SUPPORTING INFORMATION

A one-pot five component reaction for the synthesis of tetrazol-benzofuran hybrids thorough Ugi-azide/heteroannulation cascade sequence and their inhibitory activity against *Mucor lusitanicus*

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General information

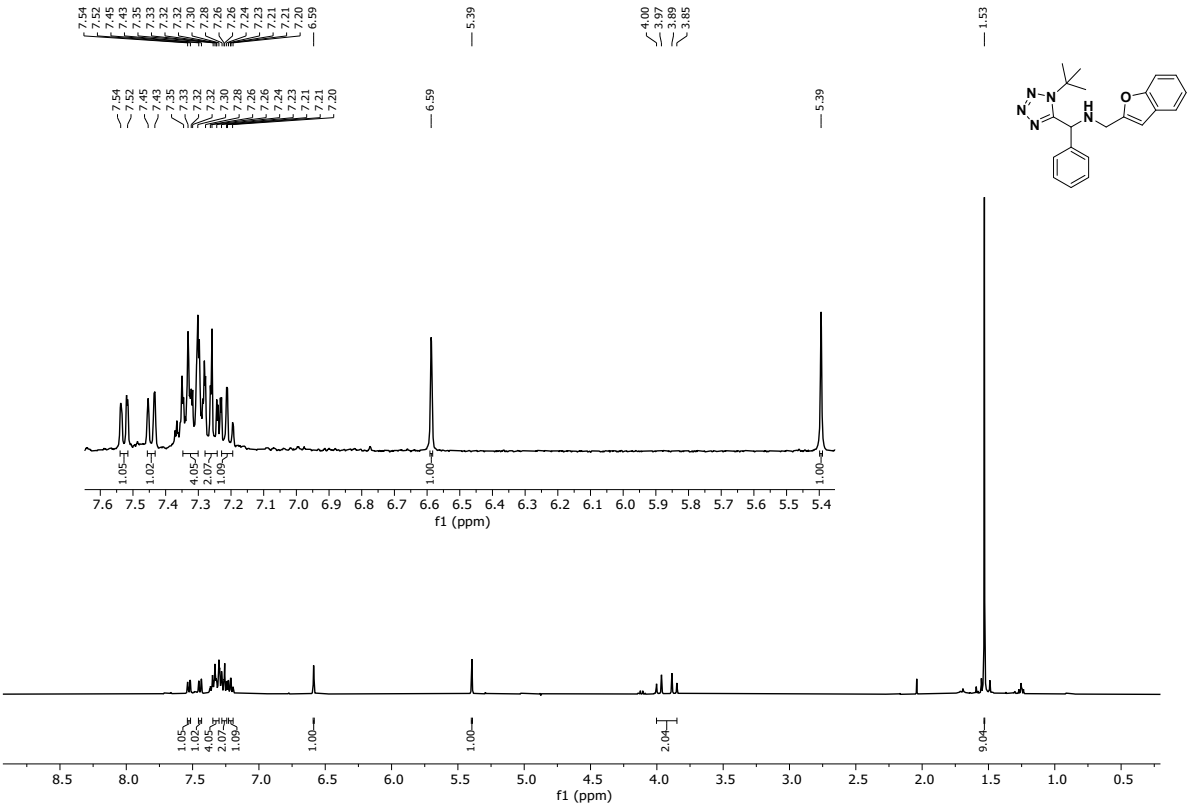
Reagents and solvents were purchased from commercial suppliers and used without further purification. Reaction progress was monitored by thin layer chromatography (TLC) using silica gel 60 F_{254} from Merck and the spots were visualized under UV light at 254 or 365 nm. Column chromatography was performed using silica gel (230-400 mesh). Chemical names and drawings were obtained using ChemDraw Professional (version 15.0.0.106). Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. HRMS spectra were acquired on a Bruker MicroTOF-II spectrometer. NMR spectra were recorded in a Varian Mercury spectrometer (400 MHz) and Bruker AMX Advance III spectrometer (500 MHz). Chemical shifts were reported as δ values (ppm). Coupling constants *J* are reported in Hertz (Hz). Internal reference for NMR spectra is in respect to TMS at 0.0 ppm. Multiplicities are reported, using the standard abbreviations, as follows: singlet (s), apparent triplet (at), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), broad signal (bs), doublet of triplets (dt), triplet of doublets (td), quartet of doublets (qd), multiplet (m), apparent triplet (at). NMR spectra were analyzed using the MestreNova software (version 6.0.2-5475). IR spectra were recorded on a Thermo Scientific NICOLET iS10 by ATR method using neat compounds. The wavelengths are reported in reciprocal centimeters (ν/cm^{-1}).

General procedure for 1,5-disubstituted tetrazol-benzofurans *bis*-heterocycles 9a-n (GP1)

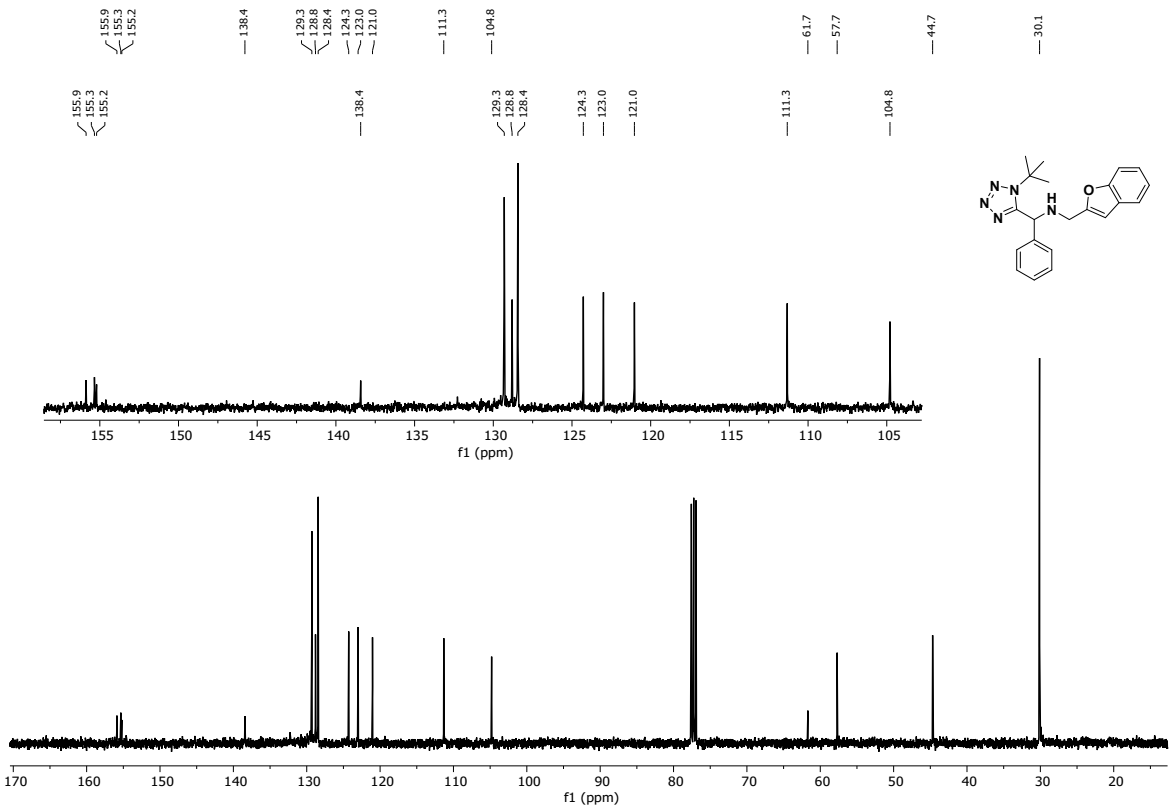
In a dry pressure tube, propargylamine (1.0 equiv.) and aldehyde (1.0 equiv.) were dissolved on TFE (1 M) and reacted for 5 min at room temperature. Sequentially, isocyanide (1.0 equiv.) and $TMSN_3$ (1.2 equiv.) were added and stirred at room temperature until complete by TLC (approximately 24 h). Later, the reaction mixture was evaporated under reduced pressure. The residue was suspended on dry DMF (0.8 M) and $(PPh_3)_2PdCl_2$ (0.1 equiv.), CuI (0.08 equiv.), Et_3N (0.1 M) and 2-iodophenol **8** (1.2 equiv) were loaded and deoxygenated via cannula under a nitrogen atmosphere. Next, the reaction mixture was heated to 70°C for 1 hour. Finally, ethyl acetate (3 mL) was added and filtration through a pad of celite and silica gel was done. The liquor mothers were poured into a mixture of water (7 mL) and ethyl acetate (5 mL). The aqueous phase was extracted twice with ethyl acetate (5 mL). The combined organic phases were washed with water (2x5 mL), dried over Na_2SO_4, filtered, and concentrated under reduce pressure. Purification by flash column chromatography Hexane:EtOAc 7:3 (v/v) afforded the 1,5-disubstituted tetrazole-benzofurans **9a-n**.

N-(benzofuran-2-ylmethyl)-1-(1-(*tert*-butyl)-1*H*-tetrazol-5-yl)-1-phenylmethanamine (**9a**)

Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), benzaldehyde (37 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), *tert*-butyl isocyanide (41.0 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9a** was obtained as a yellow semisolid (59.0 mg, 45%). R_f = 0.48 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.35–7.30 (m, 4H), 7.28–7.24 (m, 2H), 7.23-7.20 (m, 1H), 6.59 (s, 1H), 5.39 (s, 1H), 3.98 (d, *J* = 14.8 Hz, 1H), 3.87 (d, *J* = 14.8 Hz, 1H), 1.53 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.9, 155.3, 155.2, 138.4, 129.3, 128.8, 128.4, 124.3, 123.0, 121.0, 111.3, 104.8, 61.7, 57.7, 44.7, 30.1. FT-IR (ATR) ν_{max}/cm^{-1} 3063, 2985, 1739, 1450, 1249, 1110. HRMS (ESI$^+$): m/z: Calcd. for $C_{21}H_{23}N_5O$ $[M+H]^+$: 362.1975; Found: 362.1970.



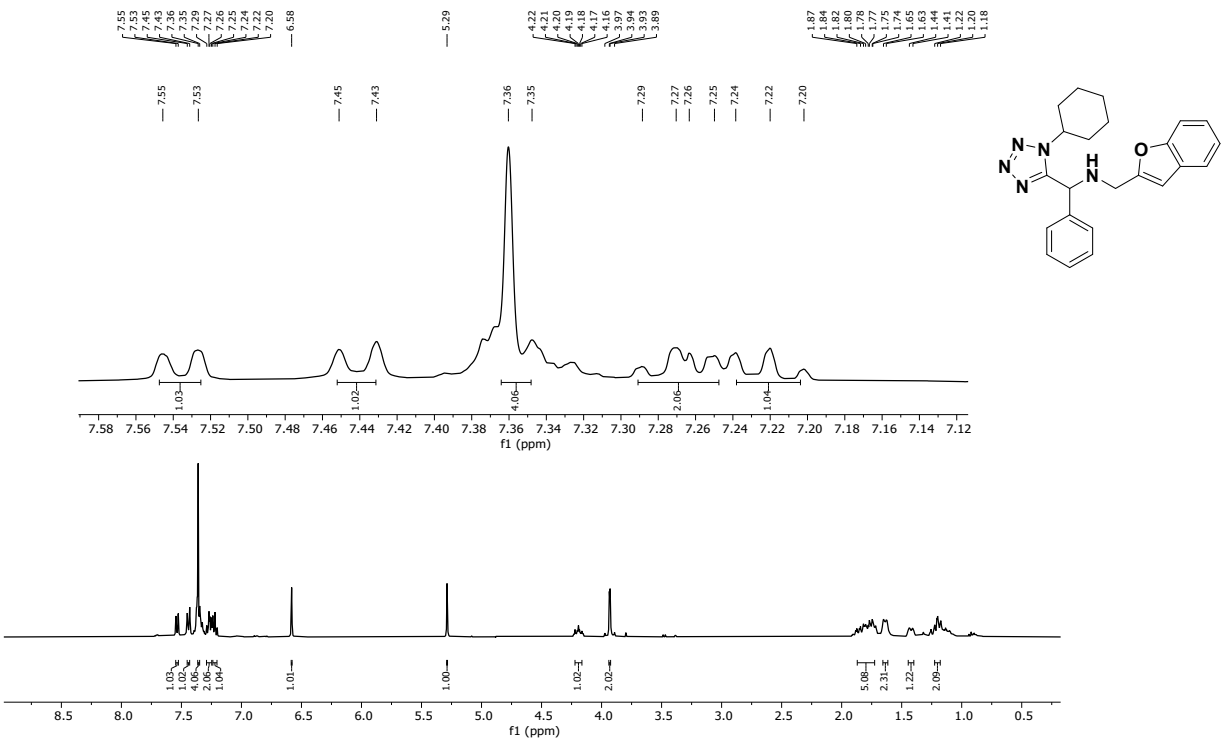
^{1}H NMR spectra of the compound **9a**.



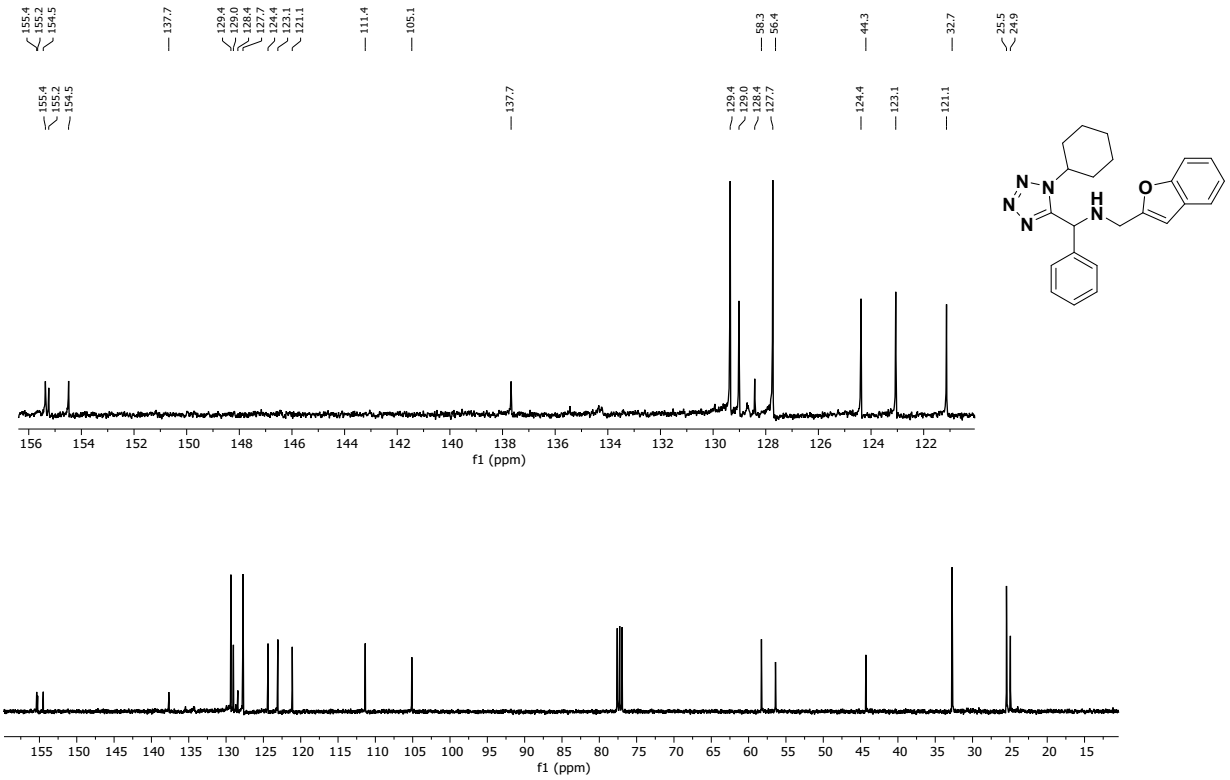
^{13}C NMR spectra of the compound **9a**.

N-(benzofuran-2-ylmethyl)-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)-1-phenylmethanamine (**9b**)

Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), benzaldehyde (37 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), cyclohexyl isocyanide (45.1 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9b** was obtained as a yellow semisolid (56.2 mg, 40%). R_f = 0.51 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.54 (d, *J* = 7.6 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.36–7.35 (m, 4H), 7.29–7.24 (m, 2H), 7.22–7.20 (m, 1H), 6.58 (s, 1H), 5.29 (s, 1H), 4.22–4.16 (m, 1H), 3.93 (d, *J* = 2.4 Hz, 2H), 1.87–1.18 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.4, 155.2, 154.5, 137.7, 129.4, 129.0, 128.4, 127.7, 124.4, 123.1, 121.1, 111.4, 105.1, 58.3, 56.4, 44.3, 32.7, 25.5, 24.9. FT-IR (ATR) ν_{max}/cm^{-1} 3065, 2938, 1456, 1257, 1102. HRMS (ESI$^+$): m/z: Calcd. for $C_{23}H_{26}N_5O[M+H]^+$: 388.2132; Found: 388.2135.



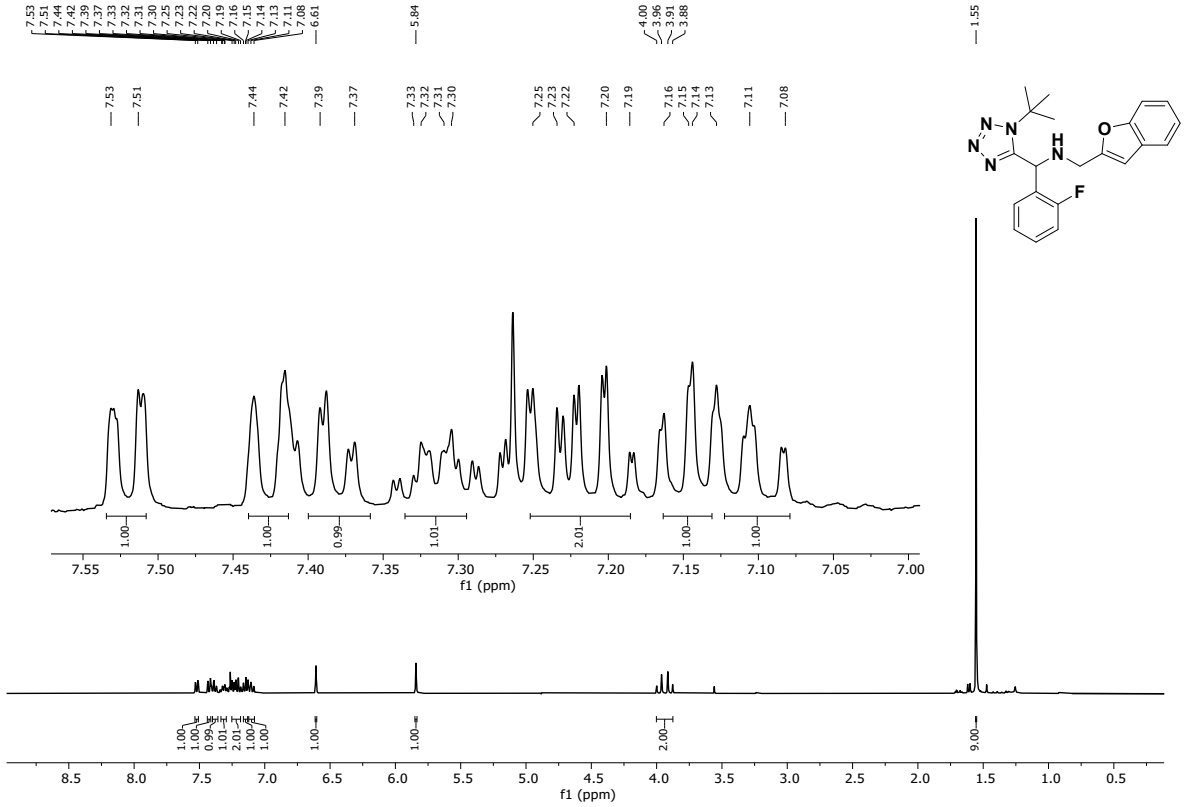
^{1}H NMR spectra of the compound **9b**.



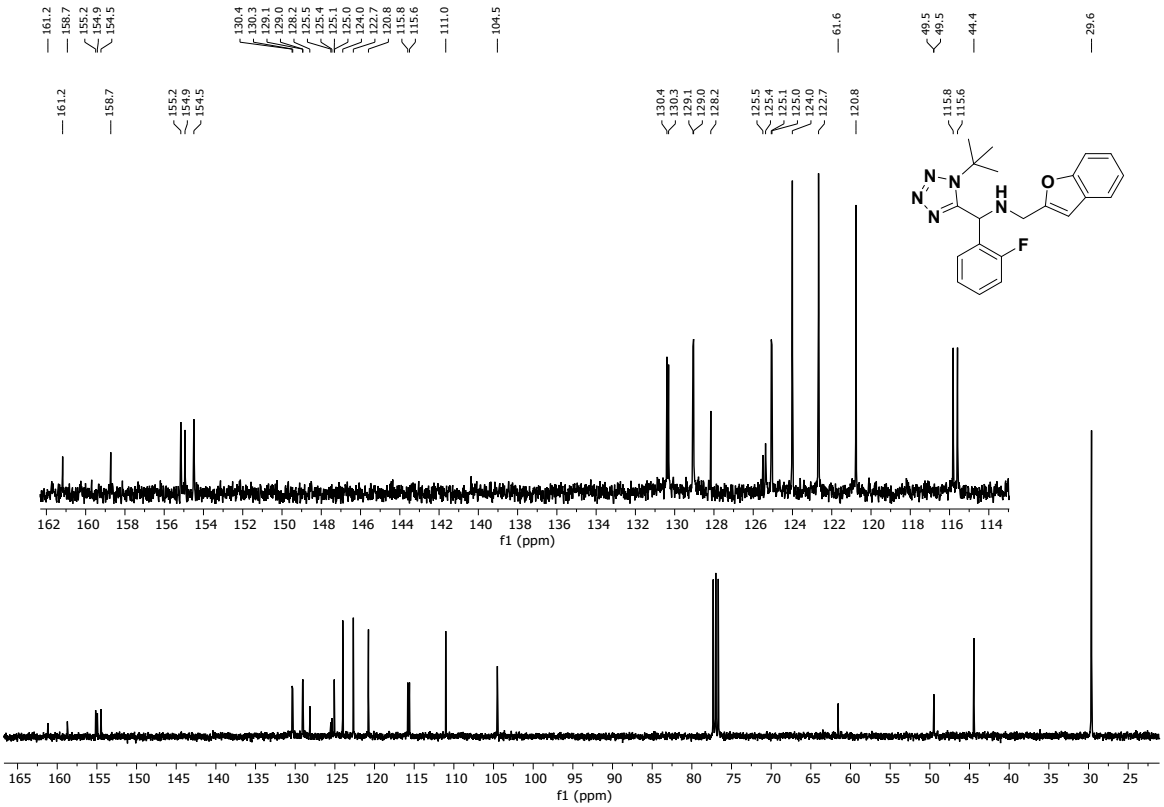
^{13}C NMR spectra of the compound **9b**.

N-(benzofuran-2-ylmethyl)-1-(1-(*tert*-butyl)-1*H*-tetrazol-5-yl)-1-(2-fluorophenyl)methanamine (**9c**)

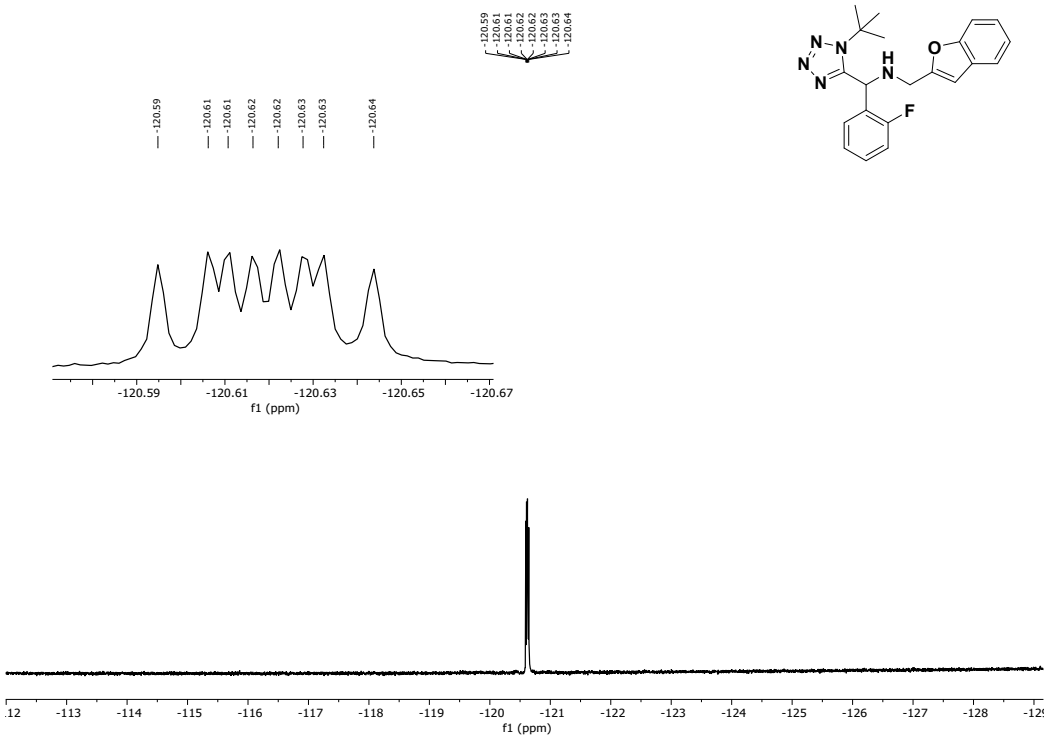
Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 2-fluorobenzaldehyde (38.2 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), *tert*-butyl isocyanide (41.0 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9c** was obtained as a semisolid yellow (59.2 mg, 43%). R_f = 0.51 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52 (d, *J* = 7.3 Hz, 1H), 7.44–7.42 (m, 1H), 7.39–7.37 (m, 1H), 7.33–7.30 (m, 1H), 7.25–7.19 (m, 2H), 7.16–7.14 (m, 1H), 7.11–7.08 (m, 1H), 6.61 (s, 1H), 5.84 (s, 1H), 3.98 (d, *J* = 14.8 Hz, 1H), 3.90 (d, *J* = 14.7 Hz, 1H), 1.55 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.9 (d, *J* = 246.8 Hz), 155.2, 154.9, 154.5, 130.4 (d, *J* = 8.4 Hz), 129.1 (d, *J* = 2.9 Hz), 128.2, 125.4 (d, *J* = 13.9 Hz), 125.1 (d, *J* = 3.5 Hz), 124.0, 122.7, 120.8, 115.7 (d, *J* = 22.2 Hz), 111.0, 104.5, 61.6, 49.5, 49.5, 44.4, 29.6. ^{19}F NMR (500 MHz, $CDCl_3$) δ = -120 (m). FT-IR (ATR) ν_{max}/cm^{-1} 2985, 2915, 1738, 1462, 1235, 1100. HRMS (ESI$^+$): m/z: Calcd. for $C_{21}H_{23}FN_5O$ $[M+H]^+$: 380.1881; Found: 380.1878.



^{1}H NMR spectra of the compound **9c**.



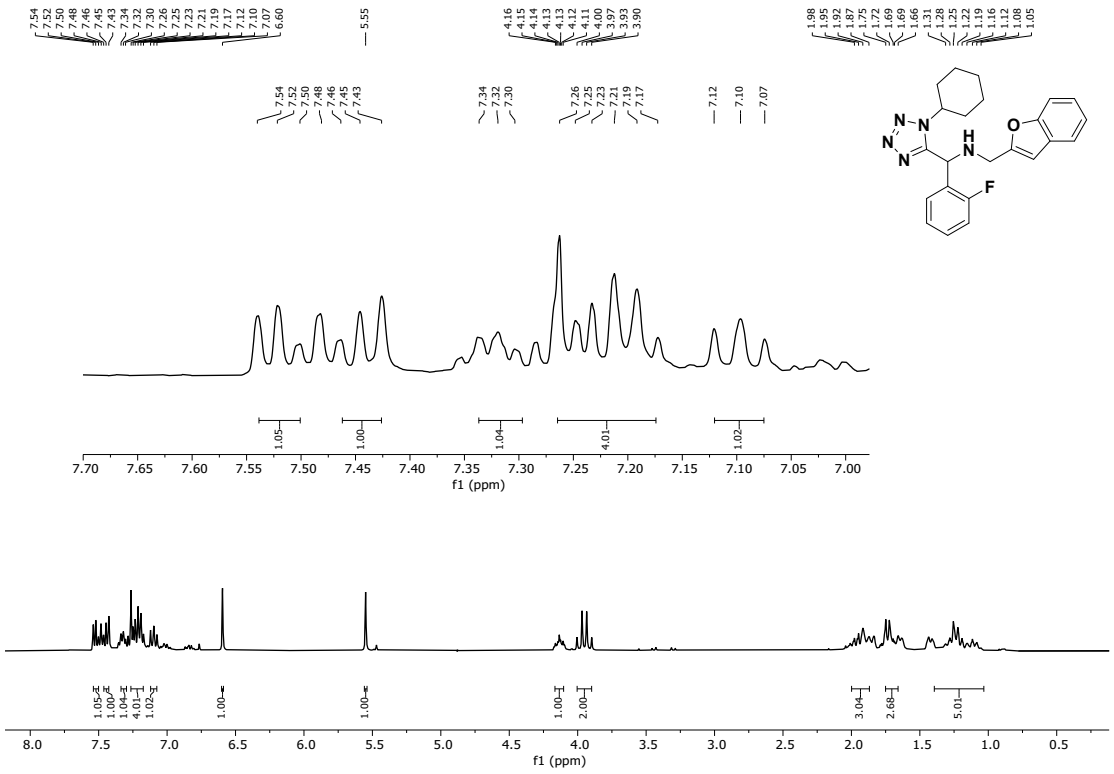
^{13}C NMR spectra of the compound **9c**.



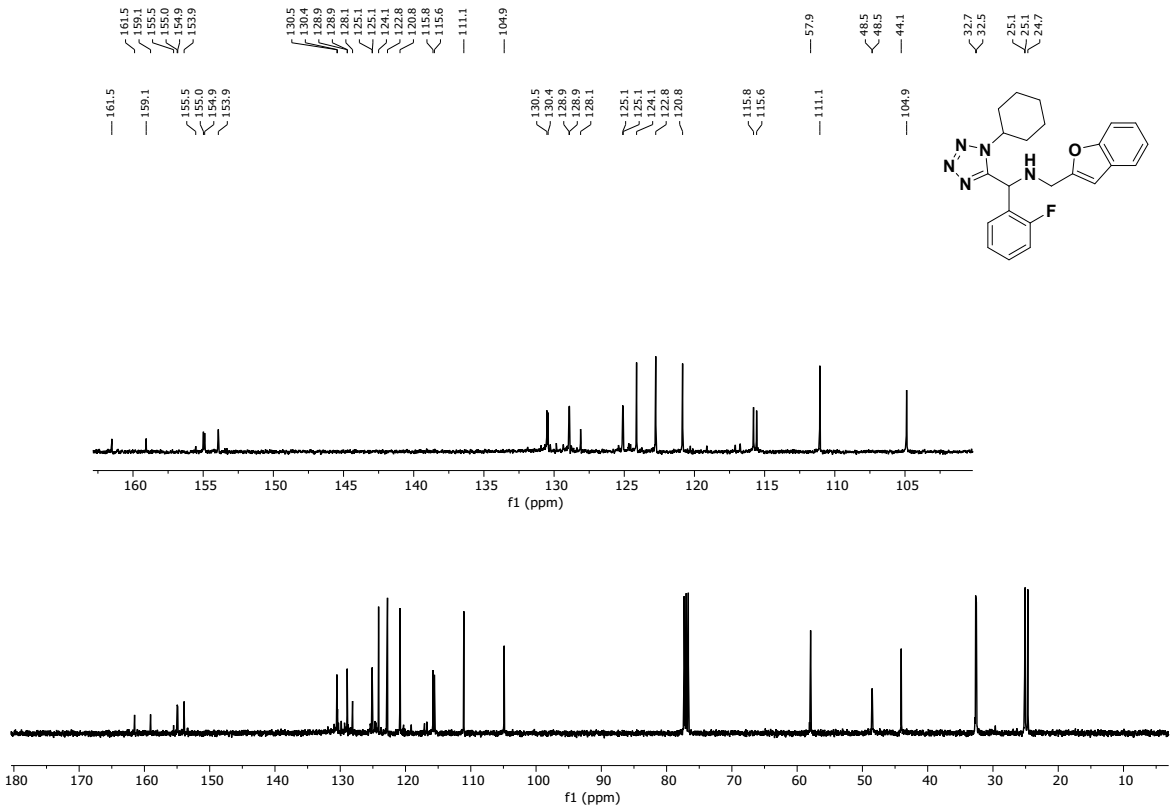
^{19}F NMR spectra of the compound **9c**.

N-(benzofuran-2-ylmethyl)-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)-1-(2-fluorophenyl)methanamine (**9d**)

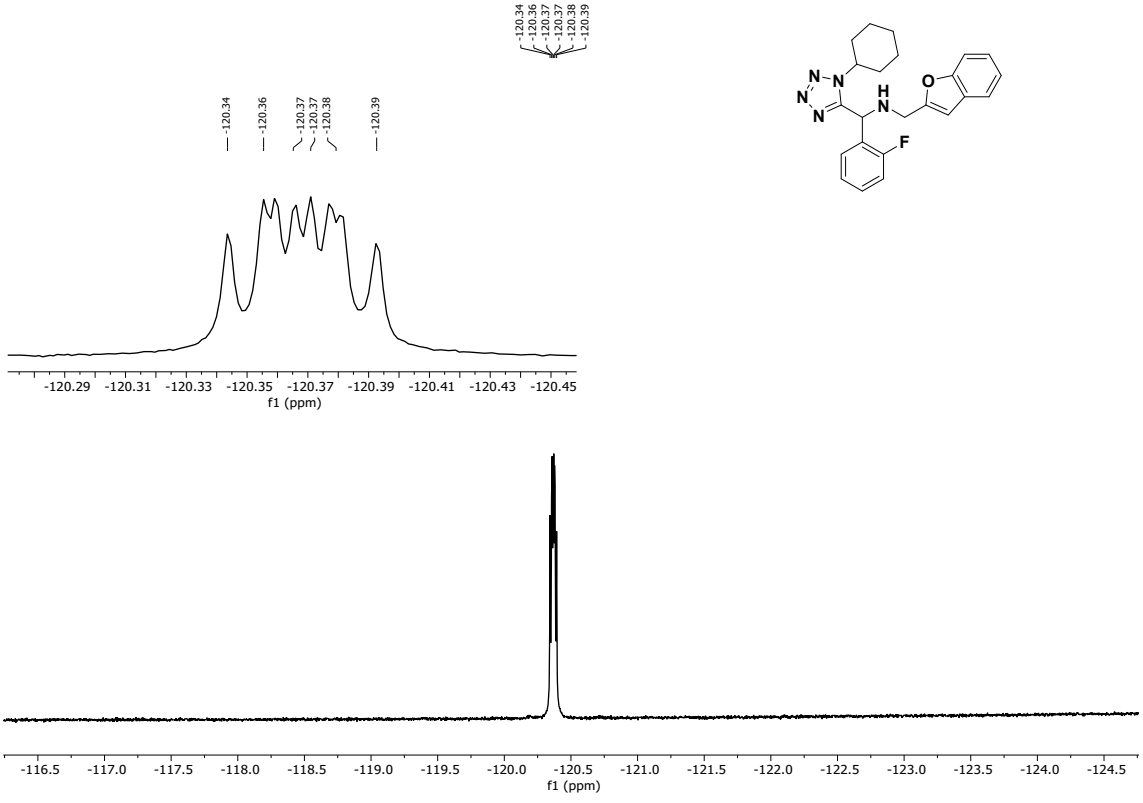
Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 2-fluorobenzaldehyde (38.2 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), cyclohexyl isocyanide (45.1 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9d** was obtained as a yellow semisolid (66.2 mg, 45%). R_f = 0.45 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (d, *J* = 7.3 Hz, 1H), 7.44 (d, *J* = 7.9 Hz, 1H), 7.34–7.30 (m, 1H), 7.26–7.17 (m, 4H), 7.12–7.07 (m, 1H), 6.60 (s, 1H), 5.55 (s, 1H), 4.16–4.11 (m, 1H), 3.95 (q, *J* = 14.7 Hz, 2H), 1.98–1.05 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.3 (d, *J* = 246.6 Hz), 155.5, 155.0, 154.9, 153.9, 130.5 (d, *J* = 8.4 Hz), 128.9 (d, *J* = 3.1 Hz), 128.1, 125.1 (d, *J* = 3.5 Hz), 124.1, 122.8, 120.8, 115.7 (d, *J* = 21.9 Hz), 111.1, 104.9, 57.9, 48.5, 48.5, 44.1, 32.7, 32.5, 25.1, 25.1, 24.7. ^{19}F NMR (500 MHz, $CDCl_3$) δ = -120 (m). FT-IR (ATR) ν_{max}/cm^{-1} 3001, 2932, 1732, 1458, 1225, 1096. HRMS (ESI$^+$): m/z: Calcd. for $C_{23}H_{25}FN_5O[M+H]^+$: 406.2038; Found: 406.2047.



^{1}H NMR spectra of the compound **9d**



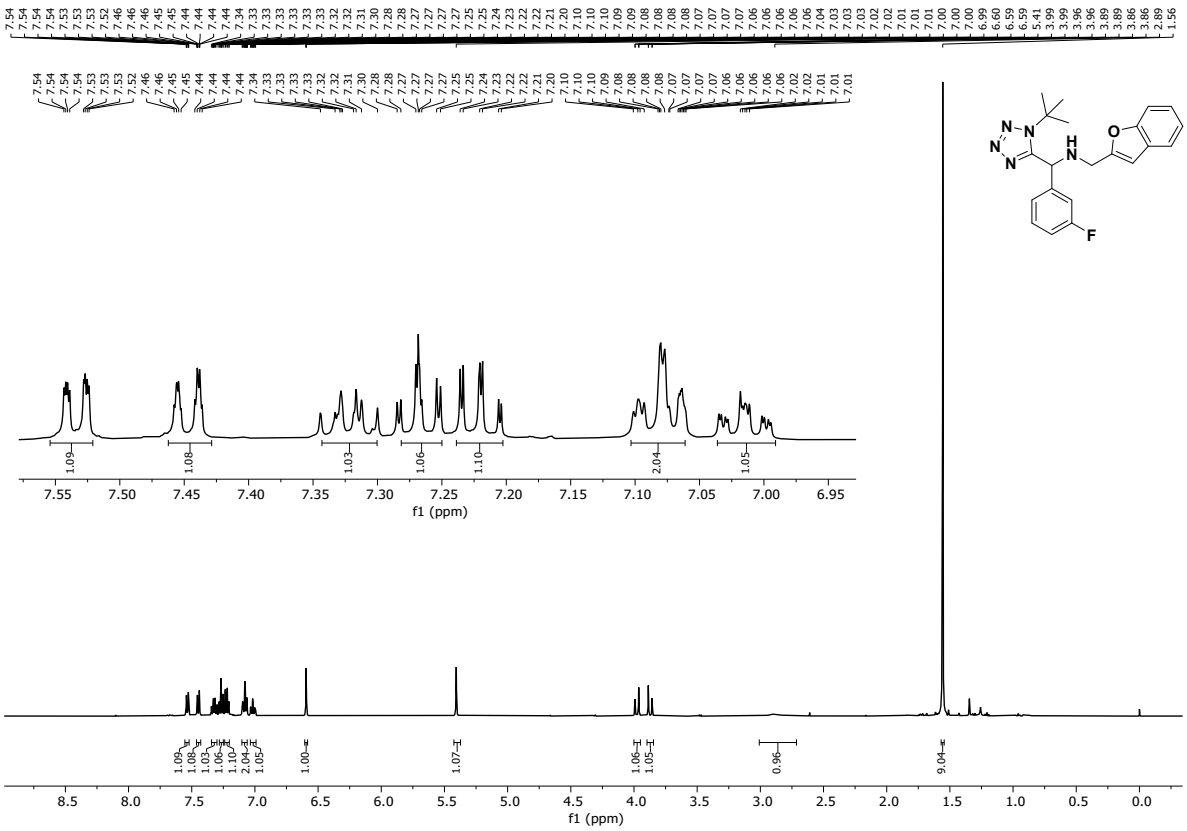
^{13}C NMR spectra of the compound **9d**



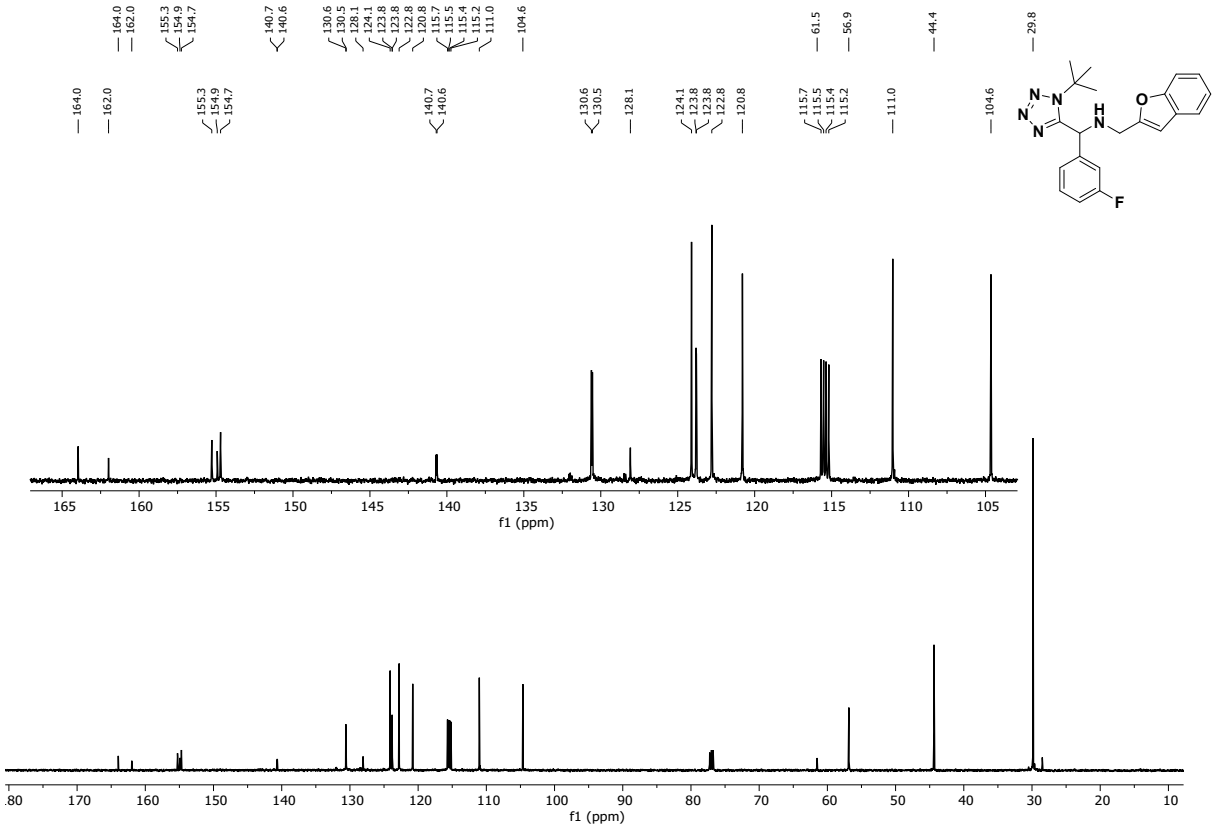
^{19}F NMR spectra of the compound **9d**.

N-(benzofuran-2-ylmethyl)-1-(1-(*tert*-butyl)-1*H*-tetrazol-5-yl)-1-(3-fluorophenyl)methanamine (**9e**)

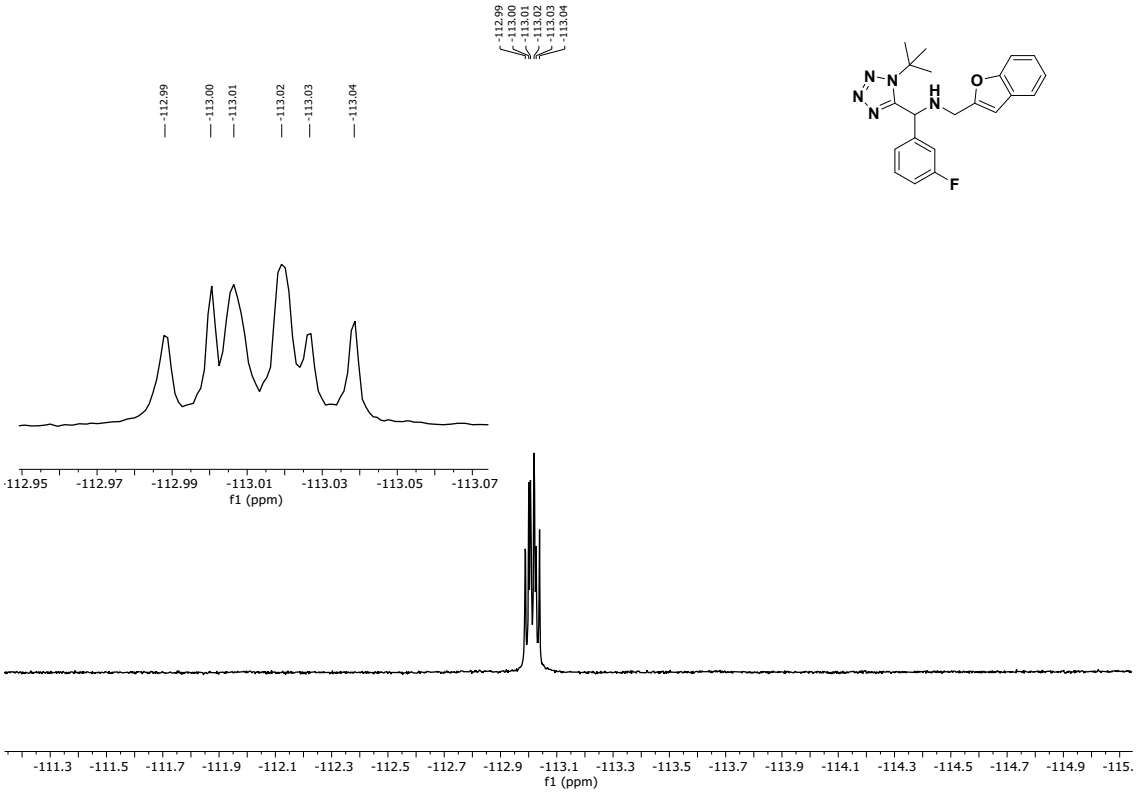
Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 3-fluorobenzaldehyde (38.4 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), *tert*-butyl isocyanide (41.0 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9e** was obtained as a semisolid yellow (50.8 mg, 67%). R_f = 0.51 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.53 (ddd, *J* = 7.5, 1.5, 0.7 Hz, 1H), 7.45 (dq, *J* = 8.2, 0.9 Hz, 1H), 7.34–7.30 (m, 1H), 7.28–7.25 (m, 1H), 7.24–7.20 (m, 1H), 7.10–7.06 (m, 2H), 7.04–6.99 (m, 1H), 6.59 (d, *J* = 0.9 Hz, 1H), 6.59 (d, *J* = 0.9 Hz, 1H), 5.41 (s, 1H), 3.98 (dd, *J* = 14.7, 0.9 Hz, 1H), 3.87 (dd, *J* = 14.7, 0.8 Hz, 1H), 2.89 (bs, 1H), 1.56 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.0 (d, *J* = 247.9 Hz), 155.3, 154.9, 154.7, 140.7 (d, *J* = 6.9 Hz), 130.6 (d, *J* = 8.2 Hz), 128.1, 124.1, 123.8, 122.8, 120.8, 115.6 (d, *J* = 21.2 Hz), 115.3 (d, *J* = 22.4 Hz), 111.0, 104.6, 61.5, 56.9, 44.4, 29.8. ^{19}F NMR (500 MHz, $CDCl_3$) δ = -113.0 (m) FT-IR (ATR) ν_{max}/cm^{-1} 3288, 2977, 1749, 1591, 1454, 1247, 1116. HRMS (ESI$^+$): m/z: Calcd. for $C_{21}H_{23}FN_5O$ $[M+H]^+$: 380.1881; Found: 380.1880.



^{1}H NMR spectra of the compound **9e**



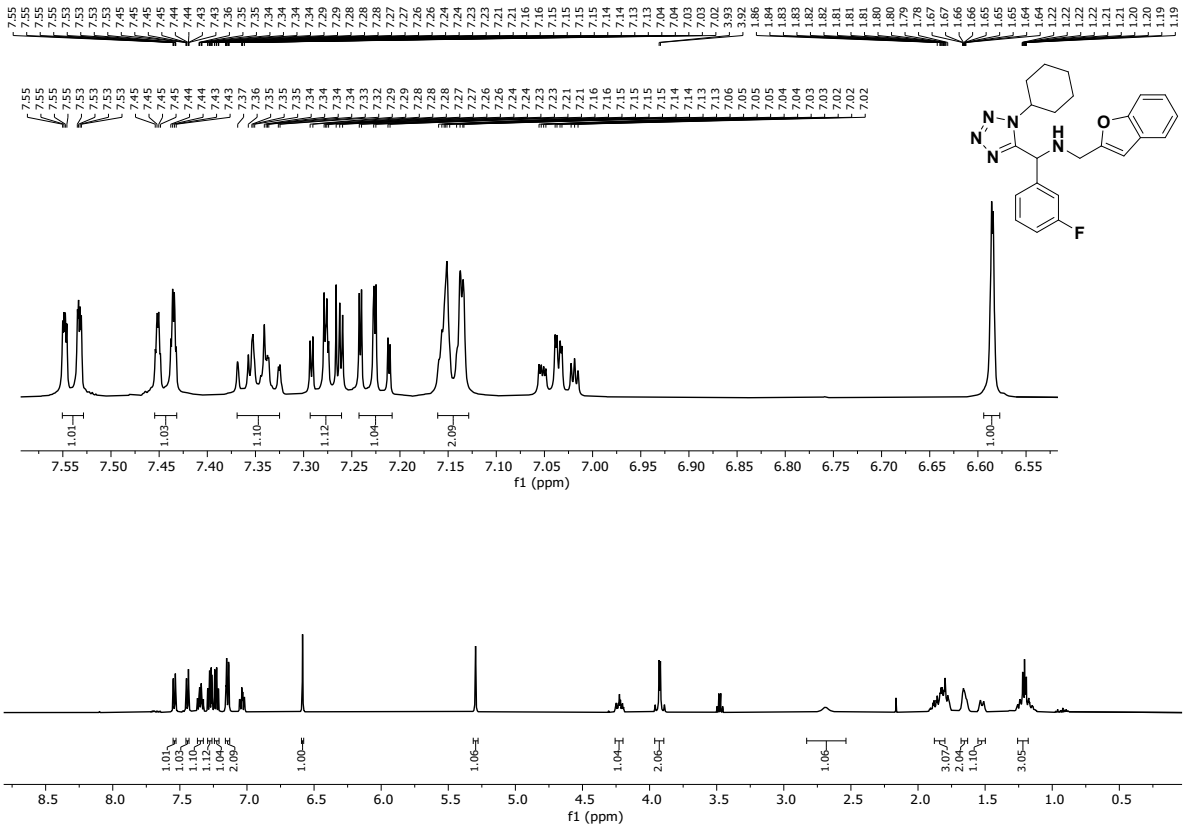
^{13}C NMR spectra of the compound **9e**



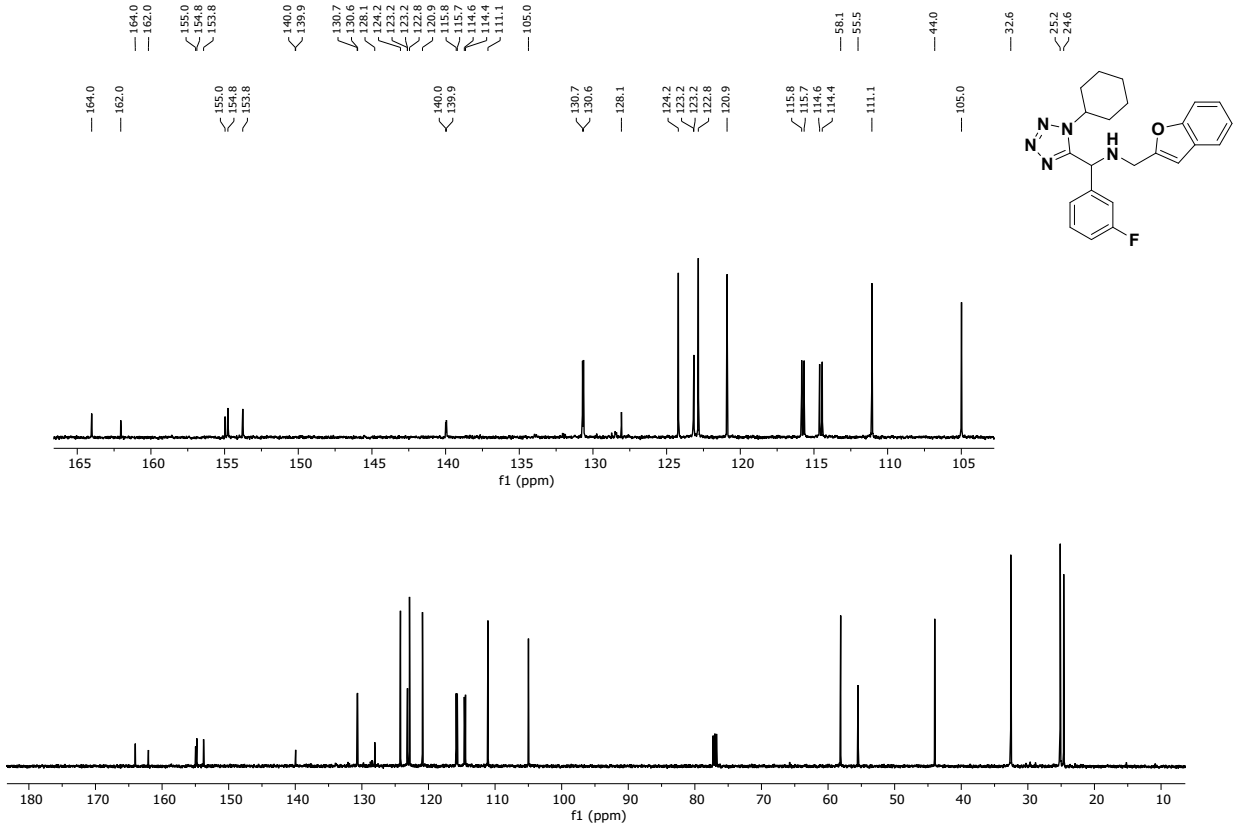
^{19}F NMR spectra of the compound **9e**.

N-(benzofuran-2-ylmethyl)-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)-1-(3-fluorophenyl)methanamine (**9f**)

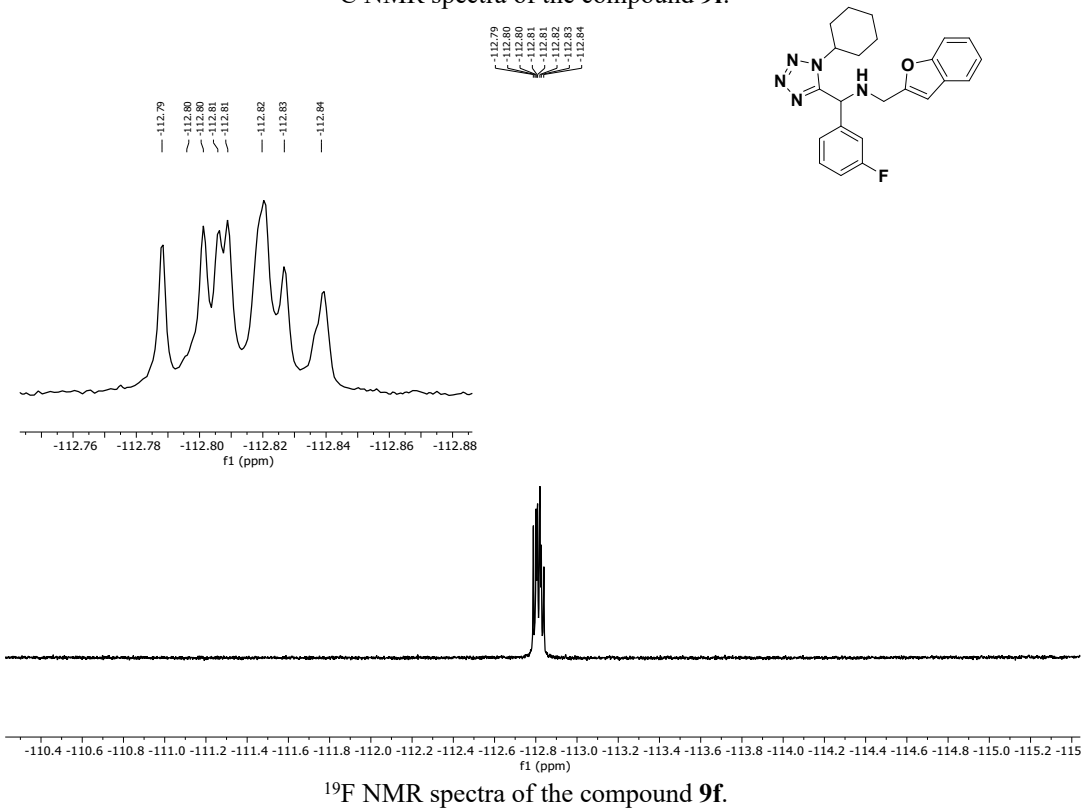
Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 3-fluorobenzaldehyde (38.4 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), cyclohexyl isocyanide (45.1 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9f** was obtained as a yellow semisolid (98.6 mg, 67%). R_f = 0.55 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.54 (ddd, *J* = 7.5, 1.5, 0.7 Hz, 1H), 7.44 (dq, *J* = 8.2, 0.9 Hz, 1H), 7.37–7.32 (m, 1H), 7.29–7.26 (m, 1H), 7.24–7.21 (m, 1H), 7.15–7.13 (m, 2H), 7.06–7.02 (m, 1H), 6.58 (d, *J* = 0.9 Hz, 1H), 5.30 (s, 1H), 4.26–4.19 (m, 1H), 3.92 (d, *J* = 4.8 Hz, 2H), 2.69 (bs, 1H), 1.89–1.17 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.0 (d, *J* = 248.2 Hz), 155.0, 154.8, 153.8, 140.0 (d, *J* = 6.8 Hz), 130.7 (d, *J* = 8.1 Hz), 128.1, 124.2, 123.2 (d, *J* = 3.0 Hz), 122.8, 120.9, 115.8 (d, *J* = 20.9 Hz), 114.5 (d, *J* = 22.5 Hz), 111.1, 105.0, 58.1, 55.5, 44.0, 32.6, 25.2, 24.6. ^{19}F NMR (500 MHz, $CDCl_3$) δ = -112.0 (m). FT-IR (ATR) ν_{max}/cm^{-1} 2934, 2860, 1749, 1591, 1448, 1249. HRMS (ESI$^+$): m/z: Calcd. for $C_{23}H_{25}FN_5O[M+H]^+$: 406.2038; Found: 406.2031.



^{1}H NMR spectra of the compound **9f**.



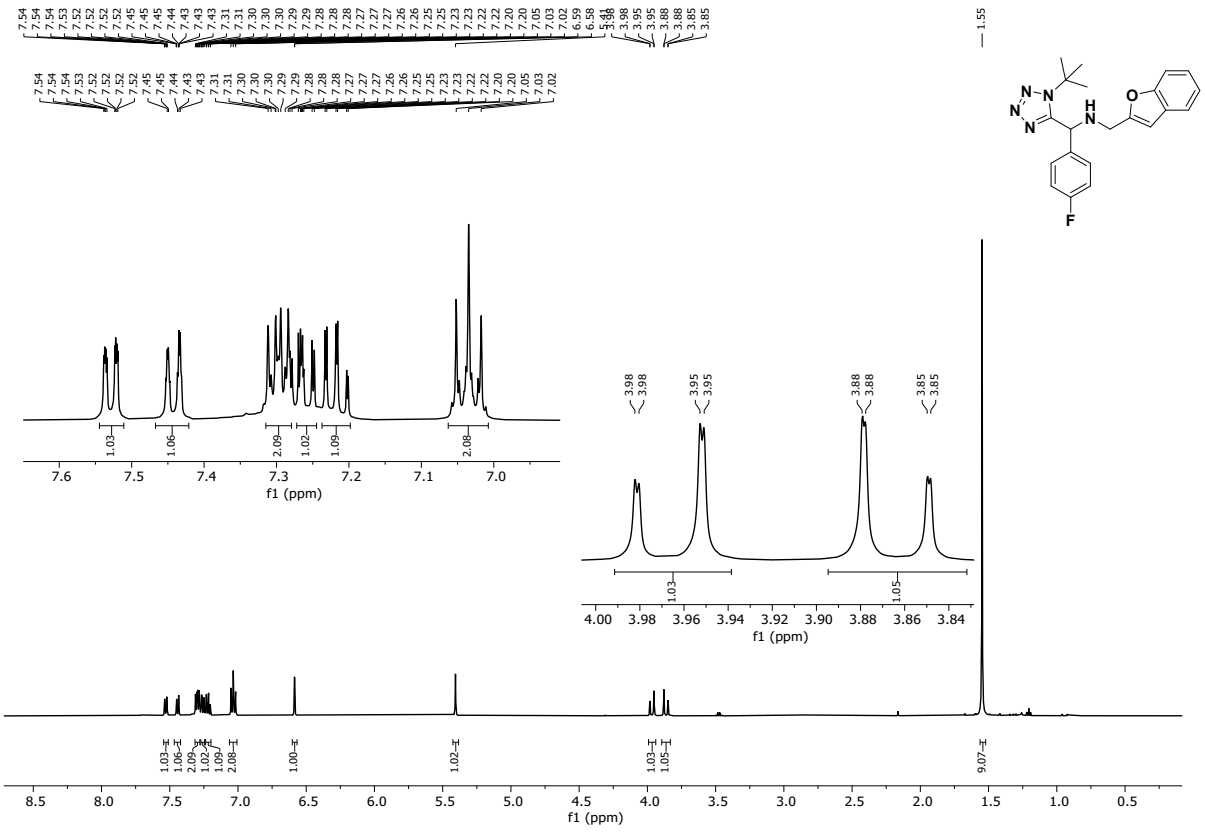
^{13}C NMR spectra of the compound **9f**.



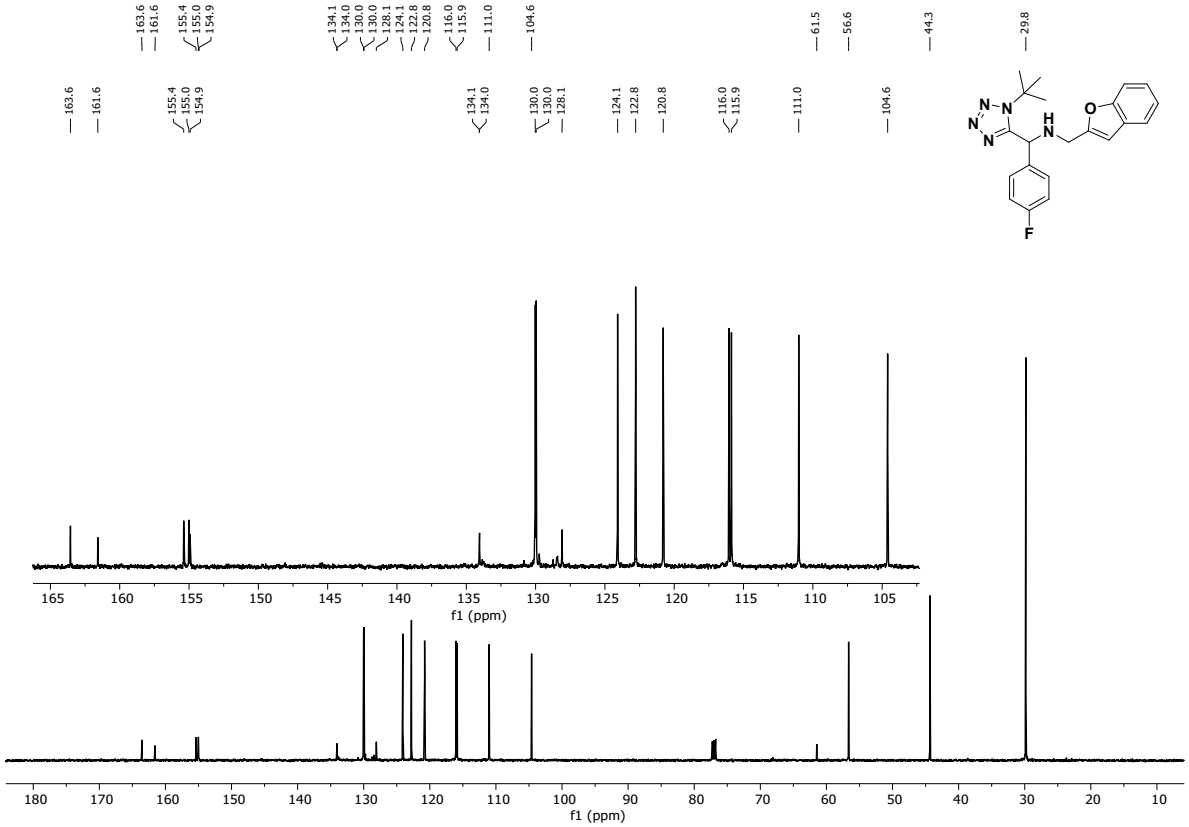
^{19}F NMR spectra of the compound **9f**.

N-(benzofuran-2-ylmethyl)-1-(1-(*tert*-butyl)-1*H*-tetrazol-5-yl)-1-(4-fluorophenyl)methanamine (**9g**)

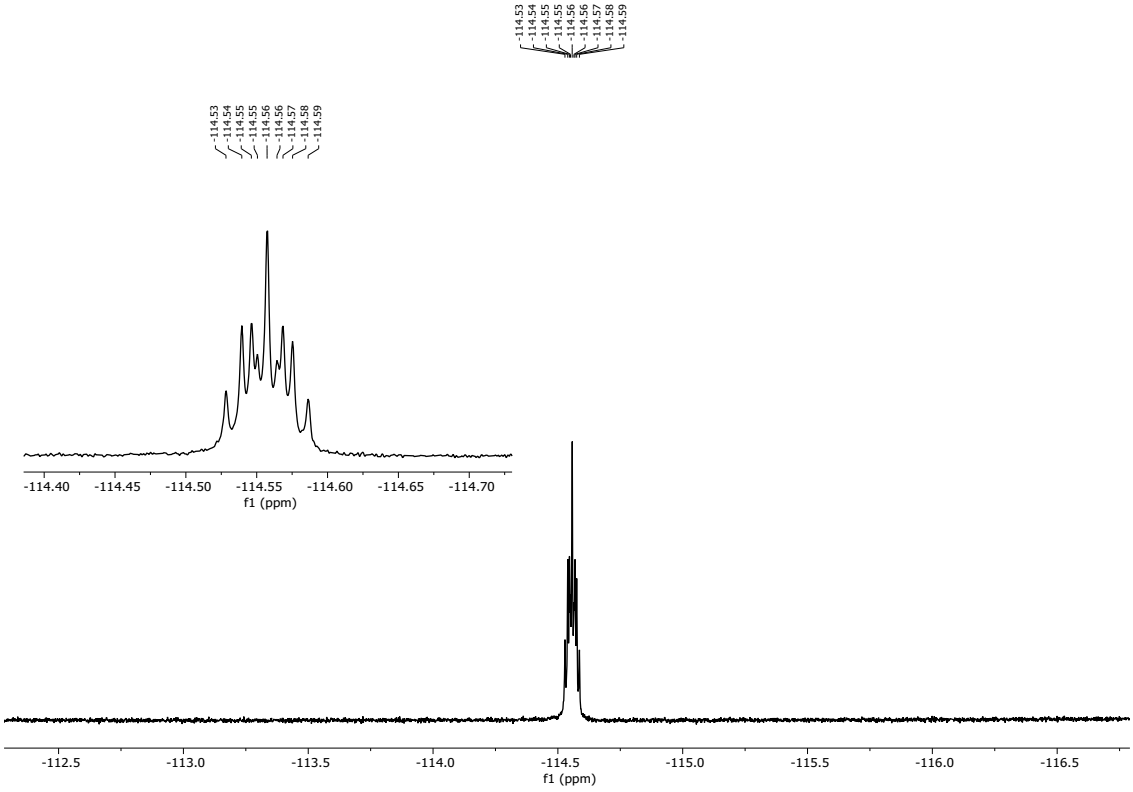
Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 4-fluorobenzaldehyde (39.0 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), *tert*-butyl isocyanide (41.0 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9g** was obtained as a oil yellow (42.6 mg, 31%). R_f = 0.48 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.53 (ddd, J = 7.5, 1.5, 0.7 Hz, 1H), 7.44 (dq, *J* = 8.2, 1.0 Hz, 1H), 7.31–7.28 (m, 1H), 7.27–7.25 (m, 1H), 7.23–7.20 (m, 1H), 7.03 (at, *J* = 8.6 Hz, 2H), 6.59 (d, *J* = 0.9 Hz, 1H), 5.41 (s, 1H), 3.97 (dd, *J* = 14.8, 1.0 Hz, 1H), 3.86 (dd, *J* = 14.8, 0.8 Hz, 1H), 1.55 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 162.6 (d, *J* = 248.0 Hz), 155.4, 155.0, 154.9, 134.1 (d, *J* = 3.2 Hz), 130.0 (d, *J* = 8.3 Hz), 128.1, 124.1, 122.8, 120.8, 116.0 (d, *J* = 21.5 Hz), 111.0, 104.6, 61.5, 56.6, 44.3, 29.8. ^{19}F NMR (500 MHz, $CDCl_3$) δ = -114.0 (m). FT-IR (ATR) ν_{max}/cm^{-1} 2989, 2925, 1749, 1597, 1513, 1452, 1214. HRMS (ESI$^+$): m/z: Calcd. for $C_{21}H_{23}FN_5O$ $[M+H]^+$: 380.1881; Found: 380.1892.



^{1}H NMR spectra of the compound **9g**.



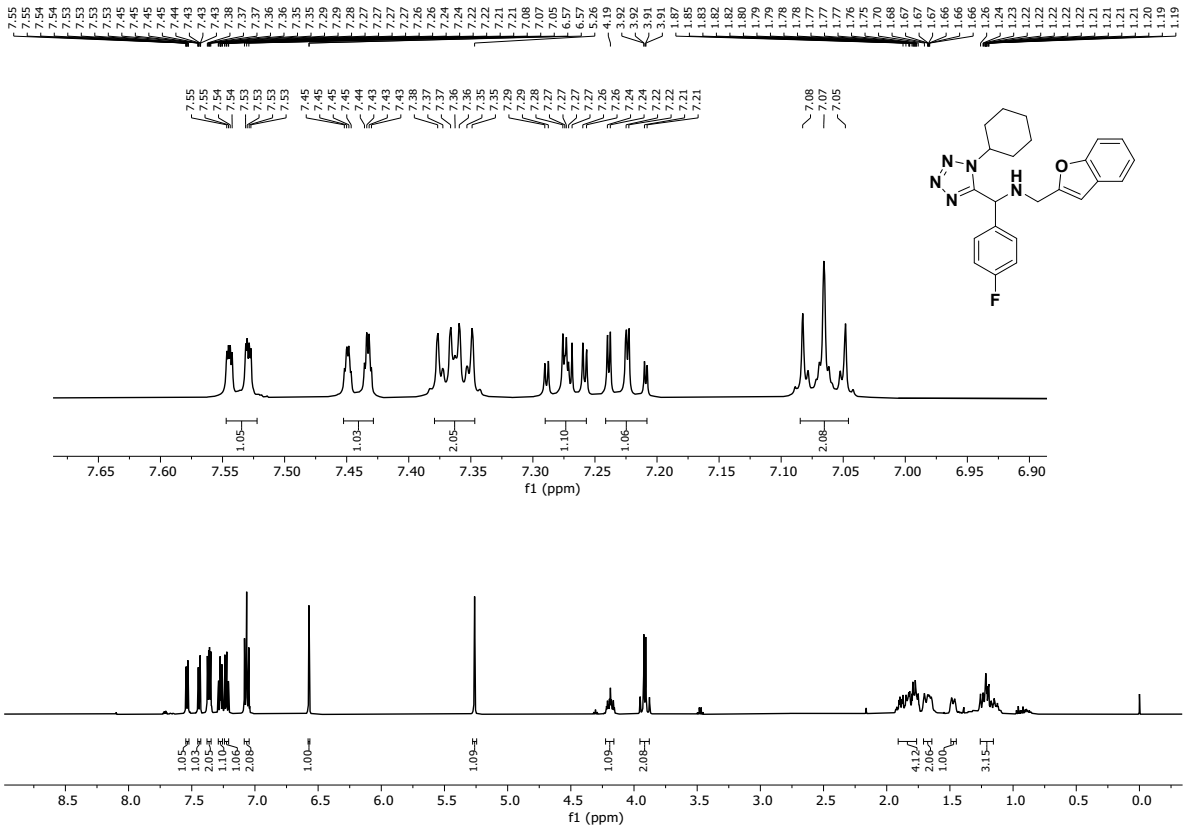
^{13}C NMR spectra of the compound **9g**.



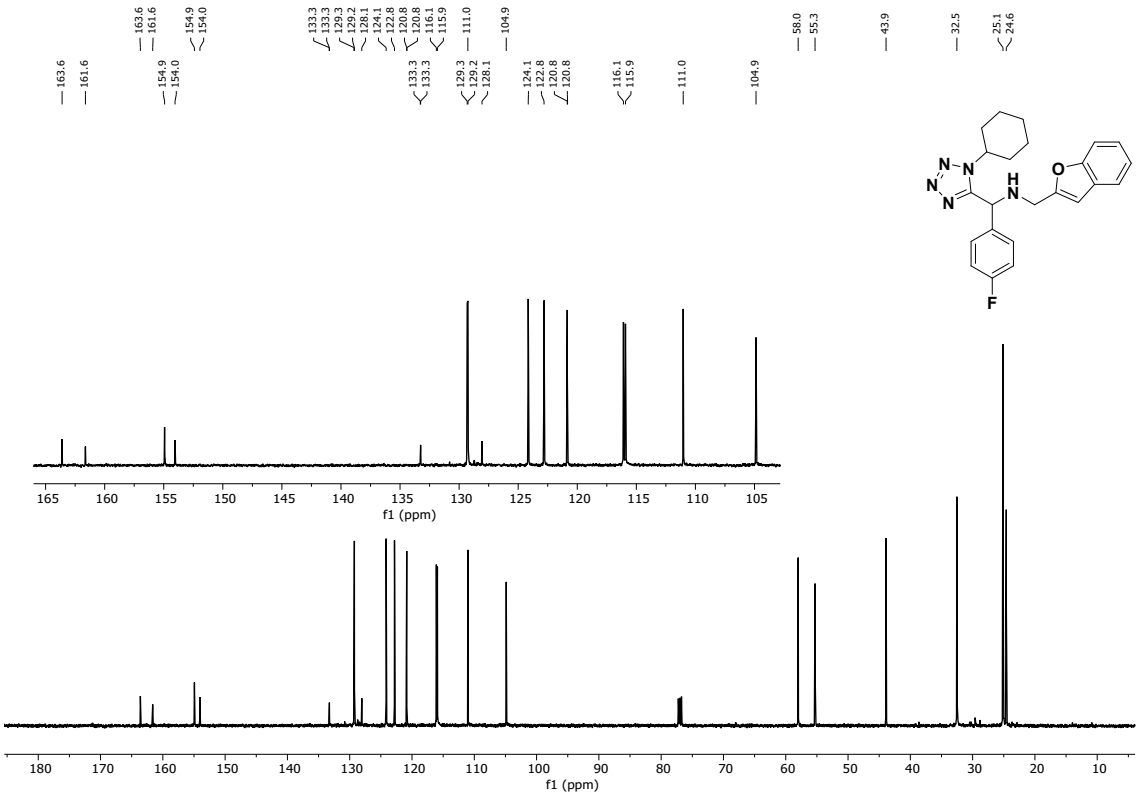
^{19}F NMR spectra of the compound **9g.**

N-(benzofuran-2-ylmethyl)-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)-1-(4-fluorophenyl)methanamine (**9h**)

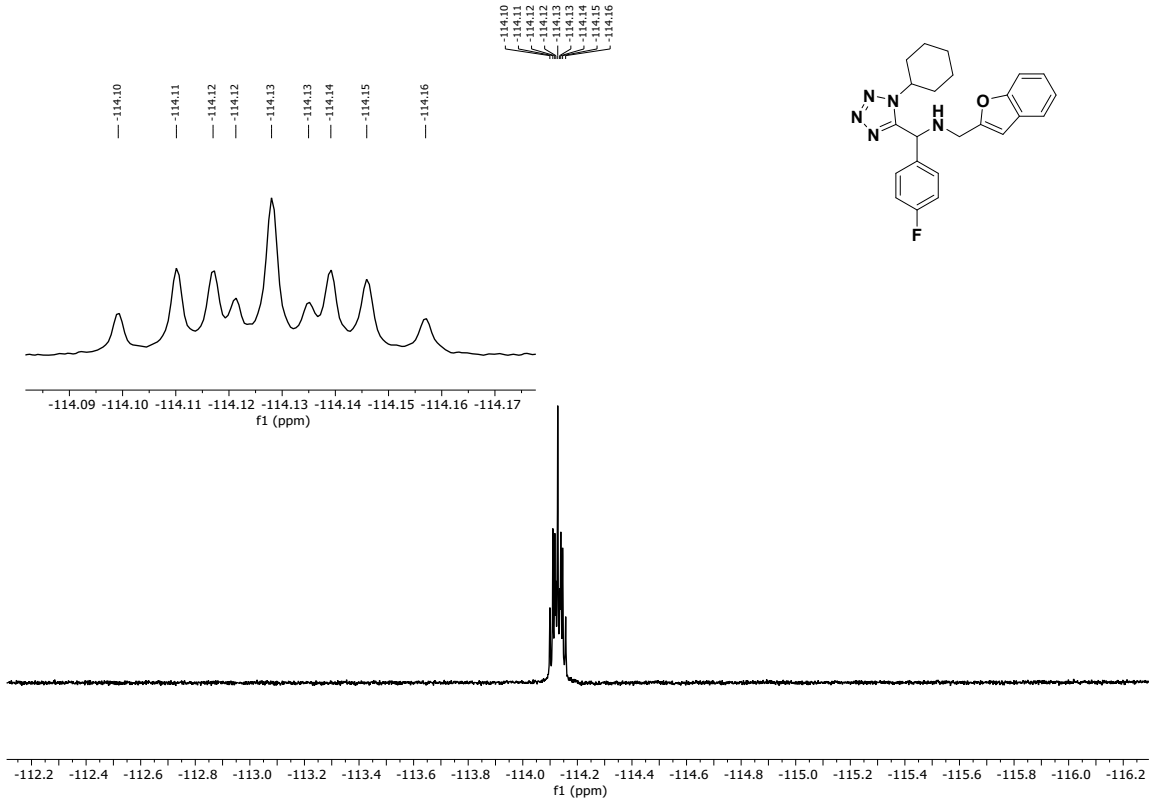
Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 4-fluorobenzaldehyde (39.0 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), cyclohexyl isocyanide (45.1 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9h** was obtained as a yellow oil (50.0 mg, 34%). R_f = 0.59 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.54 (ddd, *J* = 7.5, 1.5, 0.7 Hz, 1H), 7.44 (dq, *J* = 8.2, 0.9 Hz, 1H), 7.38–7.35 (m, 2H), 7.29–7.26 (m, 1H), 7.24–7.21 (m, 1H), 7.07 (a, t, *J* = 8.6 Hz, 2H), 6.57 (d, *J* = 0.9 Hz, 1H), 5.26 (s, 1H), 4.22–4.16 (m, 1H), 3.91 (dd, *J* = 7.2, 0.8 Hz, 2H), 1.92–1.14 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 162.6 (d, *J* = 248.5 Hz), 154.9, 154.0, 133.3 (d, *J* = 3.3 Hz), 129.3 (d, *J* = 8.2 Hz), 128.1, 124.1, 122.8, 120.8, 116.0 (d, *J* = 21.6 Hz), 111.0, 104.9, 58.0, 55.3, 43.9, 32.5, 25.1, 24.6. ^{19}F NMR (500 MHz, $CDCl_3$) δ = -114.0 (m). FT-IR (ATR) ν_{max}/cm^{-1} 2940, 1749, 1605, 1507, 1454, 1235. HRMS (ESI$^+$): m/z: Calcd. for $C_{23}H_{25}FN_5O[M+H]^+$: 406.2038; Found: 406.2045.



^{1}H NMR spectra of the compound **9h**.



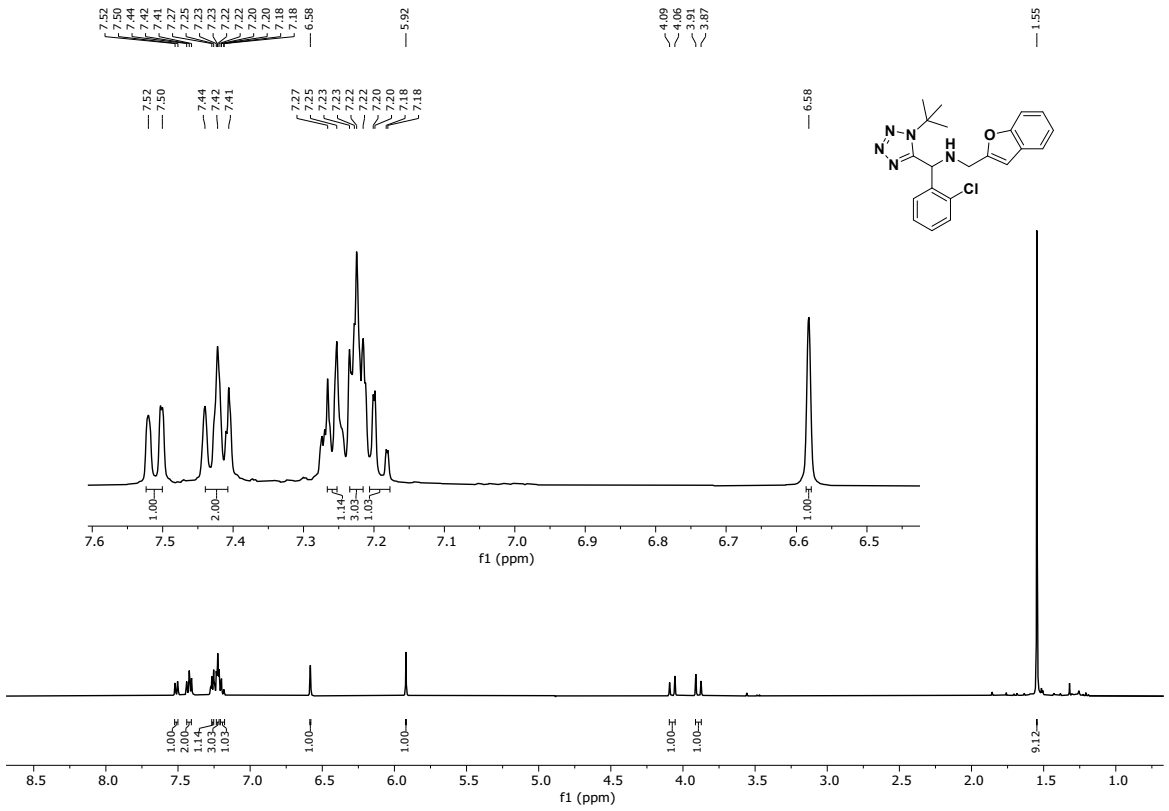
^{13}C NMR spectra of the compound **9h**.



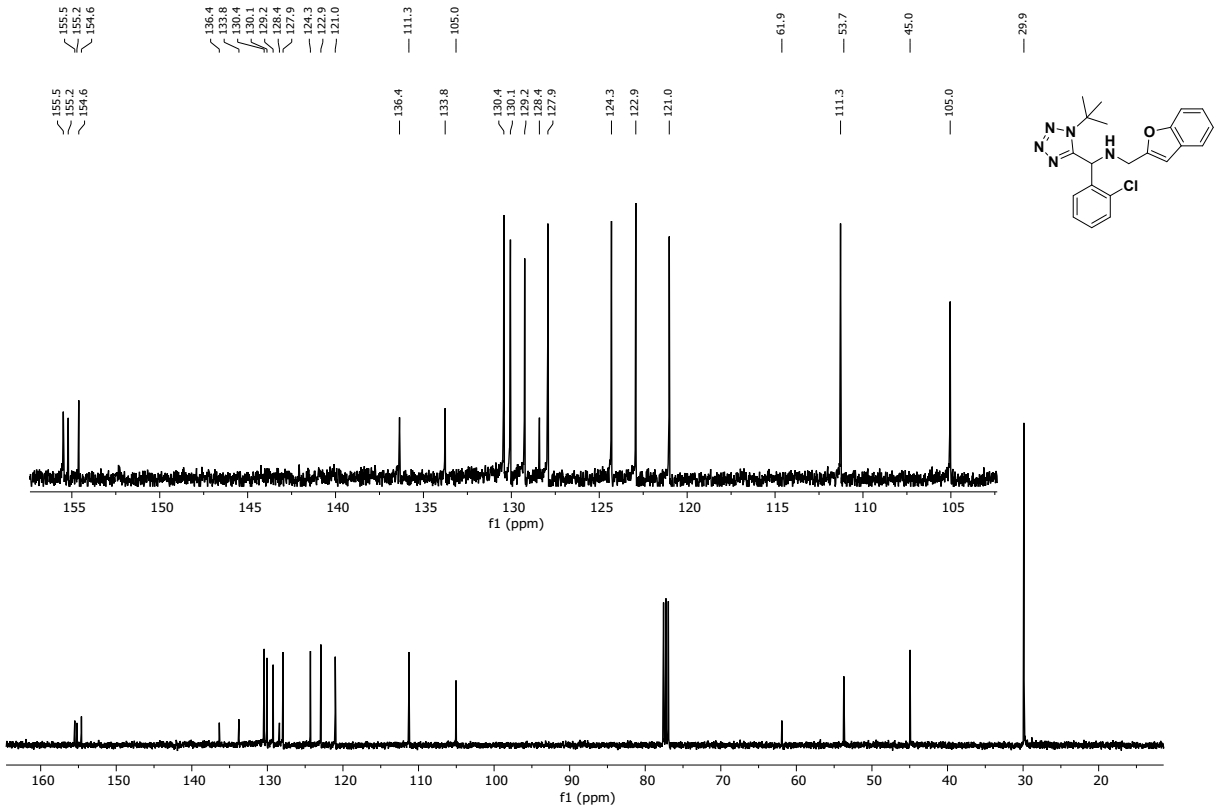
^{19}F NMR spectra of the compound **9h.**

N-(benzofuran-2-ylmethyl)-1-(1-(*tert*-butyl)-1*H*-tetrazol-5-yl)-1-(2-chlorophenyl)methanamine (**9i**)

Based on the GP1, propargylamine (23.3 μL, 0.36 mmol), 2-chlorobenzaldehyde (41.0 μL, 0.36 mmol), $TMSN_3$ (58 μL, 0.43 mmol), *tert*-butyl isocyanide (41.0 μL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9i** was obtained as an oil yellow (57.4 mg, 40%). R_f = 0.56 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.51 (d, *J* = 7.9 Hz, 1H), 7.44–7.41 (m, 2H), 7.27–7.25 (m, 1H), 7.23–7.22 (m, 3H), 7.20–7.18 (m, 1H), 6.58 (s, 1H), 5.92 (s, 1H), 4.07 (d, *J* = 14.7 Hz, 1H), 3.89 (d, *J* = 14.6 Hz, 1H), 1.55 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.5, 155.2, 154.6, 136.4, 133.8, 130.4, 130.1, 129.2, 128.4, 127.9, 124.3, 122.9, 121.0, 111.3, 105.0, 61.9, 53.7, 45.0, 29.9. FT-IR (ATR) ν_{max}/cm^{-1} 2987, 2915, 1751, 1452, 1104, 756. HRMS (ESI$^+$): m/z: Calcd. for $C_{21}H_{23}ClN_5O$ $[M+H]^+$: 396.1586; Found: 396.1578.



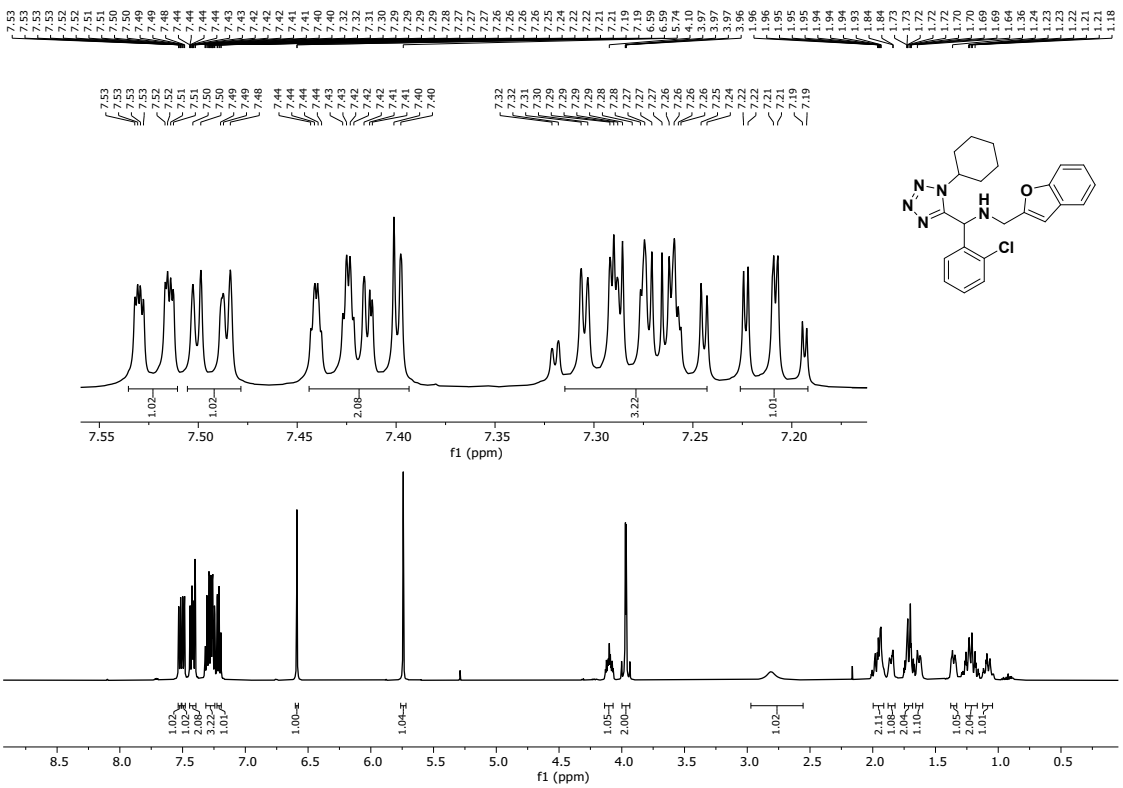
^{1}H NMR spectra of the compound **9i**.



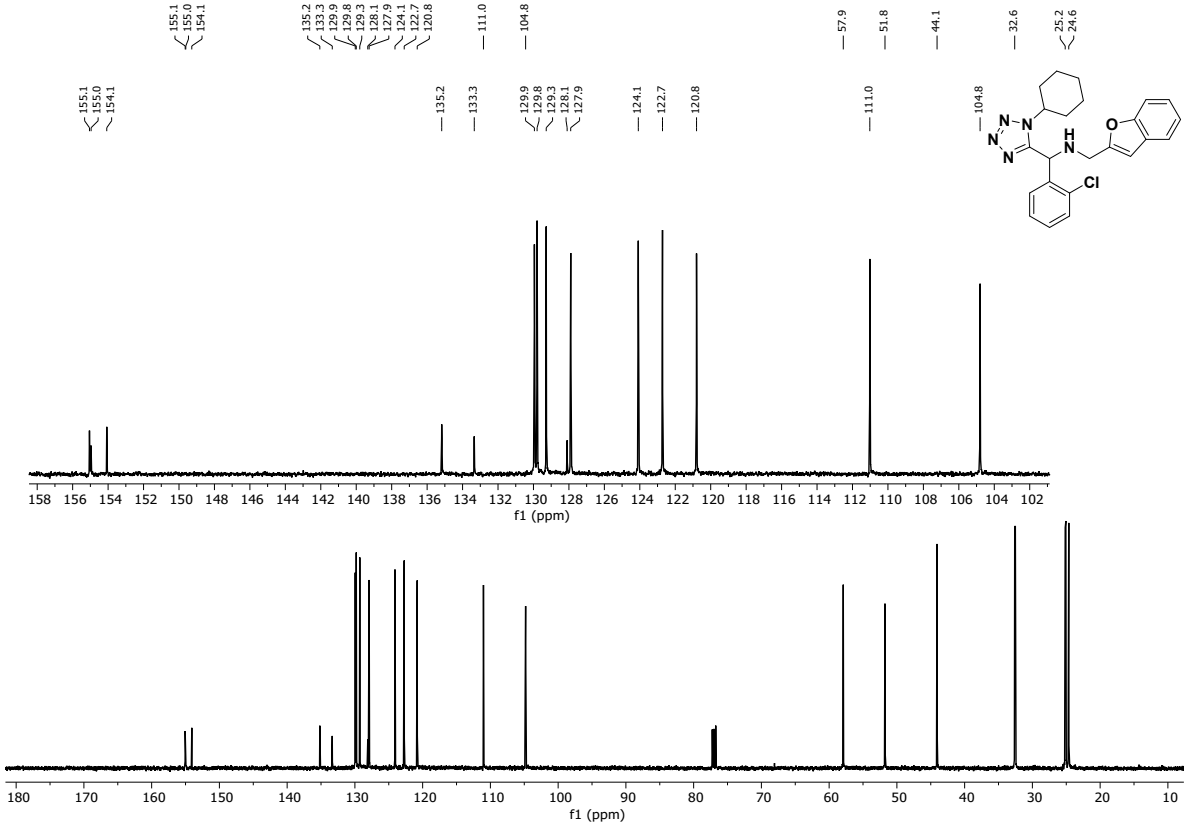
^{13}C NMR spectra of the compound **9i**.

N-(benzofuran-2-ylmethyl)-1-(2-chlorophenyl)-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)methanamine (**9j**)

Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 2-chlorobenzaldehyde (41.0 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), cyclohexyl isocyanide (45.1 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9j** was obtained as a beige solid (50.5 mg, 33%). mp = 135–138 °C. R_f = 0.53 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.52 (ddd, *J* = 7.6, 1.5, 0.7 Hz, 1H), 7.50–7.48 (m, 1H), 7.44–7.40 (m, 2H), 7.32–7.24 (m, 3H), 7.21 (td, *J* = 7.4, 1.1 Hz, 1H), 6.59 (d, *J* = 0.9 Hz, 1H), 5.74 (s, 1H), 4.13–4.07 (m, 1H), 3.97 (dd, *J* = 3.4, 0.8 Hz, 2H), 2.81 (bs, 1H), 1.96–1.18 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 155.1, 155.0, 154.1, 135.2, 133.3, 129.9, 129.8, 129.3, 128.1, 127.9, 124.1, 122.7, 120.8, 111.0, 104.8, 57.9, 51.8, 44.1, 32.6, 25.2, 24.6. FT-IR (ATR) ν_{max}/cm^{-1} 3325, 2936, 1751, 1448, 1223, 758. HRMS (ESI$^+$): m/z: Calcd. for $C_{23}H_{25}ClN_5O[M+H]^+$: 422.1742; Found: 422.1737.



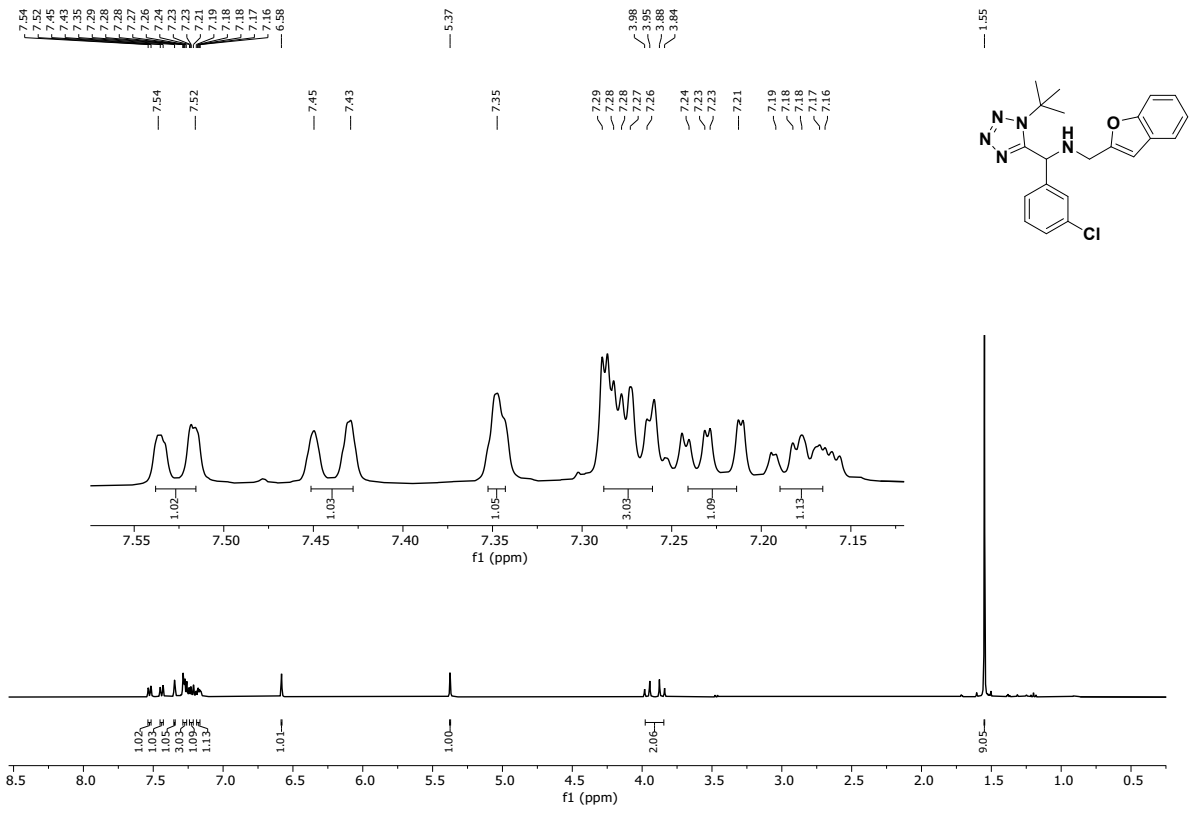
^{1}H NMR spectra of the compound **9j**.



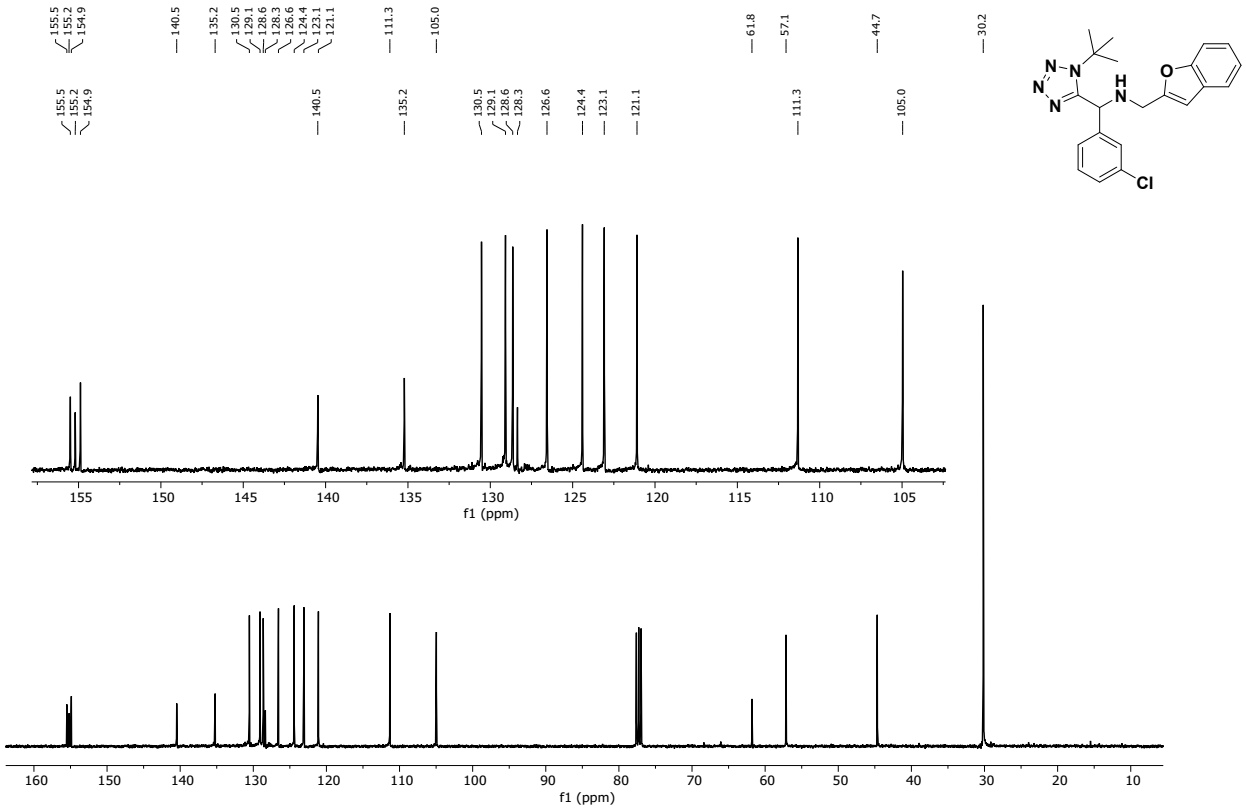
^{13}C NMR spectra of the compound **9j**.

N-(benzofuran-2-ylmethyl)-1-(1-(*tert*-butyl)-1*H*-tetrazol-5-yl)-1-(3-chlorophenyl)methanamine (**9k**)

Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 3-chlorobenzaldehyde (41.0 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), *tert*-butyl isocyanide (41.0 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9k** was obtained as an brown solid (83.3 mg, 58%). mp = 103–106 °C. R_f = 0.56 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (d, *J* = 8.3 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.35 (s, 1H), 7.29–7.26 (m, 3H), 7.24–7.21 (m, 1H), 7.19–7.16 (m, 1H), 6.58 (s, 1H), 5.37 (s, 1H), 3.96 (d, *J* = 14.8 Hz, 1H), 3.86 (d, *J* = 14.8 Hz, 1H), 1.55 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.5, 155.2, 154.9, 140.5, 135.2, 130.5, 129.1, 128.6, 128.3, 126.6, 124.4, 123.1, 121.1, 111.3, 105.0, 61.8, 57.1, 44.7, 30.2. FT-IR (ATR) ν_{max}/cm^{-1} 3292, 2981, 1734, 1450, 1372, 1120, 748. HRMS (ESI$^+$): m/z: Calcd. for $C_{21}H_{23}ClN_5O$ $[M+H]^+$: 396.1586; Found: 396.1599.



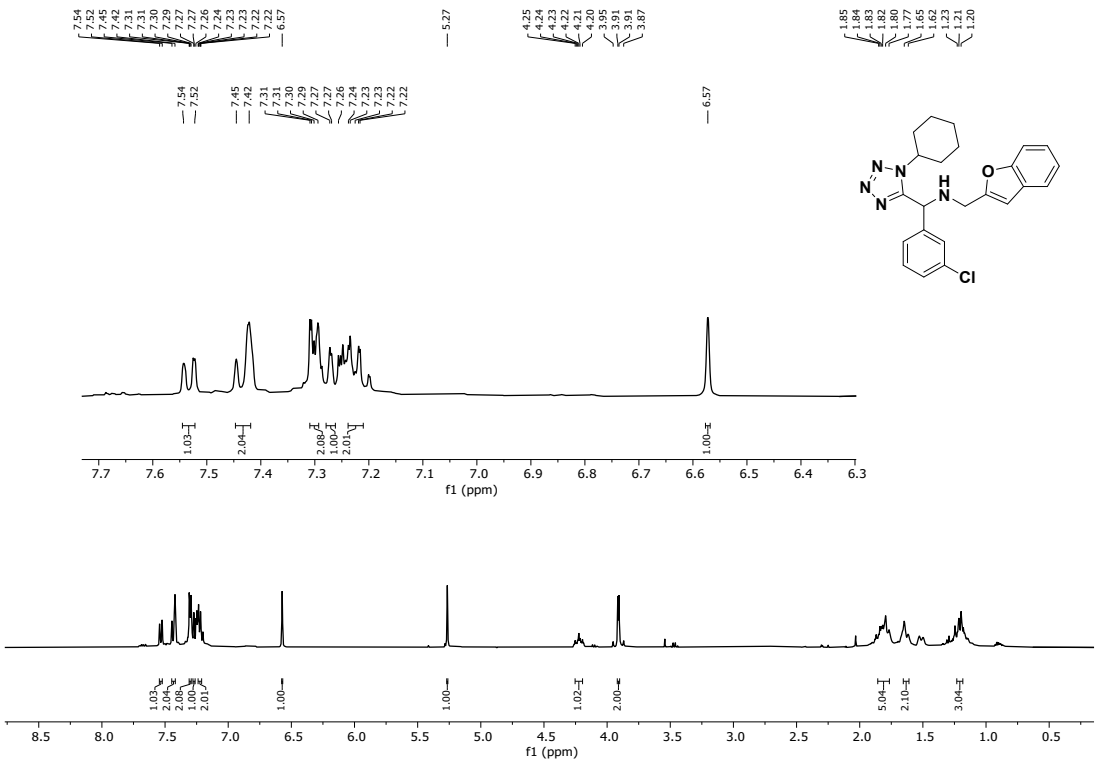
^{1}H NMR spectra of the compound **9k**.



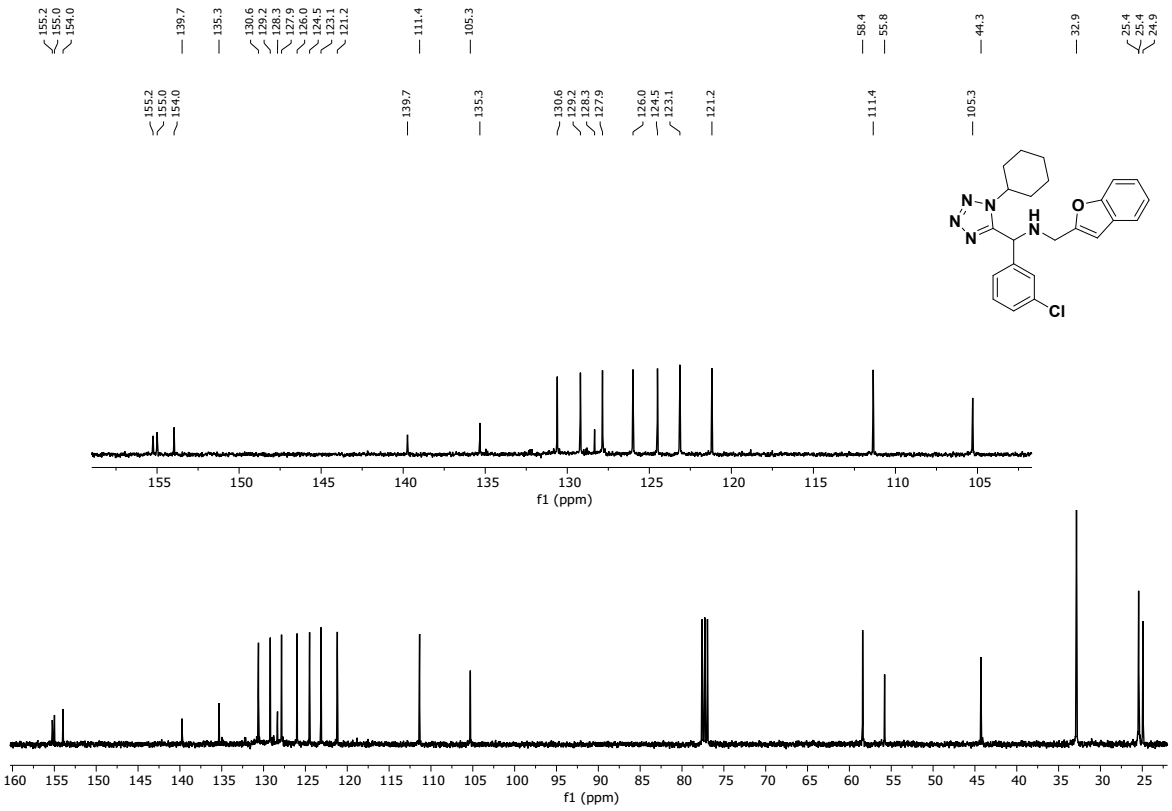
^{13}C NMR spectra of the compound **9k**.

N-(benzofuran-2-ylmethyl)-1-(3-chlorophenyl)-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)methanamine (**9l**)

Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 3-chlorobenzaldehyde (41.0 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), cyclohexyl isocyanide (45.1 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9l** was obtained as a yellow semisolid (96.5 mg, 63%). R_f = 0.60 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (d, *J* = 8.3 Hz, 1H), 7.45–7.42 (m, 2H), 7.31–7.29 (m, 2H), 7.27–7.22 (m, 3H), 6.57 (s, 1H), 5.27 (s, 1H), 4.25–4.20 (m, 1H), 3.91 (d, *J* = 3.5 Hz, 2H), 1.85–1.20 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.2, 155.0, 154.0, 139.7, 135.3, 130.6, 129.2, 128.3, 127.9, 126.0, 124.5, 123.1, 121.2, 111.4, 105.3, 58.4, 55.8, 44.3, 32.9, 25.4, 25.4, 24.9. FT-IR (ATR) ν_{max}/cm^{-1} 2938, 2852, 1456, 1255, 1100, 754. HRMS (ESI$^+$): m/z: Calcd. for $C_{23}H_{25}ClN_5O[M+H]^+$: 422.1742; Found: 422.1750.



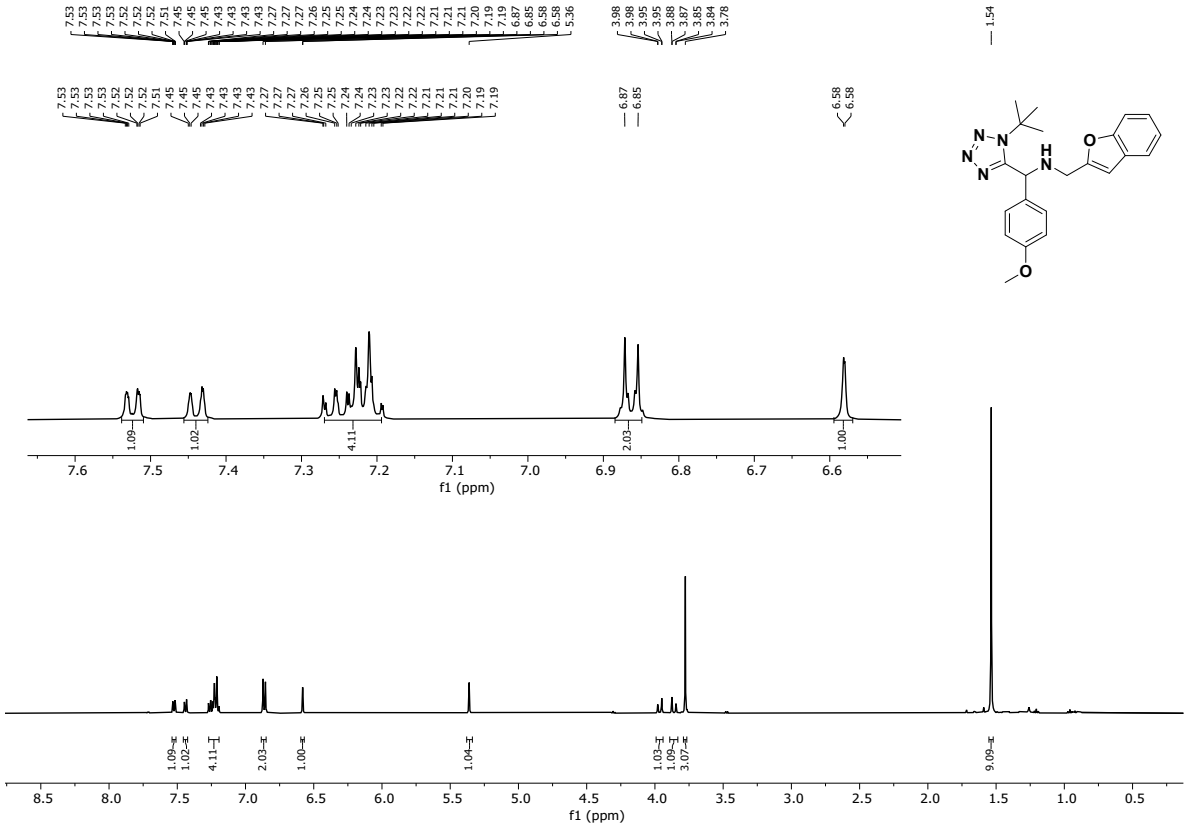
^{1}H NMR spectra of the compound **9l**.



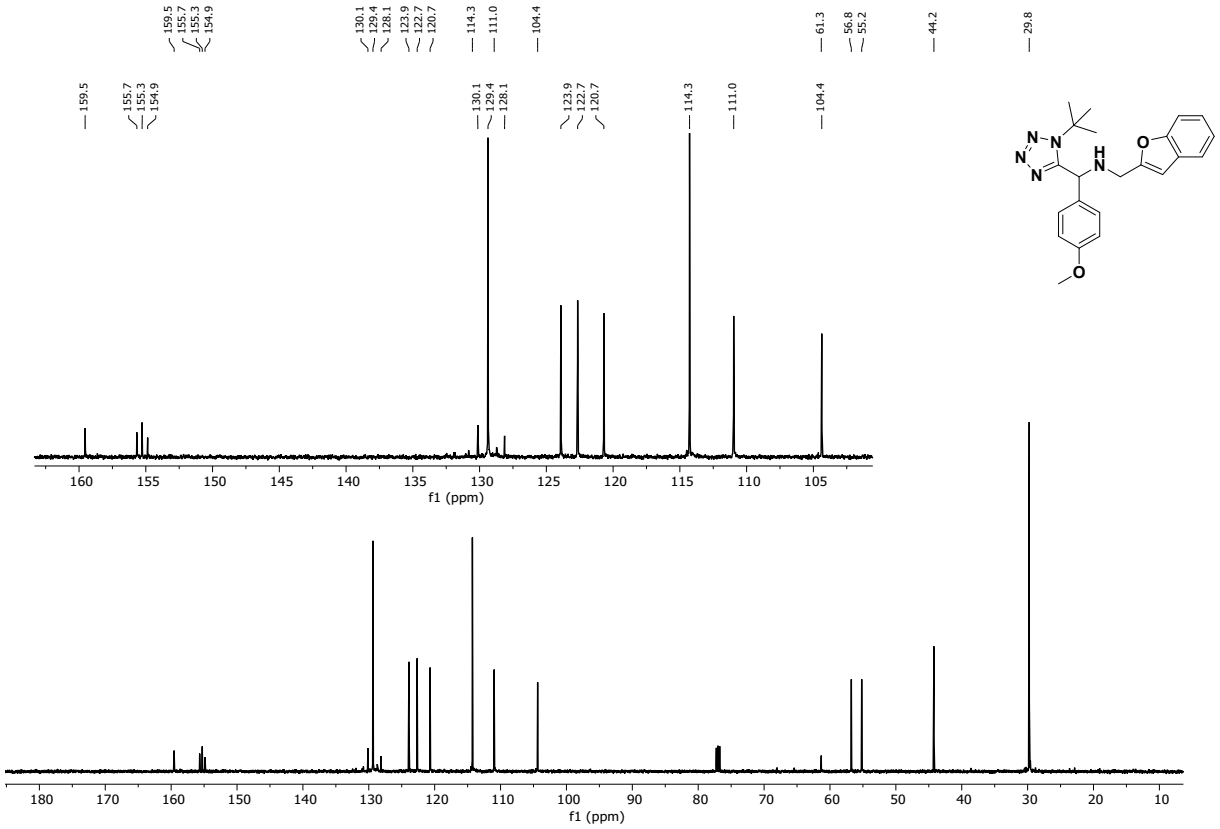
^{13}C NMR spectra of the compound **9l**.

N-(benzofuran-2-ylmethyl)-1-(1-(*tert*-butyl)-1*H*-tetrazol-5-yl)-1-(4-methoxyphenyl)methanamine (**9m**)

Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 4-methoxybenzaldehyde (44.1 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), *tert*-butyl isocyanide (41.0 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9m** was obtained as a brown semisolid (29.8 mg, 21%). R_f = 0.37 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.52 (ddd, *J* = 7.5, 1.5, 0.7 Hz, 1H), 7.45–7.43 (m, 1H), 7.27–7.19 (m, 4H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.58 (d, *J* = 0.9 Hz, 1H), 5.36 (s, 1H), 3.96 (dd, *J* = 14.8, 0.9 Hz, 1H), 3.86 (dd, *J* = 14.7, 0.8 Hz, 1H), 3.78 (s, 3H), 1.54 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 159.5, 155.7, 155.3, 154.9, 130.1, 129.4, 128.1, 123.9, 122.7, 120.7, 114.3, 111.0, 104.4, 61.3, 56.8, 55.2, 44.2, 29.8. FT-IR (ATR) ν_{max}/cm^{-1} 2921, 1728, 1607, 1448, 1251, 744. HRMS (ESI$^+$): m/z: Calcd. for $C_{22}H_{26}N_5O_2$ $[M+H]^+$: 392.2081; Found: 392.2093.



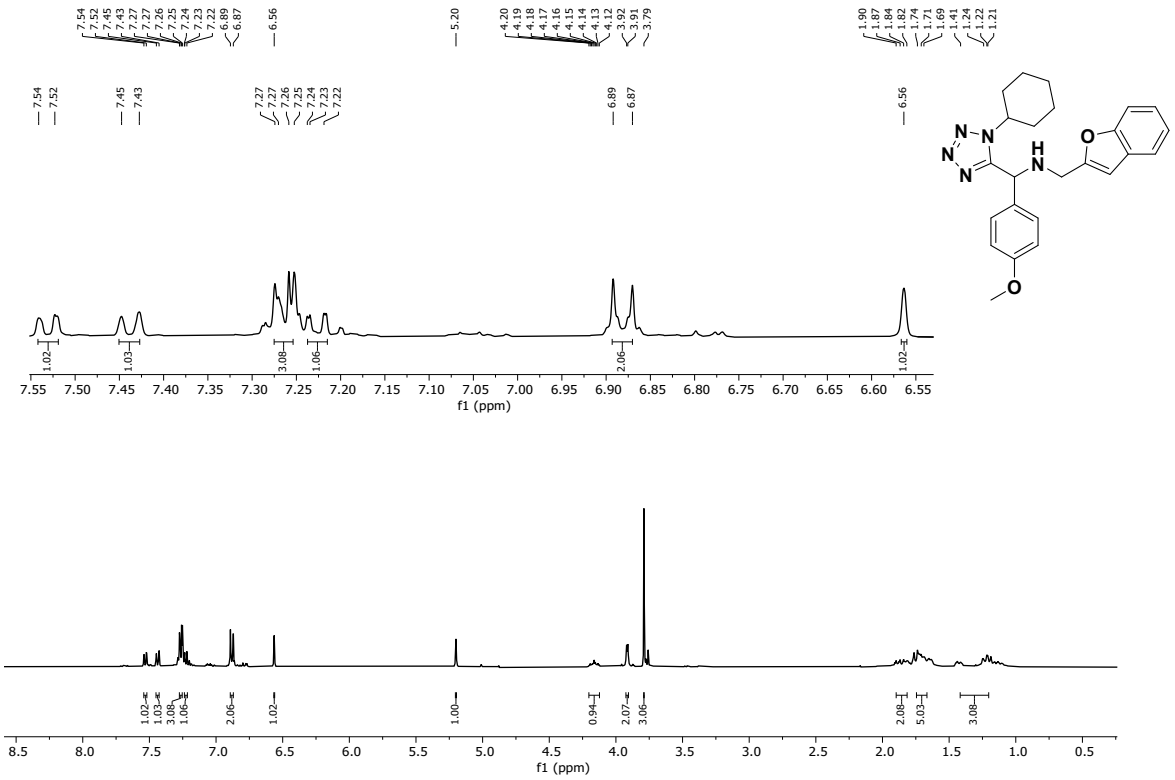
^{1}H NMR spectra of the compound **9m**.



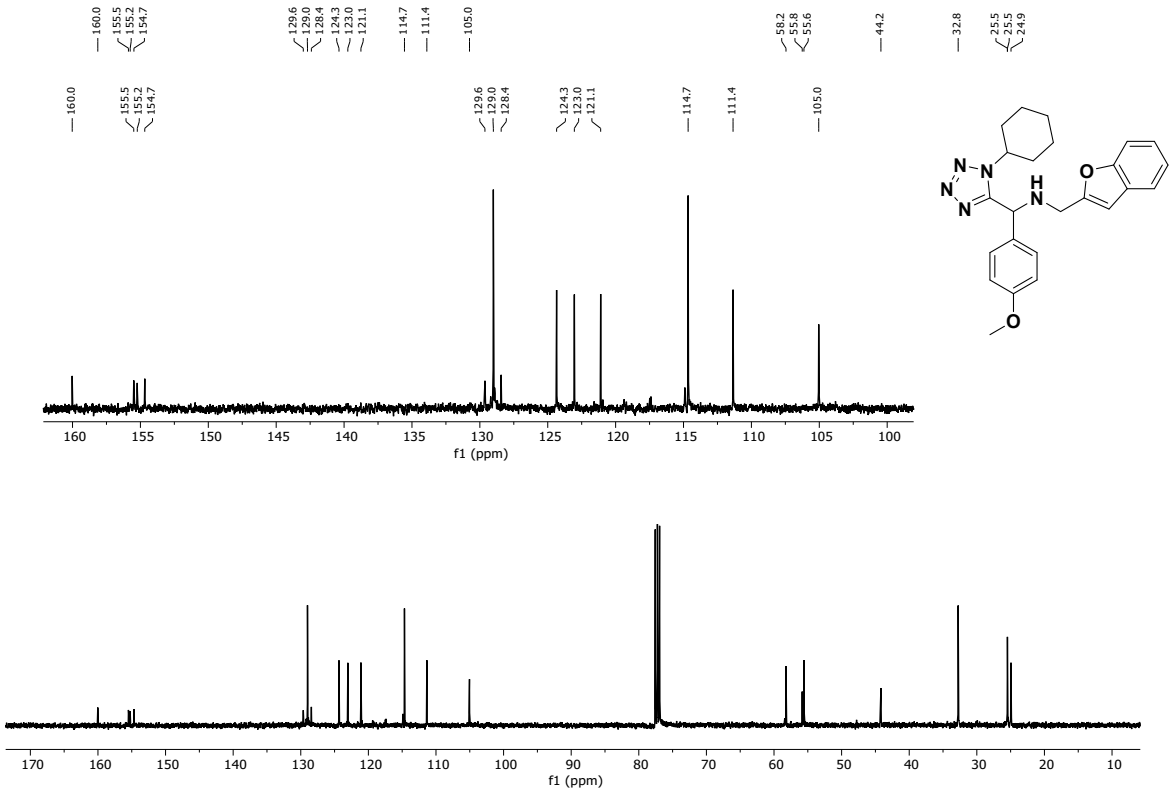
^{13}C NMR spectra of the compound **9m**.

N-(benzofuran-2-ylmethyl)-1-(1-cyclohexyl-1*H*-tetrazol-5-yl)-1-(4-methoxyphenyl)methanamine (**9n**)

Based on the GP1, propargylamine (23.3 µL, 0.36 mmol), 4-methoxybenzaldehyde (44.1 µL, 0.36 mmol), $TMSN_3$ (58 µL, 0.43 mmol), cyclohexyl isocyanide (45.1 µL, 0.36 mmol), 2-iodophenol (86.0 mg, 0.39 mmol), $(PPh_3)_2PdCl_2$ (23.0 mg, 0.03 mmol) and CuI (5.0 mg, 0.03 mmol) were used and **9n** was obtained as a yellow semisolid (36.3 mg, 24%). R_f = 0.43 (Hexane-AcOEt 7:3 v/v); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (d, *J* = 7.3 Hz, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.27–7.25 (m, 3H), 7.24–7.22 (m, 1H), 6.88 (d, *J* = 8.7 Hz, 2H), 6.56 (s, 1H), 5.20 (s, 1H), 4.20–4.12 (m, 1H), 3.92 (d, *J* = 3.5 Hz, 2H), 3.79 (s, 3H), 1.90–1.21 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.0, 155.5, 155.2, 154.7, 129.6, 129.0, 128.4, 124.3, 123.0, 121.1, 114.7, 111.4, 105.0, 58.2, 55.8, 55.6, 44.2, 32.8, 25.5, 25.5, 24.9. FT-IR (ATR) ν_{max}/cm^{-1}2930, 1456, 1251, 1094, 1018. HRMS (ESI$^+$): m/z: Calcd. for $C_{24}H_{28}N_5O_2$ $[M+H]^+$: 418.2238; Found: 418.2248.



^{1}H NMR spectra of the compound **9n**.



^{13}C NMR spectra of the compound **9n**.

General information for biological evaluation of compounds 9a-n

All the compounds (**9a-n**) at 40 μg/ml were evaluated during aerobic germination of ***M. lusitanicus*** in liquid media YNB at 2% glucose. We evaluated the germination rate by maintaining the liquid cultures at 28°C for 8 hours with constant shaking. The presence of hyphal cells in 100 spores determined the germination percentages. Only compounds (**9d**, **9h**, **9i**, **9m**, and **9b**) showed significant changes in the germination rate of *M. lusitanicus.*

Strains and culture media

All experiments used *M. lusitanicus strain MU636 (leuA-, pyrG+)*[1]. MU636 was grown in either a yeast-peptone-glucose (YPG) medium (pH 4.5, 3 g yeast extract, 10 g peptone, and 20 g glucose per liter) or Yeast Nitrogen Base (YNB), supplemented with 2% glucose (Difco, NJ, USA). We added 15 g/L agar to the media when solid media was necessary. After 5 days of growth on solid medium, spores were obtained, counted, and stored at 4°C for 2 weeks.

Spore germination under aerobic conditions

M. lusitanicus cultures were inoculated with 5×10^5 spores/mL. We obtained the growth of M. lusitanicus after aerobic growth in a 125-mL flask with 10 mL of corresponding media by incubating it in an orbital shaker at 150 rpm. Germination rates of the fungus under different growth conditions were assessed by observation under an Olympus CKX41 microscope equipped with a 40 × objective lens (Shinjuku, Tokyo, Japan), and spores or spore germination were captured with a DMC-T25 camera 9 (Panasonic, Kadoma, Japan). We calculated the spore germination rate using hyphae, yeast, and swollen spores present in 100 cells.

Hyphal length measurements

The length of each of the hyphae of 100 cells during aerobic growth was quantified using an Olympus CKX41 microscope equipped with a 40 × objective lens (Shinjuku, Tokyo, Japan).

Respiration measurements

Cell respiration was determined after 6 h of growth at 28 °C in YNB liquid culture media containing 2% glucose using 5×10^5 spores/mL of each strain, as previously described[2].

Viability Assay

Cell viability was determined using the MTT colorimetric method with thiazolyl blue tetrazolium bromide (Sigma-Aldrich Co). In summary, we inoculated spores in a 125-mL flask containing 10 mL of YNB at 2% glucose (YNB-2%) for 48 h at 28 °C, which corresponded to the YNB condition. In the **9b** without media change condition, we inoculated spores in YNB-2% with additions of 4 μg/mL and incubated them for 48 h. In the **9b** with media change condition, we inoculated spores in YNB-2% for the first 5 h in the presence of **9b** compound. Next, we recovered the cells by centrifuging them at 2,800 x g for 5 min. We then incubated the recovered cells with fresh YNB-2% without the presence of 9b for the next 43 h. Afterwards, we loaded cells equivalent to 1 mL from the previous cultures into 24-well flat-bottomed plates to assess their viability. 500 μL of 5 mg MTT per mL in PBS was added to each well and incubated for 4 h at 37 °C. Finally, 500 μL of 2-propanol/1 M HCl (19 : 1 v/v) was added to dissolve the formazan crystals. We conducted absorbance measurements at 595 nm using a microplate spectrophotometer (IMarK Microplate Reader, BIO-RAD, Hercules, CA, USA).

Minimal Inhibitory Concentration (MIC)

Spores of *M. lusitanicus* were germinated under aerobic conditions in YNB-2% medium in the presence of amphotericin B and compound **9b**, respectively. At hour 8, we quantified the percentage of germinated cells and determined the minimal inhibitory concentration that completely prevented germination.

Statistical analysis

All the data were evaluated by Analysis of Variance (ANOVA) and statistically significant differences ($P \leq 0.05$) after using the Fisher post-hoc test is indicated using asterisks.

References

1. M. I. Navarro-Mendoza, C. Pérez-Arques, S. Panchal, F. E. Nicolás, S. J. Mondo, P. Ganguly, J. Pangilinan, I. V. Grigoriev, J. Heitman, K. Sanyal and V. Garre, *Curr. Biol*., 2019, **29**, 3791-3802.
2. J. A. Patiño-Medina, M. I. Valle-Maldonado, D. Vargas-Tejeda, V. M. Chávez-Jacobo, A. R. Corrales-Escobosa, J. Ramírez-Emiliano, L. F. Ruiz-Herrera, M. I. Ramírez-Díaz, V. Garre and V. Meza-Carmen, *Fungal Biol*., 2020, **124**, 619-628.