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Synthesis and anti HSV-1 evaluation of novel indole-3,4-diones

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General

Solvents and reagents were used as received from commercial sources. ^{1}H and ^{13}C-NMR spectra were obtained on a Varian 500MHz Instrument. Chemical shifts (δ) and coupling constants (J) are expressed in ppm and hertz (Hz) respectively. Mass spectrometric analyses and microchemical analyses were carried out on a 3200 QTRAP (Applied Biosystems SCIEX) and on a Carlo Erba EA 1102, respectively. Merck Kieselgel 60F254 plates were used for TLC, and Merck Silica gel 60 (0.063–0.100 mm) for column chromatography. Estimated purity of all compounds by combustion analysis was always at least 95%.

Synthesis and analytical data of compounds 6a-c.

Glacial acetic acid (100 mmol) and 6N HCl (180 mmol) were added to **5**[1] (3.5 mmol) and the reaction mixture was heated at reflux for 4 h. After cooling (0°C), a cold 10% solution of NaOH was slowly added to reach pH 7. The aqueous solution was quickly extracted with $CHCl_3$ (3x50 ml). After the solvent was removed, the crude product was purified by column chromatography on SiO_2 (CH_2Cl_2/MeOH 98:2) to afford a light yellow oil.

6a: Yield: 42%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.53 (d, *J*=7 Hz, 3H), 2.12-2.20 (m, 2H), 2.57-2.64 (m, 2H), 2.84-2.86 (m, 2H), 4.18 (q, *J*=7 Hz, 1H), 5.39 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.7, 25.9, 37.6, 38.6, 67.2, 107.5, 164.5, 196.0; ESI-MS: *m/z* $[M+H]^+$ calcd=166.0, obsd= 166.2; Anal. Calcd for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.55; H, 6.79; N, 8.56.

6b: Yield: 45%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.11-1.15 (m, 3H), 1.52 (d, *J*=7.2 Hz, 3H), 2.20-2.30 (m, 2H), 2.40- 2.49 (m, 1H), 2.62-2.67 (m, 1H), 2.95-3.01 (m, 1H), 4.19 (q, *J*=7.2 Hz, 1H), 5.38 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 10.1, 20.0, 22.6, 32.6, 40.4, 64.6103.6, 103.8, 167.3, 197.1; ESI-MS: *m/z* $[M+H]^+$ calcd=180.1, obsd= 180.2; Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.25; H, 7.39; N, 7.76.

6c: Yield: 50%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.08 (s, 3H), 1.11 (s, 3H), 1.51 (d, *J*=7 Hz, 3H), 2.40-2.45 (m, 2H), 2.65 (s, 2H), 4.18 (q, *J*=7 Hz, 1H), 5.40 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.9, 27.5, 27.8, 31.3, 38.2, 45.9, 59.3, 107.1, 165.5, 201.0; ESI-MS: *m/z* $[M+H]^+$ calcd=194.1 obsd= 194.2; Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.25; H, 7.97; N, 7.36.

Biological Materials and Methods

*Cells and viruses***.** Vero cells (American Type Culture Collection) were propagated in Dulbecco's modified Eagle's medium supplemented with 6% FBS. HSV-1 (F) is a limited-passage prototype HSV-1 used in our laboratories. Virus stocks were titered on Vero cells and stored in aliquots at -80°C.

Cytotoxicity test (Trypan Blue dye exclusion test). The cells were incubated overnight with culture medium containing different concentrations of compounds (DMSO solutions: 1, 10, 100 μM; water solutions: 40, 80, 100 μM) for 12 h at 37°C. The medium was then removed; the cells were then trypsinized and a solution of Trypan Blue was added. The cells were counted in a Burker chamber visualized at 20x magnification with a light microscope (Leica Microsystem GmbH type 090-135.001). The percentage of death cells was calculated as follows: % surviving cells = (Total n° of white cells /Total n° of cells counted) x 100**.** The assays were performed in triplicate for each dilution.

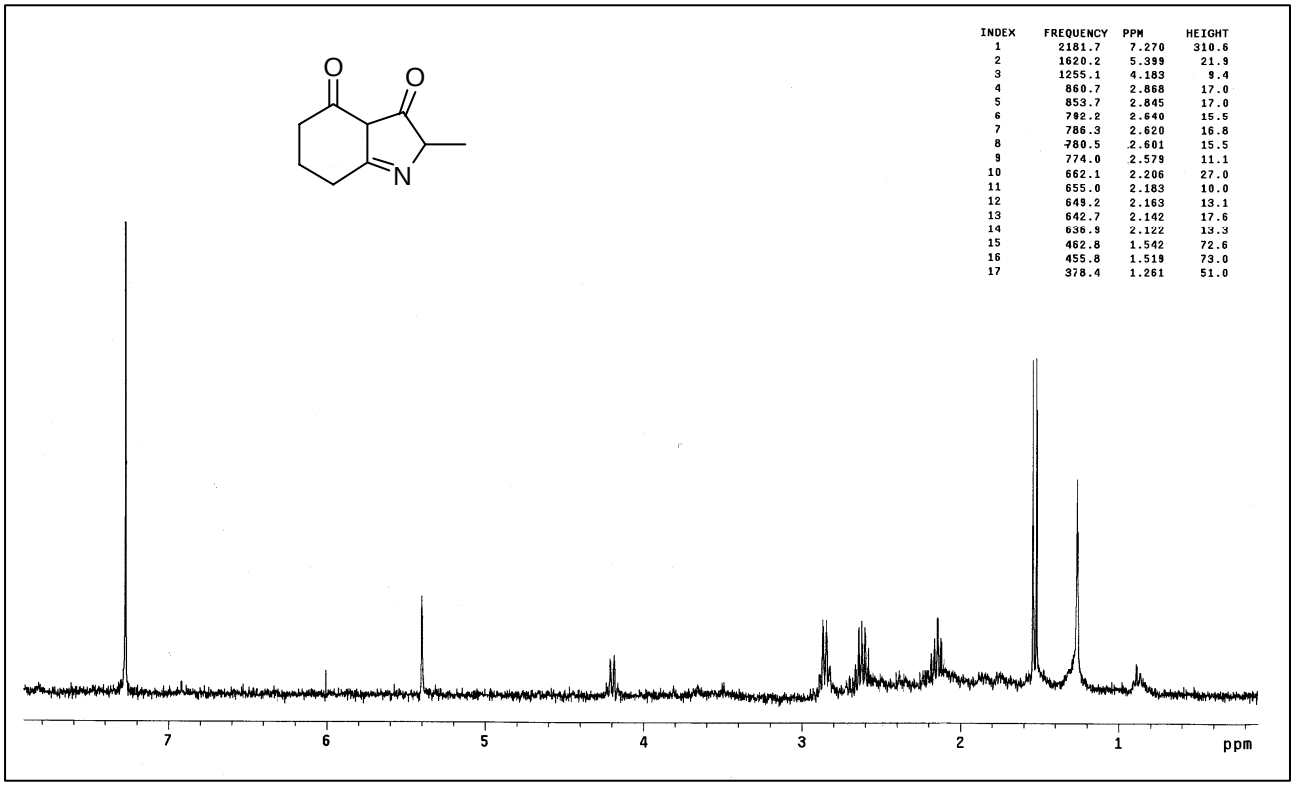
Plaque reduction assay. The virus was diluted to yield 50 plaques/250 μl. All the volumes were inoculated on monolayers of Vero cells in 6-well dishes and incubated for 1 h at 37°C. After the incubation time, the inoculum was removed and cell monolayers were covered with Dulbecco's modified Eagle's medium containing 0.3% methylcellulose and different concentrations of compounds. After 3 days of incubation at 37°C, the medium was aspirated from the wells, and the cells were then fixed, stained with crystal violet, and visualized at 20x magnification with an inverted microscope for plaque detection.

Materials. In all the spectroscopic experiments H_2O is for injection from Galenica Senese (Siena, Italy), CH_2Cl_2 is HPLC grade from Merck (Darmstadt,Germany). Dulbecco's phosphate buffered salt solutions (PBS, 1 mM at pH=7.4) from Pan Biotech GMbH (Aidenbach, Germany) was prepared in H_2O and filtered through 0.22 mm Millipore1 GSWP filters (Bedford, USA). SC6PEG was synthesized according to the general procedure.[2] In this way, highly polydispersed products can be obtained exhibiting ethylene oxide unit (EO) average number ranging between 20 and 40 EO. In this stage a product with a maximum average number of EO at 3185 m/z (M_{30EO}–Na+) was mainly obtained, as confirmed by MALDI spectra**.**

Sample preparation. Nanoaggregate dispersions of SC6PEG and **6b**/SC6PEG systems were prepared by following the conventional procedure used for liposomes.[3] An amount of SC6PEG in CH_2Cl_2 was added to solutions of **6b** in CH_2Cl_2. The organic solutions were slowly evaporated overnight and hydrated with PBS for about 15 min, sonicated for 20 min at 40 °C and analyzed. The solutions of the complexes contain respectively SC6PEG in the range of concentration between 2.5 and 40 μM and a fixed amount of **6b** ([6b]= 50 μM). A solution of **6b** free at 50 μM was also prepared for comparison by hydration of an organic film in the same conditions used for complexes.

UV/Vis Measurements. Absorption spectra were obtained with a Hewlett-Packard HP 8453 diode array spectrophotometer. Ten millimeter rectangular quartz cells (Hellma, Milano, Italy) were employed in the 200–450nm spectral range. The extinction coefficient for free molecule was measured both in H_2O (ε_{292nm} =6250 ± 10 cm^{-1} M^{-1}) and in CH_2Cl_2 (ε_{263nm} = 16240 ±130 cm^{-1} M^{-1}) by dilution of **6b** stock solutions in the range 80-10 μM. All measurements were run at least three times at 25.0 °C.

Compound 6a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6 792.2 2.640 15.5
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9 774.0 2.579 11.1
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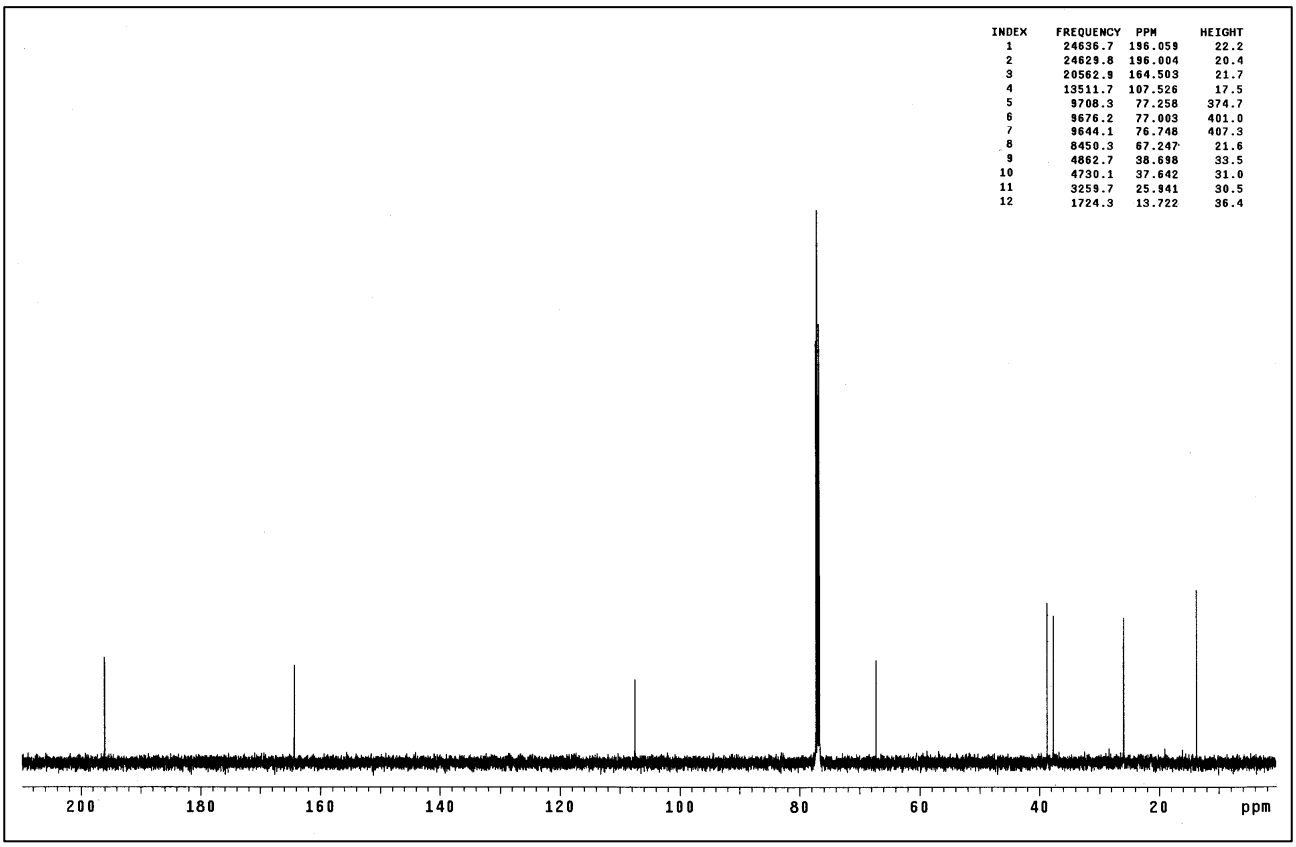
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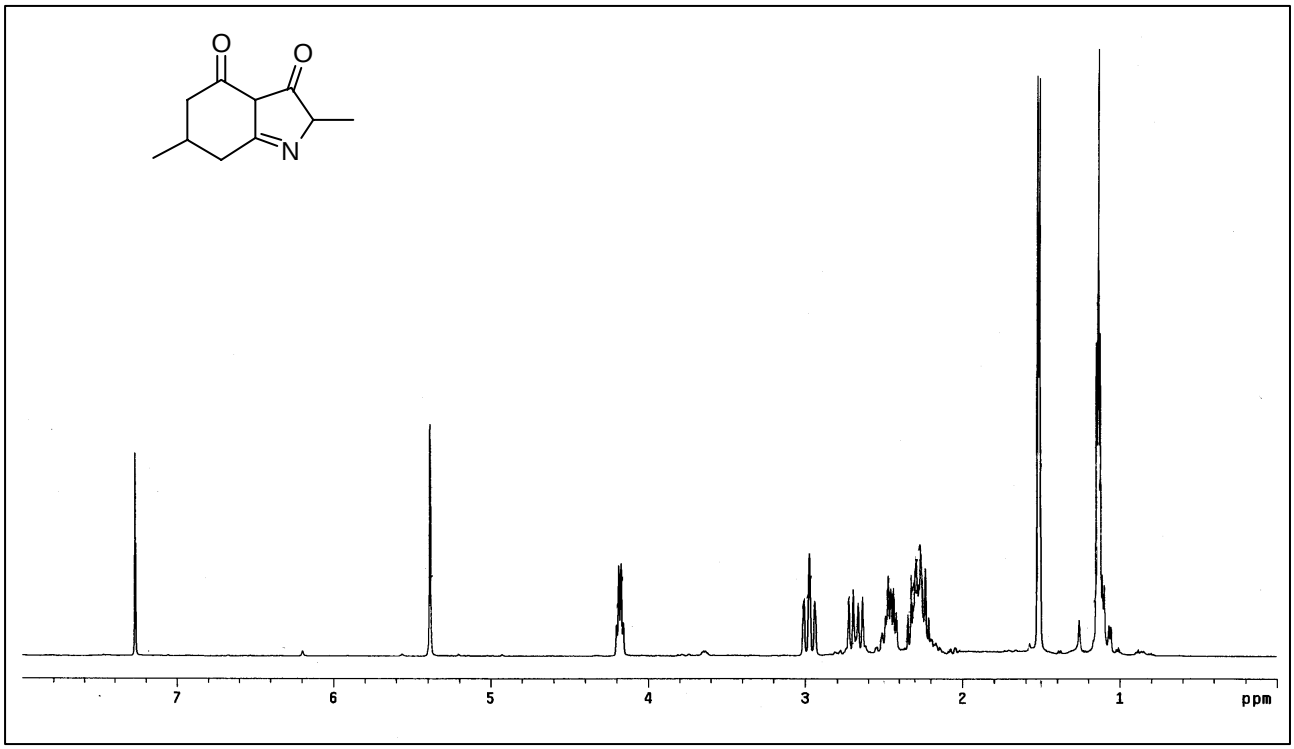
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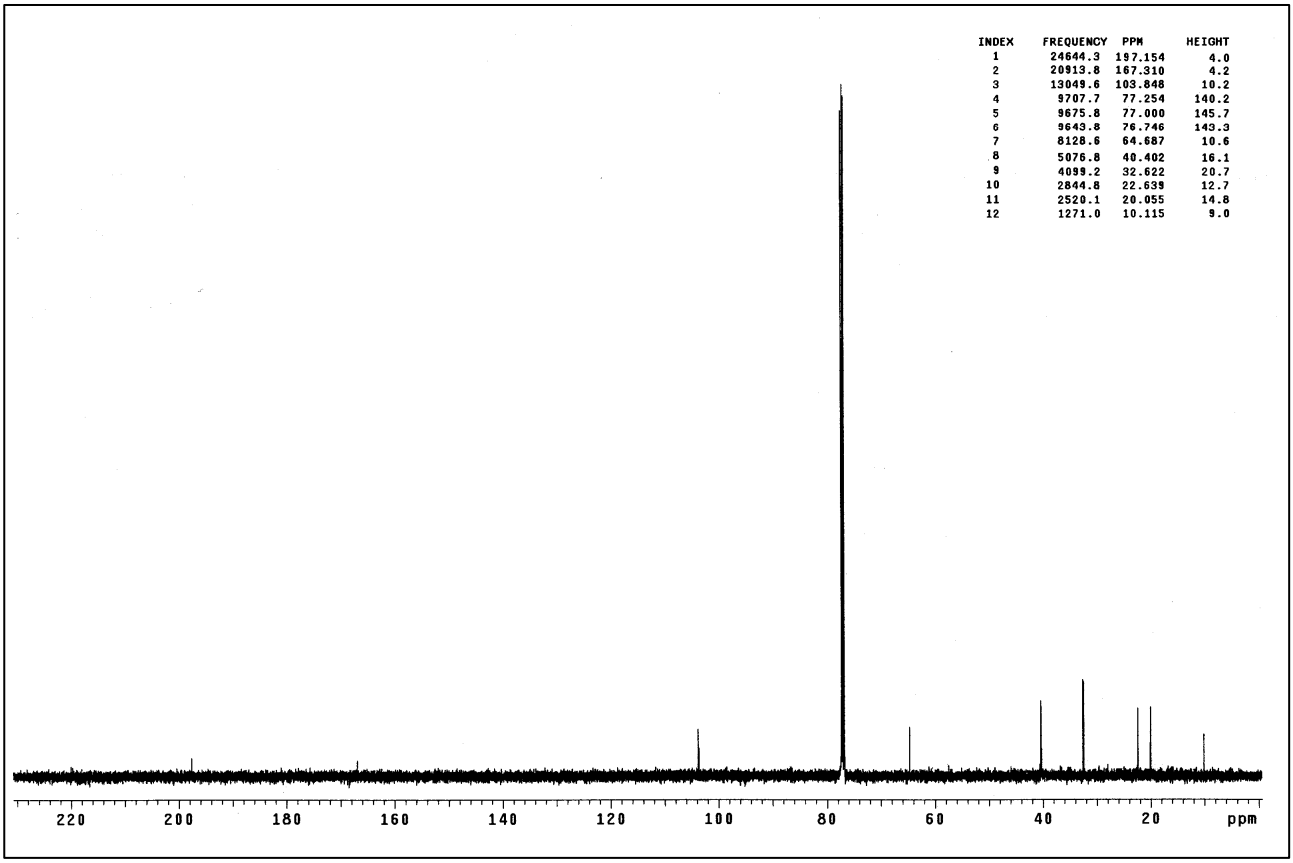
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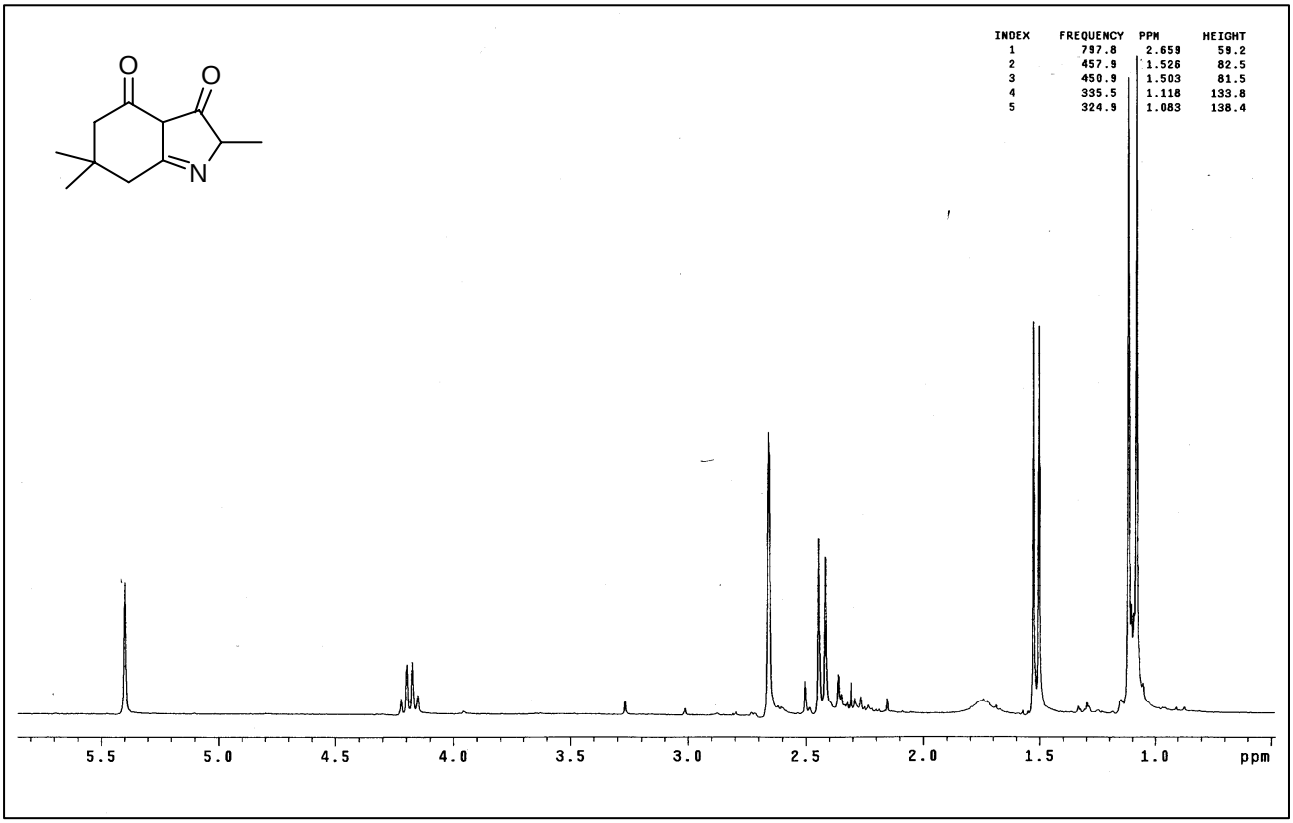
Compound 6c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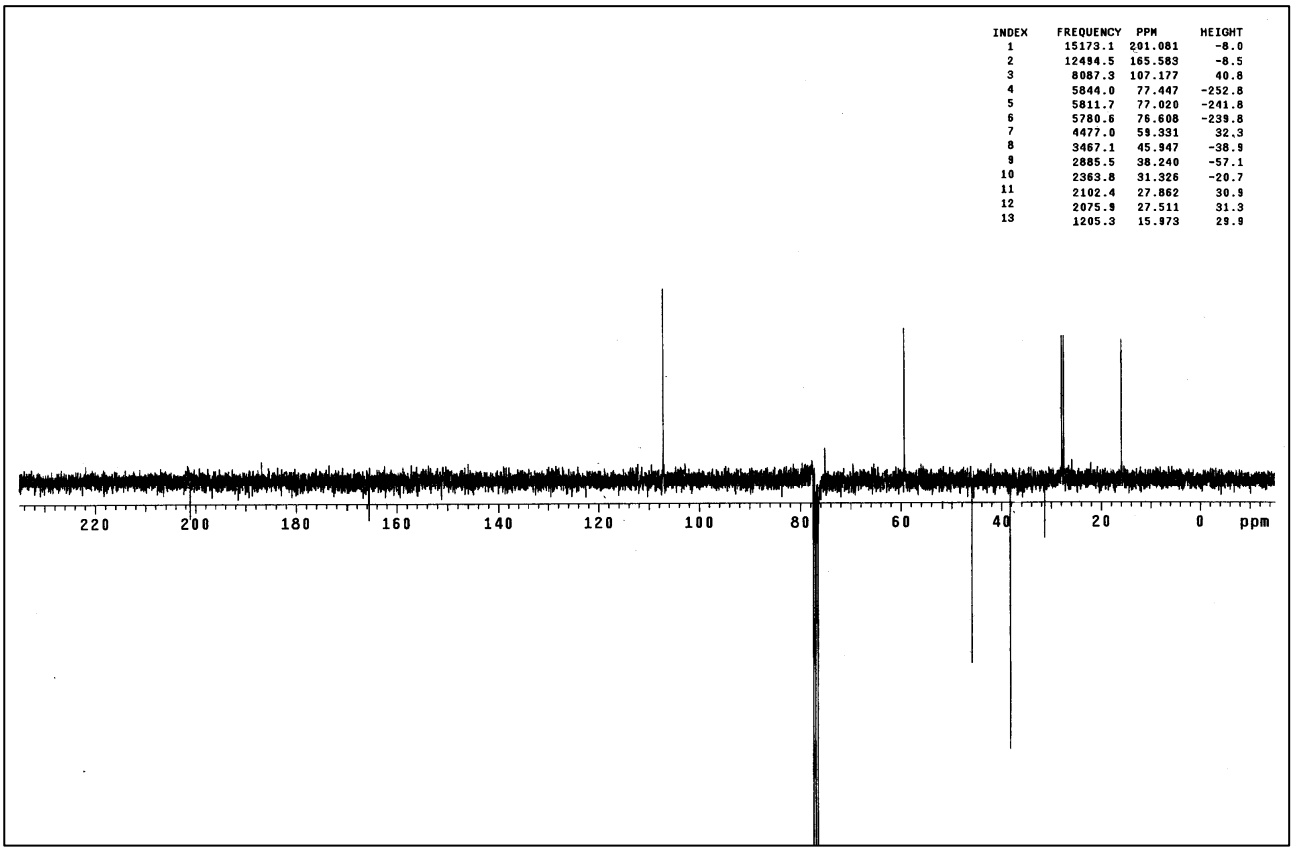
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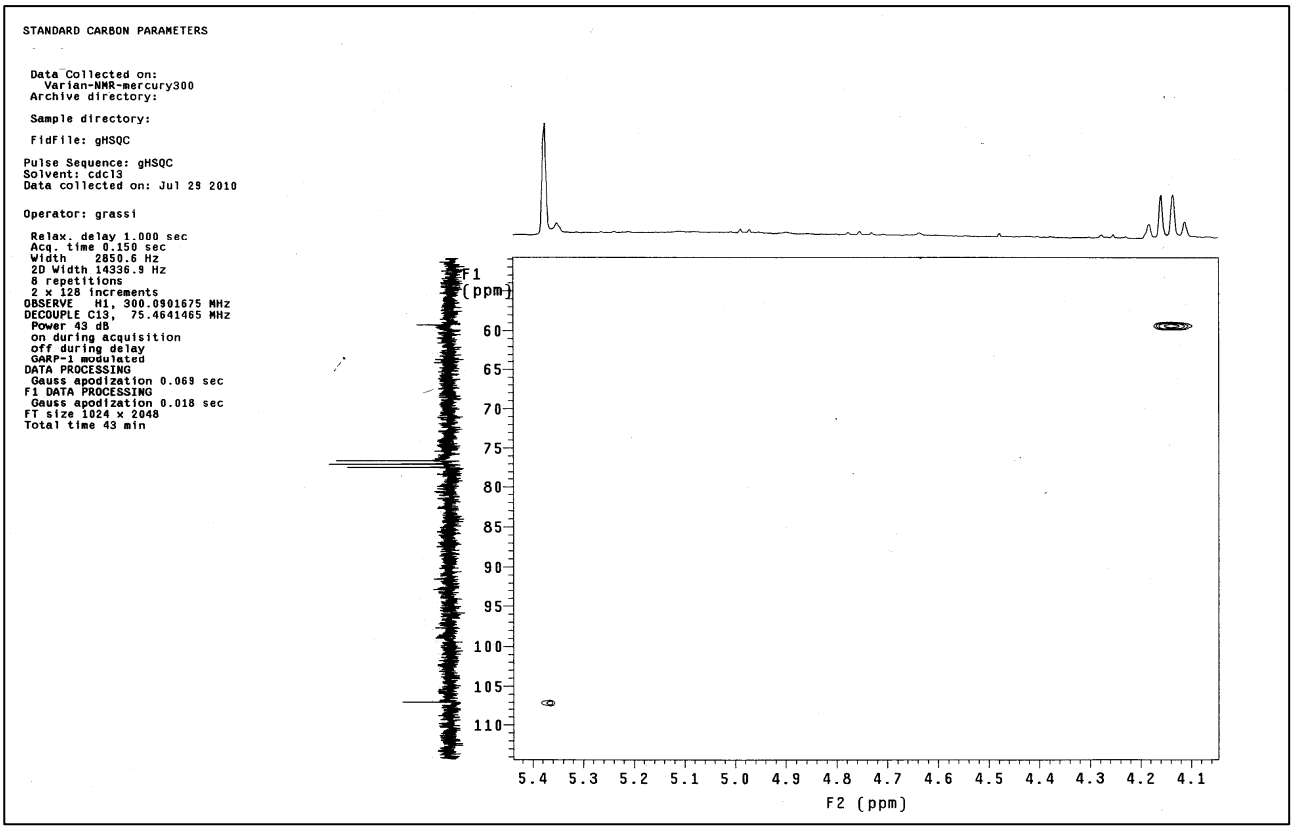
HSQC experiment on 6c



References

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(2) A. Mazzaglia, R. Donohue, B. J. Ravoo, R. Darcy, *Eur. J. Org. Chem*. 2001, 1715-1721.

(3) A. Mazzaglia, N. Angelini, R. Darcy, R. Donohue, D. Lombardo, N. Micali, M. T. Sciortino, V. Villari, L. Monsù Scolaro *Chem. Eur. J.* 2003, **9**, 5762-5769.