Supplementary Information for:

In silico peptide-directed ligand design complements experimental peptide-directed binding for protein-protein interaction modulator discovery.

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1. General Procedures

Reagents and Solvents

All chemicals were reagent grade and were purchased from Sigma Aldrich, Fisher Scientific and Tokyo Chemical Industry. Fmoc-amino acids and coupling reagents were purchased from Novabiochem or AGTC Bioproducts. Anhydrous solvents were bought from Sigma Aldrich and assumed to conform to specification.

Physical Characterisation and Spectroscopic Techniques

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz (^{1}H) or 100 MHz (^{13}C) using the specified deuterated solvent. The chemical shifts for both ^{1}H- and ^{13}C- were recorded in ppm and were referenced to the residual solvent peak of $CHCl_3$ at 7.26 ppm (^{1}H) and 77.0 ppm (^{13}C). Multiplicities in the NMR spectra are described as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, and combinations thereof; coupling constants are reported in Hz. Assignments, where conspicuous, have been confirmed by appropriate 2D NMR experiments. MALDI was performed on Kratos Analytical Axima MALDI-TOF. Low resolution mass spectra were recorded using a Shimadzu LCMS 2010EV operated under electrospray ionisation in positive (ES+) mode. Accurate mass spectra were recorded at the EPSRC National Mass Spectroscopy Service Centre, Swansea. Melting points were recorded using open capillary tubes on a Mel-Temp electrothermal melting point apparatus, melting points are uncorrected. Infra-red spectra were recorded using a PerkinElmer Spectrum BX with ATR attachment.

Chromatographic Techniques

Analytical RP-HPLC was performed on an Agilent 1200 using an Agilent eclipse XDB-C18 column, 4.6 x 150mm, 5µM and a flow rate of 1 mL/min. Solvent A = Water + 0.05% TFA and Solvent B = MeOH + 0.05% TFA. Gradient 5% B → 95% B over 20 min. Detection wavelength 214 nm and 254 nm.

Semi-preparative RP-HPLC was performed on an Agilent 1200 using an Agilent eclipse XDB-C18 column, 9.4 x 250mm, 5µM and a flow rate of 4 mL/min. Solvent A = Water + 0.05% TFA and Solvent B = MeOH + 0.05% TFA. Gradient 5% B → 95% B over 20 min. Detection wavelength 214 nm and 254 nm.

2. Protein Expression

Mcl-1

The Mcl-1 protein used in this study is the same construction as the Mcl-1 described by Yu and Wang.[1] amino acid residues 152-189 of mouse Mcl-1 fused with amino acid residues 209-327 of human Mcl-1. The plasmid was generously provided by Yu and Wang. The protein with an N-terminal 8 x His tag was expressed in *E. coli* BL21(DE3)pLysS cells. Cells were grown at 37 °C in LB medium containing 100 µg/mL ampicillin and 34 µg/mL chloramphenicol to an OD_{600} value at 0.6. Protein expression was induced by 0.4 mM IPTG at 30 °C for 4 h. Cells were lysed in 25 mM Tris-HCl, pH 8.0 buffer containing 300 mM NaCl, 5 mM βME and 0.1 mg/mL PMSF. His-TEV-Mcl-1 protein was purified from the soluble fraction using Ni-NTA resin (QIAGEN), following the manufacturer's instructions.

Bcl-2

The Bcl-2 protein used in our study has the same construction as that used in the work of Fesik *et al.*[2] (Bcl-2/Bcl-xL, isoform 2) which is composed of amino acid residues 1-34 of human Bcl-2, amino acid residues 29-44 of human Bcl-xL, and amino acid residues 92-207 of human Bcl-2, resulting in good solubility in water and maintaining the biological function of human Bcl-2.

GPLGSEFMAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDDVEENRTEAPEGTES
EVVHLTLRQAGDDFSRRYRRDFAEMSSQLHLTPFTARGRFATVVEELFRDGVNWGR
IVAFFEFGGVMCVESVNREMSPLVDNIALWMTEYLNRHLHTWIQDNGGWDAFVEL
YGPSMR.

Protein was purchased from Dundee Cell Product Ltd.

Bcl-xL

The Bcl-xL protein used in our study is a truncated construction of the full-length protein, with deletion of residues 45-84 on a large loop region and residues 210-233 at the C-terminal hydrophobic region, maintaining the biological function of Bcl-xL.

GPLGSEFMSQSNRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEGTESEAVKQAL
REAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVVNELFRDGVNWGRIVAFFSFG
GALCVESVDKEMQVLVSRIAAWMATYLNDHLEPWIQENGGWDTFVELYGNNAAA
ESRKGQER.

Protein was purchased from Dundee Cell Product Ltd.

3. Synthesis

General Click reaction of small molecules

Azide (1 equiv.), alkyne (1 equiv.), sodium ascorbate (2.5 eq), $CuSO_4.5H_2O$ (0.25 eq) and DMF (1 mL/0.5 mmol) were stirred together for 4 hours. The mixture was diluted with distilled water and extracted using diethyl ether. The organic layers were collected, washed exhaustively with water, dried with MgSO4, and evaporated under reduced pressure. Purification was achieved with semi-preparative RP-HPLC prior to testing.

Experimental Data

1 *methyl (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-(4-cyanobenzyl)-1H-1,2,3-triazol-4-yl)propanoate.*

M.P. 162 °C. ^{1}H NMR ($CDCl_3$); δ 7.75 (2H, m, 4′,5′H), 7.63-7.53 (2H, m, 1′,8′,3″,5″H), 7.44-7.36 (2H, m, 3′,6′H), 7.32-7.23 (5H, m, 2′,7′,2″,6″H,NCH), 5.78 (1H, d, ^3J = 8.2, NH), 5.56 (1H, d, ^2J = 15.6, Ph-C**H**$_A$H$_B$), 5.50 (1H, d, ^2J = 15.6, Ph-CH$_A$**H**$_B$), 4.69 (1H, m, 2H), 4.36 (2H, m, 9′-CH_2), 4.19 (1H, m, 9′H), 3.73 (3H, s, OCH_3), 3.28 (2H, m, 3-CH_2). ^{13}C NMR ($CDCl_3$); δ 171.5, 155.8, 143.8, 143.7, 141.3, 139.8, 132.8, 128.1, 127.7, 127.1, 125.1, 122.3, 120.01, 120.00, 118.0, 112.7, 67.1, 53.4, 53.3, 52.7, 47.1, 28.3. IR (neat): ν = 3420, 2953, 2362, 1705, 1517, 1343, 1210, 1049. LRMS (APCI): 508 (100, $[M+H]^+$). HRMS (ESI): $C_{29}H_{26}N_5O_4$ calcd. $[M+H]^+$ = 508.1979, found: 508.1967.

2 *methyl (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-benzyl-1H-1,2,3-triazol-4-yl)propanoate.*

M.P. 155 °C. ^{1}H NMR ($CDCl_3$); δ 7.76 (2H, m, 4′,5′H), 7.58 (2H, m, 1′,8′H), 7.43-7.18 (10H, m, NCH, Ph, 2′,3′,6′,7′H), 5.88 (1H, d, ^{3}J = 8.1, NH), 5.51 (1H, d, ^{2}J = 15, Ph-C**H$_A$**H$_B$), 5.46 (1H, d, ^{2}J = 15, Ph-CH$_A$**H$_B$**), 4.68 (1H, m, 2H), 4.35 (2H, d, ^{3}J = 7.2, 9′-CH_2), 4.20 (1H, t, ^{3}J = 7.2, 9′H), 3.69 (3H, s, OCH_3), 3.26 (2H, m, 3-CH_2). ^{13}C NMR ($CDCl_3$); δ 171.7, 143.9, 143.8, 141.3, 129.1, 128.8, 127.9, 127.7, 127.2, 127.1, 125.2, 121.8, 120.0, 67.0, 54.1, 52.6, 47.1, 28.2 (Fmoc carbonyl unobserved). IR (neat): *v* = 3421, 2362, 1743, 1719, 1517, 1443, 1349, 1204, 1049, 1006. LRMS (APCI): 483 (100, $[M+H]^+$). HRMS (ESI): $C_{28}H_{27}N_4O_4$ calcd. $[M+H]^+$ = 483.2027, found: 483.2017.

3 *methyl (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)propanoate.*

M.P. 159 °C. ^{1}H NMR ($CDCl_3$); δ 7.76 (2H, m, 4′,5′H), 7.58 (2H, m, 1′,8′H), 7.43-7.35 (2H, m, 3′,6′H), 7.32-7.28 (2H, m, 2′,7′H), 7.24 (1H, s, NCH), 7.15-7.07 (4H, m, 2″,3″,5″,6″H), 5.88 (1H, d, ^{3}J = 8.1, NH), 5.46 (1H, d, ^{2}J = 14.8, Ph-C**H$_A$**H$_B$), 5.41 (1H, d, ^{2}J = 14.8, Ph-CH$_A$**H$_B$**), 4.67 (1H, m, 2H), 4.35 (2H, d, ^{3}J = 8.1, 9′-CH_2), 4.19 (1H, t, ^{3}J = 8.1, 9′H), 3.70 (3H, s, OCH_3), 3.26 (2H, m, 3-CH_2), 2.30 (3H, s, 4″-CH_3). ^{13}C NMR ($CDCl_3$); δ 171.9, 143.8, 143.7, 141.3, 138.8, 131.3, 129.8, 128.0, 127.7, 127.1, 125.2, 122.0, 119.9, 67.1, 54.1, 53.4, 52.6, 47.1, 28.1, 21.1 (Fmoc carbonyl unobserved). IR (neat): *v* = 3342, 2968, 2362, 1747, 1693, 1521, 1435, 1260, 1214, 1083, 1041. LRMS (APCI): 497 (100, $[M+H]^+$). HRMS (ESI): $C_{29}H_{29}N_4O_4$ calcd. $[M+H]^+$ = 497.2183, found: 497.2172.

4 *methyl (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)propanoate.*

M.P. 129 °C. ^{1}H NMR ($CDCl_3$); δ 7.75 (2H, m, 4′,5′H), 7.57 (2H, m, 1′,8′H), 7.39 (2H, m, 3′,6′H), 7.32-7.25 (5H, m, 2′,7′,3″,5″H,NCH), 7.14 (2H, m, 2″,6″H), 5.84 (1H, d, ^{3}J = 7.3, NH), 5.47 (1H, d, ^{2}J = 15.2, Ph-C**H$_A$**H$_B$), 5.41 (1H, d, ^{2}J = 15.2, Ph-CH$_A$**H$_B$**), 4.68 (1H, m, 2H), 4.35 (2H, m, 9′-CH_2), 4.20 (1H, m, 9′H), 3.72 (3H, s, OCH_3), 3.27 (2H, m, 3-CH_2). ^{13}C NMR ($CDCl_3$); δ 171.8, 155.9, 143.8, 143.7, 141.3, 134.9, 132.8, 129.3, 129.2, 127.7, 127.1, 125.1, 122.1, 120.0, 67.1, 53.5, 53.4, 52.7, 47.1, 28.2. IR (neat): *v* = 3351, 2362, 1747, 1694, 1520, 1217, 1028. LRMS (APCI): 517 (100, $[M+H]^+$), 519 (37, $[M+2+H]^+$). HRMS (ESI): $C_{28}H_{26}ClN_4O_4$ calcd. $[M+H]^+$ = 517.1637, found: 517.1625.

5 *methyl (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)propanoate.*

M.P. 152 °C. ^{1}H NMR ($CDCl_3$); δ 7.75 (2H, m, 4′,5′H), 7.58 (2H, m, 1′,8′H), 7.43-7.26 (6H, m, 2′,3′,6′, 7′H,4″H,NCH), 7.20 (2H, m, 3″H), 7.05-7.15 (2H, m, 5″,6″H), 5.86 (1H, d, ^{3}J = 8.0, NH), 5.54 (2H, s, N-CH_2), 4.69 (1H, m, 2H), 4.35 (2H, d, ^{3}J = 7.3, 9′-CH_2), 4.20 (1H, t, ^{3}J = 7.3, 9′H), 3.70 (3H, s, OCH_3), 3.26 (2H, m, 3-CH_2). ^{13}C NMR ($CDCl_3$) δ 171.6, 161.7, 159.3, 155.9, 143.9, 141.3, 131.0, 130.4, 127.7, 127.1, 125.2, 124.8, 122.0, 119.9, 115.9, 115.7, 67.1, 53.4, 52.5, 47.7, 47.1, 28.2. IR (neat): *v* = 3368, 3070, 2632, 1749, 1686, 1515, 1217, 1040. LRMS (APCI): 501 (100, $[M+H]^+$). HRMS (ESI): $C_{28}H_{26}FN_4O_4$ calcd. $[M+H]^+$ = 501.1920, found: 501.1933.

6 *methyl (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)propanoate.*

M.P. 144°C. ^{1}H NMR ($CDCl_3$); δ 7.75 (2H, m, 4′,5′H), 7.58 (2H, m, 1′,8′H), 7.39 (6H, m, 3′,6′H), 7.34-7.26 (3H, m, 2′,7′H,NCH), 7.24-7.15 (2H, m, 3″,5″H), 7.05-7.15 (2H, m, 4″,6″H),

5.87 (1H, d, ^{3}J = 8.8, NH), 5.52 (1H, d, ^{2}J = 15.6, Ph-C**H**$_{A}$H$_{B}$), 5.47 (1H, d, ^{2}J = 15.6, Ph-CH$_{A}$**H**$_{B}$), 4.68 (1H, m, 2H), 4.34 (2H, m, 9′-CH$_2$), 4.20 (1H, m, 9′H), 3.70 (3H, s, OCH$_3$), 3.24 (2H, m, 3-CH$_2$), 2.24 (3H, s, 2″CH$_3$). ^{13}C NMR ($CDCl_3$); δ 171.6, 155.9, 143.9, 143.8, 141.3, 136.9, 132.6, 131.0, 129.2, 129.1, 127.7, 127.1, 126.6, 125.2, 119.9, 67.2, 52.5, 52.4, 47.1, 28.2, 18.9. IR (neat): *v* = 2929, 2355, 1717, 1506, 1197, 1058. LRMS (APCI): 497 (100, $[M+H]^+$). HRMS (ESI): $C_{29}H_{29}N_4O_4$ calcd. $[M+H]^+$ = 497.2183, found: 497.2169.

7 *(2R,3R,4S,5R)-2-(4-(4-chlorophenyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate.*

Amorphous solid. ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 1H), 5.87 – 5.80 (m, 1H), 5.47 – 5.41 (m, 1H), 5.18 (d, *J* = 4.1 Hz, 1H), 4.33 (dd, *J* = 11.7, 5.6 Hz, 1H), 3.67 – 3.57 (m, 1H), 2.09 (s, 3H), 2.07 (s, 3H), 1.90 (s, 2H). IR (neat): 2875, 2687, 1753, 1734, 1704, 1474, 1430. LRMS (APCI); 438 (100, $[M+H]^+$), 440 (33, $M+2+H]^+$). HRMS (ESI): $C_{19}H_{21}ClN_3O_7$ calcd. $[M+H]^+$ = 438.1068, found : 438.1062.

8 *(2R,3R,4S,5R)-2-(4-(4-bromophenyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate.*

Amorphous solid. ^{1}H NMR ($CDCl_3$) δ 7.97 (s, 1H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 5.86 – 5.80 (m, 1H), 5.47 – 5.41 (m, 2H), 5.19 (m, 1H), 4.33 (dd, *J* = 11.6, 5.7 Hz, 1H), 3.67 – 3.57 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 1.90 (s, 2H). IR (neat): 2815, 2698, 1742, 1738, 1711, 1425, 1400. LRMS (APCI); 482 (100, $[M+H]^+$), 484 (98, $M+2+H]^+$). HRMS (ESI): $C_{19}H_{21}BrN_3O_7$ calcd. $[M+H]^+$ = 482.0653, found : 482.0647.

4. In vitro biophysical assays

Fluorescence Anisotropy Binding Assay

Synthesis of peptides for fluorescence anisotropy assay, and procedure for assay performed as described previously.[3] Compounds were initially screened at 100 μM and 10 μM. Compounds which showed activity in initial screens were subjected to a dose response analysis.

Thermal Shift Assay

A solution of protein (10 μM) and detection dye SYPRO orange (5X) and compound (30 μM) or vehicle (10% final DMSO concentration) were added to rotor gene-Q RT-PCR sample tubes, in the presence of buffer (final volume 20 μL). A buffer screen was performed to determine the most ideal buffer to observe a thermal stability effect between Mcl-1 and DMSO. The screened buffers included sodium acetate pH 5.0, pyridine pH 5.5, sodium cacodylate pH 6.0, MES pH 6.5, sodium citrate pH 6.5, imidazole pH 7.0, bis-tris pH 7.2, MOPS pH 7.2, HEPES pH 7.5, sodium phosphate pH 7.5, tricine pH 8.0, tris pH 8.0, glycine pH 9.0, borax pH 9.0, CHES pH 9.5, ethanolamine pH 9.5. buffers contained 50 mM of buffer compound, 1 mM TCEP and 150 mM NaCl. Sodium cacodylate was highlighted as the most appropriate buffer. Samples were incubated for 30 mins at 25°C then scanned at a ramp of 0.5°C/min from 25°C to 95°C. The fluorescence intensity at an excitation of 465 nm and emission of 580 nm was recorded. All measurements were run in triplicate. The first derivate of the melting curve was analysed to identify the inflexion point, which correlated to the melting temperature.

5. Molecular Docking

The X-ray crystal structure of Mcl-1 with a modified Noxa ligand[4] (PDB ID 2NLA) was utilized for docking calculations. The ligand was removed and the protein was prepared using the Protein Preparation application from the Schrodinger Suite 2015-2.[5] The bound peptide was modified using Maestro drawing tools, and the resulting receptor was again prepared using Protein Preparation. Ligands were taken from the Sigma-Aldrich virtual library (214 azides and 896 alkynes) and prepared using the LigPrep application from the Schrodinger Suite 2015-2. Binding site grid generation was preformed using the Receptor Grid Generation application from the Schrodinger 2015-2 suite using the using the pocket left vacant by the removed section of the peptide, using the centre point of residues Arg224, Cys231 and His263 for the alkyne ligands, or the centre point of residues Asn223, Phe318 and Val 321 for the azide ligands. Covalent docking was performed using the CovDock tool from the Schrodinger Suite 2015-2,[6,7] using the following custom reaction code to generate the desired azido-alkynyl condensation.

Alkyne receptor, azide ligand:

```
LIGAND_SMARTS_PATTERN 4,CN=[N+]=[N-]
RECEPTOR_SMARTS_PATTERN 2,C#CCC=O
CUSTOM_CHEMISTRY ("<1>|<2>",("bond",1,(1,2)))
CUSTOM_CHEMISTRY ("<1>#C",("bond",2,(1,2)))
CUSTOM_CHEMISTRY ("<1>[N-]=[N+]=NC",("bond",1,(3,4)))
CUSTOM_CHEMISTRY ("<1>(=C)[N-]=[N+]N",("bond",1,(2,5)))
```

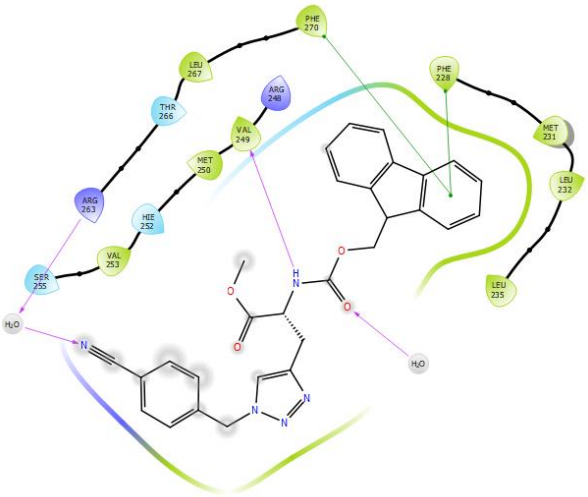
Azide receptor, alkyne ligand:

```
RECEPTOR_SMARTS_PATTERN 4,CN=[N+]=[N-]
LIGAND_SMARTS_PATTERN 2,C#C
CUSTOM_CHEMISTRY ("<1>",("charge", 0, 1))
CUSTOM_CHEMISTRY ("<1>=[N+]",("charge", 0, 2))
CUSTOM_CHEMISTRY ("<1>|<2>",("bond",1,(1,2)))
CUSTOM_CHEMISTRY ("<2>#C",("bond",2,(1,2)))
CUSTOM_CHEMISTRY ("<1>=[N]=[N]",("bond",1,(2,3)))
CUSTOM_CHEMISTRY ("<1>[N]",("bond",2,(1,2)))
CUSTOM_CHEMISTRY ("<1>([C]=[C])=[N][N]",("bond",1,(3,5)))
```

Induced fit docking was performed with protein flexibility using the Induced Fit[8] application from the Schrodinger Suite 2015-2. A total of 100 possible binding conformations were generated and grouped into clusters using a 1.0 Å root-mean-square tolerance. Conformations were then ranked based on their Glide e-model scores.[9] Figures were generated using the Chimera package from the Computer Graphics Laboratory, University of California, San Francisco (supported by NIH P41 RR-01081).

Predicted 2D ligand interaction diagrams

1



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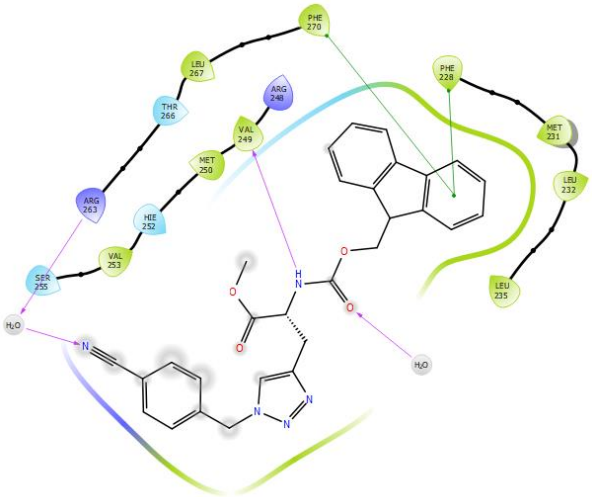

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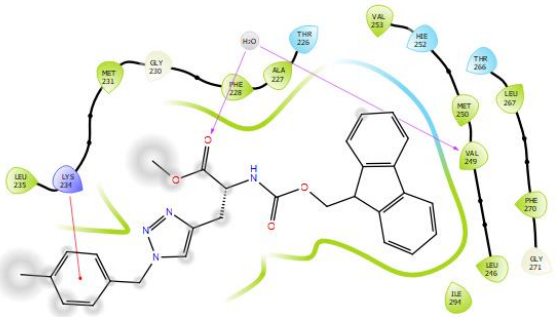

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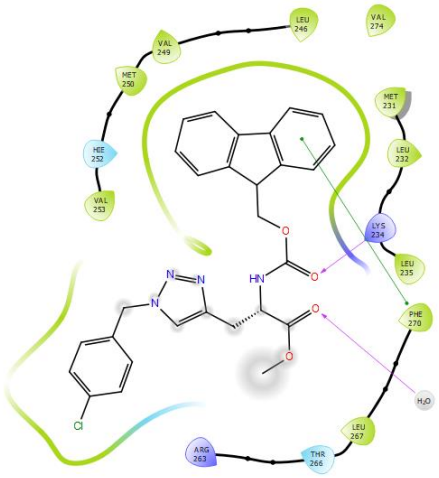

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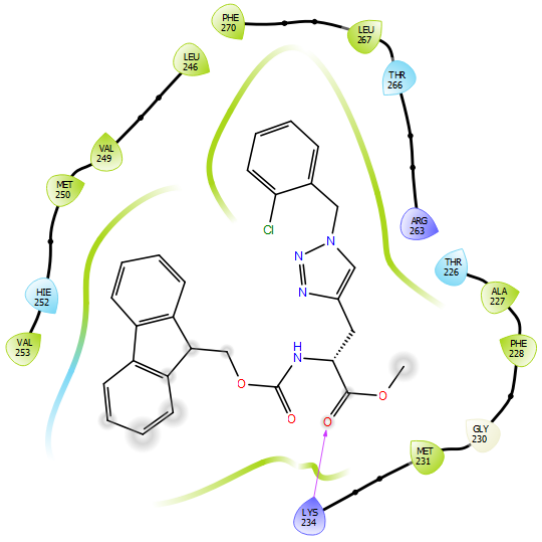

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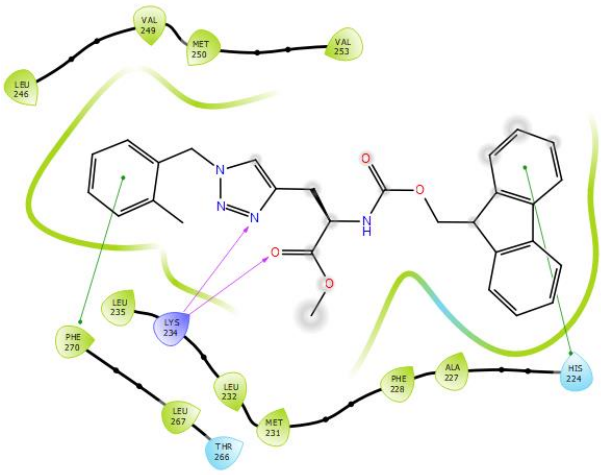
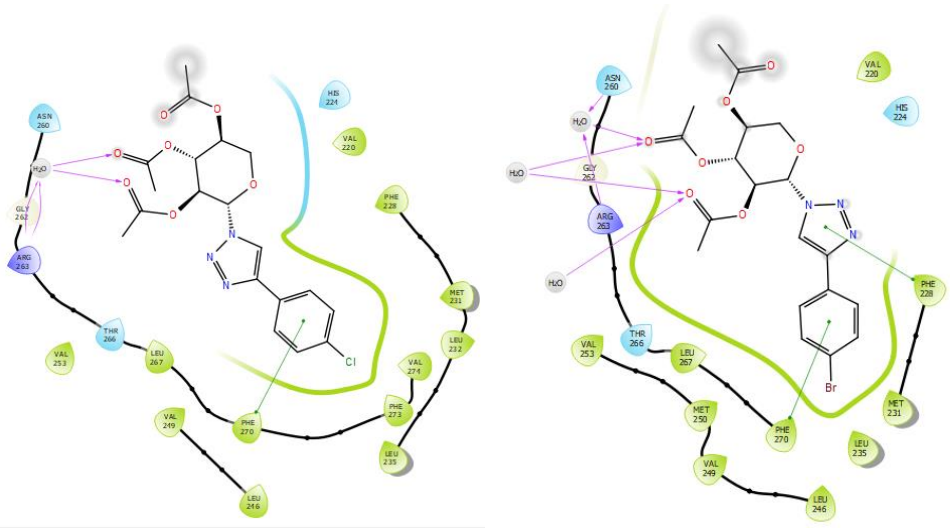


7 **8**



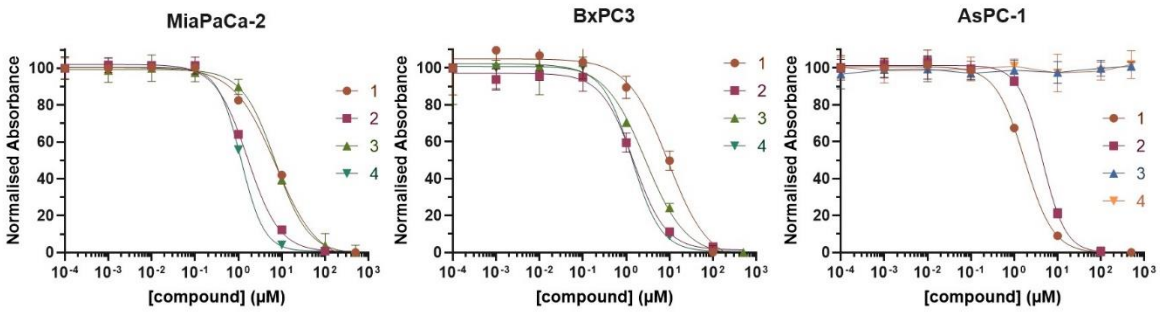
6. Cell Culture

The MiaPaCa-2 cell line, BxPC-3 cell line and AsPC-1 cell line were purchased from the European Collection of Cell Cultures (ECACC, Porton Down, UK). MiaPaCa-2 cells were cultured in DMEM media with 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and 10% FCS. BxPC-3 cells and AsPC-1 cells were cultured in RPMI-1640 media containing 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and 10% FCS. Cells were sub-cultured twice weekly and maintained at 37 °C and 5% CO2.

7. Cellular assay

MTS Cytotoxicity Assay

Antiproliferative activity was determined by MTS assay using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) and following the manufacturer's instructions. Briefly, cells (5 x 10^3 /100 µL) were seeded in 96-well plates and left untreated or treated with DMSO (vehicle control), compounds **1-8** (100 µM, 10 µM), or doxorubicin hydrochloride (10 µM), in triplicate, and allowed to incubate for 72 hr at 37 °C with 5% CO_2. Following this, MTS assay reagent was added for 4 hrs and the absorbance measured at 490 nm using the Polarstar Optima microplate reader (BMG Labtech). Doxorubicin demonstrated toxicity to all cell lines and this was used as the 100% inhibition of cell proliferation value. Compounds **5-8** showed no discernible difference to the growth and DMSO controls at all tested concentrations. Subsequently, cells were seeded at 5 x 10^3 / 100 µL in a 96-well plate and left untreated or treated with DMSO (vehicle control), or compounds **1-4** (100 µM – 10 pm, in well concentration) in triplicate for 72 h at 37 °C with 5% CO_2. Following this, MTS assay reagent was added to each well and the plates incubated for 4 h at 37 °C with 5% CO_2. The absorbance was measured at 492 nm using the BMG Labtech POLARstarOPTIMA microplate reader. IC_{50} values were calculated using GraphPad Prism Version 6.0 software, using a four-parameter logistic non-linear regression model. Errors are the 95% confidence intervals.



TMRE assay

Mitochondrial membrane potential was measured with a TMRE (tetramethylrhodamine ethyl ester) assay. BxPC-3 cells were incubated with compounds **1-4** (10 μM) for 2 hours. The positive control FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone), an uncoupler of mitochondrial oxidative phosphorylation, was applied at the concentration of 5 μM for 10 min. The cells were incubated with 500 μM TMRE for 30–45 min at 37˚C, 5% CO_2, followed by washing once with 100 μl of PBS containing 0.2% bovine serum albumin. A volume of 200 μl of PBS containing 0.2% bovine serum albumin was added to each well, and the fluorescence was measured in the CLARIOstar plate reader (BMG Labtechnologies) with excitation/emission: 544/ 590 nm.

8. References

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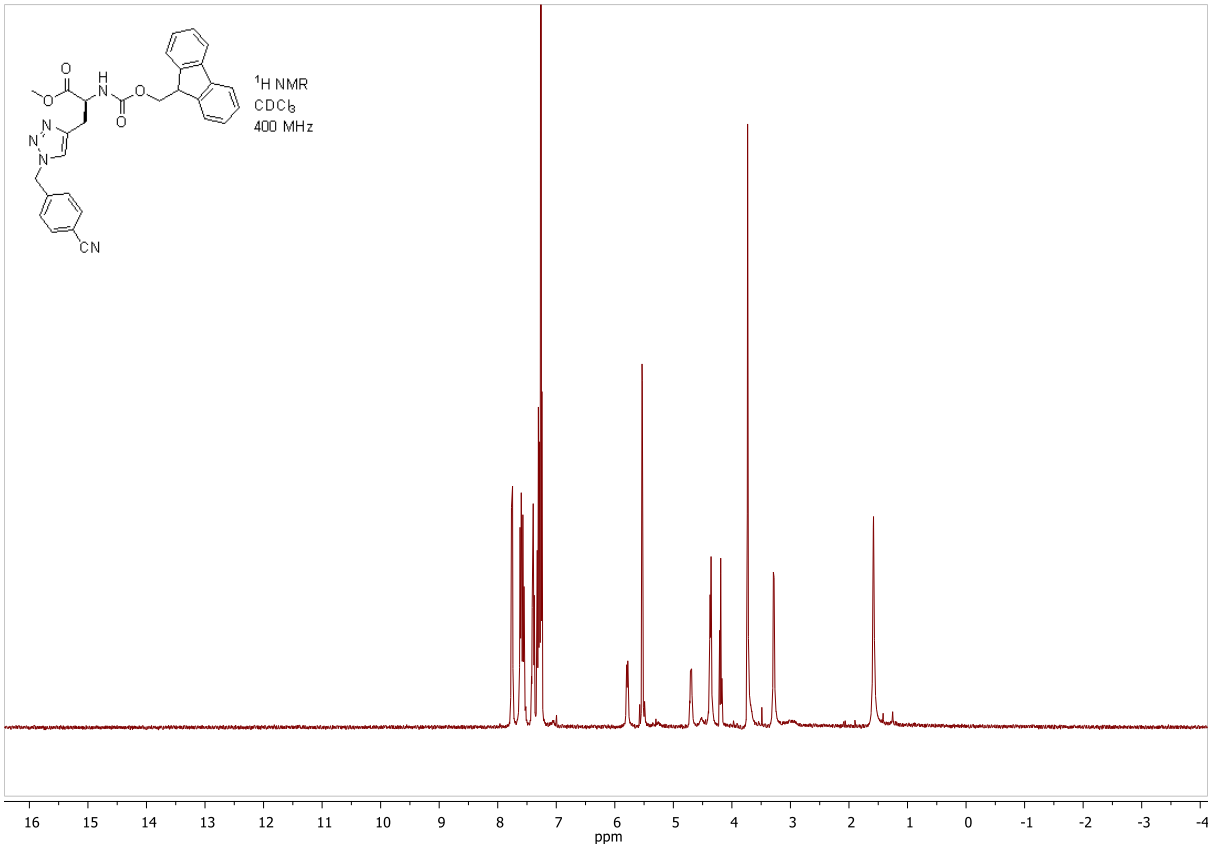
9. Spectra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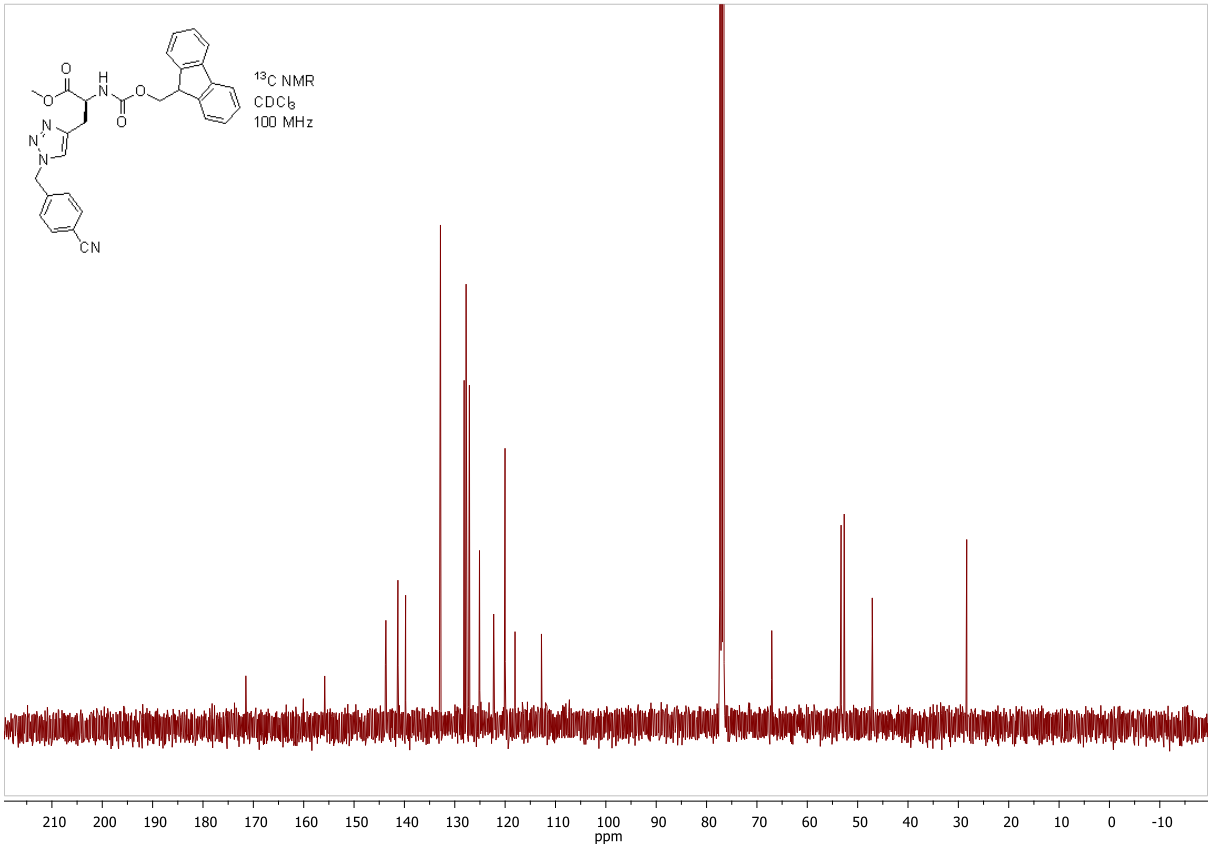
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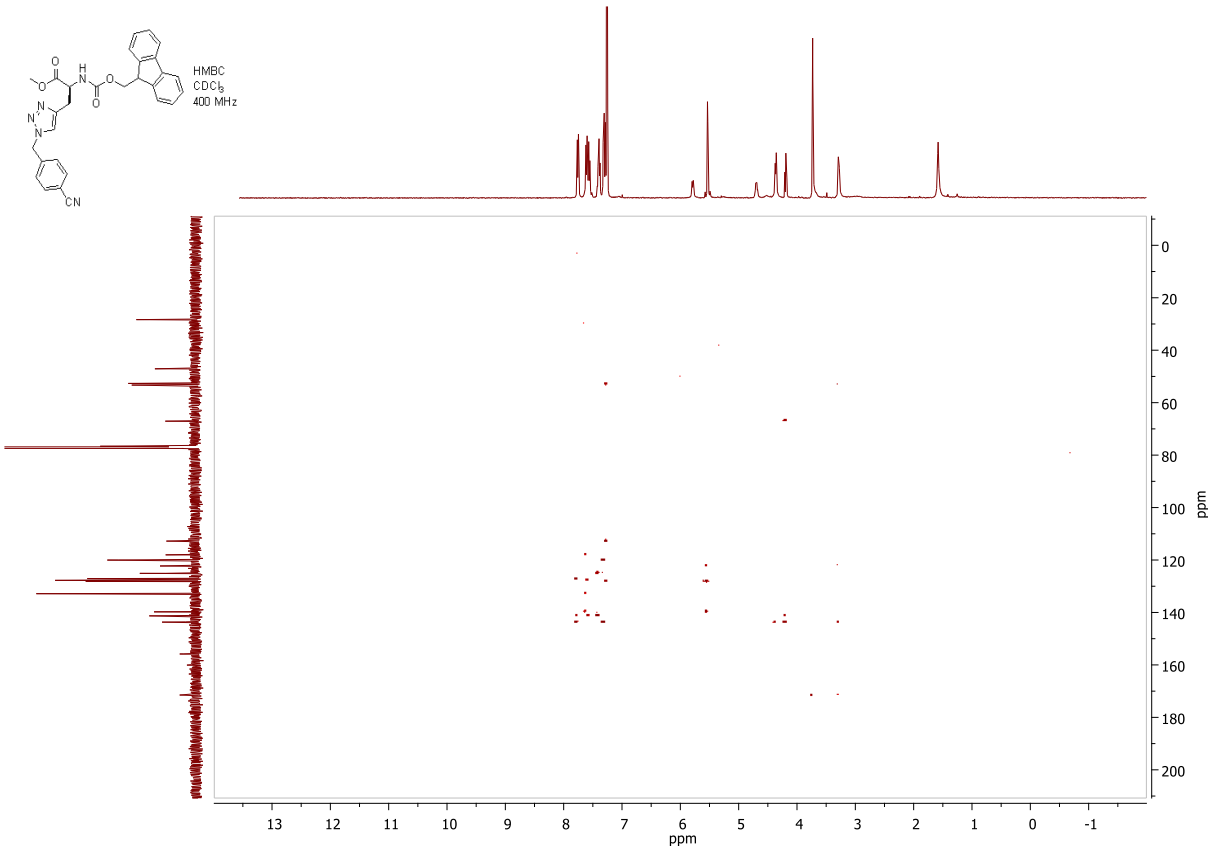
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4
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0
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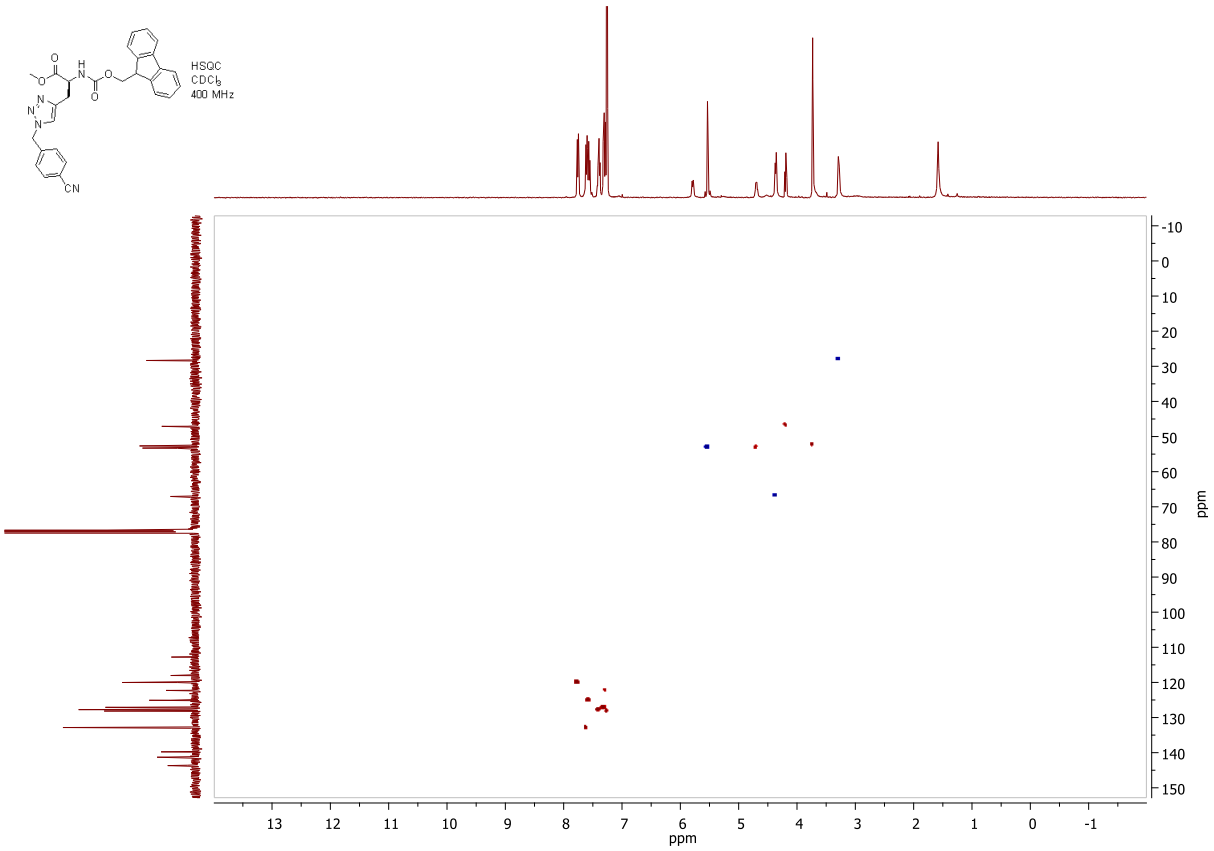
ppm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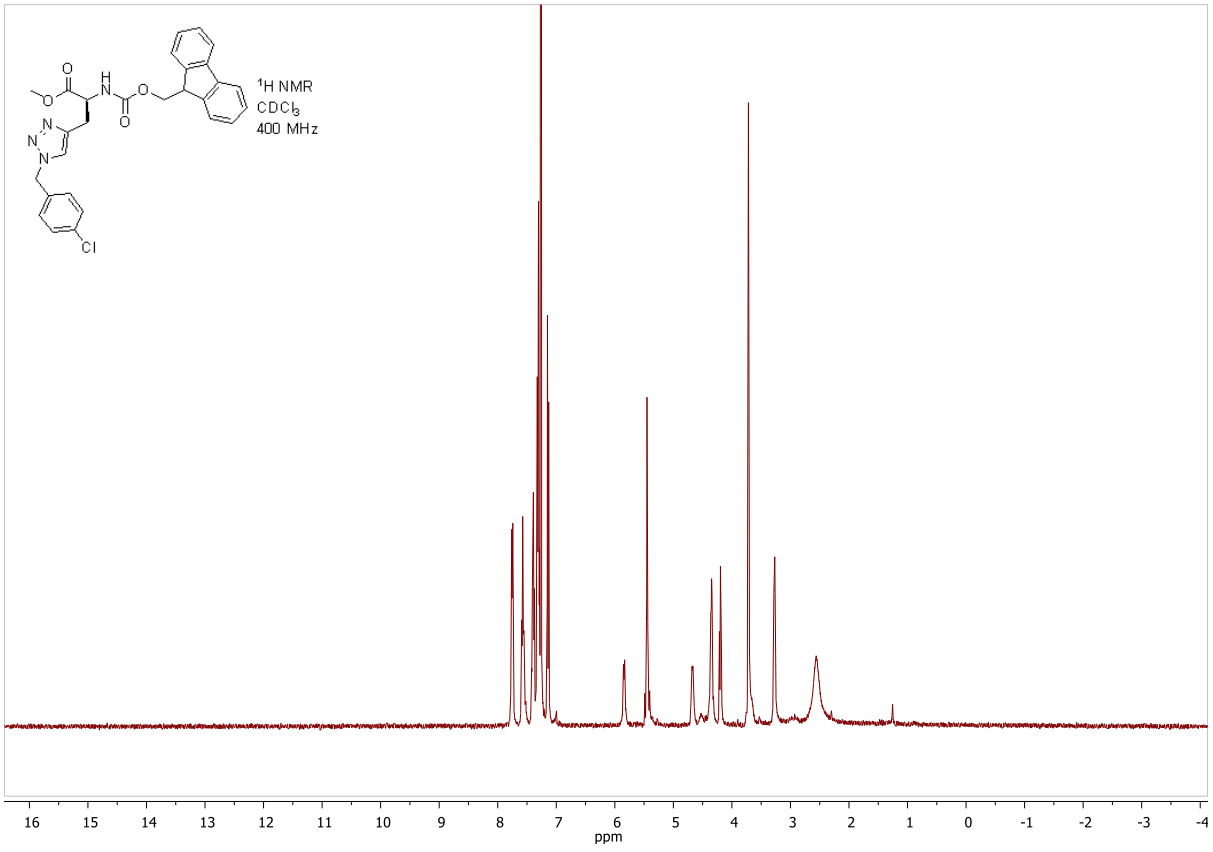
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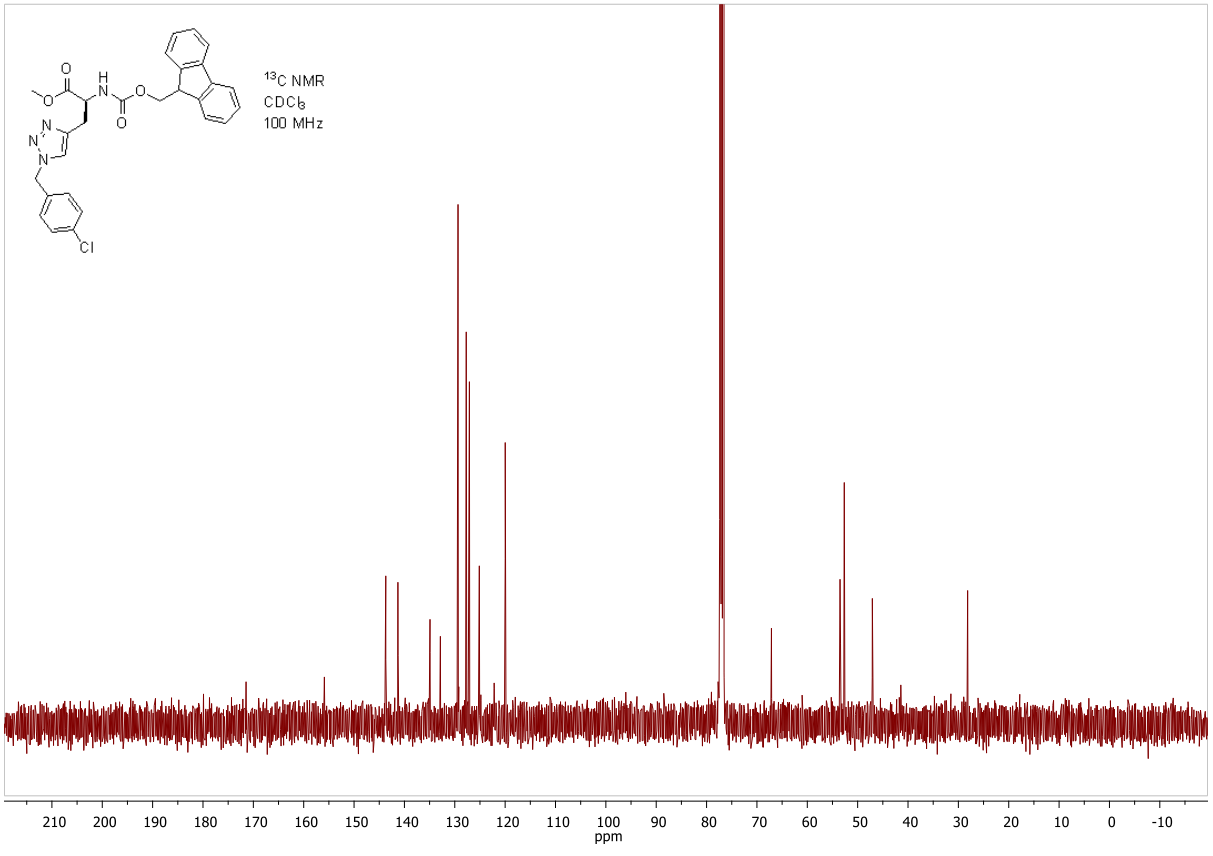
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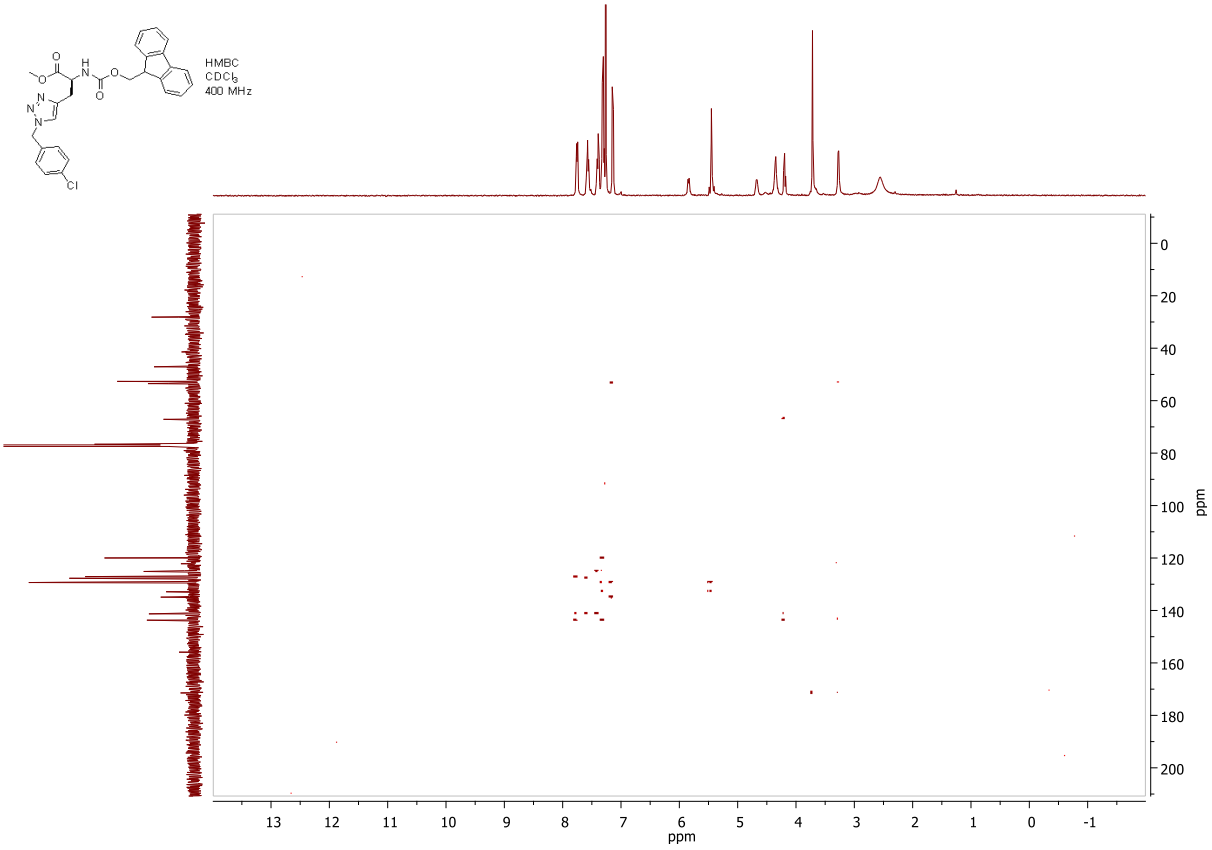
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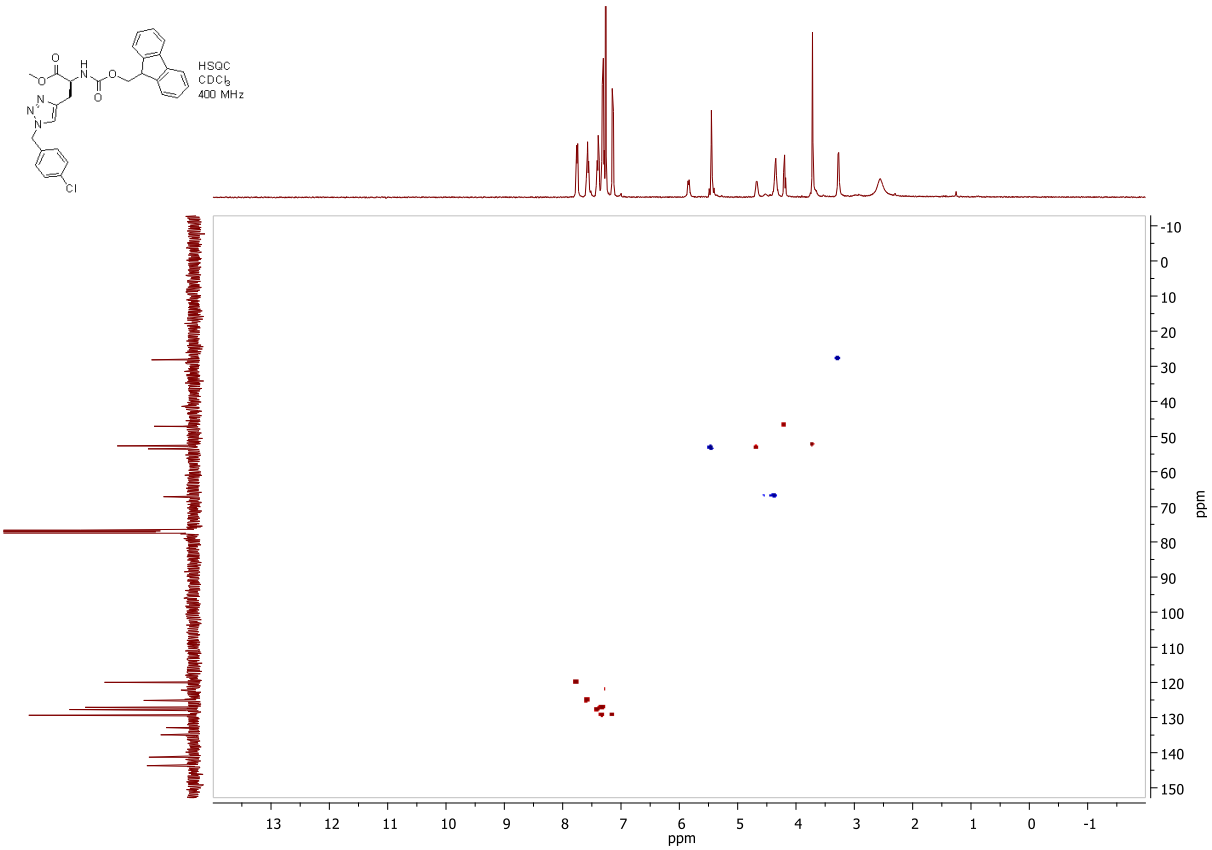
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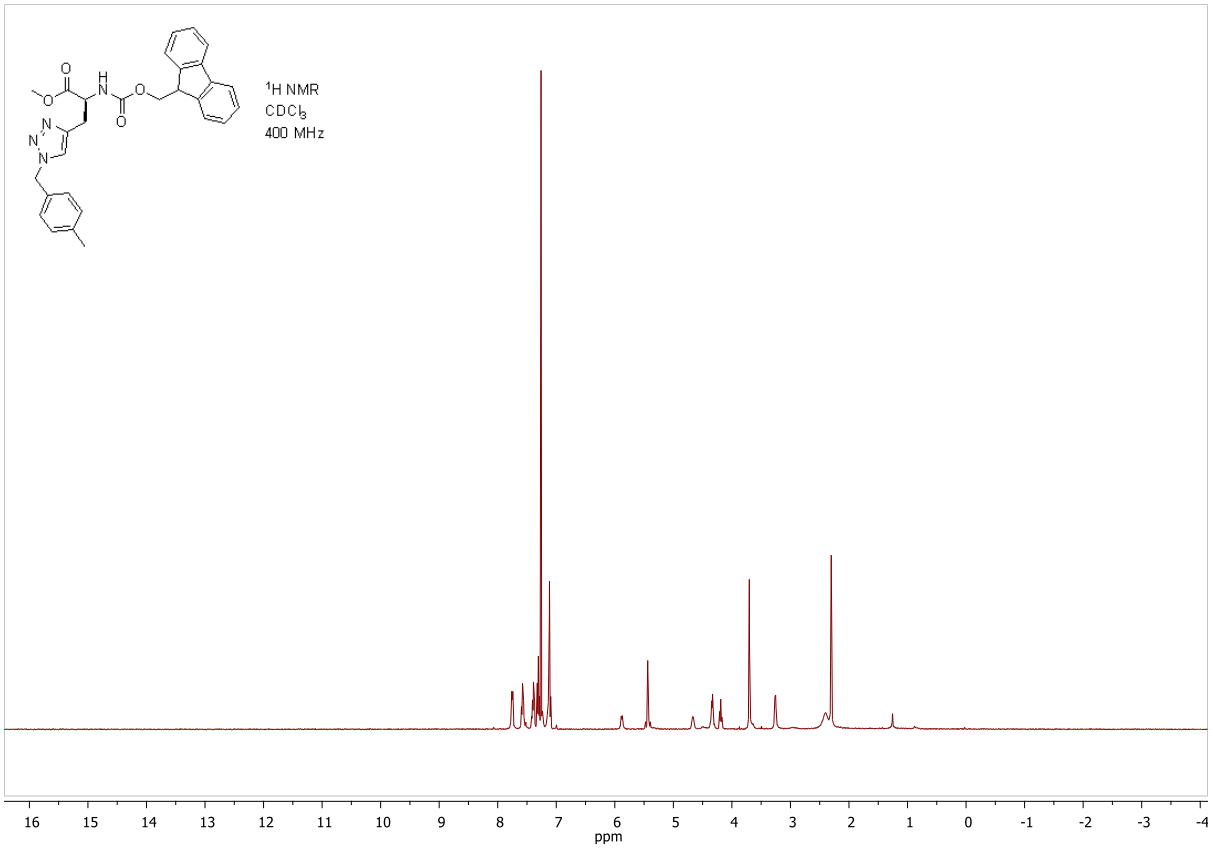
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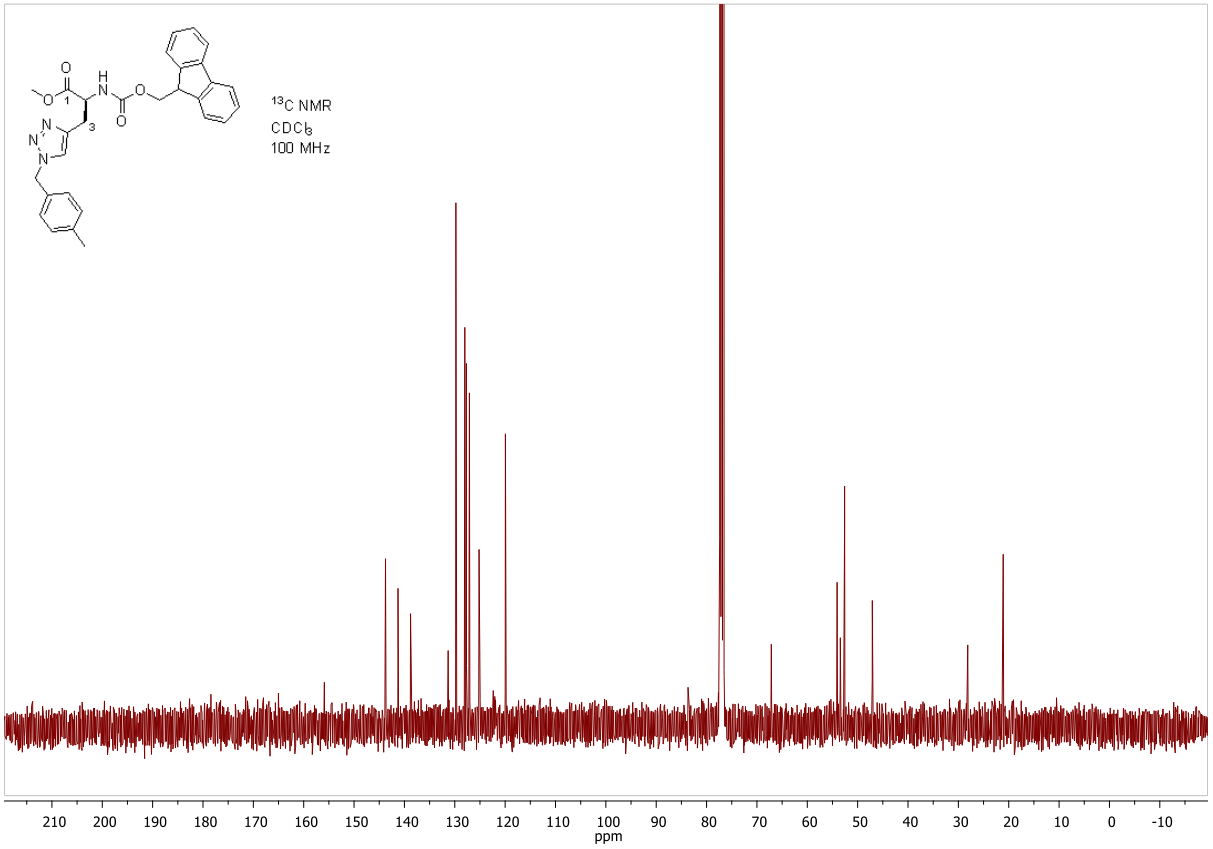
ppm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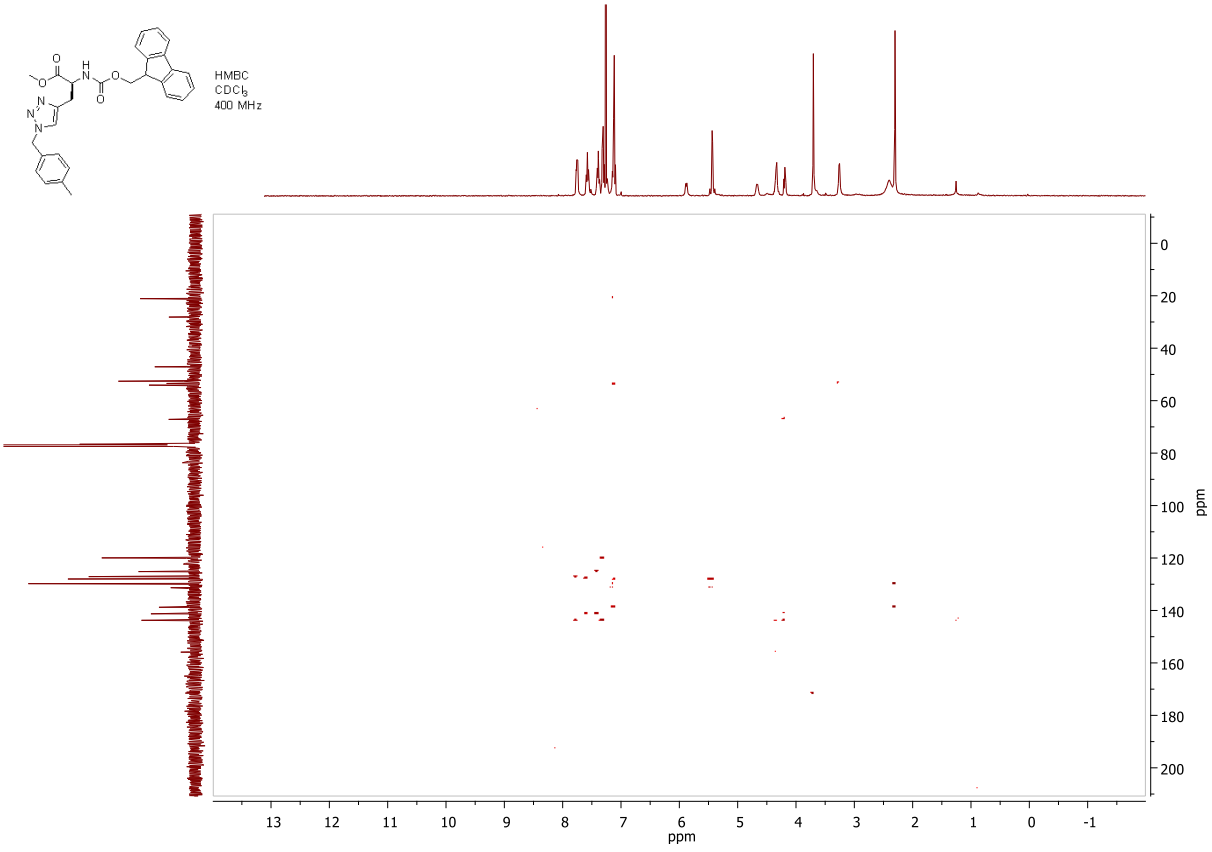
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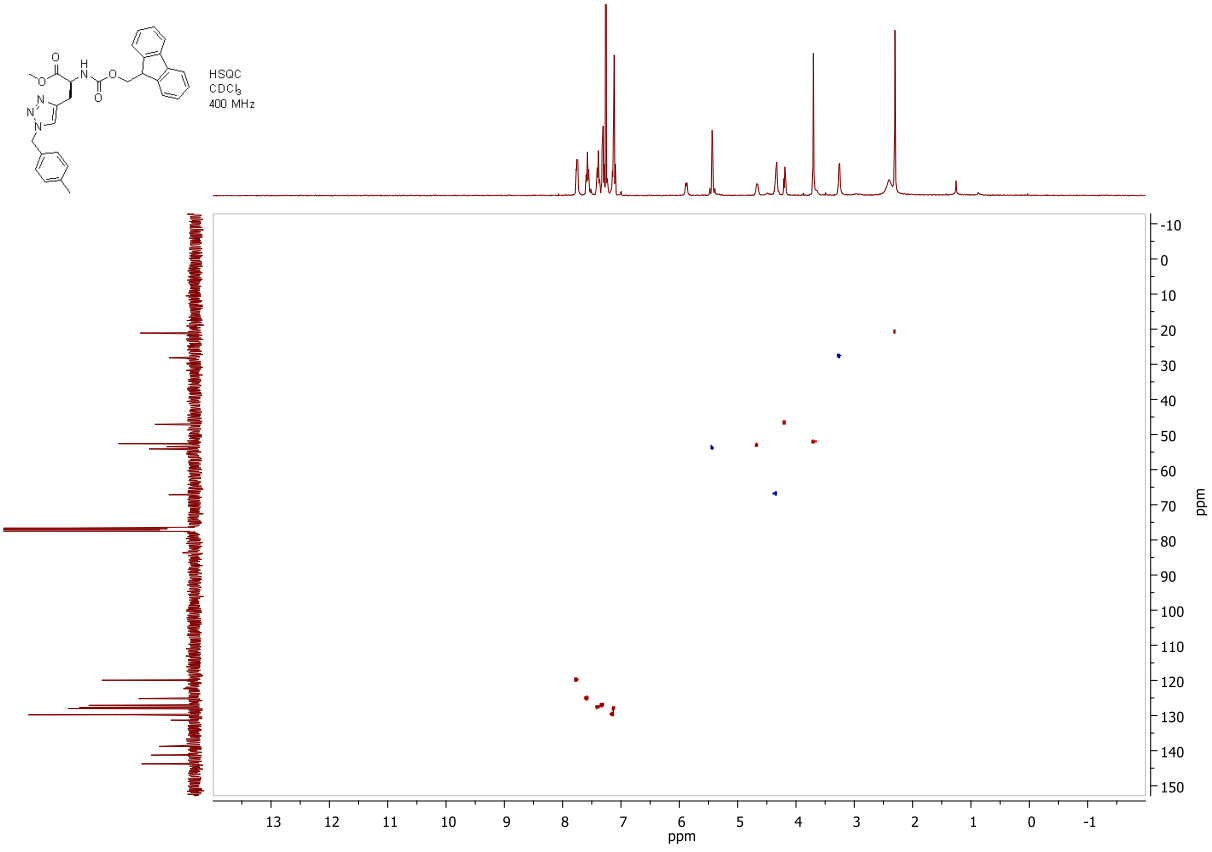
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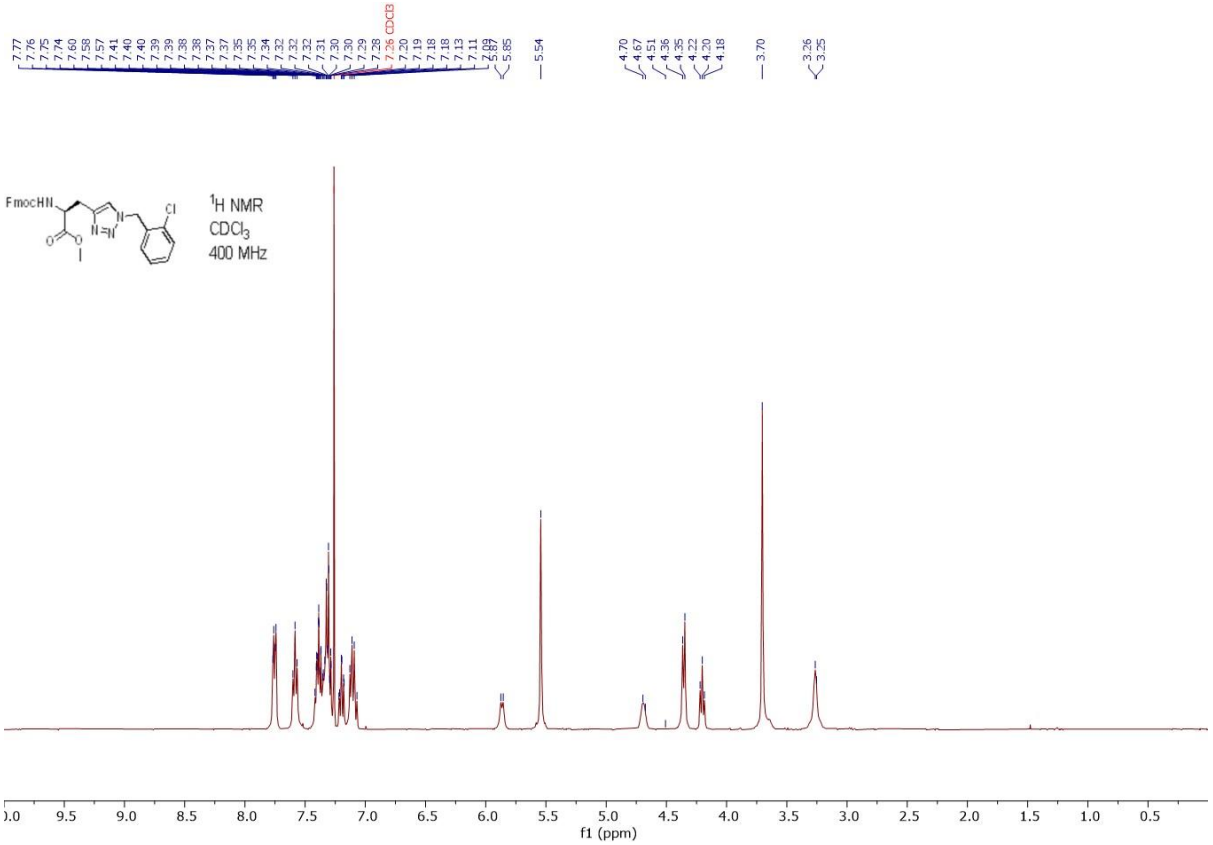
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FmocHN
Cl
1H NMR
CDCl3
400 MHz
f1 (ppm)
171.6 161.7 159.3 155.9 143.9 141.3 131.0 130.5 127.7 127.1 125.2 124.8 122.0 120.0 115.9 115.7 77.3 CDCl3 76.7 CDCl3 67.1 53.4 52.6 47.8 47.1 28.2
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Cl
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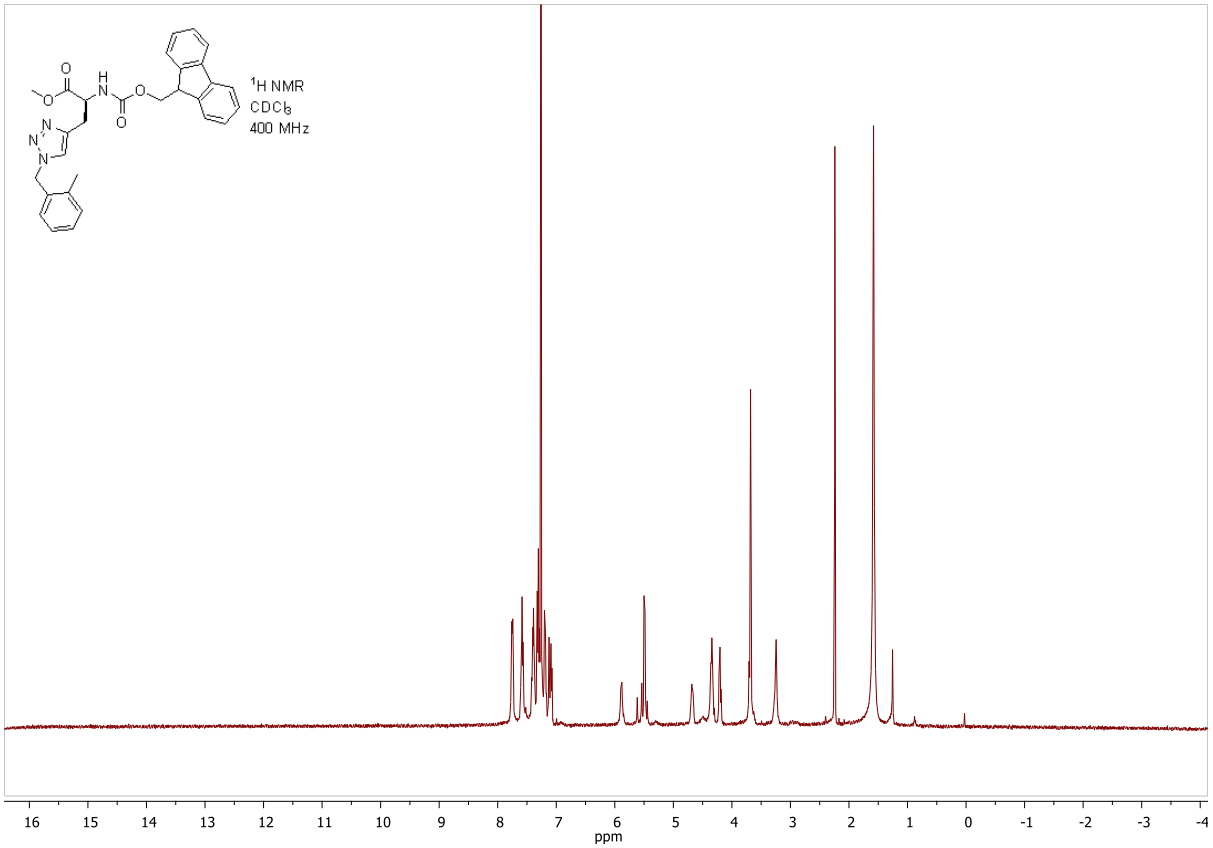
100 MHz
f1 (ppm)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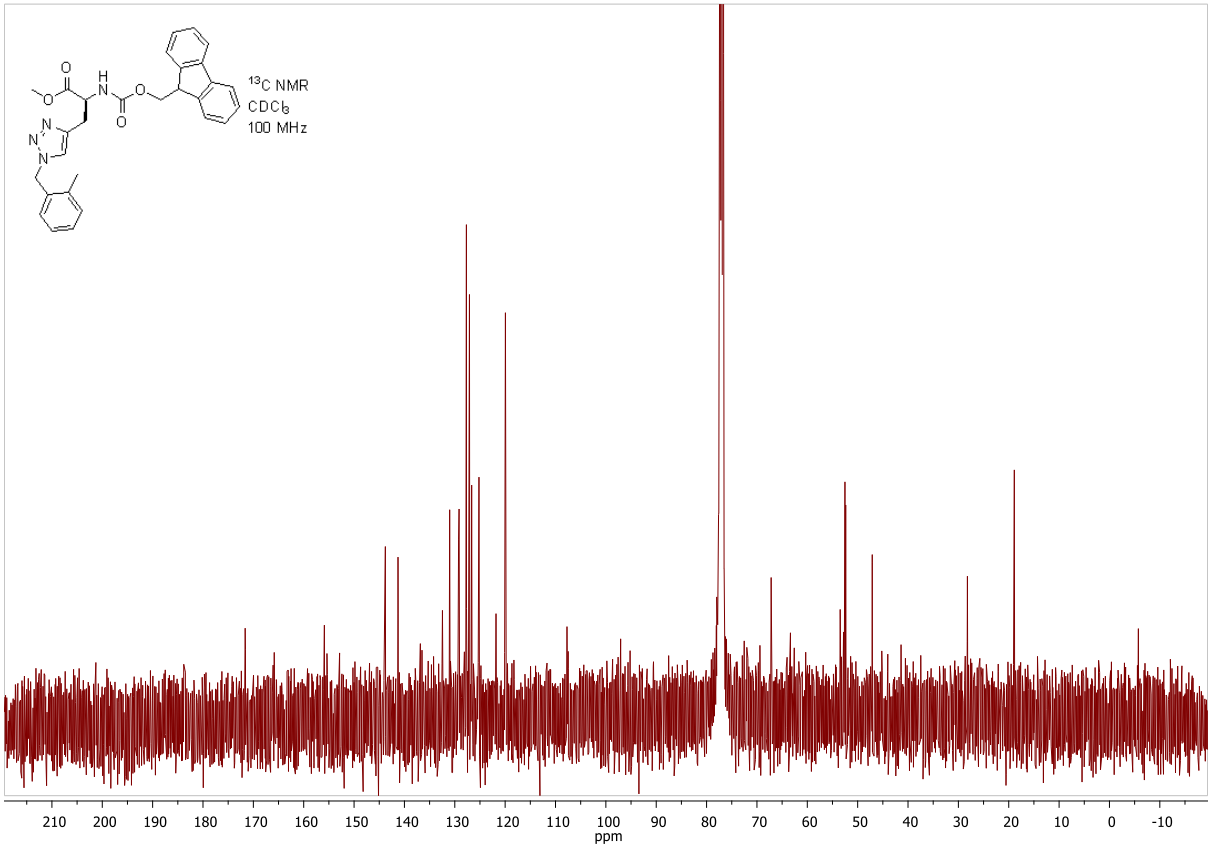
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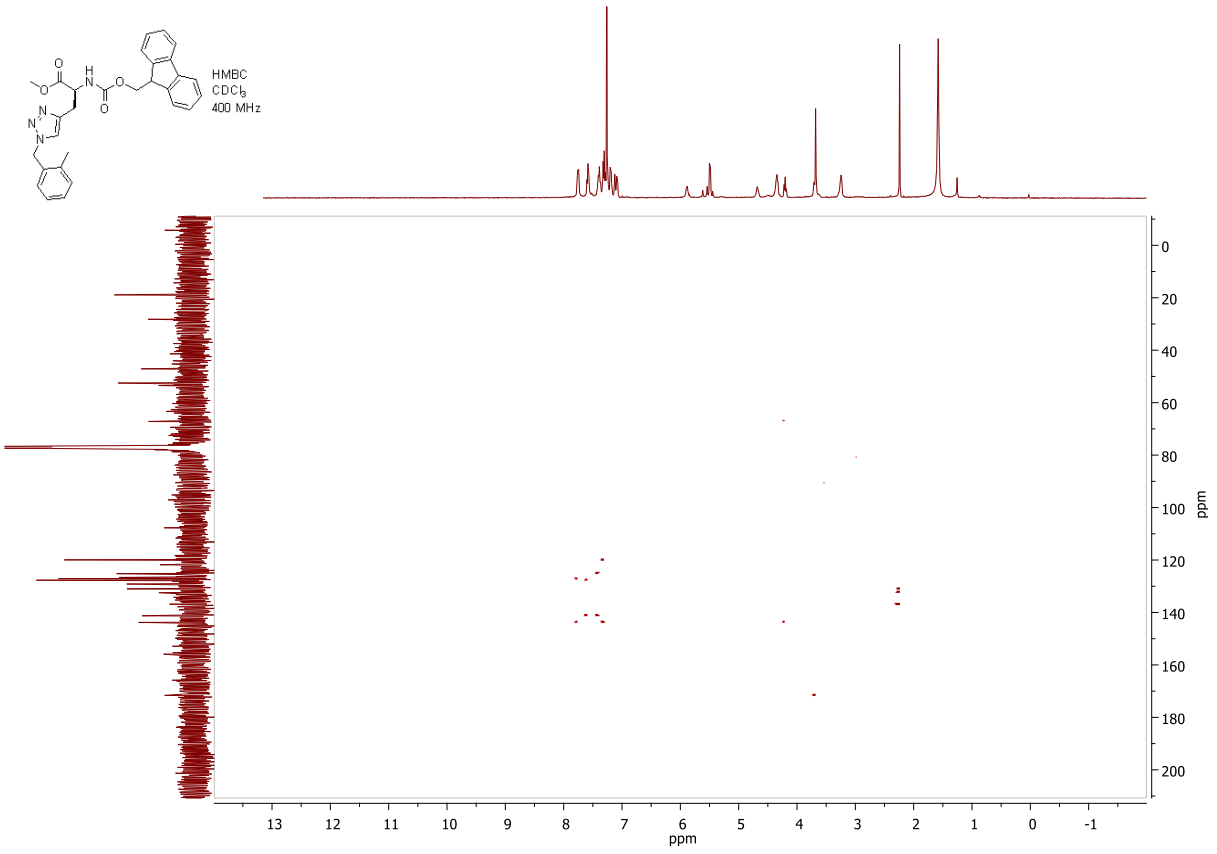
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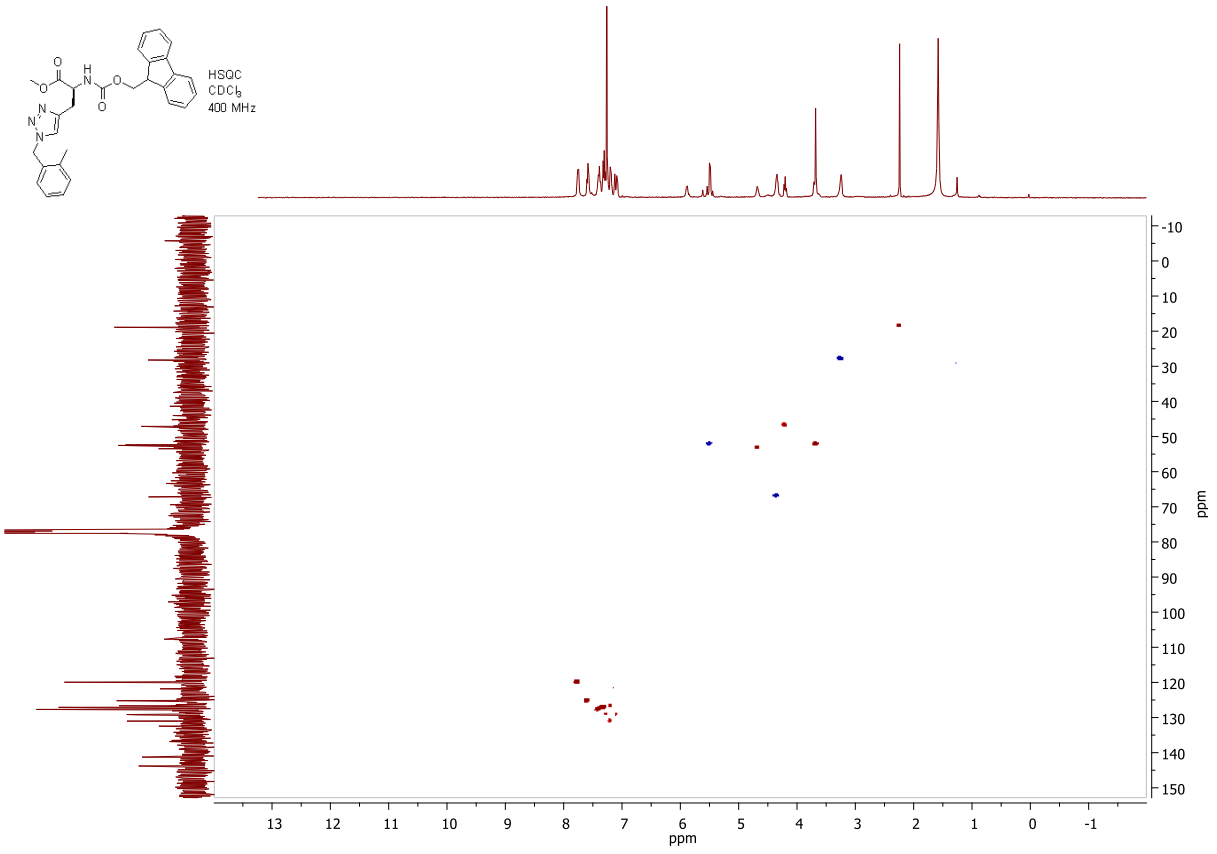
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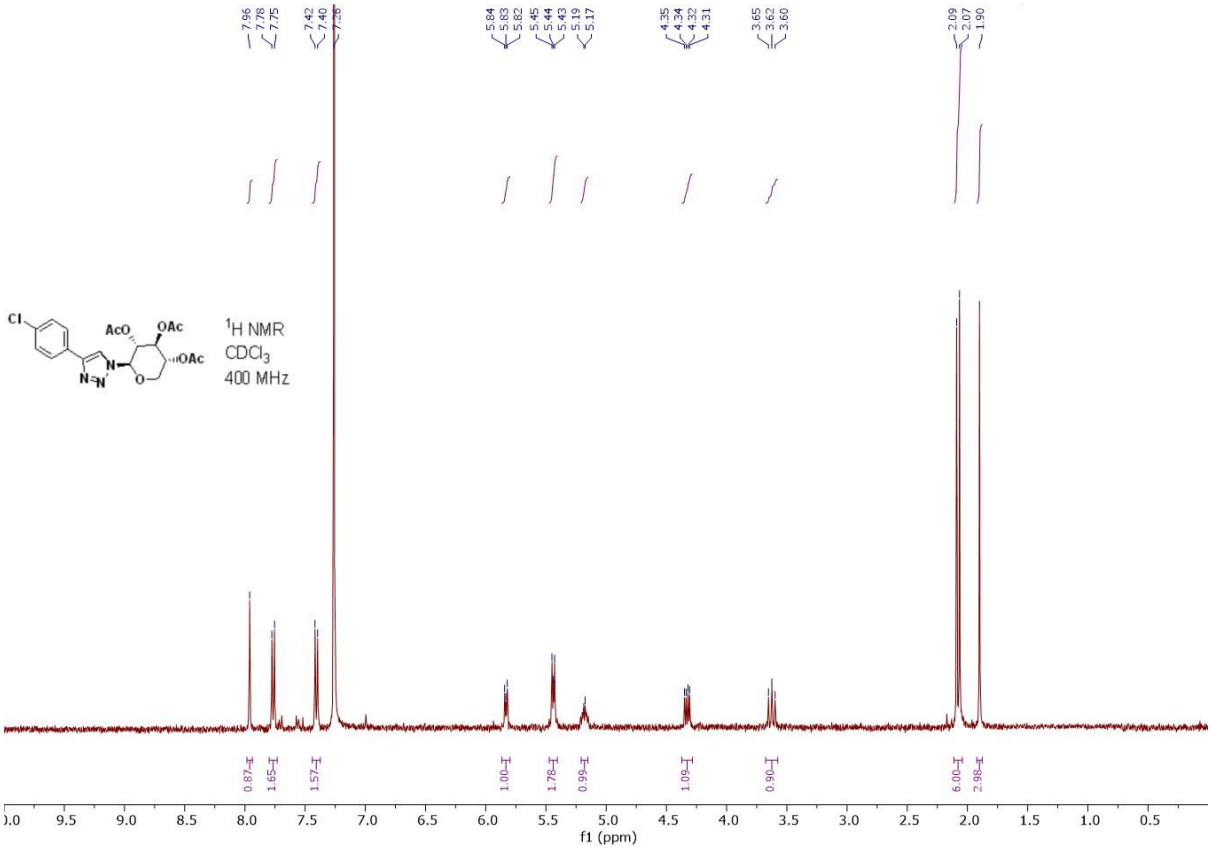
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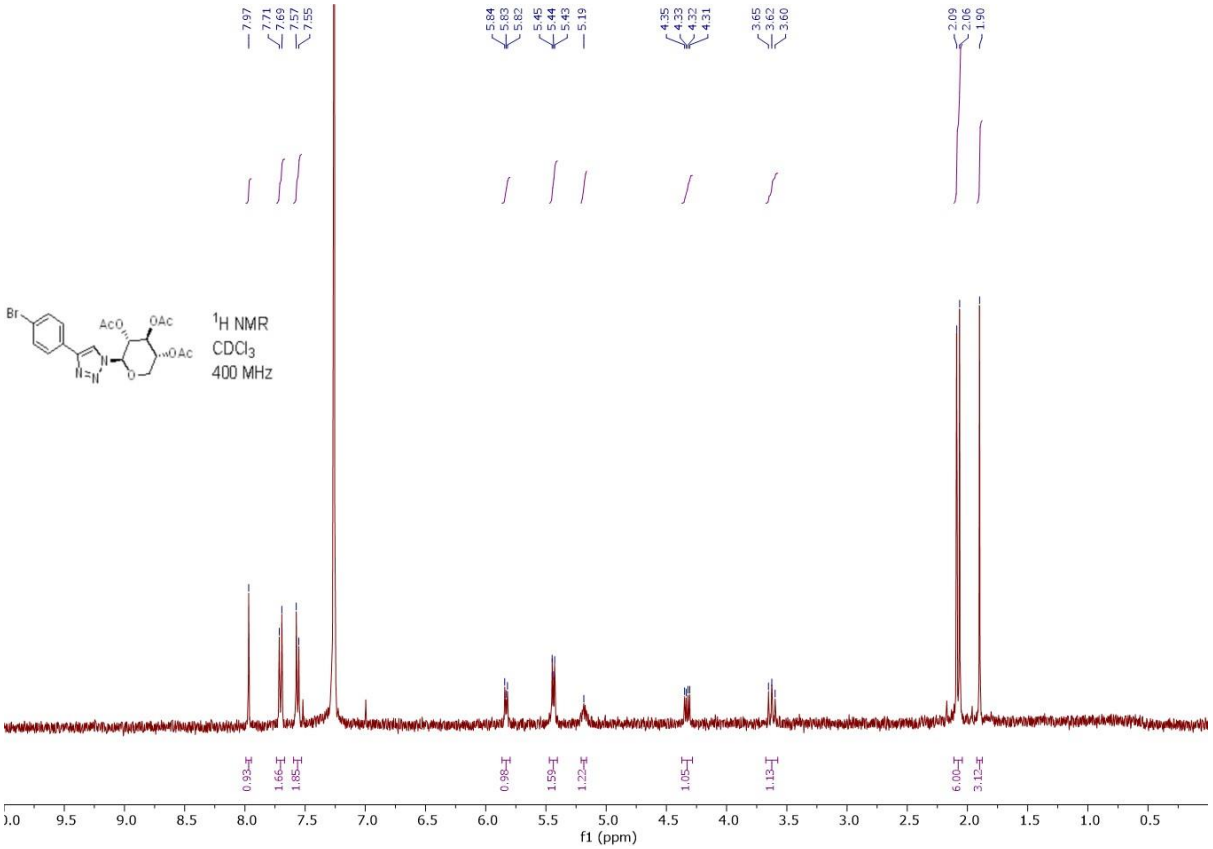
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6.00
3.12
f1 (ppm)