

**Enzymatic Tailoring of Ornithine and Elaboration to the Rhizobium Cyclic
Trihydroxamate Siderophore Vicibactin**

John R Heemstra Jr., Christopher T. Walsh* and Elizabeth S. Sattely

*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School,
Boston, Massachusetts 02115*

SUPPORTING INFORMATION

<i>Table of Contents</i>	<i>Page</i>
1. Figures S1-19	S2
2. Table S1	S21
3. Materials and General Methods for the Preparation of Synthetic Standards	S21
4. Preparation of N⁵-Hydroxy-D- and L-ornithine (D- and L-3)	S22
5. Preparation of N⁵-Hydroxy-N⁵-(D-3-hydroxybutyryl)- D- and L-ornithine (D- and L-9)	S23
6. ¹H NMR data for compounds 4 – 9	S26
7. References	S35

Figure S1. A 4-15% SDS-PAGE gel of proteins VbsOALGSC (MW = PageRulerTM Prestained Protein Ladder (Fermentas)). In gel tryptic digest and MS sequencing analysis of the two bands present in the VbsC lane indicate that both are derived from VbsC and could represent partial truncation of the parent protein following purification. This material was used for *in vitro* studies and catalyzes triacylation of desacetyl-D-vicibactin as shown in Figure 7 of the main text.

Formatted: Font: Italic

Formatted: Small caps

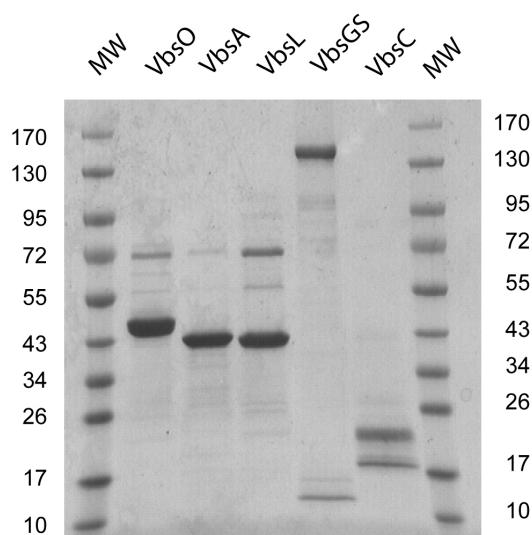
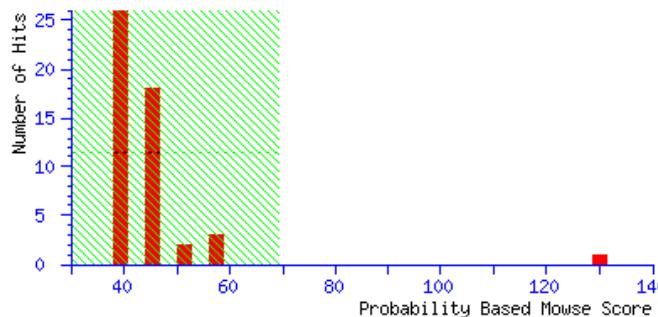


Figure S2. In gel tryptic digest of VbsG (coexpressed with VbsS) and MS (MALDI) analysis for sequence confirmation.



1. VbsG R etli CFN42 Mass: 8349 Score: 130 Expect: 4e-08 Queries matched: 16

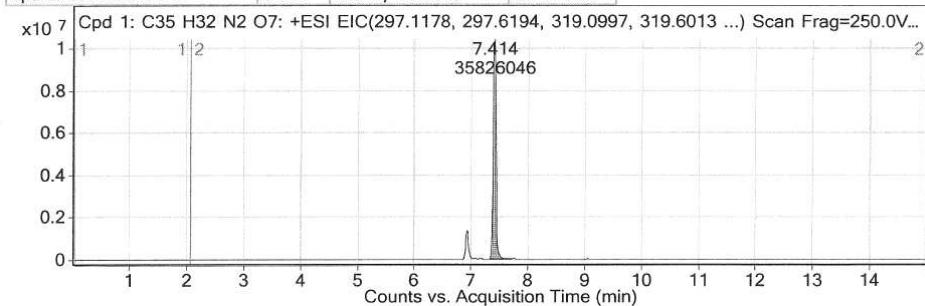
Figure S3. LC-ESI-HRMS m/z data of the Fmoc-derivatized product formed by incubation of VbsO with L-Orn, FAD and NADPH is consistent with BisFmoc- N^5 -hydroxy-L-ornithine (bisFmoc-L-3).

Data File	JRH041409-6.d	Sample Name	Sample3
Sample Type	Sample	Position	P1-C3
Instrument Name	Instrument 1	User Name	
Acq Method	HRMSquick.m	IRM Calibration Status	Success
DA Method	Default.m	Comment	

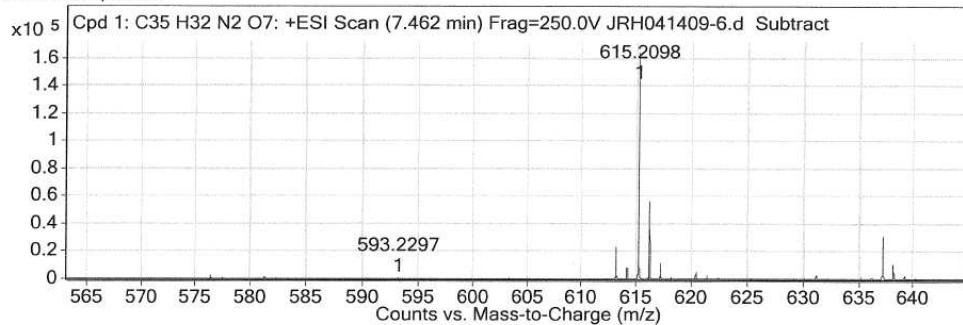
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C35 H32 N2 O7	7.414	592.2205	163890	C35 H32 N2 O7	592.221	-0.7

Compound Label	RT	Algorithm	Mass
Cpd 1: C35 H32 N2 O7	7.414	Find By Formula	592.2205



MS Zoomed Spectrum



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
177.0699				6643		
178.0774				68128		
179.0856				401254		
179.1307				5883		
179.2087				4978		
180.0887				50783		
593.2297	593.2282	2.41	1	709	C35 H33 N2 O7	(M+H)+
615.2098	615.2102	-0.66	1	164059	C35 H32 N2 Na O7	(M+Na)+
616.2128	616.2134	-0.99	1	55573	C35 H32 N2 Na O7	(M+Na)+
617.2146	617.2163	-2.85	1	11742	C35 H32 N2 Na O7	(M+Na)+

--- End Of Report ---

Figure S4. Characterization of D- and L-Lys as nonsubstrate effectors of VbsO. (A) Schematic of the incubation of L- or D-lysine with VbsO, FAD, and FADH under aerobic conditions and subsequent Fmoc-derivatized product. ESI-HRMS *m/z* data of Fmoc-derivatized assay is consistent with bisFmoc-Lys. (B) Monitoring the change in absorbance at 340 nm (due to the oxidation of NADPH to NADH⁺) with time for incubation mixtures containing 50 mM Tris pH 8.0, VbsO (5 μ M), NADPH (0.3 mM) and FAD (50 μ M). After incubation for 1 min (to ascertain the rate of NADPH oxidation in the absence of substrate) L- or D-Lys (0.5 mM) was added. Activity was monitored continuously on a Cary 50 Bio UV-visible spectrophotometer. (C) HPLC traces (263 nm) of the UV-vis reactions after derivatization with Fmoc-Cl. Conversion was determined by reverse phase HPLC analysis on a Supleco Discovery C18 column (250 x 4.6 mm) using a solvent gradient of 20 to 100% B over 40 min (solvent A, 0.1% TFA/H₂O; solvent B, 0.1% TFA/MeCN). Peaks corresponding to bisFmoc-Lys appear at *t*_R 22.4 min and bisFmoc-*N*⁵-Orn at *t*_R 21.4 min.

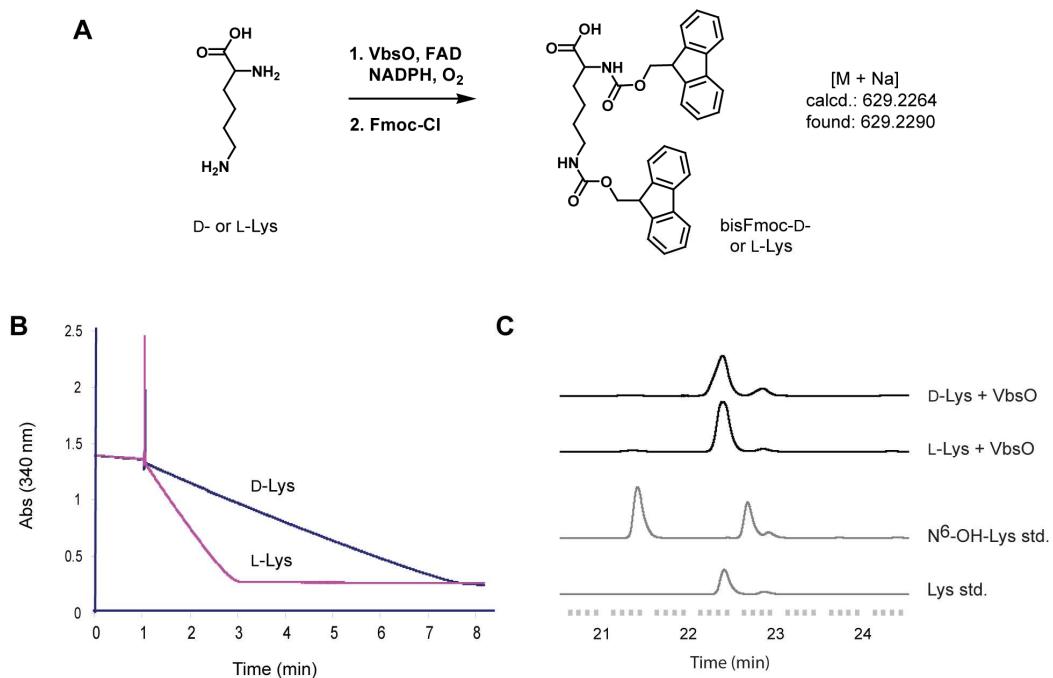


Figure S5. Characterization of *N*⁵-(*R*)-3-hydroxybutyryl-D- and L-ornithine (L- or D-**10**) as nonsubstrates for VbsO. (A) Schematic of the incubation of L- or D-**10** with VbsO, FAD, and FADH under aerobic conditions. (B) Monitoring the change in absorbance at 340 nm (due to the oxidation of NADPH to NADH⁺) with time for incubation mixtures containing 50 mM Tris pH 8.0, VbsO (5 μ M), NADPH (0.3 mM) and FAD (50 μ M). After incubation for 1 min (to ascertain the rate of NADPH oxidation in the absence of substrate) L- or D-**10** (0.5 mM) was added. Activity was monitored continuously on a Cary 50 Bio UV-visible spectrophotometer.

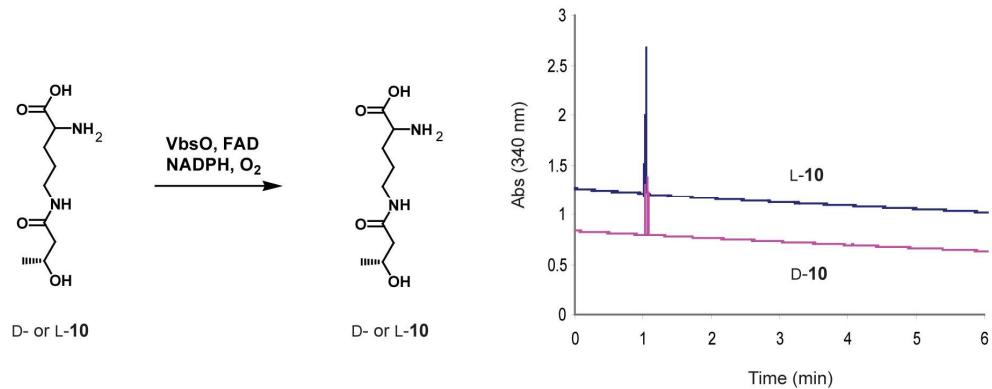
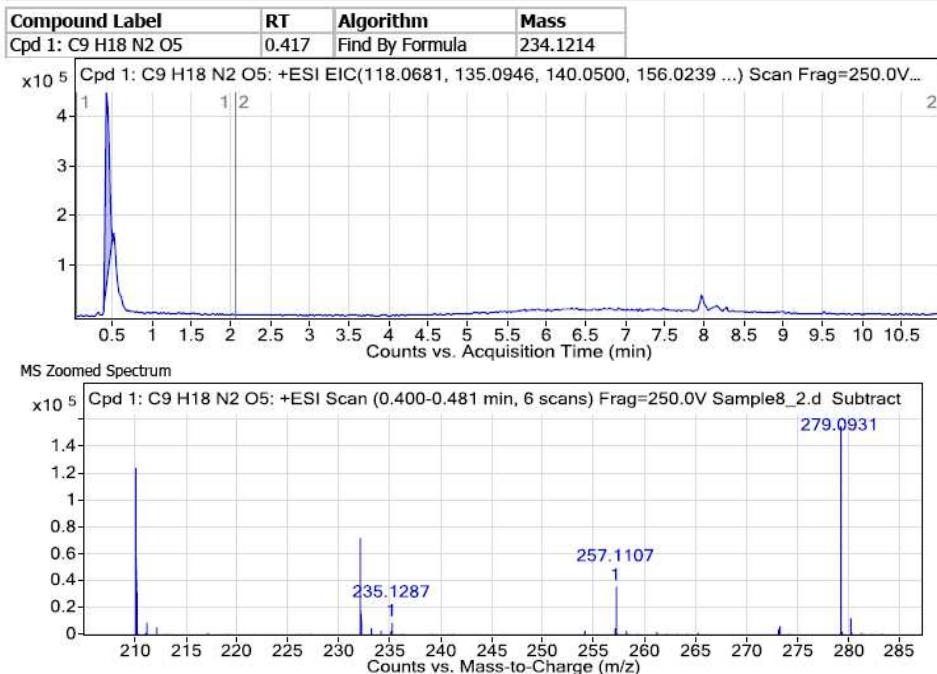


Figure S6. LC-ESI-HRMS m/z data of the product formed by incubation of VbsA with N^5 -hydroxyl-L-ornithine (**L-3**) and (R)-3-hydroxybutyryl-CoA (**11**) is consistent with N^5 -((R)-hydroxybutyryl)- N^5 -hydroxy- N^5 -L-ornithine (**L-9**) ($C_9H_{18}N_2O_5$).

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C9 H18 N2 O5	0.417	234.1214	35937	C9 H18 N2 O5	234.1216	-0.6



MS Spectrum Peak List

<i>m/z</i>	Calc <i>m/z</i>	Diff(ppm)	<i>z</i>	Abund	Formula	Ion
235.1287	235.1288	-0.49	1	9432	C9 H19 N2 O5	(M+H)+
236.1313	236.1319	-2.49	1	1114	C9 H19 N2 O5	(M+H)+
257.1107	257.1108	-0.55	1	35988	C9 H18 N2 Na O5	(M+Na)+
258.114	258.1138	0.96	1	3700	C9 H18 N2 Na O5	(M+Na)+
279.0931				155115		
279.2455				1302		
280.0957				12643		
281.0981				1622		
282.1081				536		
283.1051				1060		

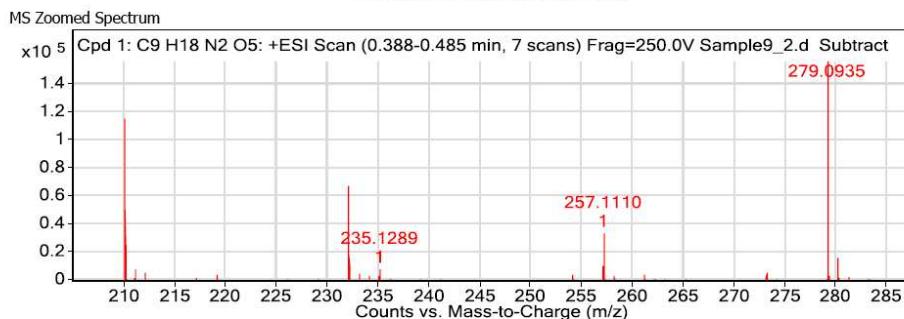
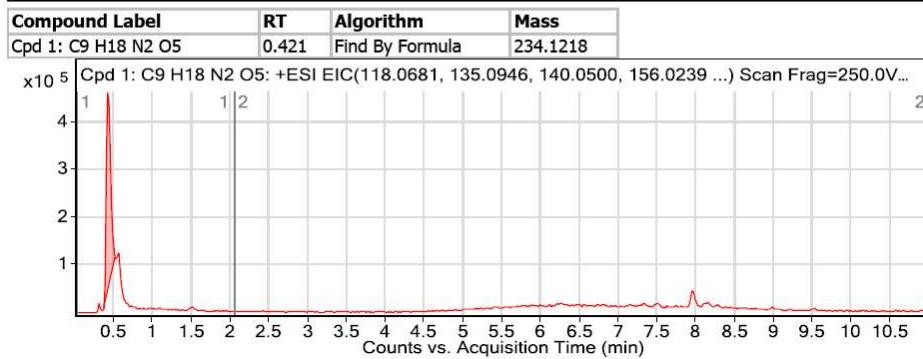
--- End Of Report ---

Figure S7. LC-ESI-HRMS m/z data of the product formed by incubation of VbsA with N^5 -hydroxy-D-ornithine (**D-9**) and (R)-3-hydroxybutyryl-CoA (**11**) is consistent with N^5 -(R)-hydroxybutyryl)- N^5 -hydroxy- N^5 -D-ornithine (**D-9**) ($C_9H_{18}N_2O_5$).

Data File	Sample9_2.d	Sample Name	Sample 9
Sample Type	Sample	Position	P1-F4
Instrument Name	Instrument 1	User Name	
Acq Method	20090604.m	IRM Calibration Status	Success
DA Method	Default.m	Comment	

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: $C_9H_{18}N_2O_5$	0.421	234.1218	33528	$C_9H_{18}N_2O_5$	234.1216	0.83



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
235.1289	235.1288	0.34	1	8187	$C_9H_{19}N_2O_5$	(M+H)+
236.1326	236.1319	3.12	1	1010	$C_9H_{19}N_2O_5$	(M+H)+
257.1111	257.1108	0.76	1	33554	$C_9H_{18}N_2NaO_5$	(M+Na)+
258.1139	258.1138	0.47	1	3175	$C_9H_{18}N_2NaO_5$	(M+Na)+
279.0935			207694			
279.2452			2404			
280.0962			16575			
281.0989			2170			
283.0692			785			
283.1053			928			

--- End Of Report ---

Figure S8. Comparison of ornithine and N^5 -hydroxyornithine (**3**) as substrates for VbsA. (A) Schematic of the VbsA-catalyzed transfer of (*R*)-3-hydroxybutyryl- from the CoA thioester (**11**) to N^5 of Orn or **3** generating the acylated product and CoASH. The reaction between the free thiol of CoASH and DTNB generates thiophenol **17** with an absorbance at 412 nm. (B) Monitoring the change in absorbance at 412 nm with time for incubation mixtures containing Tris-HCl (50 mM, pH 8.0), DTNB (50 μ M), **11** (500 μ M) and VbsA (1 μ M). After incubation for 3 min (to ascertain the rate of CoASH formation in the absence of the acceptor substrate) D-**3** (1 mM), L-**3** (1 mM), D-Orn (1 mM) or L-Orn (1 mM) was added. Activity was monitored continuously on a Cary 50 Bio UV-visible spectrophotometer. No acetyltransfer was observed with VbsA inactivated by boiling.

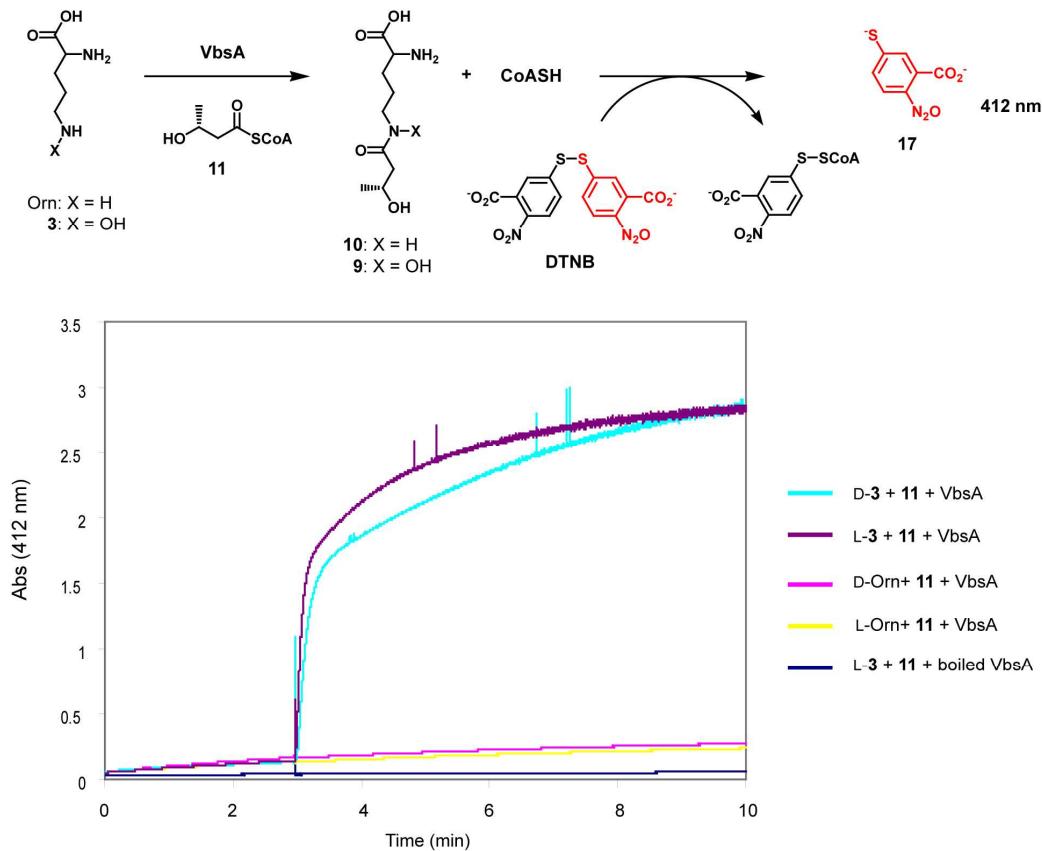


Figure S9. Characterization of (*S*)-3-hydroxybutyryl-CoA (**12**) and butyryl-CoA (**13**) as substrates for VbsA. **(A)** Schematic of the VbsA-catalyzed transfer of (*S*)-3-hydroxybutyryl- from CoA thioester **12** to N^5 of N^5 -hydroxy-L-ornithine (**L-3**). ESI-HRMS m/z data of the purified product is consistent with N^5 -((*S*)-3-hydroxybutyryl)- N^5 -hydroxy-L-ornithine (**18**). Time course HPLC traces (220 nm) showing products resulting from the incubation of **17** (1 mM) and **L-3** (1.2 mM) with VbsA (1 μ M) in 100 mM Tris, pH 7.5. Peaks corresponding to **L-18** appear at t_R 6.6 min, CoASH at t_R 8.5 min and **12** at t_R 9.4 min. **(B)** Schematic of the VbsA-catalyzed transfer of butyryl- from CoA thioester **13** to N^5 of N^5 -hydroxy-L-ornithine (**L-3**). ESI-HRMS m/z data of the purified product is consistent with N^5 -butyryl- N^5 -hydroxy-L-Orn (**20**). Time course HPLC traces (220 nm) showing products resulting from the incubation of **13** (1 mM) and **L-3** (1.2 mM) with VbsA (1 μ M) in 100 mM Tris, pH 7.5. Peaks corresponding to CoASH appear at t_R 8.5 min, **L-19** at t_R 9.4 min and **13** at t_R 12.0 min. Conversion was determined by reverse phase HPLC analysis on a Supleco Discovery C18 column (250 x 4.6 mm) using a solvent gradient of 2 to 66% B over 20 min (solvent A, 0.1% TFA/H₂O; solvent B, 0.1% TFA/MeCN).

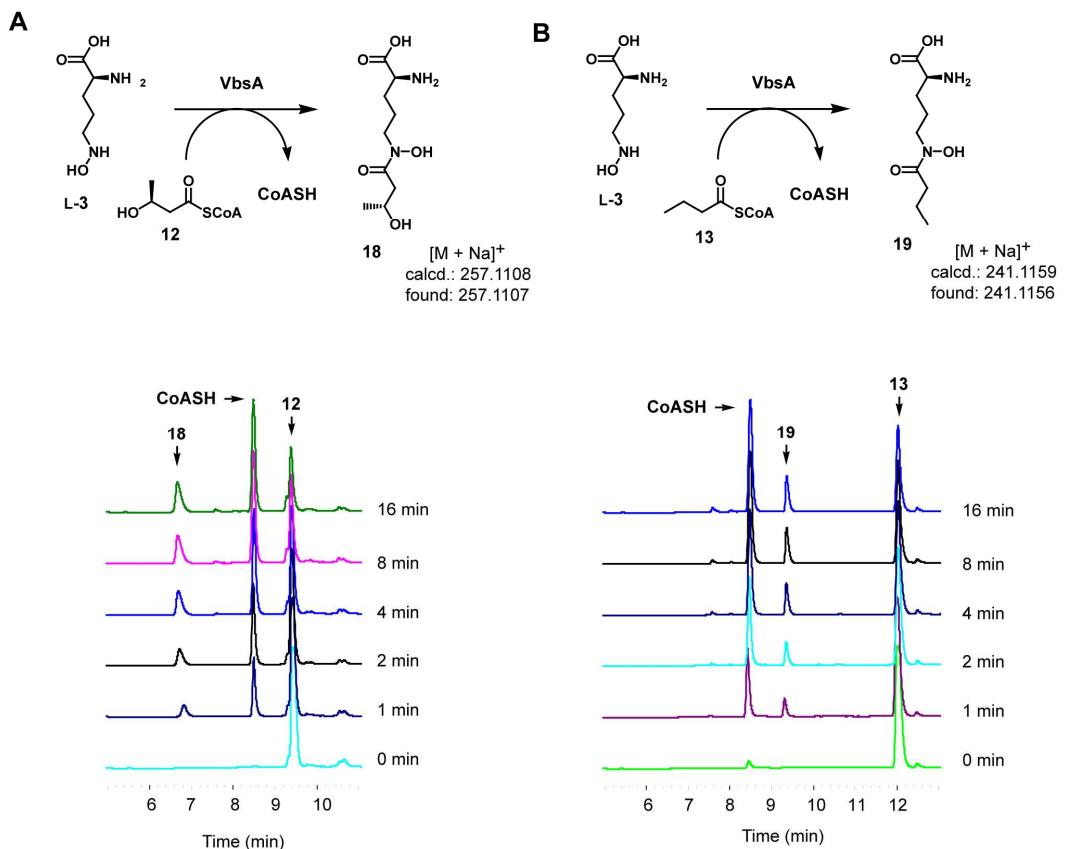


Figure S10. Characterization of VbsL as a PLP-dependent epimerase. (A) Schematic of the VbsL-catalyzed racemization of N^5 -hydroxy-L- or D-ornithine (L- or D-3) and proposed structure of the Fmoc-derivatized products (bF-L- and D-3). (B) Chiral HPLC traces (263 nm) showing products resulting from incubation with L-3 (500 μ M) with VbsL (10 μ M), PLP (50 μ M) and pH 7.75 HEPES (50 mM) followed by derivatization with Fmoc-Cl. (C) Chiral HPLC traces (263 nm) showing products resulting from incubation with D-3 (500 μ M) with VbsL (10 μ M), PLP (50 μ M) and pH 7.75 HEPES (50 mM) followed by derivatization with Fmoc-Cl. Peaks corresponding to bF-L-3 appear at t_R 16.4 min and bisFmoc-D-3 at t_R 17.9 min. Peak at t_R 21.5 min is N-Fmoc-1-aminoadamantane.

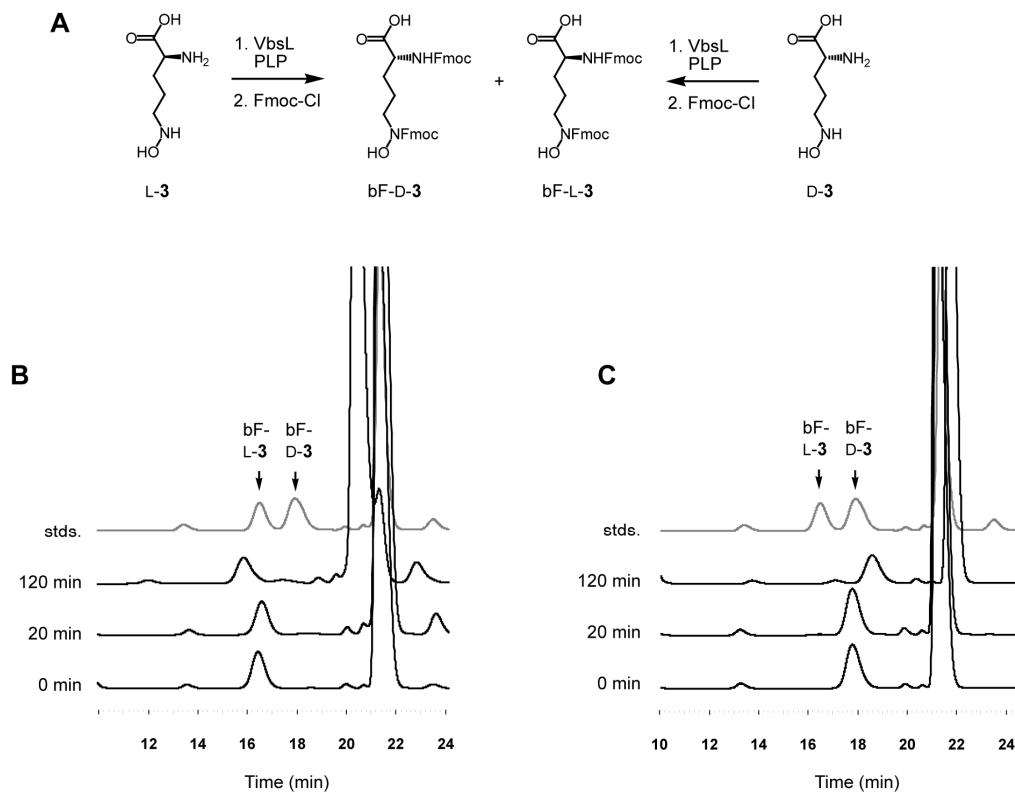
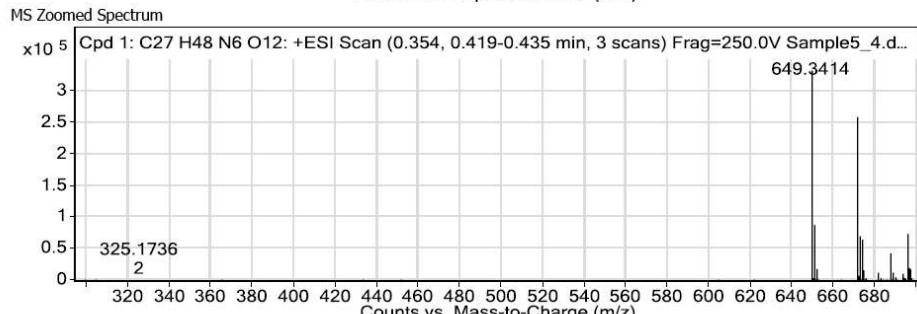
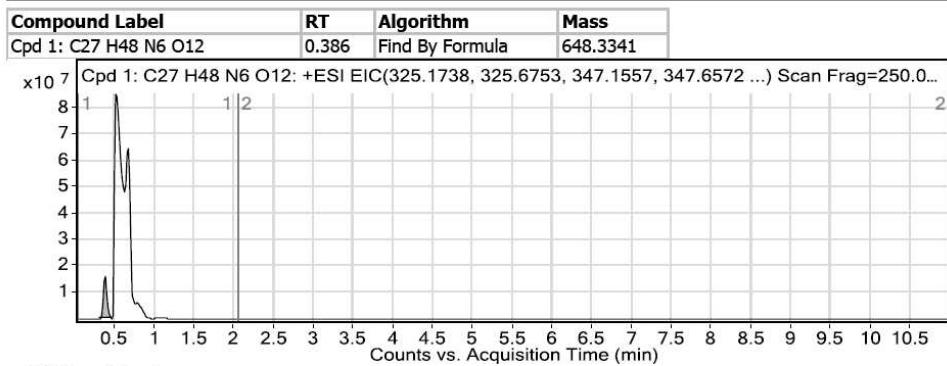


Figure S11. LC-ESI-HRMS *m/z* data of the siderophore produced by the VbsC mutant strain under iron deficient conditions is consistent with desacetylvicibactin (D-14) ($C_{27}H_{48}N_6O_{12}$).

Data File	Sample5_4.d	Sample Name	Sample 5
Sample Type	Sample	Position	P1-F2
Instrument Name	Instrument 1	User Name	
Acq Method	20090604.m	IRM Calibration Status	Success
DA Method	Default.m	Comment	

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C27 H48 N6 O12	0.386	648.3341	332103	C27 H48 N6 O12	648.333	1.64



MS Spectrum Peak List

<i>m/z</i>	Calc <i>m/z</i>	Diff(ppm)	z	Abund	Formula	Ion
325.1736	325.1738	-0.58	2	681	$C_{27}H_{50}N_6O_{12}$	$(M+2H)^{+2}$
649.3414	649.3403	1.73		332185	$C_{27}H_{49}N_6O_{12}$	$(M+H)^{+}$
649.5646				4256		
650.3444	650.3433	1.11		88633	$C_{27}H_{49}N_6O_{12}$	$(M+H)^{+}$
651.3465	651.3457	1.27		17709	$C_{27}H_{49}N_6O_{12}$	$(M+H)^{+}$
671.3236	671.3222	1.98	1	259368	$C_{27}H_{48}N_6NaO_{12}$	$(M+Na)^{+}$
672.3257	672.3252	0.7	1	70191	$C_{27}H_{48}N_6NaO_{12}$	$(M+Na)^{+}$
673.306	673.3276	-32.14	1	64650	$C_{27}H_{48}N_6NaO_{12}$	$(M+Na)^{+}$
674.3049	674.3301	-37.44	1	16577	$C_{27}H_{48}N_6NaO_{12}$	$(M+Na)^{+}$
675.3046	675.3325	-41.3	1	3592	$C_{27}H_{48}N_6NaO_{12}$	$(M+Na)^{+}$

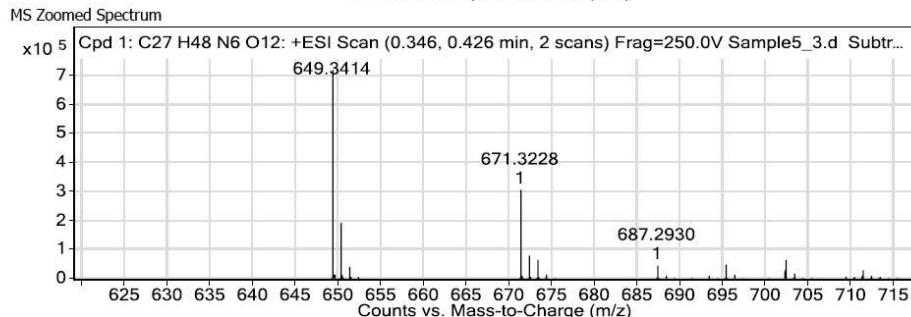
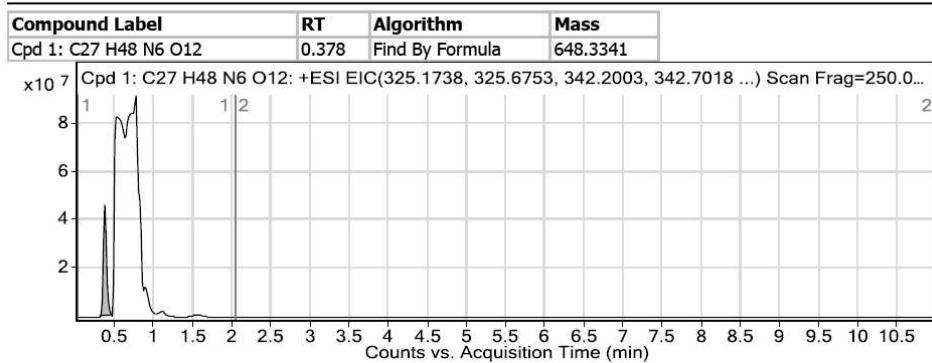
--- End Of Report ---

Figure S12. LC-ESI-HRMS *m/z* data of the siderophore produced by the VbsL mutant strain under iron deficient conditions is consistent with desacetylvicibactin (L-14) ($C_{27}H_{48}N_6O_{12}$).

Data File	Sample5_3.d	Sample Name	Unavailable
Sample Type	Unavailable	Position	Unavailable
Instrument Name	Unavailable	User Name	Unavailable
Acq Method		IRM Calibration Status	Success
DA Method	Default.m	Comment	Sample information is unavailable

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: $C_{27}H_{48}N_6O_{12}$	0.378	648.3341	718230	$C_{27}H_{48}N_6O_{12}$	648.333	1.66



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
649.3414	649.3403	1.77	725037	27	$C_{27}H_{49}N_6O_{12}$	(M+H)+
649.4684				16171		
649.5658				14339		
650.3438	650.3433	0.82	197146	27	$C_{27}H_{49}N_6O_{12}$	(M+H)+
651.348	651.3457	3.45	43244	27	$C_{27}H_{49}N_6O_{12}$	(M+H)+
671.3228	671.3222	0.77	1	305553	$C_{27}H_{48}N_6NaO_{12}$	(M+Na)+
672.3254	672.3252	0.31	1	80442	$C_{27}H_{48}N_6NaO_{12}$	(M+Na)+
673.3057	673.3276	-32.52	1	65787	$C_{27}H_{48}N_6NaO_{12}$	(M+Na)+
674.3048	674.3301	-37.63	1	17156	$C_{27}H_{48}N_6NaO_{12}$	(M+Na)+
687.293	687.2962	-4.6	1	47438	$C_{27}H_{48}K_6O_{12}$	(M+K)+

--- End Of Report ---

Figure S13. Confirmation of the stereochemistry at C² of the ornithine moiety embedded in the scaffold of the desacetylvicibactins (**14**) isolated from VbsC and VbsL mutant strains of *R. leguminosarum*. The tri-esters were saponified with 10 equiv of LiOH, derivatized with Fmoc-Cl, and analyzed by chiral HPLC (263 nm) by coelution with similarly Fmoc-derivatized N⁵-((R)-3-hydroxybutyryl)-N⁵-hydroxy-D- or L-ornithine synthetic standards (L- or D-**9**) as determined by reverse phase HPLC analysis on a chiralcel OD-RH column using a solvent gradient of 80 to 100% B over 6 min followed by 100% B for 10 min (solvent A, 0.1% TFA/H₂O; solvent B, 0.1% TFA/MeCN).

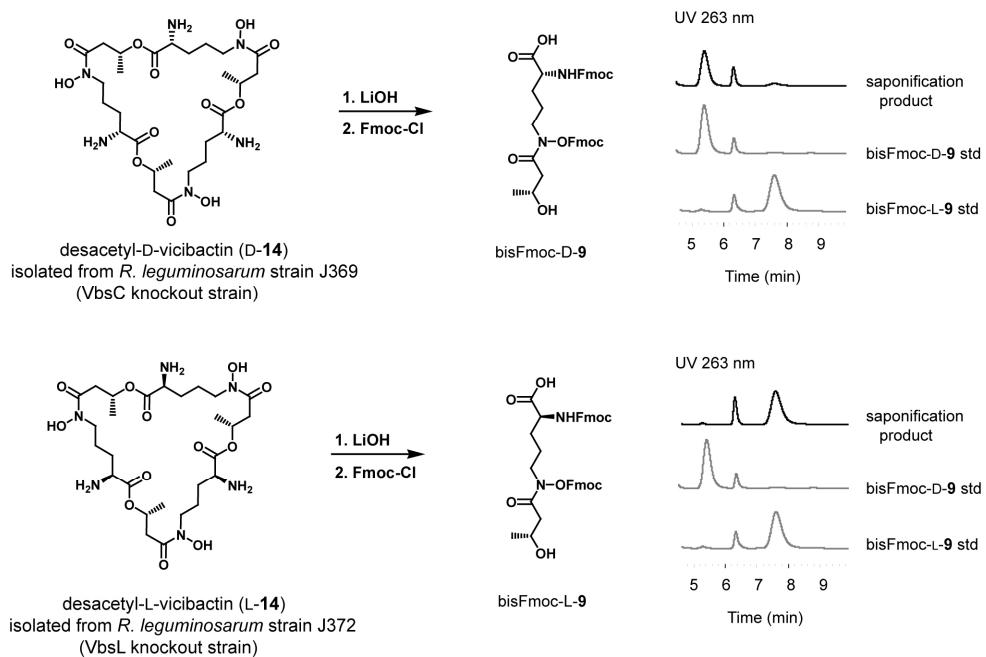
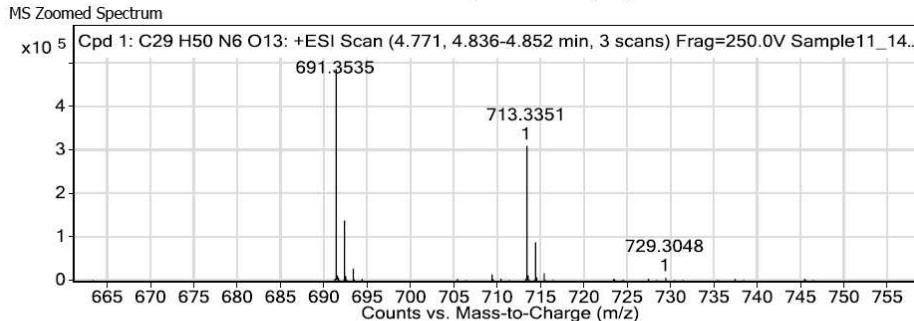
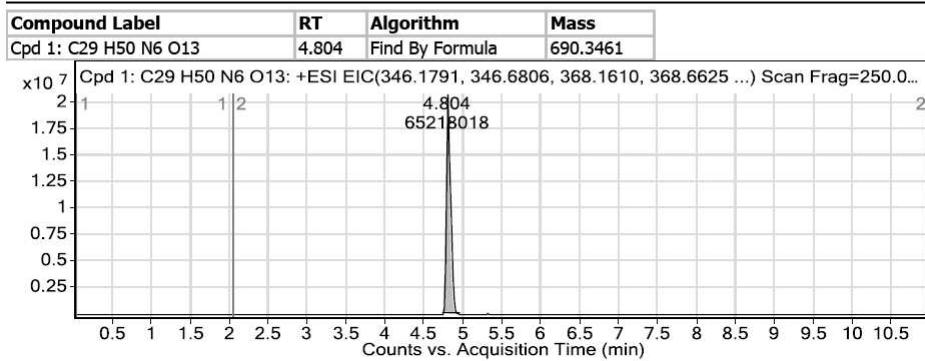


Figure S14. LC-ESI-HRMS *m/z* data of the product eluting at 11.0 min formed by incubation of VbsC with desacetyl-D-vicibactin (**D-14**) and acetyl-CoA (Figure 7) is consistent with a monoacetylated-vicibactin homolog **15** ($C_{29}H_{50}N_6O_{13}$).

Data File	Sample11_14.d	Sample Name	Unavailable
Sample Type	Unavailable	Position	Unavailable
Instrument Name	Unavailable	User Name	Unavailable
Acq Method		IRM Calibration Status	Success
DA Method	Default.m	Comment	Sample information is unavailable

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: $C_{29}H_{50}N_6O_{13}$	4.804	690.3461	485518	$C_{29}H_{50}N_6O_{13}$	690.3436	3.68



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
691.3535	691.3509	3.8		490295	$C_{29}H_{51}N_6O_{13}$	(M+H)+
691.4708				11792		
691.5837				8531		
692.3559	692.3539	2.92		140733	$C_{29}H_{51}N_6O_{13}$	(M+H)+
693.3579	693.3563	2.3		27834	$C_{29}H_{51}N_6O_{13}$	(M+H)+
694.3595	694.3588	0.92		4500	$C_{29}H_{51}N_6O_{13}$	(M+H)+
713.3351	713.3328	3.19	1	312964	$C_{29}H_{50}N_6NaO_{13}$	(M+Na)+
714.3377	714.3358	2.68	1	89891	$C_{29}H_{50}N_6NaO_{13}$	(M+Na)+
715.3375	715.3383	-1.14	1	19387	$C_{29}H_{50}N_6NaO_{13}$	(M+Na)+
729.3048	729.3067	-2.7	1	9137	$C_{29}H_{50}K_6O_{13}$	(M+K)+

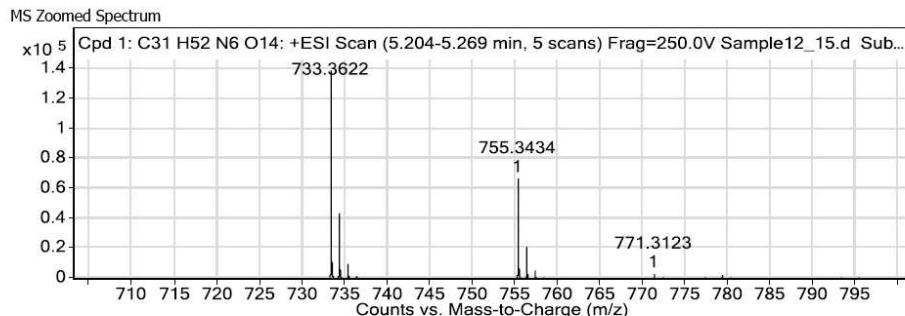
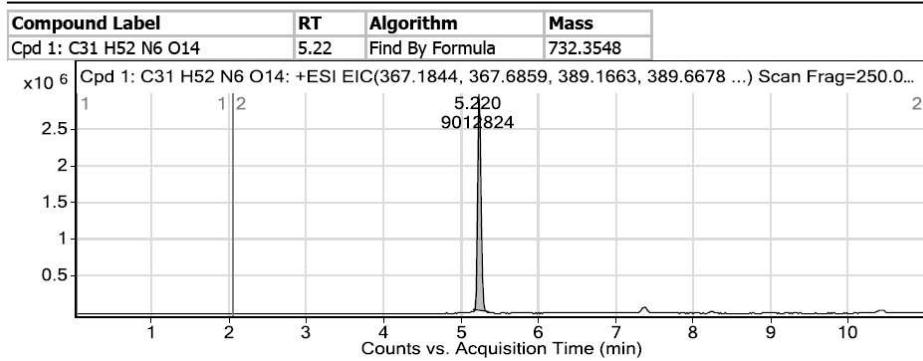
--- End Of Report ---

Figure S15. LC-ESI-HRMS m/z data of the product eluting at 11.9 min formed by incubation of VbsC with desacetyl-D-vicibactin (**D-14**) and acetyl-CoA (Figure 7) is consistent with a diacetylated-vicibactin homolog **16** ($C_{31}H_{52}N_6O_{14}$).

Data File	Sample12_15.d	Sample Name	Unavailable
Sample Type	Unavailable	Position	Unavailable
Instrument Name	Unavailable	User Name	Unavailable
Acq Method		IRM Calibration Status	Success
DA Method	Default.m	Comment	Sample information is unavailable

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: $C_{31}H_{52}N_6O_{14}$	5.22	732.3548	138502	$C_{31}H_{52}N_6O_{14}$	732.3542	0.87



MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
733.3622	733.3614	0.99		139663	$C_{31}H_{53}N_6O_{14}$	(M+H)+
733.5964				1205		
734.3645	734.3645	0.06		43984	$C_{31}H_{53}N_6O_{14}$	(M+H)+
735.3665	735.3669	-0.55		9975	$C_{31}H_{53}N_6O_{14}$	(M+H)+
736.3683	736.3695	-1.62		1673	$C_{31}H_{53}N_6O_{14}$	(M+H)+
755.3434	755.3434	-0.01	1	66967	$C_{31}H_{52}N_6NaO_{14}$	(M+Na)+
756.3469	756.3464	0.69	1	21396	$C_{31}H_{52}N_6NaO_{14}$	(M+Na)+
757.3468	757.3489	-2.7	1	4973	$C_{31}H_{52}N_6NaO_{14}$	(M+Na)+
758.349	758.3514	-3.22	1	1081	$C_{31}H_{52}N_6NaO_{14}$	(M+Na)+
771.3123	771.3173	-6.44	1	2635	$C_{31}H_{52}KNaO_{14}$	(M+K)+

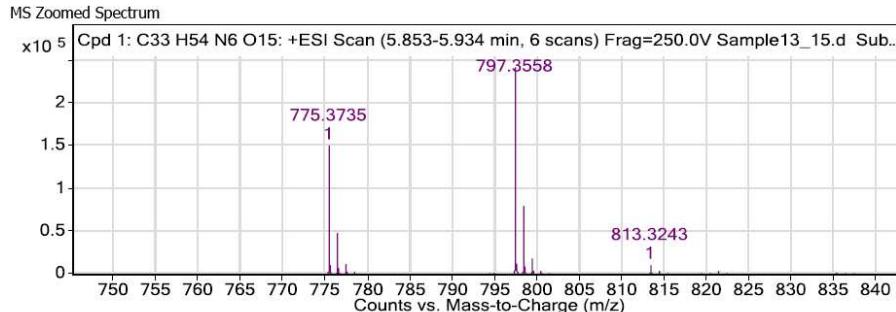
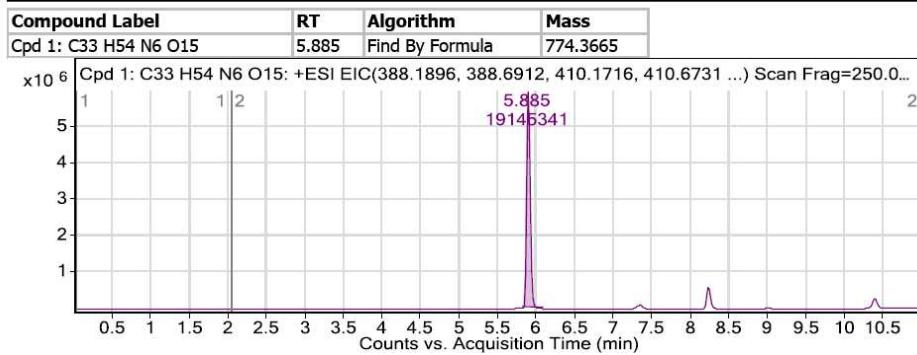
--- End Of Report ---

Figure S16. LC-ESI-HRMS m/z data of the product eluting at 12.8 min formed by incubation of VbsC with desacetyl-D-vicibactin (**D-14**) and acetyl-CoA (Figure 7) is consistent with the formation of vicibactin (**VB**) ($C_{33}H_{54}N_6O_{15}$).

Data File	Sample13_15.d	Sample Name	Unavailable
Sample Type	Unavailable	Position	Unavailable
Instrument Name	Unavailable	User Name	Unavailable
Acq Method		IRM Calibration Status	Success
DA Method	Default.m	Comment	Sample information is unavailable

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: $C_{33}H_{54}N_6O_{15}$	5.885	774.3665	241890	$C_{33}H_{54}N_6O_{15}$	774.3647	2.24



MS Spectrum Peak List

<i>m/z</i>	<i>Calc m/z</i>	Diff(ppm)	<i>z</i>	Abund	Formula	Ion
775.3735	775.372	1.9	1	150817	$C_{33}H_{55}N_6O_{15}$	(M+H)+
776.3762	776.3751	1.47	1	49500	$C_{33}H_{55}N_6O_{15}$	(M+H)+
777.378	777.3776	0.56	1	11329	$C_{33}H_{55}N_6O_{15}$	(M+H)+
797.3558	797.3539	2.29		241938	$C_{33}H_{54}N_6NaO_{15}$	(M+Na)+
797.5971				3198		
798.3583	798.357	1.58		79924	$C_{33}H_{54}N_6NaO_{15}$	(M+Na)+
799.3582	799.3595	-1.69		18756	$C_{33}H_{54}N_6NaO_{15}$	(M+Na)+
800.3566	800.362	-6.84		3772	$C_{33}H_{54}N_6NaO_{15}$	(M+Na)+
813.3243	813.3279	-4.45	1	10730	$C_{33}H_{54}K_6O_{15}$	(M+K)+
814.3277	814.3309	-3.96	1	3817	$C_{33}H_{54}K_6O_{15}$	(M+K)+

--- End Of Report ---

Figure S17. Characterization of VbsC as a CoA-dependent desacetyl-D-vicibactin (**D-14**) N^2 -acetyltransferase. (A) Schematic of the VbsC-catalyzed, tri-acetylation of **D-14** with 3 equivalents of acetyl-CoA and subsequent conversion to ferrivicibactin with $\text{Fe}(\text{ClO})_3$. LC-ESI-HRMS m/z data of purified product is consistent with ferrivicibactin (Figure S17). (B) HPLC traces (435 nm) showing the product formed upon incubation of **D-14** (1 mM) and acetylCoA (4 mM) with VbsC (10 μM) in the presence of 50 mM HEPES pH 7.75. Reaction halted after 32 min by the addition of $\text{Fe}(\text{ClO})_3$ in 0.2 M perchloric acid. Peak corresponding to ferrivicibactin appears at t_R 11.8 min. Conversion was determined by reverse phase HPLC analysis on a Supleco Discovery C18 column (250 x 4.6 mm) using a solvent gradient of 2 to 66% B over 20 min (solvent A, 0.1% TFA/H₂O; solvent B, 0.1% TFA/MeCN).

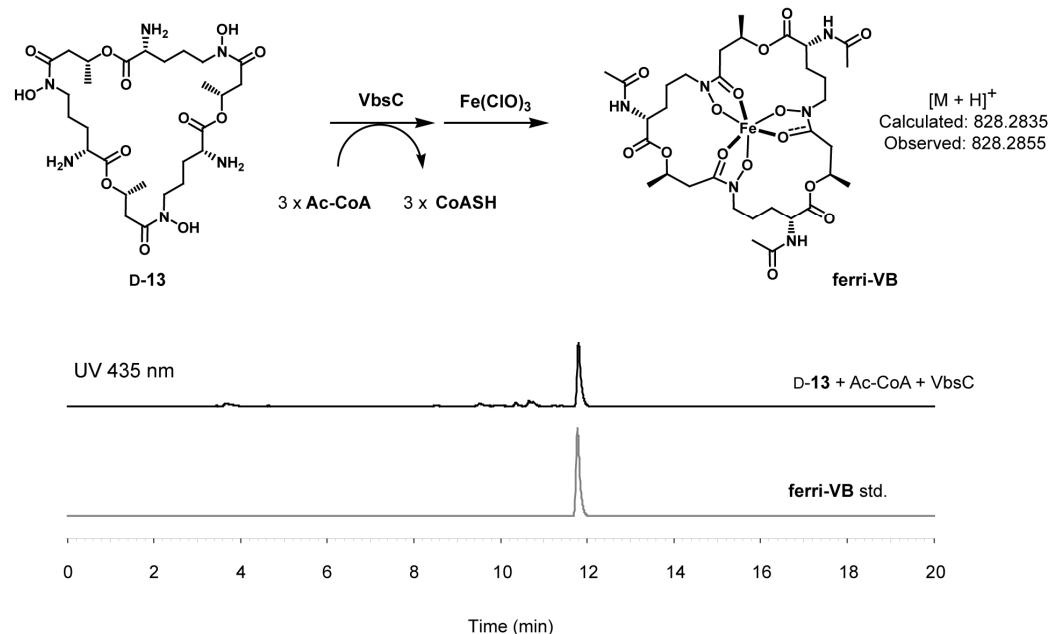


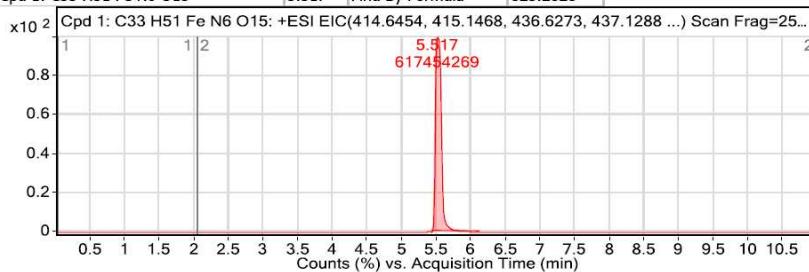
Figure S18. LC-ESI-HRMS m/z data of the product eluting at 11.8 min formed by incubation of VbsC with desacyl-D-vicibactin (**D-14**) and acetyl-CoA followed by treatment with $\text{Fe}(\text{ClO})_3$ (Figure S16) is consistent with ferricibactin ($\text{C}_{33}\text{H}_{51}\text{FeN}_6\text{O}_{15}$). [The isotopic distribution is consistent with an Fe containing complex \(\$^{54}\text{Fe}\$ isotope found at 826.287\).](#)

Data File	JRH052309_3.d	Sample Name	sample3
Sample Type	Sample	Position	P1-C3
Instrument Name	Instrument 1	User Name	
Acq Method	20090604.m	IRM Calibration Status	Success
DA Method	Default.m	Comment	

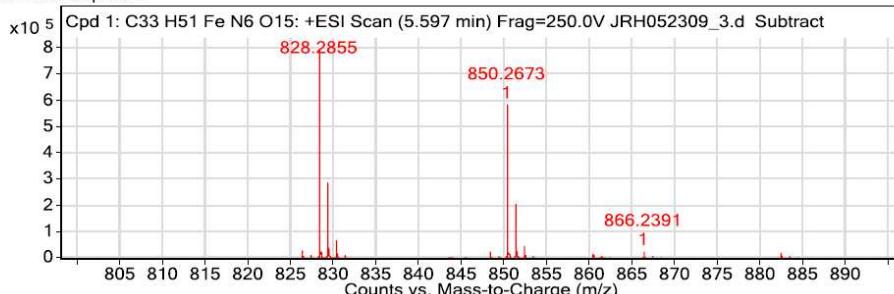
Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: $\text{C}_{33}\text{H}_{51}\text{FeN}_6\text{O}_{15}$	5.517	825.2828	795326	$\text{C}_{33}\text{H}_{51}\text{FeN}_6\text{O}_{15}$	825.2809	2.3

Compound Label	RT	Algorithm	Mass
Cpd 1: $\text{C}_{33}\text{H}_{51}\text{FeN}_6\text{O}_{15}$	5.517	Find By Formula	825.2828



MS Zoomed Spectrum

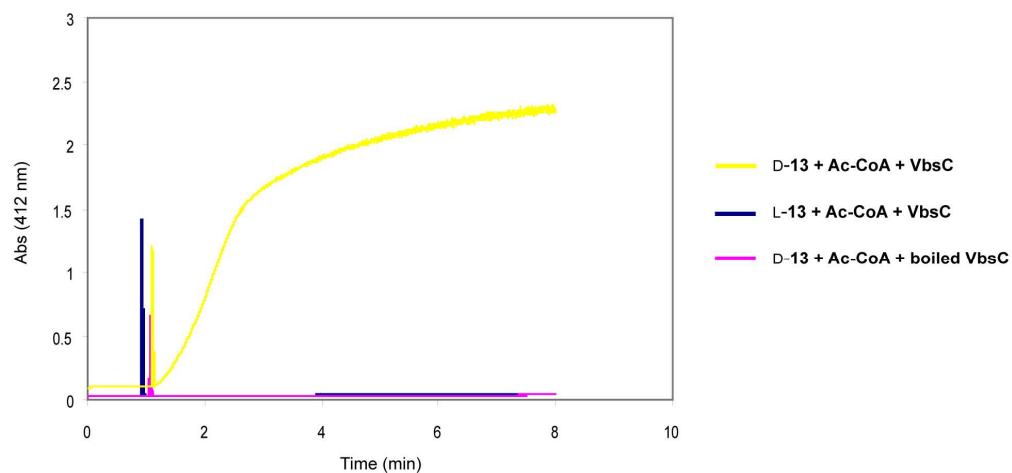
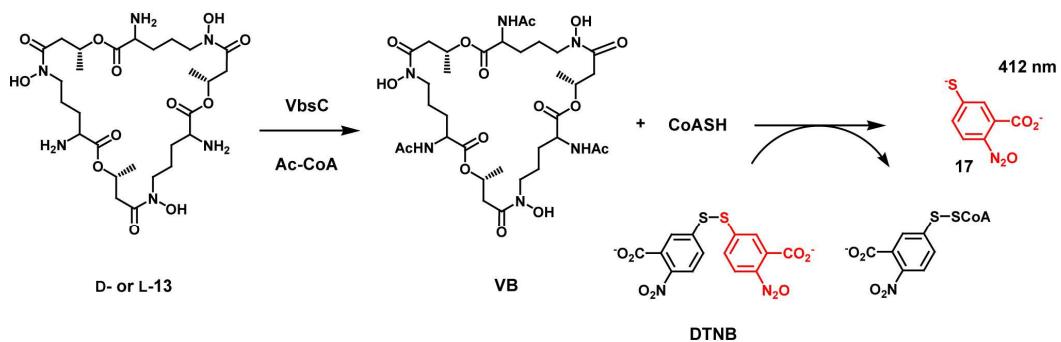


MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
826.287				32169		
827.2877				11903		
828.2855	828.2835	2.34		817986	$\text{C}_{33}\text{H}_{52}\text{FeN}_6\text{O}_{15}$	(M+H)+
828.4697				27703		
829.288	829.2864	1.94		288493	$\text{C}_{33}\text{H}_{52}\text{FeN}_6\text{O}_{15}$	(M+H)+
830.2897	830.2887	1.22		68646	$\text{C}_{33}\text{H}_{52}\text{FeN}_6\text{O}_{15}$	(M+H)+
850.2673	850.2655	2.19	1	591374	$\text{C}_{33}\text{H}_{51}\text{FeN}_6\text{O}_{15}$	(M+Na)+
851.2697	851.2683	1.55	1	210600	$\text{C}_{33}\text{H}_{51}\text{FeN}_6\text{O}_{15}$	(M+Na)+
852.2711	852.2706	0.57	1	49253	$\text{C}_{33}\text{H}_{51}\text{FeN}_6\text{O}_{15}$	(M+Na)+
866.2391	866.2394	-0.43	1	25017	$\text{C}_{33}\text{H}_{51}\text{FeK}_6\text{O}_{15}$	(M+K)+

--- End Of Report ---

Figure S19. Comparison of desacetyl-D-vicibactin (**D-14**) and desacetyl-L-vicibactin (**L-14**) as substrates for VbsC. **(A)** Schematic of the VbsC-catalyzed transfer of acetyl- from the CoA thioester to N^2 of desacetylvicibactin (**14**) generating the acetylated product and CoASH. The reaction between the free thiol of CoASH and DTNB generates thiophenol **17** with an absorbance at 412 nm. **(B)** Monitoring the change in absorbance at 412 nm with time for incubation mixtures containing Tris-HCl (50 mM, pH 8.0), DTNB (50 μ M), acetyl-CoA (500 μ M) and VbsA (1 μ M). After incubation for 1 min (to ascertain the rate of CoASH formation in the absence of the acceptor substrate) **L-14** (1 mM) or **D-14** (1 mM) was added. Activity was monitored continuously on a Cary 50 Bio UV-visible spectrophotometer. No acetyltransfer was observed with VbsC inactivated by boiling.



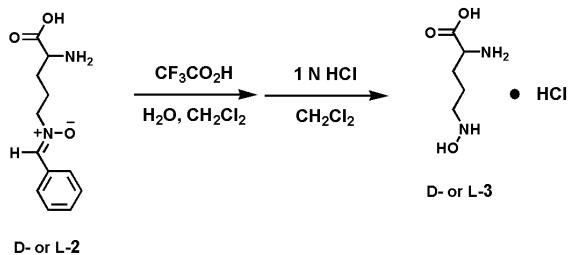
2. Table S1. Comparison of the VbsS adenylation (A) domain specificity determining residues with those from L-Orn and N⁵-OH-L-Orn activating NRPS A domains.

A-domain	substrate	Residue (according to GrsA numbering)							
		235	236	239	278	299	301	302	330
FenC-M2	L-Orn	D	V	G	E	I	G	S	I
GrsB-M2	L-Orn	D	V	G	E	I	G	S	I
TycC-M5	L-Orn	D	V	G	E	I	G	S	I
BacB-M2	L-Orn	D	V	G	E	I	G	S	I
FxbC-M1	N ⁵ -OH-L-Orn	D	M	E	N	L	G	L	I
CchH-M3	N ⁵ -OH-L-Orn	D	M	E	N	L	G	L	I
Fscl	N ⁵ -OH-L-Orn	D	M	E	N	L	G	L	I
VbsS	?	D	G	E	S	S	G	G	M

3. Materials and General Methods. N²-Boc-L-ornithine *tert*-butyl ester was purchased from Bachem bioscience Inc. All other chemicals and reagents were purchased from Sigma-Aldrich and were of the highest quality available and used without further purification. The preparation of D- and L-3-hydroxybutyryl-CoA followed the procedure of Walsh et. al.¹ The preparation of N²-Boc-L-ornithine *tert*-butyl ester followed the procedure of Moynihan et. al.² MS analysis of samples was carried out on an Agilent 6210 Time-of-Flight MS with a dual nebulizer ESI source. ¹H NMR spectra were recorded on a Varian 600 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance resulting from incomplete deuteration as the internal standard (CDCl₃ δ 7.26, D₂O δ 4.75). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), and integration.

Formatted: Justified

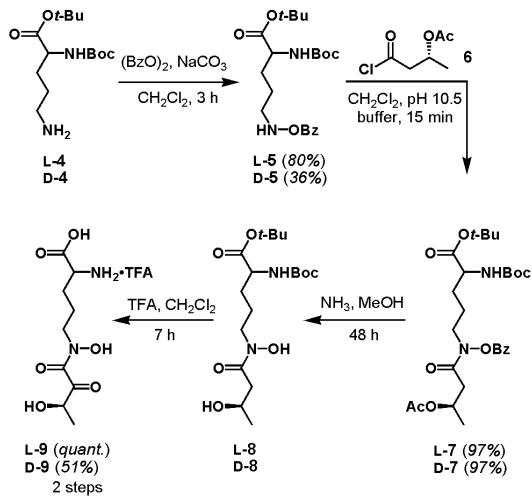
4. Preparation of Synthetic *N*⁵-Hydroxy-D- and L-Ornithine (3) Hydrochloride.



To a 50 mL round-bottomed flask was added 500 mg of nitrone **L-2** (2.1 mmol, 1.0 equiv, prepared according to a published procedure³), 9 mL trifluoroacetic acid (TFA), 1 mL of H₂O and 20 mL of CH₂Cl₂. The rapidly stirring biphasic solution was heated at 45 °C for 15 min. The volatiles were removed in *vacuo* to afford an oily residue which was dissolved in 20 mL CH₂Cl₂ and 30 mL of 1 N HCl. The resulting biphasic solution was stirred at rt. for 1 h. The aqueous layer was then removed and washed with 20 mL CH₂Cl₂ and 20 mL hexanes. The aqueous layer was concentrated to give 301 mg (78%) of **L-3** as a yellow foam. The spectroscopic data agreed with the published literature data.⁴ ¹H NMR (D₂O, 600 MHz): δ 4.01 (t, *J* = 6.2 Hz, 1 H), 3.34 (t, *J* = 7.5 Hz, 1 H), 2.05-1.86 (m, 4 H). HRMS (ESI⁺): [M+H]⁺ *m/z* calcd. for C₅H₁₂N₂O₃, 149.09207; found, 149.09205.

D-3 was prepared as described above for **L-3** to **D-3** as a yellow foam (35%). The spectroscopic data agreed with the published literature data.⁵ ¹H NMR (D₂O, 600 MHz): δ 3.97 (t, *J* = 6.3 Hz, 1 H), 3.33 (t, *J* = 7.3 Hz, 1 H), 2.01–1.82 (m, 4 H). HRMS (ESI⁺): [M+H]⁺ *m/z* calcd. for C₅H₁₃N₂O₃, 149.09207; found, 149.09162.

5. Preparation of Synthetic *N*⁵-Hydroxy-*N*⁵-(D-3-hydroxybutyryl)-D- and L-ornithine (D- and L-9).



Preparation of *N*⁵-benzoyloxy-*N*²-Boc-L-ornithine *tert*-butyl ester (**6**) was accomplished following a procedure described by Phanstiel and coworkers.⁶ To a 50 mL round-bottomed flask were added 500 mg of *N*²-Boc-L-ornithine *tert*-butyl ester **L-4** (1.54 mmol, 1 equiv) and 8 mL of aqueous sodium carbonate (pH 10.5). To this rapidly stirring solution was added 746 mg of benzoyl peroxide (70% in H₂O, 3.08 mmol) in 8 mL of CH₂Cl₂. The resulting biphasic mixture was stirred for 3 hr at room temperature, and then poured into 25 mL of H₂O. The aqueous layer was removed and extracted with CH₂Cl₂ (3 x 25 mL). The combined organic layers were dried over Na₂SO₄, filtered and the filtrate was concentrated in vacuo. The resulting residue was purified by column chromatography (SiO₂ (100 g), 4 cm diam., 7/3 hexanes/EtOAc to yield 504 mg (80%) of **L-5** as a white solid. ¹H NMR (CDCl₃, 600 MHz): δ 8.02 (d, *J* = 7.6 Hz, 2 H), 7.90 (br s, 1 H), 7.58 (t, *J* = 7.2 Hz, 1 H), 7.46 (t, *J* = 7.2 Hz, 2 H), 5.09-5.08 (m, 1 H), 4.21-4.20 (m, 1 H), 3.16 (m, 2 H), 1.96-1.89 (m, 1 H), 1.78-1.64 (m, 3 H), 1.45 (s, 9H), 1.43 (s, 9 H). HRMS (ESI⁺): [M+H]⁺ *m/z* calcd. for C₂₁H₃₃N₂O₆, 409.2333; found, 409.2341.

D-5 was prepared as described above for **L-5** to yield 100 mg (36%) of **D-5** as a white solid. ¹H NMR (CDCl₃, 600 MHz): δ 8.03 (d, *J* = 7.5 Hz, 2 H), 7.91 (br s, 1 H), 7.59 (t, *J* = 7.0

Hz, 1 H), 7.47 (t, J = 7.1 Hz, 2 H), 5.07-5.06 (m, 1 H), 4.22-4.21 (m, 1 H), 3.17-3.15 (m, 2 H), 1.98-1.91 (m, 1 H), 1.79-1.65 (m, 3 H), 1.46 (s, 9H,), 1.43 (s, 9 H).

The preparation of *N*⁵-benzoyloxy-*N*⁵-((*R*)-3-acetoxy-butyryl)-*N*²-Boc-L-ornithine *tert*-butyl ester (**D-7**) was also accomplished following a procedure described by Phanstiel and coworkers.⁵ To a 50 mL round-bottomed flask were added 90 mg of **D-5** (0.22 mmol, 1 equiv), 1.0 mL of CH₂Cl₂ and 1.0 mL of aqueous sodium carbonate (pH 10.5). To this rapidly stirring biphasic solution was added 1.1 mL of acid chloride **6** (0.2 M in CH₂Cl₂, 0.22 mmol, 1 equiv). The resulting biphasic mixture was stirred for 15 min hr at room temperature, and then poured into 25 mL of H₂O. The aqueous layer was removed and extracted with CH₂Cl₂ (3 x 25 mL). The combined organic layers were dried over Na₂SO₄, filtered and the filtrate was concentrated in vacuo. The resulting residue was purified by column chromatography (SiO₂ (25 g), 3 cm diam., 7/3 hexanes/EtOAc to yield 114 mg (97%) of **D-7** as a white solid. ¹H NMR (CDCl₃, 600 MHz): δ 8.09 (d, J = 7.4 Hz, 2 H), 7.69 (t, J = 7.2 Hz, 1 H), 7.53 (t, J = 7.6 Hz, 1 H), 5.33 (p, J = 6.6 Hz, 1 H), 5.10-5.08 (m, 1 H), 4.19-4.14 (m, 1 H) 3.83 (t, J = 6.3 Hz, 2 H), 2.74-2.63 (m, 1 H), 2.47-2.39 (m, 1 H), 2.00 (s, 3 H), 1.91-1.82 (m, 1 H), 1.75-1.62 (m, 3 H), 1.44 (s, 9H,), 1.41 (s, 9 H), 1.33-1.24 (m, 3 H). HRMS (ESI⁺): [M+H]⁺ *m/z* calcd. for C₂₇H₄₁N₂O₉, 537.2807; found, 537.2821.

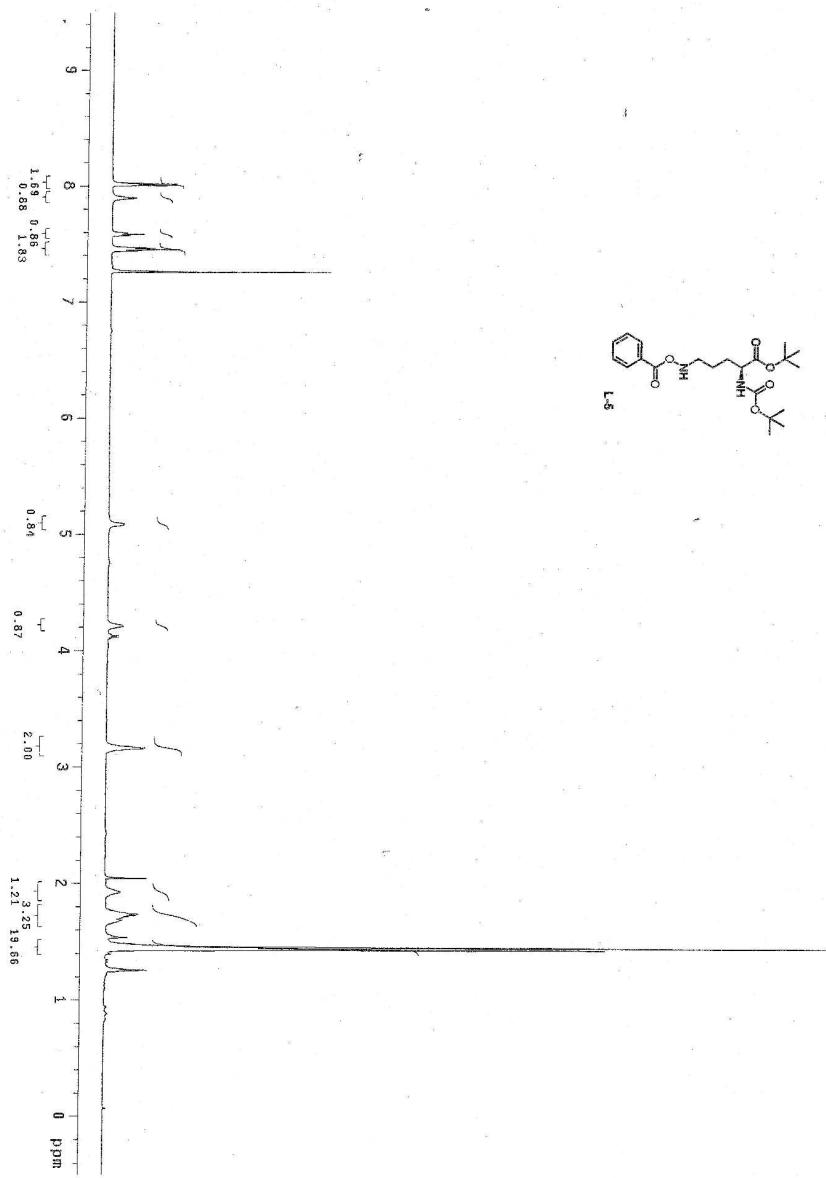
L-7 was prepared as described above for **D-7** to yield (167 mg, 0.31 mmol, 97%) of **L-7** as a white solid. ¹H NMR (CDCl₃, 600 MHz): δ 8.10 (d, J = 7.0 Hz, 2 H), 7.69 (t, J = 7.2 Hz, 1 H), 7.53 (t, J = 7.5 Hz, 1 H), 5.33 (q, J = 6.6 Hz, 1 H), 5.08-5.07 (m, 1 H), 4.19-4.12 (m, 1 H) 3.84 (br s, 2 H), 2.73-2.65 (m, 1 H), 2.47-2.38 (m, 1 H), 2.00 (s, 3 H), 1.89-1.82 (m, 1 H), 1.75-1.62 (m, 3 H), 1.43 (s, 9H,), 1.41 (s, 9 H), 1.33-1.24 (m, 3 H). HRMS (ESI⁺): [M+H]⁺ *m/z* calcd. for C₂₇H₄₁N₂O₉, 537.2807; found, 537.2803.

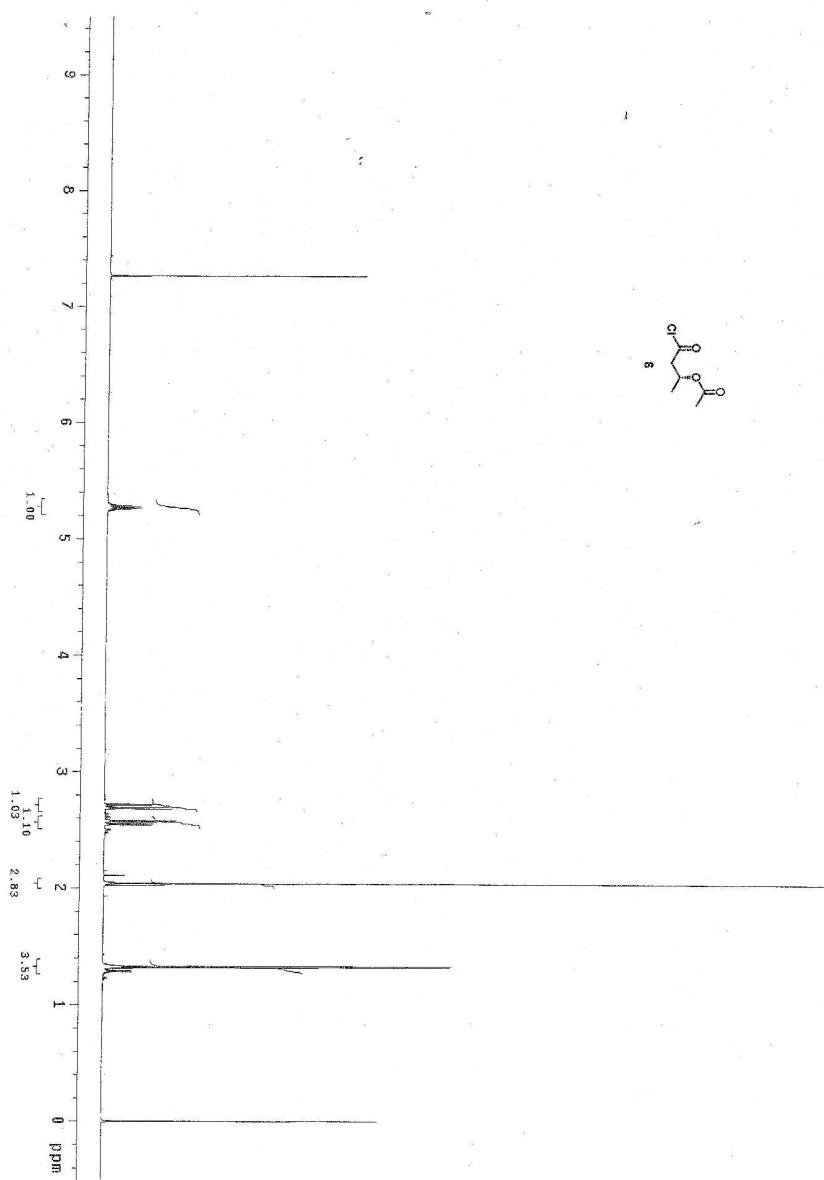
Removal of the benzoyl and acetate groups from **D-7** to afford *N*⁵-hydroxy-*N*⁵-((*R*)-3-hydroxybutyryl)-*N*²-Boc-L-ornithine *tert*-butyl ester (**D-8**) was accomplished using a solution of NH₃ in MeOH. To a 25 mL round-bottomed flask was added 75 mg of **D-7** (0.14 mmol, 1.0 equiv) and 8 mL of NH₃ in MeOH (2.0M). The mixture was stirred at rt for 48 hr and then the volatiles were removed in vacuo. The resulting residue was purified by column chromatography (lipophilic sephadex LH-20, 10 g), 3 cm diam., 7% EtOH in toluene, to yield **D-8** as a thick oil.

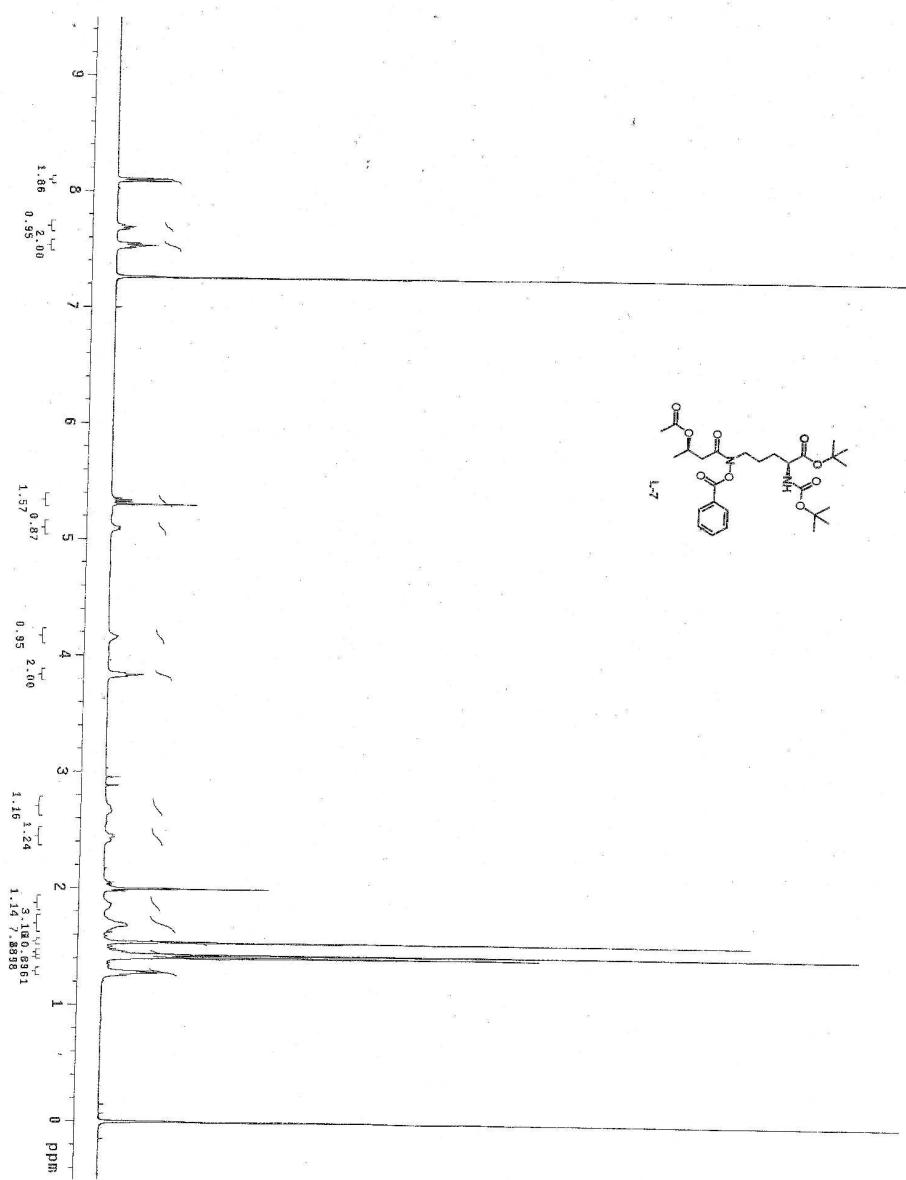
NMR data showed a complex mixture of rotomers; however, the peaks corresponding to the benzoyl and acetate groups were clearly missing. Therefore, D-8 was carried forward without any further characterization.

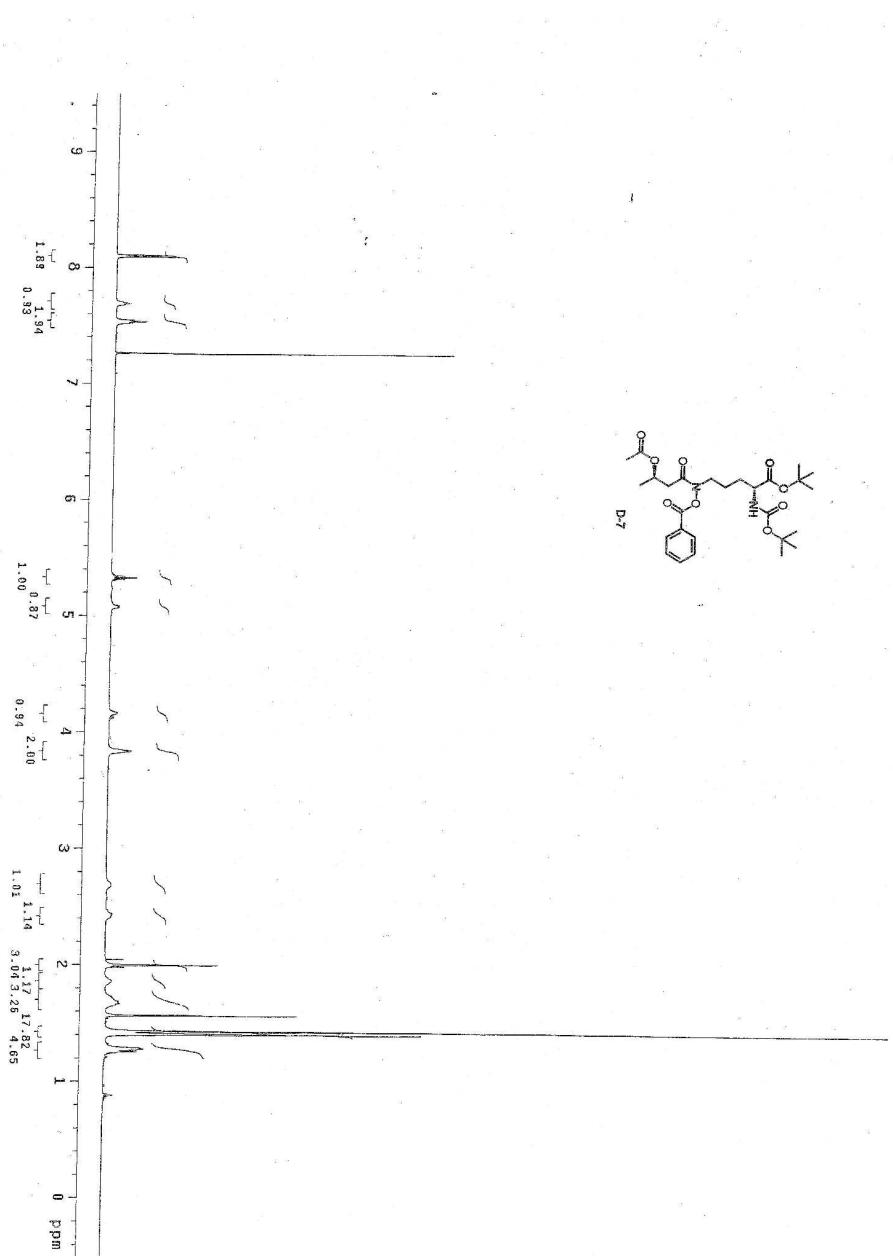
Removal of the Boc and tert-butyl groups was accomplished by treatment of a solution of D-8 in 1 mL CH₂Cl₂ with 1.0 mL of trifluoroacetic acid, followed by stirring at ambient temperature. After 7 hrs, the reaction was concentrated in vacuo to afford a light yellow oil. The resulting residue was dissolved in 3 mL of H₂O and extracted with CH₂Cl₂ (3 x 3 mL). Aqueous layer flash frozen in liquid N₂ and lyophilized to afford the TFA salt of D-9 as an amorphous solid in 51% yield. ¹H NMR (D₂O, 600 MHz): δ 4.21 (ddq, *J* = 7.8, 6.3, 5.1, Hz, 1 H), 4.02 (t, *J* = 6.3 Hz, 1 H), 3.69 (t, *J* = 6.4 Hz, 1 H), 2.77 (dd, *J* = 14.8, 8.2 Hz, 1 H), 2.59 (dd, *J* = 14.8, 5.1 Hz 1 H), 1.97-1.70 (m, 4 H), 1.22 (d, *J* = 6.3 Hz, 3 H). HRMS (ESI⁺): [M+H]⁺ *m/z* calcd. for C₉H₁₉N₂O₅, 235.1289; found, 235.1281.

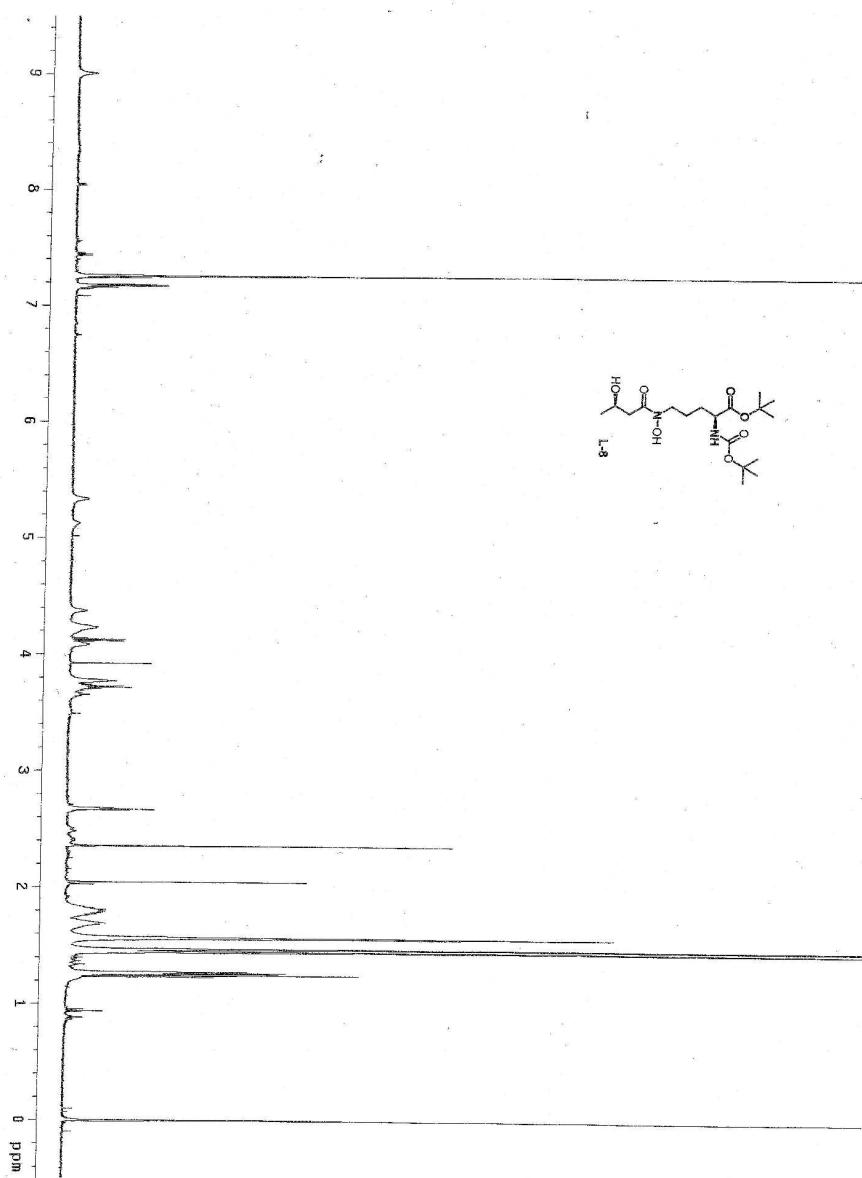
L-9 was prepared as described above for D-9 to afford the TFA salt of L-9 as in quantitative yield from L-7. ¹H NMR (D₂O, 600 MHz): δ 4.12 (ddq, *J* = 8.0, 6.4, 5.2 Hz, 1 H), 3.94 (t, *J* = 6.2 Hz, 1 H), 3.59-3.53 (m, 2 H), 2.64 (dd, *J* = 14.8, 8.1 Hz, 1 H), 2.46 (dd, *J* = 15.0, 5.3 Hz 1 H), 1.86-1.62 (m, 4 H), 1.08 (d, *J* = 6.2 Hz, 3 H). HRMS (ESI⁺): [M+H]⁺ *m/z* calcd. for C₉H₁₉N₂O₅, 235.1289; found, 235.1295.

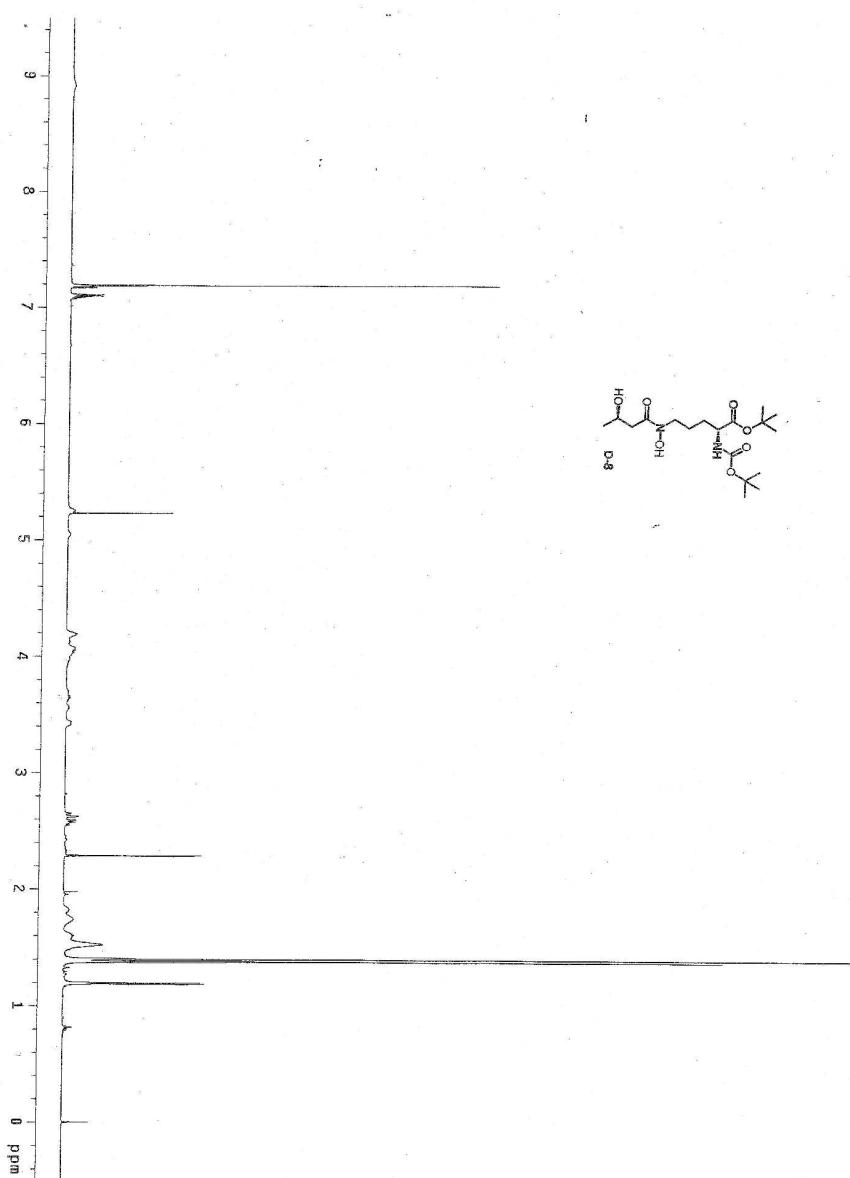


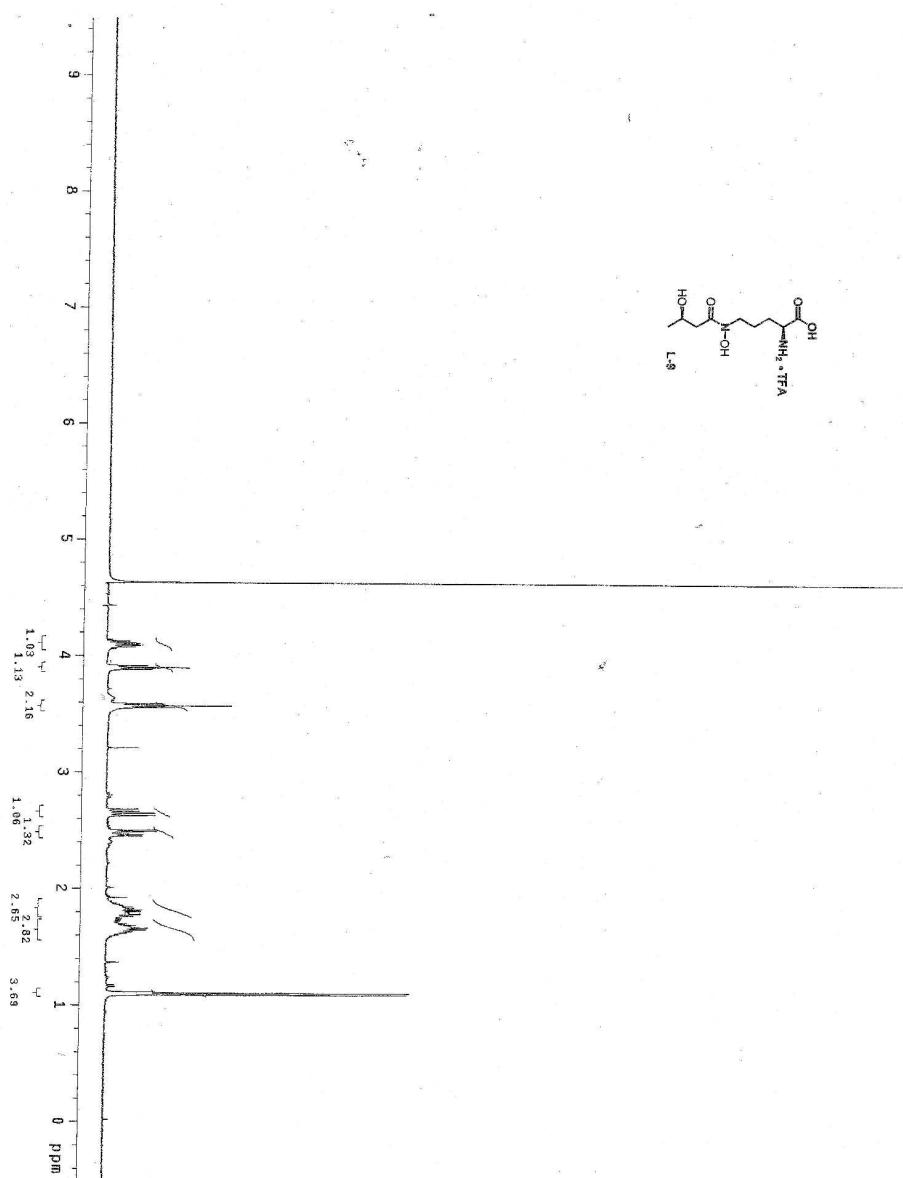


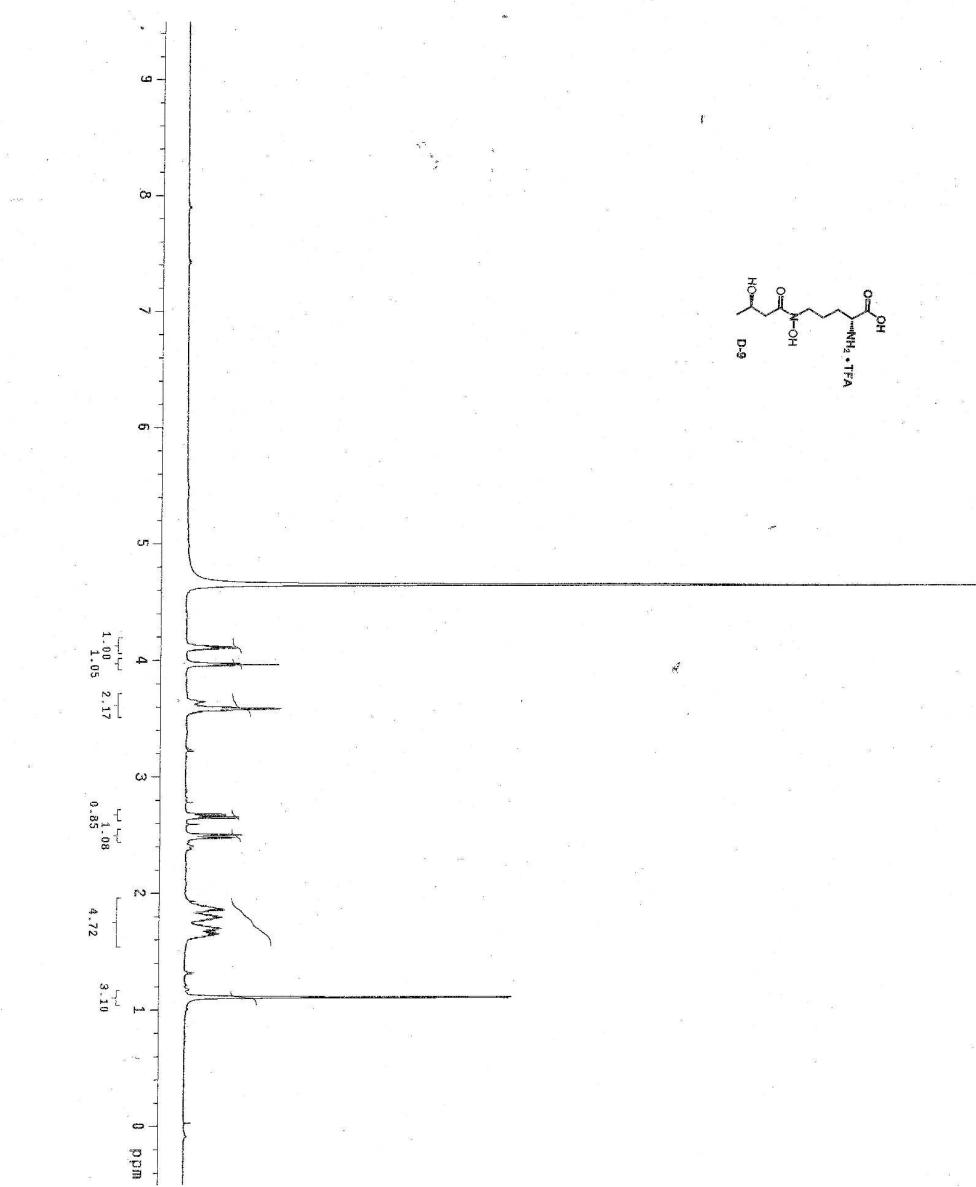












7. References

- ¹ Belshaw, P. J.; Walsh, C. T.; Stachelhaus, T. *Science*, **1999**, *284*, 486-489.
- ² Moynihan, H. A.; Roberts, S. M.; Weldon, H.; Allcock, G. H.; Anggard, E. E.; Warner, T. D. *J. Chem. Soc., Perkin Trans. I* **1994**, *7*, 769-71.
- ³ Lin, Y.-M.; Miller, M. J. *J. Org. Chem.*, **1999**, *64*, 7451-7458.
- ⁴ Maehr, H.; Leach, M. *J. Org. Chem.* **1974**, *39*, 1166-1168.
- ⁵ Maehr, H.; Leach, M. *J. Org. Chem.* **1974**, *39*, 1166-1168.
- ⁶ Wang, Q. X.; King, J.; Phanstiel IV, O. J. *J. Org. Chem.* **1997**, *62*, 8104-8108.