Supplemental Tables, Figures and Information on compound synthesis
This document includes Supplemental Tables 1-12; Supplemental Figures 1-7; Supplemental Information on compound synthesis

Supplemental Table 1 Identification and quantification of CapD reaction products generated from synthetic substrate Abz-γ-D-Glu$_{10}$-Dnp (as shown on **Fig. 3b**). Products were separated by rpHPLC using acetonitrile gradient. Compounds were recovered from HPLC fractions and subjected to MALDI-TOF MS analysis in positive and negative reflectron mode. Absorbance peak areas from HPLC chromatogram were integrated to determine the relative abundance of identified compounds (32 Karat Software Package, Beckman Coulter, Inc.).

Reaction	**Retention (min)**	**Area (%)**	**$[M+H]^+$**		**$[M-H]^-$**		**Predicted structure**
			observed	**calculated**	**observed**	**calculated**	
Hydrolysis	35-41	**10.2%**	654.14	654.20	652.28	652.20	Abz-γ-D-Glu$_4$
			n. d.	783.24	781.33	781.24	Abz-γ-D-Glu$_5$
			n. d.	912.28	910.38	910.28	Abz-γ-D-Glu$_6$
			1041.50	1041.32	1039.41	1039.32	Abz-γ-D-Glu$_7$
			n. d.	1170.36	1168.43	1168.36	Abz-γ-D-Glu$_8$
	46-48	**32.2%**	399.06	399.11	n. d.	397.11	γ-D-Glu-Dnp
			528.09	528.15	526.23	526.15	γ-D-Glu$_2$-Dnp
			657.13	657.19	655.27	655.19	γ-D-Glu$_3$-Dnp
			786.16	786.23	784.32	784.23	γ-D-Glu$_4$-Dnp
			915.20	915.27	913.36	913.27	γ-D-Glu$_5$-Dnp
			1044.24	1044.31	1042.39	1042.31	γ-D-Glu$_6$-Dnp
			1173.28	1173.35	1171.43	1171.35	γ-D-Glu$_7$-Dnp
			1302.33	1302.39	1300.47	1300.39	γ-D-Glu$_8$-Dnp
			n. d.	1431.43	1429.50	1429.43	γ-D-Glu$_9$-Dnp
Substrate*	49	**>1%**	n. d.	1679.51	1677.59	1677.51	Abz-γ-D-Glu$_{10}$-Dnp
Autotranspeptidation	50-54	**57.6%**	n. d.	647.19	645.26	645.19	Abz-γ-D-Glu$_2$-Dnp
			n. d.	776.23	774.31	774.23	Abz-γ-D-Glu$_3$-Dnp
			905.15	905.27	903.35	903.27	Abz-γ-D-Glu$_4$-Dnp
			1034.19	1034.31	1032.39	1032.31	Abz-γ-D-Glu$_5$-Dnp
			1163.23	1163.35	1161.43	1161.35	Abz-γ-D-Glu$_6$-Dnp
			1292.30	1292.39	1290.47	1290.39	Abz-γ-D-Glu$_7$-Dnp
			1421.34	1421.43	1419.51	1419.43	Abz-γ-D-Glu$_8$-Dnp
			n. d.	1550.47	1548.55	1548.47	Abz-γ-D-Glu$_9$-Dnp

n. d. Not detected
* The substrate did not form a distinct absorbance peak during HPLC. The compound was detected by MALDI-TOF MS. Non-cleaved substrate represents less than 1% of all compounds detected after CapD assay (see **Fig. 3c** and **Supplemental Table 2**).

Supplemental Table 2 Identification and quantification of CapD reaction products generated from Abz-γ-D-Glu$_{10}$-Dnp in presence of cell wall tripeptide L-Ala-γ-D-Glu-*m*-DAP (as shown on **Fig. 3c**). Products were separated by rpHPLC using acetonitrile gradient. Compounds were recovered from HPLC fractions and subjected to MALDI-TOF MS analysis in positive and negative reflectron mode. Absorbance peak areas from HPLC chromatogram were integrated to determine the relative abundance of identified compounds (32 Karat Software Package, Beckman Coulter, Inc.).

Reaction	**Retention (min)**	**Area (%)**	**$[M+H]^+$**		**$[M-H]^-$**		**Predicted structure**
			observed	**calculated**	**observed**	**calculated**	
Transpeptidation	35-41	**36.4%**	639.26	639.26	637.14	637.26	L-Ala-γ-D-Glu-*m*-DAP(γ-D-Glu-Abz)
			768.30	768.30	766.14	766.30	L-Ala-γ-D-Glu-*m*-DAP(γ-D-Glu$_2$-Abz)
			897.35	897.35	895.14	895.35	L-Ala-γ-D-Glu-*m*-DAP(γ-D-Glu$_3$-Abz)
			1026.40	1026.39	n. d.	1024.39	L-Ala-γ-D-Glu-*m*-DAP(γ-D-Glu$_4$-Abz)
Hydrolysis	44-48	**58.7%**	528.08	528.15	526.22	526.15	γ-D-Glu$_2$-Dnp
			657.11	657.19	655.26	655.19	γ-D-Glu$_3$-Dnp
			786.15	786.23	784.30	784.23	γ-D-Glu$_4$-Dnp
			915.19	915.27	913.34	913.27	γ-D-Glu$_5$-Dnp
			1044.23	1044.31	1042.38	1042.31	γ-D-Glu$_6$-Dnp
Substrate	49	**0.8%**	n. d.	1679.51	1677.08	1677.51	Abz-γ-D-Glu$_{10}$-Dnp
Autotranspeptidation	50-51	**4.2%**	776.23	776.23	774.09	774.23	Abz-γ-D-Glu$_3$-Dnp
			905.15	905.27	903.34	903.27	Abz-γ-D-Glu$_4$-Dnp
			1034.38	1034.31	1032.36	1032.31	Abz-γ-D-Glu$_5$-Dnp

n. d. Not detected

Supplemental Table 3 CID daughter ions of parent ion 639.25 shown on to **Supplemental Fig. 1** and predicted structure assignments. Program ProteinProspector was used for *m/z* calculations (http://prospector.ucsf.edu).

Ion	***Observed m/z***	***Calculated m/z***	***Predicted structure***
[M+H]	639.26	639.26	L-Ala-γ-D-Glu-*m*-DAP(-γ-D-Glu-Abz)
b4	502.33	502.21	L-Ala-γ-D-Glu-*m*-DAP(-γ-D-Glu)
b4 +H_2O	520.36	520.22	
b3	373.28	373.17	L-Ala-γ-D-Glu-*m*-DAP
b3 +H_2O	391.27	391.18	
b2	201.15	201.09	L-Ala-γ-D-Glu
a2	173.14	173.09	
a2 +H_2O	191.15	191.09	
y4	568.31	568.22	γ-D-Glu-*m*-DAP(-γ-D-Glu-Abz)
y3	439.28	439.18	*m*-DAP(-γ-D-Glu-Abz)
y3 +H_2O	457.30	457.18	
Internal [M+H]	431.28	431.18	γ-D-Glu-*m*-DAP(-γ-D-Glu)
Internal [M+H] +H_2O	449.29	449.18	
Internal [M+H]	302.22	302.13	γ-D-Glu-*m*-DAP or *m*-DAP(-γ-D-Glu)
Internal [M+H] +H_2O	320.22	320.13	

Supplemental Table 4 CID daughter ions of parent ion 1009.47 with predicted structure assignments. Programs ChemDraw (CambridgeSoft) and ProteinProspector (http://prospector.ucsf.edu) were used for *m/z* calculations. The compound was identified following the rpHPLC analysis of CapD treated and mutanolysin (muramidase) cleaved murein sacculi isolated from *B. anthracis* Ames (**Supplemental Fig. 2**) as well as in isolated capsular material [**Fig. 5c** (PDGA)] following treatment with CapD (**Fig. 5d**).

Compound *m/z* 1009.47

m/z	**Observed**	**Calculated**	**Predicted structure**
[M+H] -H_2O	1009.47	1009.41	GA-MN-L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y6	848.52	848.34	MN-L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y6'	645.42	645.27	Lac-L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y4	502.34	502.21	γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y3	373.29	373.17	*m*-DAP(-γ–D-Glu)-D-Ala
Intern [M+H]	485.40	484.20	L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)
Intern [M+H]	301.22	301.12	γ–D-Glu-*m*-DAP

Supplemental Table 5 CID daughter ions of parent ion 1051.42 with predicted structure assignments. Programs ChemDraw (CambridgeSoft) and ProteinProspector (http://prospector.ucsf.edu) were used for *m/z* calculations. The compound was identified following the rpHPLC analysis of CapD treated and mutanolysin (muramidase) cleaved murein sacculi isolated from *B. anthracis* Ames (**Supplemental Fig. 2**) as well as in isolated capsular material [**Fig. 5c** (PDGA)] following treatment with CapD (**Fig. 5d**).

Compound *m/z* 1051.53

m/z	**Observed**	**Calculated**	**Predicted structure**
[M+H] -H_2O	1051.53	1051.42	GN-MN-L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y6	848.56	848.34	MN-L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y6'	645.45	645.27	Lac-L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y4	502.36	502.21	γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y3	373.32	373.17	*m*-DAP(-γ–D-Glu)-D-Ala
Intern [M+H]	301.21	301.12	γ–D-Glu-*m*-DAP

Supplemental Table 6 CID daughter ions of parent ion 645.34 with predicted structure assignments. Programs ChemDraw (CambridgeSoft) and ProteinProspector (http://prospector.ucsf.edu) were used for *m/z* calculations. The compound was identified following the rpHPLC analysis of CapD treated and mutanolysin (muramidase) cleaved murein sacculi isolated from *B. anthracis* Ames (**Supplemental Fig. 2**) as well as in isolated capsular material [**Fig. 5c** (PDGA)] following treatment with CapD (**Fig. 5d**).

Compound *m/z* 645.34

m/z	**Observed**	**Calculated**	**Predicted structure**
[M+H] -H_2O	645.34	645.27	Lac-L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
b3 -H_2O	257.25	257.11	Lac-L-Ala-γ–D-Glu
y5	573.44	573.25	L-Ala-γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y4	502.38	502.21	γ–D-Glu-*m*-DAP(-γ–D-Glu)-D-Ala
y3	373.32	373.17	*m*-DAP(-γ–D-Glu)-D-Ala
Intern [M+H]	301.22	301.12	γ–D-Glu-*m*-DAP

Supplemental Table 7 Predicted peptidoglycan subunits isolated from encapsulated *B. anthracis* Ames cells. Compounds were separated by rpHPLC (as shown on **Fig. 5c**) using a methanol gradient in sodium phosphate buffer and desalted before MALDI-TOF MS analysis in positive and negative reflectron mode. Amino acid analysis was carried out by ion-exchange HPLC following acidic hydrolysis.

Retention	$[M+Na]^+$		$[M+H]^+$		$[M-H]^-$		Predicted structure
(min)	observed	calculated	observed	calculated	observed	calculated	
18	848.41	848.36	-	826.36	824.19	824.36	GA-MN-L-Ala-γ-D-iGln-*m*-DAP[3)]
20	890.41	890.37	-	868.37	866.17	866.37	GN-MN-L-Ala-γ-D-iGln-*m*-DAP
29	919.44	919.40	897.46	897.40	895.19	895.40	GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala[3)]
33	961.46	961.41	-	939.41	937.19	937.41	GA-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala
34	677.28	677.26	-	655.26	653.13	653.26	GA-MN-L-Ala-γ-D-iGlu
35	719.34	719.27	-	697.27	695.20	695.27	GN-MN-L-Ala-γ-D-iGlu
36	1032.52	1032.45	-	1010.45	1008.30	1008.45	GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala-D-Ala
39-40 (a)	1362.61	1362.60	-	1340.60	1338.24	1338.60	Lac-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GA-MN-L-Ala-γ-D-iGln-*m*-DAP)[3)]
39-40 (b)	1404.64	1404.61	-	1382.61	1380.26	1380.61	Lac-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GN-MN-L-Ala-γ-D-iGln-*m*-DAP)
39-40 (c)	1726.78	1726.76	-	1704.76	1702.28	1702.76	GA-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GA-MN-L-Ala-γ-D-iGln-*m*-DAP)
43-60 (a)	-	1474.65	1452.77	1452.65	-	1450.65	Lac-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala)
43-60 (b)	-	1545.69	1523.81	1523.69	-	1521.69	Lac-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala-D-Ala)
43-60 (c)	-	1838.81	1816.93	1816.81	-	1814.81	GA-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala)
43-60 (d)	-	1909.85	1887.98	1887.85	1885.32	1885.85	GA-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala-D-Ala)
43-60 (d)	-	1951.86	1929.99	1929.86	-	1927.86	GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala(GN-MN-L-Ala-γ-D-iGln-*m*-DAP-D-Ala-D-Ala)

1) HPLC fractions are identified by retention time. Collected fractions above 36 min were pooled as indicated and desalted by a second HPLC separation. Individual peaks recovered from the second HPLC are labeled with letters in parenthesis.

2) Abbreviations: GA, glucosamine; GN, *N*-acetylglucosamine; iGln, iso-glutamine; iGlu, iso-glutamate; Lac, lactoyl moiety generated during MALDI-TOF MS by cleavage of ether bond internal to *N*-acetylmuramic acid; *m*-DAP, *meso*-diaminopimelic acid; MN, *N*-acetylmuramic acid

3) Structure confirmed by amino acid composition analysis

Supplemental Table 8 PDGA fragments identified after CapD treatment of material recovered from rpHPLC peak 93-96 min (as shown on **Fig. 5d**). Compounds were separated by rpHPLC using methanol gradient in sodium phosphate buffer and desalted before MALDI-TOF MS analysis in negative reflectron mode.

$[M-H]^-$ observed*	**$[M-H]^-$ calculated**	**Glu residues**
662.13	662.20	5
791.13	791.24	6
920.13	920.28	7
1049.11	1049.32	8
1178.12	1178.36	9
1307.12	1307.40	10
1436.10	1436.44	11
1565.10	1565.48	12
1694.10	1694.52	13
1823.15	1823.56	14
1952.16	1952.60	15
2081.17	2081.64	16
2210.32	2210.68	17
2339.31	2339.72	18
2468.27	2468.76	19
2597.28	2597.80	20
2726.35	2726.84	21
2855.35	2855.88	22

* All compounds were detected as regular deprotonated mass ions $[M-H]^-$ and as water-loss deprotonated mass ions (not shown).

Supplemental Table 9 Substrate specificity of CapD and GGT. Standard assay conditions were used to test enzyme activity for the respective substrates (see **Fig. 6**).

Substrate	**Test concentration**	**Activity**	
		CapD	**GGT**
γ-glutamyl-*p*-nitroanilide	100 μM	-	+
γ-D-polyglutamate	1 mg x mL^{-1}	+	-
Abz-γ-D-Glu_5-Dnp	10 μM	+	-

Supplemental Table 10 Molecular weight determination of CapD small subunit and capsidin after incubation using electrospray ionization mass spectrometry (ESMS) following rpHPLC. The mass spectrometry analysis was carried out by the Research Resources Center at the University of Illinois in Chicago.

Protein/compound	Incubation	Average mass (*m)*	
		observed	calculated
CapD small subunit	– Capsidin	19,522.2	19,521.9
	+ Capsidin	19,564.2	19563.9
Mass difference	± Capsidin	Δm = 42.0	Δm = 42.0
Capsidin	– CapD	407.9	408.27
	+ CapD	366.0	366.24
Mass difference	± CapD	Δm = 41.9	Δm = 42.03

Supplemental Table 11 CID daughter ions of parent ion 1101.48 with structure assignments predicted using program ProteinProspector (http://prospector.ucsf.edu). Compound fragment ions were identified by tandem mass spectrometry following the rpHPLC analysis of trypsin cleaved CapD.

Compound *m/z* 1101.48

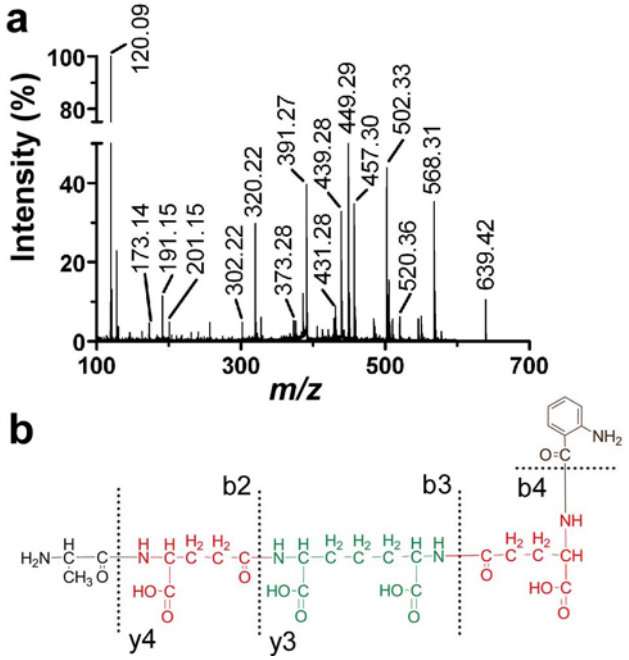
m/z	**Observed**	**Calculated**	**Predicted structure**
[M+H]	1101.48	1101.61	TTHFVIIDR
b8	927.78	927.49	TTHFVIID
b7	812.70	812.47	TTHFVII
b6	699.59	699.38	TTHFVI
b5	586.47	586.30	TTHFV
b4	487.37	487.23	TTHF
b3	340.28	340.16	TTH
b2	203.20	203.10	TT
y8	1000.93	1000.56	THFVIIDR
y7	899.81	899.51	HFVIIDR
y6	762.68	762.45	FVIIDR
y5	615.56	615.38	VIIDR
y4	516.46	516.31	IIDR
y3	403.34	403.23	IDR
y2	290.25	290.15	DR
y2 -H_2O	273.22	273.12	DR
y1	175.20	175.12	R

Supplemental Table 12 CID daughter ions of parent ion 1143.48 with structure assignments predicted using program ProteinProspector (http://prospector.ucsf.edu). Compound fragment ions were identified by tandem mass spectrometry following the rpHPLC analysis of trypsin cleaved CapD.

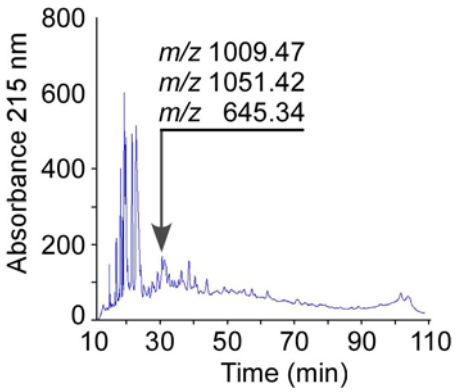
Compound *m/z* 1143.48

m/z	**Observed**	**Calculated**	**Predicted structure**
[M+H]	1143.48	1143.62	Acetyl-TTHFVIIDR
b8	969.78	969.50	Acetyl-TTHFVIID
b7	854.72	854.48	Acetyl-TTHFVII
b6	741.59	741.39	Acetyl-TTHFVI
b5	628.47	628.31	Acetyl-TTHFV
b4	529.38	529.24	Acetyl-TTHF
b3	382.29	382.17	Acetyl-TTH
b2	n. d.	245.11	Acetyl-TT
y8	1000.85	1000.56	THFVIIDR
y7	899.78	899.51	HFVIIDR
y6	762.68	762.45	FVIIDR
y5	615.56	615.38	VIIDR
y4	516.45	516.31	IIDR
y3	n. d.	403.23	IDR
y2	290.25	290.15	DR
y1	175.19	175.12	R

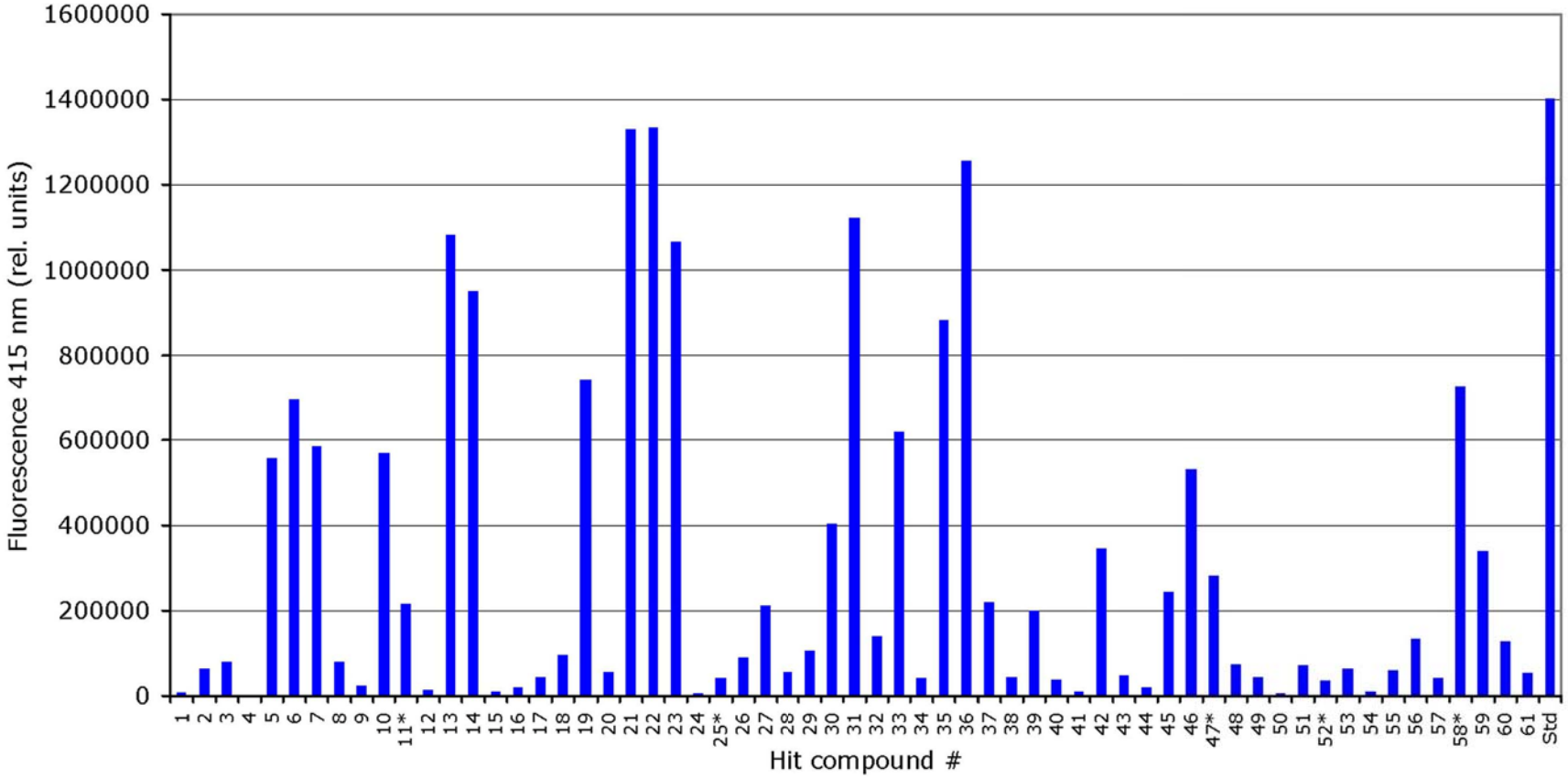
Supplemental Figures 1-7



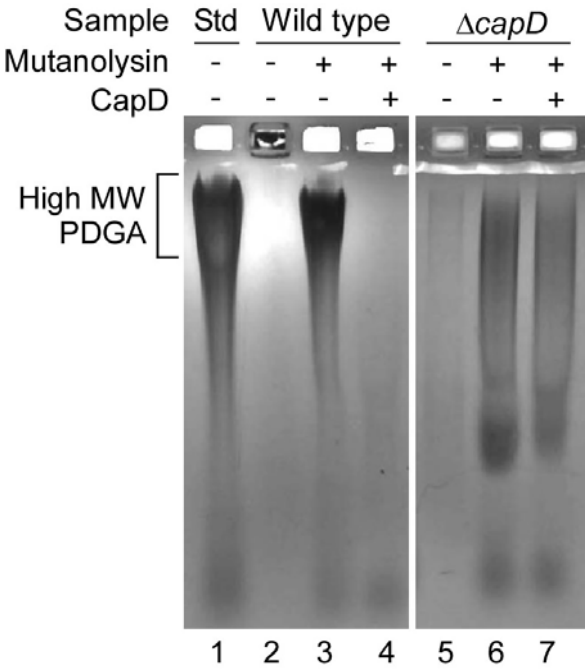
Supplemental Figure 1 CID-MS/MS analysis of smallest transpeptidase reaction product ion *m/z* 639.25 (**a**). Identified *b*- and *y*-ions are indicated on the structural diagram (**b**). The list ofdaughter ions and corresponding structure predictions is given in **Supplemental Table 3**.



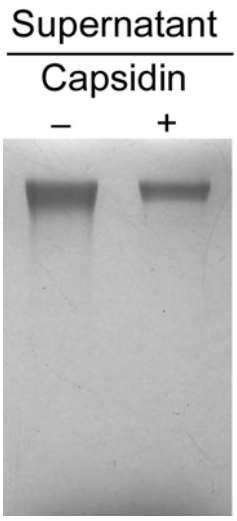
Supplemental Figure 2 PDGA anchor structures in *B. anthracis* Ames. Isolated murein sacculi of *B. anthracis* Ames were incubated with 5 µM CapD (2 hours at 37°C) to degrade capsular material and washed twice with distilled water. Peptidoglycan subunits were cleaved with mutanolysin as described in Material and Methods. Reaction products were separated by rpHPLC on a Hypersil octadecylsilane column using a linear gradient of 5% to 30% (vol/vol) acetonitrile in 0.1% (vol/vol) trifluoroacetic acid over a period of 100 min and elution monitored as absorbance at 215 nm. Fractions were dried under vacuum and compounds were analyzed by MALDI-TOF MS. rpHPLC fractions with retention times between 30 and 32 min contained *m/z* 1009.47, 1051.53, and 645.34 which were subjected to CID in tandem MS/MS experiments (**Supplemental Tables 4-6**).



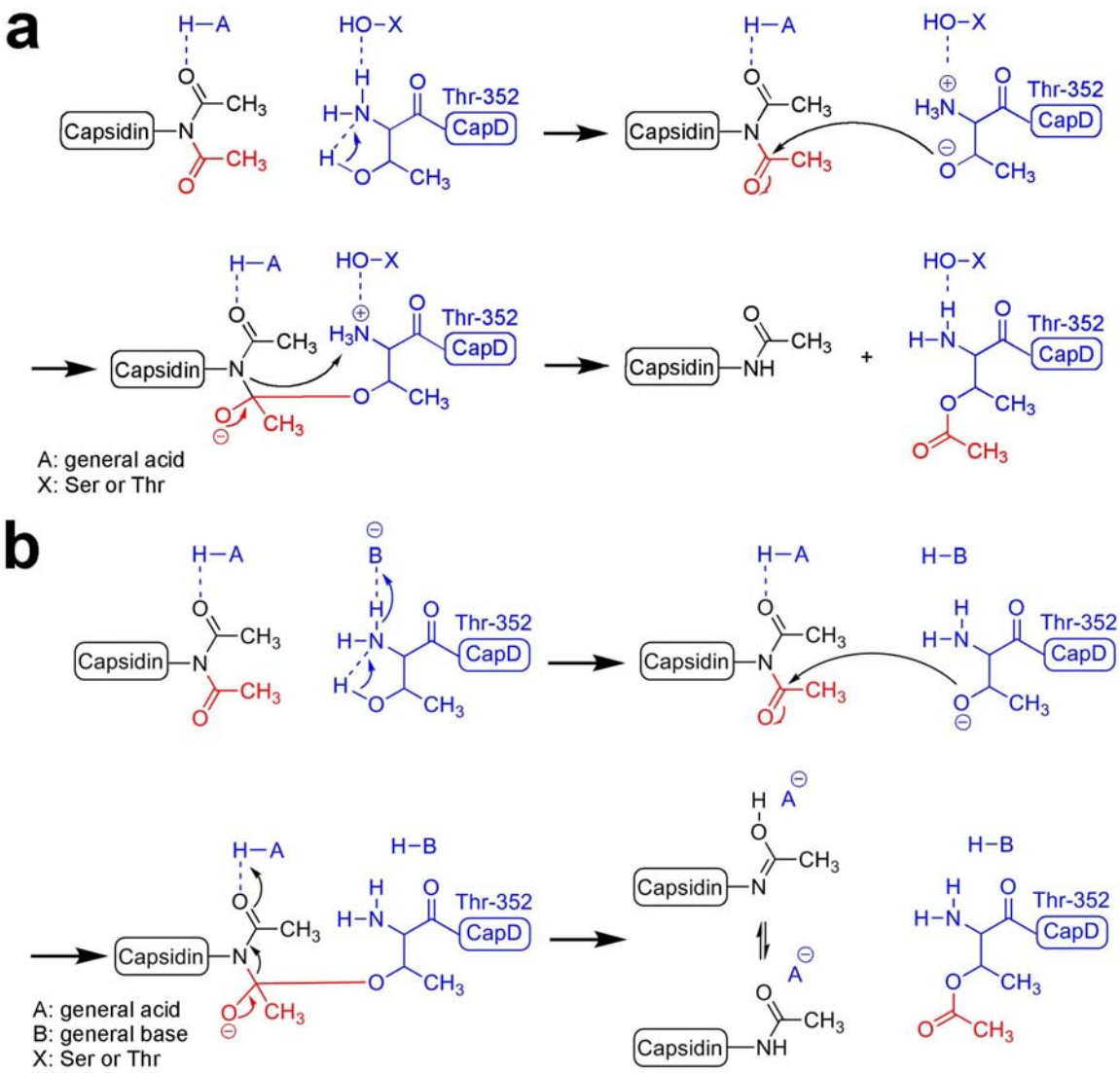
Supplemental Figure 3 Hit validation for CapD HTS. 61 strong hit compounds were tested using 1 nmol compound in 40-µL assay mix containing 20 pmol CapD with 25 mM HEPES-KOH, 01% Tween-20, pH 7.5. After pre-incubation at 22°C for 30 min, 400 pmol substrate was added and incubation continued for 90 min. To determine background fluorescence of compound and substrate combined, a mock assay without CapD was setup for every compound. Fluorescence emission at 415 nm was measured with a FluoroMax-2 (Horiba Jobin Yvon Inc., Edison, NJ). Raw data were normalized by subtracting compound-specific background and values were compared with CapD standard reaction without compound (Std). Compounds that yielded more than 50% reduction in fluorescence were subjected to a secondary screening assay and cheminformatics analysis (see text). As a result, five compounds were identified as in vitro CapD inhibitors (marked with asterisk).



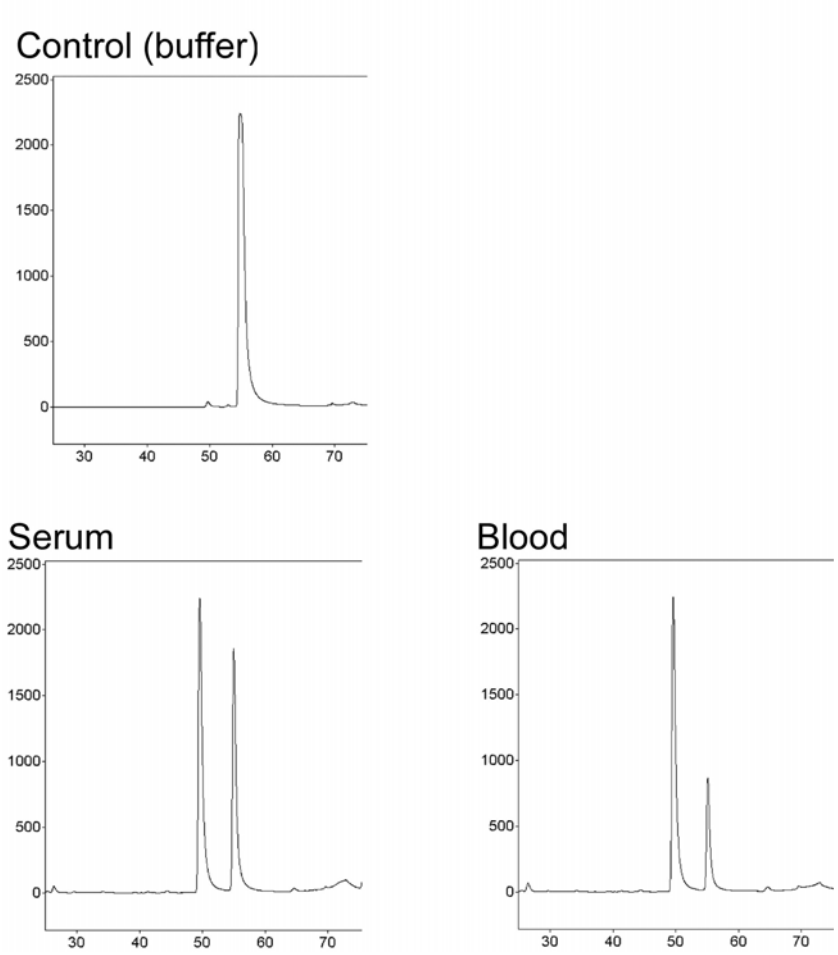
Supplemental Figure 4 Extraction of cell wall components from *B. anthracis* Ames wild type and Δ*capD* variant. Capsular material was separated by agarose gel (2.5 %) and visualized with methylene blue. Lanes on the agarose gel were loaded as follows; lane 1: purified PDGA served as standard (Std); lanes 2 and 5: insoluble murein sacculi; lanes 3 and 6: material from lanes 2 or 5 treated with mutanolysin; lanes 4 and 7: sample from lane 3 and 6 treated with recombinant CapD.



Supplemental Figure 5 *B. anthracis* Ames cells synthesize and secrete PDGA in the presence of capsidin. Cells were grown in 5% CO_2 atmosphere in absence or presence of 1 mM capsidin (see Fig. 5). The culture supernatant was treated with proteinase K and separated by agarose gel (2.5 %) followed by staining with methylene blue. Sample from supernatant without capsidin (lane 1) and with capsidin (lane 2) contains PDGA.



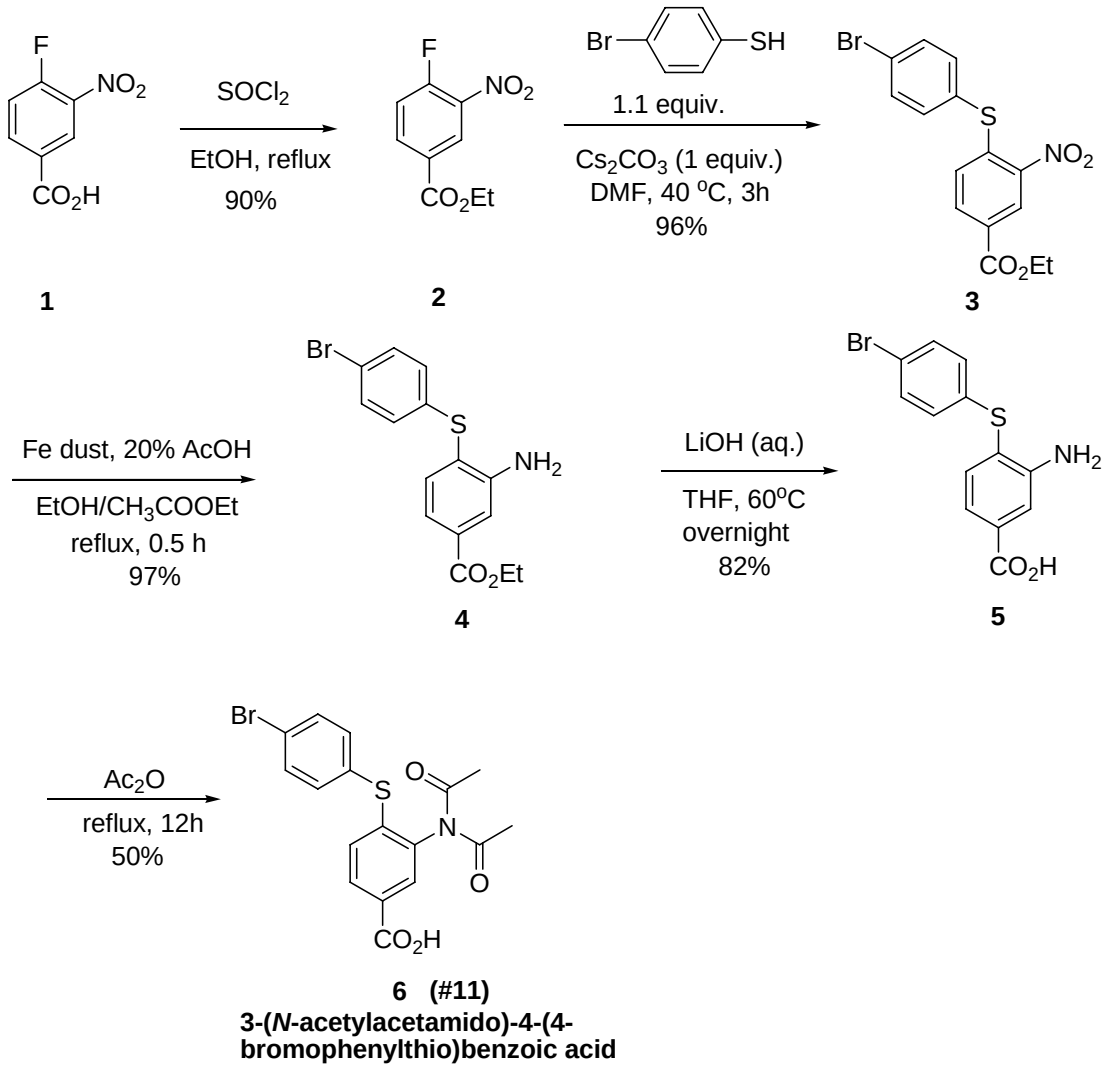
Supplemental Figure 6 Two possible catalytic mechanisms for inhibition of CapD by capsidin. (a) Nucleophilic oxygen of Thr-352 side chain donates a proton to its α-amino group. One carbonyl carbon of the diacetyl imide group in capsidin is attacked and forms a negatively charged tetrahedral intermediate. The complex is resolved by moving the proton to the nitrogen in the imide group of the inhibitor. The bond between nitrogen and carbonyl carbon is cleaved and CapD leaves the complex with an acetyl group on the side chain of Thr-352. (b) One proton moves from the nucleophilic oxygen of Thr-352 to a general base and a carbonyl carbon of capsidin is attacked. The tetrahedral intermediate is resolved by interaction with a general acid that moves one proton to the imide group nitrogen of capsidin.



Supplemental Figure 7 Capsidin (2 mM) was incubated in 1 ml of either Buffer (10 mM HEPES-KOH, 10% glycerol, pH7.5), human serum or blood for 2 hours at 37°C. All samples were filtered and concentrated over a centricon with a cut-off 6,000 Da and submitted to rpHPLC. Products were eluted using a linear gradient of 5-60% acetonitrile (vol/vol) in 0.1% formic acid (vol/vol) over 60 min. Elution profiles were recorded at 215 nm.

Synthesis of Capsidin (#11) and derivative compounds

Summary of synthesis for compound #11



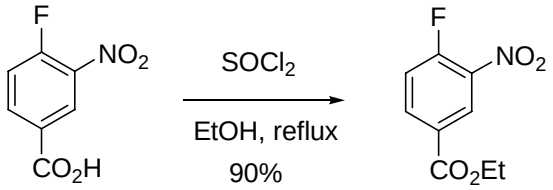
1 **2** **3**

4 **5**

6 (#11)

3-(*N*-acetylacetamido)-4-(4-bromophenylthio)benzoic acid

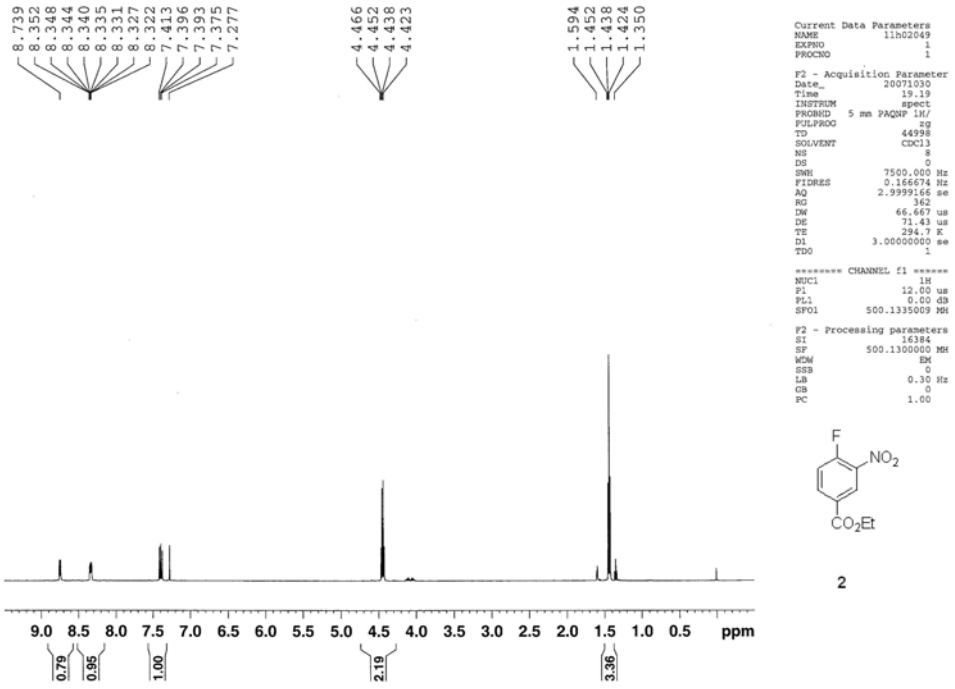
1. Synthesis of Ethyl 4-fluoro-3-nitrobenzoate (2)



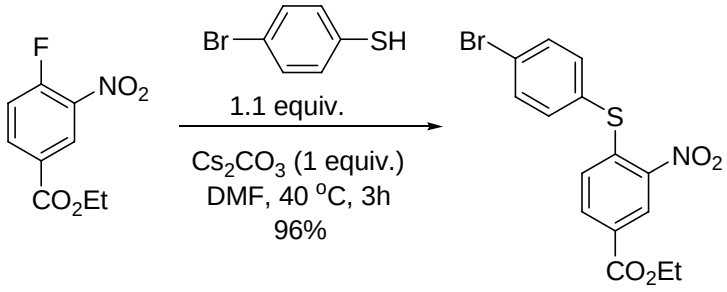
1 **2**

To a well-dried three-necked flask under N_2 were added 1.39g (7.5 mmol) of 4-fluoro-3-nitrobenzoic acid and 15 mL of EtOH. 1.65 mL (22.5 mmol, 3 equiv) of $SOCl_2$ were added slowly. After that, the solution was refluxed for 4 hr and stirred at room temperature overnight. Quench the reaction by adding saturated $NaHCO_3$ carefully to the reaction at 0 ℃. Extracted with EA (4×50 mL), washed by brine, and dried over anhydrous $MgSO_4$. After evaporation, the residue was applied to silicon gel flash chromatography (eluent: Hexanes/ethyl acetate = 5:1) to afford ethyl 4-fluoro-3-nitrobenzoate **2** (1.44g, 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.80-8.72 (m, 1 H), 8.37-8.25 (m, 1 H), 7.39 (dd, J = 10, 8.5 Hz, 1H), 4.45 (q, J = 7.0 Hz), 1.44 (t, J = 7.0 Hz).



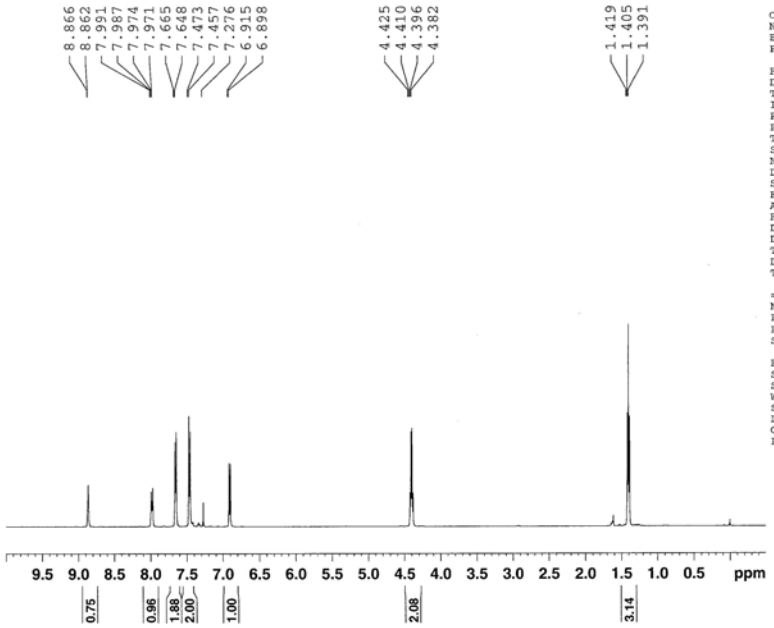
2. Synthesis of Ethyl 4-(bromophenylthio)-3-nitrobenzoate (3)



2 **3**

To a solution of compound **2** (1.427g, 6.7 mmol) and 1.1 equiv of 4-Bromobenzene thiol (1.401g, 7.4 mmol) in 20 mL of DMF, were added 2.178g (6.7 mmol, 1 equiv) of $CsCO_3$ under N_2. After stirred at 40 ℃ for 3 hr, the mixture was stirred at rt overnight. The reaction was added water to dissolve the salt and extracted with CH_2Cl_2. After dried on $MgSO_4$, the organic solvent was evaporated and the residue was purified by silicon gel flash chromatography (eluent: Hexanes/ethyl acetate = 8:1) to afford 2.456g (96%) of yellow solid compound **3**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.86 (d, J = 2.0 Hz, 1 H), 7.98 (dd, J = 8.5, 2.0 Hz, 1H), 7.66 (d, J = 8.5 Hz, 2 H), 7.46 (d, J = 8.5 Hz, 2 H), 6.91 (d, J = 8.5 Hz, 1 H), 4.40 (q, J = 7.0 Hz), 1.40 (t, J = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2, 144.2, 137.3, 133.6, 133.5, 129.2, 128.0, 127.8, 127.0, 125.4, 61.7, 14.2.

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PROCNO                      1

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PULPROG                  zg
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FIDRES             0.166674 Hz
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RG                        161
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TE                    295.0 K
D1                 3.00000000 se
TD0                         1

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PL1                     0.00 dB
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F2 - Processing parameters
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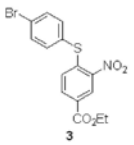
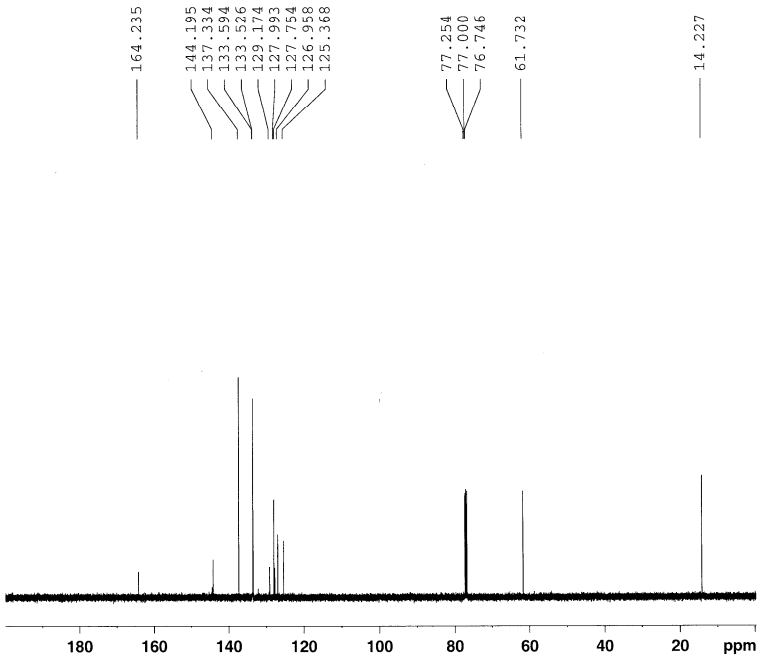


3

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PROCNO                      1

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PULPROG                zgpg
TD                     197364
SOLVENT                CDCl3
NS                         24
DS                          4
SWH                29761.904 Hz
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AQ                 3.3157651 se
RG                       2050
DW                    16.800 us
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TE                    295.8 K
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d11                0.03000000 se
DELTA              9.89999962 se
TD0                         1

======== CHANNEL f1 ======
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PL1                     1.00 dB
SFO1           125.7716224 MH

======== CHANNEL f2 ======
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NUC2                       1H
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PL13                   17.00 dB
PL2                     0.00 dB
SFO2           500.1325006 MH

F2 - Processing parameters
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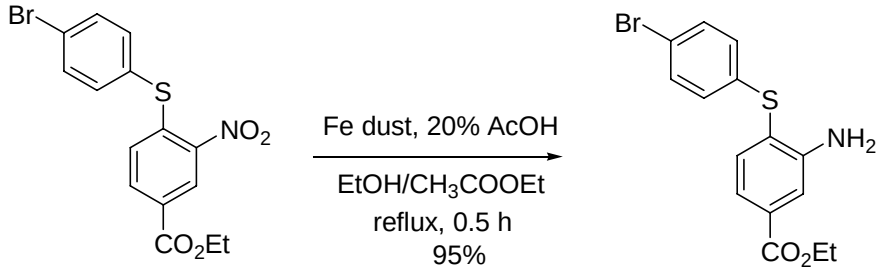
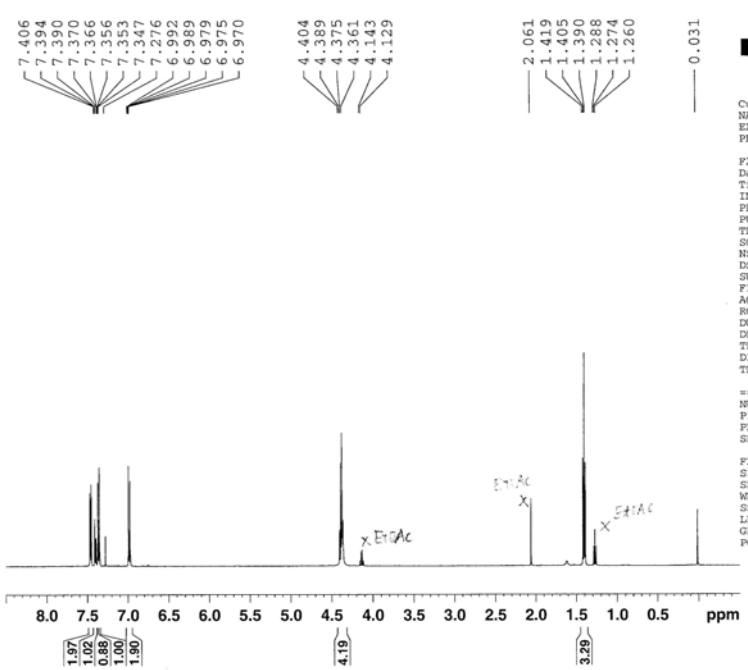
3. Synthesis of Ethyl 4-(Bromophenylthio)-3-aminobenzoate (4)



3 **4**

A solution of compound **3** (3.44 g, 9 mmol) in EtOH (10 mL) and ethyl acetate (22 mL) was treated with iron dust (4.055 g, 72 mol) and 20% acetic acid (3 mL) refluxed for 30 min with vigorous stirring. The reaction mixture was diluted with EtOAc (50 mL) and filtered over Celite. The filtrate was washed with 5% aqueous $NaHCO_3$ (30 mL), followed by water (2×30 mL), and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo and the residue was purified by silicon gel flash chromatography (eluent: Hexanes/EtOAc = 10:1) to afford 3.086g (95%) of compound **4**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42-7.39 (m, 2 H), 7.38-7.37 (m, 1H), 7.36-7.34 (m, 1 H), 7.13-7.10 (m, 1 H), 7.00-6.96 (m, 2 H), 4.38 (q, J = 7.0 Hz, 2 H), 1.04 (t, J = 7.0 Hz, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 148.0, 136.3, 134.6, 132.7, 132.0, 128.7, 119.7, 119.2, 119.1, 116.0, 61.0, 14.2.



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PROCNO                      1

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PULPROG                 zg
TD                     44998
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FIDRES             0.166674 Hz
AQ                 2.9999166 sec
RG                       203
DW                  66.667 usec
DE                  71.43 usec
TE                    295.0 K
D1                 3.00000000 sec
TD0                        1

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PL1                    0.00 dB
SFO1              500.1335009 MHz

F2 - Processing parameters
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WDW                       EM
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GB                         0
PC                      1.00
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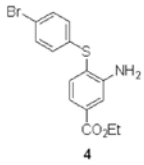

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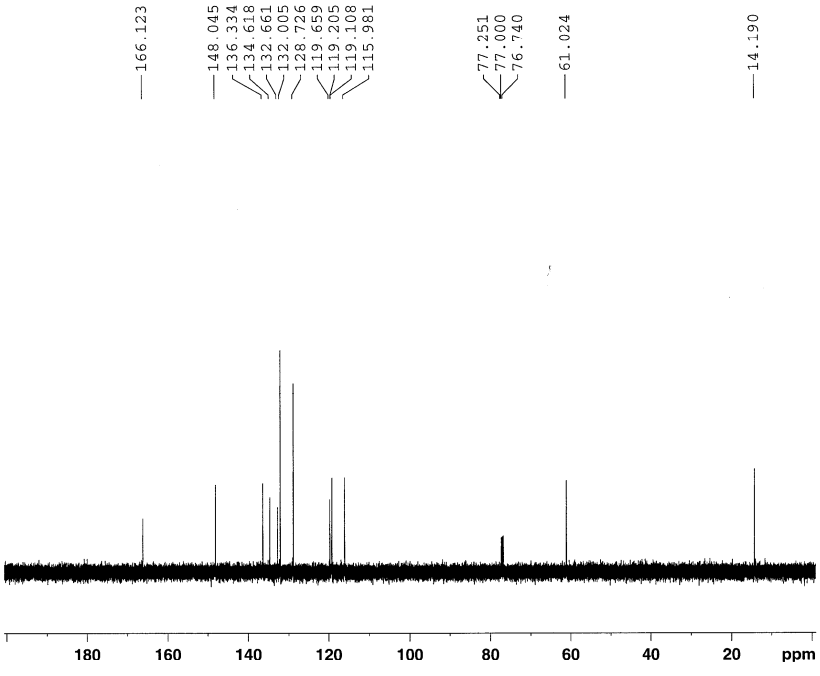


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PULPROG                zgpg
TD                    197364
SOLVENT                CDCl3
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DS                         4
SWH               32894.738 Hz
FIDRES             0.166670 Hz
AQ                 2.9999828 sec
RG                      2050
DW                   15.200 use
DE                    6.00 use
TE                    298.5 K
D1                10.00000000 sec
D11                0.03000000 sec
TD0                        1

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PL1                    1.00 dB
PL1W              72.42802429 W
SFO1             125.7716224 MHz

======== CHANNEL f2 ========
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NUC2                      1H
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PL12                  16.50 dB
PL13                  17.00 dB
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PL12W              0.54940748 W
PL13W              0.48965994 W
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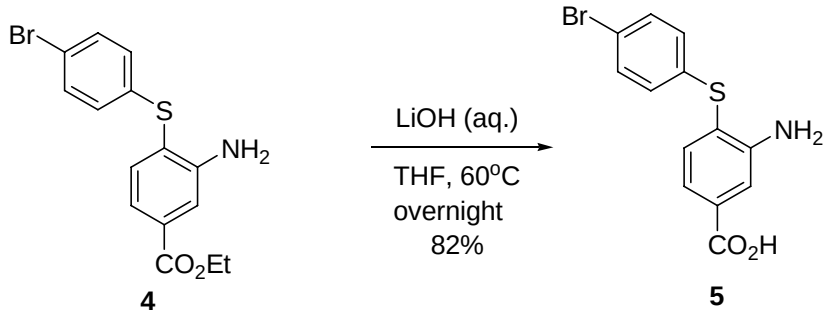
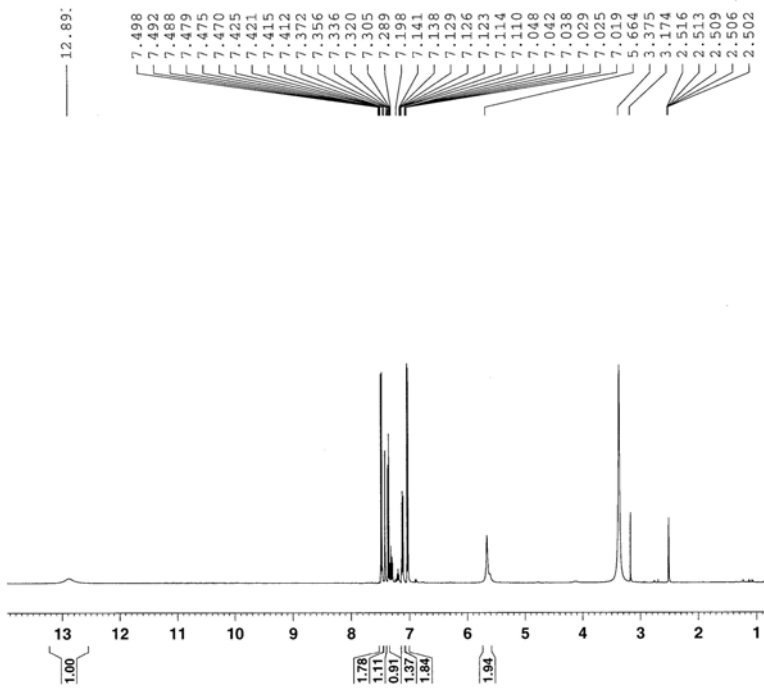
4. Synthesis of 4-(Bromophenylthio)-3-aminobenzoic acid (5)



4 **5**

To a 100 mL flask were added 1.1g (3 mmol) of compound **4** in 20 mL of THF. LiOH solution (10mL, 1.0 M in H_2O) was added and the mixture was stirred at 60 ℃ overnight. After the reaction was finished, evaporate most of the THF and extracted the residue by ethyl acetate. The aqueous layer was acidified by 2 N HCl to pH 2 and precipitation was formed. Filter to get the solid as the product **5** (0.8g, 82%) after washed with water and dried in desiccators.
^{1}H NMR (500 MHz, DMSO) δ 12.89 (bs, 1 H), 7.50-7.46 (m, 2 H), 7.43-7.40 (m, 1H), 7.36 (d, J = 8.0 Hz, 1 H), 7.14-7.10 (m, 1 H), 7.06-7.01 (m, 2 H), 5.66 (s, 2 H). ^{13}C NMR (125 MHz, DMSO) δ 167.9, 150.4, 136.8, 135.8, 133.6, 132.5, 129.7, 119.5, 117.7, 117.4, 116.3.

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FIDRES                  0.178071 Hz
AQ                      2.8079252 se
RG                           203
DW                        62.400 us
DE                        71.43 us
TE                        295.1 K
D1                    3.00000000 se
TD0                            1

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SFO1                500.1340010 MH

F2 - Processing parameters
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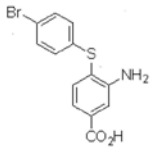

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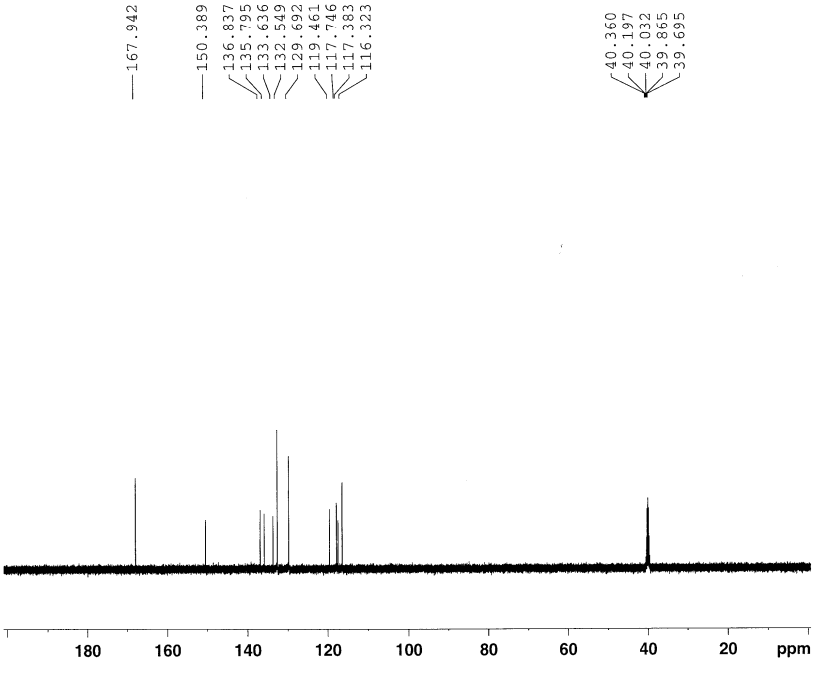


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TD                197364
SOLVENT              DMSO
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DS                      4
SWH               32894.738 Hz
FIDRES            0.166670 Hz
AQ                2.9999828 sec
RG                     2050
DW                   15.200 usec
DE                    6.00 usec
TE                   298.4 K
D1               10.00000000 sec
D11               0.03000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    13C
P1                    8.00 usec
PL1                   1.00 dB
PL1W           72.42802429 W
SFO1          125.7716224 MHz

======== CHANNEL f2 ========
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NUC2                 1H
PCPD2             80.00 usec
PL2                   0.00 dB
PL12                 16.50 dB
PL13                 17.00 dB
PL2W           24.54113007 W
PL12W           0.54940748 W
PL13W           0.48965994 W
SFO2          500.1325006 MHz
SI                 131072
SF            125.7577716 MHz
WDW                    no
SSB                     0
LB                 0.00 Hz
GB                      0
PC                   1.40
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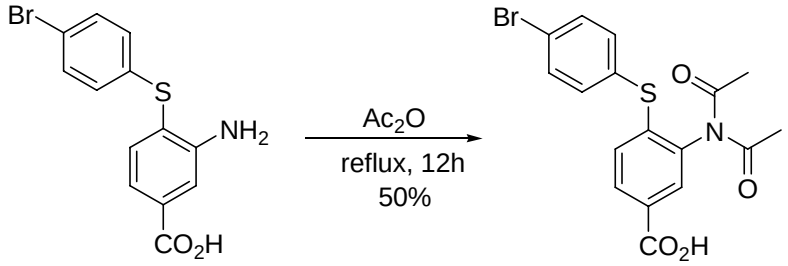
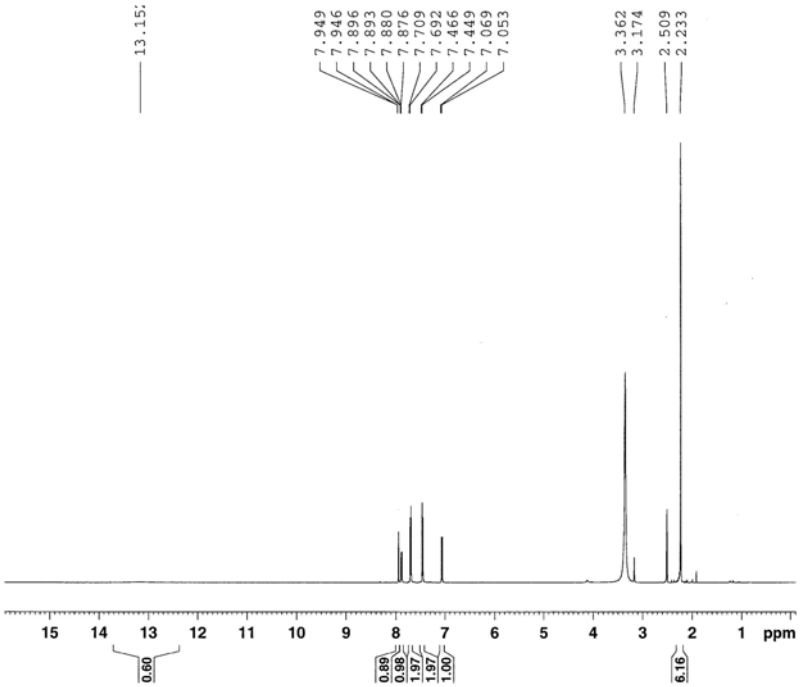
5. Synthesis of 3-(N-Acetylacetamido)-4-(4-bromophenylthio)benzoic acid (6, #11)



5

6 (#11)
3-(*N*-acetylacetamido)-4-(4-bromophenylthio)benzoic acid

In a 100 mL, round-bottom flask were placed 30 mL of acetic anhydride and 0.8 g (2.5 mmol) of compound **5**. The solution was refluxed for 12 h, then most of the acetic anhydride was removed by distillation. The residue was diluted with water, washed by CH_2Cl_2, and 30 mL of brine, and dried over anhydrous $MgSO_4$. The solution was concentrated and applied to silicon gel column to afford 3-(N-acetylacetamido)-4-(4-bromophenylthio)benzoic acid **6** (0.505 g, 50%) as a solid. ^{1}H NMR (500 MHz, DMSO) δ 13.80-12.50 (bs, 1H), 7.94 (d, J = 2.0 Hz, 1 H), 7.88 (dd, J = 8.5, 2.0 Hz, 1H), 7.70 (d, J = 8.5 Hz, 2 H), 7.46 (d, J = 8.5 Hz, 2 H), 7.06 (d, J = 8.0 Hz, 1 H), 2.23 (s, 6 H). ^{13}C NMR (125 MHz, DMSO) δ 172.3, 166.7, 143.0, 137.5, 136.6, 133.6, 131.6, 130.9, 130.6, 129.9, 129.1, 123.8, 26.8. MS (ESI) *m/z* 407.9 $[M_{Br}{}^{81}]^+$; 405.9 $[M_{Br}{}^{79}]^+$.

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FIDRES               0.178071 H
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RG                         362
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TE                     295.0 K
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======== CHANNEL f1 ========
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SFO1          500.1339510 M

F2 - Processing parameters
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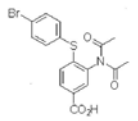
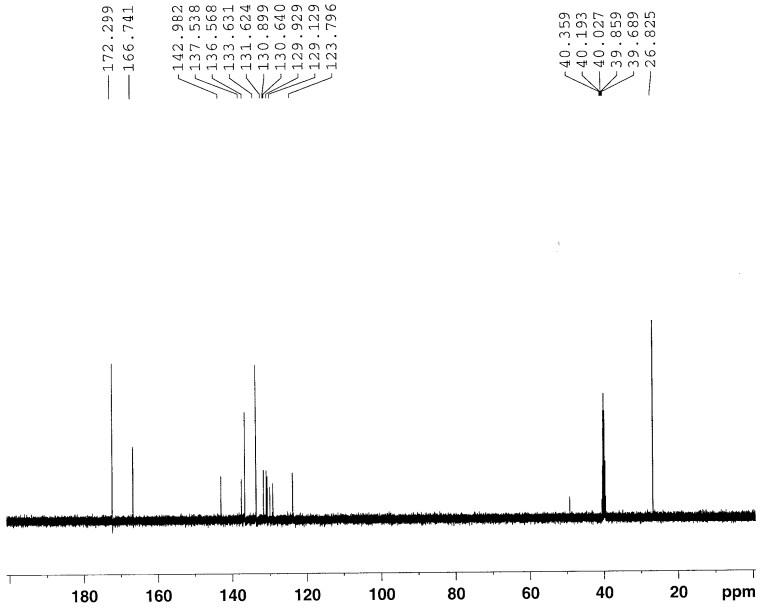


6

3-(*N*-acetylacetamido)-4-(4-bromophenylthio)benzoic acid

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PULPROG                  zgpg
TD                      197364
SOLVENT                  DMSO
NS                          12
DS                           4
SWH                32894.738 Hz
FIDRES               0.166670 Hz
AQ                   2.9999828 sec
RG                        2050
DW                      15.200 usec
DE                       6.00 usec
TE                      298.0 K
D1                   10.00000000 sec
D11                   0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
NUC1                       13C
P1                       8.00 usec
PL1                      1.00 dB
PL1W                72.42802429 W
SFO1               125.7716224 MHz

======== CHANNEL f2 ========
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NUC2                       1H
PCPD2                   80.00 usec
PL2                      0.00 dB
PL12                    16.50 dB
PL13                    17.00 dB
PL2W                24.54113007 W
PL12W                0.54940748 W
PL13W                0.48965994 W
SFO2               500.1325006 MHz
SI                      131072
SF                 125.7577770 MHz
WDW                         no
SSB                          0
LB                        0.00 Hz
GB                           0
PC                        1.40
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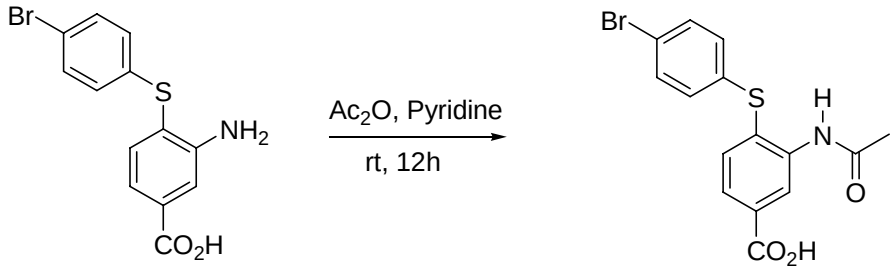
Synthesis for compound #11-2

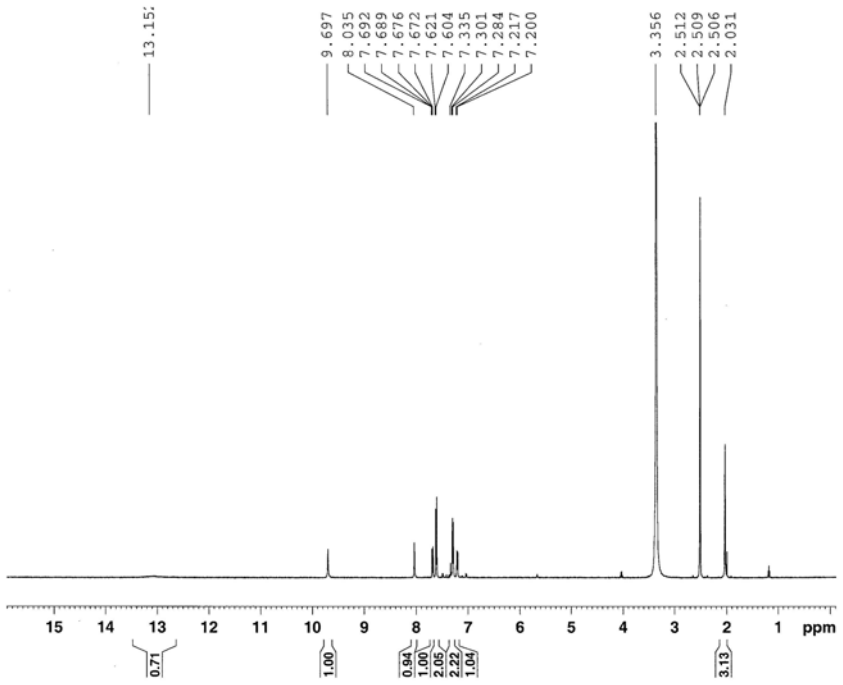
6. Synthesis of 3-Acetamido-4-(4-bromophenylthio)benzoic acid (7, #11-2)

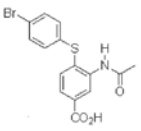


5 **7 (#11-2)**

3-acetamido-4-(4-bromophenylthio)benzoic acid

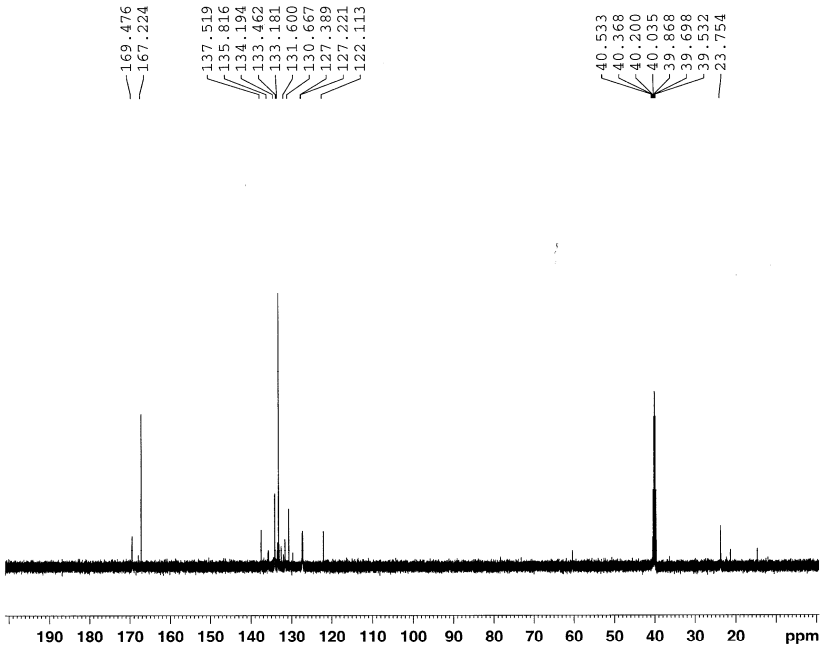
In a 50 mL flask were dissolved 1.2 g (3.7 mmol) of compound **5**, 3mL of acetic anhydride in 7 mL of pyridine. The solution was stirred at rt overnight. After remove most of the solvent, the residue was diluted by 20 mL of CH_2Cl_2, washed by water and dried on anhydrous $MgSO_4$. After evaporation, the residue was purified by column and afforded 456 mg (50%) of product **7**. ^{1}H NMR (500 MHz, DMSO) δ 13.80-12.60 (bs, 1H), 9.70 (s, 1 H), 8.04 (s, 1H),7.69 (dd, J = 8.5, 2.0 Hz, 1 H), 7.61 (d, J = 8.5 Hz, 2 H), 7.29 (d, J = 8.5 Hz, 2 H), 7.21 (d, J = 8.5 Hz, 1 H), 2.03 (s, 3 H). ^{13}C NMR (125 MHz, DMSO) δ 169.5, 167.2, 137.5, 135.8, 134.2, 133.5, 133.2, 131.6, 130.7, 127.4, 127.2, 122.1, 23.8. MS (ESI) *m/z* 365.9 $[M_{Br}{}^{81}]^+$; 363.9 $[M_{Br}{}^{79}]^+$.

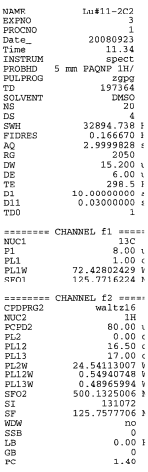




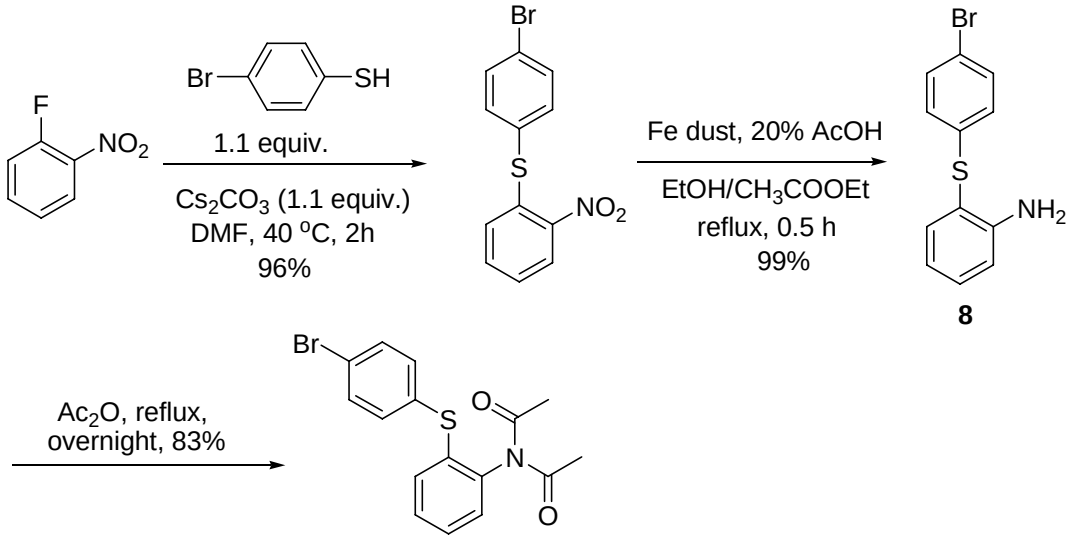
7

3-acetamido-4-(4-bromo-phenylthio)benzoic acid

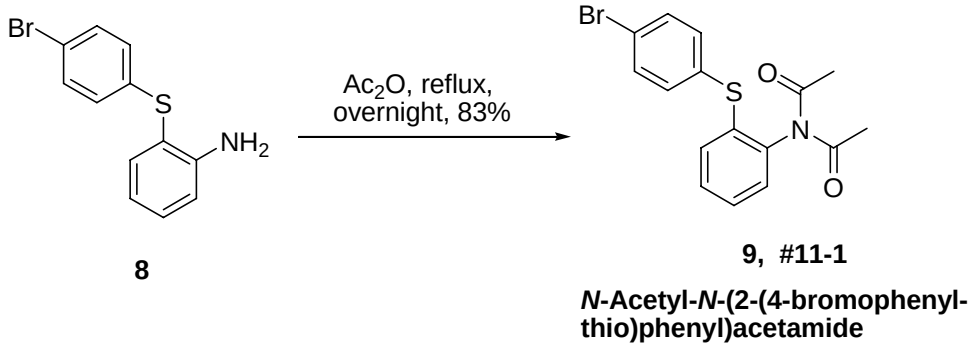




Summary of synthesis for compound #11-1



7. **Synthesis of N-Acetyl-N-(2-(4-bromophenylthio)phenyl)acetamide (9, #11-1)**



Compound **8** 2-(4-bromophenylthio)benzenamine was synthesized following the above steps same as compound **4**. In a 100 mL, round-bottom flask were placed 30 mL of acetic anhydride and 1.37 (5 mmol) of **8**. The solution was refluxed for 12 h, then most of the acetic anhydride was removed by distillation. The residue was diluted with water, washed by CH_2Cl_2, and 30 mL of brine, and dried over anhydrous $MgSO_4$. The solution was concentrated and applied to silicon gel column and afforded 51.368 g (83%) of final product **9** N-Acetyl-N-(2-(4-bromophenyl-thio)phenyl)acet- amide as a solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 8.4 Hz, 2 H), 7.35-7.31 (m, 2H), 7.29 (d, J = 8.4 Hz, 2 H), 7.20-7.15 (m, 2 H), 2.30 (s, 6 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 138.1, 136.3,

134.4, 132.5, 131.5, 131.1, 129.8, 129.6, 128.1, 122.6, 26.3. MS (ESI) *m/z* 366.0 $[M_{Br}{}^{81}+H]^+$; 364.0 $[M_{Br}{}^{79}+H]^+$.