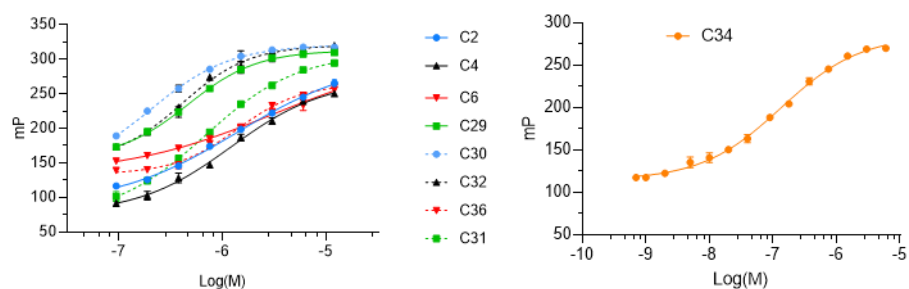
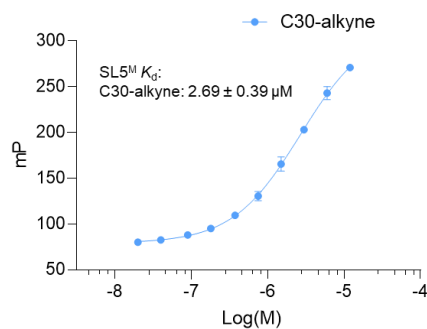


Supplementary Information For

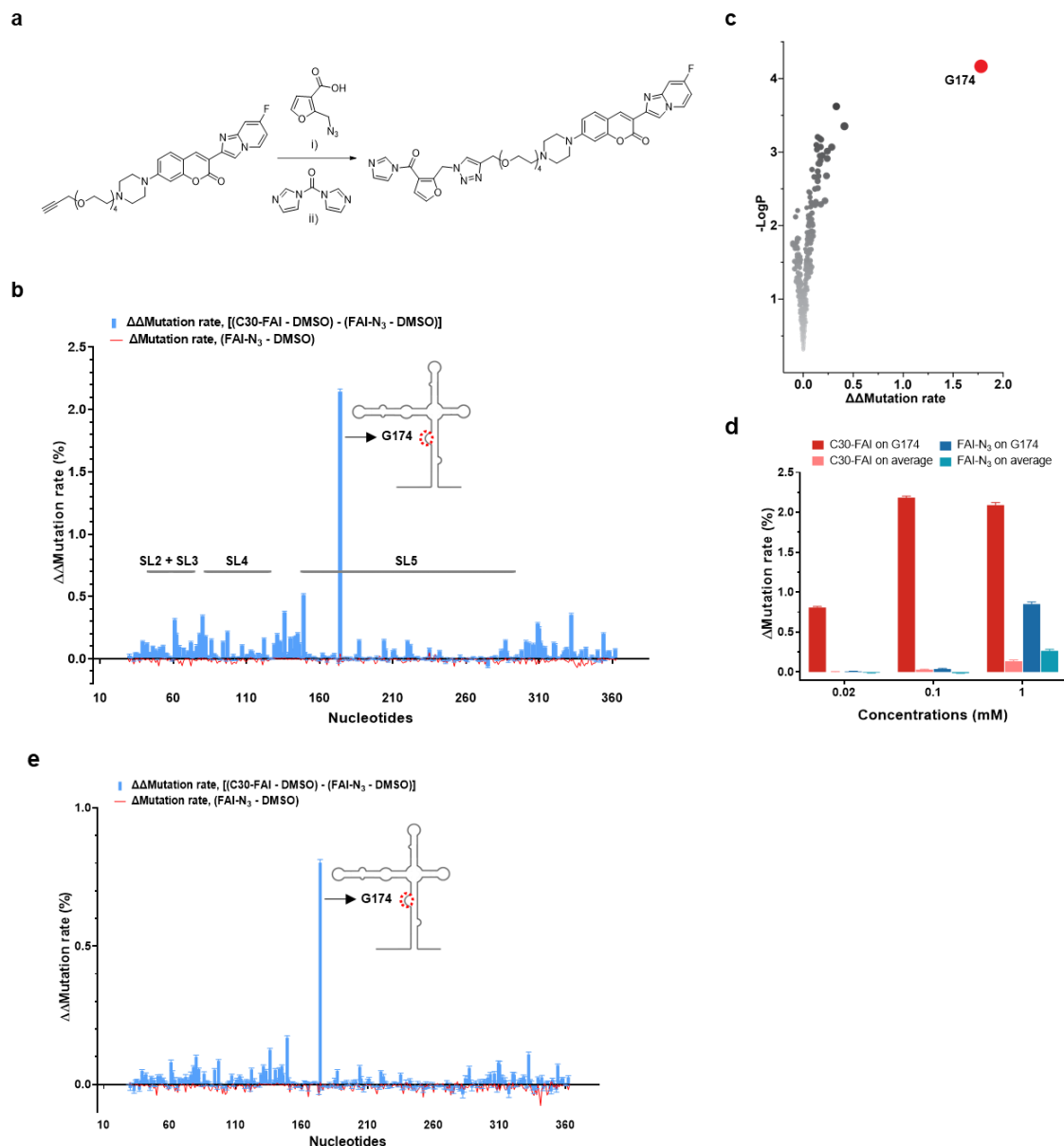
“Chemical-guided SHAPE sequencing (cgSHAPE-seq) informs the binding site of RNA-degrading chimeras targeting SARS-CoV-2 5' untranslated region”



Supplementary Fig. S1 | Binding affinity of coumarin derivatives to SL5 RNA. Dose-response curves of coumarin derivatives in fluorescence polarization assay with in vitro transcribed SL5 RNA. All compounds were used at a concentration of 80 nM. Each data point represents the mean fluorescence polarization value of two independent replicates ($n = 2$). Source data are provided as a Source Data file.

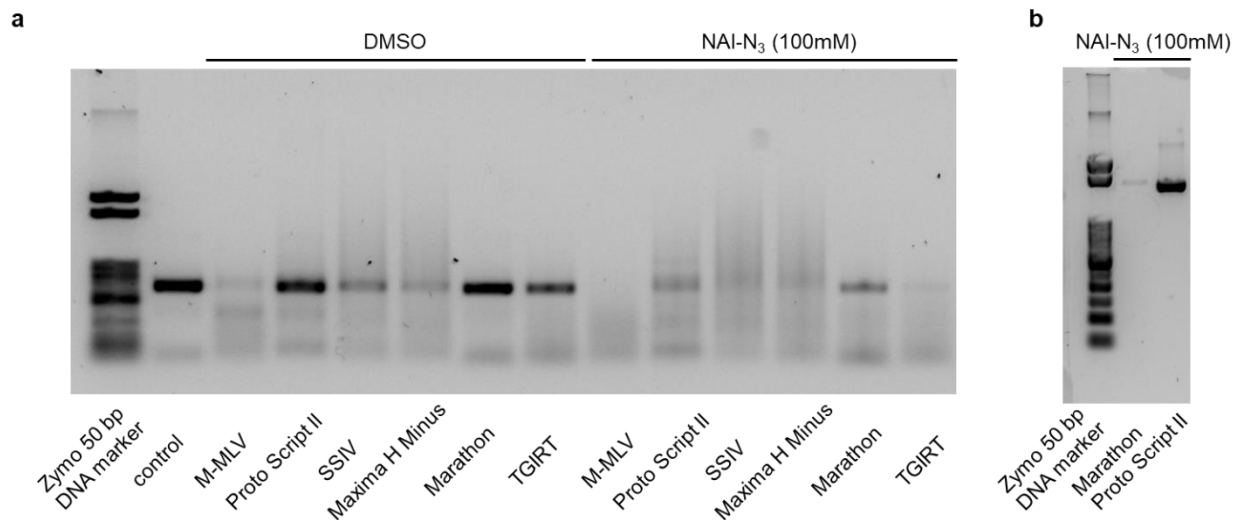


Supplementary Fig. S2 | Binding affinity of C30-alkyne to SL5^M RNA. C30-alkyne was used at a concentration of 80 nM. Each data point represents the mean fluorescence polarization value of two independent replicates ($n = 2$). Source data are provided as a Source Data file.



Supplementary Fig. S3 | Identification of the binding site by cgSHAPE-seq using C30-FAI containing imidazole.

a, cgSHAPE-seq probe (**C30-FAI**) synthetic route. Reaction conditions: i) tris(hydroxypropyltriazolylmethyl)amine, CuSO₄, sodium ascorbate, DMSO, room temperature; ii) anhydrous DMSO, room temperature. **b**, cgSHAPE-seq mutational profiling analysis of the SL5 sequence in total RNA extract treated with **C30-FAI** (0.1 mM). Δmutation rate (FAI-N₃ – DMSO) indicates the background structure-based differential acylation. ΔΔmutation rate [(C30-FAI – DMSO) – (FAI-N₃ – DMSO)] indicates the proximity-based differential acylation. **c**, Scatter plot of -LogP vs ΔΔmutation rate. *P* values are calculated from one-sided *t*-test. **d**, Comparison of the Δmutation rates of G174 and on average in RNAs treated with different concentrations of **C30-FAI** or FAI-N₃. **e**, cgSHAPE-seq mutational profiling analysis of the SL5 sequence in total RNA extract treated with **C30-FAI** (0.02 mM). Three data points (C75, G216, G285) were removed as outliers as they had an abnormally high mutation rate (Z-score > 4.0) in DMSO-treated samples. The cgSHAPE-seq experiments were performed with three replicates (*n* = 3). Source data are provided as a Source Data file.



Supplementary Fig. S4 | Comparison of reverse transcriptases. RNA was diluted in denaturing buffer (90% formamide, 5 mM EDTA), heated to 80 °C for 2 minutes and snap-cooled on ice. DMSO or NAI-N₃ was added and mixed well. The solution was heated at 80 °C for 5 minutes and cooled on ice. RNA was recovered using Qiagen RNeasy kit. RT reaction buffer: 50 mM Tris-HCl (pH 7.4), 75 mM KCl, 10 mM DTT, 3 mM MnCl₂. Manual suggested optimal reaction temperature of each enzyme was used (42 °C incubation 1 h for M-MLV, ProtoScript II, and Marathon; 50 °C incubation 1 h for SSIV and Maxima H minus). For TGIRT, reaction buffer: 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 1.5 mM MgCl₂. 50 °C incubation 1 h. Source data are provided as a Source Data file.

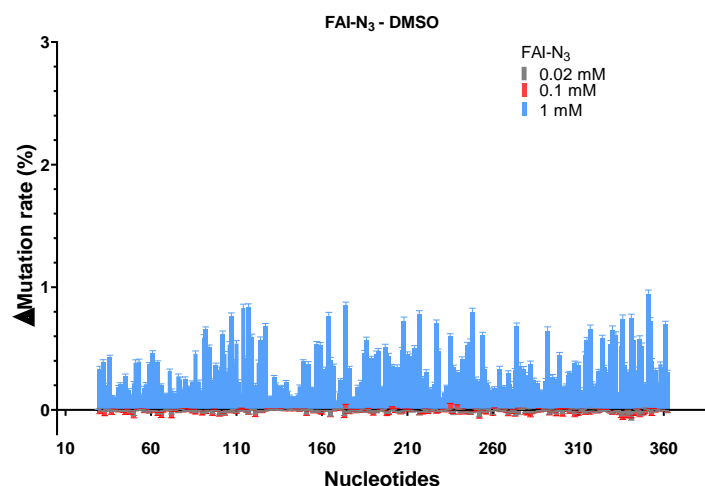
Reference sequence for **a** (378 nt) (5' to 3'):

```
aggtttataccttcccaggtaacAAACCAACCAACTTTTCGATCTCTTGTAGATCTGTTTCTCTAAACGAACTTTAAATCTGTGT
GGCTGTCACTCGGCTGCGTGTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTGCTTGACAGGACAC
GAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTT
TCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAGGGAGTCAAAGTTCTGTTT
GCCCTGATCTGCATCGCTGTGGCCGAGGCCAAGCCCACCGAGAACAACGAagacttcaacatcgtggccg.
```

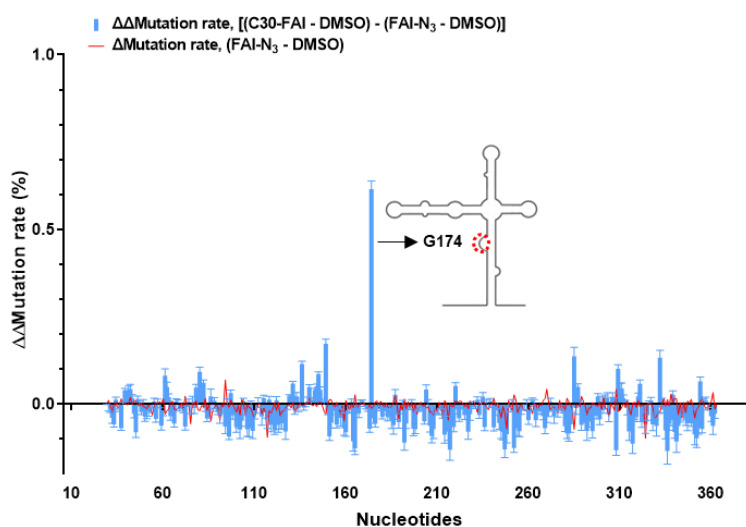
Reference sequence for **b** (827 nt) (5' to 3'):

```
aacttcctttattttccttacagGGTTTTAGACAAAATCAAAAAGAAGGAAGGTGCTCACATTCCTTAAATTAAGGAGTAAGTCT
GCCAGCATTATGAAAGTGAATCTTACTTTTGTAACCTTTATGGTTTGTGGAACAAATGTTTTTGAACATTTAAA
AAGTTTCAGATGTTAGAAAGTTGAAAGGTTAATGTAACAAATCAATATTAAGAATTTTGATGCCAAACTATTAGA
TAAAAGGTTAATCTACATCCCTACTAGAATTCTCATACTTAACTGGTTGGTTGTGTGGAAGAAACATACTTTTACA
ATAAAGAGCTTTAGGATATGATGCCATTTTATATCACTAGTAGGCAGACCAGCAGACTTTTTTTTATTGTGATATG
GGATAACCTAGGCATACTGCACTGTACACTCTGACATATGAAGTGCTCTAGTCAAGTTTAACTGGTGTCCACAGA
GGACATGGTTTAACTGGAATTCGTCAAGCCTCTGGTTCTAATTTCTCATTTGCAGGAAATGCTGGCATAGAGCAG
CACTAAATGACACCACTAAAGAAACGATCAGACAGATCTGGAATGTGAAGCGTTATAGACGATAACTGGCCTCAT
TTCTTCAAATATCAAGTGTTGGGAAAGAAAAAGGAAGTGAATGGGTAAGTCTTCTTCTGATTAAAGGTTATGTAA
TAACCAAATGCAATGTGAAATATTTTACTGGACTCTATTTTGAACCAACATCTGTAAAGACTGAGGTGGGGGTG
GGAGGCCAGCACGGTGGTGAGGCAGTTGAgaaaaagtaagtacttgacatgataagatac
```

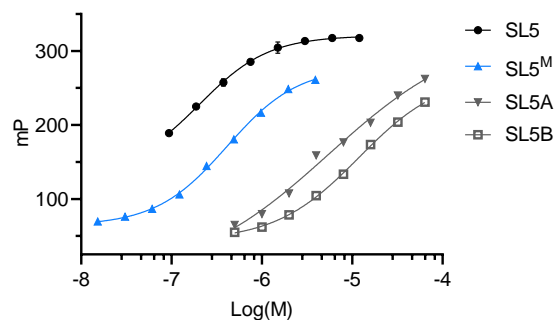
(lowercase = primer binding sequences)



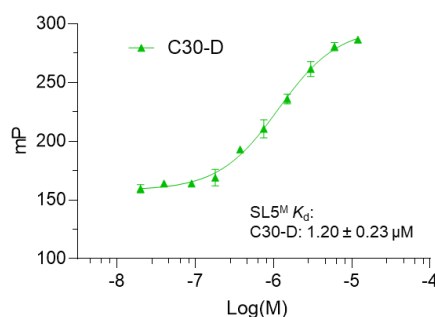
Supplementary Fig. S5 | Background Δ mutation rate (FAI-N₃ – DMSO) of the SL5 sequence in total RNA extract treated with different concentrations of FAI-N₃. Three data points (C75, G216, G285) were removed as outliers as they had an abnormally high mutation rate (Z-score > 4.0) in DMSO-treated samples. The cgSHAPE-seq experiments were performed with three replicates ($n=3$). Source data are provided as a Source Data file.



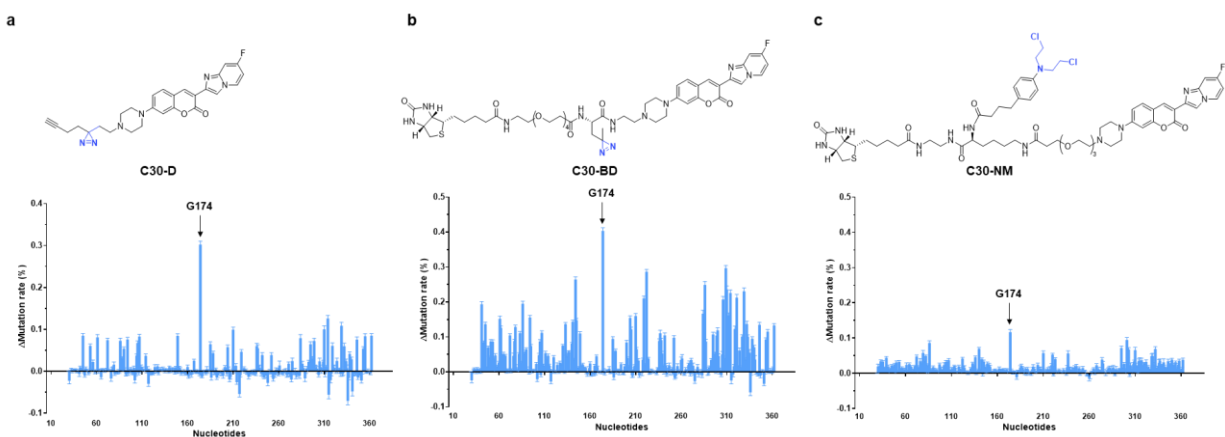
Supplementary Fig. S6 | cgSHAPE-seq mutational profiling analysis using C30-FAI (0.02mM). Δ mutation rate (FAI-N₃ – DMSO) indicates the background structure-based differential acylation. $\Delta\Delta$ mutation rate [(C30-FAI – DMSO) – (FAI-N₃ – DMSO)] indicates the proximity-based differential acylation. The cgSHAPE-seq experiments were performed with three replicates ($n=3$). Source data are provided as a Source Data file.



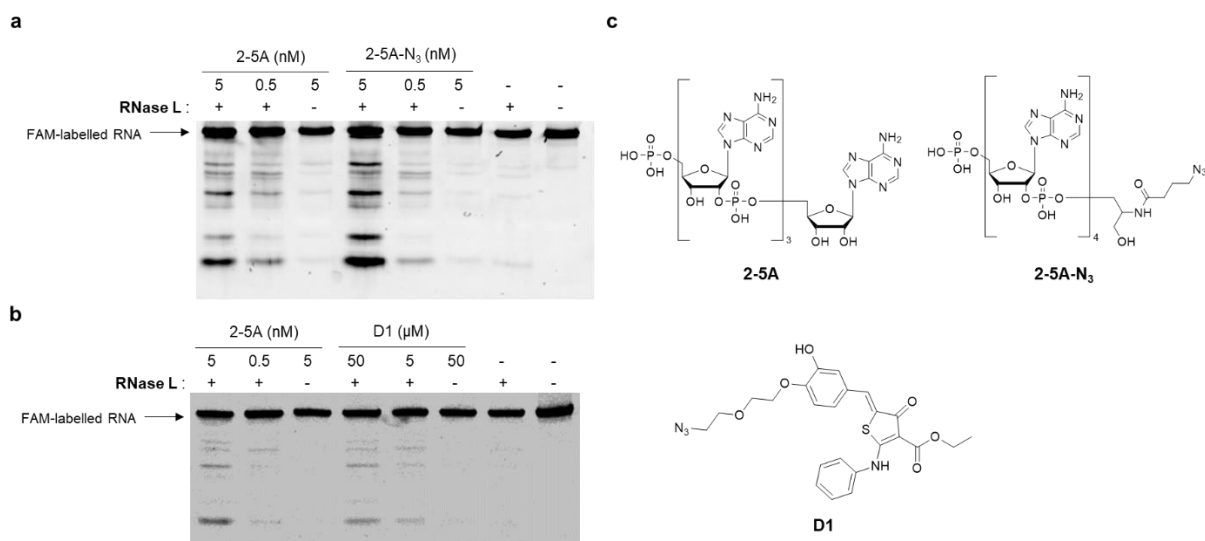
Supplementary Fig. S7 | Dose-response curves of **C30** (80 nM) in fluorescence polarization assay with SL5 RNA and its substructures. Each data point represents the mean fluorescence polarization value of two independent replicates ($n = 2$). Source data are provided as a Source Data file.



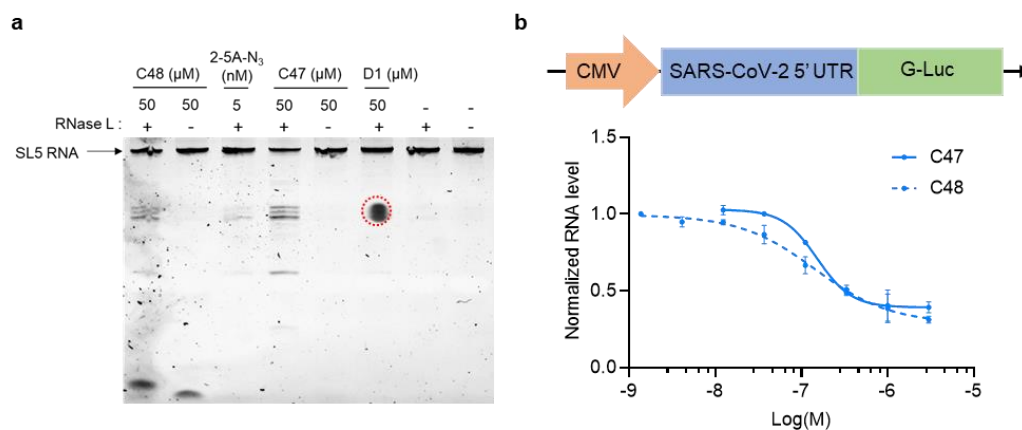
Supplementary Fig. S8 | Binding affinity of C30-D with SL5^M RNA. Each data point represents the mean fluorescence polarization value of two independent replicates ($n = 2$). Source data are provided as a Source Data file.



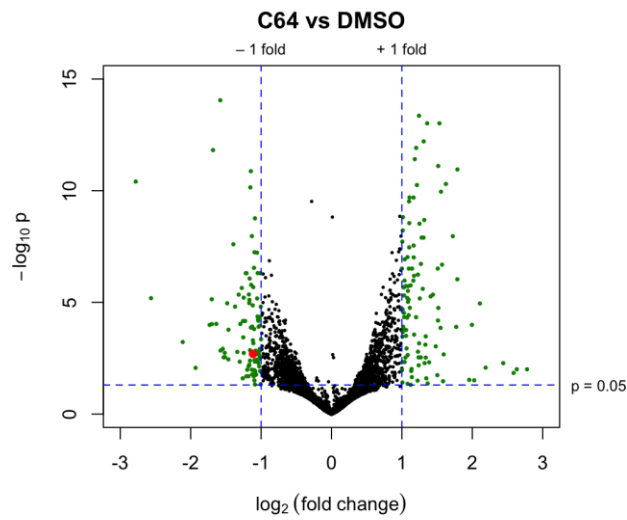
Supplementary Fig. S9 | Mutational profiling analysis of crosslinking probes. **a**, Structure of C30-D and mutational profiling analysis. **b**, Structure of C30-BD and mutational profiling analysis. **c**, Structure of C30-NM and mutational profiling analysis. Three data points (C75, G216, G285) were removed as outliers as they had an abnormally high mutation rate (Z -score > 4.0) in DMSO-treated samples. All experiments were performed with three replicates ($n = 3$). Source data are provided as a Source Data file.



Supplementary Fig. S10 | In vitro RNase L degradation assay with a 5' 6-fluorescein-tagged model RNA containing multiple RNase L cleavage sites. a, Comparison of RNA degradation activity of RNase L in the presence of **2-5A** and **2-5A-N₃** with no significant differential activity observed. **b**, Comparison of synthetic RNase L recruiter (**D1**) and **2-5A**. The activity of **D1** in RNase L activation is ~ 10,000 times weaker than **2-5A**. **c**, Chemical structures of **2-5A**, **2-5A-N₃**, and **D1**. All experiments were performed three times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. S11 | RNA degrading activity of C47 and C48. a, Comparison of two RLR moieties in the RIBOTAC modality using the in vitro RNase L degradation assay with purified SL5 RNA (red circle is a staining artifact). Experiments were performed three times with similar results. **b**, Cellular activity of **C47** and **C48** in SARS-CoV-2 5' UTR expressing cells. The dose-response curves are representative of three independent measurements ($n = 3$). Source data are provided as a Source Data file.



Supplementary Fig. S12 | Volcano plot of differential gene expression in SARS-CoV-2 5' UTR expressing cells treated with C64 (3 μM). DMSO-treated cells were used as a control. Red spot = SARS-CoV-2 5' UTR transcript. The RNA-seq analysis was performed with three biological replicates ($n = 3$). P values are calculated from one-sided t -test.

Methods

In vitro RNA transcription

In vitro RNA transcription was performed using a T7 RNA Polymerase (New England Biolabs, M0251L) following the manual's protocol. Usually, 100 μ L transcription mixture containing 2 μ g DNA template, 10 μ L 10x transcription buffer, 0.5 mM NTPs, 2 μ L RNase inhibitor (ApexBio, Cat. K1046) and 10 μ L T7 polymerase was incubated at 37 °C for 12 h. 2 μ L DNase I (10 U/ μ L, Roche) was added into the reaction mixture and incubated for 15 min at 37 °C. RNA was purified by precipitation with isopropanol or gel electrophoresis on a denaturing polyacrylamide gel. Finally, RNA was dissolved in molecular biology water and quantified by NanoDrop (ND-1000, Thermo, Waltham, MA, USA).

RNA sequences from in vitro transcription:

	sequences
SL5	UCGUUGACAGGACACGAGUAAACUCGUCUAUCUUCUGCAGGCUGCUUACGGUUUCGUCCGUGUUG CAGCCGAUCAUCAGCACAUCAAGGUUUCGUCCGGGUGUGACCGAAAGGUAAGAUGGAGAGCCUU GUCCCUUGGUUUAACGA
SL5 ^M	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAGAGC
1	ACUCAUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAGAGC
2	ACUCCUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAGAGC
3	ACUCUUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAGAGC
4	ACUCGGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAA AGGUAAGAUGGAGAGC
5	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAGAGC
6	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAAGAGC
7	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGACGAGC
8	ACUUGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAAAGC
9	ACUCGCCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGGAGC
10	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUAAGAGC
11	ACUCGUCUAUCUUCUGCAGGCUGAAACACAGCCGAUCAUCAGCACAUCAACGGGUGUGACCGAAA GGUAAGAUGGAGAGC
12	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUAAACACGUGUGACCGAA AGGUAAGAUGGAGAGC
13	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUCAACGGGUGUGACGAAAC ACGUAAGAUGGAGAGC

(Mutations in SL5^M are coloured in purple)

Fluorescence Polarization Binding Assay

Synthetic RNA oligomers were purchased from GenScript or Integrated DNA Technologies (IDT) and reconstituted in nuclease-free water (Invitrogen #AM9932). Compounds (1 mM in DMSO) were diluted in 2x assay buffer (40 mM MES, 200 mM NaCl, 0.0125% TritonX, pH 6.5) to 0.16 μ M.

A 1:2 dilution series (8 points) of each RNA was prepared in 20–30 μ L water to desired concentrations (i.e., 0.09 – 12 μ M). 20–30 μ L 2x working solution containing the 2x assay buffer and the small-molecule ligand was added to each RNA sample in 1:1 (v/v) and mixed by pipetting.

For fluorescence polarization measurement, 20 μL of the above 1 \times working solution was transferred into a 384-well, black, flat-bottom microplates (Greiner Bio-One) in duplicates or triplicates. The plate was equilibrated at room temperature for 5 min before being read using a microplate reader (SYNERGY H1, BioTek; Excitation/Emission = 360/460 nm) at 25 $^{\circ}\text{C}$. The experimental data were analysed using Prism 8.0 software (Graphpad Software, San Diego, CA, USA). The dissociation constant (K_d) was calculated with 95 % confidence interval after nonlinear curve fitting (Sigmoidal, 4 parameters).

RNA sequences from IDT (first two) or GenScript (last three):

	sequences
SL5A	GGCUGCUUACGGUUUCGUCCGUGUUGCAGCC
SL5B	CACAUCUAGGUUUCGUCCGGGUGUG
14	ACUCUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCAGCACAUACGGGUGUGACCGAAAG GUAAGAUGGAGAGC
15	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCGAUCAUCACCACAUCUACGGGUGUCUCCGAAA GGUUUGAUGGAGAGC
16	ACUCGUCUAUCUUCUGCAGGCUGCUUGCAGCCCUUCUCCACAUCUACGGGUGUGACCGAAA GGUAAGAUGGAGAGC

(Mutations in SL5^M are coloured in purple)

Surface Plasmon Resonance (SPR) Assay

SPR experiments were performed on a Biacore T200 (GE Healthcare) instrument at 25 $^{\circ}\text{C}$ using streptavidin pre-coated SA sensor chips (GE Healthcare). The running buffer composed of 10 mM HEPES, 100 mM NaCl, 0.05% Tween 20 (w/v), 5 mM EDTA, 0.1% (v/v) DMSO at pH 6.8 was prepared freshly, filtered through the 0.22 μm PVDF membrane prior to use. 3'-biotinylated RNA SL5 was prepared by 3' ligation according to the literature protocol². For immobilization of the biotinylated RNA, the sensor chip was firstly conditioned with 3 consecutive 1 min injections of high salt solution (50 mM NaOH, 1M NaCl) at a flow rate of 10 $\mu\text{L}/\text{min}$. Next, the biotinylated RNA was diluted 100 \times in running buffer (100 nM) and applied over the streptavidin sensor chip surface at a flow rate of 10 $\mu\text{L}/\text{min}$ to achieve immobilization level of about 500 RU. Finally, alkyne-PEG-biotin (50 μM in running buffer) was injected (1 min, 10 $\mu\text{L}/\text{min}$) to block remaining streptavidin surface binding sites. The kinetics analysis was performed following the BiaControl Software Wizard Kinetics protocol. Compound **C30** (HCl salt form, 10 mM in water) was diluted in the running buffer to the following concentrations (0, 0.075, 0.15, 0.3, 0.6 μM) and titrated over the immobilized RNA SL5 (contact time: 1 min, flow rate: 30 $\mu\text{L}/\text{min}$). The data analysis and plotting were performed using the instrument BiaEvaluation Software. All monitored resonance signals were subtracted with signals from a non-binding reference channel. Kinetic values (K_d , k_a , k_d) were calculated using the BiaEvaluation Software Binding Affinity protocol with 1:1 fitting. 3'-biotinylated RNA SL5 sequence: 5'-UCGUUGACAGGACACGAGUAACUCGUCUAUCUUCUGCAGGCUGCUUACGGUUUCGUCCGUGUUGCAGCCGAUCAUCAGCACAUACGGUUCGUC CGGGUGUGACCGAAAGGUAAGAUGGAGAGCCUUGUCCUGGUUUAACGA-biotin

Mutational profiling for compounds C30-D, C30-BD and C30-NM

Protocols were similar to cgSHAPE-seq except RNA modification step:

For **C30-D** and **C30-BD**, total RNA was added water and 5 \times folding/reaction buffer (500 mM HEPES pH 7.4, 500 mM KCl, 30 mM MgCl_2) to make a 47.5 μL solution. The solution was incubated at 37 $^{\circ}\text{C}$ for 30 min to refold. 2.5 μL **C30-D** or **C30-BD** (2 mM in DMSO) or DMSO (blank control) was added to the total RNA and mixed well by pipetting. The mixture was then irradiated under 365 nm UV light in a photocrosslinker (Fisher Cat. #13-245-221) for 15 min. The RNA was then recovered using a RNeasy kit (Qiagen).

For **C30-NM**, total RNA was added water and 5× folding/reaction buffer (500 mM HEPES pH 7.4, 500 mM KCl, 30 mM MgCl₂) to make a 47.5 µL solution. The solution was incubated at 37 °C for 30 min to refold. 2.5 µL **C30-NM** (2 mM in DMSO) or DMSO (blank control) was added to the total RNA and mixed well by pipetting. The mixture was then incubated at 37 °C for 12 h. The RNA was recovered using a RNeasy kit (Qiagen).

500 ng total RNA was used for reverse transcription and then PCR as described in cgSHAPE-seq protocol. All reactions were performed in triplicates.

Cytotoxicity Assay

The ACE-2 expressing A549 cells were seeded in a 384-well plate at 10,000 cells per well in 30 µl of growth media³. The cells were then treated with various concentrations of **C64** (2.44 nM–20 µM at 1:2 serial dilution) and a DMSO control (0.1%) in triplicates. After incubation for 48 h in the presence of **C64**, the cell viability was measured using CellTitre-Glo (Promega, G9242) and a luminescence plate reader (BioTek, Cytation 5). The relative viability was normalized to the DMSO control.

RNA-seq

The Stranded mRNA-Seq was performed using the Illumina NovaSeq 6000 Sequencing System at the University of Kansas Medical Center Genomics Core (Kansas City, KS). Quality control on RNA submissions was completed using the Agilent Bioanalyzer 2100 using the RNA 6000 Nano Assay kit (Agilent Technologies, 5067-1511). Total RNA (750 ng) was used to initiate the library preparation protocol. The total RNA fraction was processed by oligo dT bead capture of mRNA, fragmentation, reverse transcription into cDNA, end repair of cDNA, ligation with the appropriate Unique Dual Index (UDI) adaptors, strand selection and library amplification by PCR using the Universal Plus mRNA-seq with UDI preparation kit (NuGEN Technologies, 0508-02).

Library validation was performed using the DNA 1000 Assay kit (Agilent Technologies, 5067-1504) on the Agilent Bioanalyzer 2100. Concentration of each library was determined by qPCR using the with the Roche Lightcycler96 using FastStart Essential DNA Green Master (Roche 06402712001) and KAPA Library Quant (Illumina) DNA Standards 1-6 (KAPA Biosystems KK4903). Libraries were pooled based on equal molar amounts to 1.9 nM for multiplexed sequencing.

Pooled libraries were denatured with 0.2N NaOH (0.04N final concentration) and neutralized with 400 mM Tris-HCl pH 8.0. A dilution of the pooled libraries to 380 pM was performed in the sample tube, on instrument, followed by onboard clonal clustering of the patterned flow cell using the NovaSeq 6000 S1 Reagent Kit v1.5 (200 cycle) (Illumina 20028318). A 2x101 cycle sequencing profile with dual index reads was completed using the following sequence profile: Read 1 – 101 cycles x Index Read 1 – 8 cycles x Index Read 2 – 8 cycles x Read 2 – 101 cycles. Following collection, sequence data was converted from .bcl file format to .fastq file format using bcl2fastq software and de-multiplexed into individual sequences for data distribution. We followed the differential gene expression (DGE) analysis pipeline developed by Maude and Cebola (<https://github.com/CebolaLab/RNA-seq>). The result was used to generate Extended Data Fig. 9 and Table S1.

Supplementary Table S1. Significantly changed RNA transcripts in C64-treated SARS-CoV2-5' UTR reporter cells determined by RNA-seq analysis (| log₂FoldChange | > 2 and adjusted p value (padj) < 0.05). P values were calculated from one-sided t-test.

Gene	HGNC_symbol	baseMean	log ₂ FoldChange	lfcSE	pvalue	padj
ENSG00000117600	PLPPR4	22.3	-3.0	0.84	1.58E-05	7.60E-04
ENSG00000111344	RASAL1	71.8	-2.8	0.39	3.54E-14	3.89E-11
ENSG00000260448	LCMT1-AS1	35.1	-2.6	0.54	3.90E-08	6.07E-06
ENSG00000105392	CRX	15.5	-2.6	0.86	7.58E-05	2.51E-03
ENSG00000157601	MX1	14.2	-2.6	1.05	4.42E-04	9.29E-03
ENSG00000259030	FPGT-TNNI3K	62.9	-2.6	0.53	4.37E-08	6.49E-06
ENSG00000104320	NBN	27.3	-2.4	0.68	8.41E-06	4.64E-04
ENSG00000204116	CHIC1	43.2	-2.4	0.84	1.65E-04	4.49E-03
ENSG00000157087	ATP2B2	14.6	-2.3	1.01	6.96E-04	1.27E-02
ENSG00000050767	COL23A1	81.1	-2.1	0.59	1.17E-05	5.96E-04
ENSG00000220008	LINGO3	26.3	-2.1	0.65	5.38E-05	1.93E-03
ENSG00000243708	PLA2G4B	31.8	-2.1	0.62	3.17E-05	1.29E-03
ENSG00000287908		25.0	-2.0	0.91	6.33E-04	1.19E-02
ENSG00000156983	BRPF1	357.2	2.0	1.49	2.55E-03	3.08E-02
ENSG00000271781		24.6	2.0	0.61	3.17E-05	1.29E-03
ENSG00000275215	RNA5-8SN3	55.9	2.1	0.44	8.66E-08	1.12E-05
ENSG00000259660	DNM1P47	52.3	2.2	0.93	3.81E-04	8.37E-03
ENSG00000151702	FLI1	1074.6	2.4	0.90	2.03E-04	5.27E-03
ENSG00000277209	RPPH1	32.2	2.5	0.62	2.47E-06	1.75E-04
ENSG00000085998	POMGNT1	373.0	2.6	1.26	8.47E-04	1.45E-02
ENSG00000204970	PCDHA1	17.3	2.6	1.69	1.48E-03	2.10E-02
ENSG00000177646	ACAD9	363.3	2.6	1.13	4.85E-04	9.87E-03
ENSG00000254873		29.8	2.7	1.21	5.99E-04	1.15E-02
ENSG00000136271	DDX56	1346.0	2.8	1.20	4.88E-04	9.92E-03
ENSG00000170160	CCDC144A	15.5	2.9	1.76	1.61E-03	2.23E-02
ENSG00000288534		193.6	3.0	1.25	3.55E-04	8.00E-03
ENSG00000115155	OTOF	24.3	3.5	0.62	7.42E-10	2.27E-07
ENSG00000251357		109.3	3.6	2.18	1.17E-03	1.83E-02
ENSG00000227082	LINC02798	18.7	3.6	1.61	4.10E-04	8.83E-03
ENSG00000107669	ATE1	395.9	3.6	2.22	1.13E-03	1.79E-02

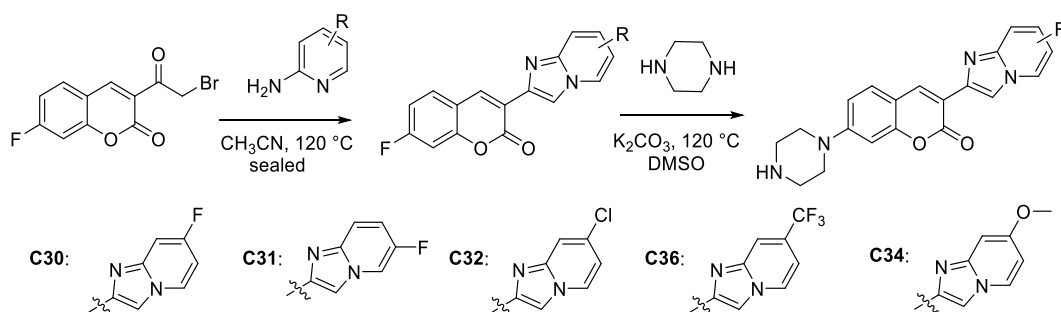
ENSG00000082438	COBLL1	434.3	3.8	1.13	2.07E-05	9.35E-04
ENSG00000070831	CDC42	1913.2	4.3	1.60	1.65E-04	4.49E-03
ENSG00000285976		1863.1	4.7	1.50	3.99E-05	1.54E-03
ENSG00000174405	LIG4	133.6	5.3	1.73	4.44E-05	1.65E-03
ENSG00000126561	STAT5A	128.2	6.1	2.11	5.94E-05	2.07E-03
ENSG00000196305	IARS1	1519.8	6.2	1.62	3.08E-06	2.07E-04

Chemistry

General Methods. Reagents and solvents were purchased from commercial sources (Fisher, Sigma-Aldrich and Combi-Blocks) and used as received. Reactions were tracked by TLC (Silica gel 60 F₂₅₄, Merck) and Waters ACQUITY UPLC-MS system (ACQUITY UPLC H Class Plus in tandem with Qda Mass Detector). Intermediates and products were purified by a Teledyne ISCO Combi-Flash system using prepacked SiO₂ cartridges. NMR spectra were acquired on a Bruker AV400 instrument (400 MHz for ¹H NMR, 100 MHz for ¹³C NMR) or Bruker AV500 instrument (500 MHz for ¹H NMR, 126 MHz for ¹³C NMR). ¹³C shifts were obtained with ¹H decoupling. MestReNova 14.0.1 developed by MESTRELAB RESEARCH was used for NMR data processing. MS-ESI spectra were recorded on Waters Qda Mass Detector. HRMS-ESI were recorded with a time-of-flight (TOF) analyzer on a Waters LCT PremierTM mass spectrometer. HPLC was performed on Waters ACQUITY UPLC H Class Plus system using Waters BEH C18 (2.1 mm × 50 mm, 1.7 μm) column and peak detection at 254nm with UV.

Compounds **C2**, **C4**, **C6** and **C29** were synthesized following procedures reported in literature and verified by NMR and Mass spectra^{2,4}.

General procedures for compounds **C30**, **C31**, **C32**, **C36** and **C34**.



Step 1: Synthesis of Coumarin intermediate

A mixture of 3-(2-bromoacetyl)-7-fluoro-2H-chromen-2-one (1 eq) and substituted aminopyridine (1.2 eq) in acetonitrile was heated to 120 °C for 20 min in a Biotage Initiator+ microwave reactor. TLC and LC-MS showed completion of reaction. The reaction mixture was filtered, and the solid was washed with acetonitrile to afford a yellow solid which was used in next step without further purification.

Step 2: Synthesis of final product

To a solution of Coumarin intermediate (1 eq) and piperazine (1.5 eq) in DMSO was added K₂CO₃ (3 eq). The reaction mixture was heated to 120 °C and stirred for 2 h. TLC and LC-MS showed completion of reaction. The mixture was cooled to room temperature, poured into ice water and

extracted with ethyl acetate. Organic layer was washed with brine, dried with anhydrous sodium sulphate and removed under vacuum. The residue was purified by silica gel column chromatography (0 – 10% CH₃OH in CH₂Cl₂) to afford product as yellow solid.

3-(7-fluoroimidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-2H-chromen-2-one (C30)

Following General procedures, from 3-(2-bromoacetyl)-7-fluoro-2H-chromen-2-one (50 mg, 0.17 mmol) and 4-fluoro-2-aminopyridine (22 mg, 0.2 mmol), 21 mg of compound **C30** was obtained as a yellow solid (two steps yield, 33%). MS-ESI (*m/z*) [M+H]⁺ 365.12. HRMS-ESI (*m/z*) [M+H]⁺ Calcd 365.1414; Found 365.1410. [M+Na]⁺ Calcd 387.1233; Found 387.1228.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.73 – 8.69 (m, 2H), 8.53 (s, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 7.39 (dd, *J* = 10.1, 2.6 Hz, 1H), 7.05 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.99 (td, *J* = 7.6, 2.6 Hz, 1H), 6.93 (d, *J* = 2.4 Hz, 1H), 3.48 (t, *J* = 5.2 Hz, 4H), 3.05 (t, *J* = 5.2 Hz, 4H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 160.2 (d, *J* = 248.0 Hz), 159.4, 154.8, 153.1, 144.3 (d, *J* = 12.5 Hz), 139.3, 138.5, 129.7, 129.3 (d, *J* = 11.8 Hz), 114.9, 112.1 (d, *J* = 28.1 Hz), 110.6, 104.2 (d, *J* = 29.8 Hz), 99.8, 99.6, 99.5, 46.0, 43.8.

¹⁹F NMR (471 MHz, DMSO-*d*₆) δ -112.80.

3-(6-fluoroimidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-2H-chromen-2-one (C31)

Following General Procedures, from 3-(2-bromoacetyl)-7-fluoro-2H-chromen-2-one (50 mg, 0.17 mmol) and 5-fluoro-2-aminopyridine (22 mg, 0.2 mmol), 23 mg of compound **C31** was obtained as a yellow solid (two steps yield, 36%). MS-ESI (*m/z*) [M+1]⁺ 365.18. HRMS-ESI (*m/z*) [M+H]⁺ Calcd 365.1414; Found 365.1429.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.84 (dd, *J* = 4.6, 2.4 Hz, 1H), 8.71 (s, 1H), 8.54 (s, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.61 (dd, *J* = 9.9, 5.2 Hz, 1H), 7.40 – 7.35 (m, 1H), 7.02 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.87 (d, *J* = 2.3 Hz, 1H), 3.34 (t, *J* = 5.2 Hz, 4H), 2.88 (t, *J* = 5.2 Hz, 4H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 159.6, 155.0, 153.7, 152.3 (d, *J* = 232.0 Hz), 142.1, 139.7, 138.7, 129.6, 117.3 (d, *J* = 26.2 Hz), 116.8 (d, *J* = 9.9 Hz), 114.4, 114.0, 113.6, 111.7, 110.1, 99.3, 47.5, 45.0.

¹⁹F NMR (471 MHz, DMSO-*d*₆) δ -141.87.

3-(7-chloroimidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-2H-chromen-2-one (C32)

Following General Procedures, from 3-(2-bromoacetyl)-7-fluoro-2H-chromen-2-one (50 mg, 0.17 mmol) and 4-chloro-2-aminopyridine (26 mg, 0.2 mmol), 31 mg of compound **C32** was obtained as a yellow solid (two steps yield, 46%). MS-ESI (*m/z*) [M+1]⁺ 381.13. HRMS-ESI (*m/z*) [M+H]⁺ Calcd 381.1118; Found 381.1135. [M+Na]⁺ Calcd 403.0938; Found 403.0949.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.70 (s, 1H), 8.67 (d, *J* = 7.3 Hz, 1H), 8.56 (s, 1H), 7.71 (d, *J* = 8.0 Hz, 1H), 7.70 (s, 1H), 7.04 – 6.98 (m, 2H), 6.88 (d, *J* = 2.3 Hz, 1H), 3.35 (t, *J* = 5.2 Hz, 4H), 2.89 (t, *J* = 5.1 Hz, 4H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 159.5, 155.0, 153.7, 143.9, 139.4, 138.9, 130.6, 129.7, 128.2, 114.7, 114.2, 113.1, 112.6, 111.7, 110.2, 99.3, 47.4, 44.9.

7-(piperazin-1-yl)-3-(7-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)-2H-chromen-2-one (C36)

Following General Procedures, from 3-(2-bromoacetyl)-7-fluoro-2H-chromen-2-one (50 mg, 0.17 mmol) and 4-trifluoromethyl-2-aminopyridine (32 mg, 0.2 mmol), 35 mg of compound **C36** was

obtained as a yellow solid (two steps yield, 48%). MS-ESI (m/z) $[M+1]^+$ 415.19. HRMS-ESI (m/z) $[M+H]^+$ Calcd 415.1382; Found 415.1398.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 9.33 (s, 1H), 8.82 (d, $J = 7.1$ Hz, 1H), 8.73 (s, 1H), 8.70 (s, 1H), 7.96 (s, 1H), 7.71 (d, $J = 8.8$ Hz, 1H), 7.19 (dd, $J = 7.1, 1.9$ Hz, 1H), 7.02 (dd, $J = 8.9, 2.5$ Hz, 1H), 6.94 (d, $J = 2.4$ Hz, 1H), 3.60 (t, $J = 5.3$ Hz, 4H), 3.16 (brs, 4H).

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 159.3, 155.0, 152.7, 141.8, 139.6, 139.5, 130.0, 129.0, 126.1 (q, $J = 33.1$ Hz), 123.5 (q, $J = 270$ Hz), 114.1, 114.1, 113.6 (q, $J = 5.7$ Hz), 112.2, 110.8, 107.9, 100.3, 54.9, 44.0.

^{19}F NMR (471 MHz, $\text{DMSO}-d_6$) δ -61.99.

3-(7-methoxyimidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-2H-chromen-2-one (C34)

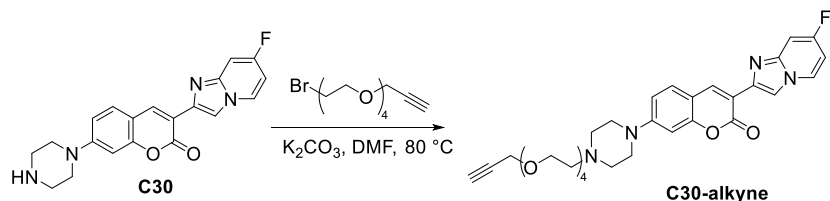
Following General Procedures, from 3-(2-bromoacetyl)-7-fluoro-2H-chromen-2-one (50 mg, 0.17 mmol) and 4-methoxypyridin-2-amine (25 mg, 0.2 mmol), 33 mg of compound **C34** was obtained as a yellow solid (two steps yield, 51%). MS-ESI (m/z) $[M+1]^+$ 377.18. HRMS-ESI (m/z) $[M+H]^+$ Calcd 377.1614; Found 377.1633.

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.61 (s, 1H), 8.45 (d, $J = 7.4$ Hz, 1H), 8.34 (s, 1H), 7.66 (d, $J = 8.8$ Hz, 1H), 7.00 (dd, $J = 8.9, 2.4$ Hz, 1H), 6.88 (d, $J = 2.5$ Hz, 1H), 6.85 (d, $J = 2.4$ Hz, 1H), 6.61 (dd, $J = 7.4, 2.5$ Hz, 1H), 3.85 (s, 3H), 3.30 (t, $J = 5.1$ Hz, 4H), 2.85 (t, $J = 5.1$ Hz, 4H).

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 160.0, 158.4, 155.2, 154.0, 146.2, 138.5, 138.2, 129.8, 128.2, 115.4, 112.1, 111.6, 110.7, 107.1, 99.8, 94.1, 56.0, 48.2, 45.6.

Synthesis of **C30** conjugates (**C30-alkyne**, **C47** and **C48**)

7-(4-(3,6,9,12-tetraoxapentadec-14-yn-1-yl)piperazin-1-yl)-3-(7-fluoroimidazo[1,2-a]pyridin-2-yl)-2H-chromen-2-one (**C30-alkyne**)



Compound **C30** (50 mg, 0.14 mmol) in DMF (1 mL) was added K_2CO_3 (37 mg, 0.27 mmol) and Propargyl-PEG₄-Br (80 mg, 0.27 mmol) and the reaction mixture was heated at 80 °C for overnight. The reaction mixture was added ice-water and filtered. The precipitate was washed with water and dried. The crude product was purified by silica gel column chromatography (0 – 5% CH_3OH in CH_2Cl_2) to afford **C30-alkyne** as a yellow solid (47 mg, 72%). MS-ESI (m/z) $[M+1]^+$ 579.22.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.71 – 8.68 (m, 2H), 8.52 (s, 1H), 7.69 (d, $J = 8.8$ Hz, 1H), 7.38 (dd, $J = 10.2, 2.6$ Hz, 1H), 7.02 (dd, $J = 8.9, 2.4$ Hz, 1H), 6.97 (td, $J = 7.6, 2.6$ Hz, 1H), 6.87 (d, $J = 2.3$ Hz, 1H), 4.15 (d, $J = 2.4$ Hz, 2H), 3.58 – 3.51 (m, 14H), 3.42 (t, $J = 2.5$ Hz, 1H), 3.37 (t, $J = 5.1$ Hz, 4H), 2.58 – 2.53 (m, 6H).

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 160.2 (d, $J = 249.5$ Hz), 159.5, 154.9, 153.4, 144.3 (d, $J = 14.9$ Hz), 139.4, 138.6, 129.6, 129.2 (d, $J = 11.9$ Hz), 114.5, 112.1, 111.7, 110.2, 104.2 (d, $J = 29.5$ Hz), 99.5 (d, $J = 23.5$ Hz), 99.4, 80.3, 77.1, 69.8, 69.7, 69.5, 68.5, 68.4, 57.5, 57.1, 52.8, 46.8.

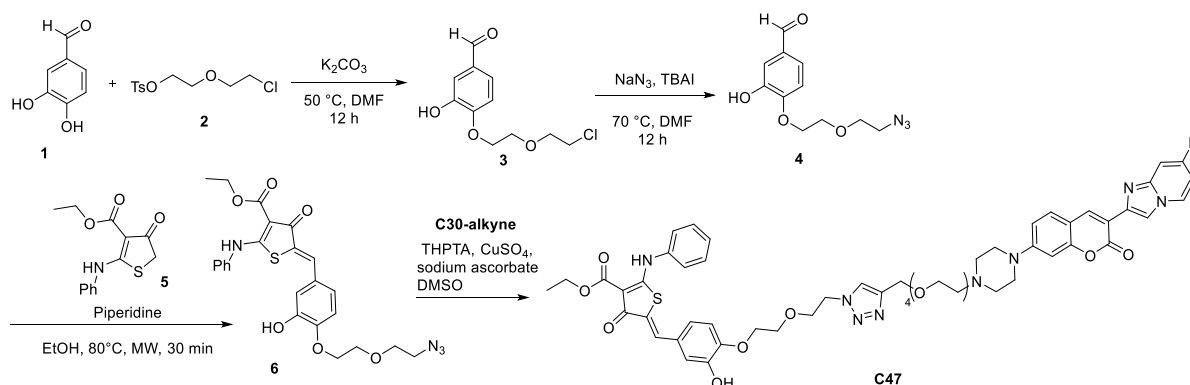
Synthesis of **C30-FAI** containing imidazole

compound **C30-FCA** (45 mg, 0.06 mmol) in anhydrous DMSO-*d*₆ (0.6 mL) was added carbonyl diimidazole (CDI, 15 mg, 0.09 mmol) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture contains ~91% **C30-FAI** and ~6% unreacted **C30-FCA** (see Supplementary Information) and was used directly in RNA modification. The stock solution was used as **91 mM** and can be stored at –80 °C for long-term storage.

¹H NMR (400 MHz, DMSO-*d*₆) δ 8.70 – 8.67 (m, 2H), 8.51 (s, 1H), 8.33 (t, *J* = 1.1 Hz, 1H), 8.17 (s, 1H), 7.93 (d, *J* = 2.1 Hz, 1H), 7.74 (t, *J* = 1.5 Hz, 1H), 7.37 (dd, *J* = 10.1, 2.6 Hz, 1H), 7.16 (dd, *J* = 1.7, 0.8 Hz, 1H), 6.95 (td, *J* = 7.6, 2.6 Hz, 1H), 6.85 (d, *J* = 2.3 Hz, 1H), 5.88 (s, 2H), 4.52 (s, 2H), 3.56 – 3.50 (m, 16H), 3.35 (t, *J* = 5.1 Hz, 4H), 2.54 (t, *J* = 5.2 Hz, 4H).

¹³C NMR (100 MHz, DMSO-*d*₆) δ 160.2 (d, *J* = 240 Hz), 160.2, 159.5, 155.1, 154.9, 153.4, 144.3, 139.4, 138.6, 137.8, 130.6, 129.6, 129.2 (d, *J* = 11.3 Hz), 124.5, 117.8, 116.9, 114.5, 112.1, 111.7, 111.4, 110.2, 104.2 (d, *J* = 29.6 Hz), 99.7, 99.4, 69.8, 69.7, 69.1, 68.3, 63.4, 57.1, 54.9, 52.7, 46.8, 44.9.

Ethyl (Z)-5-(4-(2-(2-(4-(13-(4-(3-(7-fluoroimidazo[1,2-a]pyridin-2-yl)-2-oxo-2H-chromen-7-yl)piperazin-1-yl)-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)-3-hydroxybenzylidene)-4-oxo-2-(phenylamino)-4,5-dihydrothiophene-3-carboxylate (**C47**)



3,4-dihydroxybenzaldehyde **1** (0.1 g, 0.72 mmol) in DMF (2 mL) was added 2-(2-chloroethoxy)ethyl 4-methylbenzenesulfonate **2** (0.19 g, 0.72 mmol) and potassium carbonate (0.1 g, 0.72 mmol). The reaction mixture was stirred at 50 °C for overnight. Added water to the reaction mixture, extracted with diethyl ether. The organic phase was washed with brine solution, dried (Na₂SO₄) and concentrated in vacuo to afford compound **3** as a colorless oil (0.15 g, 85%). MS-ESI (*m/z*) [*M*+1]⁺ 245.00, 247.01.

Compound **3** (0.15 g, 0.56 mmol) in DMF (2 mL) was added sodium azide (0.06 g, 0.83 mmol) and tetrabutyl ammonium iodide (0.23 g, 0.11 mmol). The reaction mixture was stirred at 70 °C for overnight. Added water to the reaction mixture, extracted with diethyl ether. The organic phase was washed with brine solution, dried (Na₂SO₄) and concentrated in vacuo. The crude product was purified by silica gel column chromatography (0 – 18% EtOAc in hexanes) to afford compound **4** as a yellow oil (0.08 g, 57%). MS-ESI (*m/z*) [*M*+1]⁺ 252.08.

^1H NMR (400 MHz, Chloroform- d) δ 9.88 (s, 1H), 7.48 (d, J = 2.0 Hz, 1H), 7.44 (dd, J = 8.2, 2.0 Hz, 1H), 7.03 (d, J = 8.2 Hz, 1H), 4.37 – 4.29 (m, 2H), 3.98 – 3.88 (m, 2H), 3.83 – 3.75 (m, 2H), 3.50 – 3.42 (m, 2H).

Compound **4** (0.05 g, 0.199 mmol) in ethanol was added ethyl 4-oxo-2-(phenylamino)-4,5-dihydro thiophene-3-carboxylate **5** (0.057 g, 0.218 mmol) and piperidine (3 μL , 0.029 mmol). The reaction mixture was heated under microwave irradiation at 100 $^\circ\text{C}$ for 45 min. Evaporated the solvent and added ethanol, heated to 50 $^\circ\text{C}$ and filtered and washed with cold ethanol to get compound **6** as a yellow solid, (0.055 g, 57%). MS-ESI (m/z) [$\text{M}+1$] $^+$ 497.08.

^1H NMR (500 MHz, CDCl_3) δ 11.52 (s, 1H), 7.76 (s, 1H), 7.55 – 7.48 (m, 2H), 7.41 (d, J = 7.7 Hz, 3H), 7.16 (d, J = 2.1 Hz, 1H), 7.06 (dd, J = 8.3, 2.2 Hz, 1H), 6.93 (d, J = 8.3 Hz, 1H), 4.44 (q, J = 7.1 Hz, 2H), 4.25 (dd, J = 5.3, 3.7 Hz, 2H), 3.91 – 3.84 (m, 2H), 3.77 (t, J = 4.8 Hz, 2H), 3.45 (t, J = 4.8 Hz, 2H), 1.47 (t, J = 7.0 Hz, 3H).

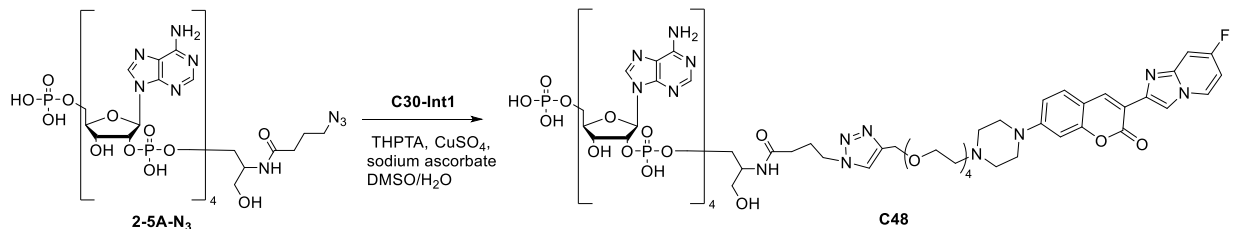
^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 177.4, 171.5, 162.3, 142.3, 142.1, 132.4, 126.7, 125.2, 123.8, 123.1, 121.2, 119.4, 114.8, 111.2, 108.9, 65.5, 64.6, 64.3, 55.9, 45.8, 9.7.

Compound **6** (9 mg, 0.018 mmol) in DMSO (0.5 mL) was added compound **C30-alkyne** (10 mg, 0.017 mmol), sodium ascorbate (40 μL , 100 mM in water, 0.004 mmol), THPTA (40 μL , 100 mM in water, 0.004 mmol) and CuSO_4 (40 μL , 100 mM in water, 0.004 mmol). The reaction vial was sealed, evacuated, and refilled with N_2 three times and stirred at room temperature for overnight. DMSO was removed under vacuum and the residue was purified by silica gel column chromatography (0 – 10% CH_3OH in CH_2Cl_2) to afford **C47** as a yellow solid (7 mg, 38%). MS-ESI (m/z) [$\text{M}/2+1$] $^+$ 538.35. HRMS-ESI (m/z) [$\text{M}+\text{H}$] $^+$ Calcd 1075.4035; Found 1075.4016. [$\text{M}+\text{Na}$] $^+$ Calcd 1097.3855; Found 1097.3812.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.24 (s, 1H), 9.44 (s, 1H), 8.71 – 8.68 (m, 2H), 8.52 (s, 1H), 8.05 (s, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.56 – 7.43 (m, 6H), 7.38 (d, J = 7.8 Hz, 1H), 7.01 – 6.95 (m, 5H), 6.86 (s, 1H), 4.54 (t, J = 5.2 Hz, 2H), 4.47 (s, 2H), 4.27 (q, J = 7.1 Hz, 2H), 4.12 – 4.07 (m, 3H), 3.88 (t, J = 5.2 Hz, 2H), 3.75 (t, J = 5.2 Hz, 2H), 3.56 – 3.47 (m, 14H), 3.18 (d, J = 5.1 Hz, 3H), 2.57-2.55 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 180.9, 175.3, 164.9, 160.2 (d, J = 252.0 Hz), 159.5, 154.9, 153.3, 148.6, 147.0, 143.8, 139.5, 138.6, 137.5, 130.0, 129.6, 129.5, 129.2 (d, J = 11.9 Hz), 128.1, 126.4, 125.5, 124.7, 124.3, 123.1, 115.9, 114.5, 113.7, 112.1, 111.7, 110.2, 104.2 (d, J = 30.2 Hz), 99.6, 99.4, 96.9, 69.8, 69.7, 69.6, 68.9, 68.8, 68.6, 67.8, 63.4, 59.5, 52.7, 49.3, 48.6, 14.4.

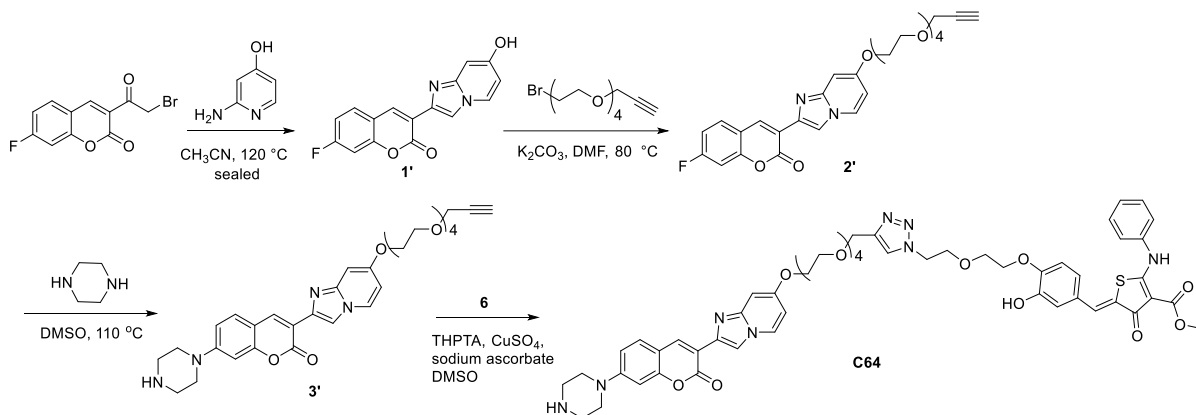
Compound C48



Compound **C30-alkyne** (2 mg, 0.003 mmol) and **2-5A-N₃** (WuXi AppTec, 5 mg, 0.003 mmol) in $\text{DMSO}/\text{H}_2\text{O}$ (0.4 mL/0.4 mL) was added sodium ascorbate (15 μL , 100 mM in water, 0.0015 mmol), THPTA (15 μL , 100 mM in water, 0.0015 mmol) and CuSO_4 (15 μL , 100 mM in water, 0.0015 mmol). The reaction vial was sealed, evacuated, and refilled with N_2 three times and stirred at room temperature for overnight. Solvent was removed by freeze-dry and the residue was purified by reverse-phase column chromatography (0 – 98% acetonitrile in 0.1% ammonium

formate water solution) to afford **C48** as a yellow solid (2 mg, 26%). MS-ESI (m/z) [($M+n$)/ n]⁺ 726.74 ($n=3$), 545.33 ($n=4$), 435.25 ($n=5$). HPLC purity: 93.39%.

Ethyl (Z)-5-(3-hydroxy-4-(2-(2-(4-(13-((2-(2-oxo-7-(piperazin-1-yl)-2H-chromen-3-yl)imidazo[1,2-a]pyridin-7-yl)oxy)-2,5,8,11-tetraoxatridecyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethoxy)benzylidene)-4-oxo-2-(phenylamino)-4,5-dihydrothiophene-3-carboxylate (C64)



Following general procedures step 1, from 3-(2-bromoacetyl)-7-fluoro-2H-chromen-2-one (200 mg, 0.7 mmol) and 2-aminopyridin-4-ol (77 mg, 0.7 mmol), 50 mg of compound **1'** was obtained as a yellow solid (yield, 24%). MS-ESI (m/z) [$M+1$]⁺ 297.03.

Compound **1'** (41 mg, 0.14 mmol) in DMF (1 mL) was added K_2CO_3 (37 mg, 0.27 mmol) and Propargyl-PEG₄-Br (60 mg, 0.21 mmol). The reaction mixture was heated at 80 °C for 6 h. The reaction mixture was added ice-water and extracted with EtOAc. The organic phase was washed with brine solution, dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica gel column chromatography (0 – 5% CH_3OH in CH_2Cl_2) to afford compound **2'** as a yellow solid (21 mg, 30%). MS-ESI (m/z) [$M+1$]⁺ 511.10.

Following general procedures step 2, from compound **2'** (21 mg, 0.7 mmol), 14 mg compound **3'** was obtained as a yellow solid (yield, 59%). MS-ESI (m/z) [$M+1$]⁺ 577.22.

¹H NMR (500 MHz, $DMSO-d_6$) δ 8.62 (s, 1H), 8.46 (d, J = 7.4 Hz, 1H), 8.35 (s, 1H), 7.67 (d, J = 9.0 Hz, 1H), 7.01 (d, J = 8.9 Hz, 1H), 6.90 (s, 1H), 6.86 (s, 1H), 6.63 (d, J = 7.5 Hz, 1H), 4.19 (t, J = 5.0 Hz, 2H), 4.14 (d, J = 2.4 Hz, 2H), 3.80 (t, J = 5.0 Hz, 2H), 3.62 – 3.60 (m, 2H), 3.57 – 3.52 (m, 10H), 3.42 (t, J = 2.5 Hz, 1H), 2.31 (t, J = 5.1 Hz, 4H), 2.86 (t, J = 5.1 Hz, 4H).

¹³C NMR (126 MHz, $DMSO-d_6$) δ 159.6, 157.0, 154.7, 153.6, 145.7, 138.1, 137.8, 129.4, 127.8, 115.0, 111.7, 111.2, 110.3, 106.7, 99.3, 94.3, 80.4, 77.1, 69.9, 69.8, 69.7, 69.5, 68.6, 68.5, 67.6, 57.5, 47.8, 45.2.

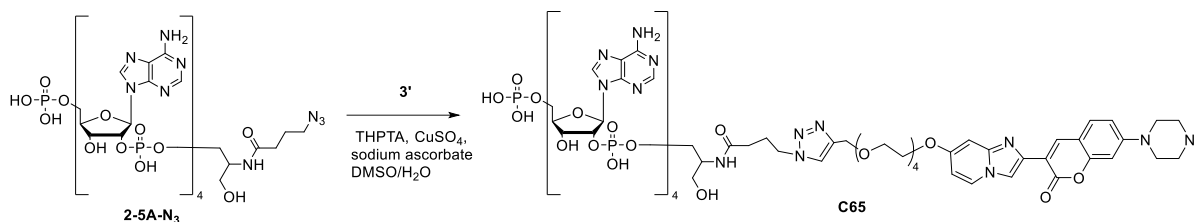
Compound **3'** (4 mg, 0.007 mmol) in DMSO (0.5 mL) was added compound **6** (3.5 mg, 0.007 mmol), sodium ascorbate (20 μ L, 100 mM in water, 0.002 mmol), THPTA (20 μ L, 100 mM in water, 0.002 mmol) and $CuSO_4$ (20 μ L, 100 mM in water, 0.002 mmol). The reaction vial was sealed, evacuated, and refilled with N_2 three times and stirred at room temperature for overnight. DMSO was removed under vacuum and the residue was purified by silica gel column chromatography (0 – 10% CH_3OH in CH_2Cl_2) to afford **C64** as a yellow solid (3 mg, 41%). MS-ESI (m/z) [$M/2+1$]⁺ 537.40. HRMS-ESI (m/z) [$M+Na$]⁺ Calcd 1095.3898; Found 1095.3895.

¹H NMR (500 MHz, $DMSO-d_6$) δ 9.42 (s, 1H), 8.62 (s, 1H), 8.45 (d, J = 7.4 Hz, 1H), 8.34 (s, 1H), 8.04 (s, 1H), 7.68 (d, J = 8.8 Hz, 1H), 7.50 – 7.47 (m, 2H), 7.39 – 7.33 (m, 4H), 7.03 – 6.88 (m,

6H), 6.61 (dd, $J = 7.4, 2.5$ Hz, 1H), 4.53 (t, $J = 5.2$ Hz, 2H), 4.47 (s, 2H), 4.24 (q, $J = 7.1$ Hz, 2H), 4.16 (t, $J = 5.2$ Hz, 2H), 4.06 (t, $J = 5.2$ Hz, 2H), 3.87 (t, $J = 5.2$ Hz, 2H), 3.78 – 3.73 (m, 4H), 3.59 – 3.47 (m, 16H), 1.28 (t, $J = 7.1$ Hz, 3H).

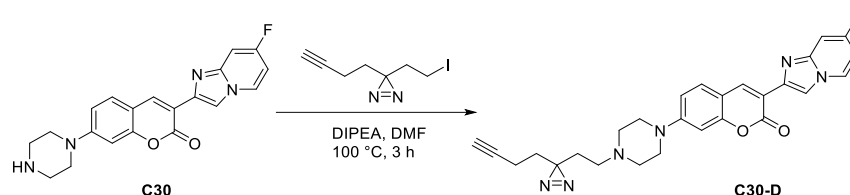
^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 181.4, 165.2, 159.5, 157.0, 154.6, 148.2, 147.0, 145.7, 143.8, 138.0, 137.7, 129.4, 127.8, 127.0, 124.6, 124.4, 122.7, 115.9, 115.4, 113.8, 111.9, 111.3, 110.6, 106.8, 99.8, 96.5, 94.3, 69.9, 69.8, 69.8, 69.7, 68.9, 68.8, 68.6, 67.8, 67.6, 63.4, 59.2, 49.3, 14.5.

Compound C65



Compound **3'** (3 mg, 0.005 mmol) and **2-5A-N₃** (WuXi AppTec, 8 mg, 0.005 mmol) in $\text{DMSO}/\text{H}_2\text{O}$ (0.5 mL/0.5 mL) was added sodium ascorbate (25 μL , 100 mM in water, 0.0025 mmol), THPTA (25 μL , 100 mM in water, 0.0025 mmol) and CuSO_4 (25 μL , 100 mM in water, 0.0025 mmol). The reaction vial was sealed, evacuated, and refilled with N_2 three times and stirred at room temperature for overnight. Solvent was removed by freeze-dry and the residue was purified by reverse-phase column chromatography (0 – 98% acetonitrile in 0.1% ammonium formate water solution) to afford **C65** as a yellow solid (3 mg, 26%). MS-ESI (m/z) $[(M+n)/n]^+$ 726.29 ($n=3$), 545.00 ($n=4$). HPLC purity: 89.28%.

7-(4-(2-(3-(but-3-yn-1-yl)-3*H*-diazirin-3-yl)ethyl)piperazin-1-yl)-3-(7-fluoroimidazo[1,2-*a*]pyridin-2-yl)-2*H*-chromen-2-one (C30-D)

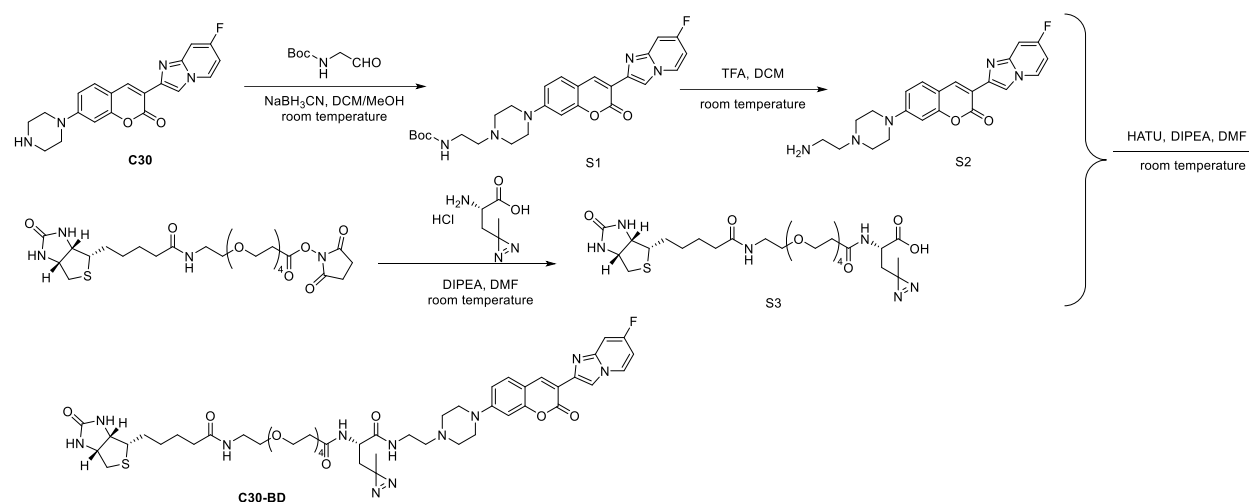


Compound **C30** (10 mg, 0.027 mmol) in DMF (0.5 mL) was added 3-(but-3-yn-1-yl)-3-(2-iodoethyl)-3*H*-diazirine (7 mg, 0.028 mmol) and DIPEA (14 μL , 0.081 mmol). The mixture was stirred at 100 °C for 3 h. The mixture was diluted with DCM and water. The organic phase was washed with brine solution, dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica gel column chromatography (0 – 10% CH_3OH in CH_2Cl_2) to afford compound **C30-D** as a yellow solid (7 mg, 52%). MS-ESI (m/z) $[M+1]^+$ 485.19. HRMS-ESI (m/z) $[M+H]^+$ Calcd 485.2101; Found 485.2119. $[M+\text{Na}]^+$ Calcd 507.1921; Found 507.1940. HPLC purity: >98%.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.63 – 8.59 (m, 2H), 8.44 (s, 1H), 7.60 (d, $J = 8.8$ Hz, 1H), 7.30 (dd, $J = 10.1, 2.6$ Hz, 1H), 6.94 (dd, $J = 8.9, 2.4$ Hz, 1H), 6.89 (td, $J = 7.6, 2.6$ Hz, 1H), 6.79 (d, $J = 2.4$ Hz, 1H), 2.77 (t, $J = 2.6$ Hz, 1H), 2.40 (t, $J = 5.1$ Hz, 4H), 2.12 (t, $J = 7.4$ Hz, 2H), 1.96 (td, $J = 7.4, 2.7$ Hz, 2H), 1.55 – 1.51 (m, 4H).

^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) δ 160.6 (d, $J = 239.4$ Hz), 159.9, 155.3, 153.8, 144.8 (d, $J = 14.6$ Hz), 139.8, 139.0, 130.0, 129.7 (d, $J = 11.6$ Hz), 115.0, 112.4 (d, $J = 42.5$ Hz), 110.7, 104.6 (d, $J = 29.8$ Hz), 100.1, 99.9, 83.8, 72.2, 52.6, 52.5, 47.3, 32.2, 29.9, 28.1, 13.2.

***N*-((*S*)-1-((2-(4-(3-(7-fluoroimidazo[1,2-*a*]pyridin-2-yl)-2-oxo-2*H*-chromen-7-yl)piperazin-1-yl)ethyl)amino)-3-(3-methyl-3*H*-diazirin-3-yl)-1-oxopropan-2-yl)-1-(5-((3*aS*,4*S*,6*aR*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)-3,6,9,12-tetraoxapentadecan-15-amide (C30-BD)**



Compound **C30** (40 mg, 0.11 mmol) in DCM (0.5 mL) and MeOH (0.5 mL) was cooled with ice. Acetaldehyde (32 μL , 0.55 mmol) was added, followed by sodium cyanoborohydride (35 mg, 0.55 mmol). The mixture was stirred at room temperature for 2 h. The mixture was diluted with DCM and water was added dropwise to quench the reaction. The organic phase was washed with brine solution, dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica gel column chromatography (0 – 5% CH_3OH in CH_2Cl_2) to afford compound **S1** as a yellow solid (40 mg, 72%). MS-ESI (m/z) [$\text{M}+1$] $^+$ 508.13.

Compound **S1** was dissolved in DCM (1 mL), TFA (0.5 mL) was added. The mixture was stirred at room temperature for 1 h. DCM and TFA were removed under vacuum. The residue was dissolved in DMF (1 mL) and used directly in the amide coupling reaction without purification.

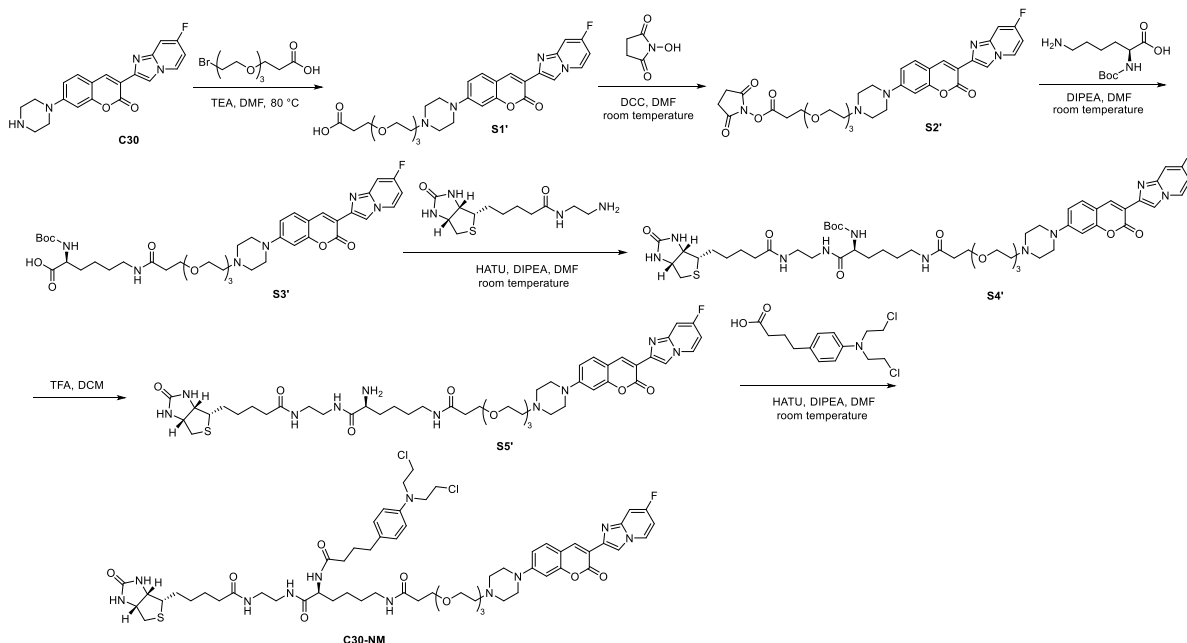
Biotin-NHS (30 mg, 0.051 mmol) was dissolved in DMF (1 mL), (*S*)-2-amino-3-(3-methyl-3*H*-diazirin-3-yl) propanoic acid hydrochloride (10 mg, 0.055 mmol) and DIPEA (44 μL , 0.25 mmol) were added. The mixture was stirred at room temperature overnight. The mixture was cooled with ice, HATU (28 mg, 0.075 mmol) was added, and stirred at room temperature for 15 min. Compound **S2** in DMF was added and the mixture was stirred at room temperature overnight. The mixture was diluted with ethyl acetate and water. The organic phase was washed with brine solution, dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica gel column chromatography (0 – 10% CH_3OH in CH_2Cl_2) to afford compound **C30-BD** as a yellow solid (10 mg, 20%). MS-ESI (m/z) [$(\text{M}+2)/2$] $^+$ 503.86. HRMS-ESI (m/z) [$\text{M}+\text{Na}$] $^+$ Calcd 1028.4440; Found 1028.4436. HPLC purity: >98%.

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 8.71 – 8.68 (m, 2H), 8.52 (s, 1H), 8.14 (d, $J = 8.4$ Hz, 1H), 7.96 (t, $J = 5.6$ Hz, 1H), 7.83 (t, $J = 5.7$ Hz, 1H), 7.70 (d, $J = 9.0$ Hz, 1H), 7.39 (dd, $J = 10.0, 2.6$ Hz, 1H), 7.03 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.98 (td, $J = 7.6, 2.6$ Hz, 1H), 6.88 (d, $J = 2.4$ Hz, 1H), 6.42 (s, 1H), 6.36 (s, 1H), 4.32 – 4.29 (m, 1H), 4.27 – 4.22 (m, 1H), 4.14 – 4.11 (m, 1H), 3.63 (td, $J = 6.6, 1.4$ Hz, 2H), 3.50 – 3.49 (m, 12H), 3.40 – 3.38 (m, 5H), 3.26 – 3.16 (m, 5H), 3.11 – 3.07 (m, 1H),

2.81 (dd, $J = 12.4, 5.1$ Hz, 1H), 2.58 (d, $J = 12.5$ Hz, 1H), 2.53 (t, $J = 5.2$ Hz, 4H), 2.42 (t, $J = 6.0$ Hz, 4H), 2.06 (t, $J = 7.5$ Hz, 2H), 1.75 (dd, $J = 14.7, 5.0$ Hz, 1H), 1.63 – 1.58 (m, 1H), 1.53 – 1.46 (m, 4H), 1.33 – 1.27 (m, 2H), 1.01 (s, 3H).

^{13}C NMR (126 MHz, DMSO- d_6) δ 172.6, 171.0, 170.4, 163.2, 160.7 (d, $J = 252$ Hz), 160.0, 155.4, 153.8, 144.8 (d, $J = 14.8$ Hz), 139.8, 139.1, 130.1, 129.7 (d, $J = 11.6$ Hz), 114.9, 112.4 (d, $J = 42.2$ Hz), 110.7, 104.6 (d, $J = 29.7$ Hz), 100.1, 99.9, 70.2, 70.1, 70.0, 69.6, 67.2, 61.5, 59.7, 57.0, 55.9, 52.7, 49.1, 47.3, 35.6, 28.7, 28.5, 25.7, 24.8, 20.0.

(S)-2-(4-(4-(bis(2-chloroethyl)amino)phenyl)butanamido)-6-(3-(2-(2-(2-(4-(3-(7-fluoroimidazo[1,2-a]pyridin-2-yl)-2-oxo-2H-chromen-7-yl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)propanamido)-N-(2-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)ethyl)hexanamide (C30-NM)



Compound **C30** (50 mg, 0.137 mmol) in DMF (1 mL) was added 3-(2-(2-(2-bromoethoxy)ethoxy)ethoxy) propanoic acid (58 mg, 0.2 mmol) and triethylamine (55 μL , 0.4 mmol). The mixture was stirred at 80 °C overnight. DMF and TEA were removed under vacuum. The crude product was purified by silica gel column chromatography (0 – 15% CH_3OH in CH_2Cl_2) to afford compound **S1'** as a yellow solid (55 mg, 70%). MS-ESI (m/z) $[\text{M}+1]^+$ 569.21.

Compound **S1'** (55 mg, 0.096 mmol) was dissolved in DMF (1 mL), N-hydroxysuccinimide (17 mg, 0.15 mmol) and DCC (31 mg, 0.15 mmol) were added. The mixture was stirred at room temperature overnight. Precipitate was filtered and DMF was removed under vacuum. The crude product was purified by silica gel column chromatography (0 – 10% CH_3OH in CH_2Cl_2) to afford compound **S2'** as a yellow solid (50 mg, 77%). MS-ESI (m/z) $[\text{M}+1]^+$ 666.27.

Compound **S2'** (50 mg, 0.075 mmol) was dissolved in DMF (1 mL), Boc-Lys-OH (27 mg, 0.11 mmol) and DIPEA (19 μL , 0.11 mmol) were added. The mixture was stirred at room temperature overnight. DMF and DIPEA were removed under vacuum. The crude product was purified by silica gel column chromatography (0 – 15% CH_3OH in CH_2Cl_2) to afford compound **S3'** as a yellow solid (45 mg, 75%). MS-ESI (m/z) $[\text{M}+1]^+$ 797.26.

Compound **S3'** (45 mg, 0.056 mmol) was dissolved in DMF (1 mL) and cooled with ice, HATU (27 mg, 0.07 mmol) and DIPEA (24 μ L, 0.14 mmol) were added. The mixture was stirred at room temperature for 15 min. Biotin-amine (20 mg, 0.07 mmol) was added and the mixture was stirred at room temperature overnight. DMF and DIPEA were removed under vacuum. The crude product was purified by silica gel column chromatography (0 – 15% CH₃OH in CH₂Cl₂) to afford compound **S4'** as a yellow solid (40 mg, 67%). MS-ESI (*m/z*) [(M+2)/2]⁺ 533.38.

Compound **S4'** (40 mg, 0.037 mmol) was dissolved in DCM (1 mL) and TFA (0.5 mL). The mixture was stirred at room temperature for 1 h. DCM and TFA were removed under vacuum. The residue was dissolved in DMF (0.5 mL) and used directly in the amide coupling reaction without purification.

Chlorambucil (12 mg, 0.04 mmol) was dissolved in DMF (0.5 mL), HATU (19 mg, 0.05 mmol) and DIPEA (34 μ L, 0.2 mmol) were added. The mixture was stirred at room temperature for 15 min. Compound **S5'** in DMF was added and the mixture was stirred at room temperature overnight. DMF and DIPEA were removed under vacuum. The crude product was purified by silica gel column chromatography (0 – 10% CH₃OH in CH₂Cl₂) to afford compound **C30-NM** as a yellow solid (20 mg, 43%). MS-ESI (*m/z*) [(M+2)/2]⁺ 626.74. HRMS-ESI (*m/z*) [M+Na]⁺ Calcd 1272.5226; Found 1272.5209. HPLC purity: 98.19%.

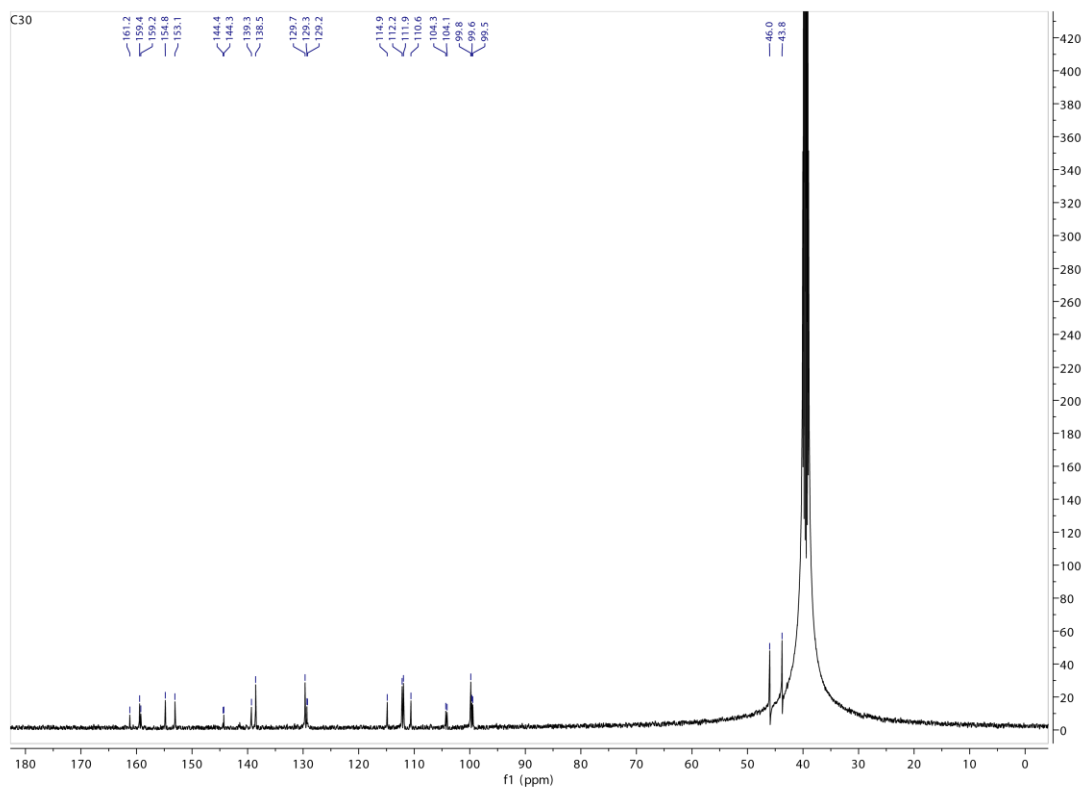
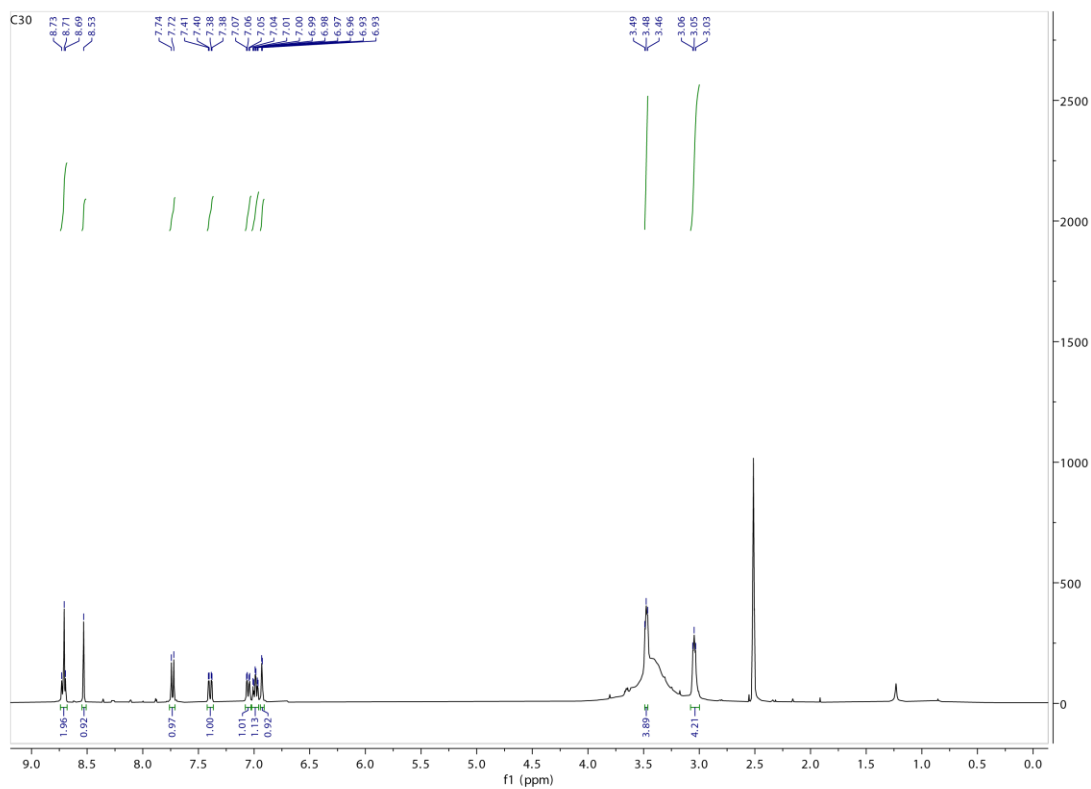
¹H NMR (500 MHz, DMSO-*d*₆) δ 8.71 – 8.68 (m, 2H), 8.52 (s, 1H), 7.91 – 7.88 (m, 2H), 7.81 (t, *J* = 5.6 Hz, 1H), 7.76 (t, *J* = 5.3 Hz, 1H), 7.69 (d, *J* = 8.9 Hz, 1H), 7.39 (dd, *J* = 10.1, 2.6 Hz, 1H), 7.04 – 6.96 (m, 4H), 6.88 (d, *J* = 2.4 Hz, 1H), 6.66 (s, 1H), 6.65 (s, 1H), 6.43 (s, 1H), 6.36 (s, 1H), 4.30 (dd, *J* = 7.7, 5.1 Hz, 1H), 4.15 – 4.10 (m, 3H), 3.72– 3.66 (m, 8H), 3.59 (t, *J* = 6.5 Hz, 2H), 3.55 (t, *J* = 5.9 Hz, 2H), 3.51 – 3.47 (m, 8H), 3.17 (d, *J* = 5.2 Hz, 1H), 3.10 – 3.07 (m, 5H), 3.03 – 2.98 (m, 2H), 2.81 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.59 – 2.53 (m, 7H), 2.42 (t, *J* = 8.0 Hz, 2H), 2.29 (t, *J* = 6.5 Hz, 2H), 2.14 (t, *J* = 7.5 Hz, 2H), 2.04 (t, *J* = 7.5 Hz, 2H), 1.75 – 1.69 (m, 2H), 1.64 – 1.57 (m, 3H), 1.52 – 1.44 (m, 4H), 1.39 – 1.24 (m, 7H).

¹³C NMR (126 MHz, DMSO-*d*₆) δ 172.8, 172.6, 172.5, 170.3, 163.2, 160.0, 155.4, 153.9, 144.8, 144.7, 139.9, 139.1, 130.5, 130.0, 129.8, 129.6, 114.9, 112.5, 112.3, 112.2, 110.7, 100.1, 99.9, 70.2, 70.0, 68.8, 67.3, 61.5, 59.7, 57.6, 55.8, 53.2, 52.7, 47.3, 35.7, 35.2, 34.1, 32.0, 29.3, 28.6, 28.5, 27.8, 25.6, 23.4.

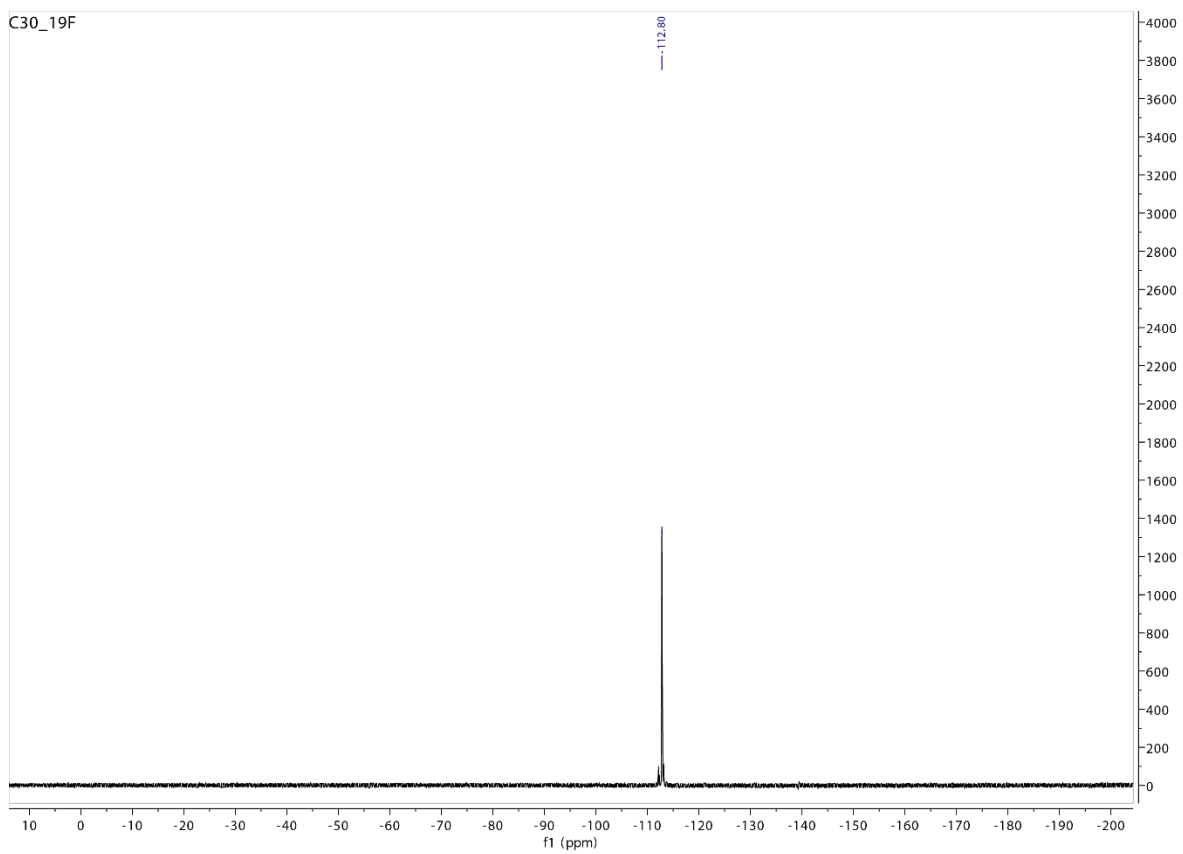
References

1. Busan, S. & Weeks, K. M. Accurate detection of chemical modifications in RNA by mutational profiling (MaP) with ShapeMapper 2. *RNA* **24**, 143–148 (2018).
2. Tang, Z. *et al.* Recognition of single-stranded nucleic acids by small-molecule splicing modulators. *Nucleic Acids Res.* **49**, 7870-7883. PMC8373063 (2021).
3. Adhikary, P. *et al.* Discovery of Small Anti-ACE2 Peptides to Inhibit SARS-CoV-2 Infectivity. *Adv. Ther.* **4**, 2100087 (2021).
4. Woll, M. G. *et al.* Discovery and Optimization of Small Molecule Splicing Modifiers of Survival Motor Neuron 2 as a Treatment for Spinal Muscular Atrophy. *J. Med. Chem.* **59**, 6070–6085 (2016).

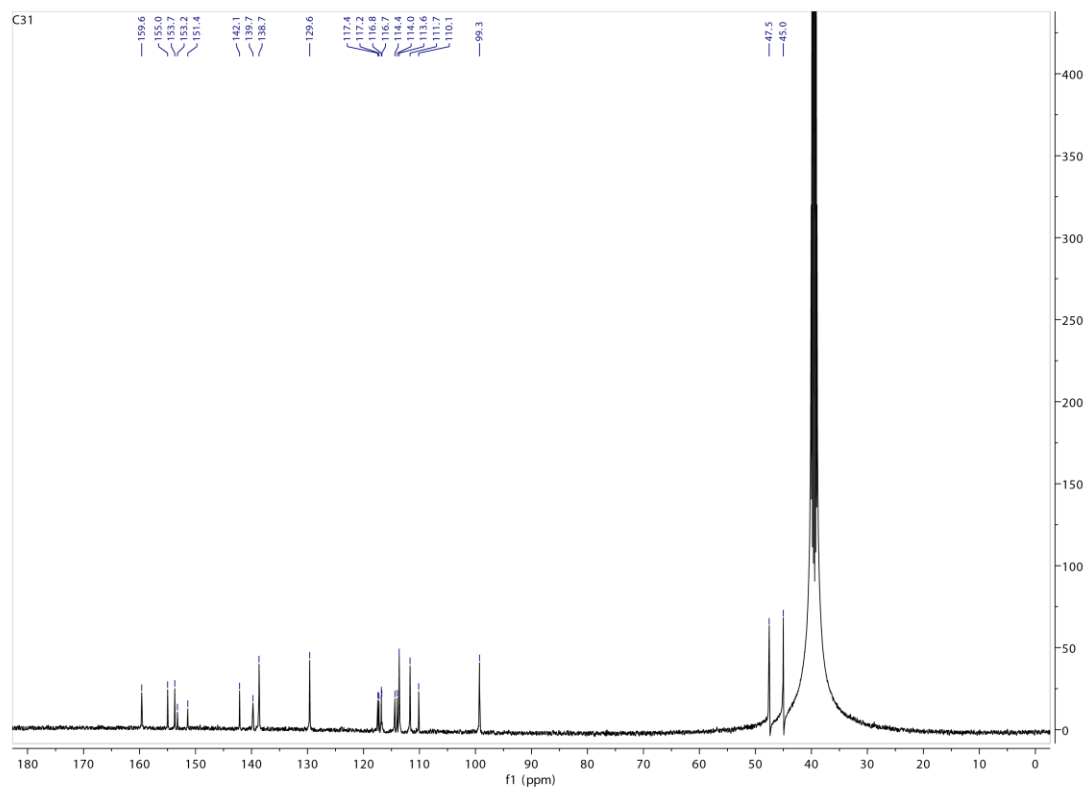
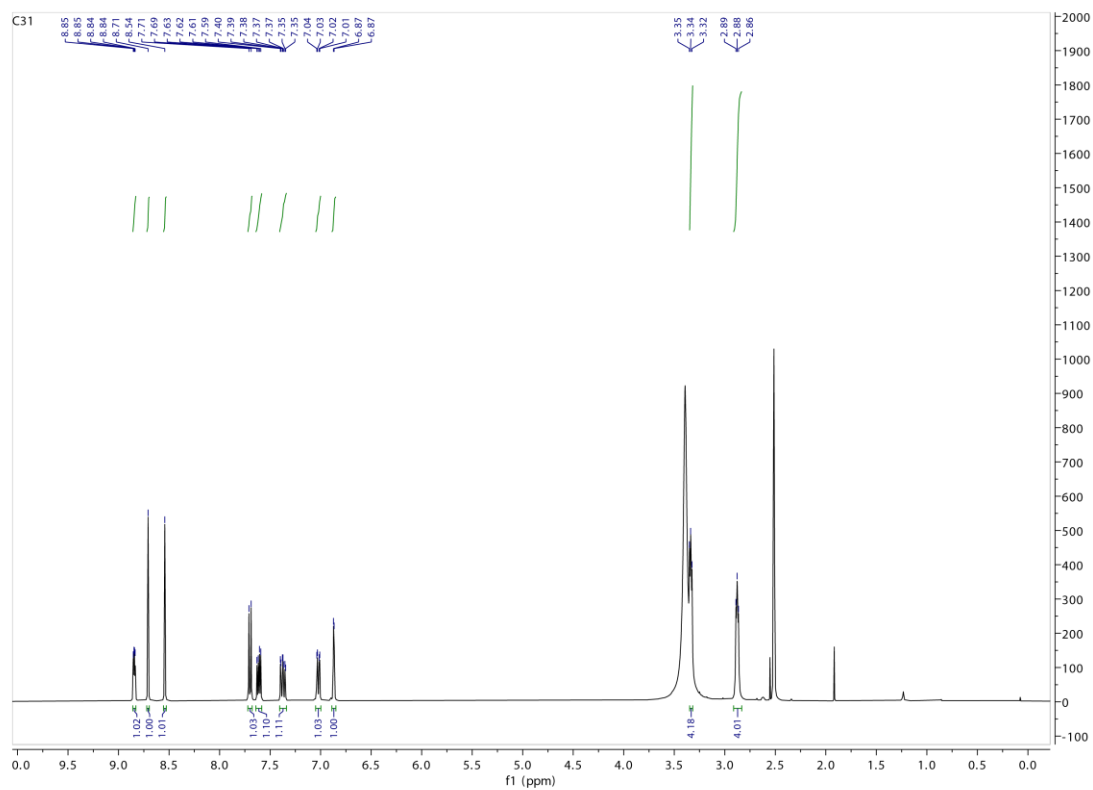
NMR and HPLC Spectra C30

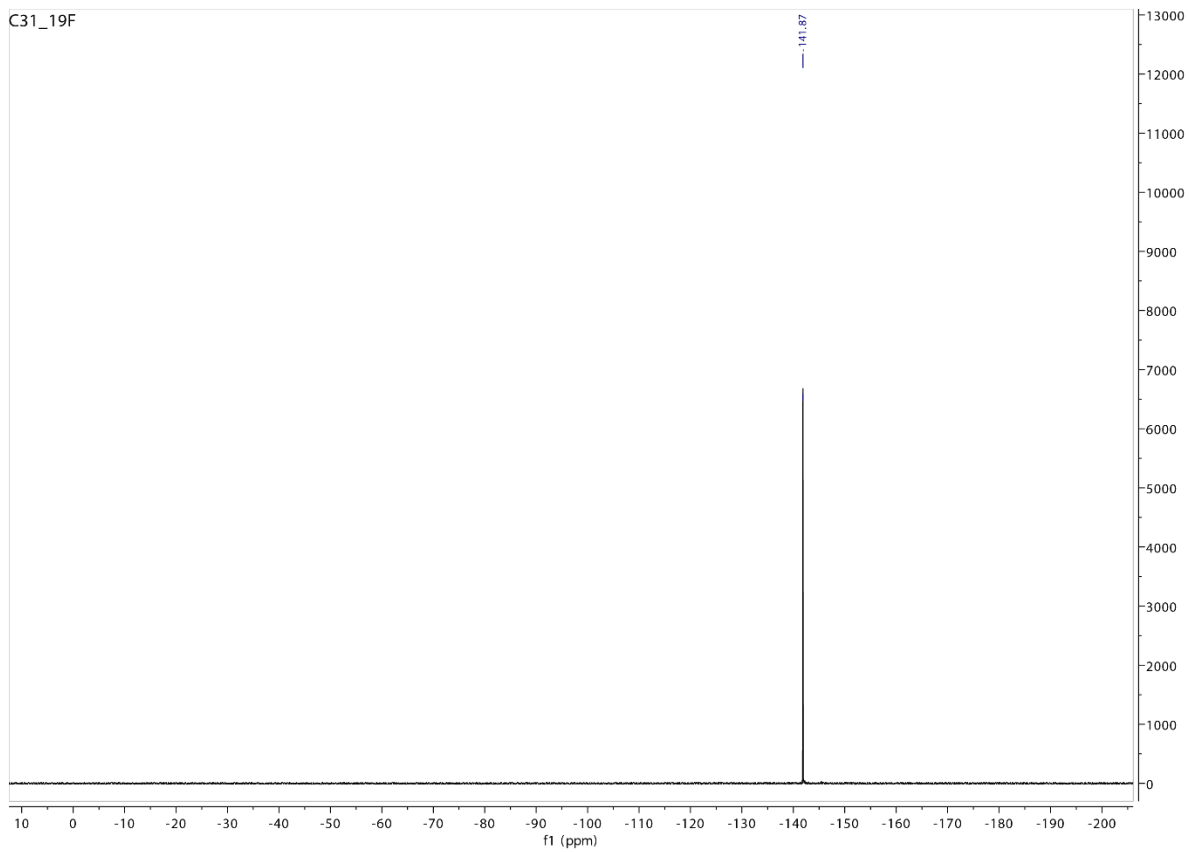


C30_19F

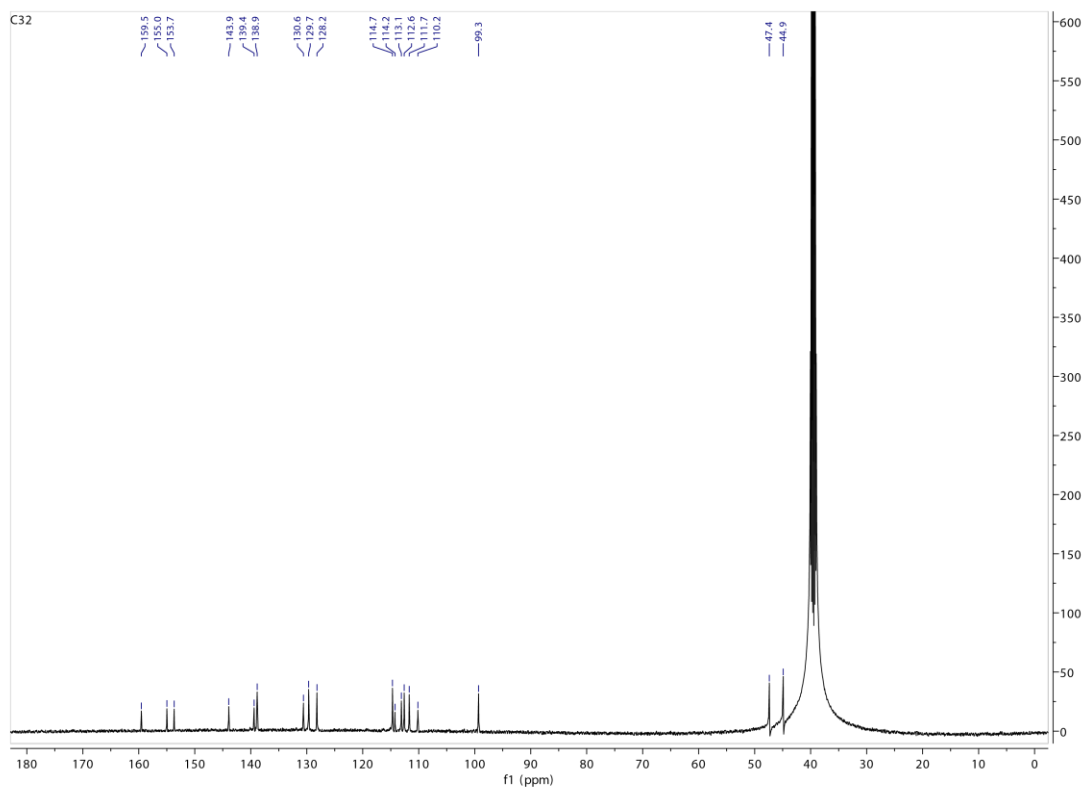
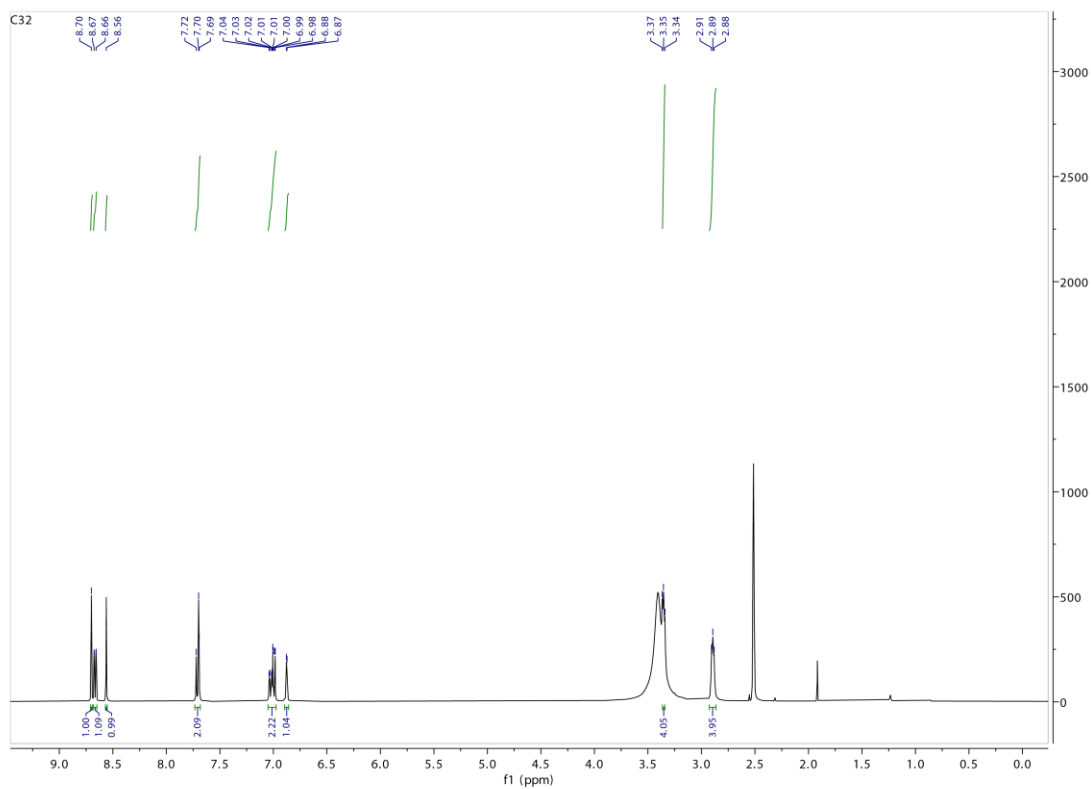


C31

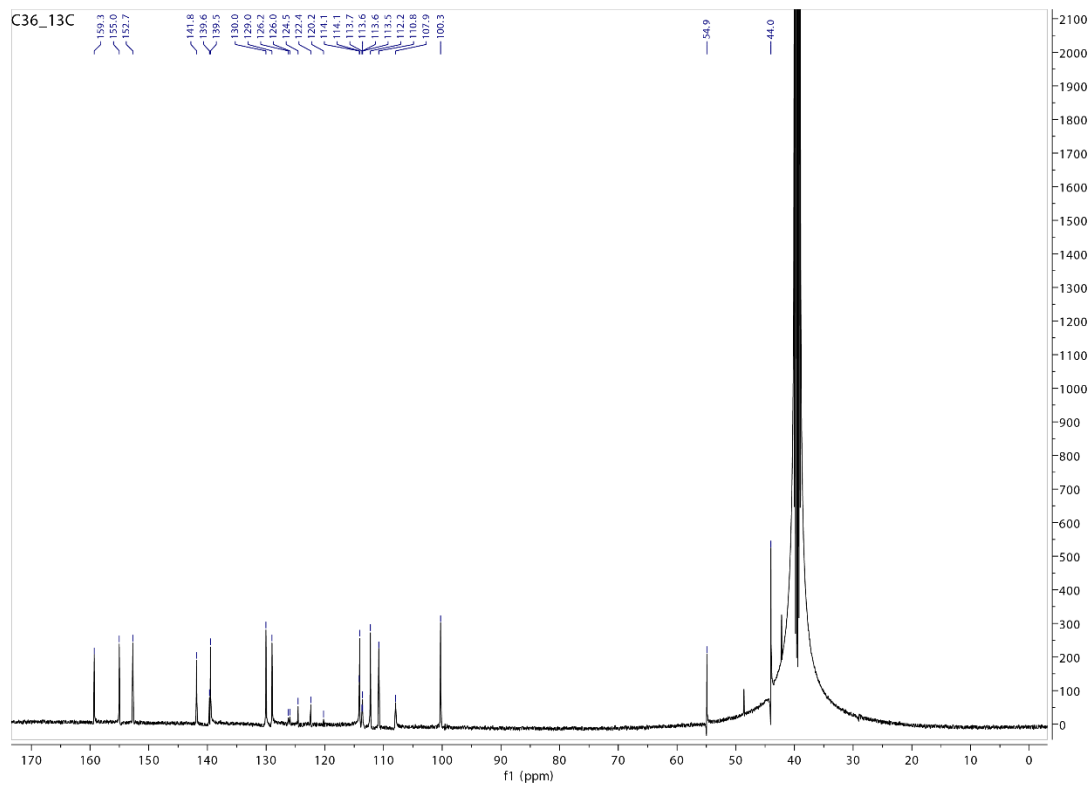
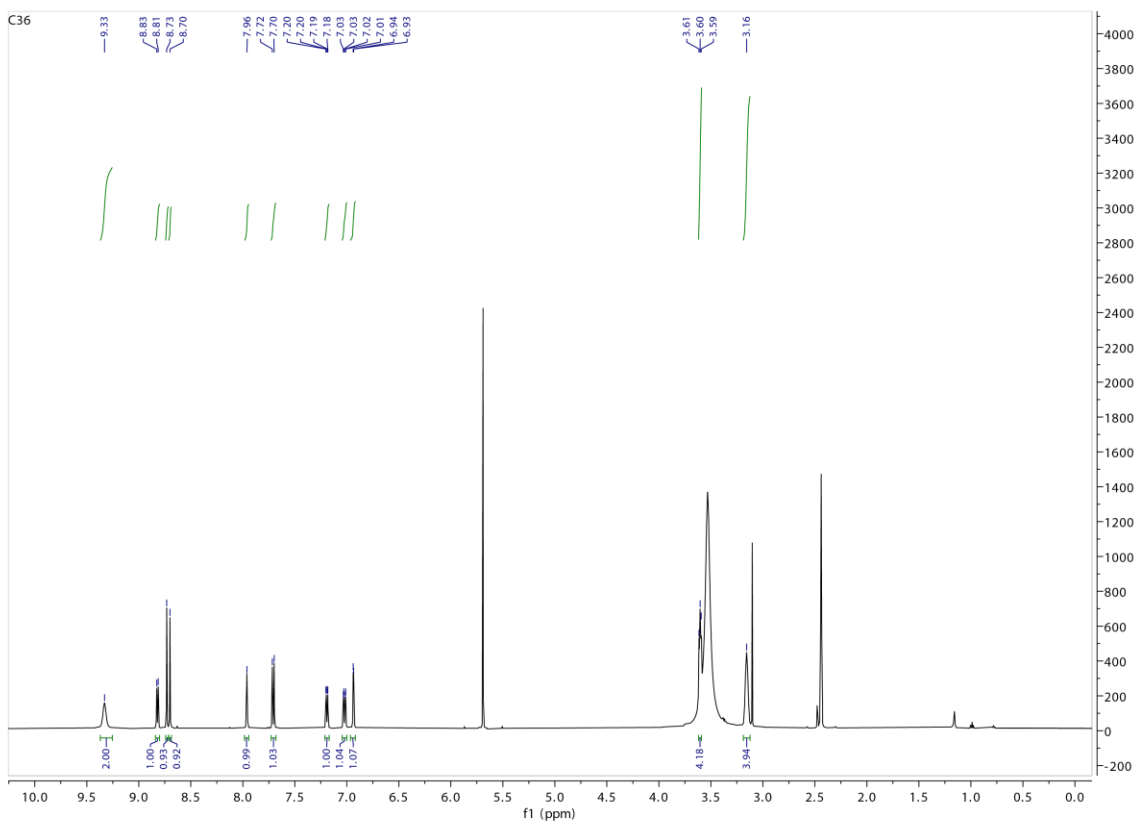


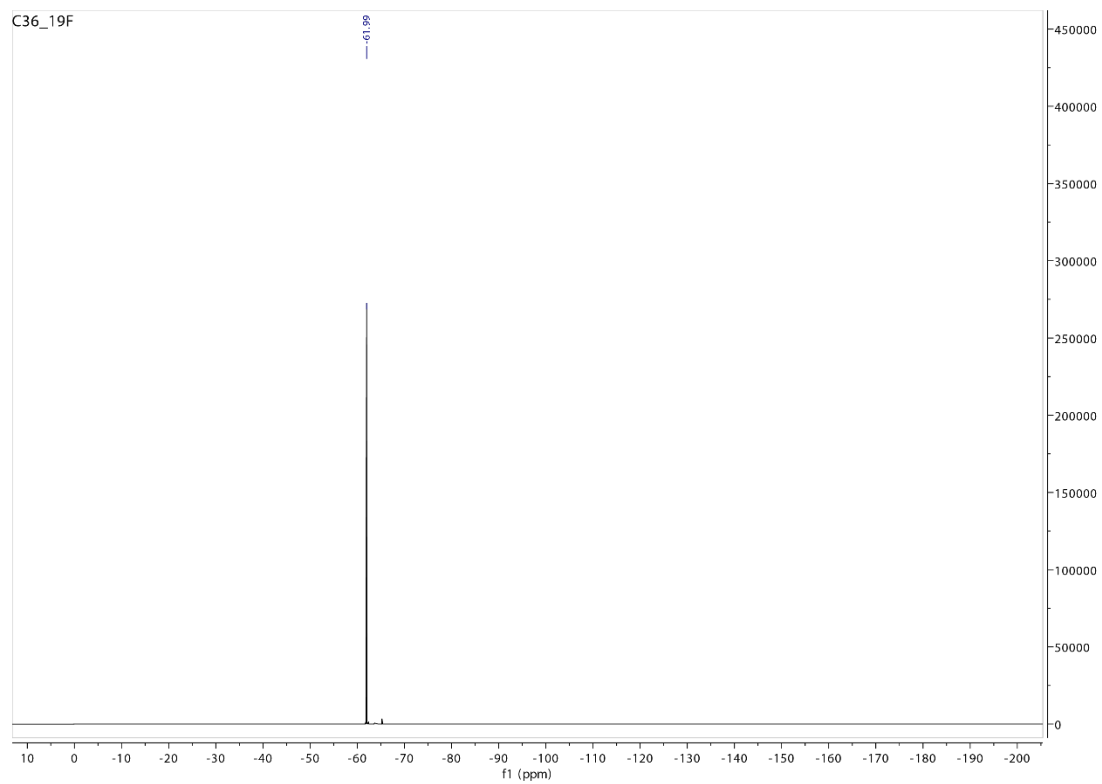


C32

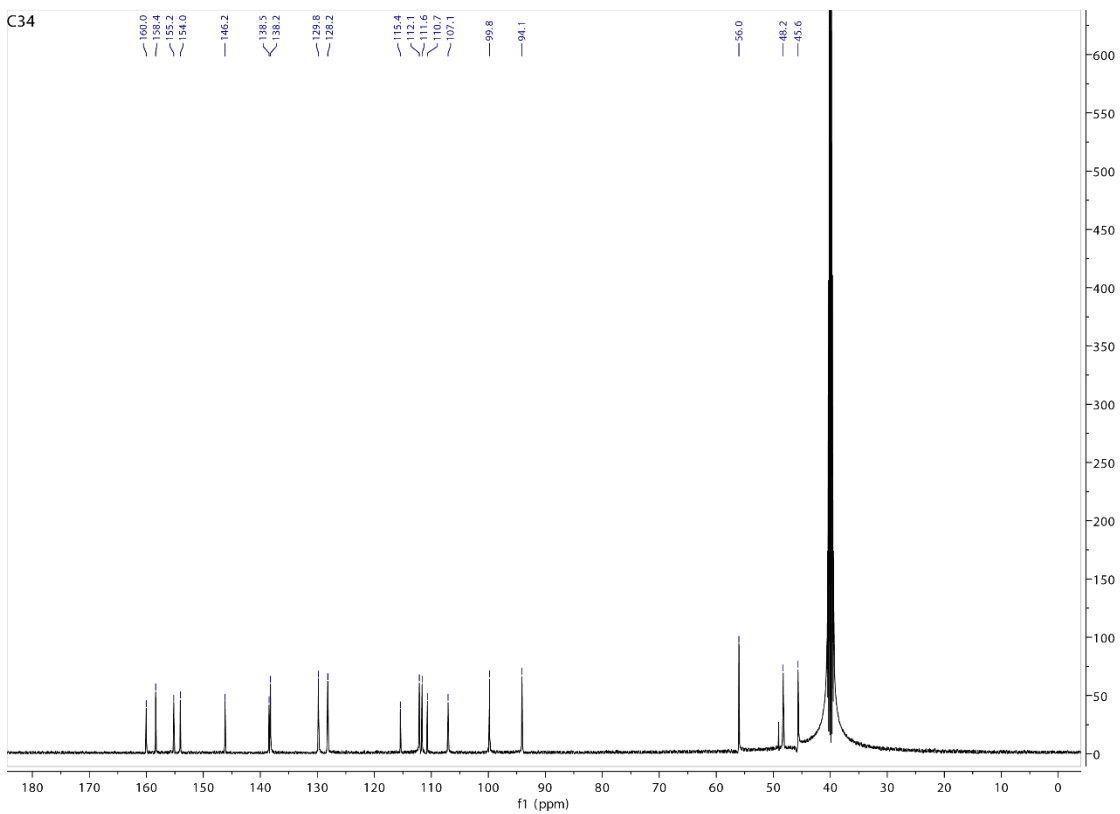
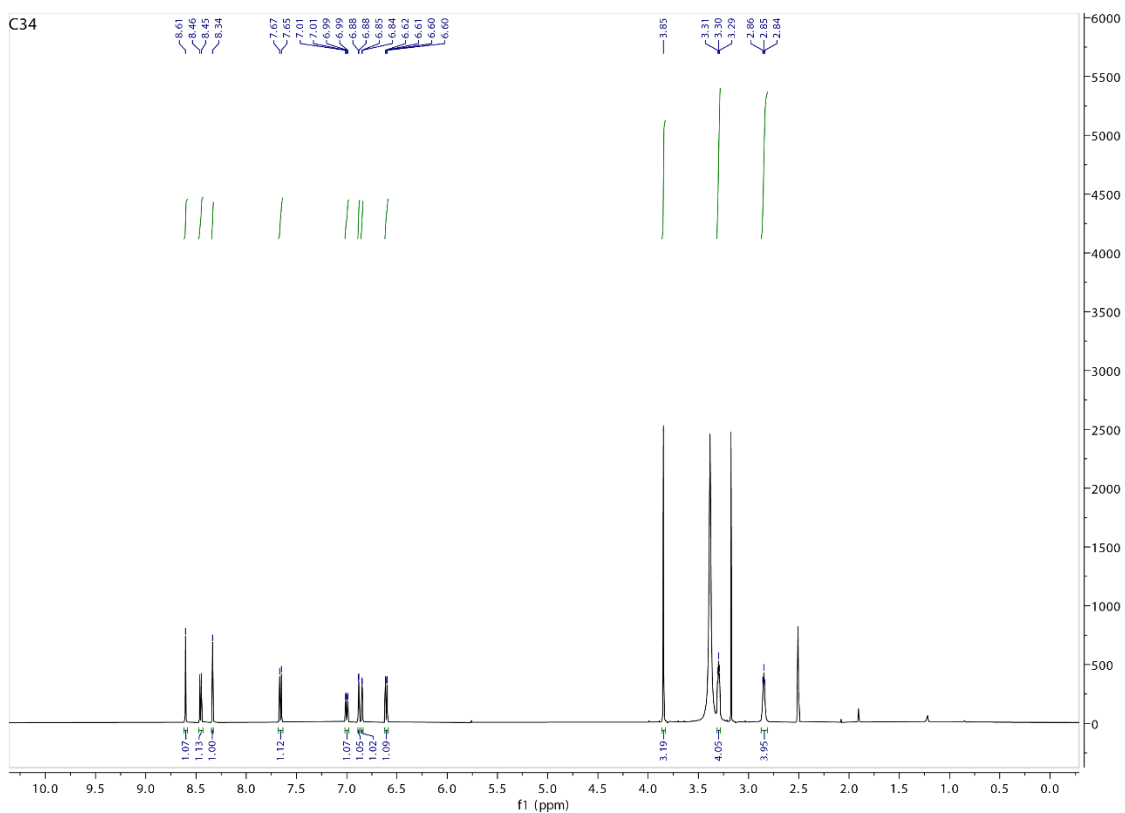


C36

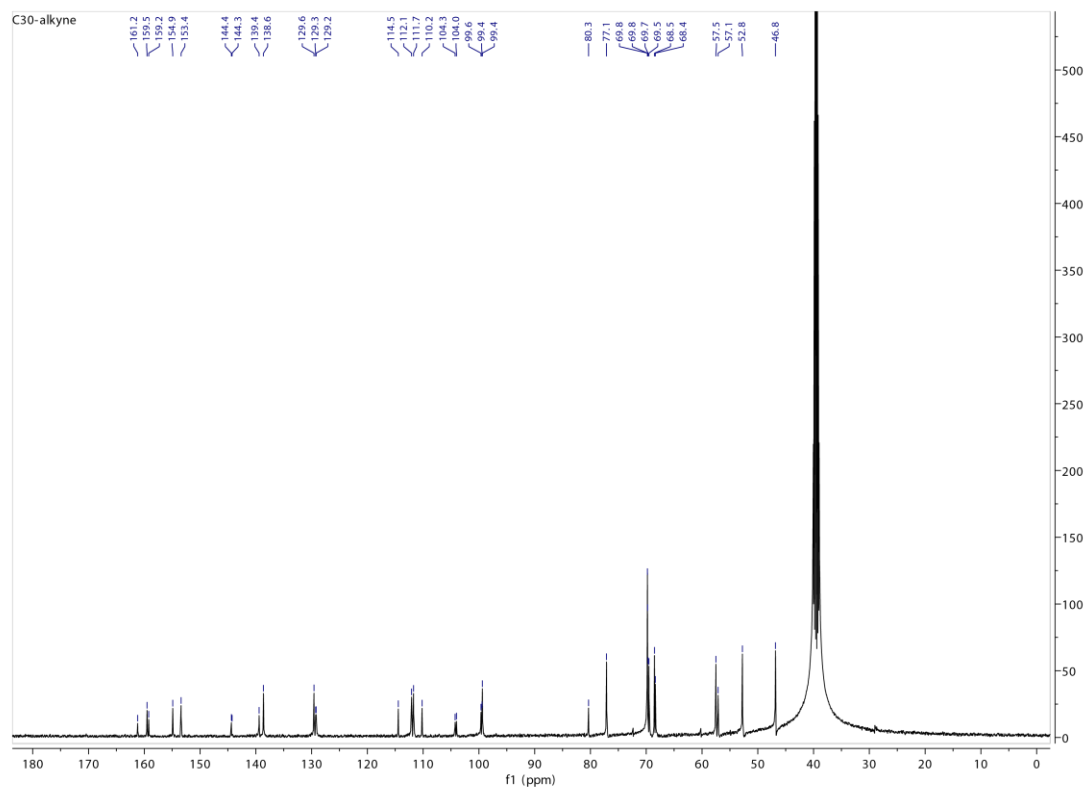
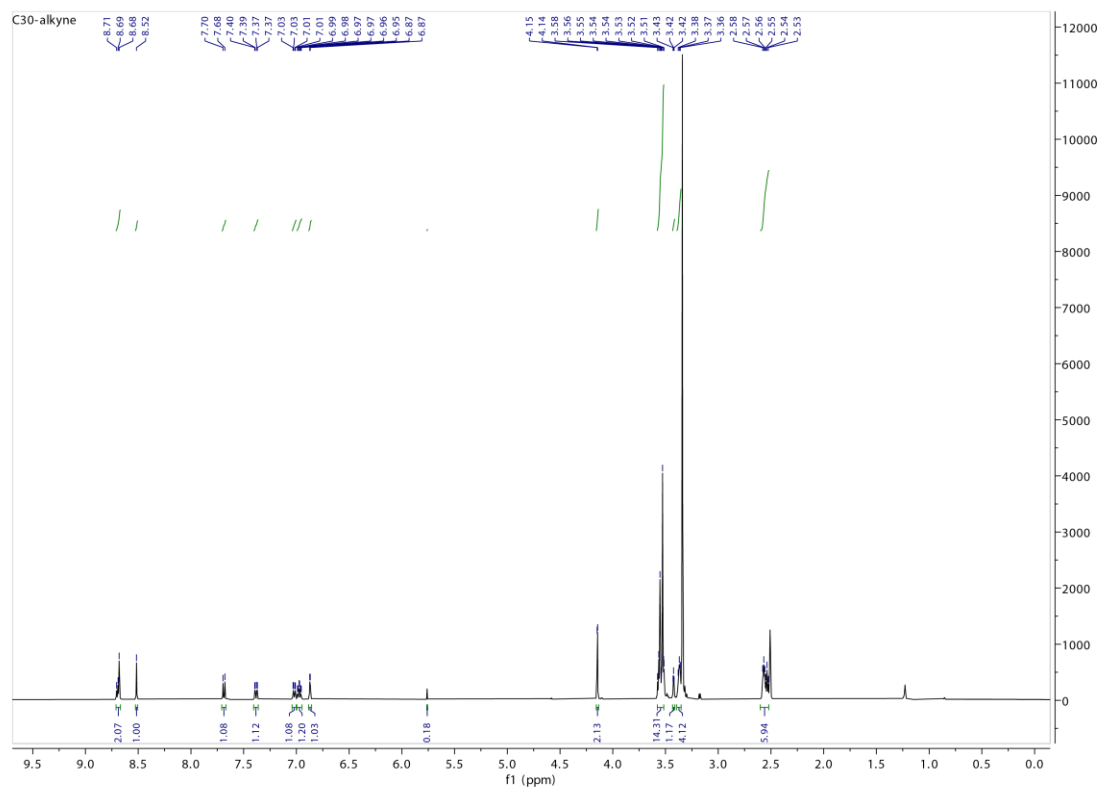




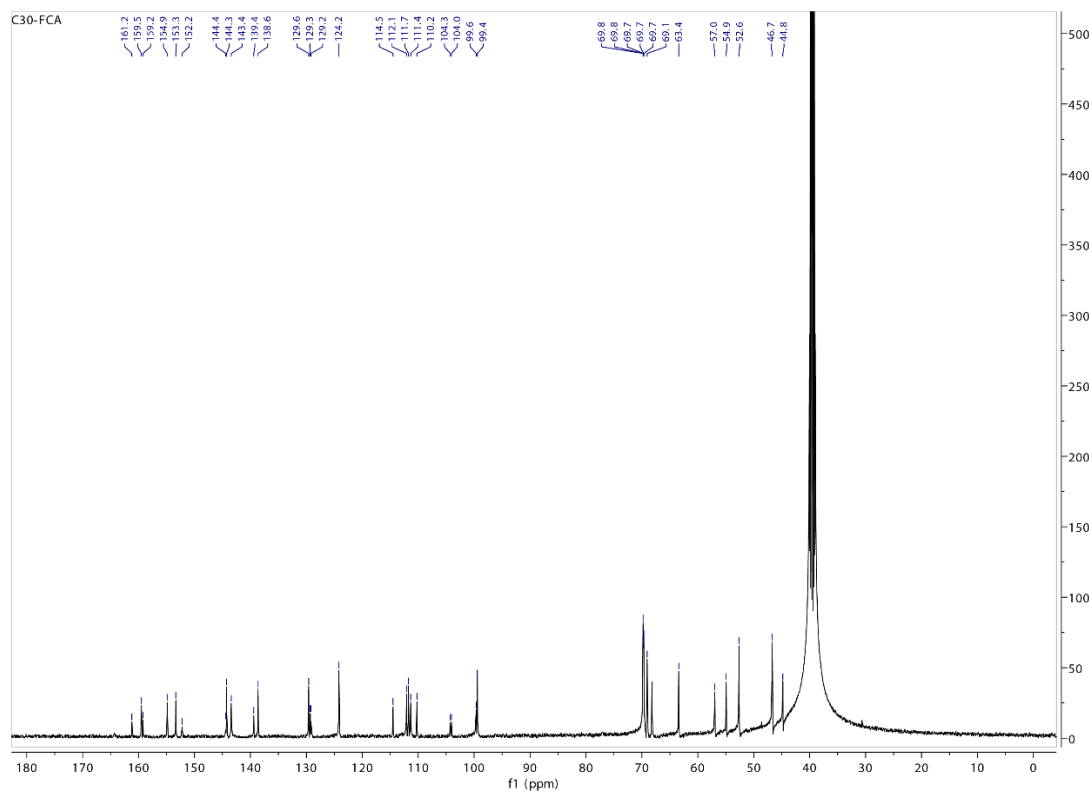
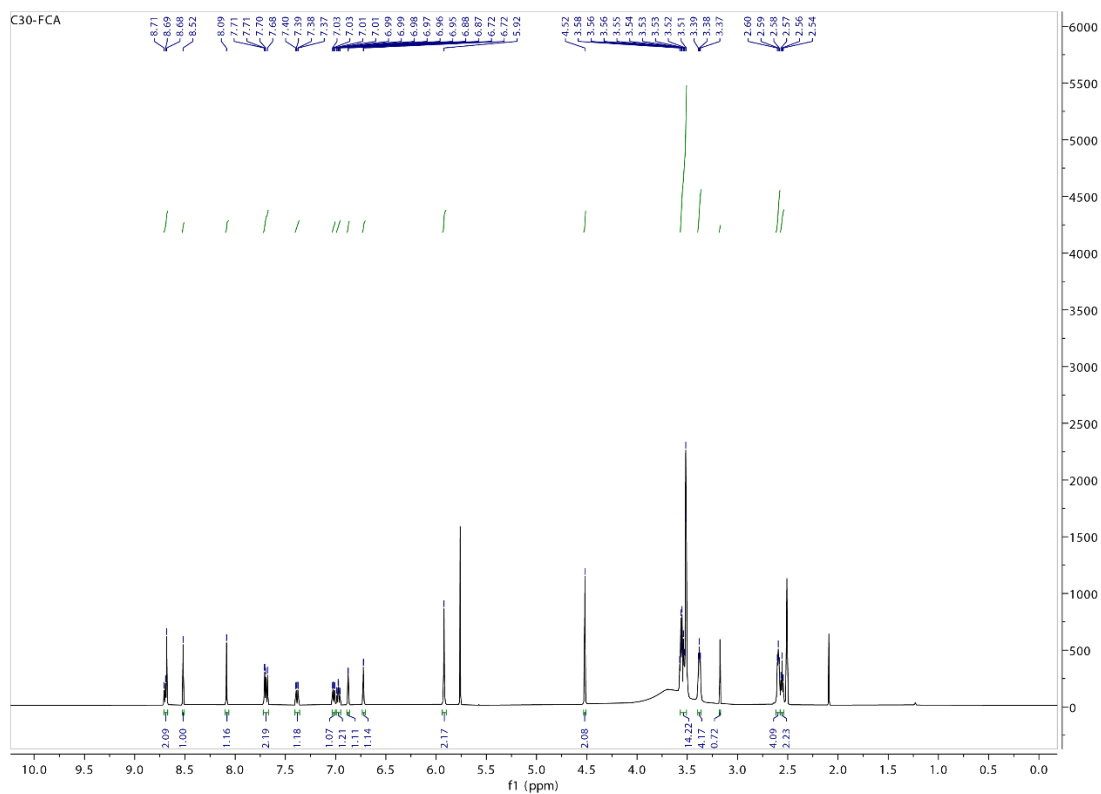
C34



C30-alkyne

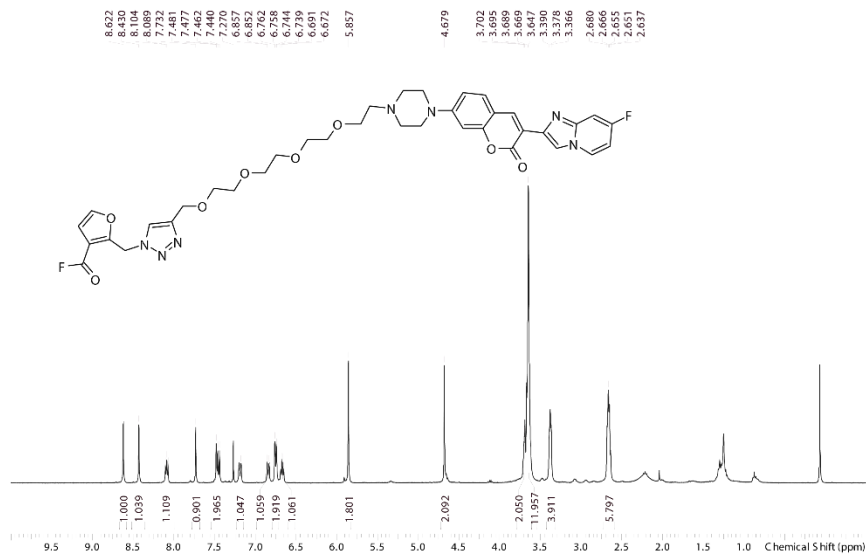


C30-FCA



C30-FCF

EW57770-1-P1A CDCl3 BRUKER_B_400MHz



Acquisition Time (sec) 2.0447
 Comment EW57770-1-P1A CDCl3 BRUKER_B_400MH z
 Date 01 Jul 2024 17:18:14 (GMT+08:00)
 Frequency (MHz) 400.1300
 Nucleus 1H
 Number of Transients 1
 Origin spect
 Original Points Count 16384
 Owner nmrsu
 Points Count 65536
 Pulse Sequence zg
 Receiver Gain 16.11
 SW(cyclical) (Hz) 8012.82
 Solvent CHLORO FORM-d
 Spectrum Offset (Hz) 2395.2576
 Spectrum Type standard
 Sweep Width (Hz) 8012.70
 Temperature (degree C) 24.528

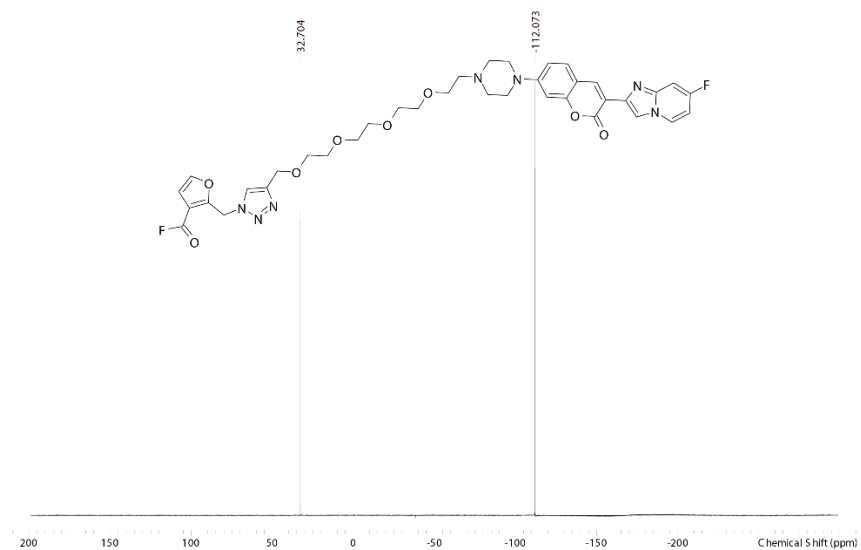
Confidential. For research information only

Operator:

Date:

Compound ID:

EW57770-1-P1A CDCl3 BRUKER_B_400MHz



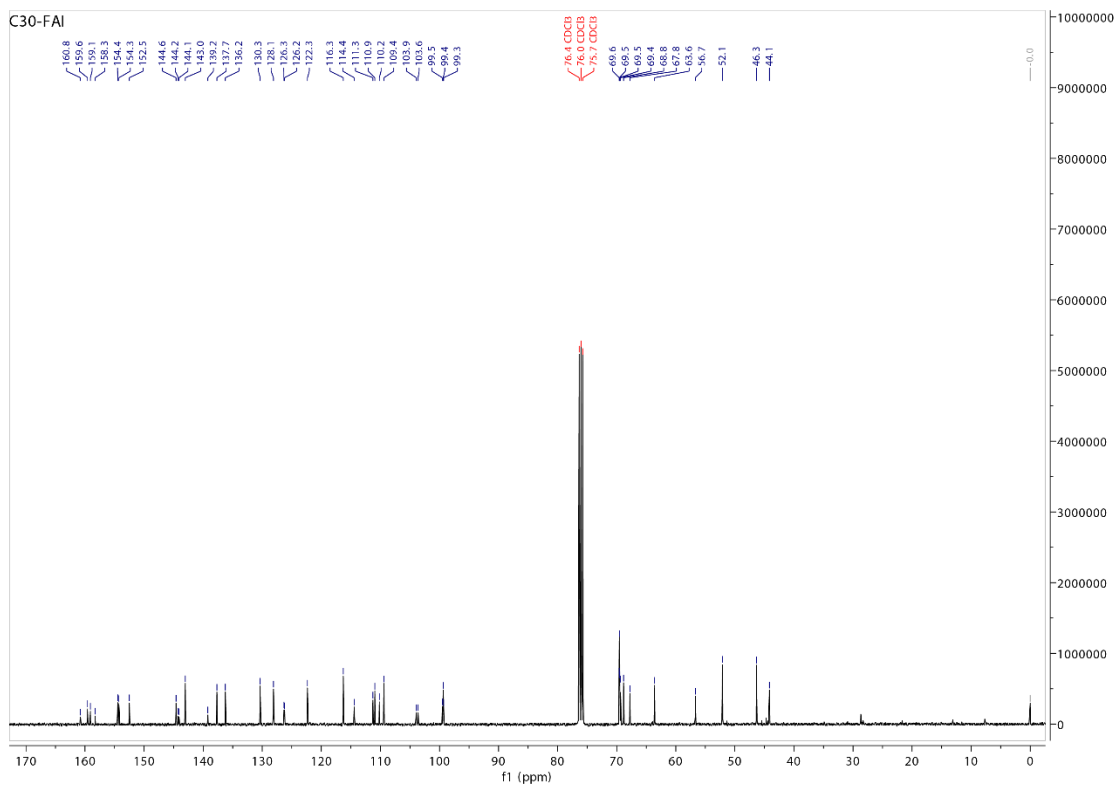
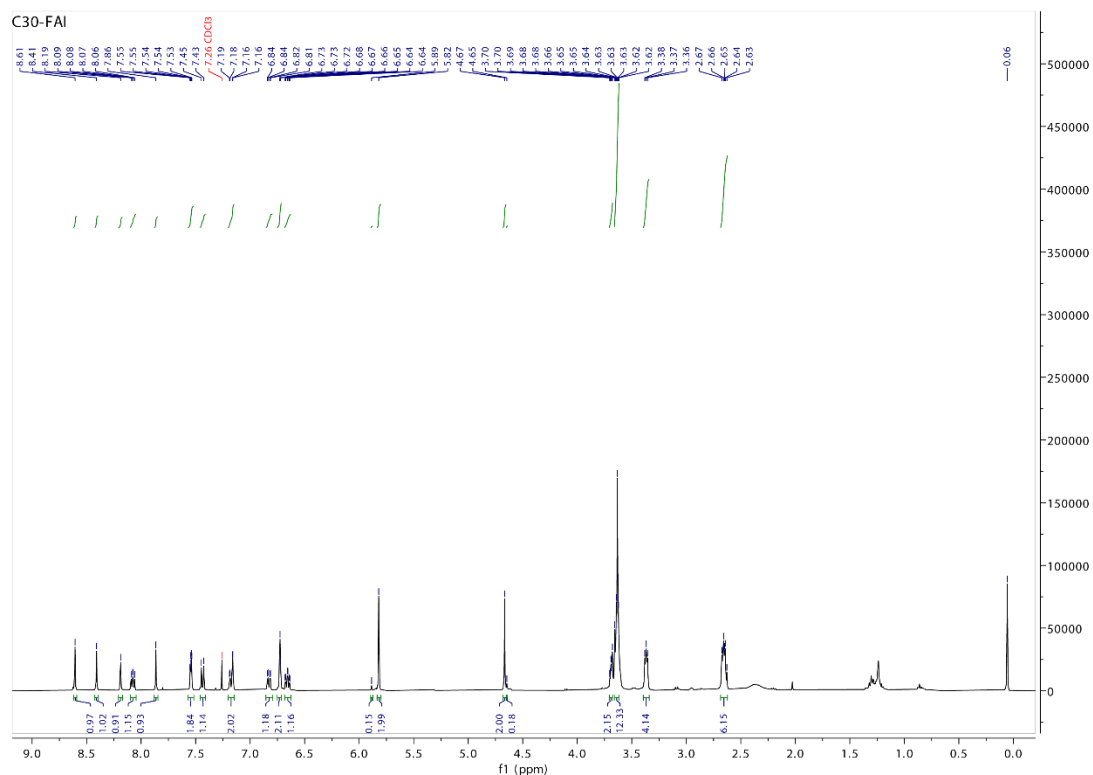
Acquisition Time (sec) 0.3550
 Comment EW57770-1-P1A CDCl3 BRUKER_B_400MH z
 Date 01 Jul 2024 17:18:01 (GMT+08:00)
 Frequency (MHz) 376.4984
 Nucleus 13C
 Number of Transients 8
 Origin spect
 Original Points Count 66560
 Owner nmrsu
 Points Count 65536
 Pulse Sequence zgfgn2
 Receiver Gain 190.49
 SW(cyclical) (Hz) 187500.00
 Solvent CHLORO FORM-d
 Spectrum Offset (Hz) -18824.90
 Spectrum Type standard
 Sweep Width (Hz) 187497.14
 Temperature (degree C) 24.491

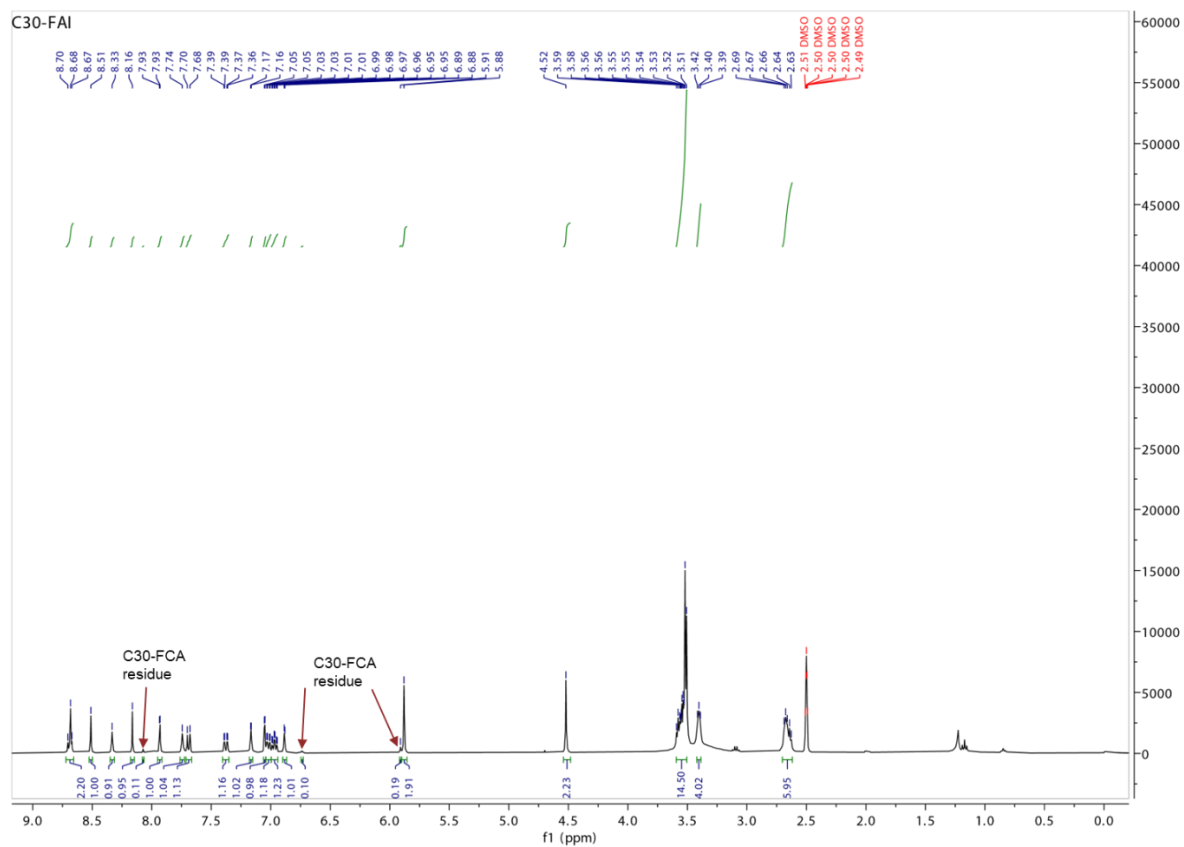
Confidential. For research information only

Operator:

Date:

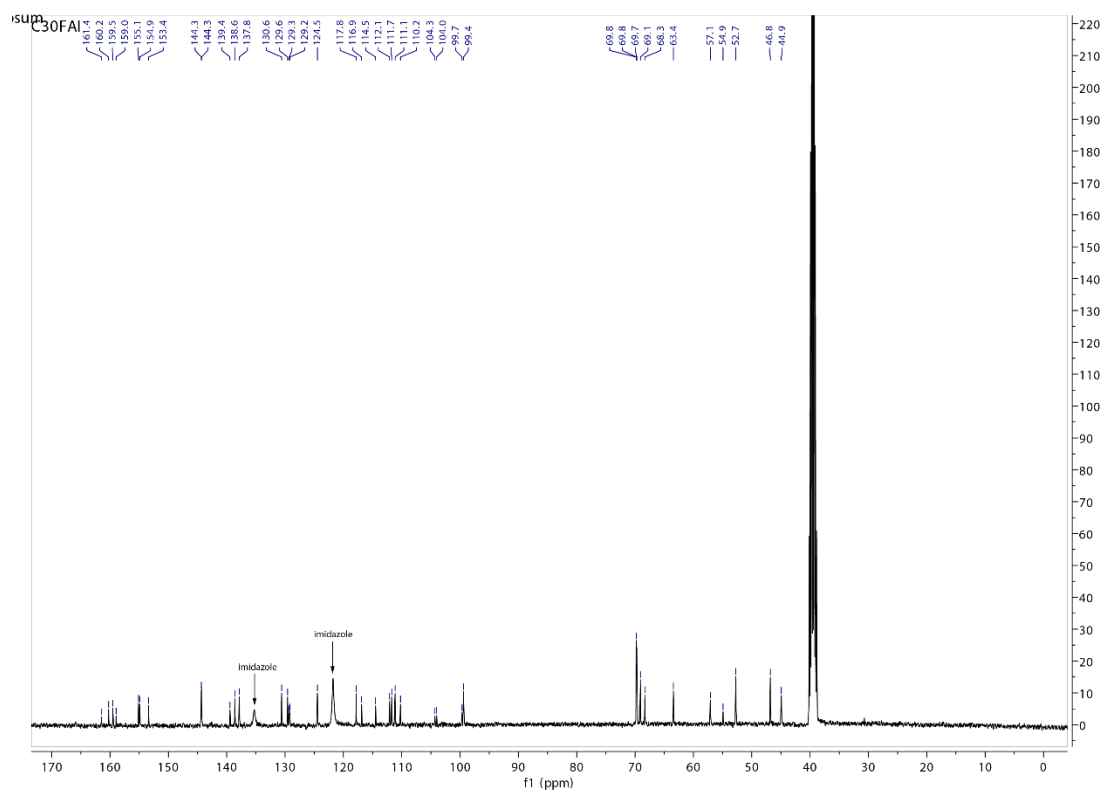
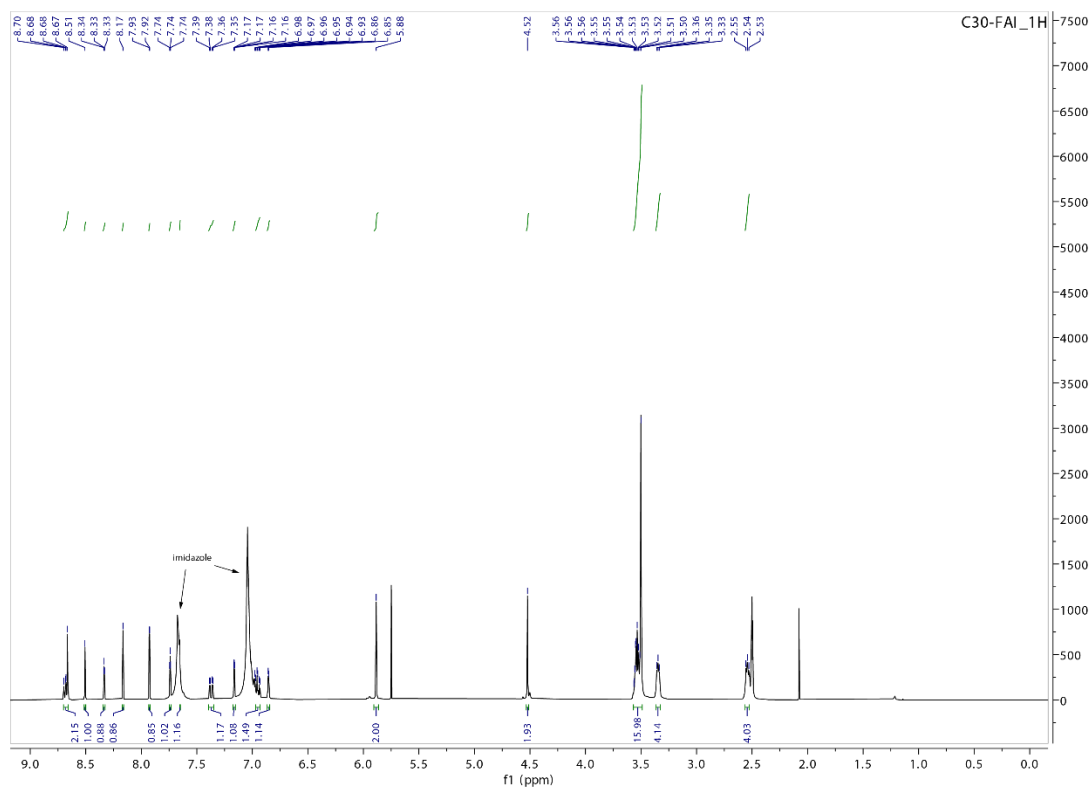
C30-FAI synthesized from C30-FCF and imidazole, no free imidazole observed.



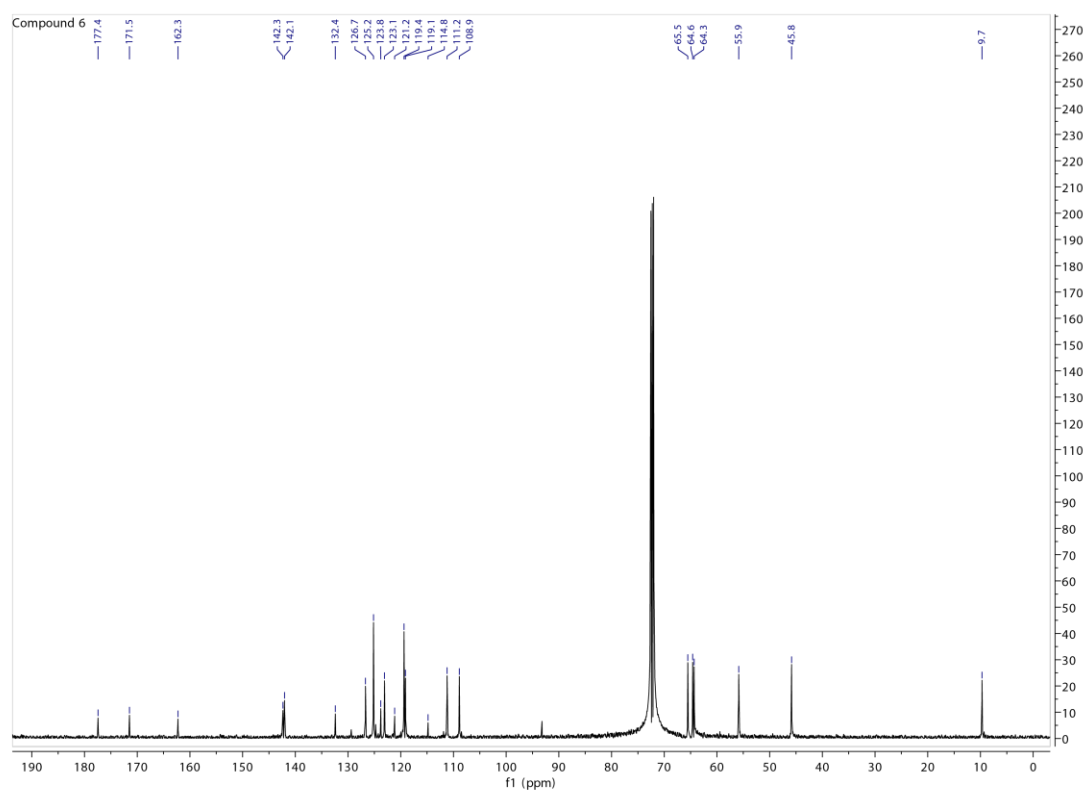
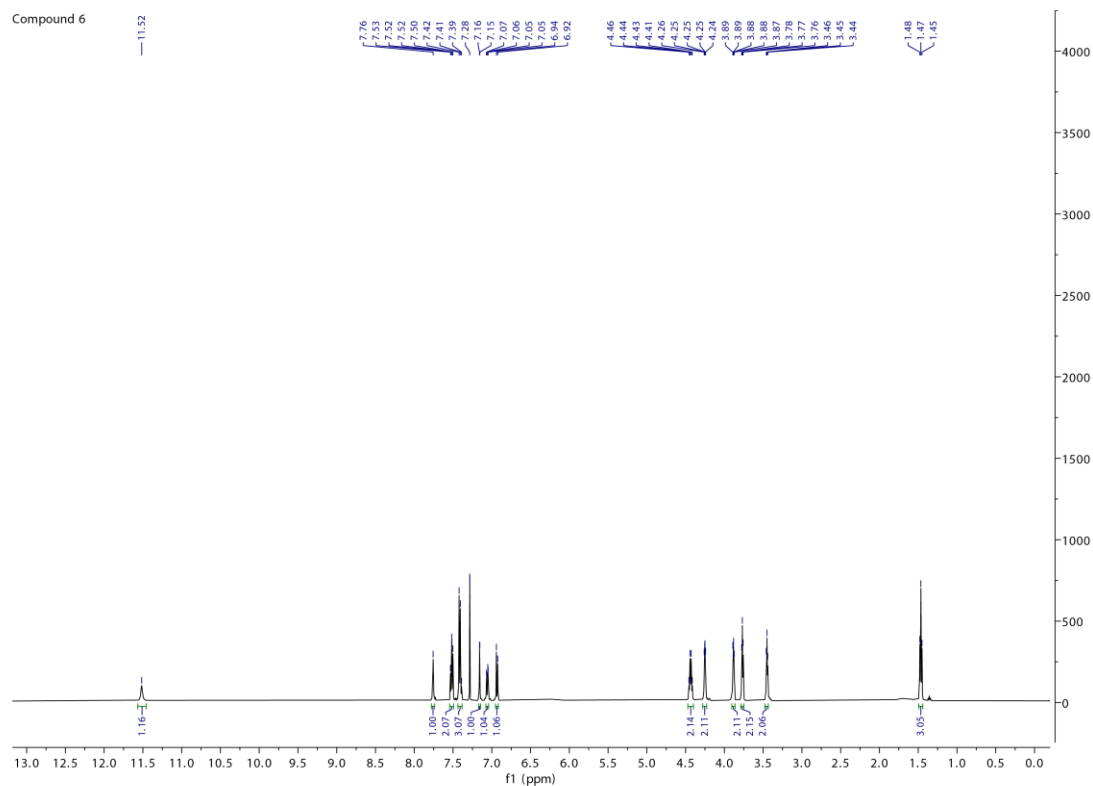


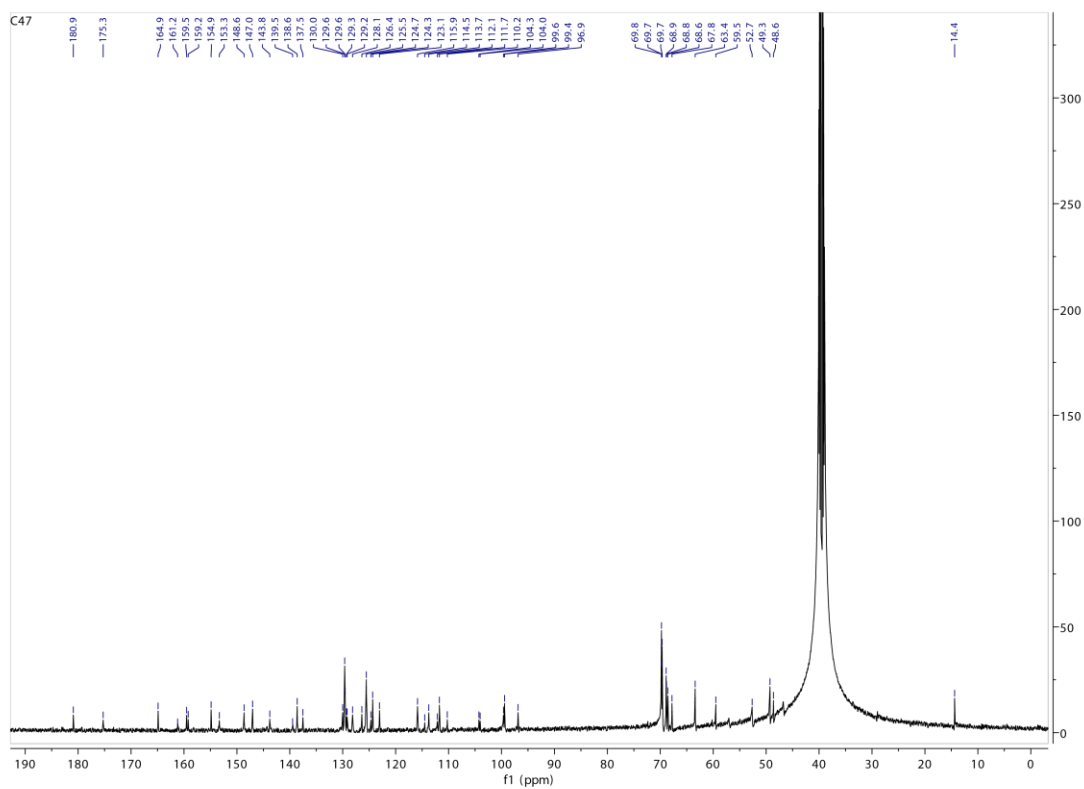
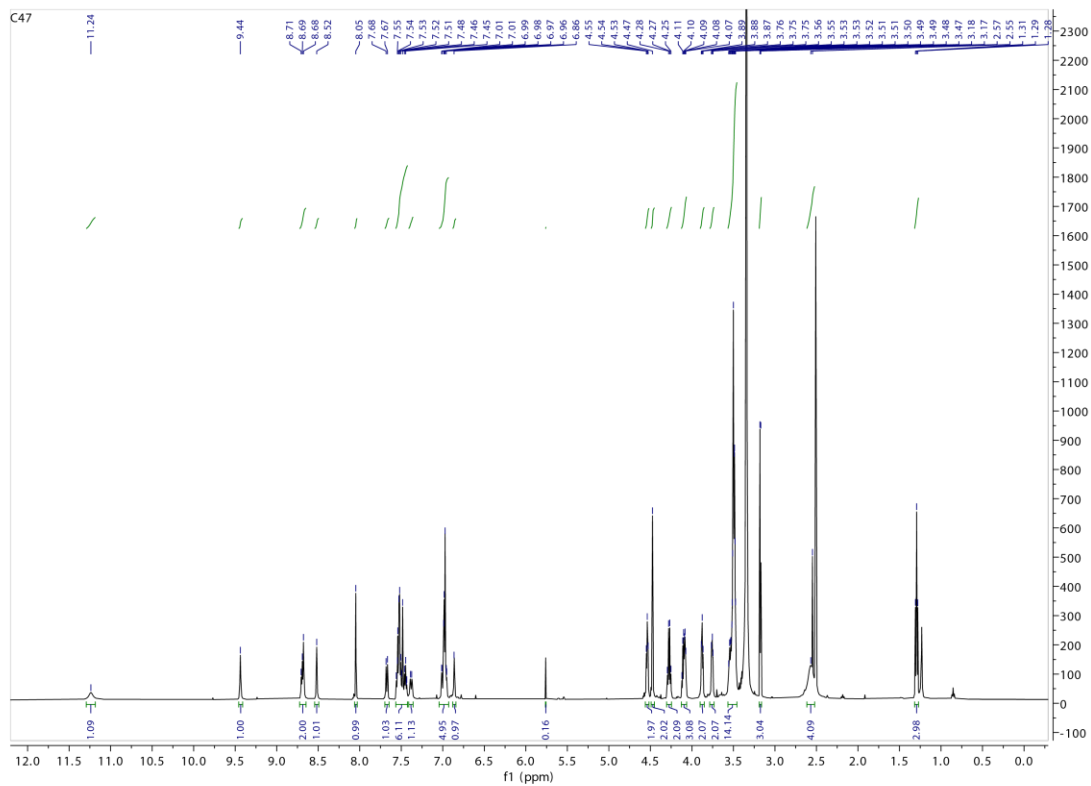
(^1H NMR of the new C30-FAI in DMSO- d_6 , the C30-FCA residue peaks were marked, the ratio of C30-FCA residue to C30-FAI is ~ 1 to 10)

C30-FAI synthesized from C30-FCA and CDI, imidazole observed as a side product.

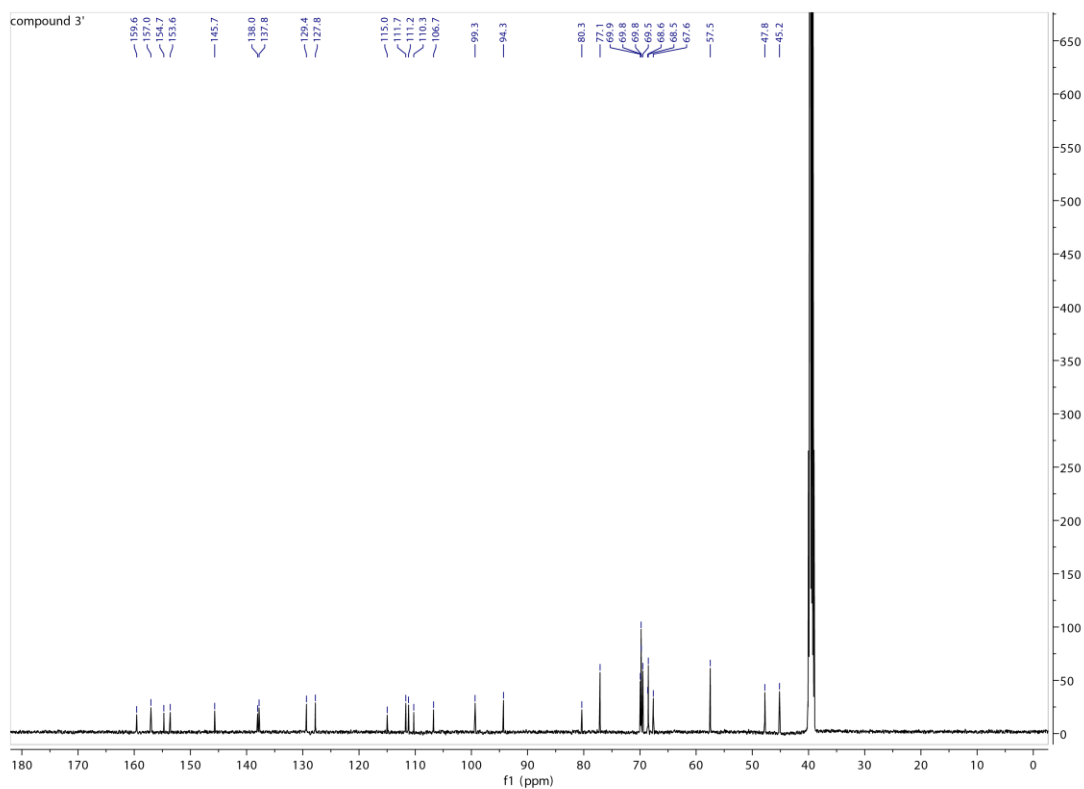
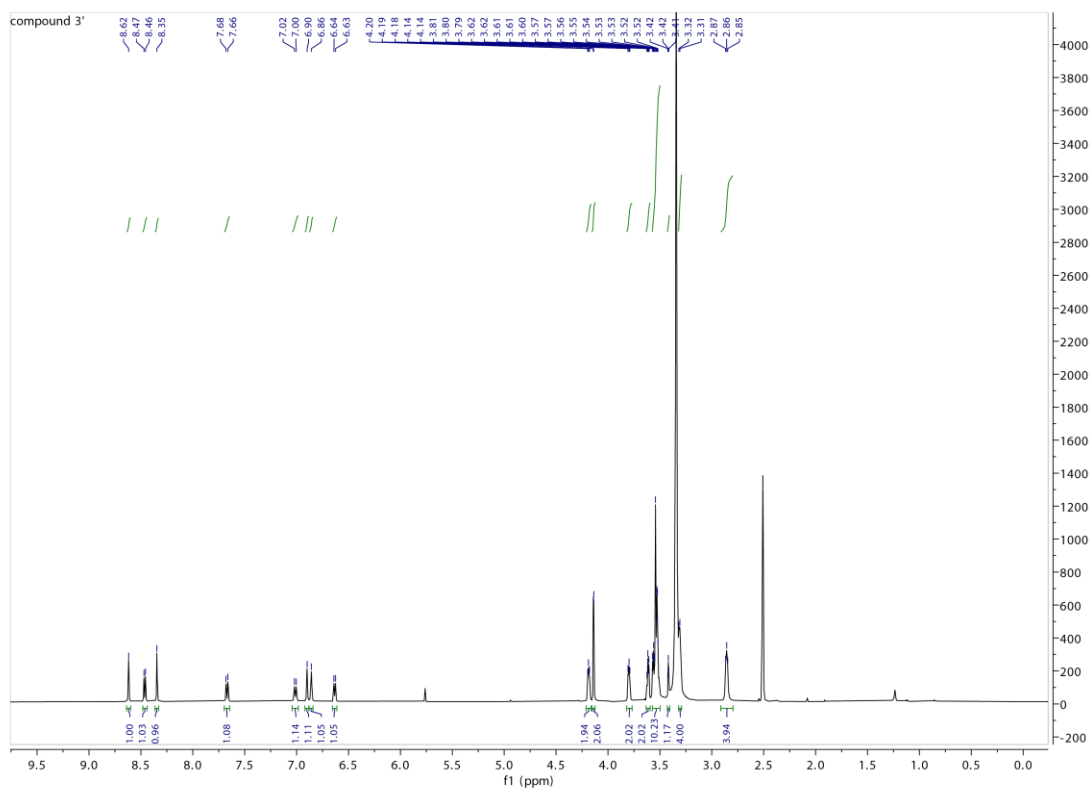


Compound 6

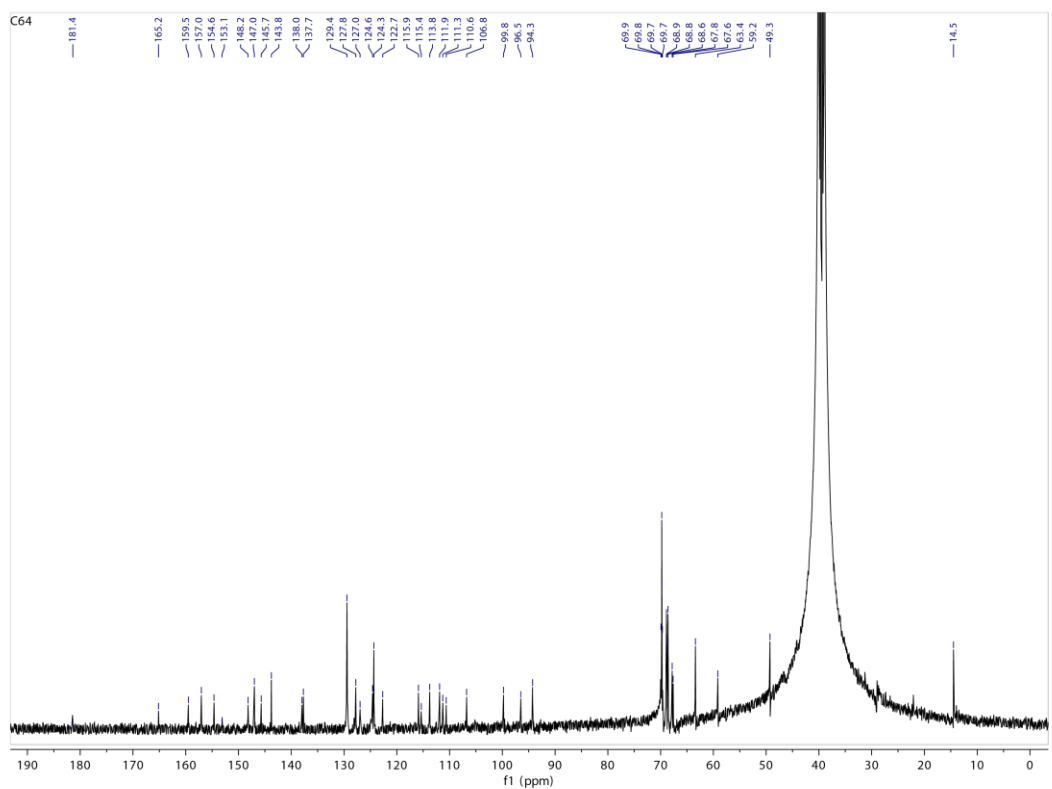
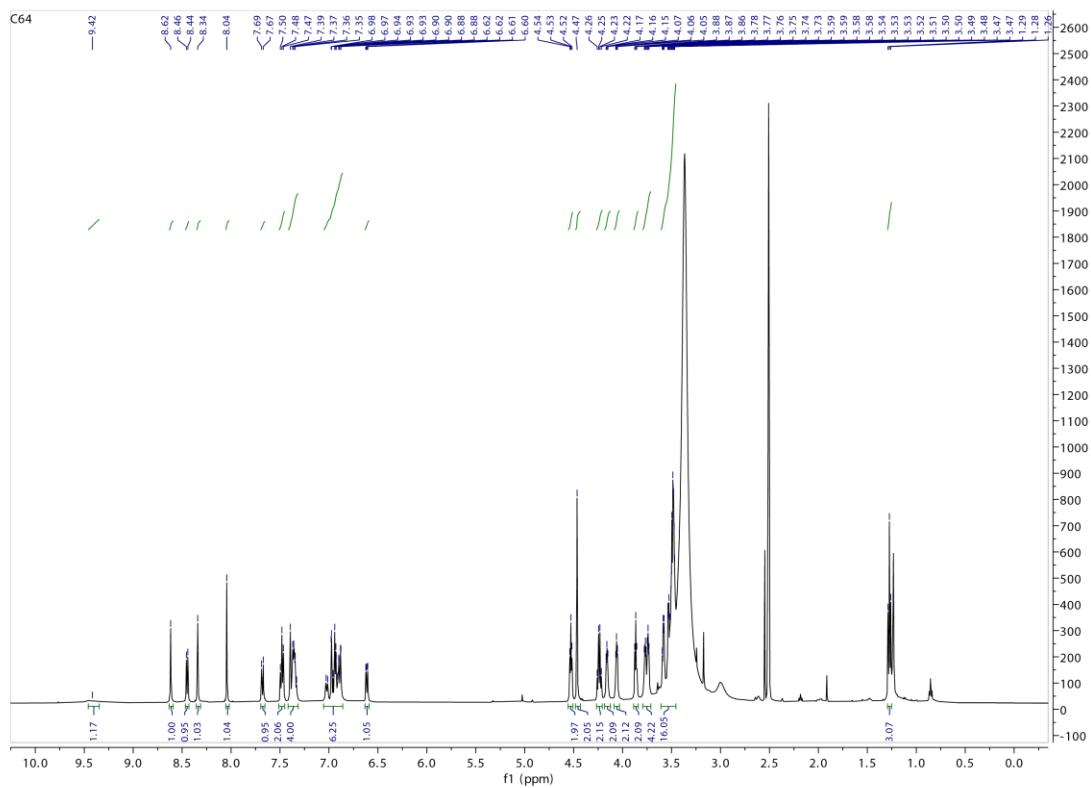




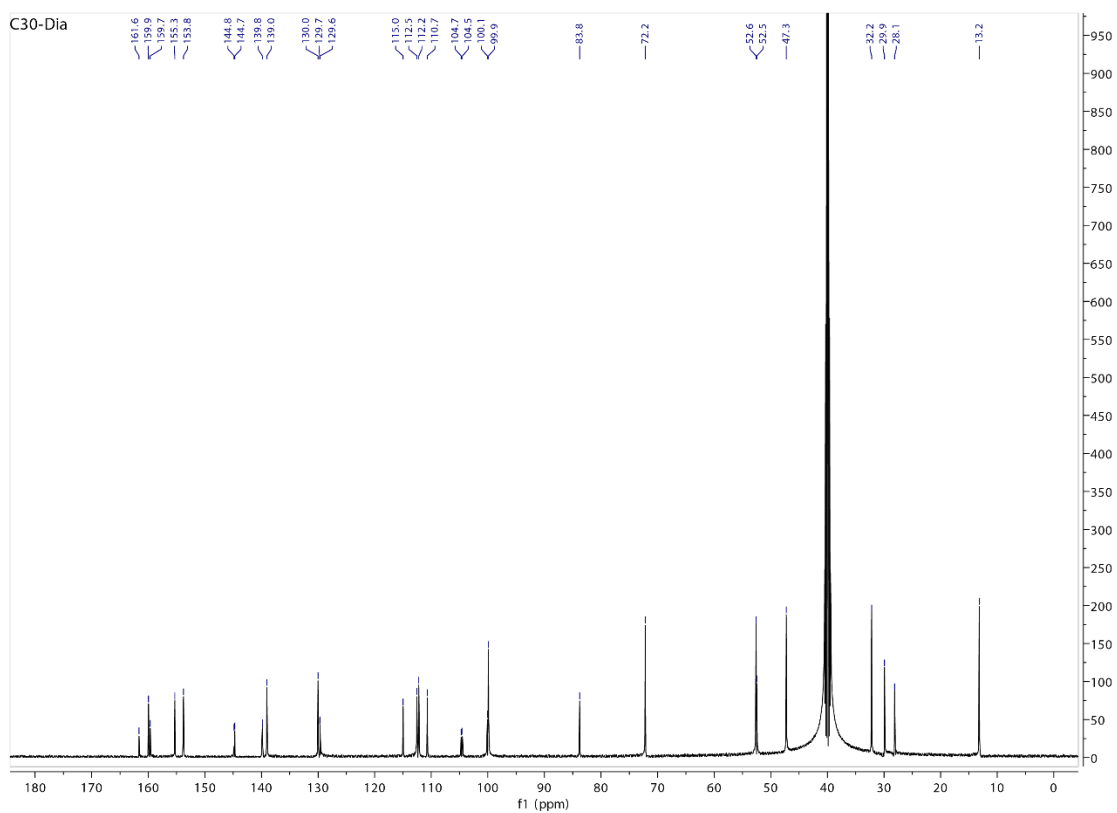
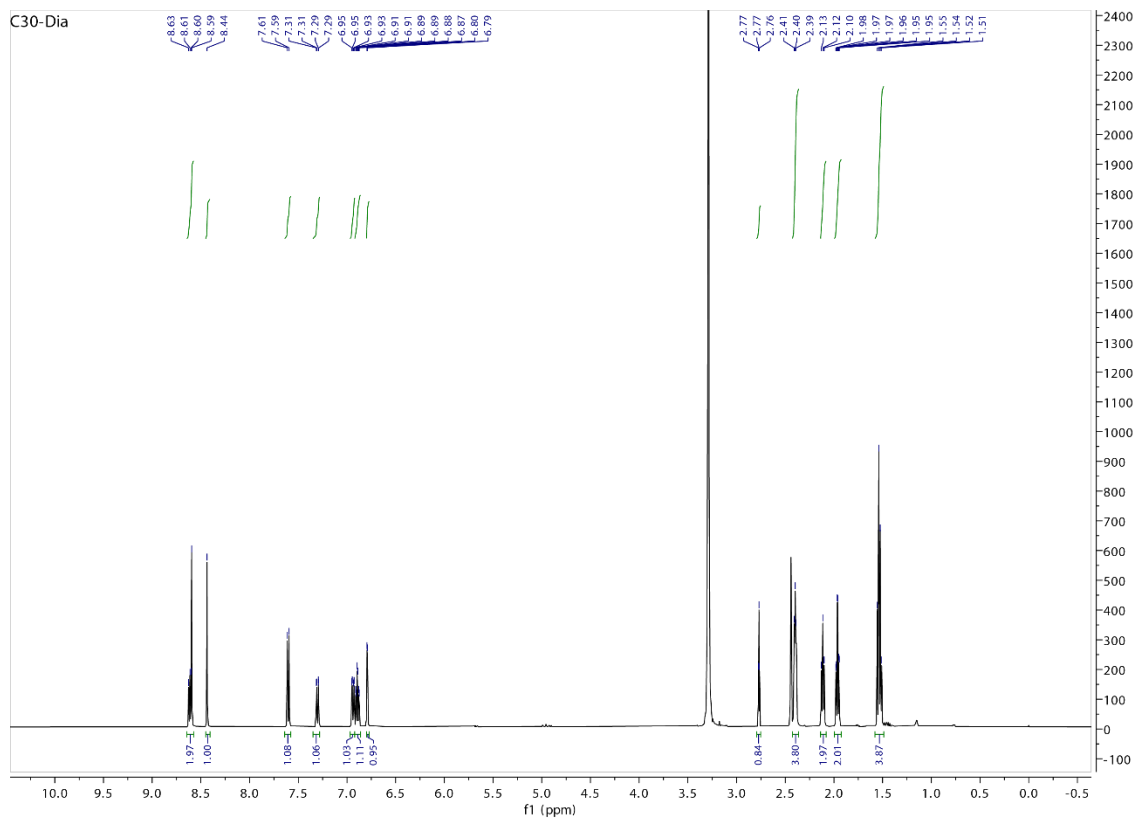
Compound 3'



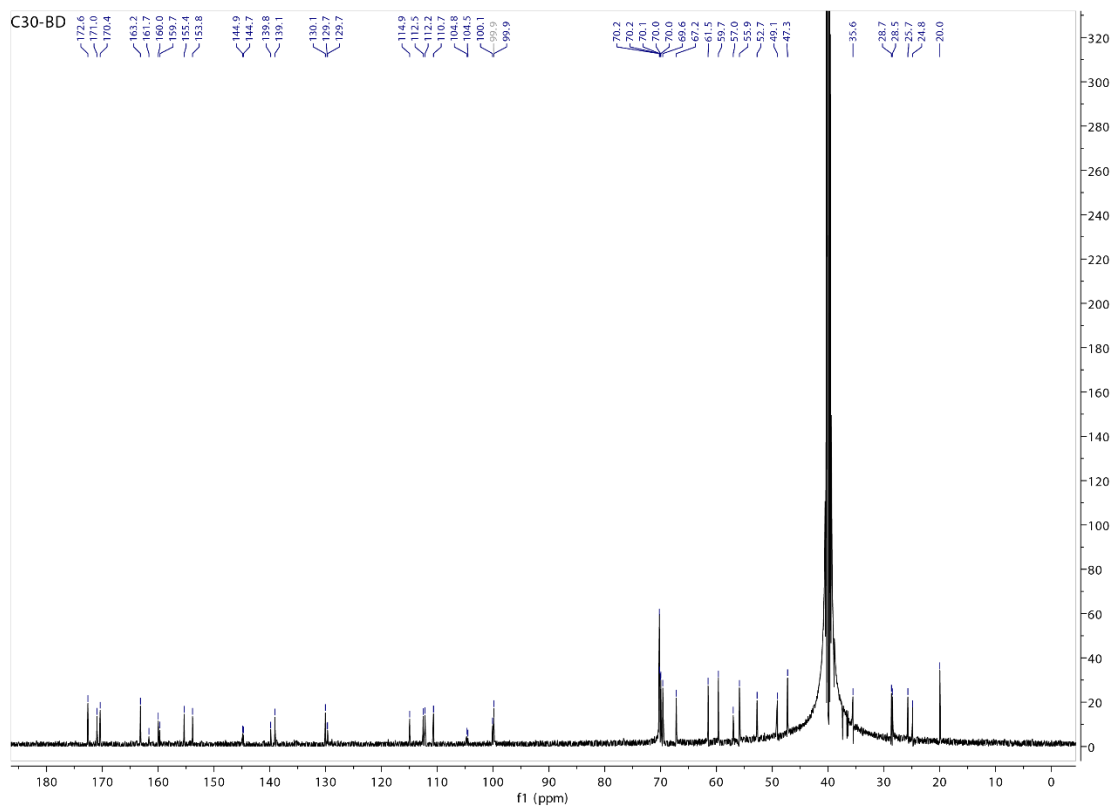
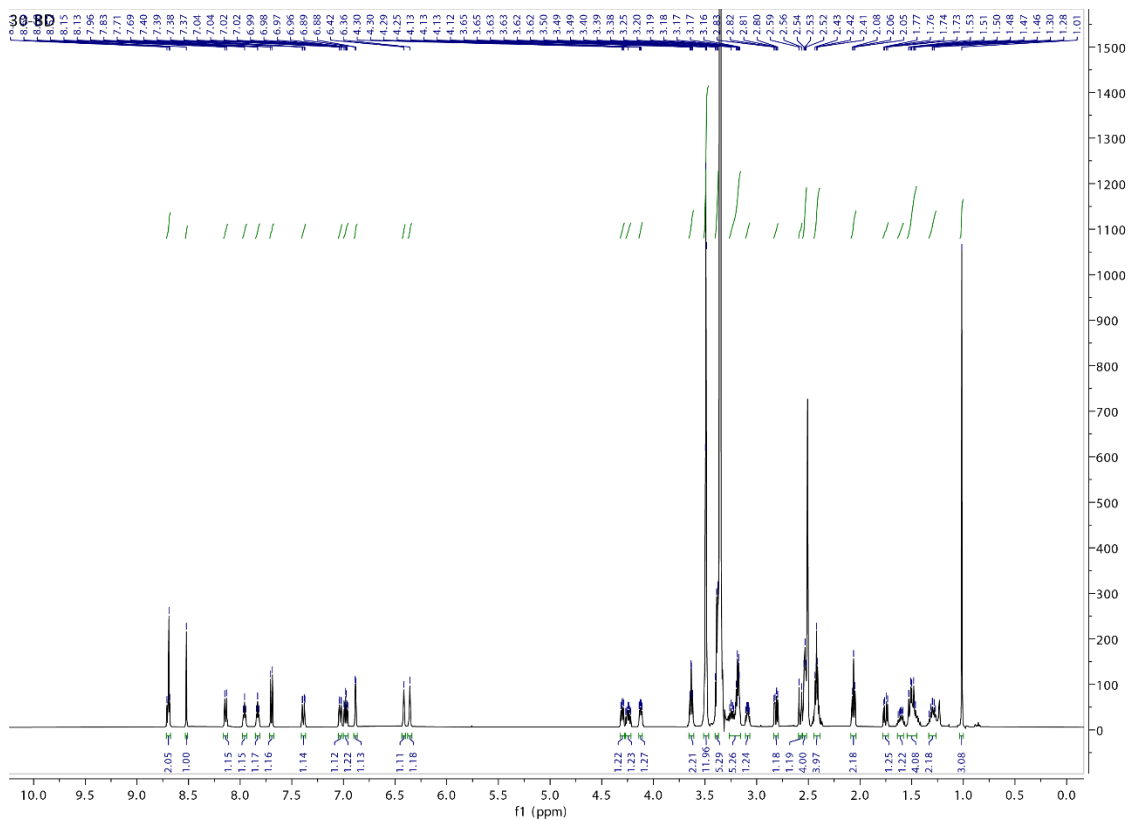
C64



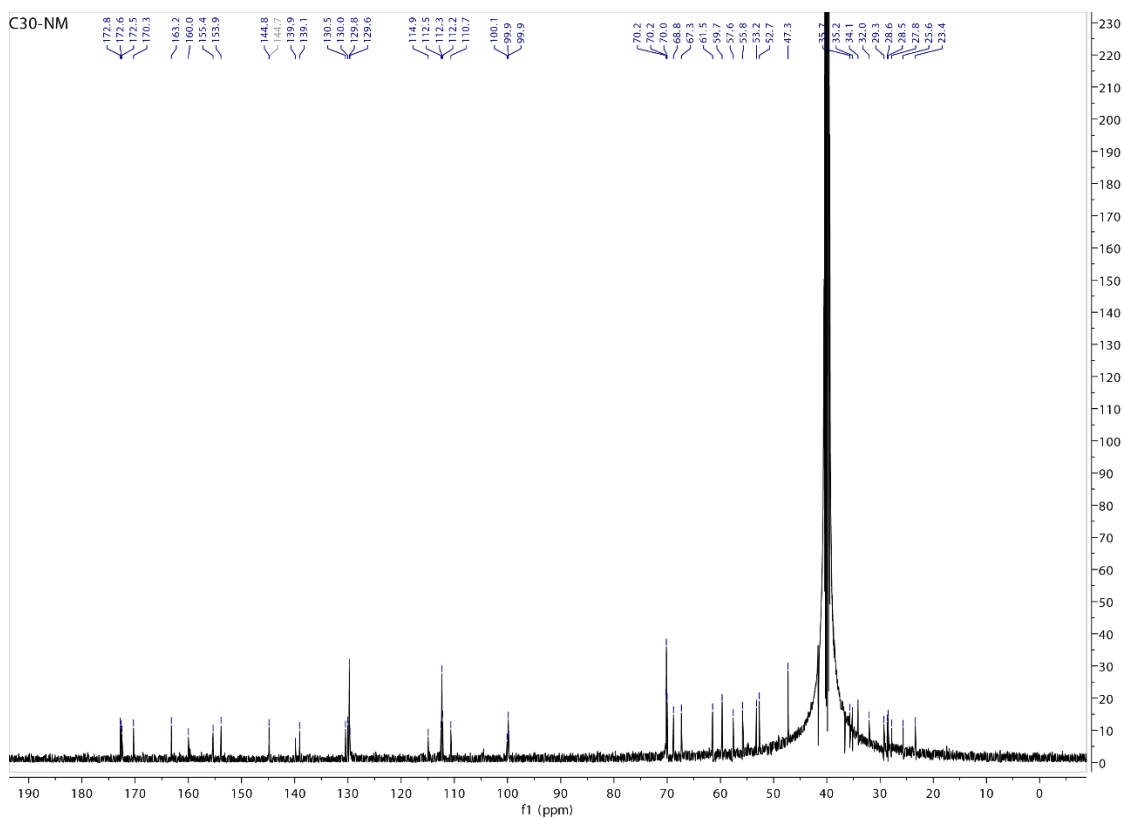
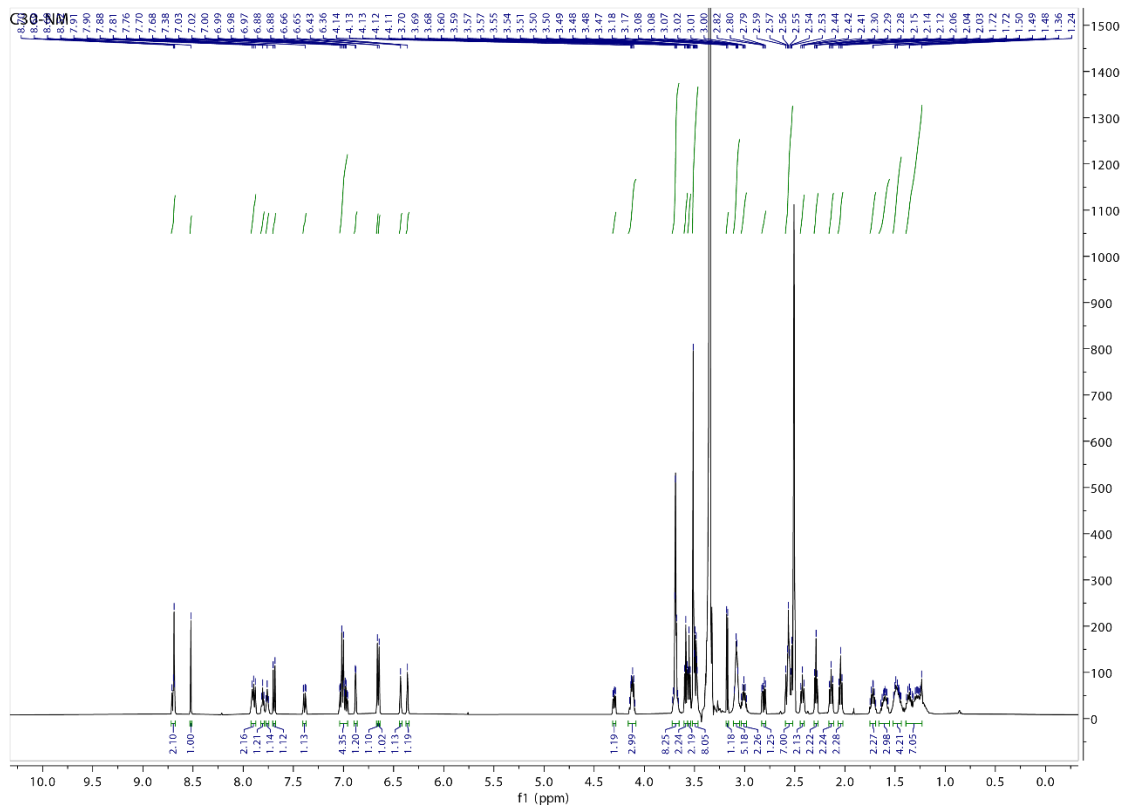
C30-D



C30-BD



C30-NM



HPLC analysis of C30-FAI containing imidazole

The purity of **C30-FAI** was determined by titration with β -mercaptoethanol and analyzed by UPLC-MS. Briefly, 5 μ l of the reaction solution (0.5 μ mol of **C30-FAI**) was added to a 95 μ l DMSO solution containing 0.4 μ l β -mercaptoethanol (5.7 μ mol). The mixture was incubated at room temperature for 4 h to form an adduct from **C30-FAI**. The mixture was then diluted in methanol and analyzed by UPLC-MS. Analysis shows the mixture contains ~91% **C30-FAI- β -mercaptoethanol** adduct (MS-ESI $[M+H]^+$ 806.27) and ~6% **C30-FCA** (MS-ESI $[M+H]^+$ 746.28). The purity of C30-FAI was then determined as 91%.

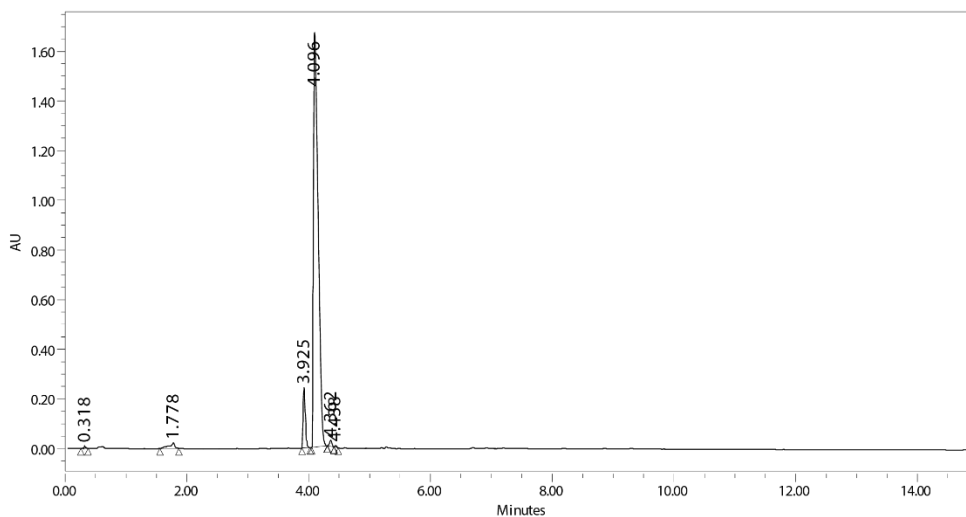
Organic: acetonitrile; aqueous: 0.1% formic acid in water. Gradient: 0-15 min, 2 – 98% acetonitrile.

EmpowerTM 3
SOFTWARE

Default Individual Report

SAMPLE INFORMATION

Sample Name:	ZT-C30FAI	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	ZTSM
Vial:	1:A,1	Acq. Method Set:	L1ABT2
Injection #:	1	Processing Method:	111DCM3
Injection Volume:	4.00 μ l	Channel Name:	PDA Ch1 254nm@4.8nm
Run Time:	15.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 254nm@4.8nm



	RT	Area	% Area	Height
1	0.318	20374	0.21	10984
2	1.778	135977	1.40	21751
3	3.925	596367	6.12	241610
4	4.096	8908232	91.48	1669778
5	4.362	63765	0.65	24263
6	4.438	12901	0.13	5805

HPLC analysis of C48

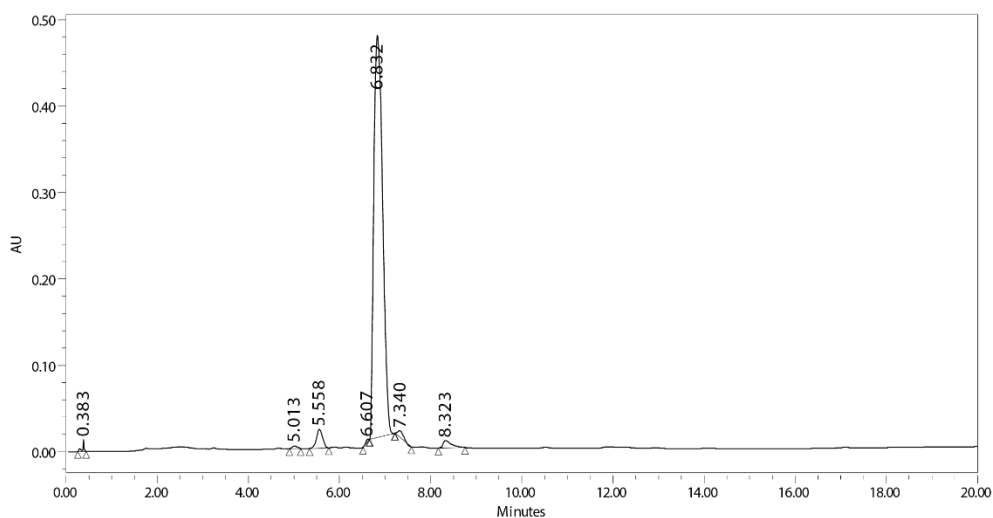
Organic: acetonitrile; aqueous: 0.1% ammonium formate in water. Gradient: 0-15 min, 2 – 50% acetonitrile; 15-20 min, 50 – 98% acetonitrile.



Default Individual Report

SAMPLE INFORMATION

Sample Name:	c48	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	ztsm
Vial:	1:F,1	Acq. Method Set:	L4ADT2_25A
Injection #:	1	Processing Method:	C48
Injection Volume:	2.00 ul	Channel Name:	PDA Ch1 254nm@4.8nm
Run Time:	20.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 254nm@4.8nm



	RT	Area	% Area	Height
1	0.383	29397	0.44	13485
2	5.013	22597	0.33	2964
3	5.558	204508	3.03	21699
4	6.607	11746	0.17	2965
5	6.832	6306966	93.39	465230
6	7.340	69013	1.02	7787
7	8.323	109469	1.62	8503

HPLC analysis of C65

Organic: acetonitrile; aqueous: 0.1% ammonium formate in water. Gradient: 0-15 min, 2 – 50% acetonitrile; 15-20 min, 50 – 98% acetonitrile.

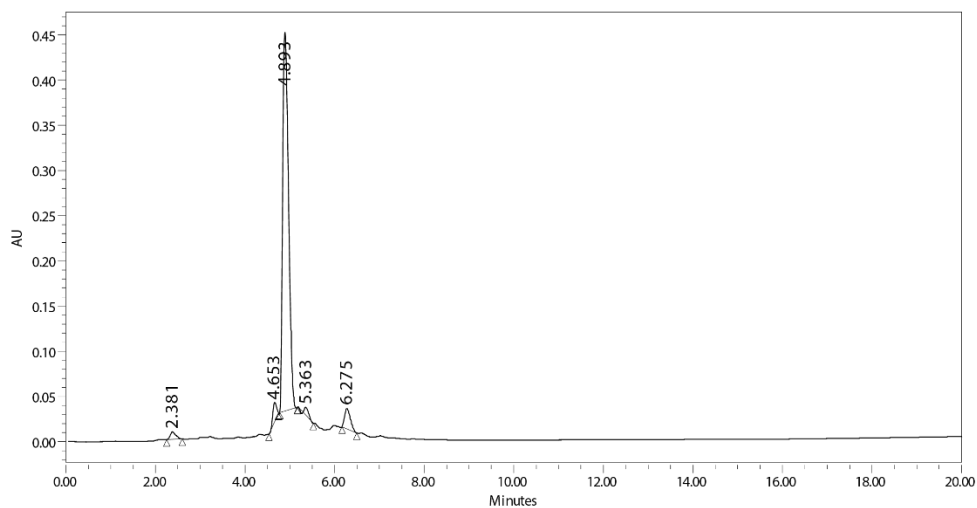
Empower[®] 3
SOFTWARE

Default Individual Report

SAMPLE INFORMATION

Sample Name: c65
Sample Type: Unknown
Vial: 1:F,2
Injection #: 1
Injection Volume: 4.00 ul
Run Time: 20.0 Minutes

Acquired By: System
Sample Set Name: ztsm
Acq. Method Set: L4ADT2_25A
Processing Method: c65
Channel Name: PDA Ch1 254nm@4.8nm
Proc. Chnl. Descr.: PDA Ch1 254nm@4.8nm



	RT	Area	% Area	Height
1	2.381	72656	1.69	8318
2	4.653	123080	2.86	21625
3	4.893	3839028	89.28	418395
4	5.363	65413	1.52	8731
5	6.275	199733	4.65	22701

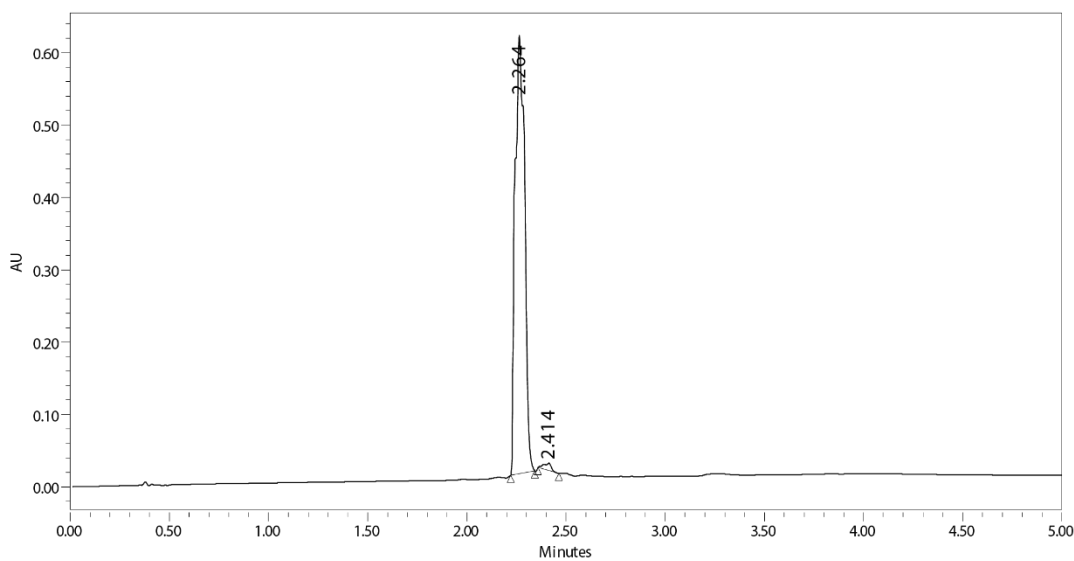
HPLC analysis of C30-D

Organic: acetonitrile; aqueous: 0.1% formic acid in water. Gradient: 0-5 min, 2 – 98% acetonitrile.



SAMPLE INFORMATION

Sample Name:	zt-4f-dia	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	ztsm
Vial:	1:F,4	Acq. Method Set:	L4ABT1
Injection #:	1	Processing Method:	DIA
Injection Volume:	5.00 ul	Channel Name:	PDA Ch1 254nm@4.8nm
Run Time:	5.0 Minutes	Proc. Chnl. Descr.:	PDA Ch1 254nm@4.8nm



	RT	Area	% Area	Height
1	2.264	2027567	98.69	605695
2	2.414	26834	1.31	10139

HPLC analysis of C30-BD

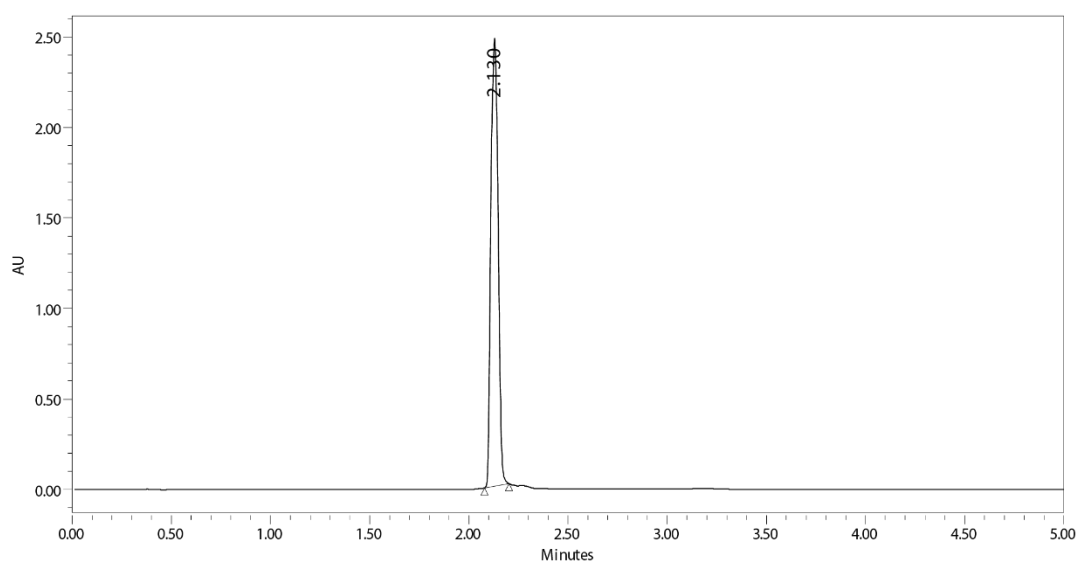
Organic: acetonitrile; aqueous: 0.1% formic acid in water. Gradient: 0-5 min, 2 – 98% acetonitrile.



SAMPLE INFORMATION

Sample Name: ZT-C30-B/D
Sample Type: Unknown
Vial: 1:E,4
Injection #: 1
Injection Volume: 3.00 ul
Run Time: 5.0 Minutes

Acquired By: System
Sample Set Name: ZTSM
Acq. Method Set: L4ABT1
Processing Method: BD
Channel Name: PDA Ch1 254nm@4.8nm
Proc. Chnl. Descr.: PDA Ch1 254nm@4.8nm



	RT	Area	% Area	Height
1	2.130	6341917	100.00	2471904

HPLC analysis of C30-NM

Organic: acetonitrile; aqueous: 0.1% formic acid in water. Gradient: 0-5 min, 2 – 98% acetonitrile.

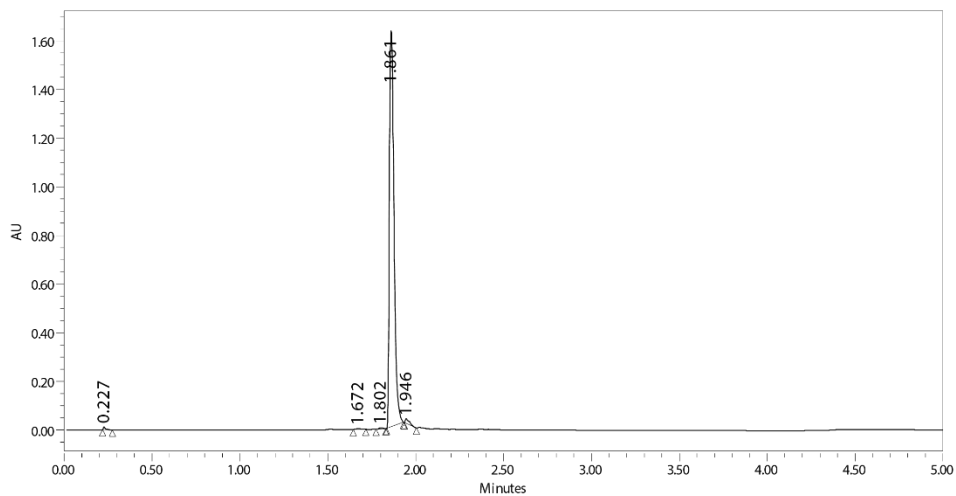
Empower[™] 3
SOFTWARE

Default Individual Report

SAMPLE INFORMATION

Sample Name: ZT-C30NM
Sample Type: Unknown
Vial: 1:A,3
Injection #: 1
Injection Volume: 5.00 ul
Run Time: 5.0 Minutes

Acquired By: System
Sample Set Name: ZTSM
Acq. Method Set: L1ABT1
Processing Method: C30NM
Channel Name: PDA Ch1 254nm@4.8nm
Proc. Chnl. Descr.: PDA Ch1 254nm@4.8nm



	RT	Area	% Area	Height
1	0.227	10826	0.36	12050
2	1.672	6769	0.22	3658
3	1.802	6453	0.21	4750
4	1.861	2959078	98.19	1627169
5	1.946	30530	1.01	18205