9-Aryl-1,2-dihydropyrrolo[3,4-*b*]indolizin-3-one (Seoul-Fluor) as a smart platform for ratiometric fluorescent pH sensors

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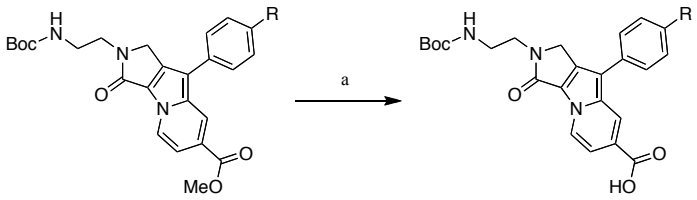
I. General Information

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-300 (Bruker Biospin, Germany) and Varian Inova-500 (Varian Assoc., Palo Alto, USA), and chemical shifts were measured in ppm downfield from internal tetramethylsilane (TMS) standard. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); dt (doublet of triplet); td (triplet of doublet); br s (broad singlet), etc. Coupling constants were reported in Hz. Routine mass analyses were performed on LC/MS system equipped with a reverse phase column (C-18, 50 x 2.1 mm, 5 μm) and photodiode array detector using electron spray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The progress of reaction was monitored using thin-layer chromatography (TLC) (silica gel 60 F_{254} 0.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the TLC plates with anisaldehyde, $KMnO_4$, and phosphomolybdic acid followed by heating. All reactions were conducted in oven-dried glassware under dry argon atmosphere, unless otherwise specified. Toluene and THF were dried by distillation from sodium- benzophenone immediately prior to use. CH_2Cl_2 was distilled from CaH_2 and TEA was distilled over KOH. Other solvents and organic reagents were purchased from commercial venders and used without further purification unless otherwise mentioned. Distilled water was polished by ion exchange and filtration. Excitation and Emission maxima were measured by Cary Eclipse Fluorescence spectrophotometer (Varian Assoc., Palo Alto, USA) and a final concentration of each fluorescent compounds in pH solution was 100 μM. The pH buffer solutions were prepared by adjusting the pH of the potassium hydrogen phthalate (for pH 3.0-5.8) and potassium dihydrogen phosphate (for pH 5.8-8.0) solution with 0.1 M hydrogen chloride or sodium hydroxide solution respectively. All the photography images of the fluorescent compounds in each pH condition (final concentration = 100 μM) were taken under irradiation at 365 nm wavelength.

II. General Synthetic Procedure and Compound Characterization

General procedure for preparing the Seoul-Fluor (pre-SF45, SF46, SF47, SF48 and SF52) is same as the procedure described in the previous report.[1] In this study, SF45, SF49, SF50 and SF51 compounds were synthesized from pre-SF45 (methyl ester form of SF45), SF03, SF11 and SF15, using saponification reaction with following method.

a. General synthetic procedure for saponification reaction of pre-SF45 (R: CF_3), SF03 (R: CN), SF11 (R: Me) and SF15 (R: OMe).

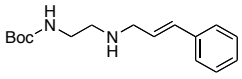


Reagents and conditions: a) LiOH (0.5 equiv.) / MeOH:H_2O (v:v = 3:1)

A solution of methyl ester form of Seoul-Fluor derivatives in 10 % LiOH solution (w/v) in MeOH:H_2O (v:v = 3:1) was stirred at room temperature. After completion of the reaction, reaction mixture was dilued with ammonium chloride (sat) and extracted with ethyl acetate for 3 times. The combined organic layer was dried over $MgSO_4$(s), and the filtrate was concentrated *in vacuo*. The desired compound was purified by silica-gel flash column chromatography.

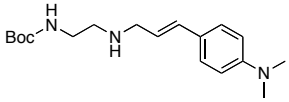
b. Compound characterization

(E)-*tert*-Butyl 2-(cinnamylamino)ethylcarbamate



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, *J* = 7.5 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.24–7.19 (m, 1H), 6.52 (d, *J* = 15.5 Hz, 1H), 6.26 (dt, *J* = 16.0, 6.3 Hz, 1H), 5.03 (br s, 1H), 3.41 (dd, *J* = 6.5, 1.5 Hz, 2H), 3.29–3.19 (m, 2H), 2.78 (t, *J* = 6.0 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.2, 137.0, 131.4, 128.6, 128.3, 127.4, 126.3, 79.1, 51.4, 48.6, 40.4, 28.5; LRMS (EI): *m*/*z* calcd for $C_{16}H_{25}N_2O_2$ $[M+H]^+$ 277.18, found 277.27.

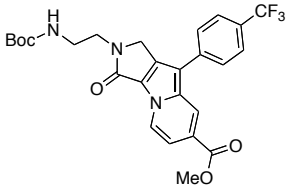
(E)-*tert*-Butyl 2-(3-(4-(dimethylamino)phenyl)allylamino)ethylcarbamate



^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, *J* = 9.0 Hz, 2H), 6.67 (d, *J* = 9.0 Hz, 2H), 6.44 (d, *J* = 16.0 Hz, 1H), 6.06 (dt, *J* = 16.0, 6.5 Hz, 1H), 5.06 (br s, 1H), 3.39 (d, *J* = 5.5 Hz, 2H), 3.28–3.24 (m, 2H), 2.95 (s, 6H), 2.79 (t, *J* = 5.8 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.3, 150.3, 133.7, 127.5, 125.0,

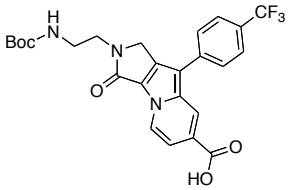
120.9, 112.5, 79.5, 51.4, 48.1, 40.6, 39.7, 28.5; LRMS (EI): *m*/*z* calcd for $C_{18}H_{30}N_3O_2$ $[M+H]^+$ 320.23, found 320.27

Methyl 2-(2-(*tert*-butoxycarbonylamino)ethyl)-3-oxo-9-(4-(trifluoromethyl)phenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylate (pre-SF45)



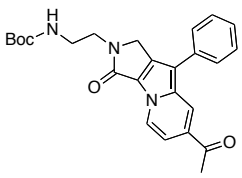
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (d, *J* = 7.0 Hz, 1H), 8.36 (s, 1H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 7.5 Hz, 2H), 7.19 (d, *J* = 6.5 Hz, 1H), 5.47 (t, *J* = 5.5 Hz, 1H), 4.52 (s, 2H), 3.92 (s, 3H), 3.72 (t, *J* = 6.0 Hz, 2H), 3.52–3.39 (m, 2H), 1.35 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.2, 161.3, 156.2, 136.8, 135.1, 134.4, 128.1 (q, $J_{C,F}$ = 33.4 Hz), 126.9, 126.0, 124.1, 124.1 (q, $J_{C,F}$ = 270.8 Hz), 122.6, 122.4, 121.0, 111.0, 110.7, 79.2, 52.4, 47.0, 42.9, 39.5, 28.2; LRMS (EI): *m*/*z* calcd for $C_{26}H_{27}F_3N_3O_5$ $[M+H]^+$ 518.18, found 518.28.

2-(2-(*tert*-Butoxycarbonylamino)ethyl)-3-oxo-9-(4-(trifluoromethyl)phenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF45)



^{1}H NMR (500 MHz, DMSO) δ 8.61 (d, *J* = 7.5 Hz, 1H), 8.47 (s, 1H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.85 (d, *J* = 8.5 Hz, 2H), 7.34 (dd, *J* = 7.0, 1.5 Hz, 1H), 6.96 (t, *J* = 6.0 Hz, 1H), 4.73 (s, 2H), 3.56 (t, *J* = 6.0 Hz, 2H), 3.24–3.20 (m, 2H), 1.28 (s, 9H); ^{13}C NMR (125 MHz, DMSO) δ 166.0, 160.3, 155.7, 137.5, 135.8, 134.1, 127.3, 126.5 (q, $J_{C,F}$ = 31.5 Hz), 126.1, 124.4 (q, $J_{C,F}$ = 270.4 Hz), 124.3, 123.7, 122.5, 120.4, 111.3, 110.0, 77.7, 46.3, 42.4, 38.6, 28.1; LRMS (EI): *m*/*z* calcd for $C_{25}H_{24}F_3N_3O_5$ $[M+H]^+$ 504.17, found 504.30.

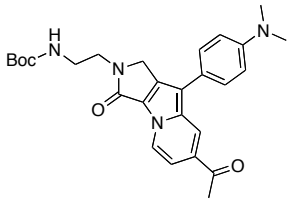
***tert*-Butyl 2-(7-acetyl-3-oxo-9-phenyl-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-yl)ethylcarbamate (SF46)**



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, *J* = 7.5, 1.0 Hz, 1H), 8.40 (s, 1H), 7.49–7.58 (m, 4H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.28 (dd, *J* = 7.5, 1.8 Hz, 1H), 4.99 (br. s, 1H), 4.59 (s, 2H), 3.74 (t, *J* = 6.0 Hz, 2H), 3.45 (q, *J* = 6.0 Hz, 2H), 2.61 (s, 3H), 1.34 (s, 9H); ^{13}C NMR (125 MHz,

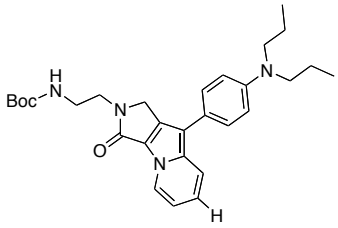
$CDCl_3$) δ 195.5, 162.0, 156.3, 149.4, 134.3, 133.8, 128.3, 124.1, 122.4, 121.8, 121.3, 114.6, 113.1, 108.9, 79.4, 47.3, 43.1, 40.6, 39.8, 28.4, 25.9; LRMS (EI): *m*/*z* calcd for $C_{25}H_{28}N_3O_4$ $[M+H]^+$ 434.20, found 434.29.

tert-Butyl 2-(7-acetyl-9-(4-(dimethylamino)phenyl)-3-oxo-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-yl)ethylcarbamate (SF47)



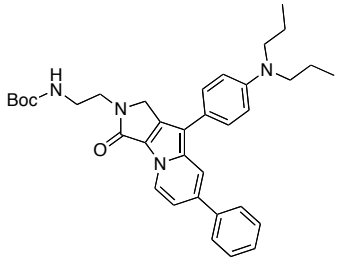
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.48 (d, *J* = 7.0 Hz, 1H), 8.35 (s, 1H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.21 (dd, 7.3, 1.8 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 5.06 (br s, 1H), 4.54 (s, 2H), 3.73 (t, *J* = 6.0 Hz, 2H), 3.46–3.43 (m, 2H), 3.04 (s, 6H), 2.59 (s, 3H), 1.35 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 195.5, 162.0, 156.3, 149.4, 134.3, 133.8, 128.3, 128.2, 124.1, 122.4, 121.8, 121.3, 114.6, 113.1, 108.9, 79.4, 47.3, 43.1, 40.6, 39.8, 28.4, 26.0; LRMS (EI): *m*/*z* calcd for $C_{27}H_{33}N_4O_4$ $[M+H]^+$ 477.24, found 477.33.

tert-Butyl 2-(9-(4-(dipropylamino)phenyl)-3-oxo-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-yl)ethylcarbamate (SF48)



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, *J* = 7.1 Hz, 1H), 7.76 (d, *J* = 9.3 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 2H), 6.90 (ddd, *J* = 9.3, 6.6, 1.0 Hz, 1H), 6.73 (d, *J* = 9.1 Hz, 2H), 6.67 (td, *J* = 6.7, 1.2 Hz, 1H), 5.05 (bs, 1H), 4.51 (s, 2H), 3.71 (t, *J* = 5.9 Hz, 2H), 3.43 (q, *J* = 5.7 Hz, 2H), 3.23–3.32 (m, 4H), 1.66 (sextet, *J* =7.5 Hz, 4H), 1.36 (s, 9H), 0.96 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.7, 156.2, 146.5, 135.7, 134.3, 128.1, 125.1, 121.1, 120.3, 119.5, 118.7, 112.1, 111.3, 109.5, 79.3, 53.0, 47.2, 42.8, 40.0, 28.4, 20.5, 11.5; LRMS (EI): *m*/*z* calcd for $C_{29}H_{39}N_4O_3$ $[M+H]^+$ 491.29, found; 491.41.

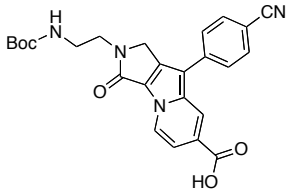
tert-Butyl 2-(9-(4-(dipropylamino)phenyl)-3-oxo-7-phenyl-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-yl)ethylcarbamate (SF49)



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (d, *J* = 8.0 Hz, 1H), 7.96 (s, 1H), 7.64 (d, *J* = 7.0 Hz, 2H), 7.45 (t, *J* = 7.8 Hz, 2H), 7.41 (d, *J* = 9.0 Hz, 2H), 7.36 (t, *J* = 7.5 Hz, 1H), 6.99 (dd, *J* = 7.3,

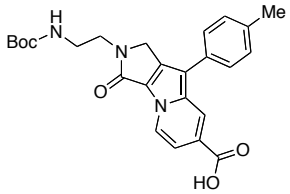
1.8 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 2H), 5.03 (br s, 1H), 4.53 (s, 2H), 3.72 (t, *J* = 5.8 Hz, 2H), 3.46–3.42 (m, 2H), 3.25 (t, *J* = 7.8 Hz, 4H), 1.67 (sextet, *J* = 7.5 Hz, 4H), 1.37 (s, 9H), 0.97 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.8, 156.3, 146.7, 139.4, 136.2, 135.1, 133.1, 129.0, 128.4, 127.8, 126.6, 125.2, 121.1, 119.5, 115.8, 112.2, 111.2, 110.4, 79.5, 53.1, 47.3, 42.9, 40.1, 28.4, 20.6, 11.6; LRMS (EI): *m*/*z* calcd for $C_{35}H_{43}N_4O_3$ $[M+H]^+$ 567.33, found 567.56.

2-(2-(*tert*-Butoxycarbonylamino)ethyl)-9-(4-cyanophenyl)-3-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF50)



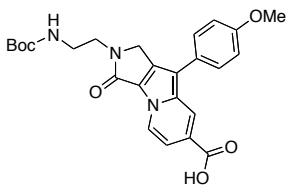
^{1}H NMR (500 MHz, DMSO) δ 8.53 (d, *J* = 6.5 Hz, 1H), 8.43 (s, 1H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.40 (d, *J* = 7.0 Hz, 1H), 6.97 (t, *J* = 5.8 Hz, 1H), 4.70 (br s, 2H), 3.55 (t, *J* = 5.8 Hz, 2H), 3.27–3.18 (m, 2H), 1.28 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3, 160.0, 155.1, 138.3, 135.5, 134.8, 132.6, 129.6, 126.4, 123.4, 121.2, 118.6, 118.5, 112.5, 107.7, 107.1, 77.2, 45.9, 41.8, 28.5, 27.6; LRMS (EI): *m*/*z* calcd for $C_{25}H_{25}N_4O_5$ $[M+H]^+$ 461.17, found 461.27.

2-(2-*(tert*-Butoxycarbonylamino)ethyl)-3-oxo-9-*p*-tolyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF51)



^{1}H NMR (500 MHz, DMSO) δ 8.52 (d, *J* = 7.0 Hz, 1H), 8.40 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 7.0 Hz, 1H), 6.96 (t, *J* = 5.8 Hz, 1H), 4.66 (s, 2H), 3.55 (t, *J* = 5.8 Hz, 2H), 3.24–3.18 (m, 2H), 2.36 (s, 3H), 1.29 (s, 9H); ^{13}C NMR (125 MHz, DMSO) δ 166.5, 160.6, 155.7, 135.8, 135.2, 133.7, 130.5, 129.8, 126.9, 124.0, 122.0, 120.8, 111.7, 111.1, 77.7, 69.8, 46.2, 42.3, 38.7, 28.1, 20.8; LRMS (EI): *m*/*z* calcd for $C_{25}H_{28}N_3O_5$ $[M+H]^+$ 450.20, found 450.29.

2-(2-*(tert*-Butoxycarbonylamino)ethyl)-9-(4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF52)



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.49 (d, *J* = 7.5 Hz, 2H), 7.55–7.42 (m, 4H), 7.33 (t, *J* = 7.0 Hz, 1H), 7.24 (dd, *J* = 7.0, 2.0 Hz, 1H),

5.19 (br s, 1H), 4.55 (s, 2H), 3.93 (s, 3H), 3.72 (t, J = 5.8 Hz, 2H), 3.48-3.39 (m, 2H), 1.34 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.9, 162.1, 156.3, 135.2, 134.8, 133.5, 129.3, 127.5, 126.8, 124.4, 122.4, 122.2, 122.1, 113.3, 110.8, 79.5, 52.5, 47.2, 43.0, 39.8, 28.4; LRMS (EI): m/z calcd for $C_{25}H_{28}N_3O_6$ $[M+H]^+$ 466.19, 466.27.

II. Supporting Figures.

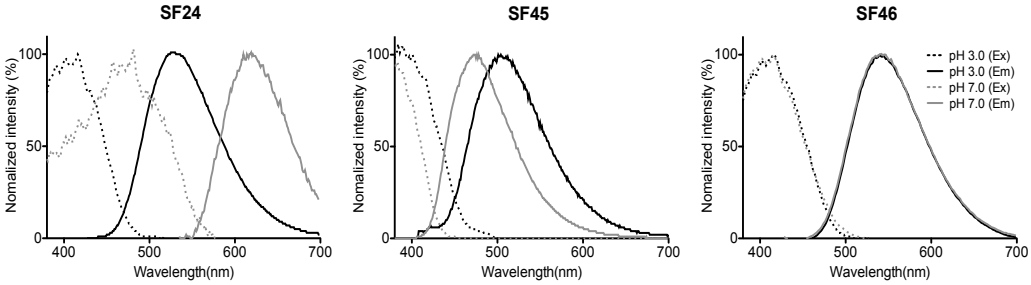


Figure S1. The pH depenent spectral changes in normalized excitation (dashed) and emission (solid) spectra of SF24, SF45, and SF46. Each spectrum was measured in pH 3.0 (black) and 7.0 (gray) buffer solution.

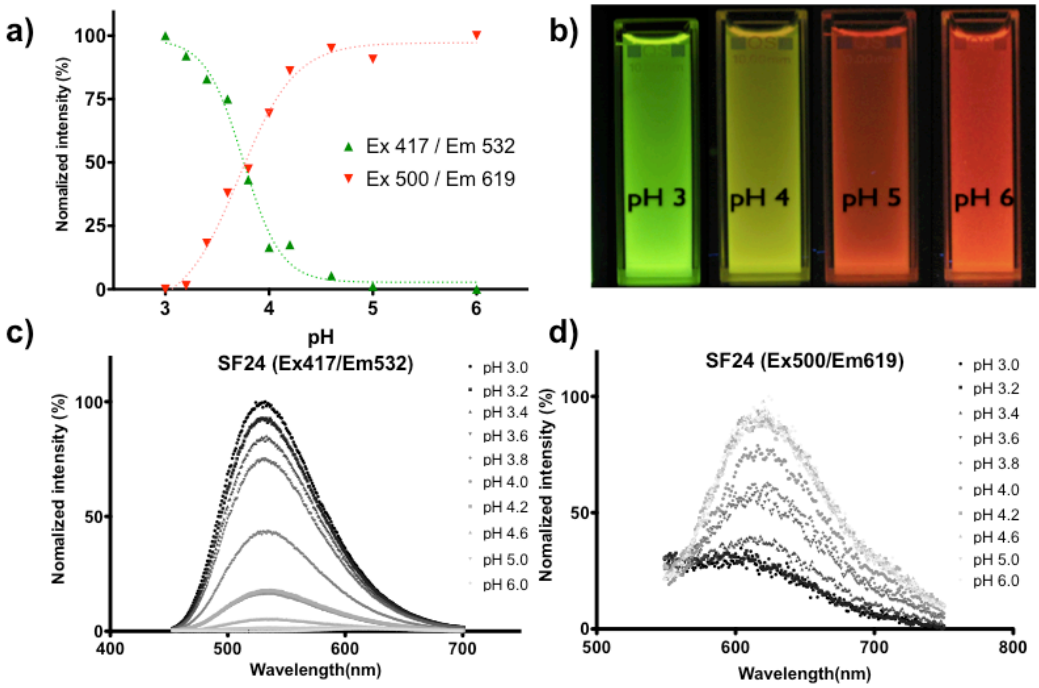


Figure S2. Scatter plots of the normalized emission intensity at two different maximum wavelength of SF24 for gradual changes in pH from 3.0 to 6.0 (a). Normalized emission spectra of SF24 for gradual changes in the pH were measured with excitation/emission wavelength at 417/532 nm (c) and at 500/619 nm (d). Each photography images of SF24 (b) were taken under irradiation at 365 nm in pH 3.0, 4.0, 5.0 and 6.0 buffer solutions.

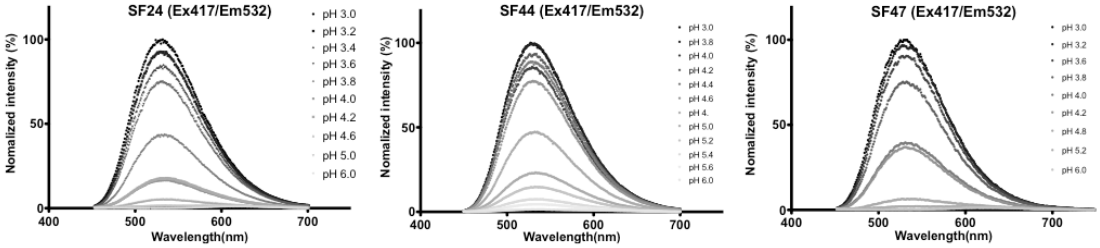


Figure S3. Normalized emission spectra of SF24, SF44, and SF47 for gradual changes in the pH changes from 3.0 to 7.0 with the excitation/emission wavelength at 417/532 nm.

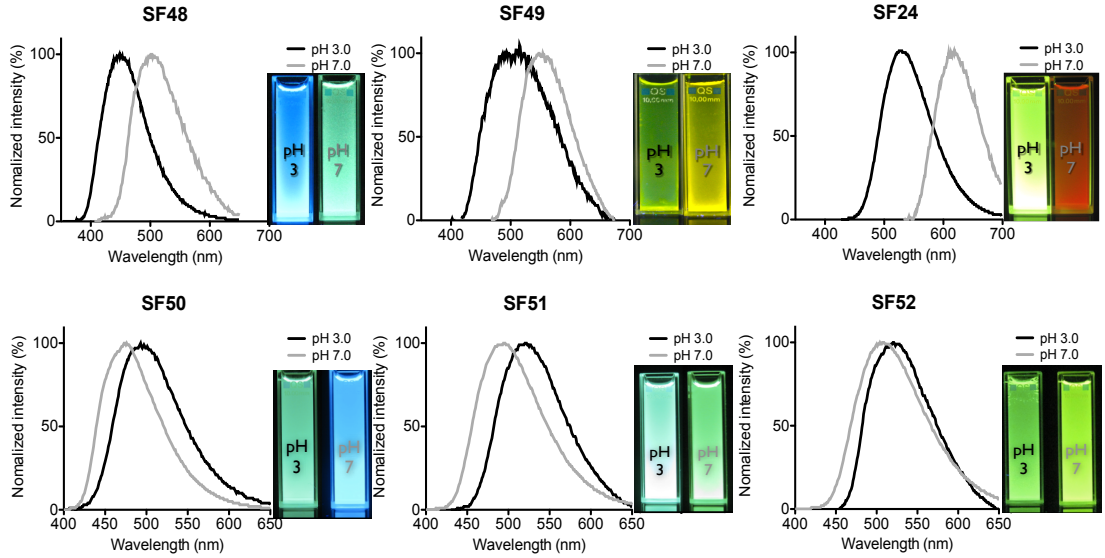
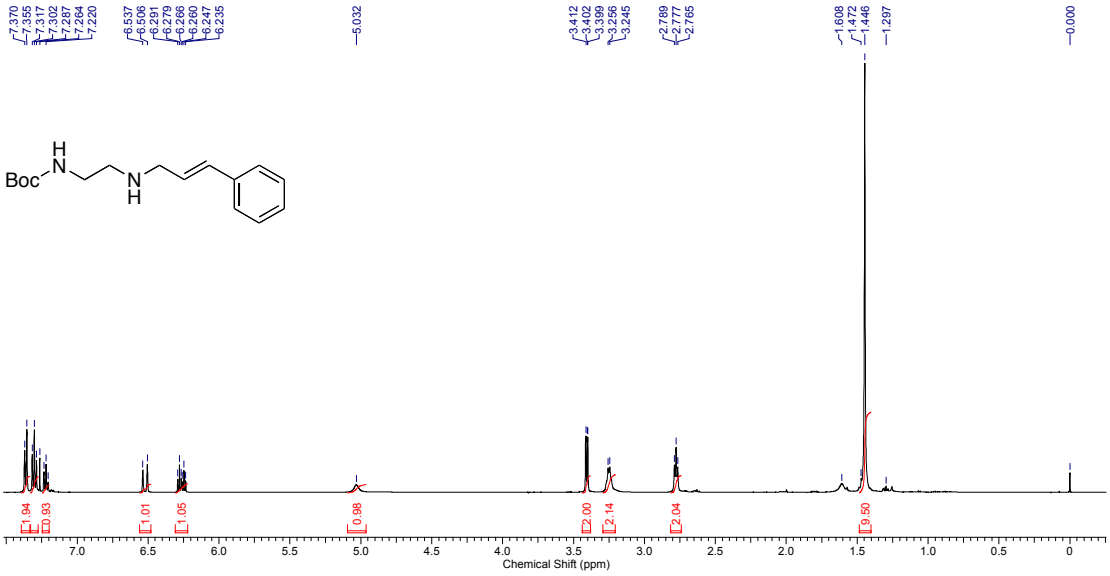
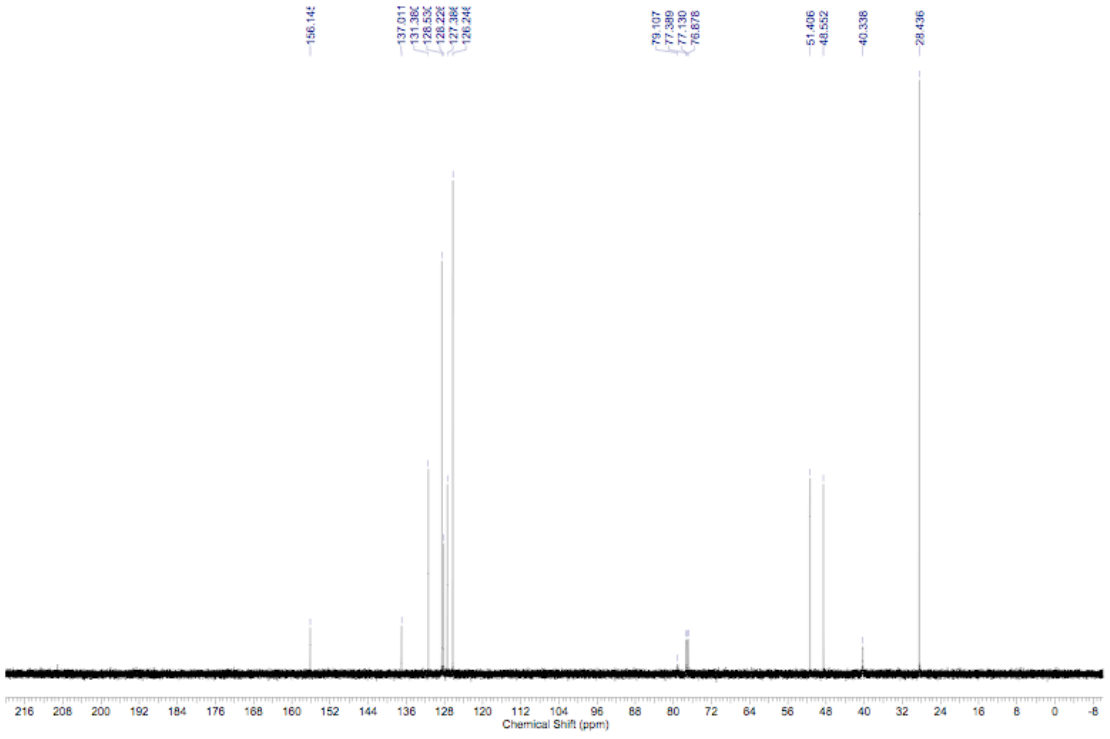


Figure S4. Spectral changes in normalized emission spectrum of SF24, and SF47-SF51 compounds for a change in the pH from 3.0 (black) to 7.0 (gray). The photography images of each compound in the buffer solution were taken under irradiation at 365 nm.

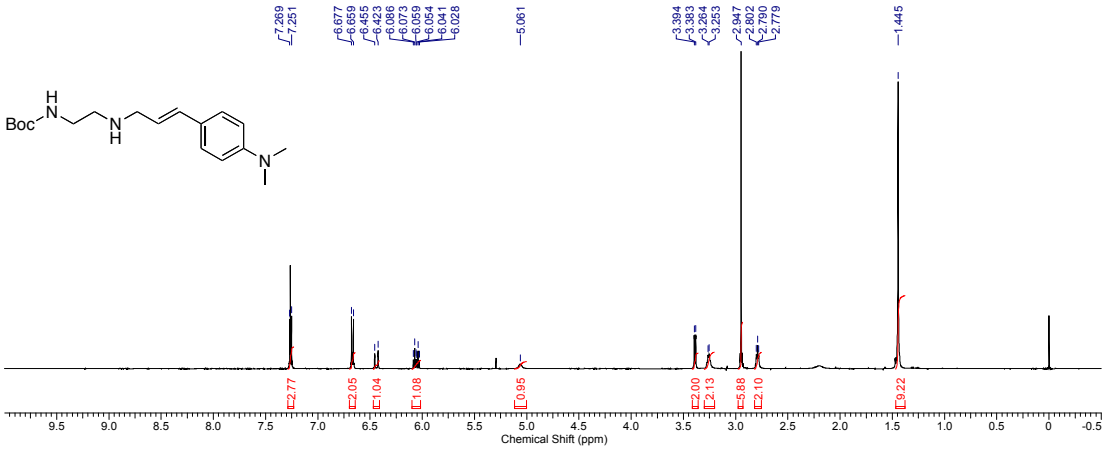
IV. Copies of ^{1}H and ^{13}C NMR Spectra of all New Compounds

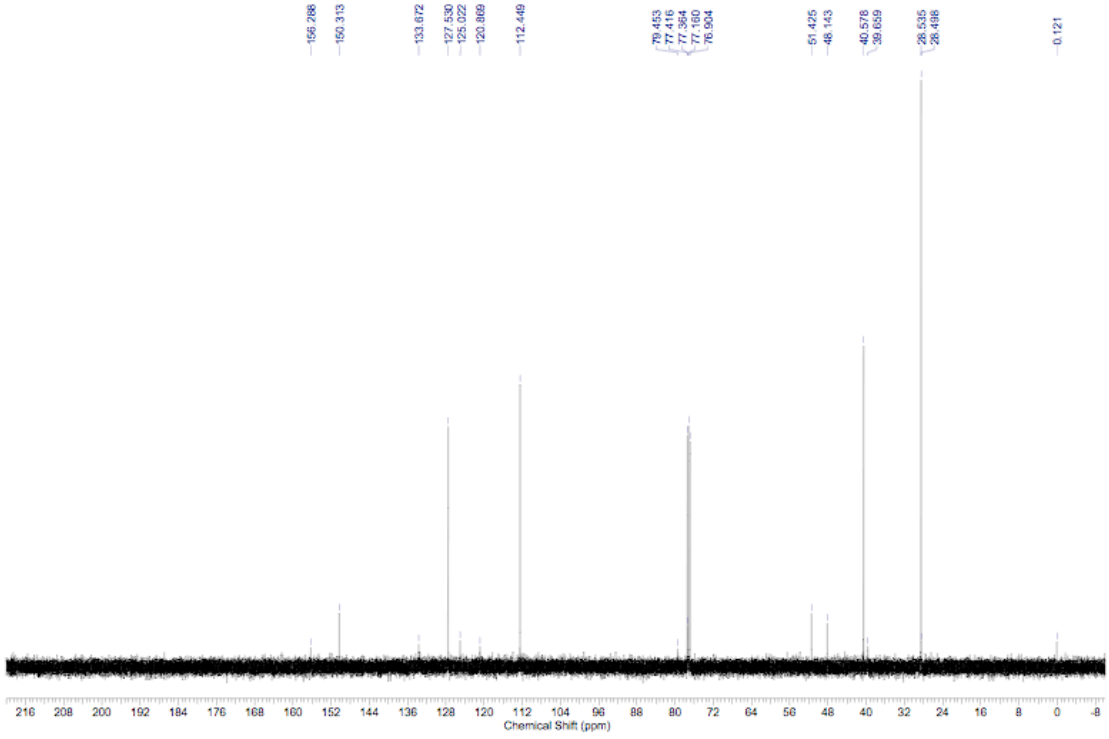
(E)-*tert*-Butyl 2-(cinnamylamino)ethylcarbamate



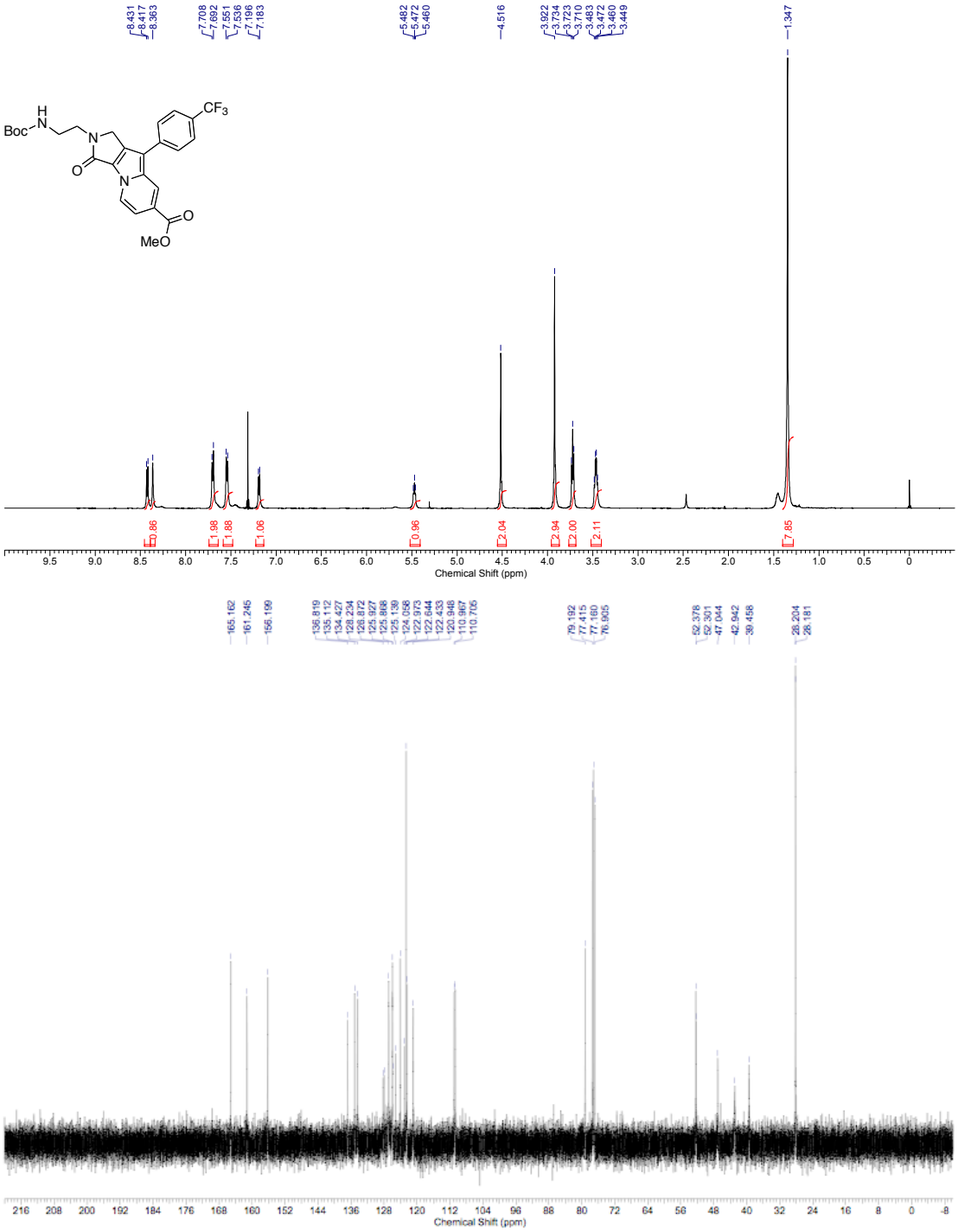


(E)-*tert*-Butyl 2-(3-(4-(dimethylamino)phenyl)allylamino)ethylcarbamate

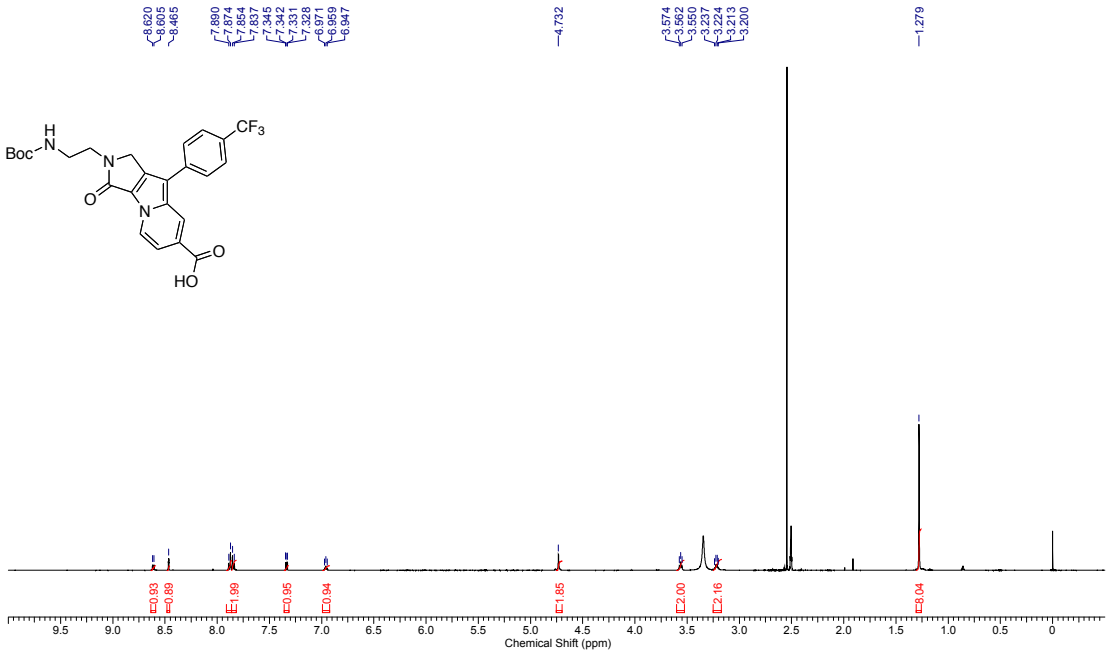


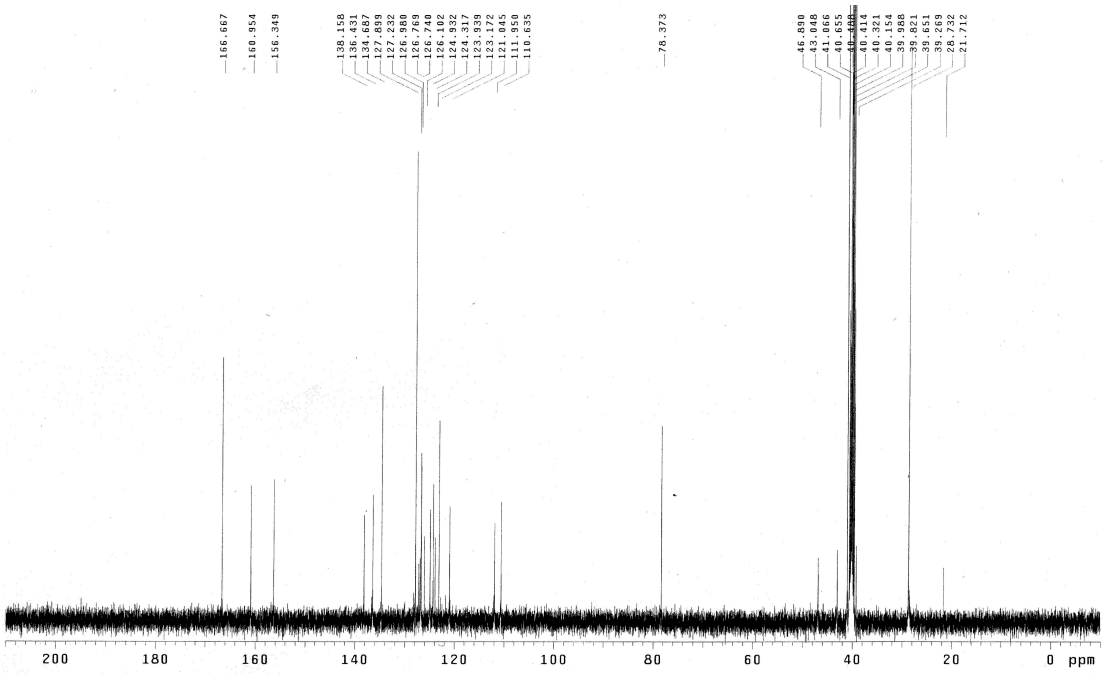


Methyl 2-(2-(*tert*-butoxycarbonylamino)ethyl)-3-oxo-9-(4-(trifluoromethyl)phenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylate (pre-SF45)

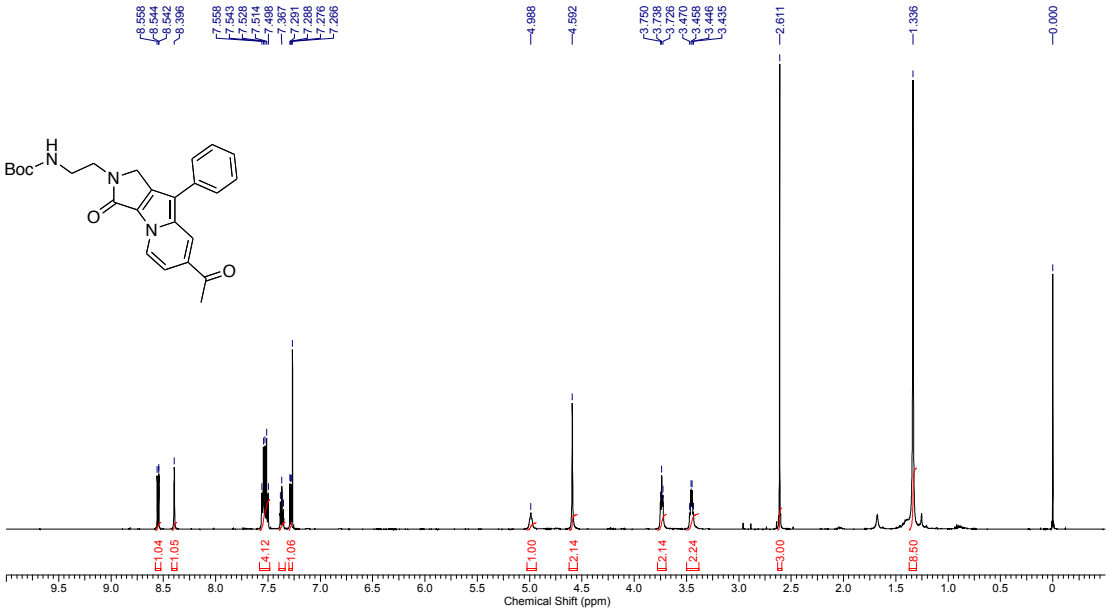


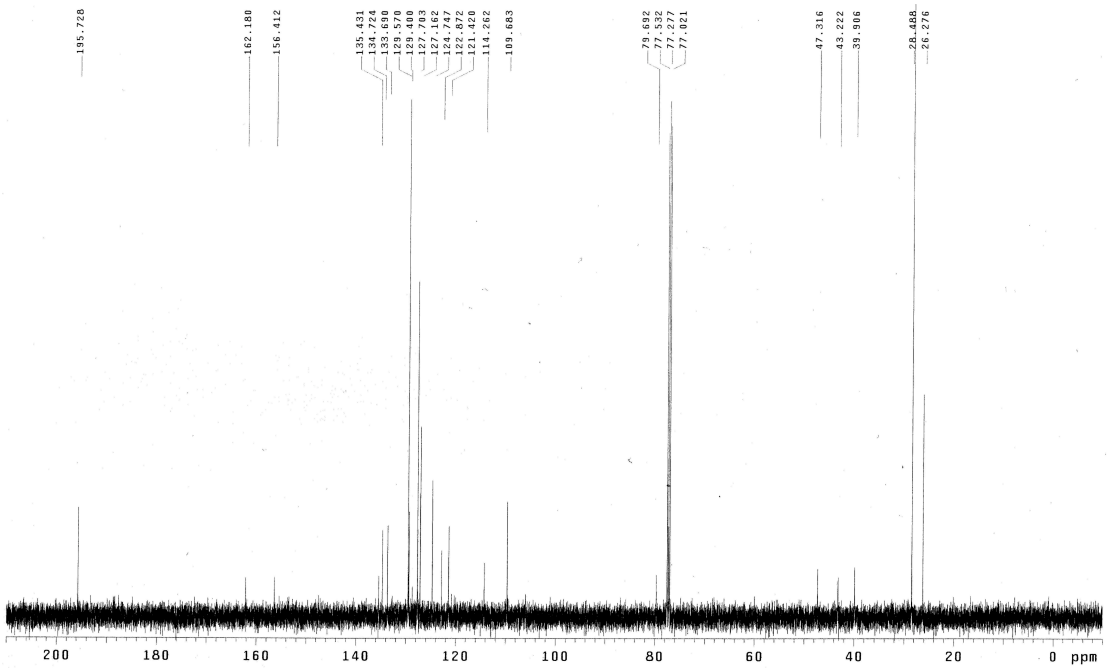
2-(2-(*tert*-Butoxycarbonylamino)ethyl)-3-oxo-9-(4-(trifluoromethyl)phenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF45)



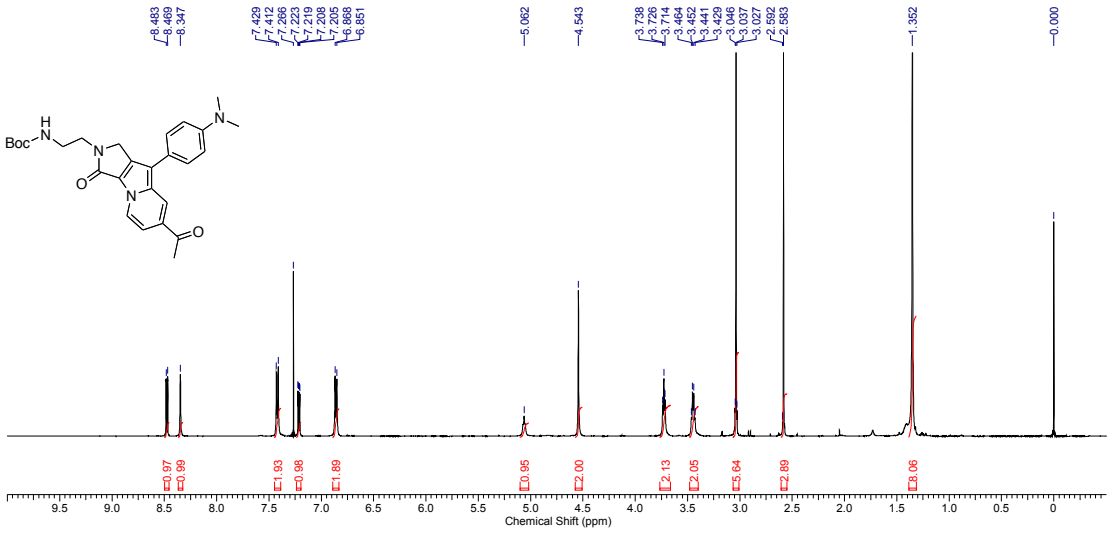


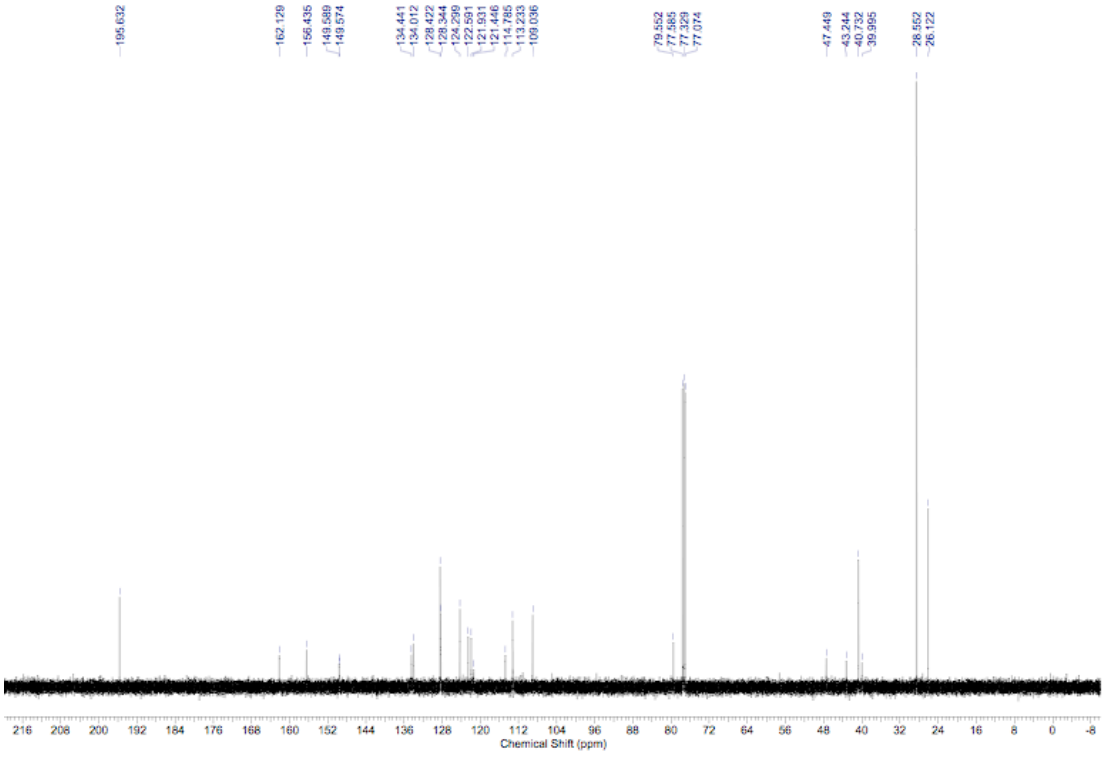
tert-Butyl 2-(7-acetyl-3-oxo-9-phenyl-1_H_-pyrrolo[3,4-_b_]indolizin-2(3_H_)-yl)ethylcarbamate (SF46)



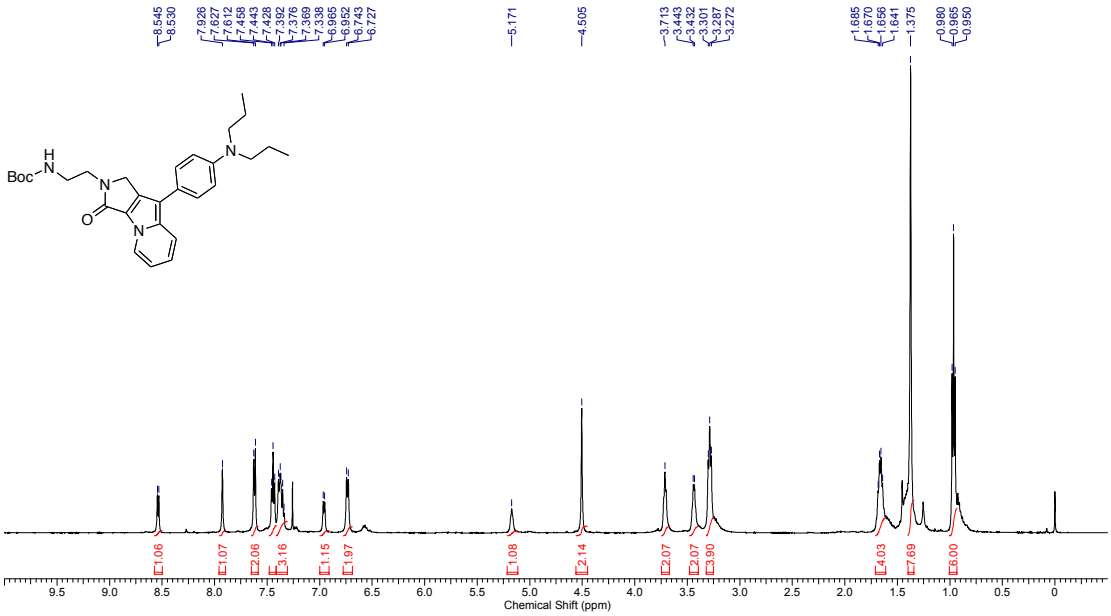


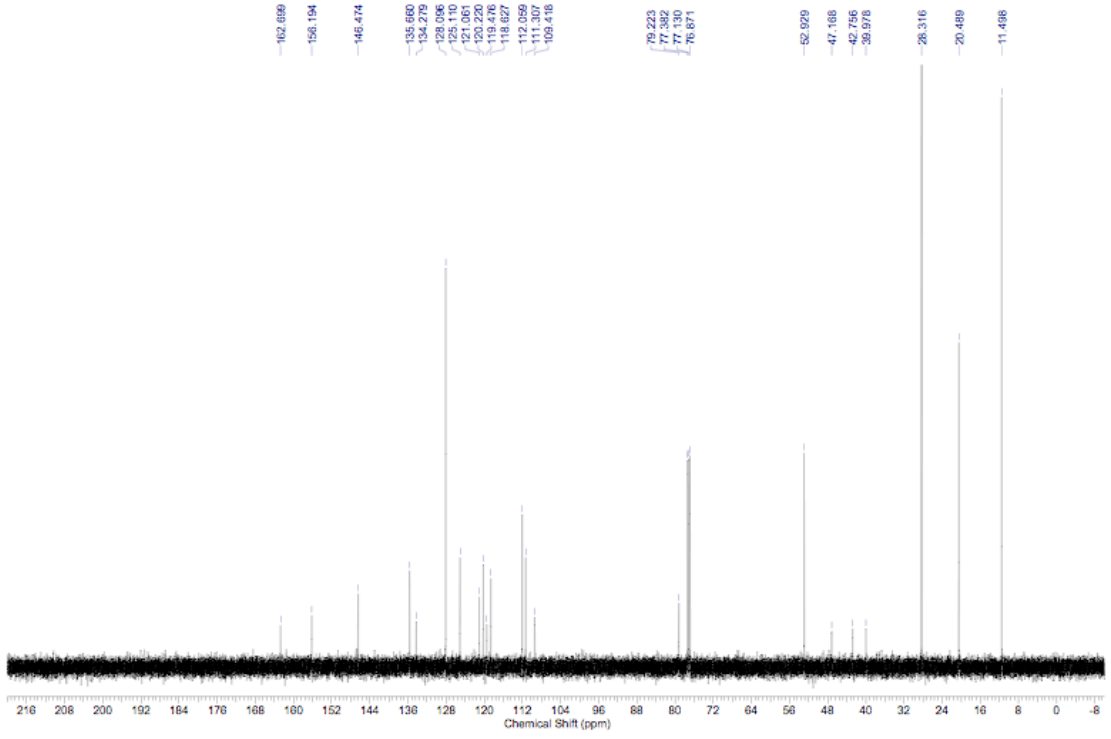
tert-Butyl 2-(7-acetyl-9-(4-(dimethylamino)phenyl)-3-oxo-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-yl)ethylcarbamate (SF47)



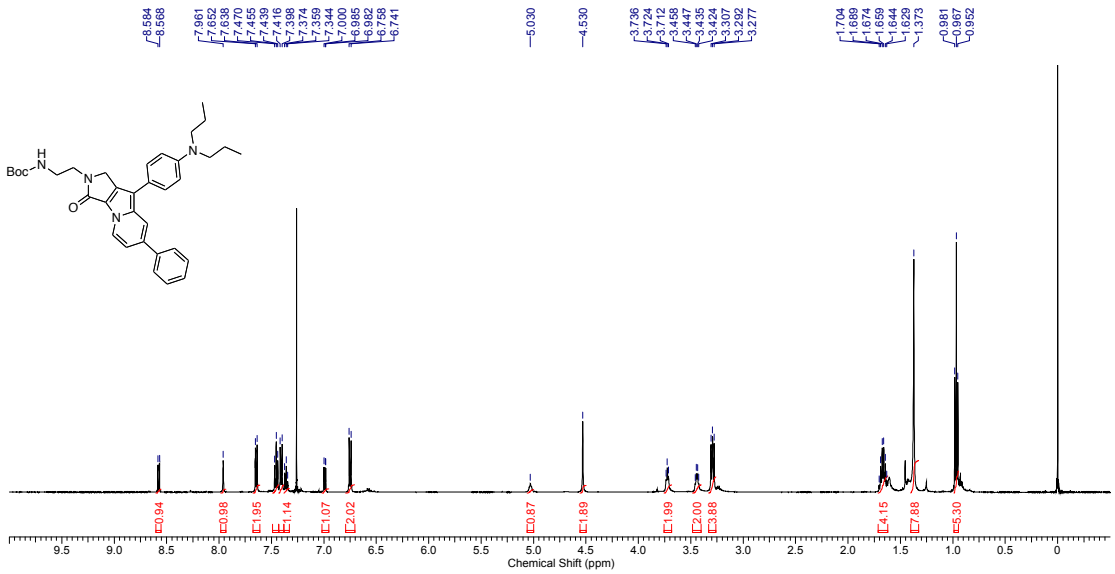


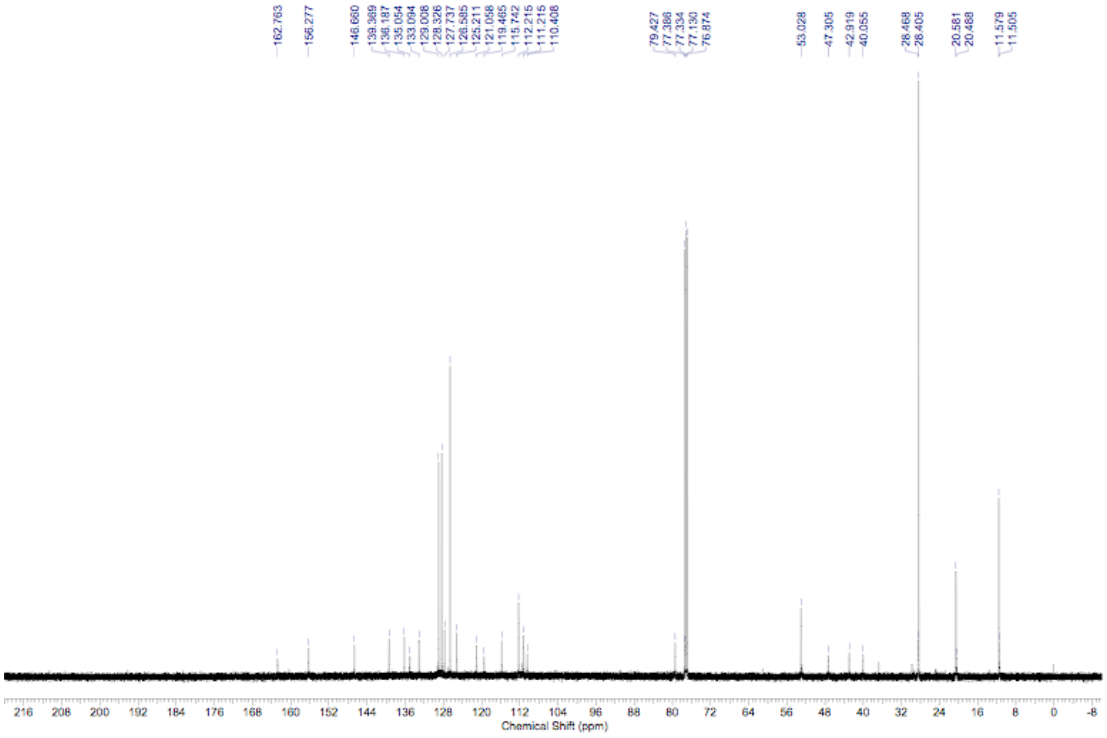
tert-Butyl 2-(9-(4-(dipropylamino)phenyl)-3-oxo-1*H*-pyrrolo[3,4-*b*]indolizin-2(3*H*)-yl)ethylcarbamate (SF48)



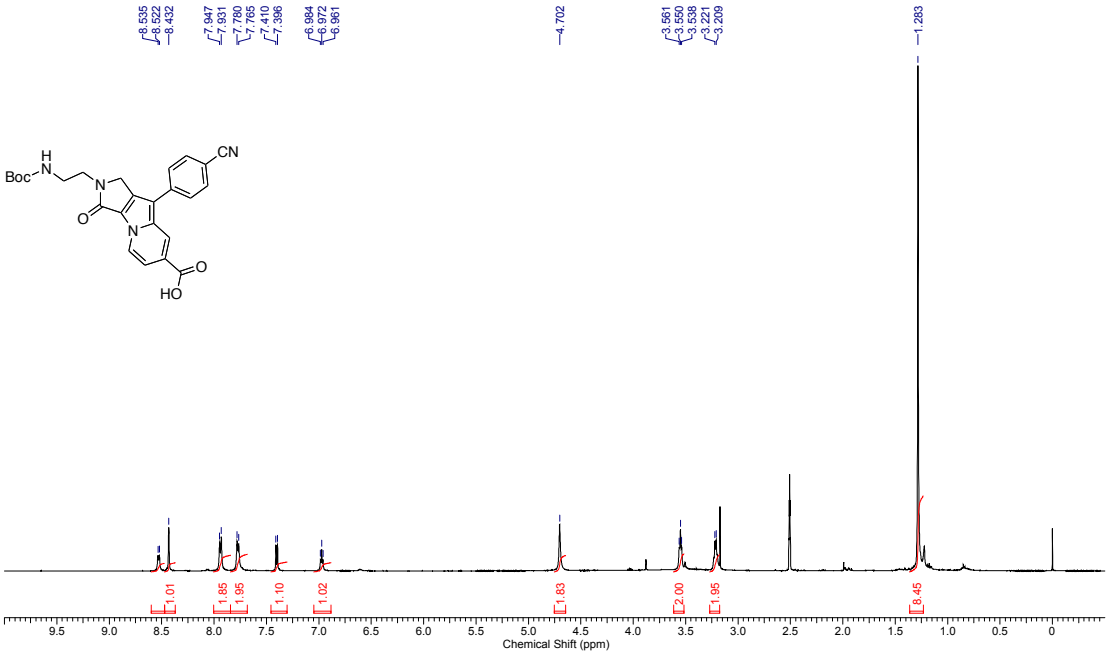


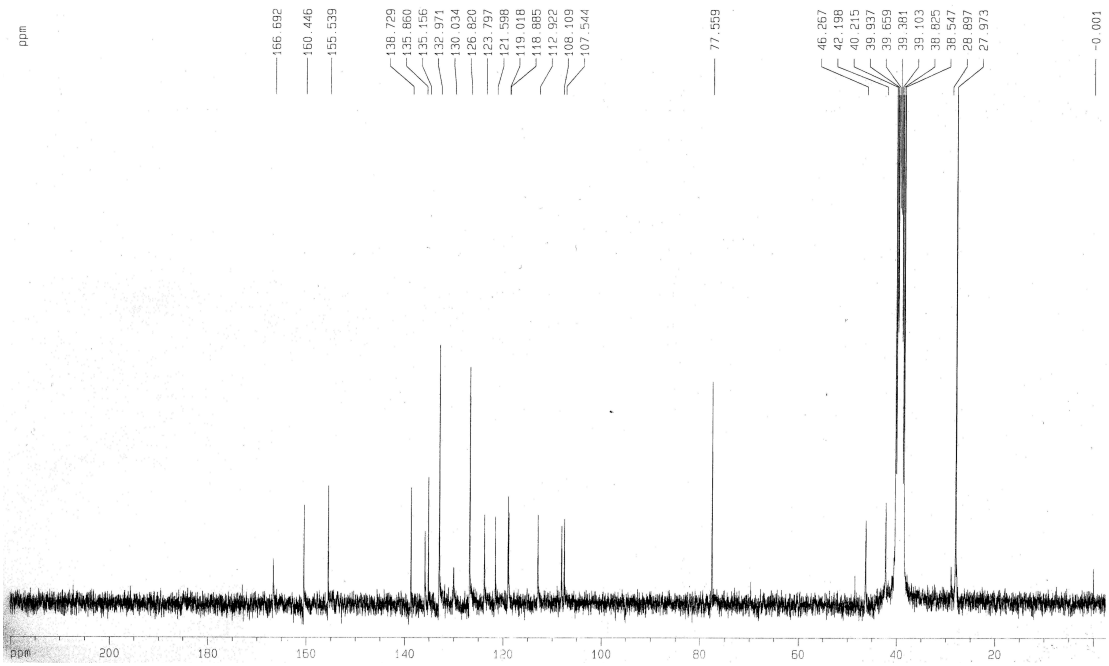
tert-Butyl 2-(9-(4-(dipropylamino)phenyl)-3-oxo-7-phenyl-1_H_-pyrrolo[3,4-_b_]indolizin-2(3_H_)-yl)ethylcarbamate (SF49)



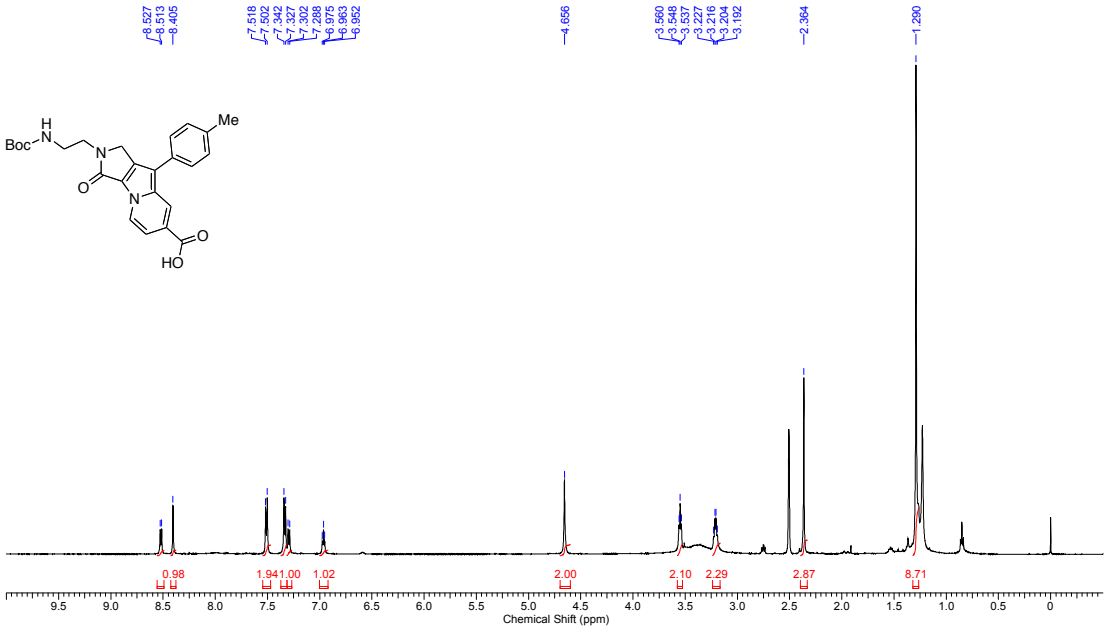


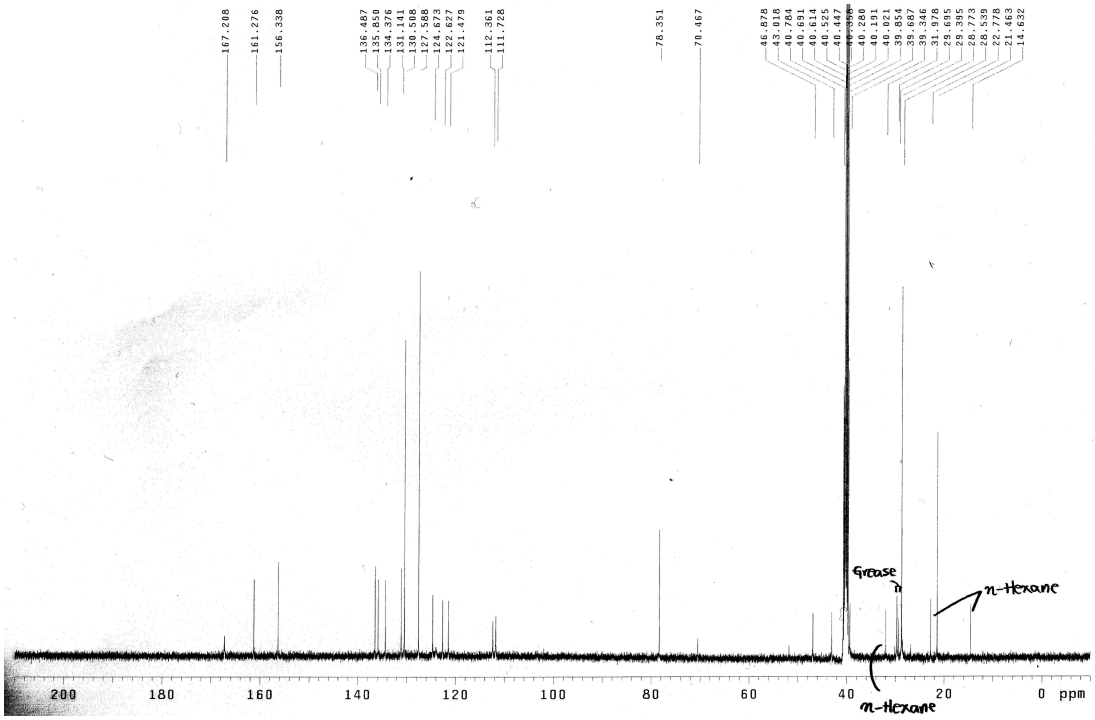
2-(2-(*tert*-Butoxycarbonylamino)ethyl)-9-(4-cyanophenyl)-3-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF50)





2-(2-*(tert*-Butoxycarbonylamino)ethyl)-3-oxo-9-*p*-tolyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF51)





2-(2-*(tert*-Butoxycarbonylamino)ethyl)-9-(4-methoxyphenyl)-3-oxo-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]indolizine-7-carboxylic acid (SF52)