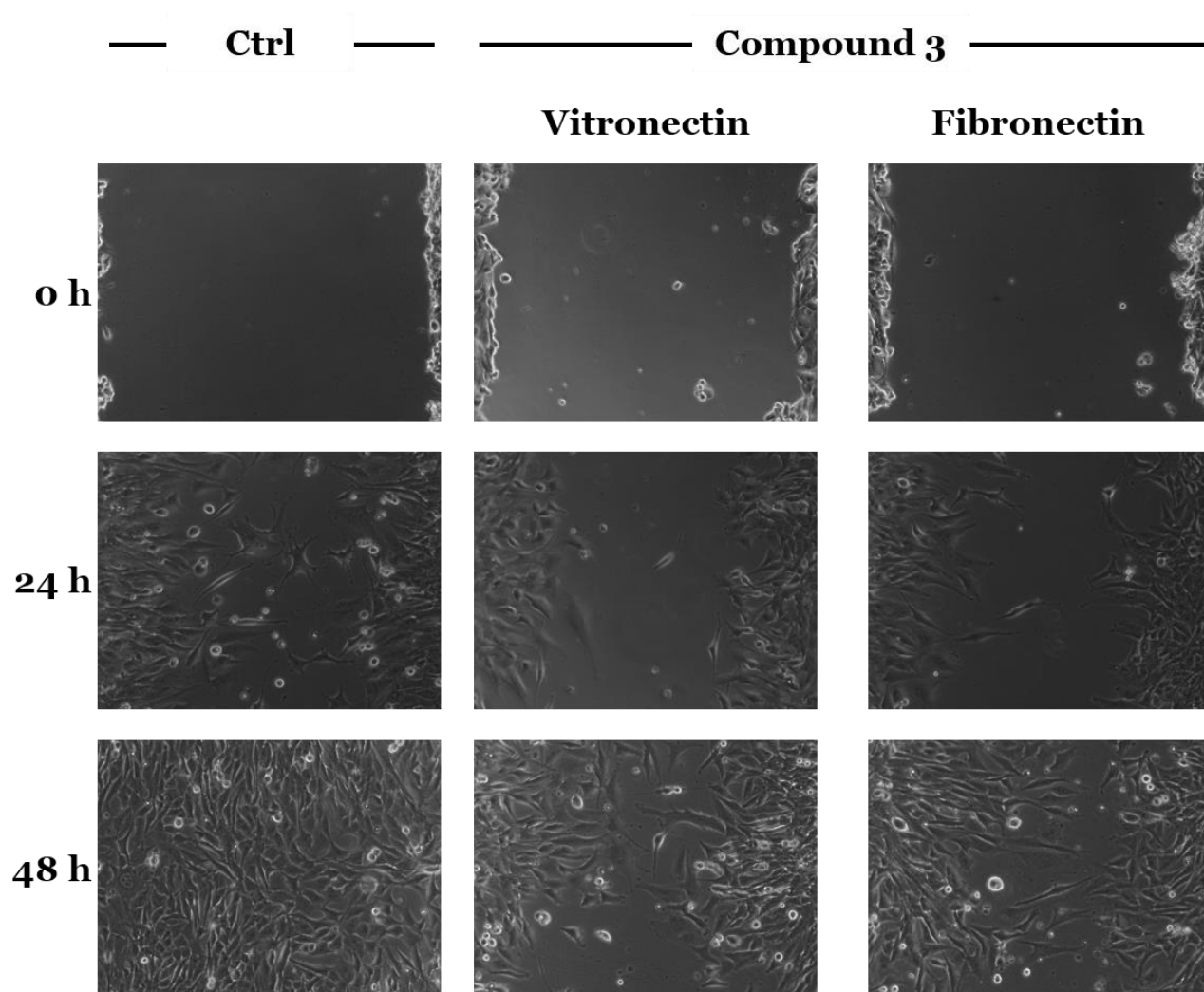


Figure S2. Wound healing assay on T98G cells. The IC₂₀ of compound **3** was incubated on T98G cells plated on vitronectin or fibronectin for 48 hours, in order to determine its anti-migratory activity. Original magnification: 10x.