Repurposing the Clinically Efficacious Anti-Fungal Agent Itraconazole as an Anti-Cancer Chemotherapeutic.

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Supporting Information – Compound synthesis and characterization, biological assay protocols, and ^{1}H and ^{13}C NMR Spectra.

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SYNTHETIC PROCEDURES

Linker Region Intermediates

1-(4-methoxyphenyl)-4-(4-nitrophenyl)piperazine (28). 1-(4-methoxyphenyl)piperazine (**26**) (8.0 g, 41.6 mmol) and 1-chloro-4-nitrobenzene (**27**) (6.3 g, 40.0 mmol) were dissolved in anhydrous DMSO (80 mL). K_2CO_3 (6.0 g, 43.5 mmol) was added and the mixture refluxed at 160°C for 12 h. The reaction was cooled to RT and a red solid precipitated. The red solid was filtered and washed with $CHCl_3$. The filtrate was concentrated to afford **28**, which was immediately utilized as the crude solid.

4-(4-(4-methoxyphenyl)piperazin-1-yl)aniline (29). 10% palladium on carbon (0.88 g, 5% mole ratio) was added to a dry 3-neck round bottom flask. Ethanol (200 mL) was added followed by slow addition of **28** (5.2 g, 16.59 mmol). Hydrazine monohydrate (5.2 mL, 165.9 mmol) was added dropwise and the mixture was stirred at reflux for 3.5 h. Upon cooling to RT, the mixture was filtered through celite. The celite was washed with ethanol (250 mL) and chloroform (1000 mL) to ensure complete elution of the aniline. The filtrate was concentrated to afford **29**, which was immediately utilized as the crude solid.

Phenyl (4-(4-(4-methoxyphenyl)piperazin-1-yl)phenyl)carbamate (30). A solution of aniline (**29**) (2.7 g, 9.51 mmol) and pyridine (12.8 mL, 158.8 mmol) in $CHCl_3$ (100 mL) was cooled to 0°C. Phenyl chloroformate (1.25 mL, 9.9 mmol) was added dropwise and the mixture stirred at RT for 3 h. Water and petroleum ether (50 mL: 50 mL) were added at which point an off-white precipitate formed. The solid was washed with H_2O and isopropyl alcohol to provide the crude carbamate **31**, which was immediately utilized without further purification.

N-(4-(4-(4-methoxyphenyl)piperazin-1-yl)phenyl)hydrazinecarboxamide (31). A solution of hydrazine hydrate (1.33 mL, 42.8 mmol) and **30** (3.2 g, 7.9 mmol) in 1.4-dioxane (70 mL) was

stirred at reflux for 3 h. The mixture was poured into H_2O (250 mL), and the resulting off-white solid was filtered and washed with H_2O and isopropyl alcohol to give carboxamide **32**, which was immediately utilized without further purification.

4-(4-(4-(4-methoxyphenyl)piperazin-1-yl)phenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (32). A solution of formamidine acetate (2.86 g, 27.5 mmol), **31** (2.1 g, 6.15 mmol), and acetic acid (4 mL) in anhydrous DMF (30 mL) was stirred at 130°C for 3 h. The mixture was cooled to room temperature and diluted with H_2O (100 mL) at which point a light brown precipitate formed. The solid was filtered and washed with $CHCl_3$ to provide triazolone **32**, which was immediately utilized without further purification.

4-(4-(4-nitrophenyl)piperazin-1-yl)phenol (68). N-(4-hydroxyphenyl)-piperazine (5.3 g, 29.7 mmol), 1-chloro-4-nitrobenzene (6.57 g, 41.7 mmol), and DIPEA (7.74 mL, 44.4 mmol) were dissolved in 50 mL anhydrous NMP and heated to 125°C for 12 h. The reaction was cooled to 80°C and isopropanol (250 mL) was added portion wise over 30 mins. The reaction mixture was cooled to room temperature and precipitate started to form. The reaction was further cooled to -20°C and stirred for 30 mins. The precipitate was vacuum filtered and washed with cold isopropanol to give the product, a brown powder, in quantitative yield. Characterization matched that previously reported.[1]

Side Chain Intermediates

(*R*)-*sec*-butyl 4-bromobenzenesulfonate (35). (*R*)-2-butanol (**33**) (3.0 g, 40.5 mmol), Et_3N (12.0 mL, 13.5 mmol), and DMAP (5.0 g, 40.5 mmol) in DCM. Cool the reaction to 0°C and slowly add a solution of 4-bromobenzene-1-sulfonylchloride (22.7 g, 89.1 mmol) in DCM. The mixture was stirred at 0°C for 4h. Dilute the reaction with H_2O and extract with DCM. Combine the organic layers, dry over sodium sulfate, filter, and concentrate. The crude residue was purified by

column chromatography (SiO_2, 0-2% EtOAc in hexanes) to afford the product (37.9%, 4.5 g). ^1H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 2H), 7.57 (m, 2H), 4.47 (m, 1H), 1.45 (m, 2H), 1.15 – 1.09 (m, 3H), 0.65 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.12, 132.83, 129.53, 128.95, 83.03, 31.99, 29.88, 23.05, 20.76, 14.51, 9.71. DART-HRMS: *m/z* calcd. for $C_{10}H_{13}BrO_3S$ $[MH]^+$, 294.9827; Found: 294.9822. IR (solid) *ν*max: 2954, 2930, 2869, 1588, 1463, 1376, 1364, 1313, 1224, 1188, 1093, 1070, 1034, 942, 903, 821, 752. $[\alpha]_D^{20}$ = -16.0 (c = 1.984, MeOH).

(*S*)-*sec*-butyl 4-bromobenzenesulfonate (36). (S)-(+)-2-butanol (**34**) (3.0 g, 40.5 mmol), Et_3N (12.0 mL, 13.5 mmol), and DMAP (5.0 g, 40.5 mmol) in DCM. Cool the reaction to 0°C and slowly add a solution of 4-bromobenzene-1-sulfonylchloride (22.7 g, 89.1 mmol) in DCM. The mixture was stirred at 0°C for 4h. Dilute the reaction with H_2O and extract with DCM. Combine the organic layers, dry over sodium sulfate, filter, and concentrate. The crude residue was purified by column chromatography (SiO_2, 0-2% EtOAc in hexanes) to afford the product (53.9%, 6.4 g). ^1H NMR (500 MHz, $CDCl_3$) δ 7.84 – 7.72 (m, 2H), 7.65 (m, 2H), 4.57 (m, 1H), 1.56 (m, 2H), 1.32 – 1.19 (m, 3H), 0.77 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 136.56, 132.31, 129.00, 128.40, 82.50, 60.22, 31.43, 29.33, 20.21, 14.07, 9.16. DART-HRMS: *m/z* calcd. for $C_{10}H_{13}BrO_3S$ $[MH]^+$, 294.9827; Found: 294.9856. IR (solid) *ν*max: 3090, 2975, 2937, 2880, 1576, 1501, 1471, 1389, 1361, 1226, 1185, 1112, 1090, 1068, 994, 895, 819, 751, 608. $[\alpha]_D^{20}$ = +11.5 (c = 4.466, MeOH).

Linker/Triazolone/Side Chain Intermediates.

General Protocol for alkylation of triazolone (propyl- or *sec*-butyl-substitutions) (39-42). To a suspension of triazolone **32** (1.0 g, 2.8 mmol) in anhydrous NMP (30 mL) was added Cs_2CO_3 (2.74 g, 8.4 mmol), and an alkylating agent (**35-38**) (8.4 - 14 mmol). The mixture was stirred at RT for 2 h, then warmed to 60º C for 12 h and finally to 110º C for 4h. The mixture was then cooled to RT, diluted with water (125 mL), and extracted with EtOAc (3 X 150 mL). The organic

layers were combined, dried ($MgSO_4$), filtered, and concentrated. The crude residue was purified by column chromatography (SiO_2, 0 to 20% EtOAc in Hex) to afford **39-42** as off-white solids (45-88%). Compounds were further purified as needed via recrystallization (EtOH) to remove a "grease-like" impurity (observed in ^{1}H NMRs at 0.88, 1.31 ppm).

(*S*)-2-(*sec*-butyl)-4-(4-(4-(4-methoxyphenyl)piperazin-1-yl)phenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (39). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.46 – 7.40 (m, 2H), 7.03 (d, *J* = 9.0 Hz, 2H), 6.96 (d, *J* = 9.0 Hz, 2H), 6.90 – 6.84 (m, 2H), 4.35 – 4.24 (m, 1H), 3.78 (s, 3H), 3.37 (m, 4H), 3.23 (m, 4H), 1.86 (m, 1H), 1.78 – 1.66 (m, 1H), 1.39 (d, *J* = 6.7 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.24, 152.07, 150.60, 145.53, 133.91, 125.96, 123.55, 118.63, 116.66, 114.57, 55.61, 52.72, 50.83, 49.31, 28.47, 19.25, 10.80. DART-HRMS: *m/z* calcd. for $C_{23}H_{29}N_5O_2$ $[MH]^+$, 408.2400; Found: 408.2368. IR (solid) *ν*max: 3120, 3053, 2967, 2935, 2877, 2821, 2765, 1686, 1549, 1507, 1446, 1388, 1240, 1222, 1154, 1117, 1030, 943, 823, 805, 720. $[\alpha]_D^{20}$ = + 12.6 (c = 0.996, DMSO).

(*R*)-2-(*sec*-butyl)-4-(4-(4-(4-methoxyphenyl)piperazin-1-yl)phenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (40). ^{1}H NMR (500 MHz, $CHCl_3$) δ 7.61 (s, 1H), 7.47 – 7.40 (m, 2H), 7.06 – 6.99 (m, 2H), 6.96 (m, 3H), 6.90 – 6.84 (m, 2H), 4.30 (m, 1H), 3.78 (d, *J* = 1.3 Hz, 2H), 3.40 – 3.28 (m, 4H), 3.24 (m, 4H), 1.94 – 1.76 (m, 1H), 1.78 – 1.67 (m, 1H), 1.39 (d, *J* = 6.7 Hz, 2H), 0.91 (t, *J* = 7.4 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.21, 152.06, 150.58, 145.50, 133.94, 125.94, 123.54, 118.62, 116.65, 114.55, 55.60, 52.70, 50.81, 49.29, 28.47, 19.29, 10.83. DART-HRMS: *m/z* calcd. for $C_{23}H_{29}N_5O_2$ $[MH]^+$, 408.2400; Found: 408.2430. IR (solid) *ν*max: 3120, 3052, 2967, 2821, 1686, 1549, 1507, 1444, 1386, 1240, 1221, 1030, 943, 824, 805, 719, 530. $[\alpha]_D^{20}$ = -12.2 (c = 0.873, DMSO).

2-(*sec*-butyl)-4-(4-(4-(4-methoxyphenyl)piperazin-1-yl)phenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (41). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, *J* = 0.6 Hz, 1H), 7.51 – 7.43 (m, 2H),

7.11 – 7.04 (m, 2H), 7.04 – 6.97 (m, 2H), 6.96 – 6.88 (m, 2H), 4.34 (m, 1H), 3.83 (s, 3H), 3.44 – 3.38 (m, 4H), 3.31 – 3.25 (m, 4H), 1.91 (m, 1H), 1.83 – 1.70 (m, 1H), 1.44 (d, *J* = 6.8 Hz, 3H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.22, 152.07, 150.60, 133.98, 125.92, 123.59, 118.66, 116.69, 114.54, 55.61, 52.69, 50.85, 49.29, 32.36, 28.49, 19.33, 10.85. DART-HRMS: *m/z* calcd. for $C_{23}H_{29}N_5O_2$ $[MH]^+$, 408.2400; Found: 408.2388. IR (solid) *ν*max: 3120, 3053, 2970, 2877, 2818, 2763, 1686, 1548, 1508, 1444, 1399, 1241, 1223, 1030, 943, 823, 805.

4-(4-(4-(4-methoxyphenyl)piperazin-1-yl)phenyl)-2-propyl-2,4-dihydro-3H-1,2,4-triazol-3-one (42): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.50 – 7.43 (m, 2H), 7.04 – 6.97 (m, 2H), 6.96 – 6.88 (m, 2H), 3.86 (m, 2H), 3.83 (s, 3H), 3.44 – 3.38 (m, 4H), 3.31 – 3.25 (m, 4H), 1.89 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.22, 152.22, 150.66, 145.51, 133.93, 125.87, 123.60, 118.64, 116.67, 114.55, 55.61, 50.83, 49.28, 47.23, 22.06, 11.13. DART-HRMS: *m/z* calcd. for $C_{22}H_{27}N_5O_2$ $[MH]^+$, 394.2243; Found: 394.2265. IR (solid) *ν*max: 3122, 3053, 2956, 2873, 2830, 1686, 1555, 1511, 1451, 1409, 1390, 1250, 1225, 1032, 942, 813, 733.

General Protocol for phenol synthesis (demethylation protocol) (43-46). Hydrogen bromide (48% aqueous solution, 10 mL) was added to a solution of methoxy-alkyl substituted triazolone **39-42** (500 mg, 1.2 mmol) in toluene (6 mL) was refluxed for 6-12 h. The reaction was cooled to RT and water (10 mL) was added. The precipitate that formed was collected by filtration and washed with copious amounts of water. ^{1}H NMR analysis indicated pure phenol in good yields (55-80%). The filtrate was neutralized with saturated $NaHCO_3$ and washed with EtOAc (2 X 150 mL). The organic layers were dried ($MgSO_4$), filtered, and concentrated to afford remaining phenol (**43-46**) in moderate purity that required chromatography.

(*S*)-2-(*sec*-butyl)-4-(4-(4-(4-hydroxyphenyl)piperazin-1-yl)phenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (43). ^{1}H NMR (500 MHz, DMSO) δ 8.38 (s, 1H), 7.58 (m, 2H), 7.53 (s, 1H), 7.19

(m, 2H), 6.91 (d, *J* = 8.4 Hz, 2H), 4.13 (m, 1H), 3.67 (s, 8H), 1.71 (m, 2H), 1.31 (d, *J* = 6.7 Hz, 3H), 0.81 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 161.5, 151.2, 148.5, 134.9, 126.4, 122.9, 122.0, 116.2, 116.1, 53.8, 51.7, 46.4, 27.8, 19.2, 10.5. DART-HRMS: *m/z* calcd. for $C_{22}H_{28}N_5O_2$ $[MH]^+$, 394.2243; Found: 394.2216. IR (solid) *v*max 3331, 2958, 2875, 2832, 2807, 1688, 1509, 1448, 1385, 1222, 1181, 1152, 965, 824, 741, 729. $[\alpha]_D^{20}$ = +13.0 (c = 0.994, DMSO).

(*R*)-2-(*sec*-butyl)-4-(4-(4-(4-hydroxyphenyl)piperazin-1-yl)phenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (44). ^{1}H NMR (500 MHz, DMSO) δ 8.38 (s, 1H), 7.58 (d, *J* = 8.9 Hz, 2H), 7.48 (s, 1H), 7.19 (d, *J* = 8.9 Hz, 2H), 6.89 (m, 2H), 4.14 (m, 1H), 3.62 (s, 8H), 1.70 (m, 2H), 1.31 (d, *J* = 6.7 Hz, 3H), 0.82 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 157.2, 151.4, 148.6, 135.1, 126.4, 123.1, 122.1, 122.0, 116.4, 116.3, 53.9, 51.9, 46.6, 28.0, 19.3, 10.7. DART-HRMS: *m/z* calcd. for $C_{22}H_{28}N_5O_2$ $[MH]^+$, 394.2243; Found: 394.2212. IR (solid) *v*max: 3241, 3055, 2965, 2928, 2855, 1670, 1509, 1451, 1378, 1234, 1185, 1110, 967, 921, 832, 807, 746. $[\alpha]_D^{20}$ = -13.7 (c = 1.254, DMSO).

2-(sec-butyl)-4-(4-(4-(4-hydroxyphenyl)piperazin-1-yl)phenyl)-2,4-dihydro-3H-1,2,4-triazol-3-one (45). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (d, *J* = 0.6 Hz, 1H), 7.41 – 7.35 (m, 2H), 7.00 – 6.94 (m, 2H), 6.86 – 6.80 (m, 2H), 6.72 – 6.67 (m, 2H), 4.31 (m, 1H), 3.37 – 3.31 (m, 4H), 3.23 – 3.17 (m, 4H), 1.87 (m, 1H), 1.73 (m, 1H), 1.40 (d, *J* = 6.7 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.72, 151.21, 151.13, 134.68, 125.80, 124.64, 119.18, 116.91, 116.38, 53.32, 51.44, 49.53, 28.85, 19.64, 11.18. DART-HRMS: *m/z* calcd. for $C_{22}H_{27}N_5O_2$ $[MH]^+$, 394.2243; Found: 394.2221. IR (solid) *v*max: 3333, 3058, 2959, 2876, 2833, 2809, 1690, 1509, 1449, 1385, 1224, 1182, 1153, 965, 825, 739.

4-(4-(4-(4-hydroxyphenyl)piperazin-1-yl)phenyl)-2-propyl-2,4-dihydro-3H-1,2,4-triazol-3-one (46): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (s, 1H), 7.44 – 7.36 (m, 2H), 7.05 – 6.98 (m, 2H),

6.90 (d, *J* = 8.4 Hz, 2H), 6.80 – 6.73 (m, 2H), 3.82 (m, 2H), 3.37 (t, *J* = 5.0 Hz, 4H), 3.22 (m, 4H), 1.82 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.40, 150.78, 150.50, 134.14, 125.58, 124.03, 118.86, 116.60, 115.99, 51.03, 49.16, 47.33, 22.05, 11.12. DART-HRMS: *m/z* calcd. for $C_{21}H_{25}N_5O_2$ $[MH]^+$, 380.2087; Found: 380.2099. IR (solid) *v*max: 3344, 2969, 2934, 2893, 1466, 1378, 1365, 1185, 1160, 1127, 1104, 950, 815.

Dioxolane Region Intermediates.

1-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethan-1-one (53). A solution of 2-chloro-2',4'-dichlorophenylacetone (**49**) (10.0 g, 45.0 mmol), 1*H*-1,2,4-triazole (**50**) (6.2 g, 89.0 mmol), $NaHCO_3$ (6.1 g, 72.0 mmol), and toluene (100 mL) were heated to reflux (110-120°C) overnight. The reaction vessel was then placed in the -20°C freezer for ~6 h. The solid was filtered, dissolved in H_2O, and extracted with ethyl acetate (~50 mL x 3). The organic layer was collected, washed with brine, and dried over sodium sulfate. The solvent was evaporated and the remaining solid was washed with EtOAc/hexanes (1:1, 100 mL) resulting in a yellow solid (7.4 g, 64.2%). Characterization matched that previously reported.[2]

(2,2-dimethyl-1,3-dioxolan-4-yl)methyl 4-methylbenzenesulfonate (55). DL-1,2-isopropylideneglycerol (**54**) (25 g, 89 mmol) was added to 300 mL of DCM with molecular sieves. Pyridine (30.5 g, 378 mmol) was then added to the mixture. The solution was cooled to 0°C and TsCl (54 g, 283.5 mmol) was added portion wise over 30 mins. The reaction stirred overnight at room temperature. The solution was washed with pH 2.0 H_2O (6M HCl) and brine. The organic layer was collected and concentrated. The crude product was purified via column chromatography (SiO_2, 0 - 25% EtOAc in hexanes) yielding a white solid in quantitative yield. Characterization matched that previously reported.[3]

2,3-dihydroxypropyl 4-methylbenzenesulfonate (56). Tosylated dioxolane **55** (9.3 g) was dissolved in 300 mL of MeOH and 30 mL of 0.5N HCl was added to the solution. The reaction was stirred and heated to reflux for 5h. The reaction was cooled to room temperature and neutralized to pH 7 with dropwise addition of saturated $NaHCO_3$. The solution was extracted (~150 mL x 1) with ethyl acetate and then washed with brine. The organic layers were collected, dried over sodium sulfate, filtered, and concentrated (7.7 g, 89.0%). Characterization matched that previously reported.[4]

(2-((1*H*-1, 2,4-triazol-1-yl) methyl)-2-(2,4-dichlorophenyl)-1,3-dioxolan-4-yl) methyl 4-methylbenzenesulfonate (59). Ketone **53** (2.0 g, 7.8 mmol) and **56** (1.98 g, 8.03 mmol) were added to a dry round bottom flask. Anhydrous toluene (15 mL) was added to the reaction. The reaction was cooled to 0°C and TfOH (2.8 mL, 31.9 mmol) was added dropwise with a glass syringe. The reaction was stirred at room temperature for 60 h. The mixture was diluted with 50 mL of ethyl acetate and slowly dropped into a solution of K_2CO_3 (5 g) in water (40 mL). The organic layer was separated with ethyl acetate (50 mL x 3). The organic layers were combined, dried over sodium sulfate, filtered, and concentrated to ~70 mL EtOAc. A solution of TsOH monohydrate (2.0 g) in EtOAc (13 mL) was slowly added at room temperature. The product precipitated as the salt (3.6 g, 70.3%). Characterization matched that previously reported.[5]

(*S*)-2,3-dihydroxypropyl 4-methylbenzenesulfonate (62). (*S*)-(+)-1,2-isopropylideneglycerol was tosylated following the above procedure for **55** and was then subjected to the acid-mediated hydrolysis procedure for **56**. Characterization matched that previously reported.

(*R*)-2,3-dihydroxypropyl 4-methylbenzenesulfonate (63). (*R*)-(-)-1,2-isopropylideneglycerol was tosylated following the above procedure for **55** and was then subjected to the acid-mediated hydrolysis procedure for **56**. Characterization matched that previously reported.

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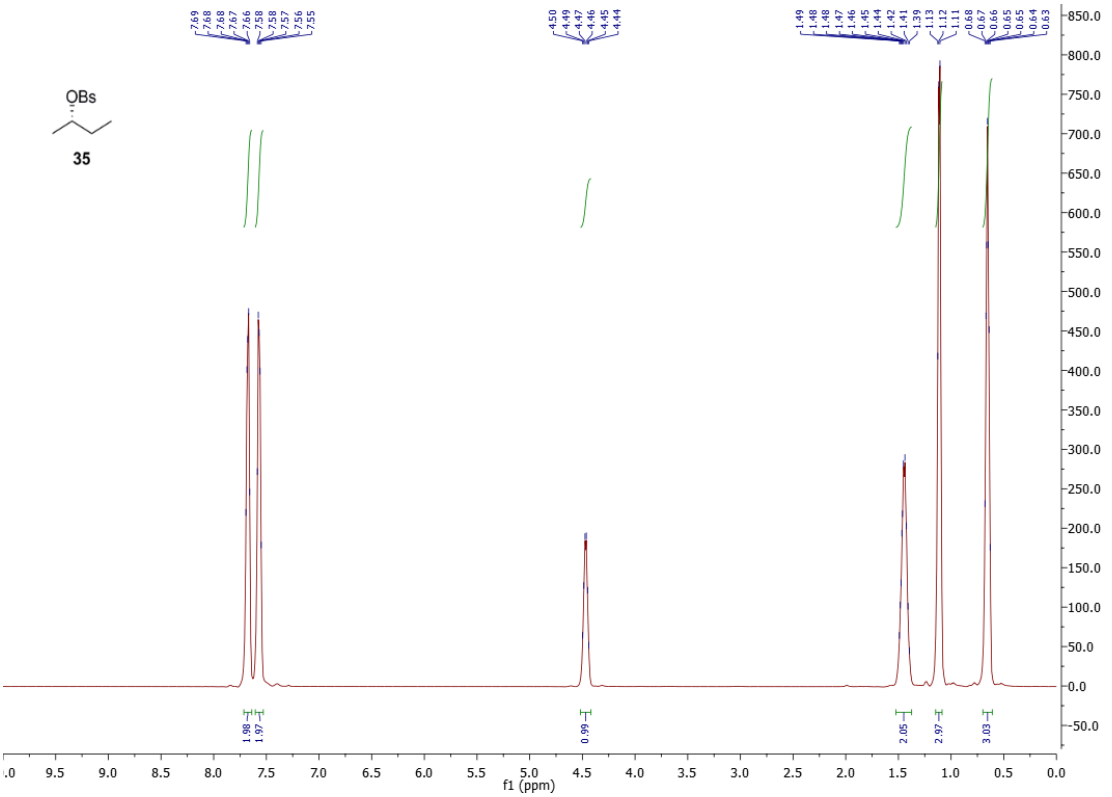
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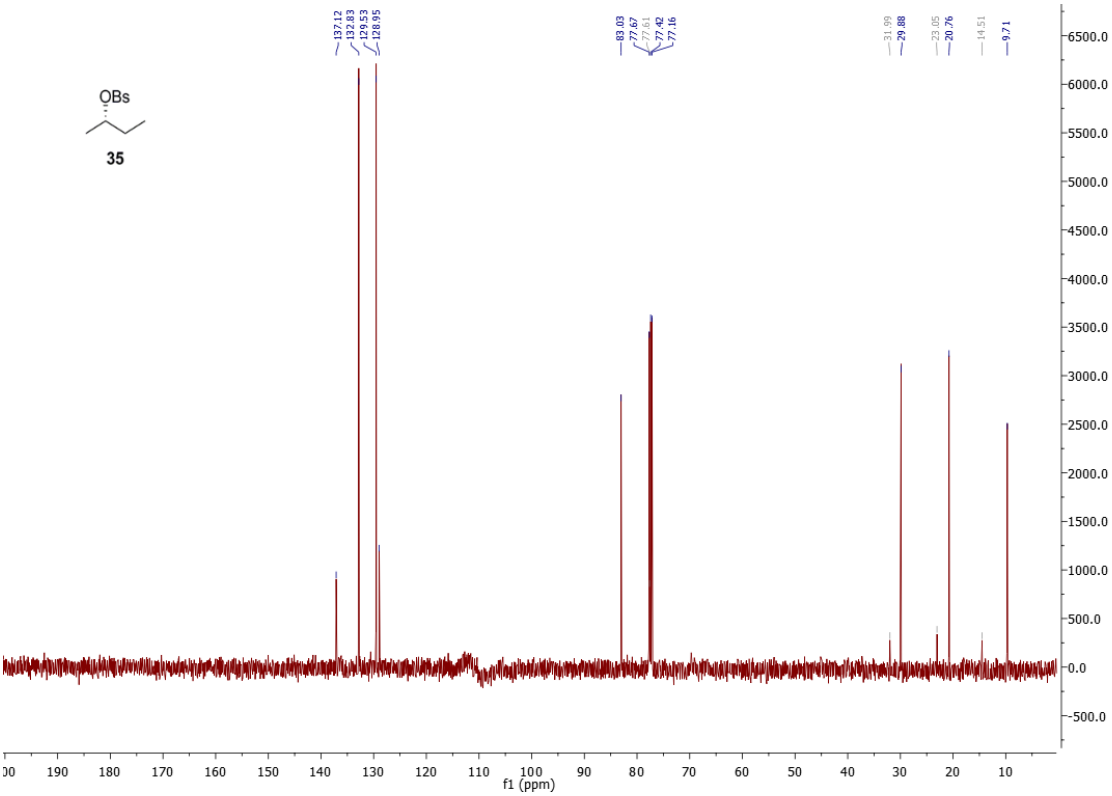
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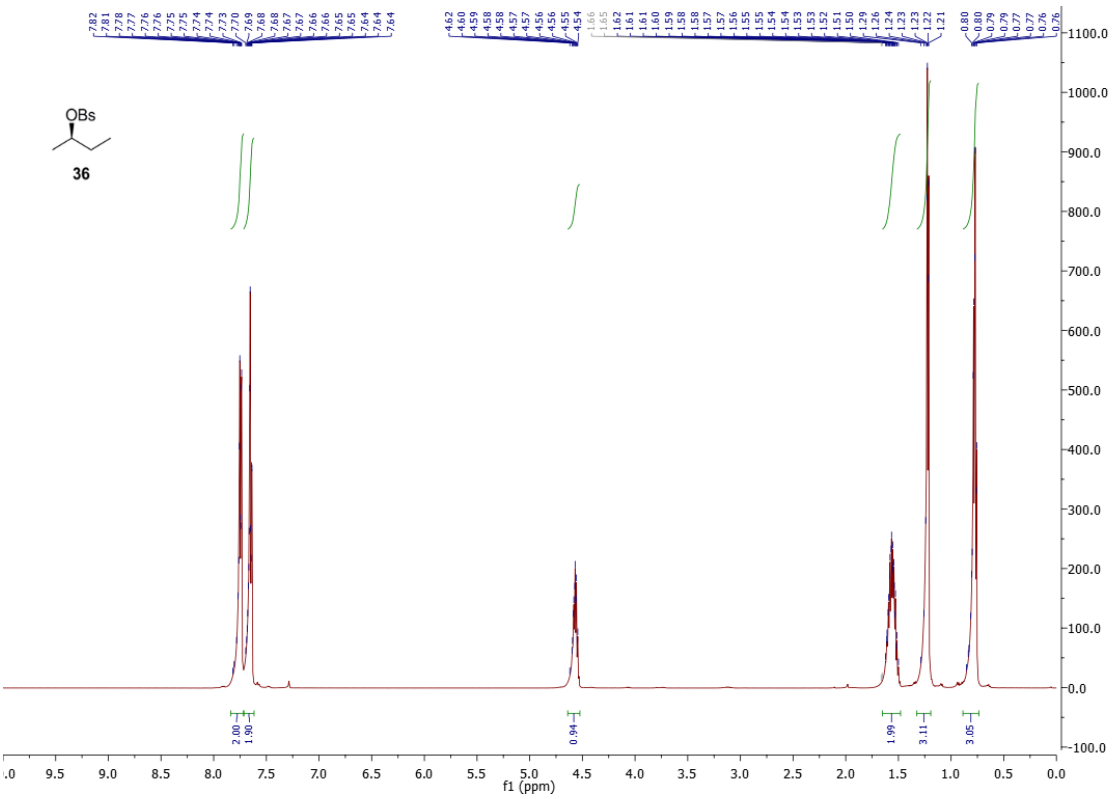
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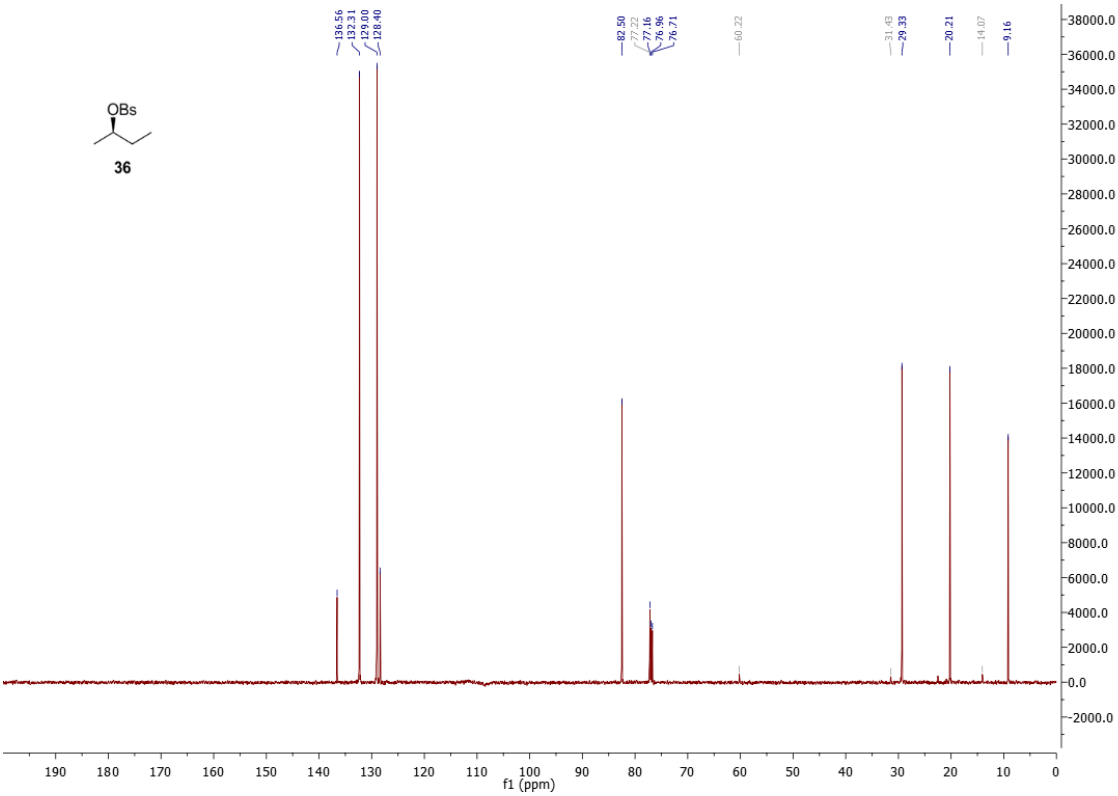
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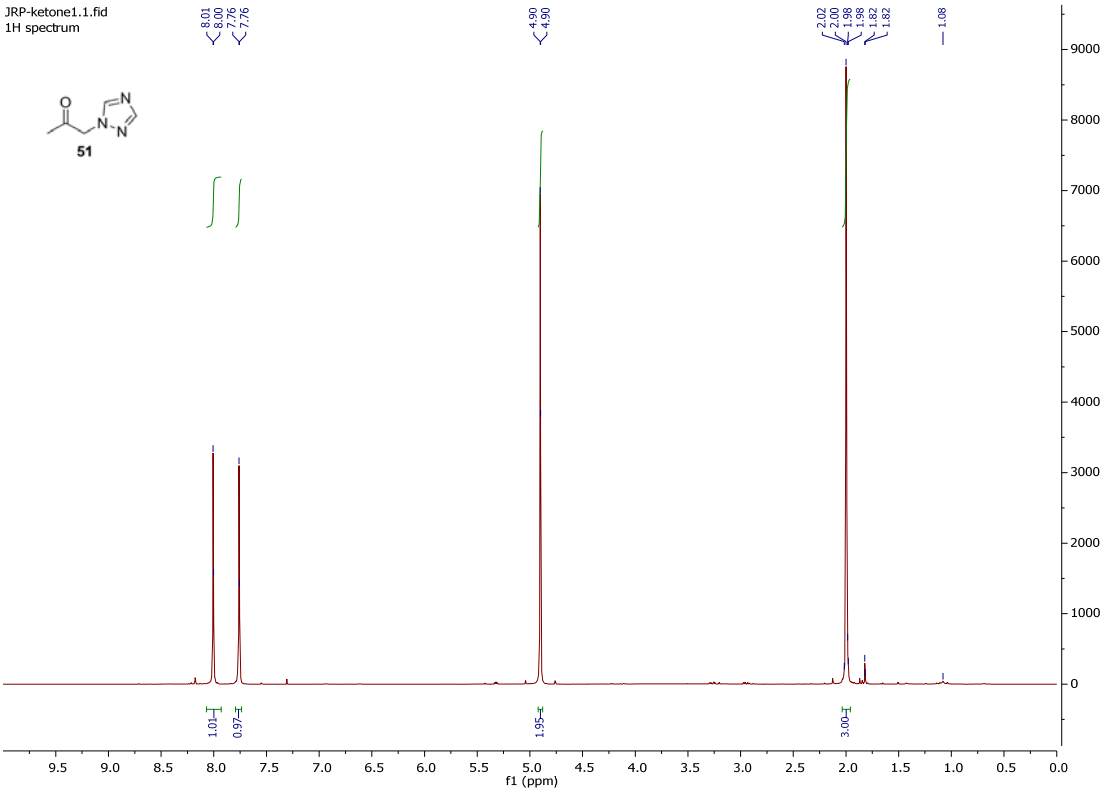
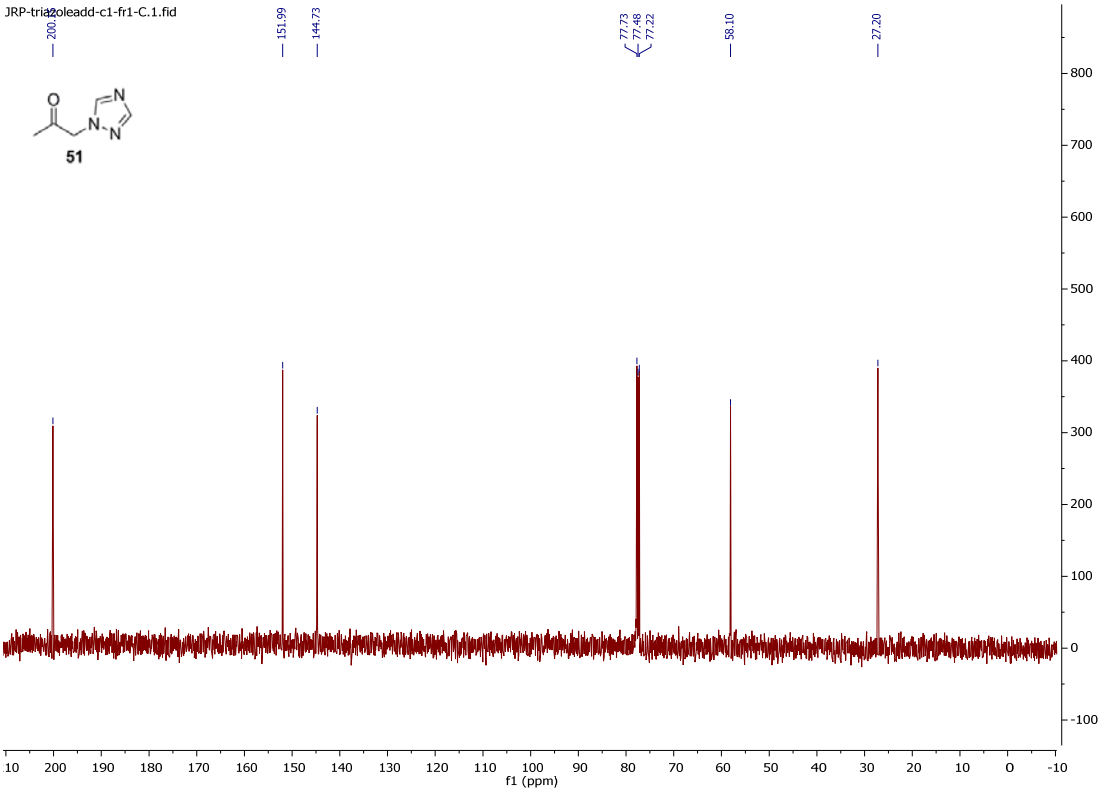
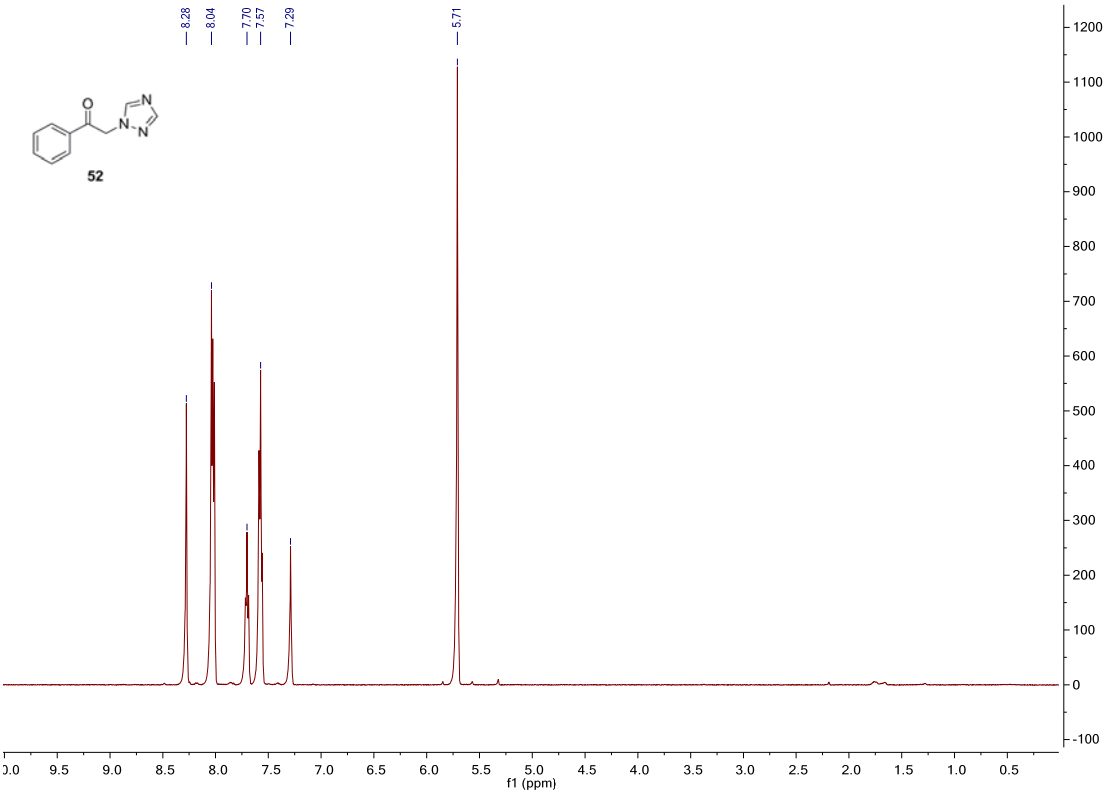
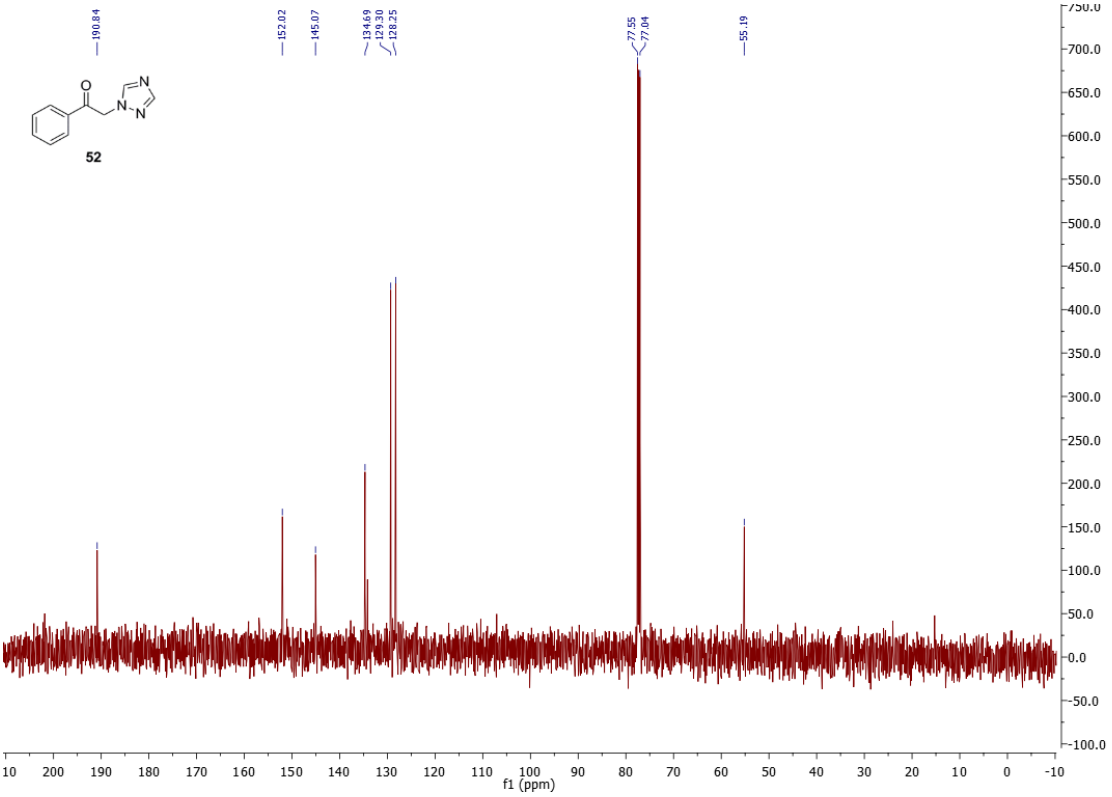
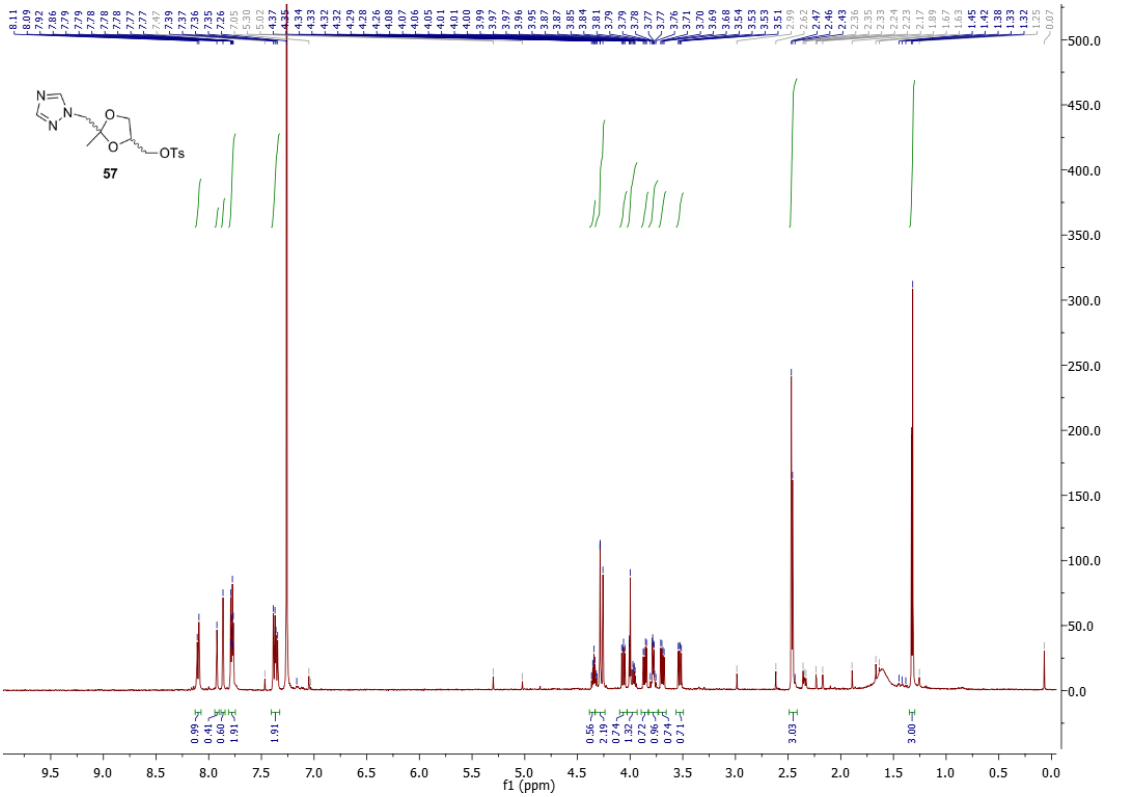
36
f1 (ppm)

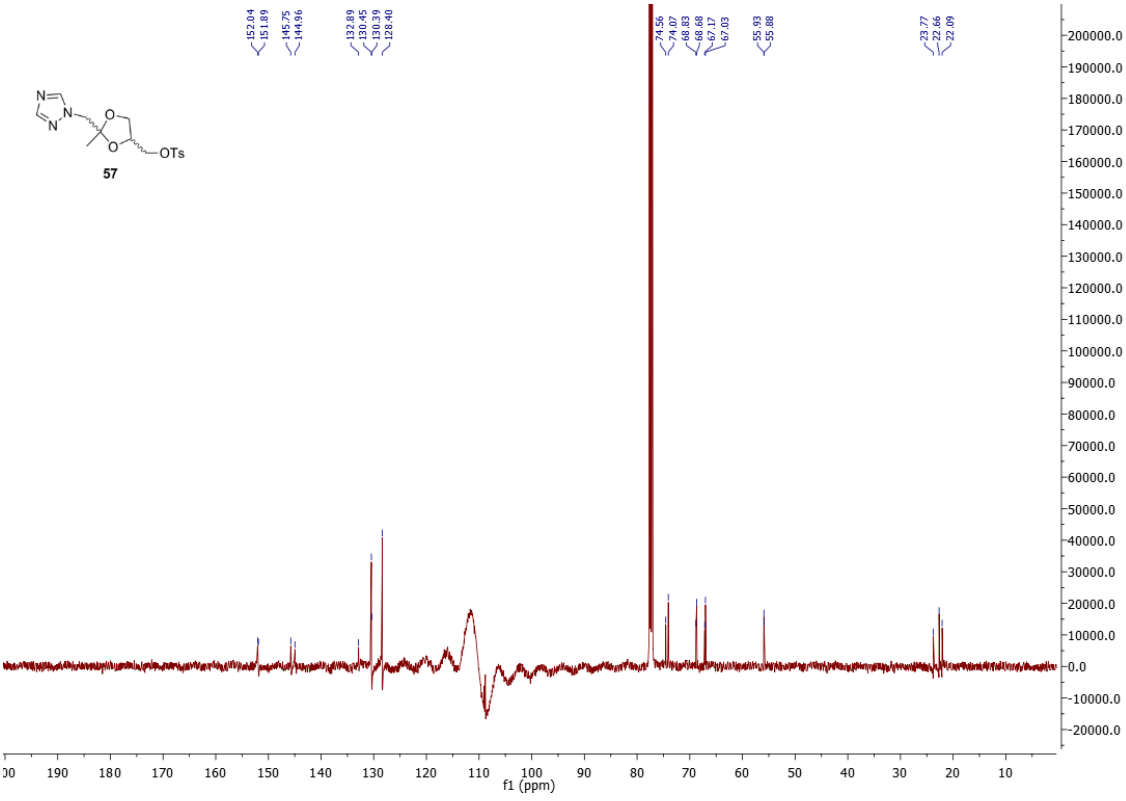

JRP-ketone1.1.fid
1H spectrum
51
f1 (ppm)

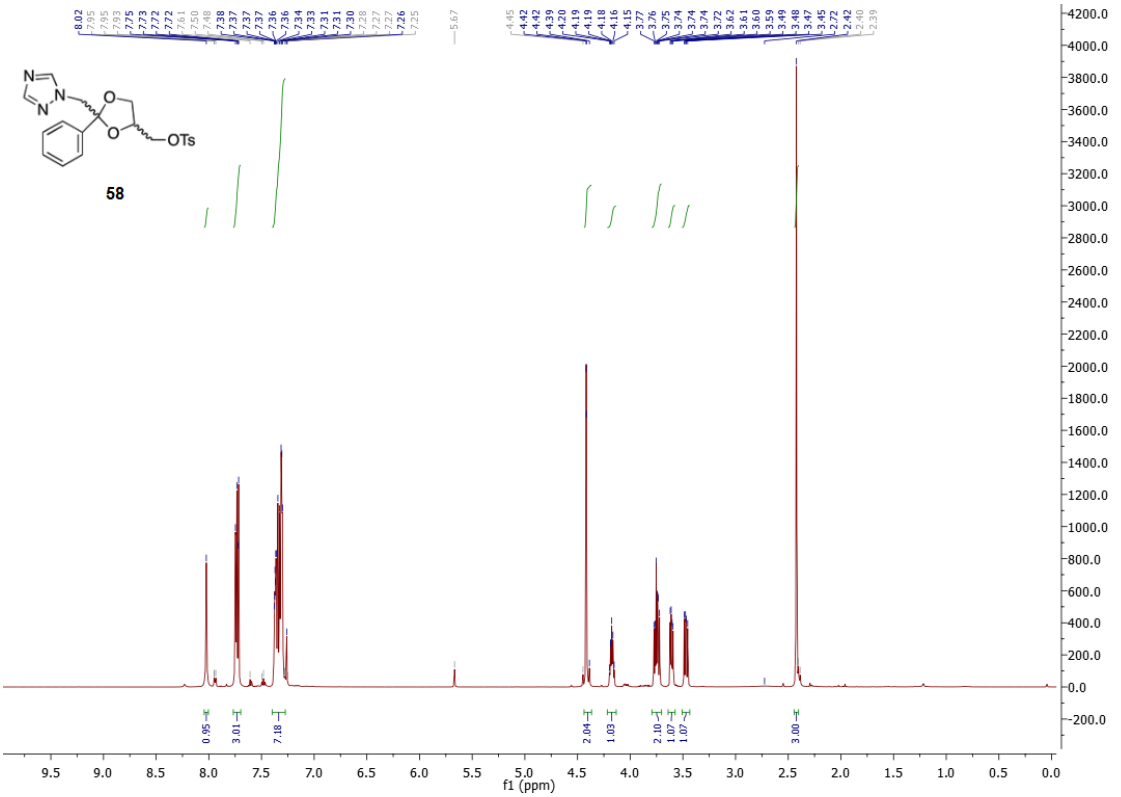

JRP-triazoleadd-c1-fr1-C.1.fid
51
f1 (ppm)

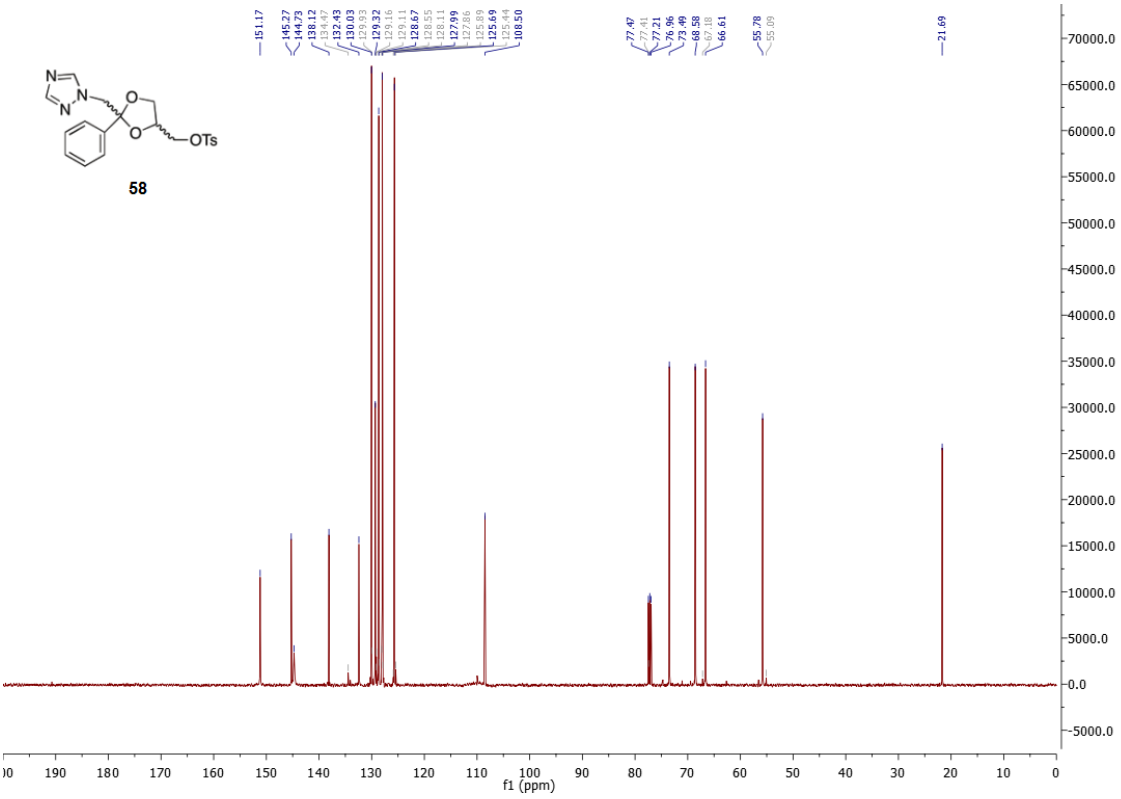

8.28
8.04
7.70
7.57
7.29
5.71
52
f1 (ppm)

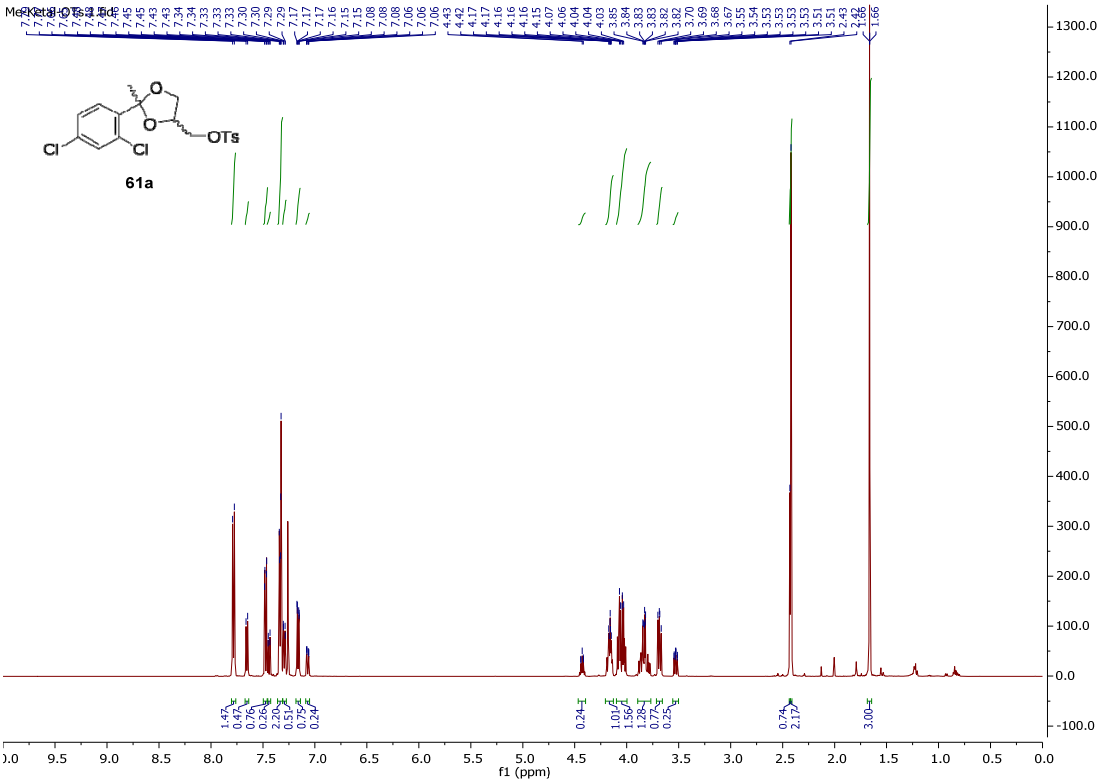

190.84
152.02
145.07
134.69
129.30
128.25
77.55
77.04
55.19
52
f1 (ppm)

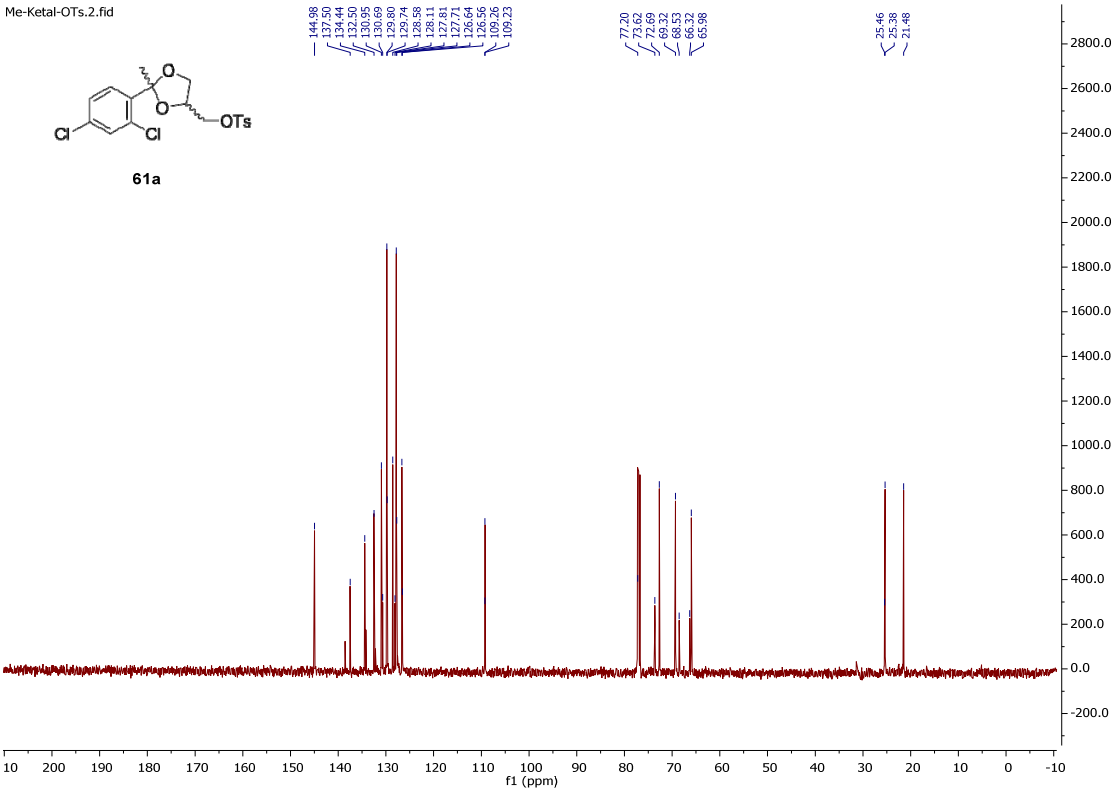

OTs
57
f1 (ppm)

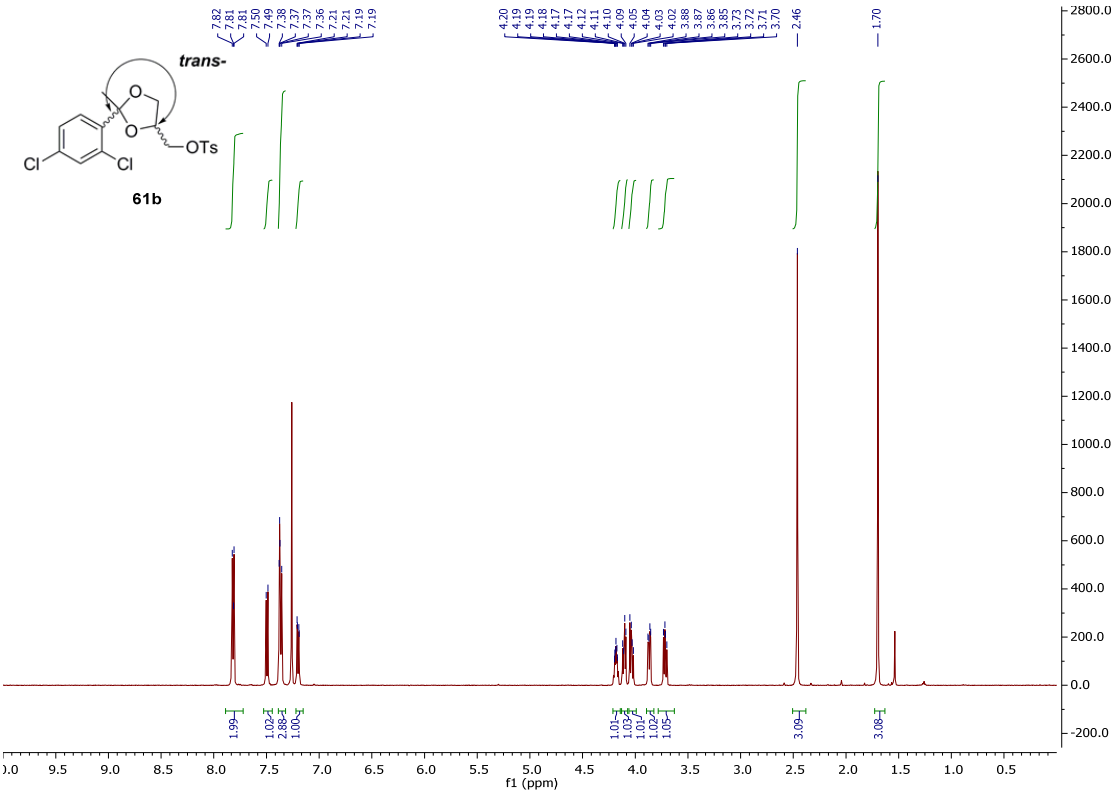

OTs
57
f1 (ppm)

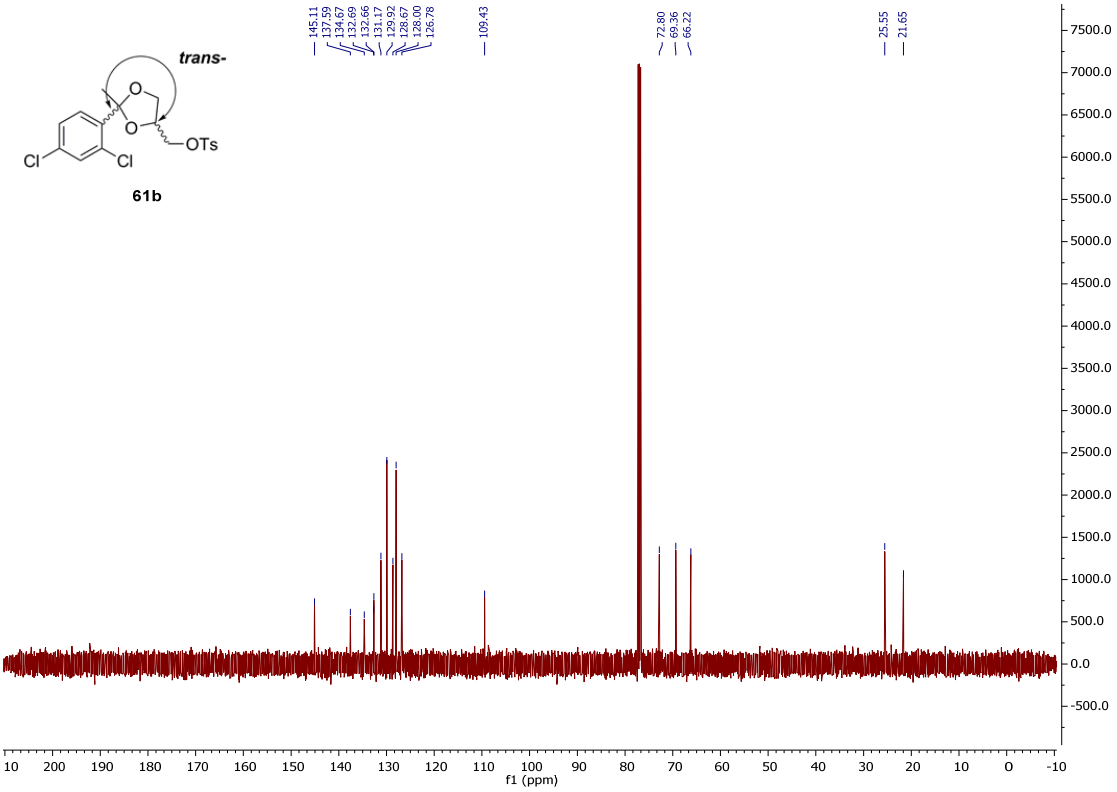

OTs
58
f1 (ppm)

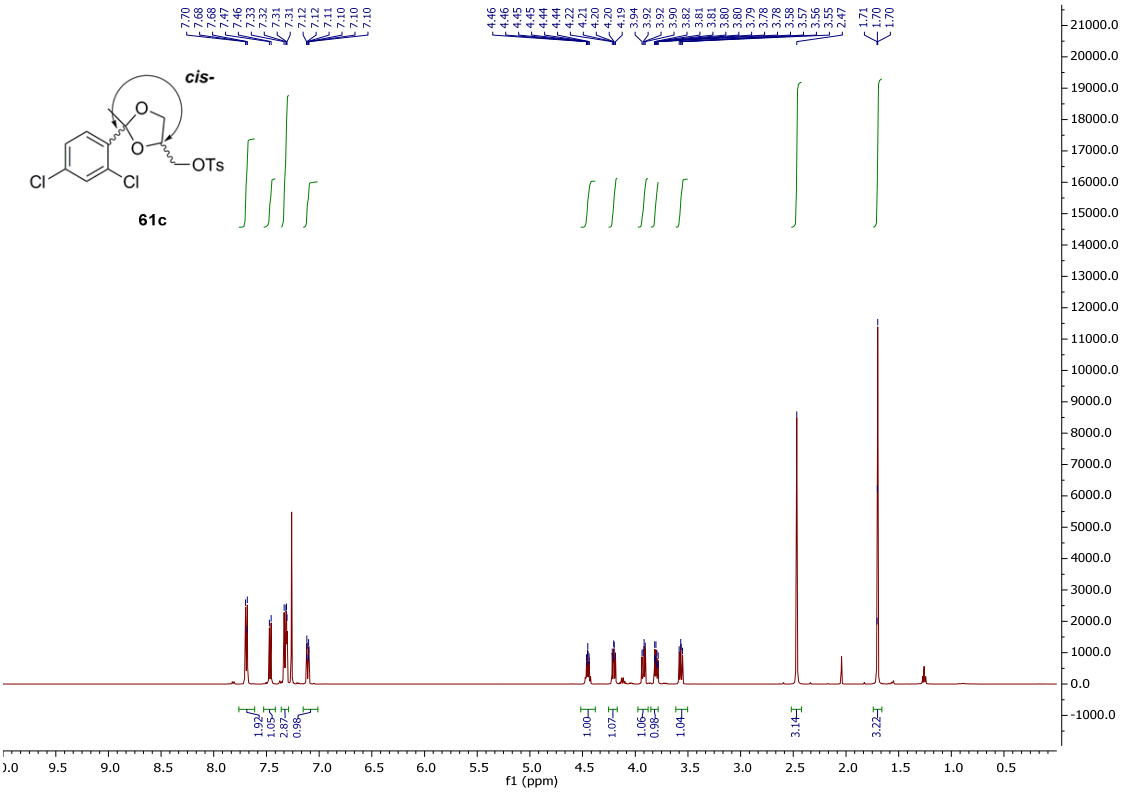

OTs
58
f1 (ppm)

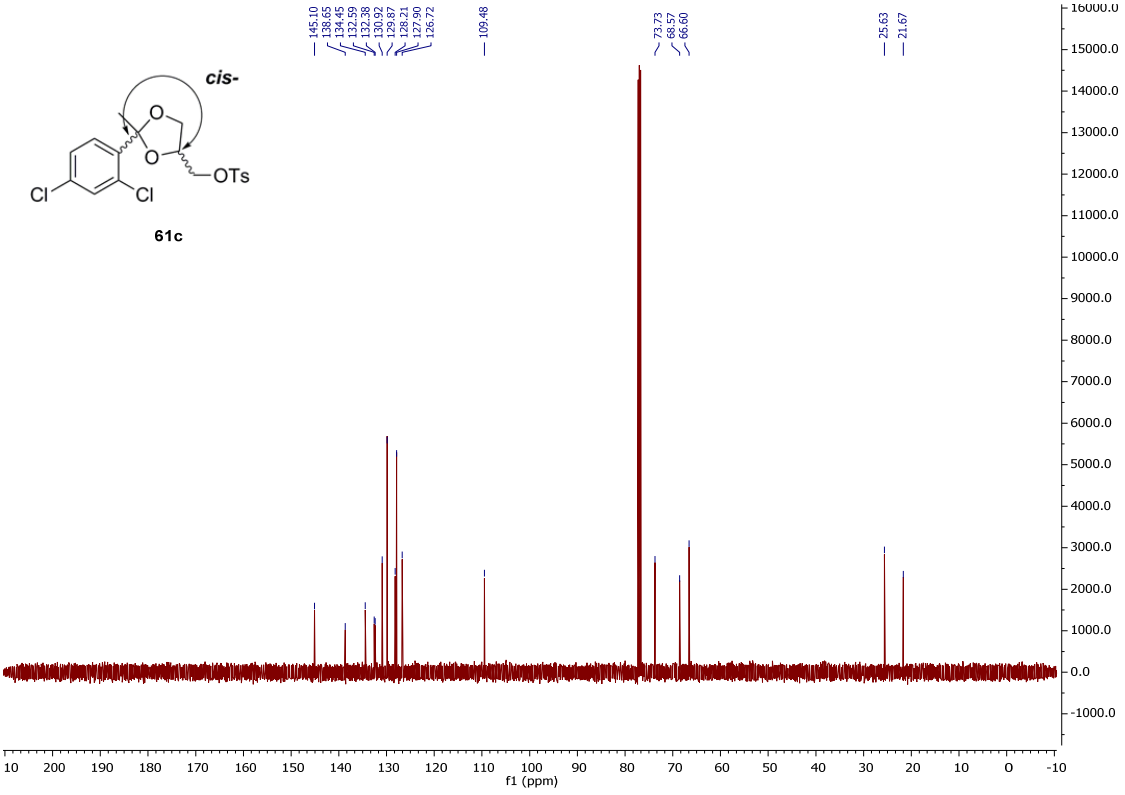

Me-Ketal-OTs.1.fid
OTs
Cl
Cl
61a
f1 (ppm)

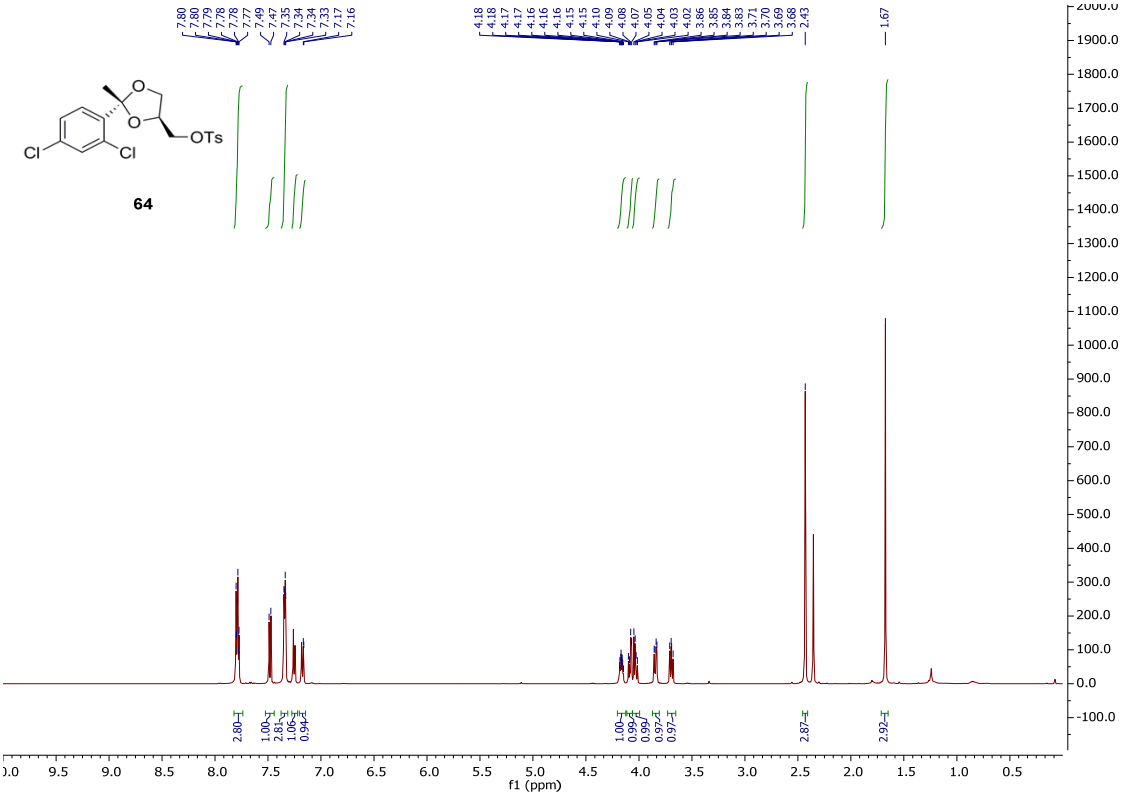

Me-Ketal-OTs.2.fid
OTs
Cl
Cl
61a
f1 (ppm)

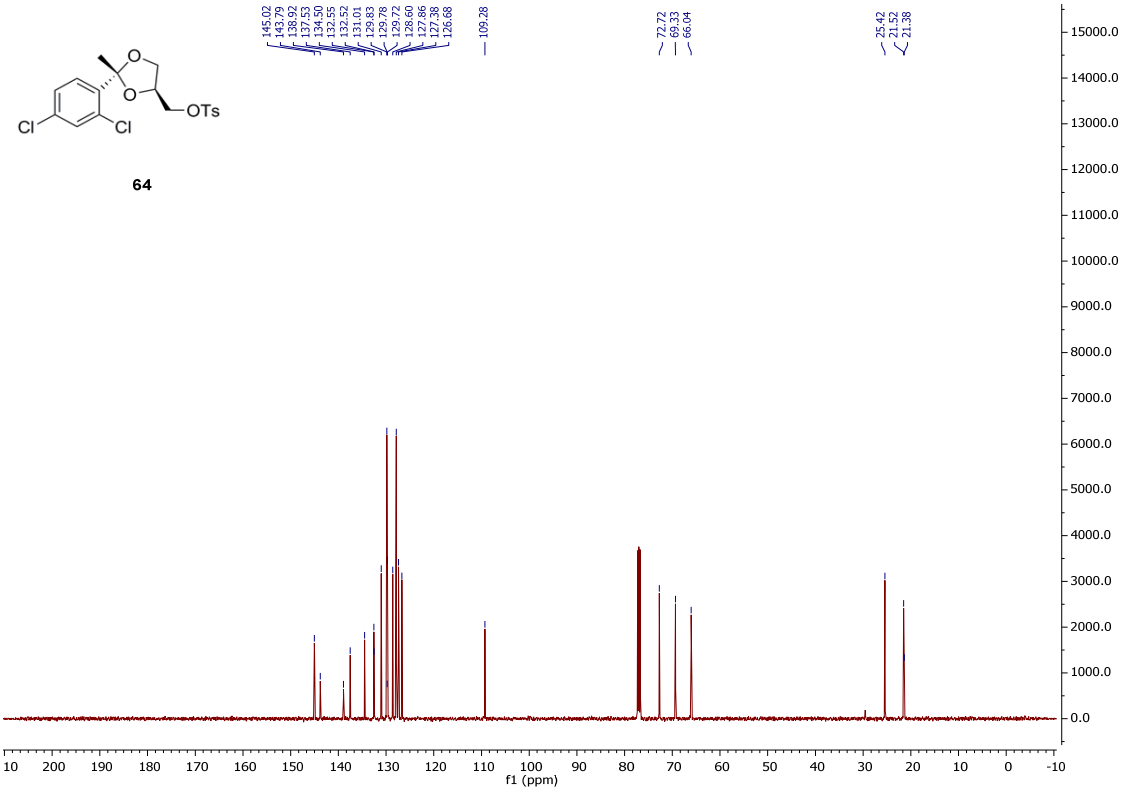

trans-
OTs
Cl
Cl
61b
7.82
7.81
7.81
7.50
7.49
7.38
7.37
7.37
7.36
7.21
7.21
7.19
7.19
4.20
4.19
4.19
4.18
4.17
4.17
4.12
4.11
4.10
4.09
4.05
4.04
4.03
4.02
3.88
3.87
3.86
3.85
3.73
3.72
3.71
3.70
2.46
1.70
1.99
1.02
2.88
1.00
1.01
1.03
1.01
1.02
1.05
3.09
3.08
f1 (ppm)

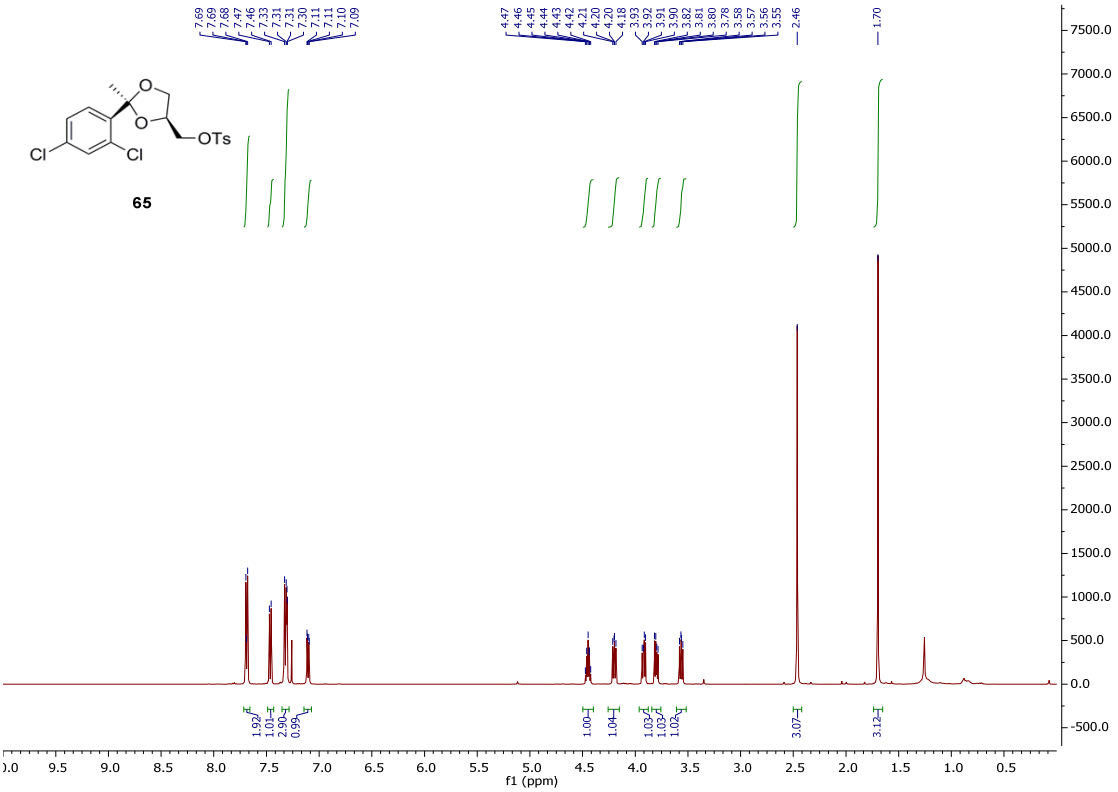

trans-
OTs
Cl
Cl
61b
145.11
137.59
134.67
132.69
132.66
131.17
129.92
128.67
128.00
126.78
109.43
72.80
69.36
66.22
25.55
21.65
f1 (ppm)

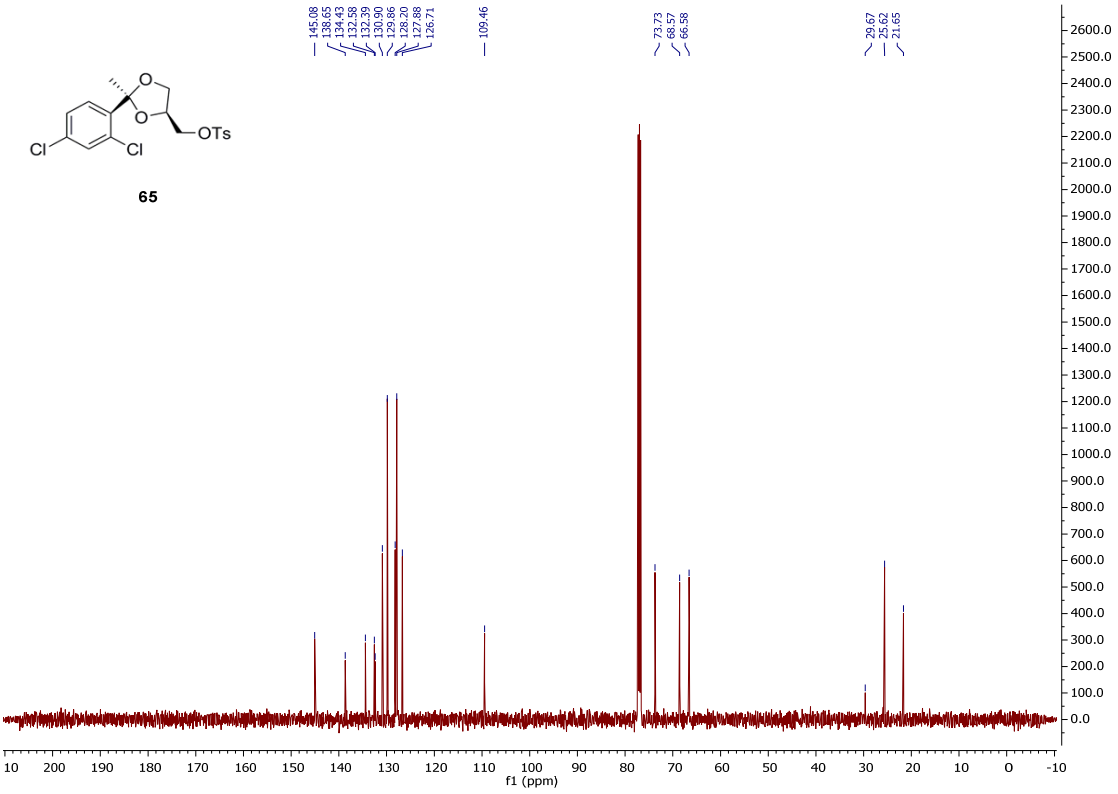

cis-
OTs
Cl
Cl
61c
f1 (ppm)

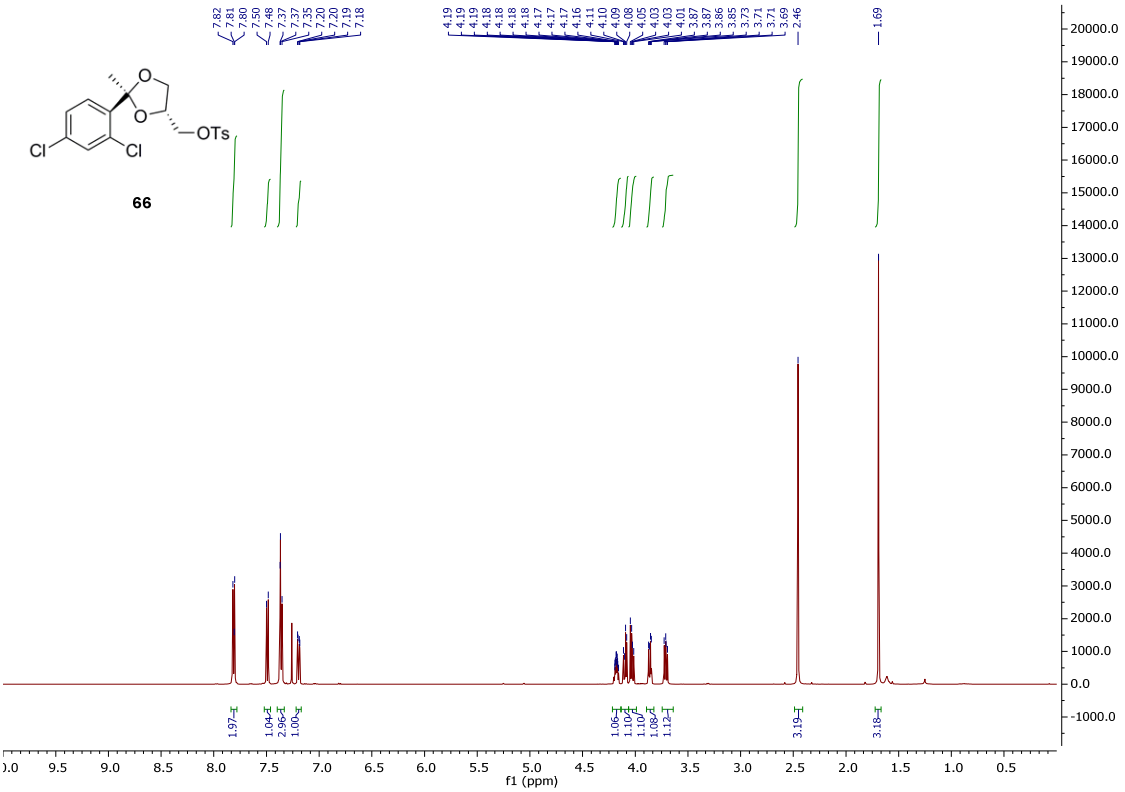

cis-
OTs
Cl
Cl
61c
f1 (ppm)

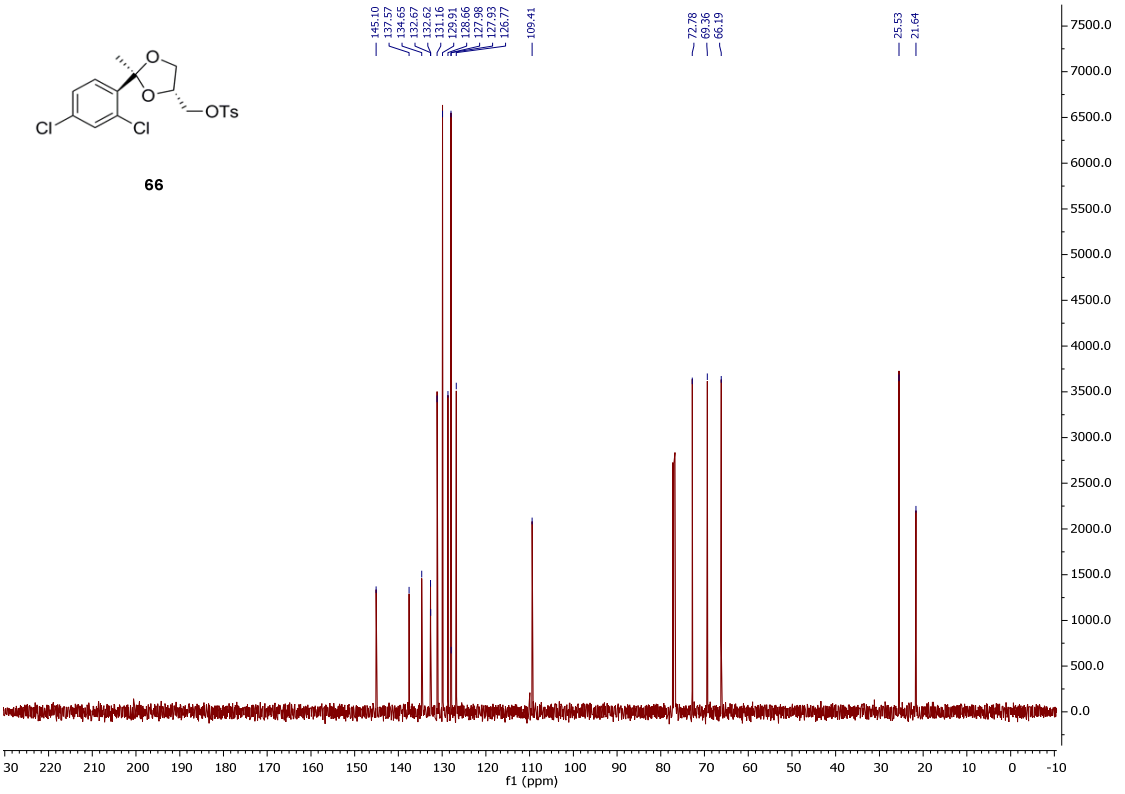

64
OTs
Cl
Cl
f1 (ppm)


64
OTs
Cl
Cl
f1 (ppm)

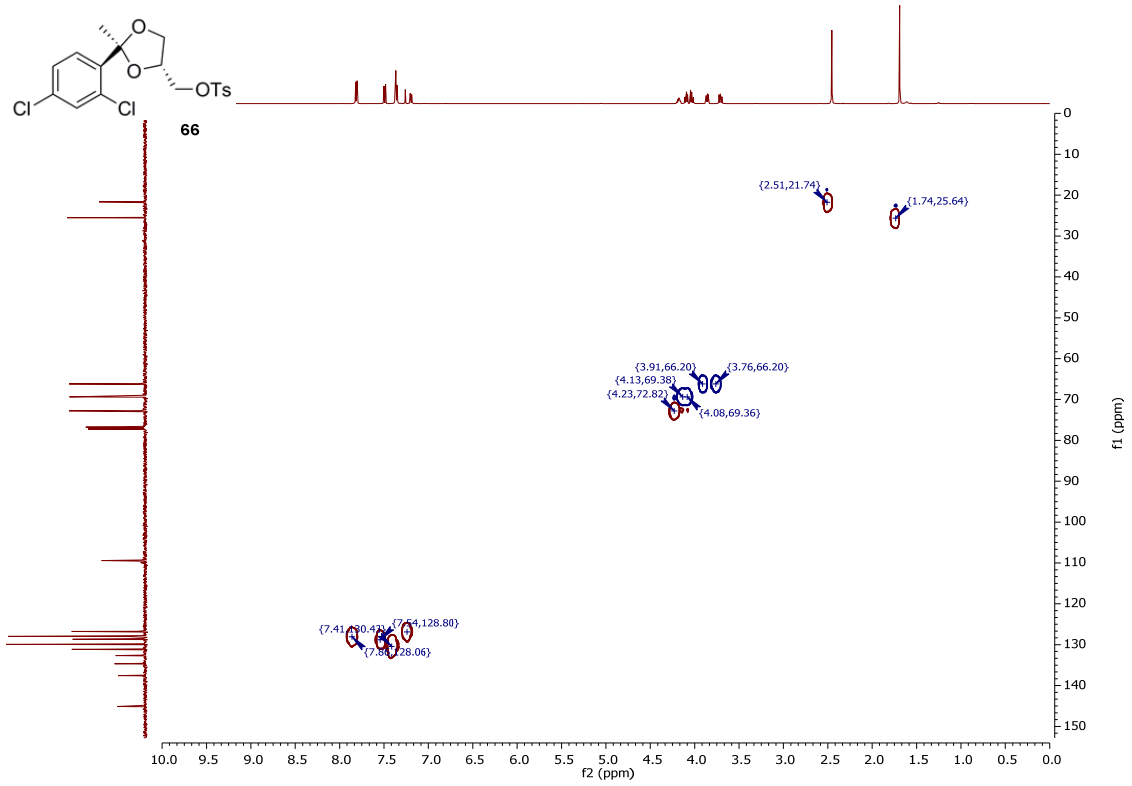

7.69
7.69
7.68
7.47
7.46
7.33
7.31
7.31
7.30
7.11
7.10
7.09
4.47
4.46
4.45
4.44
4.43
4.42
4.21
4.20
4.20
4.18
3.93
3.92
3.91
3.90
3.82
3.81
3.80
3.78
3.58
3.57
3.56
3.55
2.46
1.70
O
O
OTs
Cl
Cl
65
1.92
1.01
2.90
0.99
1.00
1.04
1.03
1.03
1.02
3.07
3.12
f1 (ppm)


145.08
138.65
134.43
132.58
132.39
130.90
129.86
128.20
127.88
126.71
109.46
73.73
68.57
66.58
29.67
25.62
21.65
O
O
OTs
Cl
Cl
65
f1 (ppm)

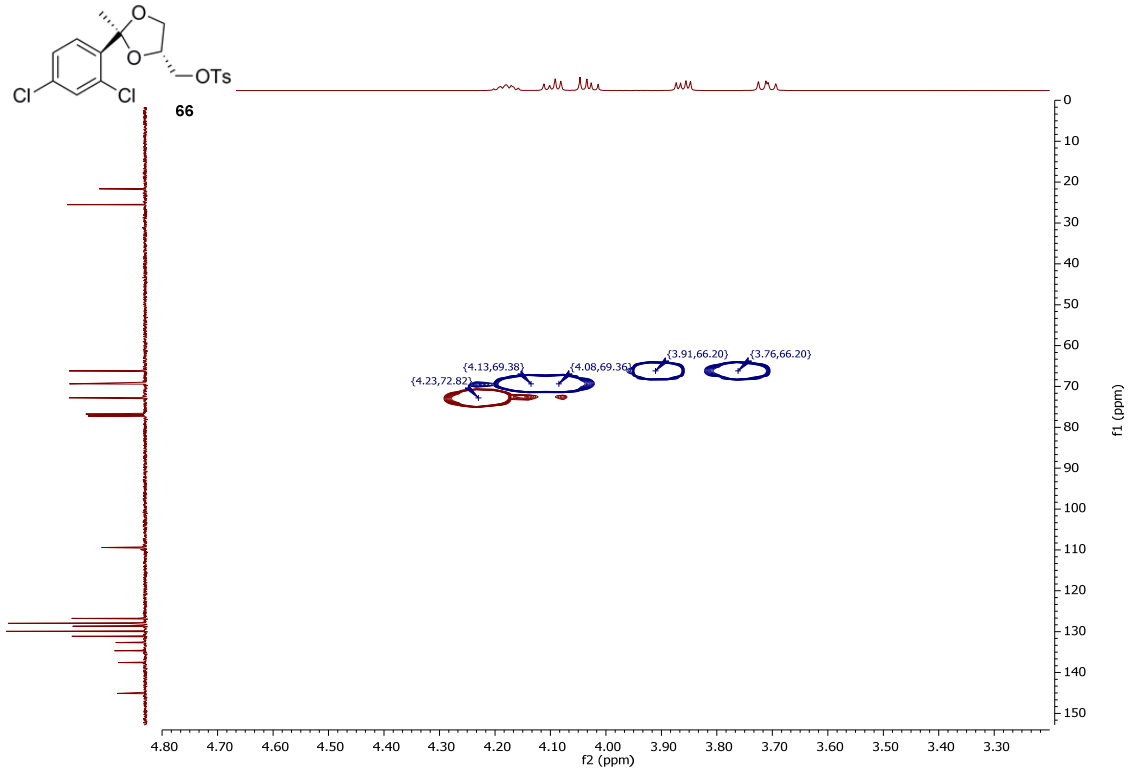

OTs
Cl
Cl
66
f1 (ppm)


OTs
Cl
Cl
66
f1 (ppm)

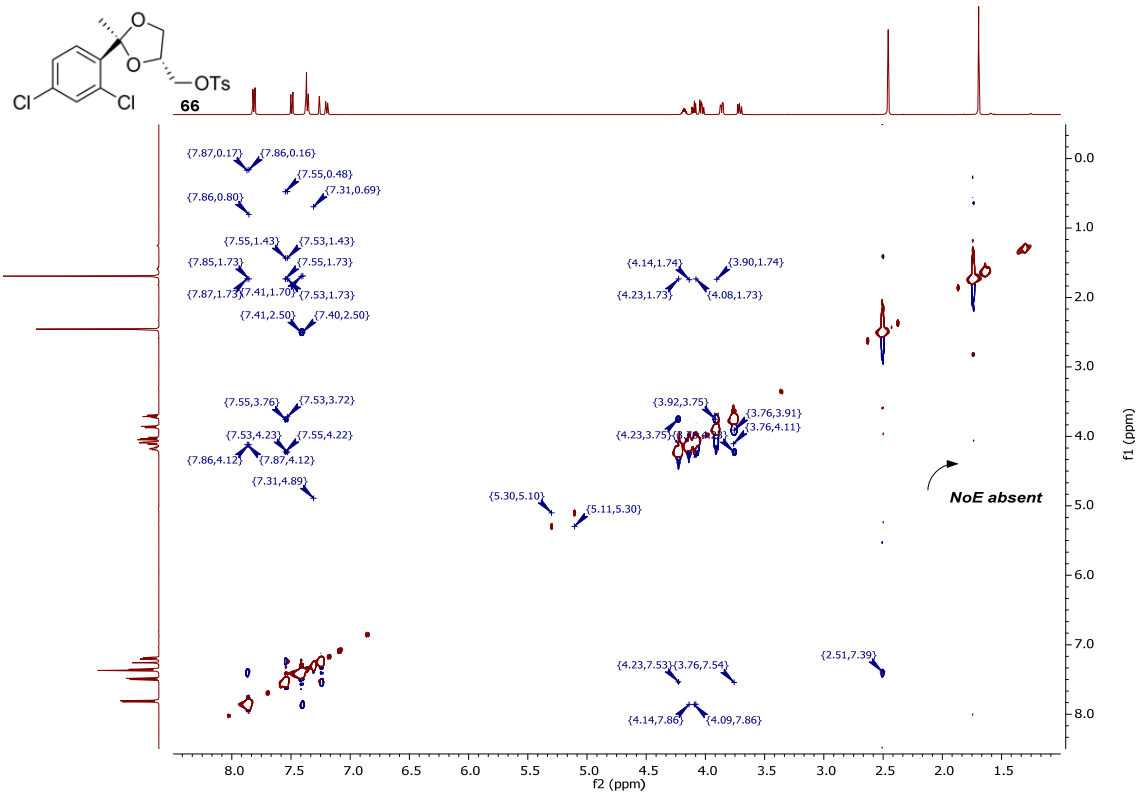
HSQC:


OTs
Cl
Cl
66
{2.51,21.74}
{1.74,25.64}
{3.91,66.20}
{3.76,66.20}
{4.13,69.38}
{4.23,72.82}
{4.08,69.36}
{7.54,128.80}
f2 (ppm)
f1 (ppm)

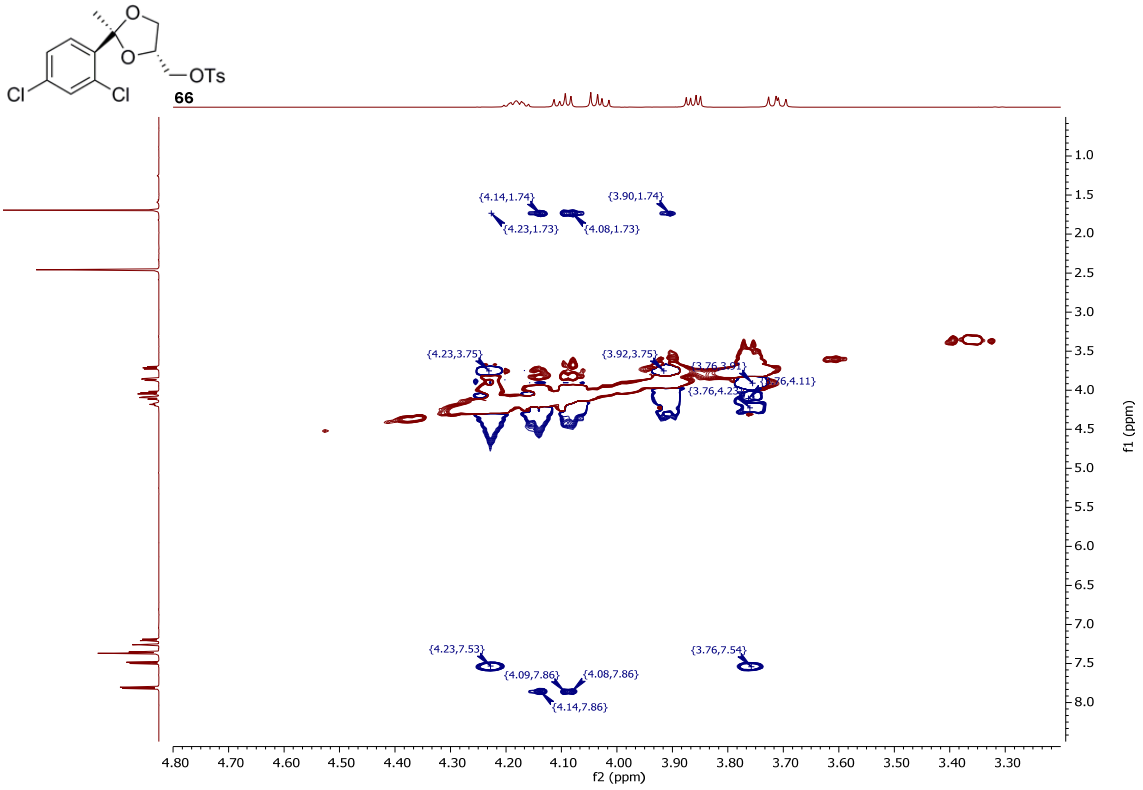
HSQC zoomed (3.2 to 4.8ppm): (1) H_{C4} (methine at C4 of dioxolane) direct correlation via HSQC experiment: 4.23→72.82ppm. (2) Remaining 1H nuclei in the expanded region below are methylene, correlating to the diastereomeric pairs at C5 of dioxolane and α-carbon to tosyl group (not specified: 3.76→66.20ppm; 3.91→66.20ppm; 4.08→69.37ppm; 4.13→69.37ppm).



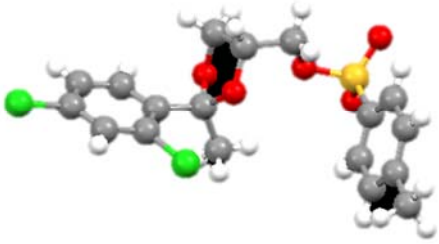
NOESY:

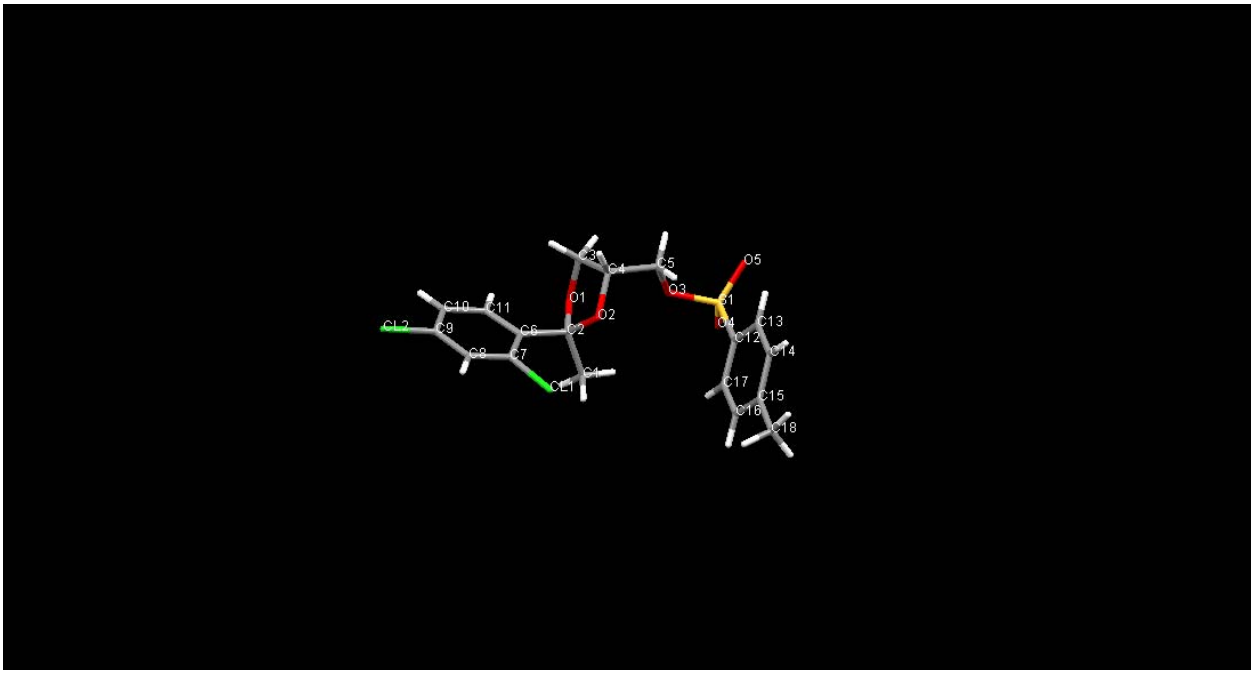

OTs
66
Cl
Cl
{7.87,0.17}
{7.86,0.16}
{7.55,0.48}
{7.31,0.69}
{7.86,0.80}
{7.55,1.43}
{7.53,1.43}
{7.85,1.73}
{7.55,1.73}
{7.87,1.73}
{7.41,1.70}
{7.53,1.73}
{7.41,2.50}
{7.40,2.50}
{4.14,1.74}
{3.90,1.74}
{4.23,1.73}
{4.08,1.73}
{7.55,3.76}
{7.53,3.72}
{7.53,4.23}
{7.55,4.22}
{7.86,4.12}
{7.87,4.12}
{7.31,4.89}
{3.92,3.75}
{3.76,3.91}
{4.23,3.75}
{3.76,4.11}
{5.30,5.10}
{5.11,5.30}
NoE absent
{4.23,7.53}
{3.76,7.54}
{2.51,7.39}
{4.14,7.86}
{4.09,7.86}
f2 (ppm)
f1 (ppm)

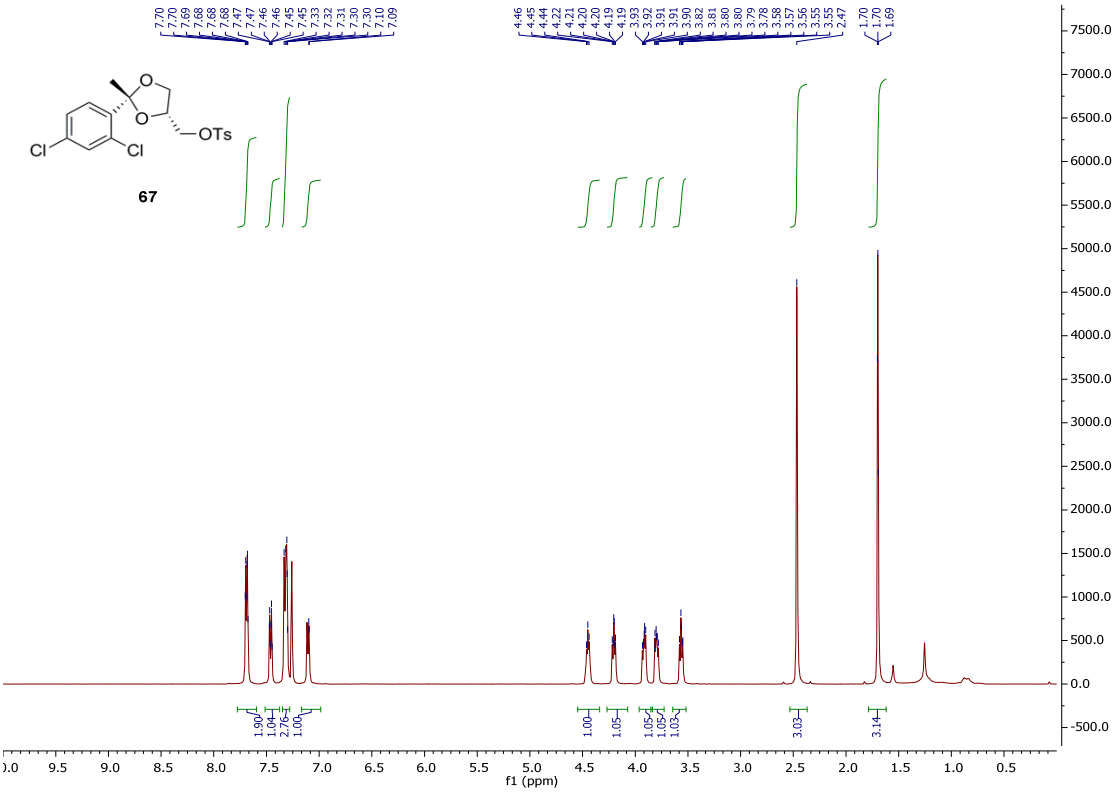
NOESY zoomed (3.2 to 4.8ppm): (1) H_{C4} (methine at C4 of dioxolane) very weak to no NoE signal with methyl at C2 of dioxolane (2,4-correlation) in experiment: 4.23→1.73ppm.

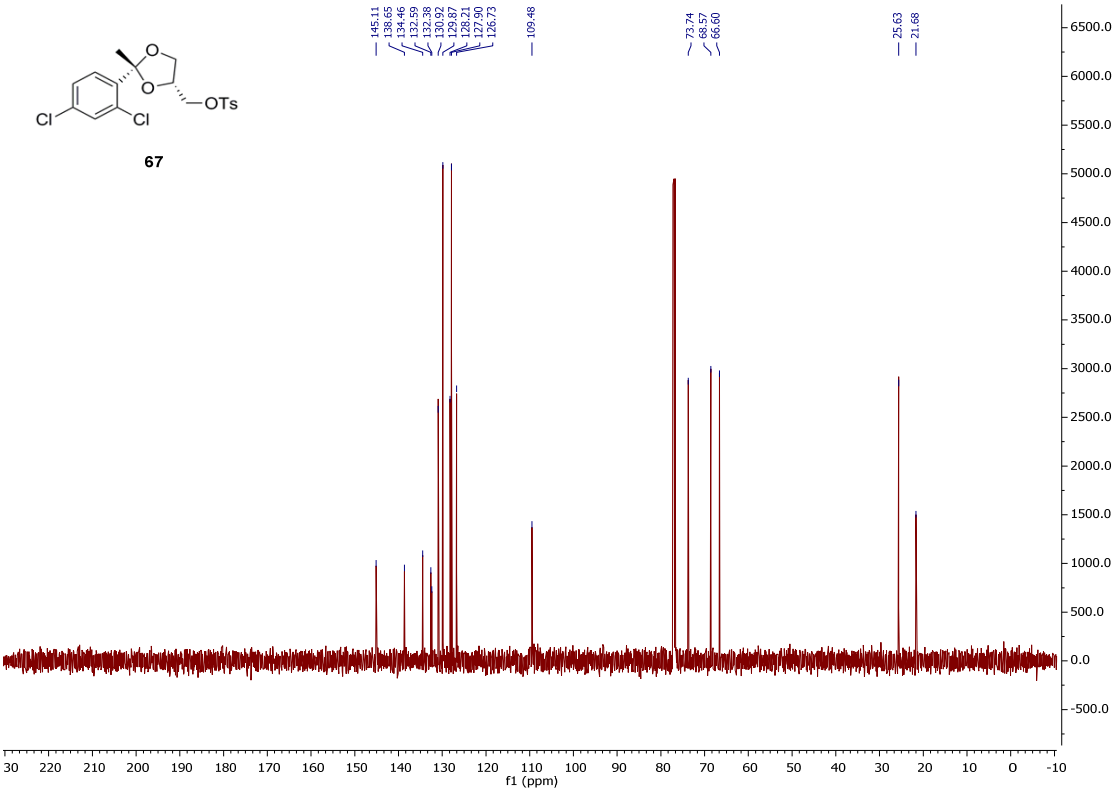


X-RAY (**66**):

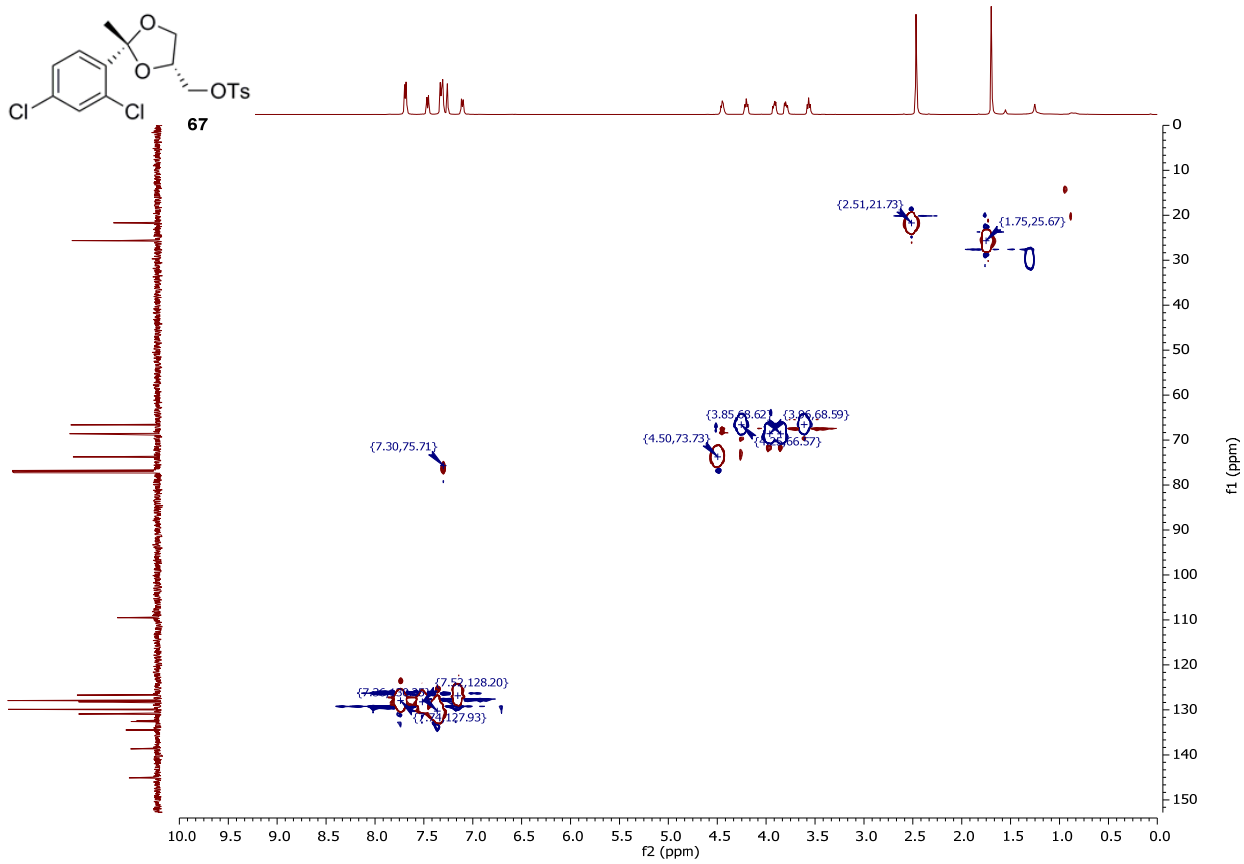



C3
C4
C5
O5
O1
O3
S1
O2
C10
C11
O4
C13
CL2
C9
C6
C2
C12
C14
C8
C7
CL1
C1
C17
C15
C16
C18

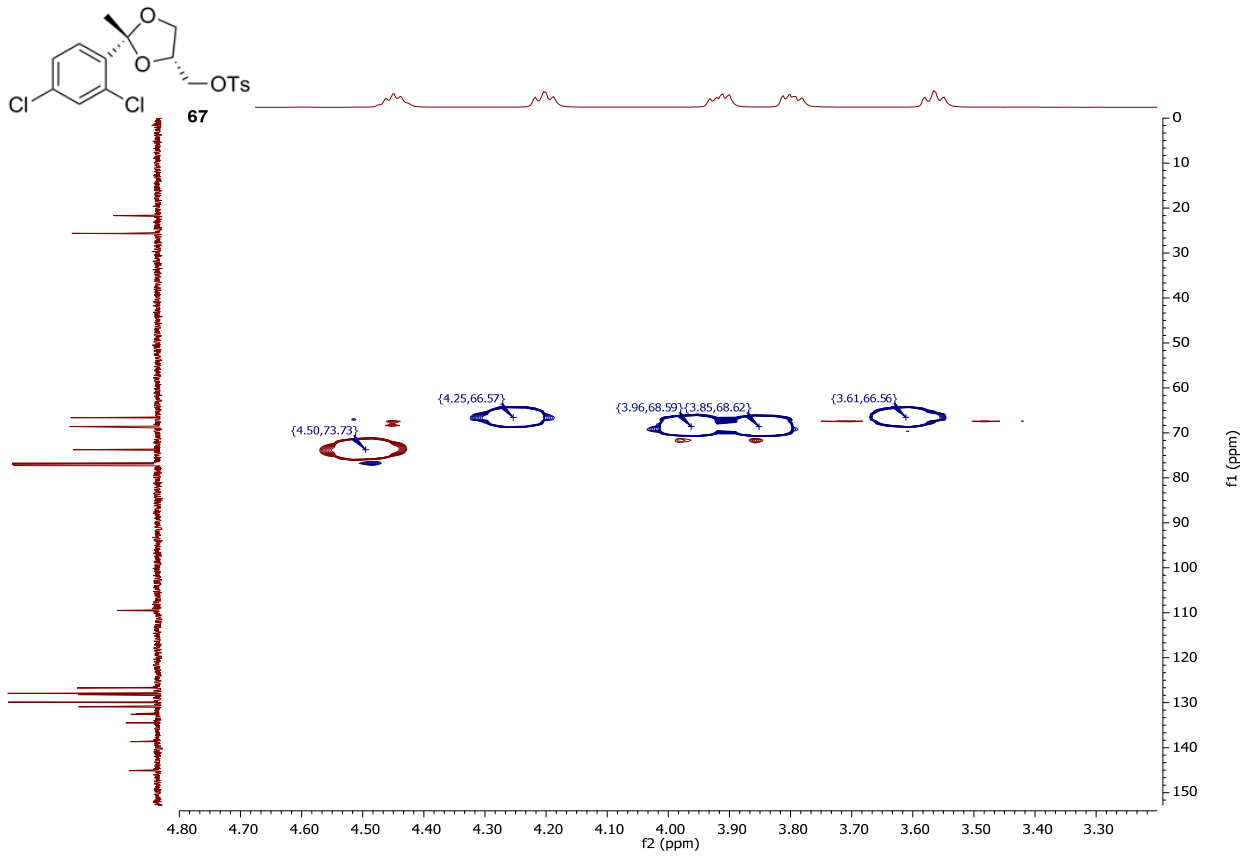

7.70
7.70
7.69
7.68
7.68
7.68
7.47
7.47
7.46
7.46
7.45
7.45
7.33
7.32
7.31
7.30
7.30
7.10
7.09
4.46
4.45
4.44
4.22
4.21
4.20
4.20
4.19
4.19
3.93
3.92
3.91
3.91
3.90
3.82
3.81
3.80
3.80
3.79
3.78
3.58
3.57
3.56
3.55
3.55
2.47
1.70
1.70
1.69
OTs
Cl
Cl
67
1.90
1.04
2.76
1.00
1.00
1.05
1.05
1.05
1.03
3.03
3.14
f1 (ppm)


145.11
138.65
134.46
132.59
132.38
130.92
129.87
128.21
127.90
126.73
109.48
73.74
68.57
66.60
25.63
21.68
OTs
Cl
Cl
67
f1 (ppm)

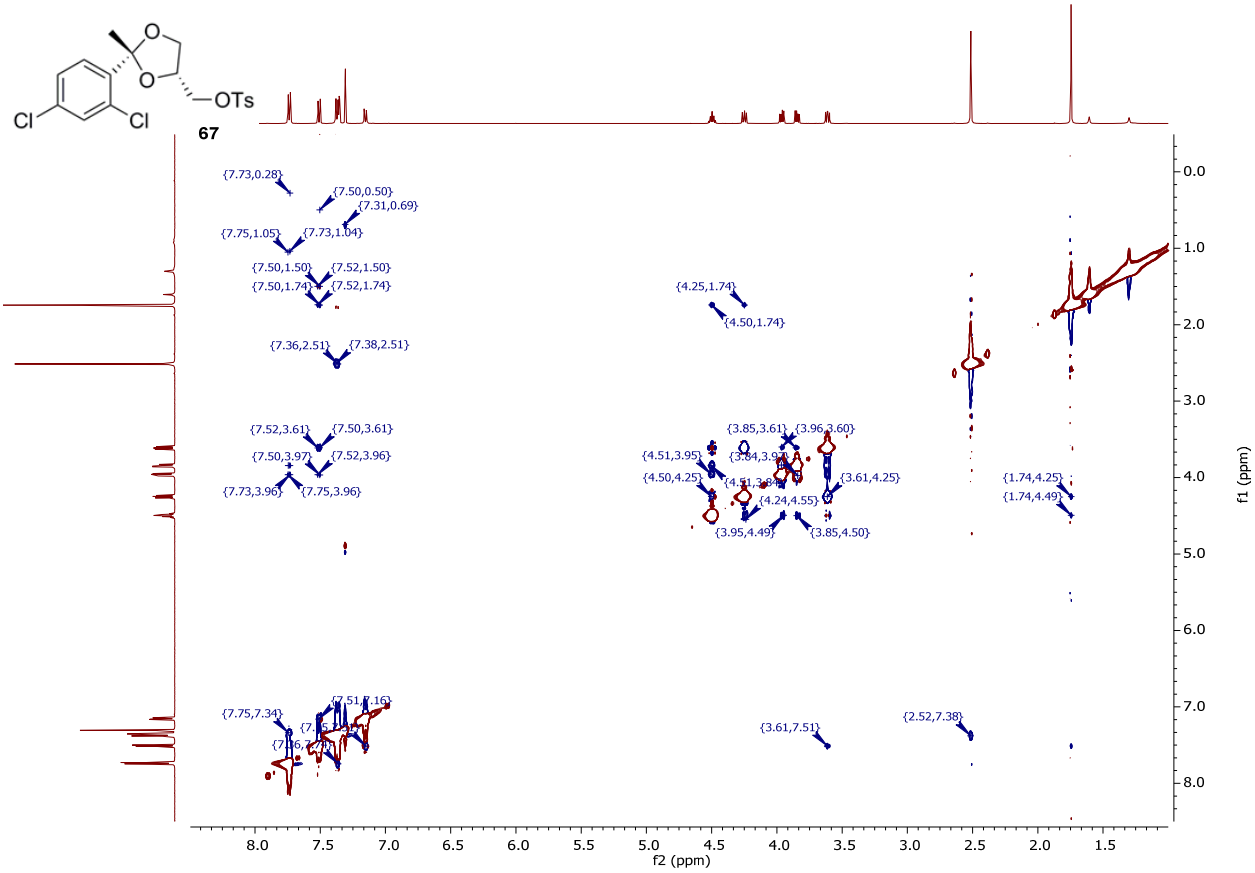
HSQC:



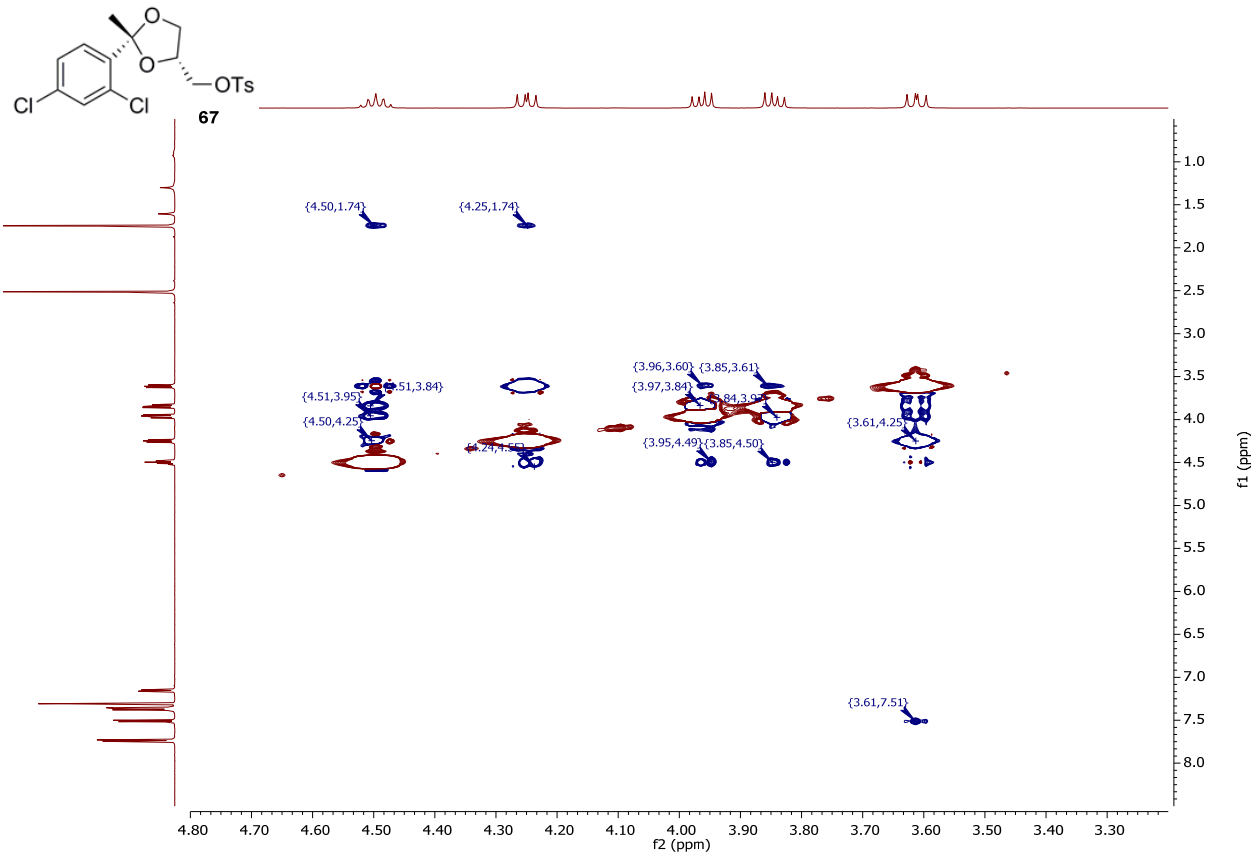
HSQC zoomed (3.2 to 4.8ppm). (1) H_{C4} (methine at C4 of dioxolane) direct correlation via HSQC experiment: 4.50→73.73ppm. (2) Remaining 1H nuclei in the expanded region below are methylene, correlating to the diastereomeric pairs at C5 of dioxolane and α-carbon to tosyl group (not specified: 3.61→66.56ppm; 3.85→68.62ppm; 3.96→68.59ppm; 4.25→66.57ppm).

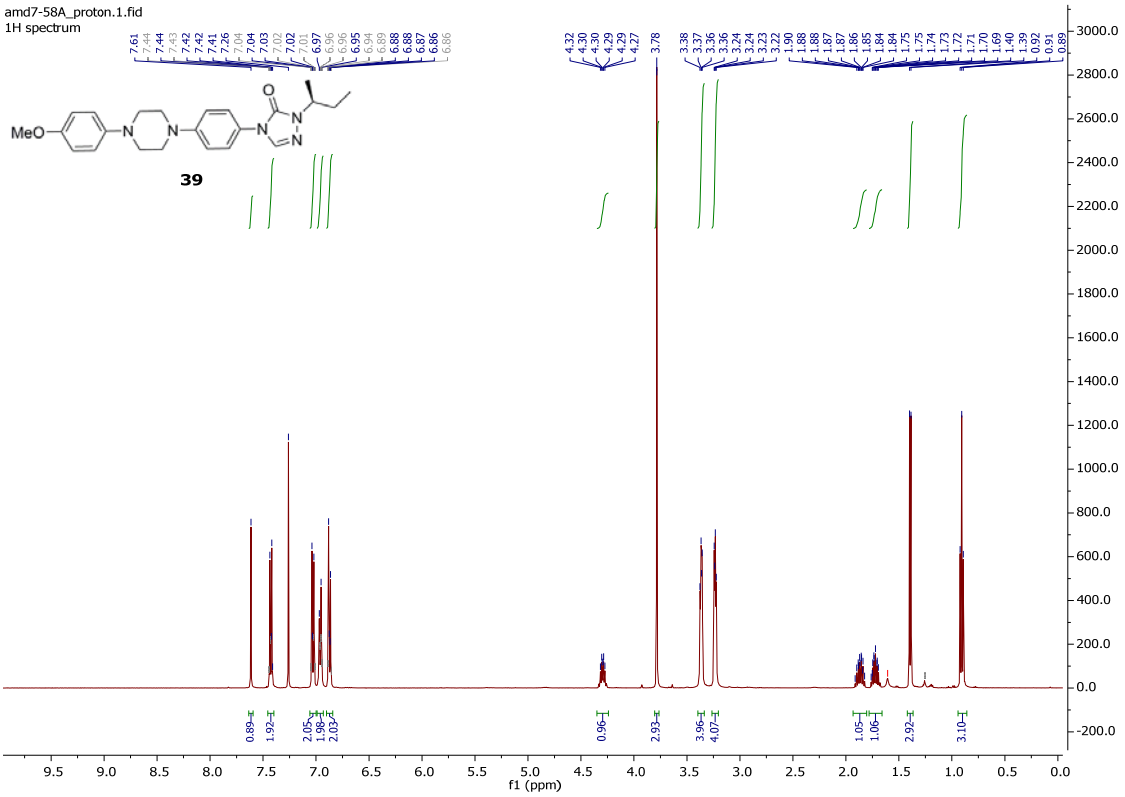


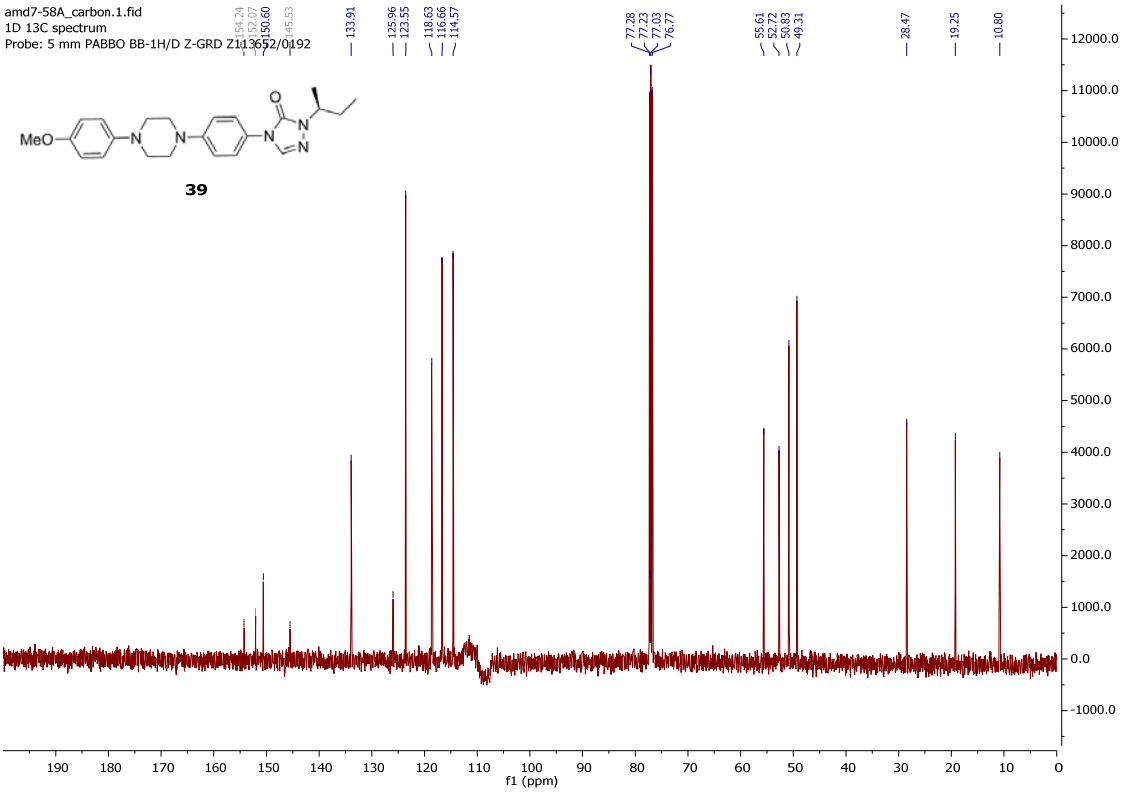
NOESY:

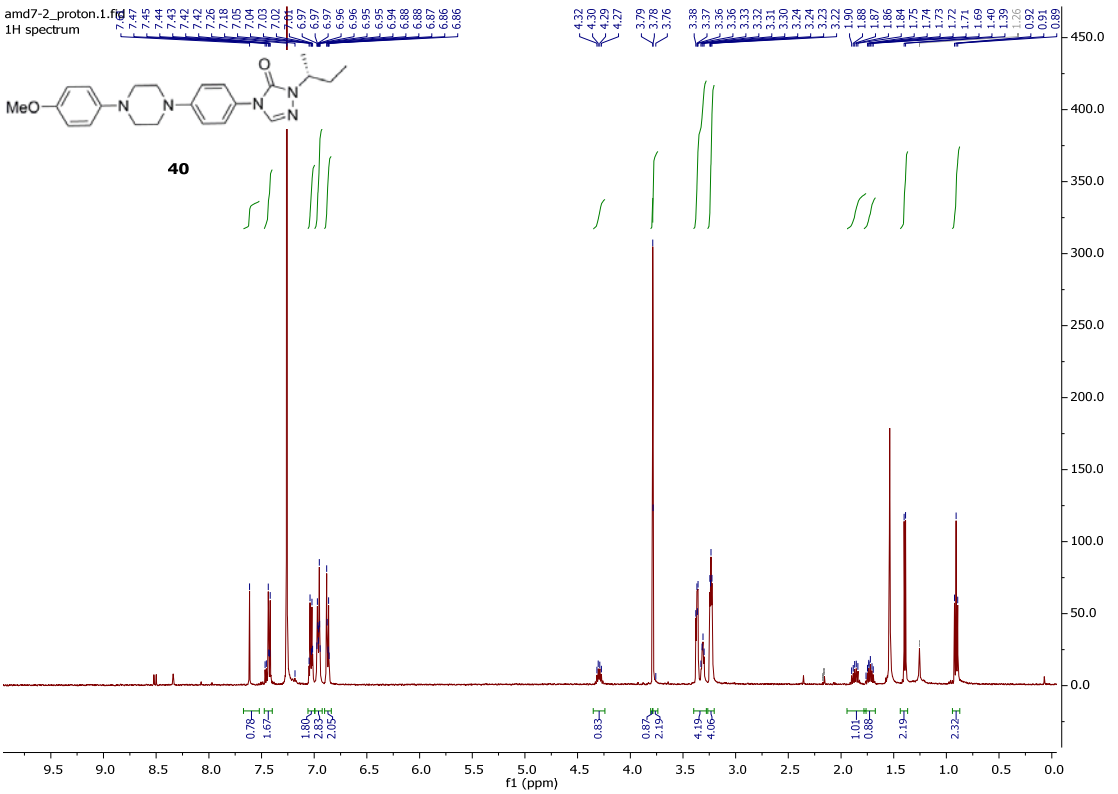

OTs
Cl
Cl
67
{7.73,0.28}
{7.50,0.50}
{7.31,0.69}
{7.75,1.05}
{7.73,1.04}
{7.50,1.50}
{7.52,1.50}
{7.50,1.74}
{7.52,1.74}
{7.36,2.51}
{7.38,2.51}
{7.52,3.61}
{7.50,3.61}
{7.50,3.97}
{7.52,3.96}
{7.73,3.96}
{7.75,3.96}
{4.25,1.74}
{4.50,1.74}
{3.85,3.61}
{3.96,3.60}
{4.51,3.95}
{4.50,4.25}
{3.61,4.25}
{4.24,4.55}
{3.95,4.49}
{3.85,4.50}
{1.74,4.25}
{1.74,4.49}
{7.75,7.34}
{3.61,7.51}
{2.52,7.38}
f2 (ppm)
f1 (ppm)

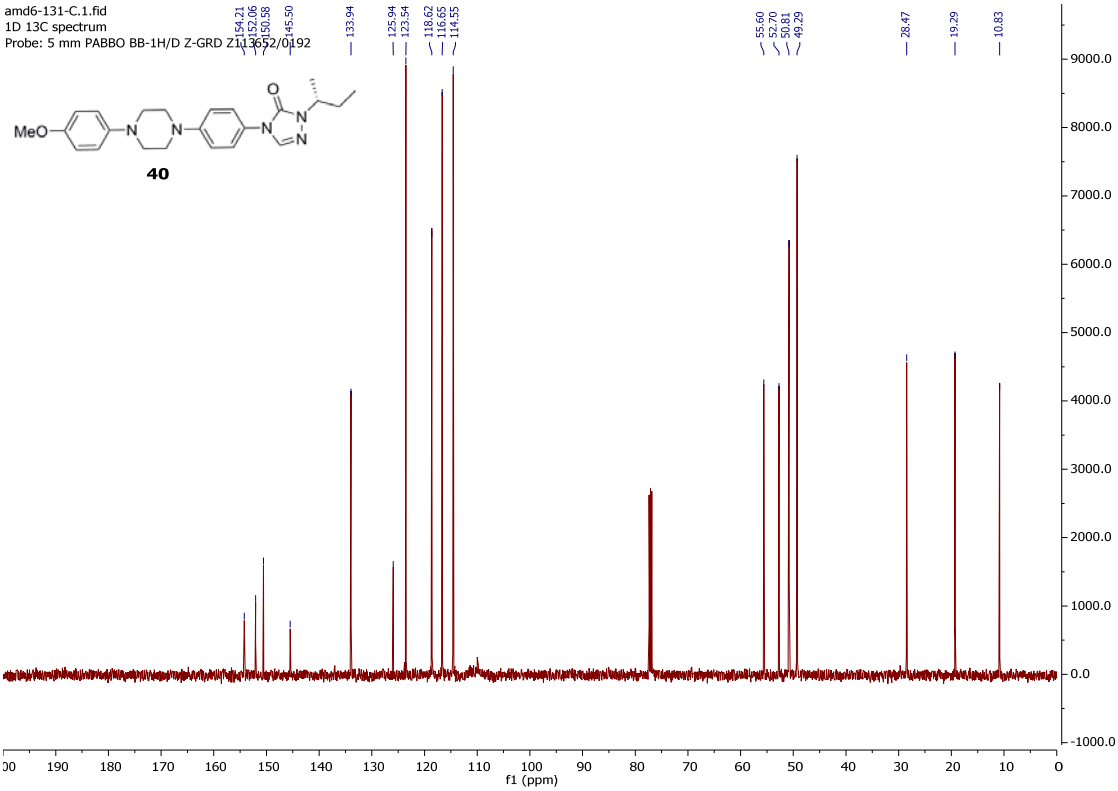
NOESY zoomed (3.2 to 4.8ppm): (1) H_{C4} (methine at C4 of dioxolane) moderate NoE signal with methyl at C2 of dioxolane (2,4-correlation) in experiment: 4.50→1.74ppm.

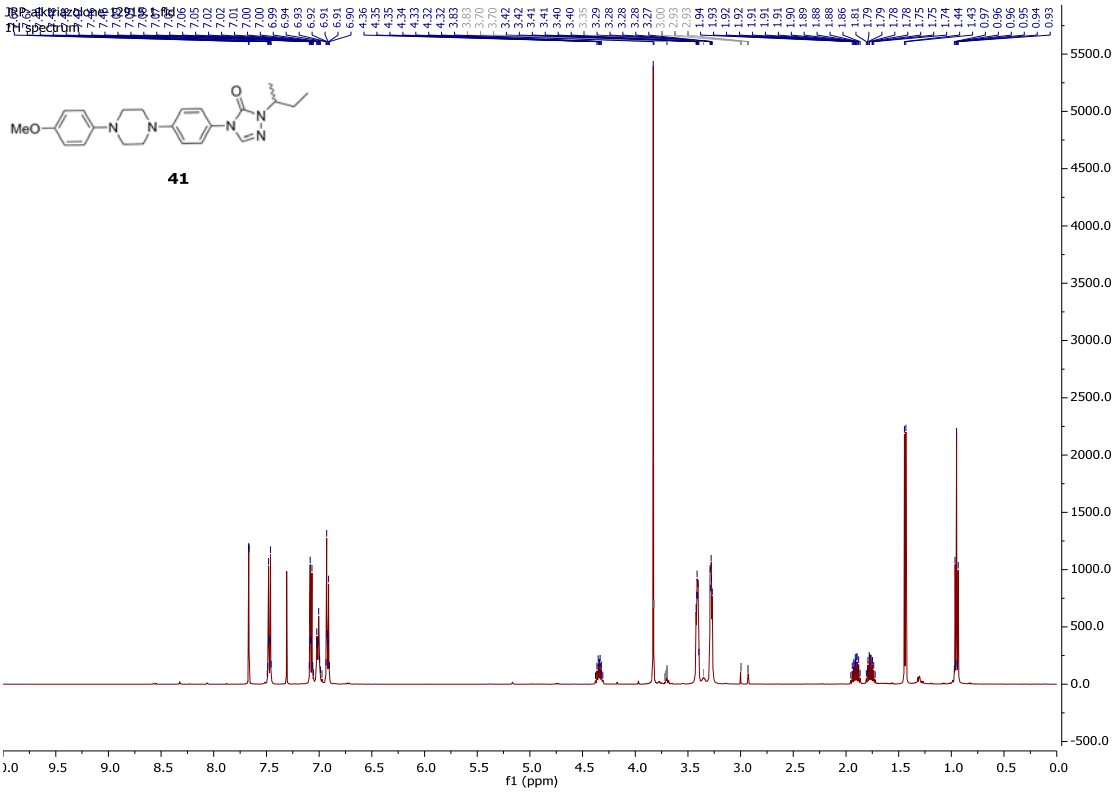


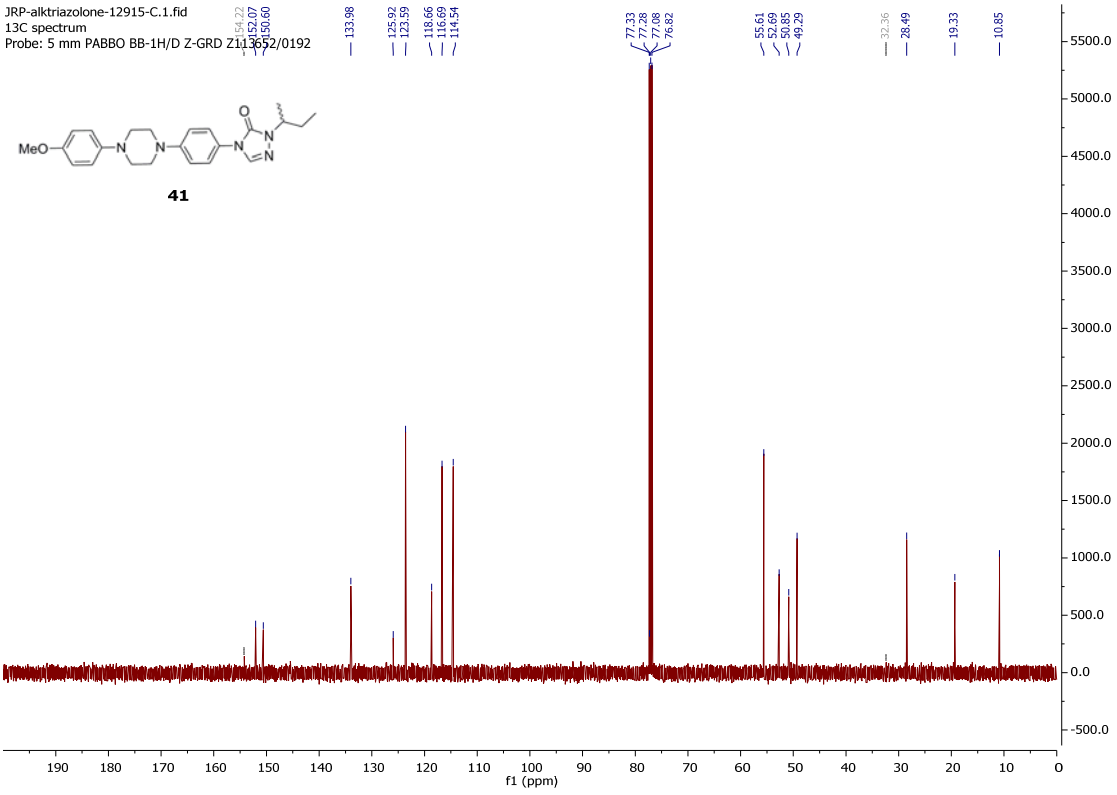

amd7-58A_proton.1.fid
1H spectrum
MeO
39
f1 (ppm)

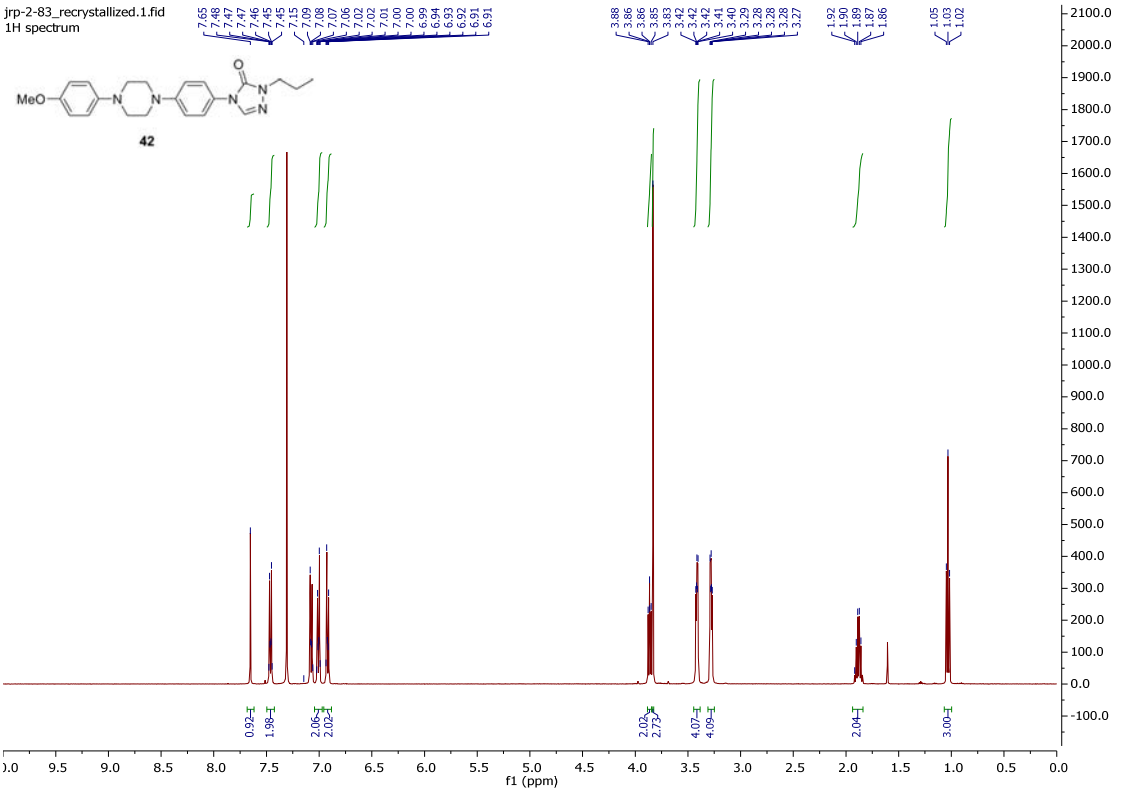

amd7-58A_carbon.1.fid
1D 13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
MeO
39
f1 (ppm)

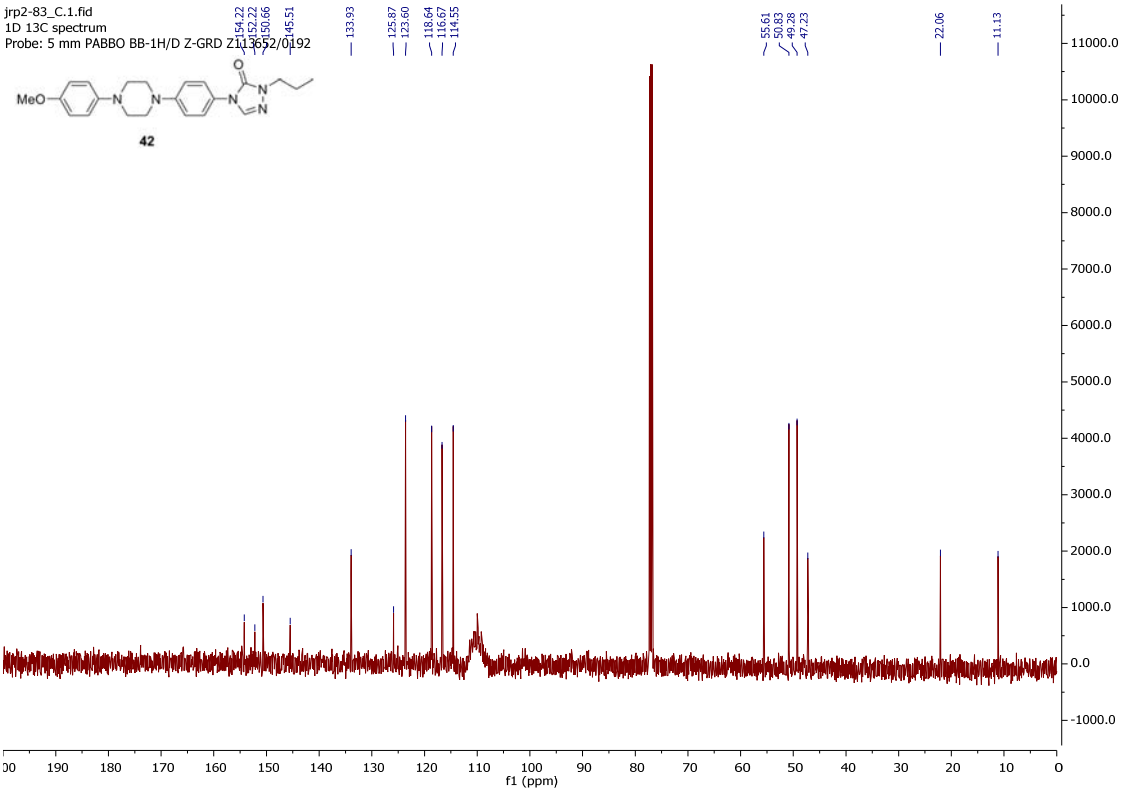

amd7-2_proton.1.fid
1H spectrum
MeO
40
f1 (ppm)

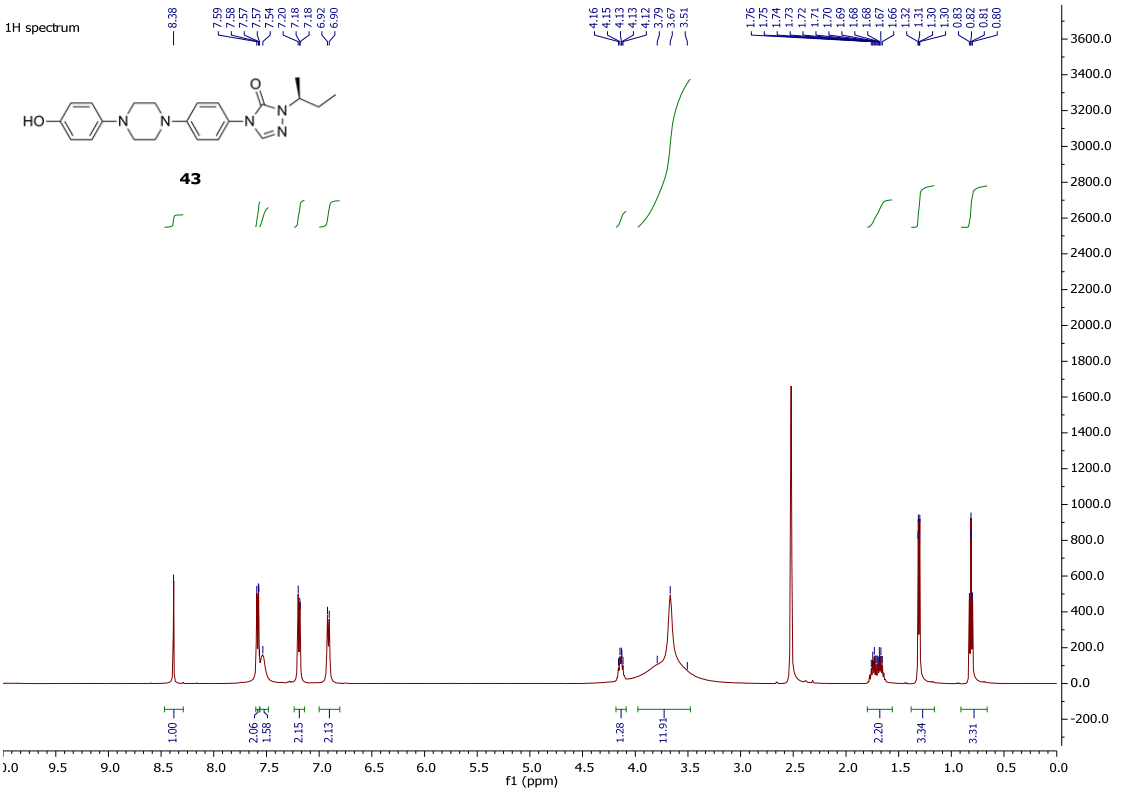

amd6-131-C.1.fid
1D 13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
MeO
40
f1 (ppm)

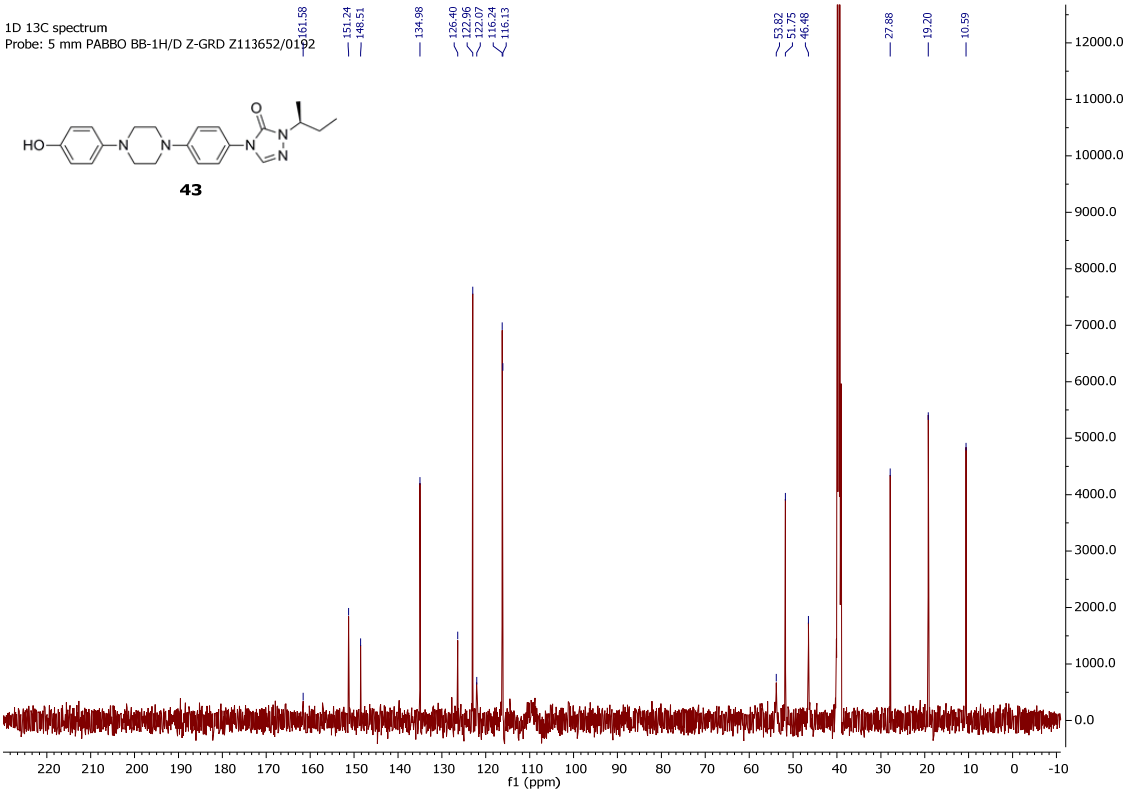

JRP-alktriazolone-12915-H.1.fid
1H spectrum
MeO
41
f1 (ppm)

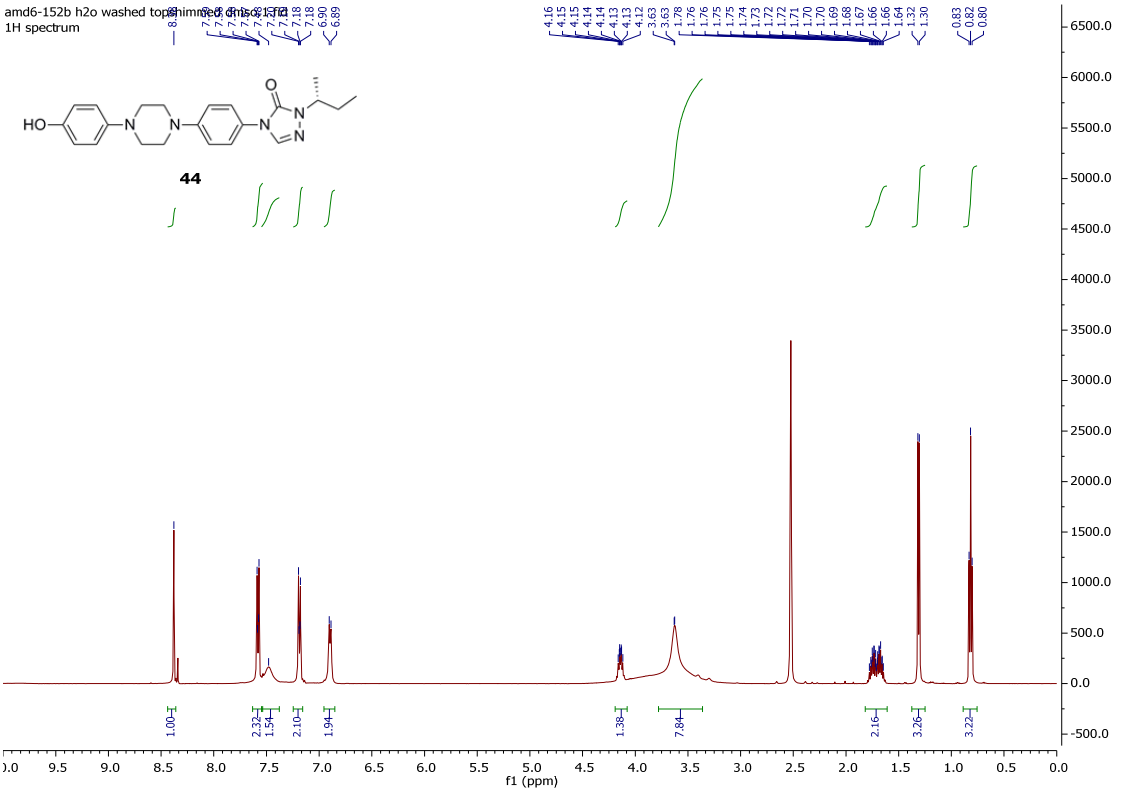

JRP-alktriazolone-12915-C.1.fid
13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
MeO
41
f1 (ppm)

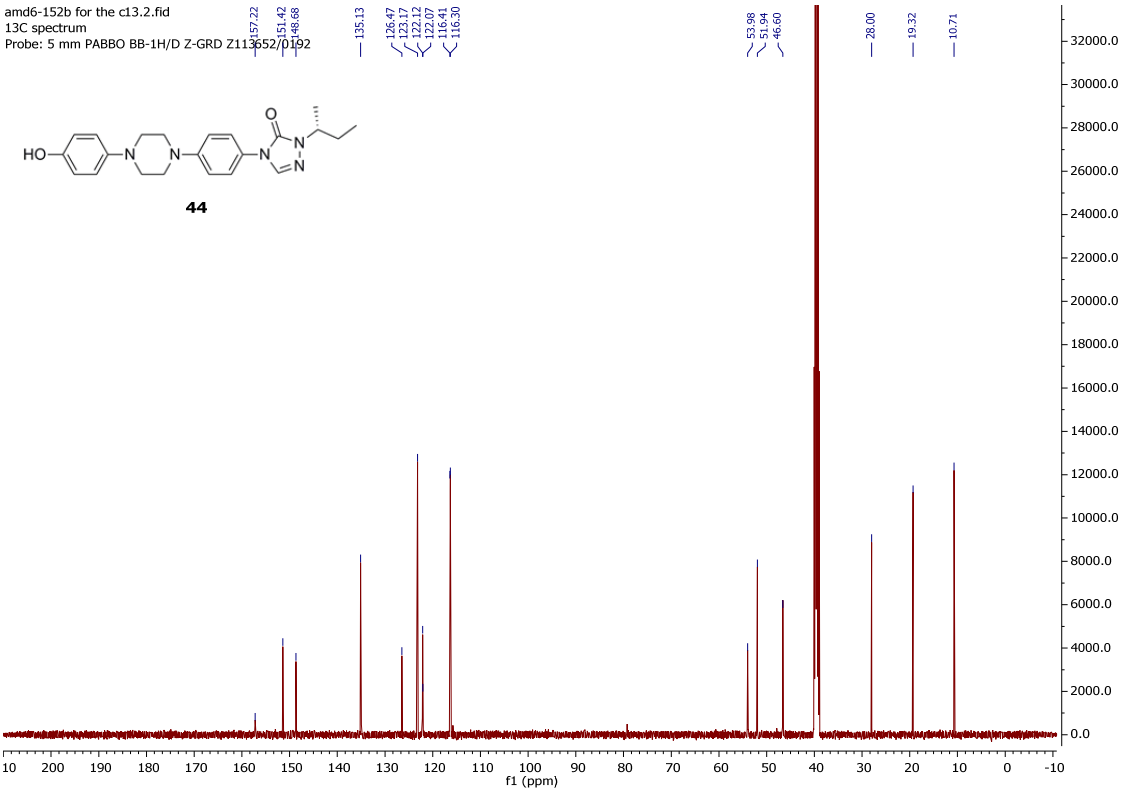

jrp-2-83_recrystallized.1.fid
1H spectrum
MeO
42
f1 (ppm)

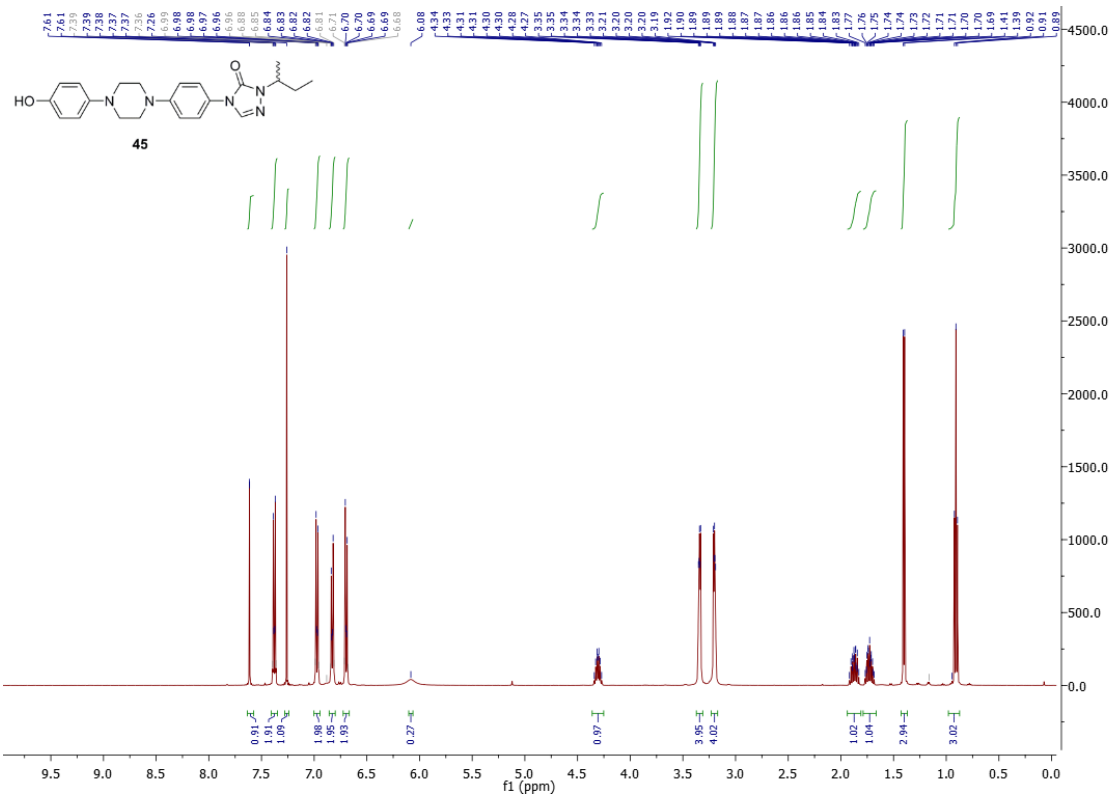

jrp2-83_C.1.fid
1D 13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
MeO
42
f1 (ppm)

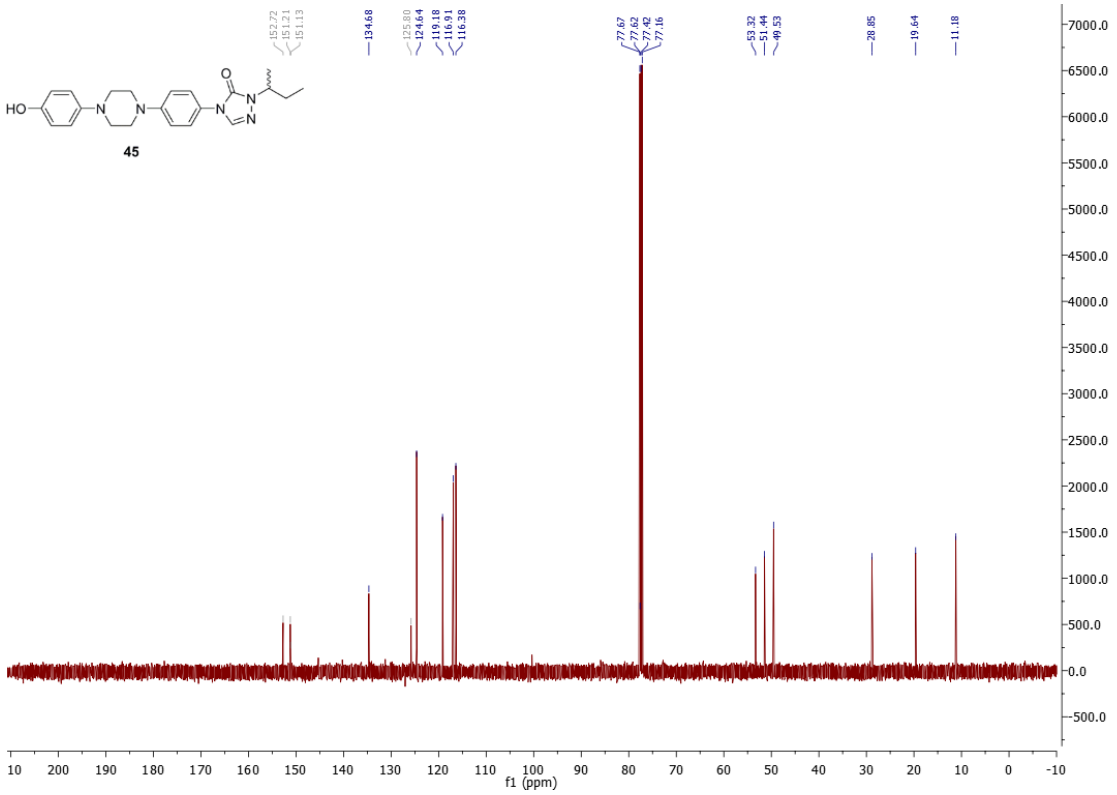

1H spectrum
8.38
7.59
7.58
7.57
7.57
7.54
7.20
7.18
7.18
6.92
6.90
4.16
4.15
4.13
4.13
4.12
3.79
3.67
3.51
1.76
1.75
1.74
1.73
1.72
1.71
1.70
1.69
1.68
1.68
1.67
1.66
1.32
1.31
1.30
1.30
0.83
0.82
0.81
0.80
HO
N
N
N
N
O
43
1.00
2.06
1.58
2.15
2.13
1.28
11.91
2.20
3.34
3.31
f1 (ppm)

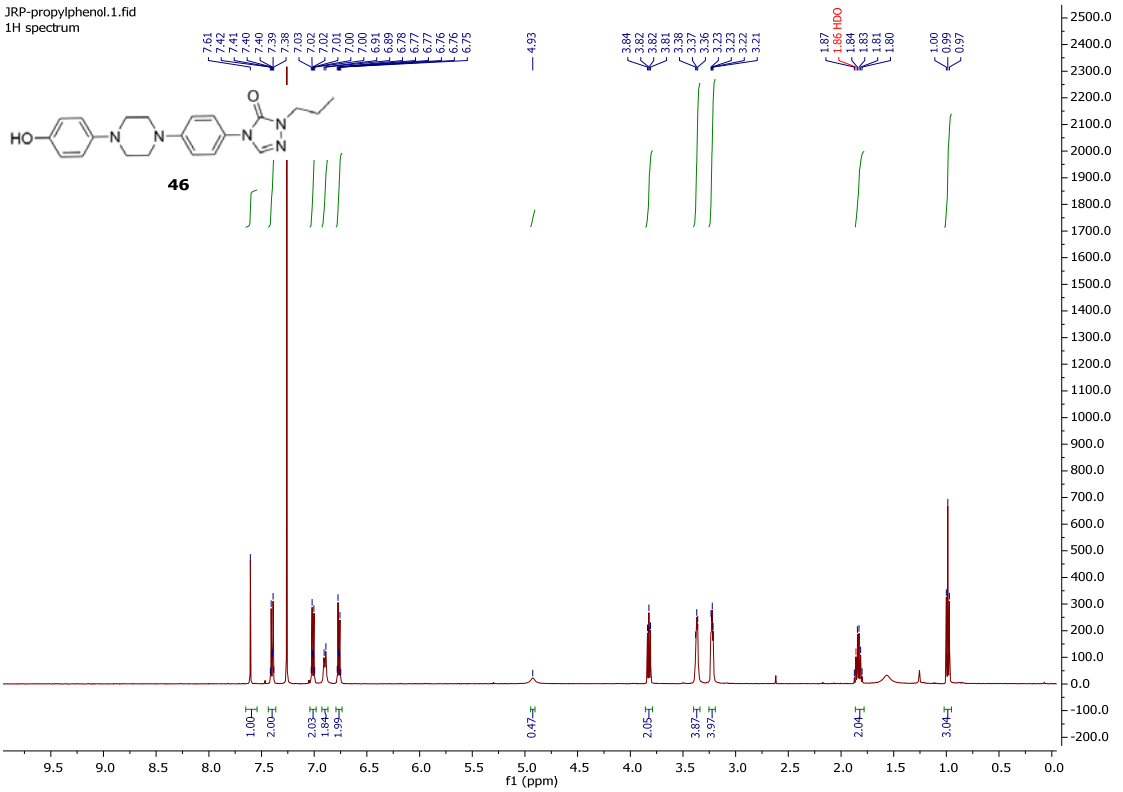

1D 13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
161.58
151.24
148.51
134.98
126.40
122.96
122.07
116.24
116.13
53.82
51.75
46.48
27.88
19.20
10.59
HO
N
N
N
N
O
43
f1 (ppm)

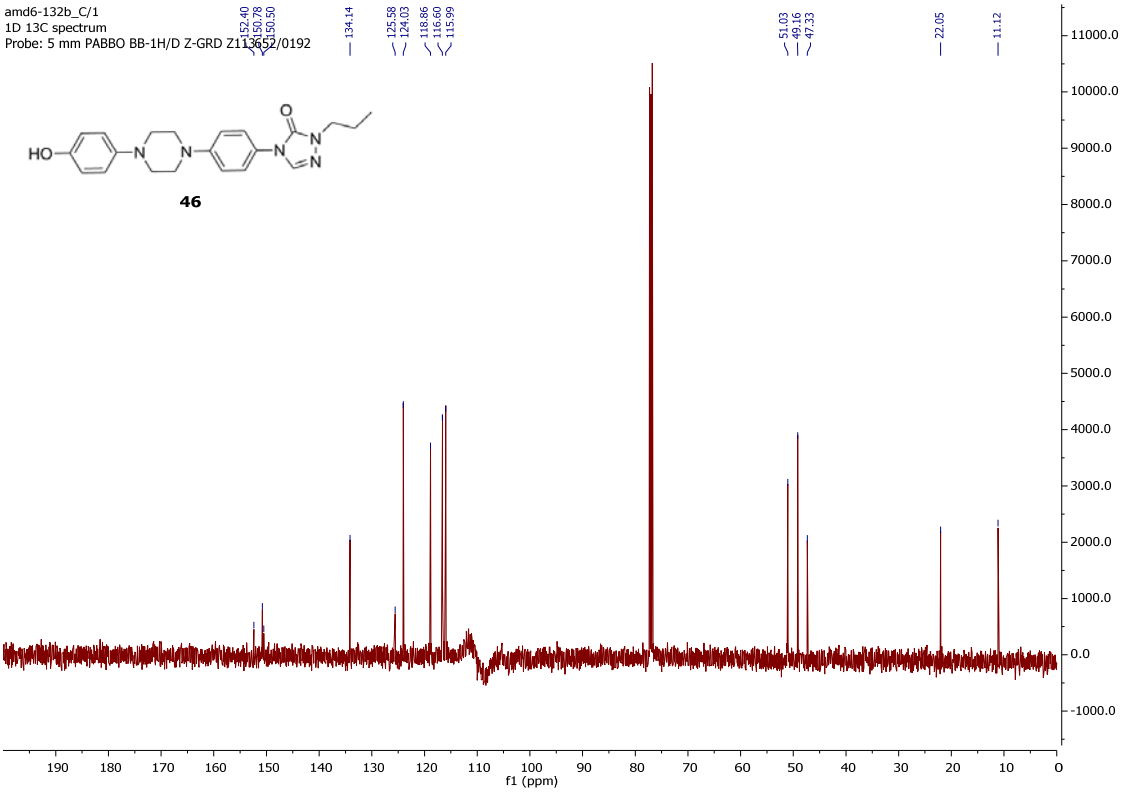

amd6-152b h2o washed top
1H spectrum
44
f1 (ppm)

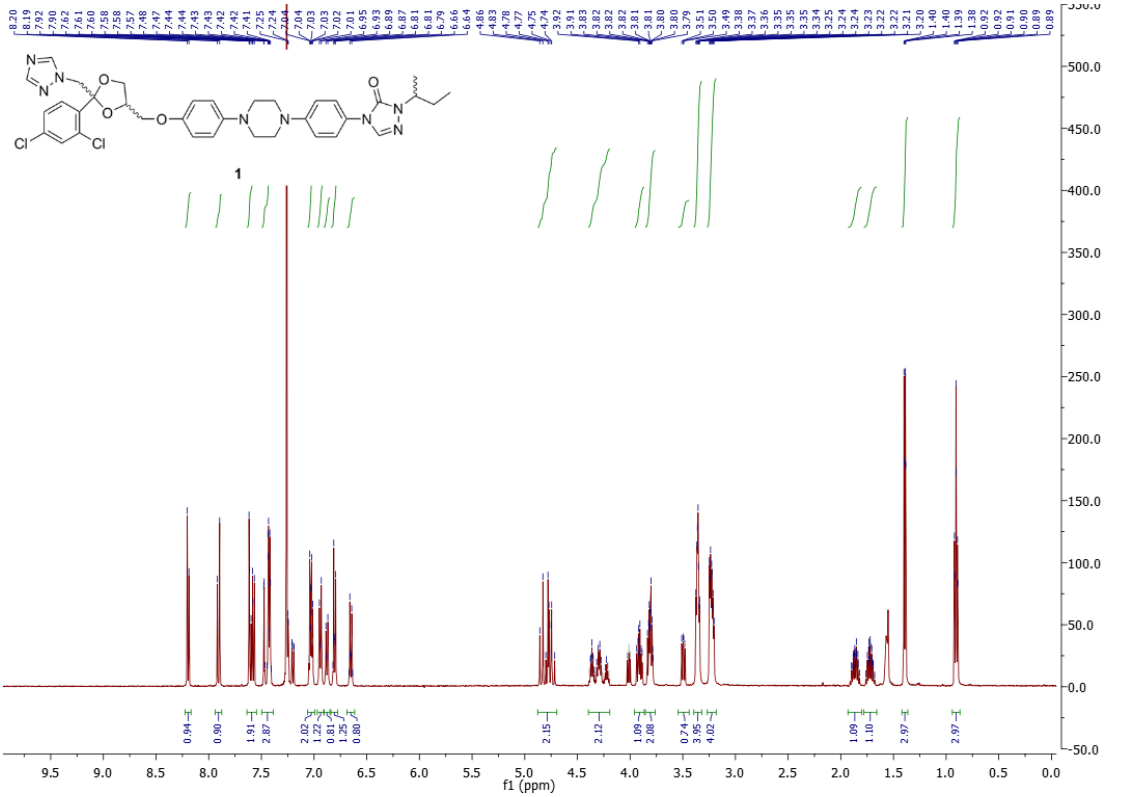

amd6-152b for the c13.2.fid
13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
44
f1 (ppm)

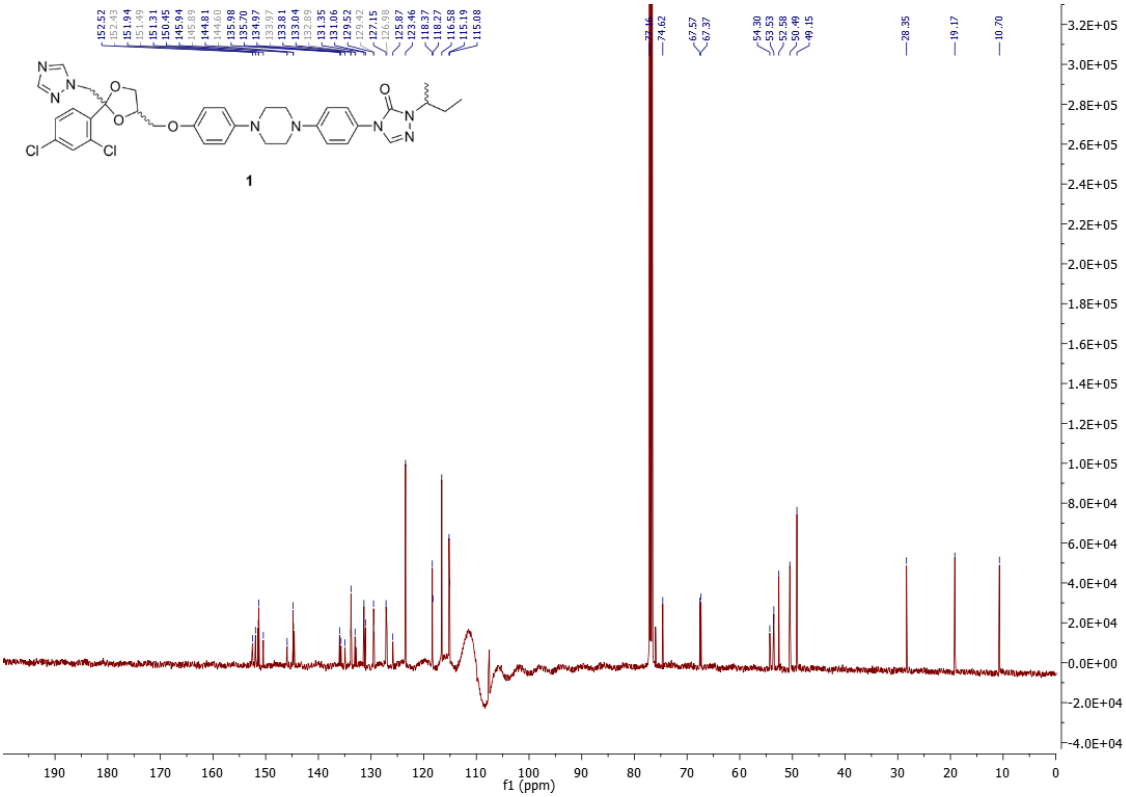

HO
45
f1 (ppm)

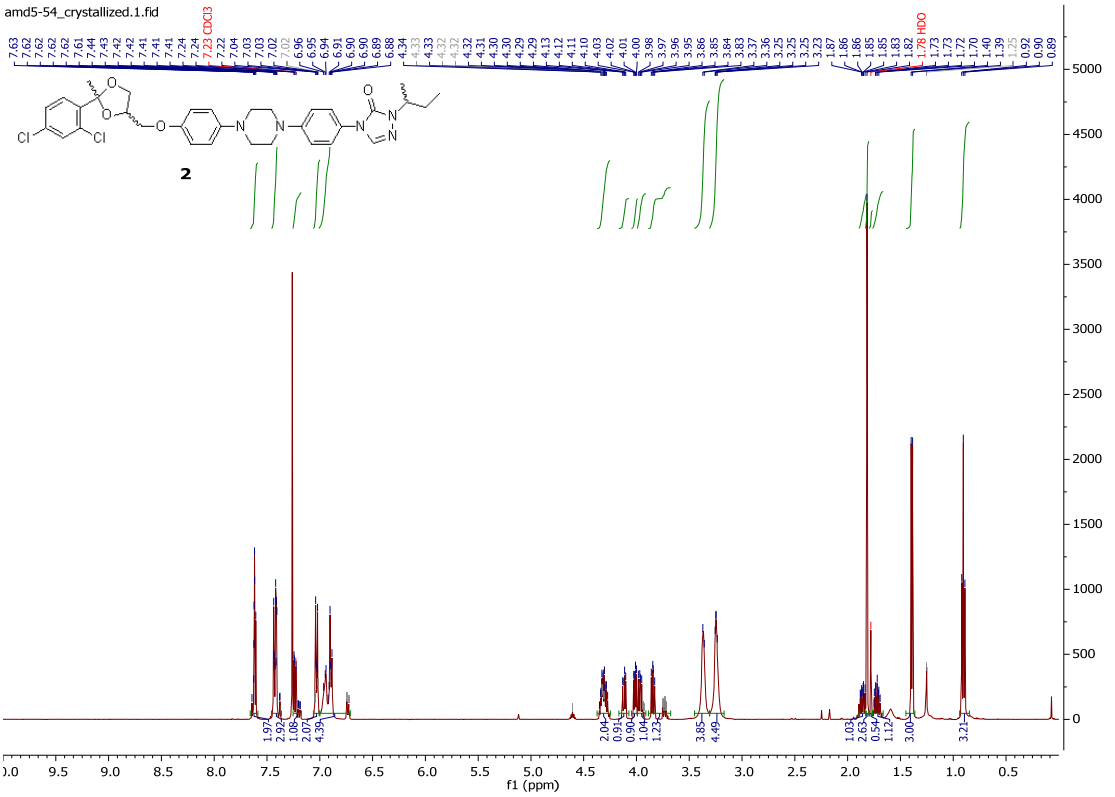

HO
45
f1 (ppm)

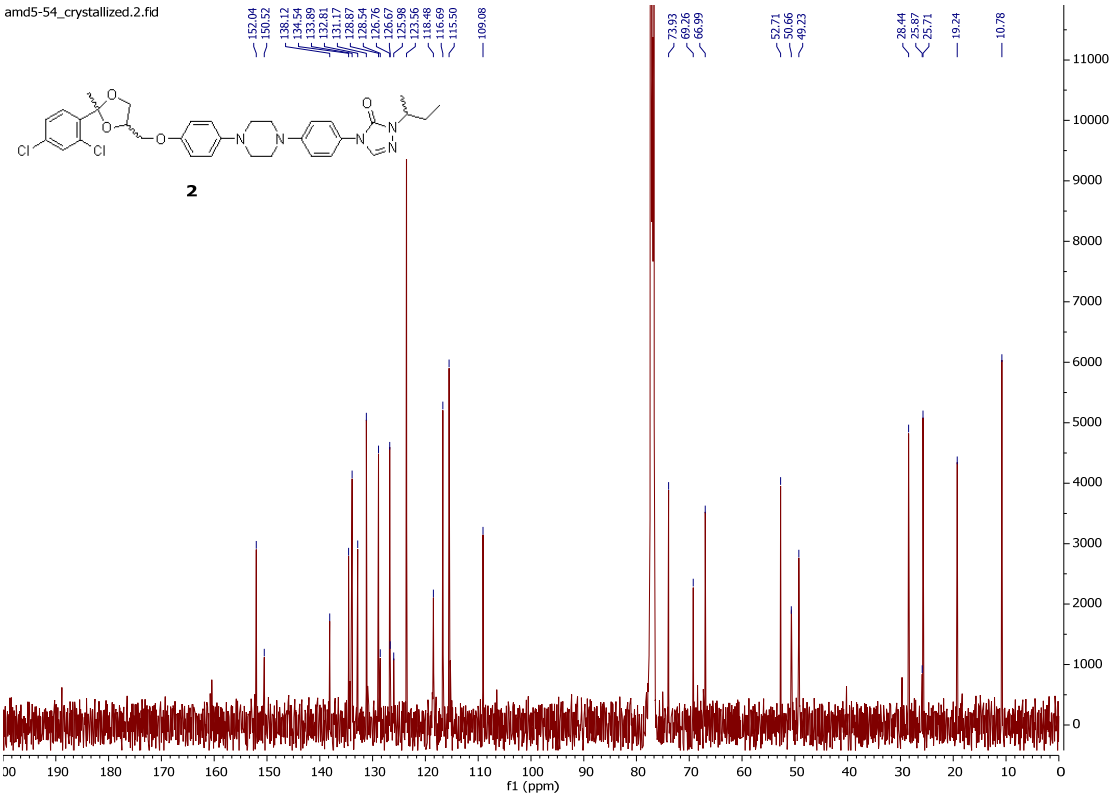

JRP-propylphenol.1.fid
1H spectrum
46
f1 (ppm)

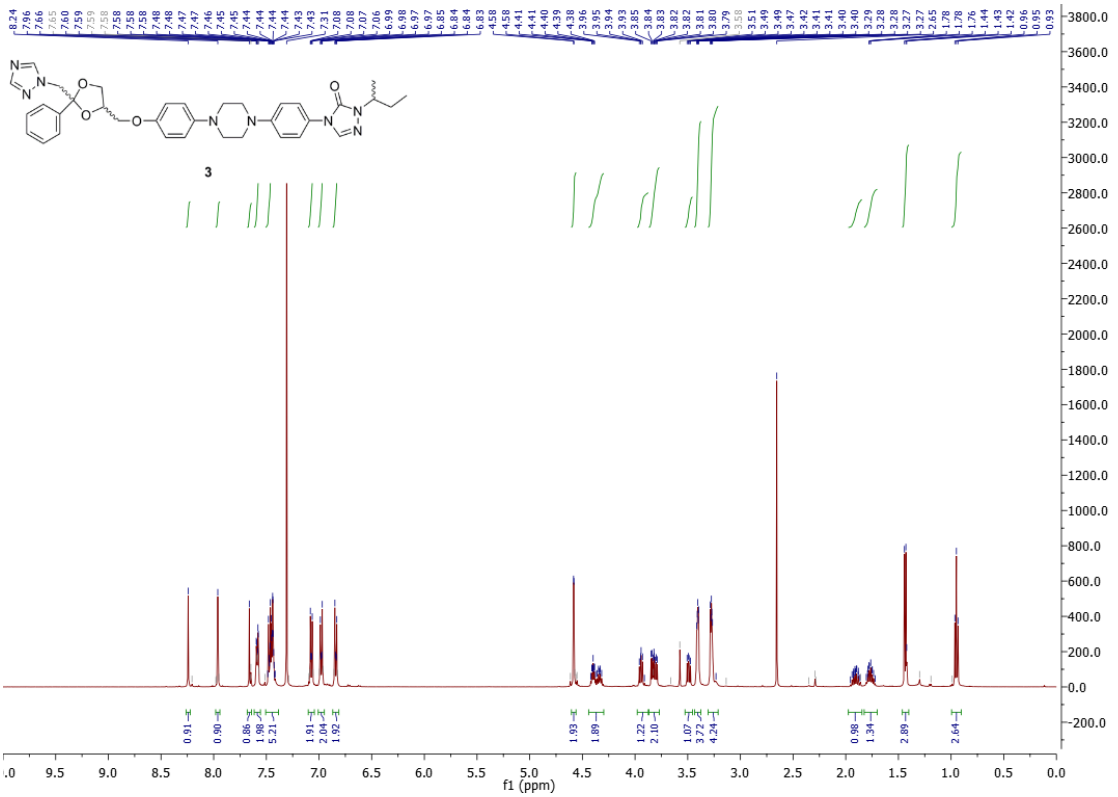

amd6-132b_C/1
1D 13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
46
f1 (ppm)

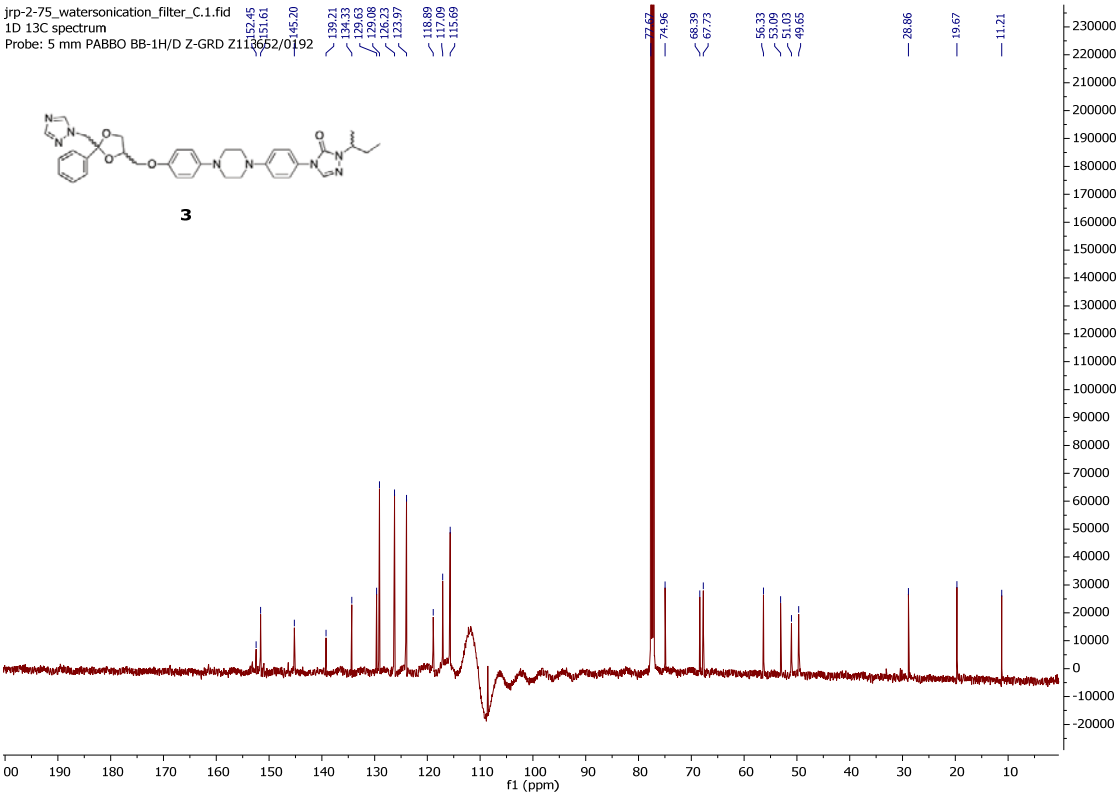

1
f1 (ppm)

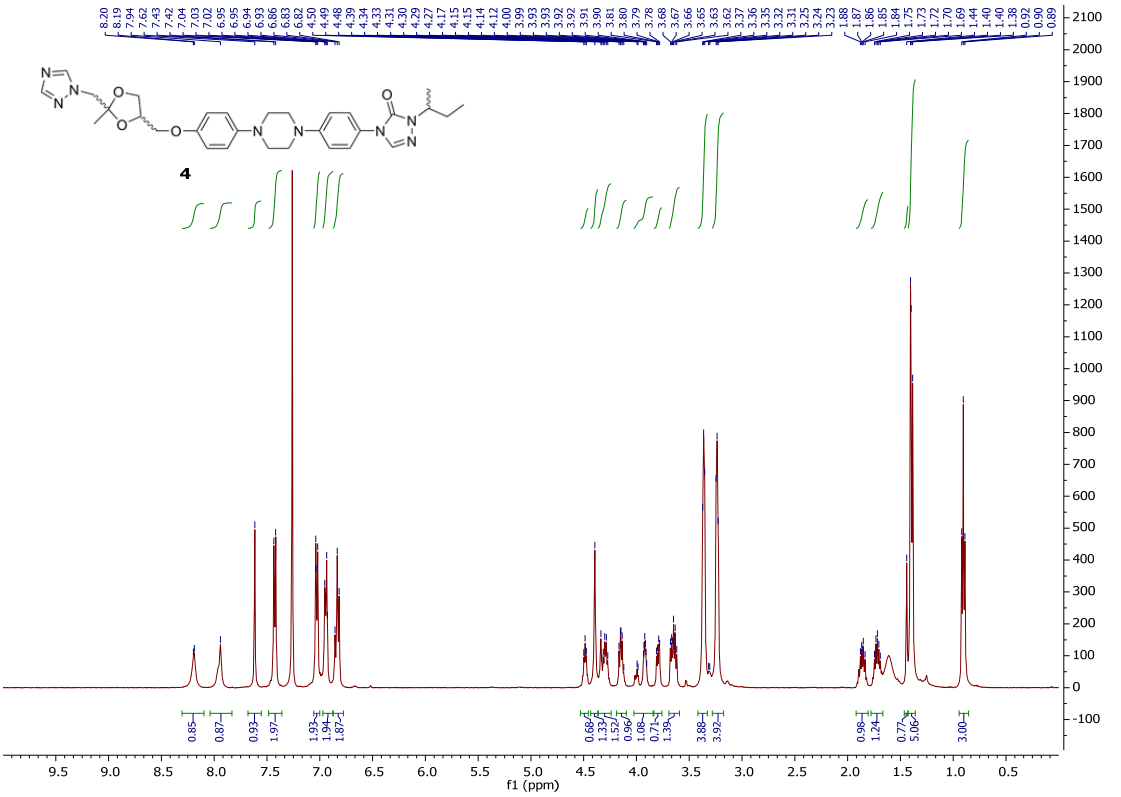

1
f1 (ppm)

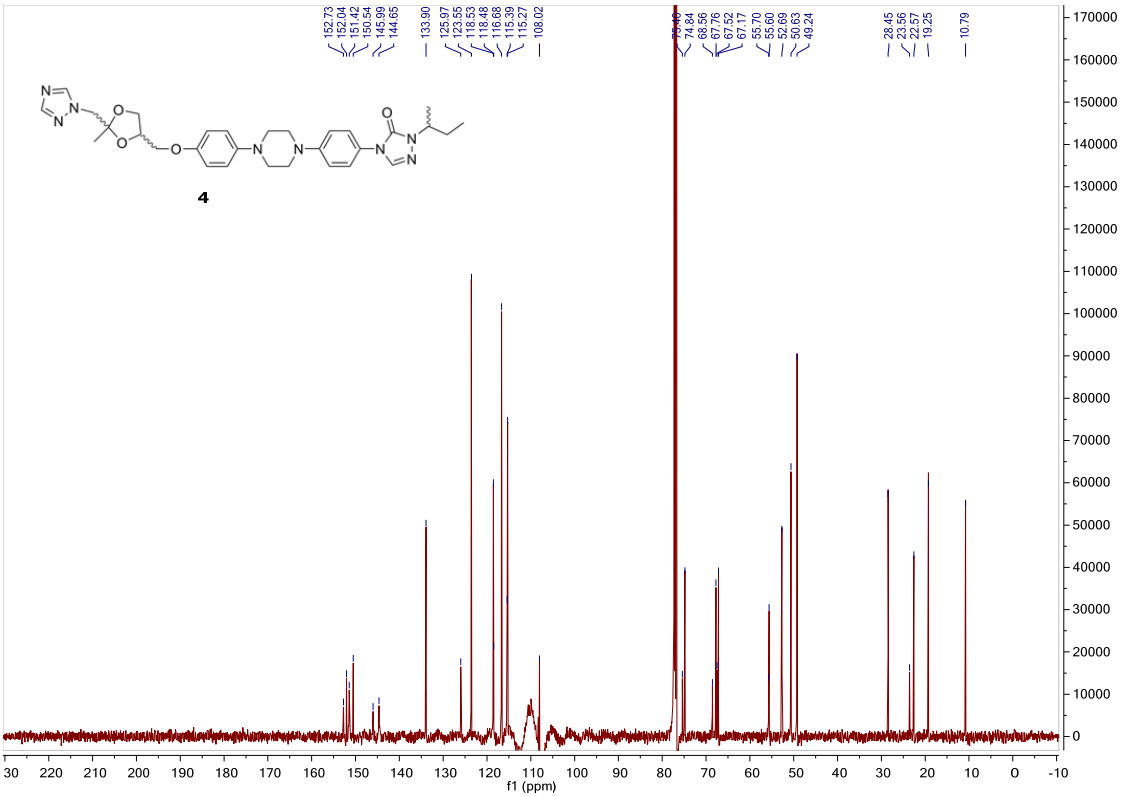

amd5-54_crystallized.1.fid
2
f1 (ppm)

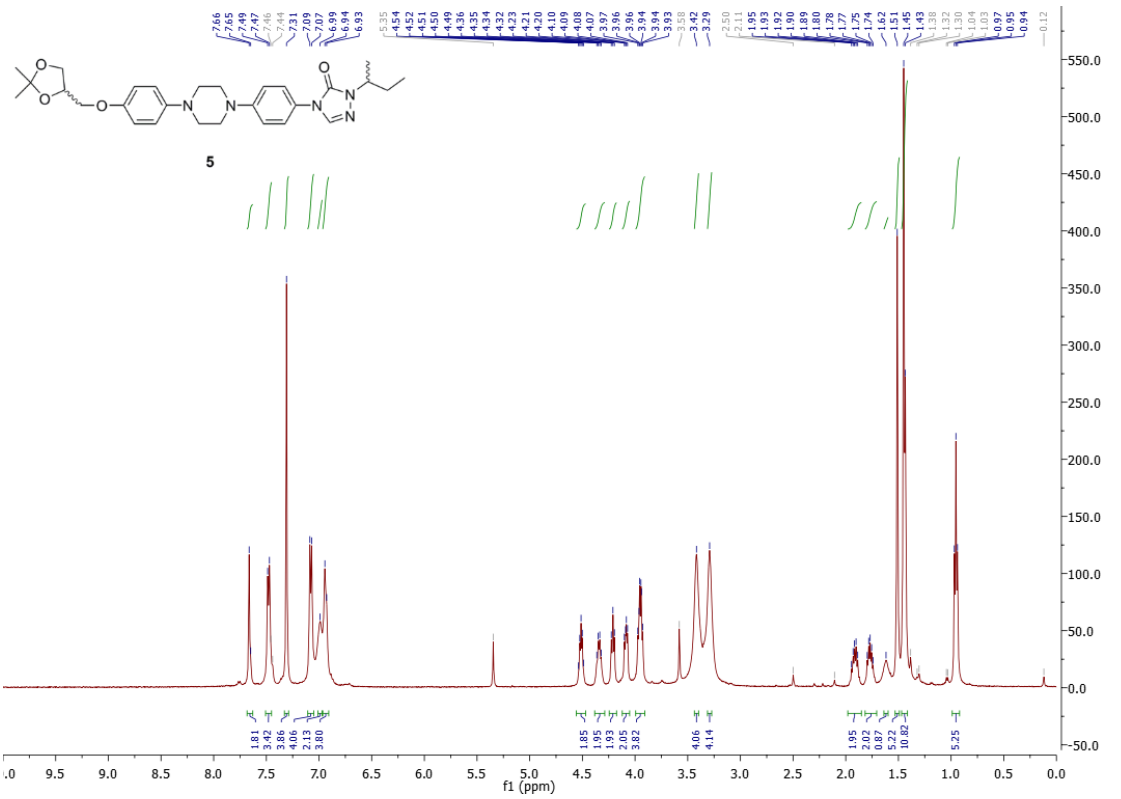

amd5-54_crystallized.2.fid
2
f1 (ppm)

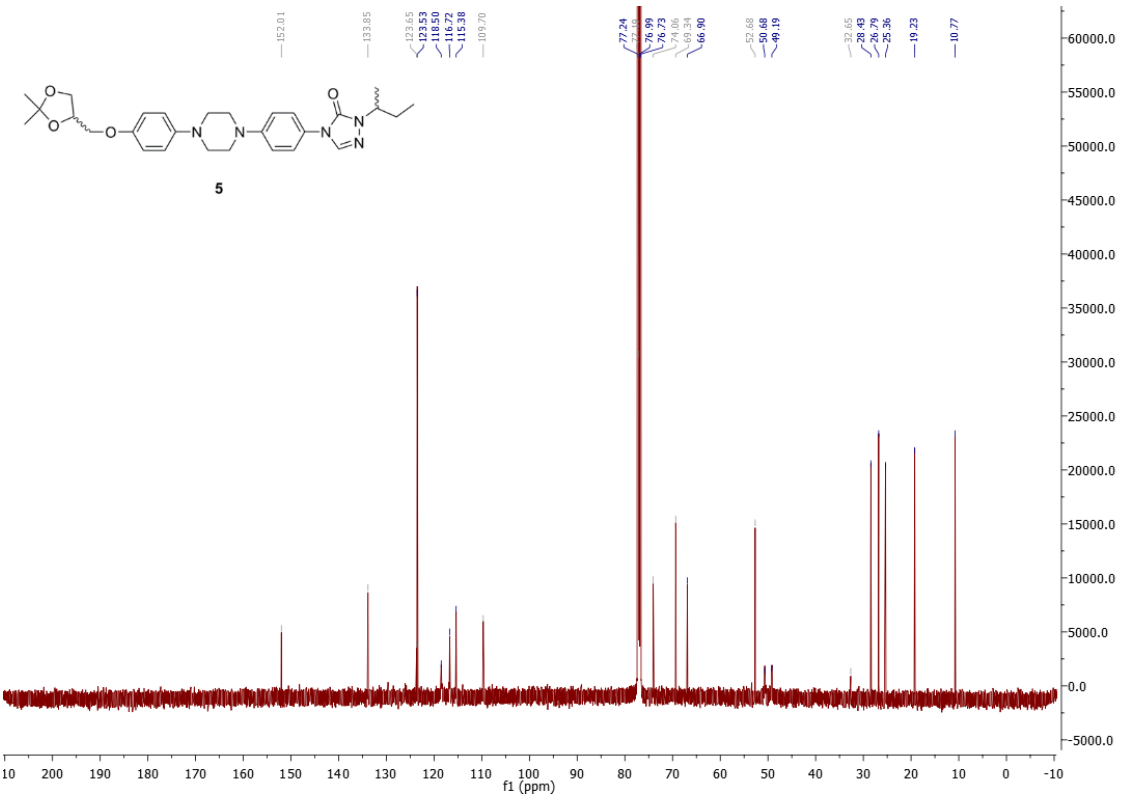

3
f1 (ppm)

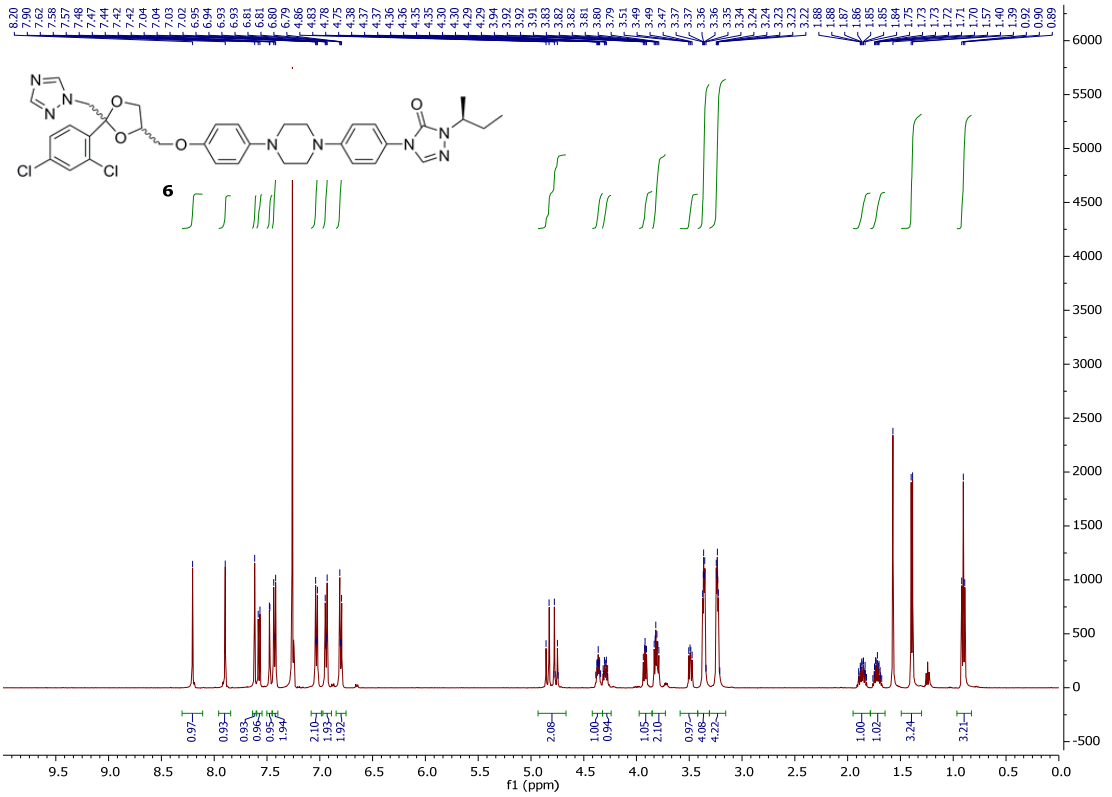

jrp-2-75_watersonication_filter_C.1.fid
1D 13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0192
3
f1 (ppm)

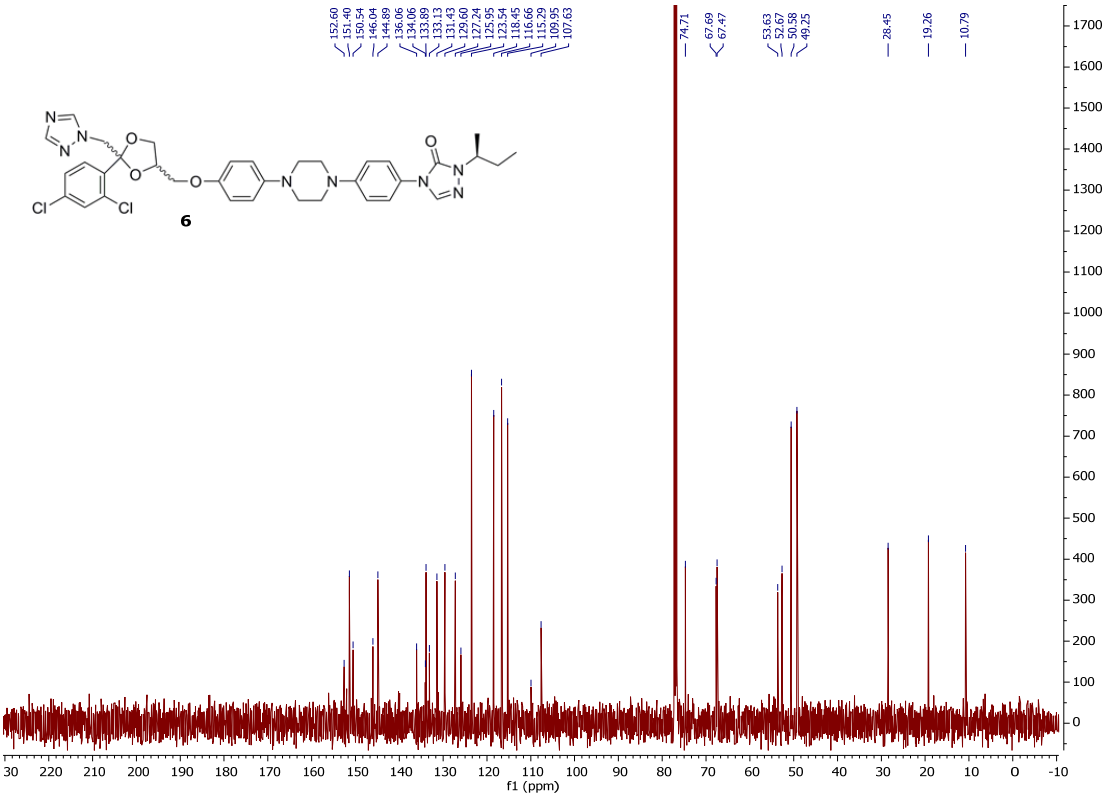

4
f1 (ppm)

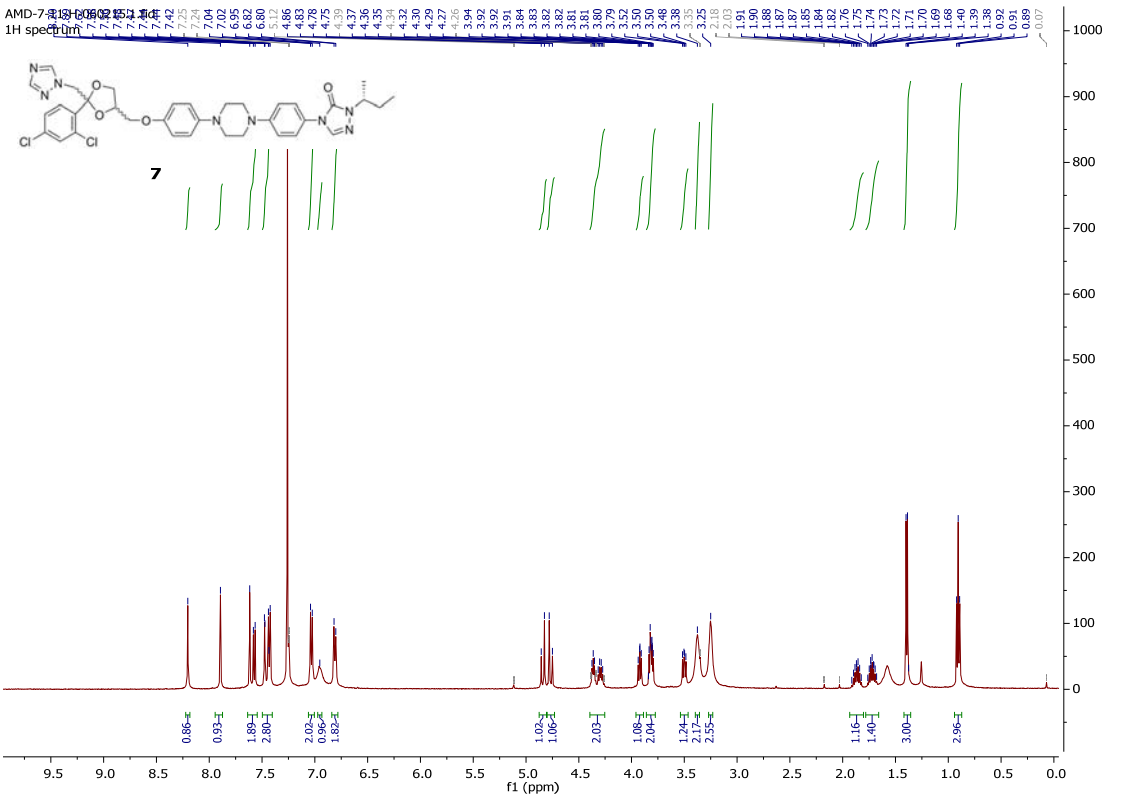

4
f1 (ppm)

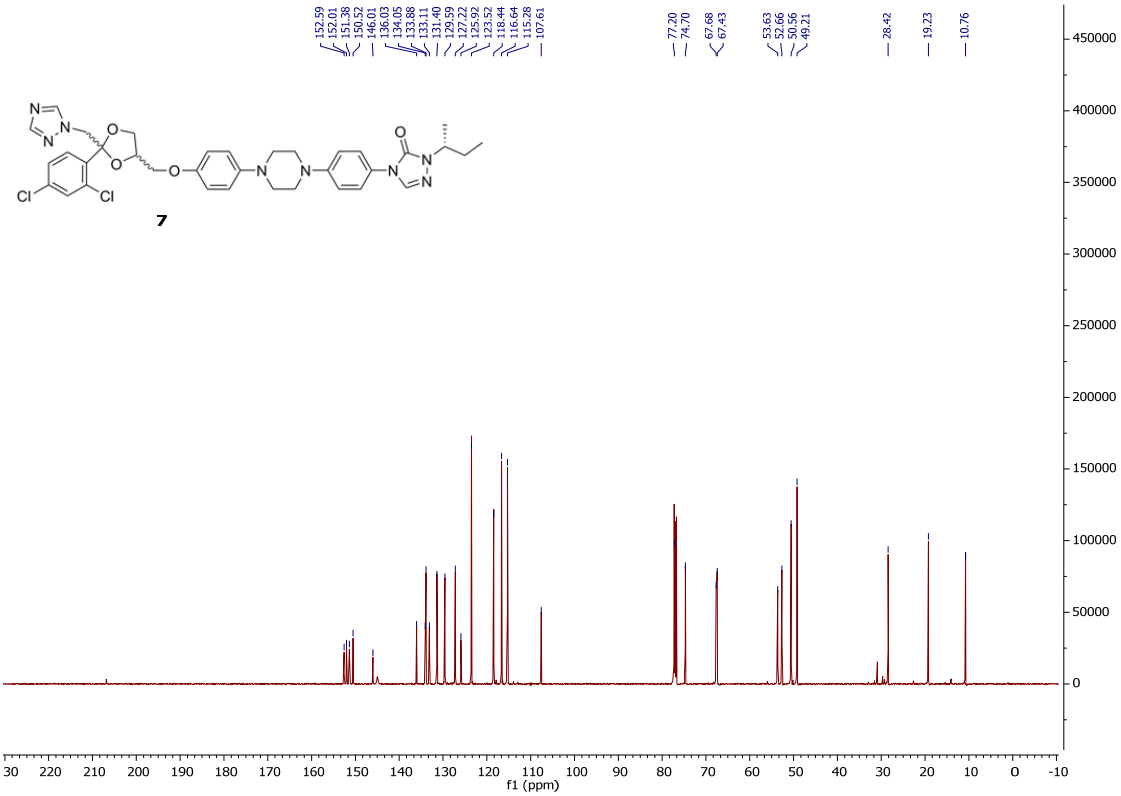

5
f1 (ppm)

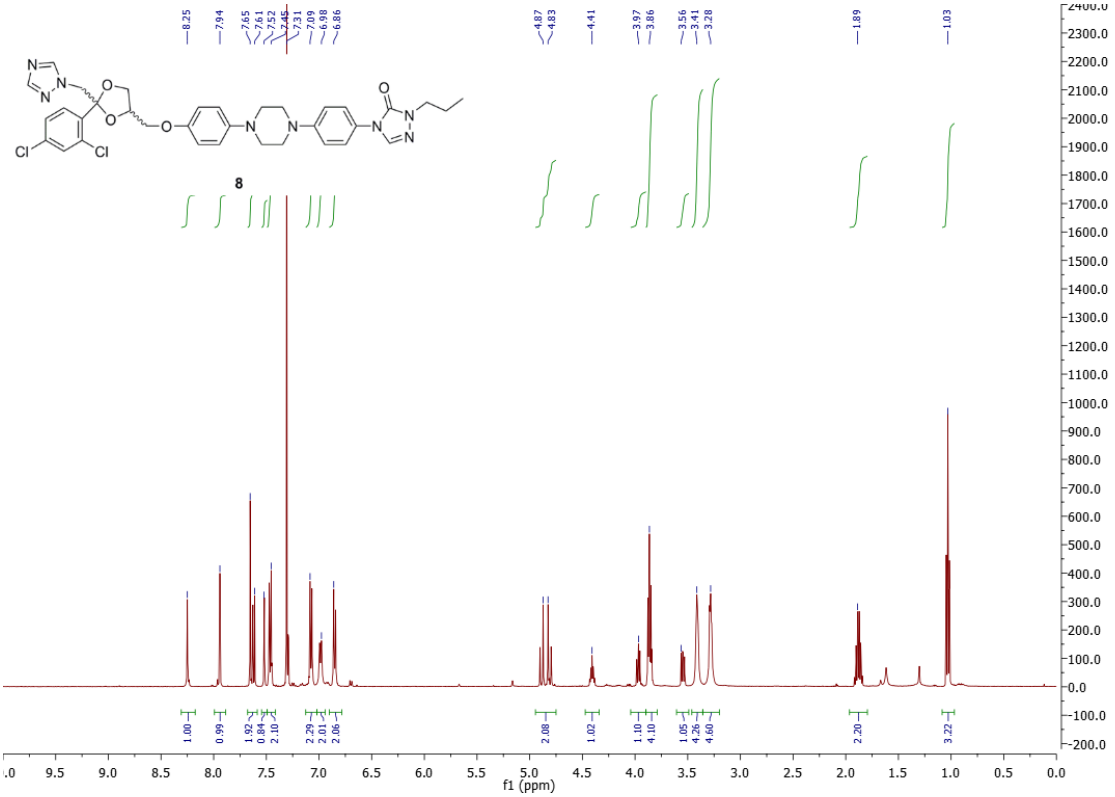

5
f1 (ppm)

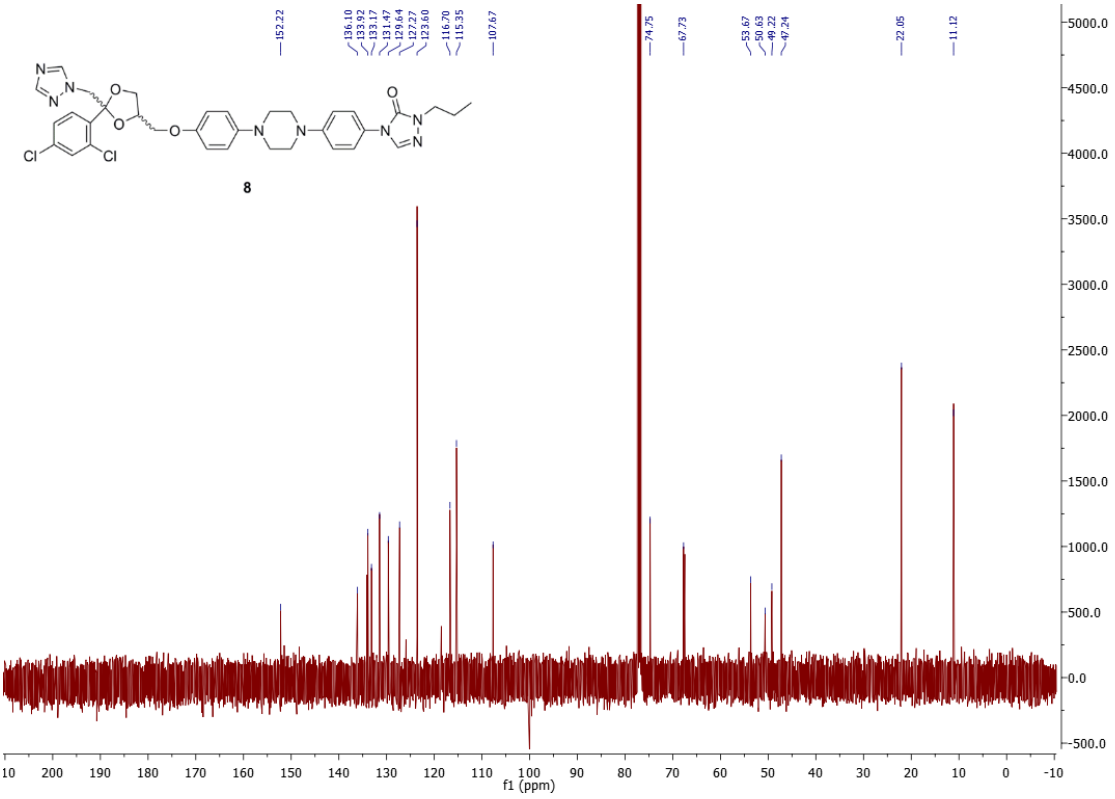

6
f1 (ppm)

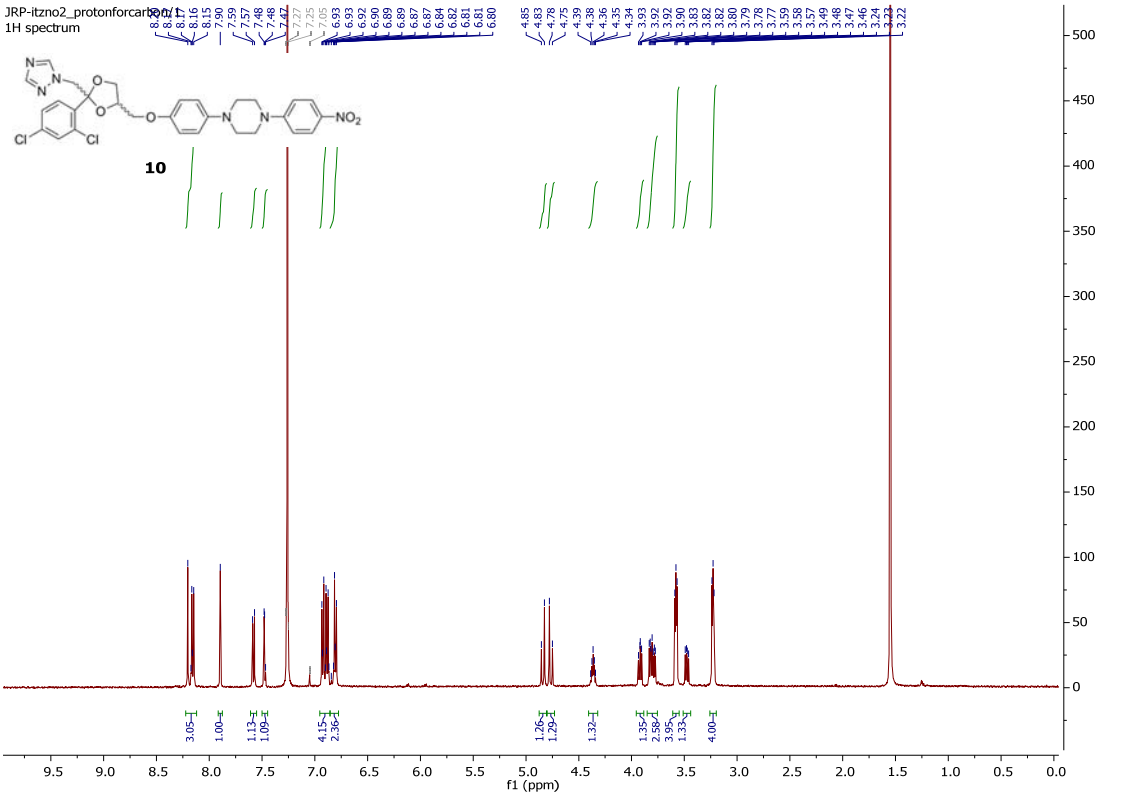

6
f1 (ppm)

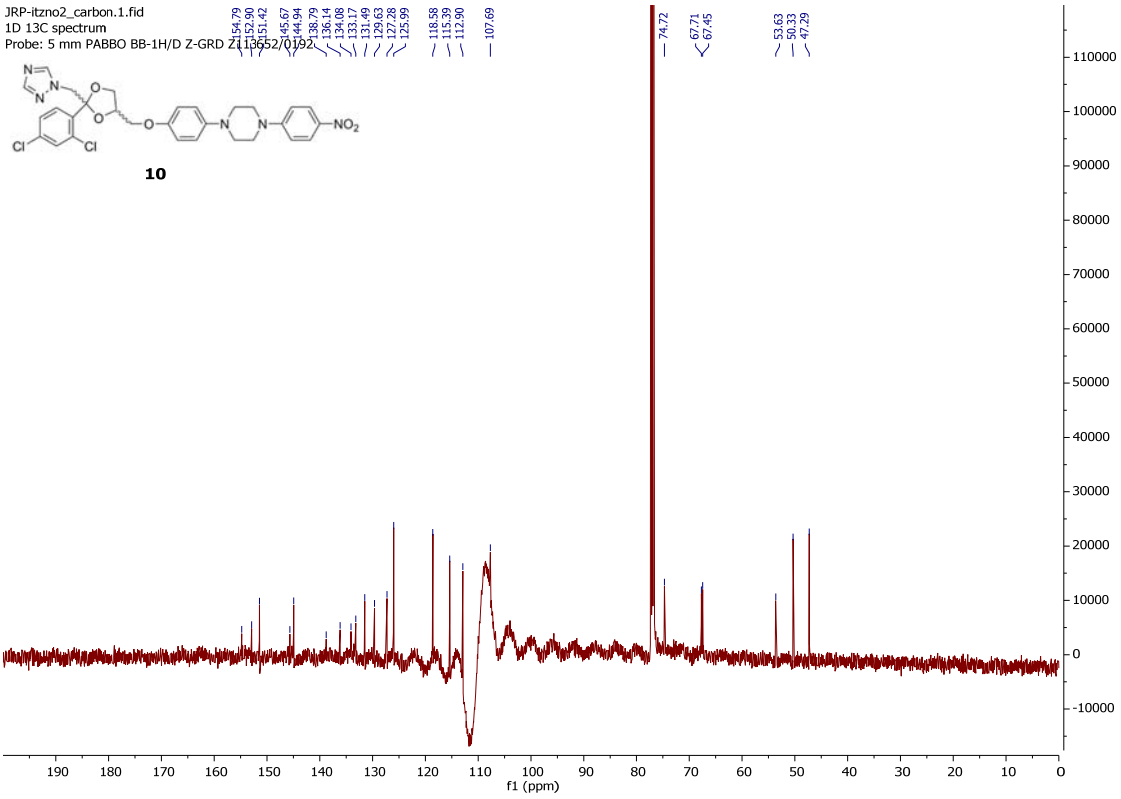

1H spectrum
7
f1 (ppm)

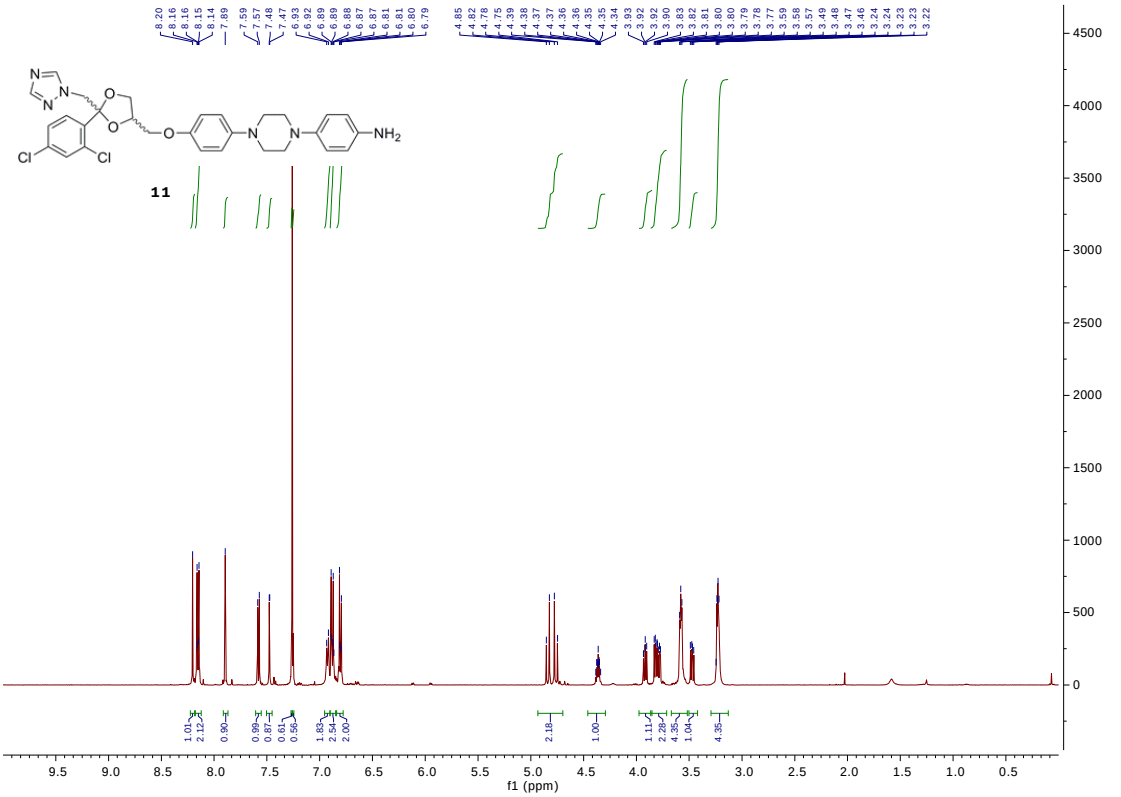

7
f1 (ppm)

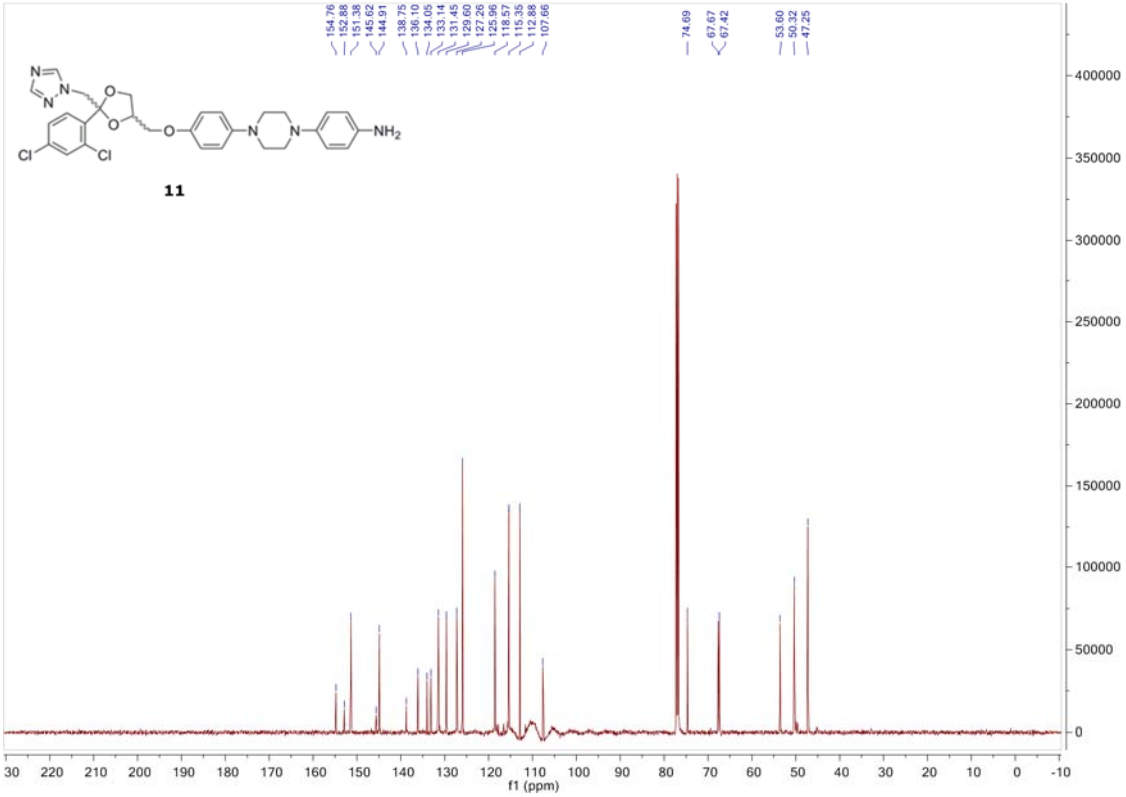

8.25
7.94
7.65
7.61
7.52
7.46
7.31
7.09
6.98
6.86
4.87
4.83
4.41
3.97
3.86
3.56
3.41
3.28
1.89
1.03
8
f1 (ppm)

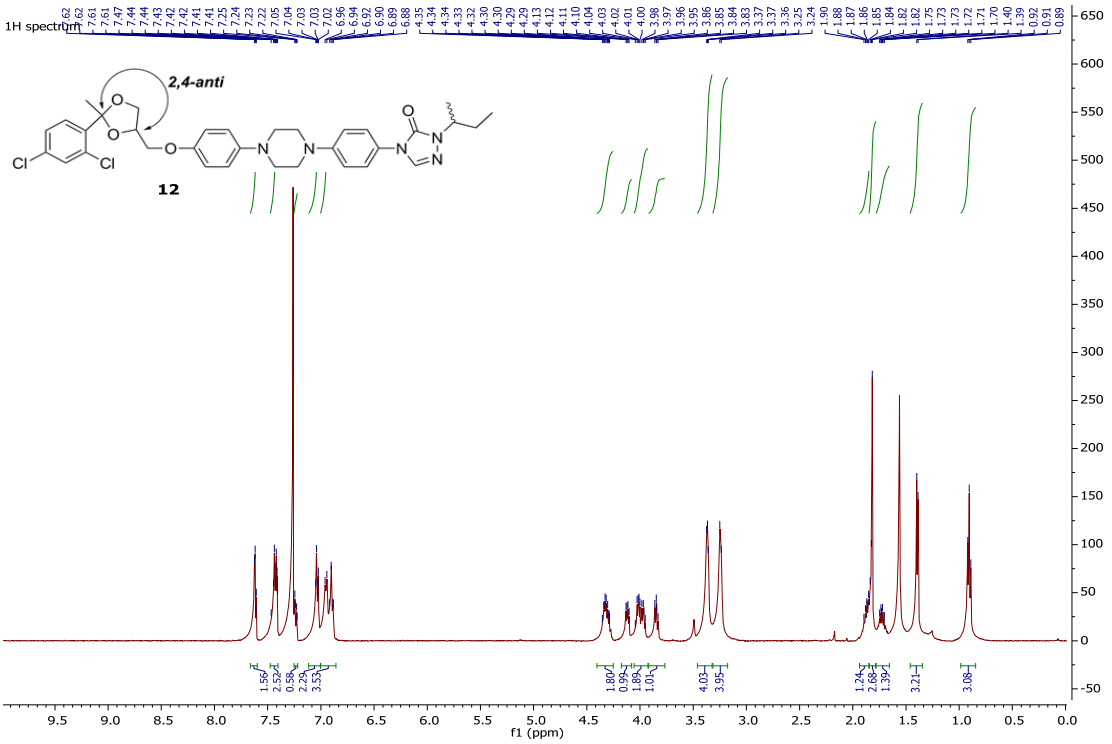

152.22
136.10
133.92
133.17
131.47
130.64
129.27
123.60
116.70
115.35
107.67
74.75
67.73
53.67
50.63
49.22
47.24
22.05
11.12
8
f1 (ppm)

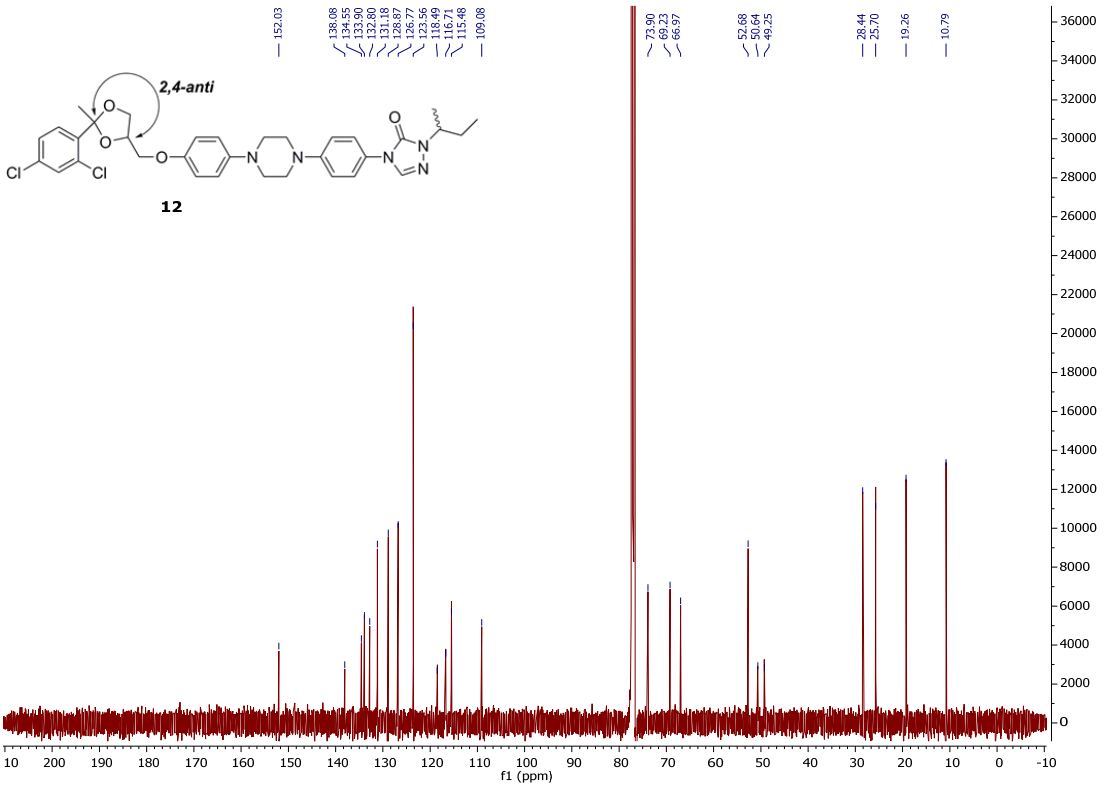

JRP-itzno2_protonforcarbon.1
1H spectrum
10
f1 (ppm)

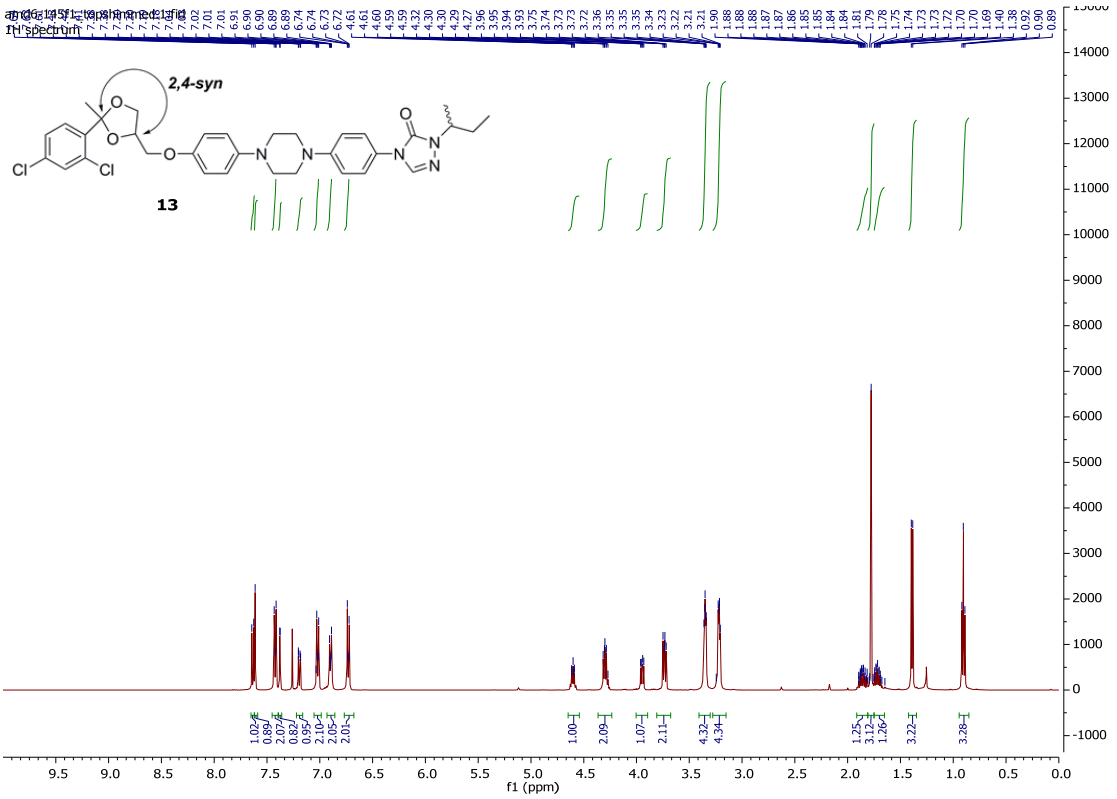

JRP-itzno2_carbon.1.fid
1D 13C spectrum
Probe: 5 mm PABBO BB-1H/D Z-GRD Z113652/0102
10
f1 (ppm)

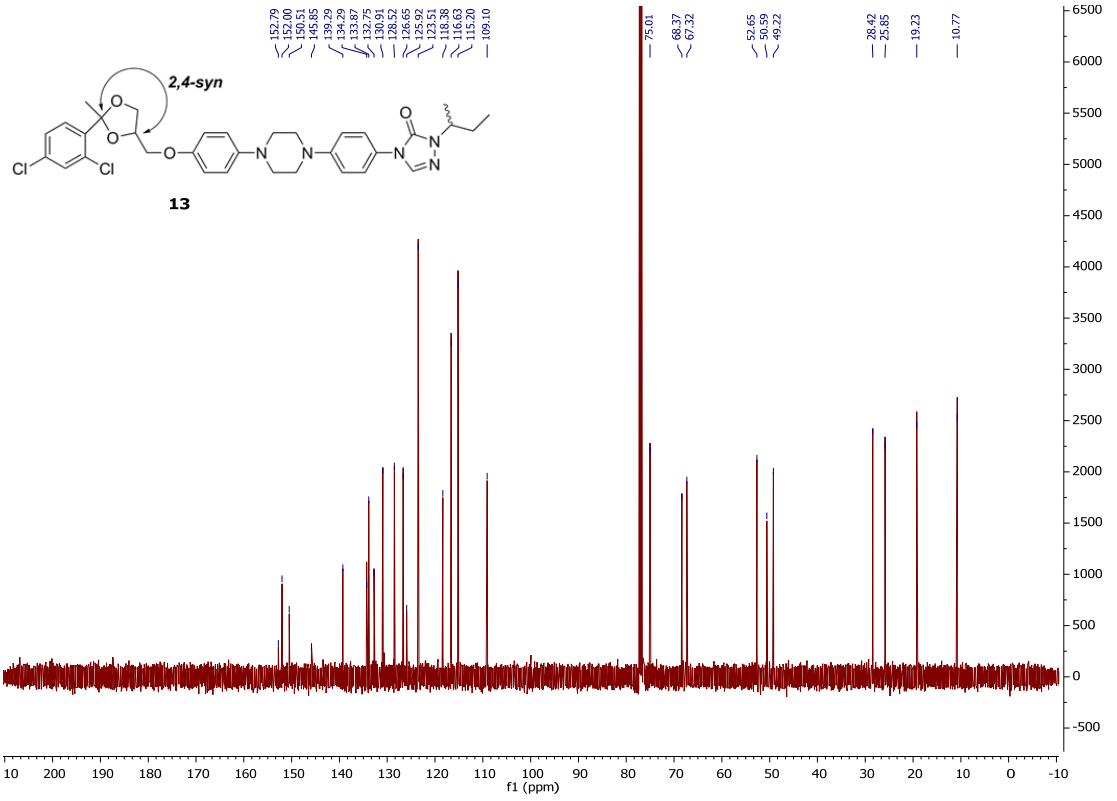

11
f1 (ppm)

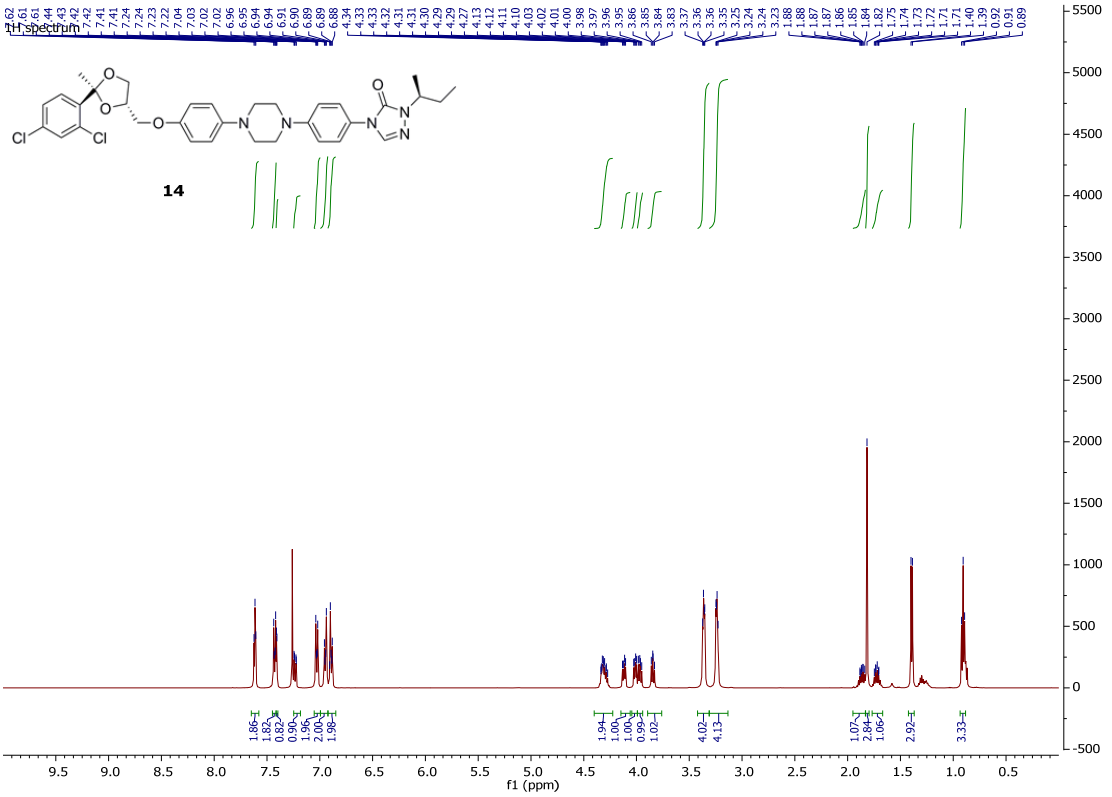

11
f1 (ppm)

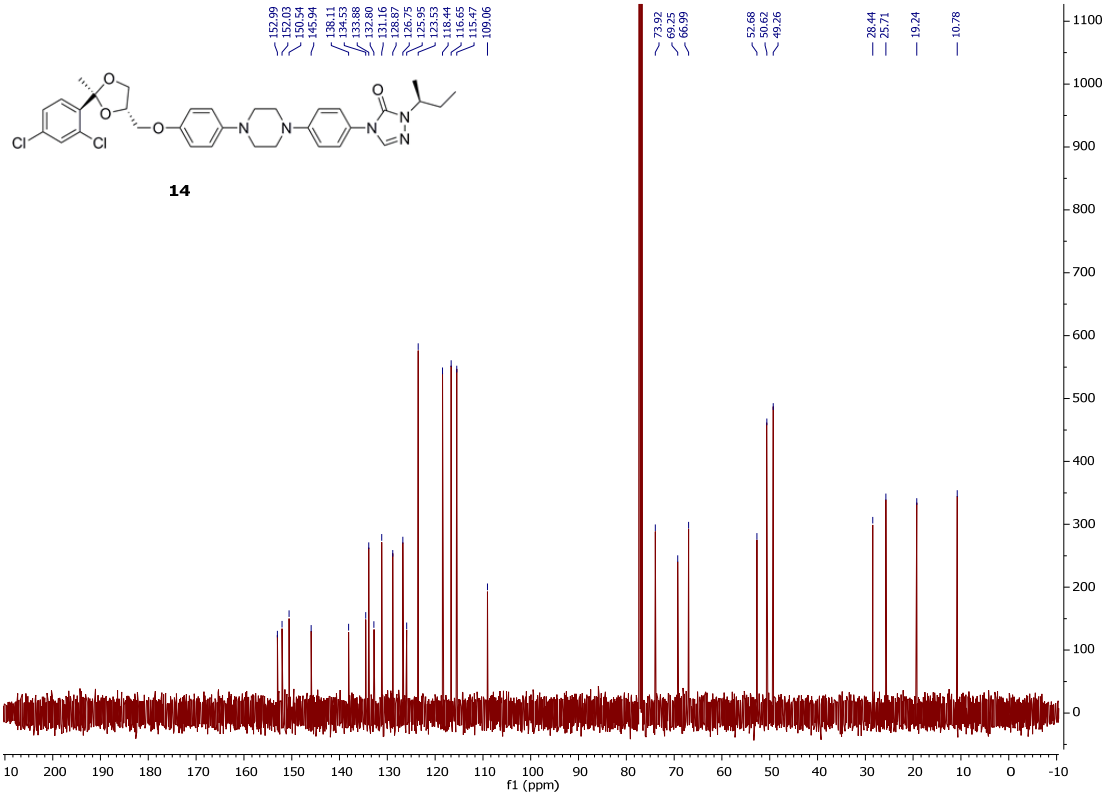

1H spectrum
2,4-anti
12
f1 (ppm)

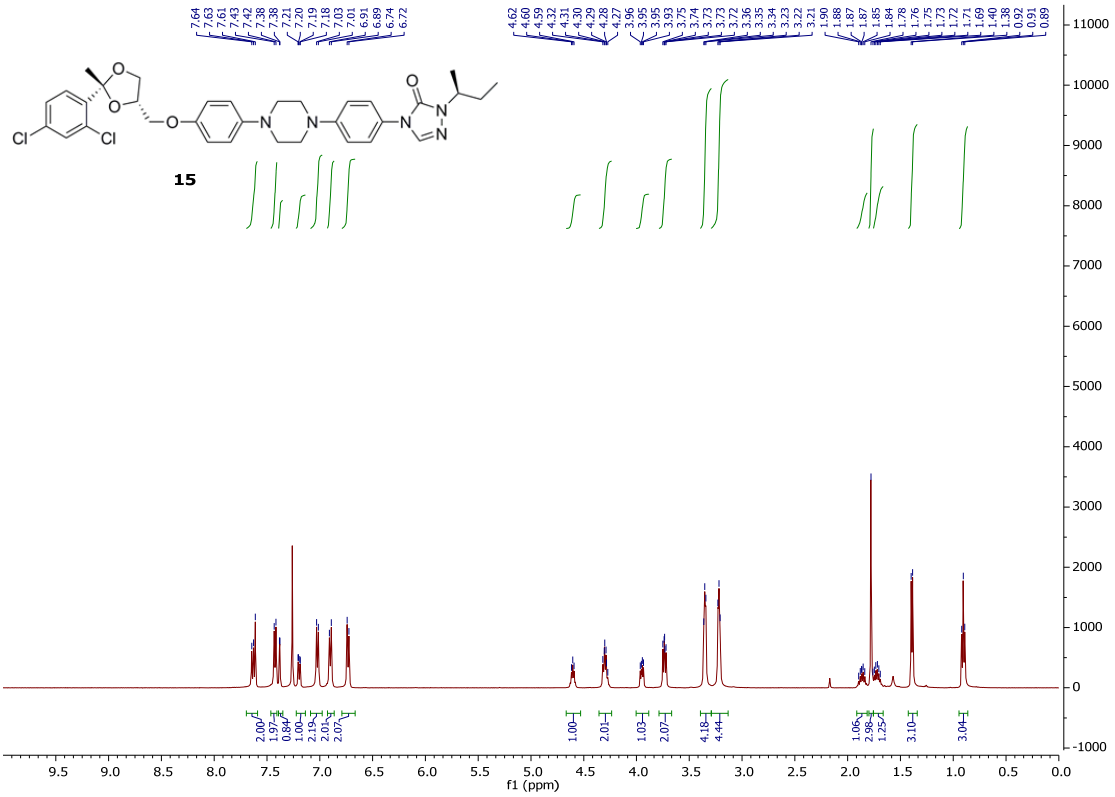

2,4-anti
12
f1 (ppm)

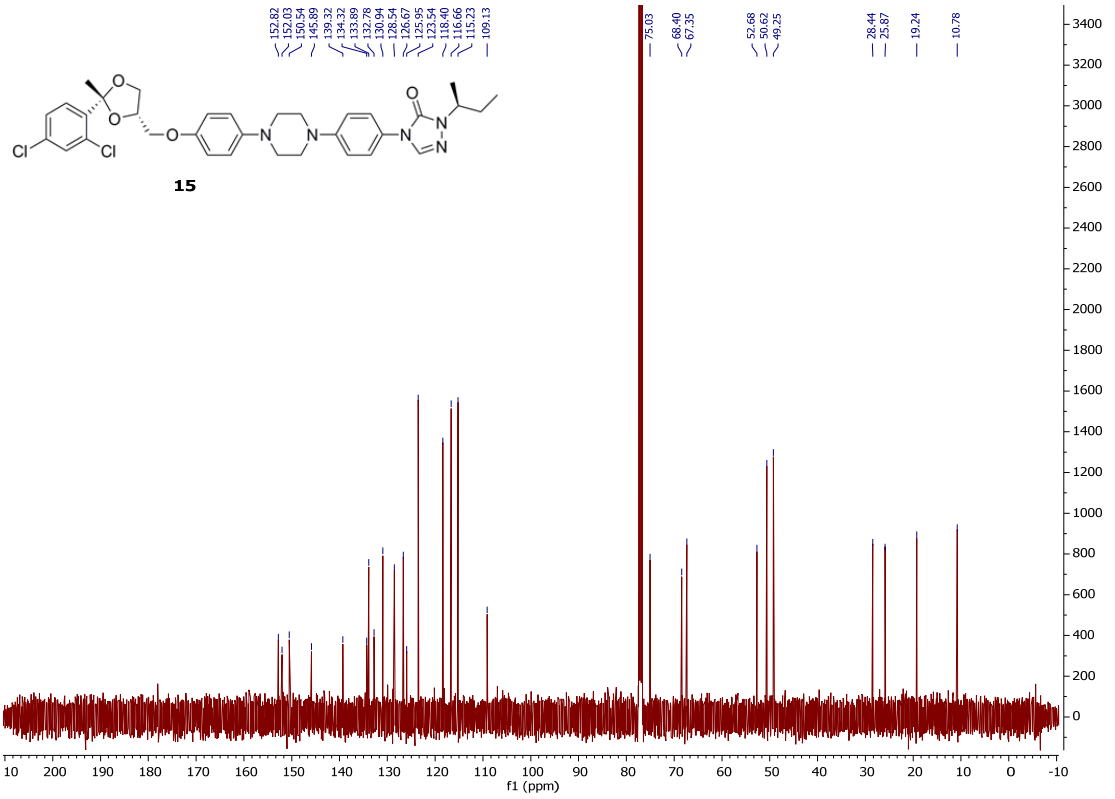

2,4-syn
13
f1 (ppm)


2,4-syn
13
f1 (ppm)

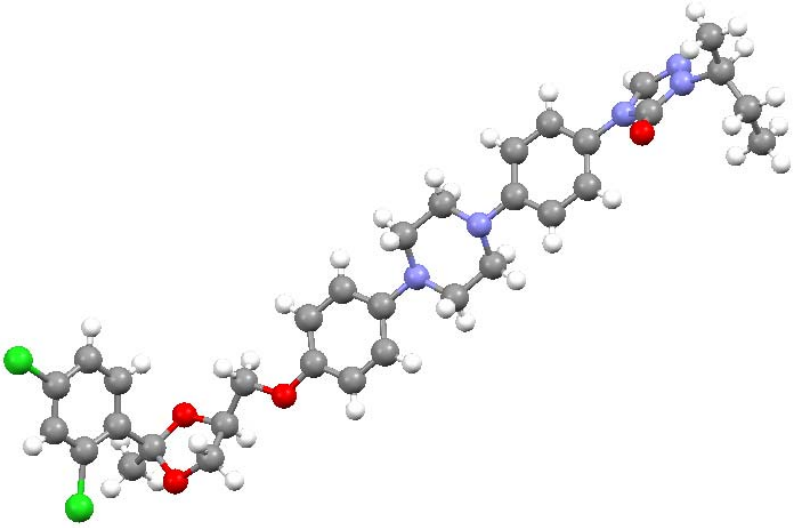

1H spectrum
14
f1 (ppm)

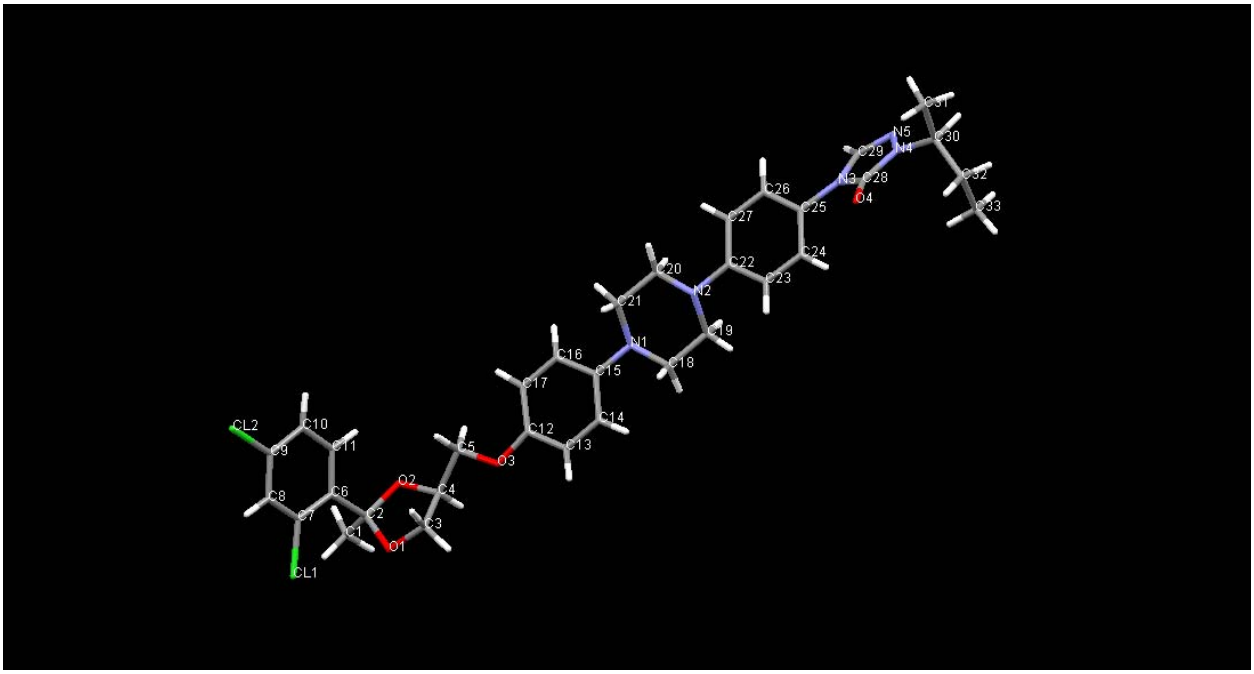

14
f1 (ppm)

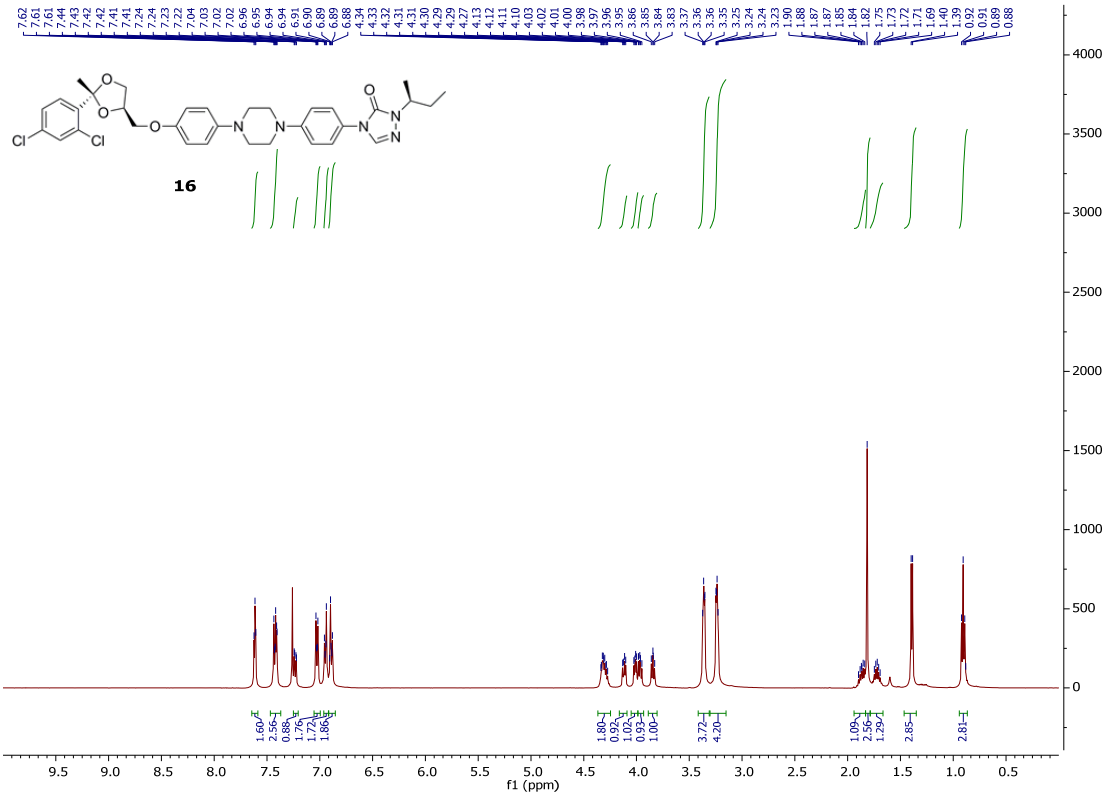

15
f1 (ppm)

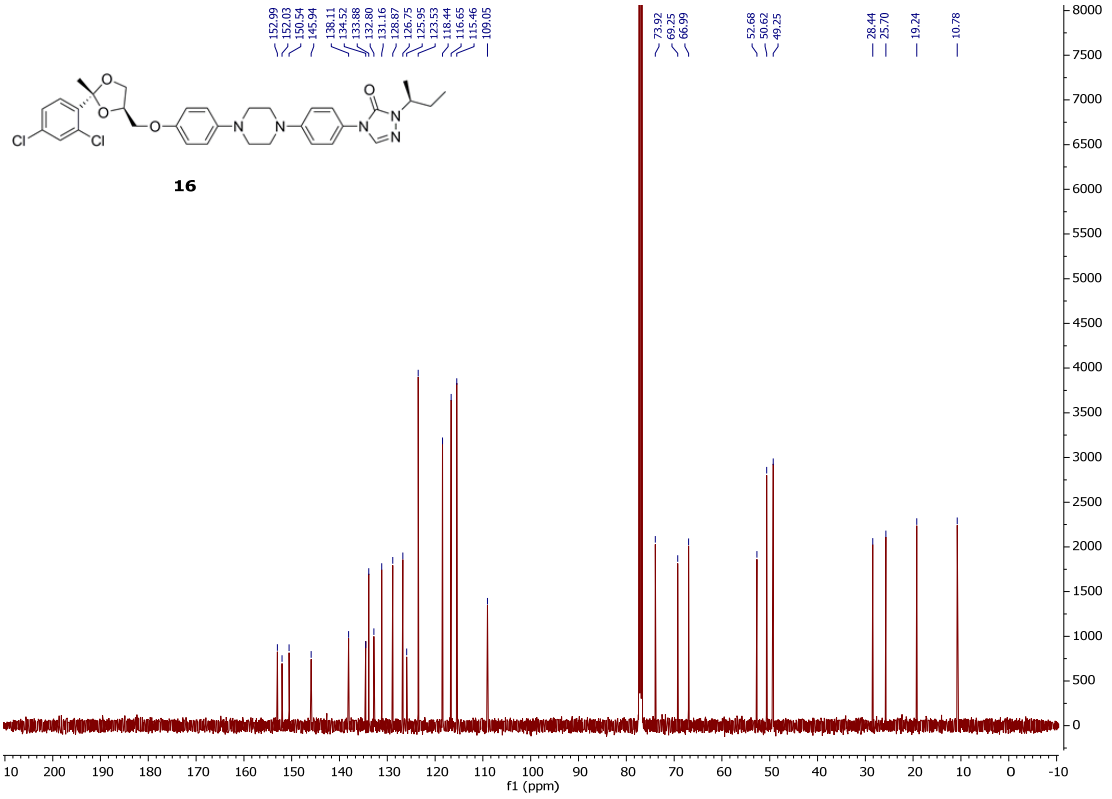

15
f1 (ppm)

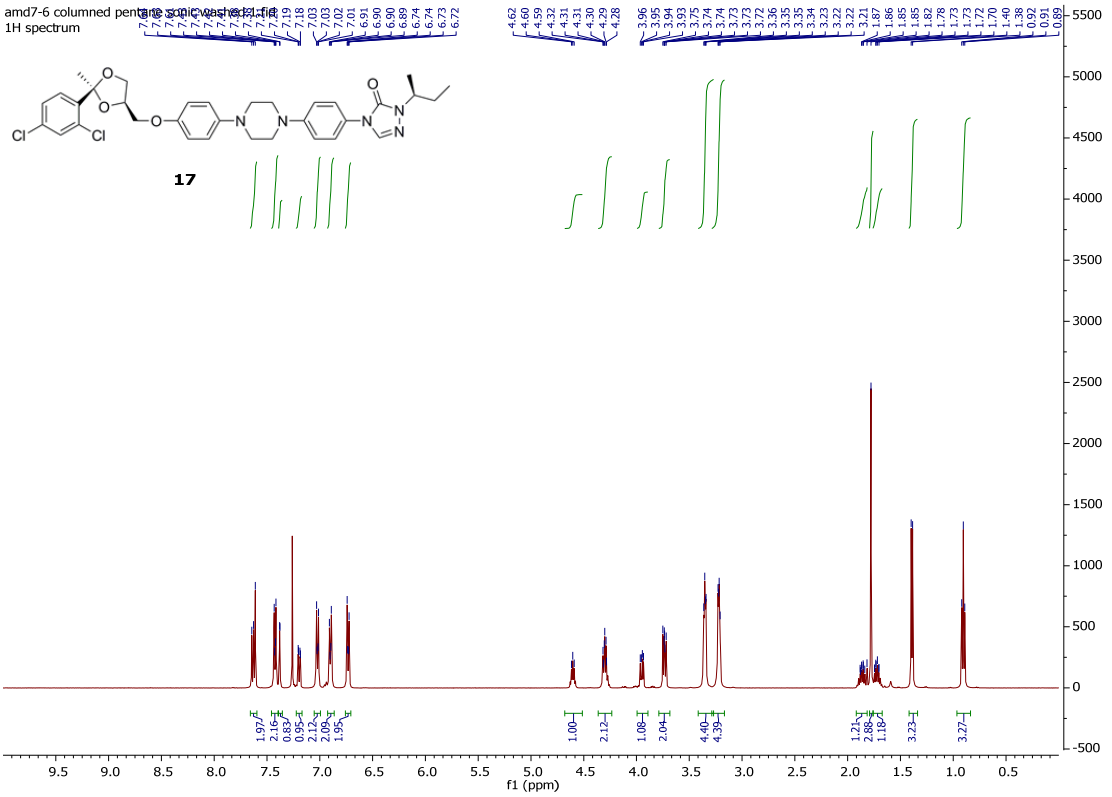
X-ray (**15**):

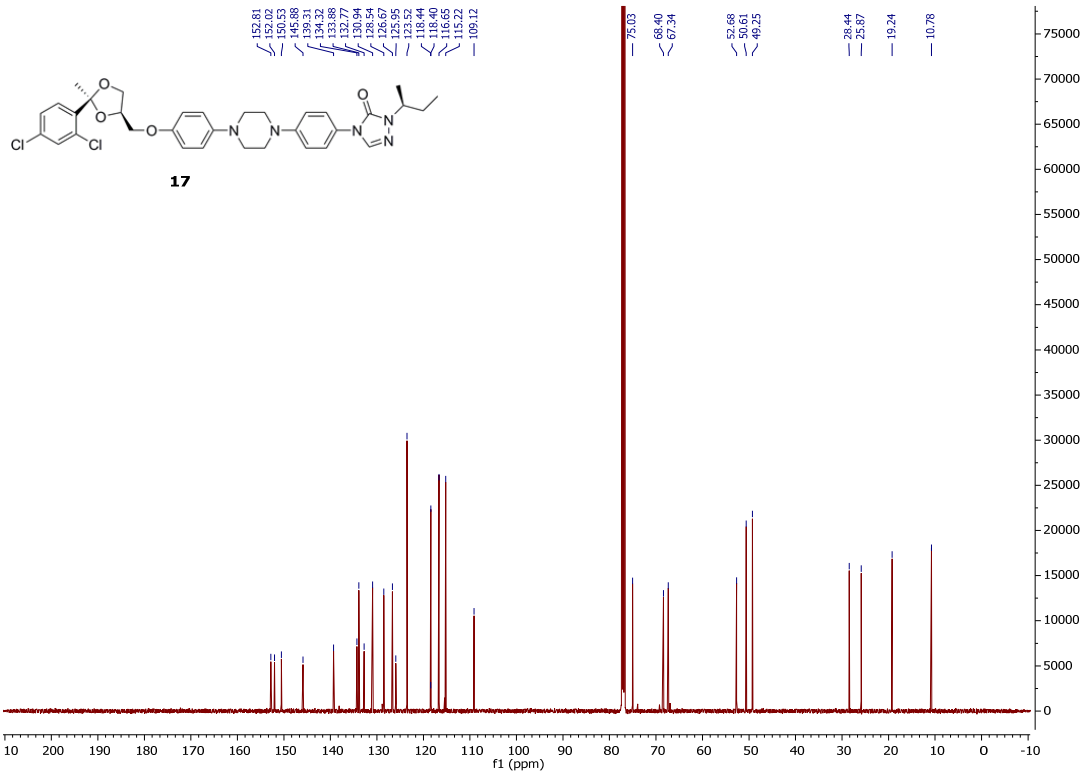


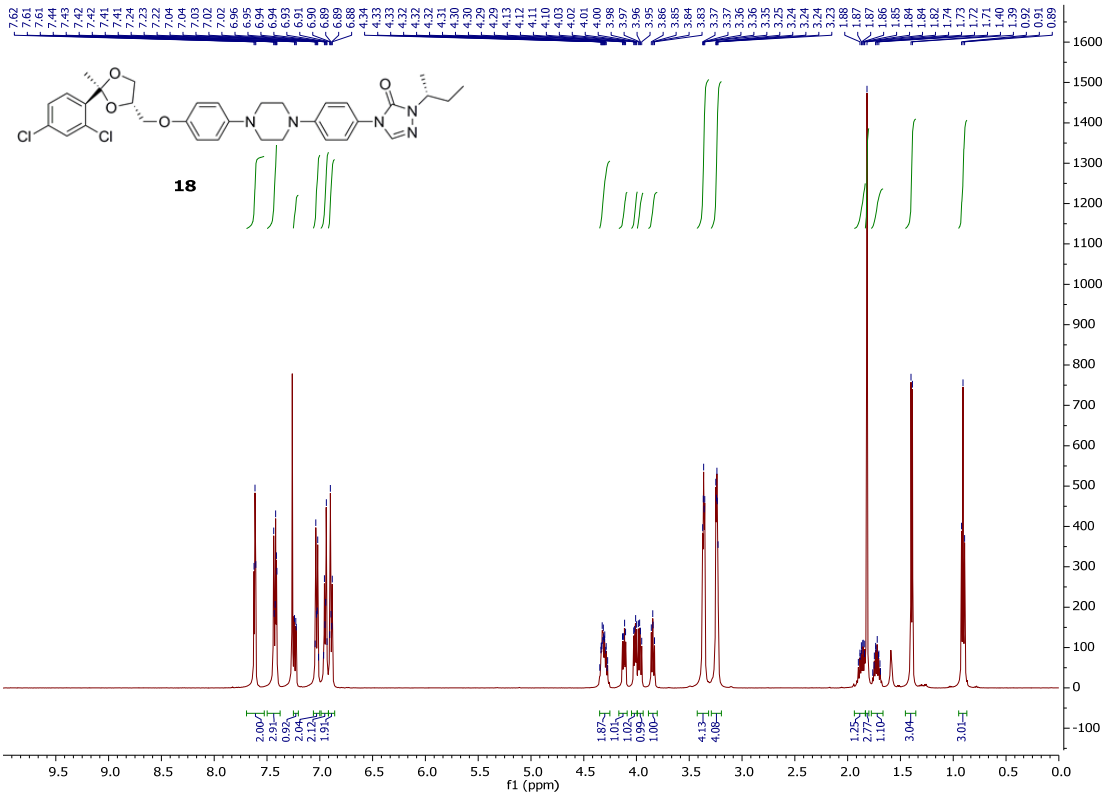

CL2
C10
C9
C11
C8
C6
C7
CL1
C1
C2
O1
O2
C3
C4
C5
O3
C12
C13
C14
C15
C16
C17
C18
N1
C19
C20
N2
C21
C22
C23
C24
C25
C26
C27
O4
C28
N3
C29
N4
N5
C30
C31
C32
C33

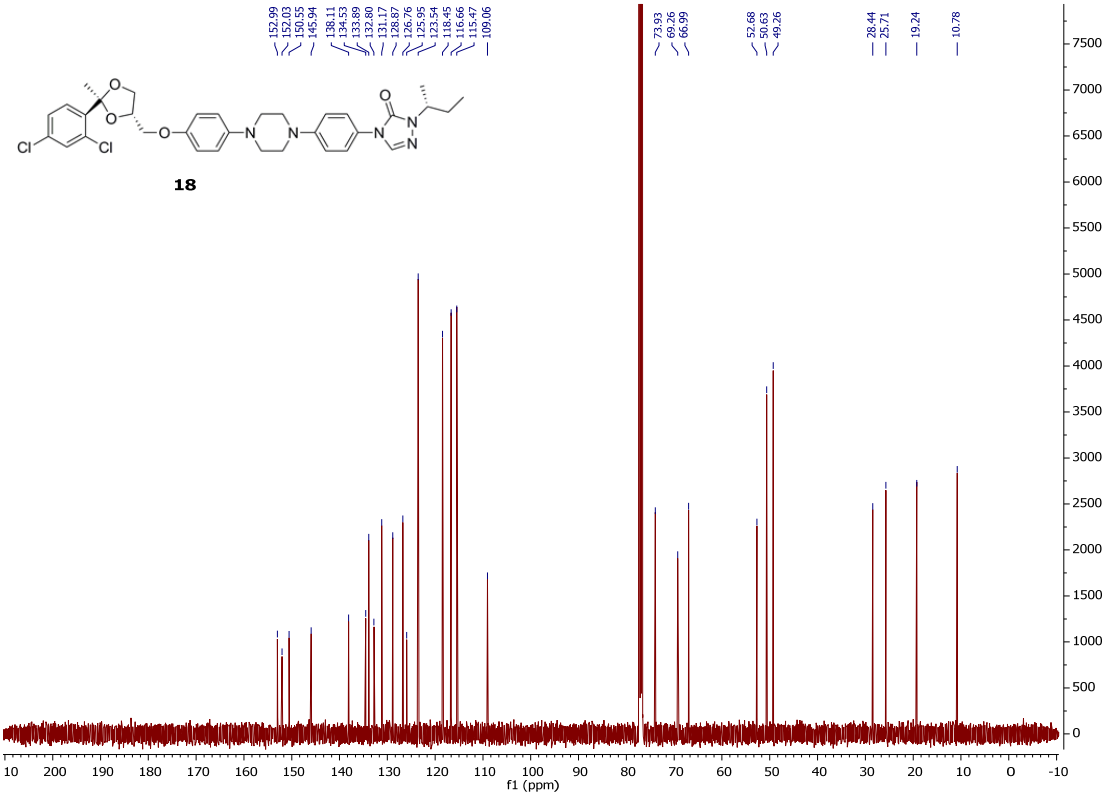

16
f1 (ppm)

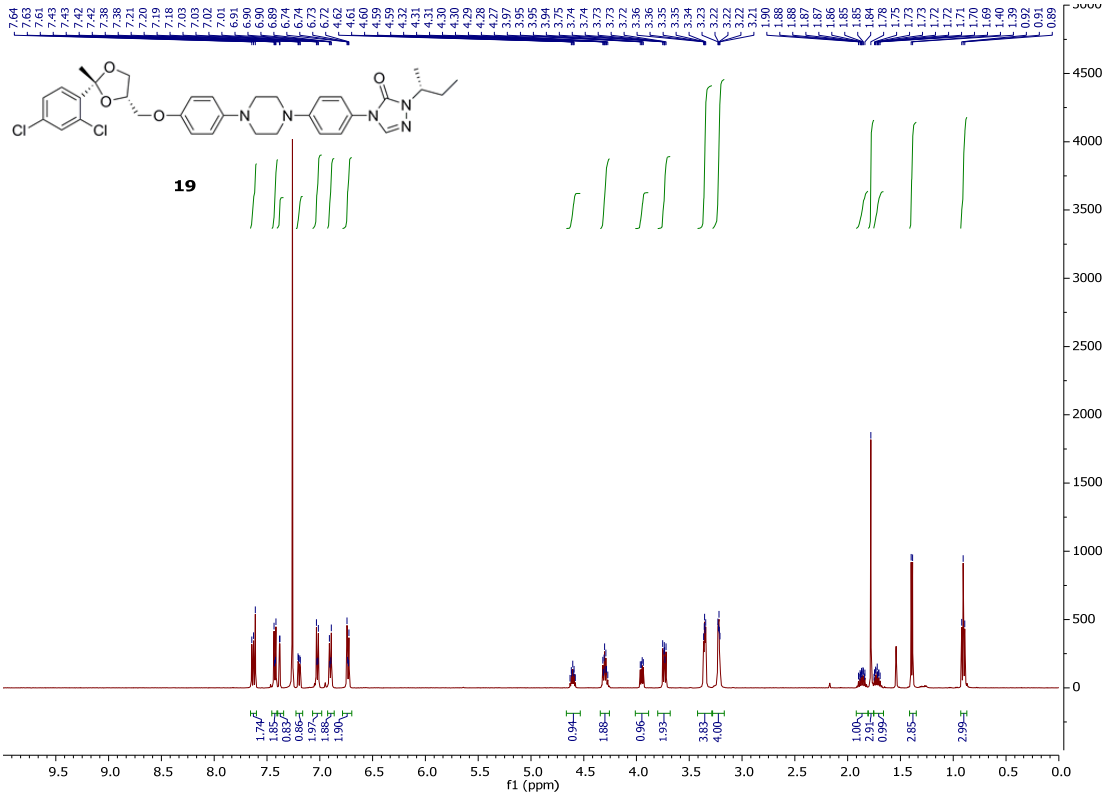

16
f1 (ppm)

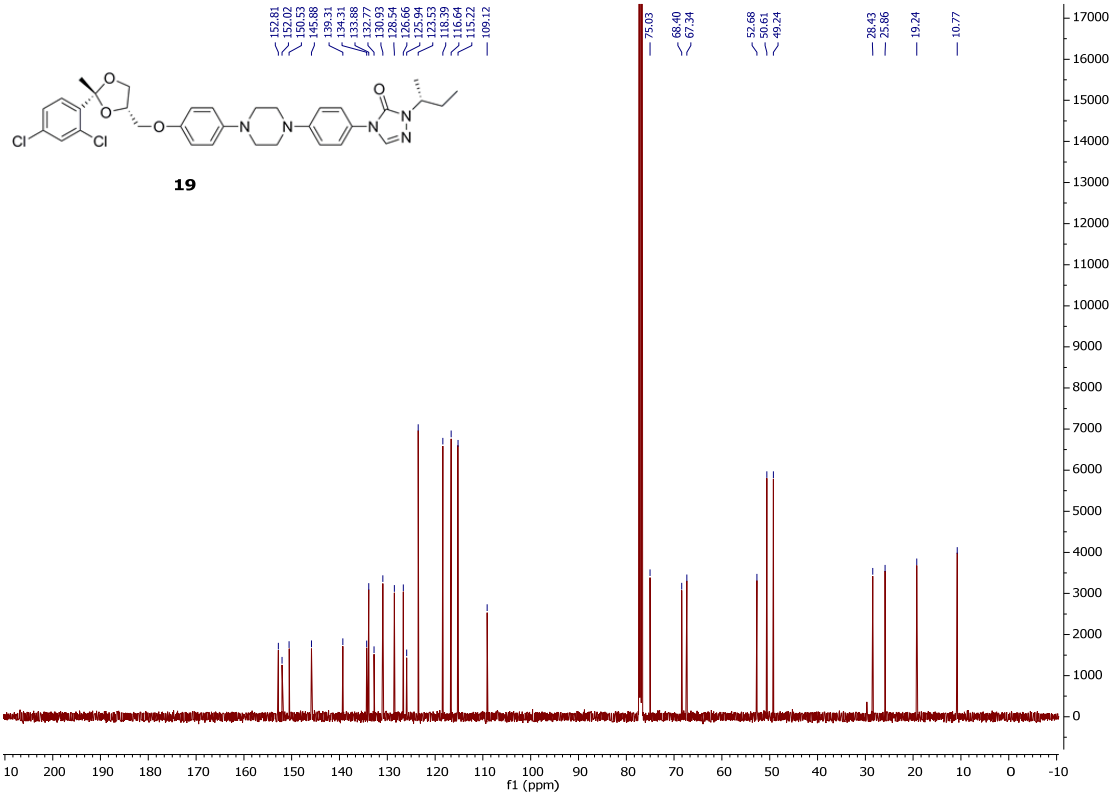

amd7-6 columned pentane sonic washed1.fid
1H spectrum
17
f1 (ppm)


17
f1 (ppm)

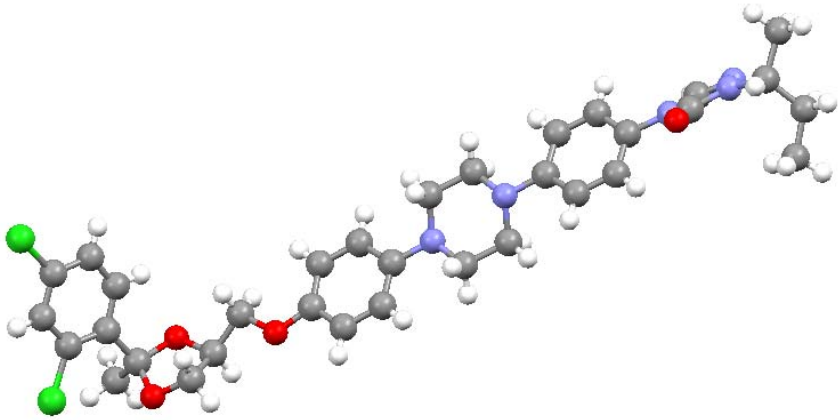

18
f1 (ppm)

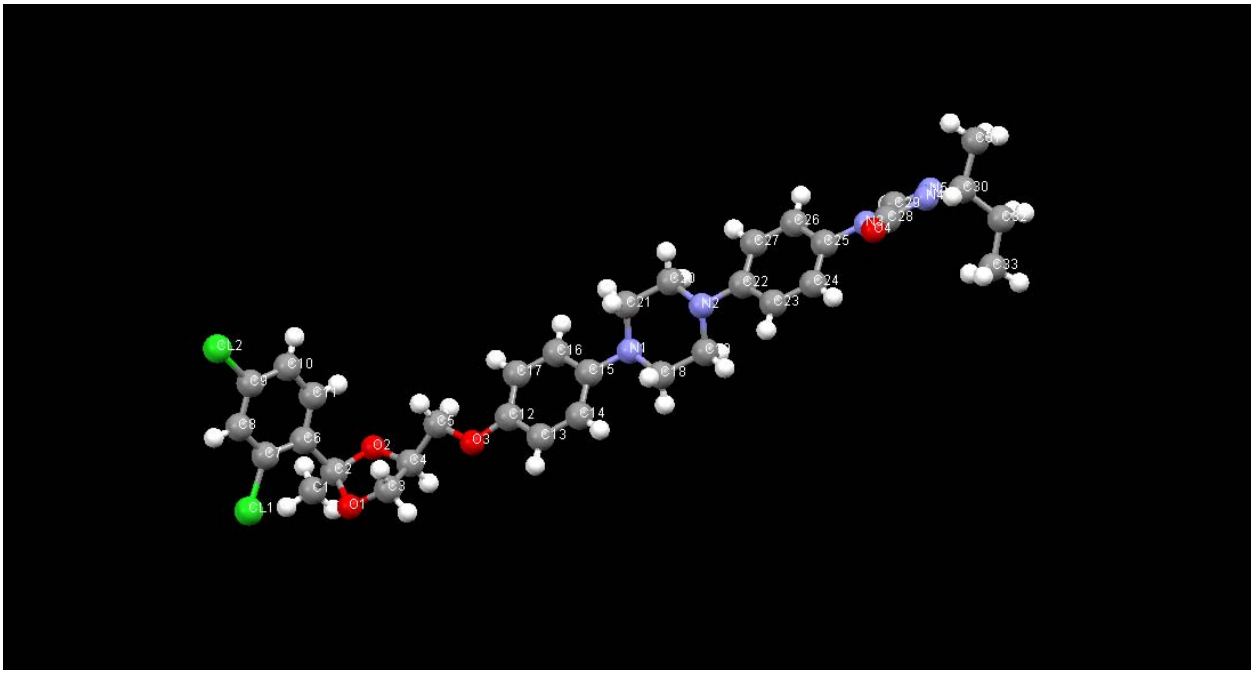

18
f1 (ppm)

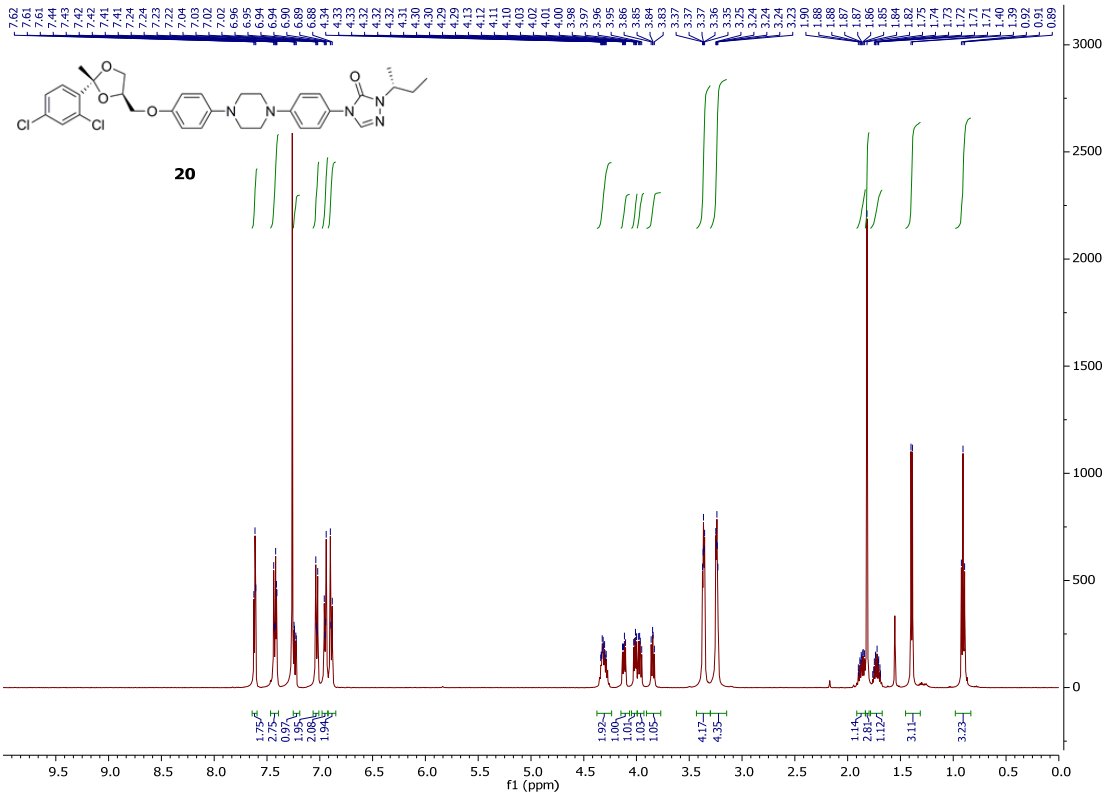

19
f1 (ppm)

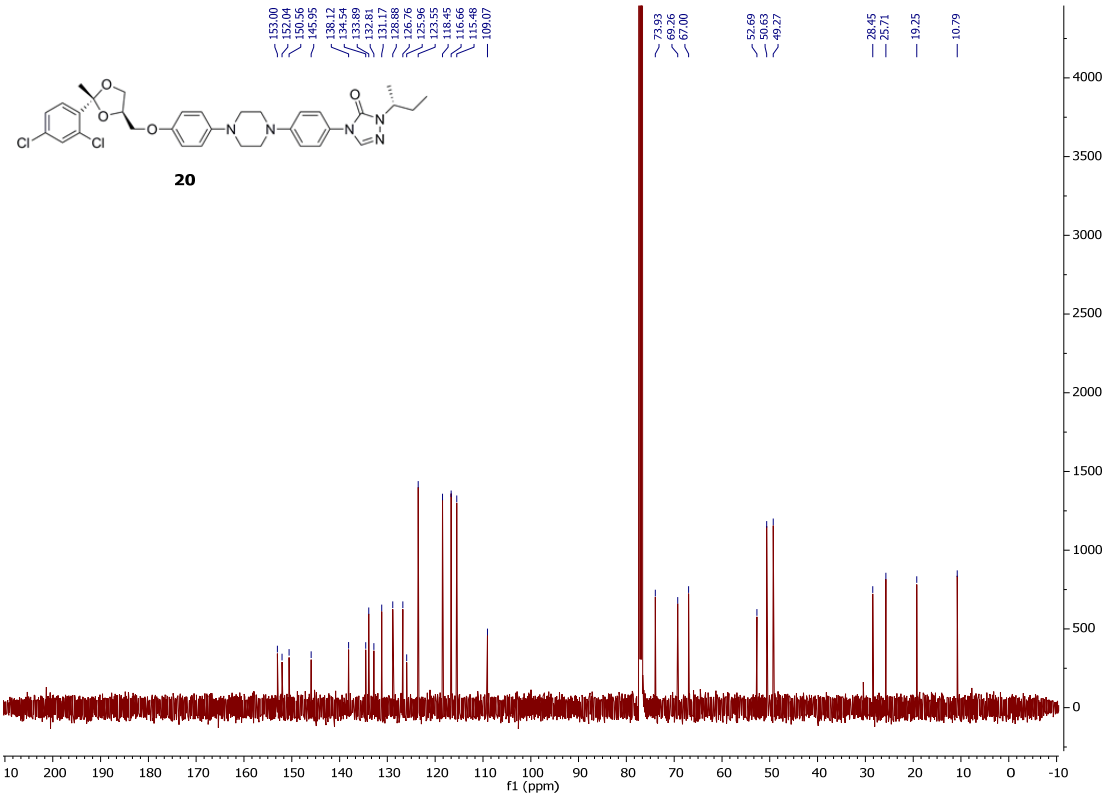

19
f1 (ppm)

X-ray (**19**):




CL2
C10
C9
C11
C8
C6
C7
O2
C5
O3
C12
C14
C13
C4
C2
C1
C3
CL1
O1
C16
C17
C15
N1
C18
C19
C21
C20
N2
C22
C23
C24
C26
C27
C25
N3
C28
N4
C29
N5
C30
C31
C32
C33


20
f1 (ppm)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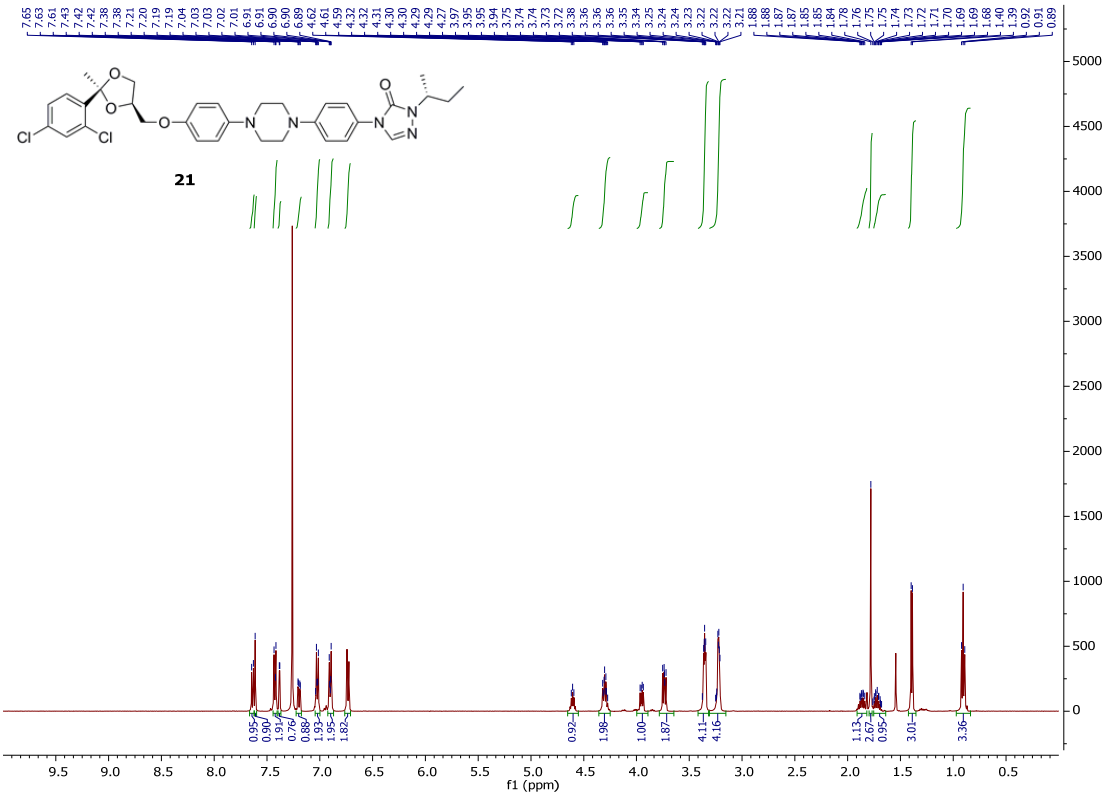
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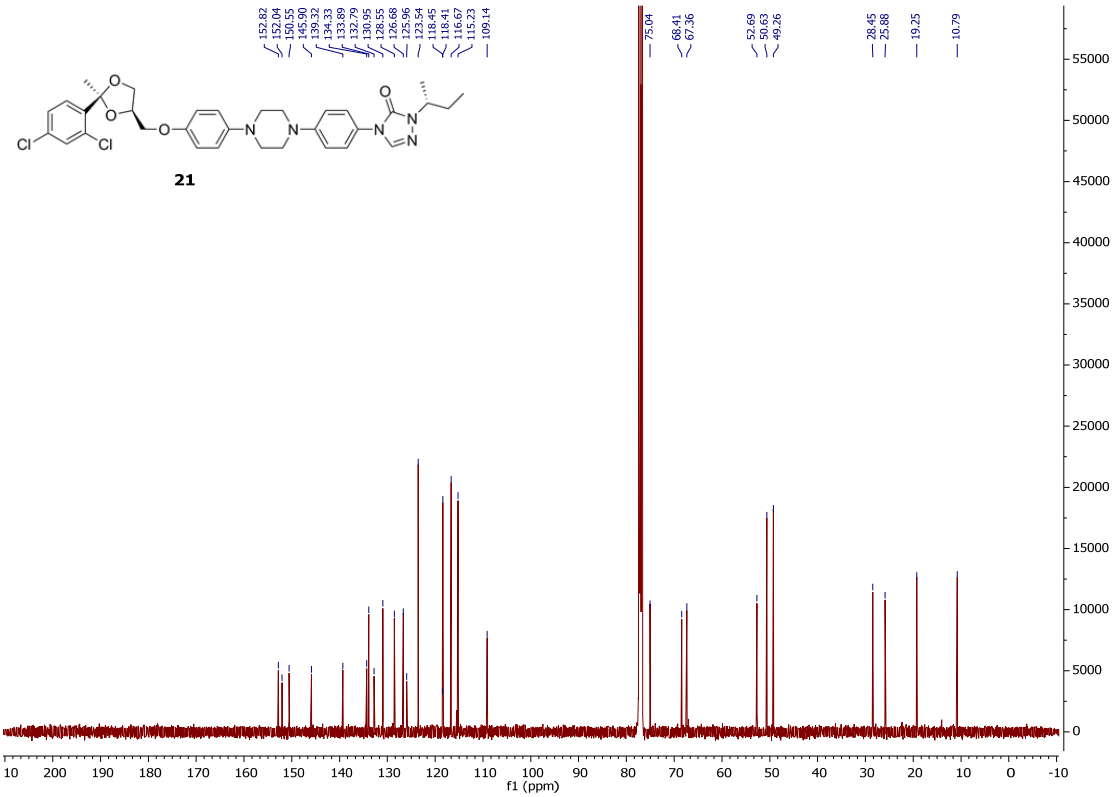
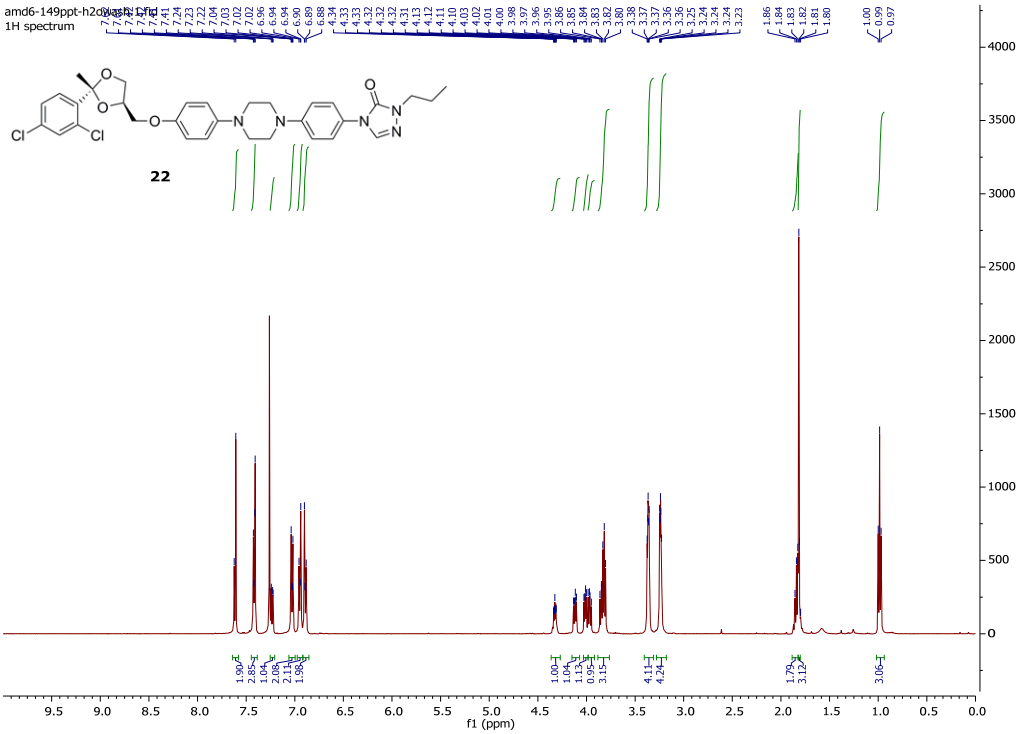
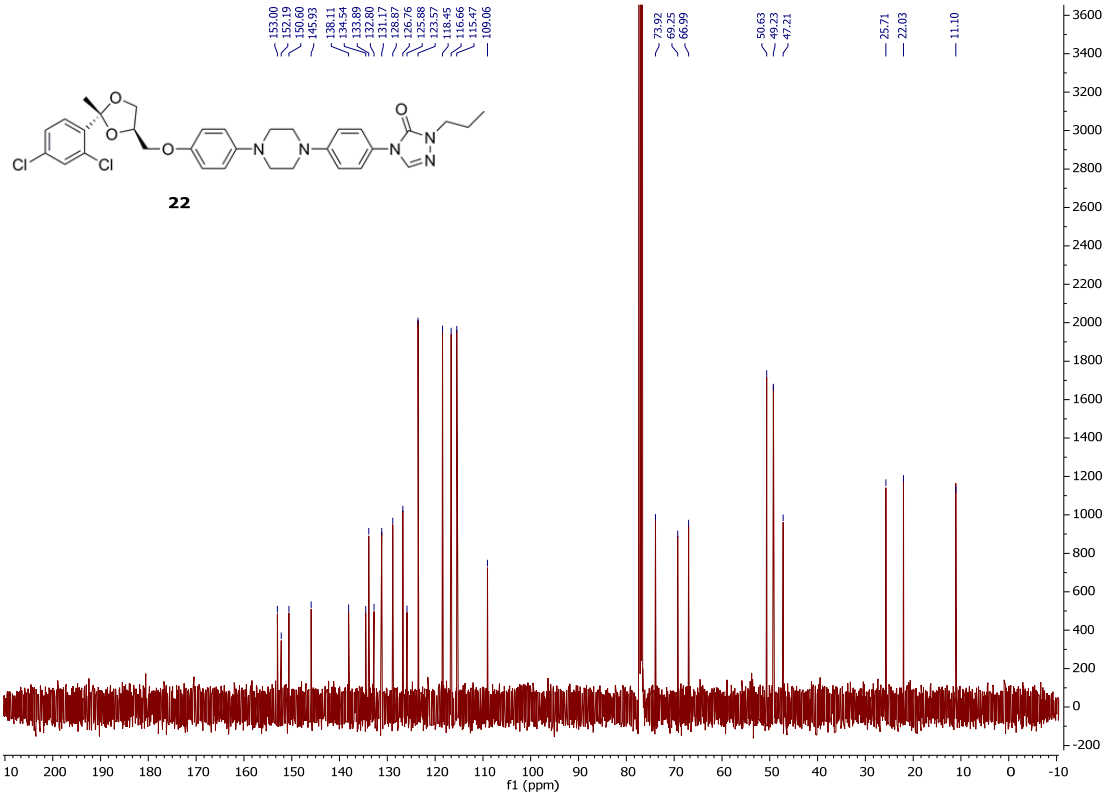
f1 (ppm)


21
f1 (ppm)


1H spectrum
22
f1 (ppm)


22
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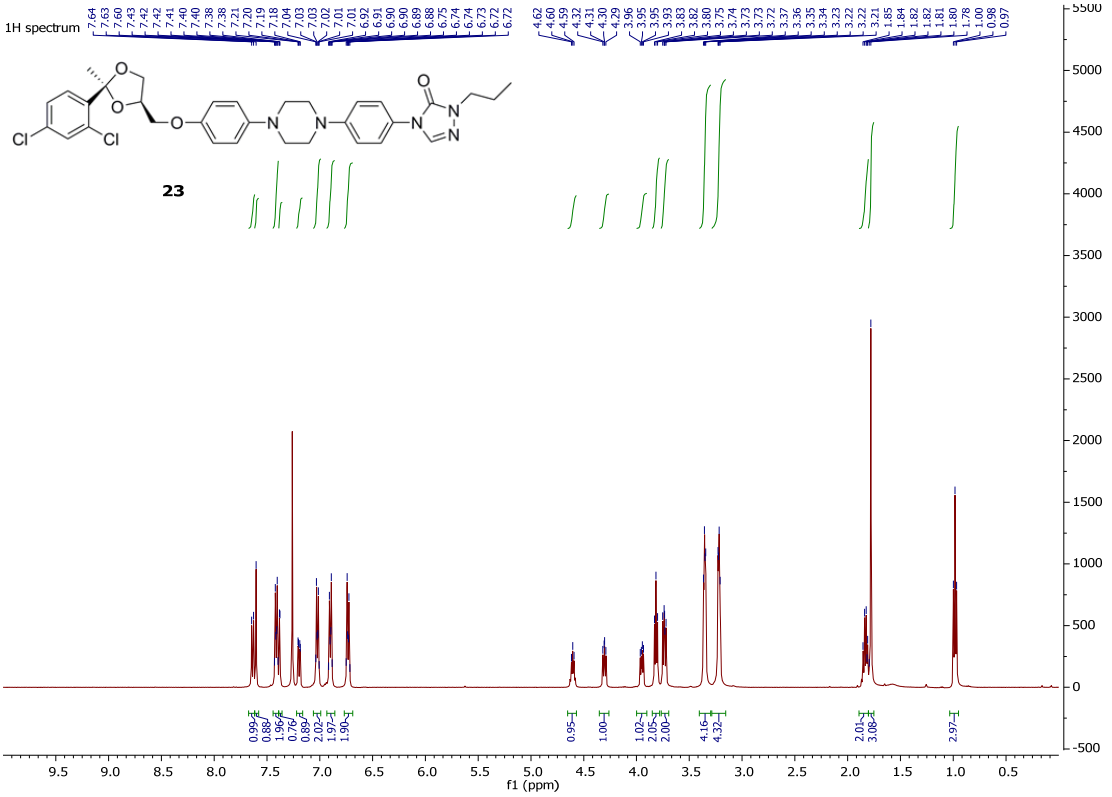
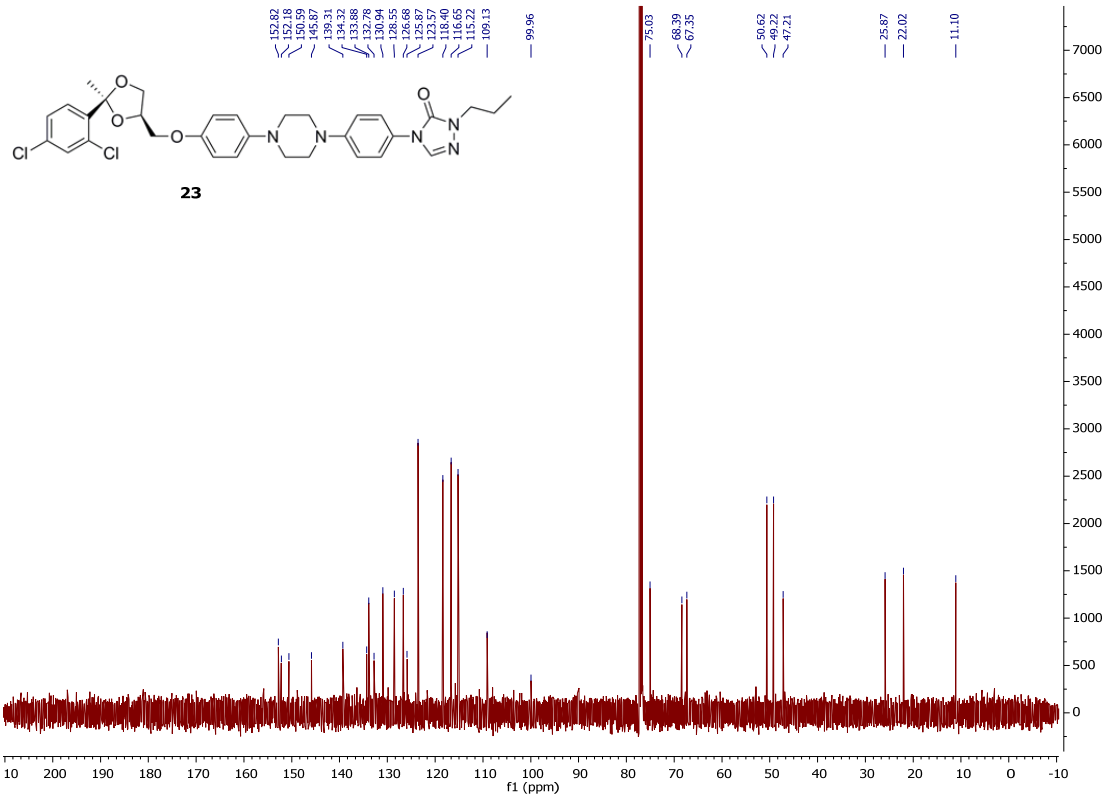
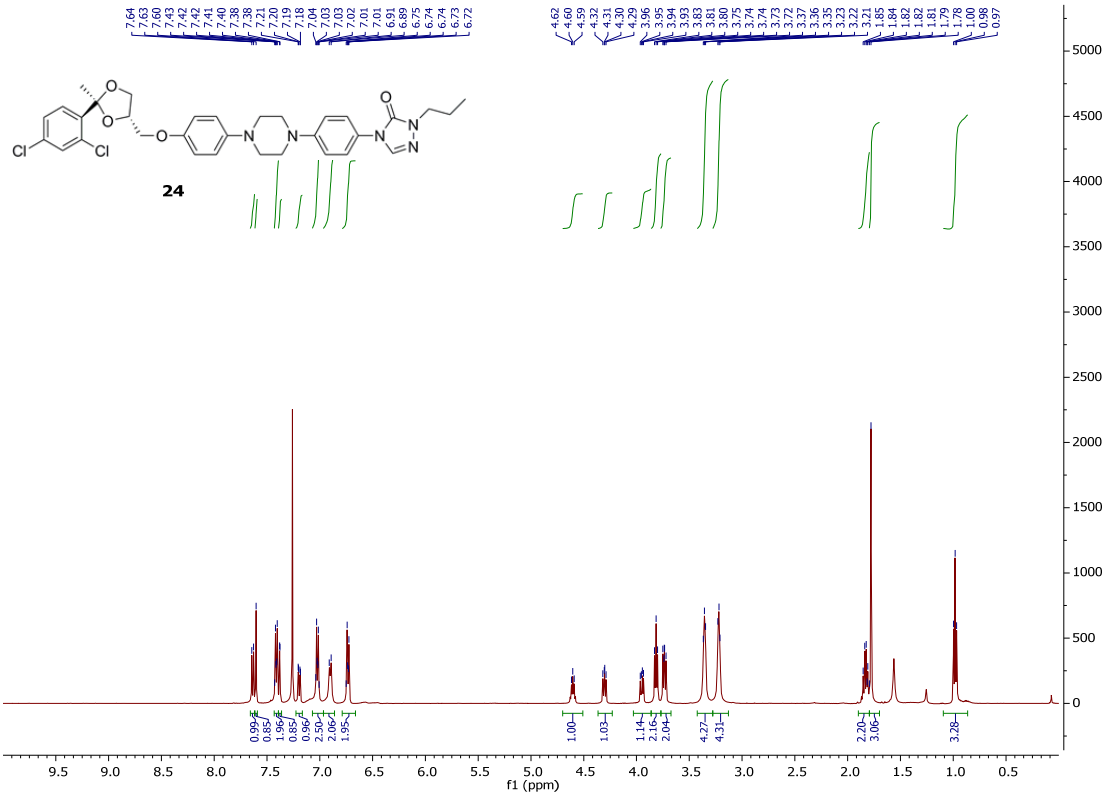
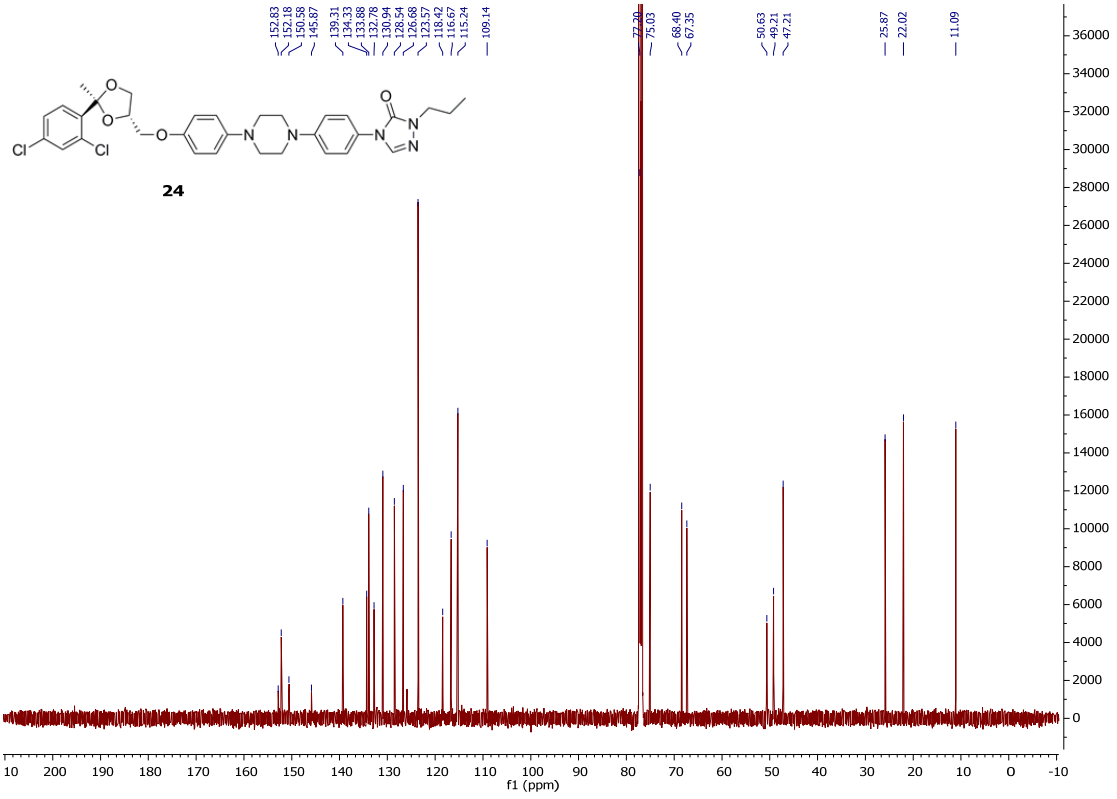
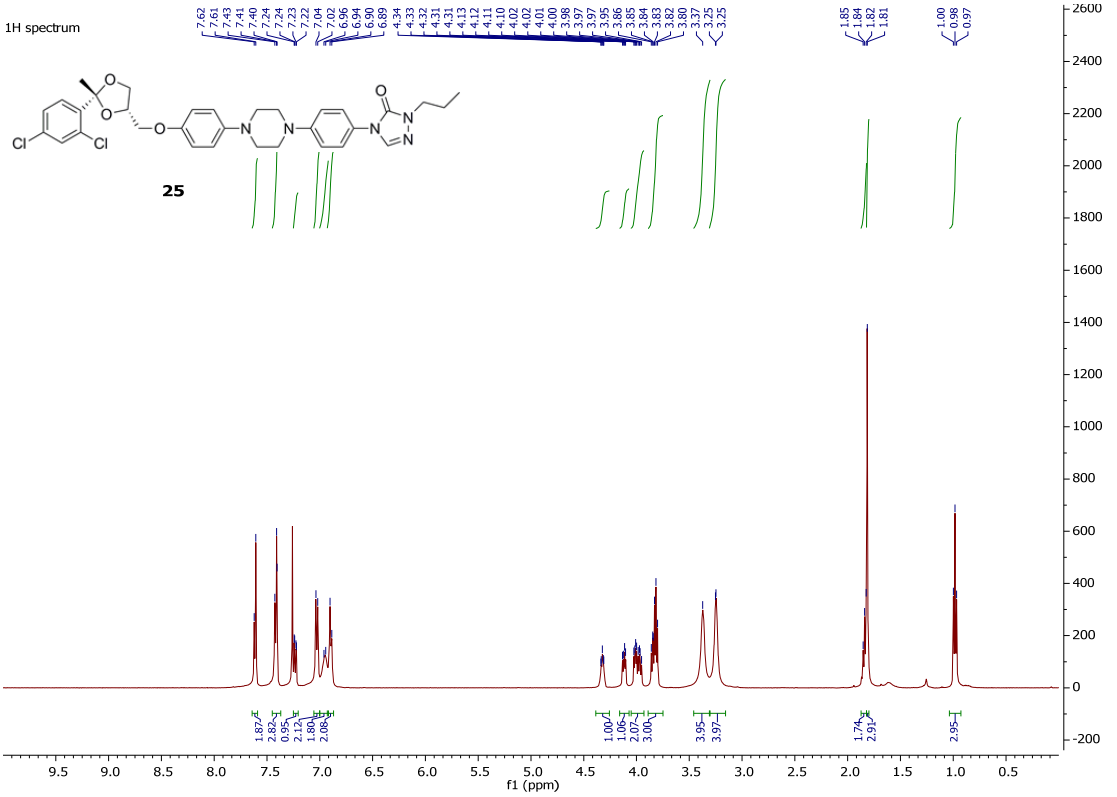
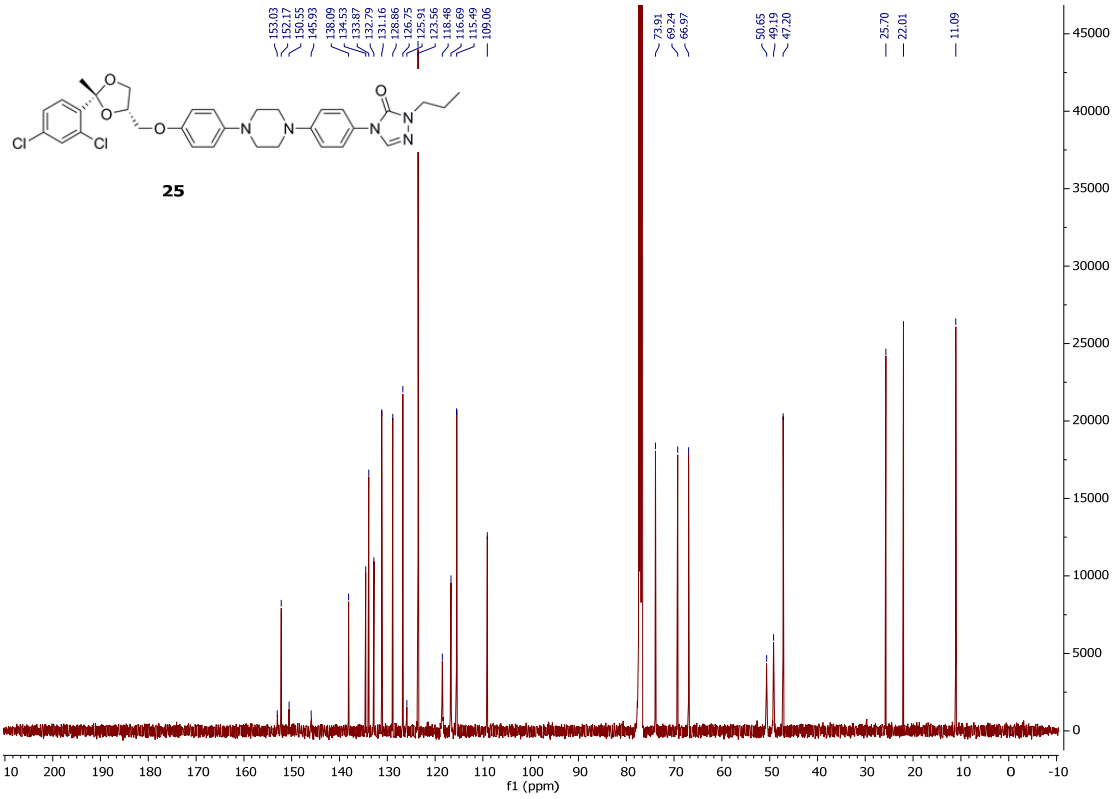

1H spectrum
23
f1 (ppm)


23
f1 (ppm)


24
f1 (ppm)

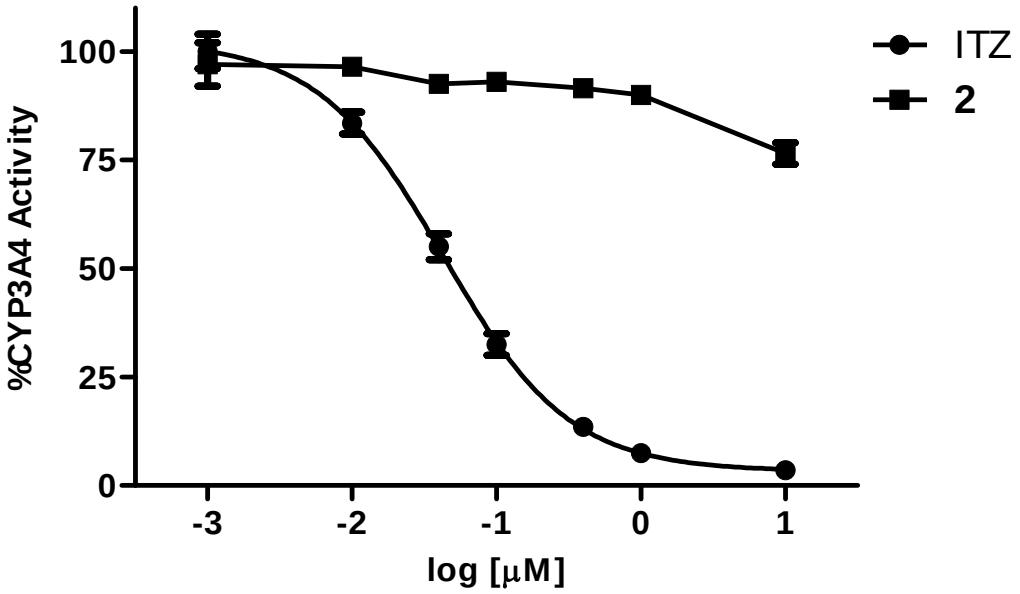

24
f1 (ppm)


1H spectrum
25
f1 (ppm)


25
f1 (ppm)

CYP3A4 Inhibition Studies.

Assay Protocol. ITZ and **2** were evaluated for their ability to inhibit CYP3A4 by Cyprotex by measuring their ability to inhibit metabolism of the known Cyp3A4 substrate testosterone via LC/MS/MS. Briefly, varying concentrations of either ITZ or **2** was mixed with microsomes (0.5 mg/mL) and testosterone (50 μM) in 50 mM potassium phosphate buffer containing 2 mM NADPH and 3 mM $MgCl_2$ at pH 7.4. Following a 10 min incubation at 37 °C, the assay was quenched with the addition of MeCN containing an internal standard. The samples were centrifuged and the quantity of testosterone metabolite in the supernatant was determined by LC/MS/MS.



Supplementary Figure 1. Inhibition of CYP3A4 by ITZ and **2**. This figure details a representative experiment performed in duplicate. Calculated IC_{50} values (μM) for inhibition of CYP3A4 activity by the two compounds are as follows: ITZ, $IC_{50} = 0.051 \pm 0.01$; **2**, $IC_{50} > 10$.