

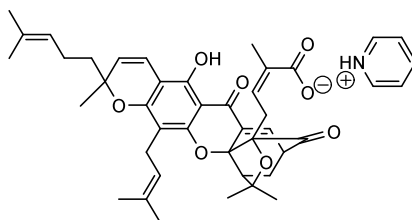
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1. General Procedures

BODIPY FL EDA was purchased from Invitrogen (Carlsbad, CA). [1,2-¹⁴C]-Ethanolamine hydrochloride (MC 407) was purchased from Moravек Biochemicals, Inc. (Brea, CA). The rest of the reagents were obtained (Aldrich, Acros) at highest commercial quality and used without further purification except where noted. Air- and moisture sensitive liquids and solutions were transferred *via* syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation below 45 °C at approximately 20 mmHg. All non-aqueous reactions were carried out under anhydrous conditions, *i.e.* using flame-dried glassware, under an argon atmosphere and in dry, freshly distilled solvents, unless otherwise noted. Yields refer to chromatographically and spectroscopically (¹H NMR, ¹³C NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) and visualized under UV light. Preparative thin-layer chromatography separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F-254). NMR spectra were recorded on Varian Mercury 400 and/or Unity 500 MHz instruments and calibrated using the residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet, b = broad. High resolution mass spectra (HRMS) were recorded on a VG7070HS mass spectrometer under chemical ionization (CI) conditions, on a VG ZAB-ZSE mass spectrometer under fast atom bombardment (FAB) conditions, or on a Bruker microTOF mass spectrometer under electrospray ionization (ESI) conditions.

2. Isolation of GA (Gambogic Acid) from Gamboge

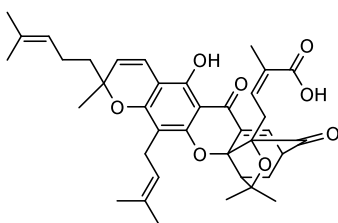
Pyridine salt of GA



Dried powder of gamboge (19.0 g) from the *G. hanburyi* tree was extracted with MeOH (80 mL) at room temperature for a day. The mixture was filtered and the residue was re-extracted two more times with MeOH (80 mL). The combined filtrate was concentrated under reduced pressure to give crude extract as a yellow powder (13.0 g, 68%). The crude extract (13.0 g) was dissolved in pyridine (13 mL), and then warm water (5 mL) was added to the stirred solution. The reaction mixture was cooled to room temperature and some precipitate was observed. Hexane (10 mL) was added to the mixture and the mixture was filtered. The solid was collected and washed with hexane and dried to yield pyridine salt of GA as a yellow solid (1.8 g, 14%). ¹H NMR (400

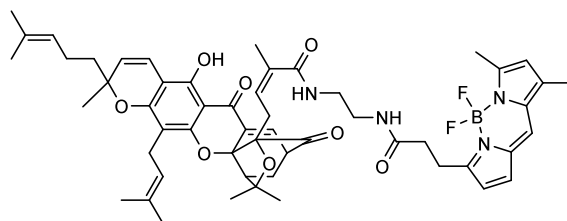
MHz, CDCl₃) δ 8.58-8.57 (m, 2H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.53 (d, *J* = 6.8 Hz, 1H), 7.33-7.30 (m, 2H), 6.55 (d, *J* = 10.1 Hz, 1H), 6.07 (t, *J* = 7.1 Hz, 1H), 5.34 (d, *J* = 10.1 Hz, 1H), 5.02 (br s, 2H), 3.47-3.45 (m, 1H), 3.29-3.26 (m, 1H), 3.14-3.12 (m, 1H), 3.02-2.97 (m, 2H), 2.50 (d, *J* = 9.2 Hz, 1H), 2.32-2.27 (m, 1H), 2.00-1.98 (m, 2H), 1.73-1.53 (m, 20H), 1.39-1.37 (m, 1H), 1.34 (s, 3H), 1.27 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 203.7, 179.2, 171.1, 161.6, 157.8 (2C), 149.1, 137.1, 136.9 (2C), 135.4, 133.6, 132.0, 131.6, 128.4, 124.6, 124.2 (2C), 124.0, 122.5, 116.1, 107.8, 102.8, 100.7, 91.2, 84.0 (2C), 81.4, 49.2, 47.0, 42.2, 30.1, 29.4, 29.1, 27.9, 25.9 (2C), 25.4, 23.0, 21.8, 21.2, 18.3, 17.8.

GA (Gambogic acid)



To a solution of the pyridine salt of GA (404.8 mg, 0.57 mmol) in ether (6 mL) was added aqueous HCl (1N, 4 mL). After 1 h of continued stirring at room temperature, the ether solution was washed with water (3 x 1 mL), dried over MgSO₄, and concentrated by rotary evaporation to give GA as a yellow solid (355.9 mg, 99%). *R_f* = 0.38 (25% EtOAc-hexane). ¹H NMR (400 MHz, CDCl₃) δ 7.54 (d, *J* = 6.9 Hz, 1H), 6.55 (d, *J* = 10.1 Hz, 1H), 6.12 (t, *J* = 7.3 Hz, 1H), 5.34 (d, *J* = 10.2 Hz, 1H), 5.05-5.01 (m, 2H), 3.49-3.46 (m, 1H), 3.31-3.25 (m, 1H), 3.13-3.10 (m, 1H), 2.98 (d, *J* = 7.3 Hz, 2H), 2.50 (d, *J* = 9.3 Hz, 1H), 2.30 (dd, *J* = 13.4, 4.6 Hz, 1H), 2.00-1.98 (m, 2H), 1.72-1.53 (m, 20H), 1.42-1.40 (m, 1H), 1.34 (s, 3H), 1.28 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 203.6, 179.0, 172.1, 161.7, 157.7, 157.5, 138.7, 135.5, 133.5, 132.0, 131.7, 127.7, 124.6, 124.1, 122.4, 116.1, 107.7, 102.9, 100.6, 91.1, 84.1, 84.0, 81.5, 49.2, 47.0, 42.2, 30.1, 29.5, 29.1, 27.9, 25.9 (2C), 25.4, 23.0, 21.8, 21.0, 18.3, 17.9. HRMS calc. for C₃₈H₄₄O₈Na (M + Na)⁺ 651.2928, found 651.2931.

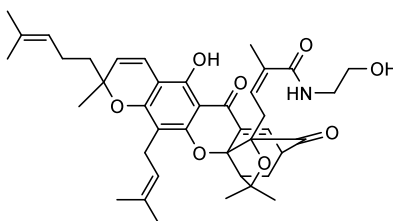
3. Synthesis of GA-Bodipy



To a solution containing GA (6.1 mg, 9.70 μmol) and BODIPY FL EDA (3.95 mg, 10.7 μmol) in CH₂Cl₂ (0.24 mL) was added DIPEA (3.38 μL, 19.4 μmol). Upon adding solid HATU (4.37 mg, 11.5 μmol) portionwise to the reaction mixture, the reaction mixture turned to pale yellow in

color within 5 min. After 24 h, the reaction mixture was diluted with EtOAc (5 mL) and washed with water (2 x 1 mL) and brine (2 mL). The organic layers were dried over MgSO₄, filtered, and concentrated by rotary evaporation. The crude material was purified by preparative TLC (silica, 100% EtOAc) to yield the amide as a red solid (7.05 mg, 77%). *R_f* = 0.38 (100% EtOAc). ¹H NMR (400 MHz, CDCl₃) δ 7.51 (d, *J* = 6.9 Hz, 1H), 7.03 (s, 1H), 6.92-6.90 (m, 1H), 6.85 (d, *J* = 4.1 Hz, 1H), 6.68 (d, *J* = 10.2 Hz, 1H), 6.62 (m, 1H), 6.27 (d, *J* = 3.9 Hz, 1H), 6.09 (s, 1H), 5.46 (d, *J* = 10.2 Hz, 1H), 5.22-5.16 (m, 1H), 5.09-4.99 (m, 2H), 3.49-3.15 (m, 9H), 2.66-2.62 (m, 3H), 2.53 (s, 3H), 2.31-2.27 (m, 3H), 2.21 (s, 3H), 2.06-2.02 (m, 2H), 1.75-1.24 (m, 27 H). ¹³C NMR (100 MHz, CDCl₃) δ 204.8, 178.9, 172.4, 169.5, 162.0, 157.9, 157.3, 135.8, 133.3, 132.1, 128.8, 125.2, 124.9, 124.0, 123.9, 122.2, 120.4, 117.9, 115.9, 108.0, 103.0, 100.5, 91.3, 84.5, 84.0, 81.8, 49.1, 46.9, 42.3, 39.9, 39.8, 35.8, 30.1, 29.9, 29.2, 29.0, 28.1, 25.9, 25.4, 24.9, 22.9, 21.8, 21.4, 18.4, 17.9, 15.1, 11.5. HRMS calc. for C₅₄H₆₄BF₂N₄O₈S (M + H)⁺ 945.4785, found 945.4824.

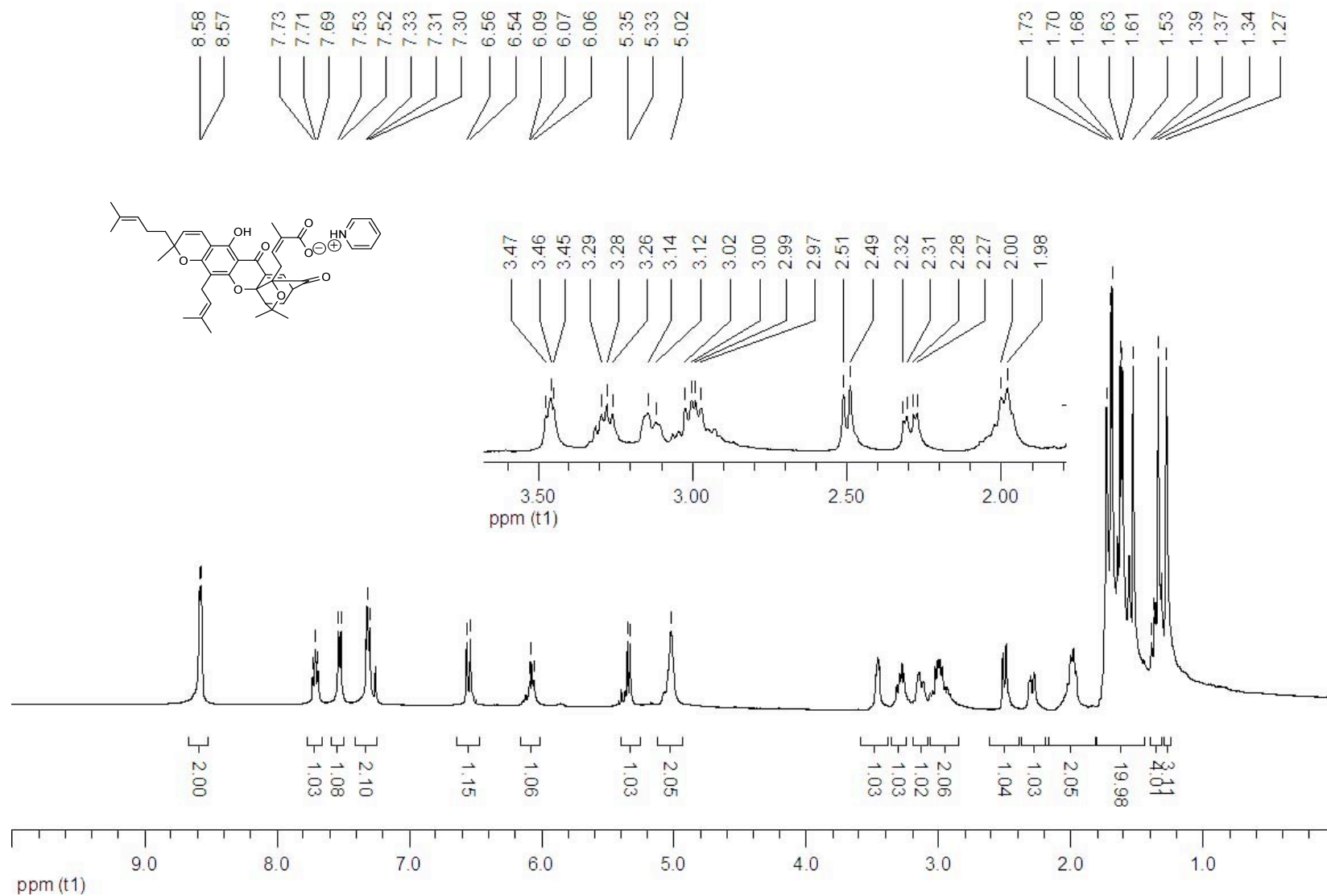
4. Synthesis of GA-ETA



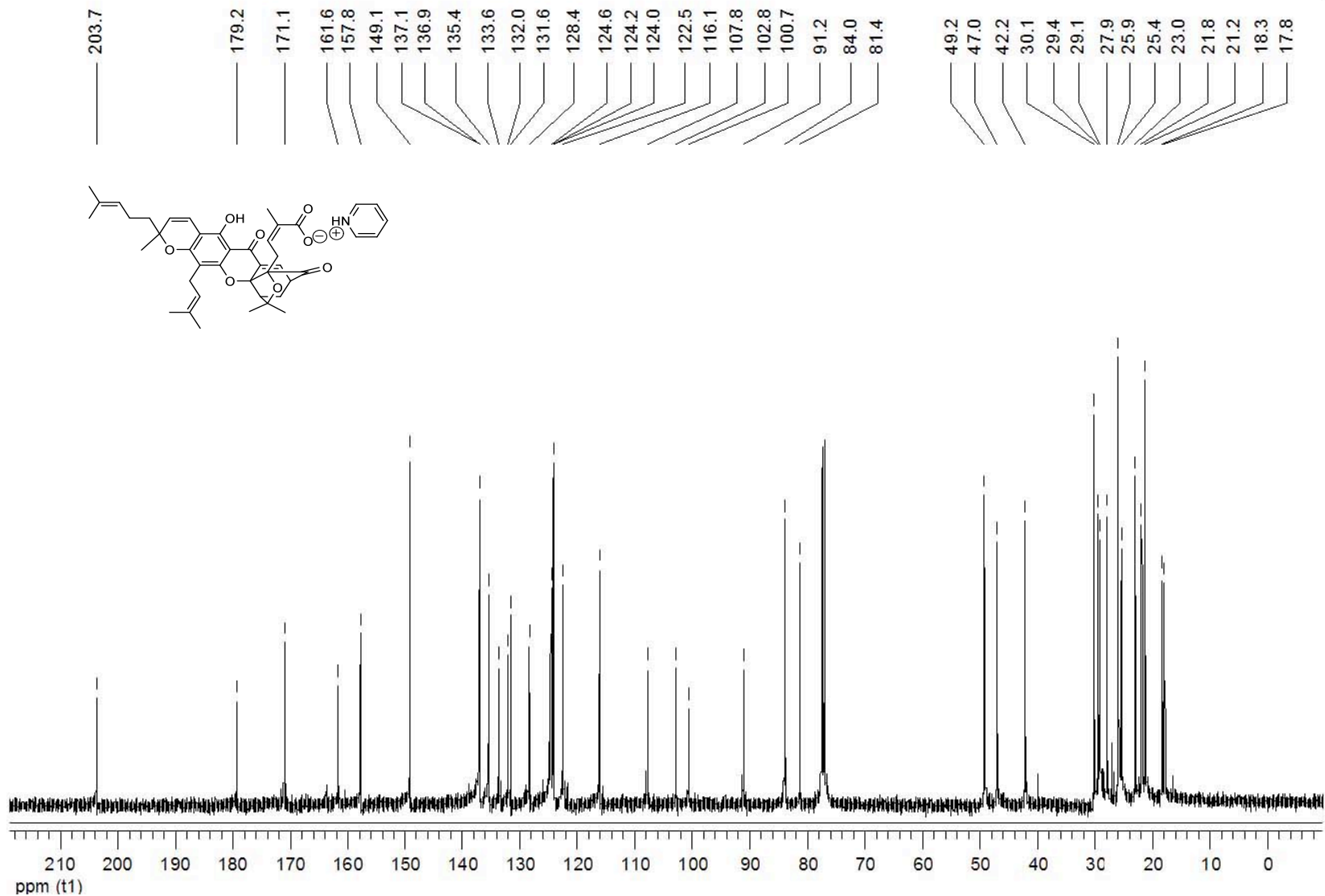
To a solution of GA (58.0 mg, 92.2 μmol) and HATU (42.1 mg, 110.6 μmol) in ACN (3 mL) was added DIPEA (64.0 μL, 369.0 μmol) via syringe. After stirring for 15 min at room temperature, ethanolamine (6.70 μL, 110.6 μmol) was added via syringe and the reaction mixture was stirred at room temperature. After 2 h, the solvent was removed and the crude mixture was diluted with ethyl acetate (5 mL). The ethyl acetate solution was washed with water (2 x 1 mL) and brine (1 mL). The organic layers were dried over MgSO₄, filtered, and concentrated by rotary evaporation. The crude material was purified through flash column chromatography (Silica, 50% EtOAc-hexane) to yield GA-ETA as a yellow solid (38.8 mg, 63%). *R_f* = 0.47 (50% EtOAc-hexane). ¹H NMR (400 MHz, CDCl₃) δ 7.57 (d, *J* = 6.9 Hz, 1H), 7.08-7.03 (m, 1H), 6.68 (d, *J* = 10.2 Hz, 1H), 5.47 (d, *J* = 10.0 Hz, 1H), 5.31-5.27 (m, 1H), 5.09-5.02 (m, 2H), 3.72-3.00 (m, 8H), 2.70 (dd, *J* = 14.8, 9.2 Hz, 1H), 2.55 (d, *J* = 9.3 Hz, 1H), 2.33 (d, *J* = 3.9 Hz, 1H), 2.30 (d, *J* = 4.1 Hz, 1H), 2.04-2.01 (m, 2H), 1.81-1.28 (m, 27H). ¹³C NMR (100 MHz, CDCl₃) δ 205.0, 179.0, 170.2, 162.1, 157.9, 157.4, 136.2, 135.8, 133.3, 132.2, 132.1, 125.2, 124.4, 123.9, 122.2, 115.9, 108.0, 103.1, 100.6, 91.3, 84.6, 84.0, 81.8, 62.8, 49.1, 47.0, 42.8, 42.3, 30.1, 29.4, 29.0, 28.1, 25.9 (2C), 25.4, 22.9, 21.8, 21.3, 18.3, 17.8. HRMS calc. for C₄₀H₅₀O₈N (M + H)⁺ 672.3531, found 672.3527.

5. Synthesis of ^{14}C -GA-ETA

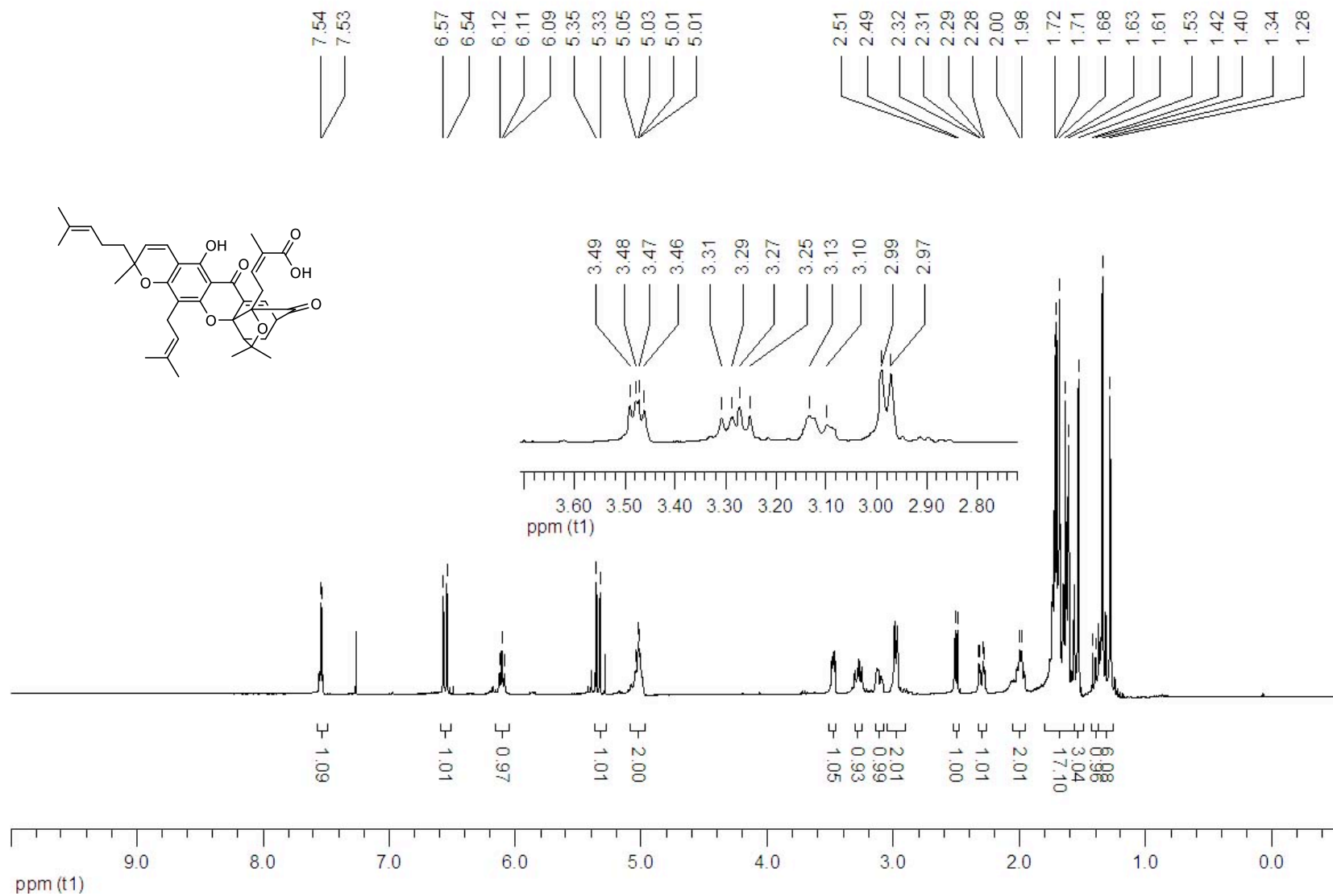
To a solution of GA (7.5 mg, 11.9 μmol) and HATU (5.44 mg, 14.3 μmol) in EtOH (0.30 mL) was added DIPEA (8.3 μL , 47.6 μmol) via syringe. After stirring for 15 min at room temperature, [1,2- ^{14}C]-ethanolamine hydrochloride (1.5 mL, EtOH solution) was added via syringe and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the crude material was purified through preparative TLC (Silica, 50% EtOAc-hexane) to yield the ^{14}C -GA-ETA as a yellow solid (4.1 mg, 51%). R_f = 0.47 (50% EtOAc-hexane). HRMS calc. for $\text{C}_{40}\text{H}_{49}\text{O}_8\text{NNa}$ ($\text{M} + \text{Na}$) $^+$ 696.3423, found 696.84796.



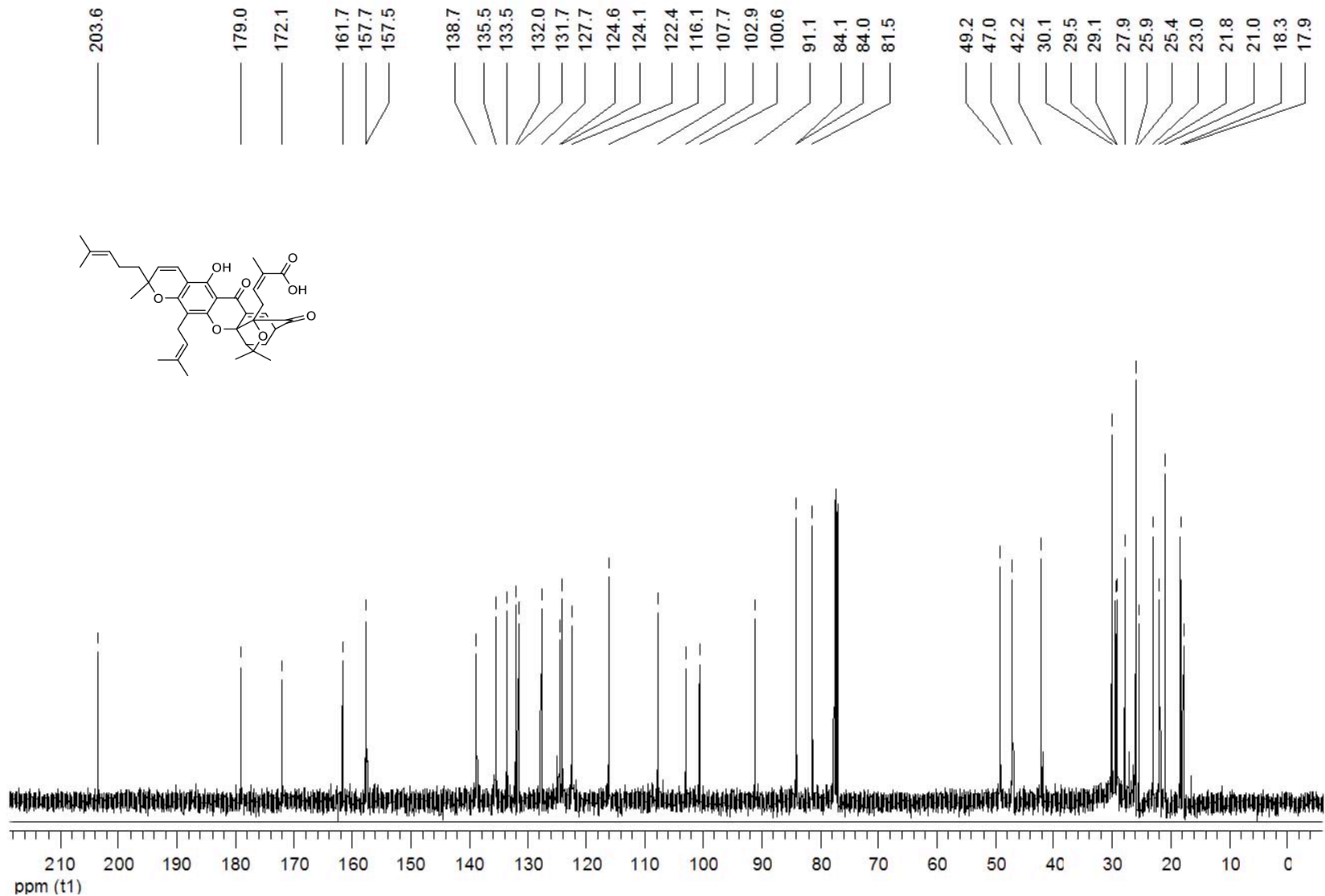
Spectrum 1: ^1H NMR (CDCl_3 , 400 MHz) of pyridine salt of GA.



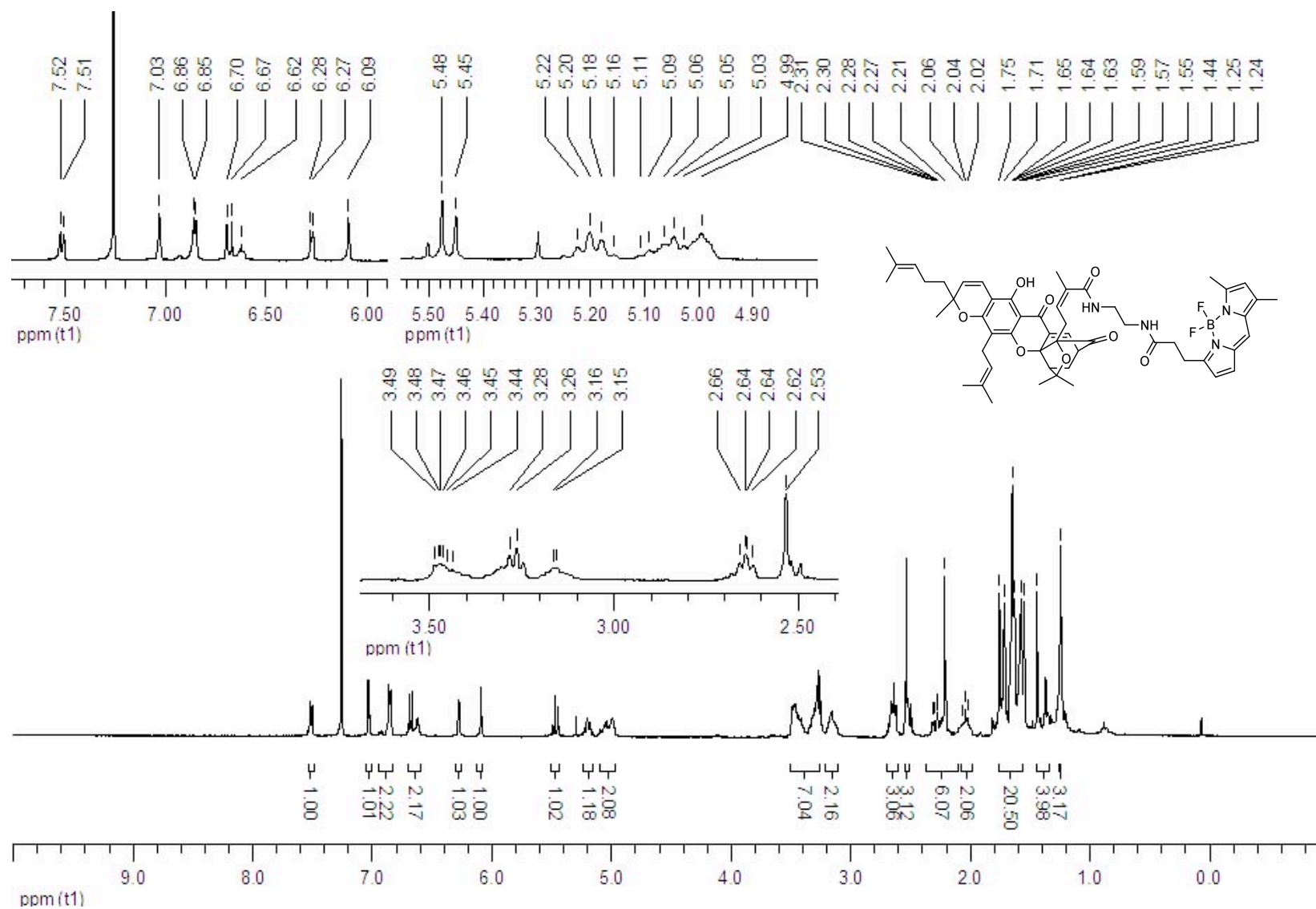
Spectrum 2: ^{13}C NMR (CDCl_3 , 100 MHz) of pyridine salt of GA.



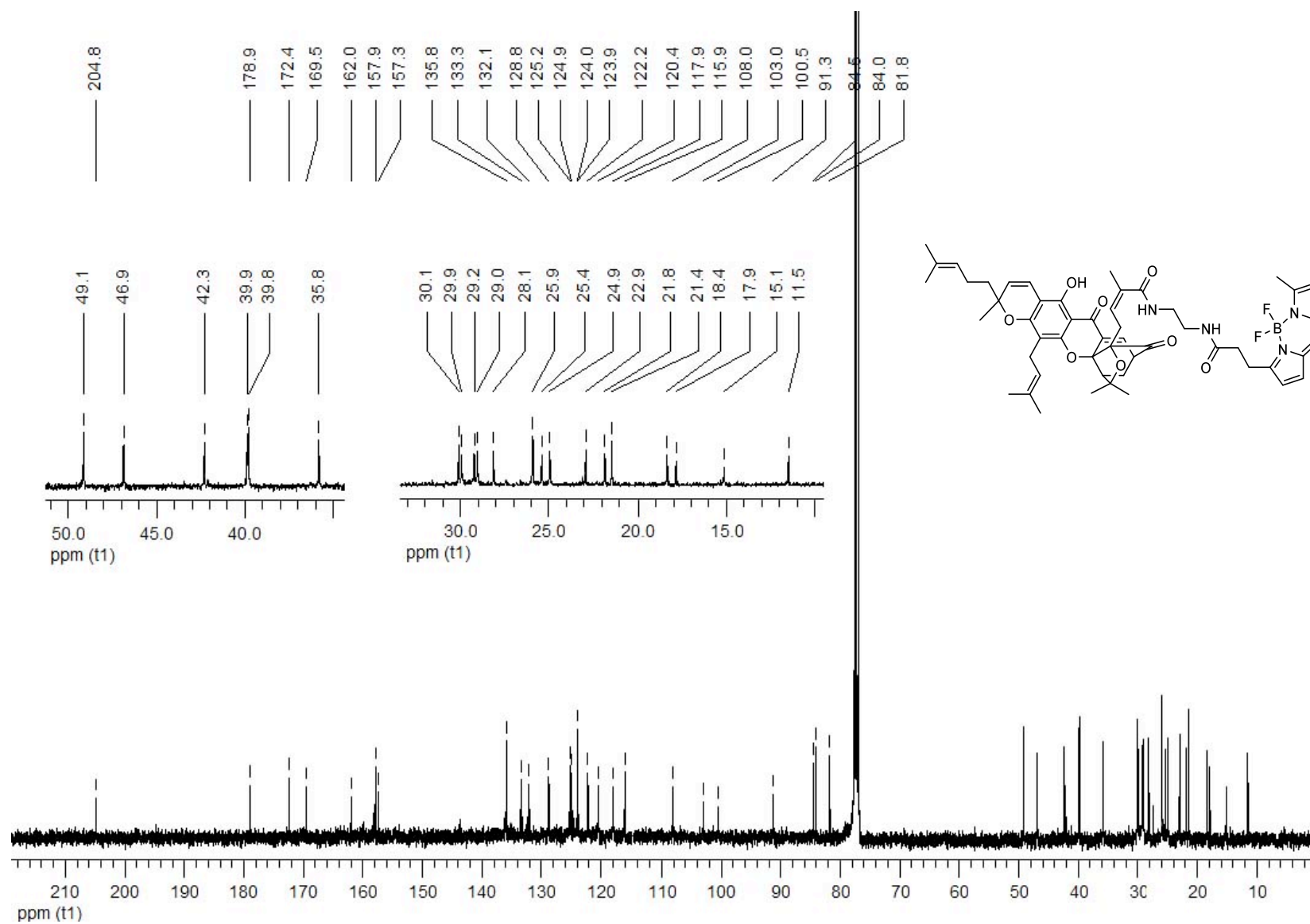
Spectrum 3: ^1H NMR (CDCl_3 , 400 MHz) of GA.



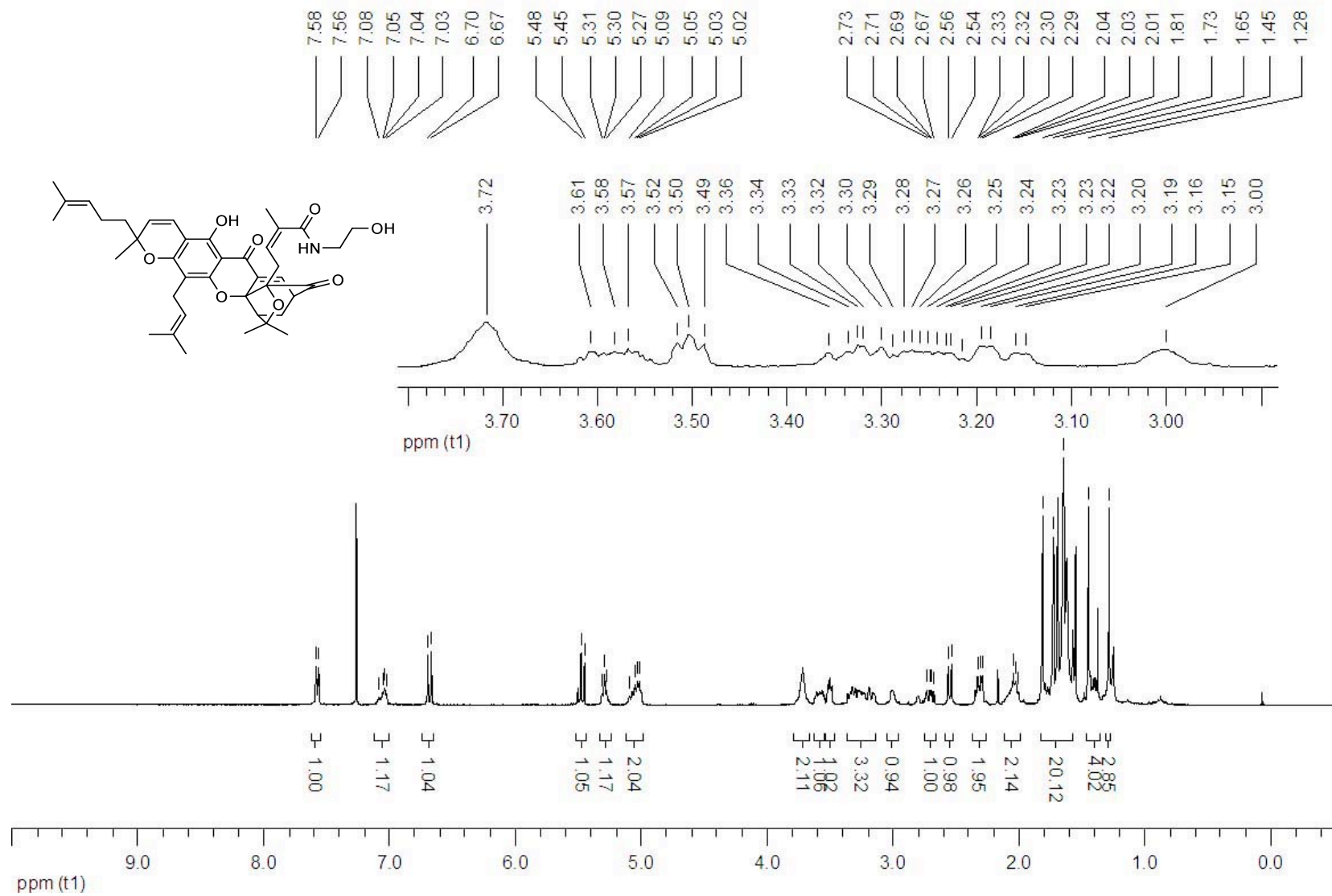
Spectrum 4: ¹³C NMR (CDCl₃, 100 MHz) of GA.



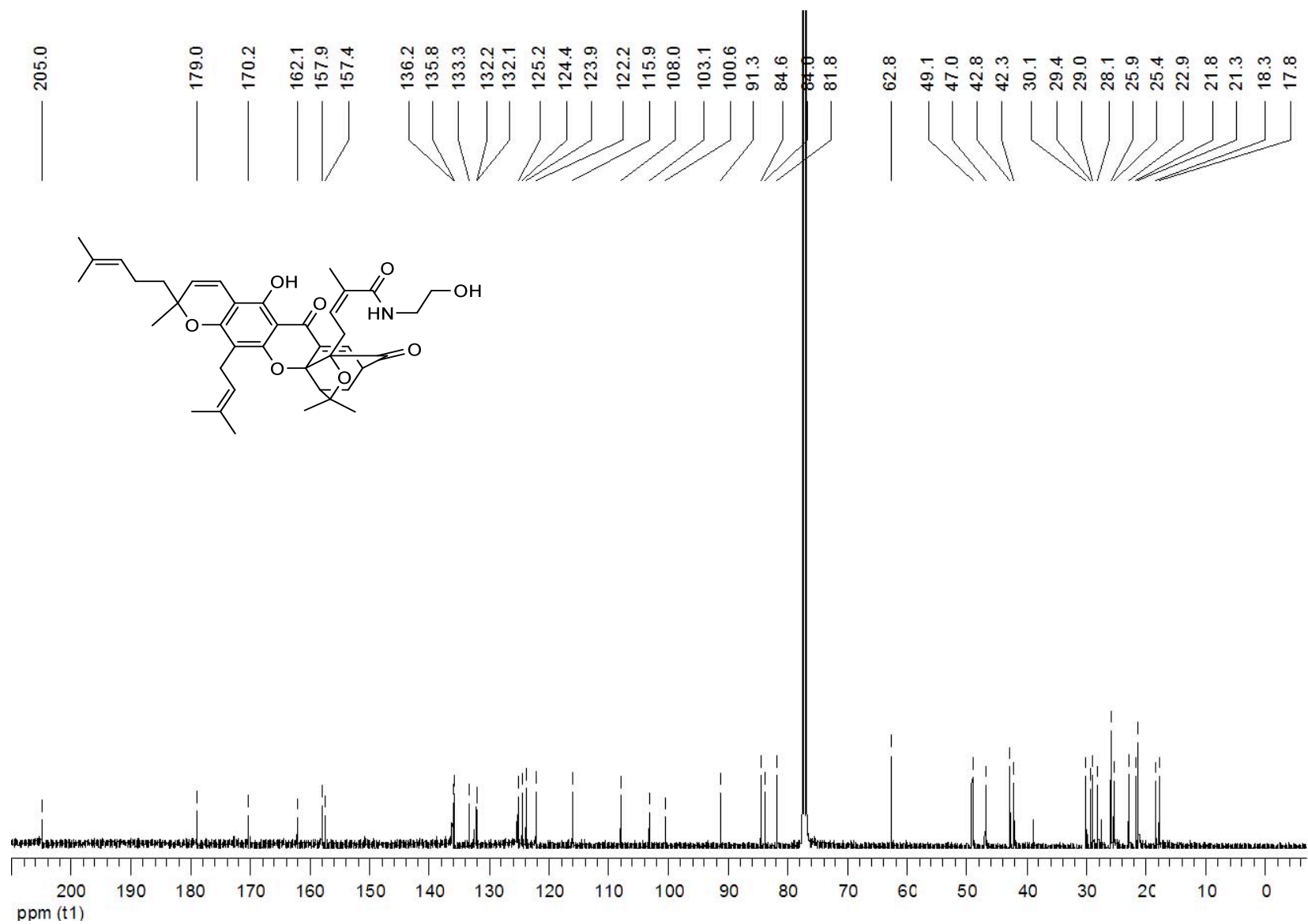
Spectrum 5: ^1H NMR (400 MHz, CDCl_3) of GA-Bodipy.



Spectrum 6: ^{13}C NMR (100 MHz, CDCl_3) of GA-Bodipy.



Spectrum 7: ^1H NMR (400 MHz, CDCl_3) of GA-ETA.



Spectrum 8: ¹³C NMR (100 MHz, CDCl₃) of GA-ETA.

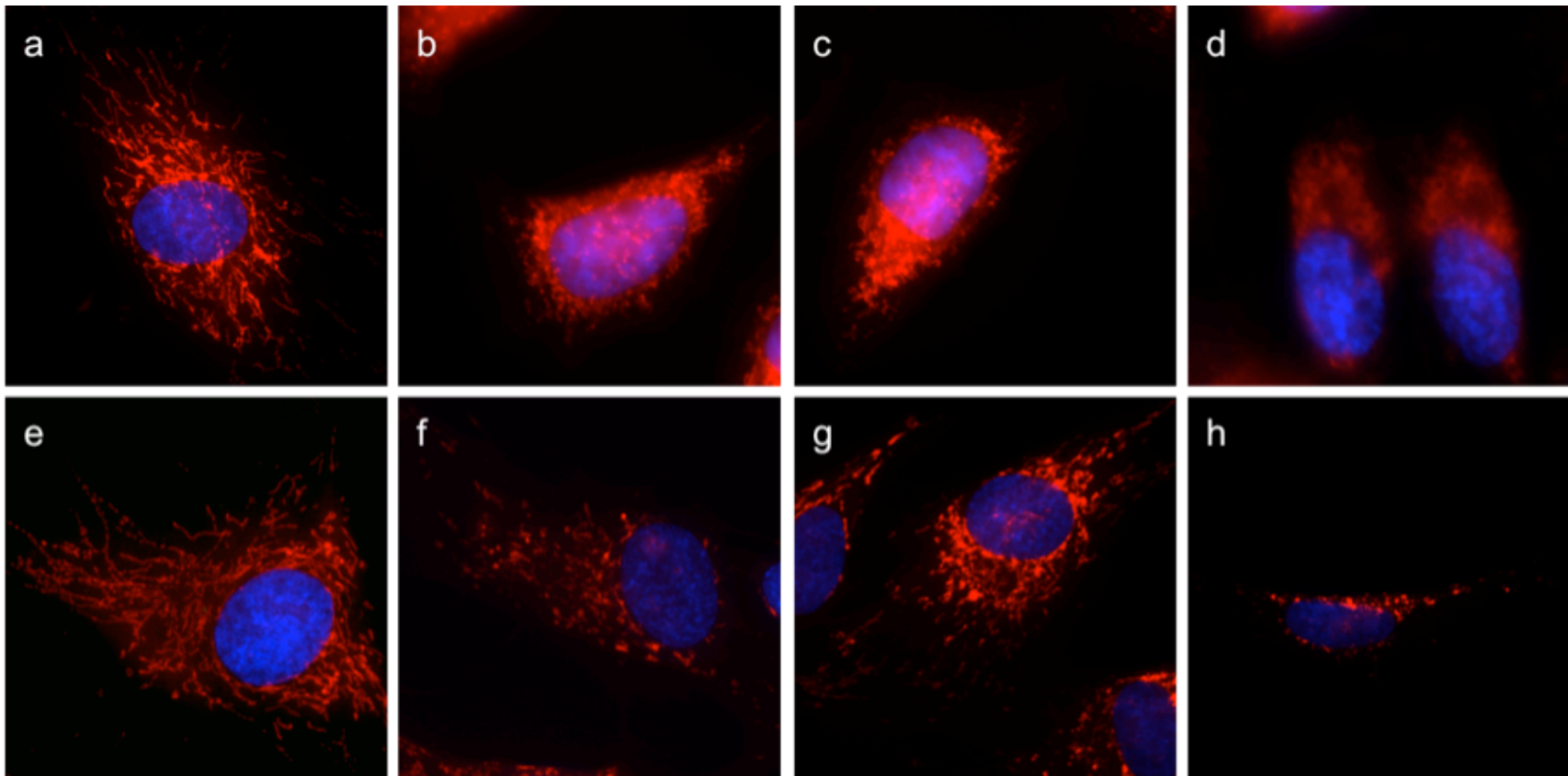


Figure S1. GA induces immediate mitochondrial fragmentation in HeLa cells at higher concentration than in normal human fibroblasts (BJ cells). HeLa cells (a-d) and BJ cells (e-h) were treated with 0, 0.5, 1 and 2 μ M GA for 2h. The cells were then processed for IF to visualize the mitochondria (shown in red; nuclei are shown in blue). Extensive loss in mitochondrial network is visible in HeLa cells already at 0.5 μ M GA, while in BJ cells μ M GA are needed to induce comparable level of fragmentation.

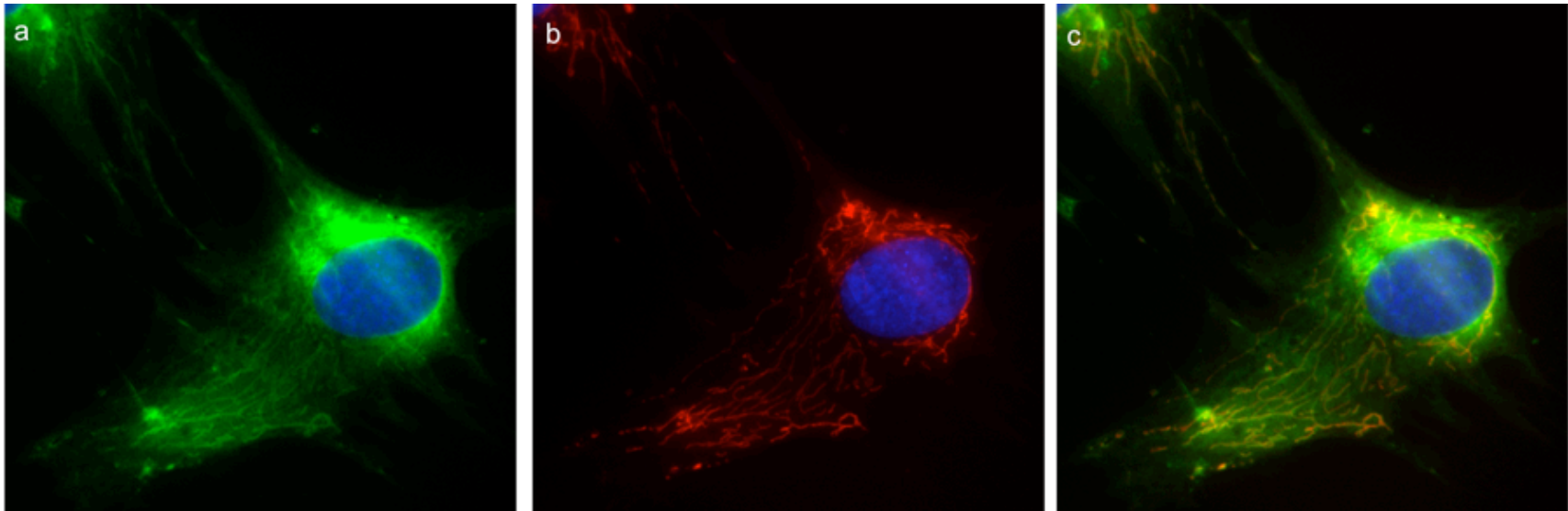


Figure S2. GA-Bodipy localizes on mitochondria in BJ cells. BJ cells are labeled to visualize mitochondria (b) and are then incubated with GA-Bodipy (a). Merged image is shown in (c). Nuclei are in blue.