

Supporting information.

Convergent synthesis of a steroidal antiestrogen-mitomycin C hybrid using “click” chemistry

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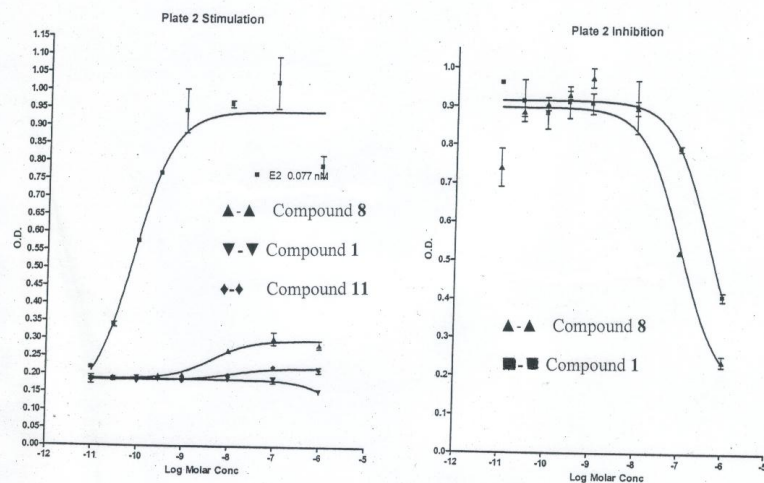
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Biological assays and graphs/figures
Spectral data for intermediates and final compound

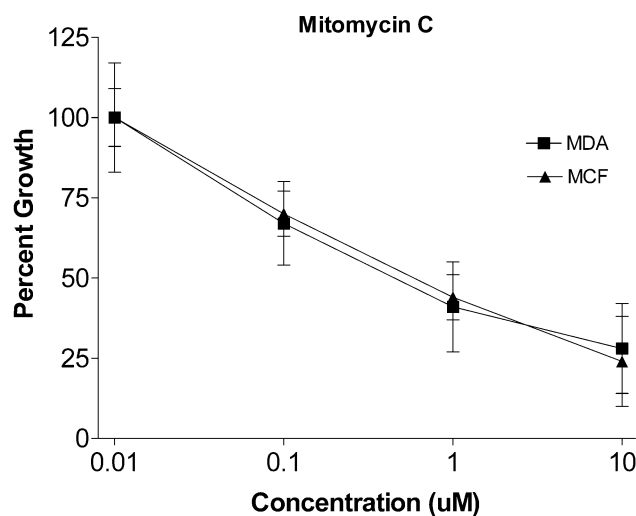
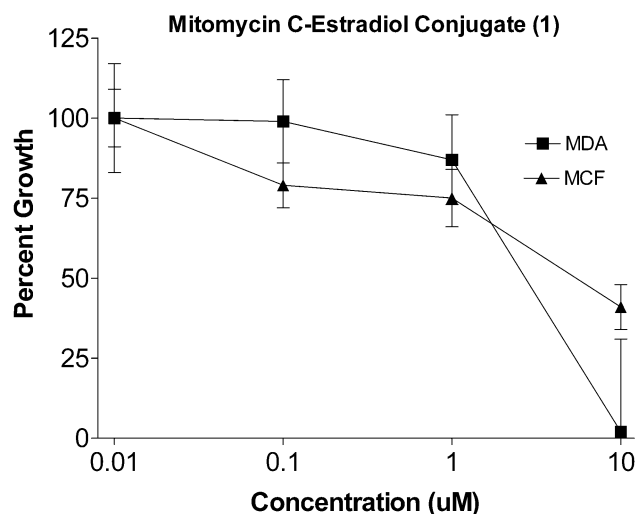
Ishikawa 12-11-06

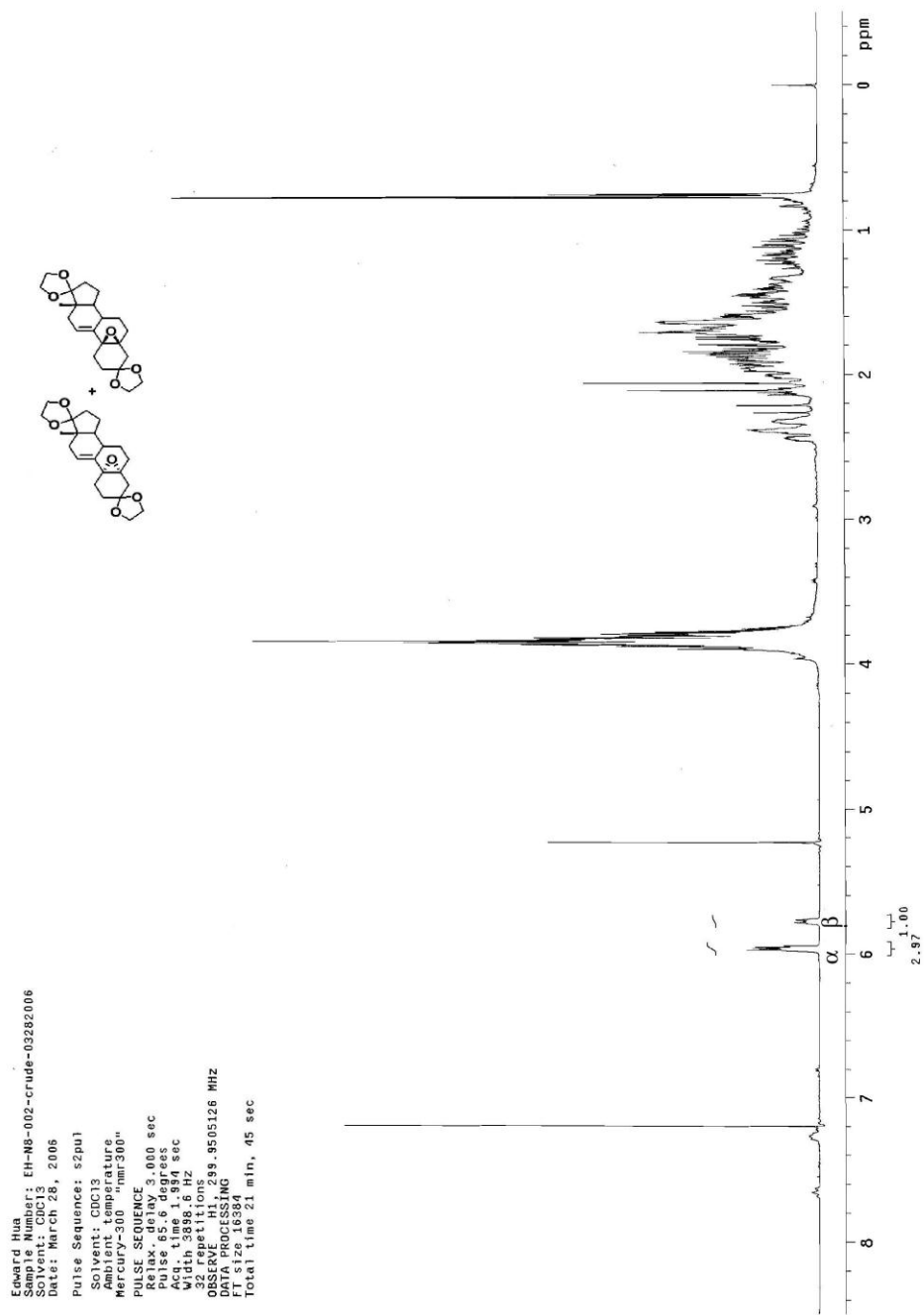


The potency of the compounds was determined in an estrogen bioassay, the induction of alkaline phosphatase in human endometrial adenocarcinoma cells (Ishikawa) grown in 96-well microtiter plates. The cells were grown in phenol red free medium with estrogen depleted (charcoal stripped) bovine serum in the presence or absence of varying amounts of the steroids, across a dose range of at least 6 orders of magnitude. For antagonists, the effect (K_i) of each compound tested at a range of 10^{-6} M to 10^{-12} M was measured for the inhibition of the action of 10^{-9} M E_2 ($EC_{50} \sim 0.2$ nM). Each compound was analyzed in at least 3 separate experiments performed in duplicate. The K_i and RSA ($RSA = \text{ratio of } 1/EC_{50} \text{ of the steroid analog to that of } E_2 \times 100$) were determined using the curve fitting program Prism.

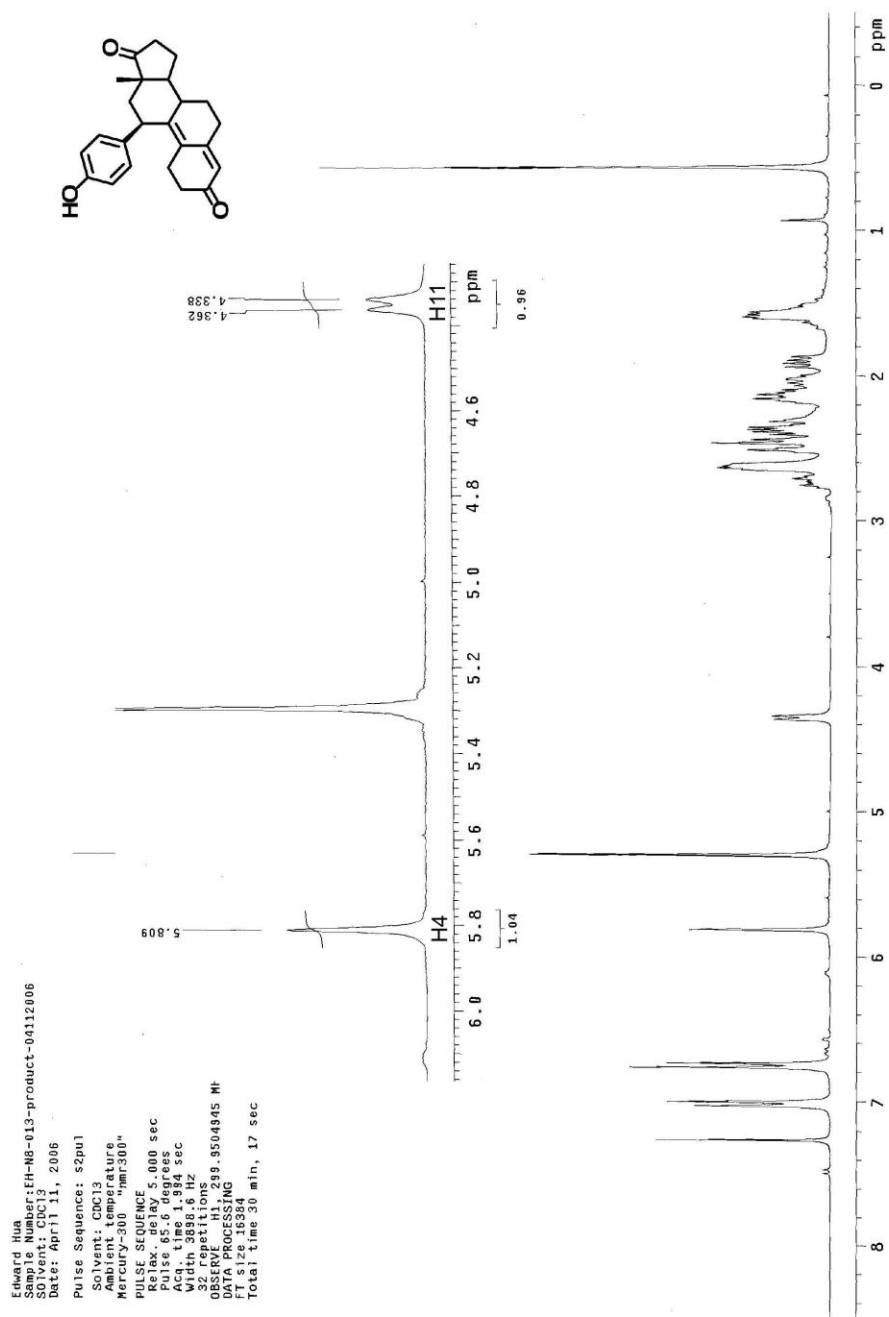
Toxicity Assay

Measurement of the toxicity of MMC and (1) toward MCF-7 (ER+) and MDA-MB231 (ER-) cell lines. Cell lines were obtained from the American Type Culture Collection, (Rockville, MD). They were grown in Minimal Essential Media (MEM) containing phenol red (GIBCO BRL, Rockville, MD) supplemented with 10% fetal bovine serum (Biowhittaker, Walkersville, MD), 2 mM glutamine and 1 mM sodium pyruvate in a 5% CO₂/95% air atmosphere. Cells were seeded at 2×10^5 per well in 6-well plates and, 48 h later, test compounds were added to the growth medium. After 24 h treatment, cells were trypsinized, suspended in Isoton and counted using a Coulter Counter.

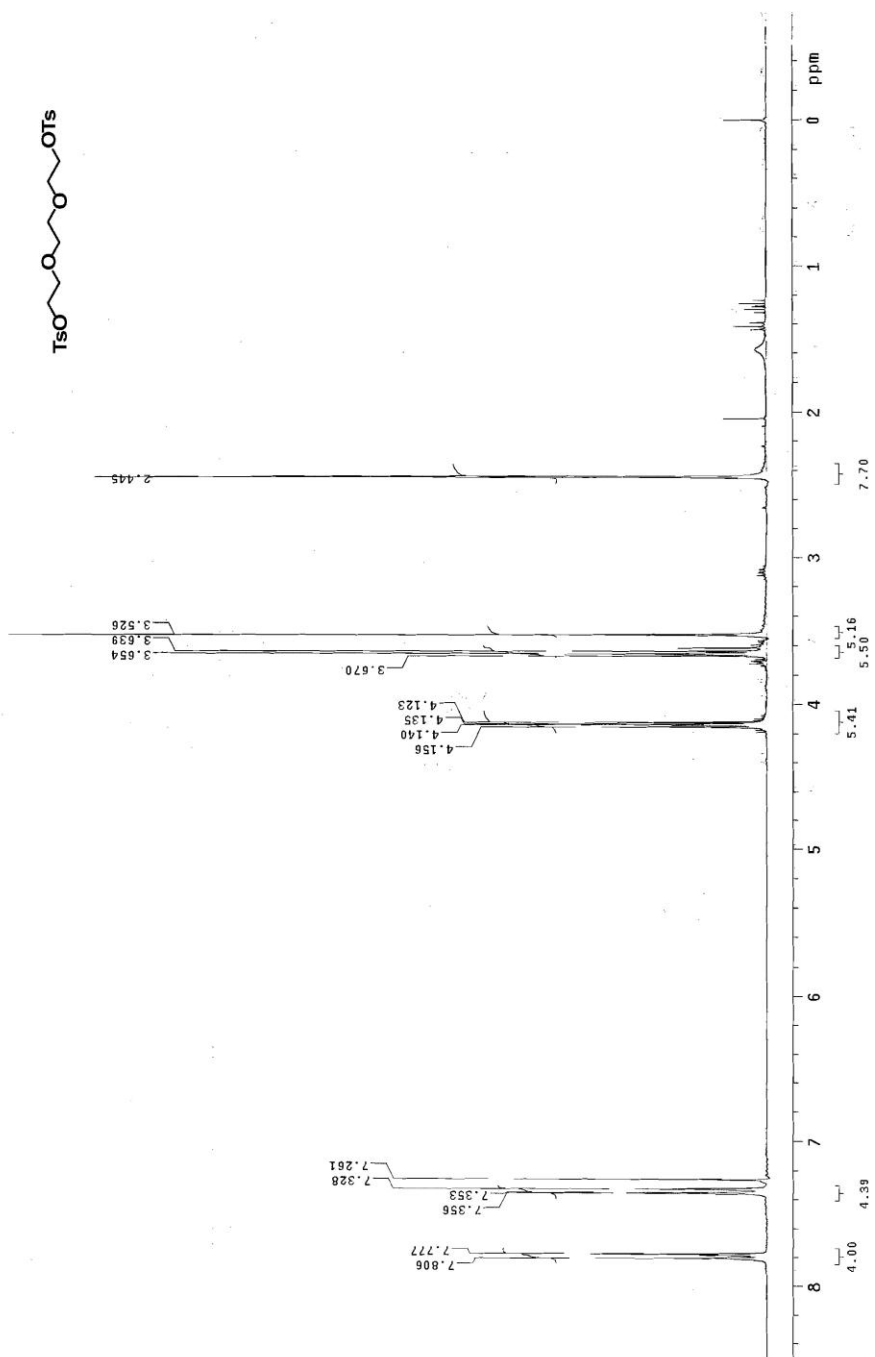




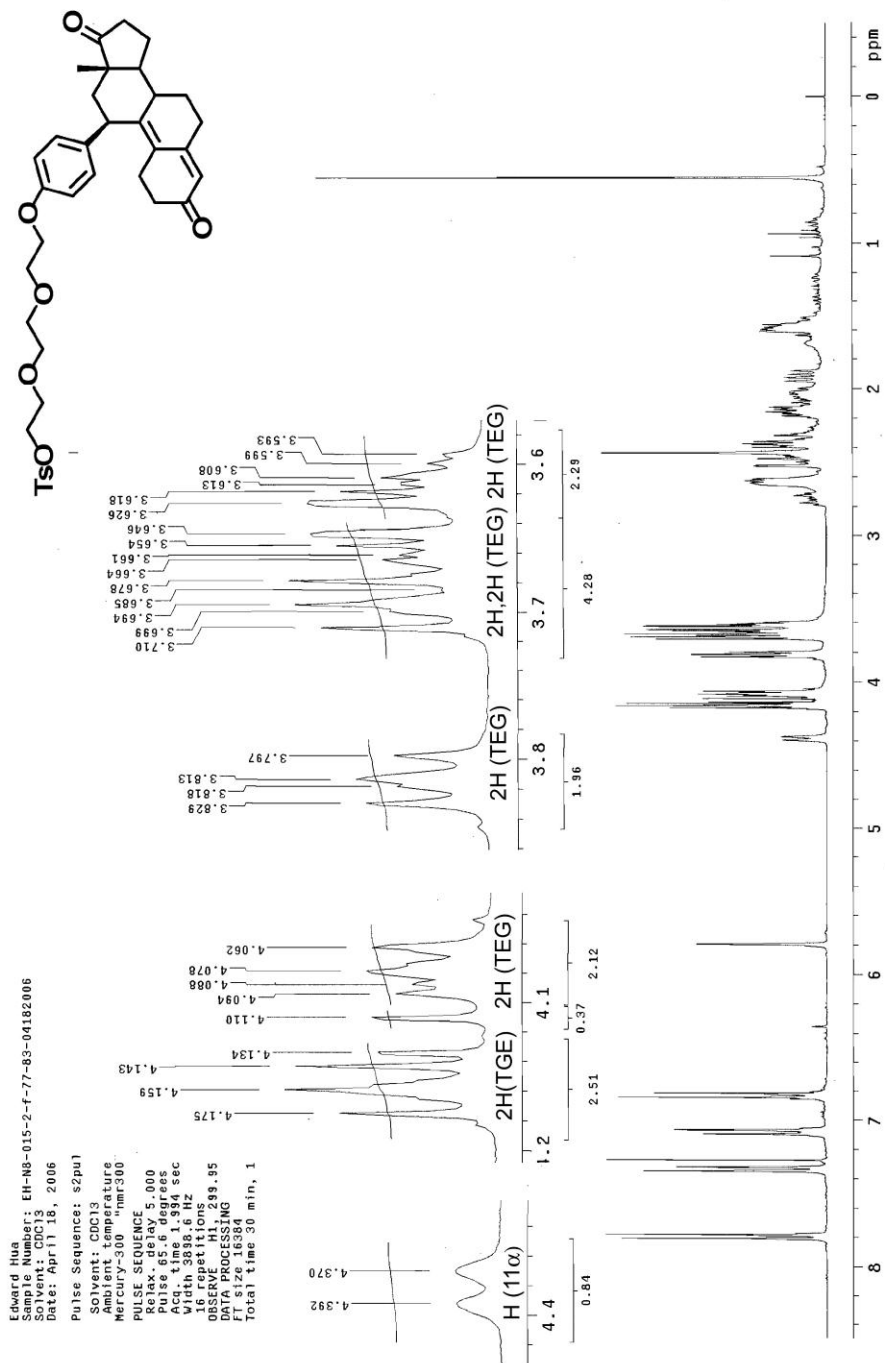
¹H NMR spectrum of mixture of compounds 3 and 4 in CDCl₃



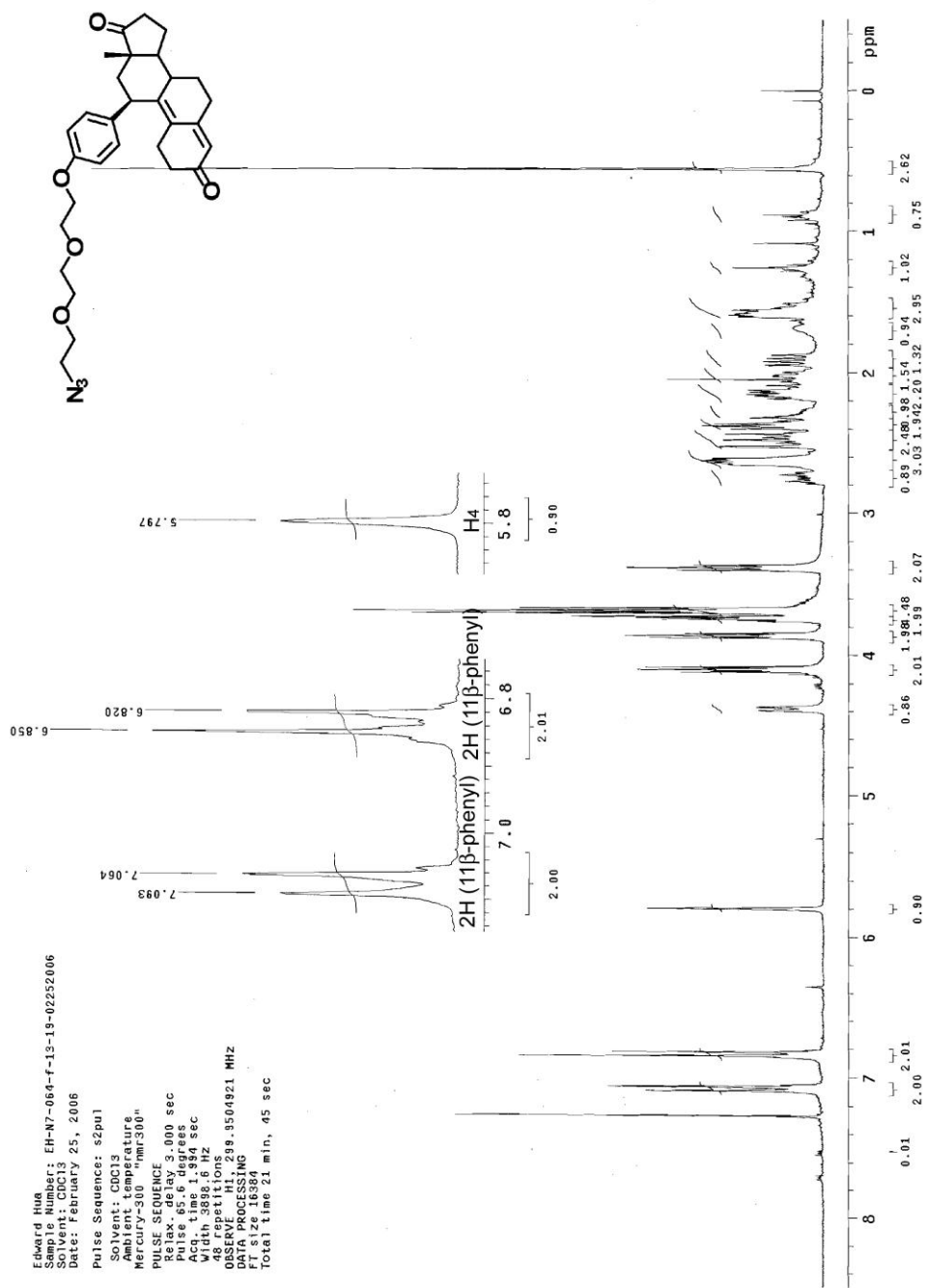
¹H NMR spectrum of compound 5 in CDCl₃

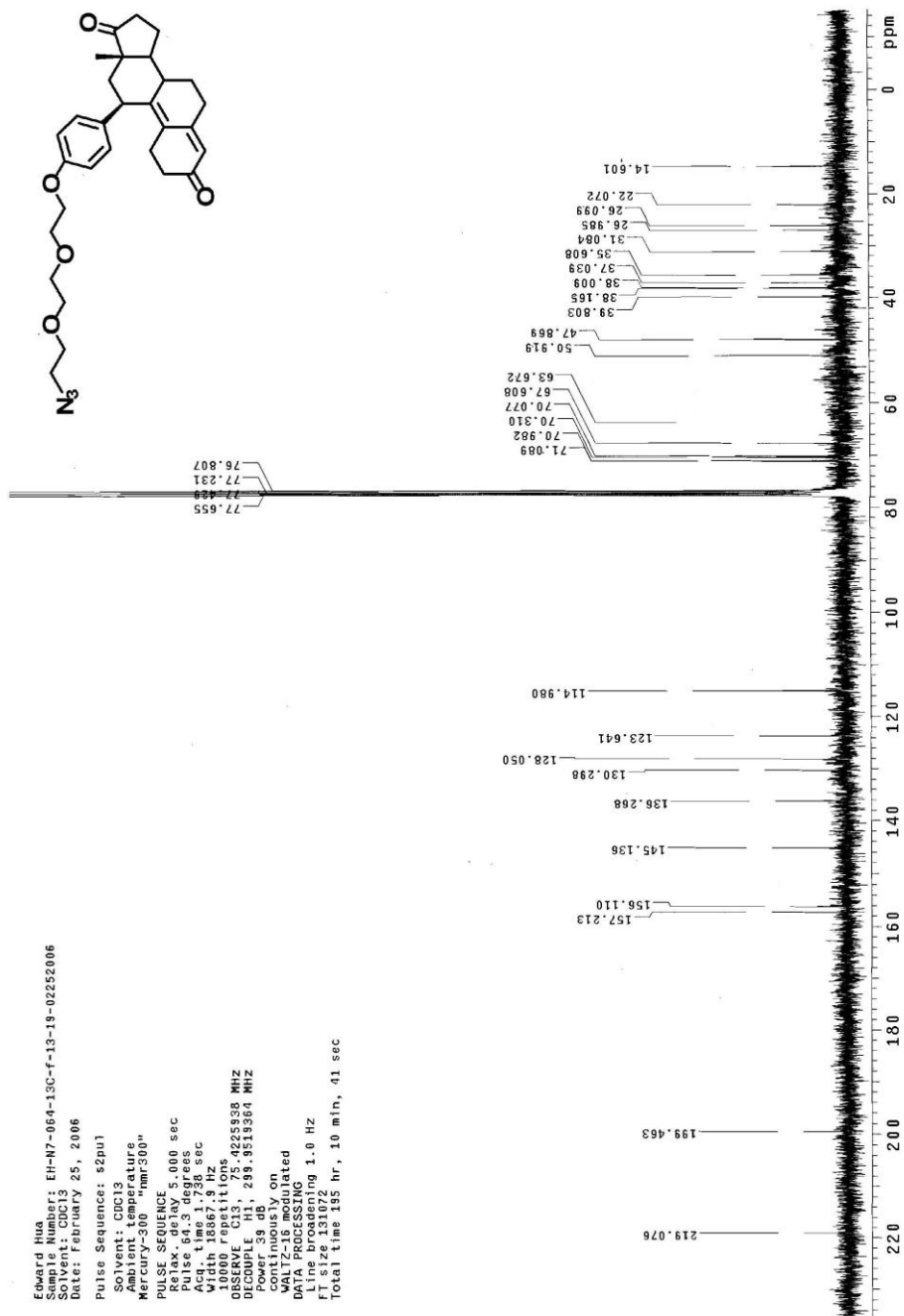


^1H NMR spectrum of compound 6 in CDCl_3

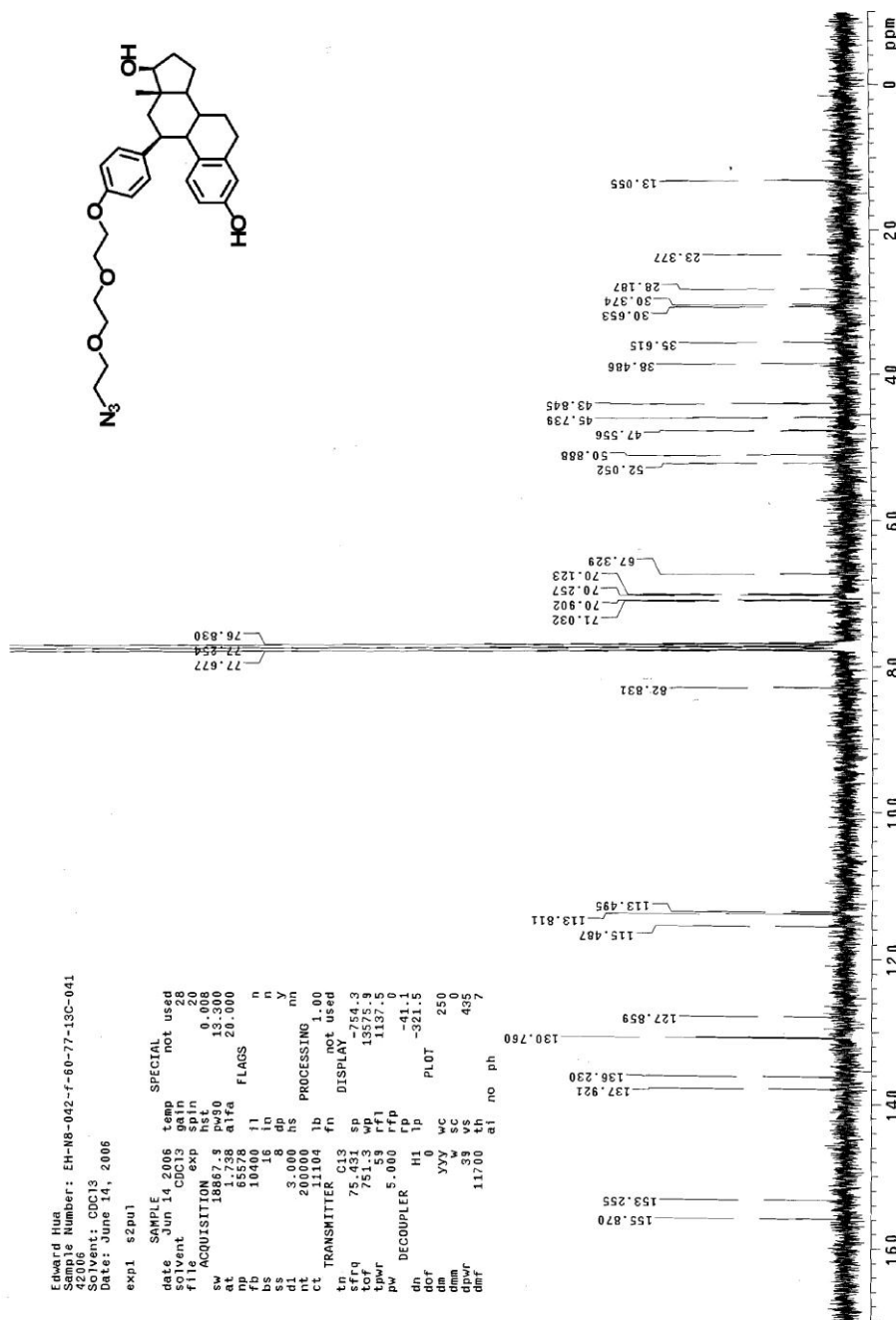


¹H NMR spectrum of intermediate in synthesis of 7, in CDCl₃ (2)

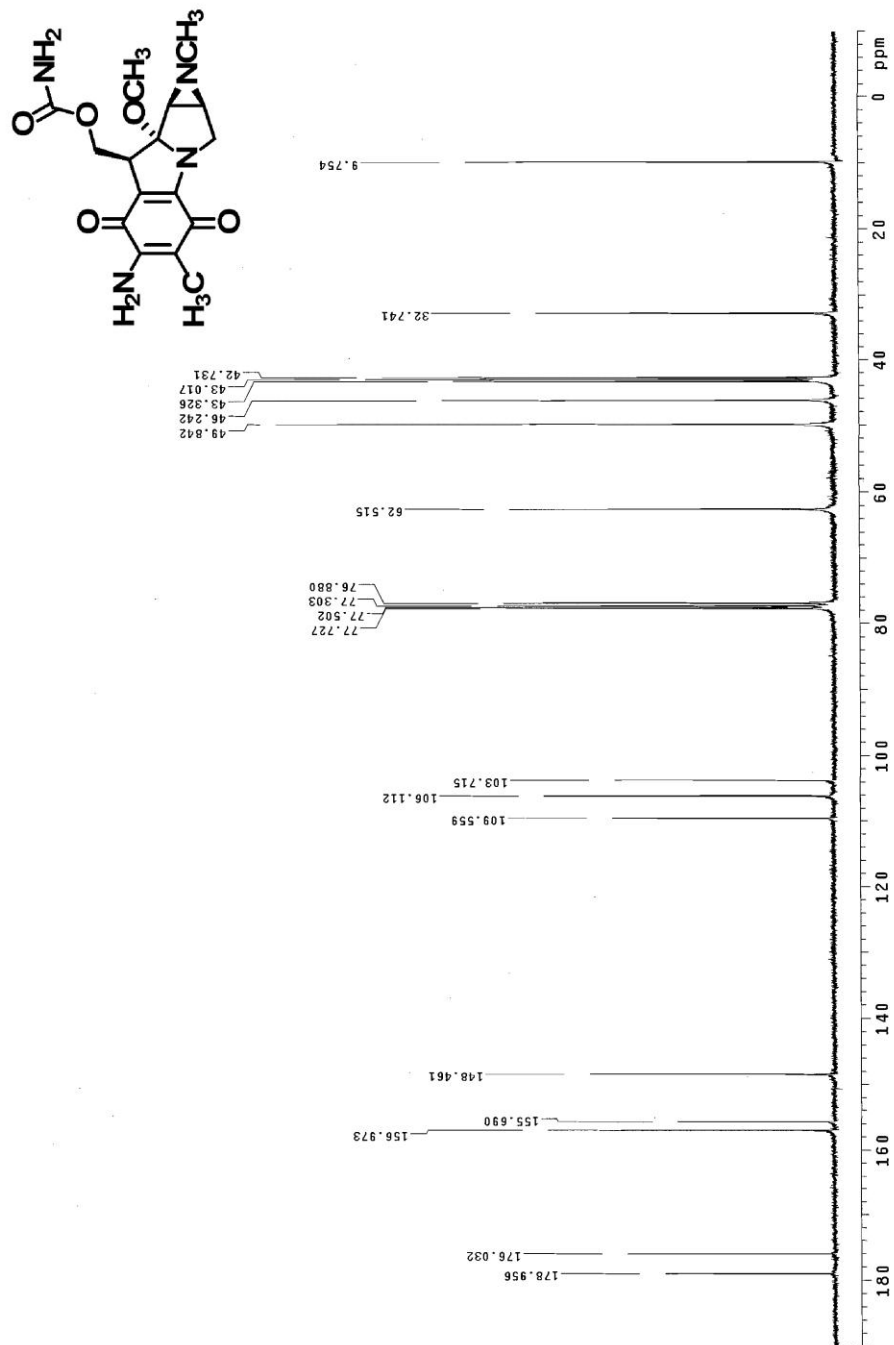




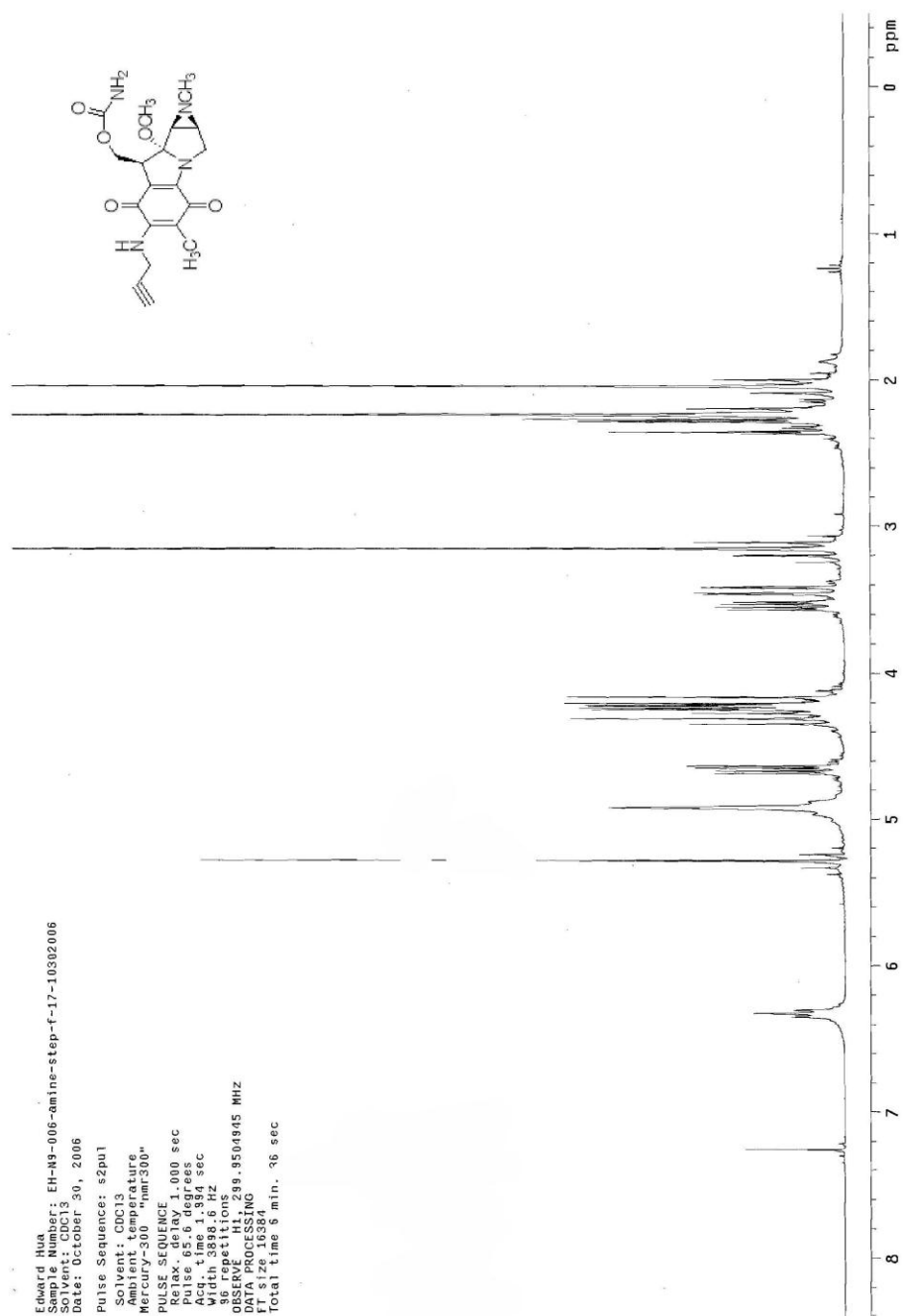
¹³C NMR spectrum of compound 7 in CDCl₃



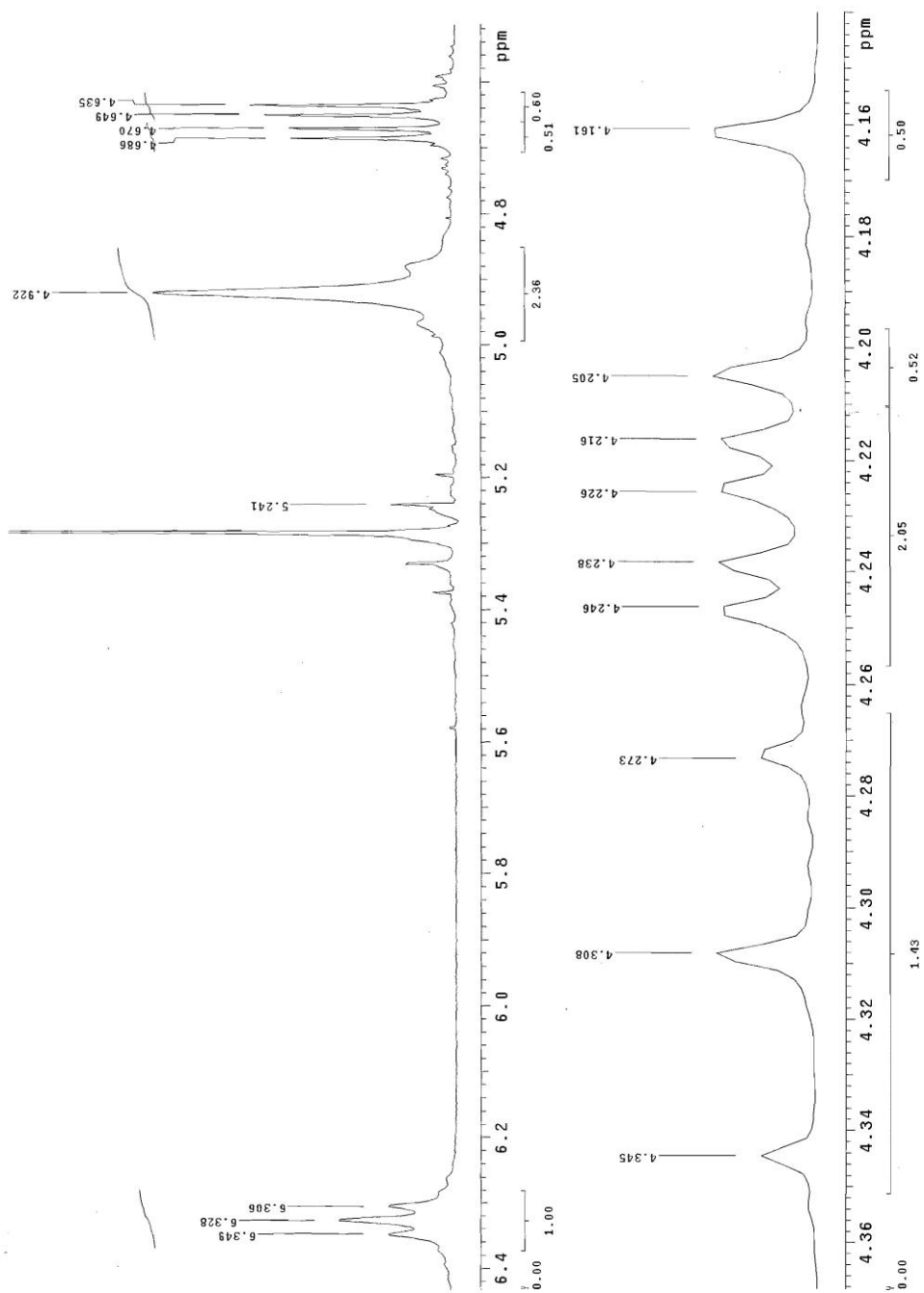
^1H NMR spectrum of compound 10 in CDCl_3



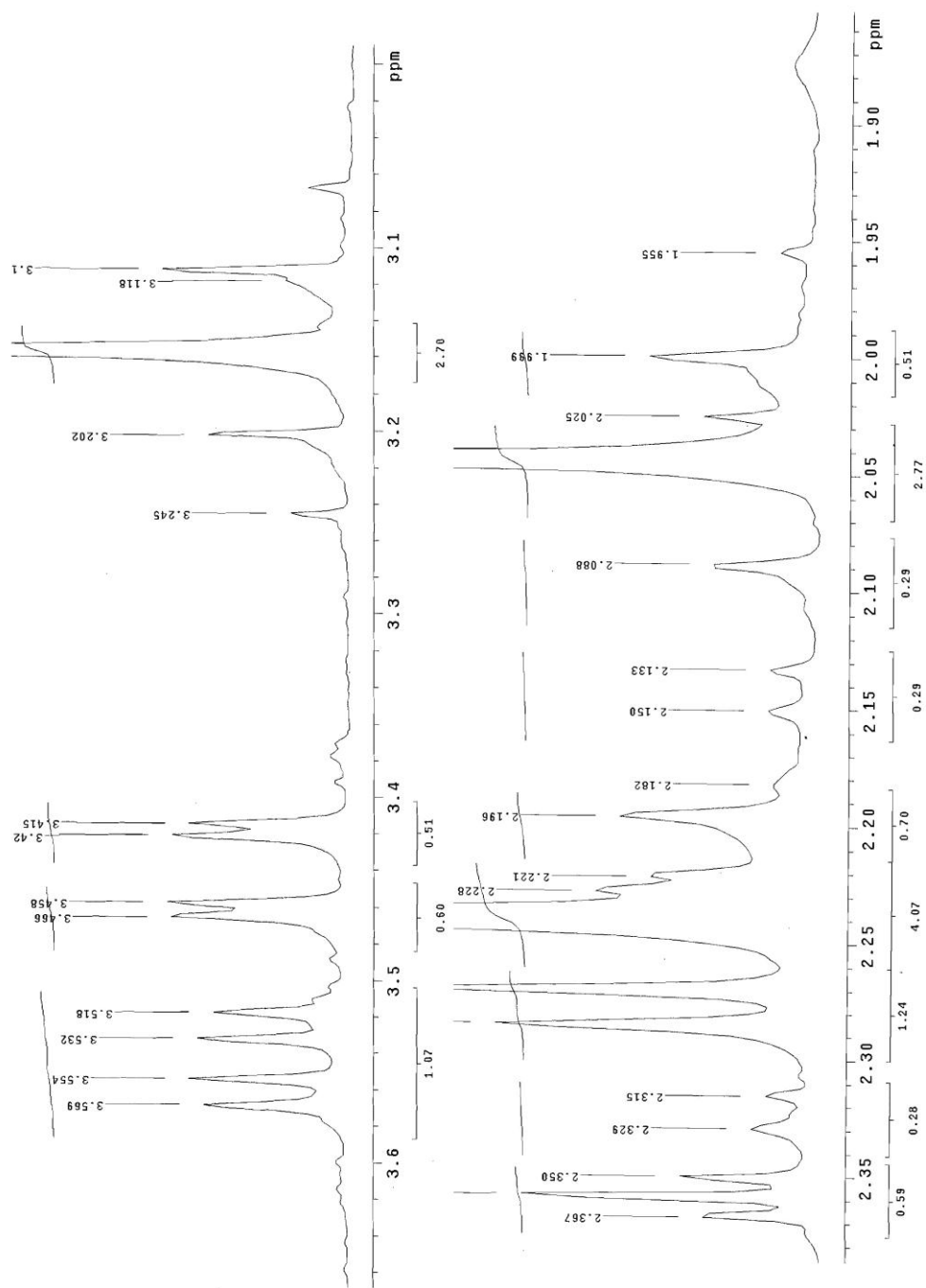
^{13}C NMR spectrum of compound 10 in CDCl_3



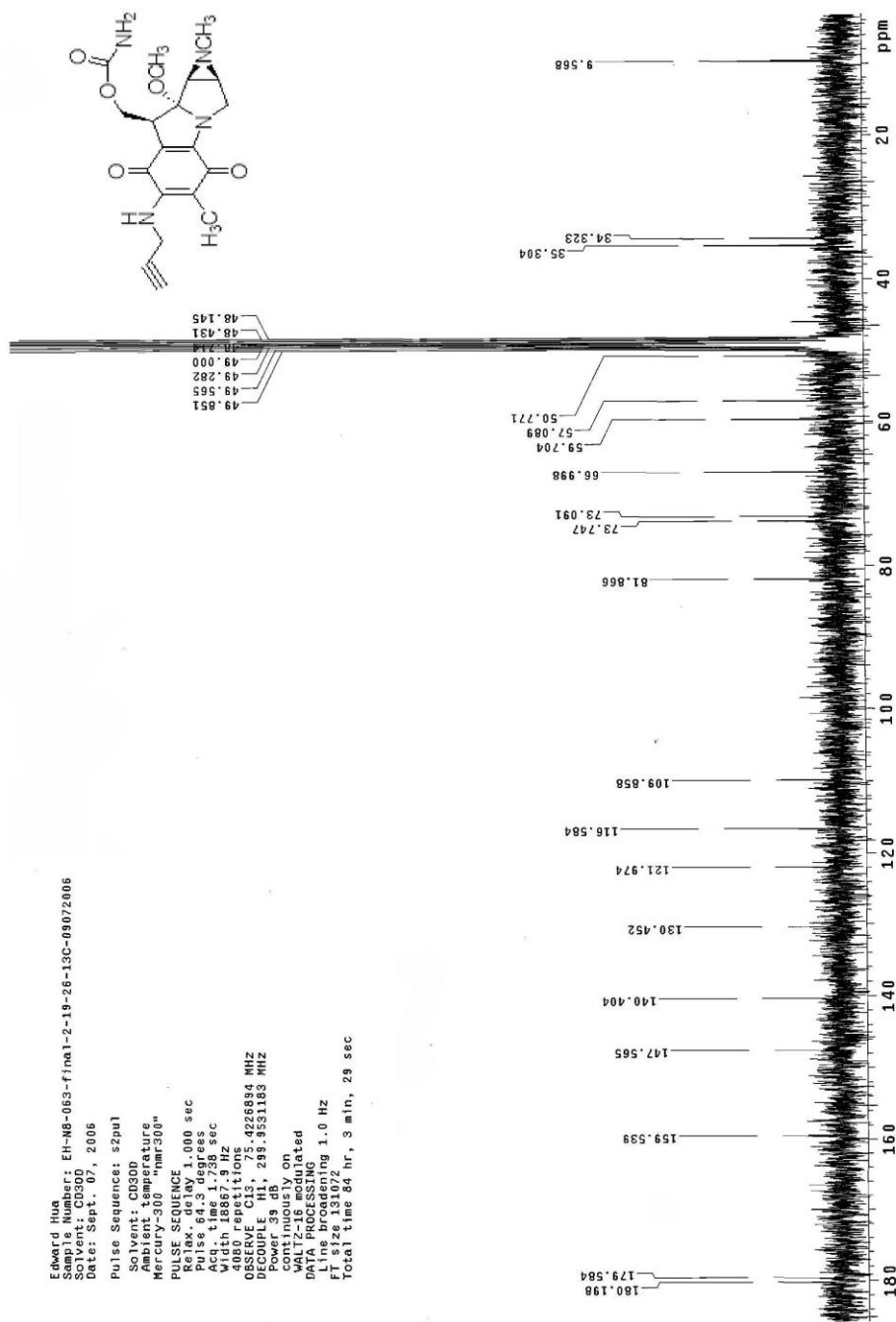
¹H NMR spectrum of compound 11 in methanol-*d*₄ (1)



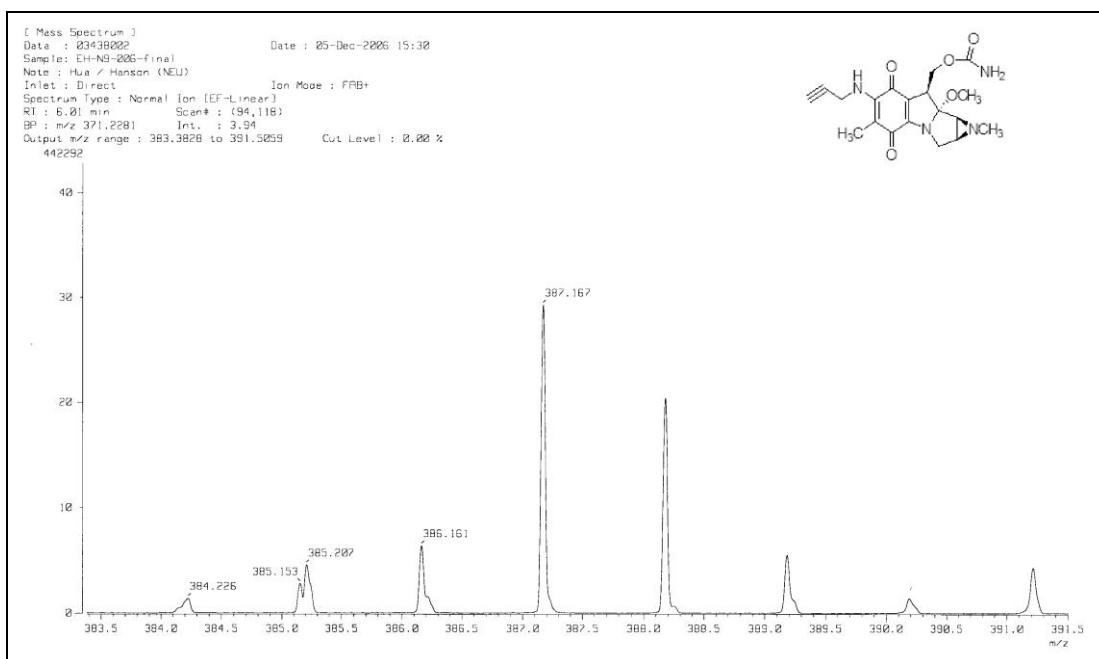
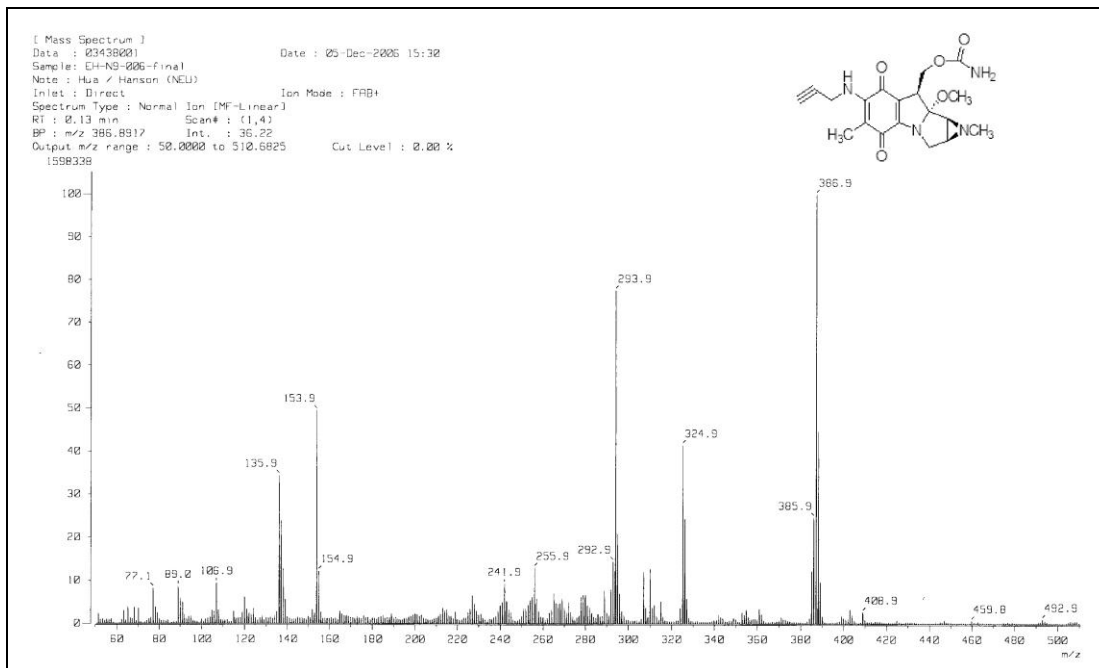
^1H NMR spectrum of compound 11 in methanol- d_4 (2)



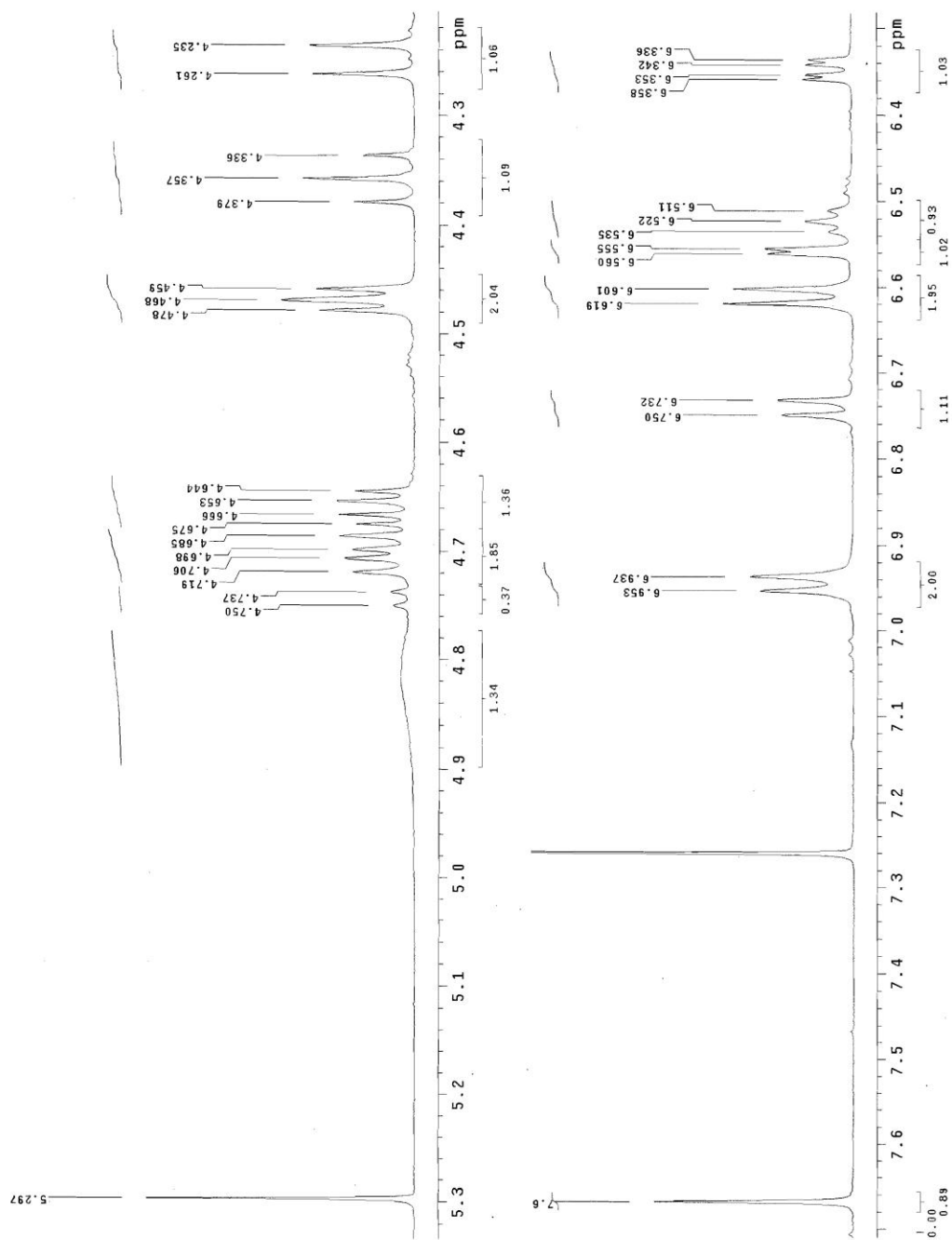
^1H NMR spectrum of compound 11 in methanol- d_4 (3)



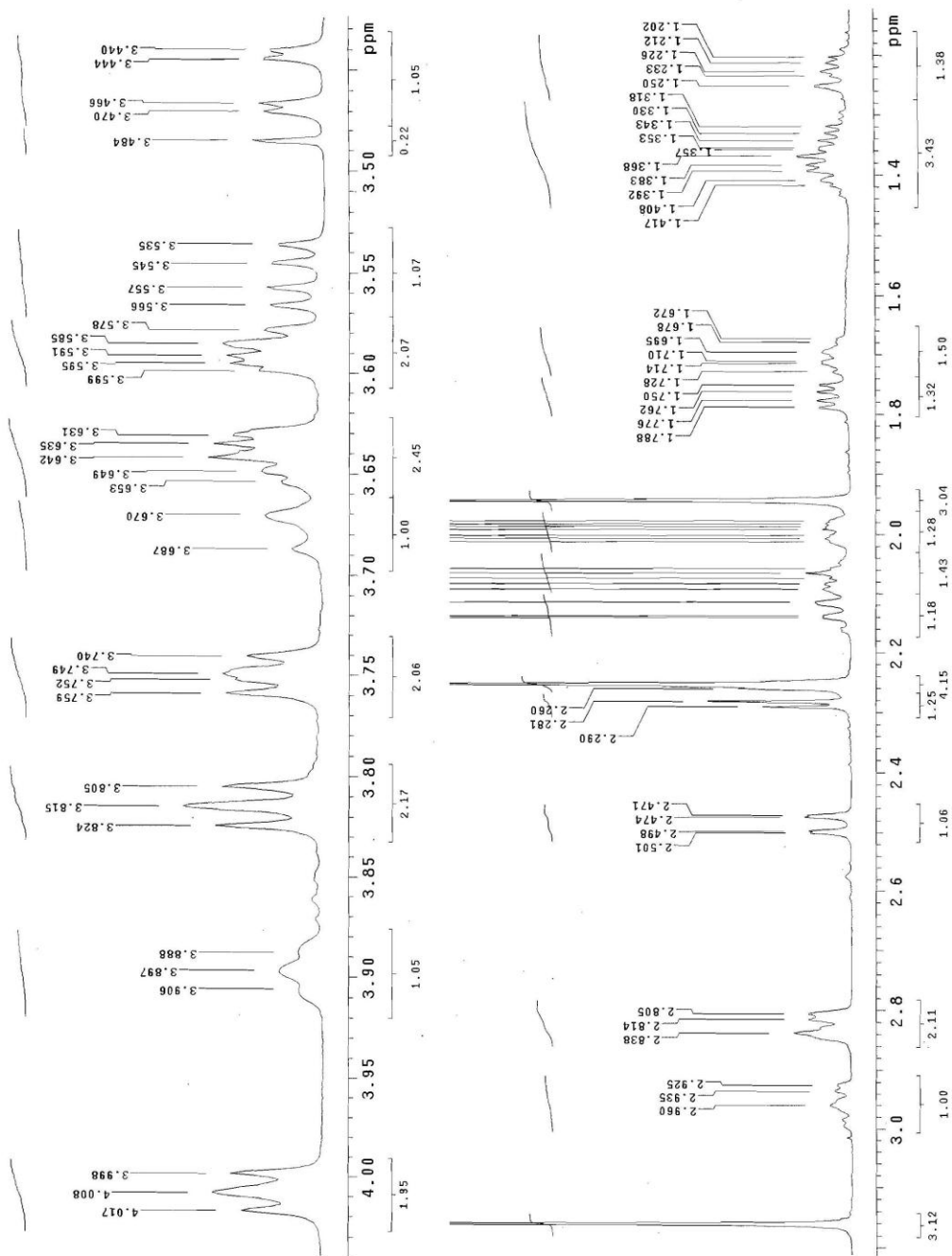
^{13}C NMR spectrum of compound **11** in methanol- d_4



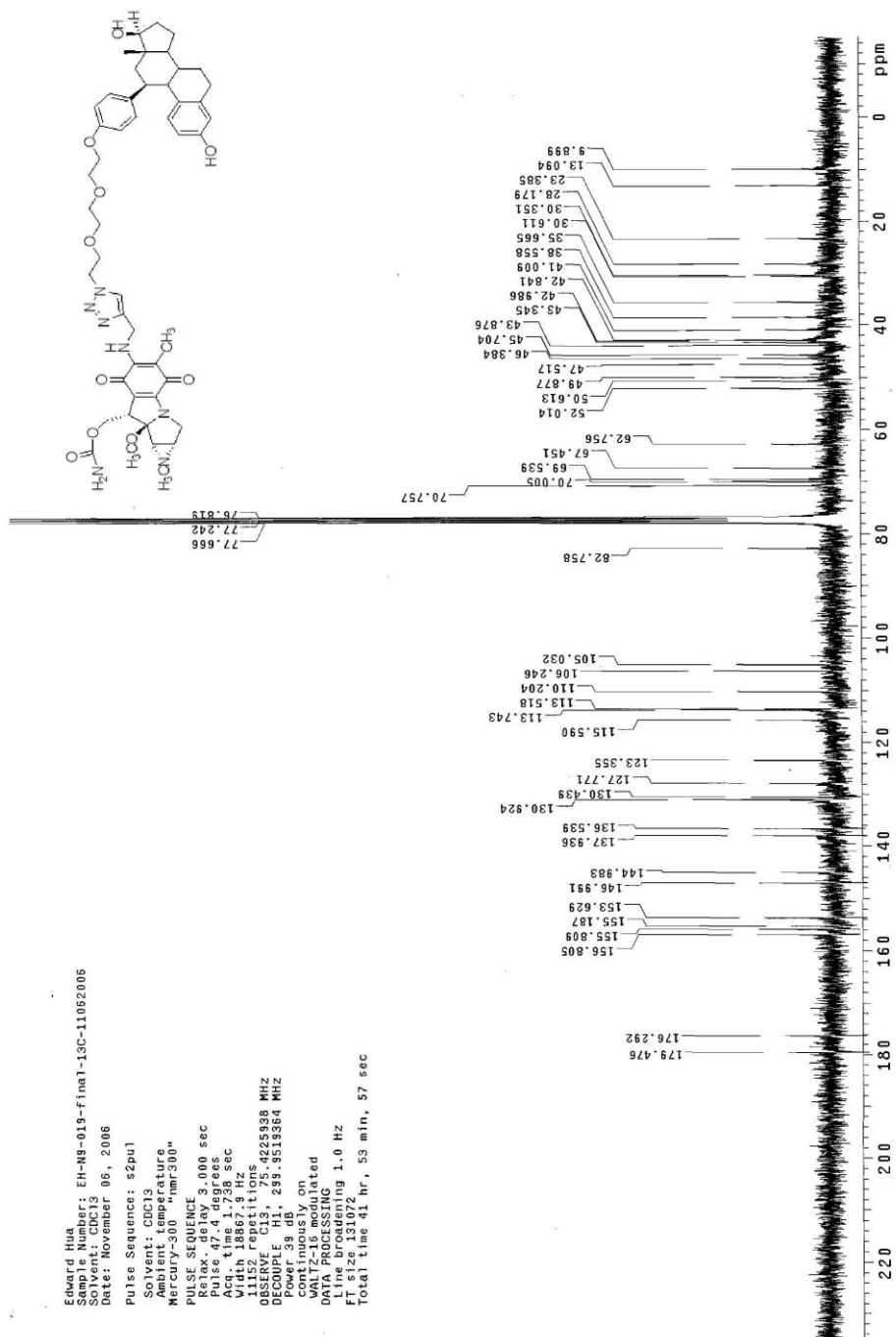
HRMS of compound 11



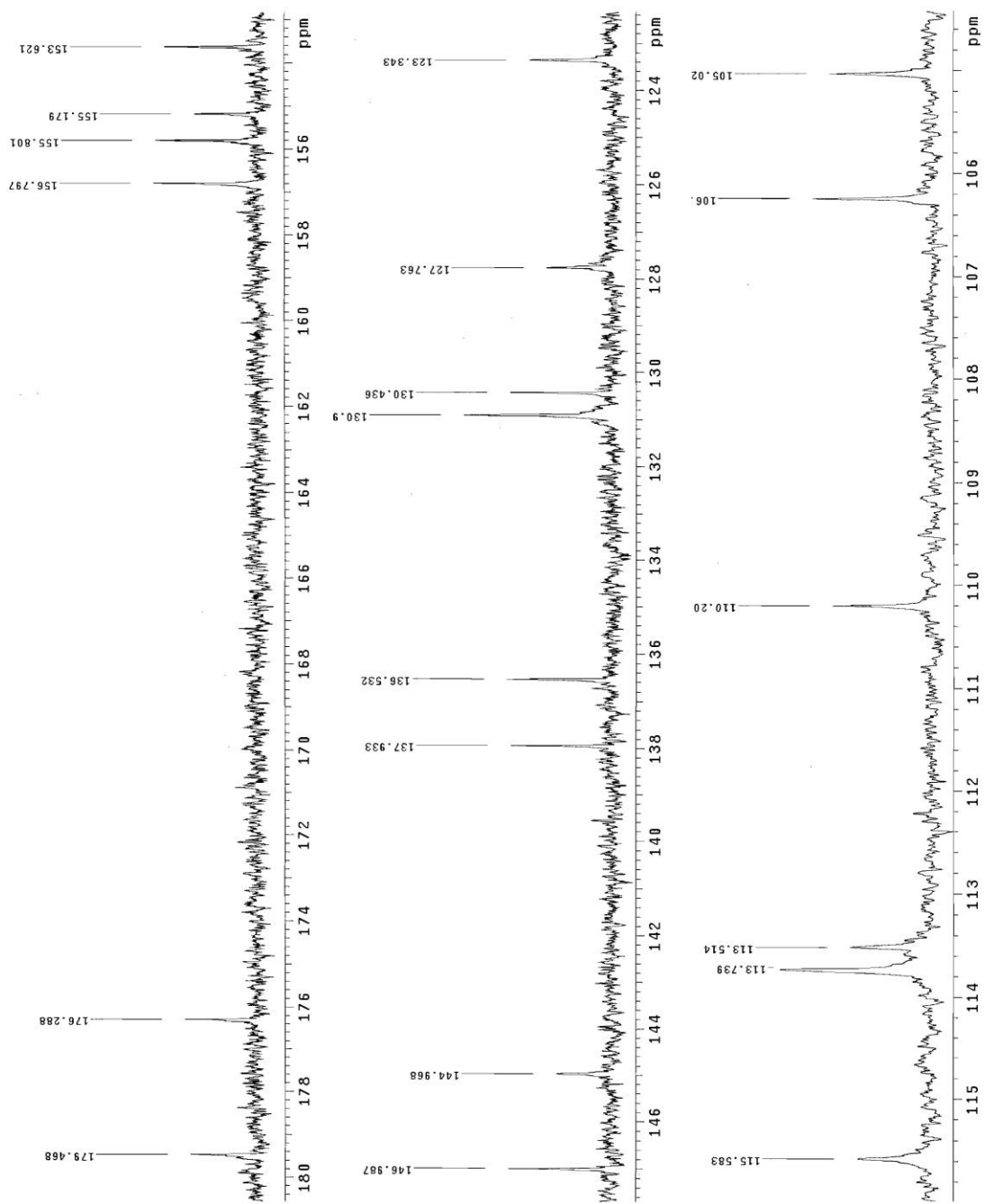
¹H NMR spectrum of compound 1 in CDCl₃



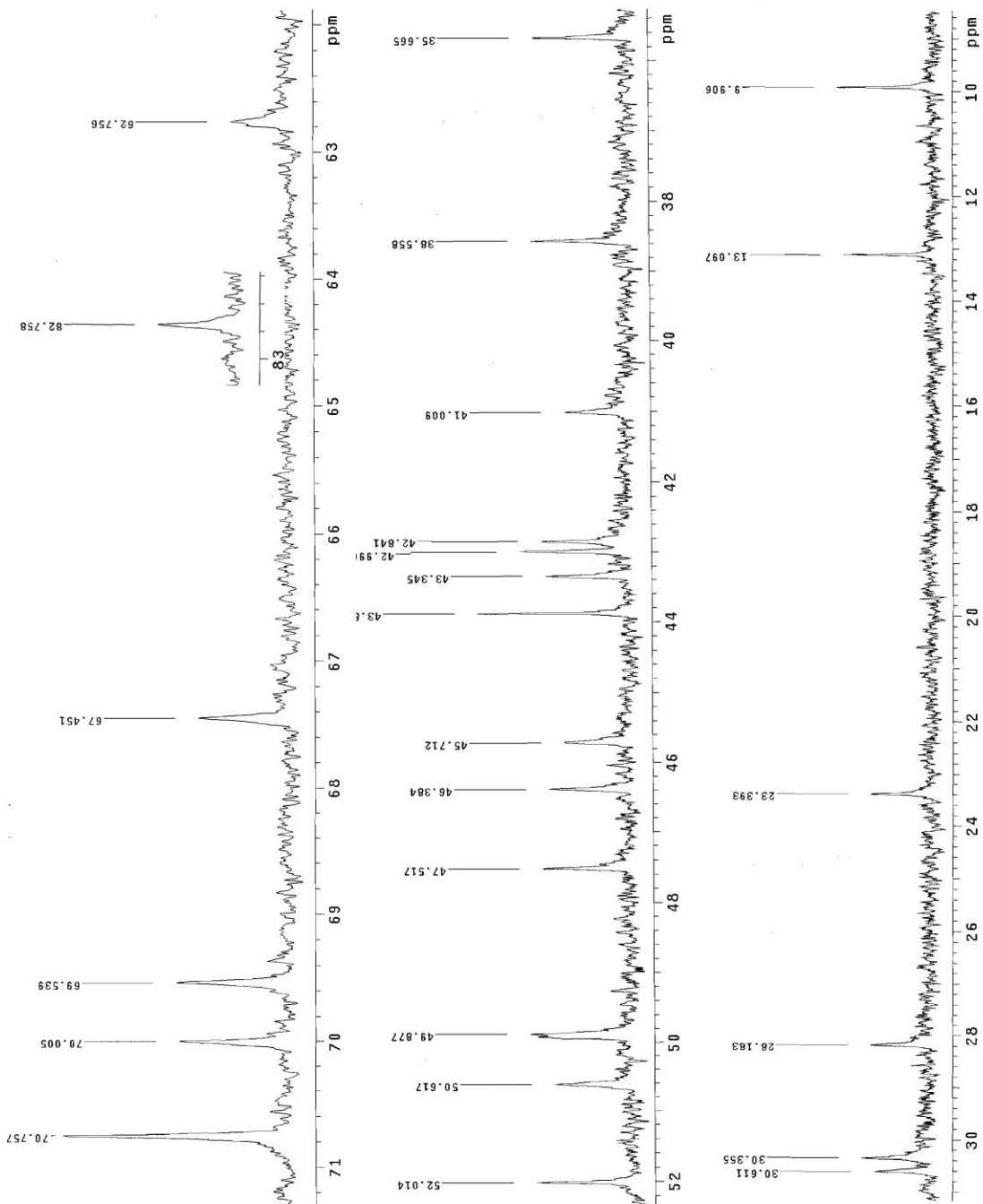
¹H NMR spectrum of compound 1 in CDCl₃



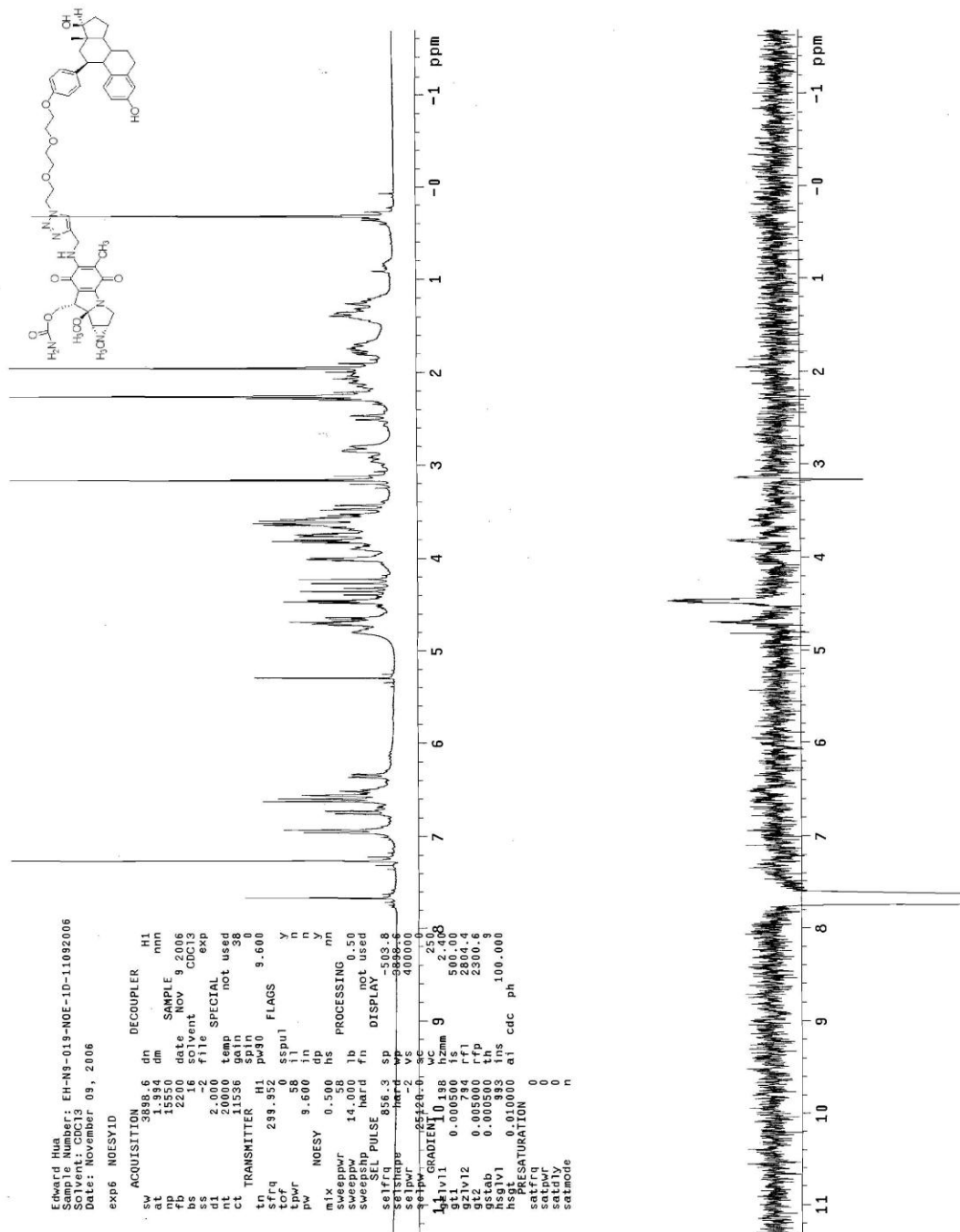
^{13}C NMR spectrum of compound 1 in CDCl_3



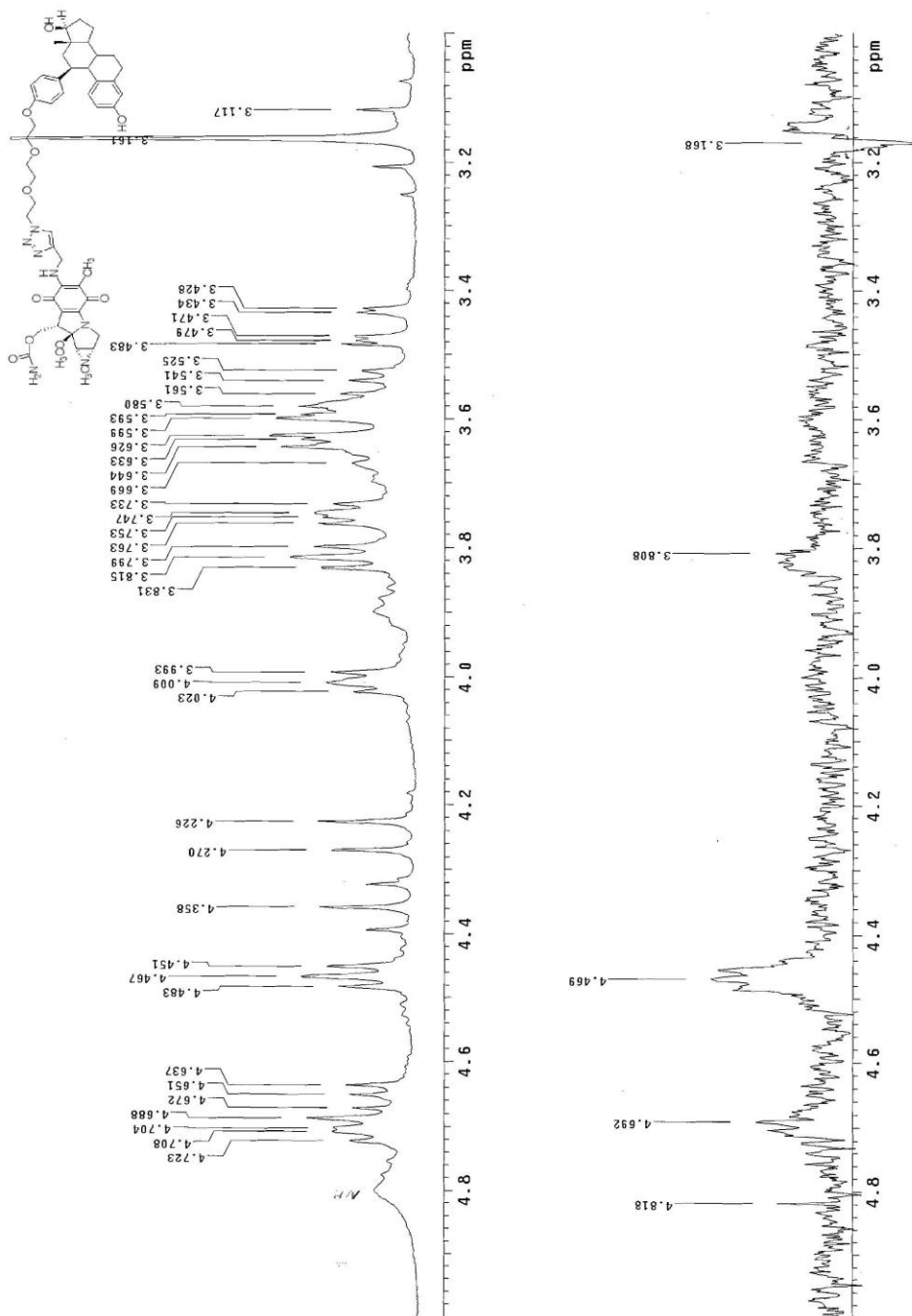
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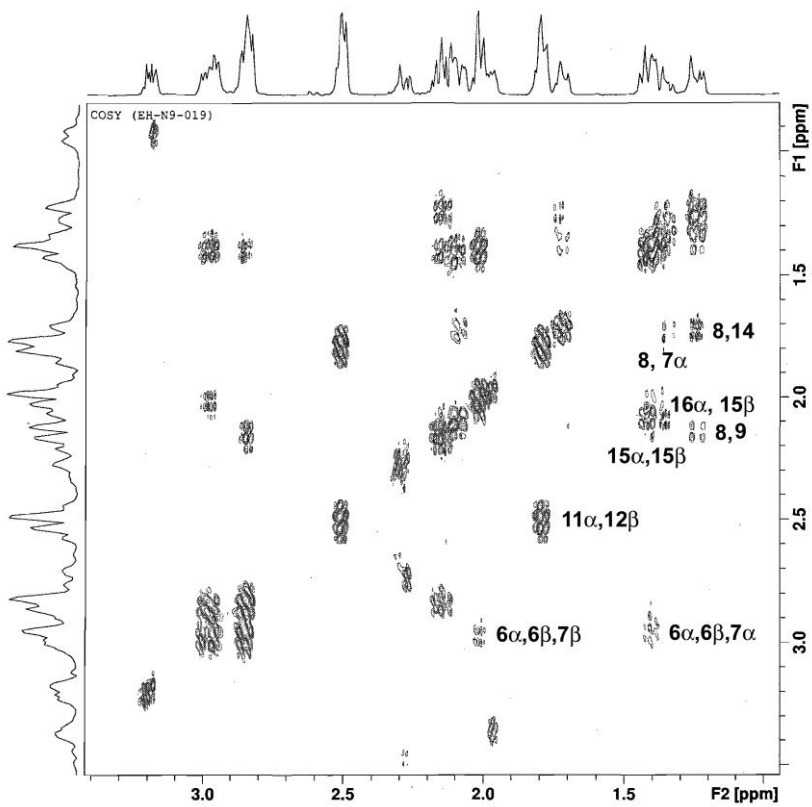
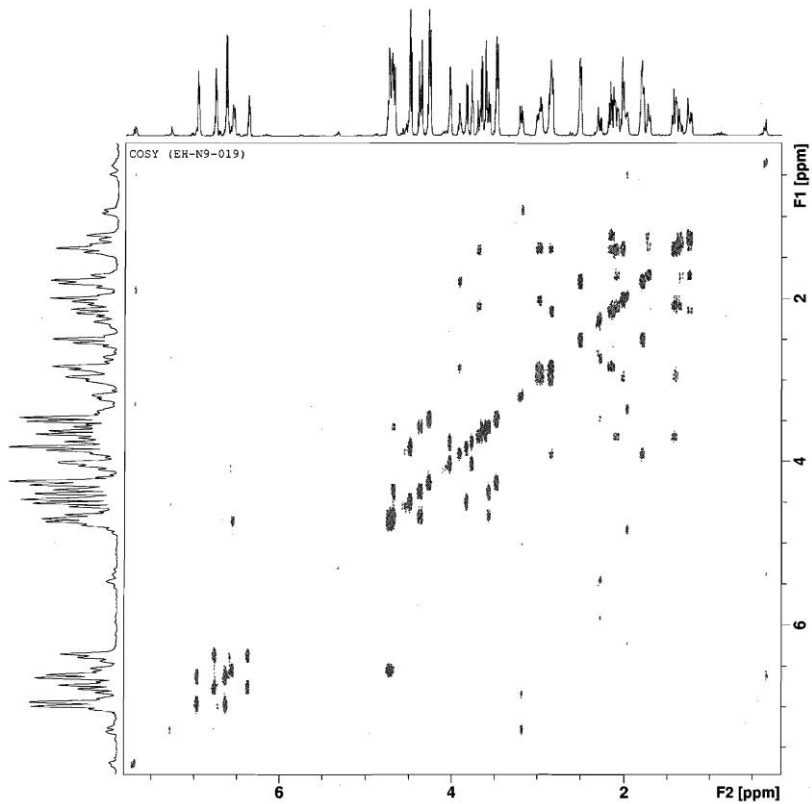
^{13}C NMR spectrum of compound 1 in CDCl_3



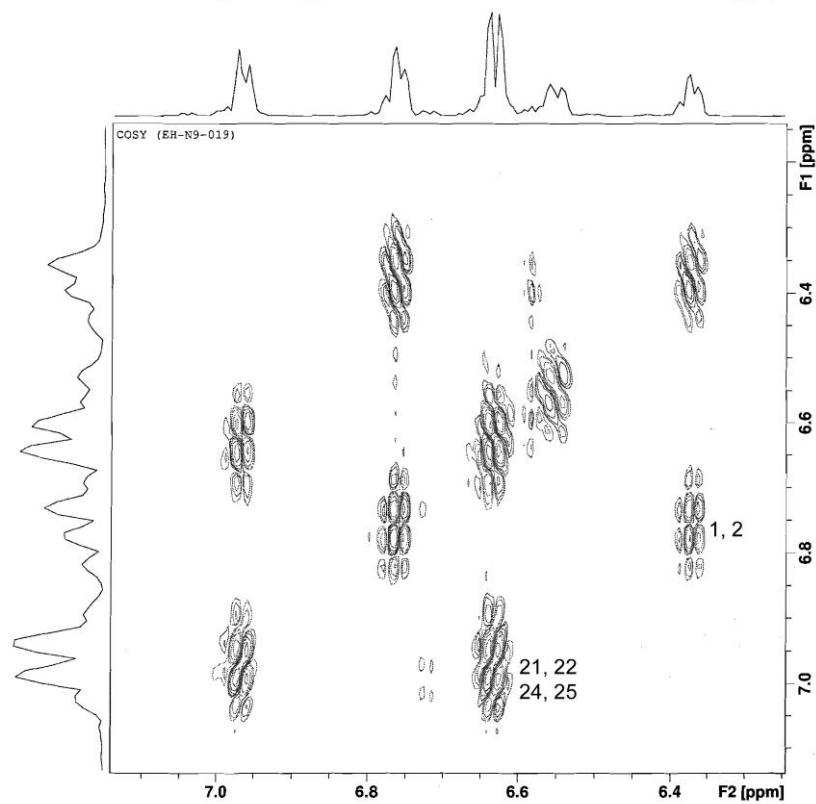
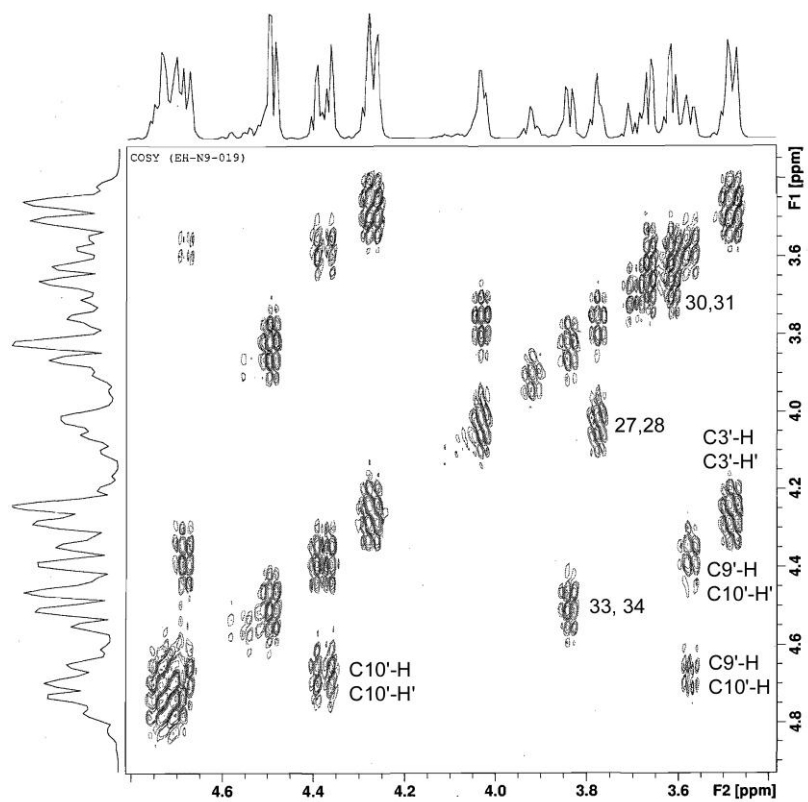
1D NOESY of compound 1 in CDCl₃



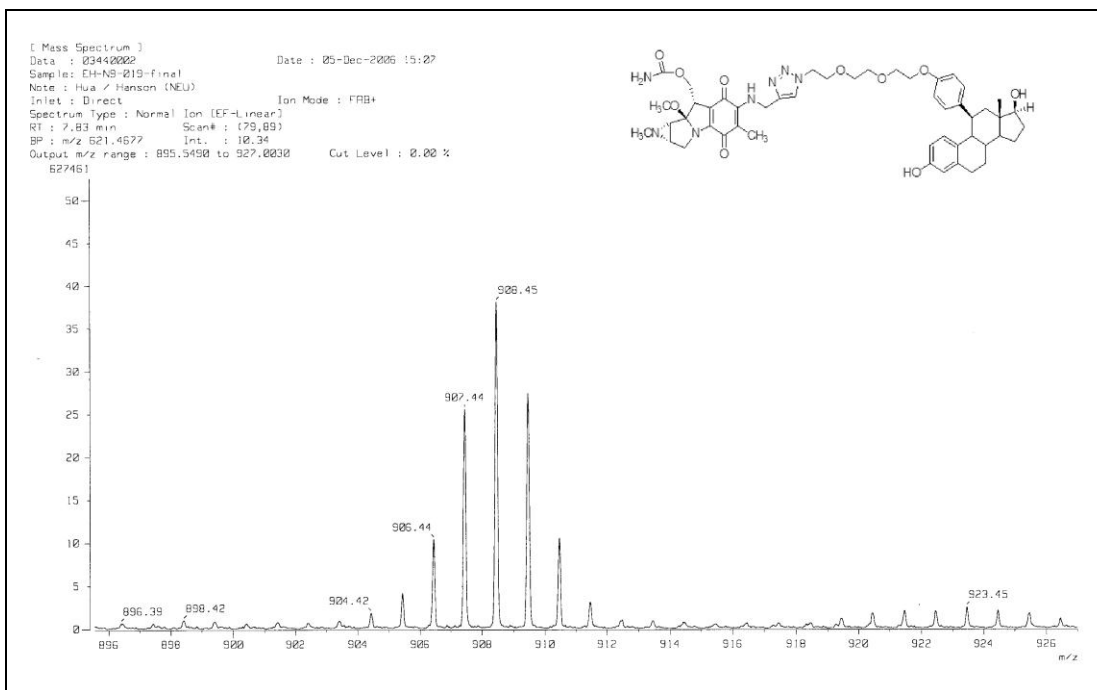
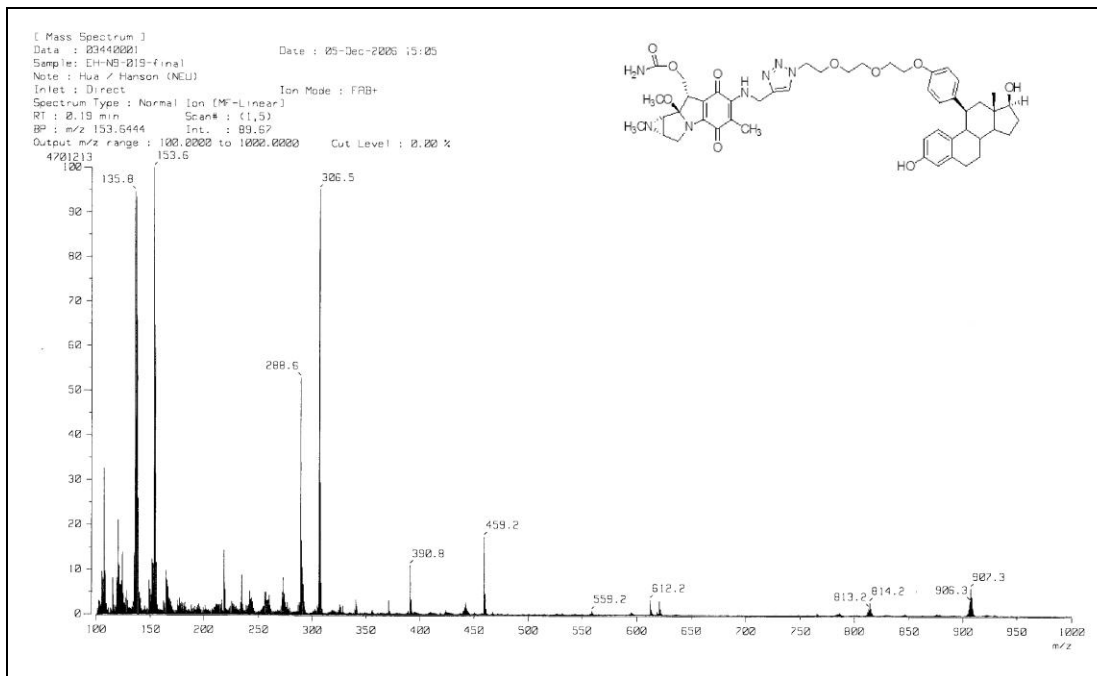
1D NOESY of compound 1 in CDCl₃



COSY of compound 1 in CDCl₃ (1)



COSY of compound **1** in CDCl₃ (2)



HRMS of compound **1**

