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**Supporting Information for**

# Syntheses and Evaluation of New Acridone Derivatives for Selective Binders of Oncogene *c-myc* Promoter i-Motif in Gene Transcriptional Regulation

Bing Shu, Jiaojiao Cao, Guotao Kuang, Jun Qiu, Meiling Zhang, Yan Zhang, Mingxue Wang, Xiaoya Li, Shuangshuang Kang, Tian-Miao Ou, Jia-Heng Tan, Zhi-Shu Huang, Ding Li*

* School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou University City, 132 Waihuan East Road, Guangzhou 510006, P. R. China

* corresponding author; Tel: 8620 3994 3058; E-mail: liding@mail.sysu.edu.cn

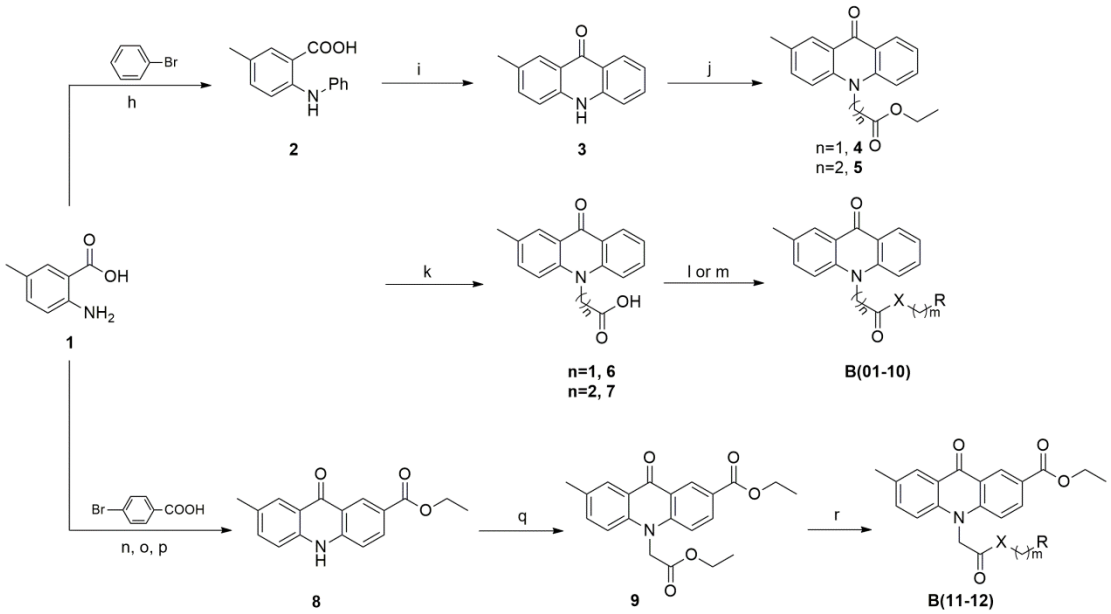
# Experiment methods and Results

## 1. Synthesis and characterization

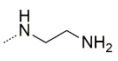
All chemicals and starting materials were purchased from commercial sources, which were analytical grade without further purification unless otherwise specified. All synthesized compounds were confirmed by $^{1}H$, $^{13}C$ NMR spectra and HRMS spectrometry. $^{1}H$ and $^{13}C$ NMR spectra were recorded using TMS as the internal standard in DMSO-$d_6$, $CD_3OD$, or $CDCl_3$ with a Bruker BioSpin GmbH spectrometer at 400 and 100 MHz, respectively. High resolution mass spectra (HRMS) were recorded on Shimadzu LCMS-IT-TOF of MAT95XP mass spectrometer (Thermo Fisher Scientific, USA). The purity of the synthesized compound was confirmed to be higher than 95% by using analytical HPLC performed with a dual pump Shimadzu LC-20 AB system equipped with an Ultimate XB-C18 column (4.6 mm × 250 mm, 5 μm), eluting with methanol-water (10:90 to 60:40) containing 0.05% TFA at a flow rate of 0.5 mL/min.

## 2. Syntheses of intermediates 2-10

The intermediates were prepared by following scheme **SI** and scheme **I**.




| | |
|---|---|
| B01 X = NH n = 1 m = 3, R = dimethylamino- | B07 X = NH n = 2 m = 2, R = morpholinyl- |
| B02 X = NH n = 1 m = 3, R = morpholinyl- | B08 X = NH n = 2 m = 2, R = dimethylamino- |
| B03 X = NH n = 1 m = 2, R = morpholinyl- | B09 X = 0 n = 1 m = 0, R = morpholinyl- |
| B04 X = NH n = 1 m = 2, R = diethylamine- | B10 X = O n = 1 m = 3, R = amino- |
| B05 X = NH n = 1 m = 2, R = ethylenediamine- | B11 X = NH m = 2, R = morpholinyl- |
| B06 X = NH n = 2 m = 2, R = (ethyl formate)-ly- | B12 X = NH m = 2, R = diethylamine- |

| R | dimethylamino- | morpholinyl- | ethylenediamine- | (ethyl formate)-ly- | diethylamine- |
|---|---|---|---|---|---|
| Structure |  |  |  |  |  |

**Scheme SI.** Reagents and conditions: (h) bromobenzene, Cu, CuI, $K_2CO_3$, DMF, 120 $^oC$, 12 h; (i) conc. $H_2SO_4$, 120 $^oC$, 3 h (yield, 38% for two steps); (j) ethyl 2-bromoacetate or ethyl 3-bromopropanoate, NaH, DMF, 0 $^oC$, 2 h (yield, 61-85%); (k) MeOH, 10 % NaOH, 60 $^oC$, 1 h (yield 88-91%); (l) TCM, $T_3P$, various alkylamine, r. t., 2 h (yield, 47-84%); (m) 1-amino-3-chloropropane hydrochloride, DMF, $K_2CO_3$, r. t., 4 h (yield, 39%); (n) 4-bromobenzoic acid, Cu, CuI, $K_2CO_3$, DMF, 120 $^oC$, 24 h; (o) conc. $H_2SO_4$, 130 $^oC$, 4 h; (p) EtOH, 80 $^oC$, 2 h (yield, 30% for three steps); (q) ethyl 2-bromoacetate, NaH, DMF, 0 $^oC$, 3 h (yield, 47%); (r) various alkylamine, 100 $^oC$, 1 h (yield, 58-69%).

2.1 5-methyl-2-(phenylamino)benzoic acid (**2**)

To a solution of 2-amino-5-methylbenzoic acid (**1**, 3.00 g, 19.9 mmol) in anhydrous dimethylformamide (40 mL), were added bromobenzene (9.36 g, 59.6 mmol), anhydrous potassium carbonate (4.11 g, 29.8 mmol), copper powder (300 mg), and copper iodide (100 mg). The mixture was heated under reflux in nitrogen atmosphere, monitored by using TLC. After cooling down, the mixture was poured into ice water and stirred, with pH adjusted to 3 - 4. Ethyl acetate (100 mL) was added and stirred for 10 mins, filtered and the residue was extracted with ethyl acetate (3×50 mL). The combined organic layer was washed with dilute hydrochloric acid, and then brine for three times, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give crude compound **2**, which was used for the next step without further purification.

2.2 2-methylacridin-9(10H)-one (**3**)

To a 100 mL round bottom flask containing residue **2**, was added 10 mL concentrated sulfuric acid. The mixture was stirred at 100 $^oC$ under nitrogen atmosphere for 2 hours, monitored by using TLC. After cooling down, the reaction mixture was poured into ice water and stirred, filtered and the solid was washed with saturated sodium bicarbonate, dried and purified by using chromatograph on silica gel with DCM/MeOH (100/1) to give **3** as a yellow solid (yield, 38% for two steps). $^1H$ NMR (400 MHz, DMSO) δ 11.70 (s, 1H), 8.23 (d, $J$ = 8.0 Hz, 1H), 8.03 (s, 1H), 7.72 (t, $J$ = 7.4 Hz, 1H), 7.62 – 7.50 (m, 2H), 7.47 (d, $J$ = 8.5 Hz, 1H), 7.24 (t, $J$ = 7.5 Hz, 1H), 2.43 (s, 3H). $^{13}C$ NMR (101 MHz, DMSO) δ 177.02, 141.24, 139.45, 135.42, 133.73, 130.58, 126.49, 125.54, 121.21, 120.83, 120.81, 117.80, 117.74, 21.09. ESI-MS (m/z) 210 $[M + H]^+$.

2.3 ethyl 2-(2-methyl-9-oxoacridin-10(9H)-yl)acetate (**4**)

To a solution of **3** (500 mg, 2.39 mmol) in anhydrous DMF (50 mL), was added NaH (200 mg, 60%, 4.78 mmol) carefully at 0 $^oC$. The mixture was stirred for 1 hour at 0 $^oC$. Then ethyl 2-bromoacetate (800 mg, 4.78 mmol) was added and stirred for 2 hours, monitored by using TLC. The reaction mixture was quenched with $NH_4Cl$ solution at 0 $^oC$. The organic layer was extracted with ethyl acetate (3×10 mL). The combined organic layer was washed with brine for three times,

dried over anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by using chromatograph on silica gel with EtOAc/hexanes (8/1) to give intermediate **4** as a yellow solid (yield, 85%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.57 (dd, *J* = 8.2, 1.7 Hz, 1H), 8.36 (d, *J* = 1.1 Hz, 1H), 7.70 (ddd, *J* = 8.7, 7.1, 1.7 Hz, 1H), 7.54 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.35 – 7.30 (m, 1H), 7.29 (d, *J* = 3.2 Hz, 1H), 7.23 (d, *J* = 8.7 Hz, 1H), 5.06 (s, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 2.48 (s, 3H), 1.30 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 178.11, 168.36, 142.19, 140.38, 135.4, 133.88, 131.54, 127.99, 127.32, 122.54, 122.48, 121.55, 114.17, 114.07, 62.12, 48.39, 20.58, 14.17. Purity: 97.9% by using HPLC. HRMS (ESI; m/z). Calcd for C18H17NO3, $[M + H]^+$ 296.1281, found 296.1279.

2.4 ethyl 3-(2-methyl-9-oxoacridin-10(9H)-yl)propanoate (**5**)

According to the procedure for **4**, replacing ethyl 2-bromoacetate with ethyl 3-bromopropanoate, a yellow solid **5** was obtained (yield, 61%). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.53 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.31 (d, *J* = 1.1 Hz, 1H), 7.68 (ddd, *J* = 8.7, 7.0, 1.8 Hz, 1H), 7.56 – 7.45 (m, 2H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.24 (ddd, *J* = 7.8, 7.0, 0.7 Hz, 1H), 4.65 (t, *J* = 8.0 Hz, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 2.86 (t, *J* = 8.0 Hz, 2H), 2.43 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3H). ESI-MS (m/z) 310 $[M + H]^+$.

2.5 2-(2-methyl-9-oxoacridin-10(9H)-yl)acetic acid (**6**)

To a solution of **6** (300 mg, 1.01 mmol) in MeOH (10 mL), was added 5 mL 10% sodium hydroxide solution. The mixture was stirred at 60 $^{o}$C for 1 hour until TLC indicated completion of reaction. After cooling down, the mixture was concentrated and 10 mL ice water was added. The residue was stirred and pH was adjusted to 5 - 6. The mixture was filtered and the solid was washed with $H_2O$, dried to give intermediate **6** as a light-yellow solid (yield, 90 %). $^{1}$H NMR (400 MHz, DMSO-d6) δ 8.33 (d, *J* = 7.9 Hz, 1H), 8.11 (d, *J* = 13.8 Hz, 1H), 7.79 – 7.72 (m, 1H), 7.66 – 7.53 (m, 3H), 7.34 – 7.26 (m, 1H), 5.11 (s, 2H), 2.43 (s, 3H). Purity: 98.1% by using HPLC. HRMS (ESI; m/z). Calcd for C16H13NO3, $[M + H]^+$, 268.0968; found, 268.0968.

2.6 3-(2-methyl-9-oxoacridin-10(9H)-yl)propanoic acid (**7**)

According to the procedure for **6**, a light-yellow solid **7** was obtained with a yield of 88%, which was used for the next step immediately.

2.7 ethyl 7-methyl-9-oxo-9,10-dihydroacridine-2-carboxylate (**8**)

According to the procedure for **2**, replacing bromobenzene with 4-bromobenzoic acid, a crude mixture was obtained. The mixture was stirred in 12 mL concentrated sulfuric acid. The mixture was stirred at 120 $^{o}$C under nitrogen atmosphere for 3 hours. The reaction was monitored by using TLC. After cooling down, ethanol (50 mL) was added dropwise at 0 $^{o}$C. The mixture was heated under reflux in nitrogen atmosphere for another 2 hours. After cooling down, the reaction mixture was poured into ice water, stirred, and filtered. The solid was washed by using saturated sodium bicarbonate, dried, and purified by using chromatograph on silica gel with DCM/MeOH (80/1) to

give **8** as a yellow solid (yield, 30% for three steps). $^{1}$H NMR (400 MHz, DMSO-d6) δ 12.01 (s, 1H), 8.83 (s, 1H), 8.19 (dd, *J* = 8.7, 1.8 Hz, 1H), 8.04 (s, 1H), 7.62 (d, *J* = 8.5 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 2.43 (s, 3H), 1.37 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, DMSO-d6) δ 176.49, 165.28, 143.45, 138.77, 135.38, 132.71, 131.23, 128.61, 125.17, 121.75, 120.77, 119.43, 117.70, 117.60, 60.59, 20.53, 14.23. ESI-MS (m/z) 282 $[M + H]^+$.

2.8 ethyl 10-(2-ethoxy-2-oxoethyl)-7-methyl-9-oxo-9,10-dihydroacridine-2- carboxylate (**9**)

According to the procedure for **4**, replacing **3** with **8**, a yellow solid **9** was obtained with a yield of 47%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.17 (d, *J* = 2.1 Hz, 1H), 8.32 (d, *J* = 1.3 Hz, 1H), 8.29 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.54 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.30 (d, *J* = 9.0 Hz, 1H), 7.23 (d, *J* = 8.7 Hz, 1H), 5.07 (s, 2H), 4.42 (q, *J* = 7.1 Hz, 2H), 4.31 (q, *J* = 7.1 Hz, 2H), 2.47 (s, 3H), 1.44 (t, *J* = 7.1 Hz, 3H), 1.29 (t, *J* = 7.1 Hz, 4H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.78, 167.93, 165.91, 144.72, 140.18, 135.79, 134.29, 132.54, 130.38, 127.50, 123.62, 122.79, 121.80, 114.43, 114.32, 62.34, 61.13, 48.55, 20.62, 14.44, 14.18. ESI-MS (m/z) 368 $[M + H]^+$.

2.9 4,4'-((2-carboxy-4-methylphenyl)azanediyl)dibenzoic acid (**10**)

According to the procedure for **2**, replacing bromobenzene with 4-iodobenzoic acid, a crude intermediate **10** was obtained and used for the next step without further purification.

## 3. Syntheses of acridone derivatives

3.1. General procedure A: preparation of **B(01-09)**

To a solution of **6** (50 mg, 0.19 mmol) or **7** (50 mg, 0.18 mmol) in anhydrous trichloromethane (5 mL), was added 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide (T3P, 71.4 mg, 0.22 mmol), and then various alkylamine (1.5 eq.). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 4 h until TLC indicated completion of reaction. The reaction was quenched with 10 mL ice water, and the organic layer was extracted with dichloromethane (3×10 mL). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by using chromatograph on silica gel with DCM/MeOH (10/1 - 5/1) to give **B(01-09)**.

*N*-(3-(dimethylamino)propyl)-2-(2-methyl-9-oxoacridin-10(9H)-yl)acetamide (**B01**). A yellow solid was obtained with a yield of 84%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.56 (d, J = 7.9 Hz, 1H), 8.35 (s, 1H), 7.74 (t, J = 7.7 Hz, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.38 (d, J = 8.7 Hz, 1H), 7.35 – 7.27 (m, 2H), 4.92 (s, 2H), 3.39 (q, J = 5.1 Hz, 2H), 2.48 (s, 3H), 2.05 (t, J = 5.1 Hz, 2H), 1.44-1.38 (m, 2H), 1.15 (s, 6H). $^{13}$C NMR (101 MHz, CDCl3) δ 178.07, 167.21, 142.18, 140.37, 135.78, 134.25, 131.93, 127.92, 127.19, 112.51, 112.47, 121.88, 114.69, 114.55, 59.71, 51.37, 44.00, 41.50, 29.72, 23.43, 20.65. Purity was determined to be 100% by using HPLC. HRMS (ESI; m/z). Calcd for C21H25N3O2, $[M + H]^+$, 352.2020; found, 352.2014.

2-(2-methyl-9-oxoacridin-10(9H)-yl)-*N*-(3-morpholinopropyl)acetamide (**B02**). A yellow solid was obtained with a yield of 57%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.22 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.01 (d, *J* = 1.2 Hz, 1H), 7.71 (ddd, *J* = 8.7, 7.0, 1.7 Hz, 1H), 7.54 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 7.23 – 7.18 (m, 1H), 4.93 (s, 2H), 3.44 (t, *J* = 6.8 Hz, 2H), 3.31 (q, *J* = 4.6 Hz, 4H), 2.41 (s, 3H), 2.26 (t, *J* = 6.8 Hz, 2H), 2.18 (t, *J* = 4.6 Hz, 4H), 1.73 – 1.64 (m, 2H). $^{13}$C NMR (101 MHz, CDCl3) δ 177.77, 167.41, 142.17, 140.36, 135.78, 134.18, 131.99, 127.83, 127.14, 122.42, 122.37, 122.03, 114.41 (d, J = 10.6 Hz), 66.45, 56.75, 53.64, 51.75, 38.70, 25.16, 20.67. Purity was determined to be 98.1% by using HPLC. HRMS (ESI; m/z). Calcd for C23H27N3O3, $[M + H]^+$, 394.2123; found, 394.2125.

2-(2-methyl-9-oxoacridin-10(9H)-yl)-*N*-(2-morpholinoethyl)acetamide (**B03**). A yellow solid was obtained with a yield of 73%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.55 (dd, J = 8.0, 1.6 Hz, 1H), 8.34 (d, J = 1.1 Hz, 1H), 7.73 (ddd, J = 8.7, 7.0, 1.7 Hz, 1H), 7.57 (dd, J = 8.7, 2.2 Hz, 1H), 7.38 (d, J = 8.7 Hz, 1H), 7.36 – 7.29 (m, 2H), 4.98 (s, 2H), 3.32 (q, J = 8.4 Hz, 2H), 3.14 (t, J = 6.5 Hz, 4H), 2.48 (s, 3H), 2.28 (t, J = 8.4 Hz, 2H), 2.10 (t, J = 6.5 Hz, 4H). $^{13}$C NMR (101 MHz, CDCl3) δ 177.72, 167.05, 141.89, 140.06, 135.69, 134.15, 132.05, 128.06, 127.38, 122.52, 122.47, 122.01, 114.37, 114.24, 66.55, 56.22, 52.85, 50.96, 35.58, 20.60. Purity was determined to be 100% by using HPLC. HRMS (ESI; m/z). Calcd for C22H25N3O3, $[M + H]^+$, 380.1896; found, 380. 1969.

*N*-(2-(diethylamino)ethyl)-2-(2-methyl-9-oxoacridin-10(9H)-yl)acetamide (**B04**). A yellow solid was obtained with a yield of 61%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 7.8 Hz, 1H), 8.34 (s, 1H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.37 – 7.30 (m, 2H), 7.05 (s, 1H), 5.00 (s, 2H), 3.31 (q, *J* = 5.5 Hz, 2H), 2.48 (s, 3H), 2.41 (t, *J* = 5.9 Hz, 2H), 2.20 (q, *J* = 6.9 Hz, 4H), 0.52 (t, *J* = 6.9 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.98, 167.02, 141.96, 140.14, 135.72, 134.20, 131.96, 128.05, 127.37, 122.54, 122.49, 121.92, 114.38, 114.38, 50.84, 50.78, 46.33, 36.35, 20.60, 11.24. Purity was determined to be 98.0% by using HPLC. HRMS (ESI; m/z). Calcd for C22H27N3O2, $[M + H]^+$, 366.2176; found, 366.2171.

*N*-(2-((2-aminoethyl)amino)ethyl)-2-(2-methyl-9-oxoacridin-10(9H)-yl)acetamide (**B05**). A yellow solid was obtained with a yield of 80%. $^{1}$H NMR (400 MHz, DMSO) δ 8.40 (t, J = 8.1 Hz, 1H), 8.34 (dd, J = 8.0, 1.4 Hz, 1H), 8.13 (s, 1H), 7.77 (dd, J = 11.3, 4.3 Hz, 1H), 7.62 (dd, J = 16.1, 5.5 Hz, 2H), 7.54 (d, J = 8.8 Hz, 1H), 7.31 (t, J = 7.4 Hz, 1H), 5.14 (s, 2H), 3.20 (q, *J* = 5.4 Hz, 2H), 2.69 – 2.52 (m, 6H), 2.44 (s, 3H). $^{13}$C NMR (101 MHz, CDCl3) δ 178.54, 168.15, 142.30, 140.53, 135.88, 134.27, 131.88, 127.56, 126.84, 122.17, 122.14, 121.84, 114.59, 114.49, 50.38, 50.18, 48.05, 40.35, 38.87, 20.46. Purity was determined to be 93.7% by using HPLC. HRMS (ESI; m/z). Calcd for C20H24N4O2, $[M + H]^+$, 353.1972; found, 353.2049.

ethyl 3-(2-(2-methyl-9-oxoacridin-10(9H)-yl)acetamido)propanoate (**B06**). A yellow solid was obtained with a yield of 47%. $^{1}$H NMR (400 MHz, CDCl3) δ 8.32 (dd, J = 8.0, 1.6 Hz, 1H), 8.11 (s, 1H), 7.74 – 7.69 (m, 1H), 7.56 – 7.53 (m, 1H), 7.33 (d, J = 8.6 Hz, 1H), 7.24 (dd, J = 11.3, 4.4

Hz, 1H), 6.92 (t, J = 5.6 Hz, 1H), 4.95 (s, 2H), 3.91 (q, J = 7.1 Hz, 2H), 3.60 (q, J = 6.3 Hz, 2H), 2.54 (t, J = 6.3 Hz, 2H), 2.44 (s, 3H), 1.09 (t, J = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.81, 171.53, 167.40, 142.01, 140.18, 135.70, 134.12, 131.94, 128.79, 127.94, 127.30, 122.43, 121.96, 114.27, 114.17, 60.67, 51.27, 35.23, 33.89, 20.57, 13.96. Purity was determined to be 95.1% by using HPLC. HRMS (ESI; m/z). Calcd for C21H22N2O4, $[M + H]^+$, 367.1650; found, 367.1652.

3-(2-methyl-9-oxoacridin-10(9H)-yl)-*N*-(2-morpholinoethyl)propanamide (**B07**). A yellow solid was obtained with a yield of 54%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.50 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.28 (d, *J* = 0.7 Hz, 1H), 7.66 (ddd, *J* = 8.7, 6.9, 1.7 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.47 (dd, *J* = 8.2, 5.4 Hz, 2H), 7.26 – 7.19 (m, 1H), 6.39 (s, 1H), 4.72 (t, *J* = 7.4 Hz, 2H), 3.59 (t, *J* = 4.6 Hz, 4H), 3.34 (q, *J* = 7.4 Hz,2H), 2.74 (t, *J* = 7.4 Hz, 2H), 2.42 (s, 3H), 2.39 – 2.33 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.74, 169.83, 141.35, 139.56, 135.51, 133.90, 131.15, 127.91, 127.16, 122.25, 122.24, 121.17, 114.53, 114.41, 66.76(2), 56.78, 53.26(2), 41.84, 35.80, 34.10, 20.54. Purity was determined to be 99.4% by using HPLC. HRMS (ESI; m/z). Calcd for C23H27N3O3, $[M + H]^+$, 394.2125; found, 394.2121.

*N*-(3-(dimethylamino)propyl)-3-(2-methyl-9-oxoacridin-10(9H)-yl)propanamide (**B08**). A yellow solid was obtained with a yield of 47%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.55 (dd, *J* = 8.0, 1.3 Hz, 1H), 8.34 (s, 1H), 7.78 – 7.69 (m, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.59 – 7.50 (m, 2H), 7.27 (t, *J* = 7.4 Hz, 1H), 4.75 (t, *J* = 6.3 Hz, 2H), 3.34 (q, *J* = 7.5 Hz, 2H), 2.74 (t, *J* = 7.8 Hz, 2H), 2.46 (s, 3H), 2.39 (t, *J* = 6.3 Hz, 2H), 2.20 (s, 6H), 1.68 – 1.58 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 182.39, 174.82, 145.39, 143.68, 139.97, 138.28, 135.42, 131.45, 130.67, 125.84, 125.39, 122.27, 118.81, 118.70, 60.41, 48.21, 46.30, 41.28, 37.75, 33.53, 24.31. Purity was determined to be 99.1% by using HPLC. HRMS (ESI; m/z). Calcd for C22H27N3O2, $[M + H]^+$, 366.2176; found, 366.2172.

2-methyl-10-(2-morpholino-2-oxoethyl)acridin-9(10H)-one (**B09**). A yellow solid was obtained with a yield of 39%. $^1$H NMR (400 MHz, DMSO) δ 8.34 (dd, *J* = 8.0, 1.5 Hz, 1H), 8.13 (s, 1H), 7.81 – 7.72 (m, 1H), 7.61 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.31 (t, *J* = 7.4 Hz, 1H), 5.48 (s, 2H), 3.82 – 3.72 (m, 4H), 3.64 (t, J = 4.5 Hz, 2H), 3.49 (t, J = 4.5 Hz, 2H), 2.44 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) δ 176.55, 165.05, 142.33, 140.64, 135.22, 133.74, 130.47, 126.43, 125.64, 121.43, 121.41, 121.01, 116.09, 115.94, 66.11, 47.19, 46.80, 44.85, 41.97, 20.17. Purity was determined to be 100% by HPLC. HRMS (ESI; m/z). Calcd for C20H20N2O3, $[M + H]^+$, 337.1543; found, 337.1547.

3.2. General procedure B: preparation of 3-aminopropyl 2-(2-methyl-9-oxoacridin-10(9H)-yl) acetate (**B10)**

To a solution of **6** (50 mg, 0.19 mmol) in anhydrous dimethylformamide (5 mL) was added 1-amino-3-chloropropane hydrochloride (73.0 mg, 0.56 mmol), and then potassium carbonate

(129 mg, 0.93 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere overnight. The reaction mixture was quenched with 10 mL ice water, and extracted with ethyl acetate (3×10 mL). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure. The residue was purified by using chromatograph on silica gel with DCM/MeOH (10/1) to give desire compound **B10** as a yellow solid with a yield of 37%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 7.9 Hz, 1H), 8.12 (s, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.26 (s, 1H), 7.23 (d, *J* = 7.8 Hz, 1H), 4.94 (s, 2H), 3.44 (t, *J* = 5.4 Hz, 2H), 3.32 (t, *J* = 5.4 Hz, 2H), 2.39 (s, 3H), 1.68 – 1.48 (m, 3H). Purity was determined to be 96.7% by using HPLC. HRMS (ESI; m/z). Calcd for C19H20N2O3, $[M + H]^+$, 325.1547; found, 325.1545.

3.3. General procedure C: preparation of **B(11-12)**

To a 25 mL round bottomed flask containing intermediate **9** (50 mg, 0.13 mmol) was added various alkylamine (10 eq.). The mixture was stirred at 100 $^{o}$C under nitrogen atmosphere for 2 hours. The reaction was monitored by using TLC. After cooling down, ice water (5 mL) was added and stirred, followed with addition of dichloromethane (10 mL). The organic layer was extracted with dichloromethane (3 × 10 mL), and the combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by using chromatograph on silica gel with DCM/MeOH (5/1) to give desire compound.

ethyl 7-methyl-10-(2-((2-morpholinoethyl)amino)-2-oxoethyl)-9-oxo-9,10-dihydroacridine-2-carboxylate (**B11**). A yellow solid was obtained with a yield of 69%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.88 (d, *J* = 2.1 Hz, 1H), 8.30 (dd, *J* = 9.0, 2.2 Hz, 1H), 8.04 (s, 1H), 7.54 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.39 (d, *J* = 9.0 Hz, 1H), 7.31 (d, *J* = 8.7 Hz, 1H), 7.01 (s, 1H), 4.98 (s, 2H), 4.42 (q, *J* = 7.1 Hz, 2H), 3.42 (q, *J* = 5.6 Hz, 2H), 3.28 (t, *J* = 4.4 Hz, 4H), 2.41 (s, 3H), 2.38 (t, *J* = 5.6 Hz, 2H), 2.22 (t, *J* = 4.4 Hz, 4H), 1.45 (t, *J* = 7.1 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.38, 166.61, 165.49, 144.51, 139.99, 136.03, 134.47, 132.87, 130.09, 127.25, 123.93, 122.57, 121.57, 114.73, 114.55, 66.68(2), 61.25, 56.53, 53.03, 51.31(2), 35.84, 20.61, 14.44. Purity was determined to be 97.7% by using HPLC. HRMS (ESI; m/z). Calcd for C25H29N3O5, $[M + H]^+$, 452.2180; found, 452.2178.

ethyl 10-(2-((2-(diethylamino)ethyl)amino)-2-oxoethyl)-7-methyl-9-oxo-9,10-dihydroacridine-2-carboxylate (**B12**). A yellow solid was obtained with a yield of 58%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 9.04 (d, *J* = 1.6 Hz, 1H), 8.32 (dd, *J* = 9.0, 1.7 Hz, 1H), 8.19 (s, 1H), 7.56 (d, *J* = 7.4 Hz, 1H), 7.41 (d, *J* = 9.0 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 1H), 7.14 (s, 1H), 5.00 (s, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 3.34 (q, *J* = 6.0 Hz, 2H), 2.45 (s, 3H), 2.42 (t, *J* = 6.0 Hz, 2H), 2.21 (q, *J* = 7.0 Hz, 4H), 1.45 (t, *J* = 7.1 Hz, 3H), 0.55 (t, *J* = 7.0 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.56, 166.47, 165.70, 144.55, 140.00, 136.04, 134.50, 132.85, 130.25, 127.37, 123.86, 122.69, 121.70, 114.75, 114.57, 61.20,

50.84, 46.35, 36.60, 20.59, 18.67, 14.42, 11.43. Purity was determined to be 97.6% by using HPLC. HRMS (ESI; m/z). Calcd for C25H31N3O4, $[M + H]^+$, 438.2387; found, 438.2390.

3.4. Synthesis of ethyl 10-(4-(ethoxycarbonyl)phenyl)-7-methyl-9-oxo-9,10-dihydroacridine-2-carboxylate (**B13**)

To a 100 mL round bottom flask containing residue **10** (4.0 g, crude) was added 12 mL concentrated sulfuric acid. The mixture was stirred at 120 $^oC$ under nitrogen atmosphere for 3 hours. The reaction was monitored by using TLC. After cooling down, ethanol (50 mL) was added dropwise at 0 $^oC$. The mixture was heated under reflux in nitrogen atmosphere for another 2 hours. After cooling down, the reaction mixture was poured into ice water, stirred and filtered. The solid was washed with saturated sodium bicarbonate, dried, and purified by using chromatograph on silica gel with EtOAc/hexanes (10/1 - 3/1) to give **B13** as a yellow solid with a yield of 17% for three steps. $^1H$ NMR (400 MHz, $CDCl_3$) δ 9.25 (d, *J* = 2.0 Hz, 1H), 8.43 (d, *J* = 8.3 Hz, 2H), 8.39 (s, 1H), 8.12 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.36 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.74 (d, *J* = 9.0 Hz, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 4.52 (q, *J* = 7.1 Hz, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 2.49 (s, 3H), 1.50 (t, *J* = 7.1 Hz, 3H), 1.45 (t, *J* = 7.1 Hz, 3H). $^{13}C$ NMR (101 MHz, $CDCl_3$) δ 177.64, 165.94, 165.40, 145.01, 142.64, 140.81, 135.21, 133.56, 132.51, 132.11, 130.13, 130.08, 126.87, 123.63, 122.08, 121.00, 116.75, 116.46, 114.28, 61.65, 61.06, 20.66, 14.39, 14.35. Purity was determined to be 95.9% by using HPLC. HRMS (ESI; m/z). Calcd for C26H23NO5, $[M + H]^+$, 430.1649; found, 430.1644.

3.5. Synthesis of 10-(4-carboxyphenyl)-7-methyl-9-oxo-9,10-dihydroacridine-2-carboxylic acid (**B14**).

According to the procedure for **6**, a light yellow solid **B14** was obtained with a yield of 91%. $^1H$ NMR (400 MHz, DMSO) δ 13.21 (s, 2H), 8.93 (s, 1H), 8.32 (d, *J* = 8.2 Hz, 2H), 8.17 (s, 1H), 8.08 (d, *J* = 9.2 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 1H), 6.81 (t, *J* = 9.2 Hz, 1H), 6.67 (d, *J* = 8.7 Hz, 1H), 2.42 (s, 3H). Purity was determined to be 95.5% by using HPLC. HRMS (ESI; m/z). Calcd for C22H15NO5, $[M + H]^+$, 374.1023; found, 374.1023.

3.6. General procedure D: preparation of **B(15-16)**

To a 25 mL round bottom flask containing intermediate **B13** (50 mg, 0.11 mmol) was added 2-morpholinoethanamine (10 eq.). The mixture was stirred at 120 $^oC$ under nitrogen atmosphere for 4 hours. The reaction was monitored by using TLC. After cooling down, ice water (5 mL) was added and stirred, followed with addition of ethyl acetate (10 mL). The organic layer was extracted with ethyl acetate (3 × 10 mL), and the combined extract was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by using chromatograph on silica gel with DCM/MeOH (40/1 - 10/1) to give two desire compounds.

ethyl 4-(2-methyl-7-((2-morpholinoethyl)carbamoyl)-9-oxoacridin-10(9H)-yl)benzoate (**B15**). A yellow solid was obtained with a yield of 35%. $^1H$ NMR (400 MHz, $CDCl_3$) δ 9.21 (s, 1H), 8.34

(s, 1H), 8.15 (d, *J* = 7.8 Hz, 2H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.49 (d, *J* = 7.8 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 1H), 7.02 (s, 1H), 6.73 (d, *J* = 9.0 Hz, 1H), 6.63 (d, *J* = 8.7 Hz, 1H), 4.40 (q, *J* = 7.0 Hz, 2H), 3.77 (t, *J* = 7.1Hz, 4H), 3.66 (q, *J* = 5.6 Hz, 2H), 2.69 (t, *J* = 5.6 Hz, 2H), 2.59 (t, *J* = 7.1 Hz, 4H), 2.45 (s, 3H), 1.42 (t, *J* = 7.0 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.69, 166.09, 165.96, 145.09, 141.38, 140.89, 136.18, 135.23, 133.55, 132.53, 130.34, 130.09, 129.97, 126.86, 123.60, 122.07, 120.99, 116.81, 116.52, 66.96, 61.10, 56.95, 53.41, 36.27, 20.68, 14.41. Purity was determined to be 99.6% by using HPLC. HRMS (ESI; m/z). Calcd for C30H31N3O5, $[M + H]^+$, 514.2336; found, 514.2330.

ethyl 7-methyl-10-(4-((2-morpholinoethyl)carbamoyl)phenyl)-9-oxo-9,10-dihydroacridine-2-carboxylate (**B16**). A yellow solid was obtained with a yield of 38%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.41 (s, 1H), 8.39 (d, *J* = 5.2 Hz, 2H), 8.09 (d, *J* = 9.0 Hz, 1H), 7.47 (d, *J* = 7.7 Hz, 2H), 7.36 (d, *J* = 8.7 Hz, 1H), 7.02 (s, 1H), 6.78 (d, *J* = 9.0 Hz, 1H), 6.65 (d, *J* = 8.7 Hz, 1H), 4.49 (q, *J* = 7.0 Hz, 2H), 3.78 (t, *J* = 7.0 Hz, 4H), 3.61 (q, *J* = 5.3 Hz, 2H), 2.66 (t, *J* = 5.3 Hz, 2H), 2.56 (t, *J* = 7.0 Hz, 4H), 2.48 (s, 3H), 1.47 (t, *J* = 7.0 Hz, 4H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.80, 166.33, 165.37, 144.21, 142.57, 140.86, 135.34, 132.85, 132.51, 132.49, 132.16, 130.10, 127.51, 126.76, 125.26, 121.89, 120.69, 117.00, 116.78, 66.84, 61.66, 57.18, 53.45, 36.29, 20.67, 14.34. Purity was determined to be 95.0% by using HPLC. HRMS (ESI; m/z). Calcd for C30H31N3O5, $[M + H]^+$, 514.2336; found, 514.2330.

3.7. General procedure E: preparation of **B(17-21)**

The procedure was similar to general preparation of **B(01-09)** except replacing intermediate **6** with **B14**. To a solution of **B14** (50 mg, 0.13 mmol) in anhydrous trichloromethane (5 mL) was added 2,4,6-tripropyl-1,3,5,2,4,6-trioxatriphosphorinane-2,4,6-trioxide ($T_3P$, 71.4 mg, 0.22 mmol), and then anhydrous alkylamine (1.5 eq.). The mixture was stirred and monitored by using TLC. The reaction mixture was quenched with 10 mL ice water, and extracted with dichloromethane (3×10 mL). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure. The residue was purified by using chromatograph on silica gel with DCM/MeOH (60/1 - 20/1) to give desire compound.

2-methyl-7-(morpholine-4-carbonyl)-10-(4-(morpholine-4-carbonyl)phenyl)acridin-9(10H)-one (**B17**). A yellow solid was obtained with a yield of 71%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 8.37 (s, 1H), 7.78 (d, *J* = 8.0 Hz, 2H), 7.65 (d, *J* = 8.8 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 8.7 Hz, 1H), 6.79 (d, *J* = 8.8 Hz, 1H), 6.66 (d, *J* = 8.7 Hz, 1H), 3.92 – 3.59 (m, 16H), 2.47 (s, 3H). Purity was determined to be 94.7% by using HPLC. HRMS (ESI; m/z). Calcd for C30H29N3O5, $[M + H]^+$, 512.2180; found, 512.2177.

7-methyl-*N*-(2-morpholinoethyl)-10-(4-((2-morpholinoethyl)carbamoyl)phenyl)-9-oxo-9,10-dihydroacridine-2-carboxamide (**B18**). A yellow solid was obtained with a yield of 78%. $^1$H NMR

(400 MHz, $CDCl_3$) δ 8.80 (d, *J* = 2.1 Hz, 1H), 8.23 (d, *J* = 1.0 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 2H), 8.01 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.34 (dd, *J* = 9.6, 3.5 Hz, 1H), 7.30 (d, *J* = 1.0 Hz, 1H), 7.25 (t, *J* = 4.9 Hz, 1H), 6.73 (d, *J* = 9.0 Hz, 1H), 6.62 (d, *J* = 8.7 Hz, 1H), 3.77 (t, *J* = 4.5 Hz, 4H), 3.74 (t, *J* = 4.6 Hz, 4H), 3.67 (q, *J* = 5.8 Hz, 2H), 3.59 (q, *J* = 5.8 Hz, 2H), 2.70 (t, *J* = 6.0 Hz, 2H), 2.64 (t, *J* = 6.1 Hz, 2H), 2.58 (t, *J* = 4.5 Hz, 4H), 2.54 (t, *J* = 4.6 Hz, 4H), 2.41 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.62, 166.37, 166.18, 144.14, 141.18, 140.84, 136.17, 135.25, 132.53, 132.32, 130.19, 130.04, 127.33, 126.50, 125.41, 121.67, 120.51, 116.92, 116.82, 66.84, 66.82, 57.20, 57.15, 53.42, 53.38, 36.38, 36.36, 20.65. Purity was determined to be 95% by using HPLC. HRMS (ESI; m/z). Calcd for C34H39N5O5, $[M + H]^+$, 598.3024; found, 598.3060.

*N*-(3-(dimethylamino)propyl)-10-(4-((3-(dimethylamino)propyl)carbamoyl)phenyl)-7-methyl-9-oxo-9,10-dihydroacridine-2-carboxamide (**B19**). A yellow solid was obtained with a yield of 74%. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.02 (t, *J* = 4.4 Hz, 1H), 8.76 (d, *J* = 2.2 Hz, 1H), 8.73 (t, *J* = 4.7 Hz, 1H), 8.26 (d, *J* = 0.9 Hz, 1H), 8.06 (d, *J* = 8.5 Hz, 2H), 8.02 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.26 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.68 (d, *J* = 9.0 Hz, 1H), 6.56 (d, *J* = 8.7 Hz, 1H), 3.57 (q, *J* = 5.7 Hz, 2H), 3.51 (q, *J* = 5.6 Hz, 2H), 2.52 – 2.44 (m, 4H), 2.36 (s, 3H), 2.34 (s, 6H), 2.26 (s, 6H), 1.80 – 1.69 (m, 4H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.81, 165.84, 165.66, 144.12, 141.05, 140.96, 136.34, 135.16, 132.65, 132.19, 130.10, 129.80, 127.68, 126.57, 125.18, 121.77, 120.74, 116.90, 116.85, 59.33, 59.25, 45.45, 45.30, 40.81, 40.75, 25.22, 25.15, 20.61. Purity was determined to be 97.3% by using HPLC. HRMS (ESI; m/z). Calcd for C32H39N5O3, $[M + H]^+$, 542.3126; found, 542.3144.

7-methyl-*N*-(3-morpholinopropyl)-10-(4-((3-morpholinopropyl)carbamoyl)phenyl)-9-oxo-9,10-dihydroacridine-2-carboxamide (**B20**). A yellow solid was obtained with a yield of 88%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.87 (d, *J* = 1.5 Hz, 1H), 8.34 (s, 1H), 8.31 (d, *J* = 4.6 Hz, 1H), 8.28 (t, *J* = 4.5 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.08 (dd, *J* = 9.0, 1.7 Hz, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.7 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 1H), 6.62 (d, *J* = 8.7 Hz, 1H), 3.82 (t, *J* = 4.4 Hz, 4H), 3.73 (t, *J* = 3.8 Hz, 4H), 3.66 (q, *J* = 5.6 Hz, 2H), 3.60 (q, *J* = 5.6 Hz, 2H), 2.66 – 2.52 (m, 12H), 2.44 (s, 3H), 1.94 – 1.80 (m, 4H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.63, 166.27, 166.14, 144.20, 141.17, 140.90, 136.45, 135.28, 132.75, 132.35, 130.19, 130.01, 127.63, 126.75, 125.20, 121.78, 120.60, 116.93, 116.76, 66.80, 66.77, 58.51, 58.27, 53.91, 53.74, 40.45, 24.37, 24.26, 20.67, 20.61. Purity was determined to be 95.6% by using HPLC. HRMS (ESI; m/z). Calcd for C36H43N5O5, $[M + H]^+$, 626.3337; found, 626.3367.

*N*-(2-(diethylamino)ethyl)-10-(4-((2-(diethylamino)ethyl)carbamoyl)phenyl)-7-methyl-9-oxo-9,10-dihydroacridine-2-carboxamide (**B21**). A yellow solid was obtained with a yield of 63%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.89 (d, *J* = 2.2 Hz, 1H), 8.34 (d, *J* = 1.1 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 2H), 8.06 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.41 – 7.33 (m, 2H), 7.30 (d, *J* = 4.8 Hz, 1H), 6.77 (d, *J* = 9.0 Hz, 1H), 6.65 (d, *J* = 8.7 Hz, 1H), 3.60 (q, *J* = 5.3 Hz, 2H), 3.54 (q, *J* =

5.7 Hz, 2H), 2.74 (t, *J* = 6.0 Hz, 2H), 2.70 (t, *J* = 6.0 Hz, 2H), 2.65 (q, *J* = 4.7 Hz, 4H), 2.60 (q, *J* = 4.7 Hz, 4H), 2.45 (s, 3H), 1.11 – 1.00 (m, 12H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.79, 166.28, 166.03, 144.18, 141.16, 140.91, 136.26, 135.26, 132.59, 132.30, 130.21, 129.98, 127.57, 126.61, 125.44, 121.78, 120.66, 116.98, 116.87, 51.52, 51.42, 46.90, 46.80, 37.51, 37.44, 20.69, 11.78, 11.73. Purity was determined to be 98.0% by using HPLC. HRMS (ESI; m/z). Calcd for C34H43N5O3, $[M + H]^+$, 570.3439; found, 570.3470.

3.8. General procedure F: preparation of **B(22-23)**. The procedure was similar to general preparation of **B(10)** except replacing intermediate **6** with **B14.**

To a solution of **B14** (50 mg, 0.13 mmol) and various alkylamine (1.5 eq.) in anhydrous trichloromethane (5 mL) was added dicyclohexylcarbodiimide (71.8 mg, 0.35 mmol), and then 4-dimethylaminopyridine (4.56 mg, 0.04 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere for 4 h. The reaction mixture was quenched with 10 mL ice water, filtered, and the filtrate was extracted with dichloromethane (3×10 mL). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated under reduced pressure. The residue was purified by using chromatograph on silica gel with DCM/MeOH (50/1 - 10/1) to give desire compound.

2-morpholinoethyl-7-methyl-10-(4-((2-morpholinoethoxy)carbonyl)phenyl)-9-oxo-9,10-dihydr oacridine-2-carboxylate (**B22**). A yellow solid was obtained with a yield of 59%. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.22 (d, *J* = 2.0 Hz, 1H), 8.40 (d, *J* = 8.5 Hz, 2H), 8.36 (d, *J* = 1.3 Hz, 1H), 8.09 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.35 (dd, *J* = 8.7, 1.9 Hz, 1H), 6.73 (d, *J* = 9.0 Hz, 1H), 6.62 (d, *J* = 8.7 Hz, 1H), 4.57 (t, *J* = 5.9 Hz, 2H), 4.49 (t, *J* = 5.9 Hz, 2H), 3.76 (t, *J* = 4.6 Hz, 4H), 3.73 (t, *J* = 4.8 Hz, 4H), 2.84 (t, *J* = 5.9 Hz, 2H), 2.81 (t, *J* = 6.0 Hz, 2H), 2.66 – 2.58 (m, 8H), 2.46 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.66, 165.80, 165.28, 145.09, 142.75, 140.79, 135.33, 133.60, 132.70, 132.59, 131.82, 130.25, 130.20, 126.91, 123.3, 122.11, 121.03, 116.76, 116.54, 66.94, 66.93, 62.82, 62.33, 57.16, 57.14, 53.87, 53.82, 20.68. Purity was determined to be 96.6% by using HPLC. HRMS (ESI; m/z). Calcd for C34H37N3O7, $[M + H]^+$, 600.2690; found, 600.2704.

2-(pyridin-2-yl)ethyl-7-methyl-9-oxo-10-(4-((2-(pyridin-2-yl)ethoxy)carbonyl)phenyl)-9,10-dih ydroacridine-2-carboxylate (**B23**). A yellow solid was obtained with a yield of 51%. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.14 (d, *J* = 2.0 Hz, 1H), 8.60 (d, *J* = 4.5 Hz, 1H), 8.57 (d, *J* = 4.5 Hz, 1H), 8.39 – 8.29 (m, 3H), 8.02 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.71 – 7.62 (m, 2H), 7.45 (d, *J* = 8.4 Hz, 2H), 7.32 (dd, *J* = 13.7, 4.9 Hz, 3H), 7.20 (dd, *J* = 7.1, 5.3 Hz, 1H), 7.16 (dd, *J* = 6.9, 5.1 Hz, 1H), 6.67 (d, *J* = 9.0 Hz, 1H), 6.58 (d, *J* = 8.7 Hz, 1H), 4.83 (t, *J* = 6.7 Hz, 2H), 4.73 (t, *J* = 6.7 Hz, 2H), 3.33 (t, *J* = 6.7 Hz, 2H), 3.28 (t, *J* = 6.7 Hz, 2H), 2.44 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 177.64, 165.76, 165.29, 158.06, 157.81, 149.63, 149.50, 145.01, 142.64, 140.75, 136.62, 136.58, 135.28, 133.55, 132.60, 132.55, 131.20, 131.84, 130.14, 126.84, 123.68, 123.47, 123.30, 122.02, 121.87,

121.74, 120.95, 116.78, 116.50, 64.80, 64.32, 37.55, 37.45, 20.69. Purity was determined to be 99.7% by using HPLC. HRMS (ESI; m/z). Calcd for C36H29N3O5, $[M + H]^+$, 584.2180; found, 584.2173.

## 4. Biophysical and biochemical evaluation experiments

4.1 DNA oligonucleotides

DNA oligonucleotides were purchased from Invitrogen (China) and Sangon (China) as salt-free sequences. All oligonucleotides were dissolved in double distilled deionized water. Their concentrations were represented as single-stranded concentrations and determined from the absorbance at 260 nm using a Nano Drop 1000 Spectrophotometer (Thermo Fisher Scientific, USA) with the Beer-Lambert Law: $A= \varepsilon \cdot C \cdot l$. Further dilutions to working concentrations were made with relevant buffers. The DNA sequences are provided in **Table S1**.

4.2 Fluorescence resonance energy transfer (FRET) melting assay

FRET melting assay was carried out on a real-time PCR apparatus following previously published procedures. The following fluorescently dual labeled oligonucleotides were used as the FRET probes. FPy27T: 5′-FAM-CCTTCCCCACCCTCCCCACCCTCCCCA-TAMRA-3′, was prepared as 10 μM solution in 1 × BPES buffer containing 30 mM ($KH_2PO_4$, $K_2HPO_4$), 1 mM EDTA, and 100 mM KCl, pH 5.5. FPu22T: 5′-FAM-TGAG GGTGGGTAGGGTGGGTAA-TAMRA-3′, was prepared as 10 μM solution in Tris-HCl buffer (10 mM, pH 7.4) containing 10 mM KCl. F10T: 5′-FAM-dTATAGCTATA-HEG-TATAGCTATA-TAMRA-3′, was prepared as 10 μM solution in Tris-HCl buffer (10 mM, pH 7.4) containing 60 mM KCl. Donor fluorophore FAM is 6-carboxyfluorescein. Acceptor fluorophore TAMRA is 6-carboxytetramethylrhodamine. HEG linker is $[(-CH_2-CH_2-O-)_6]$. These oligonucleotides were thermally annealed. Fluorescence melting curves were determined with a Roche Light Cycler 2 real-time PCR instrument, using a total reaction volume of 20 μL, with 0.2 μM dual labeled oligonucleotide with or without 3 μM disubstituted acridones derivatives for FPy27T, FPu22T, F10T, respectively. Fluorescence readings with excitation at 470 nm and detection at 530 nm were taken at intervals of 1 ℃ over the range 37-99 ℃, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value. The melting of i-motif, G-quadruplex and duplex were monitored in the absence or presence of various concentrations of compounds. Final analysis of the data was carried out using Origin8.0 (OriginLab Corp.). For the concentration-dependent FRET assay, an equal volume of different concentration of the compound was added to probes, and the fluorescence intensity at 520 nm was measured by using LS-55 luminescence spectrophotometer (Perkin-Elmer, USA). The data were corrected with the signal of the compound at the same buffer and normalized to DMSO to obtain the relative fluorescence intensity.

4.3 Surface Plasmon Resonance (SPR) measurement

SPR measurement was performed on a ProteOn XPR36 Protein Interaction Array system (Bio-Rad Laboratories, Hercules, CA) using a Neutravidin-coated GLH sensor chip. For immobilization, all DNA samples were biotinylated and attached to a reptavidin-coated sensor chip. Oligomer Py27 (5′-biotin-d[CCTTCCCCACCCTCCC CACCCTCCCCA]-3′) was diluted to 1 μM in running buffer (20 mM 2-(4-morpholino)ethanesulfonic acid, pH 5.8, 100 mM KCl and 0.05% Tween-20), Pu27 (5′-biotin-d[TGGGGAGGGTGGGGAGGGTGGGGAAGG]-3′) and duplex DNA(5′-biotin-d[TATAGCTATA-HEG-TATAGCTATA]-3′) were diluted to 1 μM in running buffer (Tris-HCl 50 mM, pH 7.4, 100 mM KCl). The DNA samples were then captured (1000 RU) in flow cells, and a blank cell was set as a control. Ligand solutions (at 0, 3.125, 6.25, 12.5, 25, 50 μM) were prepared with the running buffer through serial dilutions from stock solution (10 mM in DMSO). Six concentrations were injected simultaneously at a flow rate of 25 mL/ min for 200 s of association phase, followed with 300 s of dissociation phase at 25 $^{o}$C. The GLH sensor chip was regenerated with short injection of 50 mM NaOH between consecutive measurements. The final graphs were obtained by subtracting blank sensorgrams from the i-motif, G-quadruplex or duplex sensorgrams. Data were analyzed with ProteOn manager software.

4.4 Microscale thermophoresis (MST) experiment

The 5′-end FAM labeled Py27, Pu27 and Duplex DNA were purchased from Sangon (China). The thermophoresis movements of the fluorescently labeled nucleic acids and compound complexes were detected by monitoring the fluorescence distributions inside the capillary by using the NT.115 MST machine (NanoTemper, Germany). The concentration of DNA was held constant at 0.5 μM, and the compound was diluted at 3:4 from 10 μM for 12 times. The samples were loaded into standard-treated MST-grade glass capillaries. The intensities of the LED and laser were set as 40% and 40%, respectively. Data were analyzed using NT Analysis 1.4.23 software.

4.5 CD experiments

CD experiments were performed on a Chirascan circular dichroism spectrophotometer (Applied Photophysics). A quartz cuvette with 4 mm path length was used for the spectra recorded over a wavelength range of 230-400 nm at 1 nm bandwidth, 1 nm step size, and 0.5 s per point. The oligomer c-*myc* Py27 was diluted from stock to the required concentration (1 μM) in 1 × BPES buffer (pH 5.5 or 6.8) in the absence or presence of compounds, and then annealed by heating at 95 ℃ for 5 min, gradually cooled to room temperature, and stored at 4 ℃ overnight. Spectra were recorded three times over a wavelength range of 230-350 nm, averaged, smoothed, and baseline corrected to remove signal contribution from buffer. Final analysis of the data was carried out using Origin 8.0 (OriginLab Corp.).

4.6 NMR Studies

The DNA oligonucleotide was purchased from Sangon (China). The final NMR samples were

prepared in 10%/90% $D_2O/H_2O$ solution at pH 5.5, 6.2, 7.0, 7.3. The concentration of DNA samples was 1.0 mM. The stock solutions of compound were dissolved in *d*6-DMSO. One-dimensional $^1H$ NMR titration experiments were performed on a Bruker DRX-600 MHz spectrometer at temperatures of 5 ℃ and 25 ℃, and the water signal was suppressed in the $^1H$ NMR experiment.

4.7 Native PAGE experiments

Native PAGE experiments were carried out in 1 × TBE buffer (pH 6.6). The oligomer c-*myc* Py27 was diluted from stock to the required concentration (3 μM) in 1 × BPES buffer (pH 6.8) in the absence or presence of different concentrations of compound **B19**, and then annealed by heating at 95 °C for 5 min. Py27 (3 μM) annealed at the similar condition (1 × BPES buffer, pH 6.0) was set as a control. Then these oligomers were gradually cooled to room temperature, and incubated at 4 ℃ overnight. Electrophoresis was carried out by using 20% acrylamide (pH 6.6) at 140 V for 5 h at 5 $^oC$. The gels were then silver-stained.

4.8 Cell culture

Human cervical cancer cell line Siha, human bone osteosarcoma epithelial cell line U2OS, human colon cancer cell line HCT116, human hepatocellular carcinoma cell line Huh7 and human embryonic kidney cell line HEK293 were purchased from China Center for Type Culture Collection in Wuhan. The cell lines were maintained in RPMI-1640 or DMEM medium supplemented with 10% fetal calf serum, 100 U/mL penicillium and 100 mg/mL streptomycin at 37 $^oC$ in a humidified atmosphere with 5% $CO_2$.

4.9 MTT cytotoxicity assay

Human cervical cancer cell line Siha, human bone osteosarcoma epithelial cell line U2OS, human colon cancer cell line HCT116, human hepatocellular carcinoma cell line Huh7 and human embryonic kidney cell line HEK293 were seeded on 96-well plates ($5.0 \times 10^3$ per well) with 100 μL of culture medium and incubated for 12h at 37 $^oC$ in a humidified atmosphere with 5% $CO_2$. After the cells were incubated in the presence or absence of the indicated concentrations of the disubstituted acridones derivatives for 48 h and the control group was administered the same volume of DMSO, 20 μL of 2.5 mg/mL methyl thiazolyl tetrazolium (MTT) solution was added to each well and further incubated for 4 h. The cells in each well were then treated with dimethyl sulfoxide (200 μL) after the culture medium was siphoned off and the absorbance was recorded at 570 nm. All drug doses were parallel tested in triplicate, and the cytotoxicity was evaluated based on the percentage of cell survival in a dose dependent manner regarding to the negative control. The final $IC_{50}$ values were calculated by using the Graph Pad Prism 5.

4.10 Dual-Luciferase reporter assay

In this assay, 200 ng of constructed psiCHECK2 luciferase plasmid (Promega, USA) containing c-*myc* wild type promoters was transfected into Siha cells by using Lipofectamine 2000

(Invitrogen, USA). After 4 h, compounds were added to the cells at 10 μM concentration. The cells were incubated at 37 ℃ with $CO_2$ for 48 h, and the transfected cells were first washed with ice-cold PBS to reduce the background signals from the medium. Luciferase assays were subsequently performed according to the manufacturer's instructions using the dual-luciferase assay system (Promega, USA). After a 3 s delay, secreted luciferase signals were collected for 10 s using a microplate reader (Molecular Devices, Flex Station 3, USA). The quantification was performed using a multimode reader (Molecular Devices). The secreted Renilla luciferase activity was normalized to the firefly luciferase activity.

4.11 RNA extraction and real time polymerase chain reaction (RT-PCR)

Siha cells were seeded in 6-well plate ($2 \times 10^5$ cells/well), and incubated for 12 h at 37 $^oC$ in a humidified atmosphere with 5% $CO_2$. After the cells were incubated in the presence or absence of different concentrations of **B19** and the control group was administered the same volume of DMSO for 3 h, cells were harvested, and the RNA was extracted according to the manufacturer's instructions. Total RNA was used as a template for reverse transcription using the following protocol: each 20 μL reaction mixture contained 1 μg of total RNA, 50 μM oligo dT18 primer (2 μL), 5 × M-MLV buffer (4 μL), 2.5 mM dNTP (1 μL), 40 U/mL RNase inhibitor (0.5 μL), M-MLV reverse transcriptase (1 μL), and DEPC $H_2O$ to make final volume of 20 μL. Briefly, RNA, DEPC $H_2O$ and oligo dT18 primer were incubated at 70 ℃ for 10 min and then immediately cooled to 4 ℃. Next, the other components were added and incubated at 42 ℃ for 1 h and at 70 ℃ for 15 min, and then immediately cooled to 4 ℃ to obtained the cDNA, which was applied directly for further qPCR. The real-time PCR was performed on a real-time PCR apparatus (Roche LightCycler 480) according to the manufacturer's protocol. The total volume of 20 μL of quantitative reaction mixtures contained 10 μL of SYBR qPCR Mix (THUNDERBIRD, Japan), 7 μL of DEPC $H_2O$, 1 μL of each primer, and 2 μL of cDNA. The c-*myc* mRNA level were normalized to β-actin mRNA level of each sample. Results of real-time PCR were analyzed using the $2^{-\Delta CT}$ method.

4.12 Western Blot

Siha cells were seeded in 6-well plate ($2 \times 10^5$ cells/well) and incubated for 12h at 37 $^oC$ in a humidified atmosphere with 5% $CO_2$. After incubated in the presence of different concentrations of **B19** and the control group was administered the same volume of DMSO for 3 h, cells were harvested from each well of culture plates and lysed in 200 μL of protein extraction buffer consisting of 1 mM PMSF for 30 min. The suspension was centrifuged at 10,000 rpm at 4 ℃ for 15 min, and the protein content of supernatant was measured by using BCA assay. The same amount of protein for each sample was loaded onto 8% polyacrylamide gel, and then transferred to a microporous polyvinylidene difluoride (PVDF) membrane. Western blotting was performed by using anti-c-*myc* and anti-β-actin (cell signaling technology) antibodies, as well as horseradish

peroxidase-conjugated anti-rabbit secondary antibody. Protein bands were visualized by using chemiluminescence substrate.

4.13 Flow cytometric analysis

Siha cells were seeded in 6-well plate ($2 \times 10^5$/well) and incubated for 12h at 37 $^oC$ in a humidified atmosphere with 5% $CO_2$. After incubated in the presence of different concentrations of **B19** and the control group was administered the same volume of DMSO for 24 h, the Siha cells were then washed in PBS and centrifuged and re-suspended in Annexin V-FITC solution for 15 min at room temperature in dark. After centrifuged for 5 min, the cells were then re-suspended in Annexin V-FITC solution and mixed with PI staining solution for 10 min at 2-5 $^oC$ in dark. Then, the cells were analyzed by using flow cytometry with an Epics Elite flow cytometer (Beckman Coulter, USA).

4.14 Colony formation assay

Siha cells were subsequently seeded in 6-well culture plates (1000/well) for a 24 h pre-culture at 37 $^oC$ in a humidified atmosphere with 5% $CO_2$, and then treated with compound at different concentrations for 7 days. The cells were washed with 1× PBS and fixed with ice cold methanol for 10 min, followed by the addition of 0.5% crystal violet solution for 30 min to observe the colony formation. Finally, the plates were washed with water, dried and photographed.

4.15 Cell scrape assay

Siha cells were subsequently seeded in 6-well culture plates (300,000/well) at 37 $^oC$ in a humidified atmosphere with 5% $CO_2$. After 24 h preculture, a cross-shaped scrape was made through the monolayer Siha cells using a plastic pipet tip, and then the cells were treated with compound at different concentrations, respectively. Several wounded areas were observed and photographed using microscopy after scratching and then culturing for 0, 48 and 96 h. The edge of the cells were marked with a white line to observe obviously.

## 5. Table S1. Oligomers or primers used in this study

| Oligomer | Sequence |
|---|---|
| FPy27T | 5′-FAM-CCTTCCCCACCCTCCCCACCCTCCCCA-TAMRA-3′ |
| FPu22T | 5′-FAM-TGAGGGTGGGTAGGGTGGGTAA-TAMRA-3′ |
| F10T | 5′-FAM-TATAGCTATA-HEG-TATAGCTATA-TAMRA-3′ |
| FdT33T | 5′-FAM-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-TAMRA-3′ |
| bio-Py27 | 5′-biotin-d[CCTTCCCCACCCTCCC CACCCTCCCCA]-3′ |
| bio-Pu27 | 5′-biotin-d[TGGGGAGGGTGGGGAGGGTGGGGAAGG]-3′ |
| bio-Hairpin | 5′-biotin- d[TATAGCTATA-HEG-TATAGCTATA]-3′ |
| Fam-Py27 | 5′-FAM-CCTTCCCCACCCTCCCCACCCTCCCCA- 3′ |
| Py27 | 5′-CCTTCCCCACCCTCCCCACCCTCCCCA-3′ |

| Primer | Sequence |
|---|---|
| b-*actin*-S | 5′-CTGGAACGGTGAAGGTGACA-3′ |
| b-*actin*-A | 5′-AAGGGACTTCTGTAACAACGCA-3′ |
| actin-F-139 | 5′-CTGGAACGGTGAAGGTGACA-3′ |
| actin-R-139 | 5′-CTGGAACGGTGAAGGTGACA-3′ |
| dT18 | 5′-TTTTTTTTTTTTTTTTTTT-3′ |

## 6. Previously reported i-motif binding ligands

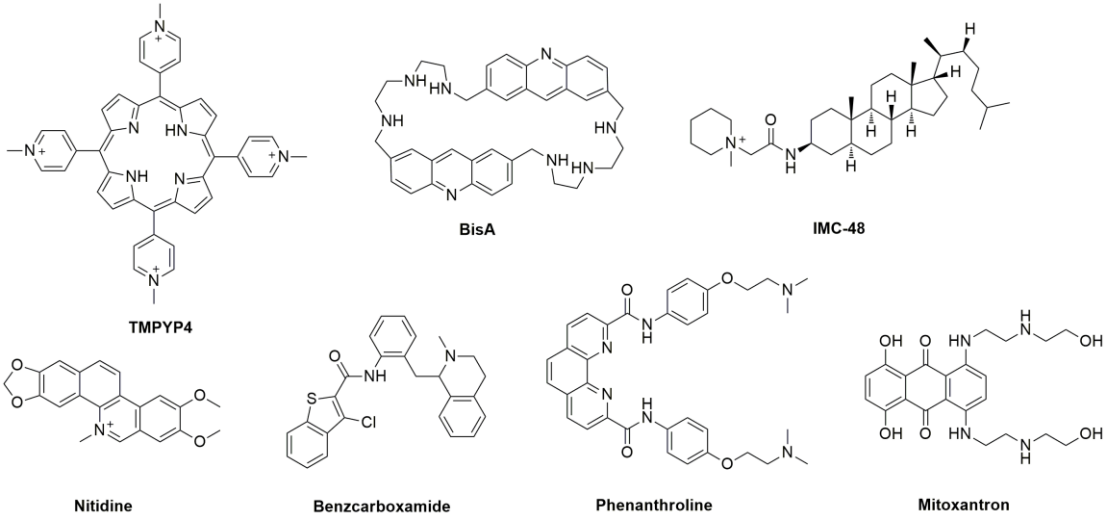



**Figure S1.** Structures of porphyrin, BisA, cholestane (IMC-48), benzcarboxamide, nitidine, phenanthroline, and mitoxantrone.

## 7. Compound 3 down-regulated c-*myc* transcription and expression

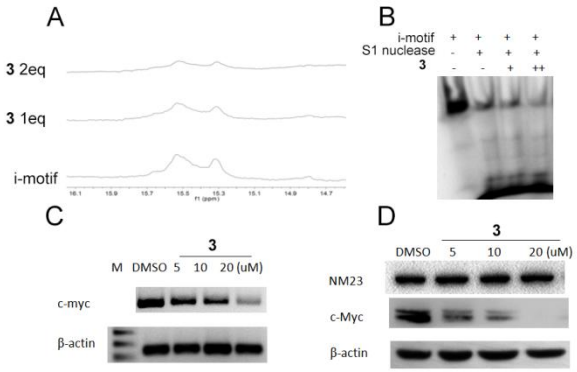



**Figure S2.** Compound **3** could bind to i-motif and down-regulate c-*myc* transcription and expression. (A) NMR titration of i-motif with **3**. (B) S1 nuclease cleavage experiment to study binding site of **3** on i-motif. (C) The mRNA levels and (D) protein levels of c-MYC in Siha cells treated with **3** determined by using RT-PCR and Western blot, respectively.

## 8. Table S2. Changes of oligomer's melting temperatures determined by using FRET-melting experiment

| Compound | $\Delta T_m$ (°C)[a] | | | Compound | $\Delta T_m$ (°C) | | |
|---|---|---|---|---|---|---|---|
| | FPy27T | FPu22T | F10T | | FPy27T | FPu22T | F10T |
| B01 | 2.1 | 1.8 | 1.9 | B07 | 2.7 | 0.1 | 1.2 |
| B02 | 2.7 | 0.2 | 1.3 | B08 | 2.8 | 0.4 | 1.3 |
| B03 | 3.1 | 2.1 | 1.6 | B09 | 2.7 | 2.4 | 1.8 |
| B04 | 4.1 | 3.1 | 1.9 | B10 | 2.6 | 1.4 | 1.0 |
| B05 | 2.9 | 5.3 | 1.1 | B11 | 3.1 | 0.1 | 1.7 |
| B06 | 2.3 | 3.4 | 1.4 | B12 | 2.1 | 1.8 | 1.9 |

[a] Δ*T*m = *T*m (DNA + ligand) - *T*m (DNA). The concentrations of FPy27T, FPu22T and F10T were 0.2 μM, and the concentrations of compounds were 3.0 μM. The melting temperatures of FPy27T, FPu22T and F10T in the absence of compounds were 53.2 °C, 66.5 °C and 59.1 °C, respectively.

## 9. FRET-melting experiments

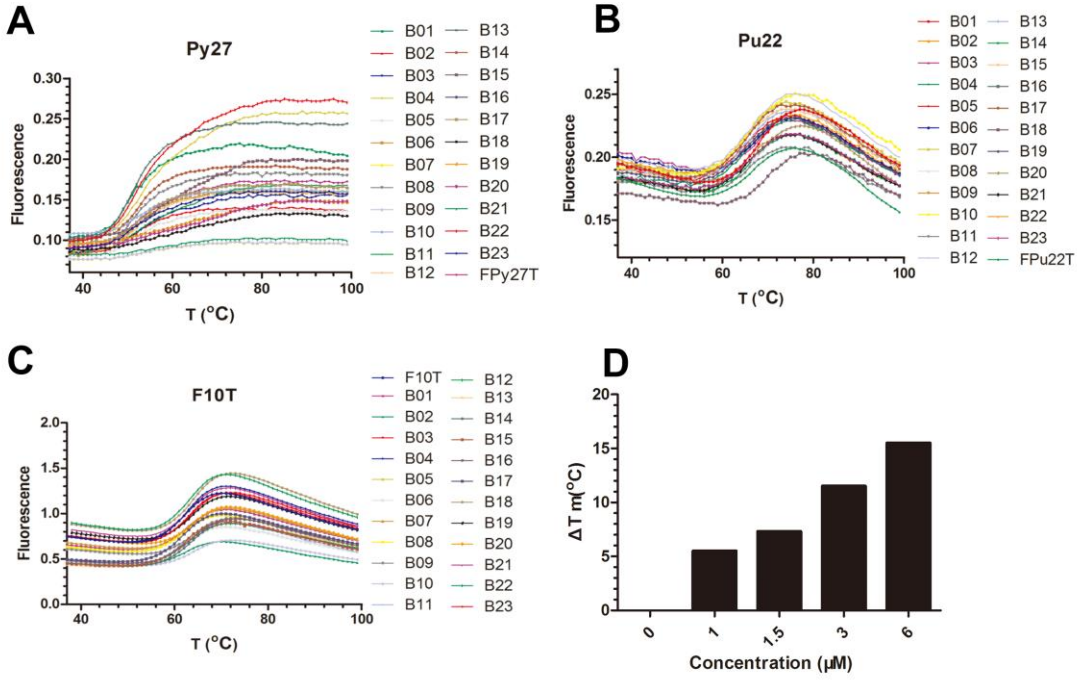



**Figure S3.** Melting curves of (A) FPy27T (0.2 μM), (B) FPu22T (0.2 μM), and (C) F10T (0.4 μM) with acridone derivatives (3 μM). (D) The dose dependent increase of Δ*T*m values of c-*myc* Py27 upon treatment with increasing concentration of **B19**.

## 10. Table S3. Equilibrium binding constants ($K_D$) determined by using SPR

| Compound | $K_D$ (μM) | | | Compound | $K_D$ (μM) | | |
|---|---|---|---|---|---|---|---|
| | Py27 | Pu27 | Duplex | | Py27 | Pu27 | Duplex |
| B01 | >50[a] | >50 | >50 | B13 | >50 | >50 | >50 |
| B02 | >50 | >50 | >50 | B14 | >50 | >50 | >50 |
| B03 | >50 | >50 | >50 | B15 | >25[b] | >50 | >50 |
| B04 | >50 | >50 | >50 | B16 | >25 | >50 | >50 |
| B05 | >50 | >50 | >50 | B17 | 14.6 | >50 | >50 |
| B06 | >50 | >50 | >50 | B18 | 11.3 | >50 | >50 |
| B07 | >50 | >50 | >50 | B19 | 7.8 | >50 | >50 |
| B08 | >50 | >50 | >50 | B20 | 8.5 | 28.6 | >50 |
| B09 | >50 | >50 | >50 | B21 | 9.1 | >50 | >50 |
| B10 | >50 | >50 | >50 | B22 | 13.6 | >50 | >50 |
| B11 | >50 | >50 | >50 | B23 | >25 | >50 | >50 |
| B12 | >50 | >50 | >50 | **3** | >25 | >50 | >50 |

[a] No significant binding was found for the addition of up to 50 μM ligand, which might indicate no specific interaction between the ligand and the DNA.

[b] The compounds showed weak binding affinity to the DNA.

## 11. SPR and MST experiments

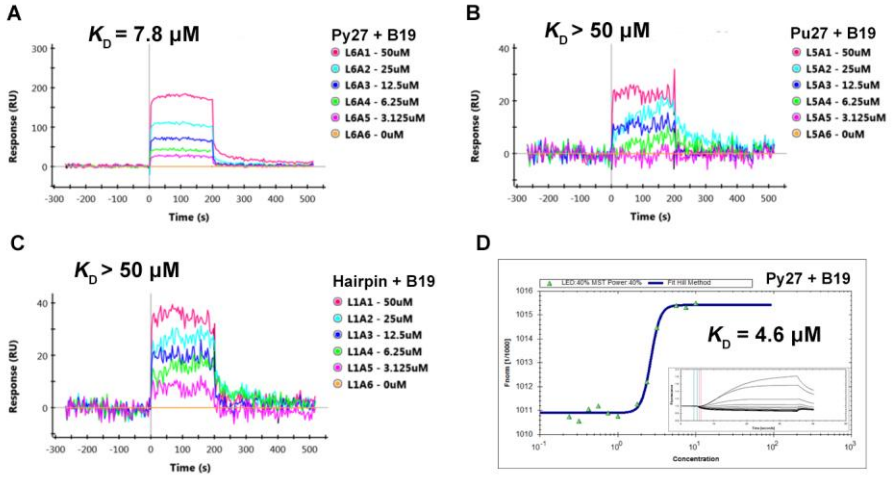



**Figure S4.** Equilibrium dissociation constants ($K_D$) were determined through SPR and MST experiments. Kinetic binding constants ($K_D$) of compound **B19** with c-*myc* i-motif (A), G-quadruplex (B) and duplex DNA (C) were determined by using SPR. The binding constant ($K_D$) of **B19** with c-*myc* i-motif (D) was also determined by using MST.

## 12. FRET experiment

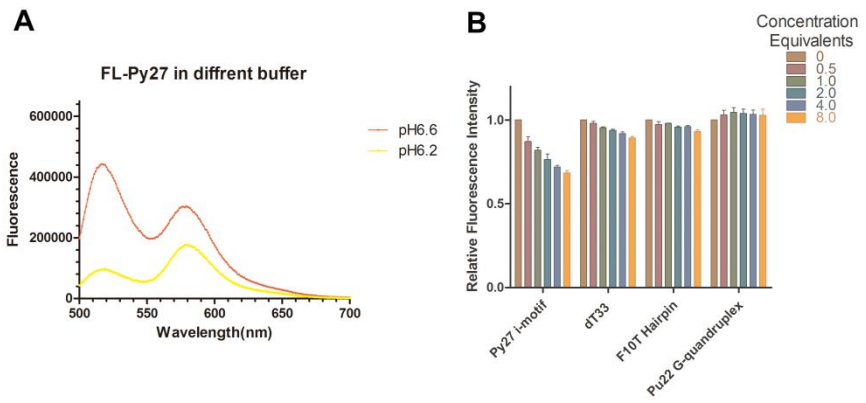



**Figure S5.** Fluorescence spectra analysis to confirm the conformational change produced by **B19**. (A) pH-dependent FRET change of c-*myc* i-motif dual labeled with FAM and TAMRA at the 5´-end and 3´-end, respectively. At low pH (pH 6.2), the $I_{579}/I_{518}$ ratio is 1.83, showing a closer distance between two dyes by folding the i-motif, while at high pH (pH 6.6) the $I_{579}/I_{518}$ ratio is 0.70, showing a longer distance between two dyes by unfolding the i-motif, confirmed by later CD assays. (B) Dose-dependent spectra changes at 518 nm for various dual labeled oligomers affected by increasing concentration of **B19**.

## 13. CD experiments

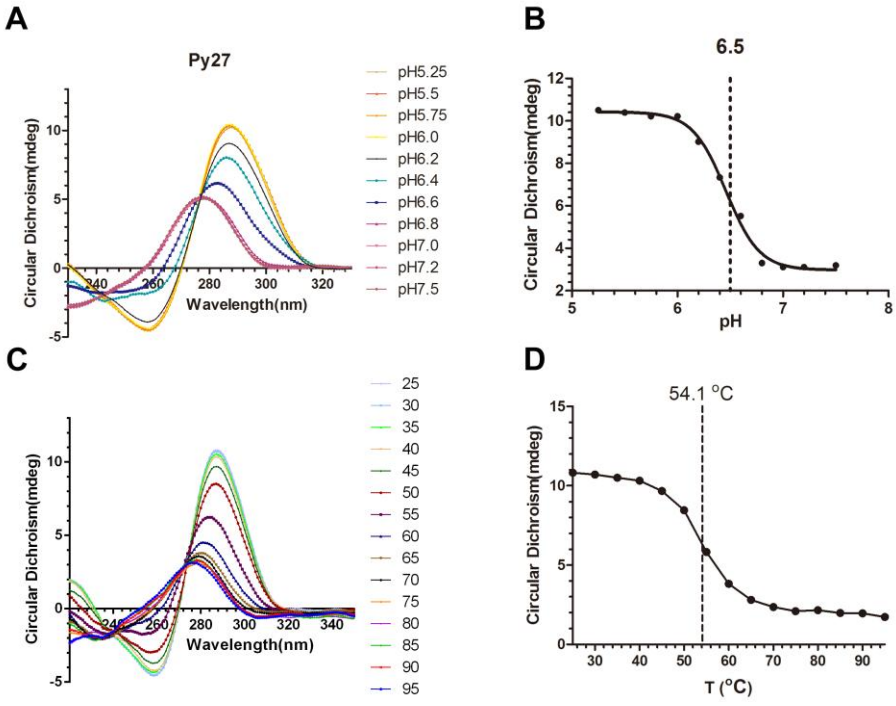



**Figure S6.** (A) CD spectra of oligomer Py27 at various pH values. (B) The molar ellipticity at 288 nm of CD spectra for oligomer Py27 versus pH values, which was used to determine the transitional pH. (C) CD spectra of oligomer Py27 at various temperatures. (D) The molar

ellipticity at 288 nm of CD spectra for oligomer Py27 versus temperatures, which was used to determine the transitional temperature.

## 14. NMR experiments

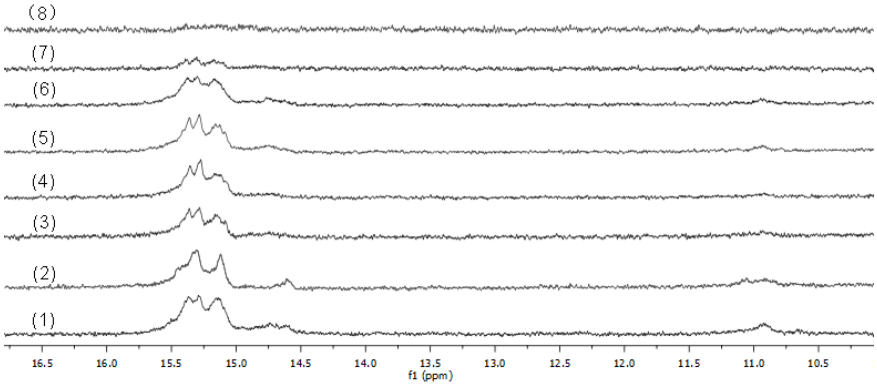



**Figure S7.** The imino proton region of $^{1}$H NMR spectra of oligomer Py27 upon addition of increasing concentration of **B19.** Spectra 1 and 2 are for oligomer Py27 only at pH 5 and pH 5.5, respectively at 25 ℃. Spectra 3-5 were recorded at pH 6.2 for oligomer Py27 plus **B19** at ratio of 1:10, 1:5, and 1:2, respectively at 5 ℃. Spectra 6-8 are for oligomer Py27 only at pH 6.2, pH 7.0, and pH 7.3, respectively at 5 ℃.

## 15. ESI-MS experiments

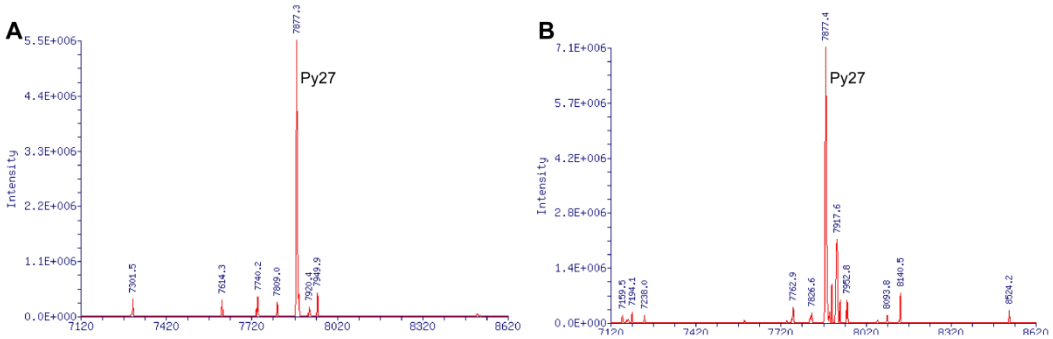



**Figure S8.** ESI-MS spectra of Py27 without or with **B19** (5 μM DNA and 20 μM ligand in 1× BPES buffer) at 25 ℃. (A) Py27 only at pH 7.0; (B) Py27 was mixed with **B19** at pH 7.0.

## 16. Table S4. $IC_{50}$ values (μM) of B15-23 against tumor cells (48 h)

| Compound | $IC_{50}$ (μM)[a] | | | | |
|---|---|---|---|---|---|
| | U2OS | HCT116 | Siha | HuH7 | HEK293 |
| B15 | >50 | >50 | >50 | >50 | >100 |
| B16 | >50 | >50 | >50 | >50 | >100 |
| B17 | >50 | >50 | >50 | >50 | >100 |

| | | | | | |
|---|---|---|---|---|---|
| B18 | 27.8 | 12.8 | 18.6 | 16.5 | 96.6 |
| B19 | 25.7 | 14.6 | 17.5 | 17.3 | 89.2 |
| B20 | 15.1 | 10.9 | 19.9 | 18.1 | 75.9 |
| B21 | 23.4 | 13.2 | 13.0 | 17.3 | 65.4 |
| B22 | >50 | 16.2 | >50 | 14.4 | 51.8 |
| B23 | >50 | 12.8 | >50 | >50 | >100 |

[a] The values given are means of three experiments

## 17. Dual luciferase reporter assays

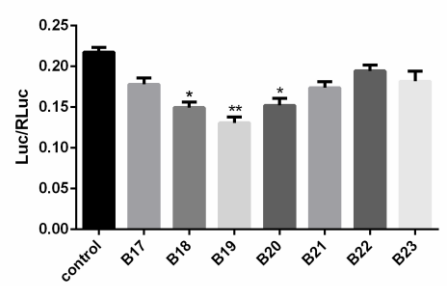



**Figure S9**. Effects of acridone derivatives (10 μM, 24 h) on c-*myc* promoter's activity via dual luciferase reporter assays. The experiments were repeated for three times: *, $P < 0.05$; **, $P < 0.01$.

## 18. Inhibition of long-term proliferation and metastasis of Siha cells

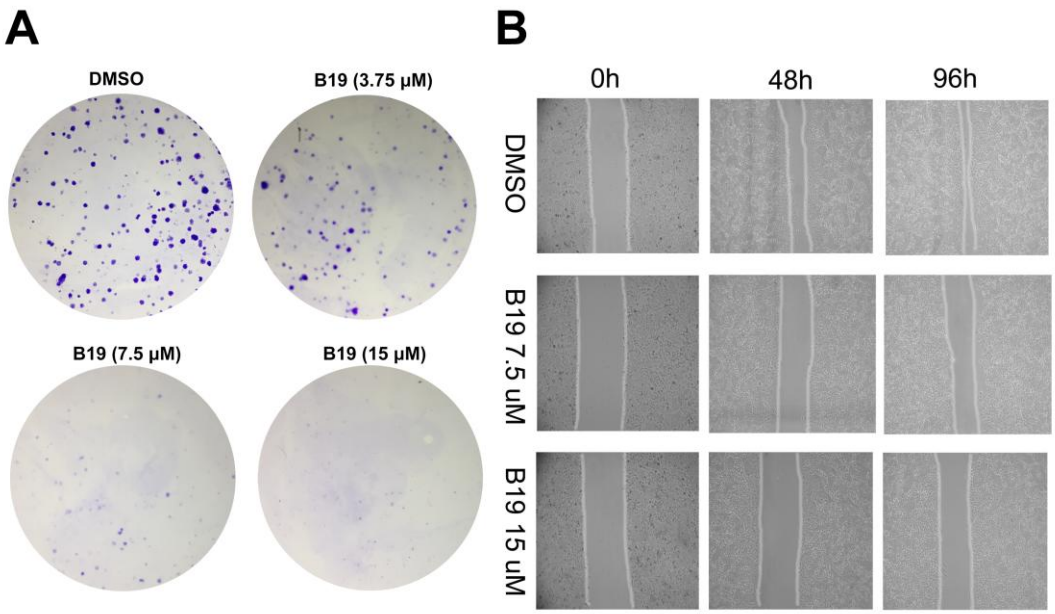
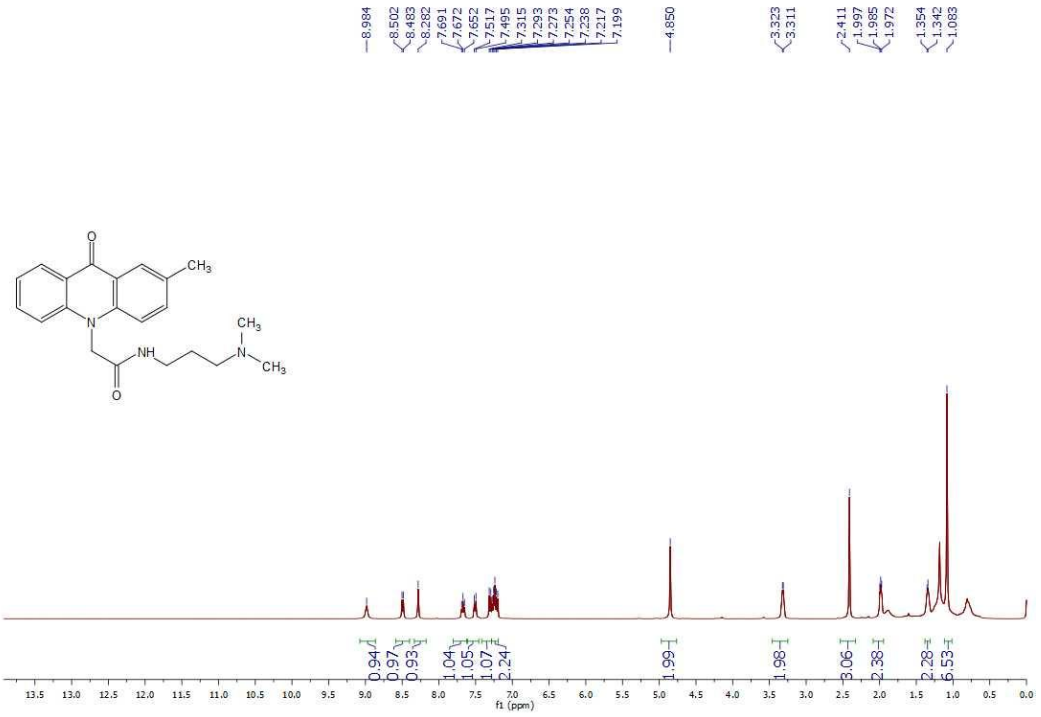
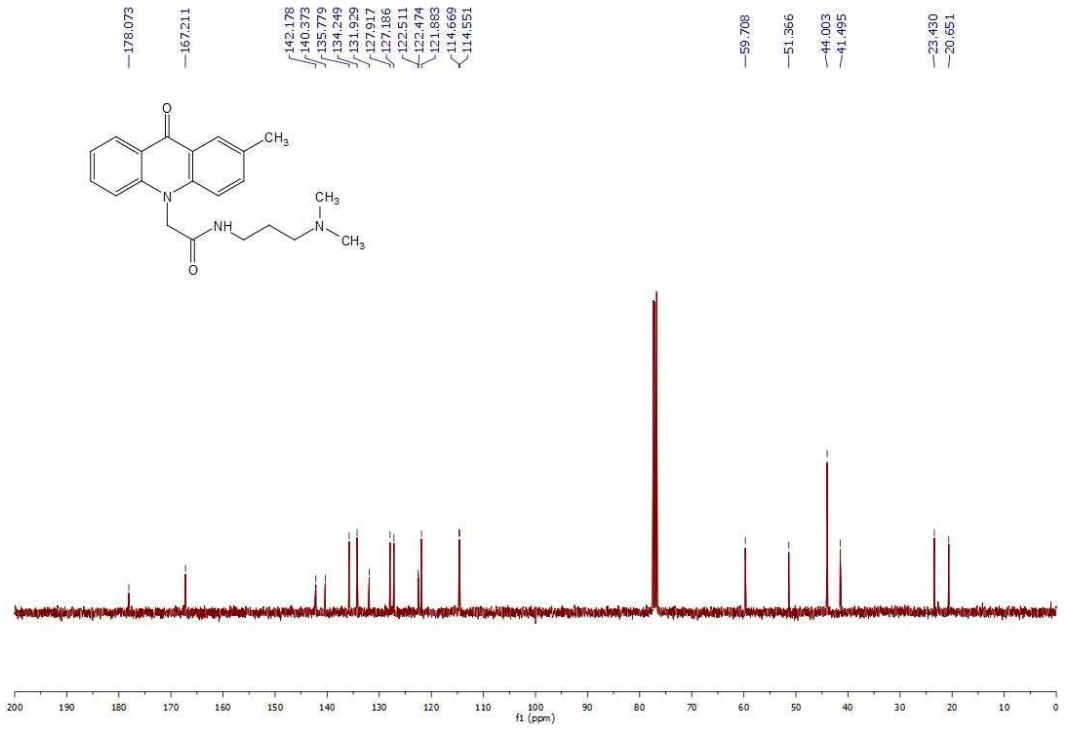


**Figure S10.** Effect of **B19** on long-term (7 days) proliferation (A) and metastasis (B) of Siha cells.

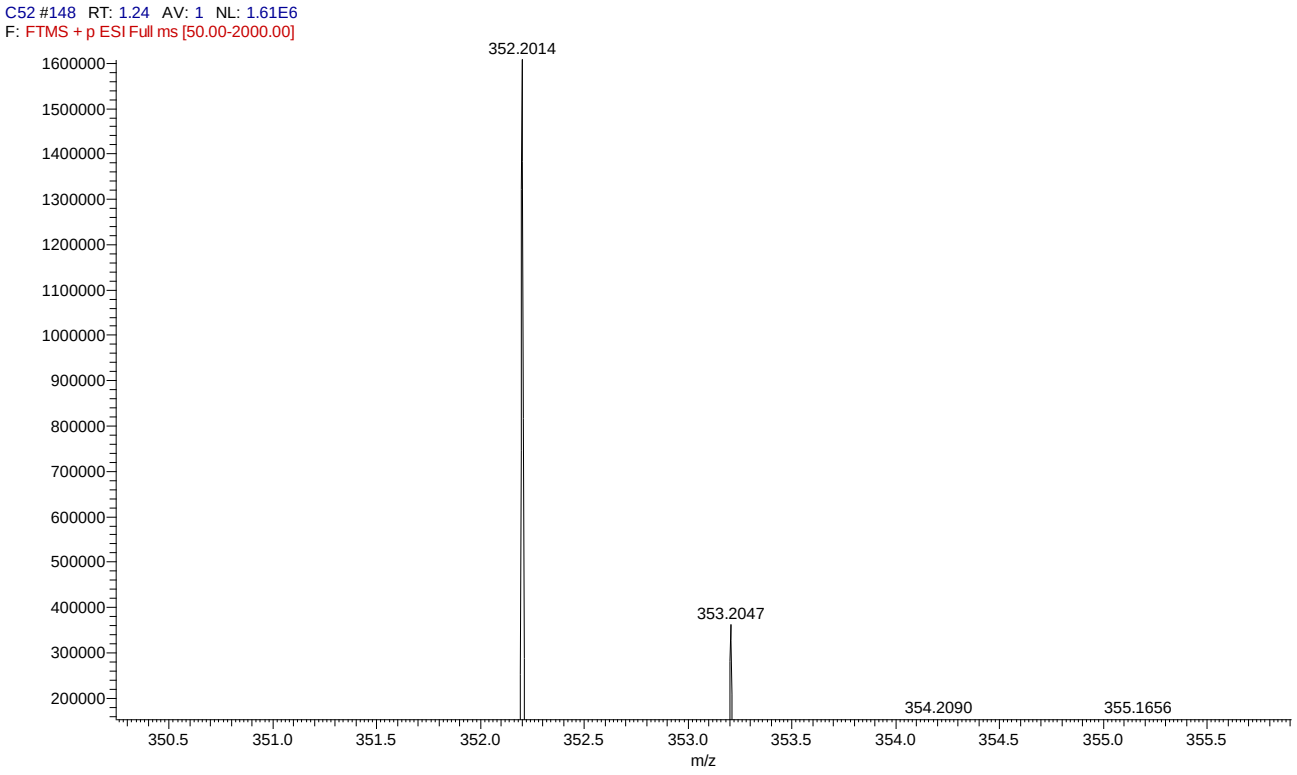
## 19. $^{1}$H NMR, $^{13}$C NMR, HRMS and HPLC spectra of ligands



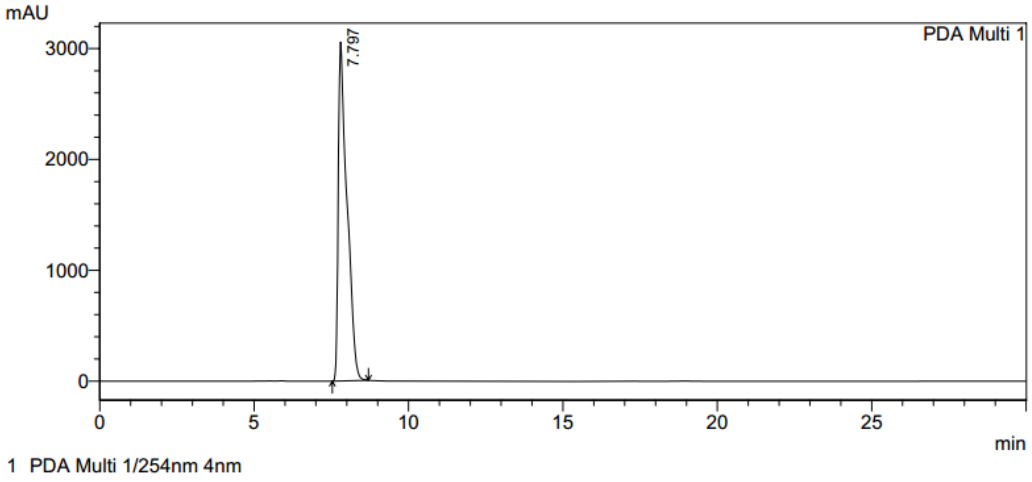

$^{1}$H NMR spectrum of **B01**




$^{13}$C NMR spectrum of **B01**




HRMS spectrum of **B01**

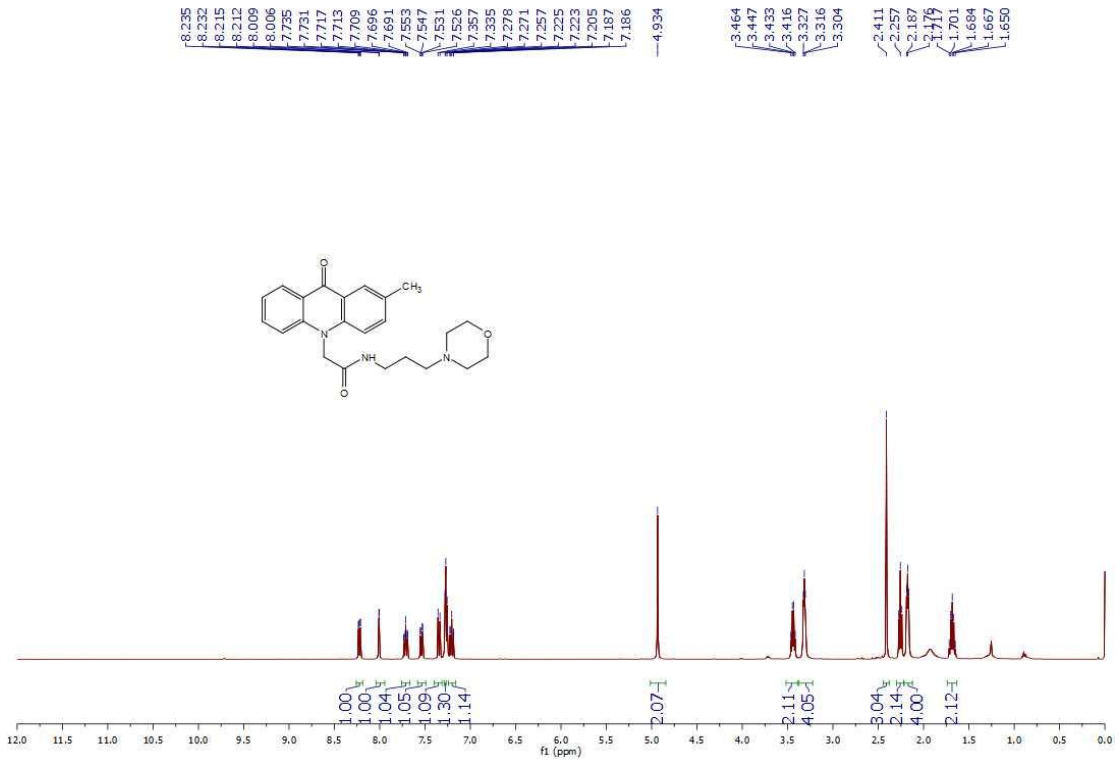



PeakTable

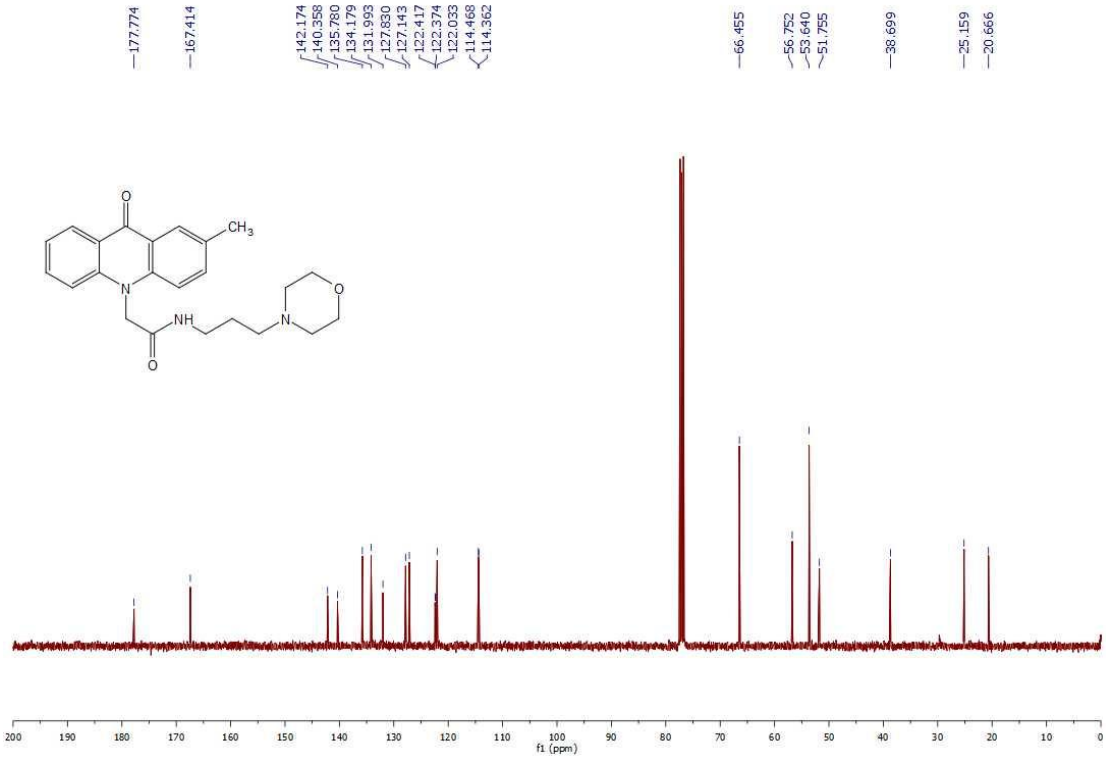
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 7.797 | 59999229 | 3056924 | 100.000 | 100.000 |
| Total | | 59999229 | 3056924 | 100.000 | 100.000 |

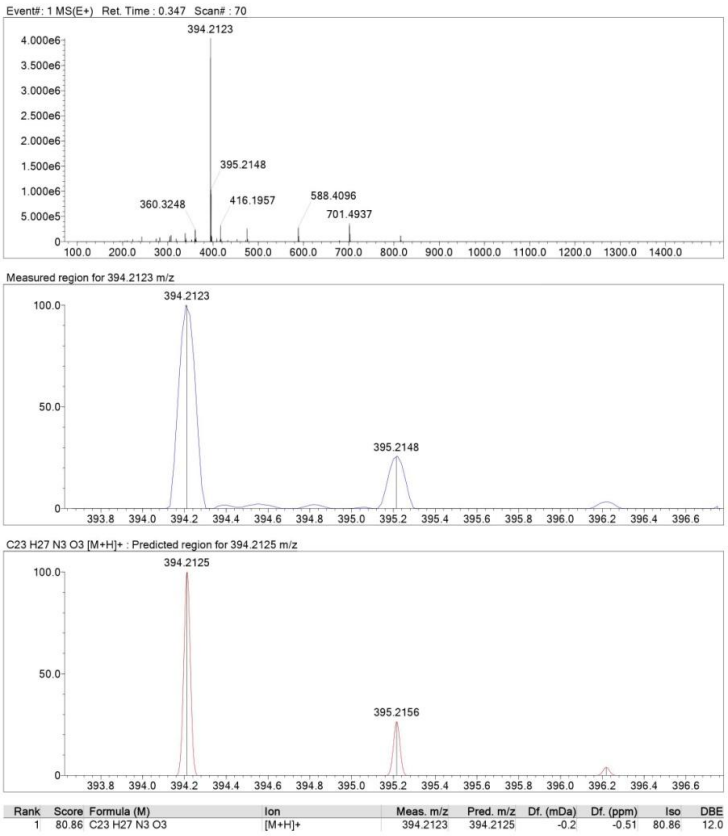
HPLC spectrum of **B01**



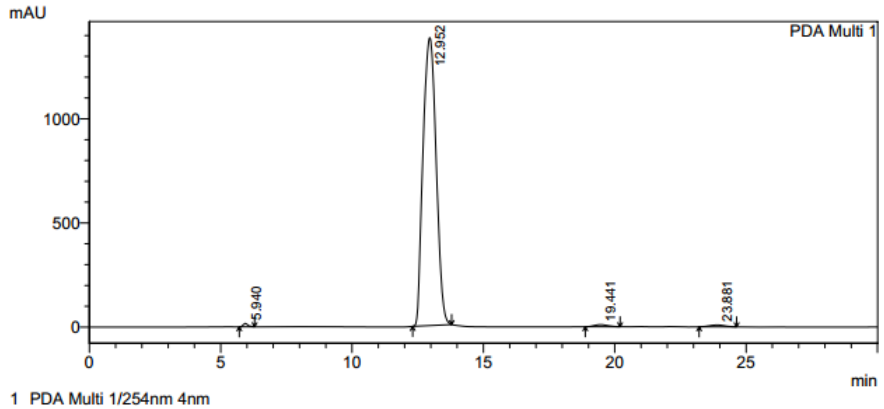

$^{1}$H NMR spectrum of **B02**




$^{13}$C NMR spectrum of **B02**




HRMS spectrum of **B02**

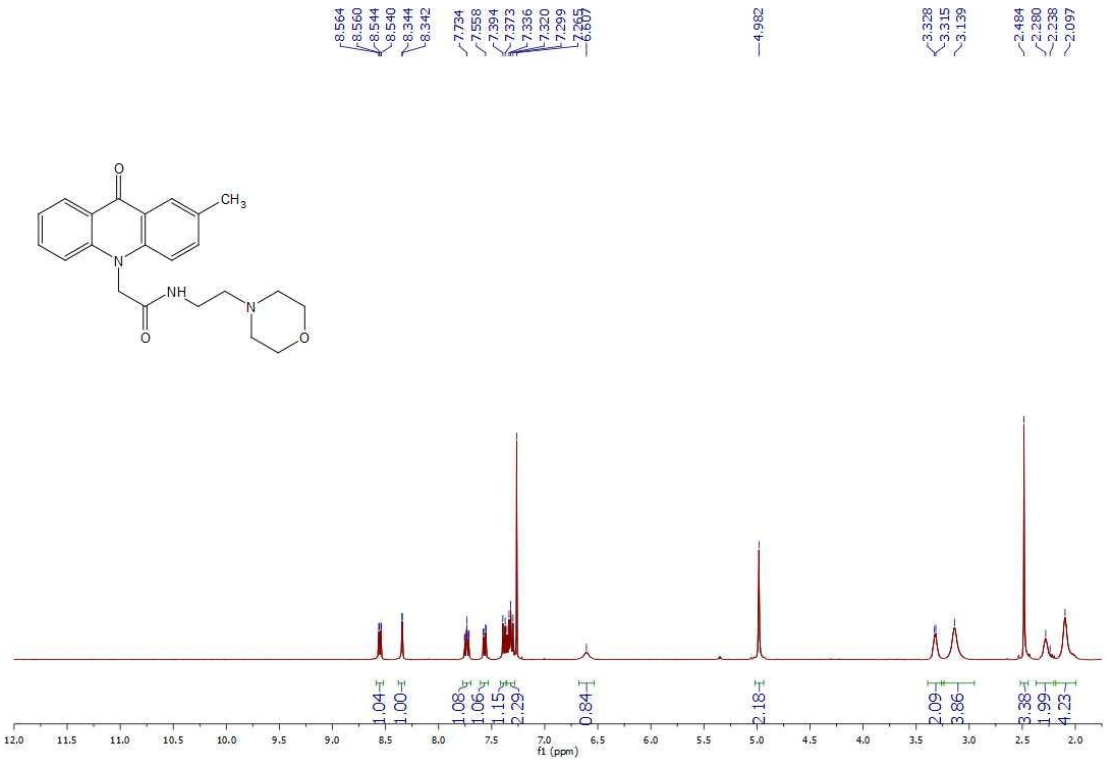



PeakTable

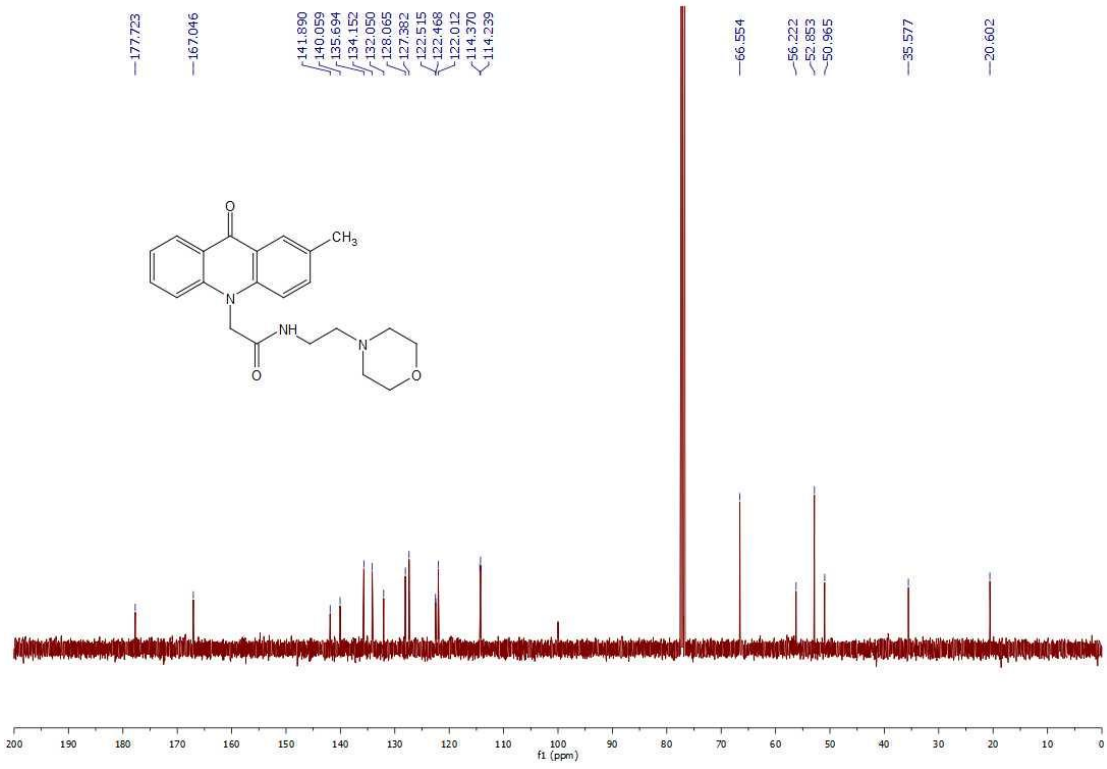
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 5.940 | 206803 | 16407 | 0.423 | 1.157 |
| 2 | 12.952 | 47942067 | 1382111 | 98.090 | 97.505 |
| 3 | 19.441 | 361380 | 9896 | 0.739 | 0.698 |
| 4 | 23.881 | 365172 | 9063 | 0.747 | 0.639 |
| Total | | 48875423 | 1417476 | 100.000 | 100.000 |

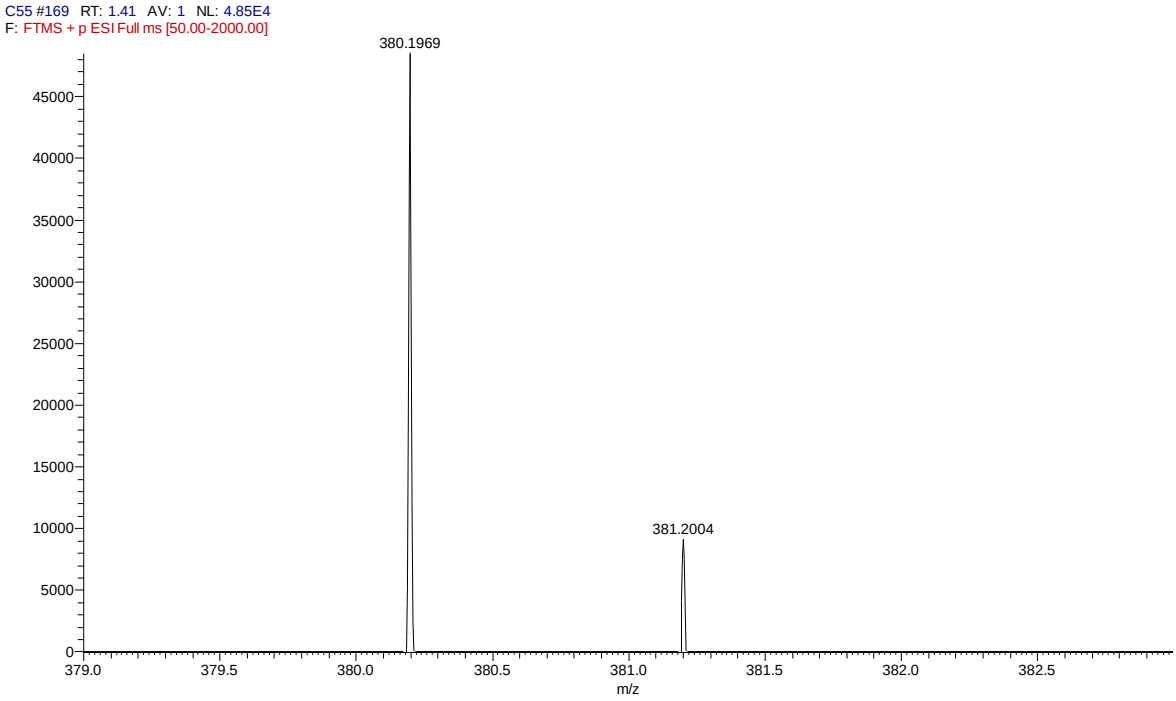
HPLC spectrum of **B02**



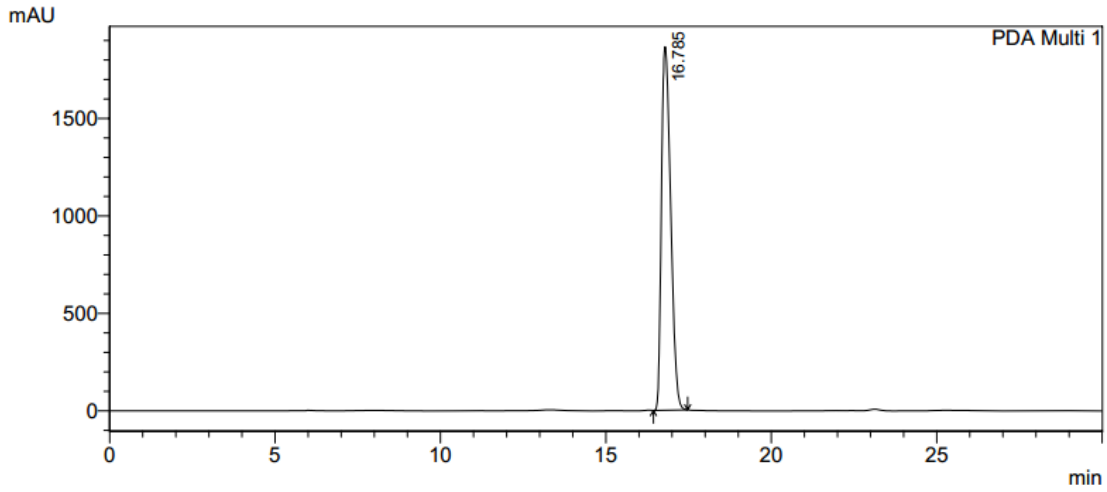

$^{1}$H NMR spectrum of **B03**




$^{13}$C NMR spectrum of **B03**




HRMS spectrum of **B03**



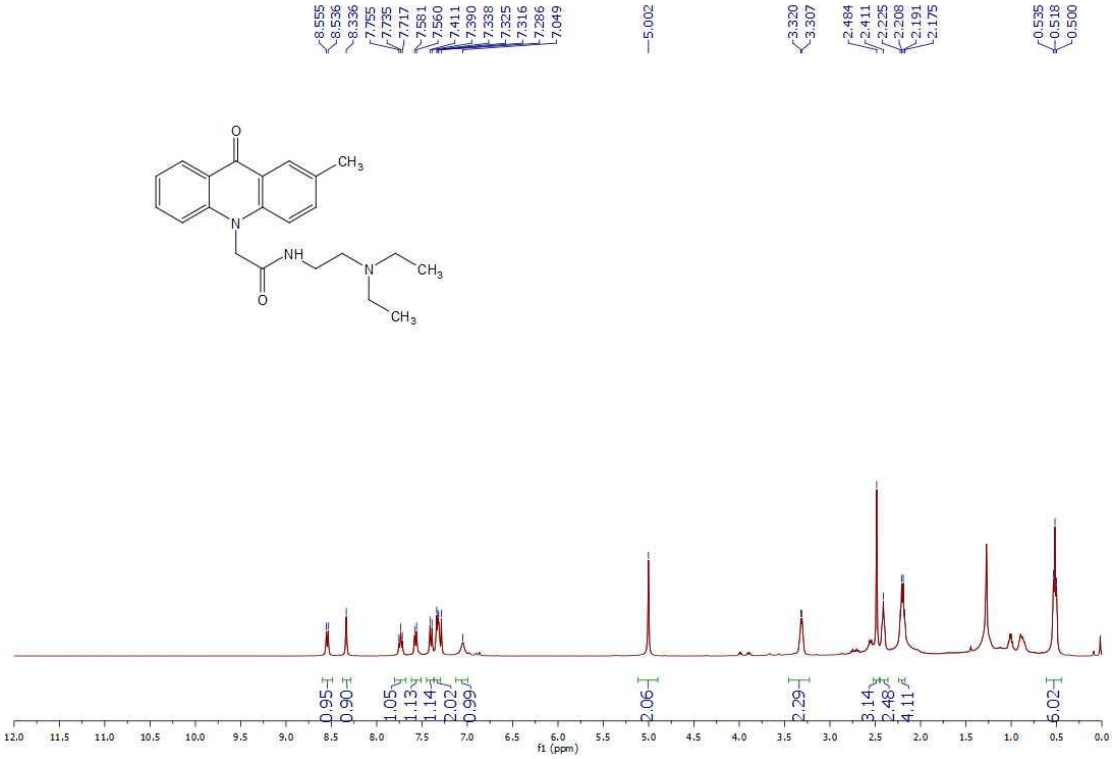

1 PDA Multi 1/254nm 4nm

PeakTable

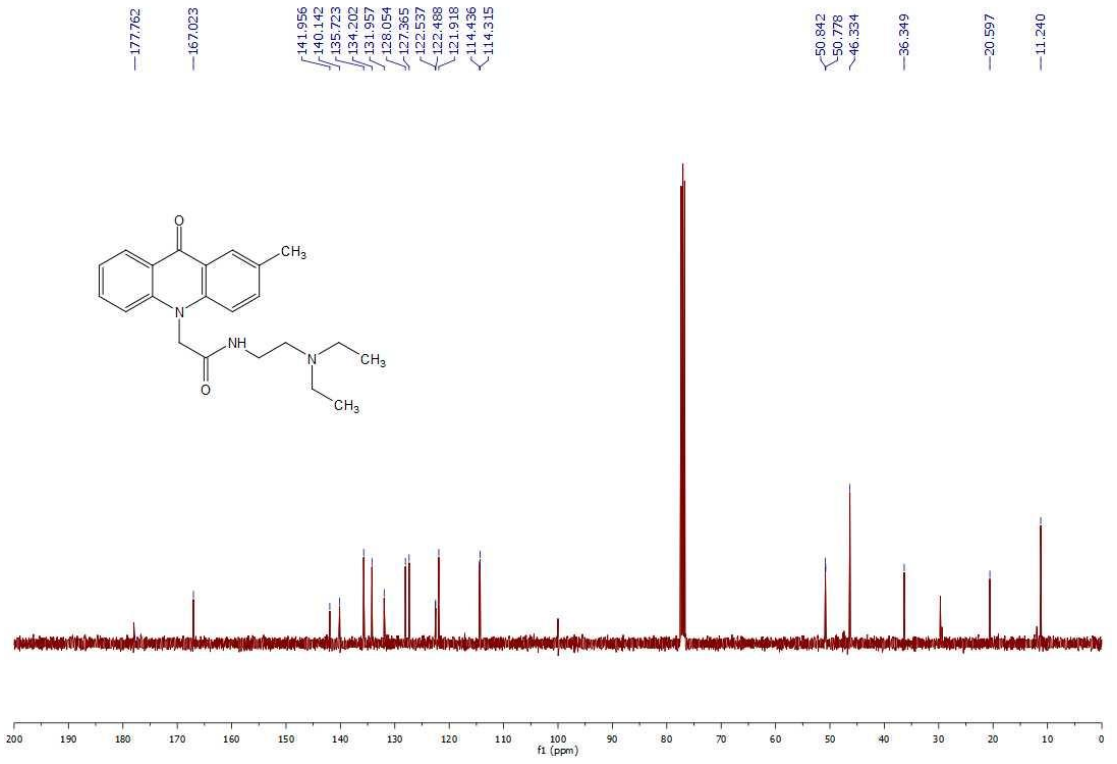
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 16.785 | 36194685 | 1865073 | 100.000 | 100.000 |
| Total | | 36194685 | 1865073 | 100.000 | 100.000 |

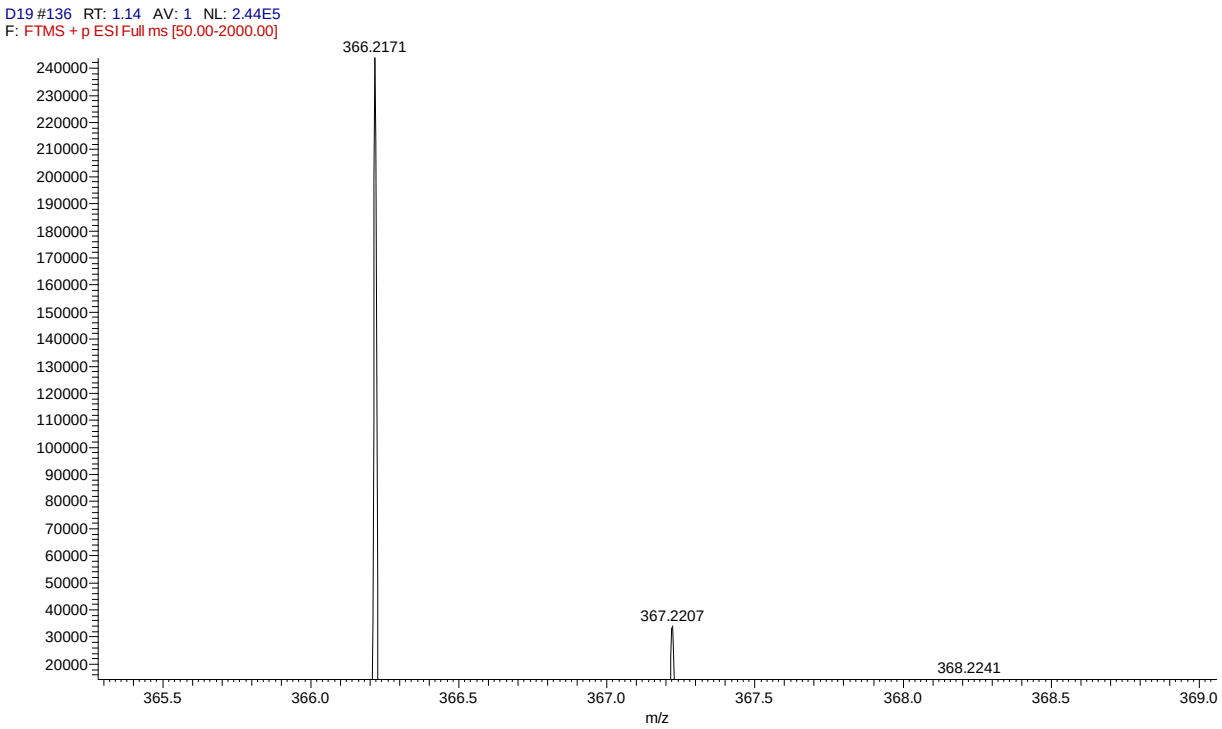
HPLC spectrum of **B03**



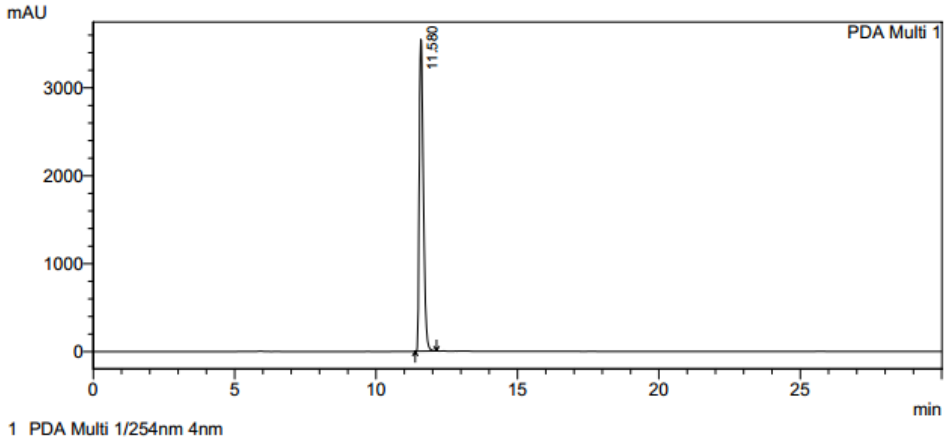

$^{1}$H NMR spectrum of **B04**




$^{13}$C NMR spectrum of **B04**




HRMS spectrum of **B04**

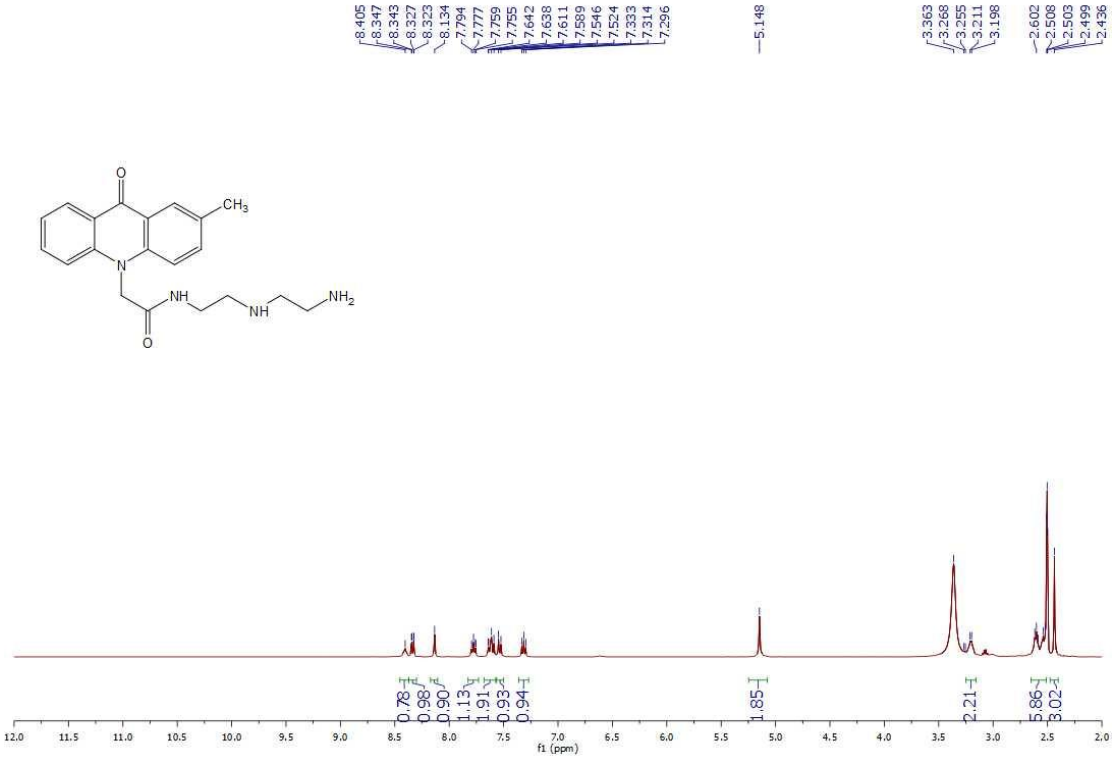



PeakTable

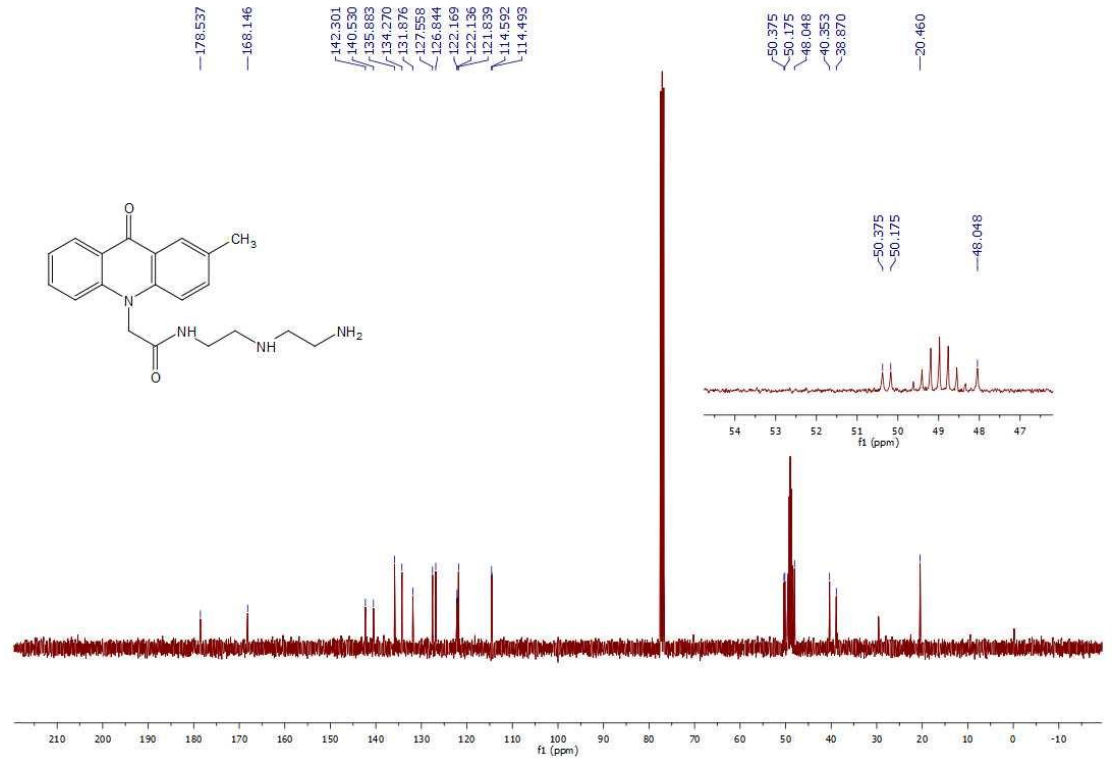
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 11.580 | 35745052 | 3546172 | 100.000 | 100.000 |
| Total | | 35745052 | 3546172 | 100.000 | 100.000 |

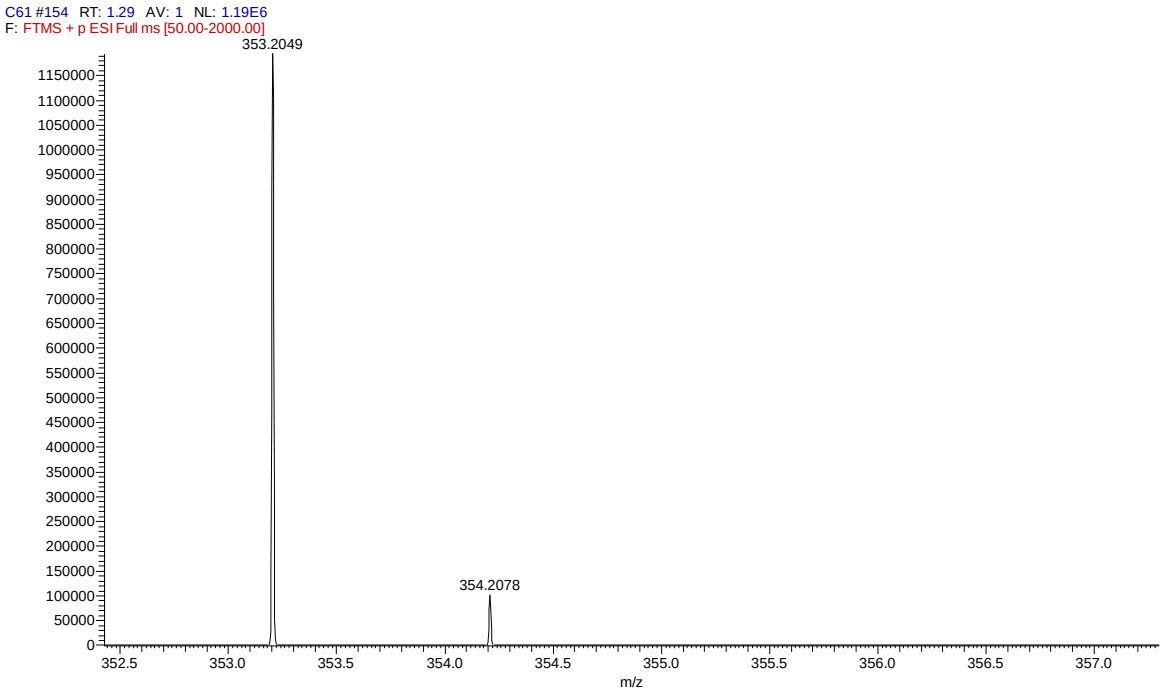
HPLC spectrum of **B04**



$^{1}$H NMR spectrum of **B05**

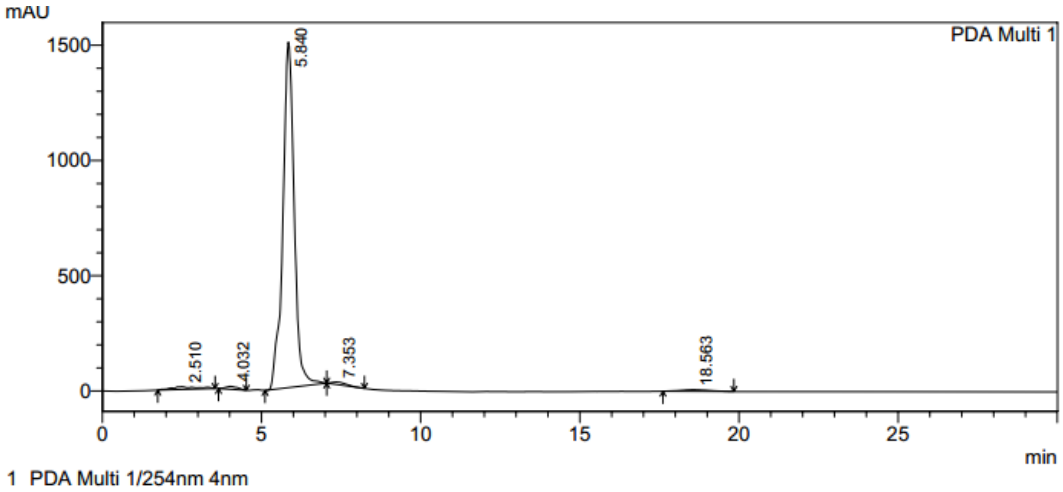


$^{13}$C NMR spectrum of **B05**




HRMS spectrum of

**B05**

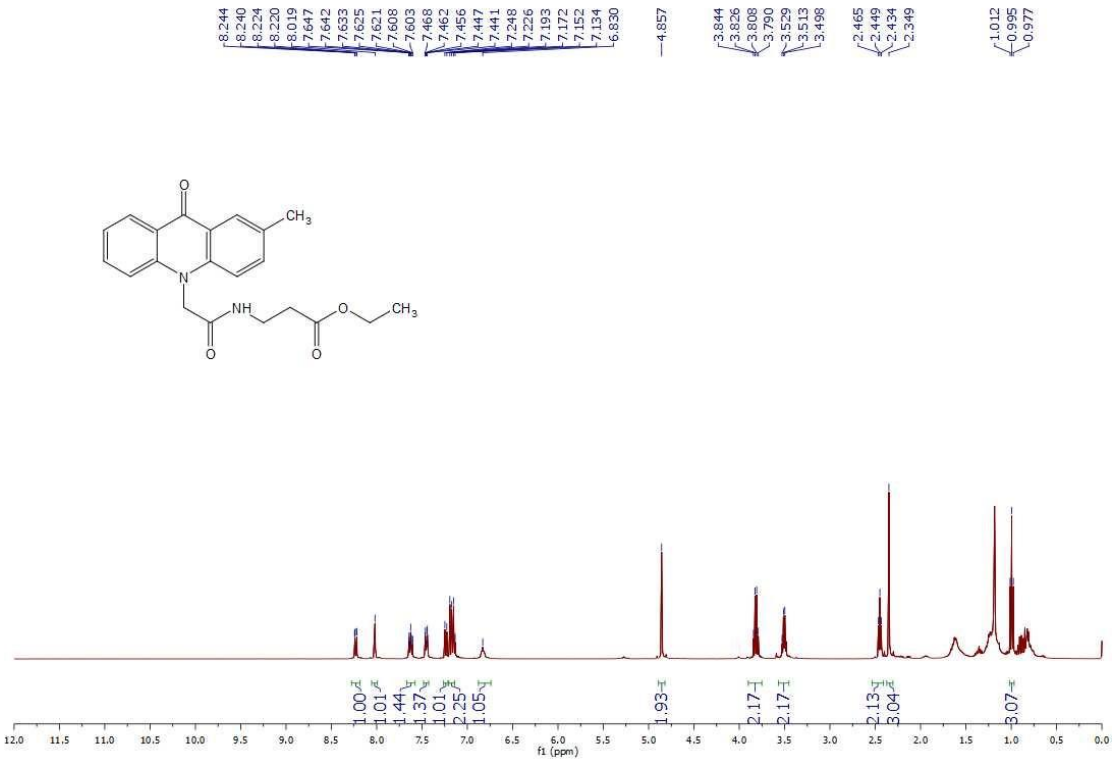



PeakTable

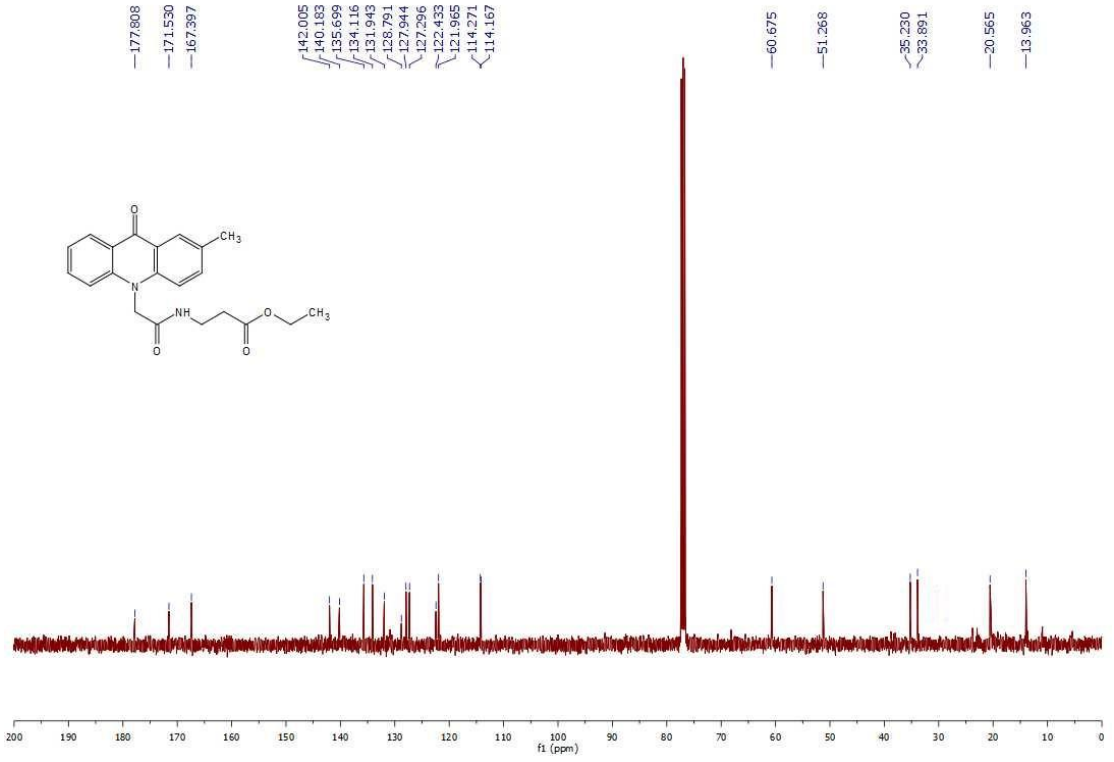
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 2.510 | 661261 | 11490 | 1.692 | 0.746 |
| 2 | 4.032 | 339140 | 12808 | 0.868 | 0.831 |
| 3 | 5.840 | 37295986 | 1499186 | 95.456 | 97.303 |
| 4 | 7.353 | 328655 | 10301 | 0.841 | 0.669 |
| 5 | 18.563 | 446442 | 6953 | 1.143 | 0.451 |
| Total | | 39071484 | 1540738 | 100.000 | 100.000 |

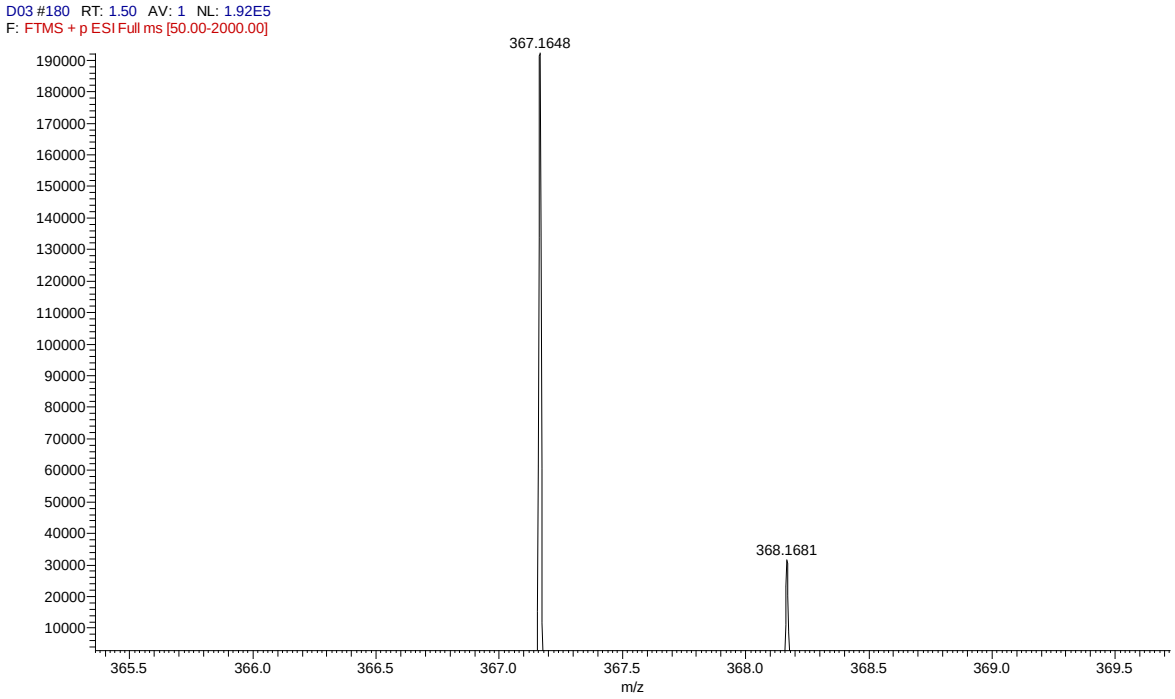
HPLC spectrum of **B05**



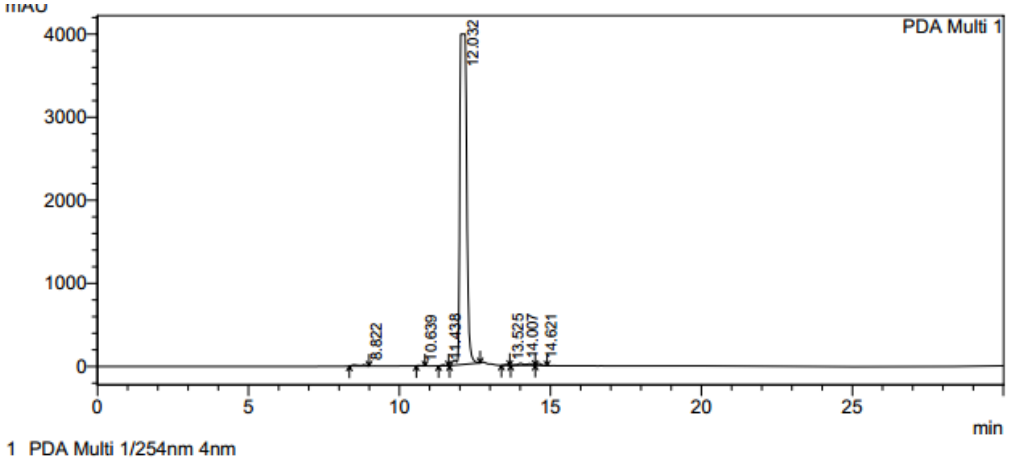

$^{1}$H NMR spectrum of **B06**




$^{13}$C NMR spectrum of **B06**




HRMS spectrum of **B06**

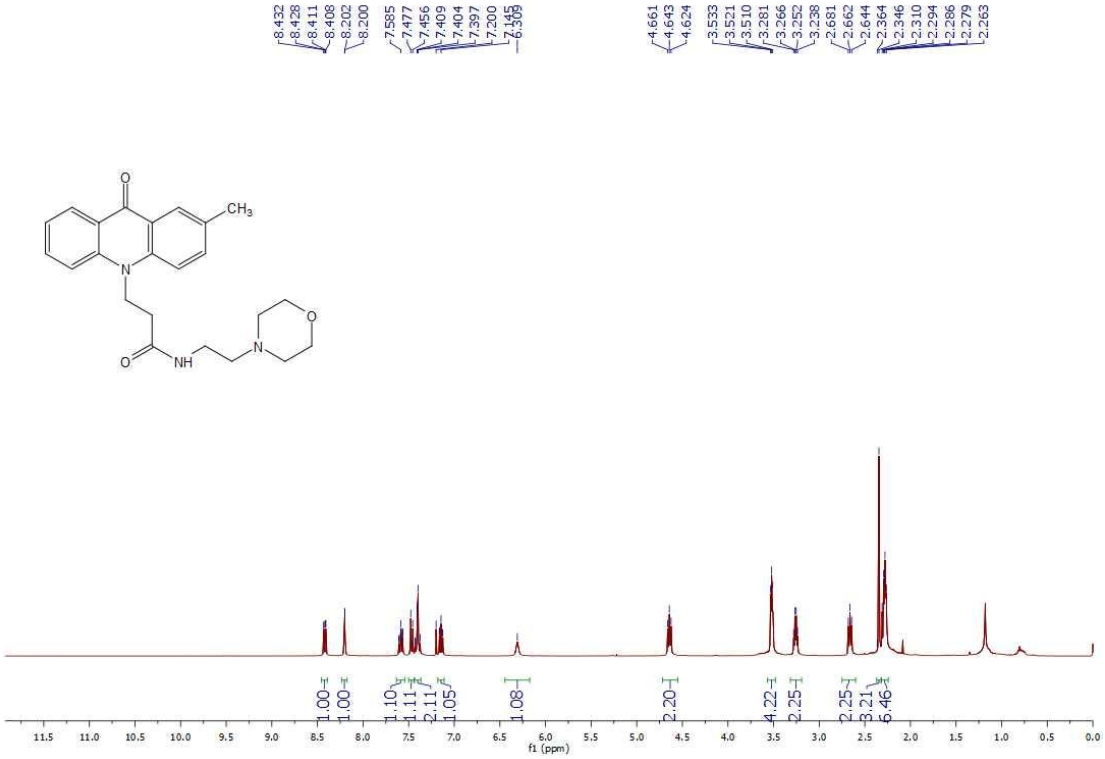



PeakTable

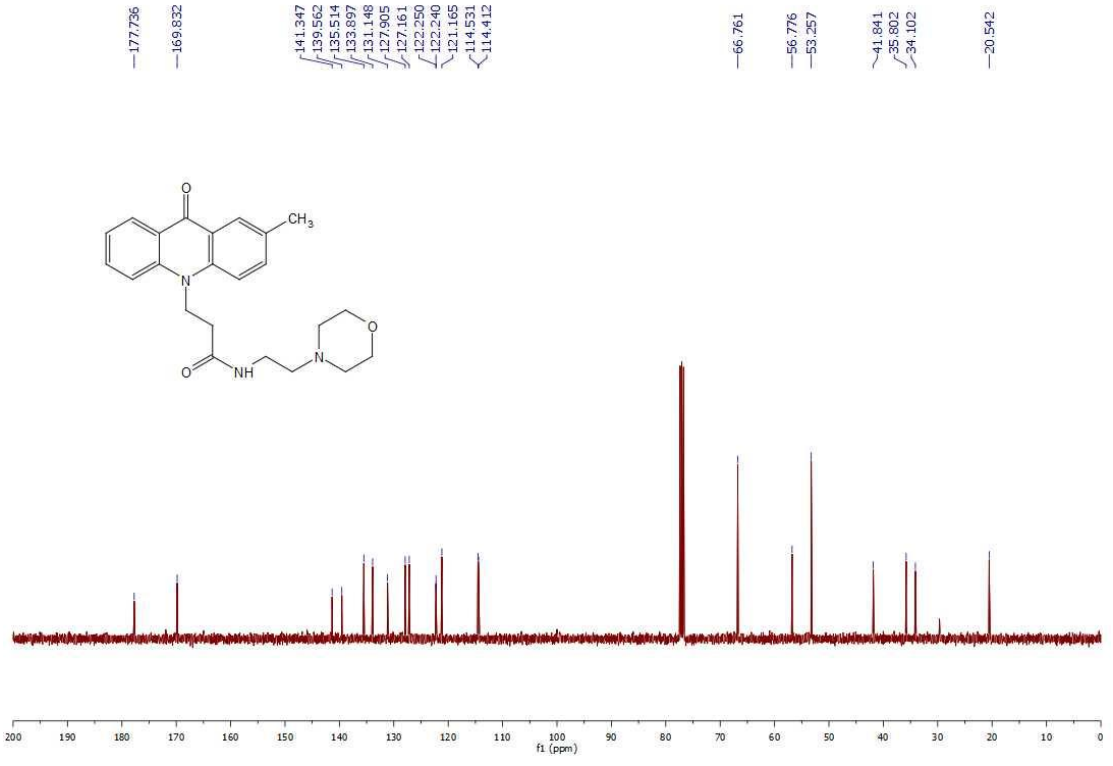
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 8.822 | 339197 | 16249 | 0.532 | 0.399 |
| 2 | 10.639 | 38382 | 5201 | 0.060 | 0.128 |
| 3 | 11.438 | 101417 | 12452 | 0.159 | 0.306 |
| 4 | 12.032 | 62409385 | 3978055 | 97.938 | 97.711 |
| 5 | 13.525 | 69824 | 8988 | 0.110 | 0.221 |
| 6 | 14.007 | 538950 | 25119 | 0.846 | 0.617 |
| 7 | 14.621 | 225923 | 25185 | 0.355 | 0.619 |
| Total | | 63723077 | 4071249 | 100.000 | 100.000 |

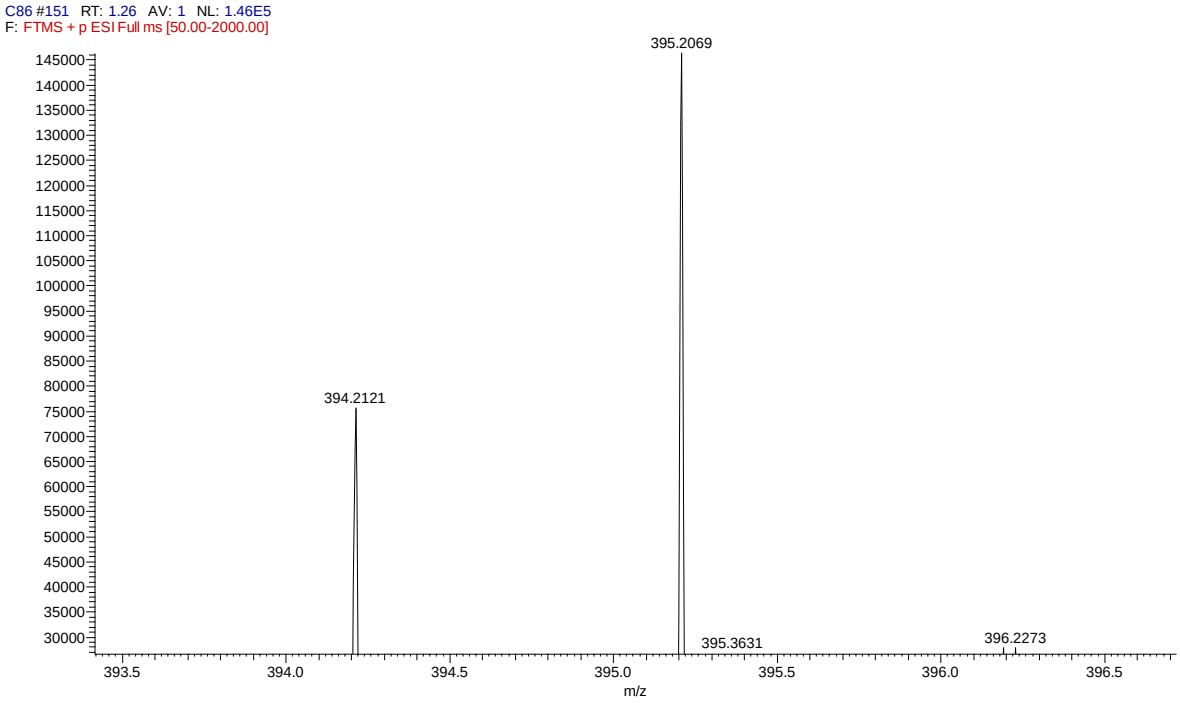
HPLC spectrum of **B06**




$^{1}$H spectrum of **B07**

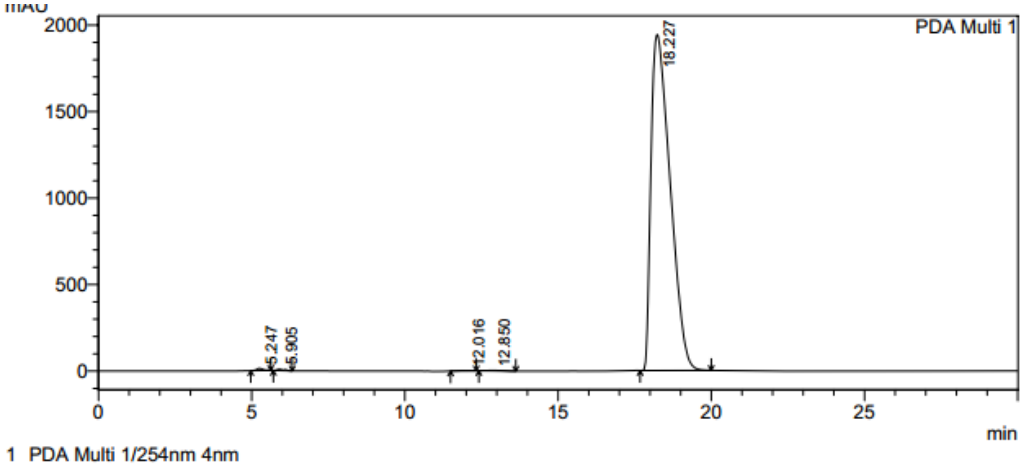



$^{13}$C spectrum of **B07**




HRMS spectrum of

**B07**

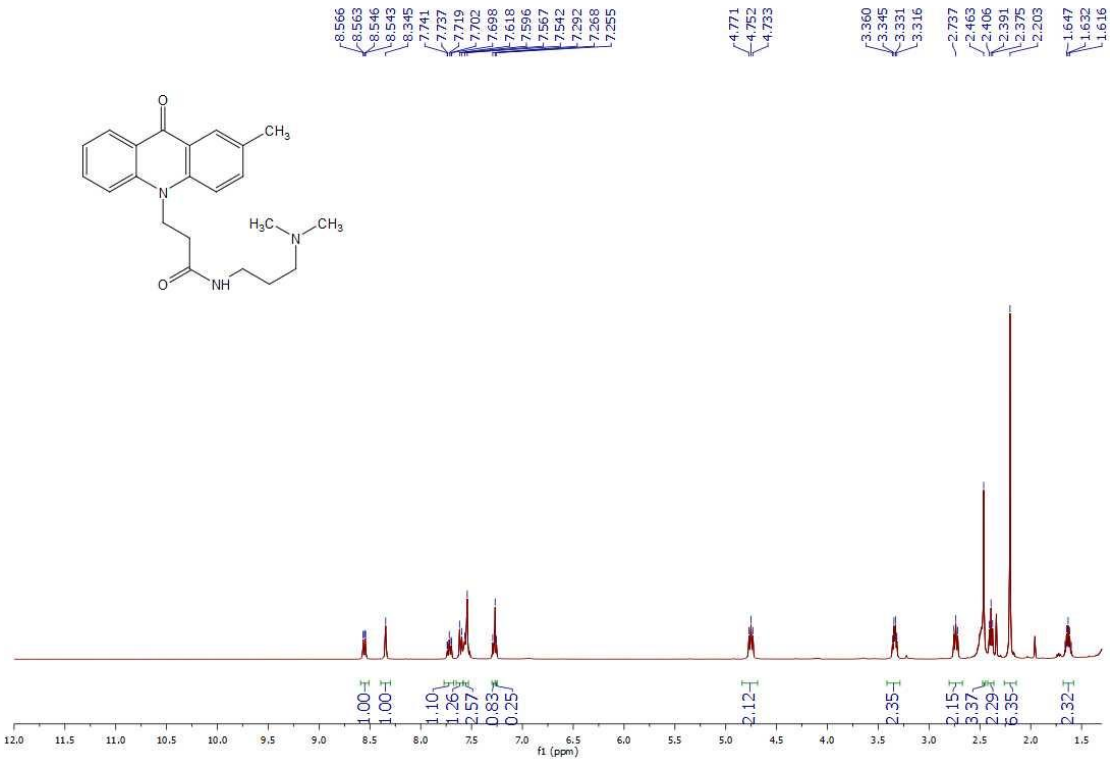



PeakTable

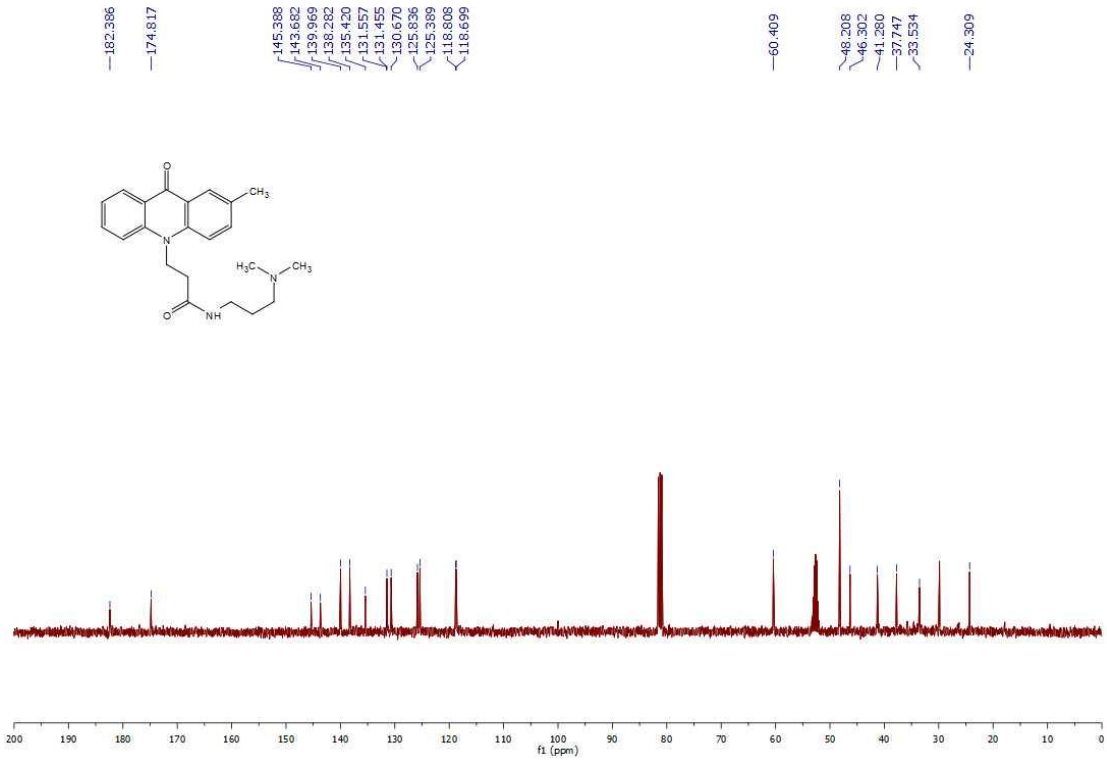
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 5.247 | 175170 | 12893 | 0.206 | 0.655 |
| 2 | 5.905 | 132028 | 7659 | 0.156 | 0.389 |
| 3 | 12.016 | 58817 | 1992 | 0.069 | 0.101 |
| 4 | 12.850 | 111763 | 2979 | 0.132 | 0.151 |
| 5 | 18.227 | 84370893 | 1941994 | 99.437 | 98.703 |
| Total | | 84848671 | 1967516 | 100.000 | 100.000 |

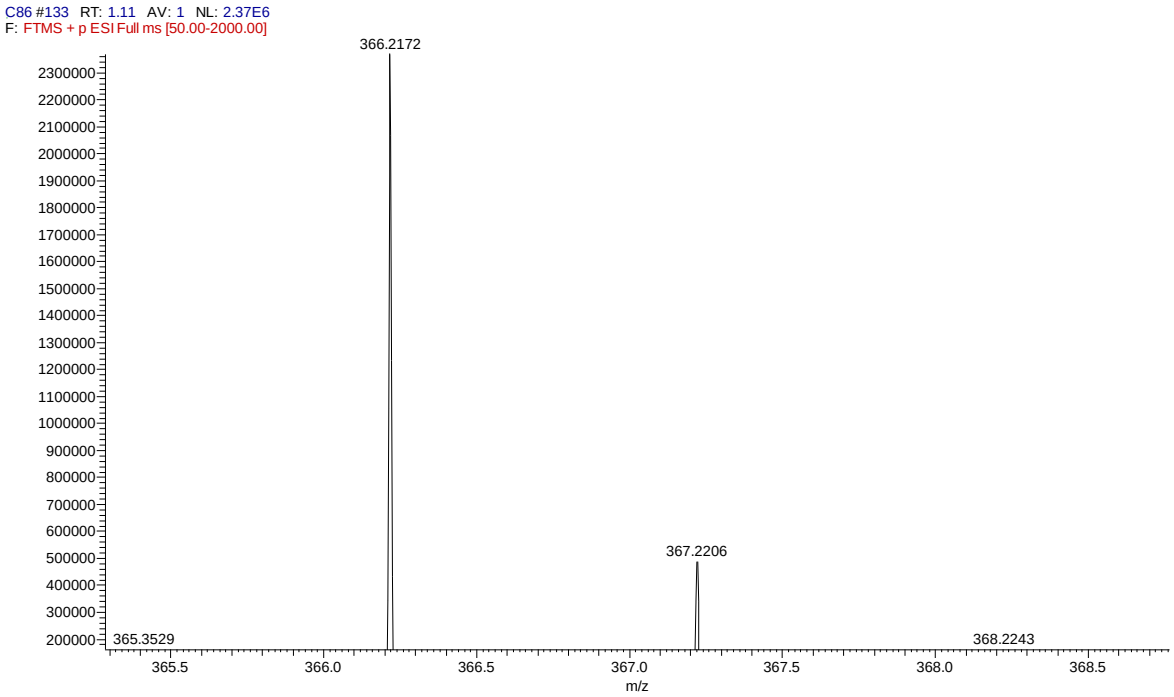
HPLC spectrum of **B07**



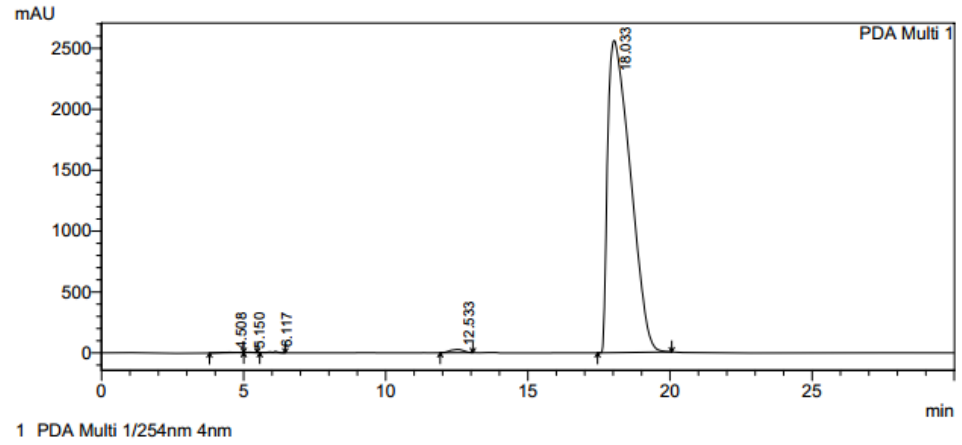

$^{1}$H NMR spectrum of **B08**




$^{13}$C NMR spectrum of **B08**




HRMS spectrum of **B08**

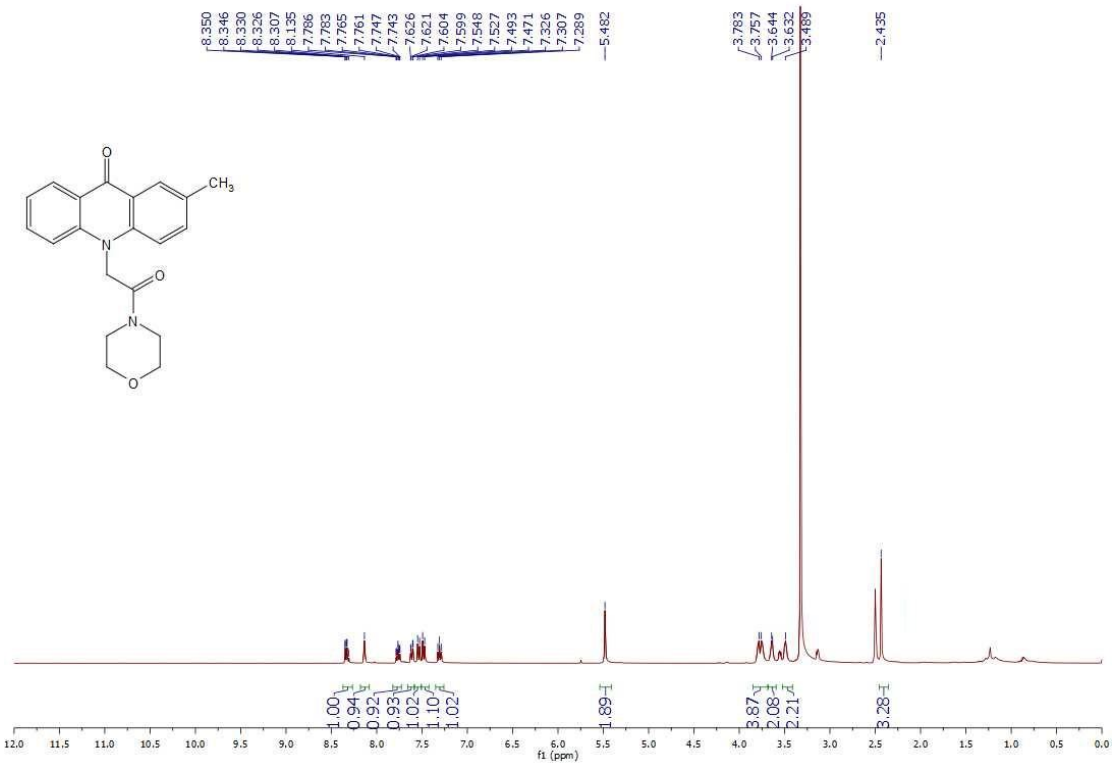



PeakTable

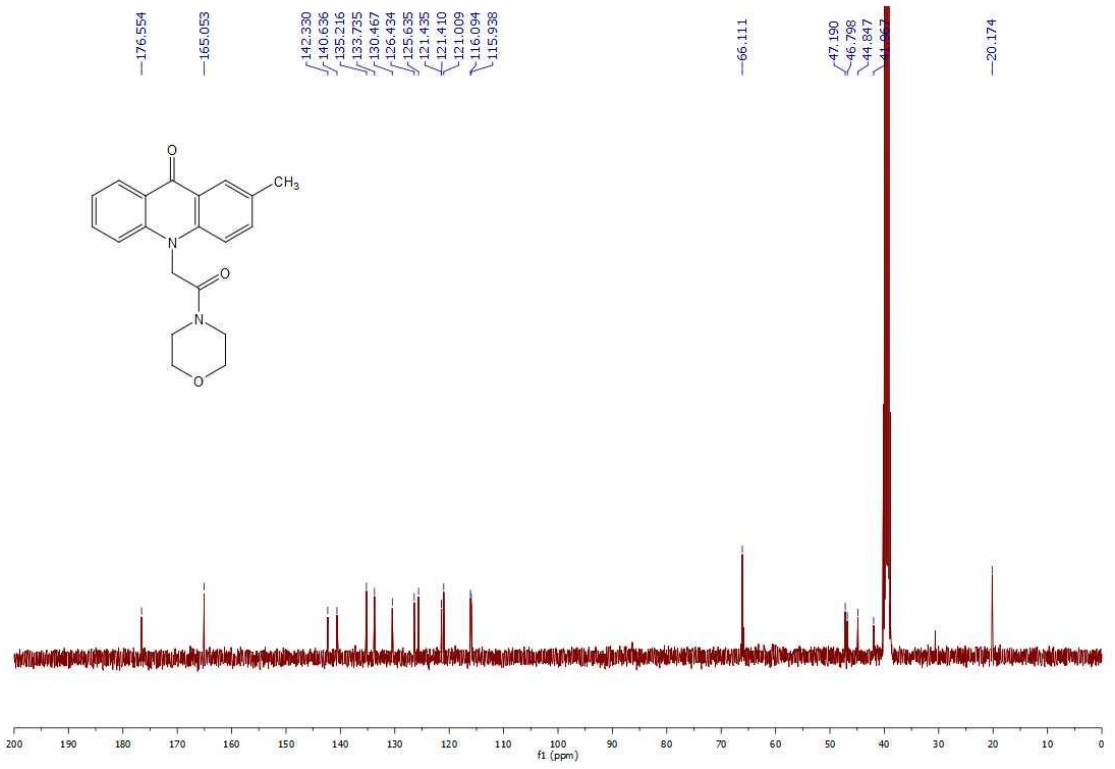
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 4.508 | 154907 | 3483 | 0.108 | 0.134 |
| 2 | 5.150 | 50413 | 3414 | 0.035 | 0.131 |
| 3 | 6.117 | 167729 | 11076 | 0.117 | 0.425 |
| 4 | 12.533 | 872401 | 25898 | 0.610 | 0.994 |
| 5 | 18.033 | 141826986 | 2562492 | 99.129 | 98.317 |
| Total | | 143072435 | 2606363 | 100.000 | 100.000 |

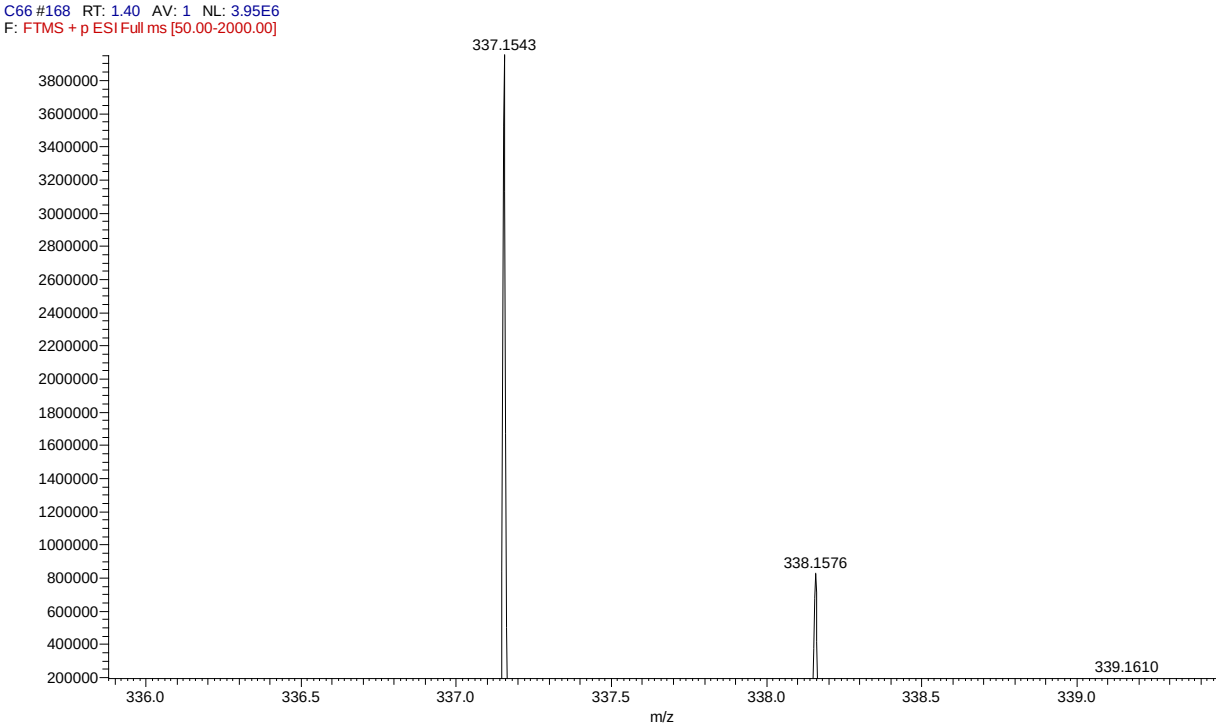
HPLC spectrum of **B08**




$^{1}$H NMR spectrum of **B09**

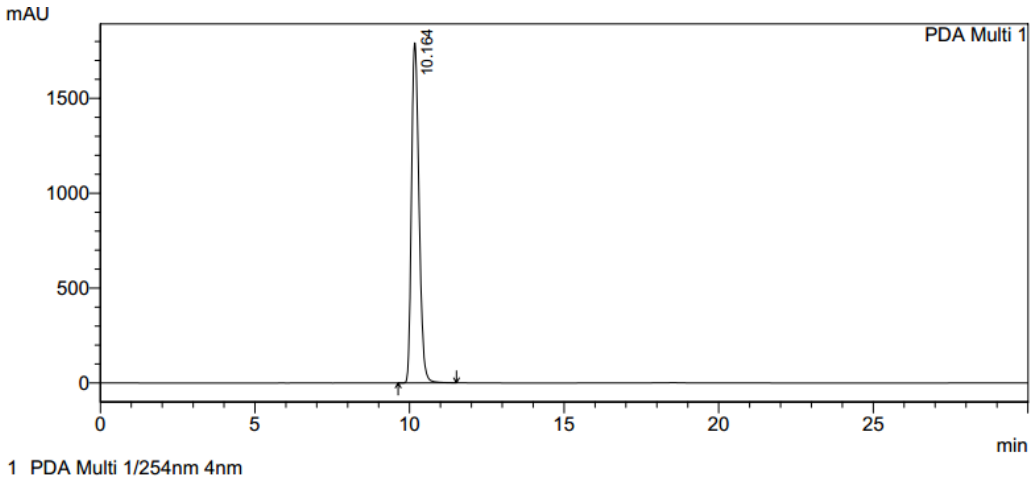



$^{13}$C NMR spectrum of **B09**




HRMS spectrum of

**B09**

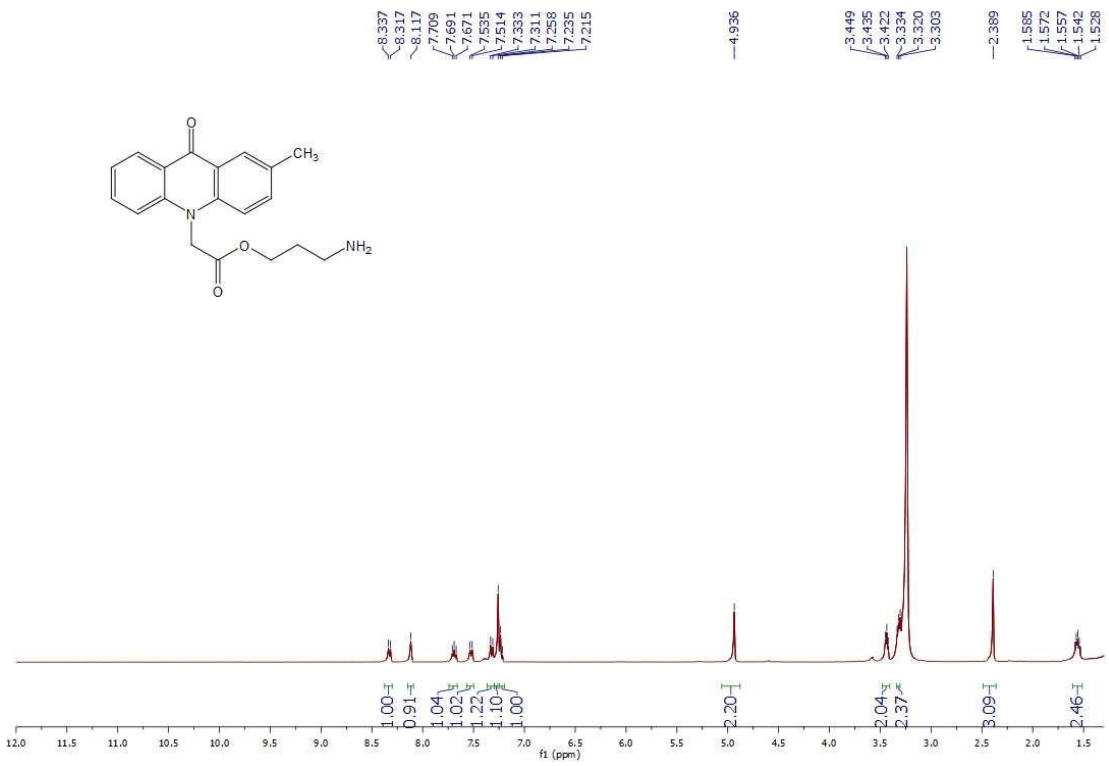



PeakTable

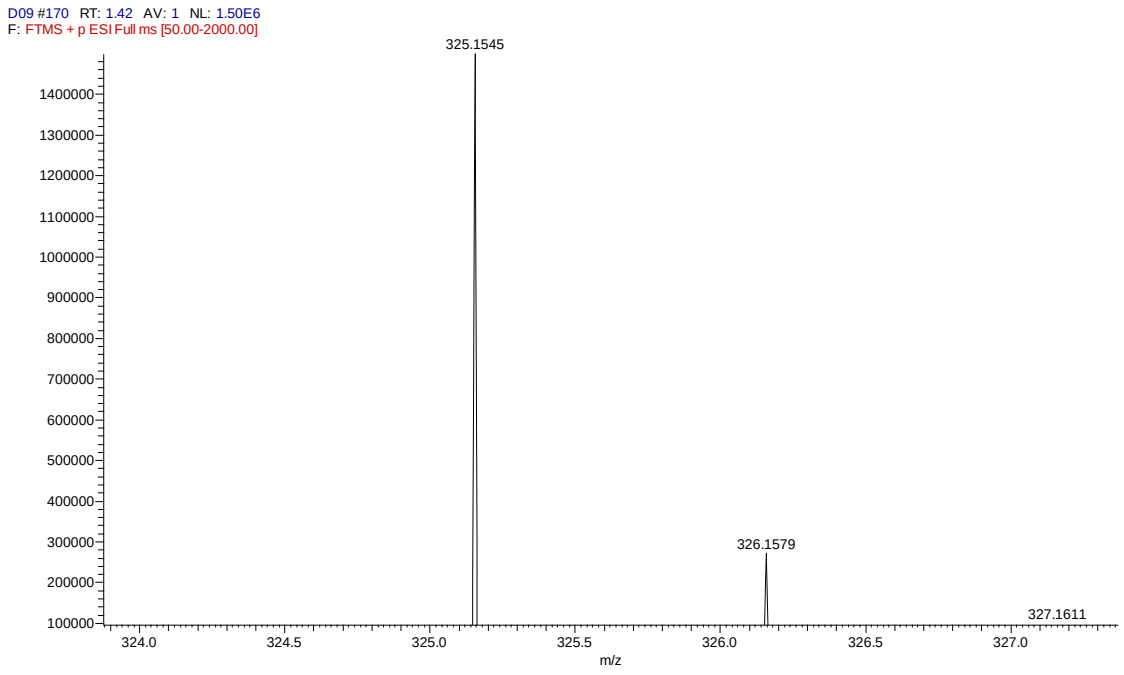
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 10.164 | 30384750 | 1791716 | 100.000 | 100.000 |
| Total | | 30384750 | 1791716 | 100.000 | 100.000 |

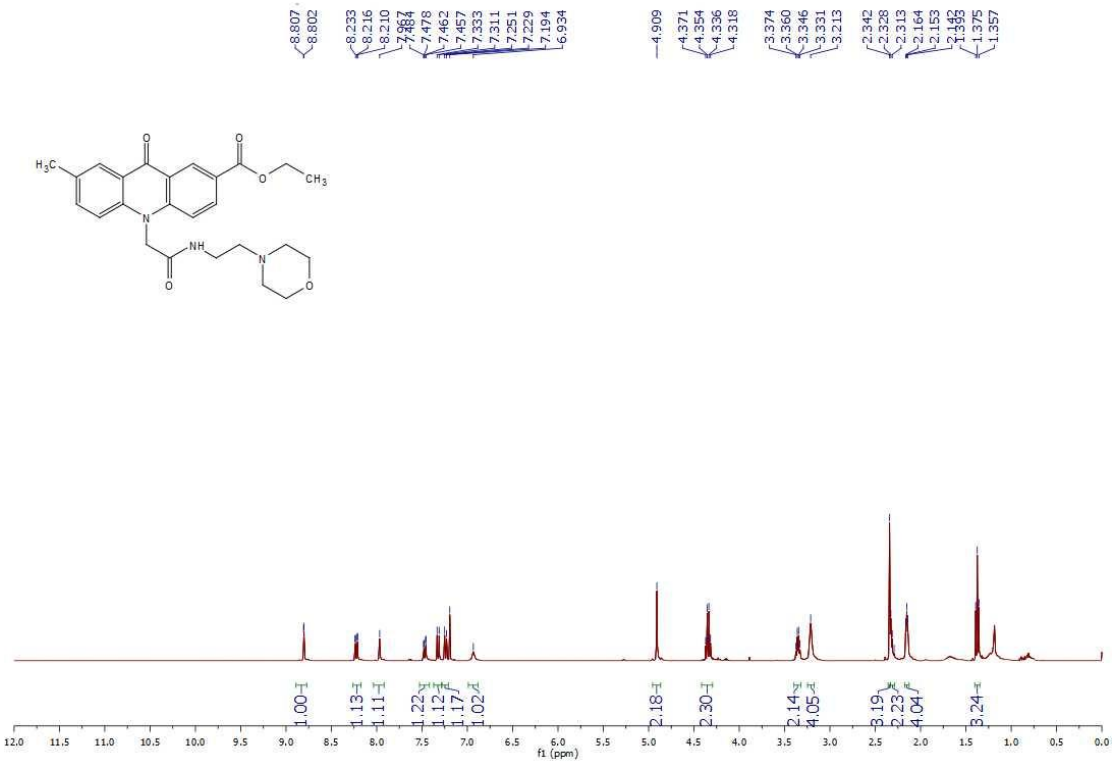
HPLC spectrum of **B09**



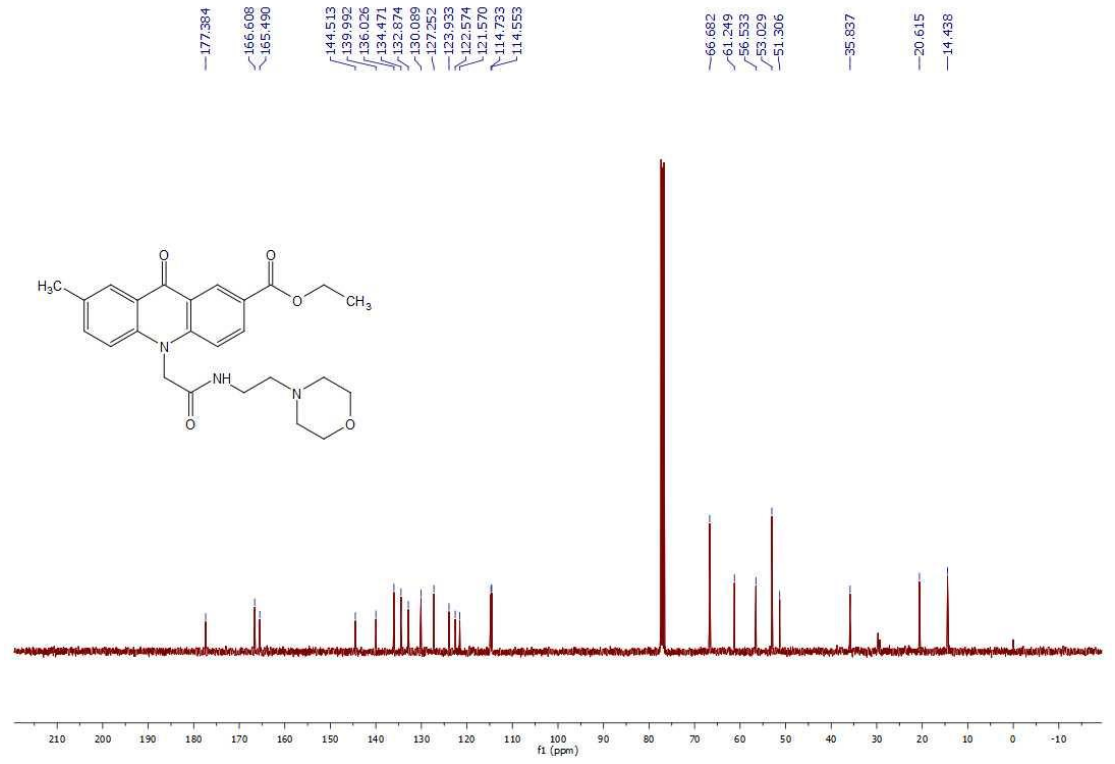

$^{1}$H NMR spectrum of **B10**



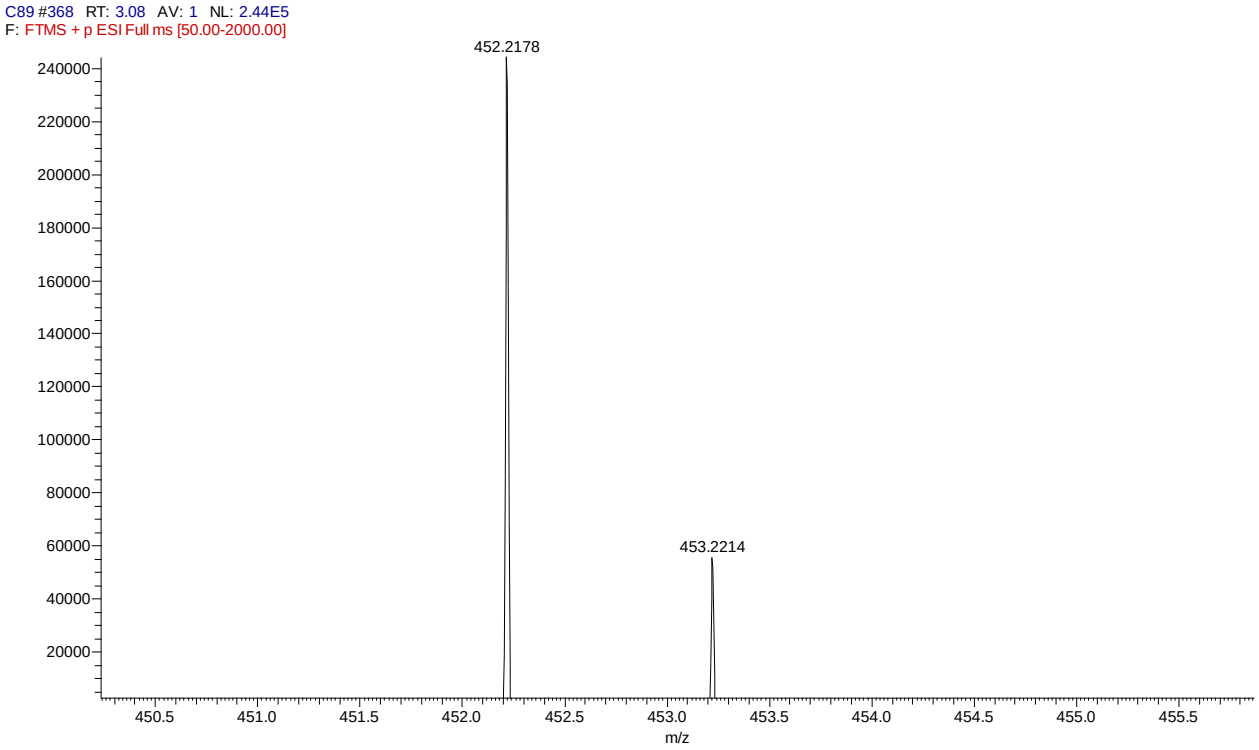

HRMS spectrum of **B10**



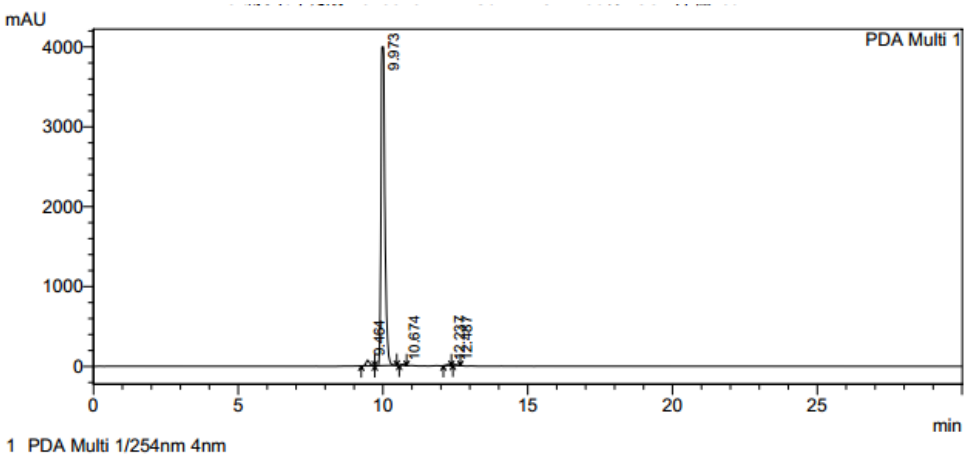
$^{1}$H NMR spectrum of **B11**



$^{13}$C NMR spectrum of **B11**




HRMS spectrum of **B11**

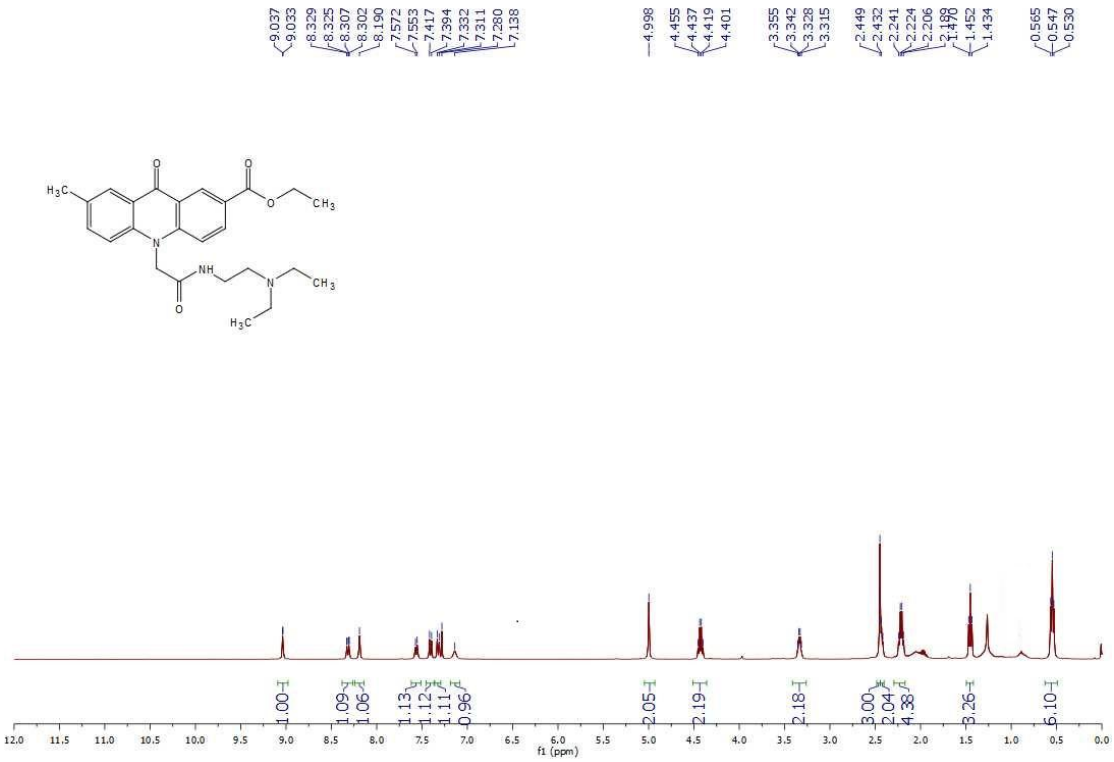



PeakTable

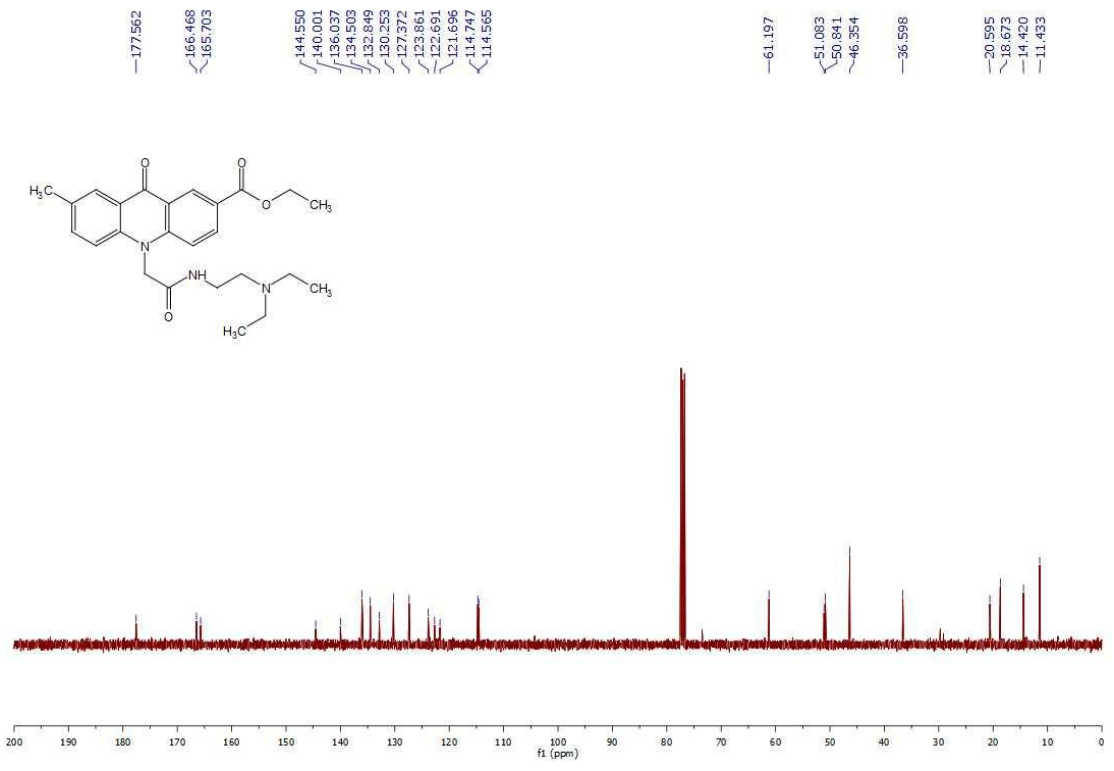
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 9.464 | 620327 | 70068 | 1.627 | 1.712 |
| 2 | 9.973 | 37244913 | 3989892 | 97.701 | 97.499 |
| 3 | 10.674 | 108096 | 12866 | 0.284 | 0.314 |
| 4 | 12.237 | 87323 | 10259 | 0.229 | 0.251 |
| 5 | 12.487 | 60832 | 9162 | 0.160 | 0.224 |
| Total | | 38121491 | 4092247 | 100.000 | 100.000 |

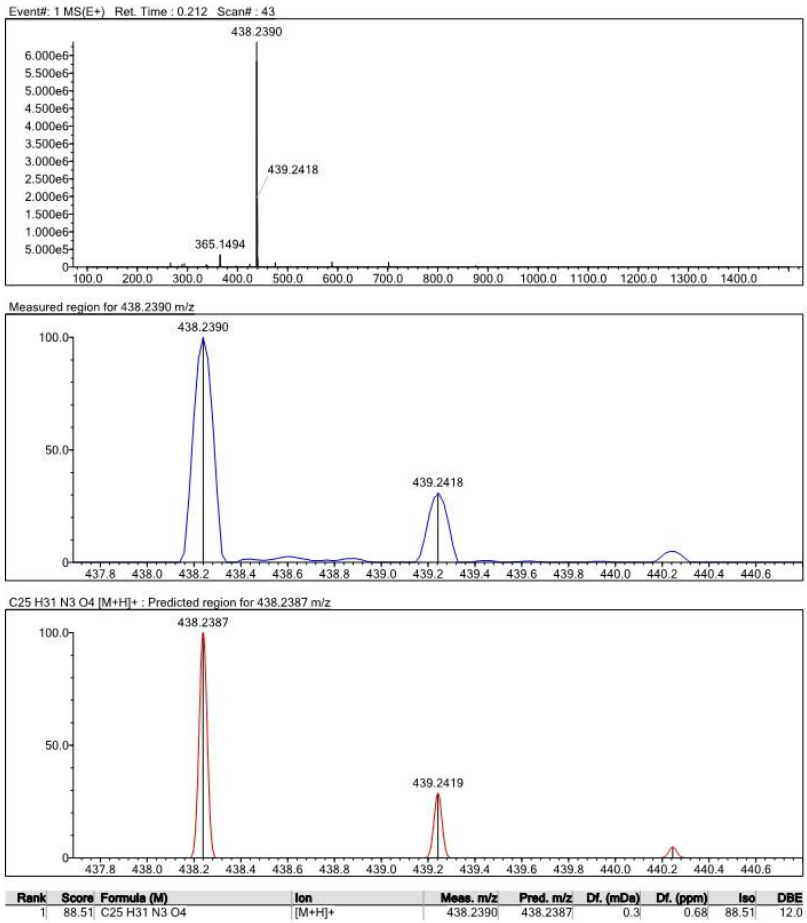
HPLC spectrum of **B11**



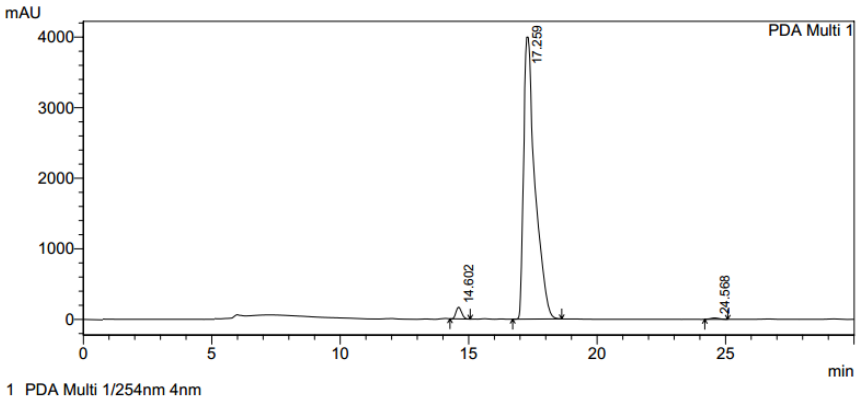
$^{1}$H NMR spectrum of **B12**



$^{13}$C NMR spectrum of **B12**




HRMS spectrum of **B12**

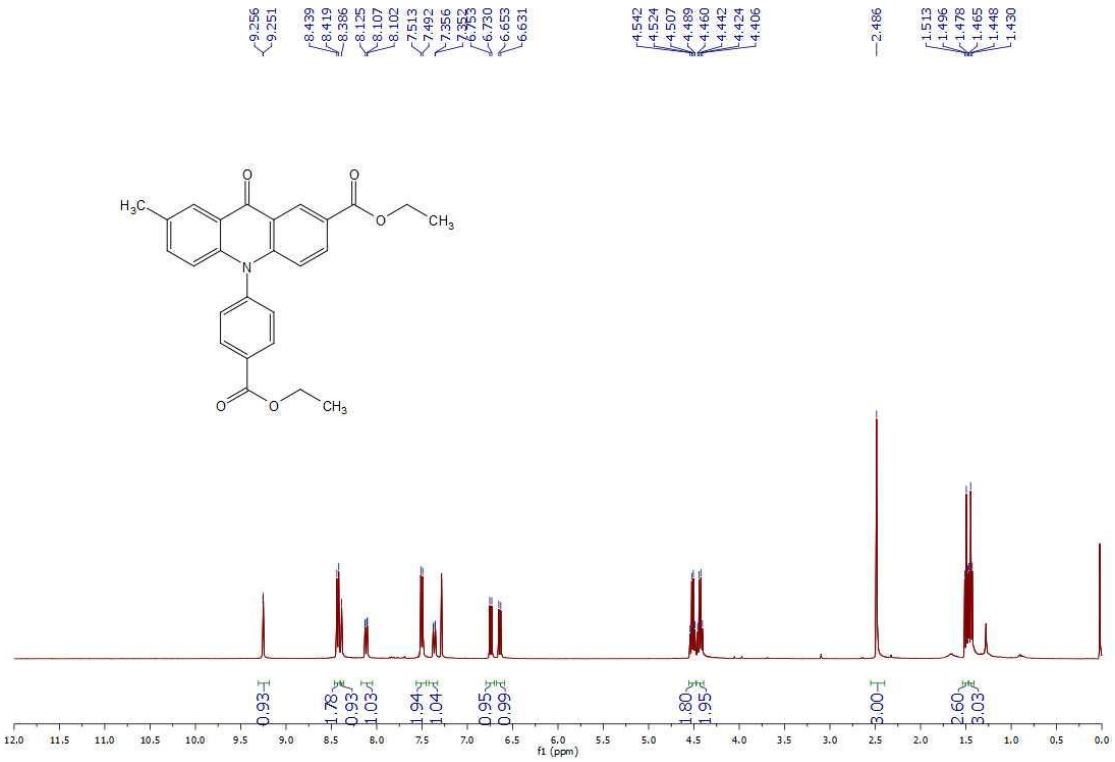



PeakTable

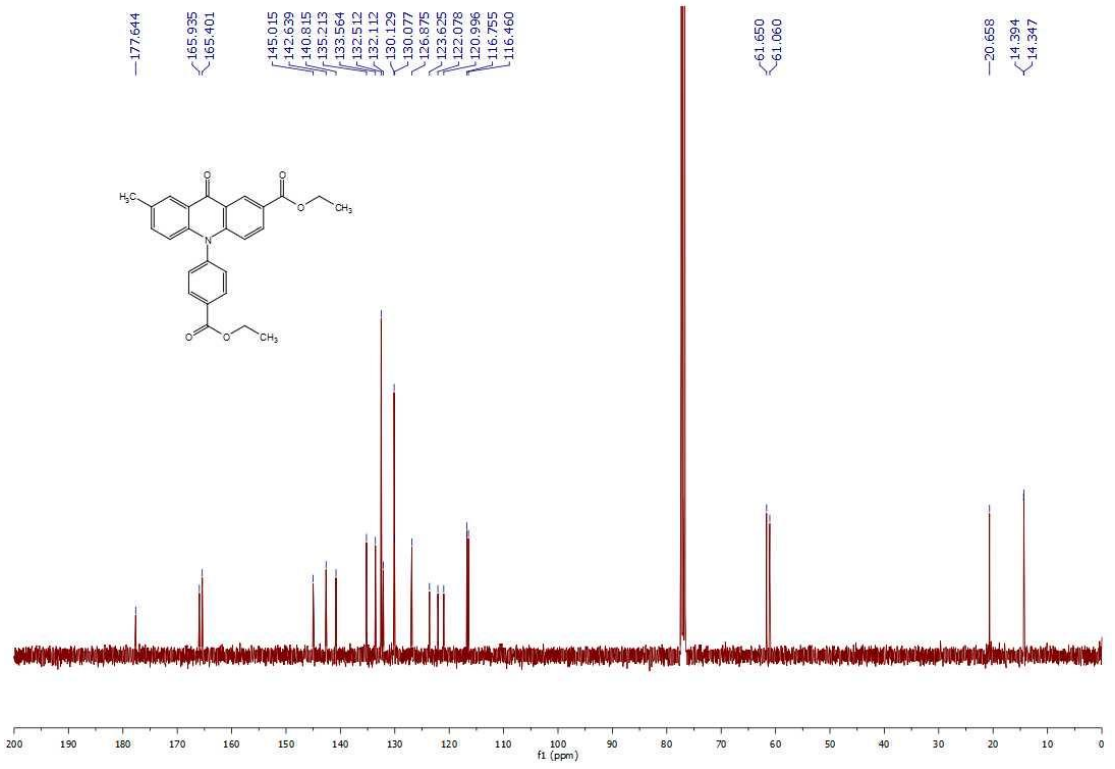
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 14.602 | 2535854 | 165861 | 2.018 | 3.968 |
| 2 | 17.259 | 122681509 | 3995692 | 97.638 | 95.584 |
| 3 | 24.568 | 431803 | 18733 | 0.344 | 0.448 |
| Total | | 125649166 | 4180286 | 100.000 | 100.000 |

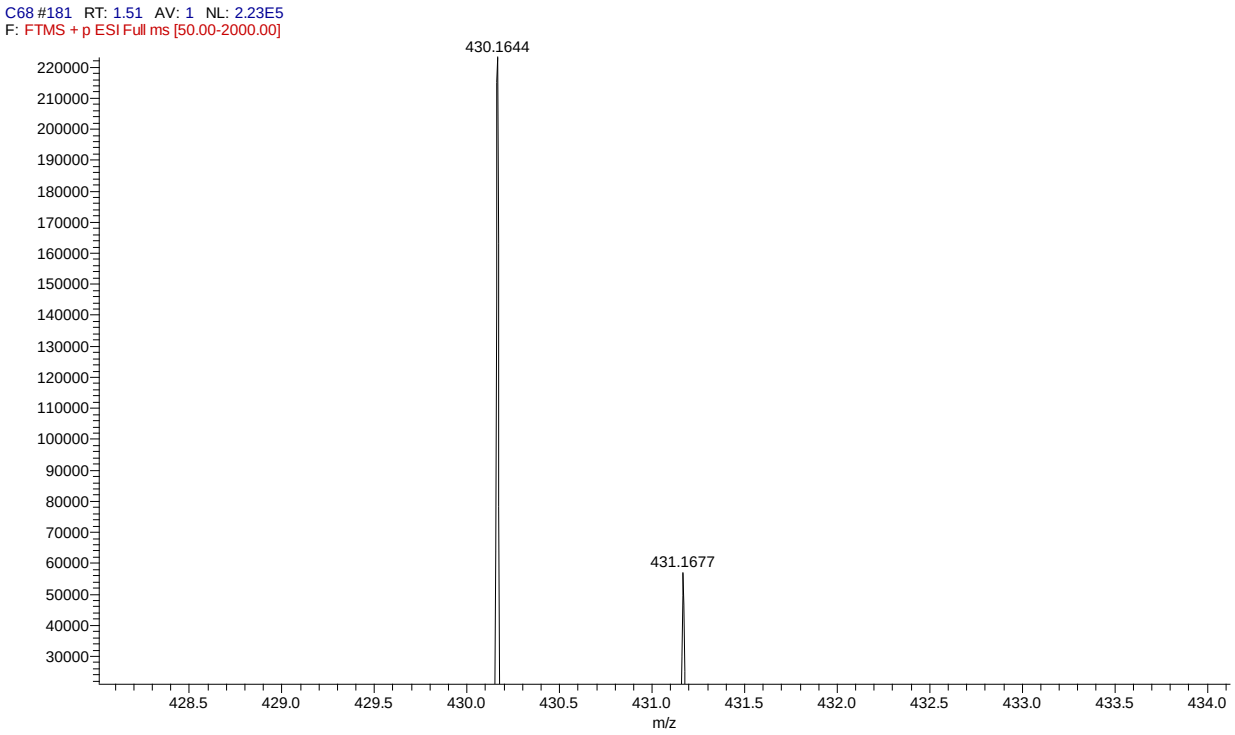
HPLC spectrum of **B12**



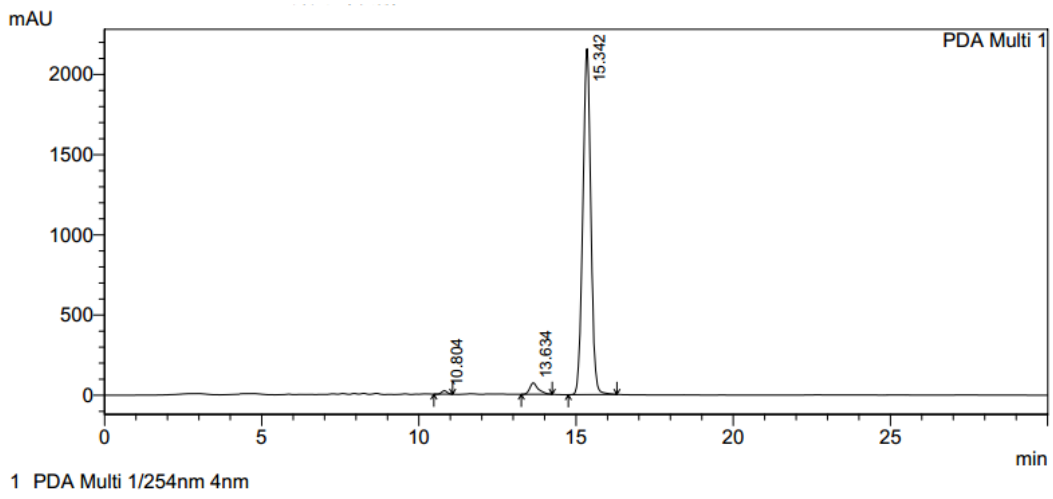

$^{1}$H NMR spectrum of **B13**




$^{13}$C NMR spectrum of **B13**




HRMS spectrum of **B13**

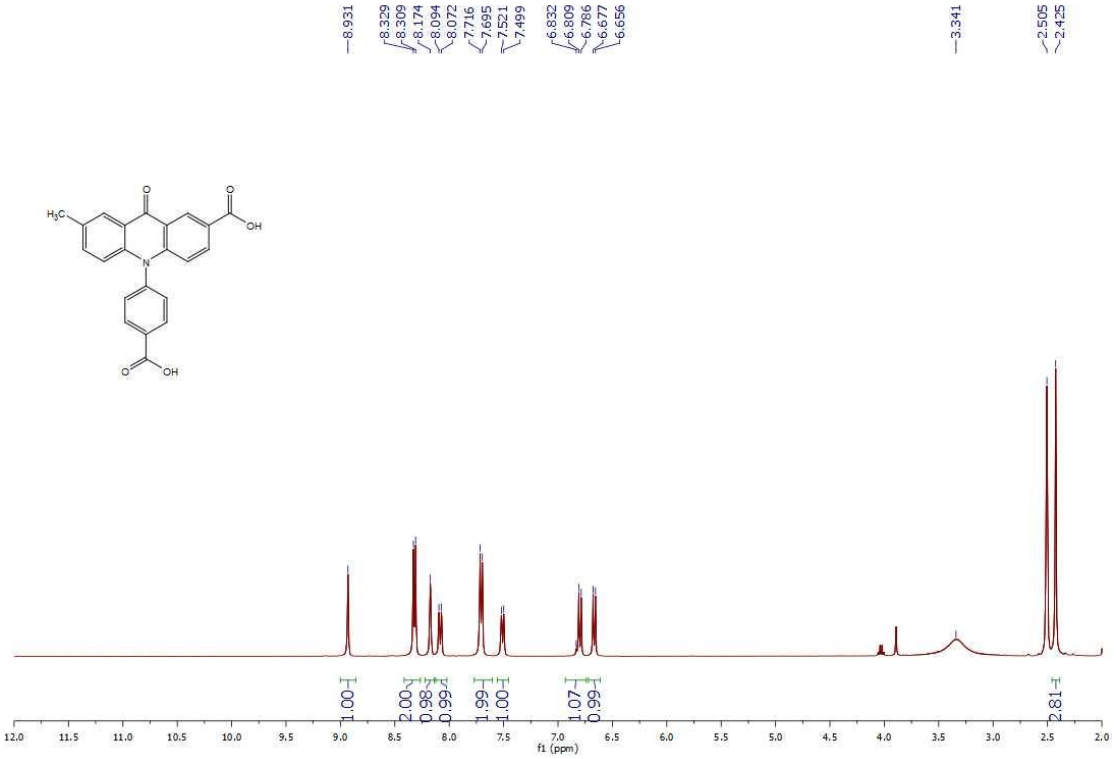



PeakTable

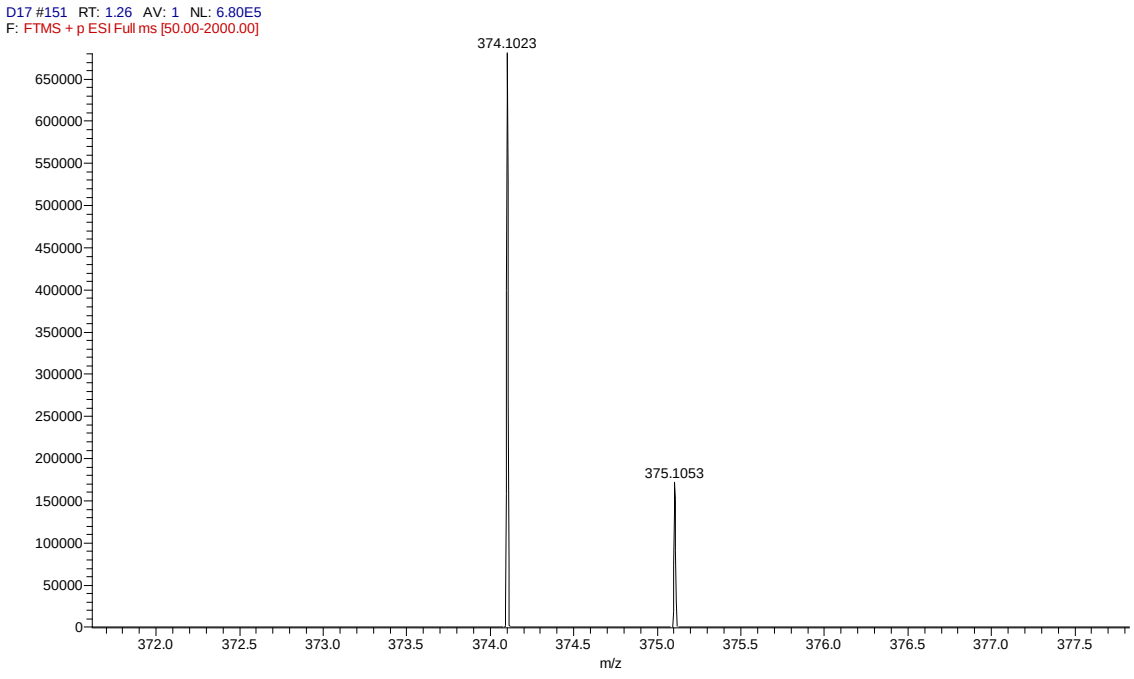
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 10.804 | 271755 | 22062 | 0.685 | 0.981 |
| 2 | 13.634 | 1337905 | 71132 | 3.370 | 3.161 |
| 3 | 15.342 | 38085154 | 2156827 | 95.945 | 95.858 |
| Total | | 39694814 | 2250020 | 100.000 | 100.000 |

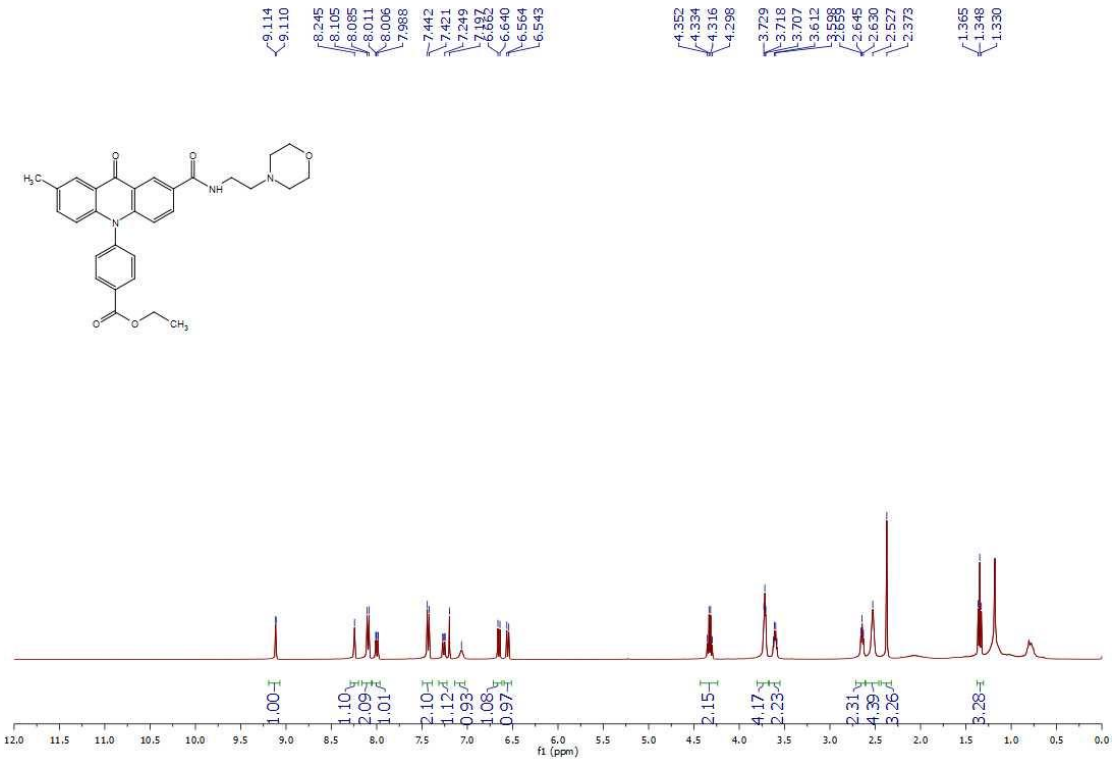
HPLC spectrum of **B13**



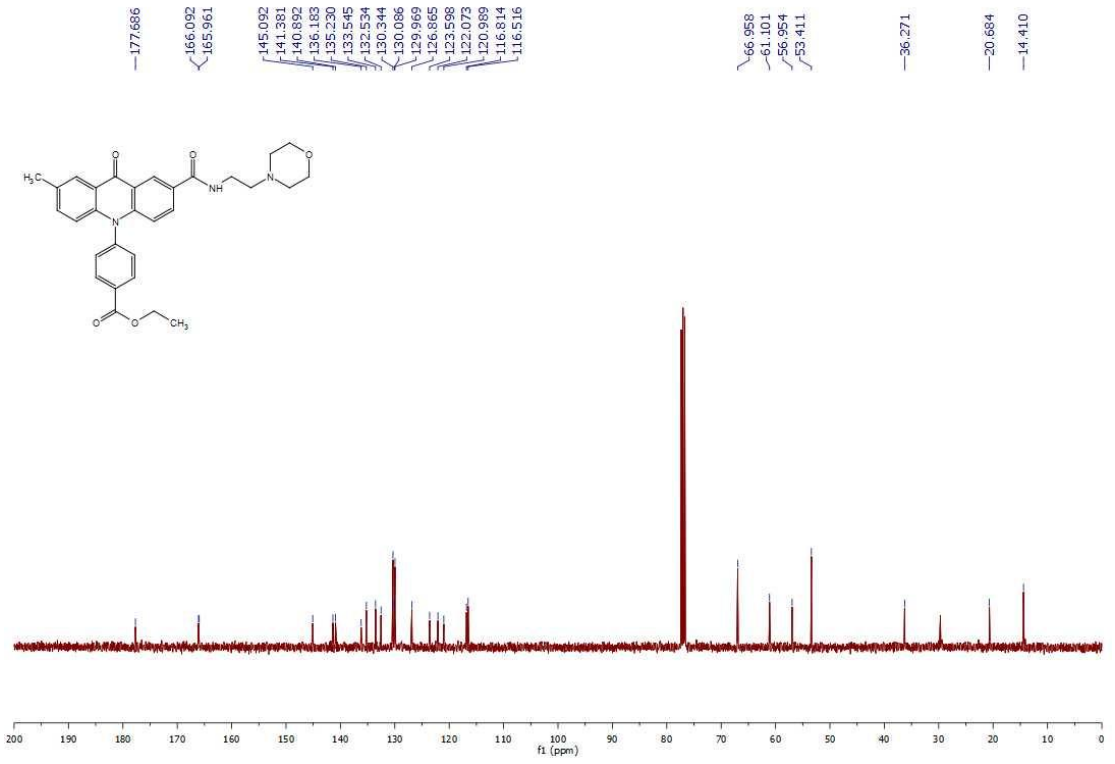

$^{1}$H NMR spectrum of **B14**



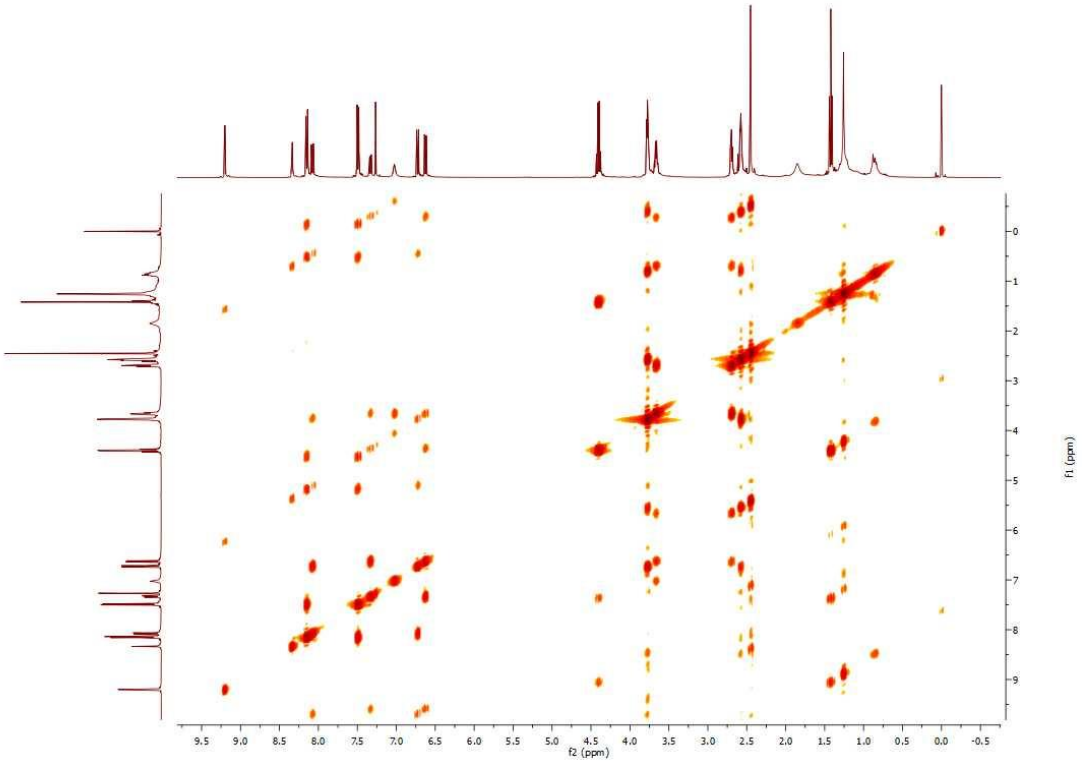

HRMS spectrum of **B14**



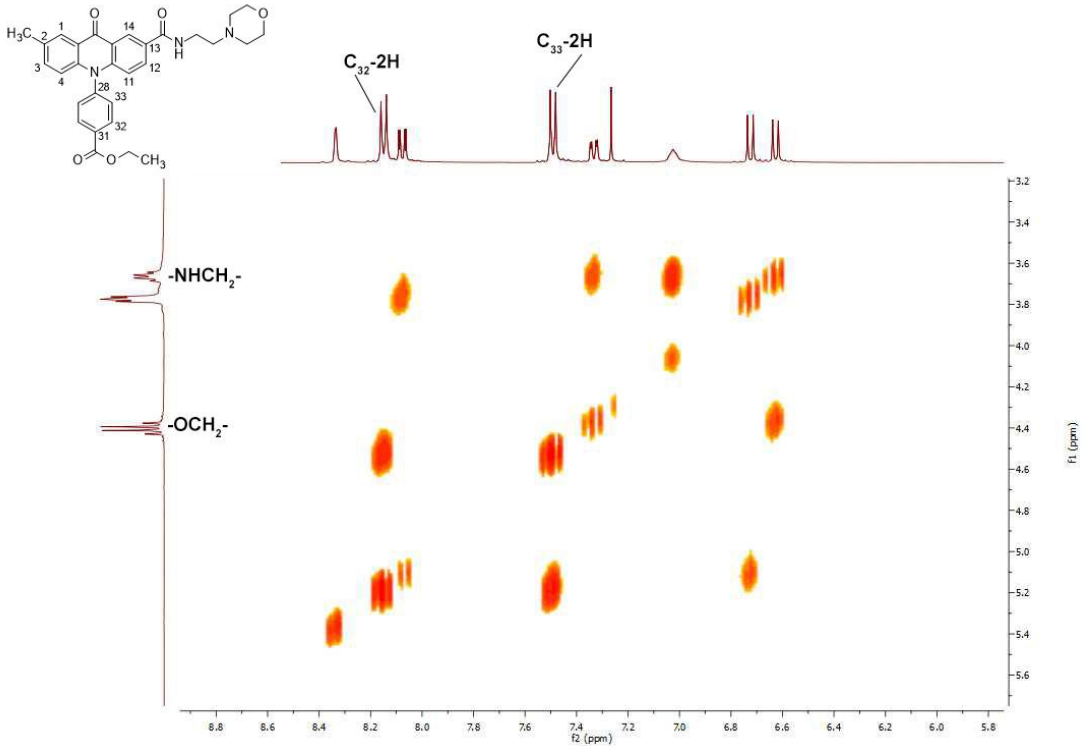

$^{1}$H NMR spectrum of **B15**



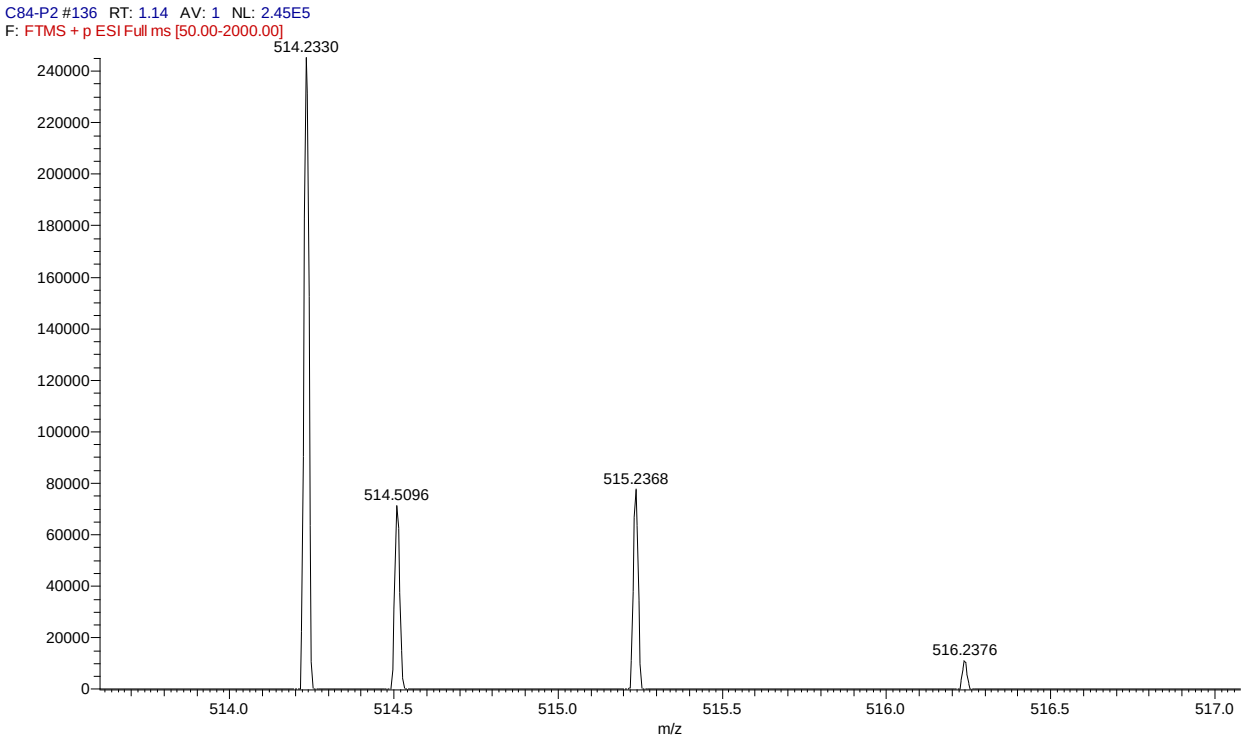

$^{13}$C NMR spectrum of **B15**




HH-COSY spectrum of **B15**

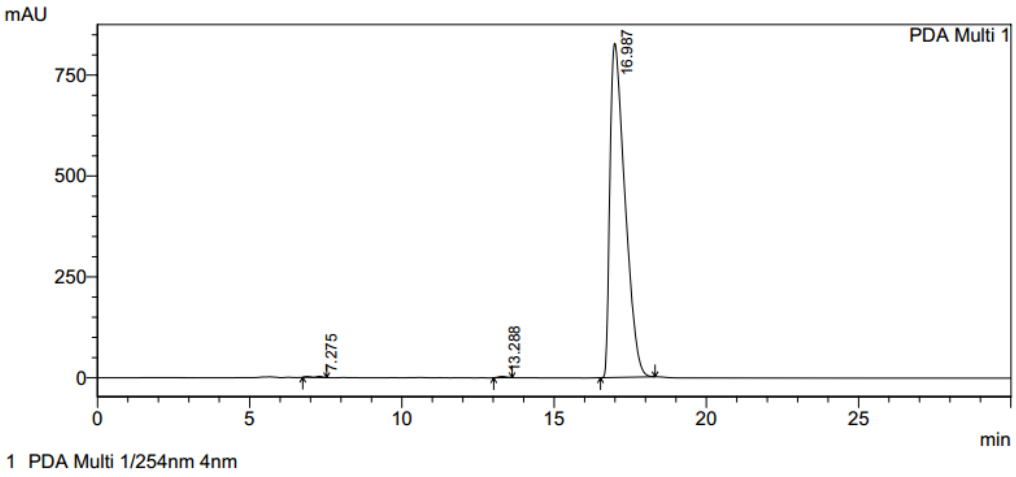



HH-COSY partial spectrum of **B15**




HRMS spectrum of

**B15**



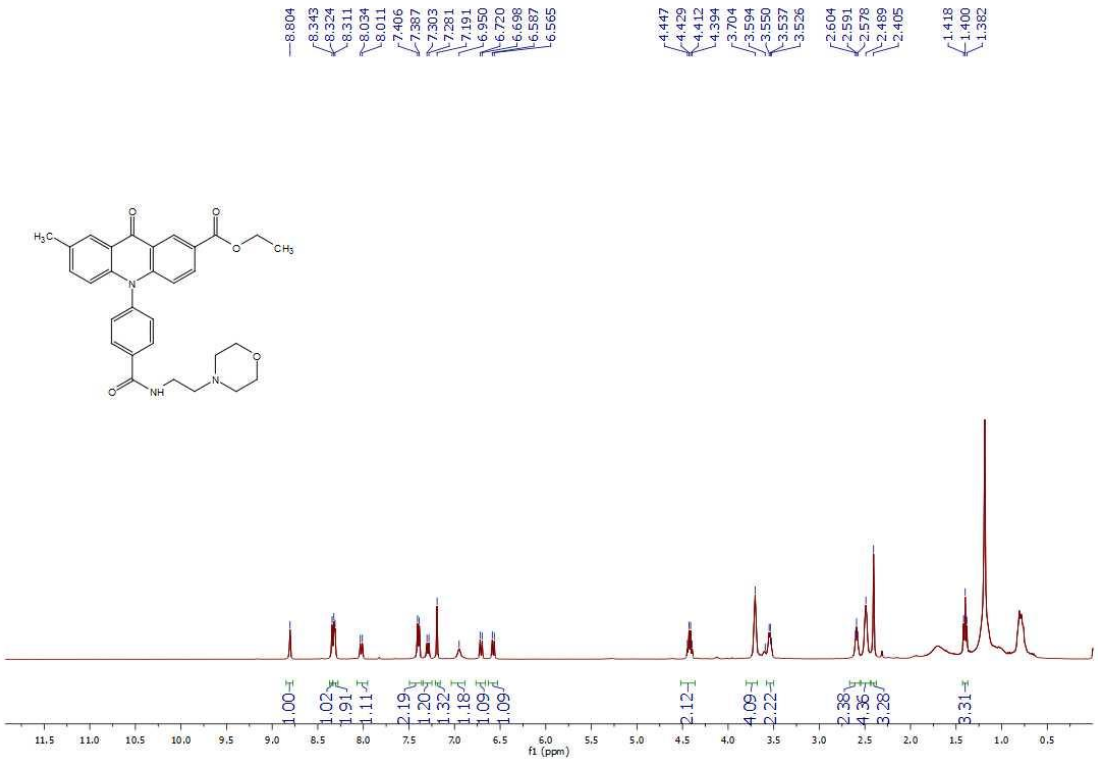

1 PDA Multi 1/254nm 4nm

PeakTable

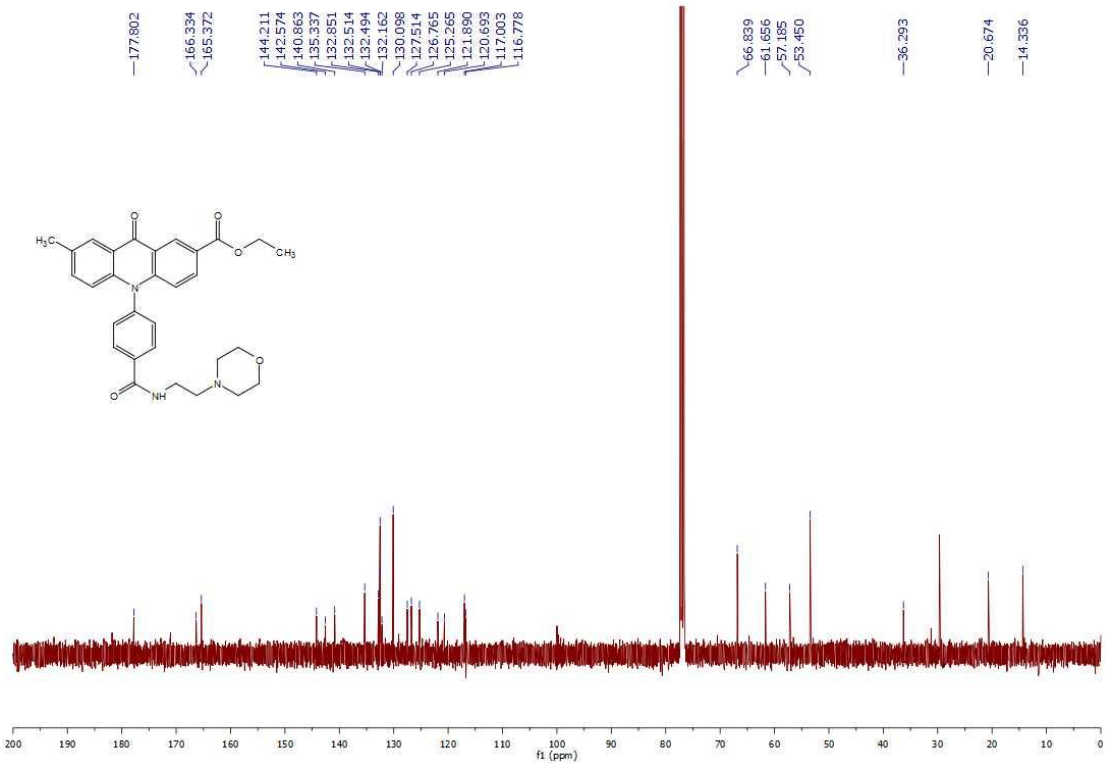
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 7.275 | 63848 | 2926 | 0.225 | 0.351 |
| 2 | 13.288 | 60028 | 3202 | 0.212 | 0.384 |
| 3 | 16.987 | 28258100 | 828256 | 99.564 | 99.266 |
| Total | | 28381976 | 834383 | 100.000 | 100.000 |

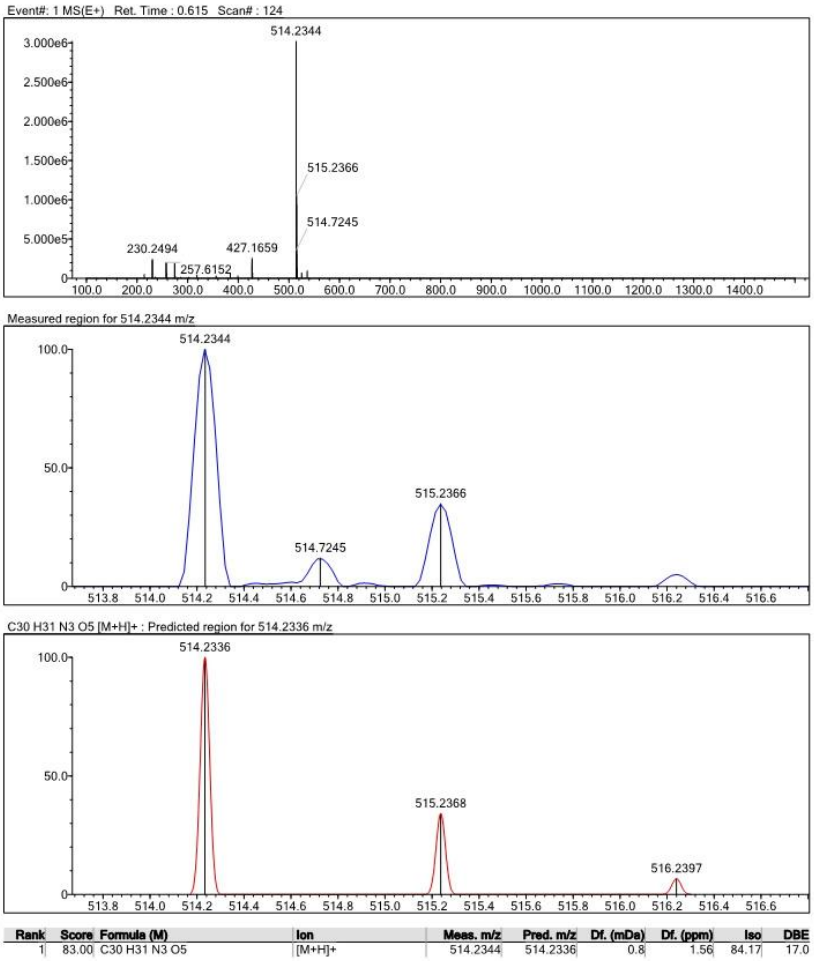
HPLC spectrum of **B15**




$^{1}$H NMR spectrum of **B16**

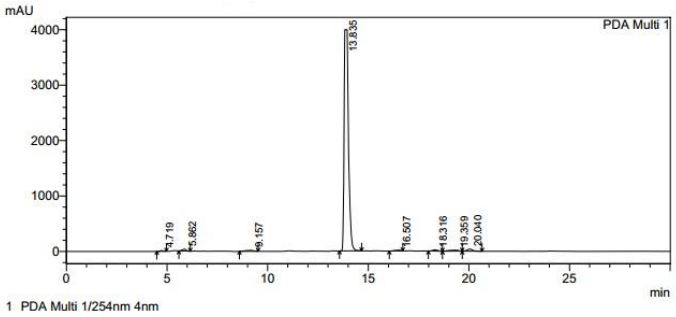



$^{13}$C NMR spectrum of **B16**




| Rank | Score | Formula (M) | Ion | Meas. m/z | Pred. m/z | Df. (mDa) | Df. (ppm) | Iso | DBE |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 83.00 | C30 H31 N3 O5 | [M+H]+ | 514.2344 | 514.2336 | 0.8 | 1.56 | 84.17 | 17.0 |

HRMS spectrum of **B16**

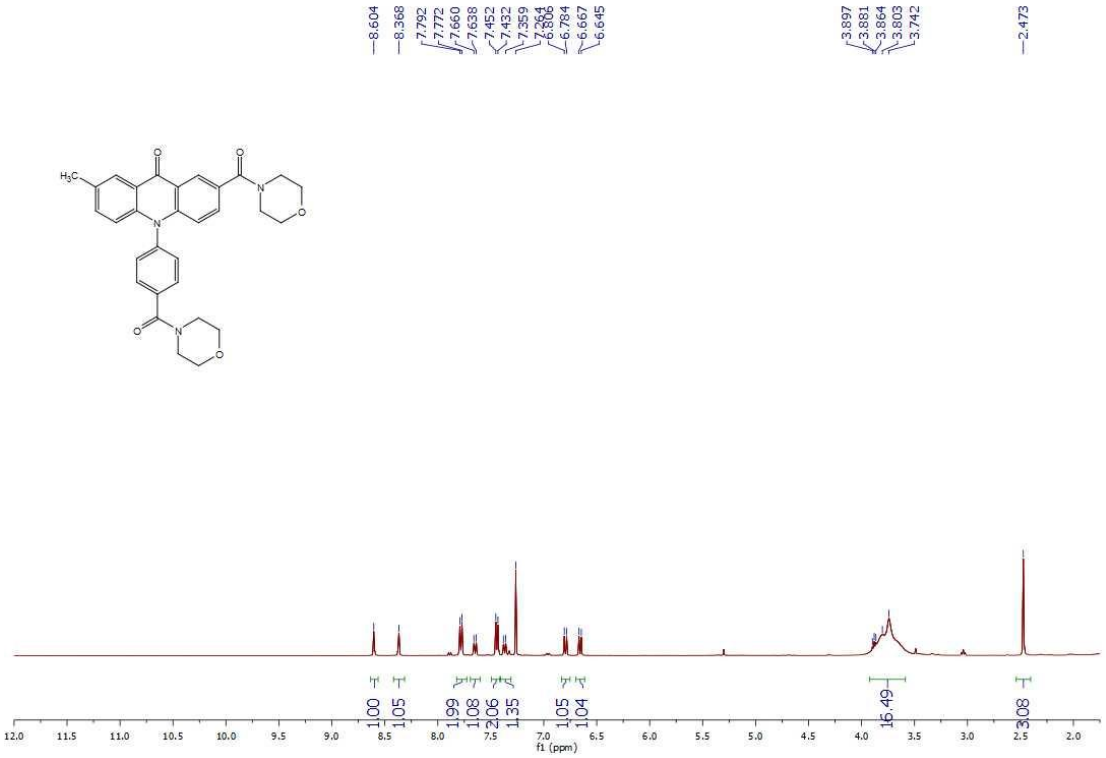



PeakTable

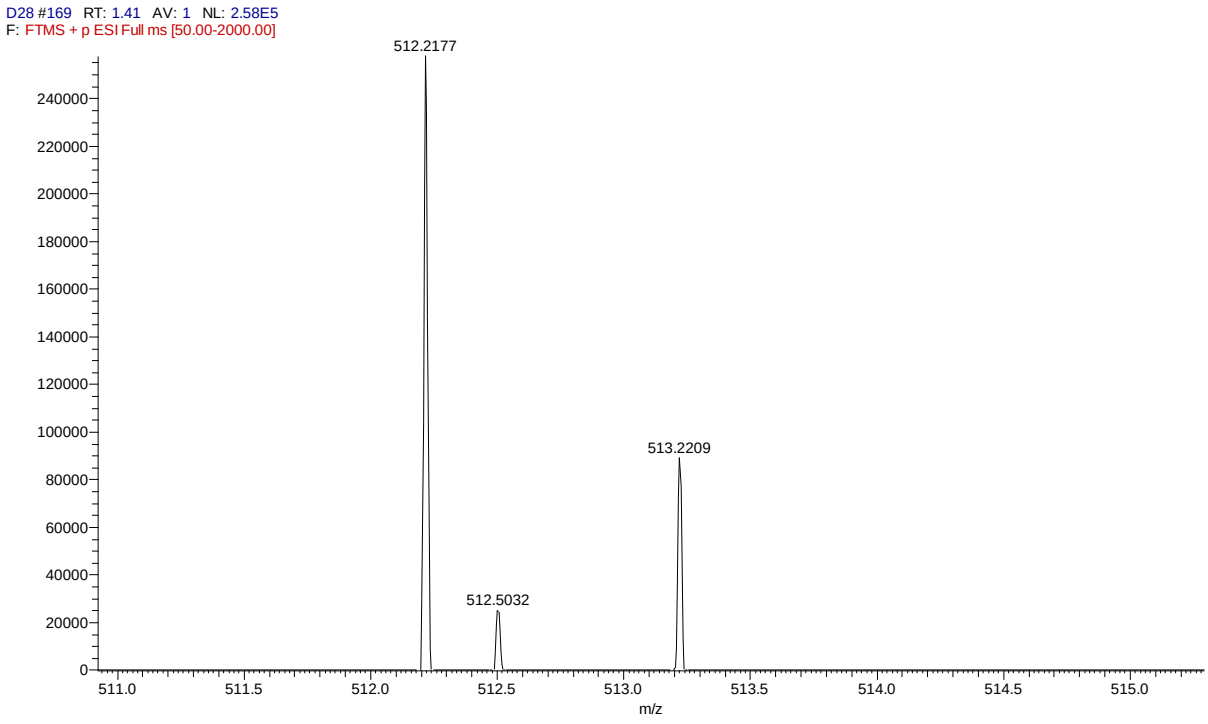
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 4.719 | 152689 | 13322 | 0.242 | 0.321 |
| 2 | 5.862 | 447912 | 34931 | 0.711 | 0.842 |
| 3 | 9.157 | 516319 | 16616 | 0.819 | 0.400 |
| 4 | 13.835 | 59891775 | 3990519 | 95.023 | 96.155 |
| 5 | 16.507 | 283437 | 14503 | 0.450 | 0.349 |
| 6 | 18.316 | 407761 | 22630 | 0.647 | 0.545 |
| 7 | 19.359 | 584493 | 18915 | 0.927 | 0.456 |
| 8 | 20.040 | 744507 | 38642 | 1.181 | 0.931 |
| Total | | 63028893 | 4150079 | 100.000 | 100.000 |

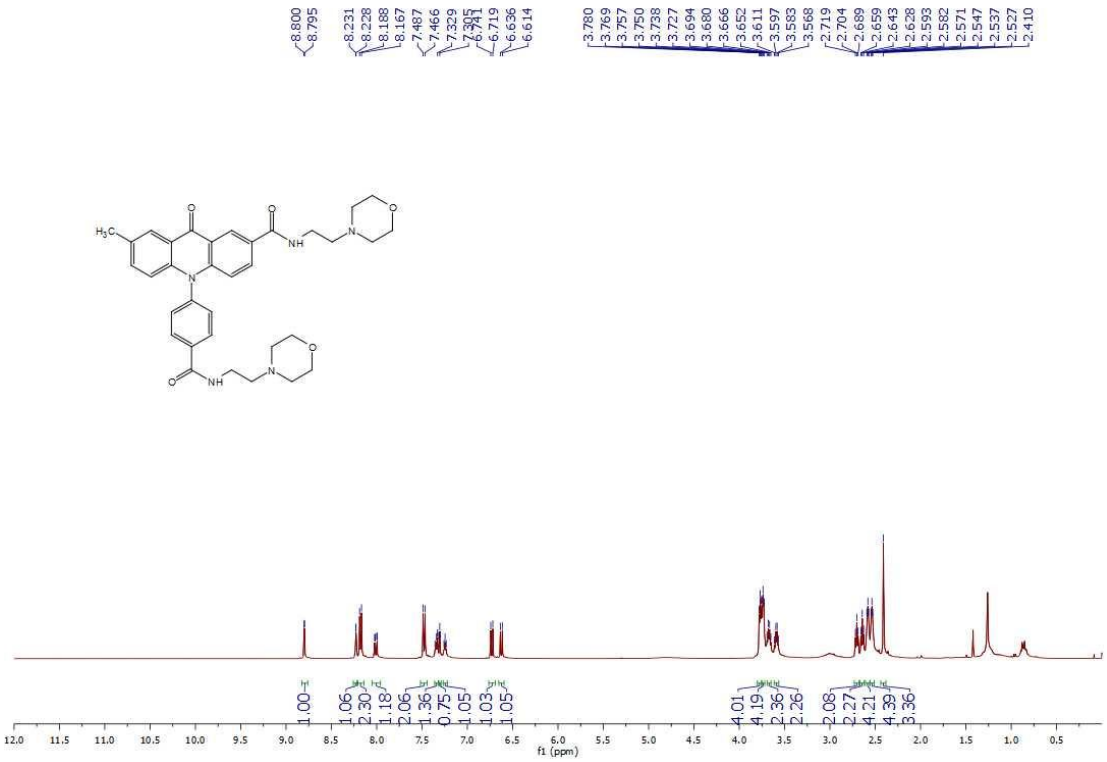
HPLC spectrum of **B16**



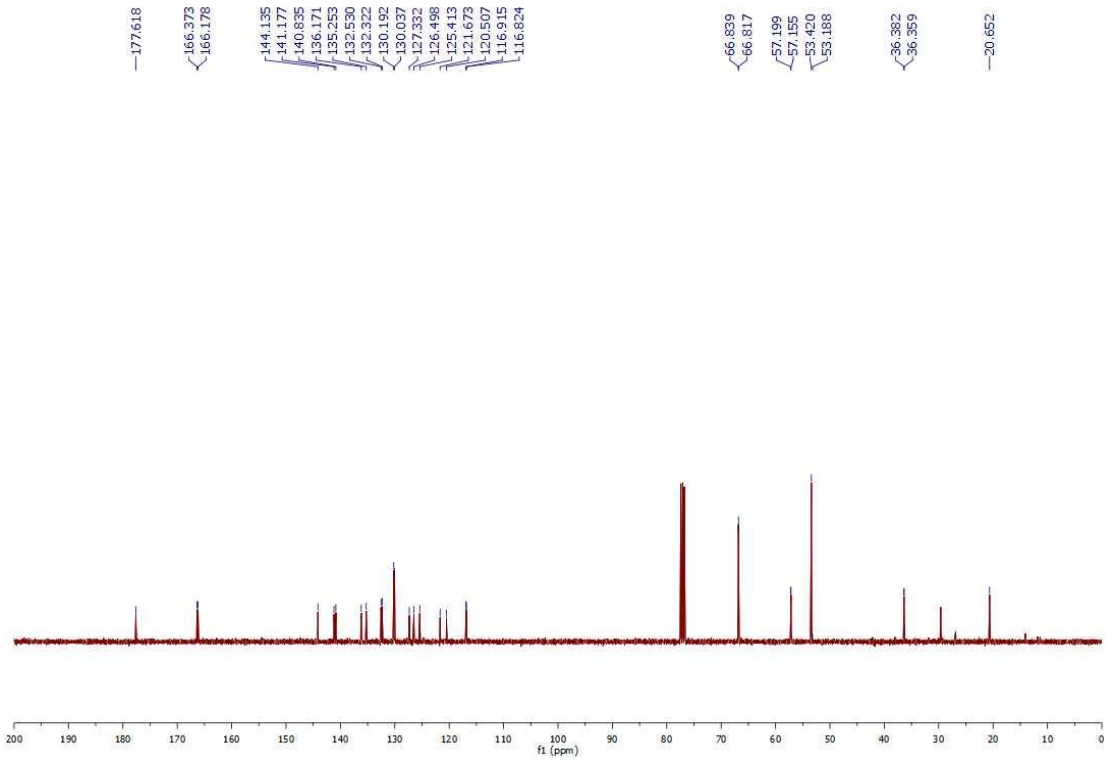

$^{1}$H NMR spectrum of **B17**



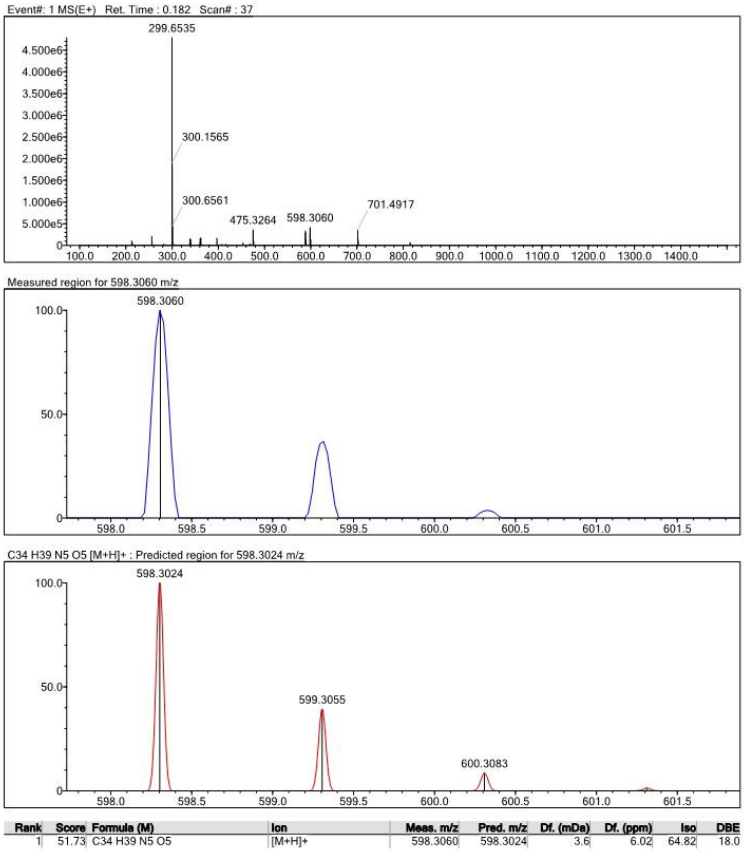

HRMS spectrum of **B17**



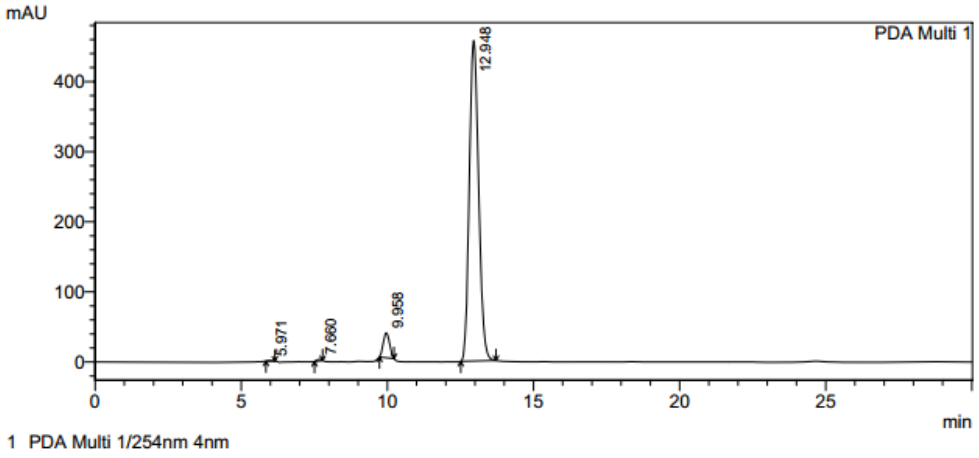
$^{1}$H NMR spectrum of **B18**



$^{13}$C NMR spectrum of **B18**




HRMS spectrum of **B18**

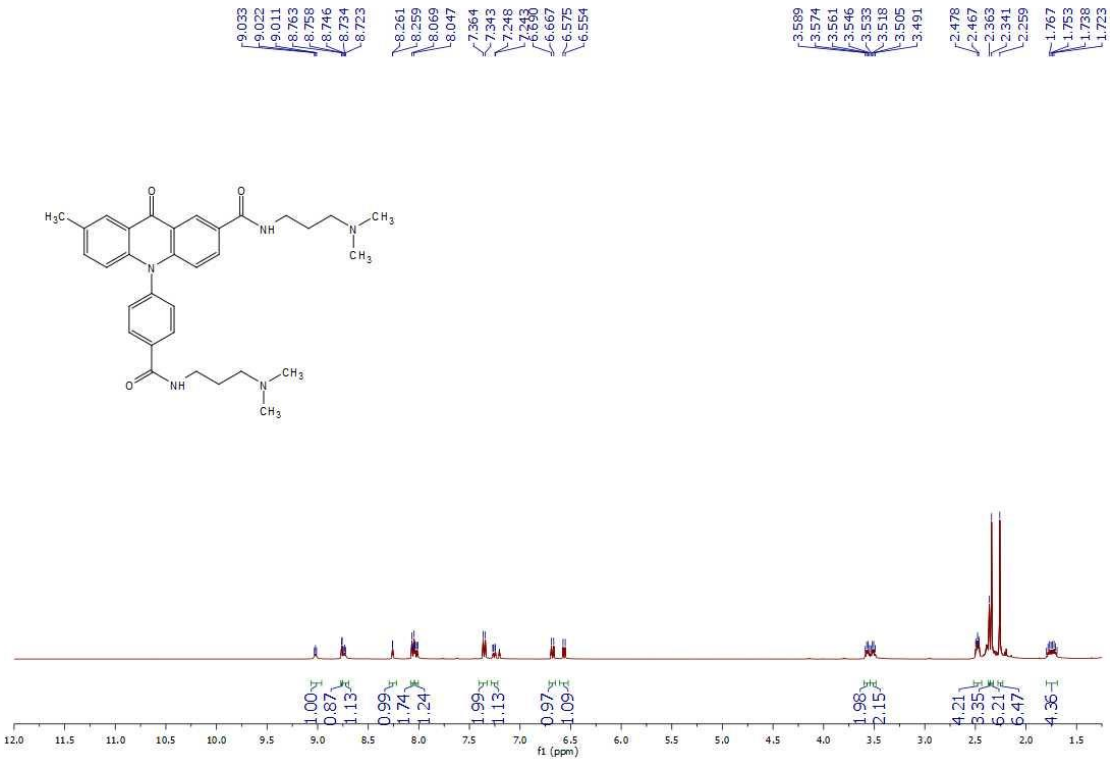



PeakTable

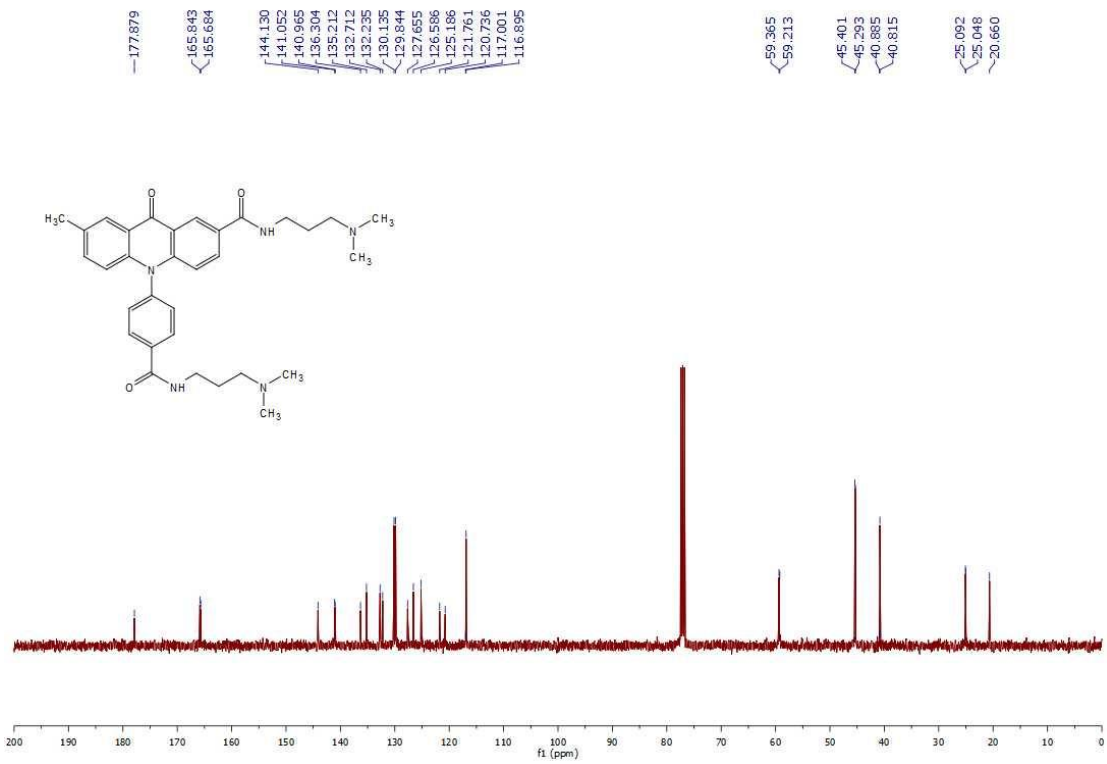
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 5.971 | 8200 | 757 | 0.077 | 0.153 |
| 2 | 7.660 | 23143 | 2330 | 0.218 | 0.470 |
| 3 | 9.958 | 535166 | 35441 | 5.034 | 7.150 |
| 4 | 12.948 | 10064407 | 457181 | 94.671 | 92.228 |
| Total | | 10630916 | 495709 | 100.000 | 100.000 |

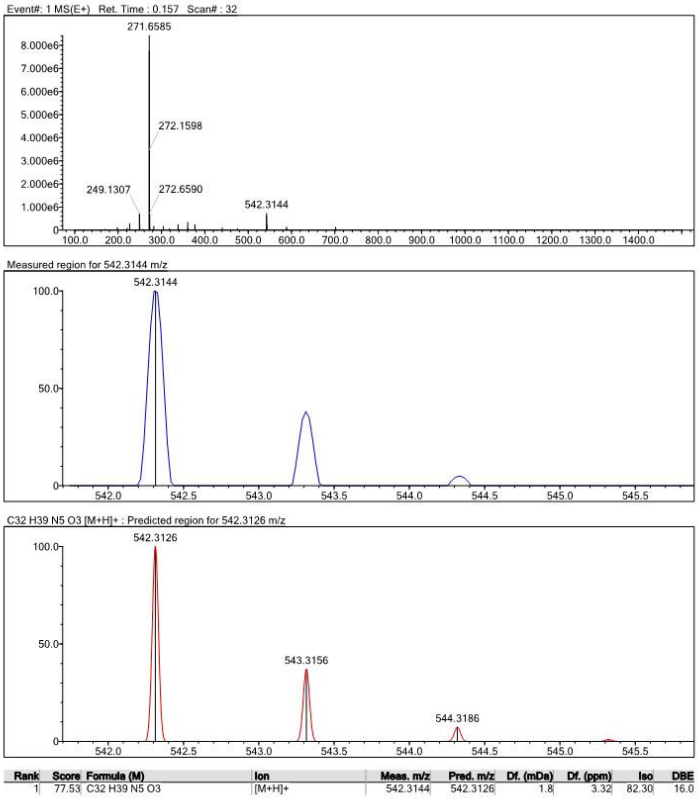
HPLC spectrum of **B18**



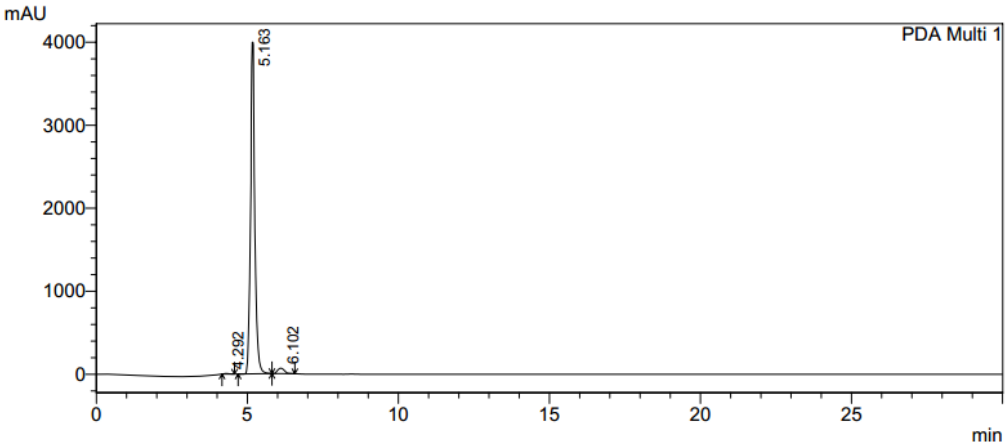
$^{1}$H NMR spectrum of **B19**



$^{13}$C NMR spectrum of **B19**




HRMS spectrum of **B19**



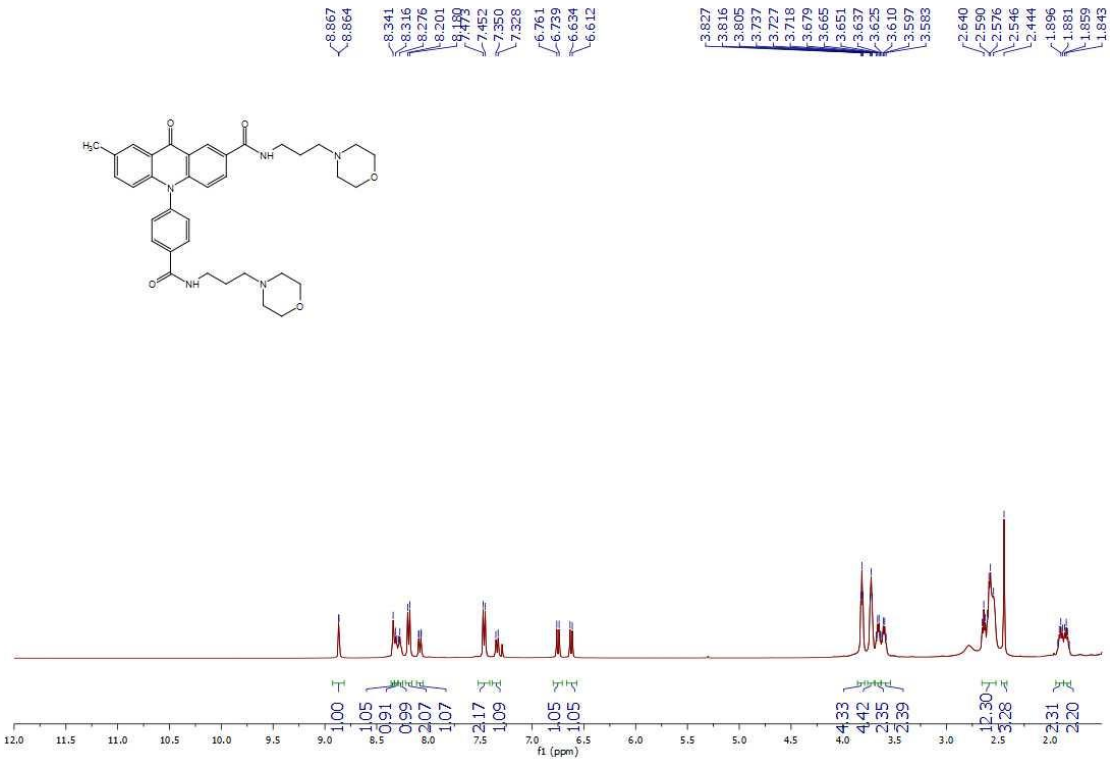

1 PDA Multi 1/254nm 4nm

PeakTable

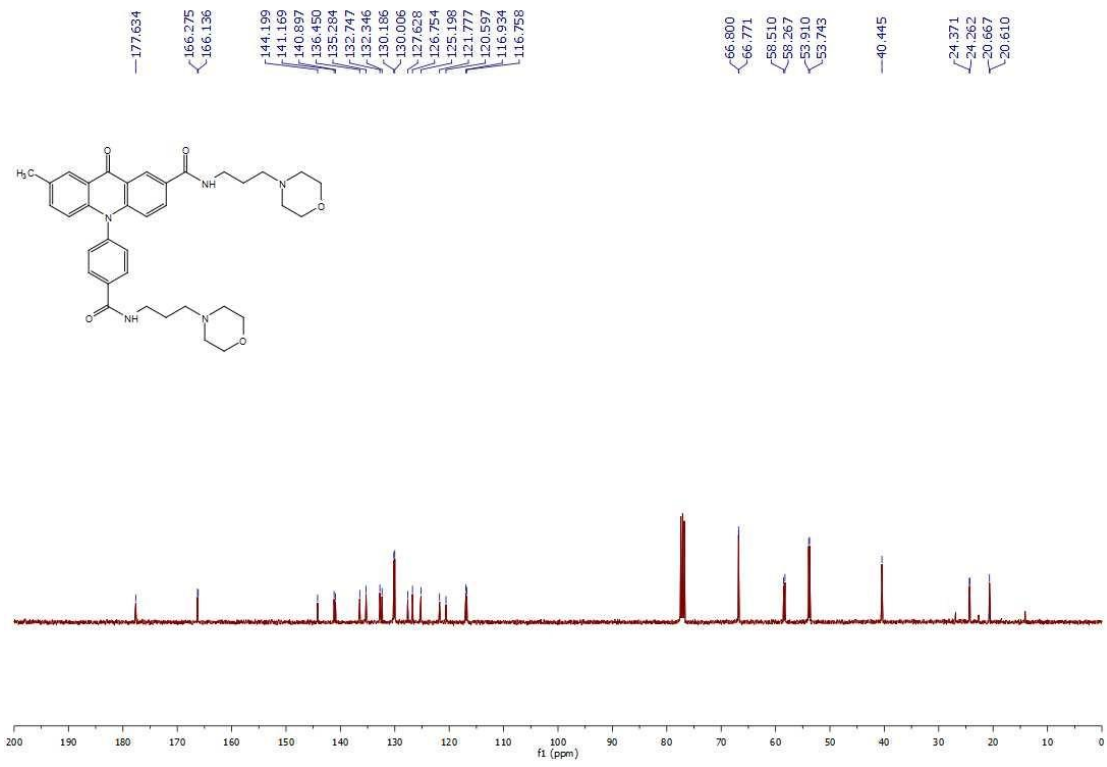
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 4.292 | 79853 | 6894 | 0.197 | 0.170 |
| 2 | 5.163 | 39446302 | 3994170 | 97.301 | 98.239 |
| 3 | 6.102 | 1014188 | 64690 | 2.502 | 1.591 |
| Total | | 40540343 | 4065754 | 100.000 | 100.000 |

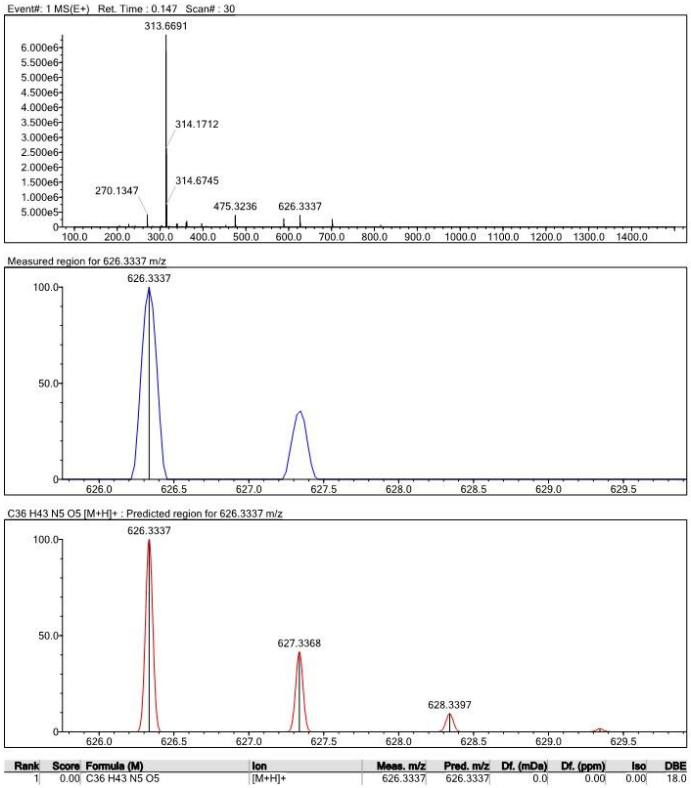
HPLC spectrum of **B19**



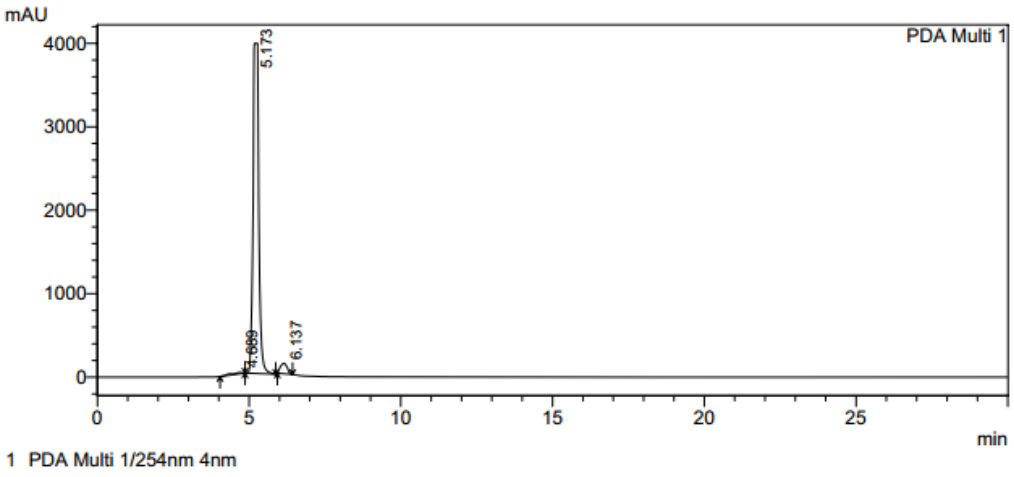
$^{1}$H NMR spectrum of **B20**



$^{13}$C NMR spectrum of **B20**




HRMS spectrum of **B20**

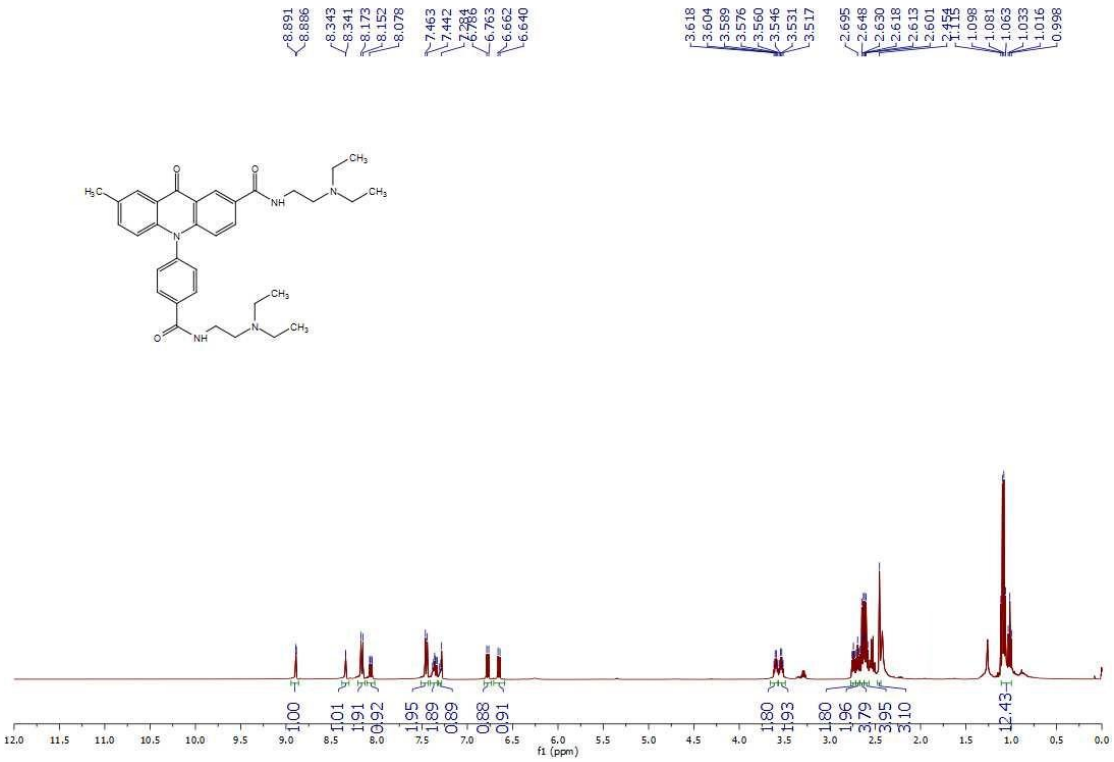



PeakTable

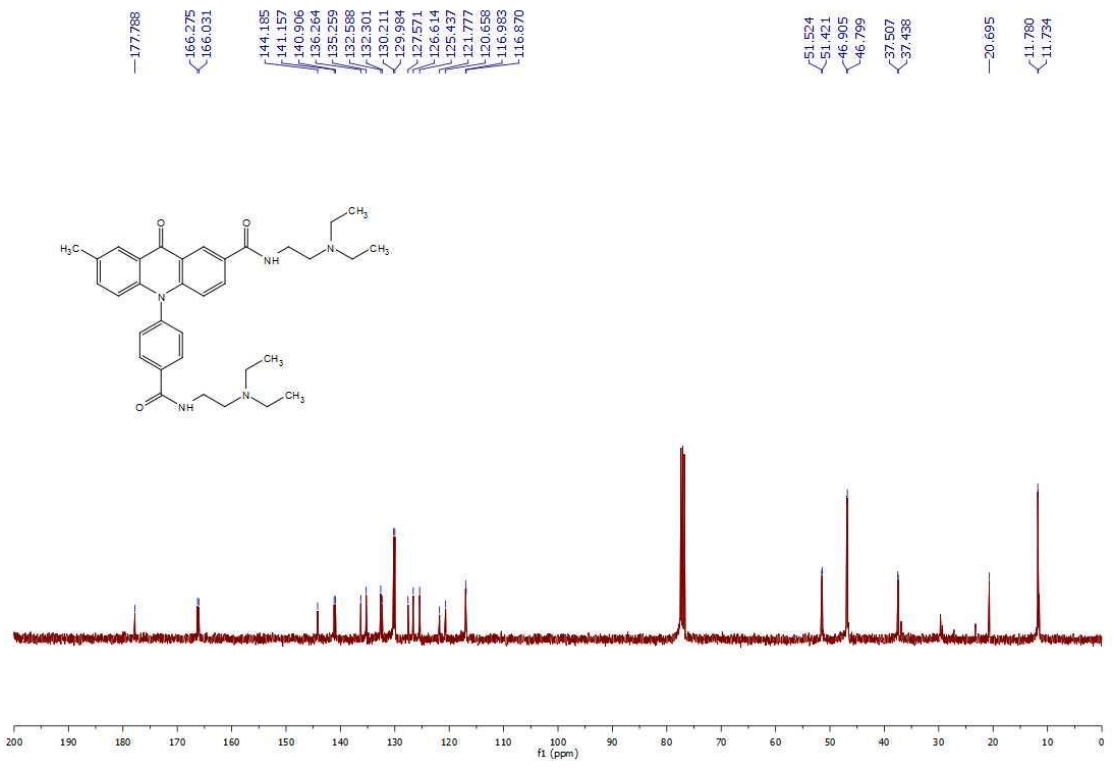
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 4.689 | 459365 | 15545 | 0.838 | 0.380 |
| 2 | 5.173 | 52382754 | 3954672 | 95.613 | 96.638 |
| 3 | 6.137 | 1944245 | 122016 | 3.549 | 2.982 |
| Total | | 54786364 | 4092234 | 100.000 | 100.000 |

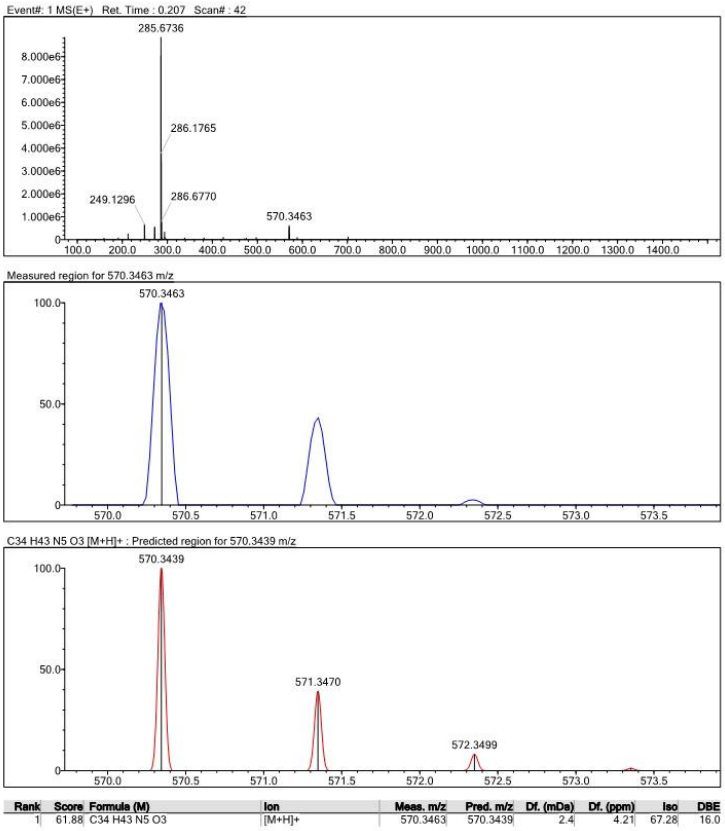
HPLC spectrum of **B20**



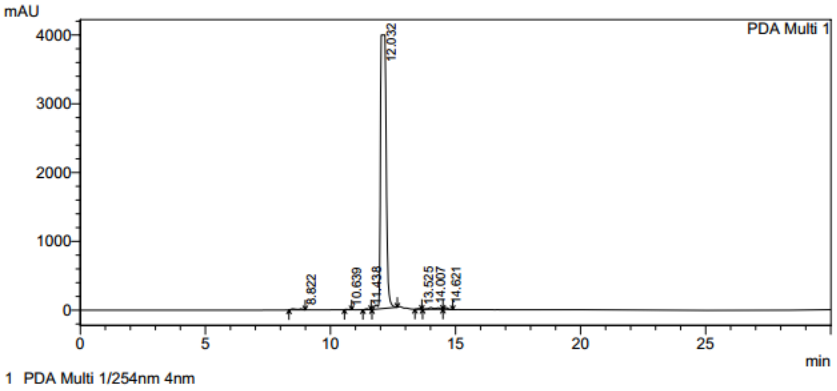
$^{1}$H NMR spectrum of **B21**



$^{13}$C NMR spectrum of **B21**




HRMS spectrum of **B21**

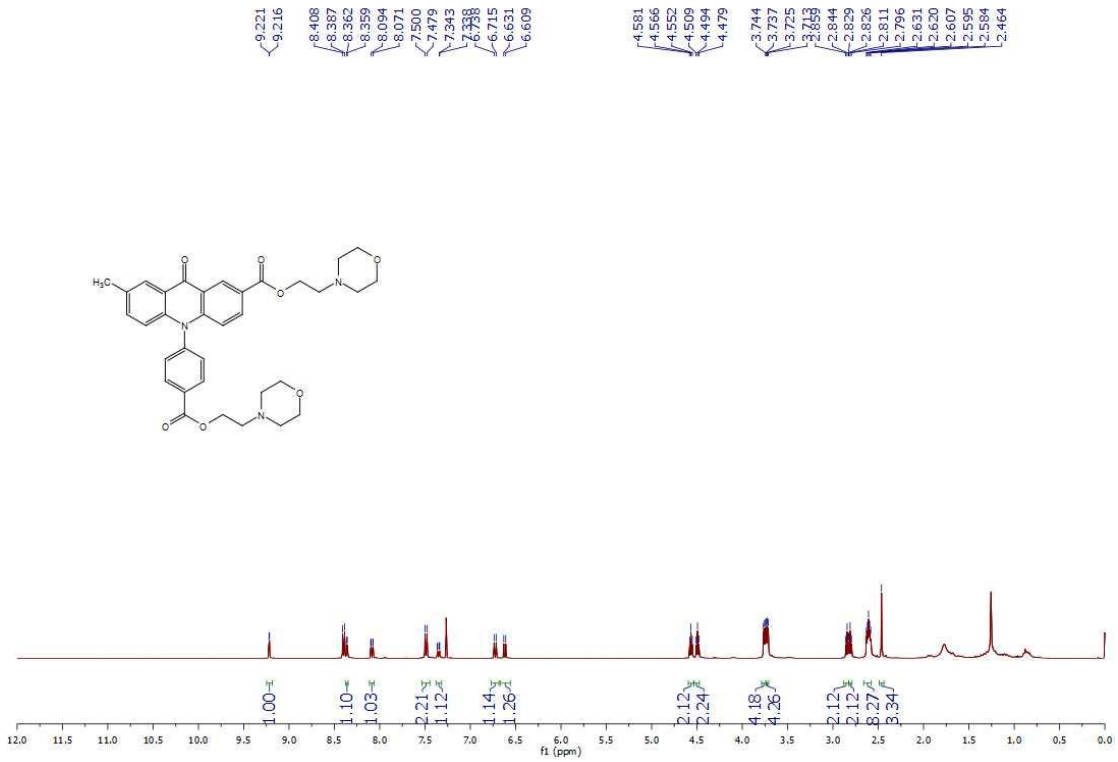



PeakTable

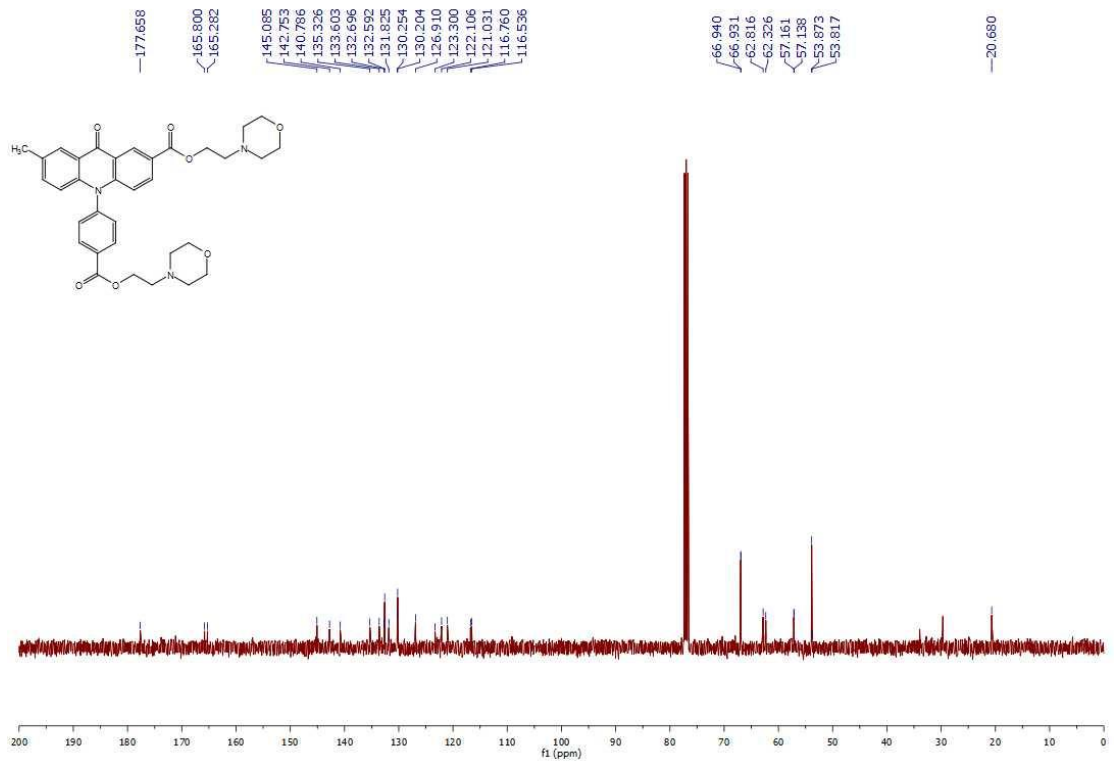
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 8.822 | 339197 | 16249 | 0.532 | 0.399 |
| 2 | 10.639 | 38382 | 5201 | 0.060 | 0.128 |
| 3 | 11.438 | 101417 | 12452 | 0.159 | 0.306 |
| 4 | 12.032 | 62409385 | 3978055 | 97.938 | 97.711 |
| 5 | 13.525 | 69824 | 8988 | 0.110 | 0.221 |
| 6 | 14.007 | 538950 | 25119 | 0.846 | 0.617 |
| 7 | 14.621 | 225923 | 25185 | 0.355 | 0.619 |
| Total | | 63723077 | 4071249 | 100.000 | 100.000 |

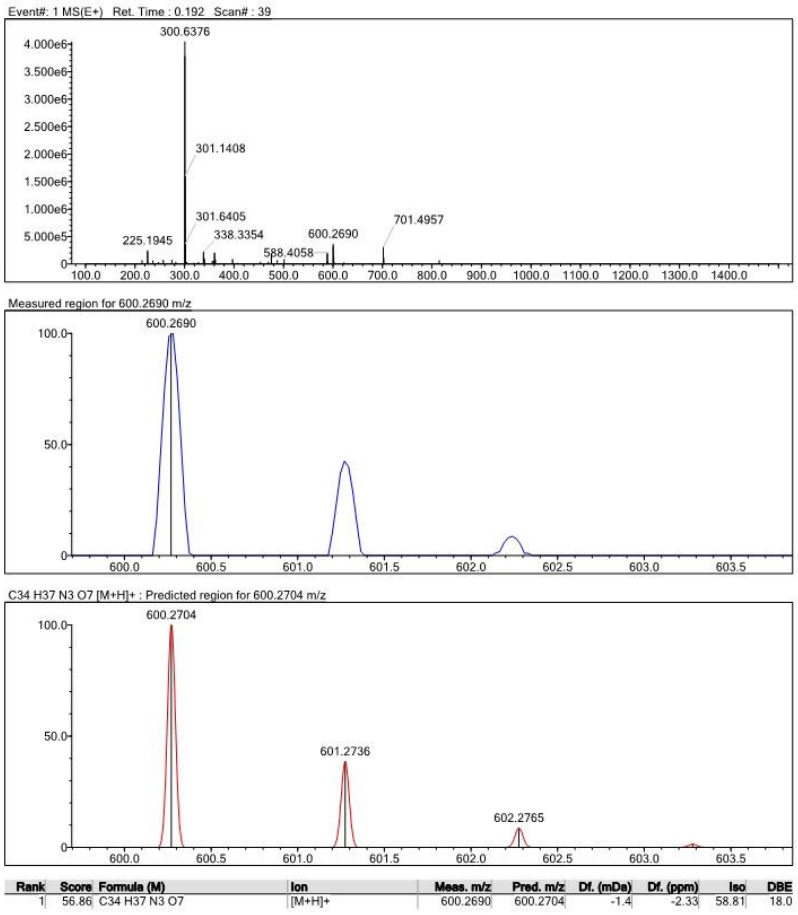
HPLC spectrum of **B21**



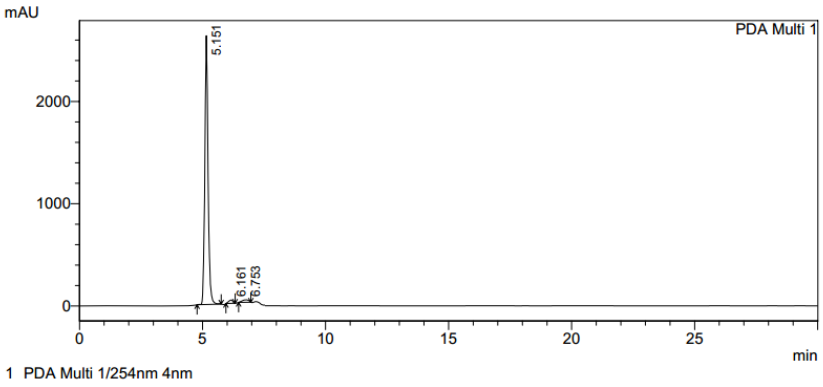
$^{1}$H NMR spectrum of **B22**



$^{13}$C NMR spectrum of **B22**




HRMS spectrum of **B22**

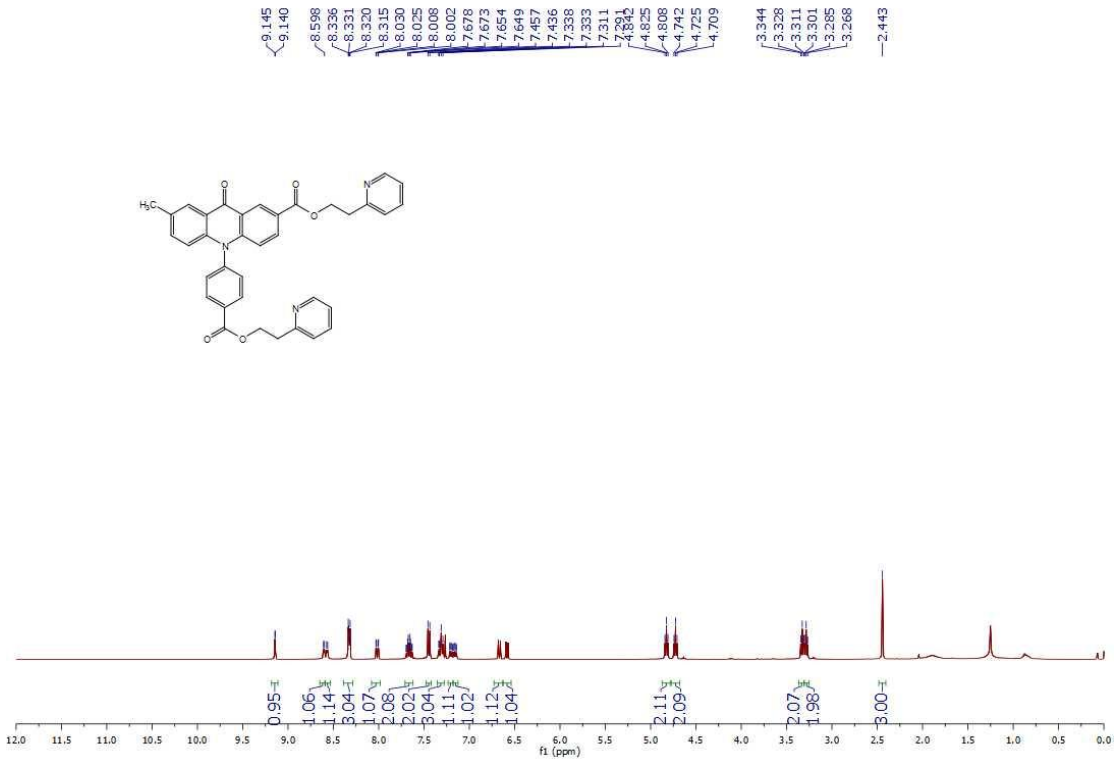



PeakTable

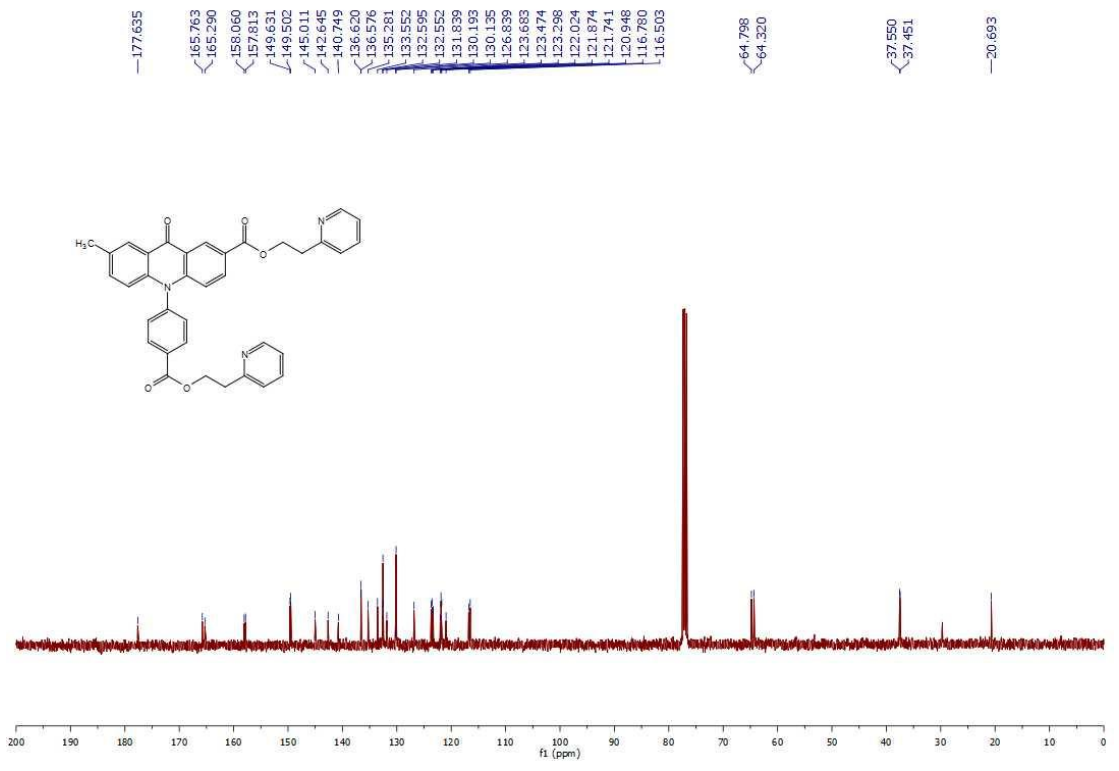
PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 5.151 | 23800898 | 2628385 | 96.661 | 97.843 |
| 2 | 6.161 | 378359 | 33479 | 1.537 | 1.246 |
| 3 | 6.753 | 443865 | 24460 | 1.803 | 0.911 |
| Total | | 24623122 | 2686324 | 100.000 | 100.000 |

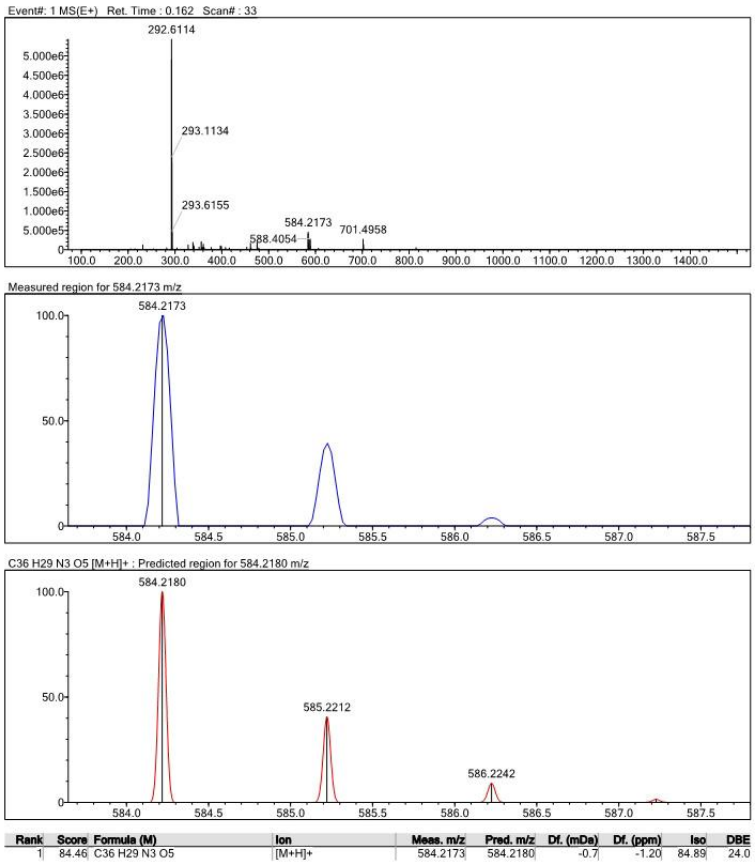
HPLC spectrum of **B22**



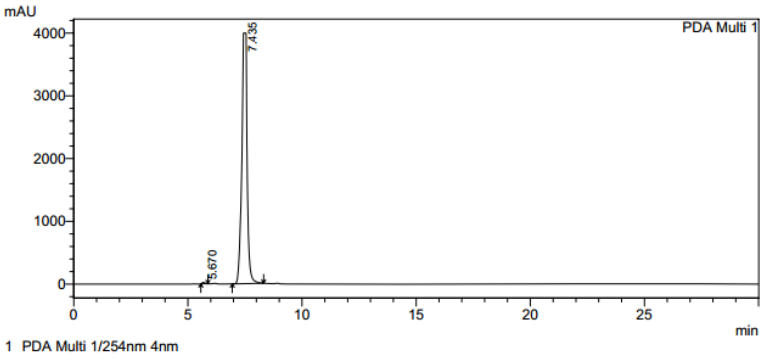

$^{1}$H spectrum of **B23**




$^{13}$C spectrum of **B23**




HRMS spectrum of **B23**




PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 5.670 | 190076 | 19927 | 0.291 | 0.497 |
| 2 | 7.435 | 65191048 | 3992442 | 99.709 | 99.503 |
| Total | | 65381124 | 4012369 | 100.000 | 100.000 |

HPLC spectrum of **B23**