Novel zerumbone-amide-triazole hybrids as potential NF-κB pathway inhibitors: design, synthesis, cytotoxicity evaluation, computational studies, and mechanistic insights

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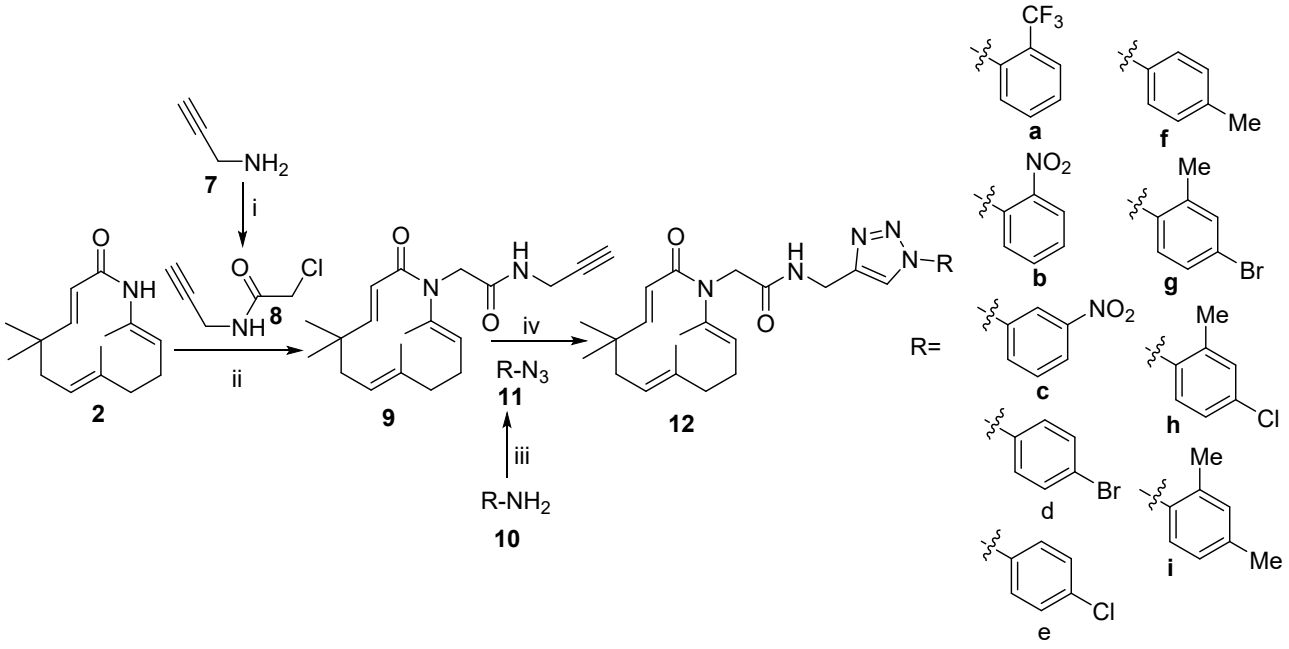
General: All reactions were performed in the appropriate oven-dried glass apparatus and under nitrogen atmosphere. Unless otherwise stated, solvents and chemicals were obtained from commercial sources and used without further purification. Column chromatography was performed using silica gel (60Å, particle size 40-60 µm). NMR spectra were recorded on a Bruker Advance I (600 MHz). Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) in hertz (Hz). High resolution mass spectrometry analysis (HRMS) was recorded on a SCIEX X500 QTOF instrument.



Scheme 1. *Reagents and conditions*: i, 2-chloroacetyl chloride (1.1 equiv.), saturated solution of $NaHCO_3$/EtOAc 1/1, v/v, 0 °C, 1 h, 91%; ii, 1.0 equiv. **8**, 3.0 equiv. NaH, THF, ultrasound 40 kHz, room temperature, 2h, 79%; iii, 3.0 equiv. $NaNO_2$, HCl 10%, room temperature, 2h, 77-96%; iv, 1.0 equiv. R-N_3(**11**), 0.1 equiv. CuI, *t*-BuOH, reflux, 12h, 65-85%.

The synthesis of 2-chloro-N-(prop-2-yn-1-yl)acetamide (TC161) (8)

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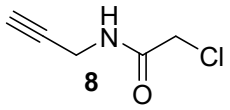
A solution of prop-2-yn-1-amine (1.0 equiv.) in saturated $NaHCO_3$/EtOAc (1:1, v/v, 15 mL) was added to 2-chloroacetyl chloride (1.1 equiv.) at 0 °C under magnetic stirring. The reaction progress was monitored by TLC (5% EtOAc in hexane). After 1 h, the reaction mixture was concentrated and extracted with CH_2Cl_2. The organic layer was washed with water and saturated brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane/EtOAc, 95:5) to afford 2-chloro-N-(prop-2-yn-1-yl)acetamide (**8**) in 91% yield.

Compound (2-chloro-N-(prop-2-yn-1-yl)acetamide) (8) (TC161)

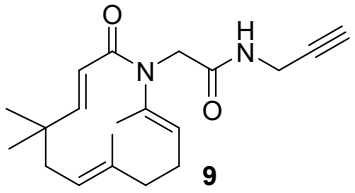


8

White powder, m.p. 61-63 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 4.10 (2H, q, *J*=2.4 Hz); 4.07 (2H, s); 2.28 (1H, t, *J*=2.4 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm: 165.6; 78.5; 72.2; 42.3; 29.6. HR-ESI-MS: Found *m/z* 132.0213; Calcd. for $C_5H_7ClNO^+$: 132.0216 $[M+H]^+$.

The synthesis of *N*-(prop-2-yn-1-yl)-2-((3*E*,7*E*,11*E*)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (9) (TC164)

A solution of azazerumbone II (**2**) (1.0 equivalent) in THF (20 mL) was added to the amide compound **8** (1.0 equivalent) followed by NaH (3.0 equivalent) at 0 °C temperature. The mixture was carried out in ultrasonic condition at room temperature and 40 kHz. After completion (2h), the mixture of the reactions was concentrated and extracted with CH_2Cl_2. The organic phase was washed with water and saturated bine. Drying of the organic phase (Na_2SO_4), filtration of the drying agent, and evaporation of the solvent in vacuo afforded crude compounds. The crude mass obtained was purified by column chromatography on silica gel (Hexane-EtOAc, 90:10) to obtain pure compounds **9** in pure form (79% yield).



9

White powder, m.p. 98-100 °C. IR (KBr) ν_{max}/cm^{-1} 3297; 3252; 3079; 2924; 2855; 2100; 1690; 1665; 1630; 1610; 1549; 1377; 1270; 1170; 977; 648. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 6.91 (1H, s); 6.45 (1H, d, *J*=16.2 Hz); 5.89 (1H, d, *J*=16.2 Hz); 5.11 (1H, t, *J*=7.2 Hz); 5.00 (1H, t, 6.0 Hz); 4.06 (2H, s); 4.00 (2H, q, *J*=3 Hz); 2.17-2.29 (6H, m); 2.18 (1H, t, *J*=2.4 Hz); 1.48 (3H, s); 1.60 (3H, s); 1.11 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$) δ ppm: 169.2; 168.3; 150.3; 134.3; 133.9; 133.1; 122.1; 79.5; 71.1; 49.8; 39.4; 38.7; 36.7; 31.2; 29.9; 28.5; 25.4; 15.2; 15.0. HR-ESI-MS: Found *m/z* 329.2228; Calcd. for $C_{20}H_{29}N_2O_2^+$: 329.2229 $[M+H]^+$.

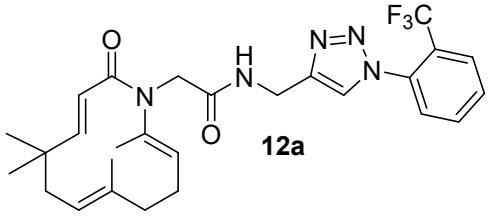
General procedure for the synthesis of compounds 11a-i

A solution of compound amine (1.0 equiv.) in 10% aqueous HCl was treated with $NaNO_2$ (3.0 equiv.) at room temperature under continuous stirring. The reaction progress was monitored by TLC using 5% EtOAc in hexane as the eluent. After 2 h, the reaction mixture was concentrated under reduced pressure and extracted with CH_2Cl_2. The organic layer was washed successively with water and saturated brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to give the crude product. Purification by silica gel column chromatography (hexane/EtOAc, 95:5) afforded the target compound **11a-i** in 77-96% yield.

General procedure for the synthesis of compounds 12a-i

A solution of compound **9** (1.0 equivalent) in THF (10 mL) was added to the azid **11a-i** (1.0 equivalent) followed by CuI (0.1 equivalent) at room temperature. The mixture was magnetically stirred at reflux and the progress of the reaction was monitored by TLC using 80% ethyl acetate in hexane. After completion (12h), the mixture of the reactions was concentrated and extracted with CH_2Cl_2. The organic phase was washed with water and saturated bine. Drying of the organic phase (Na_2SO_4), filtration of the drying agent, and evaporation of the solvent in vacuo afforded crude compounds. The crude mass obtained was purified by column chromatography on silica gel (Hexane-EtOAc, 10:90) to obtain pure compounds **12a-i** in pure form (65-85% yield).

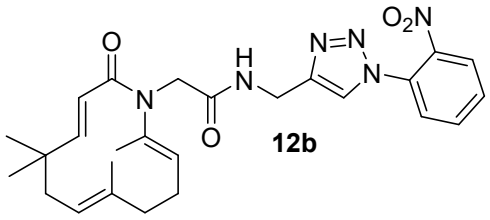
Compound 2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)-N-((1-(2-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide (12a) (TC192)



12a

White powder, m.p. 155-156 ^oC. IR (KBr) ν_{max}/cm^{-1} 3277; 3069; 2916; 2855; 1665; 1635; 1551; 1380; 1314; 1259; 1166; 1136; 768; 581. ^1H NMR (600 MHz, $CDCl_3$-d_1) δ ppm: 7.83 (1H, d, *J*=7.8 Hz); 7.80 (1H, s); 7.73 (1H, t, *J*=7.8 Hz); 7.67 (1H, t, *J*=7.8 Hz); 7.52 (1H, d, *J*=7.8 Hz); 7.15 (NH, t, *J*=5.4 Hz); 6.43 (1H, d, *J*=16.2 Hz); 5.89 (1H, d, *J*=16.2 Hz); 5.11 (1H, t, *J*=7.2 Hz); 5.00 (1H, t, *J*=6.0 Hz); 4.61 (2H, t, *J*=6.0 Hz); 4.07 (2H, bs); 2.15-2.25 (6H, m); 1.82 (3H, s); 1.59 (3H, s); 1.09 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-d_1) δ ppm: 169.6; 168.2; 150.4; 144.7; 136.0; 134.3 (q, *J*=26.0 Hz); 133.9; 133.1; 133.0; 130.4; 129.0; 127.3; 125.0; 122.0 (CF_3, q, *J*=285.0 Hz); 121.0; 120.0; 119.0; 49.6; 43.0; 39.3; 38.7; 36.9; 30.1; 29.7; 26.3; 15.2; 15.0. HR-ESI-MS: Found *m/z* 516.2583; Calcd. For $C_{27}H_{33}F_3N_5O_2^+$: 516.2586 $[M+H]^+$.

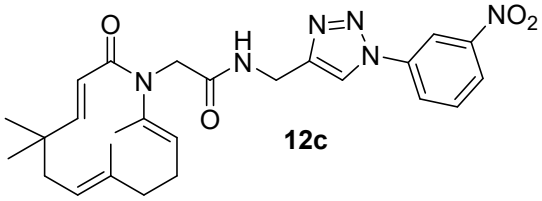
Compound N-((1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (12b) (TC184.1)



12b

White powder, m.p. 159-161 °C. IR (KBr) ν_{max}/cm^{-1} 3340; 3068; 2987; 2957; 2852; 1671; 1620; 1548; 1420 1384; 1361; 1257; 1235; 1045; 976; 849; 745. ^{1}H NMR (600 MHz, $CDCl_3$-*d_1*) δ ppm: 8.06 (1H, dd, *J*=1.8; 8.4 Hz); 7.86 (1H, s); 7.79 (1H, t, *J*=7.2 Hz); 7.76 (1H, t, *J*=7.2 Hz); 7.59 (1H, dd, *J*=1.8, 8.4 Hz); 7.11 (NH, t, *J*=5.4 Hz); 6.44 (1H, d, *J*=16.2 Hz); 5.89 (1H, d, *J*=16.2 Hz); 5.13 (1H, t, *J*=7.2 Hz); 5.01 (1H, t, *J*=6.2 Hz); 4.60 (2H, d, *J*=6.0 Hz); 4.09 (2H, s); 2.10-2.39 (6H, m); 1.83 (3H, s); 1.59 (3H, s); 1.10 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d_1*) δ ppm: 169.7; 168.2; 150.4; 145.6; 144.5; 134.2; 133.9; 133.7; 133.1; 130.6; 130.3; 127.8; 125.5; 125.0; 123.9; 122.0; 49.6; 39.3; 38.7; 36.7; 34.9; 30.1; 29.5; 25.5; 15.2; 15.0. HR-ESI-MS: Found *m/z* 493.2565; Calcd. For $C_{26}H_{33}N_6O_4^+$: 493.2563 $[M+H]^+$.

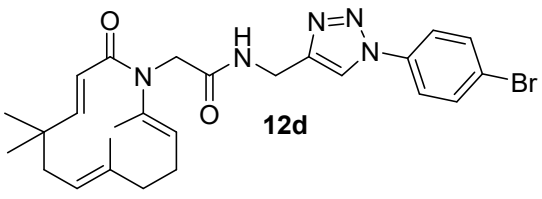
Compound *N*-((1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (12c) (TC185)



12c

White powder, m.p. 193-195 °C. IR (KBr) ν_{max}/cm^{-1} 3445; 3129; 3055; 2920; 1676; 1627; 1529; 1378; 1347; 1298; 1225; 1071; 1044; 811; 777; 741. ^{1}H NMR (600 MHz, $CDCl_3$-*d_1*) δ ppm: 8.62 (1H, t, *J*=1.8 Hz); 8.28 (1H, dd, *J*=1.2; 8.4 Hz); 8.21 (1H, s); 8.17 (1H, dd, *J*=1.2; 8.4 Hz); 7.73 (1H, *t*, J=8.4 Hz); 7.10 (NH, t, *J*=0.6 Hz); 6.46 (1H, d, *J*=16.2 Hz); 5.90 (1H, d, J=16.2 Hz); 5.12 (1H, t, *J*=7.2 Hz); 5.00 (1H, t, *J*=6.6 Hz); 4.60 (2H, d, *J*=6.0 Hz); 4.05 (2H, bs); 2.08-2.40 (6H, m); 1.85 (3H, s); 1.94 (3H, s); 1.10 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d_1*) δ ppm: 169.9; 168.2; 150.5; 149.0; 146.7; 137.8; 134.4; 133.9; 133.0; 130.8; 125.7; 123.0; 122.0; 120.7; 115.1; 49.8; 39.4; 38.7; 36.7; 35.1; 30.1; 29.9; 25.5; 15.2; 151.1. HR-ESI-MS: Found *m/z* 493.2563; Calcd. For $C_{26}H_{33}N_6O_4^+$: 493.2563 $[M+H]^+$.

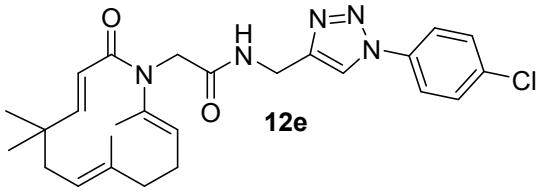
Compound *N*-((1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (12d) (TC193)



12d

White powder, m.p. 180-182 °C. IR (KBr) ν_{max}/cm^{-1} 3449; 3307; 3080; 2951; 2841; 1686; 1658; 1622; 1559; 1497; 1388; 1425; 1226; 1049; 847. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 8.05 (1H, s); 7.65 (2H, d, *J*=8.0 Hz); 7.61 (2H, d, *J*=8.0 Hz); 7.08 (NH, s); 6.43 (1H, d, *J*=16.2 Hz); 5.87 (1H, d, *J*=16.2 Hz); 5.08 (1H, t, *J*=7.2 Hz); 4.99 (1H, t, *J*=6.0 Hz); 4.58 (2H, d, *J*=6.0 Hz); 4.05 (2H, s); 2.08-2.50 (6H, m); 1.83 (3H, s); 1.58 (3H, s); 1.10 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.8; 168.2; 150.0; 146.7; 136.0; 134.3; 133.9; 133.0; 132.8 (2xC); 125.0; 122.3; 122.0; 121.8 (2xC); 120.0; 49.6; 39.4; 38.6; 36.7; 35.0; 30.0; 29.5; 25.5; 15.2; 15.0. HR-ESI-MS: Found *m/z* 526.1820 (100%); 528.1798 (94.3%); Calcd. For $C_{26}H_{33}BrN_5O_2^+$: 526.1818 (100%); 528.1797 (97.3%) $[M+H]^+$.

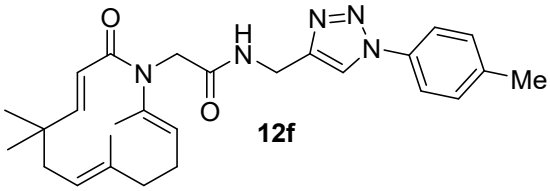
Compound N-((1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (12e) (TC190)



12e

White powder, m.p. 175-177 °C. IR (KBr) ν_{max}/cm^{-1} 3312; 3079; 2953; 2925; 1658; 1622; 1557; 1502; 1427; 1389; 1227; 1048; 849; 818. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 8.00 (1H, s); 7.68 (2H, d, *J*=8.5 Hz); 7.48 (2H, d, *J*=8.5 Hz); 7.09 (NH, t, *J*=6.0 Hz); 6.42 (1H, d, *J*=16.2 Hz); 5.88 (1H, d, *J*=16.2 Hz); 5.08 (1H, t, *J*=7.2 Hz); 4.99 (1H, t, *J*=6.0 Hz); 4.57 (2H, d, *J*=6.0 Hz); 4.05 (2H, bs); 2.10-2.30 (6H, s); 1.83 (3H, s); 1.58 (3H, s); 1.10 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.7; 168.1; 150.0; 146.0; 135.5; 134.4; 134.3; 133.9; 133.0; 129.8 (2xC); 125.0; 122.0; 121.5 (2xC); 120.5; 49.6; 39.3; 38.6; 36.6; 35.0; 30.1; 29.6; 25.5; 15.2; 15.0. HR-ESI-MS: Found *m/z* 482.2325 (100%); 484.2290 (30.65%); Calcd. For $C_{26}H_{33}ClN_5O_2^+$: 482.2323 (100.0%), 484.2293 (32.0%) $[M+H]^+$.

Compound *2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)-N-((1-(p-tolyl)-1H-1,2,3-triazol-4-yl)methyl)acetamide (12f) (TC196)*

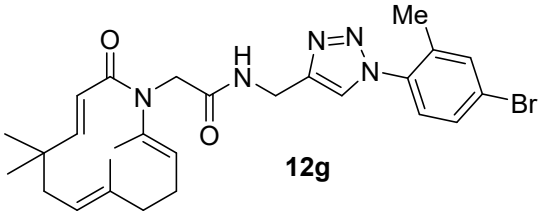


12f

White powder, m.p. 152-153 °C. IR (KBr) ν_{max}/cm^{-1} 3315; 3082; 2954; 1660; 1626; 1556; 1425; 1386; 1222; 1049; 969; 842; 819. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 7.97 (1H, s); 7.59 (2H, d, *J*=8.4 Hz); 7.28 (2H, d, *J*=8.4 Hz); 7.19 (NH, t, *J*=6.0 Hz); 6.42 (1H, d, *J*=16.2 Hz); 5.87 (1H, d, *J*=16.2 Hz); 5.09 (1H, t, *J*=7.2 Hz); 4.99 (1H, t, *J*=6.0 Hz); 4.58 (2H, d, *J*=6.0 Hz); 4.06 (2H, bs); 2.41 (3H, s); 2.10-2.40 (6H, m); 1.82 (3H, s); 1.58 (3H, s); 1.09 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.6; 168.0; 150.2; 145.5; 136.6; 134.7; 133.8; 133.0;

130.1 (2xC); 124.9; 122.0; 120.9; 120.5; 120.2 (2xC); 49.4; 40.1; 39.3; 36.6; 35.0; 29.8; 29.6; 25.4; 21.3; 15.1; 15.0. HR-ESI-MS: Found *m/z* 462.2871; Calcd. For $C_{27}H_{36}N_5O_2^+$: 462.2869 $[M+H]^+$.

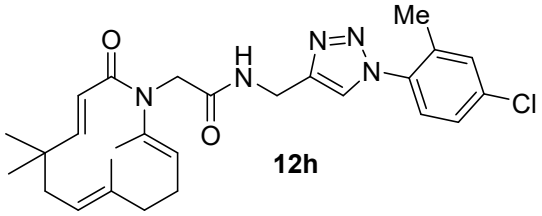
Compound N-((1-(4-bromo-2-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (12g) (TC189)



12g

White powder, m.p. 157-159 °C. IR (KBr) ν_{max}/cm^{-1} 3340; 3121; 3073; 2926; 2845; 1669; 1622; 1526; 1493; 1381; 1180; 1045; 974; 847; 822. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 7.72 (1H, s); 7.52 (1H, d, *J*=1.8 Hz); 7.45 (1H, dd, *J*=1.8; 8.4 Hz); 7.20 (1H, d, *J*=8.4 Hz); 7.13 (NH, t, *J*=6.0 Hz); 6.42 (1H, d, *J*=16.2 Hz); 5.89 (1H, d, *J*=16.2 Hz); 5.12 (1H, t, *J*=7.2 Hz); 5.00 (1H, t, *J*=6.0 Hz); 4.59 (2H, d, *J*=6.0 Hz); 4.05 (2H, bs); 2.20 (3H, s); 2.10-2.30 (6H, m); 1.83 (3H, s); 1.59 (3H, s); 1.10 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.6; 168.1; 150.3; 145.1; 135.6; 135.5; 134.3 (2xC); 133.9; 133.0; 129.9; 127.3; 125.0; 123.8; 123.6; 122.0; 49.6; 39.4; 38.6; 36.7; 35.0; 30.0; 29.5; 25.5; 17.8; 15.2; 15.0. HR-ESI-MS: Found *m/z* 540.1976 (100%); 542.1955 (93.06%); Calcd. For $C_{27}H_{35}BrN_5O_2^+$: 540.1974 (100.0%), 542.1954 (97.3%) $[M+H]^+$.

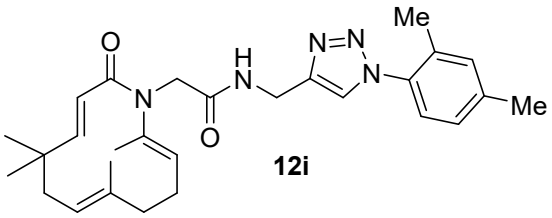
Compound N-((1-(4-chloro-2-methylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (12h) (TC183a)



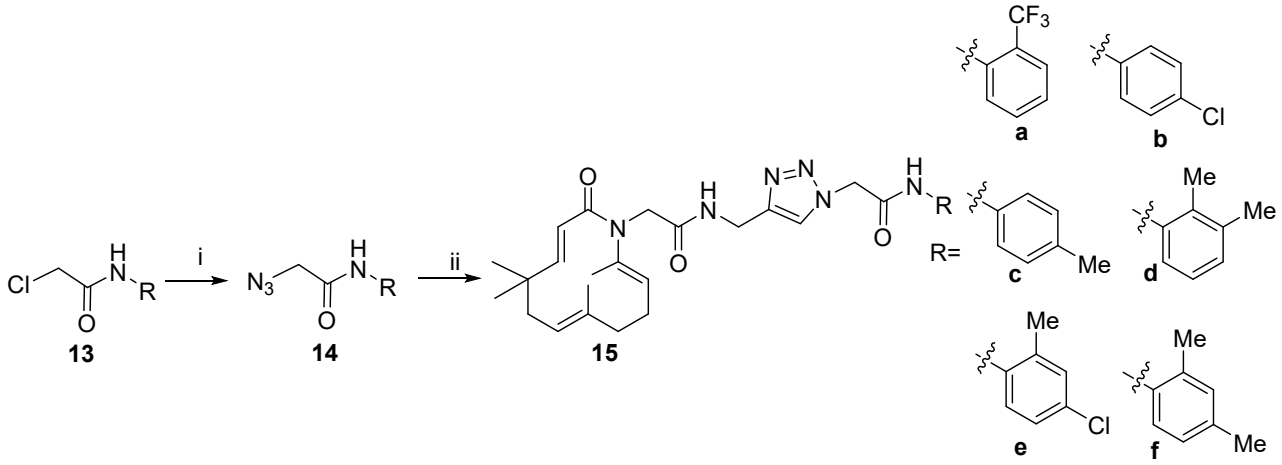
12h

White powder, m.p. 161-163 °C. IR (KBr) ν_{max}/cm^{-1} 3333; 3119; 3074; 2926; 2847; 1671; 1621; 1525; 1497; 1381; 1256; 1182; 1046; 975; 845; 822. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 7.72 (1H, s); 7.36 (1H, d, *J*=1.8 Hz); 7.30 (1H, dd, *J*=1.8; 8.4 Hz); 7.25 (1H, s); 7.12 (NH, t, *J*=6.0 Hz); 6.42 (1H, d, *J*=16.2 Hz); 5.89 (1H, d, *J*=16.2 Hz); 5.11 (1H, t, *J*=7.2 Hz); 5.00 (1H, t, *J*=6.0 Hz); 4.59 (2H, d, *J*=6.0 Hz); 4.06 (1H, bs); 2.19 (3H, s); 2.15-2.30 (6H, m); 1.83 (3H, s); 1.59 (3H, s); 1.10 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.6; 168.1; 150.4; 145.1; 135.5; 135.4; 135.0; 134.3; 133.9; 133.0; 131.3; 127.1; 127.0; 125.0; 123.9; 122.0; 49.7; 39.4; 38.6; 36.9; 35.0; 30.1; 29.9; 25.5; 17.9; 15.2; 15.9. HR-ESI-MS: Found *m/z* 496.2480; Calcd. For $C_{27}H_{35}ClN_5O_2^+$: 496.2479 $[M+H]^+$.

Compound N-((1-(2,4-dimethylphenyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (12i) (TC194)



White powder, m.p. 130-132 °C. IR (KBr) ν_{max}/cm^{-1} 3344; 3120; 3072; 2959; 2926; 1670; 1619; 1530; 1423; 1384; 1257; 1216; 1047; 973; 847; 822. ^{1}H NMR (600 MHz, $CDCl_3$-d_1) δ ppm: 7.68 (1H, s); 7.18 (1H, d, *J*=7.8 Hz); 7.11 (1H, d, *J*=7.8 Hz); 7.10 (NH, s); 6.42 (1H, d, *J*=16.2 Hz); 5.88 (1H, d, *J*=16.2 Hz); 5.17 (1H, t, *J*=7.2 Hz); 5.00 (1H, t, *J*=6.0 Hz); 4.60 (2H, d, *J*=6.0 Hz); 4.07 (2H, bs); 2.39 (3H, s); 2.15 (3H, s); 2.10-2.45 (6H, m); 1.82 (3H, s); 1.59 (3H, s); 1.10 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-d_1) δ ppm: 169.5; 168.1; 150.3; 145.1; 139.8; 134.3; 134.0; 133.9; 133.2; 133.0; 132.0; 127.3; 125.7; 125.0; 123.9; 122.0; 49.6; 39.4; 38.6; 36.7; 35.0; 29.9; 29.5; 25.5; 21.1; 17.7; 15.2; 15.0. HR-ESI-MS: Found *m/z* 476.3030; Calcd. For $C_{28}H_{38}N_5O_2^+$: 476.3026 $[M+H]^+$.



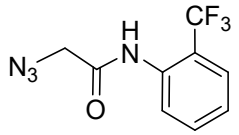
Scheme 2. *Reagents and conditions*: i, 3.0 equiv. NaN_3, acetone:H_2O 4/1, v/v, room temperature, 1.5 h, 85-90%; ii, 1.0 equiv. **9**, 0.1 equiv. CuI, *t*-BuOH, reflux, 12h, 72-93%.

General procedure for the synthesis of compounds 14a-f

A solution of compound **9** (1.0 equivalent) in acetone:H_2O (4:1, v/v; 10 mL) was added to sodium azide (3.0 equivalents) at room temperature. The reaction mixture was magnetically stirred at ambient temperature, and the progress of the reaction was monitored by TLC using 60% hexane in ethyl acetate as the mobile phase. After completion (1.5 h), the reaction mixture was concentrated under reduced pressure and subsequently extracted with CH_2Cl_2. The organic layer was washed with distilled water and saturated brine, then dried over anhydrous Na_2SO_4. After filtration of the drying agent and evaporation of the solvent under vacuum, the crude product was obtained. The residue was purified by silica gel column chromatography

using a hexane/EtOAc (80:20) eluent system to afford the target compounds **14a-f** in pure form with yields ranging from 85% to 90% (Scheme 2).

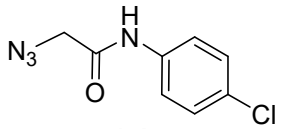
Compound 2-azido-N-(2-(trifluoromethyl)phenyl)acetamide (14a) (TC210)



14a

85%, yellow powder, m.p. 63-65 °C. IR (KBr) ν_{max}/cm^{-1} 3280; 3060; 2953; 2865; 2112; 1675; 1591; 1536; 1456; 1319; 1279; 1173; 1115; 1059; 768; 647; 596. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 8.49 (NH, bs); 8.20 (1H, d, *J*=8.4 Hz); 7.63 (1H, d, *J*=8.4 Hz); 7.56 (1H, t, *J*=8.4 Hz); 7.26 (1H, t, *J*=8.4 Hz); 4.16 (2H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 165.0; 134.2; 132.9; 126.9 (q, *J*=26.0 Hz); 126.3; 125.11; 124.9 (CF3, q, *J*=285.0 Hz); 124.7.

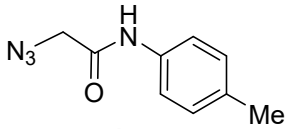
Compound 2-azido-N-(4-chlorophenyl)acetamide (14b) (TC208)



14b

88 %, white powder, m.p. 67-69 °C. IR (KBr) ν_{max}/cm^{-1} 3320; 2920; 2850; 2107; 1683; 1609; 1546; 1492; 1402; 1306; 1252; 1197; 1090; 825. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 7.99 (NH, s); 7.50 (2H, dd, *J*=1.8; 7.0 Hz); 7.31 (2H, dd, *J*=1.8, 7.0 Hz); 4.15 (2H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 164.4; 135.3; 130.1; 129.1 (2xC); 121.2 (2xC); 52.9.

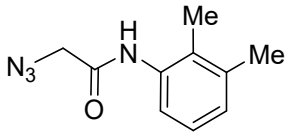
Compound 2-azido-N-(p-tolyl)acetamide (14c) (TC203)



14c

90 %, yellow powder, m.p. 98-99 °C. IR (KBr) ν_{max}/cm^{-1} 3198; 3083; 2919; 2104; 1662; 1612; 1548; 1513; 1420; 1310; 1199; 1041; 937; 815. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 8.06 (NH, s); 7.40 (2H, d, *J*=8.4 Hz); 7.12 (2H, d, *J*=8.4 Hz); 4.08 (2H, s); 2.31 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 164.5; 134.7; 134.2; 129.5 (2xC); 120.1 (2xC); 52.9; 20.8.

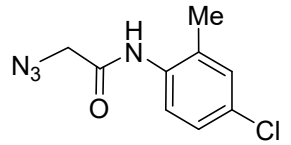
***Compound* 2-azido-N-(2,3-dimethylphenyl)acetamide (14d) (TC201)**



14d

86 %, dark yellow powder, m.p. 83-84 °C. IR (KBr) ν_{max}/cm^{-1} 3272; 3060; 2921; 2853; 2101; 1661; 1607; 1585; 1545; 1461; 1422; 1273; 1213; 996; 937; 789; 709. ^{1}H NMR (600 MHz, $CDCl_3$-*d_1*) δ ppm: 7.94 (NH, s); 7.54 (1H, d, *J*=7.8 Hz); 7.11 (1H, t, *J*=7.8 Hz); 7.04 (1H, d, *J*=7.8 Hz); 4.17 (2H, s); 2.30 (3H, s); 2.16 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d_1*) δ ppm: 164.7; 137.5; 134.2; 128.9; 127.8; 126.0; 121.5; 53.1; 20.5; 13.5. HR-ESI-MS: Found *m/z* 205.1081; Calcd. For $C_{10}H_{13}N_4O^+$: 205.1089 $[M+H]^+$.

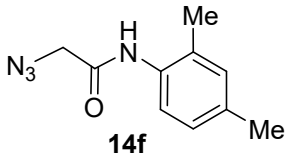
Compound 2-azido-N-(4-chloro-2-methylphenyl)acetamide (TC14e) (TC198)



14e

89 %, dark yellow powder, m.p. 99-100 °C. IR (KBr) ν_{max}/cm^{-1} 3170; 2923; 2852; 2107; 1688; 1601; 1540; 1495; 1402; 1308; 1250; 1190; 1091; 826. ^{1}H NMR (600 MHz, $CDCl_3$-*d_1*) δ ppm: 7.92 (NH, s); 7.82 (1H, d, *J*=8.4 Hz); 7.19 (1H, s); 7.18 (1H, t, *J*=8.4 Hz); 4.18 (2H, s); 2.26 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d_1*) δ ppm: 164.4; 133.3; 130.6; 130.3; 126.8; 123.6; 53.10; 17.43.

Compound 2-azido-N-(2,4-dimethylphenyl)acetamide (14f) (TC200)



14f

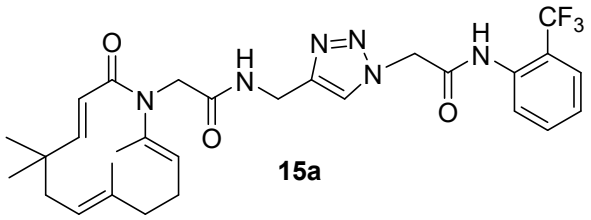
86 %, dark yellow powder, m.p. 85-87 °C. IR (KBr) ν_{max}/cm^{-1} 3281; 2923; 2853; 2103; 1661; 1596; 1546; 1531; 1498; 1446; 1273; 1036; 988; 825. ^{1}H NMR (600 MHz, $CDCl_3$-*d_1*) δ ppm: 7.85 (NH, s); 7.66 (1H, d, *J*=7.8 Hz); 7.02 (1H, d, *J*=7.8 Hz); 7.01 (1H, s); 4.16 (2H, s); 2.29 (3H, s); 2.24 (3H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d_1*) δ ppm: 164.4; 135.5; 132.0; 131.2; 129.2; 127.4; 122.9; 53.1; 20.8; 17.5.

General procedure for the synthesis of compounds 15a-f

A solution of compound **13** (1.0 equivalent) in THF (10 mL) was added to the azid **14a-f** (1.0 equivalent) followed by CuI (0.1 equivalent) at room temperature. The mixture was magnetically stirred at reflux and the progress of the reaction was monitored by TLC using 80% ethyl acetate in hexane. After completion (12h), the mixture of the reactions was concentrated and extracted with CH_2Cl_2. The organic phase was washed with water and saturated bine. Drying of the organic phase (Na_2SO_4), filtration of the drying agent, and evaporation of the solvent in vacuo afforded crude compounds. The crude mass obtained was purified

by column chromatography on silica gel (Hexane-EtOAc, 15:85) to obtain pure compounds **15a-f** in pure form (72-93% yield) (Scheme 2).

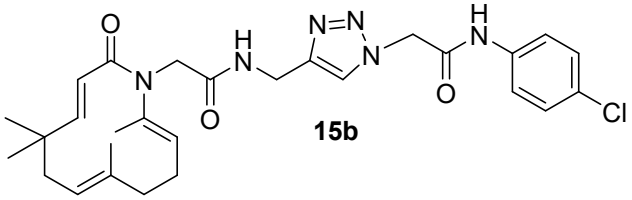
Compound N-((1-(2-oxo-2-((2-(trifluoromethyl)phenyl)amino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (15a) (TC211)



15a

Colorless viscous liquid. IR (KBr) ν_{max}/cm^{-1} 3284; 2957; 2923; 1676; 1614; 1536; 1455; 1389; 1319; 1281; 1167; 1128; 1058; 1036; 766. ^{1}H NMR (600 MHz, $CDCl_3$-d_1) δ ppm: 8.05 (1H, d, *J*=8.4 Hz), 8.04 (NH, s); 7.57 (1H, s); 7.59 (1H, t, *J*=8.4 Hz); 7.56 (1H, t, *J*=8.4 Hz); 7.28 (1H, t, *J*=8.4 Hz); 7.11 (NH, s); 6.42 (1H, d, *J*=16.2 Hz); 5.87 (1H, d, *J*=16.2 Hz); 5.21 (2H, s); 5.11 (1H, t, *J*=7.2 Hz); 4.99 (1H, t, *J*=6.0 Hz); 4.56 (2H, d, *J*=6.0 Hz); 4.03 (2H, bs); 2.10-2.40 (6H, m); 1.82 (3H, s); 1.58 (3H, s); 1.08 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-d_1) δ ppm: 169.7; 168.2; 163.5; 150.3; 134.3; 133.9; 133.0; 132.9; 131.1 (q, *J*=26.0 Hz); 129.0; 126.3; 126.2; 125.6; 125.2; 125.0 (2xC); 124.1; 122.0; 53.4; 49.6; 46.2; 39.3; 38.6; 36.6; 34.9; 29.1; 25.5; 15.2; 15.0. HR-ESI-MS: Found *m/z* 573.2796; Calcd. For $C_{29}H_{36}F_3N_6O_3^+$: 573.2801 $[M+H]^+$.

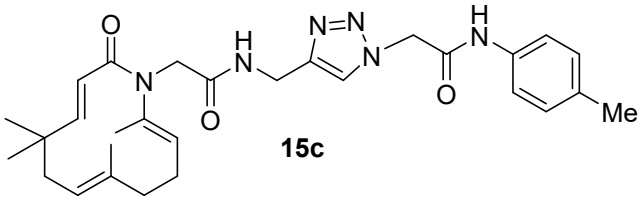
Compound N-(4-chlorophenyl)-2-(4-((2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamido)methyl)-1H-1,2,3-triazol-1-yl)acetamide (15b) (TC209)



15b

White powder, m.p. 185-186 ^{o}C. IR (KBr) ν_{max}/cm^{-1} 3447; 3060; 2922; 1699; 1651; 1618; 1554; 1493; 1399; 1252; 1170; 1090; 827. ^{1}H NMR (600 MHz, $CDCl_3$-d_1) δ ppm: 8.46 (NH, s); 7.73 (1H, s); 7.45 (2H, d, *J*=8.5 Hz); 7.27 (2H, d, *J*=8.5 Hz); 7.09 (NH, t, *J*=6.0 Hz); 6.38 (1H, d, *J*=16.2 Hz); 5.88 (1H, d, *J*=16.2 Hz); 5.16 (1H, t, *J*=7.2 Hz); 5.15 (2H, s); 4.99 (1H, t, *J*=6.0 Hz); 4.53 (2H, d, *J*=6.0 Hz); 4.03 (2H, bs); 2.10-2.40 (6H, m); 1.83 (3H, s); 1.57 (3H, s); 1.07 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-d_1) δ ppm: 169.7; 168.2; 163.0; 150.5; 134.3; 133.9; 133.1; 130.5; 130.1; 129.0 (2xC); 125.3; 124.2; 123.0; 122.0; 121.6 (2xC); 53.5; 49.7; 46.1; 39.4; 38.6; 35.1; 29.7; 25.5; 15.2; 15.1. HR-ESI-MS: Found *m/z* 539.2529; Calcd. For $C_{28}H_{36}ClN_6O_3^+$: 539.2537 $[M+H]^+$.

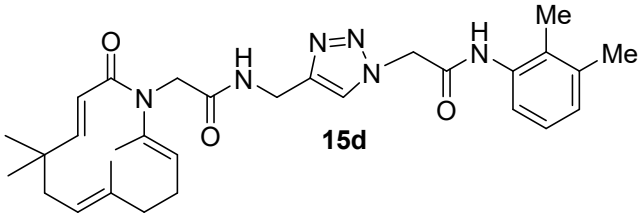
Compound N-((1-(2-oxo-2-(p-tolylamino)ethyl)-1H-1,2,3-triazol-4-yl)methyl)-2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamide (15c) (TC206)



15c

White powder, m.p. 203-204 °C. IR (KBr) ν_{max}/cm^{-1} 3340; 3258; 3193; 3059; 2948; 1695; 1657; 1618; 1555; 1516; 1384; 1255; 1216; 1171; 1055; 820. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 8.54 (NH, s); 7.71 (1H, s); 7.36 (2H, d, *J*=7.8 Hz); 7.17 (NH, t, *J*=6.0 Hz); 7.09 (2H, d, *J*=7.8 Hz); 6.39 (1H, d, *J*=16.2 Hz); 5.89 (1H, d, *J*=16.2 Hz); 5.17 (1H, t, *J*=7.2 Hz); 5.11 (2H, s); 4.98 (1H, t, *J*=6.0 Hz); 4.51 (2H, d, *J*=6.0 Hz); 4.04 (2H, bs); 2.29 (3H, s); 2.10-2.28 (6H, m); 1.82 (3H, s); 1.66 (3H, s); 1.07 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.5; 168.3; 163.0; 150.4; 134.7; 134.3; 133.9; 133.2; 130.8; 129.5 (2xC); 128.8; 125.0; 124.4; 122.2; 120.3 (2xC); 53.4; 49.6; 39.4; 38.7; 38.6; 36.7; 35.0; 28.9; 25.5; 20.8; 15.2; 15.1. HR-ESI-MS: Found *m/z* 519.3076; Calcd. For $C_{29}H_{39}N_6O_3^+$: 519.3084 $[M+H]^+$.

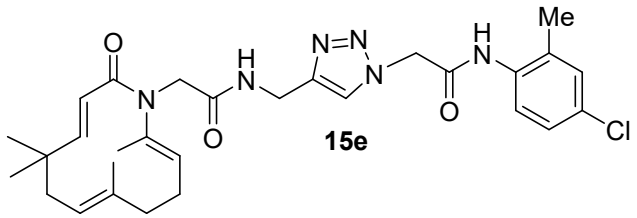
Compound N-(2,3-dimethylphenyl)-2-(4-((2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamido)methyl)-1H-1,2,3-triazol-1-yl)acetamide (15d) (TC205)



15d

White powder, m.p. 186-187 °C. IR (KBr) ν_{max}/cm^{-1} 3443; 3273; 3133; 3068; 2927; 2867; 1728; 1675; 1536; 1464; 1383; 1271; 1136; 1069; 745. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 7.93 (NH, s); 7.76 (1H, s); 7.56 (1H, d, *J*=7.8 Hz); 7.16 (NH, t, *J*=6.0 Hz); 6.98 (1H, t, *J*=7.8 Hz); 6.96 (1H, d, *J*=7.8 Hz); 6.38 (1H, d, *J*=16.2 Hz); 5.86 (1H, d, *J*=16.2 Hz); 5.16 (2H, s); 5.12 (1H, t, *J*=7.2 Hz); 4.98 (1H, t, *J*=6.0 Hz); 4.52 (2H, d, *J*=6.0 Hz); 4.02 (2H, bs); 2.26 (3H, s); 2.10-2.24 (6H, m); 2.09 (3H, s); 1.81 (3H, s); 1.58 (3H, s); 1.08 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.6; 168.2; 163.1; 150.3; 135.7; 134.4; 133.9; 133.0; 132.4; 131.2; 130.8; 128.8; 127.2; 125.0; 124.2; 123.1; 122.0; 53.9; 49.6; 39.3; 38.7; 38.6; 36.7; 35.0; 29.2; 25.5; 22.9; 20.8; 15.2; 15.0. HR-ESI-MS: Found *m/z* 533.3234; Calcd. For $C_{30}H_{41}N_6O_3^+$: 533.3240 $[M+H]^+$.

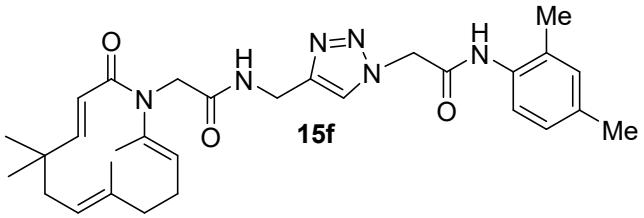
Compound N-(4-chloro-2-methylphenyl)-2-(4-((2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamido)methyl)-1H-1,2,3-triazol-1-yl)acetamide (15e) (TC207)



15e

White powder, m.p. 190-191 °C. IR (KBr) ν_{max}/cm^{-1} 3223; 3129; 3040; 2924; 2850; 1716; 1678; 1604; 1536; 1487; 1389; 1247; 1207; 1030; 965; 820; 768. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 8.18 (NH, s); 7.76 (1H, s); 7.22 (1H, d, *J*=8.5 Hz); 7.15 (1H, d, *J*=8.5 Hz); 7.14 (1H, s); 7.14 (NH, s); 6.38 (1H, dd, *J*=16.2 Hz); 5.87 (1H, d, *J*=16.2 Hz); 5.17 (2H, s); 5.13 (1H, t, *J*=7.2 Hz); 4.98 (1H, t, *J*=6.0 Hz); 4.52 (2H, d, *J*=6.0 Hz); 4.02 (1H, bs); 2.20-2.40 (6H, m); 2.12 (3H, s); 1.82 (3H, s); 1.58 (3H, s); 1.07 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.7; 168.2; 150.9; 134.3; 133.9; 133.3; 133.1; 131.4; 130.9; 130.3; 126.7; 125.5; 124.4; 124.1; 122.0; 53.5; 49.7; 39.9; 39.6; 36.6; 35.0; 29.9; 25.5; 17.5; 15.2; 15.1. HR-ESI-MS: Found *m/z* 553.2685; Calcd. For $C_{29}H_{38}ClN_6O_3^+$: 553.2694 $[M+H]^+$.

Compound N-(2,4-dimethylphenyl)-2-(4-((2-((3E,7E,11E)-5,5,8,12-tetramethyl-2-oxoazacyclododeca-3,7,11-trien-1-yl)acetamido)methyl)-1H-1,2,3-triazol-1-yl)acetamide (15f) (TC204)



15f

Colorless viscous liquid.. IR (KBr) ν_{max}/cm^{-1} 3276; 3123; 3068; 2922; 2875; 1725; 1705; 1677; 1612; 1522; 1433; 1390; 1242; 1136; 1002; 869; 823. ^{1}H NMR (600 MHz, $CDCl_3$-*d*$_1$) δ ppm: 7.93 (NH, s); 7.61 (1H, s); 7.55 (1H, d, *J*=7.8 Hz); 7.16 (NH, t, *J*=6.0 Hz); 6.97 (1H, t, *J*=7.8 Hz); 6.98 (1H, s); 6.38 (1H,d, *J*=16.2 Hz); 5.87 (1H, d, *J*=16.2 Hz); 5.16 (2H, s); 5.12 (1H, t, *J*=7.2 Hz); 4.98 (1H, t, *J*=6.0 Hz); 4.52 (2H, d, *J*=6.0 Hz);; 4.02 (2H, s); 2.21-2.60 (6H, m); 2.26 (3H, s); 2.09 (3H, s); 1.84 (3H, s); 1.58 (3H, s); 1.08 (6H, s). ^{13}C NMR (150 MHz, $CDCl_3$-*d*$_1$) δ ppm: 169.2; 168.2; 163.1; 150.3; 135.7; 134.4; 133.9; 133.0; 132.4; 131.2; 130.8; 128.8; 127.6; 125.2; 124.2; 123.1; 122.1; 53.5; 49.6; 39.3; 38.7; 38.6; 36.6; 35.0; 29.7; 25.5; 20.8; 17.5; 15.2; 15.0. HR-ESI-MS: Found *m/z* 533.3230; Calcd. For $C_{30}H_{41}N_6O_3^+$: 533.3240 $[M+H]^+$.

Cell culture and cell viability assay: The synthesized compounds (**2**, **9**, **12a-i** and **15a-f**) were evaluated for their cytotoxic activity against four human cancer cell lines: hepatocellular carcinoma (HepG2), lung carcinoma (A549), acute promyelocytic leukemia (HL-60), and gastric carcinoma (AGS), all obtained from the American Type Culture Collection (ATCC, USA). The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were used for all experiments. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which measures cellular metabolic activity as an indicator of cell proliferation and viability. Briefly, the cancer cells were seeded at a density of 1×10^5 cells/mL and treated with various concentrations of the test compounds (dissolved in DMSO): 0.125, 0.5, 2.0, 8.0, 32.0, and 128.0 µg/mL for 72 h. Following incubation, 50 µL of MTT solution (2 mg/mL) was added to each well to obtain a final amount of 0.1 mg MTT per well, and the plates were further incubated at 37°C for 4 h. The plates were then centrifuged at 1000 rpm for 10 min at room temperature, after which the supernatant was carefully removed. Subsequently, 150 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formed

formazan crystals. The absorbance was measured immediately at 540 nm using a microplate reader (TECAN GENIOUS). All experiments were performed in triplicate, and mean absorbance values were calculated. Cytotoxicity was expressed as the percentage of growth inhibition relative to untreated control cells. Dose response curves were constructed, and the half-maximal inhibitory concentration (IC_{50}) values were determined for each compound against each cell line.

Table 1 The cytotoxicity evaluation (IC_{50}) of novel zerumbone-amide-triazole hybrids.

Compounds	IC_{50} (μg/mL)			
	AGS	**HepG2**	**A549**	**HL-60**
12a	**4.72±0.14**	**4.19±0.15**	5.31±0.45	**2.60±0.12**
12b	14.23±0.55	9.34±0.42	16.46±1.29	5.35±0.22
12c	21.08±0.96	13.38±0.62	28.19±1.26	8.66±0.37
12d	**2.30±0.10**	**1.69±0.11**	**3.36±0.25**	**1.56±0.18**
12e	6.69±0.68	6.57±0.51	9.60±0.59	**3.85±0.27**
12f	**1.94±0.07**	**1.61±0.15**	**3.60±0.15**	**1.49±0.14**
12g	**1.75±0.08**	**1.19±0.03**	**2.07±0.09**	**1.31±0.45**
12h	**2.86±0.17**	**2.63±0.11**	**3.69±0.23**	**2.05±0.17**
12i	**2.15±0.06**	**1.79±0.06**	**3.43±0.21**	**1.20±0.13**
15a	5.84±0.60	6.54±0.83	7.55±0.96	**3.62±0.20**
15b	7.18±1.15	6.18±0.55	6.85±0.54	5.61±0.03
15c	**3.66±0.11**	**2.38±0.07**	**4.29±0.25**	**2.28±0.16**
15d	6.21±0.46	**2.51±0.07**	6.46±0.85	**2.35±0.11**
15e	**1.95±0.07**	**1.59±0.08**	**2.13±0.06**	**1.61±0.09**
15f	5.38±0.32	**1.85±0.10**	5.49±0.47	**1.43±0.07**
9	28.58±0.96	23.58±2.43	>100	16.60±0.83
Azazerumbone II (**2**)	43.60±2.43	35.62±3.16	68.28±3.65	14.21±1.44
Ellipticine	**0.38±0.02**	**0.33±0.02**	**0.43±0.02**	**0.32±0.01**

Table 2 Molecular docking analysis and $\Delta G_{binding}$ of protein with all ligands

Entry	Binding energy (kcal/mol)	Interactions of the ligand with NF-κB p65	Total Interactions	$\Delta G_{binding}$ (kcal/mol)
12a	-7.4	Hydrogen bonds: Glu289 (2.92 Å), Carbon hydrogen bond: Glu289 (3.74 Å), Alkyl bonds: Ala328 (3.83 Å) pi-Sigma: Ile324 (3.73Å).	4	-12.10±0.38
12b	-7	Hydrogen bond: Phe301 (2.45 & 3.08 Å); -alkyl bond: Pro303 (4.57 Å); Pi-cation: Lys337 (3.37 Å), pi-Sigma: Phe298 (3.87 Å).	5	-9.60±0.63
12c	-7.3	Hydrogen bonds: Lys326 (3.15 Å), Glu289 (2.42 Å),; pi-Alkyl bonds: Ala328 (4.78 Å), pi-cation: Lys320 (3.83Å), pi-Sigma: Ile324 (3.77Å).	5	-15.72±0.38
12d	-6.4	Hydrogen bonds: Arg258 (3.04 & 2.92Å), Alkyl/pi-alkyl bonds: Leu349 (3.91 & 4.97 Å), pi-sigma bond: Ala260 (3.71 Å)	5	-21.45±0.44
12e	-6.6	Hydrogen bonds: Phe298 (3.07 Å), Trp295 (3.01 &3.11 Å); Carbon hydrogen bonds: Phe298 (3.58 Å), Glu296 (3.44 Å), Gly297 (3.68 Å), Tyr319 (3.19 Å), Lys320 (3.19 Å); Alkyl/pi-alkyl bonds: Pro317 (5.10 & 5.21 Å), Lys318 (4.85Å).	10	-20.38±0.25
12f	-6.6	Hydrogen bonds: Phe298 (3.08 Å), Trp295 (3.07 &3.12 Å); Carbon hydrogen bonds: Phe298 (3.55 Å), Glu296 (3.41Å), Gly297 (3.68 Å), Lys320 (3.21 Å); Alkyl/pi-alkyl bonds: Pro317 (5.23 & 5.07 Å), Lys318 (4.90Å).	10	-22.27±0.32
12g	-7.3	pi-Sigma bonds: Leu272 (3.83 Å); Tyr270 (3.67 Å); pi-Alkyl bond: Ala311 (4.80 Å), pi-pi Stacked: Phe310 (4.09 & 4.31 Å).	5	-20.76±0.32
12h	-7.3	pi-Sigma bonds: Leu272 (3.90 Å); Tyr270 (3.76 Å); pi-Alkyl bond: Ala311 (4.81 Å), pi-pi Stacked: Phe310 (3.81 & 4.29 Å).	5	-16.99±0.42
12i	-7.5	Carbon hydrogen bond: Asp300 (3.60 Å), Alkyl/pi-alkyl bonds: Phe301 (4.57 Å), Pro303 (4.80 Å), Lys318 (4.64Å).	4	-28.49±0.41

15a	-6.8	Hydrogen bonds: Lys318 (3.16 Å), Phe298 (2.64 Å), Glu296 (2.87 Å); Carbon hydrogen bond: Thr316 (3.65 Å), Pi-donor hydrogen bond: Trp295 (3.64 Å), pi-pi-T-shaped: Phe298 (5.80 Å)	6	-19.84±0.34
15b	-6.8	Hydrogen bonds: Phe301 (2.30 & 3.01 Å), pi-cation bond: Lys337 (3.23 Å); pi-Sigma: Phe298 (3.75 Å), Alkyl bond: Pro303 (4.75 Å)	5	-14.45±0.33
15c	-6.8	Hydrogen bonds: Phe301 (2.38 & 3.03 Å), pi-cation bond: Lys337 (3.24 Å); pi-Sigma: Phe298 (3.75 Å), Alkyl bond: Pro303 (4.64 Å)	5	-10.19±0.78
15d	-6.5	Hydrogen bonds: Lys318 (2.93 Å), Carbon hydrogen bond: Lys318 (3.38 Å), pi-alkyl bond: : Lys318 (4.64 Å)	3	-22.27±0.67
15e	-6.8	Hydrogen bonds: Phe301 (2.98 & 2.32 Å), Carbon hydrogen bond: Pro303 (3.69 Å), pi-cation bond: Lys337 (3.21 Å); pi-Sigma: Phe298 (3.76 Å), Alkyl bond: Pro303 (4.73 Å)	6	-20.61±0.37
15f	-6.8	Hydrogen bonds: Phe301 (2.30 & 2.99 Å), pi-cation bond: Lys337 (3.24 Å); pi-Sigma: Phe298 (3.80 Å), Alkyl bond: Pro303 (4.61 Å)	5	-22.57±0.19
9	-5.3	Hydrogen bonds: Tyr319 (2.95 Å), Lys320 (2.96 Å); Alkyl bonds: Ile324 (5.38 Å), Ala328 (4.95 Å)	4	-13.92±0.32
2	-5.6	Hydrogen bonds: Gly299 (1.74 Å); Pi-sigma: Phe298 (3.73Å)	2	-13.83± 0.34
Ellipticine	-6.8	Hydrogen bonds: Gly299 (2.52 Å); pi-pi stacked bonds: Phe298 (4.98, 5.07 & 5.95 Å).	4	

Table 3. Relative protein expression levels compared to the untreated control as determined by Western blot analysis in HepG2 cells.

Sample	**Relative protein expression levels normalized to the control**			
	NF-kB (N=2)	**IKB-α (N=2)**	**p-NF-kB (N=3)**	**p-IKB-alpha (N=3)**
Control	1.00±0.16	1.00±0.06	1.00±0.07	1.00±0.11

(ĐC)				
0.5xIC_{50}	1.14±0.10	1.09±0.38	0.90±0.13	1.06±0.07
1xIC_{50}	0.95±0.05	1.02±0.32	0.87±0.10	0.96±0.04
2xIC_{50}	0.47±0.02*	0.83±0.08	0.50±0.13**	0.63±0.08**

Data are expressed as mean ± SE and normalized to the untreated control.
*Significance: *p < 0.05, *$p < 0.01$ vs. control.*

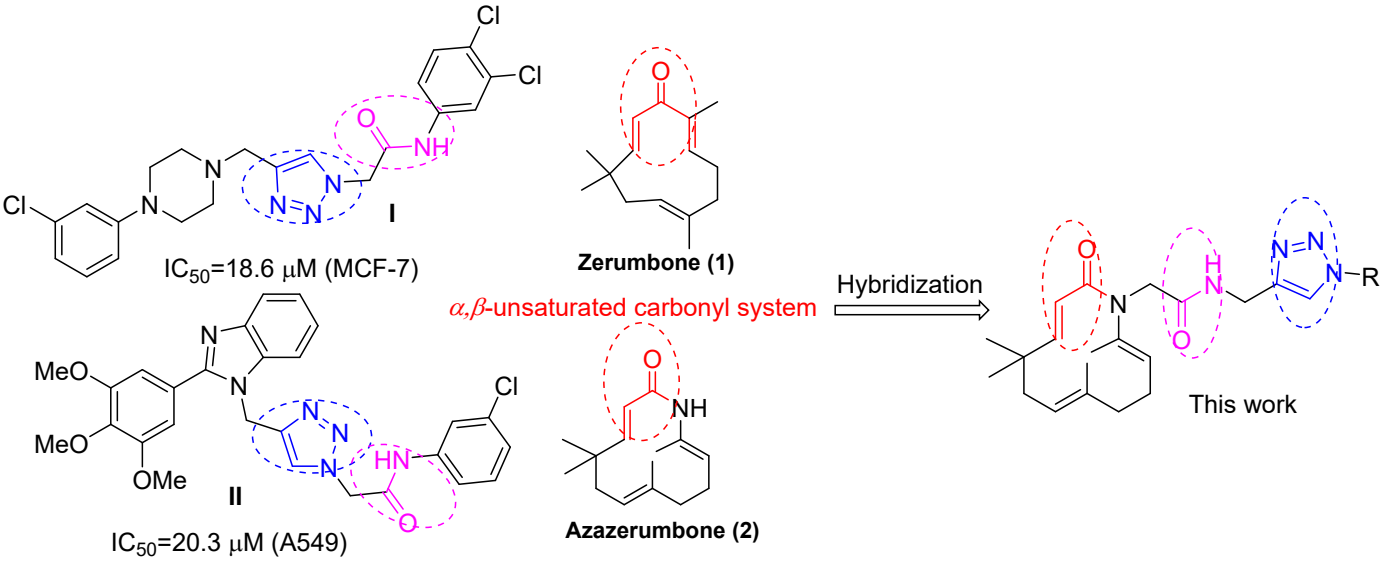
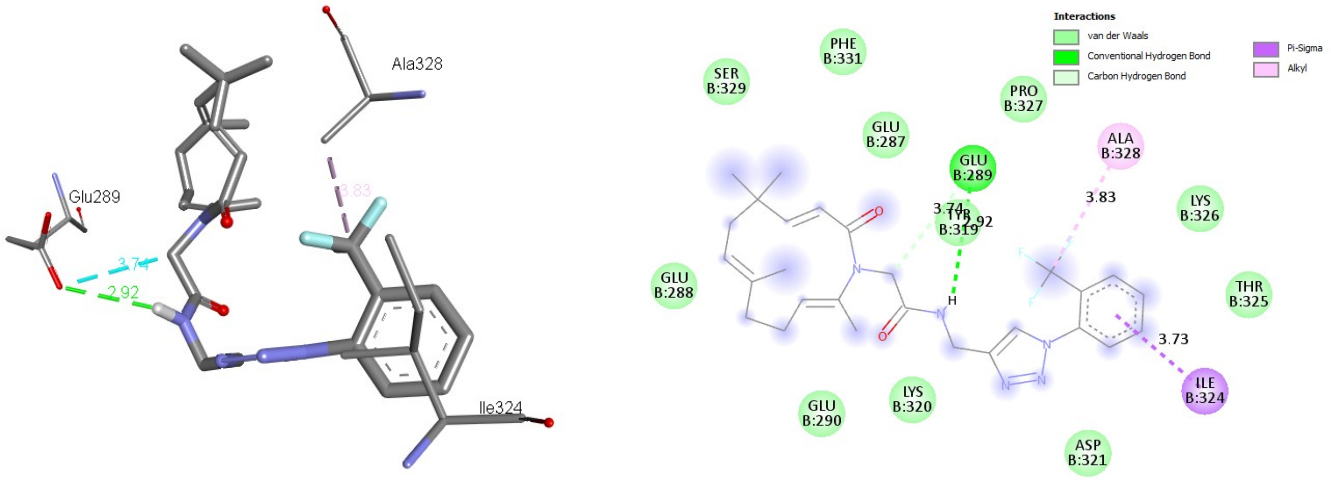


Figure 1. Design of novel zerumbone-amide-triazole hybrids as potential NF-κB pathway inhibitors



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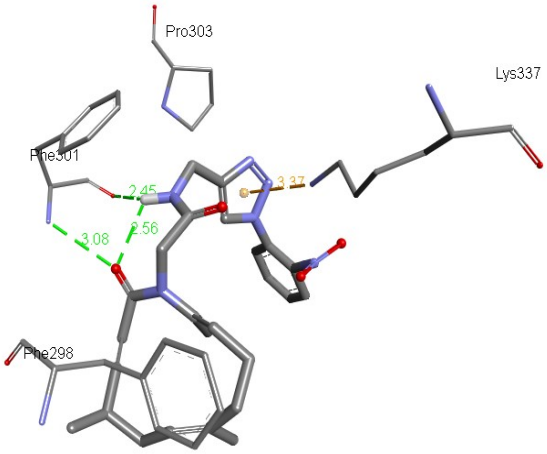
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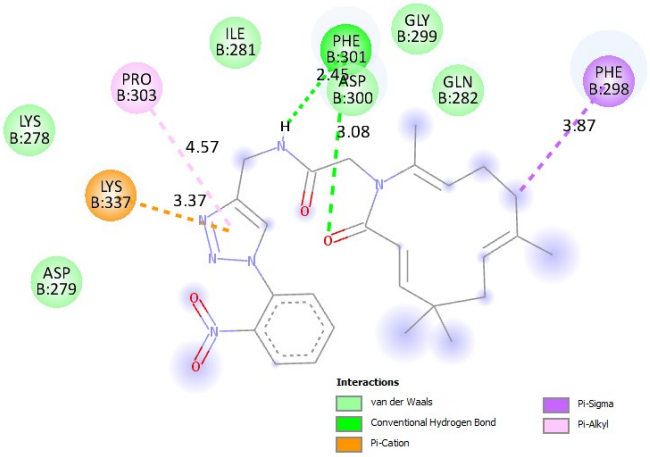
Phe298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van der Waals
Conventional Hydrogen Bond
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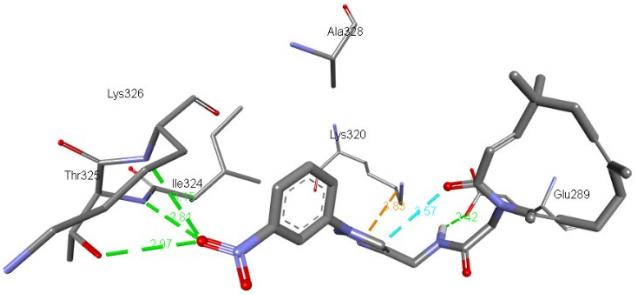
Pi-Sigma
Pi-Alkyl

12b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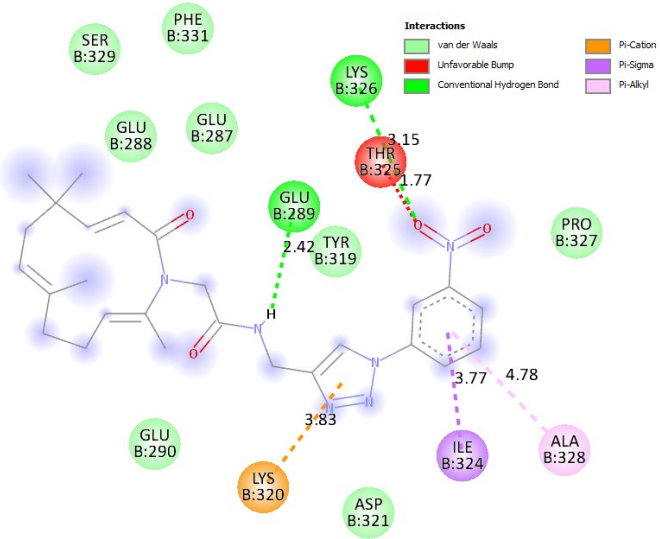
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van der Waals
Unfavorable Bump
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Pi-Sigma
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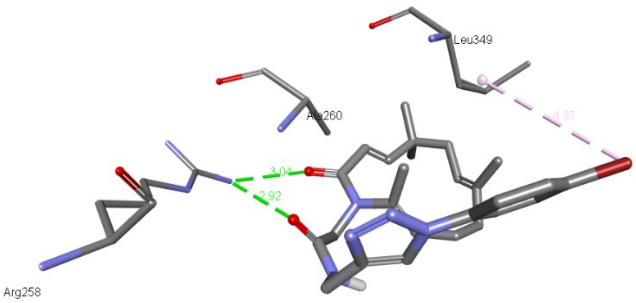
12c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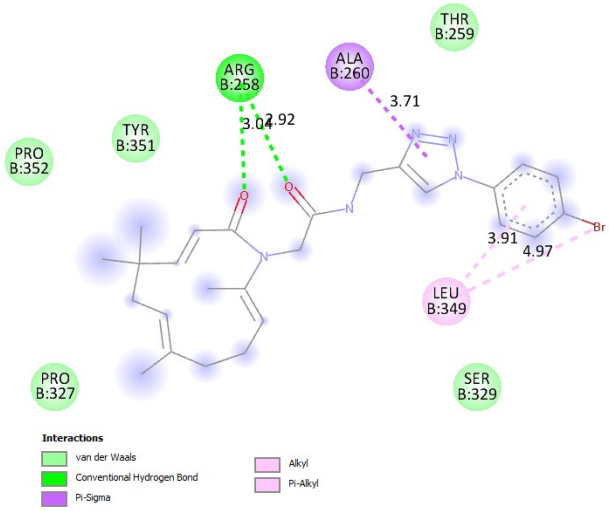
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van der Waals
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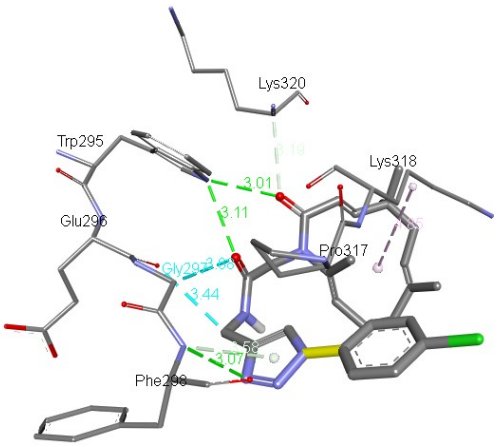
Pi-Alkyl

12d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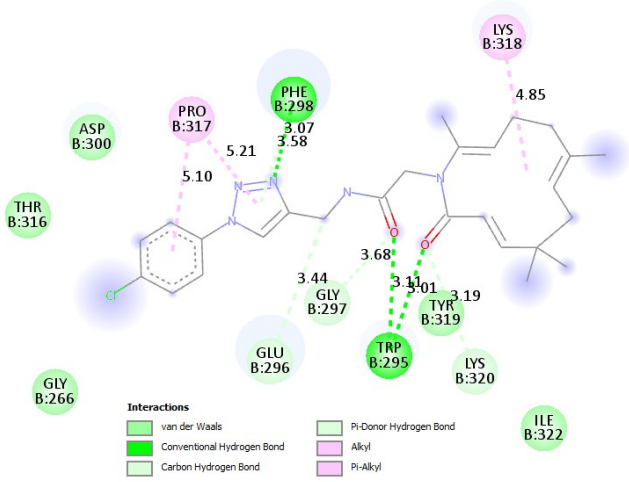
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Interactions
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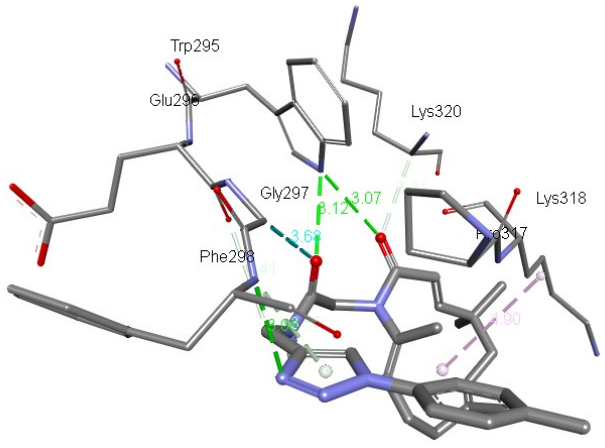
Pi-Alkyl

12e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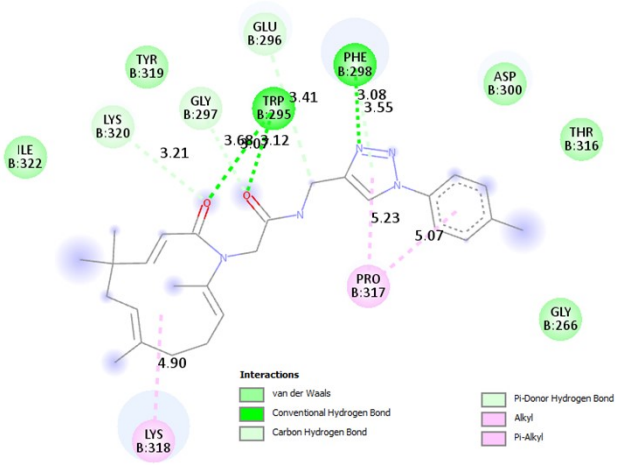
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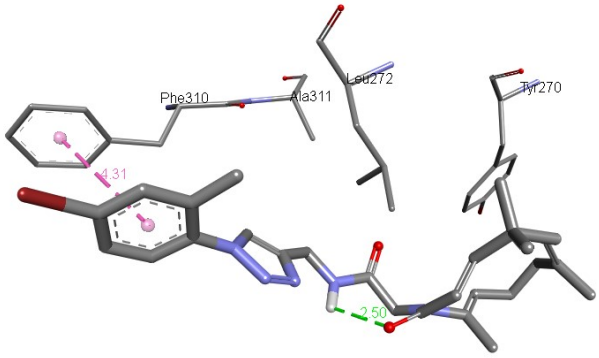
Pi-Alkyl

12f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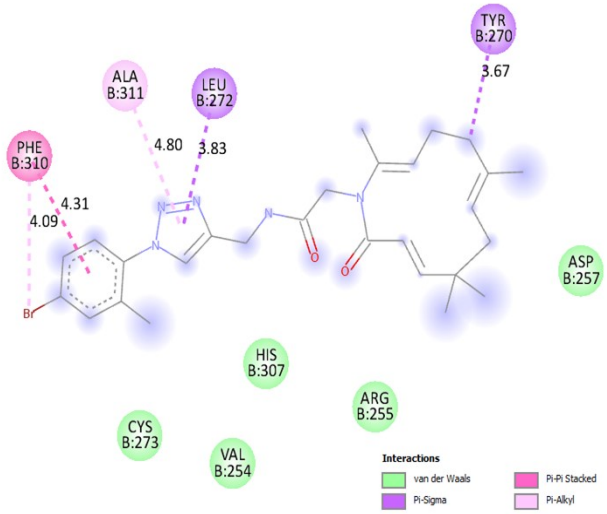
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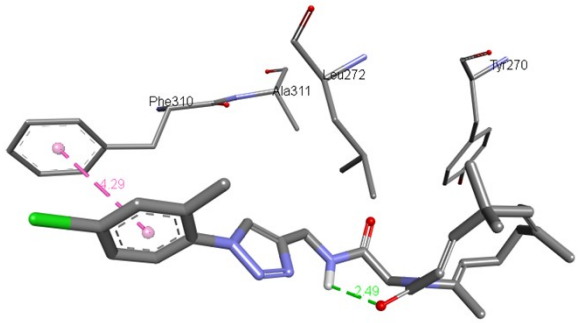
van der Waals
Pi-Pi Stacked
Pi-Sigma
Pi-Alkyl

12g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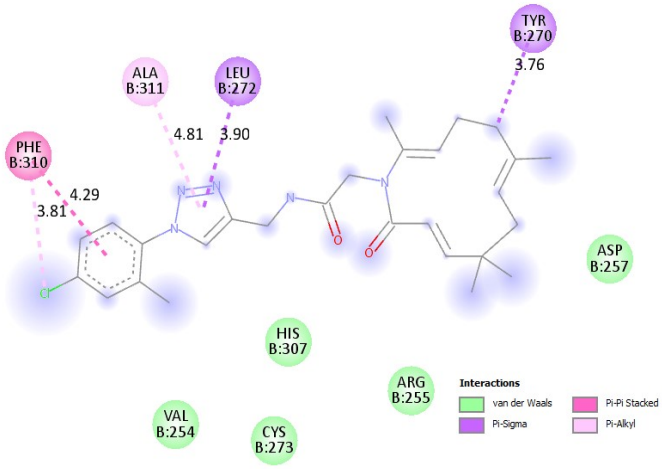
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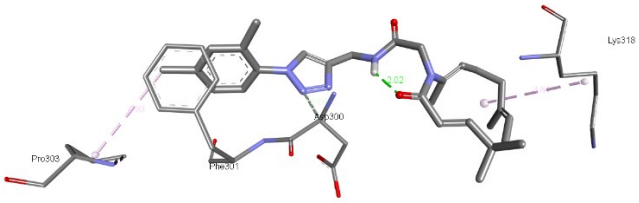
Pi-Sigma
Pi-Alkyl

12h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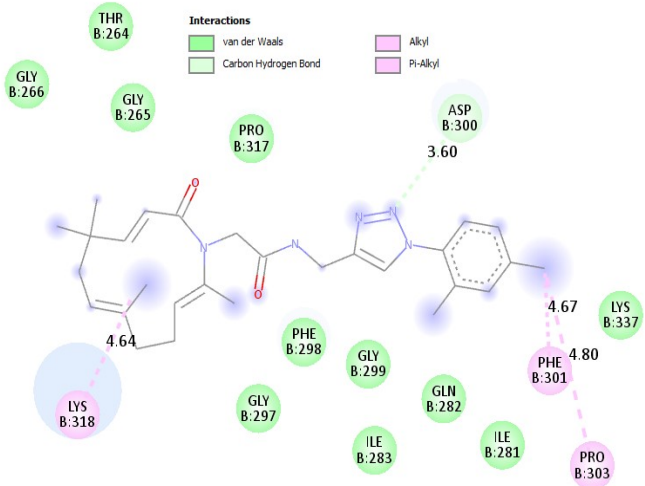
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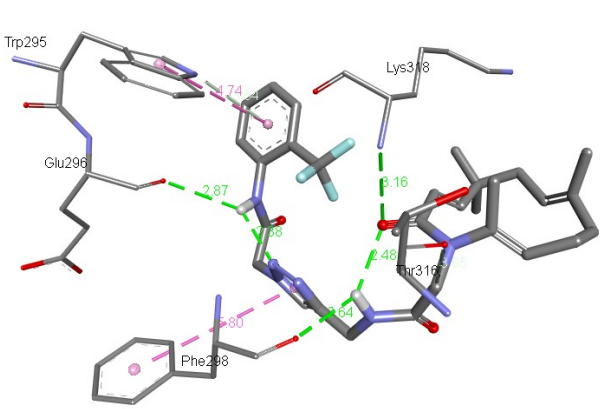
12i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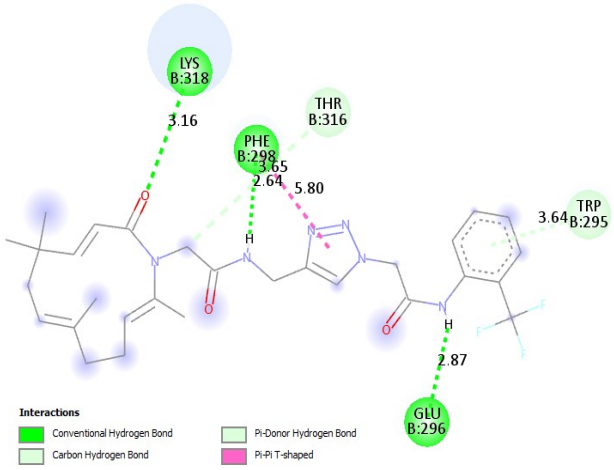
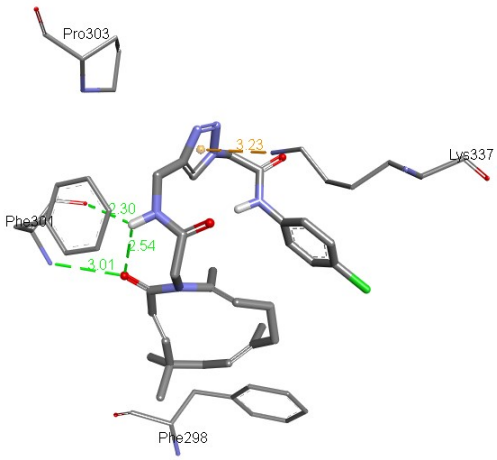
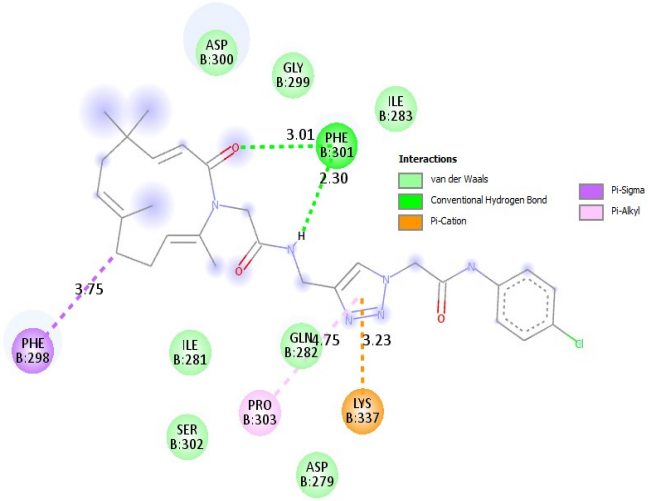
2.64
5.80

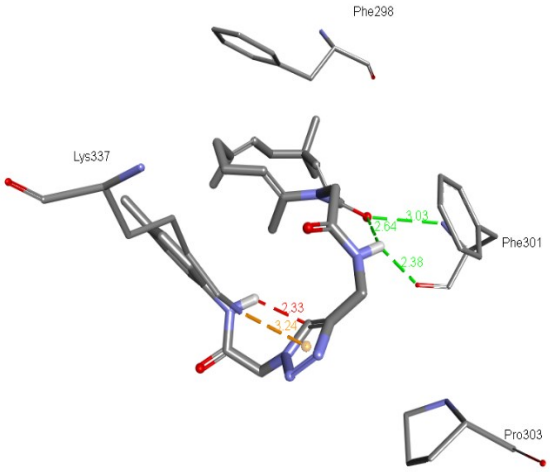

LYS B:318
THR B:316
PHE B:298
TRP B:295
GLU B:296
3.16
3.65
2.64
5.80
3.64
2.87
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Donor Hydrogen Bond
Pi-Pi T-shaped

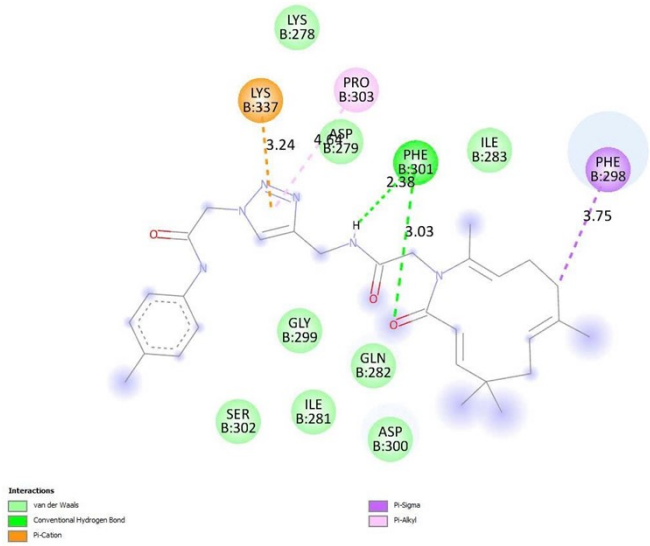
15a


Pro303
Lys337
Phe300
Phe298
3.23
2.30
2.54
3.01

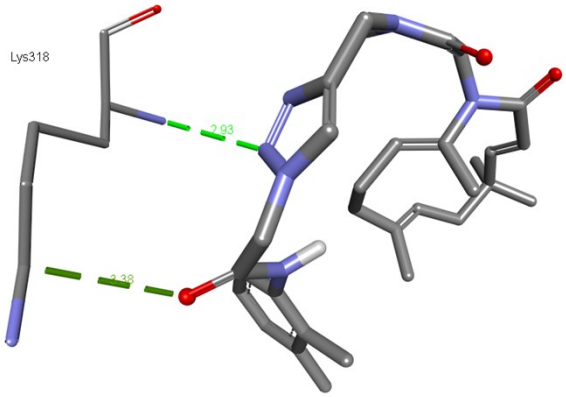

ASP B:300
GLY B:299
ILE B:283
PHE B:301
PHE B:298
ILE B:281
GLN B:282
PRO B:303
LYS B:337
SER B:302
ASP B:279
3.01
2.30
3.75
4.75
3.23
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Alkyl

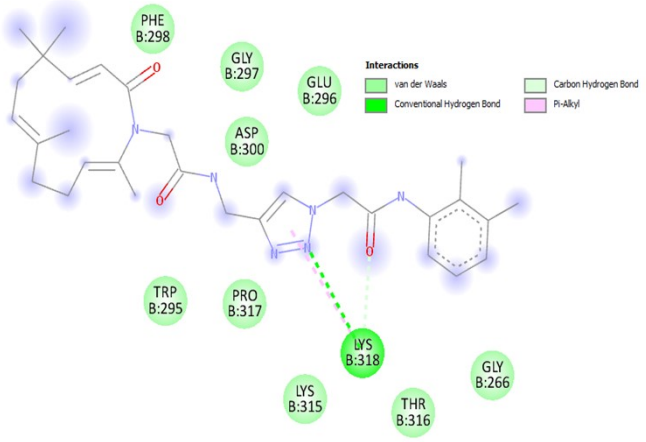
15b


Phe298
Lys337
3.03
2.64
2.38
Phe301
2.33
3.24
Pro303

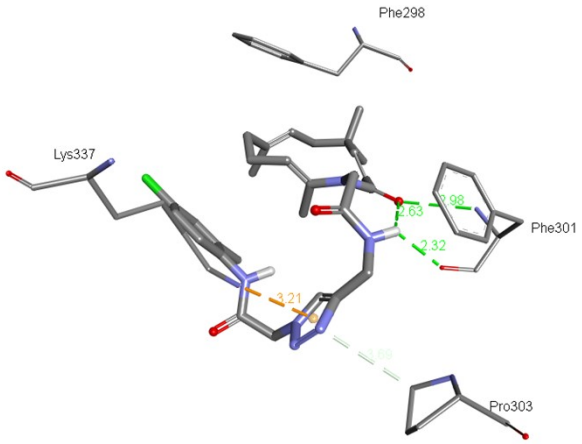

LYS B:278
LYS B:337
PRO B:303
ASP B:279
3.24
4.64
PHE B:301
2.38
ILE B:283
PHE B:298
3.75
3.03
GLY B:299
GLN B:282
SER B:302
ILE B:281
ASP B:300
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Alkyl

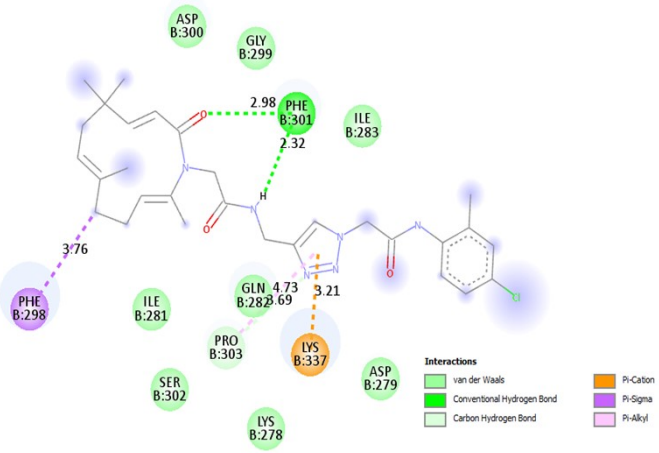
15c


Lys318
2.93
2.38

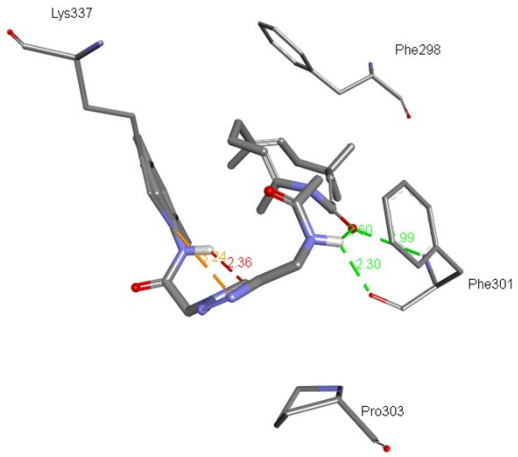

PHE B:298
GLY B:297
GLU B:296
ASP B:300
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Alkyl
TRP B:295
PRO B:317
LYS B:318
GLY B:266
LYS B:315
THR B:316

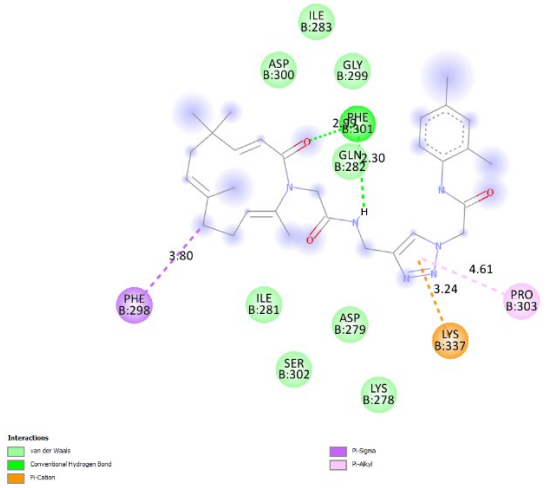
15d


Phe298
Lys337
2.98
2.63
2.32
Phe301
3.21
Pro303

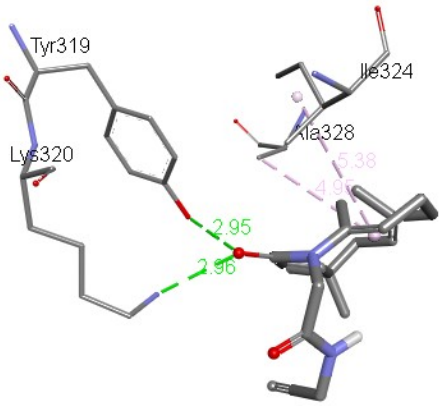

ASP B:300
GLY B:299
2.98
PHE B:301
ILE B:283
2.32
3.76
PHE B:298
ILE B:281
GLN B:282
4.73
3.69
3.21
PRO B:303
LYS B:337
SER B:302
ASP B:279
LYS B:278
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Cation
Pi-Sigma
Pi-Alkyl

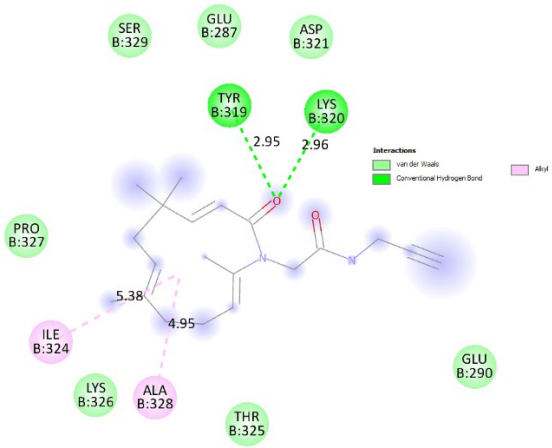
15e



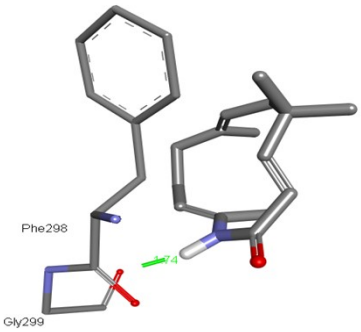


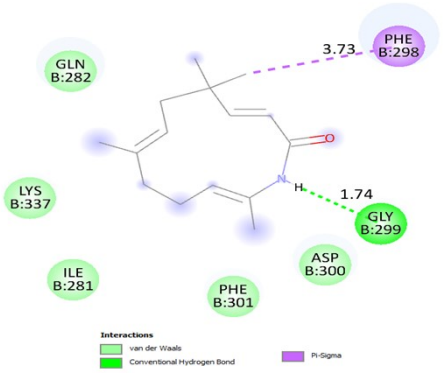
15f





9





2

Figure 2 Binding interactions of ligands into the active site of receptor: 3D (left) and 2D (right)

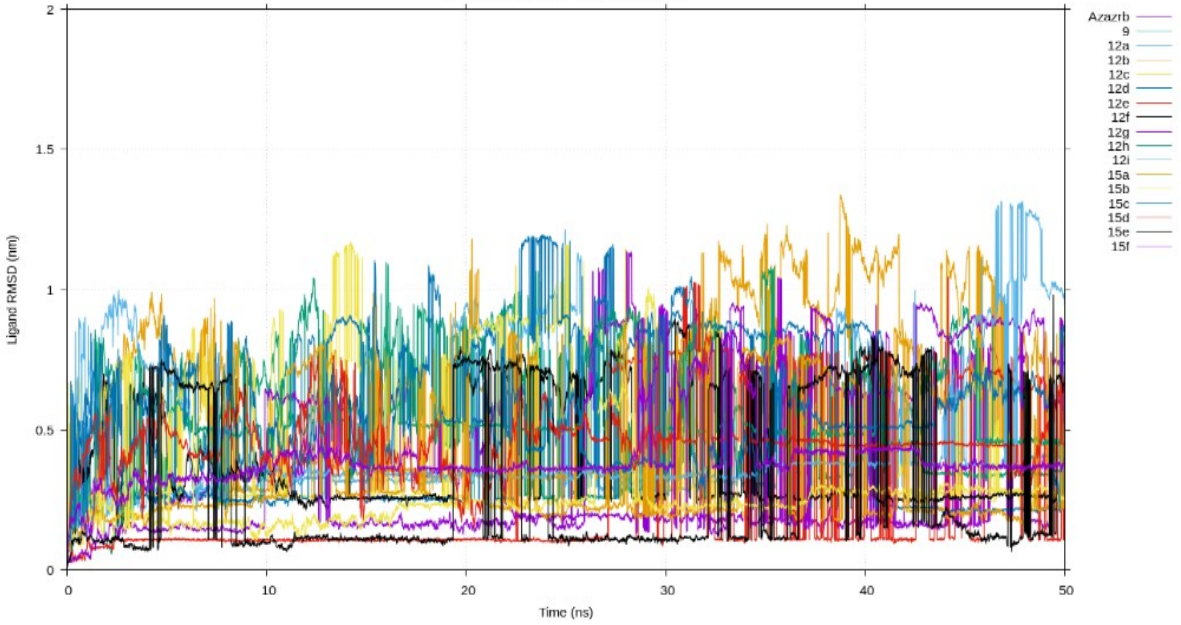


Figure 3 The RMSD during a 50 ns molecular dynamics (MD) simulation for the ligand fitting on C-α

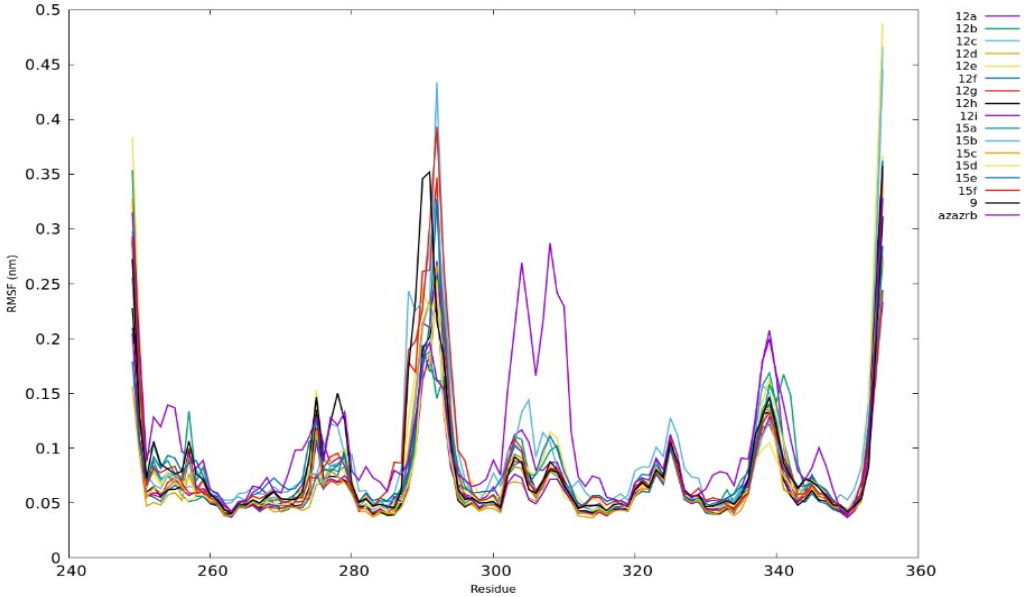


Figure 4 The RMSF during a 50 ns molecular dynamics (MD) simulation for the ligand fitting on C-α

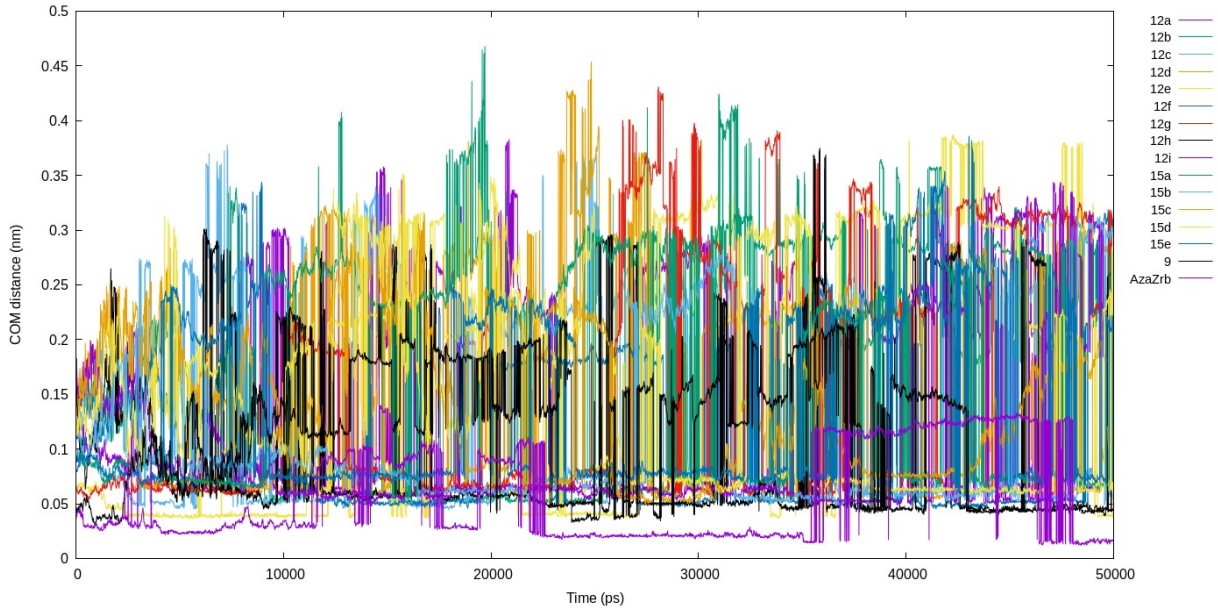


Figure 5 COM distance of all ligands

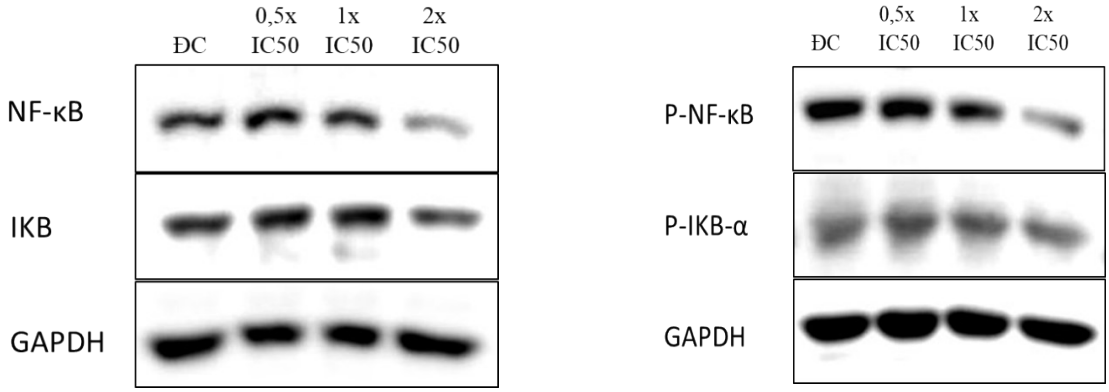


Figure 6 Expression levels of NF-κB-related proteins in HepG2 cells after 48 h of treatment with **12g**, as determined by Western blot analysis

Content

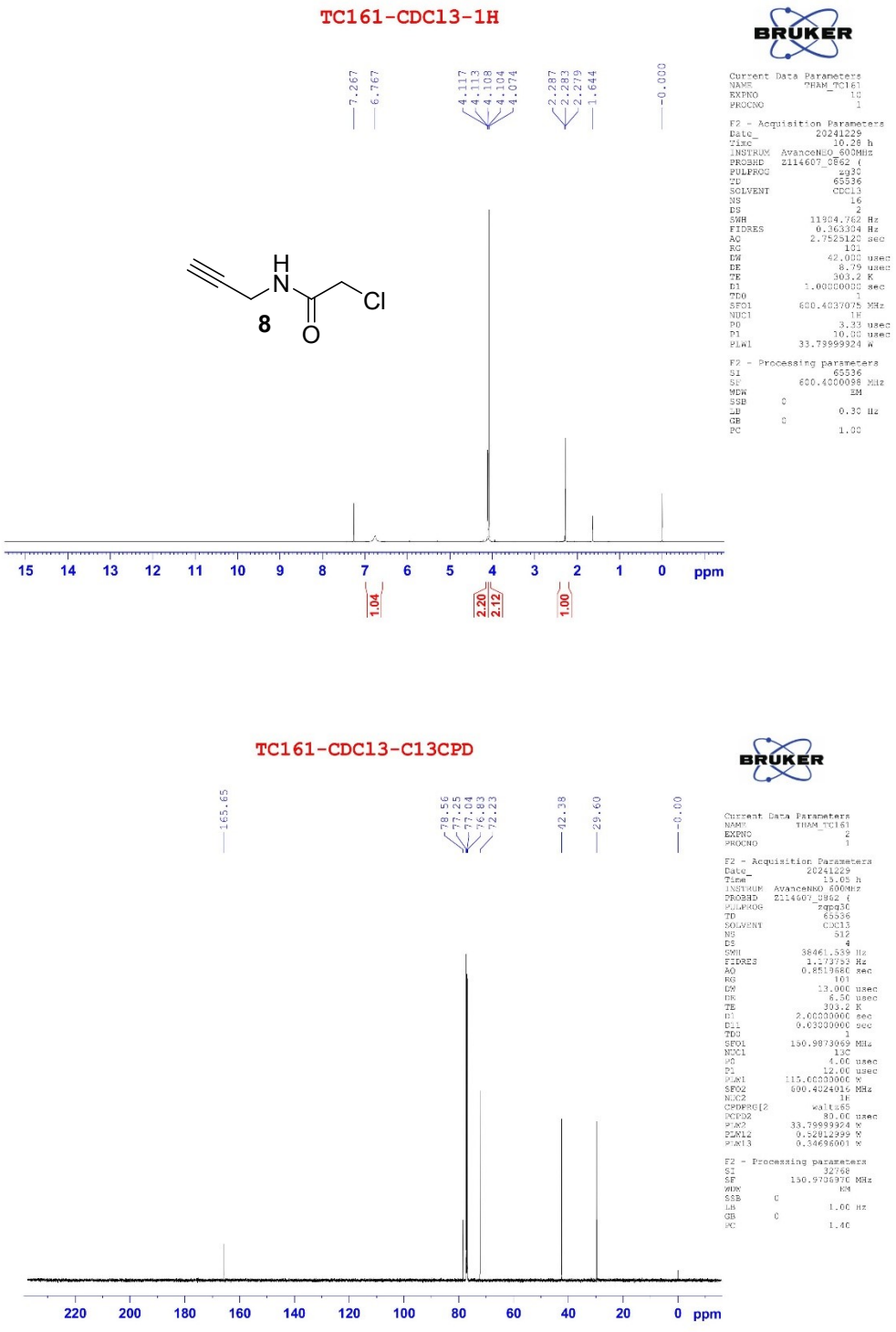


Figure S1. ^{1}H-NMR and ^{13}C-NMR spectra of compound **8**

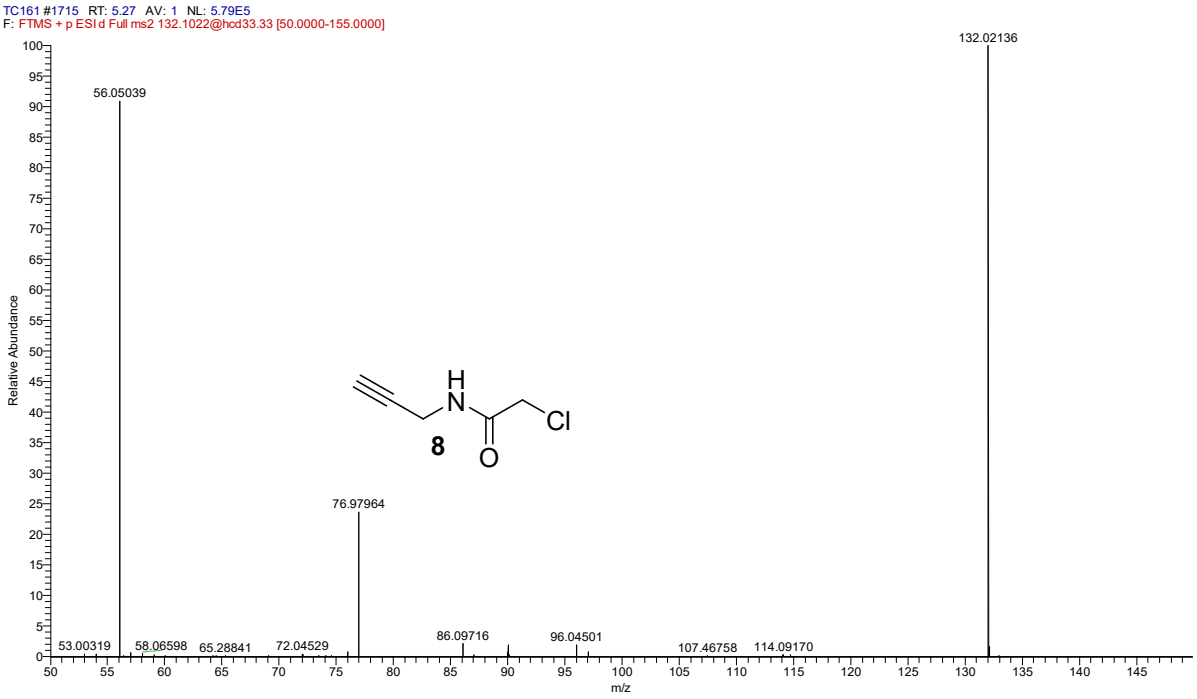


Figure S2. HRMS spectrum of compound **8**

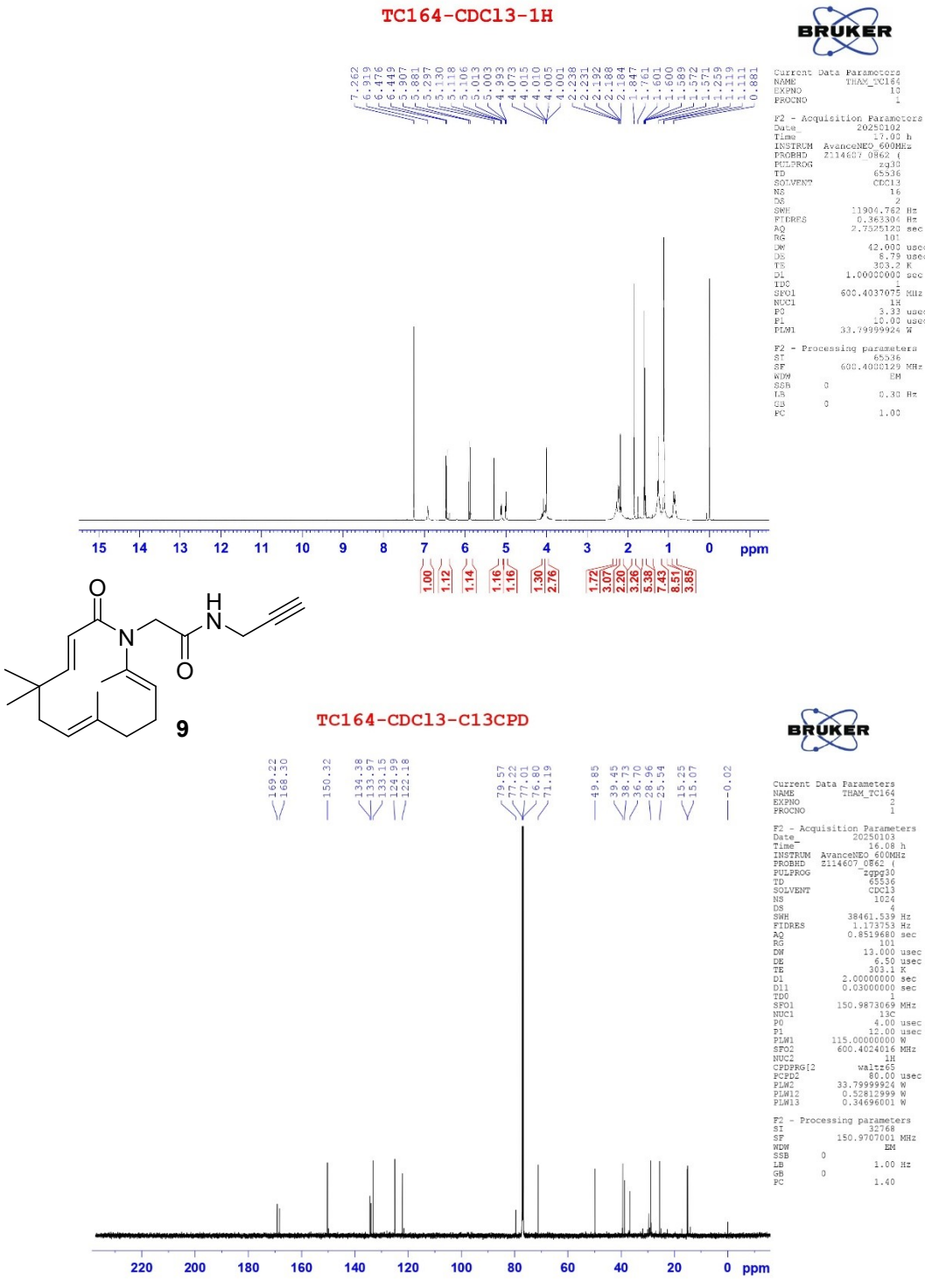
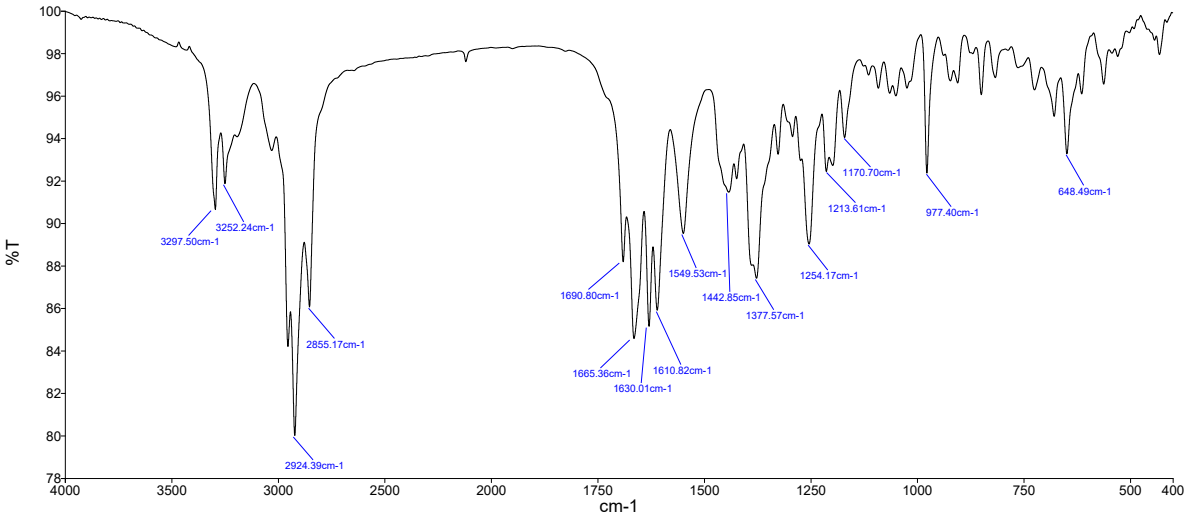


Figure S3. ^{1}H-NMR and ^{13}C-NMR spectra of compound **9**



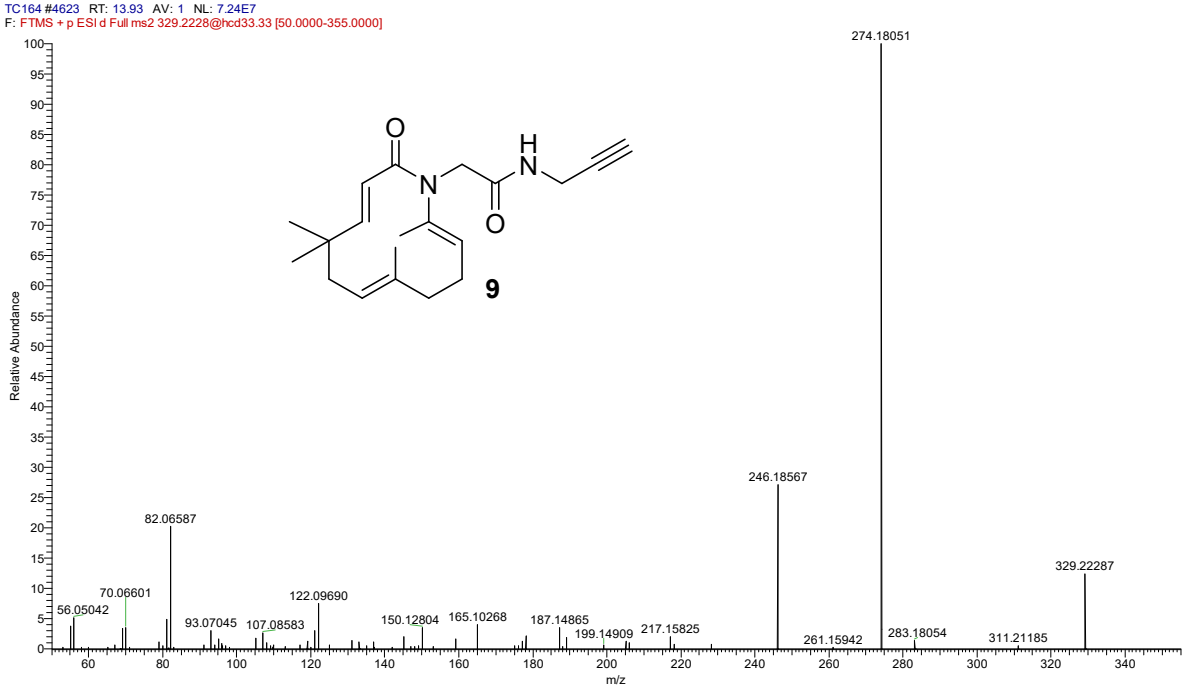


Figure S4. IR and HRMS spectra of compound **9**

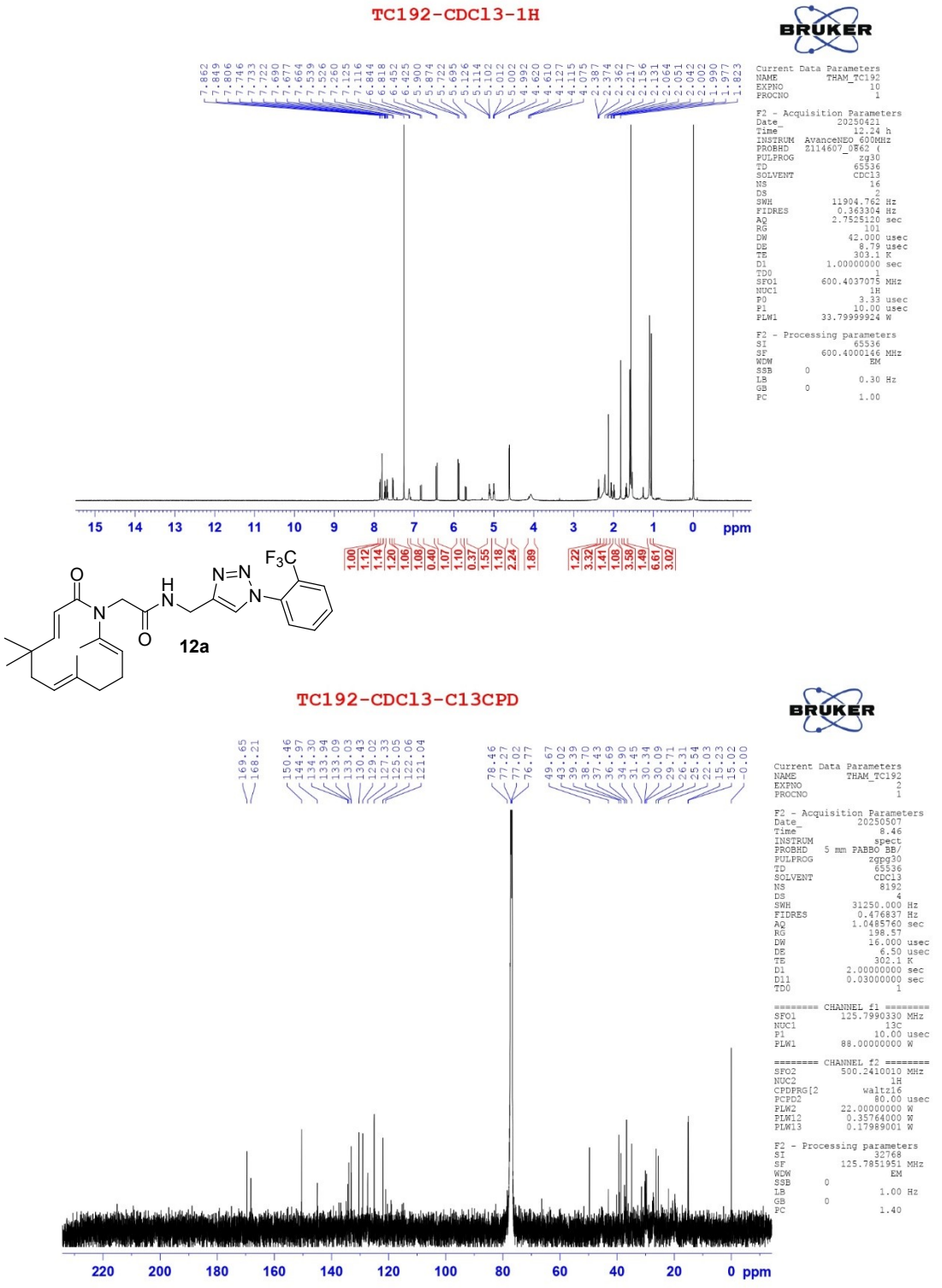
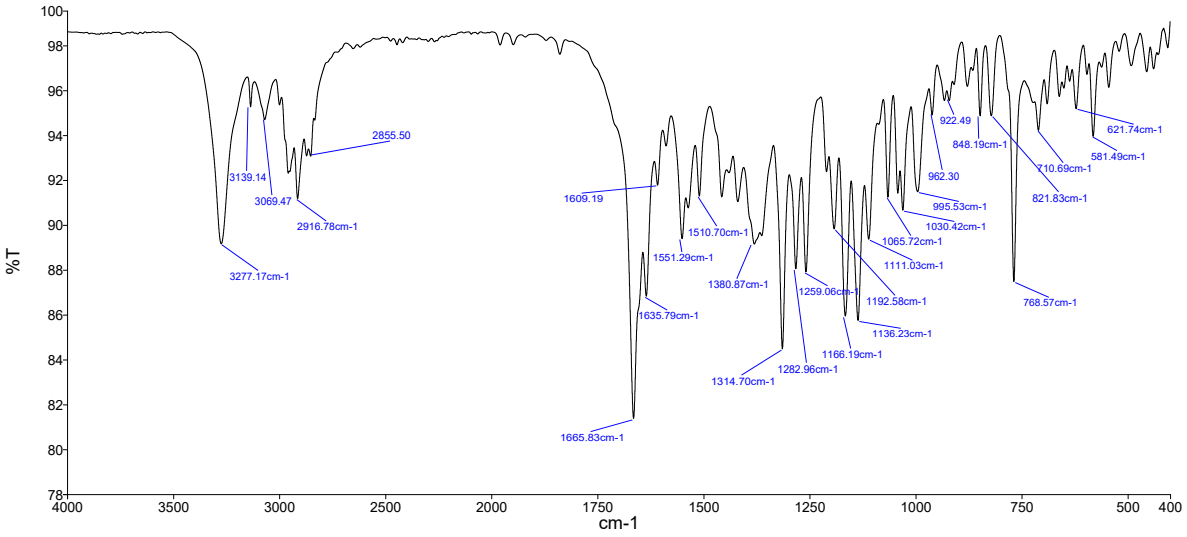


Figure S5. ^{1}H-NMR and ^{13}C-NMR spectra of compound **12a**



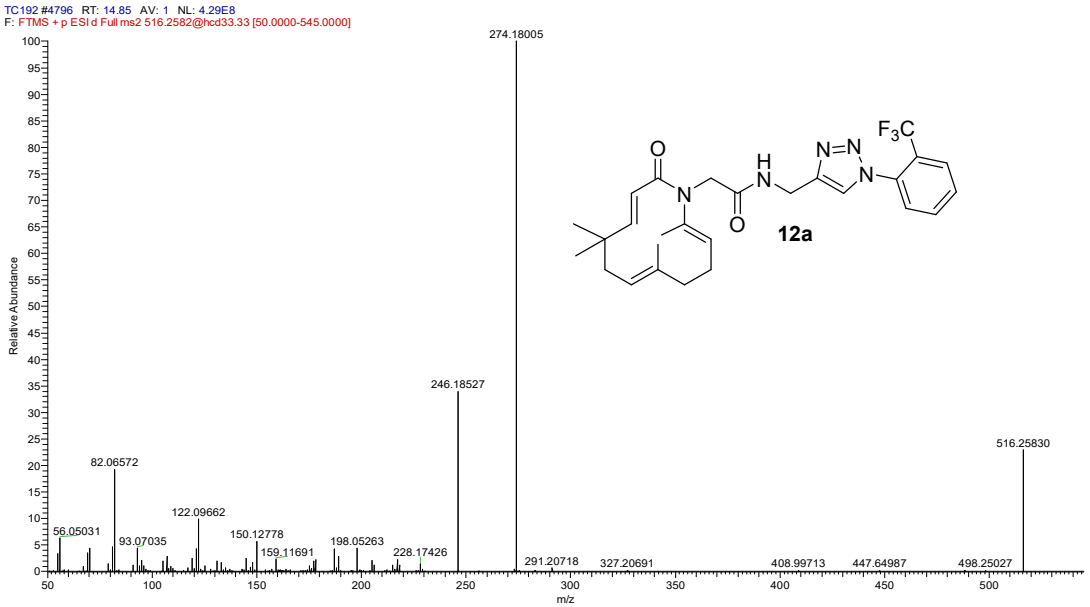


Figure S6. IR and HRMS spectra of compound **12a**

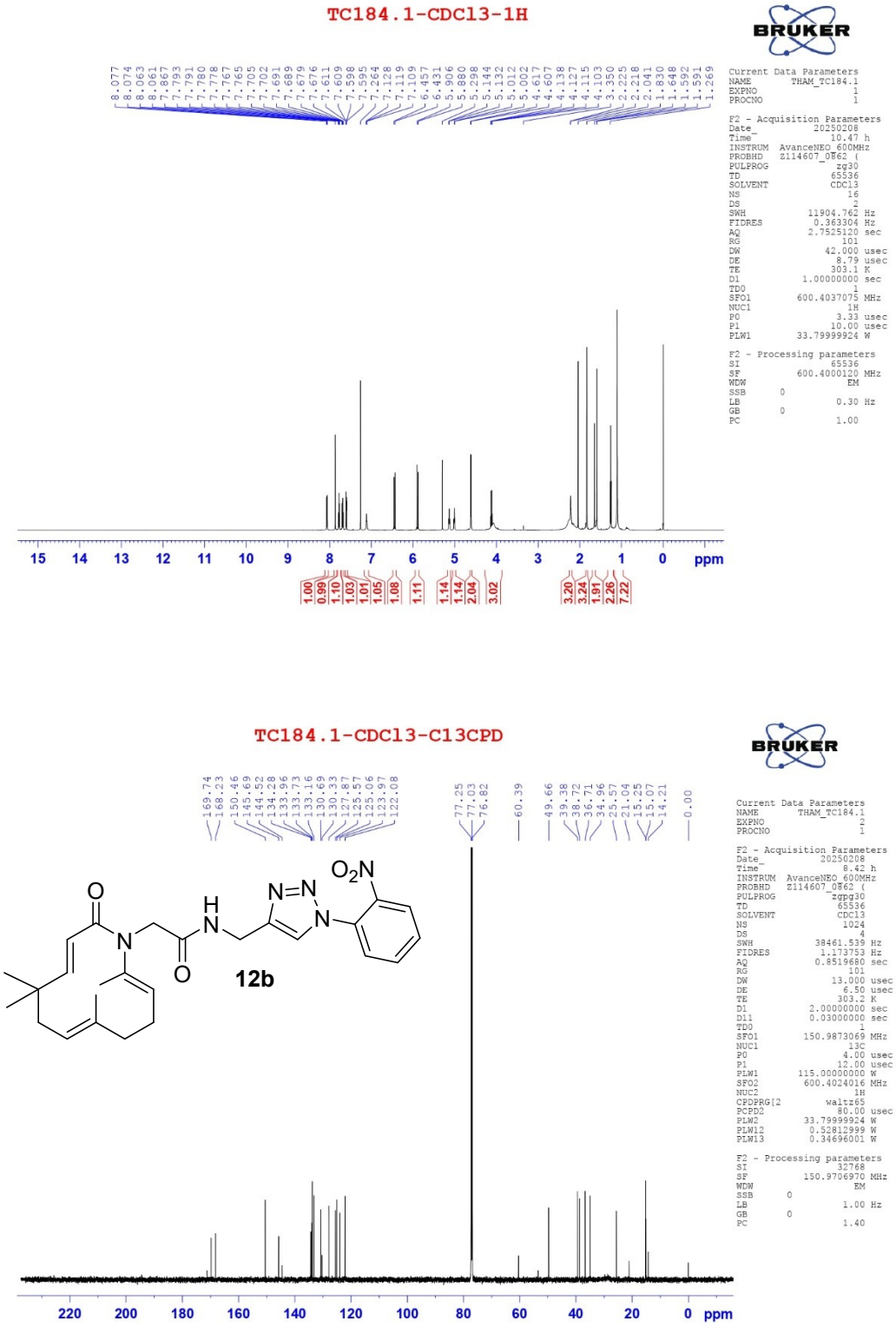
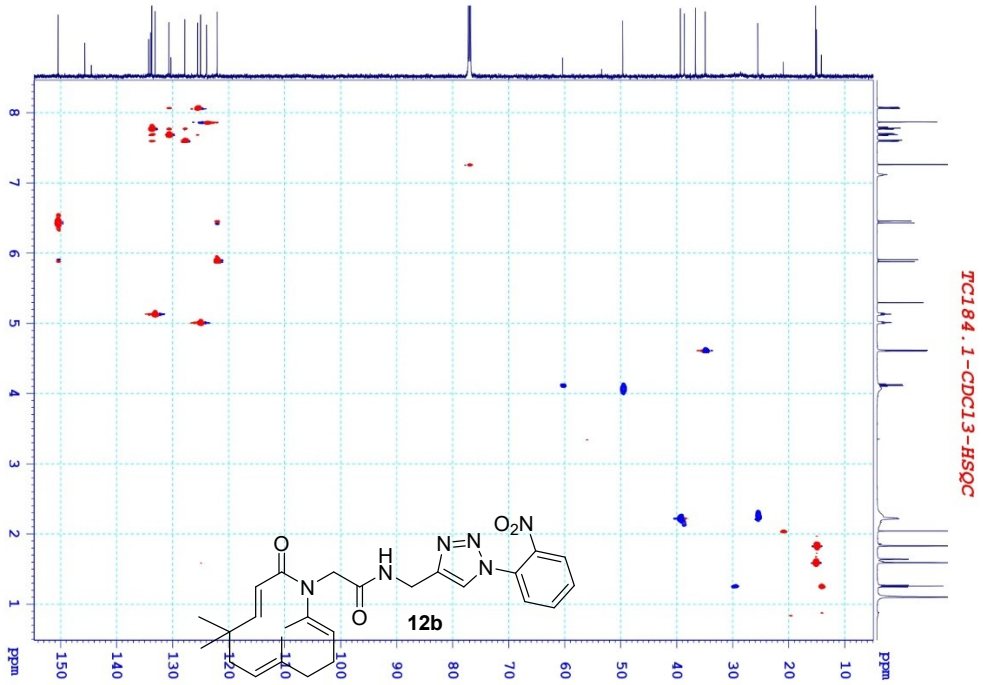


Figure S7. [1]H-NMR and [13]C-NMR spectra of compound **12b**


TC184.1-CDC13-HSQC
12b
O2N
N=N
N
H
N
O
O
ppm
ppm

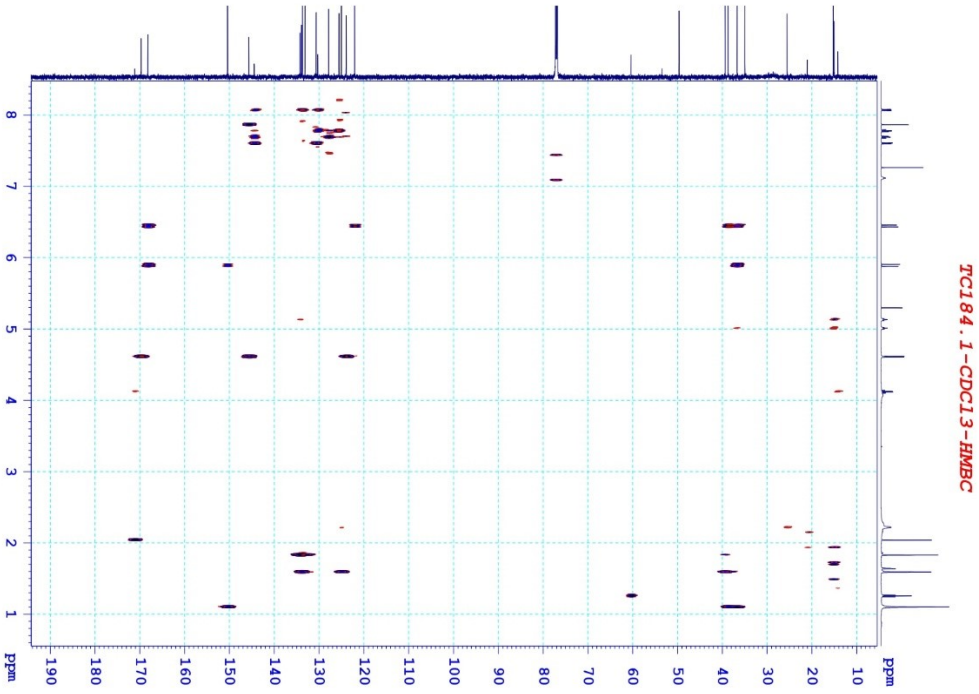
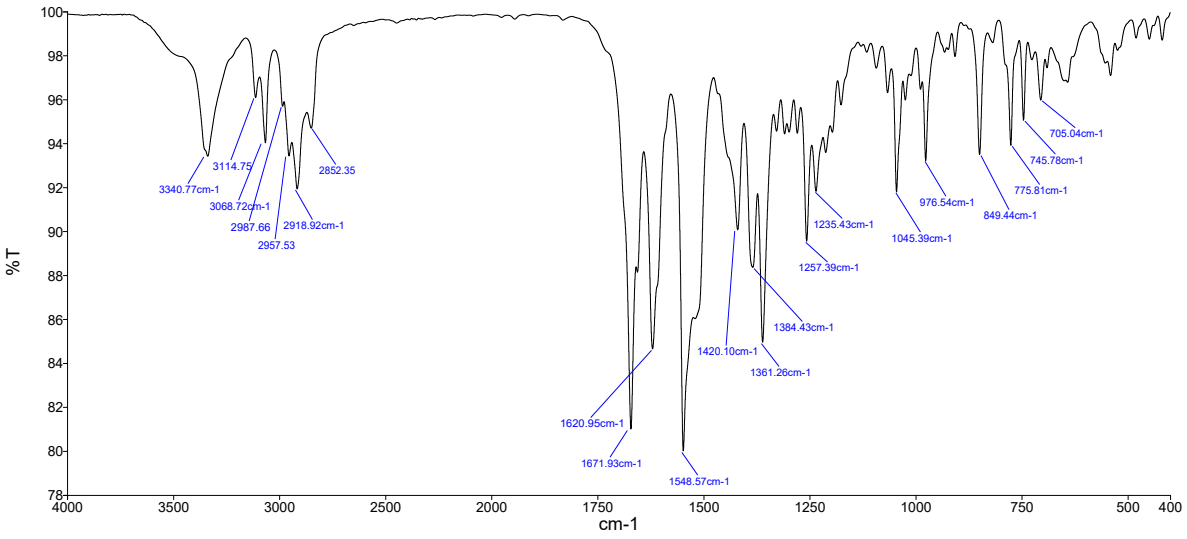


Figure S8. HSQC and HMBC spectra of compound **12b**



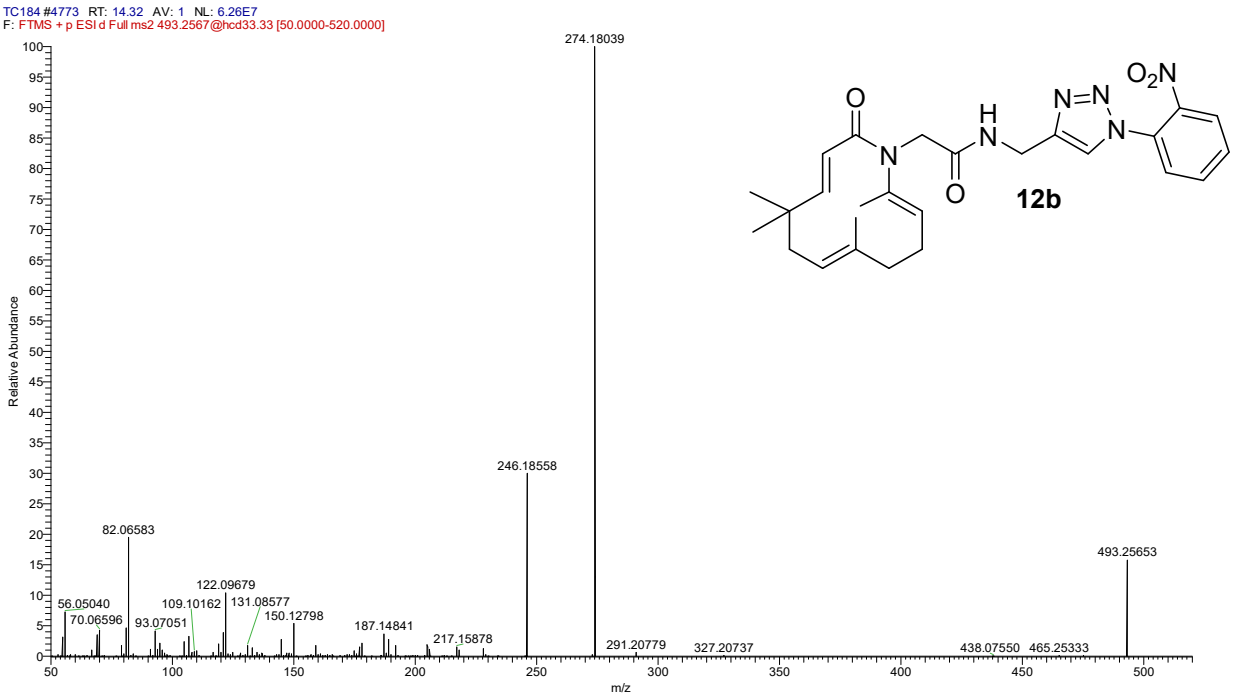


Figure S9. IR and HRMS spectra of compound **12b**

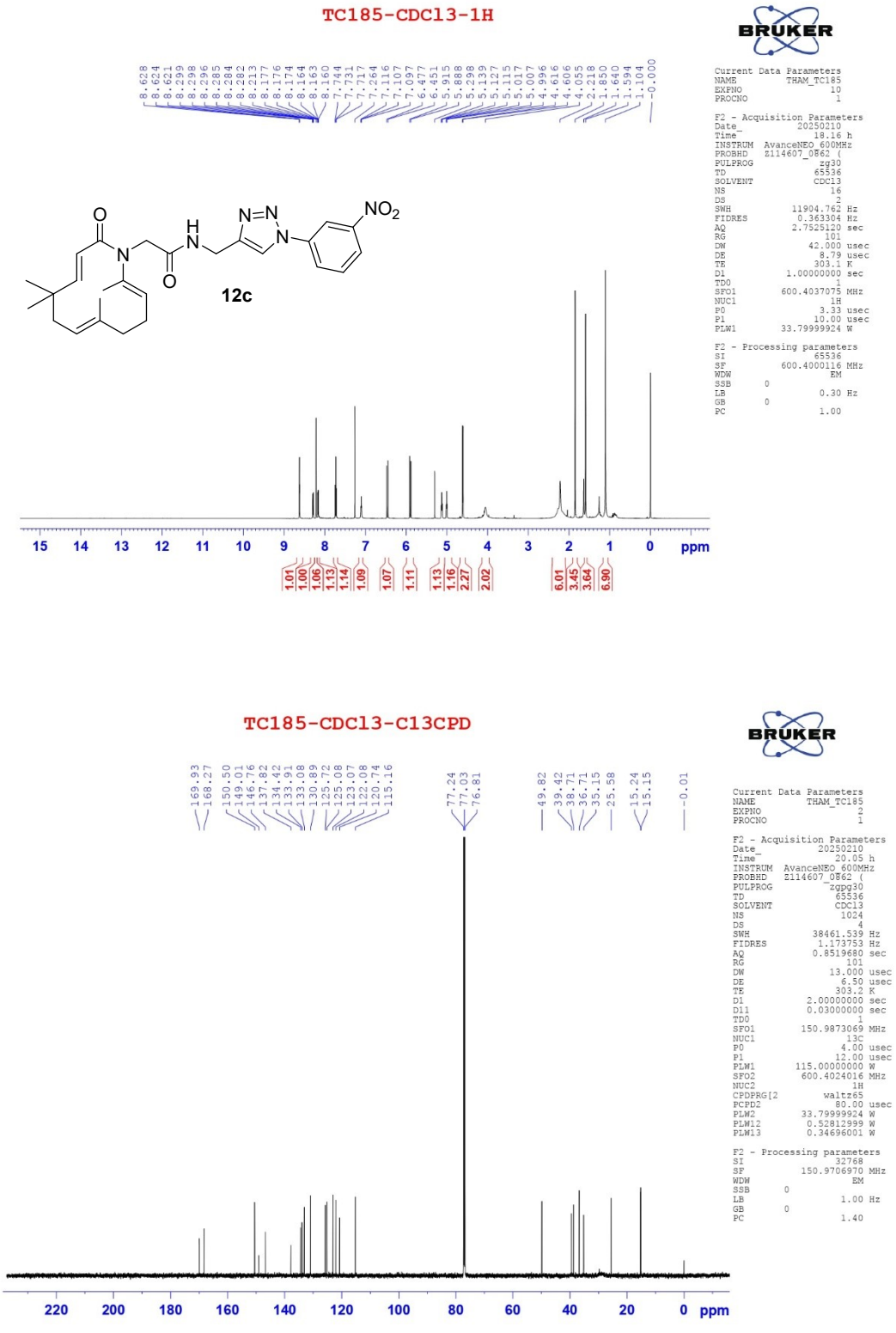
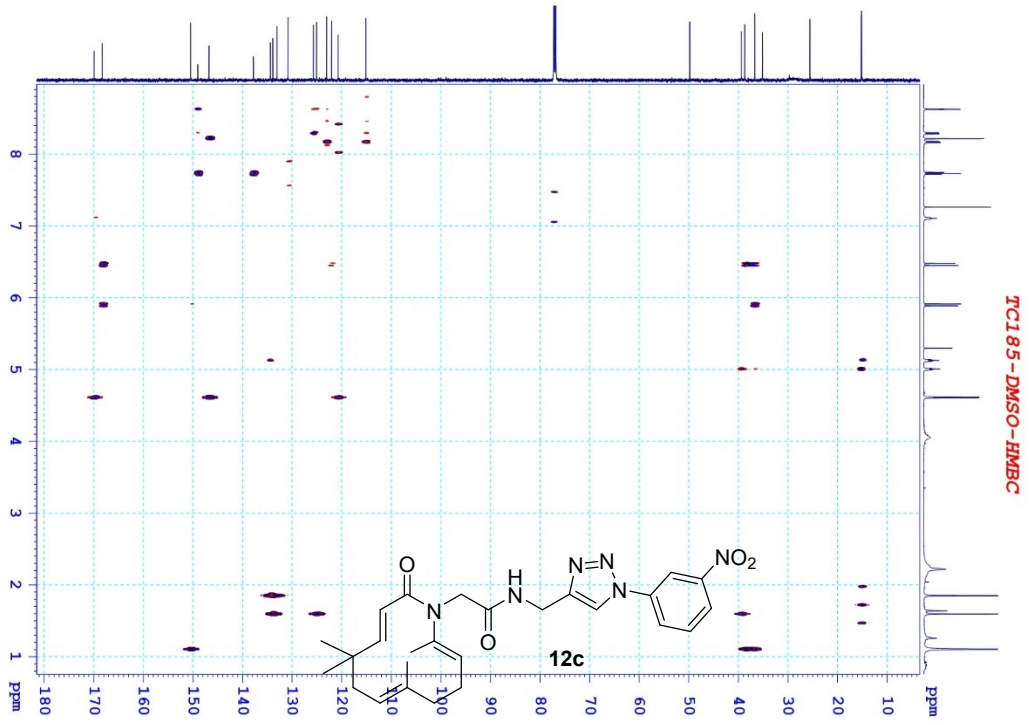


Figure S10. ^{1}H-NMR and ^{13}C-NMR spectra of compound **12c**


TC185-DMSO-HMBC
12c
NO2
ppm

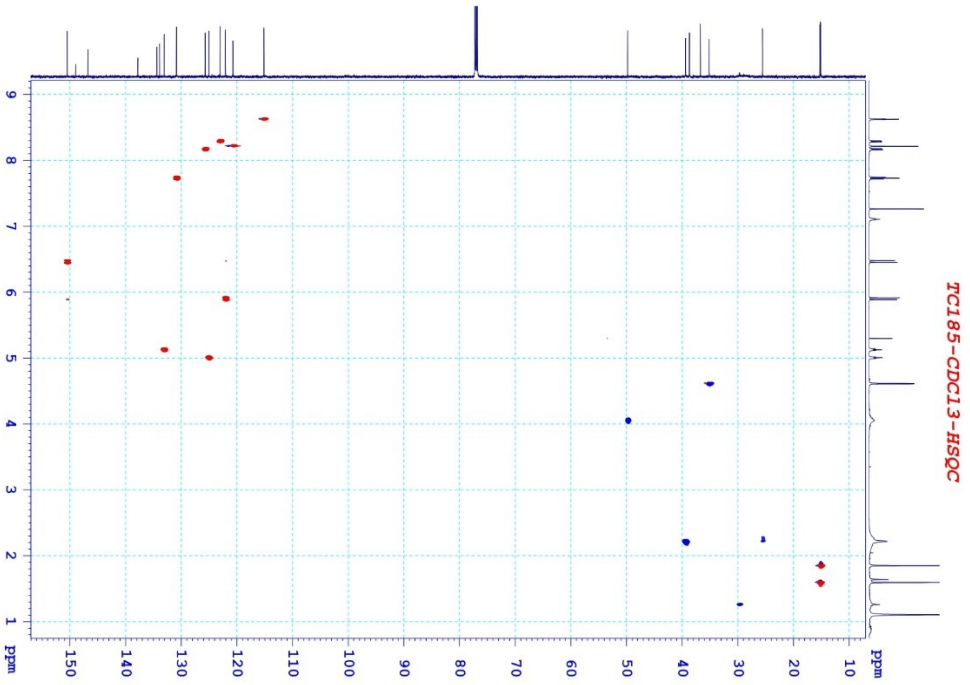
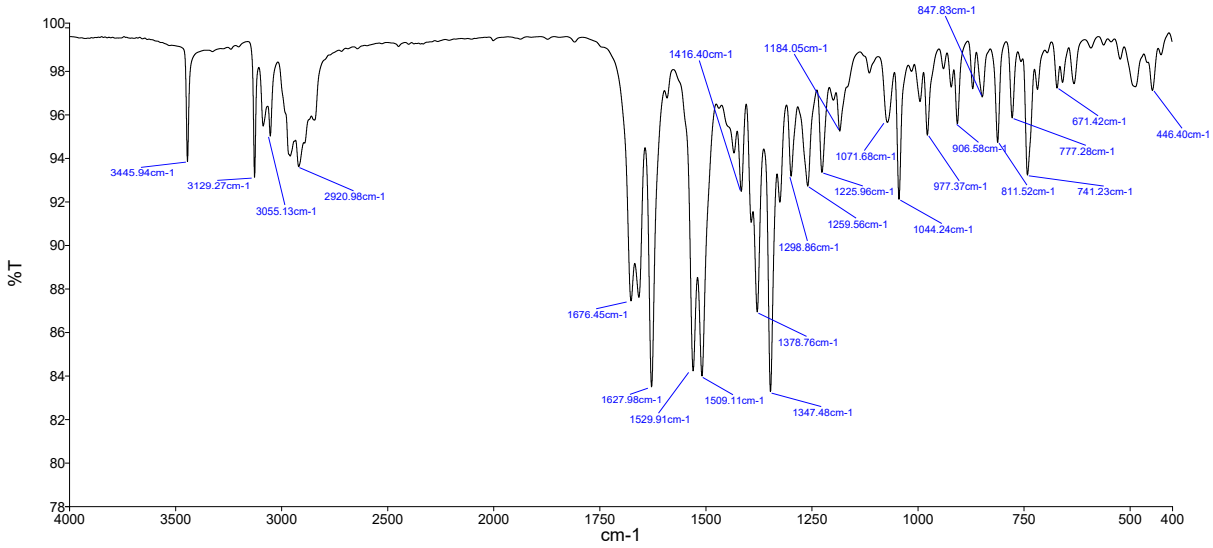


Figure S11. HSQC and HMBC spectra of compound **12c**



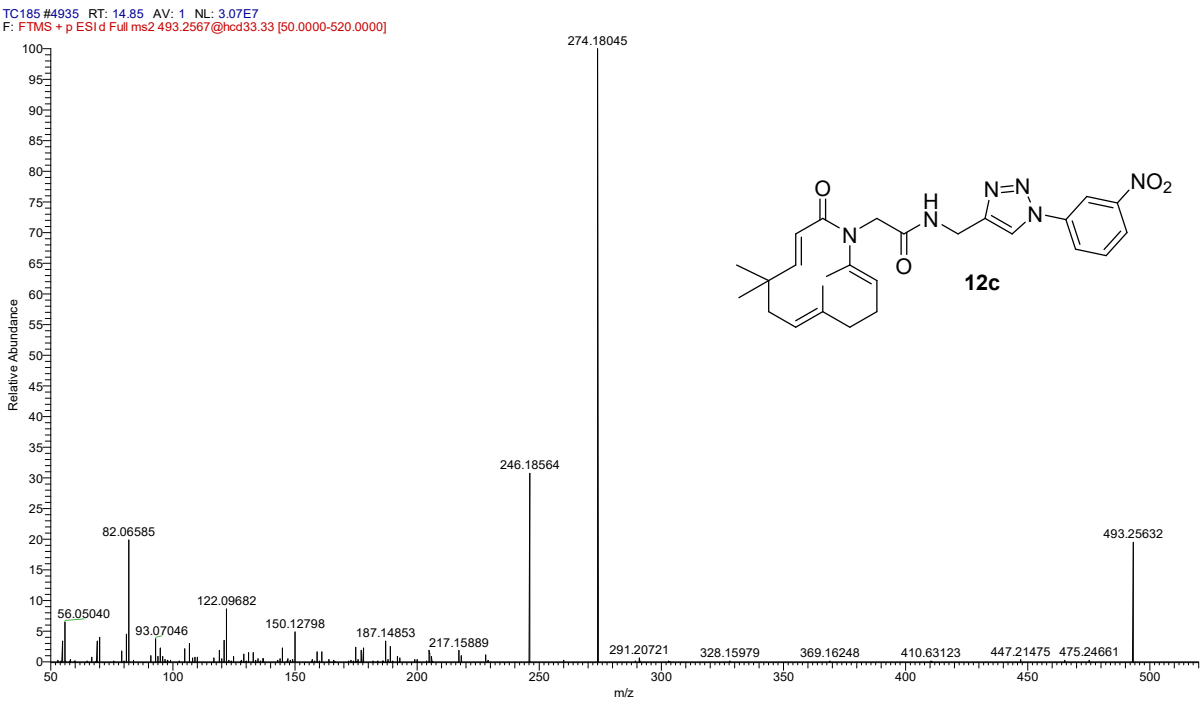


Figure S12. IR and HRMS spectra of compound **12c**

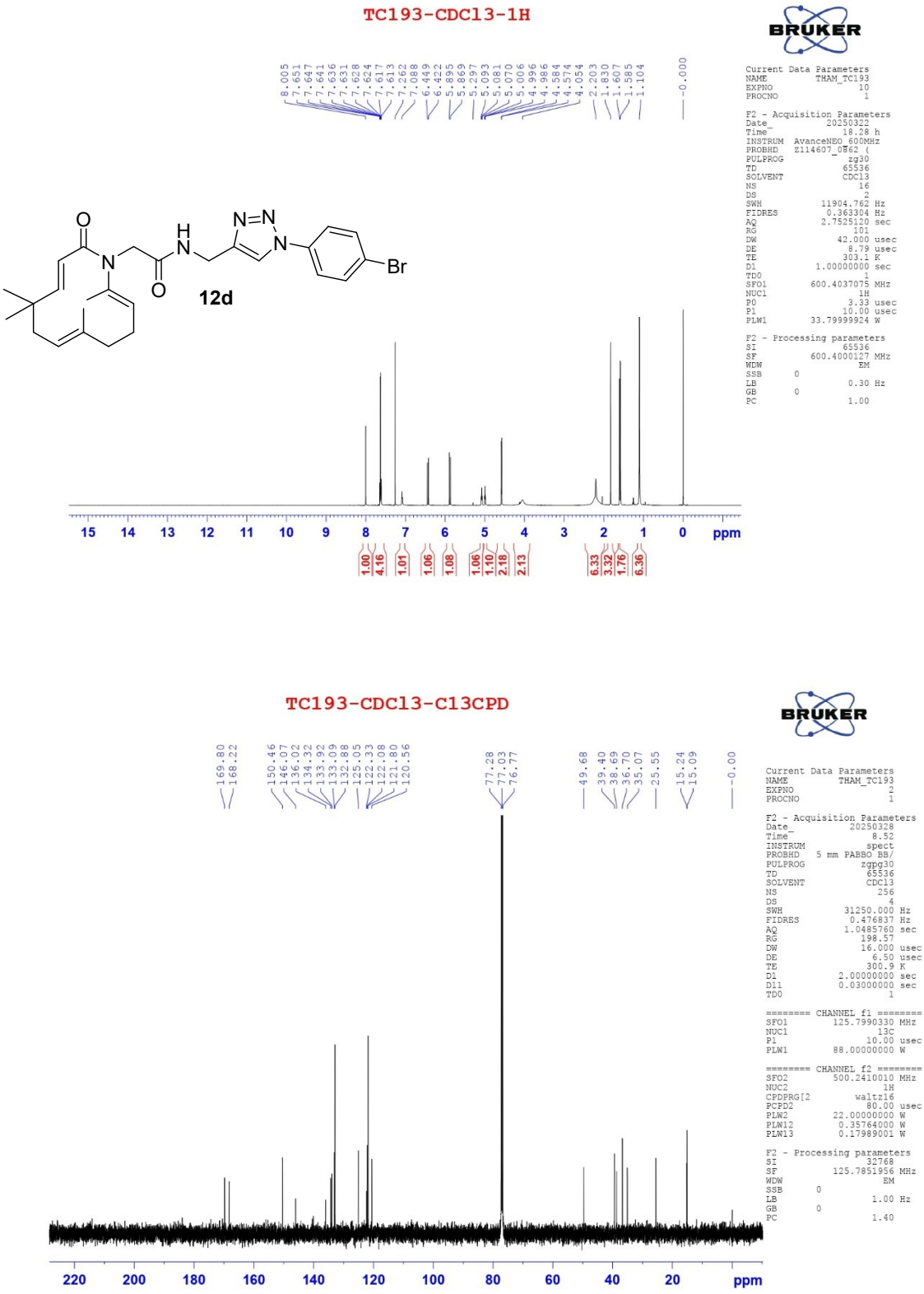
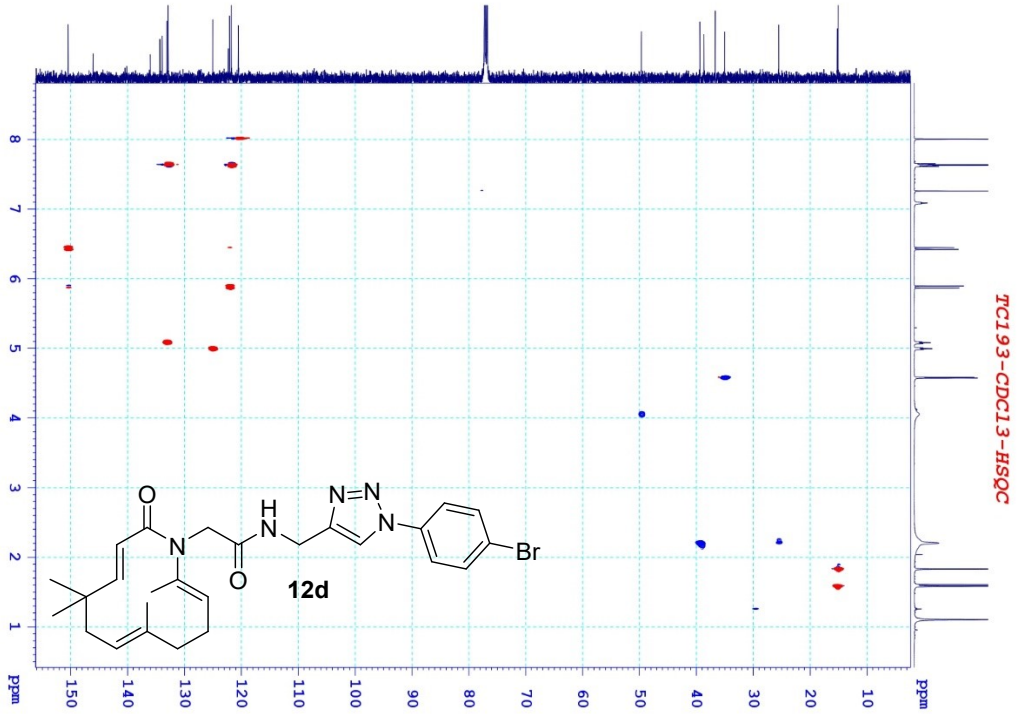


Figure S13. ^{1}H-NMR and ^{13}C-NMR spectra of compound **12d**


TC193-CDCl3-HSQC
12d
Br
ppm
ppm

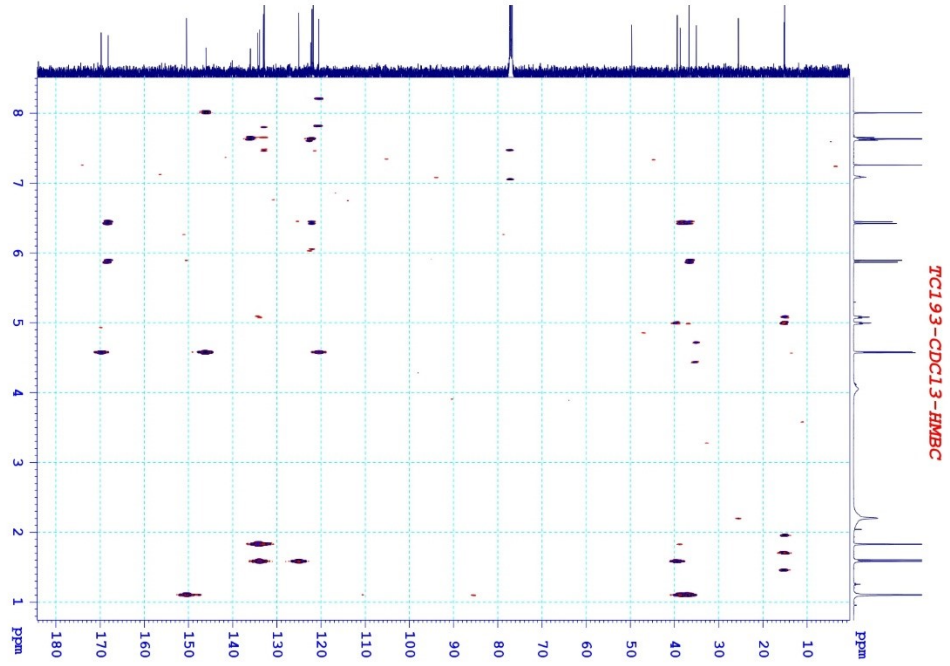
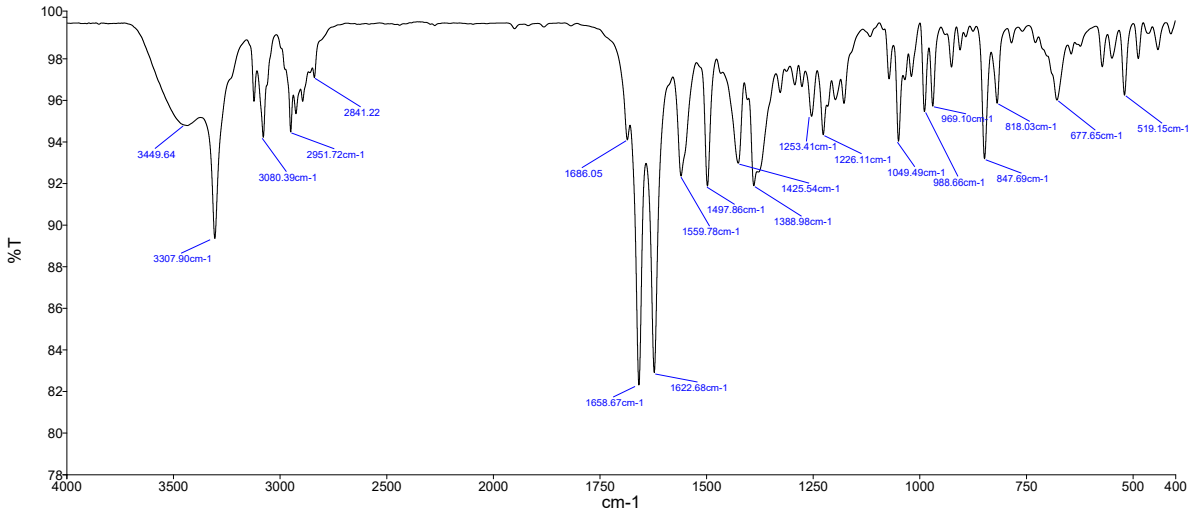


Figure S14. HSQC and HMBC spectra of compound **12d**



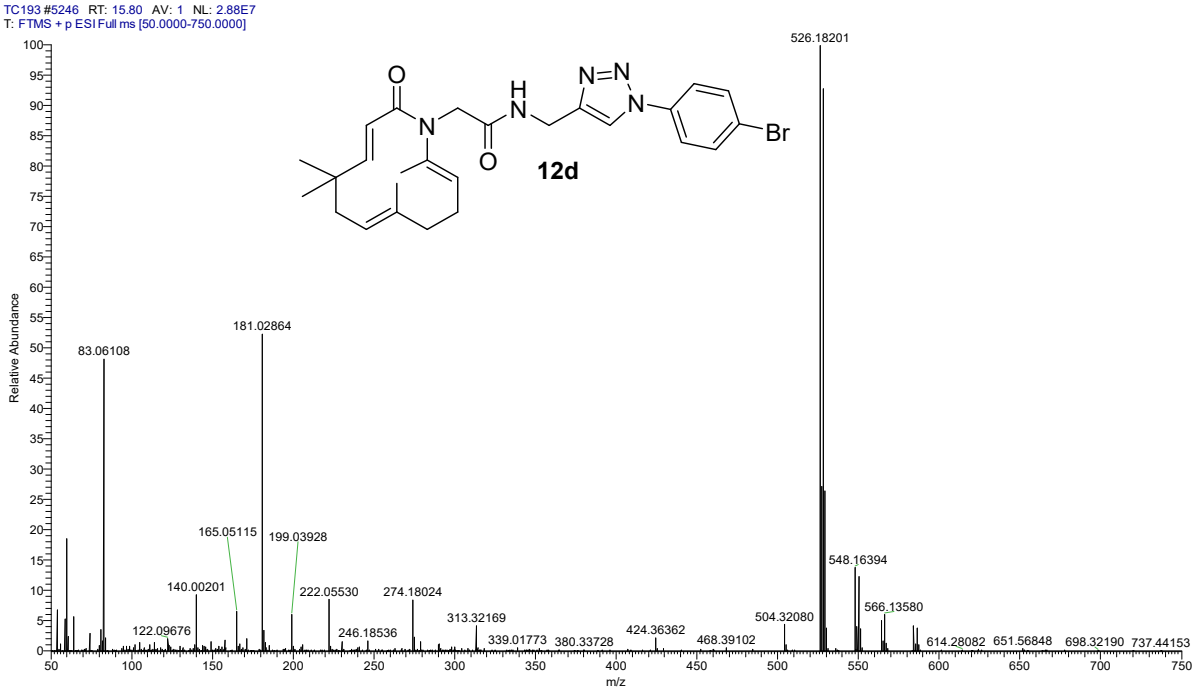


Figure S15. IR and HRMS spectra of compound **12d**

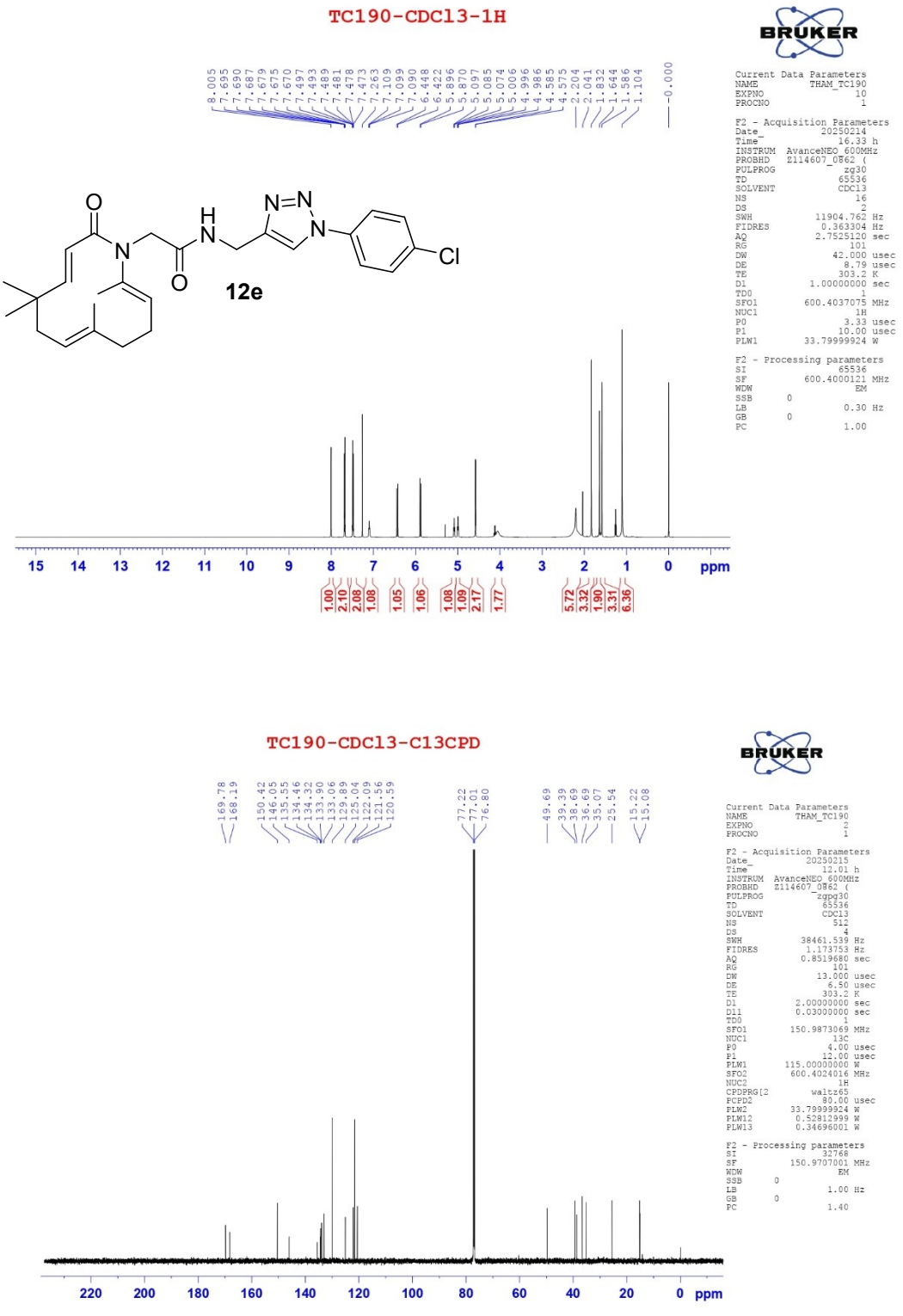


Figure S16. ^{1}H-NMR and ^{13}C-NMR spectra of compound **12e**

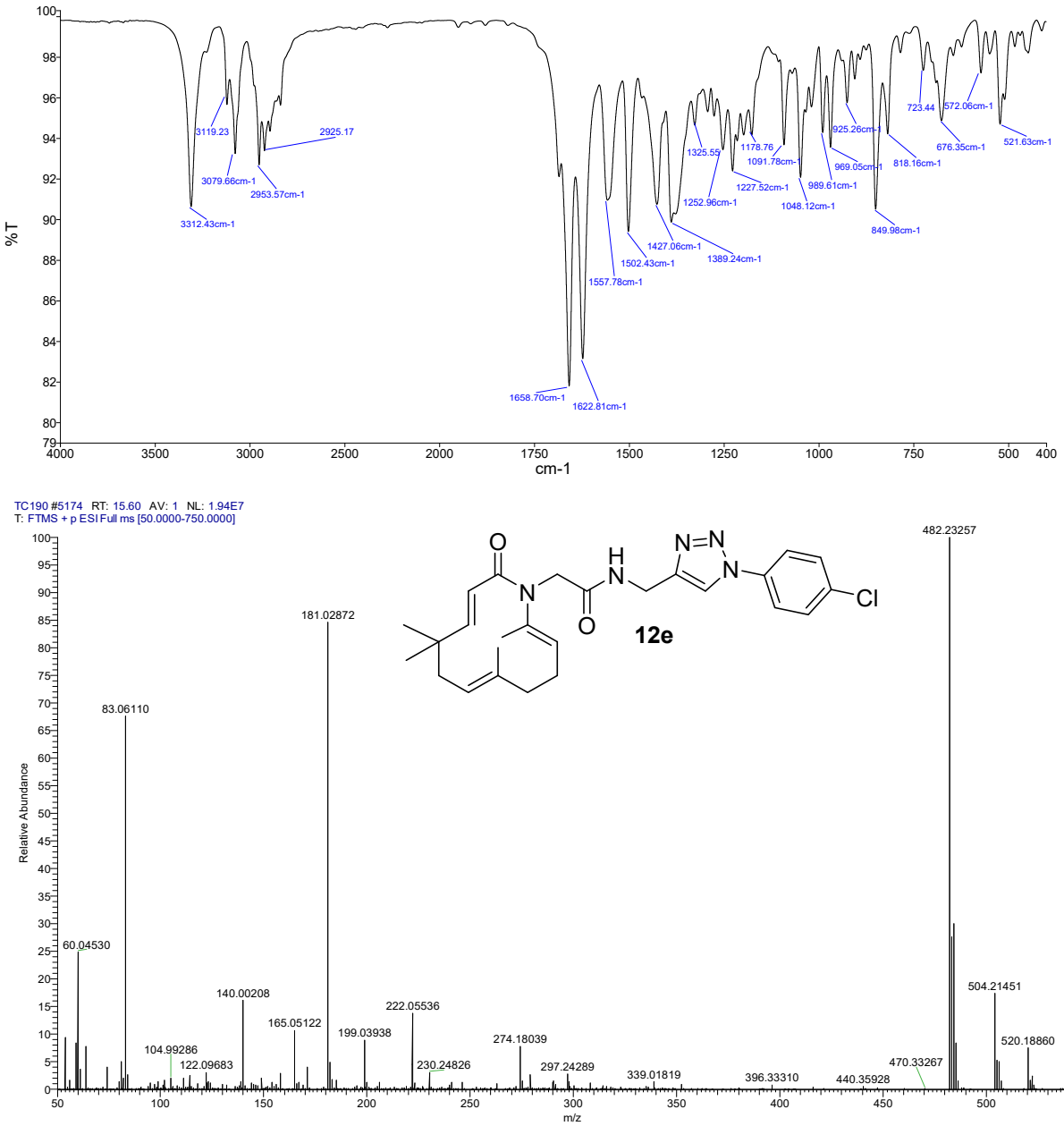
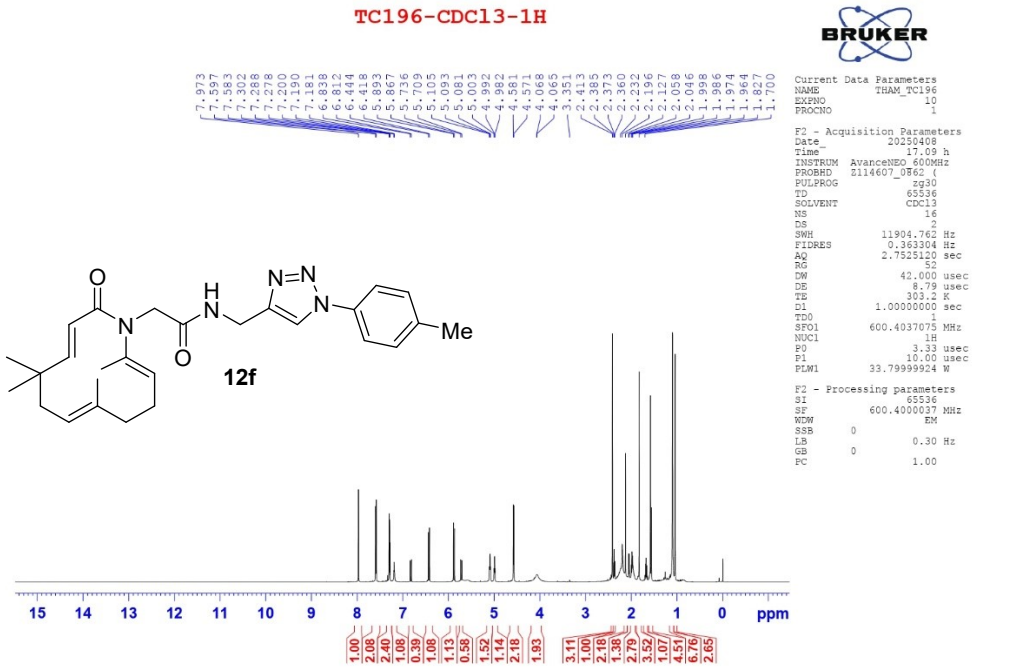


Figure S17. IR and HRMS spectra of compound **12e**



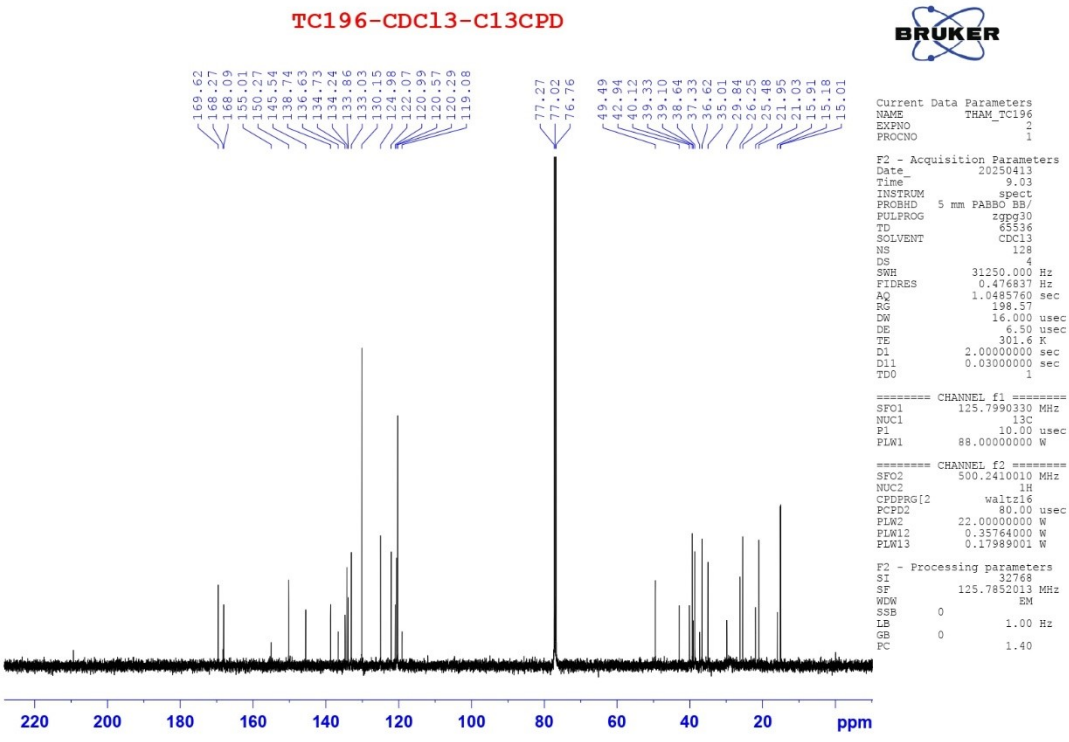
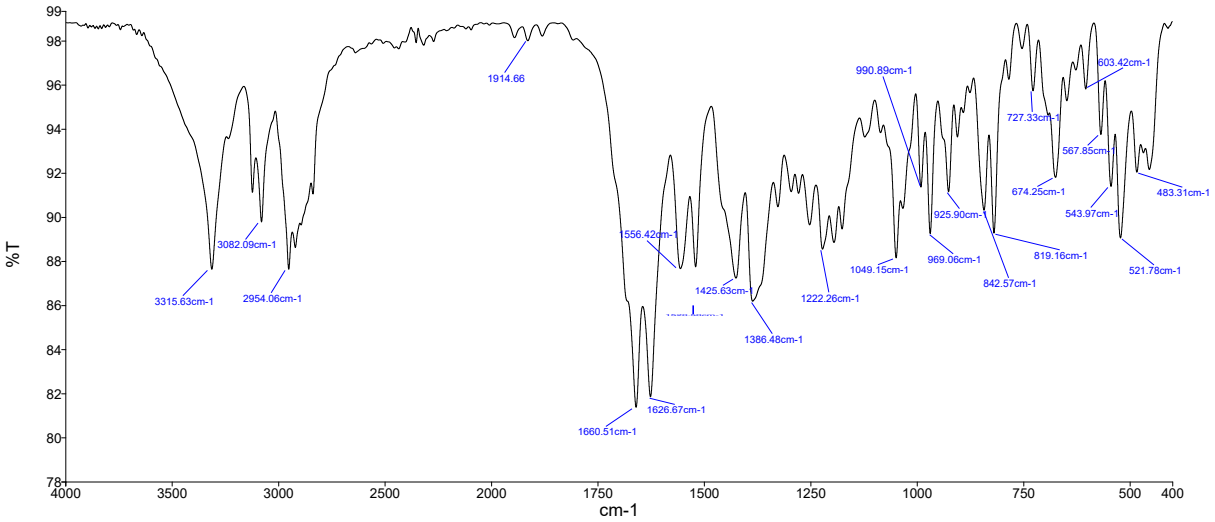


Figure S18. ^{1}H-NMR and ^{13}C-NMR spectra of compound **12f**



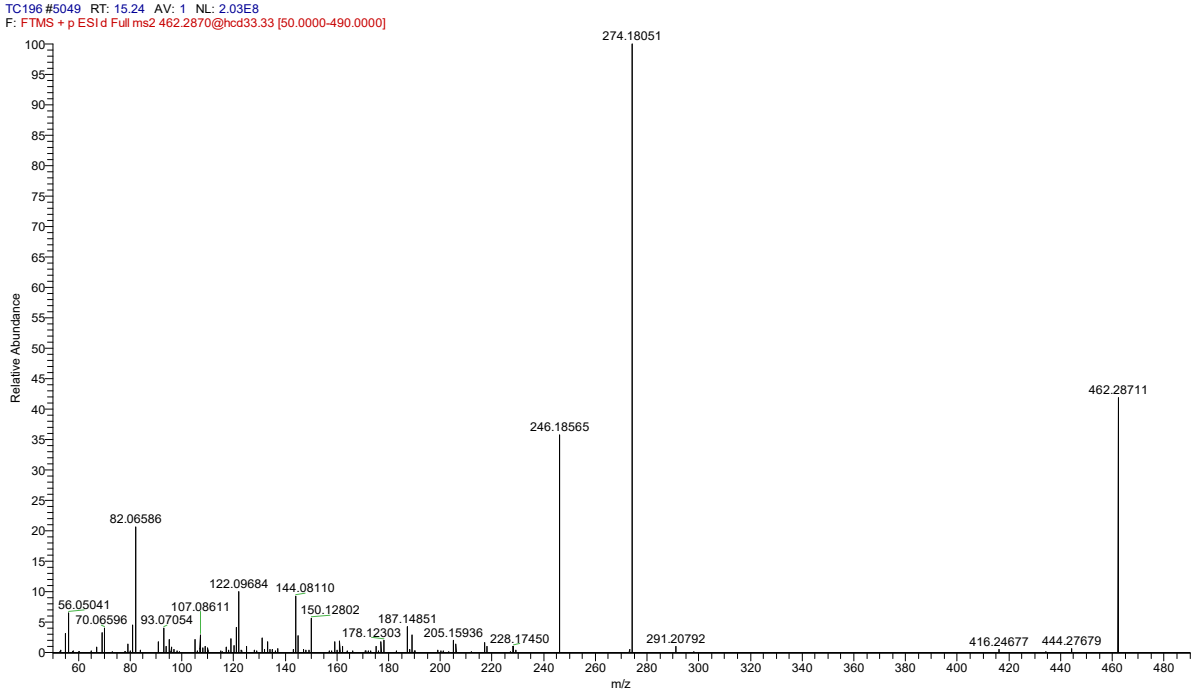


Figure S19. IR and HRMS spectra of compound **12f**

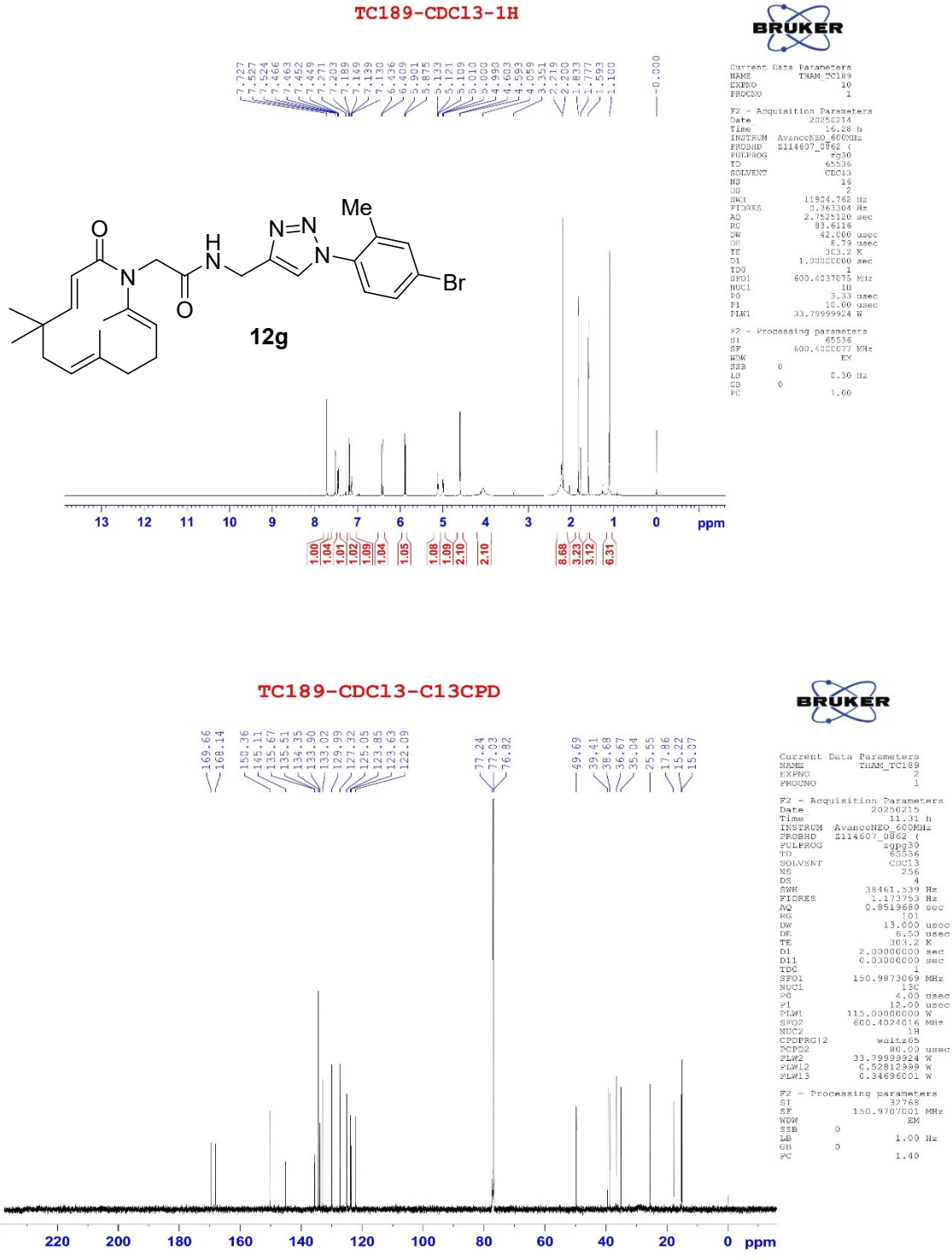


Figure S20. ^{1}H-NMR and ^{13}C-NMR spectra of compound **12g**

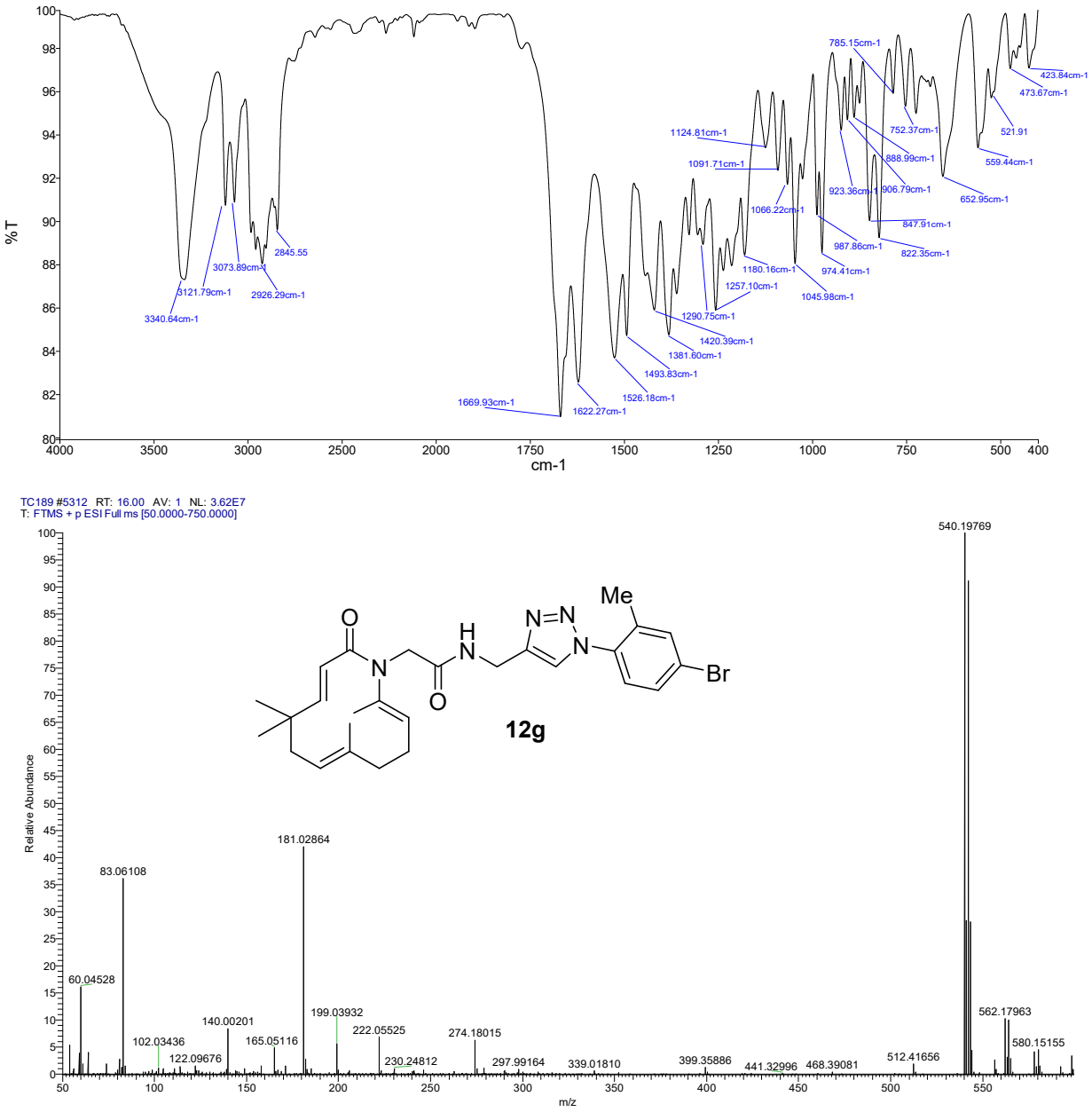


Figure S21. IR and HRMS spectra of compound **12g**

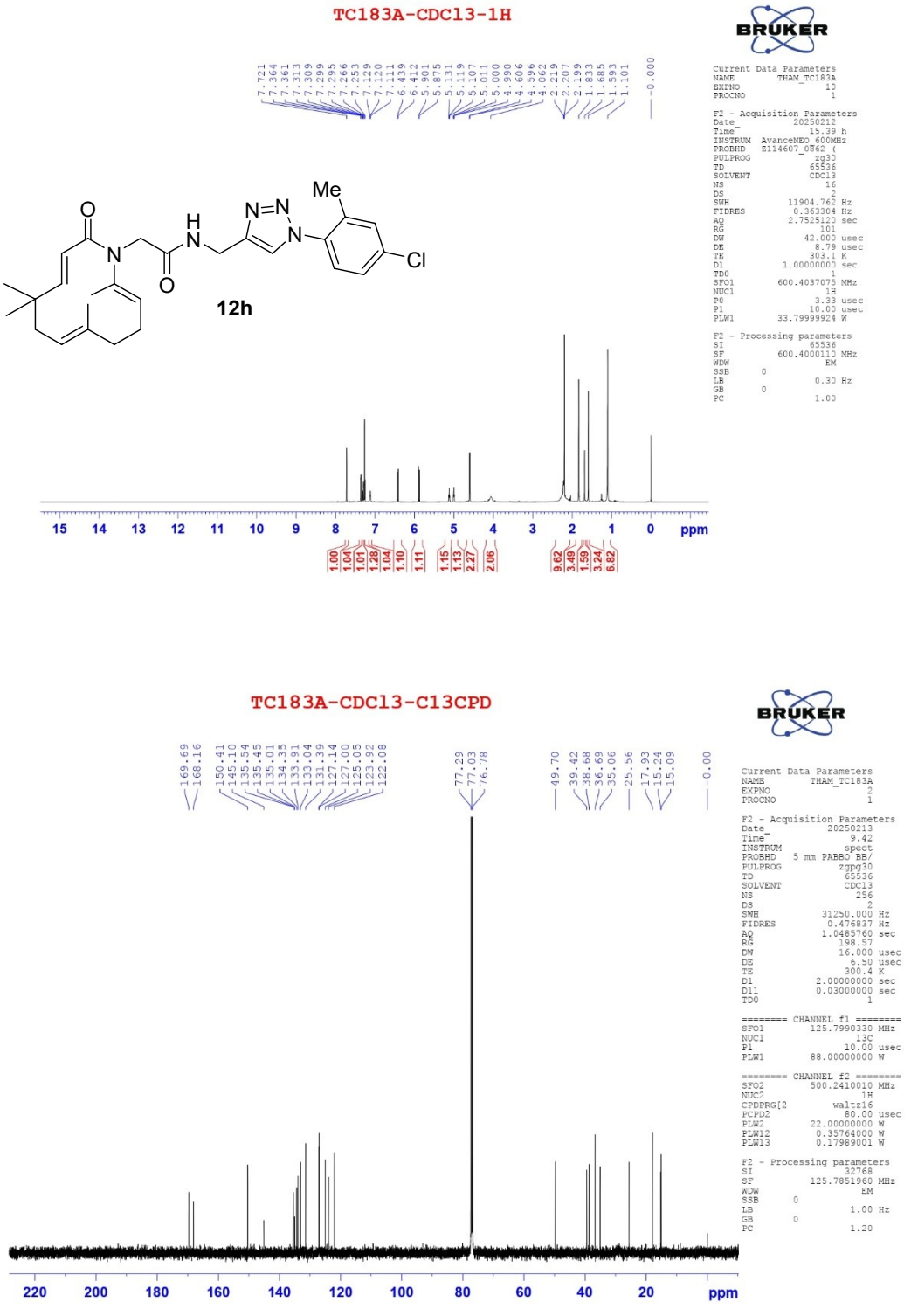


Figure S22. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **12h**

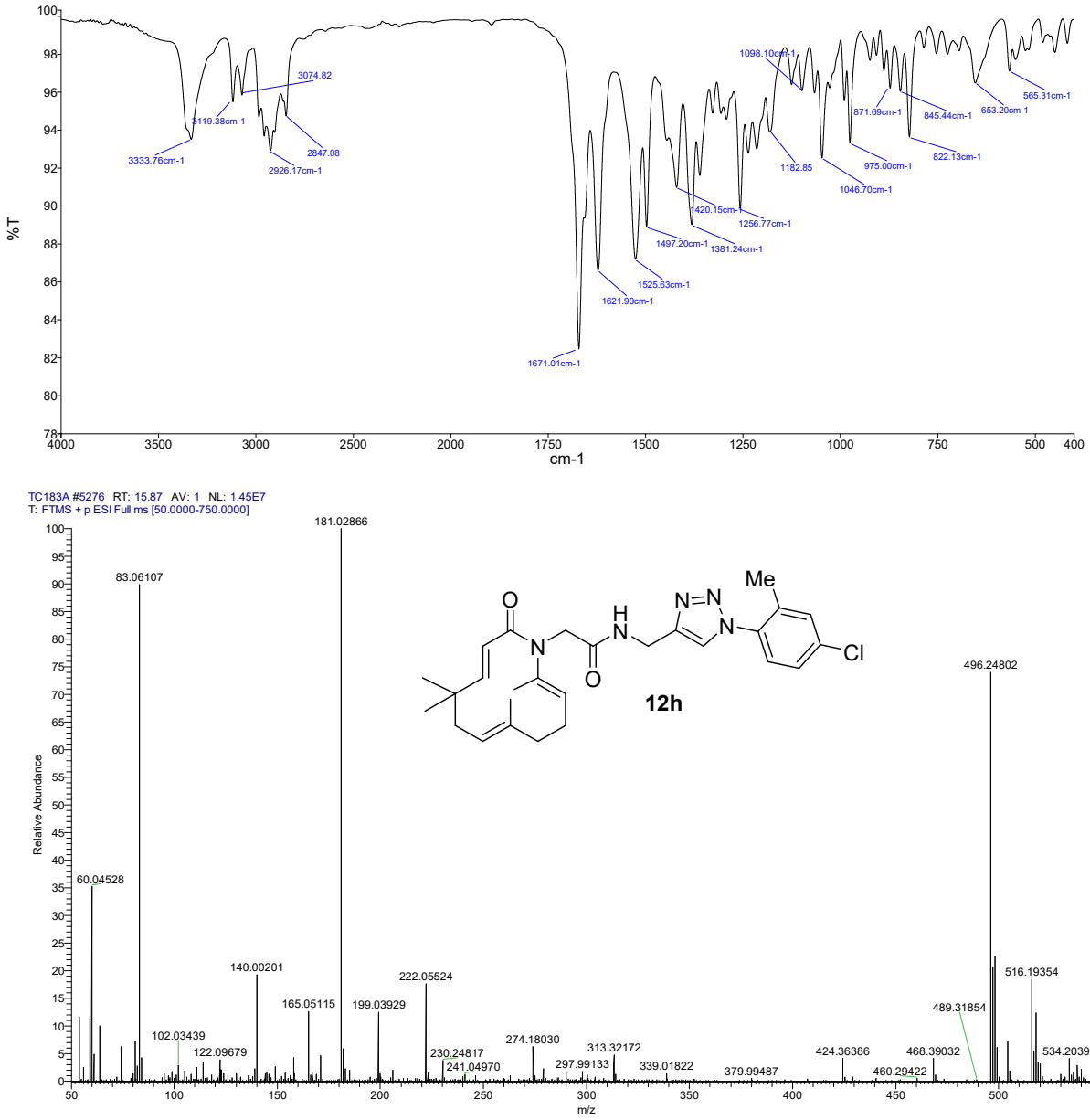


Figure S23. IR and HRMS spectra of compound **12h**

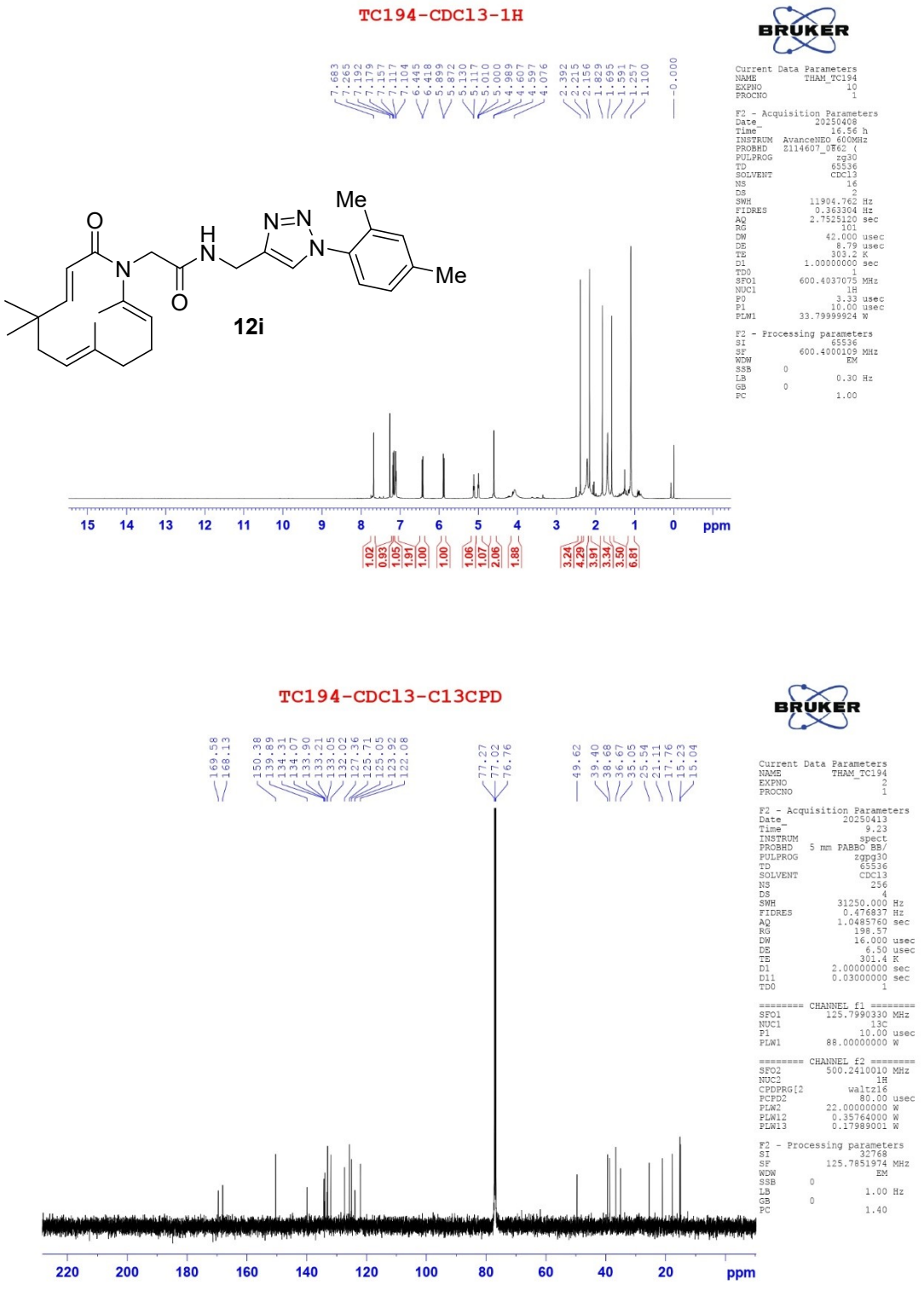
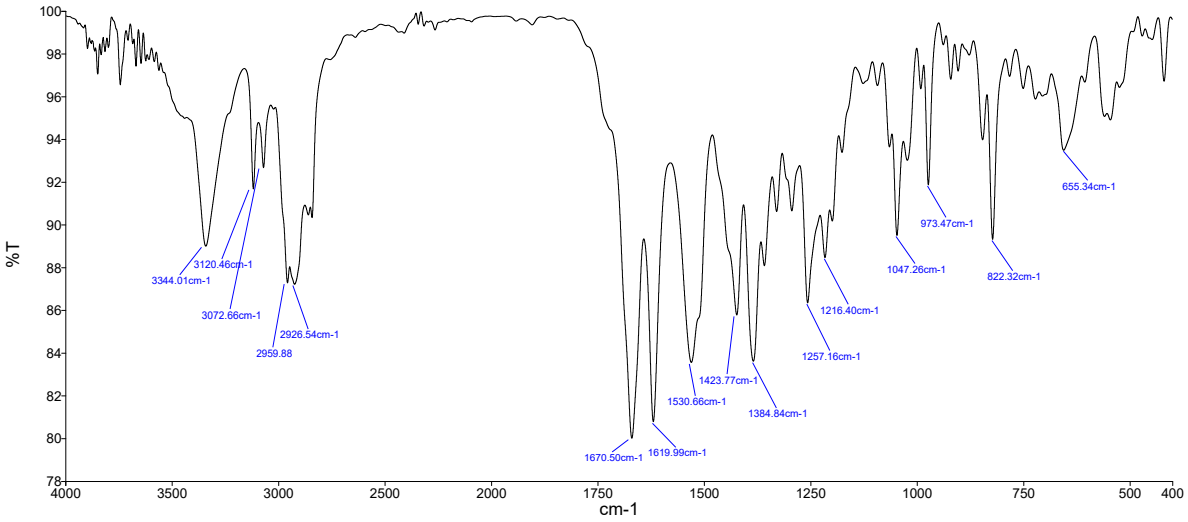


Figure S24. ^{1}H-NMR and ^{13}C-NMR spectra of compound **12i**



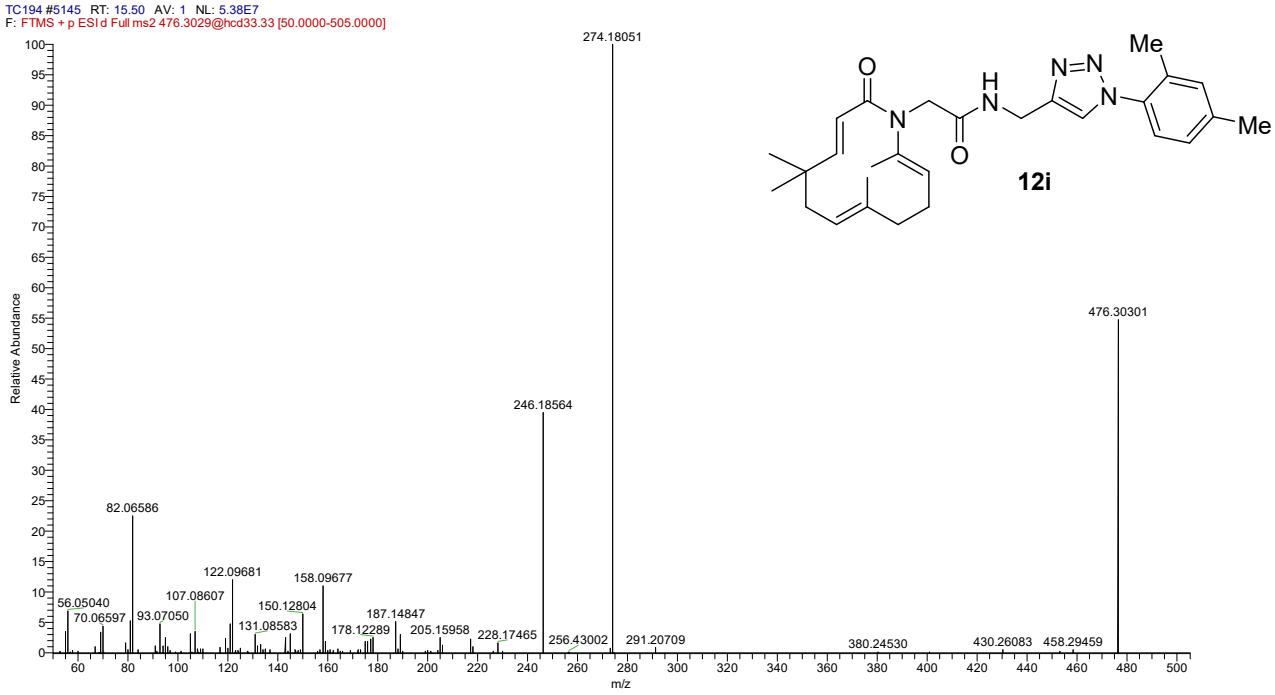


Figure S25. IR and HRMS spectra of compound **12i**

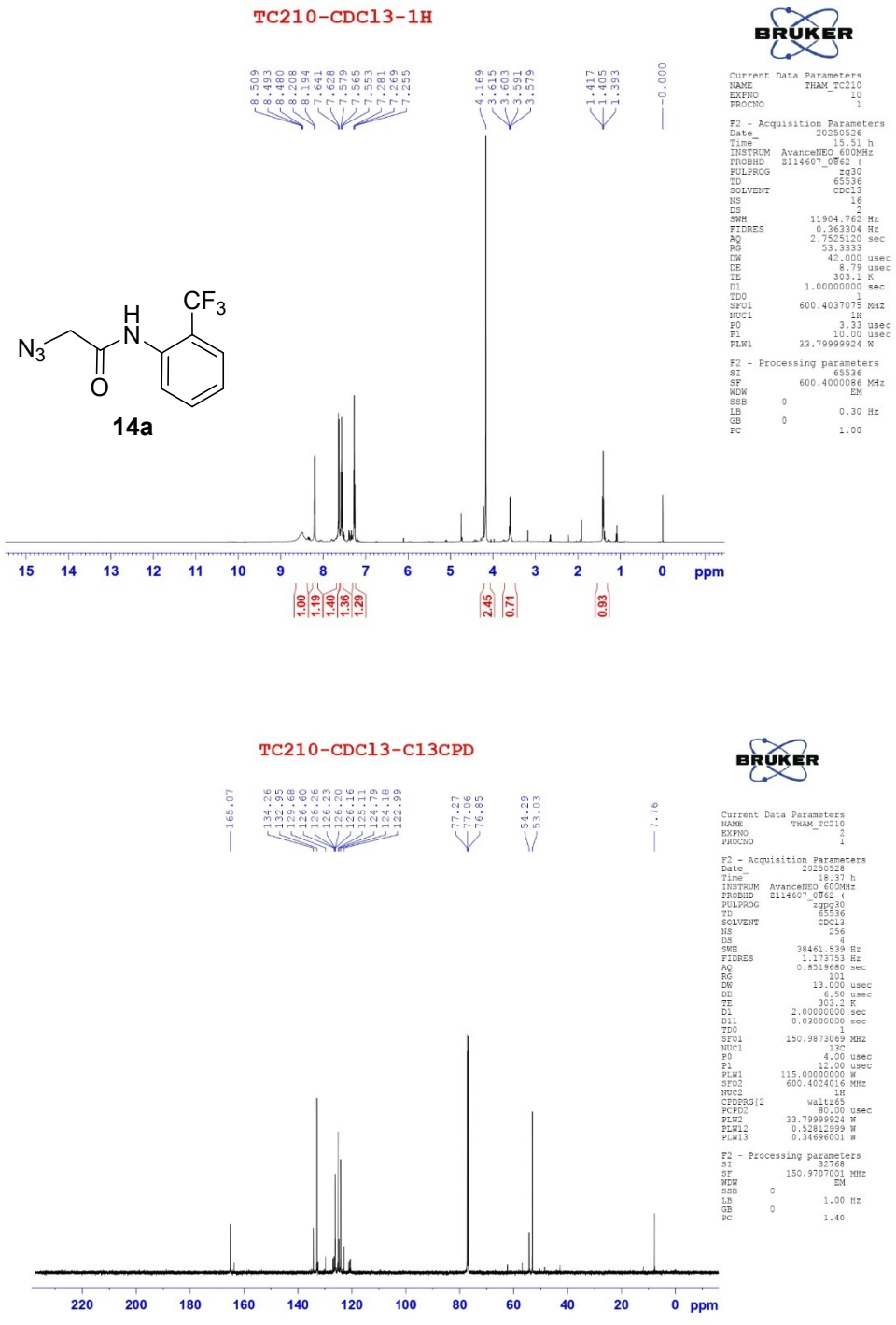


Figure S26. [1]H-NMR and [13]C-NMR spectrum of compound **14a**

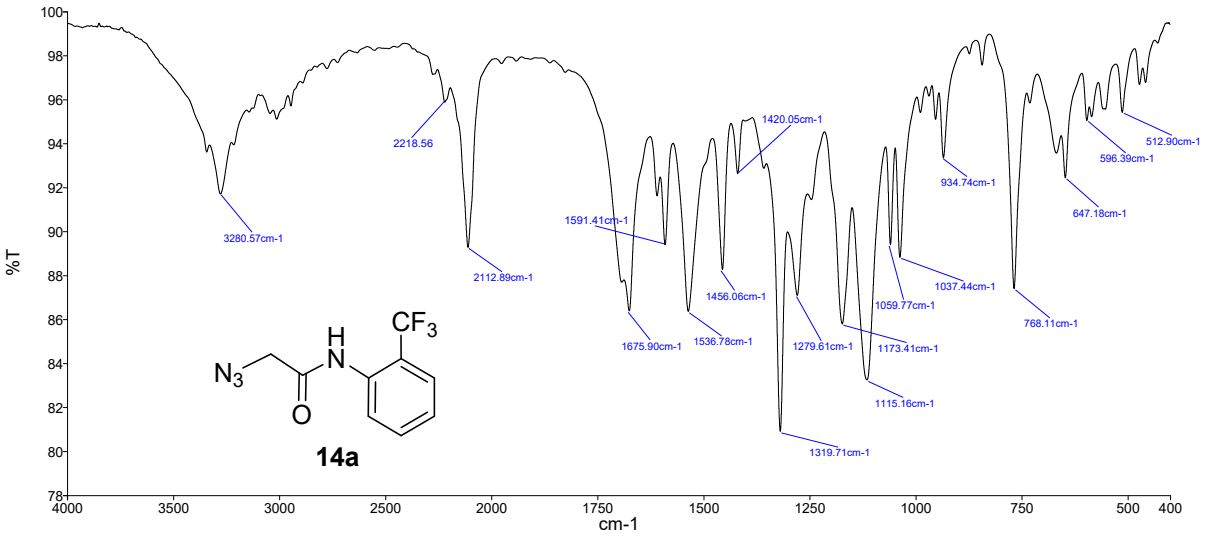


Figure S27. IR spectrum of compound **14a**

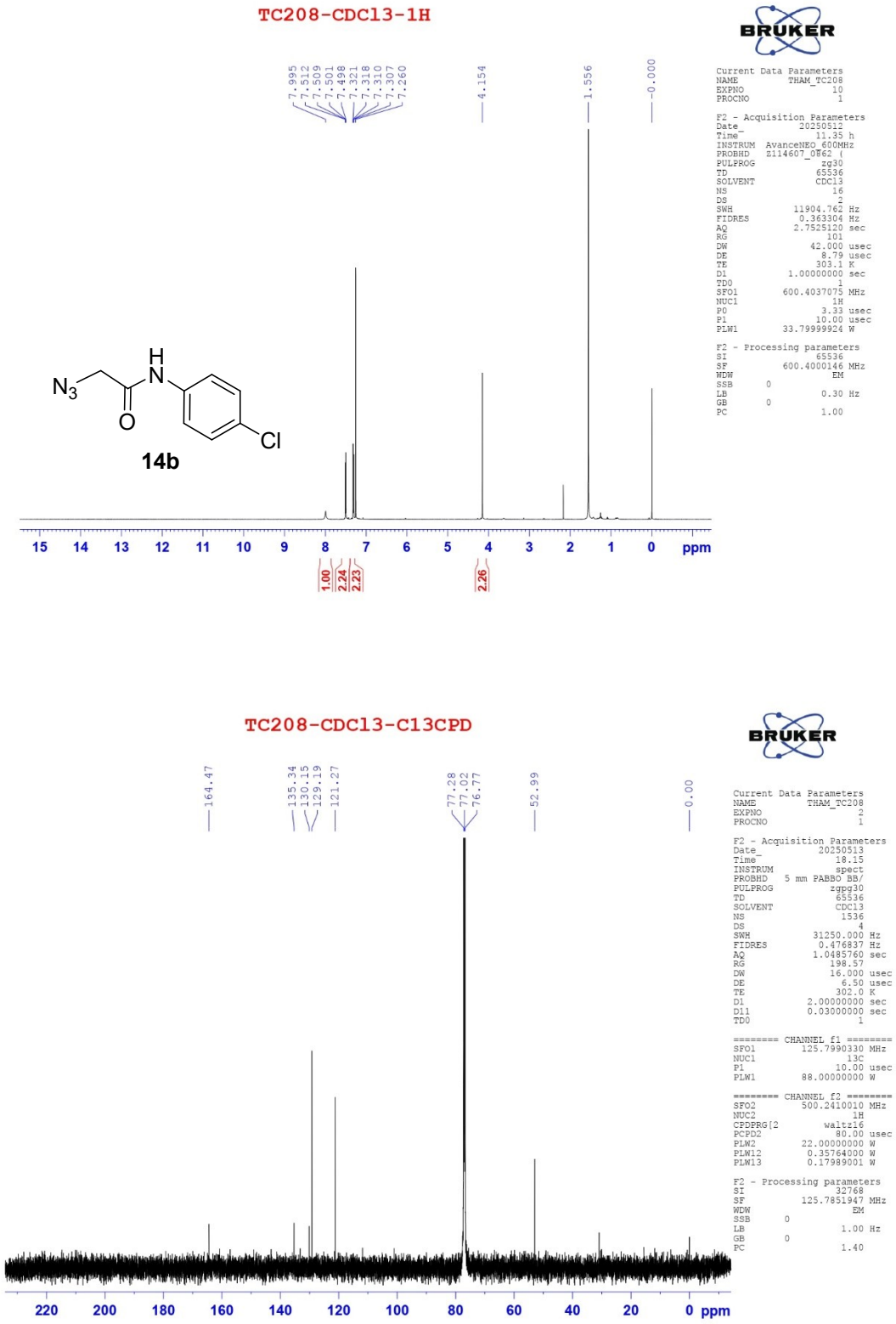


Figure S28. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **14b**

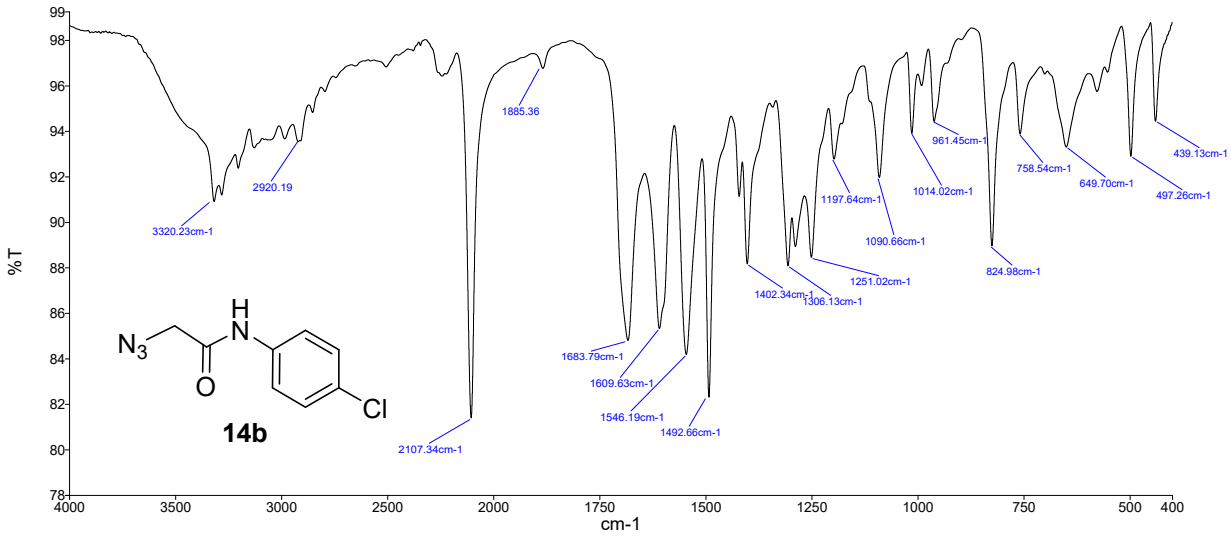


Figure S29. IR spectrum of compound **14b**

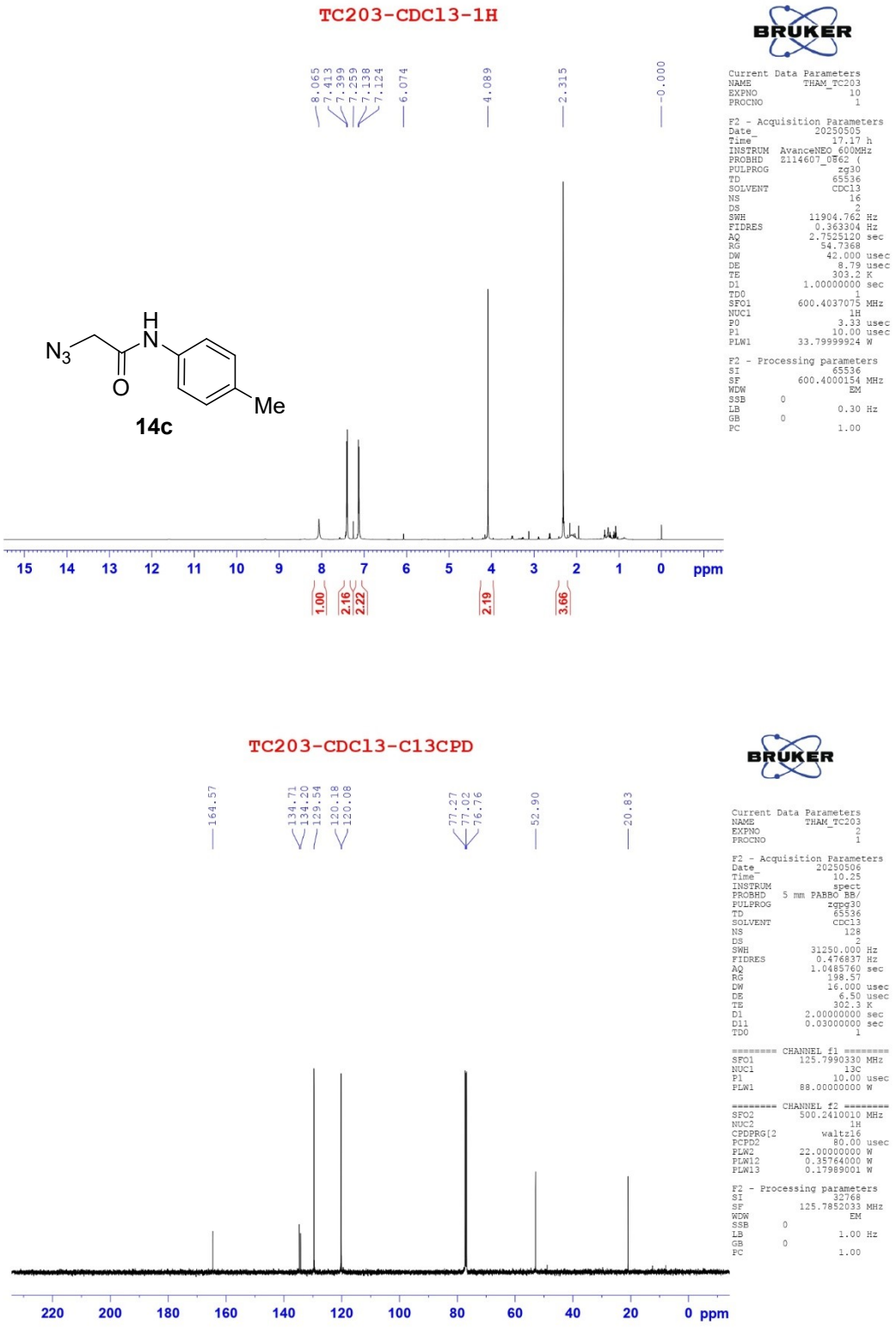


Figure S29. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **14c**

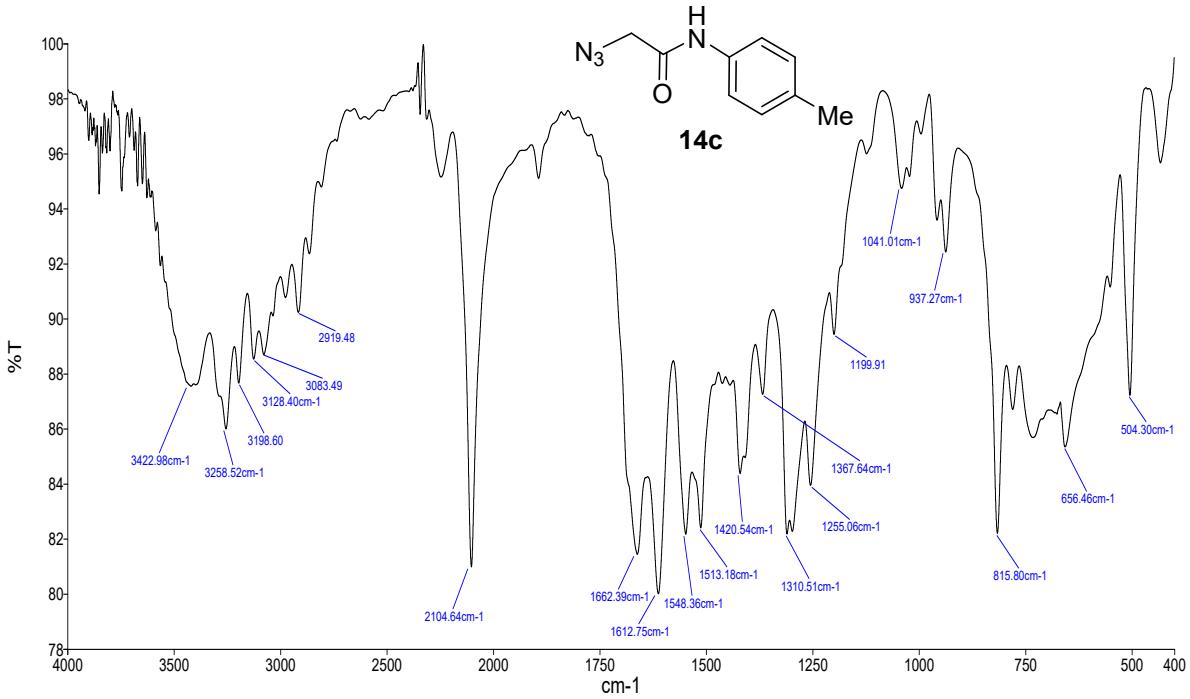


Figure S30. IR spectrum of compound **14c**

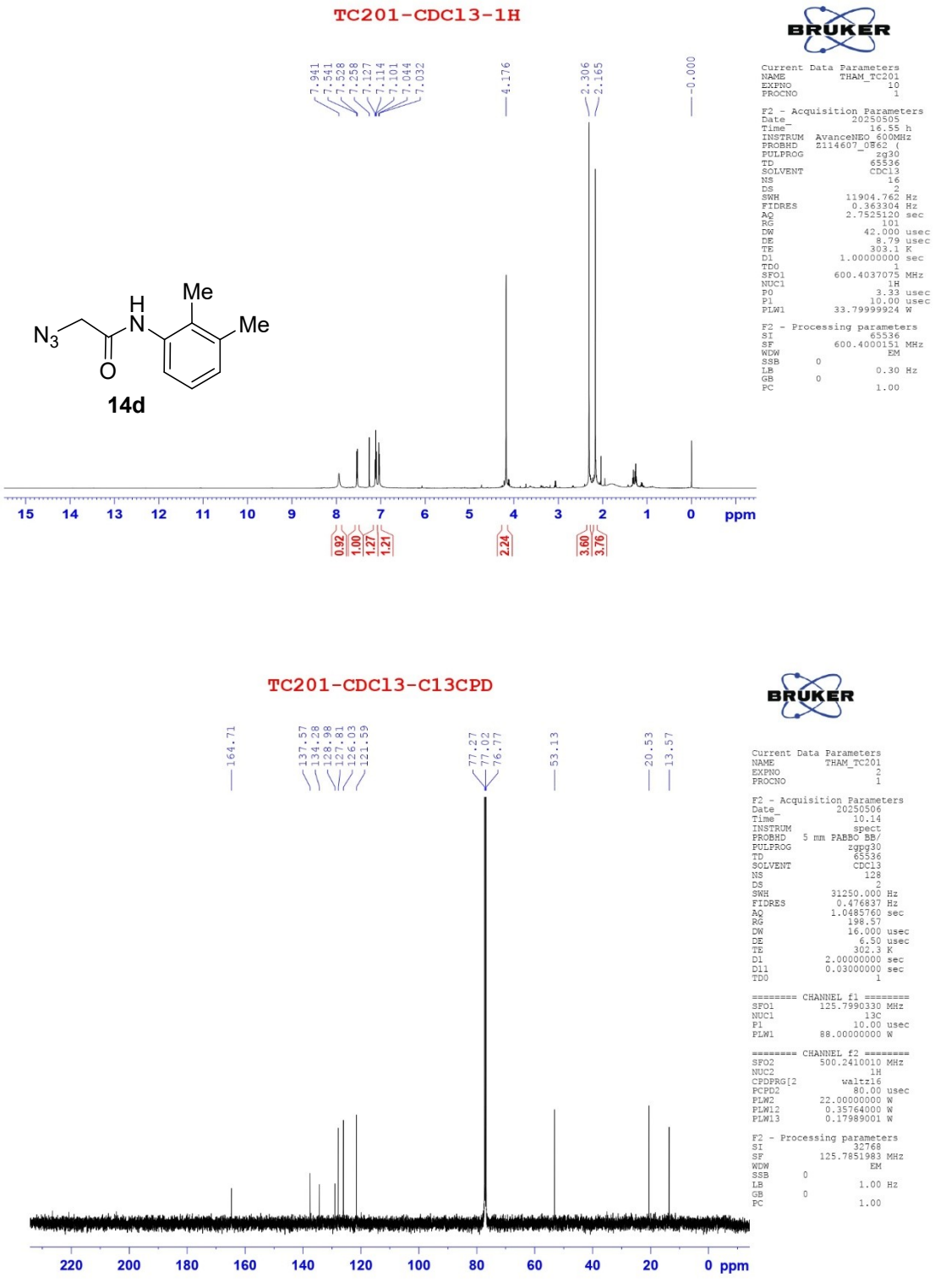


Figure S31. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **14d**

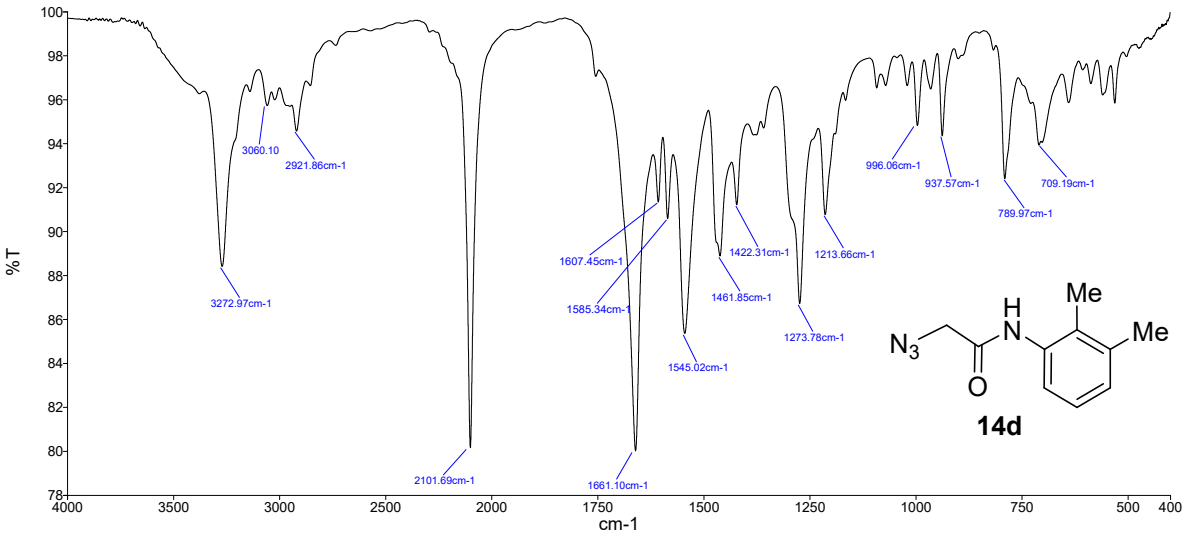


Figure S32. IR spectrum of compound **14d**

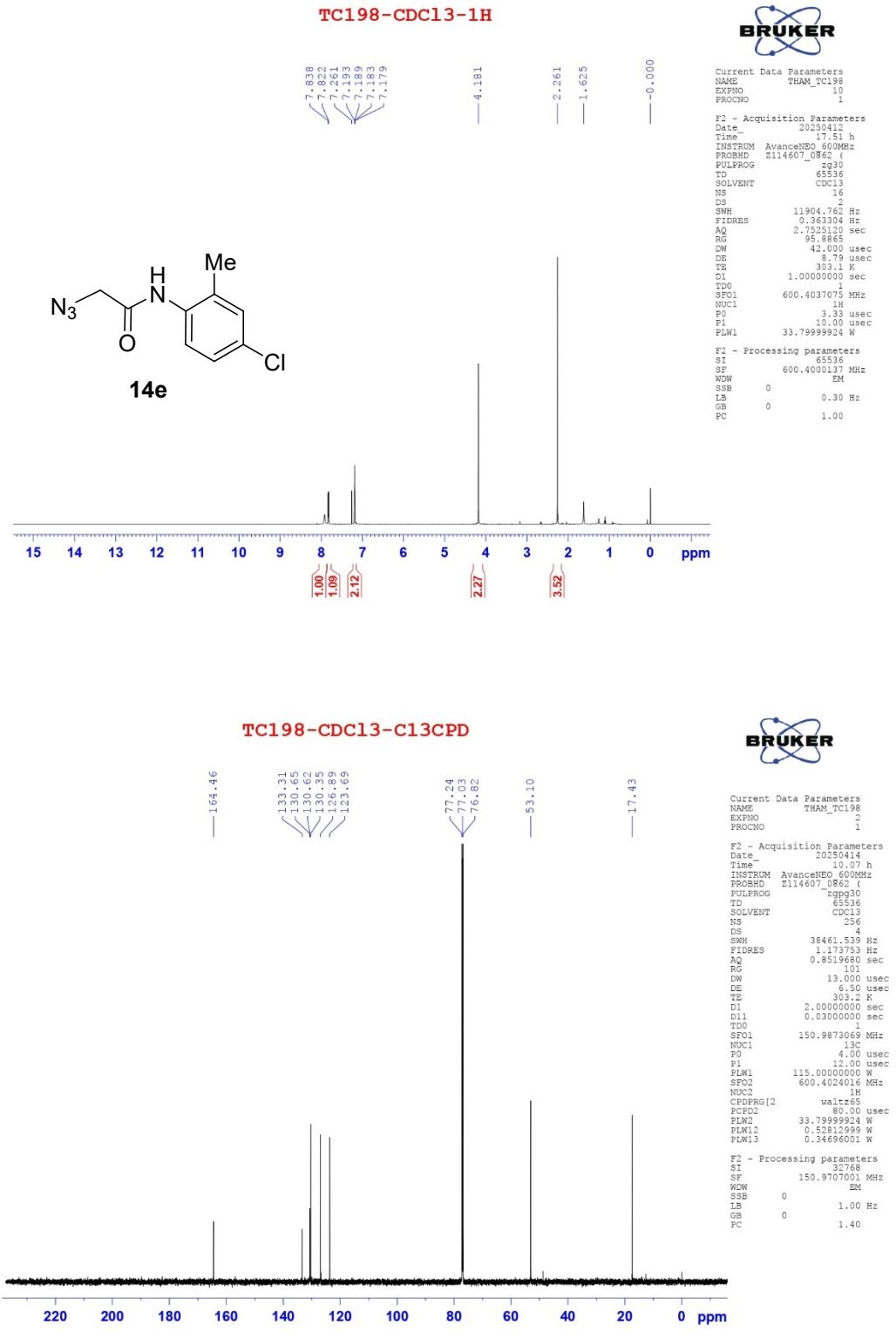


Figure S33. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **14e**

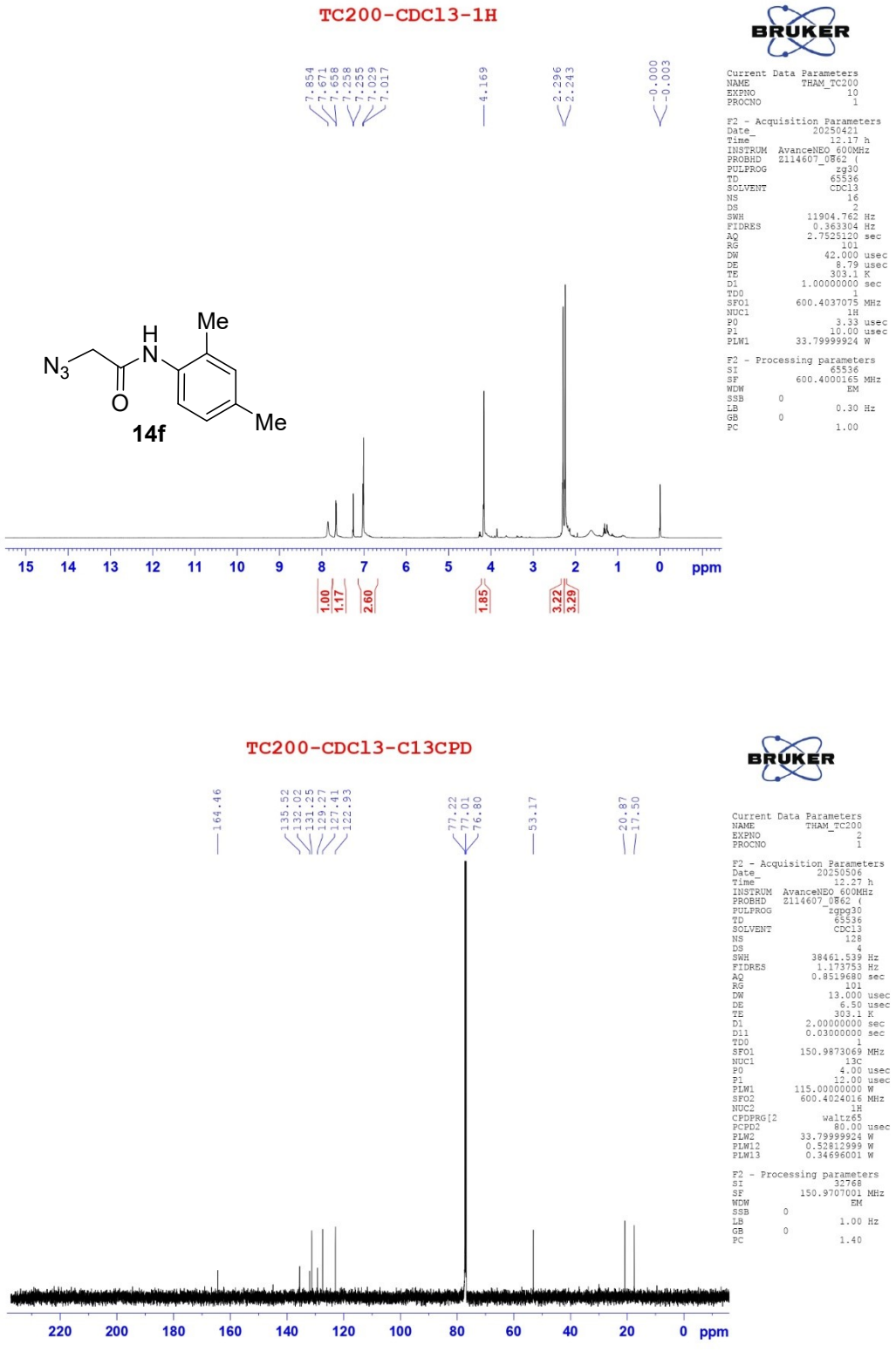


Figure S34. [1]H-NMR and [13]C-NMR spectrum of compound **14f**

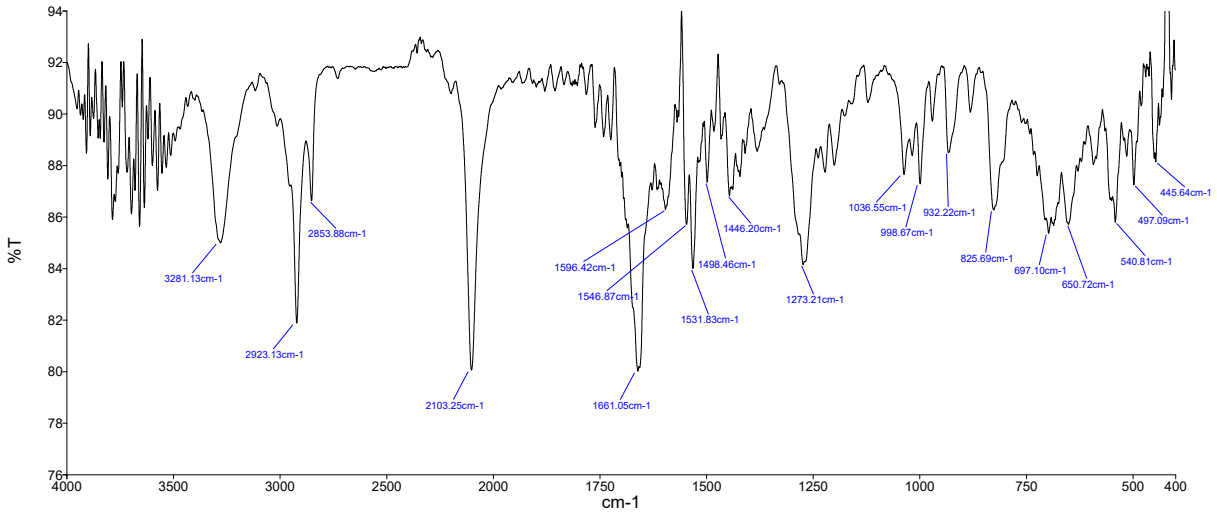


Figure S35. IR spectrum of compound **14f**

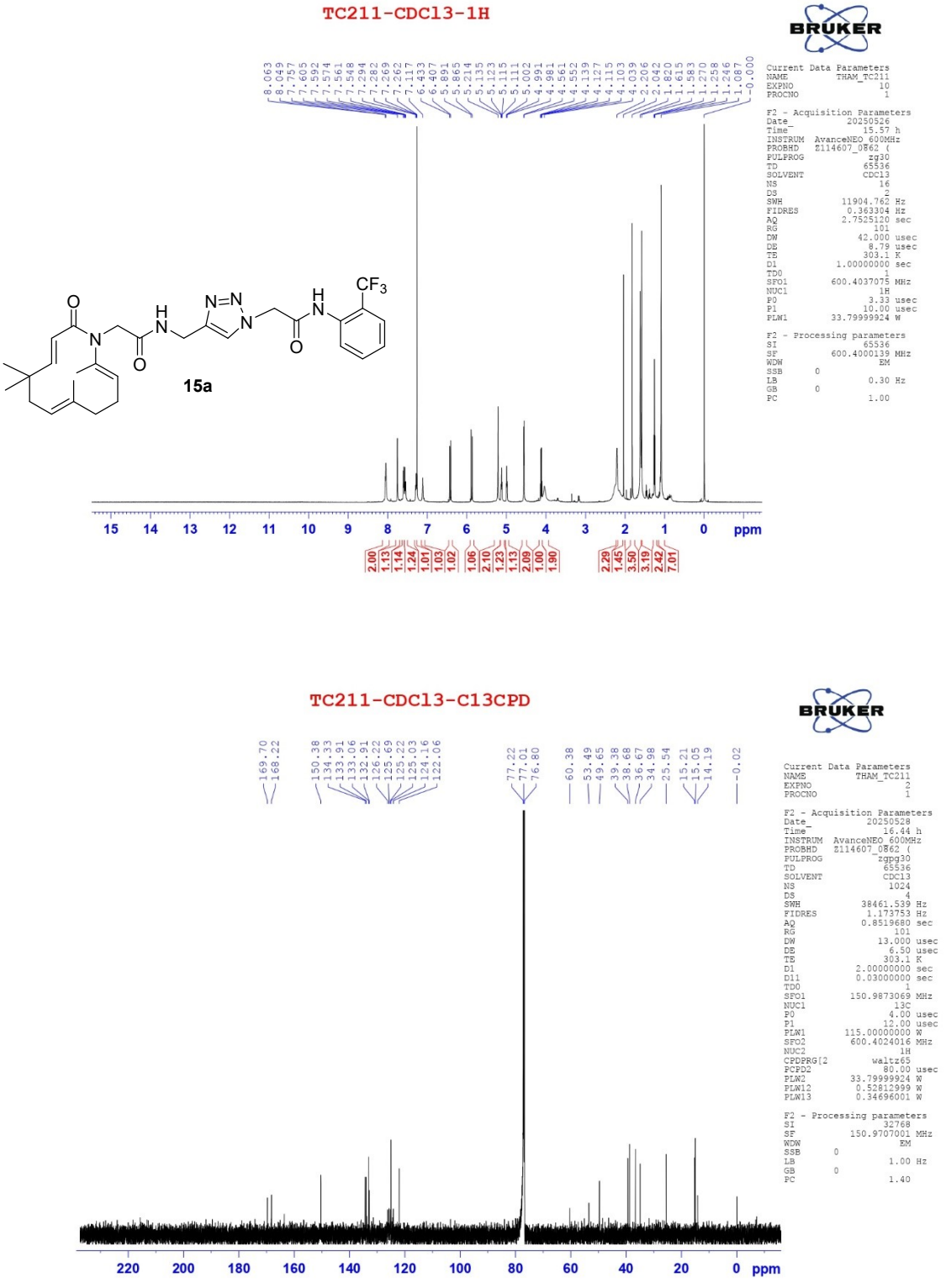
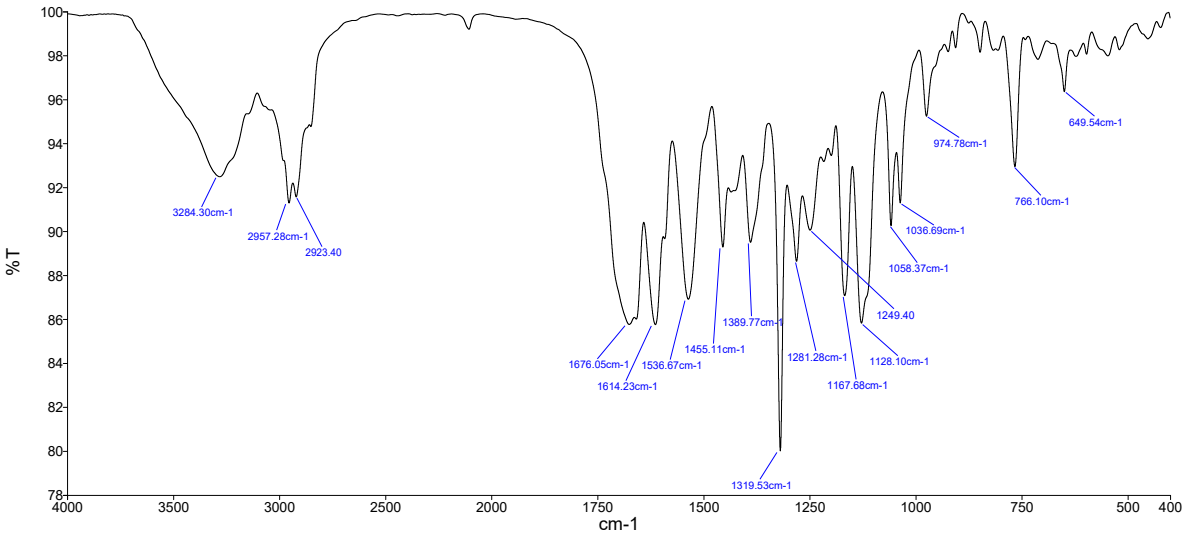


Figure S36. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **15a**



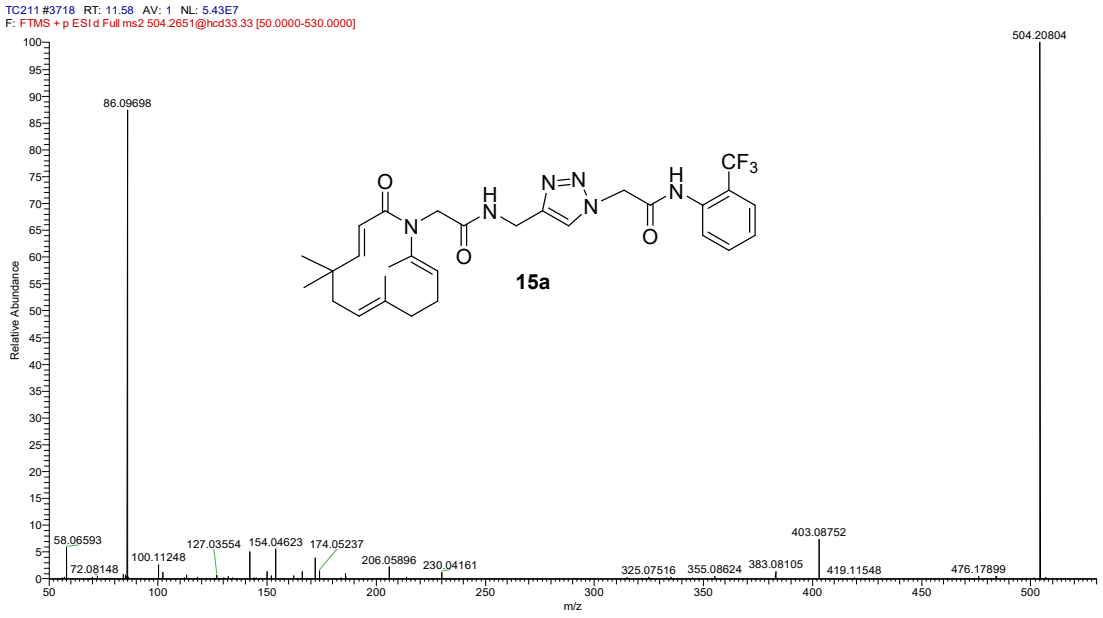


Figure S37. IR and HRMS spectrum of compound **15a**

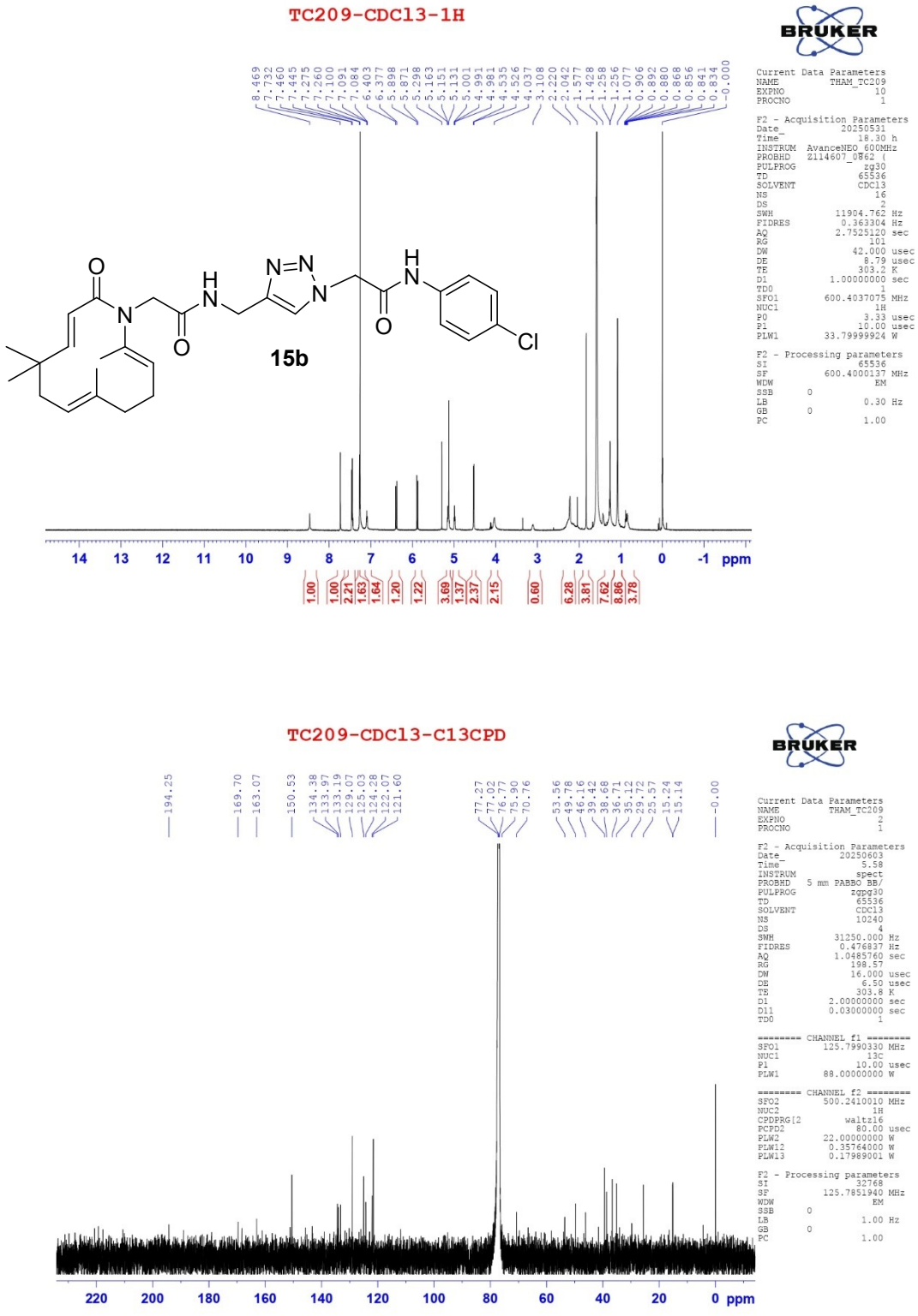


Figure S38. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **15b**

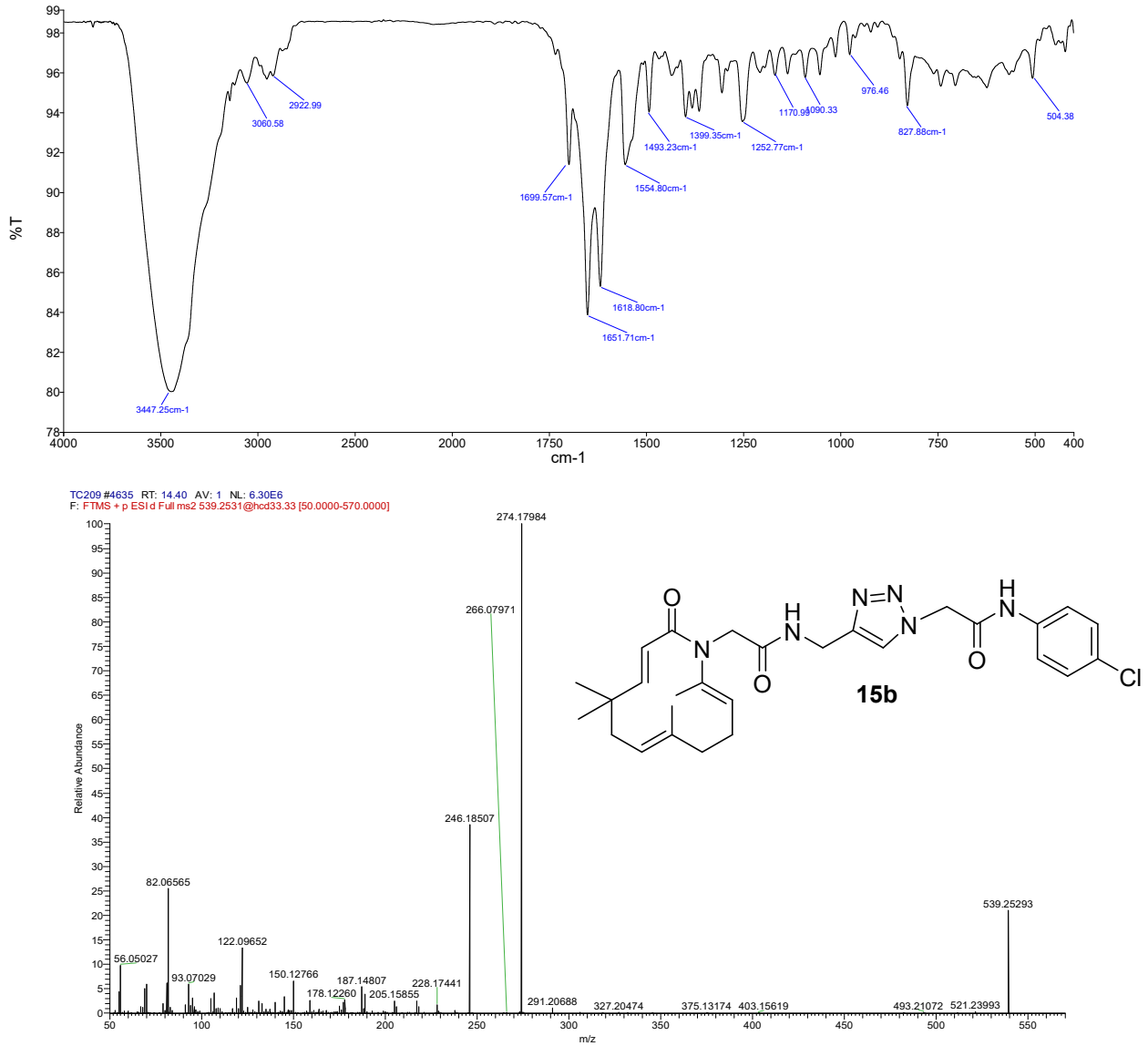


Figure S39. IR and HRMS spectrum of compound **15b**

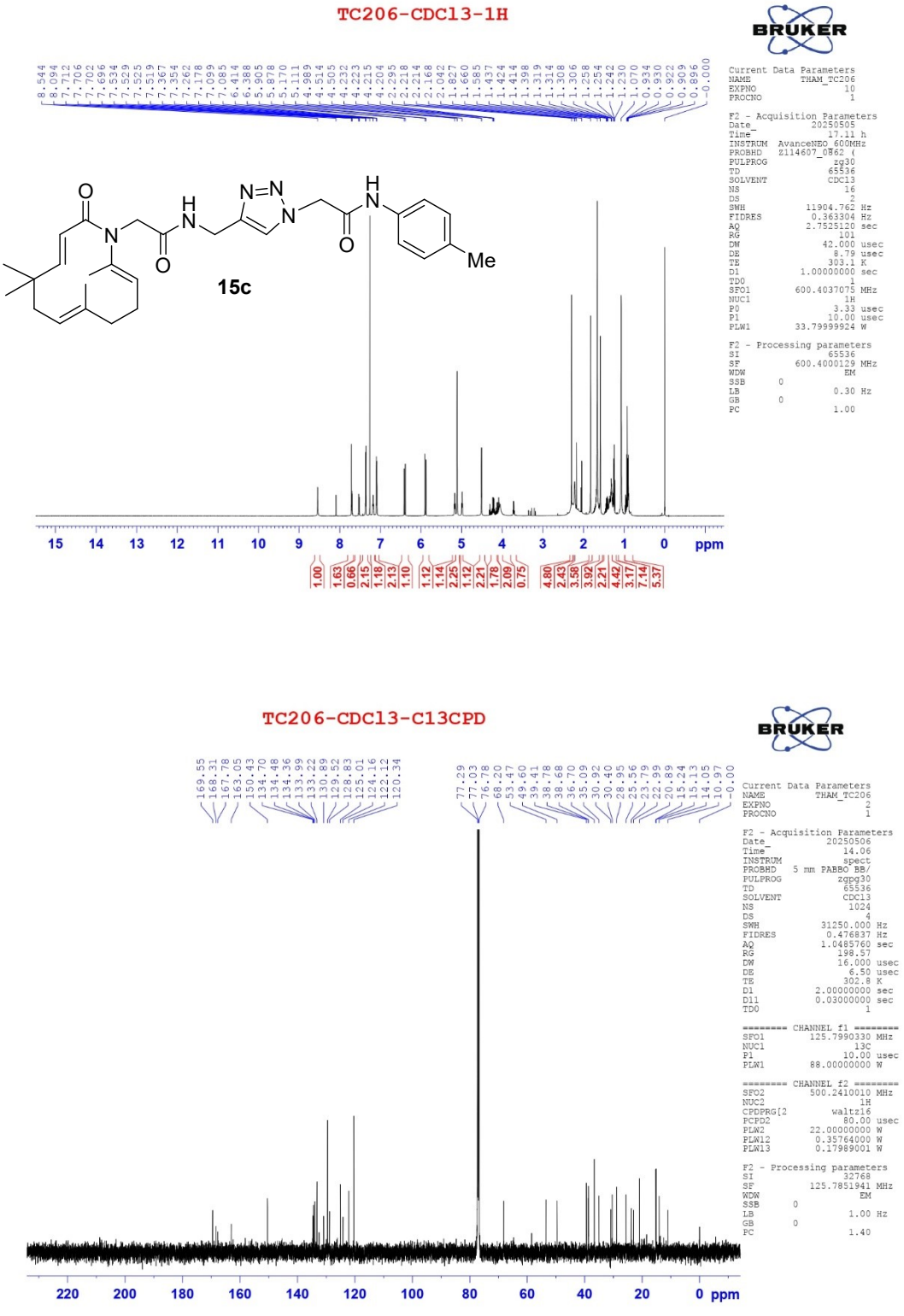


Figure S40. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **15c**

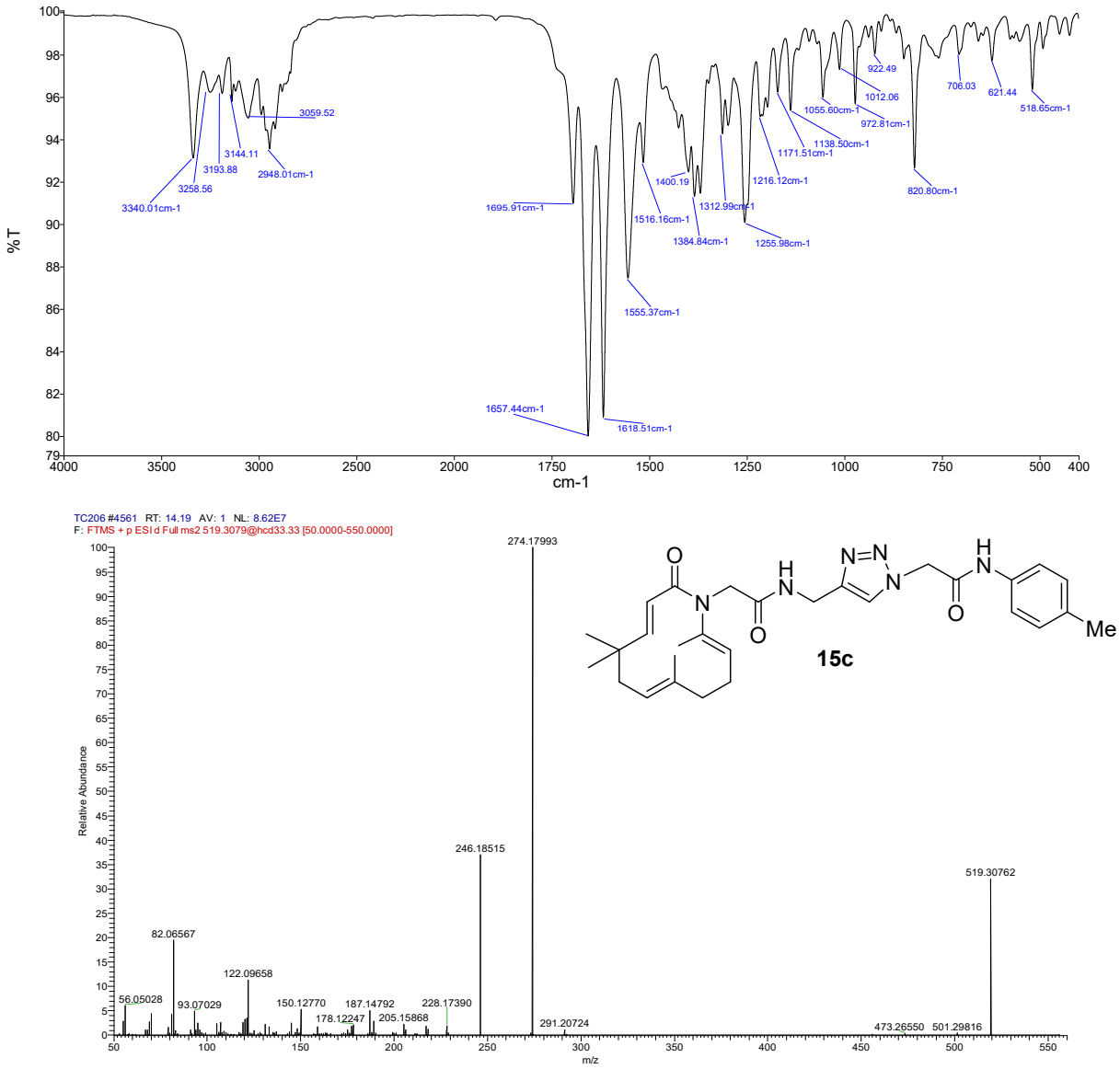


Figure S41. IR and HRMS spectrum of compound **15c**

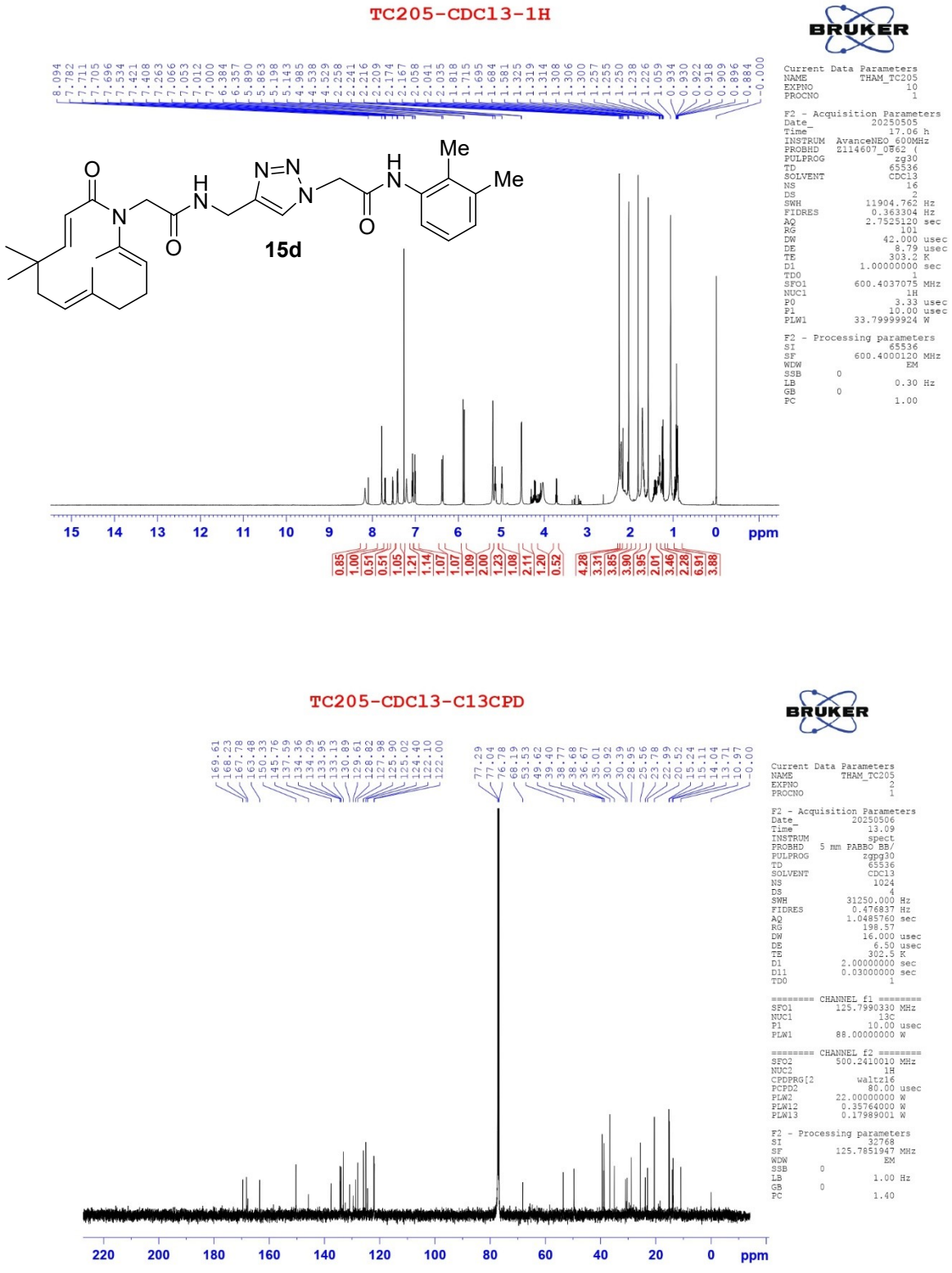


Figure S42. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **15d**

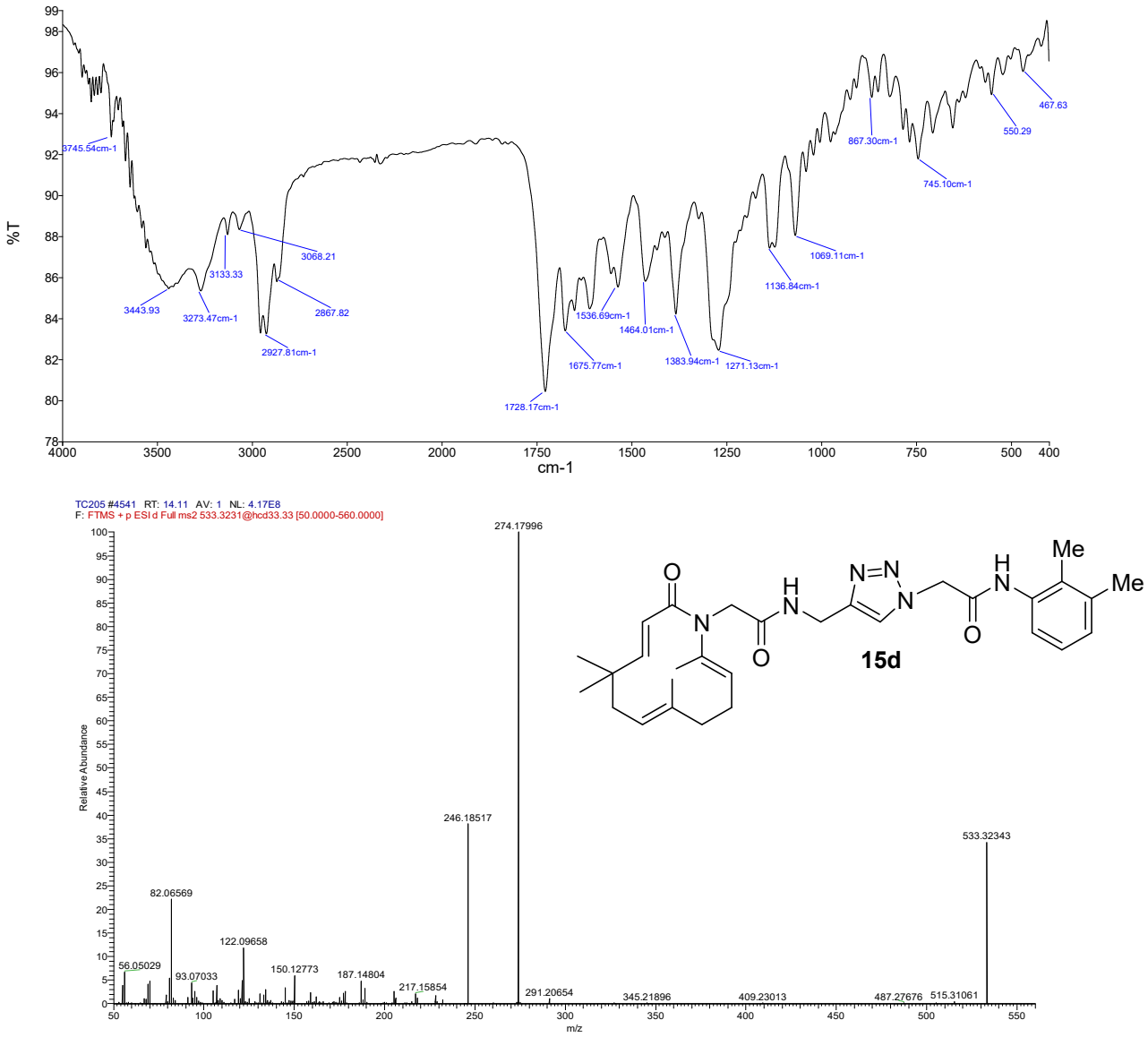


Figure S43. IR and HRMS spectrum of compound **15d**

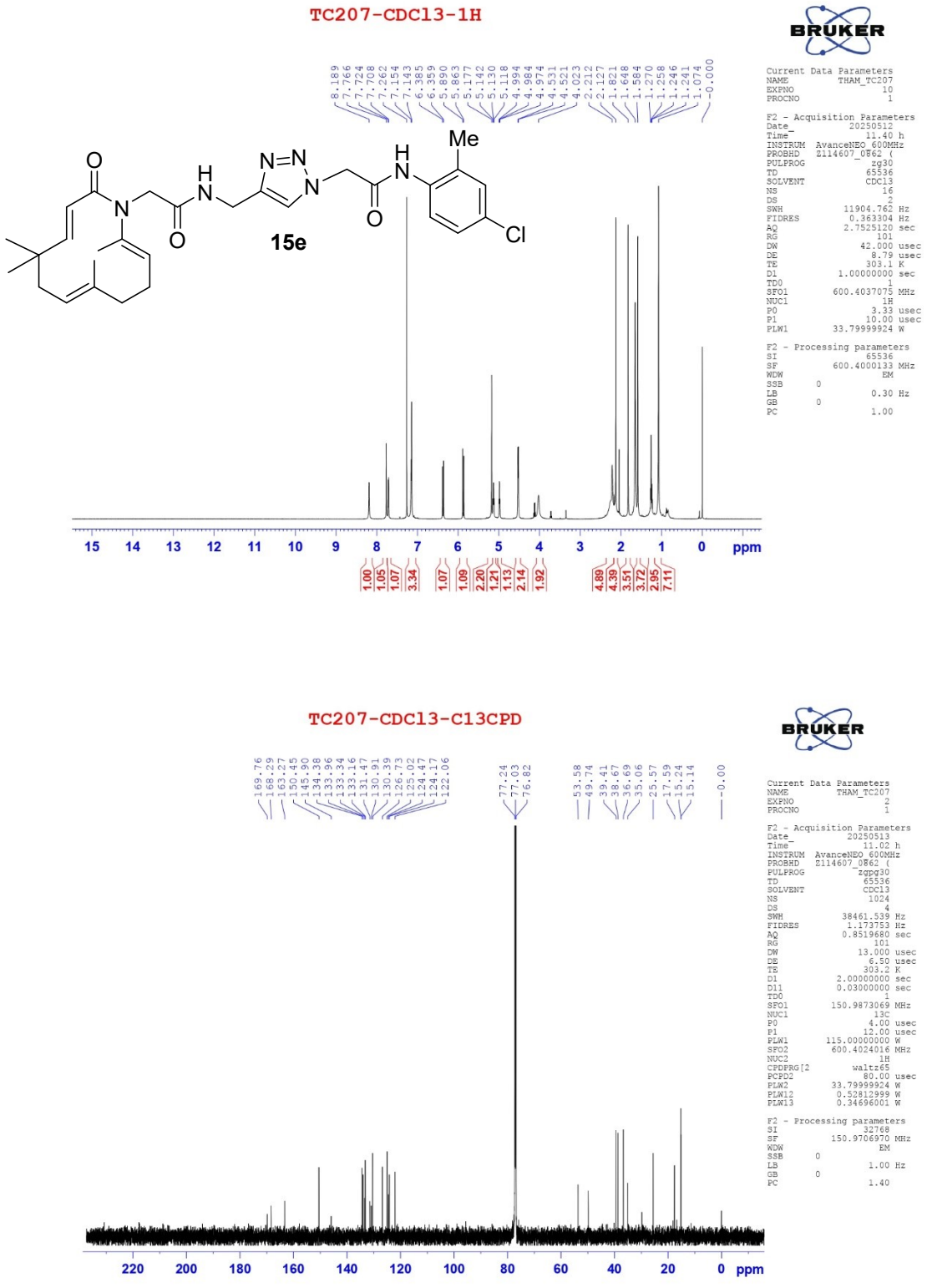
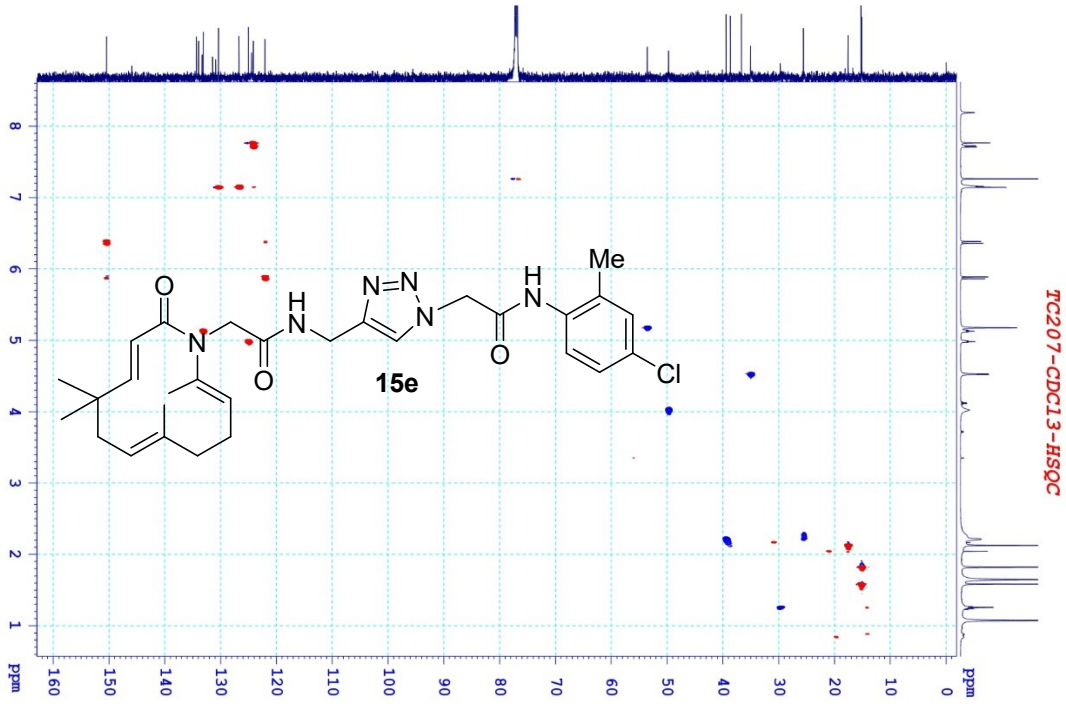


Figure S44. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **15e**


15e
TC207-CDC13-HSQC
ppm
ppm

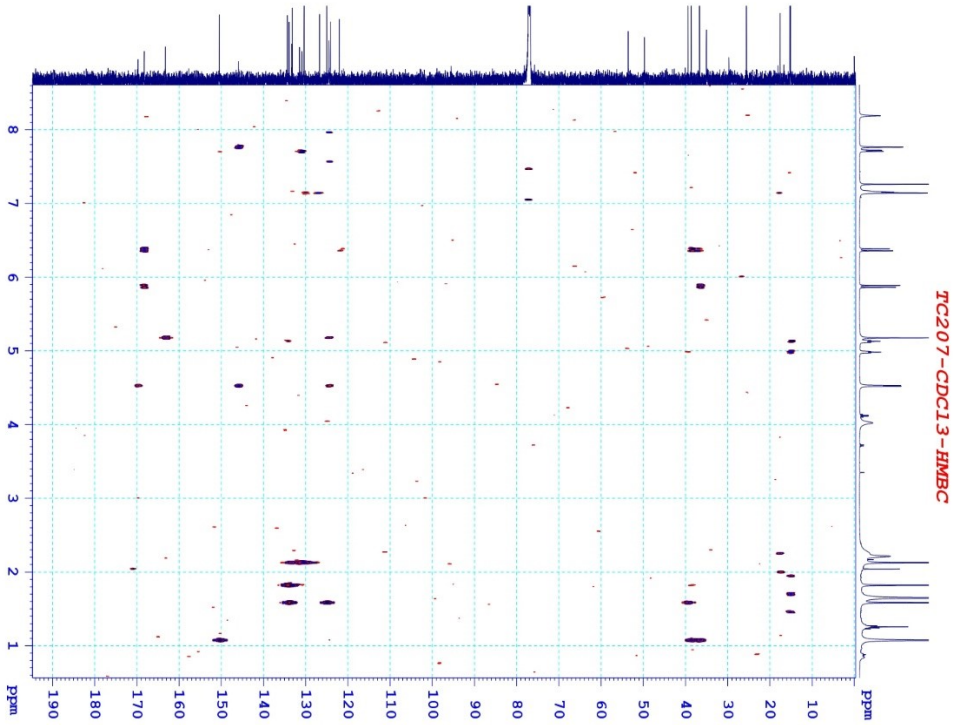
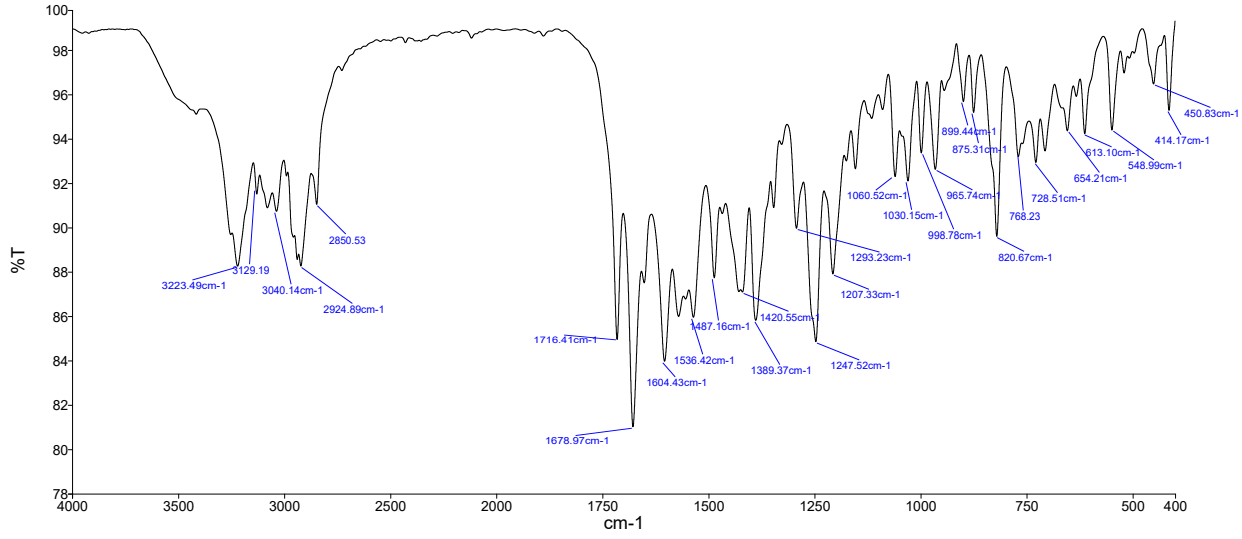


Figure S45 HSQC and HMBC spectrum of compound **15e**



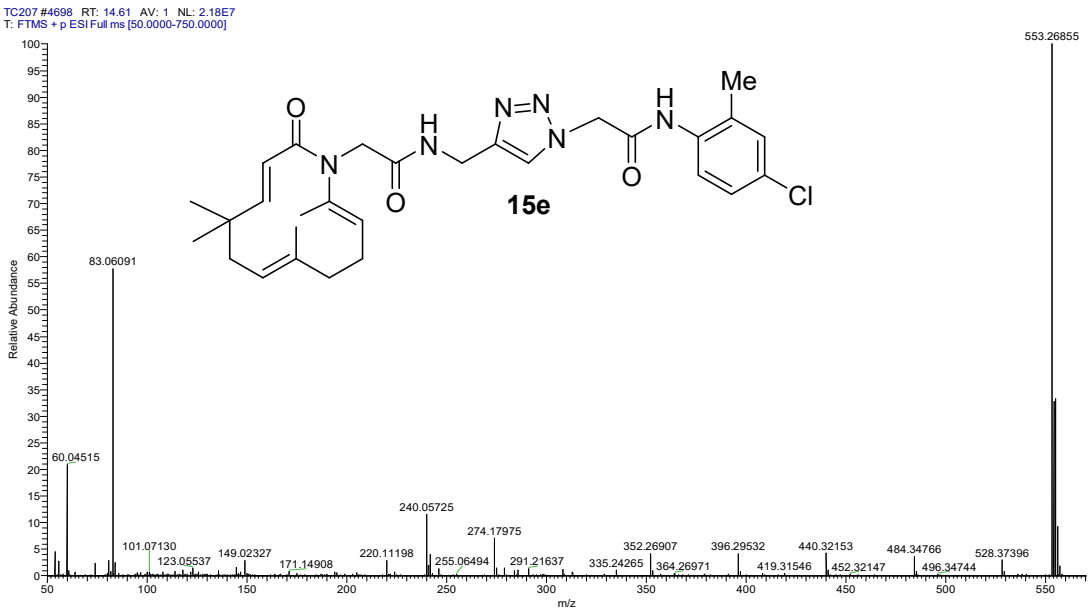


Figure S46. IR and HRMS spectrum of compound **15e**

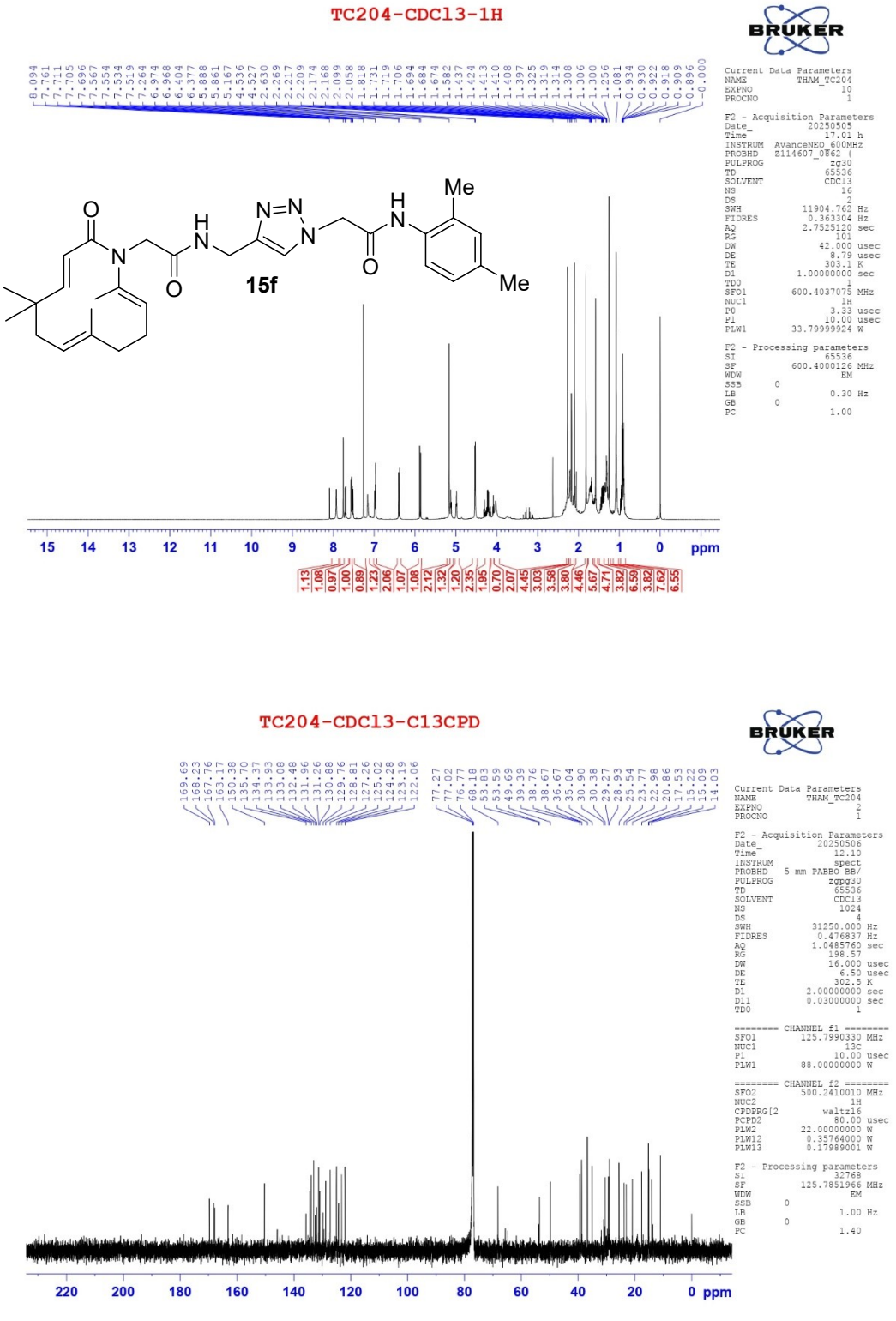
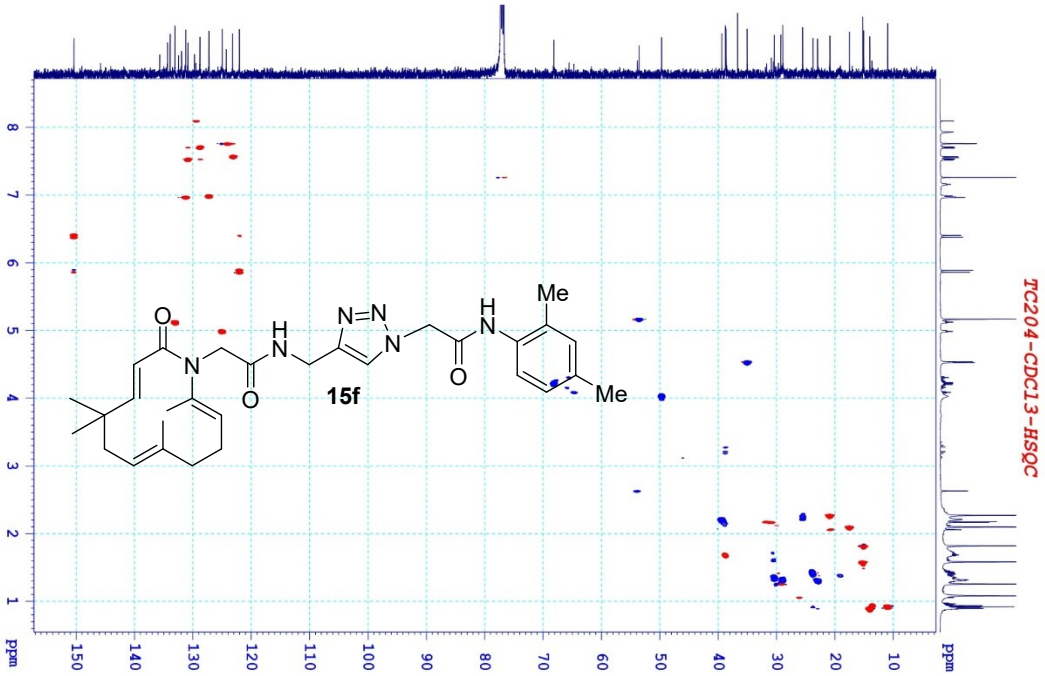


Figure S47. [1]H-NMR and [13]C-NMR spectrum of compound **15f**


TC204-CDC13-HSQC
15f
ppm
ppm

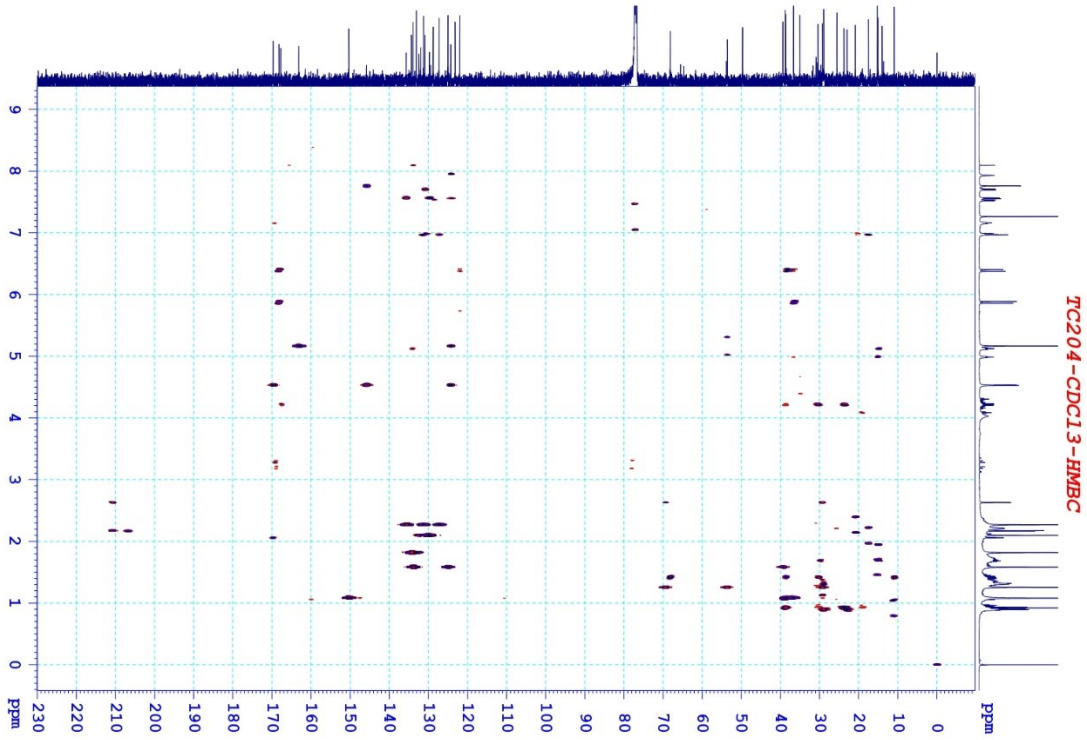


Figure S48 HSQC and HMBC spectrum of compound **15f**

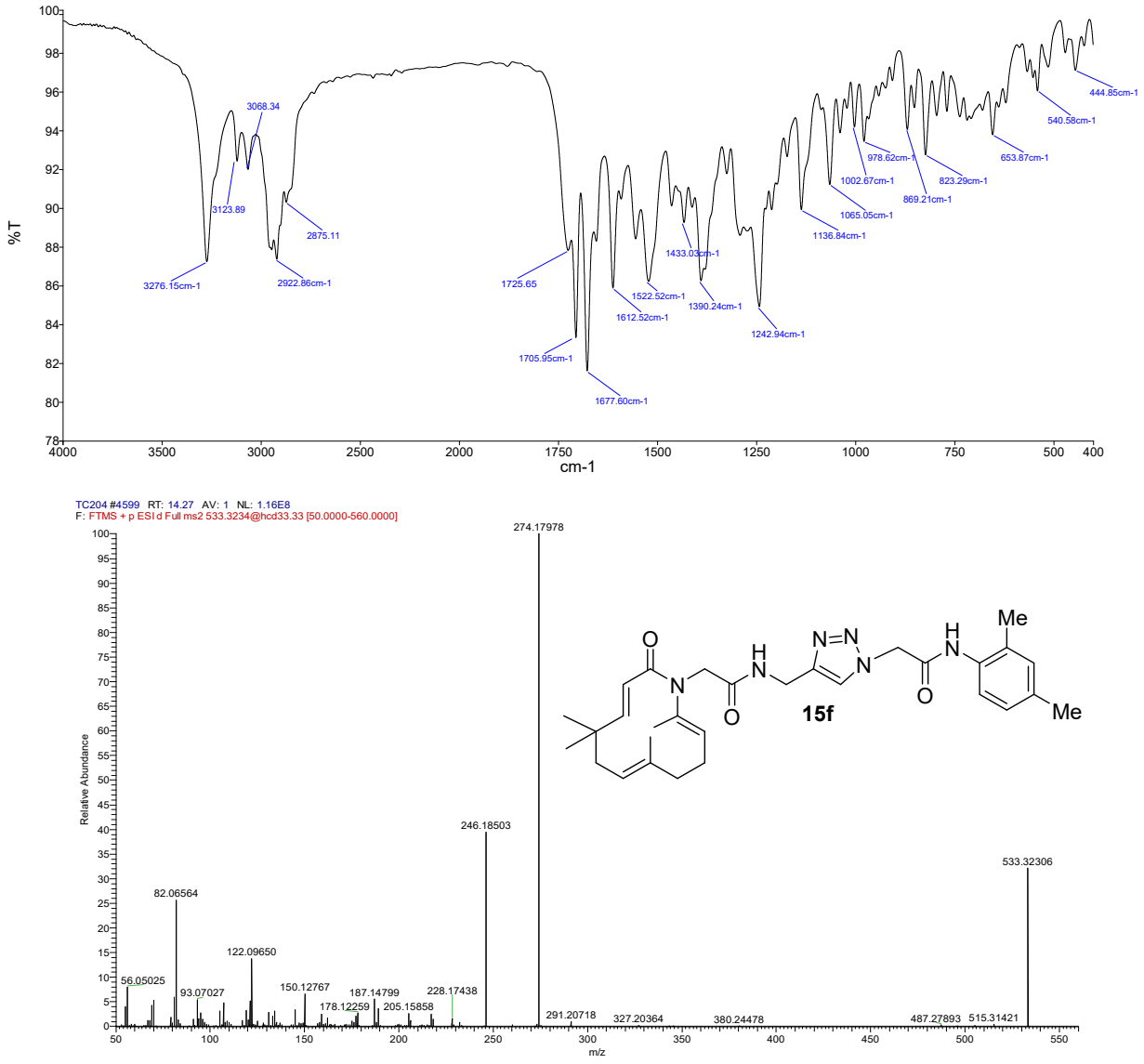


Figure S49. IR and HRMS spectrum of compound **15f**