Electronic Supplementary Information

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

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Contents

Chemistry

General. ^{1}H 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:

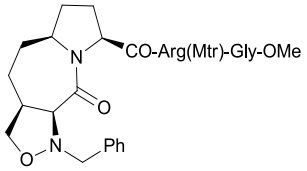
Method A. Column: Waters Atlantis 3μm 50x4.6 mm; Phase A: H_2O + 0.05% TFA, Phase B: MeCN + 0.05% TFA; Flow: 1 mL/min, Gradient: from 10% B to 90% B in 6 min, washing at 100% B for 1 min, equilibration at 10% B in the next 3 min.

Method B. Column: Supelco Ascentis-Express 2.7μm 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.

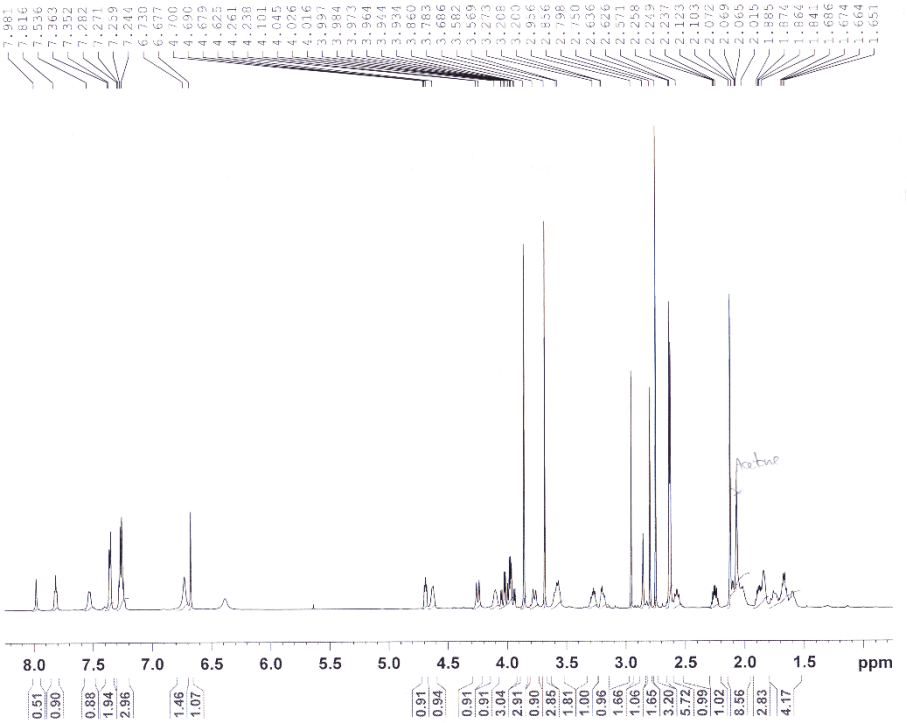
Method C. Column: Waters Atlantis 3μm 50x4.6 mm; Phase A: H_2O + 0.05% TFA, Phase B: MeCN + 0.05% TFA; Flow: 1 mL/min, Gradient: from 0% B to 30% B in 6 min, from 30% B to 90% B in 1 min, washing at 100% B for 1 min, equilibration at 0% B in the next 3 min.

Method D. Column: Supelco RP-amide 5μm 6×150 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.

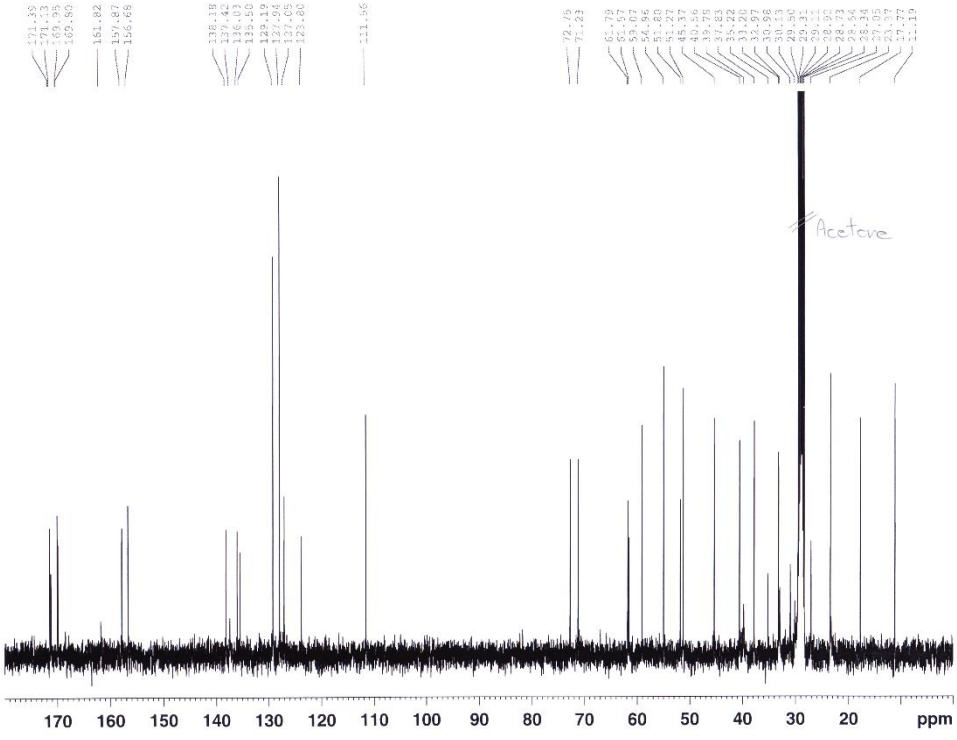
Compound 7.



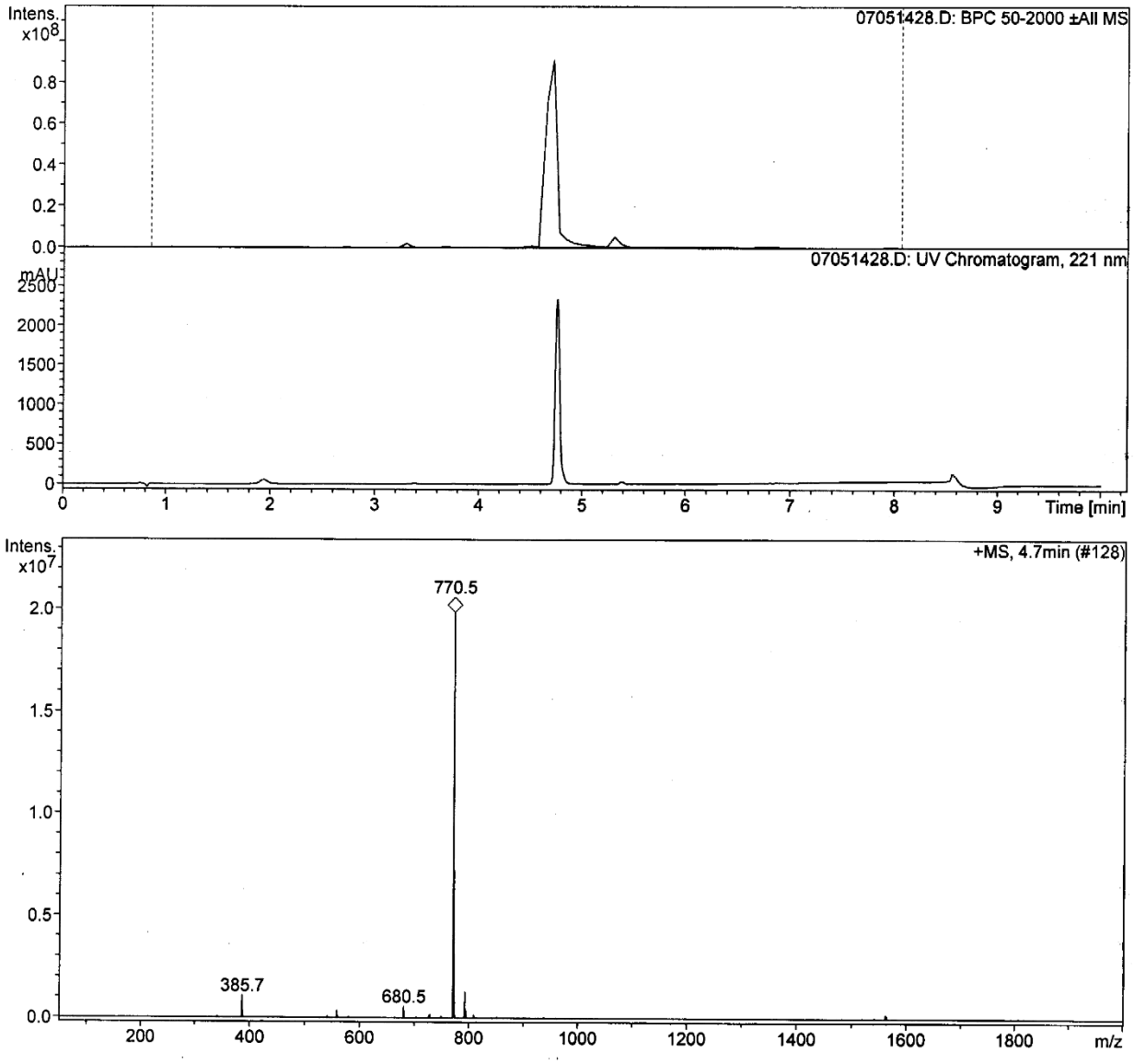
^{1}H NMR (400 MHz, *Acetone-d_6*)



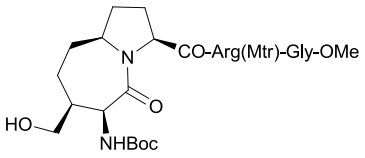
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



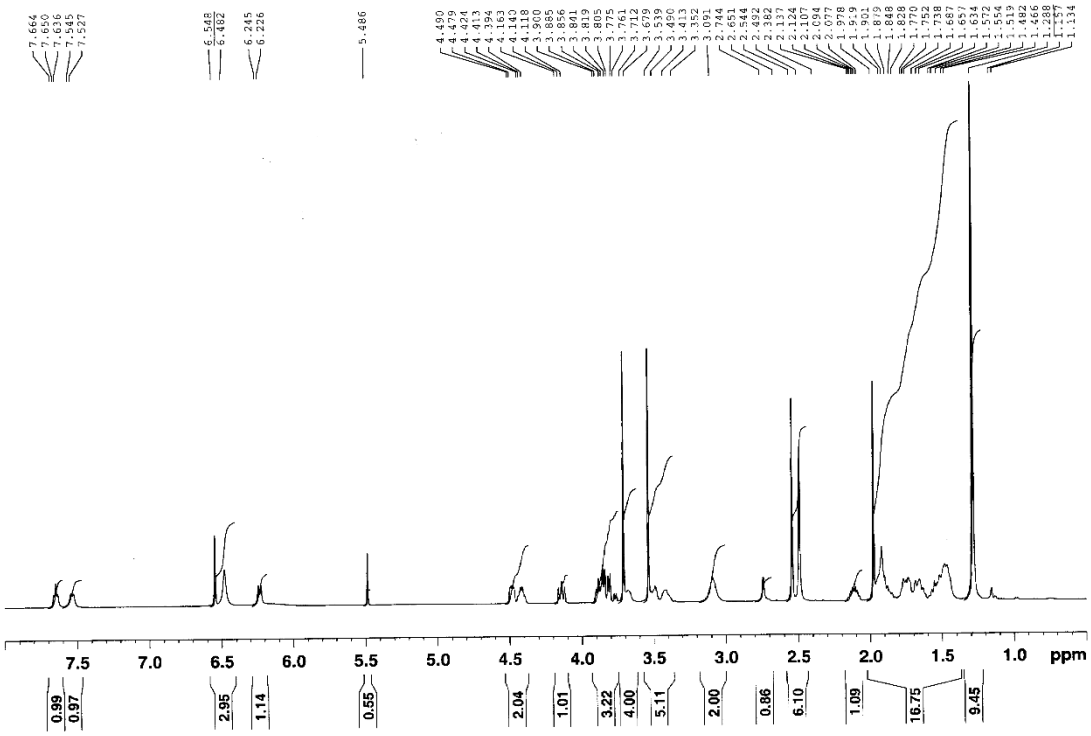
HPLC-MS (analytical method A)



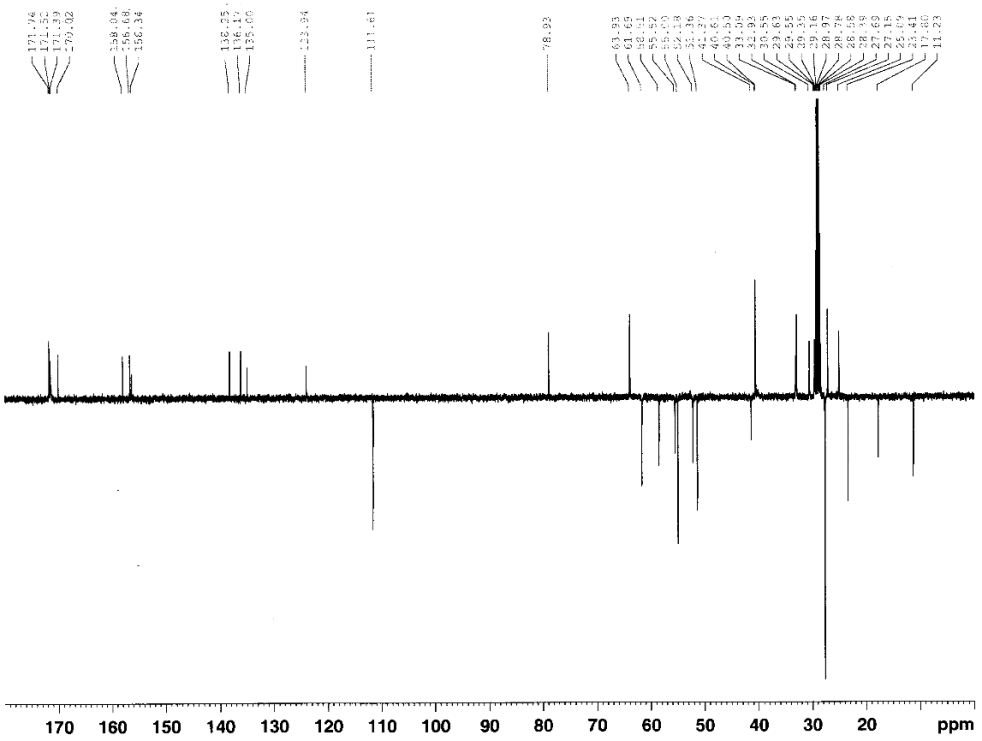
Compound 9.



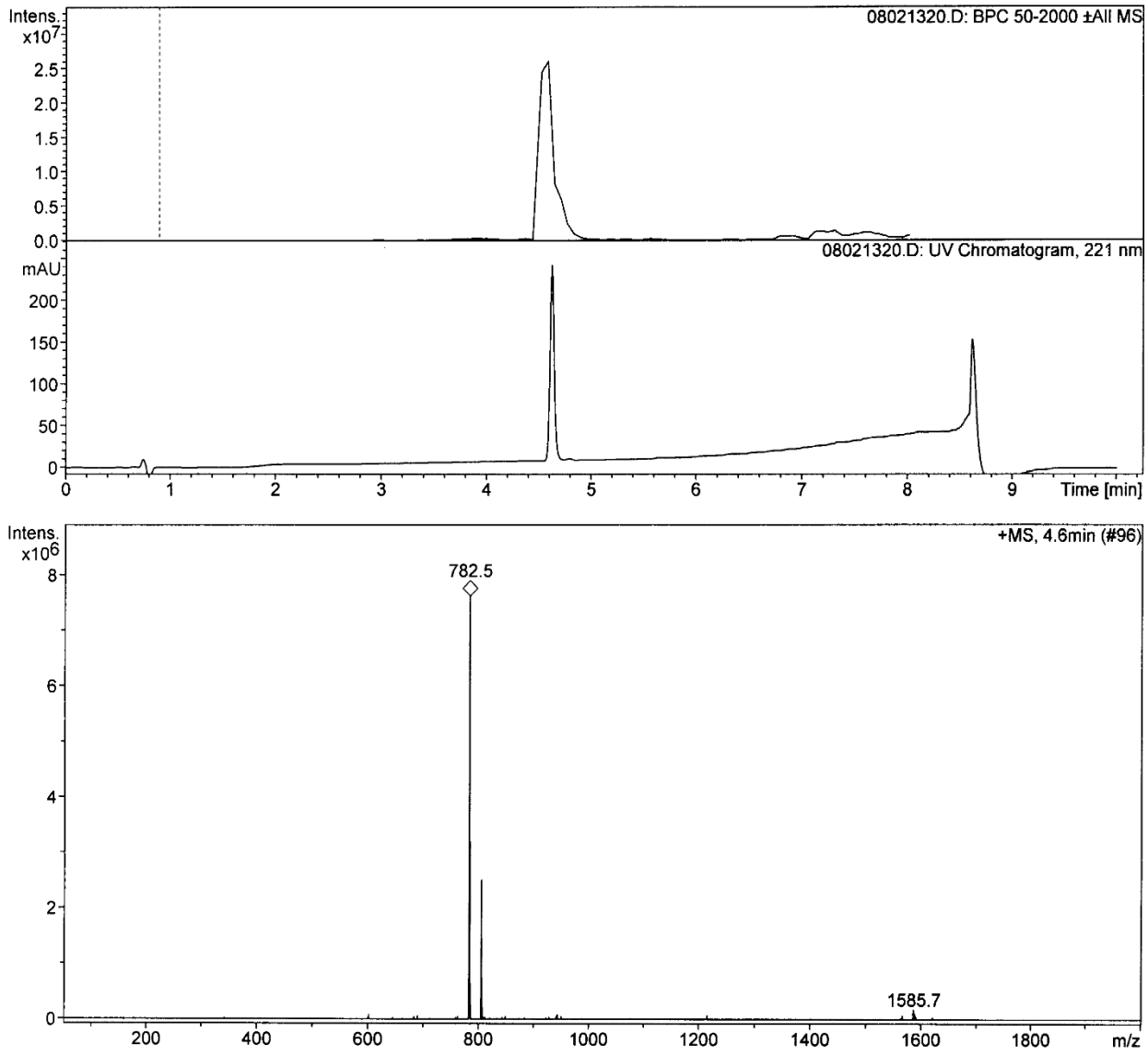
^{1}H NMR (400 MHz, *Acetone-d_6*)



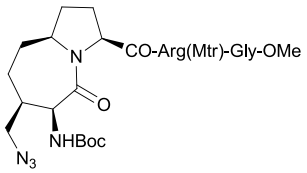
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



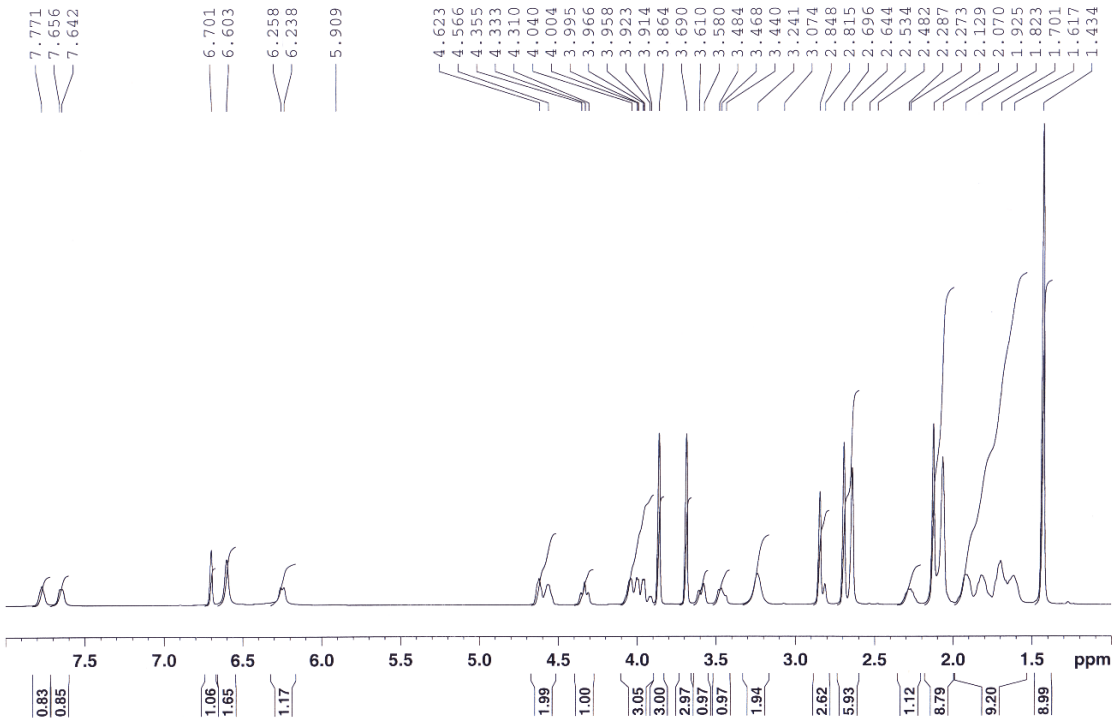
HPLC-MS (analytical method A)



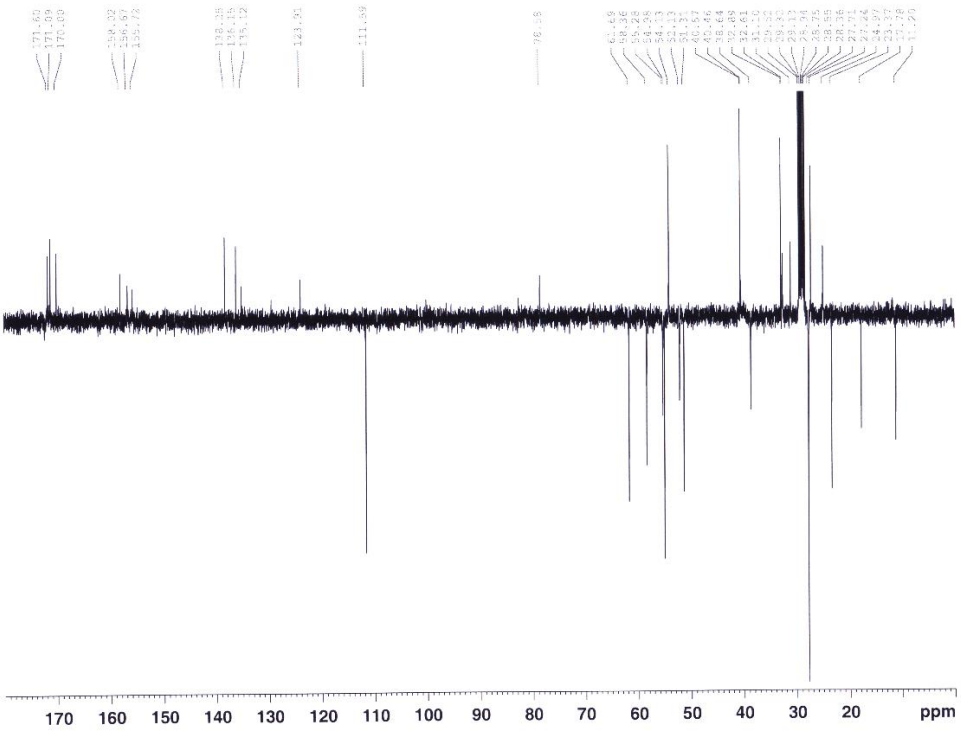
Compound 10



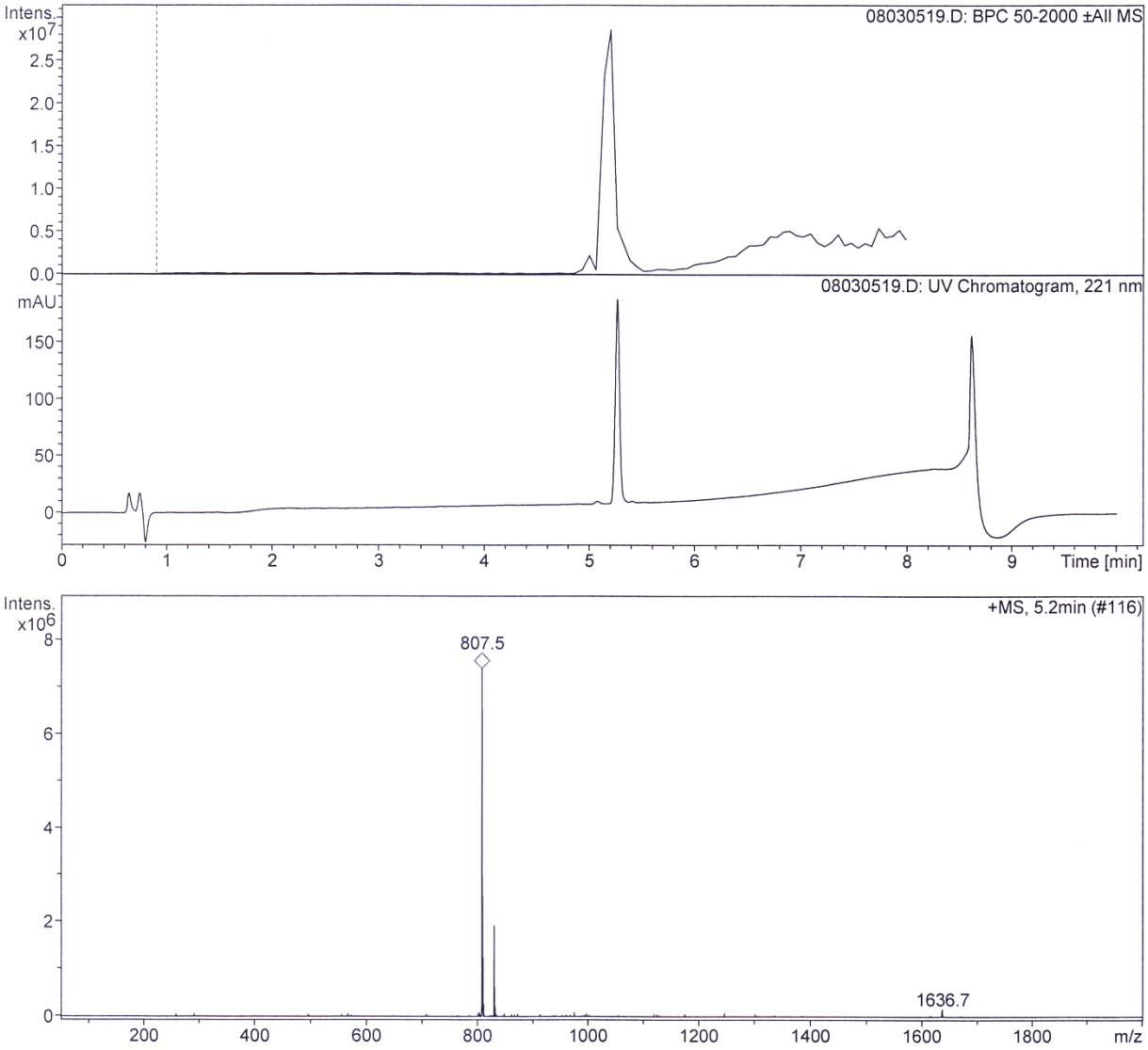
^{1}H NMR (400 MHz, *Acetone-d_6*)



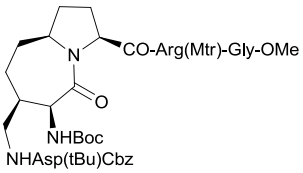
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



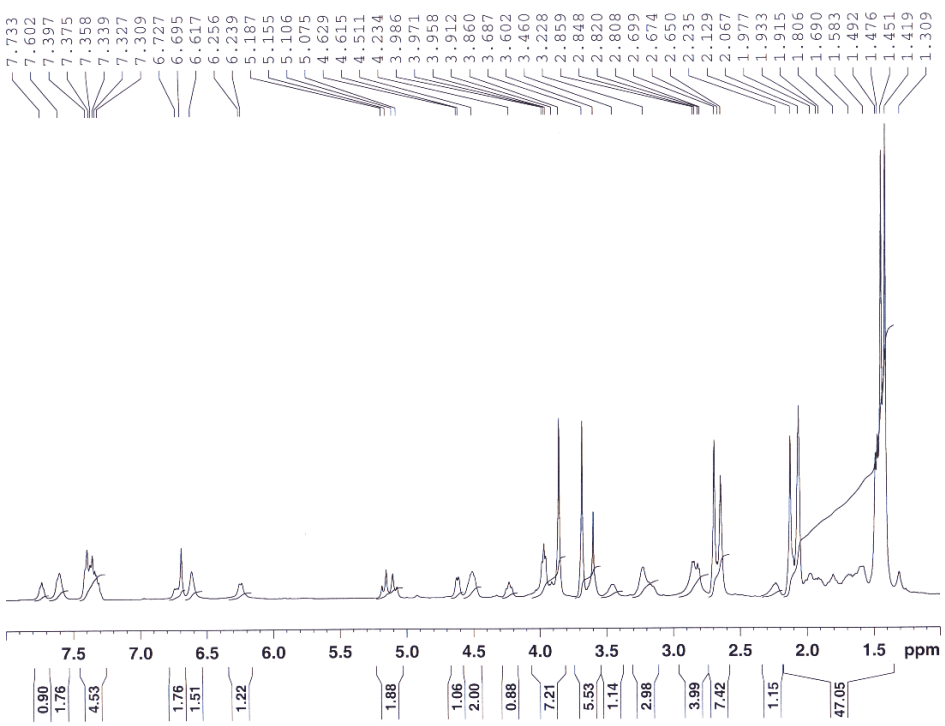
HPLC-MS (analytical method A)



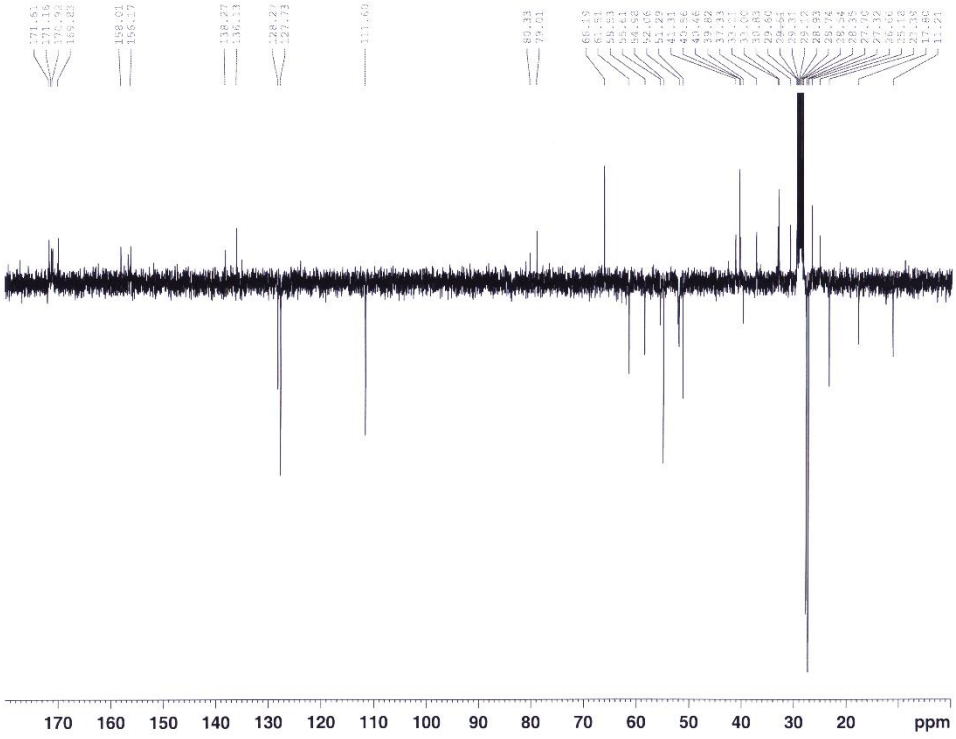
Compound 11



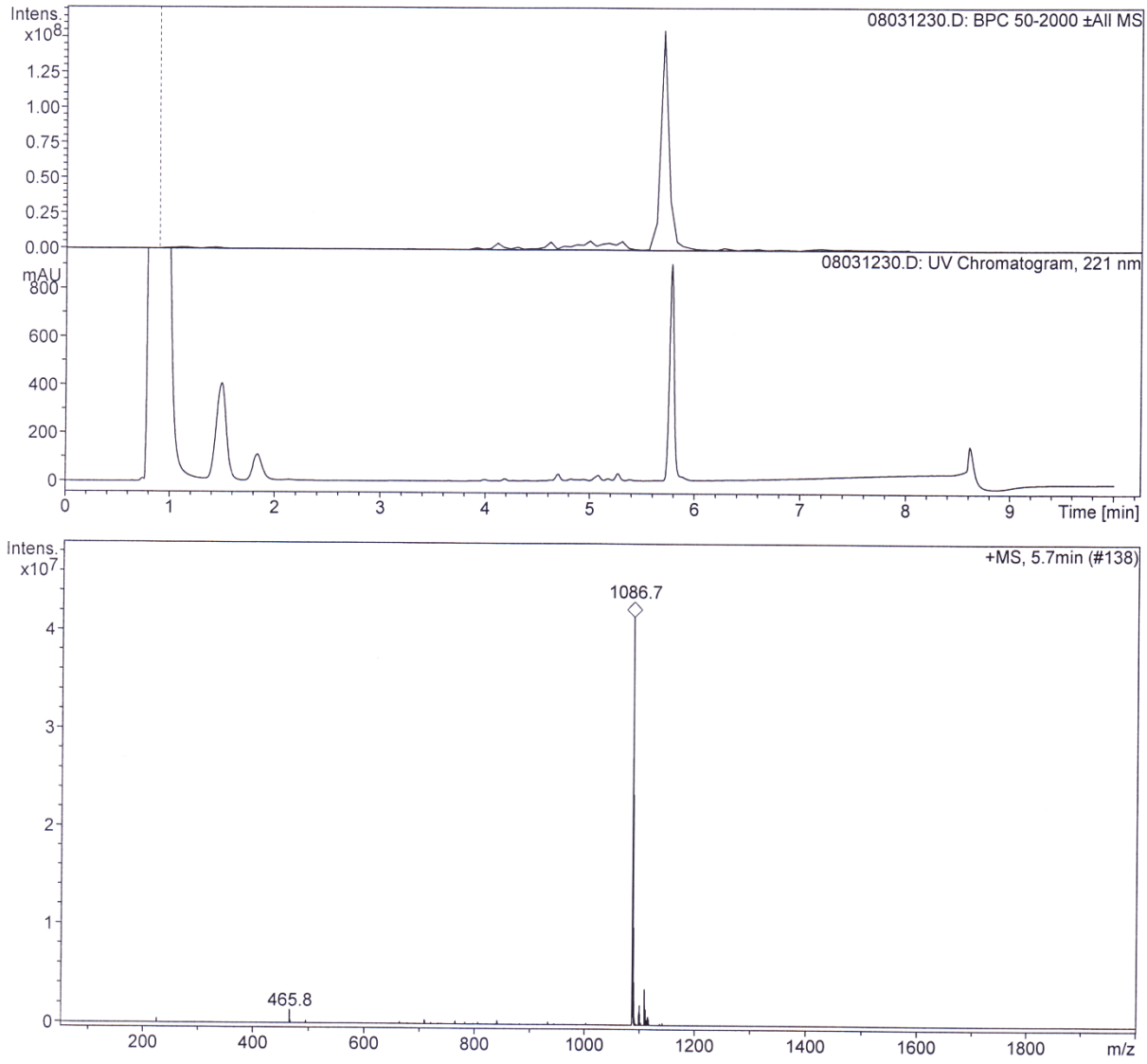
^{1}H NMR (400 MHz, *Acetone-d_6*)



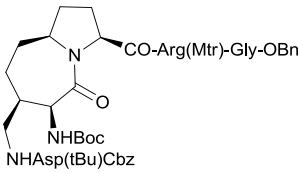
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



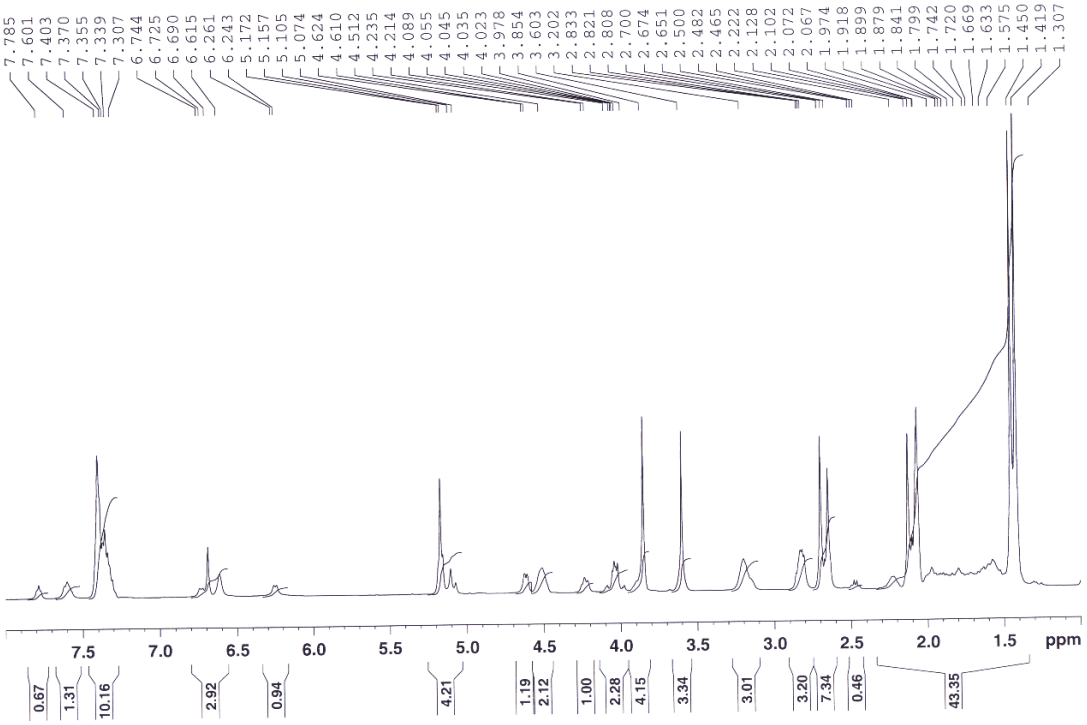
HPLC-MS (analytical method A)



Compound 12



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



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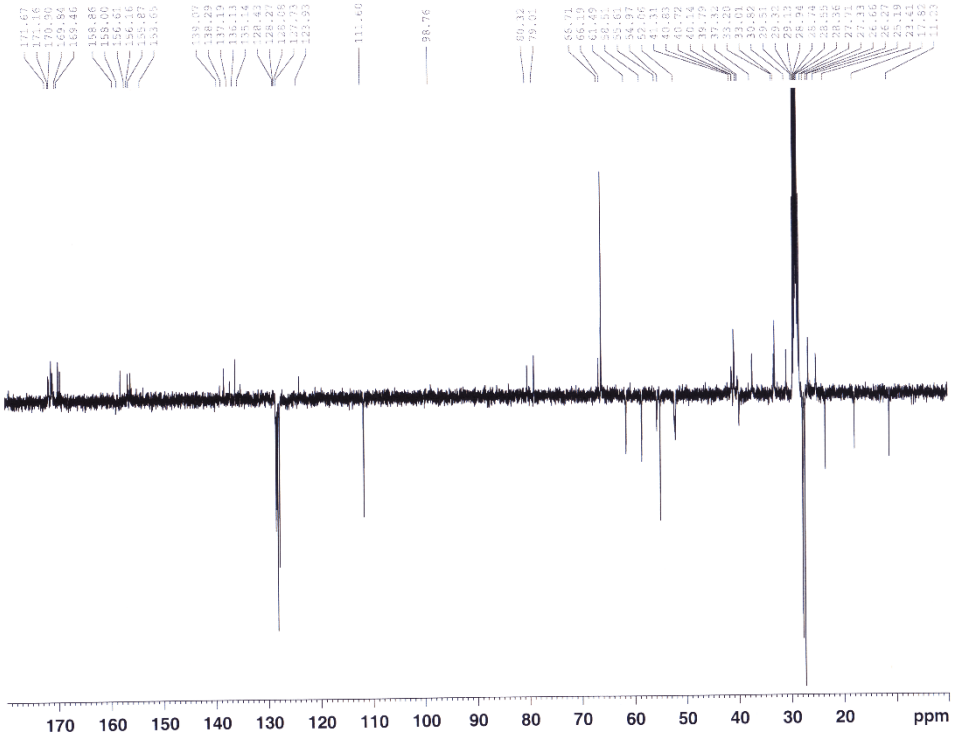
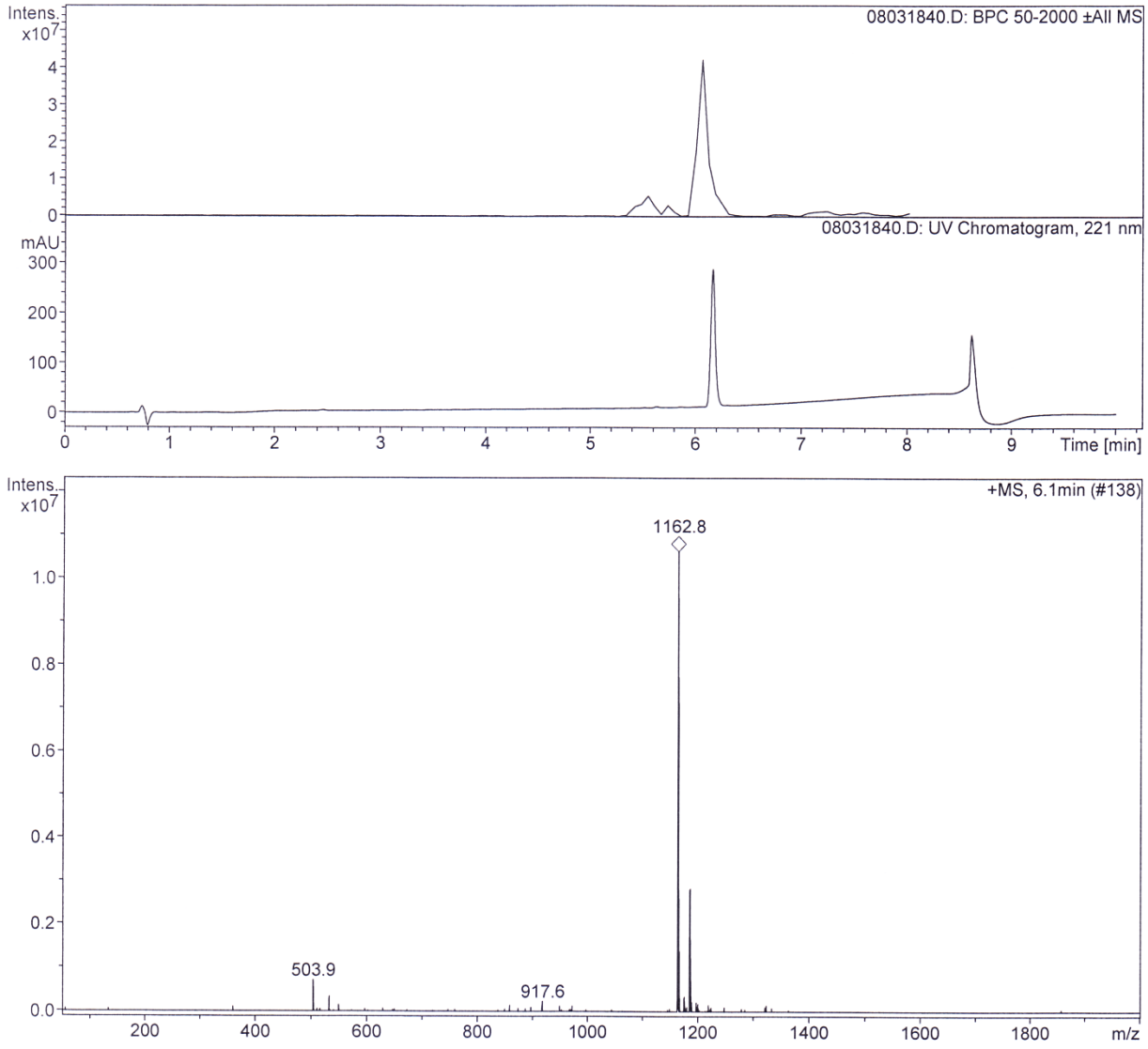
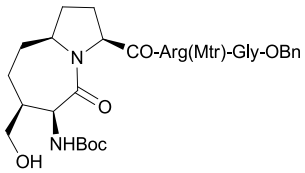


HPLC-MS (analytical method A)



Compound 13



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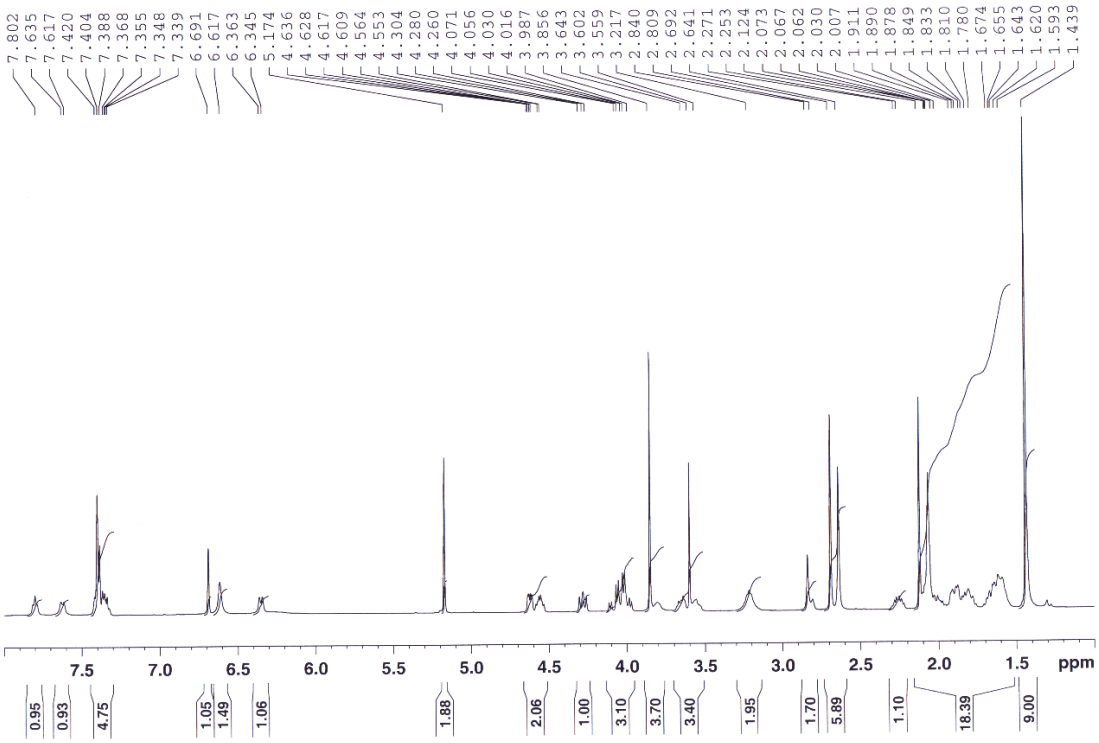

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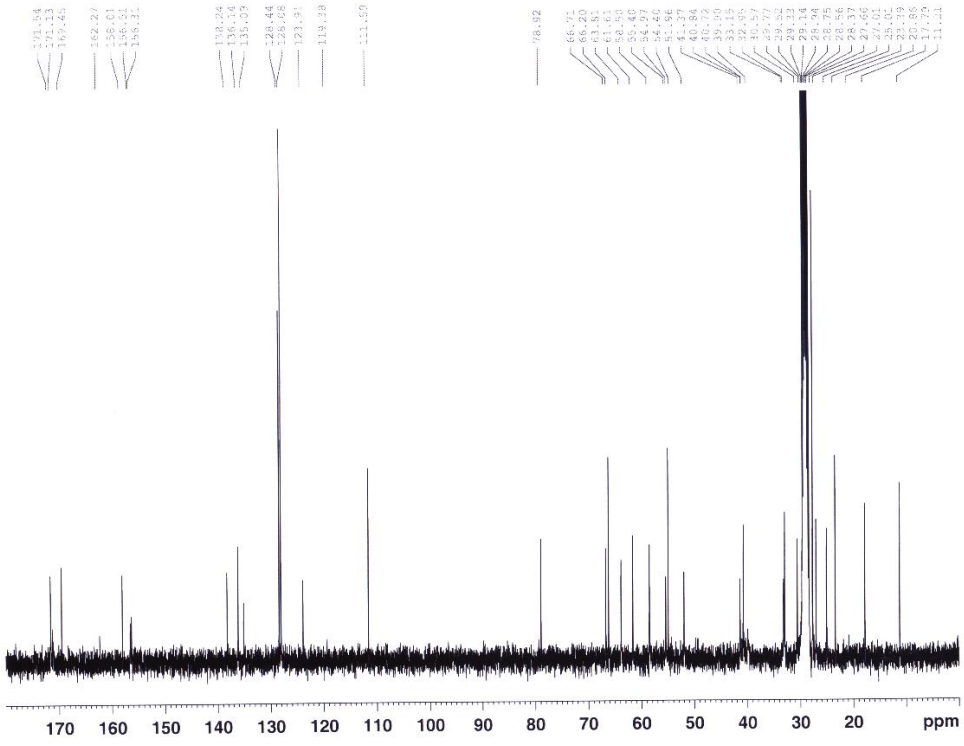

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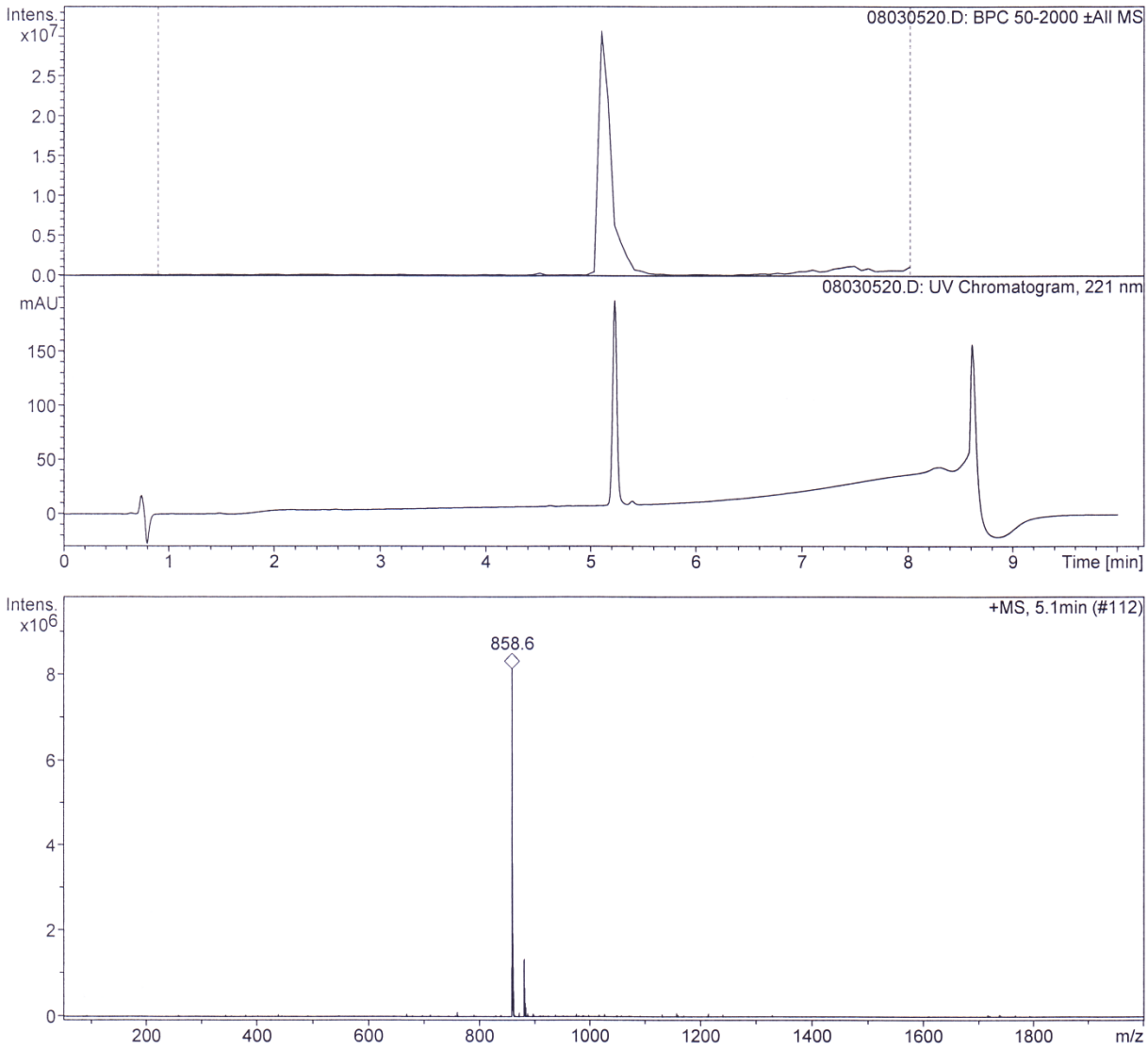
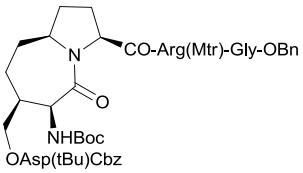
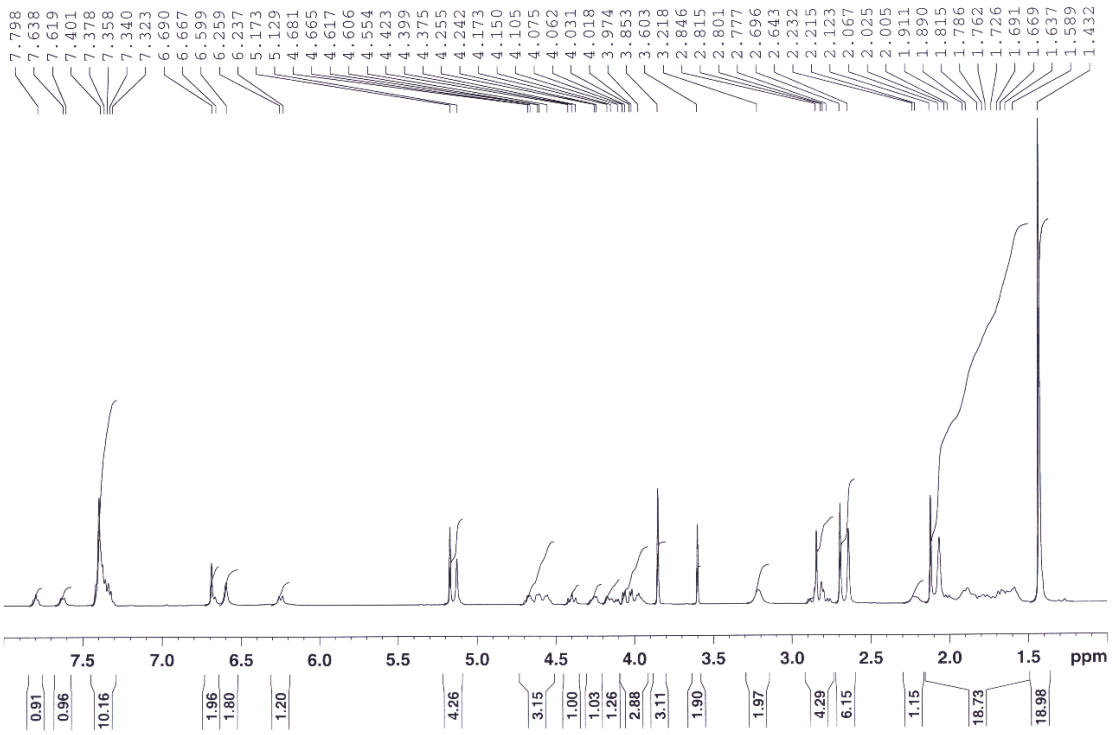


Compound 14



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



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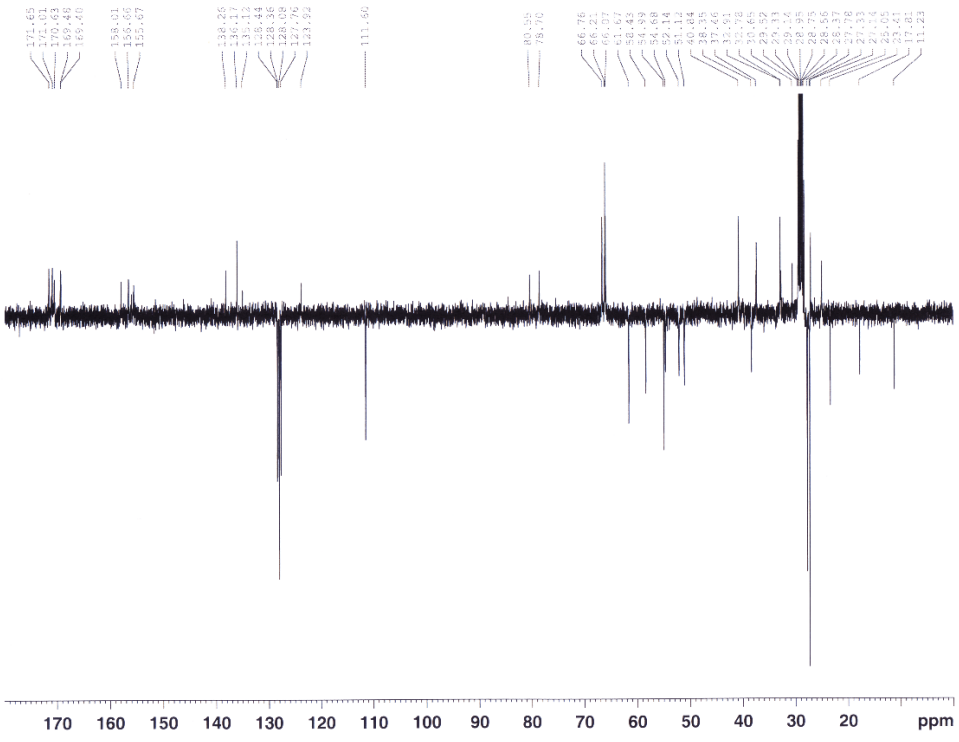
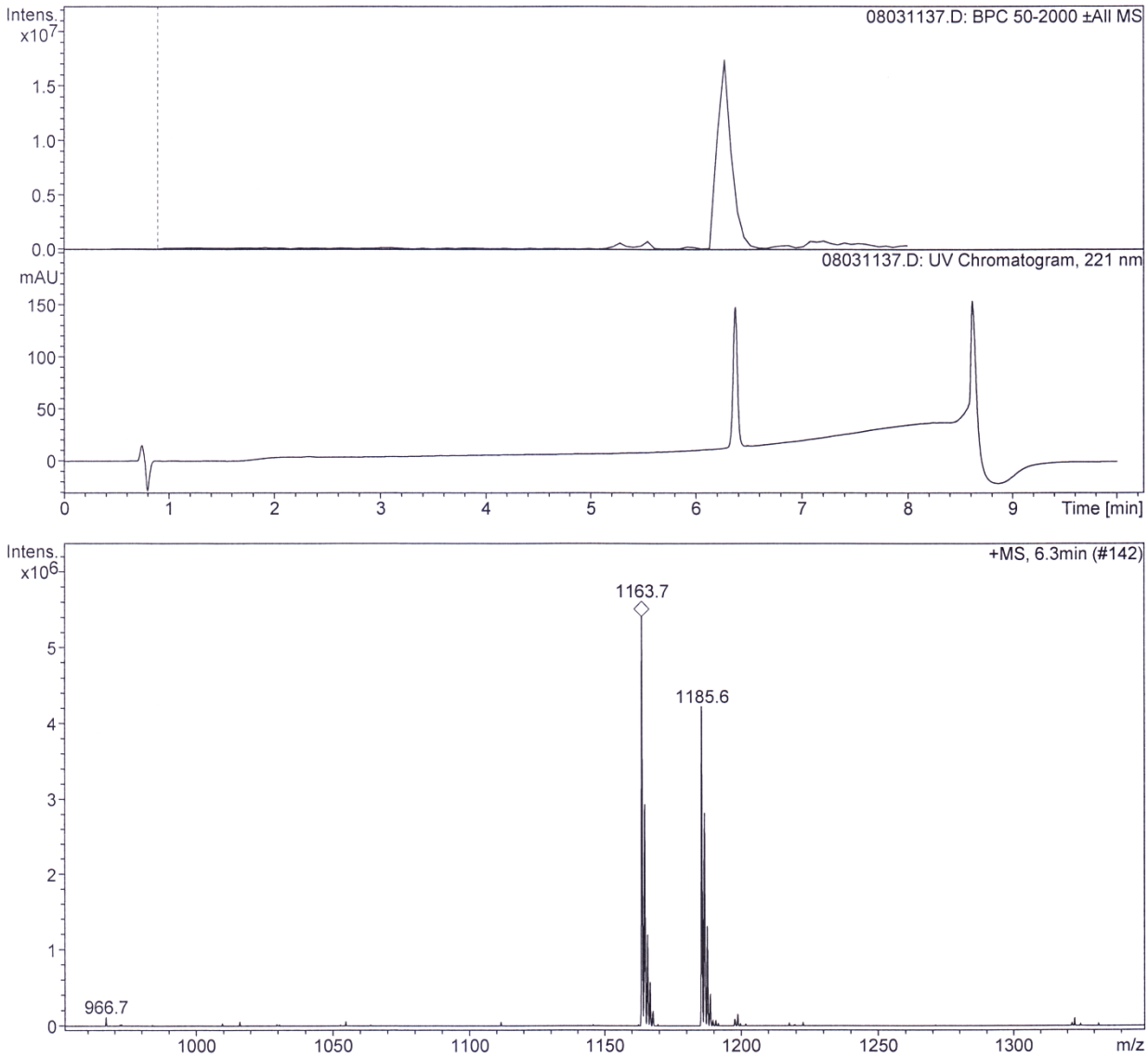
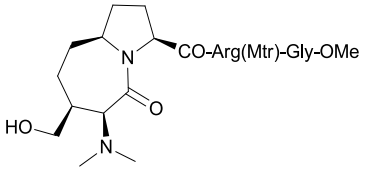


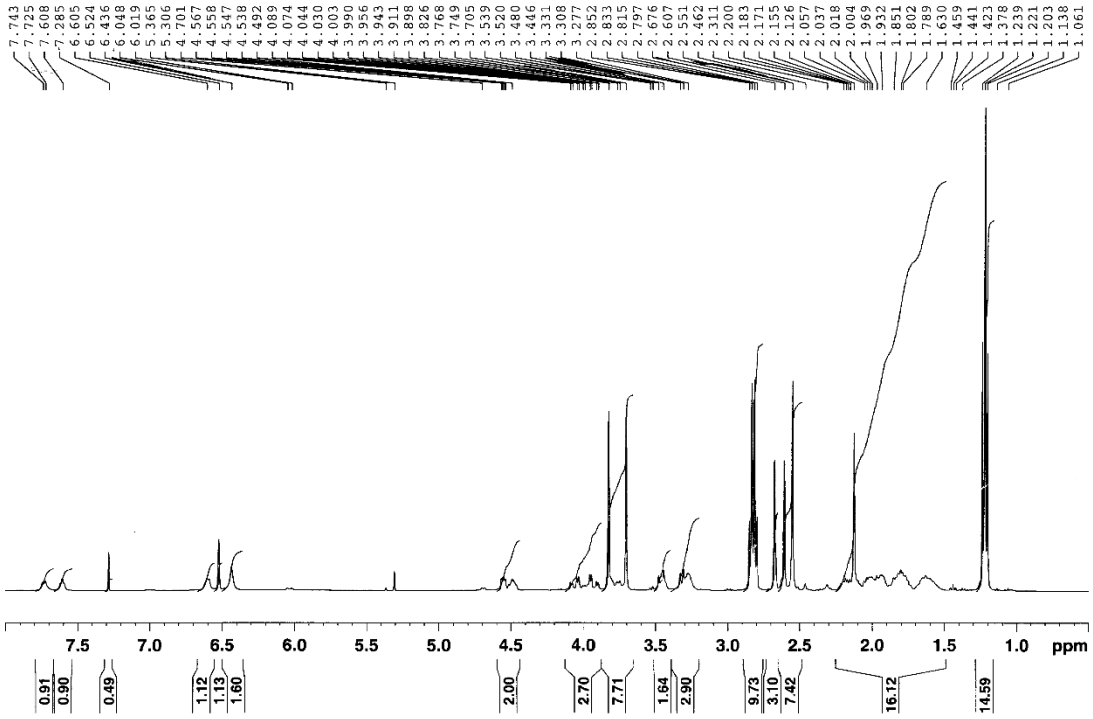
HPLC-MS (analytical method A)



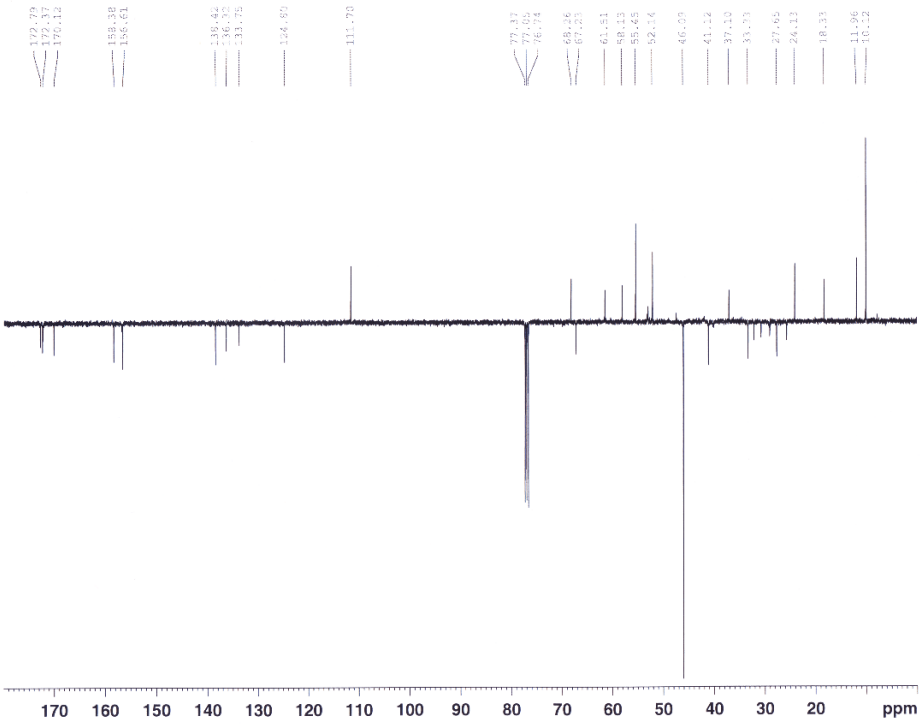
Compound 15



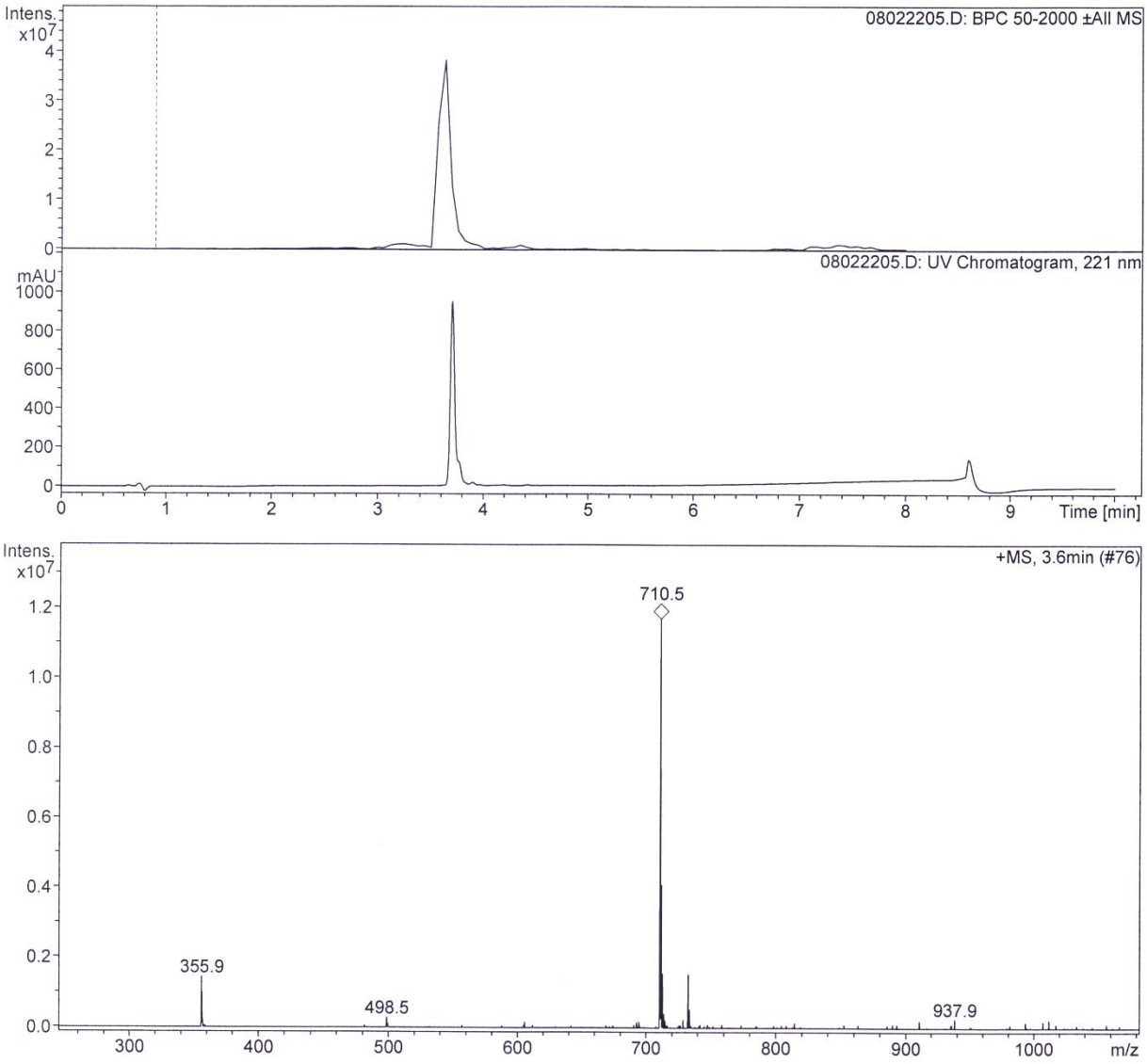
^{1}H NMR (400 MHz, *Acetone-d_6*)



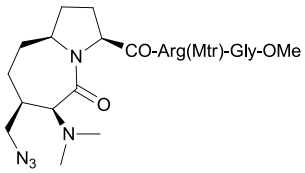
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



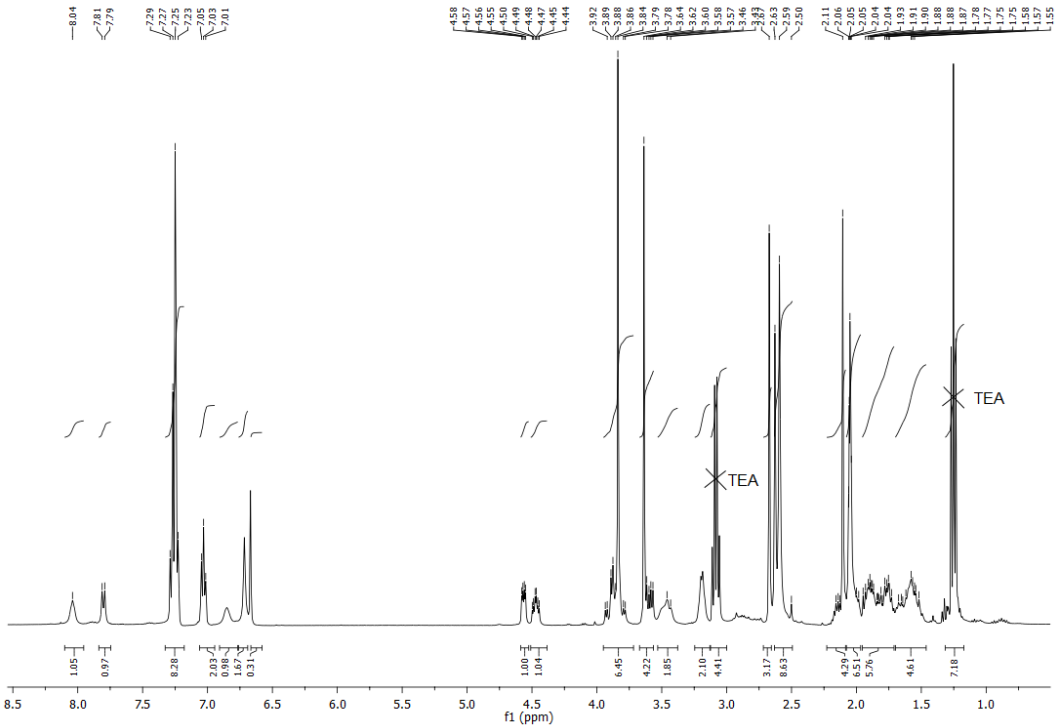
HPLC-MS (analytical method A)



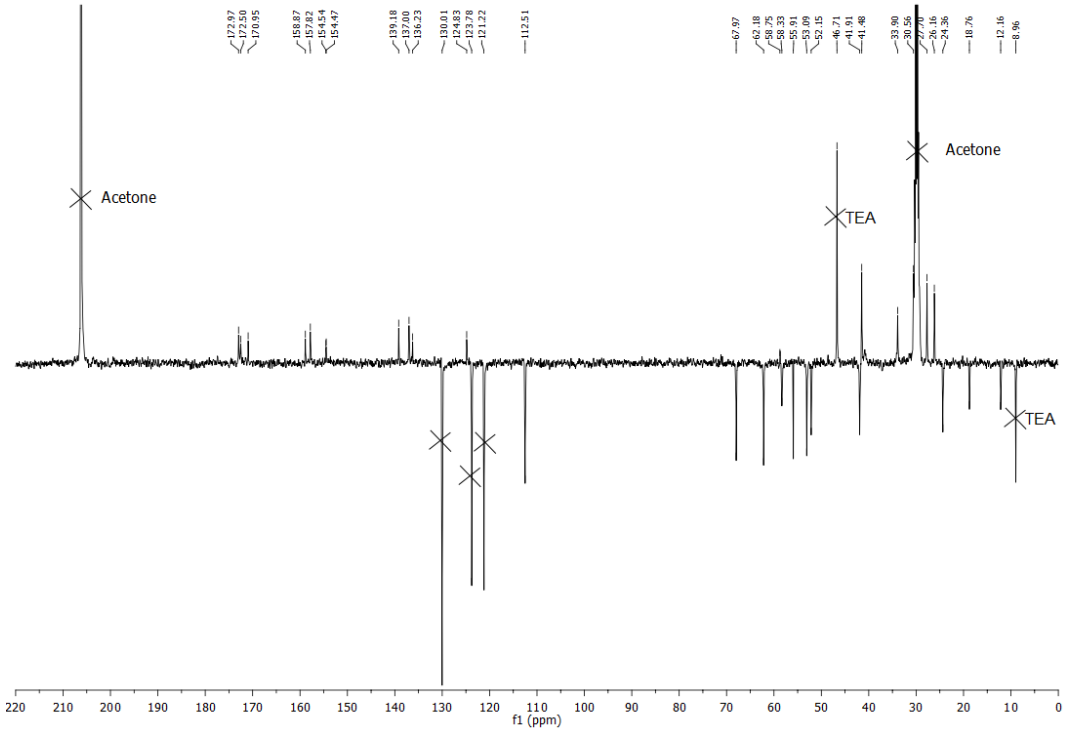
Compound 16



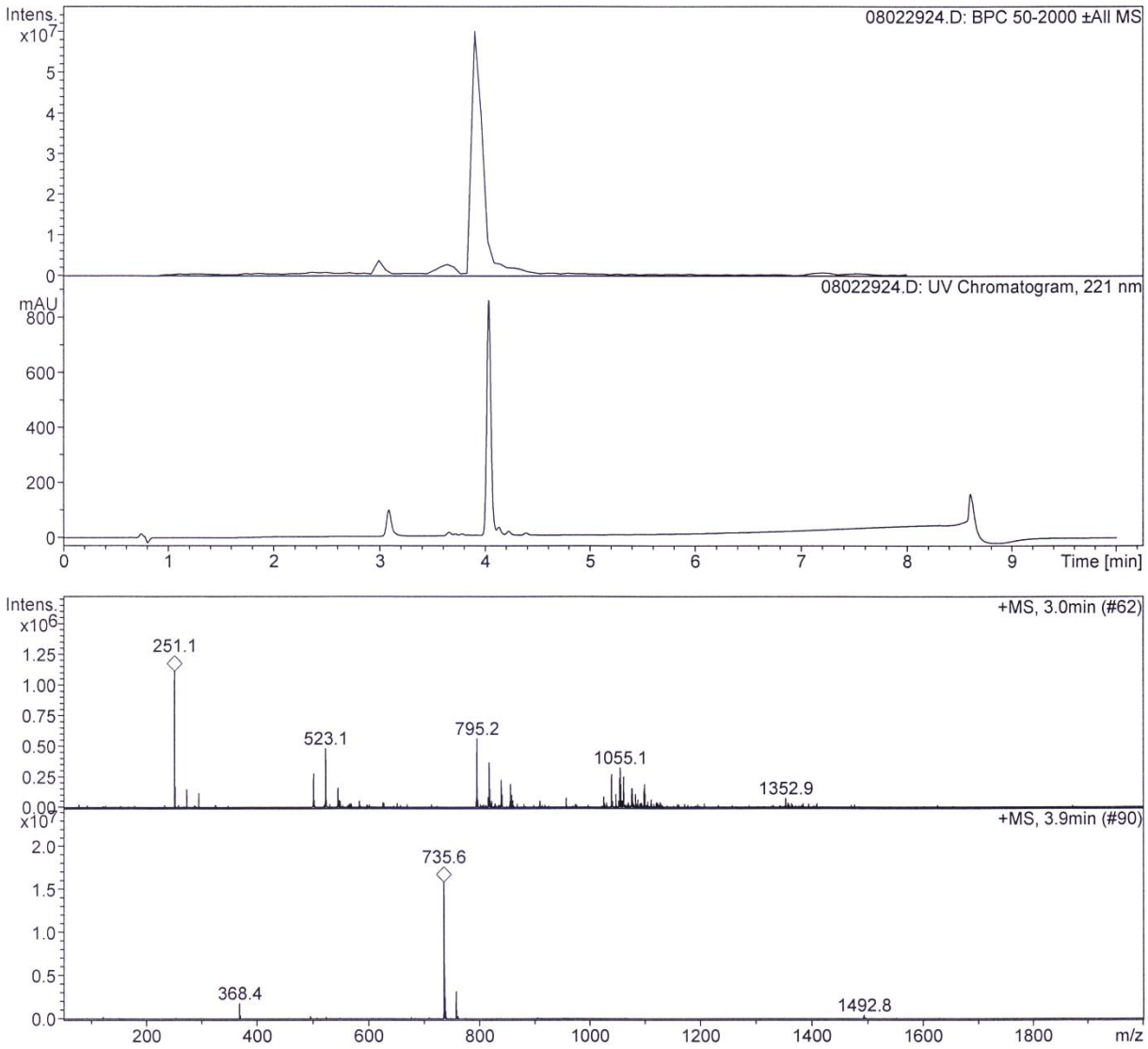
^{1}H NMR (400 MHz, *Acetone-d_6*)



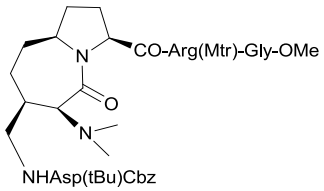
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



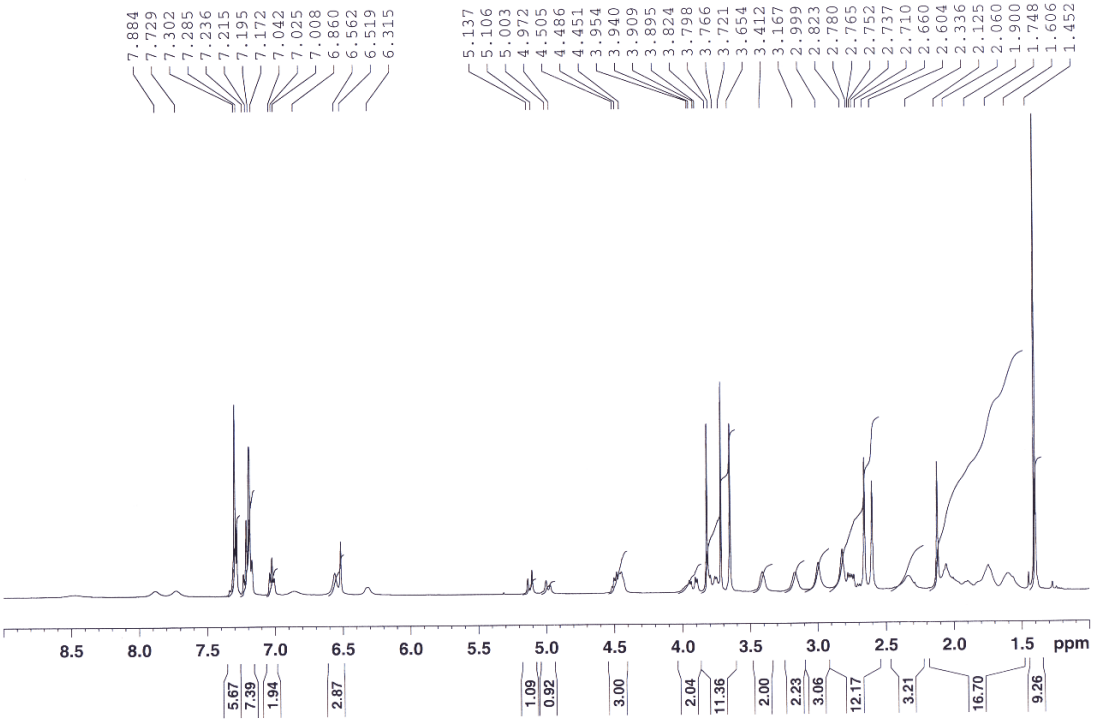
HPLC-MS (analytical method A)



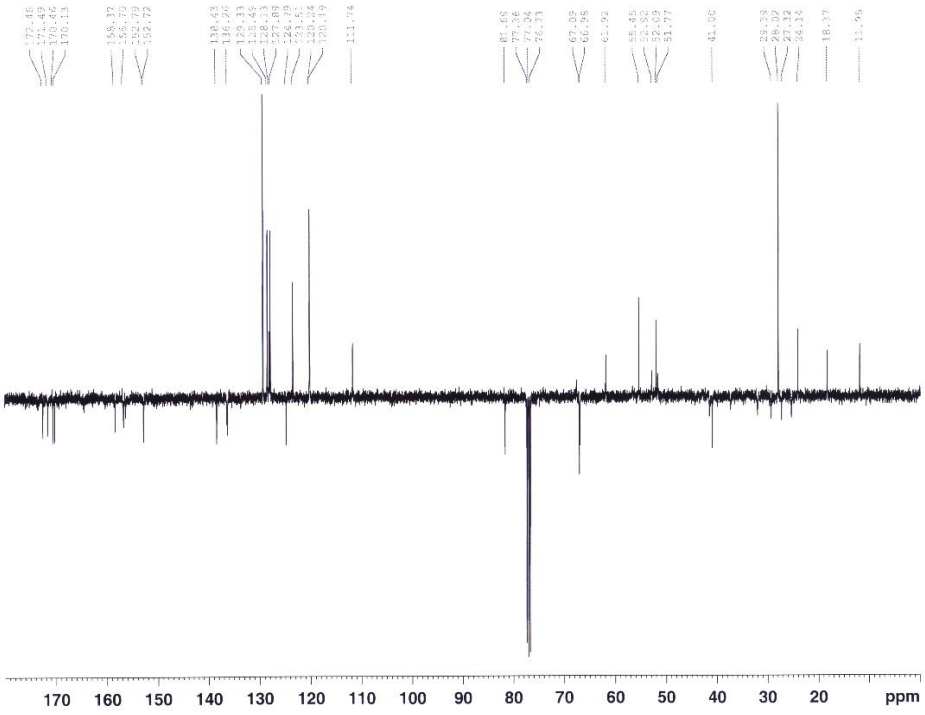
Compound 17



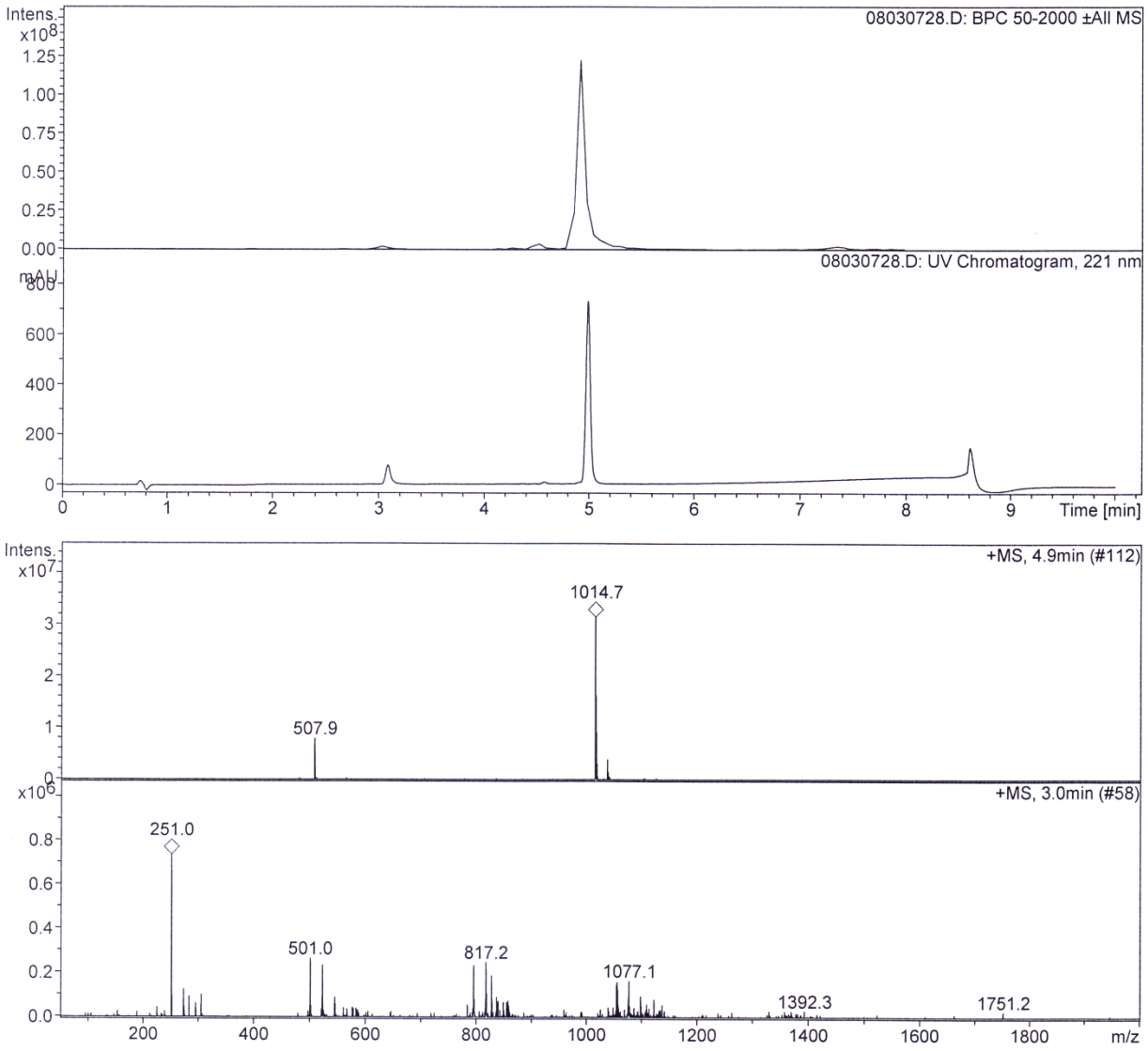
^{1}H NMR (400 MHz, $CDCl_3$)



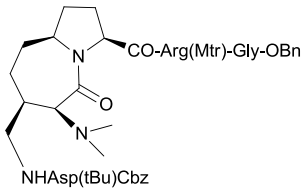
^{13}C NMR (100.6 MHz, $CDCl_3$)



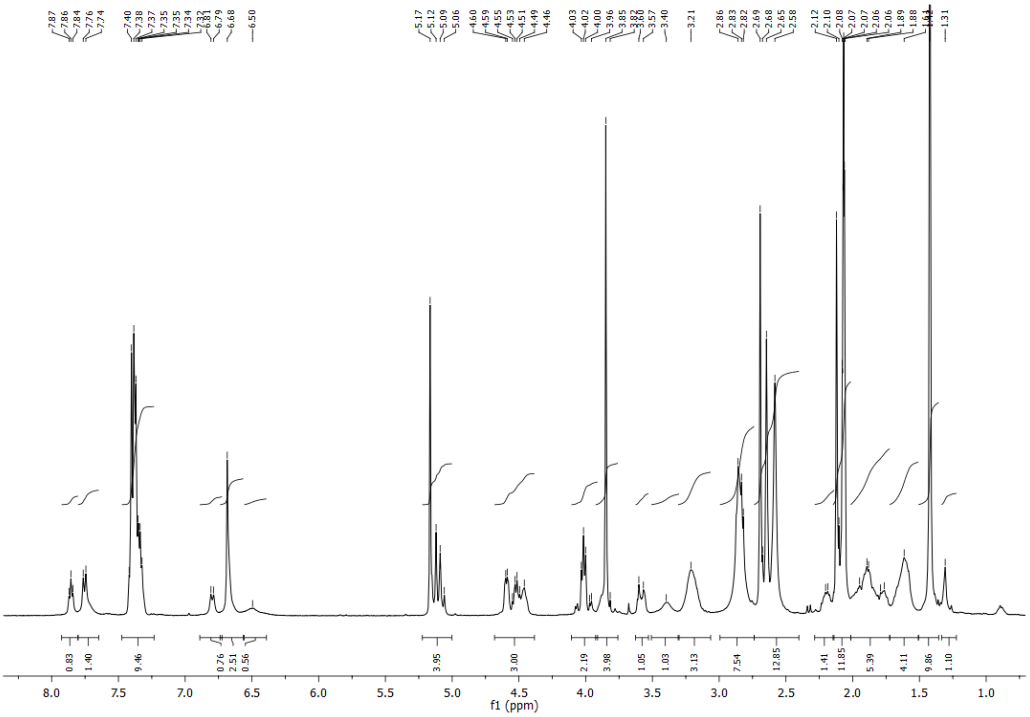
HPLC-MS (analytical method A)



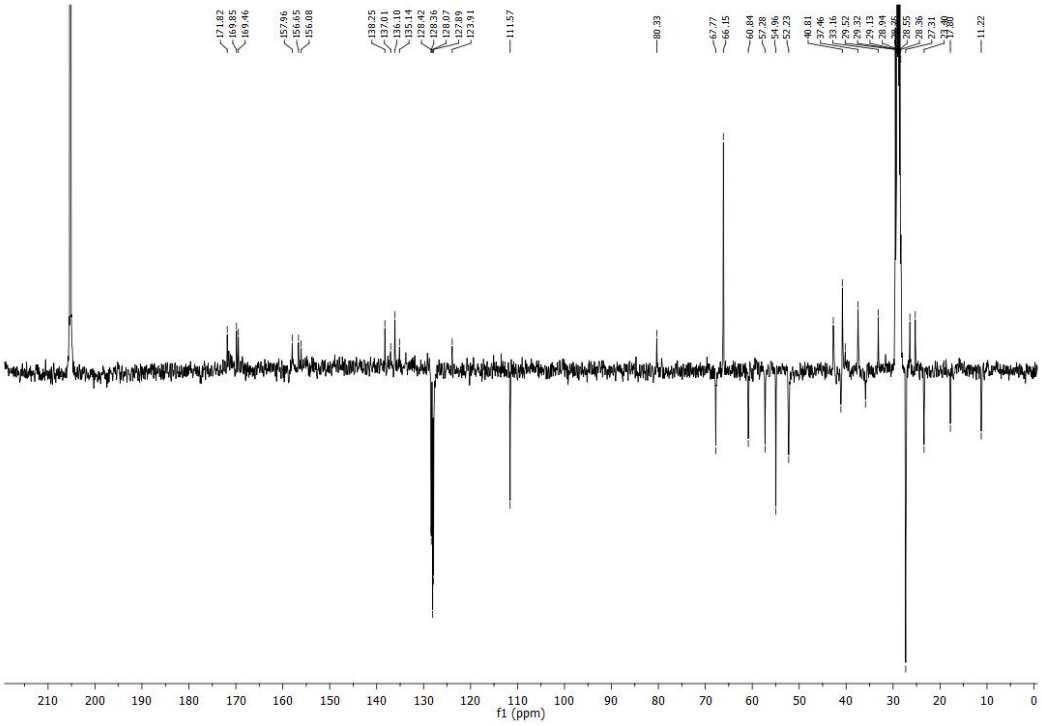
Compound 18



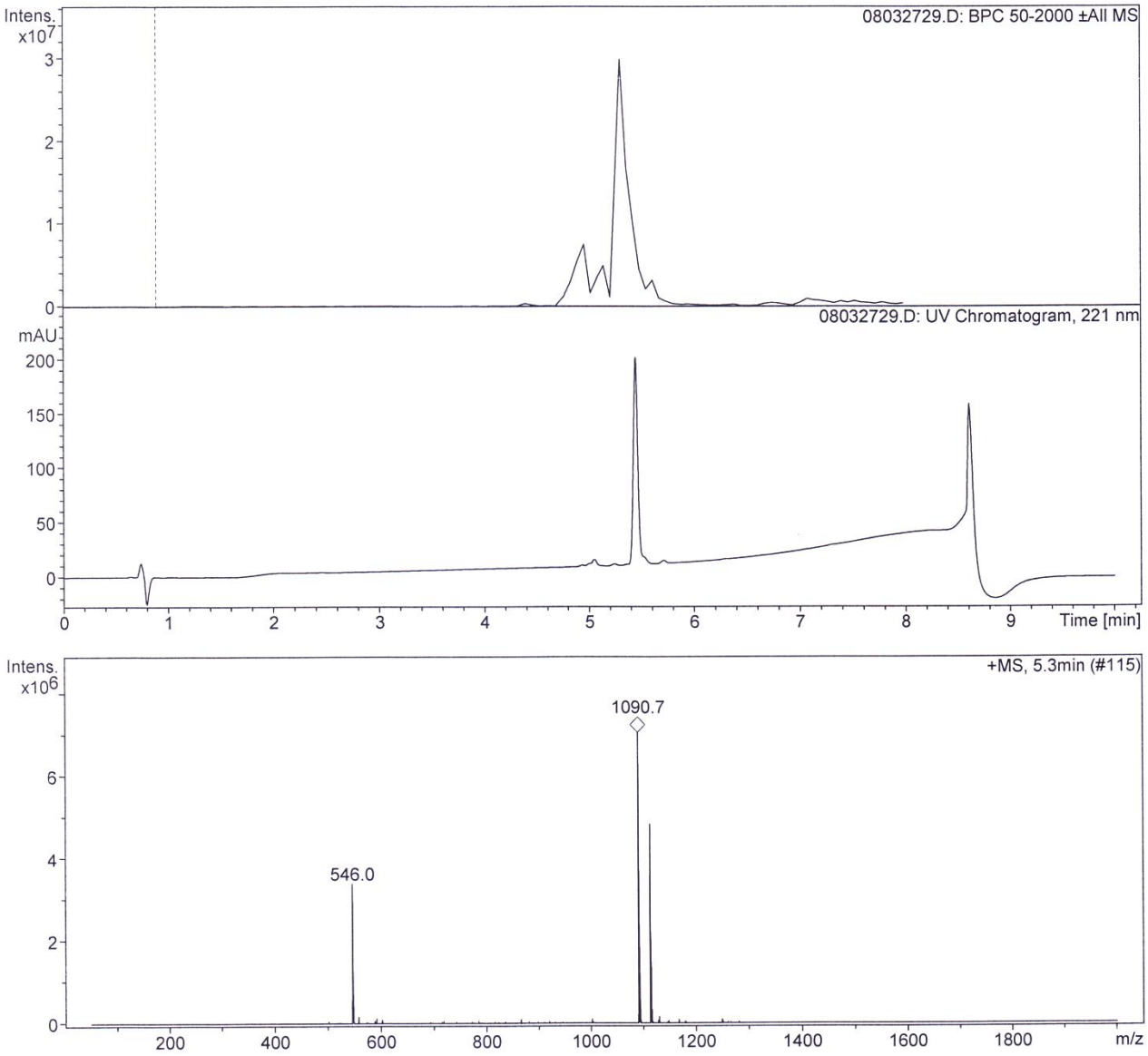
^{1}H NMR (400 MHz, *Acetone-d_6*)



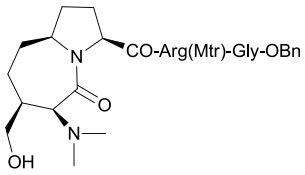
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



HPLC-MS (analytical method A)



Compound 19



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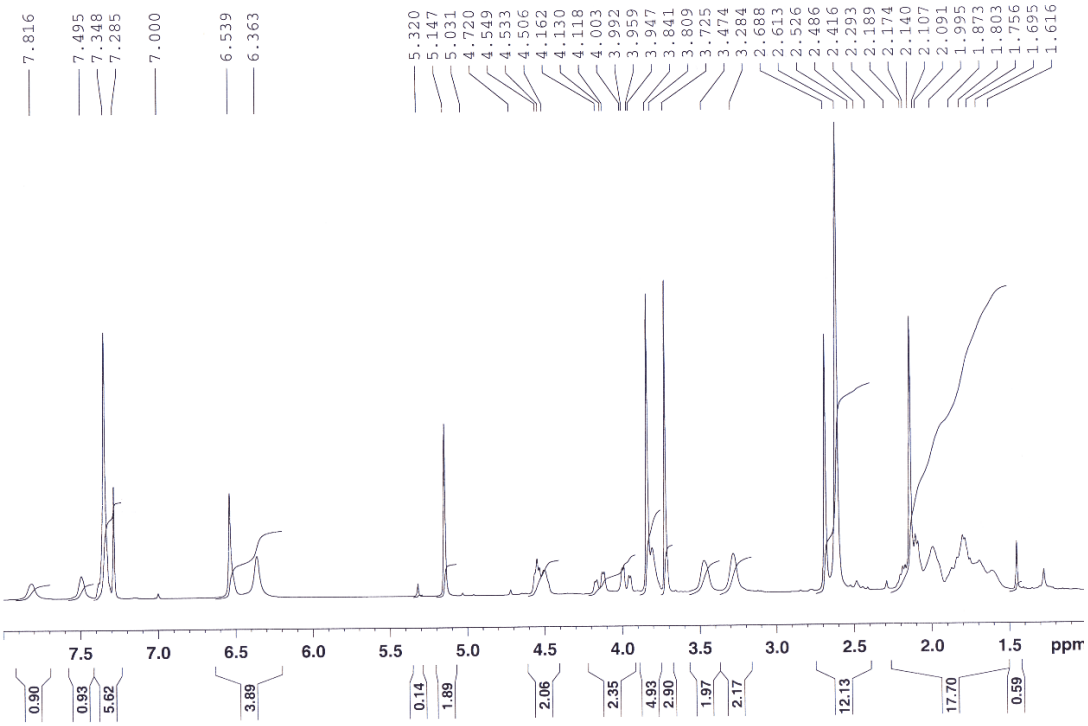

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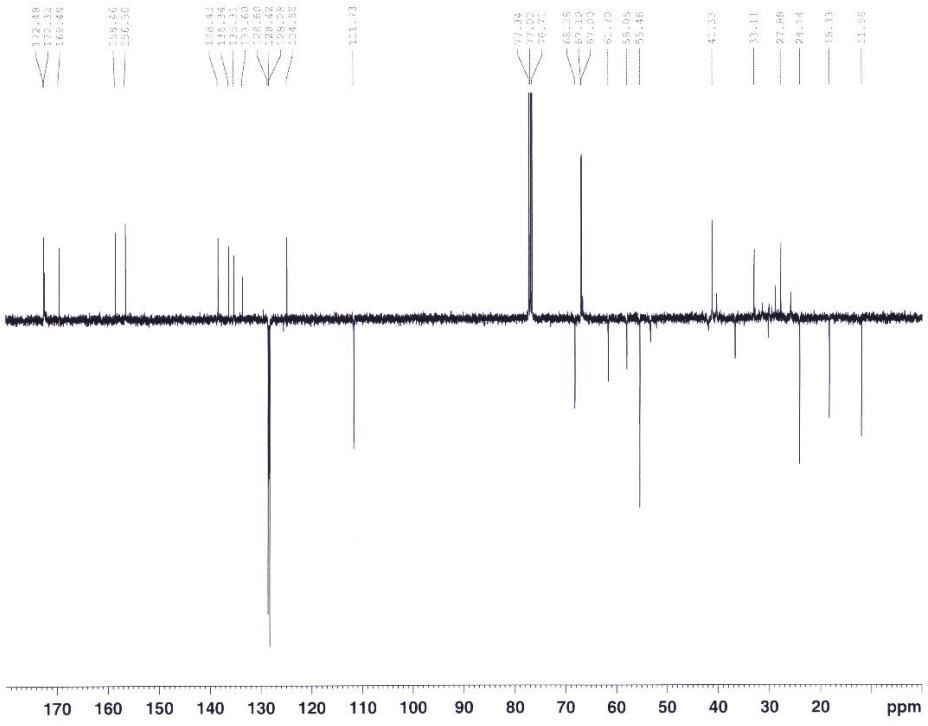
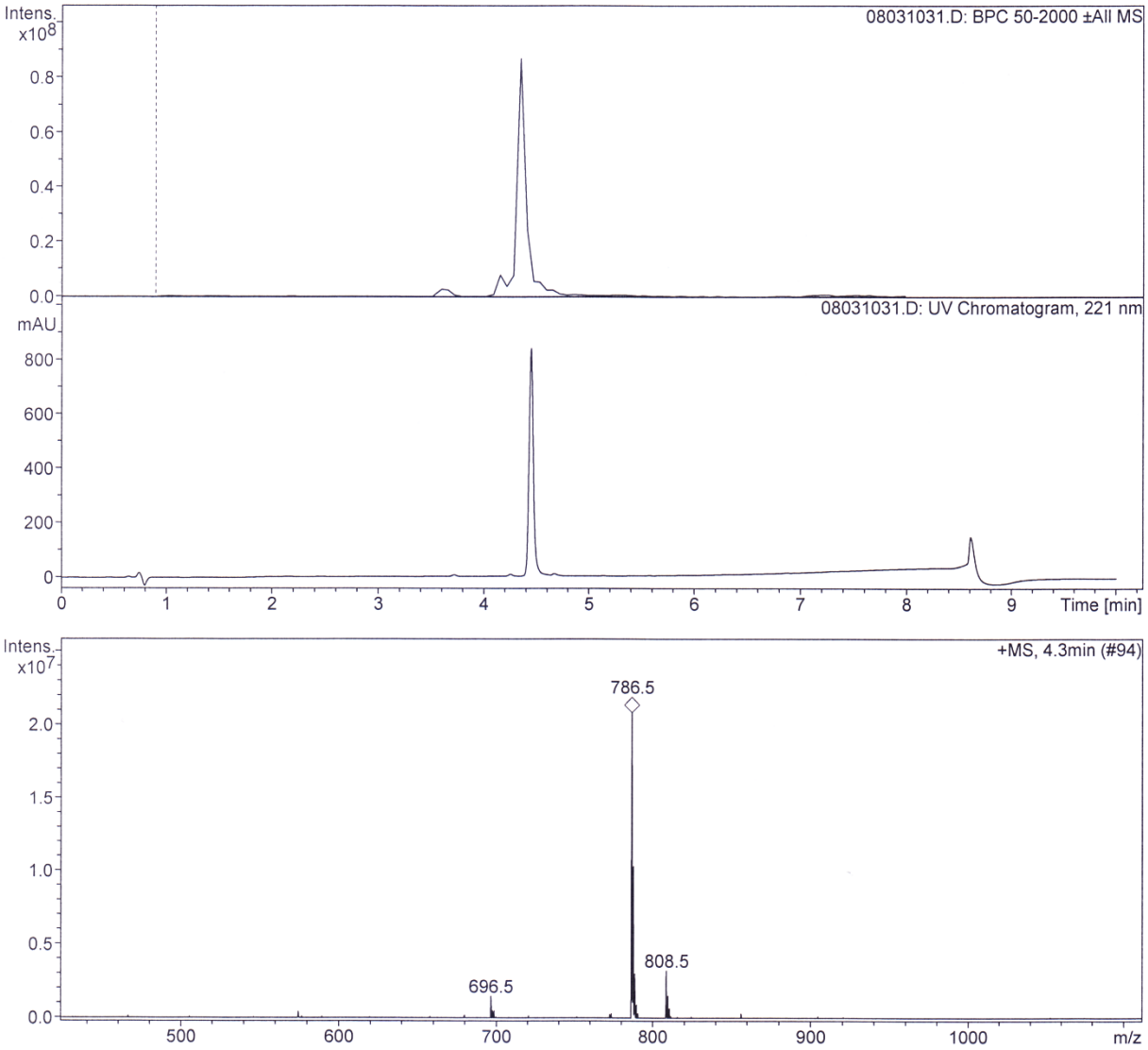
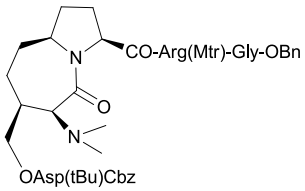


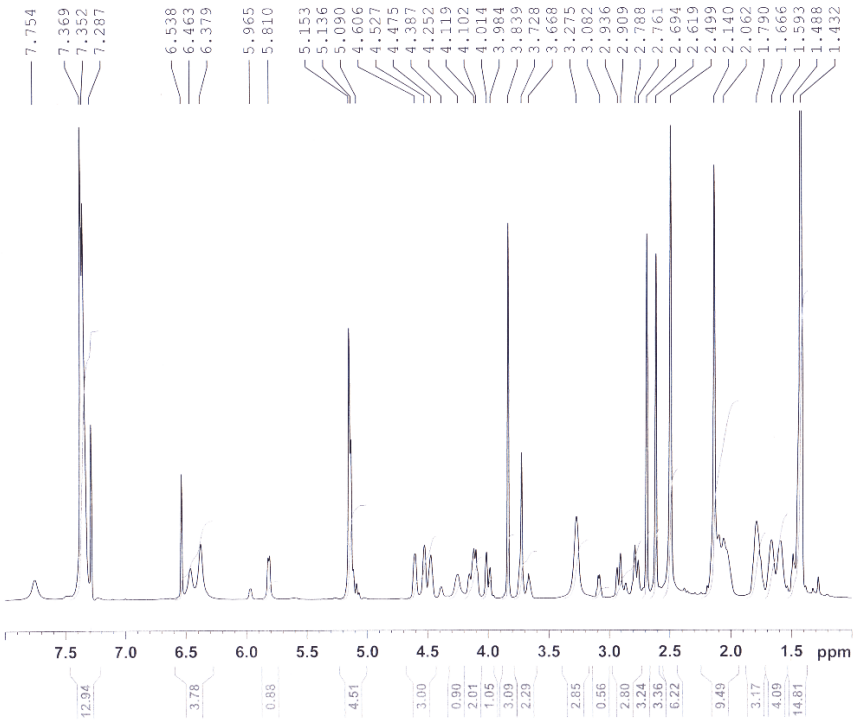
HPLC-MS (analytical method A)



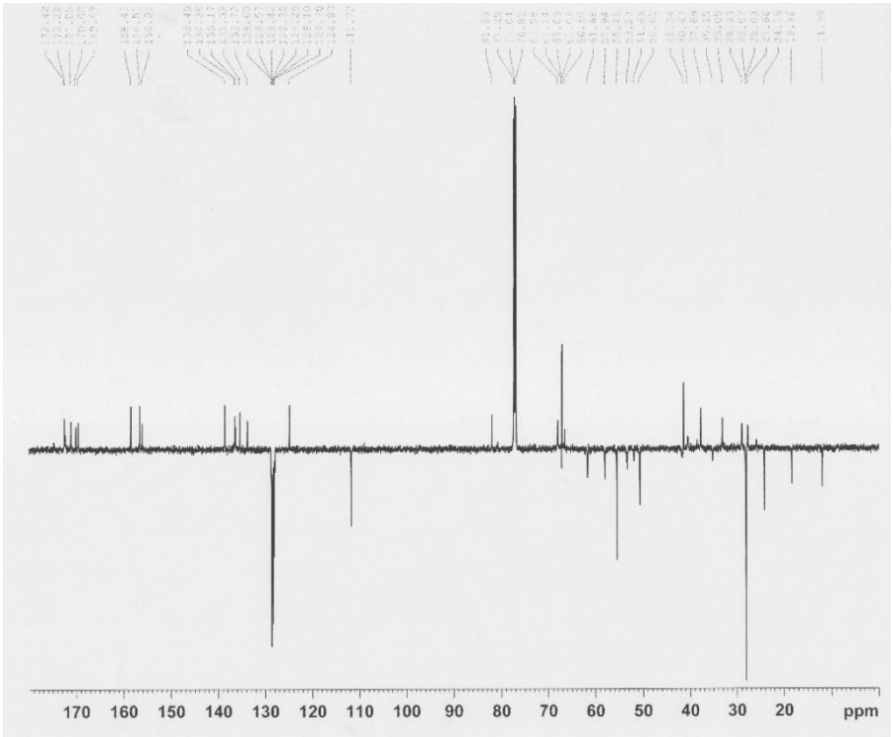
Compound 20



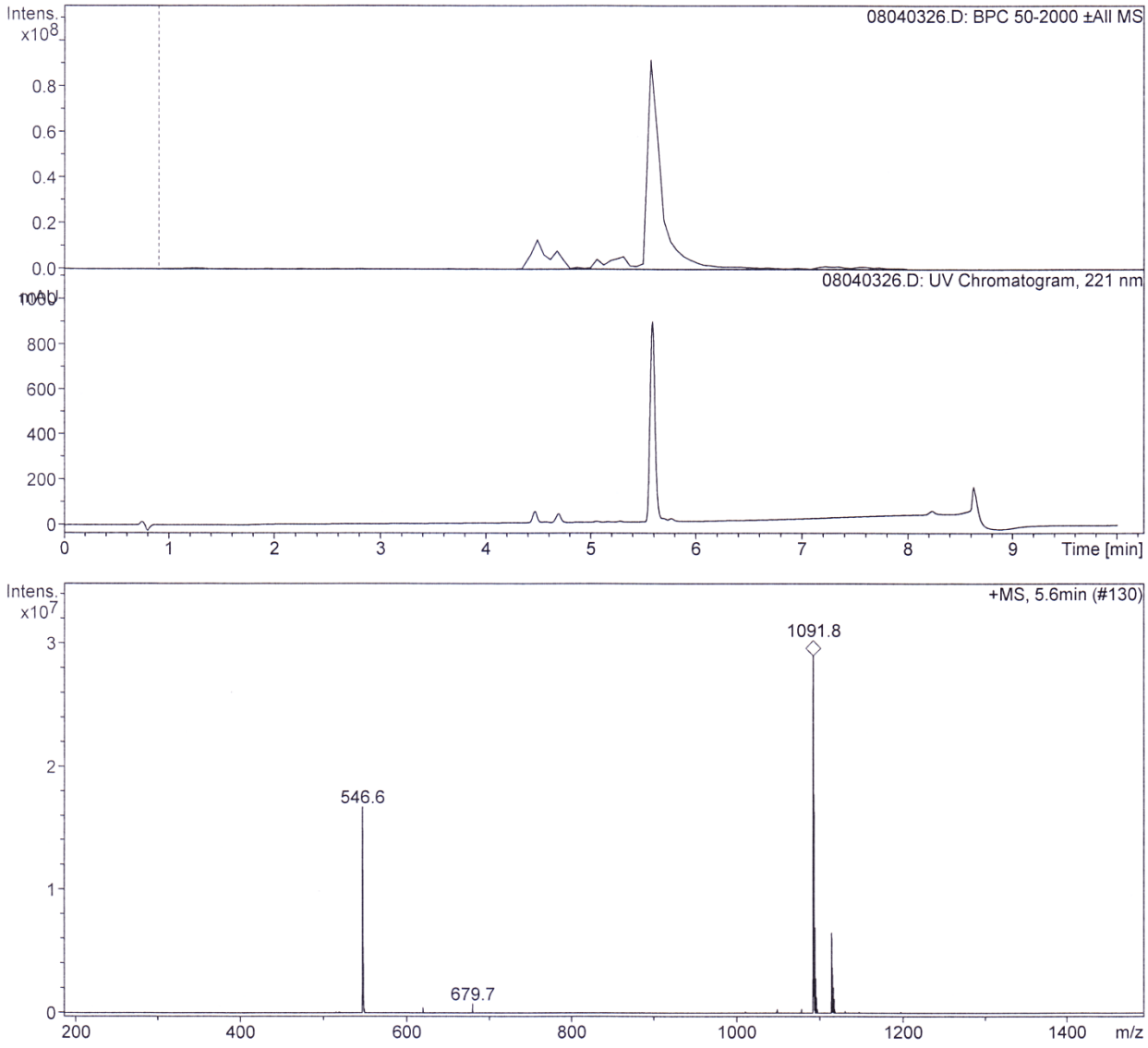
^{1}H NMR (400 MHz, $CDCl_3$)



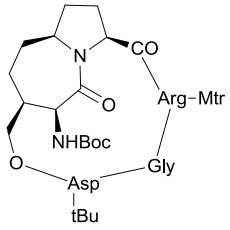
^{13}C NMR (100.6 MHz, $CDCl_3$)



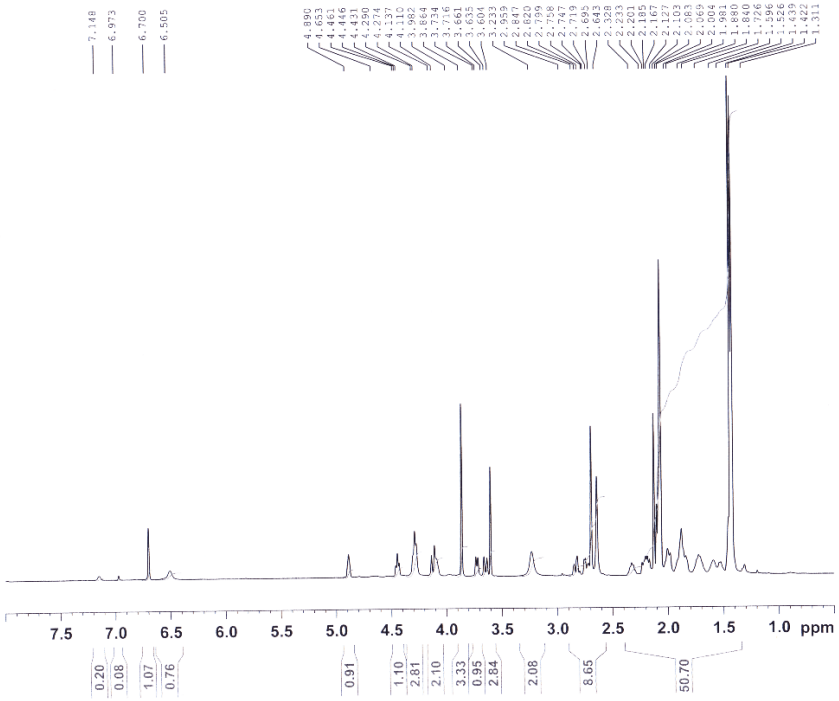
HPLC-MS (analytical method A)



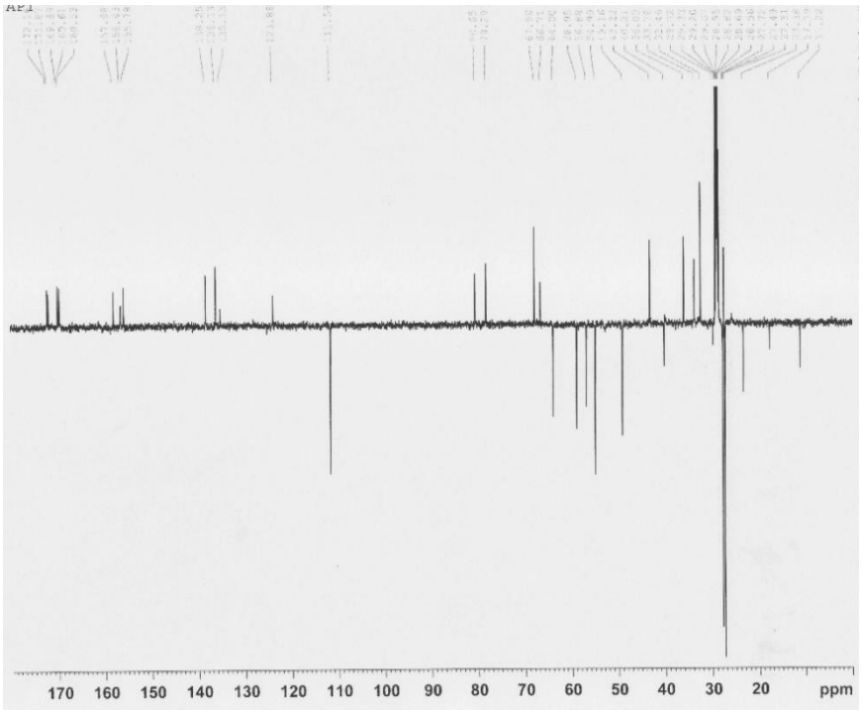
Compound 21



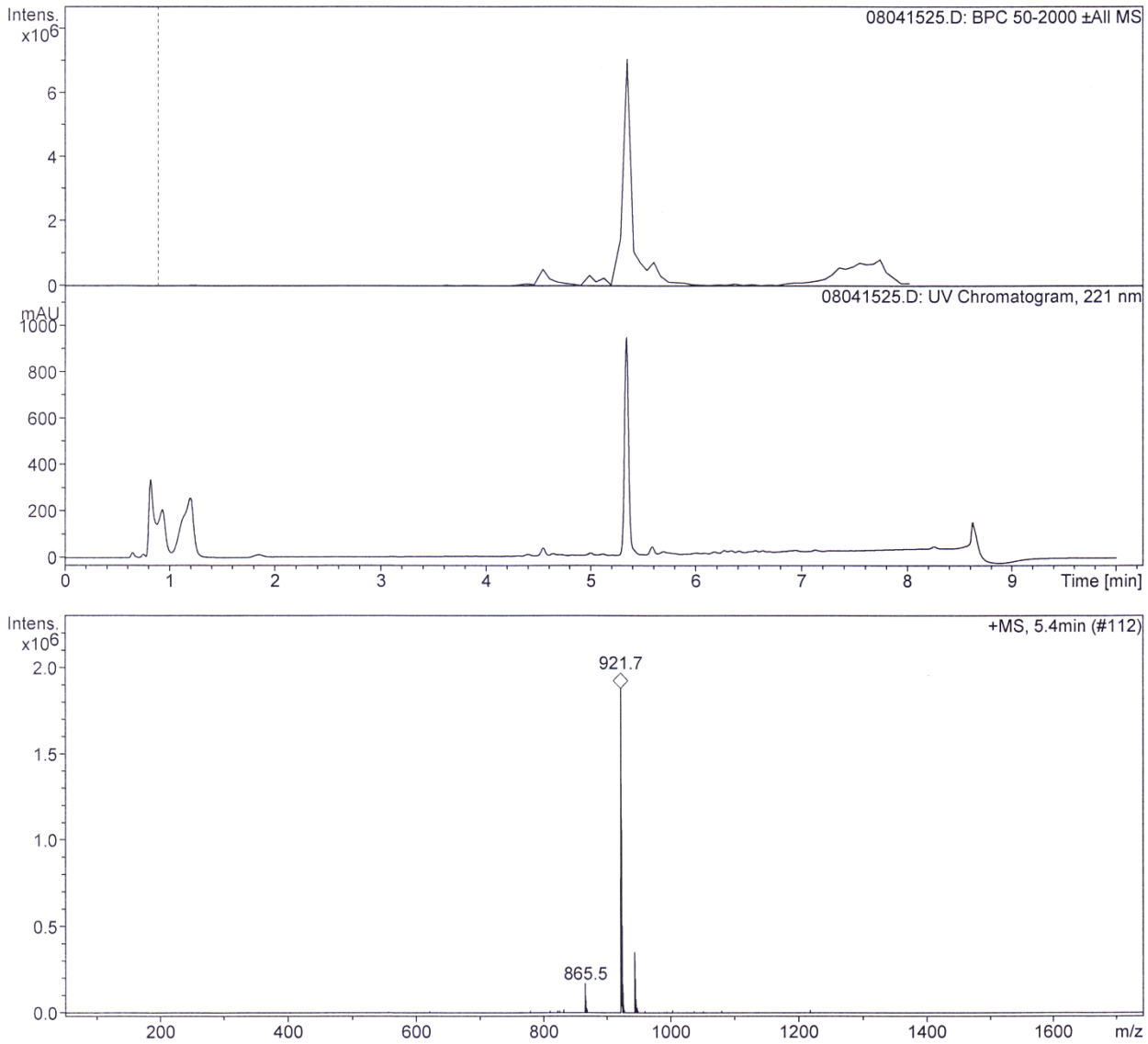
^{1}H NMR (400 MHz, *Acetone-d_6*)



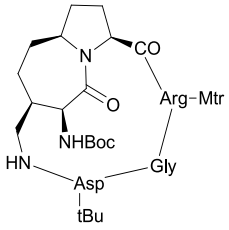
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



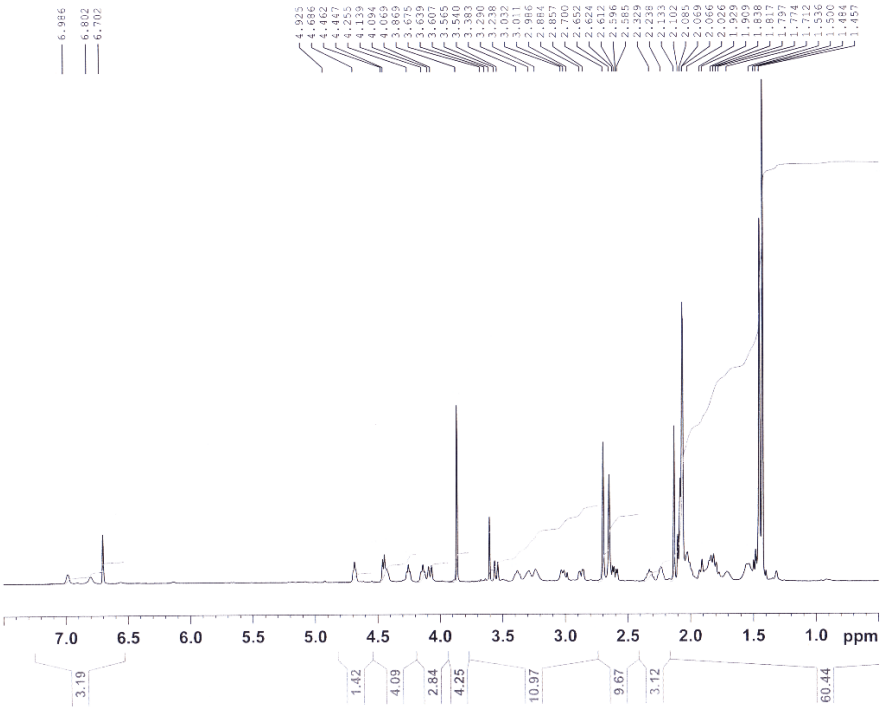
HPLC-MS (analytical method A)



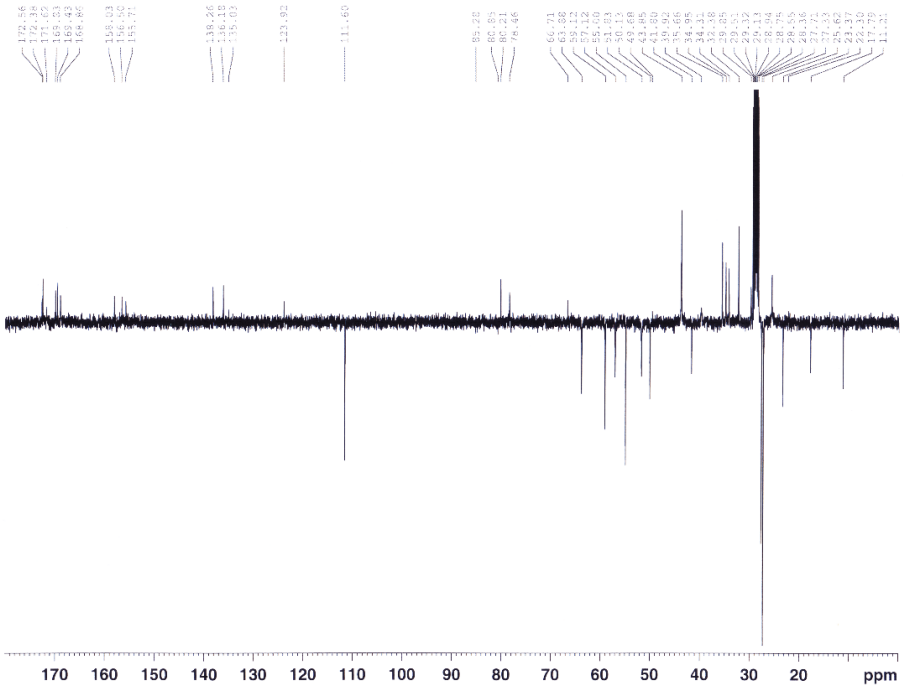
Compound 22



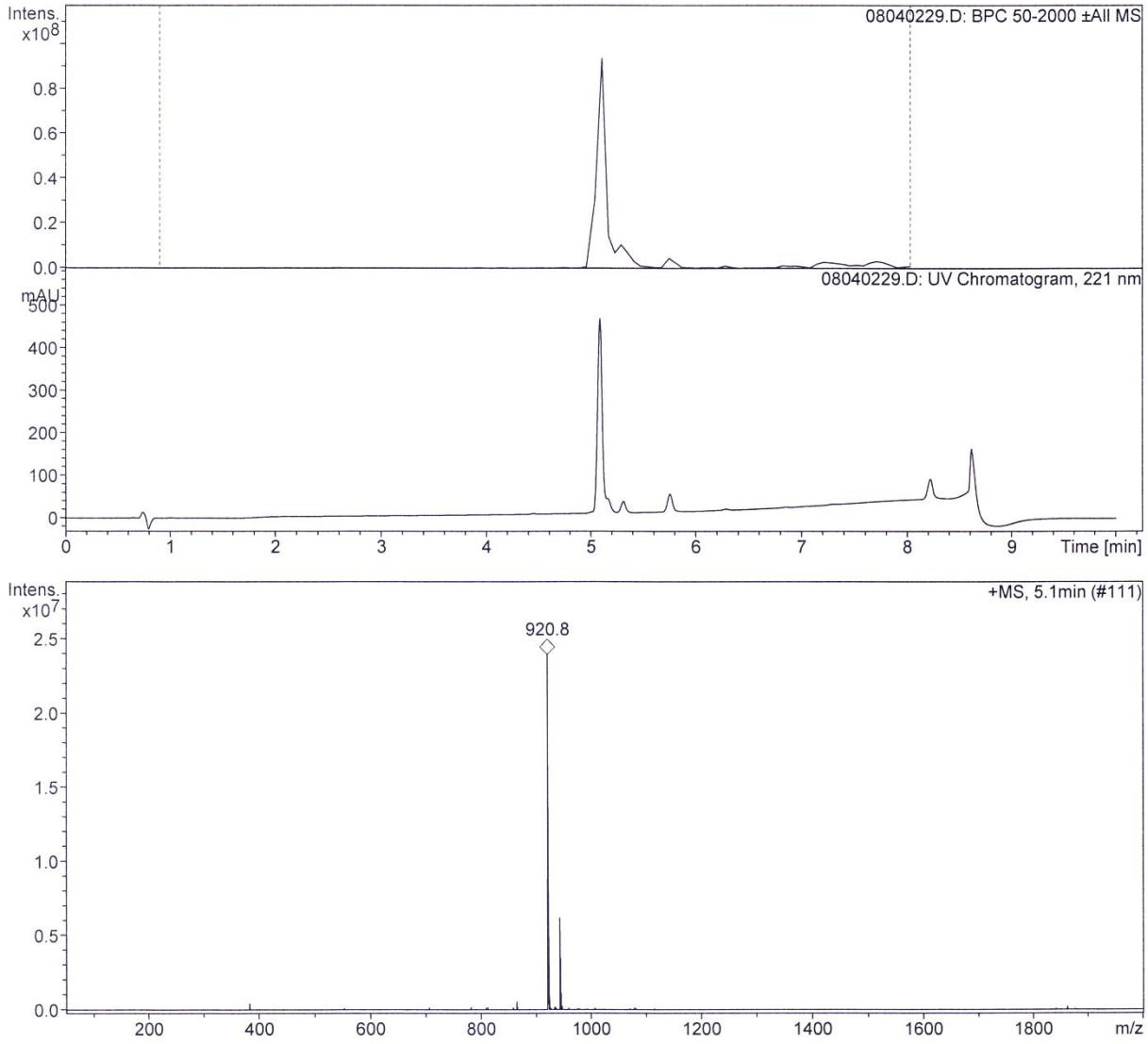
^{1}H NMR (400 MHz, *Acetone-d_6*)



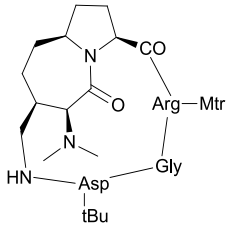
^{13}C NMR (100.6 MHz, *Acetone-d_6*)



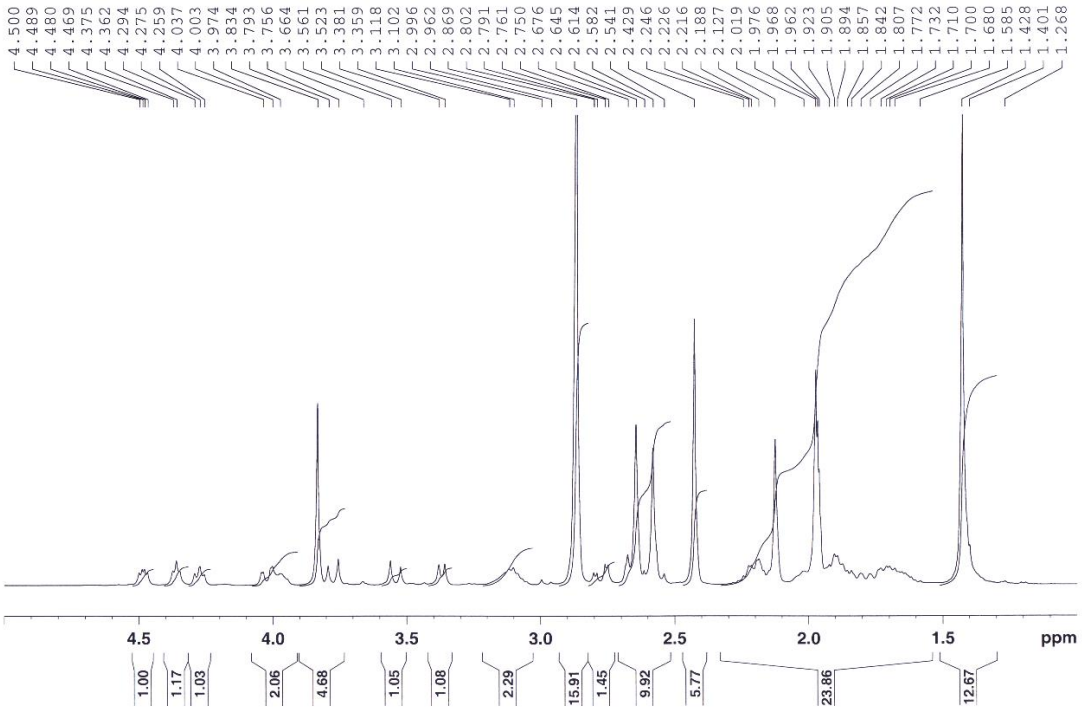
HPLC-MS (analytical method A)



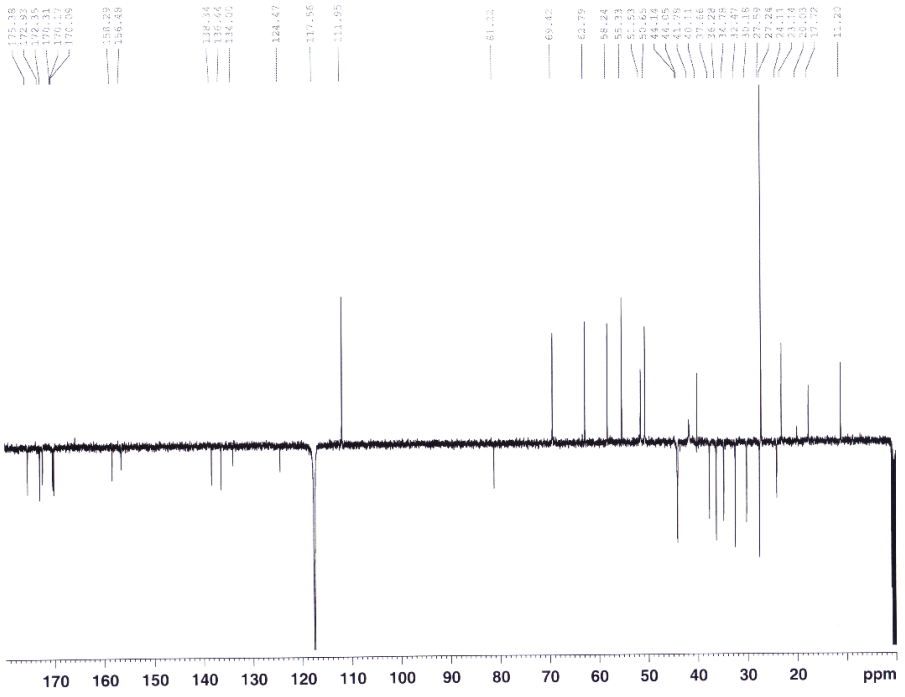
Compound 23



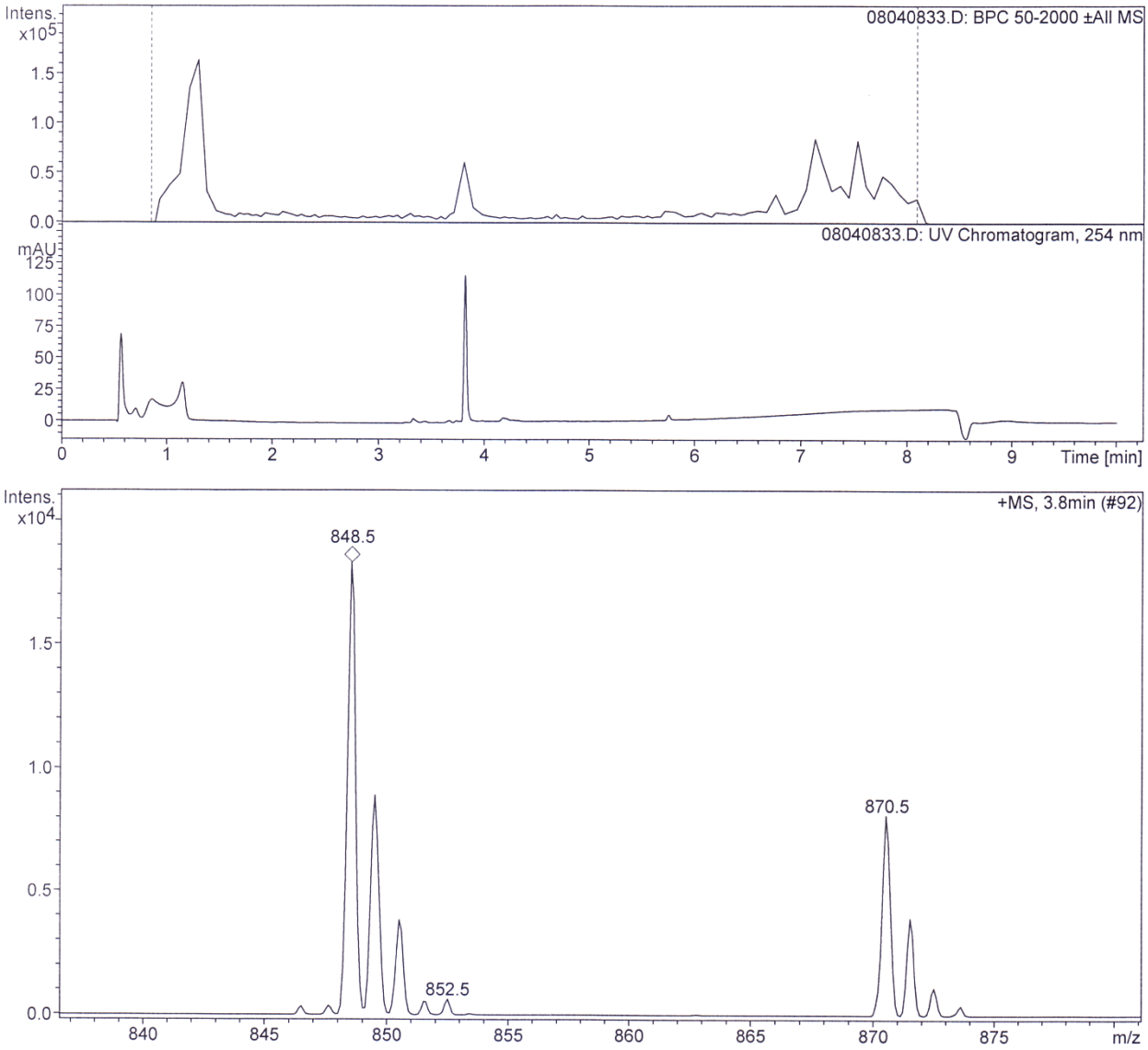
^{1}H NMR (400 MHz, $CD_3CN + D_2O$)



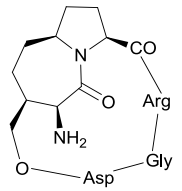
^{13}C NMR (100.6 MHz, $CD_3CN + D_2O$)



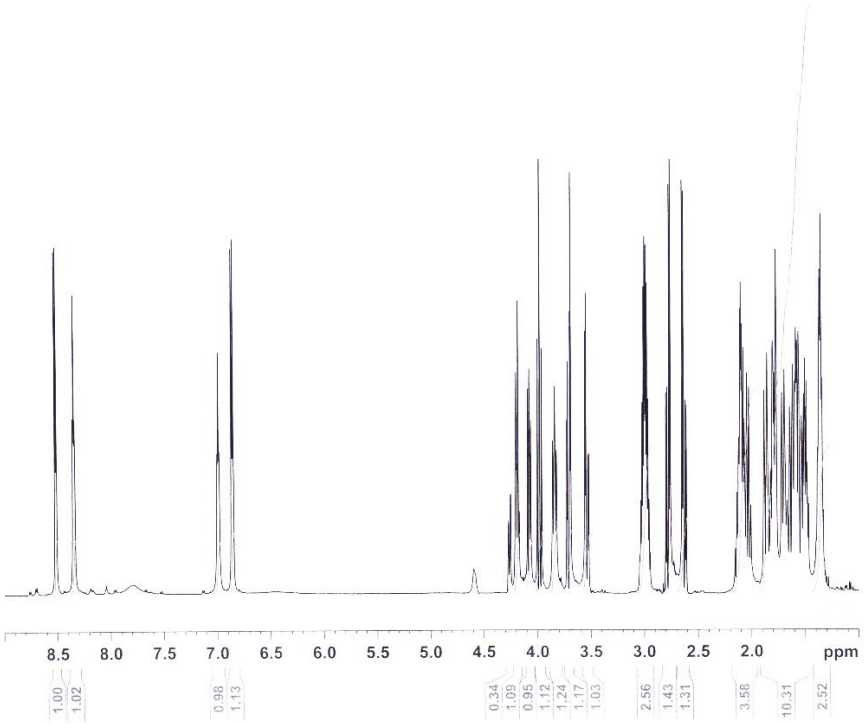
HPLC-MS (analytical method B)



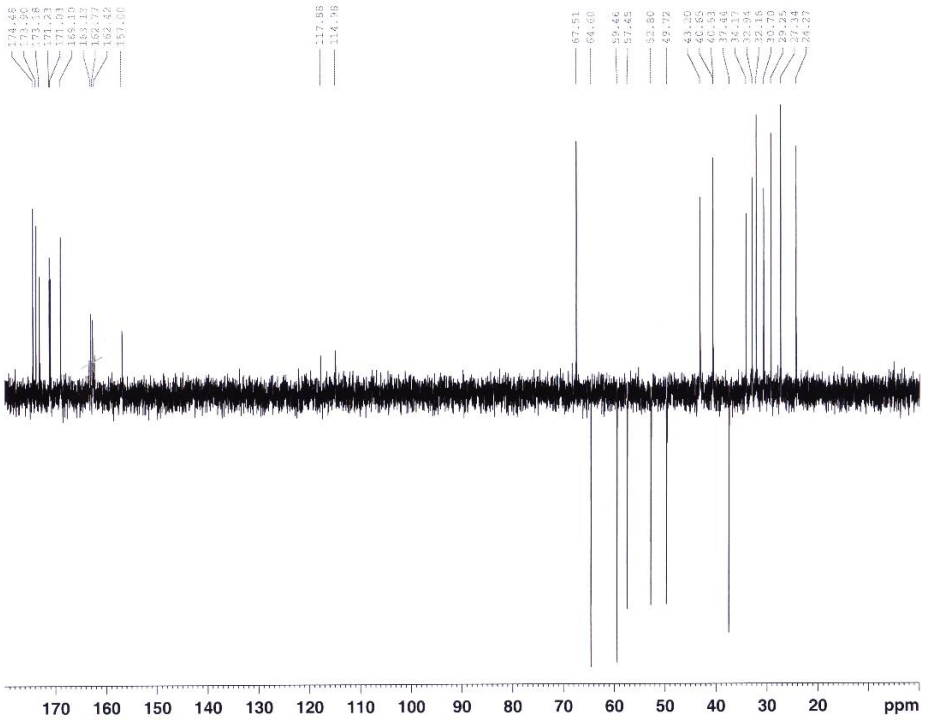
Compound 2



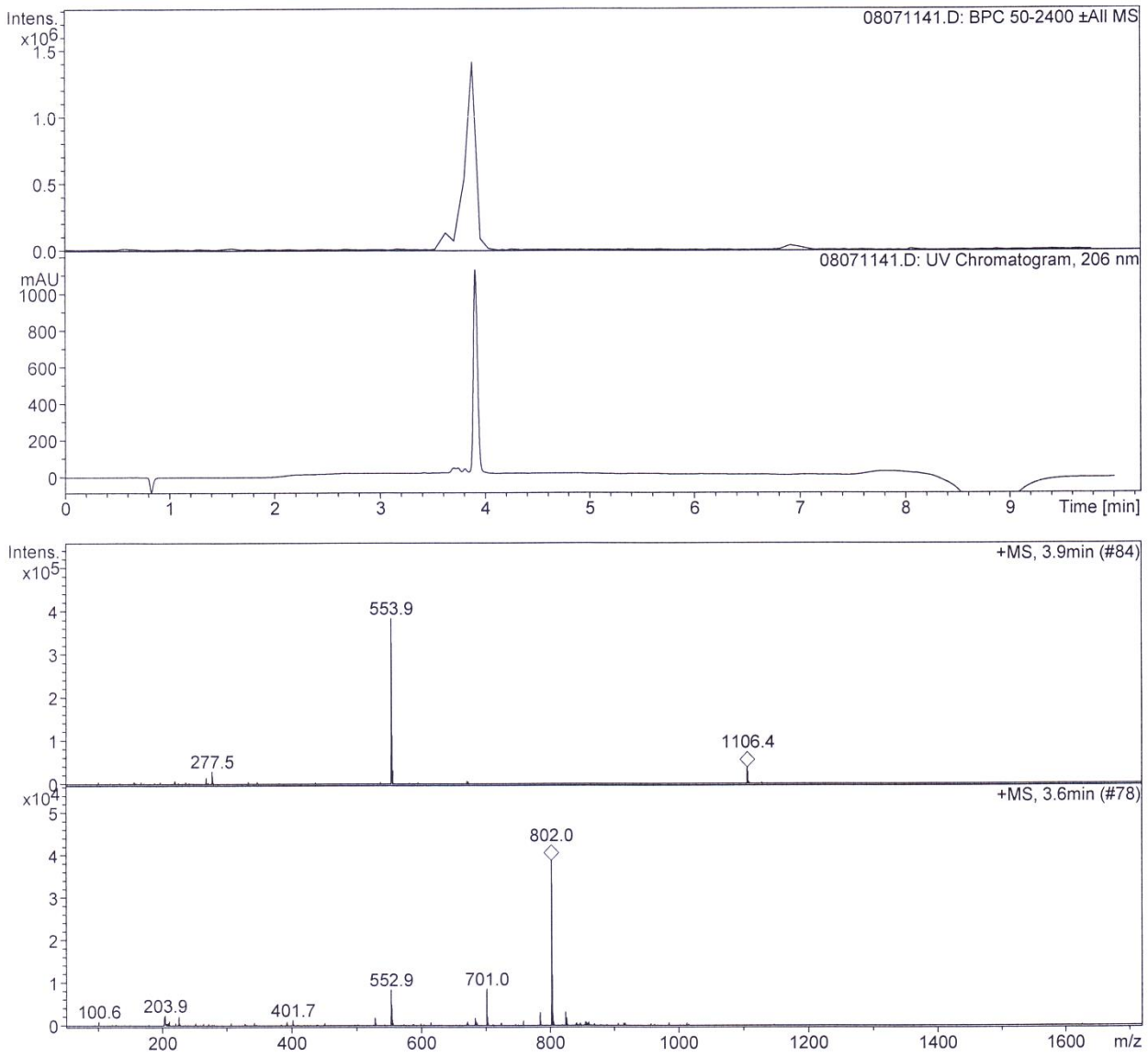
^{1}H NMR (600 MHz, D_2O + H_2O)



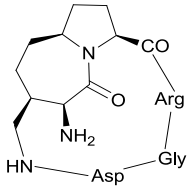
^{13}C NMR (100.6 MHz, D_2O + H_2O)



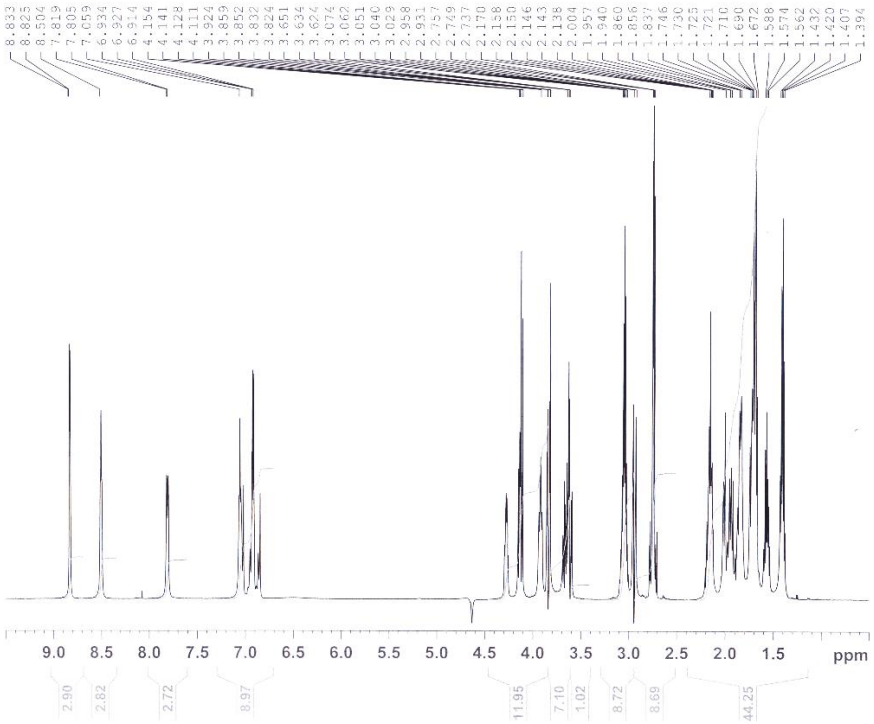
HPLC-MS (analytical method C)



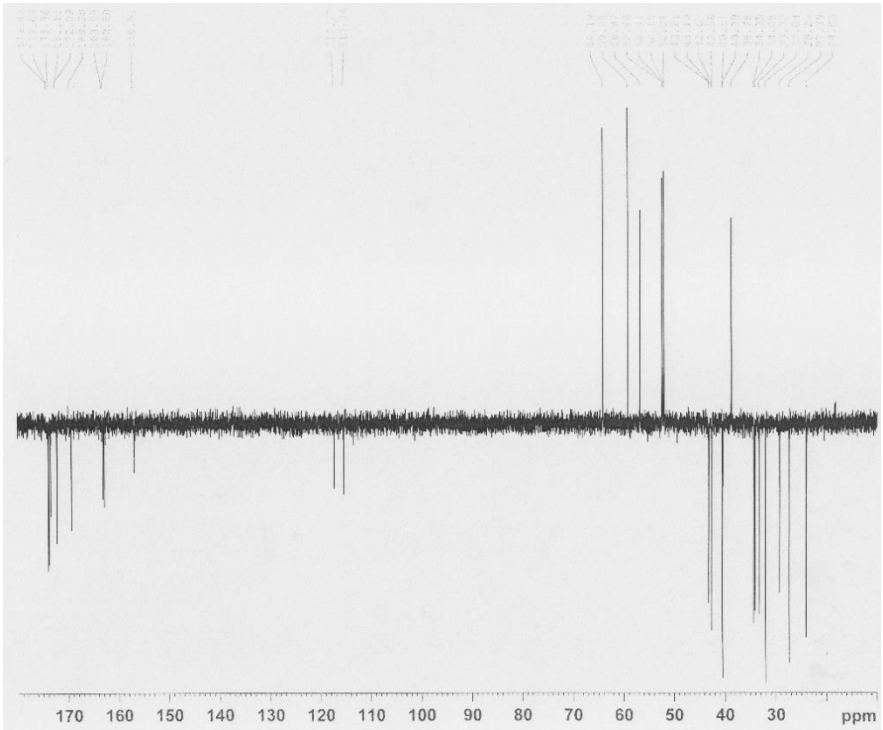
Compound 3



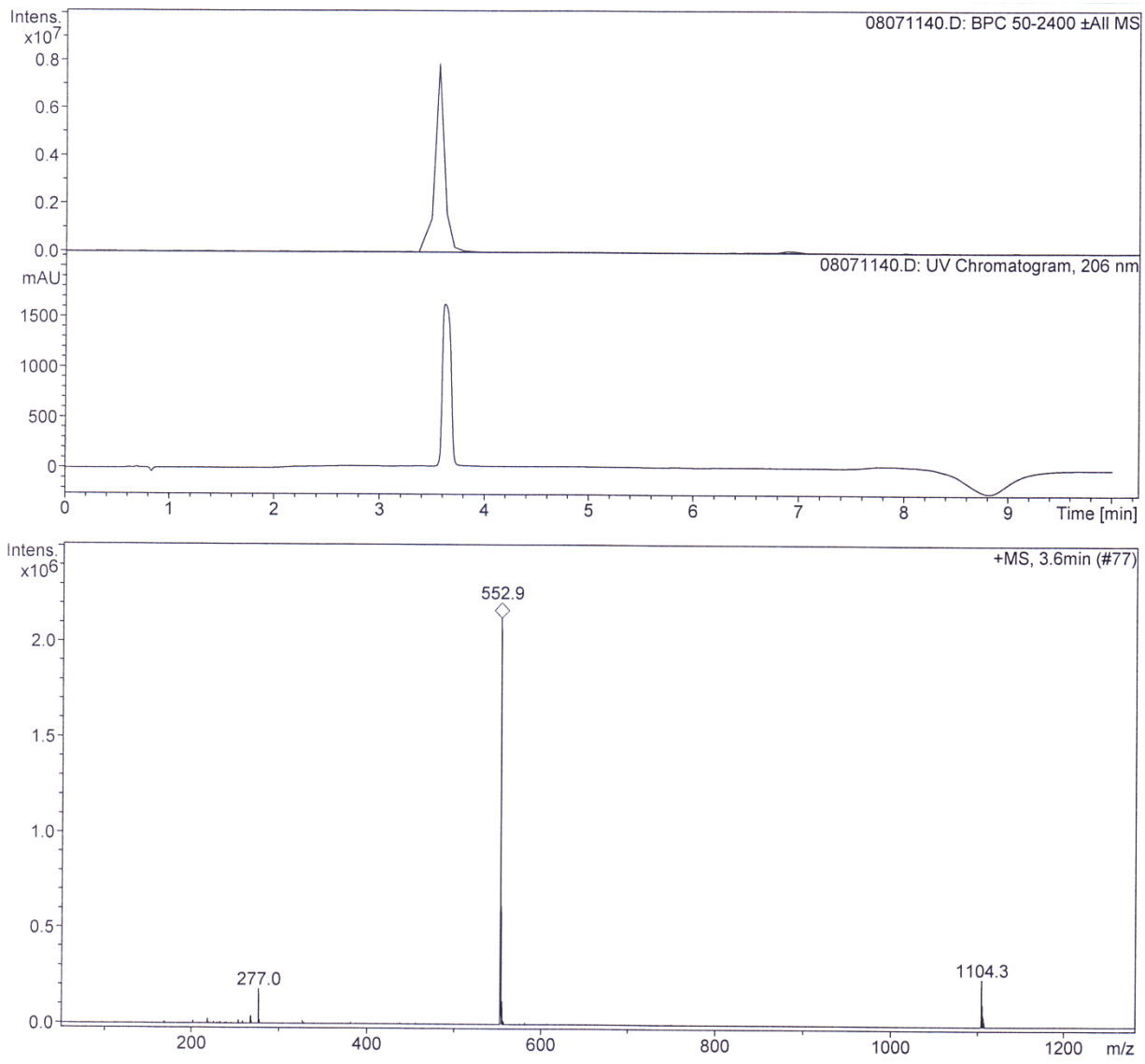
^{1}H NMR (600 MHz, D_2O + H_2O)



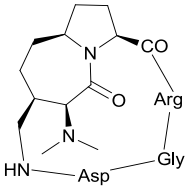
^{13}C NMR (150.95 MHz, D_2O + H_2O)



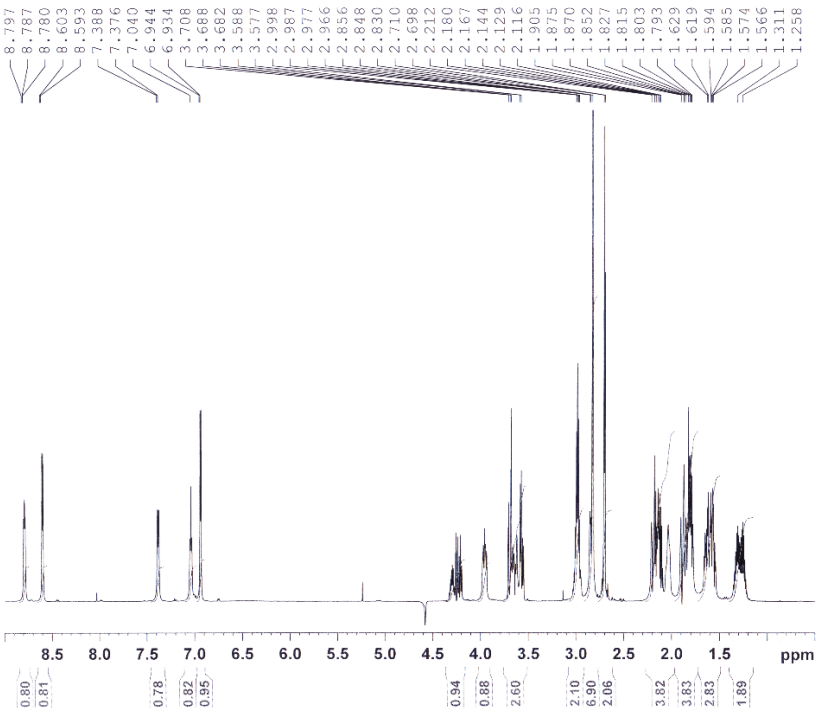
HPLC-MS (analytical method C)



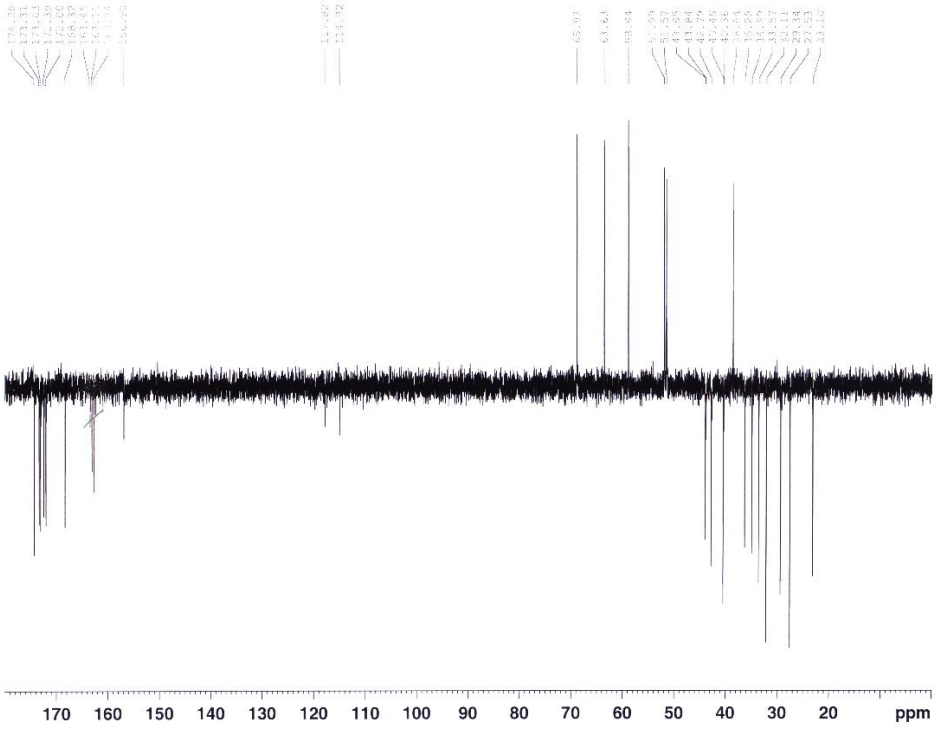
Compound 5



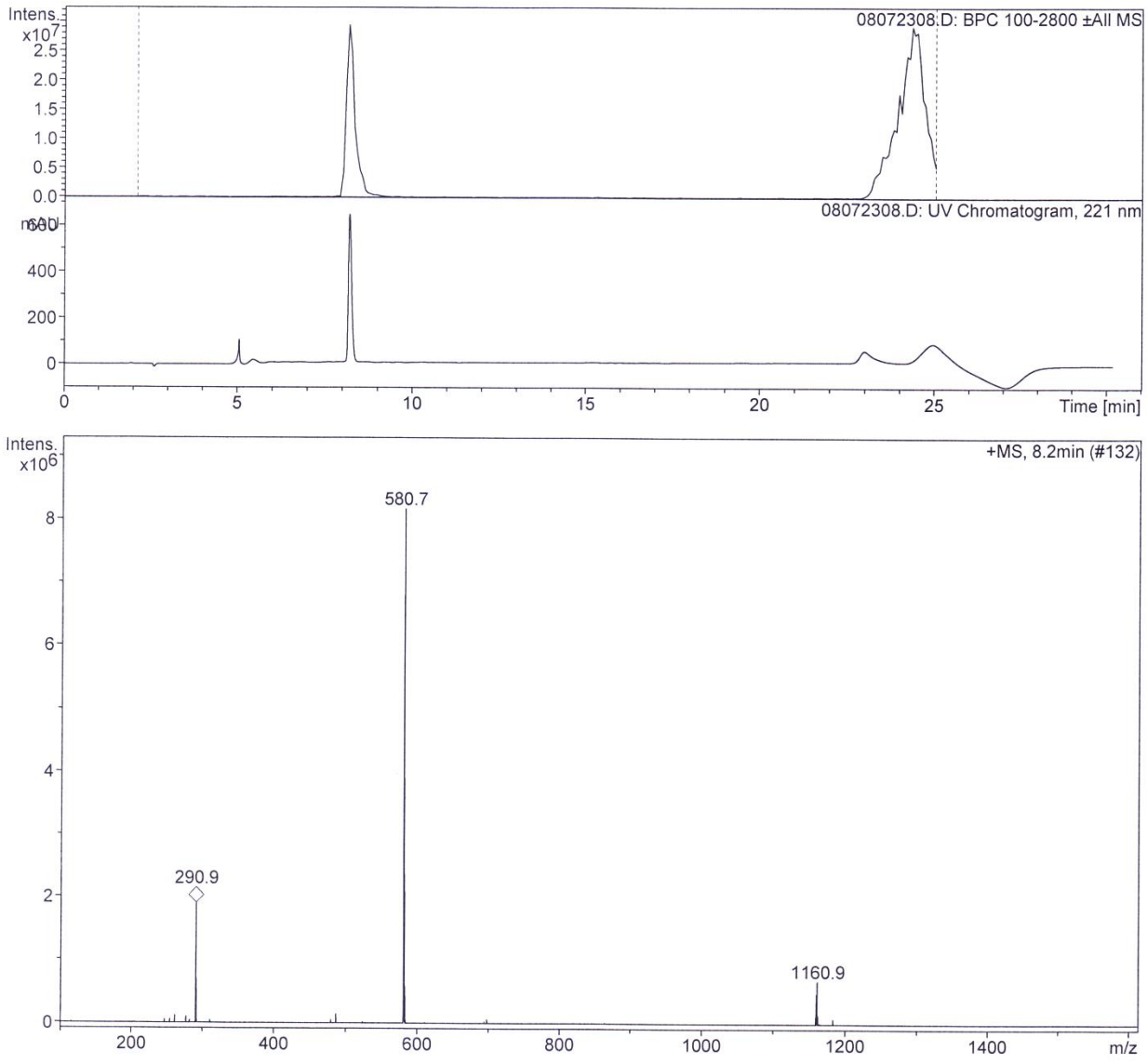
^{1}H NMR (600 MHz, D_2O + H_2O)



^{13}C NMR (150.95 MHz, D_2O + H_2O)



HPLC-MS (analytical method D)



Biology

Wound healing assay

Round bottomed 24-well plates were coated with vitronectin (Duotech) or fibronectin (Sigma) at 10 µg/mL in PBS, overnight at 37 °C. $1.2x10^4$ 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.

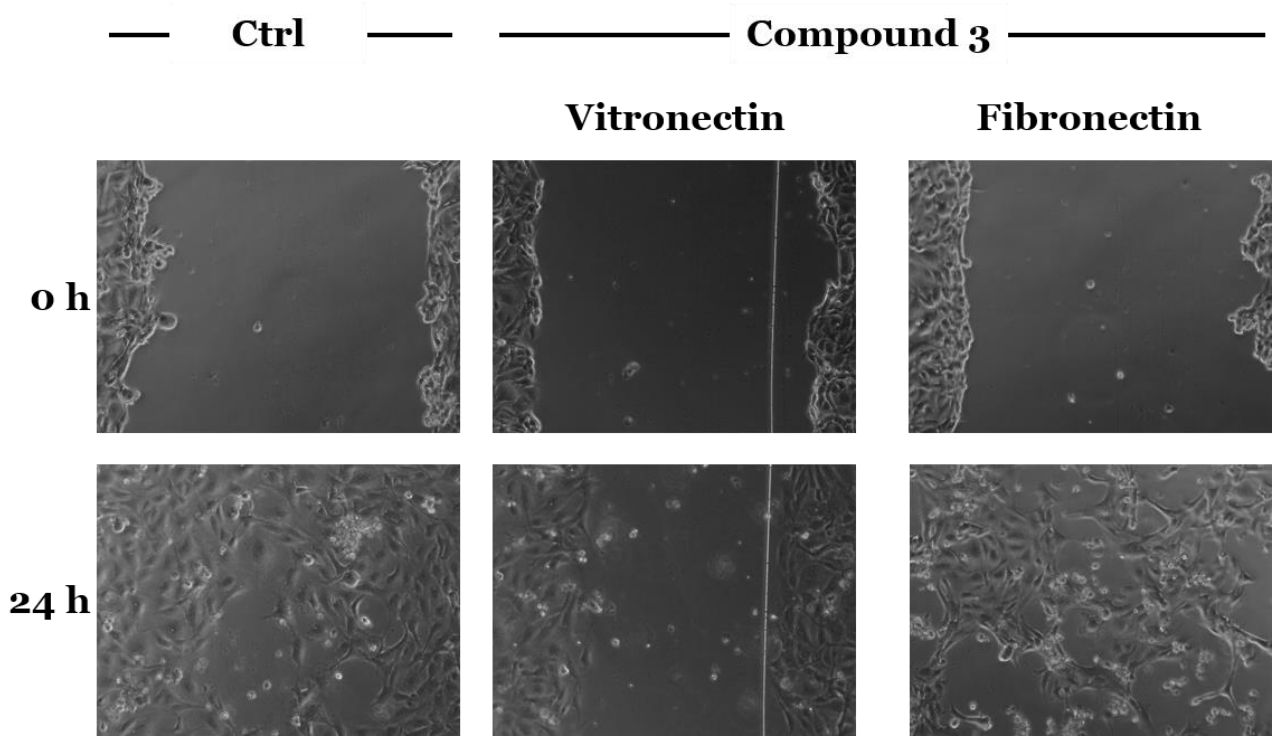


Figure S1. Wound healing assay on HUVECs. The IC_{20} of compound **3** was incubated on HUVECs plated on vitronectin or fibronectin for 24 hours, in order to determine its anti-migratory activity. Original magnification: 10x.

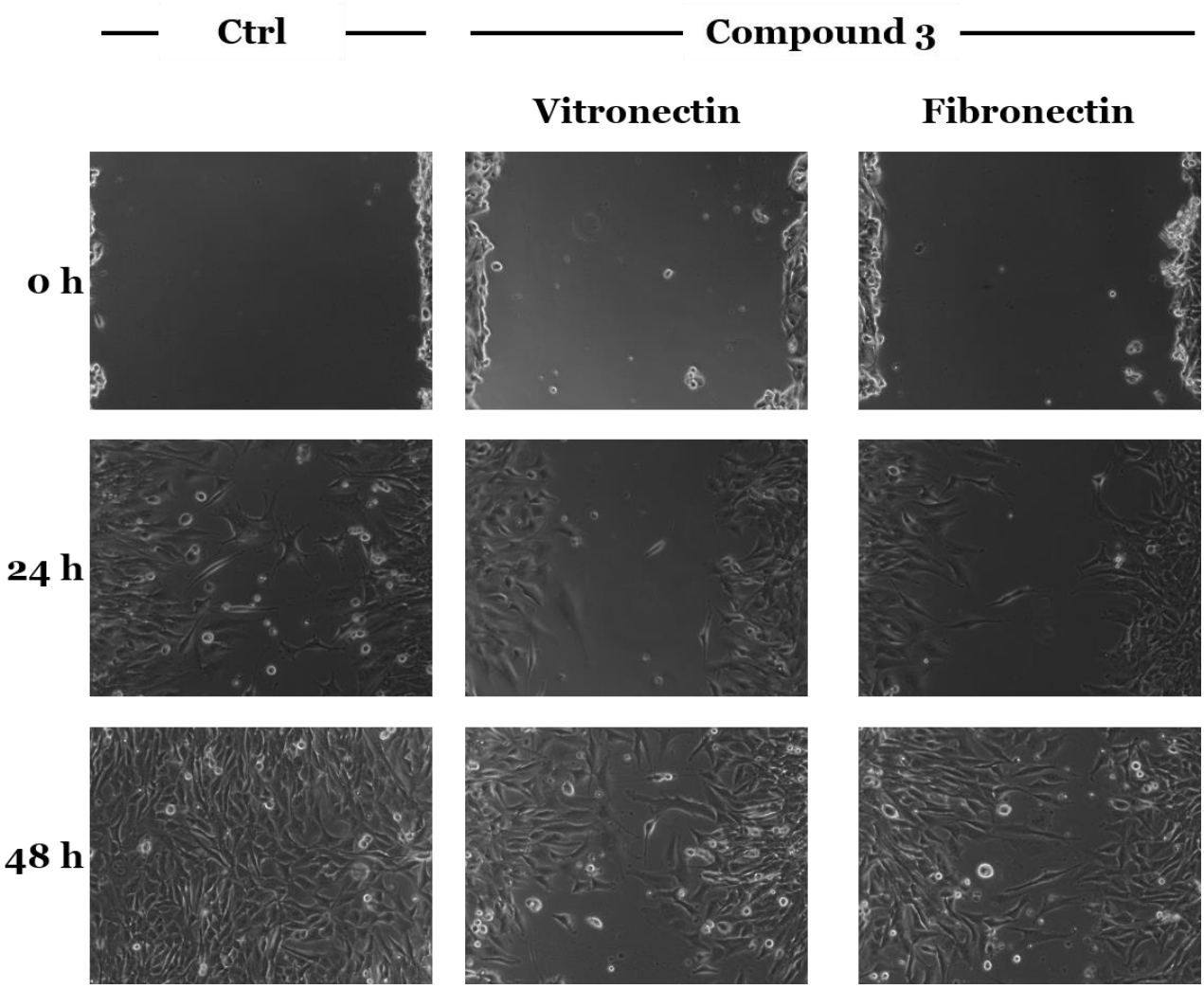


Figure S2. Wound healing assay on T98G cells. The IC_{20} of compound **3** was incubated on T98G cells plated on vitronectin or fibronectin for 48 hours, in order to determine its anti-migratory activity. Original magnification: 10x.