Supporting Information

The Contribution of Romidepsin to the Herbicidal Activity of *Burkholderia rinojensis* Biopesticide

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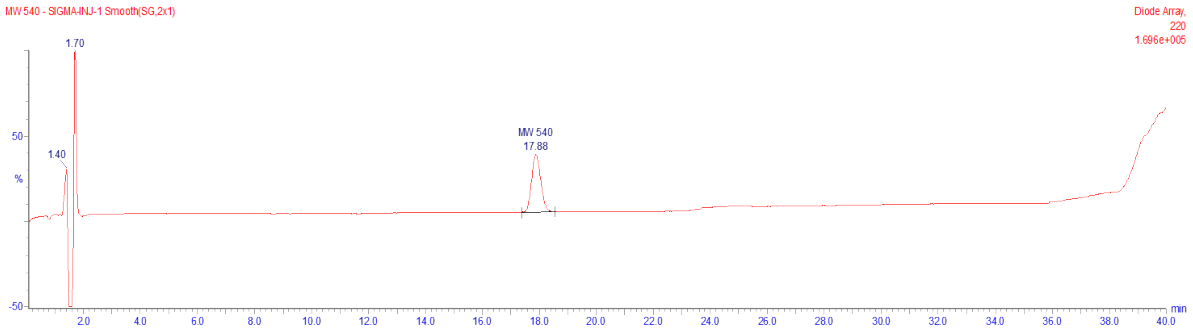
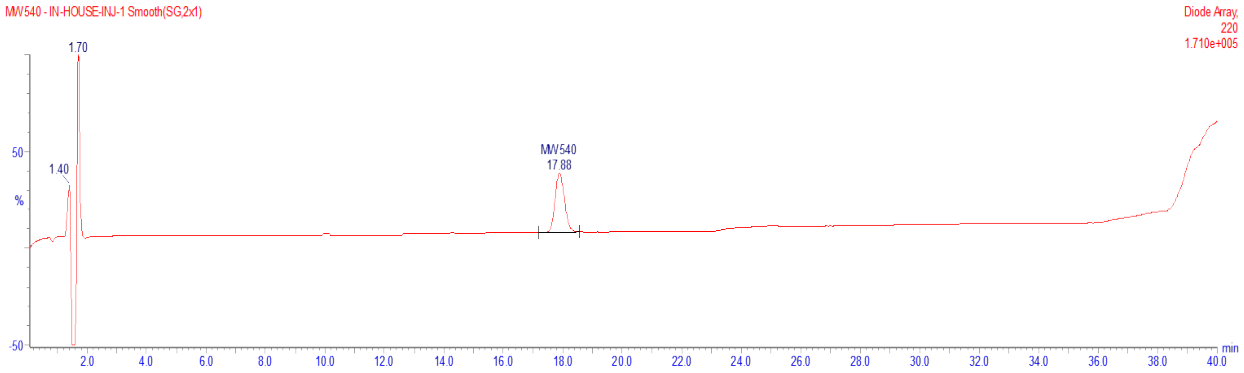
Confirmation of MW 540 isolated in-house as Romidepsin

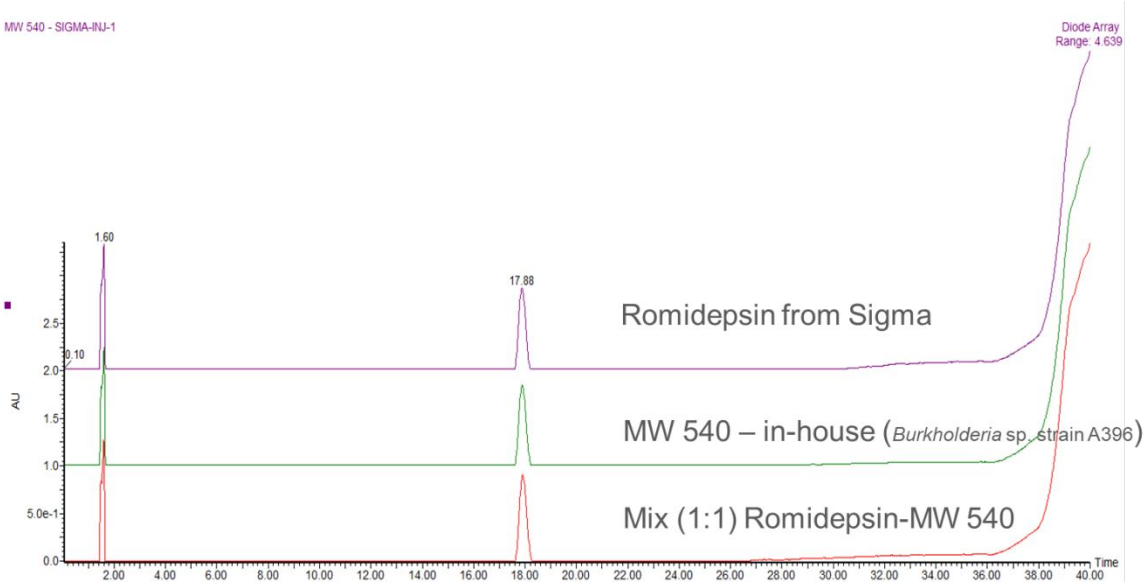
Supplement Figure 1. HPLC Chromatogram of Romidepsin (from Sigma Aldrich)



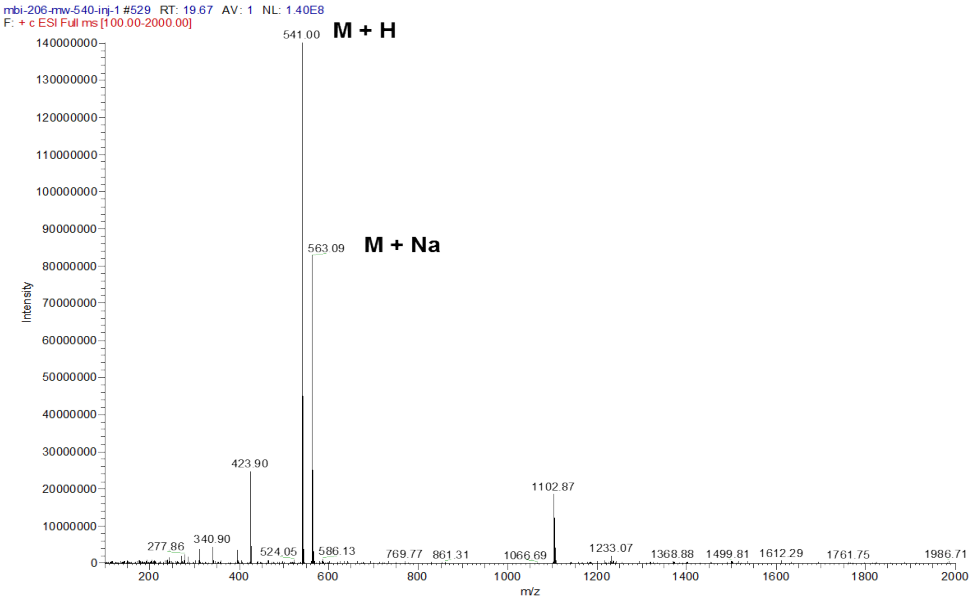
Supplement Figure 2. HPLC Chromatogram of MW 540 (in-house from *Burkholderia* sp. strain A396)



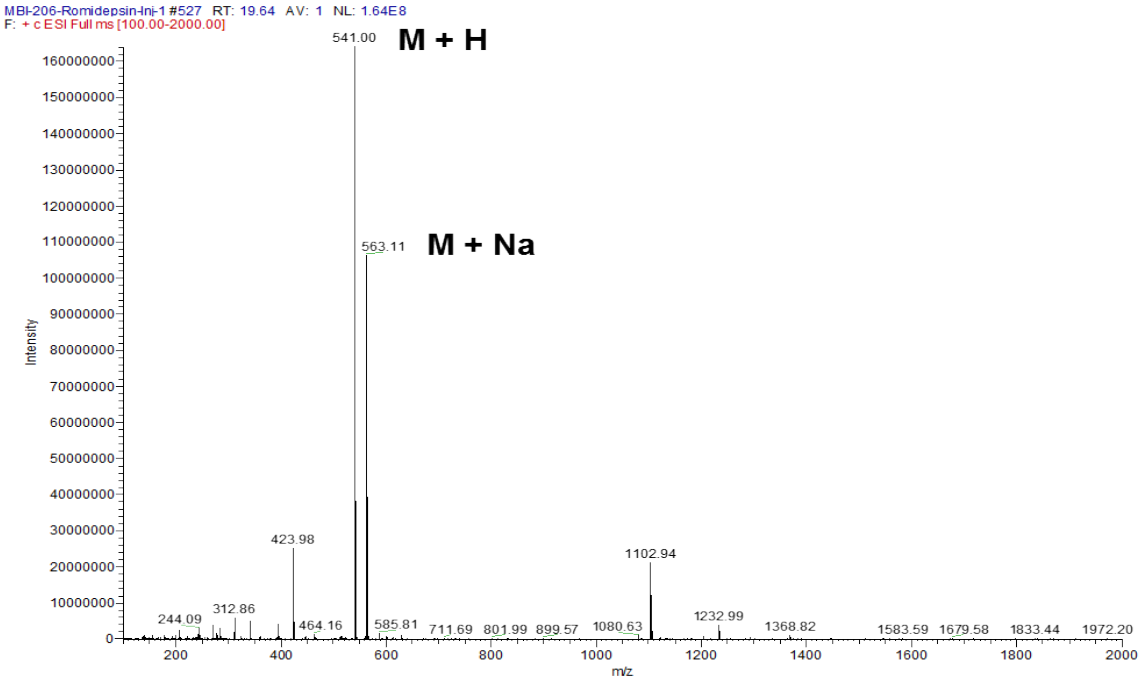
Supplement Figure 3. HPLC Chromatogram of Romidepsin, MW 540 and Mixture of Romidepsin -MW 540



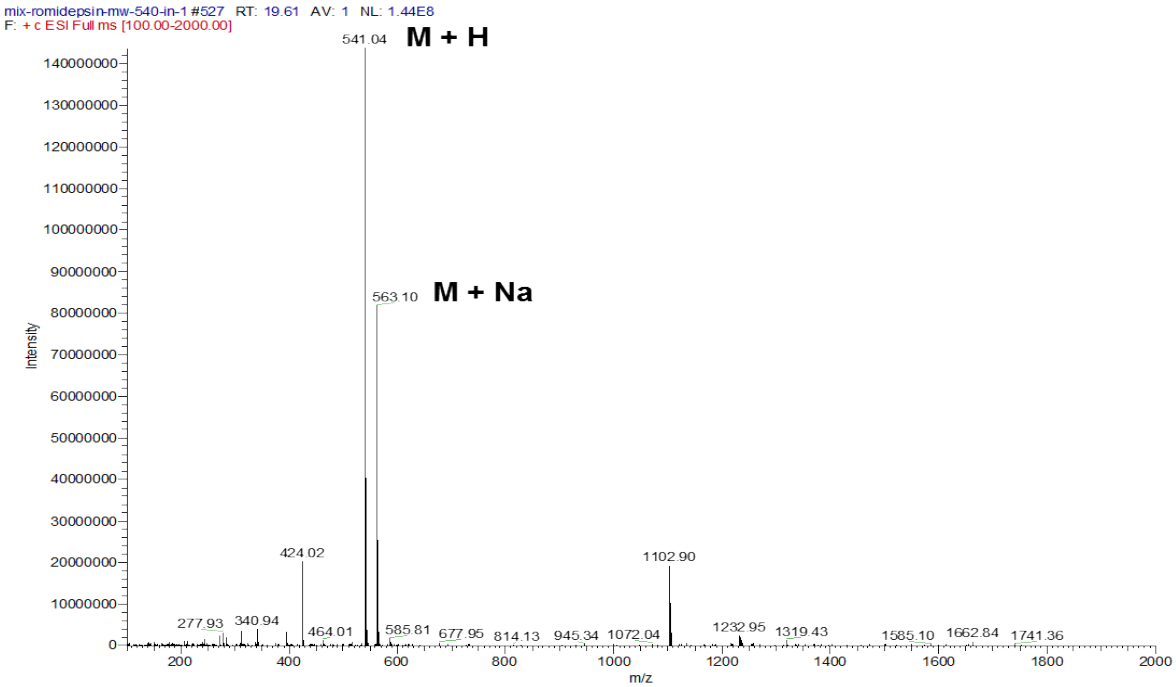
Supplement Figure 4. ESIMS data for MW 540 (isolated in-house from *Burkholderia* sp. strain A396)



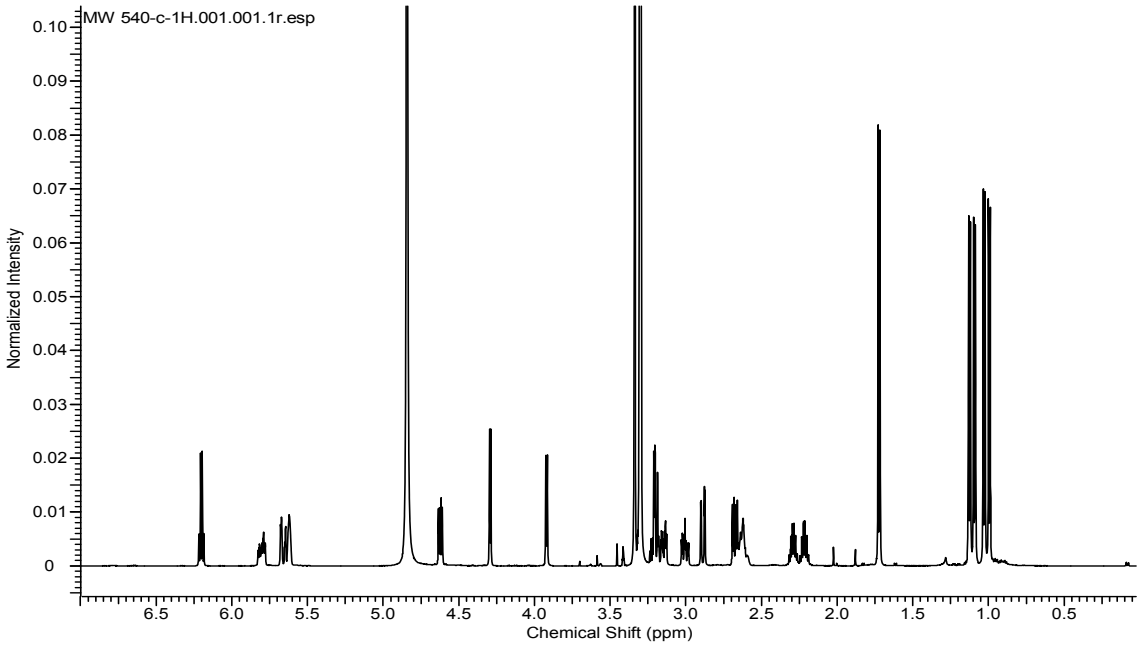
Supplement Figure 5. ESIMS data for Romidepsin



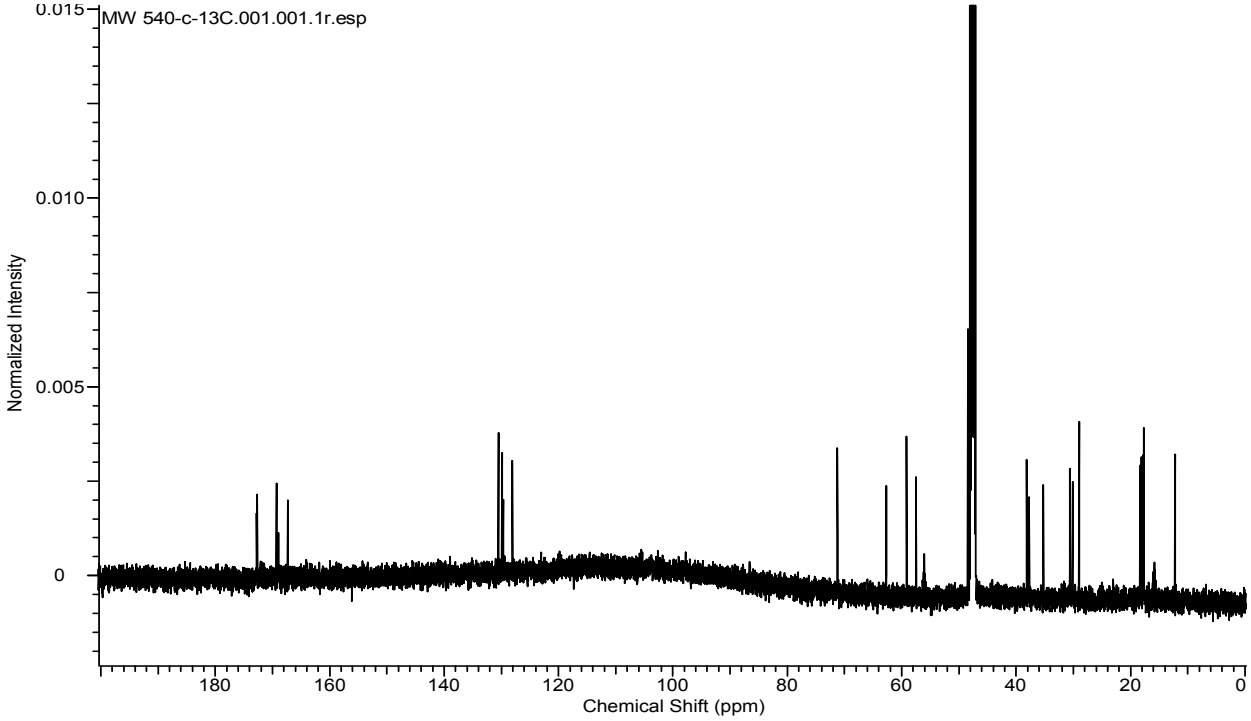
Supplement Figure 6. ESIMS data for Mixture of Romidepsin -MW 540 (1:1)



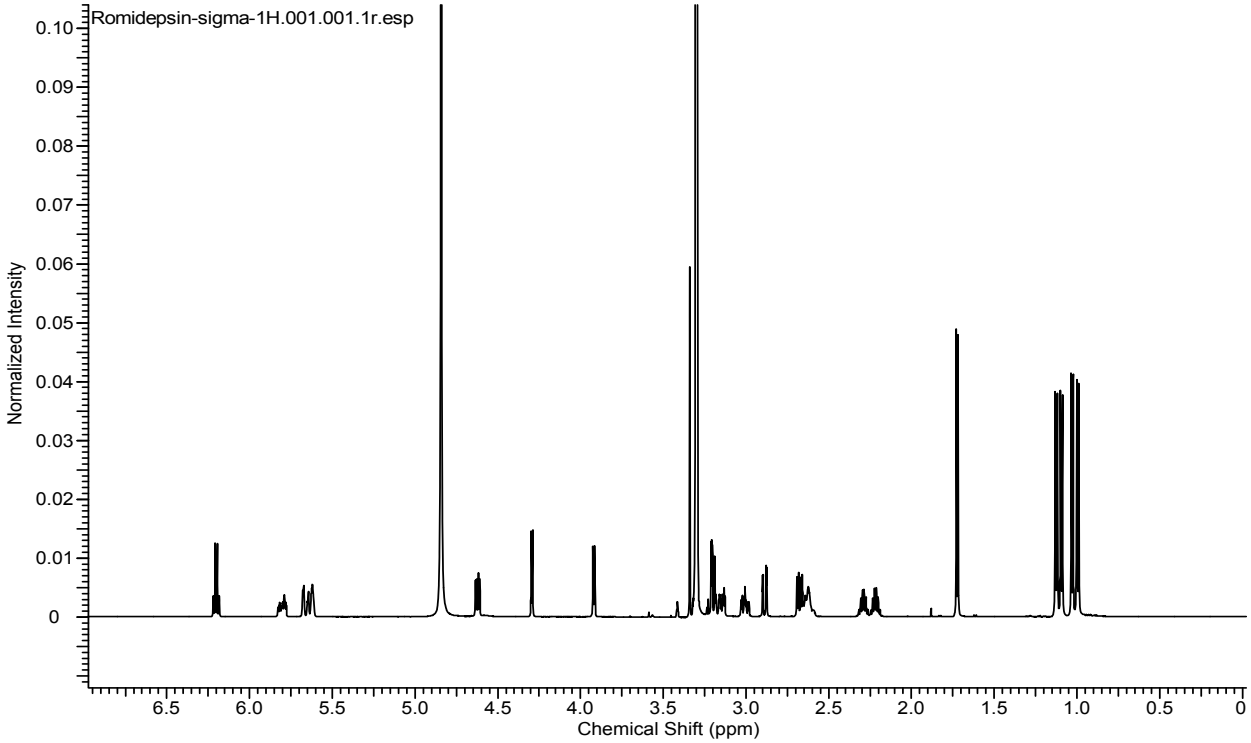
Supplement Figure 7. ^{1}H NMR Spectrum of MW 540 in CD_3OD-d_4, 600 MHz



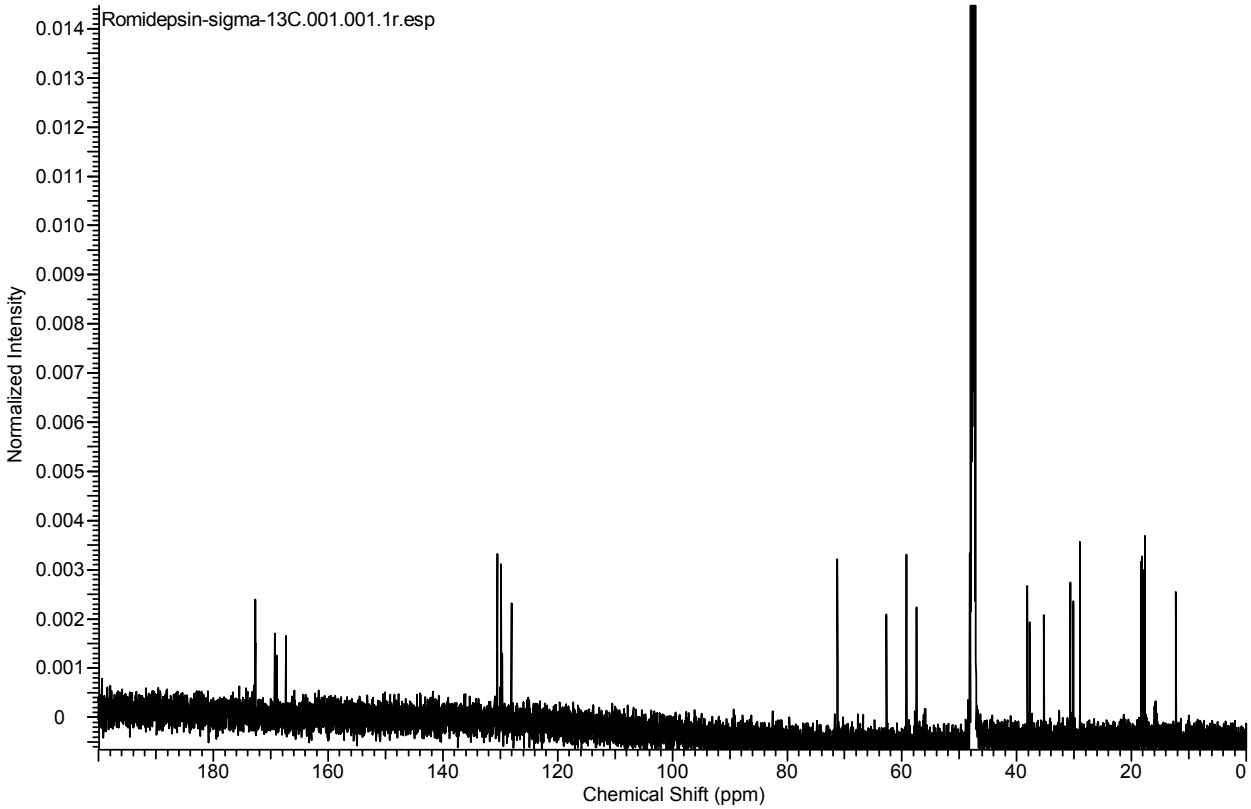
Supplement Figure 8. ^{13}C NMR Spectrum of MW 540 in CD_3OD-d_4, 150 MHz



Supplement Figure 9. ^{1}H NMR Spectrum of Romidepsin in CD_3OD-d_4, 600 MHz



Supplement Figure 10. ^{13}C NMR Spectrum of Romidepsin in CD_3OD-d_4, 150 MHz

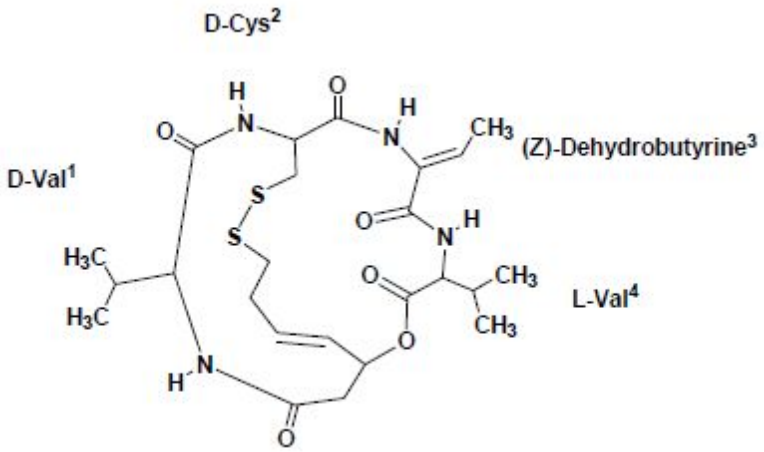


Supplement Table 1. ^{1}H and ^{13}C NMR assignment for MW 540 and Romidepsin (600 MHz in CD_3OD-d_4)

		MW 540		Romidepsin	
Assignment		δ_H (mult., *J* (Hz))	δ_C	δ_H (mult., *J* (Hz))	δ_C
Acy	1		172.74		172.74
	2	2.68 (dd, 12, 2)	37.75	2.68 (dd, 12, 2)	37.75
		2.89 (dd, 12, 2)		2.89 (dd, 12, 2)	
	3	5.62 (m)	71.24	5.62 (m)	71.24
	4	5.66 (m)	130.49	5.66 (m)	130.49
	5	5.8 (m)	129.67	5.8 (m)	129.67
	6	2.62 (2H, m)	30.11	2.62 (2H, m)	30.11
	7	3.15 (m)	38.14	3.15 (m)	38.14
		3.01 (m)		3.01 (m)	
D- Val[1]	NH				
	α	3.96 (d, 6)	62.7	3.96 (d, 6)	62.7
	β	2.22 (m)	29	2.22 (m)	29
	γ	1.13 (3H, d, 6)	18.33	1.13 (3H, d, 6)	18.33
		1.09 (3H, d, 6)	18.12	1.09 (3H, d, 6)	18.12
	CO		172.68		172.68

D-Cys[2]	NH				
	α	4.62 (dd, 12, 6)	57.48	4.62 (dd, 12, 6)	57.48
	β	3.21 (2H, m)	35.24	3.21 (2H, m)	35.24
	CO		169.32		169.32
*Deh[3]	NH				
	α		129.87		129.87
	β	6.2 (q, 6)	128.06	6.2 (q, 6)	128.06
	γ	1.72 (3H, d 6)	12.25	1.72 (3H, d 6)	12.25
	CO		167.34		167.34
L-Val[4]	NH				
	α	4.29 (d, 6)	59.17	4.29 (d, 6)	59.17
	β	2.29 (m)	30.64	2.29 (m)	30.64
	γ	0.99 (3H, d, 6)	17.83	0.99 (3H, d, 6)	17.83
		1.03 (3h, d, 6)	17.67	1.03 (3h, d, 6)	17.67
	CO		168.97		168.97

*Dehydrobutyrine



Supplemental figure 11 A) Alignment of atHDAC19 to 5iwg and 1c3r using MAFFT version 7 and visualized with ESPript

```
                                                β1        η1                α1        η2
sp|O22446|HDA19_ARATH                                  TT
                         1         10        20        30        40        50        60
sp|O22446|HDA19_ARATH    MDTGGNSLASGPDGVKRKVCYFYDPEVGNYYYGQGHPMKPHRIRMTHALLAHYGLLQHMQ
5IWG_A|PDBID|CHAIN|SEQUENCE ..........G....KKKVCYYYDGDIGNYYYGQGHPMKPHRIRMTHNLLLNYGLYRKME
1C3R_A|PDBID|CHAIN|SEQUENCE ...............MKKVKLIGTLDYGKYRYPKNHPLKIPRVSLLLRFKDAMNLIDEKE

                β2        α2        α3        η3    α4
sp|O22446|HDA19_ARATH
                         70        80        90       100       110       120
sp|O22446|HDA19_ARATH    VLKPFPARDRDLCRFHADDYVSFLRSITPETQQDQIRQLKRFNVGEDCPVFDGLYSFCQT
5IWG_A|PDBID|CHAIN|SEQUENCE IYRPHKATAEEMTKYHSDEYIKFLRSIRPDNMSEYSKQMQRFNVGEDCPVFDGLFEFCQL
1C3R_A|PDBID|CHAIN|SEQUENCE LIKSRPATKEELLLFHTEDYINTLMEAERSQSVPKGAREKYNIGGYENPVSYAMFTGSSL

                α5                β3        TT    TT  TT    TT        α6
sp|O22446|HDA19_ARATH
                        130       140       150       160       170
sp|O22446|HDA19_ARATH    YAGGSVGGSVKLNHGLCDIAINWAGGLHHAKKCEASGFCYVNDIVLAILELLKQ.HERVL
5IWG_A|PDBID|CHAIN|SEQUENCE STGGSVAGAVKLNRQQTDMAVNWAGGLHHAKKSEASGFCYVNDIVLAILELLKY.HQRVL
1C3R_A|PDBID|CHAIN|SEQUENCE ATGSTVQAIEEFLKG..NVAFNPAGGMHHAFKSRANGFCYINNPAVGIEYLRKKGFKRIL

                β4        α7        β5        TT        TT    η4    β6
sp|O22446|HDA19_ARATH
                        180       190       200       210       220       230
sp|O22446|HDA19_ARATH    YVDIDIHHGDGVEEAFYATDRVMTVSFHKFGDYFP..GTGHIQDIGYGSGKYYSLNVPLD
5IWG_A|PDBID|CHAIN|SEQUENCE YIDIDIHHGDGVEEAFYTTDRVMTVSFHKYGEYFP..GTGDLRDIGAGKGKYYAVNFPMR
1C3R_A|PDBID|CHAIN|SEQUENCE YIDLDAHHCDGVQEAFYDTDQVFVLSLHQSPEYAFPFEKGFLEEIGEGKGKGYNLNIPLP

                α8                β7    η5  TT                α9
sp|O22446|HDA19_ARATH    TT
                        240       250       260       270       280★      290
sp|O22446|HDA19_ARATH    DGIDDESYHLLFKPIMGKVMEIFRPGAVVLQCGADSLSGDRLGCFNLSIKGHAECVKFMR
5IWG_A|PDBID|CHAIN|SEQUENCE DGIDDESYGQIFKPIISKVMEMYQPSAVVLQCGADSLSGDRLGCFNLTVKGHAKCVEVVK
1C3R_A|PDBID|CHAIN|SEQUENCE KGLNDNEFLFALEKSLEIVKEVFEPEVYLLQLGTDPLLEDYLSKFNLSNVAFLKAFNIVR

                β8        α10                η6
sp|O22446|HDA19_ARATH
                        300       310       320       330       340       350
sp|O22446|HDA19_ARATH    SFNVPLLLLGGGGYTIRNVARGWCYETGVALGVEVEDKMPEHEYYEYFGPDYTLHVAPSN
5IWG_A|PDBID|CHAIN|SEQUENCE TFNLPLLMLGGGGYTIRNVARGWTYETAVALDCEIPNELPYNDYFEYFGPDFKLHISPSN
1C3R_A|PDBID|CHAIN|SEQUENCE EVFGEGVYLGGGGYHPYALARAWTLIWCELSGREVPEKLNNKAKELLKSIDFEEFDDEVD

                α11
sp|O22446|HDA19_ARATH
                        360       370       380       390       400       410
sp|O22446|HDA19_ARATH    ....MENKNSRQMLEEIRNDLLHNLSKLQHAPSVPFQERPPDTETPEVDEDQEDGDKRWD
5IWG_A|PDBID|CHAIN|SEQUENCE ....MTNQNTPEYMEKIKQRLFENLRMLP..............................
1C3R_A|PDBID|CHAIN|SEQUENCE RSYMLETLKDPWRGGEVRKEVKDTLEKAKASS...........................

sp|O22446|HDA19_ARATH
                        420       430       440       450       460       470
sp|O22446|HDA19_ARATH    PDSDMDVDDDRKPIPSRVKREAVEPDTKDKDGLKGIMERGKGCEVEVDESGSTKVTGVNP
5IWG_A|PDBID|CHAIN|SEQUENCE ............................................................
1C3R_A|PDBID|CHAIN|SEQUENCE ............................................................
```

B) Alignment of entire 5iwg with residues 15-383 of atHDAC19

```
#######################################
# Program: needle
# Rundate: Mon 25 Feb 2019 23:05:32
# Commandline: needle
#    -auto
#    -stdout
#    -asequence emboss_needle-I20190225-230531-0025-4345346-p1m.asequence
#    -bsequence emboss_needle-I20190225-230531-0025-4345346-p1m.bsequence
#    -datafile EBLOSUM62
#    -gapopen 10.0
#    -gapextend 0.5
#    -endopen 10.0
#    -endextend 0.5
#    -aformat3 pair
#    -sprotein1
#    -sprotein2
# Align_format: pair
# Report_file: stdout
#######################################

#=======================================
#
# Aligned_sequences: 2
# 1: 5iwg
# 2: hdac19
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 368
# Identity:     245/368 (66.6%)
# Similarity:   309/368 (84.0%)
# Gaps:           0/368 ( 0.0%)
# Score: 1427.0
#
#
#=======================================

5iwg               1 GKKKVCYYYDGDIGNYYYGQGHPMKPHRIRMTHNLLLNYGLYRKMEIYRP     50
                     .|:||||:||.::||||||||||||||||||||.||.:|||.:.|::.:|
hdac19             1 VKRKVCYFYDPEVGNYYYGQGHPMKPHRIRMTHALLAHYGLLQHMQVLKP     50

5iwg              51 HKATAEEMTKYHSDEYIKFLRSIRPDNMSEYSKQMQRFNVGEDCPVFDGL    100
                     ..|...::.::|:|:|:.|||||.|:...:..:|::|||||||||||||||
hdac19            51 FPARDRDLCRFHADDYVSFLRSITPETQQDQIRQLKRFNVGEDCPVFDGL    100

5iwg             101 FEFCQLSTGGSVAGAVKLNRQQTDMAVNWAGGLHHAKKSEASGFCYVNDI    150
                     :.|||...||||.|:||||....|:|:||||||||||||.||||||||||||
hdac19           101 YSFCQTYAGGSVGGSVKLNHGLCDIAINWAGGLHHAKKCEASGFCYVNDI    150

5iwg             151 VLAILELLKYHQRVLYIDIDIHHGDGVEEAFYTTDRVMTVSFHKYGEYFP    200
                     |||||||||.|:||||:||||||||||||||||.||||||||||||:|:|||
hdac19           151 VLAILELLKQHERVLYVDIDIHHGDGVEEAFYATDRVMTVSFHKFGDYFP    200

5iwg             201 GTGDLRDIGAGKGKYYAVNFPMRDGIDDESYGQIFKPIISKVMEMYQPSA    250
                     |||.::|||.|.||||::|.|:.||||||||..:||||:.||||:::|.|
```

```
hdac19          201 GTGHIQDIGYGSGKYYSLNVPLDDGIDDESYHLLFKPIMGKVMEIFRPGA    250

5iwg            251 VVLQCGADSLSGDRLGCFNLTVKGHAKCVEVVKTFNLPLLMLGGGGYTIR    300
                    ||||||||||||||||||||::||||:||:.:::||:|||:|||||||||
hdac19          251 VVLQCGADSLSGDRLGCFNLSIKGHAECVKFMRSFNVPLLLLGGGGYTIR    300

5iwg            301 NVARCWTYETAVALDCEIPNELPYNDYFEYFGPDFKLHISPSNMTNQNTP    350
                    ||||||.|||.|||..|:.:::|.::|:||||||:.||::||||.|:|:.
hdac19          301 NVARCWCYETGVALGVEVEDKMPEHEYYEYFGPDYTLHVAPSNMENKNSR    350

5iwg            351 EYMEKIKQRLFENLRMLP    368
                    :.:|:|:..|..||..|.
hdac19          351 QMLEEIRNDLLHNLSKLQ    368
```

C) Clash score results from MolProbity

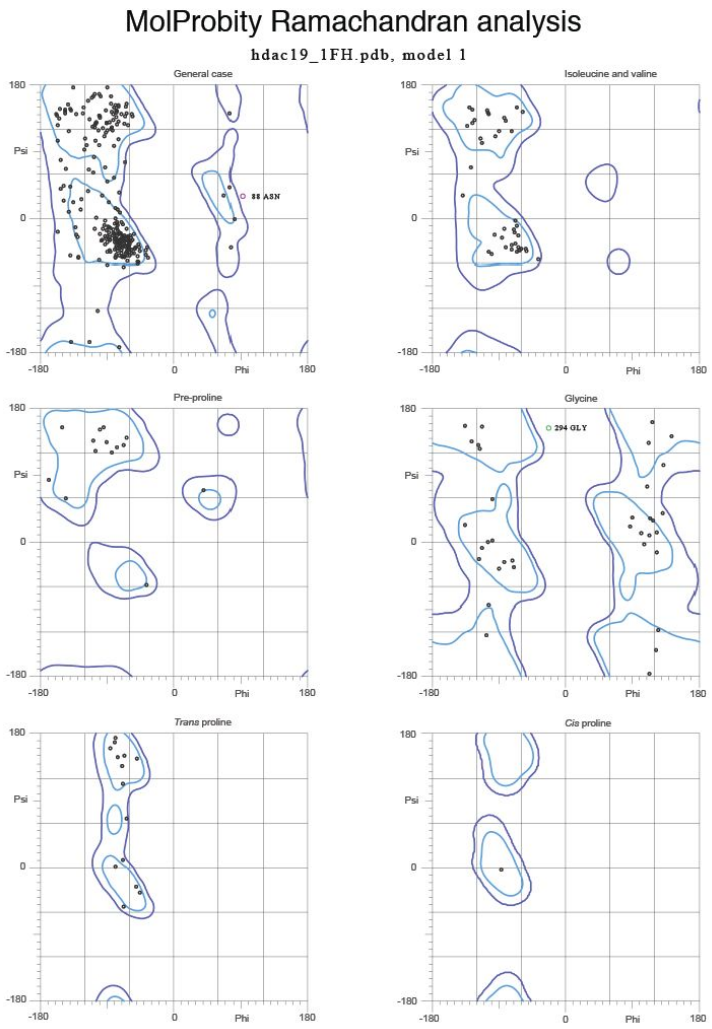
All-Atom Contacts	Clashscore, all atoms:	0		100th percentile* (N=1784, all resolutions)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.			
Protein Geometry	Poor rotamers	8	2.69%	Goal: <0.3%
	Favored rotamers	254	85.52%	Goal: >98%
	Ramachandran outliers	2	0.54%	Goal: <0.05%
	Ramachandran favored	316	86.10%	Goal: >98%
	MolProbity score^	1.47		96th percentile* (N=27675, 0Å - 99Å)
	Cβ deviations >0.25Å	37	11.11%	Goal: 0
	Bad bonds:	0 / 3023	0.00%	Goal: 0%
	Bad angles:	195 / 4087	4.77%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	1 / 15	6.67%	Expected: ≤1 per chain, or ≤5%
	Twisted Peptides:	4 / 368	1.09%	Goal: 0

In the two column results, the left column gives the raw count, right column gives the percentage.

* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.

^ MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.

D) Ramachandran plot from MolProbity



86.1% (316/367) of all residues were in favored (98%) regions.
99.5% (365/367) of all residues were in allowed (>99.8%) regions.

There were 2 outliers (phi, psi):
88 ASN (93.1, 30.8)
294 GLY (-23.7, 154.2)