Supporting Information:

Multiplexed CuAAC-Suzuki–Miyaura labelling for tandem activity-based chemoproteomic profiling

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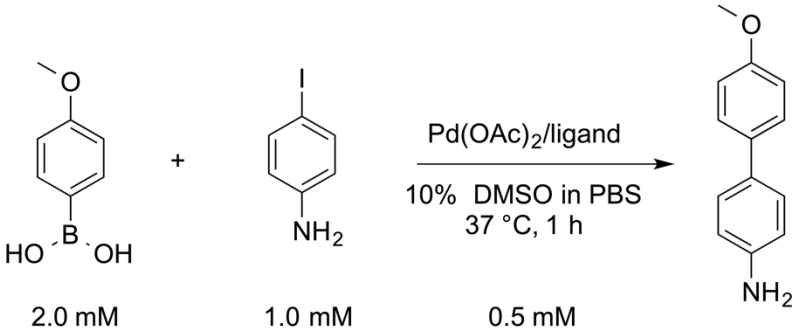
(A) Supplementary Figures.

Table S1. Sensitivity of model Suzuki–Miyaura cross-coupling reaction to additives. Reactions were conducted at 37 °C in phosphate-buffered saline (PBS) with 10% DMSO as co-solvent, a palladium loading of 50 mol%, and with addition of glutathione (1 mM), BSA (1 mg/mL), or HEK293T lysate (1 mg/mL). Peak areas reflect areas under the curve from LC-MS analysis. Calculated conversion (%) is shown in **Figure 1**.



		Peak Area			
Entry	**Ligand**	**-**	**GSH**	**BSA**	**Lysate**
1	sSPhos	2041	531	1174	531
2	(P(*t*-Bu)$_3$	1839	696	1350	696
3	DavePhos	1714	98	723	98
4	ADHP	803	69	70	69
5	DMADHP	1838	69	200	69

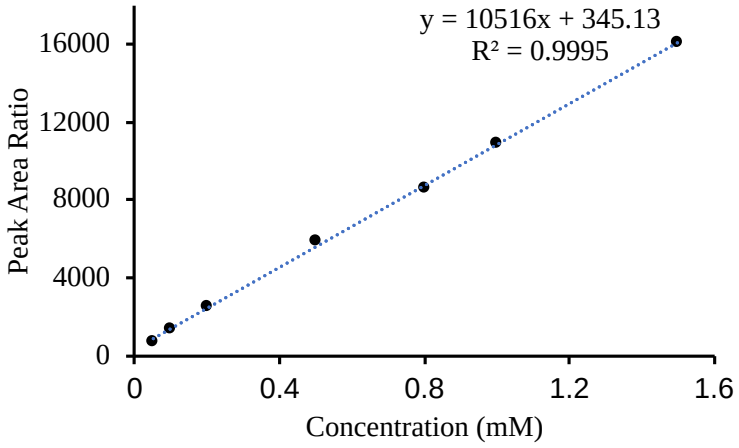
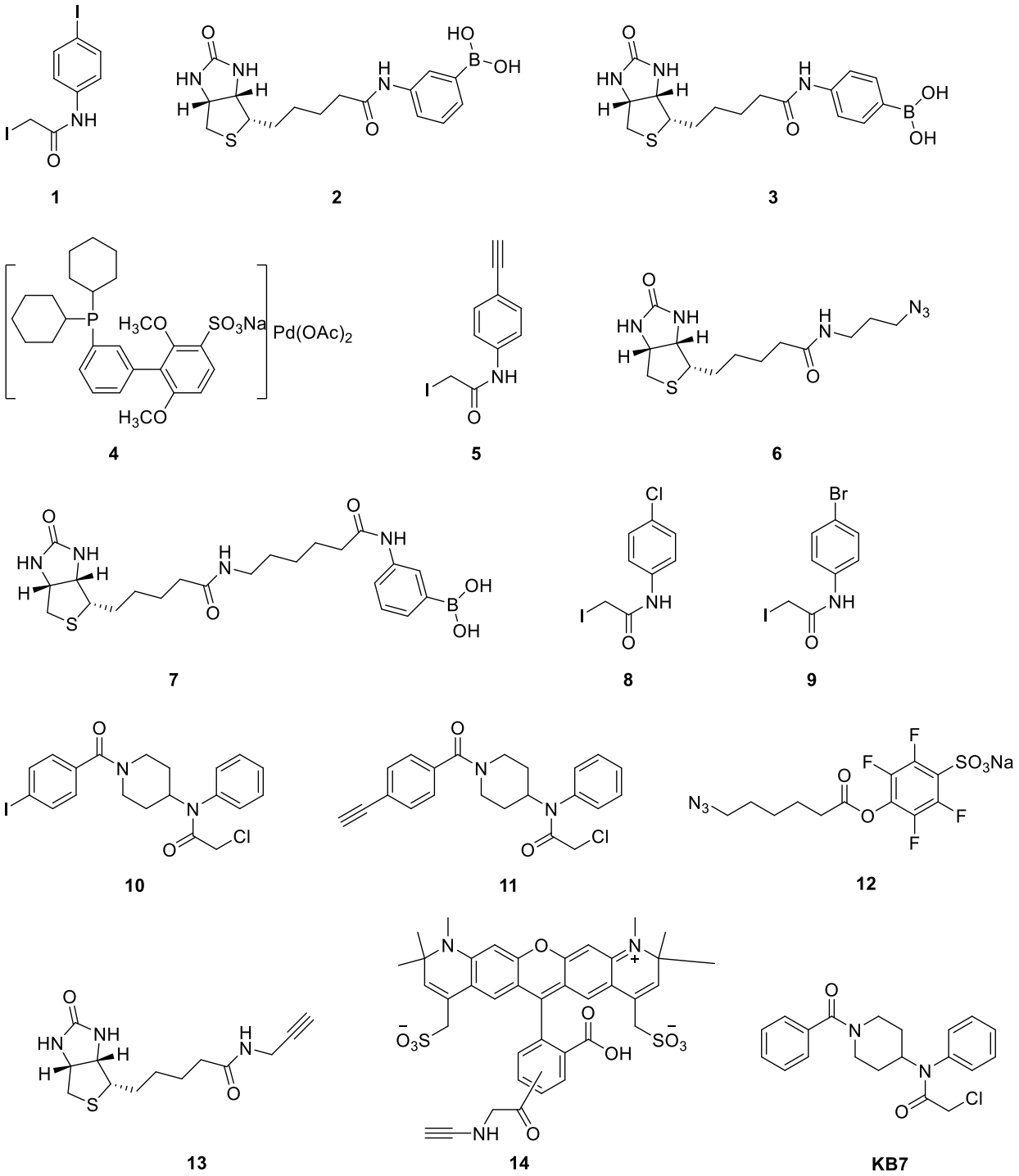


Figure S1. Standard curve generated from LC-MS analysis of the indicated concentrations of model cross-coupling product 4-(4-methoxyphenyl)aniline.



Scheme S1. Structures of probes and reagents for dual Suzuki-Miyaura-CuAAC

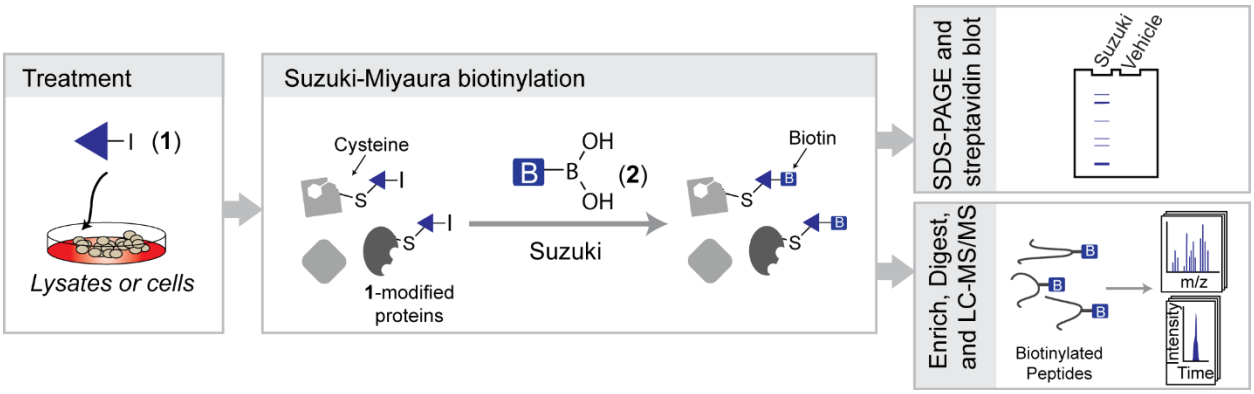


Figure S2. General workflow for Suzuki–Miyaura biotinylation of cellular lysates and detection by streptavidin blot or by LC-MS/MS.

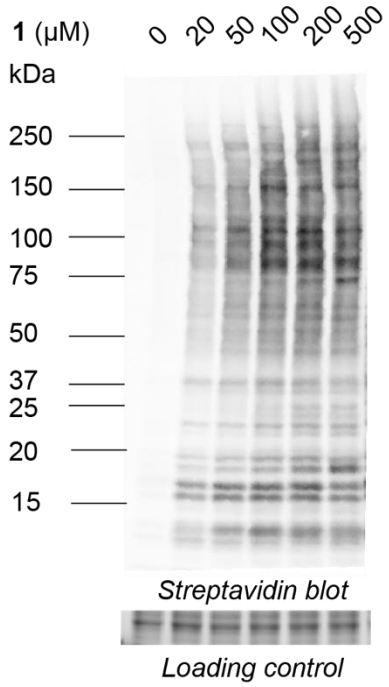


Figure S3. Dose-dependent proteome labeling by probe **1**. HEK293T lysates were labeled with indicated concentrations of probe **1** or vehicle (DMSO) at room temperature (RT) for 1h. The samples were then subjected to Suzuki–Miyaura cross-coupling conjugation to **2** (2 mM) in the presence of **4** (1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and labeling was visualized by streptavidin blot. Loading control was generated using stain-free imaging.[1]

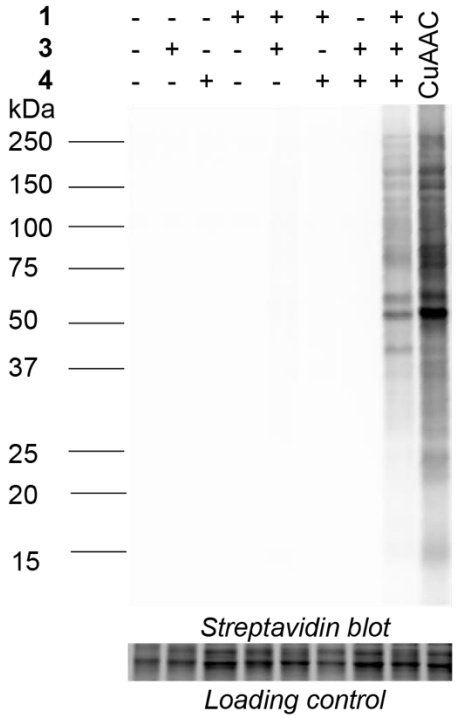


Figure S4. Gel-based ABPP using Suzuki–Miyaura cross coupling with 4-biotin-boronic acid **3**. Degassed HEK293T lysates were labeled with probe **1** (200 µM) or vehicle (DMSO) for 1h, following which the samples were subjected to Suzuki–Miyaura cross-coupling to **3** (2 mM) in the presence of **4** (1 mM) for 3h at RT. For CuAAC labeling, lysates were labeled with probe **5** (200 µM) for 1h at RT, followed by CuAAC conjugation to **6** (400 µM) for another hour at RT. The labeled proteins were resolved by SDS-PAGE gel and biotinylation visualized by streptavidin blot.

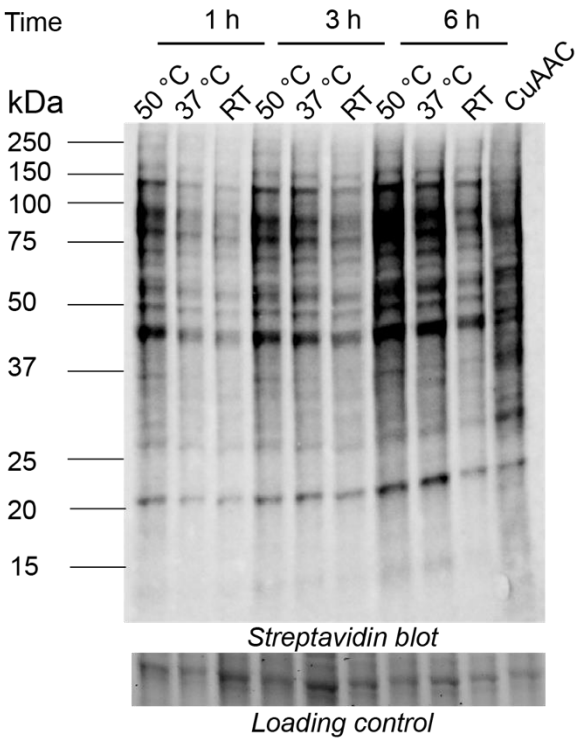


Figure S5. Time- and temperature-dependent labeling. HEK293T lysates were labeled with probe **1** (200 μM) at RT for 1h. The samples were then subjected to Suzuki–Miyaura cross-coupling to **3** (2 mM) in the presence of **4** (1 mM), varying the various temperatures (RT, 37 °C, or 50 °C) and time (1h, 3h, or 6h). The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

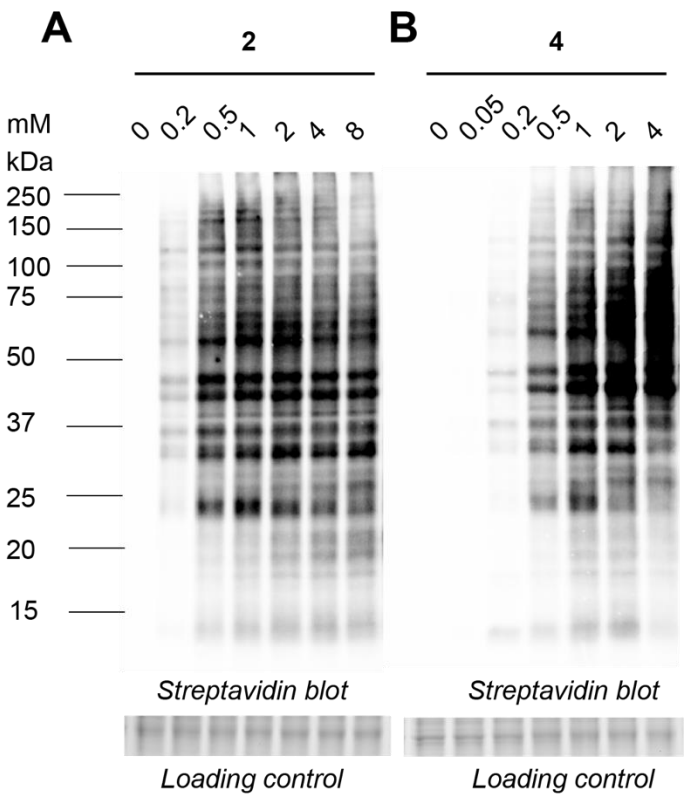


Figure S6. Concentration-dependent labeling by biotin-boronic acid **2** and catalyst **4**. HEK293T lysates were treated with probe **1** (200 µM) at RT for 1h. The samples were then subjected to Suzuki–Miyaura cross-coupling to (A) **2** (0.2, 0.5, 1, 2, 4 and 8 mM) in the presence of **4** (1 mM) or (B) **2** (2 mM) in the presence of **4** (0.05, 0.2, 1, 2 and 4 mM), both for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

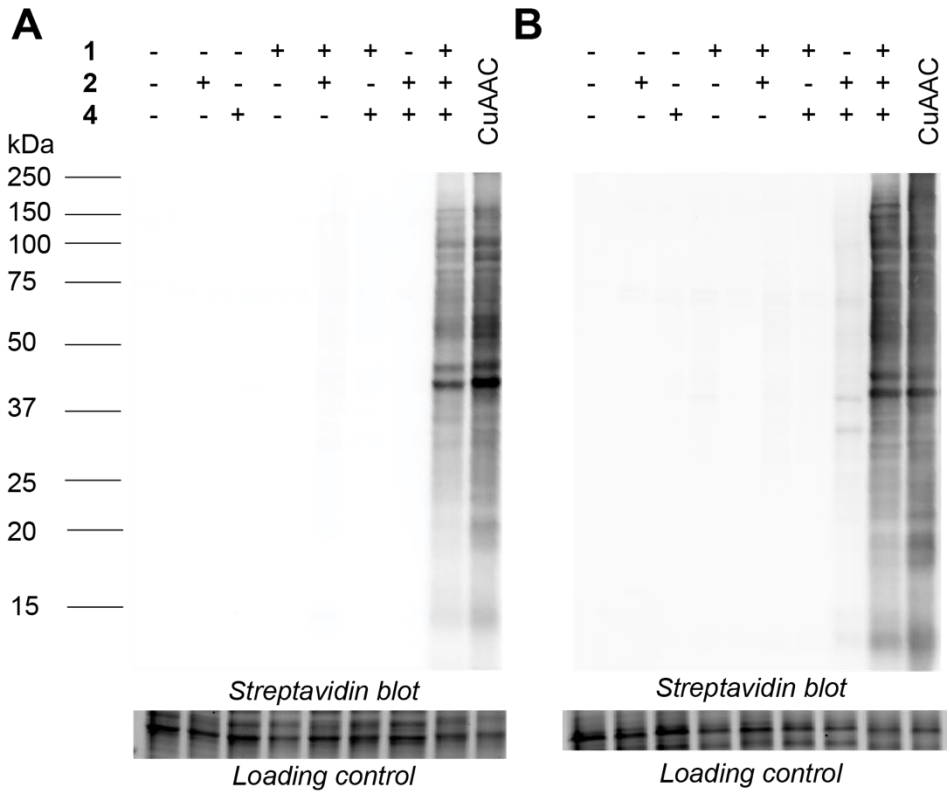


Figure S7. Sample degassing. Suzuki–Miyaura labeling of (A) cellular lysates degassed by freeze pump thaw and (B) cellular lysates processed using standard lysis procedure. HEK293T lysates were treated with probe **1** (200 μM) or vehicle (DMSO) for 1h at RT, after which the samples were subjected to Suzuki–Miyaura cross-coupling to **2 (**2 mM) in the presence of **4 (**1 mM), and both sets of reactions were incubated for 3h at 37 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

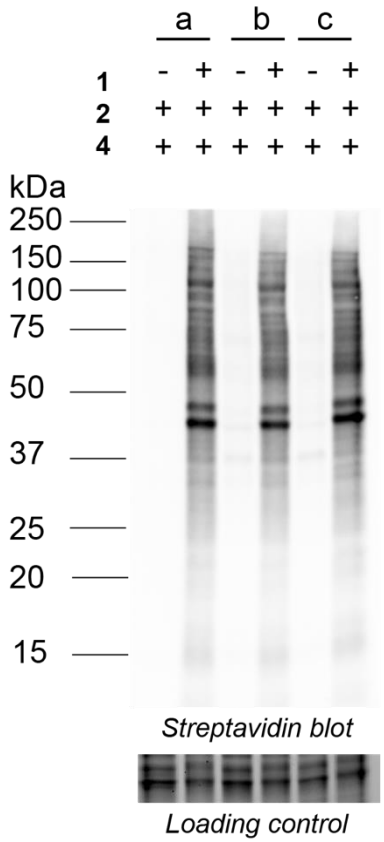


Figure S8. Evaluation of catalyst **4** from different batches. a: freshly made, b: stored at RT for one month, c: stored at RT for two months. HEK293T lysates were labeled with probe **1** (200 µM) or vehicle (DMSO for 1h at RT. The samples were then subjected to Suzuki–Miyaura cross-coupling to biotin-boronic acid **2** (2 mM) in the presence of **4** (1 mM) for 3h at 37 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

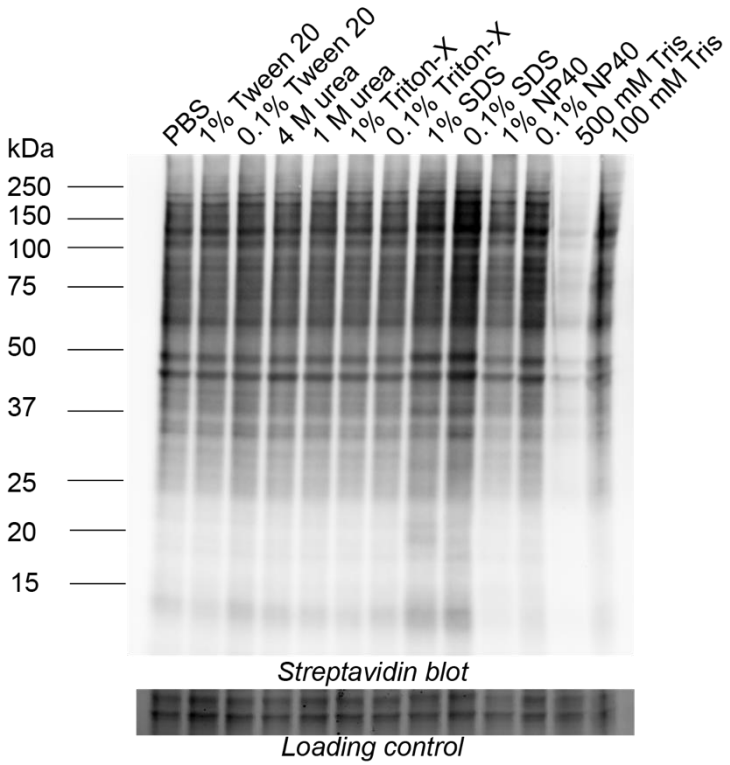


Figure S9. Suzuki–Miyaura labeling in the presence of detergents, denaturants, and free amines. HEK293T lysates (25 µL) were treated with probe **1** (200 µM) for 1h at RT. Additives in PBS (25 µL of a 2× stock) were then added into each sample to the indicated final concentrations. Next, samples were subjected to Suzuki–Miyaura cross-coupling to **2** (2 mM) in the presence of **4 (**1 mM) for 3h at 37 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

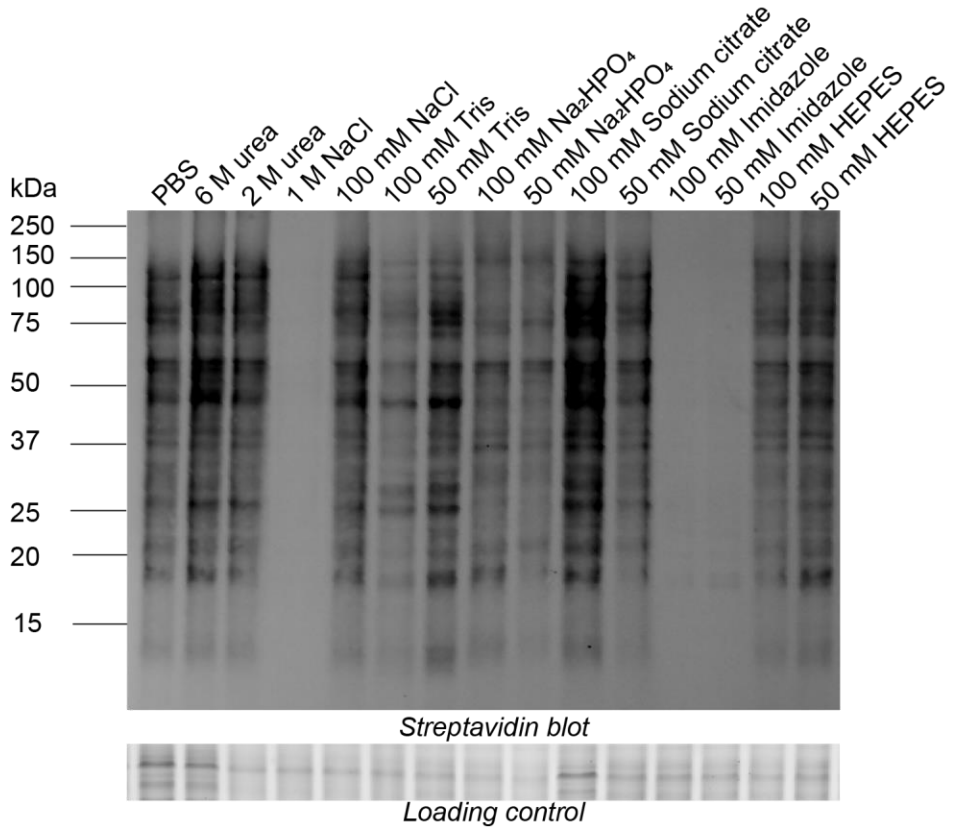


Figure S10. Suzuki–Miyaura labeling in the presence of denaturants, chaotropic agents, salts, and free amines. HEK293T lysates (25 µL) were treated with probe **1** (200 µM) for 1h at RT. Additives in PBS (25 µL of a 2× stock) were then added into each sample to the indicated final concentrations. Next, samples were subjected to Suzuki–Miyaura cross-coupling to **3** (2 mM) in the presence of **4** (1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

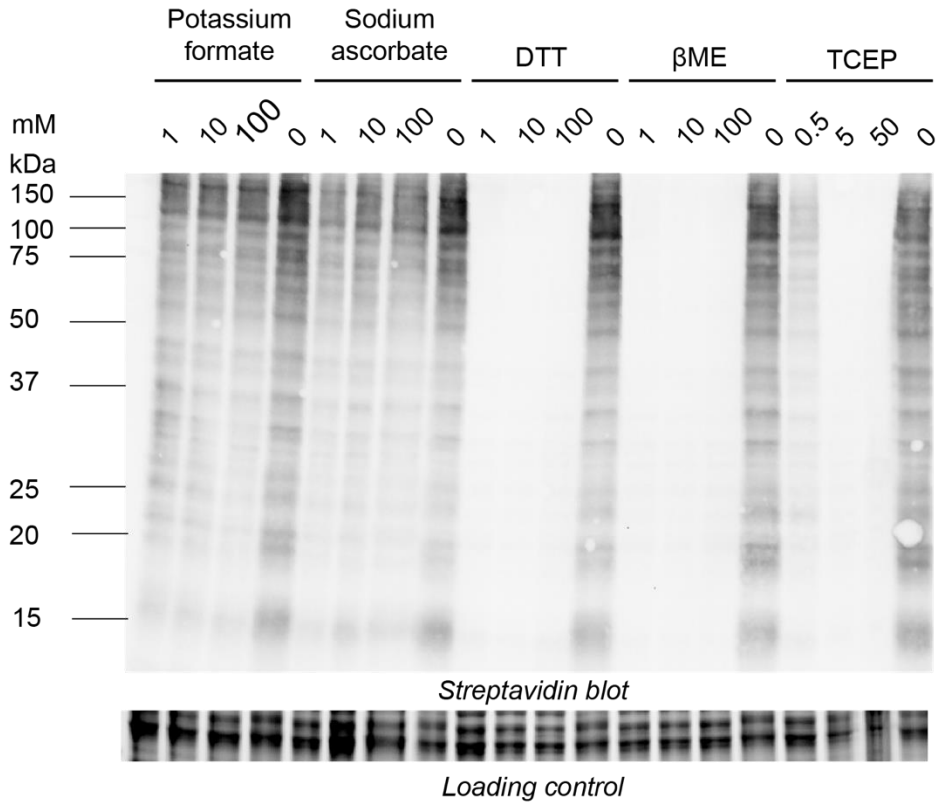


Figure S11. Suzuki–Miyaura labeling in the presence of detergents, denaturants, and free amines. HEK293T lysates were treated with probe **1** (200 μM) for 1h at RT. Reducing agents were then added to each sample at the indicated final concentrations. Next, samples were subjected to Suzuki–Miyaura cross-coupling **2** (2 mM) in the presence of **4** (1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

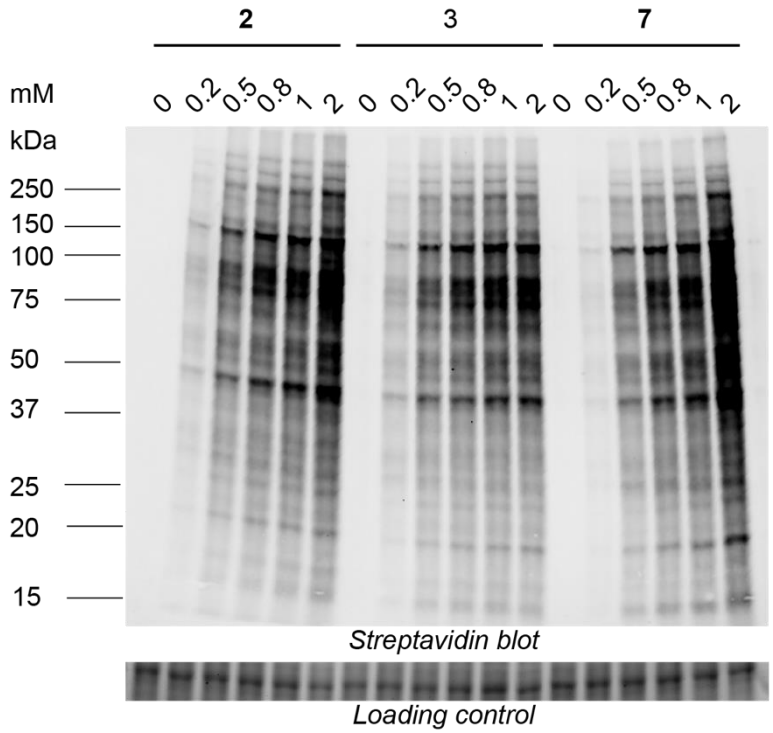


Figure S12. Comparison of biotin-boronic acids (**2**, **3** and **7**) for Suzuki–Miyaura cross-coupling. HEK293T lysates were labeled with **1** (200 µM) for 1h at RT. Next, samples were subjected to Suzuki–Miyaura cross-coupling to biotin-boronic acids **2, 3,** or **7** with the indicated concentration or vehicle (DMSO) in the presence of **4 (**1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

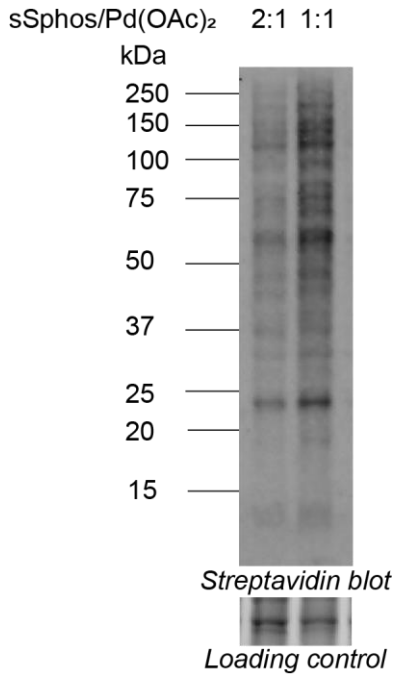


Figure S13. Evaluation of palladium to ligand ratio for Suzuki–Miyaura cross-coupling. HEK293T lysates were labeled with **1** (200 µM) for 1h at RT. Next, samples were subjected to Suzuki–Miyaura cross-coupling to **3** (2 mM) in the presence of **4** (1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot. Catalysts were prepared by heating $Pd(OAc)_2$ (0.5 mmol) with different amounts of sSPhos (1 mmol or 0.5 mmol) in DMA for 30 min at 50 °C.

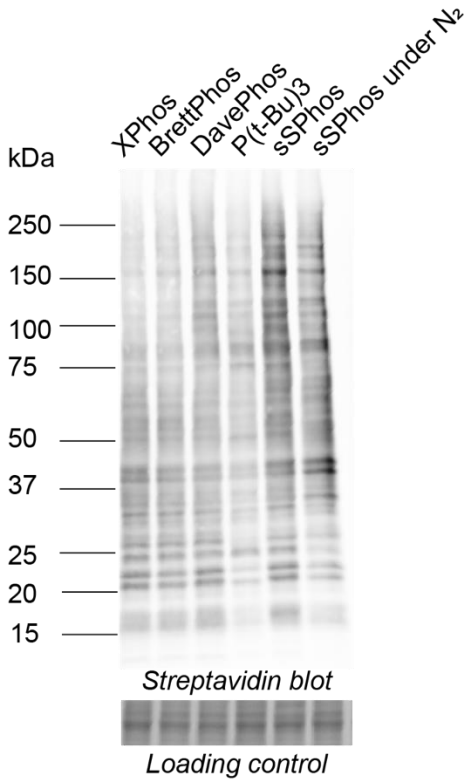


Figure S14. Scope of ligands compatible with gel-based Suzuki–Miyaura cross-coupling. HEK293T lysates were labeled with **1** (200 µM) for 1h at RT. Next, samples were subjected to Suzuki–Miyaura cross-coupling to biotin-boronic acid **2** (2 mM) in the presence of the indicated catalysts (1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot. Catalysts were prepared by heating 0.5 mmol $Pd(OAc)_2$ with the indicated ligands (0.5 mmol) in DMA for 30 min at 50 °C. sSPhos under N_2 indicates that the labeling was performed under nitrogen atmosphere.

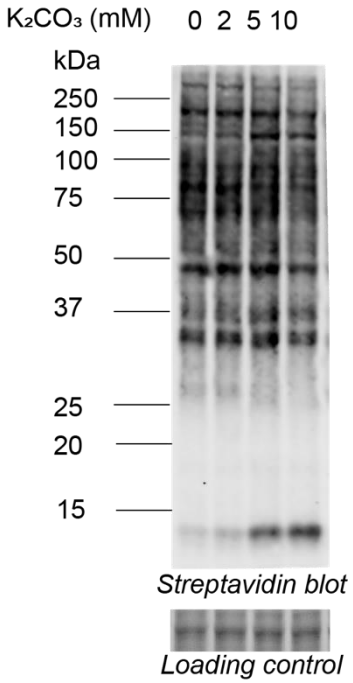


Figure S15. Suzuki–Miyaura cross-coupling in the presence of base (K_2CO_3). HEK293T lysates were labeled with **1** (200 µM) for 1h at RT. Next, samples were subjected to Suzuki–Miyaura cross-coupling to biotin-boronic acid **3** (2 mM) in the presence of **4** (1 mM) and with the indicated concentration of K_2CO_3 for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot.

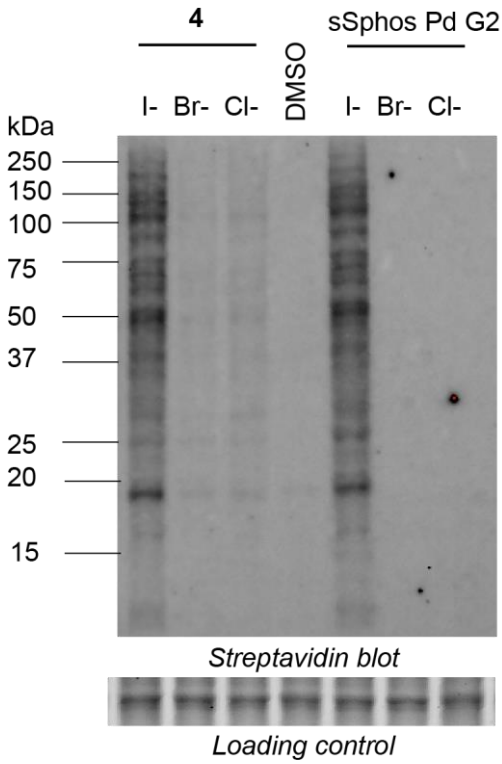
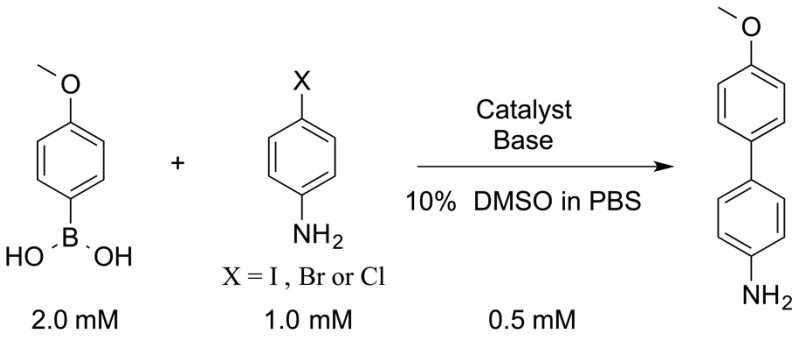


Figure S16. Evaluation of Suzuki–Miyaura labeling using chloro and bromo modified probes. HEK293T lysates were labeled with **1**, **8**, **or 9** (200 µM) or vehicle **(**DMSO) for 1h at RT. Next, samples were subjected to Suzuki–Miyaura cross-coupling to biotin-boronic acid **3** (2 mM) in the presence of the indicated catalysts (1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot. Precatalysts were mixed with 10 equiv. K_2CO_3 for 10 min at 70 °C immediately prior to lysate labeling.

Table S2. Assessment of precatalyst compatibility with PBS and aryl halogens. Model reaction between 4-iodo-, 4-bromo- or 4-iodoaniline and 4-methoxyphenyl boronic acid was monitored by LC-MS in the presence of the indicated catalyst and base.



				Yield (%)		
Entry	**Catalyst**	**Base**	**Condition**	**I**	**Br**	**Cl**
1	4	2 mM K_2CO_3	37 °C, 1 h	85	12	0
2	sSPhos Pd G2	2 mM K_2CO_3	37 °C, 1 h	96	19	0
3	XPhos Pd G3	2 mM K_2CO_3	37 °C, 1 h	94	42	0
4	BrettPhos Pd G3	2 mM K_2CO_3	37 °C, 1 h	92	6	0
5	DavePhos Pd G3	2 mM K_2CO_3	37 °C, 1 h	89	8	0
6	RuPhos Pd G3	2 mM K_2CO_3	37 °C, 1 h	91	20	0
7	N-XantPhos G3	2 mM K_2CO_3	37 °C, 1 h	94	9	0
8	XantPhos G4	2 mM K_2CO_3	37 °C, 1 h	92	38	0
9	PEPPSI™-IPr	2 mM K_2CO_3	37 °C, 1 h	95	27	0
10	(NHC)Pd(allyl)Cl	2 mM K_2CO_3	37 °C, 1 h	~100	2	0
11	sSPhos Pd G2	20 mM K_2CO_3	37 °C, 1 h	N.A.	92	7%
12	sSPhos Pd G2	100 mM CsOH	80 °C, 1 h	N.A.	96	87

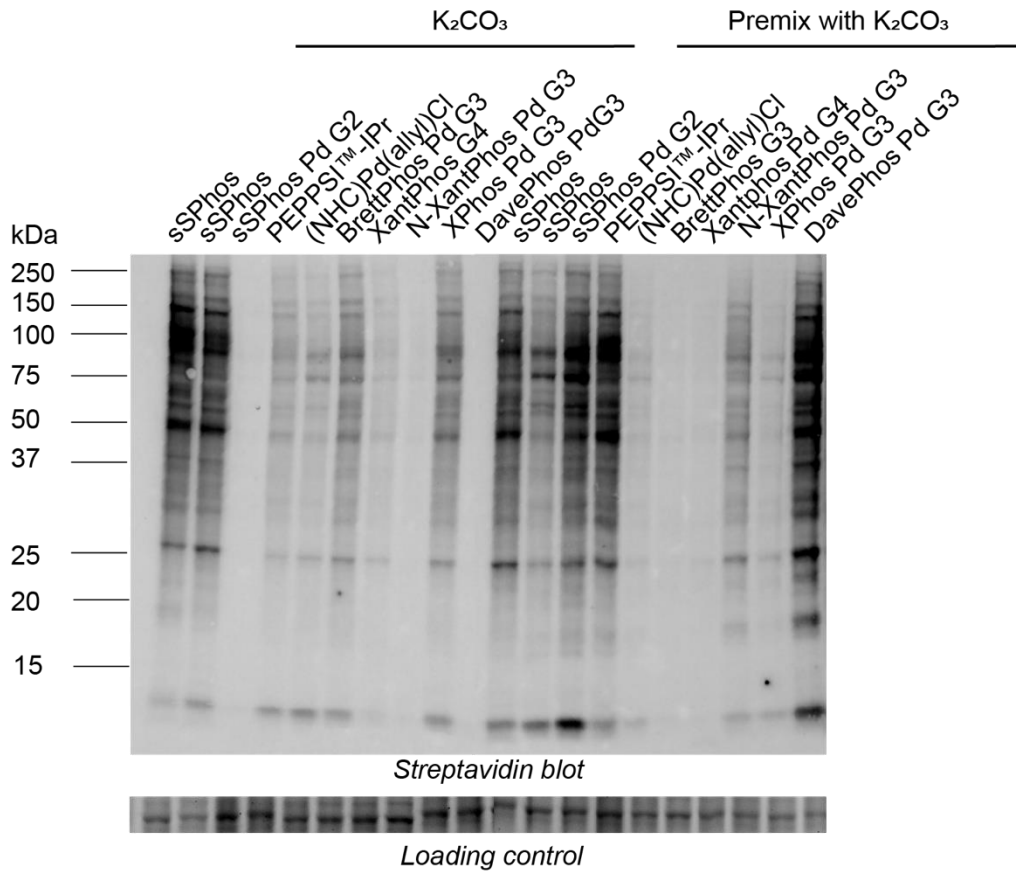


Figure S17. Evaluation of precatalysts for gel-based Suzuki–Miyaura labeling. HEK293T lysates were labeled with **1** (200 µM) for 1h at RT. Next, samples were subjected to Suzuki–Miyaura cross-coupling to biotin-boronic acid **3** (2 mM) in the presence of the indicated catalysts (1 mM) for 1h at 50 °C. The labeled proteins were resolved by SDS-PAGE gel and biotinylation was visualized by streptavidin blot. For K_2CO_3 treated samples, 10 mM of K_2CO_3 was added into reaction prior to addition of the indicated precatalyst. For the premixed samples, precatalyst or **4** in DMSO or DMA was mixed (1:1 volume/volume) with K_2CO_3 (10 mM stock in water), and the resulting mixture was heated for 10 min at 75 °C. Samples only treated with **4** without adding or premixing with K_2CO_3 were also included.

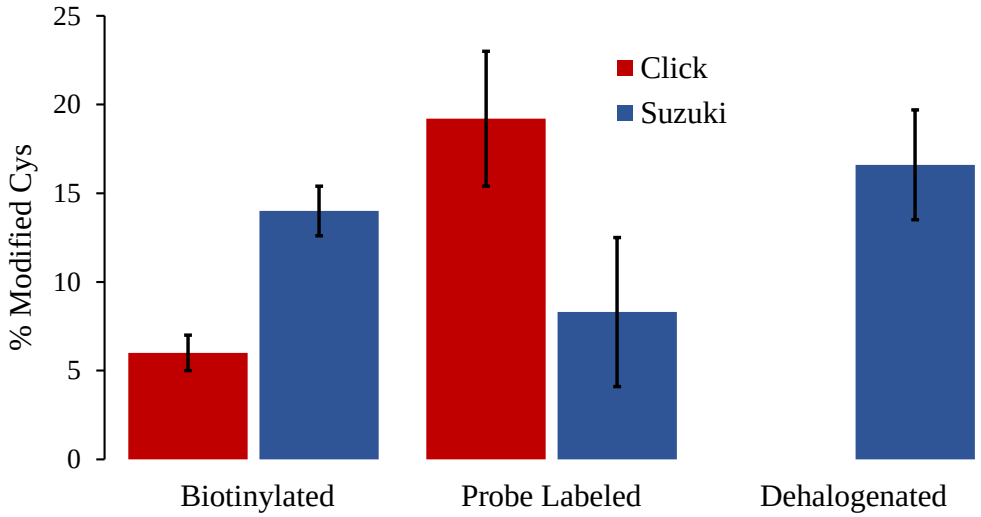


Figure S18. Comparison of Suzuki–Miyaura and click labeling of the recombinant protein BSA. BSA in PBS was labelled with **5** (400 µM) or **1** (400 µM) for 1h, followed by CuAAC (400 µM **6**, 100 µM TBTA, 1 mM CuSO4 and 1 mM TCEP) at RT for another l h or Suzuki–Miyaura cross-coupling (1 mM **4** and 2 mM of **2**) for 3h at 50 °C. The protein was then precipitated (chloroform/methanol), reduced, alkylated, digested, subjected to LC-MS/MS analysis, and the data searched using differential modifications for biotinylated, probe-labeled and dehalogenation (*N*= 3 for each probe).

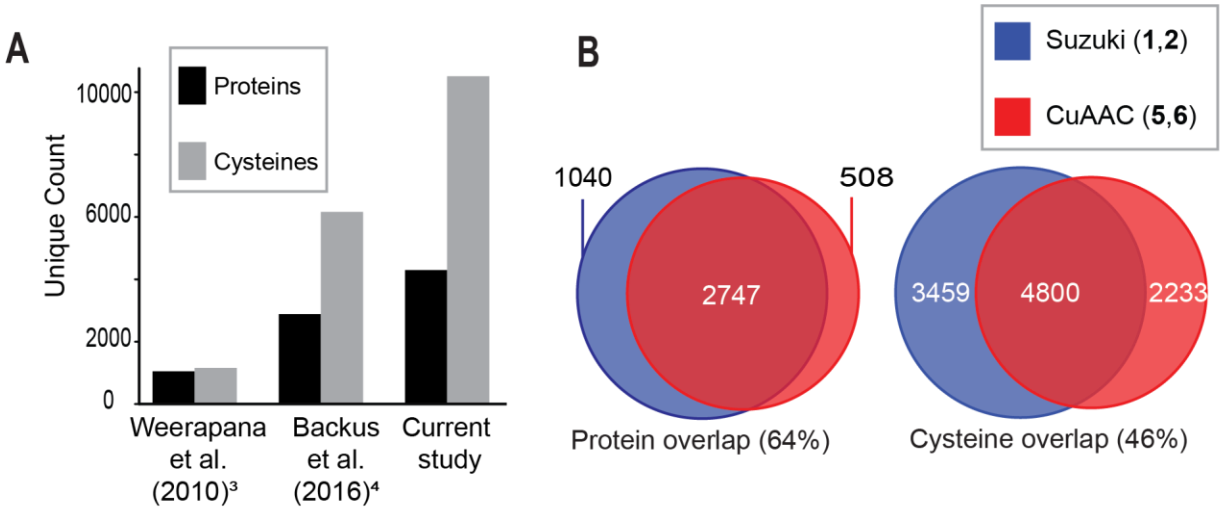


Figure S19. Comparison of CuAAC and Suzuki–Miyaura for chemoproteomic analysis of probe-labeled cysteines. For Suzuki–Miyaura, HEK293T lysates were labeled by cysteine-reactive probe **1** (200 µM) for 1h followed by cross-coupling to biotin-boronic acid **2** (2 mM) in the presence of **4** (1 mM) at 50 °C for 3h. For CuAAC, lysates were labeled with alkyne-modified **5** (200 µM), which was subjected to CuAAC labeling with azido-biotin **6** (400 µM)**.** (A) Total number of unique proteins and cysteines identified in prior and current (aggregate Suzuki and CuAAc datasets) study. (B) Overlap of unique proteins and biotinylated cysteine residues identified in replicate chemoproteomic experiments (*N*=3 for each probe).

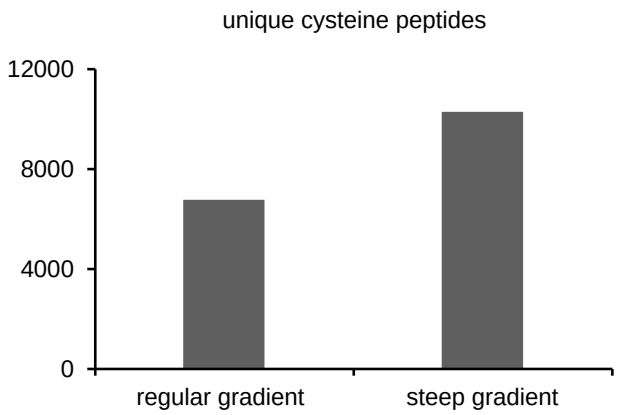


Figure S20. Comparison of solvent gradient during reverse phase chromotography for chemoproteomic analysis of probe-labeled cysteines. Lysates were labeled with alkyne-modified **5** (200 μM), which was subjected to CuAAC labeling with azido-biotin **6** (400 μM)**.** The digested and enriched peptides were subjected to LC-MS/MS analysis using different solvent gradients.

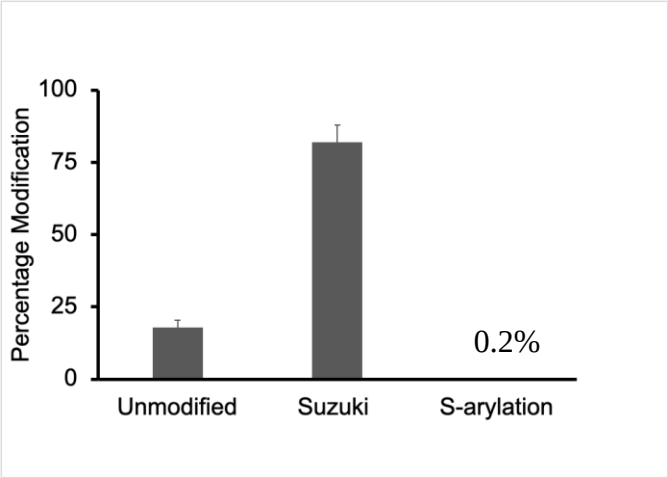


Figure S21. Percentage modification of Suzuki–Miyaura cross-coupling for chemoproteomic analysis. For Suzuki–Miyaura cross-coupling, HEK293T lysates were labeled by cysteine-reactive probe **1** (200 μM) for 1h followed by cross-coupling to biotin-boronic acid **2** (2 mM) in the presence of **4** (1 mM) at 50 °C for 3h. The protein went through SP3 cleanup, reduction, alkylation, digestion, enrichment, and subjected to LC-MS/MS analysis. The result was subjected to MSFragger open search and the unmodified, Suzuki modified and s-arylation were identified and plotted as a bar graph. Error bars are ± S.D.

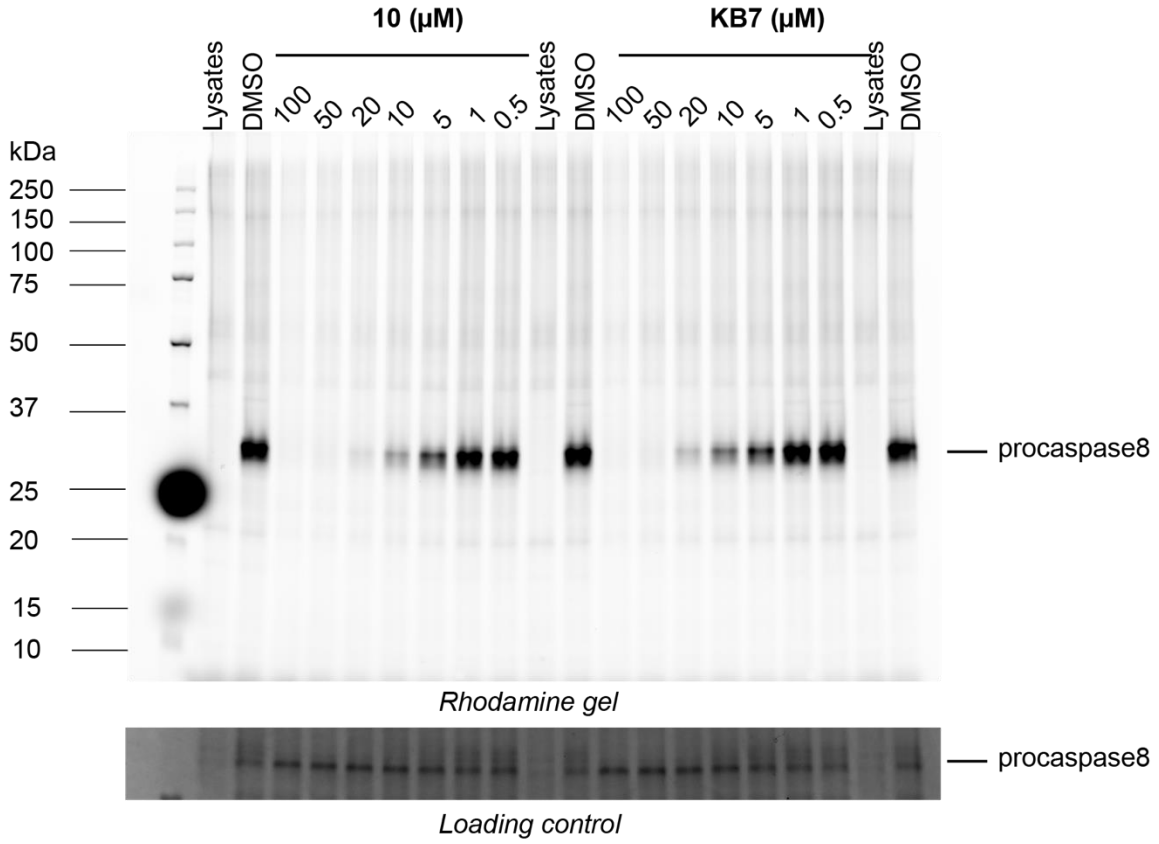


Figure S22. Representative full-length gel for IC50 competitive labeling experiments quantified in **Figure 2B**. Recombinant procaspase-8 (D374A, D384A, C409S and C433S) was added to HEK293T soluble lysates to a final protein concentration of 500 nM. The protein-containing lysates were then treated with **10** or **KB7** or vehicle (DMSO) at the indicated concentrations for 1h, followed by labeling with **11** (10 μM) for 1h and CuAAC conjugation to rhodamine-azide.

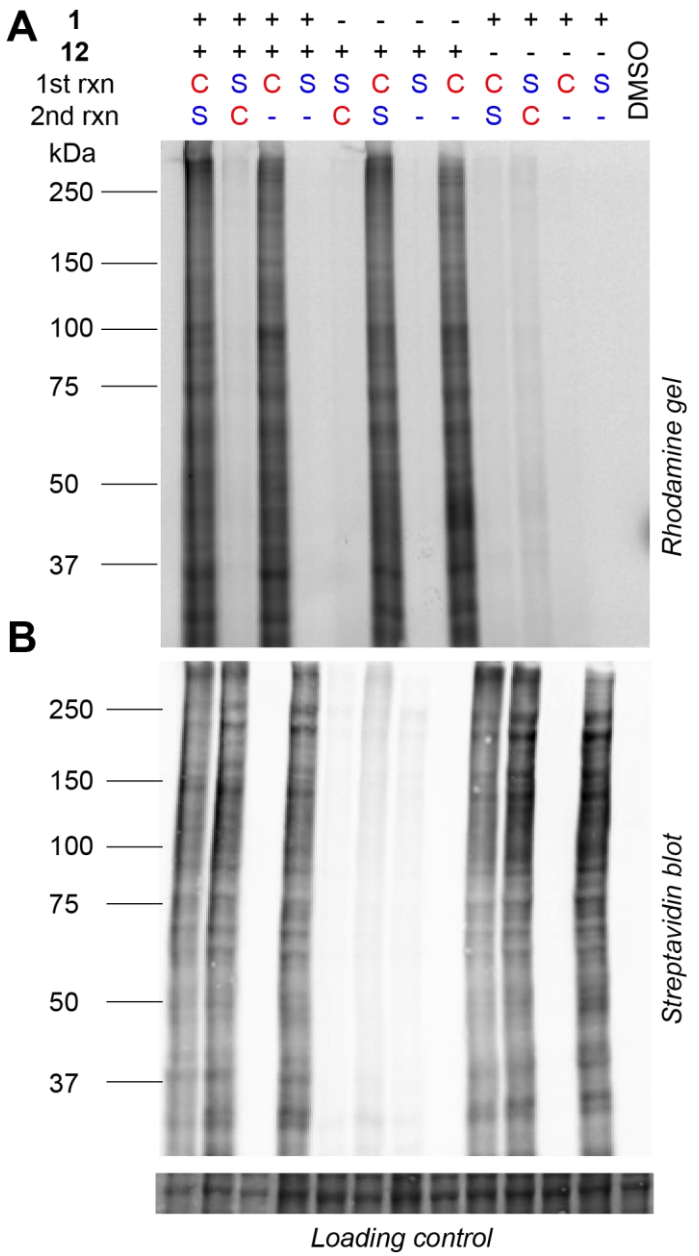
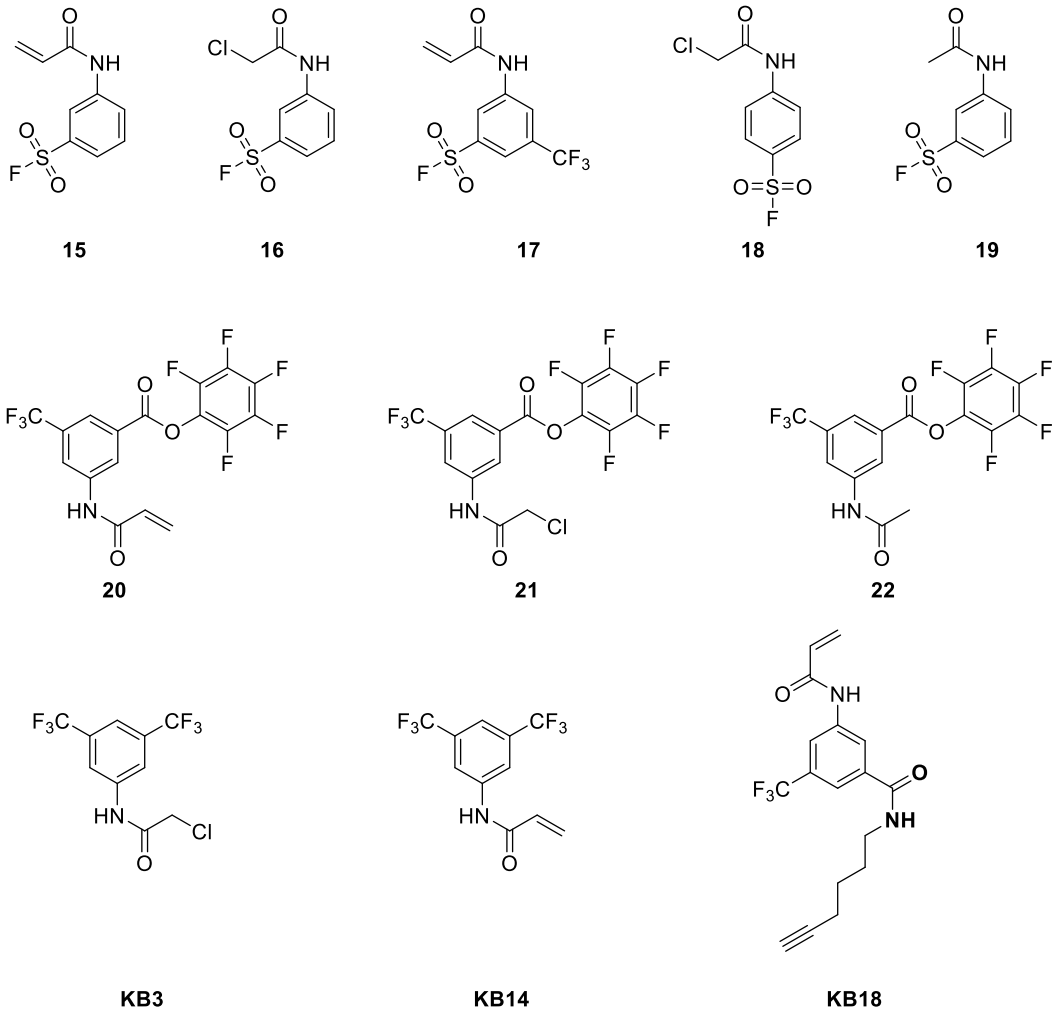


Figure S23. Tandem labeling of cysteines and lysines by combining Suzuki–Miyaura cross-coupling and click chemistry. HEK293T proteome was labeled with cysteine-reactive probe **1** (200 µM) and a lysine reactive probe **12** (50 µM), followed by one pot sequential labeling with CuAAC conjugation to **14** (AF594 alkyne, 25 µM) and Suzuki–Miyaura cross-coupling to biotin-boronic acid **2** (2 mM) in the presence of **4** (1 mM), respectively. Order of reactions is indicated. (A) The labeling is resolved by SDS-PAGE gel and click-labeling visualized by in-gel fluorescence. (B) After transfer to nitrocellulose membrane, biotinylation was visualized by streptavidin blot. Loading control was generated using ponceau stain.



Scheme S2. Structures of dual cysteine- and lysine- electrophilic fragments.

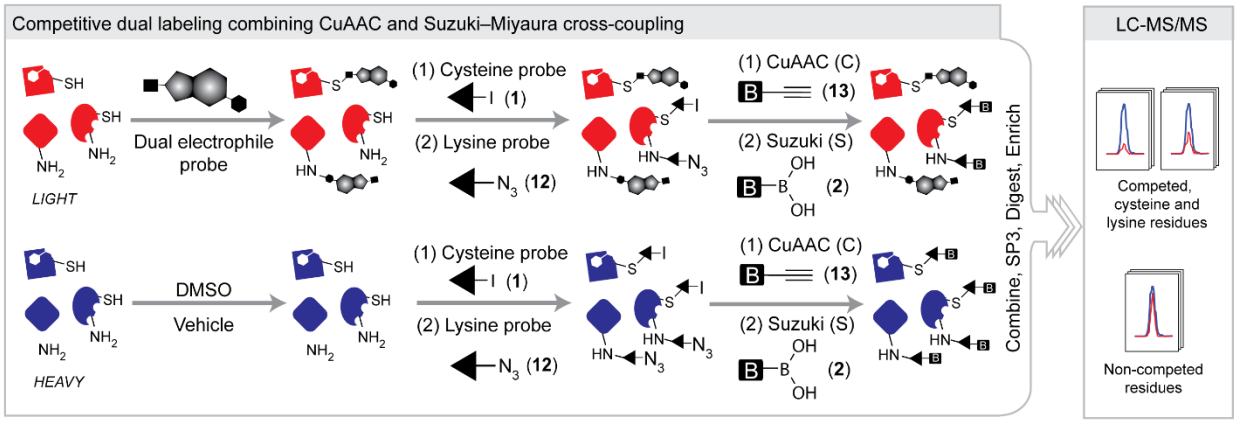


Figure S24. General workflow for competitive screening of bifunctional cysteine- and lysine-reactive compounds. Isotopically labeled cell lysates are treated with either compound or vehicle, followed by labeling with cysteine- and lysine reactive probes **1** and **12**. The samples are then subjected to CuAAC followed by Suzuki-Miyaura cross-coupling to biotin-azide **13** and biotin-boronic acid **2**. The samples are then combined pairwise, proteins captured on SP3 resin, followed by on-resin tryptic digest, elution and enrichment on neutravidin resin. Biotinylated peptides are then analyzed by LC-MS/MS and Dual electrophile probe-labeled peptides identified by ratio of Heavy/Light MS1 chromatographic peak areas.

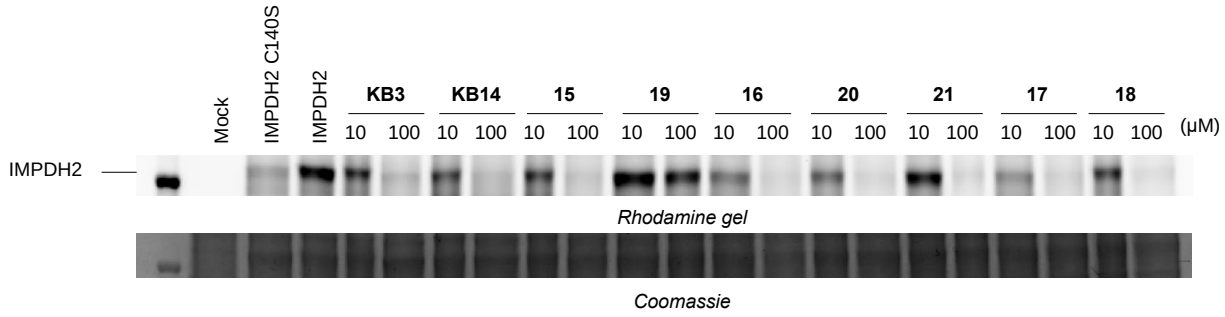


Figure S25. Gel-based competitive ABPP profiling of the labeling of the protein IMPDH2 by dual electrophile probes. Recombinant protein, expressed in E. coli was spiked into HEK 293T cell lysates followed by treatment with the indicated concentration of compounds for 1h followed by subsequent labeling with alkyne probe **KB18**, visualized by CuAAC conjugation to rhodamine azide. Loading control was generated using Coomassie instant blue.

Table S5. Representative search results of crosslinking sites of protein IMPDH2 with bifunctional compounds.

Compound	**15**		**16**		**17**		**19**		**20**	
Crosslinking sites	Y110	C140	Y110	C140	K109	C140	K134	C140	K134	C140
	K134	C140	K109	C140	Y430	C331	K511	C331	K109	C140
	K208	C140	K134	C140	Y110	C140				
	Y233	C140	K208	C140	K134	C140				
	Y459	C331	K228	C140	K208	C140				
	Y459	C468	Y430	C140	Y233	C140				
	K242	C246	K238	C246	K238	C246				
	K238	C246	K241	C246	K242	C246				
	K109	C140	K242	C246						
	Y509	C331	Y233	C246						
	Y430	C140	Y459	C339						
			Y459	C331						
			K109	C173						
			K110	C173						

(B) Biological Methods.

Cell culture and preparation of cell lysates. Cell culture reagents including Dulbecco's phosphate-buffered s aline (DPBS), Dulbecco's modified Eagle's medium (DMEM)/high glucose media, Roswell Park Memorial Institute (RPMI) media, trypsin-EDTA and penicillin/streptomycin (Pen/Strep) were purchased from Fisher Scientific. Fetal Bovine Serum (FBS) were purchased from Avantor Seradigm (lot # 214B17).

All cell lines were obtained from ATCC and were maintained at a low passage number (< 20 passages). HEK293T (ATCC: CRL-3216) cells were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (Penn/Strep, 100 U/mL). Jurkat A3 (ATCC: CRL-2570) cells were cultured in RPMI supplemented with 10% FBS and 1% antibiotics (Pen/Strep, 100 U/mL). Media was filtered (0.22 µm) prior to use. Cells were maintained in a humidified incubator at 37 °C with 5% CO_2. Cell lines were tested for mycoplasma using the Mycoplasma Detection Kit (InvivoGen).

For *in vitro* labeling, cells were grown to 90 – 95% confluence for HEK293T cells or until cell density reached 1.5 million cells per mL for Jurkat cells. Cells were harvested by centrifugation (4500*g*, 5 min, 4 °C), washed twice with cold DPBS, resuspended in 300 µL DPBS, sonicated, and clarified by centrifuging (21,000 *g*, 10 min, 4 °C). The lysates were then transferred to a new microcentrifuge tube. Protein concentrations were determined using a Bio-Rad DC protein assay kit using reagents from Bio-Rad Life Science (Hercules, CA) and the lysate diluted to the working concentrations indicated below.

Freeze pump thaw degassing of cellular lysates. Cell lysates (5 mL of 2 mg/mL, prepared as described above) were aliquoted into a Schlenk flask with stopcock closed before attaching to a Schlenk line. The lysates were frozen by submerging the flask into a Dewar filled with liquid nitrogen. When the lysates were frozen, the stopcock on the Schlenk flask was opened and the valve on the Schlenk line was switched on to vacuum for ~ 10 min, followed by closing the stopcock and letting the lysates thaw in a warm water bath. Once the lysates thawed, the warm water bath was replaced with the liquid nitrogen bath and freeze pump thaw cycle was repeated

two additional times until no gas bubbles were observed upon solution thaw, upon which the Schlenk flask was disconnected and sealed under nitrogen before use.

Gel-based ABPP with CuAAC. HEK293T proteome (50 µL of 2 mg/mL, prepared as described above) was labeled with **5** (1 µL of 10 mM stock solution in DMSO, final concentration = 200 µM) for 1h at ambient temperature followed by copper-mediated azide-alkyne cycloaddition (CuAAC). CuAAC was performed with **6** (2 µL of 10 mM stock in DMSO, final concentration = 400 µM), tris(2-carboxyethyl)phosphine hydrochloride (TCEP; 1 µL of fresh 50 mM stock in water, final concentration = 1 mM), Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 3 µL of 1.7 mM stock in DMSO/*t*-butanol 1:4, final concentration = 1 00 µM), and $CuSO_4$ (1 µL of 50 mM stock in water, final concentration = 1 mM). Samples were allowed to react for 1h at ambient temperature at which point the reactions were quenched with 4× Laemmli buffer (20 µL). Samples were then denatured (5 min, 95 °C) and analyzed by SDS-PAGE, using Criterion™ TGX Stain-Free™ gels obtained from Bio-Rad. Loading control images were obtained using the stain-free workflow on a Bio-Rad ChemiDoc™ Imager.

Gel-based ABPP combining CuAAc and Palladium catalyzed Suzuki–Miyaura cross-coupling. HEK293T proteome (50 µL of 1.5 mg/mL, prepared as described above) was labeled with **1** (1 µL of 10 mM stock solution in DMSO, final concentration = 200 µM) for 1h at RT followed by labeling with **12** (Click Chemistry Tools, 1401-5, 1 µL of 2.5 mM stock solution in DMSO, final concentration = 50 µM) for an additional hour at RT. Next, the samples were subjected to CuAAC with **14** (AF594 alkyne, Click Chemistry Tools, 1297-1, 1 µM of 1.25 mM stock in DMSO, final concentration = 25 µM), TCEP (1 µL of fresh 50 mM stock in water, final concentration = 1 mM), TBTA (3 µL of 1.7 mM stock in DMSO/*t*-butanol 1:4, final concentration = 100 µM), and $CuSO_4$ (1 µL of 50 mM stock in water, final concentration = 1 mM) for 1h at RT. Suzuki–Miyaura cross-coupling reagents were then added after click reaction with **2** (1 µL of 100 mM stock in DMSO, final concentration = 2 mM), and **4** (1 µL of 50 mM stock of $Pd(OAc)_2$/sSPhos in DMA, final concentration = 1 mM). Samples were allowed to react for another 1h at 50°C before quenching with 4× Laemmli buffer (20 µL). Samples were denatured (5 min, 95 °C) and then resolved by SDS-PAGE. SDS-PAGE gels were transferred to a nitrocellulose membrane using a TransBlot Turbo (Bio-Rad) following the manufacturer's instructions. Loading was assessed by Ponceau stain. The membrane was then probed as described for streptavidin blots, below. Blot was imaged on the Bio-Rad ChemiDoc™ Imager using 800 NIR channel for streptavidin-IRDye® 800CW and rhodamine channel for AF594 alkyne **14**.

Streptavidin blot. Gels were transferred to either polyvinylidene difluoride (PVDF, Bio-Rad, 1620177) or nitrocellulose (Bio-Rad, 1704271) membranes using a Trans-Blot Turbo transfer system (Bio-Rad) following the manufacturer's instructions. After transfer, the membranes were then blocked (2% w/v of BSA in TBS-T, 30 min) and probed with streptavidin-IRDye® 800CW (Fisher, NC0883593, 1:4000) in TBS-T. Blots were incubated overnight at 4 °C with rocking and were then washed (3 × 5 min, TBS-T). The membranes were then imaged with a Bio-Rad ChemiDoc™ Imager using the 800 NIR channel.

Proteomic sample preparation with bovine serum albumin (BSA). BSA (200 µL, 2 mg/mL in PBS) was labelled with **5** (8 µL of 10 mM stock in DMSO, final concentration = 400 µM) or **1** (8 µL of 10 mM stock in DMSO, final concentration = 400 µM) for 1h, followed by CuAAC including **6** (8 µL of 10 mM stock in DMSO, final concentration = 400 µM), TCEP (4 µL of fresh

50 mM stock in water, final concentration = 1 mM), TBTA (12 µL of 1.7 mM stock in DMSO/*t*-butanol 1:4, final concentration = 100 µM), and $CuSO_4$ (4 µL of 50 mM stock in water, final concentration = 1 mM) for 1h at RT or Suzuki–Miyaura cross-coupling with **2** (4 µL of 100 mM stock in DMSO, final concentration = 2 mM), and **4** (4 µL of 50 mM stock of $Pd(OAc)_2$/sSPhos in DMA, final concentration = 1 mM) for 3h at 50 °C. The protein was then subjected to chloroform/methanol precipitation following which the pellet was resuspended with sonication in 300 µL 8 M urea (660 mg/mL in PBS). To this was added DTT (6 µL of 200 mM stock in water, final concentration = 4 mM) and the reaction was incubated at 65 °C for 15 min following which iodoacetamide (6 µL of 400 mM stock in water, final concentration = 8 mM) was added and the reaction was incubated for 30 min at 37 °C with shaking. The samples were diluted with PBS (400 µL). Trypsin (Worthington Biochemical, LS003740, 20 µL, 1 mg/mL in 666 µL of 50 mM acetic acid and 334 µL of 100 mM $CaCl_2$, final weight = 20 ng) was then added and digest was allowed to proceed overnight at 37 °C with shaking. The samples were then acidified to a final concentration of 5% (v/v) formic acid (FA). Samples were then desalted using C18 Tips (Pierce, 87782), following the manufacturer's instructions. The samples were then dried (SpeedVac) and then reconstituted in Molecular Biology Grade (MB) water containing 5% acetonitrile and 1% FA. The samples were then analyzed by LC-MS/MS, as described below.

Proteomic sample preparation using Suzuki–Miyaura cross-coupling. HEK293T proteome (200 µL of 2 mg/mL, prepared as described above) was labeled with **1** (4 µL of 10 mM stock solution in DMSO, final concentration = 200 µM) for 1h at ambient temperature. Suzuki–Miyaura cross-coupling was performed with biotin-boronic acid **2** (4 µL of 100 mM stock in DMSO, final concentration = 2 mM) and catalyst **4** (4 µL of 50 mM stock in DMA, final concentration = 1 mM). 2 µL of 10% SDS in PBS was also added into the solution (final concentration 0.1% SDS). Reactions were allowed to proceed for 3h at 50 °C. The samples were then subjected to either chloroform/methanol precipitation sample preparation or SP3 sample preparation and LC-MS/MS analysis, as described below. Experiments were conducted in triplicate.

Proteomic sample preparation using CuAAC. HEK293T proteome (200 µL of 2 mg/mL, prepared as described above) was labeled with **5** (4 µL of 10 mM stock solution in DMSO, final concentration = 200 µM) for 1h at ambient temperature. CuAAC was performed with biotin-azide **6** (8 µL of 100 mM stock in DMSO, final concentration = 400 µM), TCEP (4 µL of fresh 50 mM stock in water, final concentration = 1 mM), TBTA (12 µL of 1.7 mM stock in DMSO/*t*-butanol 1:4, final concentration = 100 µM), and $CuSO_4$ (4 µL of 50 mM stock in water, final concentration = 1 mM). Samples were allowed to react for 1h at ambient temperature. The samples were then subjected to either chloroform/methanol precipitation sample preparation or SP3 sample preparation and LC-MS/MS analysis, as described below. Experiments were conducted in triplicate.

Cellular labeling in Jurkat cells for mass spectrometry. 20 mL Jurkat cells in complete RPMI (1 million cells/mL) were treated with **10** or **11** (40 µL of 50 mM stock solution in DMSO, final concentration = 100 µM) for 1 h. Control cells were treated with DMSO. Cells were then harvested by centrifugation (4,200g, 10 min, 4 °C) and washed twice in cold PBS (4,200*g*, 10 min, 4 °C). The cell pellets were then lysed in cold PBS (300 µL) with sonication, clarified (21,000 rpm, 10 min) and supernatants removed to new microcentrifuge tubes. Protein concentrations were adjusted to 1.5 mg/mL and 200 µL cell lysates were then transferred to a new microcentrifuge tube

for Suzuki–Miyaura cross-coupling or CuAAC reactions, which were performed as described above. The samples were then subjected to SP3 sample preparation and LC-MS/MS analysis, as described below. Experiments were conducted in triplicate.

Proteomic sample preparation combining Suzuki–Miyaura cross-coupling and CuAAC. HEK293T proteome (200 µL of 2 mg/mL, prepared as described above) was labeled with **1** (4 µL of 10 mM stock solution in DMSO, final concentration = 200 µM) for 1h at RT followed by labeling with **12** (Click Chemistry Tools, 1401-5, 4 µL of 2.5 mM stock solution in DMSO, final concentration = 50 µM) for another hour at RT. Labeled lysates were subjected to CuAAC labeling with **6** (8 µL of 10 mM stock in DMSO, final concentration = 400 µM), TCEP (4 µL of fresh 50 mM stock in water, final concentration = 1 mM), TBTA (12 µL of 1.7 mM stock in DMSO/*t*-butanol 1:4, final concentration = 100 µM), and $CuSO_4$ (4 µL of 50 mM stock in water, final concentration = 1 mM) and reactions were allowed to proceed for 1h at RT. After CuAAC, **2** (4 µL of 100 mM stock in DMSO, final concentration = 2 mM) and **4** (4 µL of 50 mM stock of $Pd(OAc)_2$/sSPhos in DMA, final concentration = 1 mM) were added directly into the sample and Suzuki–Miyaura cross-coupling allowed to proceed for 2h at 50 °C. 30 µL 10% SDS in PBS was then added into the reaction solutions, followed by SP3 sample preparation and LC-MS/MS analysis, as described below. Experiments were conducted in triplicate.

Chloroform/methanol precipitation for proteomic sample preparation. After CuAAC and/or Suzuki–Miyaura labeling, 3 mL methanol, 1 mL chloroform, and 3 mL water were added into each sample followed by centrifugation (4,200g, 10 min, 4 °C). The upper liquid was aspirated and the resulting pellet was further washed with methanol (2 x 3 mL). The pellet was solubilized in PBS containing 0.6 % SDS (0.5 mL) with sonication and heating (5 min, 95 ºC) and any insoluble material was removed by an additional centrifugation step at ambient temperature (14,000 g, 1 min).

NeutrAvidin enrichment of labeled proteins. For each chloroform/methanol precipitated sample, 100 µL of NeutrAvidin-agarose beads slurry (Pierce, 29200) was washed twice in 10 mL PBS and then resuspended in 2.5 mL PBS. The SDS-solubilized proteins were added to the suspension of streptavidin-agarose beads (final concentration 0.1 % SDS in PBS) and the bead mixture was rotated for 2h at ambient temperature. After incubation, the beads were pelleted by centrifugation (1,400 g, 3 min) and were washed (2 × 10 mL PBS and 2 × 10 mL water).

Trypsin digest on NeutrAvidin-agarose beads. The beads were transferred to an eppendorf tube with 1 mL water, centrifuged (21,000 g, 2 min), and resuspended in PBS containing 6 M urea (500 µL). DTT (10 µL of 200 mM stock in water, final concentration = 10 mM) was added into each sample and the sample was incubated at 65 °C for 15 min. To this iodoacetamide (10 µL of 400 mM stock in water, final concentration = 20 mM) was added and the solution was incubated for 30 min at 37 °C with shaking. The bead mixture was diluted with 900 µL PBS, pelleted by centrifugation (21,000 g, 1 min), and resuspended in PBS containing 2M Urea (75 µL) followed by the addition of trypsin solution (Worthington Biochemical, LS003740, 10 µL, 1 mg/mL in 666 µL of 50 mM acetic acid and 334 µL of 100 mM $CaCl_2$, final weight = 10 ng). The digestion was allowed to proceed overnight at 37 ºC with shaking. The beads were separated from the digest with Micro Bio-Spin columns (Bio-Rad) by centrifugation (21,000 g, 1 min), washed (2 × 1 mL PBS and 3 × 1 mL water) and then transferred to fresh eppendorf tubes with 1 mL water. Bound peptides

were eluted twice with 60 µL of 80% acetonitrile in MB water containing 0.1% FA (first at RT for 10 min, second at 72 °C for 10 min). After the first 10 min incubation at RT, the sample was centrifuged (21,000 rpm, 1 min) and the top liquid layer was transferred into a Micro Bio-Spin columns (Bio-Rad) with a receiving eppendorf tube and the bottom beads were then re-dissolved in 60 µL of 80% acetonitrile in water containing 0.1% FA. After the 10 min incubation at 72 °C, the mixture was transferred into the same Bio-Spin column. Elution was collected by centrifugation (21,000 rpm, 1 min). The combined elution was dried (SpeedVac) and resuspended in 100 µL water containing 0.1% FA. Samples were then desalted using C18 Tips (Pierce, 87782), following the manufacturer's instructions. The samples were dried (SpeedVac) and then reconstituted in Molecular Biology Grade (MB) water containing 5% acetonitrile and 1% FA. The samples were then analyzed by LC-MS/MS, as described below.

SP3 proteomic sample preparation.[2] After CuAAC and/or Suzuki–Miyaura labeling, each sample was then treated with 0.5 µL benzonase (Fisher Scientific, 70-664-3) for 30 min at 37 °C. DTT (10 µL of 200 mM stock in water, final concentration = 10 mM) was added into each sample and the sample was incubated at 65 °C for 15 min. To this iodoacetamide (10 µL of 400 mM stock in water, final concentration = 20 mM) was added and the solution was incubated for 30 min at 37 °C with shaking. SP3 sample cleanup was performed at a bead/protein ratio of 10:1 (wt/wt). For each 200 µL sample (2 mg/mL protein concentration), 40 µL Sera-Mag SpeedBeads Carboxyl Magnetic Beads, hydrophobic (GE Healthcare, 65152105050250, 50 µg/µL, total 2 mg) and 40 µL Sera-Mag SpeedBeads Carboxyl Magnetic Beads, hydrophilic (GE Healthcare, 45152105050250, 50 µg/µL, total 2 mg) were aliquoted into a single microcentrifuge tube and gently mixed. Tubes were then placed on a magnetic rack until the beads settled to the tube wall, and the supernatants were removed. The beads were removed from the magnetic rack, reconstituted in 400 µL of MB water, and gently mixed. Tubes were then returned to the magnetic rack, beads allowed to settle, and the supernatants removed. Washes were repeated for two more cycles, and then the beads were reconstituted in 80 µL MB water. The bead slurries were then transferred to the Suzuki–Miyaura cross-coupling or CuAAC samples, and the samples incubated for 5 min at RT with shaking. Absolute ethanol (400 µL) was added to each sample, and the samples were incubated for 5 min at RT with shaking. Samples were then placed on a magnetic rack, and beads allowed to settle. Supernatants were then removed and discarded. Using the magnetic rack as described above, the beads were further washed three times with ethanol (400 µL of 80% solution in water). Beads were then resuspended in 100 µL PBS containing 2 M urea followed by addition trypsin solution (Worthington Biochemical, LS003740, 10 µL, 1 mg/mL in 666 µL of 50 mM acetic acid and 334 µL of 100 mM $CaCl_2$, final weight = 10 ng). Digest was allowed to proceed overnight at 37 ºC with shaking. The samples were then acidified to a final concentration of 3% (v/v) FA. After incubation for 5 min at RT with shaking, ~ 2 mL acetonitrile (> 95% of the final volume) was added to each sample and the mixtures were then incubated for an additional 10 min at RT with shaking. Supernatants were then removed and discarded using the magnetic rack, and the beads were washed (3 × 500 µL acetonitrile). Peptides were then eluted from SP3 beads with 100 µL of 2% DMSO in MB water for 30 min at 37 °C with shaking. The elution can be used for NeutrAvidin enrichment or can be dried (SpeedVac) before NeutrAvidin enrichment.

For proteomic sample preparation combining Suzuki–Miyaura cross-coupling and CuAAC, each sample was loaded into SP3 beads after reaction followed by protein binding with ethanol (400

µL). Beads were washed with 80% ethanol solution (3 x 400 µL) and resuspended in PBS containing 2M urea and 0.5% SDS (200 µL). DTT (10 µL of 200 mM stock in water, final concentration = 10 mM) was added into each beads slurry solution and the sample was incubated at 65 °C for 15 min. To this iodoacetamide (10 µL of 400 mM stock in water, final concentration = 20 mM) was added and the mixture was incubated for 30 min at 37 °C with shaking. Ethanol (400 µL) was then added to induce protein-bead binding following which the sample was washed with 80% ethanol solution (3 x 400 µL). The washed beads were resuspended in PBS containing 2M urea followed by addition trypsin solution (Worthington Biochemical, LS003740, 10 µL, 1 mg/mL in 666 µL of 50 mM acetic acid and 334 µL of 100 mM $CaCl_2$, final weight = 10 ng). After tryptic digest, each sample was subjected to the remaining procedures as detailed for SP3 proteomic sample preparation.

NeutrAvidin enrichment of labelled peptides. For each sample, 50 µL of NeutrAvidin® Agarose resin slurry (Pierce, 29200) was washed twice in 10 mL IAP buffer (50 mM MOPS pH 7.2, 10 mM sodium phosphate, and 50 mM NaCl buffer) and then resuspended in 500 µL IAP buffer. Peptide solutions eluted from SP3 beads were then transferred to the NeutrAvidin® Agarose resin suspension, and the samples were then rotated for 2h at RT. After incubation, the beads were pelleted by centrifugation (21,000 g, 1 min) and washed by centrifugation (6 × 700 µL water). Bound peptides were eluted with 60 µL of 80% acetonitrile in MB water containing 0.1% FA (10 min at RT). The samples were then harvested by centrifugation (21,000 g, 1 min) and residual beads separated from supernatants using Micro Bio-Spin columns (Bio-Rad). The remaining peptides were then eluted from pelleted beads with 60 µL of 80% acetonitrile in water containing 0.1% FA (10 min, 72 °C). Beads were then separated from the eluants using the same Bio-Spin column. Eluants were then collected by centrifugation (21,000 g, 1 min) and the combined eluants were dried (SpeedVac). The samples were then reconstituted in 40 µL water containing 5% acetonitrile and 1% FA and analyzed by LC-MS/MS.

Liquid-chromatography tandem mass-spectrometry (LC-MS/MS) analysis of Suzuki–Miyaura cross-coupling enriched samples. The samples were analyzed by liquid chromatography tandem mass spectrometry using an Q Exactive™ mass spectrometer (Thermo Scientific) coupled to an Easy-nLC™ 1000 pump. Peptides were resolved on a C18 reversed phase column (3 µM, 100Å pores), packed in-house, with 100 µm internal diameter and 18 cm of packed resin. The peptides were eluted using a 140 min gradient of Buffer B in Buffer A (Buffer A: water with 3% DMSO and 0.1% FA; Buffer B: acetonitrile with 3% DMSO and 0.1% FA) and a flow rate of 220 nL/min with electrospray ionization of 2.2 kV. The regular gradient includes 0 – 5 min from 1% to 5%, 15 – 130 min from 5% to 27%, 15 – 137 min from 27% to 35%, and 137 – 138 min from 35% to 80% buffer B in buffer A. The steep gradient for this study includes 0 – 5 min from 1% to 5%, 5 – 20 min from 5% to 15%, 20 – 130 min from 15% to 35% and from 130 – 135 min from 35 to 95% buffer B in buffer A. Data was collected in data-dependent acquisition mode with dynamic exclusion (15 s), and charge exclusion (1,7,8,>8) was enabled. Data acquisition consisted of cycles of one full MS scan (400 − 1800 m/z at a resolution of 70,000) followed by 12 MS2 scans of the nth most abundant ions at resolution of 17,500.

Peptide and protein identification. The MS2 spectra data were extracted from a raw file using RAW Xtractor (version 1.1.0.22; available at http://fields.scripps.edu/rawconv/). MS2 spectra data were searched using the ProLuCID algorithm (publicly available at

http://fields.scripps.edu/yates/wp/?page_id=17 using a reverse concatenated, nonredundant variant of the Human UniProt database (release-2020_01). Cysteine residues were searched with a static modification for carboxyamidomethylation (+57.02146) and one differential modification at cysteine residues, which is +393.1511 for Suzuki enrichment and +426.18375 for CuAAC enrichment. Peptides were required to have at least one tryptic terminus and to contain the biotin modification. ProLuCID data was filtered through DTASelect (version 2.0) to achieve a peptide false-positive rate below 1%.

Proteomic data processing. Custom python and R scripts were implemented to filter and compile labeled peptide datasets. Peptides with one tryptic terminus and those sequenced only once (singletons) out of three experiments were filtered out before further analysis. Unique proteins, unique residues (cysteines or lysines) and unique peptide-spectrum matches (PSMs) were quantified for each dataset, using unique identifiers. Unique proteins were established based on UniProt protein ID. Unique residues were classified by an identifier consisting of a UniProt protein ID and the residue number of the modified cysteine/lysine; residue numbers were found by aligning the peptide sequence to the corresponding UniProt protein sequence. Unique peptides were found based on sequences containing modified residue location. If a peptide was labeled at multiple residues, an identifier was generated for each protein ID and modified residue location. The overlap between datasets represented the number of common identifiers shared between both methods. The current data was compared to previously published datasets from Weerapana et al.[3] and Backus et al.,[4] which were processed to remove peptides no longer found in UniProt database (release-2020_01), due to database updates. Identifiers from each dataset were averaged and plotted as mean +/- SD. Unpaired, two-tailed (student's or welch) t-tests were performed between replicates, where p-values < 0.05 were considered significant.

BSA data processing. Cysteine residues were searched with a static modification for carboxyamidomethylation (+57.02146) and one differential modification at cysteine residues, which is +393.1511 for Suzuki enrichment and +426.18375 for CuAAC enrichment. Modifications of +100.0310, +201.96794, +76.0313 were used to identify only CuAAC click-probe **5** labeled cysteines, Suzuki-probe **1** labeled cysteines, and dehalogenated Suzuki-probe 1 labeled cysteines, respectively. Peptides with one tryptic terminus and those sequenced only once (singletons) out of three experiments were filtered out before further analysis. Unique peptide-spectrum matches were totaled and averaged for each replicate. Percentages for each modification were calculated using the count of modified unique peptides divided by the combined count of unmodified and modified unique peptides.

Proteomic data processing for in cellular labeling. Cysteine residues were searched with a static modification for carboxyamidomethylation (+57.02146) and one differential modification at cysteine residues, +580.25082 for compound **10** modification and +613.28351 for compound **11** modification. Peptides with one tryptic terminus and those sequenced only once (singletons) out of three experiments were filtered out before further analysis. Uniprot-ID_cysteine-position identifiers were constructed to find unique overlapping cysteines between **10** and **11** datasets. In totaling overlapping protein, each unique peptide was assigned to only one Uniprot ID for peptides mapping to multiple proteins. The longest tryptic peptide for all identified cysteines is reported from both datasets.

Proteomic data processing for multiplexed proteomics combining Suzuki–Miyaura cross-coupling and CuAAC. Cysteine residues were searched with a static modification for carboxyamidomethylation (+57.02146) and a differential modification (+393.1511) for Suzuki labeling. Lysine residues were searched with a differential modification (+420.19436) for CuAAC labeling. Peptides containing only one tryptic terminus were removed during processing. The proteomic data processing workflow was used to obtain unique protein, peptides, and residues for both cysteine and lysine labeling and including labeling overlap.

Recombinant procaspase-8 expression. Procaspase-8 (D374A, D384A, C409S and C433S) was overexpressed as an N-terminal His6-tag fusion from a pET23b vector, as described previously.[5] Briefly, transformed BL21(DE3) chemically competent E. coli (DE3, NEB) cells were grown in LB media supplemented with 100 μg/mL ampicillin at 37 °C to an OD600 nm of 0.6–0.8. Flasks were then transferred to an 18 °C incubator, and protein expression was induced with 1.0 mM IPTG for 4 h. Cells were immediately harvested and resuspended in ice cold 100 mM Tris, pH 8.0 and 100 mM NaCl (Buffer A) and subjected to 2 cycles of lysis by microfluidization (Avestin EmulsiFlex-C3). The cell lysate was clarified by centrifugation (14,500*g*, 15 min, 4 °C), and clarified lysate was then loaded onto a 5 mL HisTrap FF crude Ni-NTA affinity column (GE Amersham) pre-equilibrated with buffer A and eluted with buffer A containing 250 mM of imidazole. Imidazole was removed from the collected eluted sample using a disposable PD-10 Desalting Column (GE) to buffer exchange to size exclusion (SEC) buffer (100 mM Tris, pH 8.0 and 100 mM NaCl and 1 mM DTT). The eluted protein was purified by FPLC (GE HiLoad 16/600 Superdex 200 pg) with size exclusion (SEC) buffer. Fractions corresponding to procaspase-8 were pooled and immediately stored at −80 °C.

Calculation of apparent IC50s. HEK293T proteomes (25 μL) containing procaspase-8 (D374A, D384A, C409S, C433S) at 500 nM final concentration were treated with the indicated compounds (1 μL of 25 × stock solution in DMSO) for 1h at RT, followed by 10 μM of probe **11** (1 μL of 25 × stock solution in DMSO). labeled with probe **11** for another 1 h). Subsequently, the samples were subjected to CuAAC conjugation to rhodamine-azide for 1h at ambient temperature. CuAAC was performed with rhodamine-azide (Click Chemistry Tools, AZ109-5, 0.5 μL of 1.25 mM stock in DMSO, final concentration = 25 μM), TCEP (1.5 μL of fresh 50 mM stock in water, final concentration = 1 mM), TBTA (0.5 μL of 1.7 mM stock in DMSO:t-butanol 1:4, final concentration = 100 μM), and $CuSO_4$ (0.5 μL of 50 mM stock in water, final concentration = 1 mM). Samples were then quenched with 10 μL 4× SDS-PAGE loading buffer, denatured for 5 min at 95 °C, resolved by SDS-PAGE, and visualized by in-gel fluorescence. The percentage labeling was determined by quantifying the integrated optical intensity of the bands using ImageJ software14. Nonlinear regression analysis was used to determine the apparent IC50 values from a dose–response curve generated using GraphPad Prism 6. Experiments were conducted in triplicate.

Competitive ABPP of procaspase-8 labeling using Suzuki–Miyaura cross-coupling. 50 μL of HEK293T proteome (1 mg/mL) containing procaspase-8 (2 μM final concentration) was treated with **KB7** (1 μL of 2.5 mM stock in DMSO**)** or vehicle (DMSO) for 1h, followed by labeling with **10** or **11** (1 μL of 500 μM stock in DMSO) for 1h. **10-** and **11**-treated samples were conjugated to biotin-boronic acid **2** (1 mM**)** or biotin-azide **6** (200 μM), respectively using Suzuki–Miyaura cross-coupling for 3h at 37 °C and CuAAC for 1h at RT, as described above. Samples were heated

at 95 °C for 5 min and resolved by SDS-PAGE. Gels were imaged with Stain-Free imaging and then processed as described above for Streptavidin blots.

IMPDH2 expression and purification. IMPDH2 in a pET28a vector were expressed in BL21(DE3) Chemically Competent Cells (NEB), grown on LB broth supplemented with the desired antibiotic (100 g/ml ampicillin) to OD600 of 0.6–0.8 and induced with 0.5 mM IPTG for 16 h at 18 °C. Cells were immediately harvested by centrifugation (7,000 g) for 30 min and the pellet was resuspended in 30 mL cold buffer A (25 mM Tris, pH 8.0, 200 mM NaCl, 10% glycerol, 1 mM BME), supplemented with lysozyme (Sigma), DNAase (NEB) and complete protease inhibitor tablets (Roche), sonicated, and centrifuged (15,000 g, 20 min). The soluble fractions were collected and rotated for 1 h with 1 mL Ni-NTA slurry (Qiagen) at 4 °C. The slurry was then transferred to a 50 mL volume fritted column and collected by gravity flow. The resin was then washed with 100 mL buffer A containing 20 mM imidazole and eluted with 10 mL buffer A containing 200 mM imidazole. The eluant was concentrated to 2.5 mL (Amicon-Ultra-15, 10 kDa MW cutoff), buffer exchanged using PD10 columns (GE Amersham) into the storage buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 10% glycerol, 1 mM BME) and further concentrated (Amicon-Ultra-4, 10 kDa MW cutoff) to a final concentration of approximately 30 µM protein. Protein concentration was determined using the Bio-Rad DC™ protein assay kit. Protein purity was assayed by SDS-PAGE under reducing conditions and were >95% pure.

Crosslinking mass spectrometry sample preparation using recombinant IMPDH2. 20 µL purified recombinant IMPDH2 was treated with 500 µM bifunctional crosslinking probes (1 µL of 10 mM stock in DMSO). After 1 h incubation, reaction mixture was diluted with 8M urea (20 µL) and 10% SDS (2 µL). To this was added DTT (5 µL of 200 mM stock in water) and the reaction was incubated at 65 °C for 15 min following which iodoacetamide (5 µL of 400 mM stock in water) was added and the reaction was incubated for 30 min at 37 °C with shaking. The samples were diluted with PBS (40 µL). Trypsin (Worthington Biochemical, LS003740, 5 µL, 1 mg/mL in 666 µL of 50 mM acetic acid and 334 µL of 100 mM $CaCl_2$, final weight = 20 ng) was then added and digest was allowed to proceed overnight at 37 °C with shaking. The samples were then acidified to a final concentration of 5% (v/v) formic acid (FA). Samples were then subjected to SP3 cleanup and LC-MS/MS analysis. Crosslinking sites were identified with SIM-XL search[6].

SILAC dual proteomics to identify bifunctional compound labeling sites. All SILAC dual proteomic experiments were performed using isotopically labeled human HEK293T cells generated by 8 passages in either light (100 µg/ml each of L-arginine and L-lysine) or heavy (100 µg/mL each of [$^{13}C_6{}^{15}N_4$] L-arginine and [$^{13}C_6{}^{15}N_2$]L-lysine) SILAC DMEM media (Thermo Scientific) supplemented with 10% dialyzed fetal calf serum, penicillin, streptomycin and glutamine. 100 µL of light and heavy cellular lysates (2 mg/mL) were treated with crosslinking compounds (2 µL of 25 mM stock solution in DMSO, final concentration = 500 µM) or DMSO, respectively, for 1 h. Next the lysates were labeled with **1** (4 µL of 10 mM stock solution in DMSO, final concentration = 200 µM) for 1h at RT followed by labeling with **12** (Click Chemistry Tools, 1401-5, 4 µL of 2.5 mM stock solution in DMSO, final concentration = 50 µM) for another hour at RT. Labeled lysates were subjected to CuAAC labeling with **6** (8 µL of 10 mM stock in DMSO, final concentration = 400 µM), TCEP (4 µL of fresh 50 mM stock in water, final concentration = 1 mM), TBTA (12 µL of 1.7 mM stock in DMSO/*t*-butanol 1:4, final concentration = 100 µM), and $CuSO_4$ (4 µL of 50 mM stock in water, final concentration = 1 mM) and reactions were allowed

to proceed for 1h at RT. After CuAAC, **2** (4 μL of 100 mM stock in DMSO, final concentration = 2 mM) and **4** (4 μL of 50 mM stock of $Pd(OAc)_2$/sSPhos in DMA, final concentration = 1 mM) were added directly into the sample and Suzuki–Miyaura cross-coupling allowed to proceed for 2h at 50 °C. 30 μL 10% SDS in PBS was then added into the reaction solutions. 100 μL light proteome and 100 μL heavy proteome were then combined followed by SP3 sample preparation and LC-MS/MS analysis, as described below. Experiments were conducted in duplicate.

(C) General Synthetic Methods.

General Methods. All reactions were performed in oven dried glassware under inert atmosphere unless stated otherwise. Silica gel P60 (SiliCycle) was used for column chromatography and SiliCycle 60 F254 silica gel (precoated sheets, 0.25 mm thick) was used for analytical thin layer chromatography. Plates were visualized by fluorescence quenching under UV light or by staining with iodine. Other reagents were purchased from Sigma-Aldrich (St. Louis, MO), Alfa Aesar (Ward Hill, MA), EMD Millipore (Billerica, MA), Fisher Scientific (Hampton, NH), Oakwood Chemical (West Columbia, SC), Combi-blocks (San Diego, CA), Click Chemistry Tools (Scottsdale, AZ) and Cayman Chemical (Ann Arbor, MI) and used without further purification. ^{1}H NMR and ^{13}C NMR spectra for characterization of new compounds and monitoring reactions were collected in $CDCl_3$, CD_3OD, CD_6CO or DMSO-d6 (Cambridge Isotope Laboratories, Cambridge, MA) on a Bruker AV 400 or 500 MHz spectrometer in the Department of Chemistry & Biochemistry at University of California, Los Angeles. All chemical shifts are reported in the standard notation of parts per million using the peak of residual proton signals of the deuterated solvent as an internal reference. Coupling constant units are in Hertz (Hz). Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets. High-resolution mass spectroscopy was performed on a Shimadzu IT-TOF (ESI source) at University of California, Irvine.

(D) Catalyst Preparation and Reaction Screening

The following compounds were purchased from the indicated vendors. sSPhos (Sigma-Aldrich, 677280-2G), $Pd(OAc)_2$ (Oakwood Chemical, 50231507), DavePhos (Sigma-Aldrich, 638021-1G), 2-amino-4,6-dihydroxypyrimidine (VWR, AAA1181314), 2-(dimethylamino)pyrimidine-4,6-diol (Matrix Scientific, MFCD01935970), tri-*tert*-butylphosphonium tetrafluoroborate (Combi-Blocks, OR-6368), 4-iodoaniline (Combi-Blocks, AN-3814), 4-chloroaniline (Combi-Blocks, QA9461), 4-bromoaniline (Combi-Blocks, OS-7881), 4-methoxyphenylboronic acid (Combi-Blocks, BB-2155). All precatalysts and the rest of ligands were purchased from Sigma-Aldrich.

4: $Pd(OAc)_2$ (11.2 mg, 0.05 mmol, 1.0 equiv.) and [1,1′-Biphenyl]-3-sulfonic acid, 2′-(dicyclohexylphosphino)-2,6-dimethoxy-sodium salt (sSPhos, 25.6 mg, 0.05 mmol, 1.0 equiv.) were mixed in 1 mL degassed dimethylacetamide (DMA). The mixture was heated to 50 °C for 30 min until the solution become dark brown. The mixture was then used directly as a 50 mM catalyst solution for Suzuki–Miyaura cross-coupling.

$Pd(OAc)_2$/DavePhos: $Pd(OAc)_2$ (11.2 mg, 0.05 mmol, 1.0 equiv.) and 2-dicyclohexylphophino-2'-(*N*,*N*-dimethylamino)biphenyl (DavePhos, 19.7 mg, 0.05 mmol, 1.0 equiv.) were mixed in 1 mL degassed dimethylacetamide (DMA), and the mixture was heated to 50 °C for 30 min until the

solution become dark brown. The mixture can be directly used as a 50 mM catalyst solution for Suzuki–Miyaura cross-coupling.

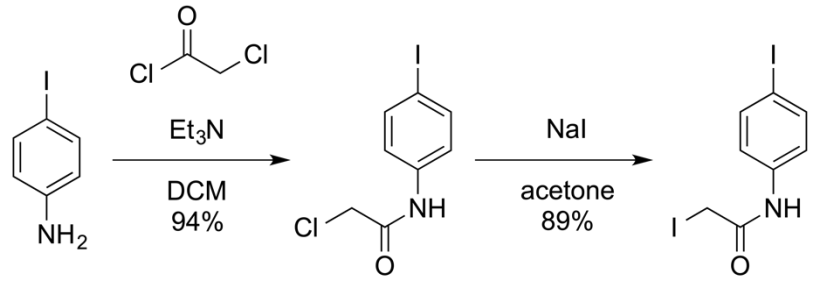
ADHP and DMADPH: The preparation for ADHP and DMADHP were adopted from previous literature.[7] For ADHP, 2-amino-4,6-dihydroxypyrimidine (13 mg, 0.1 mmol, 1.0 equiv.) was dissolved in NaOH (0.1 M, 2 mL) at 65 °C. $Pd(OAc)_2$ (11.2 mg, 0.05 mmol, 0.5 equiv.) was added and allowed to stir at 65 °C for 30 min. The solution was allowed to cool to ambient temperature and diluted with 5.0 mL of distilled water to give a final catalyst concentration of 0.01 M. For DMADPH, 2-(dimethylamino)pyrimidine-4,6-diol (15.5 mg, 0.1 mmol, 1 equiv.) was dissolved in NaOH (0.1 M, 2 mL) at 65 °C. $Pd(OAc)_2$ (11.2 mg, 0.05 mmol, 0.5 equiv.) was added and allowed to stir at 65 °C for 30 min. The resulting orange solution was allowed to cool to ambient temperature and diluted with 5.0 mL of distilled water to give a final catalyst concentration of 0.01 M.

Precatalyst preparation: Precatalyst were either used directly from stocks (50 mM) or premixed with 10 equiv. K_2CO_3 for 10 min at 70 °C before adding to reaction samples as indicated above in figure legends.

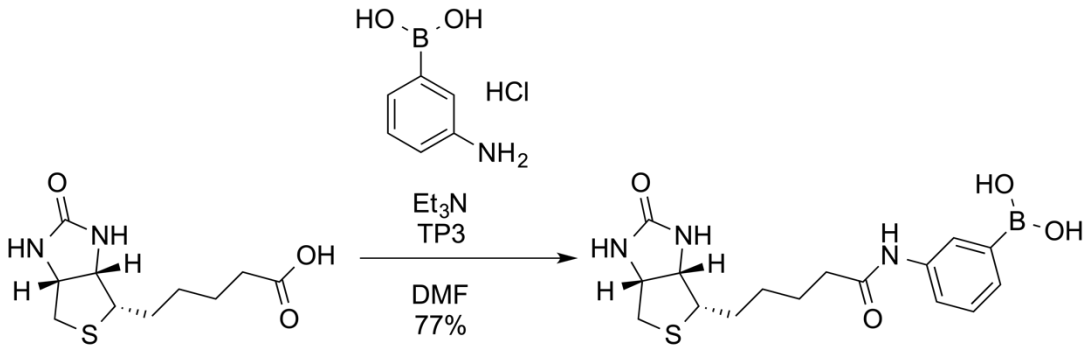
Reaction screening. Serial dilutions were performed on 4'-methoxy-[1,1'-biphenyl]-4-amine to achieve concentrations of 0.05 mM, 0.1 mM, 0.2 mM, 0.5 mM, 0.8 mM, 1.0 mM, and 1.5 mM. Areas under the peaks were taken from the 254 nm wavelength of the LC-MS trace to generate the calibration curve.

The reaction screening was conducted by making separate stock solutions of PBS, 1 mM glutathione (GSH) in PBS, 1 mg/mL bovine serum albumin (BSA) in PBS, and 1 mg/mL HEK293T cell lysate in PBS. sSPhos, DavePhos, tri-*tert*-butylphosphonium tetrafluoroborate ($(P(t\text{-}Bu)_3)$), 2-amino-4,6-dihydroxypyrimidine (ADHP) and 2-(dimethylamino)pyrimidine-4,6-diol (DMADHP) were used as ligands to generate palladium catalysts. To each stock solution was added 1 mM of 4-iodoaniline (5.0 µL of 100 mM stock solution in DMSO) and 2 mM of 4-methoxyphenylboronic acid (10.0 µL of 100 mM stock solution in DMSO). Catalysts were then added to give a final concentration of 0.5 mM and a final solution volume of 500 µL. All twenty reactions were allowed to react at 37 °C for 1 h. The reaction mixtures were then extracted with 1.0 mL ethyl acetate. 500 µL of the solution was then added to 500 µL of MeOH for LC-MS. The extent of each of the reactions was determined by taking the area under the curve of the 254 nm wavelength UV trace. Concentrations were calculated by referencing the standard.

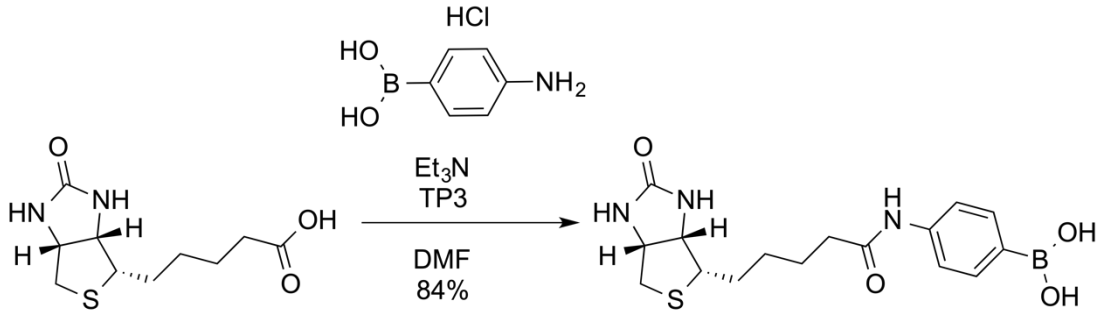
(E) Synthesis of Probes.



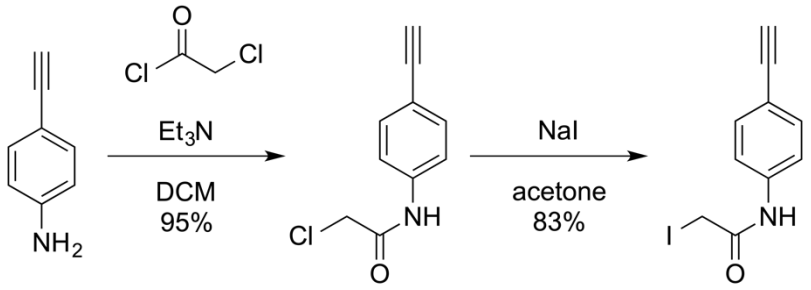
***N*-(4-iodophenyl)-2-iodoacetamide** (**1**). The procedure was adopted from previous literature with modifications.[8] 4-Iodoaniline (150.0 mg, 0.68 mmol, 1.0 equiv.) was dissolved in 3 mL THF, followed by the addition of TEA (95 µL, 0.68 mmol, 1.0 equiv.). Chloroacetyl chloride (109 µL, 1.37 mmol, 2 equiv.) was then added dropwise into the reaction mixture at 0 °C. The reaction was then stirred for 2h at RT. The reaction was quenched with 20 mL saturated NH_4Cl (aq), extracted with EtOAc (2 × 30 mL), and evaporated under reduced pressure to afford , 2-chloro-*N*-(4-iodophenyl)acetamide as a pale yellow oil (189.0 mg, 94%), which was used without further purification. 2-chloro-*N*-(4-iodophenyl)acetamide (150 mg, 0.51 mmol, 1 equiv.) and NaI (120 mg, 0.80 mmol, 1.57 equiv.) were dissolved in 10 mL acetone. After stirring for 3h at 65 °C, the mixture was concentrated under reduced pressure. Purification by silica column chromatography (20% – 33% EtOAc/hexanes) yielded the title compound as a white solid (175.6 mg, 84% overall over two steps). ^{1}H NMR (500 MHz, CD_3OD) δ 7.65 (d, 2 H, J = 8.9 Hz), 7.36 (d, 2H, J = 8.9 Hz), 3.83 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD) δ 168.1, 138.3, 137.6, 121.4, 86.8, −2.6; HRMS-ESI (m/z) calculated for $C_8H_6I_2NO$ $[M-H]^-$: 385.8539; found 385.8550.



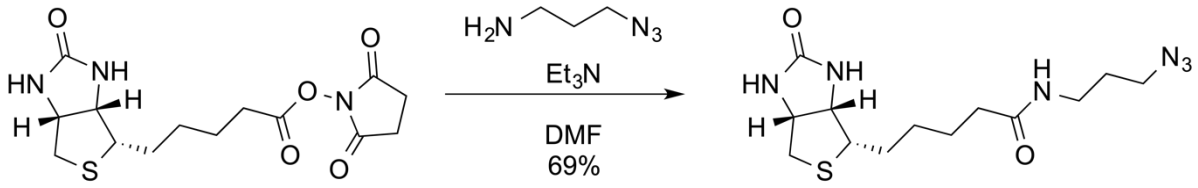
(3-(5-((4*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)phenyl)boronic acid[9] (**2**). D-Biotin (324.4 mg, 1.33 mmol, 1.0 equiv.) and 3-aminophenylboronic acid (200.0 mg, 1.46 mmol, 1.1 equiv.) were dissolved in 10 mL THF, followed by the addition of TEA (0.556 mL) and propylphosphonic anhydride (TP3, 1.58 mL, 0.6 mL, 50 % w/w soln. in acetonitrile). The reaction was stirred for 22h at RT. The reaction mixture was concentrated and purified by silica column chromatography (5 : 1: 0.1 MeOH : DCM : H_2O) to afford product (371.9 mg, 77%) as a colorless solid. ^{1}H NMR (500 MHz, CD3OD): δ 7.78 (s, 1H), 7.61 (dd, J = 8.6 Hz, 1.6, 1H), 7.28 (m, 2H), 4.46 (m, 1H), 4.27 (m, 1H), 3.18 (m, 1H), 2.89 (dd, J = 12.8 Hz, 4.9 Hz, 1H), 2.67 (d, J = 12.7 Hz, 1H), 2.38 (t, J = 7.3 Hz, 2H), 1.73 (m, 3H), 1.61 (m, 1H), 1.48 (m, 2H); ^{13}C NMR (125 MHz, CD3OD): δ 176.4, 173.1, 164.7, 137.7, 128.9, 127.7, 124.9, 121.3, 61.9, 60.2, 55.6, 39.7, 36.3, 28.4, 28.1, 25.4. HRMS-ESI (m/z) calculated for $C_{16}H_{22}BN_3O_4NaS$ $[M+Na]^+$: 386.1325; found: 386.1304.



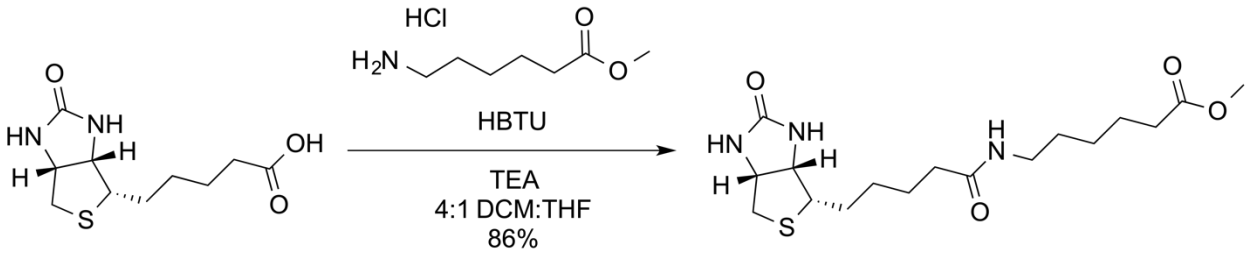
(4-(5-((4*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)phenyl)boronic acid (**3**). D-Biotin (150.0 mg, 0.61 mmol, 1.0 equiv.) and 4-aminophenylboronic acid hydrochloride (127.4 mg, 0.73 mmol, 1.2 equiv.) were dissolved in 3 mL DMF, followed by the addition of TEA (0.85 mL) and propylphosphonic anhydride (TP3, 0.6 mL, 50+% w/w soln. in acetonitrile). The reaction was stirred for 18h at RT. The reaction mixture was concentrated and purified by silica column chromatography (5% – 20% MeOH/DCM) to afford title compound (187.0 mg, 84%) as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.60 (m, 4H), 4.50 (m, 1H), 4.32 (m, 1H), 3.23 (m, 1H), 2.95 (dd, J = 12.8 Hz, 4.9 Hz, 1H), 2.72 (d, J = 12.7 Hz, 1H), 2.42 (t, J = 7.3 Hz, 2H), 1.77 (m, 3H), 1.65 (m, 1H), 1.49 (m, 2H); ^{13}C NMR (125 MHz, CD3OD): δ 173.2, 164.7, 134.11, 122.0, 118.7, 114.8, 61.9, 60.2, 55.6, 39.7, 36.3, 28.4, 28.1, 25.4, 7.9. HRMS-ESI (m/z) calculated for $C_{16}H_{22}BN_3O_4NaS$ $[M+Na]^+$: 386.1325; found: 386.1318.



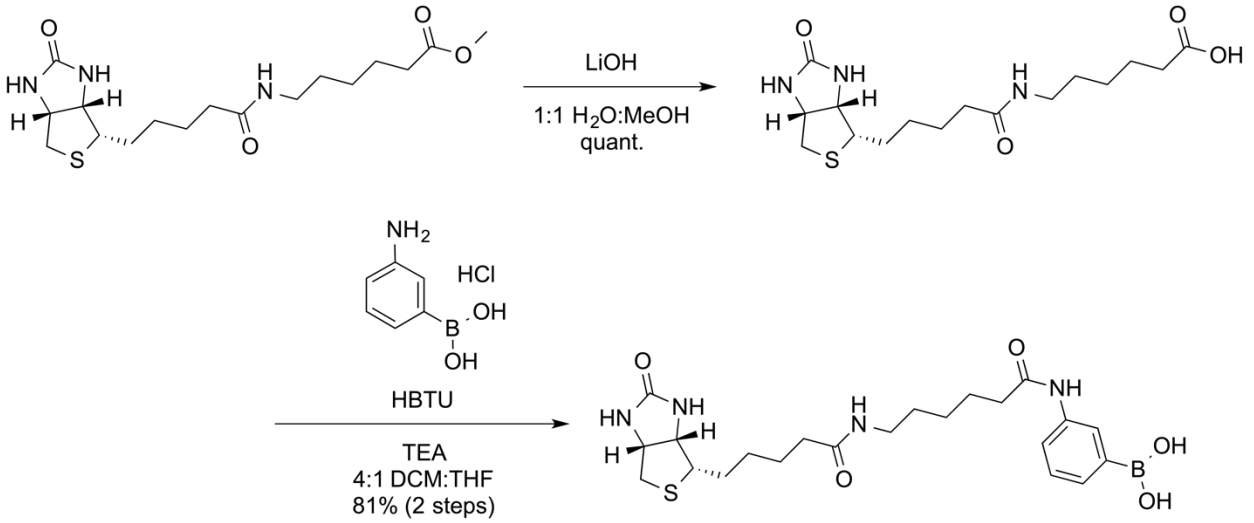
***N*-(4-ethynylphenyl)-2-iodoacetamide (5)**. 4-Ethynylaniline (300.0 mg, 2.56 mmol, 1.0 equiv.) was dissolved in 3 mL THF, followed by the addition of TEA (714 µL, 5.12 mmol, 2.0 equiv.). Then, chloroacetyl chloride (509 µL, 6.40 mmol, 2.5 equiv.) was added dropwise into the reaction mixture at 0 °C. The reaction was stirred for another 2h at RT. The reaction was quenched with 20 mL saturated NH_4Cl (aq), extracted with 2 x 30 mL EtOAc, and evaporated under reduced pressure to afford a pale yellow oil, 2-chloro-*N*-(4-iodophenyl)acetamide with no further purification (470.0 mg, 95%). 2-chloro-*N*-(4-iodophenyl)acetamide (150 mg, 0.78 mmol, 1 equiv.) and NaI (234.0 mg, 1.56 mmol, 2 equiv.) were dissolved in 10 mL acetone. After 3 h stirring at 70 °C, the mixture was concentrated. Purification by silica column chromatography (1:6 – 1:5 EtOAc/hexanes) yielded *N*-(4-ethynylphenyl)-2-iodoacetamide (184.5 mg, 79% overall yield in two steps) as a white solid. ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ 7.69 (s, 1H), 7.48 (m, 4H), 3.85 (s, 2H), 3.06 (s, 1H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 165.2, 138.0, 133.6, 119.7, 118.8, 83.5, −0.5. HRMS-ESI (m/z) calculated for $C_{10}H_8INNaO$ $[M+Na]^+$: 307.9548; found: 307.9540.



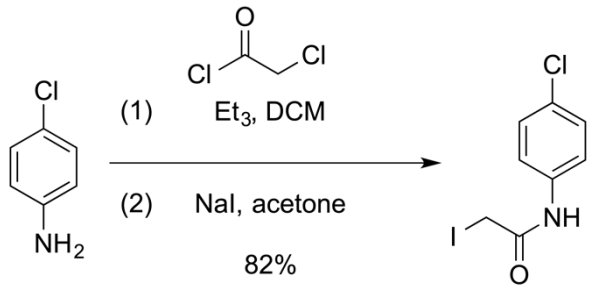
***N*-(3-azidopropyl)-5-((4*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (6)**.[10] (+)-Biotin N-hydroxysuccinimide ester were prepared according to previous reported procedure[11] with 73% yield. (+)-Biotin N-hydroxysuccinimide ester (500 mg, 1.47 mmol, 1.0 equiv.) was suspended in 5 mL of DMF. 3-azidopropan-1-amine (147 mL, 1.54 mmol, 1.05 equiv.) was added, followed by TEA (306 mL, 2.20 mmol, 1.5 equiv.). The reaction was left to stir at room temperature until all solids were fully dissolved, indicating reaction completion. Product was recrystallized as a white solid in excess ether, filtered off, and dried in *vacuo* to afford product as a white powder (330 mg, 69%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.83 (s, 1H), 6.42 (s, 1H), 6.35 (s, 1H), 4.34 – 4.26 (m, 1H), 4.16 – 4.09 (m, 1H), 3.34 (d, J = 6.9 Hz, 3H), 3.08 (q, J = 6.4 Hz, 3H), 2.82 (dd, J = 12.4, 5.1 Hz, 1H), 2.59 (s, 1H), 2.05 (t, J = 7.4 Hz, 2H), 1.68 – 1.21 (m, 9H);^{13}C NMR (126 MHz, DMSO-d_6): δ 172.0, 162.7, 61.0, 59.2, 55.42, 48.4, 39.5, 35.7, 35.2, 28.5, 28.2, 28.0, 25.3; HRMS-ESI (m/z) calculated for $C_{13}H_{22}N_6O_2NaS$ $[M+Na]^+$: 349.1423; found: 349.1429.



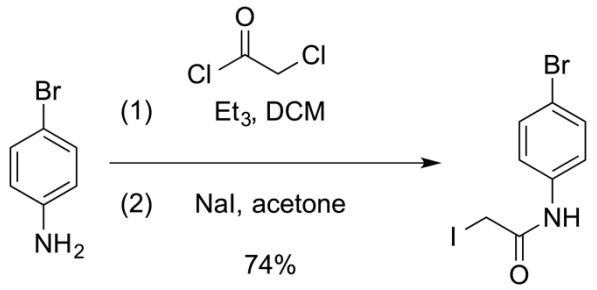
Methyl 6-(5-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)hexanoate[12] **(15)**. D-biotin (300.0 mg, 1.23 mmol, 1.0 equiv.) and methyl 6-aminohexanoate hydrochloride (267.7 mg, 1.47 mmol, 1.2 equiv.) were dissolved in 5 mL 4:1 DCM/THF, followed by the addition of TEA (1.5 mL, 11.07 mmol, 9.0 equiv.) and HBTU (700mg, 1.85 mmol, 1.5 equiv.). The reaction mixture was stirred for 18h at RT. The reaction mixture was concentrated and purified by silica column chromatography (5% – 8% MeOH/DCM) to afford the title compound (305.9 mg, 67%) as a white solid. ^{1}H NMR (500 MHz, (CD_3OD): δ 4.48 (m, 1 H), 4.30 (m, 1 H), 3.19 (m, 3H), 2.92 (dd, J = 12.8 Hz, 4.9 Hz, 1H), 2.70 (d, J = 12.7 Hz, 1H), 2.33 (t, J = 7.4 Hz, 2H), 2.19 (t, J = 7.4 Hz, 2H), 1.61 (m, 3H), 1.46 (m, 3H), 1.31 (m, 7H); ^{13}C NMR (125 MHz, CD3OD): δ 174.6, 174.4, 164.7, 61.97, 60.2, 55.6, 50.6, 46.5, 39.7, 38.9, 35.4, 33.3, 28.7, 28.4, 28.1, 7.9; HRMS-ESI (m/z) calculated for $C_{17}H_{29}N_3O_4NaS$ $[M+Na]^+$: 394.1776; found: 394.1763.



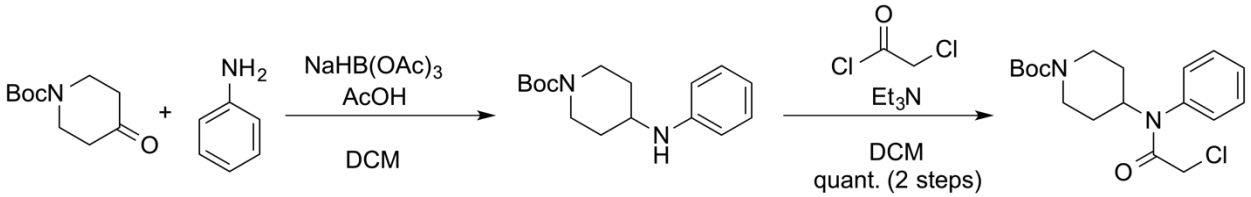
(4-(6-(5-((4*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexanamido)phenyl)boronic acid (7). To a solution of methyl 6-(5-((4S)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)hexanoate (200 mg, 0.54 mmol, 1 equiv.) in 2 mL 1:1 MeOH/H_2O, lithium hydroxide monohydrate (68.0 mg, 1.62 mmol, 3 equiv.) was added. The reaction was stirred for 4h at RT. The formation of the hydrolyzed product was monitored by TLC and LC-MS. The resulting solution was neutralized with 1M HCl and concentrated. Without further purification, the hydrolyzed product (115 mg, 0.32 mmol, 1 equiv.) was re-dissolved in 3 mL DMF, followed by the addition of HBTU (146.4 mg, 0.39 mmol, 1.2 equiv.), 3-aminophenylboronic acid hydrochloride (78 mg, 0.45 mmol, 1.4 equiv.) and TEA (136.0 µL, 1.62 mmol, 3 equiv.). This solution was stirred for 3h and concentrated. Purification by silica gel chromatography (6:1:0.1 DCM/MeOH/H_2O) to afford the title compound as a white solid (123.0 mg, 81%). ^{1}H NMR (500 MHz, (CD_3OD): δ 7.7 (Br, 1H), 7.58 (d, J = 7.95 Hz, 1H), 7.34 (Br, 1H), 7.26 (t, J = 7.65 Hz, 1H), 4.45 (m, 1 H), 4.26 (m, 1 H), 3.17 (m, 3H), 2.89 (dd, J = 12.8 Hz, 4.9 Hz, 1H), 2.67 (d, J = 12.7 Hz, 1H), 2.37 (t, J = 7.4 Hz, 2H), 2.17 (t, J = 7.4 Hz, 2H), 1.62 (m, 8H), 1.40 (m, 4H); ^{13}C NMR (125 MHz, CD3OD): δ = 174.6, 173.1, 164.7, 137.6, 129.1, 127.5, 125.0, 115.0, 62.0, 60.2, 55.6, 39.6, 38.8, 36.4, 35.4, 28.8, 28.4, 28.1, 26.2, 25.5, 25.2; HRMS-ESI (m/z) calculated for $C_{22}H_{33}BN_4O_5NaS$ $[M+Na]^+$: 499.2167; found: 499.2202.



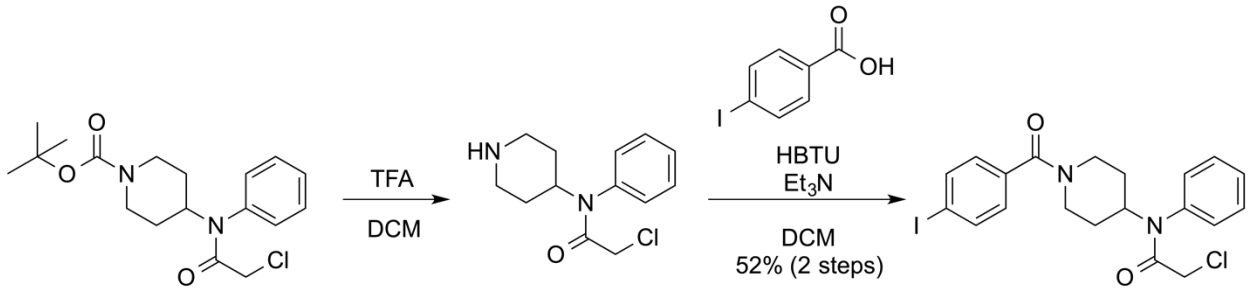
***N*-(4-chlorophenyl)-2-iodoacetamide**[8] **(8).** The same procedure was followed as for the synthesis of *N*-(4-iodophenyl)-2-iodoacetamide (1) to afford the title compound (208 mg, 82% overall yield in two steps). ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ 10 (s, 1H), 8.98 (m, 4H), 2.86 (s, 2H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 167.3, 154.4, 150.0, 149.9, 133.6, 1.9; HRMS-ESI (m/z) calculated for C_8H_6ClINO $[M-H]^-$: 293.9183; found: 293.9181.



***N*-(4-bromophenyl)-2-iodoacetamide (9).** The same procedure was followed as for the synthesis of *N*-(4-iodophenyl)-2-iodoacetamide (1) to afford the title compound (128 mg, 74% overall yield in two steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.44 (m, 4H), 3.85 (s, 2H)**;** HRMS-ESI (m/z) calculated for C_8H_6BrINO $[M-H]^-$: 337.8677; found: 337.8674.

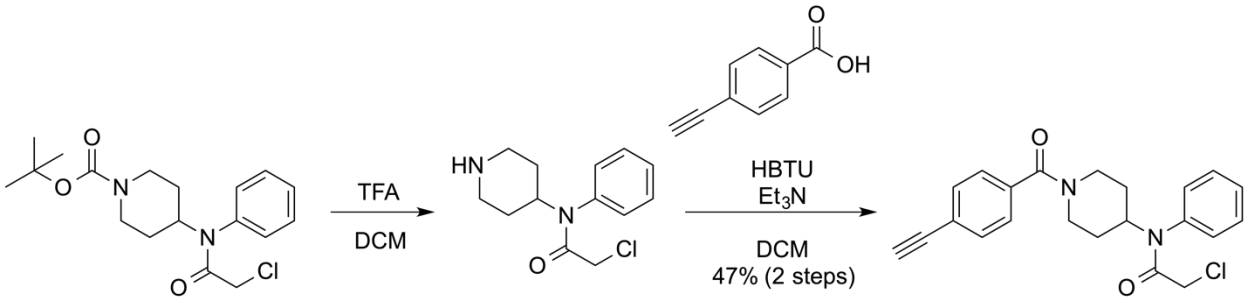


***tert*-butyl 4-(2-chloro-*N*-phenylacetamido)piperidine-1-carboxylate (16)**.[4] The title compound was prepared according to Backus *et al*, Nature. 2016, 534, 570-574.[4] in two steps with quantitative yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 – 7.35 (m, 3H), 7.20 – 7.01 (m, 2H), 4.77 – 4.61 (m, 1H), 4.05 (s, 2H), 3.69 (s, 2H), 2.74 (s, 2H), 1.85 – 1.67 (m, 2H), 1.35 (s, 9H), 1.27 – 1.12 (m, 2H); HRMS-ESI (m/z) calculated for $C_8H_7BrINNaO$ $[M+Na]^+$: 375.1451; found: 375.1446.

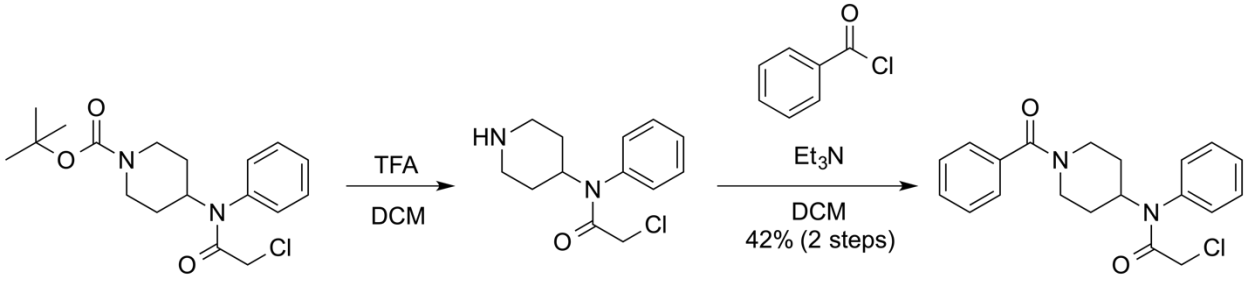


2-chloro-*N*-(1-(4-iodobenzoyl)piperidin-4-yl)-*N*-phenylacetamide (10). To a solution of *tert*-butyl 4-(2-chloro-*N*-phenylacetamido)piperidine-1-carboxylate (240 mg, 0.68 mmol, 1 equiv) in 5 mL DCM was added trifluoroacetic acid (2 mL). The reaction was allowed to react for 2h at RT. The formation of product was monitored by LC-MS and TLC (1:1 Hexane/EtOAc) showing starting material is gone and forming a new baseline spot. After complete consumption of starting material was observed the resulting solution was concentrated under a stream of nitrogen until no further evaporation was observed, affording the deprotected amine as its trifluoroacetate salt. This viscous gum was then treated with triethylamine in ethyl acetate (30% v/v, 5 mL; solution smokes upon addition) and concentrated. Without further purification, the deprotected amine was dissolved in 18 mL DCM, followed by addition of TEA (800 µL), 4-iodobenzoic acid (248 mg, 1.0 mmol, 1.5 equiv.) and HBTU (760 mg, 2.00 mmol, 3.0 equiv.). This solution was stirred overnight, quenched with saturated aqueous sodium bicarbonate, and extracted with Et_2O (3 × 10

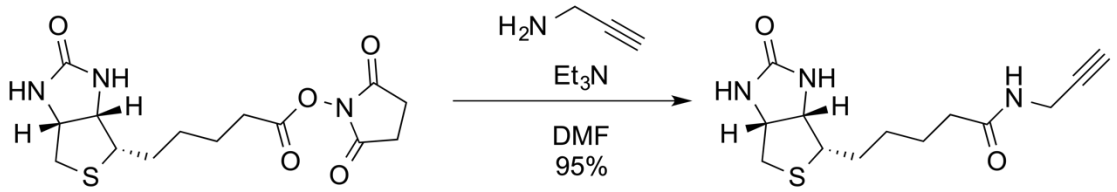
mL). The resulting solution was dried over magnesium sulfate, filtered, and concentrated. The resulting oil was purified by silica gel chromatography (20 – 50% EtOAc/hexanes) to afford the product as a pale yellow solid (170 mg, 52%). ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ 7.69 (d, J = 8.25 Hz, 2H), 7.46 (m, 3H), 7.12 (Br, 1H), 7.02 (d, J = 8.25 Hz, 2H), 4.82 (tt, J = 12.15 Hz, 3.85 Hz, 2H), 3.69 (s, 2H), 2.20 (m, 2H), 1.86 (br, 2H), 1.37 (m, 1H), 1.24 (m, 1H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 169.3, 166.0, 165.7, 137.6, 136.9, 135.0, 130.0, 129.6, 128.6, 95.9, 60.4, 53.2, 42.4, 38.6. HRMS-ESI (m/z) calculated for $C_{20}H_{20}ClIN_2NaO_2$ $[M+Na]^+$: 505.0156; found: 505.0161.



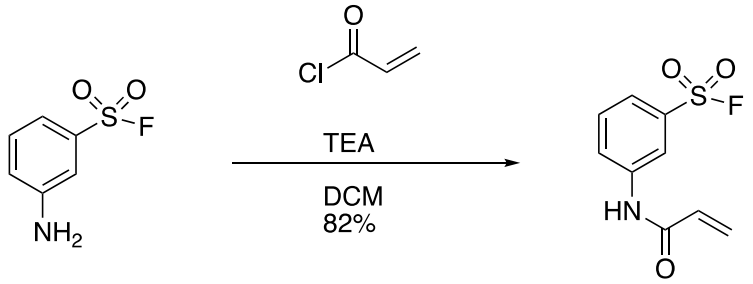
2-chloro-*N*-(1-(4-ethynylbenzoyl)piperidin-4-yl)-*N*-phenylacetamide (**11**). Deprotected amine in DCM (6 mL) was obtained according to the same procedure detailed above with tert-butyl 4-(2-chloro-N-phenylacetamido)piperidine-1-carboxylate (230 mg, 0.68 mmol, 1 equiv.) followed by the addition of TEA (661 µL), HBTU (455 mg, 1.2 mmol, 1.8 equiv.) and 4-ethynyl benzoic acid (146 mg, 0.95 mmol, 1.4 equiv.). This solution was stirred overnight, quenched with saturated aqueous sodium bicarbonate, and extracted with Et_2O (3 × 10 mL). The resulting solution was dried over magnesium sulfate, filtered, and concentrated. The resulting oil was purified by silica gel chromatography (20 – 50% EtOAc/hexanes) to afford the title compound as a yellow oil (122 mg, 47%). ^{1}H NMR (500 MHz, $(CD_3)_2CO$): δ 7.46 (m, 5H), 7.24 (d, J = 8.46, 2H), 7.12 (Br, 1H), 4.82 (tt, J = 12.15 Hz, 3.85 Hz, 2H), 3.69 (s, 2H), 3.09 (s, 1H),2.09 (m, 2H), 1.86 (br, 2H), 1.32 (Br, 1H), 1.24 (m, 2H); ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 169.4, 166.0, 165.6, 137.0, 135.8, 132.2, 130.0, 129.6, 126.9, 123.7, 82.7, 78.7, 60.4, 53.3, 42.4, 38.6; HRMS-ESI (m/z) calculated for $C_{22}H_{21}ClN_2NaO_2$ $[M+Na]^+$: 403.1189; found: 403.1169.



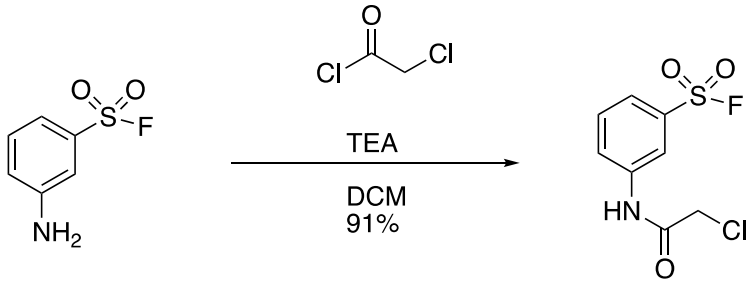
***N*-(1-benzoylpiperidin-4-yl)-2-chloro-*N*-phenylacetamide (KB7)**. KB7 was obtained following the literature procedure[4] to afford KB7 (85 mg, 42%).



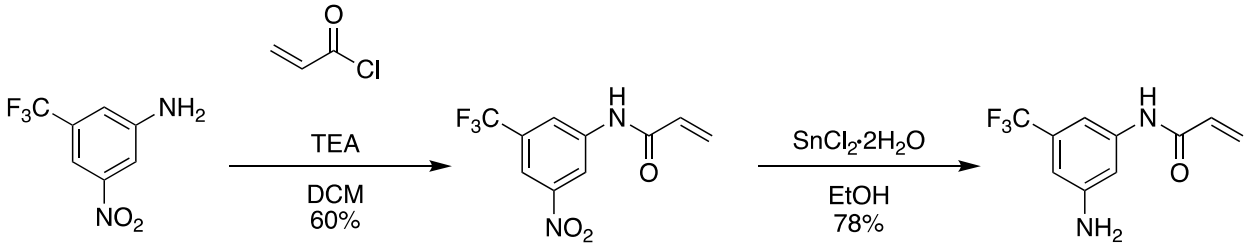
5-((4*S*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-*N*-(prop-2-yn-1-yl)pentanamide[11] (13). (+)-Biotin N-hydroxysuccinimide ester[11] (500 mg, 1.47 mmol, 1.0 equiv.) was dissolved in 5 mL of DMF in a 25 mL round bottom flask. TEA (306 mL, 2.20 mmol, 1.5 equiv.) and propargylamine (103 mL, 1.61 mmol, 1.1 equiv.) were added and the reaction was left to stir for 6h, at which point the white suspension turned into a faint yellow gel. Excess diethyl ether was added to the gel to precipitate out the product as white crystals. The white crystals were filtered off and collected in a scintillation vial. 1 mL of 1:1 H_2O/acetonitrile was added to the crystals and the suspension was frozen at -80 °C. The crystals were dried via lyophilization, yielding a white powder (393 mg, 95% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.42 (d, *J* = 5.8 Hz, 1H), 6.35 (s, 1H), 4.34 – 4.24 (m, 1H), 4.12 (s, 1H), 3.83 (d, *J* = 5.4 Hz, 1H), 3.08 (s, 2H), 2.82 (dd, *J* = 12.4, 5.0 Hz, 1H), 2.59 (s, 1H), 2.19 (t, *J* = 7.4 Hz, 1H), 2.08 (t, *J* = 7.4 Hz, 1H), 1.68 – 1.19 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 171.8, 162.7, 81.4, 72.8, 61.1, 59.2, 55.4, 39.5, 34.9, 33.6, 28.2, 28.1, 28.1, 28.0, 27.7, 25.2, 25.1, 24.6; HRMS-ESI (m/z) calculated for $C_{13}H_{19}N_3O_2NaS$ $[M+Na]^+$: 304.1096; found: 304.1106

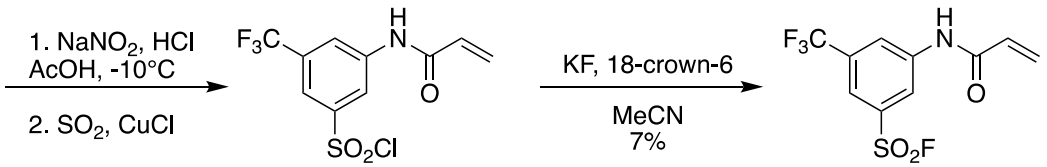


3-acrylamidobenzenesulfonyl fluoride (15). To a solution of 3-aminobenzenesulfonyl fluoride hydrochloride (100 mg, 0.47 mmol, 1 equiv.) in 2 mL DCM was added TEA (198 µL), acryloyl chloride (77 µL) in an ice bath. Next the reaction was allowed to react for 3h at RT. The reaction was quenched with 30 mL saturated $NaHCO_3$ (aq), extracted with DCM (2 × 20 mL), and was concentrated and purified by silica column chromatography (2:1 hexanes/ EtOAc) to afford product (88.9 mg, 82%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.45 (s, 1H), 8.27 (s, 1H), 8.04 (d, J = 8.1 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.55 (t, J = 8.1 Hz, 1H), 6.46 (dd, J = 16.9, 1.05 Hz, 1H), 6.32 (dd, J = 16.9, 10.2 Hz, 1H), 5.83 (dd, J = 10.2, 1.05 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.1, 139.4, 133.6, 133.4, 130.5, 126.4, 123.6, 118.9, 25.5; ^{19}F-NMR (376 MHz, $CDCl_3$) δ 65.7. HRMS-ESI (m/z) calculated for $C_9H_7FNO_3S$ $[M-H]^-$: 228.0131; found: 228.0133.



3-(2-chloroacetamido)benzenesulfonyl fluoride (16). To a solution of 3-aminobenzenesulfonyl fluoride hydrochloride (100 mg, 0.47 mmol, 1 equiv.) in 2 mL DCM was added TEA (198 µL), 2-chloroacetyl chloride (75 µL) in an ice bath. Next the reaction was allowed to react for 3h at RT. The reaction was quenched with 30 mL saturated $NaHCO_3$ (aq), extracted with DCM (2 × 20 mL), and was concentrated and purified by silica column chromatography (2:1 hexanes/ EtOAc) to afford product (108.1 mg, 91%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.23 (t, J = 2.0 Hz 1H), 8.02 (d, J = 8.3 Hz 1H), 7.81 (d, J = 8.0 Hz 1H), 7.63 (t, J = 8.0 Hz, 1H), 4.24 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.2, 138.1, 134.1, 133.9, 130.6, 126.5, 124.6, 119.4, 42.7; ^{19}F-NMR (376 MHz, $CDCl_3$) δ 65.7. HRMS-ESI (m/z) calculated for $C_8H_6ClFNO_3S$ $[M-H]^-$: 249.9741; found: 249.9757.

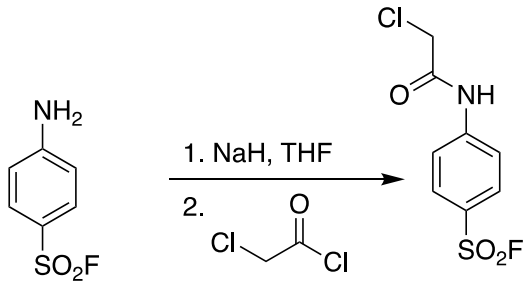




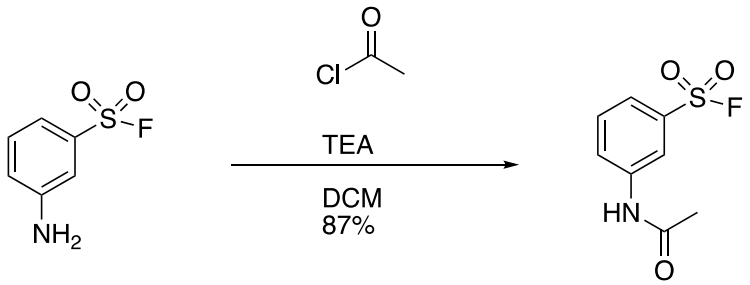
***N*-(3-nitro-5-(trifluoromethyl)phenyl)acrylamide**. To an oven-dried 25 mL round-bottom flask equipped with stir bar was added 3-nitro-5-(trifluoromethyl)aniline (0.5 g, 2.42 mmol). The flask was then capped with rubber septa and purged with nitrogen. To this was added methylene chloride (10 mL) followed by triethylamine (0.67 mL, 4.85 mmol). The solution was cooled to 0°C and acryloyl chloride (0.44mL, 4.85 mmol) was added dropwise over a 5 minute period. Upon complete addition the ice bath was removed and the reaction was allowed to stir at ambient temperature. Once complete conversion was achieved as determined by TLC the reaction mixture was diluted with saturated sodium bicarbonate solution, extracted with DCM (3x) and the combined organic layers washed with brine and dried over Na_2SO_4. After flash column chromatography (EtOAc:Hex 1:99 to 1:3) the desired compound was obtained as a yellow solid (0.42 g, 60%). ^{1}H-NMR (400MHz, $CDCl_3$) δ 8.66 (t, 1H), 8.34 (t, 1H) 8.23 (t, 1H), 6.55 (dd, 1H), 6.29 (dd, 1H), 5.92 (dd, 1H). ^{13}C-NMR (100MHz, $CDCl_3$) δ 163.8, 148.7, 139.8, 133.1, 132.8, 130.2, 129.8, 121.8, 117.3, 115.9.

N-(3-amino-5-(trifluoromethyl)phenyl)acrylamide. To an oven-dried 15mL pressure tube equipped with stir bar was added *N*-(3-nitro-5-(trifluoromethyl)phenyl)acrylamide (100mg, 0.38 mmol) and tin (II) chloride dihydrate (269 mg, 1.19 mmol). The tube was purged with nitrogen gas and ethanol (2.74 mL) was added, the tube capped, and allowed to stir at reflux. After 1h the solution was allowed to cool to ambient temperature, diluted with saturated sodium bicarbonate solution, and extracted with ethyl acetate (3x). The combined organic layers washed with brine and dried over Na_2SO_4. After flash column chromatography (EtOAc:Hex 1:3 to 1:1) the desired compound was obtained as a yellow oil (69mg, 78%). ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.37 (s, 1H), 6.97 (s, 1H), 6.62 (s, 1H), 6.41 (dd, 1H), 6.26 (dd, 1H), 5.76 (dd, 1H), 3.66 (br, 2H). ^{13}C-NMR (100MHz, $CDCl_3$) δ 163.8, 148.7, 139.8, 133.1, 132.8, 130.2, 129.8, 121.8, 117.3, 115.9

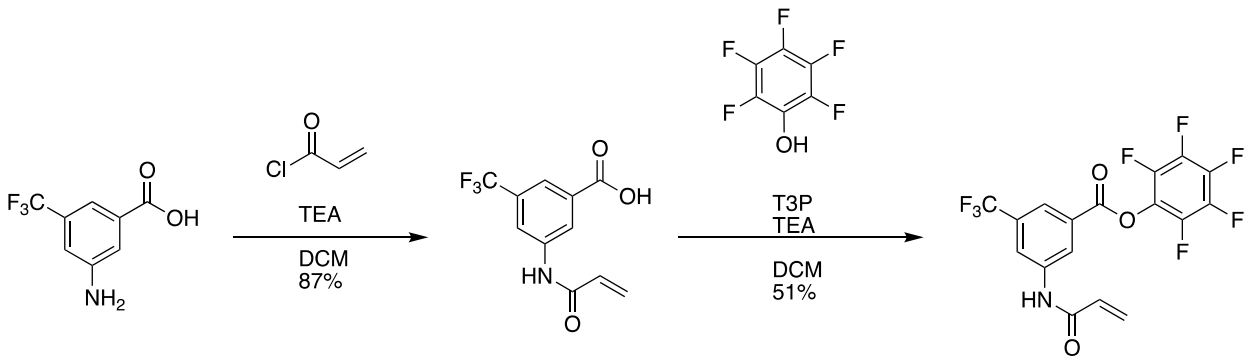
3-acrylamido-5-(trifluoromethyl)benzenesulfonyl fluoride (17). To **N-(3-amino-5-(trifluoromethyl)phenyl)acrylamide** (69 mg, 0.3 mmol) in a 3 mL microwave vial was added a mixture of concentrated hydrochloric acid (0.13 mL) and glacial acetic acid (0.13 mL) with vigorous stirring. Once the white hydrochloride salt precipitated, the beaker was transferred to a salt-ice bath and gradual decrease in the temperature of the reaction mixture was monitored carefully. When the temperature of the reaction mixture had reached -10°C, a solution of sodium nitrite (23 mg, 0.33 mmol) in 0.4 mL of water was added in such a rate that the temperature of the reaction mixture did not exceed 5°C. After complete addition of the sodium nitrite solution, the mixture was stirred for additional 45 mins at -10°C. Separately at the same time, in a 3 mL microwave vial equipped with magnetic stirrer, was added glacial acetic acid (0.3 mL).Sulfur dioxide was bubbled through the glacial acetic acid. Cuprous chloride was added to this solution. The sulfur dioxide was bubbled until the color of the suspension changed from yellow–green to blue-green. The whole process took approximately 30 min for completion. The reaction mixture was then placed in an ice bath and the temperature was monitored carefully, when the S9 temperature reached 10°C, the diazotization reaction mixture was added in portions over 30-min duration to the sulfur dioxide solution gradually and carefully. The excess foaming of the reaction mixture was prevented by addition of a drop of diethyl ether. After the complete addition of the diazotization mixture to the round bottom flask, the contents of the round bottom flask was poured into ice water (1:1, 20 mL), and stirred magnetically until the melting of the ice. The crude product was extracted with 20-mL portions of ether until the ether washings was colorless. The combined organic layer was neutralized with saturated aqueous sodium bicarbonate until neutral and washed once with brine followed by drying with sodium sulfate. The resulting sulfonyl chloride was used without further purification. To a 3mL microwave vial was added sulfonyl chloride (55 mg), KF (14 mg, 0.23 mmol), and 18-crown-6 (4 mg, 0.009 mmol). The vial was capped with a rubber septa, purged with nitrogen, and acetonitrile was subsequently added. After 18h the reaction mixture was diluted with water and extracted with Et_2O (3x). The combined organic layers were washed once with brine and dried over Na_2SO_4. The desired sulfonyl fluoride was afforded after flash column chromatography (EtOAc:Hex 1:9 to 1:3) as a white solid (6 mg, 7% over 2 steps). ^{1}H-NMR (500MHz, $CDCl_3$) δ 8.45 (t, 1H), 8.39 (t, 1H), 7.98 (t, 1H), 6.54 (dd, 1H), 6.28 (dd, 1H), 5.93 (dd, 1H); ^{13}C-NMR (125MHz, $CDCl_3$) δ 163.8, 140.1, 134.9, 134.7, 133.3, 130.4, 129.7, 122.8, 121.8, 120.3; ^{19}F-NMR (376 MHz, $CDCl_3$) δ -63.1 (3H) , 65.9 (1H). HRMS-ESI (m/z) calculated for $C_{10}H_6F_4NO_3S$ $[M-H]^-$: 296.0005; found: 296.0018.



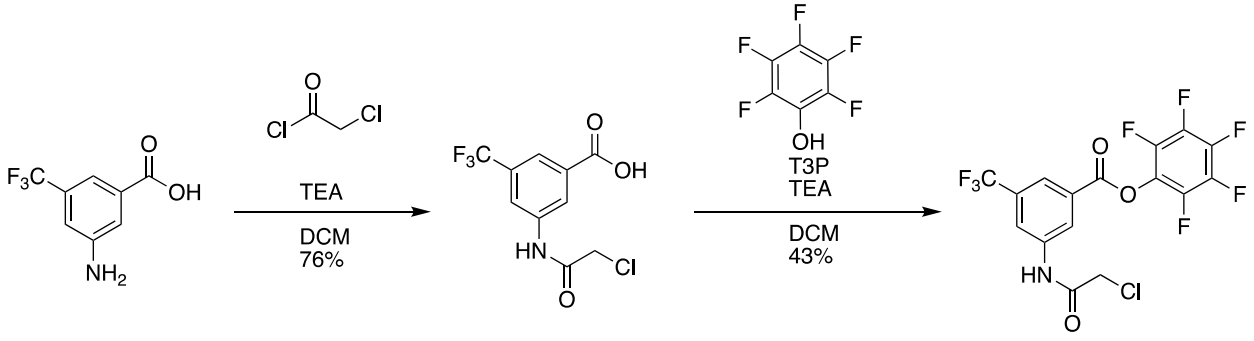
4-(2-chloroacetamido)benzenesulfonyl fluoride (18). To an oven-dried 3mL microwave vial equipped with stir bar was added sodium hydride (60% in mineral oil) (14mg, 0.34mmol). The flask was then capped with rubber septa and purged with nitrogen. To this was added 4-aminobenzenesulfonyl fluoride (50 mg, 0.28 mmol) in THF (1.12mL) and allowed to stir for 30 minutes. The solution was then cooled to 0°C Then chloroacetyl chloride (34uL, 0.43mmol) was added dropwise over a five minute period. Upon complete the reaction was allowed to warm to ambient temperature and stir until complete conversion was achieved as determined by TLC. The reaction mixture was then quenched with saturated sodium bicarbonate solution, extracted with DCM (3x) and the combined organic layers washed with brine and dried over Na_2SO_4 to furnish the desired product as an off-white solid (54mg, 77%). **^{1}H-NMR** (500MHz, $CDCl_3$) δ 8.57 (br, 1H), 7.99 (d, 2H), 7.85 (d, 2H), 4.23 (s, 2H); **^{13}C-NMR** (125MHz, $CDCl_3$) δ 164.50, 143.30, 130.28, 128.69 (d), 120.03, 42.97; **^{19}F-NMR** (XXX MHz, $CDCl_3$) δ 66.7 (1H); HRMS (ESI-MS) $[M-H]^-$ Calculated = 249.9741; Found = 249.9741



3-acetamidobenzenesulfonyl fluoride (19). To a solution of 3-aminobenzenesulfonyl fluoride hydrochloride (100 mg, 0.47 mmol, 1 equiv.) in 2 mL DCM was added TEA (198 µL), acetyl chloride (67.3 µL) in an ice bath. Next the reaction was allowed to react for 3h at RT. The reaction was quenched with 30 mL saturated $NaHCO_3$ (aq), extracted with DCM (2 × 20 mL), and was concentrated and purified by silica column chromatography (2:1 hexanes/ EtOAc) to afford product (89.3 mg, 87%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.16 (s, 1H), 8.00 (m, 1H), 7.70 (t, J = 8.1 Hz, 1H), 2.2 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.4, 139.3, 133.6, 133.4, 130.5, 130.3, 129.5, 126.8, 123.8, 119.4; ^{19}F-NMR (376 MHz, $CDCl_3$) δ 65.8.. HRMS-ESI (m/z) calculated for $C_8H_7FNO_3S$ $[M-H]^-$: 216.0131; found: 216.0149.

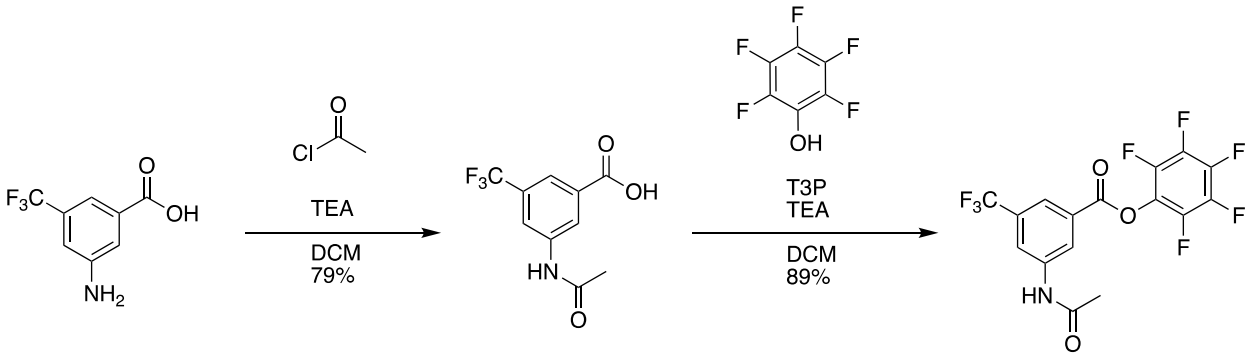


Perfluorophenyl 3-acrylamido-5-(trifluoromethyl)benzoate (20). To a solution of 3-aminobenzenesulfonyl fluoride hydrochloride (300 mg, 1.46 mmol, 1 equiv.) in 6 mL DCM was added TEA (0.73 mmol, 612 µL, 3 equiv.), acryloyl chloride (2.92 mmol, 236 µL, 2 equiv.) in an ice bath. Next the reaction was allowed to react for 3h at RT. The reaction was quenched with 30 mL saturated $NaHCO_3$ (aq), extracted with DCM (2 × 20 mL), and was concentrated to afford product as a brown solid without further purification. Partial of the brown solid (149 mg, 0.58 mmol, 1 equiv.) was redissolved in 4 ml DCM. 2,3,4,5,6-pentafluorophenol (106 mg, 0.58 mmol, 1 equiv.) was added into the solution, followed by the addition of TEA (0.24 mL) and propylphosphonic anhydride (TP3, 0.4 mL, 50+% w/w soln. in acetonitrile). The reaction was stirred for 40 min at RT. The reaction mixture was concentrated and purified by silica column chromatography (2:1 hexanes/ EtOAc) to afford title compound (125.8 mg, 51%) as a pale oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.53 (s, 1H), 8.37 (s, 1H)， 8.18 (s, 1H), 7.75 (s, 1H), 6.52 (dd, J = 16.9, 1.05 Hz, 1H), 6.28 (dd, J = 16.9, 10.2 Hz, 1H), 5.88 (dd, J = 10.2, 1.05 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.0, 161.3, 139.48, 133.0, 130.28, 129.9, 128.9, 124.6, 124.3, 124.1, 123.1, 122.9, 122.4, 122.2, 122.1; ^{19}F-NMR (376 MHz, $CDCl_3$) δ 63.0 . HRMS-ESI (m/z) calculated for $C_{17}H_6F_8NO_3$ $[M-H]^-$: 412.022; found: 412.0201.



Perfluorophenyl 3-(2-chloroacetamido)-5-(trifluoromethyl)benzoate (21). To a solution of 3-aminobenzenesulfonyl fluoride hydrochloride (300 mg, 1.46 mmol, 1 equiv.) in 6 mL DCM was added TEA (0.73 mmol, 612 µL, 3 equiv.), 2-chloroacetyl chloride (2.92 mmol, 232 µL, 2 equiv.) in an ice bath. Next the reaction was allowed to react for 3h at RT. The reaction was quenched with 30 mL saturated $NaHCO_3$ (aq), extracted with DCM (2 × 20 mL), and was concentrated to afford 3-(2-chloroacetamido)-5-(trifluoromethyl)benzoic acid which was used without further purification. Partial of the product (50 mg, 0.18 mmol, 1 equiv.) was redissolved in 1 ml DCM. 2,3,4,5,6-pentafluorophenol (33.2 mg, 0.18 mmol, 1 equiv.) was added into the solution, followed by the addition of TEA (60 µL) and propylphosphonic anhydride (TP3, 0.15 mL, 50+% w/w soln. in acetonitrile). The reaction was stirred for 40 min at RT. The reaction mixture was concentrated

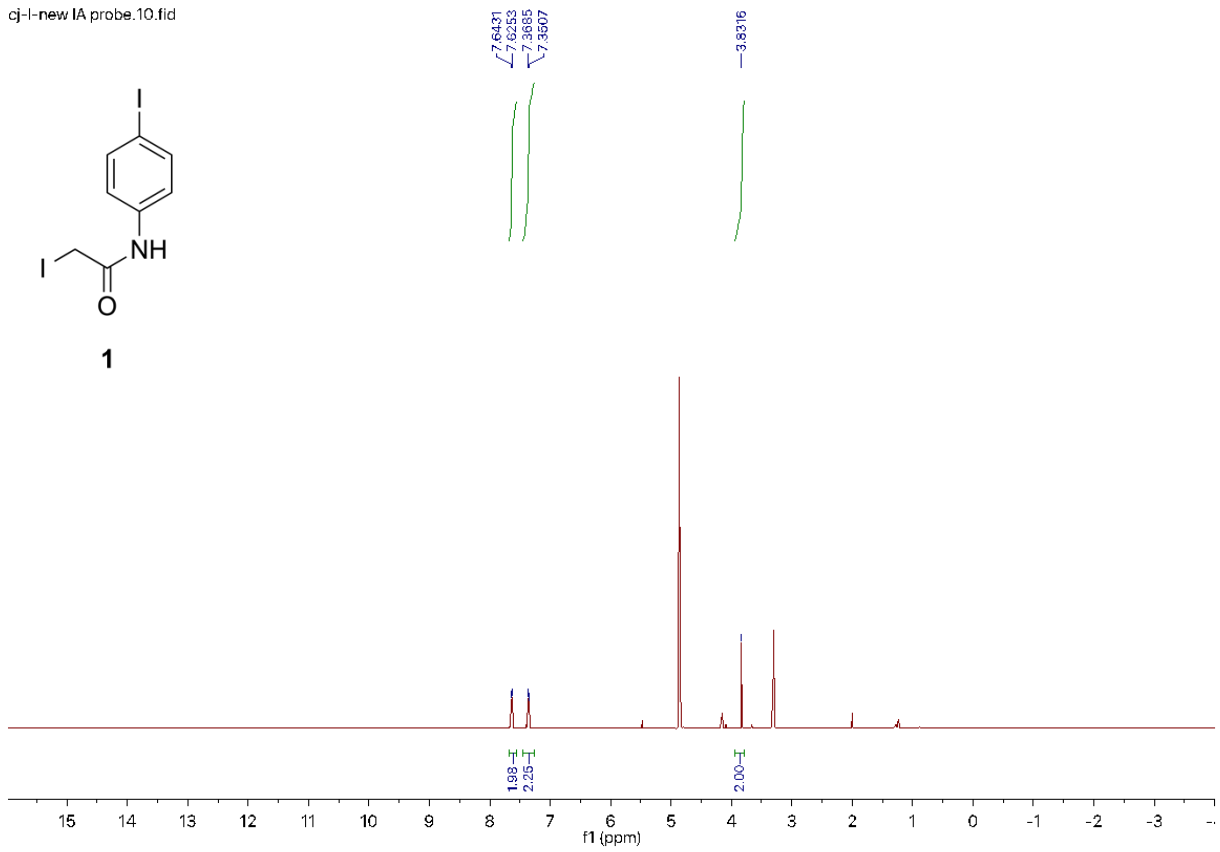
and purified by preparative thin layer chromatography (PTLC) (2:1 hexanes/ EtOAc) to afford title compound (34.7 mg, 43%) as brown oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 (s, 1H), 8.50 (s, 1H), 8.32 (s, 1H), 8.24 (s, 1H), 4.25 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.4, 161.0, 138.3, 132.9, 132.7, 128.9, 124.6, 124.1, 123.7, 123.6, 122.4, 122.3, 121.9, 42.7; ^{19}F-NMR (376 MHz, $CDCl_3$) δ 62.9. HRMS-ESI (m/z) calculated for $C_{16}H_6F_8NO_3$ $[M-H]^-$: 412.022; found: 412.022.



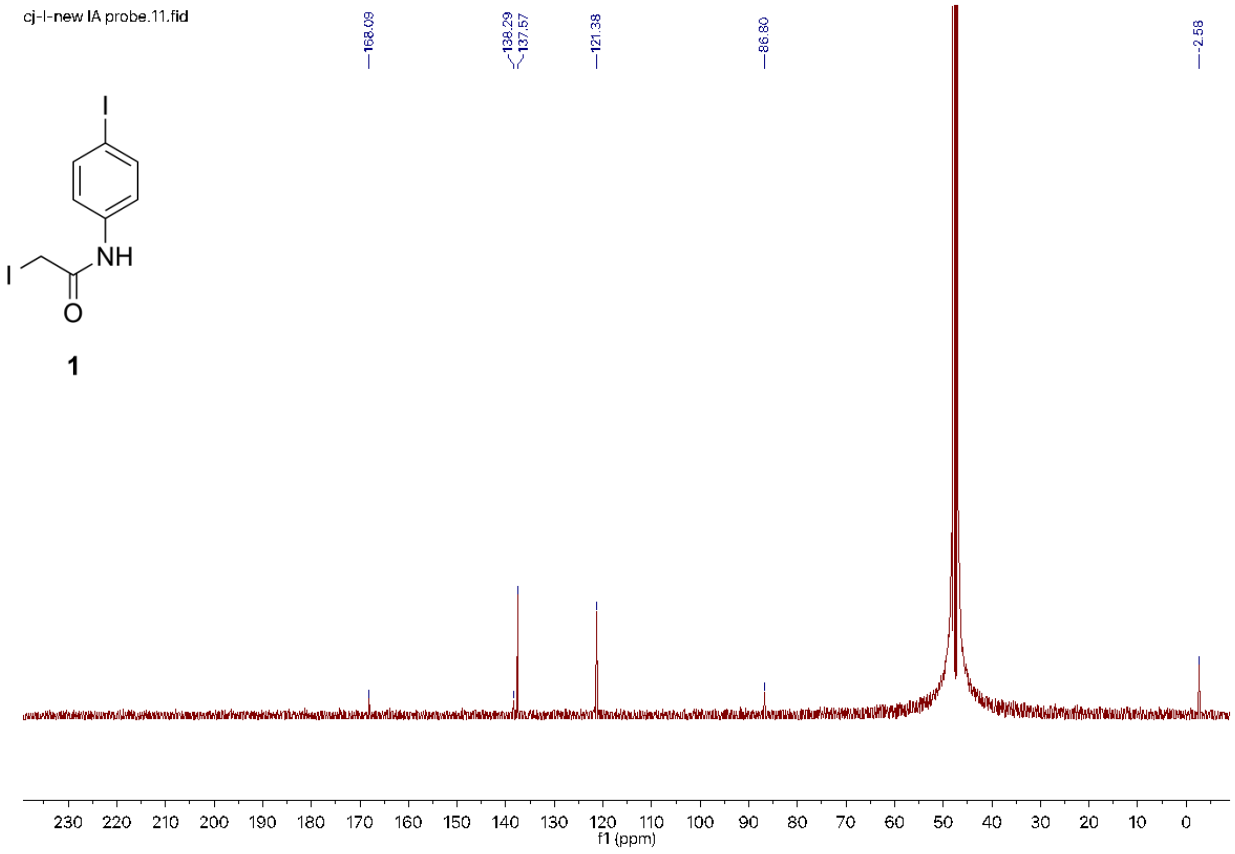
Perfluorophenyl 3-acetamido-5-(trifluoromethyl)benzoate (22). To a solution of 3-aminobenzenesulfonyl fluoride hydrochloride (300 mg, 1.46 mmol, 1 equiv.) in 6 mL DCM was added TEA (0.73 mmol, 612 µL, 3 equiv.), acetyl chloride (2.92 mmol, 208 µL, 2 equiv.) in an ice bath. Next the reaction was allowed to react for 3h at RT. The reaction was quenched with 30 mL saturated $NaHCO_3$ (aq), extracted with DCM (2 × 20 mL), and was concentrated to afford 3-acetamido-5-(trifluoromethyl)benzoic acid which was used without further purification. Partial of the product (40 mg, 0.16 mmol, 1 equiv.) was redissolved in 1 ml DCM. 2,3,4,5,6-pentafluorophenol (29.7 mg, 0.16 mmol, 1 equiv.) was added into the solution, followed by the addition of TEA (67 µL) and propylphosphonic anhydride (TP3, 0.15 mL, 50+% w/w soln. in acetonitrile). The reaction was stirred for 40 min at RT. The reaction mixture was concentrated and purified by preparative thin layer chromatography (PTLC) (2:1 hexanes/ EtOAc) to afford title compound (58.9 mg, 89%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.43 (s, 1H), 8.28 (s, 1H), 8.16 (s, 1H), 7.68 (s, 1H), 2.25 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.7, 161.2, 139.4, 132.7, 132.4, 128.6, 124.2, 122.8, 122.7, 122.1, 122.0, 121.9, 24.6; ^{19}F-NMR (376 MHz, $CDCl_3$) δ 63.0. HRMS-ESI (m/z) calculated for $C_{16}H_5ClF_8NO_3$ $[M-H]^-$: 445.983; found: 445.9832.

(F) ^{1}H, ^{13}C NMR and ^{19}F spectra.

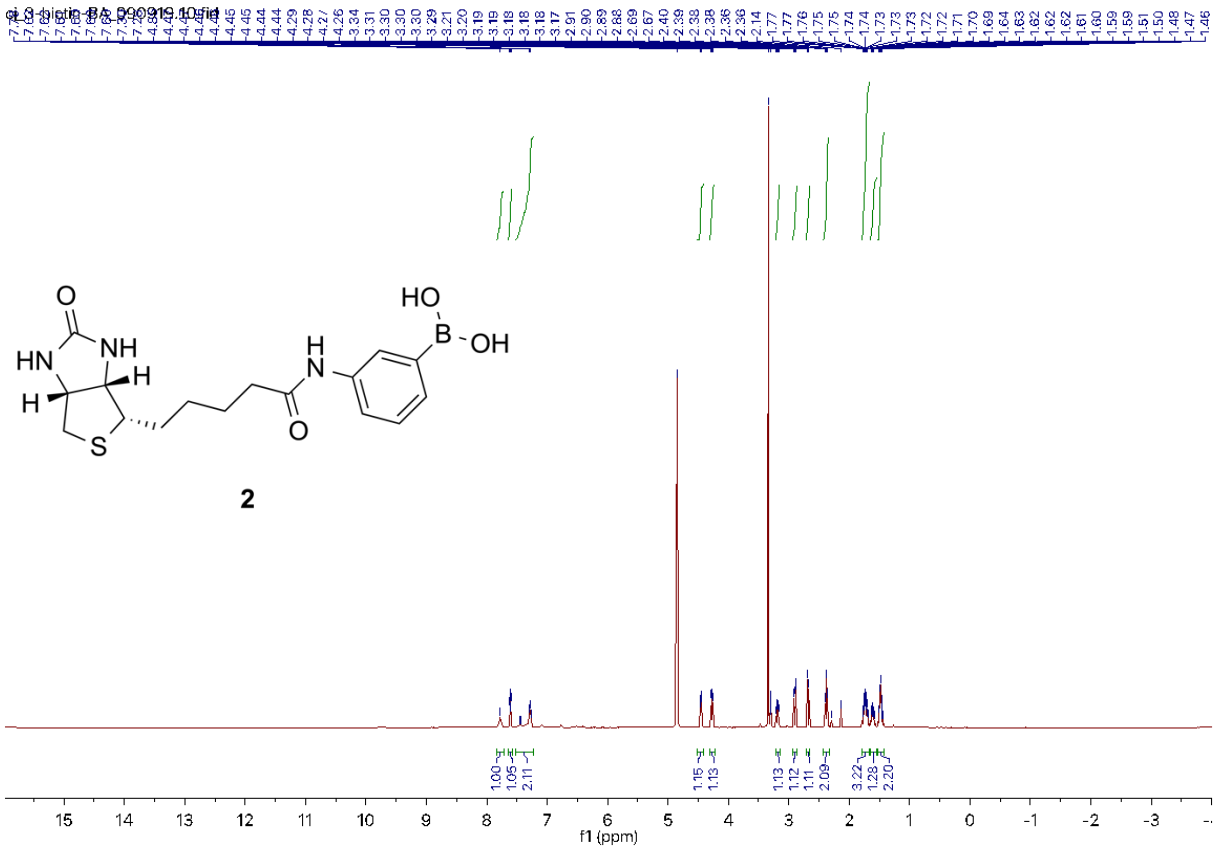
^{1}H-NMR for 1.



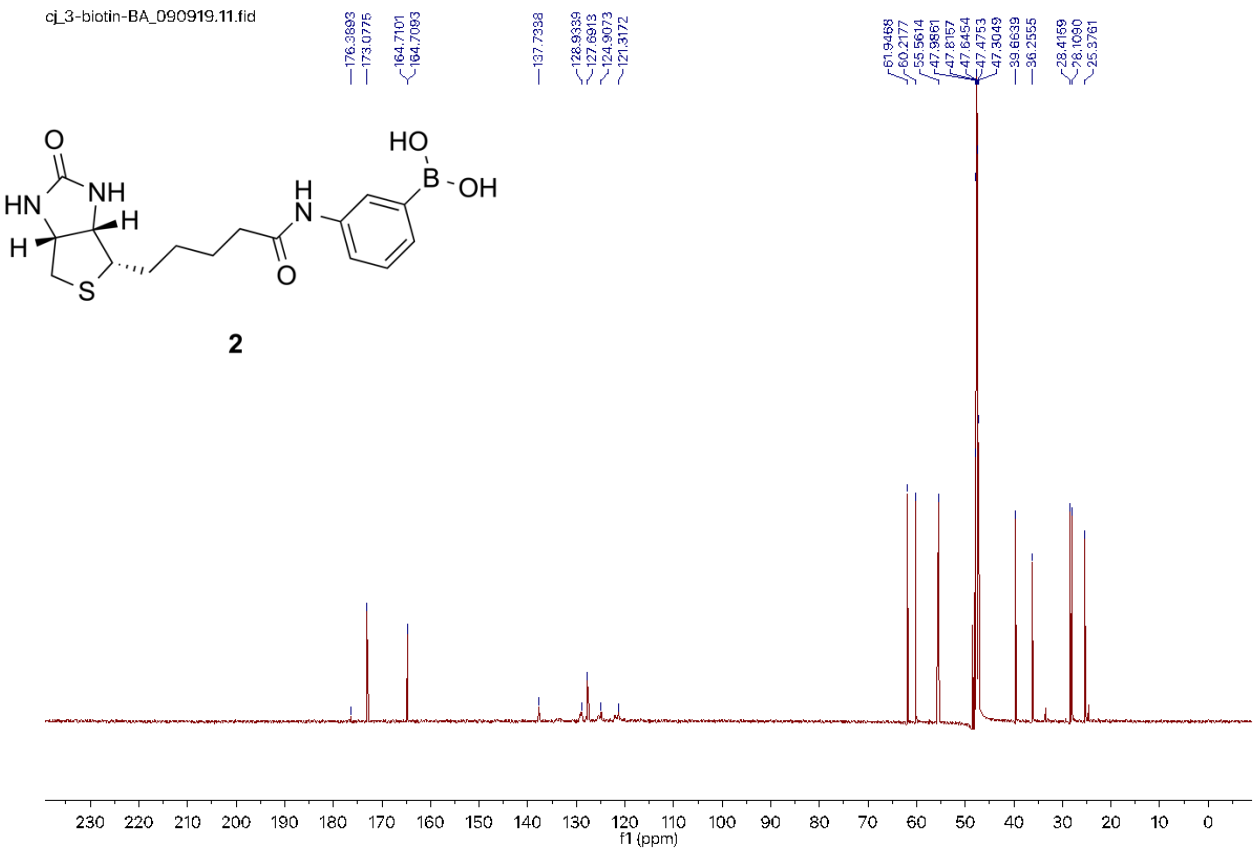
^{13}C-NMR for 1.


cj-l-new IA probe.11.fid
168.09
138.29
137.57
121.38
86.80
2.58
I
NH
I
O
1
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

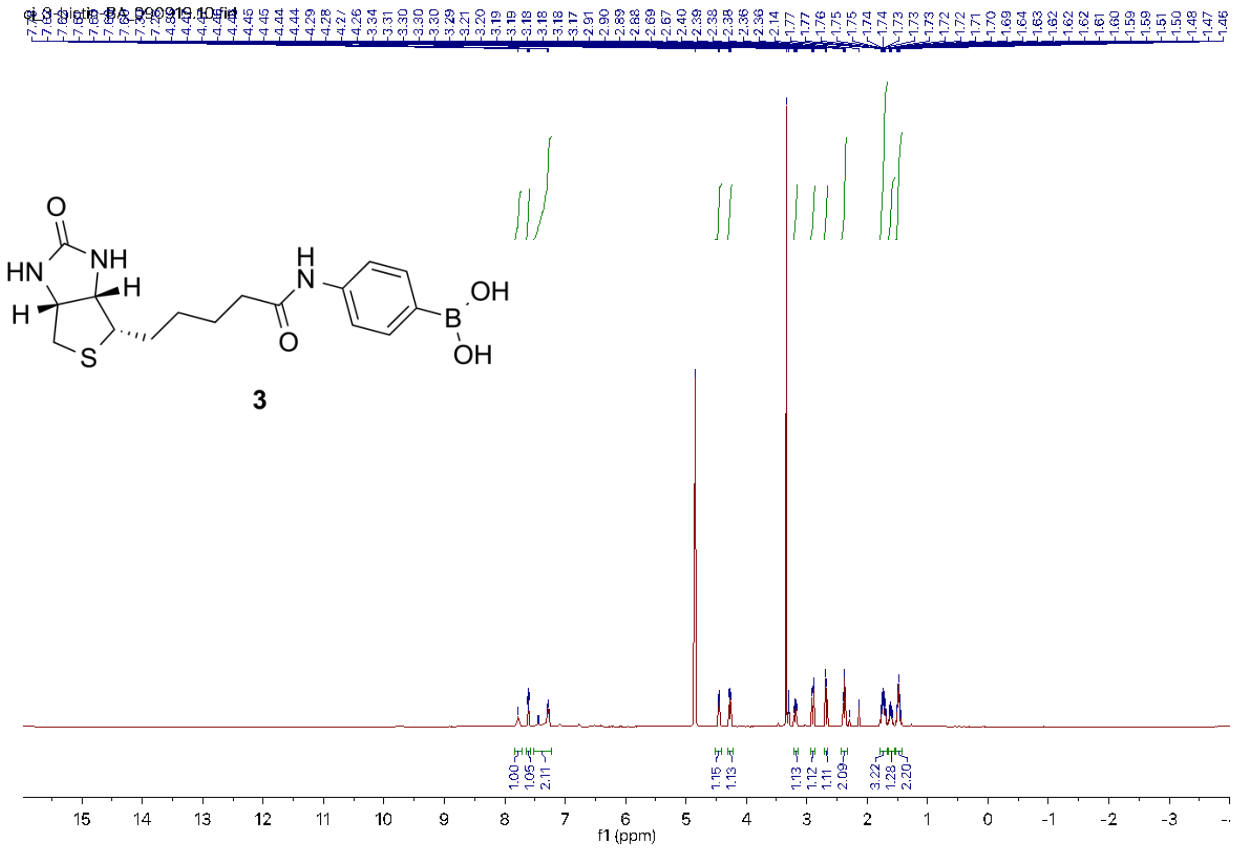
^{1}H-NMR for 2.


HO
B
OH
H
N
O
HN
NH
H
H
S
O
2
1.00
1.05
2.11
1.15
1.13
1.13
1.12
1.11
2.09
3.22
1.23
2.20
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
f1 (ppm)

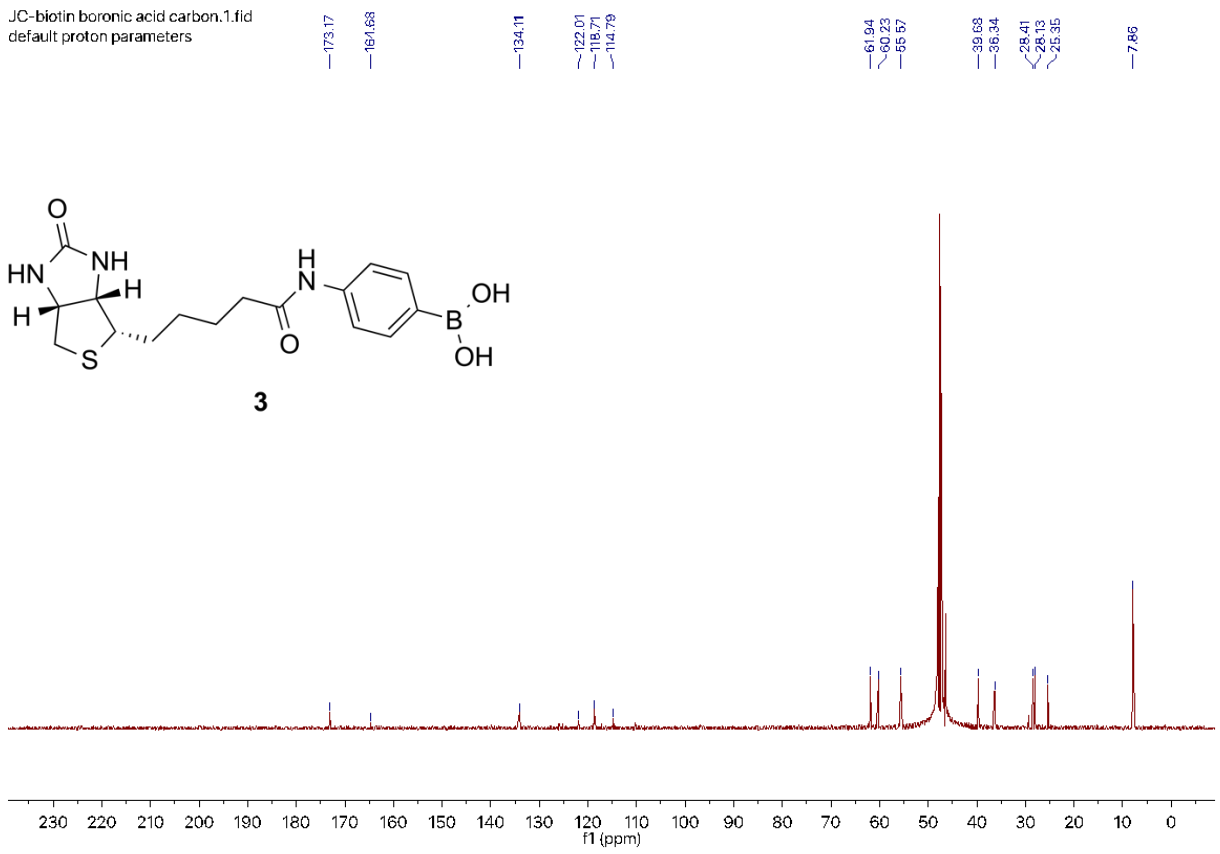
^{13}C-NMR for 2


cj_3-biotin-BA_090919.11.fid
176.3893
173.0775
164.7101
164.7093
137.7338
128.9339
127.6913
124.9073
121.3172
61.9468
60.2177
55.5614
47.9861
47.8157
47.6454
47.4753
47.3049
39.6639
36.2555
28.4159
28.1090
25.3761
O
HN
NH
H
H
S
O
H
N
HO
B
OH
2
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

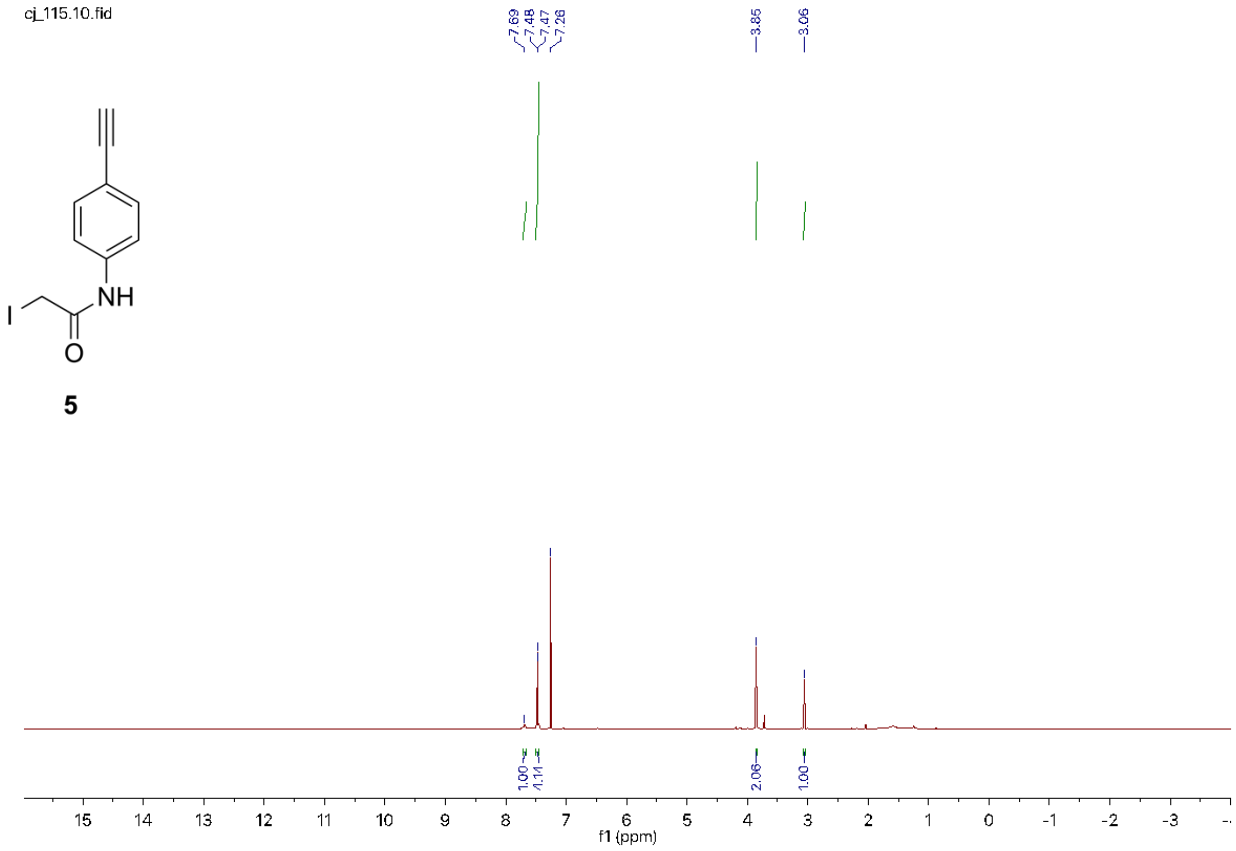
^{1}H-NMR for 3.



^{13}C-NMR for 3.

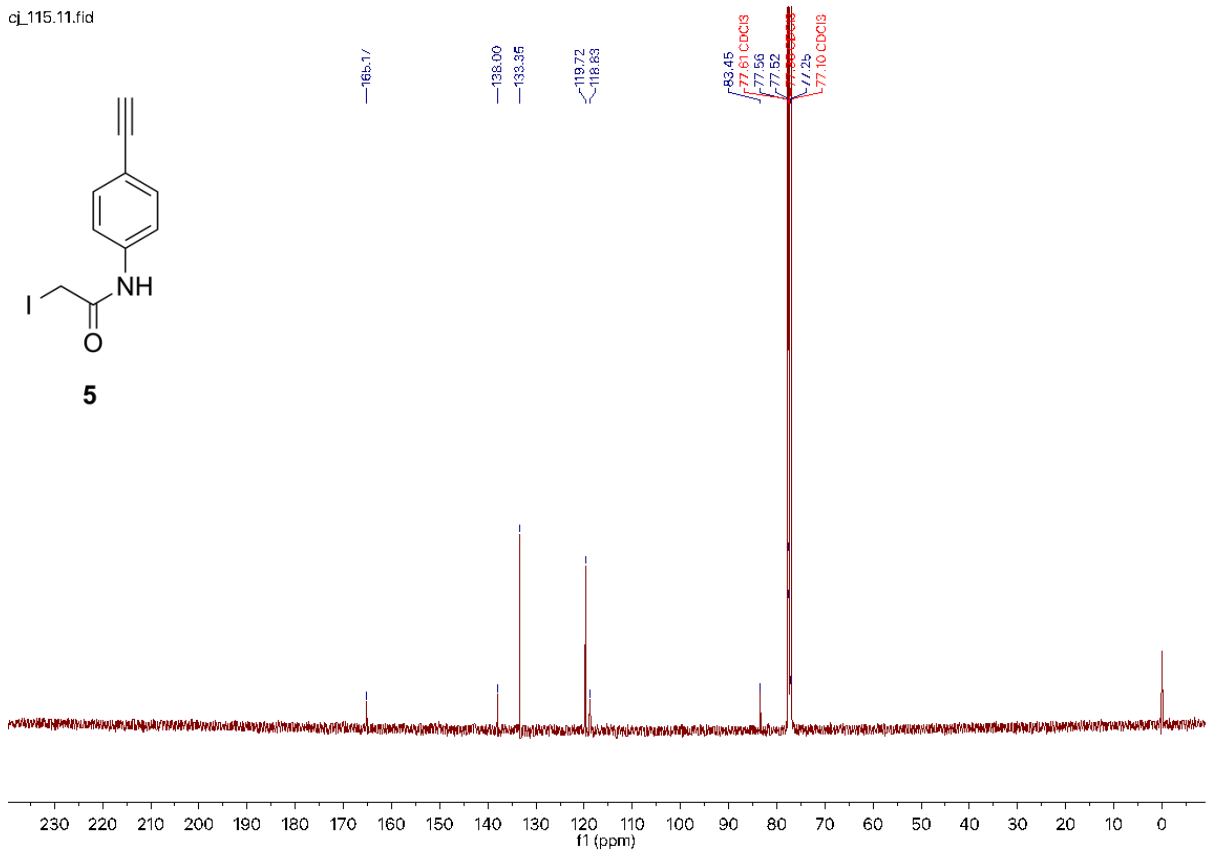

JC-biotin boronic acid carbon.1.fid
default proton parameters
173.17
164.63
134.11
122.01
118.71
114.79
61.94
60.23
55.57
39.68
36.34
28.41
28.13
25.35
7.86
O
HN
NH
H
H
S
O
H
N
OH
B
OH
3
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

^{1}H-NMR for 5.

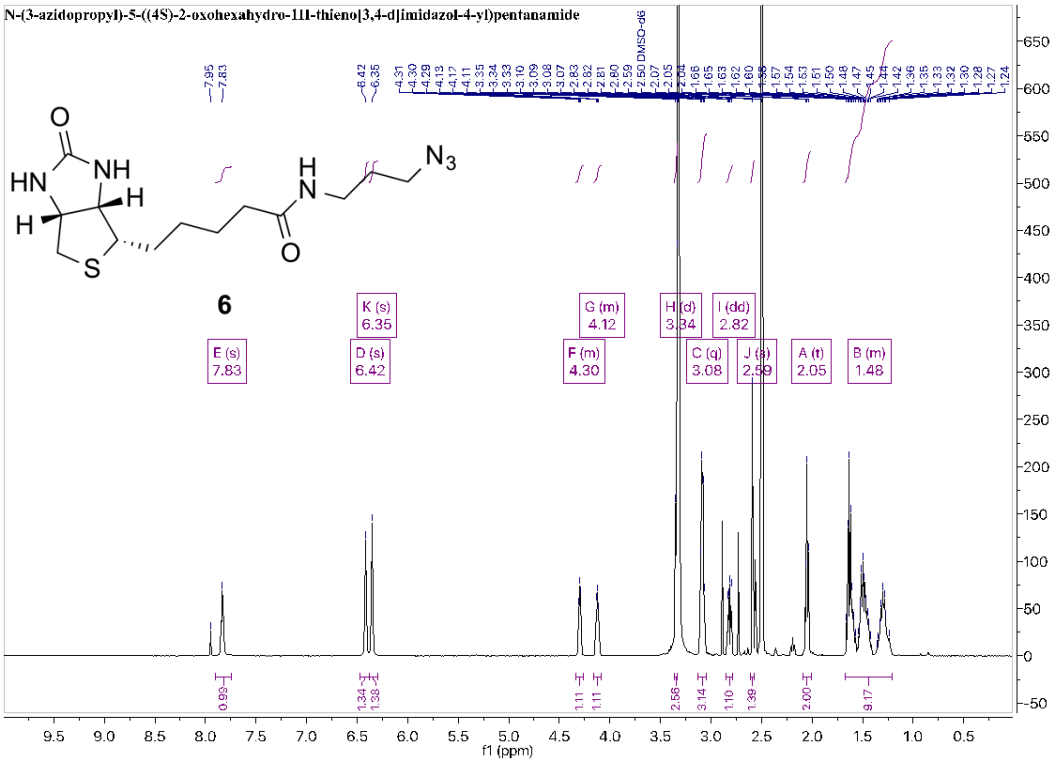

cj_115.10.fid
7.69
7.48
7.47
7.28
3.85
3.06
NH
I
O
1.00
1.14
2.06
1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)

5

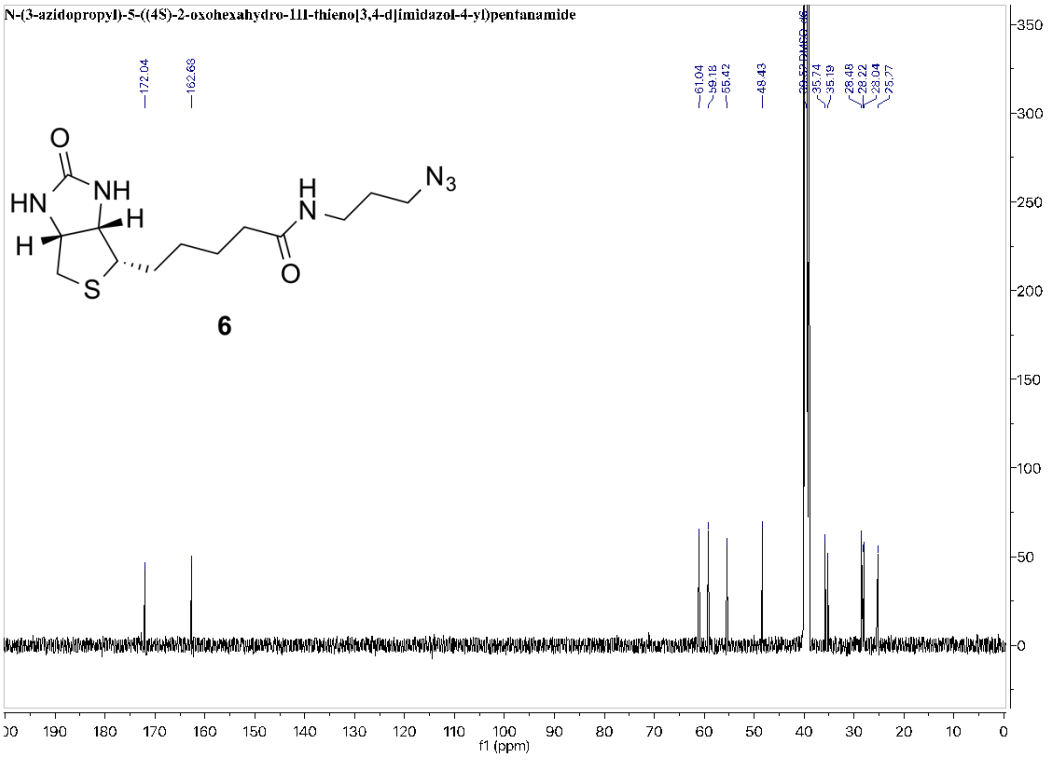
^{1}H-NMR for 5.


cj_115.11.fid
166.17
138.00
133.35
119.72
118.83
83.45
77.61 CDCl3
77.56
77.52
77.25
77.10 CDCl3
NH
I
O
5
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

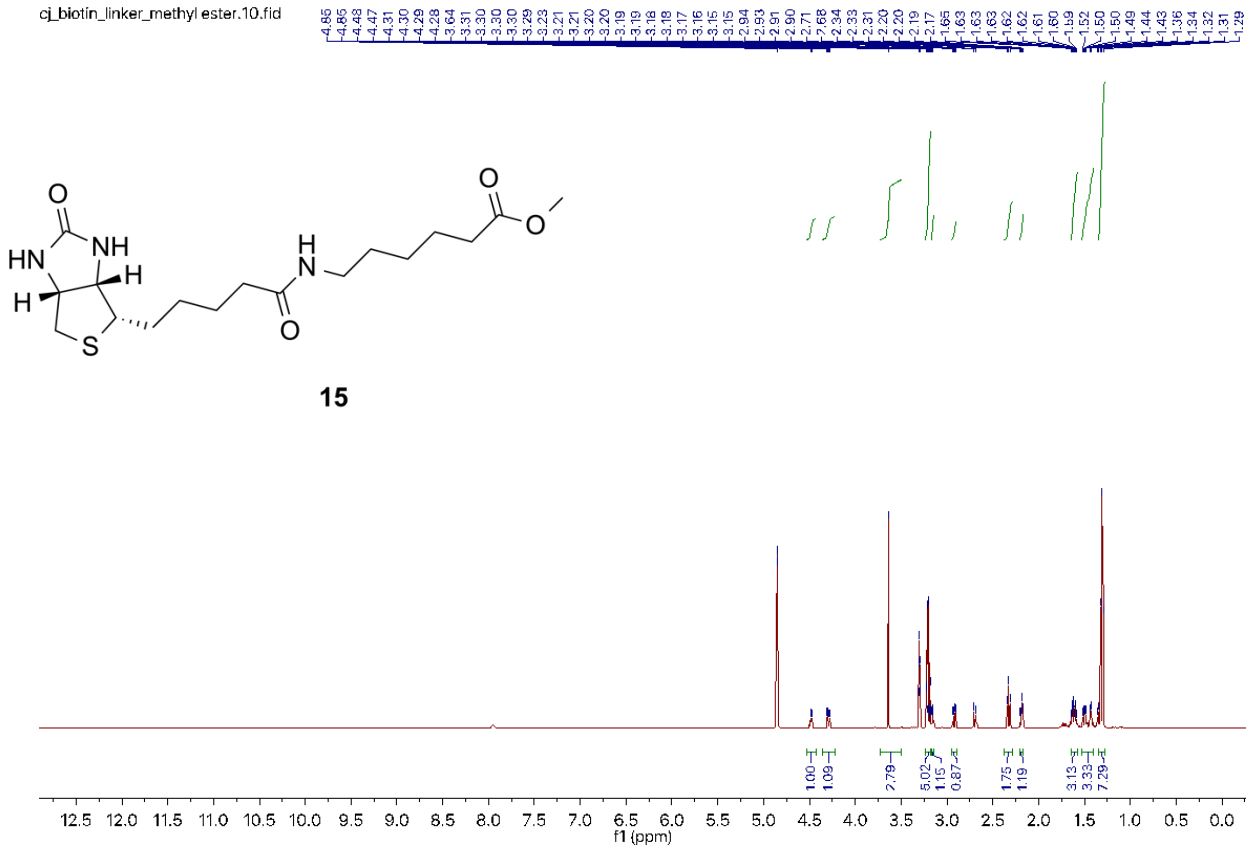
^{1}H-NMR for 6.



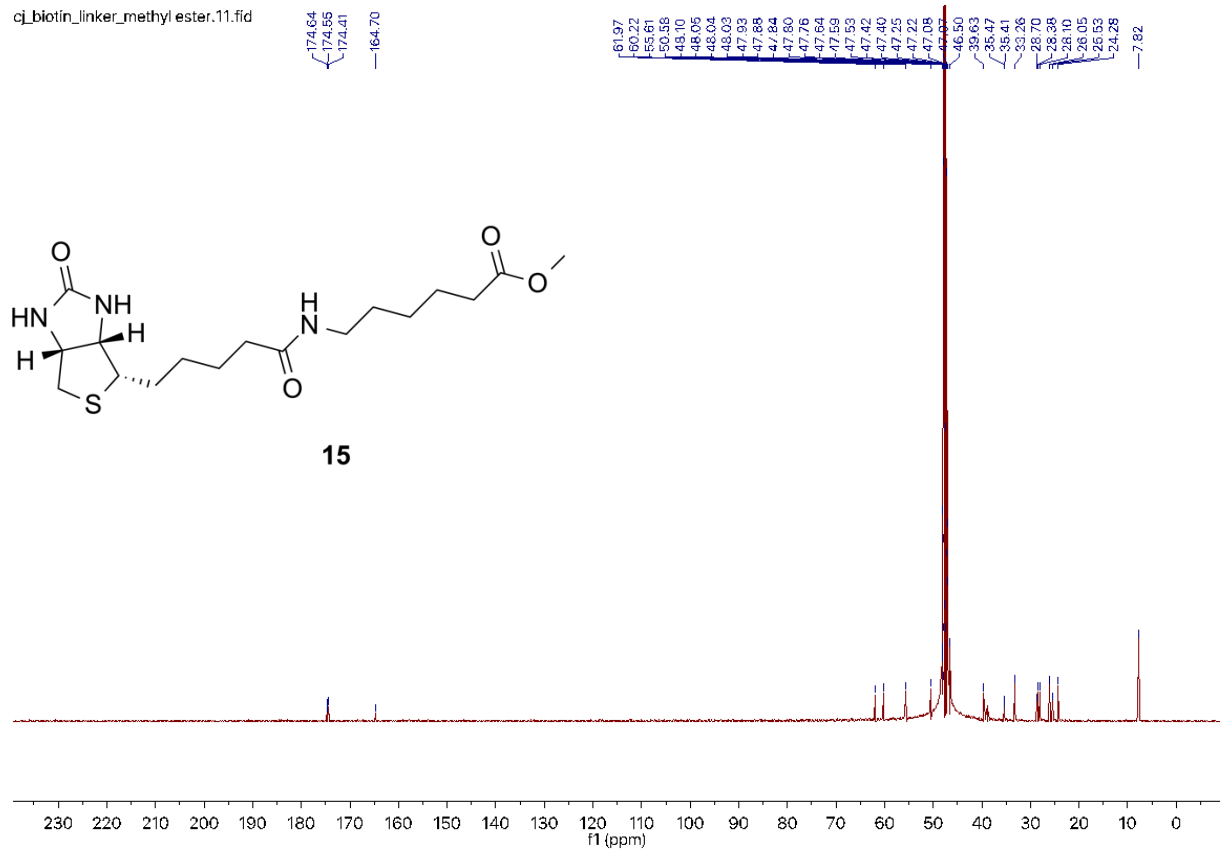
^{13}C-NMR for 6.



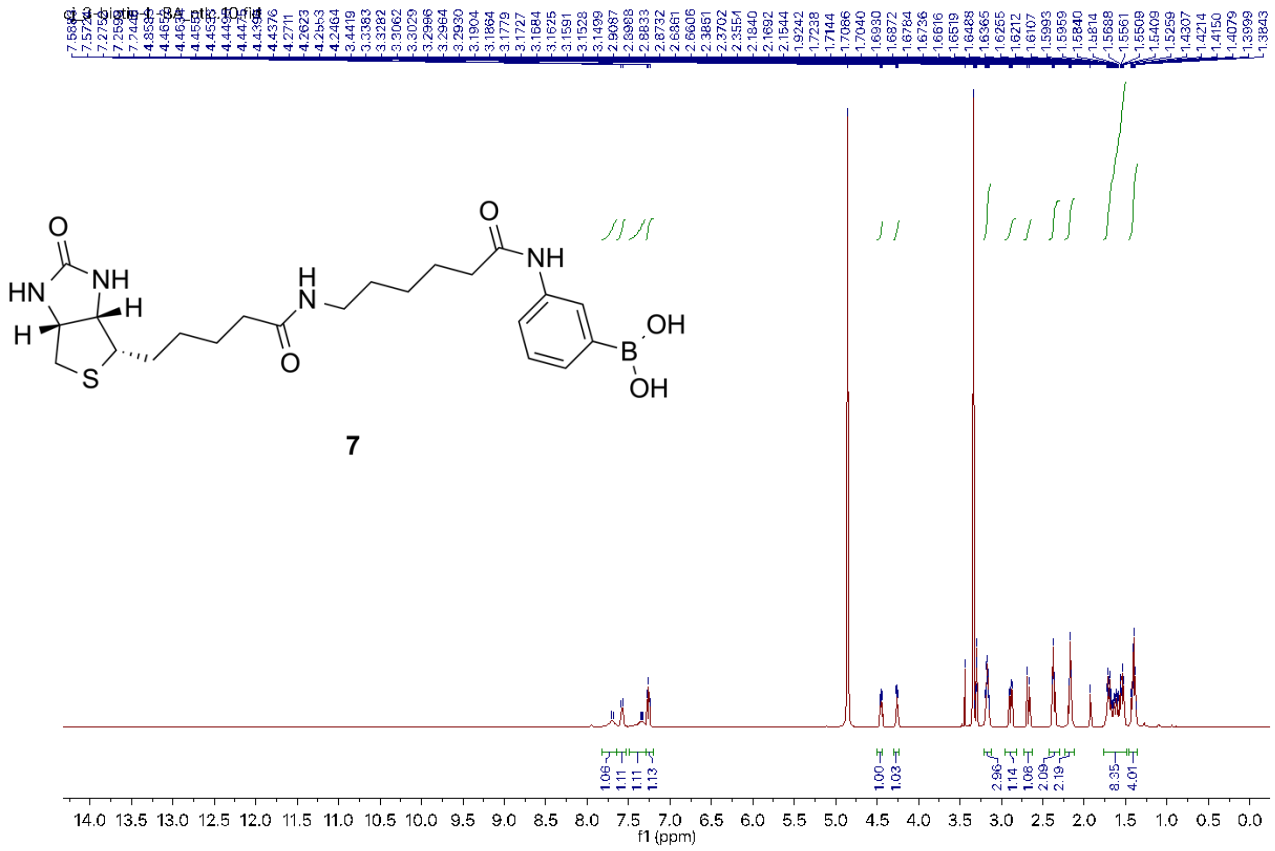
^{1}H-NMR for 15.



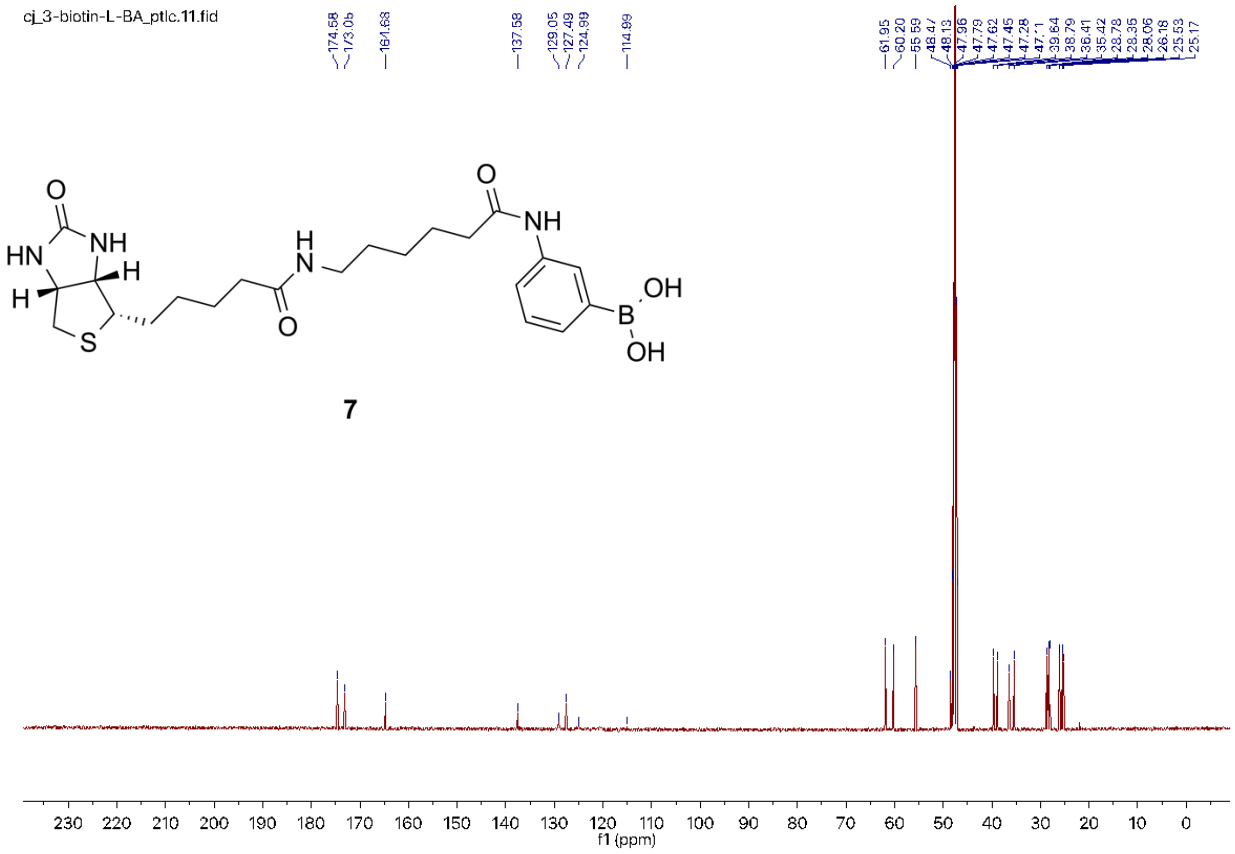
^{13}C-NMR for 15.



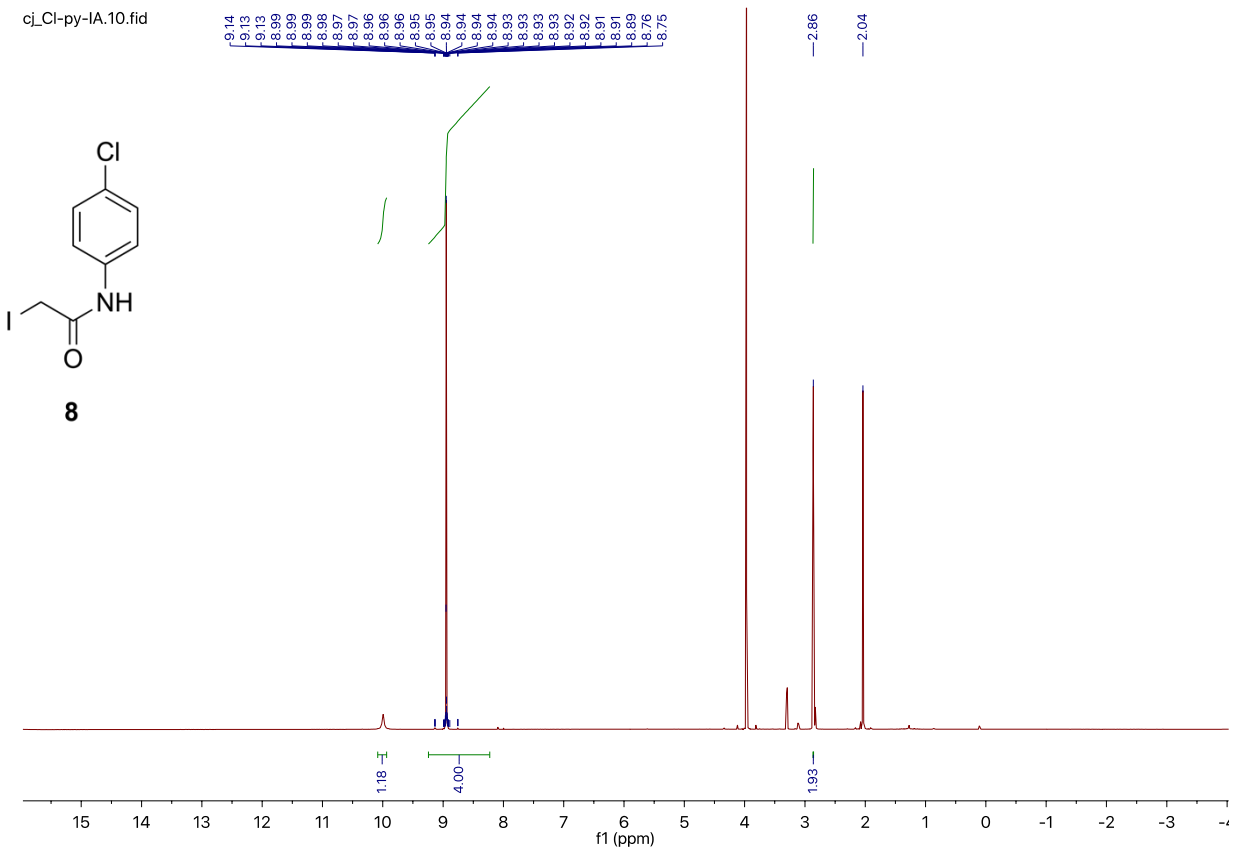
^{1}H-NMR for 7.



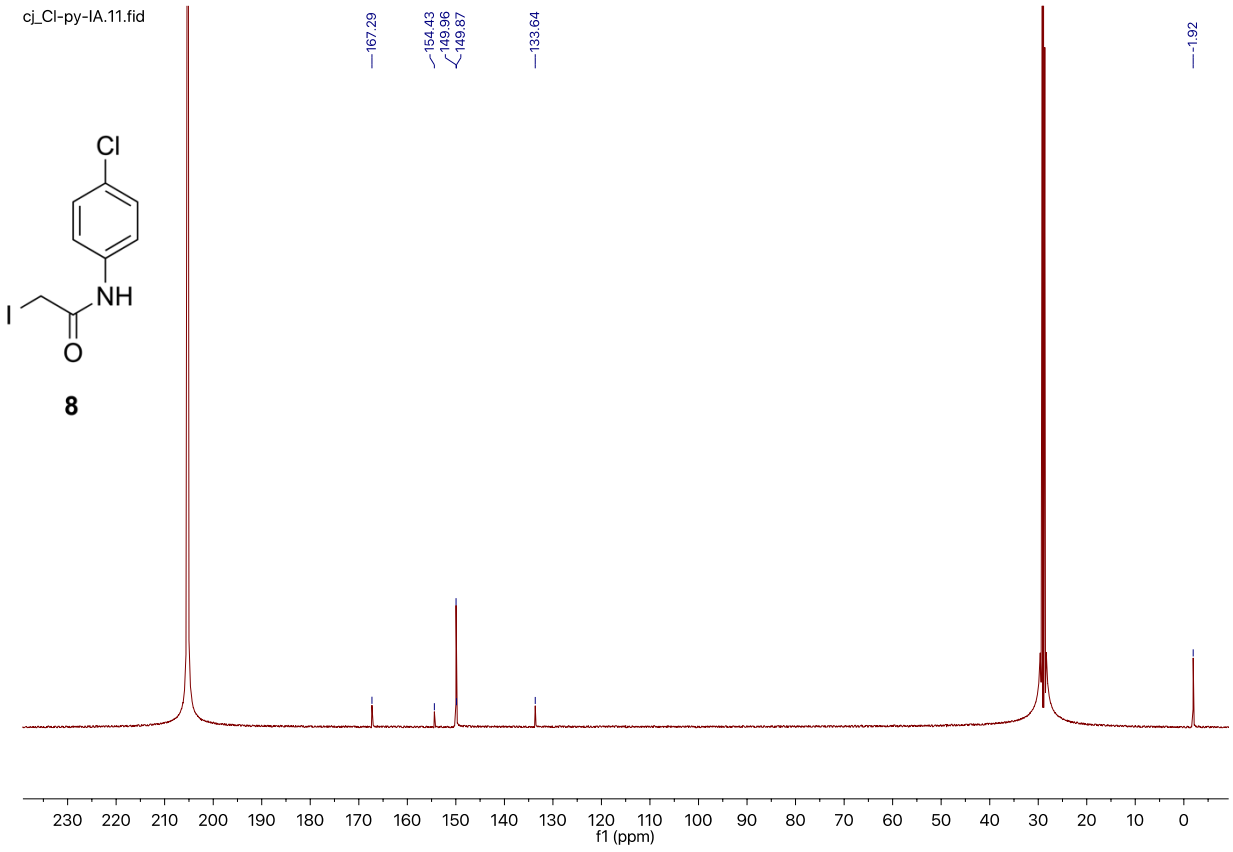
^{13}C-NMR for 7.


cj_3-biotin-L-BA_ptlc.11.fid
174.58
173.05
161.63
137.58
129.05
127.49
124.99
114.99
61.95
60.20
55.59
48.47
48.13
47.96
47.79
47.62
47.45
47.28
47.10
39.64
38.79
36.41
35.42
28.78
28.35
28.06
26.18
25.53
25.17
O
NH
HN
H
H
S
H
N
O
O
NH
OH
B
OH
7
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

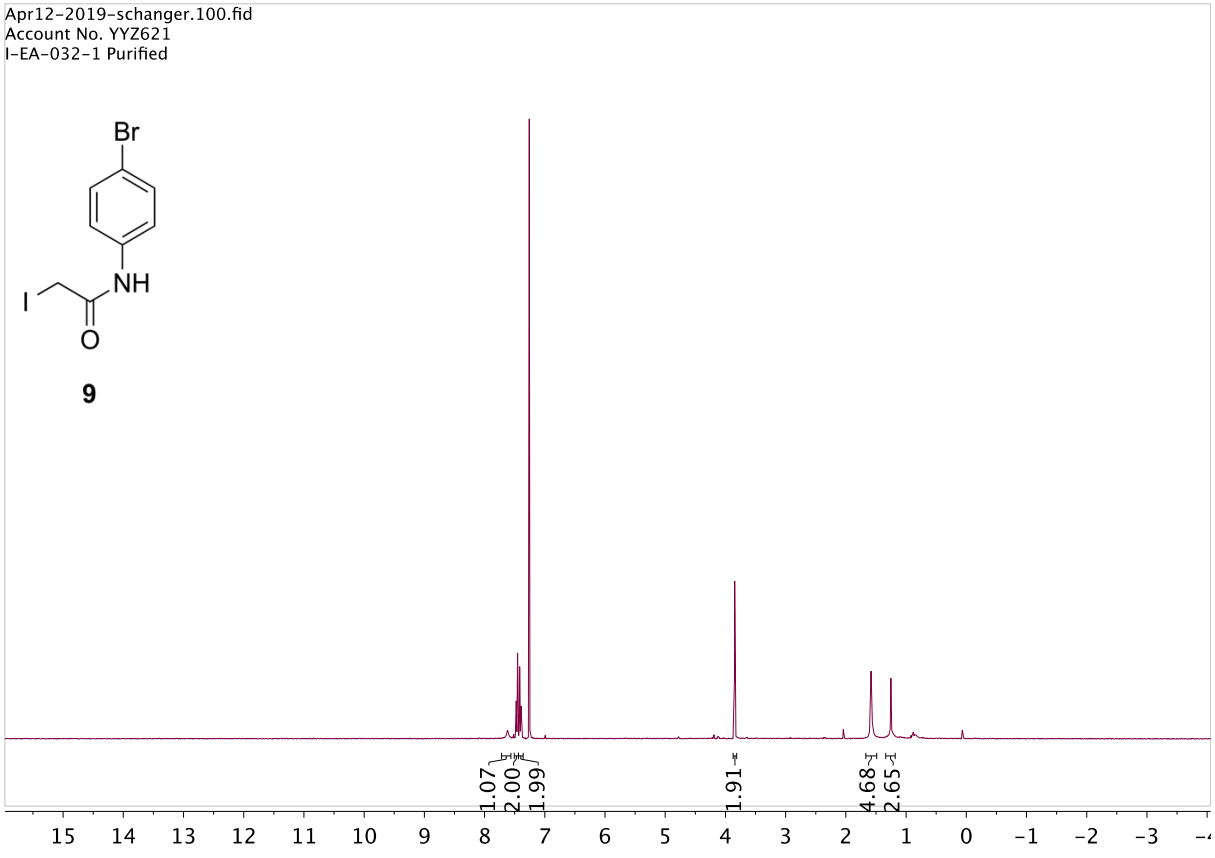
^{1}H-NMR for 8.


cj_Cl-py-IA.10.fid
9.14
9.13
9.13
8.99
8.99
8.99
8.98
8.97
8.97
8.96
8.96
8.96
8.95
8.95
8.94
8.94
8.94
8.94
8.94
8.93
8.93
8.93
8.93
8.92
8.92
8.91
8.91
8.89
8.76
8.75
2.86
2.04
Cl
NH
I
O
8
1.18
4.00
1.93
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
f1 (ppm)

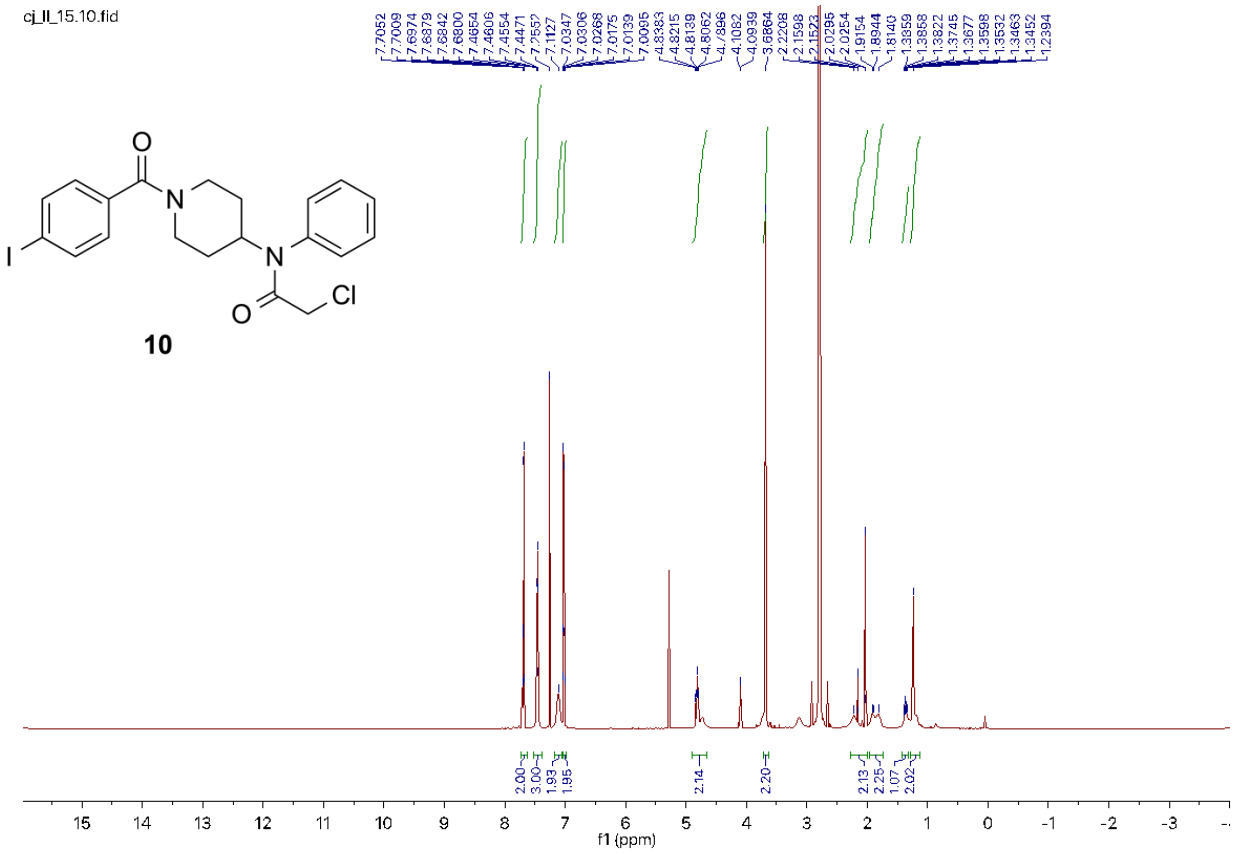
^{13}C-NMR for 8.


cj_Cl-py-IA.11.fid
167.29
154.43
149.96
149.87
133.64
-1.92
Cl
NH
I
O
8
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

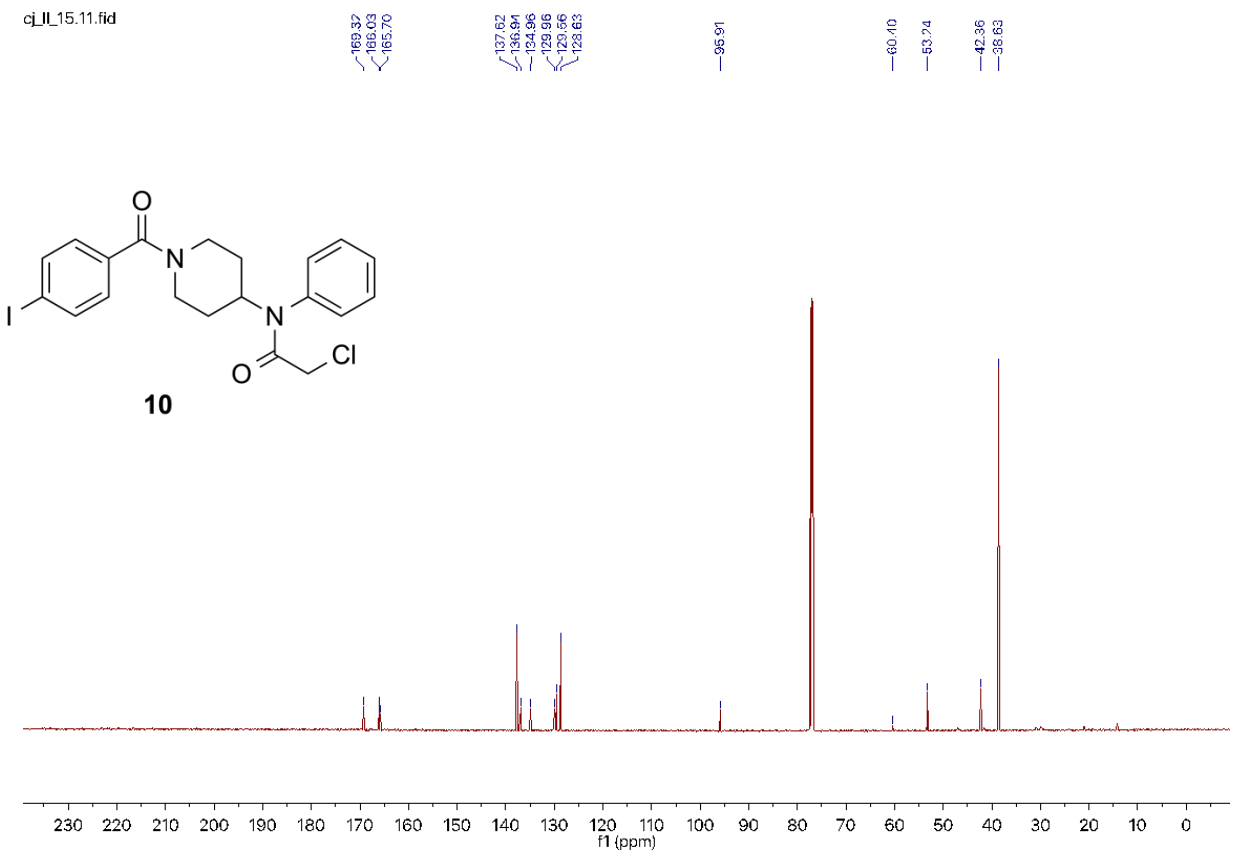
^{1}H-NMR for 9.


Apr12-2019-schanger.100.fid
Account No. YYZ621
I-EA-032-1 Purified
Br
NH
I
O
9
1.07
2.00
1.99
1.91
4.68
2.65
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 −1 −2 −3

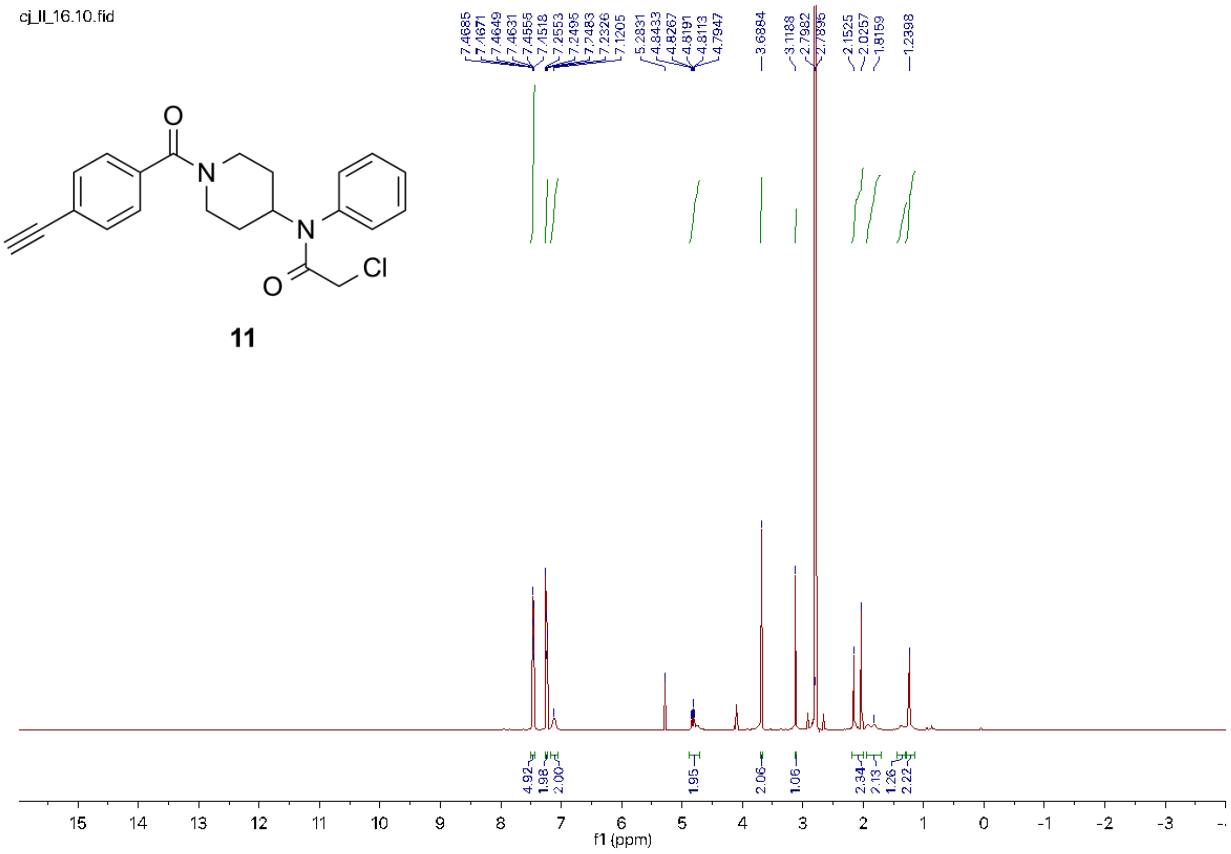
^{1}H-NMR for 10.



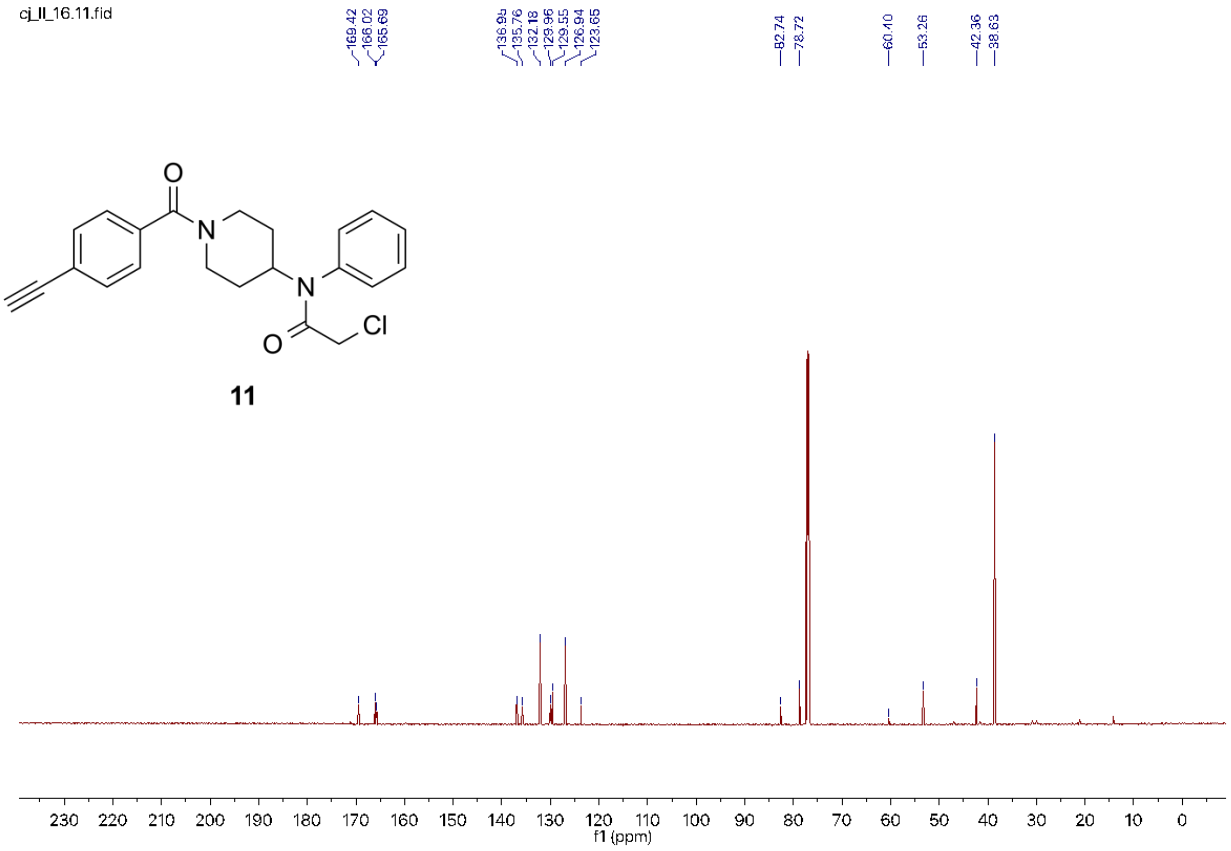
^{13}C-NMR for 10.



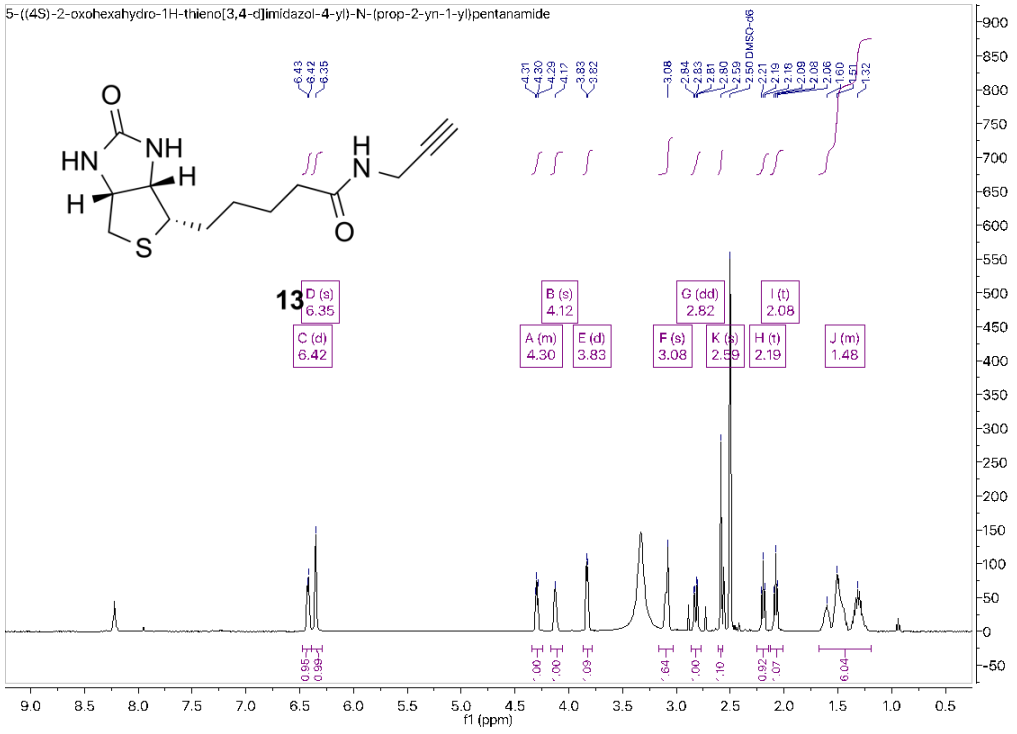
^{1}H-NMR for 11.


cj_II_16.10.fid
7.4685
7.4671
7.4649
7.4631
7.4555
7.1518
7.2553
7.2495
7.7483
7.2326
7.1205
5.2831
4.8433
4.8267
4.8191
4.8113
4.7947
3.6884
3.1188
2.7982
2.7896
2.1525
2.0257
1.8159
1.2398
O
N
N
O
Cl
11
4.92
1.98
2.00
1.95
2.06
1.06
2.84
2.13
1.26
2.22
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)

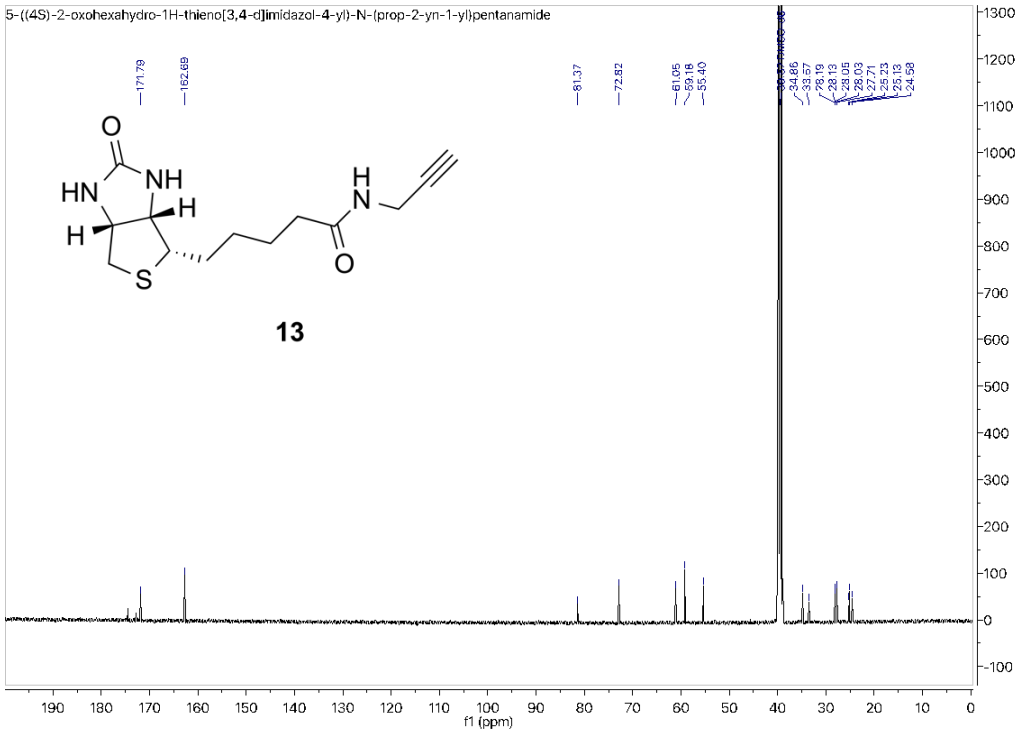
^{13}C-NMR for 11.



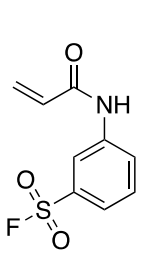
^{1}H-NMR for 13.



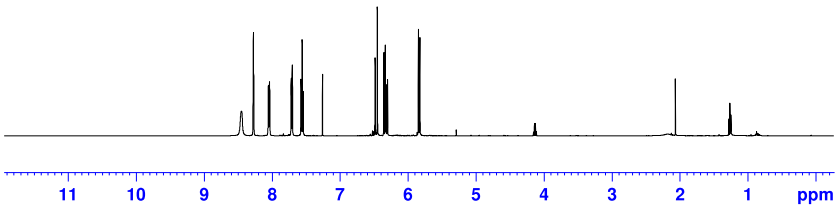
^{13}C-NMR for 13.



^{1}H-NMR for 15.



15

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PROCNO                 1

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Time              14.06 h
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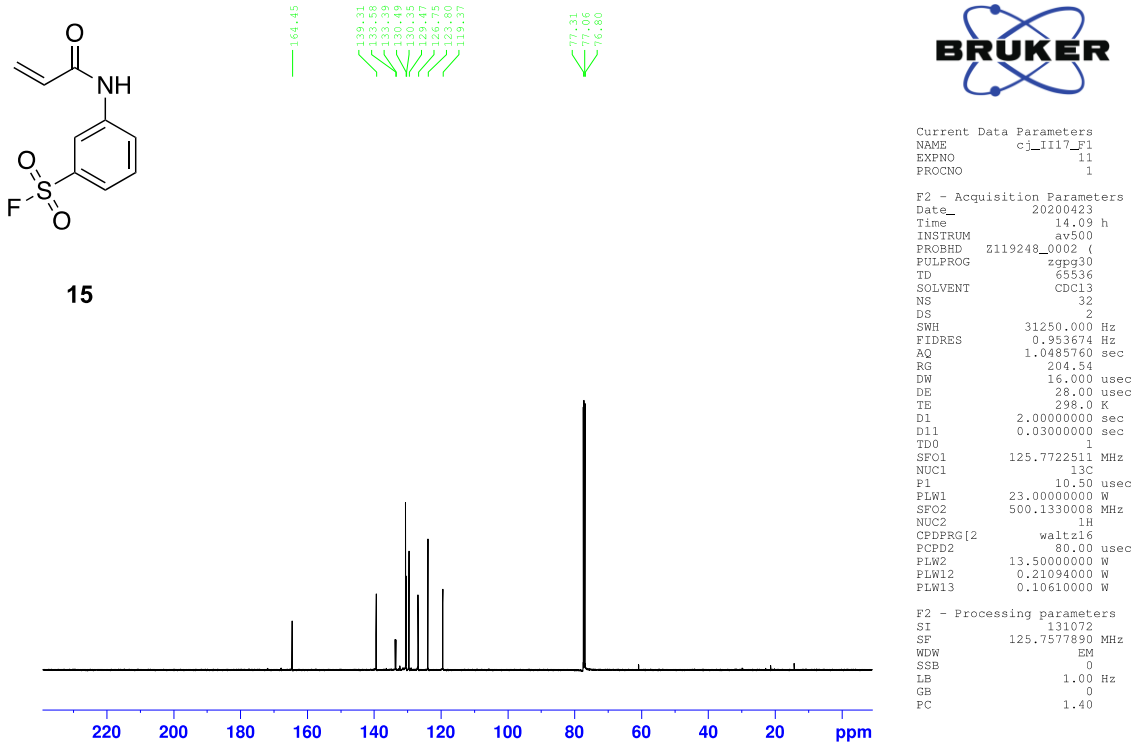
PROBHD   Z119248_0002 (
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     8
DS                     0
SWH           10000.000 Hz
FIDRES         0.305176 Hz
AQ             3.2767999 sec
RG                 12.14
DW                50.000 usec
DE                10.00 usec
TE                298.0 K
D1             2.00000000 sec
TD0                    1
SFO1         500.1330008 MHz
NUC1                  1H
P1                 10.00 usec
PLW1        13.50000000 W

F2 - Processing parameters
SI                 65536
SF           500.1300146 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

^{13}C-NMR for 15.

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Current Data Parameters
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EXPNO                 11
PROCNO                 1

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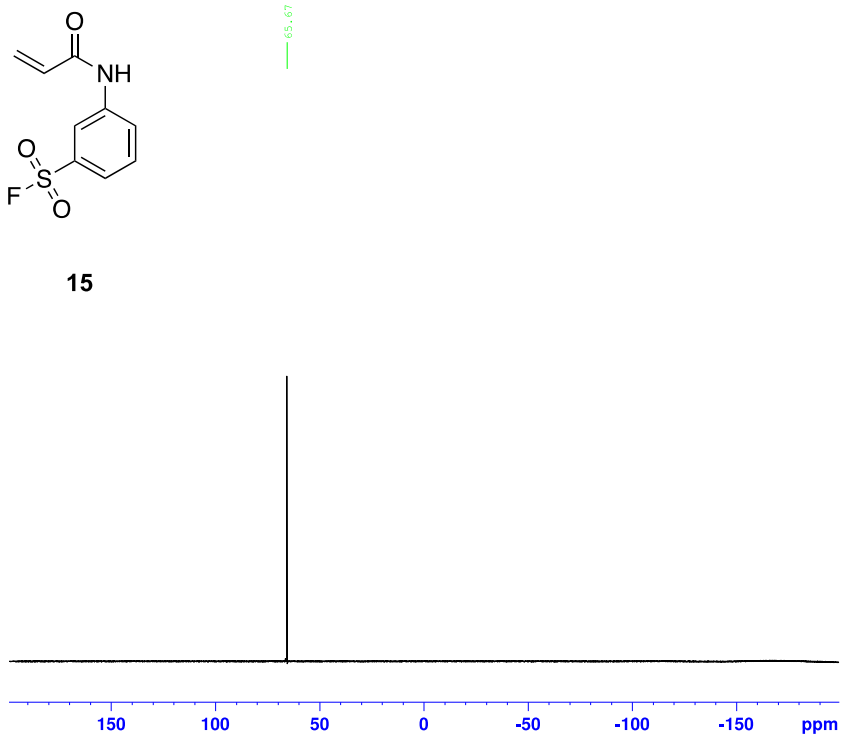
PULPROG            zgpg30
TD                  65536
SOLVENT             CDCl3
NS                     32
DS                      2
SWH             31250.000 Hz
FIDRES              0.953674 Hz
AQ                1.0485760 sec
RG                   204.54
DW                  16.000 usec
DE                  28.00 usec
TE                  298.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1
SFO1          125.7722511 MHz
NUC1                  13C
P1                  10.50 usec
PLW1          23.00000000 W
SFO2          500.1330008 MHz
NUC2                  1H
CPDPRG[2          waltz16
PCPD2               80.00 usec
PLW2          13.50000000 W
PLW12          0.21094000 W
PLW13          0.10610000 W

F2 - Processing parameters
SI                  131072
SF            125.7577890 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

^{19}F-NMR for 15.

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Current Data Parameters
NAME         Jian's NMRs
EXPNO                100
PROCNO                 1

F2 - Acquisition Parameters
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Time              14.08
INSTRUM           av400
PROBHD   5 mm PABBO BB/
PULPROG      zgfhigqn.2
TD               262144
SOLVENT            CDCl3
NS                    32
DS                     0
SWH           150000.000 Hz
FIDRES          0.572205 Hz
AQ              0.8738133 sec
RG                189.85
DW                  3.333 usec
DE                  6.50 usec
TE                296.5 K
D1            1.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         376.4983660 MHz
NUC1                 19F
P1                 14.50 usec
PLW1         17.00000000 W

======== CHANNEL f2 ========
SFO2         400.1324008 MHz
NUC2                 1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2         13.00000000 W
PLW12         0.36111000 W

F2 - Processing parameters
SI               262144
SF           376.4983660 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.00
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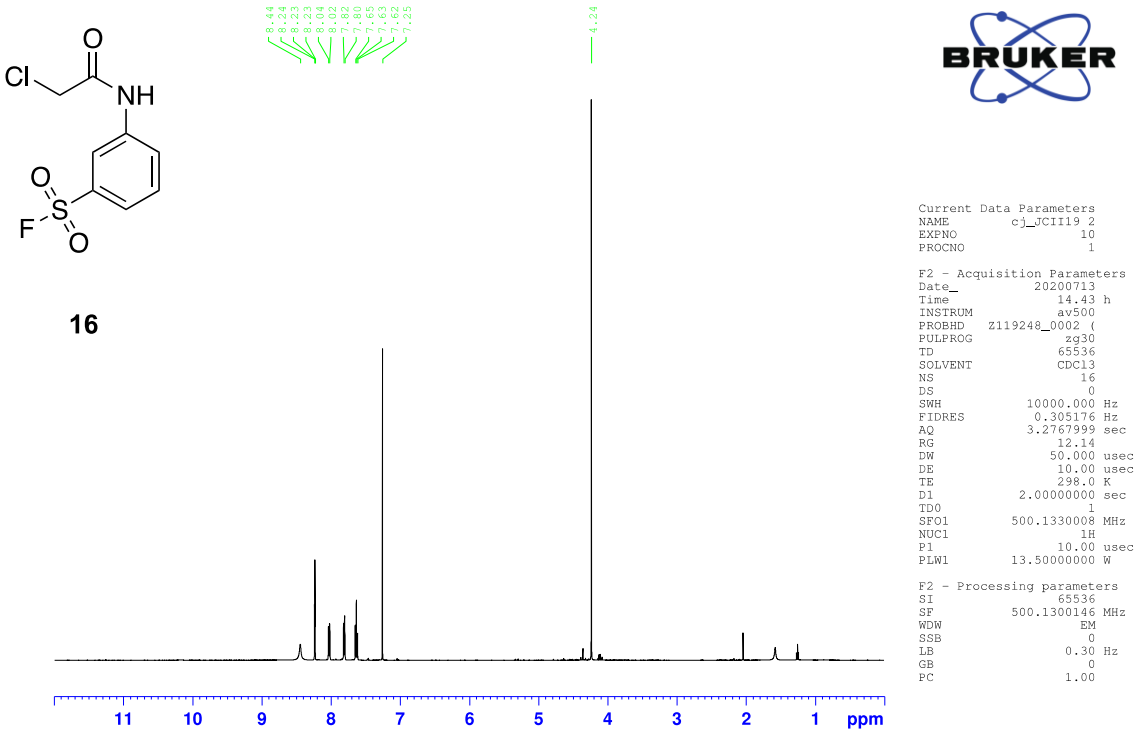
[1]H-NMR for 16.

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Current Data Parameters
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EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
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TD                 65536
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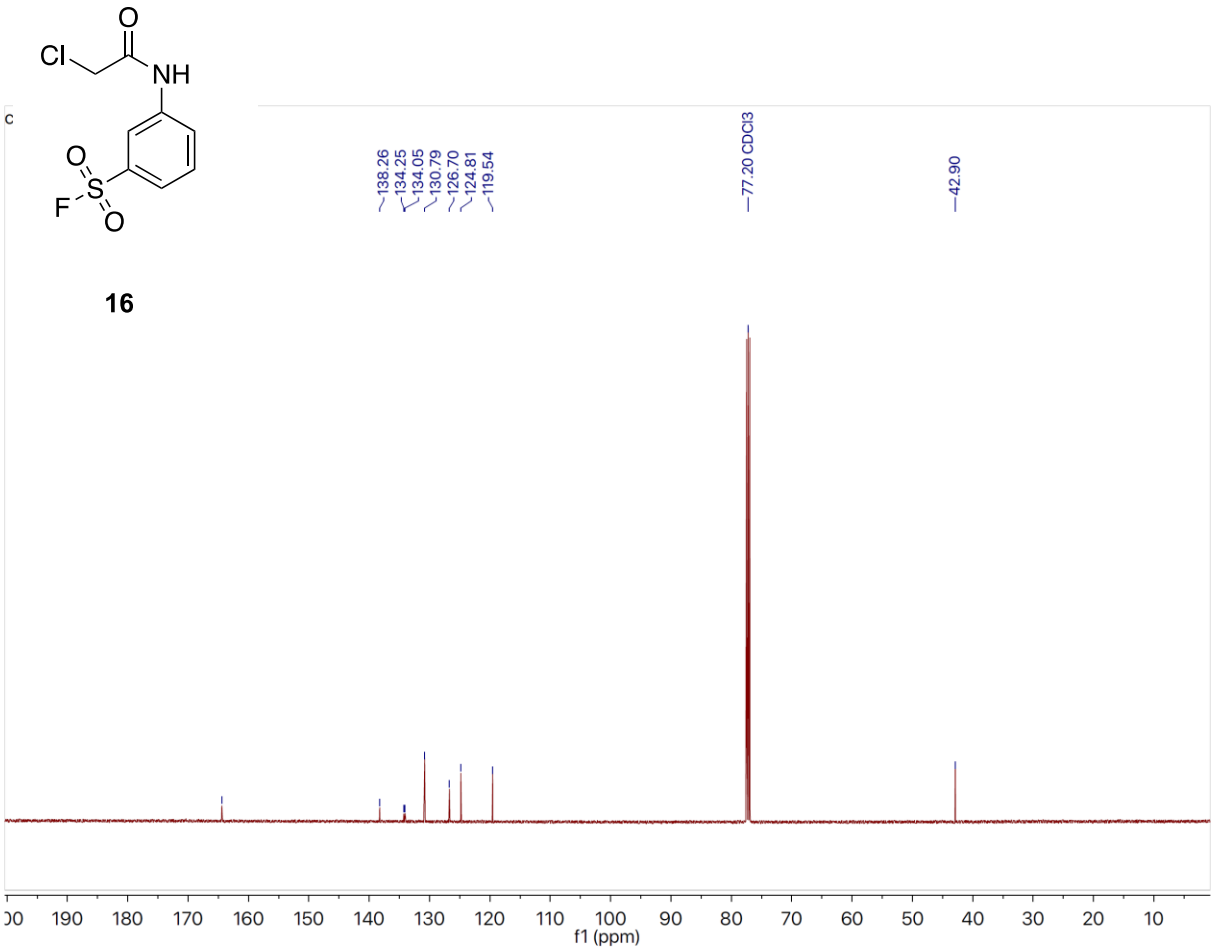
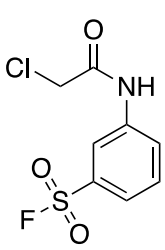
NS                    16
DS                     0
SWH           10000.000 Hz
FIDRES            0.305176 Hz
AQ             3.2767999 sec
RG                 12.14
DW                50.000 usec
DE                10.00 usec
TE                298.0 K
D1             2.00000000 sec
TD0                    1
SFO1          500.1330008 MHz
NUC1                  1H
P1                 10.00 usec
PLW1          13.50000000 W

F2 - Processing parameters
SI                 65536
SF            500.1300146 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

^{13}C-NMR for 16.





16


```
Current Data Parameters
NAME         Jian's NMRs
EXPNO                120
PROCNO                 1

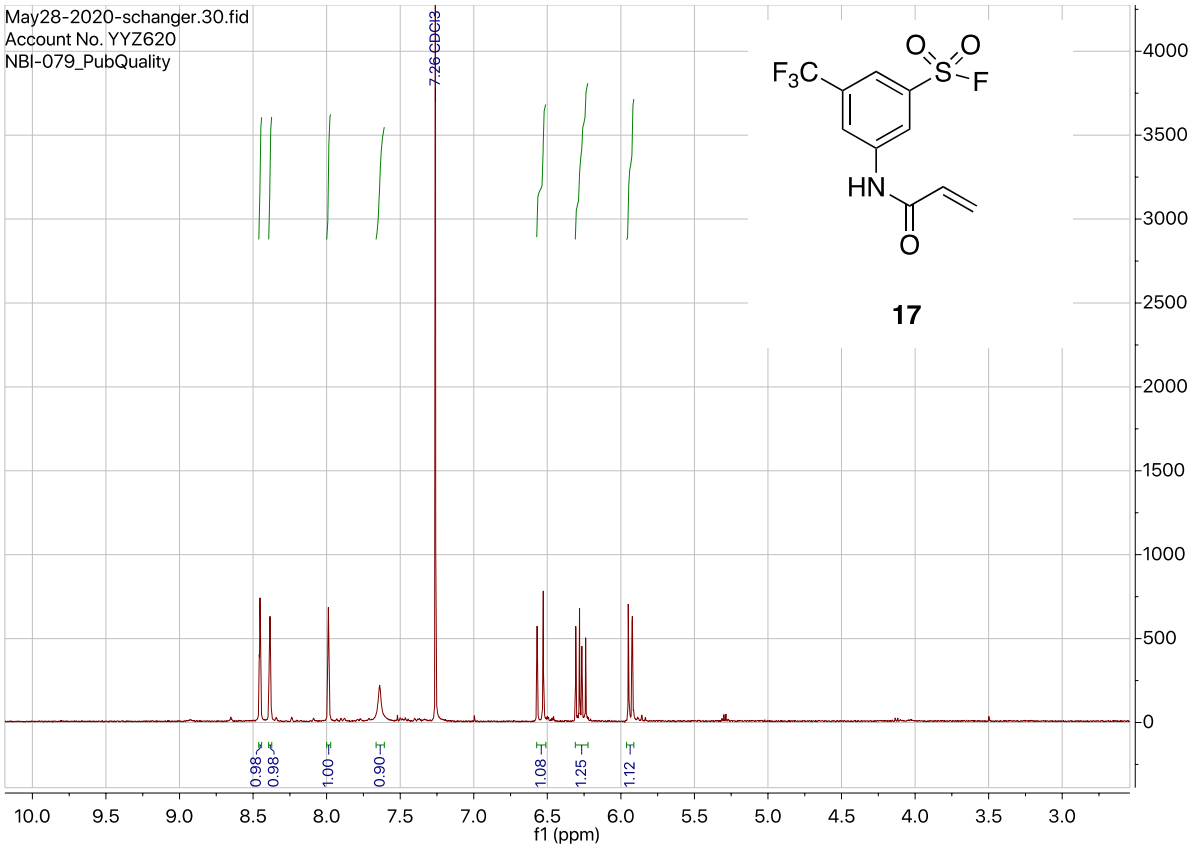
F2 - Acquisition Parameters
Date_           20201028
Time               14.20
INSTRUM            av400
PROBHD   5 mm PABBO BB/
PULPROG      zgfhigqn.2
TD                262144
SOLVENT           CDCl3
NS                    32
DS                     0
SWH           150000.000 Hz
FIDRES          0.572205 Hz
AQ             0.8738133 sec
RG                189.85
DW                 3.333 usec
DE                 6.50 usec
TE                296.5 K
D1            1.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          376.4983660 MHz
NUC1                  19F
P1                 14.50 usec
PLW1         17.00000000 W

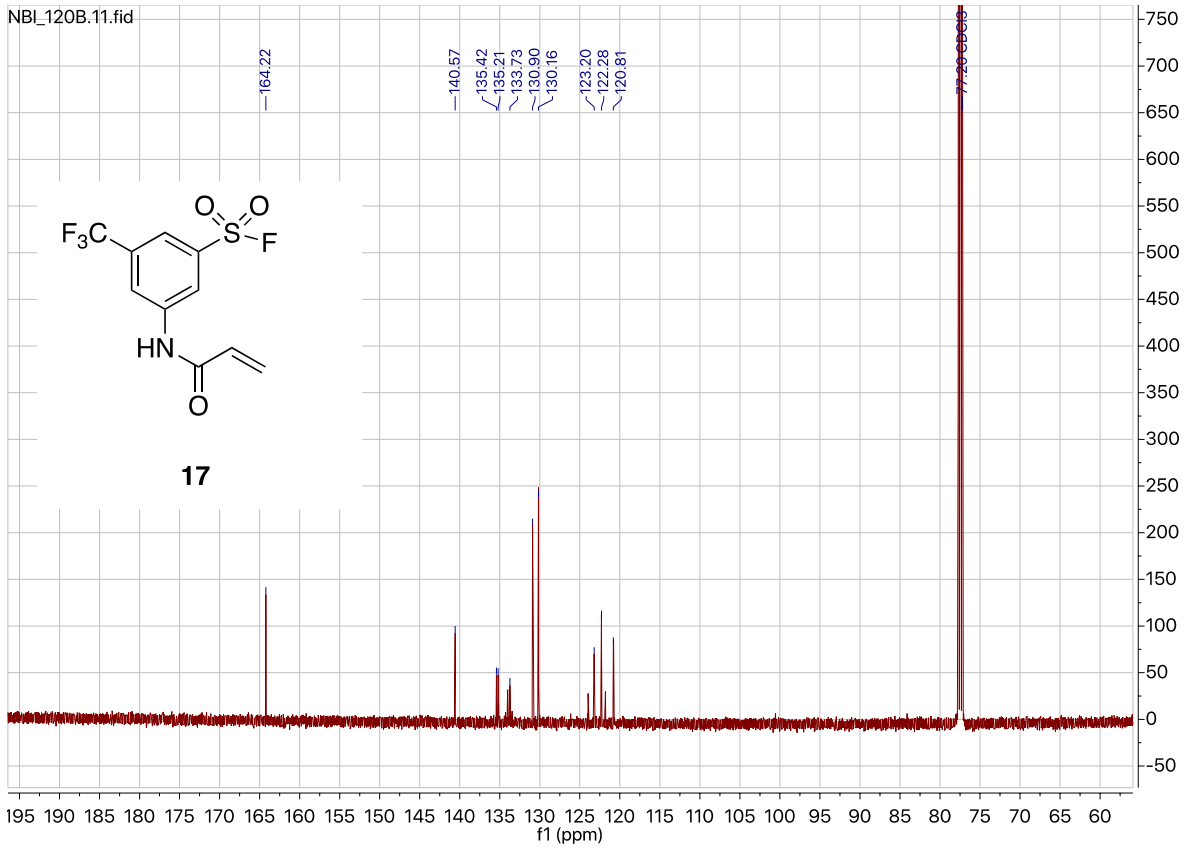
======== CHANNEL f2 ========
SFO2          400.1324008 MHz
NUC2                  1H
CPDPRG[2         waltz16
PCPD2              90.00 usec
PLW2         13.00000000 W
PLW12         0.36111000 W

F2 - Processing parameters
SI                262144
SF          376.4983660 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.00
```

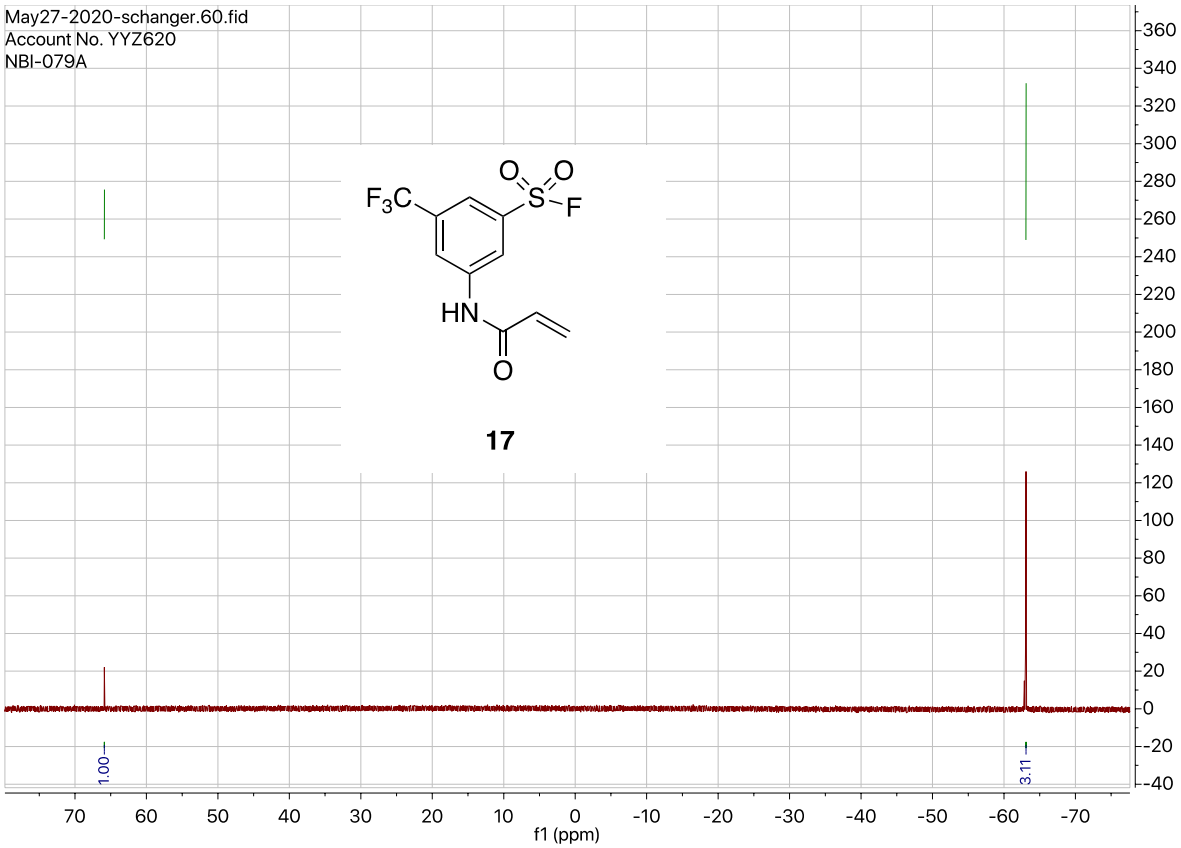
^{1}H-NMR for 17.


May28-2020-schanger.30.fid
Account No. YYZ620
NBI-079_PubQuality
7.26 CDCl3
F3C
O
O
S
F
HN
O
17
0.98
0.98
1.00
0.90
1.08
1.25
1.12
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
f1 (ppm)
4000
3500
3000
2500
2000
1500
1000
500
0

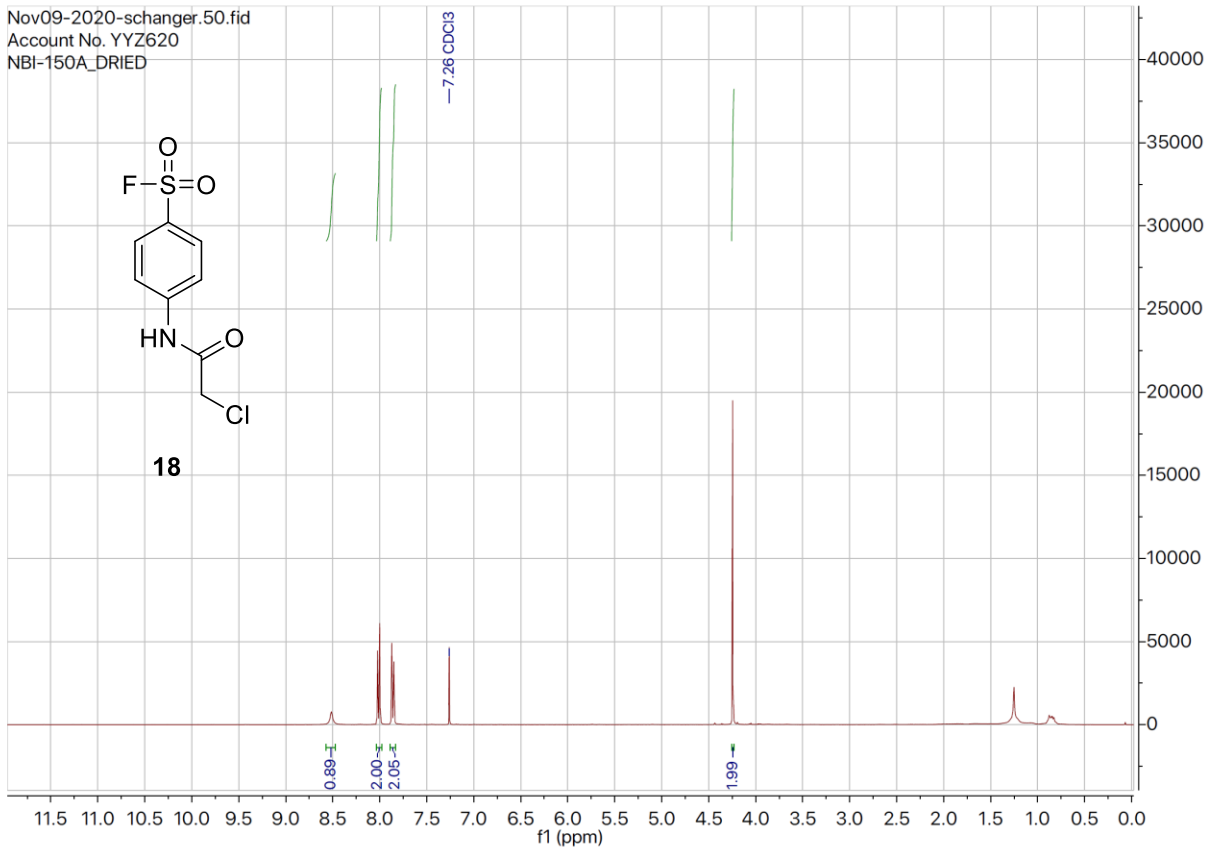
^{13}C-NMR for 17.



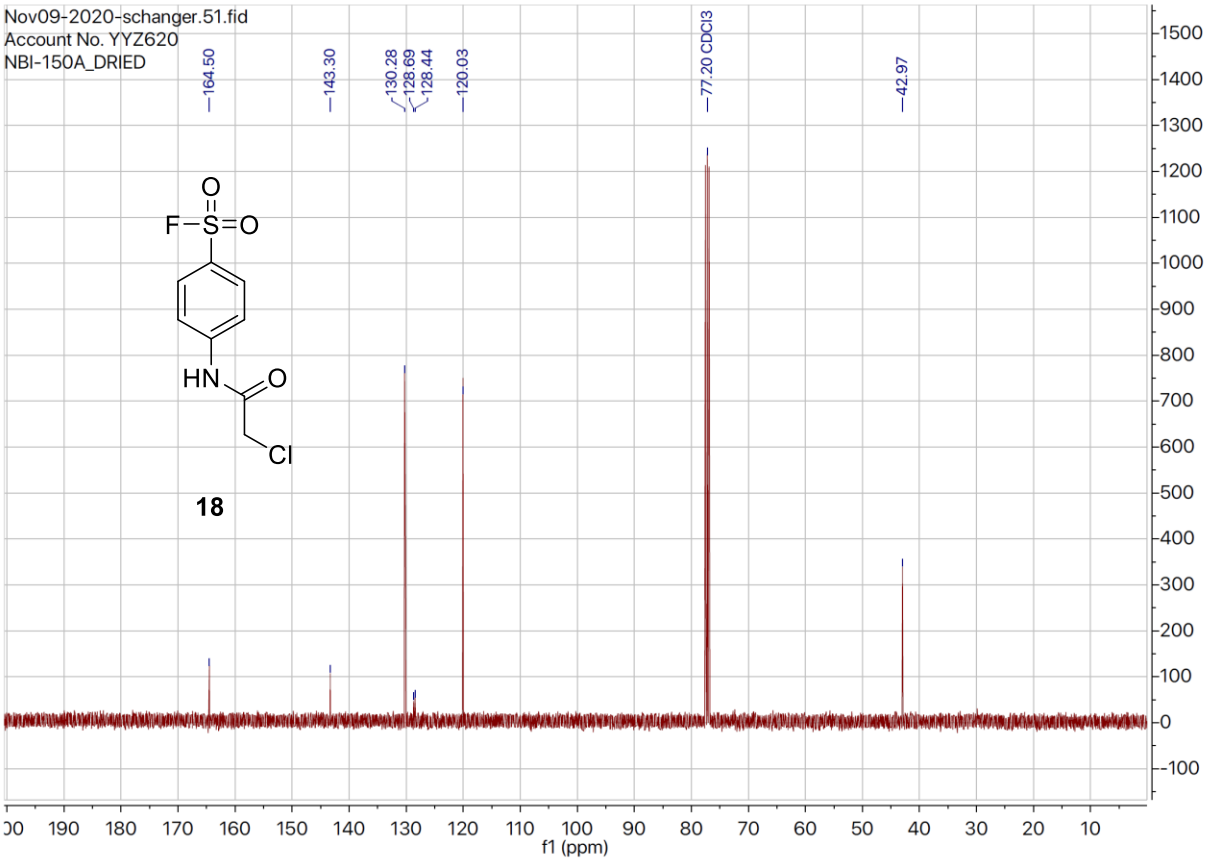
^{19}F-NMR for 17.



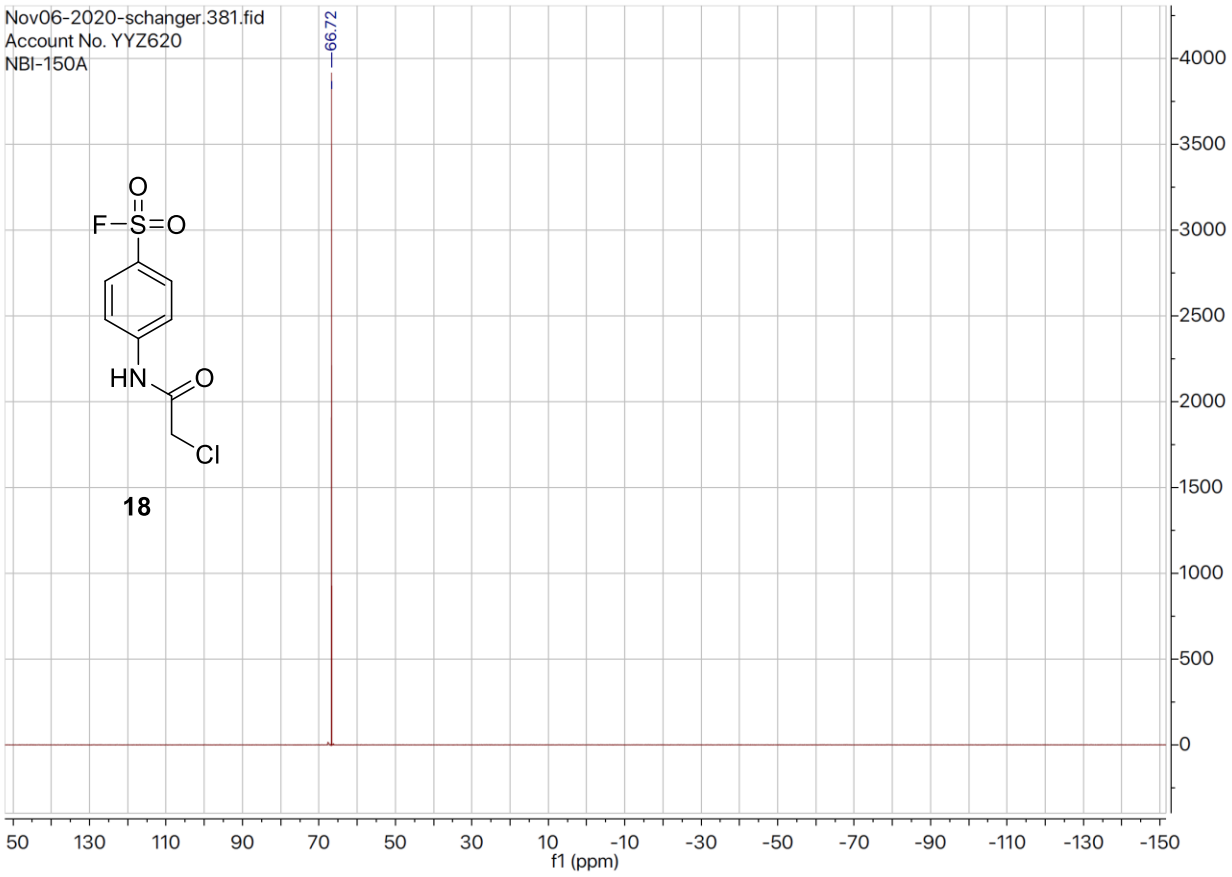
^{1}H-NMR for 18.


Nov09-2020-schanger.50.fid
Account No. YYZ620
NBI-150A_DRIED
7.26 CDCl3
18
0.89
2.00
2.05
1.99
f1 (ppm)

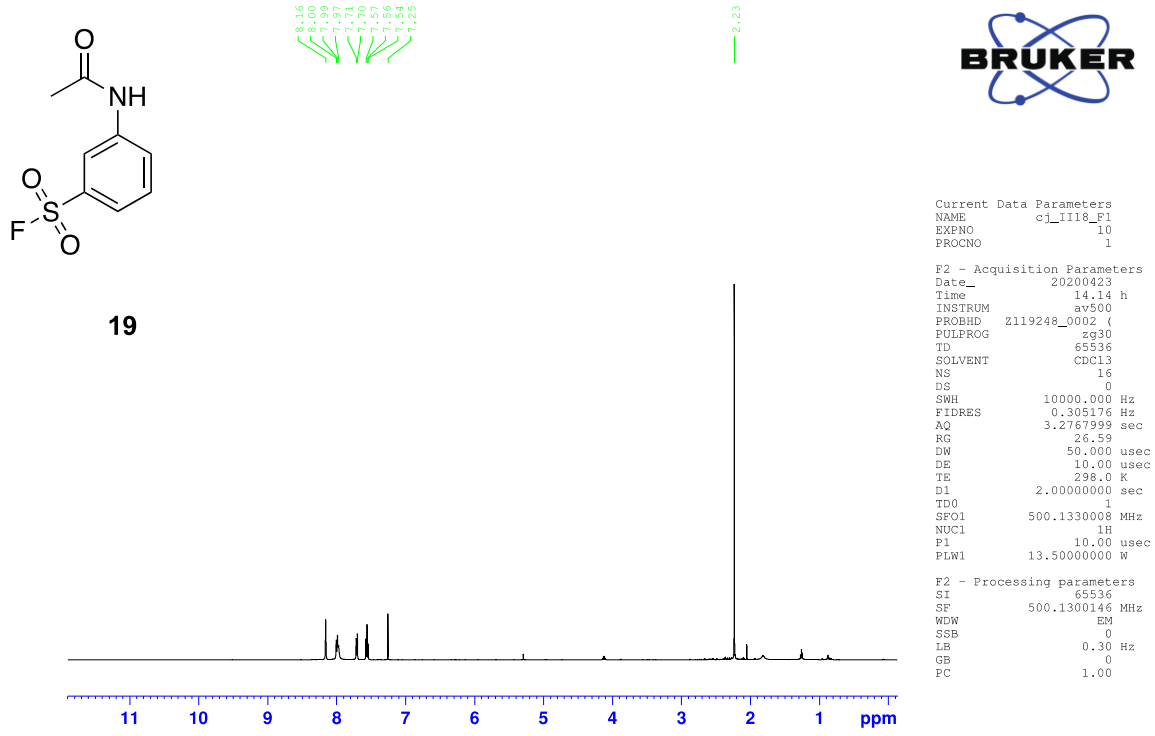
^{13}C-NMR for 18.



^{19}F-NMR for 18.



^{1}H-NMR for 19.

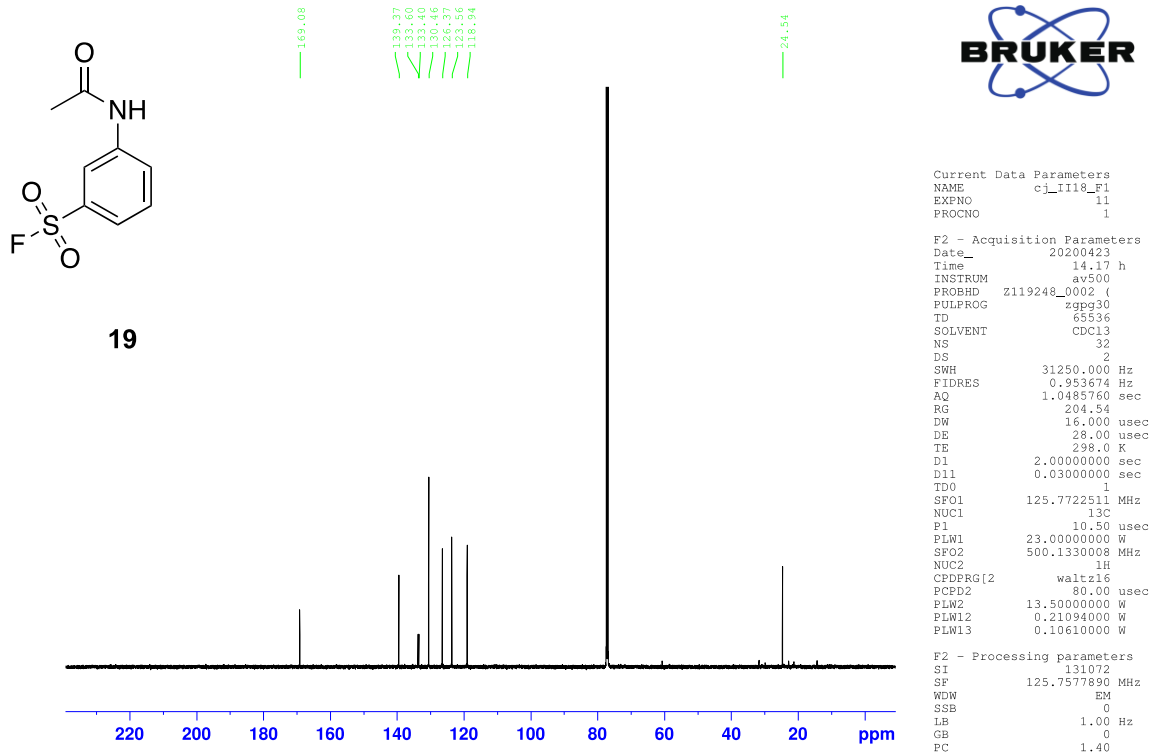



```
Current Data Parameters
NAME          cj_III8_F1
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200423
Time              14.14 h
INSTRUM             av500
PROBHD   Z119248_0002 (
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                    16
DS                     0
SWH           10000.000 Hz
FIDRES            0.305176 Hz
AQ              3.2767999 sec
RG                 26.59
DW                50.000 usec
DE                10.00 usec
TE                298.0 K
D1             2.00000000 sec
TD0                    1
SFO1          500.1330008 MHz
NUC1                  1H
P1                 10.00 usec
PLW1          13.50000000 W

F2 - Processing parameters
SI                 65536
SF            500.1300146 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

^{13}C-NMR for 19.

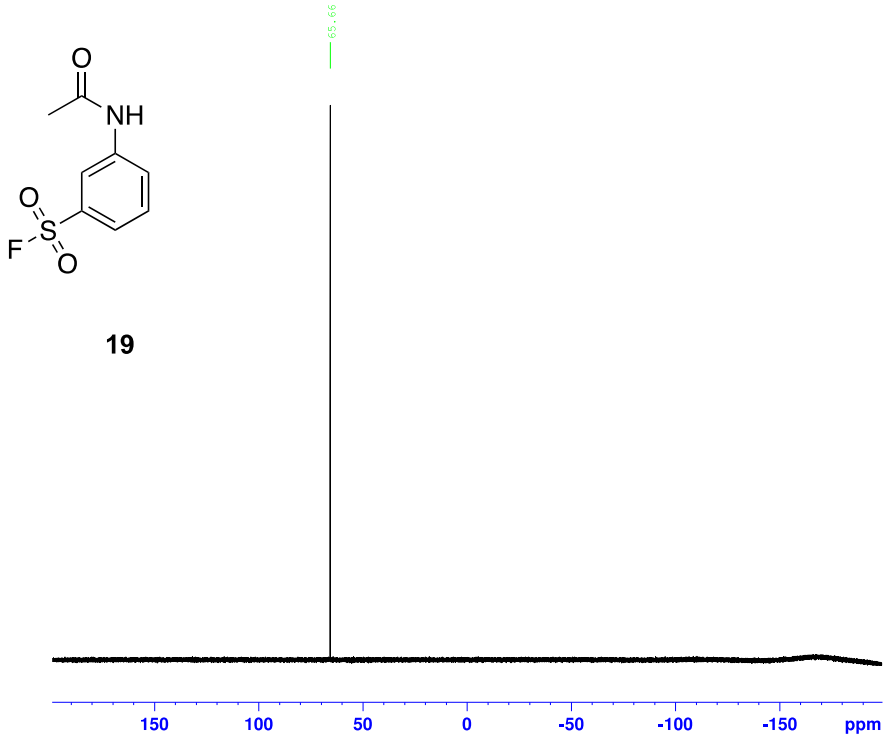



```
Current Data Parameters
NAME          cj_II18_F1
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200423
Time              14.17 h
INSTRUM             av500
PROBHD   Z119248_0002 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                    32
DS                     2
SWH           31250.000 Hz
FIDRES         0.953674 Hz
AQ            1.0485760 sec
RG               204.54
DW               16.000 usec
DE                28.00 usec
TE                298.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1        125.7722511 MHz
NUC1                13C
P1                10.50 usec
PLW1         23.00000000 W
SFO2        500.1330008 MHz
NUC2                1H
CPDPRG[2         waltz16
PCPD2             80.00 usec
PLW2         13.50000000 W
PLW12         0.21094000 W
PLW13         0.10610000 W

F2 - Processing parameters
SI                131072
SF          125.7577890 MHz
WDW                 EM
SSB                  0
LB                1.00 Hz
GB                   0
PC                1.40
```

^{19}F-NMR for 19.



BRUKER

```
Current Data Parameters
NAME        Jian's NMRs
EXPNO               110
PROCNO                1

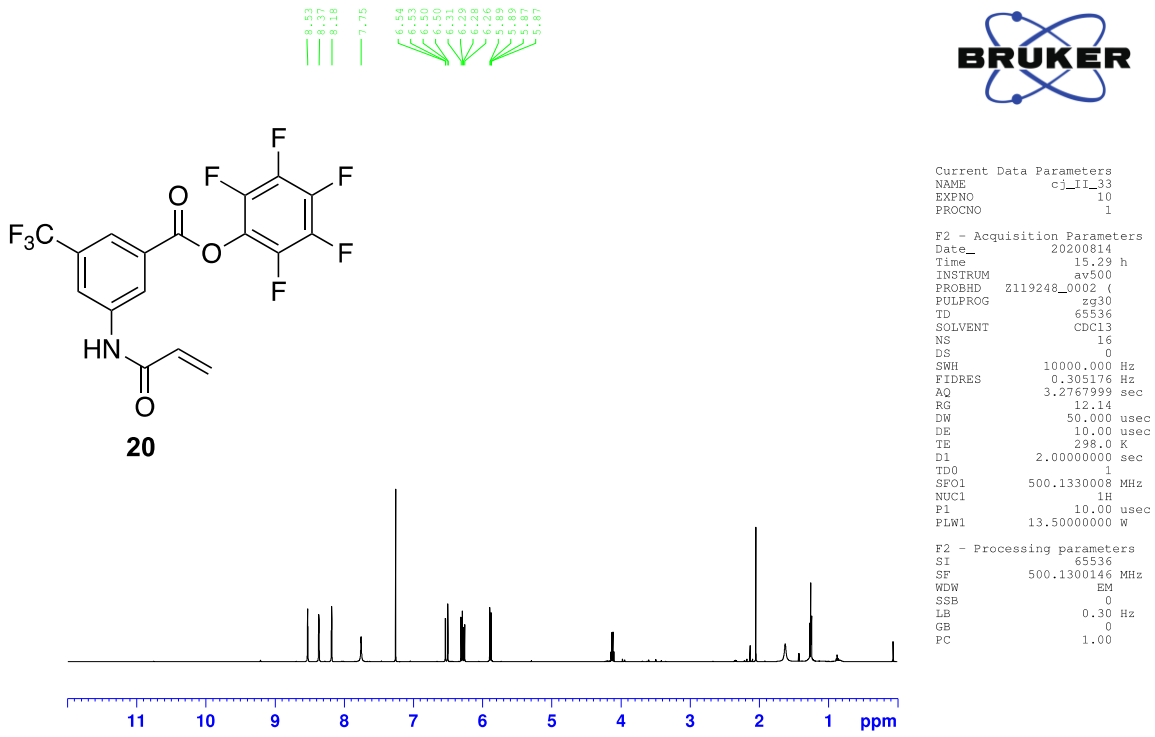
F2 - Acquisition Parameters
Date_          20201028
Time              14.15
INSTRUM           av400
PROBHD   5 mm PABBO BB/
PULPROG      zgfhigqn.2
TD               262144
SOLVENT            CDCl3
NS                    32
DS                     0
SWH           150000.000 Hz
FIDRES          0.572205 Hz
AQ              0.8738133 sec
RG                189.85
DW                3.333 usec
DE                 6.50 usec
TE                296.5 K
D1            1.00000000 sec
D11           0.03000000 sec
D12           0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         376.4983660 MHz
NUC1                 19F
P1                14.50 usec
PLW1        17.00000000 W

======== CHANNEL f2 ========
SFO2         400.1324008 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2        13.00000000 W
PLW12        0.36111000 W

F2 - Processing parameters
SI               262144
SF           376.4983660 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.00
```

[1]H-NMR for 20.

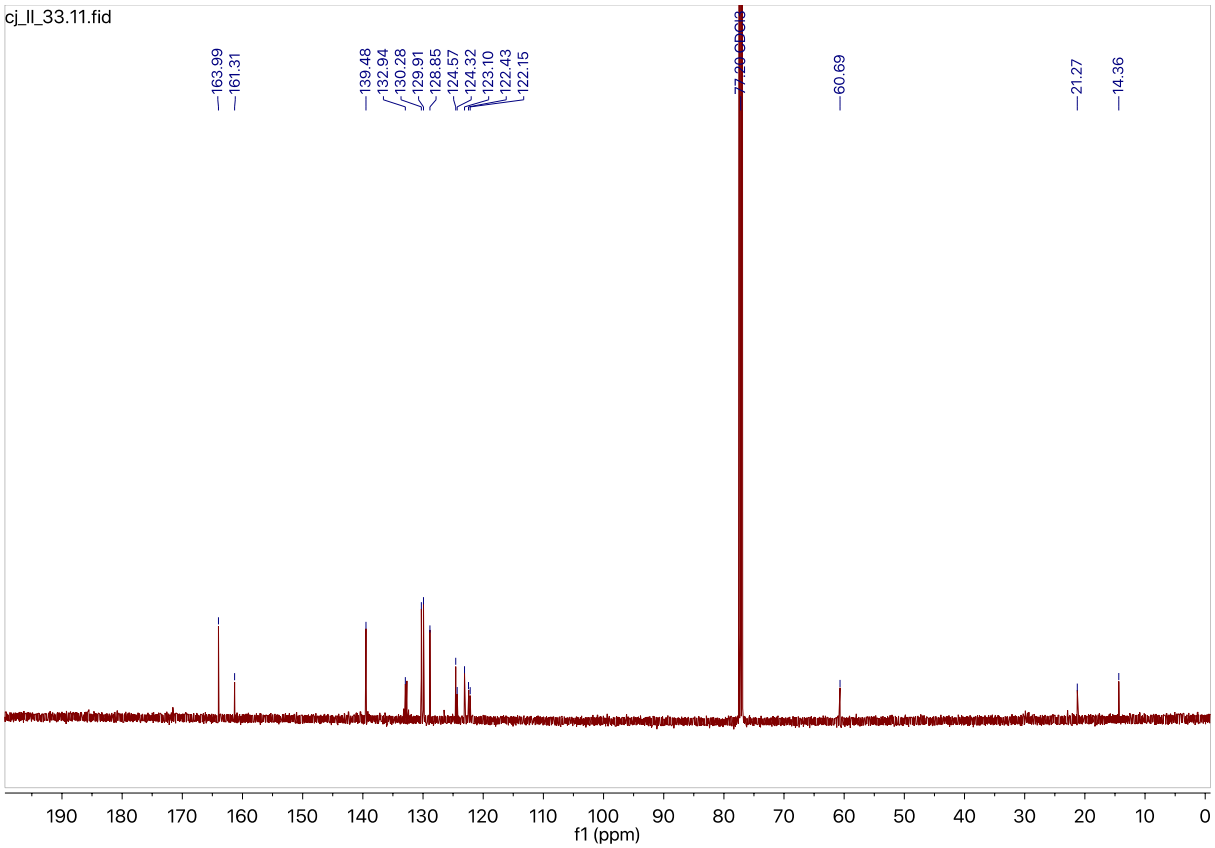



```
Current Data Parameters
NAME                  cj_II_33
EXPNO                       10
PROCNO                       1

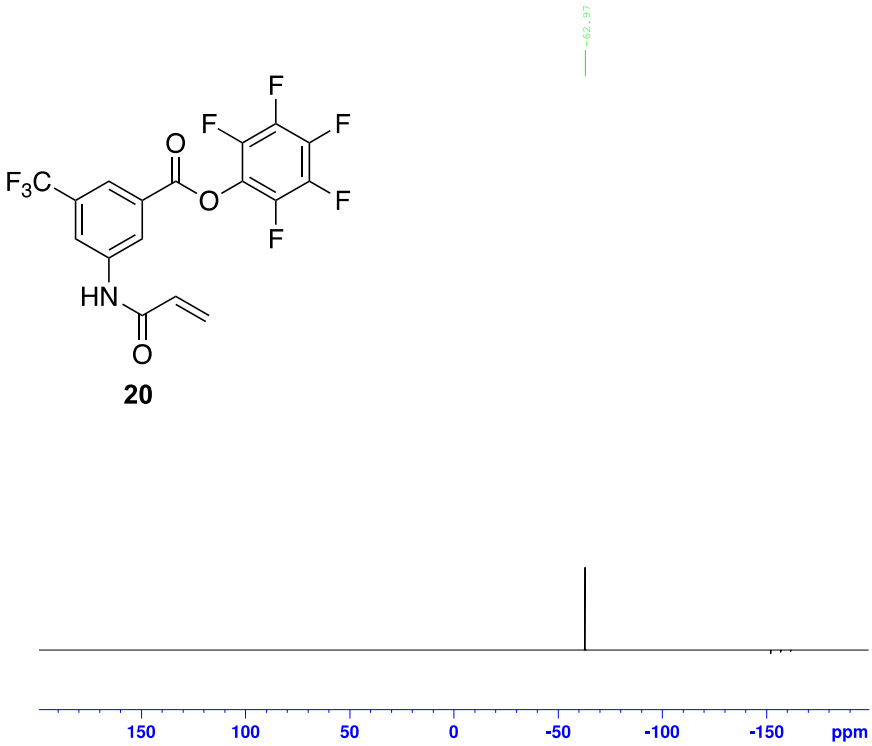
F2 - Acquisition Parameters
Date_               20200814
Time                   15.29 h
INSTRUM                av500
PROBHD    Z119248_0002 (
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                        16
DS                         0
SWH               10000.000 Hz
FIDRES                0.305176 Hz
AQ                 3.2767999 sec
RG                        12.14
DW                    50.000 usec
DE                    10.00 usec
TE                    298.0 K
D1                 2.00000000 sec
TD0                        1
SFO1           500.1330008 MHz
NUC1                      1H
P1                    10.00 usec
PLW1          13.50000000 W

F2 - Processing parameters
SI                     65536
SF             500.1300146 MHz
WDW                       EM
SSB                        0
LB                    0.30 Hz
GB                         0
PC                    1.00
```

^{13}C-NMR for 20.



^{19}F-NMR for 20.




```
Current Data Parameters
NAME          Jian's NMRs
EXPNO                 240
PROCNO                  1

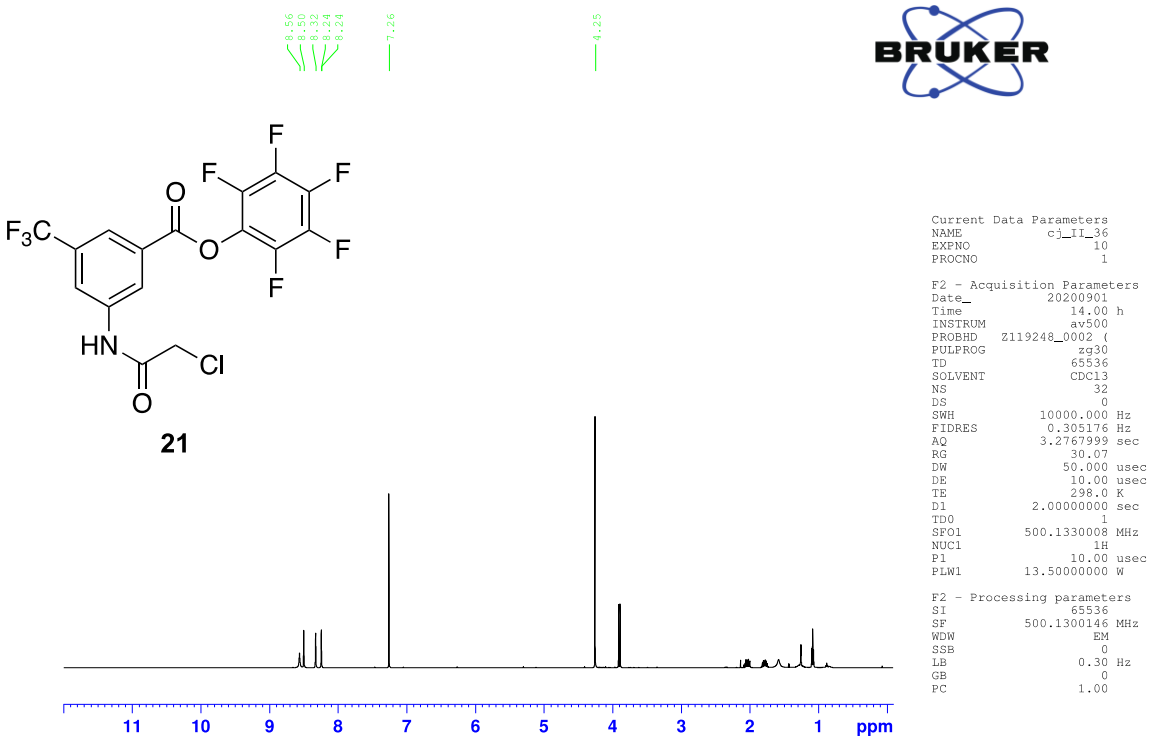
F2 - Acquisition Parameters
Date_              20201029
Time                 15.48
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG       zgfhigqn.2
TD                   262144
SOLVENT              CDCl3
NS                      32
DS                       0
SWH             150000.000 Hz
FIDRES            0.572205 Hz
AQ                0.8738133 sec
RG                   189.85
DW                   3.333 usec
DE                    6.50 usec
TE                   296.6 K
D1              1.00000000 sec
D11             0.03000000 sec
D12             0.00002000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          376.4983660 MHz
NUC1                   19F
P1                   14.50 usec
PLW1          17.00000000 W

======== CHANNEL f2 ========
SFO2          400.1324008 MHz
NUC2                   1H
CPDPRG[2           waltz16
PCPD2                90.00 usec
PLW2          13.00000000 W
PLW12          0.36111000 W

F2 - Processing parameters
SI                   262144
SF            376.4983660 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.00
```

^{1}H-NMR for 21.

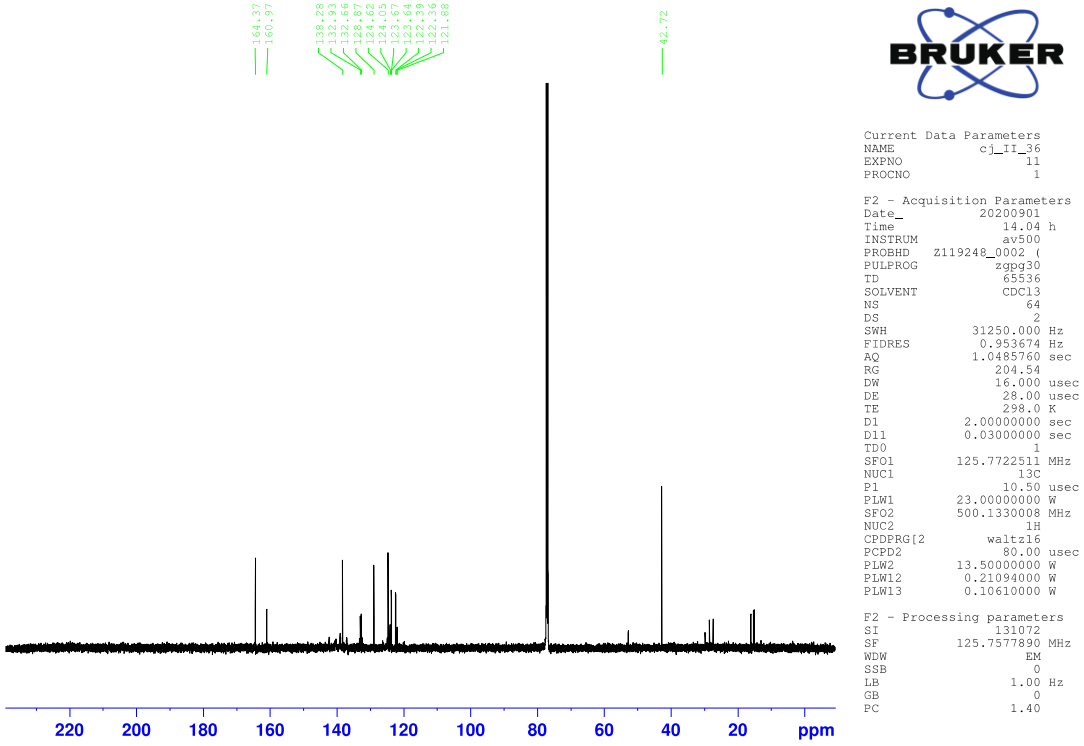



```
Current Data Parameters
NAME                  cj_II_36
EXPNO                       10
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20200901
Time                     14.00 h
INSTRUM                  av500
PROBHD    Z119248_0002 (
PULPROG                  zg30
TD                      65536
SOLVENT                 CDCl3
NS                         32
DS                          0
SWH                 10000.000 Hz
FIDRES                0.305176 Hz
AQ                    3.2767999 sec
RG                        30.07
DW                       50.000 usec
DE                       10.00 usec
TE                       298.0 K
D1                  2.00000000 sec
TD0                         1
SFO1                500.1330008 MHz
NUC1                        1H
P1                       10.00 usec
PLW1                13.50000000 W

F2 - Processing parameters
SI                      65536
SF                  500.1300146 MHz
WDW                        EM
SSB                         0
LB                       0.30 Hz
GB                          0
PC                       1.00
```

^{13}C-NMR for 21.

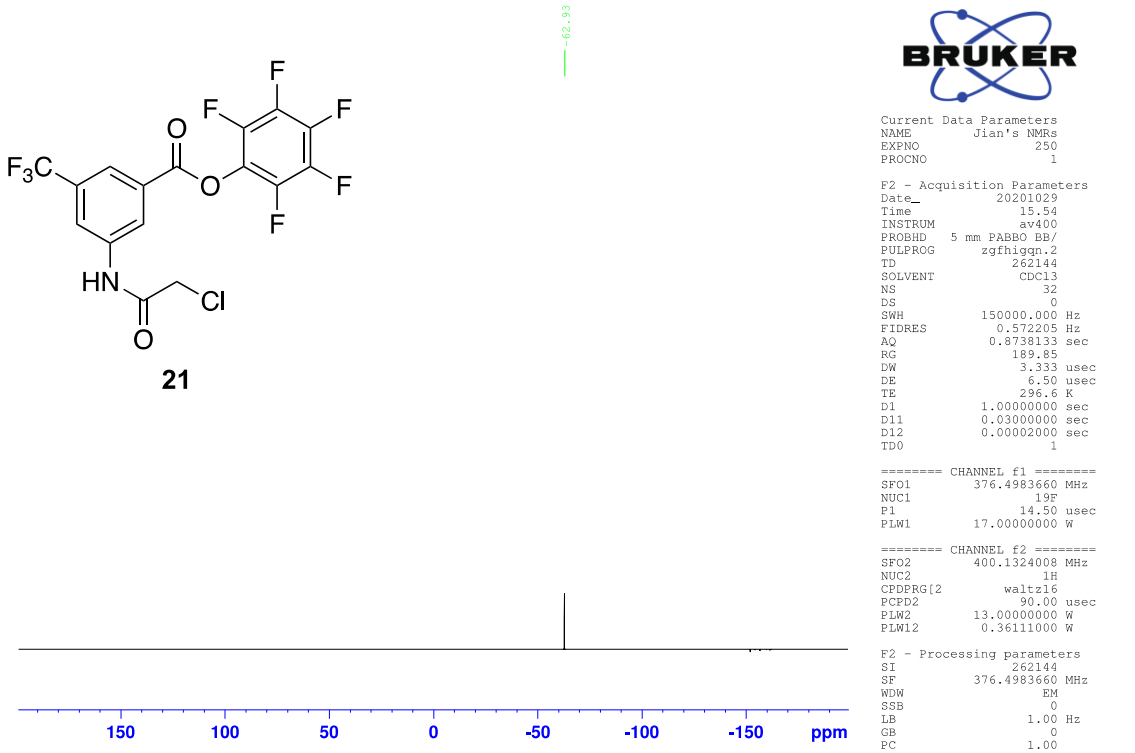



```
Current Data Parameters
NAME            cj_II_36
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20200901
Time                14.04 h
INSTRUM             av500
PROBHD    Z119248_0002 (
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                    64
DS                     2
SWH            31250.000 Hz
FIDRES          0.953674 Hz
AQ              1.0485760 sec
RG                204.54
DW                16.000 usec
DE                28.00 usec
TE                298.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1
SFO1         125.7722511 MHz
NUC1                 13C
P1                 10.50 usec
PLW1          23.00000000 W
SFO2         500.1330008 MHz
NUC2                 1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          13.50000000 W
PLW12          0.21094000 W
PLW13          0.10610000 W

F2 - Processing parameters
SI                131072
SF           125.7577890 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

^{19}F-NMR for 21.




```
Current Data Parameters
NAME         Jian's NMRs
EXPNO                250
PROCNO                 1

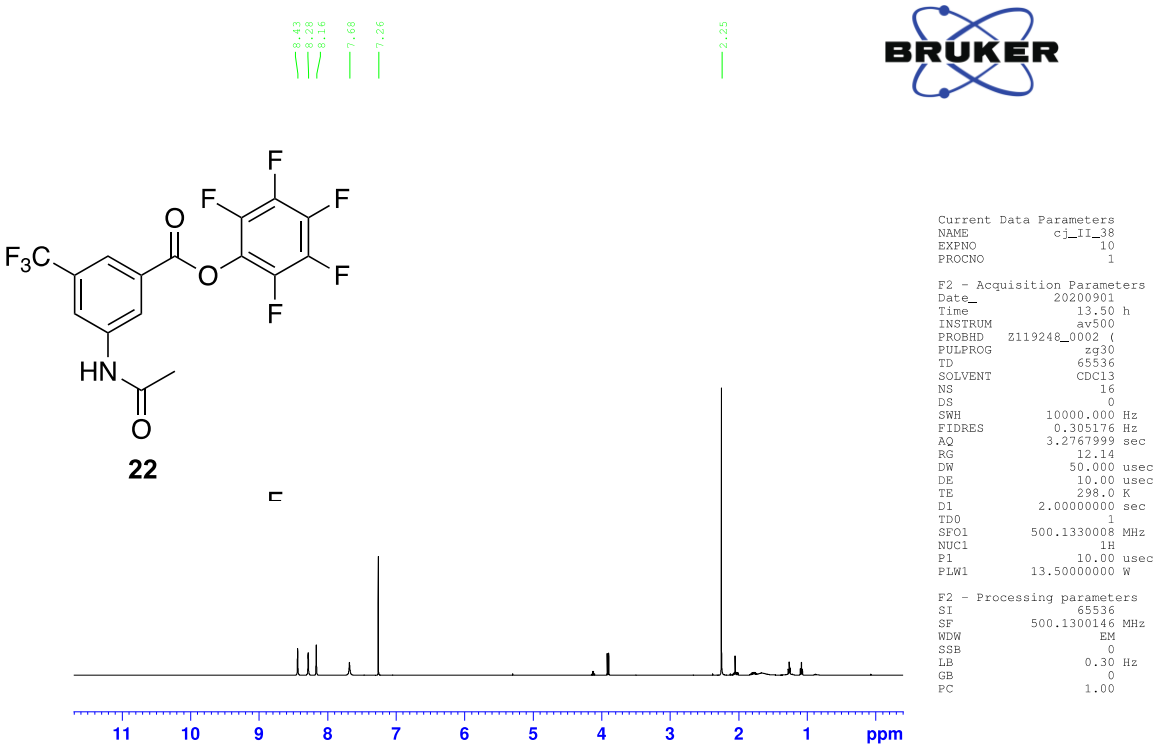
F2 - Acquisition Parameters
Date_           20201029
Time               15.54
INSTRUM           av400
PROBHD   5 mm PABBO BB/
PULPROG      zgfhigqn.2
TD                262144
SOLVENT            CDCl3
NS                    32
DS                     0
SWH           150000.000 Hz
FIDRES          0.572205 Hz
AQ              0.8738133 sec
RG                189.85
DW                3.333 usec
DE                6.50 usec
TE                296.6 K
D1             1.00000000 sec
D11            0.03000000 sec
D12            0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          376.4983660 MHz
NUC1                  19F
P1                 14.50 usec
PLW1         17.00000000 W

======== CHANNEL f2 ========
SFO2          400.1324008 MHz
NUC2                  1H
CPDPRG[2           waltz16
PCPD2             90.00 usec
PLW2         13.00000000 W
PLW12         0.36111000 W

F2 - Processing parameters
SI                262144
SF          376.4983660 MHz
WDW                   EM
SSB                    0
LB                 1.00 Hz
GB                     0
PC                 1.00
```

[1]H-NMR for 22.

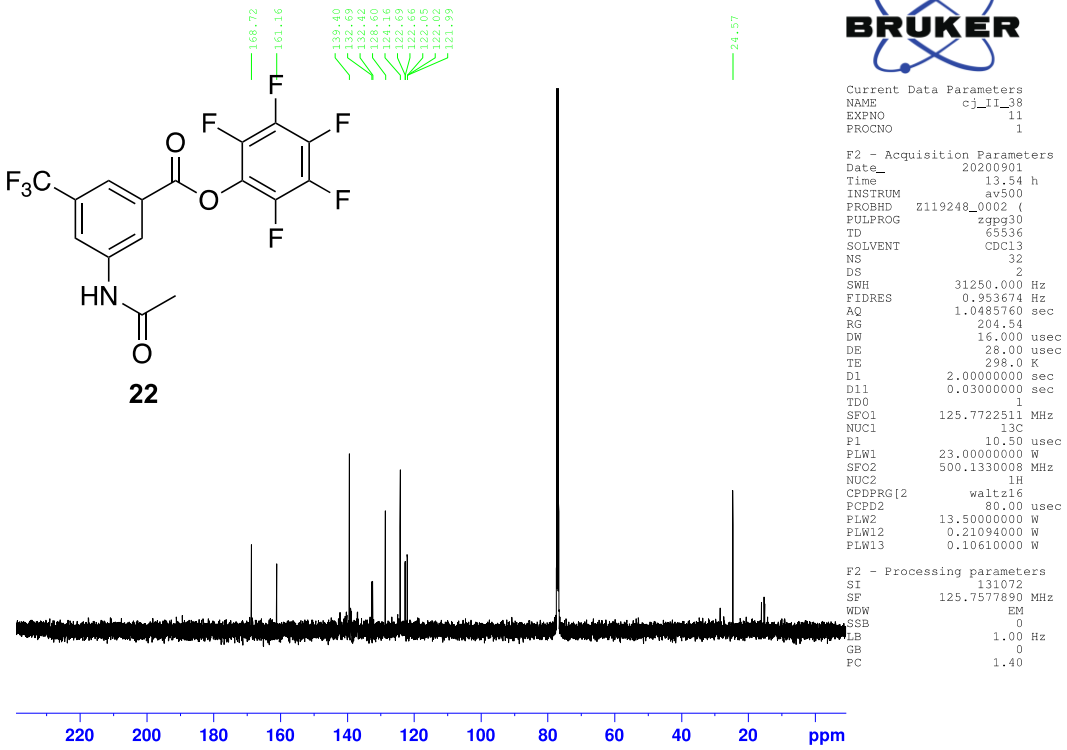



```
Current Data Parameters
NAME            cj_II_38
EXPNO                 10
PROCNO                 1

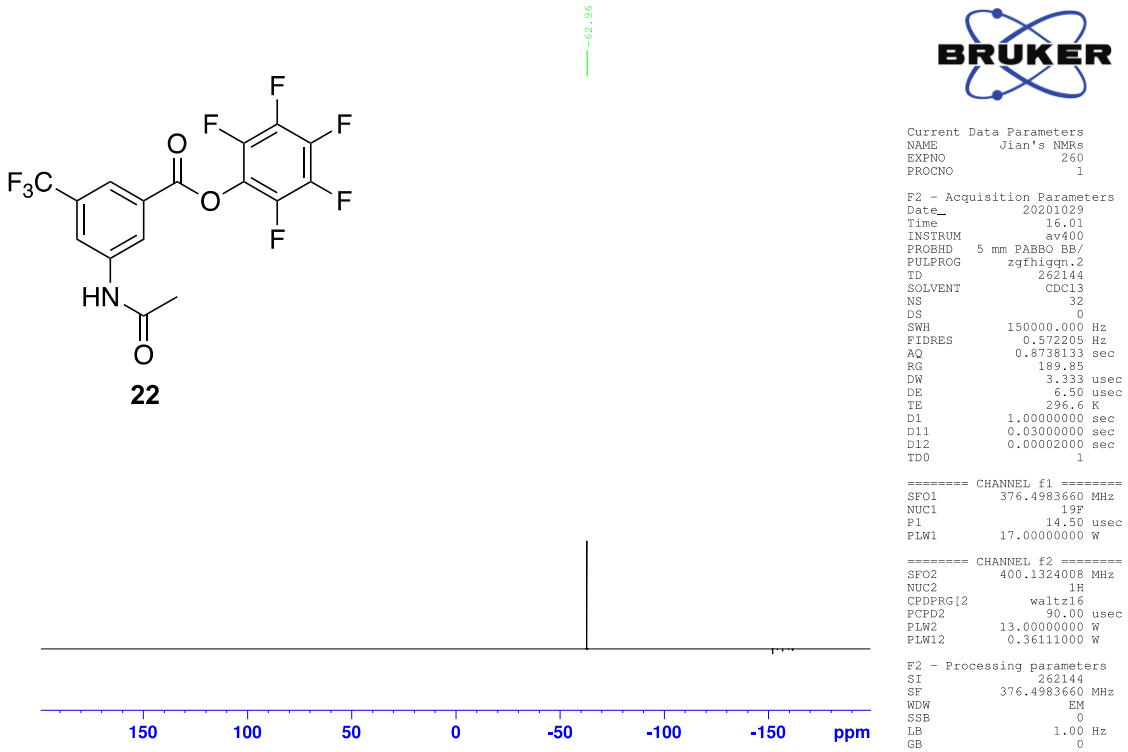
F2 - Acquisition Parameters
Date_           20200901
Time              13.50 h
INSTRUM             av500
PROBHD    Z119248_0002 (
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                     16
DS                      0
SWH           10000.000 Hz
FIDRES         0.305176 Hz
AQ             3.2767999 sec
RG                 12.14
DW                50.000 usec
DE                10.00 usec
TE                298.0 K
D1             2.00000000 sec
TD0                    1
SFO1          500.1330008 MHz
NUC1                  1H
P1                 10.00 usec
PLW1          13.50000000 W

F2 - Processing parameters
SI                  65536
SF            500.1300146 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

^{13}C-NMR for 22.



^{19}F-NMR for 22.




```
Current Data Parameters
NAME          Jian's NMRs
EXPNO                 260
PROCNO                  1

F2 - Acquisition Parameters
Date_              20201029
Time                 16.01
INSTRUM              av400
PROBHD   5 mm PABBO BB/
PULPROG       zgfhigqn.2
TD                 262144
SOLVENT             CDCl3
NS                     32
DS                      0
SWH           150000.000 Hz
FIDRES          0.572205 Hz
AQ             0.8738133 sec
RG                189.85
DW                 3.333 usec
DE                 6.50 usec
TE                296.6 K
D1             1.00000000 sec
D11            0.03000000 sec
D12            0.00002000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          376.4983660 MHz
NUC1                   19F
P1                  14.50 usec
PLW1          17.00000000 W

======== CHANNEL f2 ========
SFO2          400.1324008 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              90.00 usec
PLW2          13.00000000 W
PLW12          0.36111000 W

F2 - Processing parameters
SI                 262144
SF            376.4983660 MHz
WDW                    EM
SSB                     0
LB                  1.00 Hz
GB                      0
PC                  1.00
```

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