Supporting information

Lysine-Targeted Reversible Covalent Ligand Discovery for Proteins via Phage Display

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I. Supplementary Figures

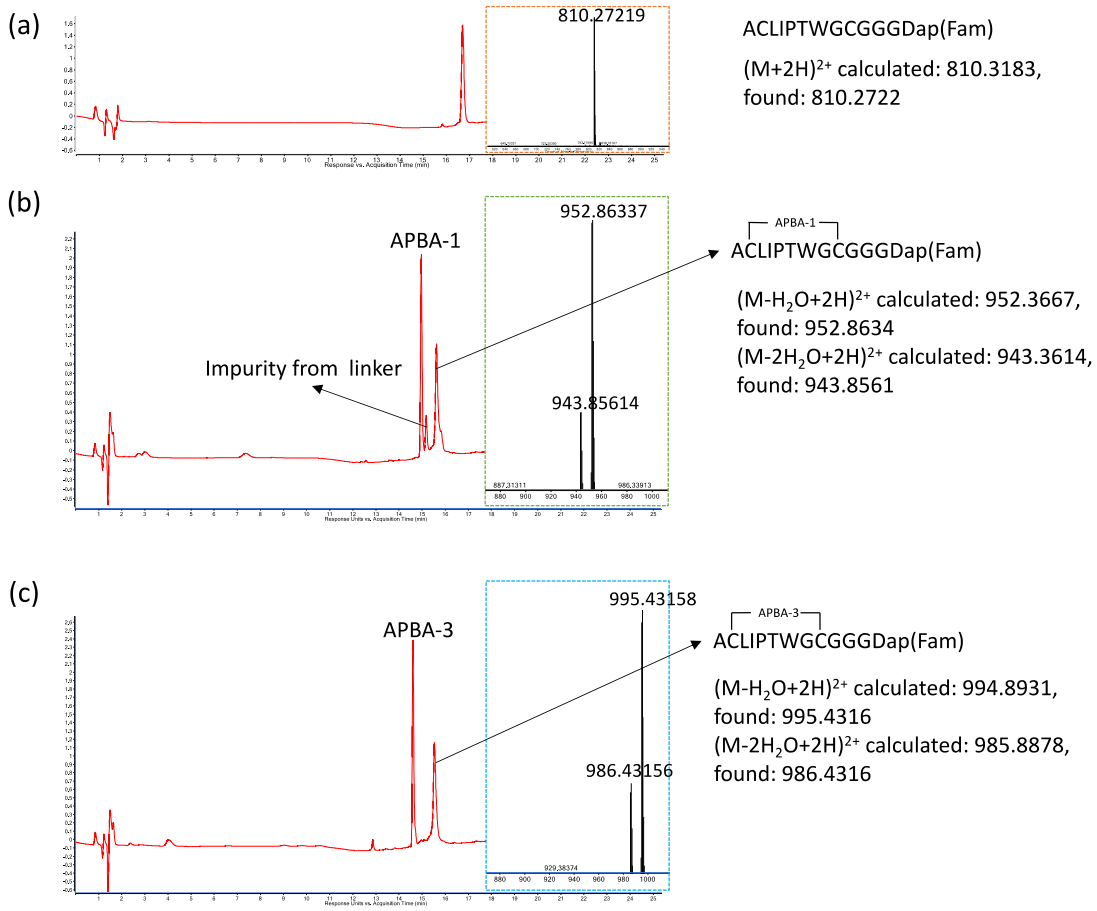


Figure S1. LC-MS analysis of 50 μM peptide ACLIPTWGCGGGDap(Fam) alone (a), conjugated with 0.3 mM **APBA-1** (b) and **APBA-3** (c) over 2 h at room temperature. These chromatograms (recorded with absorbance at 254 nm) show quantitative peptide stapling by **APBA-1/3** under the experimental conditions.

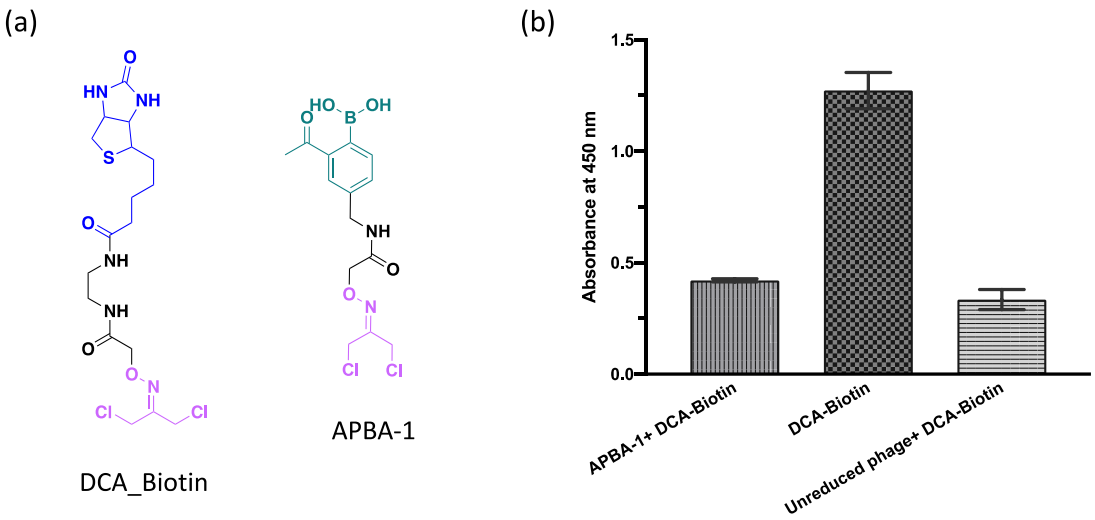


Figure S2. Confirmation of phage labeling by DCA derivatives. (a) Chemical structure of **DCA-Biotin** and **APBA-1**. (b) Confirmation of **DCA-Biotin** labeling of phage library via streptavidin pulldown (experimental details see S24 of the SI). Treating the non-reduced and a TCEP reduced phage library respectively with **DCA-Biotin** gives little versus complete streptavidin pulldown, affirming the validity of this assay for evaluating the percentage of phage biotinylation. Consistently, treating the reduced library with **APBA-1** first and then by **DCA-biotin** resulted in little pulldown, confirming highly efficient **APBA-1** modification.

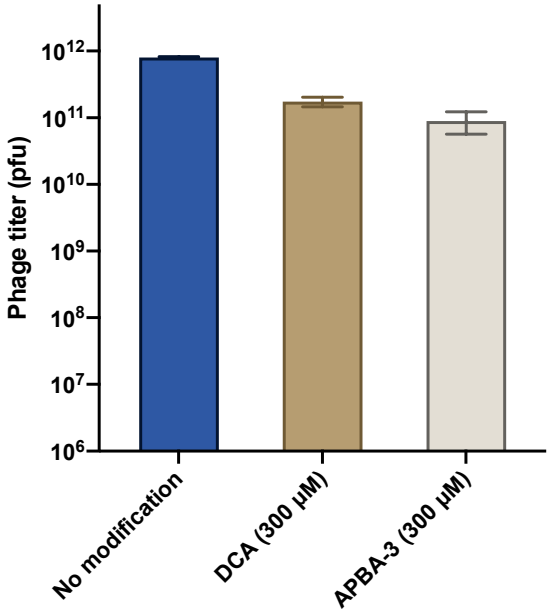


Figure **S3**. Titers of phage treated with **DCA** and **APBA-3** respectively. In comparison to the untreated control, a slight reduction (less than one order magnitude) of phage count was observed for the chemically modified phage, suggesting the crosslinkers are suitable for phage modification. Note the phage count reduction can be partially accounted by imperfect phage precipitation performed after the chemical modification step.

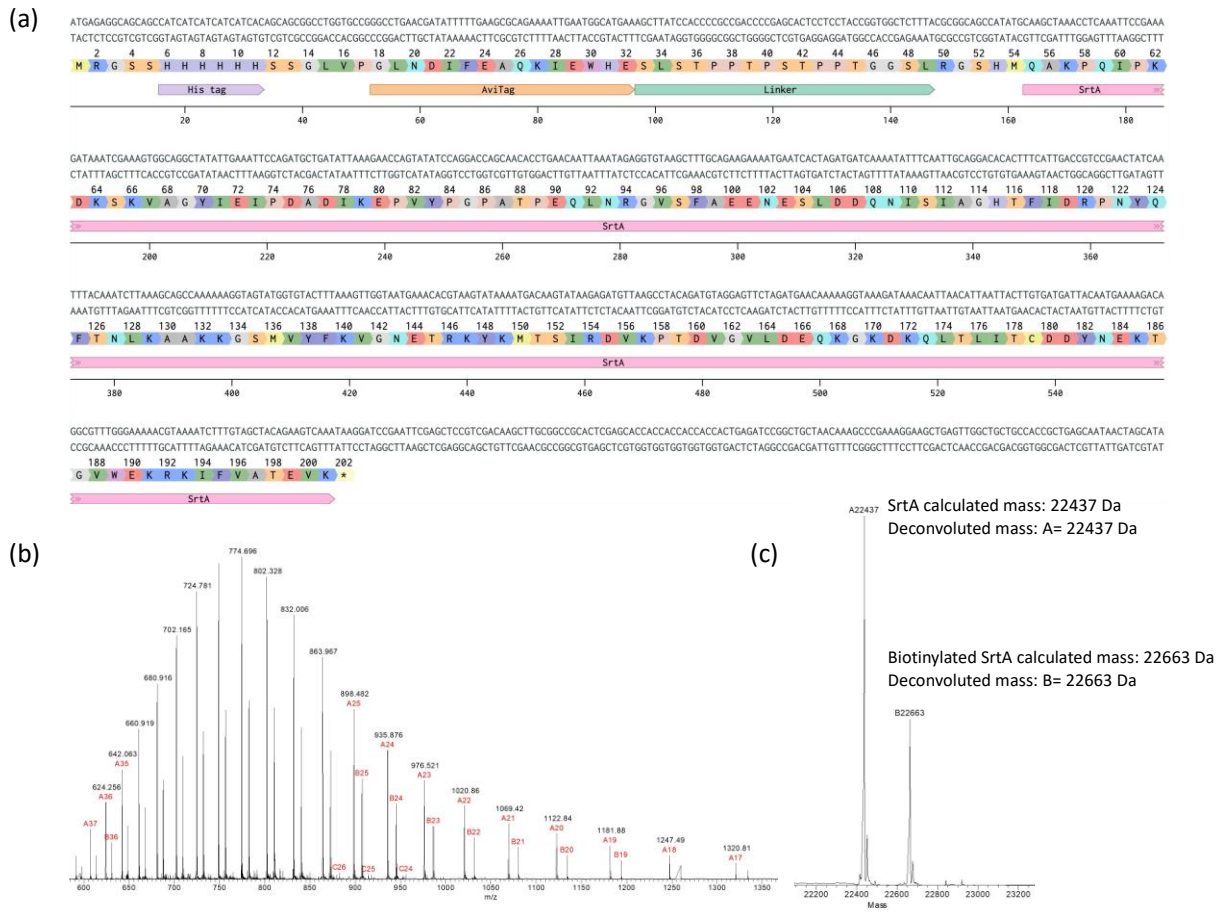


Figure S4. Recombinant expression of biotinylated SrtA. (a) Expression construct for producing biotinylated SrtA using AviTag. A HisTag is used for protein purification, an AviTag is for protein biotinylation in vivo and a rigid proline linker is used to improve BirA biotinylation efficiency. (b) Raw MS data of expressed biotinylated SrtA after purification. (c) Deconvolution mass data of biotinylated SrtA showing a biotinylation efficiency around 40%.

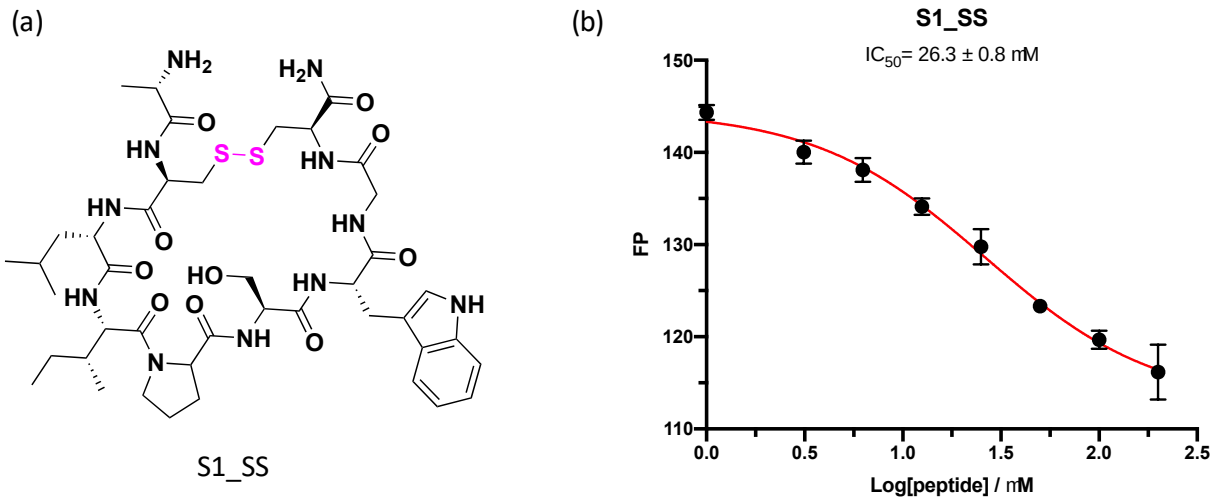


Figure S5. SrtA binding of the peptide hit **S1_SS**. (a) The chemical structure of **S1_SS**. (b) Fluorescence polarization-based competition assay showing **S1_SS** binds SrtA at low micromolar concentrations. A fluorophore-labeled SrtA ligand (**W7-SS***: ACLIPTWGGCGGGDap(Fam)) was used as a competitive binder to enable fluorescence polarization measurement.

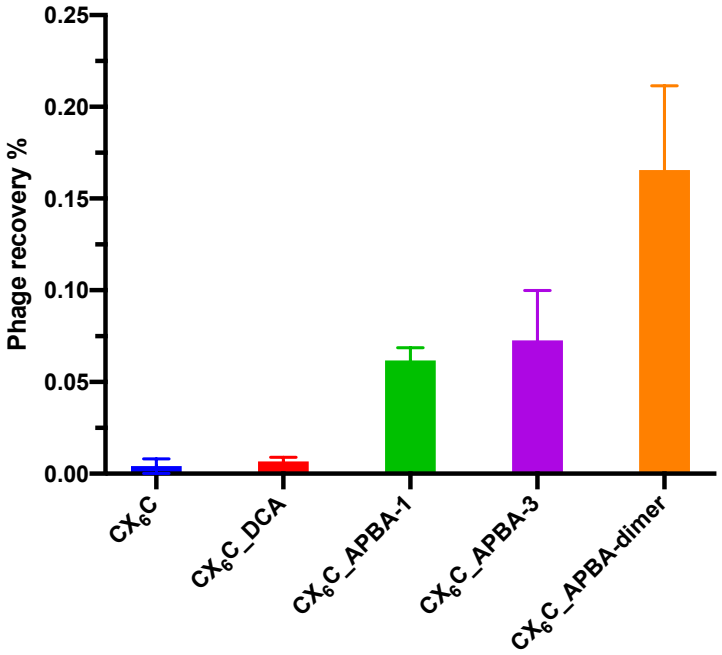


Figure S6. Percentage of phage recovery after the first round of panning against SrtA. The percentages were determined via titering of the input and output phage populations. The APBA-bearing libraries showed higher percentages of retention, presumably due to nonspecific binding, which is eliminated with increasing the stringency of washing: washing buffer containing 0.5% tween20 was used instead of 0.1%.

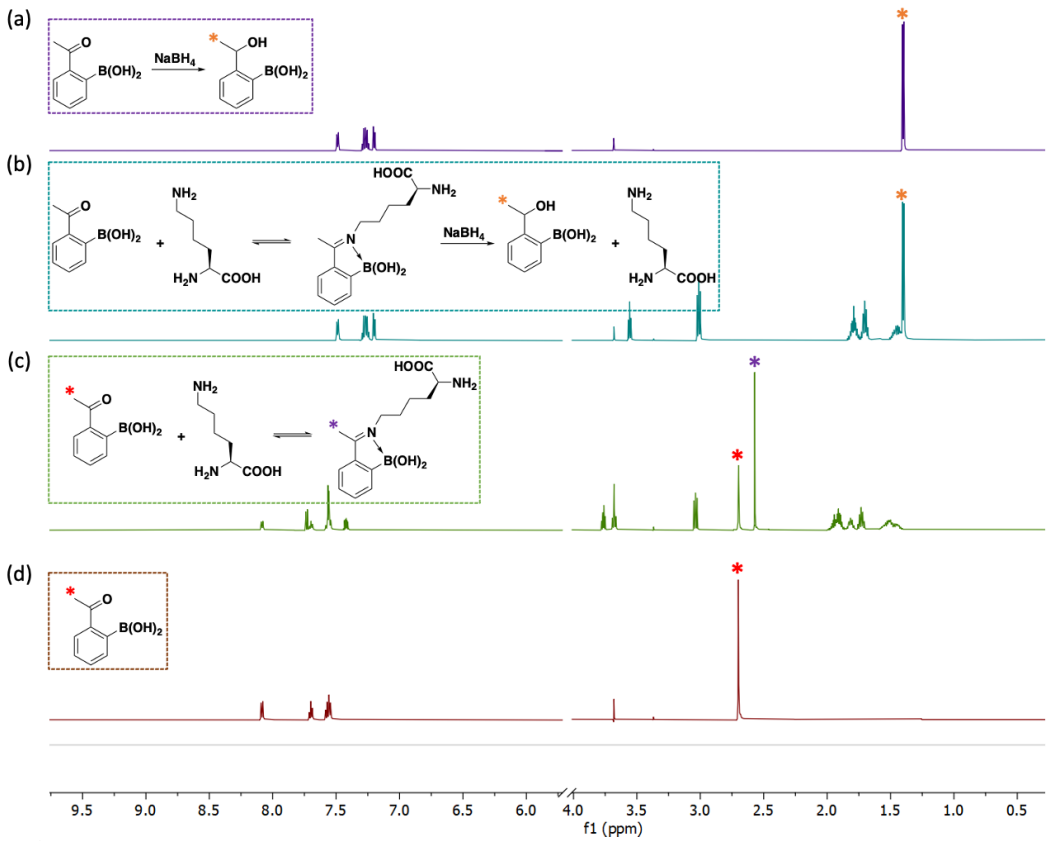


Figure S7. ^{1}H-NMR characterization data of APBA-lysine conjugate upon reduction with $NaBH_4$. (a) 2-APBA (10 mM) incubated with $NaBH_4$ (10 mM) for 1 h; (b) 2-APBA (10 mM) incubated with lys (10 mM) for 1 h, followed by adding $NaBH_4$ (10 mM) for 1 h; (c) 2-APBA (10 mM) incubated with lys (10 mM) for 1 h; (d)) 2-APBA (10 mM) alone.

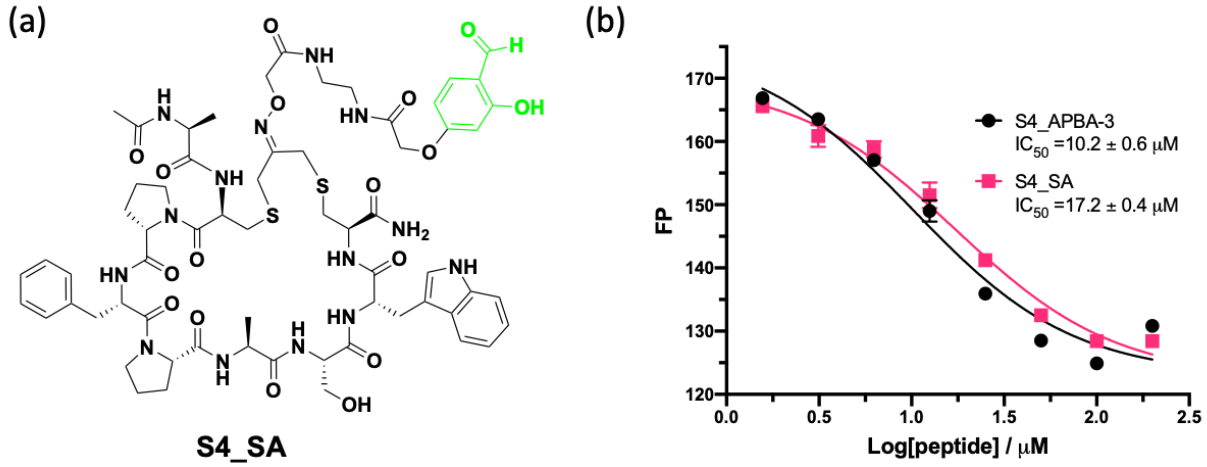


Figure **S8**. (a) Chemical structure of **S4_SA**. (b) IC_{50} of S4 hits measured through competition assay using **W7-F** (ACLIPTWGGCGGGDap(Fam)) as a fluorescence reporter. A similar potency was observed for **S4_SA** in comparison to **S4_APBA-3**.

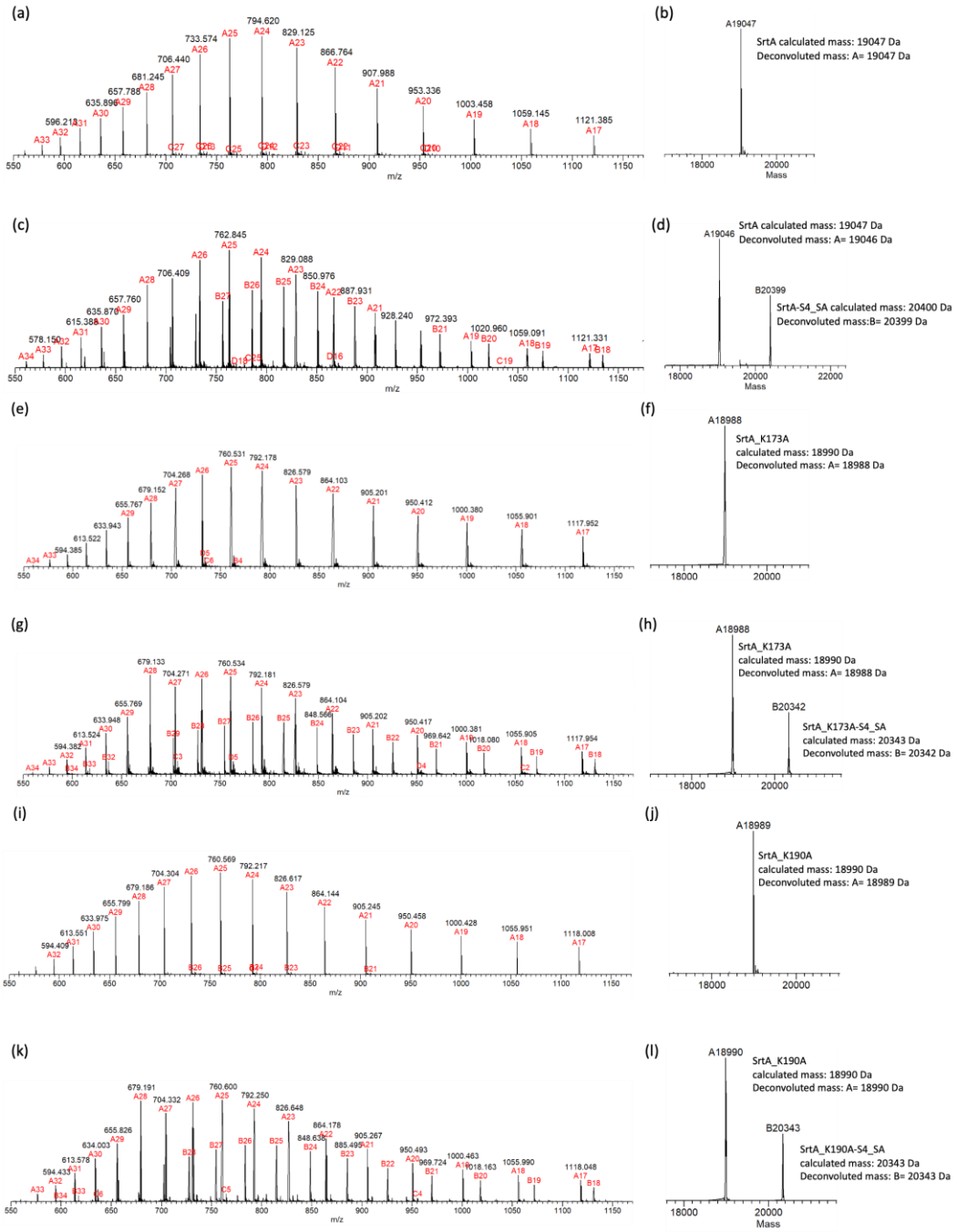


Figure **S9**. Mass spectrometry analysis of SrtA and its covalent adduct with **S4_SA**. (a) and (b) are raw and deconvoluted mass data of expressed SrtA; (c) and (d) are raw and deconvoluted mass data demonstrating Srt-**S4_SA** conjugation formation; (e) and (f) are raw and deconvoluted mass data of expressed SrtA_K173A; (g) and (h) are raw and deconvoluted mass data demonstrating Srt_K173A-**S4_SA** conjugation formation; (i) and (j) are raw and deconvoluted mass data of expressed SrtA_K190A; (k) and (l) are raw and deconvoluted mass data demonstrating Srt_K190A-**S4_SA** conjugation formation. SrtA or Srt A mutants (50 μM) and peptide **S4_SA** (100 μM) were incubated at room temperature overnight, followed by incubation with $NaBH_4$ (5 mM) for 2 h. The mixture was then diluted by water and injected for LC-MS analysis directly.

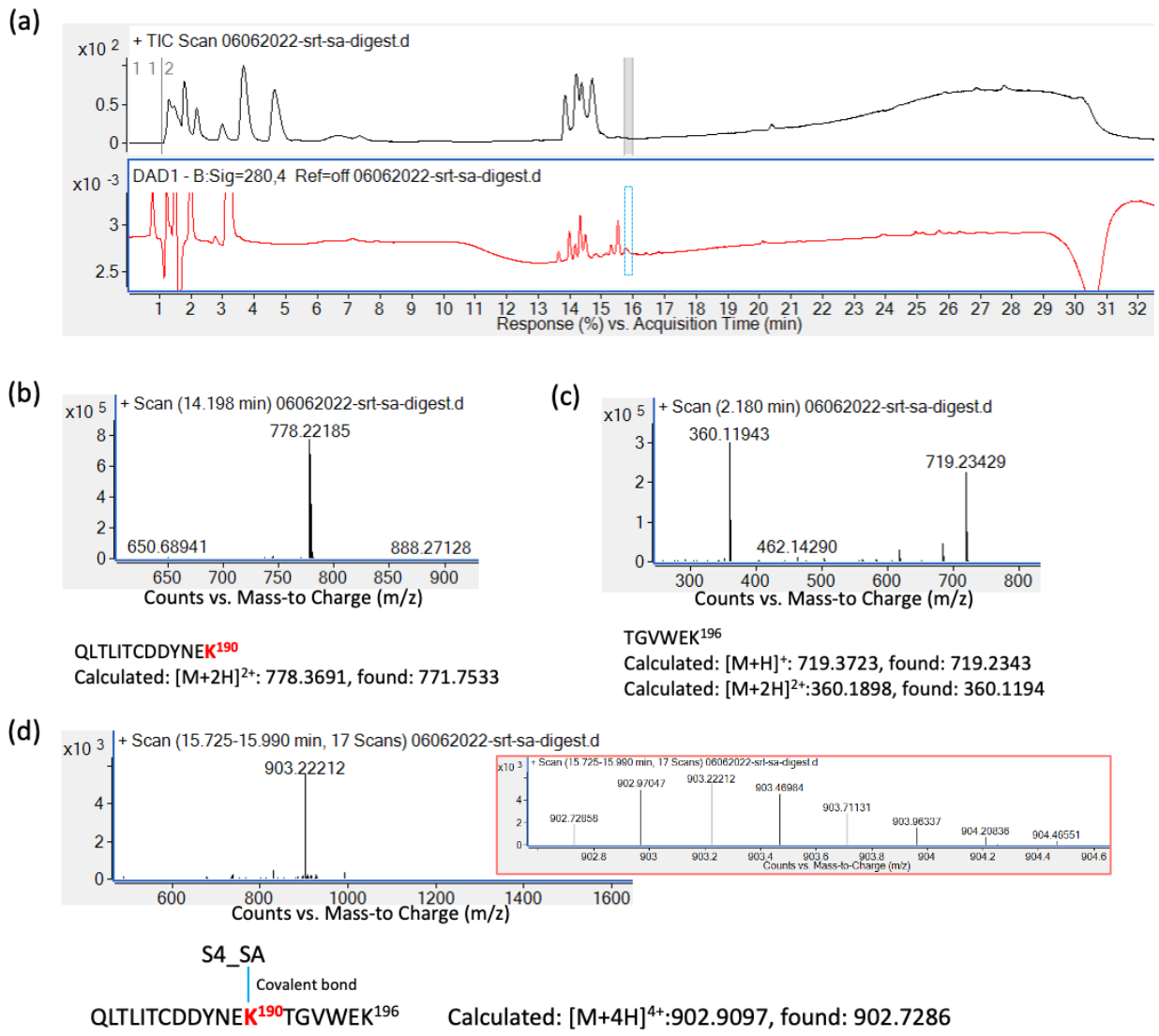


Figure **S10**. Peptide mapping to identify the conjugation site of **S4_SA** on SrtA. (a) The TIC and LC trace (280 nm) of trypsin digested SrtA-**S4_SA** conjugate. The boxes highlight the region where the **S4_SA** adduct was observed. (b) Mass-spec data of peptide fragment QLTLICDDYNEK^{190}. (c) Mass-spec data of peptide fragment TGVWEK^{196}. (d) Mass-spec data corresponds to the **S4_SA** conjugate with QLTLICDDYNEK^{190}TGVWEK^{196}, which indicated that peptide S4_SA conjugate with K^{190} to make it no longer susceptible to trypsin cleavage. The **S4_SA** adduct with K173 could not be identified from this experiment.

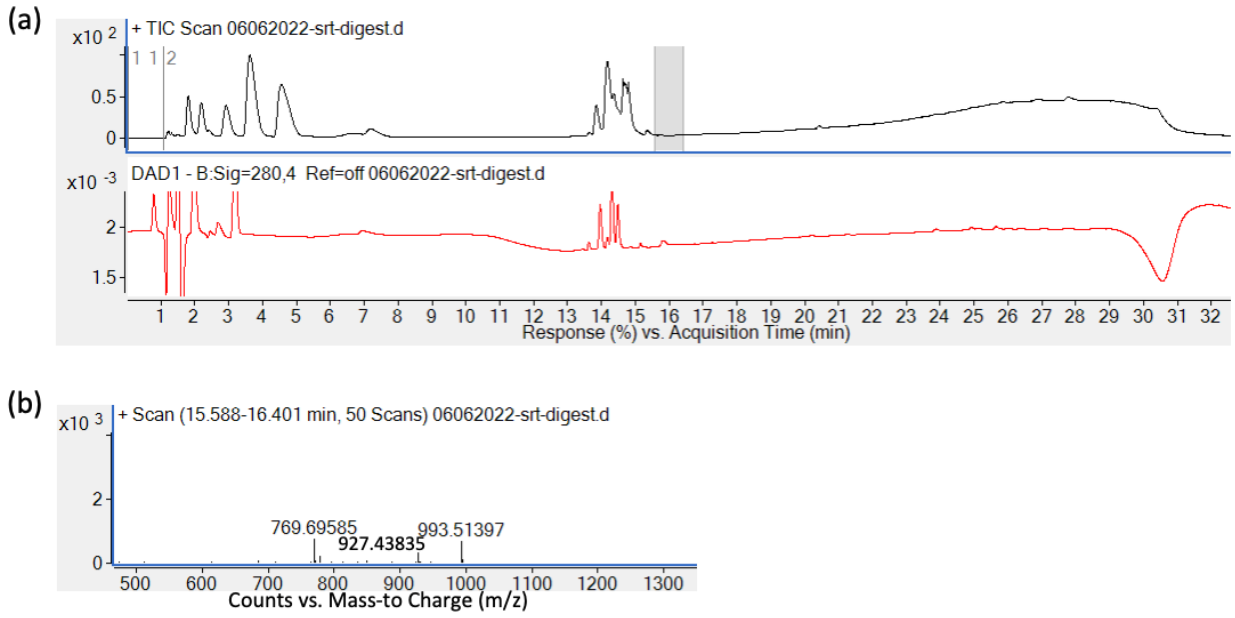


Figure **S11**. Peptide mapping of SrtA alone. (a) The TIC and LC trace of trypsin digested SrtA alone. (b) Mass-spec data of trypsin digested SrtA alone at 15.6-15.9 min, showing the absence of the mass 903.22, which we assigned as the **S4_SA** conjugate with QLTLICDDYNEK^{190}TGVWEK^{196} in Figure S10.

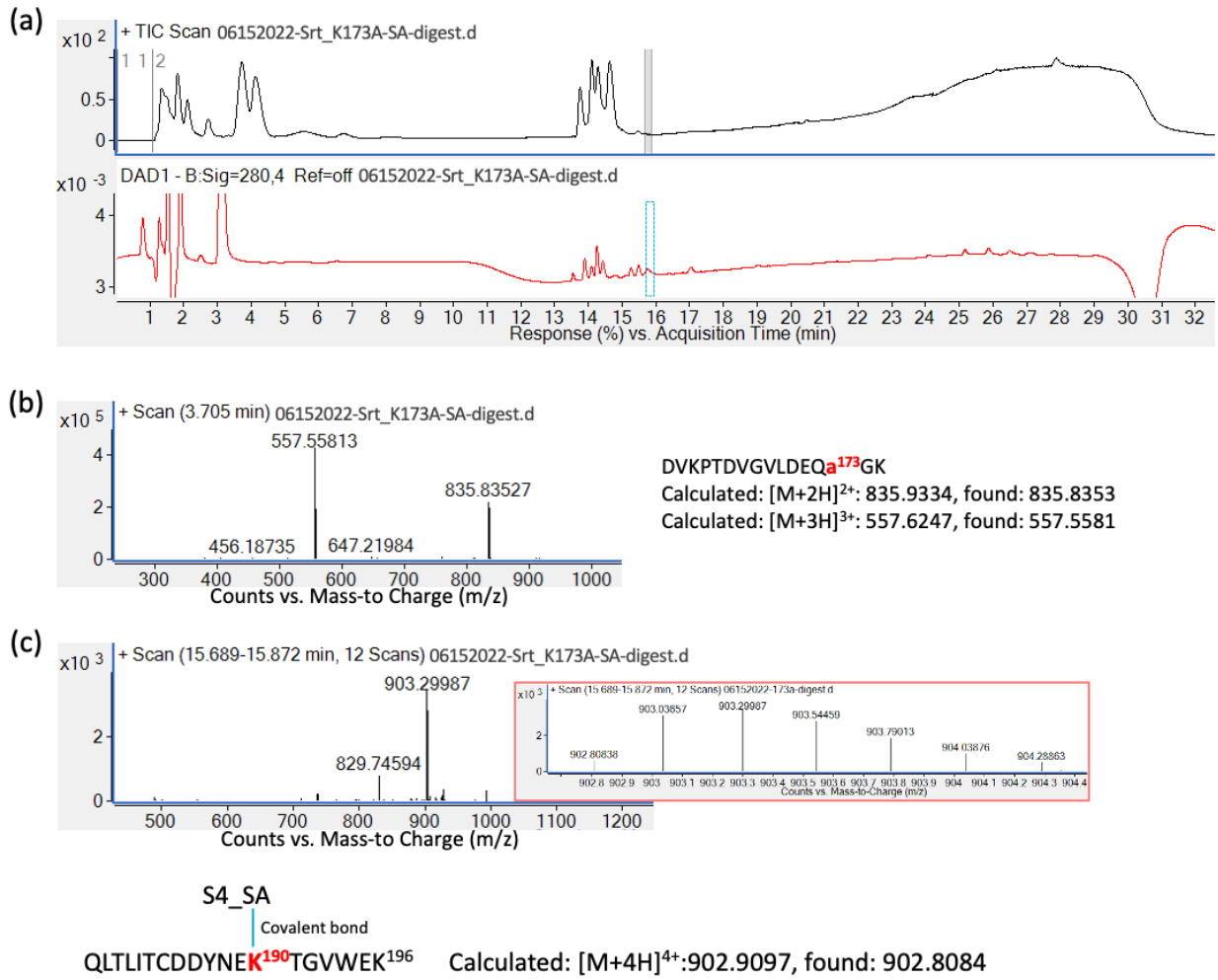


Figure **S12**. Peptide mapping to identify the conjugation site of **S4_SA** on SrtA_K173A. (a) The TIC and LC trace of trypsin digested SrtA_K173A-**S4_SA** conjugate. (b) Mass-spec data of peptide fragment DVKPTDVGVLDEQA^{173}GK to further confirmed the K173A mutation. (c) Mass-spec data corresponds to the **S4_SA** conjugate with QLTLICDDYNEK^{190}TGVWEK^{196}, the adduct consistent with the peptide mapping results of wild type SrtA shown in Figure S10.

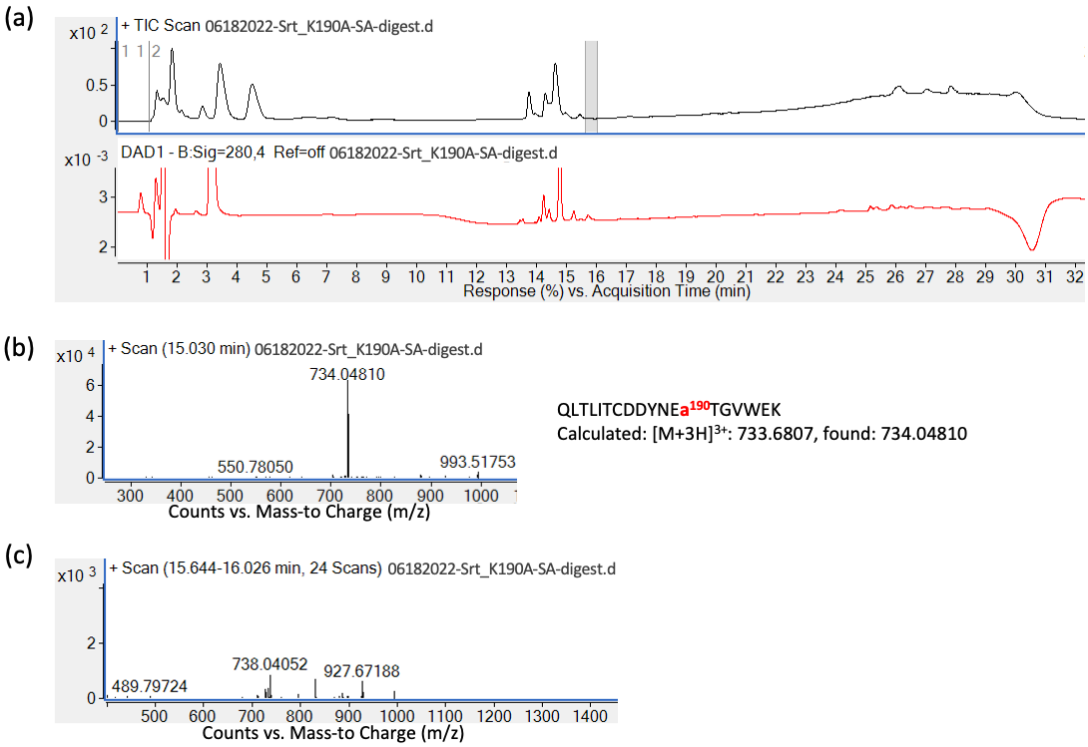


Figure **S13**. Peptide mapping to identify the conjugation site of **S4_SA** on SrtA_K190A. (a) The TIC and LC trace of trypsin digested SrtA_K190A-S4_SA conjugate. (b) Mass-spec data of peptide fragment DQLTLITCDDYNEA^{190}TGVWEK to confirm the K190A mutation. (c) Mass-spec data at 15.6-16.0 min showing the absence of 903.22, mass of the **S4_SA** conjugate with QLTLICDDYNEK^{190}TGVWEK^{196}. We failed to identify the **S4_SA** adduct with K173, although the **S4_SA** adduct with the intact SrtA_K190A was observed in Figure S9.

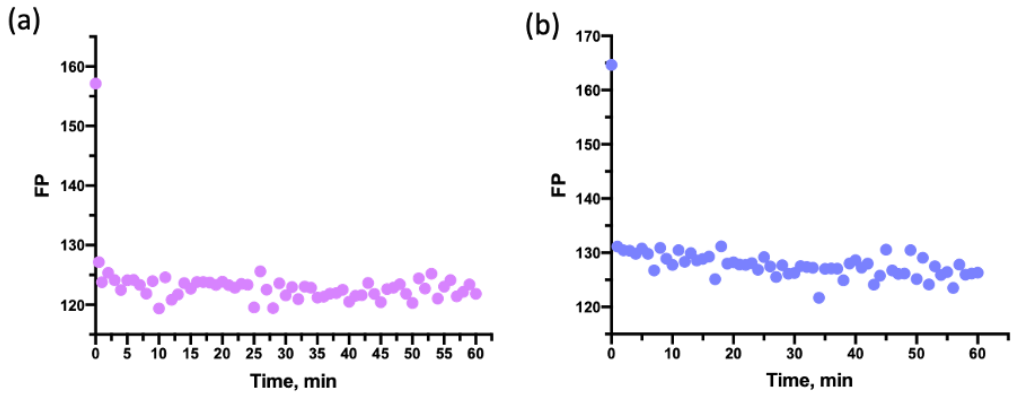


Figure **S14**. SrtA binding kinetics of **S4_APBA-3** recorded via a competition assay. (a) For the association experiments, 200 nM reporter peptide **W7-F**(ACLIPTWGGCGGGDap(Fam)-SS) was incubated with 3 μM of SrtA for 30 min, followed by addition of 50 μM of peptide **S4_APBA-3** and fluorescence polarization was recorded with time. (b) For the dissociation experiments, 200 nM **S4_APBA-3*** (with fluorescein label) was incubated with 3 μM of SrtA for 30 min, followed by addition of 10 μM of peptide **W7** (ACLIPTWGGC_SS) and fluorescence polarization was recorded with time. Instantaneous equilibrium was reached in both cases as expected for the fast kinetics of iminoboronate formation.

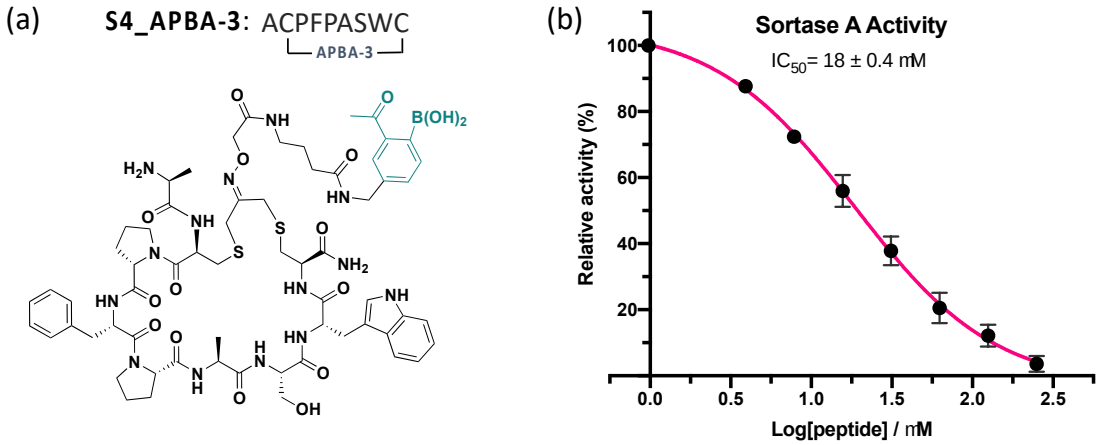


Figure S15. S4_APBA-3 inhibits SrtA catalytic activity. (a) Chemical structure of **S4_APBA-3**. (b) IC_{50} determination of SrtA inhibition by **S4_APBA-3**. The extent of inhibition was determined by using a fluorogenic substrate for SrtA.

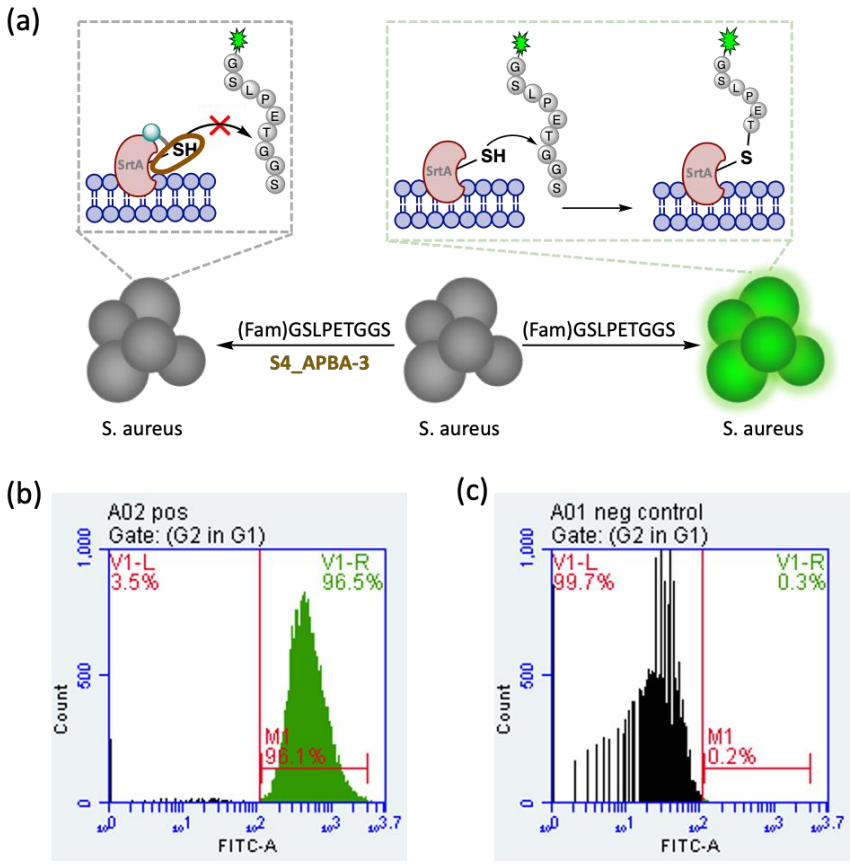


Figure S16. Flow cytometry analysis of SrtA mediated labeling of *S. aureus* cells. (a) Diagram illustration of SrtA-mediated fluorescence labeling of *S. aureus* cells. (b) Positive control: 0.3 mM (Fam)GSLPETGGS incubated with *S. aureus* cells at room temperature for 6 h. (c) Negative control: *S. aureus cells* only.

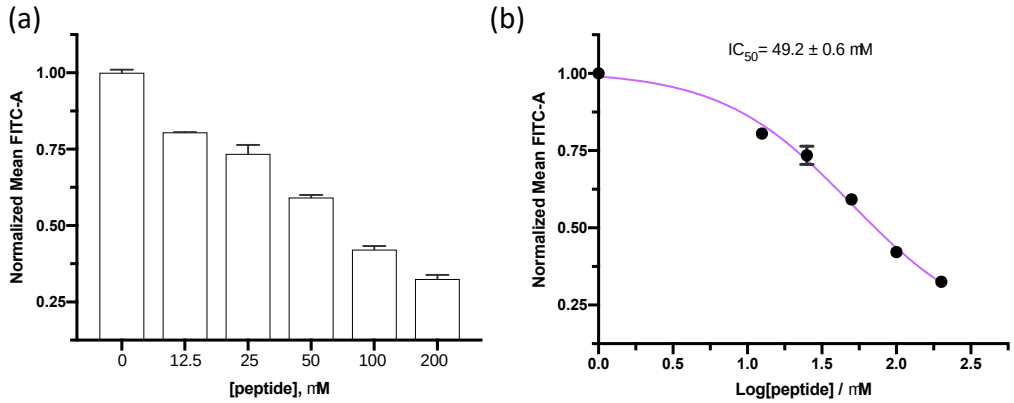


Figure S17. **S4_APBA-3** inhibits SrtA on live bacterial cells. (a) Concentration profile of **S4_APBA-3** in SrtA inhibition on live *S. aureus* cells. The bar graph plots mean ± SD of three independent measurements for each concentration; (b) Curve fitting yields an IC_{50} of 49 μM for live cell SrtA inhibition by **S4_APAB-3**.

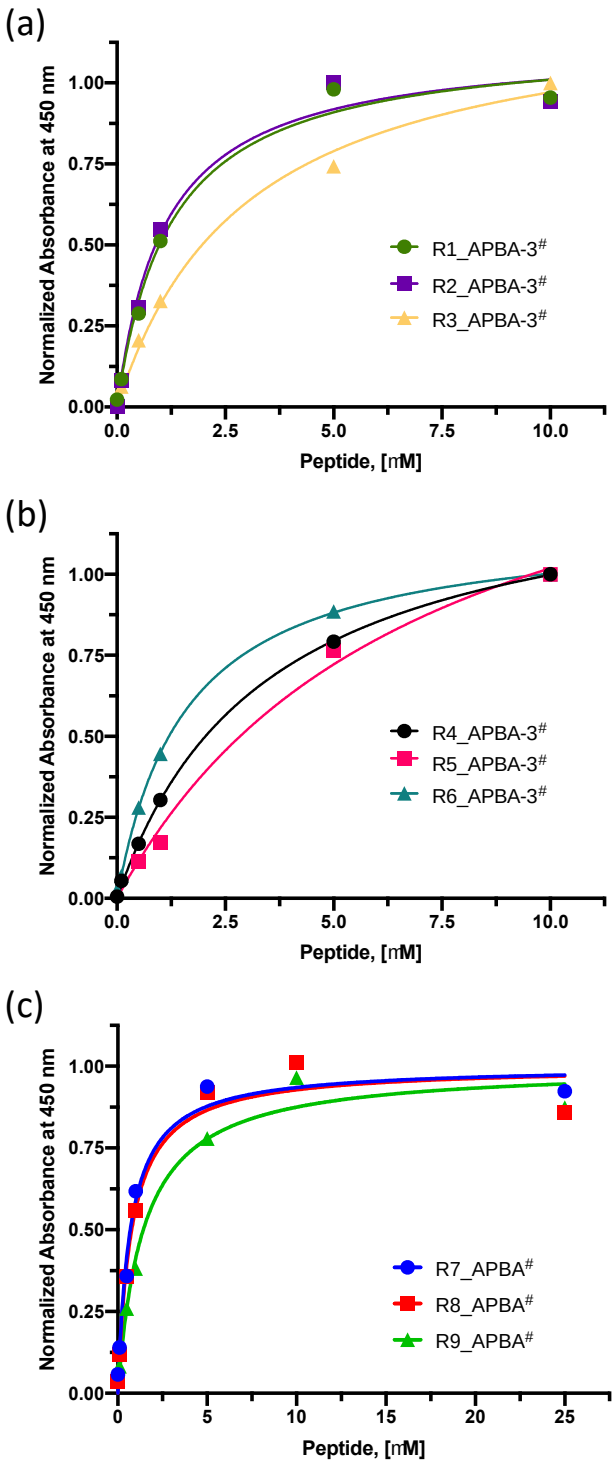


Figure S18. RBD binding curves of the peptide hits generated through the ELISA assay. (a)(b) Binding curves of **APBA-3** cyclized peptides selected from CX_9C_APBA-3 library. Data were background subtracted using background signals obtained without RBD immobilization; (c) Binding curves of the APBA-dimer peptides selected from CX_9C_APBA-dimer library. Each data point presents the mean value of three intendent measurements. # indicates biotin labeled peptides.

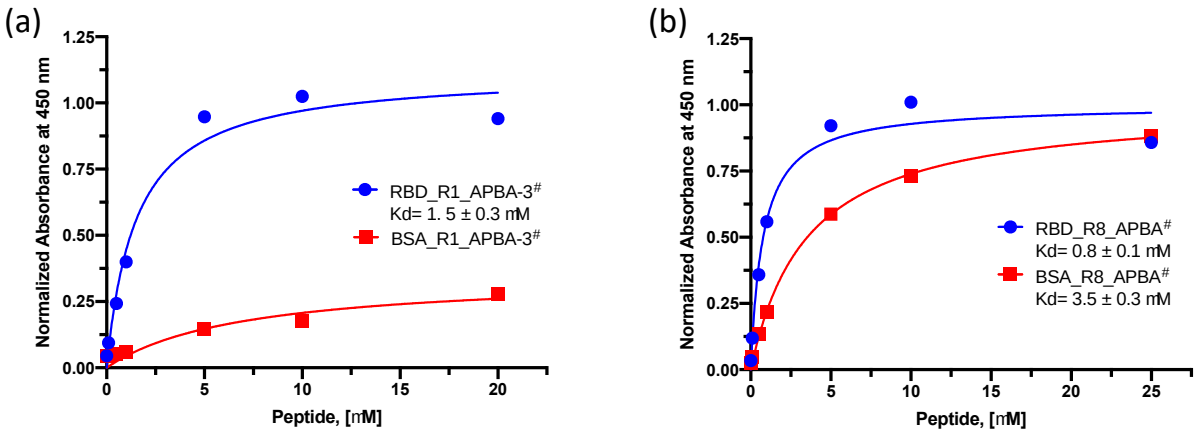


Figure S19. Evaluating the APBA modified peptides for RBD binding specificity. (a) A representative **APBA-3** cyclized peptide showing high selectivity for RBD over BSA. (b) A representative APBA-dimer peptide showing significant binding to BSA.

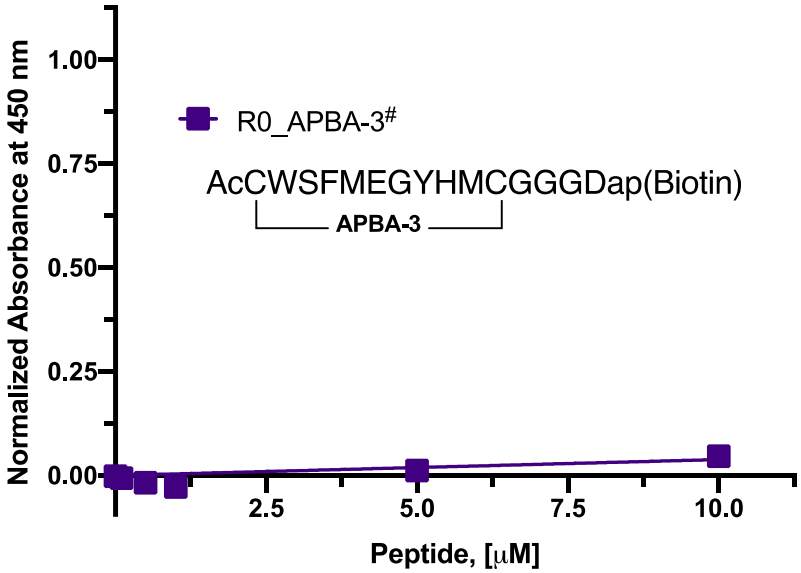


Figure S20. Peptide **R0_APBA-3#**, a random peptide sequence synthesized as a negative control, showed no binding to RBD as revealed by the ELISA assay.

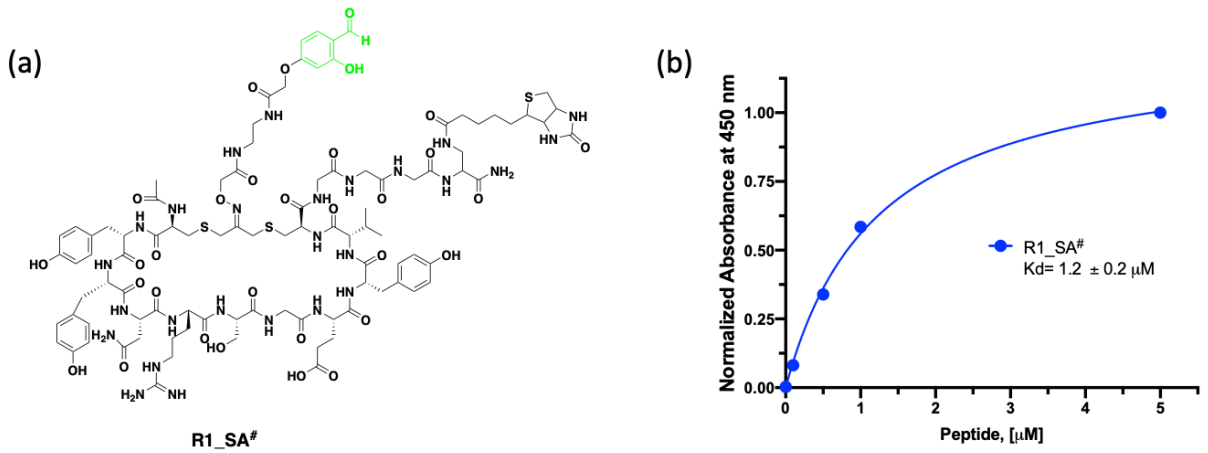


Figure **S21**. (a) Chemical structure of **R1_SA#**. (b) Binding curve of peptide **R1_SA#** by ELISA assay. Data were background subtracted using background signals obtained without RBD immobilization. The peptide shows a similar potency as **R1_APBA-3#**.

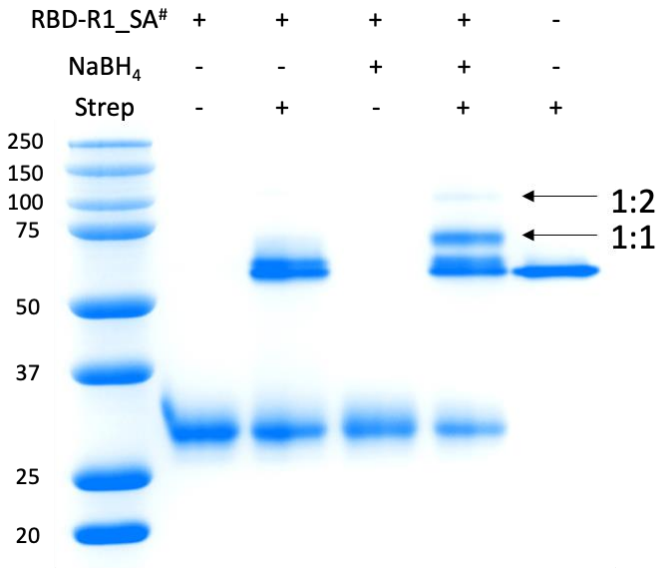


Figure **S22**. Gel-shift assay to confirm the covalent conjugation of **R1_SA#** and RBD. Peptide **R1_SA#** (50 μM) was mixed with RBD (17 μM) at room temperature for overnight, and then $NaBH_4$ (5 mM) was added and incubated at room temperature. After 2 h, 5 μL reaction mixture were mixed with 5 μL streptavidin (1 mg/mL, 1.6 eq). Samples were subject to SDS-PAGE gel analysis. The formation of 1:1 and 1:2 streptavidin:RBD complexes indicated the formation of irreversible covalent bond between peptide **R1_SA#** and RBD.

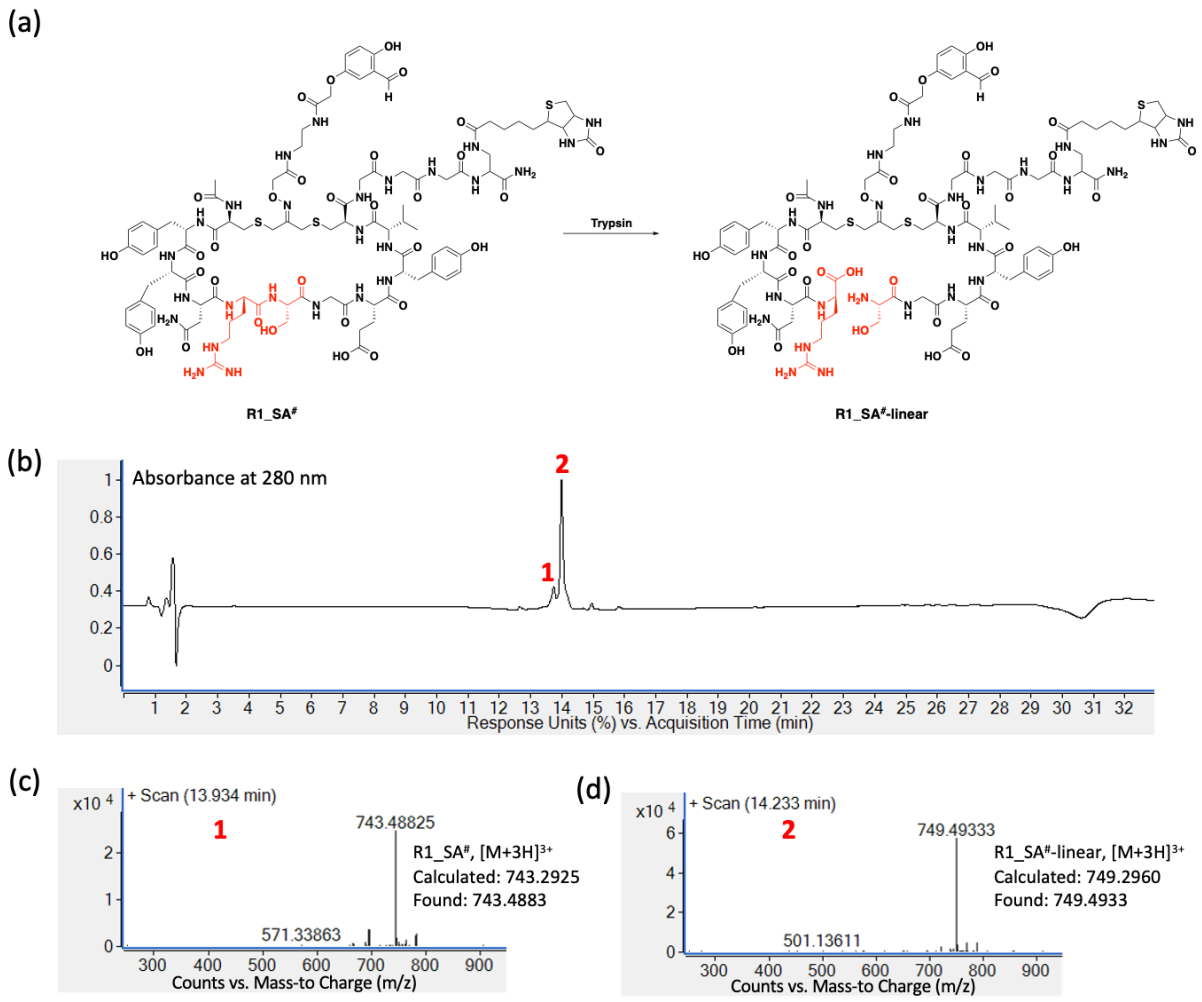


Figure **S23**. Trypsin digestion of peptide **R1_SA**$^{\#}$. (a) Illustration of the cyclic peptide **R1_SA**$^{\#}$ gives a linear peptide by trypsin digestion. (b) LC trace of trypsin digested peptide **R1_SA**$^{\#}$. (c) mass-spec data of peak 1 corresponding to peptide **R1_SA**$^{\#}$. (d) mass-spec data of peak 2 corresponding to peptide **R1_SA**$^{\#}$**-linear**. These data indicate that cyclic peptide **R1_SA**$^{\#}$ was efficiently cleaved by trypsin to form a linear peptide.

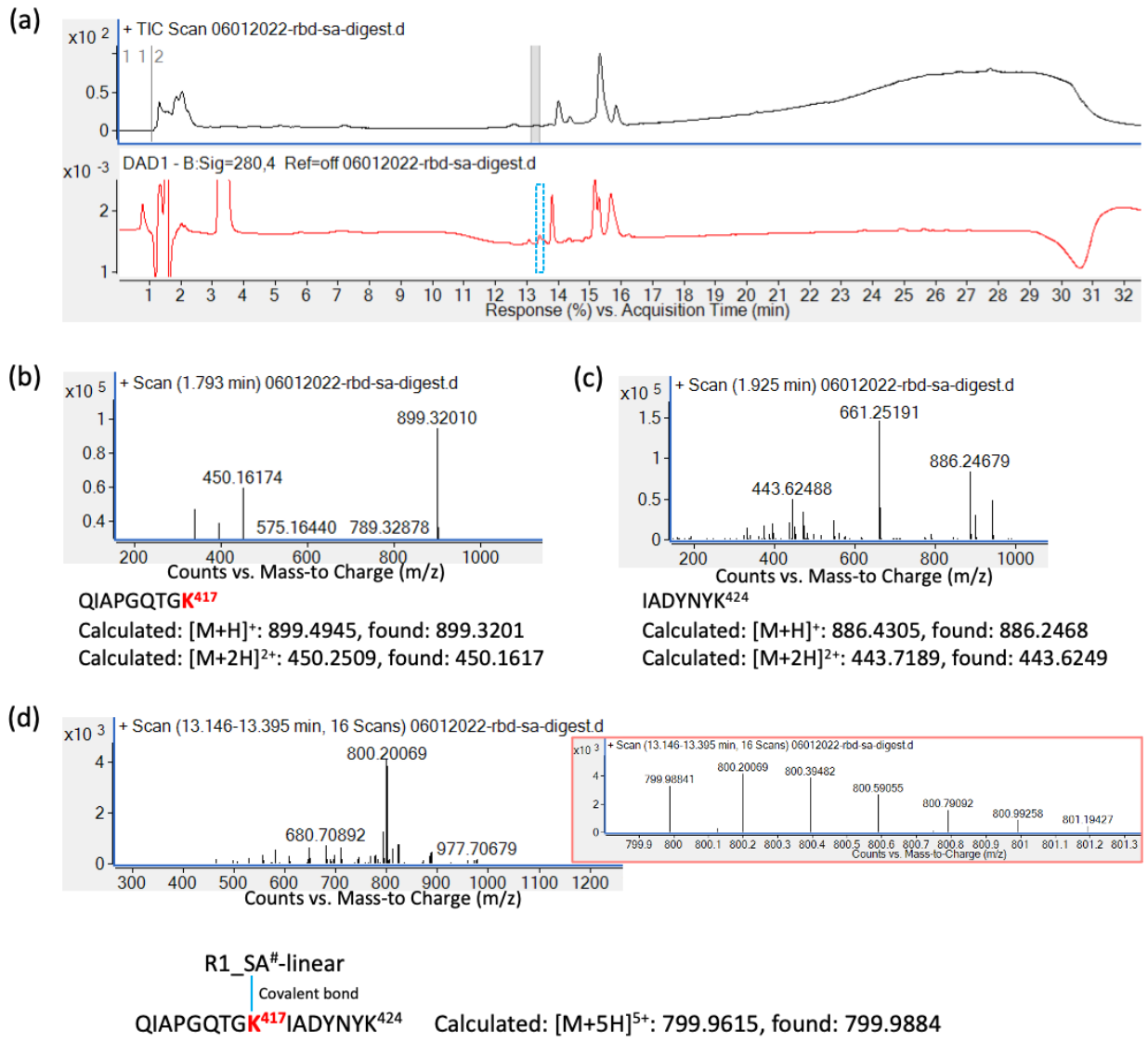


Figure **S24**. Peptide mapping to identify the conjugation site of **R1_SA**$^{\#}$ on RBD. (a) The TIC and LC trace of trypsin digested RBD-**R1_SA**$^{\#}$ conjugate. The boxes indicate the region where the **R1_SA**$^{\#}$ adduct (shown in part (d)) was observed. (b) Mass-spec data of peptide fragment QIAPGQTGK^{417}. (c) Mass-spec data of peptide fragment IADYNYK^{424}. (d) Mass-spec data corresponds to the **R1_SA**$^{\#}$**-linear** conjugate with QIAPGQTGK^{417}IADYNYK^{424}, which indicated that peptide **R1_SA**$^{\#}$ conjugate with K^{417} to make it no longer susceptible to trypsin cleavage.

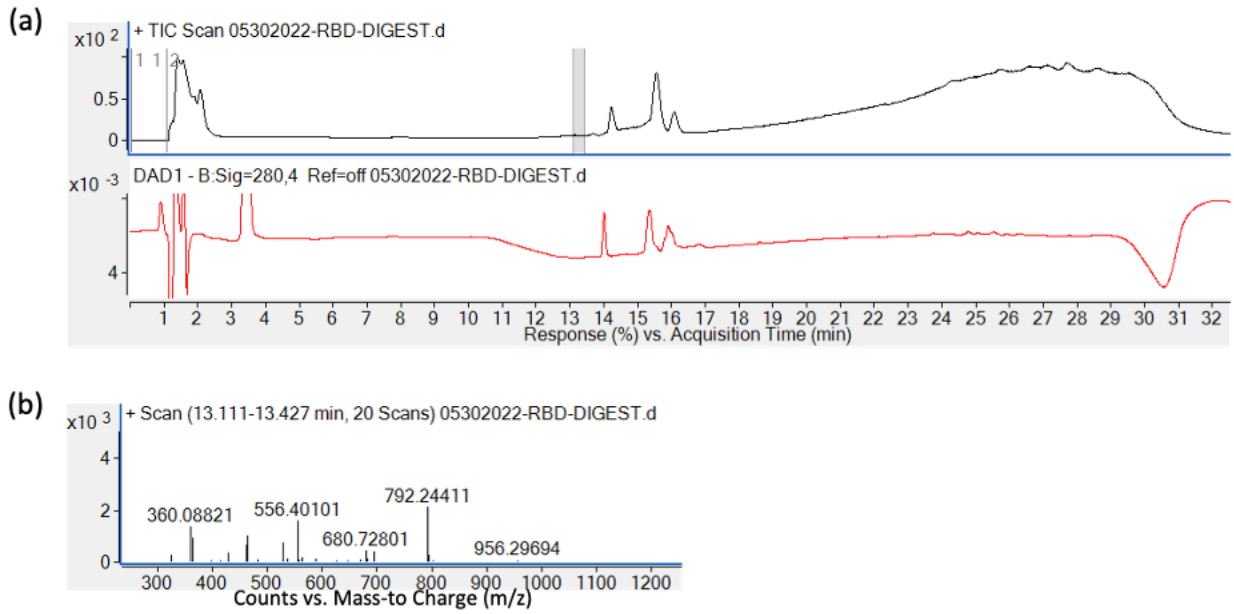


Figure **S25**. Peptide mapping of RBD alone. (a) The TIC and LC trace of trypsin digested RBD alone. (b) Mass-spec data of trypsin digested RBD alone at 13.1-13.4min showing the absence of 800.20, which we assigned as the **R1_SA**$^{\#}$ conjugate with the RBD peptide QIAPGQTGK^{417}IADYNYK^{424} in Figure S24.

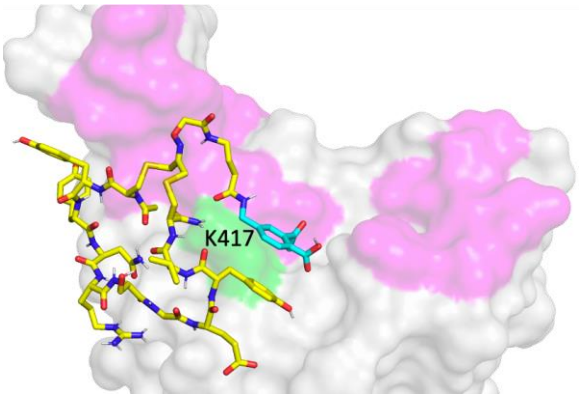


Figure **S26**. A docking structure of **R1_APBA-3** on RBD. RBD-hACE2 interface highlight in magenta. An analogue of APBA warhead was used for docking in which the -$B(OH)_2$ group is replaced with -COOH.

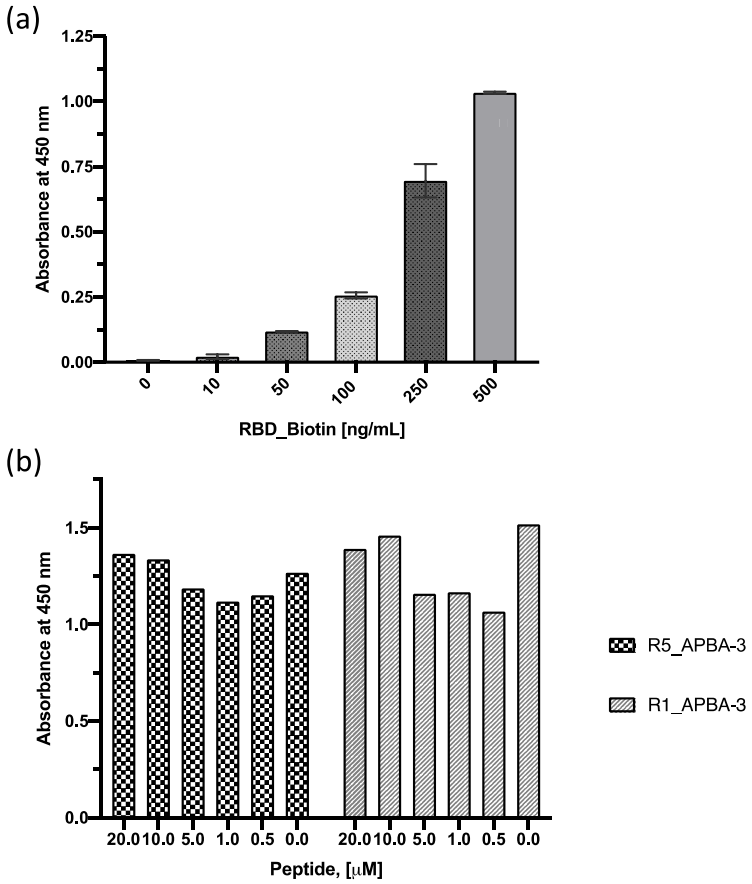


Figure S27. Assessing the peptides' inhibition of the RBD-hACE2 interaction. (a) A titration experiment showing concentration dependent binding of a biotinylated RBD to hACE2. The experimental setup is as follows. The hACE2 protein were costed on the plate and blocked, then biotinylated RBD with different concentration was added in the solution, the amount of bound RBD was quantified using streptavidin-HRP. The concentration dependent absorbance at 450 nm confirming the validity of this assay. (b) ELISA assay results showing a lack of inhibition of RBD-hACE2 interaction for the peptide hits. The hACE2 protein were costed on the plate and blocked, then biotinylated RBD (0.5 μg/mL) with different concentration of peptide ligands were added in the solution, the amount of bound RBD was quantified using streptavidin-HRP.

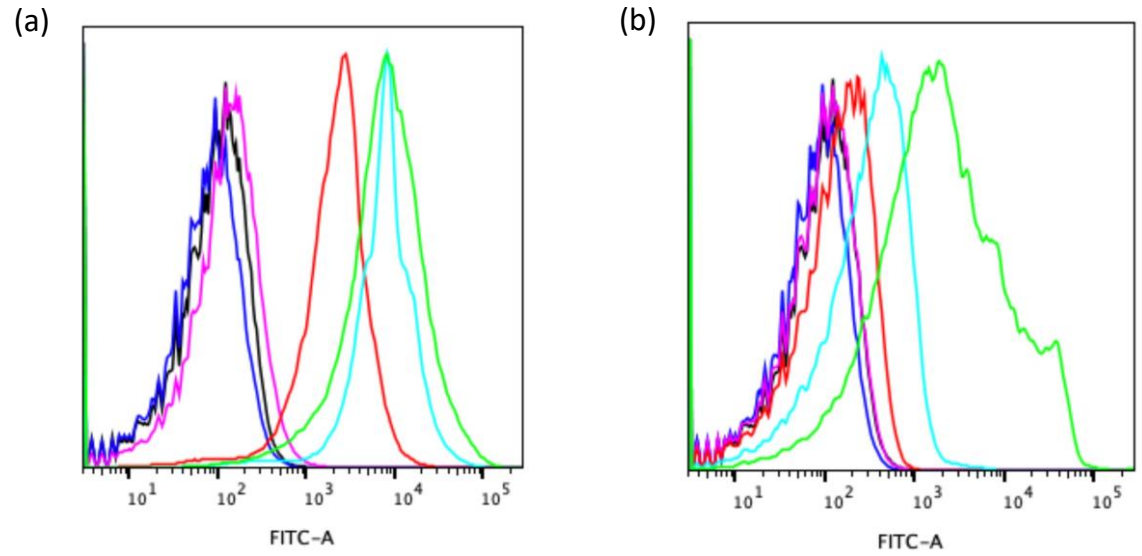


Figure S28. Flow cytometry analysis of (a) **R1_APBA-3**[#] (b) no peptide coated beads incubated with varied concentration of a fluorophore-labeled spike protein. Spike protein concentration: 0 mg/mL, black; 1 pg/mL, blue; 10 pg/mL, magenta; 100 pg/mL, red; 1 ng/mL, cyan; 10 ng/mL, green.

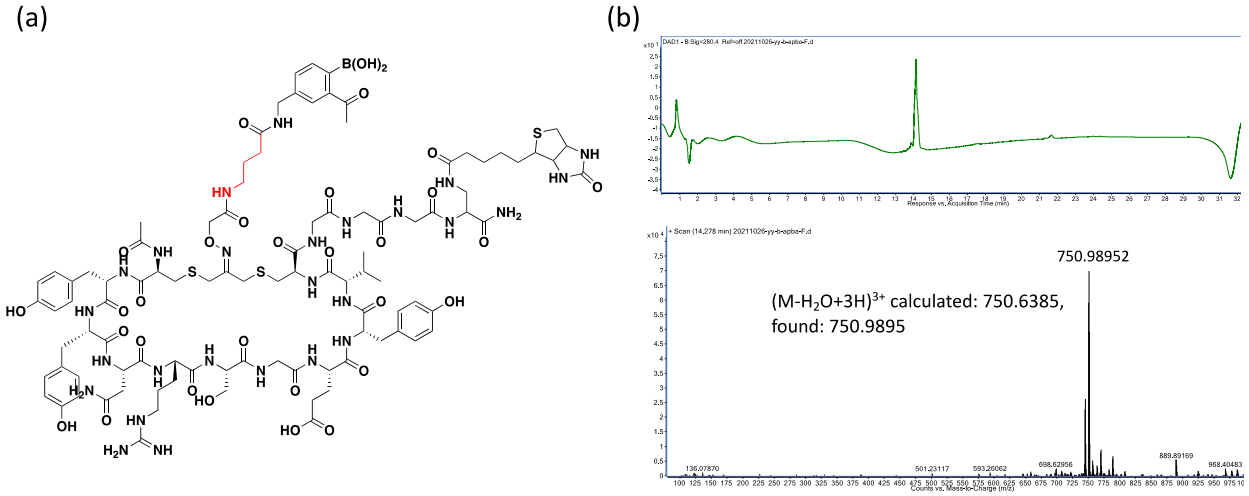


Figure S29. Structure (a) and LC-MS characterization (b) of **R1_APBA-3**[#], shown as an example to demonstrate the purity and integrity of the peptides used for this study.

II. Supplementary Tables

Table S1. Sequencing results of the CX_6C library after four rounds of amplification.

CX_6C sequence
CHAARPNC
CHALSFTC
CHSSLNTC
CLTHPKTC
CLYVDSFC
CNTRTAHC
CQKPSKVC
CRLAPLNC
CSRTGDNC
CHAARPNC

Table S2. Peptides sequences isolated after three rounds of selection against SrtA using the CX_6C library.

Peptide	CX_6C sequence	No
S1	CLIPSWGC	7
S2	CLLPIWGC	2
	CLIALWGC	1
	CPLFASTC	1
	CRTSDNLC	1

Table S3. Peptides sequences isolated after four rounds of selection against SrtA using the CX_6C_DCA library.

Peptide	CX_6C sequence	No
S3	CPFPPSWC	5
S4	CPFPASWC	1
S1	CLIPSWGC	1
	CLIALWGC	1
	CGPRTPLC	1
	CLFLPPHC	1
	CNLQYKTC	1
	CVLEQLKC	1

Table S4. Peptides sequences isolated after five rounds of selection against SrtA using the CX_6C_APBA-1 library.

CX_6C sequence
Blank (6)
CQSSSXFC
CRQPHDAC
CSPHVGEC
CSSDNTIC
CSSFTPMC
CRQPHDAC
CSPHVGEC
CSSDNTIC
CSSFTPMC

Table S5. Peptides sequences isolated after five rounds of selection against SrtA using the CX_6C_APBA-dimer library.

CX_6C sequence
CKAQSHMC
CKNDARSC
CLGPISYC
CNVNNQKC
CNWPWDWC
CPAPNPAC
CPMSISDC
CPYGPYAC

Table S6. Peptides sequences isolated after fifth rounds of selection against SrtA using the CX_6C_APBA-3 library.

Peptide	CX_6C sequence	No
S4	CPFPASWC	8
	CDNQSQHC	1
	CHSQHHDC	1
	CPDNSRTC	1
	CPGAHKVC	1

Table S7. Peptides sequences isolated after fifth rounds of selection against RBD using the CX_9C_APBA-3 library.

Peptide	CX_9C sequence	No	Peptide	CX_9C sequence	No
	CDASLLAWHRC	1		CDPALSGNMTC	1
	CDASLWRAREC	1		CDPLLIPGTTC	1
R7	CDATLYVNNSC	1	R9	CDPLLYPGSVC	1
	CDATLYYNNMC	1		CDPRLVPNGSC	1
	CDDTLVLWRTC	1		CDQRLLAREMC	1
	CDGETWASSNC	1		CDSNLYFYSVC	1
	CDGNLYSNITC	1		CDTAFLASARC	1
R8	CDGQLYSHSQC	2		CNNTFYSPYSC	1
	CDGRLYATSVC	1		CPDADTNYYTC	1
	CDGTLYASVSC	1		CSHELHQWQVC	1
	CDNPFDPTESC	1		CSPSNQLENSC	1
	CDPALRPMRLC	1		CYSGPIYKNYC	1

Table S8. Peptides sequences isolated after fifth rounds of selection against RBD using the CX_9C_APBA dimer library.

Peptide	CX_9C sequence	No
R1	CYYNRSGEYVC	10
R2	CHFNIHGEMIC	2
R3	CFNRHGEPEMC	1
R4	CHSDAPLEMSC	3
R5	CTAEAPLEYHC	3
R6	CHYDRAGNFHC	3
	CGYTDNGTMYC	1
	CHRQKLFESTC	1
	CNRHDNLPRSC	1
	CSNCMLDYCAC	1
	CTHLNANYGLC	1
	CTNQPDYFLTC	1

Table S9. Kd values of various SrtA-binding peptides identified from phage display. Values are given as the mean ± standard deviation of three independent experiments.

Peptide	K_d (μM)	Peptide	K_d (μM)
S1_SS*	5.2 ± 0.6	S4_SS*	n.d.
S1_DCA*	6.3 ± 0.8	S4_DCA*	16 ± 1
S1_APBA-3*	64 ± 7	S4_DCAoxime*	11 ± 2
S3_SS*	n.d.	S4_APBA-1*	19 ± 0.6
S3_DCA*	22 ± 1	S4_APBA-3*	2.9 ± 0.5
S3_APBA-3*	12 ± 1	S4_APBA-3-O*	12 ± 0.4

Table S10. Mass-spec data of peptides for Sortase A.

Name	Peptide sequence	Calculated Mass	Observed Mass
S1_SS*	AC†LIPSWGC†GGGDap(Fam)	$[M+2H]^{2+}$: 781.2974	781.2705
S1_SS	AC†LIPSWGC†	$[M+H]^{+}$: 946.4274	946.4080
S1_DCA*	AC†LIPSWGC†GGGDap(Fam)	$[M+2H]^{2+}$: 809.3105	809.2824
S1_APBA-3*	AC†LIPSWGC†GGGDap(Fam)	$[M-H_2O+2H]^{2+}$: 966.8800	966.8462
S3_SS*	AC†PFPPSWC†GGGDap(Fam)	$[M+2H]^{2+}$: 810.2896	810.2707
S3_DCA*	AC†PFPPSWC†GGGDap(Fam)	$[M+2H]^{2+}$: 838.3027	838.2851
S3_APBA-3*	AC†PFPPSWC†GGGDap(Fam)	$[M-H_2O+2H]^{2+}$: 995.8722	995.81273
S4_SS*	AC†PFPASWC†GGGDap(Fam)	$[M+2H]^{2+}$: 797.2818	797.2546
S4_APBA-3	AC†PFPASWC†	$[M+H]^{+}$: 1367.5719	1367.5328
S4_SA	AC†PFPASWC†	$[M+H]^{+}$: 1369.5340	1369.3140
S4_DCA*	AC†PFPASWC†GGGDap(Fam)	$[M+2H]^{2+}$: 825.2949	825.2822
S4_APBA-1*	AC†PFPASWC†GGGDap(Fam)	$[M-H_2O+2H]^{2+}$: 940.3380	940.2971
S4_APBA-3*	AC†PFPASWC†GGGDap(Fam)	$[M-H_2O+2H]^{2+}$: 982.8644	982.8707
S4_DCAoxime*	AC†PFPASWC†GGGDap(Fam)	$[M+2H]^{2+}$: 861.8031	861.7736
S4_APBA-3-O*	AC†PFPASWC†GGGDap(Fam)	$[M+2H]^{2+}$: 977.8636	977.8366

C†: cysteine is cyclized by disulfide bond formation; C†: cysteine is cyclized by DCA modification; C†: cysteine is cyclized by DCA derivatives modification.

Table S11. Mass-spec data of peptides for Spike protein.

Name	Peptide sequence	Calculated Mass	Observed Mass
R0_APBA-3$^{\#}$	AcC†WSFMEGYHMC†GGGDap(Biotin)	$[M-H_2O+3H]^{3+}$: 762.9528	763.2616
R1_APBA-3$^{\#}$	AcC†YYNRSGEYVC†GGGDap(Biotin)	$[M-H_2O+3H]^{3+}$: 750.6385	750.9895
R1_APBA-3	AcC†YYNRSGEYVC†GGGDap(alloc)	$[M-H_2O+3H]^{3+}$: 703.2863	703.5982
R1_APBA-3-O$^{\#}$	AcC†YYNRSGEYVC†GGGDap(Biotin)	$[M+3H]^{3+}$: 747.3047	747.6194
R1_DCA$^{\#}$	AcC†YYNRSGEYVC†GGGDap(Biotin)	$[M+2H]^{2+}$: 967.8846	968.4055
R1_SA$^{\#}$	AcC†YYNRSGEYVC†GGGDap(Biotin)	$[M+3H]^{3+}$: 743.2925	743.4883
R1_IA$^{\#}$	AcC‡YYNRSGEYVC‡GGGDap(Biotin)	$[M+2H]^{2+}$: 997.9008	998.3689
R2_APBA-3$^{\#}$	AcC†HFNIHGEMIC†GGGDap(Biotin)	$[M-H_2O+3H]^{3+}$: 732.9729	733.2810
R3_APBA-3$^{\#}$	AcC†FNRHGEPEMC†GGGDap(Biotin)	$[M-H_2O+3H]^{3+}$: 739.2960	739.6011
R4_APBA-3$^{\#}$	AcC†HSDAPLEMSC†GGGDap(Biotin)	$[M-H_2O+3H]^{3+}$: 695.9413	695.8826
R5_APBA-3$^{\#}$	AcC†TAEAPLEYHC†GGGDap(Biotin)	$[M-H_2O+3H]^{3+}$: 710.6277	710.9008
R5_APBA-3	AcC†TAEAPLEYHC†GGGDap(alloc)	$[M-H_2O+2H]^{2+}$: 994.4097	994.3434
R6_APBA-3$^{\#}$	AcC†HYDRAGNFHC†GGGDap(Biotin)	$[M-H_2O+3H]^{3+}$: 739.2987	739.5770
R7_APBA$^{\#}$	AcC‡DATLYVNNSC‡GGGDap(Biotin)	$[M-2H_2O+2H]^{2+}$: 1078.9281	1079.8893
R7_APBA	AcC‡DATLYVNNSC‡GGGDap(alloc)	$[M-3H_2O+2H]^{2+}$: 998.8946	998.8526
R8_APBA$^{\#}$	AcC‡DGQLYSHSQC‡GGGDap(Biotin)	$[M-3H_2O+2H]^{2+}$: 1088.9180	1089.3665
R8_APBA	AcC‡DGQLYSHSQC‡GGGDap(alloc)	$[M-3H_2O+2H]^{2+}$: 1017.8898	1017.8409
R9_APBA$^{\#}$	AcC‡DPLLYPGSVC‡GGGDap(Biotin)	$[M-3H_2O+2H]^{2+}$: 1051.9430	1052.3980
R9_APBA	AcC‡DPLLYPGSVC‡GGGDap(alloc)	$[M-3H_2O+2H]^{2+}$: 980.9148	980.8662

C†: cysteine is cyclized by DCA derivatives modification; C‡: cysteine is alkylated by iodoacetamide (IA), C‡: cysteine is alkylated by APBA-IA.

III. General Information

Unless noted otherwise, all chemicals were obtained from commercial sources and used as received without further purification. All Fmoc-protected amino acids and HBTU were purchased from Chem-Impex International (Wood Dale, IL) or Advanced Chemtech (Louisville, KY). 5(6)-Carboxyfluorescein was purchased from Acros Organics (Germany). Rink Amide MBHA resin was purchased from NovaBiochem (San Diego, CA). Peptide synthesis was carried out on a Tribute peptide synthesizer (Protein Technologies, Tucson, AZ). Peptides were purified on a Waters PrepLC system using a Phenomenex Jupiter C18 column (Torrance, CA). ^{1}H NMR spectra were collected using a VNMRS 500 MHz or 600 MHz NMR spectrometer. NMR data were processed using MestReNova software. Mass spectrometry data were collected using an Agilent 6230 LC TOF mass spectrometer.

Biotinylated SARS-CoV-2 Spike RBD (L452R, E484Q), His, Avitag™ (cat. no. SPD-C82Ec), SARS-CoV-2 Spike RBD (L452R, E484Q), His Tag (cat. no. SPD-C52Hv) and human ACE2 / ACEH Protein, Fc Tag (cat. no. AC2-H5257) were purchased from Acro Biosystems (Newark, DE, USA). Dynabeads™ M-280 Streptavidin (cat. no. 11205-D) was purchased from Invitrogen (USA).

IV. Sortase A Expression

Sortase A (SrtA) carrying an N-terminal His tag was expressed in *E. coli* strain BL21(DE3) using the cytoplasmic expression plasmid pET28a-SrtAdelta59 (Addgene, #51138). For protein expression, a single colony from a plate was inoculated into a culture tube with 5 mL LB medium containing 50 μg/mL Kanamycin, which was then incubated overnight at 37°C with shaking at 250 rpm. Then this overnight culture was inoculated into 500 mL of the same medium in a 2.5 L flask. The cell culture was incubated at 37°C with shaking at 250 rpm until the O.D. reached 0.4-0.6. IPTG (1 mM) was then added to induce SrtA expression. After shaking at room temperature for 16 h, the cells were harvested by centrifugation (5,000 g for 15 min) and then lysed in a lysis buffer (20 mM Tris, 300 mM NaCl, 10 mM imidazole) via sonication on ice. The insoluble cell debris was removed by centrifuging at 10,000 g for 15 min and the clear supernatant was purified

with Ni-NTA agarose resin. Finally, the protein was desalted by running through a NAP-10 column (GE healthcare) and stored in DPBS buffer at -80 °C until further use.

Expression of SrtA K-to-A mutants

The expression vectors for the SrtA mutants were built using a Q5 site-directed mutagenesis kit. The primers for each mutant are as follows:

K162A_F: 5'-AAGAGATGTTgccCCTACAGATGTAGG-3'

K162A_R:5'-ATACTTGTCATTTTATACTTACG-3'

K173A_F: 5'-AGATGAACAAgcgGGTAAAGATAAACAATTAAC-3'

K173A_R: 5'-AGAACTCCTACATCTGTAG-3'

K190A_F: 5'-TTACAATGAAgccACAGGCGTTTGG-3'

K190A_R: 5'- TCATCACAAGTAATTAATGTTAATTG-3'

The sequence of the mutated plasmid vector was verified by Sanger sequencing. The plasmid was transformed into BL21(DE3) competent cells. The expression and purification protocol for the mutant proteins was the same as the wild type SrtA as described above.

Expression of biotinylated SrtA

The biotinylated SrtA (biotin-SrtA) expression vector pET28a-AviTag-Linker-SrtAdelta59 was built by inserting the AviTag and proline-rich linker sequence into the pET28a-SrtAdelta59 through a Q5 mutagenesis kit in two steps. First, the AviTag was inserted with primers

AviTag_F: 5'-GCAGAAAATTGAATGGCATGAACGCGGCAGCCATATGCAAGC-3'

AviTag_R: 5'-GCTTCAAAAATATCGTTCAGGCCCGGCACCAGGCCGCTGCT-3'

The sequence of the plasmid was verified by Sanger sequencing, and then the linker coding sequence was inserted in between the AviTag and SrtA with the following primers:

Linker_F: 5'-CACTCCTCCTACCGGTGGCTCTTTACGCGGCAGCCATATGCAA-3'

Linker_R: 5'CTCGGGGTCGGCGGGGTGGATAAGCTTTCATGCCATTCAATTTTCTGCGC-3'

The sequence of the pET28a-AviTag-Linker-SrtAdelta59 vector was verified by Sanger sequencing. The plasmid was transformed into CVB-T7 POL competent cells for expression. An overnight preculture in 5 mL LB medium containing 50 μg/mL Kanamycin and 10 μg/mL Chloramphenicol was used for inoculating 500 mL LB medium containing 50 μg/mL Kanamycin. The cells were cultured at 37°C with shaking at 250 r.p.m. until the O.D. reaches 0.4-0.6. IPTG (1 mM) and biotin (50 μM) were then added to induce the expression of BirA and SrtA. After shaking

at room temperature for 16 h, the cells were harvested by spinning at 5,000 g for 15 min. The purification was done following the same prototol described above for other SrtA variants.

V. Peptide Synthesis

All peptides were synthesized on a peptide synthesizer using a Rink Amide resin and the standard Fmoc/tBu protocol. Fmoc-Dap(Alloc)-OH was used to introduce an Alloc-protected residue on the C-terminus to facilitate on-resin coupling of fluorophore or biotin. The Dap residue was preceded by a triple glycine linker and then the peptide hit sequence at the N-terminus. 5(6)-FAM or biotin was conjugated to the peptide on resin by removing the Alloc protecting group with $Pd(PPh_3)_4$ (50 mg) and phenylsilane (0.3 mL) in DCM (2 mL) followed by subsequent HBTU-mediated coupling in 0.4 M NMM/DMF. The peptides were cleaved off resin with a cleavage cocktail containing 88% TFA, 5% H_2O, 2% triisopropylsilane, 5% phenol. The crude peptides obtained from ether precipitation were purified by RP-HPLC. For peptide modification, the peptide was treated with **DCA**, **APBA-1/3** or **DCA-SA** (1.1 equiv) in the presence of TCEP (2 eq) in an acetonitrile/ ammonium bicarbonate buffer (v/v=1/4, pH 7.4) for 2 hours at room temperature and purified via RP-HPLC. All peptides were characterized with LC-MS to confirm their identities and excellent purities (>95%) (Figure S16, Table S10-S11).

For the synthesis of **S4_APBA-3-O*** and **R1_APBA-3-O$^{\#}$**, 1 mg of **S4_PBA-3*** or **R1_APBA-3$^{\#}$** was dissolved in 1 mL PBS buffer, then 1 mM H_2O_2 was added, monitored the reaction by LC-MS and purified via via RP-HPLC. All peptides were characterized with LC-MS to confirm their identities and excellent purities (>95%).

For the APBA dimer peptide R7-R9, each peptide hit was treated with 2.5 equivalents of APBA-IA in the presence of DIPEA (3 eq) in DMF/PBS Buffer (v/v=1/4, pH 7.4) for 2 hours at room temperature and purified via RP-HPLC. All peptides were characterized with LC-MS to confirm their identity and excellent purity (>95%).

VI. Construction of Phage Libraries

The Ph.D. Peptide Display Cloning System (NEB #E8101S) was used to construct the peptide libraries. To obtain phage libraries that carries a K10R mutation to the pIII protein, the M13KE

expression vector was mutated through a Q5 site-directed mutagenesis kit. The primers used for this mutation are shown below and the mutation was verified by Sanger sequencing.

K10R_F: 5'-TTGTTTAGCACGCTCCCATACAGAAAATTCATTTAC-3'
K10R_R: 5'-CTTTCAACAGTTTCGGCC-3'

For ACX_6C library construction, the following library-coding sequence was used: CATGTTTCGGCCGAACCTCCACCGCANNNNNNNNNNNNNNNNNNACAAGCAGAGT GAGAATAGAAAGGTACCCGGG. The library-coding DNA was prepared by annealing the purchased oligonucleotide with an M13 extension primer. Then the annealed duplex was extended using Klenow fragment (NEB #M0210), digested with EagI-HF (NEB #R3505)/KpnI-HF (NEB #R3142) and then purified by gel. The purified DNA was ligated with gel-purified, EagI-HF/KpnI-HF digested M13KE_K10R vector. The ligation mixture was then electroporated into TOP10 competent cells. Titering of the electroporation sample revealed a library size of 9×10^7 pfu, which exceeds the theoretical diversity of the library. Finally, phage amplification was accomplished by incubating the electroporated cells at 37 °C with shaking at 250 r.p.m for 4.5-5 h. Phage was recovered from the supernatant by adding 1/6 volume of 20% PEG/2.5 M NaCl. The precipitated phage was resuspended in TBS buffer pH 7.4 and stored at 4°C until further use.

The CX_9C library construction followed the same protocol described above. The library-coding sequence used is shown below:

CATGTTTCGGCCGAACCTCCACCACAMNNMNNNMNNNMNNNMNNNMNNNMNNNMNN ACACCGGCCCTCAATTGCAGCGTAGTCTGGAACGTCGTAGGGGTAAGAGTGAGAAT AGAAAGGTACCCGGG

VII. Chemical Modification of Phage Libraries

20 μL of the prepared phage library (10^{13} pfu/mL) was reduced with 1 mM TCEP in 80 μL of buffer R (20 mM ammonium bicarbonate, pH= 8.0). The reaction was carried out at room temperature for 1 h. The reduced phage particles were precipitated with 1/6 volume of 20% PEG/2.5 M NaCl on ice for 1 h, then the precipitated phage was pelleted at 4°C by centrifugation

at 10,000 g for 20 min. For APBA cyclic peptide library, the supernatant was discarded and the phage pellet was re-suspended in 80 μL of buffer R, then 20 μL of DCA derivatives (3 mM in acetonitrile) was added. The reaction was mixed and incubated at 30°C for 2.5 h. On the other hand, for APBA-dimer library, the supernatant was discarded and the phage pellet was re-suspended in 100 μL of PBS buffer (pH= 8.0), then 1 μL of APBA-IA (100 mM in DMF) was added, the reaction was mixed and incubated at room temperature for 2 h. The phage was precipitated using 1/6 volume of 20% PEG/2.5 M NaCl solution and the phage pellet was re-suspended in 100 μL DPBS for phage selection.

We used a phage ELISA assay to quantitatively assess the efficiency of phage modification by DCA-biotin with and without pretreatment with other phage modifiers. Briefly, streptavidin (0.1 mg/mL in 0.1 M $NaHCO_3$ pH 8.6, 100 μL/well) was added into 96-well plate (Corning^{TM}, 3361) and incubated overnight at 4°C. Wells incubated with buffer only were used as a negative control. After incubation, the solution in wells was removed and the wells were blocked with 5mg/mL BSA in PBS (room temperature, 1h) and then washed six times with PBST. Chemically modified phage samples (100 μL, 10^9 pfu/mL) were added to the wells and incubated for 1 h at room temperature. After 6 times of washing with PBST, an anti-M13 antibody-HRP fusion (1:10,000 dilution) was added and incubated at room temperature for 1 h. The wells were washed with PBST for 6 times and then 100 μL TMB (Thermo scientific, 34028) was added into the plate. After incubation at room temperature for 15 min, 100 μL 2M sulfuric acid was added to stop the reaction. The absorbance at 450 nm was read for all treated wells. All experiments were repeated at least three times and consistent results were obtained.

VIII. Phage Panning Against Target Proteins

20 μL of streptavidin-coated magnetic beads (Dynabeads M-280 streptavidin, Invitrogen) were washed three times with 1 mL of DPBS. The beads were re-suspended completely with 100 μL DPBS, to which a biotinylated target protein (0.4 μg of biotinylated Sortase A or 1.4 μg of biotinylated spike RBD) was added. The mixture was incubated on a slowly rotating wheel for 30 min at room temperature. The beads were washed three times with 1 mL of DPBS, re-suspended in 450 μL of a blocking buffer (see below for detailed composition), and then incubated on a slowly rotating wheel for 30 min at room temperature. In parallel, a phage library was also blocked for 30

min at room temperature. Then the library was mixed with the magnetic beads and incubated at room temperature for 30 min. After that, the unbounded phage was removed. The beads were washed eight times with a washing buffer (see below for detailed composition) and twice with 1 mL DPBS. After washing, the beads were re-suspended in 100 μL of elution buffer (0.2 M glycine-HCl, pH= 2.2). After 5-10 min incubation, the supernatant was separated from the beads, neutralized with 400 μL of neutralization buffer (1 M Tris-HCl, pH=9.0). A small aliquot of the supernatant was subjected to titering to quantify the output phage population and the remaining was amplified to prepare for next rounds of selection.

For CX_6C and CX_6C_DCA library:
Blocking buffer: 1 mg/mL BSA, 0.1% Tween-20 in DPBS, pH=7.4
Washing buffer: 0.1% Tween-20 in DPBS, pH=7.4

For CX_6C_APBAs library:
Blocking buffer: 1 mg/mL BSA, 0.5% Tween-20 in DPBS, pH=7.4
Washing buffer: 0.5% Tween-20 in DPBS, pH=7.4

IX. Characterization of Peptide Ligands

a. Fluorescence polarization assay

Both fluorescence polarization binding assay and competition assay were performed in 0.1% DPBST buffer (pH 7.4), and the fluorescence polarization values were measured in a black 96-well plate (Corning™ 3915) using a SpectraMax M5 Microplate Reader (Molecular Devices). The experiments were conducted in triplicates, and the results from the triplicates were averaged and analyzed. Each experiment was repeated at least three times, which gave consistent results. Representative results are presented in the paper with the FP value normalized against a fitted maximum.

For the fluorescence polarization binding assay, a FAM labeled peptide was mixed with varying concentration of SrtA (0 to 50 μM). The final peptide concentration was 200 nM in all samples. After incubated at room temperature for 30 min, the fluorescence polarization values were

measured. Dissociation constants (K_d) were determined by plotting the fluorescence polarization value as a function of protein concentration, and the plots were fitted to the following equation.

$y=FPmin+(FPmax-FPmin)*(K_d+Lst+x-sqrt((K_d+Lst+x)^2-4*Lst*x))/(2*Lst)$.

The Lst is the concentration of the FAM-labeled peptide and the x stands for the concentration of the protein.

For the competition assay, the concentration of the SrtA and a FAM-labeled peptide was kept at 3 μM and 200 nM, respectively. This mixture was incubated (30 min) with a peptide competitor at varied concentrations and then the fluorescence polarization value was recorded. The IC_{50} was determined by fitting the dose-response curves to absolute IC_{50} via nonlinear regression using Prism. We note that under our experimental conditions, the IC_{50} values can be approximated as K_d as the reporter peptide was used as much lower concentrations.

b. SrtA inhibition with recombinant proteins

SrtA Inhibition activity was determined by mixing 500 μM triglycine, 20 μM fluorogenic substrate (Dabcyl-LPETG-Edans), varying concentrations of **S4_APBA-3** (0 to 250 μM) and 2.5 μM SrtA in a reaction buffer (50 mM Tris, 150 mM NaCl and 5 mM $CaCl_2$, pH=7.4). The reaction mixture was incubated at 37°C for 2 h. Fluorescence intensity was measured with a SpectraMax M5 Microplate Reader (excitation at 350 nm, emission at 480 nm). The experiments were conducted in triplicates, and the results from the triplicates were averaged and analyzed. The IC_{50} was determined by fitting dose-response curves to absolute IC_{50} via nonlinear regression using Prism.

c. SrtA inhibition on live cells

An overnight S. aureus culture was diluted 1:100 with LB broth and then allowed to grow at 37°C until OD reached ~1.0. The cell culture was then incubated with 0.3 mM Fluo-GSLPETGGS and varied concentrations of **S4_APBA-3** (0 to 250 μM) at room temperature. After 6 h incubation, cells were pelleted and washed three times with cold DPBS buffer. The fluorescence intensity of the labeled cells was measured using a flow cytometer (BD, Accuri C6 Plus). The experiment was repeated at least three times, which gave consistent results. The IC_{50} was determined by fitting

dose-response curves to absolute IC_{50} via nonlinear regression using Prism.

d. ELISA assay for peptide binding of the spike protein

Coating buffer (100 mM $NaHCO_3$, 7.7 mM NaN_3, pH=8.5)
Washing buffer (0.1% Tween-20 in PBS, pH=7.4)
Blocking buffer (2% BSA in washing buffer)

A 96-well plate (Corning™, 3361) was washed with coating buffer (1x 100 μL/well) and then coated with 100 μL/well of SARS-CoV-2 Spike RBD (L452R, E484Q) in the coating buffer (100 nM) or 100 μL/well of the coating buffer only (negative control well). After overnight incubation at 4°C, the supernatant was removed and the wells were washed three times using the washing buffer (150 μL/well). Then, the plate was treated with the blocking buffer (150 μL/well) at room temperature for three hours. The supernatant was removed and biotinylated peptides in different concentrations (20, 10, 5, 1, 0.5, 0.1, 0 μM) was added to the wells (100 μL/well). After one-hour incubation, three washes were conducted, and then the wells were incubated for one hour with streptavidin-HRP (R&D systems, cat. no. DY998) diluted (1:100) into the blocking buffer. The wells were washed three times using the washing buffer before the addition of 50 μL/well TMB, the HRP substrate (Thermo Scientific, cat. no. 34028). The plate was kept in the dark for up to 15 min. The reaction was stopped using 2M sulfuric acid and the absorbance at 450 nm was read for all treated wells. The absorbance readout was plotted against peptide concentration to give the biding curves, fitting of which using Prism yielded the K_d values for the peptide's binding to the target protein.

e. ELISA assay for assessing inhibition of spike-hACE2 interaction

A 96-well plate (Corning, 3361) was washed with coating buffer (1x100 μL/well) and then coated overnight at 4°C with 100 μL/well of hACE2 (1 μg/mL) in the coating buffer, or with the coating buffer alone as a negative control. After three washes with the washing buffer (150 μL/well), the wells were blocked with 150 μL/well of the blocking buffer for two hours at room temperature. Then, the supernatant was removed followed by the addition of biotinylated RBD (0.5 μg/mL) and

a test peptide (20, 10, 5, 1, 0.5, 0 μM) in PBS buffer to give a total volume of 100 μL/well. After one-hour incubation, three washes were conducted followed by one-hour incubation with streptavidin-HRP (R&D systems, cat. no. DY998) diluted (1:100) in the blocking buffer. The wells were washed three times before the addition of 50 μL/well TMB ELISA substrate (Thermo Scientific, cat. no. 34028) and then the plate was kept in the dark for up to 15 min. The reaction was stopped using 2M sulfuric acid and the absorbance at 450 nm was read for all treated wells. The data analysis was performed using the same protocol as described above in d.

X. Peptide Mediated Spike Protein Detection via Flow Cytometry

AlexaFluor 488 labeled spike protein was obtained by mixing biotinylated spike protein (Acro Biosystems, SPN-C82E9) with AlexaFluor 488-conjugated streptavidin (Jackson ImmunoResearch Labs), the mixture was used directly without purification.

50 μL of streptavidin-coated magnetic beads (Acro Biosystems, SMB-B01) were washed two times with PBS buffer, and then the beads were re-suspended in 50 μL PBS buffer, to which 2.5 μL of the **R1_APBA-3**$^{\#}$ stock solution was added (1 mM). The mixture was incubated on a slowly rotating wheel at room temperature for 2 h. The beads were washed twice with PBS and then blocked with 1% BSA for overnight at 4°C. As a negative control, 50 μL of streptavidin-coated magnetic beads were washed twice with PBS buffer and then directly blocked by incubating with 1% BSA overnight at 4°C. On the second day, the blocked beads were separated into multiple tubes and washed with PBS, then incubated with different concentrations of AlexaFluor 488 labeled spike protein (0 to 10 ng/mL), which was prepared either in 10% saliva or in 0-20% of human serum. The samples were incubated at room temperature for 45 min and then subjected to analysis by flow cytometry. Each experiment was repeated three times and consistent results were observed.

XI. Protocols for Trypsin Digestion

For peptide mapping experiment of SrtA, 50 μM SrtA was incubated with 100 μM **S4_SA** for overnight at room temperature, followed by incubation with sodium borohydride (5 mM) for 2 h. The mixture was then diluted by water and injected for LC-MS analysis directly. The result indicates that ~40% of conjugates were formed. After that, the mixture was desalted and

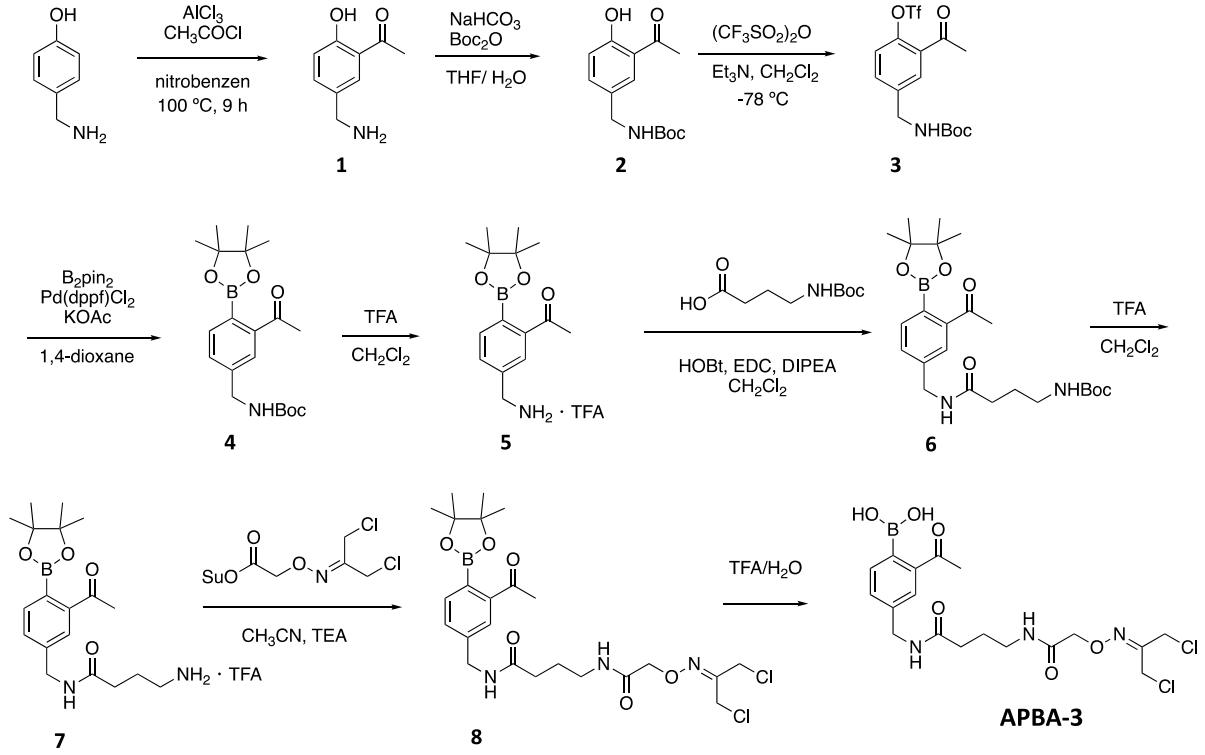
concentrated by centrifugal filters. Then the sample was incubated with DTT(5 mM) at 37 °C for 1 h, followed by treating with Trypsin Gold (V5280, Promega) at a ratio of 1:20 (enzyme: substrate) for 6 h at 37 °C. The mixture was diluted by water and injected for LC-MS directly. For the control experiment, SrtA alone was digested with trypsin under the same condition and then subjected to LC-MS analysis.

For peptide mapping experiment of RBD, similar protocol was used except the concentration of RBD is 17 μM and the concentration of **R1_SA**$^{\#}$ was 50 μM. In addition, the formation of the conjugate was confirmed by SDS-Page gel analysis, which indicated that ~50% of conjugates were formed.

XII. Protocols for Peptide Docking

Peptide models were prepared using the Avogadro program and saved in pdb-file format. To avoid complications, the APBA warhead was replaced with an analogue that carries an -COOH group instead of -$B(OH)_2$. The Auto Dock Tool utility program prepare_ligand4.py (v 1.10) was used to create the ligand input file for the docking calculations in pdb qt file format. The target protein structures were prepared from the Brookhaven Protein Databank file with code 1T2W for SrtA and 6M0J for the spike RBD. The molecular docking program Vina (AutoDockVina 1.1.2 (May 11, 2011) was used for the molecular docking calculations. The molecular docking space was chosen to be 40 by 40 by 40 Å, encompassing the entire target protein. All molecular docking parameters were set to their default values.

XIII. Chemical Synthesis of APBA Derivatives



Scheme S1. Synthetic scheme of **APBA-3**.

Synthesis of **2**. 2.5 g (20 mmol) of 4-hydroxybenzylamine was dissolved in 80 mL of nitrobenzene. $AlCl_3$ (10.8 g, 81 mmol) was added slowly to the mixture followed by the addition of acetyl chloride (1.7 mL, 24 mmol). The mixture was heated on oil bath at 100 °C for 9 hours while being vigorously stirred. The reaction was quenched with 250 mL of 2N HCl. The nitrobenzene layer was discarded and the aqueous layer was washed with EtOAc (2× 100 mL). The aqueous layer was concentrated to ~50 mL to get a light-yellow mixture. The mixture was neutralized by 6 M NaOH solution followed by the addition of 11 g (105 mmol) of $NaHCO_3$. 4.36 g of Boc anhydride (20 mmol) dissolved in 100 mL of THF was slowly added to the solution on ice. The reaction was allowed to stir at room temperature overnight. THF was then evaporated and the residue was extracted by EtOAc (3× 80 mL). The organic layers were combined and washed by saturated brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified flash column chromatography (hexane : ethyl acetate = 10:1). The product was obtained as white solid (3.5 g, 65% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.19 (s, 1H), 7.63 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 1H), 4.90 (brs, 1H), 4.24 (s, 2H), 2.62 (s, 3H),

1.46 (s, 9H).^{13}C NMR (101 MHz, $CDCl_3$) δ 204.51, 161.79, 156.02, 136.02, 129.73, 119.56, 118.82, 79.89, 44.11, 28.63, 28.52, 26.78. HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{14}H_{19}NNaO_4$ $[M+Na]^+$ 288.1212, found 288.1125.

Synthesis of **3**. To a stirred solution of **2** (902 mg, 3.4 mmol) in dry dichloromethane was added triethyl amine (1.9 mL, 13.6 mmol) and the resulting mixture was cooled to -78 °C with dried ice/acetone bath. Tf_2O was added to the mixture at -78 °C and the reaction mixture was warmed to room temperature and stirred for 30 min. The reaction was quenched with saturated $NaHCO_3$ aqueous solution and extracted with dichloromethane. The combined organic layer was washed with brine, dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified flash column chromatography (hexane : ethyl acetate = 3:1). The product was obtained as pale-yellow solid (1.23 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71, 7.71, 7.52, 7.52, 7.51, 7.50, 7.30, 7.29, 4.99, 4.38, 4.37, 2.63, 1.47. ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.70, 156.06, 145.84, 140.43, 132.38, 132.27, 129.57, 122.96, 118.72, 80.26, 43.67, 29.60, 28.44. ^{19}F NMR (470 MHz, $CDCl_3$) δ -73.25. HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{15}H_{18}F_3NNaO_6S$ $[M+Na]^+$ 420.0705, found 420.0564.

Synthesis of **4**. Under N_2 atmosphere, the mixture of **3** (1.59 g, 4 mmol), B_2Pin_2 (2.54 g, 2.5 eq), $Pd(dppf)_2Cl_2{\cdot}CH_2Cl_2$ (0.326 g, 10 mol%), KOAc (1.18 g, 3 eq), 4Å MS (1 g) in 1,4-dioxane (8 mL) was bulbed with N_2 for 30 min. Then the mixture was heated at 80 °C for 6 hours. Upon completion, the reaction was quenched with water and extracted with ethyl acetate. The combined organic layer was washed with brine and dried over anhydride sodium sulfate. The solvent was removed under reduced pressure and the residue was purified flash column chromatography (hexane : ethyl acetate = 2:1). The obtained yellow oil was washed with hexane to give a white solid. The boronic ester product was obtained as a white solid (0.8 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.50 – 7.42 (m, 2H), 4.93 – 4.81 (m, 1H), 4.35 (d, *J* = 5.8 Hz, 2H), 2.60 (s, 3H), 1.46 (s, 9H), 1.43 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.82, 156.03, 151.18, 141.29, 140.26, 132.66, 131.49, 127.58, 83.85, 79.86, 44.46, 28.49, 25.76, 24.99. HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{20}H_{30}BNNaO_5$ $[M+Na]^+$ 398.2115, found 398.1961.

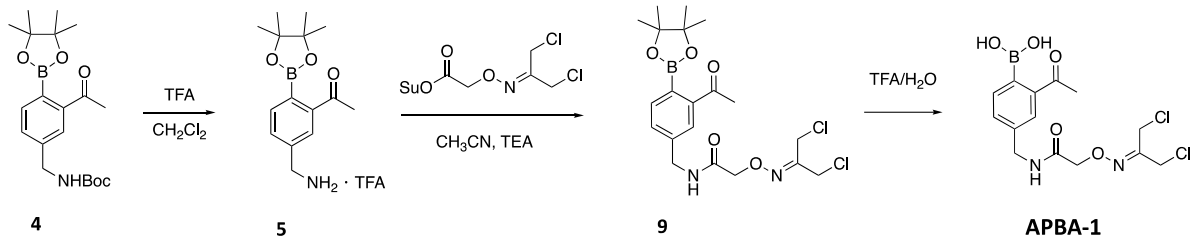
Synthesis of **6**. 75 mg (0.2 mmol, 1.1 eq) of **4** was dissolved in 1 mL of trifluoroacetic acid/dichloromethane (1/1, v/v). After 2 hour, the reaction mixture was concentrated under reduced

pressure. The residue was dissolved with dichloromethane and concentrated. Repeat this procedure three times to remove the trifluoroacetic acid completely. The obtained Bpin-containing amine was used directly for the next steps. The solution of γ-(Boc-amino)butyric acid (36.6 mg, 1 eq) in 1 mL of DCM was cooled to 0 °C. To the solution was added HOBt (37.5 mg, 1.5 eq), EDC (51.5 mg, 1.5 eq), and DIPEA (34 μL, 2 eq). After stirring at 0 °C for 30 min, a solution of Bpin-containing amine in DCM was added and the mixture was stirred overnight. Upon completion, the DCM was removed under reduced pressure and the residue was dissolved in ethyl acetate. The mixture was washed with 1N HCl, sat. $NaHCO_3$, brine and dried over anhydride sodium sulfate. The solvent was removed under reduced pressure and the residue was purified flash column chromatography (hexane : ethyl acetate = 1:4). The product was obtained as a white solid (48.1 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.46 (s, 2H), 4.49 (d, *J* = 5.6 Hz, 2H), 3.12 (t, *J* = 6.4 Hz, 2H), 2.58 (s, 3H), 2.26 (t, *J* = 6.9 Hz, 2H), 1.78 (m, 2H), 1.42 (s, 9H), 1.42 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.87, 172.99, 156.95, 141.39, 139.65, 132.76, 131.92, 128.02, 83.89, 79.79, 43.40, 39.66, 33.40, 28.56, 28.52, 26.94, 25.81, 25.00. HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{24}H_{37}BN_2NaO_6$ $[M+Na]^+$ 483.2642, found 483.2428.

Synthesis of **8**. 46 mg (0.1 mmol, 1.0 eq) of **6** was dissolved in 1 mL of trifluoroacetic acid/dichloromethane (1/1, v/v). After 2 hour, the reaction mixture was concentrated under reduced pressure. The residue was dissolved with dichloromethane and concentrated. Repeat this procedure three times to remove the trifluoroacetic acid completely. The obtained Bpin-containing amine was used directly for the next steps. The NHS ester (29.7 mg, 0.1 mmol) was dissolved in 2 mL of acetonitrile, followed by triethylamine (0.042 mL, 2 eq), and the mixture was cooled to 0 °C. The solution of obtained Bpin-containing amine in DCM was added and the mixture was stirred at the same temperature for 1 hour. The solvent was removed under reduced pressure and dissolved in ethyl acetate. The mixture was washed with 1N HCl, sat. $NaHCO_3$, brine and dried over anhydride sodium sulfate. The solvent was removed under reduced pressure. The product was obtained as a white solid (46.7 mg, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.47 – 7.41 (m, 2H), 6.83 (t, *J* = 5.9 Hz, 1H), 6.76 (t, *J* = 5.9 Hz, 1H), 4.61 (s, 2H), 4.46 (d, *J* = 5.9 Hz, 2H), 4.39 (s, 2H), 4.23 (s, 2H), 3.31 (q, *J* = 6.2 Hz, 2H), 2.57 (s, 3H), 2.25 (dd, *J* = 7.6, 5.7 Hz, 2H), 1.83 (p, *J* = 6.5 Hz, 2H), 1.41 (s, 12H).^{13}C NMR (126 MHz, $CDCl_3$) δ 199.89, 172.75, 169.38, 153.82, 141.43, 139.61, 132.78, 131.79, 127.93, 83.89, 73.87, 60.50, 43.36, 41.96, 38.73, 33.86, 33.12, 29.80,

25.82, 25.60, 24.98, 14.30. HRMS (TOF-ESI+) *m/z* calc. for $C_{24}H_{35}BCl_2N_3O_6$ $[M+H]^+$ 542.1996, found 542.1893.

Synthesis of **APBA-3**. 10.8 mg of **8** (0.02 mmol) was dissolved in 1 mL of trifluoroacetic acid/water (1/1, v/v) and the solution was stirred for 2 hours. The mixture was concentrated under reduced pressure and the residue was purified by RP-HPLC to give **APBA-3** as a white solid (1.8 mg, 20%). ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.86 (s, 1H), 7.73 (s, 1H), 7.47 (s, 2H), 7.44 (s, 1H), 6.73 (s, 1H), 4.57 (s, 2H), 4.56 (s, 2H), 4.46 (d, *J* = 6.1 Hz, 2H), 4.38 (s, 2H), 3.26 (t, *J* = 6.3 Hz, 2H), 2.54 (s, 3H), 2.30 (t, *J* = 7.0 Hz, 2H), 1.82 -1.77 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 199.56, 171.94, 167.73, 152.89, 140.24, 139.29, 131.48, 130.47, 127.28, 73.23, 42.41, 41.79, 38.10, 34.15, 32.84, 26.37, 25.37. HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{18}H_{23}BCl_2N_3O_5$ $[M-H_2O+H]^+$ 442.1108, found 442.1069.

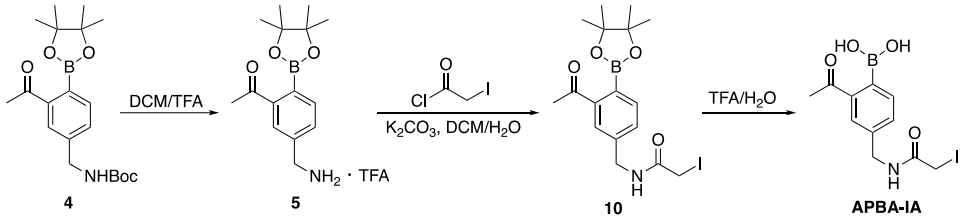


Scheme S2. Synthetic scheme of **APBA-1**.

Synthesis of **9**. 18.8 mg (0.05 mmol, 1.2 eq) of **4** was dissolved in 1 mL of trifluoroacetic acid/dichloromethane (1/1, v/v) and the solution was stirred for 2 hours. The reaction mixture was concentrated under reduced pressure. The residue was dissolved with dichloromethane and concentrated. Repeat this procedure three times to remove the trifluoroacetic acid completely. The obtained amine salt was used directly for the next steps. The NHS ester (12.5 mg, 0.042 mmol, 1.0 eq) was dissolved in 1 mL of dry acetonitrile, followed by triethylamine (17.6 µL, 2 eq), and the mixture was cooled to 0 °C. The solution of obtained amine salt in DCM was added and the mixture was stirred at the same temperature for 1 hour. The solvent was removed under reduced pressure and dissolved in ethyl acetate. The mixture was washed with 1N HCl, sat. $NaHCO_3$, brine and dried over anhydride sodium sulfate. The solvent was removed under reduced pressure. The product was obtained as a white solid (13 mg, 68%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (s, 1H),

7.48 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 1H), 6.56 (t, *J* = 6.2 Hz, 1H), 4.72 (s, 2H), 4.55 (d, *J* = 6.2 Hz, 2H), 4.28 (s, 2H), 4.21 (s, 2H), 2.59 (s, 3H), 1.43 (s, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 199.71, 168.74, 153.97, 141.55, 138.88, 132.93, 131.72, 127.85, 83.94, 74.01, 42.84, 41.96, 32.77, 25.84, 25.01. HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{20}H_{28}BCl_2N_2O_5$ $[M+H]^+$ 457.1468, found 457.1409.

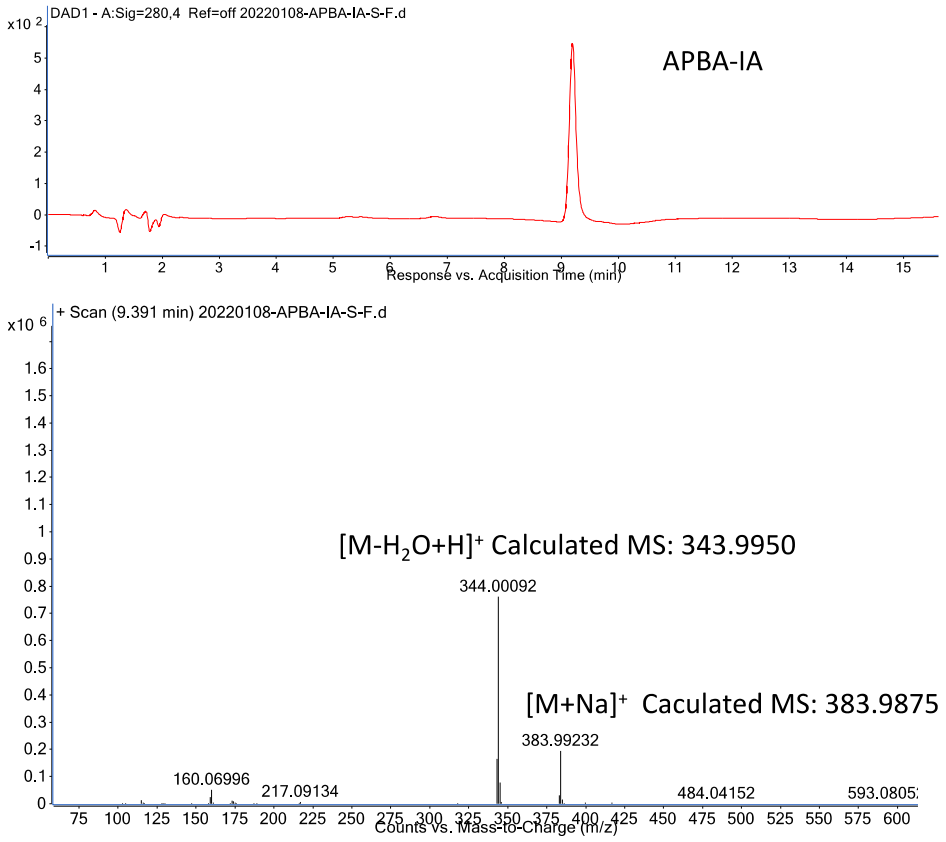
Synthesis of **APBA-1**. 13 mg of starting boronic ester was dissolved in 1 mL of trifluoroacetic acid/water (1/1, v/v). After deprotection was completed (monitored by LC-MS), the mixture was concentrated and the residue was purified by RP-HPLC to give **APBA-1** as a white solid. ^{1}H NMR (600 MHz, DMSO-*d*$_6$) δ 8.45 (t, *J* = 6.2 Hz, 1H), 7.78 (s, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.34 (d, *J* = 7.4 Hz, 1H), 4.63 (s, 2H), 4.54 (d, *J* = 3.8 Hz, 2H), 4.41 (s, 2H), 4.38 (d, *J* = 6.2 Hz, 2H), 2.53 (s, 3H). ^{13}C NMR (151 MHz, DMSO-*d*$_6$)) δ 199.49, 168.01, 152.97, 140.22, 138.84, 131.45, 130.30, 127.18, 73.23, 42.38, 41.46, 34.12, 26.36. HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{14}H_{16}BCl_2N_2O_4$ $[M-H_2O+H]^+$ 357.0580, found 357.0432.

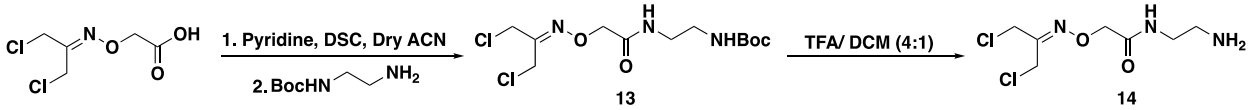


Scheme S3. Synthetic scheme of **APBA-IA**.

Synthesis of **APBA-IA**. 4 (37.5 mg, 0.1 mmol) was dissolved in 1 mL of DCM, to which was added 1 mL TFA. The reaction was stirred at room temperature for 2 hr. After solvent removal, K_2CO_3 (70 mg, 0.5 mmol) was added to the residue. The mixture was dissolved in DCM/H_2O (2:1, 3 mL) and kept on ice for 20 min. Iodoacetyl choloride (92 mg, 0.45 mmol) was added slowly during 5 min to the reaction. The mixture was allowed to stir at room temperature for 2 hr. The solution was acidified to pH 3 by 1 N HCl and the product was extracted with DCM (3× 20 mL). The combined organic layer was washed with brine (50 mL) and dried over sodium sulfate. DCM was removed and the residue was treated with TFA/H_2O for 2 h. After solvent removal, the crude material was re-dissolved in 3 mL Acetonitrile/H_2O (2:3) solution and purified via RP-HPLC. The product is a white solid after lyophilization (11 mg, 30% yield over three steps). ^{1}H NMR (600

MHz, DMSO-d_6) δ 8.81 (t, J = 6.0 Hz, 1H), 7.79 (s, 1H), 7.41 (d, J = 6.0 Hz, 1H), 7.34 (d, J = 6.0 Hz, 1H), 4.32 d, J = 6.0 Hz, 2H), 3.70 (s, 2H), 2.54 (s, 3H). HRMS (TOF-ESI$^+$) *m/z* calc. for $C_{11}H_{12}BINO_3^+$ $[M\text{-}H_2O+H]^+$ 343.9950, found 344.0009; $C_{11}H_{13}BINNaO_4^+$ $[M+Na]^+$ 383.9875, found 383.9923. LC-MS result of purified **APBA-IA** is as following:





Scheme S4. Synthetic scheme of **14**.

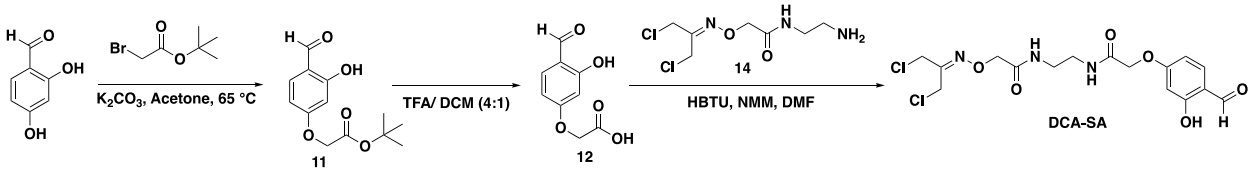
Synthesis of **13**.

DCA-Oxime acid (400 mg, 2 mmol) was dissolved in 5 mL of dry ACN. To this solution, pyridine (161 uL, 2 mmol) was added under N_2 atmosphere and stirred for about 15 min at 0 °C. Then N,N'-disuccinimidyl carbonate (1.3 g, 5 mmol) was added at 0 °C and stirred for another about 4h at room temperature. After formation of the active ester (confirmed by TLC), *N*-Boc-

ethylenediamine (480 mg, 3 mmol) was added and stirred for about 16 h at room temperature under N_2 atmosphere. After completion of the reaction (confirmed by TLC), acetonitrile was evaporated under *vacuum* and organic layer was extracted using ethyl acetate (3 × 20 mL). The combined organic layer was washed with brine (3 × 15 mL) and dried over anhydrous Na_2SO_4. Then the organic layer was evaporated under vacuum and crude compound was purified through Ethyl acetate/Hexane (477 mg, 70%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.88 (*b*s, 1H), 4.85 (*b*s, 1H), 4.65 (s, 2H), 4.43 (s, 2H), 4.27 (s, 2H), 3.43 (q, J = *6* Hz, 2H), 3.28 (q, J = *6* Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.28, 156.95, 153.78, 79.92, 73.90, 41.91, 40.49, 40.32, 33.10, 28.47. MS-ESI$^+$: *m/z* calculated for $C_7H_{13}Cl_2N_3O$ [M-Boc+H]$^+$ 242.0463, found 242.0278.

Synthesis of **14**.

13 (342 mg, 1mmol) was dissolved in 3 mL of TFA/DCM (4:1) and stirred for 2 h at room temperature. After completion of the reaction (confirmed by TLC), TFA was evaporated under vacuum. The compound was directly used without any further purification steps (170 mg, 70%). MS-ESI$^+$: *m/z* calculated for $C_7H_{13}Cl_2N_3O$ [M+H]$^+$ 242.0463, found 242.0245.



Scheme S5. Synthetic scheme of **DCA-SA**.

Synthesis of **11**.

Briefly, commercially available 2,4-dihydroxylbenzaldehyde (1.38 g, 10 mmol) was dissolved in 15 mL of acetone. To this solution, K_2CO_3 (1.38 g, 10 mmol) was added and stirred for 5 mins. After that, *tert*-butyl bromoacetate (1.4 mL, 10 mmol) was added dropwise. Then the reaction mixture was refluxed at 65 °C for about 16 h. After completion of the reaction (confirmed by TLC), acetone was evaporated and the organic layer was extracted with ethyl acetate (3 × 30 mL). Then the combined organic layer was washed with brine (3 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated under *vacuum*. The crude compound was purified through silica gel column chromatography using EA/Hexane as solvent. (1.2 g, 49%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.43 (s, 1H), 9.73 (s, 1H), 7.46 (d, *J* = 9 Hz, 1H), 6.58 (dd, *J* = 8.7, 2 Hz, 1H), 6.37 (d, *J* = 2.5 Hz, 1H),

4.56 (s, 3H), 1.49 (s, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 194.67, 166.99, 165.06, 164.46, 135.55, 115.88, 108.66, 101.61, 83.18, 65.66, 28.18. MS-ESI$^+$: *m/z* calculated for $C_{13}H_{17}O_5$ $[M+H]^+$ 253.1076, found 253.0880.

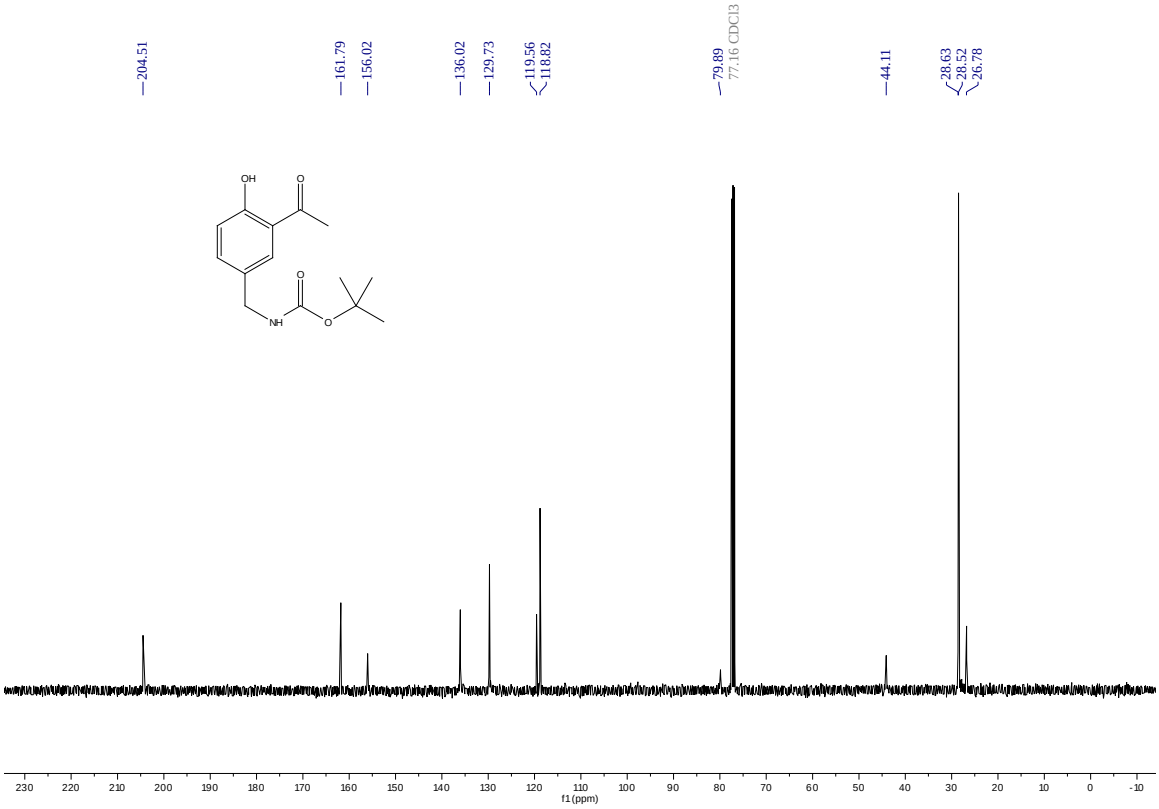
Synthesis of **12**.

11 (252 mg, 1 mmol) was dissolved in 5 mL of TFA/DCM (4:1) and stirred for 2 h at room temperature. After completion of the reaction (confirmed by TLC), TFA was evaporated under *vacuum*. The compound was used without any further purification steps (158 mg, 80%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.15 (bs, 1H), 10.95 (s, 1H), 10.02 (s, 1H), 7.62 (d, *J* = 9 Hz, 1H), 6.56 (dd, *J* = 9, 2.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 4.76 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.86, 169.56, 164.32, 162.83, 131.98, 116.64, 107.60, 101.52, 64.64. MS-ESI$^+$: *m/z* calculated for $C_9H_9O_5$ $[M+H]^+$ 197.0450, found 197.0423.

Synthesis of **DCA-SA**.

12 (39 mg, 0.2 mmol) was dissolved in 1 mL of DMF and cooled to 0 °C. To this solution, HBTU (75.8 mg, 0.2 mmol) and N-methylmorpholine (44 uL, 0.4 mmol) were added under N_2 atmosphere. Then the reaction mixture was stirred for 10 mins at 0 °C. After that compound **14** (72.6 mg, 0.3 mmol) was added and stirred for another 1 h at room temperature. After completion of the reaction (confirmed by LC-MS analysis), 10 mL of brine solution was added. Then the organic layer was extracted with ethyl acetate (3 × 15 mL) and washed with brine (3 × 20 mL). The combined organic layer was evaporated under *vacuum*. The crude compound was purified by RP-HPLC on a C18 column using ACN/ H_2O gradient system starting from 95% H_2O and 5% ACN and reached 95% ACN and 5% H_2O in 50 mins. The product is a white solid after lyophilization (25 mg, 30%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.98 (s, 1H), 10.03 (s, 1H), 8.25 (t, *J* = 6 Hz, 1H), 7.90 (d, *J* = 5.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 6.59 (dd, *J* = 9, 2.4 Hz, 1H), 6.48 (d, *J* = 2.4 Hz, 1H), 4.54 (s, 2H), 4.53 (s, 2H), 4.51 (s, 2H), 4.39 (s, 2H), 3.22 – 3.19 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 190.87, 168.11, 167.32, 164.14, 162.78, 152.91, 131.94, 116.72, 107.69, 101.82, 73.17, 66.86, 42.36, 38.16, 38.13, 34.13. MS-ESI$^+$: *m/z* calculated for $C_{16}H_{20}Cl_2N_3O_6$ $[M+H]^+$ 420.0729, found 420.0546.

XIV. ^{1}H NMR and ^{13}C NMR Spectra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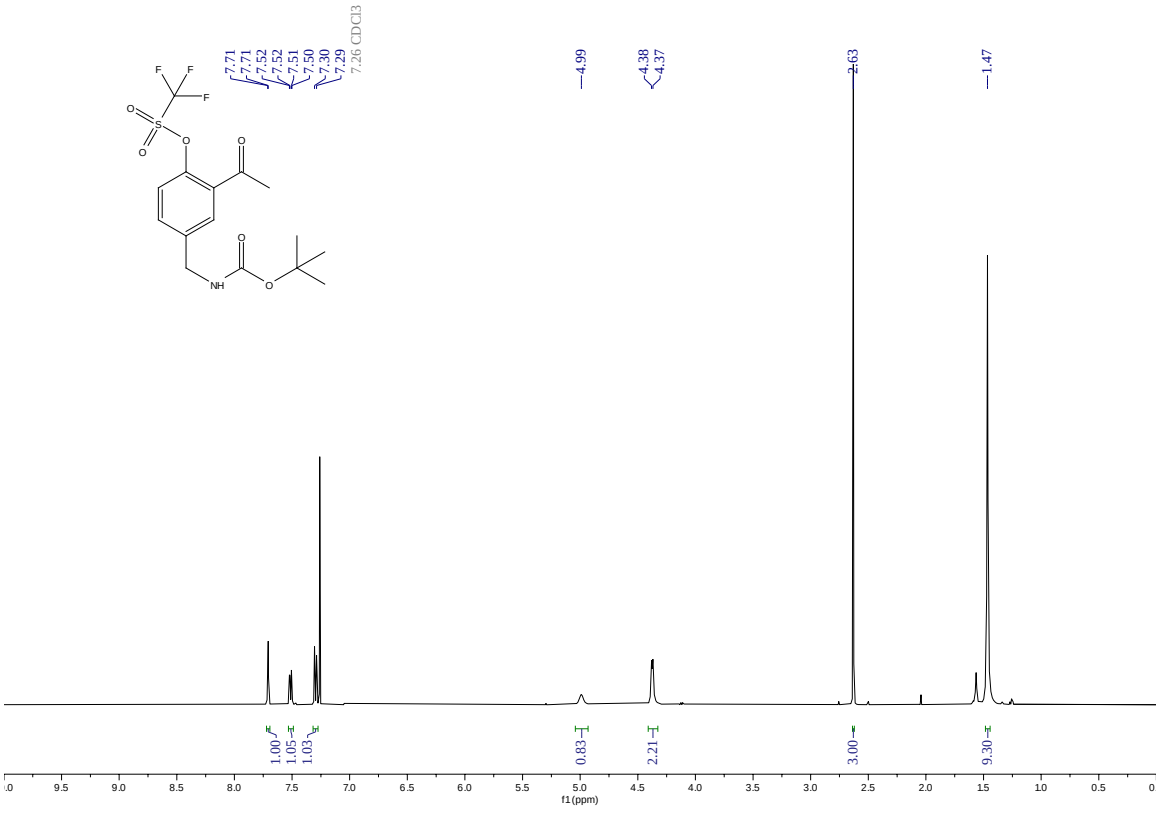
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1.47
F
F
F
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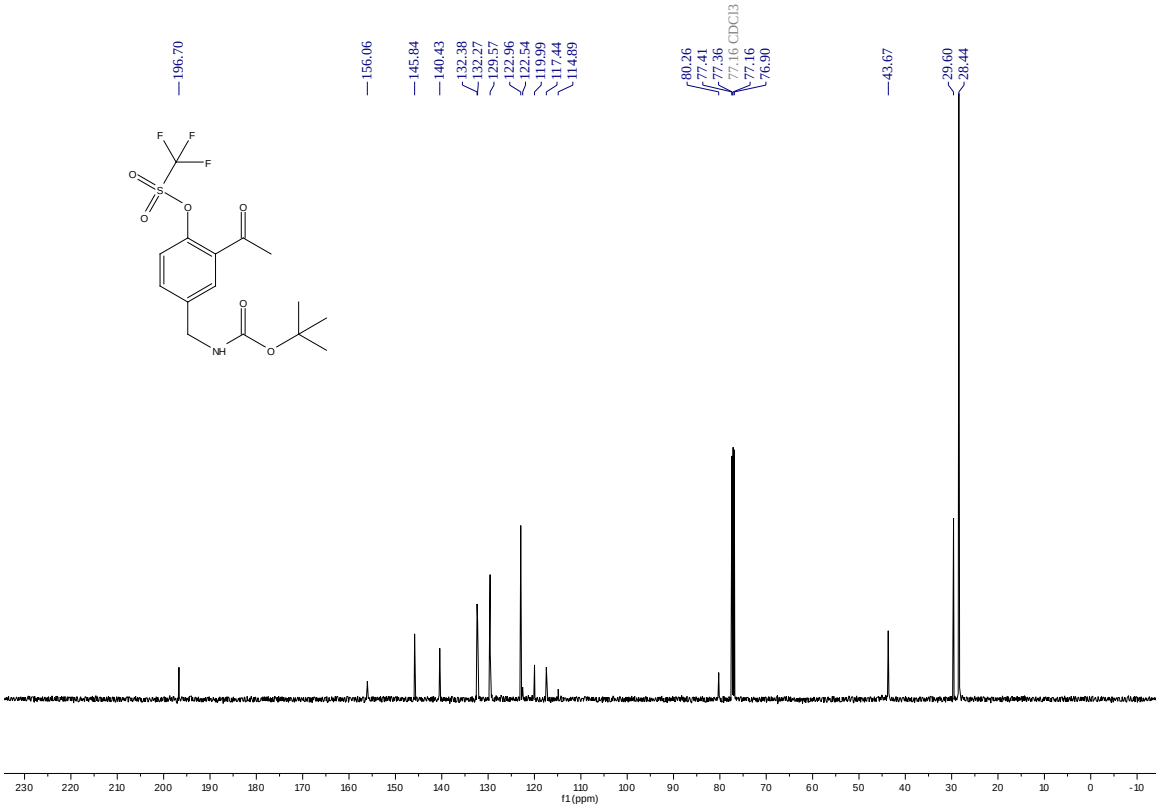
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77.16 CDCl3
77.16
76.90
43.67
29.60
28.44
f1 (ppm)


7.73
7.49
7.47
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7.44
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4.35
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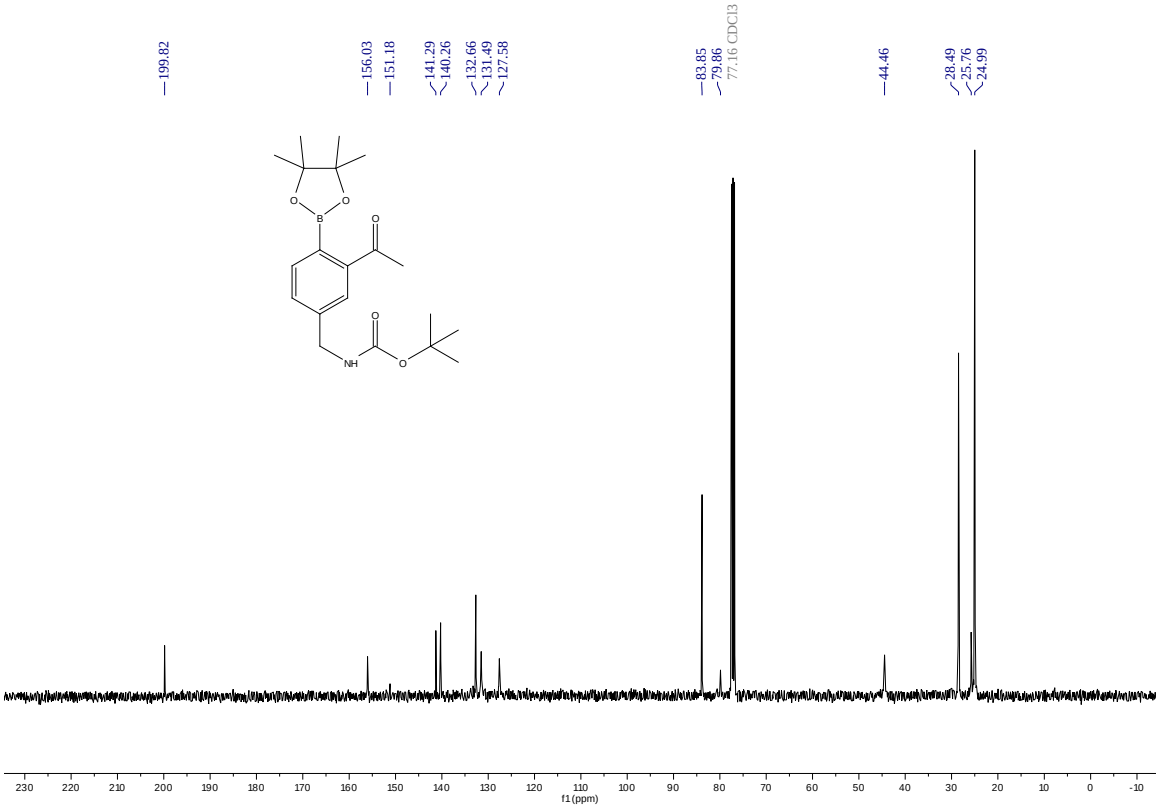
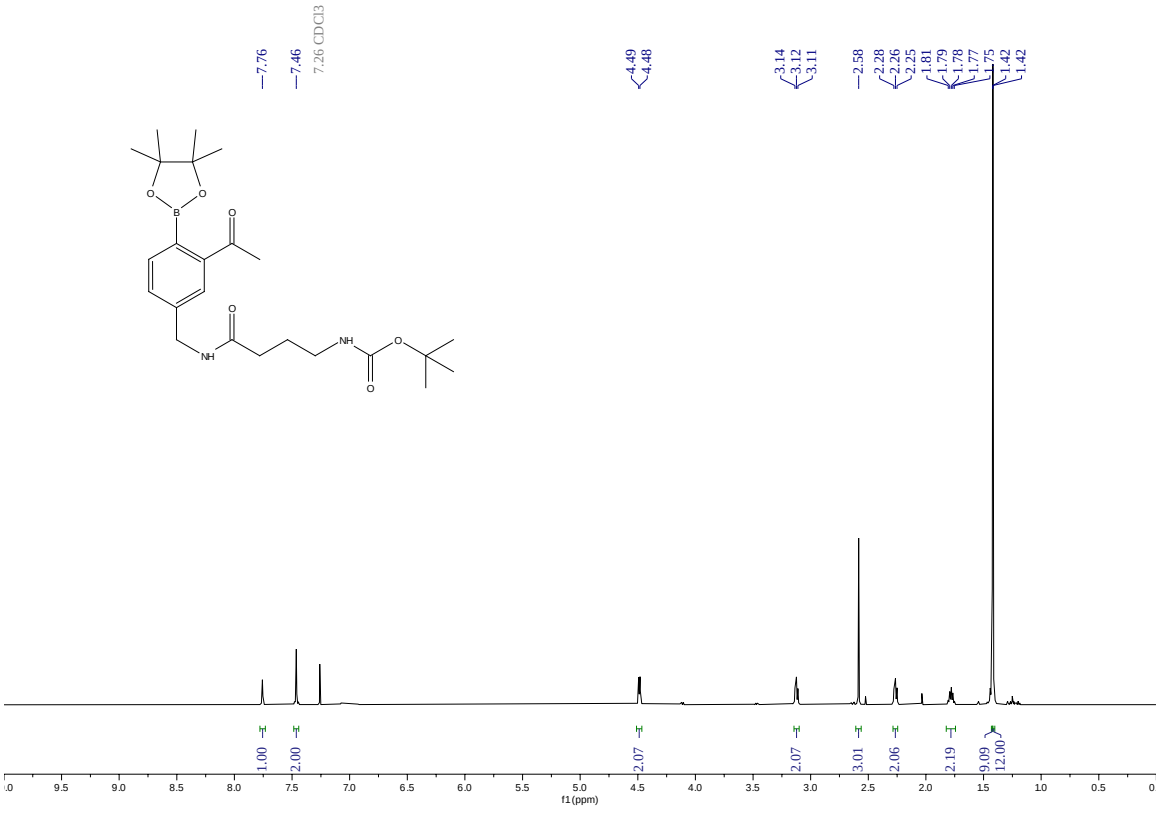
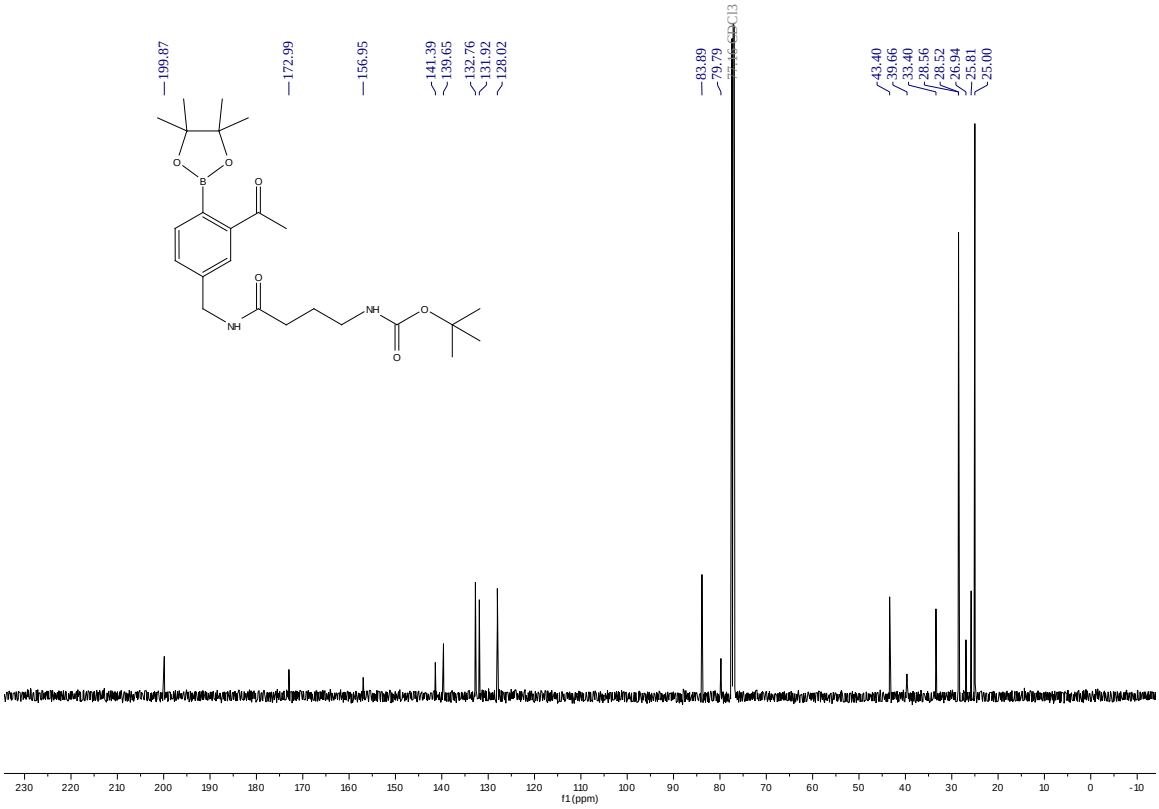
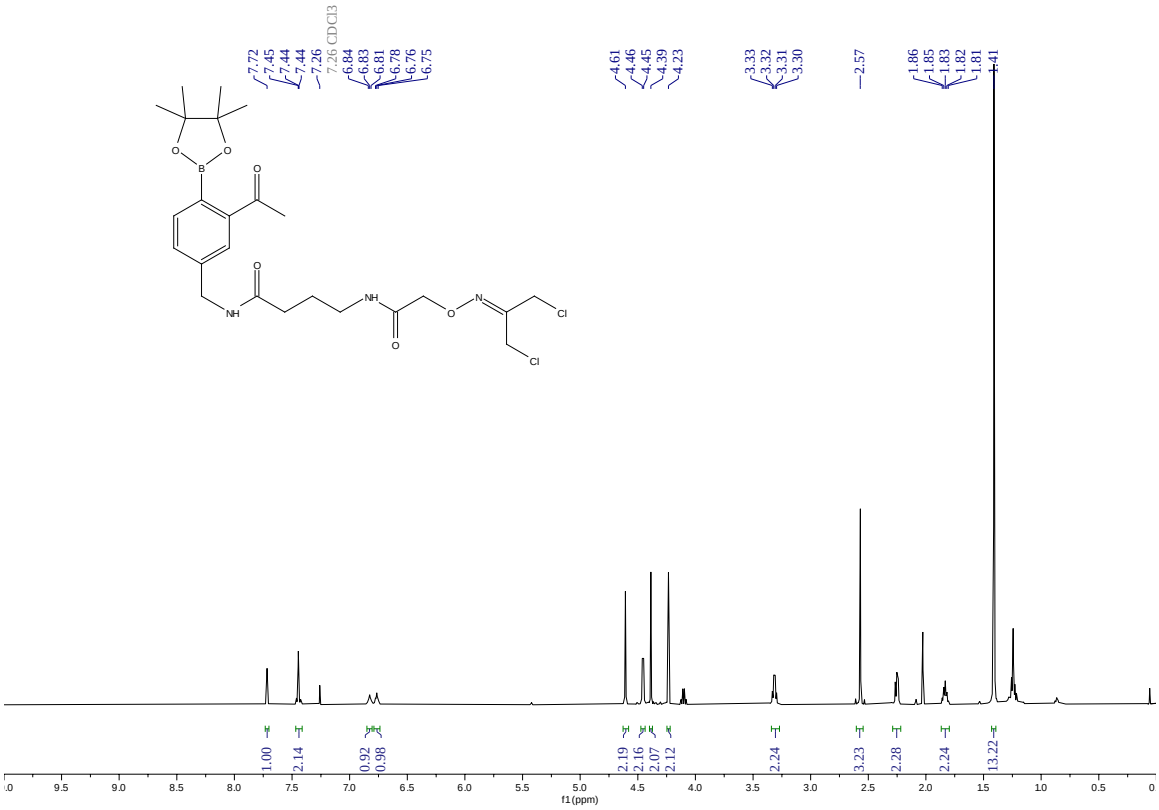
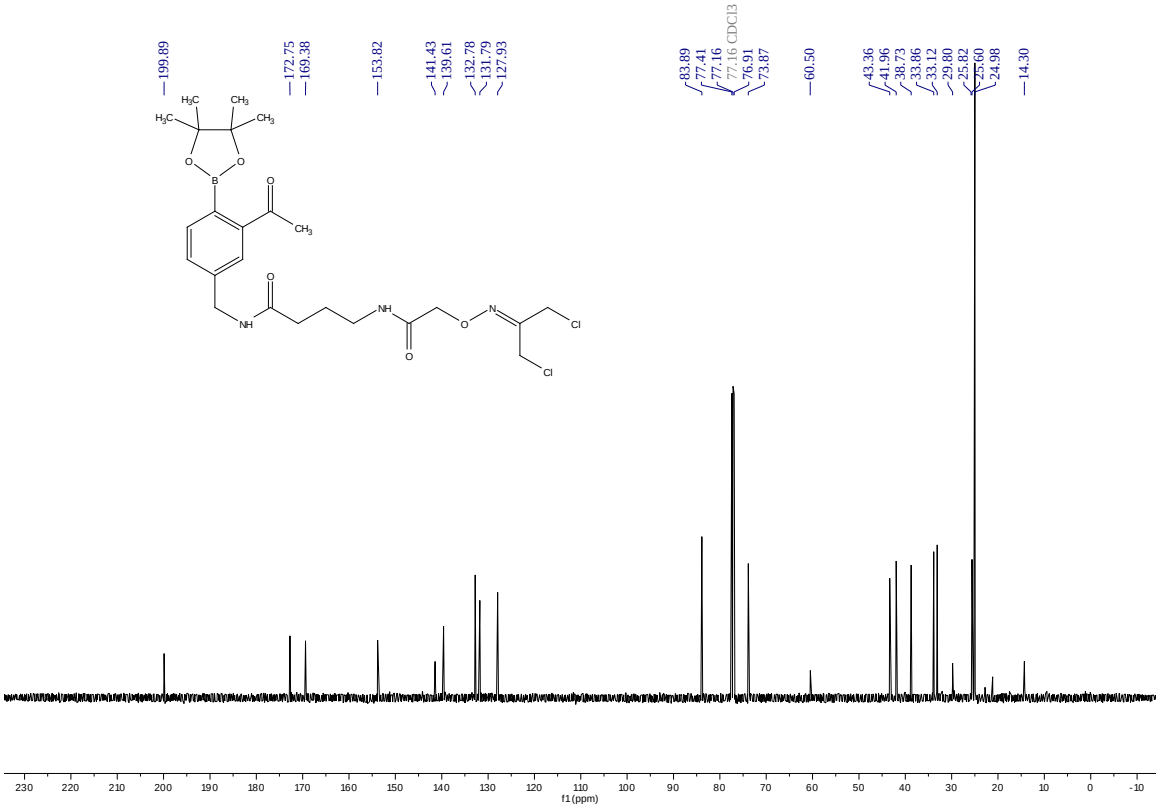
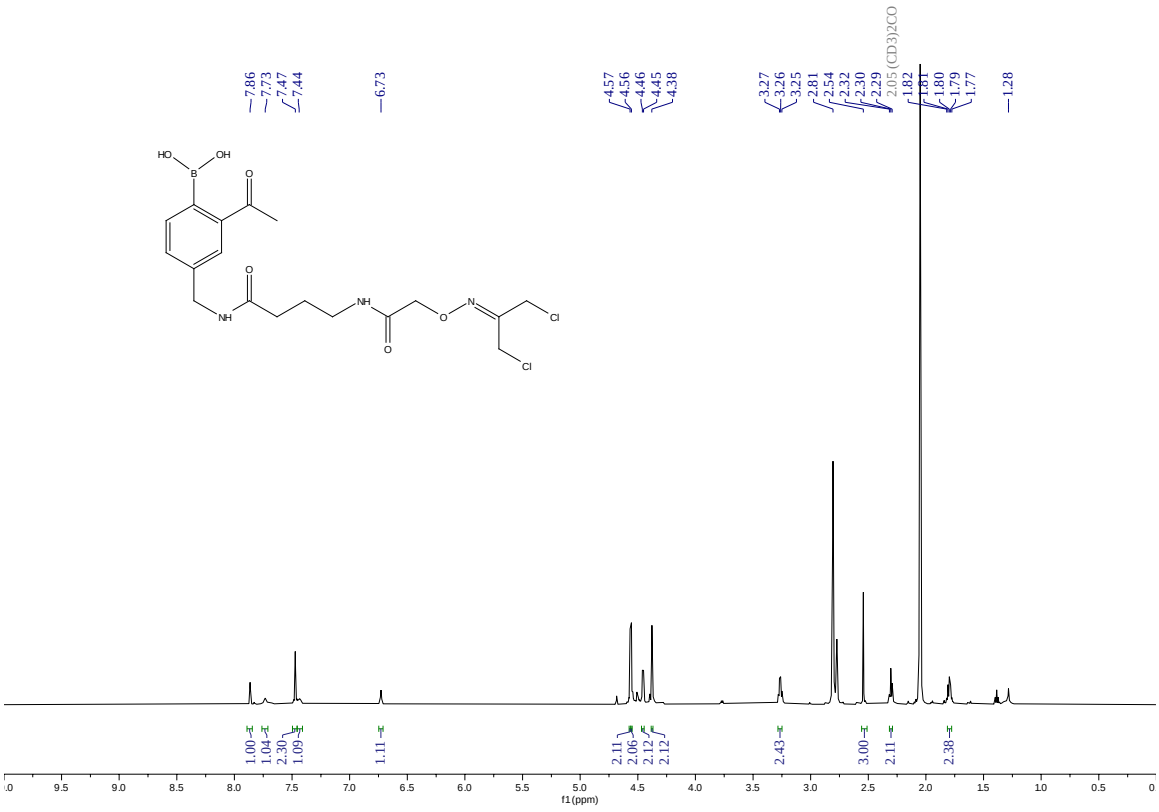

199.82
156.03
151.18
141.29
140.26
132.66
131.49
127.58
83.85
79.86
77.16 CDCl3
44.46
28.49
25.76
24.99
f1 (ppm)

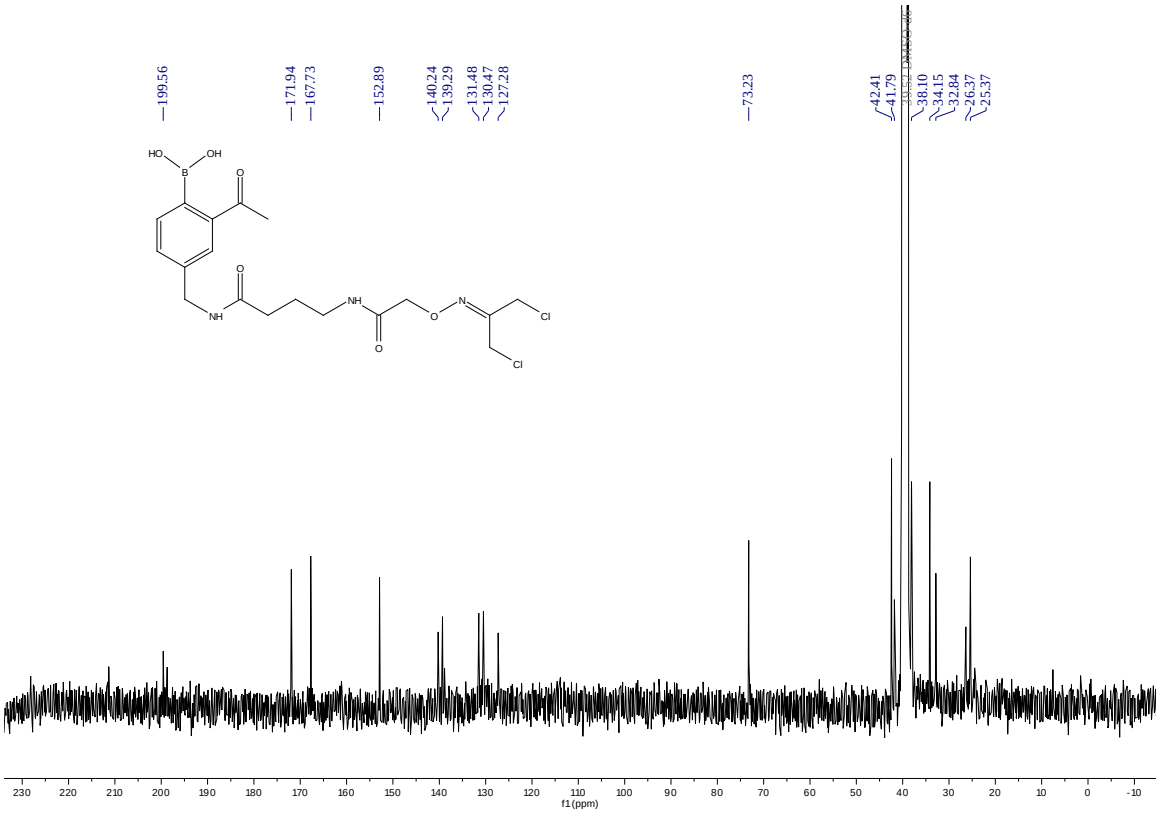

7.76
7.46
7.26 CDCl3
4.49
4.48
3.14
3.12
3.11
2.58
2.28
2.26
2.25
1.81
1.79
1.78
1.77
1.75
1.42
1.42
1.00
2.00
2.07
2.07
3.01
2.06
2.19
9.09
12.00
f1 (ppm)


199.87
172.99
156.95
141.39
139.65
132.76
131.92
128.02
83.89
79.79
77.48
77.16
76.84
CDCl3
43.40
39.66
33.40
28.56
28.52
26.94
25.81
25.00
f1 (ppm)

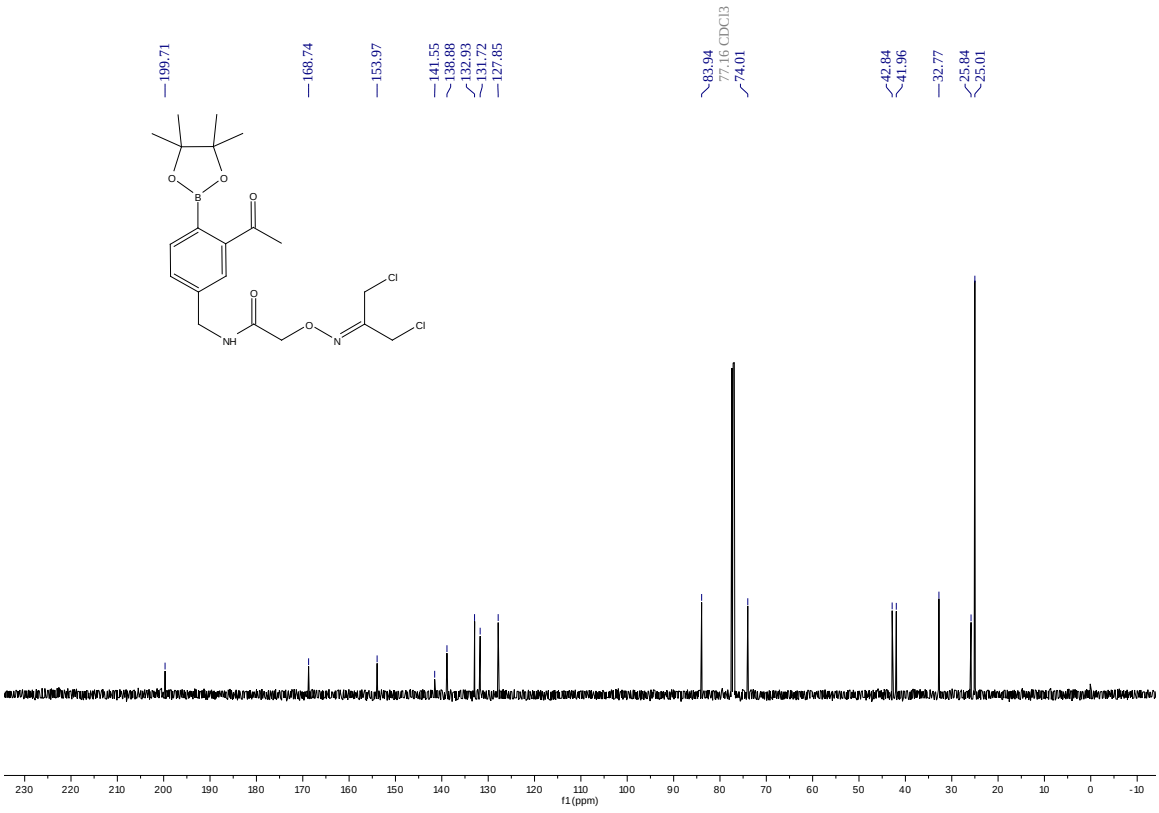

7.72
7.45
7.44
7.44
7.26
7.26
CDCl3
6.84
6.83
6.81
6.78
6.76
6.75
4.61
4.46
4.45
4.39
4.23
3.33
3.32
3.31
3.30
2.57
1.86
1.85
1.83
1.82
1.81
1.41
1.00
2.14
0.92
0.98
2.19
2.16
2.07
2.12
2.24
3.23
2.28
2.24
13.22
f1 (ppm)

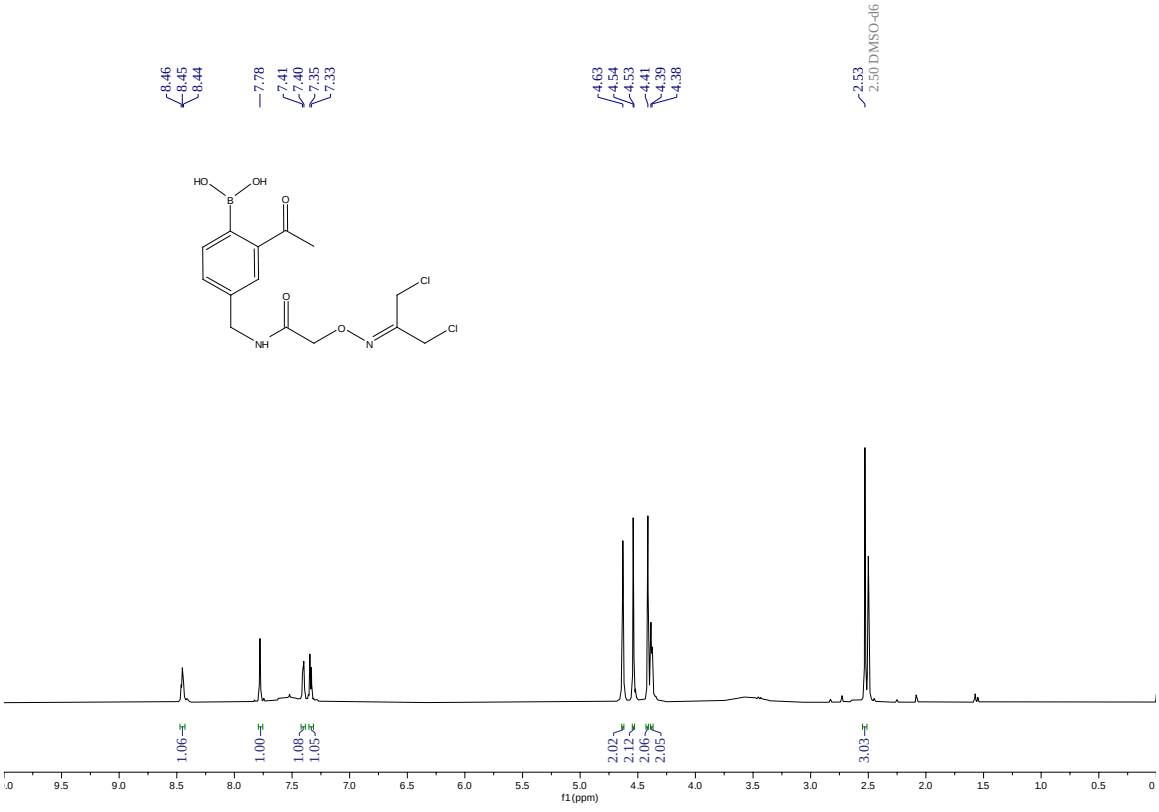

199.89
172.75
169.38
153.82
141.43
139.61
132.78
131.79
127.93
83.89
77.41
77.16
77.16 CDCl3
76.91
73.87
60.50
43.36
41.96
38.73
33.86
33.12
29.80
25.82
25.60
24.98
14.30
f1 (ppm)

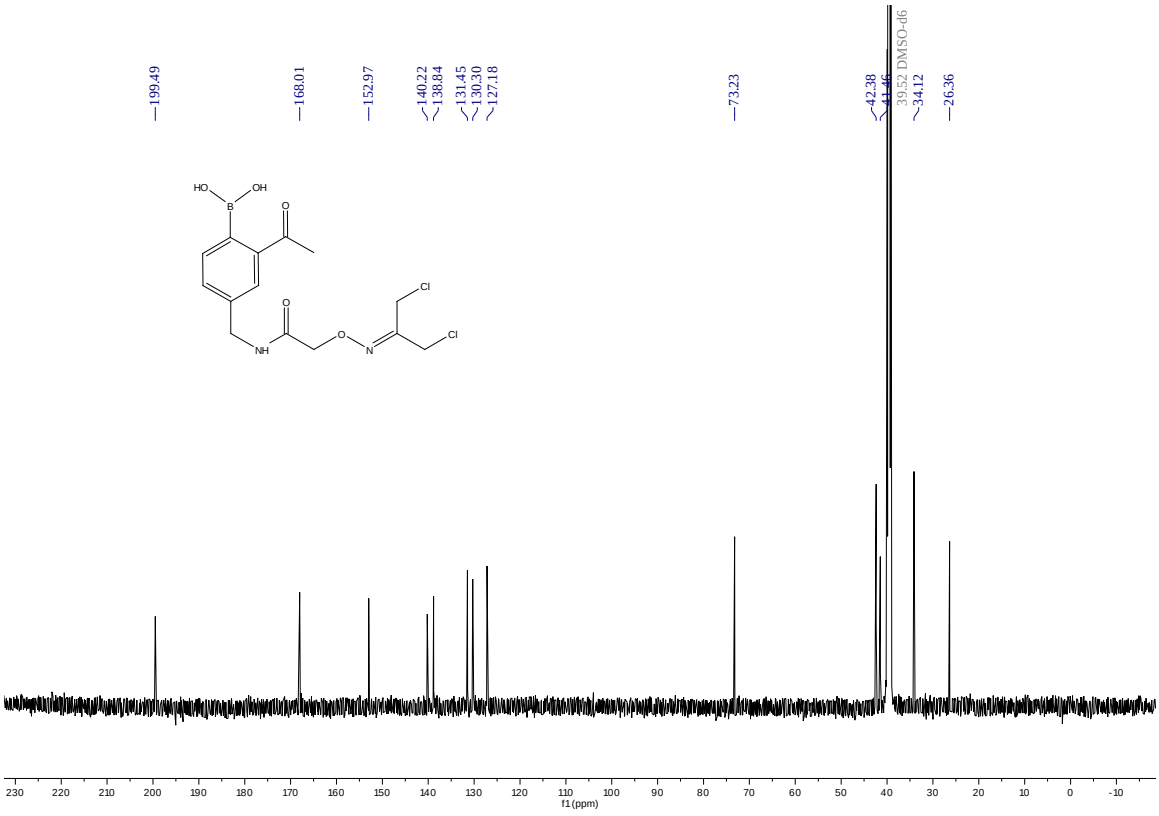

7.86
7.73
7.47
7.44
6.73
4.57
4.56
4.46
4.45
4.38
3.27
3.26
3.25
2.81
2.54
2.32
2.30
2.29
2.05 (CD3)2CO
1.82
1.81
1.80
1.79
1.77
1.28
1.00
1.04
2.30
1.09
1.11
2.11
2.06
2.12
2.12
2.43
3.00
2.11
2.38
f1 (ppm)

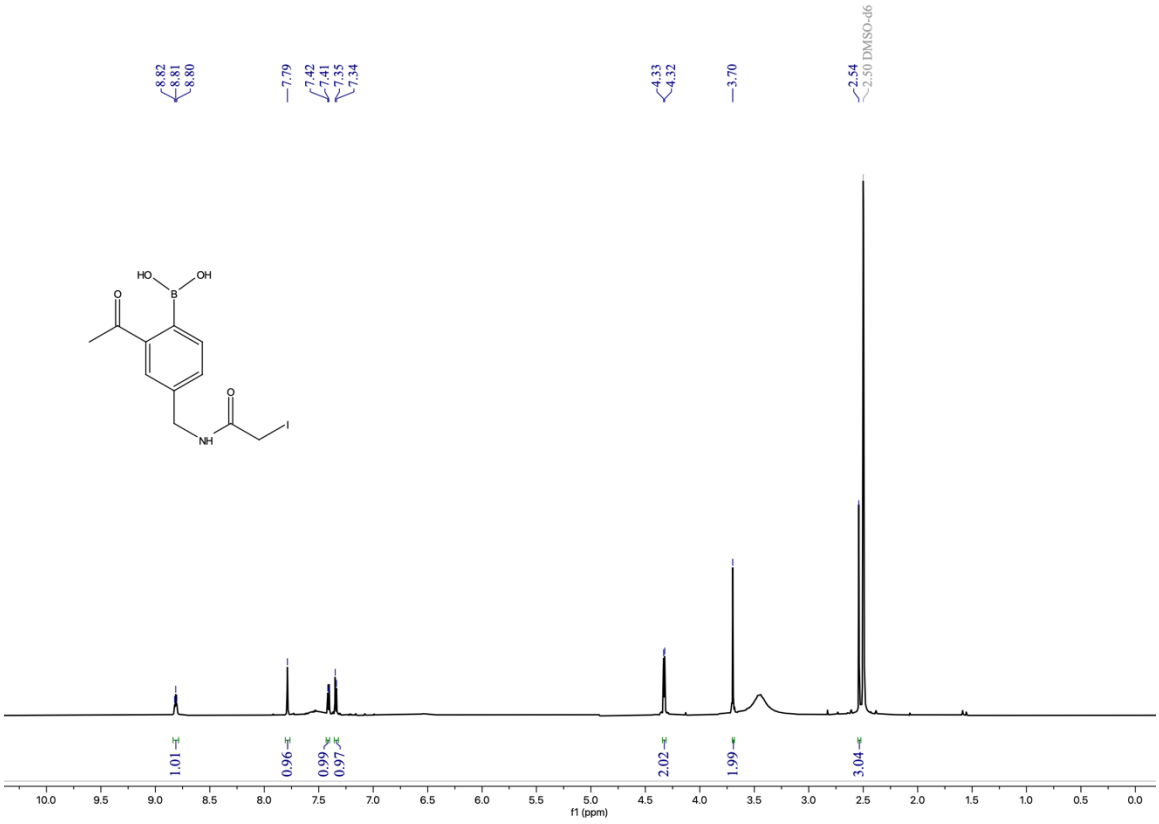

199.56
171.94
167.73
152.89
140.24
139.29
131.48
130.47
127.28
73.23
42.41
41.79
38.10
34.15
32.84
26.37
25.37
f1 (ppm)

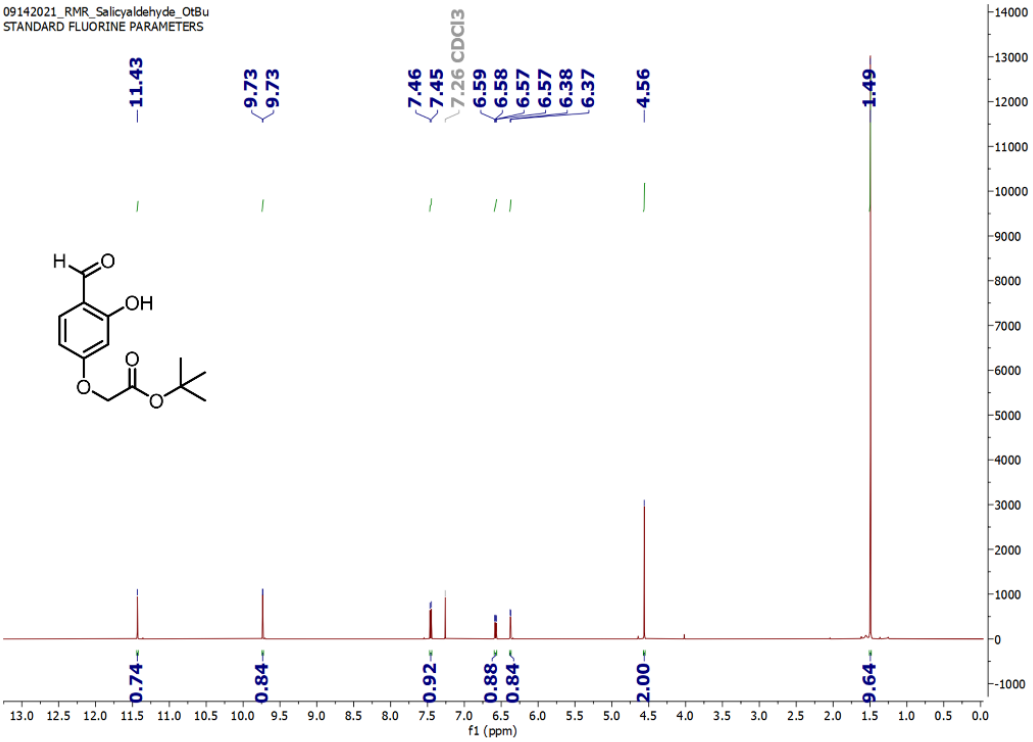

7.70
7.49
7.47
7.44
7.43
7.26 CDCl3
7.26
6.57
6.56
6.54
4.71
4.55
4.54
4.27
4.21
2.59
1.42
0.94
0.93
1.03
0.96
2.01
2.23
1.98
1.90
3.05
12.66
f1 (ppm)

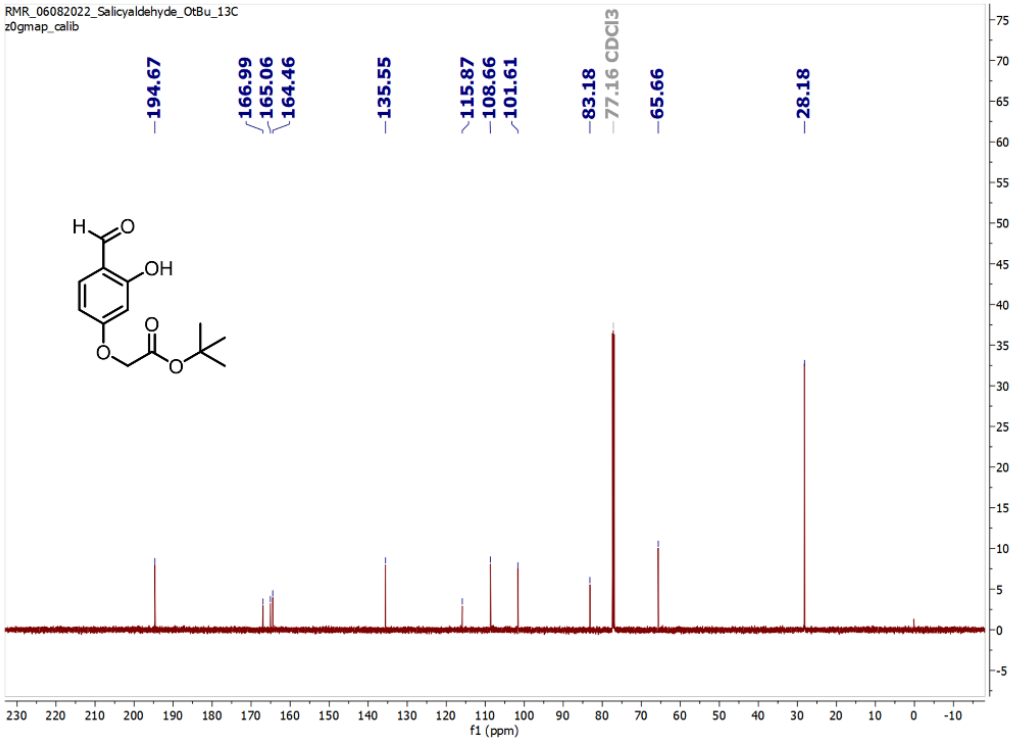

199.71
168.74
153.97
141.55
138.88
132.93
131.72
127.85
83.94
77.16 CDCl3
74.01
42.84
41.96
32.77
25.84
25.01
f1 (ppm)

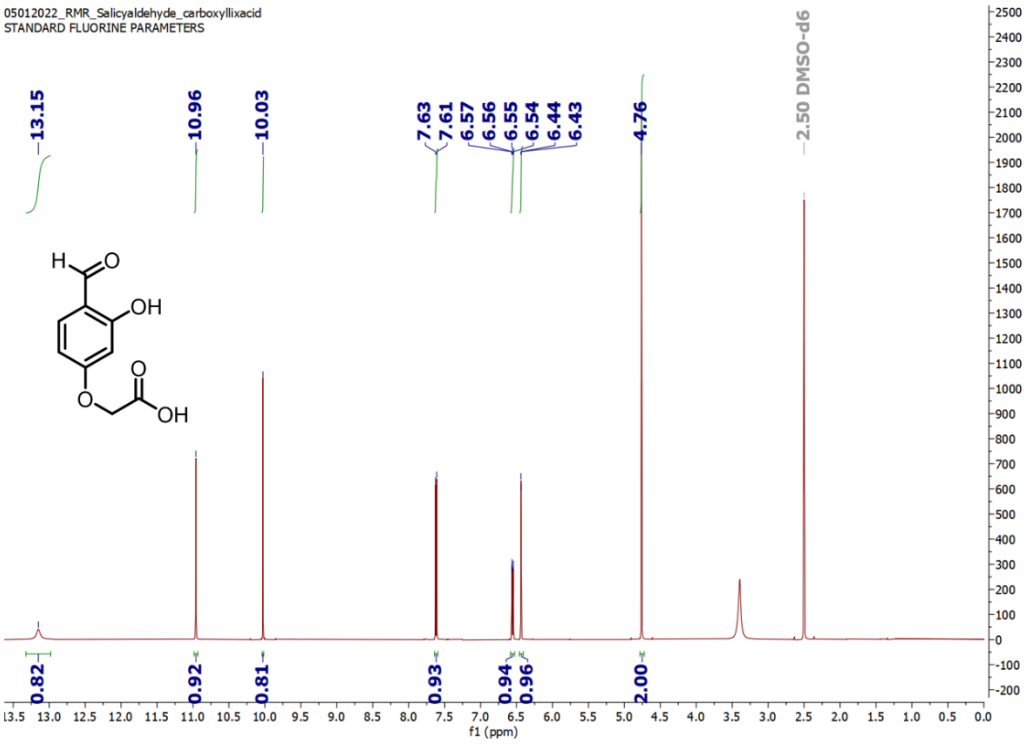

8.46
8.45
8.44
7.78
7.41
7.40
7.35
7.33
4.63
4.54
4.53
4.41
4.39
4.38
2.53
2.50 DMSO-d6
1.06
1.00
1.08
1.05
2.02
2.12
2.06
2.05
3.03
f1 (ppm)

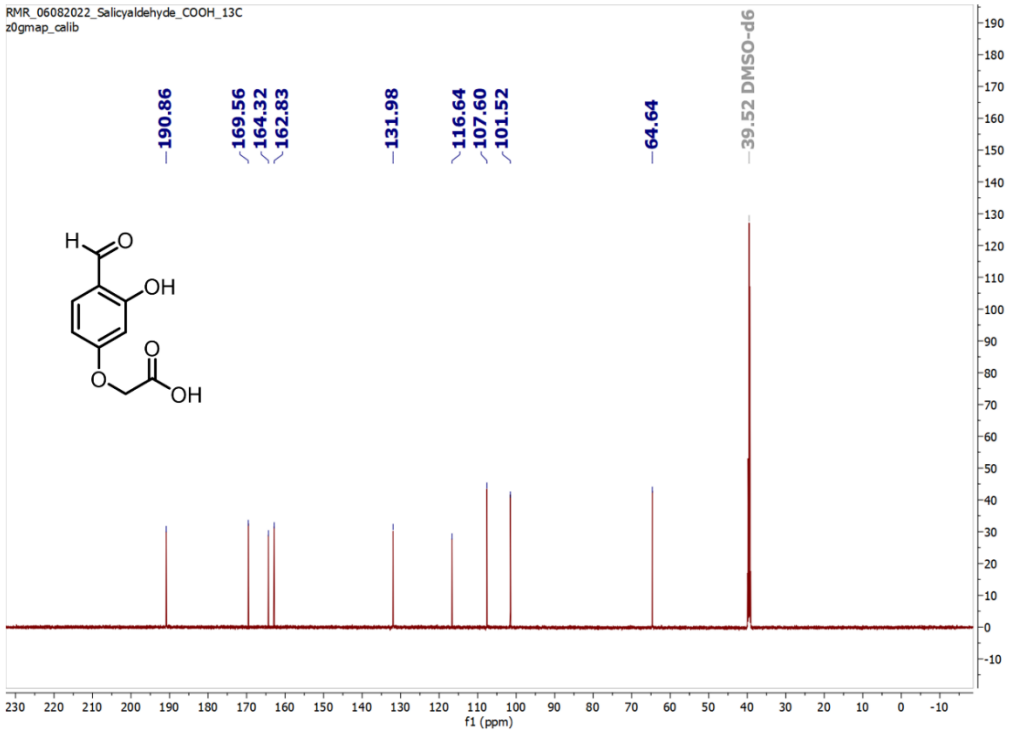

199.49
168.01
152.97
140.22
138.84
131.45
130.30
127.18
73.23
42.38
41.46
39.52 DMSO-d6
34.12
26.36
HO
B
OH
O
Cl
O
O
N
Cl
NH
f1 (ppm)

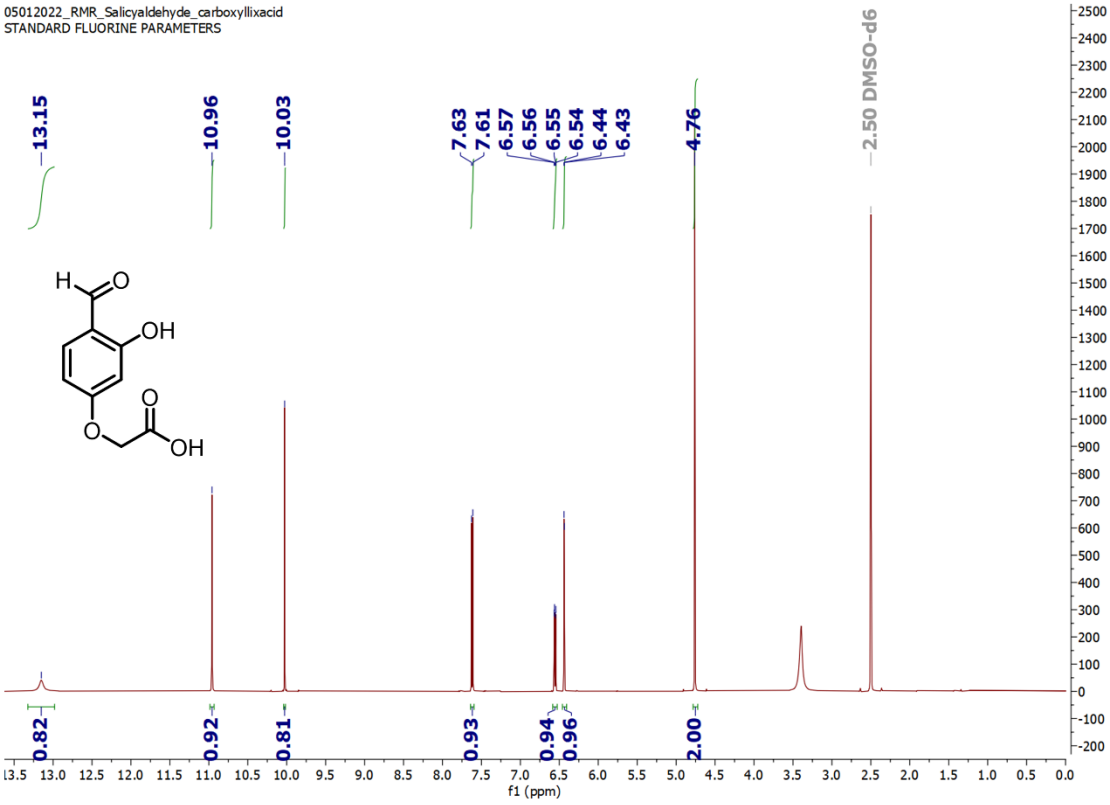

8.82
8.81
8.80
7.79
7.42
7.41
7.35
7.34
4.33
4.32
3.70
2.54
2.50 DMSO-d6
HO
B
OH
O
O
NH
I
1.01
0.96
0.99
0.97
2.02
1.99
3.04
f1 (ppm)

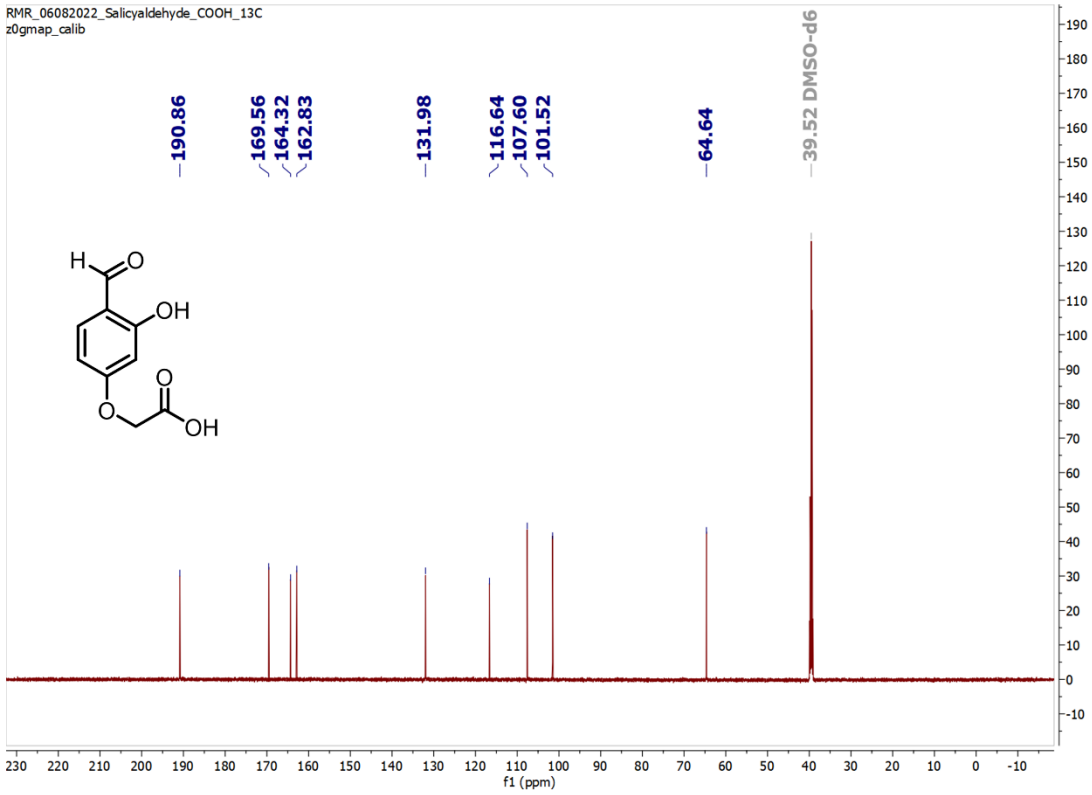

09142021_RMR_Salicyaldehyde_OtBu
STANDARD FLUORINE PARAMETERS
11.43
9.73
9.73
7.46
7.45
7.26 CDCl3
6.59
6.58
6.57
6.57
6.38
6.37
4.56
1.49
0.74
0.84
0.92
0.88
0.84
2.00
9.64
f1 (ppm)

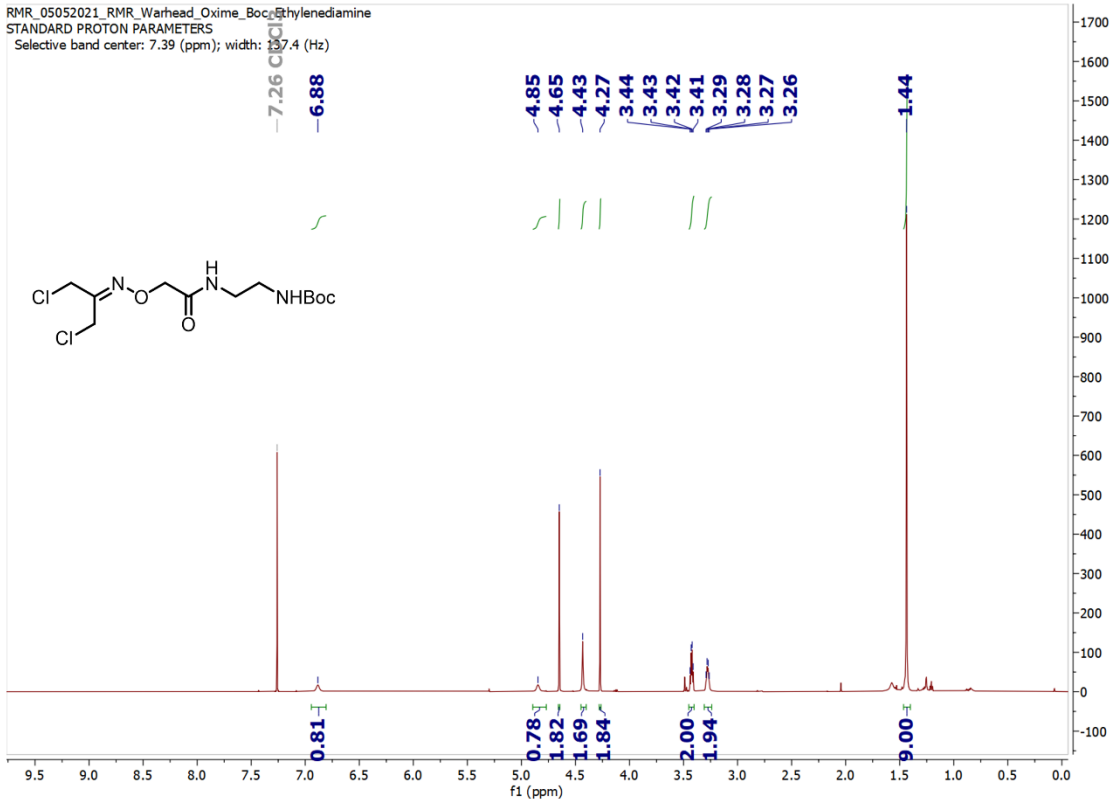

RMR_06082022_Salicyaldehyde_OtBu_13C
z0gmap_calib
194.67
166.99
165.06
164.46
135.55
115.87
108.66
101.61
83.18
77.16 CDCl3
65.66
28.18
f1 (ppm)

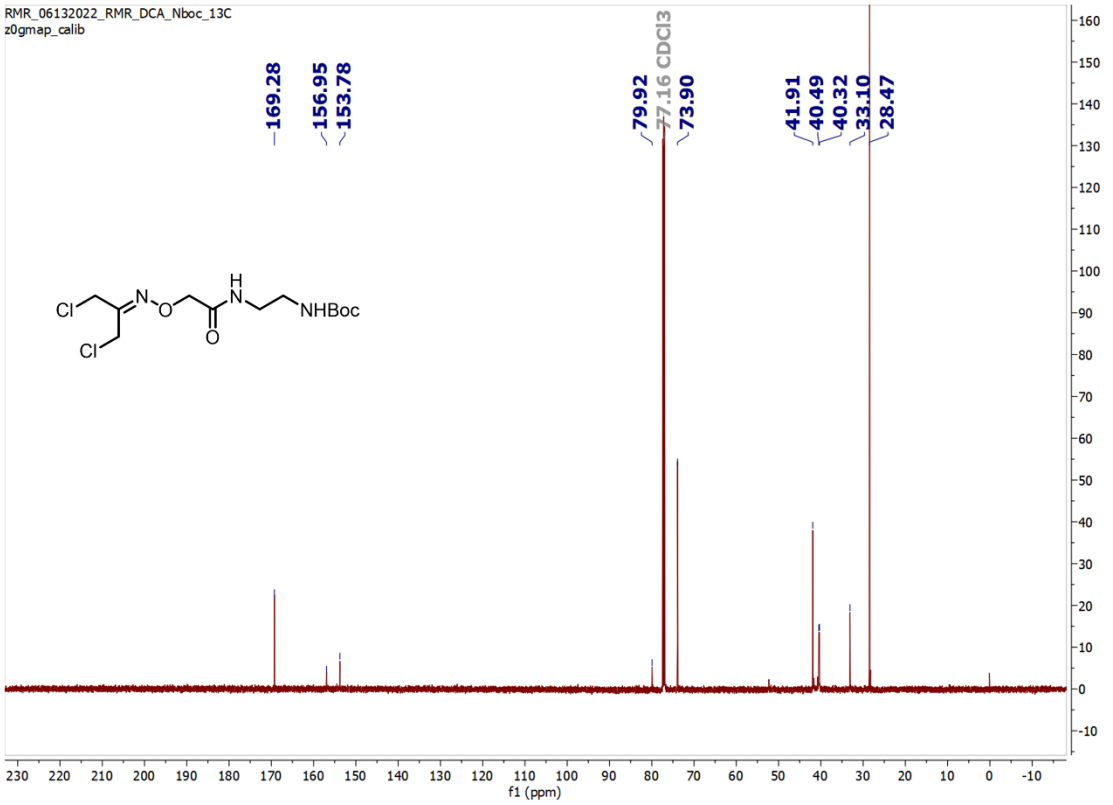

05012022_RMR_Salicyaldehyde_carboxyllixacid
STANDARD FLUORINE PARAMETERS
13.15
10.96
10.03
7.63
7.61
6.57
6.56
6.55
6.54
6.44
6.43
4.76
2.50 DMSO-d6
0.82
0.92
0.81
0.93
0.94
0.96
2.00
f1 (ppm)

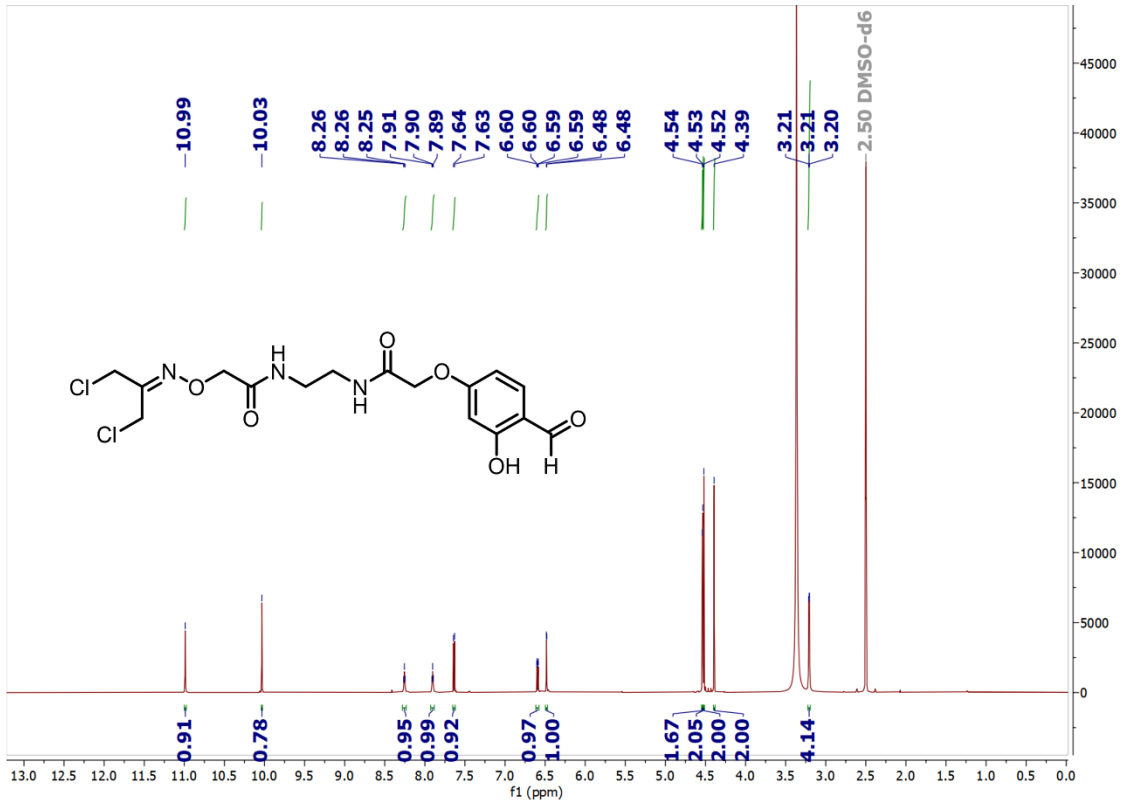

RMR_06082022_Salicyaldehyde_COOH_13C
z0gmap_calib
190.86
169.56
164.32
162.83
131.98
116.64
107.60
101.52
64.64
39.52 DMSO-d6
f1 (ppm)

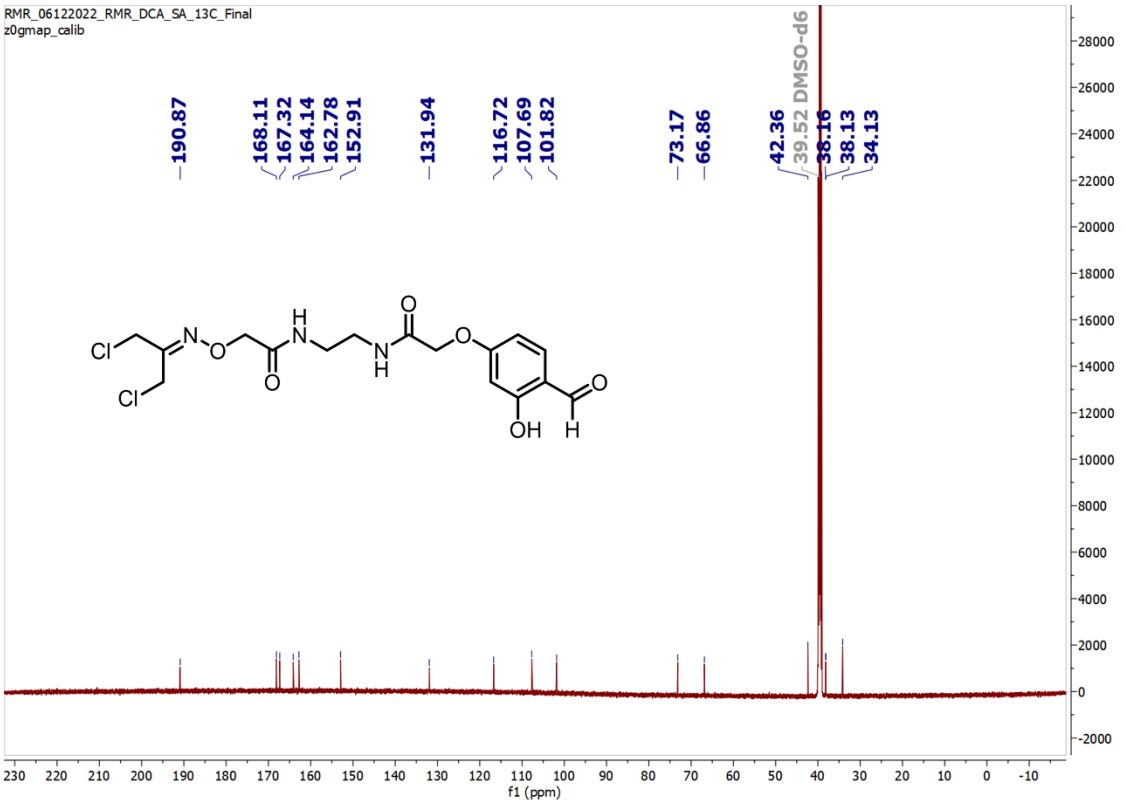

05012022_RMR_Salicyaldehyde_carboxyllixacid
STANDARD FLUORINE PARAMETERS
13.15
10.96
10.03
7.63
7.61
6.57
6.56
6.55
6.54
6.44
6.43
4.76
2.50 DMSO-d6
0.82
0.92
0.81
0.93
0.94
0.96
2.00
f1 (ppm)


RMR_06082022_Salicyaldehyde_COOH_13C
z0gmap_calib
190.86
169.56
164.32
162.83
131.98
116.64
107.60
101.52
64.64
39.52 DMSO-d6
f1 (ppm)


RMR_05052021_RMR_Warhead_Oxime_Boc_Ethylenediamine
STANDARD PROTON PARAMETERS
Selective band center: 7.39 (ppm); width: 137.4 (Hz)
7.26 CDCl3
6.88
4.85
4.65
4.43
4.27
3.44
3.43
3.42
3.41
3.29
3.28
3.27
3.26
1.44
Cl
N
O
H
N
NHBoc
O
Cl
0.81
0.78
1.82
1.69
1.84
2.00
1.94
9.00
f1 (ppm)


RMR_06132022_RMR_DCA_Nboc_13C
z0gmap_calib
169.28
156.95
153.78
79.92
77.16 CDCl3
73.90
41.91
40.49
40.32
33.10
28.47
Cl
N
O
H
N
NHBoc
O
Cl
f1 (ppm)


10.99
10.03
8.26
8.26
8.25
7.91
7.90
7.89
7.64
7.63
6.60
6.60
6.59
6.59
6.48
6.48
4.54
4.53
4.52
4.39
3.21
3.21
3.20
2.50 DMSO-d6
0.91
0.78
0.95
0.99
0.92
0.97
1.00
1.67
2.05
2.00
2.00
4.14
f1 (ppm)


RMR_06122022_RMR_DCA_SA_13C_Final
z0gmap_calib
190.87
168.11
167.32
164.14
162.78
152.91
131.94
116.72
107.69
101.82
73.17
66.86
42.36
39.52 DMSO-d6
38.16
38.13
34.13
f1 (ppm)