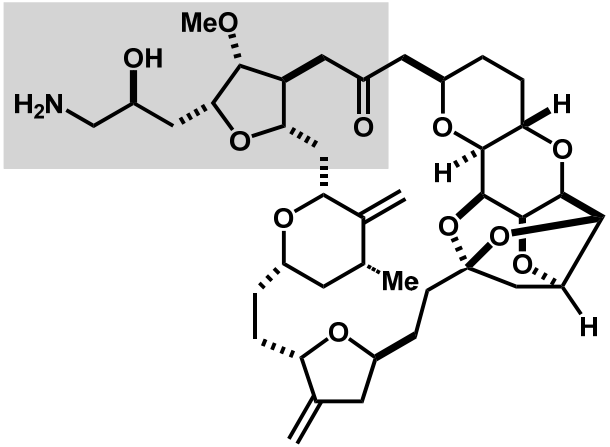
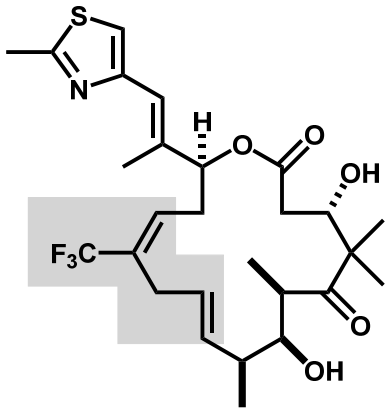
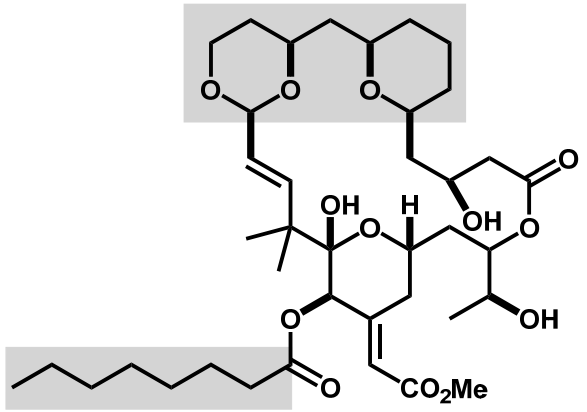
Artificial bioactive compounds based on natural products



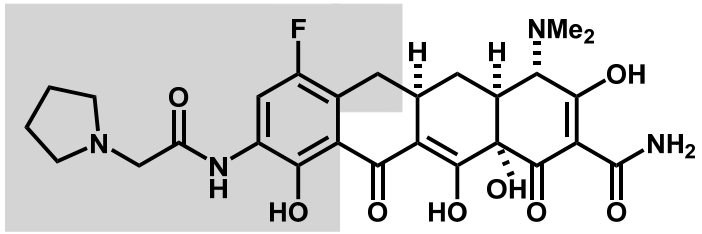
E 7389



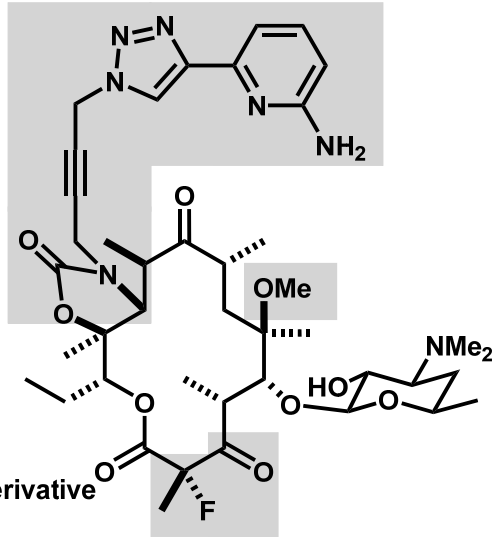
epothilone derivative



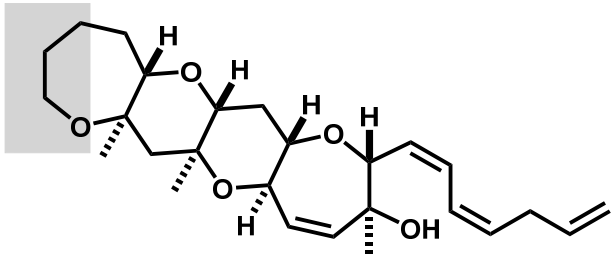
simplified bryostatin



tetracycline derivative

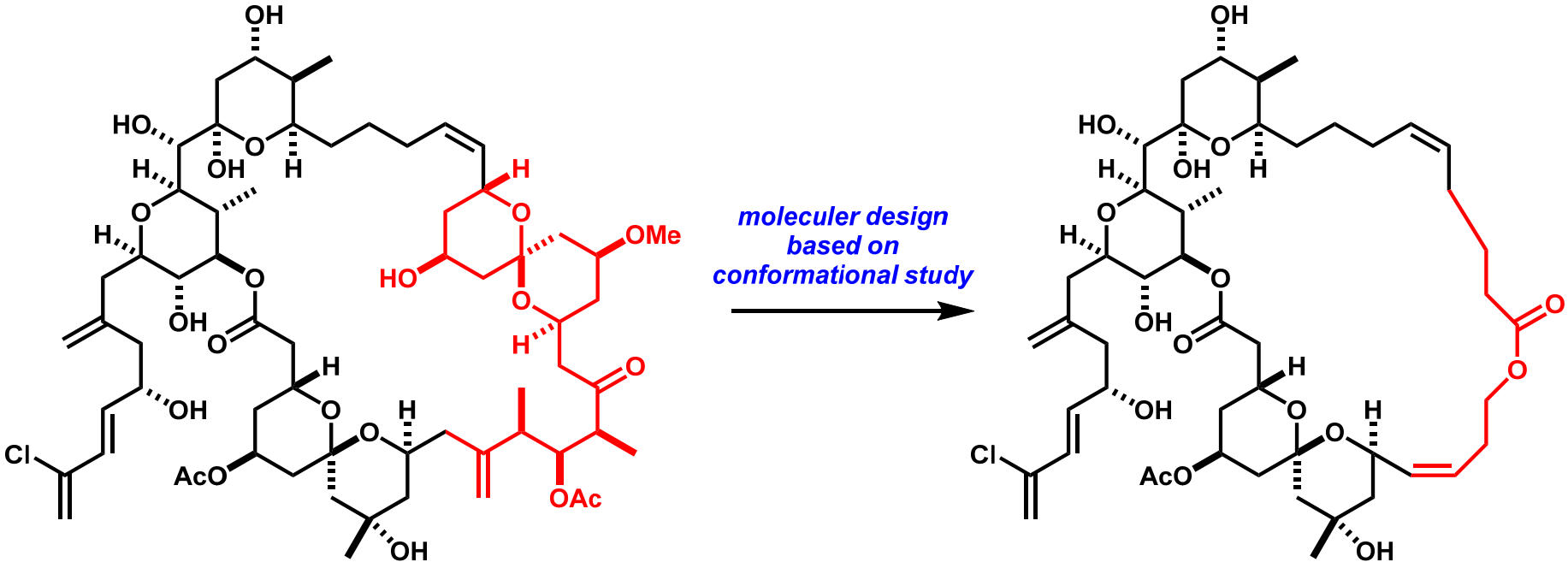


erythromycin derivative



simplified gambierol

Smith's effort to create spongistatin-based anti-cancer agent

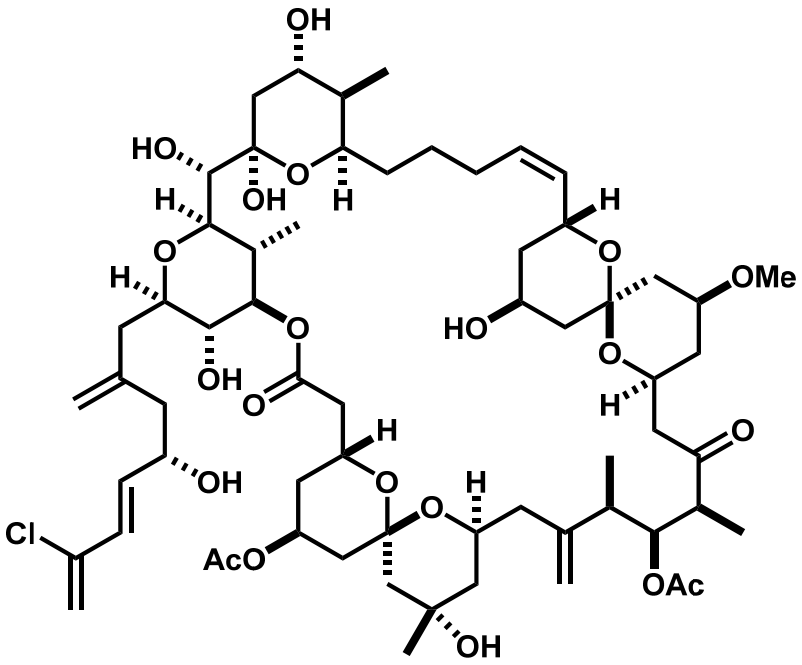


spongistatin 1

ABEF analog

J. Am. Chem. Soc. 2011, 133, 14042.

Spongistatin



spongistatin 1

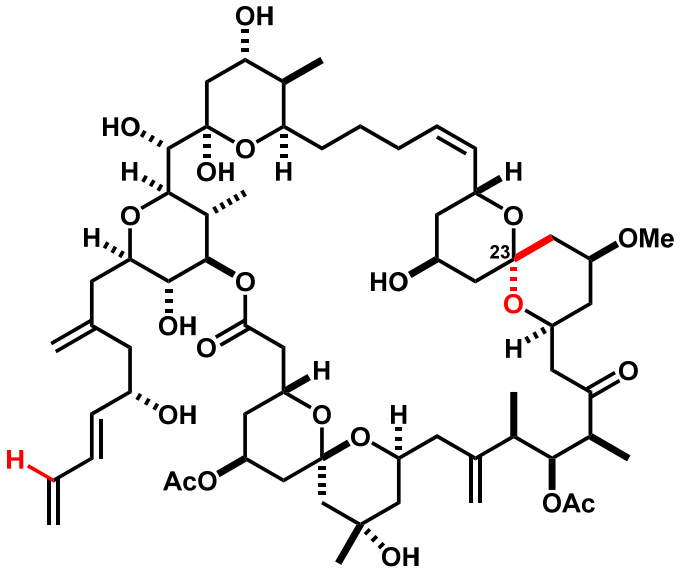
Isolation: the genus *Songia* sp. by Pettit, Fusetani, Kitagawa

Bioactivity: avg. IC_{50} value 0.12 nM against the NCI panel of 60 human cancer cell lines
inhibition of tubulin polymerization

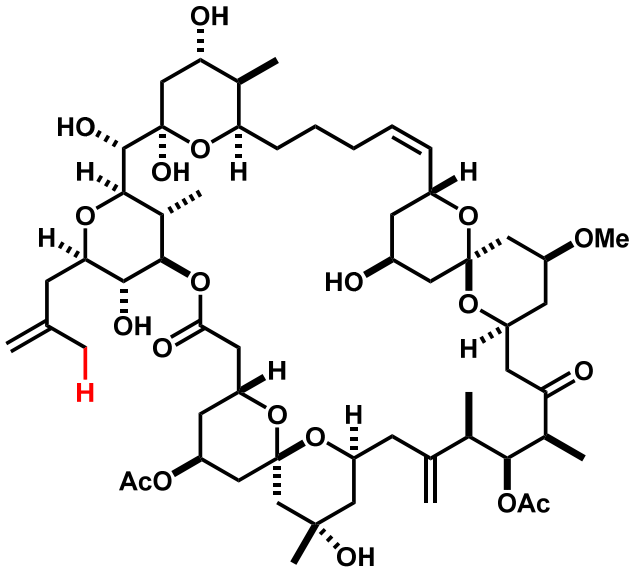
Structural feature: two 6,6-spiroketal, 42-membered ring, chlorinated unsaturated side chain
24 stereocenters

Total synthesis of related compounds:
Evans, Kishi, Crimmins, Heathcock, Paterson, Nakata, Smith, Ley

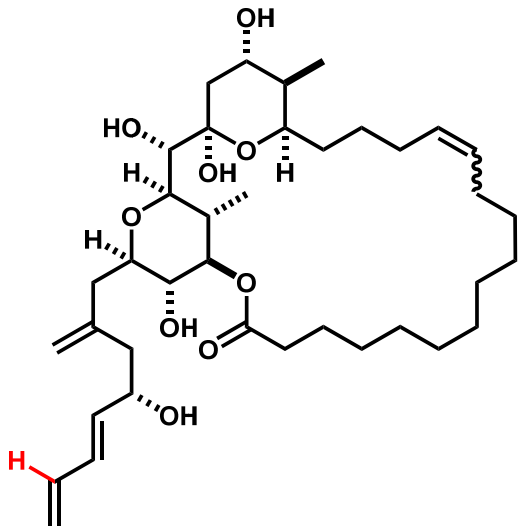
Songistatin analogs from intermediates for total synthesis



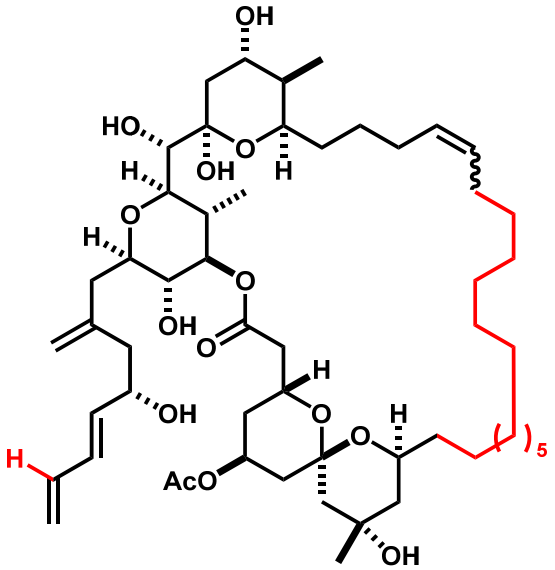
Kishi: C23-*epi*-spongistatin 2
(avg. GI_{50} value 200 nM)



Paterson: analog lacking side chain
(GI_{50} value 587 and 407 nM against the MIP101 and HCT116 colon carcinoma cell lines)

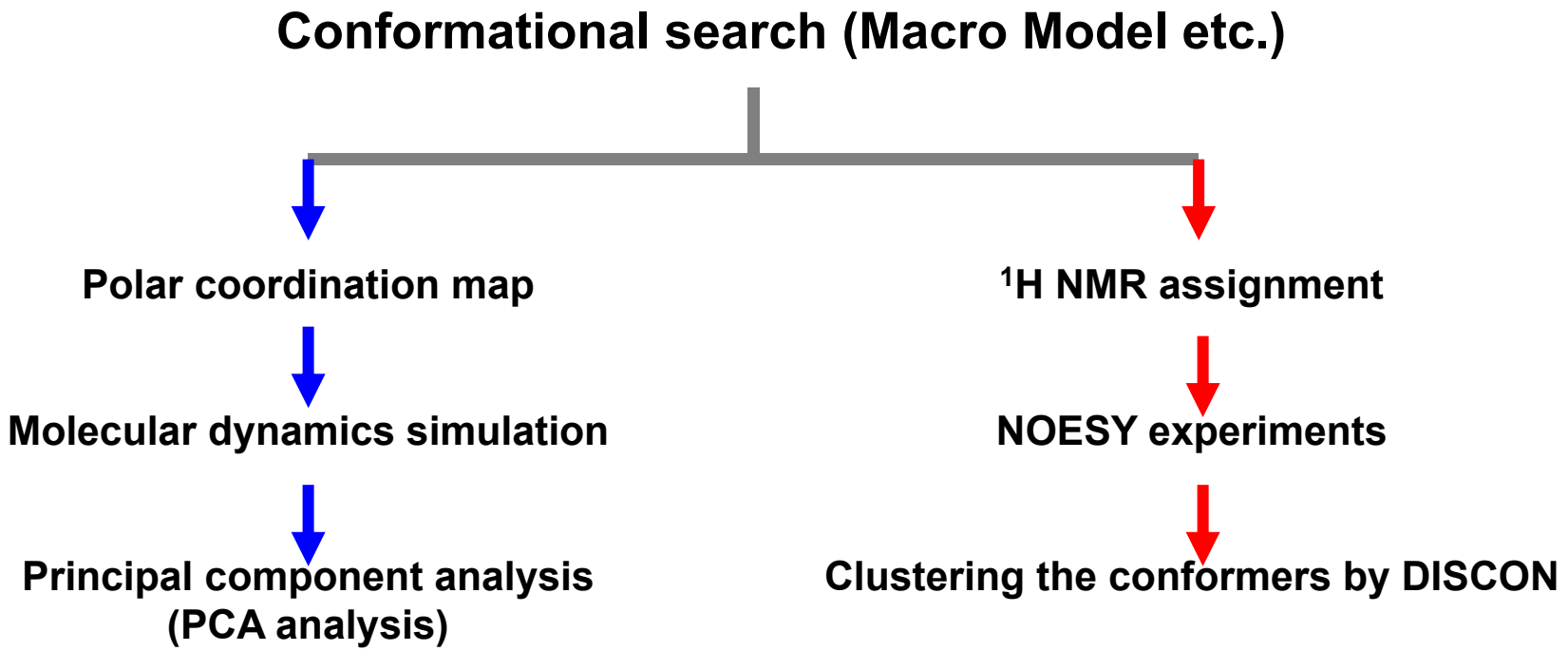


Paterson: analog lacking ABCD-rings of spongistatin 2
(GI_{50} value 480 nM against HCT116 colon carcinoma cell lines)



Paterson: analog lacking CD-rings of spongistatin 2
(GI_{50} value 460 nM against HCT116 colon carcinoma cell lines)

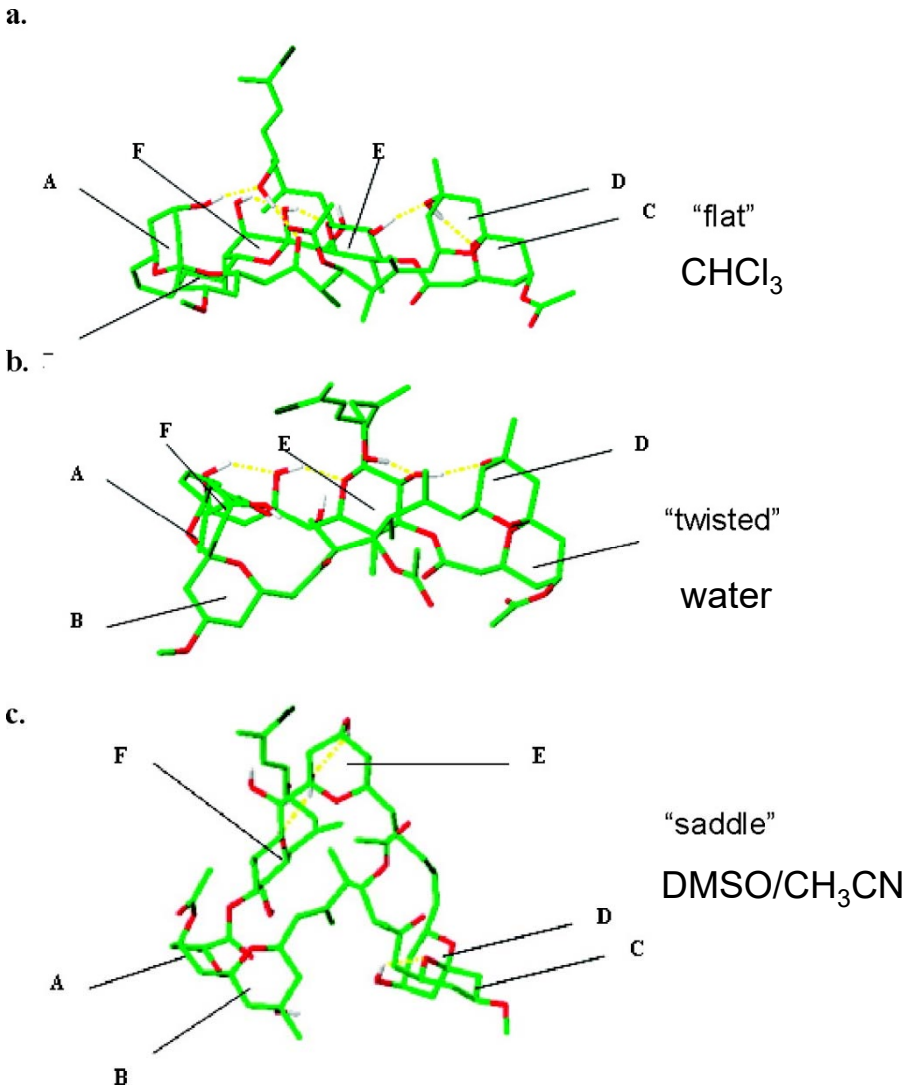
An approach to the conformational analysis of flexible molecule

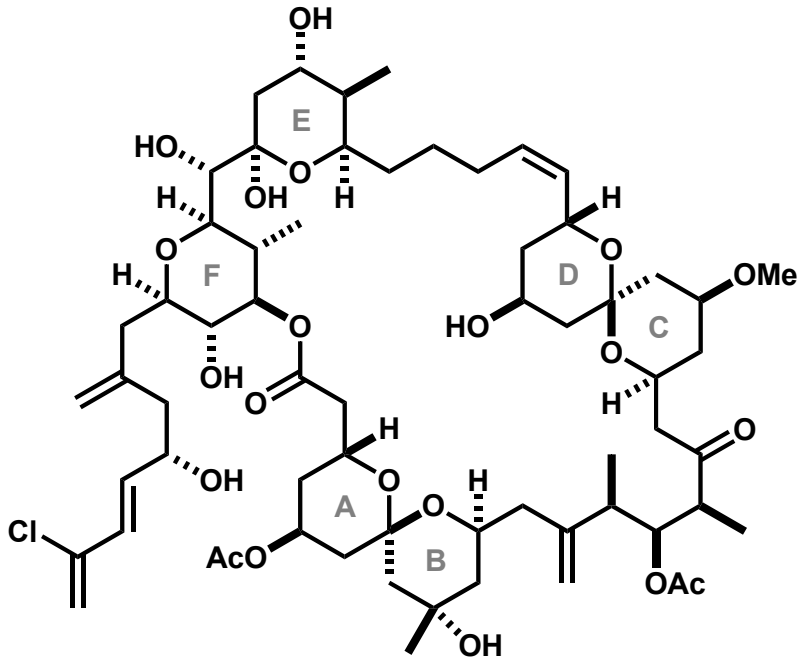


Identification of flexible component

Determination of solution conformation

The lowest energy conformation by conformational search



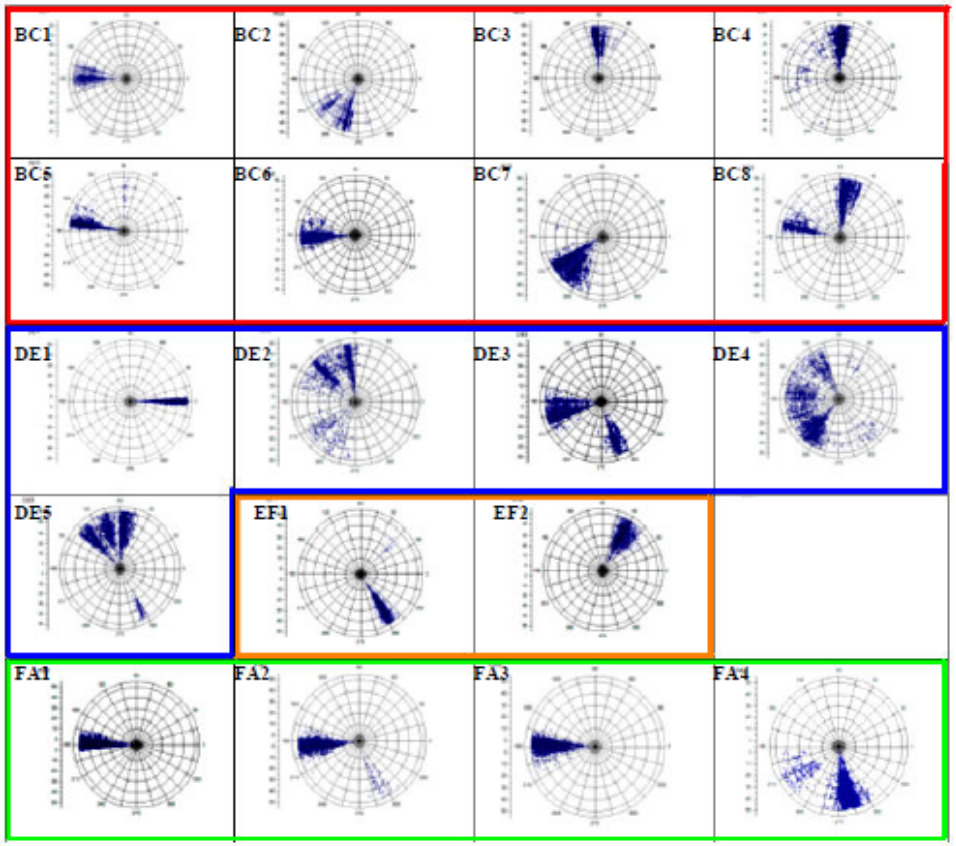


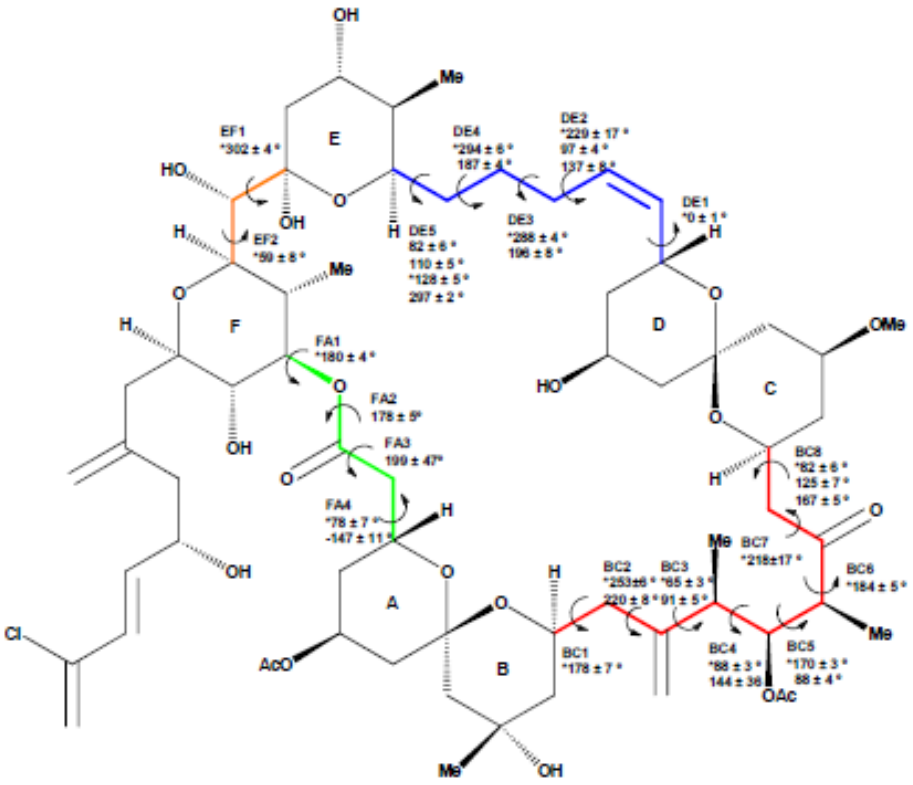
spongistatin 1

Method: 30000-step Monte Carlo searches (MMFF force field), GB/SA solvation models

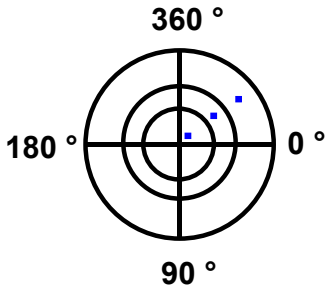
Calculations were repeated from different initial geometries until no additional distinct conformational families were obtained within a 100 kJ/mol energy difference of the global minima.

PCM approach to identify the flexibility of dihedral angles -1

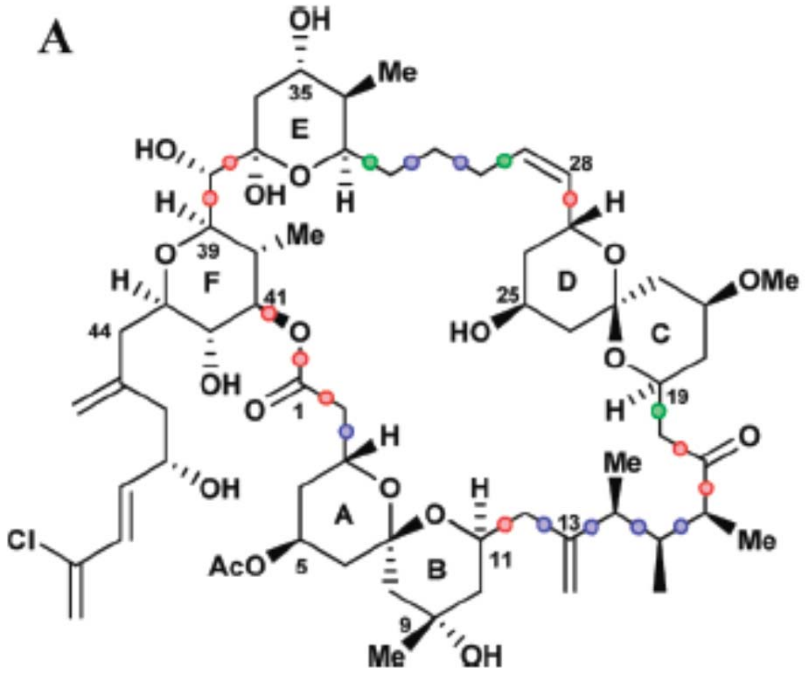




Polar maps drawn for each backbone dihedral angle. Each dot is a conformer. Center in each map represents the lowest energy.



PCM approach to identify the flexibility of dihedral angles -2

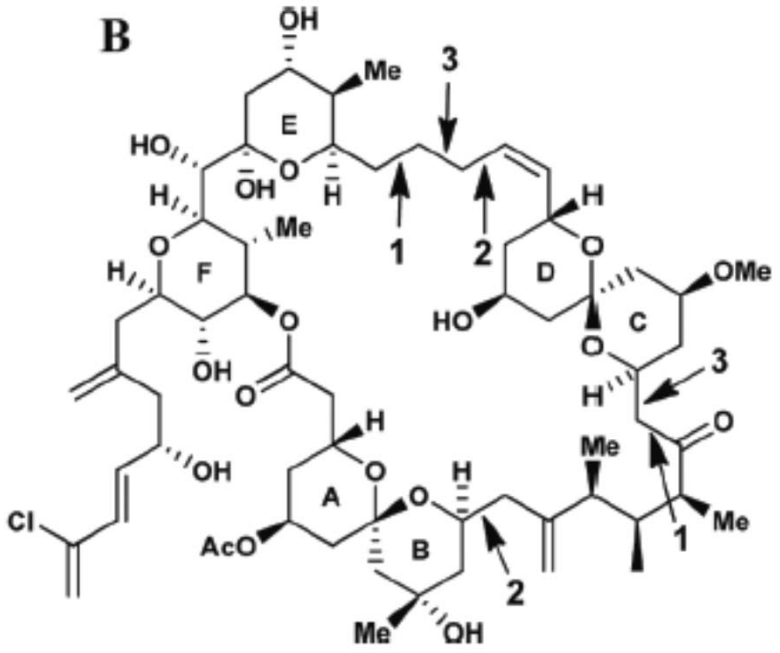


red: rigid, green: flexible, blue: intermediately flexible

Identification of the long-range movements of dihedral angles

Molecular dynamics simulation
Structures at every 150 fs were extracted from five nanosecond simulation (1.5 fs time step, 300K).
The structures were minimized first and clustered by Xcluster according to their backbone torsional angles eliminated the redundant structures and yielded 2921 distinct structures.

PCA analysis
The analysis revealed that while the molecule adapts to different conformations, several bonds have coupled conformational changes.

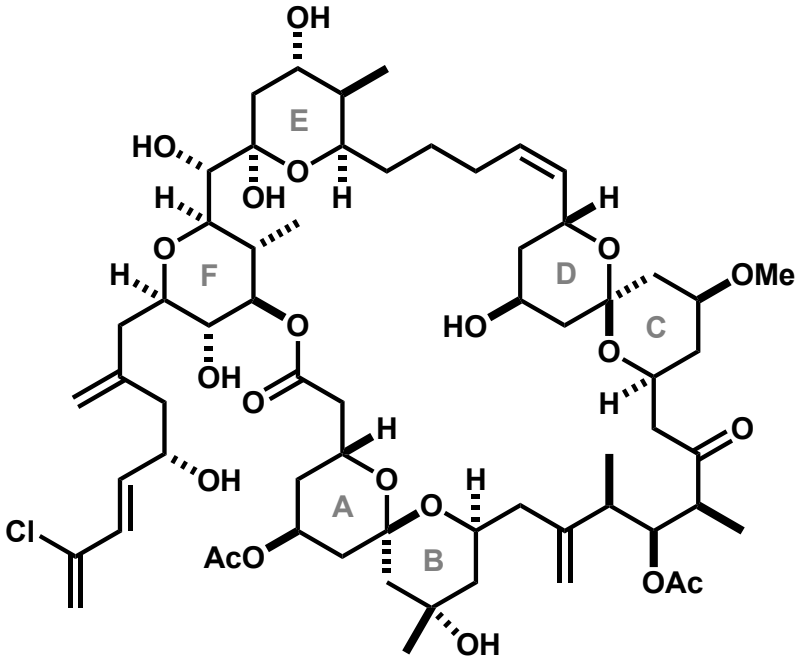


long-range movements from PCA analysis

^{1}H NMR assignment:
Non-overlapping protons in the ^{1}H spectra, including the four hydroxyl hydrogens, were assigned via a combination of COSY, TOCSY, HSQC, and HMBC in DMSO and acetonitrile.

The coupling constants for adjacent protons were obtained from spectra in acetonitrile when the corresponding resonances in DMSO could not be resolved.

Due to the similarity in the polarities of DMSO and acetonitrile, a similar conformational preference would be anticipated. This hypothesis was confirmed by comparison of the ^{1}H NMR coupling constants and by conformational search calculations in the two solvents, which yielded the same low energy conformational families.



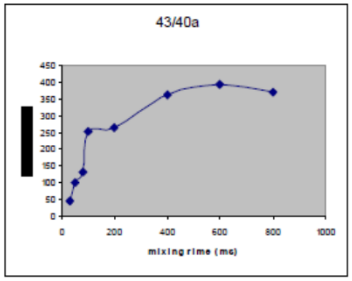
spongistatin 1

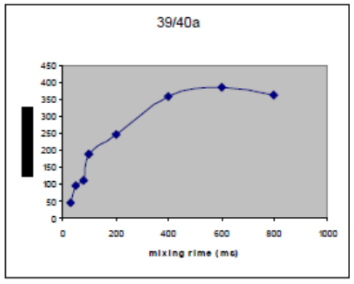
NOESY experiments:

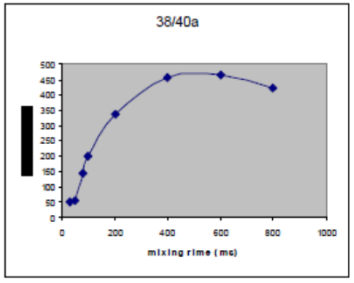
NOESY experiments in DMSO at various mixing times were performed.

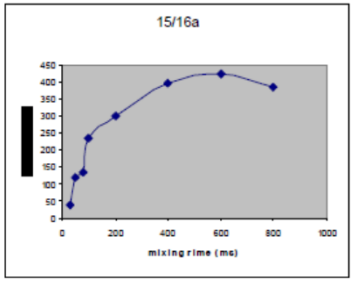
Buildup curves revealed a mixing time optimum of 600 ms.

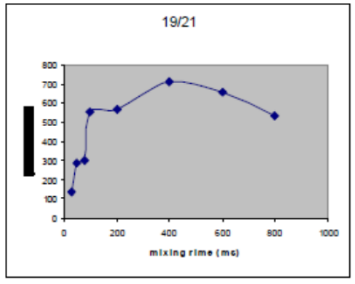
The NOE peak volumes were integrated and normalized by diagonal peaks. Calibrations were performed for each proton independently according to the known distances in the spiroketals.











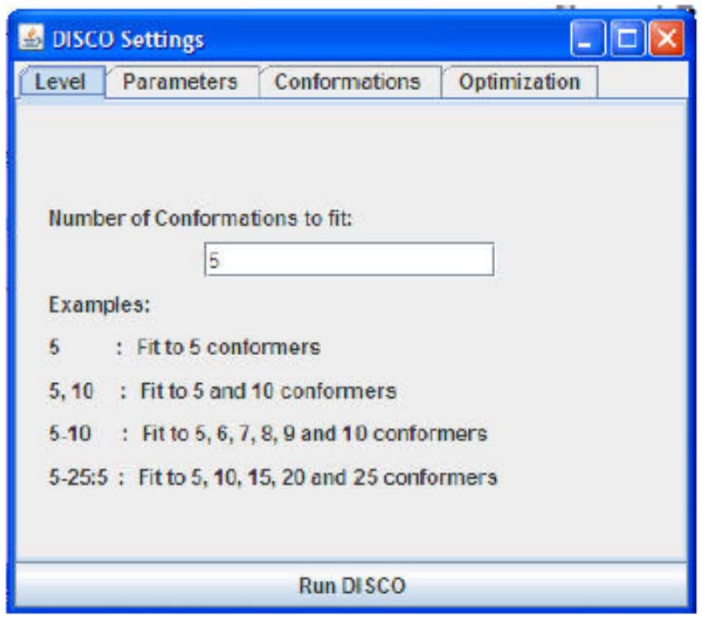
H1	H2	NOE distance in angstroms
40Me	38	2.82
40Me	42	4.8
40Me	16	4.08
40Me	14	4.94
40Me	16Me	4.06
34Me	21	5.11
34Me	38	5.56
14Me	35OH	4.23
9Me	21	3.82
9Me	21OMe	6.08
16Me	39	3.46
16Me	43	4.75
16Me	38	6.3
16Me	41	3.25
34Me	21OMe	6.79

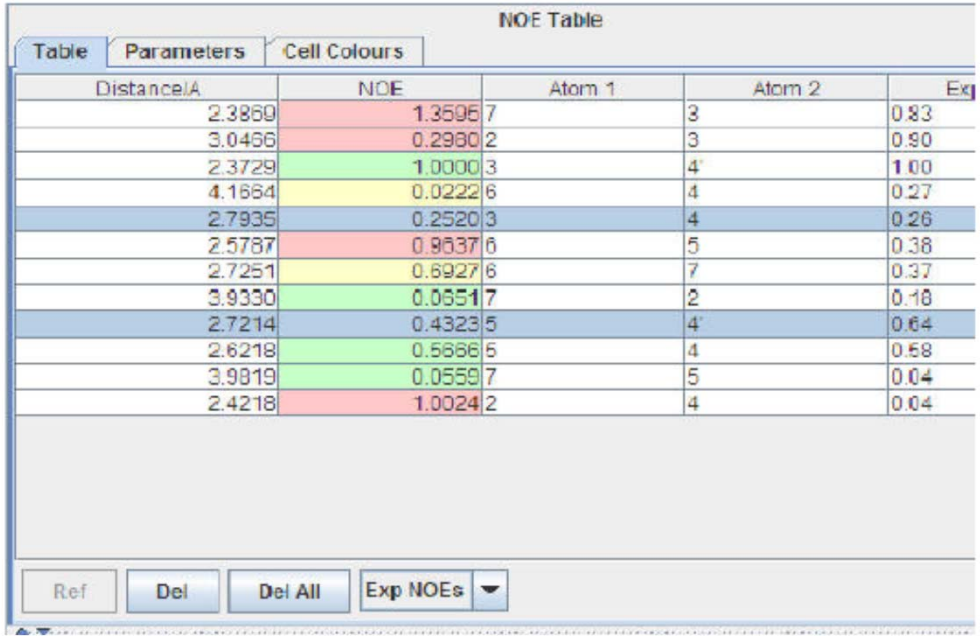
DISCON:

DISCON (Distribution of Solution Conformations) is a multiplatform application for calculating the solution conformation distributions of organic molecules and small peptides from NOEs, coupling constants and a set of pre-calculated conformations.

http://discon.sourceforge.net/

Examples of the setup

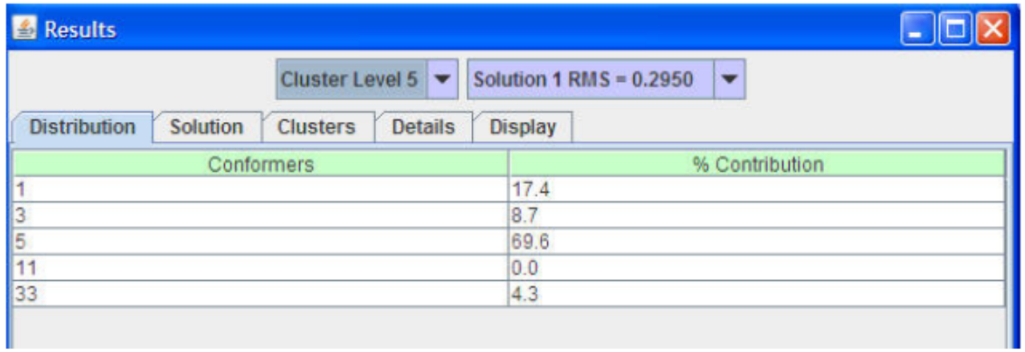




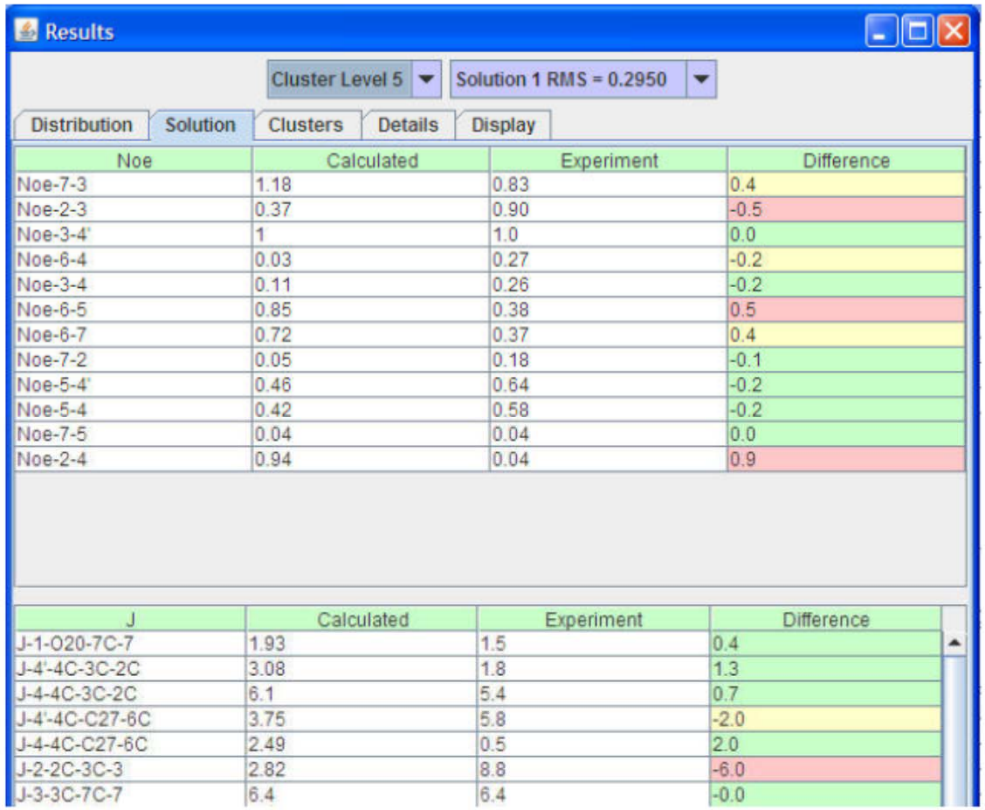
DISCON:

DISCON (Distribution of Solution C olution conformation distributions of organi s and a set of pre-calculated conformations.
http://discon.sourceforge.net/

Examples of the result

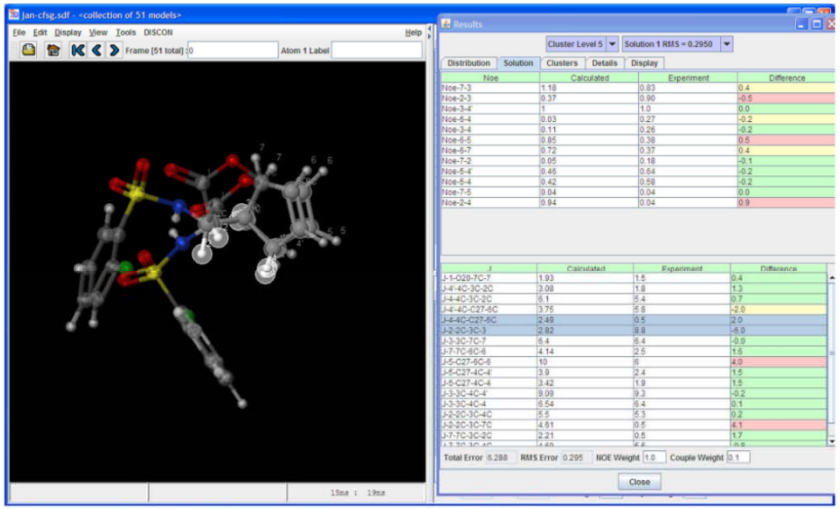


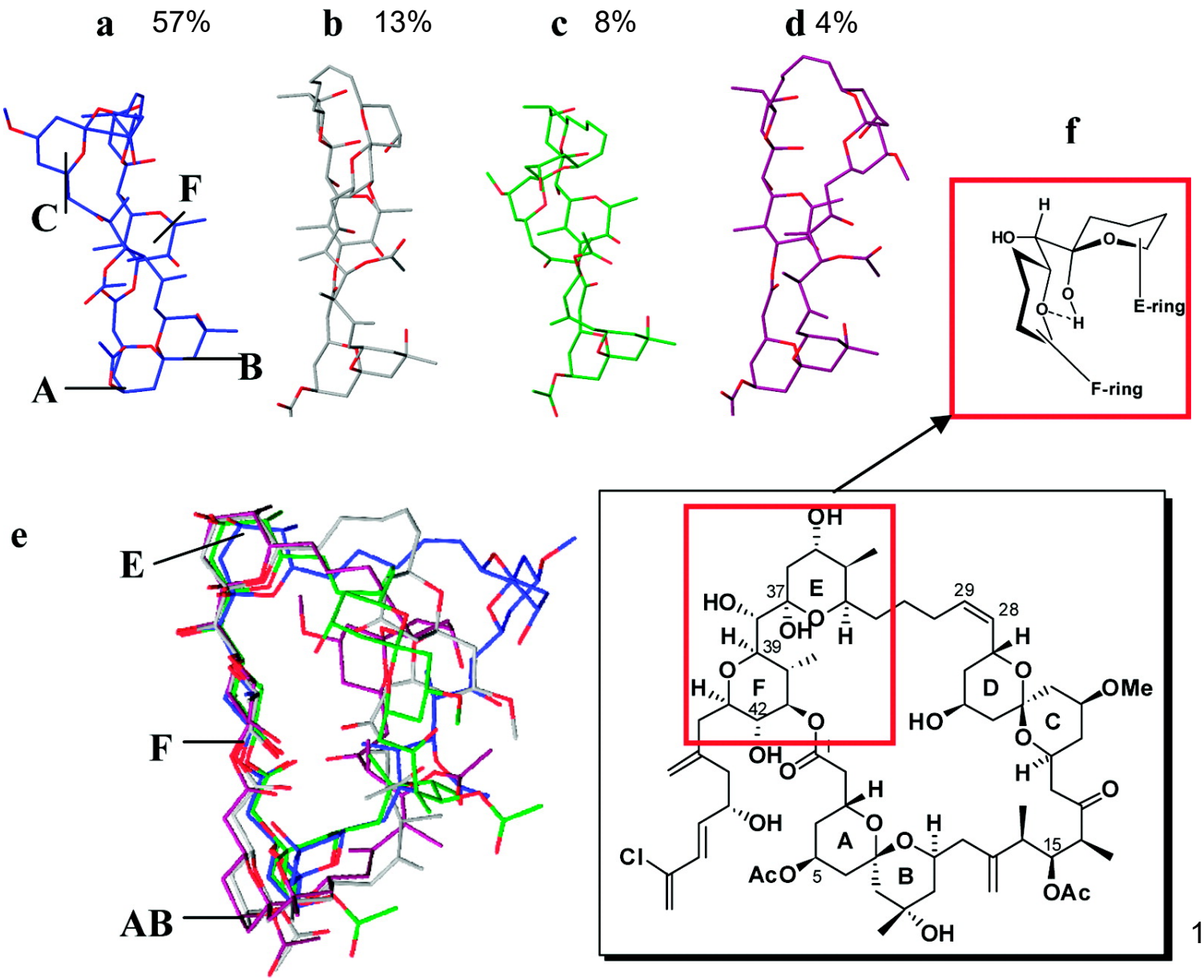
Conformers	% Contribution
1	17.4
3	8.7
5	69.6
11	0.0
33	4.3



Noe	Calculated	Experiment	Difference
Noe-7-3	1.18	0.83	0.4
Noe-2-3	0.37	0.90	-0.5
Noe-3-4'	1	1.0	0.0
Noe-6-4	0.03	0.27	-0.2
Noe-3-4	0.11	0.26	-0.2
Noe-6-5	0.85	0.38	0.5
Noe-6-7	0.72	0.37	0.4
Noe-7-2	0.05	0.18	-0.1
Noe-5-4'	0.46	0.64	-0.2
Noe-5-4	0.42	0.58	-0.2
Noe-7-5	0.04	0.04	0.0
Noe-2-4	0.94	0.04	0.9

J	Calculated	Experiment	Difference
J-1-O20-7C-7	1.93	1.5	0.4
J-4'-4C-3C-2C	3.08	1.8	1.3
J-4-4C-3C-2C	6.1	5.4	0.7
J-4'-4C-C27-6C	3.75	5.8	-2.0
J-4-4C-C27-6C	2.49	0.5	2.0
J-2-2C-3C-3	2.82	8.8	-6.0
J-3-3C-7C-7	6.4	6.4	-0.0




a 57%
b 13%
c 8%
d 4%
C
F
A
B
e
E
F
AB
f
HO
H
O
E-ring
F-ring
OH
HO
37
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Cl
OH
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B
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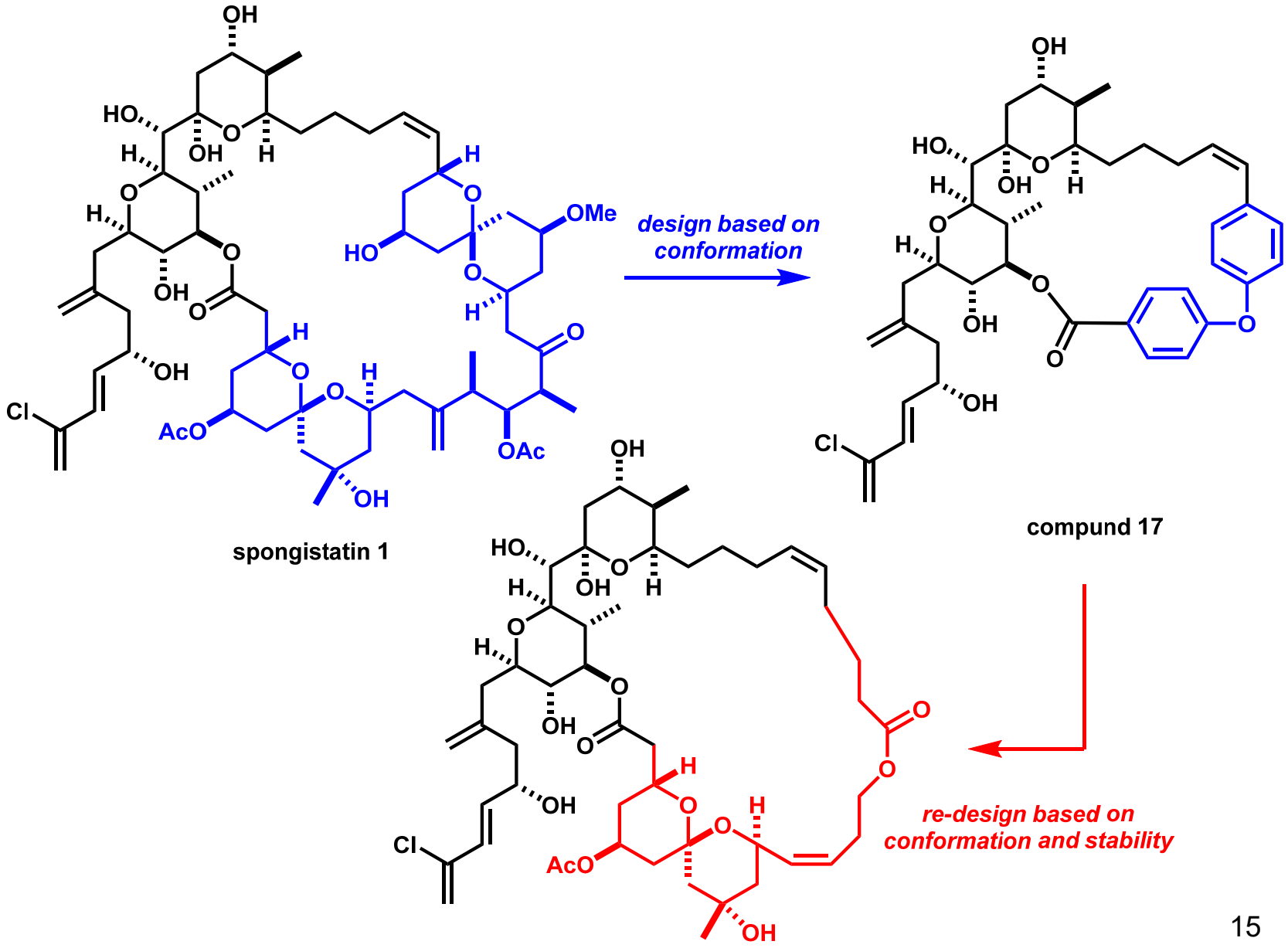
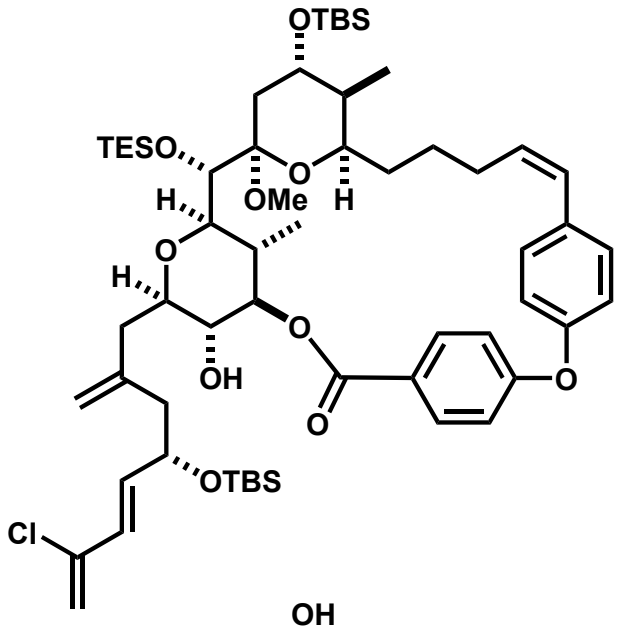
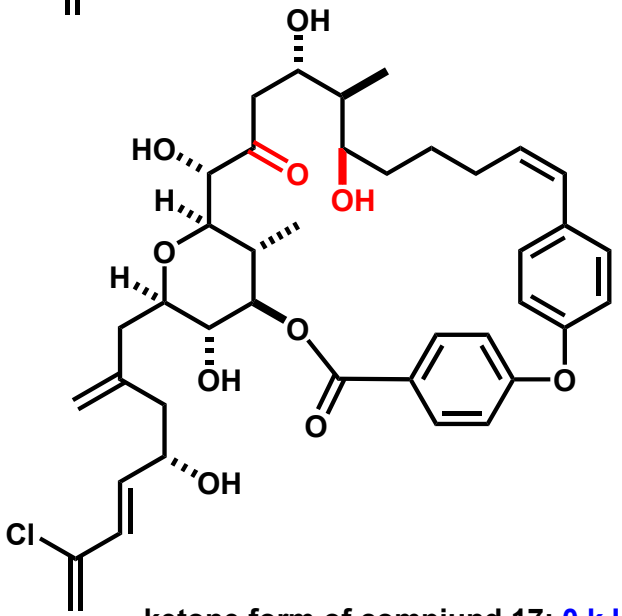
OAc


design based on conformation
spongistatin 1
compund 17
re-design based on conformation and stability
ABEF analog

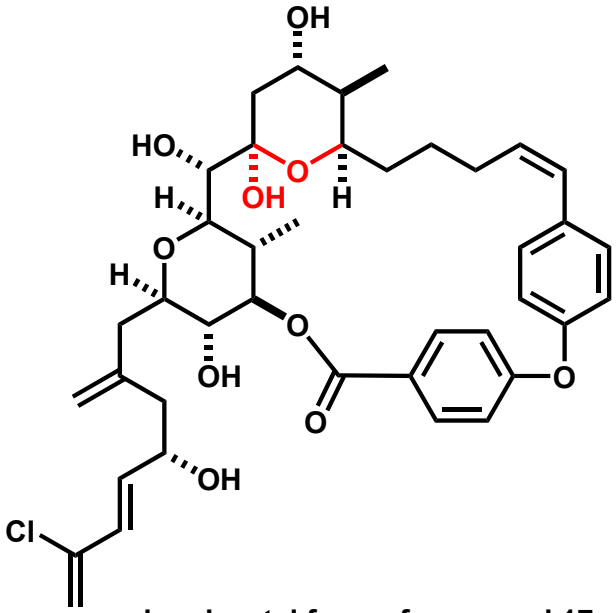


HF aq, CH_3CN
-20 °C

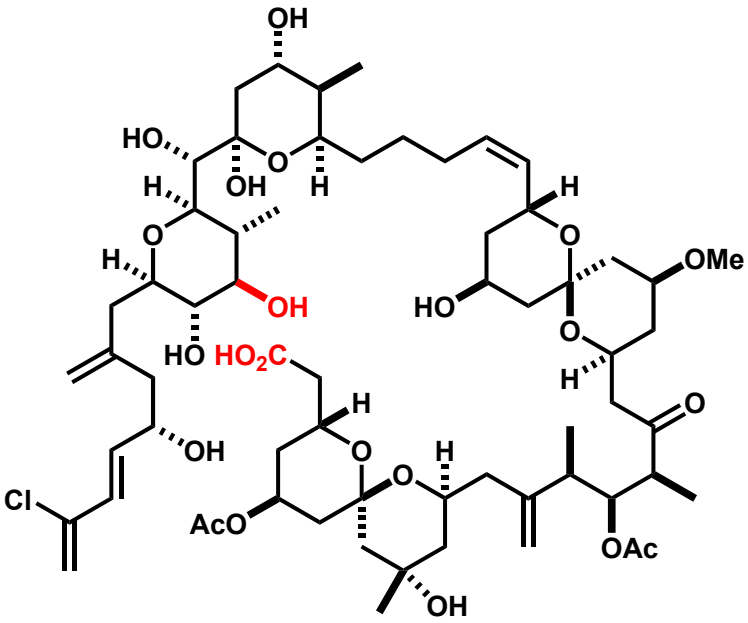
42%
ketone form : hemicacetal form
= 11 :1



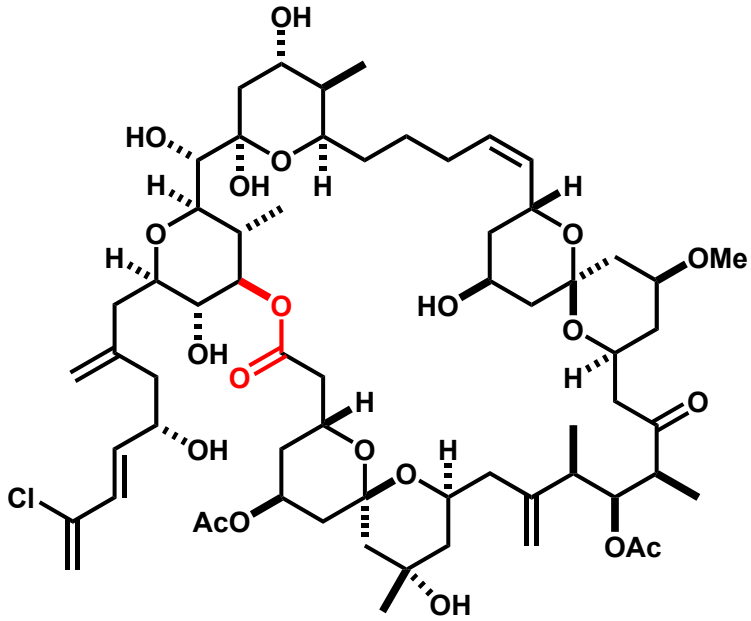
ketone form of compiund 17: 0 kJ/mol



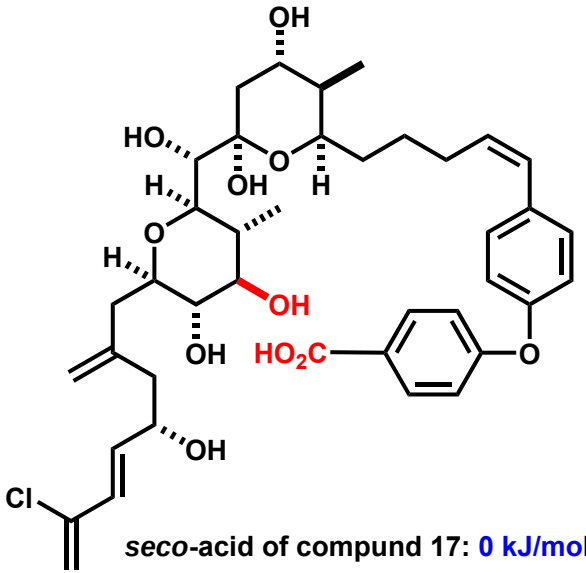
hemiacetal form of compund 17: 11.2 kJ/mol



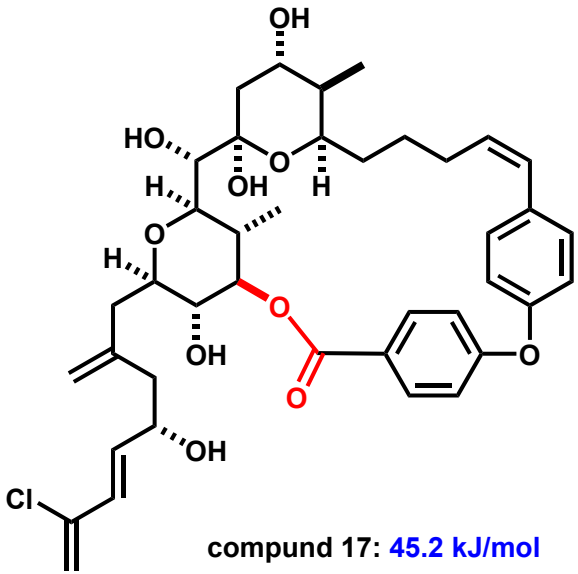
seco-acid of spongistatin 1: 0 kJ/mol



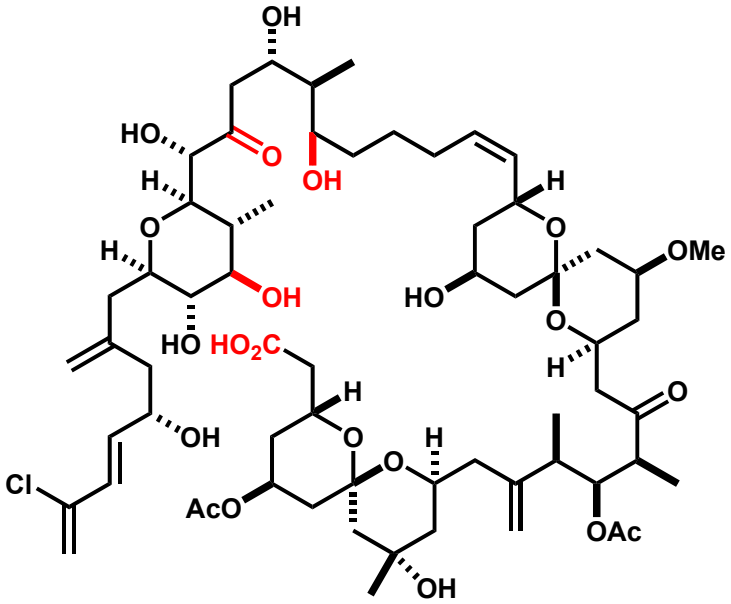
spongistatin 1: 5.8 kJ/mol



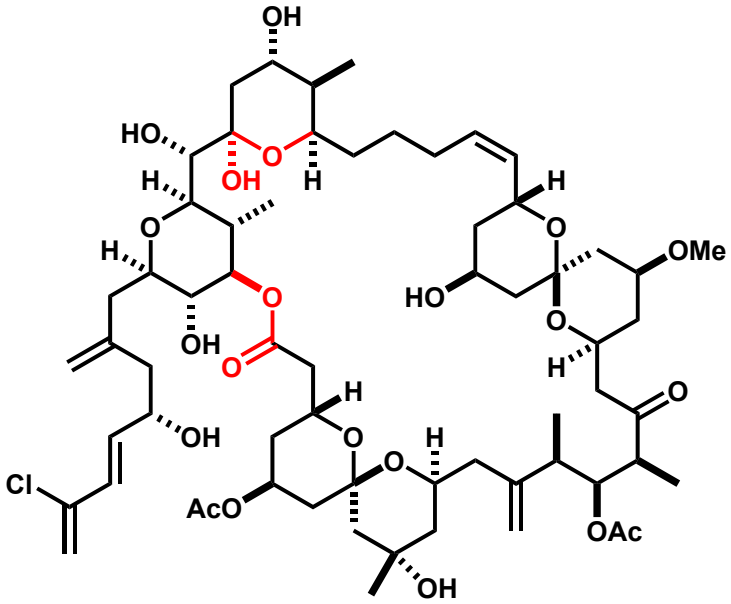
seco-acid of compund 17: 0 kJ/mol



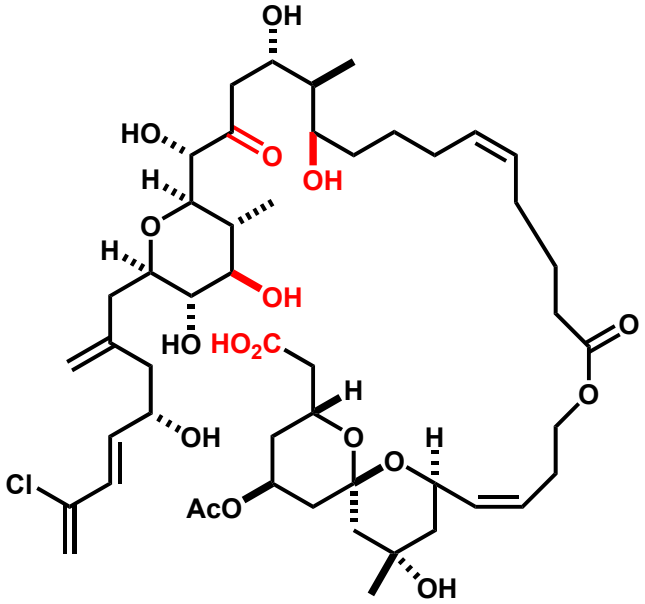
compund 17: 45.2 kJ/mol



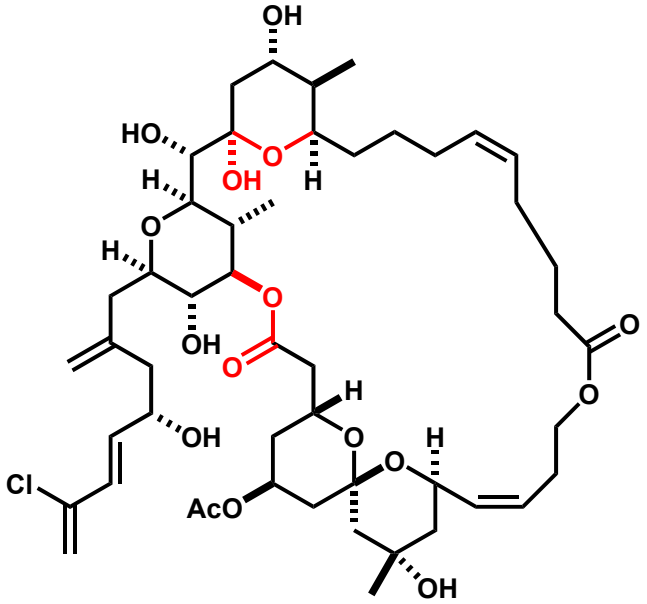
open form of spongistatin 1: 0 kJ/mol



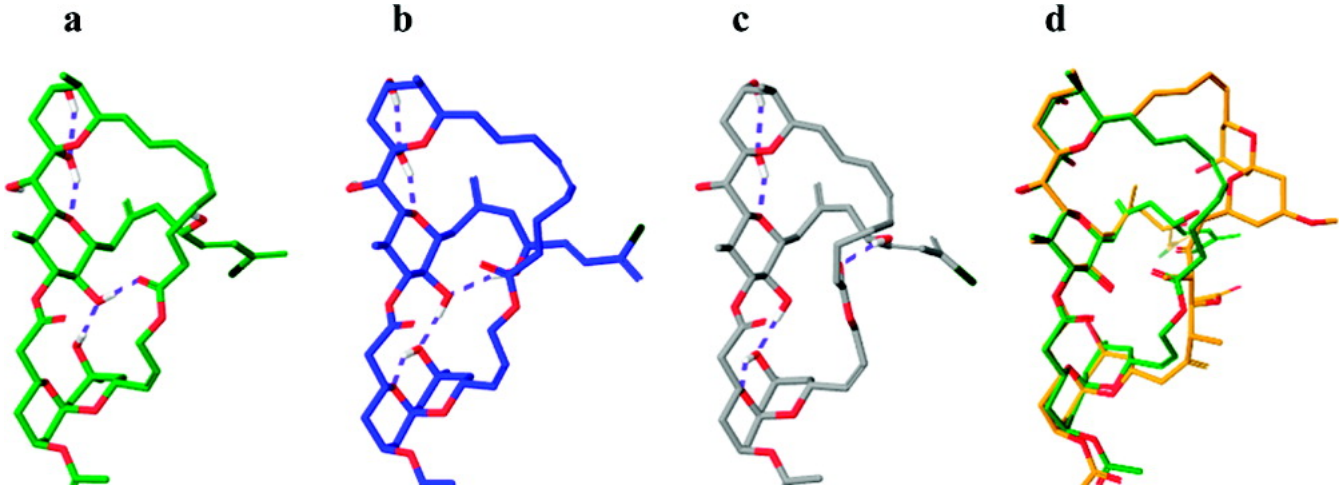
spongistatin 1: 8.3 kJ/mol



open form of ABEF analog: 0 kJ/mol



ABEF analog: 9.1 kJ/mol

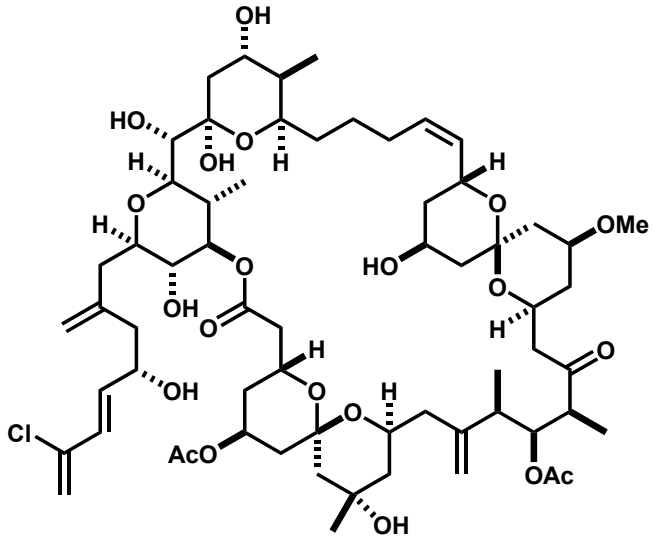


59%

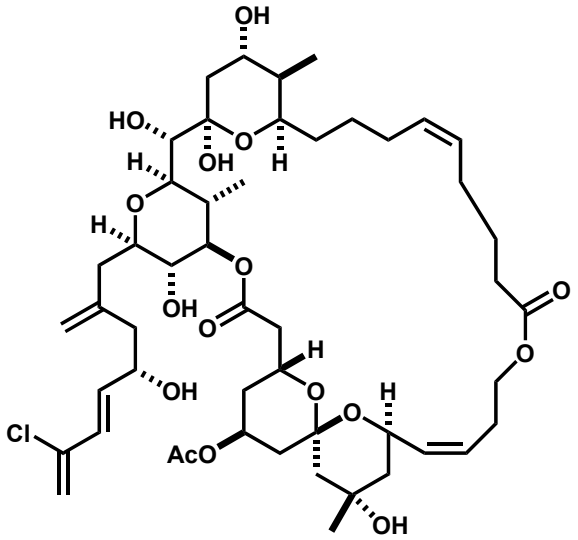
33%

8%

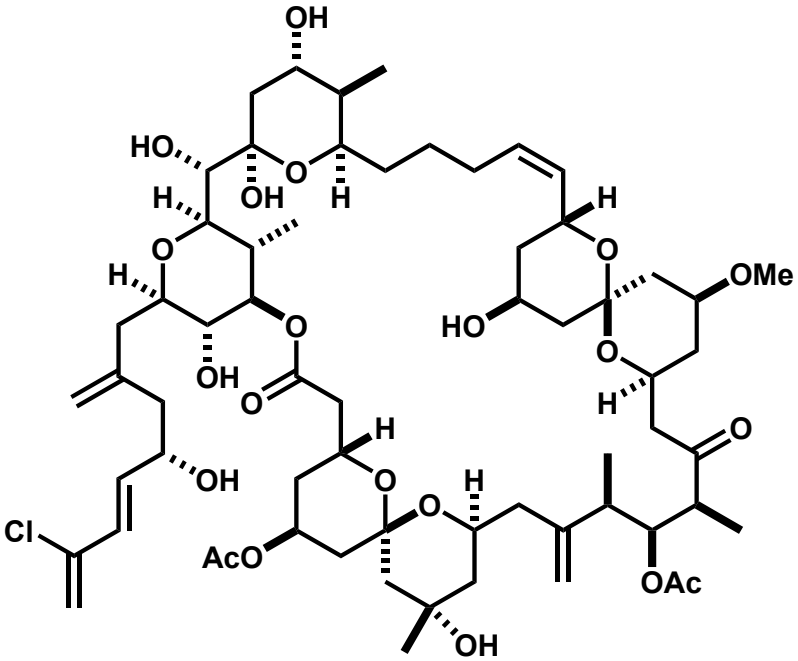
overlay of conformer **a** of ABEF analog and spongstatin 1



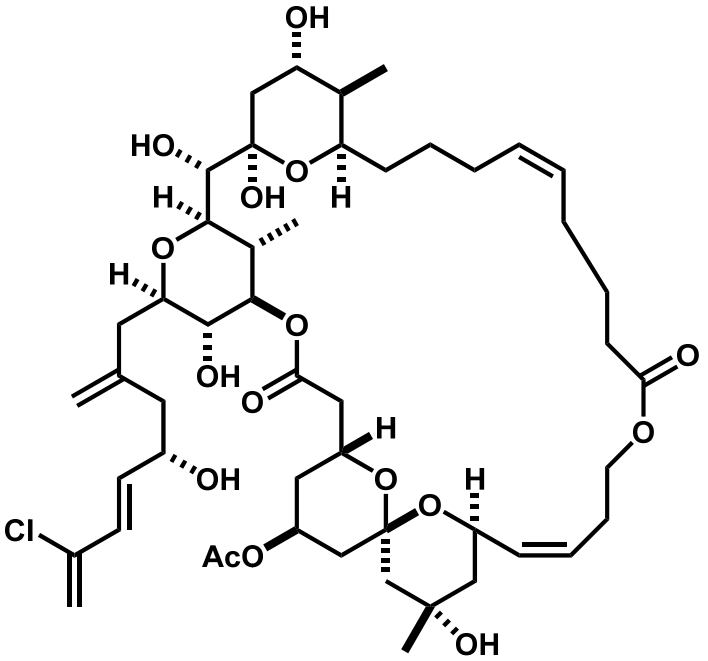
spongistatin 1



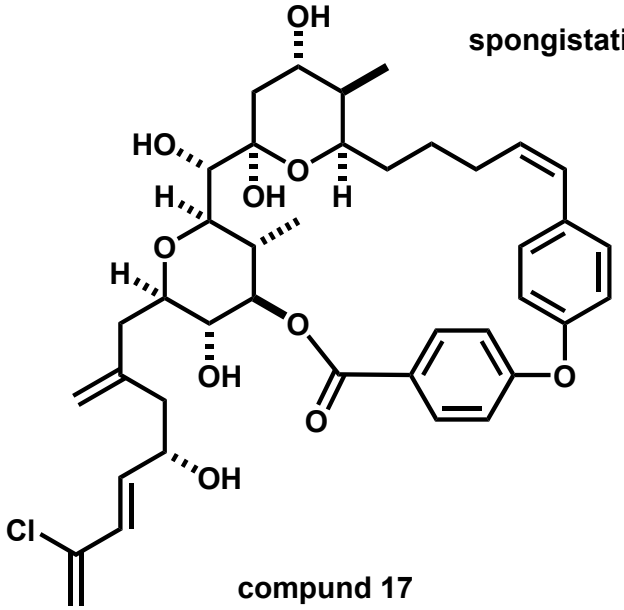
ABEF analog



spongistatin 1



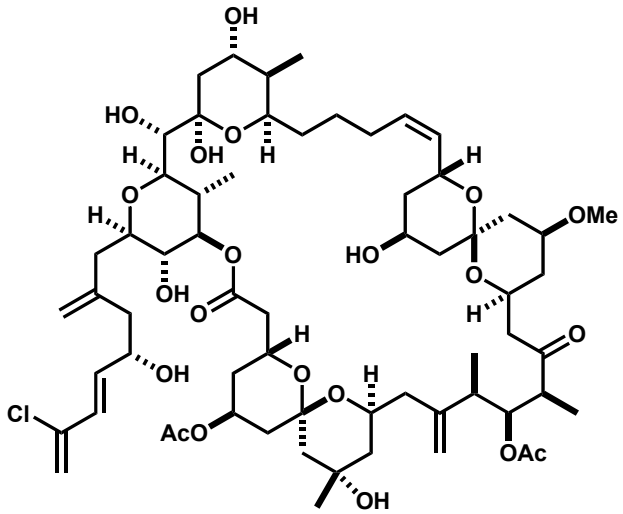
ABEF analog



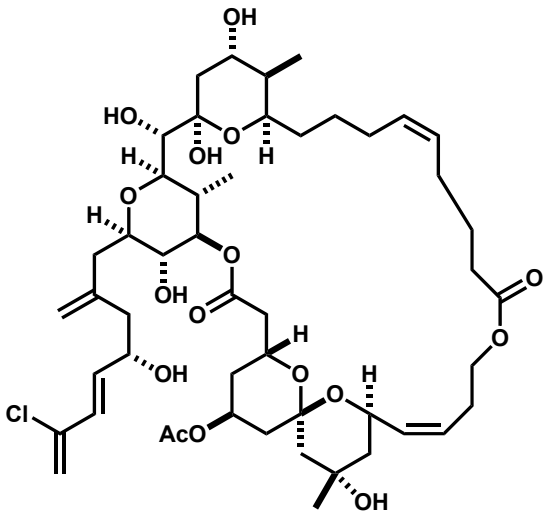
compund 17

Table 3. Cell Growth Inhibition (IC_{50}, nM) with Spongistatin 1 and the Synthesized Analogues

	MDA-MB-435	HT-29	H522-T1	U937
(+)-1	0.0225	0.058	0.16	0.059
(−)-38	82.8	161.2	297.2	60.5
17	>1000	>1000	>1000	>1000



spongistatin 1

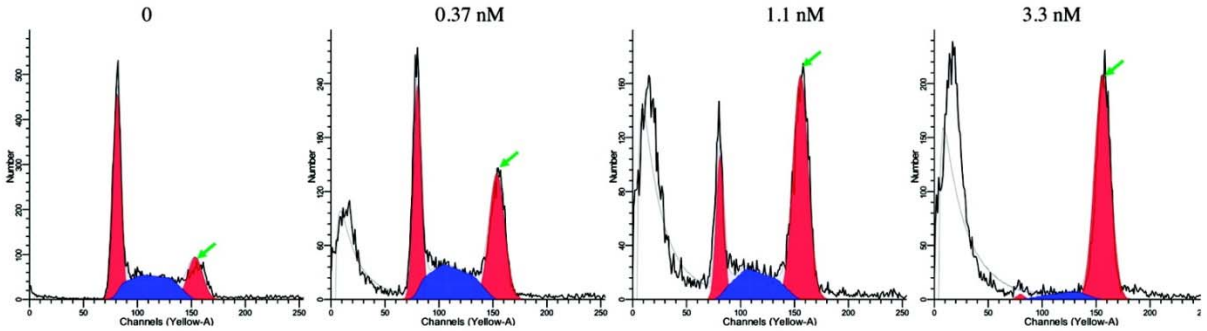


ABEF analog

A.

B.

(+)-Spongistatin 1



ABEF ring analog (–)-**38**