# Generation of Candidate Ligands for Nicotinic Acetylcholine Receptors via *In Situ* Click Chemistry with a Soluble Acetylcholine Binding Protein Template

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## Supporting Information

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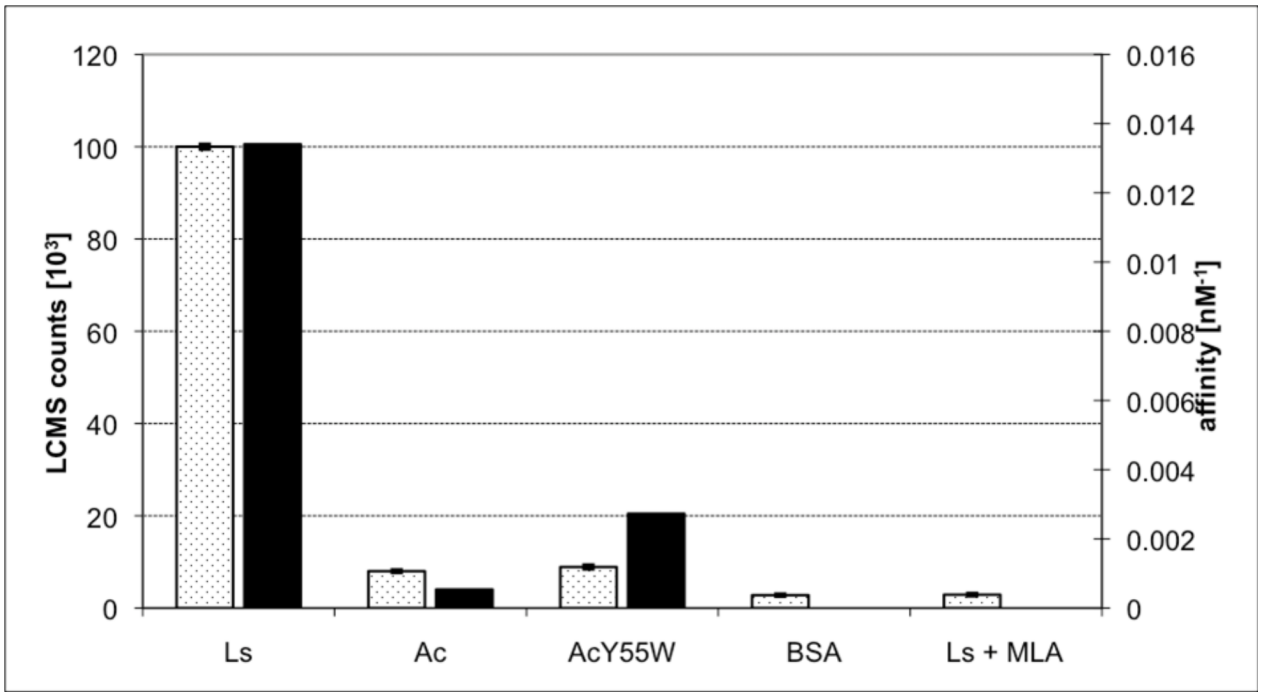
## *In situ* click screens

**Table S1: LCMS data – *in situ* click chemistry proof of concept run**

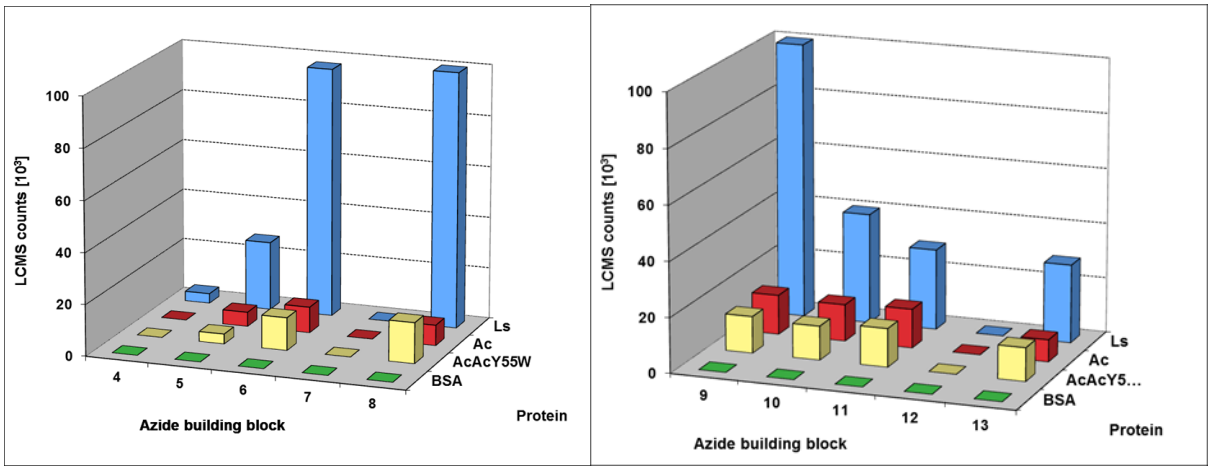
| Protein | Cmpd # | Run 1 [counts] | Run2 [counts] | Run3 [counts] | Average [counts] | Standard Deviation | Standard error | Standardized [counts] | ± |
|---|---|---|---|---|---|---|---|---|---|
| *Ls* | **2** | 396748 | 395310 | 392222 | 394760 | 2313 | 1335 | 100.0 | 0.3 |
| *Ac* | **2** | 32164 | 31753 | 31058 | 31658 | 559 | 323 | 8.0 | 0.1 |
| *Ac*Y55W | **2** | 36796 | 33579 | 35472 | 35282 | 1617 | 933 | 8.9 | 0.2 |
| BSA | **2** | 11145 | 10662 | 11165 | 10991 | 285 | 164 | 2.8 | 0.0 |
| *Ls* + MLA | **2** | 10745 | 11620 | 11394 | 11253 | 454 | 262 | 2.9 | 0.1 |




**Standardized LCMS counts (dotted) compared to affinity towards the respective AChBP.**

**Table S2: LCMS data- *in situ* click chemistry screen of library 1a**

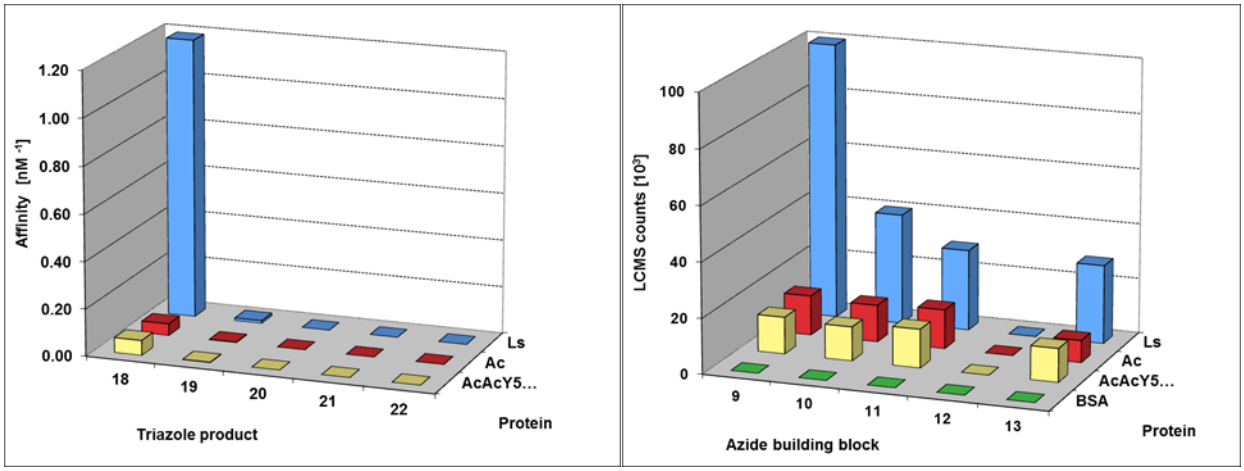
| Protein | Cmpd # | Run 1 [counts] | Run 2 [counts] | Run 3 [counts] | Average [counts] | Standard Deviation | Standard error | Standardized [counts] | ± |
|---|---|---|---|---|---|---|---|---|---|
| *Ls* | **2** | 16265 | 15088 | 16791 | 16048 | 872 | 503 | 3.8 | 0.1 |
| *Ac* | **2** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| *Ac*Y55W | **2** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| BSA | **2** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| *Ls* | **14** | 441443 | 410258 | 411660 | 421120 | 17614 | 10169 | 100.0 | 2.4 |
| *Ac* | **14** | 34251 | 32840 | 33599 | 33563 | 706 | 408 | 8.0 | 0.1 |
| *Ac*Y55W | **14** | 66415 | 67796 | 66470 | 66894 | 782 | 451 | 15.9 | 0.1 |
| BSA | **14** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| *Ls* | **15** | 429438 | 416561 | 382930 | 409643 | 24013 | 13864 | 97.3 | 3.3 |
| *Ac* | **15** | 42763 | 42740 | 42121 | 42541 | 364 | 210 | 10.1 | 0.0 |
| *Ac*Y55W | **15** | 52099 | 55744 | 53859 | 53901 | 1823 | 1052 | 12.8 | 0.2 |
| BSA | **15** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| *Ls* | **16** | 115527 | 107630 | 113806 | 112321 | 4153 | 2398 | 26.7 | 0.6 |
| *Ac* | **16** | 24040 | 22750 | 23576 | 23455 | 653 | 377 | 5.6 | 0.1 |
| *Ac*Y55W | **16** | 2350 | 22555 | 24468 | 16458 | 12255 | 7075 | 3.9 | 1.7 |
| BSA | **16** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| *Ls* | **17** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| *Ac* | **17** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| *Ac*Y55W | **17** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| BSA | **17** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |




**Comparison of LCMS counts of *in situ* click screen with AChBP affinities of the triazole products – library 1a**

**Table S3: LCMS data- *in situ* click chemistry screen of library 1b**

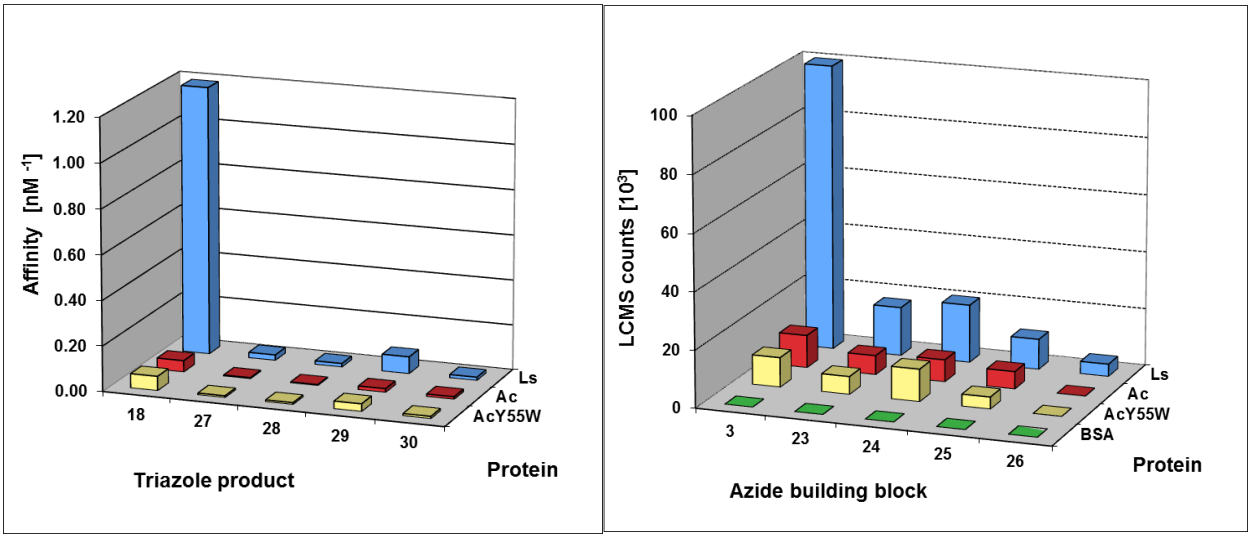
| Protein | Cmpd # | Run 1 [counts] | Run 2 [counts] | Run 3 [counts] | Average [counts] | Standard Deviation | Standard error | Standardized [counts] | ± |
|---|---|---|---|---|---|---|---|---|---|
| Ls | **18** | 296691 | 296691 | 300771 | 298051 | 2356 | 1360 | 100.0 | 0.5 |
| Ac | **18** | 42574 | 42617 | 43031 | 42741 | 252 | 146 | 14.3 | 0.0 |
| AcY55W | **18** | 38150 | 40327 | 40691 | 39723 | 1374 | 793 | 13.3 | 0.3 |
| BSA | **18** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **19** | 123595 | 123595 | 110082 | 119091 | 7802 | 4504 | 40.0 | 1.5 |
| Ac | **19** | 38205 | 42986 | 38088 | 39760 | 2795 | 1614 | 13.3 | 0.5 |
| AcY55W | **19** | 35820 | 36079 | 38307 | 36735 | 1367 | 789 | 12.3 | 0.3 |
| BSA | **19** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **20** | 90441 | 90441 | 80586 | 87156 | 5690 | 3285 | 29.2 | 1.1 |
| Ac | **20** | 40907 | 41469 | 44052 | 42143 | 1677 | 968 | 14.1 | 0.3 |
| AcY55W | **20** | 40497 | 41143 | 43258 | 41633 | 1444 | 834 | 14.0 | 0.3 |
| BSA | **20** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **21** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ac | **21** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| AcY55W | **21** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| BSA | **21** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **22** | 86823 | 86823 | 80722 | 84789 | 3522 | 2034 | 28.4 | 0.7 |
| Ac | **22** | 23015 | 24760 | 24011 | 23929 | 875 | 505 | 8.0 | 0.2 |
| AcY55W | **22** | 36921 | 34330 | 37269 | 36173 | 1606 | 927 | 12.1 | 0.3 |
| BSA | **22** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |




**Comparison of LCMS counts of *in situ* click screen with AChBP affinities of the triazole products – library 1b**

**Table S4: LCMS data- *in situ* click chemistry screen of alkynes versus azide 10**

| Protein | Cmpd # | Run 1 [counts] | Run 2 [counts] | Run 3 [counts] | Average [counts] | Standard Deviation | Standard error | Standardized [counts] | ± |
|---|---|---|---|---|---|---|---|---|---|
| Ls | **18** | 235245 | 227407 | 217881 | 226844 | 8696 | 5020 | 100.0 | 2.2 |
| Ac | **18** | 24008 | 26823 | 26466 | 25766 | 1533 | 885 | 11.4 | 0.4 |
| AcY55W | **18** | 24516 | 23189 | 22508 | 23404 | 1021 | 590 | 10.3 | 0.3 |
| BSA | **18** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **27** | 38984 | 39170 | 37786 | 38647 | 751 | 434 | 17.0 | 0.2 |
| Ac | **27** | 14019 | 16280 | 15198 | 15166 | 1131 | 653 | 6.7 | 0.3 |
| AcY55W | **27** | 14140 | 13515 | 14673 | 14109 | 580 | 335 | 6.2 | 0.1 |
| BSA | **27** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **28** | 47103 | 44413 | 46436 | 45984 | 1401 | 809 | 20.3 | 0.4 |
| Ac | **28** | 19838 | 16470 | 16036 | 17448 | 2081 | 1202 | 7.7 | 0.5 |
| AcY55W | **28** | 24750 | 25039 | 27016 | 25602 | 1233 | 712 | 11.3 | 0.3 |
| BSA | **28** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **29** | 24019 | 21630 | 26540 | 24063 | 2455 | 1418 | 10.6 | 0.6 |
| Ac | **29** | 15480 | 12790 | 13027 | 13766 | 1489 | 860 | 6.1 | 0.4 |
| AcY55W | **29** | 10071 | 8890 | 9407 | 9456 | 592 | 342 | 4.2 | 0.2 |
| BSA | **29** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| Ls | **30** | 9930 | 9929 | 9487 | 9782 | 255 | 148 | 4.3 | 0.1 |
| Ac | **30** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| AcY55W | **30** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| BSA | **30** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |

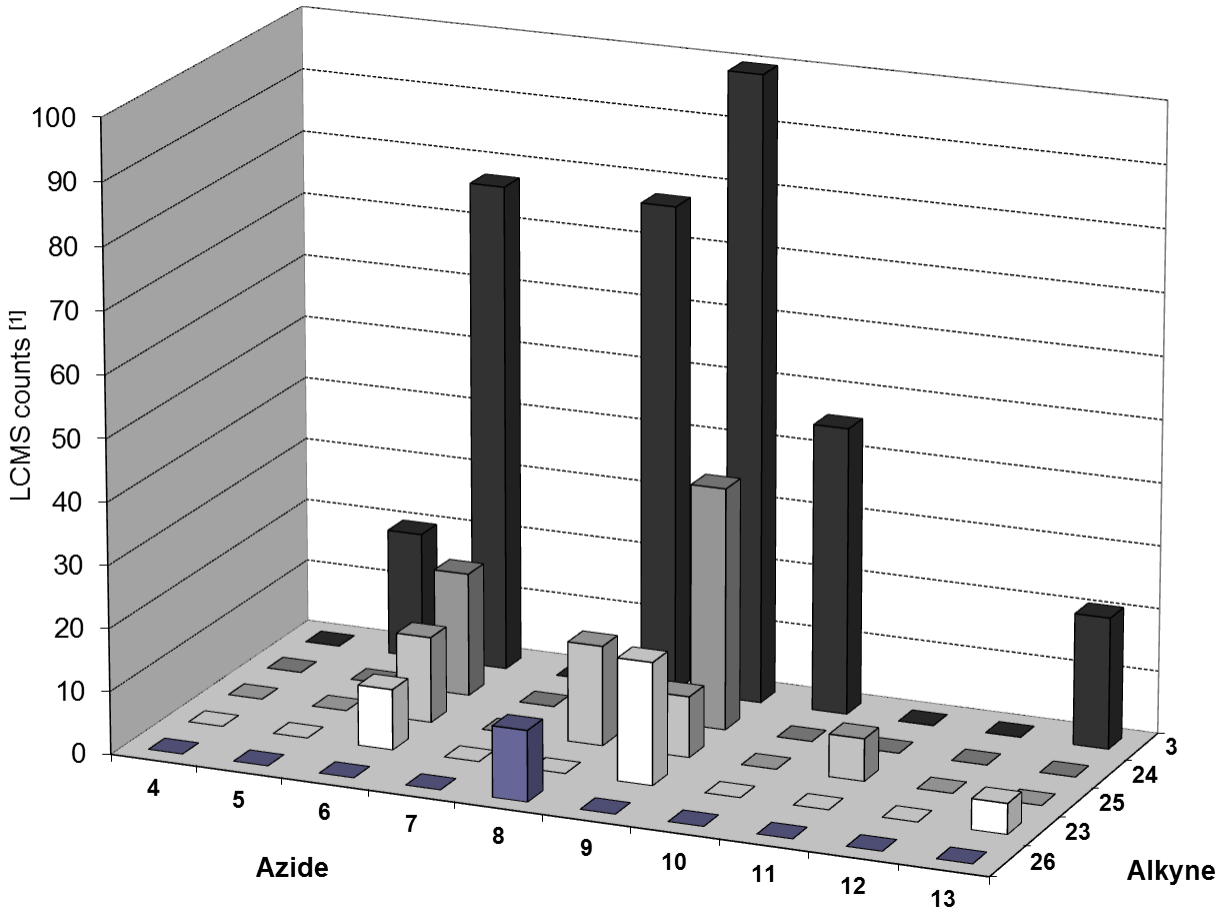



**Comparison of LCMS counts of *in situ* click screen with AChBP affinities of the triazole products – library 2**

**Table S5: LCMS data- *in situ* click chemistry screen of alkyne-azide pool against *Ls***

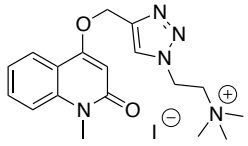
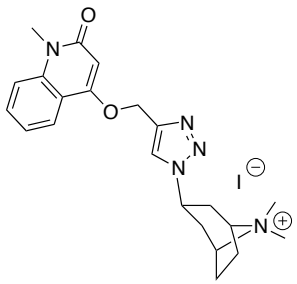
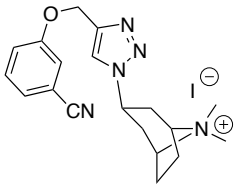
| Alkine # | Azide # | Run 1 [counts] | Run 2 [counts] | Run 3 [counts] | Average [counts] | Standard Deviation | Standard error | Standardized [counts] | ± |
|---|---|---|---|---|---|---|---|---|---|
| **3** | **4** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **3** | **5** | 9170 | 9348 | 9920 | 9479 | 392 | 226 | 20.1 | 0.5 |
| **3** | **6** | 36836 | 37065 | 36199 | 36700 | 449 | 259 | 77.7 | 0.5 |
| **3** | **7** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **3** | **8** | 37990 | 37305 | 39059 | 38118 | 884 | 510 | 80.7 | 1.1 |
| **3** | **9** | 47573 | 47313 | 46858 | 47248 | 362 | 209 | 100.0 | 0.4 |
| **3** | **10** | 21953 | 19361 | 23536 | 21617 | 2108 | 1217 | 45.8 | 2.6 |
| **3** | **11** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **3** | **12** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **3** | **13** | 9189 | 10595 | 9836 | 9873 | 704 | 406 | 20.9 | 0.9 |
| **23** | **4** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **23** | **5** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **23** | **6** | 4690 | 4269 | 4703 | 4554 | 247 | 143 | 9.6 | 0.3 |
| **23** | **7** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **23** | **8** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **23** | **9** | 9139 | 9286 | 9191 | 9205 | 75 | 43 | 19.5 | 0.1 |
| **23** | **10** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **23** | **11** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **23** | **12** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **23** | **13** | 2219 | 2348 | 2339 | 2302 | 72 | 42 | 4.9 | 0.1 |
| **24** | **4** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **24** | **5** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **24** | **6** | 9164 | 9439 | 9160 | 9254 | 160 | 92 | 19.6 | 0.2 |
| **24** | **7** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **24** | **8** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **24** | **9** | 17626 | 17835 | 19142 | 18201 | 822 | 474 | 38.5 | 1.0 |
| **24** | **10** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **24** | **11** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **24** | **12** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **24** | **13** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **25** | **4** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **25** | **5** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **25** | **6** | 6095 | 6361 | 6863 | 6440 | 390 | 225 | 13.6 | 0.5 |
| **25** | **7** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **25** | **8** | 7707 | 7292 | 7514 | 7504 | 208 | 120 | 15.9 | 0.3 |
| **25** | **9** | 4678 | 4439 | 4620 | 4579 | 125 | 72 | 9.7 | 0.2 |
| **25** | **10** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **25** | **11** | 3104 | 3426 | 3053 | 3194 | 202 | 117 | 6.8 | 0.2 |
| **25** | **12** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **25** | **13** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **4** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **5** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **6** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **7** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **8** | 5621 | 4881 | 5519 | 5340 | 401 | 232 | 11.3 | 0.5 |
| **26** | **9** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |

| Alkine # | Azide # | Run 1 [counts] | Run 2 [counts] | Run 3 [counts] | Average [counts] | Standard Deviation | Standard error | Standardized [counts] | ± |
|---|---|---|---|---|---|---|---|---|---|
| **26** | **10** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **11** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **12** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |
| **26** | **13** | n.d. | n.d. | n.d. | n.a | n.a | n.a | n.d | n.a |




**LCMS counts of triazoles (standardized to largest peak) formed *in situ* click in screen of azides (4-13) and alkynes (3 and 24-26) against *Ls***

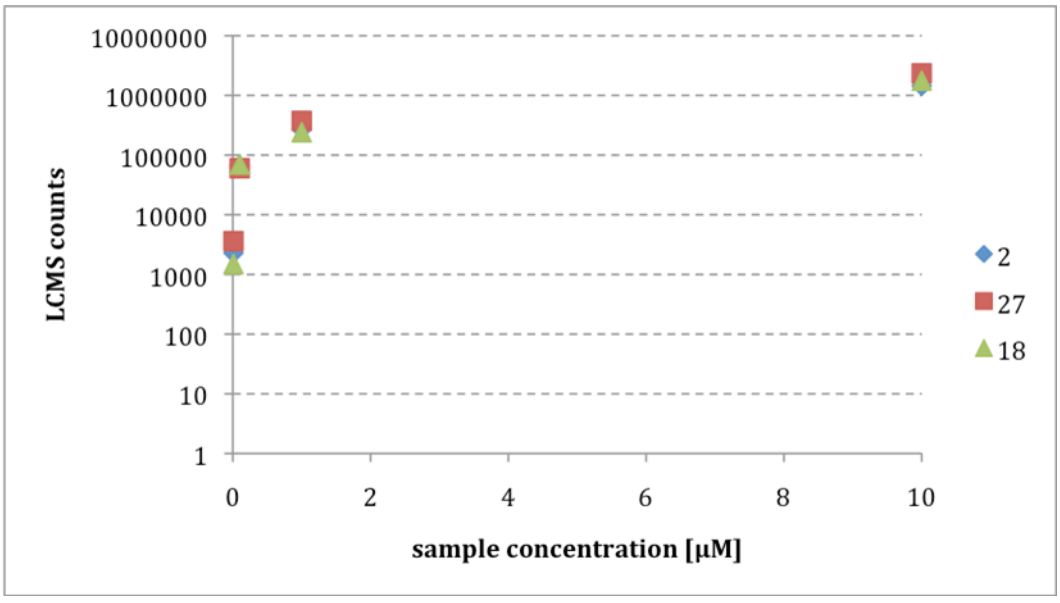
## Comparison of Mass Spectrum Response

**2**:  **18**:  **27**: 

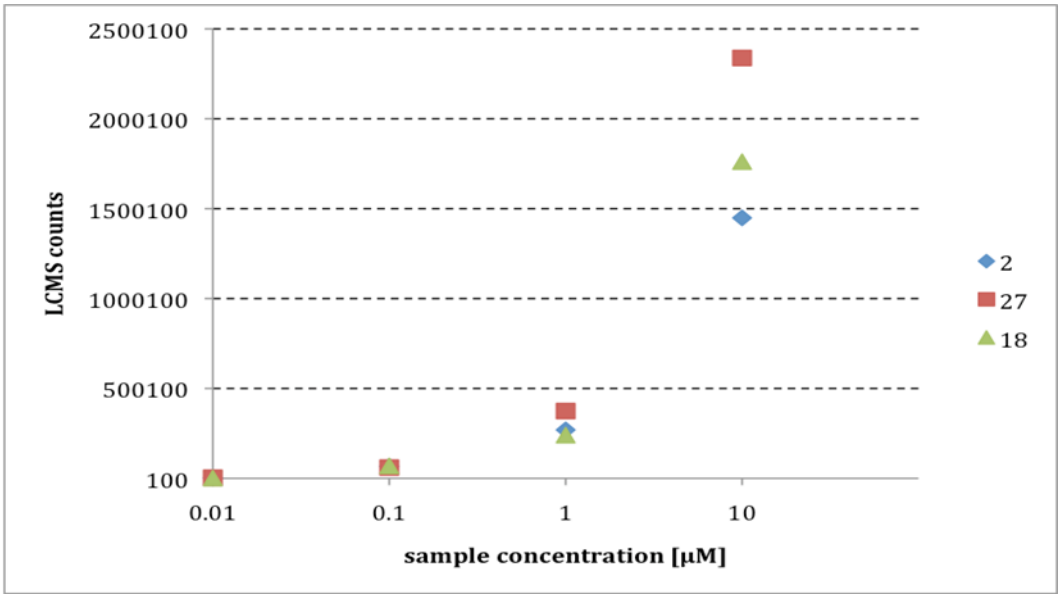
Compounds **2** (starting compound), **18** (varying azide portion) and **27** (varying alkyne portion) were dissolved in water at 1 mM. The solutions were combined and serially diluted to yield the following concentrations: 10 $\mu$M, 1$\mu$M, 0.1 $\mu$M, 0.01 $\mu$M and 0.001 $\mu$M with respect to each component. Three samples (25 $\mu$L) at each concentration were directly injected into the LC/MS instrument to perform LC/MS-SIM analysis: Zorbax, 4.6 mm x 3 cm, SB-C18 (rapid resolution) reverse phase column, preceded by a Phenomenex C18 guard column, flow rate 0.5 mL/min, using a gradient elution as follows: ($H_2O$ + 0.05% TFA):(MeCN + 0.05 % TFA) 100: 00 to 0:100 over 8 min; then 100 % MeCN + 0.05 % TFA for 2 min with a post run time of 4 min using the starting solvent ratio. Detection was by ESI-MS using positive selected-ion monitoring, tuned to the molecular weights of **2**, **18** and **27** ($M^+$). The mass response for each peak was integrated (Table S6) and graphed against the concentration (see below). It should be noted that, although the desired peaks were visible to the naked eye at 0.001 $\mu$M, they were too small to be integrated accurately, and therefore were not included. These data demonstrate that for these closely related compounds the mass spec response can be used to gauge the amount of compound present in the *in situ* reaction mixtures.

**Table S6: LCMS data – compounds 2, 18 and 27 at different concentrations**

| Concentration [µM] | LCMS counts | | | Average [LCMS counts] | Compound |
|---|---|---|---|---|---|
| 10 | 1476750 | 1404670 | 1464700 | 1448706.667 | **2** |
| 1 | 272439 | 262922 | 273307 | 269556 | **2** |
| 0.1 | 75681 | 59282 | 65099 | 66687.33333 | **2** |
| 0.01 | 2064 | 1937 | 2874 | 2291.666667 | **2** |
| 0.001 | N/A | N/A | N/A | 0 | **2** |
| | A | B | C | Average | |
| 10 | 2430050 | 2288850 | 2295840 | 2338246.667 | **27** |
| 1 | 382072 | 374389 | 366273 | 374244.6667 | **27** |
| 0.1 | 66523 | 57867 | 55889 | 60093 | **27** |
| 0.01 | 3985 | 3282 | 3582 | 3616.333333 | **27** |
| 0.001 | N/A | N/A | N/A | 0 | **27** |
| | A | B | C | Average | |
| 10 | 1703600 | 1843820 | 1736990 | 1761470 | **18** |
| 1 | 251013 | 236817 | 231924 | 239918 | **18** |
| 0.1 | 85989 | 60709 | 60601 | 69099.66667 | **18** |
| 0.01 | 1349 | 1344 | 1767 | 1486.666667 | **18** |
| 0.001 | N/A | N/A | N/A | 0 | **18** |




**LCMS counts (logarithmic scale) of triazoles 2, 18 and 27 as a function of concentration**




**LCMS counts of triazoles 2, 18, and 27 as a function of concentration (logarithmic scale)**

## Experimental

### General Information

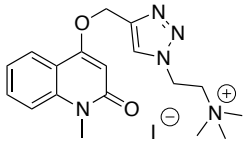
$^{1}H$ and $^{13}C$ NMR spectra were recorded on either a Varian Mercury-300 or a Bruker DRX-600 equipped with a DCH cryoprobe and calibrated using residual undeuterated solvent as an internal reference. The following notation is used: br – broad, s – singlet, d – doublet, t – triplet, q – quartet, quin – quintet, m – multiplet, dd – doublet of doublets, ddd – doublet of doublets of doublets, dt – doublet of triplets, td – triplet of doublets, app - apparent. Infrared spectra were recorded on a Nicolet Avatar 370 Fourier transform infrared spectrometer. LCMS analysis was performed on an Agilent 1100 LC/MSD with an Agilent 1100SL mass spectrometer (ES) eluting with 0.1% trifluoroacetic acid in $H_2O$ and 0.05% trifluoroacetic acid in $CH_3CN$. High resolution mass spectra (HRMS) were recorded on an Agilent Mass spectrometer using ESI-TOF (electrospray ionization-time of flight). Melting points were recorded using a Thomas Hoover Capillary Melting Point apparatus and are reported uncorrected. Unless otherwise noted, analytical grade solvents and commercially available reagents were used without further purification.

### General Procedure for Copper Catalyzed Alkyne Azide Cycloaddition

Amino-azide (1 eq, 0.5 mmol) and alkyne (1 eq, 0.5 mmol) were stirred together in *t*-butanol/water (2:1, yielding a final azide/alkyne concentration of 0.1 M). To this suspension was added aq. copper sulfate (0.5 M, 1 mol %) and aq sodium ascorbate (0.1 M, 10 mol %). The reaction was stirred at room temperature until LCMS analysis showed complete consumption of the starting materials. The reaction was subsequently basified with sat aq $K_2CO_3$, diluted with water and extracted with ethyl acetate (x3). The organic layers were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resulting residue was dissolved in methyl iodide (1 M) and stirred at room temperature until LC-MS analysis showed complete conversion of the amine intermediate to the desired quaternary ammonium derivative. The reaction was subsequently evaporated to dryness under reduced pressure and the residue purified by

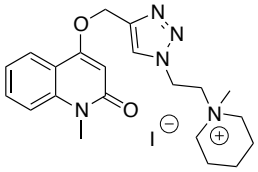
reverse phase column chromatography (Biotage Snap Cartridge KP-C18-HS) to yield the desired salt.

***N,N,N*-trimethyl-2-(4-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethanaminium iodide** (**2**)



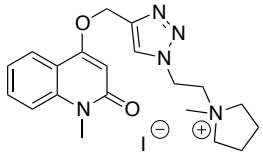
**2**: (199 mg, 85%) white solid; mp = 161-163 ˚C; $^{1}$H NMR (300 MHz, $D_2O$) δ ppm 8.22 (s, 1H), 7.10-7.02 (br m, 2H), 6.69-6.66 (m, 2H), 5.17 (s, 1H), 5.07 (t, *J* = 6.7 Hz, 2H), 4.57 (s, 2H), 4.08 (t, *J* = 6.7 Hz, 2H), 3.28 (s, 9H), 2.85 (s, 3H); $^{13}$C NMR (75 MHz, $D_2O$) δ ppm 163.8, 160.9, 143.2, 137.6, 131.6, 131.5, 125.3, 122.48, 114.6, 114.3, 95.2, 63.9, 62.1, 54.0, 44.4, 29.3; FTIR 3450, 1634, 1582, 1234, 1154, 1117, 750 cm$^{-1}$; HRMS *m/z* 342.1932 $[M]^+$, calculated for $C_{18}H_{24}N_5O_2^+$ 342.1025.

**1-methyl-1-(2-(4-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)piperidin-1-ium iodide** (**15**)



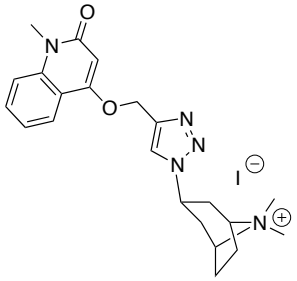
**15**: (198 mg, 73%) white solid; mp = 150-152 ˚C; $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ ppm 8.48 (s, 1H), 7.82 (d, *J* = 7.5 Hz, 1H), 7.69 (t, *J* = 8.5 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.25 (t, *J* = 8.5 Hz, 1H), 6.30 (s, 1H), 5.39 (s, 2H), 5.01 (t, *J* = 6.9 Hz, 2H), 3.95 (t, *J* = 6.9 Hz, 2H), 3.57 (s, 3H), 3.40 (t, *J* = 5.5 Hz, 4H), 3.11 (s, 3H), 1.86-1.71 (m, 4H), 1.61-1.46 (m, 2H); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ ppm 162.1, 160.3, 142.2, 139.4, 131.6, 125.6, 122,7, 121.5, 115.4, 114.8, 97.6, 61.8, 60.1, 42.6, 40.4, 40.1, 28.5, 20.5, 19.2; FTIR 3442, 1637, 1580, 1217, 689 cm$^{-1}$; HRMS *m/z* 382.2251 $[M+H]^+$, calculated for $C_{21}H_{28}N_5O_2^+$ 382.2238.

**1-methyl-1-(2-(4-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)pyrrolidin-1-ium iodide** (**16**)



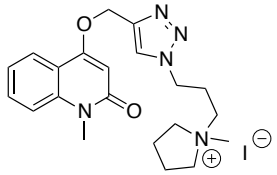
**16**: (202 mg, 82%) off-white solid; mp = 148-151 ˚C; $^{1}$H NMR (600 MHz, $D_2O$) δ ppm 8.34 (s, 1H), 7.68 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.55 (td, *J* = 8.6, 1.5 Hz, 1H), 7.30 (app d, *J* = 8.6 Hz, 1H), 7.18 (app t, *J* = 8.1 Hz, 1H), 5.86 (s, 1H), 5.22 (s, 2H), 5.10 (t, *J* = 6.4 Hz, 2H), 4.05 (t, *J* = 6.4 Hz, 2H), 3.53-3.46 (m, 4H), 3.38 (s, 3H), 3.11 (s, 3H), 2.17 (m, 4H); $^{13}$C NMR (150 MHz, $D_2O$) δ ppm 165.5, 162.6, 143.9, 139.2, 132.8, 126.6, 123.7, 123.5, 116.4, 115.7, 96.7, 66.0, 62.8, 62.2, 48.6, 45.7, 30.1, 21.9; FTIR 3362, 1630, 1576, 1236, 1117, 751 cm$^{-1}$; HRMS *m/z* 368.2089 $[M]^+$, calculated for $C_{20}H_{26}N_5O_2^+$ 368.2081.

**8,8-dimethyl-3-(4-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)-8-azabicyclo[3.2.1]octan-8-ium iodide** (**18**)



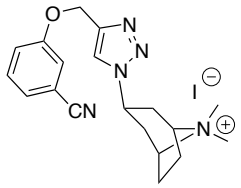
**18**: (229 mg, 88%) white solid; mp = 186-188 ˚C; $^{1}$H NMR (600 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 8.65 (s, 1H), 7.85 (app d, *J* = 8.0 Hz, 1H), 7.64 (app t, *J* = 8.5 Hz, 1H), 7.51 (app d, *J* = 8.5 Hz, 1H), 7.23 (app t, *J* = 8.0 Hz, 1H), 6.28 (s, 1H), 5.33 (s, 2H), 4.85 (m, 1H), 3.94 (m, 2H), 3.55 (s, 3H), 3.20 (s, 3H), 3.01 (s, 3H), 2.85-2.81 (m, 4H), 2.23-2.15 (m, 2H), 1.75-1.69 (m, 2H); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ 163.5, 161.7, 143.7, 140.7, 132.9, 126.0, 124.1, 122.9, 116.7, 116.1, 99.0, 67.4, 63.3, 54.0, 51.1, 48.9, 44.8, 31.1, 25.0; FTIR 3448, 1637, 1577, 1240, 765, 750 cm$^{-1}$; HRMS *m/z* 394.2245 $[M]^+$, calculated for $C_{22}H_{28}N_5O_2^+$ 394.2243.

**1-methyl-1-(2-(4-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)pyrrolidin-1-ium iodide** (**19**)



**19**: (218 mg, 86%) white solid; mp = 156-158 ˚C; $^{1}$H NMR (600 MHz, $D_2O$) δ ppm 8.21 (s, 1H), 7.58 (dd, *J* = 8.1, 1.4 Hz, 1H), 7.47 (td, *J* = 8.6, 1.48 Hz, 1H), 7.20 (app d, *J* = 8.1 Hz, 1H), 7.13 (app t, *J* = 8.6 Hz, 1H), 5.78 (s, 1H), 5.13 (s, 2H), 4.61 (t, *J* = 6.5 Hz, 2H), 3.52-3.36 (m, 6H), 3.30 (s, 3H), 3.03 (s, 3H), 2.54-2.45 (m, 2H), 2.14-2.03 (m, 4H); $^{13}$C NMR (150 MHz, $D_2O$) δ ppm 165.4, 162.4, 143.3, 139.1, 132.7, 126.1, 123.5, 123.4, 116.3, 115.6, 96.5, 65.3, 62.2, 61.5, 48.8, 48.0, 30.0, 24.9, 21.9; FTIR 3367, 1636, 1578,1461, 1237, 751 cm$^{-1}$; HRMS *m/z* 382.2243 $[M+H]^+$, calculated for $C_{21}H_{28}N_5O_2^+$ 382.2246.

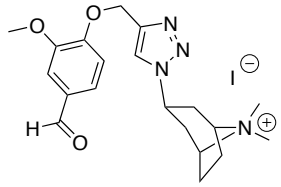
**3-(4-((3-cyanophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-8,8-dimethyl-8-azabicyclo-[3.2.1]octan-8-ium iodide** (**27**)



**27**: (214 mg, 92%) white solid; mp = 225-228 ˚C; $^{1}$H NMR (600 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 8.41 (s, 1H), 7.58-7.56 (m, 1H), 7.52 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.45 (app dt, *J* = 7.6, 1.1 Hz, 1H), 7.39 (ddd, *J* = 8.3, 2.5, 0.8 Hz, 1H), 5.25 (m, 3H), 4.06 (br s, 2H), 3.32 (s, 3H), 3.10 (s, 3H), 2.65-2.60 (m, 2H), 2.42-2.40 (m, 2H), 2.36-2.31 (m, 2H), 2.22-2.19 (m, 2H); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 158.1, 141.9, 130.9, 124.9, 124.2, 120.5, 118.7, 117.5, 112.2, 68.0, 60.9, 50.2, 48.3, 44.2, 31.8, 24.4;

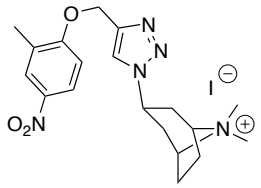
FTIR 3026, 2239, 1678, 1593, 1255, 1025, 797 $cm^{-1}$; HRMS *m/z* 338.1985 $[M]^+$, calculated for $C_{19}H_{24}N_5O^+$ 338.1975.

**8,8-dimethyl-3-(4-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)-8-azabicyclo[3.2.1]octan-8-ium iodide** (**28**)



**28**: (268 mg, 93%) white solid; mp = 210-212 ˚C; $^1$H NMR (600 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 9.86 (s, 1H), 8.41 (s, 1H), 7.57 (dd, *J* = 6.0, 1.8 Hz, 1H), 7.42 (s, 1H), 7.41 (d, *J* = 6.0 Hz, 1H), 5.28 (s, 2H), 5.26-5.21 (m, 1H), 4.04 (br s, 2H), 3.81 (s, 3H), 3.12 (s, 3H), 3.09 (s, 3H), 2.64-2.60 (m, 2H), 2.42-4.40 (m, 2H), 2.34-2.31 (m, 2H), 2.21-2.19 (m, 2H); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 191.5, 152.8, 149.3, 141.9, 129.9, 124.6, 112.5, 109.7, 67.9, 61.7, 55.6, 49.9, 48.2, 47.5, 43.7, 31.9, 24.6; FTIR 2959, 1678, 1586, 1514, 1271, 1151, 995 $cm^{-1}$; HRMS *m/z* 371.2090 $[M]^+$, calculated for $C_{20}H_{27}N_4O_3^+$ 371.2078.

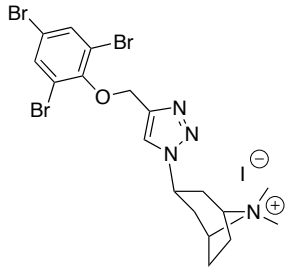
**8,8-dimethyl-3-(4-((2-methyl-4-nitrophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-8-azabicyclo[3.2.1]octan-8-ium iodide** (**29**)



**29**: (280 mg, 89%) yellow solid; mp = 200-202 ˚C; $^1$H NMR (600 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 8.41 (s, 1H), 8.15 (dd, *J* = 9.0, 2.8 Hz, 1H), 8.11 (d, *J* = 2.8 Hz, 1H), 7.40 (d, *J* = 9.0 Hz, 1H), 5.37 (s, 2H), 5.22 (m, 1H), 4.05 (br s, 2H), 3.31 (s, 3H), 3.09 (s, 3H), 2.65-2.60 (m, 2H), 2.43-2.39 (m, 2H), 2.34-2.30 (m, 2H), 2.22 (s, 3H), 2.20-2.17 (m, 2H); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 161.5, 141.9, 140.4, 127.5, 125.7,

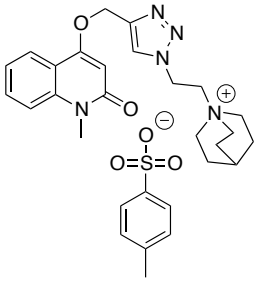
124.3, 123.6, 111.7, 67.9, 62.1, 50.0, 48.2, 43.8, 31.9, 24.4, 16.0; FTIR 3453, 1590, 1521, 1334, 1274, 1094, 749 $cm^{-1}$; HRMS *m/z* 372.2037 $[M]^+$, calculated for $C_{19}H_{26}N_5O_3^+$ 372.2030.

**8,8-dimethyl-3-(4-((2,4,6-tribromophenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-8-azabicyclo[3.2.1]octan-8-ium iodide (30)**



**30**: (255 mg, 76%) tan solid; mp = 223-225 ˚C; $^1$H NMR (600 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 8.47 (s, 1H), 7.98 (s, 2H), 5.28-5.22 (m, 1H), 5.11 (s, 2H), 4.05 (br s, 2H), 3.32 (s, 3H), 3.10 (s, 3H), 2.67-2.59 (m, 2H), 2.42-2.39 (m, 2H), 2.33-2.28 (m, 2H), 2.22-2.18 (m, 2H); $^{13}$C NMR (150 MHz, DMSO-$d_6$ + 1 drop $CDCl_3$) δ ppm 151.6, 141.4, 135.0, 124.8, 119.1, 117.4, 68.0, 66.1, 50.2, 47.9, 44.1, 31.7, 24.3; FTIR 3055, 1438, 1234, 949, 734 $cm^{-1}$; HRMS *m/z* 546.9344 $[M]^+$, calculated for $C_{18}H_{22}Br_3N_4O^+$ 546.9338.

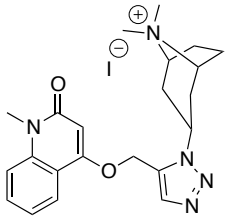
**1-(2-(4-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)ethyl)quinuclidin-1-ium 4-methylbenzenesulfonate** (**14**)



2-Azidoethyl 4-methylbenzenesulfonate (120 mg, 0.5 mmol) and alkyne **3** (106 mg, 0.5 mmol) were stirred together in *t*-butanol/water (2:1, 5 mL). To this suspension was added copper sulfate (0.5 M in water, 12.5 μL, 1 mol%) and aq. sodium ascorbate (0.1 M, 500 μL, 10 mol%). The reaction was stirred at room temperature for 16 h. Water (20

mL) was added and the mixture was extracted with ethyl acetate (X3, 20 mL). The organic layers were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was suspended in toluene (10 mL) and quinuclidine (56 mg, 0.5 mmol) added. The reaction was stirred at reflux for 2 h, then allowed to cool to room temperature and evaporated under reduced pressure. The residue was purified by reverse phase column chromatography (Biotage Snap Cartridge KP-C18-HS) to yield the salt **14** as an off white solid (259 mg, 92%); mp = 202-205 ˚C; $^1$H NMR (600 MHz, $D_2O$) δ ppm 8.24 (s, 1H), 7.57 (d, *J* = 8.2 Hz, 2H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.38 (t, *J* = 8.5 Hz, 1H), 7.17 (d, *J* = 8.2 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 1H), 7.02 (t, *J* = 8.0 Hz, 1H), 5.64 (s, 1H), 5.01 (s, 2H), 4.96 (t, *J* = 6.7 Hz, 2H), 3.74 (t, *J* = 6.7 Hz, 2H), 3.42-3.34 (m, 9H), 3.19 (s, 3H), 2.23 (quin, *J* = 3.3 Hz, 1H), 1.98-1.90 (m, 6H); $^{13}$C NMR (150 MHz, $D_2O$) δ ppm 165.2, 162.2, 143.6, 142.9, 140.4, 138.9, 132.6, 130.1, 126.5, 126.0, 123.4, 123.2, 116.6, 116.0, 93.4, 62.4, 62.2, 56.1, 44.2, 29.9, 24.0, 21.2, 19.5; FTIR 3489, 1638, 1589, 1209, 1186, 1010, 678 cm$^{-1}$; HRMS *m/z* 394.2243 $[M]^+$, calculated for $C_{22}H_{28}N_5O_2^+$ 394.2243.

**8,8-dimethyl-3-(5-(((1-methyl-2-oxo-1,2-dihydroquinolin-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)-8-azabicyclo[3.2.1]octan-8-ium iodide** (**18a**)

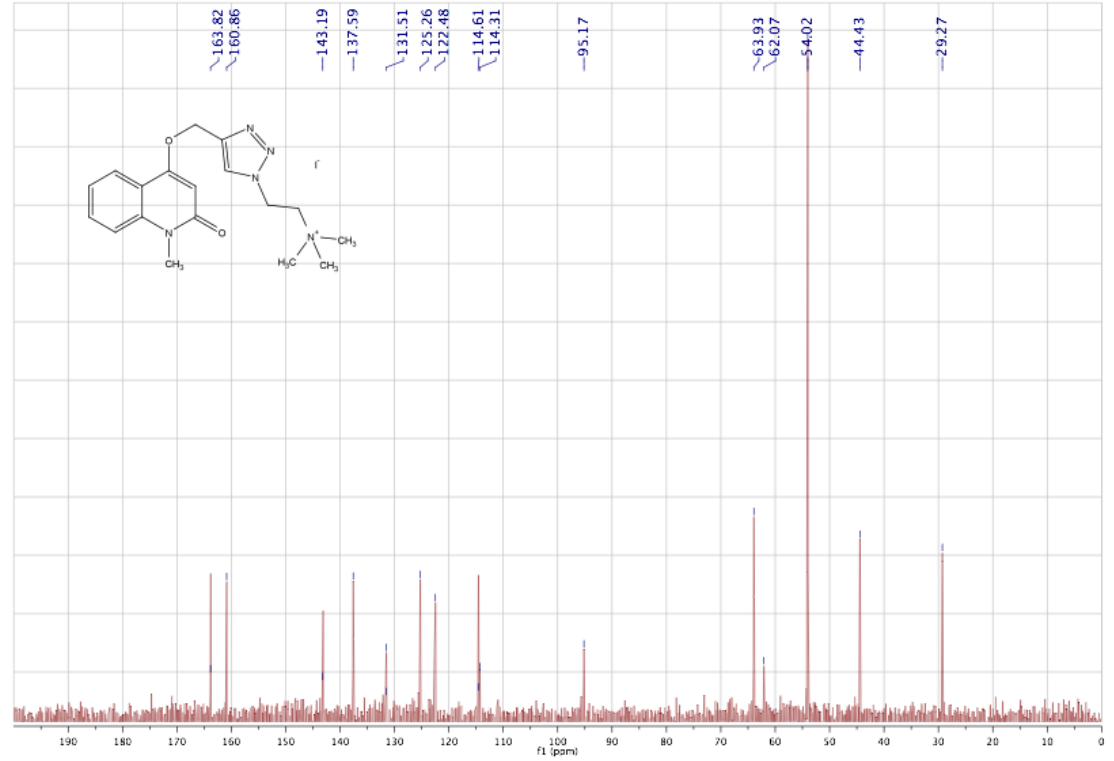



A solution of alkyne **3** (106 mg, 0.5 mmol) and azide **31** (83 mg, 0.5 mmol) in dioxane (600 μL) was added to $Cp^*RuCl(PPh_3)_2$ (12 mg, 0.015 mmol) dissolved in dioxane (3 mL). The vial was purged with nitrogen, sealed and heated at 60 ˚C. After 16 h, water (20 mL) was added and the mixture was extracted with ethyl acetate (X3, 10 mL). The organic layers were combined, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The resultant residue was dissolved in methyl iodide (500 μL) and stirred at room temperature for 1 hour, then evaporated to dryness under reduced pressure and purified by reverse phase column chromatography (Biotage Snap

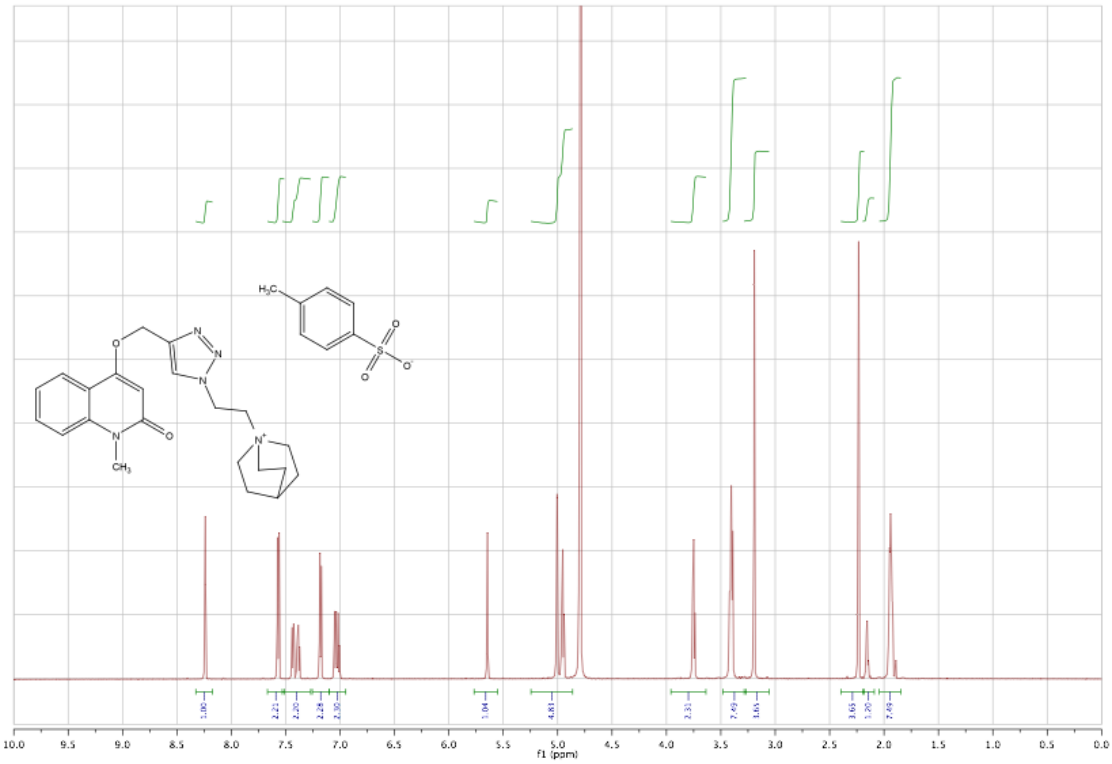
Cartridge KP-C18-HS) yielding **18a** as a brown solid (187 mg, 72%); mp = 194-196 ˚C; $^{1}$H NMR (600 MHz, DMSO-$d_6$) δ ppm 8.02 (s, 1H), 7.90 (app d, $J$ = 7.9 Hz, 1H), 7.67 (app t, $J$ = 8.4 Hz, 1H), 7.53 (app d, $J$ = 8.9 Hz, 1H), 7.27 (app t, $J$ = 7.4 Hz, 1H), 6.36 (s, 1H), 5.62 (s, 2H), 5.11 (m, 1H), 4.08 (m, 2H), 3.57 (s, 3H), 3.37 (s, 3H), 3.10 (s, 3H) 2.75 (t, $J$ = 12.24 Hz, 2H), 2.40 (m, 2H), 2.30 (m, 2H), 2.219 (m, 2H); $^{13}$C NMR (150 MHz, DMSO-$d_6$) δ ppm 161.4, 161.2, 140.8, 133.9, 133.0, 123.8, 123.1, 116.4, 116.2, 99.3, 69.1, 60.4, 51.2, 47.7, 45.1, 33.7, 32.3, 30.1, 25.6; FTIR 3407, 1631, 1580, 1457, 1231, 1114, 753 cm$^{-1}$; HRMS *m/z* 394.2245 $[M]^+$, calculated for $C_{22}H_{28}N_5O_2^+$ 394.2248.

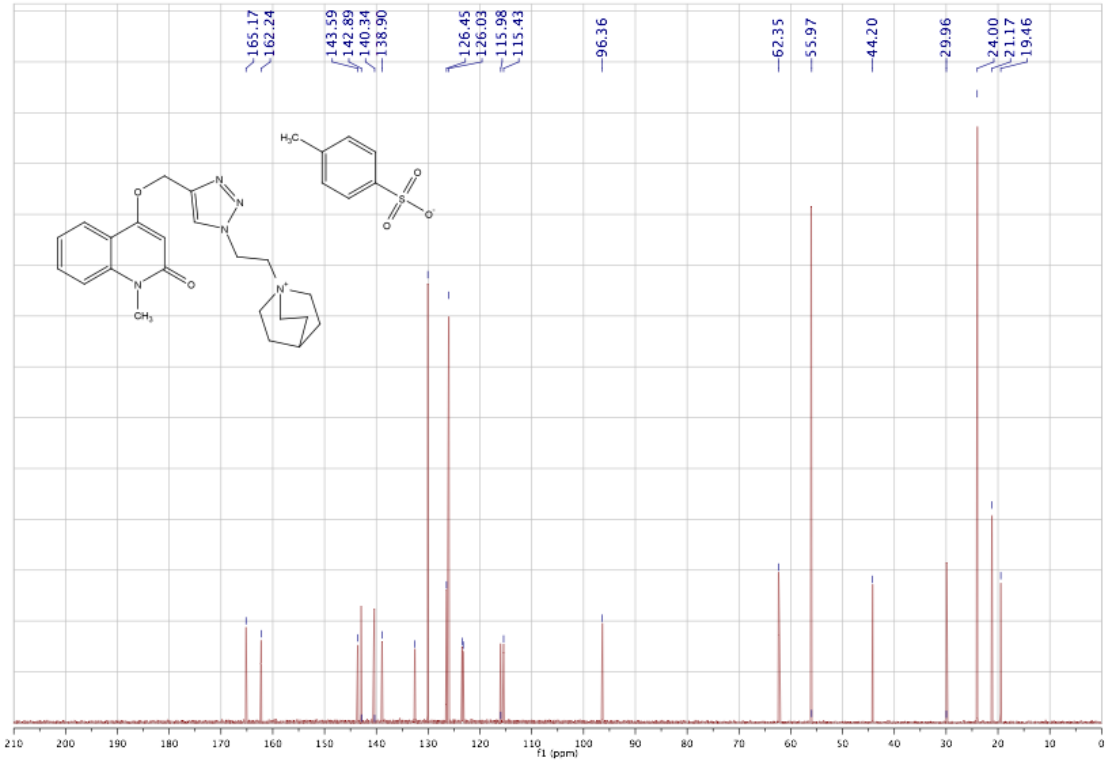
## NMR Spectra

**2:**

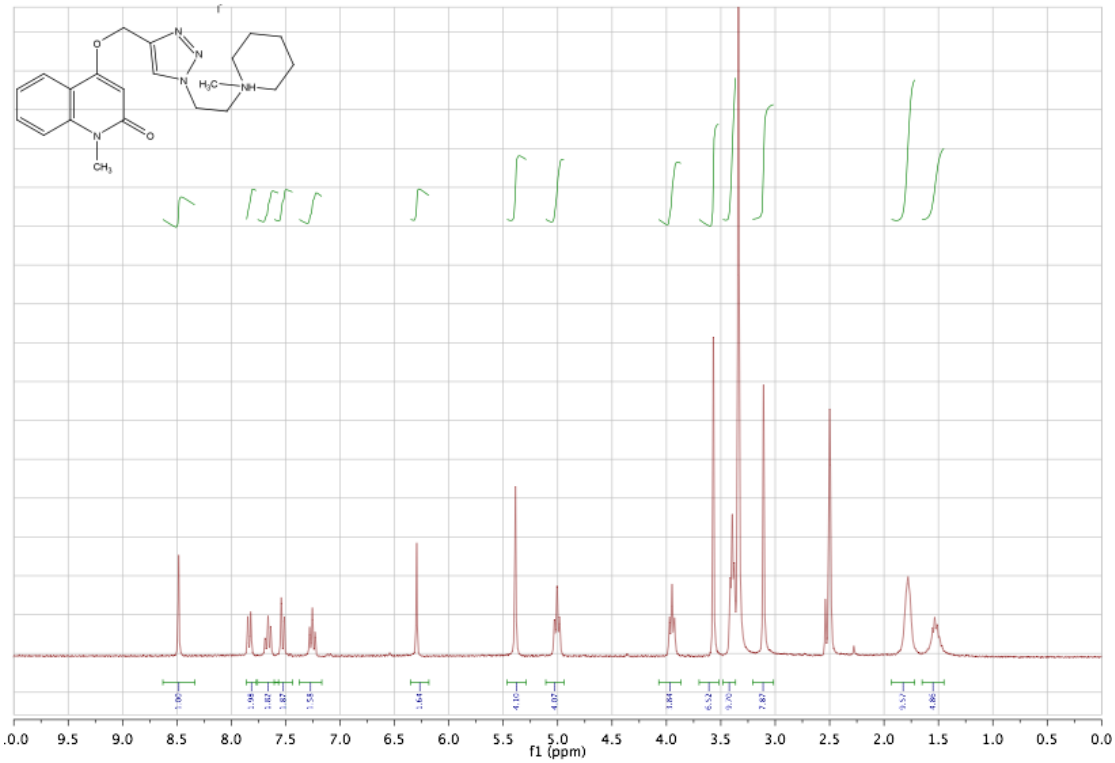



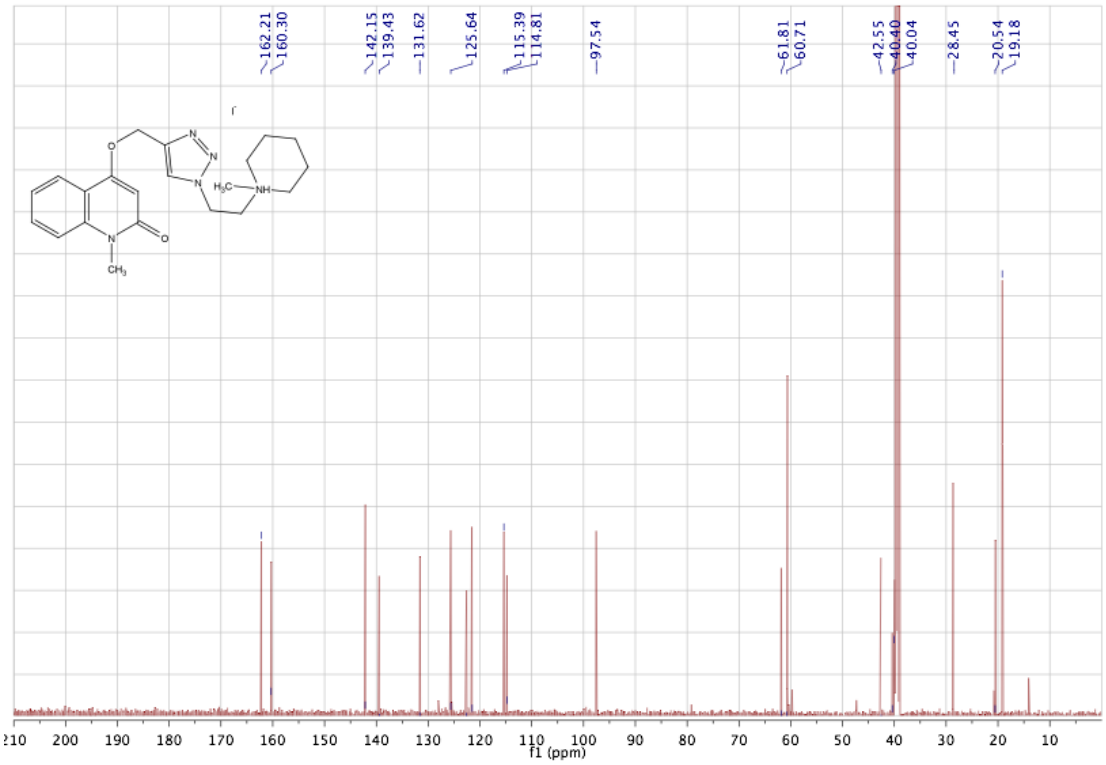
**14:**


f1 (ppm)

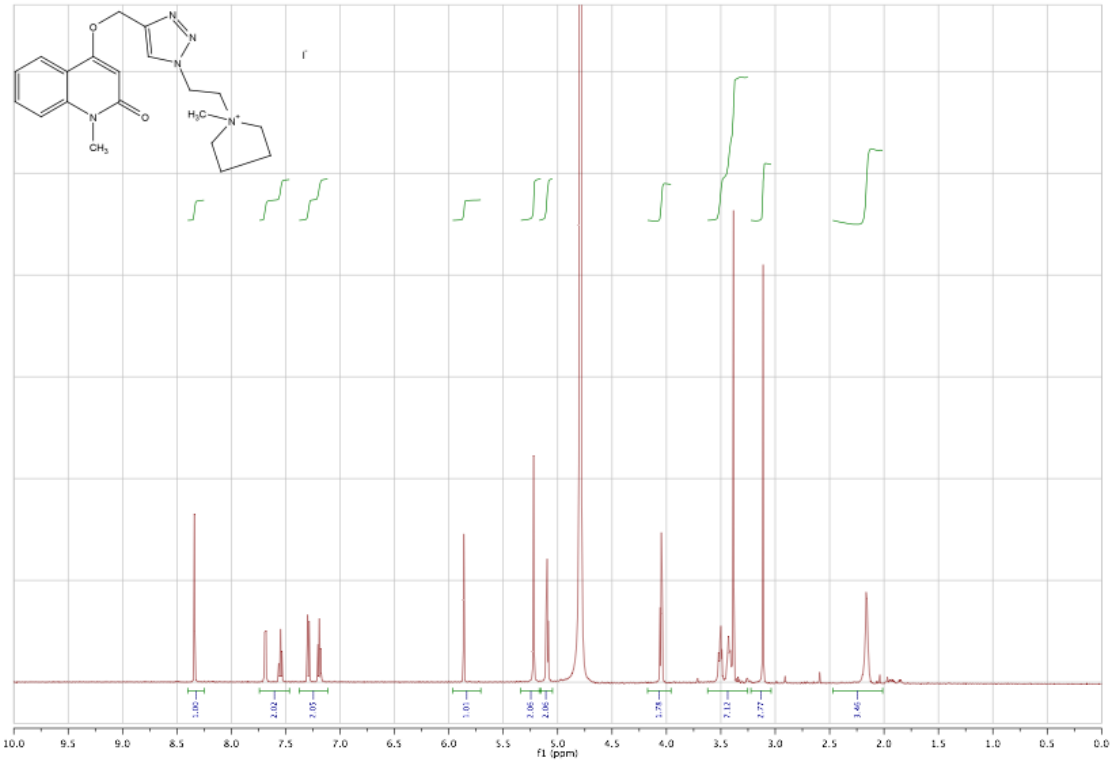

165.17
162.24
143.59
142.89
140.34
138.90
126.45
126.03
115.98
115.43
96.36
62.35
55.97
44.20
29.96
24.00
21.17
19.46
f1 (ppm)

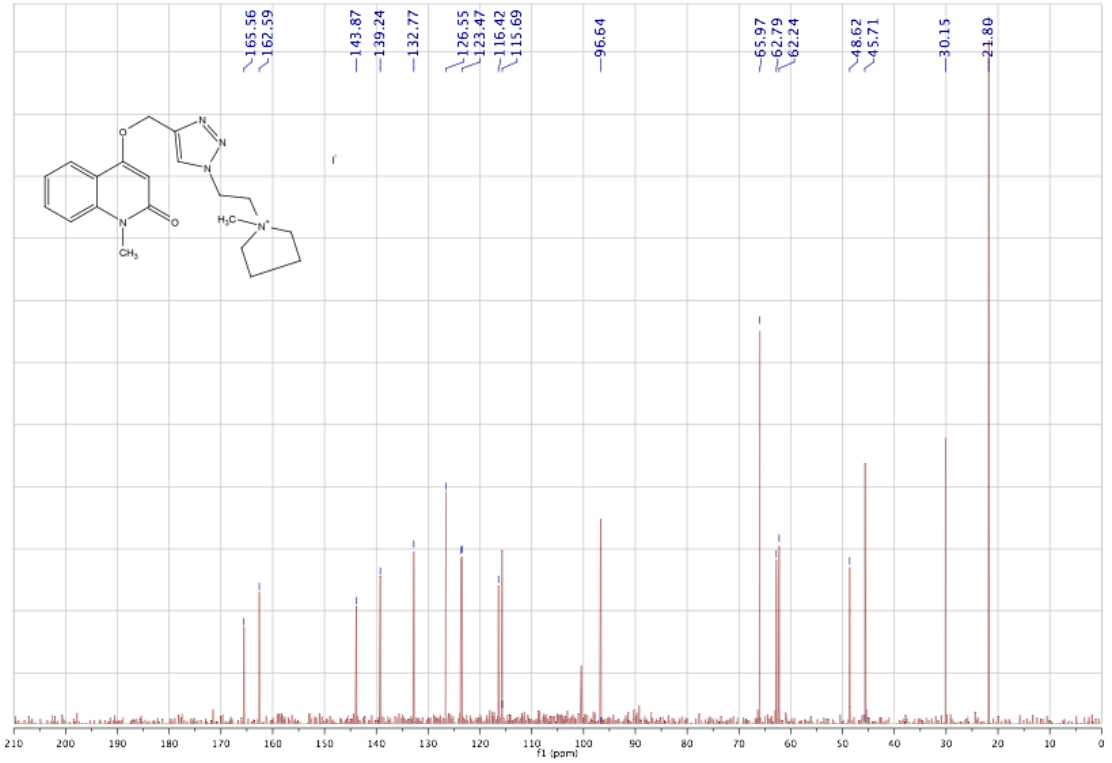
**15:**


f1 (ppm)

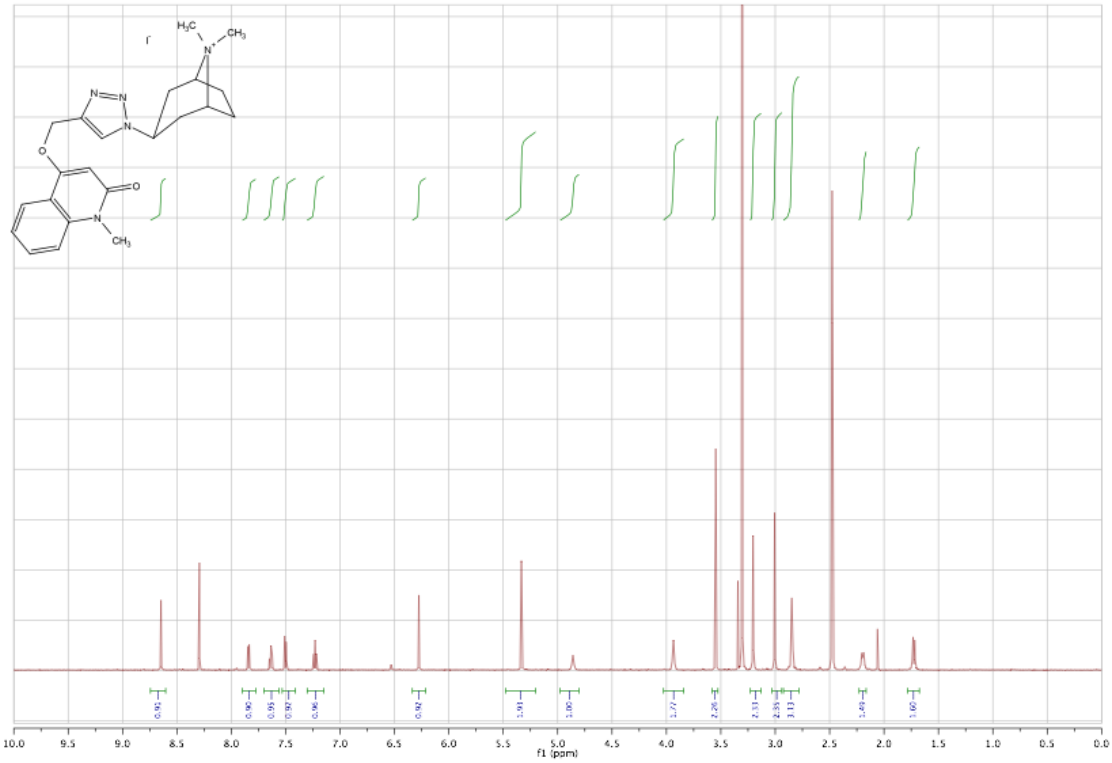

162.21
160.30
142.15
139.43
131.62
125.64
115.39
114.81
97.54
61.81
60.71
42.55
40.40
40.04
28.45
20.54
19.18
f1 (ppm)

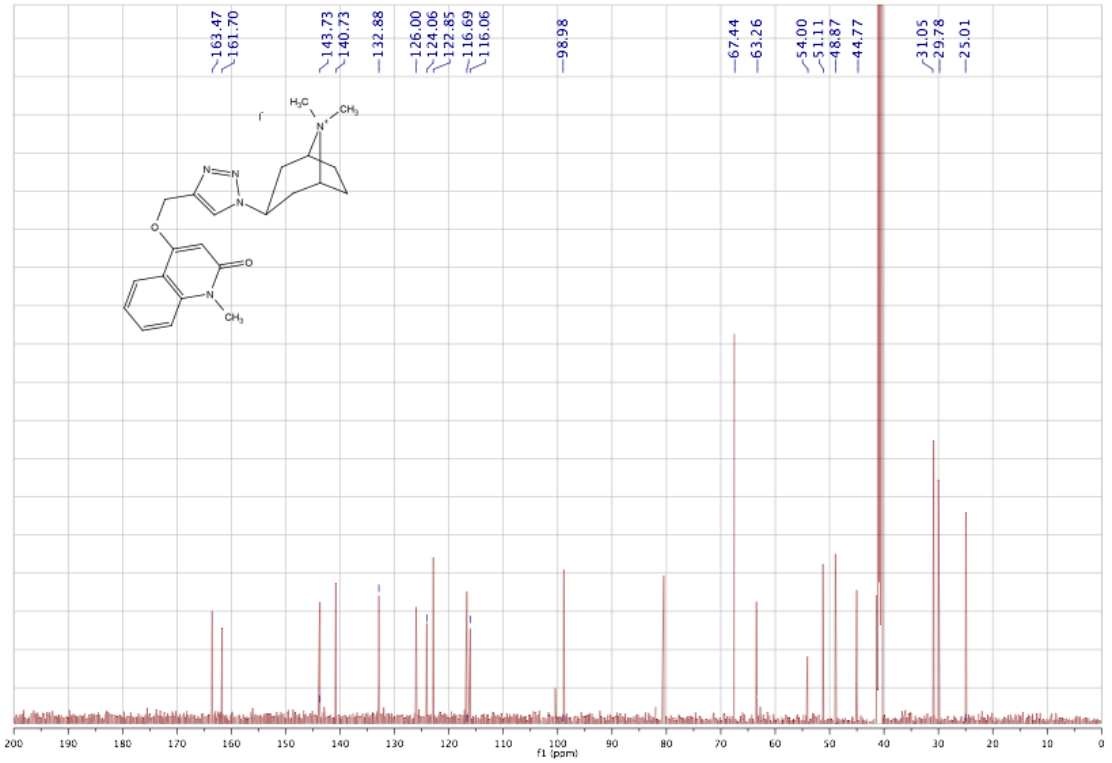
**16:**


f1 (ppm)

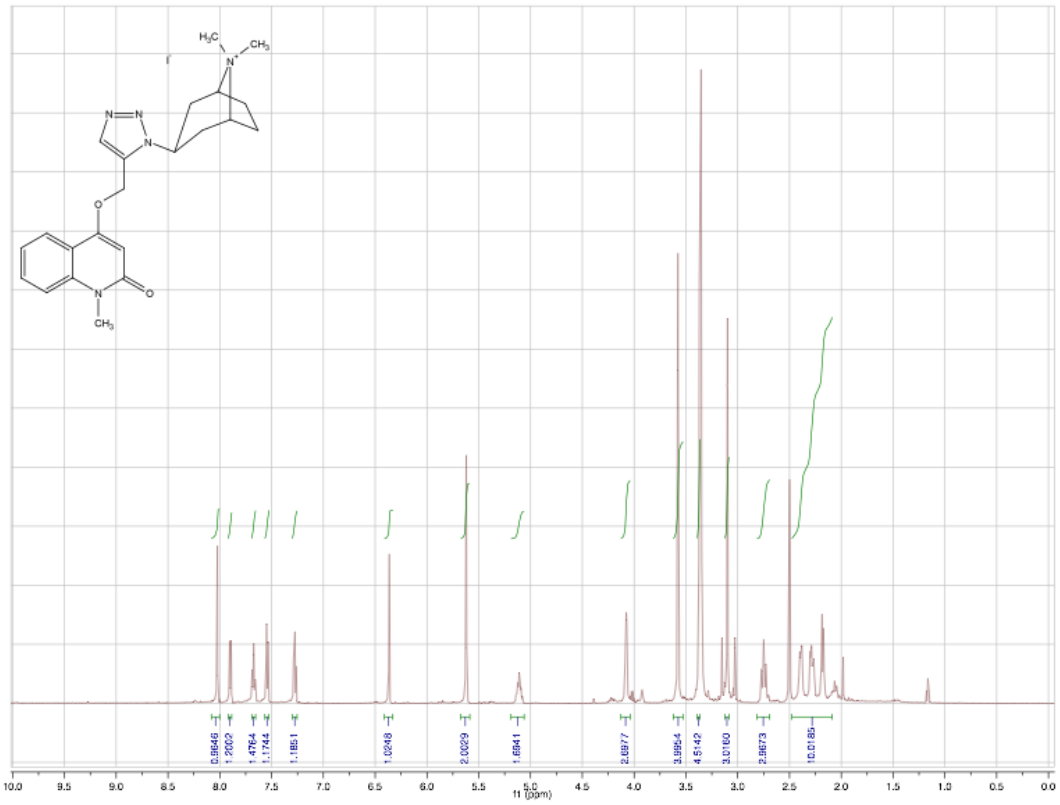

165.56
162.59
143.87
139.24
132.77
126.55
123.47
116.42
115.69
96.64
65.97
62.79
62.24
48.62
45.71
30.15
21.80
f1 (ppm)

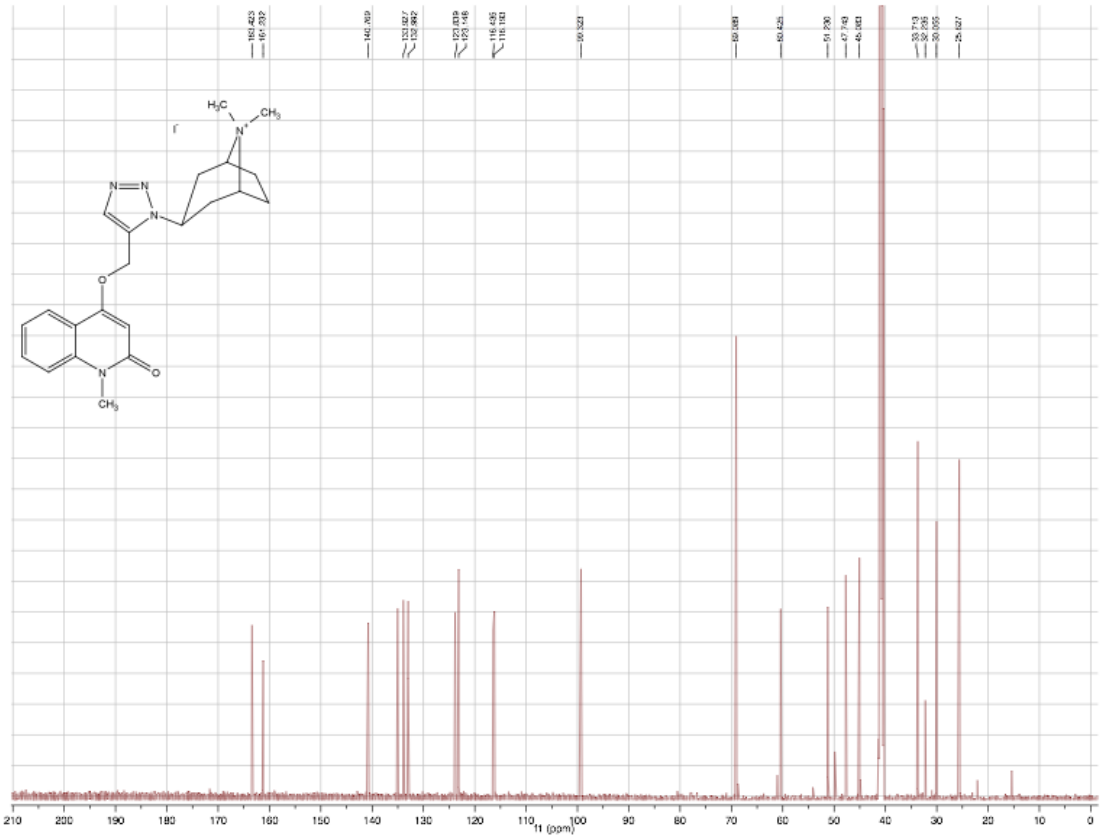
**18:**


f1 (ppm)

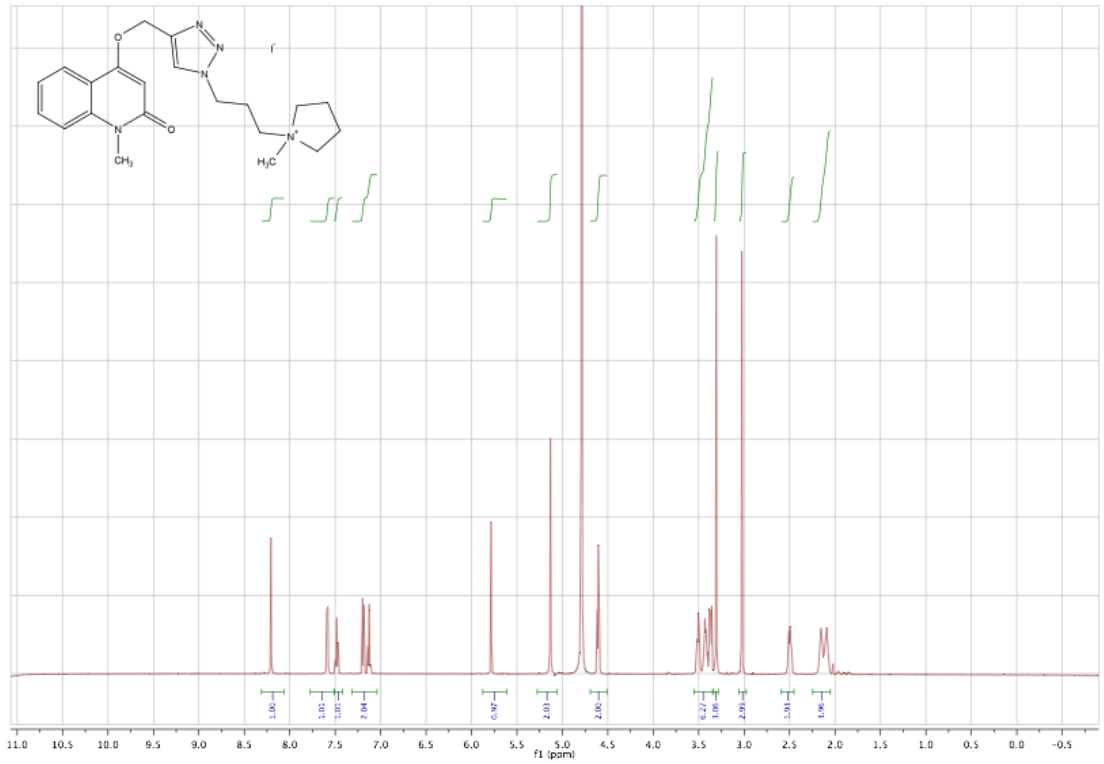

163.47
161.70
143.73
140.73
132.88
126.00
124.06
122.85
116.69
116.06
98.98
67.44
63.26
54.00
51.11
48.87
44.77
31.05
29.78
25.01
f1 (ppm)

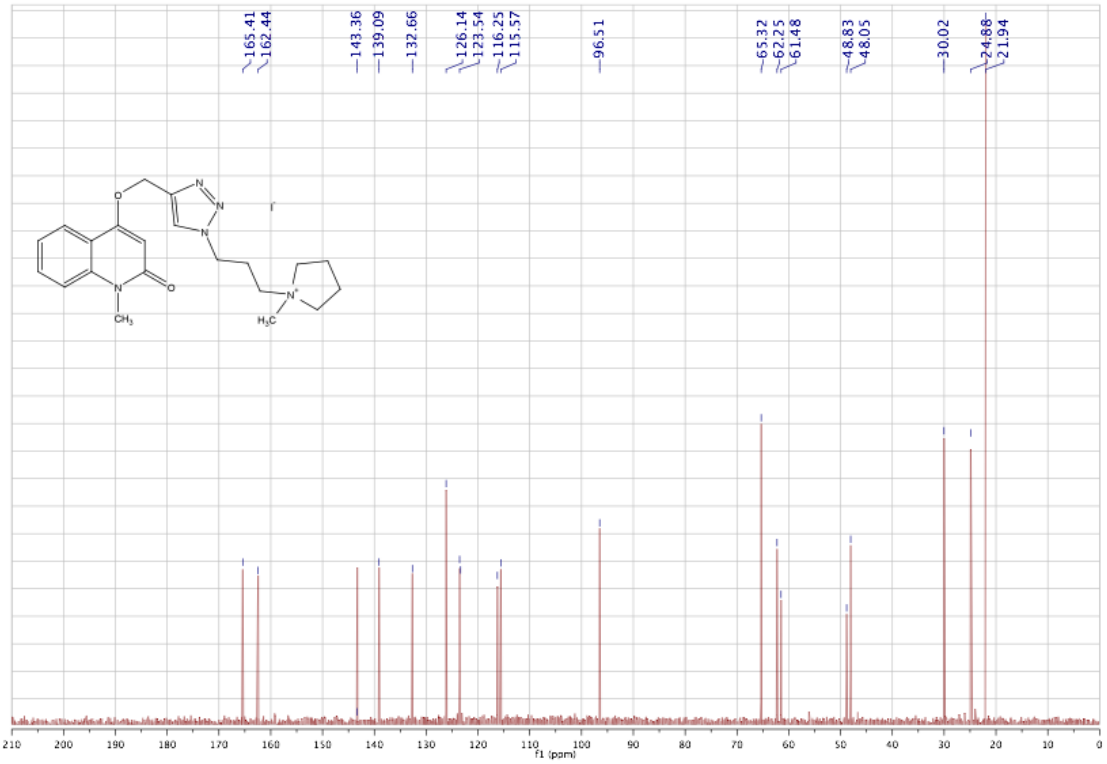
**18a:**


0.9646
1.2002
1.4764
1.1744
1.1851
1.0248
2.0029
1.6841
2.6977
3.9954
4.5142
3.0160
2.0673
10.0185
11 (ppm)

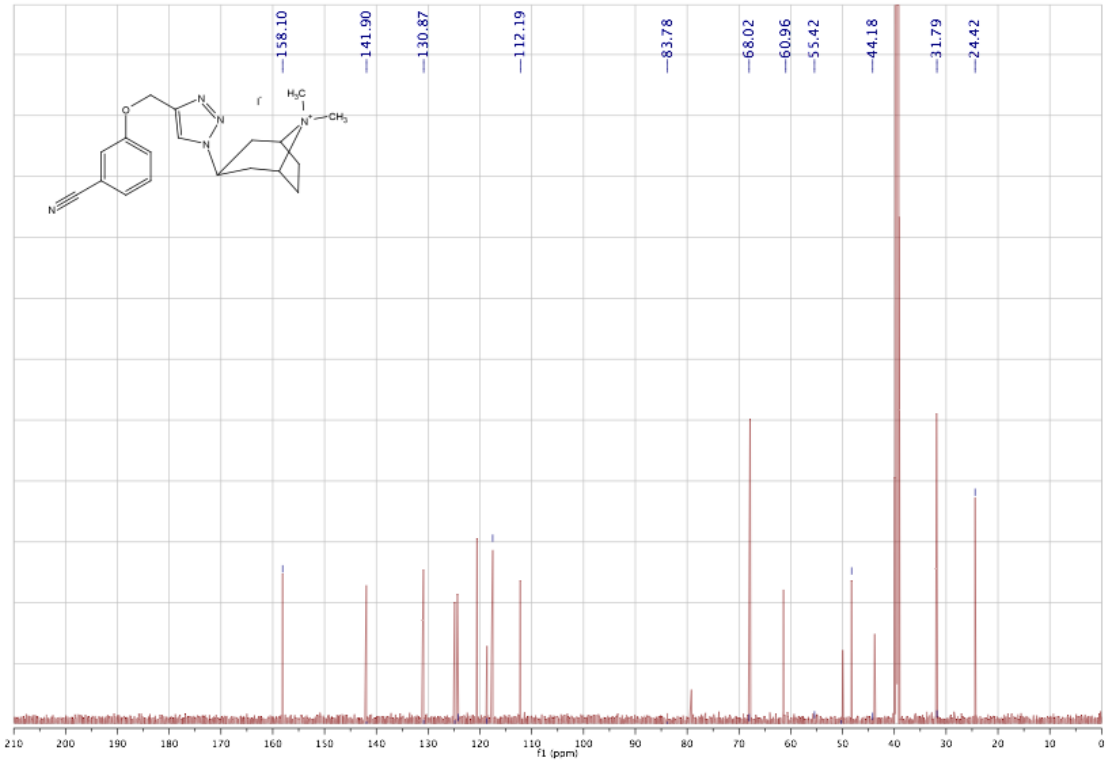

11 (ppm)

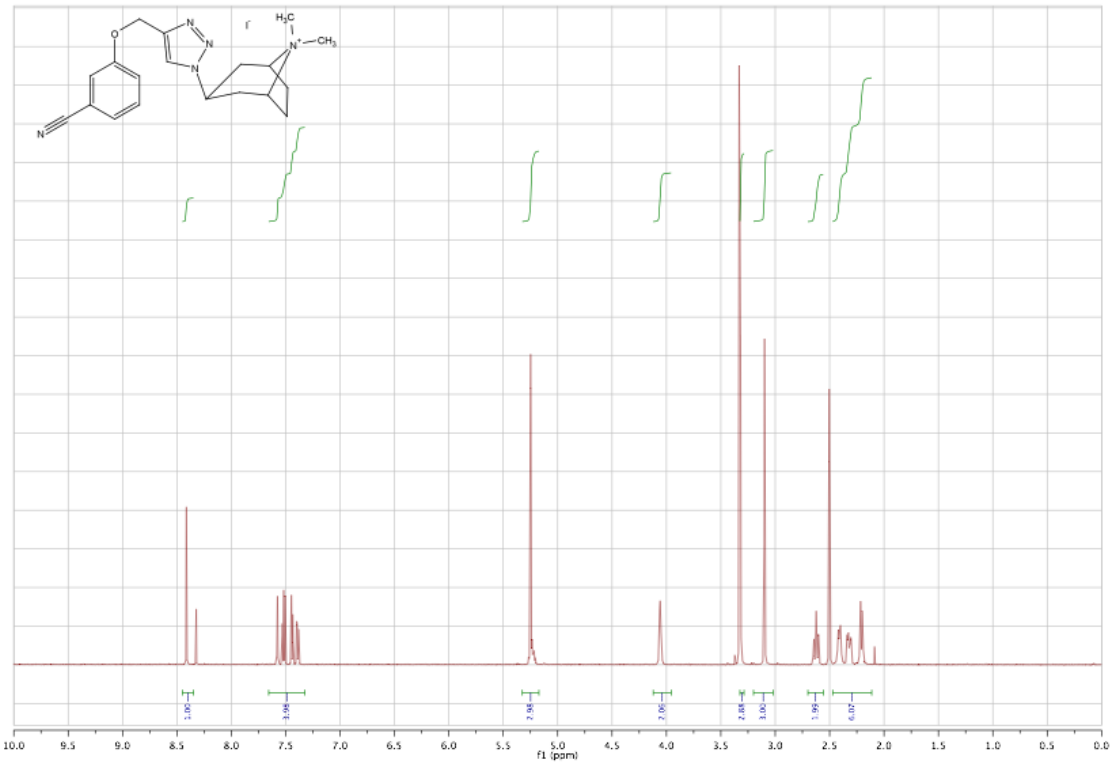
**19:**


f1 (ppm)

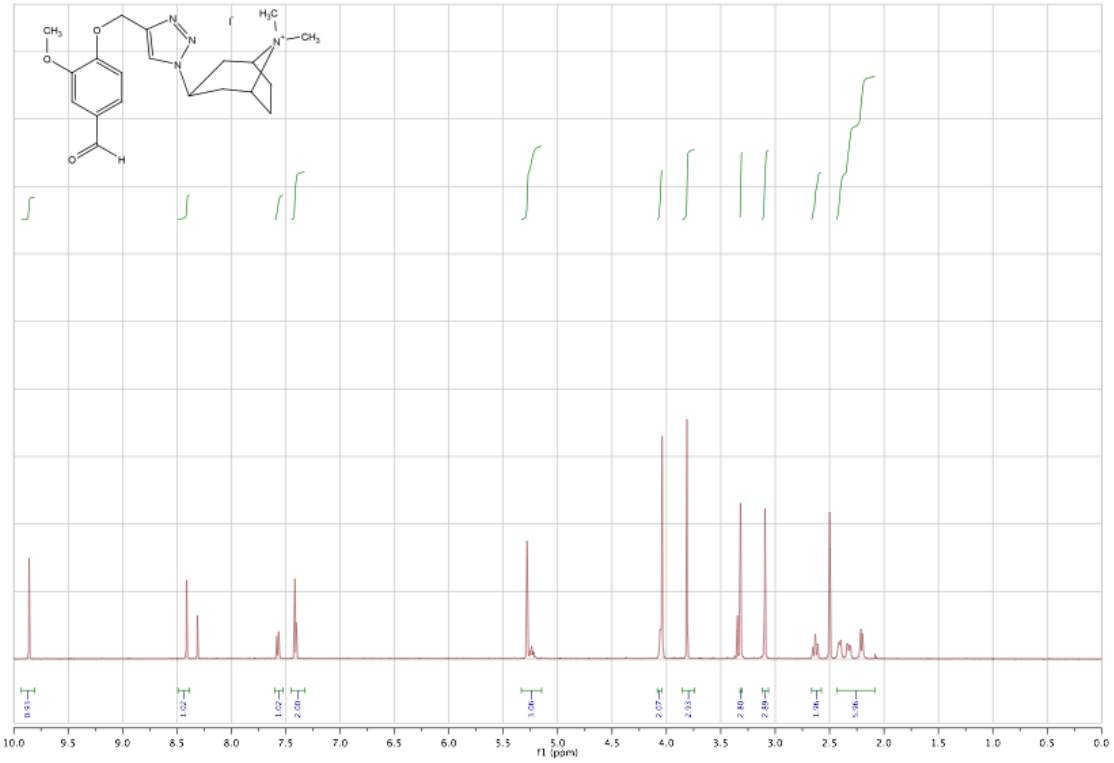

165.41
162.44
143.36
139.09
132.66
126.14
123.54
116.25
115.57
96.51
65.32
62.25
61.48
48.83
48.05
30.02
24.88
21.94
f1 (ppm)

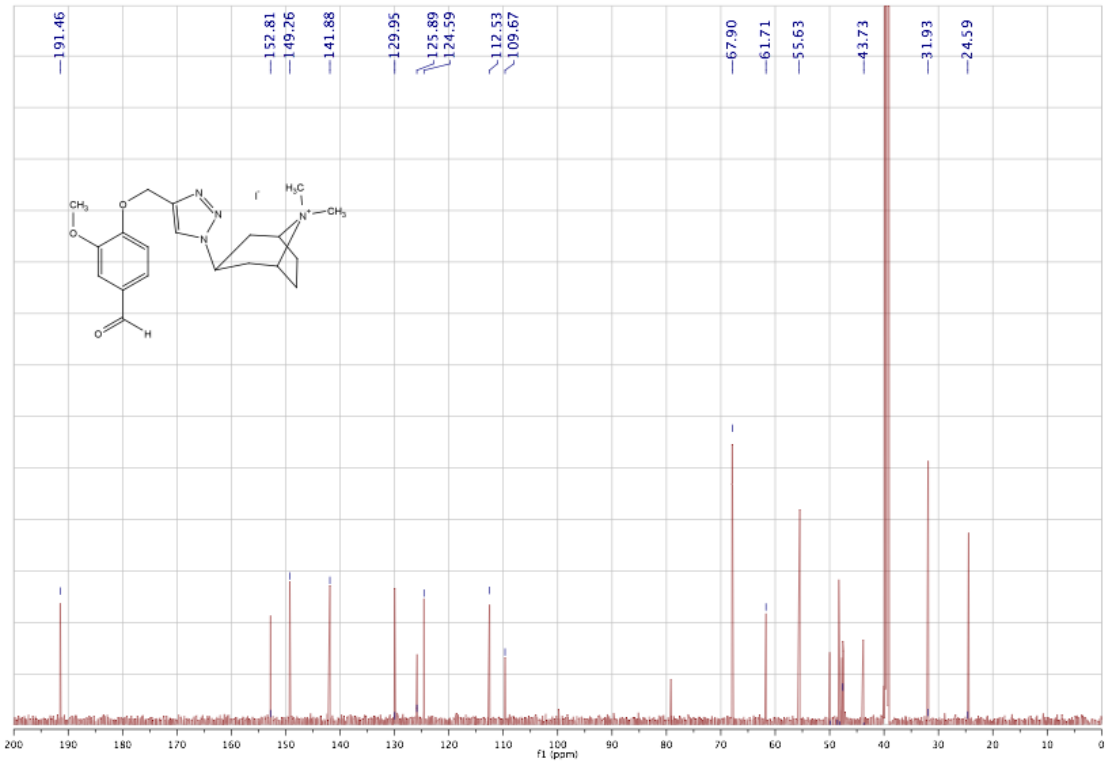
**27:**


158.10
141.90
130.87
112.19
83.78
68.02
60.96
55.42
44.18
31.79
24.42
f1 (ppm)

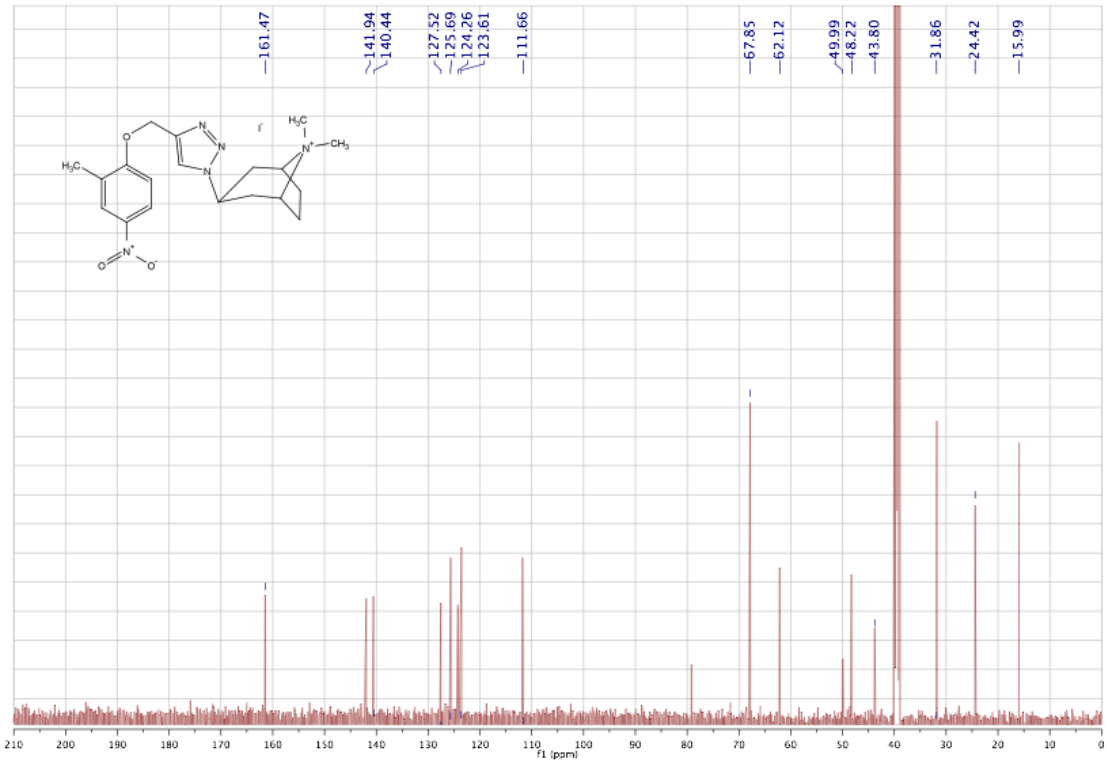

f1 (ppm)

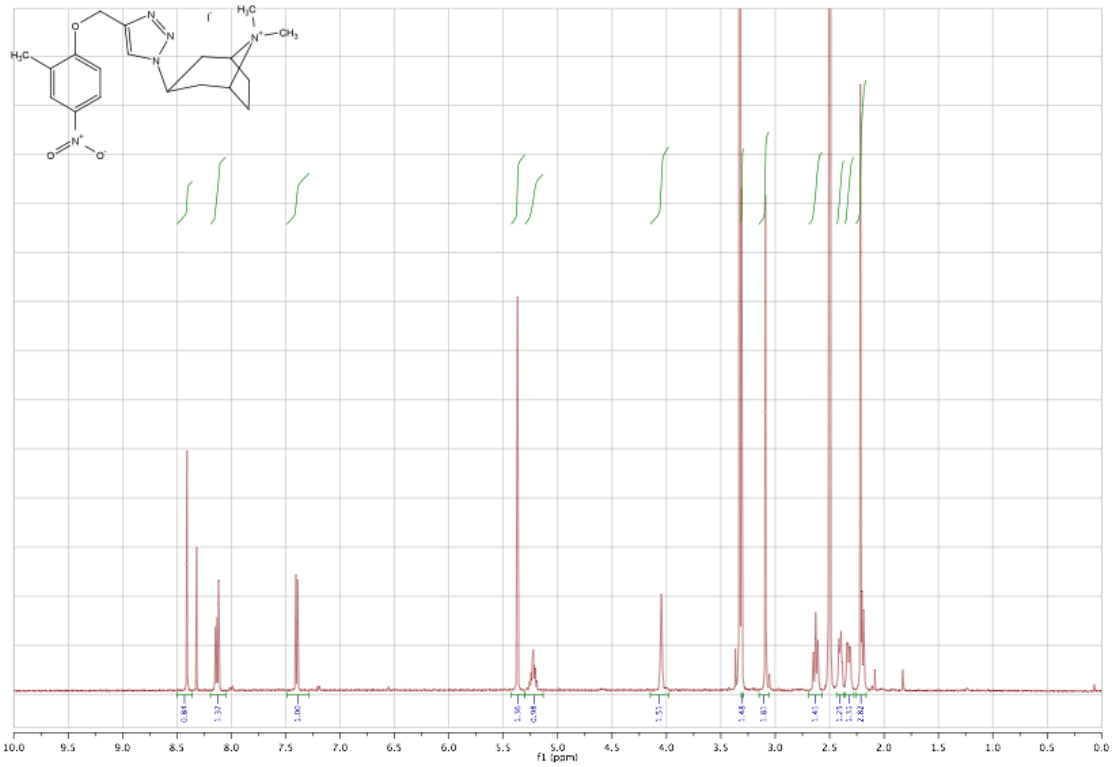
**28:**


f1 (ppm)

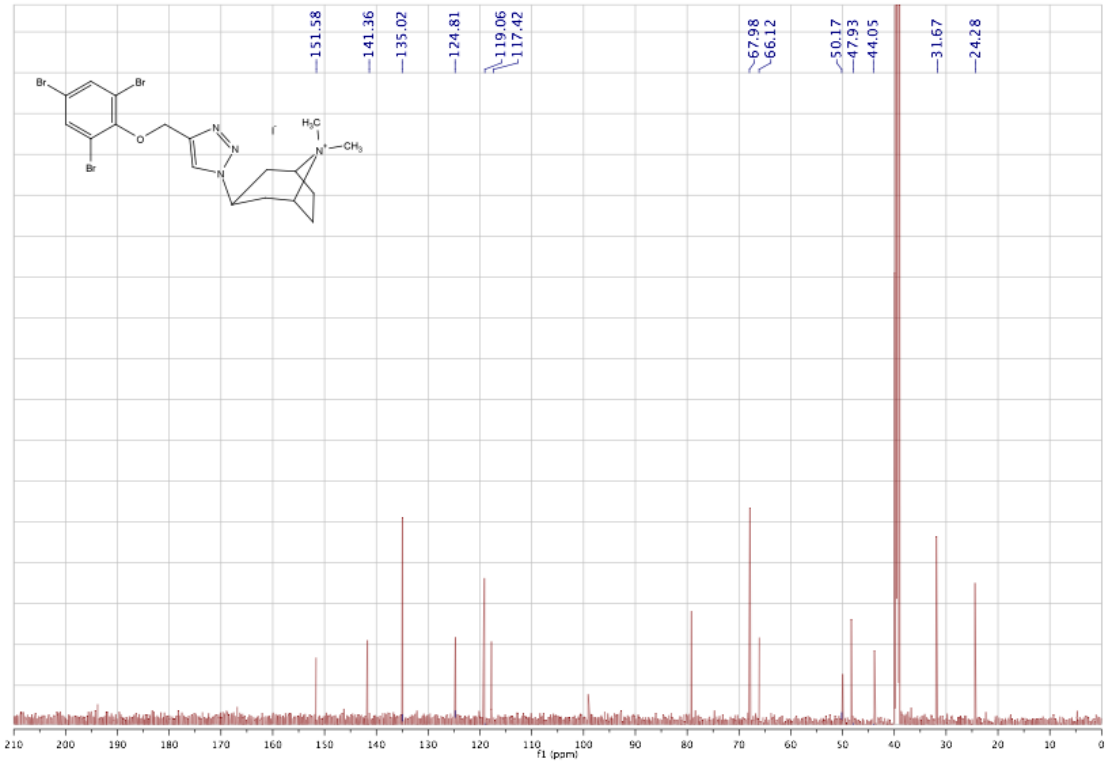

191.46
152.81
149.26
141.88
129.95
125.89
124.59
112.53
109.67
67.90
61.71
55.63
43.73
31.93
24.59
f1 (ppm)

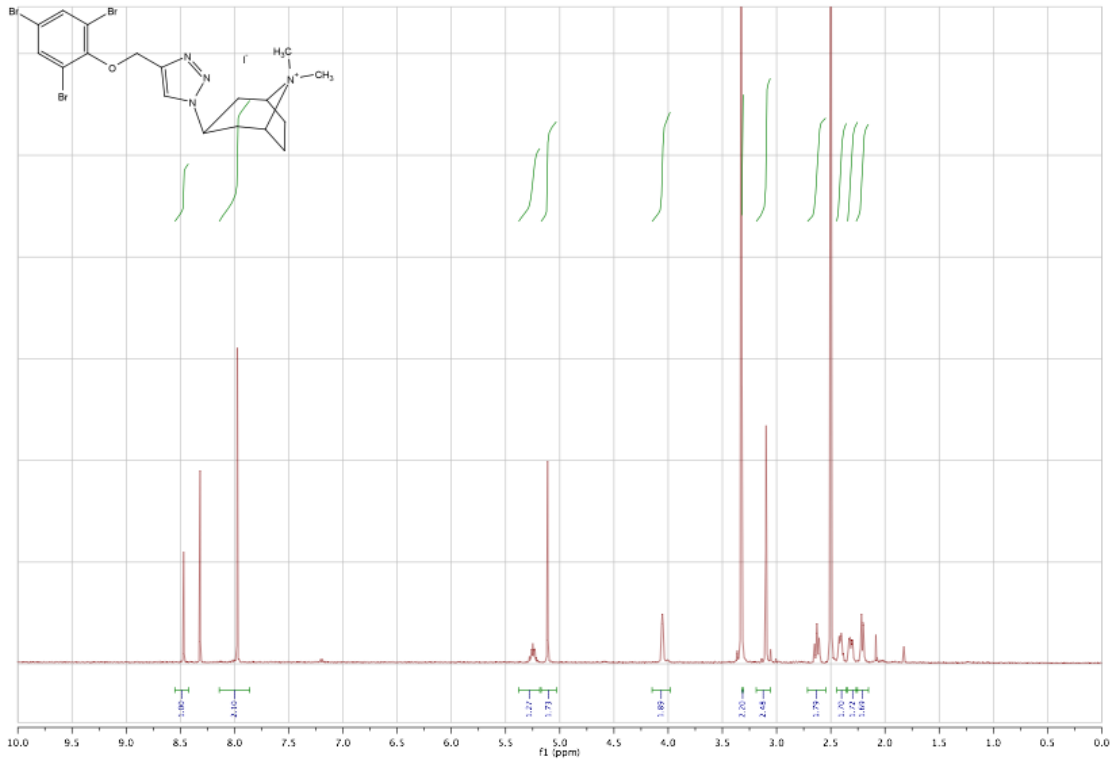
**29:**


161.47
141.94
140.44
127.52
125.69
124.26
123.61
111.66
67.85
62.12
49.99
48.22
43.80
31.86
24.42
15.99
f1 (ppm)



**30:**


151.58
141.36
135.02
124.81
119.06
117.42
67.98
66.12
50.17
47.93
44.05
31.67
24.28


Br
Br
Br
O
N
N
N
I⁻
H3C
N+
CH3
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

## Protein crystallography

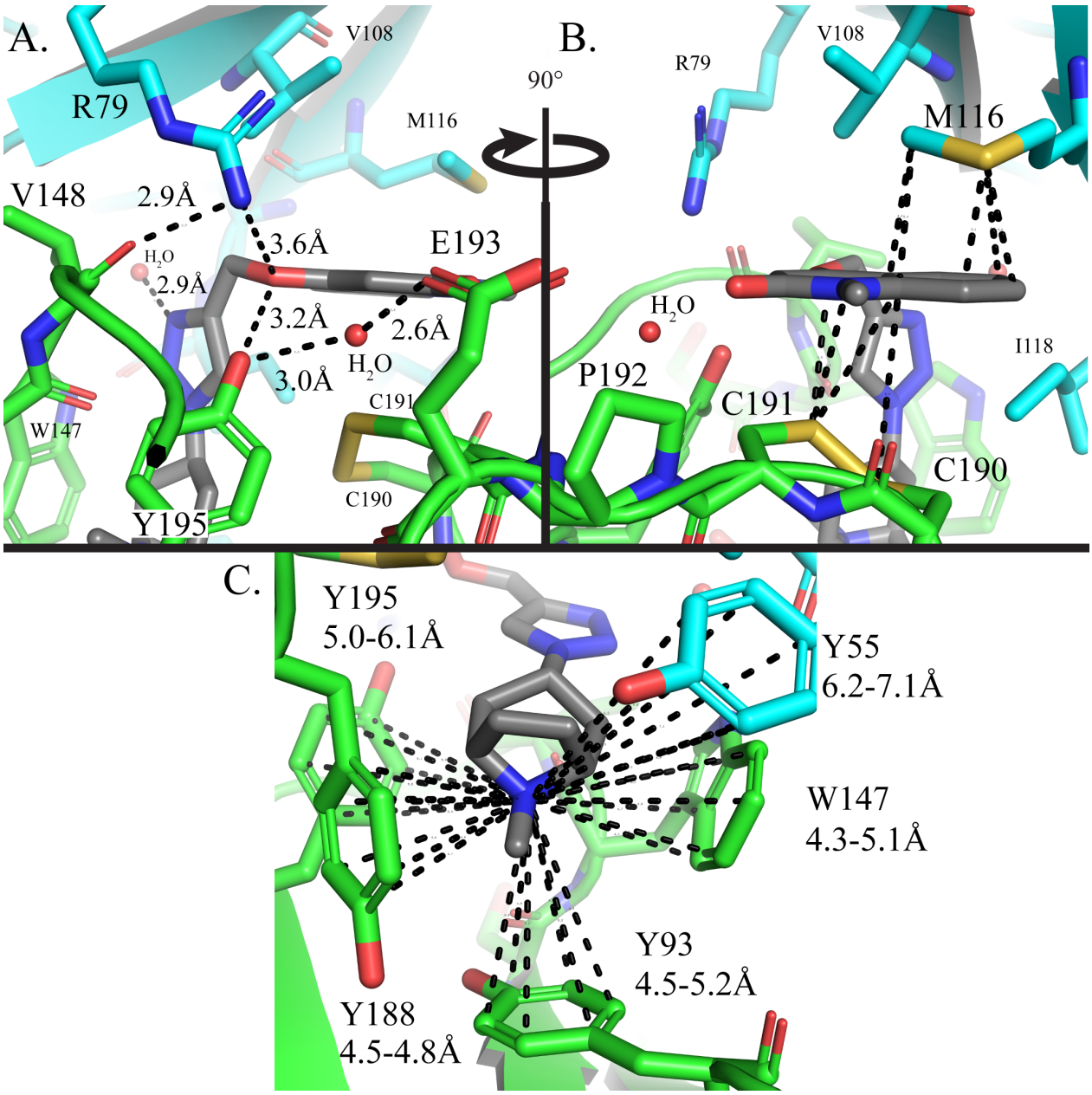
| Data set | *Ac*-AChBP/(**18**) |
|---|---|
| Space group | $P2_12_12_1$ |
| Cell constants (Å) | a=88.3 b=115.1, c=131.4 α=β=γ=90 |
| Wavelength (Å) | 1.0 |
| Resolution (Å) | 43.29 – 2.3 |
| Total/unique reflections | 472547/57215 |
| Average redundancy | 8.0(6.6) |
| Completeness (%) | 98.5 (94.9) |
| $\langle I \rangle / \langle \sigma_I \rangle$ | 28.0 (2.56) |
| $R_{sym}$[◊] | 0.075 (0.473) |
| $R_{work}$ (%) | 19.42 (24.68) |
| $R_{free}$[¶] (%) | 25.18 (33.95) |
| Overall B value( Å$^2$) | 36.15 |
| Rmsd bond length (Å) | 0.013 |
| Rmsd bond angle(°) | 1.185 |

[◊] $R_{sym} = \Sigma_h \Sigma_i |I(h) - I(h)_i| I \Sigma_h \Sigma_i I(h)_i$, where $I(h)$ is the mean intensity after rejections.

[§] Numbers in parentheses correspond to the highest resolution shell of data, which were 2.34 to 2.30 for reflections and 2.357 to 2.3 for refinement

[‖] $R_{work} = \Sigma_h ||F_{obs}(h)| - |F_{calc}(h)|| I \Sigma_h |F_{obs}(h)|$; no $I/\sigma$ cutoff was used during refinement.

[¶] 5.0% of the observed intensities was excluded from refinement for cross validation purposes.




**Supplementary Figure 1: Molecular interactions of complex 18 with *Ac*-AChBP from binding site with best occupancy and electron density.**
The primary subunit is colored in green, whereas the complementary subunit is in cyan. Only residues within 4Å of ligand **18** are shown. A.) Hydrogen bonding network from ether oxygen of **18**. The length of the distances from Arg79 and Tyr195 show a very weak interaction with the ligand, elaborated by the weak density found for the quinolinone in many of the binding pockets (Figure 3A). B.) Select distances shown range from 3.1-5.0 Å, to demonstrate hydrophobic interactions between disulfide of Cys190-191, quinolinone of ligand, and Met116. C.) Aromatic nest of residues interacting with aza-nitrogen in the dimethyl-tropane. Distances are the ranges found from the nitrogen to each carbon on the aromatic ring. Tyr93, Tyr188, and Trp147 are within cation-π distances (4.0-5.0 Å nitrogen to center of aromatic ring), whereas Tyr55 and Tyr195 are more distant.