

Supplemental Information

Depurinating Acylfulvene-DNA Adducts: Characterizing Cellular Chemical Reactions of a Selective Antitumor Agent

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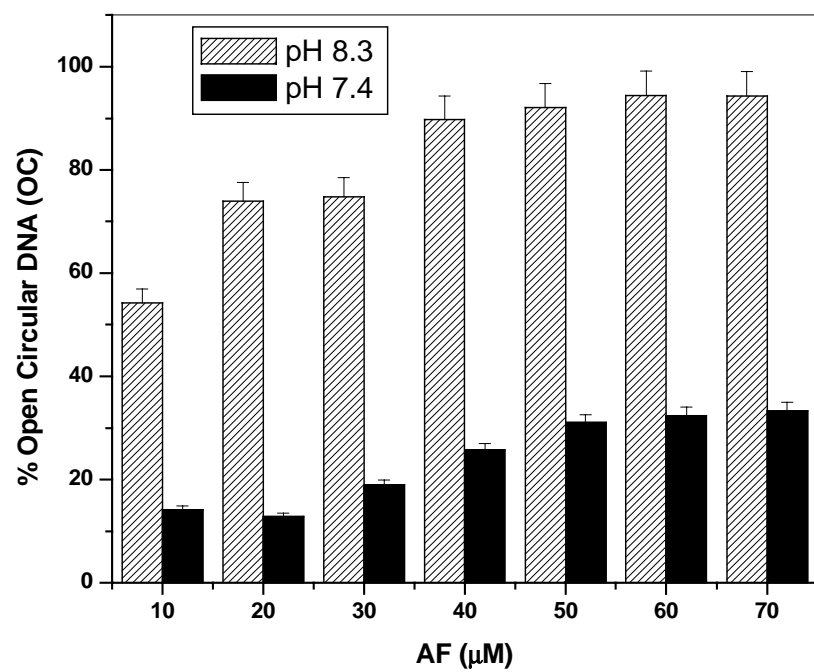


Figure S1. Relative levels of open circular DNA (OC DNA) (%) vs. AF (μM) at varying pH.

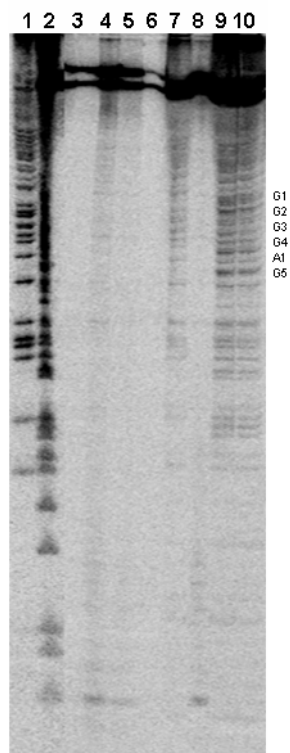


Figure S2. DNA alkylation by acylfulvenes (AF). Lane 1: Maxam-Gilbert G reaction; lane 2: G + A; lane 3: DNA alone; lane 4: DNA + AOR (10 ng); lane 5: DNA + NADPH (200 μ M); lane 6: DNA +AF (20 μ M); lane 7: DNA +AF (40 μ M); lane 8: DNA + AOR + NADPH (200 μ M); lane 9: DNA + AF (20 μ M) + AOR (10 ng) + NADPH (200 μ M); lane 10: DNA + AF (40 μ M) + AOR (10 ng) + NADPH (200 μ M)

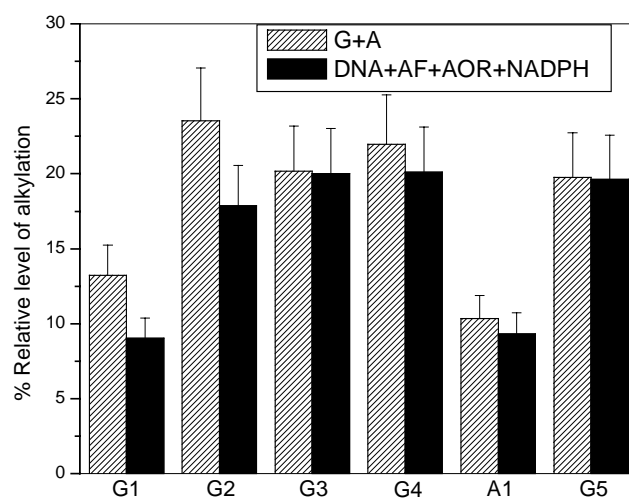


Figure S3. Relative efficiencies of DNA alkylation by AF. The height of each bar represents the relative level of alkylation of DNA by AF in the presence of AOR and NADPH. Efficiency of alkylation is compared with standard G + A reaction

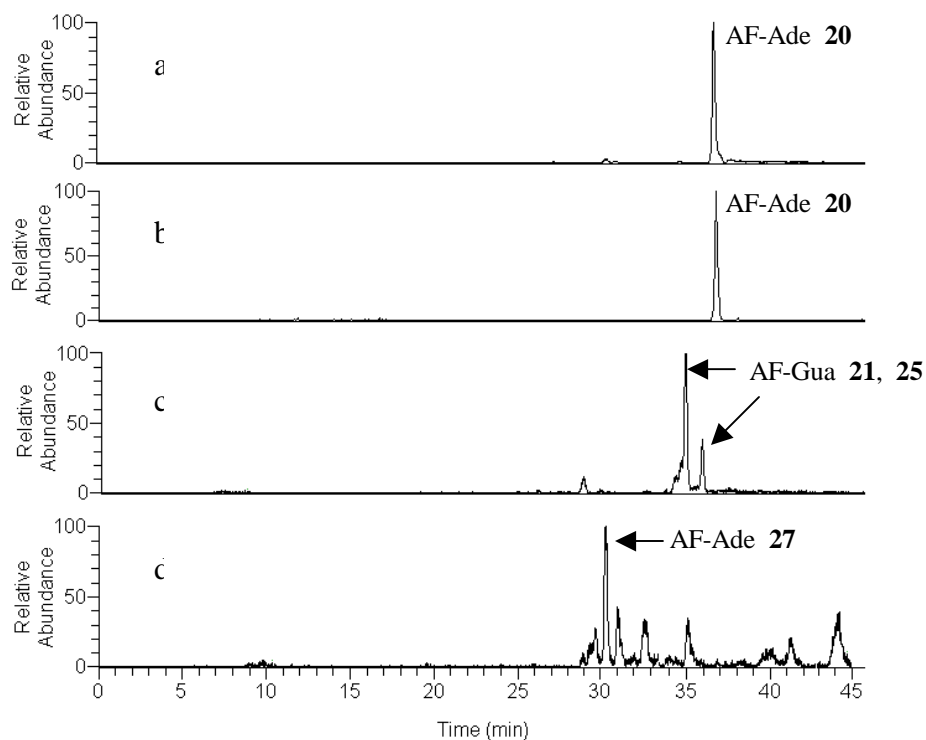


Figure S4. Extracted ion chromatographs from HPLC-ESI-MS/MS analysis of hydrolyates of DNA samples isolated from cells (a) **20**, transition of m/z 336 $[M + 1]^+$ to m/z 201 $[M - Ade]^+$; (b) Co-injection of synthetic **20** with DNA sample, transition of m/z 336 $[M + 1]^+$ to m/z 201 $[M - Ade]^+$; (c) **21** and **25**, transition of m/z 352 $[M + 1]^+$ to m/z 201 $[M - Gua]^+$; (d) **27**, transition of m/z 352 $[M + 1]^+$ to m/z 217 $[M - Ade]^+$.

Chemical Synthesis

2,4-dimethyl-4-(2-methyl-cyanoethyl)phenol (10): Anhydrous AlCl_3 (6.5 g, 49.2 mmol) was added slowly at 25 °C to a vigorously stirred mixture of methacrylonitrile (7.9 g, 98.4 mmol) and 2, 4-dimethylphenol (10.0 g, 82.0 mmol). The mixture was heated at 90 °C and dry HCl was bubbled through the solution continuously for 30 min. A HCl-saturated atmosphere was maintained with a HCl-filled balloon, which was charged periodically to maintain pressure. After stirring for 6 h, the reaction was quenched by adding 100 mL ice water and extracted with 150 mL ethyl acetate. The organic extract was washed successively with saturated aqueous NaCl and H_2O , dried, and the solvent was evaporated under reduced pressure. Unreacted 2, 4-dimethylphenol was removed by vacuum distillation (0.1 Barr, 80 °C) and the residue was purified by flash chromatography (5% ethyl acetate/hexanes), yielding **10** (7.5 g, 75%) as a white solid. Mp 68-70 °C; ^1H NMR (500 MHz, acetone- d_6) δ 1.29 (d, J = 6.5 Hz, 3H), 2.19 (s, 3H), 2.25 (s, 3H), 2.75 (m, 2H), 2.91 (d, J = 9.5 Hz, 1H), 6.85 (s, 1H), 6.91 (s, 1H); ^{13}C NMR (125 MHz, acetone- d_6) δ 32.8, 33.4, 38.4 50.0, 52.5, 141.6, 143.4, 144.5, 148.5, 166.7, 189.4; HRMS calcd for $\text{C}_{12}\text{H}_{15}\text{NO}$ $[\text{M}-1]^+$ = 188.1075, found 188.1071.

2,4-dimethyl-4-(2-methyl-carboxylpropyl)phenol (11): A solution of **10** (3.0 g, 15.9 mmol) in 20 mL MeOH and 80 mL 10% aq. NaOH was heated at 100 °C for 2 d. After cooling to 25 °C, the mixture was acidified by adding 45 mL of 20% aqueous HCl and extracted twice with 100 mL ethyl acetate. The organic extract was washed successively with saturated aq NaCl and H_2O , dried, and the solvent was evaporated under reduced pressure. The residue was recrystallized in a 1:1 mixture of acetone/hexanes to afford yielding **11** (3.0 g, 90%) as a white solid. Yield: 95%. Mp 108-110 °C; ^1H NMR (500

MHz, acetone- d_6) δ 1.21 (d, J = 7.5 Hz, 3H), 2.18 (s, 3H), 2.20 (s, 3H), 2.67 (m, 1H), 2.87 (s, 1H), 3.02 (s, 1H), 6.78 (s, 2H), 7.25 (s, 1H), 10.81 (s, 1H); ^{13}C NMR (125 MHz, acetone- d_6) δ 26.4, 27.6, 30.3, 44.3, 50.4, 135.0, 136.5, 138.8, 139.7, 140.2, 161.4, 188.8; HRMS calcd for $\text{C}_{12}\text{H}_{16}\text{O}_3$ $[\text{M}-1]^-$ = 207.1021, found 207.1050.

2,3-dihydro-2,5,7-trimethyl-4-hydroxy-1H-inden-1-one (12): A mixture of anhydrous AlCl_3 (20.0 g, 150 mmol), NaCl (10.0g, 169 mmol), and **11** (4.0 g, 19.1 mmol) was heated at 200 °C for 1 h. After cooling to 25 °C, the dark solid was dissolved in 200 mL ice water and extracted with 300 mL ethyl acetate. The organic extract was washed successively with saturated aq NaCl and H_2O , dried, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (20% EtOAc/hexanes) yielding **12** (3.3 g, 90%) as a white solid. Mp 170-172 °C; ^1H NMR (500 MHz, acetone- d_6) δ 1.23 (d, J = 7.5 Hz, 3H), 2.28 (s, 3H), 2.46 (s, 3H), 2.61 (m, 2H), 3.33 (s, 1H), 6.91 (s, 2H), 7.76 (s, 1H); ^{13}C NMR (125 MHz, acetone- d_6) δ 34.1, 34.4, 34.9, 49.3, 60.6, 110.7, 147.2, 148.8, 150.6, 151.2, 158.4, 168.7; HRMS calcd for $\text{C}_{12}\text{H}_{14}\text{O}_2$ $[\text{M}-1]^-$ = 189.0916, found 189.0914.

2,3-dihydro-2,5,7-trimethyl-4-hydroxy-6-chloromethyl-1H-inden-1-one (13): To a solution of **12** (2.5 g, 13.2 mmol) in 50 mL dry CH_2Cl_2 was added MOMCl (3.16 g, 39.6 mmol). SnCl_4 (6.8 g, 26.4 mmol) was then added slowly. The resulting dark solution was stirred at 25 °C for 24 h, and quenched by adding 100 mL ice water. Ethyl acetate extraction followed by flash chromatography (20% EtOAc/hexanes) yielding **13** (2.2 g, 70%) as a white solid. Mp 225-227 °C; ^1H NMR (500 MHz, acetone- d_6) δ 1.25 (d, J = 8.0 Hz, 3H), 2.42 (s, 3H), 2.59 (m, 2H), 2.61 (s, 3H), 3.35 (m, 1H), 4.85 (s, 2H); ^{13}C

NMR (125 MHz, acetone- d_6) δ 30.0, 34.3, 49.0, 59.2, 60.7, 110.7, 147.5, 148.9, 151.0, 154.0, 158.9, 168.7; HRMS calcd for $C_{13}H_{15}ClO_2$ $[M-1]^- = 237.0682$, found 237.0697.

2,3-dihydro-2,5,7-trimethyl-4-hydroxy-6-cyanomethyl-1H-inden-1-one (14): To a solution of **13** (1.80 g, 7.6 mmol) in 20 mL dimethylformide (DMF), was added KCN (0.49 g, 7.6 mmol). The mixture was stirred at 25 °C for 16 h, then partitioned between 80 mL H_2O and 150 mL ethyl acetate. The organic extract was washed successively with saturated aq NaCl and H_2O , dried, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (20% EtOAc/hexanes) yielding **14** (1.6 g, 88%) as a white solid. Mp 178-180 °C; 1H NMR (500 MHz, acetone- d_6) δ 1.24 (d, $J = 7.0$ Hz, 3H), 2.40 (s, 3H), 2.50 (m, 2H), 2.64 (s, 3H), 3.35 (m, 1H), 3.91 (s, 2H), 8.05 (s, 1H); ^{13}C NMR (125 MHz, acetone- d_6) δ 12.0, 12.2, 15.7, 16.8, 30.4, 42.2, 75.5, 117.5, 128.0, 129.7, 130.0, 132.5, 139.8, 150.4; HRMS calcd for $C_{14}H_{15}NO_2$ $[M-1]^- = 228.1025$, found 228.1013.

2,3-dihydro-2,5,7-trimethyl-4-hydroxy-6-carboxylethyl-1H-inden-1-one (15): A solution of **14** (2.20 g, 17.1 mmol) in 20 mL MeOH and 80 mL 10% aq NaOH was heated at 100 °C for 1 d. After cooling to 25 °C, the mixture was acidified with 45 mL of 20% aqueous HCl and extracted twice with 100 mL ethyl acetate. The organic extract was washed successively with saturated aq NaCl and H_2O , dried, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (20% EtOAc/hexanes) yielding **15** (2.3 g, 95%) as a white solid. Mp 196-198 °C; 1H NMR (500 MHz, DMSO- d_6) δ 1.15 (d, $J = 7.5$ Hz, 3H), 2.17 (s, 3H), 2.42 (m, 1H), 2.49 (s, 3H), 2.59 (m, 1H), 3.22 (m, 1H), 3.62 (s, 2H), 9.79 (s, 1H), 12.34 (s, 1H); ^{13}C NMR (125 MHz, acetone- d_6) δ 13.1, 13.5, 16.8, 31.1, 35.0, 42.2, 104.5, 128.0, 131.2, 131.8,

134.4, 139.3, 150.2, 172.700; HRMS calcd for C₁₄H₁₆O₄ [M-1]⁻ = 247.0970, found 247.1017.

2,4,6-trimethyl-5-hydroxyethyl-7-hydroxyl-1*H*-indene (8): To a solution of **15** (0.5 g, 3.34 mmol) in 20 mL dry THF, was added BH₃ (1.0 M solution in THF, 6.7 mL, 6.7 mmol) at 0 °C. The mixture was warmed to 25 °C and stirred for 24 h. It was then cooled to 0 °C and quenched by adding 30 mL 5 % H₂SO₄ solution. The mixture was extracted twice with 50 mL ethyl acetate and the organic extract was washed successively with saturated aq NaCl and H₂O, dried, and the solvent was evaporated under reduced pressure. The residue was crystallized in a 1:1 mixture of acetone/hexanes yielding **8** (0.26 g, 60%) as a white solid. ¹H NMR spectrum is consistent with previously published data, in which the characterization data includes mp, UV, ¹H NMR, HRMS, and an X-Ray crystal structure.¹ ¹H NMR (500 MHz, CDCl₃) δ 2.17 (s, 3H), 2.23 (s, 3H), 2.28 (s, 3H), 3.05 (t, *J* = 7.5 Hz, 2H), 3.20 (s, 2H), 3.75 (t, *J* = 7.5 Hz, 2H), 4.46 (s, 1H), 6.54 (s, 1H). ¹³C NMR (125 MHz, acetone-*d*₃) δ 11.6, 14.9, 16.4, 34.0, 40.2, 61.4, 119.5, 119.6, 125.6, 126.2, 134.7, 143.5, 143.8, 148.4. IR (cm⁻¹) 3372, 2960, 2905, 1700, 1460, 1253, 1082, 1047.

2-(7-hydroxy-2,4,6-trimethyl-1*H*-inden-5-yl)ethyl-2,4,6-trimethylbenzenesulfonate (16) To a solution of **8** (0.20 g, 0.92 mmol) in 1 mL dry pyridine was slowly added a solution of 2-mesitylenesulfonylchloride (0.22 g, 1.1 mmol) in 0.5 mL pyridine and the mixture was stirred at 25 °C for 12 h. Solvent was removed under reduced pressure, and the residue was purified by flash chromatography (20% EtOAc/hexanes) yielding **16** (0.28 g, 70%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 2.10 (s, 3H), 2.18 (s, 3H), 2.30 (s, 3H), 2.40 (s, 3H), 2.65 (s, 6H), 3.12 (t, *J* = 7.5 Hz, 2H), 3.25 (s, 2H), 4.10 (t, *J* =

7.5 Hz, 2H), 4.46 (s, 1H), 6.54 (s, 1H), 7.35 (s, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 12.5, 15.4, 25.6, 29.5, 30.9, 35.8, 43.1, 64.2, 74.5, 109.5, 113.2, 122.4, 128.5, 130.5, 131.4, 134.2, 135.1, 138.5, 149.9; HRMS calcd for $\text{C}_{23}\text{H}_{27}\text{O}_4\text{S}$ $[\text{M}-1]^- = 399.1624$, found 399.1620.

2-(7-hydroxy-2,4,6-trimethyl-1H-inden-5-yl)ethyl-4-methylbenzenesulfonate (17) To a solution of **8** (0.20 g, 0.92 mmol) in 1 mL dry pyridine was slowly added a solution of *p*-toluenesulfonylchloride (0.21 g, 1.1 mmol) in 0.5 mL pyridine and the mixture was stirred at 25 °C for 12 h. Solvent was removed under reduced pressure, and the residue was purified by flash chromatography (20% EtOAc/hexanes) yielding **17** (0.28 g, 70%) as a colorless oil. ^1H NMR (500 MHz, CDCl_3) δ 2.12 (s, 3H), 2.22 (s, 3H), 2.35 (s, 3H), 2.52 (s, 3H), 3.16 (t, $J = 7.5$ Hz, 2H), 3.28 (s, 2H), 4.14 (t, $J = 7.5$ Hz, 2H), 4.52 (s, 1H), 6.44 (s, 1H), 7.78 (s, 2H); ^{13}C NMR (125 MHz, CDCl_3) δ 12.8, 15.8, 25.0, 32.1, 35.8, 43.9, 64.0, 77.4, 104.5, 113.2, 122.4, 128.5, 129.1, 129.4, 130.2, 138.1, 142.5, 150.5; HRMS calcd for $\text{C}_{21}\text{H}_{23}\text{O}_4\text{S}$ $[\text{M}-1]^- = 371.1312$, found 371.1307.

2,4,6-trimethyl-5-bromoethyl-7-hydroxyl-1H-indene (18): To a solution of **17** (0.21 g, 0.57 mmol) in 2 mL DMF was added NaBr (0.47 g, 4.6 mmol). The suspension was stirred at 25 °C for 12 h. The mixture was partitioned between 10 mL H_2O and 20 mL ethyl acetate, and the organic layer was washed successively with saturated aq NaCl and H_2O , dried, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (20% EtOAc/hexanes) yielded **18** (0.08 g, 50%) as a white solid. Mp 161-163 °C; ^1H NMR (500 MHz, acetone- d_6) δ 2.13 (s, 3H), 2.24 (s, 3H), 2.30 (s, 3H), 3.19 (t, $J = 6.5$ Hz, 2H), 3.26 (s, 2H), 3.46 (t, $J = 6.5$ Hz, 2H), 6.57 (m, 1H), 7.43 (s, 1H); ^{13}C NMR (125 MHz, acetone- d_6) δ 12.3, 15.8, 30.3, 34.1, 42.1, 62.2, 73.5,

107.5, 128.5, 130.4, 132.0, 134.0, 138.5, 149.9; HRMS calcd for C₁₄H₁₇BrO [M-1]⁻ = 279.0385, found 279.0381.

2,4,6-trimethyl-5-iodoethyl-7-hydroxyl-1*H*-indene (19) To a solution of **17** (0.21 g, 0.57 mmol) in 20 mL acetone was added NaI (0.85 g, 5.7 mmol). The suspension was stirred at 70 °C for 12 h. Solvent was removed under the reduced pressure, and the residue was washed with CH₂Cl₂. The organic solution was filtered and concentrated, and was purified by flash chromatography (15% EtOAc/hexanes) yielded **19** (0.17 g, 90%) as a white solid. Mp 155-157 °C; ¹H NMR (500 MHz, CDCl₃) δ 2.16 (s, 3H), 2.24 (s, 3H), 2.30 (s, 3H), 3.20 (s, 2H), 3.24 (m, 4H), 4.43 (s, 1H), 6.55 (s, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 11.1, 14.3, 16.0, 35.4, 40.0, 61.6, 71.7, 107.0, 118.8, 125.9, 137.3, 143.9, 144.1, 148.5; HRMS calcd for C₁₄H₁₇IO [M-1]⁻ = 327.0240, found 327.0238.

2,4,6-trimethyl-5-[2-(6-amino-3*H*-purin-3-yl)ethyl]-7-hydroxyl-1*H*-indene (20) A mixture of **18** (50 mg, 0.16 mmol) and adenine (43 mg, 0.32 mmol) in 1 mL DMF was heated at 80 °C for 2 d. Solvent was removed under reduced pressure. The residue was purified first by normal phase flash chromatography (10% MeOH/CH₂Cl₂), and then using a Biotage Sp1 high performance flash chromatography system (Biotage, Charlottesville, VA) equipped with a Biotage C18 reverse phase flash column (12 + M). This system was pre-equilibrated with 100% water for 2 min at 12 mL/min. After sample introduction, the column was eluted with 100% water for 2 min followed by a linear gradient to 40% acetonitrile in water over the course of 15 min. The product **20** was eluted in the 20 to 30% acetonitrile fractions. Yield: 2%. Mp 237-240 °C; UV λ_{max} (nm) (50% acetonitrile in H₂O) 268, 284, 312; ¹H NMR (500 MHz, DMSO-*d*₆) δ 2.07 (s, 3H), 2.12 (s, 3H), 2.18 (s, 3H), 3.21 (s, 2H), 3.24 (t, *J* = 7.5 Hz, 2H), 4.35 (t, *J* = 7.5 Hz, 2H),

6.56 (s, 1H), 7.80 (s, 2H), 7.82 (s, 1H), 8.08 (s, 1H), 8.37 (s, 1H). ^{13}C NMR (125 MHz, DMSO- d_6) 12.2, 15.8, 30.3, 41.6, 49.4, 64.0, 70.5, 120.1, 121.9, 126.4, 129.8, 134.0, 143.0, 144.2, 144.6, 149.1, 150.1; HRMS calcd for $\text{C}_{19}\text{H}_{21}\text{N}_5\text{O}$ $[\text{M}+1]^+ = 336.1824$, found 336.1825.

2-amino-7-(2-(7-hydroxy-2,4,6-trimethyl-1H-inden-5-yl)ethyl)-1H-purin-6(7H)-one

(21) A mixture of **19** (105 mg, 0.32 mmol) and dGuo (171 mg, 0.64 mmol) in 2 mL DMA was stirred at 25 °C for 12 h, and was then heated at 80 °C for 12 h. Solvent was removed under reduced pressure. The residue was purified using a Biotage Sp1 high performance flash chromatography system (Biotage, Charlottesville, VA) equipped with a Biotage C18 reverse phase flash column (12 + M), using a solvent method same as that for compound **20**. The product **21** was eluted in the 20 to 30% acetonitrile fractions. Yield: 5%. Mp 225-227 °C; UV λ_{max} (nm) (50% acetonitrile in H_2O) 270, 290, 308; ^1H NMR (500 MHz, DMSO- d_6) δ 2.07 (s, 3H), 2.12 (s, 3H), 2.19 (s, 3H), 3.04 (t, $J = 7.5$ Hz, 2H), 3.18 (s, 2H), 4.21 (t, $J = 7.5$ Hz, 2H), 6.12 (s, 2H), 6.53 (s, 1H), 7.71 (s, 1H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 11.8, 14.8, 16.7, 31.7, 40.5, 45.8, 108.1, 119.0, 119.7, 125.9, 133.3, 143.0, 143.2, 143.5, 148.0, 152.7, 152.9, 154.5, 160.0; HRMS calcd for $\text{C}_{19}\text{H}_{21}\text{N}_5\text{O}_2\text{Na}$ $[\text{M}+\text{Na}]^+ = 374.1587$, found 374.1565.

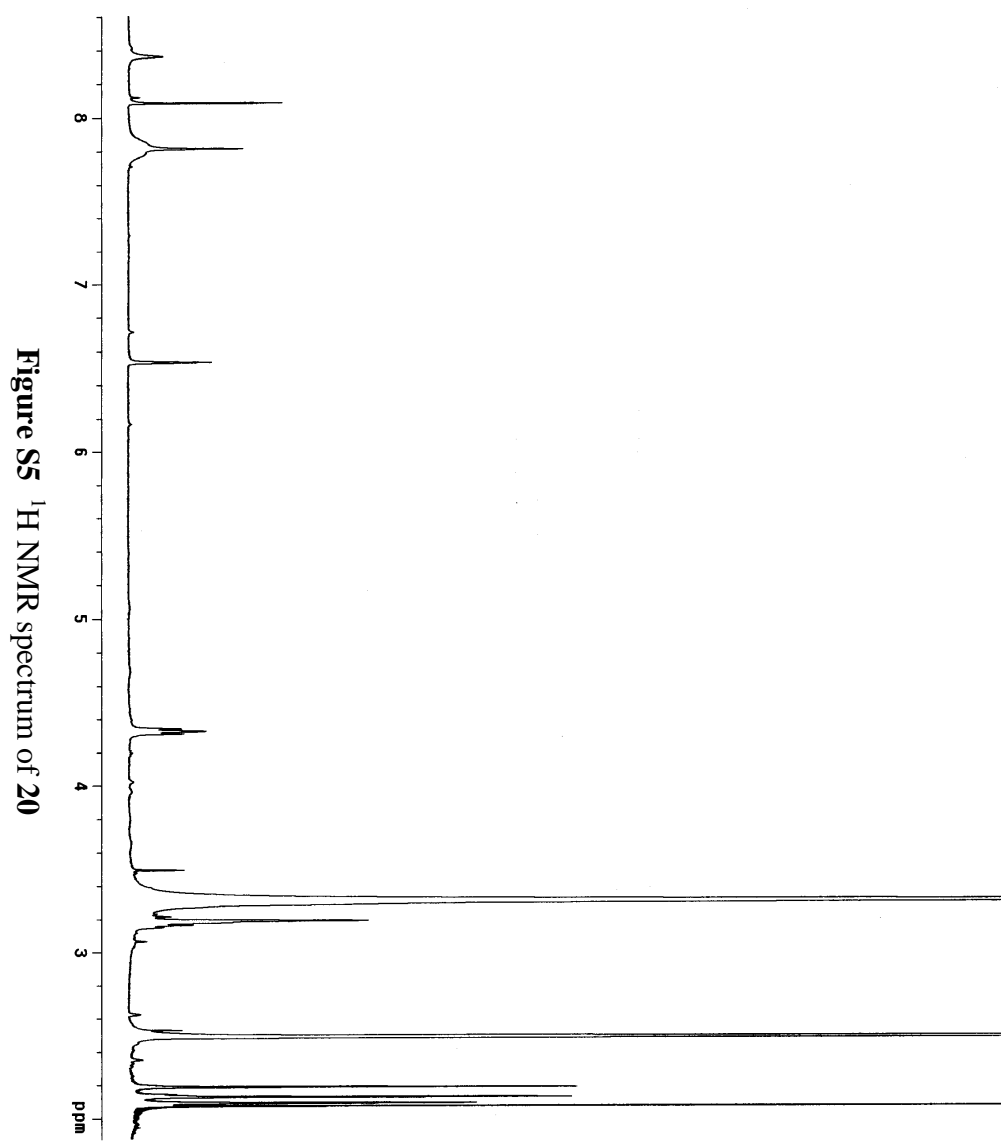


Figure S5 ^1H NMR spectrum of **20**

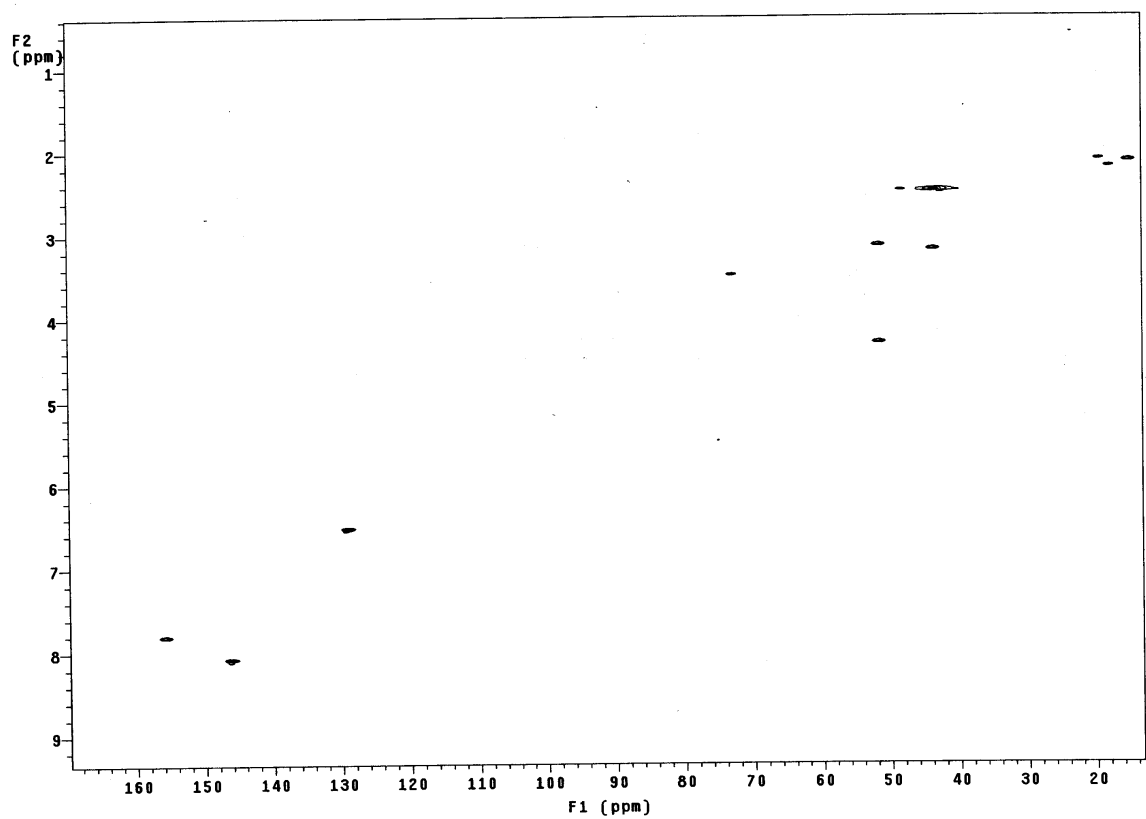


Figure S6 HMQC NMR spectrum of **20**

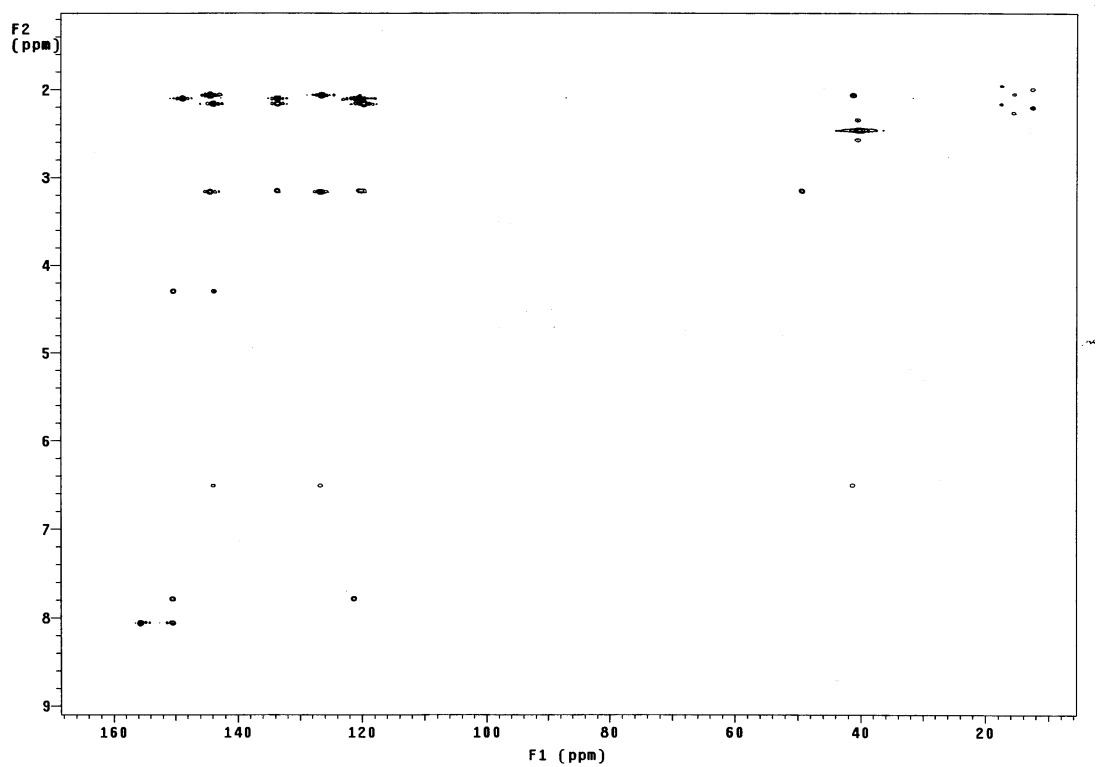


Figure S7 gHMBC NMR spectrum of **20**

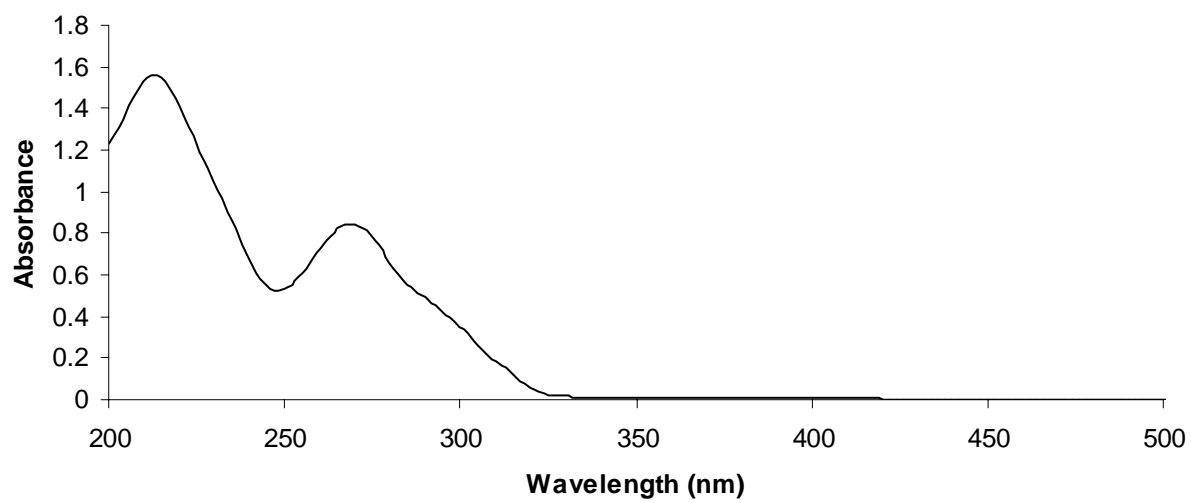


Figure S8 UV-vis spectrum of **20**

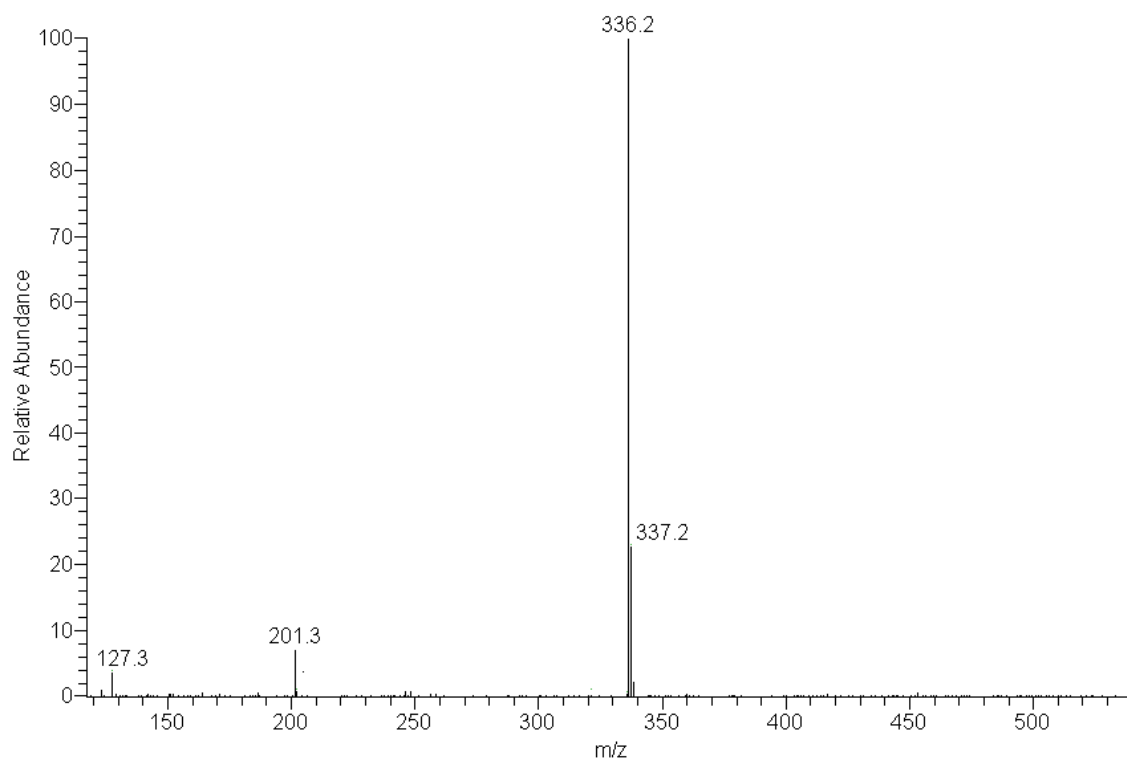


Figure S9 Mass Spectrum of **20**

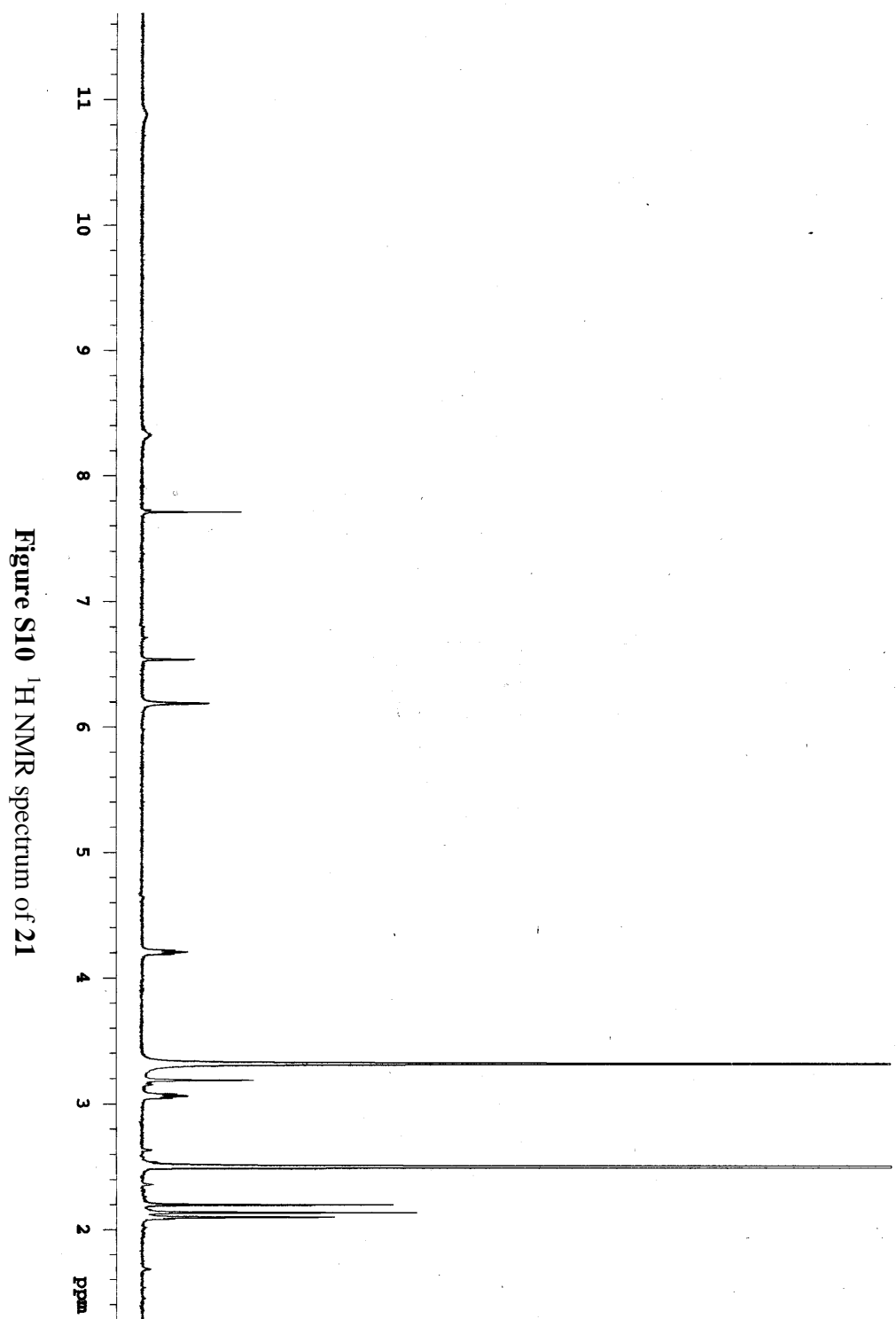
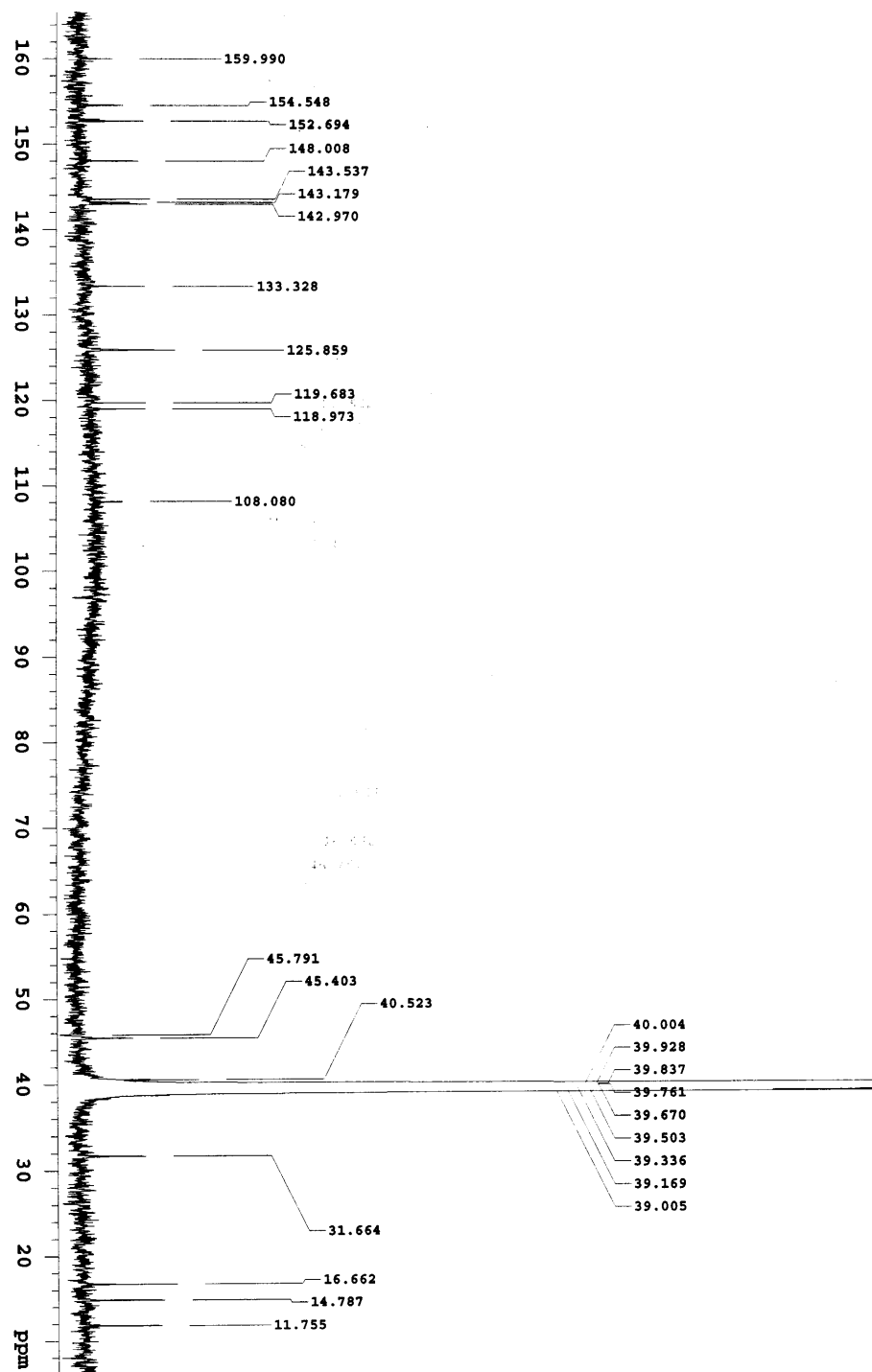


Figure S10 ^1H NMR spectrum of 21

Figure S11 ^{13}C NMR spectrum of 21



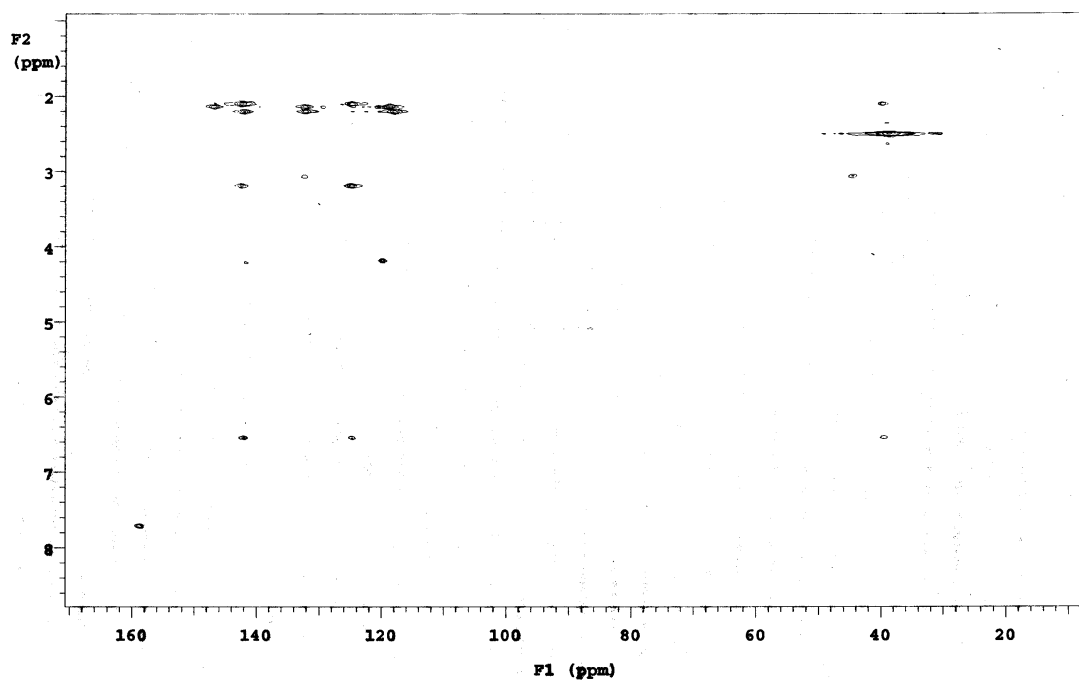


Figure S12 gHMBC NMR spectrum of **21**

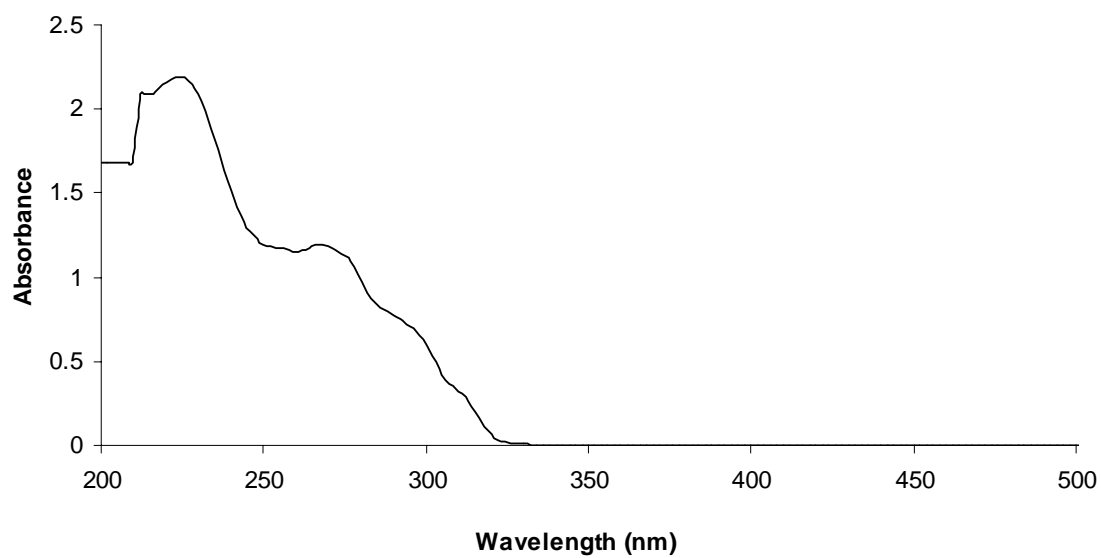


Figure S13 UV-vis spectrum of **21**

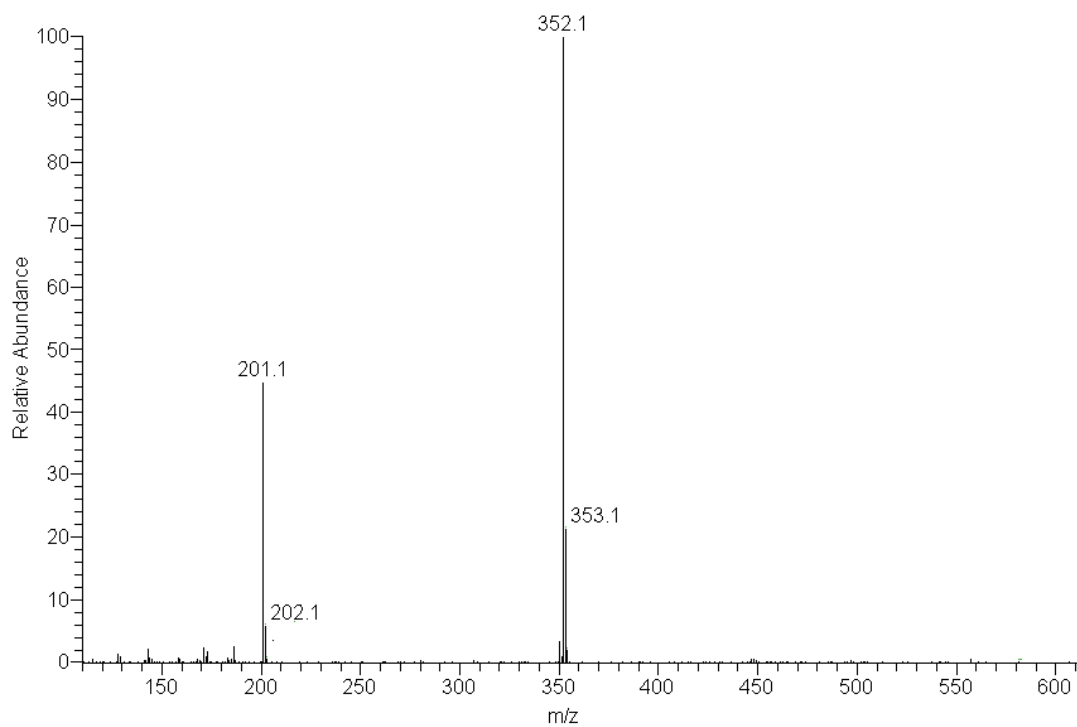


Figure S14 Mass spectrum of **21**

Reference List

- (1) McMorris, T. C.; Elayadi, A. N.; Yu, J.; Kelner, M. J. *Biochem.Pharmacol.* **1999**, *57*, 83-88.