Supporting Information

Targeting cytotoxic agents through EGFR-mediated covalent binding and release

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Supplementary Figures

Table S1. WT and DM EGFR potencies determined by TR FRET (n=1, 2 technical replicates).

Compound	DM EGFR pIC_{50}	WT EGFR pIC_{50}
30	6.8	5.7
35	5.5	<4.0
39	3.7	<4.0
48	6.7	6.6
49	6.4	5.6
50	6.6	5.9
51	n.d.	6.6

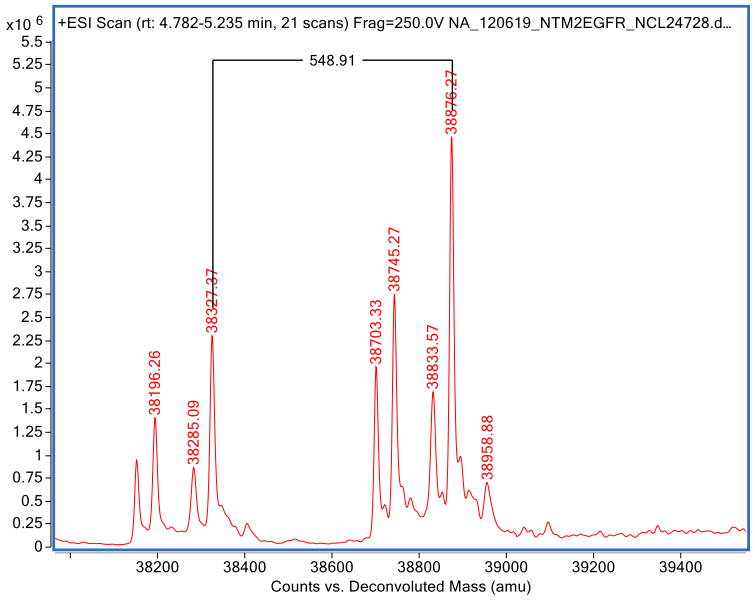


Figure S1. Protein mass spectrometry spectrum showing the mass addition of 548 to the protein, corresponding to the addition of the intact hybrid **30** without 5FU release.

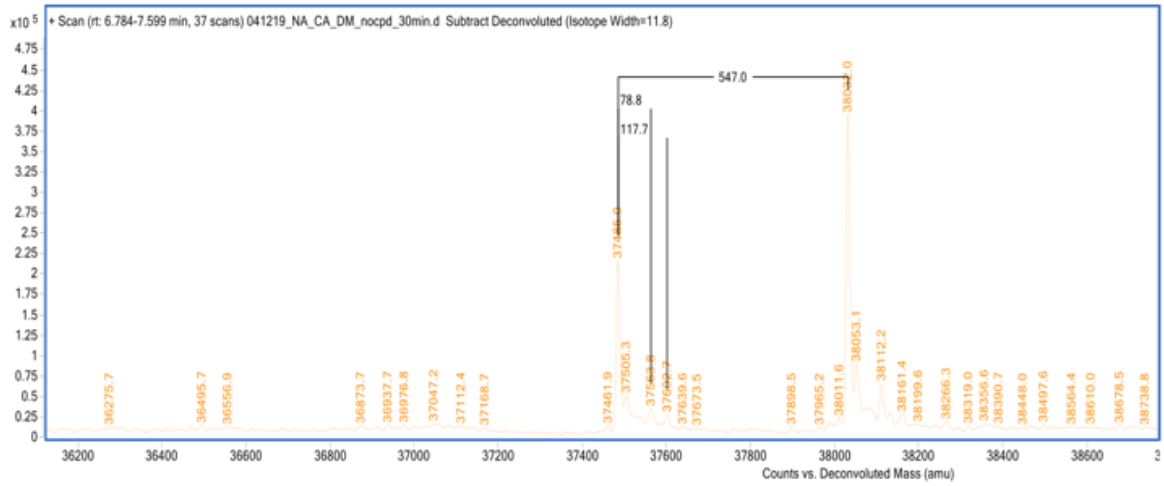


Figure S2. Protein mass spectrometry spectrum showing the mass addition of 547 to the protein, corresponding to the addition of the intact hybrid **35** without 5FU release.

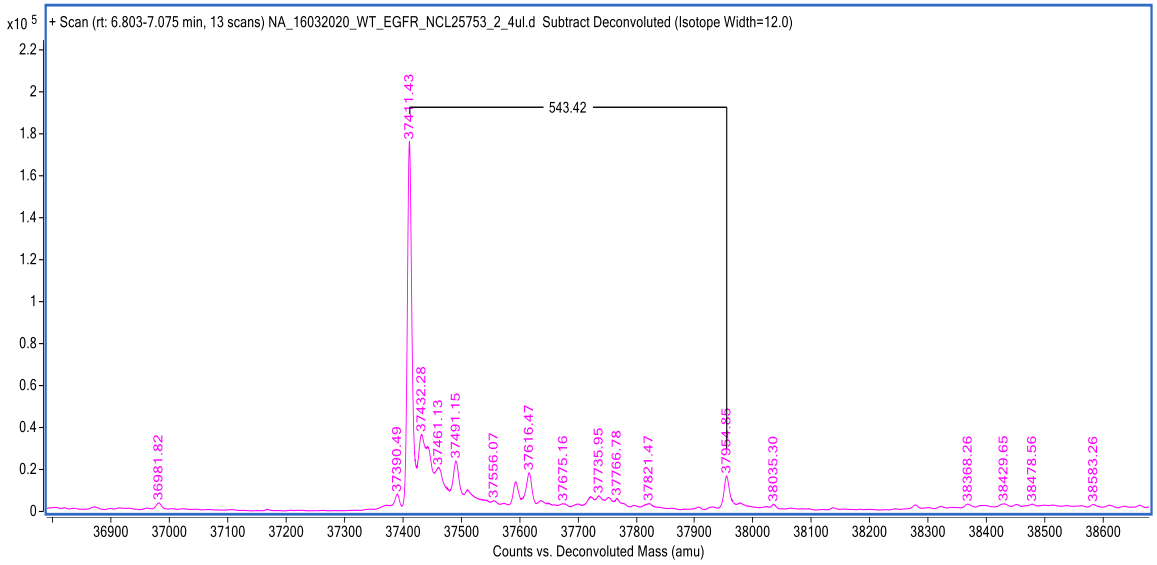


Figure S3. Protein mass spectrometry spectrum showing the addition of 543 Daltons to the protein, corresponding to the addition of the hybrid **39** without release of 5FU.

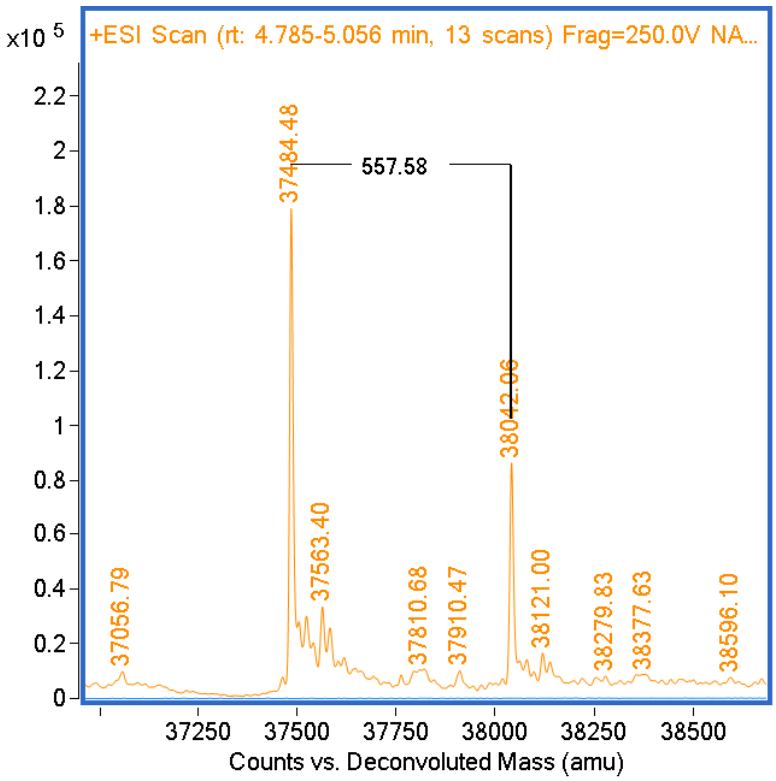


Figure S4. Protein mass spectrometry spectrum showing the addition of 557 Daltons to the protein, corresponding to the addition of the conjugate **48** without release of 5FU.

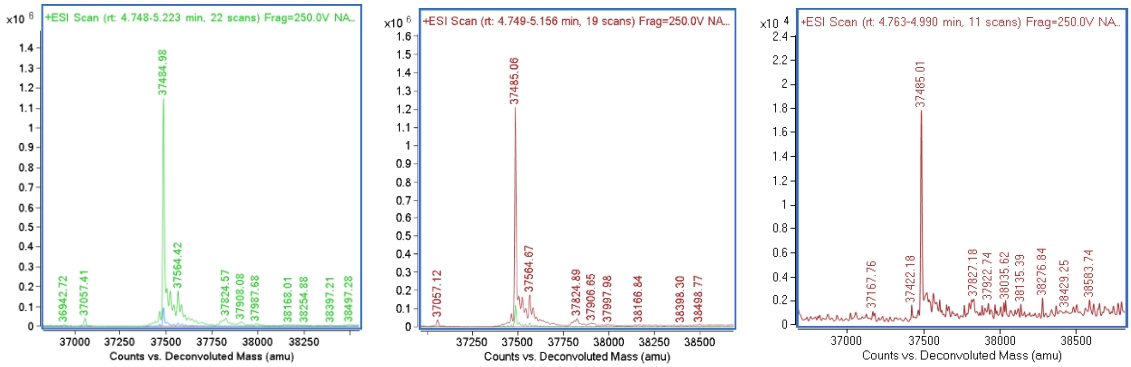
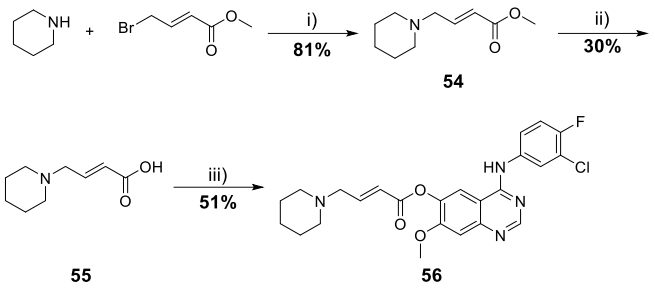


Figure S5. Protein mass spectrometry spectra of incubation of **49-51** with EGFR protein without release of 5FU.



Scheme S1. Synthesis of the acrylate analogue of dacomitinib, **56**. Reagents and conditions: i) K_2CO_3, DCM, rt, 4.5 h; ii) 2 M aq. NaOH, THF, rt, 18 h; iii) %FU, DCC, DMAP; DMF, 0 °C-rt, 18 h.

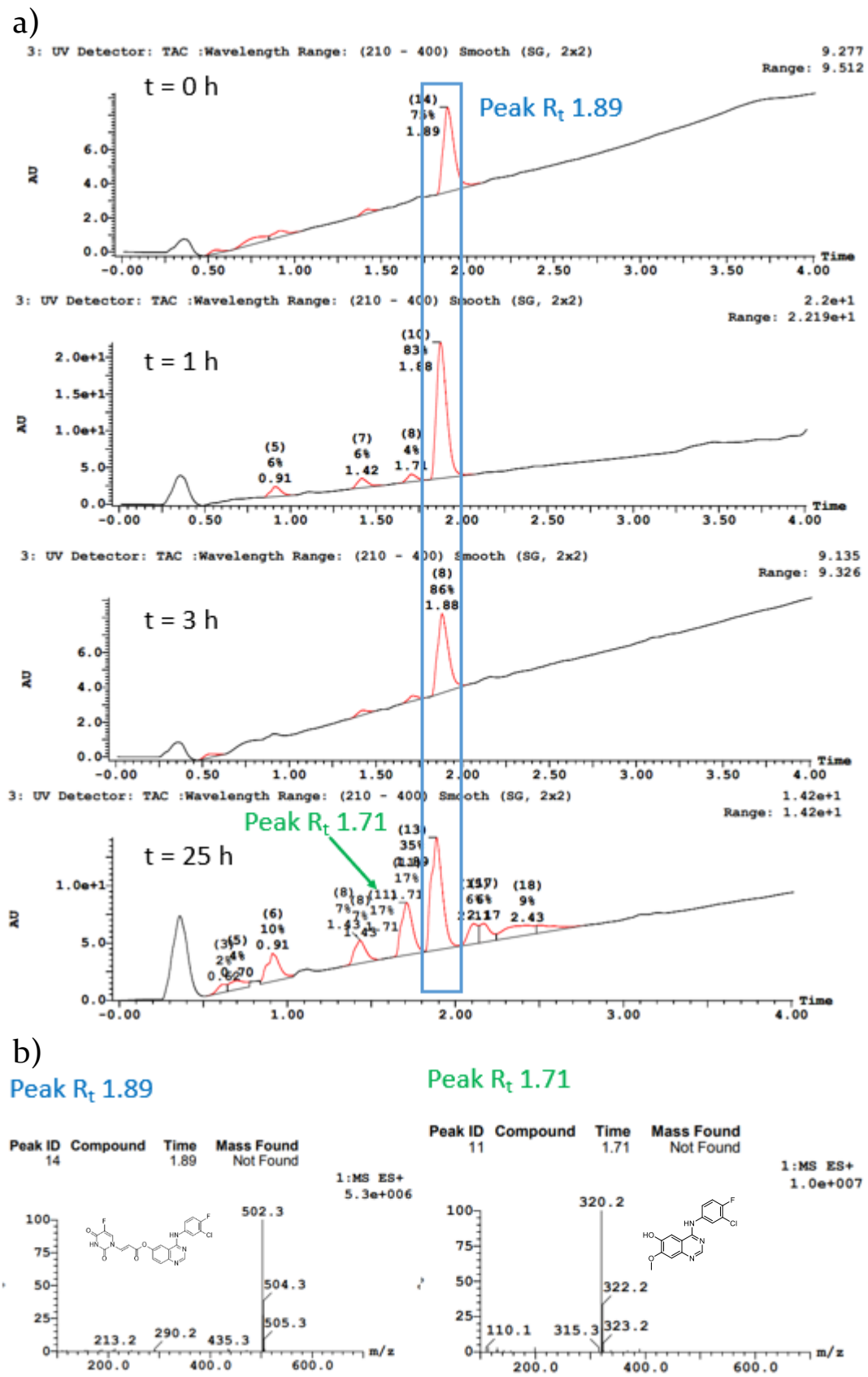
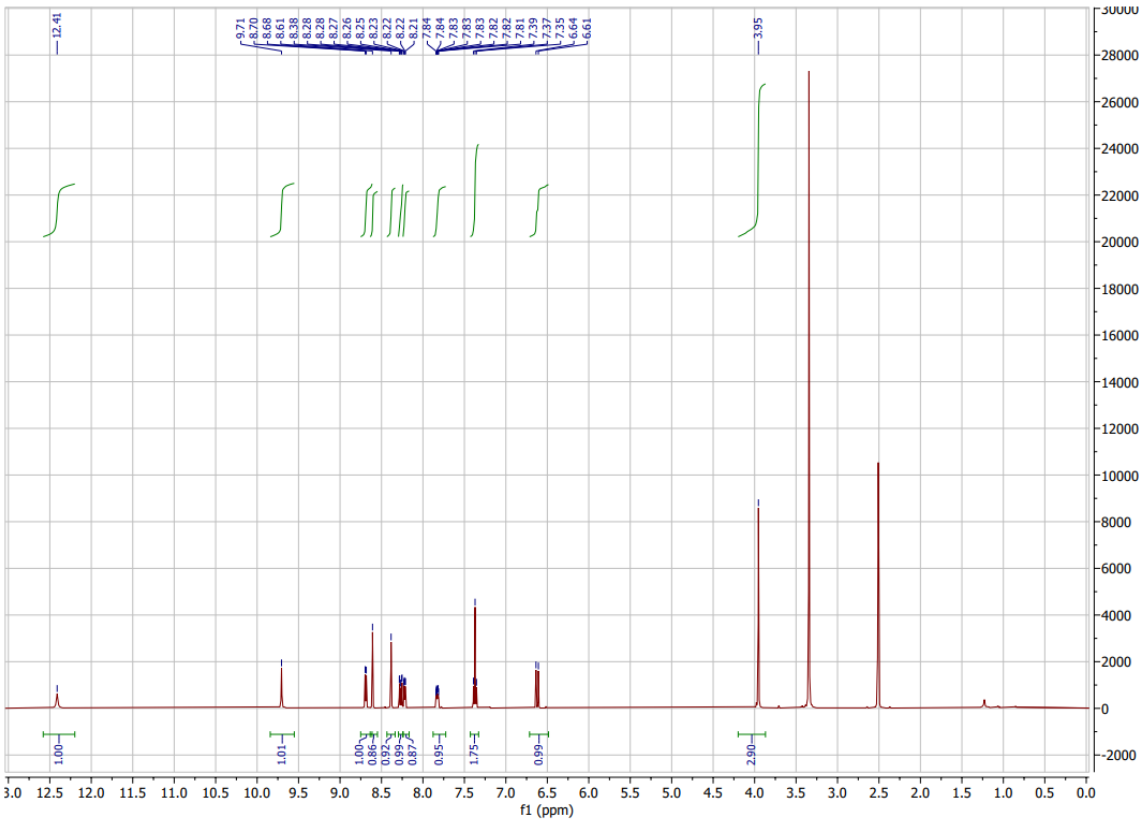
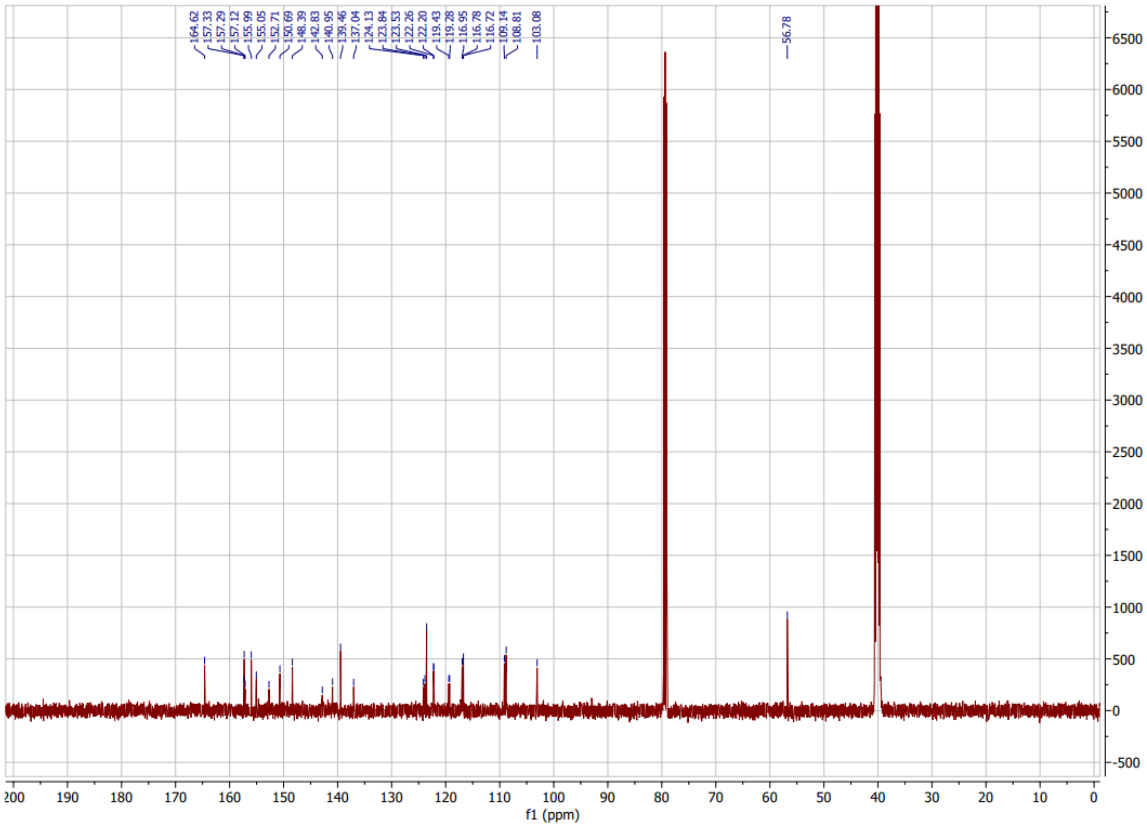


Figure S6. a) LCMS traces of compound **53** incubated in media and analyzed at t = 0, 1, 3 & 25 hours by LCMS analysis; b) mass spectra of main peaks and corresponding structures.

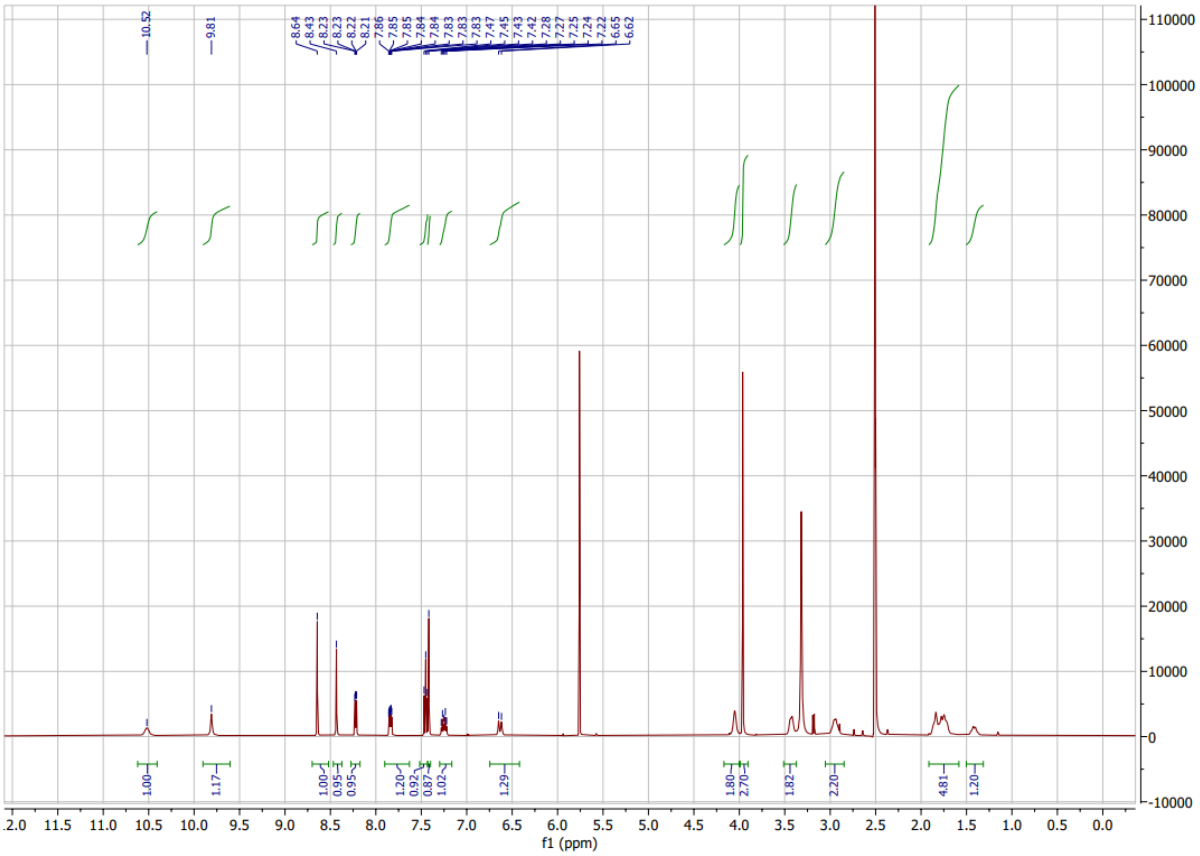
NMR Spectra

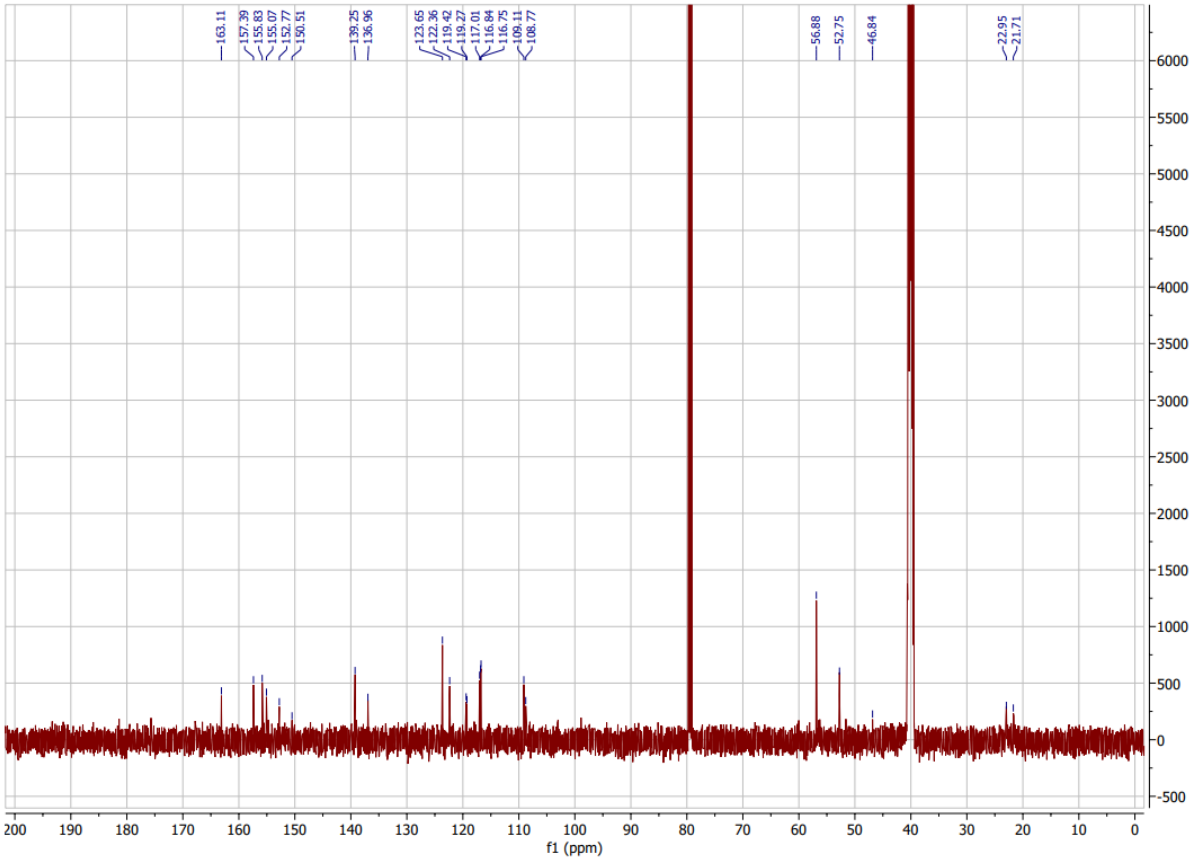
NMR data for compound 53



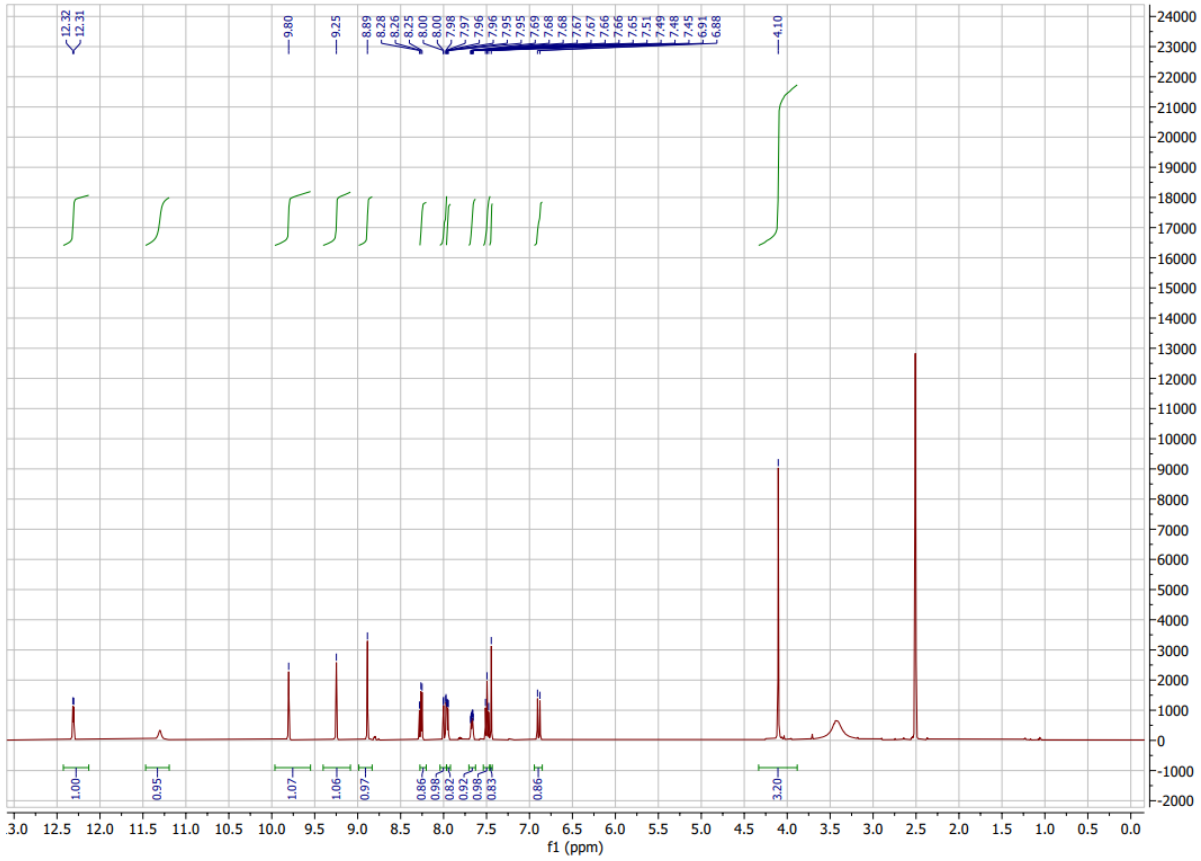


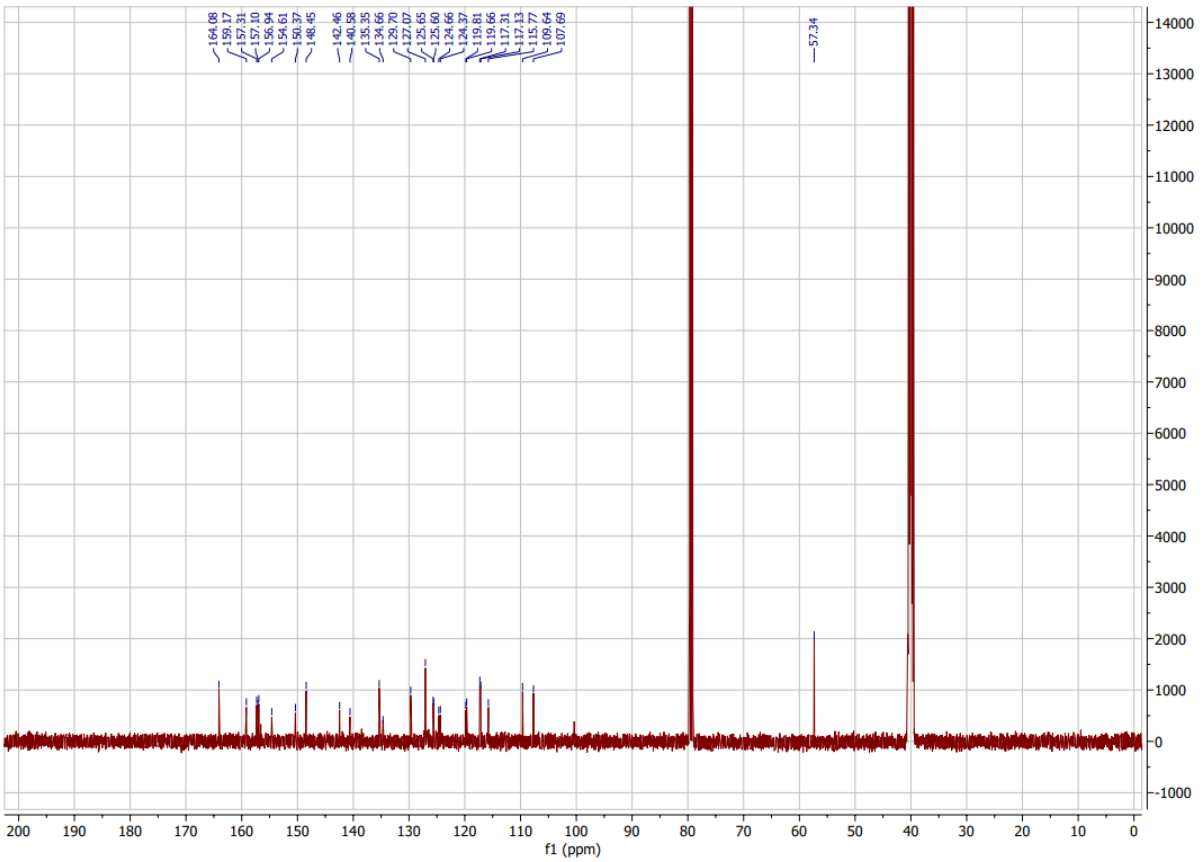
NMR data for compound 56



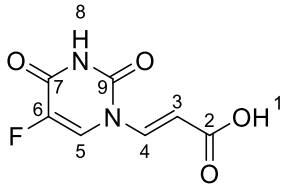


NMR data for compound 57

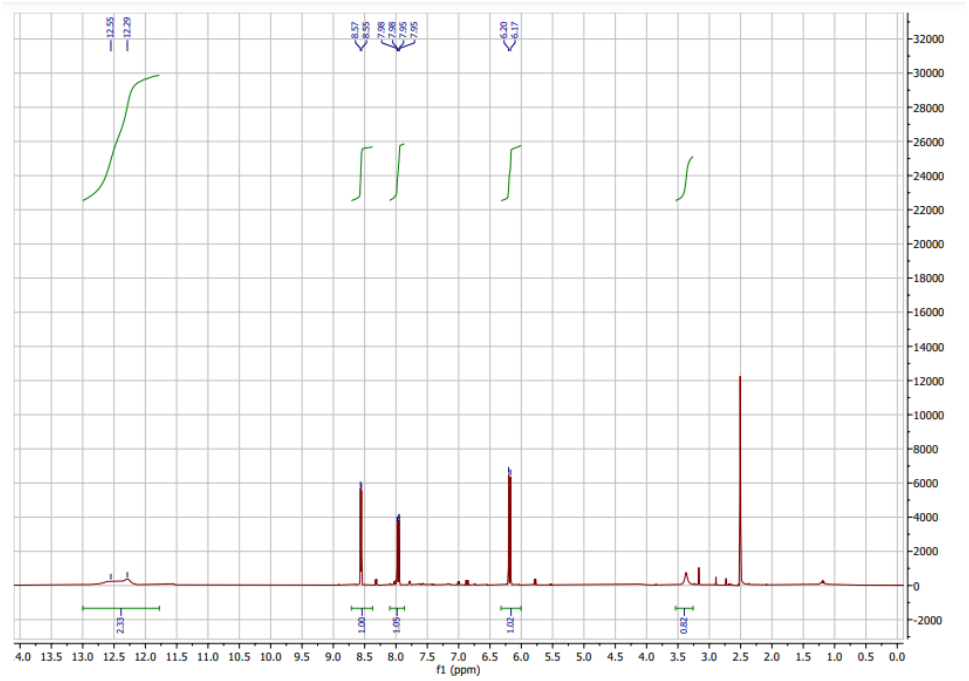




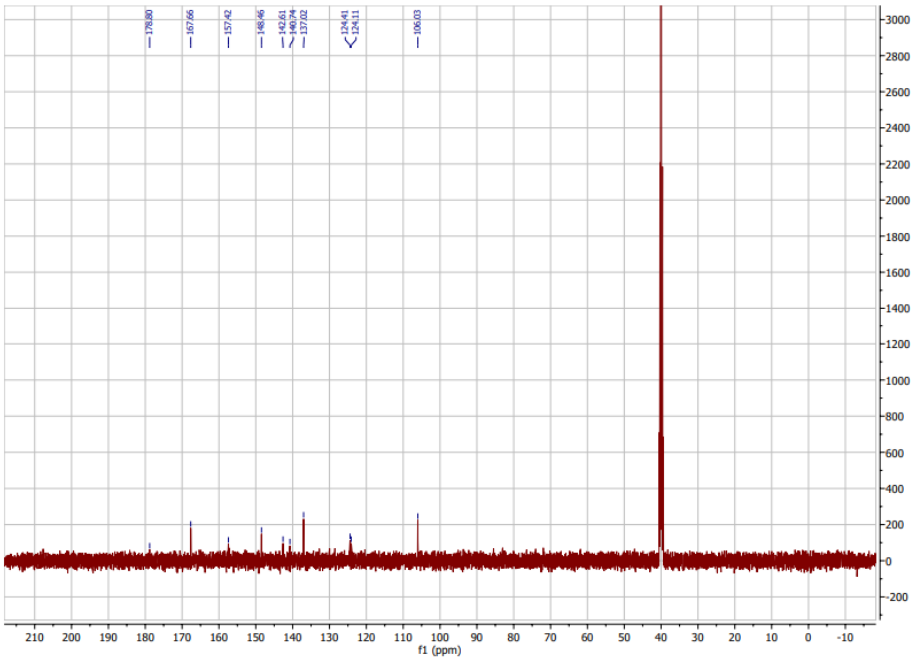
Assignment of 5FU addition – Compound **16**

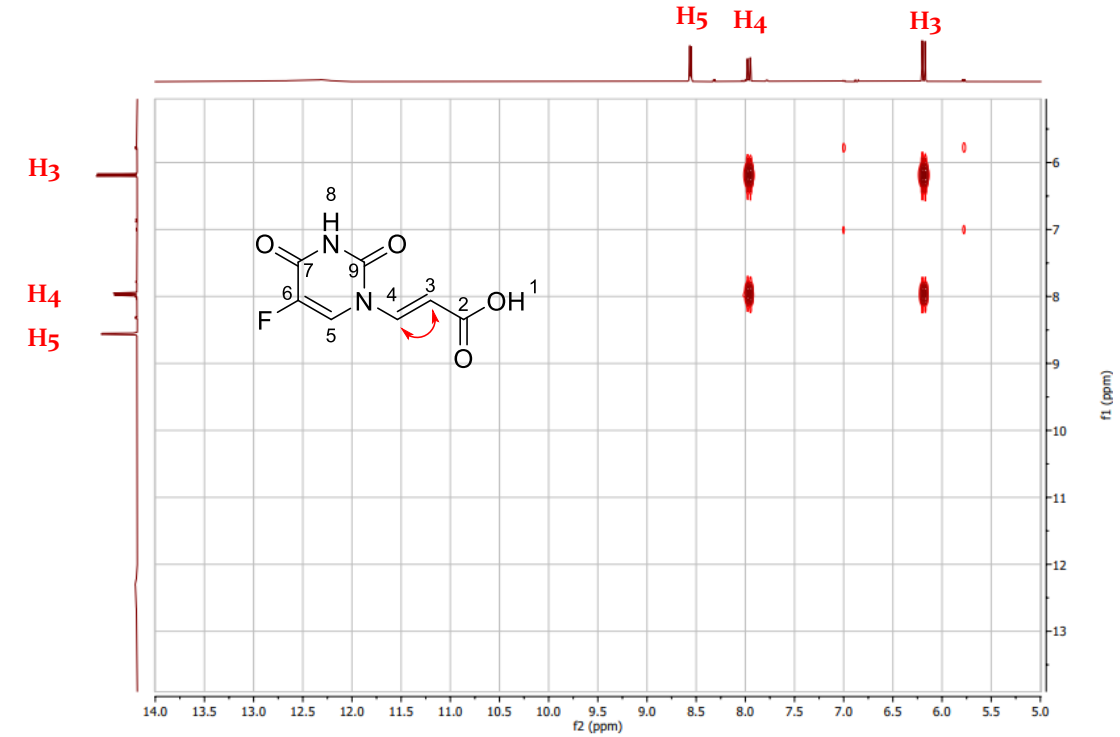


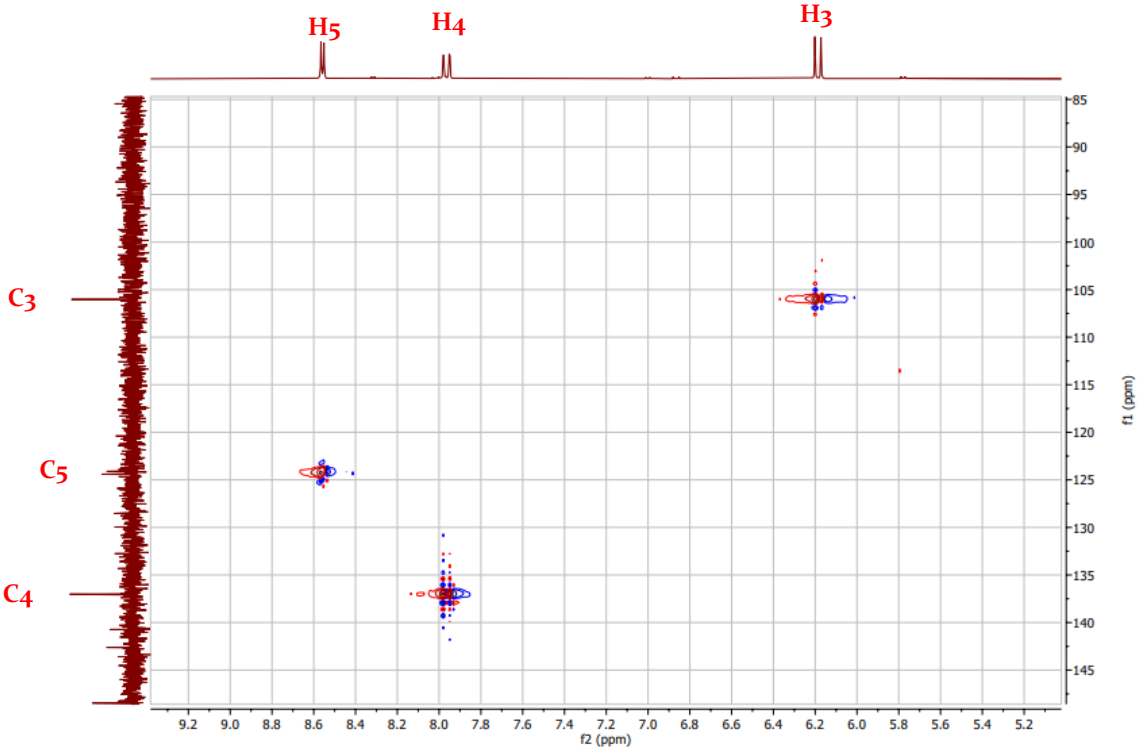
^{1}H NMR (500 MHz, DMSO) δ 12.55 (s, 1H, H1/2), 12.29 (s, 1H, H1/2), 8.56 (d, *J* = 7.0 Hz, 1H, H5), 7.96 (dd, *J* = 14.6, 1.8 Hz, 1H, H4), 6.19 (d, *J* = 14.5 Hz, 1H, H3).

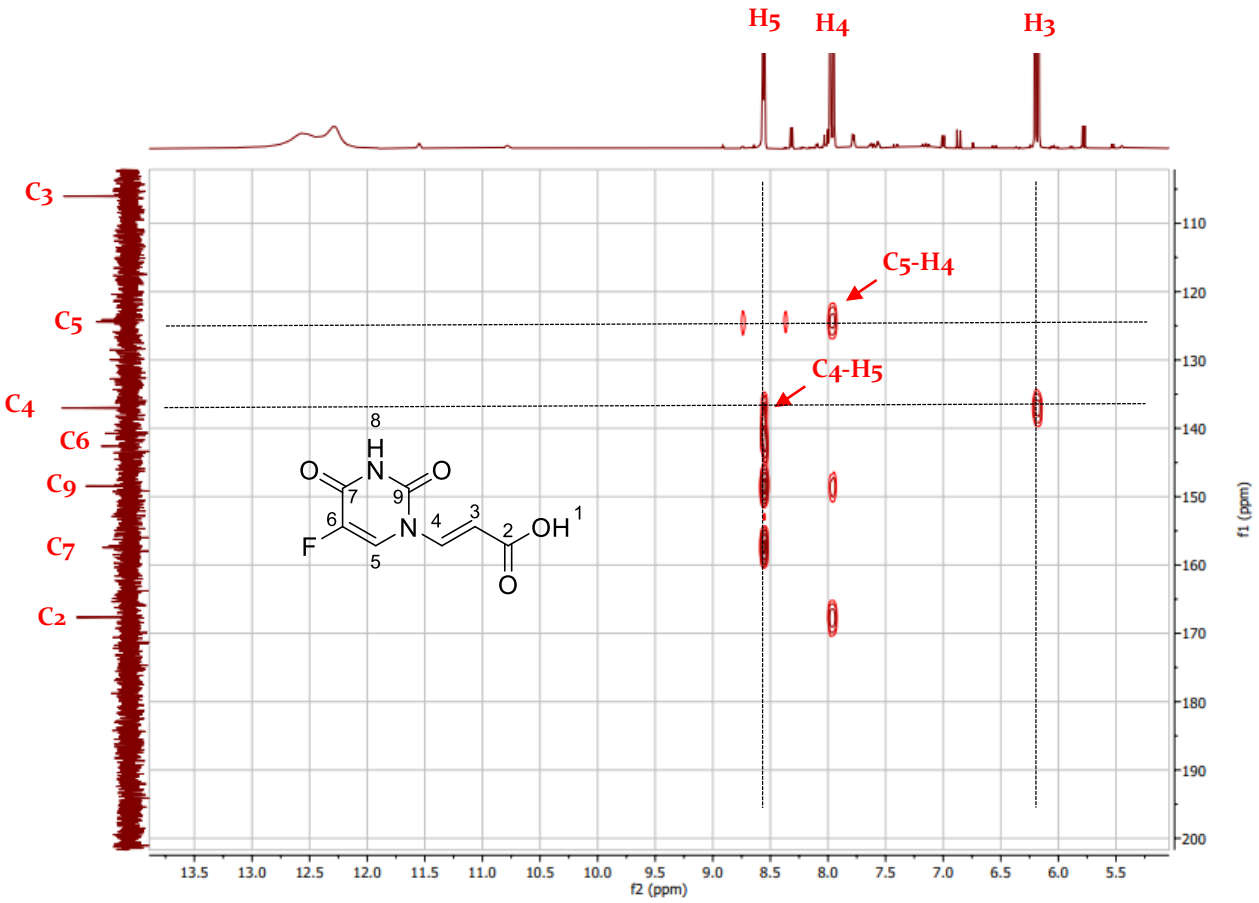


^{13}C NMR (126 MHz, DMSO) δ 167.7 (C2), 157.4 (d, J = 26.5 Hz, C7), 148.5 (C9), 142.6 (d, *J* = 235.7 Hz, C6), 137.0 (C4), 124.3 (d, *J* = 36.8 Hz, C5), 106.03 (C3).



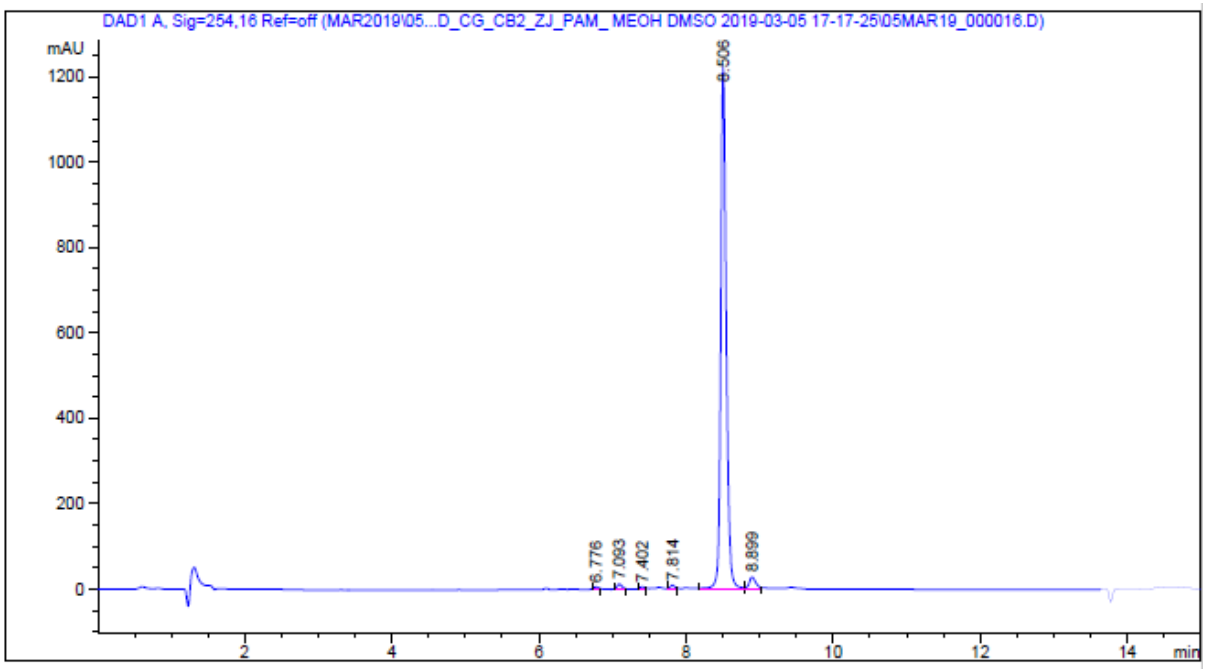

H5
H4
H3
H3
H4
H5
f2 (ppm)
f1 (ppm)


H5
H4
H3
C3
C5
C4
f2 (ppm)
f1 (ppm)


H5
H4
H3
C3
C5
C4
C6
C9
C7
C2
C5-H4
C4-H5
f2 (ppm)
f1 (ppm)

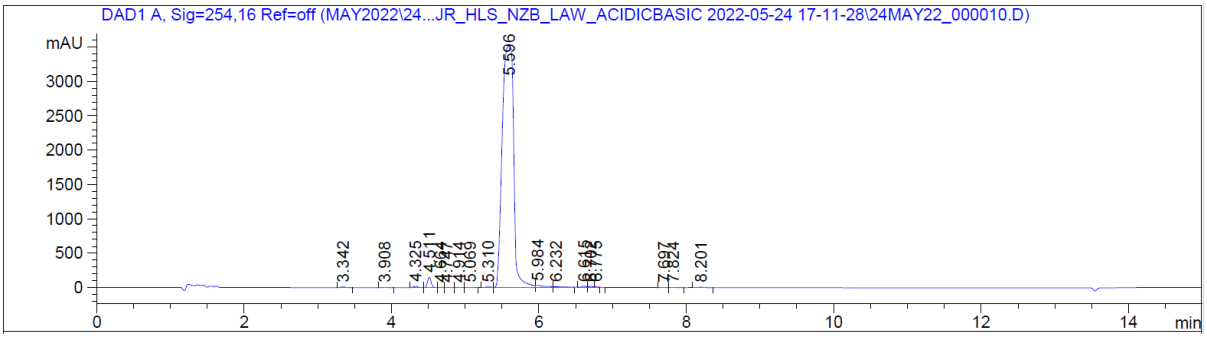
Analytical HPLC Traces

HPLC trace for compound 2



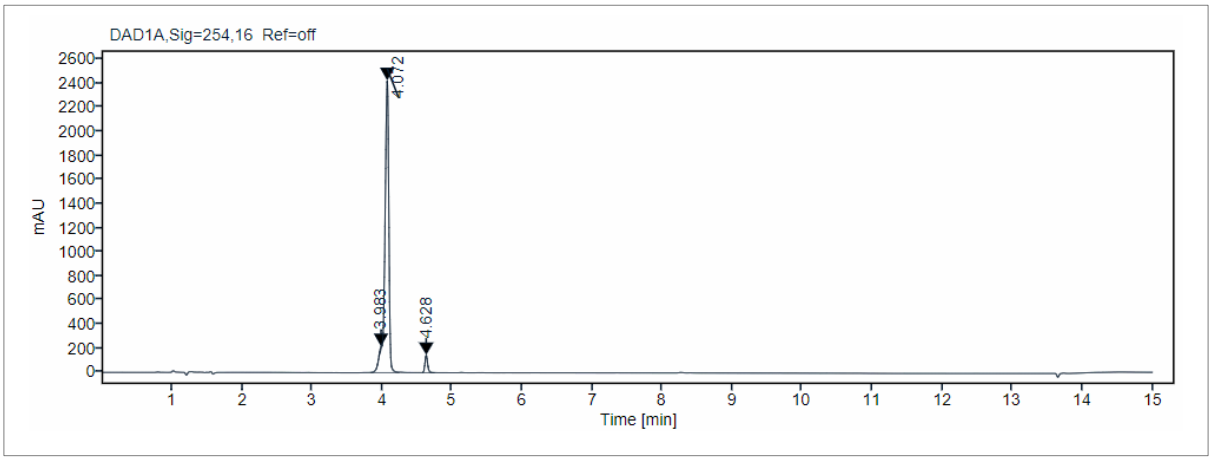
Peak area 95.0%

HPLC trace for compound 53



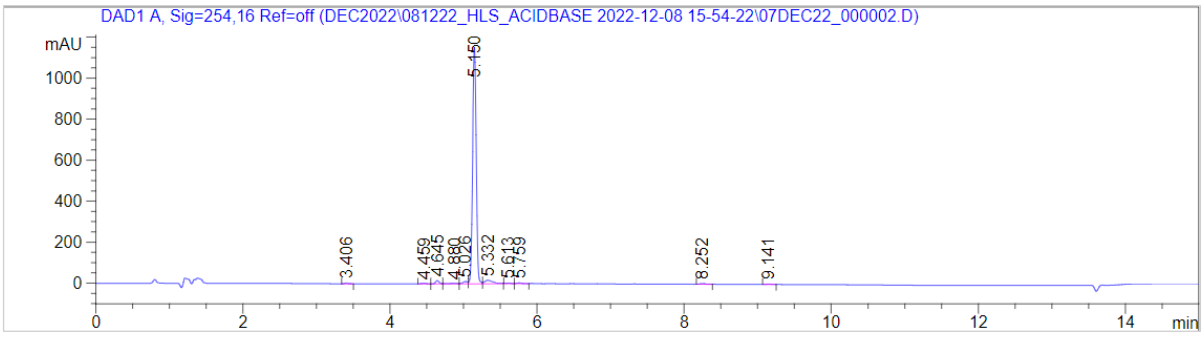
Peak area 96.2%

HPLC trace for compound 56



Peak area 95.6%

HPLC trace for compound 57



Peak area 94.2%