The Synthesis of a c(RGDyK) Targeted SN38 Prodrug with an Indolequinone Structure for Bioreductive Drug Release

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Supporting Information

All ^{1}H NMR spectra were measured on 300, 400, or 500 MHz Bruker NMR system equipped with a multinuclear 5-mm probe. ^{1}H chemical shifts are reported in parts per million from TMS. Mass spectra were performed on a Waters 1525 mass spectrometer. A thermometer was used without further calibration. Anhydrous solvents were obtained from Acros Organics or Sigma-Aldrich and used as received. Chemicals were purchased from Sigma-Aldrich. SN38 hydrochloride was purchased from 3B Medical Systems Inc. c(RGDyK) was purchased from Peptides International Inc. DT-diaphorase, FAD and NADH were purchased from Sigma. Silica gel 60, particle size 0.040–0.063 mm, 230–400 mesh ASTM was obtained from EM Sciences. Thin layer chromatography (TLC) was performed using Whatman Adsorption plates, 60 Å silica gel, 250 µm layer thickness. Deionized water (18.2MΩ) was made using a Nanopure Infinity DI water system.

The release of SN38 from prodrug 1: 0.63 mg of prodrug **1** was dissolved in DMSO (17.7µL), and PBS buffer (17.7µL, 20mM, pH=7.0) was added to make a 10nM stock solution. 2.0µL of this stock solution was diluted with 98.0µL PBS buffer. DTD was diluted with PBS buffer to make a 40mU/µL solution. NADH was dissolved in PBS buffer to make a 4mM solution. FAD was dissolved in PBS buffer to make a 100µM solution. The following spreadsheet shows the experiments performed for the drug release study.

Entry	Conditions	H_2O	Buffer	Prodrug **1**	NADH	FAD	DTD
1	Control	8.0µL	4.0 µL	60.0 µL	4.0 µL	4.0 µL	0
2	With DTD	4.0 µL	4.0 µL	60.0 µL	4.0 µL	4.0 µL	4.0 µL
Final concentration			25mM	0.15mM	0.2mM	5.0 µM	2.0 µU/µL

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) analysis was carried out on a Waters Delta 600 HPLC system equipped with a Waters 2996 photodiode array (PDA) detector, a Waters 717 Plus auto sampler, and a Waters Fraction collector III. The instrument is controlled by Empower 2 software. For analysis, aliquots were taken from the incubated samples and run on a C5 silica-based RP-HPLC column (250 × 4.6 mm, 300 Å) connected to a C5 guard column (4 × 3 mm). The mobile phase used for elution was a linear gradient beginning with 90:10 (v/v) water/acetonitrile at a flow rate of 1 mL/min. Trifluoroacetic acid at a 0.14 wt % concentration in water as well as in acetonitrile was used as a counter ion. The data were collected at λ_{max}=280 nm.

***In vitro* cytotoxicity testing by XTT assay.**

For the cytotoxicity experiments, KB cells were seeded in 96-well microtiter plates (3000 cells/well) in RPMI (folate free) medium containing 10% fetal bovine serum. Two days after plating, the cells were treated with the different concentrations of the prodrug and free SN38 in the presence and absence of 2 mU/µl recombinant DTD (Sigma), 133 µM NADH and 3.3 µM FAD, added into the medium. A colorimetric "XTT" (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonicacid hydrate) assay (Roche Molecular Biochemicals, Indianapolis, IN) was performed following the vendor's protocol. After incubation with the XTT labeling mixture added into the medium, the microtiter plates were read on an ELISA reader (Synergy HT, BioTek) at 492 nm with the reference wavelength at 690 nm. The data is presented as the percent mean OD of for the respective

control cells obtained in the absence of any drugs.

3-Formyl-5-methoxy-2-methylindole (2) $POCl_3$ (0.85mL, 9.28mmol) was added to an ice water-cooled DMF (3.0mL, 38.9mmol) while stirring. After the addition, the mixture was stirred at 0°C for 10 minutes to form the Vilsmeier reagent. The 5-methoxy-2-methylindole (1.04g, 6.45mmol) was dissolved in 3 mL anhydrous DMF and cooled to 0°C. The previous made Vilsmeier reagent was added dropwise. The reaction was stirred at 0°C for an additional 30 minutes after the addition. The reaction mixture was then added dropwise to an ice-cooled 2M NaOH (50mL) solution. DCM (100mL) was added and separated. The aqueous layer was extracted one more time with DCM (50mL). Organic layers were combined and washed with brine and dried with Na_2SO_4. The solvent was removed and the residue was washed with cooled EtOAc to give 1.05g (90%) of **2** as a pale brown solid: mp 198.0-199.2°C; ^{1}H NMR ($CDCl_3$ 400MHz) δ 2.61(s, 3H, 2-C*H*$_3$), 3.78 (s, 3H, C*H*$_3$O), 6.76 (d, 1H, *J*=8.0 Hz, Ar-6*H*), 7.18 (d, 1H, *J*=8.0 Hz, Ar-7*H*), 7.60 (s, 1H, Ar-4*H*), 9.90 (s, 1H, C*H*O); ^{13}C NMR ($CDCl_3$ 100.5 MHz) 10.20, 54.60, 102.57, 110.00, 111.41, 112.12, 114.06, 126.54, 130.44, 156.34, 184.77ppm; MS(EI$^+$) found (M+H) 190.1, calc ($C_{11}H_{11}NO_2$ m/z) 189.08

1-(ethoxycarbonyl methyl)-3-formyl-5-methoxy-2-methylindole (3) Compound **2** (1.78g, 9.40mmol) was added gradually and under argon to a stirred suspension of NaH (0.564g of 60% in mineral oil, 14.11mmol). The mixture was stirred at room temperature for 2 hours and cooled to 0°C with an ice-water bath. Ethyl bromoacetate (1.25mL, 11.28mmol) was added dropwise. The reaction was allowed to warm up to room temperature and was stirred for an additional 2 hours. Water (50mL) was added to the mixture and extracted with DCM (40mL × 3). The organic layers were combined and washed with water (50mL) and brine (50mL) and then dried with Na_2SO_4. After solvent removal, the residue was purified on silica, eluting with EtOAc/hexanes (1:2, R_f =0.25) to give 2.22g (86.0%) of **3** as a pale white solid: mp 120.0-121.5°C; ^{1}H NMR ($CDCl_3$ 300MHz) δ 1.27(t, 3H, *J*=7.2 Hz, CH_2C*H*$_3$), 2.66(s, 3H, 2-C*H*$_3$), 3.89 (s, 3H, C*H*$_3$O), 4.23 (q, 2H, *J*=7.2 Hz, C*H*$_2$CH$_3$), 4.79 (s, 2H, N-C*H*$_2$CO), 6.91 (d, 1H, *J*=8.0

Hz, Ar-6*H*), 7.11 (d, 1H, *J*=8.0 Hz, Ar-7*H*), 7.81 (s, 1H, Ar-4*H*), 10.18 (s, 1H, C*H*O); ^{13}C NMR ($CDCl_3$ 100.5 MHz) 10.35, 14.07, 44.86, 55.80, 62.19, 103.12, 109.48, 113.35, 114.96, 126.36, 131.38, 156.74, 167.16, 184.31 ppm; MS(EI$^+$) found (M^+) 275.2, calc ($C_{15}H_{17}NO_4$ m/z) 275.29

4-Amino-1-(ethoxycarbonyl formate methyl)-3-formyl-5-methoxy-2-methylindole (5) Compound **3** (1.48g, 5.38mmol) was dissolved in acetic acid (88.7mL) and was cooled to 10-15°C. Nitric acid solution (3.2mL nitric acid in 18.8mL acetic acid) was added dropwise during a 45 minutes period while the temperature was maintained at 10-15°C. After the addition, the mixture was allowed to warm to room temperature and was stirred for an additional 2 hours. The reaction mixture was then poured into ice (150g) and extracted with DCM (150mL × 3). The combined organic layer was washed with water (200mL) and brine (200mL) and dried with Na_2SO_4. The solvent was removed to give the 4- and 6-nitro products as a mixture. The mixture (1.67g) was suspended in EtOH (100mL) and powdered Tin (2.23g, 18.8mmol) was added, followed by HCl (3.0M, 23mL). The reaction was heated under gentle reflux for 1 hour. Water (20mL) was added and the solution was neutralized with $NaHCO_3$ (aq) and extracted with DCM (200mL × 3). The organic layers were combined and washed with water (100mL) and brine (50mL) and dried with Na_2SO_4. After solvent removal, the residue was purified on silica, eluting with EtOAc/hexanes (1:1, R_f =0.15) to give 0.43g (47%, two steps) of **5** as a yellow solid: mp 128.2-130.0°C; ^{1}H NMR ($CDCl_3$ 300MHz) δ 1.26(t, 3H, *J*=7.2 Hz, CH_2CH_3), 2.60(s, 3H, 2-C*H*$_3$), 3.86 (s, 3H, C*H*$_3$O), 4.22 (q, 2H, *J*=7.2 Hz, C*H*$_2$CH$_3$), 4.72 (s, 2H, N-C*H*$_2$CO), 5.95 (s, br, 1H, N*H*$_2$), 6.38 (d, 1H, *J*=8.4 Hz, Ar-6*H*), 6.84 (d, 1H, *J*=8.4 Hz, Ar-7*H*), 9.85 (s, 1H, C*H*O); ^{13}C NMR ($CDCl_3$ 100.6 MHz) 10.44, 14.07, 44.83, 57.03, 62.11, 95.48, 110.06, 113.02, 116.26, 132.26, 133.98, 141.83, 149.56, 167.21, 183.57 ppm; MS(EI$^+$) found (M+H) 291.1, calc ($C_{15}H_{18}N_2O_4$ m/z) 290.13

1-(ethoxycarbonyl methyl)-3-formyl-5-methoxy-2-methylindole-4,7-dione (6) To a solution of compound **5** (0.13g, 0.45mmol) in 18.9mL acetone was added a solution of Fremy's salt (0.59g, 2.2mmol) in NaH_2PO_4/Na_2HPO_4 buffer (18.9mL, 0.3M, pH 6.0) and the solution was stirred at room

temperature for 3 hours. Solvent was removed and the residue was extracted with DCM (20mL × 2). The organic layers were combined and washed with water (20mL × 2) and brine (10mL) and dried with Na_2SO_4. The solvent was removed to give **6** (0.134g, 97%) as a red solid without further purification. mp 282.0-183.2°C; ^{1}H NMR ($CDCl_3$ 300MHz) δ 1.31(t, 3H, *J*=7.2 Hz, CH_2CH_3), 2.57(s, 3H, 2-CH_3), 3.85 (s, 3H, CH_3O), 4.26 (q, 2H, *J*=7.2 Hz, CH_2CH_3), 5.16 (s, 2H, N-CH_2CO), 5.70 (s, 1H, 6-*H*), 10.56 (s, 1H, C*H*O); ^{13}C NMR ($CDCl_3$ 100.6 MHz) 10.89, 14.07, 46.06, 56.68, 62.33, 106.35, 119.91, 122.73, 129.14, 142.52, 159.91, 166.69, 177.62, 178.85, 188.10 ppm; MS(EI^+) found (M+H) 306.1, calc ($C_{15}H_{15}NO_6$ m/z) 305.09

1-(ethoxycarbonyl methyl)-3-(hydroxymethyl)-5-methoxy-2-methylindole-4,7-dione (7) Compound **6** (0.60g, 1.97mmol) was dissolved in a mixture of anhydrous MeOH (60mL) and anhydrous THF (60mL). The solution was cooled to 0°C with an ice-water bath. $NaBH_4$ (0.373g, 9.85mmol) was added as a solid and the reaction was stirred for 8 minutes at 0°C. The reaction was then quenched with NH_4Cl solution (10mL saturated NH_4Cl in 10mL water). Solvent was removed and the residue was taken up in DCM (20mL), washed with water (15mL) and brine (15mL), and dried with Na_2SO_4. After solvent removal, the residue was purified on silica, eluting with EtOAc/hexanes (1:1, R_f =0.21) to give 0.41g (67.7%) of **7** as a bright red solid: mp 186.0-186.5°C; ^{1}H NMR ($CDCl_3$ 300MHz) δ 1.30(t, 3H, *J*=7.2 Hz, CH_2CH_3), 2.19(s, 3H, 2-CH_3), 3.83 (s, 3H, CH_3O), 4.25 (q, 2H, *J*=7.2 Hz, CH_2CH_3), 4.64 (s, 2H, CH_2OH), 5.11 (s, 2H, N-CH_2CO), 5.63 (s, 1H, 6-*H*); ^{13}C NMR ($CDCl_3$ 100.6 MHz) 9.26, 14.10, 46.43, 55.85, 56.58, 62.05, 106.80, 122.38, 122.71, 129.35, 134.39, 159.80, 167.26, 178.50, 179.24 ppm; MS(EI^+) found (M+Na) 330.1, calc ($C_{15}H_{17}NO_6$ m/z) 307.11

1-(carboxyl methyl)-3-(hydroxymethyl)-5-methoxy-2-methylindole-4,7-dione (8) Compound **7** (100mg, 0.325mmol) was dissolved in MeOH (6mL) and THF (6mL), and a solution of LiOH (14.3mg, 0.341mmol in 5mL water) was added dropwise. After the addition, the reaction was allowed to warm to room temperature and was stirred for 1 hour. The mixture was neutralized with 1N HCl. After solvent

removal, the residue was purified on silica, eluting with EtOAc/MeOH (1:1, R_f =0.33) to give 0.92g (93.2%) of **8** as a bright red solid: mp 250°C (decompose); ^{1}H NMR (D_2O, 400MHz) δ 2.04(s, 3H, 2-C*H*$_3$), 3.66 (s, 3H, C*H*$_3$O), 4.52 (s, 2H, C*H*$_2$OH), 4.76 (s, 2H, N-C*H*$_2$CO), 5.54 (s, 1H, 6-*H*); ^{13}C NMR (D_2O 100.5 MHz) 8.14, 48.75, 53.40, 56.58, 106.17, 119.90, 120.89, 128.60, 138.35, 159.54, 174.39, 179.04, 179.54 ppm; MS(EI$^+$) found (M+Na) 302.1, calc ($C_{13}H_{13}NO_6Na$ m/z) 302.07

N-(2-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-2-(3-(hydroxymethyl)-5-methoxy-2-methyl-4,7-dioxo-4,7-dihydro-1H-indol-1-yl)acetamide (9) Compound **8** (84mg, 0.30mmol), TEA (50mg, 0.493mmol) and 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanamine (98.2mg, 0.45mmol) was dissolved in anhydrous DMF under argon. The mixture was cooled to 0°C with an ice-water bath. Then *O*-(7-Azabenzotriazol-1-yl)-N,N,N'N'-tetramethyluronium hexafluorophosphate (HATU, 136.8mg, 0.36mmol) was added. The reaction was stirred at room temperature for 18 hours. The solvent was removed and the residue was taken up in DCM (10mL). The organic layer was washed with water (10mL), 2% HCl (10mL), water (10mL), sat. $NaHCO_3$ (10mL), and brine (10mL). The solution was dried with Na_2SO_4 and solvent was removed. The product was purified with silica with 2%-5% methanol in DCM to give 63.6mg (40%) of **9** as a red solid: mp 102.5-103.2°C; ^{1}H NMR ($CDCl_3$, 400MHz) δ 2.29(s, 3H, 2-C*H*$_3$), 3.01(s, br, 2H), 3.40-3.69(m, 18H, C*H*$_2$C*H*$_2$O), 3.83(s, 3H, C*H*$_3$O), 4.63 (s, 2H, C*H*$_2$OH), 4.99(s, br, 2H, N-C*H*$_2$CO), 5.64 (s, 1H, 6-*H*), 6.60(s, br, 1H, CON*H*) ; ^{13}C NMR ($CDCl_3$ 100.5 MHz) 9.44, 39.43, 48.27, 50.62, 55.76, 56.60, 69.44, 69.93, 70.25, 70.48, 70.56, 70.60, 106.87, 122.47, 122.94, 128.88, 135.65, 159.73, 166.15, 178.55, 179.12 ppm; MS(EI$^+$) found (M+H) 480.24, calc ($C_{21}H_{29}N_5O_8$ m/z) 479.20

(1-(14-azido-2-oxo-6,9,12-trioxa-3-azatetradecyl)-5-methoxy-2-methyl-4,7-dioxo-4,7-dihydro-1*H*-indol-3-yl)methyl methyl(2-(methylamino)ethyl)carbamate (10)

a. Compound **9** (45mg, 0.094mmol) and pyridine (76μL) were dissolved in anhydrous DCM (1.0mL). The solution was cooled to 0°C with an ice-water bath. A solution of 4-nitrophenyl chloroformate

(189mg, 0.94mmol) in DCM (0.5mL) was added slowly while stirring. The reaction was allowed to warm to room temperature and was stirred for an additional 1 hour. Solvent was removed and the residue was taken up in DCM (5mL). The organic layer was washed with water (5mL) and brine (5mL) and dried with Na_2SO_4. After solvent removal, the residue was purified on silica, eluting with DCM /MeOH (100:3, R_f =0.64) to give the activated compound **9a** as a reddish-yellow solid: (55.2mg, 91%). mp 118.0-119.0°C; ^{1}H NMR ($CDCl_3$, 400MHz) δ 2.39(s, 3H, 2-C*H*$_3$), 3.38-3.70(m, 18H, C*H*$_2$C*H*$_2$O), 3.82(s, 3H, C*H*$_3$O), 5.02(s, 2H, N-C*H*$_2$CO), 5.46 (s, 2H, 6-*H*), 5.64 (s, 1H), 6.66(s, br, 1H, CON*H*), 7.39(d, *J*=9.4Hz, 2H, aromatic H), 8.26(d, *J*=9.4Hz, 2H, aromatic H); ^{13}C NMR ($CDCl_3$ 100.5 MHz) 9.57, 39.52, 48.27, 50.62, 56.56, 61.17, 69.41, 69.96, 70.28, 70.48, 70.57, 70.61, 106.51, 114.35, 121.79, 122.17, 125.19, 128.93, 139.30, 145.22, 152.36, 155.59, 159.83, 166.07, 177.49, 178.90 ppm; **b**. N, N'-methylethylenediamine (82mg, 0.93mmol) was dissolved in anhydrous THF (8mL) and cooled to 0°C. A solution of compound **9a** (200mg, 0.31mmol in 2mL THF) was added dropwise. The mixture was stirred at 0°C for 30 minutes and was then allowed to warm to room temperature and was stirred overnight. Saturated $NaHCO_3$ (5mL) was added and the mixture was extracted with DCM (10mL × 3). The combined organic layer was washed with brine (20mL) and dried with Na_2SO_4. After solvent removal, the residue was purified on silica, eluting with CH_2Cl_2 /MeOH (2:1, R_f =0.18) to give compound **10** as a reddish-brown oil: (121.1mg, 66%). ^{1}H NMR ($CDCl_3$, 400MHz) δ 2.36(s, 3H, 2-C*H*$_3$), 2.46(s, 2H, Me-NH-C*H*$_2$), 2.62(m, br, 2H, Me-NH-CH$_2$-C*H*$_2$), 2.79(m, br, 2H, Me-NH-C*H*$_2$-CH$_2$), 3.28-3.59(m, 18H, C*H*$_2$C*H*$_2$O), 3.68(s, 6H, C*H*$_3$N), 3.80(s, 3H, C*H*$_3$O), 5.00 (s, 2H, C*H*$_2$-OCO), 5.23(s, br, 2H, N-C*H*$_2$CO), 5.61 (s, 1H, 6-*H*), 6.68(s, br, 1H, CON*H*) ; ^{13}C NMR ($CDCl_3$ 100.5 MHz) 9.57, 25.55, 36.18, 39.45, 48.35, 48.63, 49.64, 50.62, 56.48, 57.25, 67.91, 69.45, 69.99, 70.30, 70.51, 70.57, 70.64, 106.43, 117.12, 122.37, 128.57, 159.78, 166.25, 177.36, 178.86 ppm; MS(EI$^+$) found (M+H) 580.30, calc ($C_{25}H_{37}N_7O_9$ m/z) 579.26

(*S*)-(1-(14-azido-2-oxo-6,9,12-trioxa-3-azatetradecyl)-5-methoxy-2-methyl-4,7-dioxo-4,7-dihydro-1*H*-indol-3-yl)methyl (4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1*H*-

pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) ethane-1,2-diylbis(methylcarbamate) (12)

Compound **10** (57mg, 0.096mmol) was dissolved in anhydrous DCM (3.0mL) and cooled with an ice-water bath. TEA (142µL) was added followed by the addition of triphosgene (28.5mg, 0.096mmol). The reaction was stirred at 0°C for 30 min. Solvent was then removed and the residue was dried under high vacuum for 1 hour to give compound **11**. The crude **11** was dissolved in anhydrous DCM (2mL) and was cooled with an ice-water bath. The solution was used for the next reaction without further purification. SN38 was suspended in anhydrous DCM (5.0mL) and TEA (65 µL) was added. The previously made solution was added to this SN38 solution dropwise at room temperature under argon. The reaction was stirred for 24 hours. Then the reaction was diluted with DCM (5mL) and washed successively with saturated NH_4Cl (10mL × 3), water (10mL), 5% $NaHCO_3$ (10mL), water (10ml) and brine(10mL). Organic layer was dried with Na_2SO_4. After solvent removal, the residue was purified on silica, eluting with DCM /MeOH (2%-4%, R_f =0.32) to give compound **12** as a reddish-brown solid: (60.1mg, 77%). Mp 118.5-124.0°C; ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.02(t, 3H), 1.39(t, 3H), 1.89(m, 2H), 2.04(s, br, 1H), 2.30(m, br, 2H), 2.36(s, br, 1H), 2.96(m, 5H), 3.15(m, 4H), 3.36-3.64(m, 10H), 3.65(m, 16H), 3.78(s, 3H), 3.98(d, 1H), 4.81(s, 1H), 4.94(m, 1H), 5.22-5.32(m, 6H), 5.52(m, 1H), 5.75(d, 1H), 6.71(br, 1H), 7.46-7.59(m, 1H), 7.65(d, 1H), 7.82(s, 1H), 8.19(t, 3H); ^{13}C NMR ($CDCl_3$ 100.5 MHz) 7.79, 9.55, 13.98, 23.11, 31.55, 34.98, 39.47, 47.18, 48.21, 49.37, 50.63, 56.49, 57.31, 66.28, 69.47, 69.96, 70.27, 70.48, 70.55, 70.62, 72.76, 97.91, 106.37, 118.38, 127.09, 127.42, 128.61, 131.68, 146.92, 147.10, 150.16, 151.50, 154.49, 157.60, 166.15, 173.85 ppm; MS(EI^+) found (M+H) 1012.3, (M+Na) 1034.2, calc ($C_{49}H_{57}N_9O_{15}$ m/z) 1011.37

5-oxo-5-(prop-2-ynylamino)pentanoic acid (13) Propargyl amine (530mg, 9.6mmol) and TEA (971mg, 9.6mmol) was dissolved in anhydrous acetonitrile (12mL) and cooled to 0°C. A solution of glutaric anhydride (912.8mg, 8.0mmol) in anhydrous acetonitrile (8ml) was added dropwise. The reaction was then allowed to warm to room temperature and stirred for 3 hours. Solvent was then removed and the residue was take up in EtOAc(100ml), washed with 0.5N HCl (50ml × 2) and brine

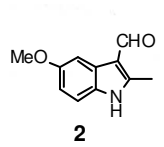
(25mL). The solution was dried with Na_2SO_4. Solvent was then removed to give **13** as a pale white solid. (640mg, 38%). Mp: 75.5-76.2 °C. ^{1}H NMR (CD_3OD, 400MHz) δ 1.91(pent, 2H, *J*=6Hz), 2.28(t, 2H, *J*=6Hz), 2.33(t, 2H, *J*=6Hz), 2.58(t, 1H, *J*=2Hz), 3.95(d, 2H, *J*=2Hz). ^{13}C NMR (CD_3OD, 100.6MHz) δ 20.66, 27.95, 32.57, 34.34, 70.65, 79.21, 173.55, 175.33 ppm.

Alkyne modified c(RGDyK) (14) Compound **13** (12mg, 70.84μmol) was dissolved in anhydrous acetonitrile (0.3mL) and DIPEA (20μL) was added. Then TSTU (14.7mg, 49.1μmol) was added and the mixture was stirred at room temperature for 30 min. This mixture was then added to a solution of c(RGDyK) (4.0mg, 6.45μmol) in anhydrous DMF (0.3mL) and DMSO (0.15mL). The reaction mixture was stirred under argon over night. Crude MS showed that no free c(RGDyK) was left. Solvent was removed and the residue was purified using a semi-prep HPLC. Collected fractions were combined and solvent was removed. The purified product was dissolved in DI water and lyophilized to give **14** as a fluffy solid. (3.27mg, 66%); MS(EI$^+$): found (M+H) 771.3, calc ($C_{35}H_{50}N_{10}O_{10}$ m/z) 770.37. HPLC retention time: 17.4min.

Compound 1

Compound **12** (11.13mg, 11.0μmol) was dissolved in *t*-butanol (0.3mL) and DI water (0.3mL). Then compound **14** (2.12mg, 2.75 μmol) was added and mixed well. Then a solution of $CuSO_4$ (0.1N, 0.028ml) and a solution of sodium ascorbate (0.2N, 0.028mL) was added. The reaction mixture was stirred over night. Solvent was removed and the residue was dissolved in HPLC eluent (0.14%TFA in DI water/acetonitrile) and purified using a semi-prep HPLC. Collected fractions were combined and solvent was removed. The purified product was dissolved in DI water and lyophilized to give **1** as a fluffy solid. (2.34mg, 48%); MS(EI$^+$): found (M+H) 1782.1, (M+H+Na) 902.5. calc ($C_{84}H_{107}N_{19}O_{25}$ m/z) 1781.76. HPLC retention time: 25.1min.

^{1}H and ^{13}C NMR spectra for **2**



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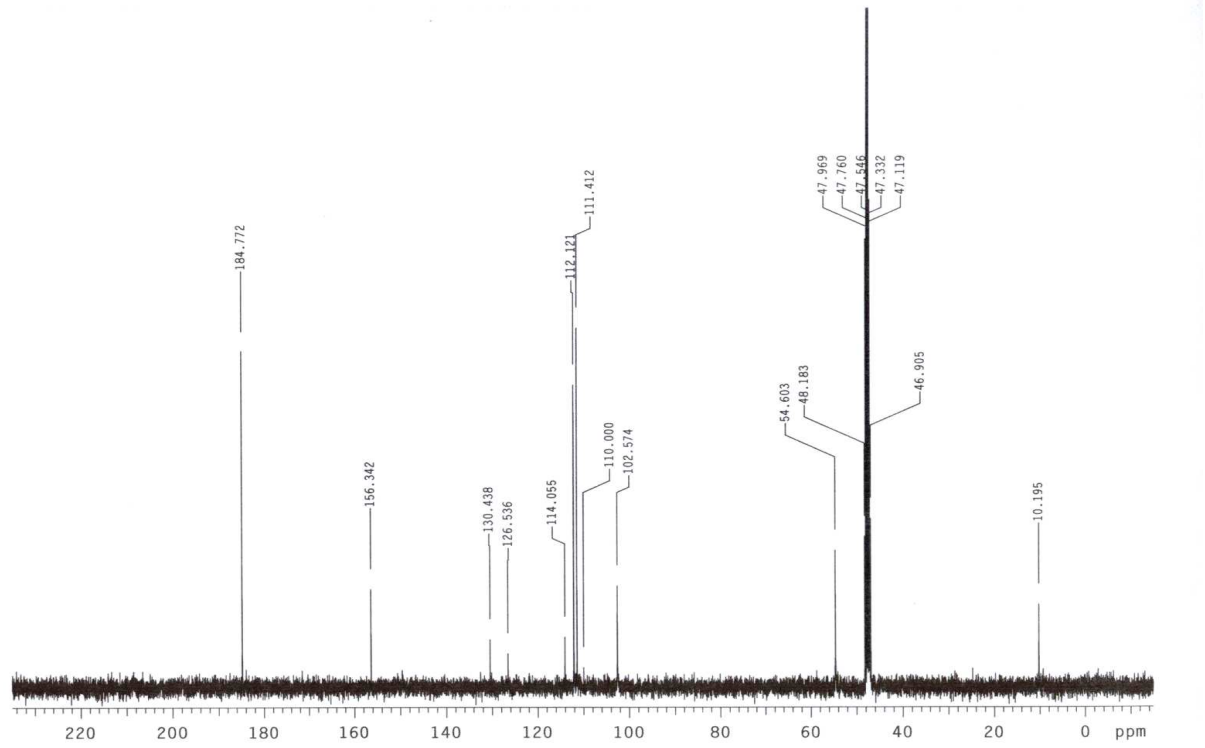
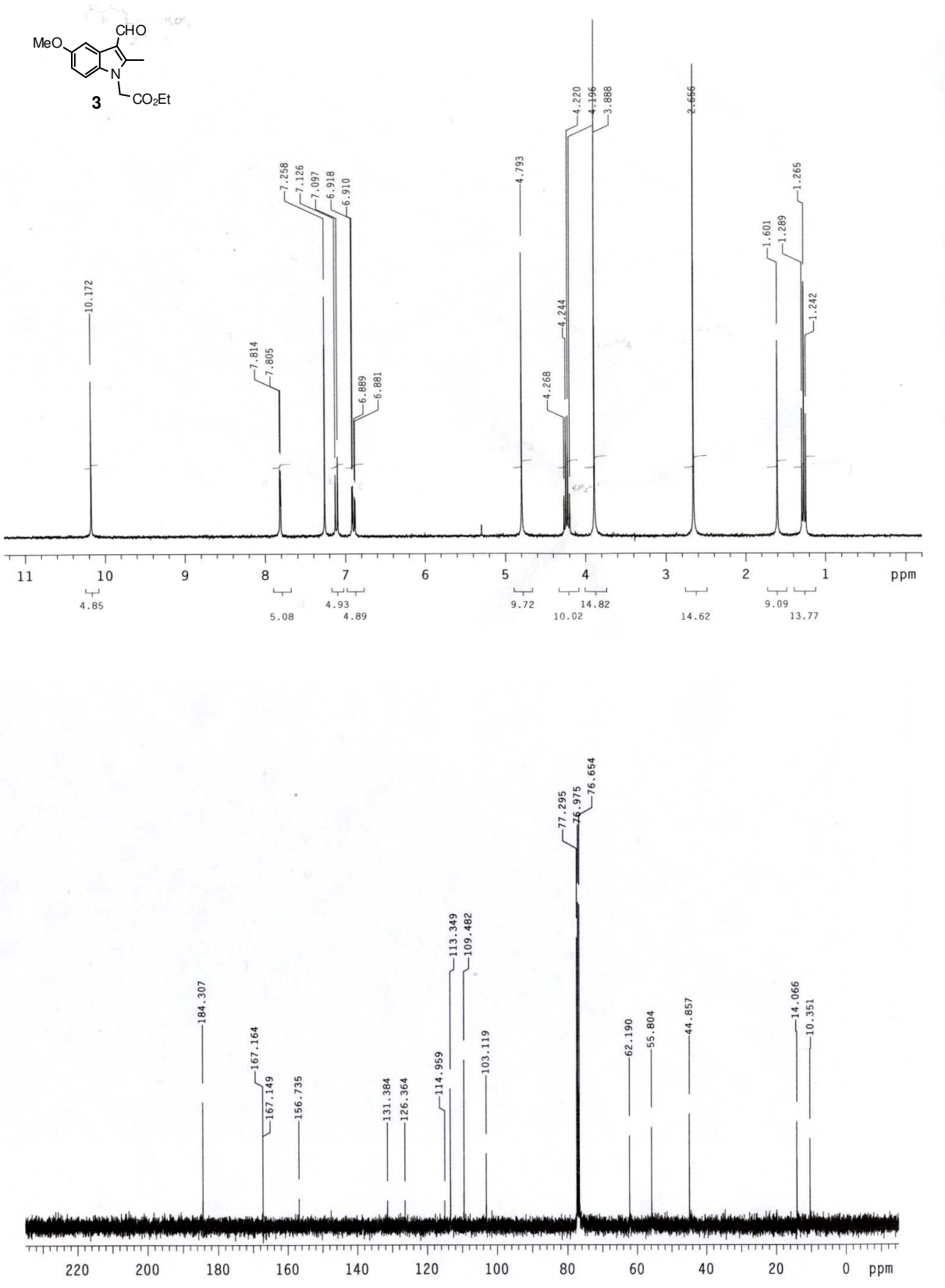
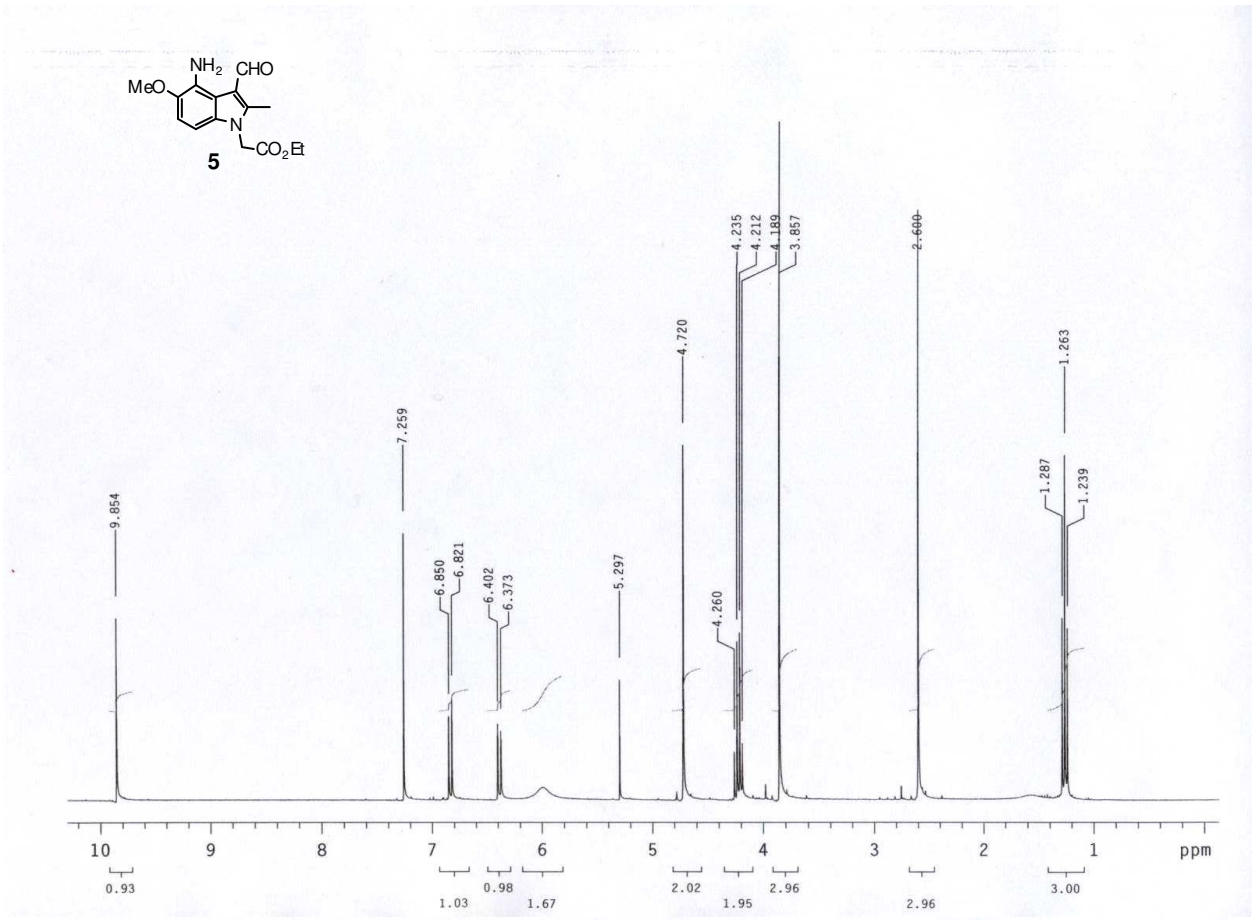
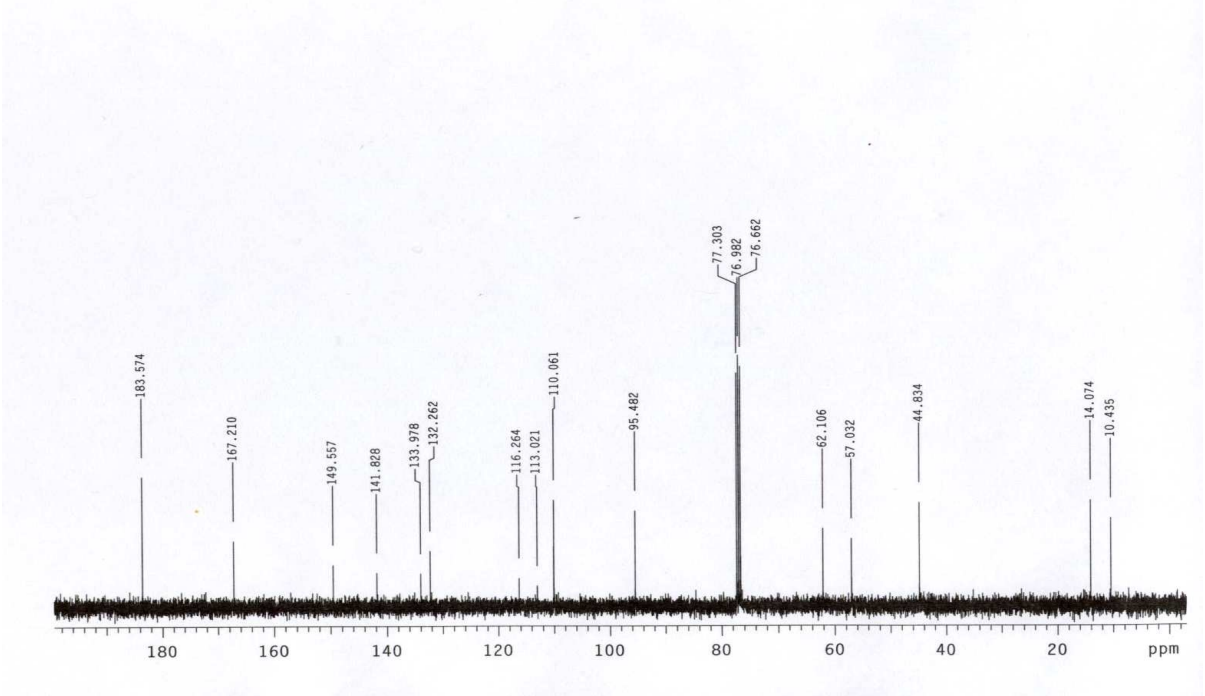


^{1}H and ^{13}C NMR spectra for **3**

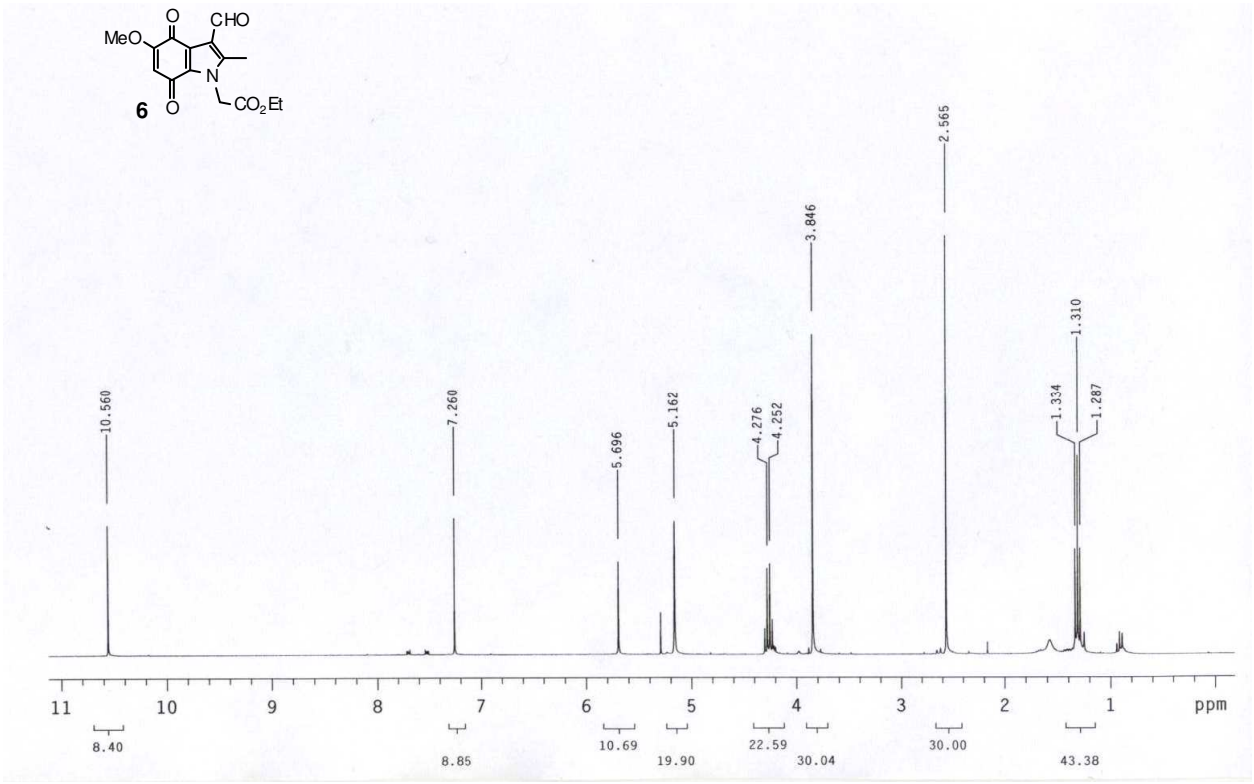


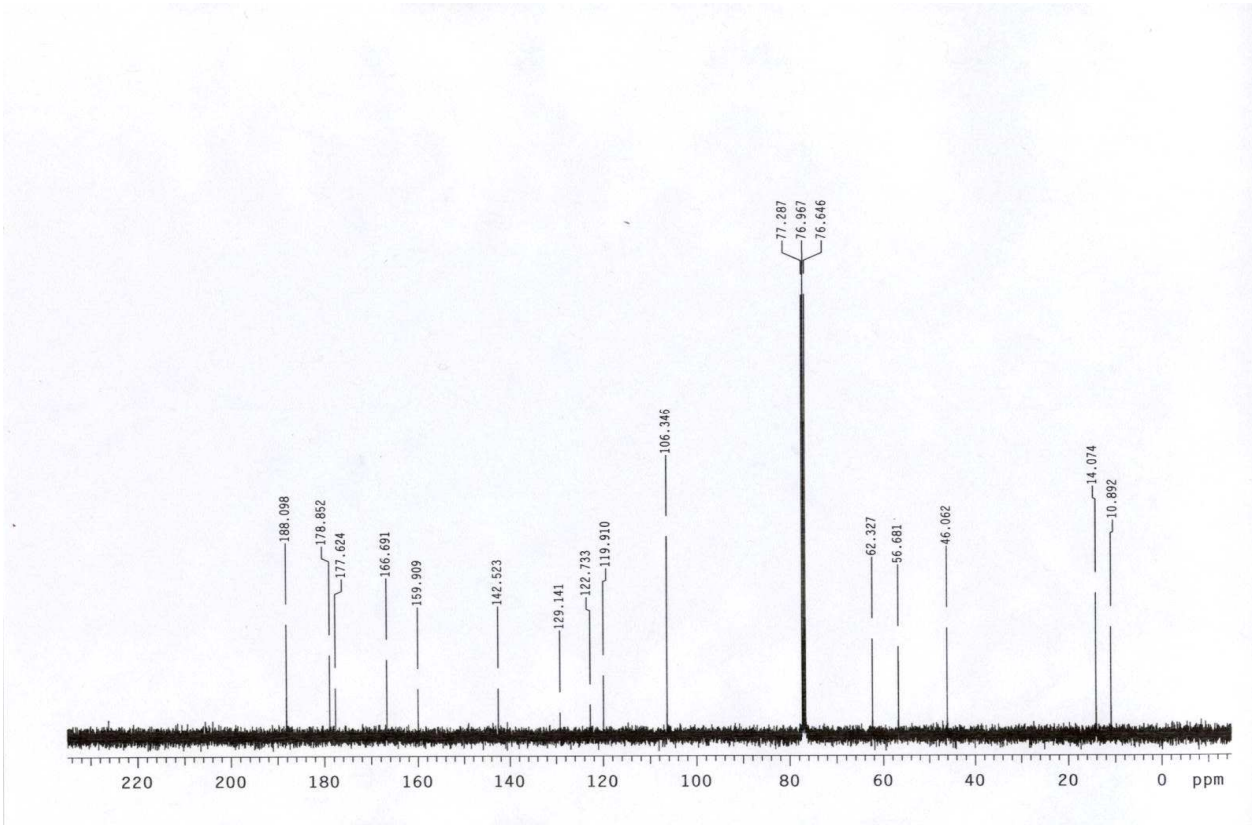
^{1}H and ^{13}C NMR spectra for **5**



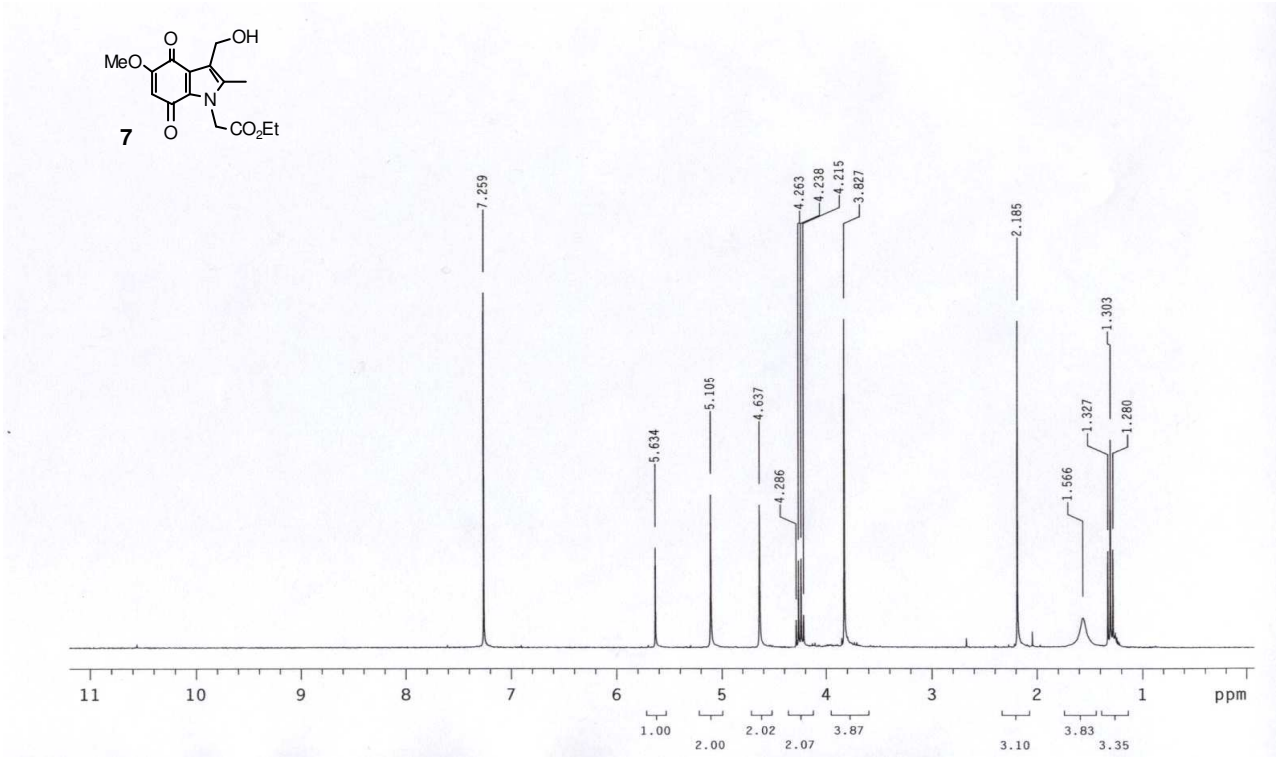


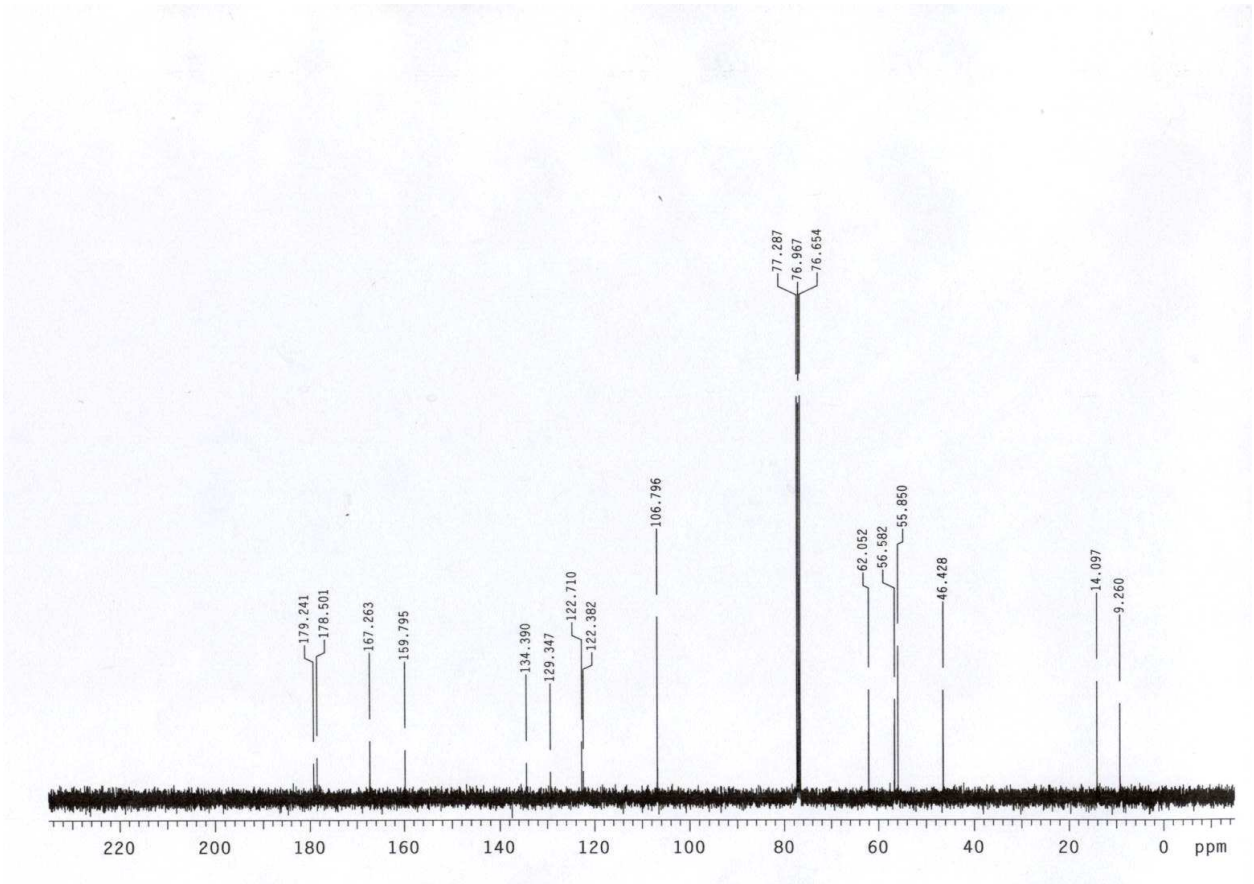
^{1}H and ^{13}C NMR spectra for **6**



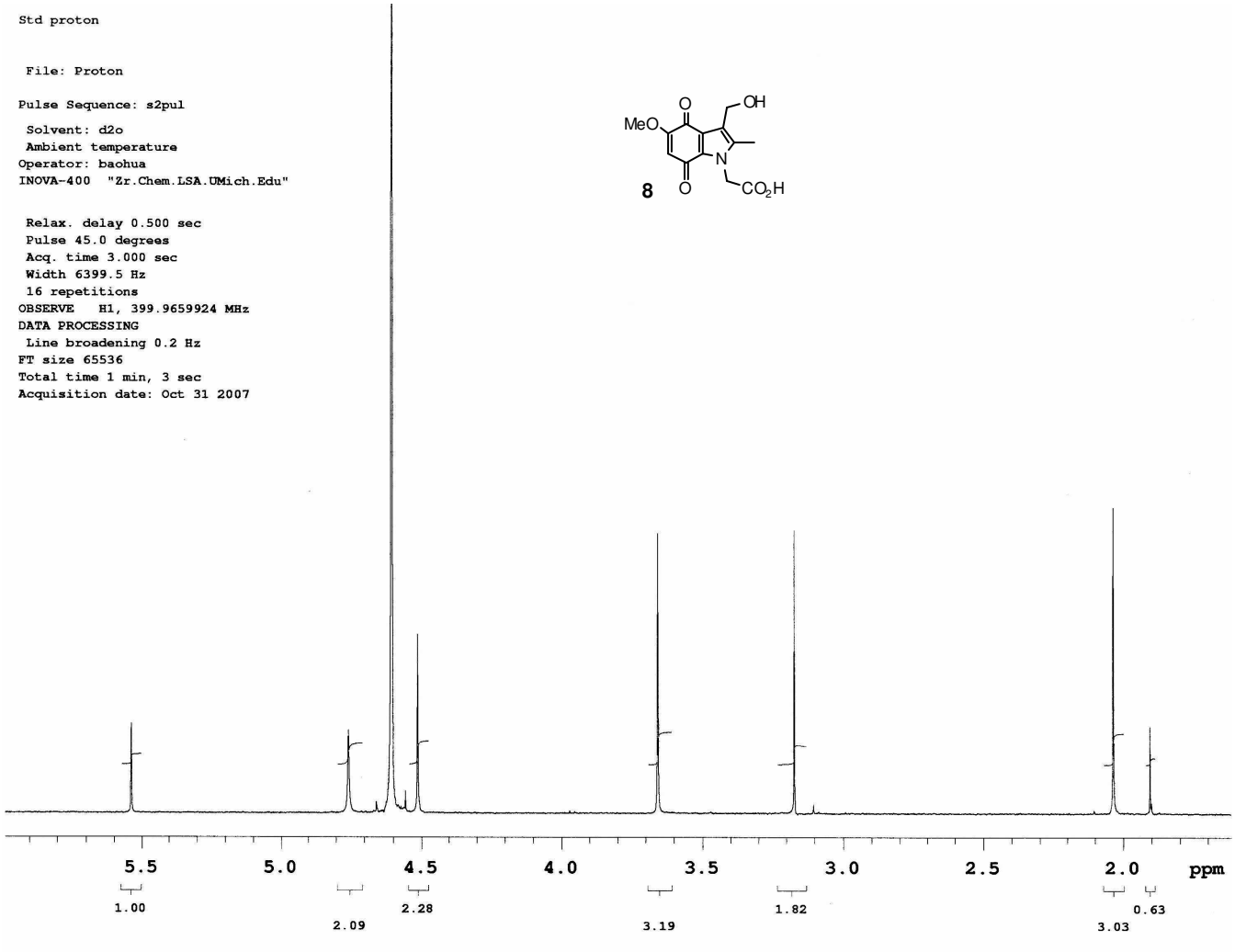


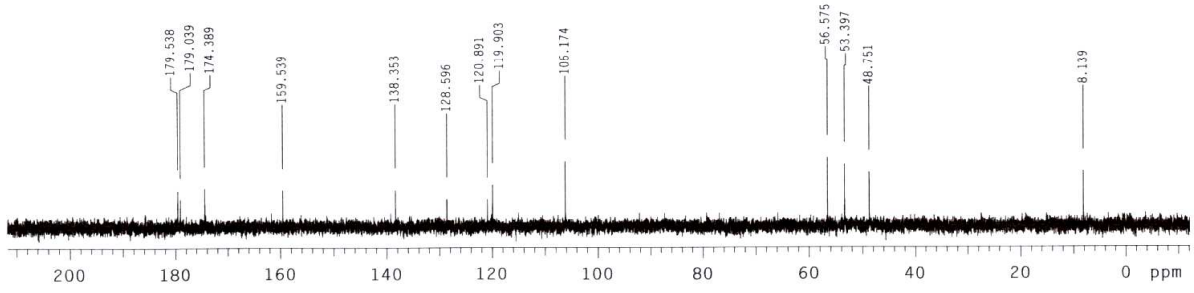
^{1}H and ^{13}C NMR spectra for **7**



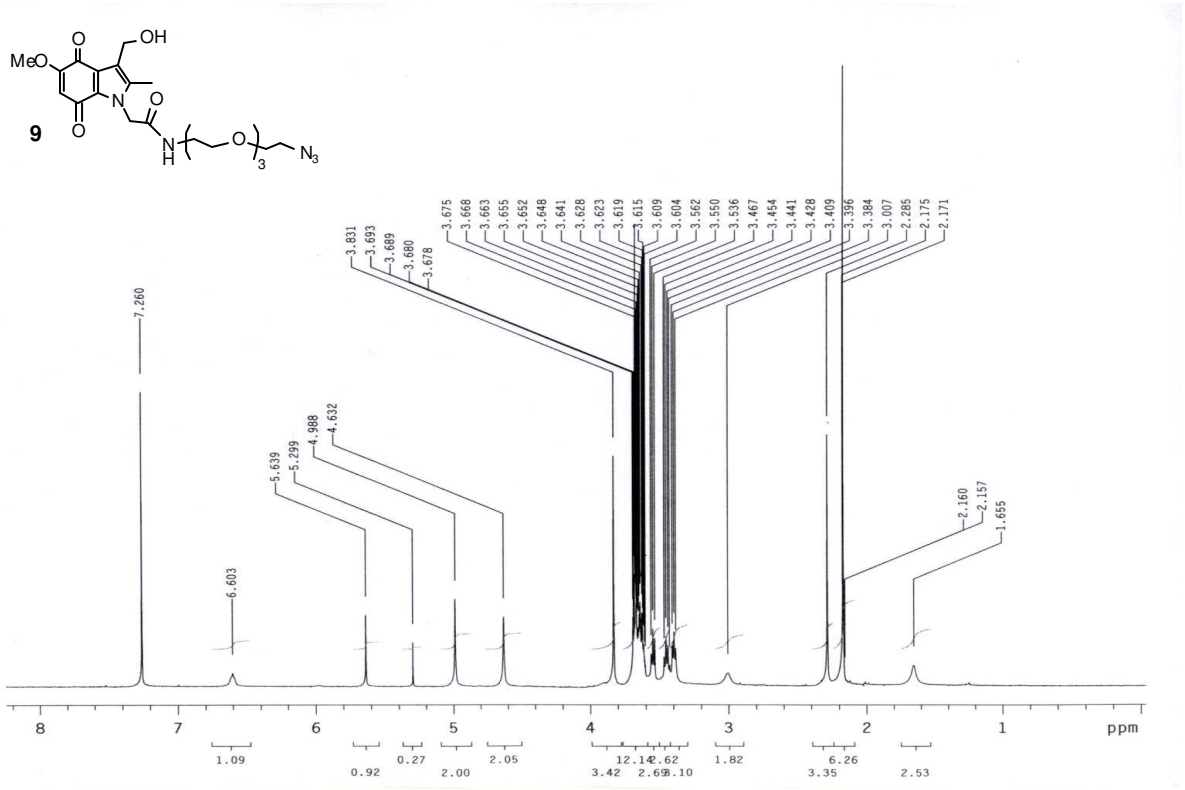


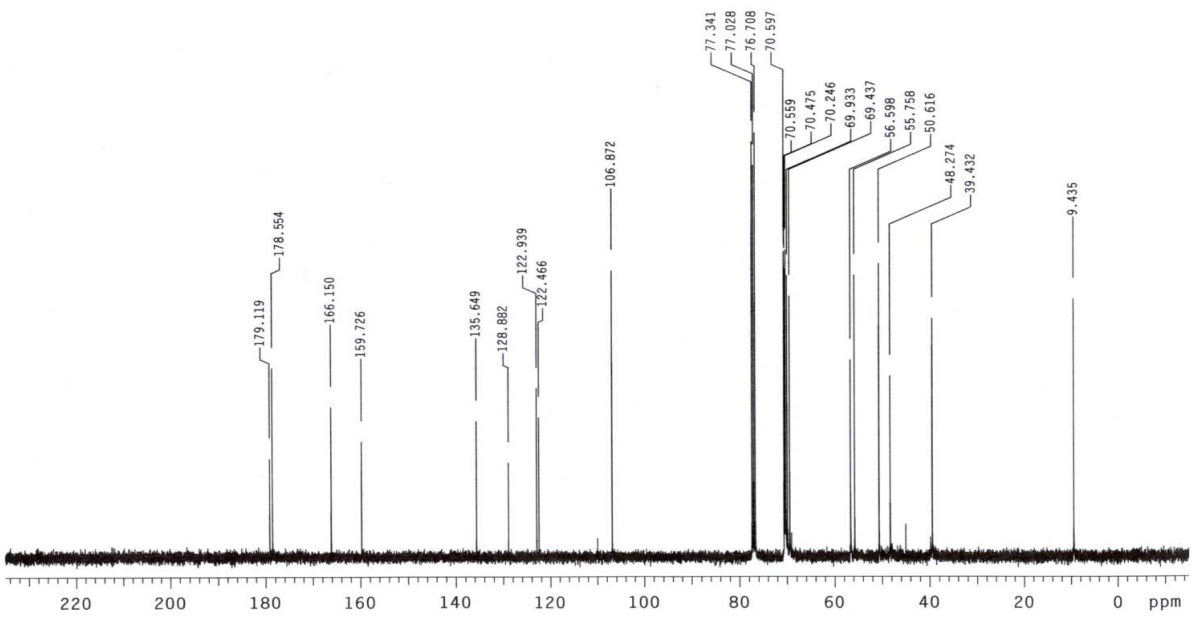
^{1}H and ^{13}C NMR spectra for **8**



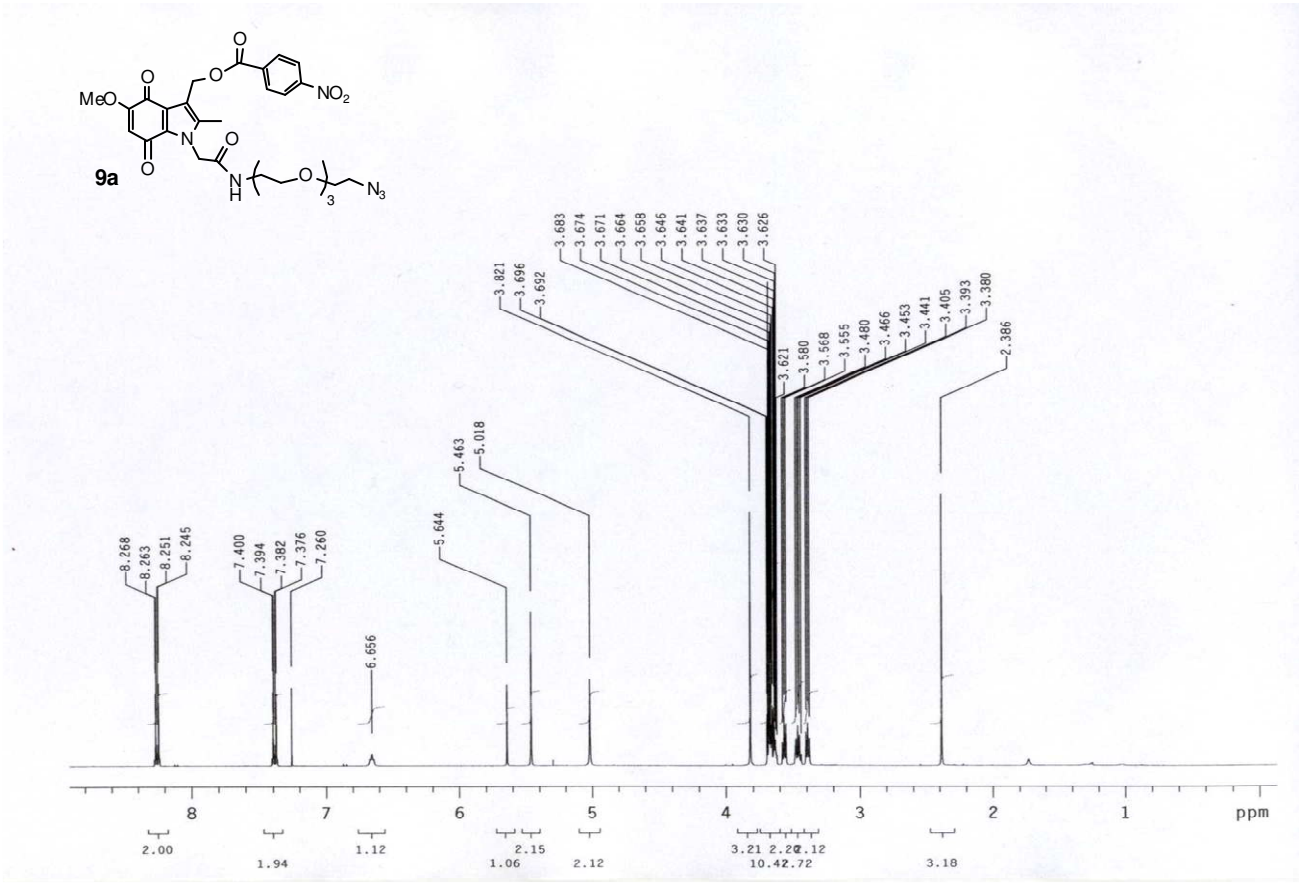


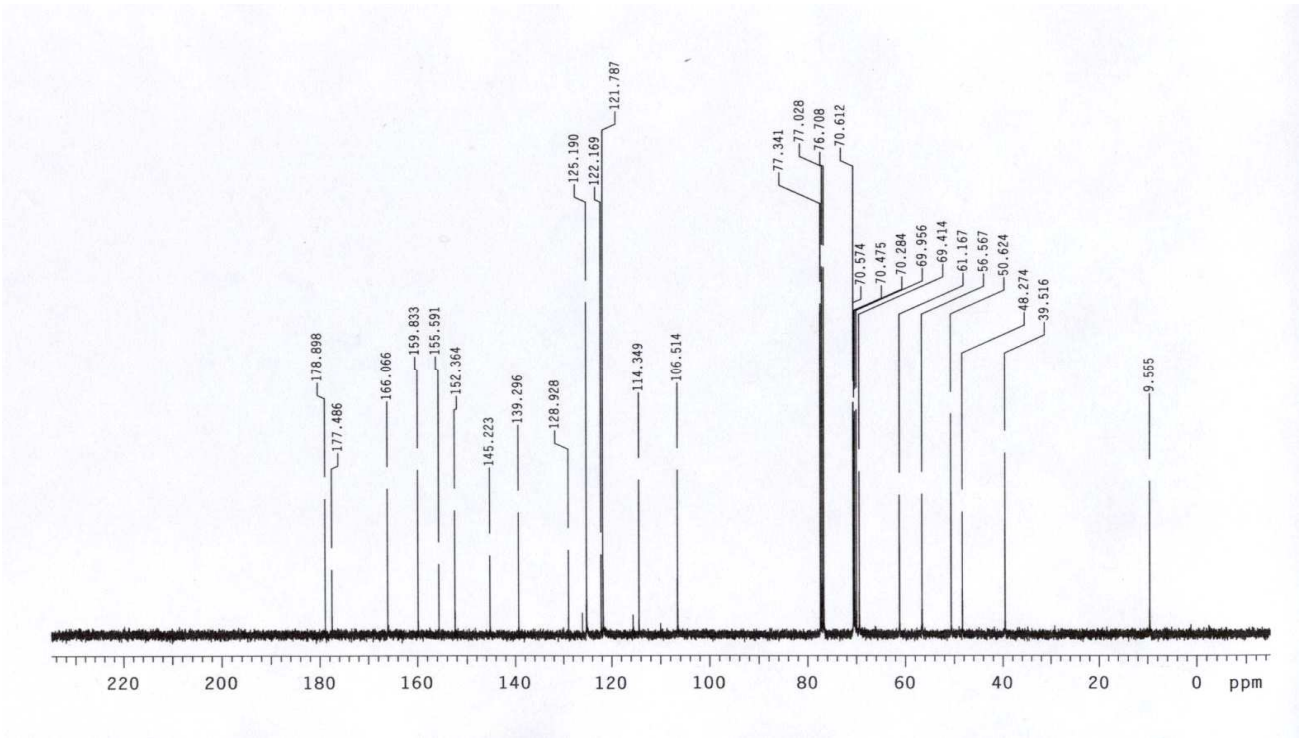
^{1}H and ^{13}C NMR spectra for **9**



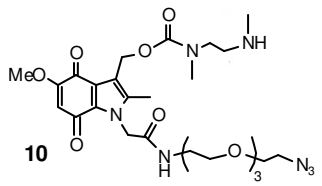


^{1}H and ^{13}C NMR spectra for **9a**

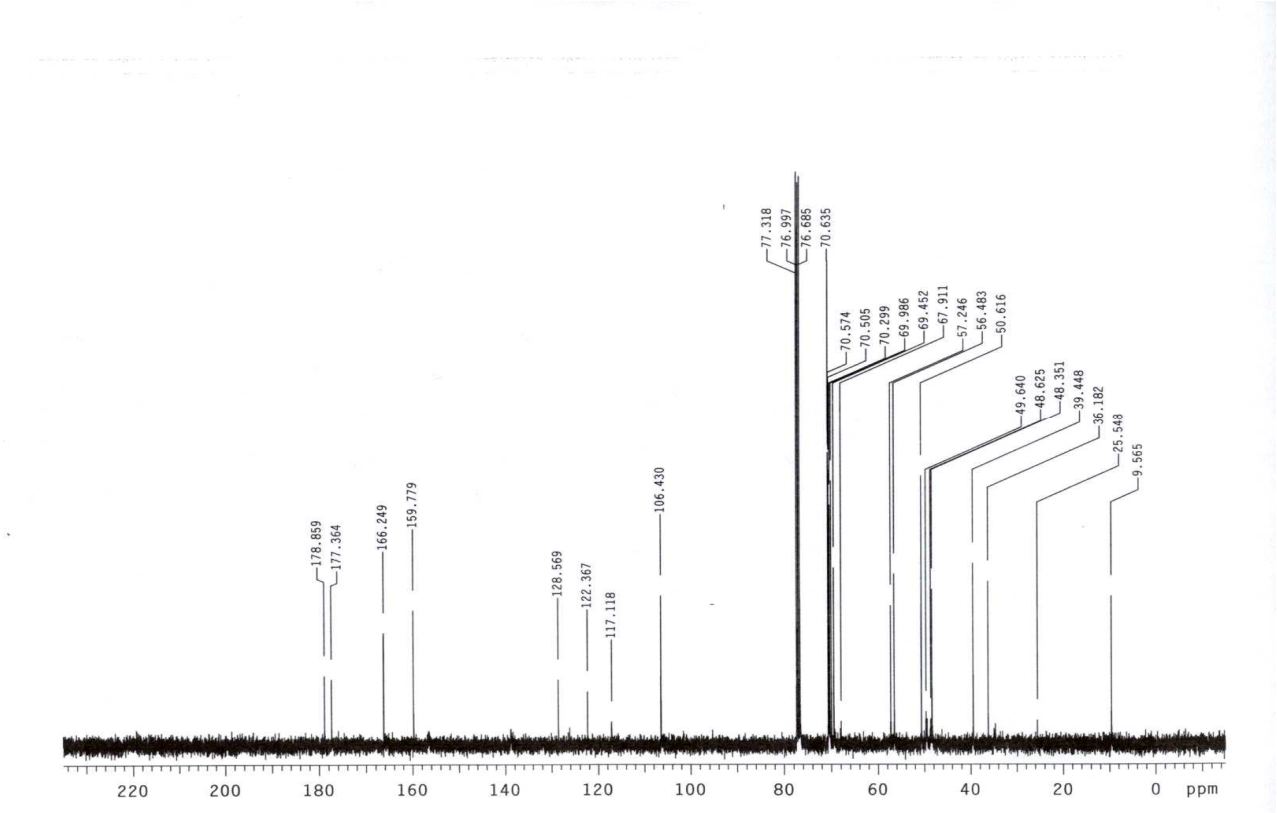




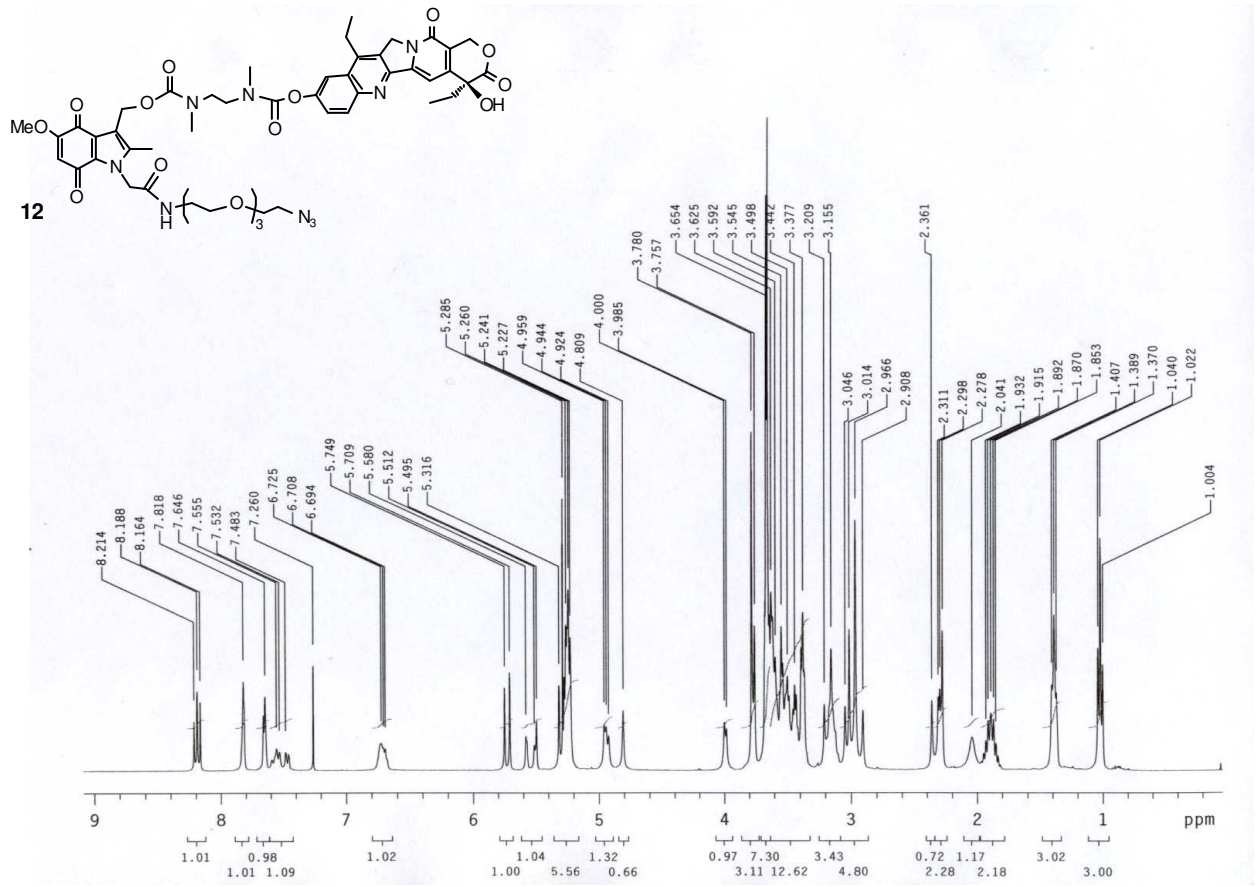
^{1}H and ^{13}C NMR spectra for **10**

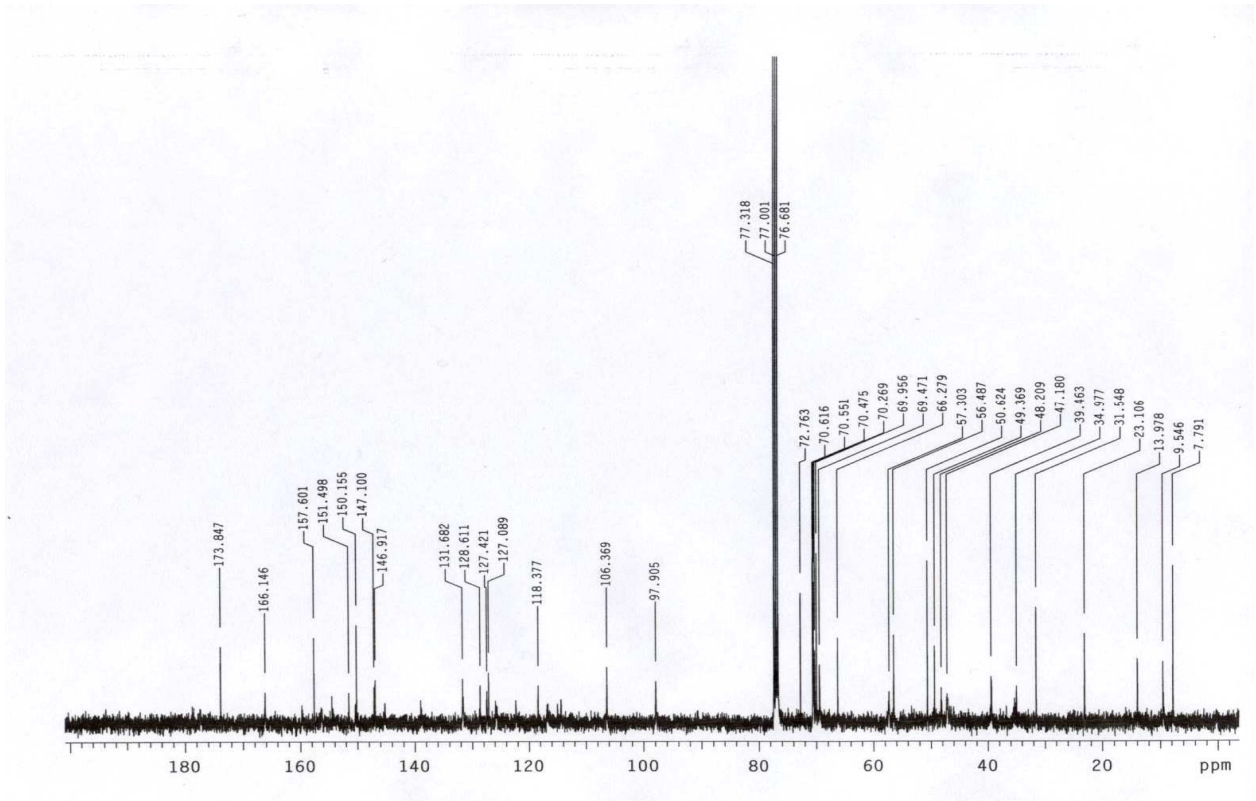


10

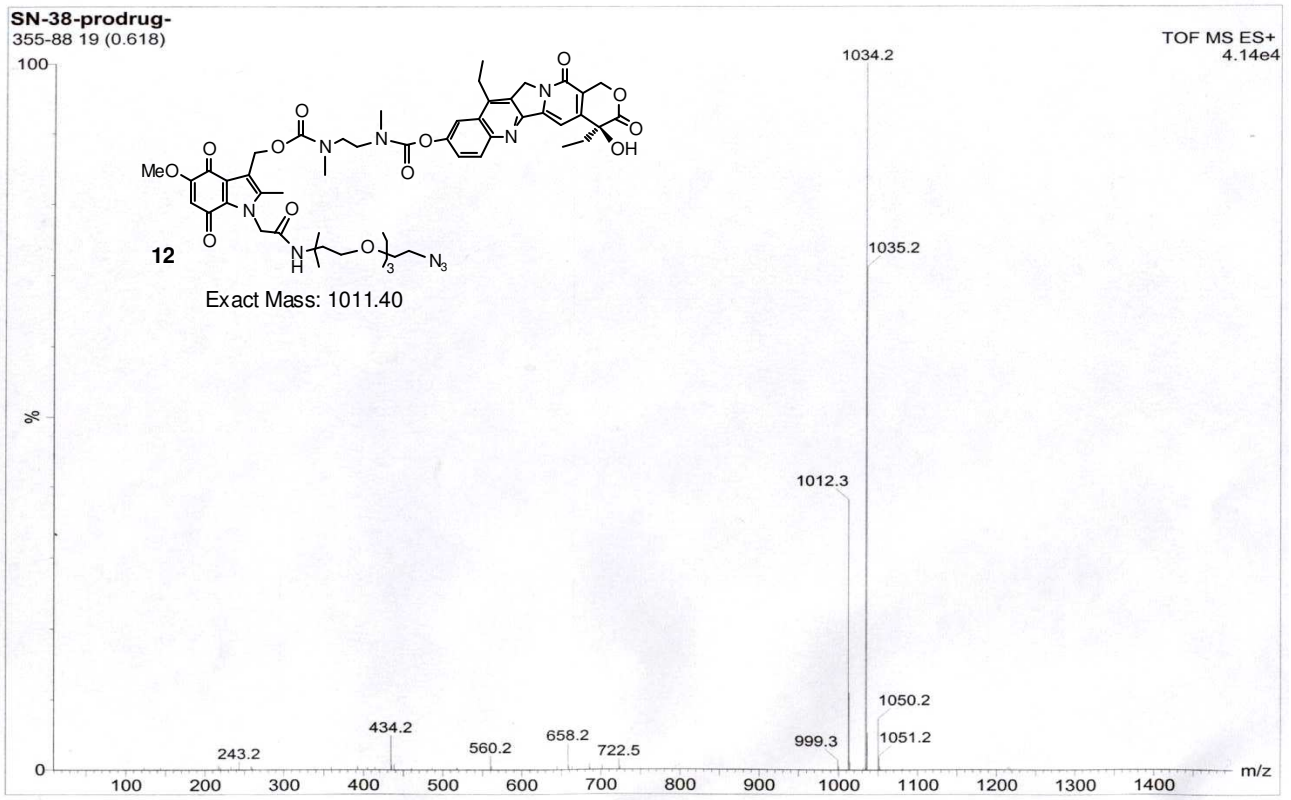


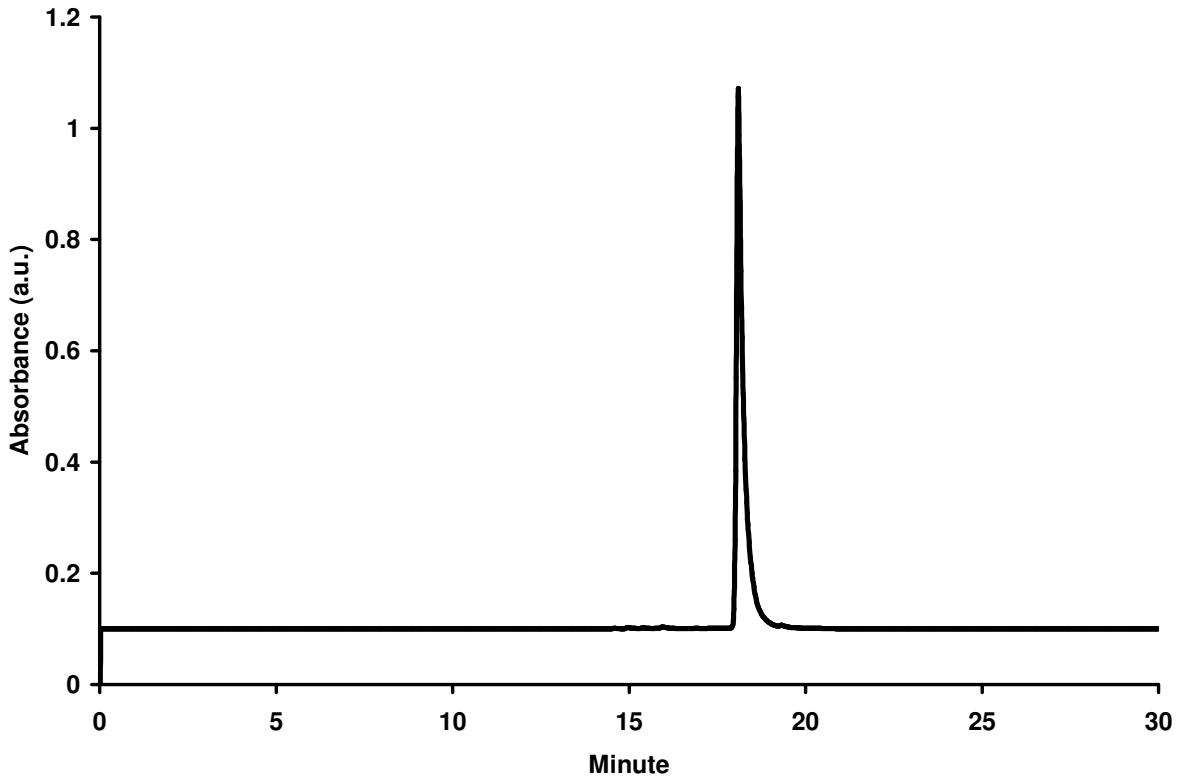
^{1}H and ^{13}C NMR spectra for **12**



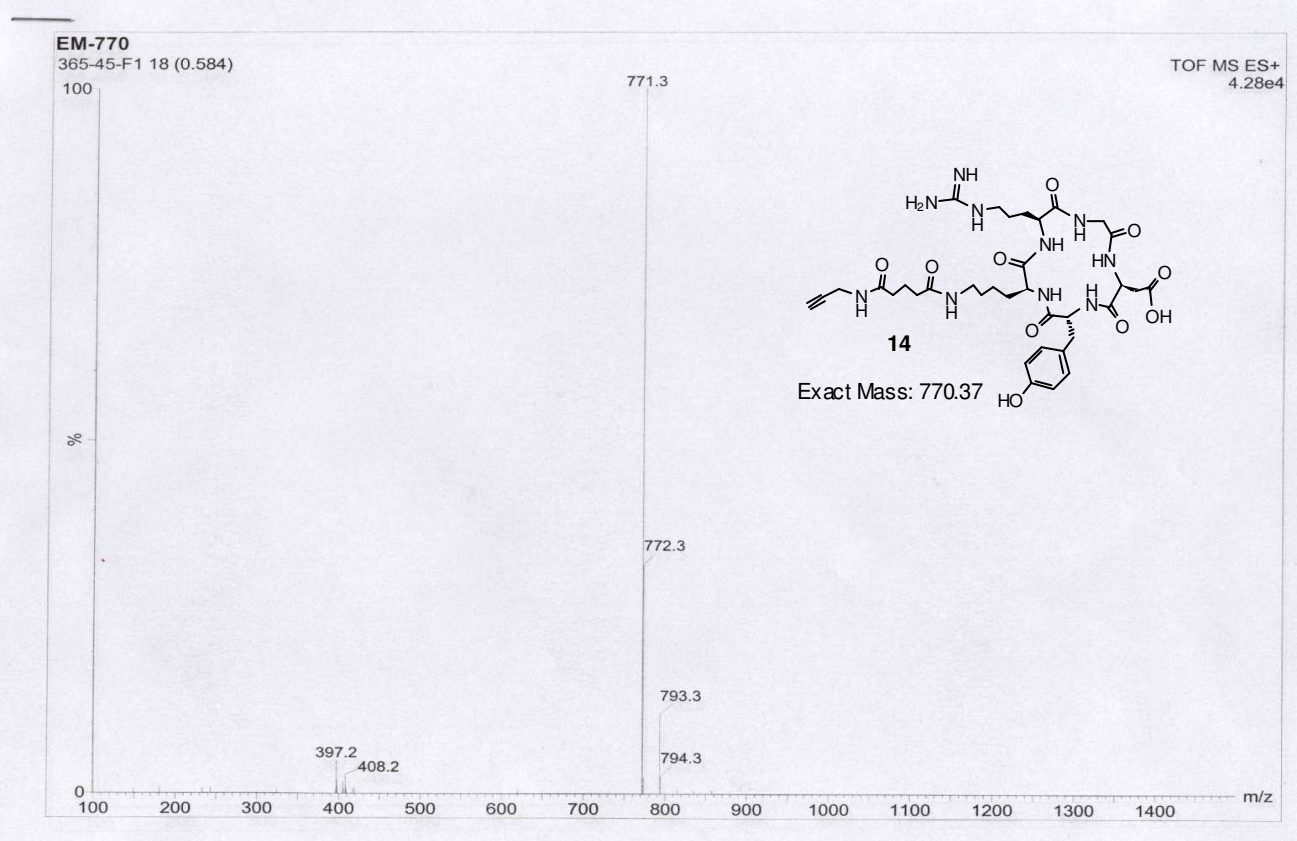


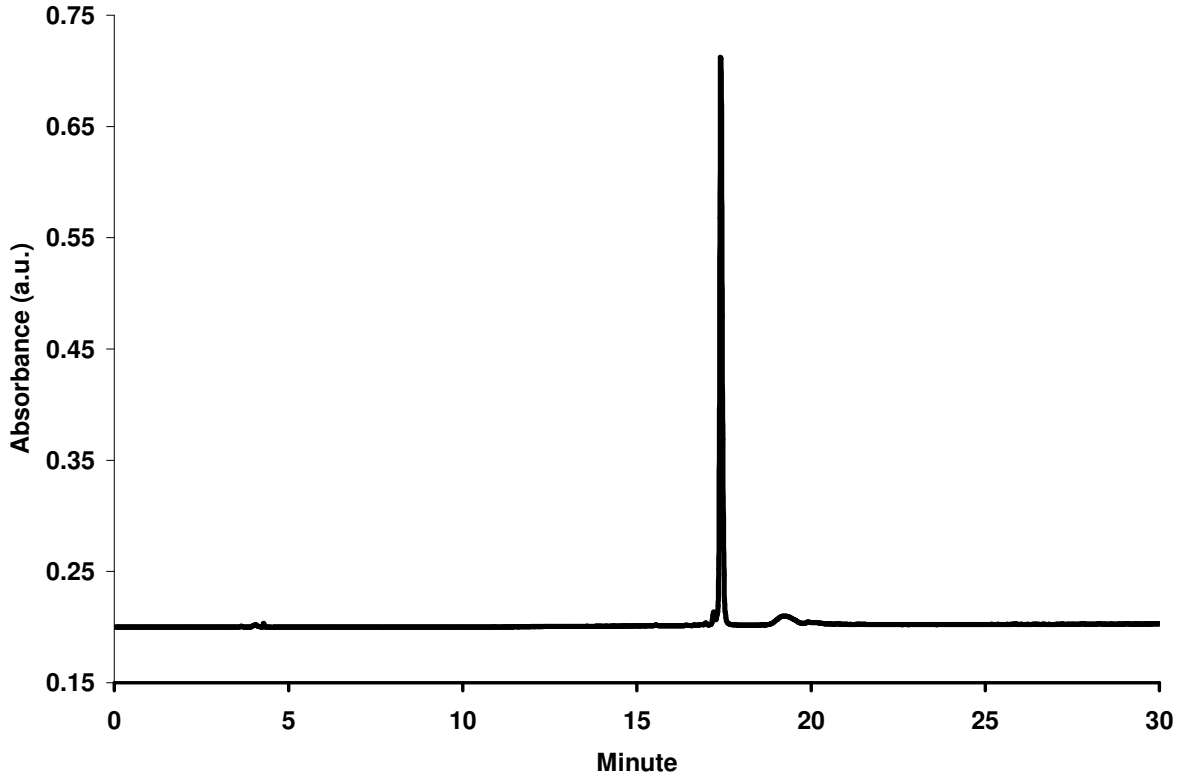
Mass spectrum and HPLC profile for **12**





Mass spectrum and HPLC profile for **14**





Mass spectrum and HPLC profile for **1**