

Supporting Information

Design and Synthesis of a Mitochondria-Targeting Carrier for Small Molecule Drugs

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1. Materials and general procedures:

All chemistry experiments were performed under an atmosphere of nitrogen except indicated otherwise. Proton (¹H) and carbon (¹³C) nuclear magnetic resonance (NMR) spectra were obtained with a Bruker 600 MHz and 150 MHz magnetic resonance spectrometer, respectively. Solvent peaks of CDCl₃ at 7.26 ppm and 77.1 ppm were used as internal standards for ¹H and ¹³C NMR, respectively. Data of ¹H NMR spectra are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, b = broad, m = multiplet), coupling constants, and number of protons. Low resolution mass spectra (LRMS) were recorded on a quadrupole spectrometer using electrospray ionization (ESI). Analytical thin layer chromatography (TLC) was conducted on glass sheets coated with silica gel 60 F254. Flash chromatography was performed on regular grade silica gel (60 Å, Fisher Scientific). Mito Tracker Deep Red (MDR) and NucBlue® Live ReadyProbes® Reagent (Hoechst 33324) were purchased from Life Technologies (Carlsbad, CA).

2. Cell cultures:

A drug-sensitive human uterine sarcoma cell lines MES-SA and DOX-resistant MES-SA/Dx5 cell lines were obtained from American Type Culture Collection (ATCC, CRL-1976). MES-SA & MES-SA/Dx5 cells were cultured in ATCC-formulated McCoy's 5A Medium (ATCC, 30-2007) supplemented with 10% (v/v) fetal bovine serum (vendor) at 37 °C humidified with 5% CO₂.

3. Preparation of compounds **1**, **QCy7HA**, **QCy7MH** and **QCy7HA-DOX**

Synthesis of compound **1**

A solution of compound 4-hydroxyisophthalaldehyde (262 mg, 1.75 mmol), methyl 6-bromohexanoate (548 mg, 2.62 mmol), K_2CO_3 (290 mg, 2.10 mmol) in anhydrous DMF (15 mL) was stirred at room temperature for 15 h. The solution was diluted with H_2O (50 mL), and the aqueous phase was washed with EtOAc (25 mL x 3). The combined organic solvent was washed with H_2O (20 mL x 1) and brine (20 mL x1), dried over anhydrous Na_2SO_4 , and finally removed under reduced pressure. The crude residue was purified by regular silica gel chromatography eluting with hexane/EtOAc (2/1) to yield the desired product as white solid (296 mg) in 61% yield. $R_f = 0.30$ (Hexane/EtOAc = 2/1); 1H NMR ($CDCl_3$, 600 MHz) δ 10.48 (s, 1 H), 9.92 (s, 1 H), 8.31 (d, $J = 2.4$ Hz, 1 H), 8.09 (dd, $J = 9.0, 2.4$ Hz, 1 H), 7.10 (d, $J = 9.0$ Hz, 3.0 Hz, 1 H), 4.19 (t, $J = 6.0$ Hz, 2 H), 3.67 (s, 3 H), 2.36 (t, $J = 7.2$ Hz, 2 H), 1.94-1.89 (m, 2 H), 1.75-1.70 (m, 2 H), 1.57-1.52 (m, 2 H); ^{13}C NMR ($CDCl_3$, 180 MHz) δ 190.2, 188.5, 173.8, 165.4, 135.6, 131.8, 129.5, 124.8, 113.1, 67.0, 51.6, 33.8, 28.6, 25.5, 24.5; LRMS calculated for $C_{15}H_{18}O_5Na$ ($M + Na^+$) $^+$ 301.1, found 301.0, and $C_{16}H_{22}O_6Na$ ($M + MeOH + Na^+$) $^+$ 333.1, found 333.0.

Synthesis of compound **QCy7MH**

A mixture of **1** (26 mg, 0.044 mmol), 1, 2, 3, 3-tetramethyl-3H-indolium iodide (27 mg, 0.089 mmol), NaOAc (13 mg, 0.016 mmol) in Ac_2O (0.4 mL) was stirred at 80 °C for 30 mins. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and the insoluble NaOAc was removed by filtration. The organic solvent was removed under reduced pressure. The residue was dissolved in small amount of CH_2Cl_2 , and the product precipitated with addition of diethyl ether. Finally, the desired product was isolated as an orange solid by

filtration (38 mg, 73% yield). ^1H NMR (CDCl_3 , 600 MHz) δ 9.74 (d, $J = 1.8$ Hz, 1 H), 9.00 (dd, $J = 9.0$ Hz, 1.8 Hz, 1 H), 8.81 (d, $J = 16.2$ Hz, 1 H), 8.66 (d, $J = 16.2$ Hz, 1 H), 8.33 (d, $J = 16.2$ Hz, 1 H), 8.09 (d, $J = 16.2$ Hz, 1 H), 7.59-7.49 (m, 8 H), 7.24 (d, $J = 9.0$ Hz, 2 H), 4.54 (s, 3 H), 4.47 (s, 3 H), 4.24 (t, $J = 6.0$ Hz, 2 H), 3.64 (s, 3 H), 2.36 (t, $J = 7.2$ Hz, 2 H), 1.98 (s, 3 H), 1.96-1.91 (m, 2 H), 1.84 (s, 6 H), 1.75-1.71 (m, 2 H), 1.59-1.55 (m, 2 H). ^{13}C NMR (CDCl_3 , 180 MHz) δ 183.4, 182.8, 173.8, 162.9, 154.4, 148.2, 143.8, 143.2, 141.4 (2 C), 139.7, 135.0, 130.2, 129.9, 129.8, 129.4, 128.2, 123.3, 122.8 (2 C), 114.7, 114.3, 114.2, 113.6, 112.5, 69.5, 52.9, 52.4, 51.7, 37.4, 33.9, 28.9, 28.2, 27.3, 27.0, 25.7, 24.7. LRMS calculated for $\text{C}_{39}\text{H}_{46}\text{N}_2\text{O}_3\text{I}$ ($\text{M}^{2+} + \text{I}^-$) $^+$ 717.3, found 717.1, and calculated for $\text{C}_{39}\text{H}_{46}\text{N}_2\text{O}_3$ (M) $^{2+}$ 295.2, found 295.3.

Synthesis of compound QCy7HA

A solution of compound QCy7MH in HCl (0.5 M, THF:H₂O, 1:1 v:v by volume) solution was stirred at room temperature for overnight. The solvent was removed under reduced pressure and the crude compound is directly used for the next step without further purification. LRMS calculated for $\text{C}_{38}\text{H}_{44}\text{N}_2\text{O}_{32}^+$ (M) $^{2+}$, 288.2, found 288.2.

Synthesis of compound QCy7HA-DOX

A mixture of QCy7HA (6.0 mg, 0.009 mmol), N-hydroxysuccinimide (NHS, 1.0 mg, 0.010 mmol), N, N'-dicyclohexylcarbodiimide (DCC, 3.0 mg, 0.013 mmol) in DMF:CH₂Cl₂ (2:1, 0.5 mL) solution was stirred at room temperature for overnight. Then doxorubicin hydrochloride (8.0 mg, 0.009 mmol) and Et₃N (3.0 μL) was added to the solution and stirred for additional overnight. The solvent was removed under reduced pressure and the residue was purified by reverse phase HPLC (20 mins gradient 10-90% solvent B) to give the desired product as an

orange solid (1.5 mg, 13% yield). LRMS calculated for $C_{65}H_{70}N_3O_{13}^+ (M - H)^+$, 1100.5, found 1100.2.

4. Confocal microscopy

Cell images were acquired using a confocal microscope (Fluo View TM 1000 Confocal Microscope). The emission of DOX and QCy7HA-DOX was collected through band pass filter set with excitation at 488 nm and emission at 582-622 nm. The fluorescence of MDR was obtained through the CY5 filter set (excitation: 638 nm, emission: 663–738 nm), whereas Hoechst 33342 taken via the DAPI filter set (excitation: 405 nm, emission: 425–475 nm).

5. Live cell co-localization staining and imaging under microscope

MES-SA cells (60,000 cells per well) were cultured on 35 mm Fluorodish Cell Culture Dish (World Precision Instruments, FD35-100) in complete media for 36 hrs. The cells were incubated in the presence of DOX (4 μ M) or QCy7HA-Dox (4 μ M) and MDR (300 nM) in the media for 1 hr in incubator. After three times of PBS washing, the cell nuclei were labeled by NucBlue Live ReadyProbes Reagent (Hoechst 33342, Life technologies, R37605) for 20 min at 25 °C. The cells were washed and kept in PBS buffer before the cell images were acquired using a confocal microscope.

6. MTT cytotoxicity assay

MES-SA and MES-SA/Dx5 cells (30,000 cells per well) were seeded in wells of 96-well plates and allowed to grow for 24 hrs at 37 °C. Cells were incubated for 72 hrs with drugs at concentrations ranging from 5×10^{-9} to 1×10^{-4} M, and every concentration was prepared 6 wells for improving the reliability of the studies. MTT dye (30 μ L, 2 mg/ml in PBS, Sigma Aldrich

M5655) was added to the cell growth media in each well. After 3 hrs of incubation, the medium was removed from each well and replaced with DMSO (100 μ L, Sigma Aldrich D8418). Fifteen minutes later, the absorbance at wavelength of 544 nm was measured using a FLUOstar OPTIMA (BMG LABTECH) for calculation of cell viability. The mean percentage of cell survival was defined to be relative to untreated cells.

7. Figure S1

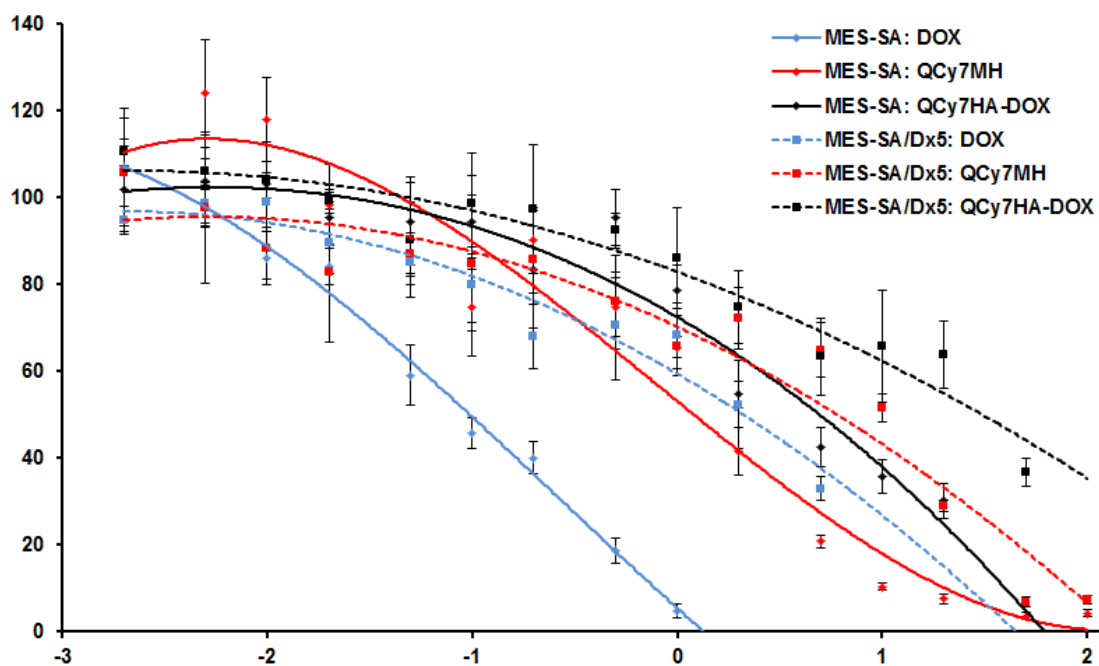
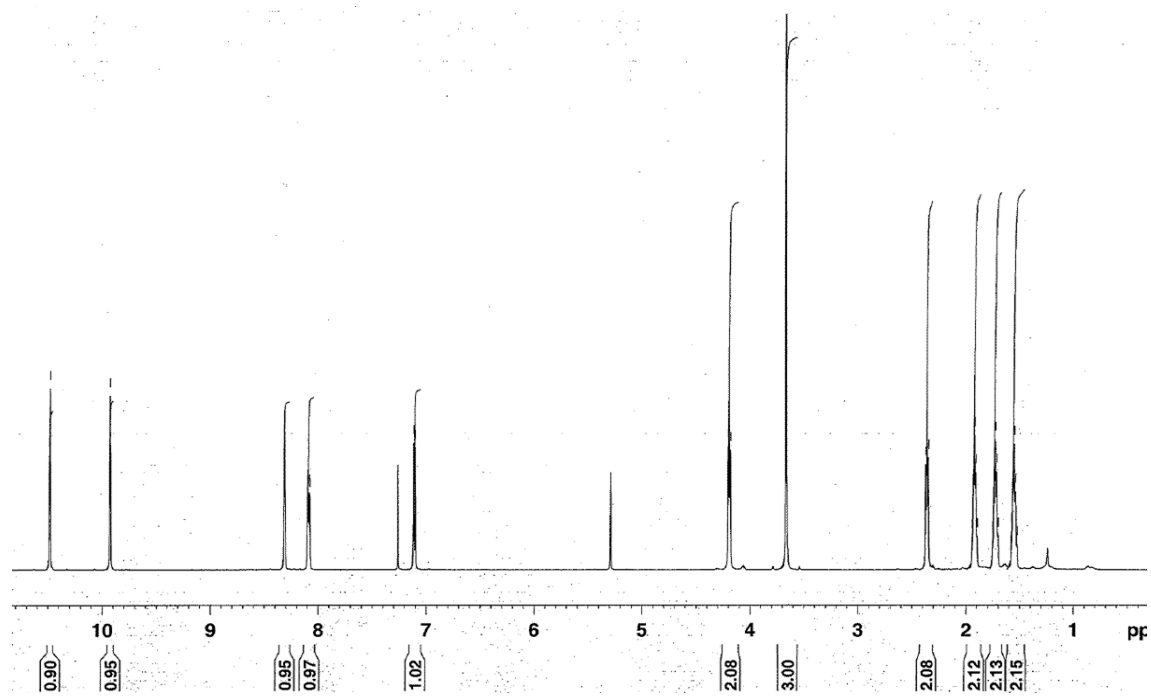
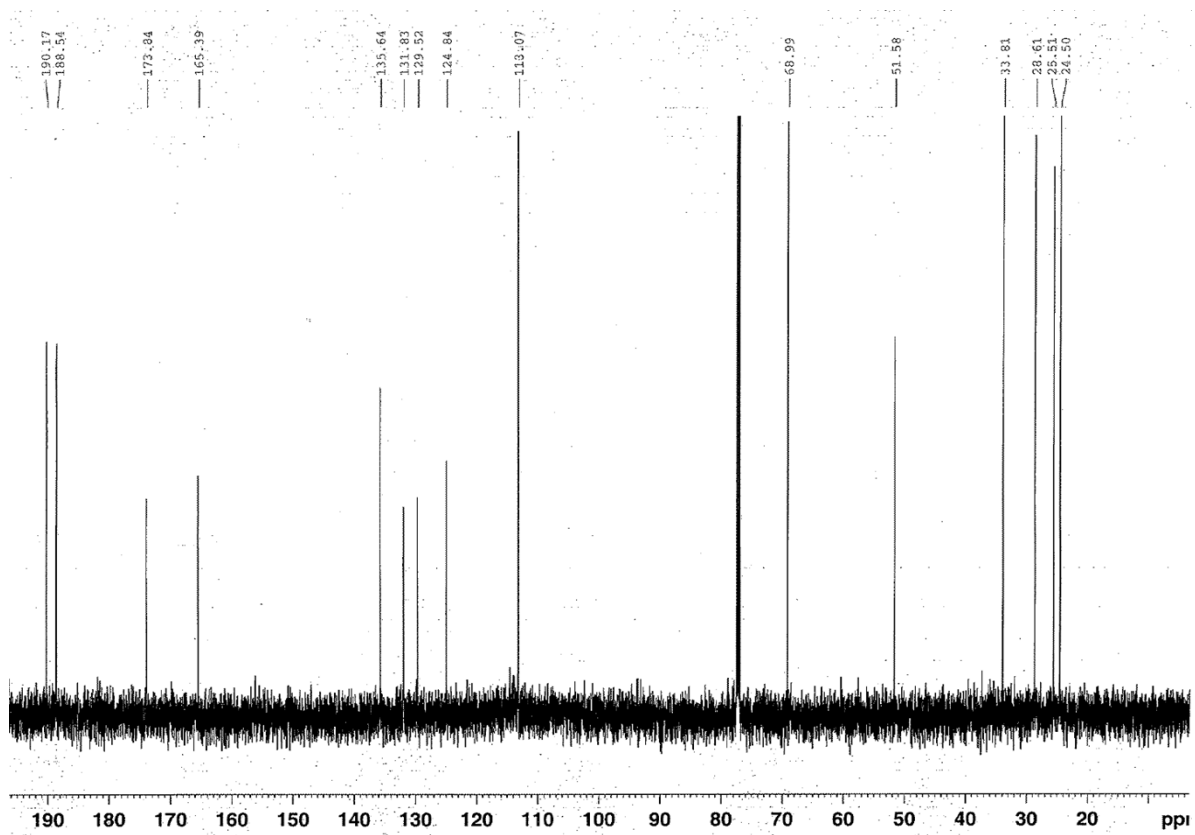


Figure S1. The toxicities of QCY7MH, DOX and QCy7HA-DOX at various concentrations to drug-sensitive MES-SA and multidrug-resistant MES-SA/Dx5 cells analysed by MTT assays. Error bars are the standard deviations from six measurements.

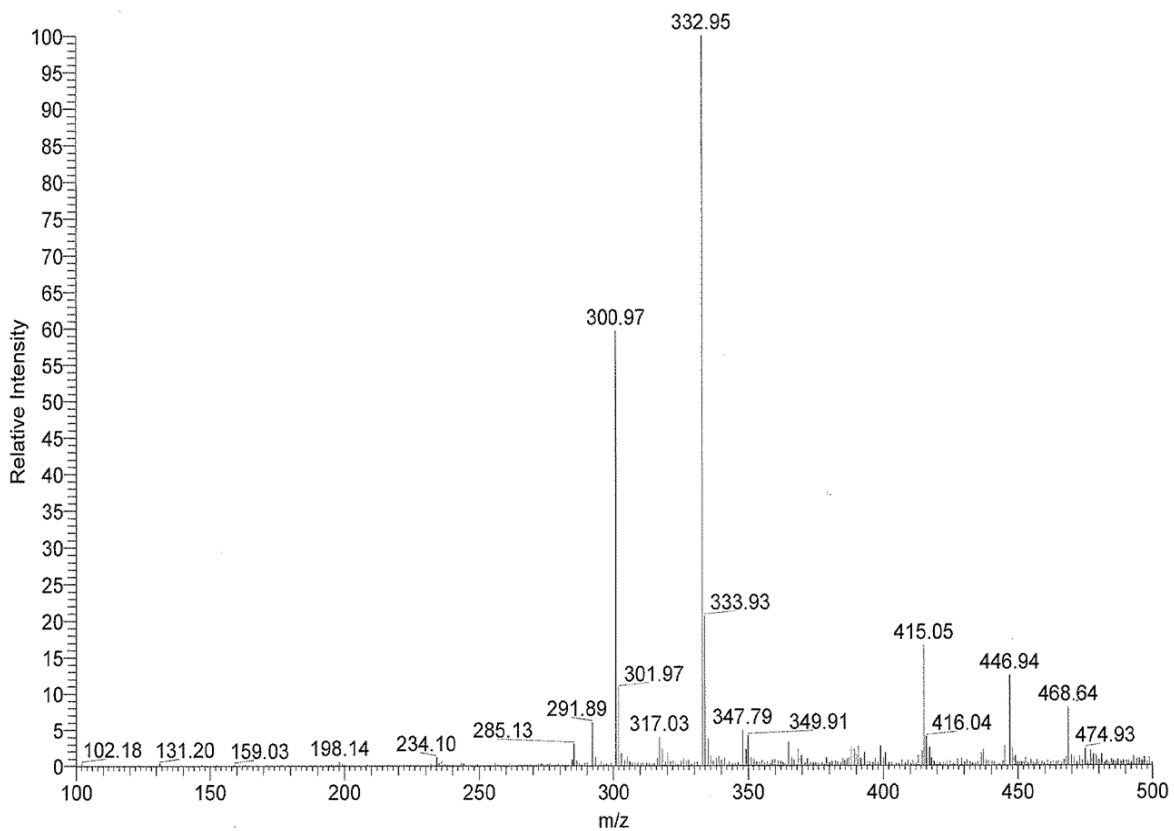
^1H NMR of compound 1 (500 MHz, CDCl_3)



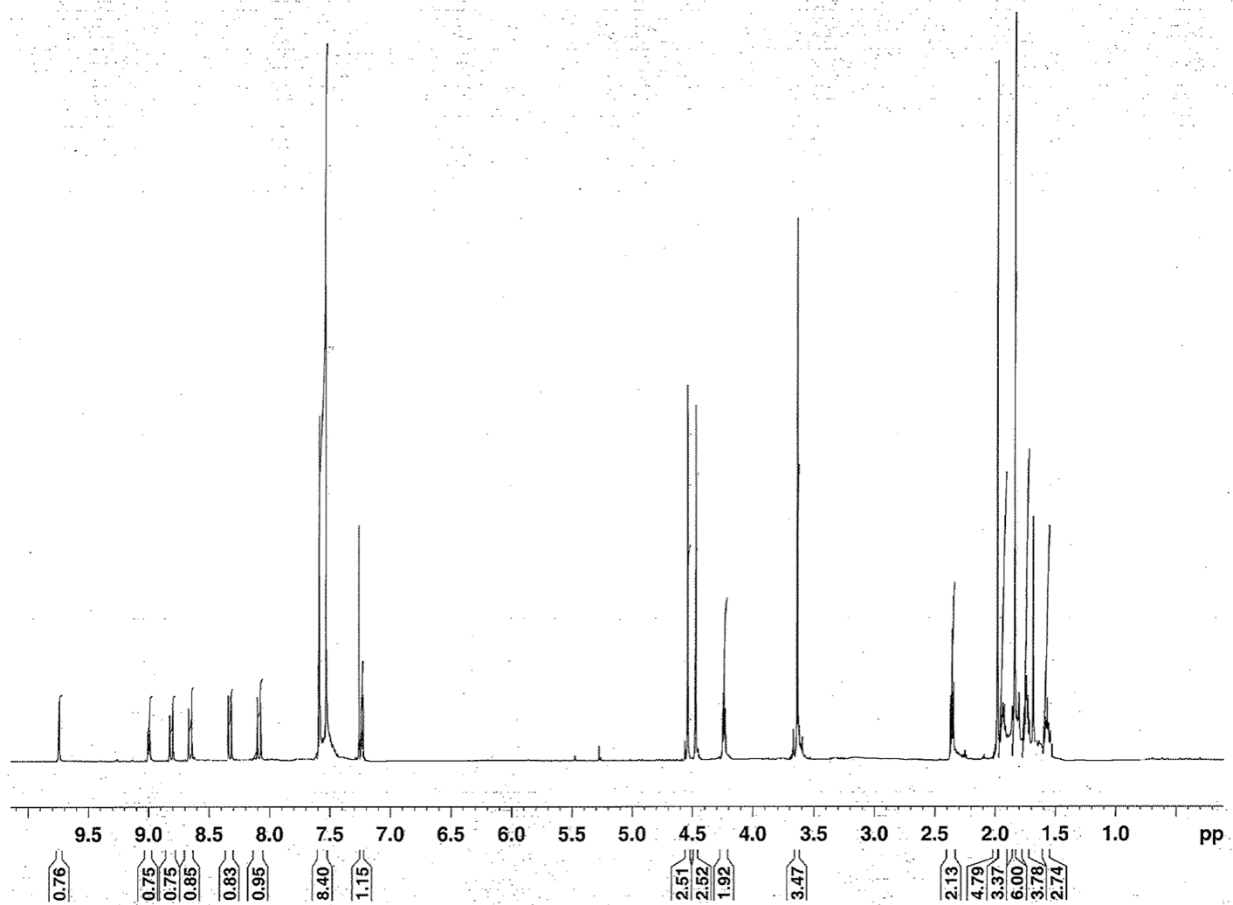
^{13}C NMR of compound 1 (125 MHz, CDCl_3)



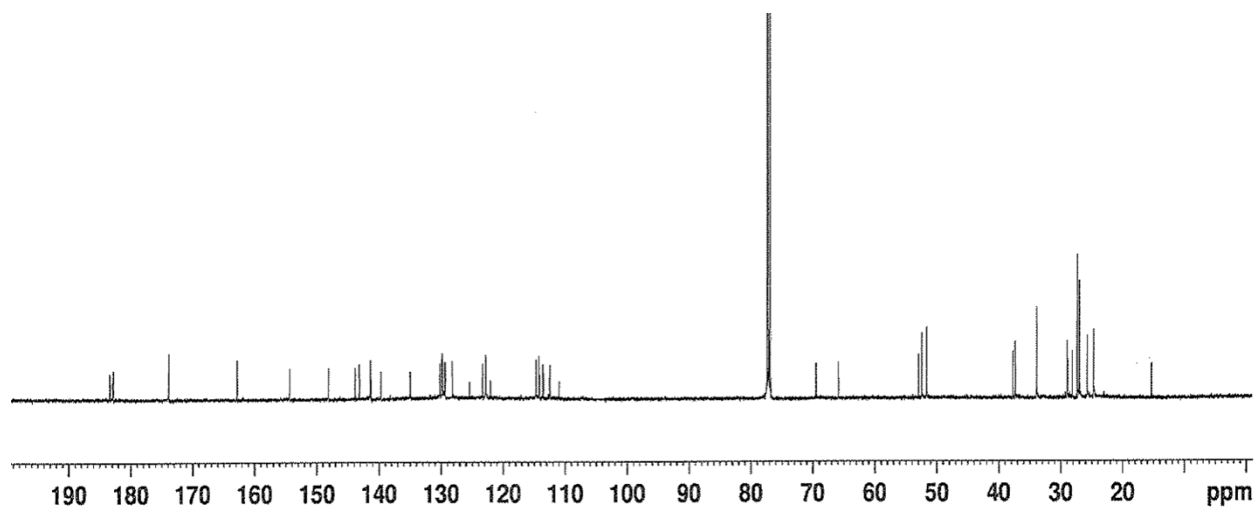
MS spectra of compound 1 (ESI)



^1H NMR of QCy7MH (CDCl_3 , 600 MHz)



^{13}C NMR of QCy7MH (CDCl_3 , 150 MHz)



MS spectra of QCy7MH (ESI)

