Supplementary material

Alkylamino and aralkylamino derivatives of avarone and its mimetic as selective agents against non-small cell lung cancer cells, their antibacterial and antifungal potential

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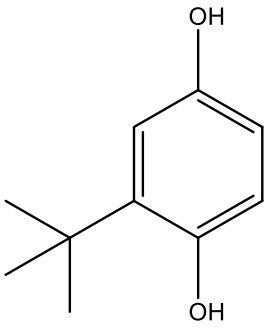
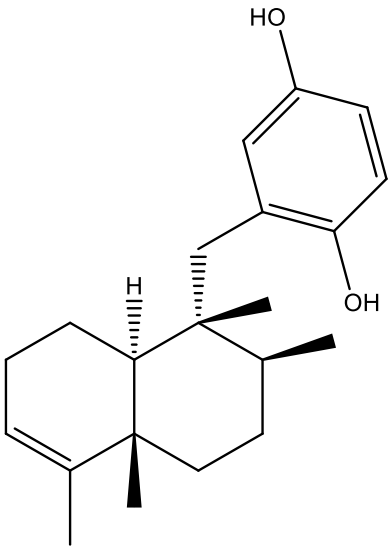


tert-butylhydroquinone



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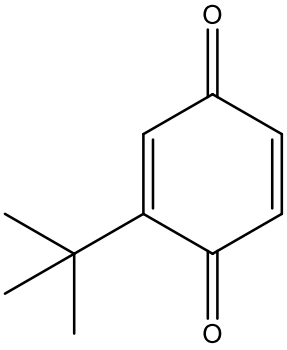
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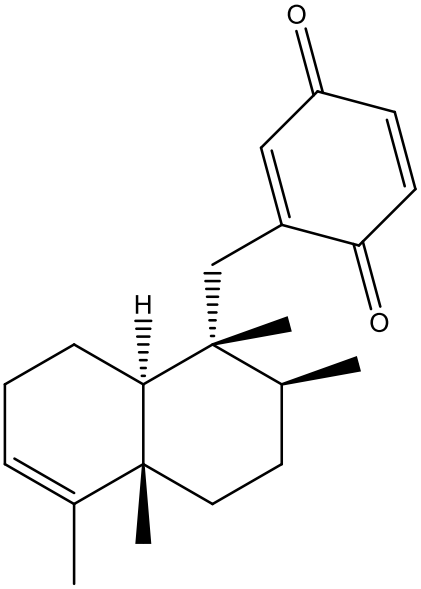
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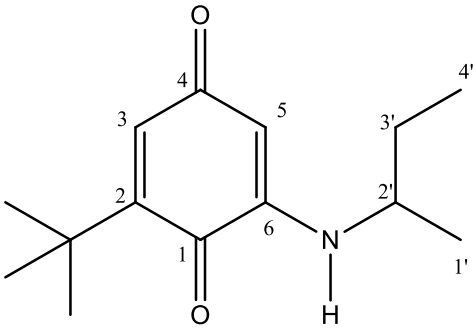
O
H

1 **2**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N
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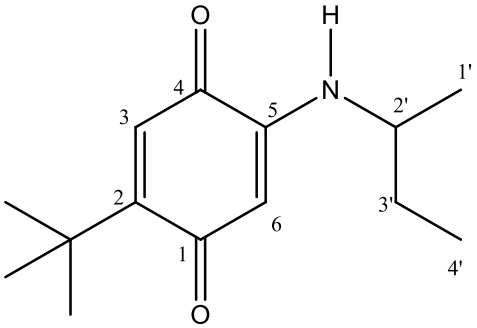
3'
2'
1'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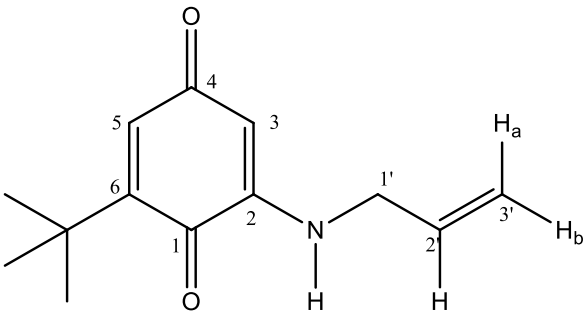
3a **3b**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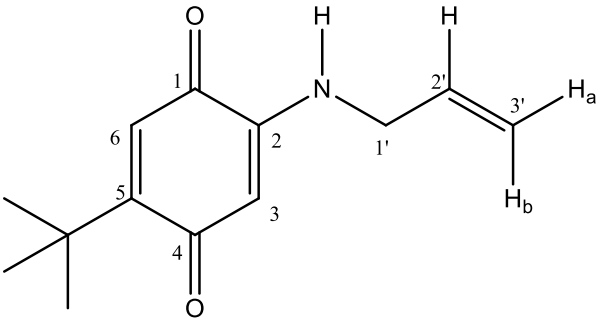
Ha
Hb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H
H
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2'
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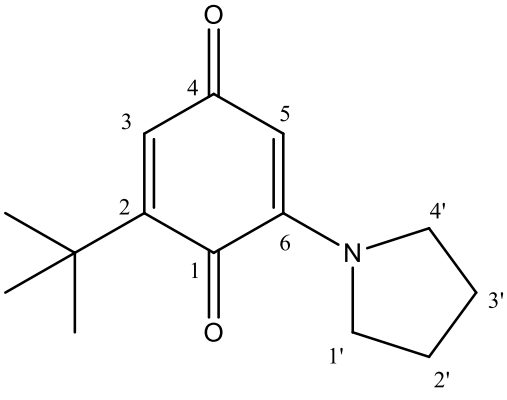
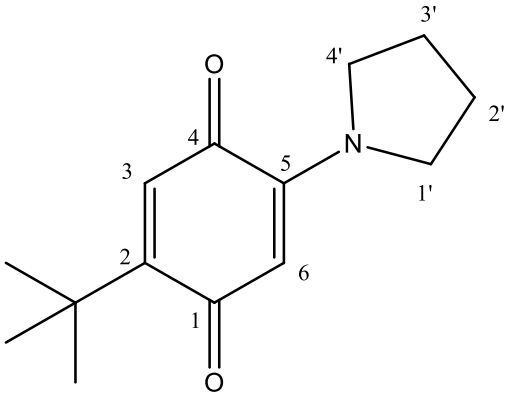
4
O
Hb

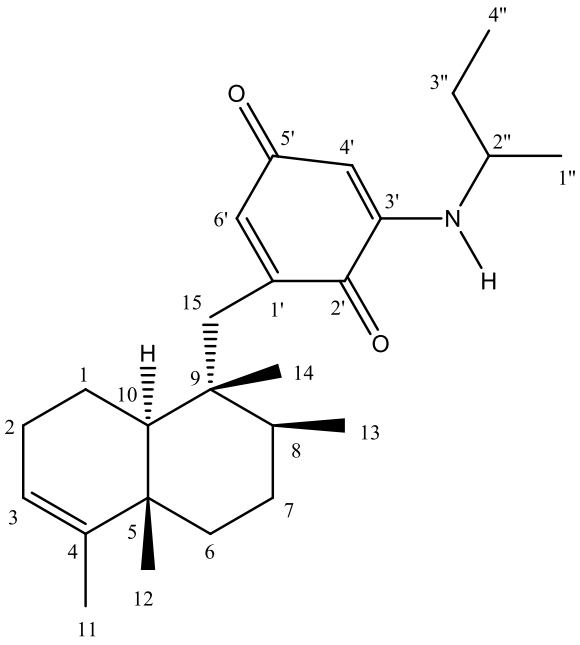
4a **4b**

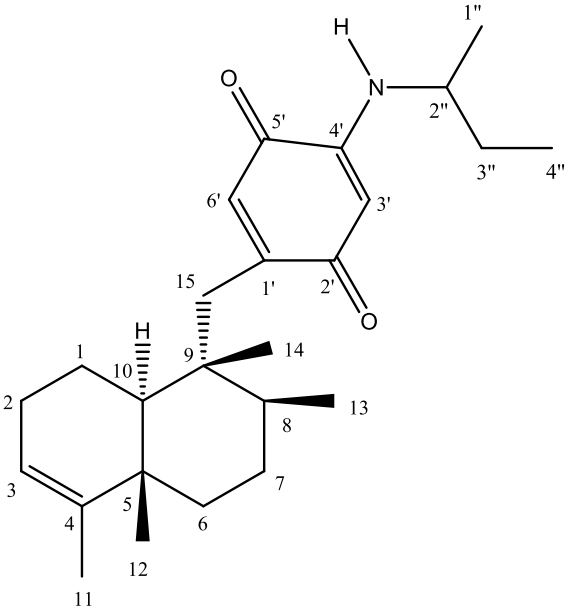




34

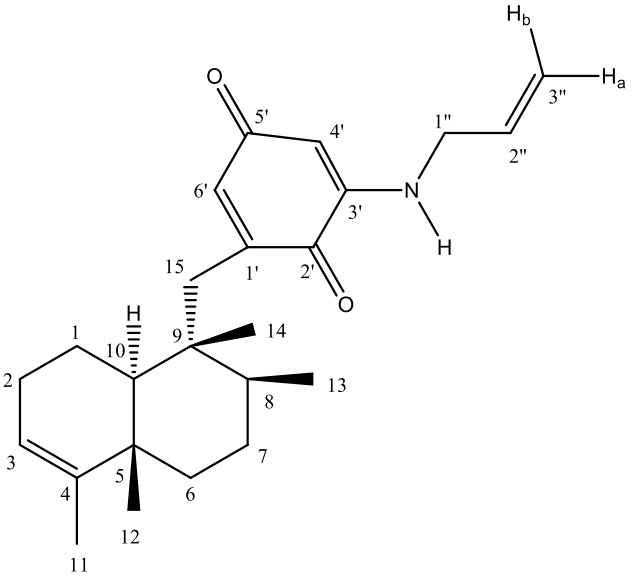
35 **5a** **5b**





36

37 **7a** **7b**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1"
3"
2"
6'
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3'
H
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8
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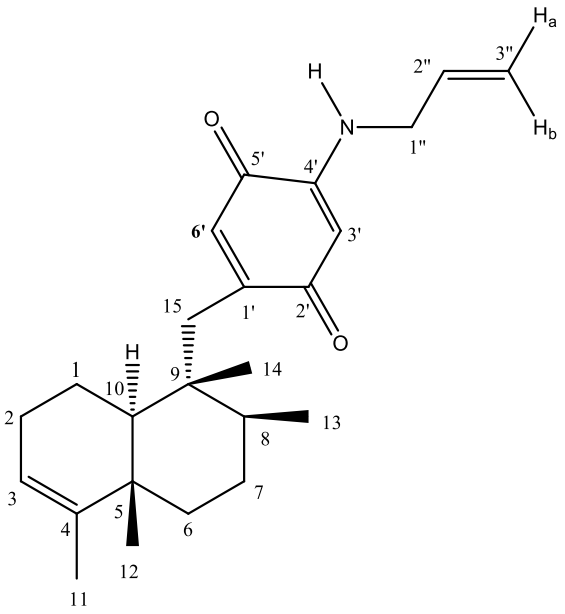
4
6
12
11

8a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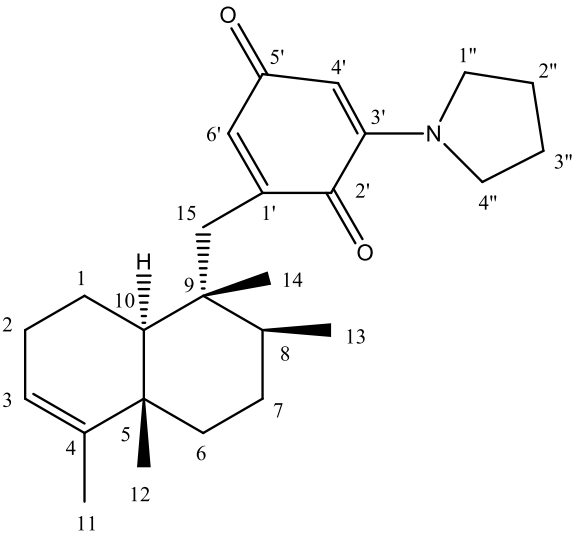
8b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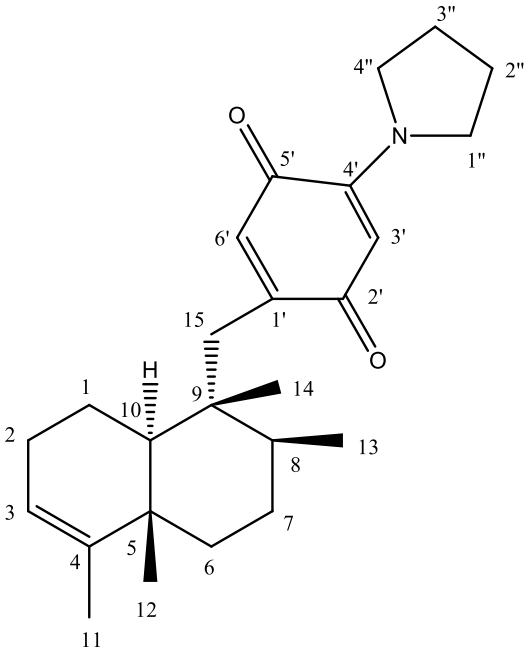
12
11

9a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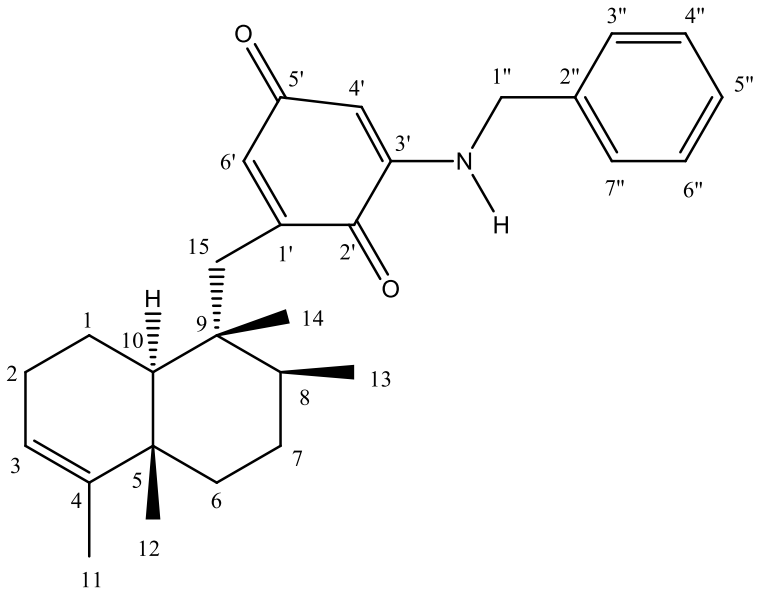
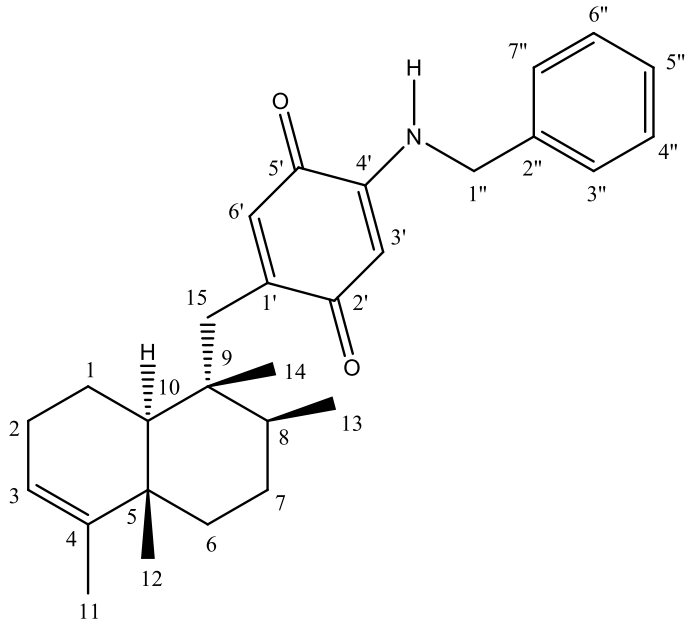
9b

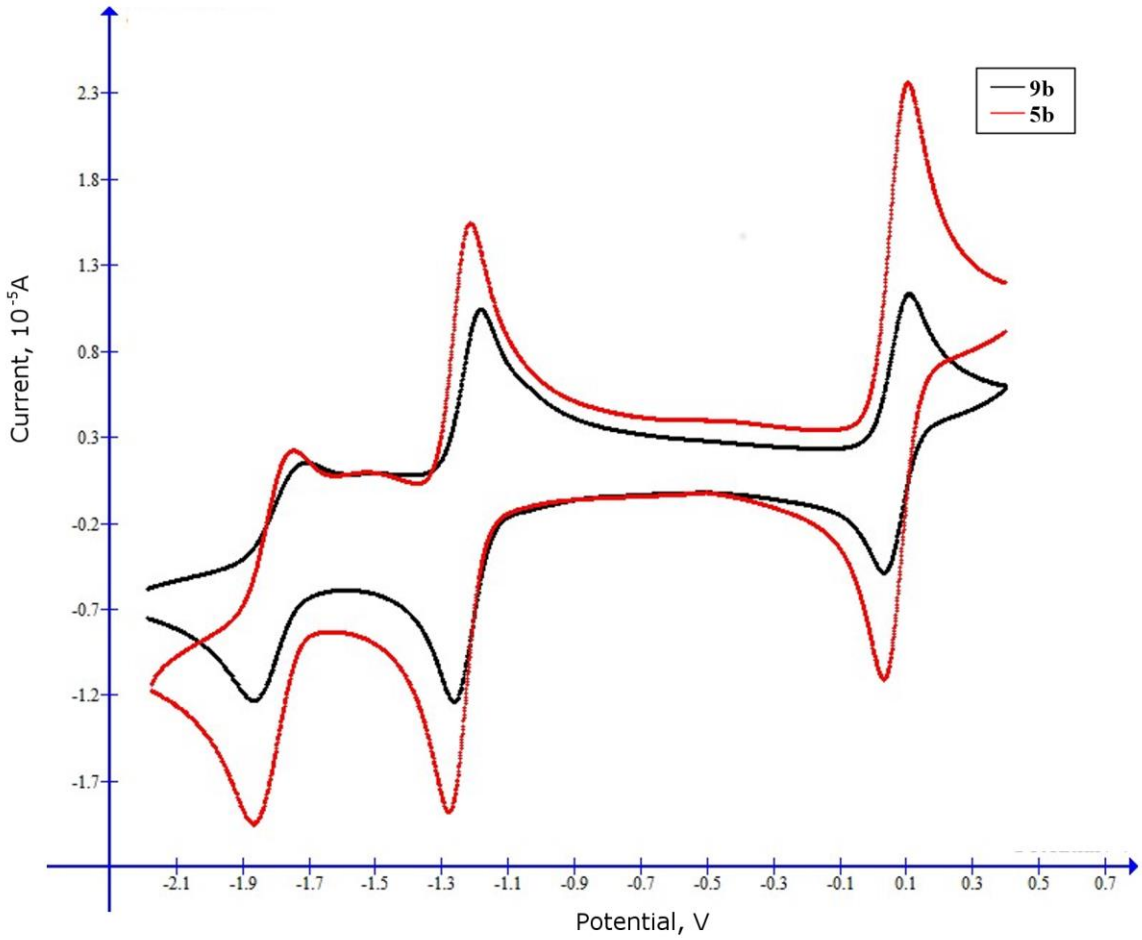


10a



10b

Scheme S1. Structures of the derivatives



57

58 Fig. S1. Cyclic voltammogram of compounds **5b** and **9b**

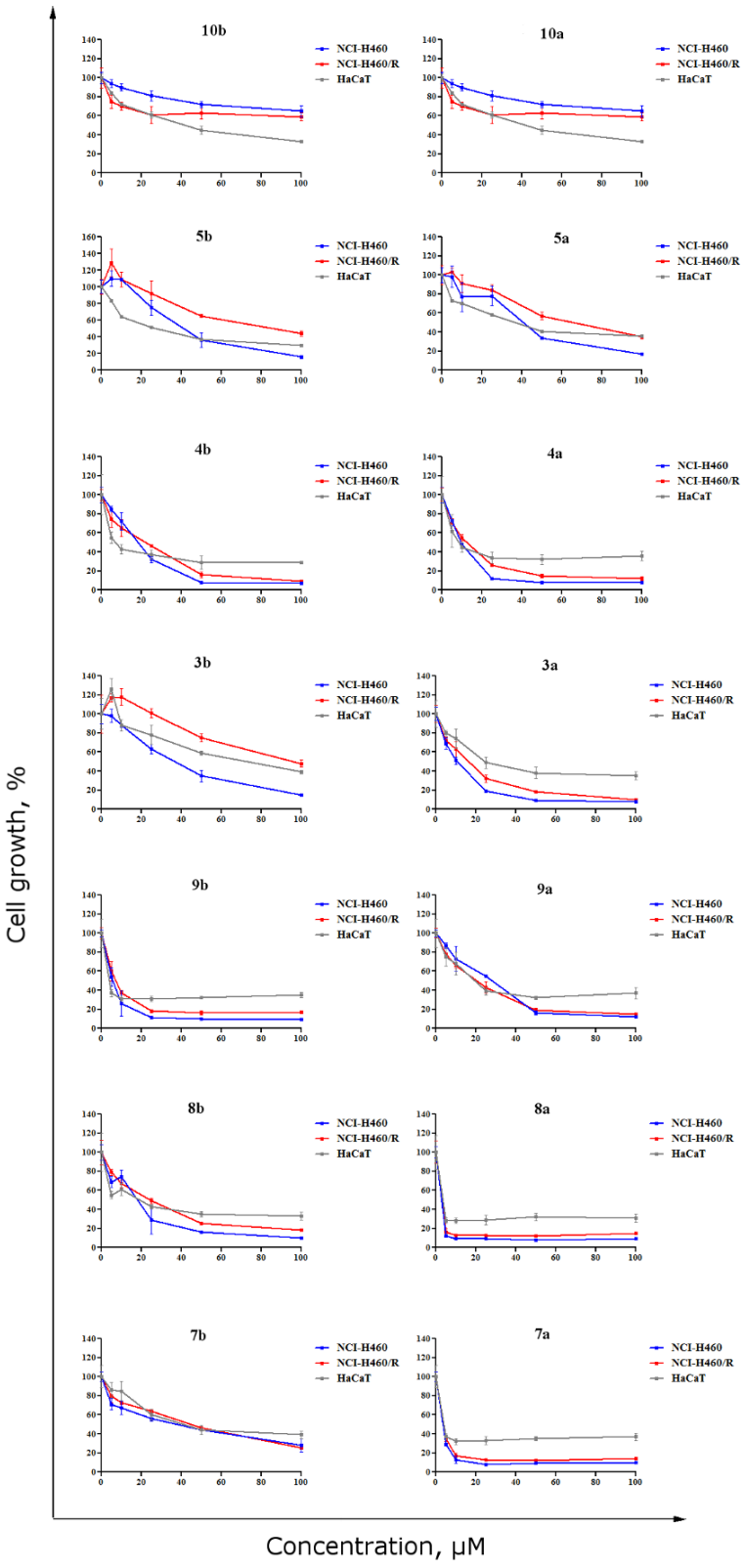


Fig. S2. Cell growth inhibition by TBQ and avarone derivatives. Inhibitory potential of 14 compounds was studied in NCI-H460, NCI-H460/R and HaCaT cells after 72 h by MTT assay

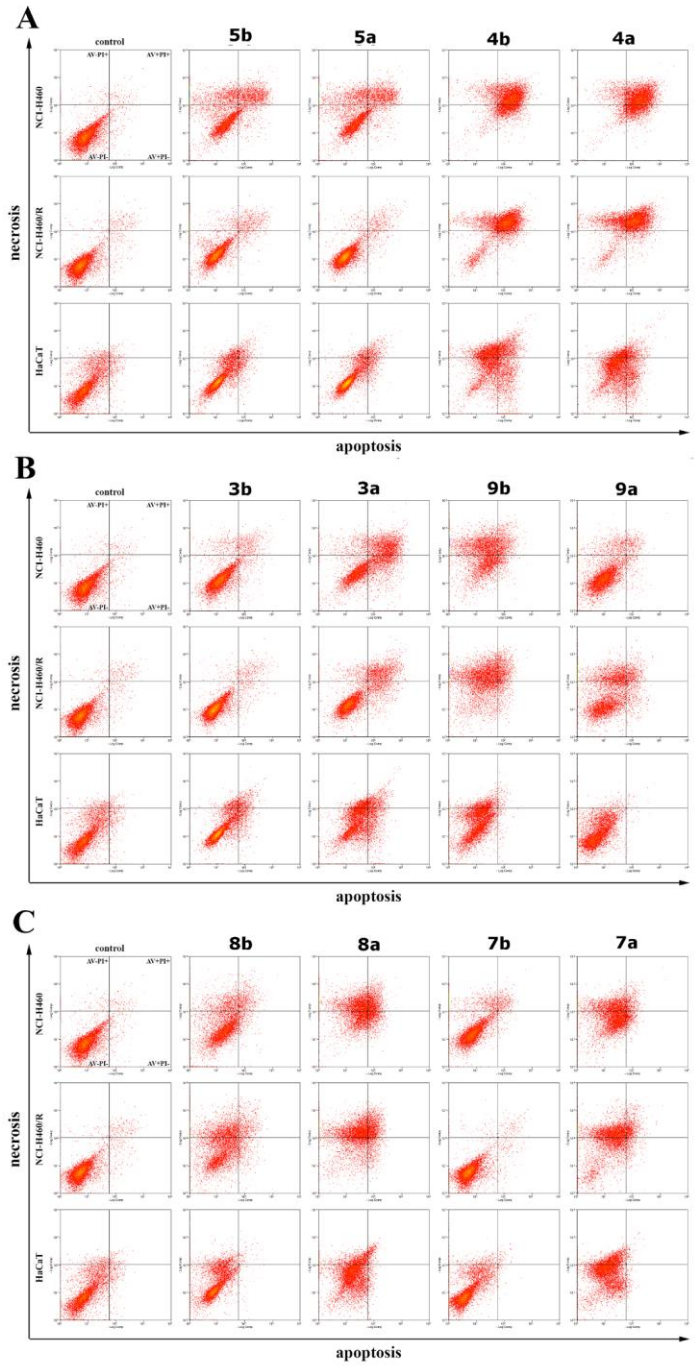


Fig. S3. Cell death induction by TBQ and avarone derivatives. Cell death type was investigated in NCI-H460, NCI-H460/R and HaCaT cells after 72 h by flow cytometry (AV/PI staining). (**A**) Treatment with 25 µM **5b**, **5a**, **4b** and **4a**. (**B**) Treatment with 25 µM **3b**, **3a**, **9b** and **9a**. (**C**) Treatment with 25 µM **8b**, **8a**, **7b** and **7a**. The assay distinguishes viable cells (AV-PI-), early apoptotic cells (AV+PI-), late apoptotic cells (AV+PI+) and necrotic cells (AV-PI+). The x-axis represents FL1-H channel and y-axis represents red FL2-H channel.

2-tert-*Butyl-6-(*sec-*butylamino)-1,4-benzoquinone (**3a**)*

The compound was separated from its regioisomer by column chromatography using toluene:ethyl acetate (9:1) as eluent (Rf = 0.46) and purified by preparative thin-layer chromatography using hexane:acetone (9:1) as eluent (Rf = 0.36). The product was obtained as reddish brown oil. Yield 139.2 mg; 32.4 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.46 (d, 1H, *J* = 2 Hz, C3-**H**), 5.58 (d, 1H, *J* = 6 Hz, C6-N**H**), 5.42 (d, 1H, *J* = 2 Hz, C5-**H**), 3.29 (m, 1H, C2'-**H**), 1.57 (m, 2H, C3'-$\mathbf{H_2}$), 1.27 (s, 9H, C2-C(C$\mathbf{H_3}$)$_3$), 1.12 (d, 3H, *J* = 6 Hz, C1'-$\mathbf{H_3}$), 0.95 (t, 3H, *J* = 7-8 Hz, C4'-$\mathbf{H_3}$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 186.0 (**C4**), 183.4 (**C1**), 151.2 (**C2**), 146.7 (**C6**), 135.1 (**C3**), 97.0 (**C5**), 49.6 (**C2'**), 34.7 (C2-**C**$(CH_3)_3$), 29.0 (3C, C2-C(**C**$H_3)_3$), 28.7 (**C3'**), 19.0 (**C1'**), 10.2 (**C4'**). IR (ATR): 3379, 3288, 2966, 2874, 1670, 1633, 1586, 1508, 1457, 1344, 1264, 1171, 1009, 906, 808, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 274 ($6.61x10^4$), 486 ($2.63x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{14}H_{21}NO_2$ +H^+] 236.16451, observed 236.16441; calculated for [$C_{14}H_{21}NO_2$ +K^+] 274.12039, observed 274.12284. E_{c1}= -1.210 V, E_{a1}= -1.126 V, E_{c2}= -1.890 V, E_{a2}= -1.741 V, E^0_1/Fc = -1.170 V.

2-tert-*Butyl-5-(*sec-*butylamino)-1,4-benzoquinone (**3b**)*

The compound was separated from its regioisomer by column chromatography using toluene:ethyl acetate (9:1) as eluent (Rf = 0.70) and purified by preparative thin-layer chromatography using hexane:acetone (9:1) as eluent (Rf = 0.53). The product was obtained as reddish brown oil. Yield 41.2 mg; 9.6 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.43 (s, 1H, C3-**H**), 5.38 (s, 1H, C6-**H**), 5.28 (bs, 1H, C5-N**H**), 3.28 (m, 1H, C2'-**H**), 1.57 (m, 2H, C3'-$\mathbf{H_2}$), 1.30 (s, 9H, C2-C(C$\mathbf{H_3}$)$_3$), 1.19 (d, 3H, *J* = 6 Hz, C1'-$\mathbf{H_3}$), 0.93 (t, 3H, *J* = 8 Hz, C4'-$\mathbf{H_3}$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.9 (**C1**), 185.0 (**C4**), 160.0 (**C2**), 144.5 (**C5**), 127.4 (**C3**), 100.2 (**C6**), 49.3 (**C2'**), 35.7 (C2-**C**$(CH_3)_3$), 29.6 (3C, C2-C(**C**$H_3)_3$), 28.8 (**C3'**), 19.2 (**C1'**),

10.2 (**C4'**). IR (ATR): 3379, 3348, 2965, 2875, 1670, 1627, 1588, 1515, 1484, 1457, 1387, 1344, 1223, 1189, 1047, 1017, 895, 834, cm^{-1}). UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 270 ($7.86x10^4$), 482 ($2.24x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{14}H_{21}NO_2+H^+$] 236.16451, observed 236.16475; calculated for [$C_{14}H_{21}NO_2+K^+$] 274.12039, observed 274.12345. E_{c1}= -1.218 V, E_{a1}= -1.154 V, E_{c2}= -1.868 V, E_{a2}= -1.744 V, E^0_1/Fc = -1.186 V.

*2-(Allylamino)-6-*tert*-butyl-1,4-benzoquinone (**4a**)*

The compound was separated from its regioisomer by column chromatography using toluene:ethyl acetate (9:1) as eluent (Rf = 0.35) and purified by preparative thin-layer chromatography using hexane: acetone (8:2) as eluent (Rf = 0.45). The product was obtained as reddish brown crystals, m.p. 64°C. Yield 168 mg; 41.9 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.47 (d, 1H, *J* = 2 Hz, C5-**H**), 5.86 (m, 2H, C2-N**H**, C2'-**H**), 5.45 (d, 1H, *J* = 2 Hz, C3-**H**), 5.31 (dd, 1H, J_1 = 8 Hz, J_2 = 1 Hz, C3'-$\mathbf{H_a}$), 5.24 (s, 1H, C3'-$\mathbf{H_b}$),3.75 (t, 2H, *J* = 6 Hz, C1'-$\mathbf{H_2}$), 1.27 (s, 9H, C6-C(C$\mathbf{H_3}$)$_3$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 186.2 (**C4**), 183.1 (**C1**), 151.3 (**C6**), 147.3 (**C2**), 134.8 (**C2'**), 131.7 (**C5**), 118.1 (**C3'**), 97.8 (**C3**), 44.9 (**C1'**), 34.7 (C6-**C**$(CH_3)_3$), 28.9 (3C, C6-C(**C**$H_3)_3$). IR (ATR): 3285, 3073, 2990, 2961, 2909, 2871, 1673, 1627, 1579, 1499, 1432, 1367, 1339, 1293, 1250, 1204, 1164, 1073, 991, 915, 804, 703, 644, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 272 ($6.55x10^4$), 476 ($2.33x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{13}H_{17}NO_2+H^+$] 220.13321, observed 220.13319. E_{c1}= -1.180 V, E_{a1}= -1.121 V, E_{c2}= -1.812 V, E_{a2}= -1.712 V, E^0_1/Fc = -1.149 V.

*2-(Allylamino)-5-*tert*-butyl-1,4-benzoquinone (**4b**)*

The compound was separated from its regioisomer by column chromatography using toluene:ethyl acetate (9:1) as eluent (Rf = 0.57) and purified by preparative thin-layer chromatography using hexane: acetone (8:2) as eluent (Rf = 0.54). The product was obtained

as reddish brown crystals, m.p. 39°C. Yield 38 mg; 9.5 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.45 (s, 1H, C6-**H**), 5.84 (m, 1H, C2'-**H**), 5.54 (bs, 1H, C2-N**H**), 5.41 (s, 1H, C3-**H**), 5.28 (d, 1H, *J* = 6 Hz, C3'-$\mathbf{H_a}$), 5.22 (s, 1H, C3'-$\mathbf{H_b}$), 3.73 (t, 2H, *J* = 6 Hz, C1'-$\mathbf{H_2}$), 1.30 (s, 9H, C5-C(C$\mathbf{H_3}$)$_3$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 186.0 (**C4**), 184.8 (**C1**), 159.5 (**C5**), 145.1 (**C2**), 131.8 (**C2'**), 127.5 (**C6**), 118.0 (**C3'**), 101.1 (**C3**), 44.7 (**C1'**), 35.7 (C5-**C**$(CH_3)_3$), 29.6 (3C, C5-C(**C**$H_3)_3$). IR (ATR): 3388, 3067, 3004, 2961, 2916, 2870, 1670, 1627, 1589, 1513, 1456, 1390, 1339, 1248, 1225, 1187, 1017, 992, 930, 896, 837, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 270 ($6.52x10^4$), 474 ($1.47x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{13}H_{17}NO_2$+H^+] 220.13321, observed 220.13311. E_{c1}= -1.210 V, E_{a1}= -1.152 V, E_{c2}= -1.848 V, E_{a2}= -1.733 V, E^0_1/Fc = -1.181 V.

2-tert-*Butyl-6-(pyrrolidin-1-yl)-1,4-benzoquinone (**5a**)*

The compound was separated from its regioisomer by column chromatography using toluene:ethyl acetate (8:2) as eluent (Rf = 0.31) and purified by two preparative thin-layer chromatographies, first by using toluene:ethyl acetate (8:2) as eluent, and then by using hexane:acetone (7:3) as eluent (Rf = 0.45). The product was obtained as reddish brown crystal, m.p. 106-107°C. Yield 63 mg; 14.8 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.45 (d, 1H, *J* = 2 Hz, C3-**H**), 5.41 (d, 1H, *J* = 2 Hz, C5-**H**), 3.53 (bs, 4H, C1'-$\mathbf{H_2}$, C4'-$\mathbf{H_2}$), 1.95 (m, 4H, C2'-$\mathbf{H_2}$, C3'-$\mathbf{H_2}$), 1.25 (s, 9H, C2-C(C$\mathbf{H_3}$)$_3$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.5 (**C4**), 185.4 (**C1**), 151.8 (**C2**), 149.3 (**C6**), 133.8 (**C3**), 101.0 (**C5**), 50.8 (2C, **C1'**, **C4'**), 34.9 (C2-**C**$(CH_3)_3$), 29.2 (3C, C2-C(**C**$H_3)_3$), 25.1 (2C, **C2'**, **C3'**). IR (ATR): 2963, 2873, 1670, 1632, 1598, 1563, 1480, 1458, 1413, 1366, 1336, 1314, 1287, 1250, 1174, 1156, 1120, 1049, 999, 907, 796, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 274 ($4.80x10^4$), 500 ($3.20x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{14}H_{19}NO_2$+H^+] 234.14886, observed 234.14892. E_{c1}= -1.276 V, E_{a1}= -1.214 V, E_{c2}= -1.876 V, E_{a2}= -1.694 V, E^0_1/Fc = -1.244 V.

2-tert-*Butyl-5-(pyrrolidin-1-yl)-1,4-benzoquinone (**5b**)*

The compound was separated from its regioisomer by column chromatography using toluene:ethyl acetate (9:1) as eluent (Rf = 0.49) and purified by preparative thin-layer chromatography using hexane:acetone (7:3) as eluent (Rf = 0.56). The product was obtained as reddish brown crystals, m.p. 136°C. Yield 48.6 mg; 11.4 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.31 (s, 1H, C3-**H**), 5.38 (s, 1H, C6-**H**), 3.51 (m, 4H, C1'-**H**$_2$, C4'-**H**$_2$), 1.93 (m, 4H, C2'-**H**$_2$, C3'-**H**$_2$), 1.28 (s, 9H, C2-C(C**H**$_3$)$_3$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 186.3 (**C1**), 185.1 (**C4**), 157.8 (**C2**), 146.2 (**C5**), 128.5 (**C3**), 104.2 (**C6**), 50.2 (2H, **C1'**, **C4'**), 35.2 (C2-**C**$(CH_3)_3$), 29.5 (3C, C2-C(**C**$H_3)_3$), 25.4 (2C, **C2'**, **C3'**). IR (ATR): 3360, 3191, 3047, 2956, 2924, 2855, 1739, 1660, 1626, 1582, 1455, 1427, 1370, 1343, 1324, 1260, 1196, 1055, 1019, 930, 899, 838, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^{2}mol^{-1})): 276 (6.64x10^4), 502 (3.09x10^4). (+)ESI-HRMS m/z: calculated for [$C_{14}H_{19}NO_2$+H^+] 234.14886, observed 234.14952. E_{c1}= -1.276 V, E_{a1}= -1.214 V, E_{c2}= -1.865 V, E_{a2}= -1.749 V, E^0_1/Fc = -1.243 V.

*2-(*sec-*Butylamino)-6-(((1*R*,2*S*,4a*S*,8a*S*)-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**7a**)*

The compound was separated from its regioisomer by low-bar chromatography using petroleum ether:ethyl acetate (9:1) as eluent (Rf = 0.26) and purified by preparative thin-layer chromatography using petroleum ether:ethyl acetate (9:1) as eluent. The product was obtained as reddish brown oil. Yield 63.9 mg; 17.3 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.37 (d, 1H, *J* = 2 Hz, C6'-**H**), 5.52 (d, 1H, *J* = 8 Hz, C3'-N**H**), 5.42 (d, 1H, *J* = 2 Hz, C4'-**H**), 5.14 (s, 1H, C3-**H**), 3.28 (m, 1H, C2"-**H**), 2.61 (dd, 1H, J_1 = 2 Hz, J_2 = 14 Hz, C15-**H**$_a$), 2.37 (dd, 1H, J_1 = 4 Hz, J_2 = 14 Hz, C15-**H**$_b$), 1.80-2.10 (m, 4H, C2-**H**$_2$, C6-**H**$_2$), 1.10-1.70 (m, 14H, C1-**H**$_2$, C7-**H**$_2$, C8-**H**, C10-**H**, C11-**H**$_3$, C1"-**H**$_3$, C3"-**H**$_2$), 0.80-1.10 (m, 12H, C14-**H**$_3$,

C13-**H$_3$**, C4"-**H$_3$**, C12-**H$_3$**). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.5 (**C5'**), 184.1 (**C2'**), 146.0 (**C3'**), 144.0 (**C4**), 142.0 (**C1'**), 139.8 (**C6'**), 120.6 (**C3**), 97.6 (**C4'**), 49.6 (**C2"**), 46.7 (**C10**), 42.0 (**C5**), 38.4 (**C9**), 36.6 (**C8**), 36.0 (**C6**), 35.0 (**C15**), 28.7 (**C3"**), 27.4 (**C7**), 26.5 (**C2**), 20.0 (**C12**), 19.3 (**C1**), 19.0 (**C1"**), 18.0 (**C11**), 17.7 (**C14**), 16.7 (**C13**), 10.2 (**C4"**). IR (ATR): 3378, 3291, 2962, 2929, 1669, 1634, 1585, 1506, 1453, 1380, 1340, 1288, 1261, 1190, 1149, 1099, 1048, 912, 802, 446, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 286 ($2.91x10^4$), 492 ($1.28x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{25}H_{37}NO_2$+H^+] 384.28971, observed 384.29014. E_{c1}= -1.193 V, E_{a1}= -1.127 V, E_{c2}= -1.881 V, E_{a2}= -1.774 V, E^0_1/Fc = -1.158 V. $[\alpha]^{20}$ = –120 (*c* = 0.083 mg/mL in MeOH).

*2-(*sec*-Butylamino)-5-(((1*R*,2*S*,4a*S*,8a*S*)-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**7*b***)*

The compound was separated from its regioisomer by low-bar chromatography using petroleum ether:ethyl acetate (9:1) as eluent (Rf = 0.49) and purified by preparative thin-layer chromatography using petroleum ether:ethyl acetate (9:1) as eluent. The product was obtained as reddish brown oil. Yield 62.2 mg; 16.9 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.36 (s, 1H, C6'-**H**), 5.38-5.42 (m, 2H, C4'-N**H**, C3'-**H**), 5.17 (s, 1H, C3-**H**), 3.29 (m, 1H, C2"-**H**), 2.64 (dd, 1H, J_1 = 2 Hz, J_2 = 12 Hz, C15-**H$_a$**), 2.48 (dd, 1H, J_1 = 2 Hz, J_2 = 14 Hz, C15-**H$_b$**), 1.80-2.10 (m, 4H, C2-**H$_2$**, C6-**H$_2$**), 1.10-1.70 (m, 14H, C1-**H$_2$**, C7-**H$_2$**, C8-**H**, C10-**H**, C11-**H$_3$**, C1"-**H$_3$**, C3"-**H$_2$**), 0.80-1.10 (m, 12H, C14-**H$_3$**, C13-**H$_3$**, C4"-**H$_3$**, C12-**H$_3$**). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.2 (**C2'**), 183.8 (**C5'**), 151.2 (**C1'**), 145.2 (**C4'**), 144.0 (**C4**), 131.8 (**C6'**), 120.7 (**C3**), 98.2 (**C3'**), 49.3 (**C2"**), 47.1 (**C10**), 43.1 (**C5**), 38.5 (**C9**), 37.0 (**C8**), 36.0 (**C6**), 35.6 (**C15**), 28.7 (**C3"**), 27.5 (**C7**), 26.4 (**C2**), 20.0 (**C2**), 19.4 (**C1**), 19.1 (**C1"**), 18.0 (**C11**), 17.7 (**C14**), 16.8 (**C13**), 10.2 (**C4"**). IR (ATR): 3327, 2964, 2927, 1665, 1627, 1587, 1518, 1450, 1380, 1321, 1267, 1215, 1126, 1098, 1032, 999, 978, 898, 846, 792, 696, 636, 463, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 288 ($4.54x10^4$), 488

($1.27x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{25}H_{37}NO_2+H^+$] 384.28971, observed 384.29020. E_{c1}= -1.201 V, E_{a1}= -1.133 V, E_{c2}= -1.859 V, E_{a2}= -1.713 V, E^0_1/Fc = -1.168 V. $[\alpha]^{20} = 0$ (*c* = 0.083 mg/mL in MeOH).

*2-(Allylamino)-6-(((1*R*,2*S*,4a*S*,8a*S*)-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**8a**)*

The compound was separated from its regioisomer by low-bar chromatography using petroleum ether:ethyl acetate (85:15) as eluent (Rf = 0.27) and purified by preparative thin-layer chromatography using petroleum ether:ethyl acetate (8:2) as eluent (Rf = 0.44) three times, due to close proximity with avarol as side product (Rf = 0.36), resulting in low yield. The product was obtained as reddish brown oil. Yield 25.3 mg; 7.2 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.37 (d, 1H, *J* = 2 Hz, C6'-**H**), 5.70-6.00 (m, 2H, C2"-**H**, C4'-N**H**), 5.44 (d, 1H, *J* = 2 Hz, C3'-**H**), 5.31 (m, 1H, C3"-$\mathbf{H_a}$), 5.23 (t, 1H, *J* = 2 Hz, C3"-$\mathbf{H_b}$), 5.14 (s, 1H, C3-**H**), 3.74 (t, 2H, *J* = 6 Hz, C1"-$\mathbf{H_2}$), 2.63 (d, 1H, *J* = 14 Hz, C15-$\mathbf{H_a}$), 2.38 (d, 1H, *J* = 12 Hz, C15-$\mathbf{H_b}$), 1.80-2.10 (m, 4H, C2-$\mathbf{H_2}$, C6-$\mathbf{H_2}$), 1.10-1.70 (m, 9H, C1-$\mathbf{H_2}$, C7-$\mathbf{H_2}$, C8-**H**, C10-**H**, C11-$\mathbf{H_3}$), 0.80-1.10 (m, 9H, C14-$\mathbf{H_3}$, C13-$\mathbf{H_3}$, C12-$\mathbf{H_3}$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.6 (**C5'**), 183.9 (**C2'**), 146.6 (**C3'**), 144.0 (**C4**), 142.2 (**C1'**), 139.6 (**C6'**), 131.7 (**C2"**), 120.6 (**C3**), 118.3 (**C3"**), 98.5 (**C4'**), 46.7 (**C10**), 45.0 (**C1"**), 42.0 (**C5**), 38.4 (**C9**), 36.6 (**C8**), 36.0 (**C6**), 35.0 (**C15**), 27.4 (**C7**), 26.5 (**C2**), 20.0 (**C12**), 19.3 (**C1**), 18.0 (**C11**), 17.6 (**C14**), 16.7 (**C13**). IR (ATR): 3390, 3298, 2960, 1670, 1633, 1585, 1501, 1441, 1380, 1344, 1289, 1247, 1189, 1093, 1028, 914, 801, 632, 442, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 286 ($2.19x10^4$), 484 ($0.97x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{24}H_{33}NO_2+H^+$] 368.25841, observed 368.25854; calculated for [$C_{24}H_{33}NO_2+Na^+$] 390.24035, observed 390.24116. E_{c1}= -1.170 V, E_{a1}= -1.113 V, E_{c2}= -1.830 V, E_{a2}= -1.741 V, E^0_1/Fc = -1.142 V. $[\alpha]^{20} = –50$ (*c* = 0.083 mg/mL in MeOH).

*2-(Allylamino)-5-(((1*R,*2*S,*4a*S,*8a*S)*-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**8b**)*

The compound was separated from its regioisomer by low-bar chromatography using petroleum ether:ethyl acetate (9:1) as eluent (Rf = 0.35) and purified by preparative thin-layer chromatography using petroleum ether:ethyl acetate (8:2) as eluent (Rf = 0.61). The product was obtained as reddish brown oil. Yield 58.5 mg; 16.6 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.37 (s, 1H, C6'-**H**), 5.85 (m, 1H, C2"-**H**), 5.61 (bs, 1H, C4'-N**H**), 5.46 (s, 1H, C3'-**H**), 5.30 (m, 1H, C3"-**H$_a$**), 5.24 (t, 1H, *J* = 2 Hz, C3"-**H$_b$**), 5.15 (s, 1H, C3-**H**), 3.73 (t, 2H, *J* = 6 Hz, C1"-**H$_2$**), 2.65 (d, 1H, *J* = 14 Hz, C15-**H$_a$**), 2.47 (d, 1H, *J* = 14 Hz, C15-**H$_b$**), 1.80-2.10 (m, 4H, C2-**H$_2$**, C6-**H$_2$**), 1.10-1.70 (m, 9H, C1-**H$_2$**, C7-**H$_2$**, C8-**H**, C10-**H**, C11-**H$_3$**), 0.80-1.10 (m, 9H, C14-**H$_3$**, C13-**H$_3$**, C12-**H$_3$**). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.4 (**C2'**), 183.5 (**C5'**), 151.1 (**C1'**), 145.8 (**C4'**), 144.0 (**C4**), 131.8 (2C, **C6'**, **C2"**), 120.7 (**C3**), 118.2 (**C3"**), 99.1 (**C3'**), 47.2 (**C10**), 44.7 (**C1"**), 43.1 (**C5**), 38.5 (**C9**), 37.1 (**C8**), 36.1 (**C6**), 35.7 (**C15**), 27.5 (**C7**), 26.5 (**C2**), 20.0 (**C12**), 19.4 (**C1**), 18.0 (**C11**), 17.7 (**C14**), 16.8 (**C13**). IR (ATR): 3332, 3083, 2925, 1663, 1624, 1586, 1506, 1448, 1379, 1339, 1312, 1256, 1224, 1205, 1099, 1037, 916, 841, 799, 567, 460, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 286 ($4.14x10^4$), 480 ($1.05x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{24}H_{33}NO_2$+H^+] 368.25841, observed 368.2534; calculated for [$C_{24}H_{33}NO_2$+Na^+] 390.24035, observed 390.24053. E_{c1}= -1.177 V, E_{a1}= -1.117 V, E_{c2}= -1.799 V, E_{a2}= -1.689 V, E^0_1/Fc = -1.150 V. $[\alpha]^{20}$ = +70 (*c* = 0.083 mg/mL in MeOH).

*2-(Pyrrolidin-1-yl)-6-(((1*R,*2*S,*4a*S,*8a*S)*-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**9a**)*

The compound was separated from its regioisomer by low-bar chromatography using petroleum ether:ethyl acetate (8:2) as eluent (Rf = 0.18) and purified by preparative thin-layer

chromatography using petroleum ether:ethyl acetate (7:3) as eluent (Rf = 0.22). The product was obtained as reddish brown crystals, m.p. 121°C. Yield 96.4 mg; 26.3 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.36 (d, 1H, *J* = 2 Hz, C6'-**H**), 5.42 (d, 1H, *J* = 2 Hz, C4'-**H**), 5.13 (s, 1H, C3-**H**), 3.52 (m, 4H, C1"-$\mathbf{H_2}$, C4"-$\mathbf{H_2}$), 2.67 (d, 1H, *J* = 4 Hz, C15-$\mathbf{H_a}$), 2.37 (d, 1H, *J* = 6 Hz, C15-$\mathbf{H_b}$), 1.90-2.10 (m, 8H, C2-$\mathbf{H_2}$; C6-$\mathbf{H_2}$; C2"-$\mathbf{H_2}$; C3"-$\mathbf{H_2}$), 1.10-1.70 (m, 9H, C1-$\mathbf{H_2}$; C7-$\mathbf{H_2}$; C8-**H**; C10-**H**; C11-$\mathbf{H_3}$), 0.80-1.10 (m, 9H, C14-$\mathbf{H_3}$; C13-$\mathbf{H_3}$; C12-$\mathbf{H_3}$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.6 (**C5'**), 185.0 (**C2'**), 148.6 (**C3'**), 144.3 (**C4**), 143.1 (**C1'**), 138.5 (**C6'**), 120.8 (**C3**), 101.8 (**C4'**), 50.9 (2C, **C1"**, **C4"**), 47.0 (**C10**), 42.2 (**C5**), 38.9 (**C9**), 36.9 (**C8**), 36.2 (**C6**), 35.3 (**C15**), 27.6 (**C7**), 26.8 (2C, **C2"**, **C3"**), 20.2 (**C2**), 19.4 (**C12**), 18.3 (**C1**), 17.9 (**C11**), 17.0 (**C14**), 16.9 (**C13**). IR (ATR): 3323, 2961, 1670, 1635, 1589, 1559, 1454, 1415, 1379, 1335, 1284, 1262, 1189, 1098, 1025, 912, 861, 800, 755, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 292 ($2.79x10^4$), 506 ($2.14x10^4$). (+)ESI-HRMS m/z: calculated for $[C_{25}H_{35}NO_2+H)^+]$ 382.27406, observed 382.27388. E_{c1}= -1.267 V, E_{a1}= -1.163 V, E_{c2}= -1.883 V, E_{a2}= -1.710 V, E^0_1/Fc = -1.217 V. $[\alpha]^{20}$ = +60 (*c* = 0.083 mg/mL in MeOH).

*2-(Pyrrolidin-1-yl)-5-(((1*R,*2*S,*4a*S,*8a*S)*-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**9b**)*

The compound was separated from its regioisomer by low-bar chromatography using petroleum ether:ethyl acetate (9:1) as eluent (Rf = 0.32) and purified by preparative thin-layer chromatography using petroleum ether:ethyl acetate (8:2) as eluent (Rf = 0.45). The product was obtained as reddish brown crystals, m.p. 42°C. Yield 48.6 mg; 13.2 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 6.26 (s, 1H, C6'-**H**), 5.44 (s, 1H, C3'-**H**), 5.15 (s, 1H, C3-**H**), 3.54 (m, 4H, C1"-$\mathbf{H_2}$, C4"-$\mathbf{H_2}$), 2.63 (d, 1H, *J* = 6 Hz, C15-$\mathbf{H_a}$), 2.46 (d, 1H, *J* = 6 Hz, C15-$\mathbf{H_b}$), 1.90-2.10 (m, 8H, C2-$\mathbf{H_2}$; C6-$\mathbf{H_2}$; C2"-$\mathbf{H_2}$; C3"-$\mathbf{H_2}$), 1.10-1.70 (m, 9H, C1-$\mathbf{H_2}$; C7-$\mathbf{H_2}$; C8-**H**; C10-**H**; C11-$\mathbf{H_3}$), 0.80-1.10 (m, 9H, C14-$\mathbf{H_3}$; C13-$\mathbf{H_3}$; C12-$\mathbf{H_3}$). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.3

(**C2'**), 184.8 (**C5'**), 149.5 (**C1'**), 147.0 (**C4'**), 144.2 (**C4**), 133.2 (**C6'**), 121.0 (**C3**), 102.3 (**C3'**), 50.6 (2C, **C1"**, **C4"**), 47.4 (**C10**), 43.1 (**C5**), 38.7 (**C9**), 37.2 (**C8**), 36.4 (**C6**), 35.5 (**C15**), 27.8 (**C7**), 26.8 (2C, **C2"**, **C3"**), 20.2 (**C2**), 19.6 (**C12**), 18.3 (**C1**), 18.0 (**C11**), 17.0 (**C14**), 14.4 (**C13**). IR (ATR): 3330, 2961, 1662, 1630, 1562, 1451, 1380, 1260, 1227, 1096, 1024, 801, 757, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 294 ($2.79x10^4$), 506 ($2.14x10^4$). (+)ESI-HRMS m/z: calculated for [$C_{25}H_{35}NO_2+H^+$] 382.27406, observed 382.27420. E_{c1}= -1.259 V, E_{a1}= -1.177 V, E_{c2}= -1.861 V, E_{a2}= -1.704 V, E^0_1/Fc = -1.218 V. $[\alpha]^{20}$ = +30 (*c* = 0.083 mg/mL in MeOH).

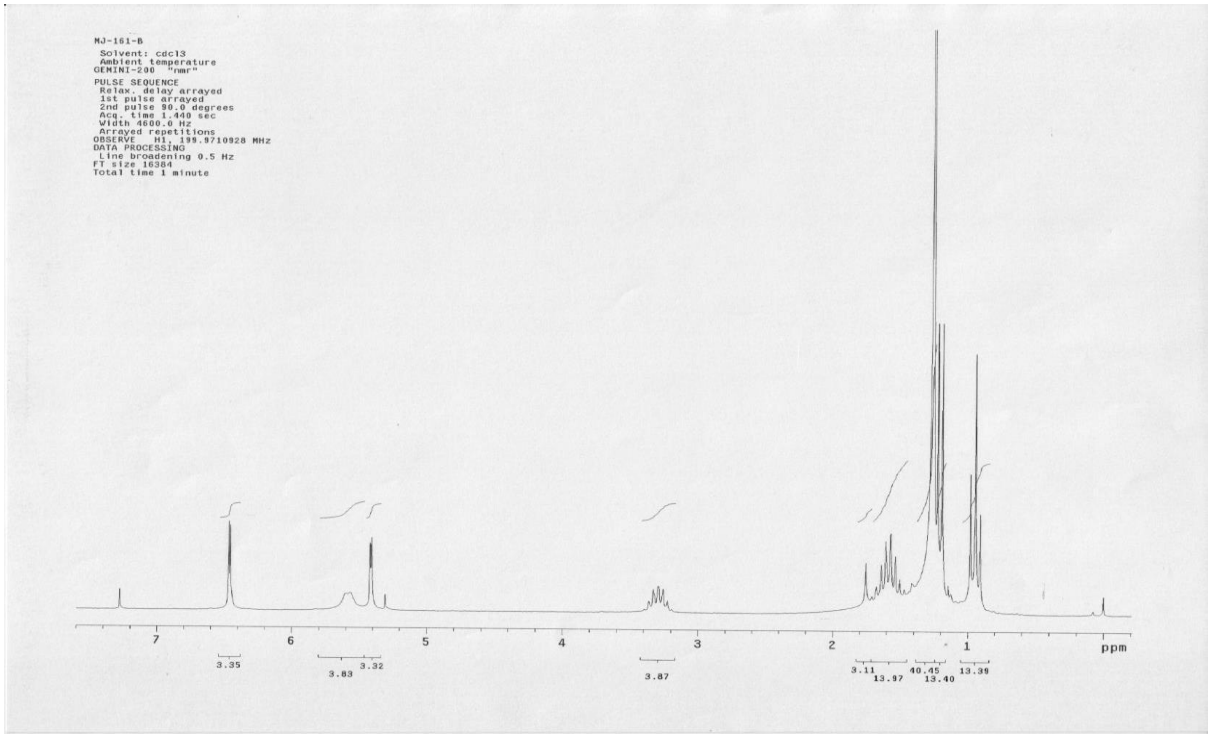
*2-(Benzylamino)-6-(((1*R,*2*S,*4a*S,*8a*S)*-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**10a**)*

The compound was separated from its regioisomer by column chromatography using toluene as eluent (Rf = 0.19) and purified by preparative thin-layer chromatography using hexane:acetone (7:3) as eluent (Rf = 0.61). The product was obtained as reddish brown crystals, m.p. 85°C. Yield 24.8 mg; 6.2 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.20-7.50 (m, 5H, C3''-**H**, C4''-**H**, C5''-**H**, C6''-**H**, C7''-**H**), 6.38 (d, 1H, *J* = 2.4 Hz, C6'-**H**), 5.93 (bs, 1H, C3'N-**H**), 5.49 (d, 1H, *J* = 1.6 Hz, C4'-**H**), 5.14 (s, 1H, C3-**H**), 4.27 (d, 2H, *J* = 5.6 Hz, C1''-**H$_2$**), 2.63 (d, 1H, *J* = 14 Hz, C15-**H$_b$**), 2.39 (d, 1H, *J* = 14 Hz, C15-**H$_a$**), 1.80-2.20 (m, 4H, C2-**H$_2$**, C6-**H$_2$**), 1.20-1.80 (m, 12H, C1-**H$_2$**, C7-**H$_2$**, C8-**H**, C10-**H**, C11-**H$_3$**, C12-**H$_3$**), 0.80-1.1-0 (m, 6H, C13-**H$_3$**, C14-**H$_3$**). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.7 (**C5'**), 183.9 (**C2'**), 146.6 (**C3'**), 144.0 (**C4**), 142.2 (**C1'**), 139.6 (**C6'**), 135.9 (**C2''**), 129.0 (2C, **C4''**, **C6''**), 128.1 (2C, **C3''**, **C7''**), 127.7 (**C5''**), 120.6 (**C3**), 98.6 (**C4'**), 46.9 (**C1''**), 46.7 (**C10**), 42.1 (**C5**), 38.4 (**C9**), 36.6 (**C8**), 36.0 (**C6**), 35.0 (**C15**), 27.3 (**C7**), 26.5 (**C2**), 20.0 (**C12**), 19.3 (**C1**), 18.0 (**C11**), 17.7 (**C14**), 16.6 (**C13**). IR (ATR): 3388, 2960, 1670, 1635, 1587, 1503, 1455, 1380, 1346, 1251, 1189, 1095, 1072, 1028, 913, 803, 736, 699, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 288 ($4.21x10^4$), 488 ($1.89x10^4$). (+)ESI-

HRMS m/z: calculated for [$C_{28}H_{35}NO_2$+H^+] 418.27406, observed 418.27452. E_{c1}= -1.115 V, E_{a1}= -1.043 V, E_{c2}= -1.865 V, E_{a2}= -1.733 V, E^0_1/Fc = -1.147V. $[\alpha]^{20}$ = +10 (*c* = 0.083 mg/mL in MeOH).

*2-(Benzylamino)-5-(((1*R,*2*S,*4a*S,*8a*S)*-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (**10b**)*

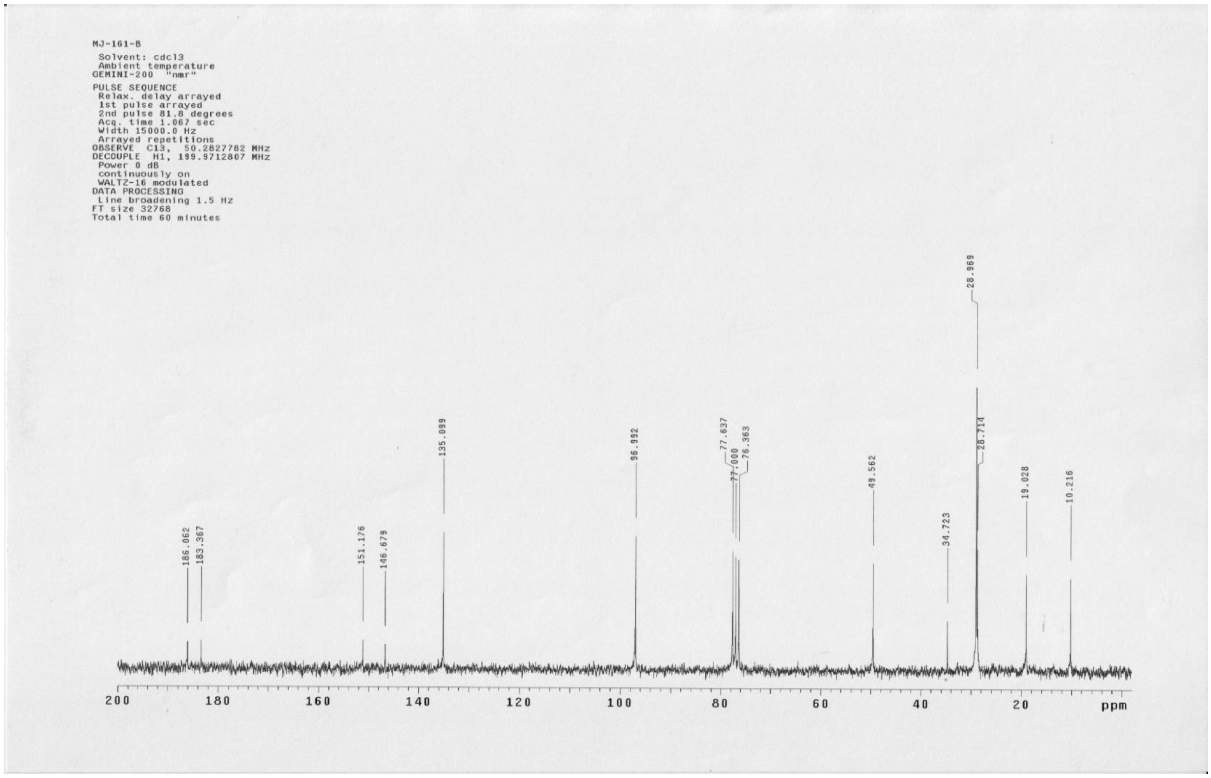
The compound was separated from its regioisomer by column chromatography using toluene as eluent (Rf = 0.36) and purified by preparative thin-layer chromatography using hexane:acetone (7:3) as eluent (Rf = 0.65). The product was obtained as reddish brown crystals, m.p. 107°C. Yield 24.7 mg; 6.2 %. ^{1}H NMR (200 MHz, $CDCl_3$, δ): 7.20-7.50 (m, 5H, C3''-**H**, C4''-**H**, C5''-**H**, C6''-**H**, C7''-**H**), 6.38 (s, 1H, C6'-**H**), 5.80 (bs, 1H, C4'N-**H**), 5.50 (s, 1H, C3'-**H**), 5.16 (s, 1H, C3-**H**), 4.26 (d, 1H, *J* = 5.8 Hz, C1''-**H$_2$**), 2.65 (d, 1H, *J* = 12.8 Hz, C15-**H$_b$**), 2.47 (d, 1H, *J* = 12.8 Hz, C15-**H$_a$**), 1.80-1.20 (m, 4H, C2-**H$_2$**, C6-**H$_2$**), 1.20-1.80 (m, 12H, C1-**H$_2$**, C7-**H$_2$**, C8-**H**, C10-**H**, C11-**H$_3$**, C12-**H$_3$**), 0.80-1.10 (m, 6H, C13-**H$_3$**, C14-**H$_3$**). ^{13}C NMR (50 MHz, $CDCl_3$, δ): 185.4 (**C2'**), 183.5 (**C5'**), 151.1 (**C4'**), 145.8 (**C4**), 144.0 (**C1'**), 136.0 (**C2''**), 131.9 (**C6'**), 129.0 (2C, **C4''**, **C6''**), 128.1 (2C, **C3''**, **C7''**), 127.8 (**C5''**), 120.7 (**C3**), 99.2 (**C3'**), 47.1 (**C10**), 46.6 (**C1''**), 43.1 (**C5**), 38.5 (**C9**), 37.0 (**C8**), 36.0 (**C6**), 35.7 (**C15**), 27.5 (**C7**), 26.4 (**C2**), 20.0 (**C12**), 19.3 (**C1**), 18.0 (**C11**), 17.7 (**C14**), 16.8 (**C13**). IR (ATR): 3383, 2933, 1666, 1628, 1530, 1512, 1454, 1380, 1314, 1245, 1222, 1099, 1030, 901, 839, 736, 699, cm^{-1}. UV-Vis (c = 0.083 mg/mL in MeOH, λ_{max}/nm, (ε/dm^2mol^{-1})): 290 (7.57x10^4), 484 (2.02x10^4). (+)ESI-HRMS m/z: calculated for [$C_{28}H_{35}NO_2$+H^+] 418.27406, observed 418.27438. E_{c1}= -1.123 V, E_{a1}= -1.054 V, E_{c2}= -1.840 V, E_{a2}= -1.685 V, E^0_1/Fc = -1.157 V. $[\alpha]^{20}$ = +50 (*c* = 0.083 mg/mL in MeOH).



313

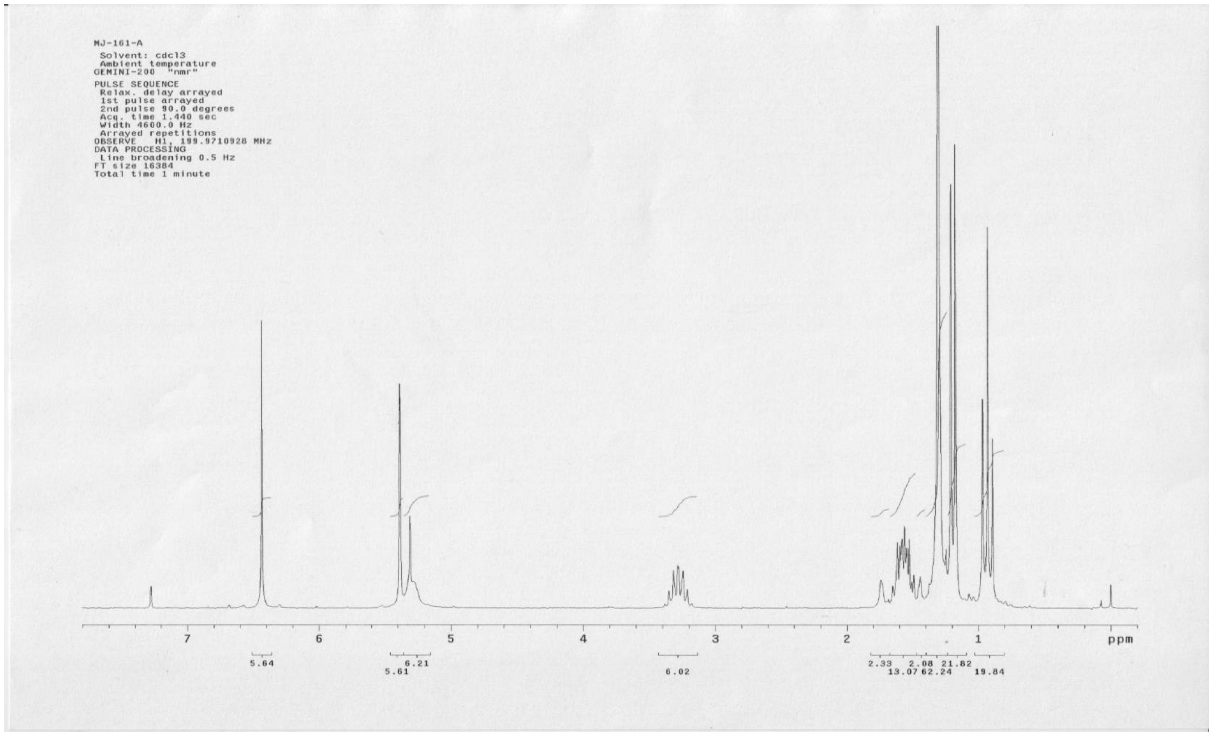
314 ^{1}H NMR spectrum of compound **3a**

315

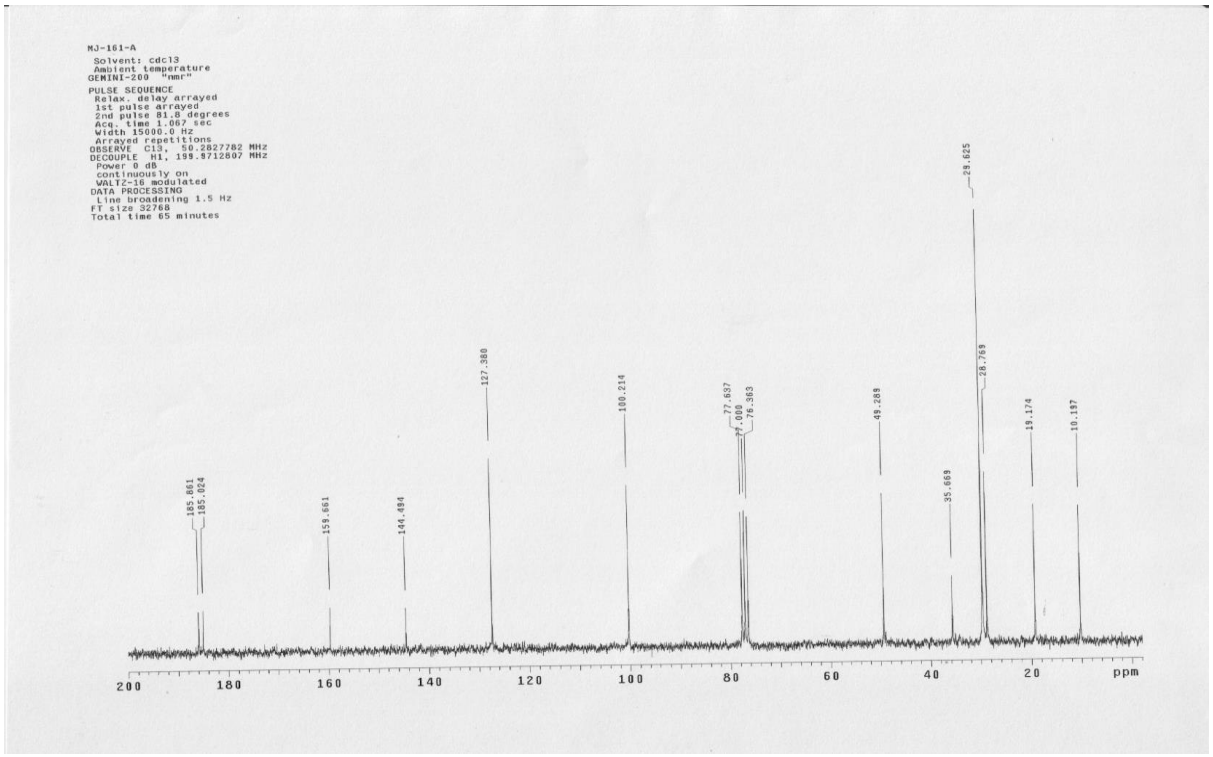


316

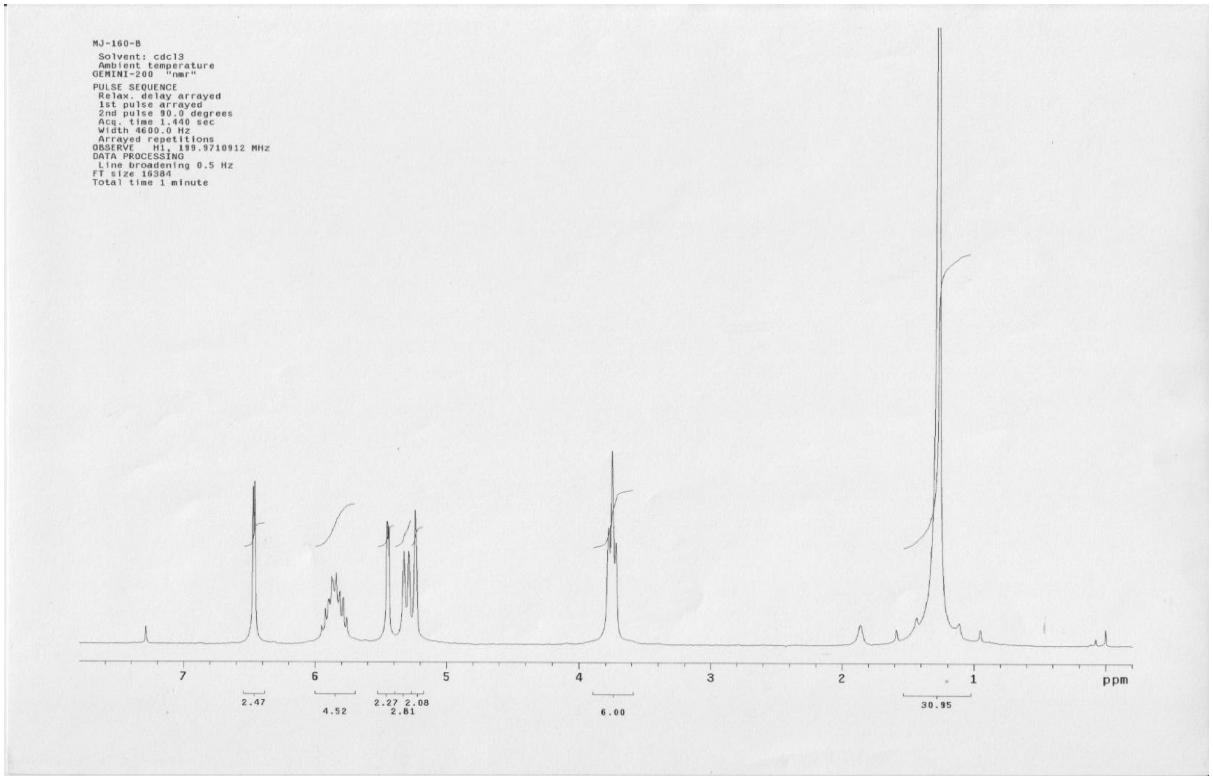
317 ^{13}C NMR spectrum of compound **3a**



[1]H NMR spectrum of compound **3b**



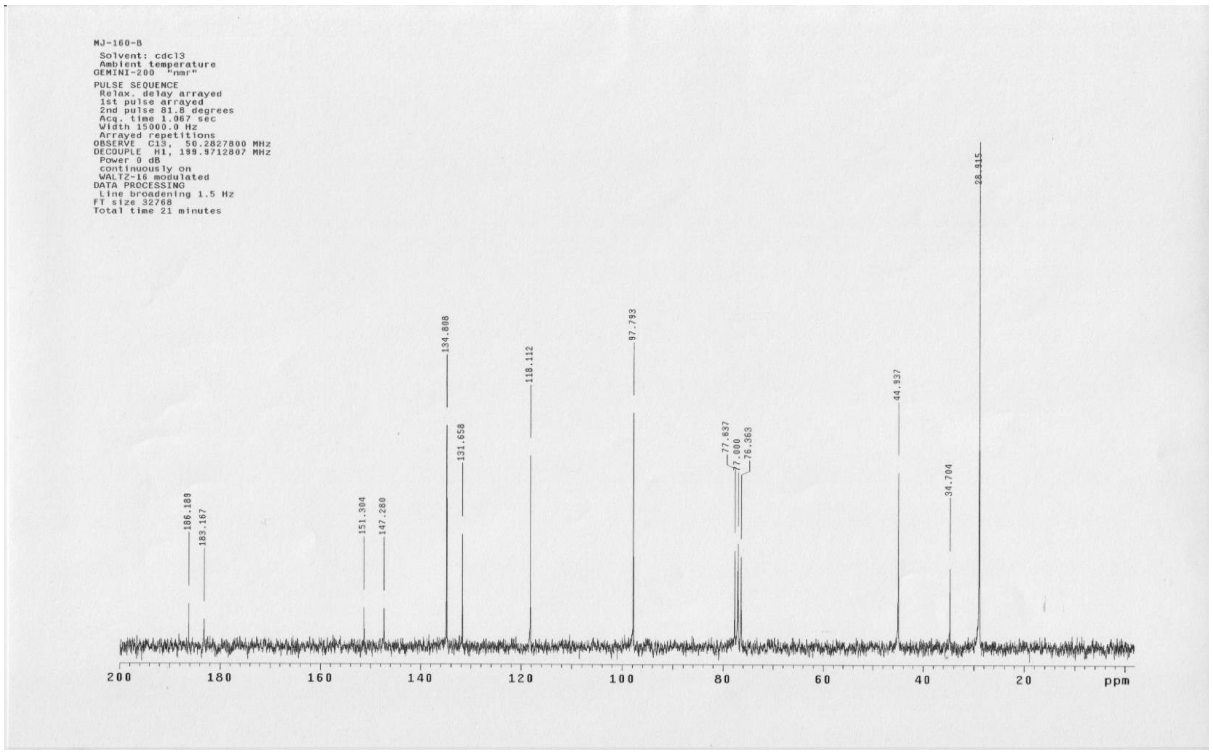
[13]C NMR spectrum of compound **3b**



324

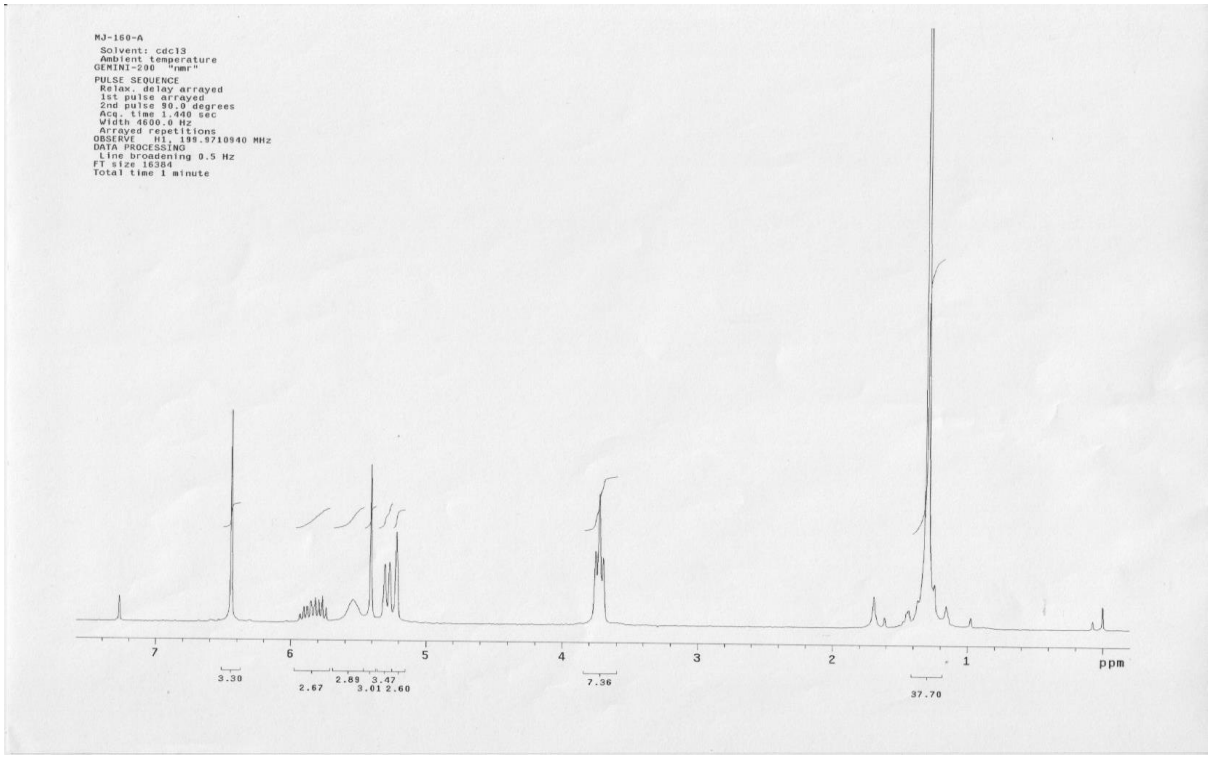
325 ^{1}H NMR spectrum of compound **4a**

326



327

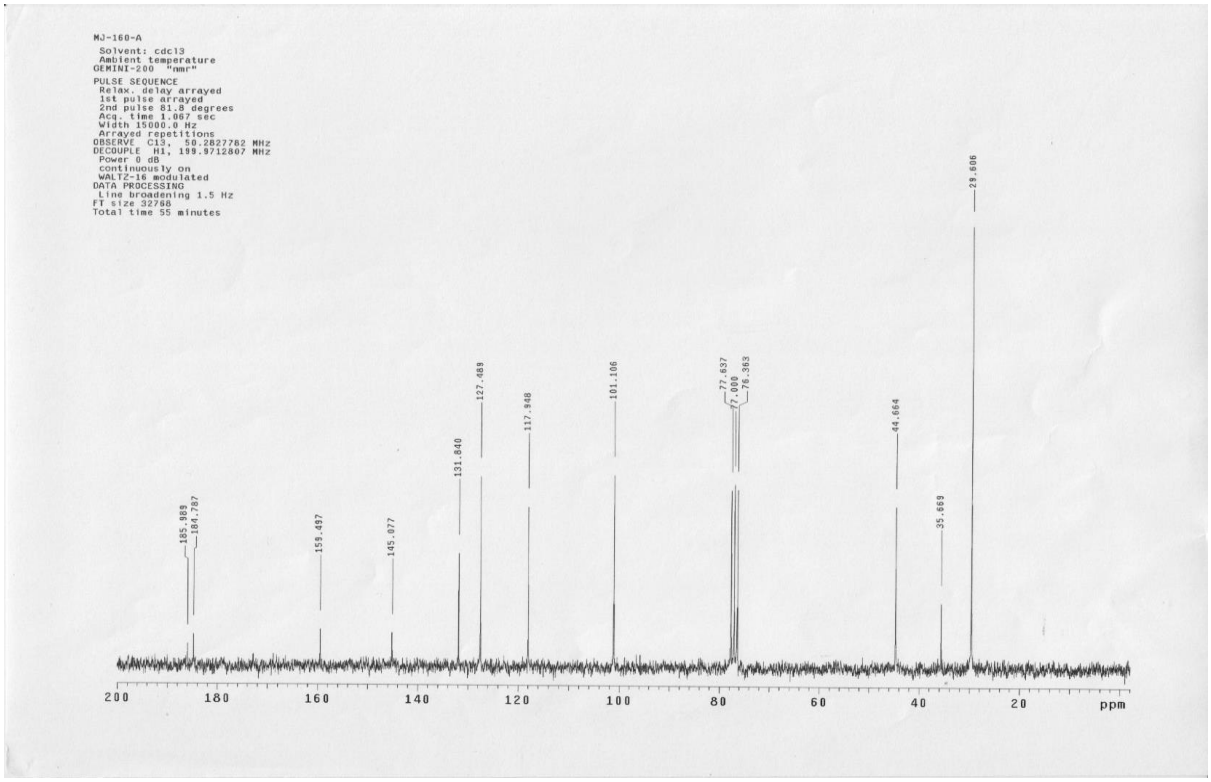
328 ^{13}C NMR spectrum of compound **4a**



329

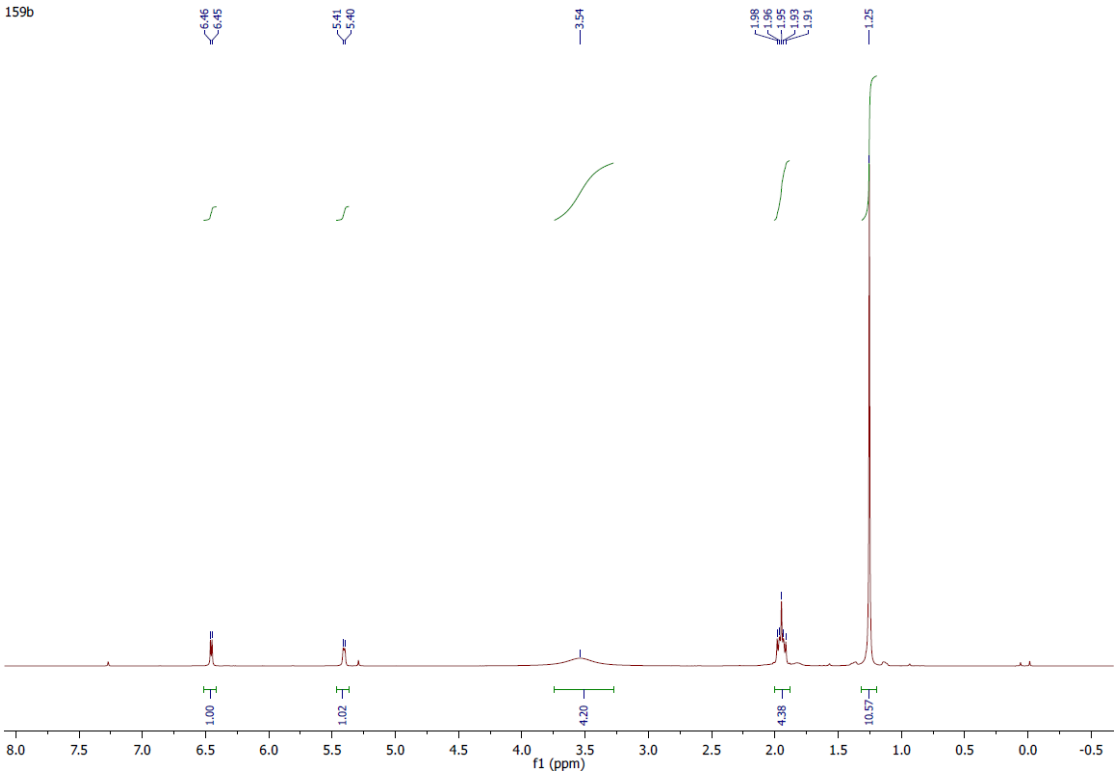
330 ^{1}H NMR spectrum of compound **4b**

331



332

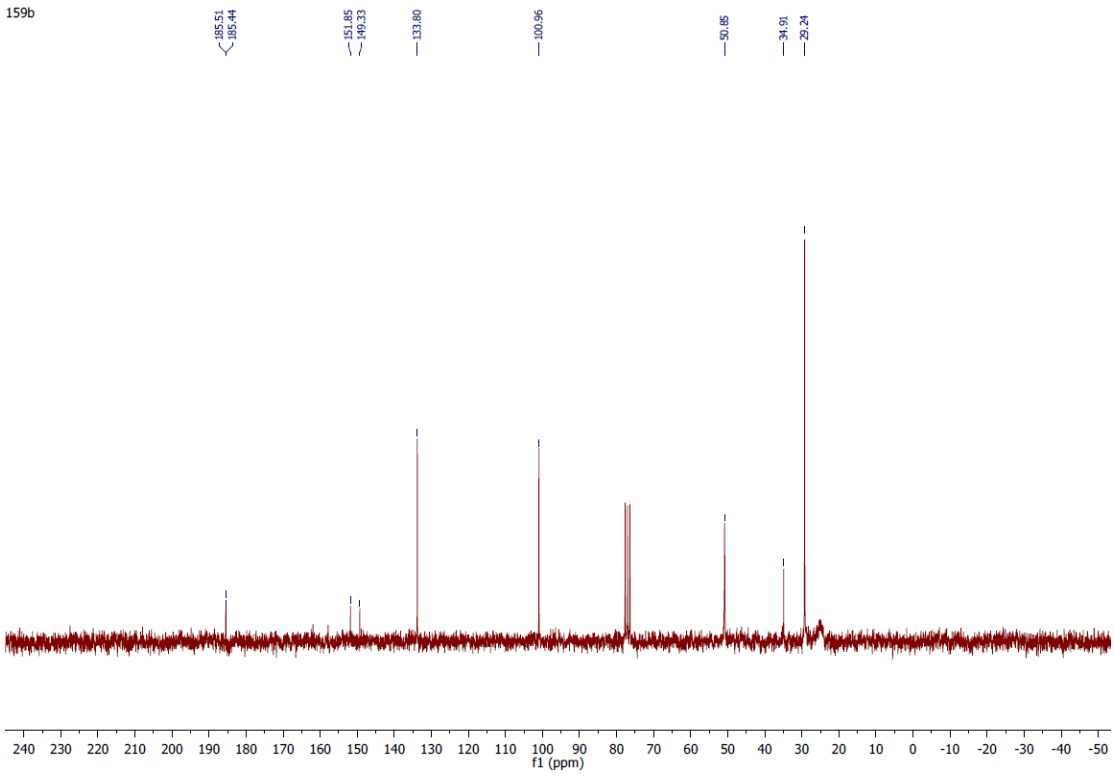
333 ^{13}C NMR spectrum of compound **4b**



334

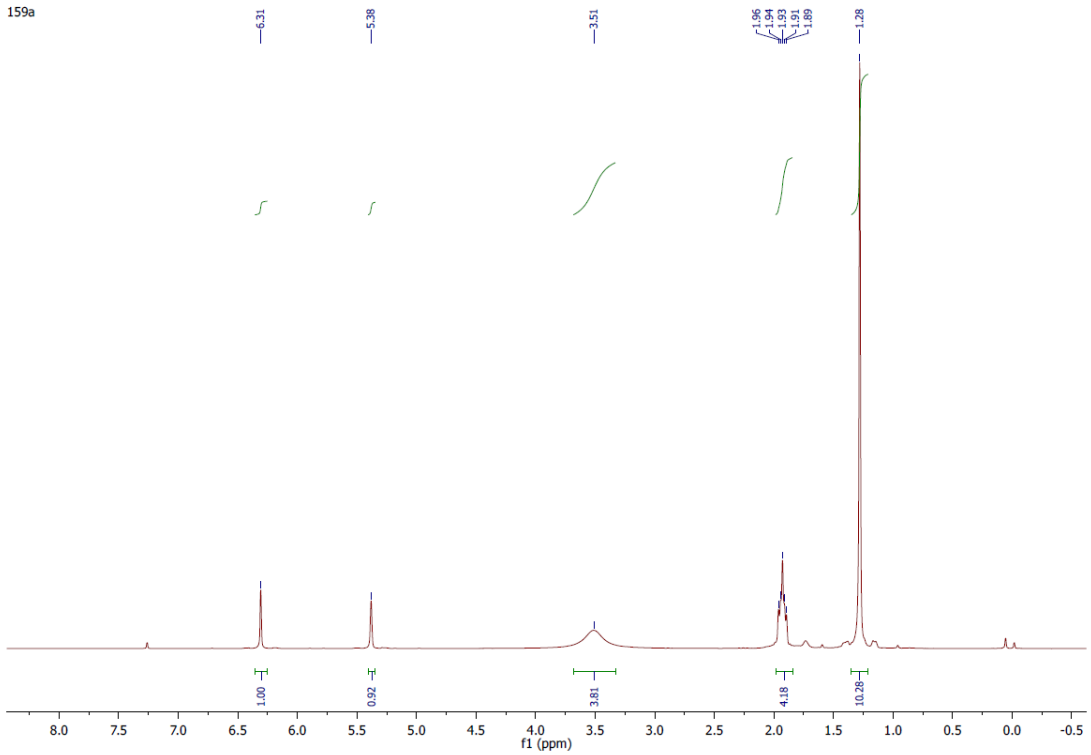
335 ^{1}H NMR spectrum of compound **5a**

336



337

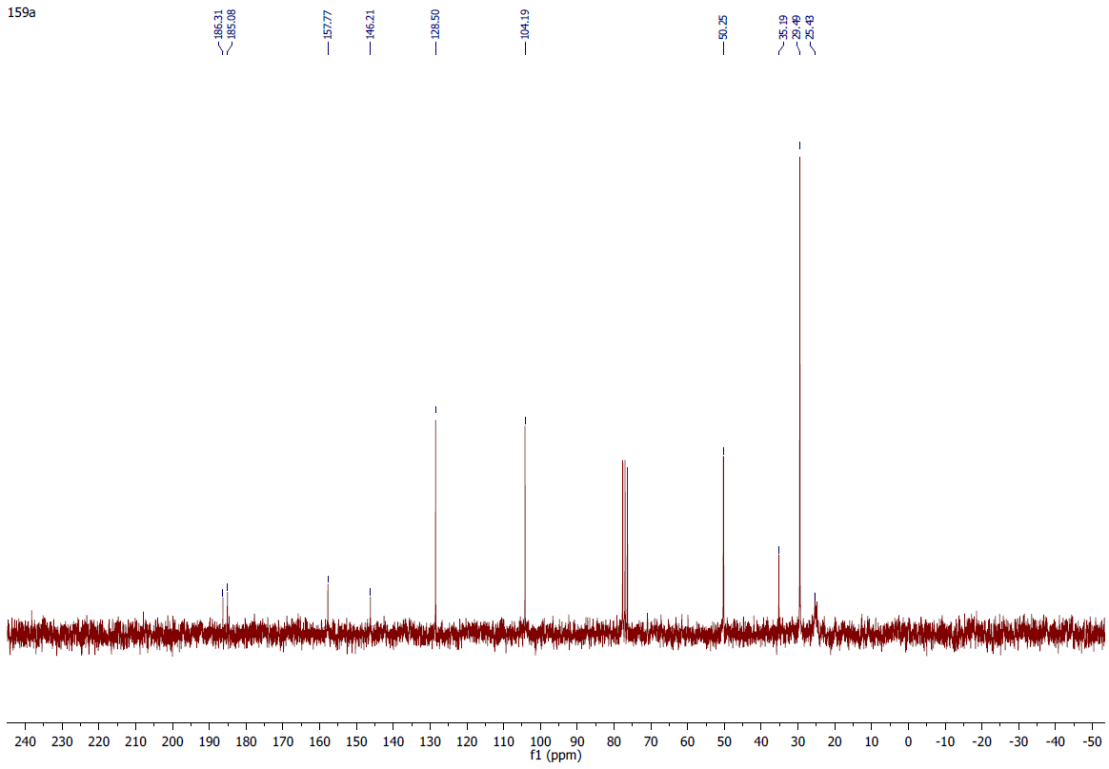
338 ^{13}C NMR spectrum of compound **5a**



339

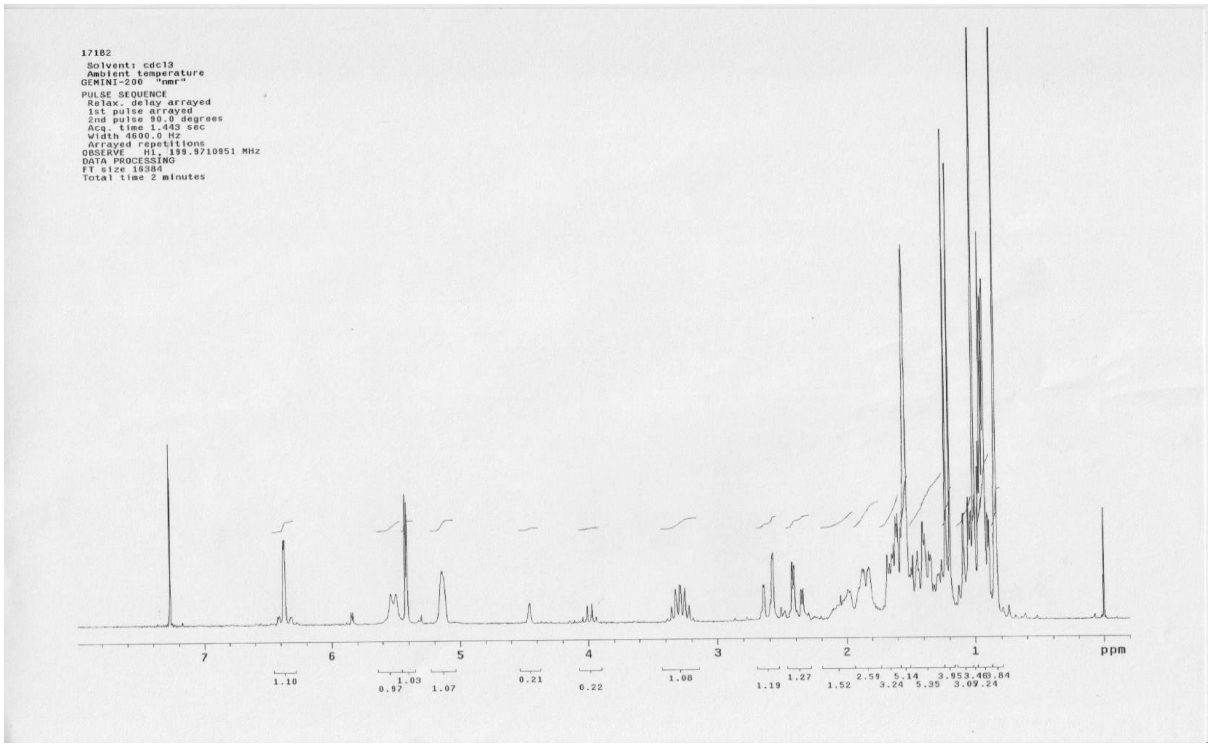
340 ^{1}H NMR spectrum of compound **5b**

341

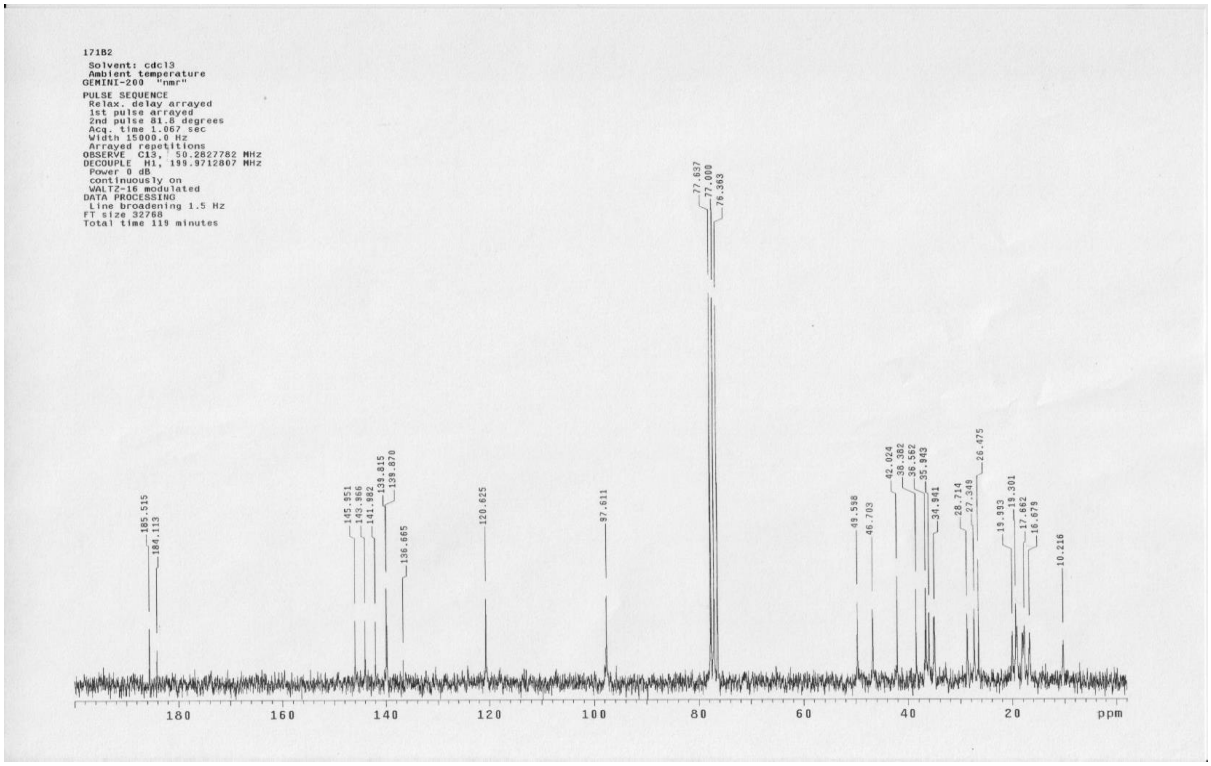


342

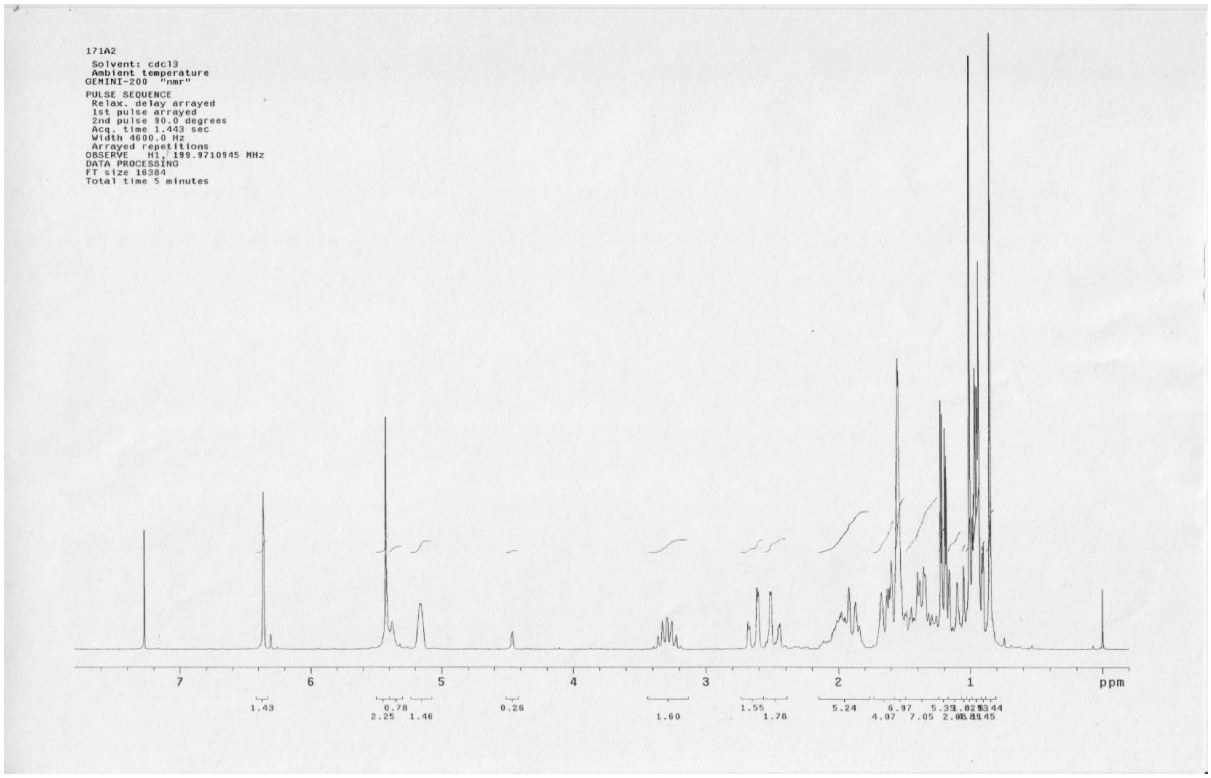
343 ^{13}C NMR spectrum of compound **5b**



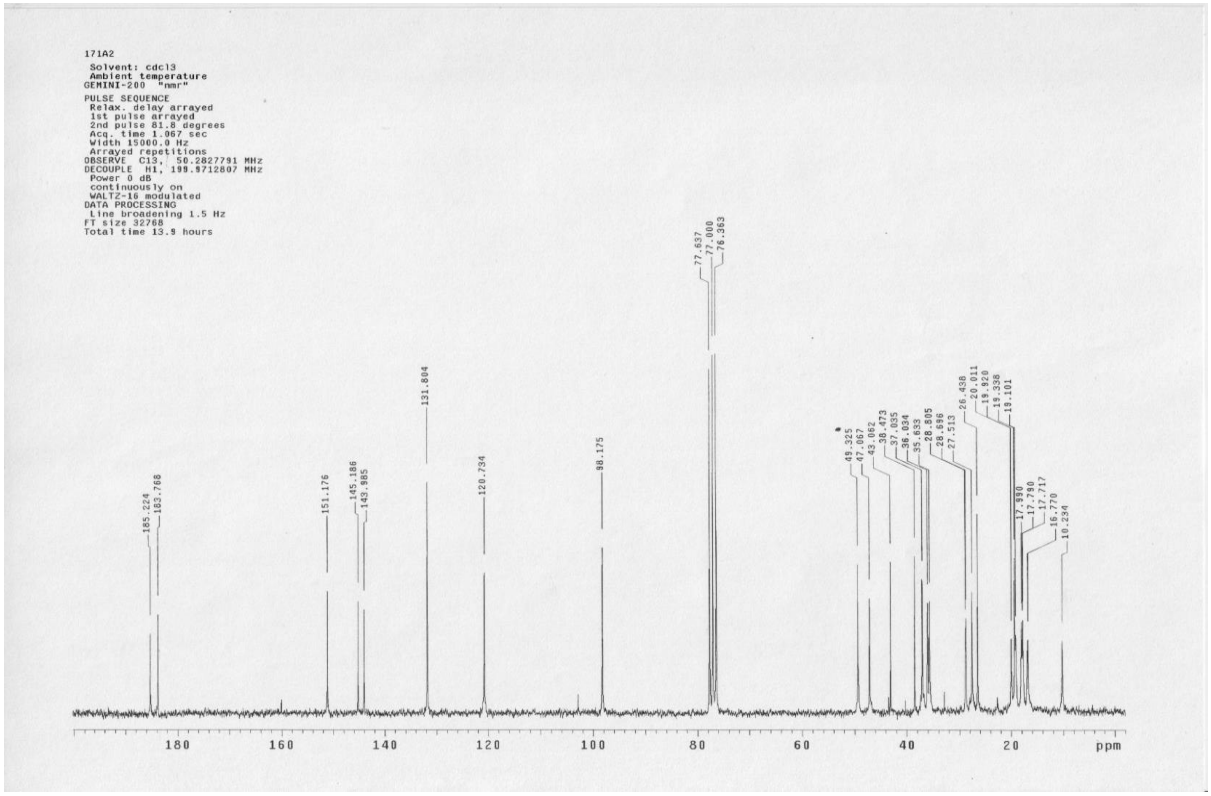
344
345
346 ^{1}H NMR spectrum of compound **7a**
347



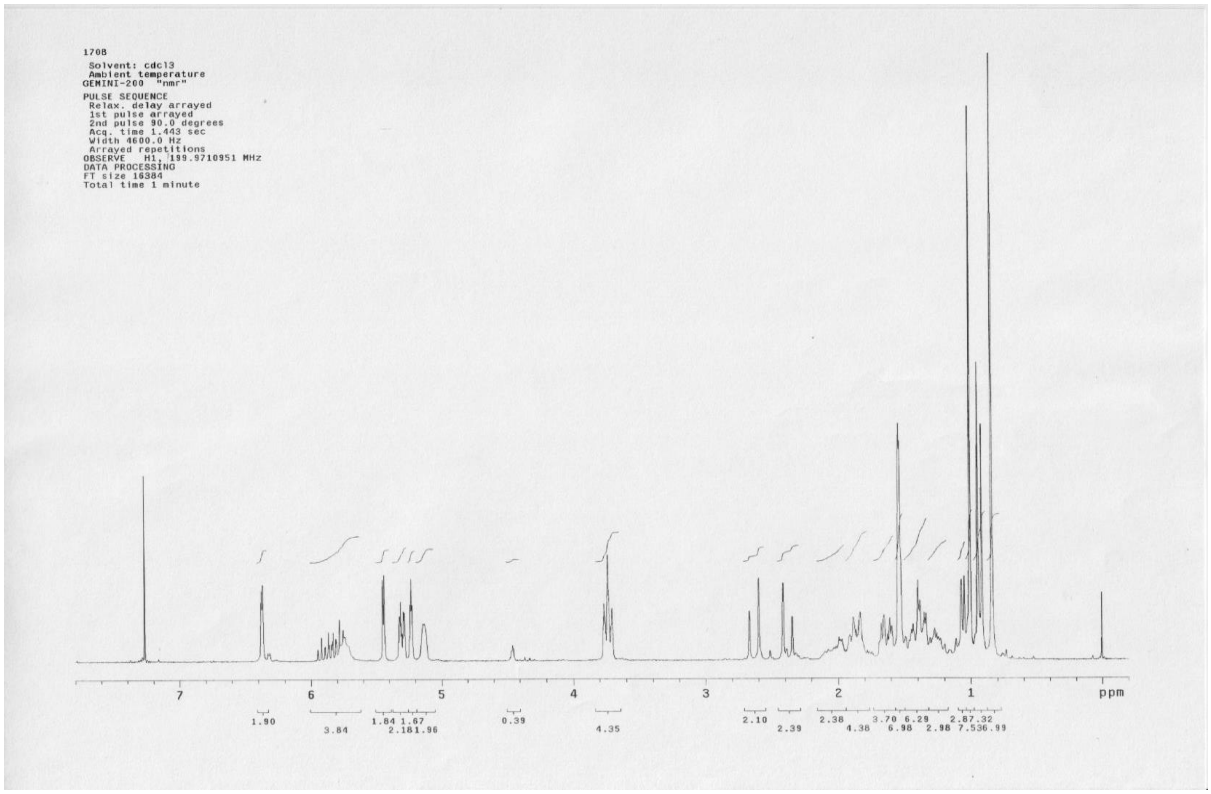
348
349
350 ^{13}C NMR spectrum of compound **7a**



^{1}H NMR spectrum of compound **7b**



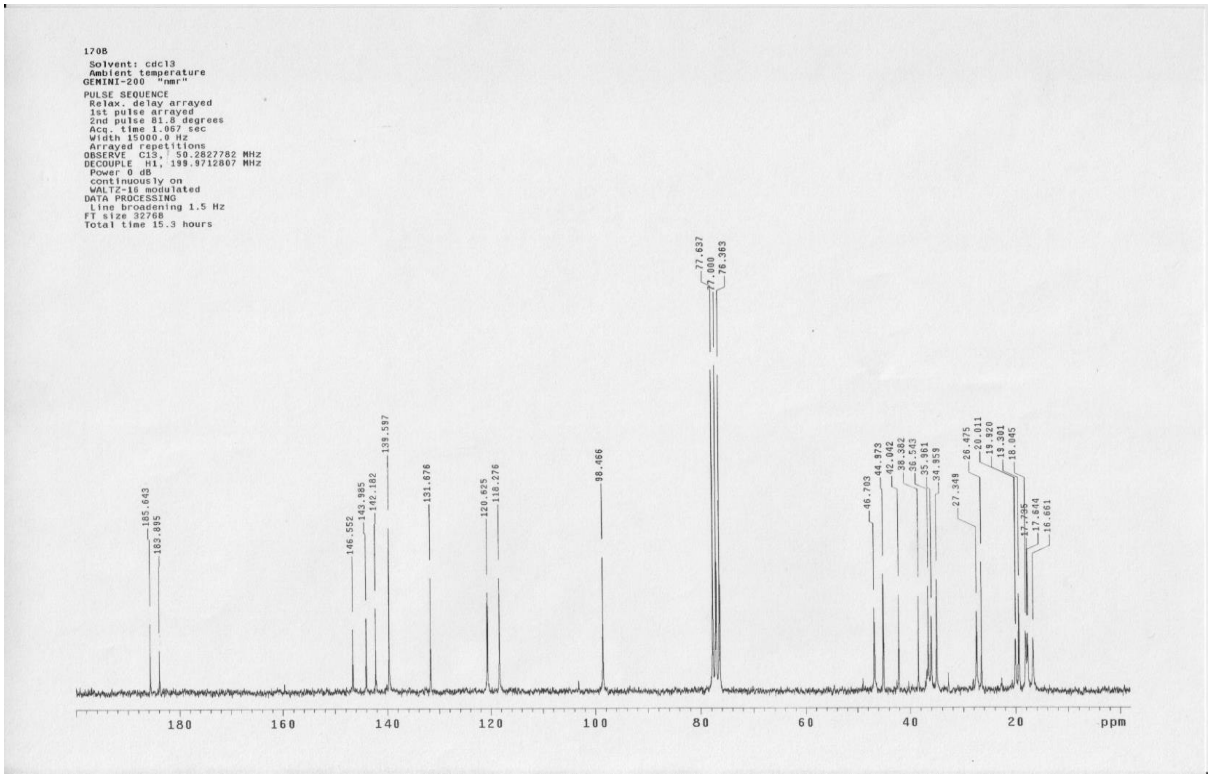
^{13}C NMR spectrum of compound **7b**



358

359 ^{1}H NMR spectrum of compound **8a**

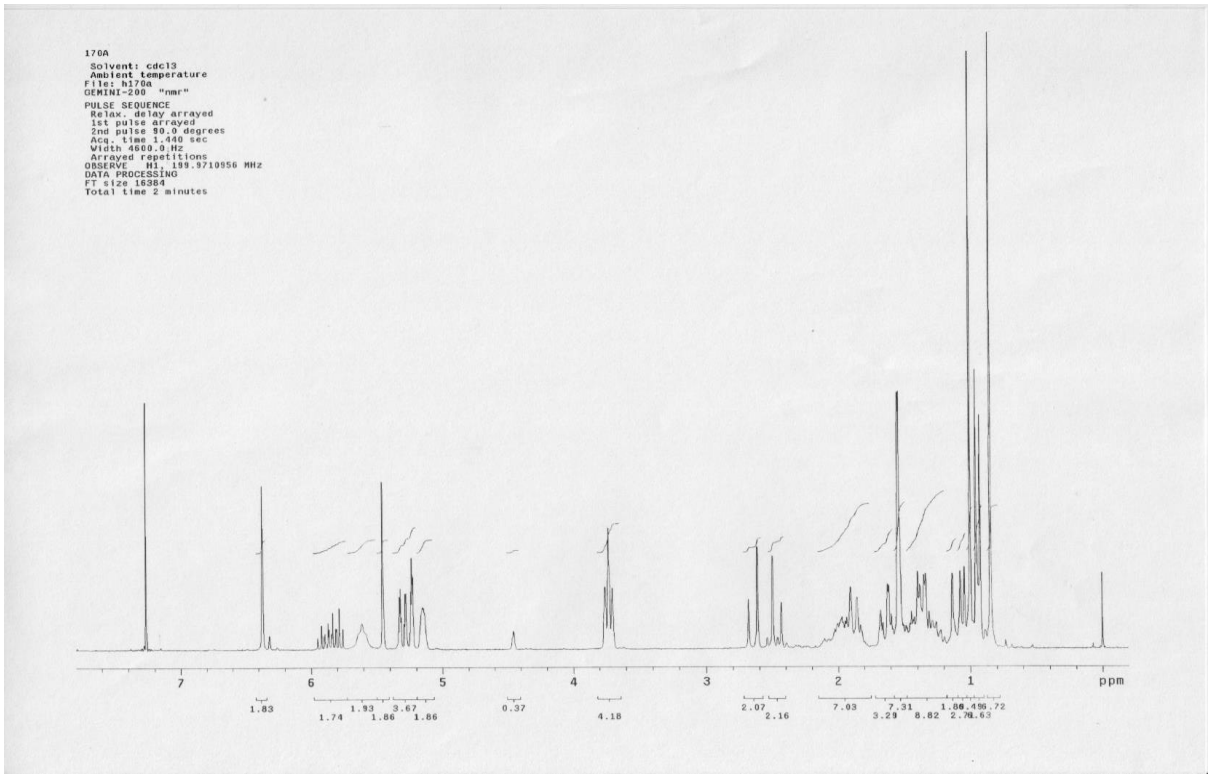
360



361

362

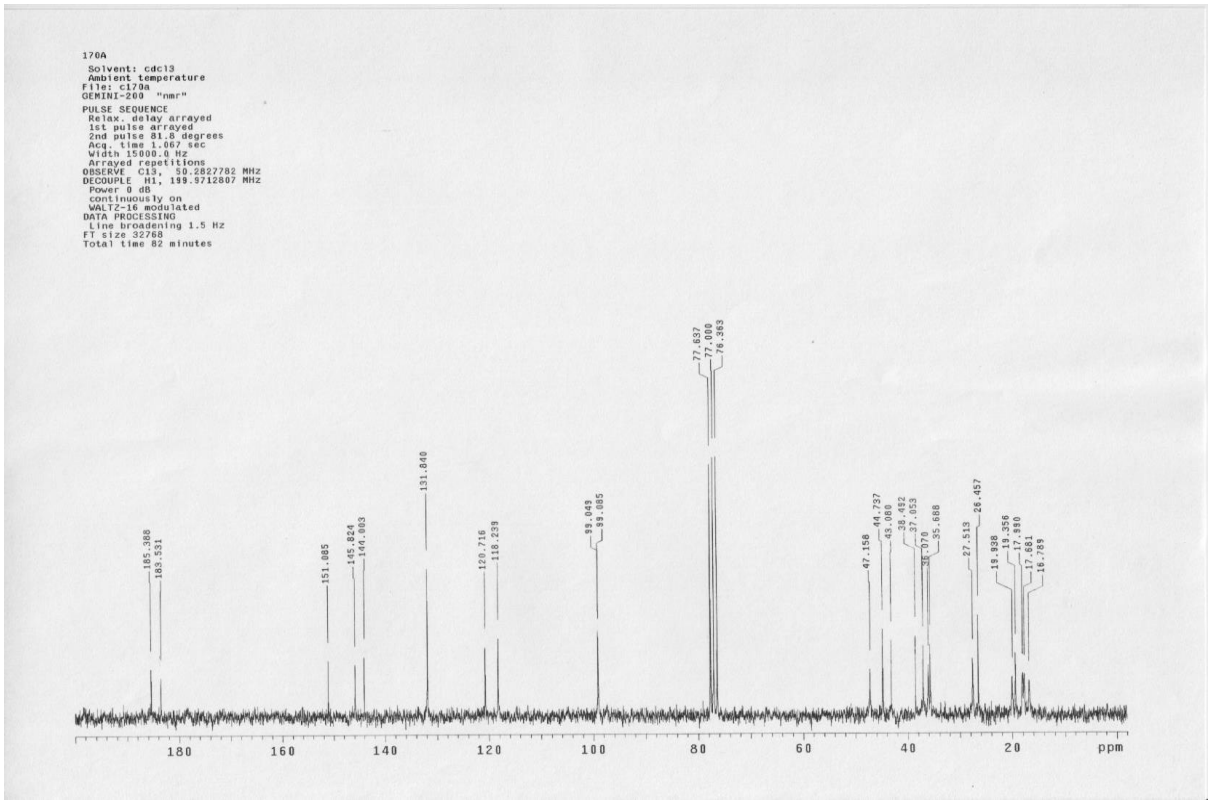
363 ^{13}C NMR spectrum of compound **8a**



364

365 ^{1}H NMR spectrum of compound **8b**

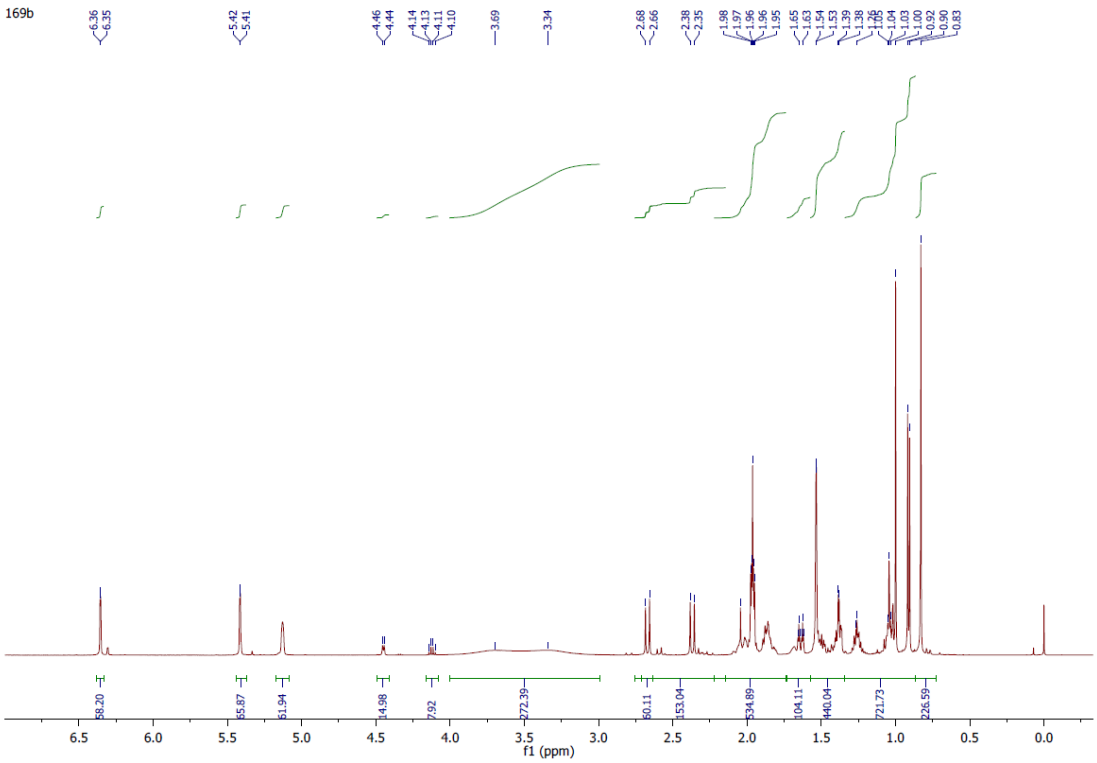
366



367

368

369 ^{13}C NMR spectrum of compound **8b**

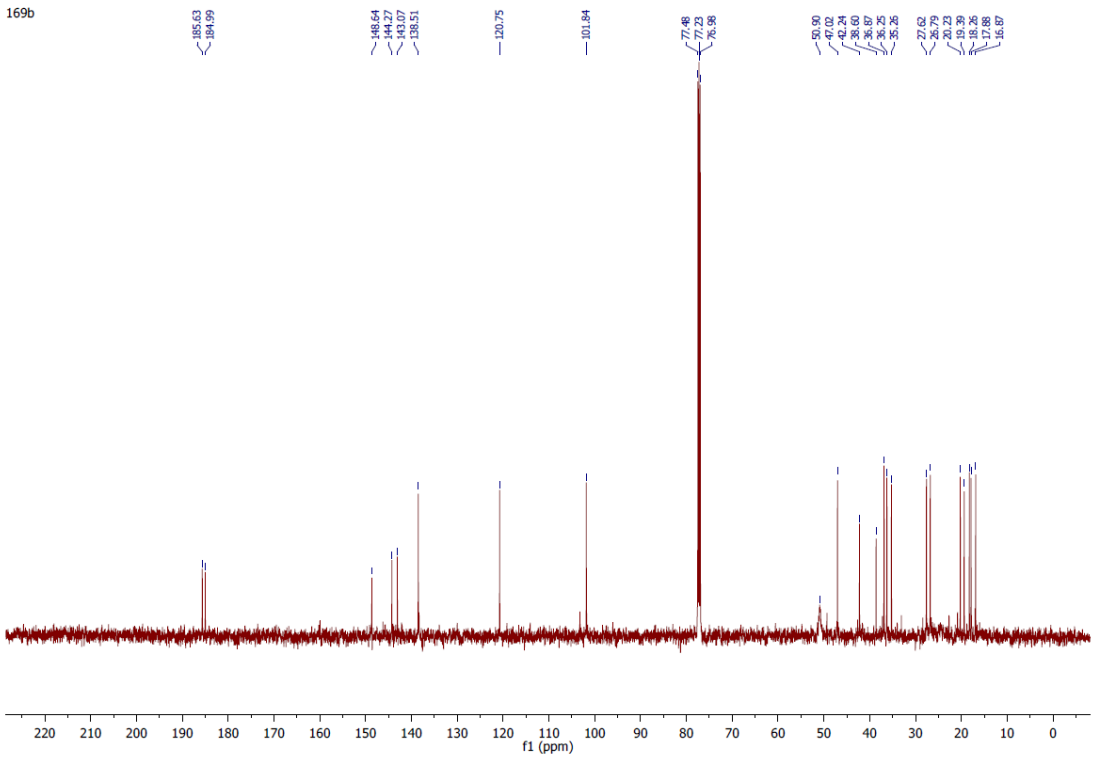


370

371 ^{1}H NMR spectrum of compound **9a**

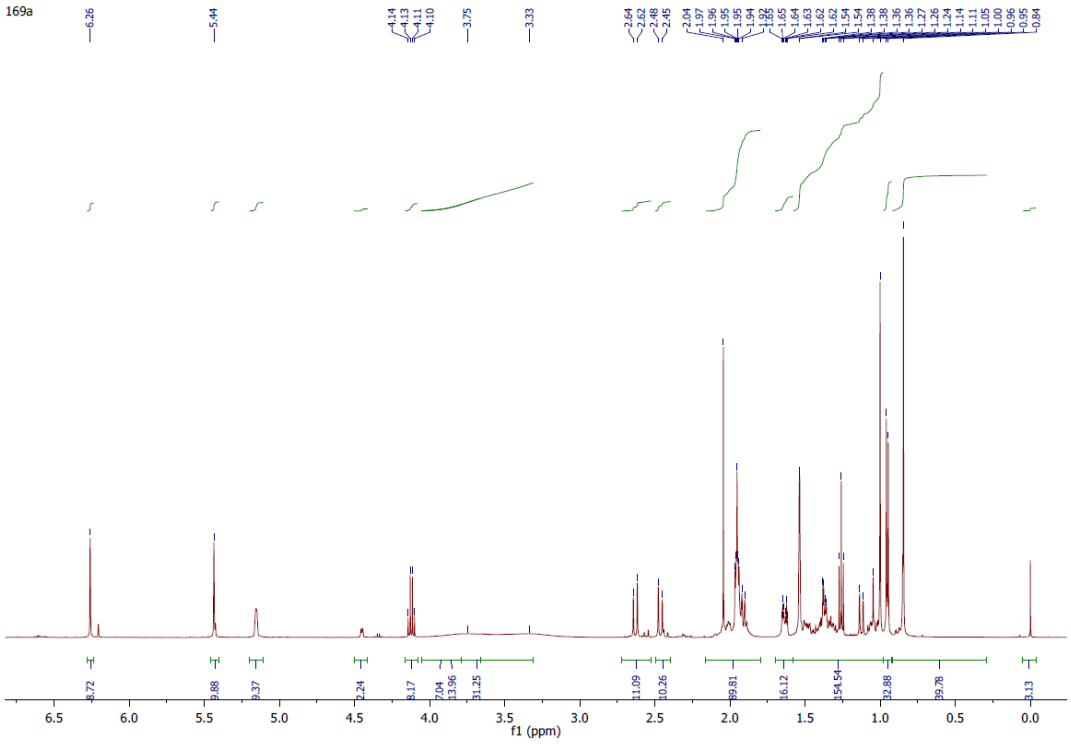
372

373



374

375 ^{13}C NMR spectrum of compound **9a**



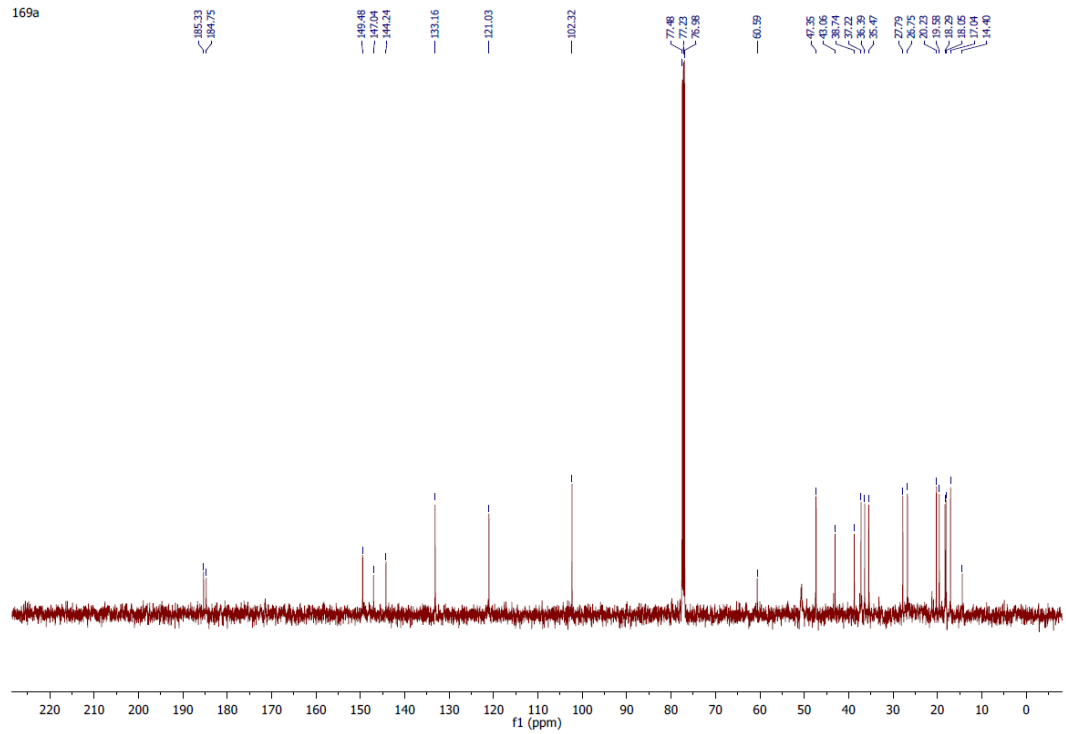
376

377

378 ^{1}H NMR spectrum of compound **9b**

379

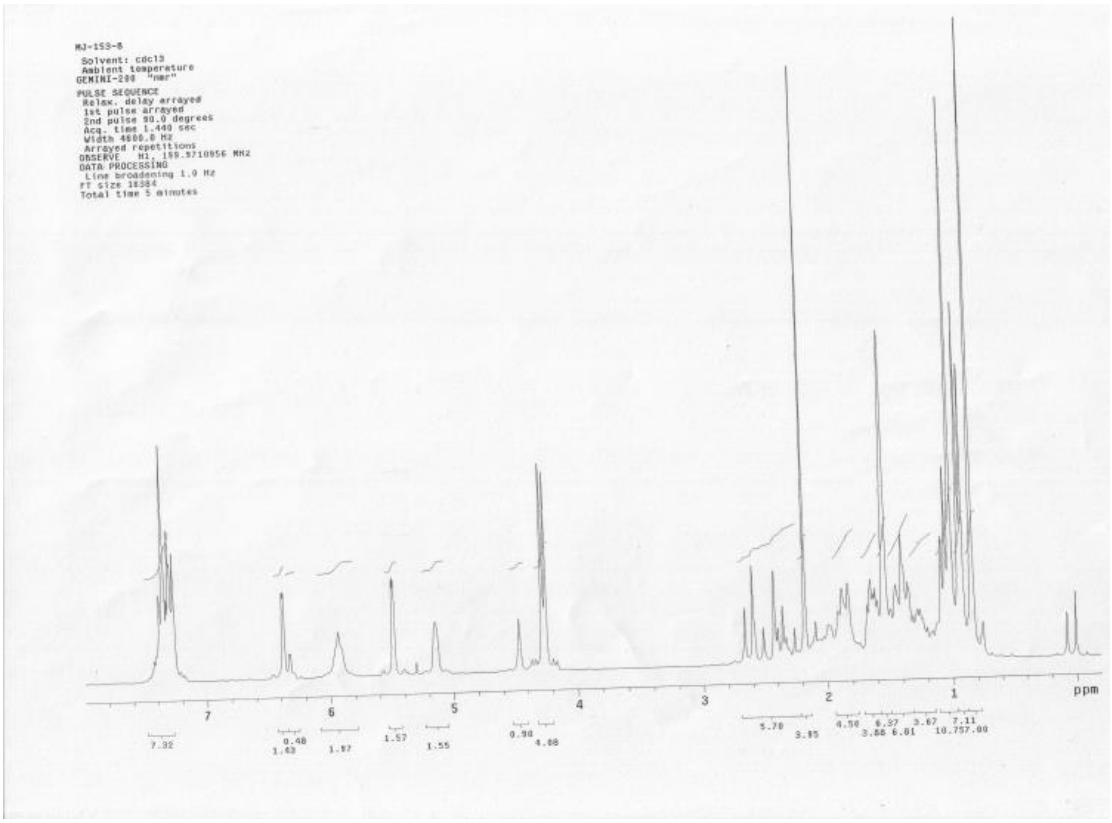
380



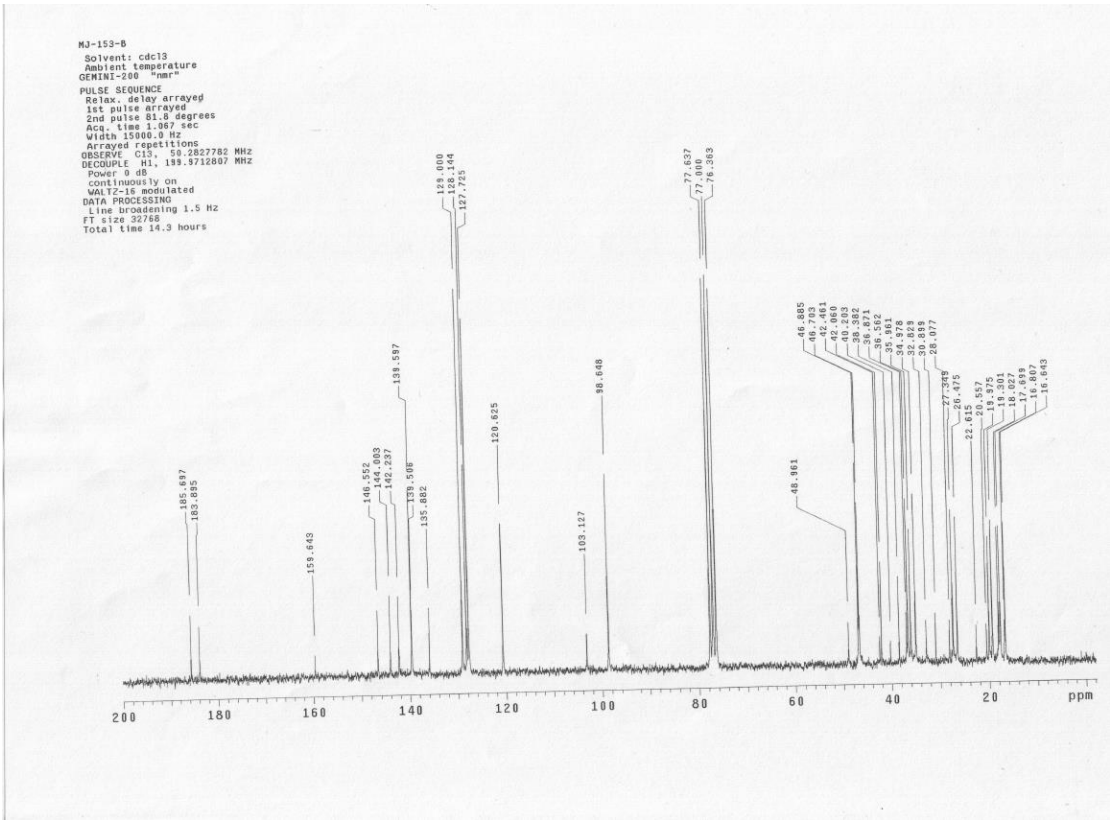
381

382

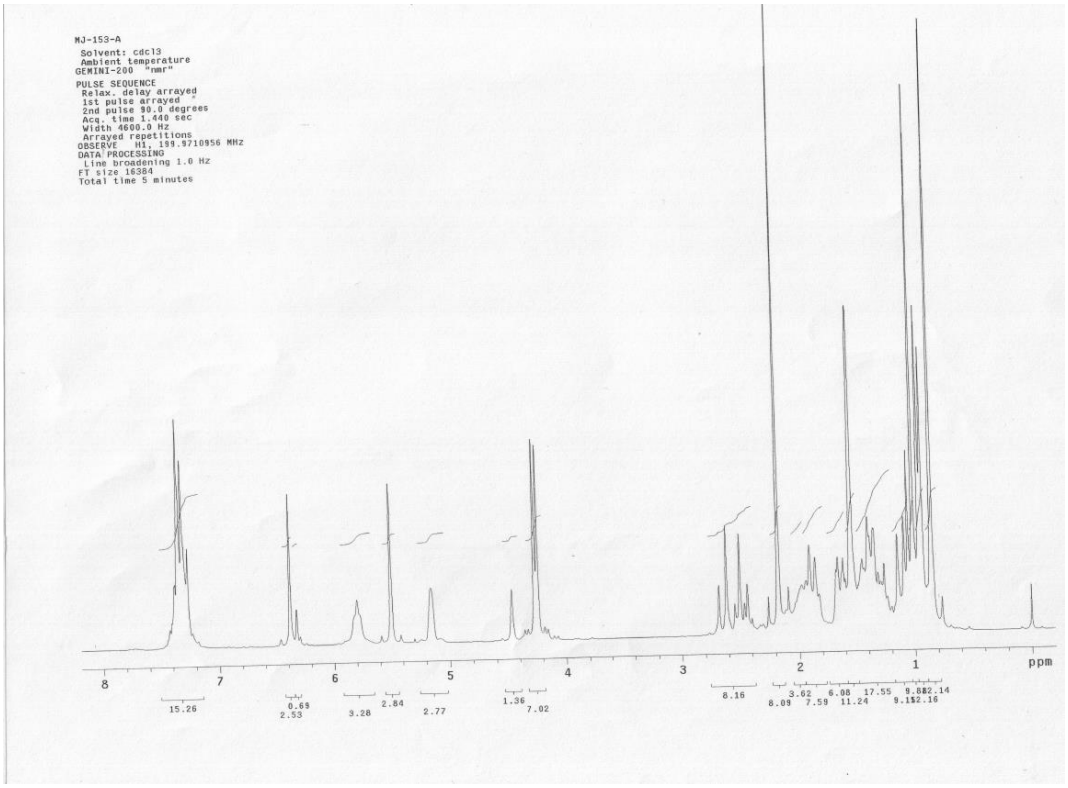
383 ^{13}C NMR spectrum of compound **9b**



^{1}H NMR spectrum of compound **10a**



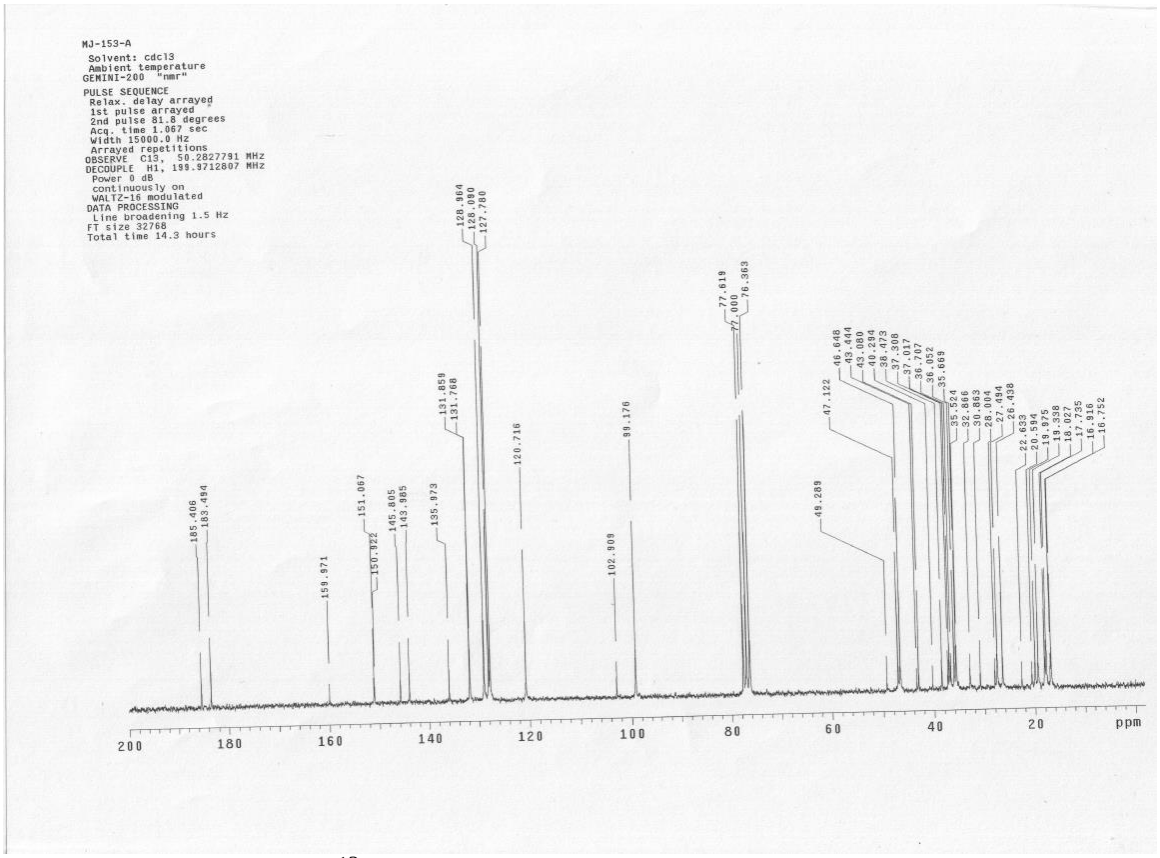
^{13}C NMR spectrum of compound **10a**



391

392 ^{1}H NMR spectrum of compound **10b**

393



394

395 ^{13}C NMR spectrum of compound **10b**