

## Supporting Information

### Computational prediction and experimental validation of a bridged cation intermediate in akantomycin biosynthesis

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# Experimental Procedures

## 1. Strains and culture conditions

*A. nidulans* FGSC A1145 was obtained from the Fungal Genetics Stock Center (<http://www.fgsc.net/>). *A. nidulans* was maintained on Czapek-Dox (CD) agar for sporulation or in liquid CD–ST medium for gene overexpression, compound production and RNA extraction (<http://www.fgsc.net/>).

## 2. General DNA manipulation techniques

*Escherichia coli* TOP10 was used for cloning, following standard recombinant DNA techniques. DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs, NEB). PCR was performed using Phusion High-Fidelity DNA Polymerase (NEB). The gene-specific primers are listed in the Supplementary Information. PCR products were confirmed by DNA sequencing. *E. coli* BL21(DE3) (Novagen) was used as the *E. coli* host for protein expression. GeneArt Seamless Cloning and Assembly kit (Thermo Fisher Scientific) was used for the construction of pET28b-derived protein expression vectors. *In vivo* homologous recombination using *Saccharomyces cerevisiae* was used for the construction of the *A. nidulans* overexpression plasmids.

For preparation of overexpression plasmids for *A. nidulans*, full length *sdxC* with the terminator (~500 bp) was amplified by PCR with three sets of primers of pMO90008-f1/r1, pMO90008-f2/r2, pMO90008-f3/r3 using the genomic DNA of *Sarocladium oryzae* as the template. The three overlapping DNA fragments and NotI/SwaI-digested pYTU expression vector were transformed into yeast to generate pMO90008 by yeast homologous recombination. The *sdxD* gene with the terminator sequence was amplified with a primer pair of pMO90002-f1/r1, and ligated into expression vector pYTR (pdxD) in the sites of BamHI/SwaI to create plasmids pMO90002(pdxD). The *pogpdA* (from *Penicillium oxalicum*) promoter and *sdxA* gene with the terminal sequence were amplified with the primer pairs of pMO90009-f1/r1 and pMO90009-f2/r2, respectively. The two overlapping DNA fragments and NotI-digested pMO90002 expression vector were transformed into yeast to generate pMO90009 by yeast homologous recombination.

### 3. Heterologous expression of the *sdx* gene cluster in *A. nidulans*

*A. nidulans* A1145 was initially grown on oatmeal agar plates containing 10 mM uracil, 0.5 µg/mL pyridoxine HCl and 2.5 µg/mL riboflavin at 30 °C for 5 days. Fresh spores of *A. nidulans* were inoculated into 45 mL liquid CD media (1 L: 10 g Glucose, 50 mL 20 x Nitrate salts, 1 mL Trace elements, pH 6.5) in 125-mL Erlenmeyer flask and germinated at 30 °C and 250 rpm for approximately 16 h. For the preparation of 20 x Nitrate salts, 120 g NaNO<sub>3</sub>, 10.4 g KCl, 10.4 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 30.4 g KH<sub>2</sub>PO<sub>4</sub> were dissolved in 1 L double distilled water. The 100 mL trace elements with pH 6.5 contains 2.20 g ZnSO<sub>4</sub> · 7H<sub>2</sub>O, 1.10 g H<sub>3</sub>BO<sub>3</sub>, 0.50 g, MnCl<sub>2</sub> · 4H<sub>2</sub>O, 0.16 g FeSO<sub>4</sub> · 7H<sub>2</sub>O, 0.16 g CoCl<sub>2</sub> · 5H<sub>2</sub>O, 0.16 g CuSO<sub>4</sub>·5H<sub>2</sub>O, and 0.11 g (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> · 4H<sub>2</sub>O. Mycelia were harvested by centrifugation at 4000 g for 10 min, and washed with 10 mL Osmotic buffer (1.2 M MgSO<sub>4</sub>, 10 mM sodium phosphate, pH 5.8). Then the mycelia were transferred into 10 mL of Osmotic buffer containing 100 mg lysing enzymes from Trichoderma and 60 mg Yatalase in a 125-mL flask. The flask was kept in shaker at 80 rpm for 4 h at 30 °C. Cells were collected in a 30 mL Corex tube and overlaid gently by 10 mL of Trapping buffer (0.6 M sorbitol, 0.1 M Tris-HCl, pH 7.0). After centrifugation at 3,500 rpm for 15 min at 4 °C, protoplasts were collected in the interface of the two buffers. The protoplasts were then transferred to a sterile 15-mL falcon tube and washed by 10 mL STC buffer (1.2 M sorbitol, 10 mM CaCl<sub>2</sub>, 10 mM Tris-HCl, pH 7.5). The protoplasts were resuspended in 1 mL STC buffer for transformation. Then, the plasmids were added to 60 µL protoplast suspension and the mixture incubated 60 min on ice. Then 600 µL of PEG solution at pH 7.5 (60% PEG, 50 mM calcium chloride and 50 mM Tris-HCl) was added to the protoplast mixture, and the mixture was incubated at room temperature for additional 20 min. The mixture was spread on the regeneration dropout solid medium (CD solid medium with 1.2M sorbitol and appropriate supplements) and incubated at 30 °C for 2 to 3 days. The transformants were grown in liquid CD-ST (1 L: 20 g starch, 20 g casamino acids, 50 mL 20 x nitrate salts, 1 mL trace elements) medium for 3 to 4 days before extraction.

[Isolation of compound **12**]: The *Aspergillus nidulans* ΔEM expression host co-transformed with plasmids pMO90008, pMO90009, and pYTP was selected on CD sorbitol agar. The harvested spores were used to inoculate CDST broth. The cultures were in 4 baffled 2L Erlenmeyer flasks containing 1 L CD-ST media at 28 °C with shaking at 250 rpm for 96 h. The broth was separated from mycelial mass with miracloth and extracted twice with equal vols. of ethyl acetate, and solvent removed with vacuum. The mycelial mass was extracted with acetone, and after removal of solvent, the residual mass was combined with the broth extract, and separated by silica flash chromatography on a CombiFlash instrument with hexanes / acetone solvent system. Fractions were screened by LCMS and the fractions containing **12** were pooled and further purification was performed by several runs over a semi-preparative reverse phase HPLC column with gradient elution with water/acetonitrile as mobile phase. **12** was collected and analyzed by HRMS, and 1D and 2D NMR experiments.

#### 4. Expression and purification of SDR enzymes AdxG, PdxG, and EpiG

Activity assays for AdxG, PdxG, and EpiG towards **12** in HEPES buffer (100 mM HEPES, pH 8.0) were performed at 50- $\mu$ l scale with 2 mM NADPH, 30  $\mu$ M AdxG, PdxG, or EpiG, at 30 °C for 96 hours. Then the reactions were quenched with 100  $\mu$ l cold acetonitrile. Protein was precipitated and removed by centrifugation, and the supernatant was analyzed by LC-MS.

## 5. Chemical reactions with NaBH<sub>4</sub>

NaBH<sub>4</sub> (5.21 mg, 137 mmol) was added to the solution of compound **12** (4.01 mg, 14.5 μmol) in 400 μl EtOH at room temperature and the mixture was stirred at 30 °C for 24 hours. The reaction mixture was extracted with 0.5 ml ethyl acetate twice. The extract was concentrated, and dissolved in a 1000 μl mixture of DMSO : H<sub>2</sub>O (2:98) and allowed to react for 72 hours. The reaction mixture was then subjected to HPLC, dried and characterized by NMR, HR-MS, and optical rotation.

**1a**: <sup>1</sup>H NMR (500 MHz, CH<sub>3</sub>OD): δ 7.15 (d, *J* = 7.0 Hz, 1H), 6.07 (d, *J* = 7.0 Hz, 1H), 2.78 (dd, *J* = 11.0, 5.5 Hz 1H), 2.62 (m, 1H), 2.04 (m, 1H), 1.71 (m, 2H), 1.60-1.57 (m, 2H), 1.09 (s, 3H), 0.95 (m, 1H), 0.92 (overlap, 3H), 0.90 (overlap, 3H), 0.64 (d, *J* = 6.5 Hz, 3H).

**1b**: <sup>1</sup>H NMR (500 MHz, CH<sub>3</sub>OD): δ 7.12 (d, *J* = 7.0 Hz, 1H), 5.99 (d, *J* = 7.0 Hz, 1H), 2.94 (dd, *J* = 11.0, 5.5 Hz 1H), 2.48 (m, 1H), 2.04 (m, 1H), 1.71 (m, 2H), 1.64-1.61 (m, 2H), 1.15 (s, 3H), 1.00 (m, 1H), 0.92 (overlap, 3H), 0.90 (overlap, 3H), 0.64 (d, *J* = 6.5 Hz, 3H).

HRMS (ESI, M<sup>+</sup>H<sup>+</sup>) *m/z* calculated for C<sub>16</sub>H<sub>26</sub>NO<sub>3</sub>, 280.1913; found 280.1917.

[α]<sub>D</sub><sup>22</sup> +18 (c 0.1, MeOH).

The optical rotation value of deoxykanthomycin was reported to be: [α]<sub>D</sub><sup>20</sup> +0.8 (c 0.1, MeOH).

## 6. Computational Methods

Conformational searches were conducted using XTB<sup>1</sup> and CREST 2.7.1<sup>2</sup> on all calculated ground states and transition states. Ground state structures were built and optimized using XTB using a `-o loose` criteria then reoptimized using `-o tight`. The XTB optimized structures were then used as an input for a CREST conformational search using the default keywords with `-metac`

Transition states were located at the  $\omega$ B97X-D/def2-svp<sup>3,4</sup> level of theory and then subjected to constrained conformational searching by freezing the forming bond distances using the following keywords:

```
-metac -rthr 0.5
```

Bonds were frozen using the `.constraints` file with a force constant of `1.0`.

The outputs of conformational searches were optimized using density functional theory paired with a Pople basis set and the CPCM polarizable conductor calculation model<sup>5,6</sup> with H<sub>2</sub>O,  $\omega$ B97X-D-CPCM(H<sub>2</sub>O)/6-311+G(d,p),<sup>3,7,8</sup> as implemented in Gaussian 16, revision A.03 sse4.<sup>9</sup> This functional was chosen for its ability to calculate geometries of asynchronous Diels–Alder reactions.<sup>10</sup> Structures were characterized as minima or transition states by frequency calculations. Single point energy calculations were then conducted with the recommended basis set for the  $\omega$ B97X-D functional<sup>11</sup> at the  $\omega$ B97X-D-CPCM(H<sub>2</sub>O)/def2-QZVPP level of theory.

Energetics were calculated using GoodVibes 3.0.0 and 3.0.1<sup>12</sup> with quasi-harmonic enthalpy<sup>13</sup> and entropy corrections<sup>14</sup> with the following keywords:

```
-c 1 -q --spc SP
```



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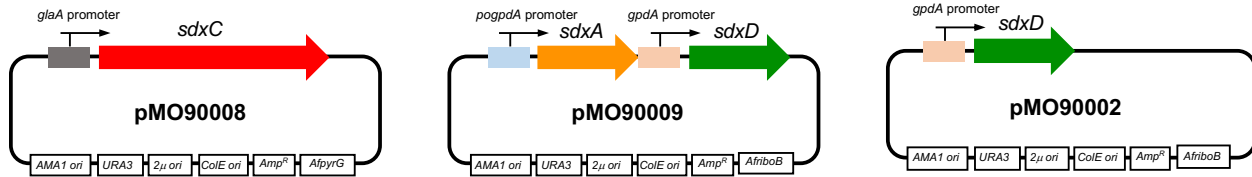
## Supplementary Tables

**Table S1.** Bioinformatic analysis of *sdx* gene cluster

<b>Protein</b>	<b>Size (aa)</b>	<b>Proposed Function</b>	<b>Homologue (Identity/Positive)</b>	<b>Strains</b>
SdxG	275	short-chain dehydrogenase/reductase	Q00278.1 (38%/58%)	<i>Aspergillus parasiticus</i> SU-1
SdxI	460	O-methyltransferase	S0E608.1 (28%/47%)	<i>Fusarium fujikuroi</i> IMI 58289
Orf3	515	MFS transporter	F5HN69.1 (45%/68%)	<i>Aspergillus oryzae</i>
SdxF	497	cytochrome P450	A0A3S9NM20.1 (39%/57%)	<i>Talaromyces wortmannii</i>
Orf5	725	transcription factor	B8NJG9.1 (31%/50%)	<i>Aspergillus flavus</i> NRRL3357
Orf6	132	cytochrome B5	Q9P5L0.2 (45%/62%)	<i>Neurospora crassa</i> OR74A
SdxC	4114	polyketide synthase nonribosomal peptide synthetase	B8NJG3.1 (44%/61%)	<i>Aspergillus flavus</i> NRRL3357
SdxD	413	enoylreductase	A0A482N9T9.1 (41%/55%)	<i>Talaromyces variabilis</i>
SdxE	801	transcriptional factor	B8NJG9.1 (30%/46%)	<i>Aspergillus flavus</i> NRRL3357
SdxB	528	cytochrome P450	B8NJG8.1 (36%/59%)	<i>Aspergillus flavus</i> NRRL3357
SdxA	533	cytochrome P450	Q5ATG9.1 (57%/73%)	<i>Aspergillus nidulans</i> FGSC A4

**Table S2.** Primers and plasmids used in this study

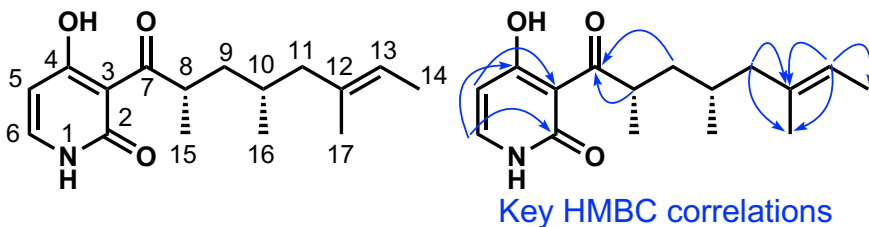
Primers	Sequences (5' → 3')
pMO90002-f1	TAACCATTACCCCGCCACATAGACACATCTAAACAATGCCCCATCAGACGGAGACTTTG
pMO90002-r1	AGGGTATCATCGAAAGGGAGTCATCCAATTTAAATTGTGCGGTAGCGGTGGTCATAG
pMO90008-f1	TAACCTCGCGGGTGTTCCTTGACGATGGCATCCTGCACTCCGGTGAATTGATTTGGGTG
pMO90008-r1	CAAGCAGATCCGATGATGCCTACTGGCTTCTGCATTGTTAGATGTGTCTATGTGGCGG
pMO90009-f1	CGCGGGTGTTCCTTGACGATGGCATCCTGCGGCCGCTTTGCTCCAGGAATACATGTGAGC
pMO90009-r1	TTCAGCTTCTTGAACACCATTTTTGCGATTGTTTGAAGTGTCTG
pMO90009-f2	CACTTCAAACAATCGAAAAATGGTGTTC AAGAAGCTGAATCTCAAC
pMO90009-r2	GGTCTCTCCCGTCACCCAAATCAATTCACCGGAGTCTACGACGGCTCGACTTCTGAC



**Table S3.** Spectroscopic data of compound **12**

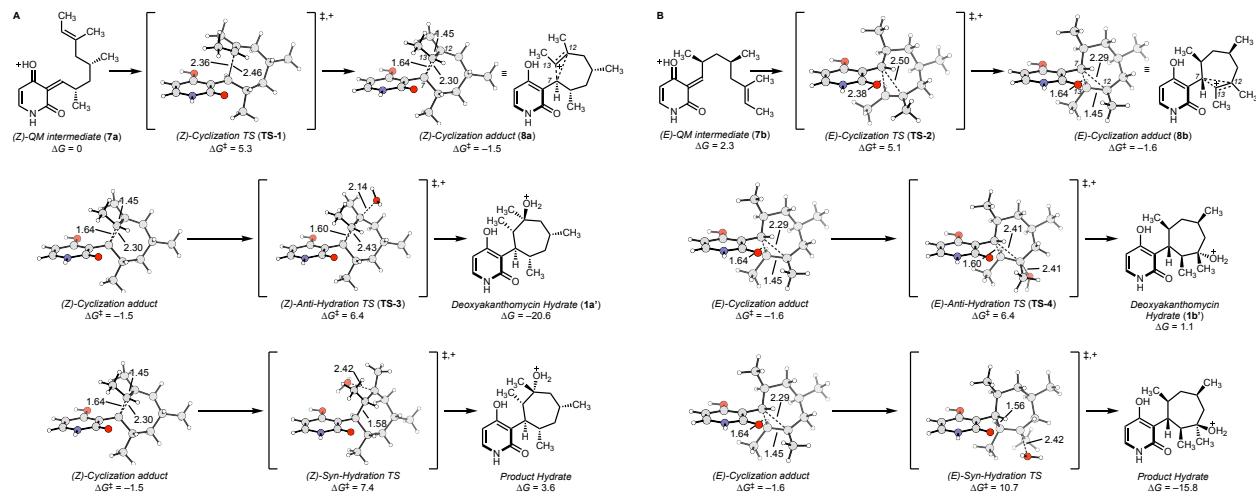
HRMS (ESI,  $M^+H^+$ )  $m/z$  calculated for  $C_{16}H_{24}NO_3$ , 278.1756; found 278.1752.

The absolute stereochemistry is estimated based on that of deoxyakanthomycin (**1a/1b**)

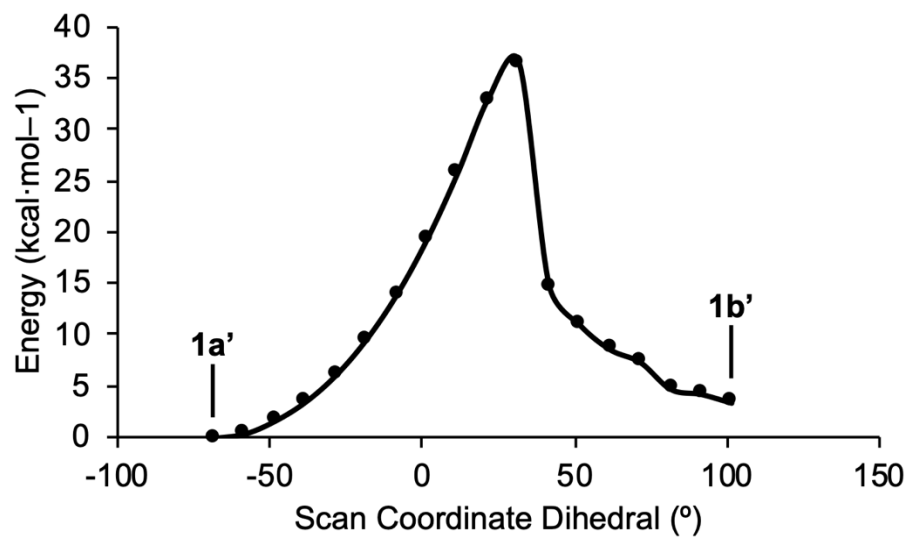


position	$\delta_H$ , mult ( $J$ in Hz)	$\delta_C$	COSY
1			
2		164.4	
3		106.8	
4		178.6	
5	6.01, d (7.5)	101.7	H6
6	7.32, d (7.5)	140.0	H5
7		212.7	
8	4.35, m	40.6	H9, H14
9	1.81, m 1.10, m	40.8	H8, H10
10	1.66, overlap	28.6	H9
11	2.15, m 1.66, overlap	47.5	H12
12		134.6	H9, H11
13	5.17, m	120.0	H8
14	1.57, overlap	13.4	H13
15	1.15, d (7.0)	17.4	H8
16	0.79, d (6.5)	20.1	H10
17	1.58, overlap	15.4	

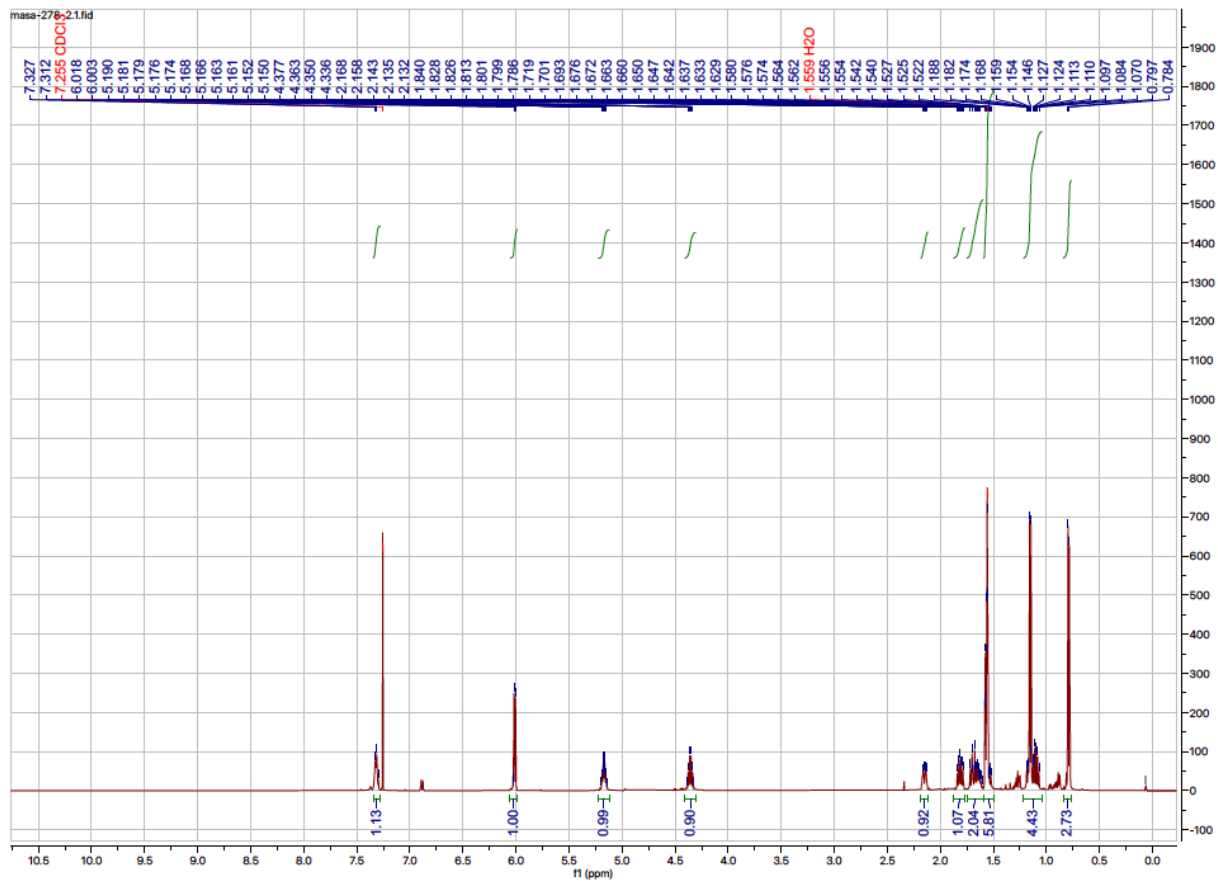
## Supplementary Figures



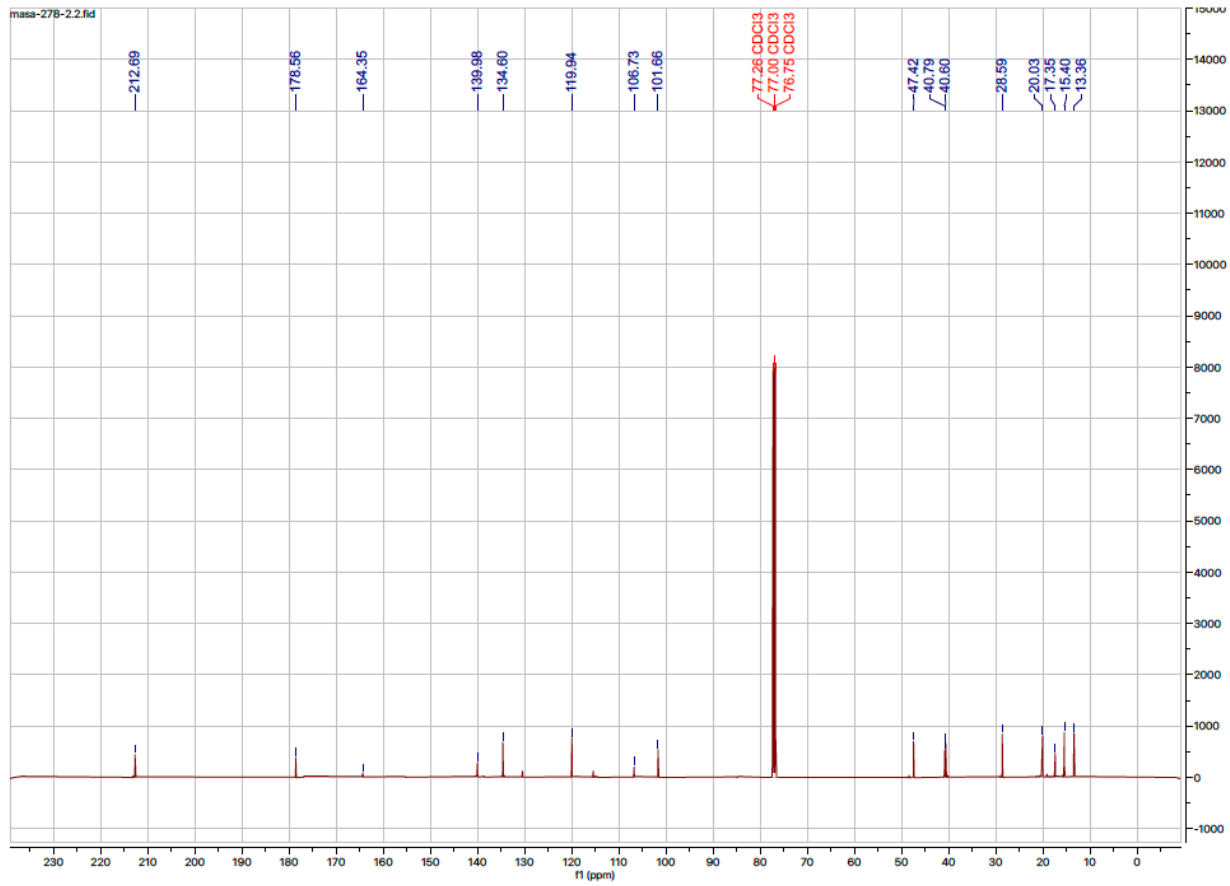
**Figure S1.** Calculated pathway for proposed cationic cyclization from (Z)-QM (a) and (E)-QM (b).



**Figure S2.** Barrier of interconversion between atropisomers **1a'** and **1b'** calculated at the  $\omega$ B97X-D-CPCM(H<sub>2</sub>O)/6-311+G(d,p) level of theory.

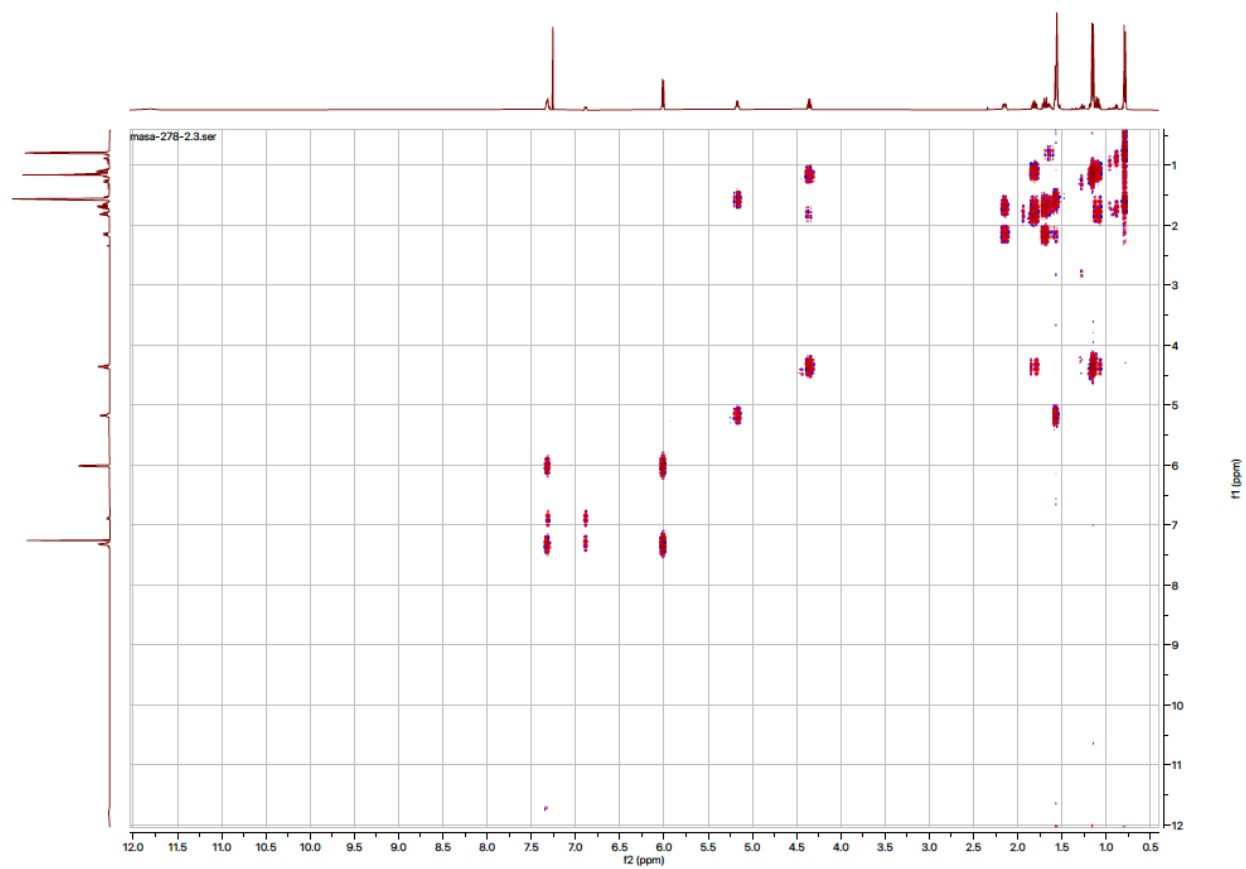


**Figure S3.**  $^1\text{H}$  NMR spectrum of compound **12** in  $\text{CDCl}_3$ .

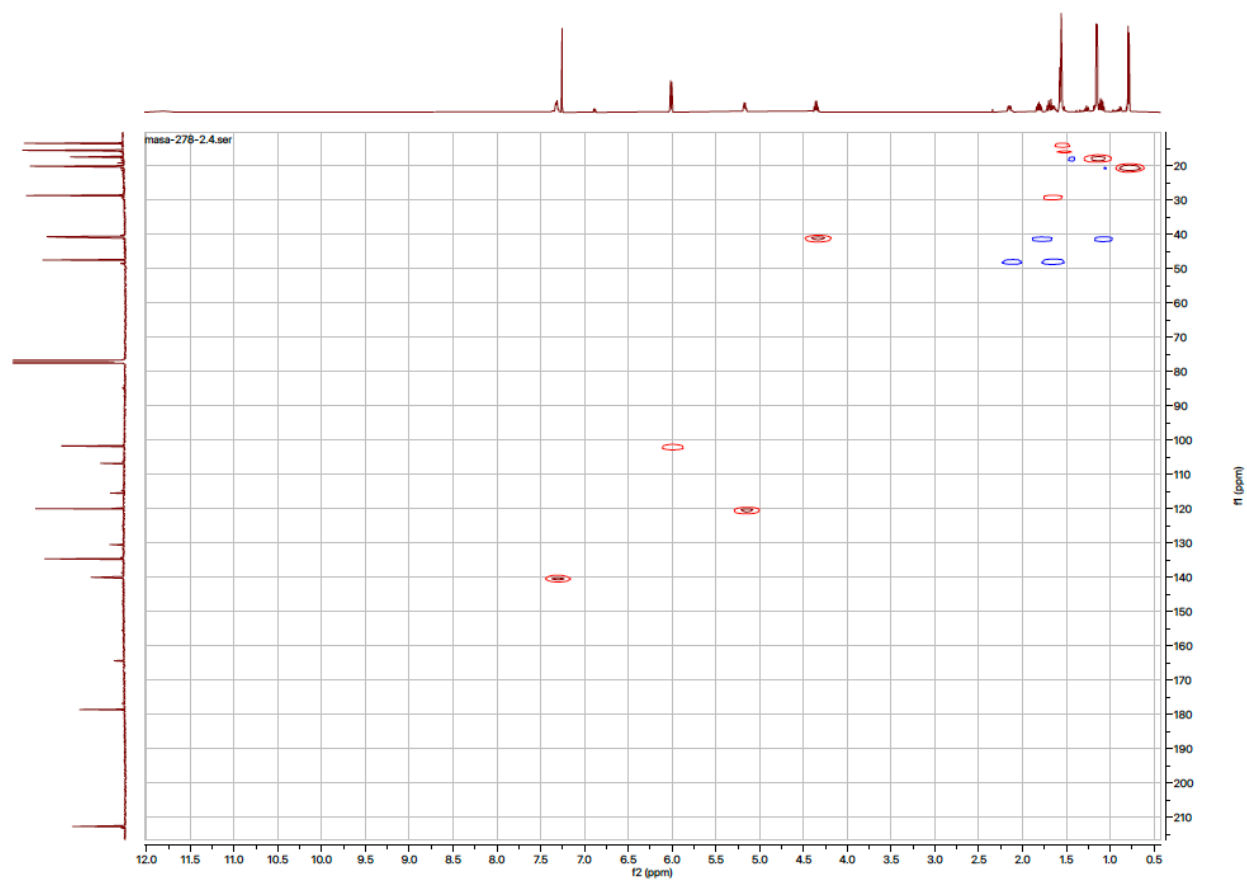


**Figure S4.**  $^{13}\text{C}$  NMR spectrum of compound **12** in  $\text{CDCl}_3$ .

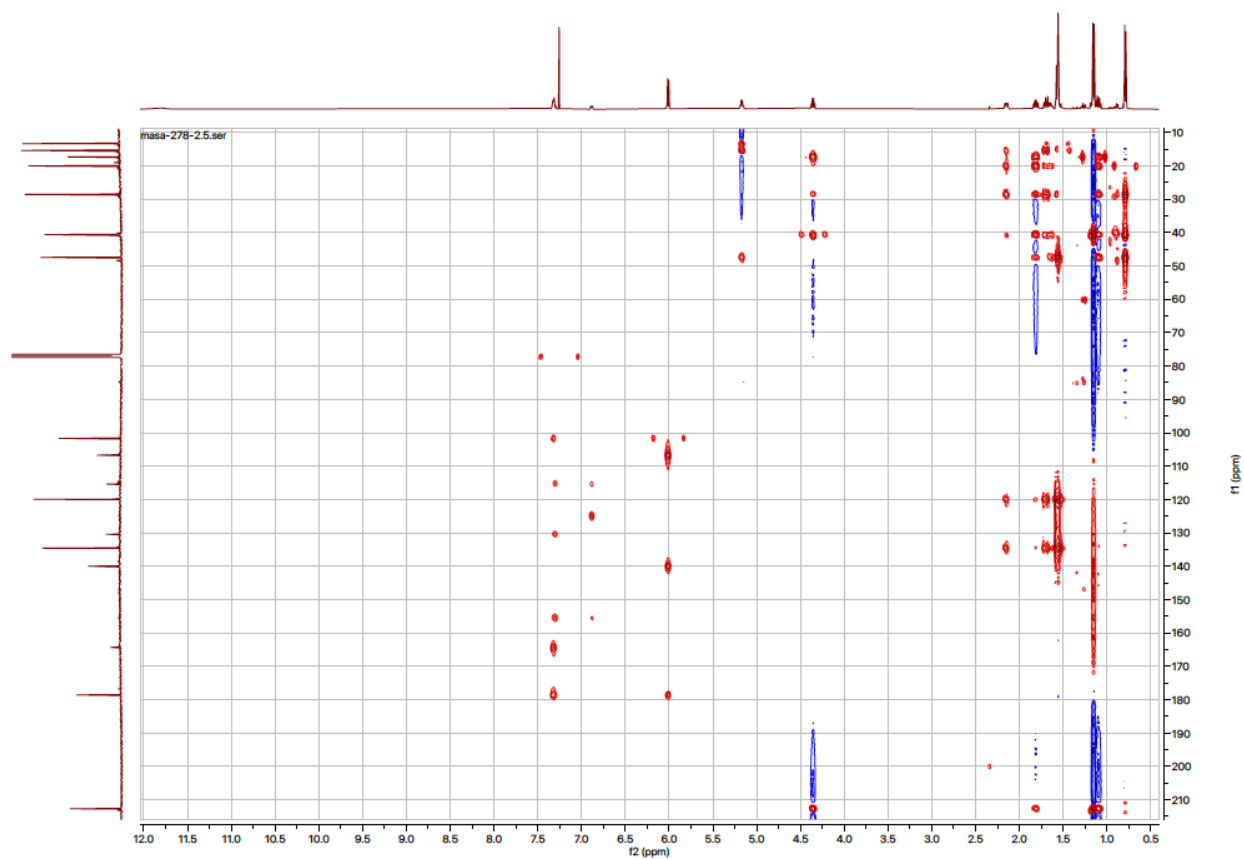




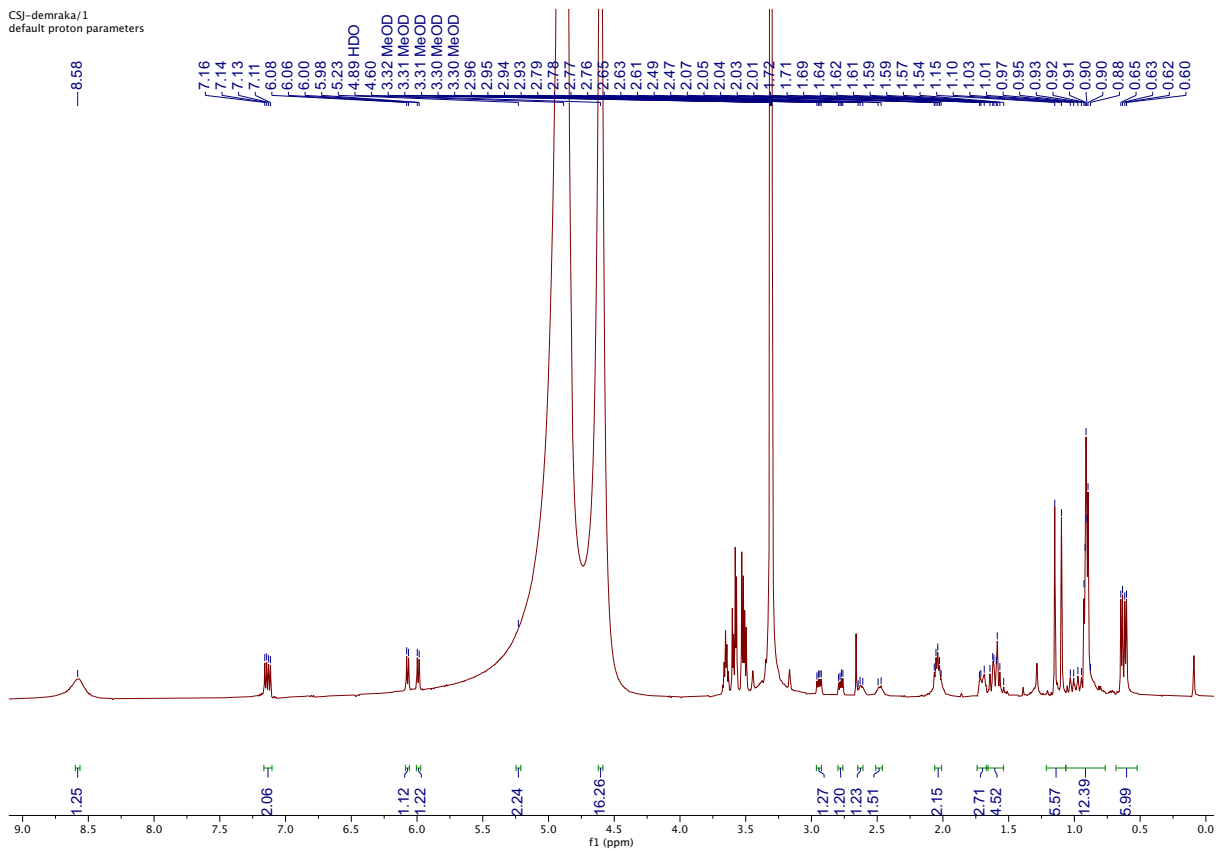
**Figure S5.**  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of compound **12** in  $\text{CDCl}_3$ .



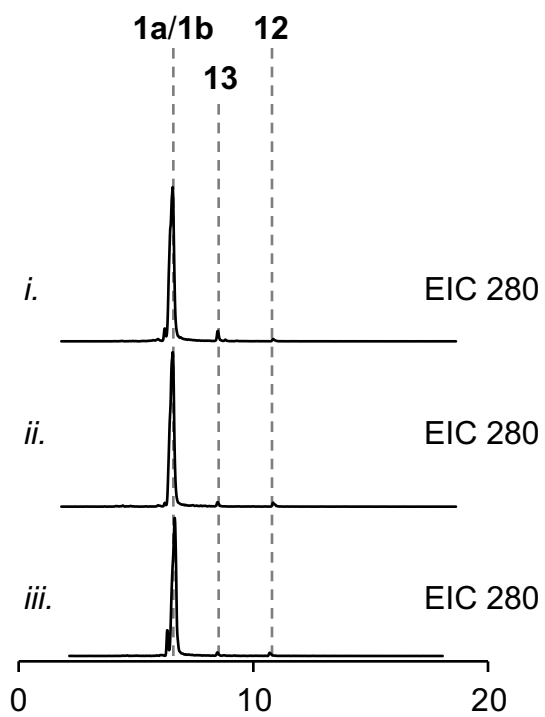
**Figure S6.**  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of compound **12** in  $\text{CDCl}_3$ .



**Figure S7.**  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of compound **12** in  $\text{CDCl}_3$ .



**Figure S8.**  $^1\text{H}$  NMR spectrum of compound **1a/1b** in  $\text{CD}_3\text{OD}$ .



**Figure S9.** *In vitro* reactions of **12** with various SDR enzymes after 24 h: *i.* PdxG, *ii.* AdxG, *iii.* EpiG.

## Supplementary Data

Cartesian coordinates and energies of all calculated structures

### (Z)-Cyclization Adduct **8a**

E -829.163127

#### Cartesian coordinates

C	0.142542	-0.110437	-0.174697
C	-1.358234	-0.110527	-0.119369
C	-2.096159	0.639537	-1.009224
C	-3.516334	0.618831	-1.006158
C	-4.138596	-0.151776	-0.081563
N	-3.421890	-0.870850	0.812916
C	-2.034510	-0.892729	0.881814
O	-1.482953	-1.552301	1.767127
H	-3.902434	-1.425355	1.508160
H	-5.214463	-0.227828	-0.006200
H	-4.089864	1.200948	-1.714418
O	-1.431736	1.411666	-1.888240
H	-2.039865	1.865735	-2.478076
H	0.397450	0.392611	-1.114465
C	0.813031	-1.501802	-0.234928
C	2.291473	-1.432620	-0.646298
H	2.670507	-2.459055	-0.644236
C	3.233152	-0.583821	0.214452
C	4.692398	-0.907172	-0.110988
H	4.906185	-1.957497	0.097926
H	5.376087	-0.295299	0.483269
H	4.903786	-0.722810	-1.169286
C	3.002613	0.909825	-0.011814
C	1.706226	1.502106	0.328046
C	0.723595	0.764617	1.089262
C	-0.292800	1.596732	1.857029
H	0.229238	2.225120	2.582578
H	-0.964428	0.940073	2.407134
H	-0.890774	2.238561	1.209294
H	1.193350	0.032231	1.744181
C	1.415663	2.864237	-0.143543
H	1.279585	3.509144	0.732827
H	2.194151	3.263810	-0.788589
H	0.451330	2.868542	-0.664226
H	3.275985	1.208281	-1.031820
H	3.682209	1.513461	0.622353
H	3.067424	-0.819513	1.271302
H	2.359235	-1.084932	-1.686940
C	0.075136	-2.402461	-1.229075

H	0.602250	-3.354535	-1.329748
H	-0.945462	-2.616807	-0.908957
H	0.030976	-1.936414	-2.219157
H	0.743092	-1.952690	0.757926

(E)-Syn Product Hydrate

E -905.651720

Cartesian coordinates

C	-0.015514	0.363691	-0.049177
C	1.483071	0.165856	-0.091166
C	2.414014	0.808529	0.728424
C	3.799633	0.613832	0.557114
C	4.224803	-0.267948	-0.385505
N	3.321509	-0.958606	-1.112605
C	1.989508	-0.788232	-0.973653
O	1.272970	-1.588107	-1.733208
H	3.632892	-1.658416	-1.775223
H	5.265398	-0.474870	-0.586517
H	4.518274	1.146107	1.165115
O	1.942705	1.610348	1.679296
H	2.654473	2.023905	2.178422
H	-0.336026	0.063425	-1.049855
C	-0.524980	1.819551	0.066133
H	-0.488394	2.116531	1.118321
C	-1.980839	1.925700	-0.436643
C	-3.039508	0.882045	-0.036223
C	-4.402052	1.345918	-0.559716
H	-4.400677	1.387804	-1.654572
H	-5.194926	0.659327	-0.250933
H	-4.650111	2.342922	-0.185792
H	-3.112942	0.831504	1.056423
C	-2.730178	-0.518943	-0.596842
C	-1.793002	-1.405970	0.245070
C	-2.612816	-2.273814	1.193081
H	-1.993357	-2.976332	1.754860
H	-3.140301	-1.640443	1.910880
H	-3.355185	-2.843899	0.630260
C	-0.726290	-0.571941	0.984553
H	-1.259653	0.072786	1.686809
C	0.217657	-1.428010	1.831992
H	0.959131	-0.804143	2.333167
H	-0.334695	-1.949758	2.614440
H	0.753771	-2.177368	1.242257
O	-1.106937	-2.267388	-0.712402
H	0.355916	-1.749812	-1.380927
H	-0.951679	-3.126523	-0.310495
H	-2.306475	-0.407924	-1.600074
H	-3.665078	-1.069125	-0.734796
H	-2.347465	2.914220	-0.139878
H	-1.950361	1.933839	-1.535895



C	0.310811	2.806543	-0.753557
H	1.318521	2.936759	-0.358238
H	-0.168730	3.789076	-0.759118
H	0.391294	2.470983	-1.793199

(Z)-Anti Deoxykanthomycin Hydrate (1a')

E -905.656948

Cartesian coordinates

C	-0.171488	0.477540	0.408218
C	-1.613313	0.108546	0.101587
C	-2.670687	0.694744	0.805809
C	-4.017928	0.391034	0.517962
C	-4.285004	-0.495864	-0.474090
N	-3.267190	-1.071111	-1.147234
C	-1.964357	-0.807573	-0.893292
O	-1.154258	-1.478902	-1.668820
H	-3.462269	-1.742370	-1.879894
H	-5.282727	-0.784753	-0.770097
H	-4.827260	0.854957	1.065190
O	-2.361870	1.570929	1.760628
H	-3.151216	1.927373	2.179649
H	-0.241796	1.206322	1.216541
C	0.477627	1.284179	-0.745889
C	-0.393567	2.502180	-1.074141
H	0.050705	3.071020	-1.894483
H	-0.473311	3.167046	-0.207282
H	-1.404659	2.220602	-1.377990
H	0.550300	0.671300	-1.647898
C	1.893524	1.756284	-0.377018
H	2.098506	2.665718	-0.951628
H	1.921759	2.058743	0.679347
C	3.030243	0.774590	-0.666930
C	4.379972	1.489682	-0.568711
H	4.433596	2.325565	-1.271599
H	4.534313	1.888331	0.440117
H	5.206811	0.808567	-0.789419
C	3.051017	-0.454391	0.241298
C	1.798271	-1.337348	0.278960
C	2.168092	-2.699418	0.872057
H	2.882124	-3.206974	0.216940
H	1.284716	-3.332896	0.980941
H	2.647004	-2.590618	1.846281
C	0.584364	-0.750531	1.038126
C	0.910268	-0.427804	2.501056
H	1.378715	-1.266862	3.017817
H	1.572244	0.438474	2.579621
H	-0.012675	-0.186813	3.033381
H	-0.133308	-1.577984	1.061017
O	1.318903	-1.561700	-1.068785
H	-0.186016	-1.463180	-1.405412

H	1.922799	-2.124226	-1.560226
H	3.281286	-0.147043	1.267576
H	3.889053	-1.088266	-0.072116
H	2.907244	0.428012	-1.699922

(E)-Anti Deoxykanthomycin Hydrate (**1b'**)

E -905.624452

Cartesian coordinates

C	-0.188254	0.469050	0.387700
C	-1.632158	0.127180	0.080893
C	-2.022386	-0.740808	-0.901672
C	-3.378178	-1.057908	-1.170389
C	-4.322788	-0.459886	-0.406757
N	-3.963655	0.402250	0.573573
C	-2.658300	0.753096	0.888610
O	-2.454793	1.552167	1.805760
H	-4.679475	0.841694	1.135944
H	-5.382280	-0.632949	-0.532402
H	-3.655453	-1.749902	-1.953517
O	-1.054595	-1.334242	-1.667236
H	-1.431952	-1.936005	-2.317288
H	-0.252421	1.164225	1.227639
C	0.483005	1.309135	-0.728234
C	-0.396758	2.523278	-1.047203
H	0.060223	3.120802	-1.839752
H	-0.508747	3.160430	-0.163711
H	-1.394299	2.230600	-1.381833
H	0.580330	0.729979	-1.651364
C	1.880646	1.793811	-0.310547
H	2.093473	2.713686	-0.864569
H	1.870877	2.078534	0.750917
C	3.040491	0.834601	-0.578271
C	4.377161	1.559817	-0.404541
H	4.446426	2.413094	-1.083895
H	4.484411	1.934300	0.619115
H	5.220406	0.894589	-0.609578
C	3.047669	-0.414075	0.303976
C	1.825697	-1.322027	0.299157
C	2.189510	-2.708915	0.808522
H	2.951982	-3.164520	0.171509
H	1.317061	-3.362970	0.853713
H	2.622090	-2.628177	1.805622
C	0.536359	-0.804796	0.963593
C	0.752740	-0.605136	2.470409
H	1.151336	-1.493803	2.961155
H	1.423040	0.235305	2.666500
H	-0.209298	-0.375704	2.932101
H	-0.166097	-1.638868	0.868660
O	1.456131	-1.522160	-1.182619
H	0.464323	-1.514385	-1.363203

H	1.867923	-2.302513	-1.579353
H	3.208002	-0.124298	1.348351
H	3.914198	-1.023358	0.028077
H	2.968823	0.509840	-1.622574

(E)-Cyclization Adduct (8b)

E -829.163981

Cartesian coordinates

C	0.150676	-0.139606	-0.149825
C	-1.351123	-0.132117	-0.119413
C	-2.105562	-0.909502	0.730357
C	-3.525240	-0.902345	0.681117
C	-4.128670	-0.079439	-0.209964
N	-3.394284	0.718777	-1.020457
C	-2.009956	0.780894	-1.016178
O	-1.434935	1.597058	-1.747351
H	-3.861484	1.349441	-1.657397
H	-5.202450	-0.011362	-0.315179
H	-4.112913	-1.532438	1.334734
O	-1.459890	-1.680688	1.619316
H	-2.080194	-2.180577	2.157572
H	0.390536	0.387472	-1.082488
C	0.847731	-1.515518	-0.238279
C	2.318095	-1.398380	-0.670852
H	2.727676	-2.412845	-0.685357
C	3.249168	-0.529965	0.181986
C	4.710732	-0.803563	-0.177488
H	5.387240	-0.177578	0.410271
H	4.894061	-0.600095	-1.237494
H	4.961488	-1.849079	0.013846
C	2.969050	0.959577	-0.017522
C	1.660327	1.507154	0.352957
C	0.721594	0.723916	1.125088
C	-0.310123	1.498627	1.929933
H	-0.933497	2.142794	1.308893
H	0.201276	2.118013	2.670643
H	-0.957961	0.805458	2.464623
H	1.233357	-0.001074	1.756214
C	1.306895	2.854852	-0.112289
H	2.091574	3.312625	-0.709842
H	0.382664	2.773299	-0.702865
H	1.057753	3.483490	0.748623
H	3.216940	1.282335	-1.036489
H	3.639658	1.572058	0.617210
H	3.114012	-0.785006	1.238735
H	2.357293	-1.038327	-1.708621
C	0.122586	-2.422095	-1.236391
H	0.679011	-3.354218	-1.362419
H	-0.885348	-2.675280	-0.905936
H	0.048562	-1.939709	-2.216682

H            0.812269    -1.986730    0.747457

(Z)-Cyclization TS **TS-1**

E -829.149689  
Imag. Freq. -183.808

Cartesian coordinates

C	-0.115811	0.336160	-0.494278
C	1.286198	0.261390	-0.288270
C	2.052856	-0.627161	-1.070075
C	3.447649	-0.721489	-0.916385
C	4.043653	0.058324	0.028647
N	3.332146	0.898177	0.800075
C	1.948599	1.063434	0.729361
O	1.400098	1.832924	1.505064
H	3.802699	1.454939	1.501602
H	5.110874	0.037282	0.203671
H	4.038208	-1.391055	-1.525336
O	1.410906	-1.364293	-1.964463
H	2.012551	-1.936012	-2.452529
H	-0.456695	-0.264193	-1.333569
C	-0.962033	1.553371	-0.237240
C	-2.446815	1.352162	-0.559966
H	-2.926057	2.328342	-0.437388
C	-3.231483	0.329353	0.267347
C	-4.726545	0.469276	-0.028808
H	-5.312980	-0.234868	0.567313
H	-4.932308	0.269848	-1.085913
H	-5.076176	1.479669	0.197453
C	-2.794867	-1.106076	-0.030974
C	-1.421502	-1.544957	0.401086
C	-0.737309	-0.927623	1.403082
C	0.492605	-1.457552	2.067427
H	1.204681	-1.896876	1.366529
H	0.208141	-2.245555	2.772691
H	0.996345	-0.674453	2.633693
H	-1.203626	-0.082179	1.895104
C	-0.947437	-2.814435	-0.238311
H	-1.051968	-2.763372	-1.324384
H	0.082599	-3.066554	0.004722
H	-1.589648	-3.632342	0.106600
H	-2.896423	-1.286777	-1.108704
H	-3.491678	-1.810278	0.443040
H	-3.087796	0.545689	1.331849
H	-2.548615	1.093685	-1.623278
C	-0.396134	2.662725	-1.152456
H	-1.013336	3.556969	-1.041257
H	0.630403	2.917922	-0.888650



H	-0.424482	2.358589	-2.202613
H	-0.837520	1.883872	0.792909

(E)-Syn Hydration TS

E -829.140254  
Imag. Freq. -137.241

Cartesian coordinates

C	0.134124	-0.268285	-0.467703
C	-1.286682	-0.254824	-0.211336
C	-1.932452	-0.675038	0.954126
C	-3.341009	-0.659669	1.054840
C	-4.064797	-0.207630	-0.000337
N	-3.461328	0.224170	-1.128048
C	-2.088813	0.256901	-1.317155
O	-1.627019	0.698801	-2.364653
H	-4.020268	0.569941	-1.896445
H	-5.145286	-0.165491	0.008384
H	-3.821281	-0.997663	1.960666
O	-1.307172	-1.109937	2.046900
C	1.050787	-1.387177	0.025504
H	1.163826	-1.402039	1.115541
C	0.385605	-2.711856	-0.404749
H	-0.585992	-2.866996	0.064066
H	1.038793	-3.538918	-0.121513
H	0.251373	-2.736952	-1.489258
C	2.462975	-1.322183	-0.573531
C	3.384230	-0.259387	0.016181
C	4.760708	-0.310489	-0.643610
H	5.210941	-1.299151	-0.524549
H	4.685272	-0.099912	-1.715336
H	5.435217	0.426346	-0.200044
H	3.517100	-0.473499	1.082476
C	2.778325	1.135766	-0.143087
C	1.452158	1.351026	0.530491
C	0.428556	1.928945	-0.175354
C	-0.781911	2.565683	0.412040
H	-0.508949	3.597062	0.664112
H	-1.135517	2.089141	1.325446
H	-1.595408	2.616315	-0.310940
H	0.610729	2.146762	-1.223406
C	1.400756	1.197179	2.018409
H	1.864487	2.088003	2.456258
H	1.979260	0.340998	2.365737
H	0.387793	1.134515	2.413684
H	2.694649	1.375003	-1.207868
H	3.459545	1.876396	0.294207
H	2.927473	-2.300909	-0.425667
H	2.380168	-1.178216	-1.658787

H	0.333587	-0.039911	-1.510844
H	-1.880042	-1.714691	2.524073

(Z)-Syn Hydration TS

E -829.146740  
Imag. Freq. -174.540

Cartesian coordinates

C	0.096359	-0.316180	-0.510325
C	-1.310599	-0.266822	-0.207573
C	-2.209914	0.069995	-1.226736
C	-3.605108	0.058670	-1.017258
C	-4.060126	-0.261259	0.223264
N	-3.209105	-0.555117	1.225544
C	-1.820353	-0.561128	1.116963
O	-1.145903	-0.796938	2.113620
H	-3.572965	-0.769892	2.144294
H	-5.113548	-0.290552	0.466458
H	-4.297139	0.298059	-1.812363
O	-1.697922	0.401590	-2.409500
H	-2.388214	0.609832	-3.046955
C	1.007117	-1.358156	0.121139
H	1.036095	-1.238665	1.204195
C	0.345099	-2.721136	-0.179717
H	-0.637325	-2.812974	0.282565
H	0.983993	-3.513917	0.214823
H	0.239773	-2.874973	-1.257760
C	2.427068	-1.366451	-0.463384
C	3.373168	-0.287953	0.055110
C	4.758988	-0.434651	-0.570408
H	5.175152	-1.423906	-0.363358
H	4.709387	-0.309996	-1.657144
H	5.449917	0.314642	-0.175052
H	3.478672	-0.420428	1.137516
C	2.819930	1.111820	-0.225152
C	1.490522	1.411590	0.399856
C	0.465755	1.877977	-0.379136
C	-0.747333	2.578869	0.132787
H	-0.496906	3.641592	0.224936
H	-1.071942	2.229519	1.112358
H	-1.578553	2.507229	-0.569116
H	0.669720	2.006588	-1.438287
C	1.404820	1.393212	1.893504
H	1.662804	2.395769	2.254134
H	2.116172	0.694258	2.331611
H	0.407899	1.144727	2.252500
H	2.768768	1.271881	-1.306866
H	3.517089	1.859130	0.175528
H	2.870408	-2.340468	-0.236428

H	2.368023	-1.305337	-1.558876
H	0.304698	-0.193895	-1.568085

(E)-Cyclization TS TS-2

E -829.149198  
Imag. Freq. -171.085

Cartesian coordinates

C	0.100048	-0.350370	-0.486886
C	-1.297266	-0.261005	-0.280843
C	-2.027129	-1.025146	0.650647
C	-3.424833	-0.903592	0.757965
C	-4.059151	-0.002272	-0.042556
N	-3.384327	0.758880	-0.921969
C	-2.003872	0.717761	-1.104298
O	-1.480622	1.467625	-1.914263
H	-3.883964	1.427362	-1.493467
H	-5.129846	0.147048	-0.005514
H	-3.987255	-1.496920	1.464991
O	-1.354069	-1.855100	1.431859
H	-1.939074	-2.335898	2.027548
H	0.422331	0.269771	-1.320494
C	0.964012	-1.557826	-0.245766
C	2.441623	-1.336564	-0.589692
H	2.929143	-2.311676	-0.494639
C	3.233207	-0.327550	0.248319
C	4.727388	-0.478000	-0.048389
H	5.319418	0.217331	0.552497
H	4.935082	-0.273172	-1.104085
H	5.068667	-1.492716	0.171222
C	2.811652	1.115699	-0.033662
C	1.442589	1.568818	0.398358
C	0.750447	0.950070	1.392380
C	-0.485916	1.466862	2.053786
H	-1.142853	2.014575	1.377533
H	-0.200410	2.149501	2.861209
H	-1.051794	0.653576	2.510133
H	1.218450	0.106865	1.888039
C	0.988149	2.837707	-0.255296
H	0.956501	2.715693	-1.340730
H	0.013109	3.180692	0.082663
H	1.725800	3.618632	-0.041631
H	2.917995	1.308519	-1.109032
H	3.516307	1.805577	0.449798
H	3.087221	-0.554861	1.310371
H	2.523739	-1.056268	-1.648898
C	0.400992	-2.672322	-1.157556
H	1.033227	-3.557374	-1.059754
H	-0.618267	-2.944940	-0.883455

H	0.409993	-2.361734	-2.206009
H	0.872664	-1.895556	0.785649

(E)-QM (7b)

E -829.150880

Cartesian coordinates

C	-0.003465	-0.621761	1.283925
C	1.141202	-0.698435	0.550535
C	1.438493	-1.678701	-0.473119
C	2.574036	-1.550460	-1.265788
C	3.428832	-0.498253	-1.037830
N	3.223273	0.403859	-0.077755
C	2.136549	0.375960	0.804966
O	2.061471	1.198642	1.685693
H	3.893819	1.148428	0.067598
H	4.321607	-0.361467	-1.634708
H	2.796694	-2.260307	-2.049885
O	0.596060	-2.667421	-0.624617
H	0.858703	-3.265952	-1.335235
C	-1.240537	-1.438852	1.295320
H	-1.119381	-2.362797	0.736377
C	-2.351098	-0.590846	0.626330
H	-3.298609	-1.096025	0.837999
H	-2.398051	0.377620	1.133613
C	-2.185784	-0.405122	-0.896981
C	-3.208164	-1.244317	-1.663218
H	-3.041689	-1.180256	-2.742026
H	-4.225805	-0.897039	-1.455064
H	-3.146044	-2.296931	-1.371772
H	-1.194937	-0.767771	-1.190428
C	-2.271284	1.079420	-1.301837
C	-1.084375	1.897129	-0.844226
C	-1.174921	2.695616	0.224618
C	-0.120789	3.574629	0.827131
H	-0.429391	4.624445	0.779095
H	0.018775	3.332859	1.884988
H	0.848565	3.485215	0.336551
H	-2.141533	2.741185	0.725585
C	0.150995	1.744517	-1.691733
H	1.038360	2.198232	-1.249768
H	0.366736	0.688791	-1.888126
H	-0.007081	2.208963	-2.671186
H	-3.200110	1.499867	-0.901011
H	-2.342016	1.136563	-2.394694
C	-1.607734	-1.760982	2.751168
H	-1.787916	-0.845706	3.322233
H	-2.520997	-2.359523	2.765395
H	-0.817896	-2.329201	3.246795



H      -0.026714      0.226773      1.967790

Water

E -76.440394

Cartesian coordinates

O	-0.000000	0.000000	0.117280
H	-0.000000	0.759217	-0.469121
H	-0.000000	-0.759217	-0.469121

(Z)-Anti Hydration TS **TS-3**

E -905.610172  
Imag. Freq. -118.498

Cartesian coordinates

C	-0.090139	0.299526	0.198214
C	-1.582779	0.106944	0.096919
C	-2.301250	-0.396232	1.157691
C	-3.714654	-0.544987	1.105497
C	-4.350656	-0.190470	-0.036054
N	-3.649773	0.281647	-1.093851
C	-2.270432	0.442839	-1.121777
O	-1.733612	0.844749	-2.159816
H	-4.134643	0.525442	-1.946409
H	-5.421519	-0.266640	-0.163696
H	-4.271723	-0.929250	1.948957
O	-1.629182	-0.759003	2.268424
H	-2.233359	-1.076977	2.944796
H	0.146114	0.145621	1.254890
C	0.383369	1.732663	-0.155355
C	1.811315	2.035558	0.321614
H	2.064828	3.033063	-0.051305
C	2.942785	1.081797	-0.078613
C	4.297131	1.714810	0.248175
H	5.121101	1.060348	-0.048242
H	4.385756	1.905898	1.322839
H	4.414132	2.667096	-0.274026
C	2.837321	-0.258638	0.649076
C	1.801945	-1.228747	0.216428
C	0.689812	-0.799726	-0.666228
C	-0.166982	-1.949835	-1.186267
H	-0.729635	-2.442086	-0.391296
H	0.460129	-2.695492	-1.679388
H	-0.874374	-1.575428	-1.924110
H	1.106665	-0.255833	-1.515585
C	1.634144	-2.424133	1.072297
H	2.545224	-2.673304	1.613413
H	0.861702	-2.147245	1.804527
H	1.251323	-3.285345	0.528012
H	2.639521	-0.086461	1.718943
H	3.787553	-0.799453	0.640648
H	2.909638	0.908882	-1.160551
H	1.808226	2.114412	1.418419
C	-0.548519	2.785621	0.451699
H	-0.146023	3.787042	0.277864
H	-1.549526	2.749567	0.019728

H	-0.640375	2.644977	1.534206
H	0.348934	1.831706	-1.243600
O	3.053296	-2.159738	-1.252855
H	2.983161	-3.118770	-1.300605
H	2.957084	-1.843294	-2.157393

(Z)-Syn Hydration TS

E -905.609360  
Imag. Freq. -40.358

Cartesian coordinates

C	-0.026787	0.276336	-0.101737
C	1.477586	0.142956	-0.070560
C	2.155280	-0.552048	-1.042680
C	3.573008	-0.644775	-1.062640
C	4.262845	-0.028915	-0.073767
N	3.607827	0.640387	0.903999
C	2.228250	0.760516	0.994071
O	1.736581	1.361471	1.954475
H	4.133472	1.081217	1.646187
H	5.341884	-0.041153	-0.009273
H	4.091913	-1.186592	-1.841497
O	1.438226	-1.171292	-2.013989
H	2.019519	-1.584112	-2.659499
H	-0.335912	-0.107189	-1.075216
C	-0.511108	1.750147	-0.053383
C	-1.947271	1.909578	-0.564666
H	-2.221476	2.961126	-0.433246
C	-3.048563	1.063829	0.074660
C	-4.420406	1.549049	-0.397980
H	-5.227094	0.983748	0.074937
H	-4.516504	1.439523	-1.482912
H	-4.555022	2.604543	-0.150910
C	-2.929619	-0.441614	-0.267679
C	-1.979034	-1.230920	0.543939
C	-0.707440	-0.633409	0.995692
C	0.208905	-1.609206	1.734793
H	0.590847	-2.379879	1.062528
H	-0.322311	-2.091342	2.556054
H	1.048045	-1.076381	2.174234
H	-1.102156	0.066384	1.760056
C	-2.492472	-2.474928	1.130002
H	-1.723652	-3.201268	1.372287
H	-2.969256	-2.150567	2.070862
H	-3.277767	-2.911803	0.514789
H	-2.594887	-0.538109	-1.309475
H	-3.908238	-0.921809	-0.221743
H	-3.010036	1.179082	1.163982
H	-1.960363	1.724808	-1.648160
C	0.384643	2.664110	-0.895288
H	-0.043572	3.668803	-0.940618
H	1.391521	2.751717	-0.485790

H	0.466388	2.288952	-1.921200
H	-0.465395	2.087407	0.985889
O	-0.907251	-2.573841	-1.157085
H	-1.487034	-2.865399	-1.865436
H	-0.125038	-2.199370	-1.588688

(E)-Syn Hydration TS

E	-905.605078
Imag. Freq.	-95.376

Cartesian coordinates

C	-0.053440	-0.261332	-0.128157
C	-1.559158	-0.121843	-0.078428
C	-2.304428	-0.391170	1.042939
C	-3.720179	-0.267868	1.058588
C	-4.333135	0.138357	-0.078765
N	-3.607181	0.421046	-1.186545
C	-2.224108	0.331190	-1.271347
O	-1.664896	0.642824	-2.330764
H	-4.077304	0.735388	-2.023934
H	-5.404205	0.259181	-0.162574
H	-4.295468	-0.489379	1.947191
O	-1.644282	-0.773434	2.154314
H	-2.254883	-0.933305	2.879133
H	0.201870	-0.013191	-1.163343
C	0.423402	-1.719566	0.144012
C	1.821374	-2.023267	-0.410797
H	2.066410	-3.052395	-0.129174
C	2.983403	-1.140816	0.036943
C	4.315243	-1.692761	-0.470403
H	5.154847	-1.092183	-0.113426
H	4.337085	-1.699510	-1.564368
H	4.454185	-2.717922	-0.119126
C	2.819418	0.321549	-0.503655
C	2.047215	1.093646	0.476064
C	0.636203	0.784721	0.793233
C	-0.202038	2.059990	1.043760
H	-0.628576	2.413710	0.105523
H	0.388876	2.867658	1.469516
H	-1.015997	1.835514	1.729638
H	0.769104	0.323630	1.792428
C	2.827034	1.935465	1.400526
H	2.245855	2.405423	2.187890

H	3.577496	1.270575	1.849690
H	3.395521	2.678884	0.838478
H	2.304390	0.270056	-1.463376
H	3.798371	0.778006	-0.647252
H	3.020789	-1.124564	1.132459
H	1.780103	-2.007347	-1.508461
C	-0.539350	-2.753688	-0.449937
H	-0.121726	-3.758235	-0.344063
H	-1.512576	-2.744839	0.041417
H	-0.697528	-2.567333	-1.517603
H	0.447928	-1.865436	1.229464
O	1.608603	2.857129	-1.118735
H	2.386421	3.040603	-1.653626
H	0.909138	2.671896	-1.752680

(E)-Anti Hydration TS **TS-4**

E -905.610757  
Imag. Freq. -180.301

Cartesian coordinates

C	0.074543	0.341143	-0.155941
C	1.567633	0.143586	-0.101215
C	2.369190	0.554223	0.937806
C	3.778242	0.365160	0.918257
C	4.322991	-0.263230	-0.150438
N	3.538176	-0.703405	-1.162589
C	2.158802	-0.569011	-1.204479
O	1.532279	-1.063032	-2.151604
H	3.955829	-1.193764	-1.941229
H	5.383327	-0.449275	-0.249746
H	4.402539	0.707223	1.732516
O	1.782147	1.141565	1.996330
H	2.435520	1.392213	2.655325
H	-0.166686	0.125430	-1.201671
C	-0.432160	1.777959	0.118780
C	-1.857983	2.004332	-0.410266
H	-2.161351	3.006305	-0.090436
C	-2.969861	1.026757	-0.007456
C	-4.329535	1.592553	-0.424539
H	-5.143113	0.926560	-0.125257
H	-4.378960	1.720938	-1.510954
H	-4.500297	2.567409	0.037798
C	-2.791065	-0.348684	-0.655414
C	-1.736834	-1.255407	-0.129152
C	-0.688003	-0.719353	0.771831
C	0.194559	-1.780173	1.419600
H	0.795530	-2.325447	0.690248
H	-0.417494	-2.497817	1.969655
H	0.870307	-1.311947	2.134017
H	-1.167904	-0.133308	1.557292
C	-1.459597	-2.470648	-0.924764
H	-2.322723	-2.785493	-1.508528
H	-0.650387	-2.178021	-1.611787
H	-1.072026	-3.290712	-0.322441
H	-2.561765	-0.228846	-1.725283
H	-3.722021	-0.921941	-0.646490
H	-2.980097	0.916146	1.082974
H	-1.820438	2.035022	-1.508733
C	0.478819	2.827053	-0.524400
H	0.039870	3.822117	-0.413547
H	1.470271	2.847036	-0.070922



H	0.598350	2.631306	-1.595427
H	-0.433212	1.940323	1.200540
O	-2.996036	-2.191802	1.291957
H	-2.769422	-3.105043	1.496488
H	-3.076437	-1.743978	2.141005

(Z)-QM (7a)

E -829.151163

Cartesian coordinates

C	0.734910	-0.283675	1.287809
C	1.736447	0.160777	0.479708
C	2.143251	1.553488	0.571701
C	3.174370	2.052912	-0.210993
C	3.805908	1.196507	-1.082224
N	3.467689	-0.086734	-1.206312
C	2.438767	-0.714341	-0.486709
O	2.206783	-1.881256	-0.695561
H	3.960571	-0.675397	-1.866834
H	4.616134	1.536391	-1.714534
H	3.484764	3.086094	-0.152158
O	1.482122	2.295730	1.422753
H	1.796784	3.207882	1.434439
C	0.078616	-1.608029	1.373471
C	-1.317232	-1.449382	0.711750
H	-1.906810	-2.328986	0.994941
H	-1.822481	-0.585282	1.158699
C	-1.301440	-1.320483	-0.815720
C	-1.053518	-2.668988	-1.490721
H	-1.869760	-3.364443	-1.267255
H	-0.117386	-3.119632	-1.153848
H	-0.995781	-2.554783	-2.576815
C	-2.617706	-0.702599	-1.322842
C	-2.885429	0.695434	-0.816181
C	-3.990707	0.964792	-0.115117
C	-4.415507	2.275512	0.477132
H	-3.720397	3.088590	0.264547
H	-4.504613	2.190553	1.565412
H	-5.402890	2.566825	0.104206
H	-4.676906	0.138282	0.066775
C	-1.820317	1.710608	-1.143072
H	-1.456860	1.568427	-2.165649
H	-0.953895	1.594290	-0.481854
H	-2.168610	2.738819	-1.046880
H	-3.448976	-1.362796	-1.049323
H	-2.575602	-0.681240	-2.419681
H	-0.486694	-0.646900	-1.107343
H	0.649850	-2.362146	0.838275
C	-0.070321	-2.006302	2.848016
H	-0.685243	-1.282428	3.390899
H	-0.556861	-2.982020	2.908624
H	0.900586	-2.076620	3.342913

H	0.315460	0.460474	1.963046
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