CHEMMEDCHEM
Chemistry & Drug Discovery

Supporting Information

Rational Design of Amyloid Binding Agents Based on the Molecular Rotor Motif

Jeyanthy Sutharsan,[a] Marianna Dakanali,[a] Christina C. Capule,[a] Mark A. Haidekker,[b] Jerry Yang,*[a] and Emmanuel A. Theodorakis*[a]

cmdc_200900440_sm_miscellaneous_information.pdf

Supporting Information

Experimental part

General notes: All the reagents were obtained (Aldrich, Acros) at highest commercial quality and used without further purification except where noted. Air- and moisture-sensitive liquids and solutions were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation below 45 °C at approximately 20 mmHg. All non-aqueous reactions were carried out under anhydrous conditions. Yields refer to chromatographically and spectroscopically (^{1}H NMR, ^{13}C NMR) homogeneous materials, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) and visualized under UV light and/or developed by dipping in solutions of 10% ethanolic phosphomolybdic acid (PMA) or p-anisaldehyde and applying heat. E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash chromatography. Preparative thin-layer chromatography separations were carried out on 0.25 or 0.50 mm E. Merck silica gel plates (60F-254). NMR spectra were recorded on Varian Mercury 300 or 400 MHz instruments and calibrated using the residual non-deuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. High resolution mass spectra (HRMS) were recorded on a VG 7070 HS mass spectrometer under electron spray ionization (ESI) or electron impact (EI) conditions. Fluorescence spectroscopy data were recorded on a MD-5020 Photon Technology International Spectrophotometer at 25 °C.

General procedure for the preparation of fluorescence probes.

To a round bottom flask containing a solution of aldehyde (5.0 mmol) and 2-(2-(2-methoxyethoxy) ethoxy)ethyl 2-cyanoacetate (5.5 mmol) in 20 ml of THF was added 0.50 mmol of piperidine and the mixture was heated at 50 °C. The reaction was monitored by TLC and was completed within 21 hours. The crude mixture was concentrated under reduced pressure and the product was purified via flash chromatography (10-30% ethyl acetate in hexane).

(E)-2-(2-(2-methoxyethoxy)ethoxy)ethyl 2-cyano-3-(4- (dimethylamino)phenyl)acrylate (8a). 98% ; yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.93 (d, 2H, *J*= 9.0 Hz), 6.69 (d, 2H, *J*= 9.1 Hz), 4.41 (m, 2H), 3.81- 3.79 (m, 2H), 3.73-3.65 (m, 6H), 3.56 -3.54 (m, 2H), 3.37 (s, 3H), 3.10 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 154.7, 153.6, 134.1, 119.3, 117.4, 111.4, 93.6, 71.9, 70.8, 70.6, 70.5, 68.9, 65.0, 59.0, 40.0; HRMS Calc for $C_{19}H_{26}N_2O_5$ $(M)^+$ 362.1836 found 362.1841.

(E)-2-(2-(2-methoxyethoxy)ethoxy)ethyl2-cyano-3-(4-(dimethylamino)-2-methoxyphenyl)acrylate (8b). 98% yield ; yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s,1H), 8.39 (d, 1H, *J*= 9.2 Hz), 6.63 (dd, 1H, *J*= 2.3 Hz, *J*= 9.2 Hz), 6.01 (s, 1H), 4.40 (m, 2H), 3.87 (s, 3H), 3.81- 3.78 (m, 2H), 3.73- 3.65 (m, 6H), 3.56- 3.53 (m, 2H), 3.36 (s, 3H), 3.10 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.0, 162.2, 155.9, 148.5, 131.3, 118.4, 109.7, 105.4, 93.0, 92.0, 72.2, 71.1, 70.9, 70.8, 69.2, 65.1, 59.3, 55.6, 40.4; HRMS Calc for $C_{20}H_{28}N_2O_6$ $(M+Na)^+$ 415.1840 found 415.1836.

(Z)-2-(2-(2-methoxyethoxy)ethoxy)ethyl 2-cyano-3-(4-(diethylamino)phenyl)acrylate (8c). 90% yield; orange liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.92 (d, 2H, *J*= 9.1 Hz), 6.67 (d, 2H, *J*= 9.2 Hz), 4.42 (m, 2H), 3.82- 3.79 (m, 2H), 3.73-3.72 (m, 2H), 3.69-3.65 (m, 4H), 3.57-3.54 (m, 2H), 3.45 (q, 4H, *J*= 7.1 Hz), 3.37 (s, 3H), 1.23 (t, 6H, *J*= 7.1 Hz); ^{13}CNMR (100 MHz, $CDCl_3$) δ 164.7, 154.8, 151.9, 134.8, 119.0, 117.8, 111.4, 93.0, 72.2, 71.1, 70.9, 70.8, 69.2, 65.2, 59.3, 45.0, 12.8; HRMS Calc for $C_{21}H_{30}N_2O_5$ $(M+Na)^+$ 413.2047 found 413.2053.

(Z)-2-(2-(2-methoxyethoxy)ethoxy)ethyl-2-cyano-3-(4-(dibutylamino)phenyl)acrylate (8d). 78% yield; yellow liquid; ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.87 (d, 2H, *J*= 9.0 Hz), 6.60 (d, 2H, *J*= 9.2 Hz), 4.38 (m, 2H), 3.78- 3.76 (m, 2H), 3.71-3.69 (m, 2H), 3.66-3.62 (m, 4H), 3.53-3.51 (m, 2H), 3.34- 3.30 (m, 7H), 1.57 (m, 4H), 1.34 (m, 4H), 0.94 (t, 6H, *J*= 7.3 Hz); ^{13}CNMR

(100 MHz, $CDCl_3$) δ 164.7, 154.7, 152.2, 134.6, 118.9, 117.9, 111.5, 92.8, 72.1, 71.0, 70.8, 69.1, 65.2, 59.2, 51.1, 29.5, 20.4, 14.1; HRMS Calc for $C_{25}H_{38}N_2O_5$ $(M+Na)^+$ 469.2673 found 469.2677.

6-(piperidin-1-yl)-2-naphthaldehyde (10). To a 50 ml round bottom flask containing benzene (3 mL), HMPA (3 mL) and piperidine (1.65 ml, 16.7 mmol) n-BuLi (1.6 M in hexane, 10.4 mL, 16.7 mmol) was added via syringe, at 0 °C. After stirring for 15 min, the reaction mixture was treated with a solution of 6-methoxy-2-naphthaldehyde (390 mg, 2.09 mmol) in benzene: HMPA 1:1 (2 ml). The reaction mixture was warmed to room temperature, left stirring for 12 hours and then it was poured into cold 5% aqueous NaCl (30 ml). The mixture was extracted with diethyl ether (3 x 20 mL), dried over $MgSO_4$ and concentrated. The product was purified via flash chromatography (20% EtOAc in hexanes) to give compound **9**. **9**: 35% yield, yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.02 (s, 1H), 8.14 (s, 1H), 7.88-7.73 (m, 2H), 7.67 (d, 1H, *J*= 8.6 Hz), 7.32 (dd, 1H, *J*= 2.5 Hz, *J*= 9.1 Hz), 7.08 (d, 1H, *J*= 2.4 Hz), 3.42-3.32 (m, 4H), 1.85-1.57 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.2, 152.2, 138.8, 134.7, 131.6, 130.7, 127.5, 126.5, 123.6, 119.7, 109.0, 49.8, 25.8, 24.6; HRMS calc for $C_{16}H_{17}NO$ $(M+H)^+$ 240.1383 found 240.1387.

(E)-2-(2-(2-methoxyethoxy)ethoxy)ethyl-2-cyano-3-(6-(piperidin-1-yl)naphthalen-2-yl)acrylate (11). 82% yield; red liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 8.22 (d, 1H, *J*= 1.2 Hz), 8.10 (dd, 1H, *J*= 1.8 Hz, *J*= 8.8 Hz), 7.76 (d, 1H, *J*= 9.2 Hz), 7.65 (d, 1H, *J*= 8.8 Hz), 7.29 (dd, 1H, *J*= 2.4 Hz, *J*= 9.2 Hz), 7.05 (d, 1H, *J*= 2.2 Hz), 4.47 (m, 2H), 3.85-3.82 (m, 2H), 3.74-3.66 (m, 6H), 3.57- 3.54 (m, 2H), 3.42-3.38 (m, 4H), 3.37 (s, 3H), 1.74- 1.67 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.4, 155.5, 151.9, 137.8, 134.7, 130.6, 127.3, 126.4, 126.0, 125.7, 119.3, 116.4, 108.4, 98.7, 71.9, 70.8, 70.6, 70.5, 68.8, 65.4, 59.0, 49.4, 25.5, 24.3; HRMS Calc for $C_{26}H_{32}N_2O_5$ $(M+H)^+$ 453.2384 found 453.2390.

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-cyanoacetate (12). To a solution of 2-cyanoacetic acid (1.02 g, 12 mmol), the acetal (2,2-dimethyl-1,3-dioxolan-4-yl)methanol (1.32 g, 10 mmol) in

5ml of DCM and DMAP (61 mg, 0.50 mmol) was added dropwise at 0 °C. Finally, EDC 1.86 g (12 mmol) was added and the reaction mixture was stirred at 0 °C for 6 hours. The reaction was diluted with 15mL of DCM and the formed DCU was filtered off. The filtrate was dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The residue was purified by flash chromatography (Hex: EtOAc ; 10:1) to give compound **12**. **12**: 71% yield; colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34- 4.32 (m, 1H), 4.28- 4.17 (m, 2H), 4.07 (dd, 1H, *J*= 6.5 Hz, *J*= 8.5 Hz), 3.75 (dd, 1H, *J*= 5.8 Hz, *J*= 8.5 Hz), 3.51 (s, 2H), 1.41 (s, 3H), 1.34 (s, 3H); HRMS Calc for $C_9H_{13}NO_4$ $(M+H)^+$ 200.0923 found 200.0931.

(E)-(2,2-dimethyl-1,3-dioxolan-4-yl)methyl2-cyano-3-(4-(dimethylamino)phenyl)acrylate (13). To a round bottom flask containing a solution of aldehyde **6a** (0.75 g, 5.0 mmol) and compound **12** (1.2 g, 5.5 mmol) in 20 ml of THF was added 0.50 mmol of piperidine and the mixture was heated at 50 °C. The crude mixture was concentrated under reduced pressure and the product was purified via flash chromatography (10-30% ethyl acetate in hexane) to give compound **13**. **13:** 91% yield; yellow solid; ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.94 (d, 2H, *J*= 9.0 Hz), 6.69 (d, 2H, *J*= 9.2 Hz), 4.42- 4.29 (m, 3H), 4.13 (dd, 1H, *J*= 6.2 Hz, *J*= 8.6 Hz), 3.89 (dd, 1H, *J*= 5.9 Hz, *J*= 8.5 Hz), 3.11 (s, 6H), 1.46 (s, 3H), 1.38 (s, 3H); ^{13}CNMR (400 MHz, $CDCl_3$) δ 164.3, 155.3, 153.9, 134.5, 119.5, 117.5, 111.7, 110.1, 93.3, 73.7, 66.7, 65.6, 40.3, 26.9, 25.7; HRMS Calc for $C_{18}H_{22}N_2O_4$ $(M+H)^+$ 331.1658 found 331.1691.

(E)-2,3-dihydroxypropyl 2-cyano-3-(4-(dimethylamino)phenyl)acrylate (14). Compound **13** (0.5 g, 1.5 mmol) was dissolved in a mixture of THF/ MeOH (1:1) and DOWEX-H^+ resin (0.10 g) was added and the heterogeneous mixture was stirred for 20 hours. The DOWEX-H^+ resin was removed by filtration and triethylamine (50 mg, 0.5 mmol) was added and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (100% ether) to give compound **14**. **14**: 75% yield; bright yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.94 (d, 2H, *J*= 9.1 Hz), 6.69 (d, 2H, *J*= 9.2 Hz), 4.42- 4.32 (m, 2H), 4.05 (m, 1H), 3.80-

3.70 (m, 2H), 3.12 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.0, 164.8, 155.5, 154.0, 134.6, 119.4, 117.9, 111.8, 111.7, 92.8, 70.3, 70.2, 66.9, 66.8, 63.6, 63.5, 40.3, 40.2; HRMS Calc for $C_{15}H_{18}N_2O_4$ $(M+H)^+$ 291.1345 Found 291.1361.

Diethyl 4-bromobenzylphosphonate (16). 1-bromo-4-(bromomethyl) benzene (5.0 g, 20 mmol) and triethyl phosphite (51 mL, 300 mmol) were mixed in a round bottom flask and refluxed at 90 ^{o}C for 19 hours. Excess triethyl phosphite was removed under reduced pressure and the product purified by flash chromatography (1:1 Hexane/ EtOAc) to give compound **16**. **16:** 98 % yield ; colorless liquid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, 2H, *J*= 7.5 Hz), 7.05 (d, 2H, *J*= 7.6 Hz), 3.99- 3.88 (m, 4H), 2.99 (s, 1H), 2.94 (s, 1H), 1.12 (t, 6H, *J*= 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 131.7, 131.6, 131.5, 121.0, 62.3, 34.0, 32.0, 16.5; HRMS Calc for $C_{11}H_{16}BrO_3P$ ($M+H)^+$ 307.0097 found 307.0093.

(E)-4-(4-bromostyryl)-N,N-dimethylaniline (17). DMF (anhydrous) (10.5 mL) was added to sodium methoxide (176 mg, 3.26 mmol) and the color was changed to pink. To the above solution diethyl 4-bromobenzylphosphonate (1.0 g, 3.26 mmol) in DMF (6.5 ml) was added dropwise over 2 minutes, followed by 4 (dimethylamino)benzaldehyde (486 mg, 3.26 mmol). The reaction mixture was stirred at room temperature for 24 hours. Deionized water (17 mL) was added. The product was filtered out through vacuum filtration and recrystallized with DCM/ hexane to give compound **17**. **17**: 74%. Yield; tan solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47- 7.32 (m, 6H), 7.04 (d, 1H, *J*= 12.5 Hz), 6.83 (d, 1H, *J*= 16.3 Hz), 6.71 (d, 2H, *J*= 8.9 Hz), 2.99 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.5, 137.4, 136.1, 132.1, 131.8, 129.7, 128.3, 128.2, 127.9, 127.7, 125.5, 123,2, 120.3, 112.6, 40.7; HRMS Calc for $C_{16}H_{16}BrN$ 302.0541 found 302.0539.

4-(4-(dimethylamino)styryl)benzaldehyde (18). To a round bottom flask compound **17** (300 mg, 1 mmol) was transferred followed by THF (5 mL). The heterogeneous solution was cooled at –78 °C and n-BuLi (1.6M in hexane, 1 mmol) was added dropwise over 5 min, followed by DMF

(1.5 mL). The reaction mixture was stirred at –78 °C for 3 hours then it was quenched by water (1 mL) and the mixture was extracted with ether (2 x 25 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure to give compound **18**. **18**: 60% yield; yellow powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.96 (s, 1H), 7.83 (d, 2H, *J*= 8.2 Hz), 7.60 (d, 2H, *J*= 8.2 Hz), 7.44 (d, 2H, *J*= 8.8 Hz), 7.22 (d, 1H, *J*= 16.2 Hz), 6.94 (d, 1H, *J*= 16.2 Hz), 6.72 (d, 2H, *J*= 8.8 Hz), 3.01 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.8, 150.8, 144.7, 134.7, 134.6, 132.7, 130.4, 128.4, 126.4, 124.9, 122.8, 112.4, 40.5; HRMS calc for $C_{17}H_{17}NO$ 252.1384 found 252.1383.

(E)-2-(2-(2-methoxyethoxy)ethoxy)ethyl-cyano-3-(4-(4(dimethylamino)styryl)phenyl)acrylate (19). 97% yield; red solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.98 (d, 2H, *J*= 8.4 Hz), 7.57 (d, 2H, *J*= 8.4 Hz), 7.45 (d, 2 H, *J*= 8.7 Hz), 7.20 (d, 1H, *J*= 16.2 Hz), 6.92 (d, 1H, *J*=16.2 Hz), 6.72 (d, 2H, *J*= 8.7 Hz) , 4.47 (m, 2H), 3.84- 3.82 (m, 2H), 3.74-3.72 (m, 2H), 3.70- 3.66 (m, 4H), 3.57- 3.55 (m, 2H), 3.37 (s, 3H), 3.02 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9, 133.0, 132.2, 128.6, 128.5, 126.7, 122.7, 112.4, 72.2, 71.1, 70.8, 69.0, 65.8, 59.3, 40.6, 40.5, 29.9, 28.2; HRMS calc for $C_{27}H_{32}N_2O_5$ $(M+Na)^+$ 487.2203 found 487.2201.

Fluorescence studies with aggregated Aβ peptides: Aggregated Aβ peptide was prepared by dissolving Aβ(1-42) in PBS pH 7.4 to a final concentration of 100 μM. This solution was magnetically stirred at 1200 rpm for 3 days at room temperature. The 100 μM Aβ(1-42) stock solution in PBS was aliquoted and frozen at –80 °C for up to 4 weeks without noticeable change in its property. 150 μL of pre-aggregated Aβ(1-42) was added to 2.85 mL of probe to attain a final concentration of 5 μM Aβ(1-42) and 4 μM of probe. The solution was transferred to 3 mL cuvette and the fluorescence measured at 25 °C.

Determination of binding constant: Pre-aggregated Aβ(1-42) (5 μM final concentration) was mixed with various concentrations of probes (10, 5, 2.5, 1.25 μM) in PBS buffer (pH 7.4) and their fluorescence was measured. The negative inverse of the x-intercept of the linear regression, that was drawn between the double reciprocal of the fluorescence intensity maximum and concentration of the probe, represents the probe binding constant (K_d) to Aβ(1-42).

Determination of K_d's from fluorescence measurements.

In order to quantify the dissociation constants (K_d's) for the binding of fluorescent probes with aggregated β-amyloid peptides, we used the method described by LeVine (see H. LeVine III, *Protein Sci.* **1993**, *2*, 404-410). This method is similar to the method described by Benesi-Hildebran (see C. Yang, L. Liu, T. W. Mu, Q. X. Guo, *Anal. Sci.* **2000**, *16*, 537-539). Here, the fluorescence of the probe was measured with and without the addition of the aggregated peptides in solution. The relative fluorescence enhancement of the probe upon binding to aggregated β-amyloid peptides was determined by taking the difference between *F* (fluorescence after the addition of aggregated peptides) and F_O (fluorescence before the addition of aggregated peptides).

In order to estimate the binding constant (K_d) for the probe-Aβ complexes from the fluorescence studies, we made the following assumptions:

1. All probes are completely in solution and free of any significant competing binding process such as self-aggregation.

2. The concentration of unbound probes can be approximated as close to the total concentration of the probes.
3. The binding sites in the aggregated Aβ peptides are not completely occupied at the concentration of Aβ binding probes used for the fluorescence studies (i.e., the experiments are carried out under non-saturated binding conditions).

According to the Beer- Lambert law (see J. W. Robinson, "Atomic spectroscopy", **1996**), we can obtain two expressions that relate the concentration of bound probe ([*HG*]), free probe ([*G*]), and free binding sites on the amyloid peptides ([*H*]) with either 1) the measured fluorescence of the probe in solution before the addition of the aggregated peptides (F_O), or 2) the measured fluorescence of the probe in the presence of the amyloid peptides (*F*):

$$F_O = \varepsilon_G l[G_O] \qquad (1)$$

$$F = \varepsilon_{HG} l[HG] + \varepsilon_H l[H] + \varepsilon_G l[G] \qquad (2)$$

where

[G_O] = total concentration of probes

[*G*] = unbound probe concentration

[*HG*] = probe-Aβ complex concentration

[H_O] = total concentration of binding sites on the Aβ peptides

[*H*] = concentration of free binding sites on the aggregated peptide

ε_G = absorption coefficient of the probes

ε_{HG} = absorption coefficient of probe-Aβ complex

ε_H = absorption coefficient of *H*

l = path length

Substituting $[G_O] = [G] + [HG]$ into equation 1, and making the approximation that $\varepsilon_{HG} l[HG] + \varepsilon_G l[G] \gg \varepsilon_H l[H]$, we can arrive at a simplified expression for the relative fluorescence of bound probe (*DF*):

$$\Delta F = F - F_O = \varepsilon_{HG} l[HG] + \varepsilon_G l[G] - \varepsilon_G l[G] - \varepsilon_G l[HG] \qquad (3)$$

$$\text{or } \Delta F = \Delta\varepsilon l[HG] \qquad (4)$$

where $\Delta\varepsilon = \varepsilon_{HG} - \varepsilon_G$

In order to obtain a relationship between the change in measured fluorescence of the probe (ΔF) with the binding constant of the probe to aggregated β-amyloid peptides (K_d's), we used the standard equation for a binding isotherm to obtain a relationship between [HG] and K_d:

$$[HG] = \frac{[H_O][G]}{K_d + [G]} \qquad (5)$$

Combining equations 4 and 5, we obtained a relationship between ΔF and K_d:

$$\Delta F = \frac{[H_O][G]}{K_d + [G]} \Delta \varepsilon l \qquad (6)$$

In order to estimate the K_d of the probe bound to aggregated Aβ peptides from the measured change in fluorescence, we take the reciprocal of the equation 6 to give:

$$\frac{1}{\Delta F} = \frac{K_d}{\Delta \varepsilon l\,[H_O]} \frac{1}{[G]} + \frac{1}{\Delta \varepsilon l [H_O]} \qquad (7)$$

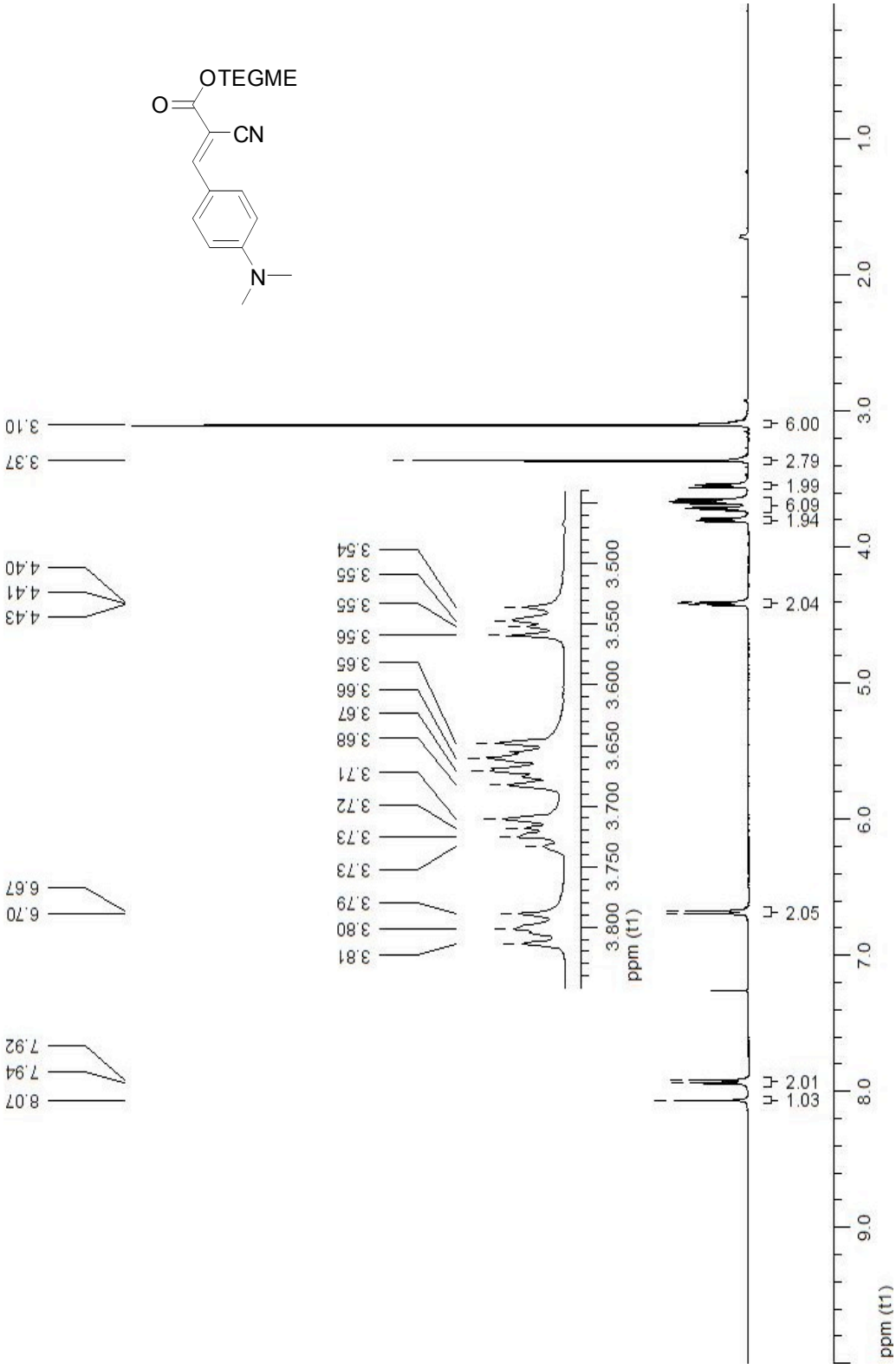
Equation 7 suggests that a double reciprocal plot of ΔF and [G] should yield a straight line with x-intercept equal to -1/K_d. Figure 4 in the main text, and the figure on page S43 of the supporting information, shows a double reciprocal plot of the measured fluorescence versus total concentration of probe [G_O]. Assuming that [G] can be approximated as close to [G_O] (assumption 2), we can obtain estimates for the K_d's of the probe-Aβ complexes from the x-intercept of the linear fits of the data for each probe. The estimated K_d's for all probes are given in Table 2 in the main text.

ELISA assay: Aggregated Aβ peptides were generated from synthetic Aβ(1-42) peptides by dissolving 30 μg of peptide in 90 μL of nanopure water (pH 5-6) and incubating at 37 °C for ≥ 72 h without agitation. Each well of a 96-well plate (well volume 0.4 mL; clear, flat bottom polypropylene) was coated for 3 h at 25 °C with 50 μL of 1.3 *μ*M solution of Aβ peptides in phosphate-buffered saline (PBS, 10 mM NaH_2PO_4/Na_2HPO_4, 138 mM NaCl, 2.7 mM KCl, pH 7.4). After removal of the excess sample, 50 μL solutions of probes in PBS buffer (various concentrations were obtained by diluting a stock solution with PBS buffer) were incubated in the wells for 12 h. Probes that did not dissolve in PBS buffer were dissolved in DMSO and diluted in PBS buffer to give a final solution of 5% DMSO in PBS buffer. The excess solutions were then removed and all wells were blocked for 30 min by adding 300 μL of a 1% (w/v) solution of bovine serum albumin in PBS buffer (BSA/PBS). On occasion, an additional blocking step was performed prior to incubation with solutions of small molecules. The blocking solution was discarded and the wells were washed once with 300 μL of PBS buffer. Wells were incubated for 1 h with 50 μL of a 1.1 nM solution (in 1% BSA/PBS, dilution 1:6000) of anti-Aβ IgG (clone 6E10, monoclonal, mouse), followed by removal of the solution. The wells were washed twice with 300 μL of PBS buffer and incubated for 60 min with 50 μL of the secondary IgG (anti- mouse IgG H+L, polyclonal, rabbit) conjugated with alkaline phosphatase (6.8 nM in 1% BSA/PBS, dilution 1:1000). The solution was discarded, and the wells were washed twice with 300 μL PBS buffer. Bound secondary IgGs were detected by the addition of 50 μL of a *p*-nitrophenyl phosphate solution (2.7 mM, in 100 mM diethanol amine/0.5 mM magnesium chloride, pH 9.8). Absorbance intensities were determined at 405 nm using a UV-vis spectroscopic plate reader (Sprectramax 190, Molecular Devices, Sunnyvale, CA). Each run was performed five times and averaged. Error bars represent standard deviations. Graphs were plotted and fitted with the sigmoid curve fitting.

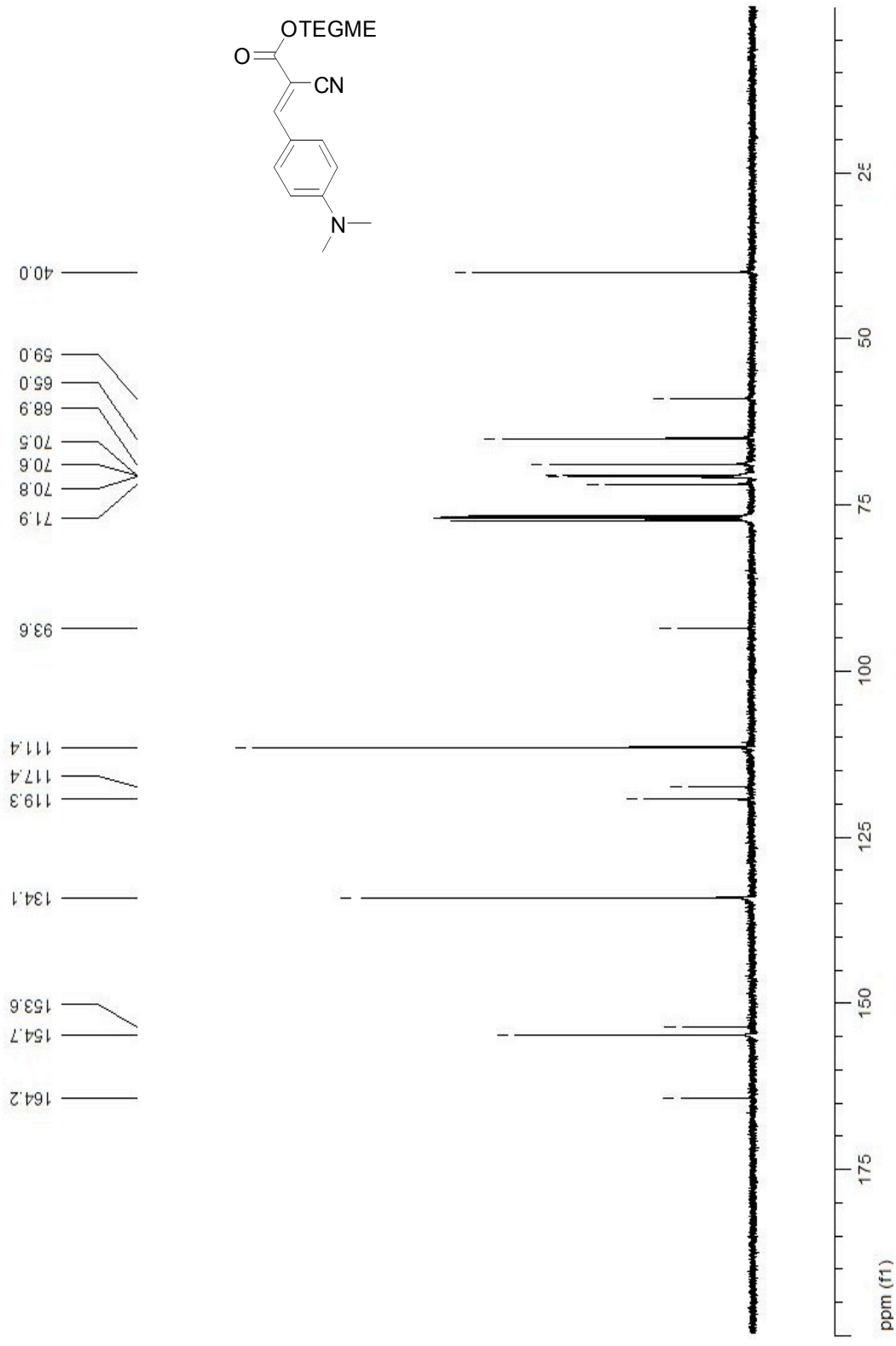
Fluorescence studies with monomeric Aβ. Aβ (Biopeptide, Inc.) was initially solubilized in hexafluoroisopropanol at 1 mM concentration, vortexed, sonicated and vortexed. The vial was covered in foil and was incubated for 21 hours at 25 °C on a shaker, with 3 times of vortexing

throughout the incubation period. The solution was sonicated and vortexed again then diluted with cold nanopure water (2:1 H_2O:HFIP), fractionated in desired amounts into small glass vials, and immediately frozen in a CO_2/acetone bath. Each fraction was covered with parafilm that was punctured to allow solvent vapors to escape. The fractions were lyophilized for 2 days to obtain monomeric Aβ (91% monomer by 12% Tris-bis PAGE gel analysis). 1.8 µL (8.42 µM) of this monomeric Aβ(1-42) was added to 3 µL of 4 µM concentration of small molecules that was prepared by dissolving in PBS buffer pH 7.4 to attain a final concentration of 5 µM of Aβ(1-42) and 4 µM of the probe. The solution was transferred to 3 mL cuvettes and the fluorescence was measured at 25 °C.

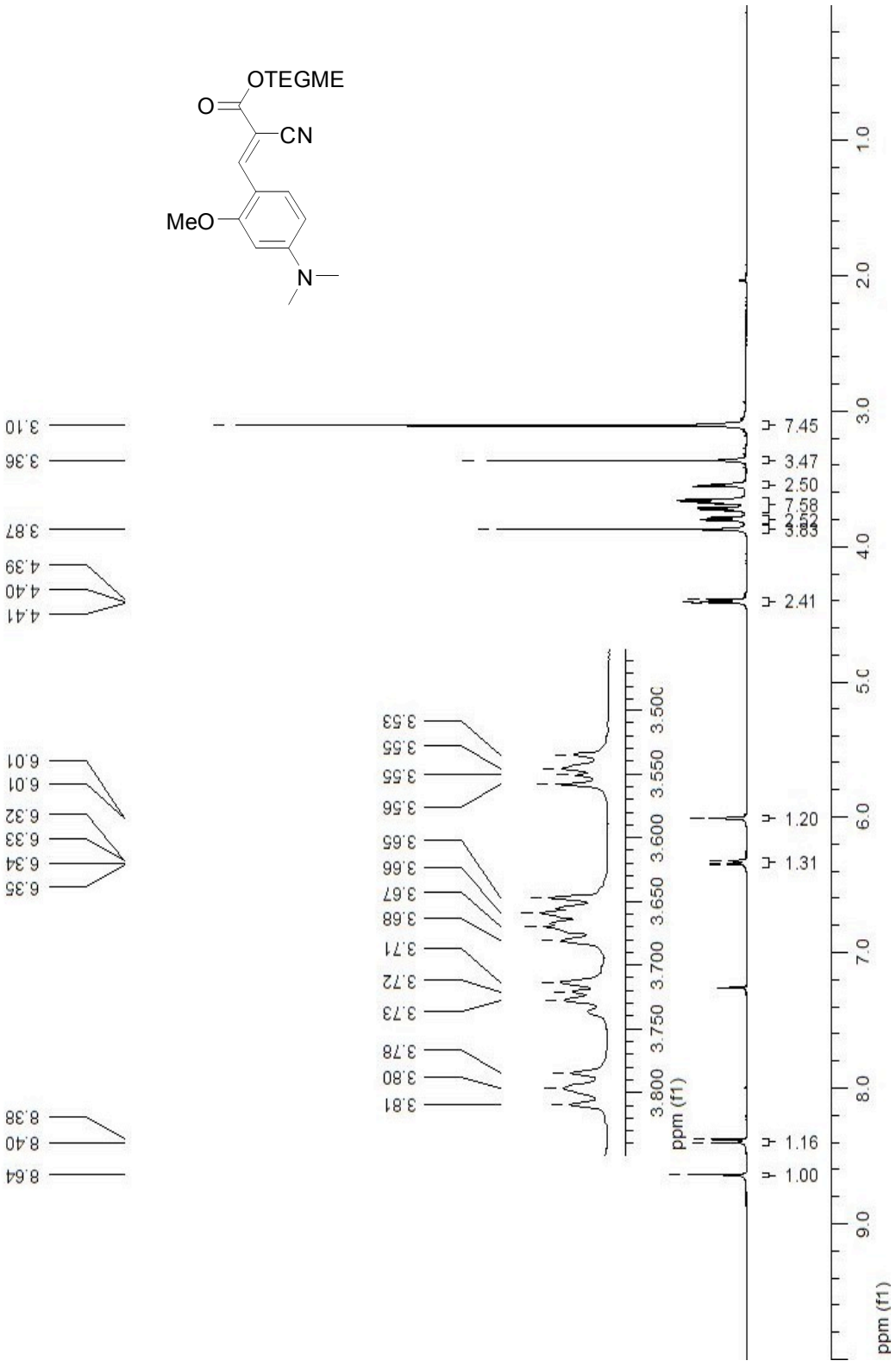
Evaluation of rigid rotors for cytotoxic activity against SH-SY5Y human neuroblastoma cells (MTT assay): SH-SY5Y human neuroblastoma cells, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell proliferation kit, Eagle's Minimum Essential Medium (EMEM), Ham's F12 nutrient mixture, and Fetal Bovine Serum (FBS) were all purchased from ATCC (Manassas, VA). Briefly, SH-SY5Y cells (in 1:1 EMEM:Ham's F-12 with 10% FBS) were seeded on 96-well plates at a density of $5x10^4$ cells/well. Plates were incubated overnight (in a humidified atmosphere of 95% air, 5% CO_2, at 37 °C) to promote attachment of cells to the wells. Cells were then treated with various concentrations of compound **8a**, **8b**, **8c**, **8d**, **11**, or **14** and incubated for 24 hours (humidified atmosphere of 95% air, 5% CO_2, at 37 °C). MTT reagent (20 µL) was added to the medium and incubated for additional 4 hours. After incubation, 100 µL of detergent reagent was added and the plates were covered with aluminum foil and left at room temperature overnight. The amount of solubilized MTT formazan was measured by spectrophotometric absorbance at 570 nm (Spectramax 190, Molecular Devices, Sunnyvale, CA). MTT assay was not performed on compound **19** due to its poor solubility in aqueous media. All data are presented as the mean ± S.D, N= 3 for each concentration. The Student's *t*-test was employed for all analyses. A *p*-value of <0.05 was considered statistically significant compared to control cells.



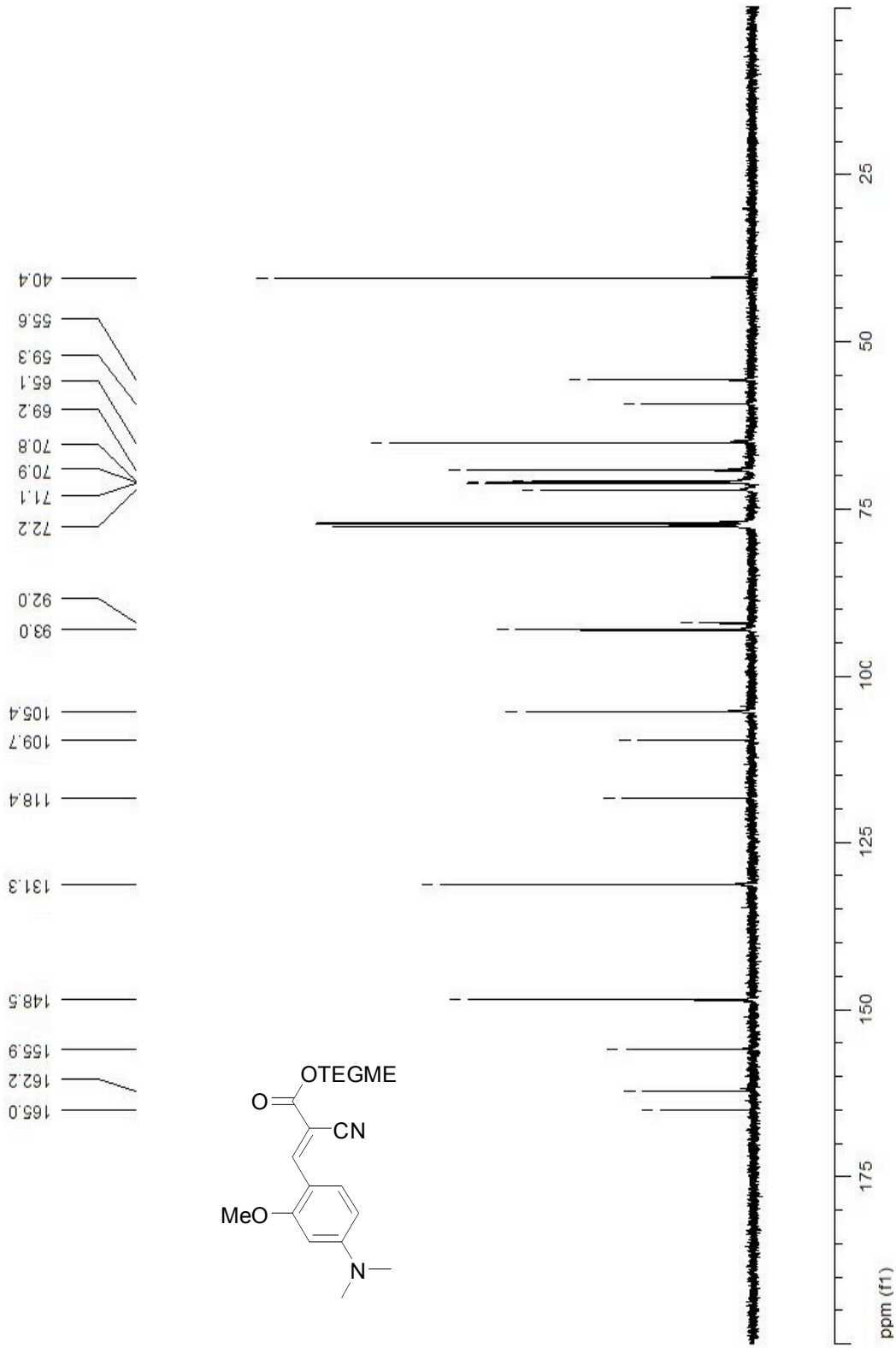
Spectrum 1. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **8a**



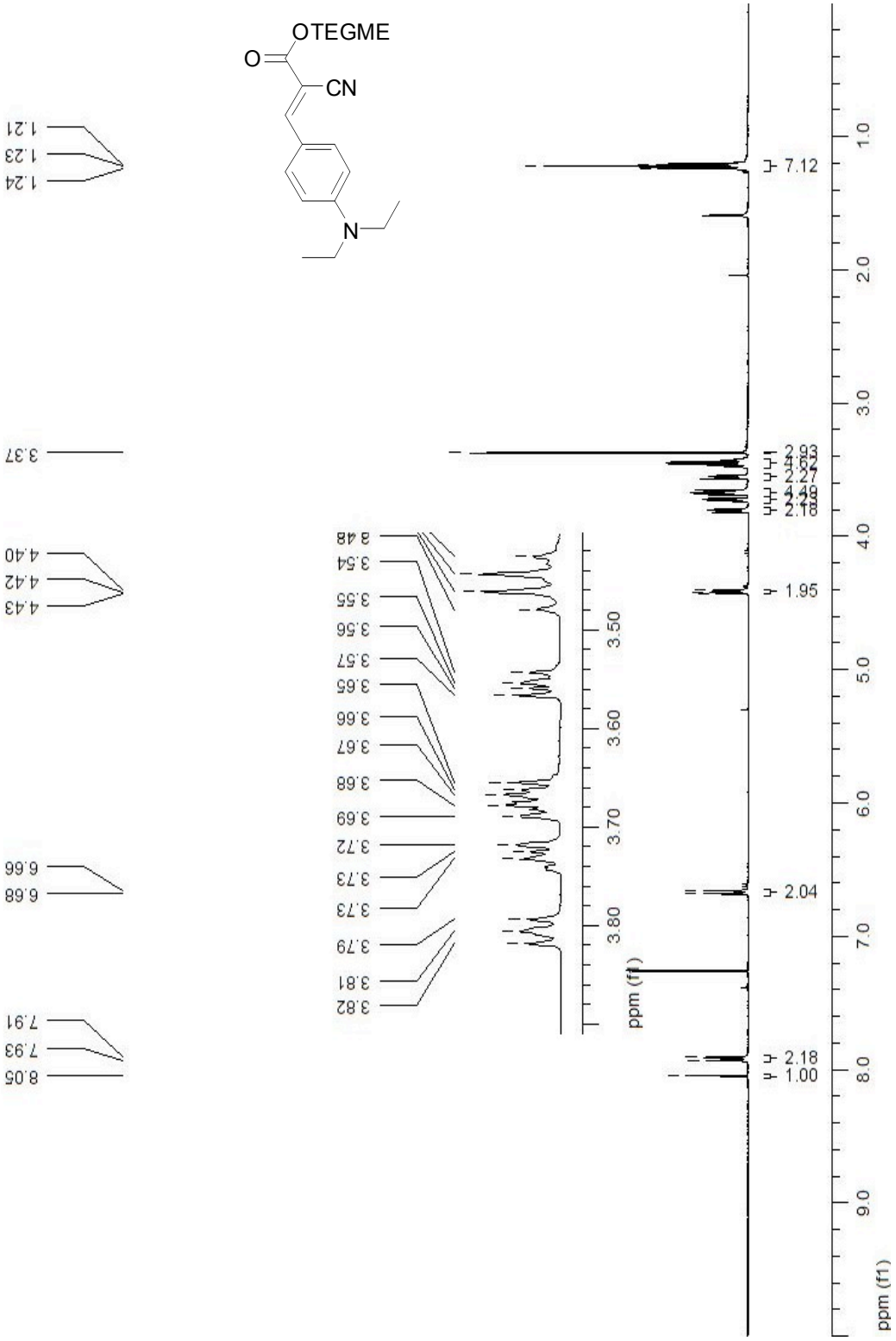
Spectrum 2. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **8a**



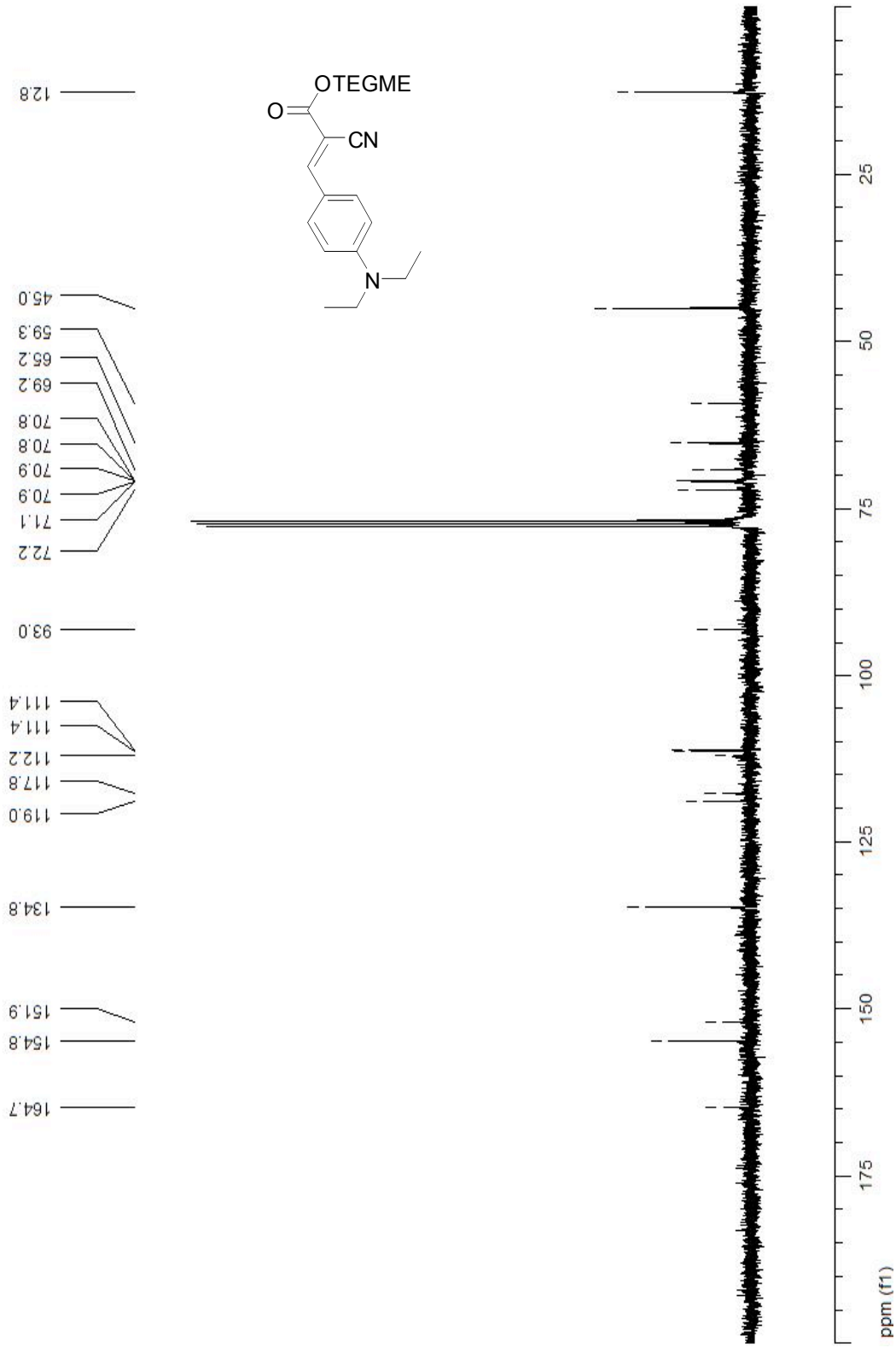
Spectrum 3. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **8b**



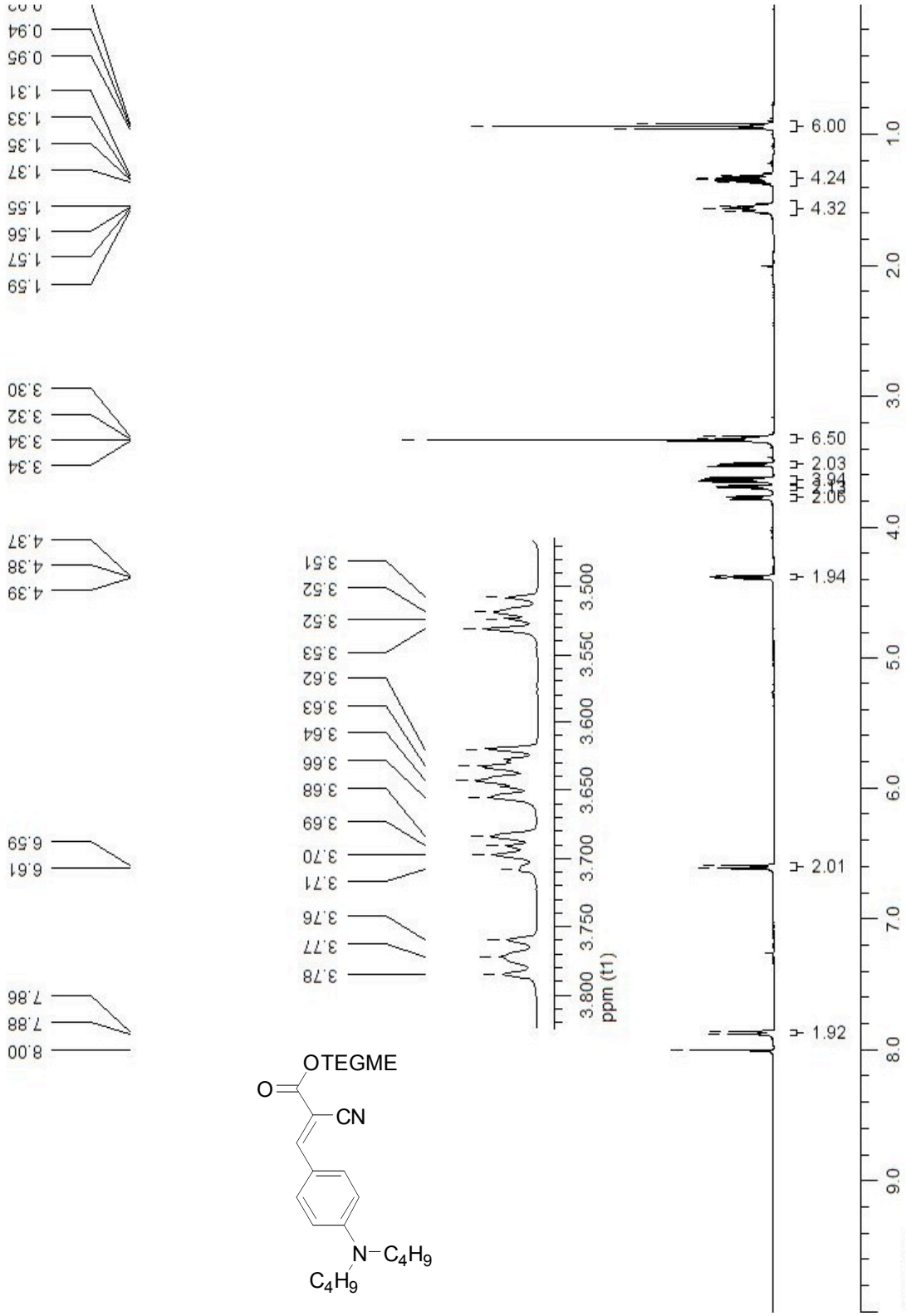
Spectrum 4. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **8b**



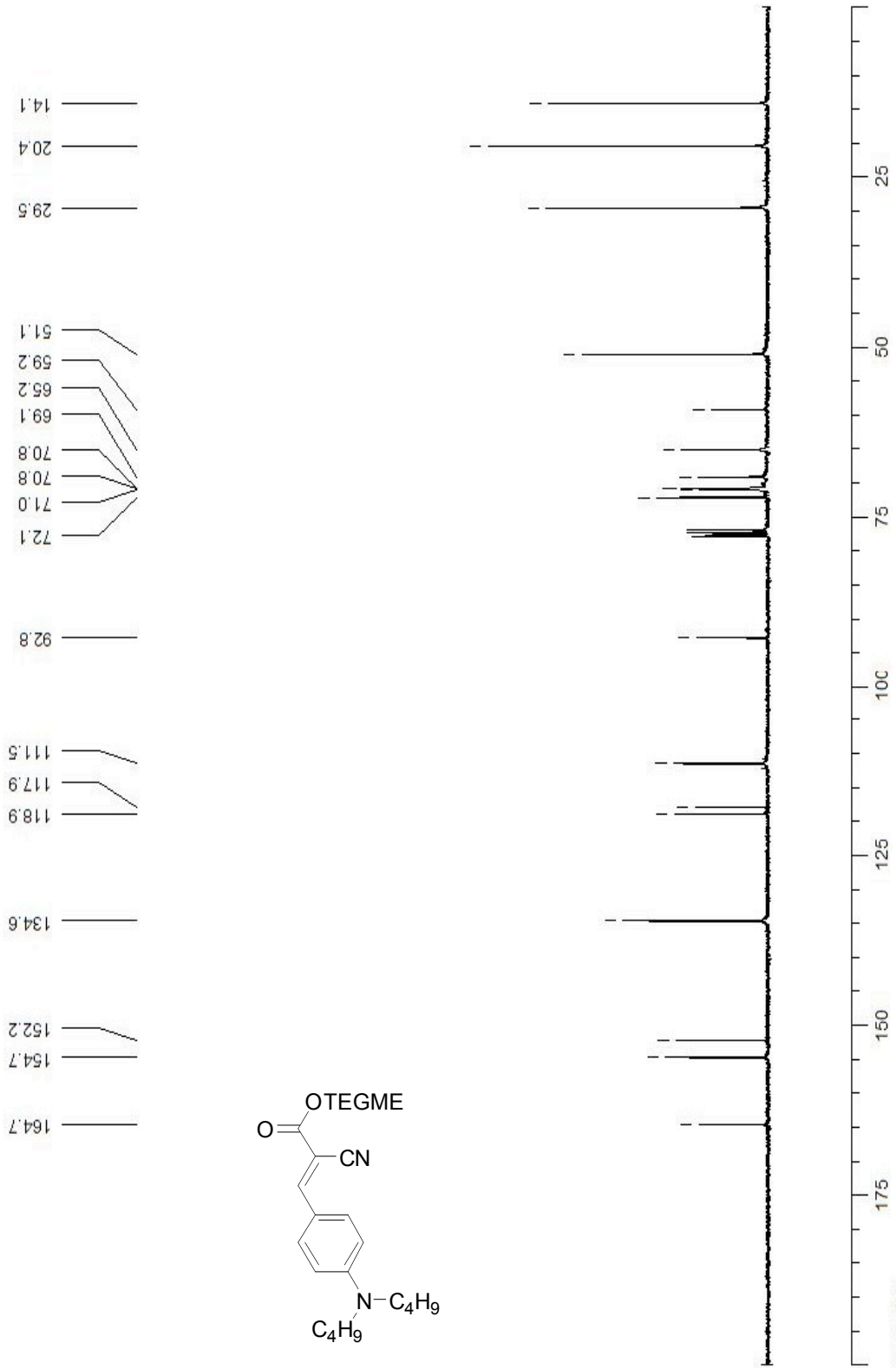
Spectrum 5. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **8c**



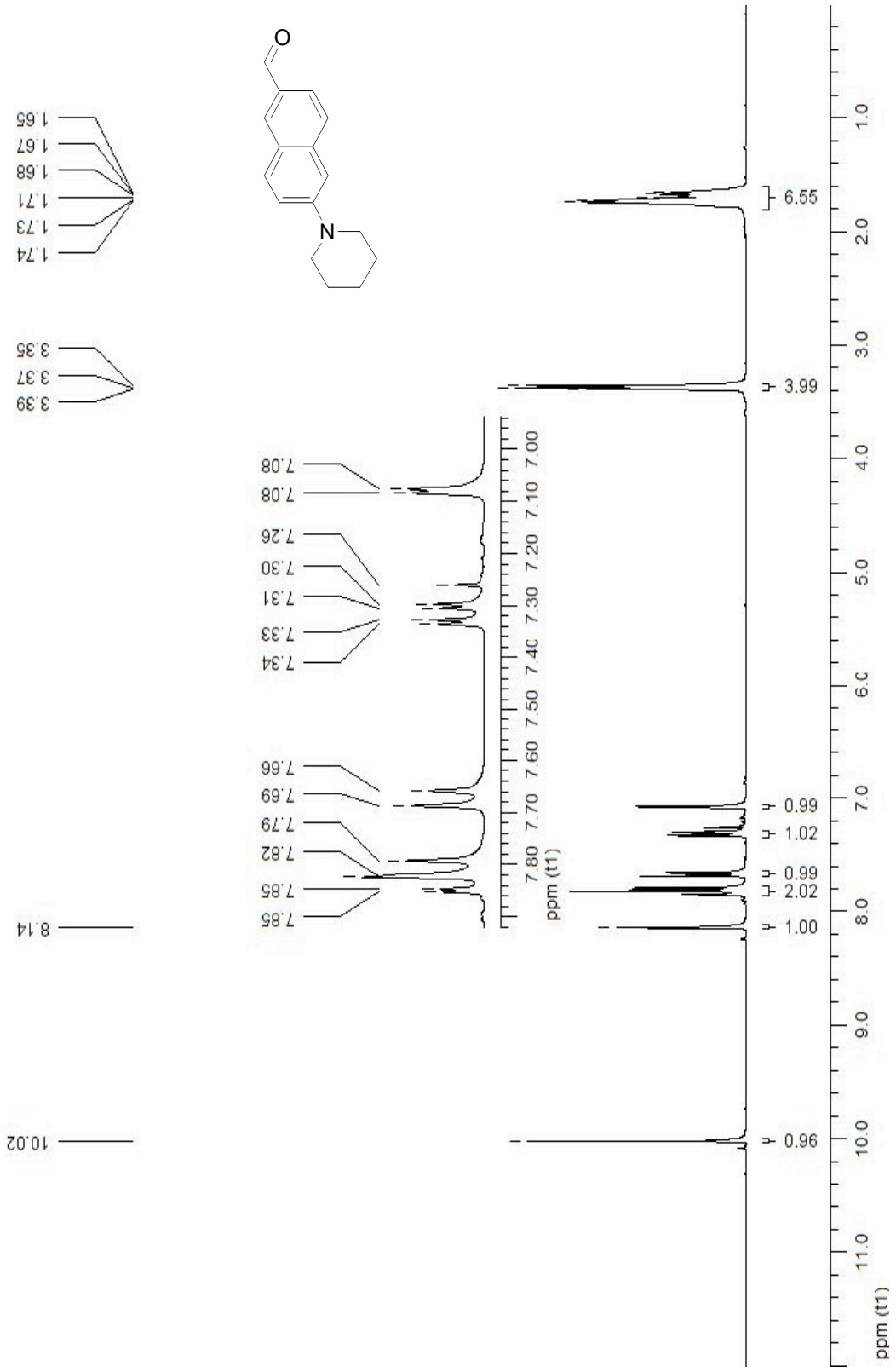
Spectrum 6. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **8c**



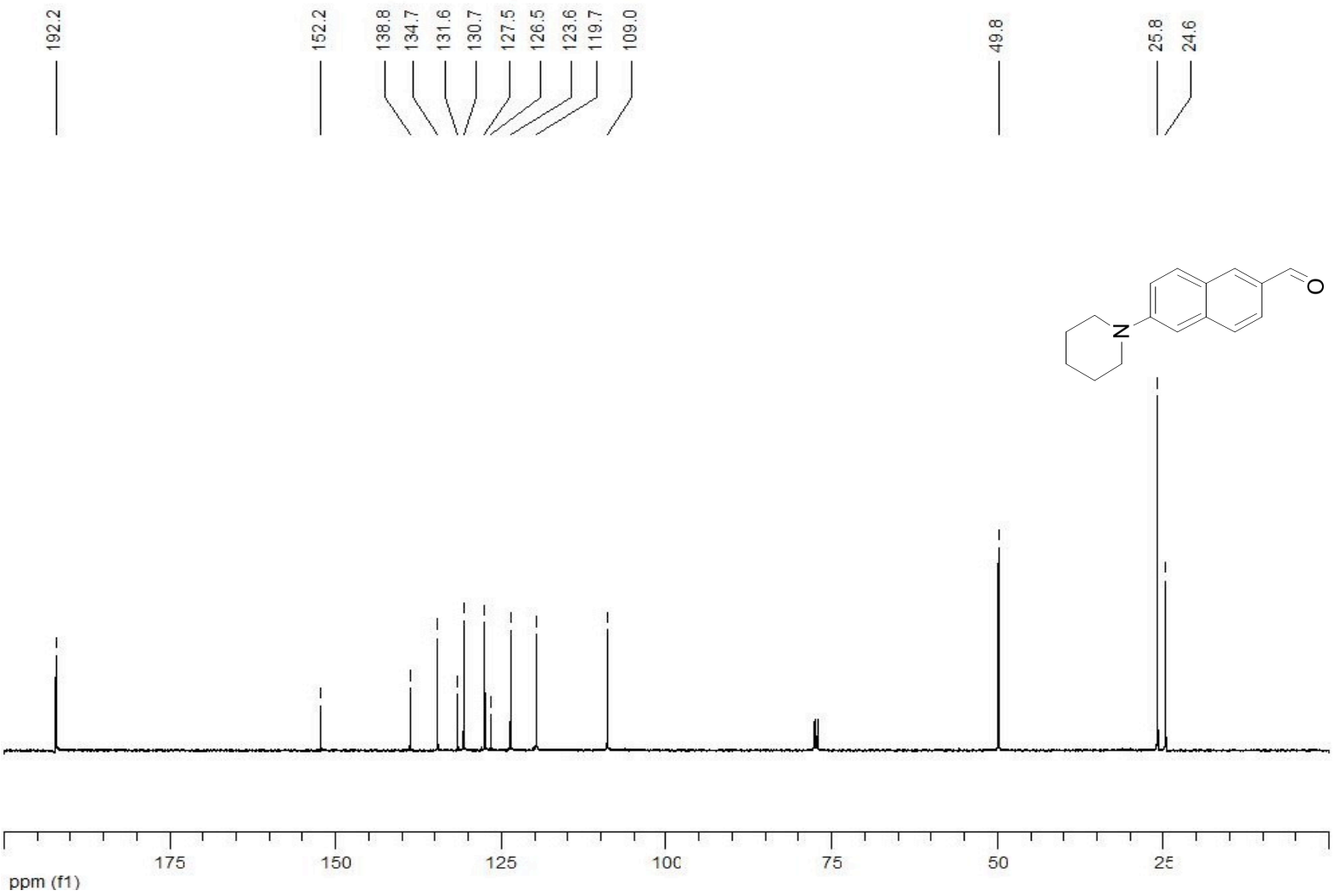
Spectrum 7. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **8d**



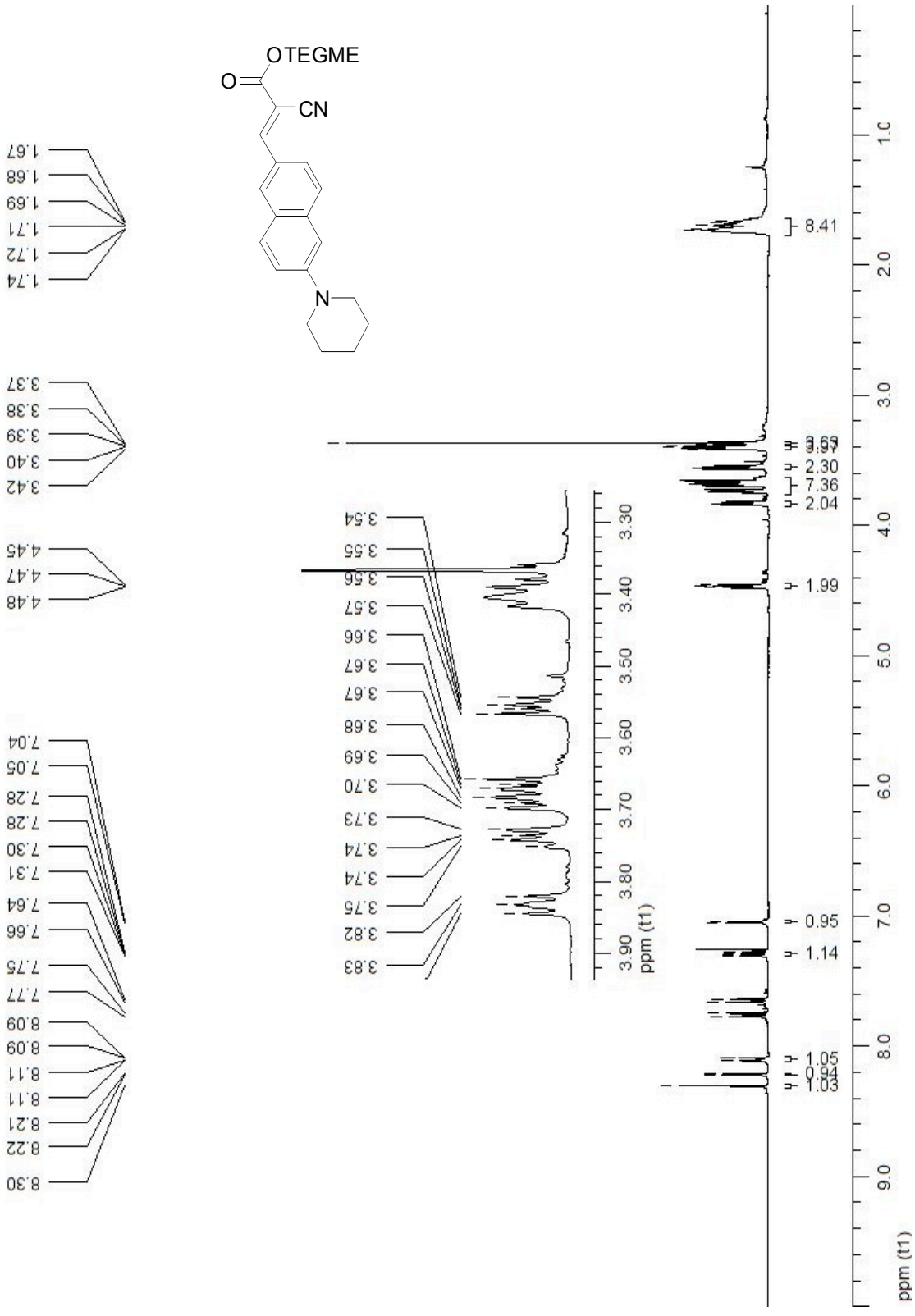
Spectrum 8. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **8d**



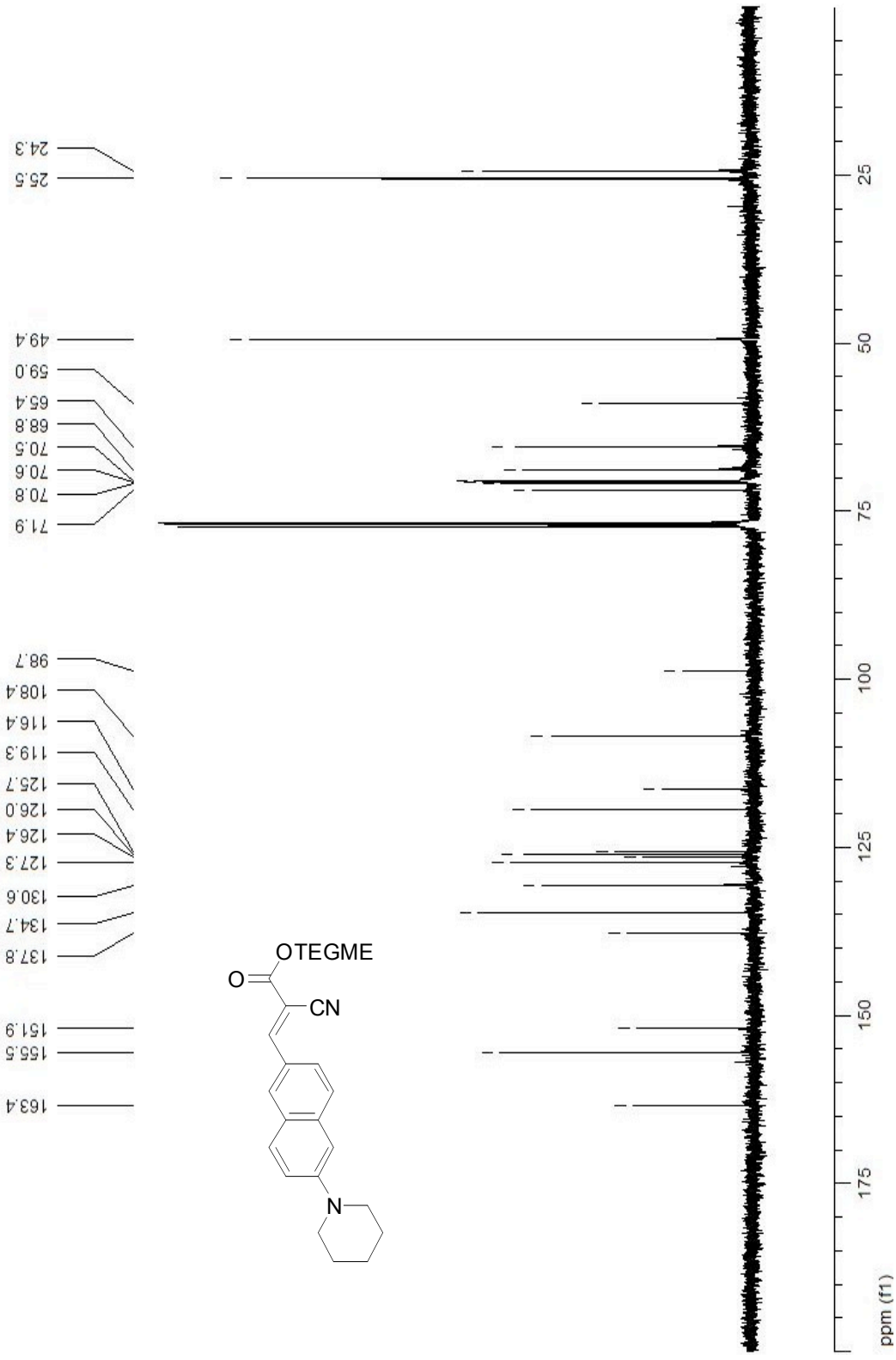
Spectrum 9. ^{1}HNMR ($CDCl_3$, 300MHz) of compound **10**



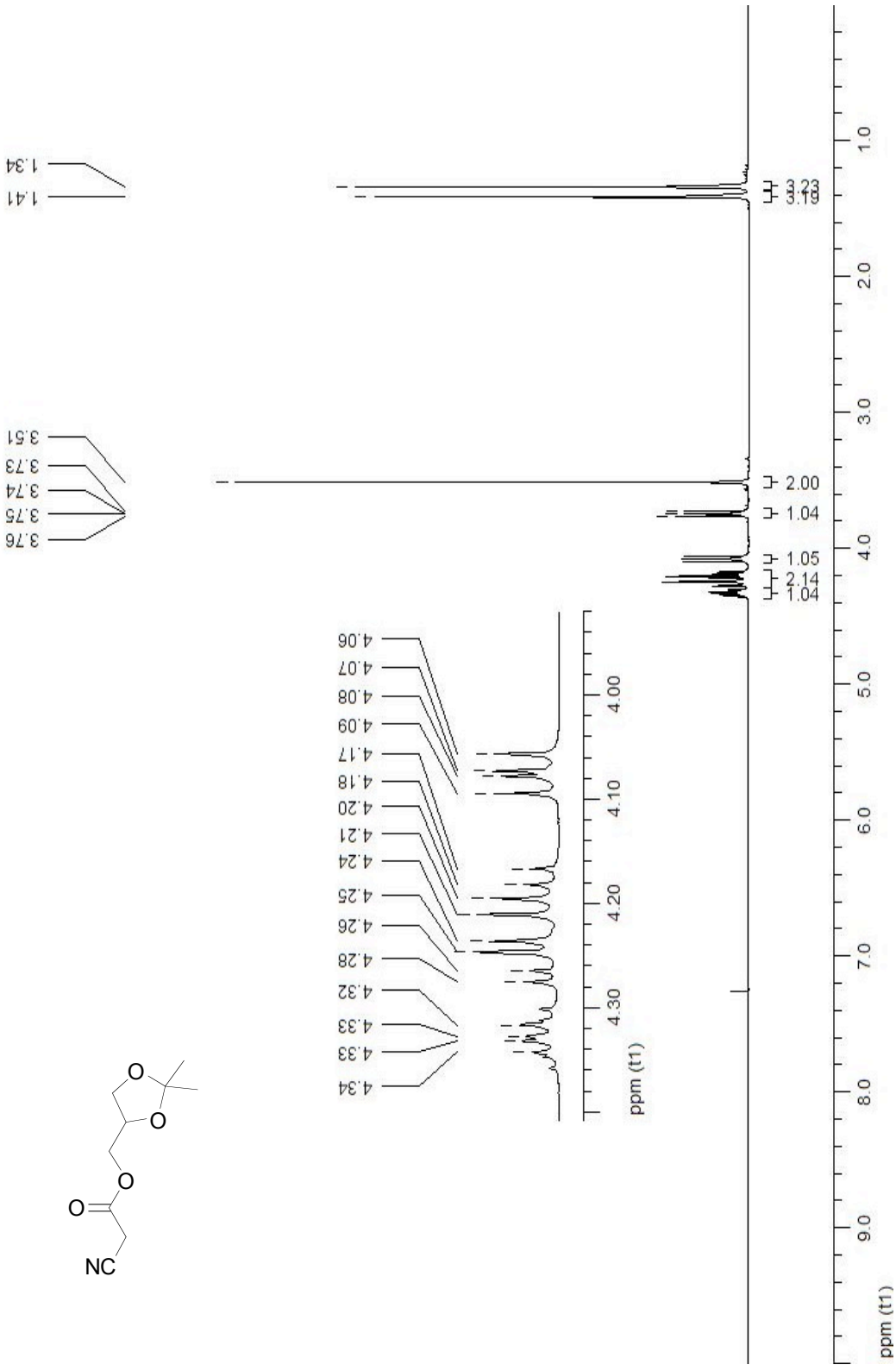
Spectrum 10. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **10**



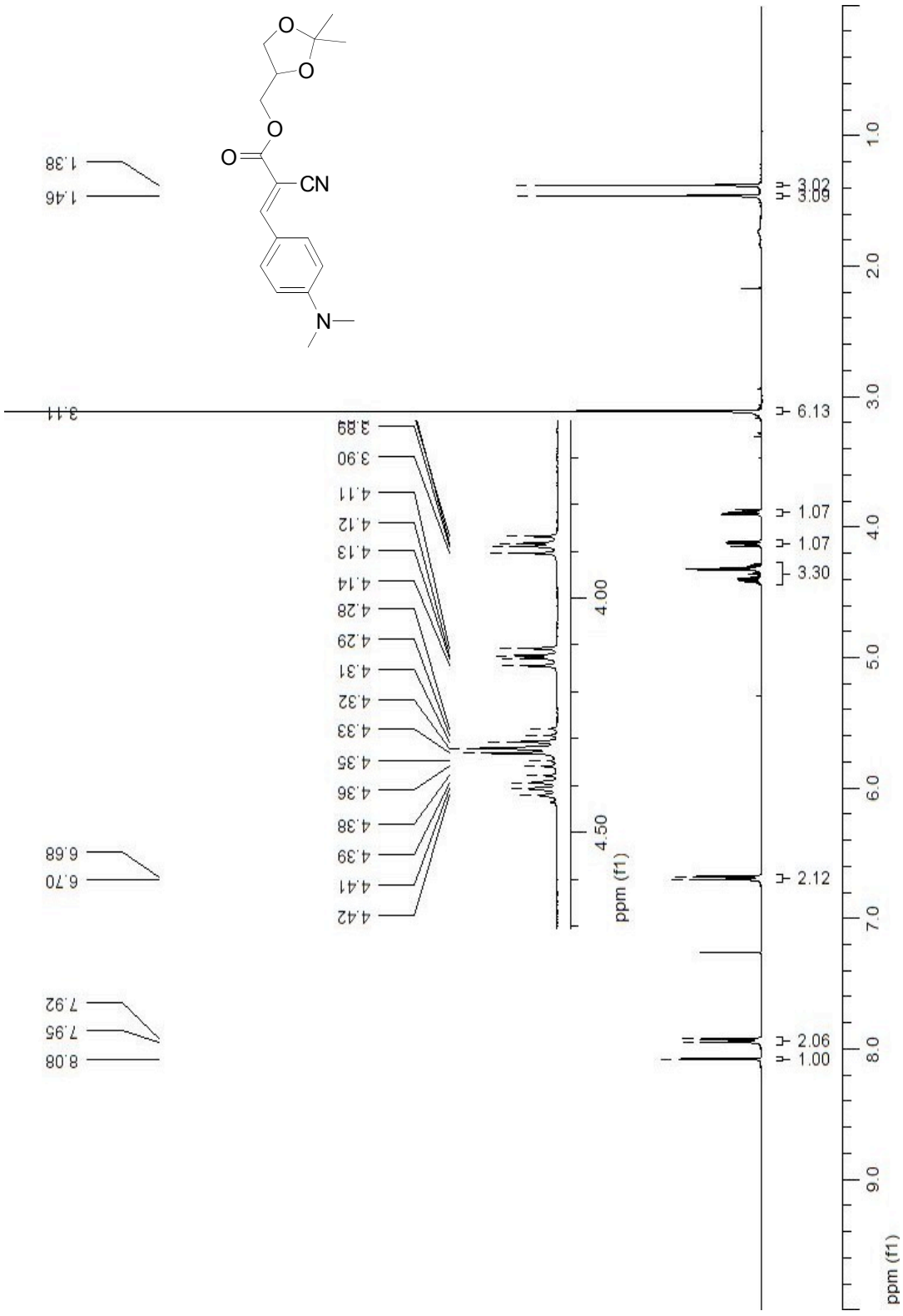
Spectrum 11. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **11**



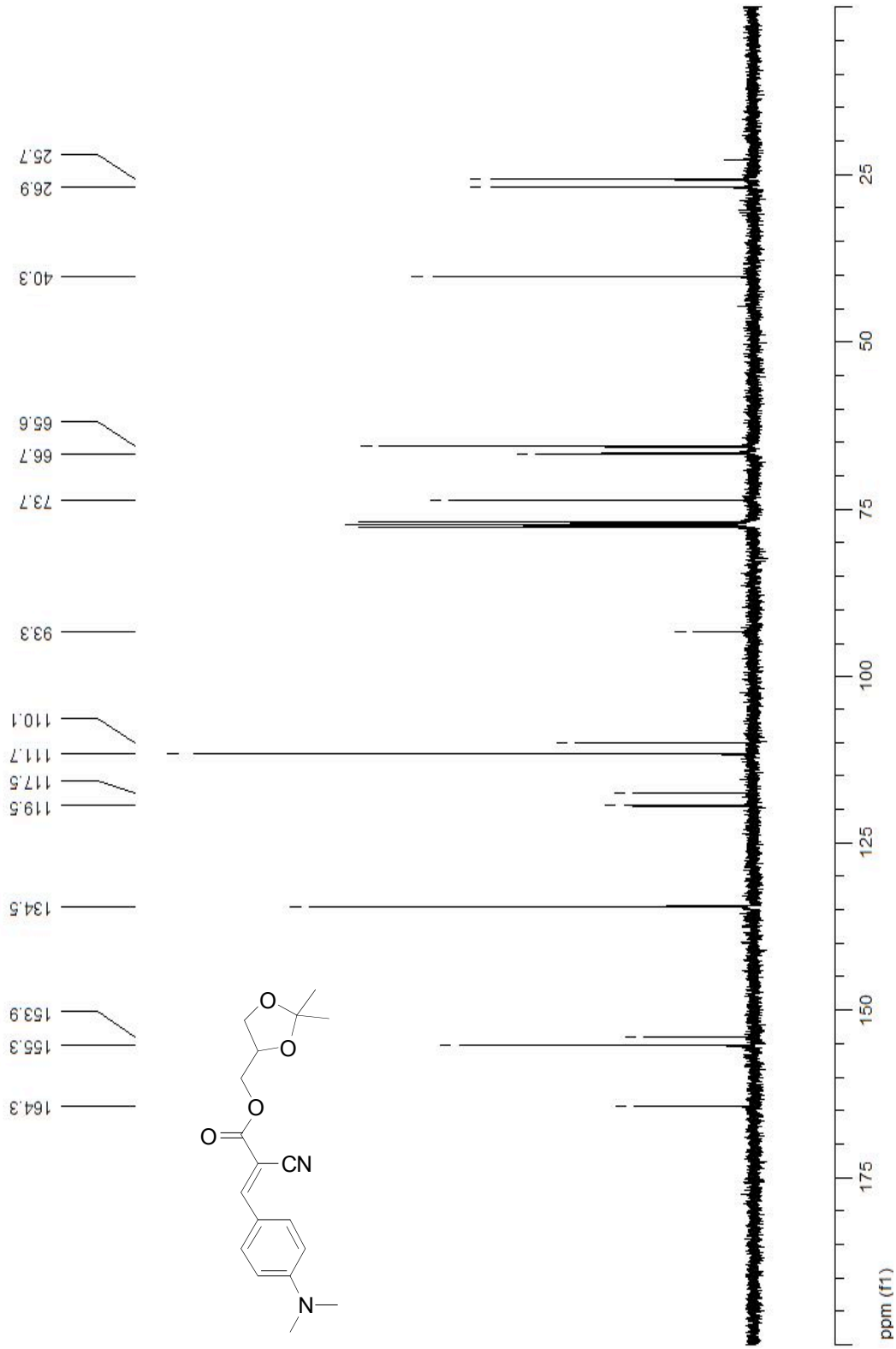
Spectrum 12. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **11**



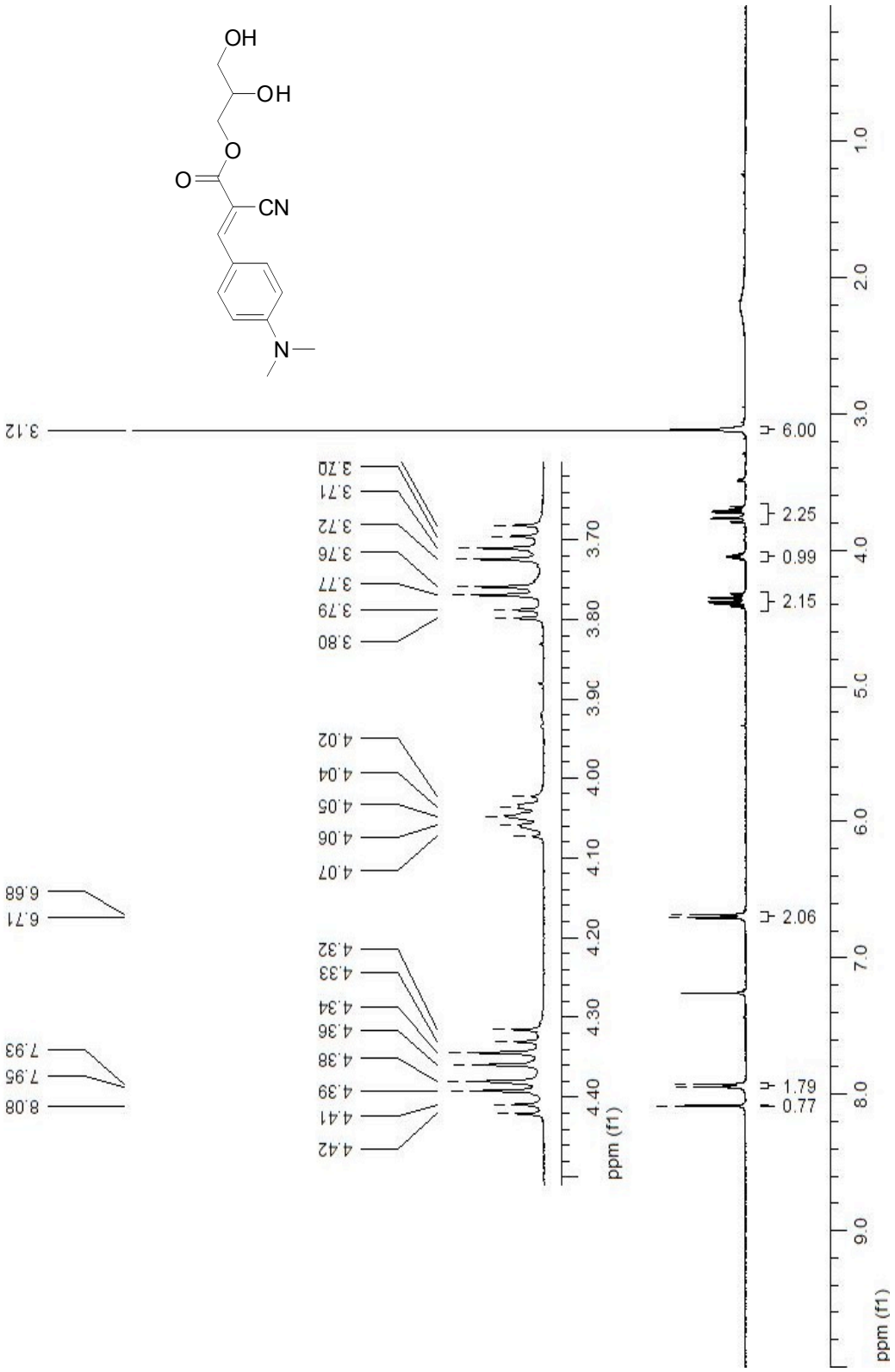
Spectrum 13. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **12**



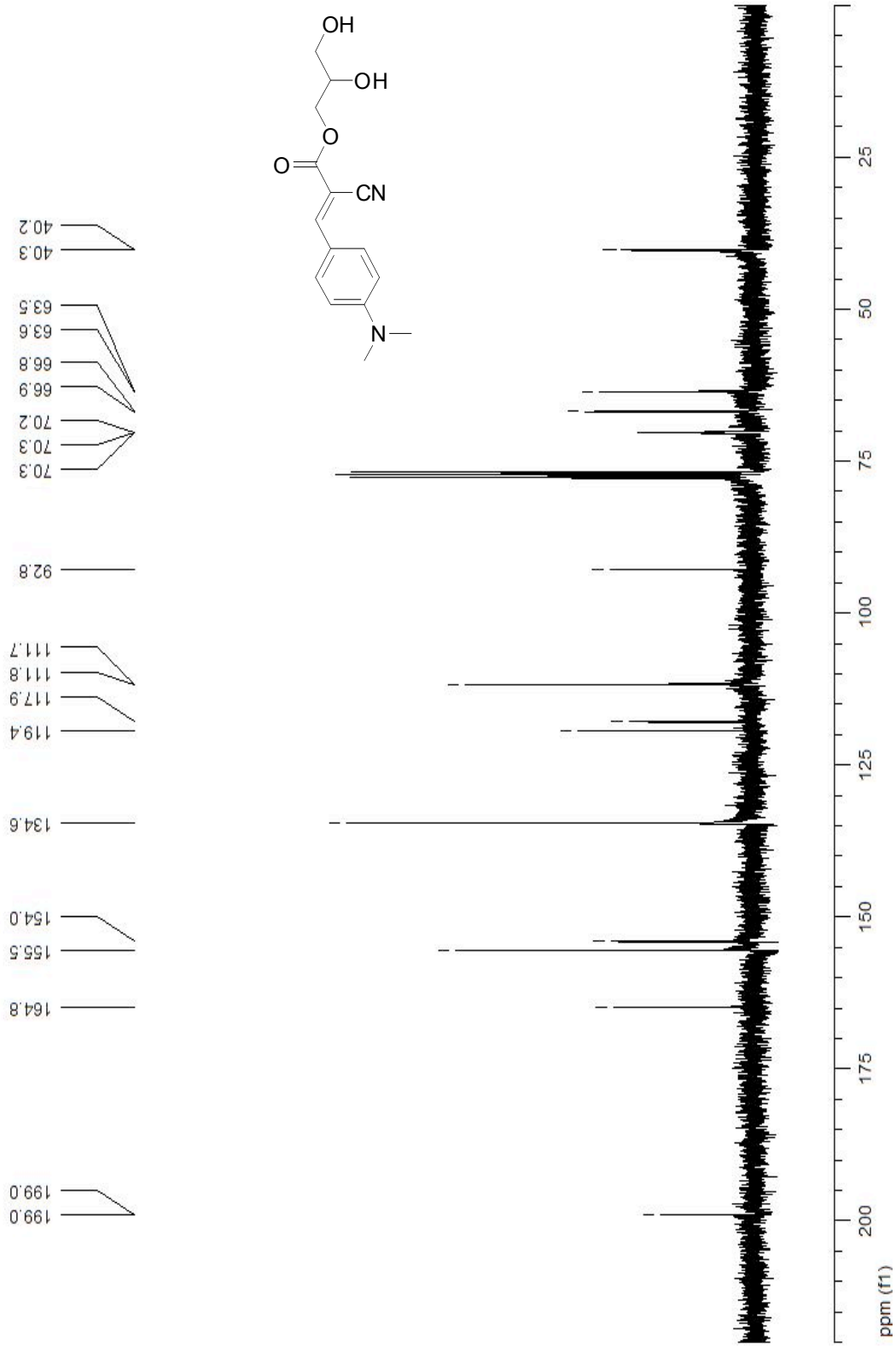
Spectrum 14. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **13**



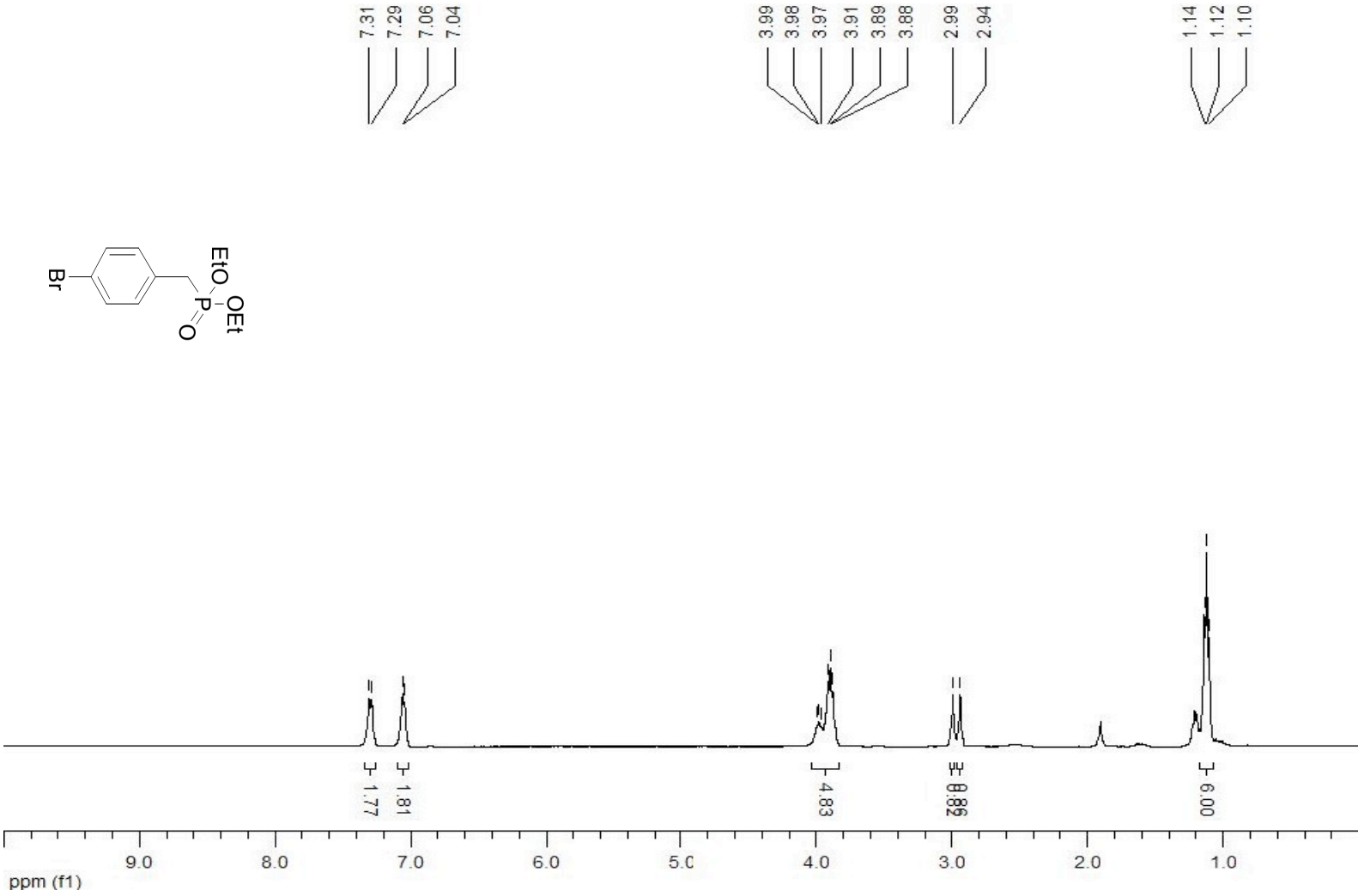
Spectrum 15. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **13**



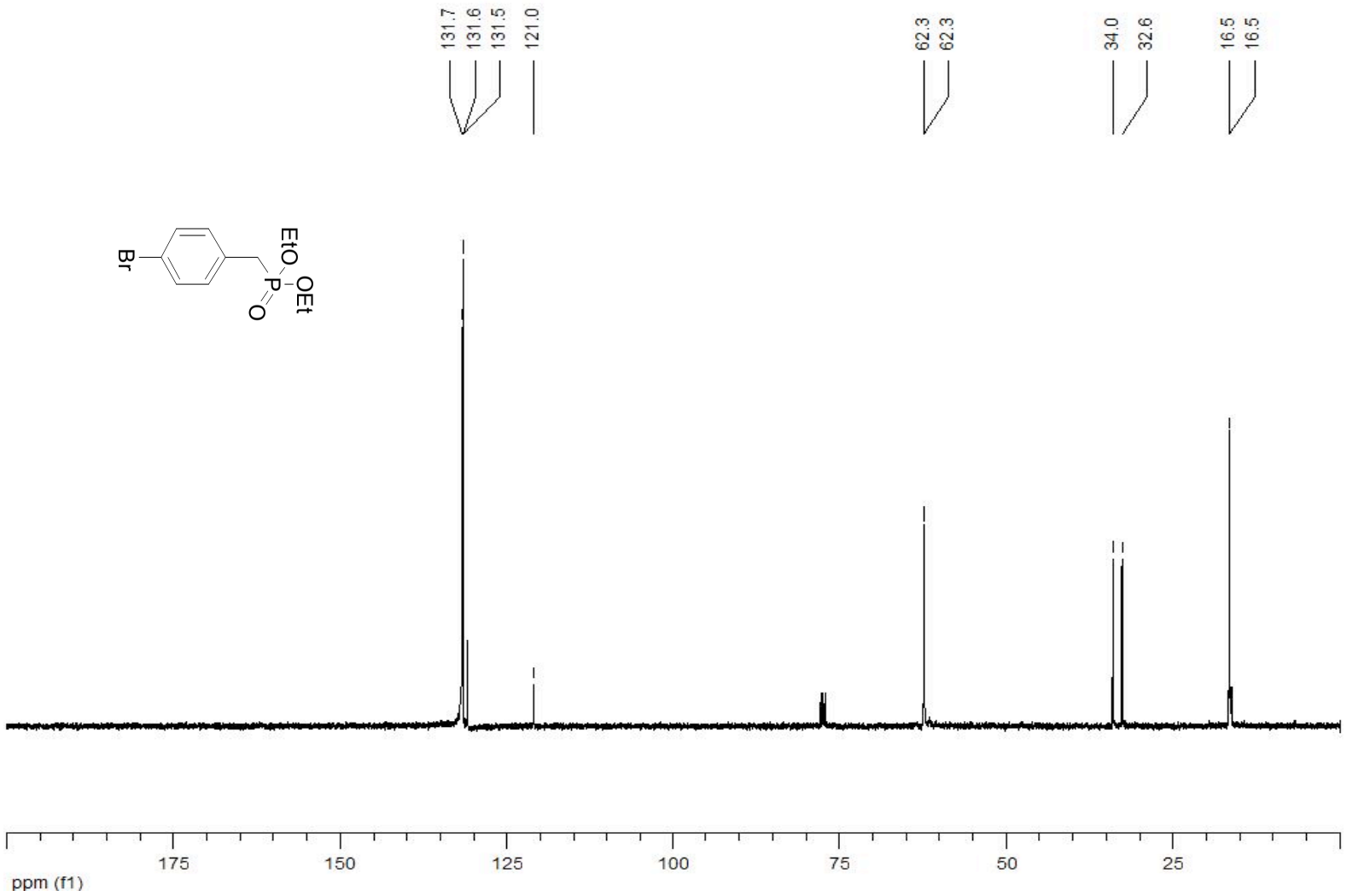
Spectrum 16. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **14**



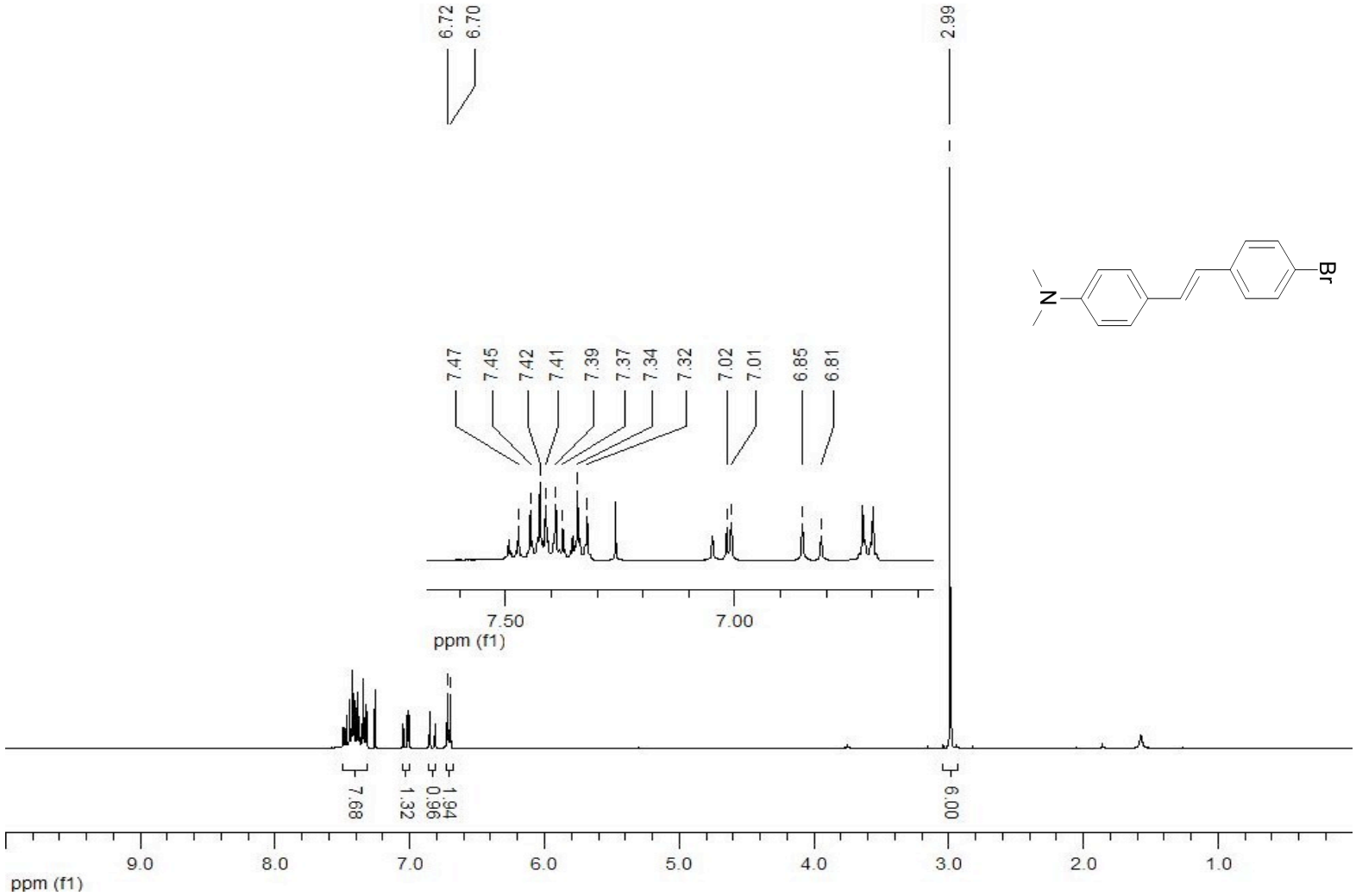
Spectrum 17. ^{13}CNMR ($CDCl_3$, 75 MHz) of compound **14**



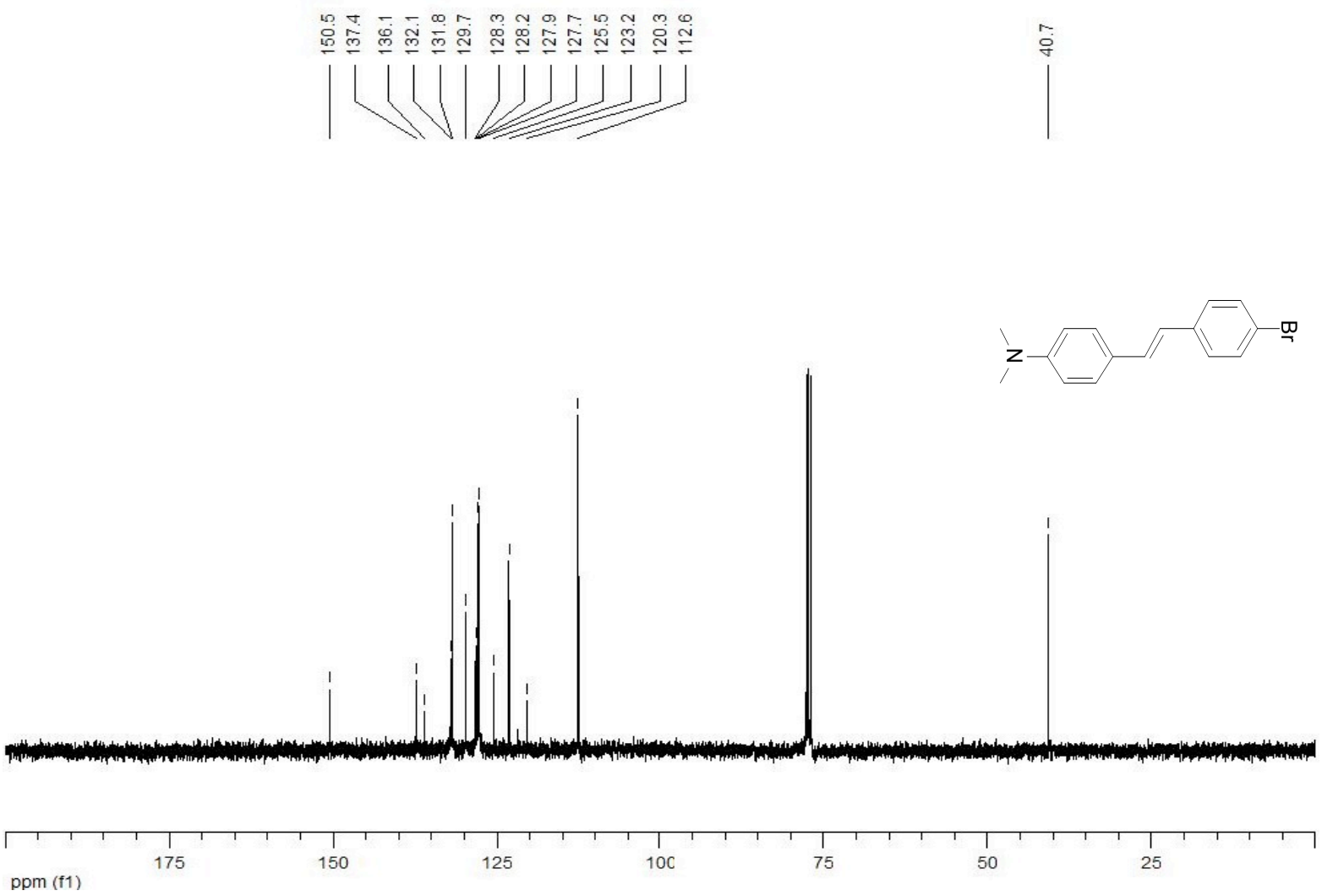
Spectrum 18. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **16**



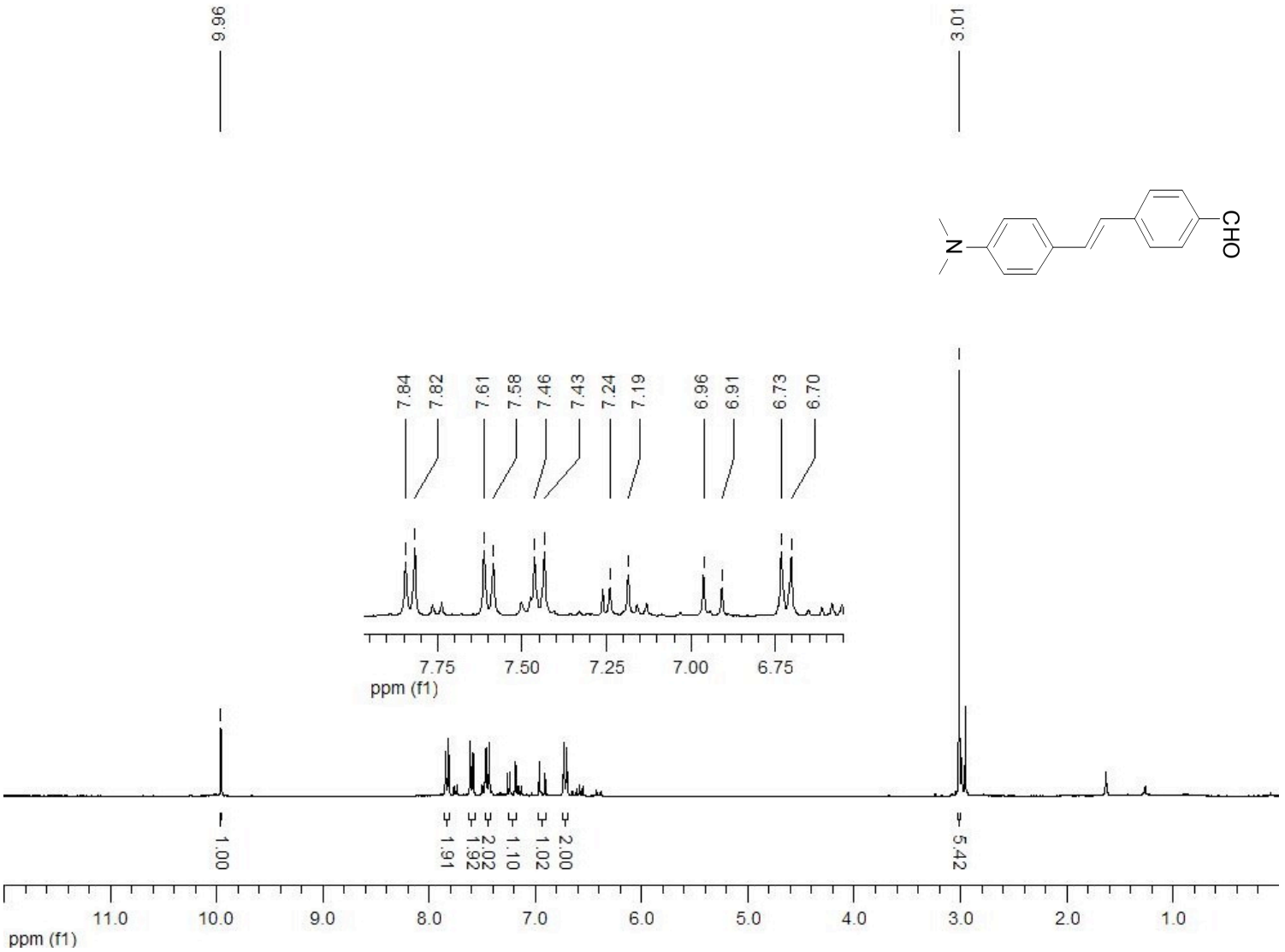
Spectrum 19. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **16**



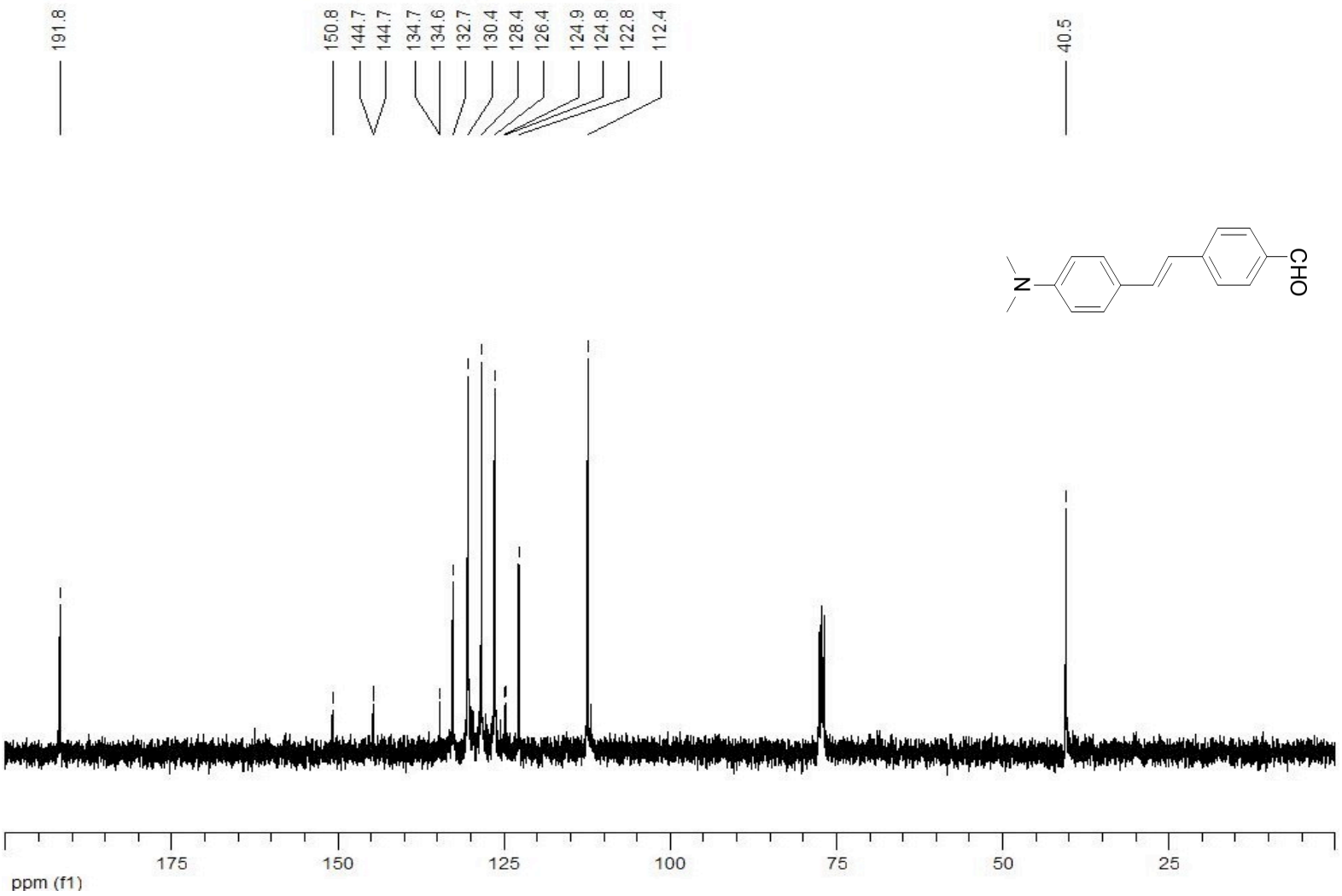
Spectrum 20. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **17**



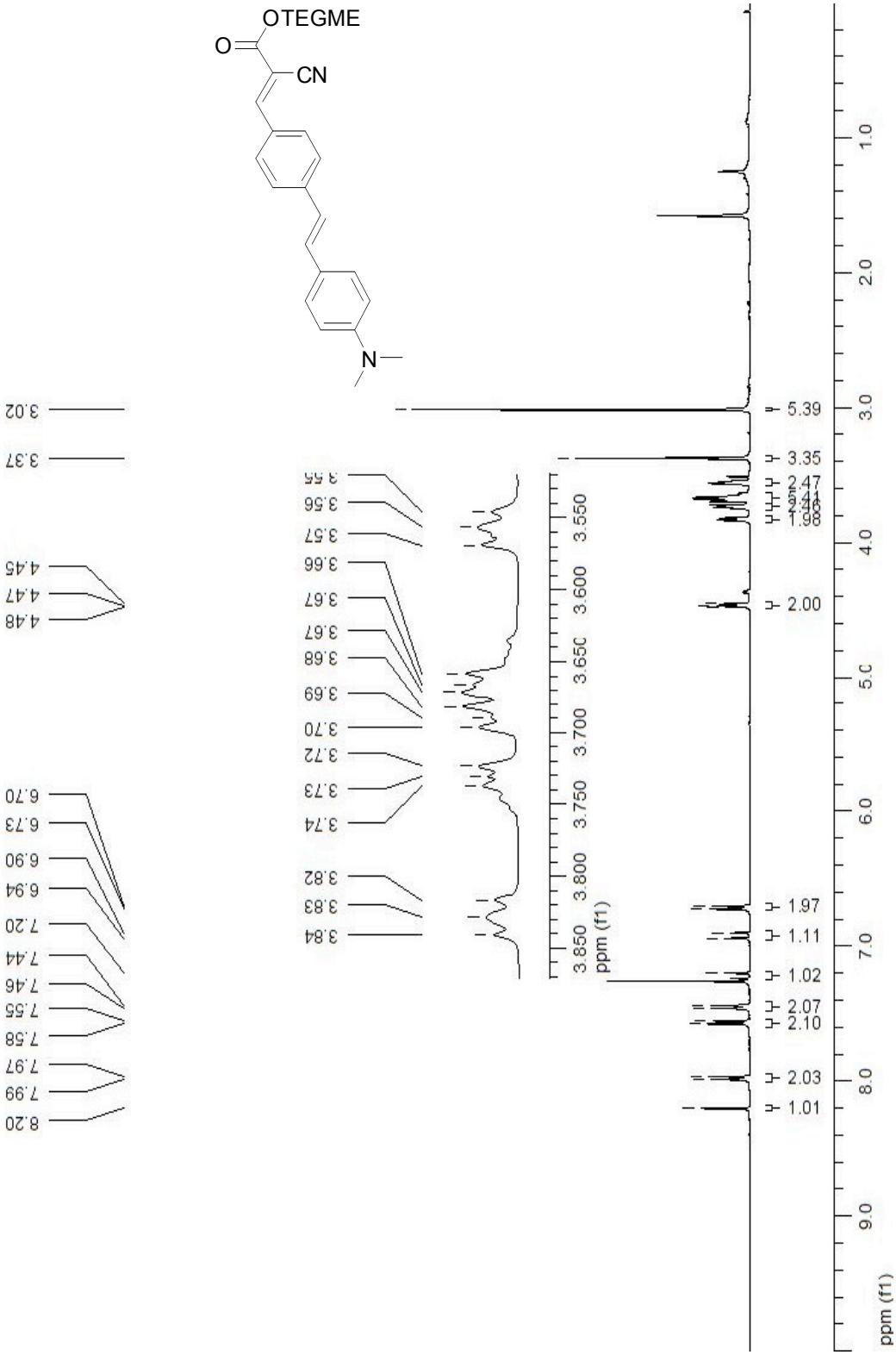
Spectrum 21. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **17**



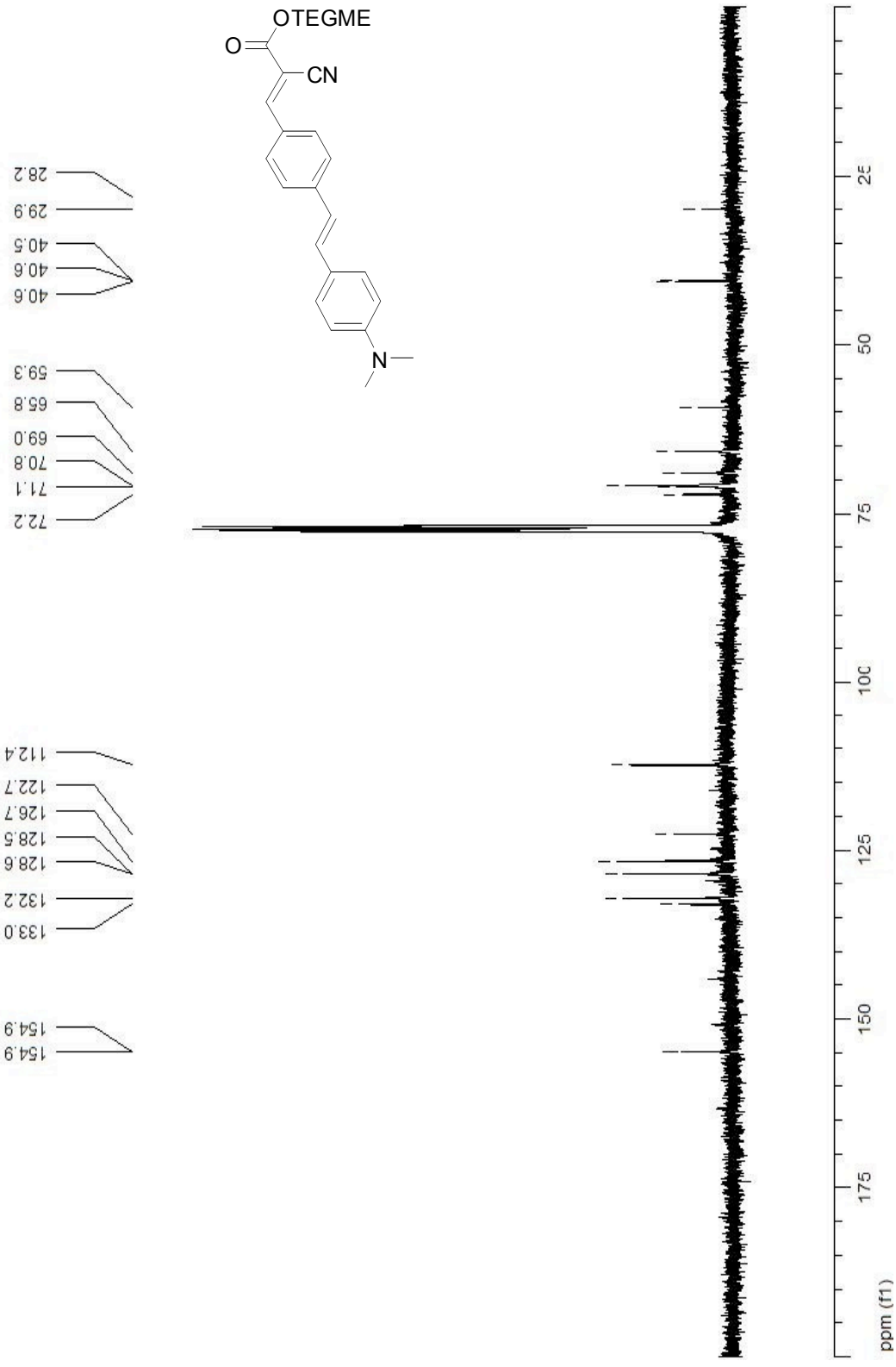
Spectrum 22. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **18**



Spectrum 23. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **18**

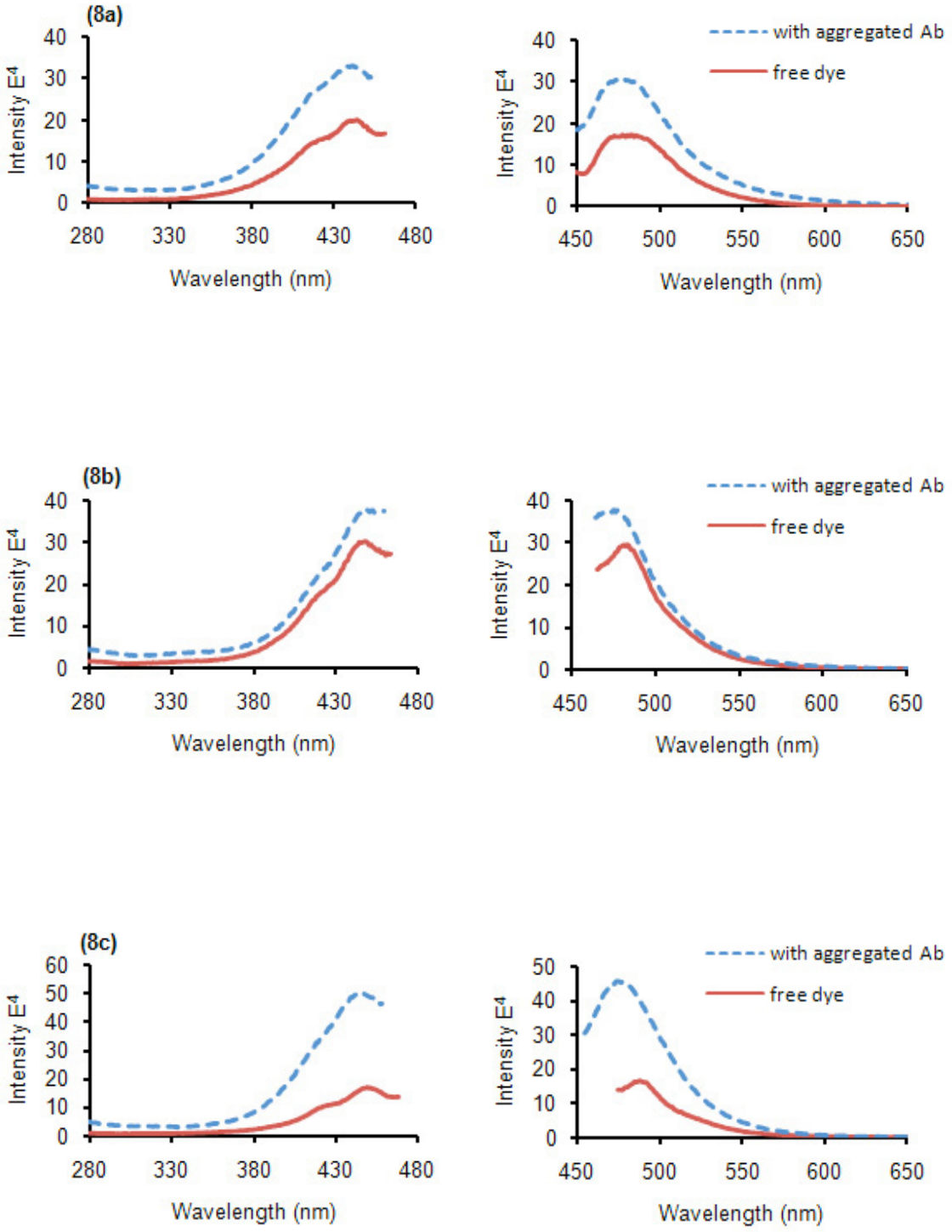


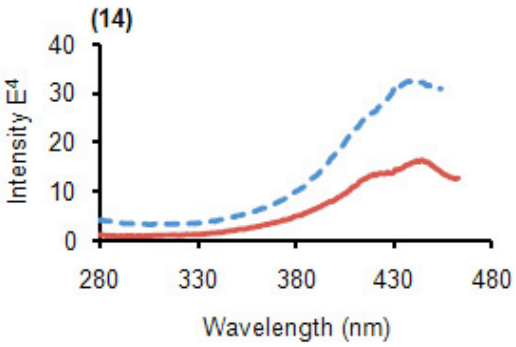
Spectrum 24. ^{1}HNMR ($CDCl_3$, 400MHz) of compound **19**

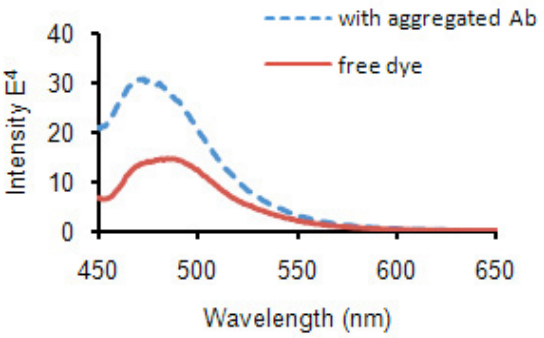


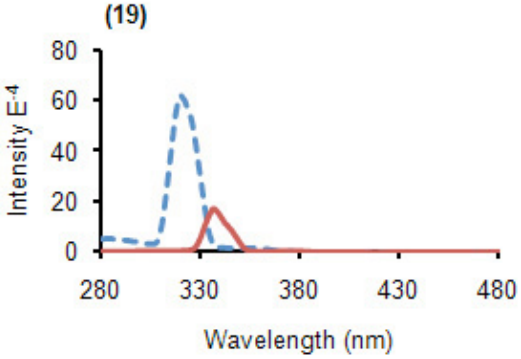
Spectrum 25. ^{13}CNMR ($CDCl_3$, 100MHz) of compound **19**

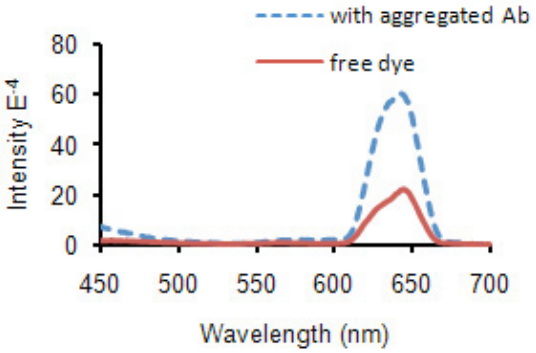
Fluorescence excitation spectra (left) and emission spectra (right) for compounds 8a-8c, 14 and 19 with aggregated Aβ(1-42) fibril



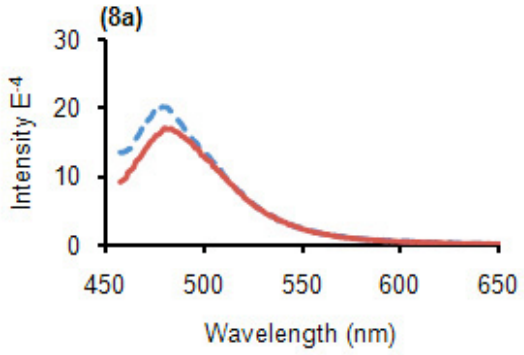

(14)
Intensity E-4
40
30
20
10
0
280
330
380
430
480
Wavelength (nm)

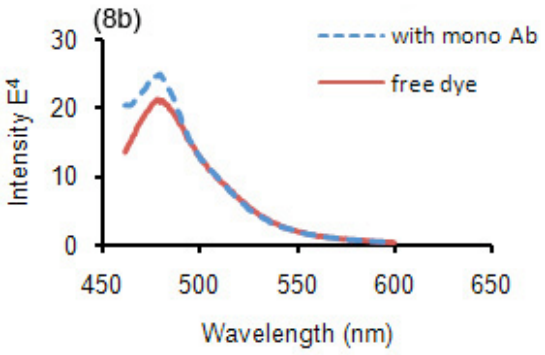

with aggregated Ab
free dye
Intensity E-4
40
30
20
10
0
450
500
550
600
650
Wavelength (nm)

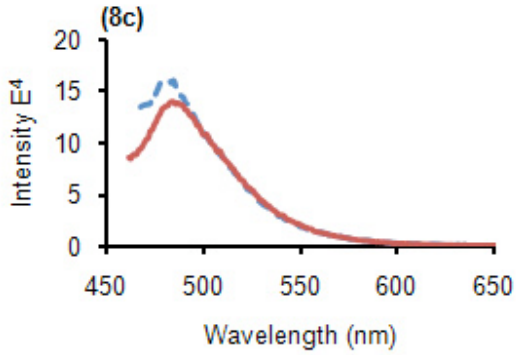

(19)
Intensity E-4
80
60
40
20
0
280
330
380
430
480
Wavelength (nm)

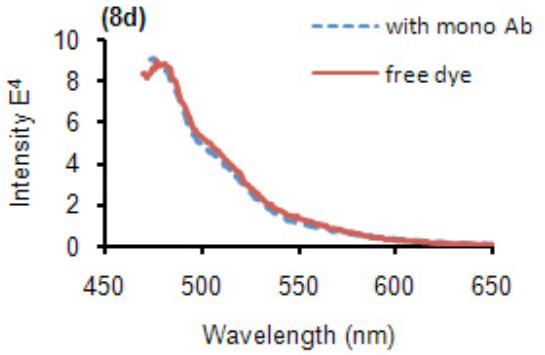

with aggregated Ab
free dye
Intensity E-4
80
60
40
20
0
450
500
550
600
650
700
Wavelength (nm)

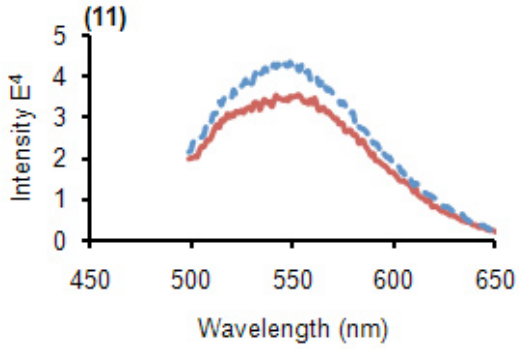
Fluorescence emission spectra of compounds 8a-8d, 11, 14 and 19 with monomeric Aβ(1-42) fibril

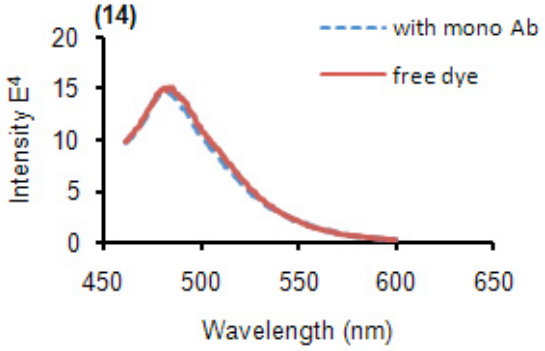


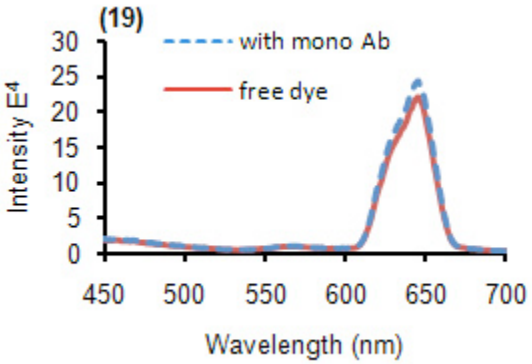




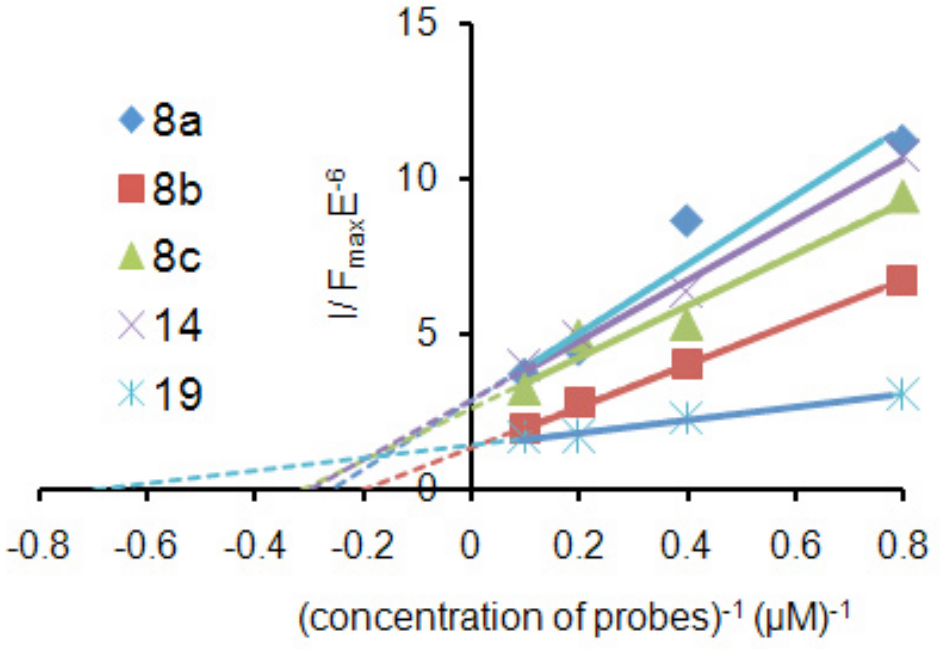




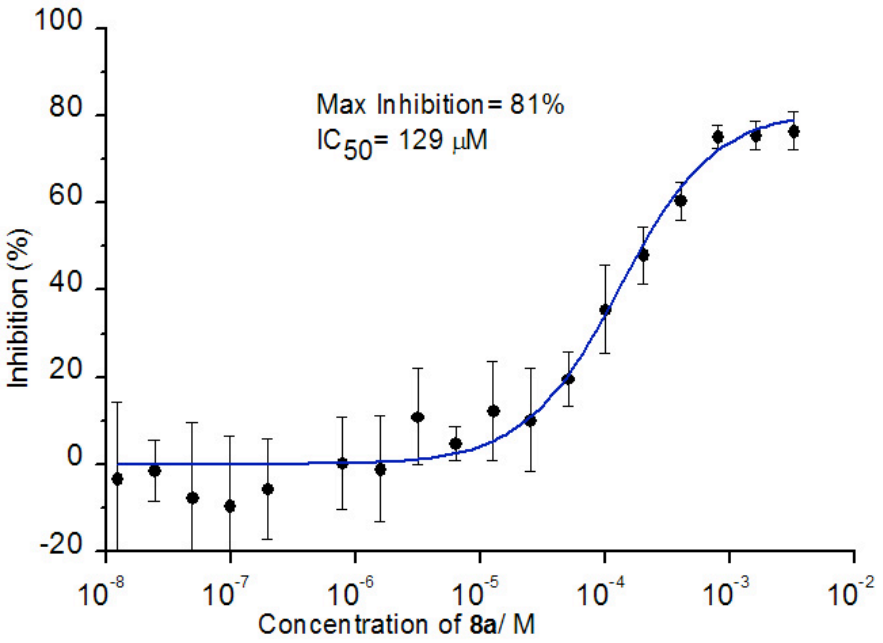


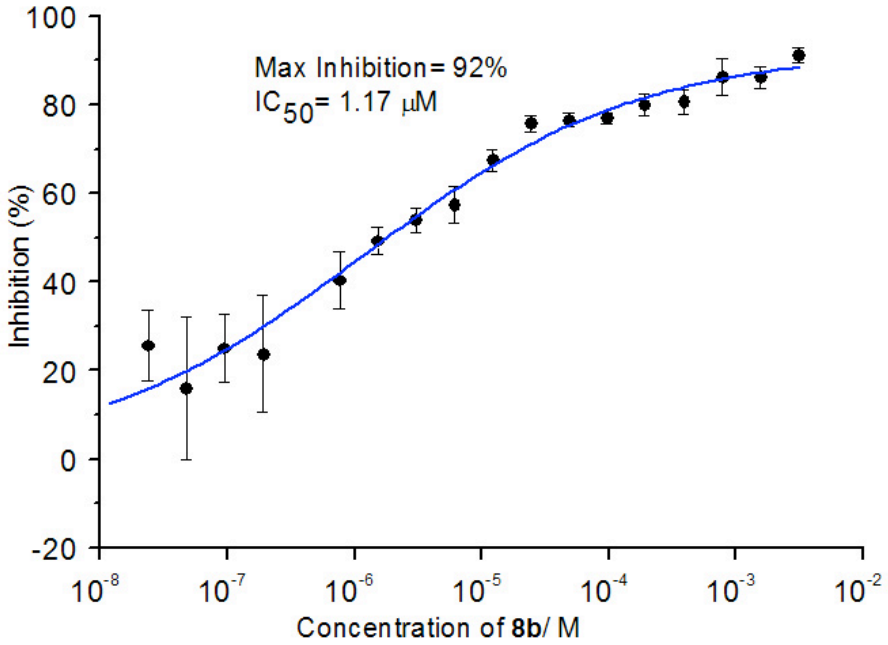

(19)
with mono Ab
free dye
Intensity E4
30
25
20
15
10
5
0
450
500
550
600
650
700
Wavelength (nm)

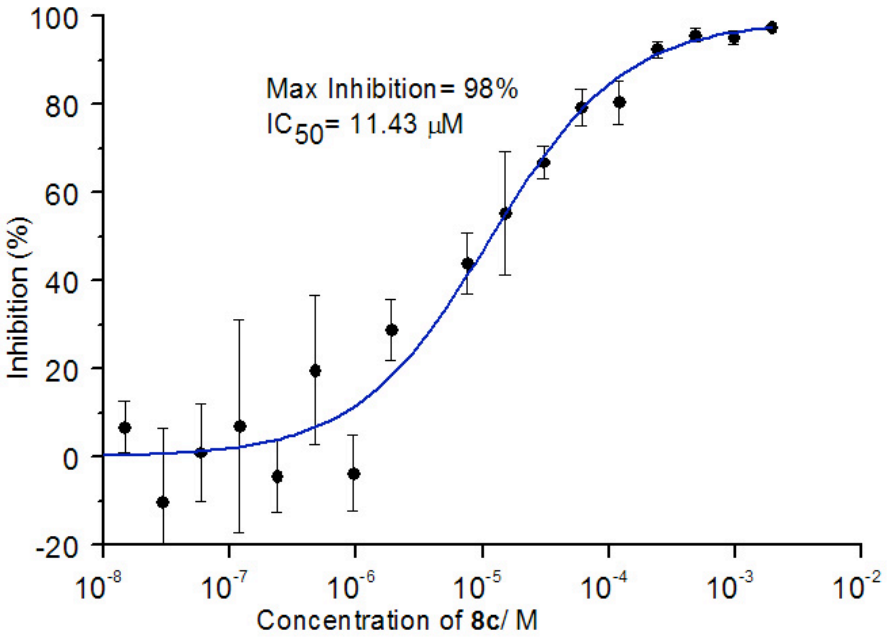
Double reciprocal of fluorescence maxima and concentration of compounds 8a-8c, 14 and 19

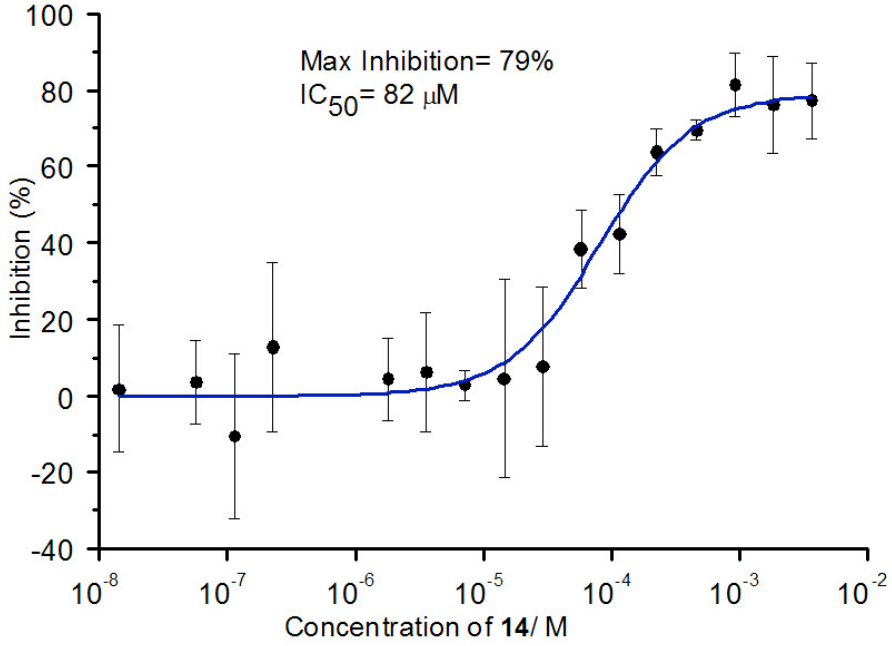


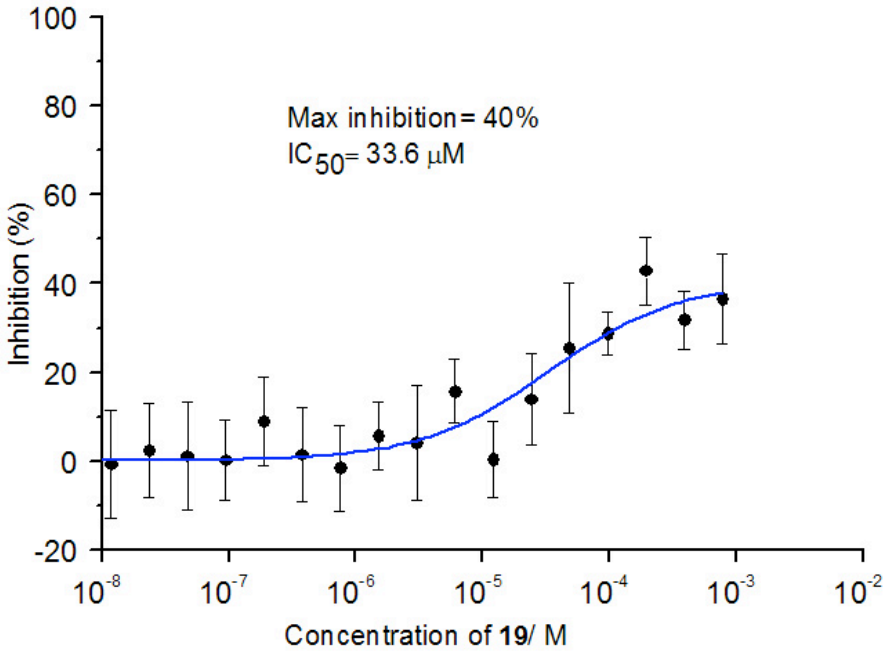
Inhibition maximum and IC_{50} of compounds 8a-8c, 14 and 19






Max Inhibition= 98%
IC50= 11.43 μM
Inhibition (%)
Concentration of 8c/ M


Max Inhibition= 79%
IC50= 82 μM
Inhibition (%)
Concentration of 14/ M


Max inhibition = 40%
IC50 = 33.6 μM
Inhibition (%)
100
80
60
40
20
0
-20
10-8
10-7
10-6
10-5
10-4
10-3
10-2
Concentration of 19/ M

Cytotoxicity data of compounds 8a- 8d, 11 and 14 on SH-SY5Y human neuroblastoma cells as determined by MTT assay