Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2020

Supporting Information

Synthesis of 3-alkyl-6-methyl-1,2,4,5-tetrazines via a Sonogashira-type cross-coupling reaction

Enric Ros,[a] Amparo Prades,[a] Dominique Forson,[a] Jacqueline Smyth,[a] Xavier Verdaguer,[a, b] Lluís Ribas de Pouplana*[a, c] and Antoni Riera*[a, b]

[a.] *Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology, Baldiri Reixac 10, 08028 Barcelona, Spain.*

[b.] *Departament de Química Inorgànica i Orgànica, Secció Orgànica. Universitat de Barcelona, Martí i Franquès 1, Barcelona E-08028, Spain.*

[c.] *Institució Catalana de Recerca i Estudis Avançats (ICREA), Passeig Lluís Companys, 23, Barcelona 08010, Spain.*

e-mail: antoni.riera@irbbarcelona.org; lluis.ribas@irbbarcelona.org.

Table of contents

1. General methods and instrumentation

General procedures and materials:

Unless otherwise indicated, materials were obtained from commercial suppliers (Merck, Fluorochem) and used without further purification. All reactions that required anhydrous conditions were performed in a degassed sealed vial under a dry nitrogen atmosphere. Dichloromethane and tetrahydrofuran were degassed and dried with a solvent purification system (SPS PS-MD-3). Anhydrous toluene was purchased from Merck. Solvents were removed under reduced pressure with a rotary evaporator. Silica gel chromatography was performed using an automated chromatography system (PuriFlash® 430, Interchim), using a 0-100% ethyl acetate gradient in hexane.

Instrumentation:

NMR spectroscopy: ^{1}H and ^{13}C were recorded on the NMR spectrometers of the *Centres Científics i Tecnològics* of Universitat de Barcelona. The employed spectrometers were a Varian Mercury 400 or 500 MHz, specified for each case. Chemical shifts (δ) were referenced to internal solvent resonance. The coupling constants (J) are reported in Hertz (Hz). The following abbreviations are used to define multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), ddd (doublet of doublet of doublets), dt (doublet of triplets), td (triplet of doublets), tt (triplet of triplets) and m (multiplet).

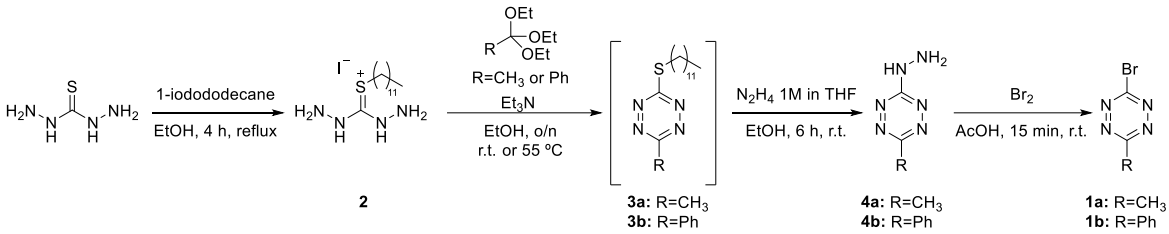
High Resolution Mass Spectrometry (HRMS): High resolution ESI-MS spectra were recorded either in an LC/MSD-TOF G1969A (Agilent Technologies) of the *Centres Científics i Tecnològics* of Universitat de Barcelona or in a LTQ-FT Ultra (ThermoFisher Scientific) at the *Mass Spectrometry and Proteomics Facility* of the IRB Barcelona.

IR spectroscopy: IR spectra were measured in a Thermo Nicolet 6700 FT-IR spectrometer using an ATR system.

Melting points were determined using a Büchi melting point apparatus and were not corrected.

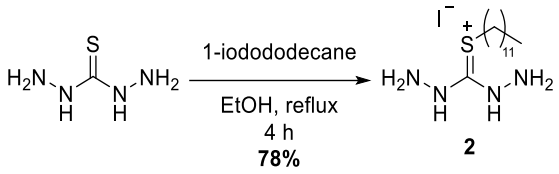
2. Experimental procedures and characterization data

2.1. Synthetic route for 3-bromo-6-methyl-1,2,4,5-tetrazine (1a) and 3-bromo-6-phenyl-1,2,4,5-tetrazine (1b)



2 **3a:** R=CH_3 **3b:** R=Ph **4a:** R=CH_3 **4b:** R=Ph **1a:** R=CH_3 **1b:** R=Ph

Dodecyl-thiocarbohydrazide iodide salt (2)



2

Same experimental procedure as previously reported was followed.[1] Briefly, commercially available thiocarbohydrazide 98% (Merck; 10.00 g, 92.32 mmol, 1 equiv) was suspended in EtOH (470 mL, 0.2 M), and the suspension was heated under reflux. 1-iodododecane 98% (Merck; 25.9 mL, 101.55 mmol, 1.1 equiv) was dissolved in EtOH (25 mL) and added dropwise, and the reaction mixture was heated at reflux temperature for 4 h.

After cooling down the reaction mixture, the solvent was concentrated under reduced pressure until half the initial volume, and fresh EtOH (≈ 150 mL) was added. The yellow suspension was filtered to remove the unreacted thiocarbohydrazide (4.90 g, NMR consistent with starting material). The filtrate solution was fully evaporated, the resulting residue was re-suspended in hexane and subjected to filtration to afford the desired product as a white solid (15.08 g, 51% conversion, 78% yield).

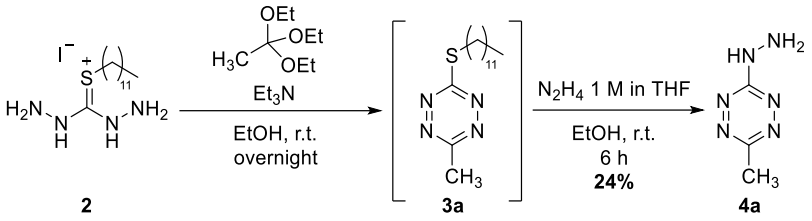
^{1}H was in excellent agreement with the reported characterisation.[1]

^{1}H NMR (400 MHz, DMSO-d_6) δ 2.90 (t, *J* = 7.4 Hz, 2H), 1.62–1.50 (m, 2H), 1.42 – 1.17 (m, 20H), 0.85 (t, *J* = 6.9 Hz, 3H) ppm

^{13}C NMR (101 MHz, DMSO-d_6) δ 31.7, 29.5–29.4, 29.3, 29.1, 28.9, 28.7, 28.5, 22.5, 14.4 ppm

[1] Ros, E.; Bellido, M.; Verdaguer, X.; Ribas de Pouplana, L.; Riera, A. *Bioconjugate Chem.* **2020**, *31*, 933.

3-hydrazineyl-6-methyl-1,2,4,5-tetrazine (4a)



Salt **2** (11.21 g, 27.86 mmol, 1 equiv) was suspended in EtOH (200 mL, 0.14 M) at room temperature, and triethyl orthoacetate (Merck; 10.55 mL, 55.716 mmol, 2 equiv) was added, turning the solution clear yellow. After 10 min, triethylamine (15.5 mL, 111.431 mmol, 4 equiv) was added, immediately turning the solution light red and eventually magenta. The reaction mixture was stirred vigorously overnight at room temperature, and TLC (50:50 Hexane/EtOAc) showed the desired product (pink, front) and a by-product (yellow, baseline). The solvent was evaporated under reduced pressure, the resulting crude diluted in water and extracted with diethyl ether (x3). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to 20 mL. The resulting crude was purified by column chromatography (50:50 Hexane/Et_2O) to afford 4.20 g of **3a** as an impure pink oil.

Then, to a stirred solution of **3a** (4.20 g) in EtOH (90 mL) was added hydrazine 1 M in THF (14.20 mL, 14.20 mmol, 1 equiv considering **3a** as pure). The reaction mixture was stirred at room temperature and TLC (100% EtOAc) analysis showed completion after 6 h. The solvent was evaporated under reduced pressure and the resulting residue was diluted in EtOAc and dry-loaded to an 80 g silica column using a 0-100% EtOAc gradient in hexane to obtain **4a**, 838 mg (24% from **2**), as a red solid.

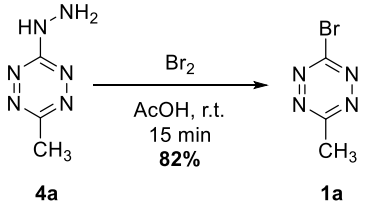
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 4.50 (s, 2H), 2.70 (s, 3H) ppm

^{13}C NMR (101 MHz, DMSO-d_6) δ 163.5, 160.7, 19.5 ppm

HRMS (ESI$^+$) calculated for $C_3H_6N_6$ $[M+H]^+$: 127.07267 / found 127.07272

IR U_{max}: 3268, 3199, 2924, 1540, 1496, 909 cm^{-1}

3-bromo-6-methyl-1,2,4,5-tetrazine (1a)



To a stirred solution of **4a** (480 mg, 3.806 mmol, 1 equiv) in 2 mL glacial acetic acid was added a solution of bromine (0.2 mL) in glacial acetic acid (4 mL) dropwise. After 15 mins, TLC (90:10

Hexane/EtOAc) showed completion. The reaction mixture was diluted in water, extracted with DCM (x3) and the combined organic layers were washed with water (x3). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to obtain **1a**, 544 mg (82% yield), as a pink crystalline solid.

Mp: 86.0 °C**.**

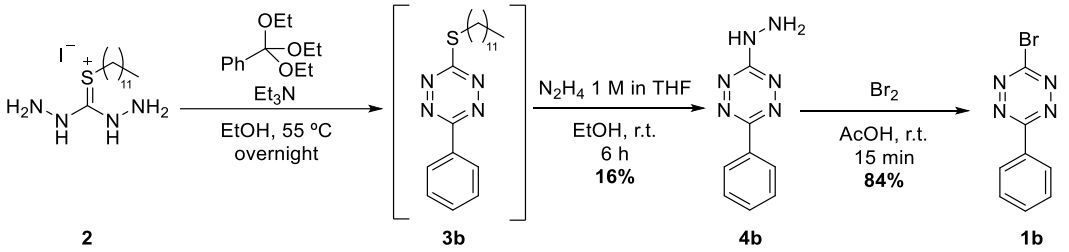
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1, 161.2, 20.6 ppm

HRMS (APCI) calculated for $C_3H_4BrN_4$ $[M+H]^+$: 174.9614 and 176.9593 / found: 174.9608 and 176.9588

IR U_{max}: 2920, 2846, 1305, 1140, 865 cm^{-1}

3-bromo-6-phenyl-1,2,4,5-tetrazine (1b)



The same experimental procedure was followed for the synthesis of **1b**, with the only exception of the temperature in the reaction from **2** to **3a**, which was set at 55 °C. Starting from **2** (2.87 g, 7.13 mmol), **1b** was finally obtained as a pink solid (219.8 mg, 0.927 mmol, 13% overall yield). Spectroscopic data are in full agreement with the reported[2]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 – 8.53 (m, 2H), 7.71 – 7.56 (m, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 164.74, 161.11, 133.62, 130.61, 129.64, 128.49 ppm

[2] A. Counotte-Potman and H. van der Plas, *J. Heterocycl. Chem.*, 1981, **18**, 123–127

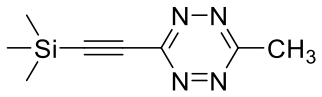
2.2. Screening of Sonogashira reaction conditions

To a degassed reaction vessel with **1a** (10.0 mg, 0.057 mmol, 1 equiv), PPh_3 (1.5 mg, 0.006 mmol, 0.1 equiv), CuI (5.5 mg, 0.006 mmol, 0.1 equiv) and the corresponding Pd-catalyst (0.003 mmol, 0.05 equiv), the required solvent (0.4 mL, for a final reaction concentration of 0.15 M), TMS-acetylene (15.8 µL, 0.114 mmol, 2 equiv) and diisopropylamine (16.0 µL, 0.114 mmol, 2 equiv) were added.

The reaction mixture was stirred overnight at the corresponding temperature, after which the suspension was filtered through Celite, the filtrate concentrated under reduced pressure, re-suspended in $CDCl_3$ and mesitylene (7.9 µL, 0.057 mmol, 1 equiv) added as an internal standard.

^{1}H NMR yield was determined through the ratio of the integration between mesitylene peak at 6.79 ppm (s, 3H) and the product 3-methyl-6-((trimethylsilyl)ethynyl)-1,2,4,5-tetrazine (**5a**) methyl peak at 3.07 ppm (s, 3H). Compound **5a** was also purified and characterized following reaction conditions specified in **section 2.3.**

3-methyl-6-((trimethylsilyl)ethynyl)-1,2,4,5-tetrazine (5a)



Pink oil (39.6 mg, 72%).

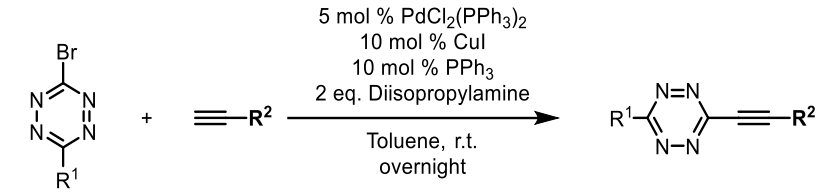
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.07 (s, 3H), 0.34 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.66, 156.49, 106.10, 96.93, 21.65, -0.60 ppm

HRMS (ESI$^+$) calculated for $C_{13}H_{13}N_4Si$ $[M+H]^+$: 193.0906 / found 193.0904

IR υ_{max}: 2960, 2900, 2066, 1390, 1349, 1274, 1250, 1076 cm^{-1}

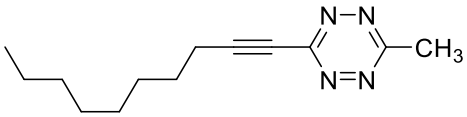
2.3. Substrate scope of Sonogashira coupling between terminal alkynes and 1a-b



To a degassed reaction vessel with **1a** (50.0 mg, 0.286 mmol, 1 equiv) or **1b** (67.8 mg, 0.286 mmol, 1 equiv), the corresponding alkyne (0.572 mmol, 2 equiv), $PdCl_2(PPh_3)_2$ (10.1 mg, 0.014 mmol, 0.05 equiv), PPh_3 (7.5 mg, 0.029 mmol, 0.1 equiv) and CuI (5.5 mg, 0.029 mmol, 0.1 equiv), toluene (2 mL, for a final reaction concentration of 0.15 M) and diisopropylamine (80.2 µL, 0.572 mmol, 2 equiv) were added. The reaction mixture was stirred overnight at room temperature.

The reaction crude was evaporated under reduced pressure, and the resulting residue extracted from water with ethyl acetate (3x). The organic phases were combined, dried with $MgSO_4$, filtered and concentrated under vacuum. The desired products were then purified with flash chromatography in 12 g of silica, using hexane with increasing concentrations of ethyl acetate.

3-(dec-1-yn-1-yl)-6-methyl-1,2,4,5-tetrazine (5b)



Pink oil (43.5 mg, 65%).

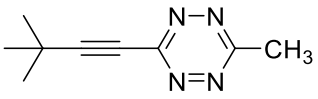
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (s, 3H), 2.58 (t, *J* = 7.2 Hz, 2H), 1.75 – 1.66 (m, 2H), 1.57 – 1.40 (m, 2H), 1.38 – 1.19 (m, 8H), 0.92 – 0.81 (m, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.57, 157.06, 101.44, 75.26, 32.02, 29.33, 29.26, 29.18, 27.98, 22.86, 21.63, 19.90, 14.30 ppm

HRMS (ESI$^+$) calculated for $C_{13}H_{21}N_4$ $[M+H]^+$: 233.176 / found 233.1761

IR U_{max}: 2924, 2854, 2235, 1458, 1402, 1362, 1273, 1078, 1035 cm^{-1}

3-(3,3-dimethylbut-1-yn-1-yl)-6-methyl-1,2,4,5-tetrazine (5c)



Pink oil (49.8 mg, 99%).

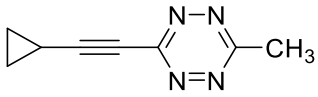
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (s, 3H), 1.42 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.46, 157.01, 108.39, 73.95, 30.28, 28.58, 21.53 ppm

HRMS (ESI$^+$) calculated for $C_9H_{13}N_4$ $[M+H]^+$: 177.1135 / found 177.1133

IR U_{max}: 2935, 2830, 2230, 1443, 1405, 1355, 1050, cm^{-1}

3-(cyclopropylethynyl)-6-methyl-1,2,4,5-tetrazine (5d)



Pink oil (25.2 mg, 55%).

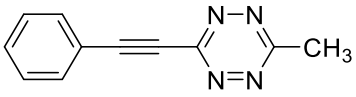
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.03 (s, 3H), 1.68 – 1.57 (m, 1H), 1.12 – 1.01 (m, 4H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.23, 156.94, 104.60, 70.42, 21.51, 9.70, 0.57 ppm

HRMS (ESI$^+$) calculated for $C_8H_9N_4$ $[M+H]^+$: 161.0818 / found 161.0822

IR U_{max}: 3011, 2923, 2234, 1410, 1336, 1274, 1182, 1077, 1058, 1033 cm^{-1}

3-methyl-6-(phenylethynyl)-1,2,4,5-tetrazine (5e)



Pink solid (27.5 mg, 49%). **Mp =** 119 °C

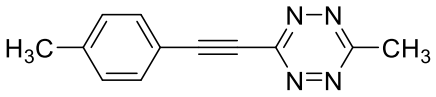
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (dd, *J* = 8.3, 1.5 Hz, 2H), 7.57 – 7.39 (m, 3H), 3.10 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.45, 157.34, 132.89, 132.84, 128.83, 120.38, 97.92, 83.01, 21.62 ppm

HRMS (ESI$^+$) calculated for $C_{13}H_{13}N_4Si$ $[M+H]^+$: 193.0906 / found 193.0904

IR U_{max}: 2960, 2900, 2066, 1390, 1349, 1274, 1250, 1076 cm^{-1}

3-methyl-6-(*p*-tolylethynyl)-1,2,4,5-tetrazine (5f)



Pink solid (49.3 mg, 82%). **Mp =** 157 °C

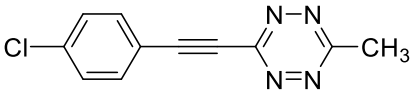
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.2 Hz, 2H), 7.25 – 7.20 (m, 2H), 3.08 (s, 3H), 2.40 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.26, 157.36, 141.52, 132.82, 129.58, 117.27, 98.48, 82.68, 21.88, 21.57 ppm

HRMS (ESI$^+$) calculated for $C_{12}H_{11}N_4$ $[M+H]^+$: 211.0978 / found 211.0977

IR U_{max}: 3040, 2953, 2923, 2850, 2213, 1509, 1404, 1365, 1274, 1158, 1036 cm^{-1}

3-((4-chlorophenyl)ethynyl)-6-methyl-1,2,4,5-tetrazine (5g)



Pink solid (41.5 mg, 63%). **Mp =** 166 °C

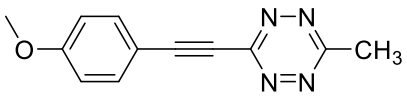
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 – 7.60 (m, 2H), 7.46 – 7.38 (m, 2H), 3.09 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.55, 157.20, 137.26, 134.04, 129.30, 118.81, 96.49, 83.81, 21.64 ppm

HRMS (ESI$^+$) calculated for $C_{11}H_8ClN_4$ $[M+H]^+$: 231.0432 / found 231.0433

IR U_{max}: 2233, 2205, 1701, 1482, 1402, 1365, 1086 cm^{-1}

3-((4-methoxyphenyl)ethynyl)-6-methyl-1,2,4,5-tetrazine (5h)



Orange solid (23.3 mg, 36%). **Mp =** 163 °C

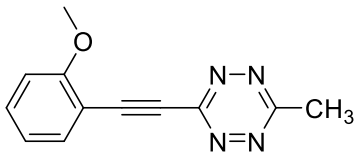
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (dd, *J* = 9.0, 0.8 Hz, 2H), 6.93 (dd, *J* = 9.0, 0.9 Hz, 2H), 3.85 (s, 3H), 3.07 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.12, 161.69, 157.41, 134.71, 114.53, 112.23, 98.80, 82.46, 55.56, 21.55 ppm

HRMS (ESI$^+$) calculated for $C_{12}H_{11}N_4O$ $[M+H]^+$: 227.0927 / found 227.0929

IR U_{max}: 3098, 2965, 2834, 2204, 1600, 1418, 1403, 1366, 1252, 1197, 1154, 1023 cm^{-1}

3-((2-methoxyphenyl)ethynyl)-6-methyl-1,2,4,5-tetrazine (5i)



Pink solid (40.1 mg, 62%). **Mp =** 134 °C

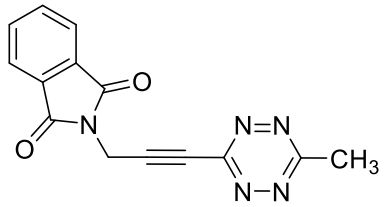
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.45 (ddd, *J* = 8.5, 7.5, 1.8 Hz, 1H), 7.06 – 6.91 (m, 2H), 3.96 (s, 3H), 3.09 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.21, 161.47, 157.46, 134.71, 132.55, 120.75, 111.03, 109.66, 95.16, 86.84, 56.04, 21.58 ppm

HRMS (ESI$^+$) calculated for $C_{12}H_{11}N_4O$ $[M+H]^+$: 227.0927 / found 227.0928

IR U_{max}: 3406, 2971, 2213, 1714, 1694, 1519, 1494, 1455, 1392, 1274, 1248, 1167, 1075, 1044, 1010 cm^{-1}

2-(3-(6-methyl-1,2,4,5-tetrazin-3-yl)prop-2-yn-1-yl)isoindoline-1,3-dione (5j)



Pink oil (44.7 mg, 56%).

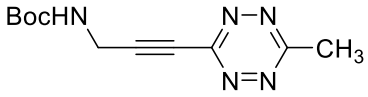
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.77 (dd, *J* = 5.5, 3.1 Hz, 2H), 4.84 (s, 2H), 3.06 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.86, 166.02, 156.49, 134.60, 132.06, 123.94, 91.77, 27.77, 21.65 ppm

HRMS (ESI^+) calculated for $C_{14}H_9N_5O_2$ $[M+H]^+$: 280.0830 / found 280.0829

IR U_{max}: 3203, 3078, 2975, 2924, 2216, 1753, 1598, 1475, 1387, 1288, 1053 cm^{-1}

***Tert*-butyl (6-(6-methyl-1,2,4,5-tetrazin-3-yl)hex-5-yn-1-yl)carbamate (5k)**



Pink oil (49.2 mg, 69%).

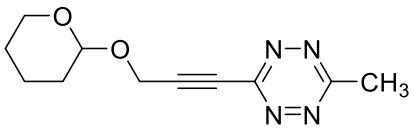
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93 (s, 1H), 4.33 (d, *J* = 5.7 Hz, 2H), 3.08 (s, 3H), 1.47 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.95, 156.62, 155.26, 95.10, 80.74, 77.39, 31.24, 28.46, 21.63 ppm

HRMS (ESI^+) calculated for $C_{11}H_{16}N_5O_2$ $[M+H]^+$: 250.12985 / found 250.13180

IR U_{max}: 3338, 2978, 2932, 2255, 1698, 1519, 1403, 1366, 1250, 1167, 1048 cm^{-1}

3-methyl-6-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-yn-1-yl)-1,2,4,5-tetrazine (5l)



Pink oil (45.5 mg, 68%).

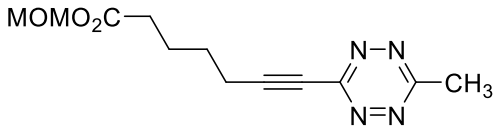
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (t, *J* = 3.1 Hz, 1H), 4.63 (s, 2H), 3.89 (ddd, *J* = 11.1, 9.3, 3.1 Hz, 1H), 3.63 – 3.56 (m, 1H), 3.08 (s, 3H), 1.89 – 1.61 (m, 6H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 165.94, 156.68, 97.51, 94.92, 79.43, 62.20, 54.41, 30.26, 25.42, 21.65, 18.97 ppm

HRMS (ESI^+) calculated for $C_{11}H_{14}N_4O_2$ $[M+H]^+$: 235.11908 / found 235.11895

IR U_{max}: 3326, 2943, 2831, 1448 cm^{-1}

Methoxymethyl 7-(6-methyl-1,2,4,5-tetrazin-3-yl)hept-6-ynoate (5m)



Pink oil (52.8 mg, 70%).

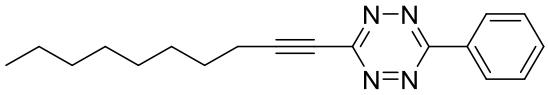
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (s, 2H), 3.47 (s, 3H), 3.06 (s, 3H), 2.64 (t, J = 6.9 Hz, 2H), 2.44 (t, J = 7.2 Hz, 2H), 1.97 – 1.68 (m, 4H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.84, 165.59, 156.89, 100.17, 90.53, 75.56, 57.81, 33.80, 27.24, 24.13, 21.55, 19.57 ppm

HRMS (ESI$^+$) calculated for $C_{12}H_{17}N_4O_3$ $[M+H]^+$: 265.12952 / found 265.13156

IR U_{max}: 2941, 2237, 1741, 1590, 1405, 1364, 1137, 1093 cm^{-1}

3-(dec-1-yn-1-yl)-6-phenyl-1,2,4,5-tetrazine (5n)



Pink oil (59.8 mg, 71%).

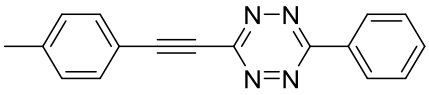
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (dd, J = 8.2, 1.6 Hz, 2H), 7.66 – 7.54 (m, 3H), 2.62 (t, J = 7.1 Hz, 2H), 1.74 (p, J = 7.2 Hz, 2H), 1.56 – 1.46 (m, 2H), 1.37 – 1.23 (m, 8H), 0.88 (t, J = 7.0 Hz, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.74, 156.89, 133.11, 131.61, 129.47, 128.49, 102.03, 75.65, 31.96, 29.27, 29.20, 29.13, 27.94, 22.79, 19.95, 14.23 ppm

HRMS (ESI$^+$) calculated for $C_{18}H_{23}N_4$ $[M+H]^+$: 295.1917 / found 295.1914

IR U_{max}: 2923, 2853, 2233, 1727, 1600, 1463, 1391, 1087 cm^{-1}

3-phenyl-6-(p-tolylethynyl)-1,2,4,5-tetrazine (5o)



Pink oil (73.2 mg, 94%).

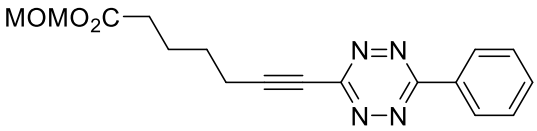
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (dd, *J* = 8.2, 1.5 Hz, 2H), 7.69 – 7.54 (m, 5H), 7.26 (d, *J* = 8.0 Hz, 2H), 2.43 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.48, 157.31, 141.60, 133.20, 132.92, 131.60, 131.04, 129.65, 129.51, 128.57, 117.45, 99.22, 83.28, 21.95 ppm

HRMS (ESI$^+$) calculated for $C_{17}H_{13}N_4$ $[M+H]^+$: 273.1135 / found 273.1131

IR U_{max}: 3066, 2952, 2920, 2213, 1723, 1598, 1509, 1395, 1166, 1076 cm^{-1}

Methoxymethyl 7-(6-phenyl-1,2,4,5-tetrazin-3-yl)hept-6-ynoate (5m)



Pink oil (70.0 mg, 75%).

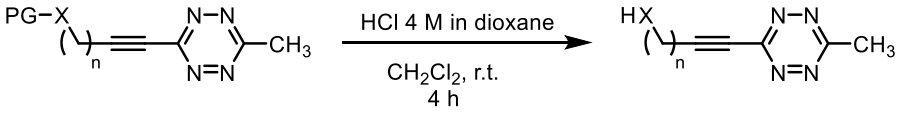
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 – 8.55 (m, 2H), 7.70 – 7.49 (m, 3H), 5.24 (d, *J* = 0.5 Hz, 2H), 3.47 (d, *J* = 0.5 Hz, 3H), 2.67 (t, *J* = 6.9 Hz, 2H), 2.46 (t, *J* = 7.2 Hz, 2H), 1.98 – 1.73 (m, 4H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.83, 161.76, 156.77, 133.15, 131.53, 129.46, 128.50, 100.75, 90.51, 76.02, 57.80, 33.80, 27.26, 24.14, 19.67 ppm

HRMS (ESI^+) calculated for $C_{17}H_{19}N_4O_3$ $[M+H]^+$: 327.1452 / found 327.1452

IR U_{max}: 2938, 2360, 2233, 1735, 1600, 1391, 1133, 1089 cm^{-1}

2.4. Deprotection of protecting groups:



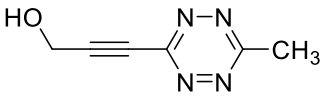
PG-X = Boc-NH, **5k**
PG-X = THP-O, **5l**
PG-X = MOM-CO_2, **5m**

X = NH, **6a***
X = O, **6b**
X = CO_2, **6c**

The corresponding amount of protected alkynyl-tetrazine (0.100 mmol) was placed in a glass vial, which was sealed, degassed and placed under N_2 atmosphere. DCM was then added (to reach a final concentration of 0.1 M), and HCl 4 M in dioxane (50 µL, 2 equiv) was added. The reaction mixture was stirred at room temperature for 4 h, after which the reaction mixture was concentrated under reduced pressure, diluted in water and extracted with ethyl acetate (3x). The combined organic phases were washed once with brine, dried with $MgSO_4$ and concentrated under high vacuum to afford the corresponding products.

* Deprotection of **5k** was verified, but compound **6a** could not be isolated.

3-(6-methyl-1,2,4,5-tetrazin-3-yl)prop-2-yn-1-ol (6b)



Pink oil (11.0 mg, 73%).

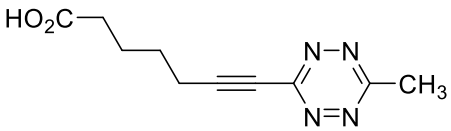
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.66 (s, 2H), 3.09 (s, 3H), 2.00 (s, 1H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 166.08, 156.61, 96.17, 79.36, 51.50, 21.66 ppm

HRMS (ESI$^+$) calculated for $C_6H_6N_4O$ $[M+H]^+$: 151.06101 / found 151.06144

IR U_{max}: 3189 (broad), 2991, 2926, 2843, 2156, 1497, 1302, 1178 cm^{-1}

7-(6-methyl-1,2,4,5-tetrazin-3-yl)hept-6-ynoic acid (6c)



Pink oil (21.8 mg, 99%). **Mp** = 102.4 °C

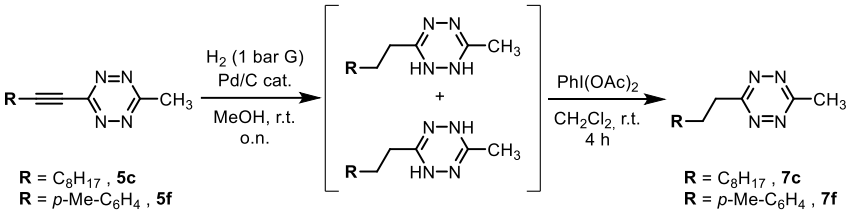
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (s, 3H), 2.64 (t, *J* = 6.8 Hz, 2H), 2.45 (t, *J* = 7.1 Hz, 2H), 1.92 – 1.69 (m, 4H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 178.62, 165.60, 156.88, 100.16, 75.56, 33.40, 27.18, 24.02, 21.56, 19.56 ppm

HRMS (ESI$^+$) calculated for $C_{10}H_{13}N_4O_2$ $[M+H]^+$: 221.10330 / found 221.10503

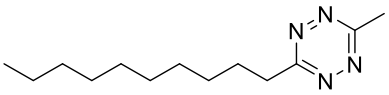
IR U_{max}: 2926, 2860, 2231, 1703, 1406, 1263 cm^{-1}

2.5. Tandem hydrogenation + oxidation of 5c and 5f



Glass vials equipped with a PTFE-coated stirring bar were charged with the corresponding substrate (0.187 mmol, 1.0 equiv.). Commercially available 10% palladium on carbon, 50% wet (Fluorochem) was added (2 mol % final Pd concentration), and MeOH (1 mL/0.1 mmol substrate) was added as the reaction solvent. Each reaction vial was placed on a reactor, which was purged with N_2, charged with 1 bar(g) of H_2 and left stirring overnight at room temperature. Afterwards, the reaction mixture was filtrated through a short plug of Celite and concentrated under reduced pressure to afford the corresponding crude mixture, which was dissolved in DCM. $PhI(OAc)_2$ (90.4 mg, 0.281 mmol, 1.5 equiv.) was added, and the reaction mixture was stirred for 4 h at room temperature until TLC showed completion. The reaction mixture was washed with water (3x), the organic phase was dried with $MgSO_4$ and evaporated under vacuum. The desired product was then purified with flash chromatography in 12 g of silica, using hexane with increasing concentrations of ethyl acetate as the mobile phase.

3-decyl-6-methyl-1,2,4,5-tetrazine (7c)



Pink oil (44.1 mg, >99%).

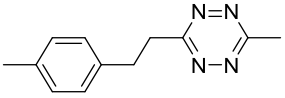
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.27 (t, *J* = 91.1 Hz, 2H), 3.03 (s, 3H), 1.92 (tt, *J* = 7.8, 6.5 Hz, 2H), 1.47 – 1.19 (m, 14H), 0.95 – 0.82 (m, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.39, 167.47, 34.88, 32.02, 29.68, 29.58, 29.43, 29.39, 29.29, 28.50, 22.81, 21.22, 14.24 ppm

HRMS (ESI$^+$) calculated for $C_{13}H_{25}N_4$ $[M+H]^+$: 237.20737 / found 237.20925

IR U_{max}: 2957, 2926, 2855, 1730, 1464, 1405, 1284, 1125, 1074 cm^{-1}

3-methyl-6-(4-methylphenethyl)-1,2,4,5-tetrazine (7f)



Pink solid (40.0 mg, >99%). **Mp** = 84 °C

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (q, J = 8.1 Hz, 4H), 3.68 – 3.50 (m, 2H), 3.29 – 3.19 (m, 2H), 3.03 (s, 3H), 2.31 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.41, 167.54, 136.93, 136.14, 129.42, 128.42, 36.60, 33.73, 21.23, 21.14 ppm

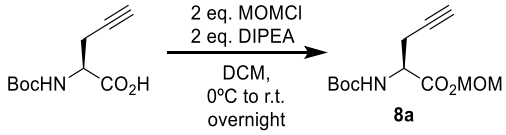
HRMS (ESI^+) calculated for $C_{12}H_{15}N_4$ $[M+H]^+$: 215.12912 / found 215.13082

IR U_{max}: 2925, 2848, 1593, 1516, 1431, 1408, 1348, 1045 cm^{-1}

2.6. Synthesis of unnatural amino acids

2.6.1. Preparation of substrates 8a and 8b

Synthesis of methoxymethyl (*S*)-2-((*tert*-butoxycarbonyl)amino)pent-4-ynoate (8a)



Commercially available *N*-Boc-L-2-propargylglycine (Merck; 400 mg, 1.876 mmol, 1 equiv) was suspended in DCM (10 mL) at 0°C, and DIPEA (654 µL, 3.752 mmol, 2 equiv) was added, turning the suspension a clear solution. Chloromethyl methyl ether (285 µL, 3.752 mmol, 2 equiv) was added dropwise, generating a thick white gas that was totally removed with N_2 flow. The reaction mixture was allowed to warm at room temperature and stirred overnight. The reaction mixture was then diluted with water, extracted with DCM (3x), the combined organics washed once with brine, dried over $MgSO_4$ and evaporated *in vacuo* to afford **8a**, 480 mg, 99% yield, as a colourless oil.

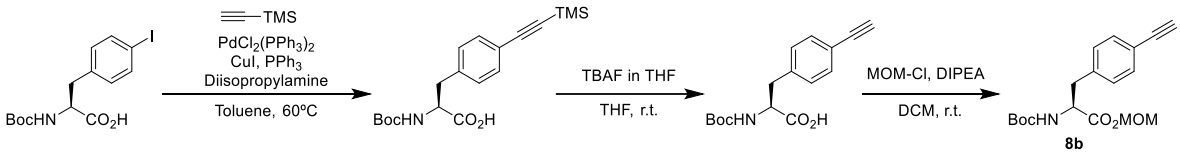
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (dd, *J* = 35.6, 5.9 Hz, 2H), 4.50 (dt, *J* = 8.7, 4.7 Hz, 1H), 3.48 (s, 3H), 2.93 – 2.61 (m, 2H), 2.05 (t, *J* = 2.6 Hz, 1H), 1.45 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 170.49, 155.23, 91.67, 80.43, 78.52, 71.92, 58.00, 52.16, 28.42, 22.91 ppm

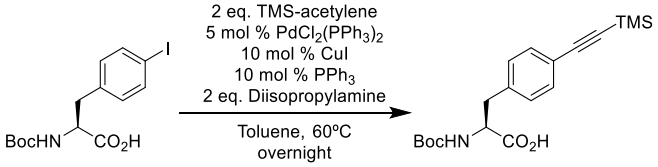
HRMS (ESI$^+$) calculated for $C_{12}H_{20}NO_5$ $[M+H]^+$: 258.13360 / found 258.13570

IR υ_{max}: 3375, 3292, 2977, 2934, 1751, 1715, 1505, 1367, 1251, 1162, 1251, 1162, 1097, 1061 cm^{-1}

Synthesis of methoxymethyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-ethynylphenyl)propanoate (8b)



(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-((trimethylsilyl)ethynyl)phenyl)propanoic acid:

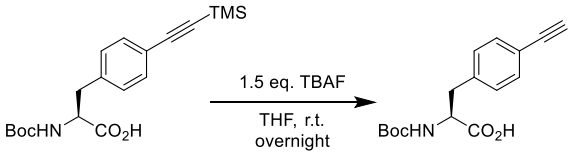


Commercially available *N*-Boc-4-iodo-L-phenylalanine (Fluorochem; 500 mg, 1.278 mmol, 1 equiv) was placed in a reaction vial together with $PdCl_2(PPh_3)_2$ (44.8 mg, 0.064 mmol, 0.05 equiv), PPh_3 (33.6 mg, 0.128 mmol, 0.1 equiv) and CuI (24.4 mg, 0.128 mmol, 0.1 equiv). Toluene (8 mL,

for a final reaction concentration of 0.16 M), TMS-acetylene (354 µL, 2.556 mmol, 2 equiv) and diisopropylamine (358 µL, 2.556 mmol, 2 equiv) were added. The reaction mixture was stirred overnight at 60 °C, and then it was filtered through Celite and the filtrate evaporated under reduced pressure. The resulting residue was re-suspended in a HCl 0.1 M aqueous solution, extracted with ethyl acetate (3x), the combined organics washed once with brine, dried over $MgSO_4$ and evaporated to yield a brown oil, from which the desired product (401 mg, 1.109 mmol, 87% yield) was purified as a colorless oil.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.17 (d, *J* = 7.7 Hz, 2H), 7.10 (d, *J* = 7.5 Hz, 2H), 6.72 (d, *J* = 7.6 Hz, 1H), 4.09 (s, 1H), 2.86 (dd, *J* = 70.6, 12.5 Hz, 2H), 1.42 (s, 9H), -0.01 (s, 9H) ppm

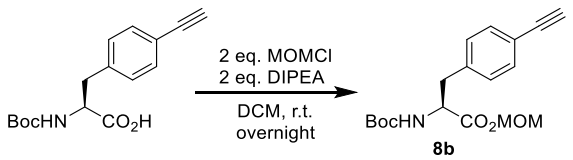
(*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-ethynylphenyl)propanoic acid:



The corresponding product from previous reaction (400 mg, 1.106 mmol, 1 equiv) was dissolved in THF (6 mL), and tetrabutylammonium fluoride solution 1.0 M in THF (1.66 mL, 1.660 mmol, 1.5 equiv) was added dropwise. The reaction mixture was stirred overnight at room temperature, moment in which TLC showed completion. The reaction mixture was concentrated *in vacuo*, the resulting residue re-suspended in HCl 0.1 M aqueous solution and extracted with ethyl acetate (3x). The combined organics were washed once with brine, dried over $MgSO_4$ and evaporated under vacuum to afford the desired product (263.8 mg, 0.912 mmol, 82%) as a brown oil, which was taken onto next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.62 (s, 1H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 8.3 Hz, 2H), 5.03 (d, *J* = 9.3 Hz, 1H), 4.60 (d, *J* = 6.9 Hz, 1H), 3.20 (dd, *J* = 14.1, 5.4 Hz, 1H), 3.09 (dd, *J* = 14.1, 5.4 Hz, 1H), 3.06 (s, 1H), 1.41 (s, 9H) ppm

Methoxymethyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-ethynylphenyl)propanoate (8b):



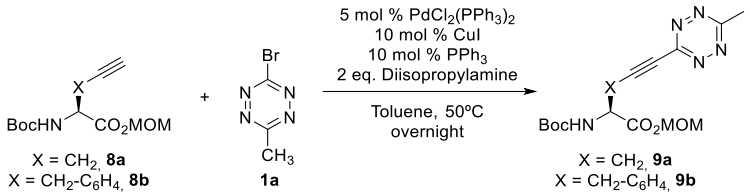
The corresponding N-Boc-4-acetylene-L-phenylalanine (263.8 mg, 0.912 mmol, 1 equiv) was suspended in DCM at 0 °C, and DIPEA (318 µL, 1.824 mmol, 2 equiv) was added. Chloromethyl methyl ether (139 µL, 1.824 mmol, 2 equiv) was added dropwise, generating a thick white gas that was totally removed with N_2 in-flow. The reaction mixture was allowed to warm at room temperature and stirred overnight. The reaction mixture was then diluted with water, extracted

with ethyl acetate (3x), the combined organics washed once with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The resulting residue was purified with flash chromatography in 12 g of silica, using hexane with increasing concentrations of ethyl acetate. The relevant fractions were combined and concentrated to obtain **8b**, 107 mg, 0.320 mmol, 35% yield, as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.2 Hz, 2H), 7.13 (d, *J* = 8.1 Hz, 2H), 5.38 – 5.12 (m, 2H), 4.97 (d, *J* = 8.3 Hz, 1H), 4.60 (d, *J* = 7.7 Hz, 1H), 3.41 (s, 3H), 3.16 (dd, *J* = 13.9, 5.9 Hz, 1H), 3.09 (dd, *J* = 13.9, 5.9 Hz, 1H), 3.06 (s, 1H), 1.41 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.52, 155.16, 136.98, 132.45, 129.51, 121.05, 91.52, 83.47, 80.28, 77.48, 58.08, 54.41, 38.30, 28.41 ppm

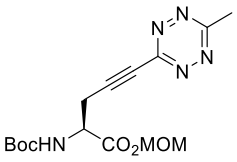
2.6.2. Sonogashira coupling between 8a-b and 1a:



To a degassed reaction vessel with **1a** (50.0 mg, 0.286 mmol, 1 equiv), the corresponding double protected amino acid **8a** or **8b** (0.572 mmol, 2 equiv), $PdCl_2(PPh_3)_2$ (10.1 mg, 0.0143 mmol, 0.05 equiv), PPh_3 (7.5 mg, 0.029 mmol, 0.1 equiv) and CuI (5.5 mg, 0.029 mmol, 0.1 equiv), toluene (2 mL, for a final reaction concentration of 0.15 M) and diisopropylamine (80.2 µL, 0.572 mmol, 2 equiv) were added. The reaction mixture was stirred overnight at 50 °C.

The reaction crude was evaporated under reduced pressure, and the resulting residue extracted from water with ethyl acetate (3x). The organic phases were combined, dried with $MgSO_4$, filtered and concentrated under vacuum. The desired tetrazine-containing protected amino acids were then purified with flash chromatography in 12 g of silica, using hexane with increasing concentrations of ethyl acetate.

Methoxymethyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-5-(6-methyl-1,2,4,5-tetrazin-3-yl)pent-4-ynoate (9a)



Pink oil (49.2 mg, 49%).

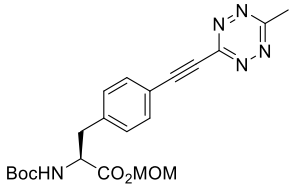
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.50 (d, *J* = 7.8 Hz, 1H), 5.36 (dd, *J* = 21.6, 5.9 Hz, 2H), 4.71 – 4.63 (m, 1H), 3.51 (s, *J* = 1.3 Hz, 3H), 3.21 (d, *J* = 5.8 Hz, 2H), 3.06 (s, 3H), 1.46 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 169.97, 165.81, 156.62, 155.11, 94.41, 92.07, 80.77, 58.22, 52.03, 28.42, 24.19, 21.60 ppm

HRMS (ESI$^+$) calculated for $C_{15}H_{22}N_5O_5$ $[M+H]^+$: 352.1615 / found 352.1624

IR U_{max}: 3339, 2976, 2922, 2359, 2239, 1713, 1614, 1503, 1366, 1259, 1149, 1054 cm^{-1}

Methoxymethyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-((6-methyl-1,2,4,5-tetrazin-3-yl)ethynyl)phenyl)propanoate (9b)



Pink oil (78.2 mg, 64%).

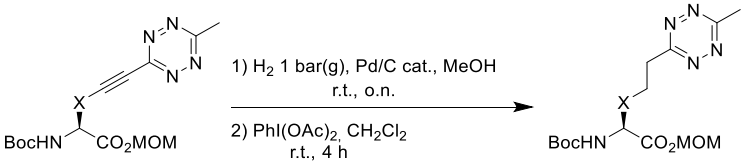
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, 2H), 7.26 (d, 2H), 5.28 (dd, 2H), 5.02 (d, *J* = 8.3 Hz, 1H), 4.64 (m, 1H), 3.43 (s, 3H), 3.27 – 3.11 (m, 2H), 3.10 (s, 3H), 1.42 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 171.39, 165.42, 157.32, 155.12, 139.42, 133.05, 129.83, 119.12, 97.70, 91.62, 83.24, 80.39, 58.16, 54.36, 38.58, 28.41, 21.63 ppm

HRMS (ESI$^+$) calculated for $C_{42}H_{50}N_{10}NaO_{10}$ $[2M+Na]^+$: 877.3604 / found 877.3604

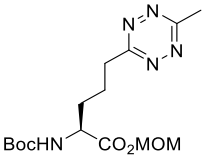
IR U_{max}: 3361, 2973, 2929, 2218, 1744, 1704, 1508, 1404, 1365, 1156, 1091, 1053, 1019 cm^{-1}

2.6.3. Tandem hydrogenation-oxidation of 9a-b:



The same experimental protocol described in **Section 5.2.** was followed. The amount of starting material employed is detailed in each case.

Methoxymethyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-5-(6-methyl-1,2,4,5-tetrazin-3-yl)pentanoate (11a)



Pink oil (36.7 mg, 97% starting from 37.4 mg of **9a**).

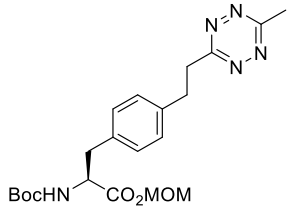
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28 (dd, *J* = 32.3, 6.0 Hz, 2H), 5.07 (d, *J* = 8.4 Hz, 1H), 4.40 (s, 1H), 3.47 (s, 3H), 3.34 (td, *J* = 7.5, 3.2 Hz, 2H), 3.04 (s, 3H), 2.11 – 1.97 (m, 2H), 1.86 – 1.77 (m, 1H), 1.60 (m, 1H), 1.44 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.31, 169.49, 167.70, 155.49, 91.43, 80.26, 58.05, 53.36, 34.30, 32.22, 28.45, 23.98, 21.25 ppm

HRMS (ESI$^+$) calculated for $C_{15}H_{26}N_5O_5$ $[M+H]^+$: 356.1928 / found 356.1932

IR U_{max}: 3354, 2971, 2929, 2360, 2341, 1709, 1507, 1456, 1405, 1365, 1249, 1148, 1088, 1049 cm^{-1}

Methoxymethyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(2-(6-methyl-1,2,4,5-tetrazin-3-yl)ethyl)phenyl)propanoate (11b)



Pink oil (21.3 mg, 95% starting from 22.3 mg of **9b**).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 – 7.15 (m, 2H), 7.07 (dd, *J* = 17.2, 7.8 Hz, 2H), 5.30 (d, *J* = 5.9 Hz, 1H), 5.22 (d, *J* = 6.0 Hz, 1H), 4.95 (d, *J* = 8.3 Hz, 1H), 4.58 (m, 1H), 3.70 (s, 1.4H), 3.59 (ddd, *J* = 8.0, 7.0, 1.7 Hz, 2H), 3.41 (s, 1.6H), 3.25 (dd, *J* = 9.1, 6.9 Hz, 2H), 3.14 – 3.04 (m, 2H), 3.03 (s, 3H), 1.41 (s, 9H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.46, 171.74, 169.29, 167.62, 155.21, 138.85, 134.25 (d, *J* = 15.9 Hz), 129.75, 129.70, 128.77, 91.43, 80.14, 58.04, 54.53, 52.34, 38.02 (d, *J* = 16.8 Hz), 36.39, 33.69, 28.43, 21.25 ppm

HRMS (ESI$^+$) calculated for $C_{21}H_{30}N_5O_5$ $[M+H]^+$: 432.2241 / found 432.2240

IR U_{max}: 3353 (broad), 2973, 2927, 2854, 1743, 1707, 1514, 1404, 1365, 1160, 1093 cm^{-1}

2.6.4. Deprotection of amino acids 9a-b and 11a-b:

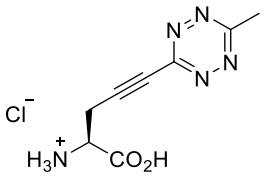
The corresponding amount of double protected amino acid (specified for each substrate) was placed in a glass vial, which was sealed, degassed and placed under N_2 atmosphere. DCM was then added (to reach a final concentration of 0.1 M), and HCl 4 M in dioxane (2 equiv) was added. The reaction mixture was stirred at room temperature for 4 h, after which the reaction mixture was concentrated under reduced pressure. Two different separation methods were employed:

Method A: the resulting residue was washed with DCM, and the supernatant decanted.

Method B: the resulting residue resuspended in DCM and filtered through a C-type crucible filter.

Each final amino acid was recovered in its hydrochloride salt form.

(*S*)-1-carboxy-4-(6-methyl-1,2,4,5-tetrazin-3-yl)but-3-yn-1-aminium chloride (10a)



Purple solid (17.9 mg, 92% starting from 28.0 mg of **9a**). Isolated using **Method A**.

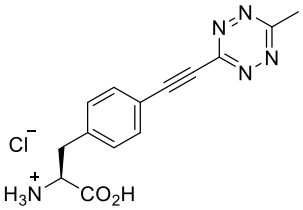
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 4.39 (dd, *J* = 8.7, 5.2 Hz, 1H), 3.42 (ddd, *J* = 14.9, 5.2, 1.0 Hz, 1H), 3.25 (ddd, *J* = 14.9, 8.7, 0.7 Hz, 1H), 3.03 (s, 3H) ppm

^{13}C NMR (101 MHz, $CDCl_3$) δ 167.70, 165.02, 140.61, 136.47, 124.55, 52.22, 43.37, 21.23 ppm

HRMS (ESI^+) calculated for $C_8H_{11}ClN_5O_2$ $[M+H]^+$: 244.0596 / found 244.0602

IR U_{max}: 3338, 2929, 2241, 1709, 1509, 1402, 1365, 1275, 1251, 1157, 1058, 1025 cm^{-1}

(*S*)-1-carboxy-2-(4-((6-methyl-1,2,4,5-tetrazin-3-yl)ethynyl)phenyl)ethan-1-aminium chloride (10b)



Pink solid (15.8 mg, 96% starting from 22.0 mg of **9b**). Isolated using **Method B**.

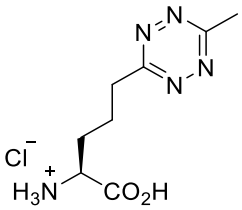
^{1}H NMR (400 MHz, CD_3OD) δ 7.76 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 4.33 (m, 1H), 3.40 (dd, *J* = 14.6, 5.8 Hz, 1H), 3.25 (dd, *J* = 14.6, 5.8 Hz, 1H), 3.04 (s, 3H) ppm

^{13}C NMR (101 MHz, CD_3OD) δ 170.99, 167.09, 158.21, 138.98, 134.21, 131.19, 121.06, 96.89, 84.27, 54.78, 37.30, 21.45 ppm

HRMS (ESI^+) calculated for $C_{14}H_{14}N_5O_2$ $[M-Cl]^+$: 284.1142 / found 284.1144

IR U_{max}: 3412, 2920, 2215, 1724, 1510, 1403, 1205, 1159, 1111, 1076, 1019 cm^{-1}

(*S*)-1-carboxy-4-(6-methyl-1,2,4,5-tetrazin-3-yl)butan-1-aminium chloride (12a)



Pink solid (15.6 mg, 89% starting from 25.1 mg of **11a**). Isolated using **Method A**.

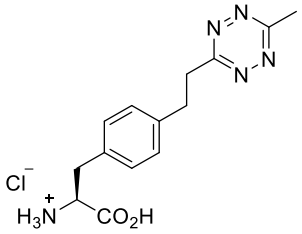
^{1}H NMR (400 MHz, CD_3OD) δ 4.06 (t, *J* = 5.7 Hz, 1H), 3.37 (t, *J* = 6.9 Hz, 2H), 2.99 (s, 3H), 2.23 – 2.04 (m, 4H) ppm

^{13}C NMR (101 MHz, CD_3OD) δ 171.58, 170.28, 169.00, 53.64, 34.76, 30.84, 24.18, 21.03 ppm

HRMS (ESI^+) calculated for $C_8H_{14}N_5O_2$ $[M+H]^+$: 212.11420 / found 212.11588

IR U_{max}: 3339, 3155, 2934, 1742, 1598, 1404, 1216, 1216, 1084, 1043 cm^{-1}

(*S*)-1-carboxy-2-(4-(2-(6-methyl-1,2,4,5-tetrazin-3-yl)ethyl)phenyl)ethan-1-aminium chloride (12b)



Pink solid (12.1 mg, 95% starting from 17.0 mg of **11b**). Isolated using **Method B**.

^{1}H NMR (400 MHz, CD_3OD) δ 7.31 – 7.14 (m, 7H), 4.23 (dd, *J* = 7.9, 5.3 Hz, 1H), 3.80 (d, *J* = 0.8 Hz, 1H), 3.58 (t, *J* = 8.6 Hz, 2H), 3.30 – 3.25 (m, 2H), 3.11 (dd, *J* = 14.6, 7.8 Hz, 2H), 2.97 (s, 3H) ppm

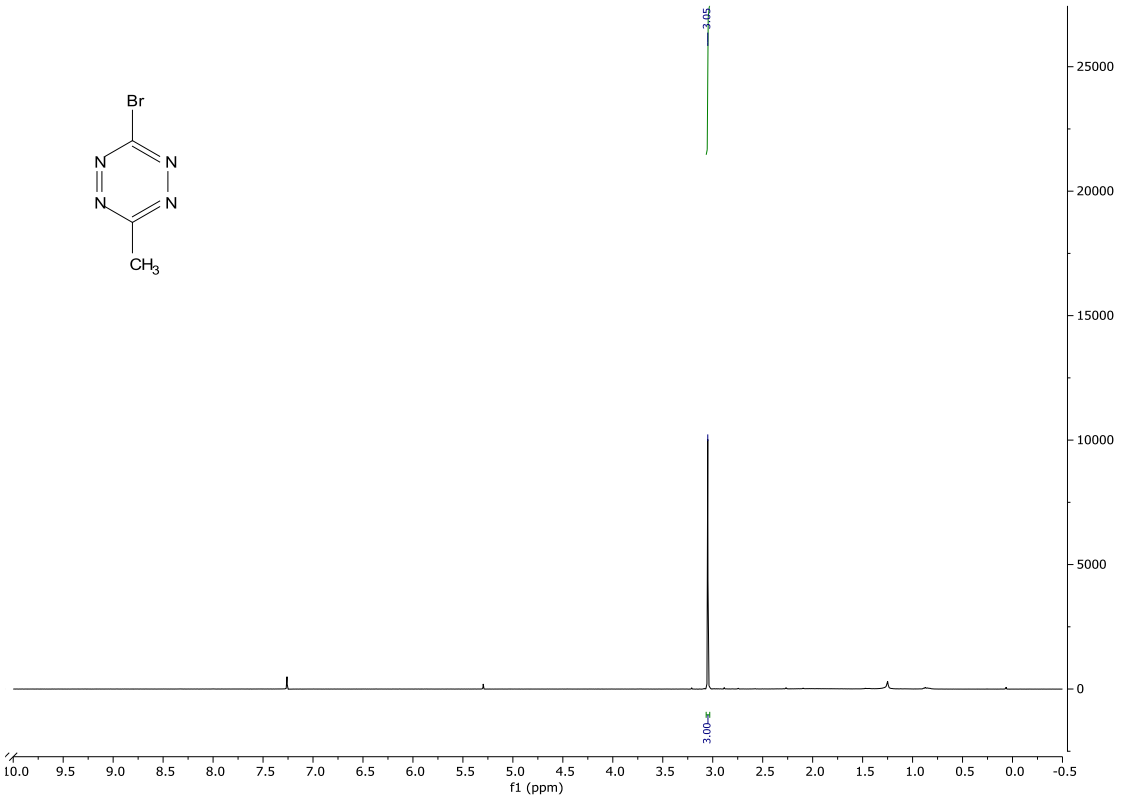
^{13}C NMR (101 MHz, CD_3OD) δ 171.18, 170.33, 168.89, 141.31, 133.62, 130.70, 130.33, 55.09, 37.15, 36.93, 34.35, 21.00 ppm

HRMS (ESI^+) calculated for $C_{14}H_{18}N_5O_2$ $[M+H]^+$: 288.14550 / found 288.14781

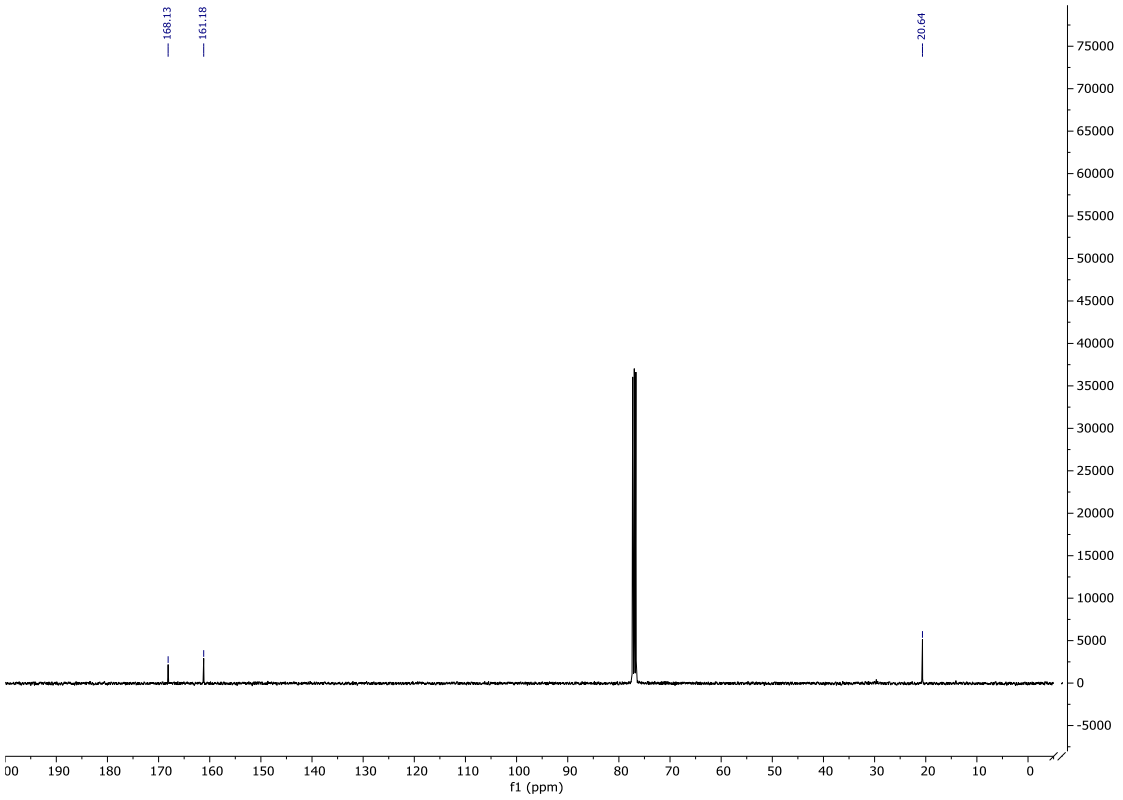
IR U_{max}: 3412, 2930, 1742, 1726, 1587, 1482, 1407 cm^{-1}

3. ^{1}H NMR and ^{13}C NMR spectra

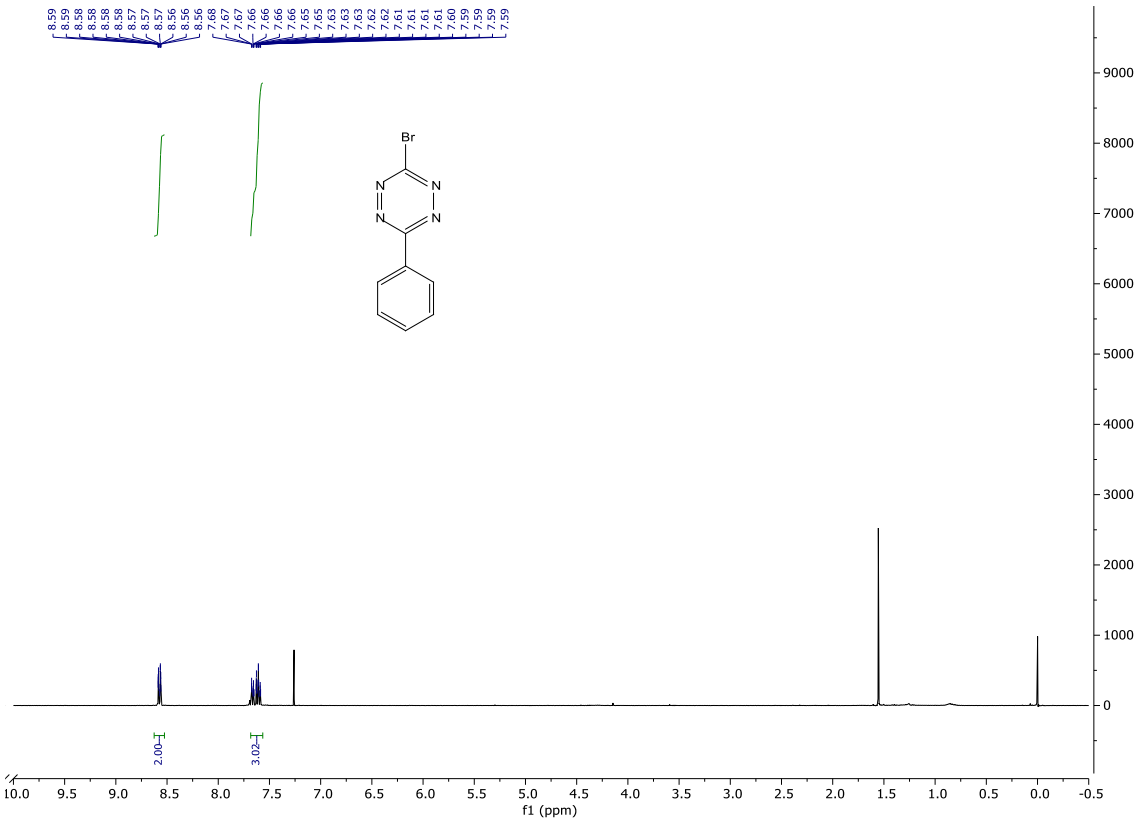
^{1}H NMR (400 MHz, $CDCl_3$) of 1a



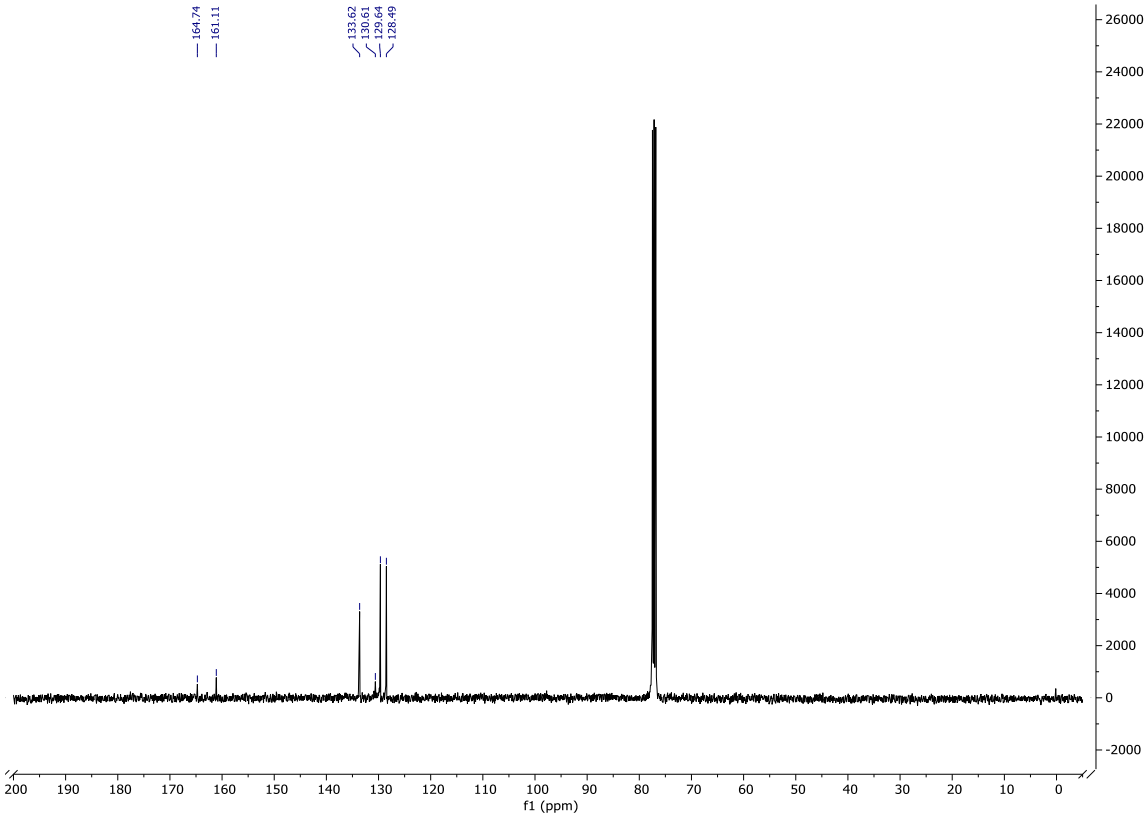
^{13}C NMR (101 MHz, $CDCl_3$) of 1a



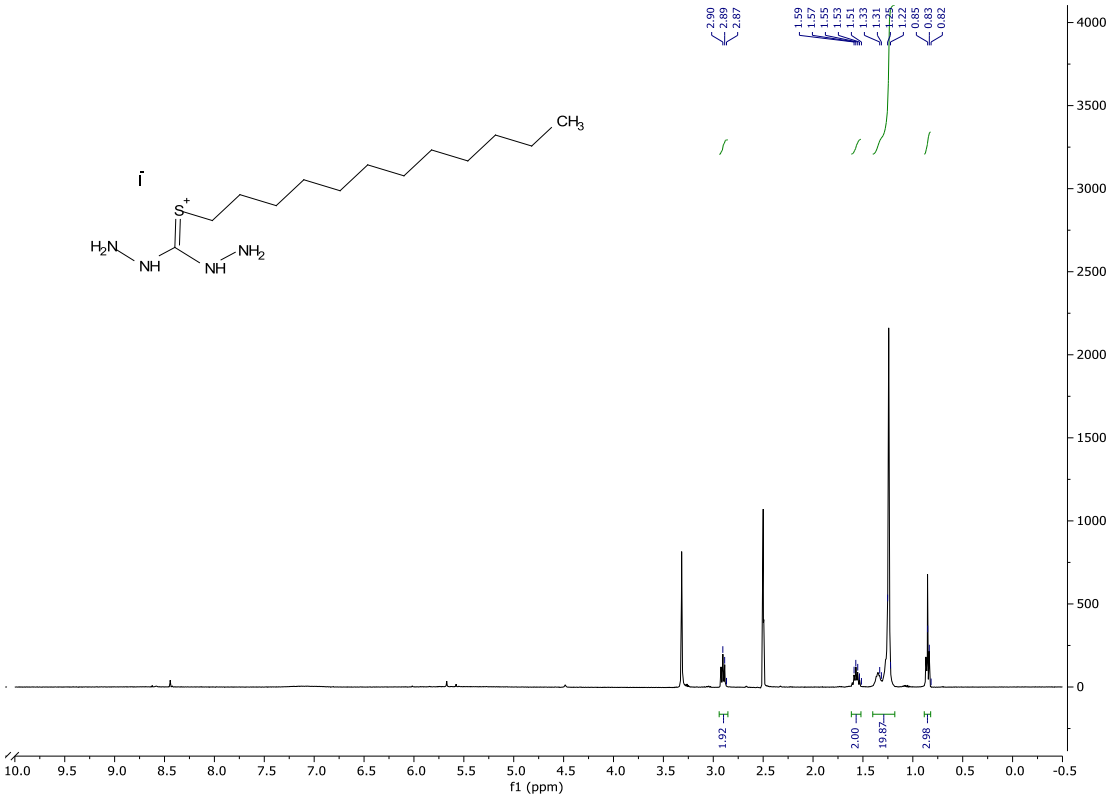
^{1}H NMR (400 MHz, $CDCl_3$) of 1b



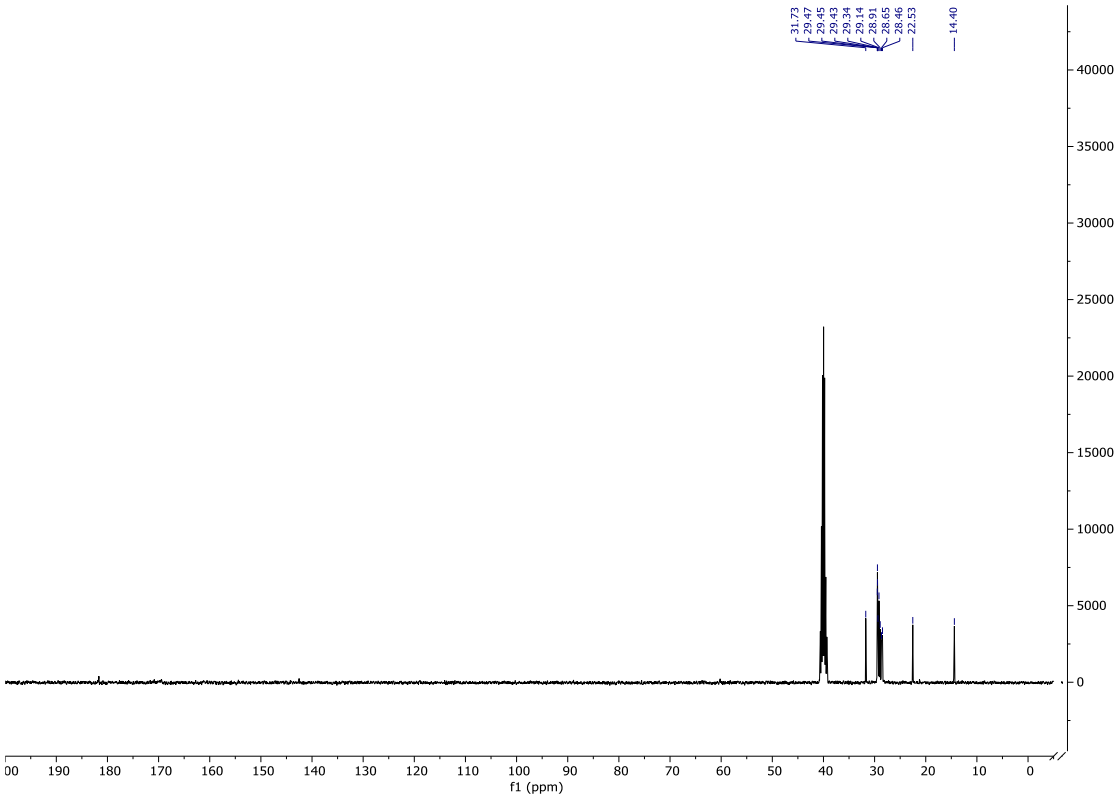
^{13}C NMR (101 MHz, $CDCl_3$) of 1b



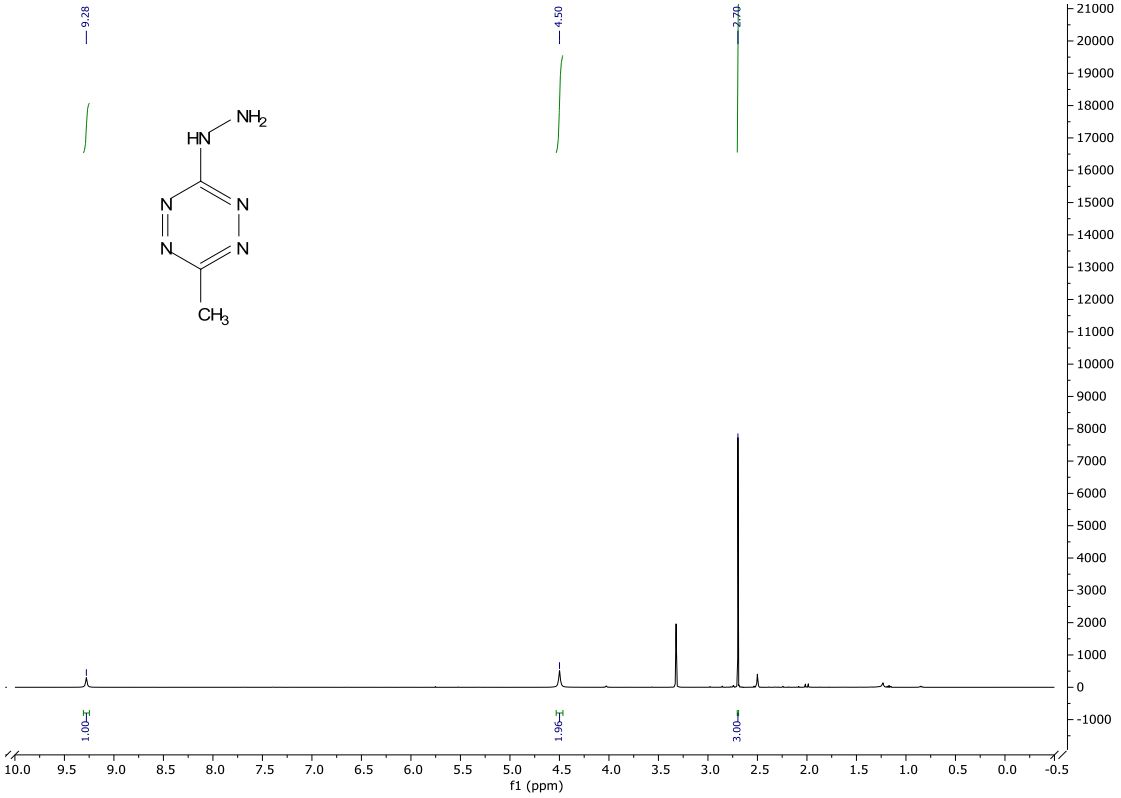
^{1}H NMR (400 MHz, DMSO-d_6) of 2



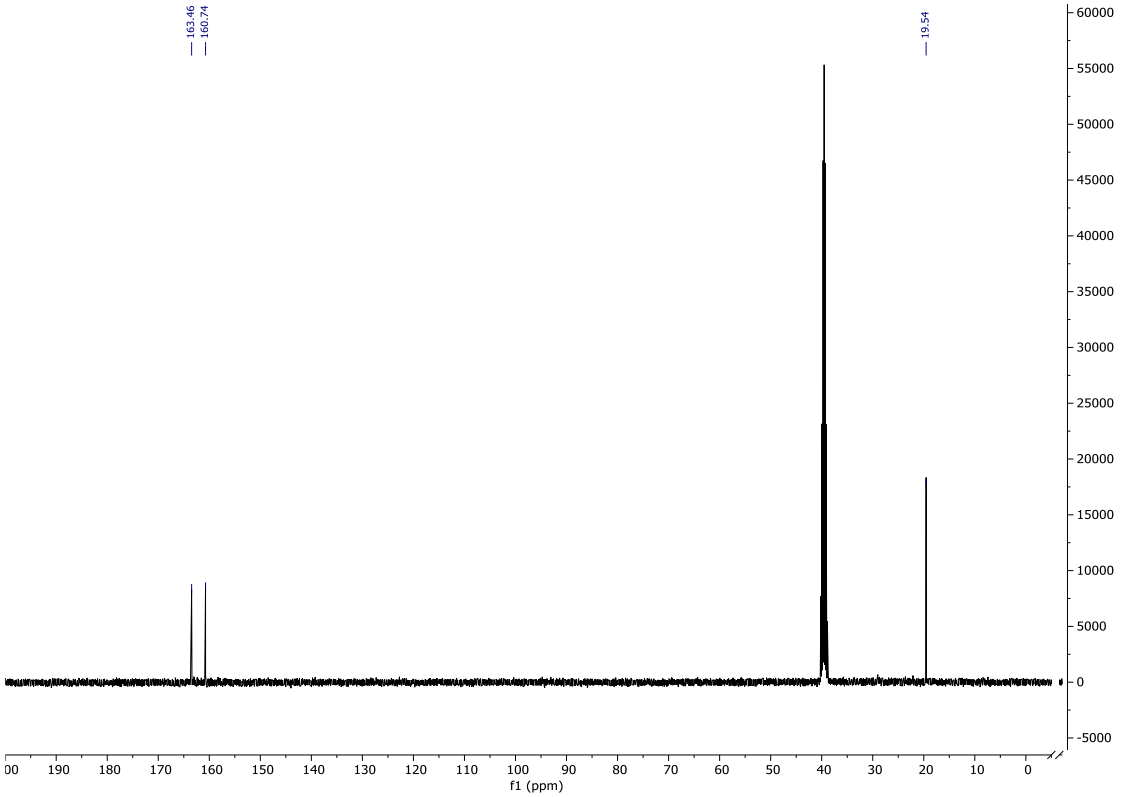
^{13}C NMR (101 MHz, DMSO-d_6) of 2



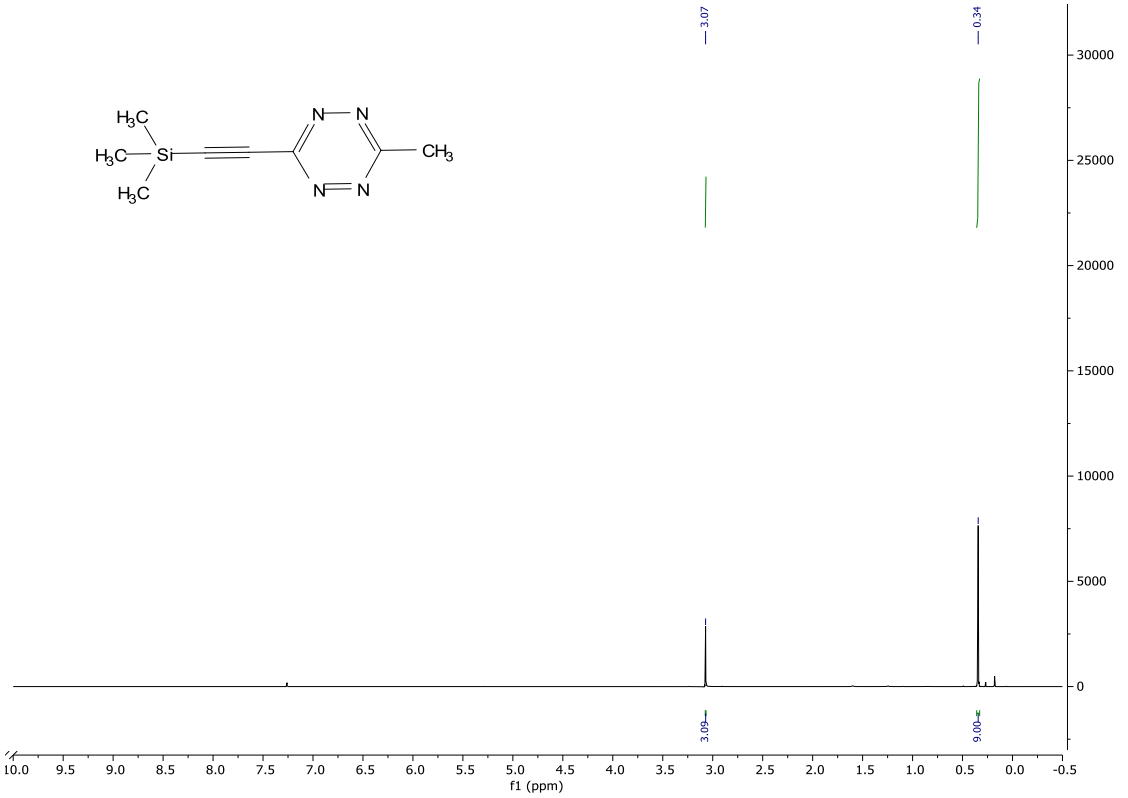
^{1}H NMR (400 MHz, DMSO-d_6) of 4a



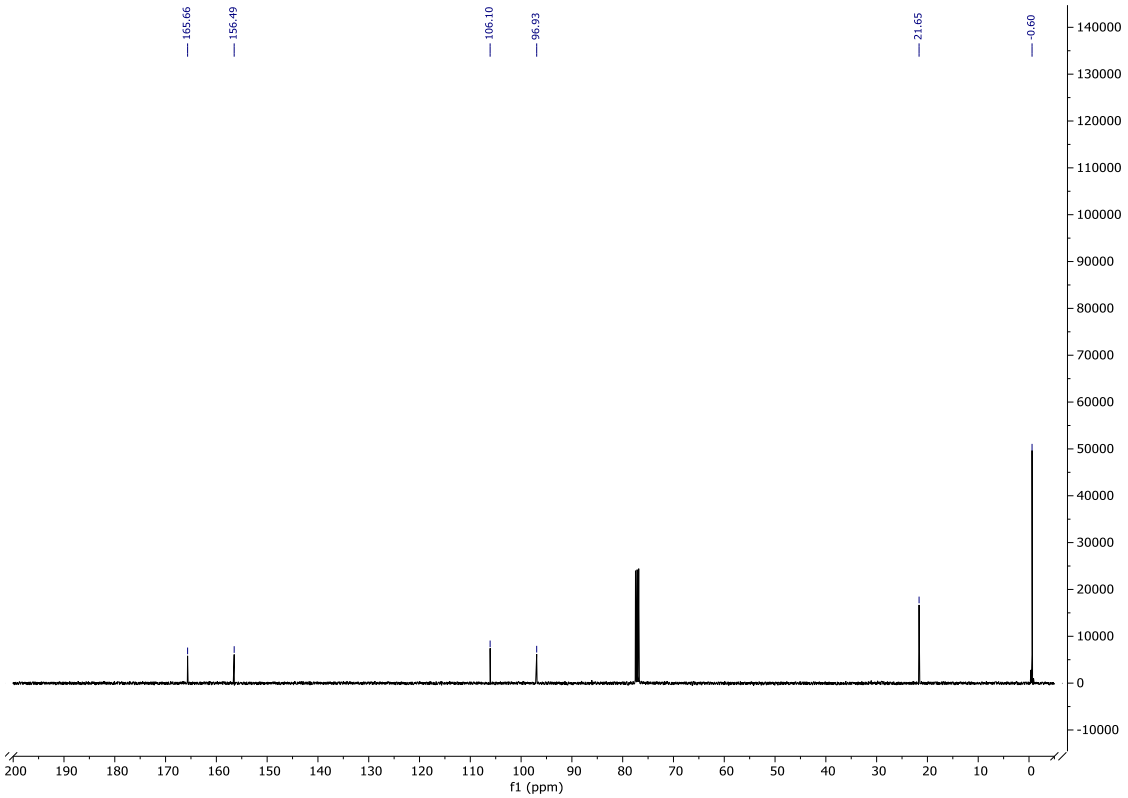
^{13}C NMR (101 MHz, DMSO-d_6) of 4a



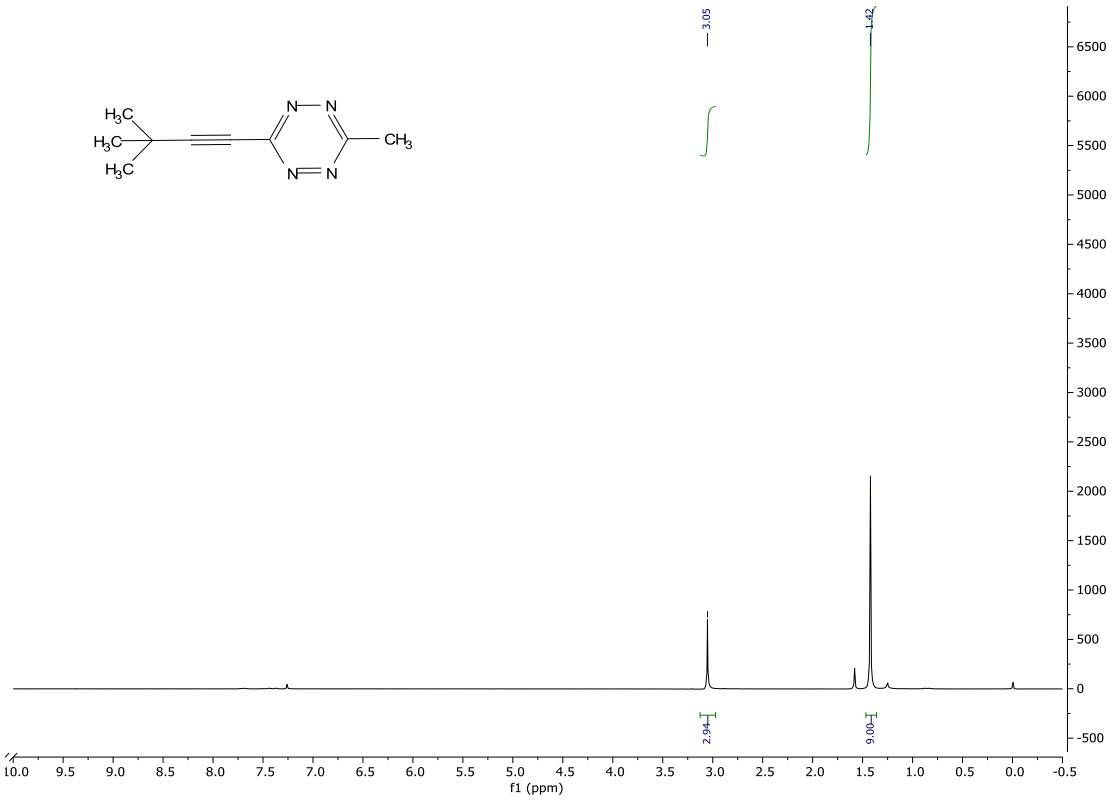
^{1}H NMR (400 MHz, $CDCl_3$) of 5a



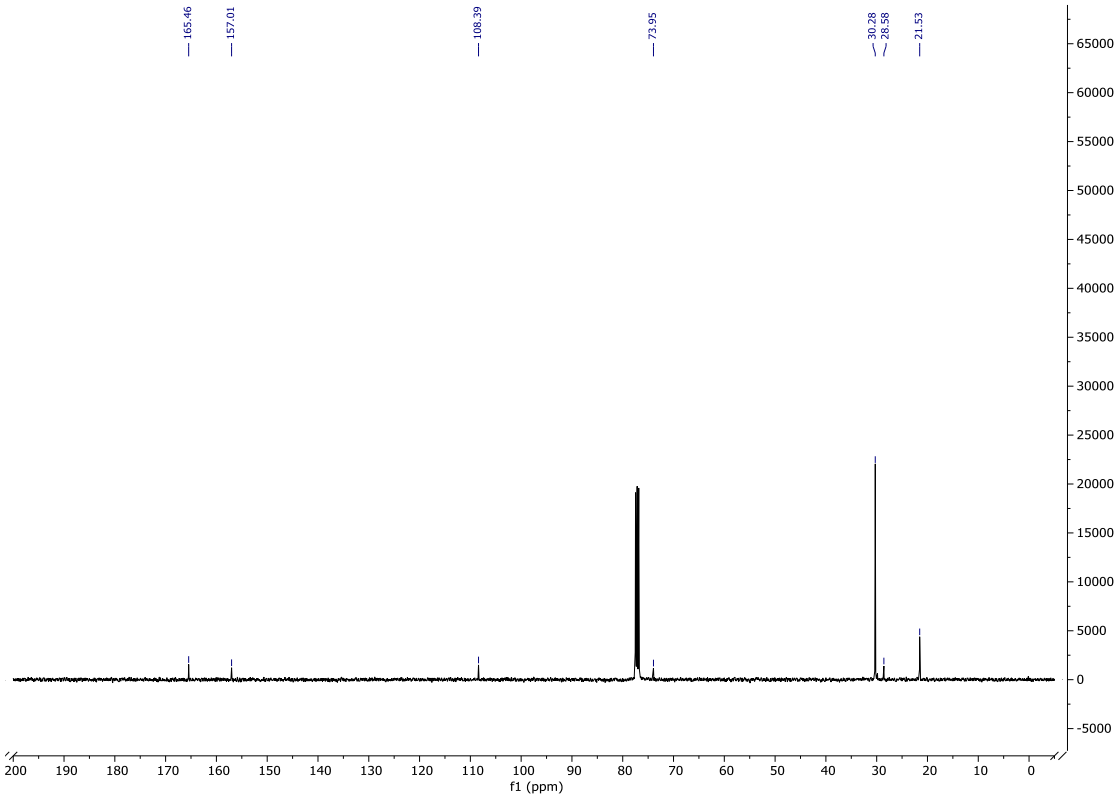
^{13}C NMR (101 MHz, $CDCl_3$) of 5a



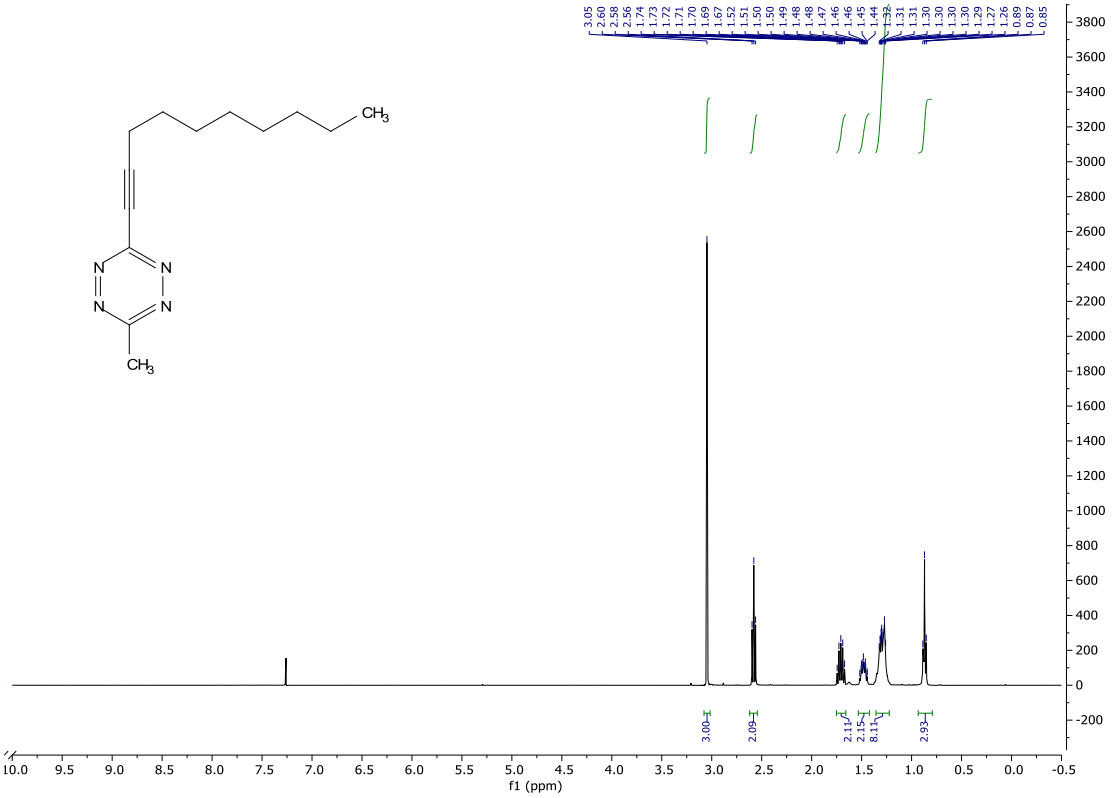
^{1}H NMR (400 MHz, $CDCl_3$) of 5b



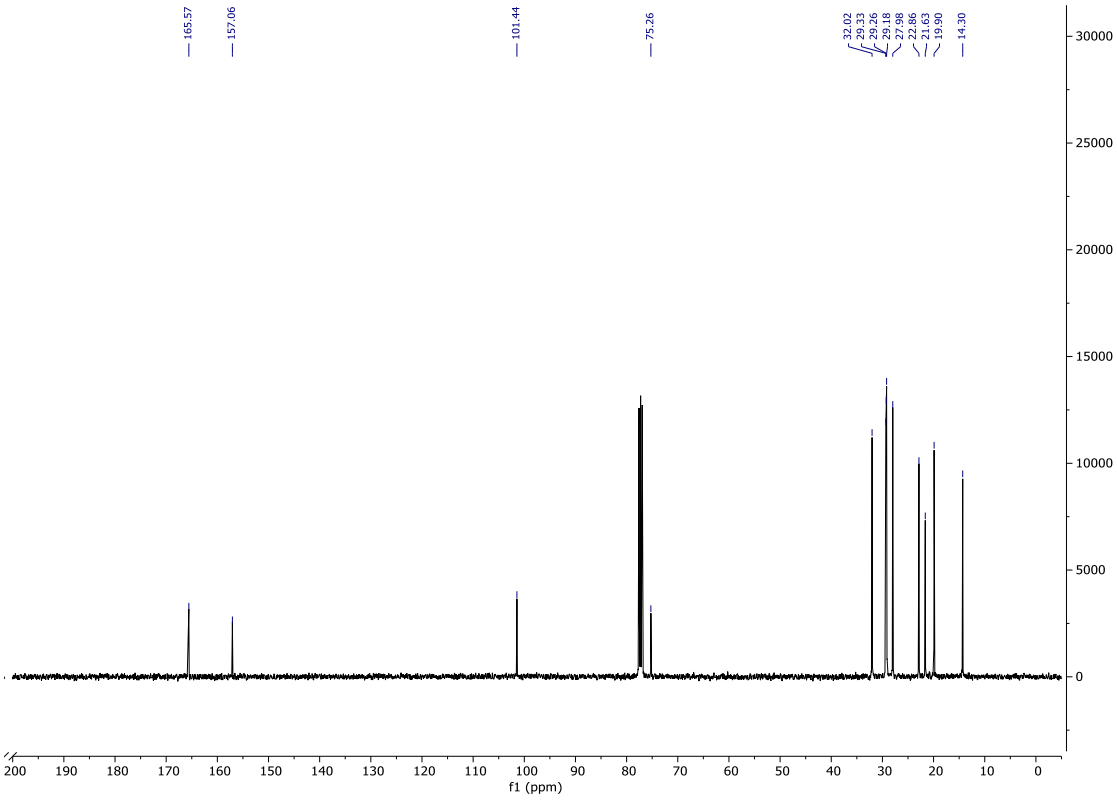
^{13}C NMR (101 MHz, $CDCl_3$) of 5b



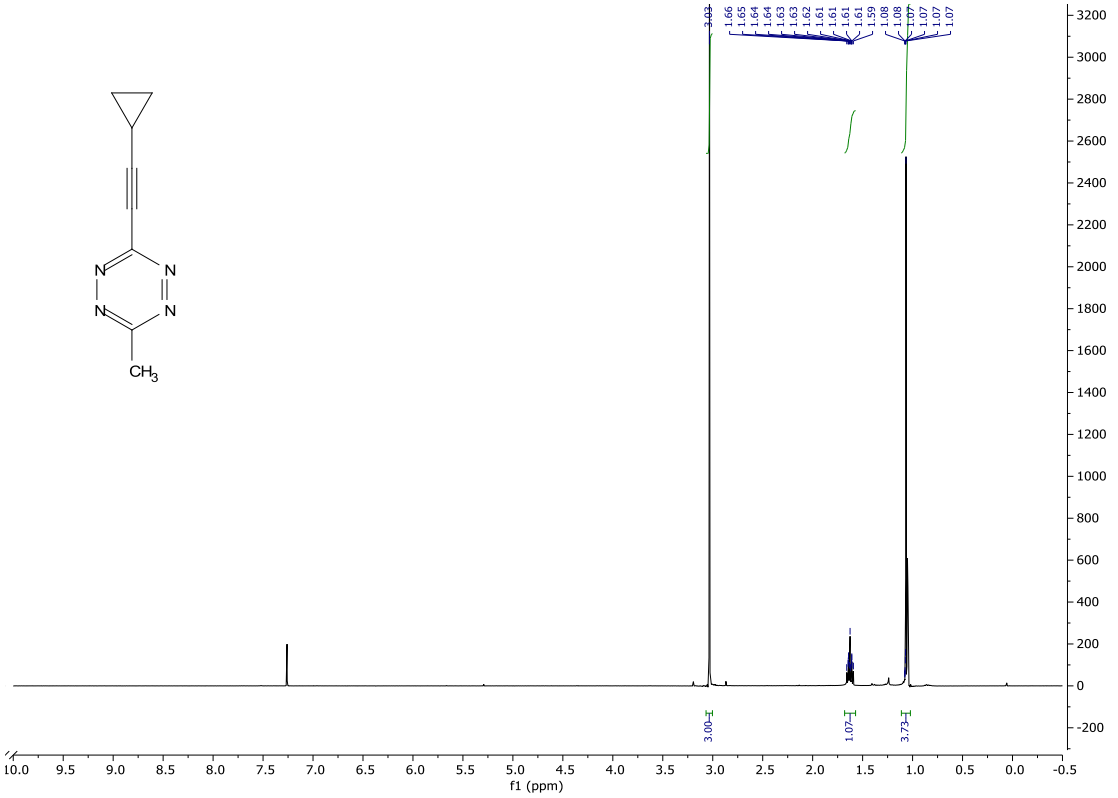
^{1}H NMR (400 MHz, $CDCl_3$) of 5c



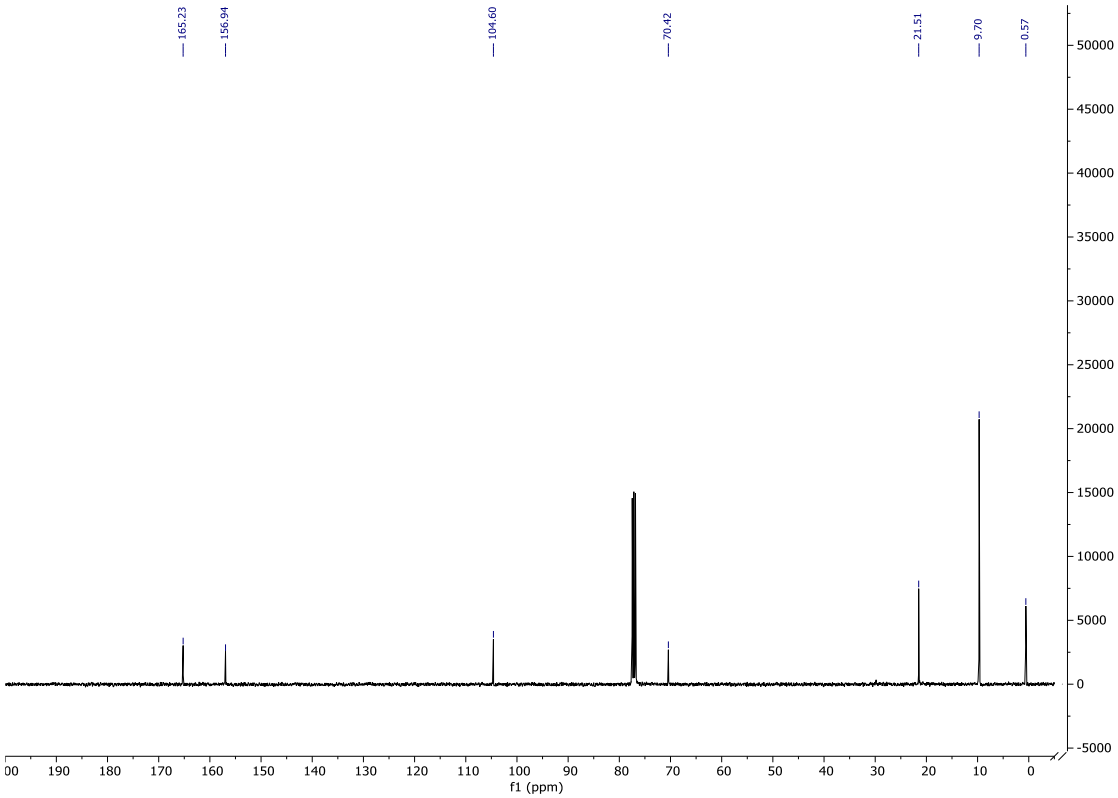
^{13}C NMR (101 MHz, $CDCl_3$) of 5c



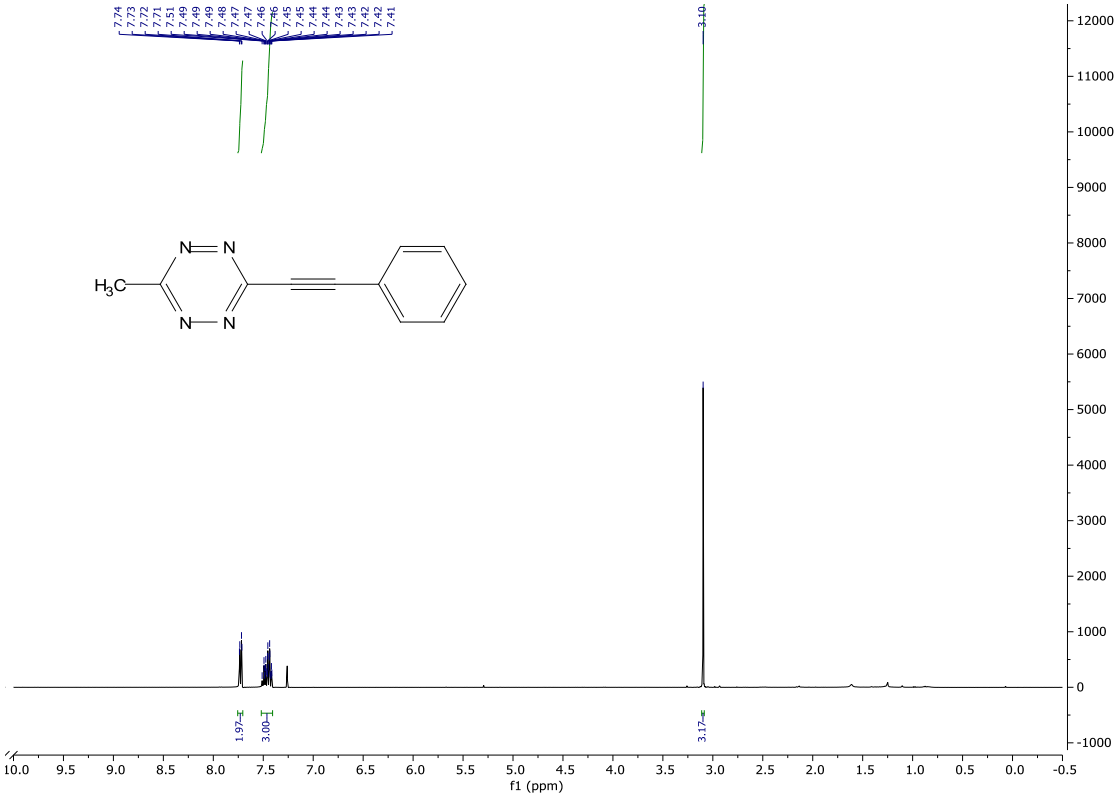
^{1}H NMR (400 MHz, $CDCl_3$) of 5d



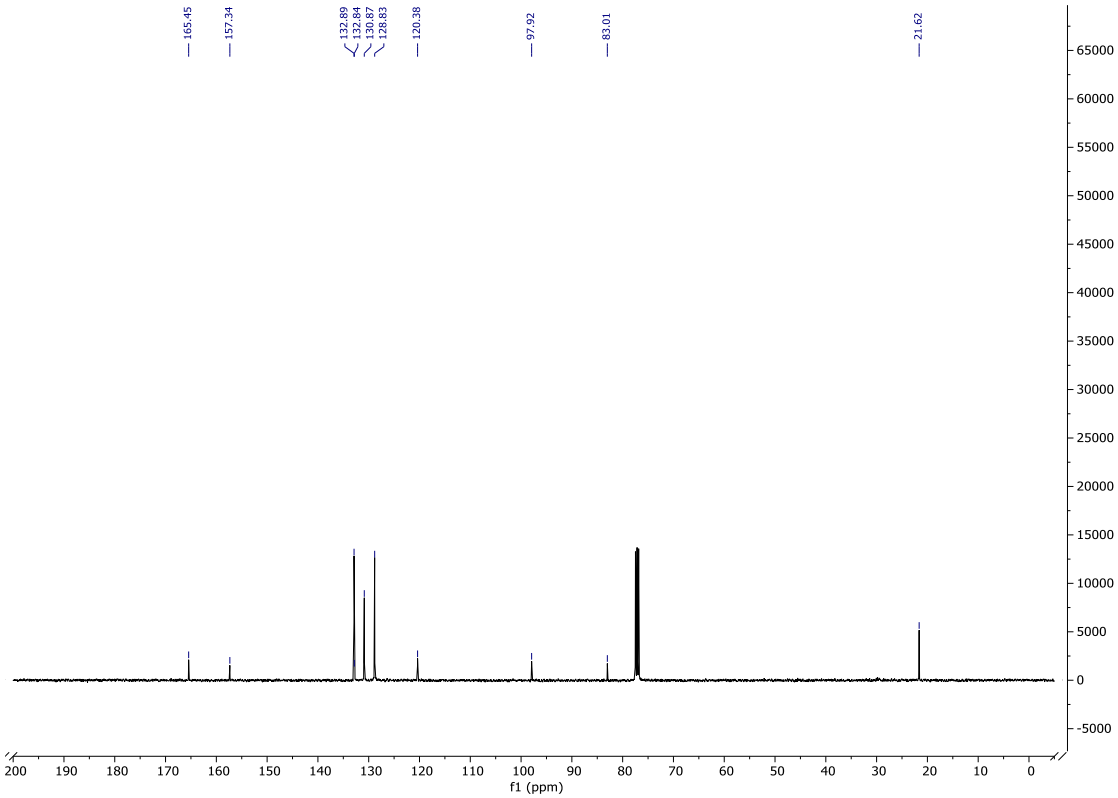
^{13}C NMR (101 MHz, $CDCl_3$) of 5d



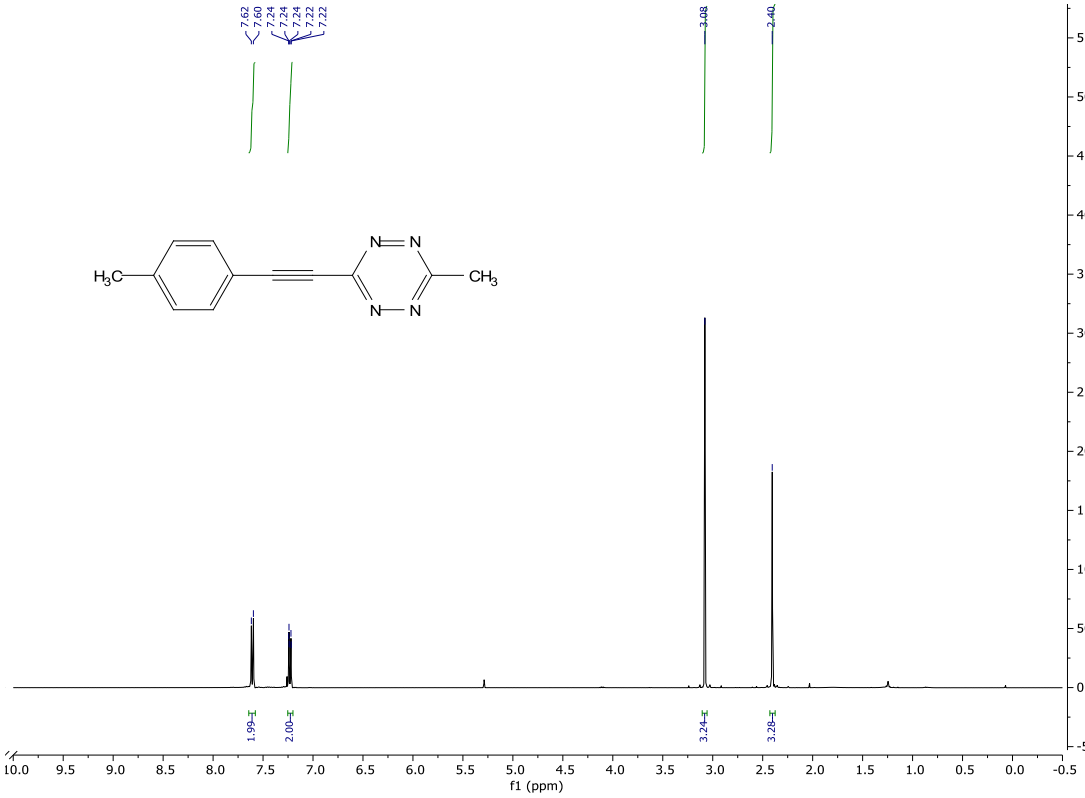
^{1}H NMR (400 MHz, $CDCl_3$) of 5e



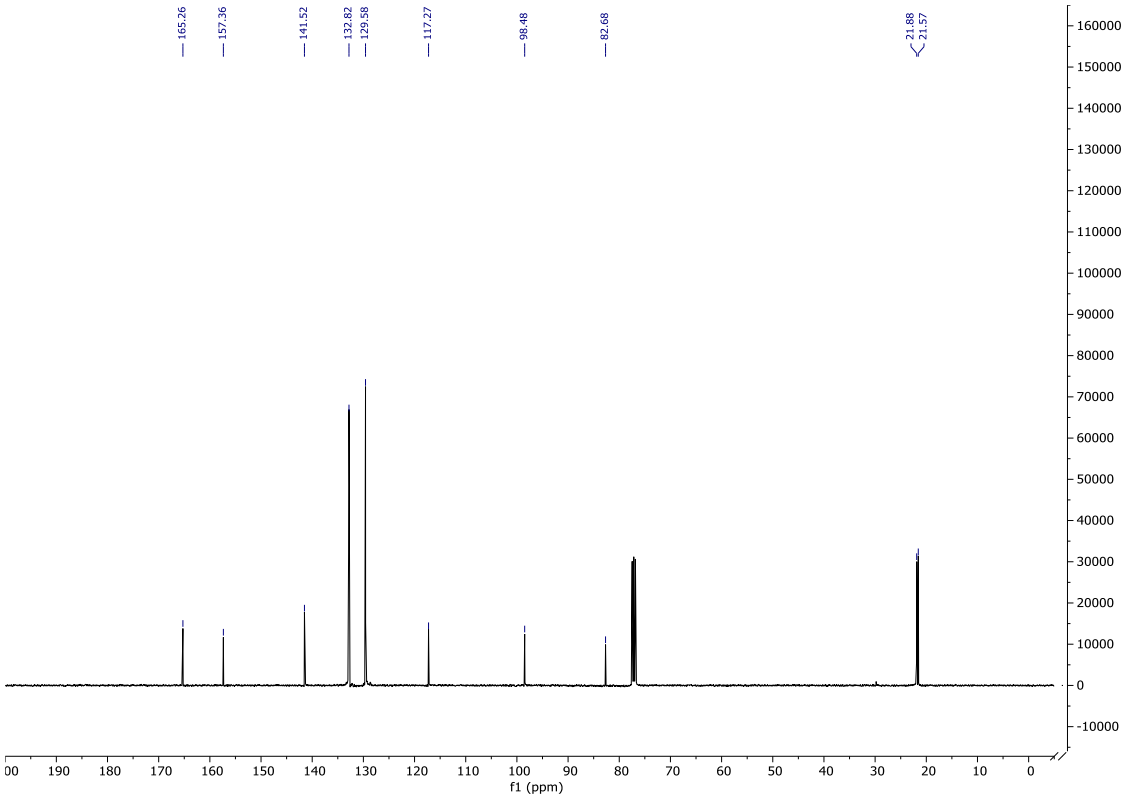
^{13}C NMR (101 MHz, $CDCl_3$) of 5e



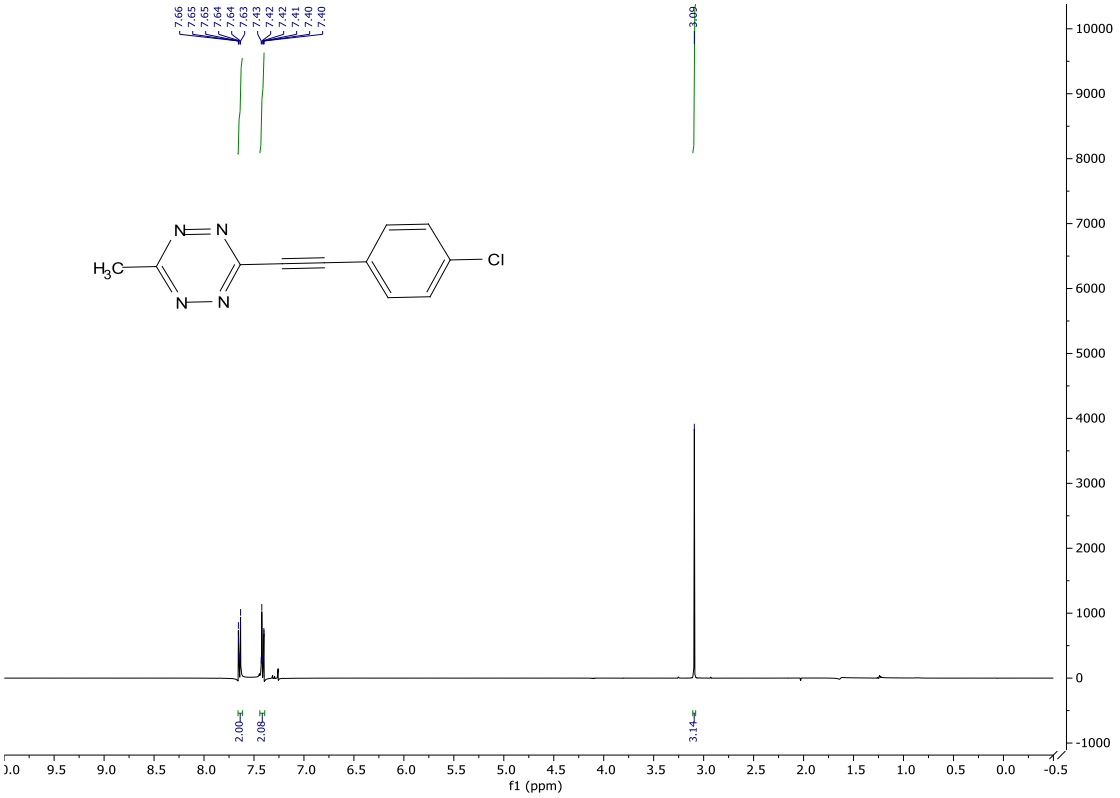
^{1}H NMR (400 MHz, $CDCl_3$) of 5f



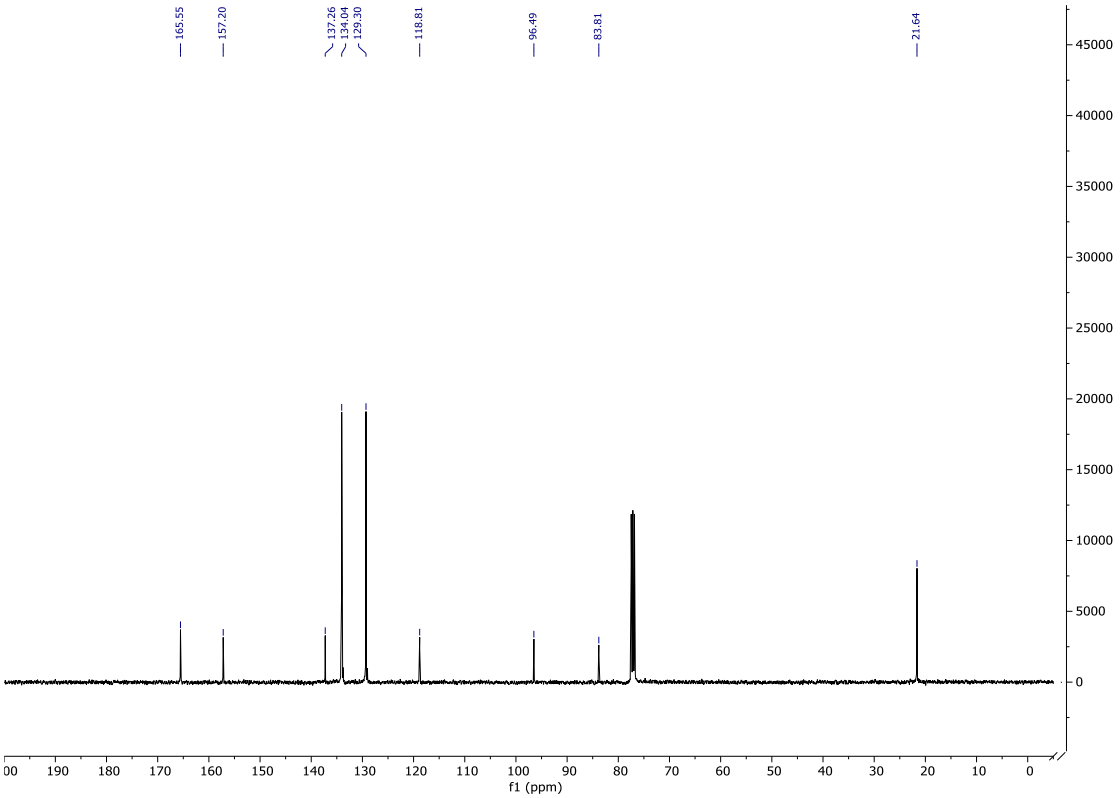
^{13}C NMR (101 MHz, $CDCl_3$) of 5f



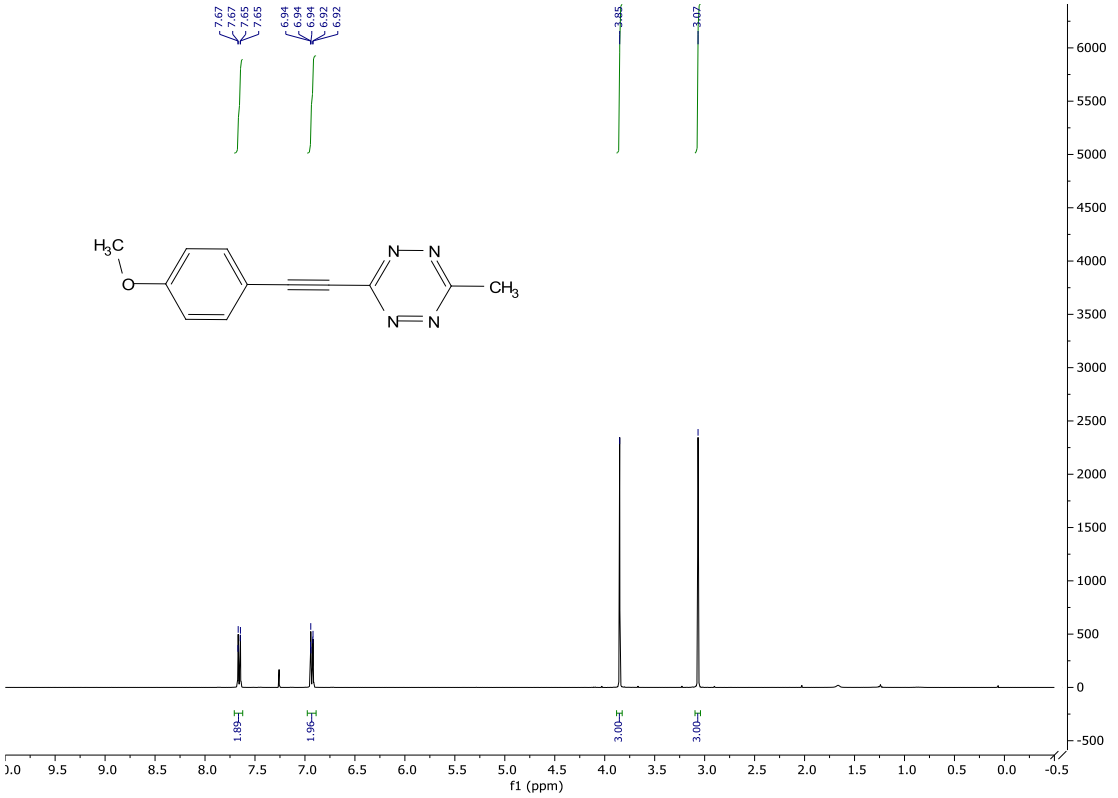
^{1}H NMR (400 MHz, $CDCl_3$) of 5g



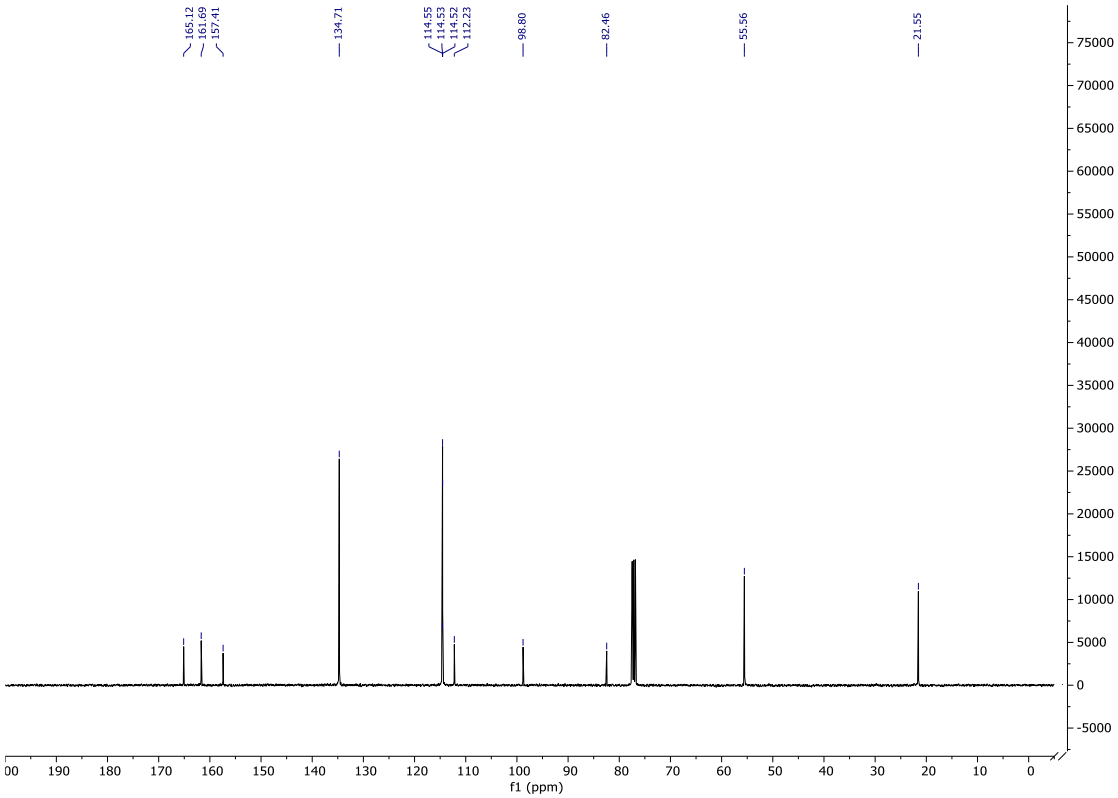
^{13}C NMR (101 MHz, $CDCl_3$) of 5g



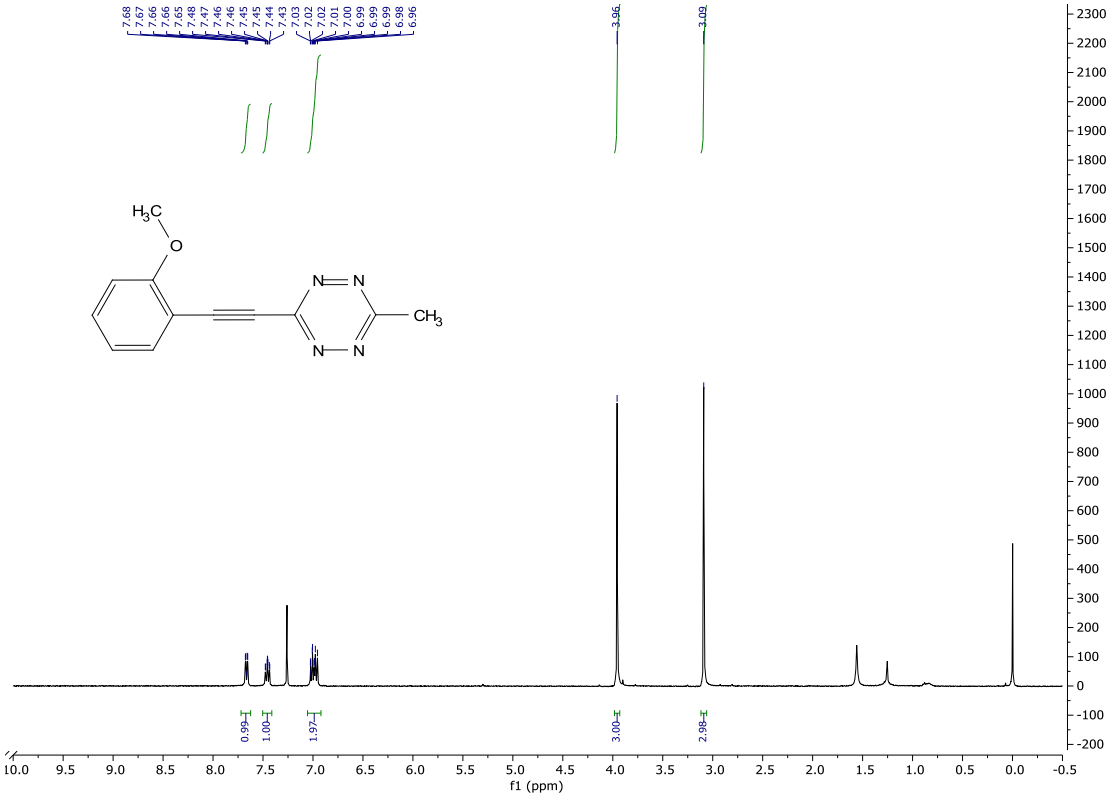
^{1}H NMR (400 MHz, $CDCl_3$) of 5h



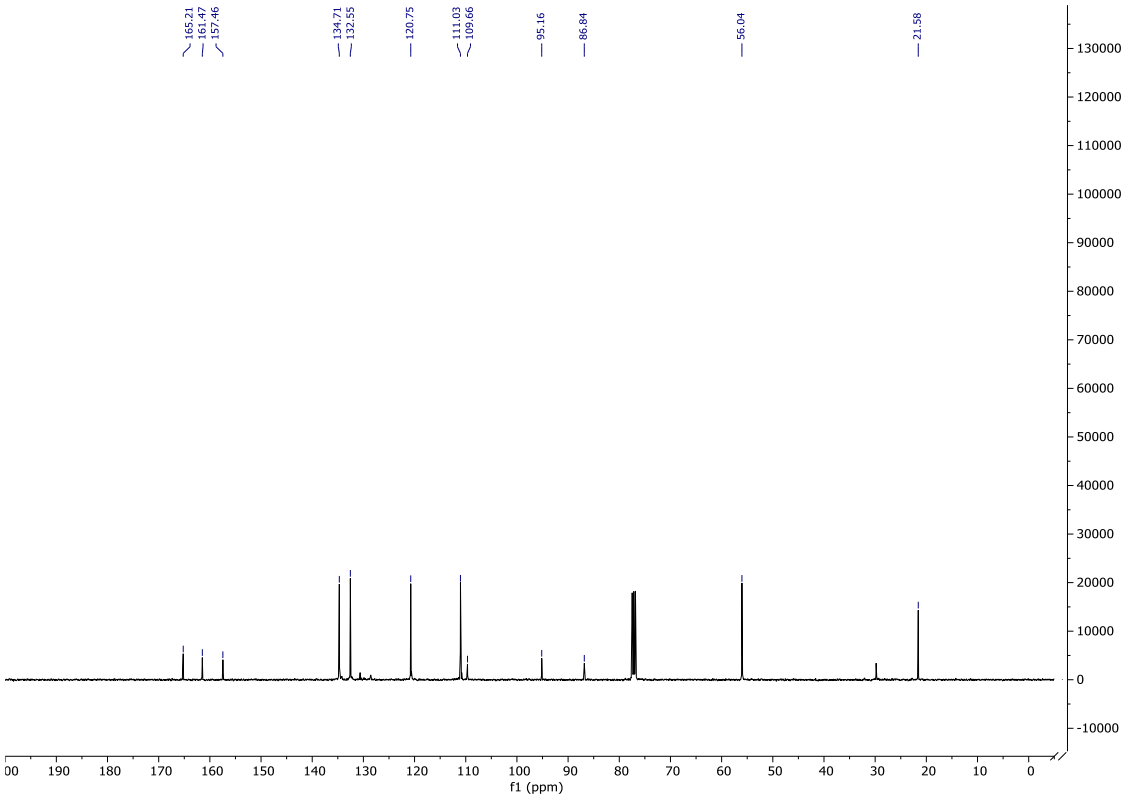
^{13}C NMR (101 MHz, $CDCl_3$) of 5h



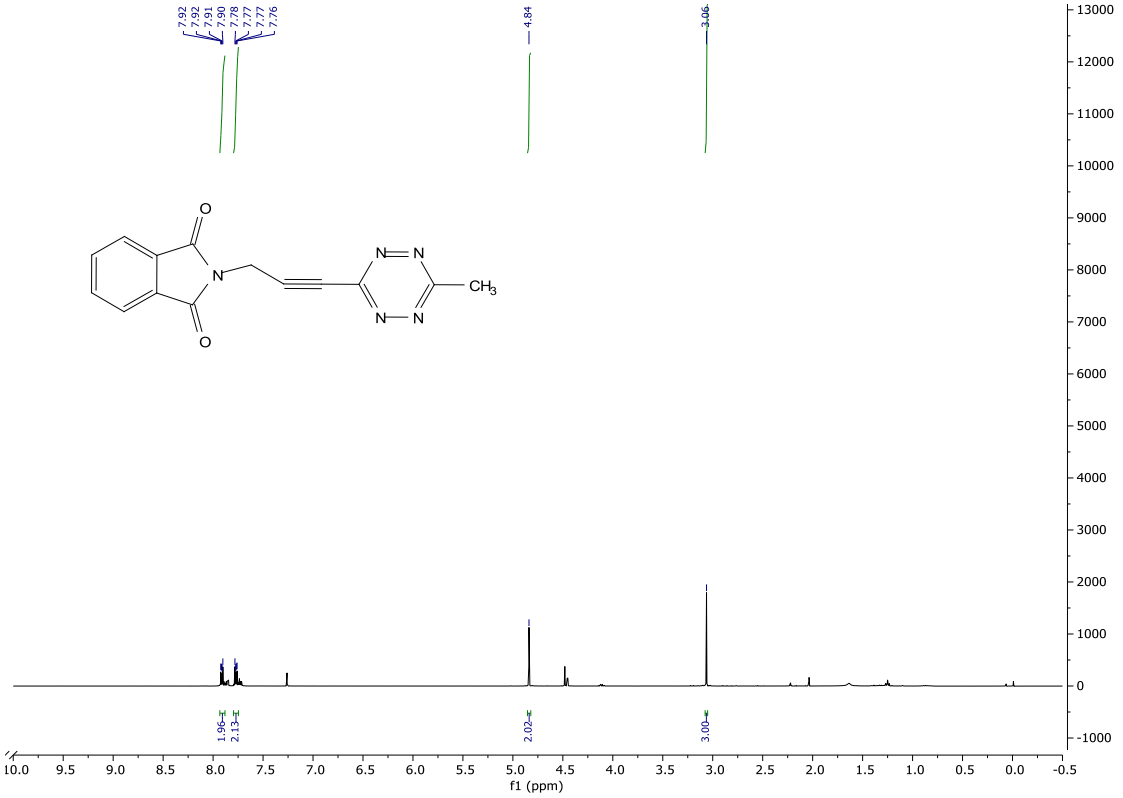
^{1}H NMR (400 MHz, $CDCl_3$) of 5i



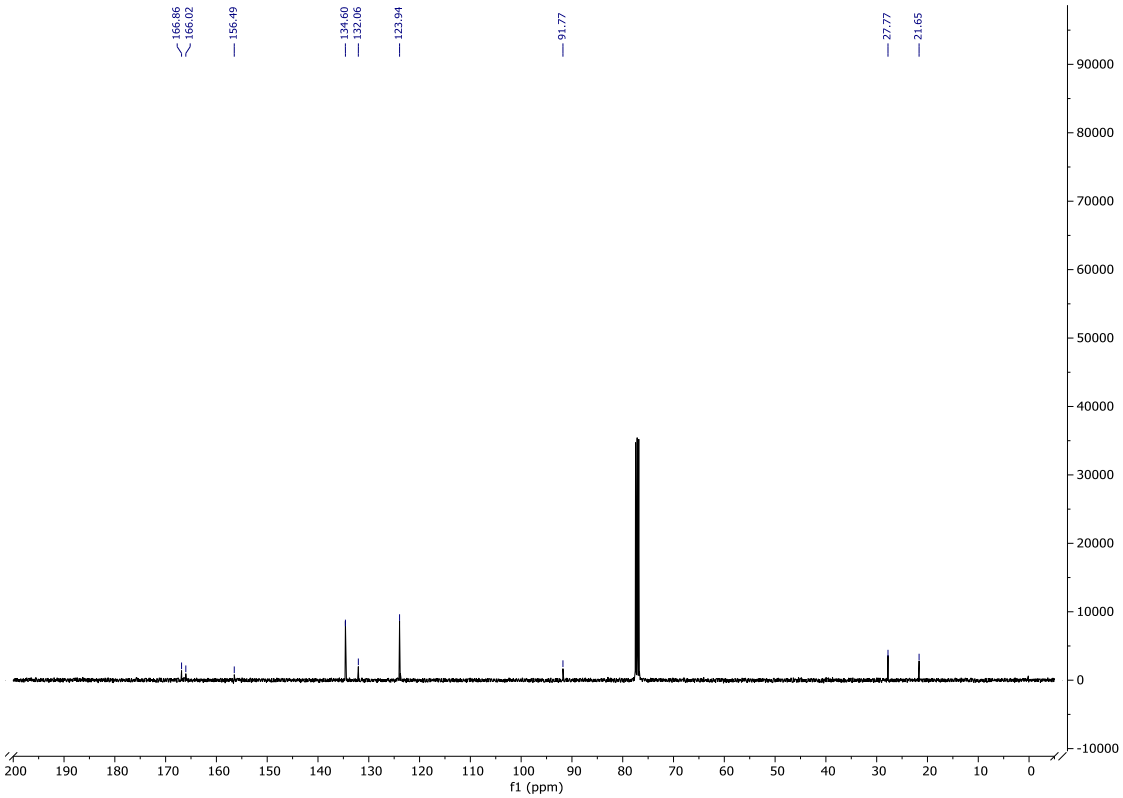
^{13}C NMR (101 MHz, $CDCl_3$) of 5i



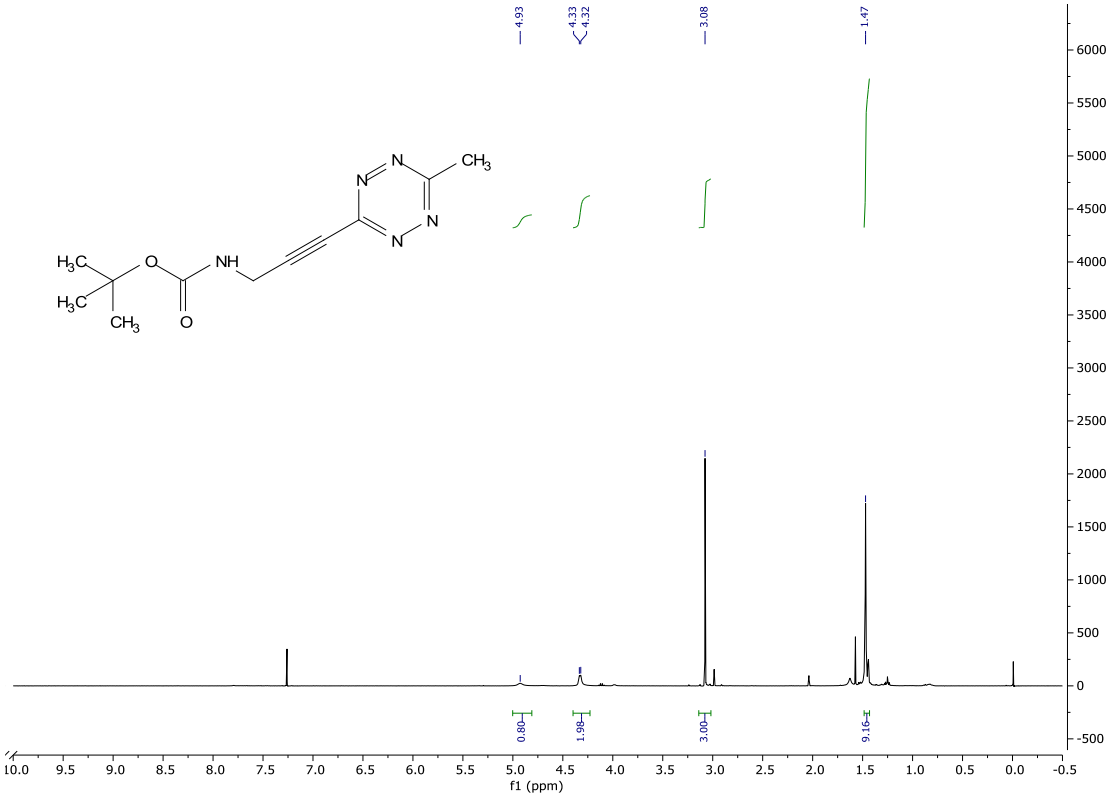
^{1}H NMR (400 MHz, $CDCl_3$) of 5j



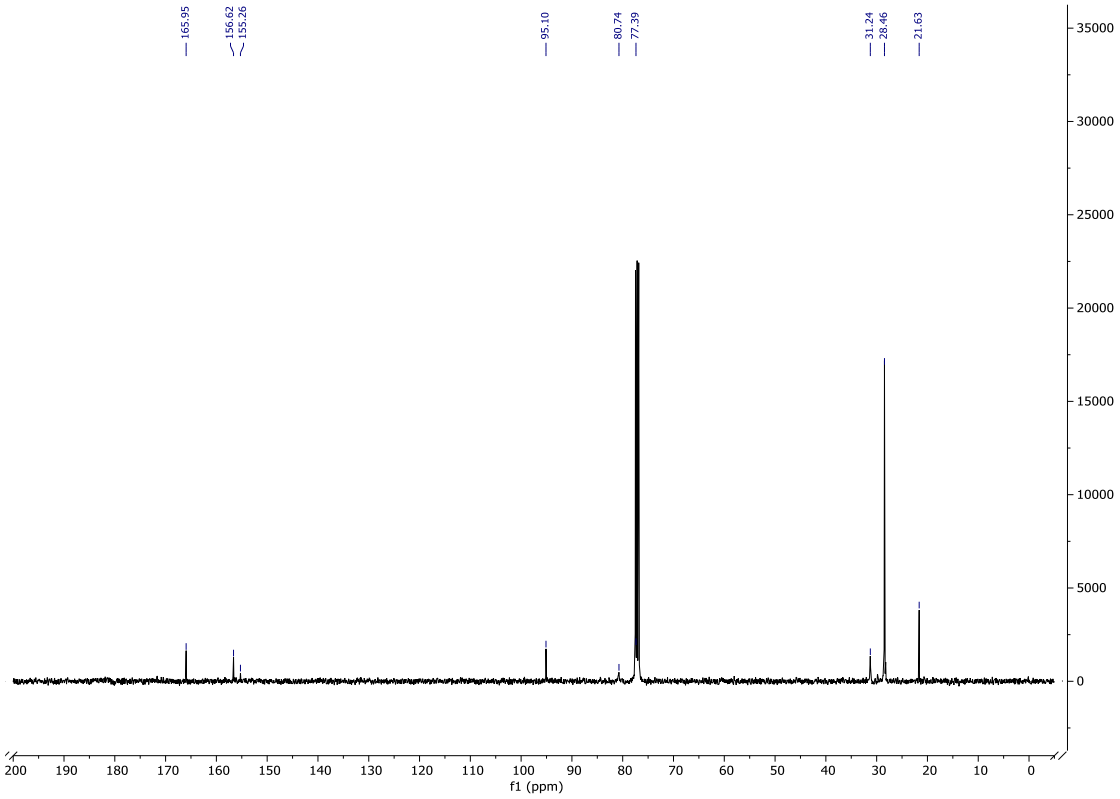
^{13}C NMR (101 MHz, $CDCl_3$) of 5j



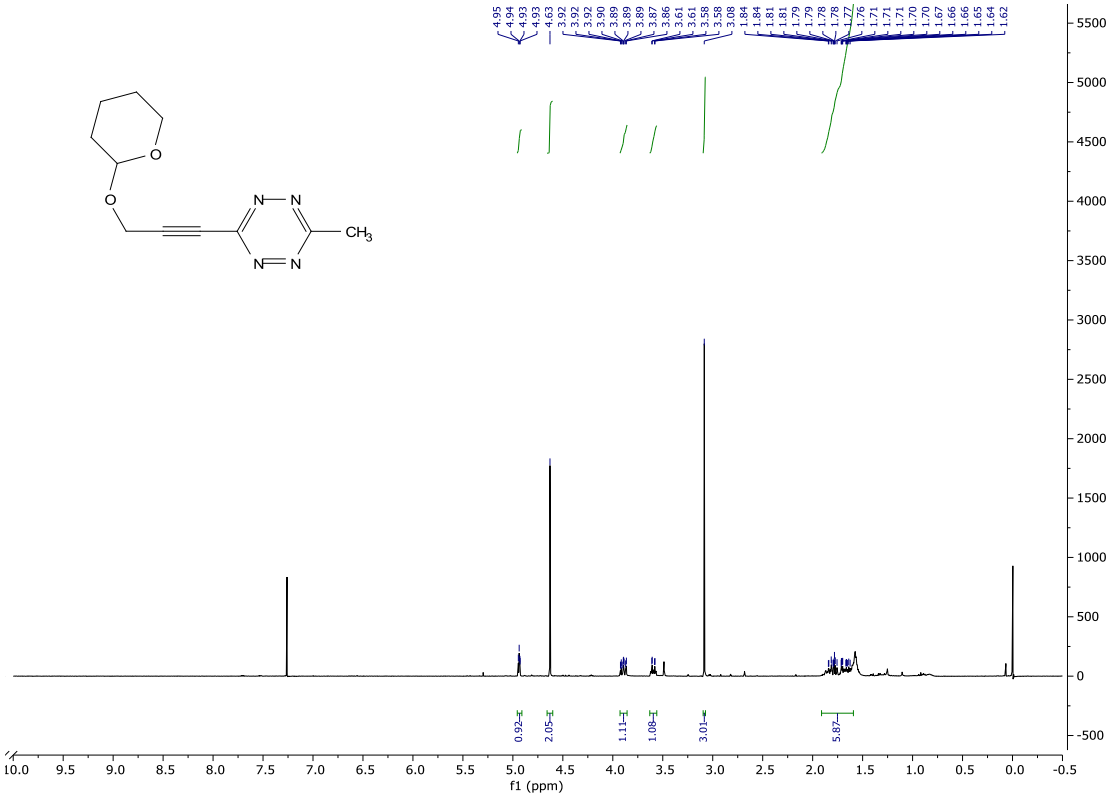
^{1}H NMR (400 MHz, $CDCl_3$) of 5k



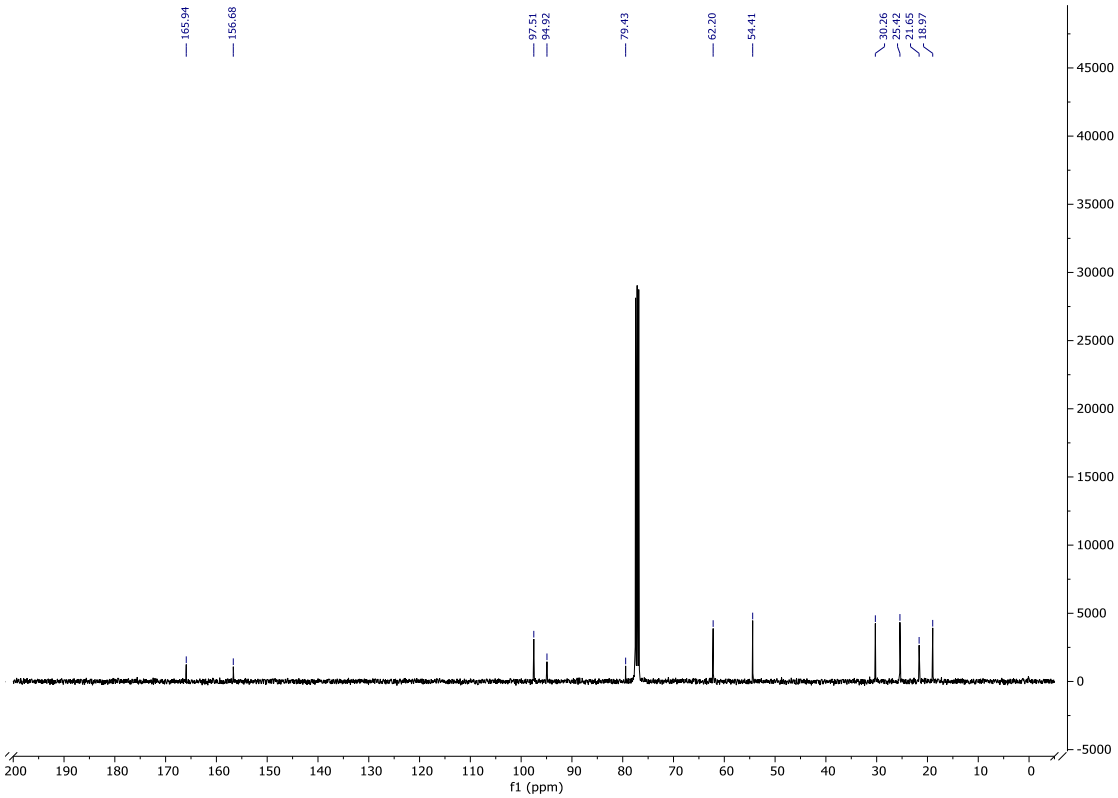
^{13}C NMR (101 MHz, $CDCl_3$) of 5k



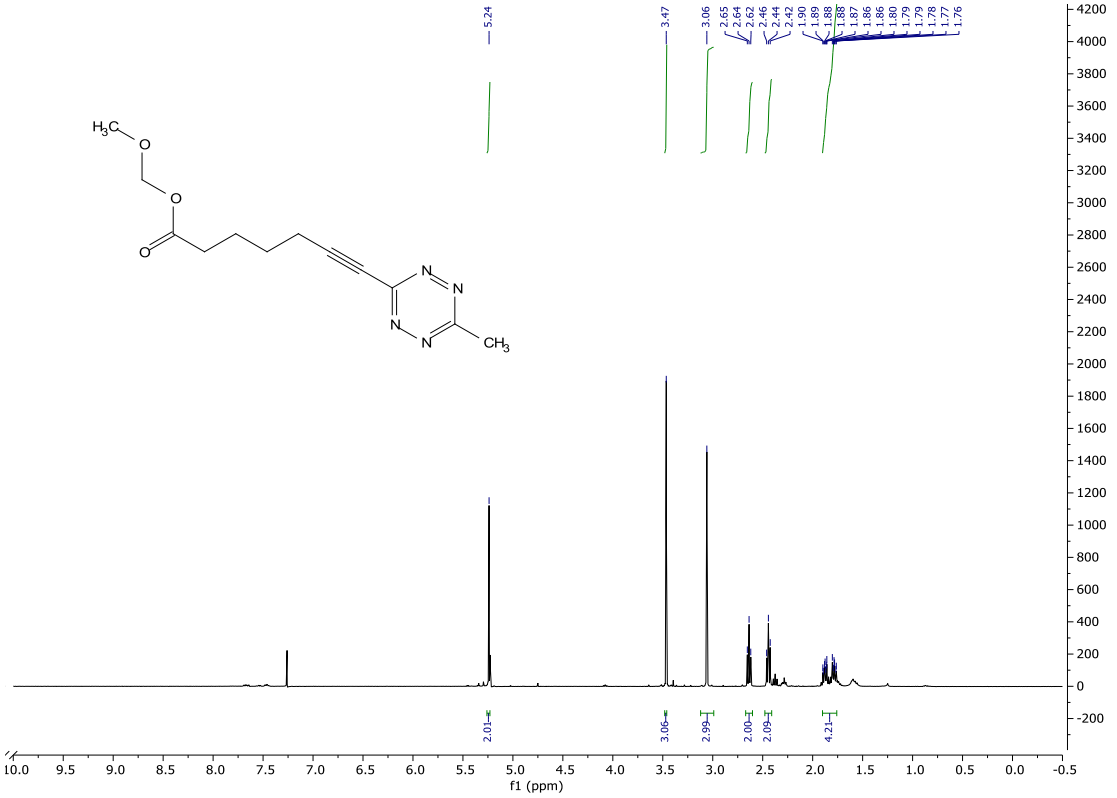
^{1}H NMR (400 MHz, $CDCl_3$) of 5l



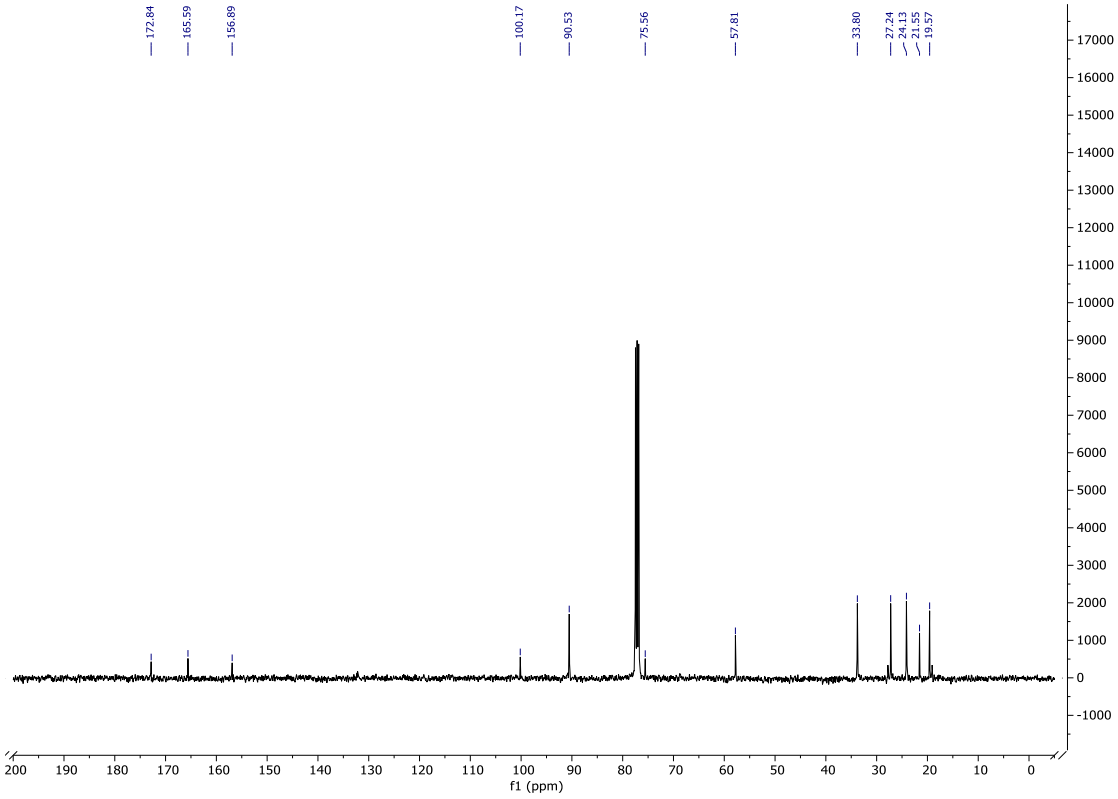
^{13}C NMR (101 MHz, $CDCl_3$) of 5l



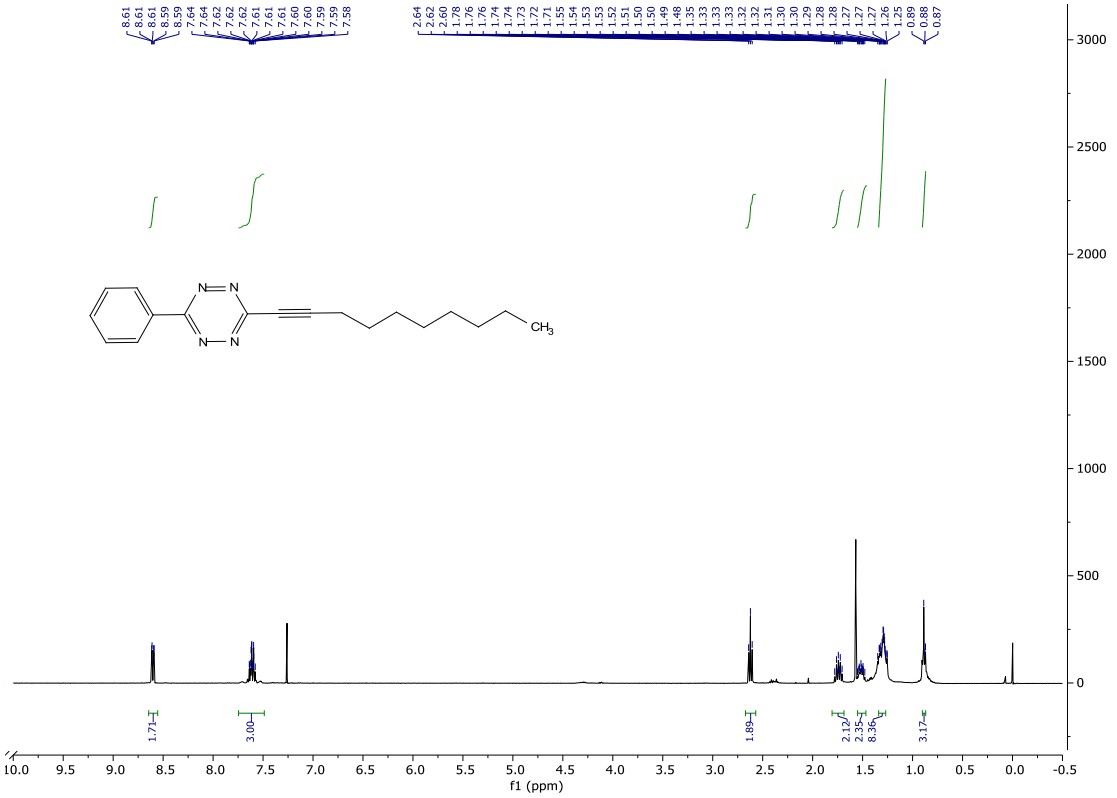
^{1}H NMR (400 MHz, $CDCl_3$) of 5m



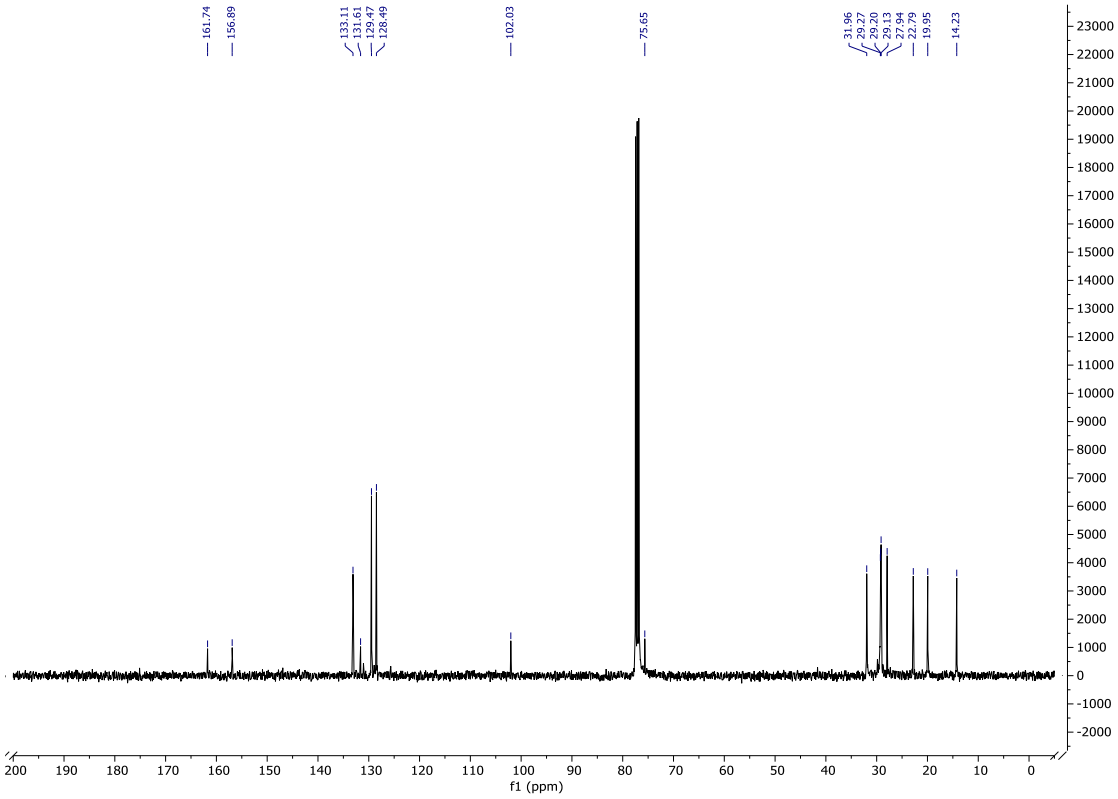
^{13}C NMR (101 MHz, $CDCl_3$) of 5m



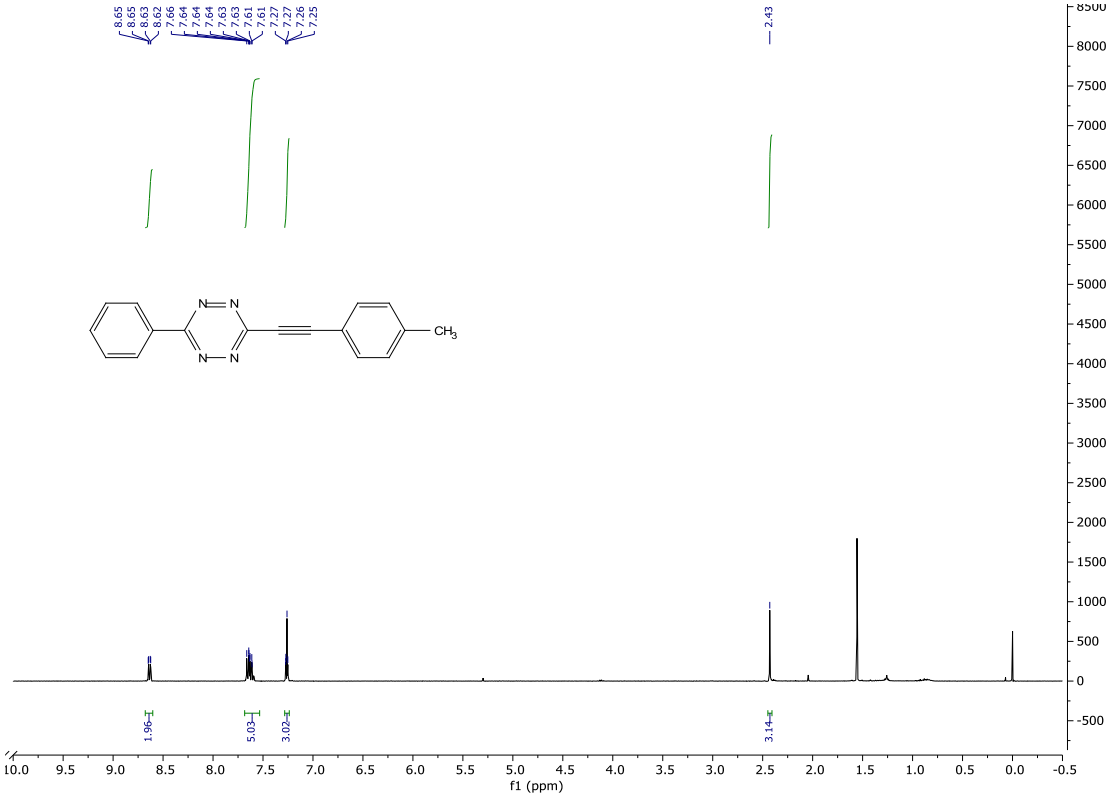
^{1}H NMR (400 MHz, $CDCl_3$) of 5n



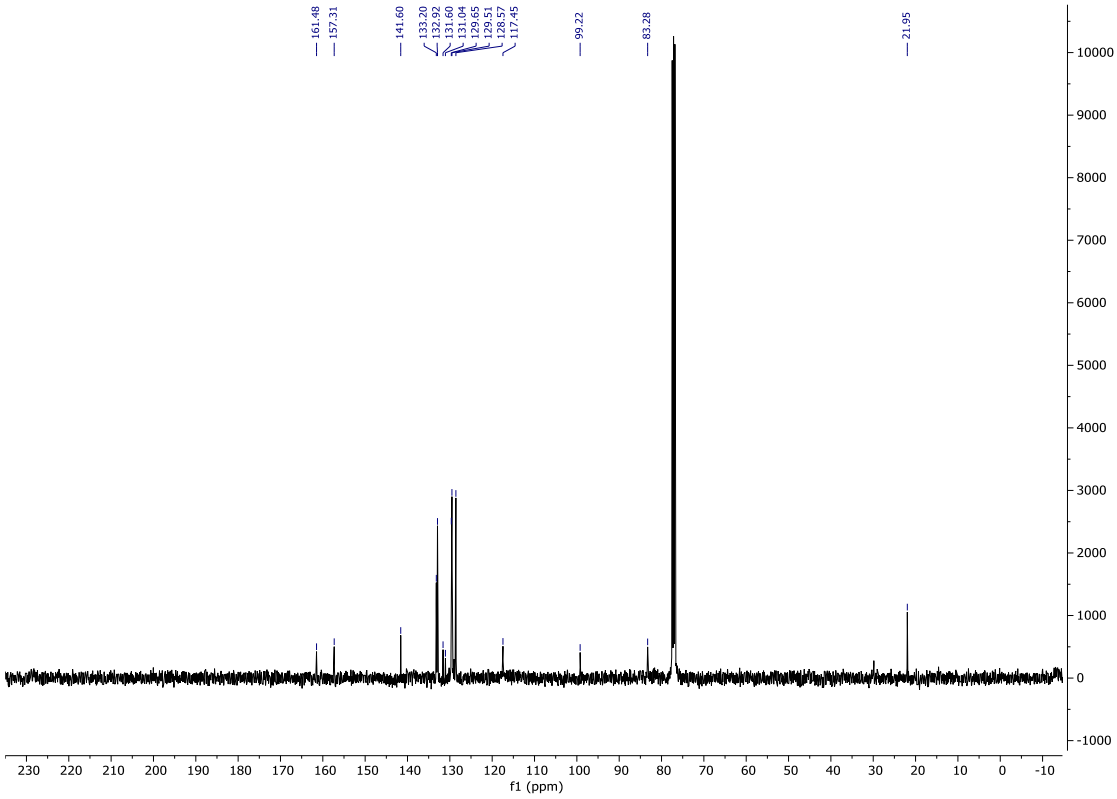
^{13}C NMR (101 MHz, $CDCl_3$) of 5n



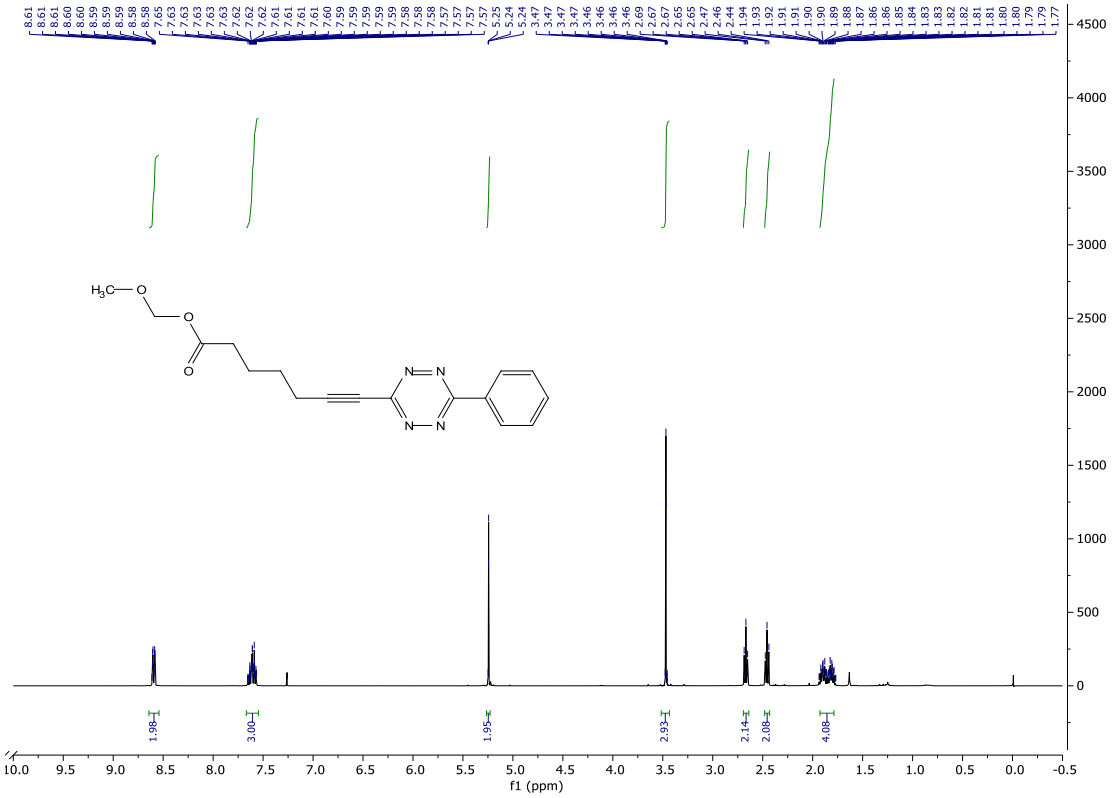
^{1}H NMR (400 MHz, $CDCl_3$) of 5o



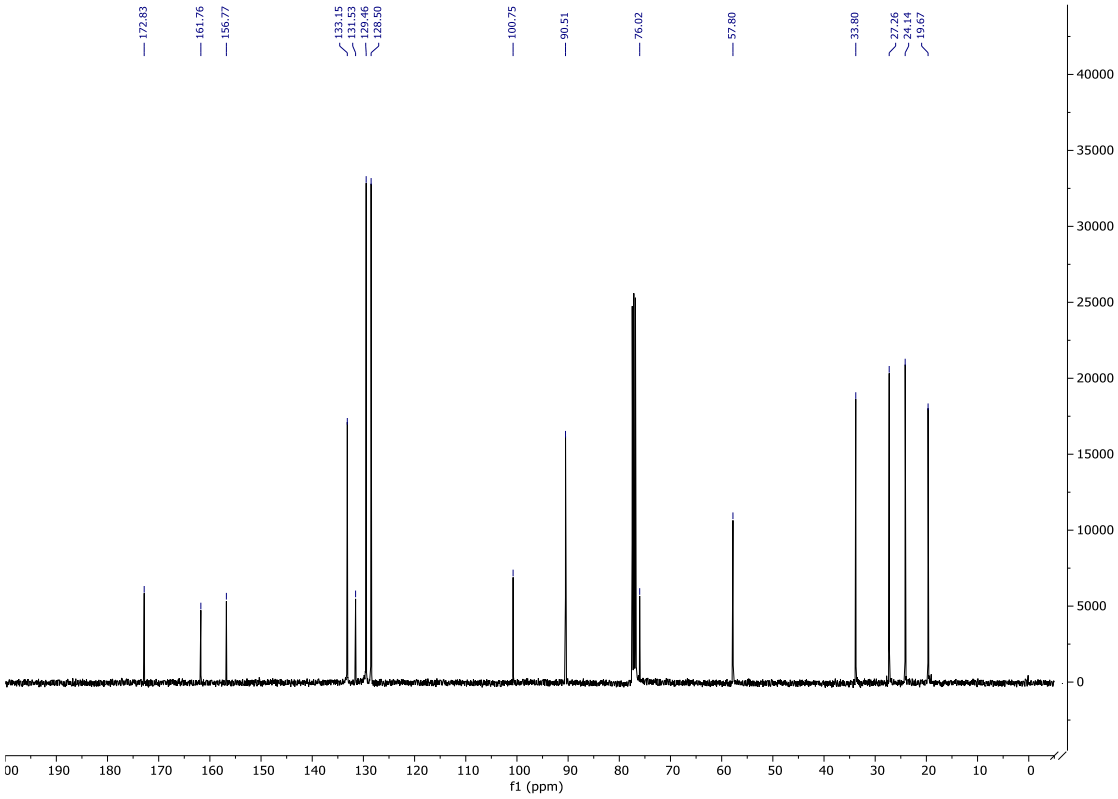
^{13}C NMR (101 MHz, $CDCl_3$) of 5o



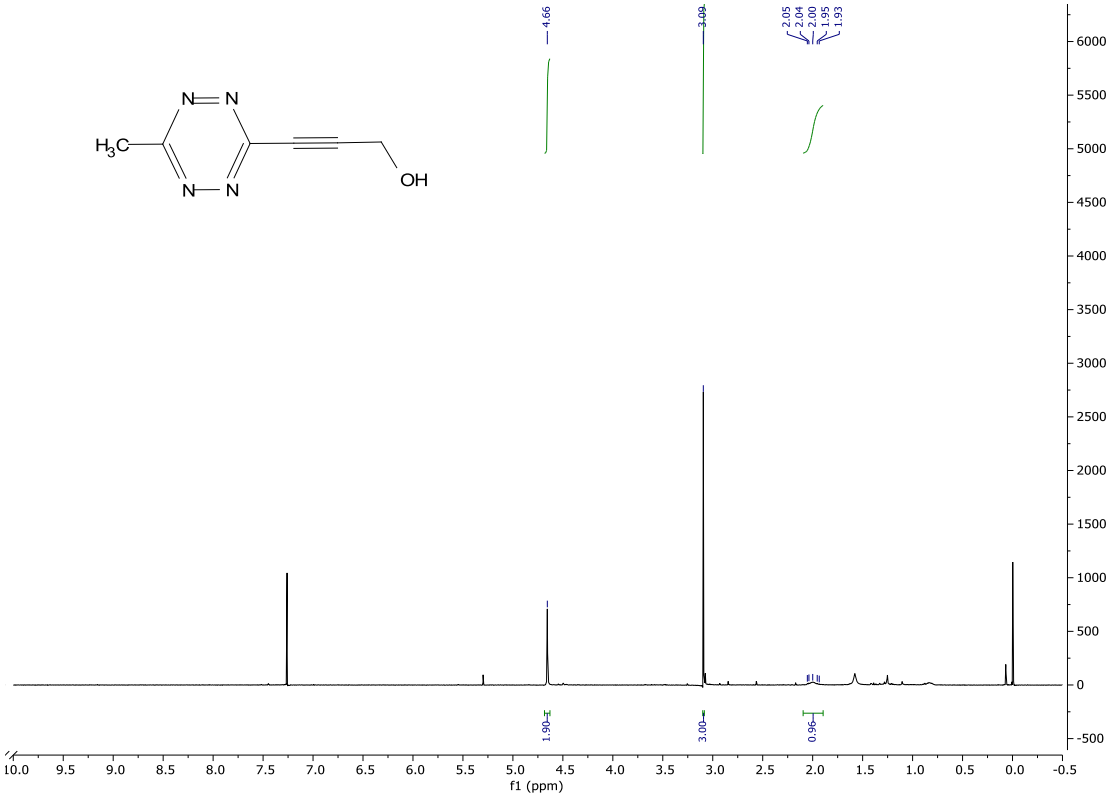
^{1}H NMR (400 MHz, $CDCl_3$) of 5p



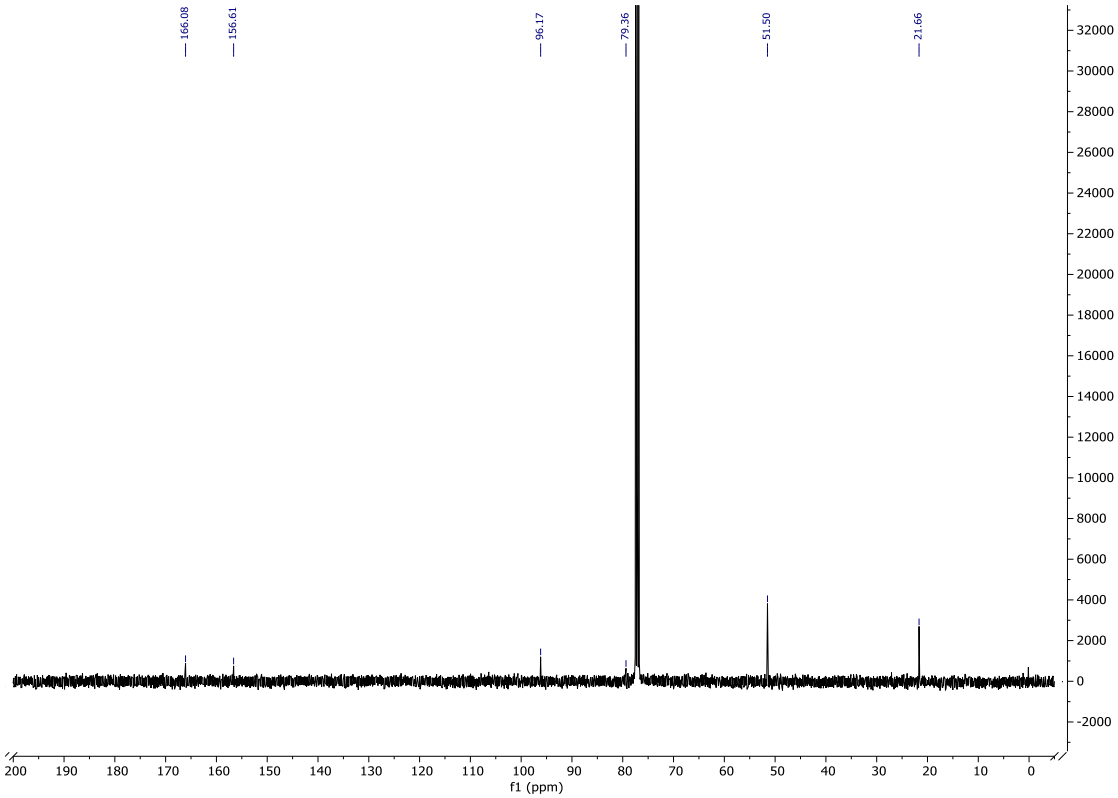
^{13}C NMR (101 MHz, $CDCl_3$) of 5p



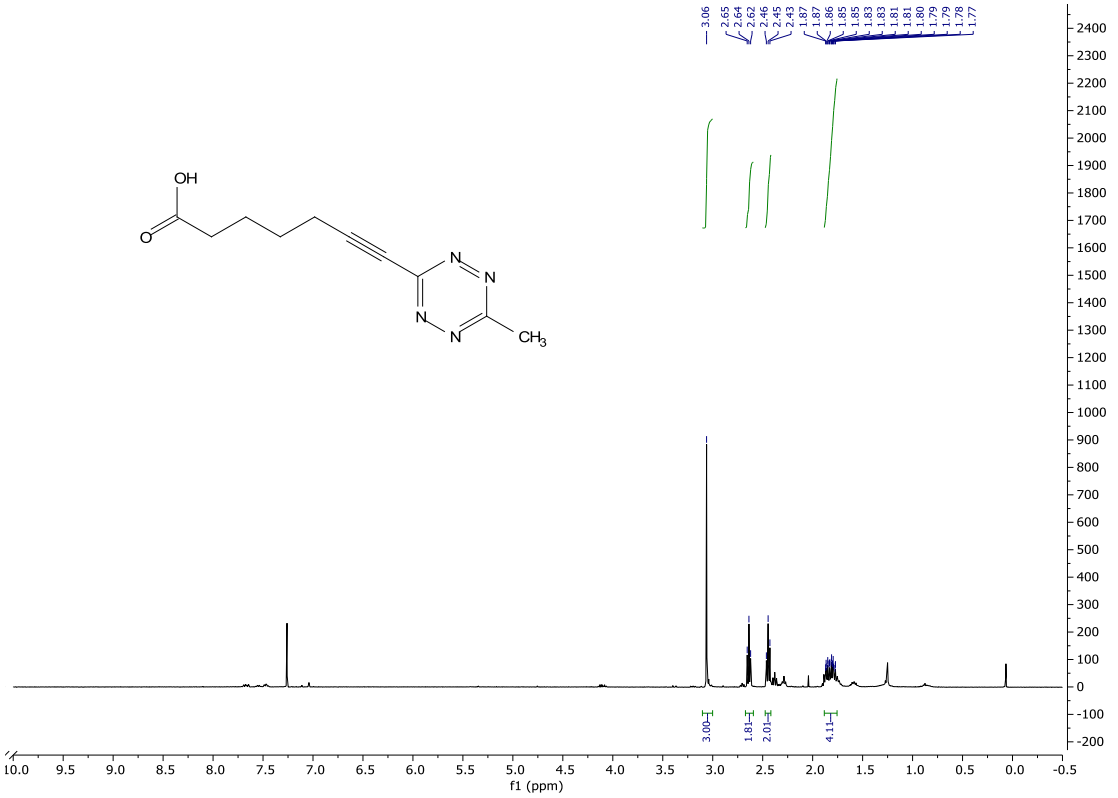
^{1}H NMR (400 MHz, $CDCl_3$) of 6b



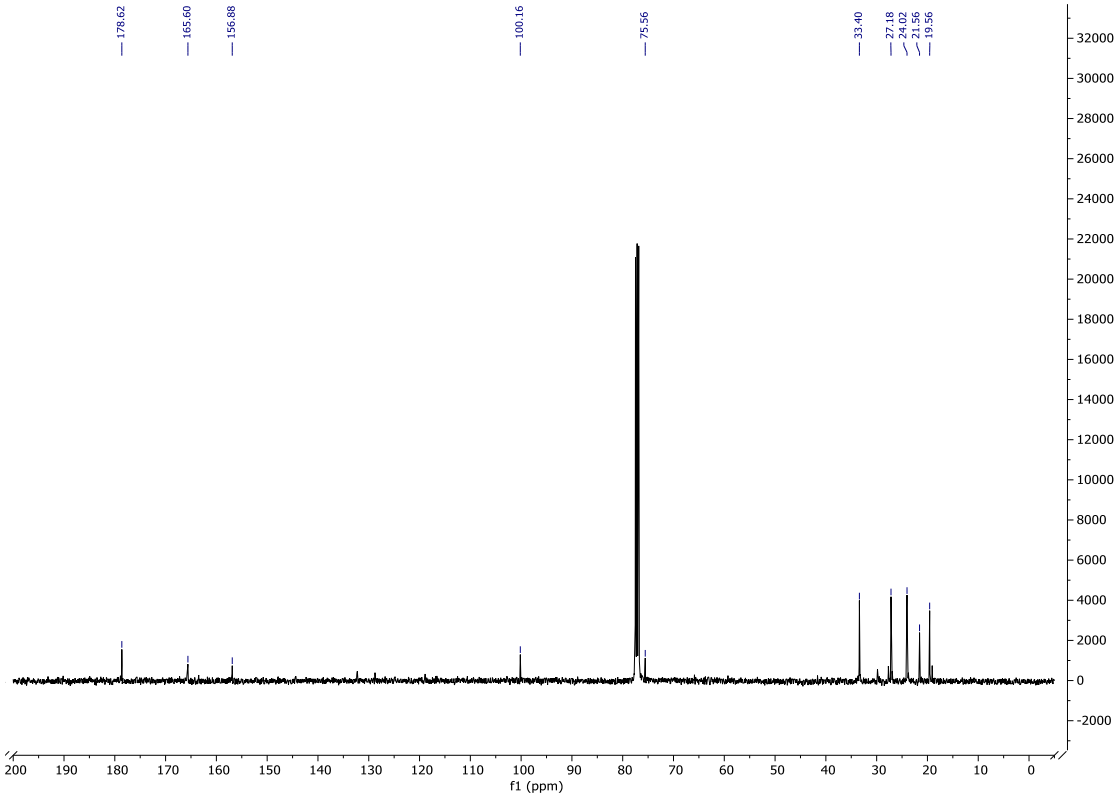
^{13}C NMR (101 MHz, $CDCl_3$) of 6b



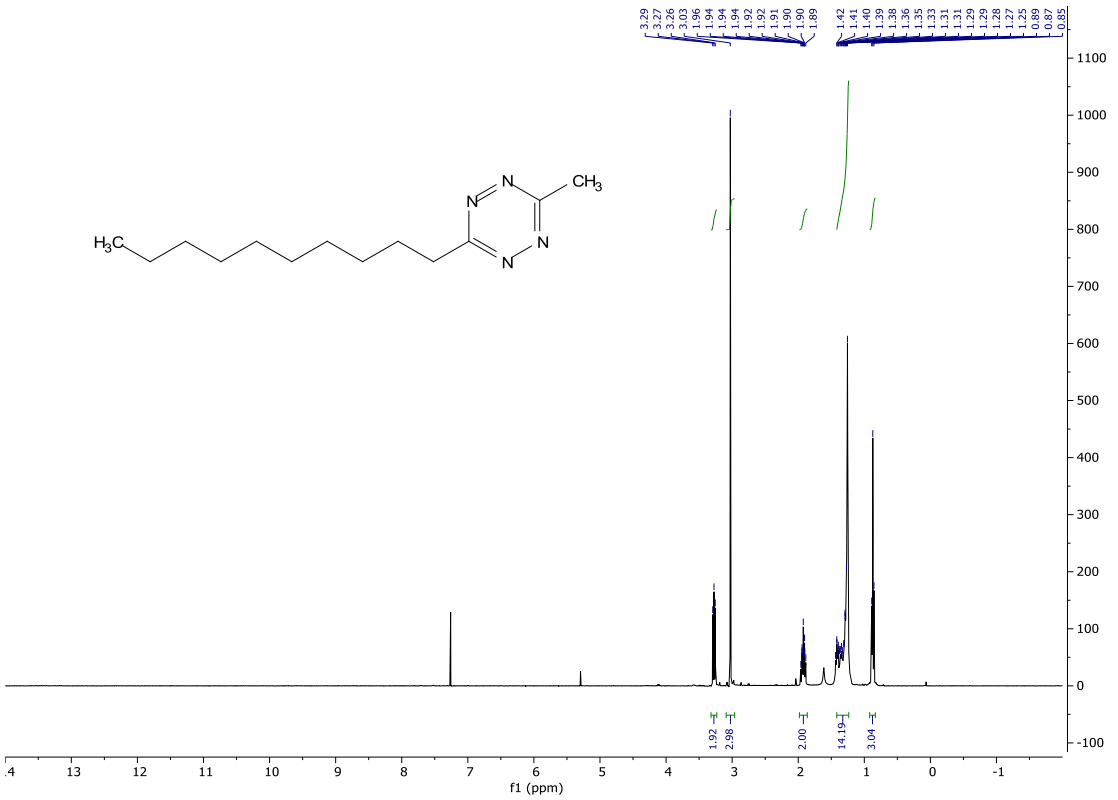
^{1}H NMR (400 MHz, $CDCl_3$) of 6c



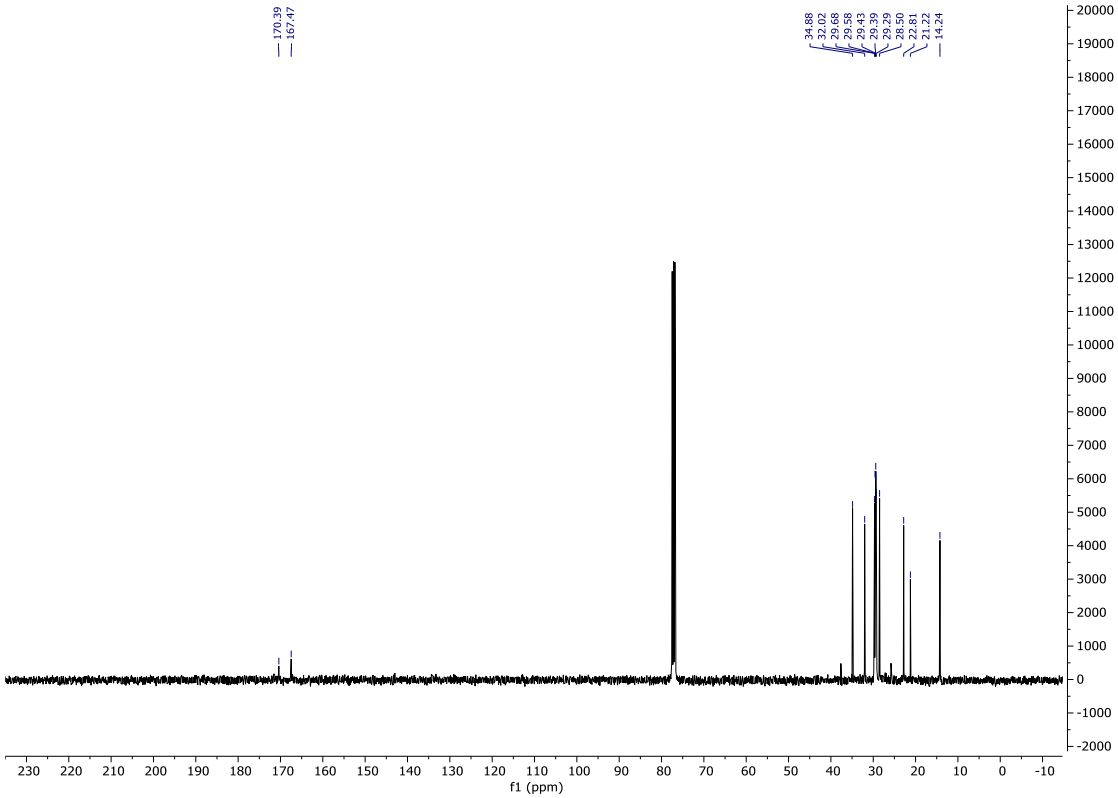
^{13}C NMR (101 MHz, $CDCl_3$) of 6c



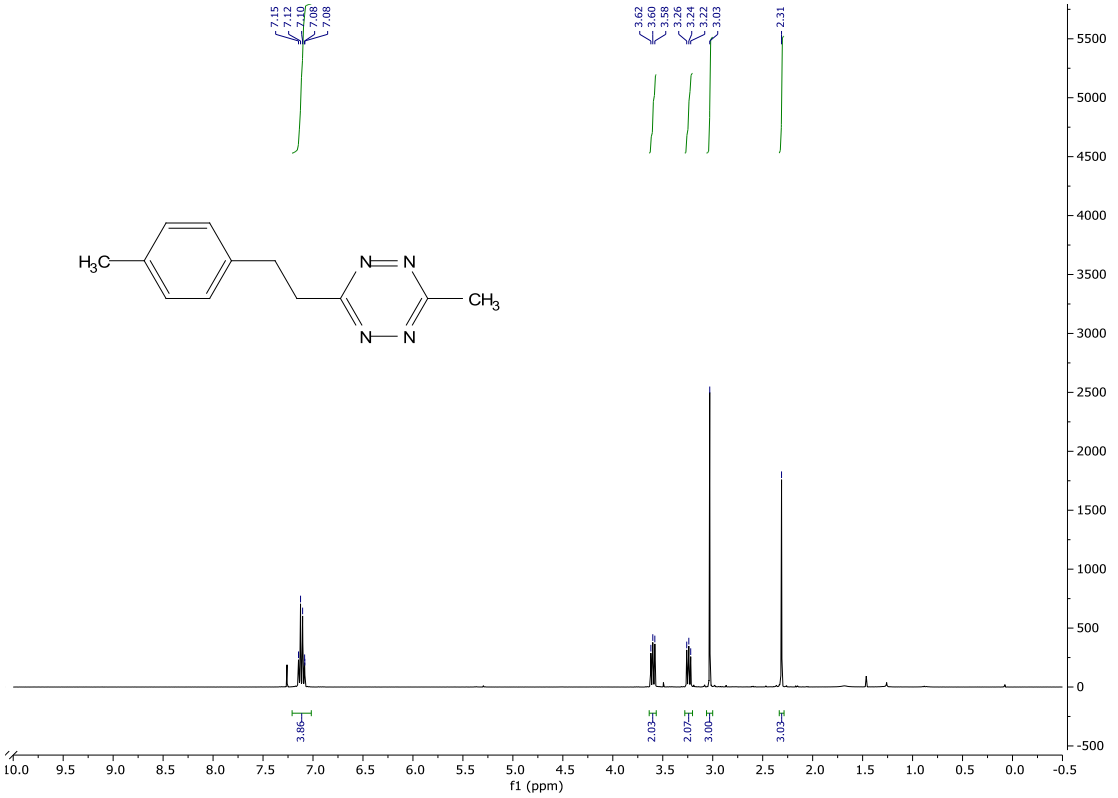
^{1}H NMR (400 MHz, $CDCl_3$) of 7c



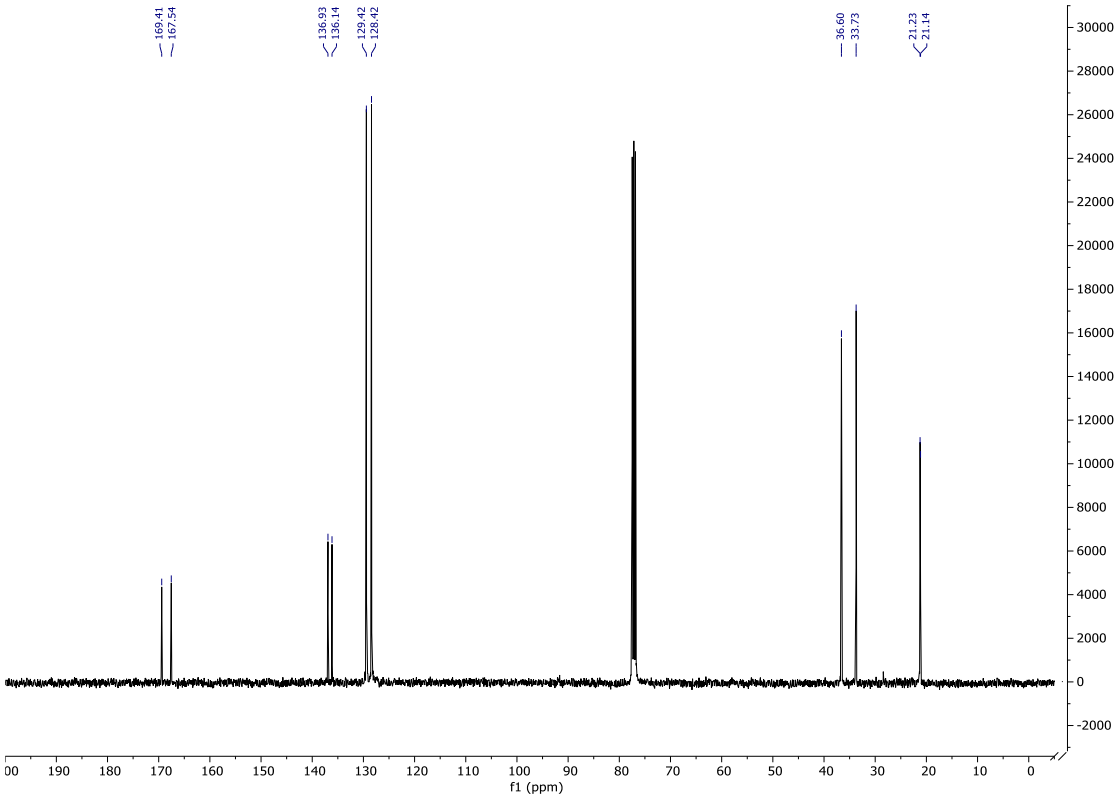
^{13}C NMR (101 MHz, $CDCl_3$) of 7c



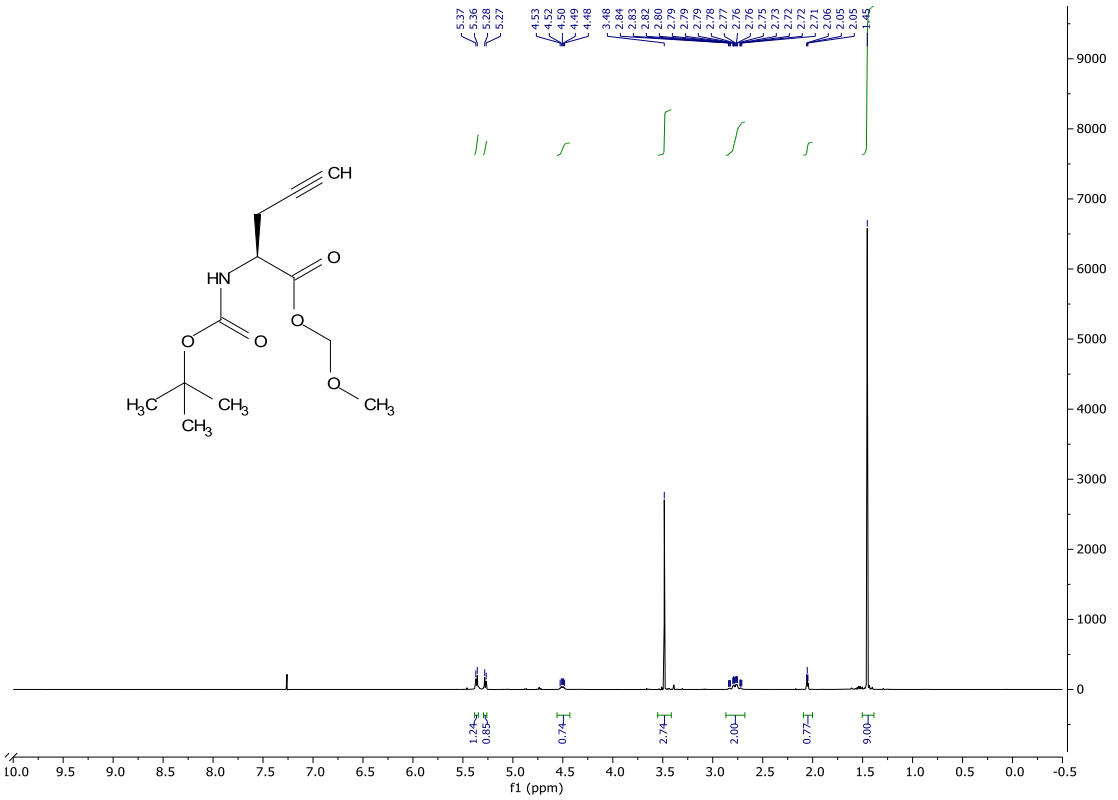
^{1}H NMR (400 MHz, $CDCl_3$) of 7f



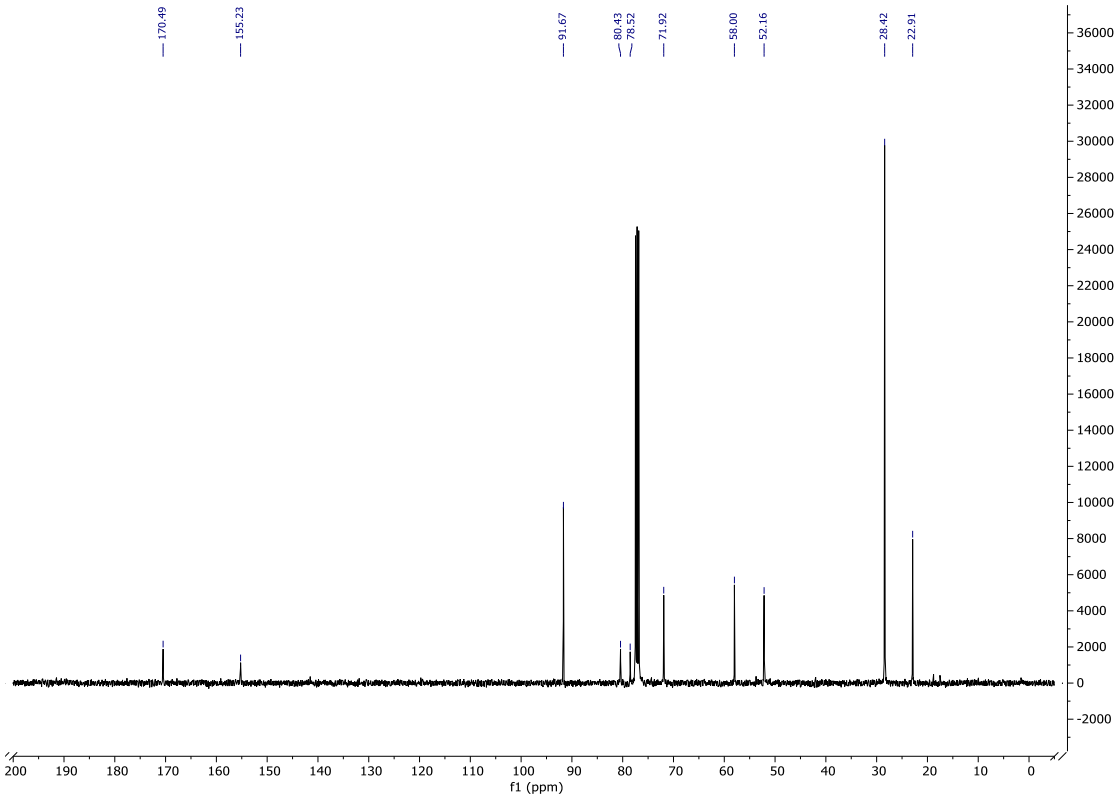
^{13}C NMR (101 MHz, $CDCl_3$) of 7f



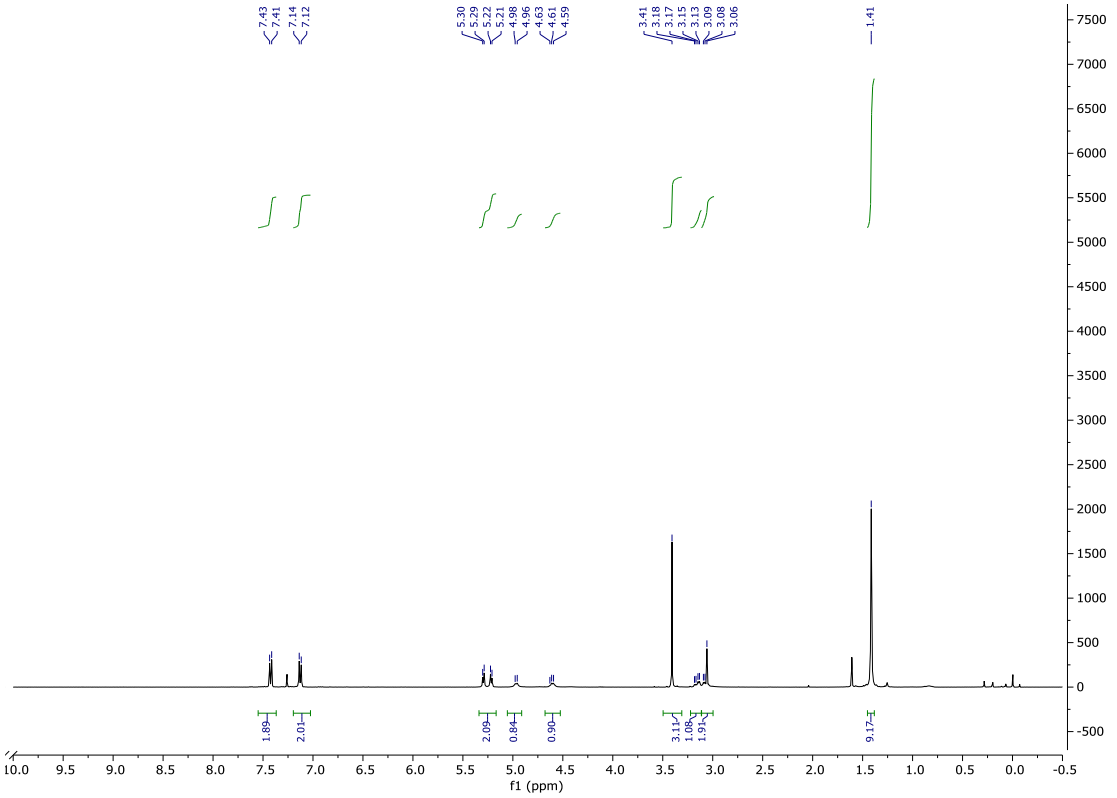
^{1}H NMR (400 MHz, $CDCl_3$) of 8a



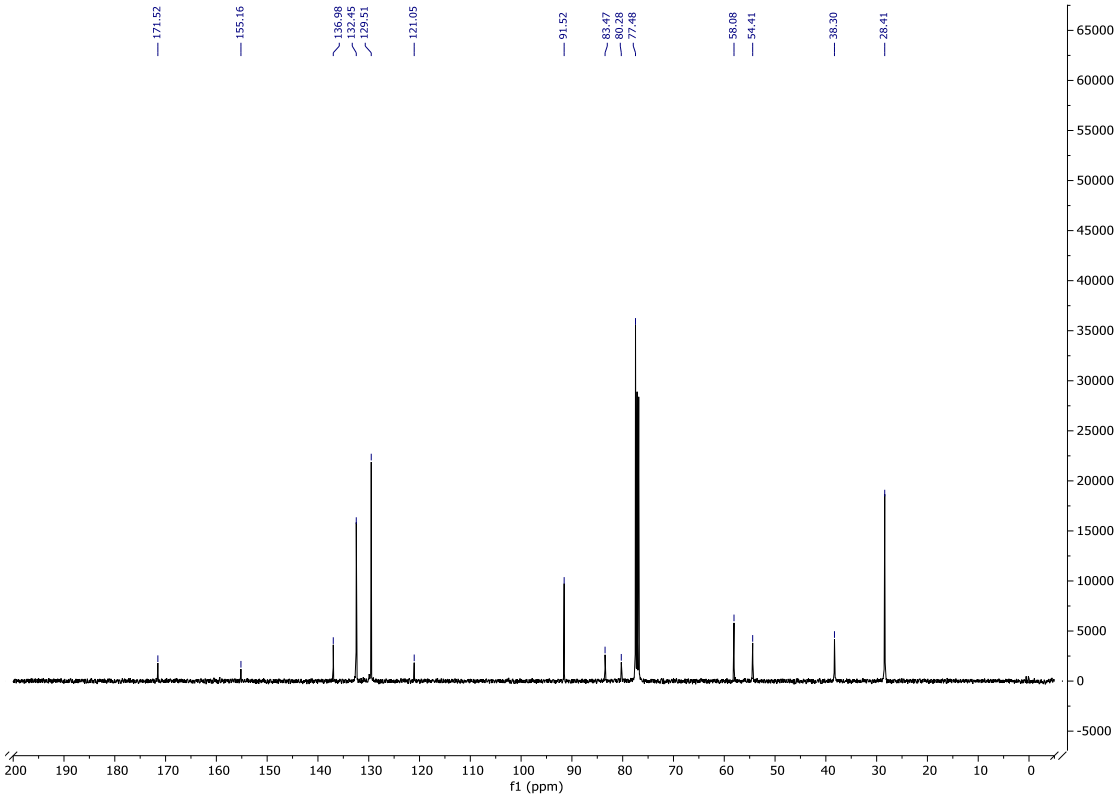
^{13}C NMR (101 MHz, $CDCl_3$) of 8a



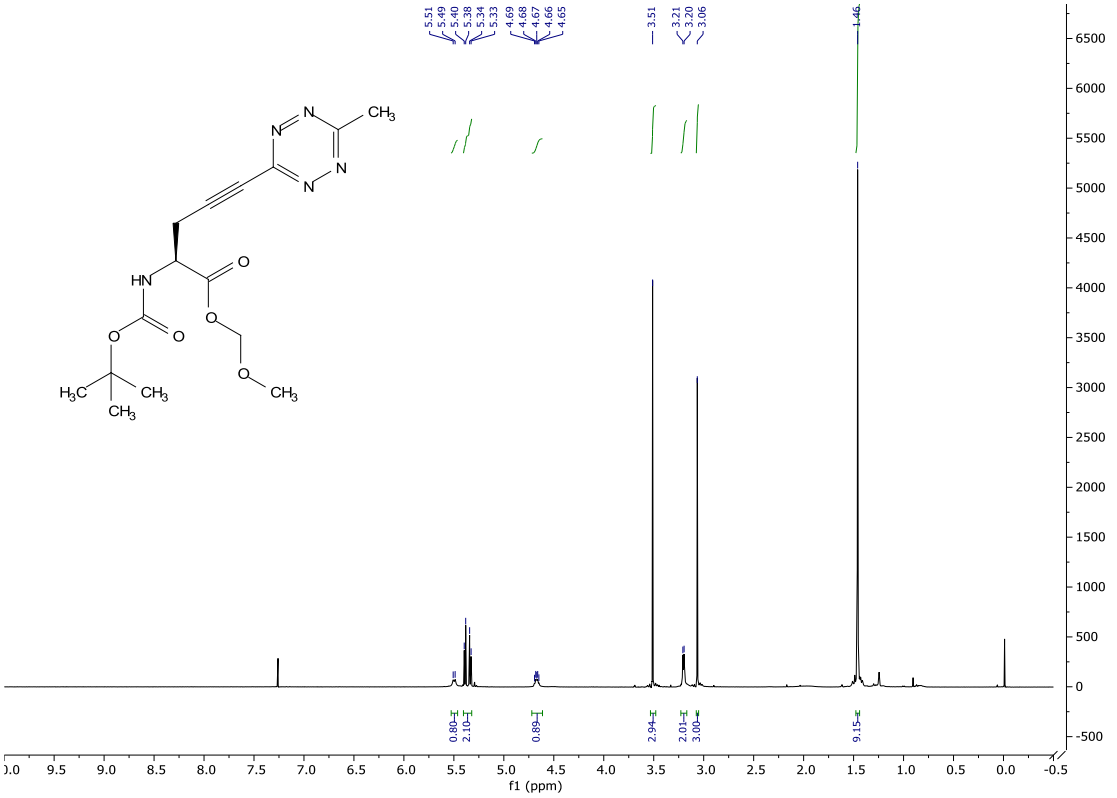
^{1}H NMR (400 MHz, $CDCl_3$) of 8b



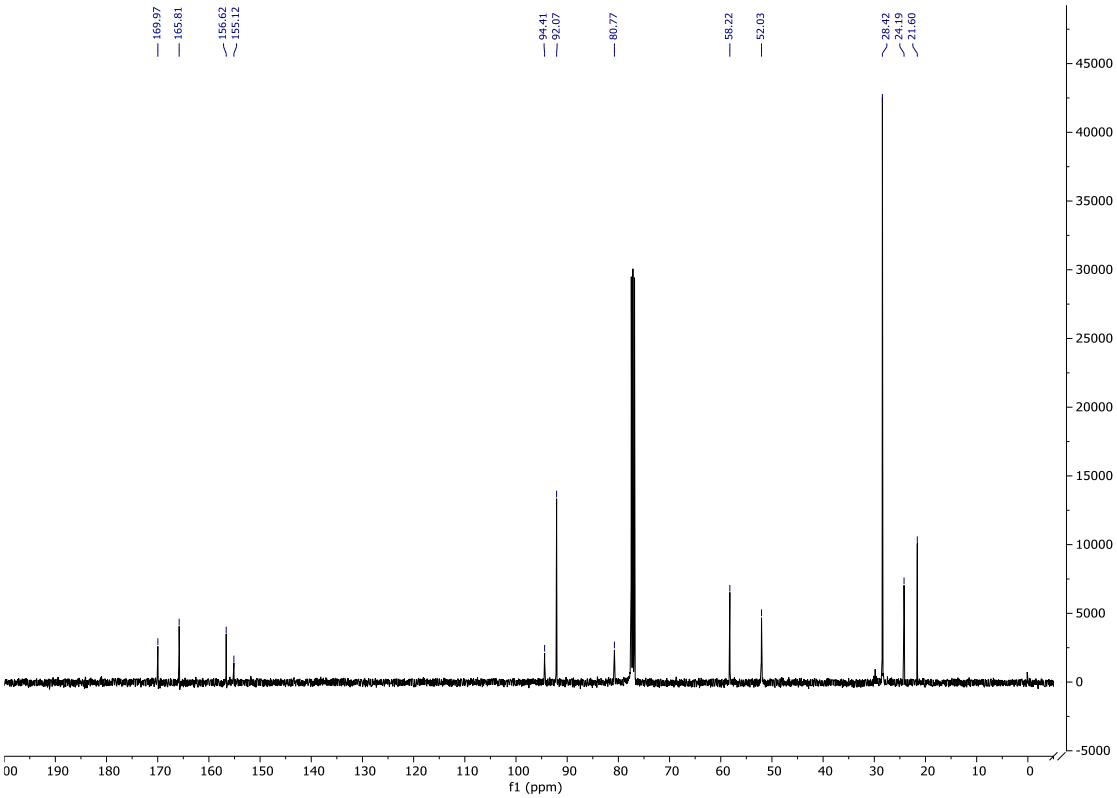
^{13}C NMR (101 MHz, $CDCl_3$) of 8b



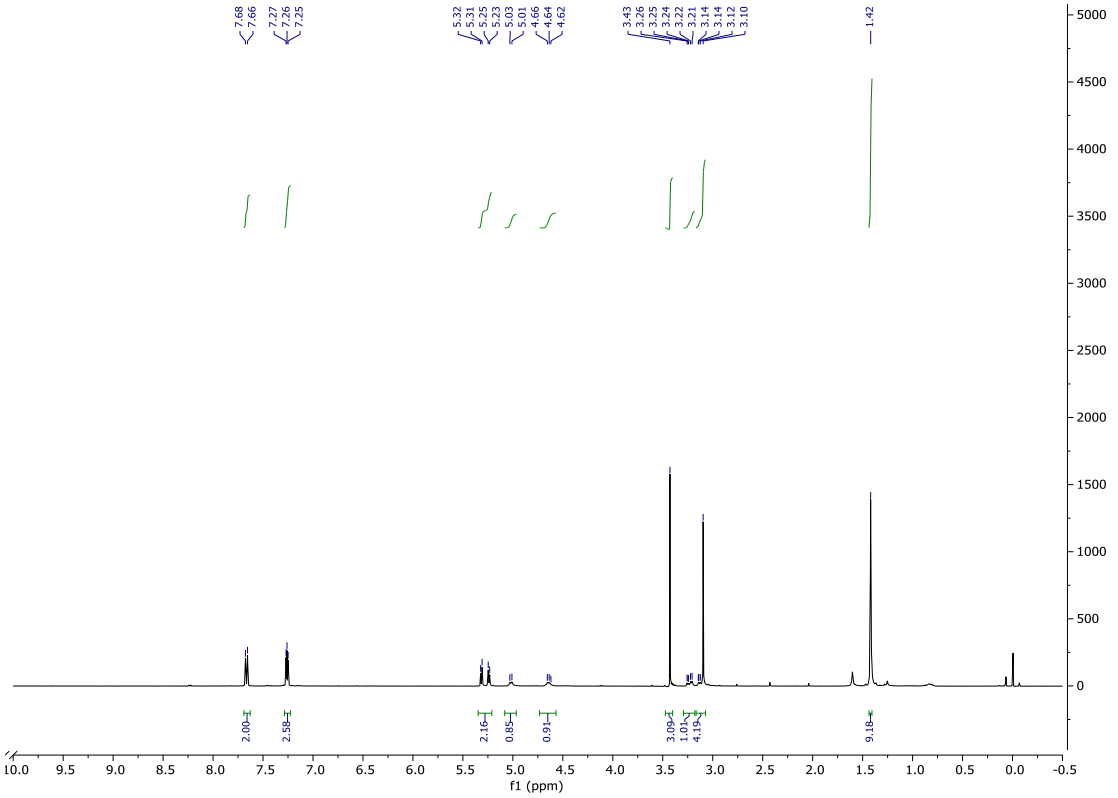
^{1}H NMR (400 MHz, $CDCl_3$) of 9a



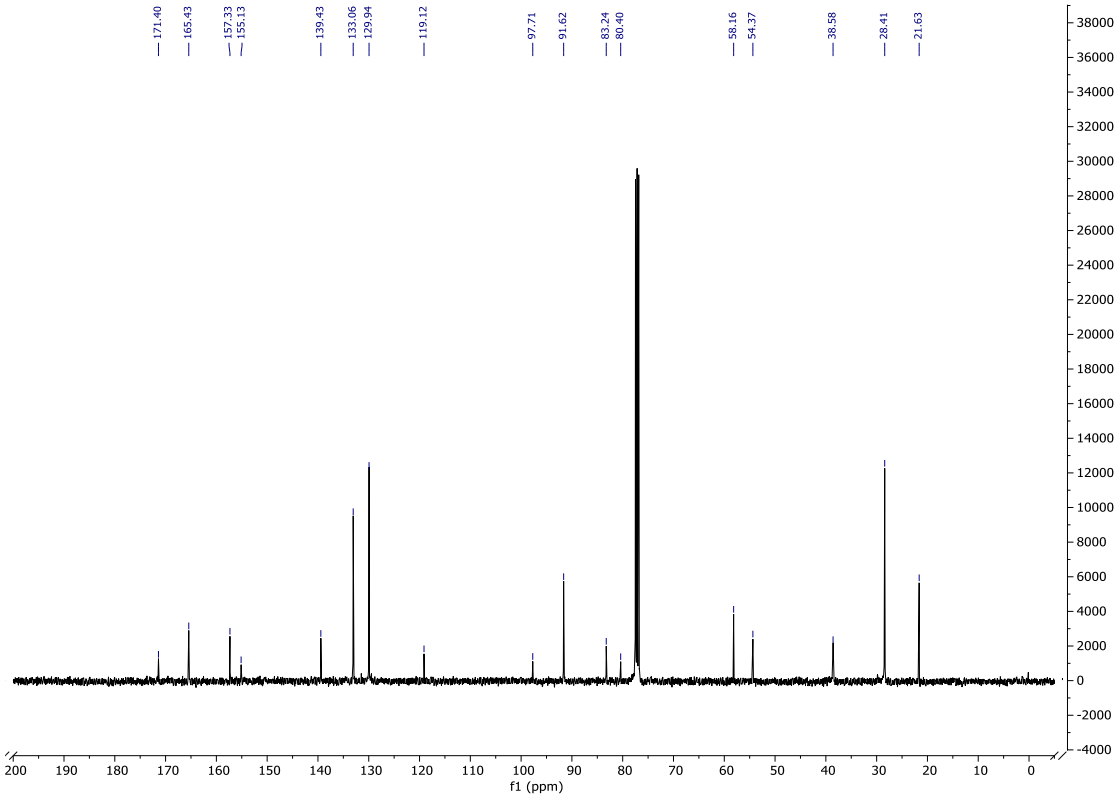
^{13}C NMR (101 MHz, $CDCl_3$) of 9a



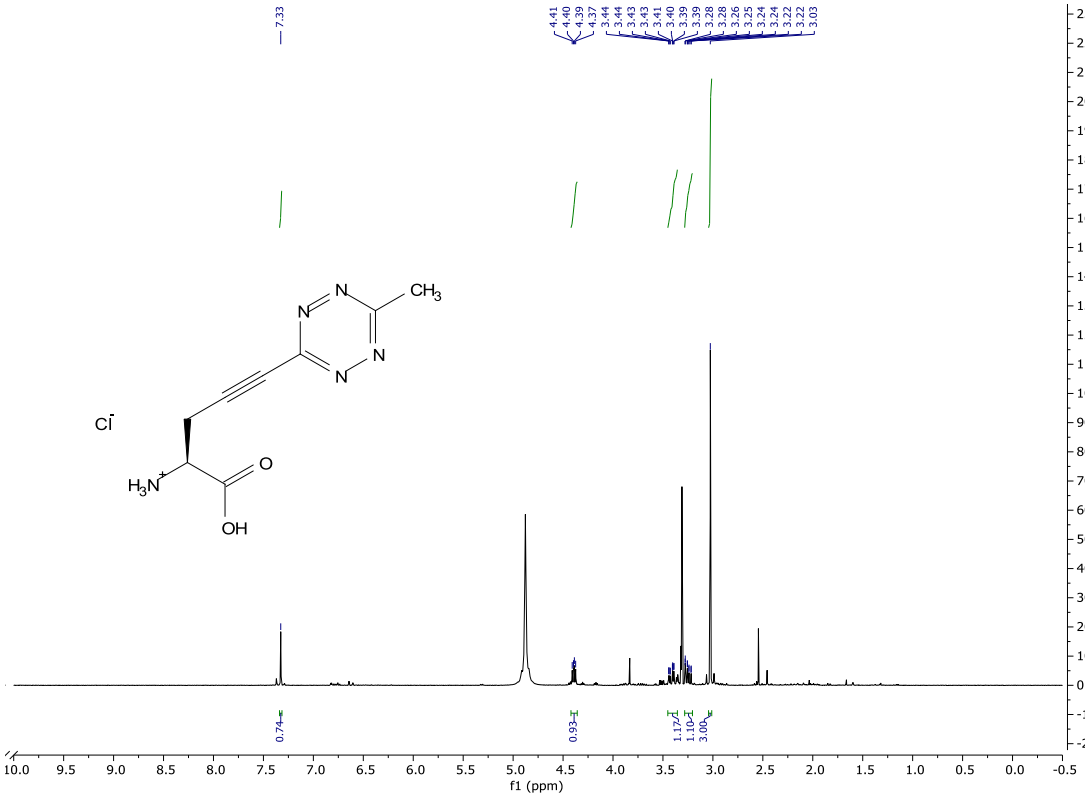
^{1}H NMR (400 MHz, $CDCl_3$) of 9b



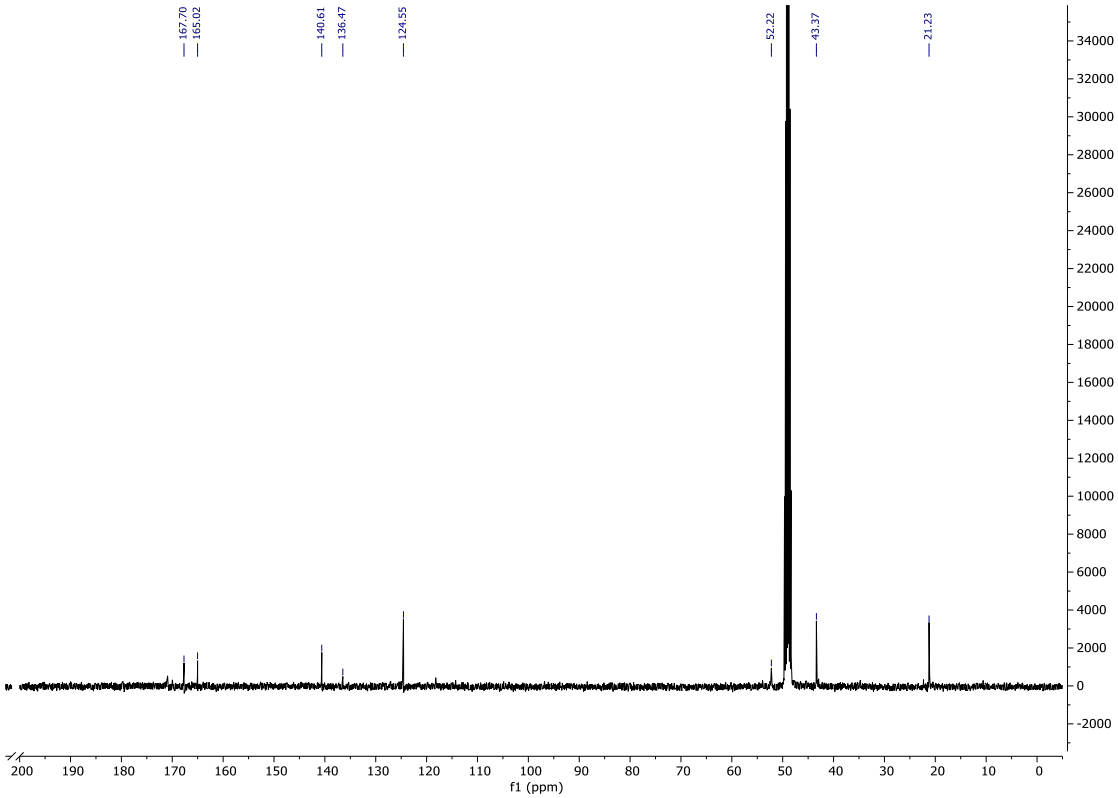
^{13}C NMR (101 MHz, $CDCl_3$) of 9b



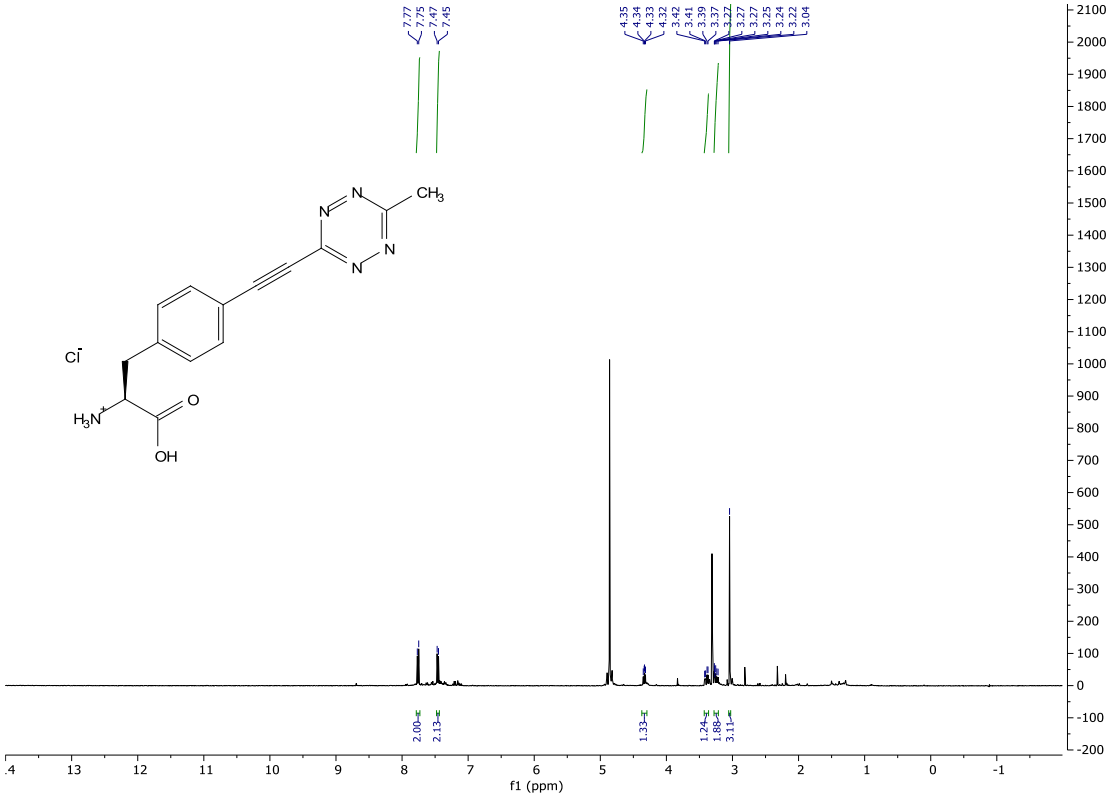
^{1}H NMR (400 MHz, CD_3OD) of 10a



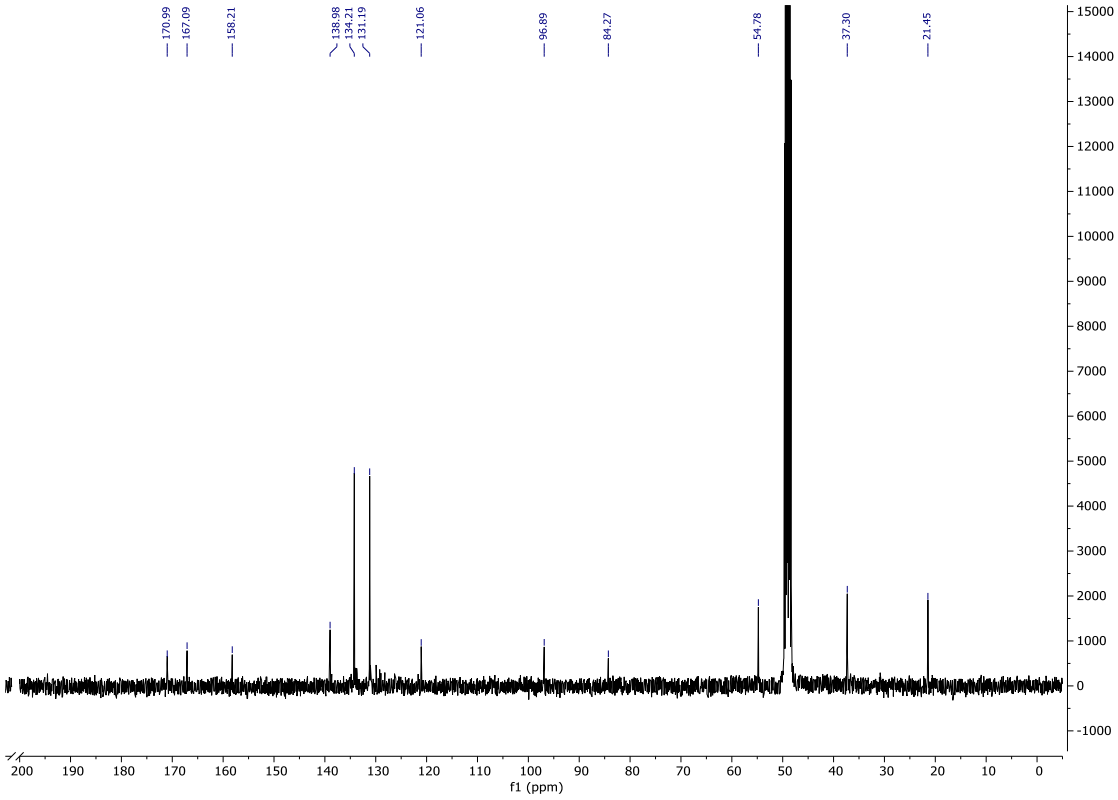
^{13}C NMR (101 MHz, CD_3OD) of 10a



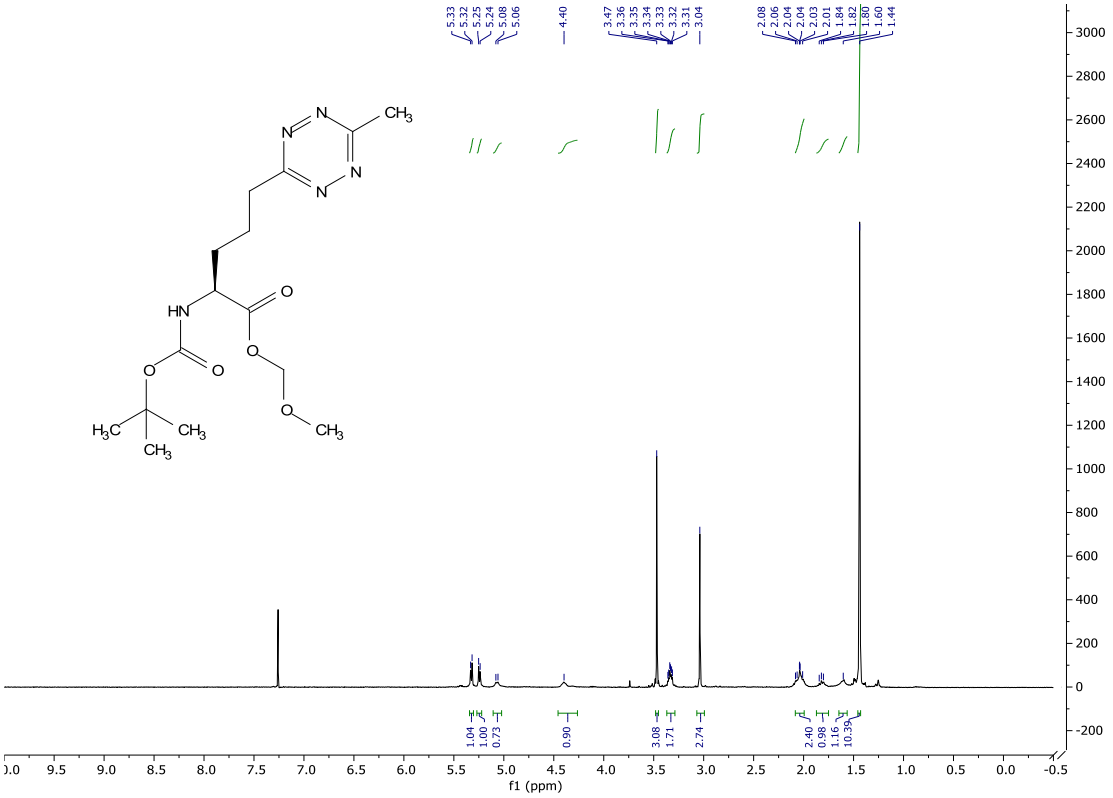
^{1}H NMR (400 MHz, CD_3OD) of 10b



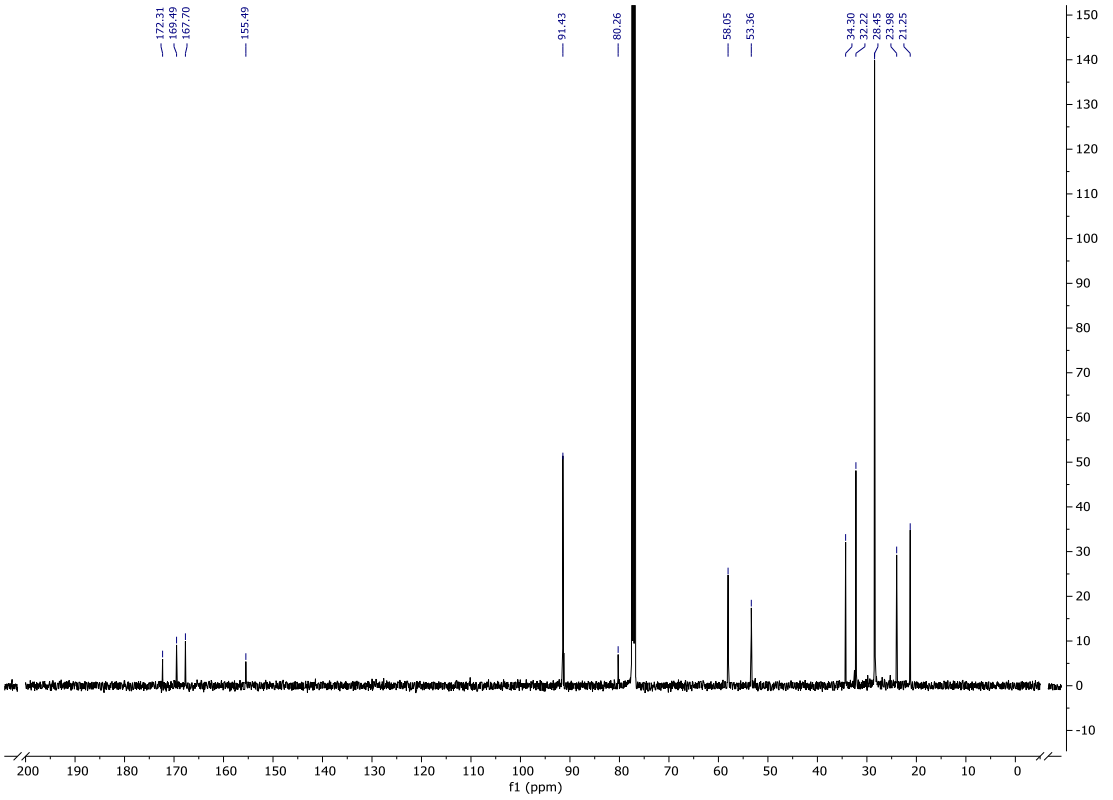
^{13}C NMR (101 MHz, CD_3OD) of 10b



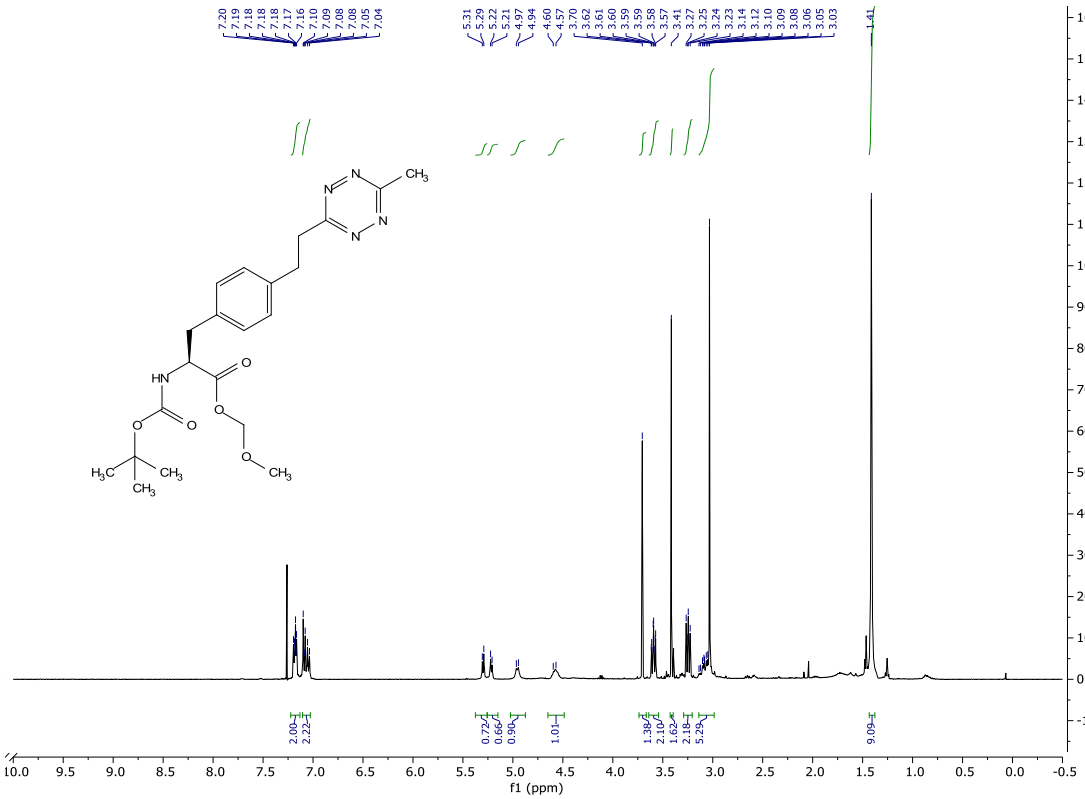
^{1}H NMR (400 MHz, $CDCl_3$) of 11a



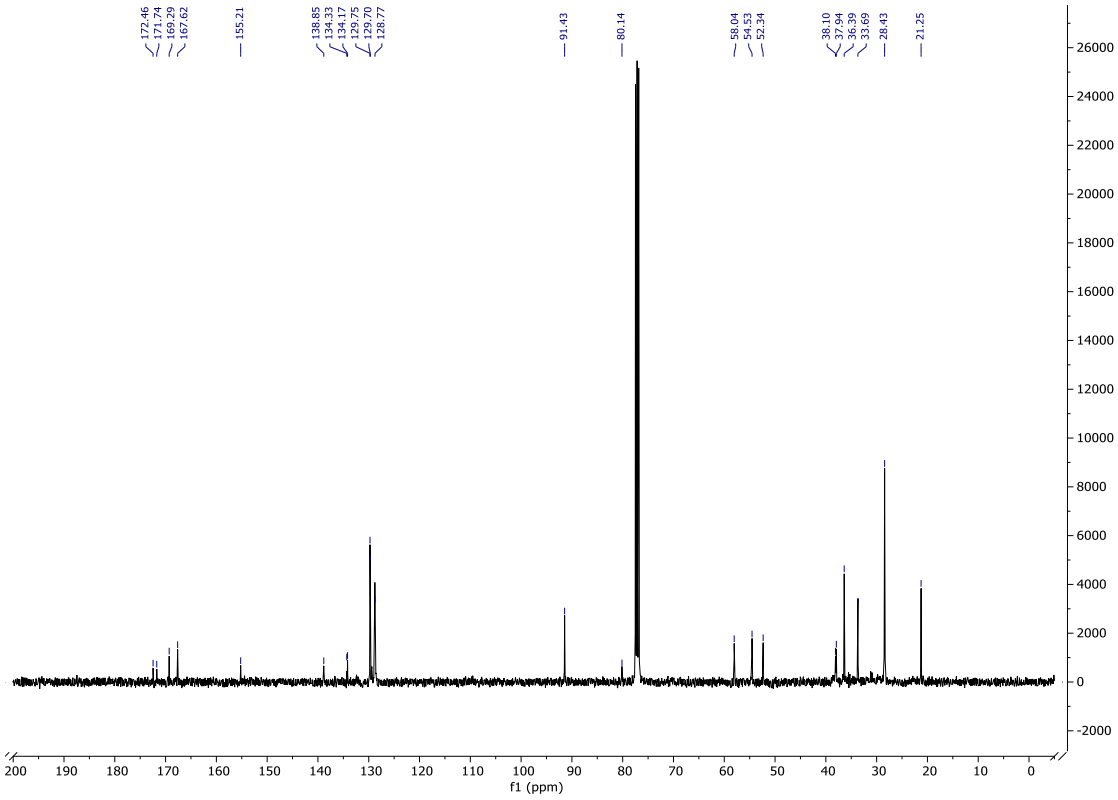
^{13}C NMR (101 MHz, $CDCl_3$) of 11a



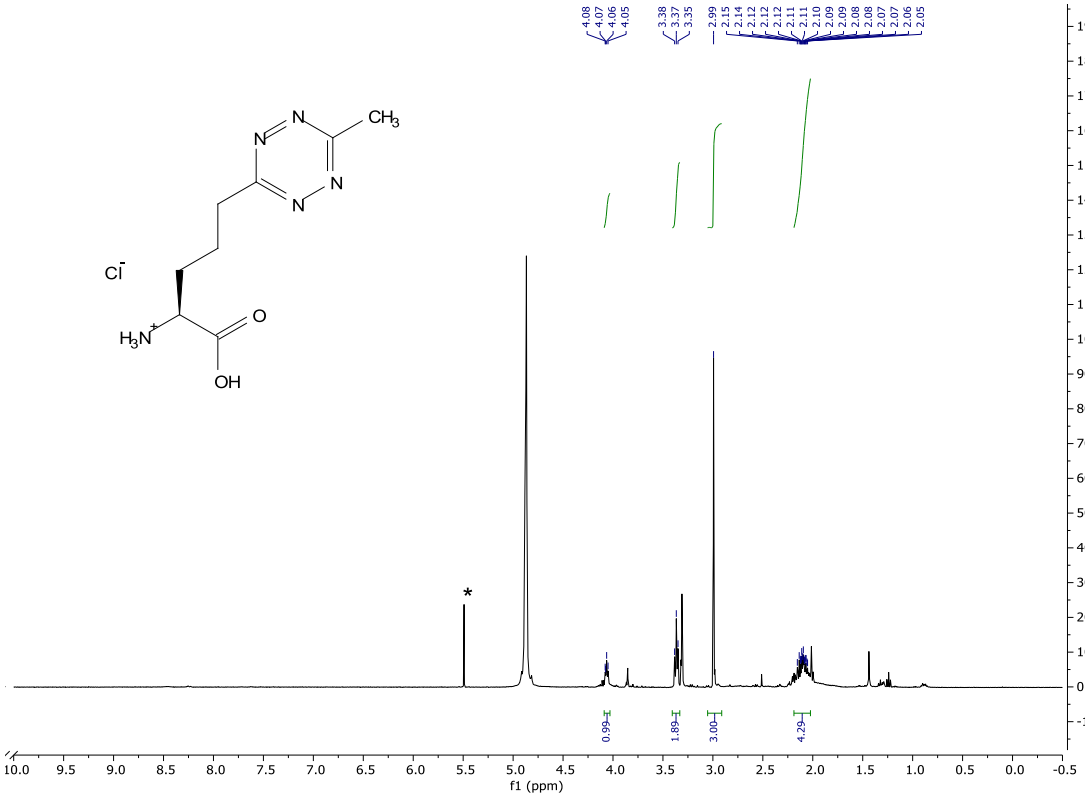
^{1}H NMR (400 MHz, $CDCl_3$) of 11b



^{13}C NMR (101 MHz, $CDCl_3$) of 11b

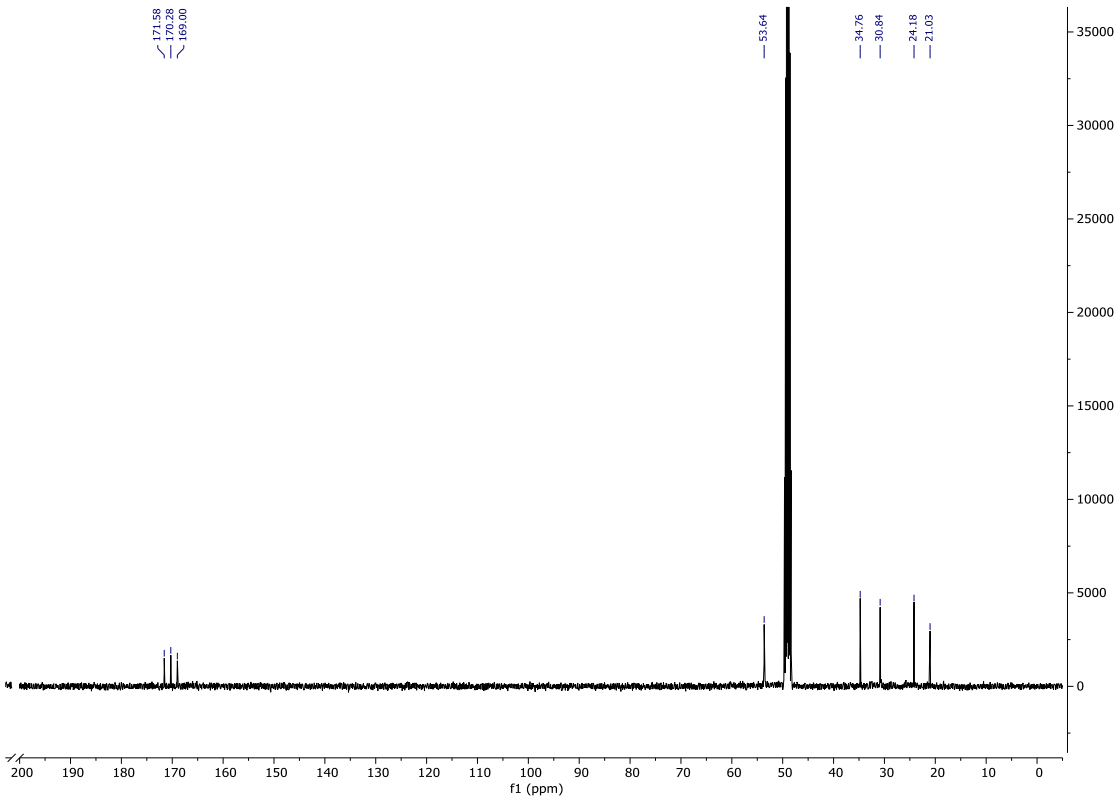


^{1}H NMR (400 MHz, CD_3OD) of 12a

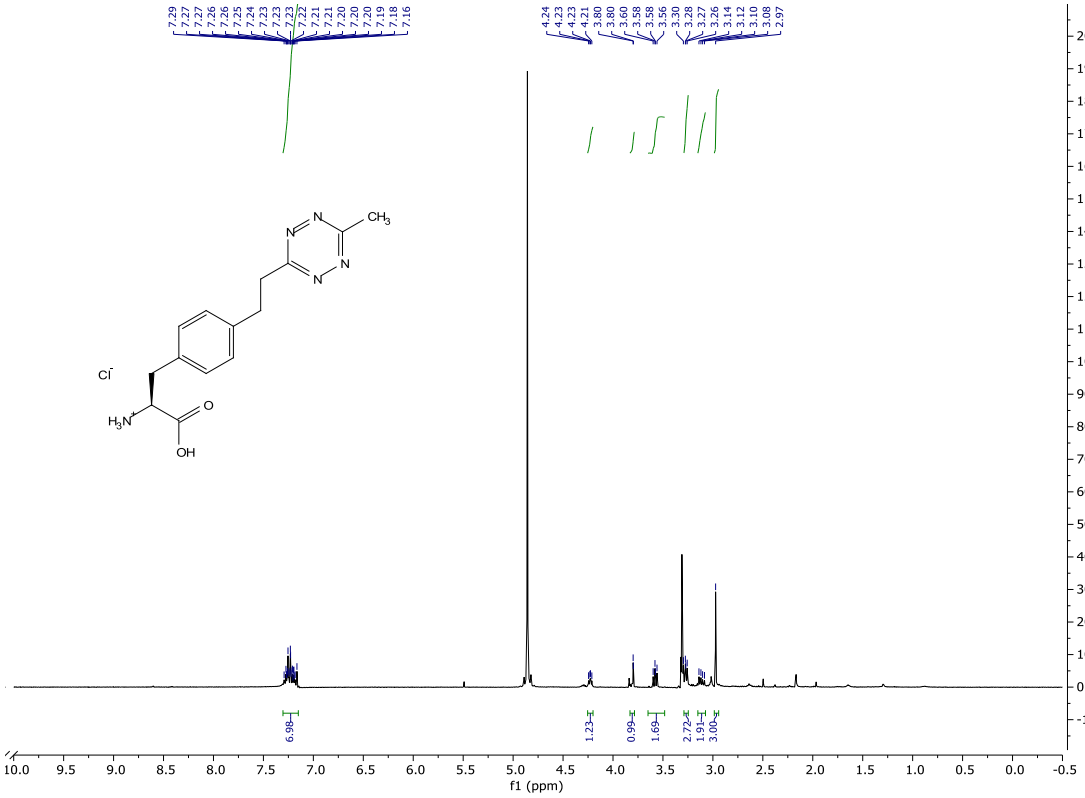


* = signals consistent with residual dichloromethane

^{13}C NMR (101 MHz, CD_3OD) of 12a



^{1}H NMR (400 MHz, CD_3OD) of 12b



^{13}C NMR (101 MHz, CD_3OD) of 12b