Chasing Molecules that Were Never There: Misassigned Natural Products and the Role of CASE Systems in Modern Structure Elucidation

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Natural Products Synthesis

Chasing Molecules That Were Never There: Misassigned Natural Products and the Role of Chemical Synthesis in Modern Structure Elucidation

K. C. Nicolaou and Scott A. Snyder*

K. C. Nicolaou was born in Cyprus and educated in England and the USA. He is currently Chairman of the Department of Chemistry at The Scripps Research Institute, and is also Professor of Chemistry at the University of California, San Diego. His impact on chemistry, biology, and medicine is reflected in nearly 600 publications and 57 patents, and he has trained hundreds of graduate students and postdoctoral fellows. His Classics in Total Synthesis series, co-authored with Erik J. Sorensen and Scott A. Snyder, is a source of inspiration for students and organic chemists around the world.

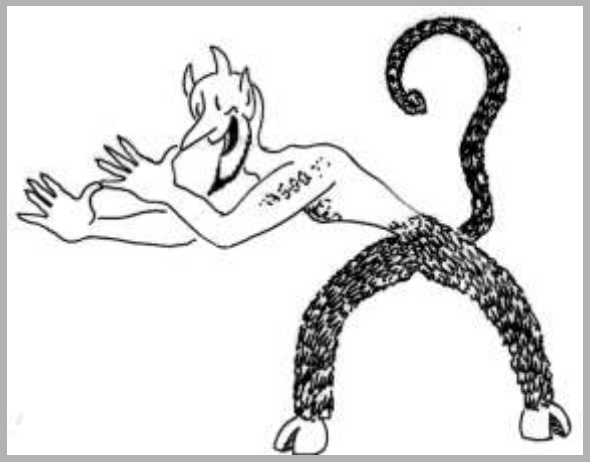
Scott A. Snyder, born in Palo Alto, California, received his BA in chemistry from Williams College in 1999. He completed his PhD in May 2004 at The Scripps Research Institute with K. C. Nicolaou on the total synthesis of diazonamide A and is currently an NIH postdoctoral fellow with E. J. Corey at Harvard University. He is co-author of Classics in Total Synthesis II and has contributed to over 30 publications, review articles, book chapters, and patents. He received predoctoral fellowships from the National Science Foundation, Pfizer, and Bristol-Myers Squibb.

K.C. NICOLAOU **S.A. SNYDER**

***Angewandte Chemie, Int. Ed.*, 2005**, *44*, 1012-1044

About 1000 articles were published last 15 years, where originally determined structures were revised

Journal Of Erroneous Structure



Journal Of Revised Structure

The cost of erroneous structure assignment

40 - 45 issues
of J. Erron. Struct.

> 40-50 issues
of J. Revis. Struct.

There exists a scientific problem:
Reduce the stream of articles containing mistaken structures.

«…There is a long way to go before natural product characterization can be considered a process devoid of … unavoidable pitfalls”.

K.C. NICOLAOU, S.A. SNYDER

Main Postulates of the Structure Elucidation Philosophy

- **Statements used for Structure Elucidation can be considered as either "axioms" or hypotheses.**
- **Setting axioms and hypotheses associated with a given problem is equivalent to the creation of some *particular axiomatic theory***
- **To obtain a valid solution to the problem, the set of axioms must be true, consistent, and complete.**

I. Axioms and hypotheses reflecting characteristic spectral features

A typical axiom confirmed by scientific experience:

If a molecule contains fragment A_i then its signals are observed in spectral ranges $X_1, X_2, \ldots X_j, \ldots X_m$ which are characteristic for this fragment.

CH2 → [1450 cm^{-1}], CH3 → [1380 cm^{-1}] /\ [1450cm^{-1}]

"no band at [1450 cm^{-1}] → fragment CH2 is absent"

A typical hypothesis related to a given problem only:

If a signal is observed in a spectrum range X_j then the molecule contains at least one fragment of the set $A_i(X_j)$, $A_k(X_j)$, ... $A_l(X_j)$.

1450 cm^{-1} → CH2 \/ CH3

II. Axioms (hypotheses) of 2D NMR Spectroscopy. *"Standard Correlations"*

COSY

- **If a peak (H-1, H-2) is observed in COSY, then a molecule contains the chemical bond**

  (C-1)–––(C-2)

HMBC

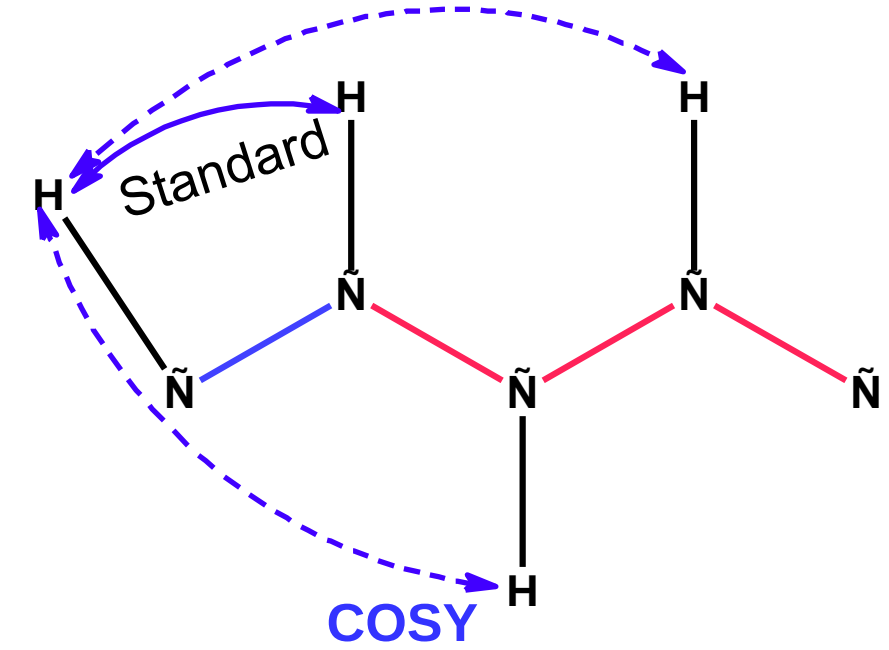
- **If a peak (H-1, C-*2*) is observed in HMBC, then atoms C-1 and C-*2* are separated in the structure by ONE or TWO chemical bonds:**

  (C-1)–––(C-2) or (C-1)––(X)––(C-*2*), X=C, O, N…

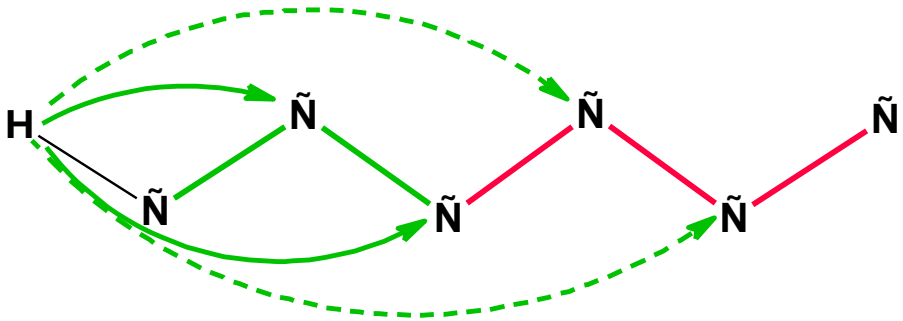
NOESY

- **If a peak (H-1, H-2) is observed in NOESY, then the distance between H-1 и H-2 <u>in space</u> is less than 5Å**

II. Axioms (hypotheses) of 2D NMR Spectroscopy. *"Nonstandard Correlations", NSCs*



COSY



HMBC

- **Signals of "*Standard*" and "*Nonstandard*" correlations are experimentally not distinguishable in 2D NMR.**
- **If the 2D NMR data contain *Standard* and *Nonstandard* correlations the set of axioms becomes contradictory.**

III. Structural axioms

necessary for structure assembling

- **Allowable chemical composition: CHO, CHNO, CHNOS…**
- **Possible molecular formula (formulae)**
- **Valences of atoms: N(3,4,5), S(2, 4, 6), P(3, 5)**
- **Hybridization of each carbon atom : *sp^3*, *sp^2*, *sp*, *sp^3* or *sp^2*, *not defined***
- **Carbon neighborhood with heteroatoms (C-X):**

  fb* (X is forbidden), *ob* (X is obligatory), *not defined
- **List of obligatory fragments**
- **List of forbidden fragments**

Properties of information used for the structure elucidation

- **Information is fuzzy by the nature (2 or 3 bonds between H and C in HMBC)**
- **Not all expected correlations are observed in spectra, i.e. information is incomplete**
- **Presence of nonstandard correlations (NSCs) frequently makes information contradictory**
- **Number of NSCs and their lengths are *unknown*. Signal overlapping leads to the appearance of *ambiguous* correlations. Information is otherwise uncertain**
- **Information can be false if a mistaken hypothesis is suggested**
- **"Structural axioms" reflects *subjective* opinion of the researcher. Information is in addition subjective.**

Consequences:

- **The human expert is frequently unable to explicitly take into account all "axioms" and derive all plausible structural hypotheses.**
- **As a result different researchers may come to different structures from the same experimental data!**
- **Algorithms and software are necessary that would be capable of inferring a valid solution from an equivocal information.**

Expert system Structure Elucidator - a Tool for Deriving and Verifying Structural Hypotheses from Equivocal Information.

- **The system was specially developed for processing the fuzzy, contradictory, incomplete, uncertain, subjective and false information.**
- **In frames of the system, each problem is formulated in terms of a partial axiomatic theory.**
- **The system includes an inference machine capable of generating all (without any exception) structural hypotheses in a reasonable time.**
- **The Most Probable Structure is selected using fast algorithms of ^{13}C NMR spectrum prediction**

See Structure Elucidator overview in:

“Computer-assisted methods for molecular structure elucidation:

Realizing a spectroscopist’s dream”.

M. Elyashberg, K. Blinov, S. Molodtsov,

Y. Smurnyy, A. Williams, T. Churanova

***Journal of Cheminformatics* 2009, 1:3**

doi:10.1186/1758-2946-1-3

(free assessed)

Structure Elucidator as a "Polygraph Detector."

EXAMPLES

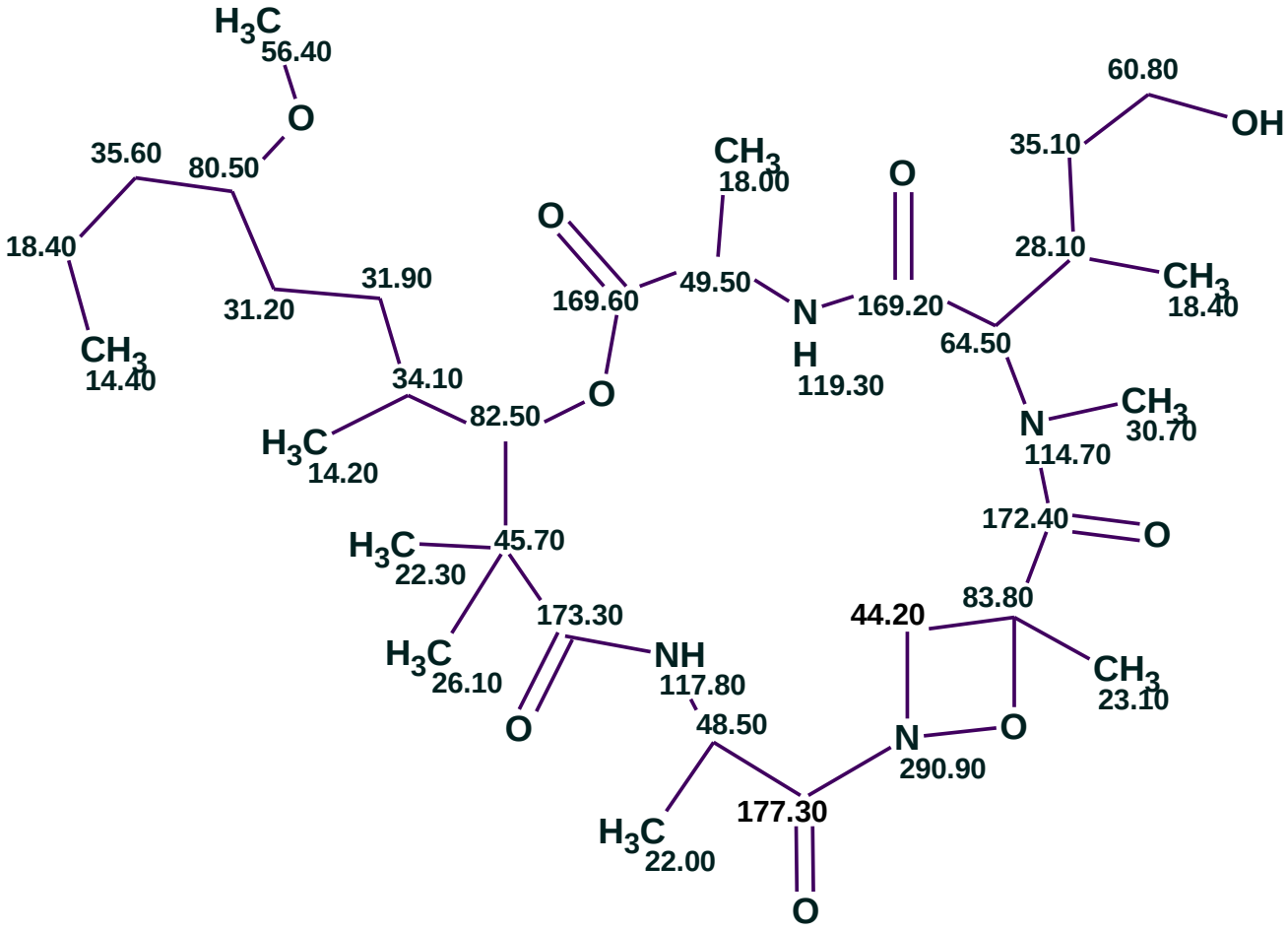
“Halipeptins A and B: Two Novel Potent Anti-inflammatory Cyclic Depsipeptides from the Vanuatu Marine Sponge *Haliclona* species”.

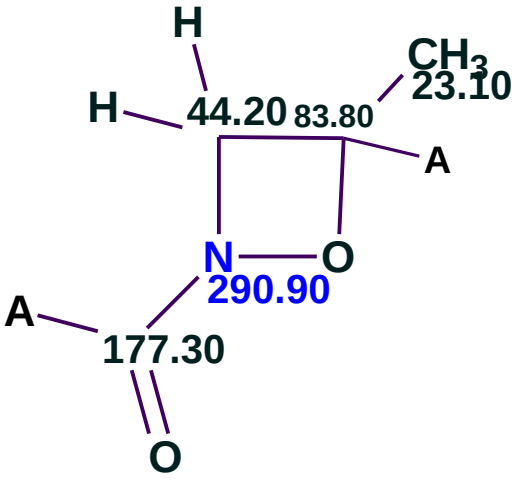
A. Randazzo, G. Bifulco, C. Giannini, M. Bucci, C. Debitus, G. Cirino, L. Gomez-Paloma.

***J. Am. Chem. Soc*. 2001, 123, 10870**

Hypotheses utilized by authors:
Elemental composition CHNO, $C_{31}H_{54}N_4O_9$, N(3)
1D and 2D NMR data

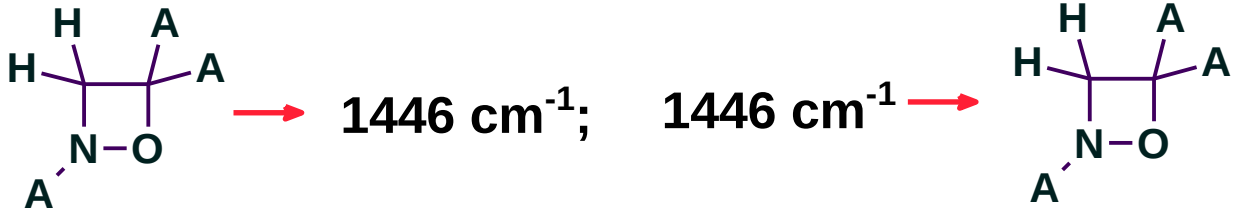
The derived structure:





"…a rather intriguing moiety, being an unprecedented four-membered N-O cyclic form …"

1. "The presence of N-O was inferred from a characteristic IR band at 1446 cm-1 (stretchings in this range have already been observed in these systems <axiom>)".

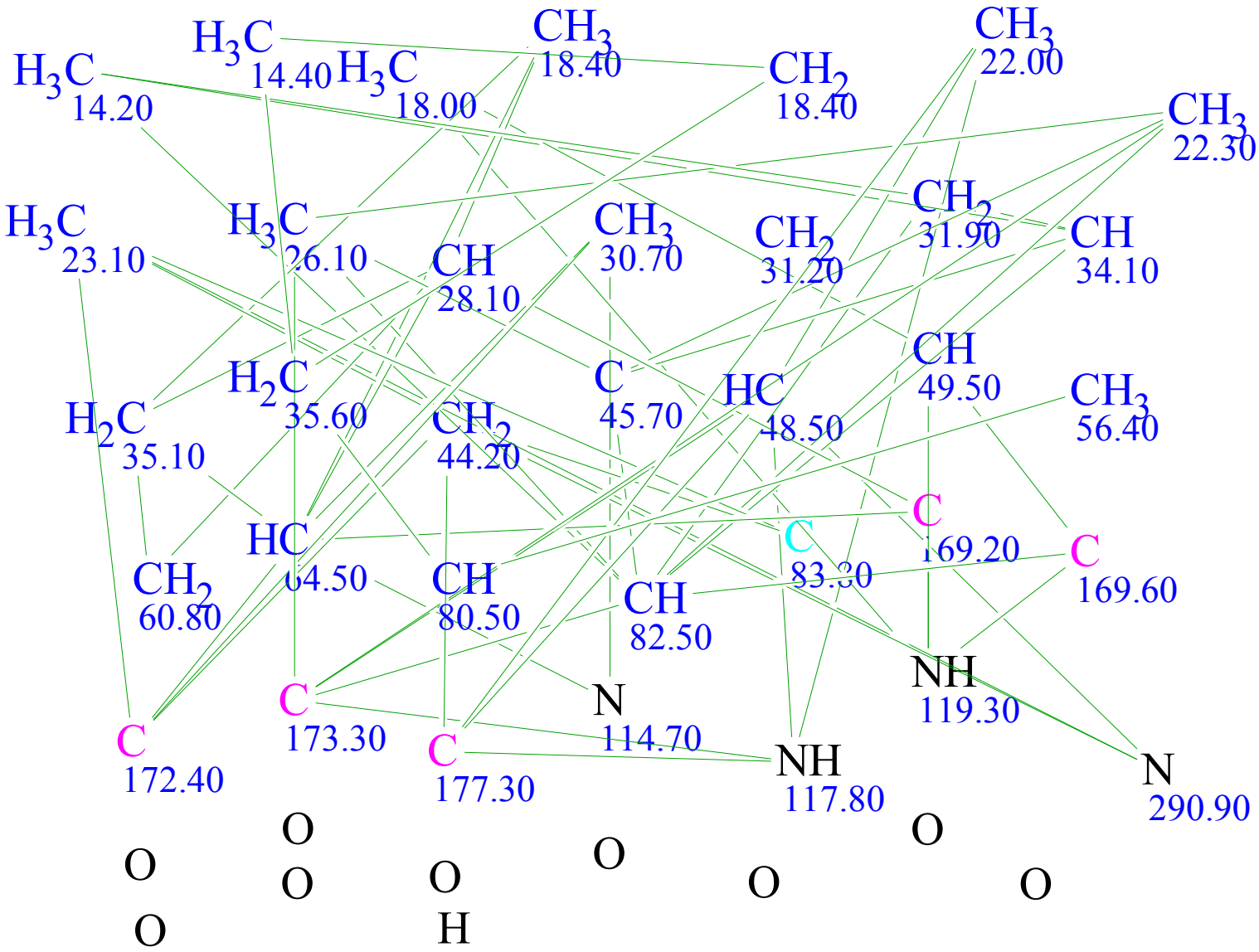


1446 cm^{-1} → CH2 \/ CH3

2. "The unusual chemical shift of nitrogen (291, typical 110-120 ppm) could be explained in terms of an effect by ring strain"

Application of StrucEluc to author's hypotheses.

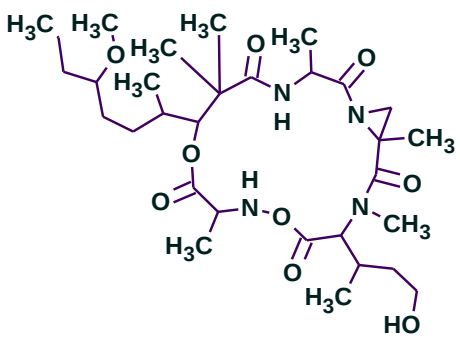
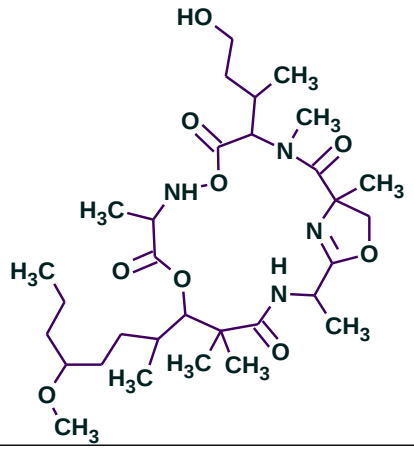
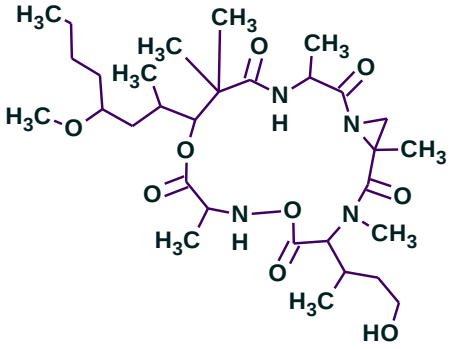
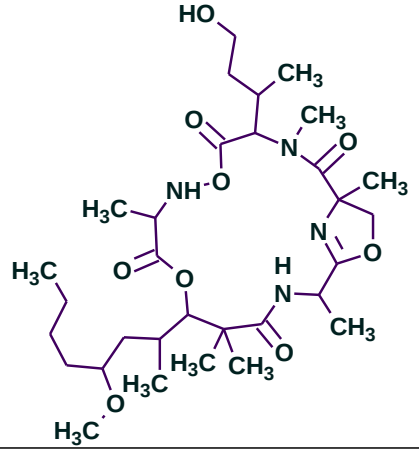
Molecular Connectivity Diagram (MCD)



Strict Structure Generation using hypotheses:

$C_{31}H_{54}N_4O_9$, no NSCs, N(3), No X≡Y, N-O bond allowed.

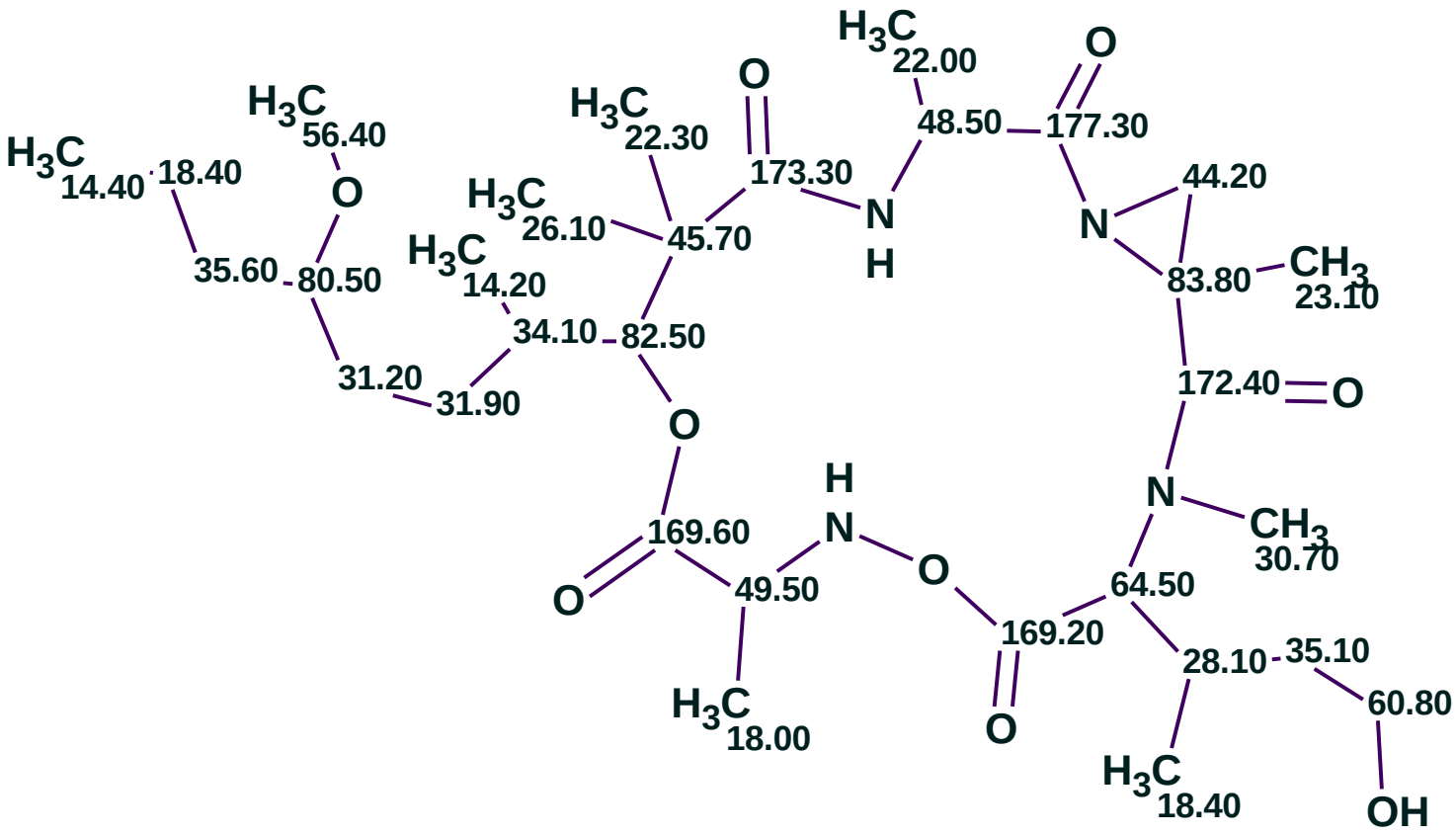
Results: *k* = 6 → 4, t_g = 0.1 s

1 **Best** 	2 
$d_A(^{13}C)$: 3.606	$d_A(^{13}C)$: 3.725
3 	4 
$d_A(^{13}C)$: 4.323	$d_A(^{13}C)$: 4.452

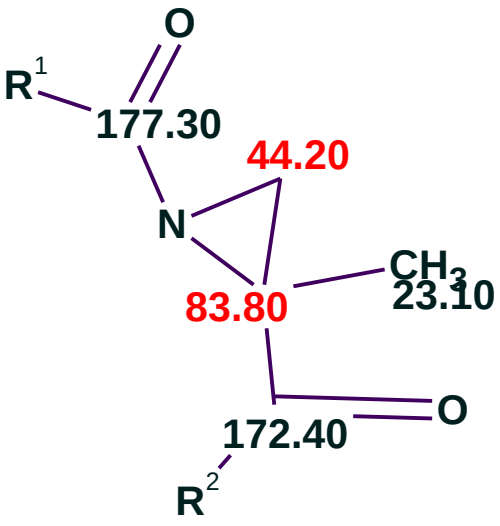
Average deviations of the Best Structure in the ranked file:

d_A =d(HOSE) = 3.60 ppm
d_N = d(NN) = 3.62 ppm
d_I = d(Inc) = 3.43 ppm

Prediction Accuracy
1.6-1.8 ppm

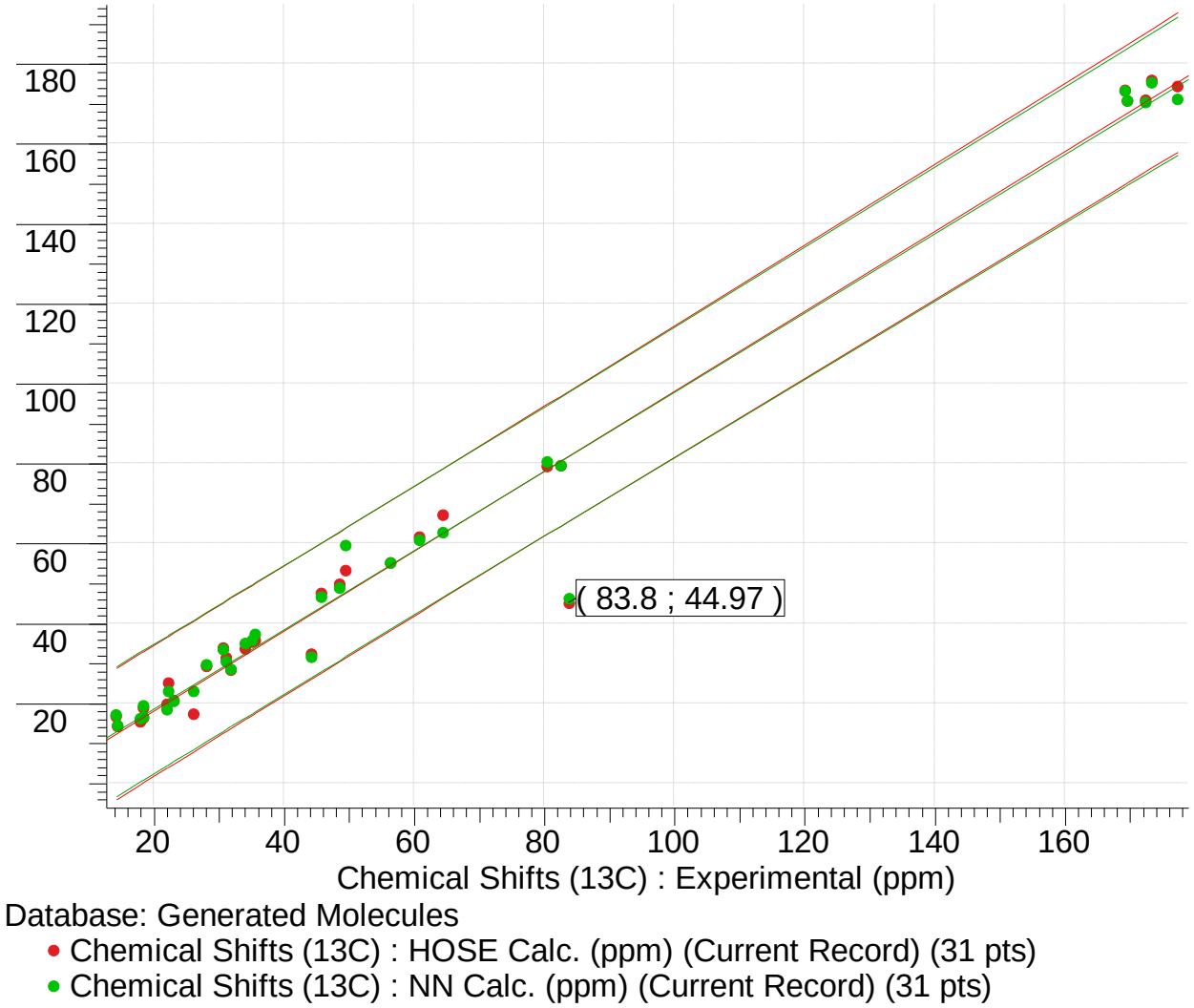


Calculated 13C chemical shifts (Y) vs. experimental ones (X).

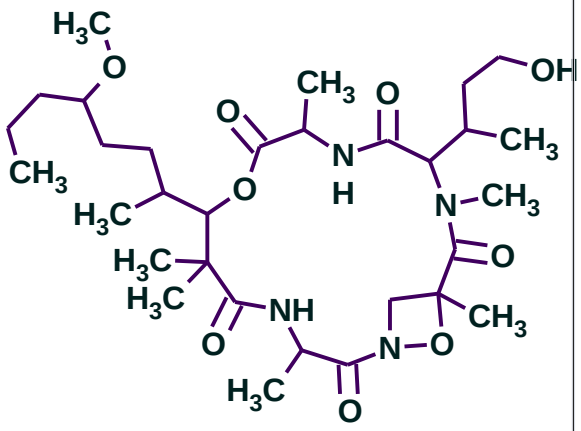
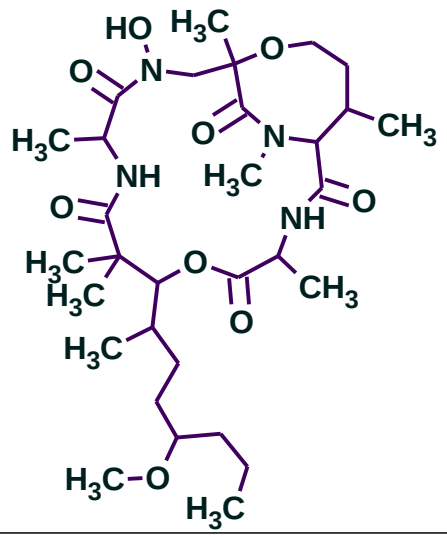
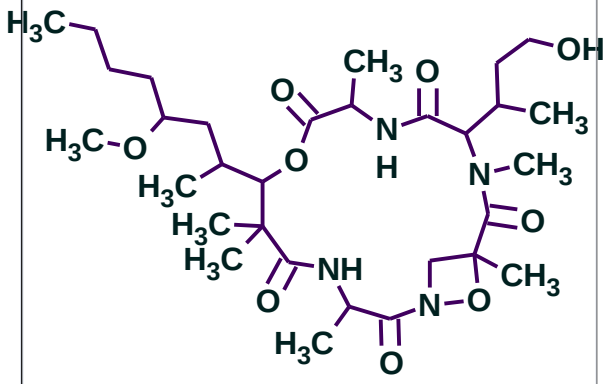


d_A^{max} = 40 ppm !

A hint to the presence of NSCs in 2D NMR!



Fuzzy Structure Generation: m=1-15, a=x
Results: k=304→284→183, t_g = 35 s

1 Original	2	3
		
$d_A(^{13}C)$: 1.526 $d_I(^{13}C)$: 1.714 $d_N(^{13}C)$: 1.879	$d_A(^{13}C)$: 2.140 $d_I(^{13}C)$: 2.217 $d_N(^{13}C)$: 2.207	$d_A(^{13}C)$: 2.218 $d_I(^{13}C)$: 2.456 $d_N(^{13}C)$: 2.480

$d_A(^{13}C)$: 1.526
$d_I(^{13}C)$: 1.714
$d_N(^{13}C)$: 1.879

Prediction Accuracy
1.6-1.8 ppm

“Structural Revision of Halipeptins: Synthesis of the Thiazoline Unit and Isolation of Halipeptin”.

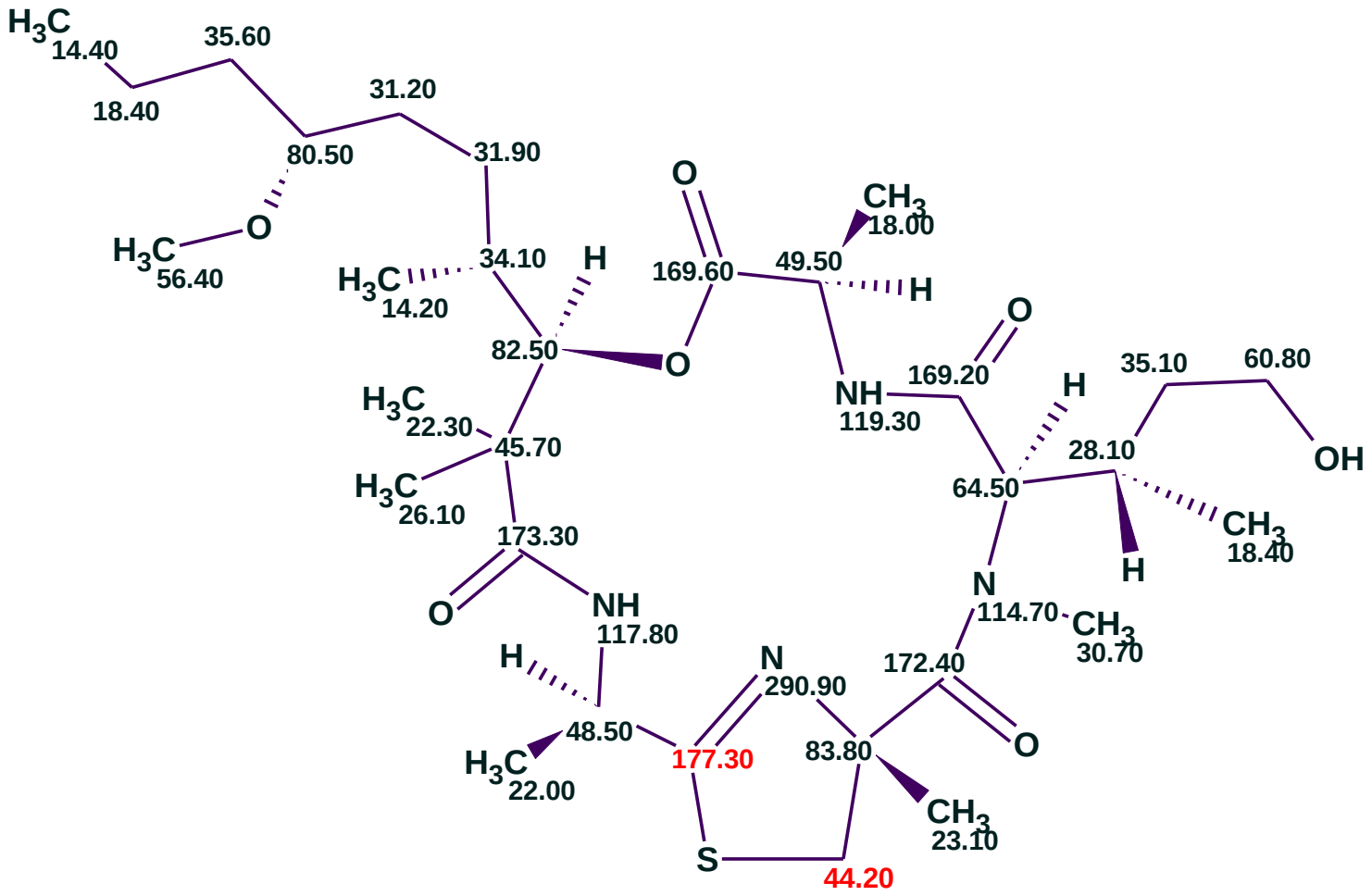
C. D. Monico, A. Randazzo, G. Bifulco, P. Cimino, M. Aquino, I. Izzo, F. De Riccardis, L. Gomez-Paloma.

***Tetrahedron Lett*. 2002, 43, 5707.**

New hypothesis from higher MS resolution (20,000).

Allowed composition: CHNOS**, $C_{31}H_{54}N_4O_7S$, M=626.8481**

For comparison: $C_{31}H_{54}N_4O_9$, M=626,7819

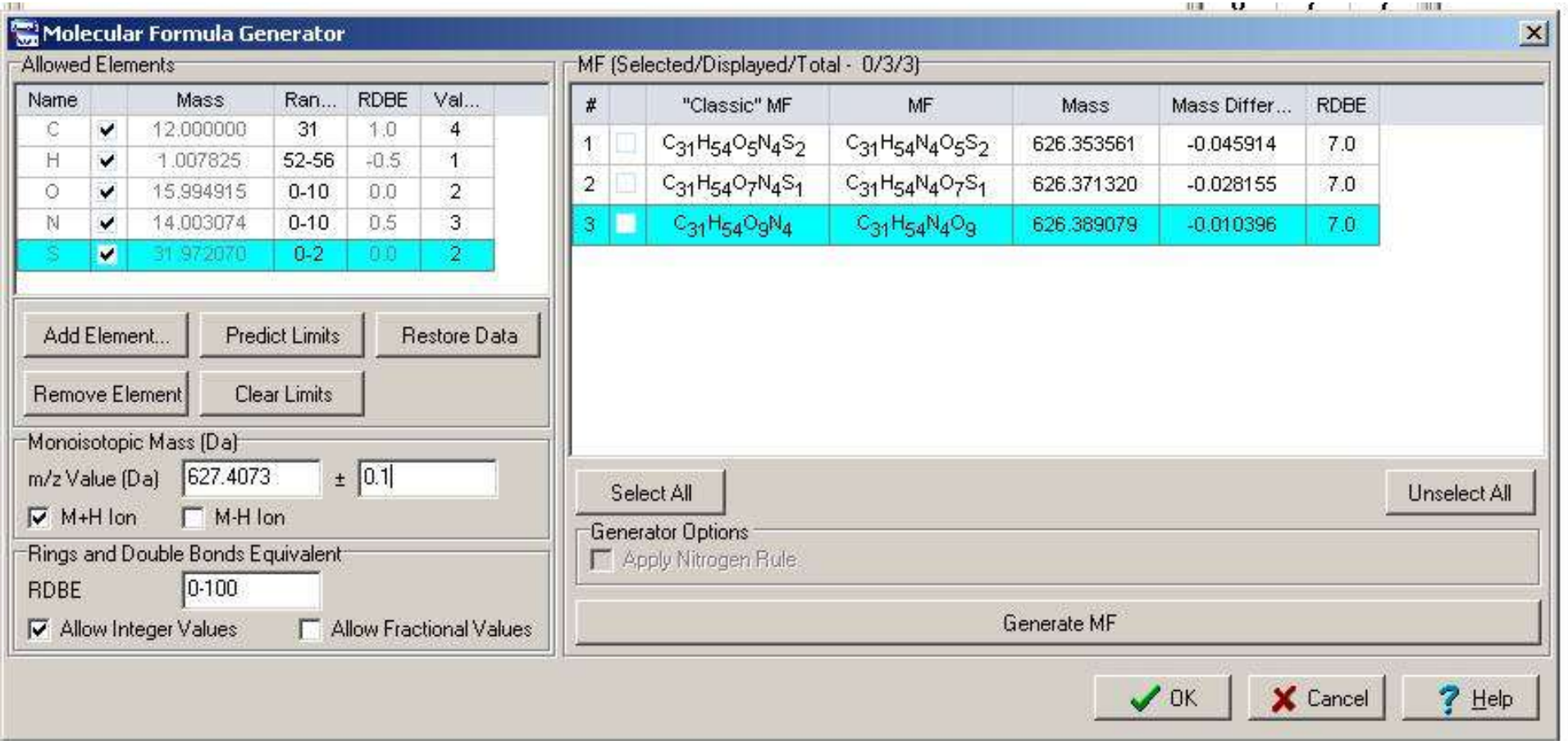


What would happen if StrucEluc was used from the very beginning?

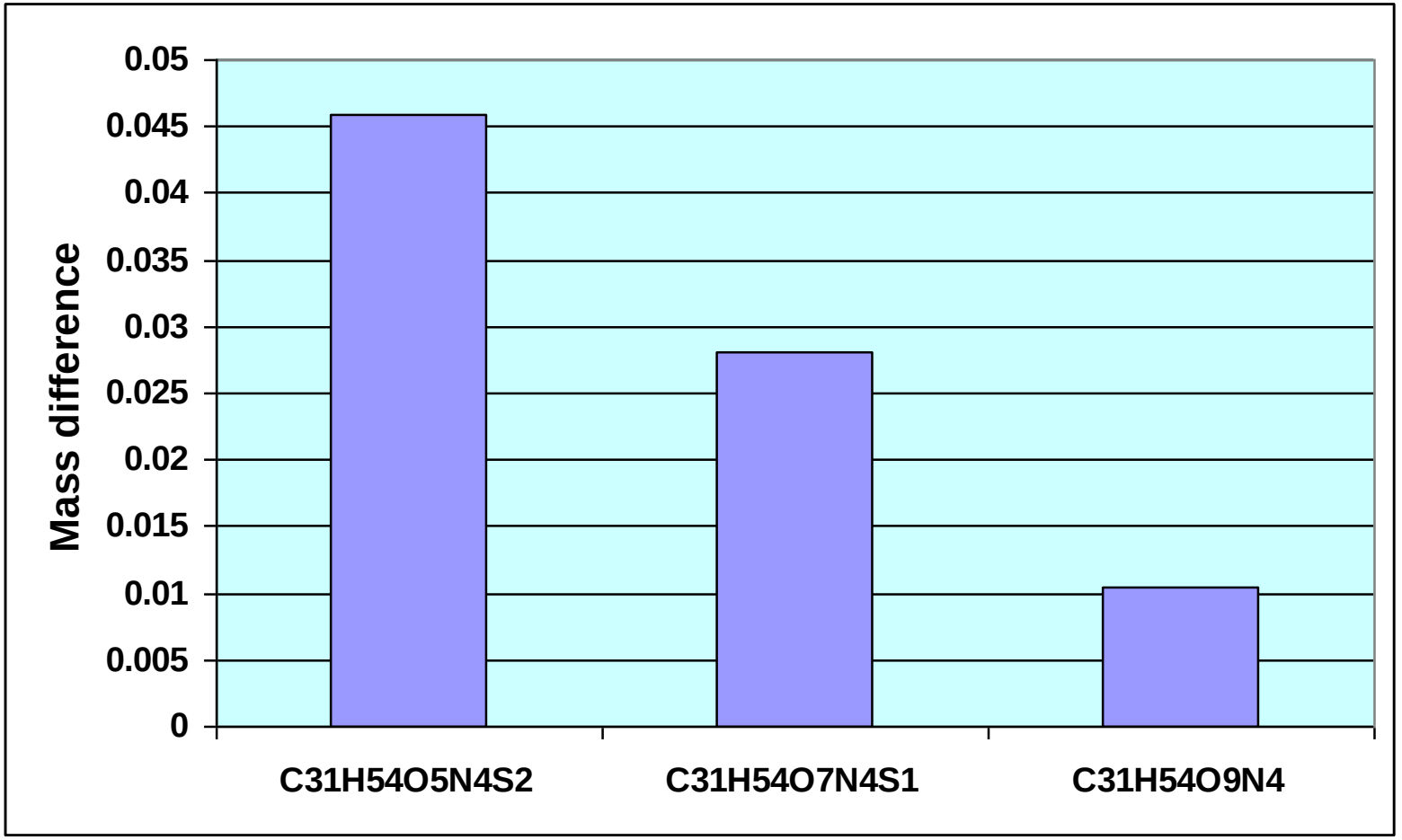
Hypotheses (constraints) on elemental composition:

C(31), H(52-56), O(0-10), N(0-10), S(0-2)

Modest MS resolution, m/z $(M+H)^+$ = 627.4073



Mass differences $|m/z_{(exp)} - M_{(calc)}|$ for three possible molecular formulae

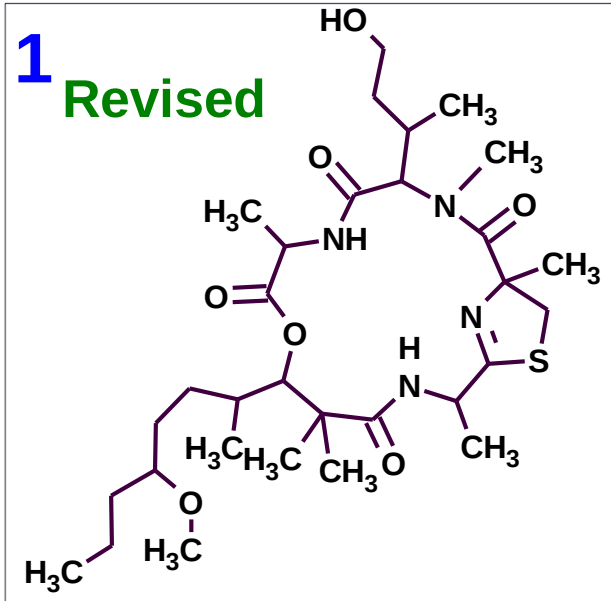
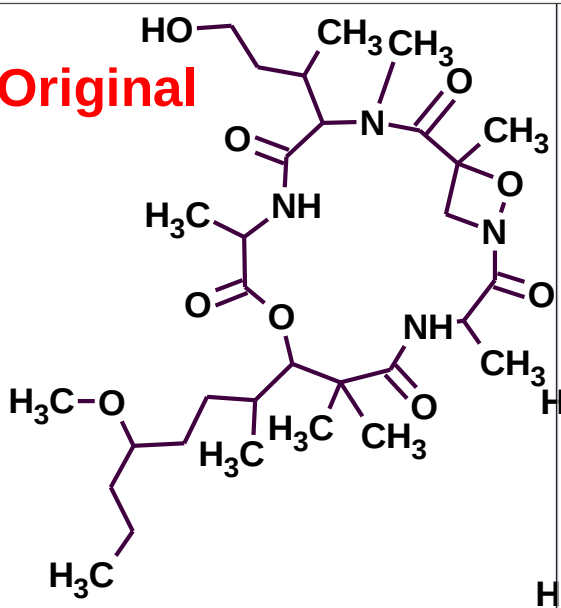
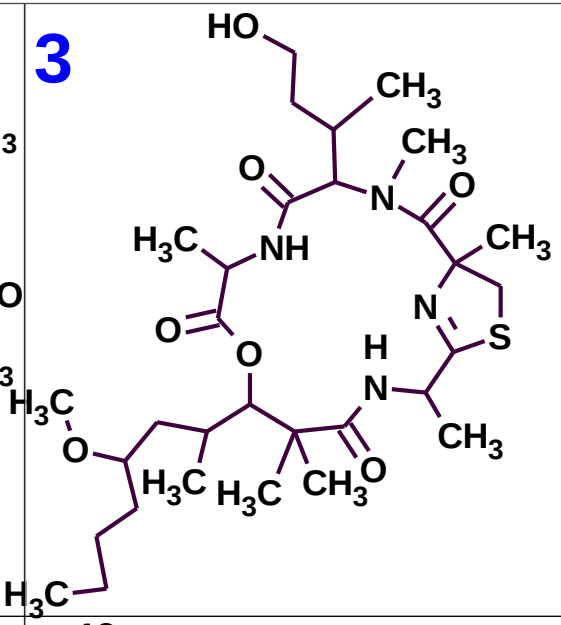


Structure generation from $C_{31}H_{54}N_4O_7S$ and $C_{31}H_{54}N_4O_9$

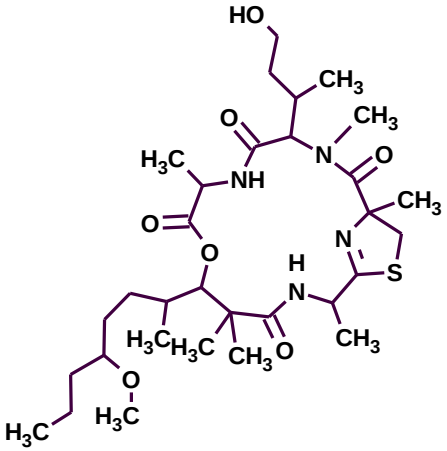
Hypotheses: N(3),S(2). No X≡Y. N-O possible.

No User intervention.

Results: k=303, t_g= 36 s

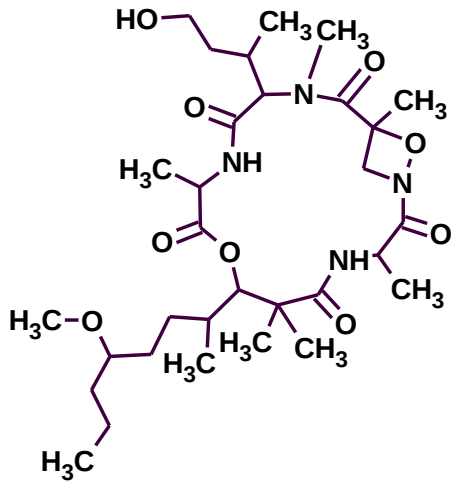
1 Revised	2 Original	3
		
$d_A(^{13}C)$: 1.112 $d_I(^{13}C)$: 1.642 $d_N(^{13}C)$: 1.539	$d_A(^{13}C)$: 1.526 $d_I(^{13}C)$: 1.714 $d_N(^{13}C)$: 1.879	$d_A(^{13}C)$: 1.803 $d_I(^{13}C)$: 2.342 $d_N(^{13}C)$: 2.142
$C_{31}H_{54}N_4O_7S$	$C_{31}H_{54}N_4O_9$	$C_{31}H_{54}N_4O_7S$

«Accuracy» and «Precision» in the choice of the Most Probable Solution.



d=1.11

Precise and accurate



d=1.52

Merely precise…

"A New Inhibitor of 5′-Hydroxyaverantin Dehydrogenase, an Enzyme Involved in Aflatoxin Biosynthesis, from *Trichoderma hamatum*"

E. Sakuno, K. Yabe, T. Hamasaki, H. Nakajima

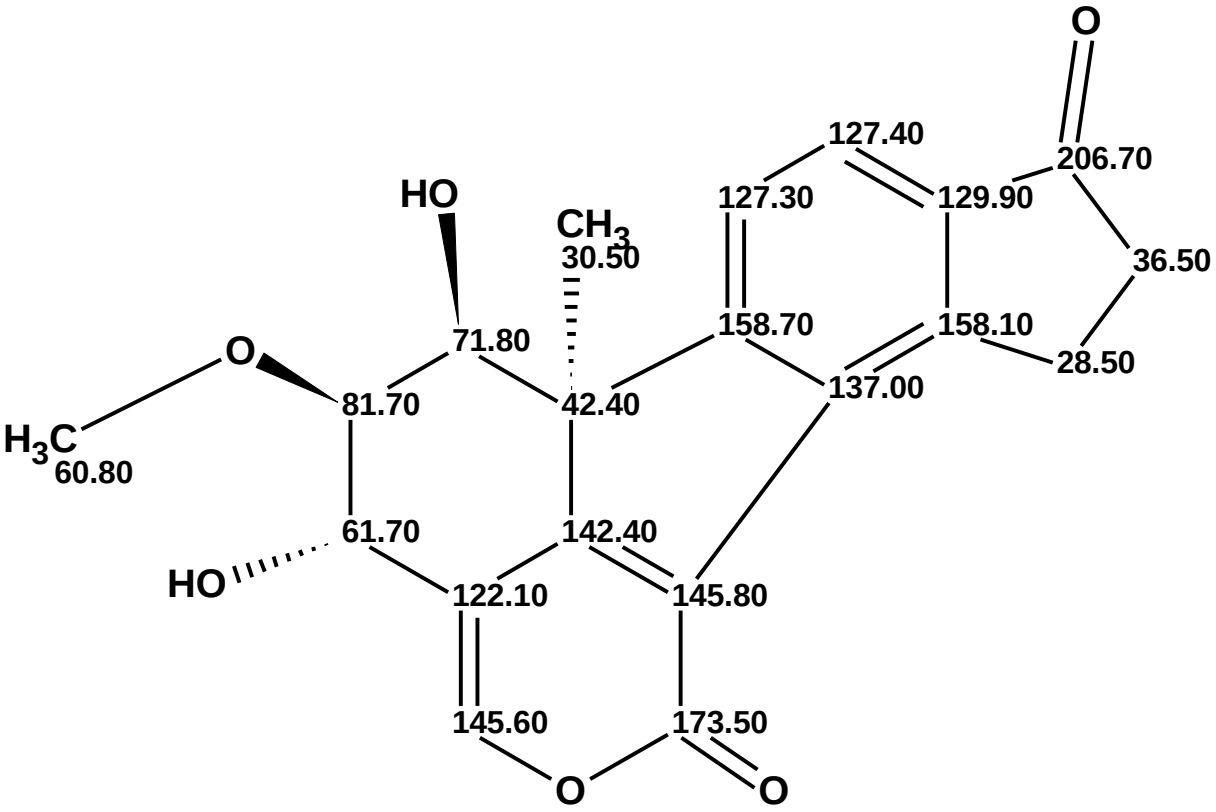
***J. Nat. Prod*. 2000, 63, 1677-1678**

“One ketone and one ester group were indicated by carbon resonances at δ 206.7 and 173.4 and IR bands at 1671 and 1707 cm^{-1}.”

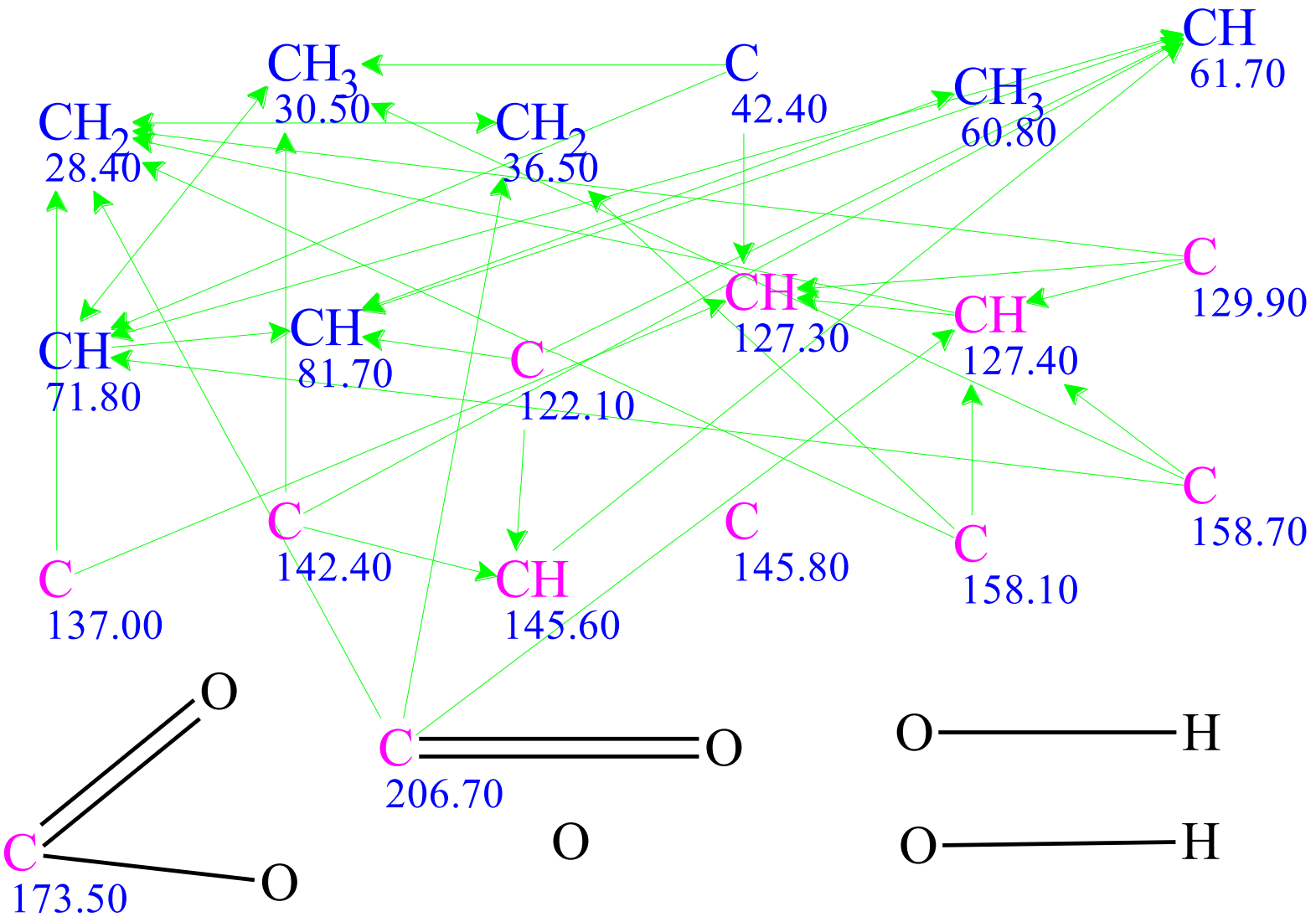
Hypotheses: O-C=O, (C)-C=O(C), 2 (O-H) are present

MF = $C_{20}H_{18}O_6$

The structure derived by authors:

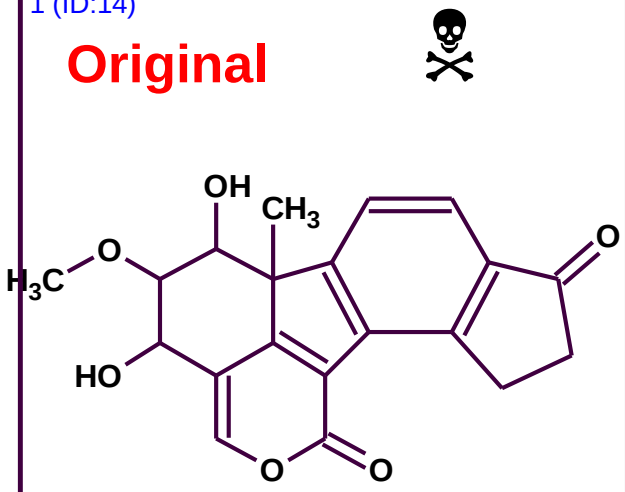
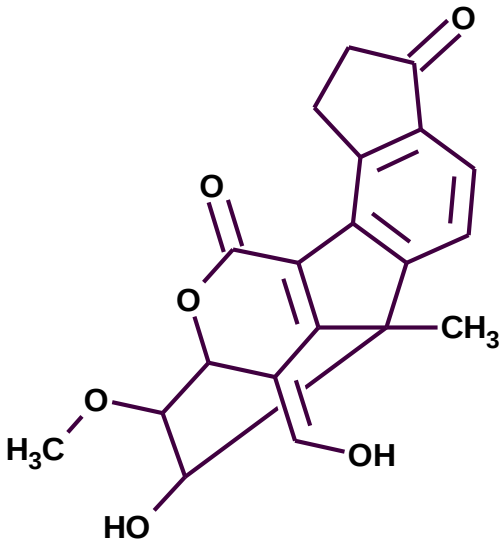
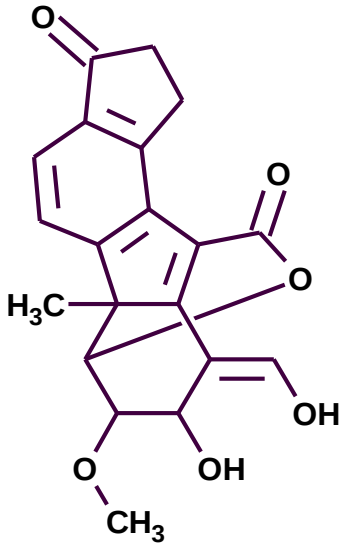


MCD corresponding to the hypotheses postulated by Sakuno *et al*.



Fuzzy Structure Generation: k=174→80→60, t_g = 30 s

Three best structures:

1 (ID:14) Original	2 (ID:13)	3 (ID:15)
		
$d_I(^{13}C)$: 4.486 $d_N(^{13}C)$: 4.137	$d_I(^{13}C)$: 6.176 $d_N(^{13}C)$: 5.453	$d_I(^{13}C)$: 6.410 $d_N(^{13}C)$: 5.565

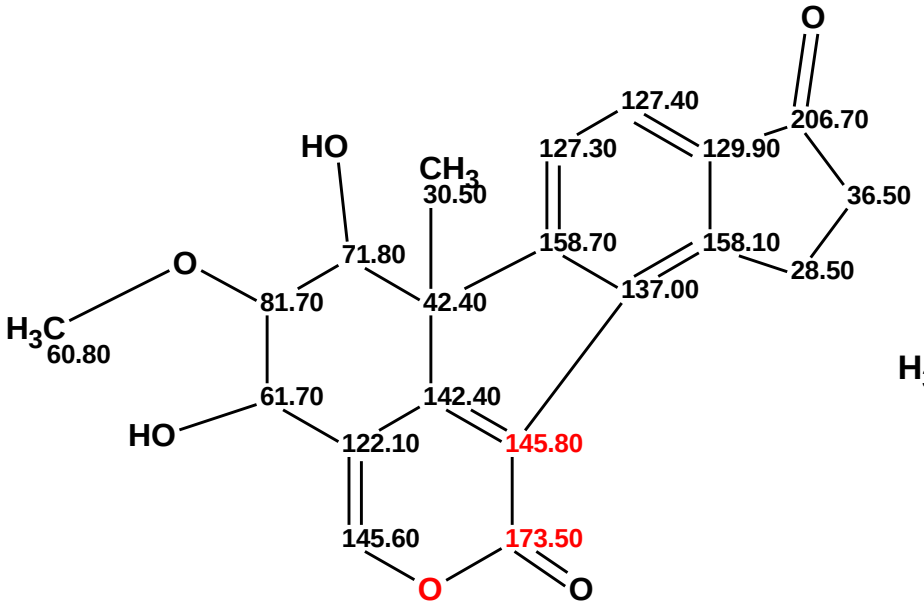
“Structure Reassignment of the Fungal Metabolite TAEMC161 as the Phytotoxin Viridiol”

P. Wipf, A.D. Kerekes

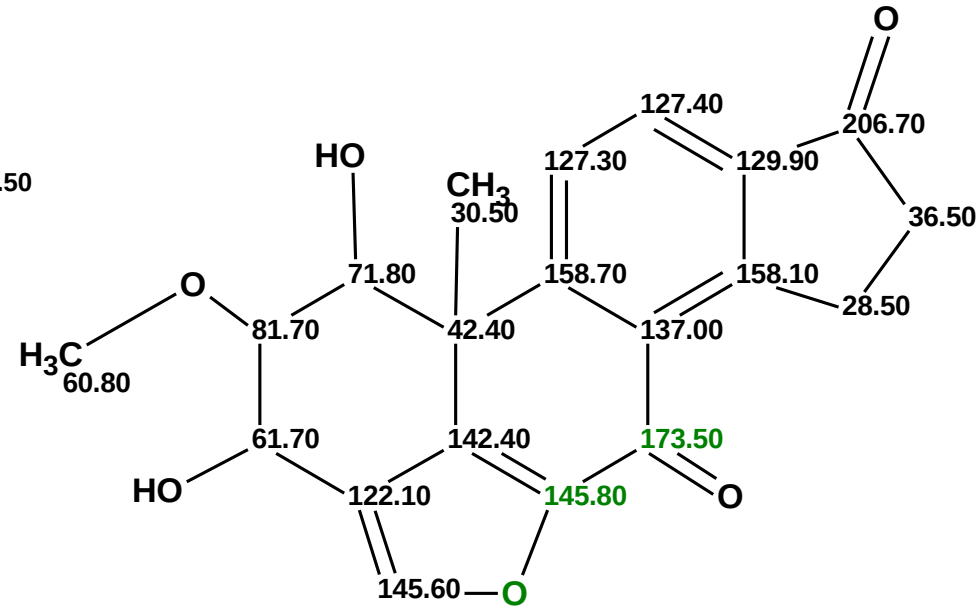
***J. Nat. Prod*. 2003, 66, 716-718**

Structure revision

Original

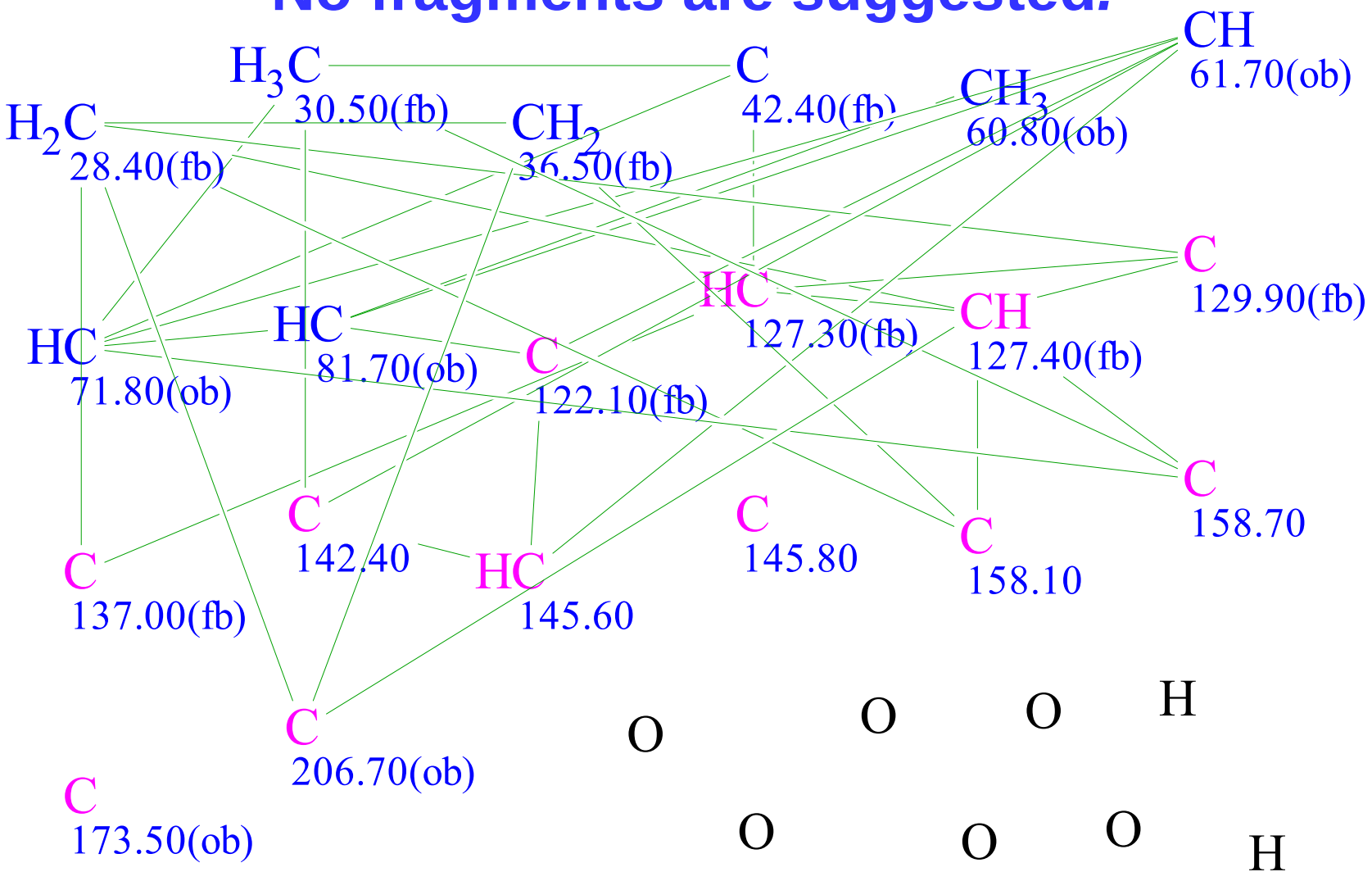


Revised using QM ^{13}C NMR prediction

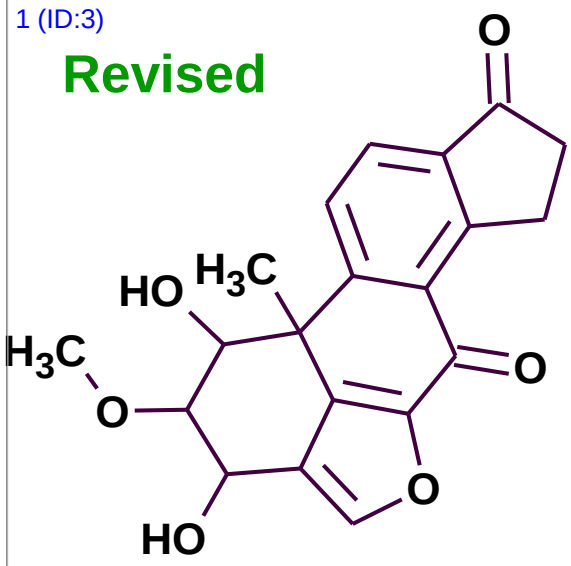
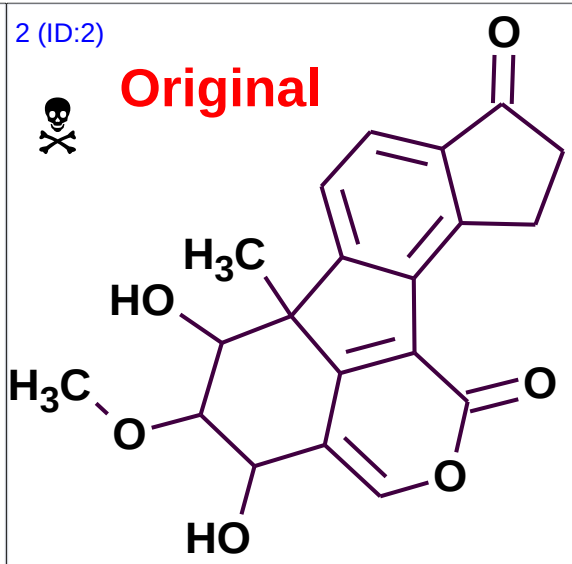
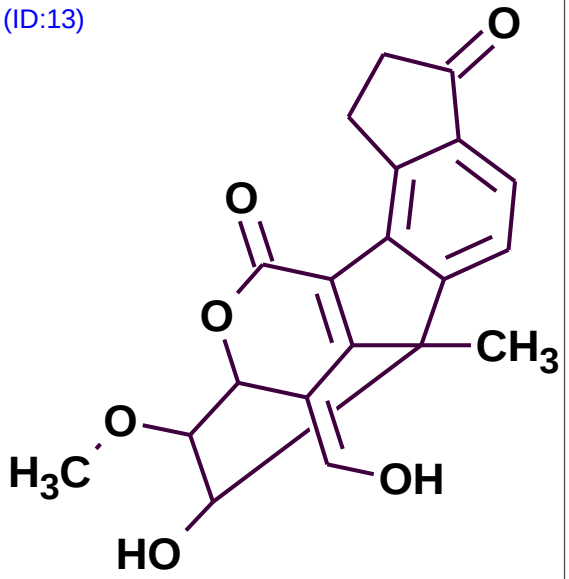


What would happen if StrucEluc was used from the very beginning?

No fragments are suggested.



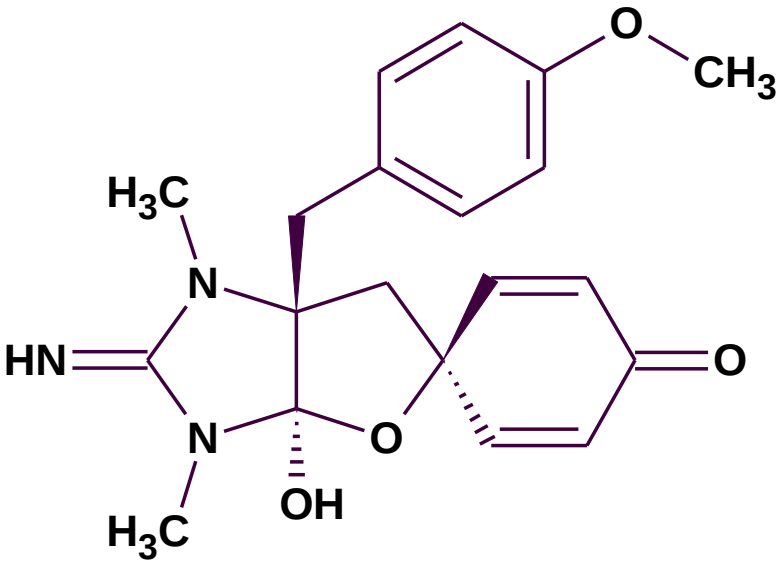
Fuzzy Structure Generation:
m=0-20, a=x, stop at m=m_g
k=494 → 398 → 272, t_g = 1 m 40 s

1 (ID:3) Revised	2 (ID:2) Original	3 (ID:13)
		
$d_I(^{13}C)$: 2.137 $d_N(^{13}C)$: 2.316	$d_I(^{13}C)$: 4.486 $d_N(^{13}C)$: 4.137	$d_I(^{13}C)$: 6.383 $d_N(^{13}C)$: 5.453

"A New Structural Theme in the Imidazole-Containing Alkaloids from a Calcareous Leucetta Sponge"

P. Ralifo, P. Crews, University of California, Santa Cruz

***J. Org. Chem*. 2004, 69, 9025-9029**

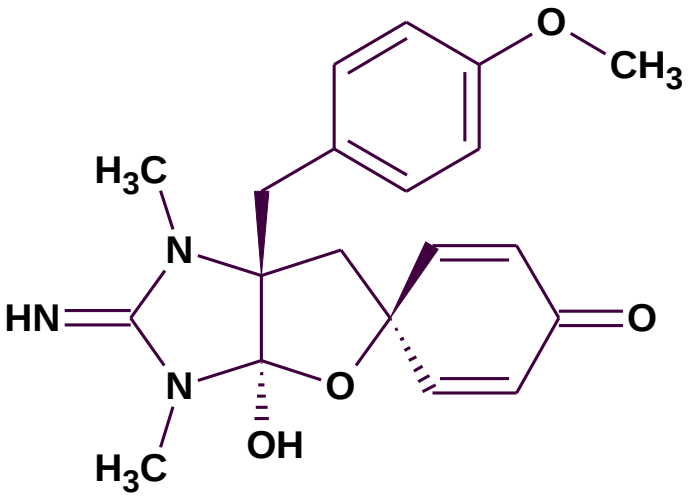


$C_{20}H_{23}N_3O_4$, DBE = 11

"NMR analysis allowed the unambiguous 2D structural assignment of the molecule, its relative stereochemistry was determined by ROESY data."

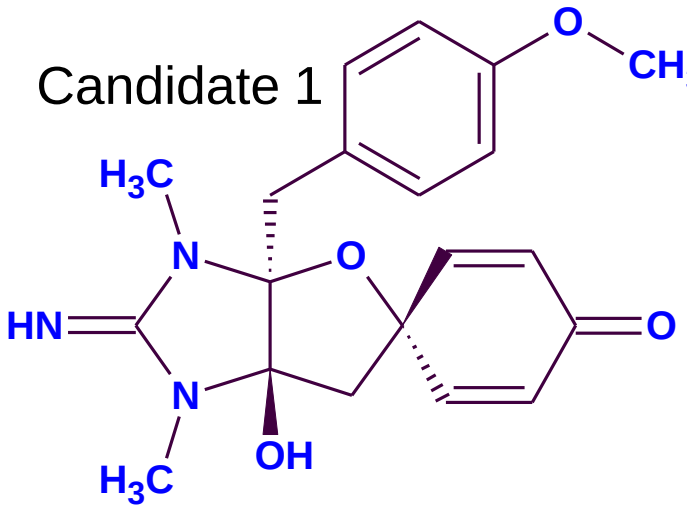
1. Li, C. M.; Danishefsky, S. J. *Tetrahedron Lett.* 2006, *47*, 385.
2. Chang, J. J.; Chan, B.; Ciufolini, M. A. *Tetrahedron Lett.* 2006, *47*, 3599.
3. Aberle, N.; Ovenden, S. P. B.; Lessene, G.; Watson, K. G.; Smith, B. J. *Tetrahedron Lett.* 2007, *48*, 2199.

Original



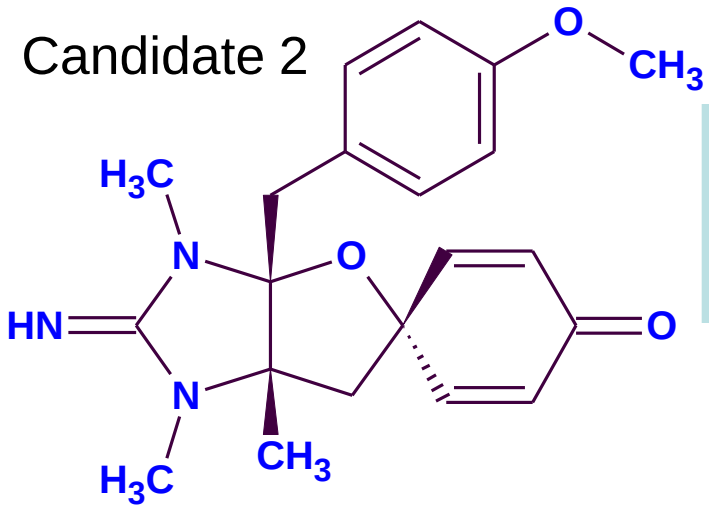
[1-2]

Candidate 1



[3]

Candidate 2



[3]

"Structure Revision of Spiroleucettadine, a Sponge Alkaloid with a Bicyclic Core Meager in H-Atoms"

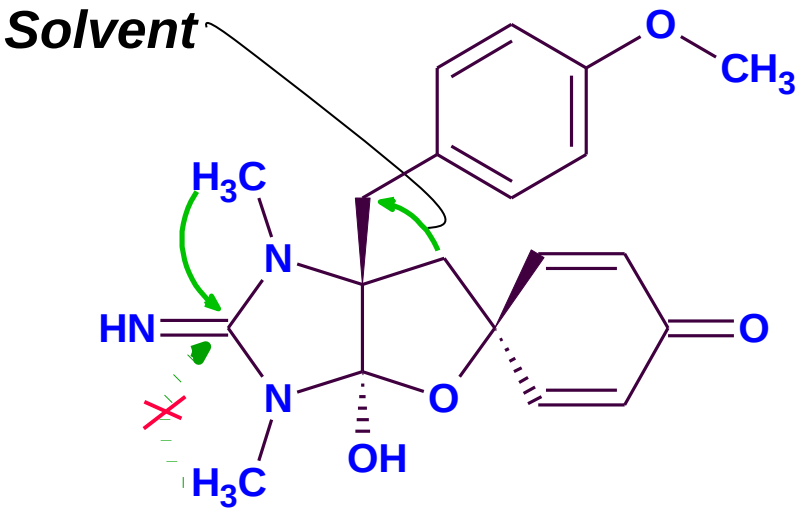
K. White, T. Amagata, A. Oliver, K. Tenney, P. Wenzel, P. Crews

J. Org. Chem. 2008, *73*, 8719–8722

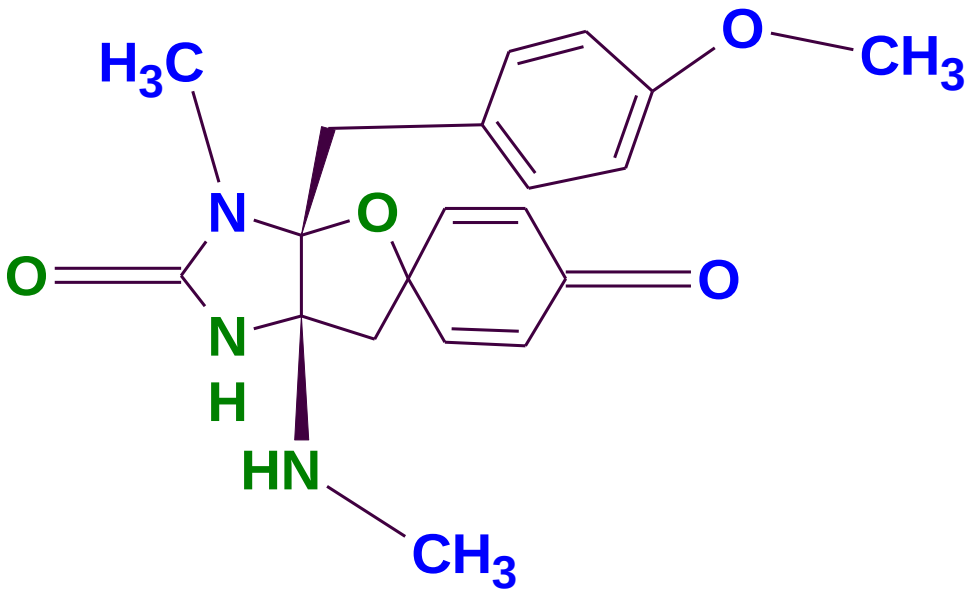
Structure was reisolated and determined by X-ray.

Confirmed by 2D NMR data and QM DFT ^{13}C calculations

Original



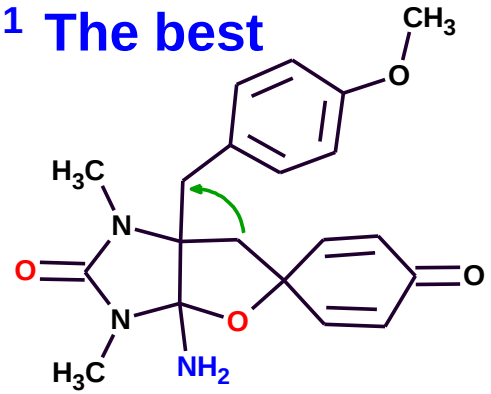
Revised



Structure Generation from OLD (erroneous) 2D NMR data

k=117 → 83 → 79, t_g = 10 s

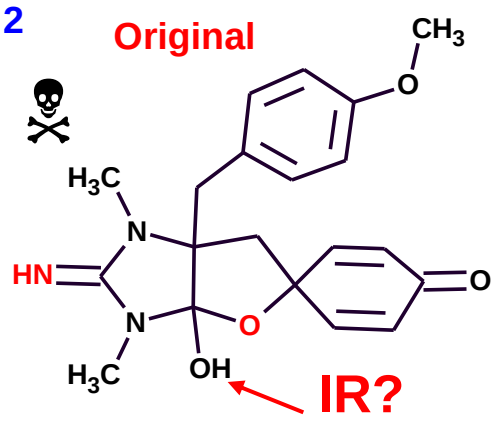
1 The best



$d_A(^{13}C)$: 2.684

$d_N(^{13}C)$: 2.190

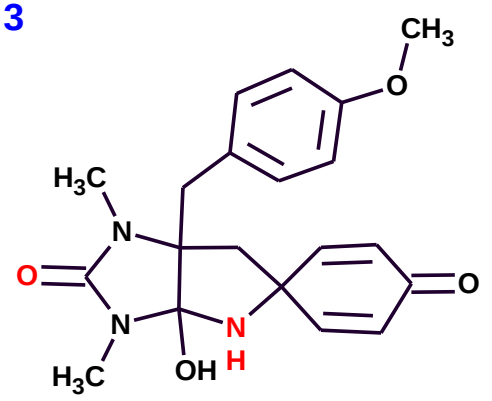
2 Original



$d_A(^{13}C)$: 3.168

$d_N(^{13}C)$: 3.078

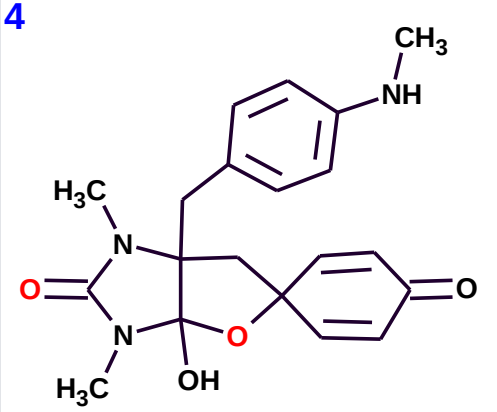
3



$d_A(^{13}C)$: 3.304

$d_N(^{13}C)$: 3.395

4

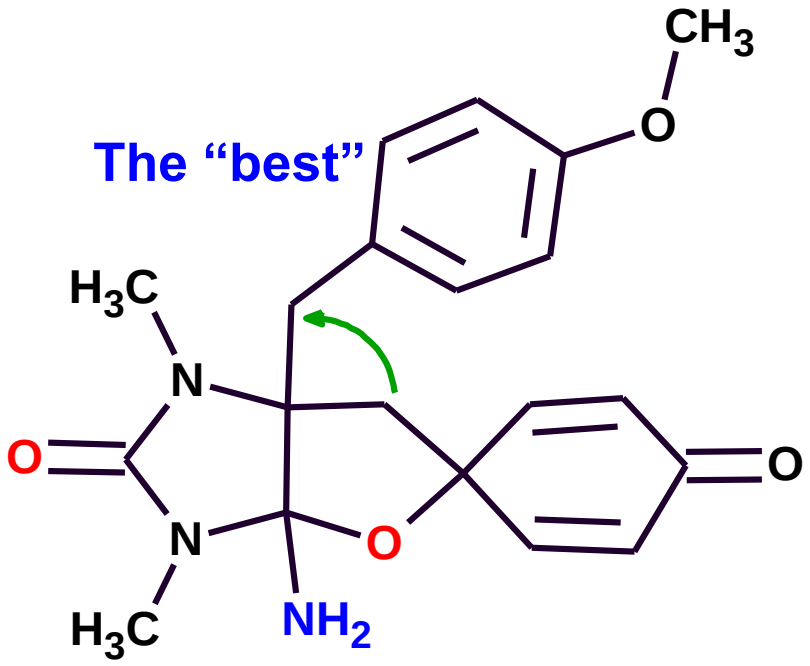


$d_A(^{13}C)$: 4.600

$d_N(^{13}C)$: 3.787

Program detected that the Original Structure was wrong. Hypotheses should be revised

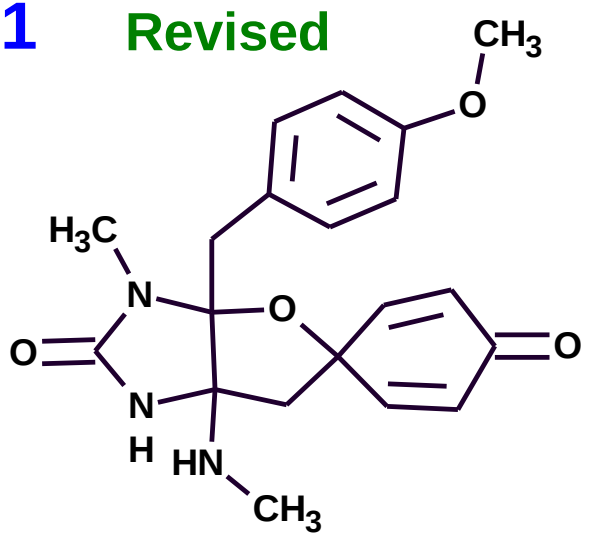
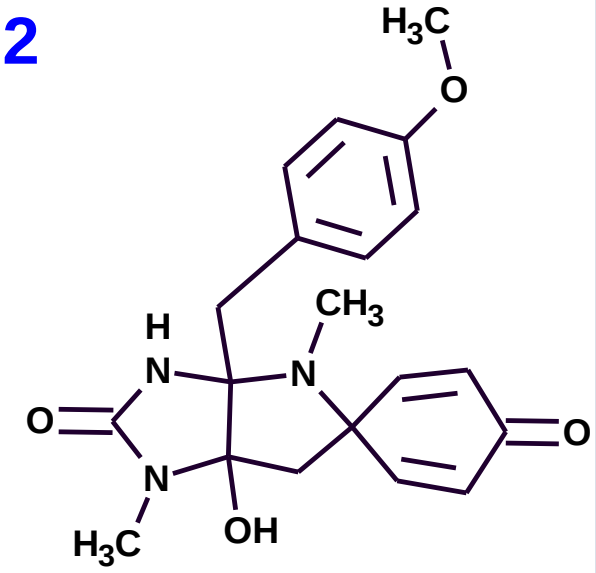
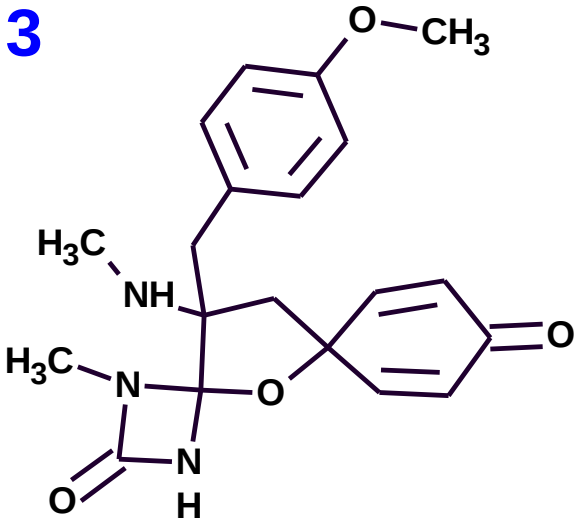
The "best"



***d* (1) = 2.69, 2.19 ppm**

***d* (2) = 3.17, 3.08 ppm**

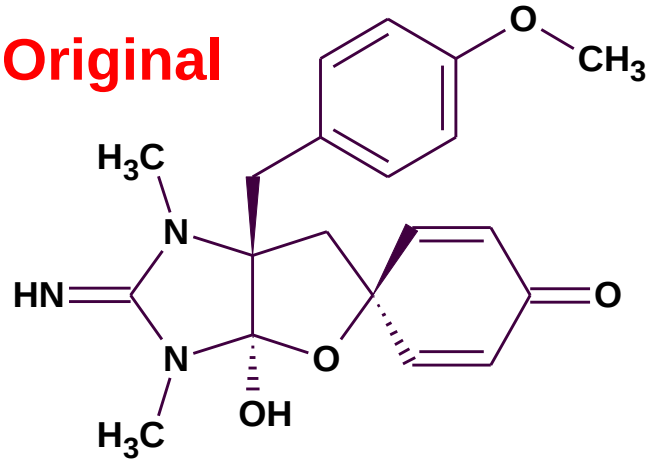
Structure Generation from NEW (correct) 2D NMR data with "liberal" atom properties at DBE = 11. k = 342 → 256, t_g = 8 h 2 min ("overnight")

1 Revised 	**2** 	**3** 
d_A(^{13}C) 1.762 d_N(^{13}C) 1.513	d_A(^{13}C) 2.745 d_N(^{13}C) 2.290	d_A(^{13}C) 2.681 d_N(^{13}C) 2.395

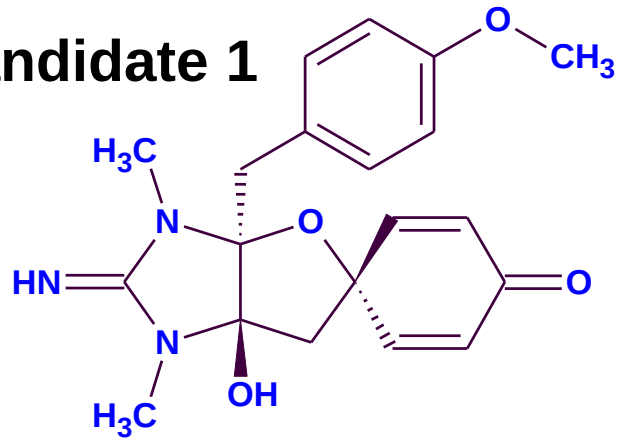
The Revised Structure and its Stereochemistry.

QM chemical shift calculations were performed by Crews group for 16 stereoisomers of all involved proposed structures.

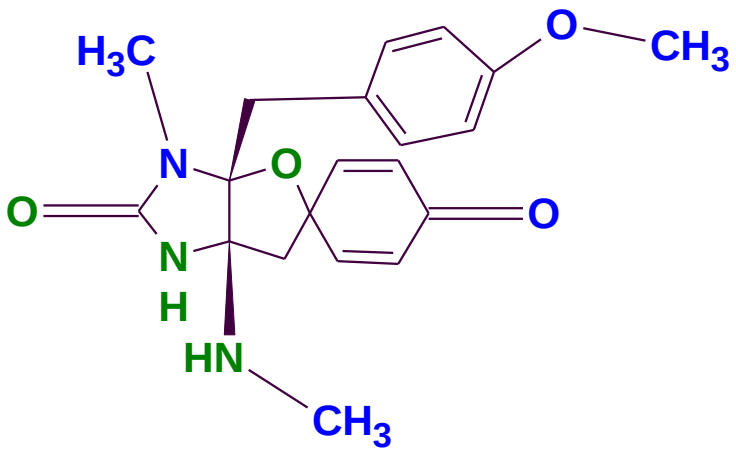
Original



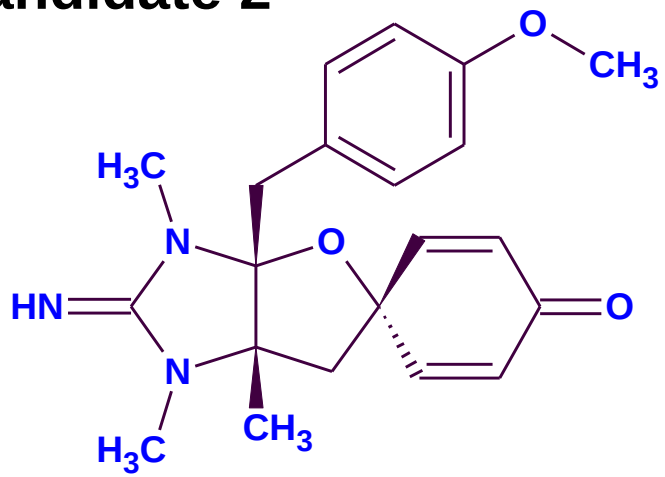
Candidate 1



Revised

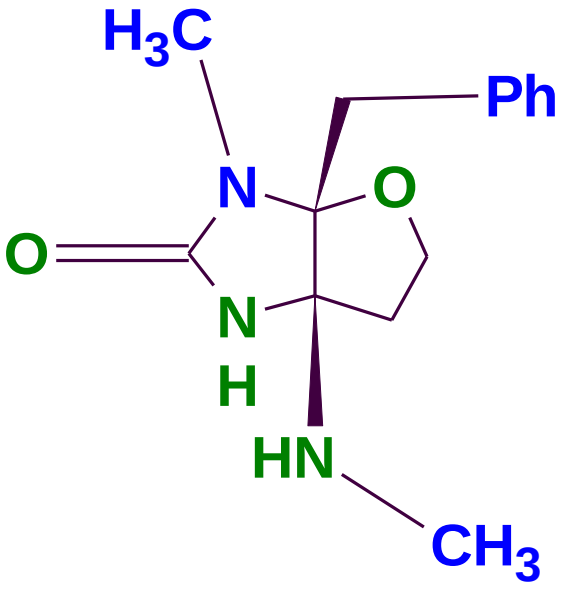


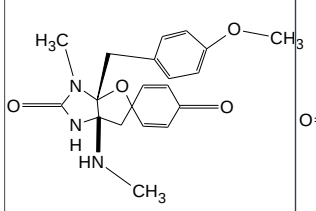
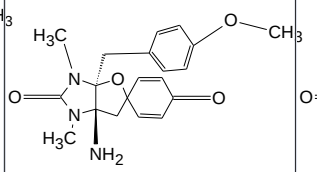
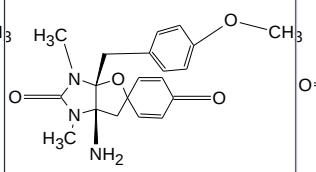
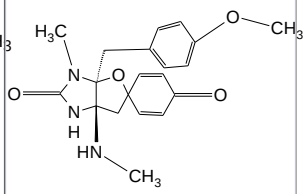
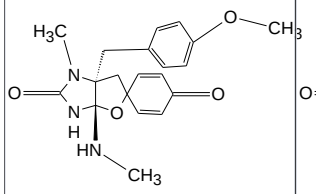
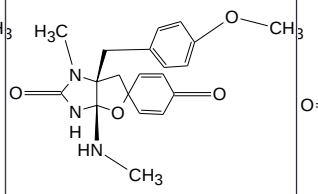
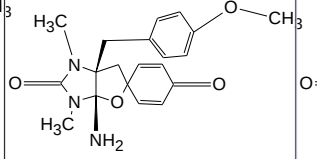
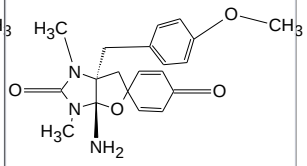
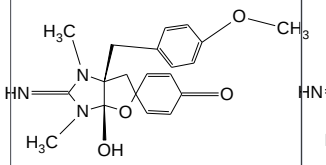
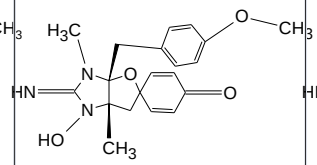
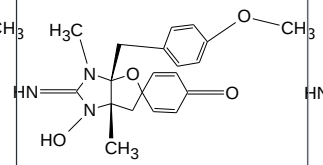
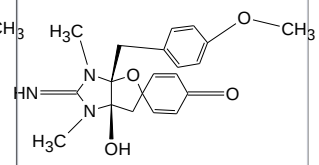
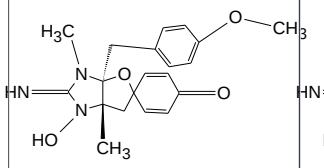
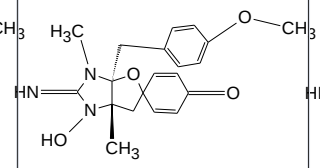
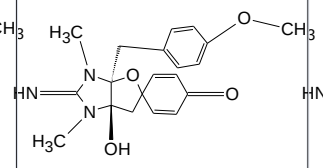
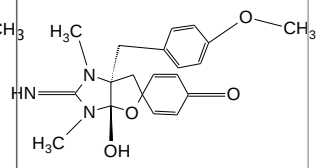
Candidate 2



A HARD WORK…

Results of ^{13}C NMR chemical shift prediction by the aid of StrucEluc



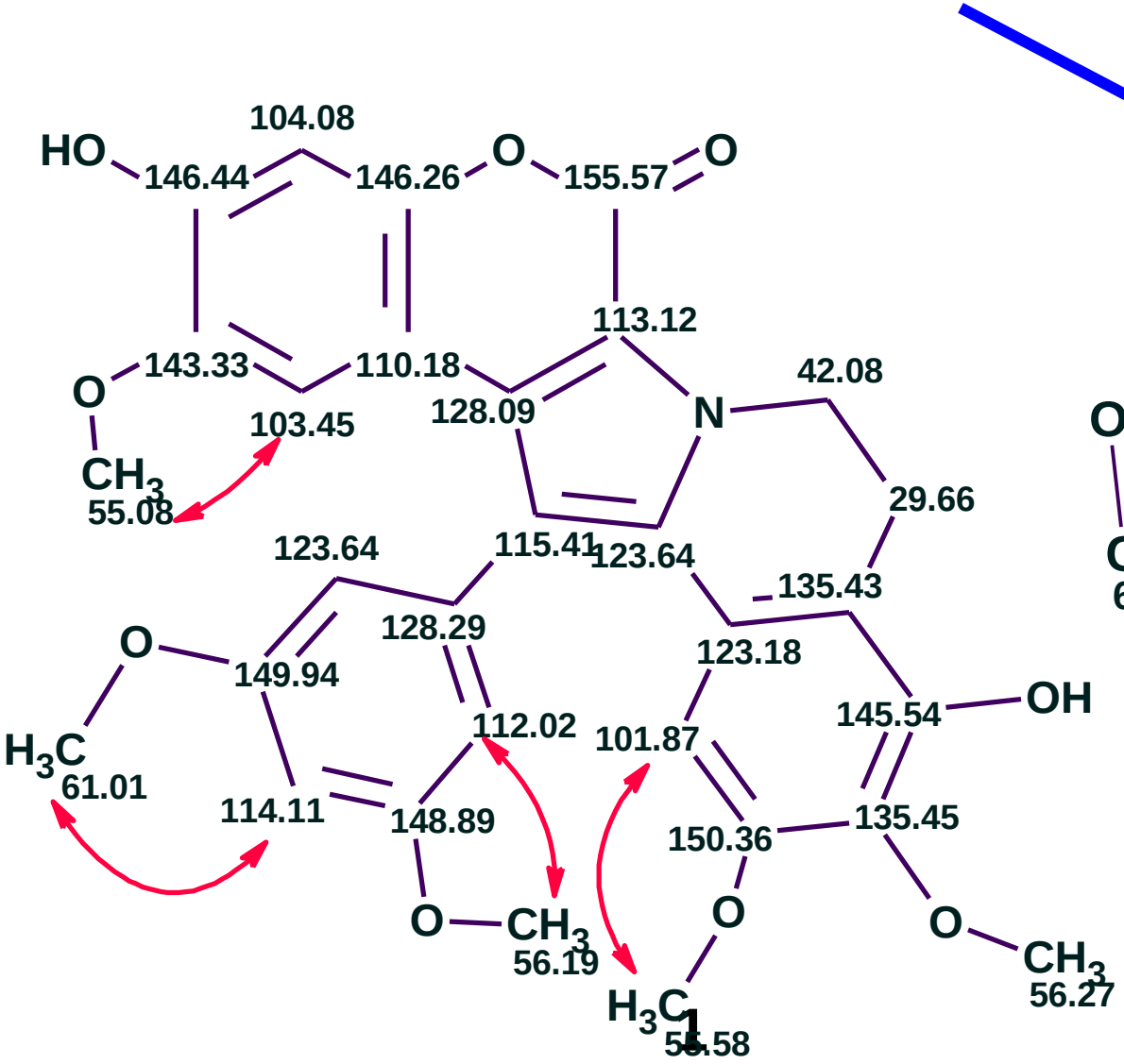
1 (ID:9) I, **Revised** 	2 (ID:12) L 	3 (ID:11) K 	4 (ID:10) J 
$d_A(^{13}C)$ 1.799	$d_A(^{13}C)$ 1.811	$d_A(^{13}C)$ 1.853	$d_A(^{13}C)$ 1.952
5 (ID:14) N 	6 (ID:13) M 	7 (ID:15) O 	8 (ID:16) P 
$d_A(^{13}C)$ 2.152	$d_A(^{13}C)$ 2.178	$d_A(^{13}C)$ 2.412	$d_A(^{13}C)$ 2.506
9 (ID:1) A 	10 (ID:7) G 	11 (ID:5) E 	12 (ID:3) C 
$d_A(^{13}C)$ 2.666	$d_A(^{13}C)$ 2.783	$d_A(^{13}C)$ 2.783	$d_A(^{13}C)$ 2.877
13 (ID:8) H 	14 (ID:6) F 	15 (ID:4) D 	16 **B, Original** 
$d_A(^{13}C)$ 2.937	$d_A(^{13}C)$ 2.937	$d_A(^{13}C)$ 2.970	$d_A(^{13}C)$ 3.039

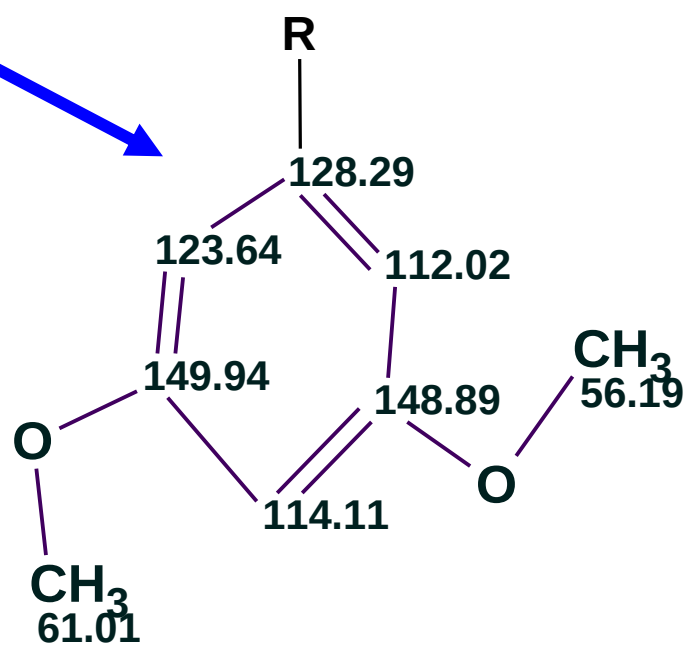
“New lamellarin alkaloids from the indian *ascidian didemnum obscurum* and their antioxidant Properties”

Krishnaiah, P.; Reddy, V. L. N.; Venkataramana, G.; Ravinder, K.; Srinivasulu, M.; Raju, T. V.; Ravikumar, K.; Chandrasekar, D.; Ramakrishna, S.; Venkateswarlu, Y.

***J. Nat. Prod*. 2004, 67, 1168-1171.**

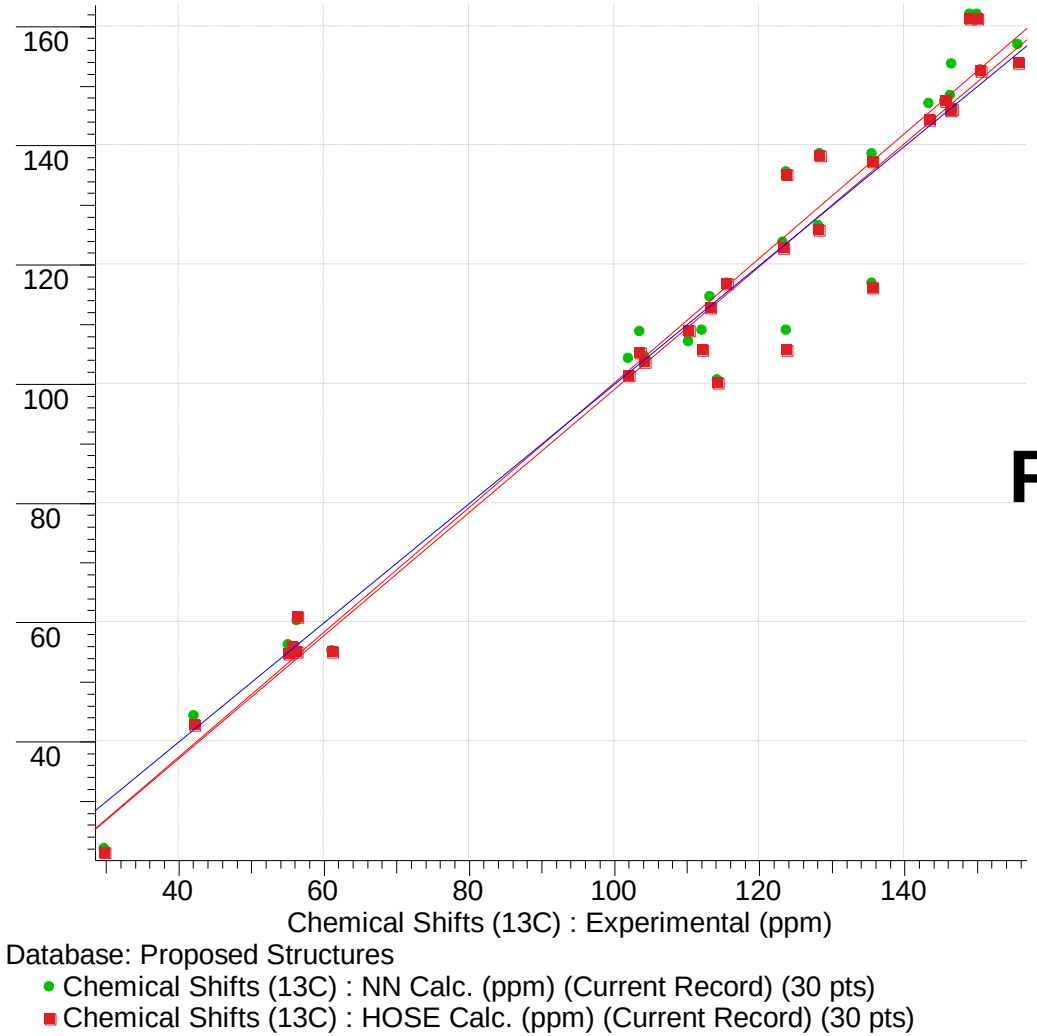
Assumptions: All 2D NMR correlations are standard
Symmetry violation is allowed (?)





d(HOSE) = 4.70ppm,
d(NN) = 5.29ppm,

Correlation between calculated ^{13}C chemical shifts (Y) and experimental ones (X).



$R^2 = 0.965$

Applications of Computer-Aided Methods of Structure Elucidation and Revision.
I. Structure Revision of Lamellarin γ.

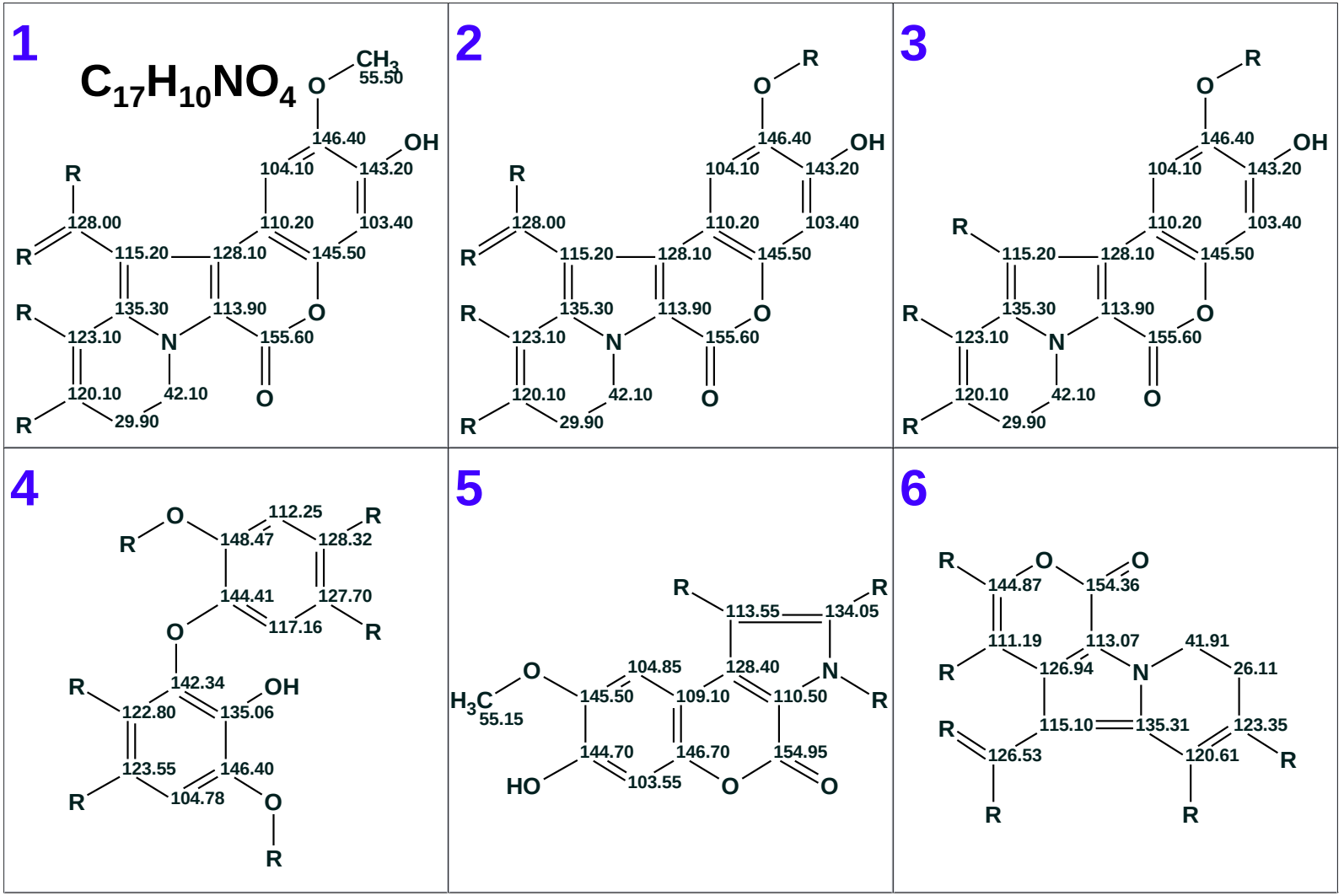
M. E. Elyashberg, K. A. Blinov, S.G. Molodtsov, T.S. Churanova, A.W. Williams.

ChemSpider Journal of Chemistry. 2009,
Published:3/18/2009 11:20:33 AM
http://www.chemmantis.com/Article.aspx?id=889

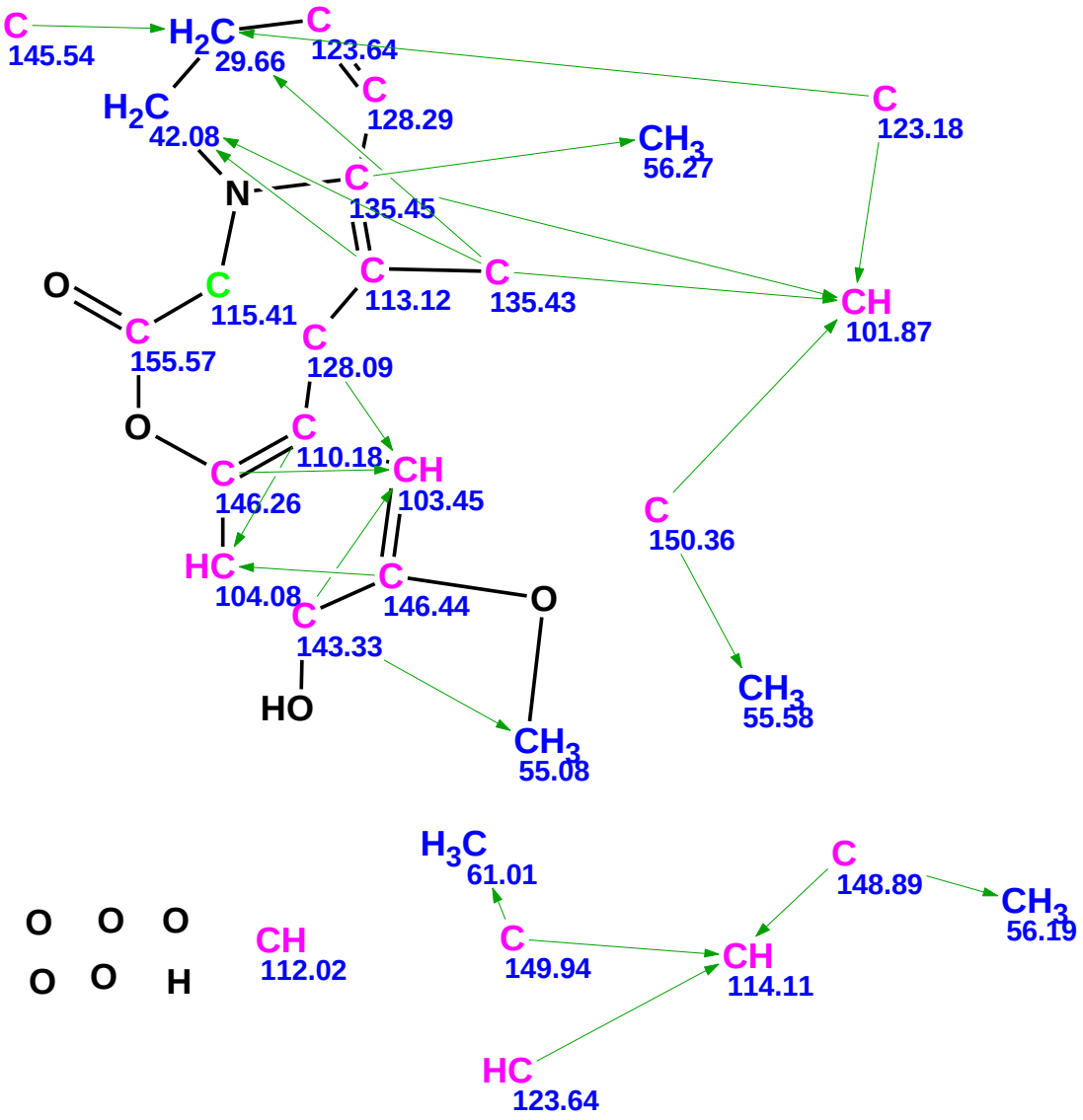
Molecular formula: $C_{30}H_{27}O_9N$, DBE = 18

Assumption: Fragment DB contains an appropriate fragment.

Number of Fragments Found by ^{13}C: *L* = 2 318; *p* = 10; n(MCD) = 192



One of 192 MCDs having different shift assignments of fragment atoms.

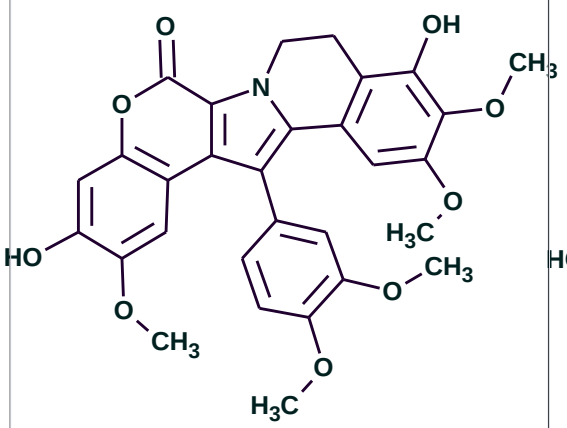
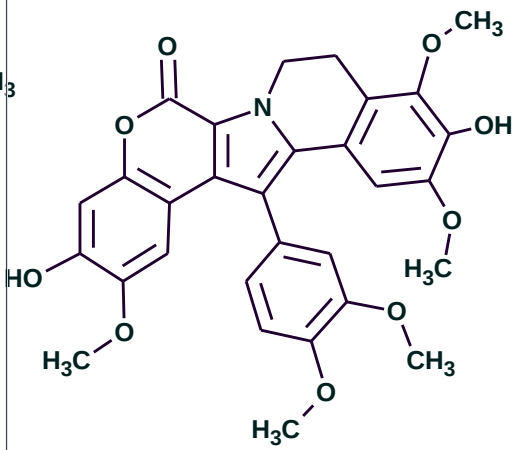
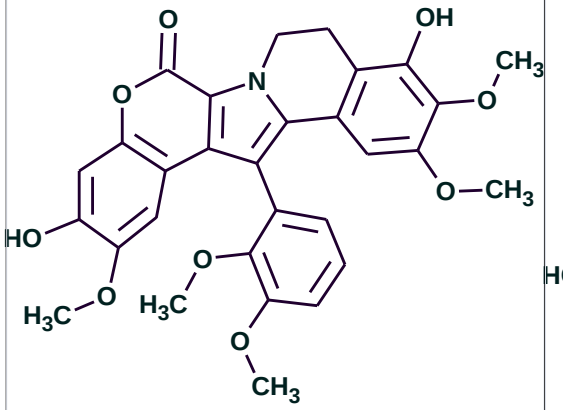
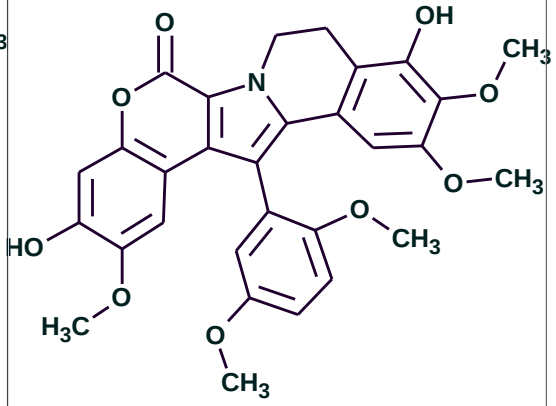


Fuzzy Structure Generation from 192 MCDs

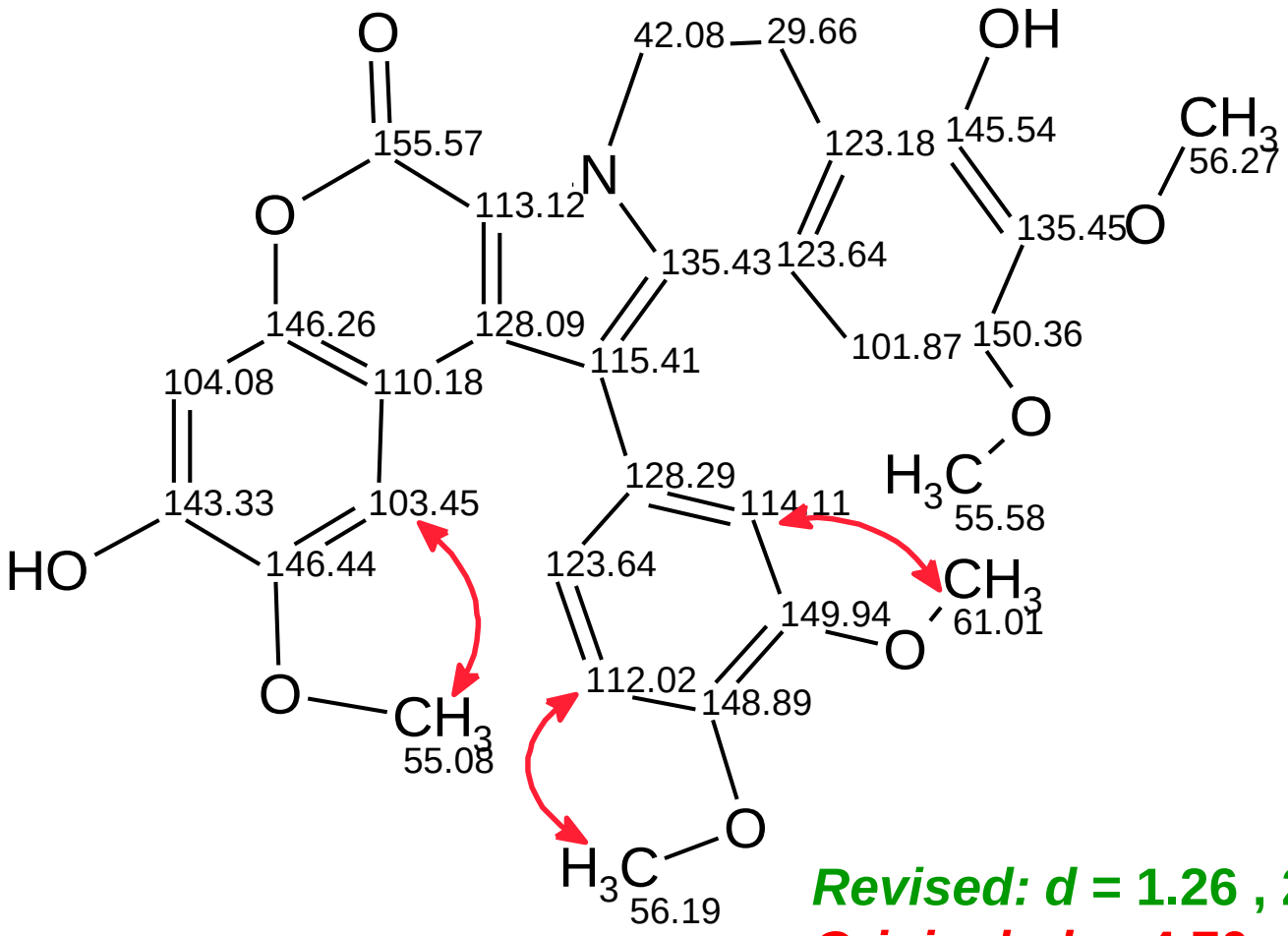
Result: k= 133, 504 → 120, 816; t_g=11 min

- **Time of ^{13}C spectrum calculation for ~121 thousand structures - 11 min**
- **Removal of duplicates: 120 816 →1 530**

Top structures of the ranked output file

1 Revised 	2 
$d_A(^{13}C)$ 1.264 $d_N(^{13}C)$ 2.550	$d_A(^{13}C)$ 1.715 $d_N(^{13}C)$ 2.574
3 	4 
$d_A(^{13}C)$ 2.068 $d_N(^{13}C)$ 2.931	$d_A(^{13}C)$ 2.120 $d_N(^{13}C)$ 2.909

Structure meets HMBC, NOE and Symmetry.

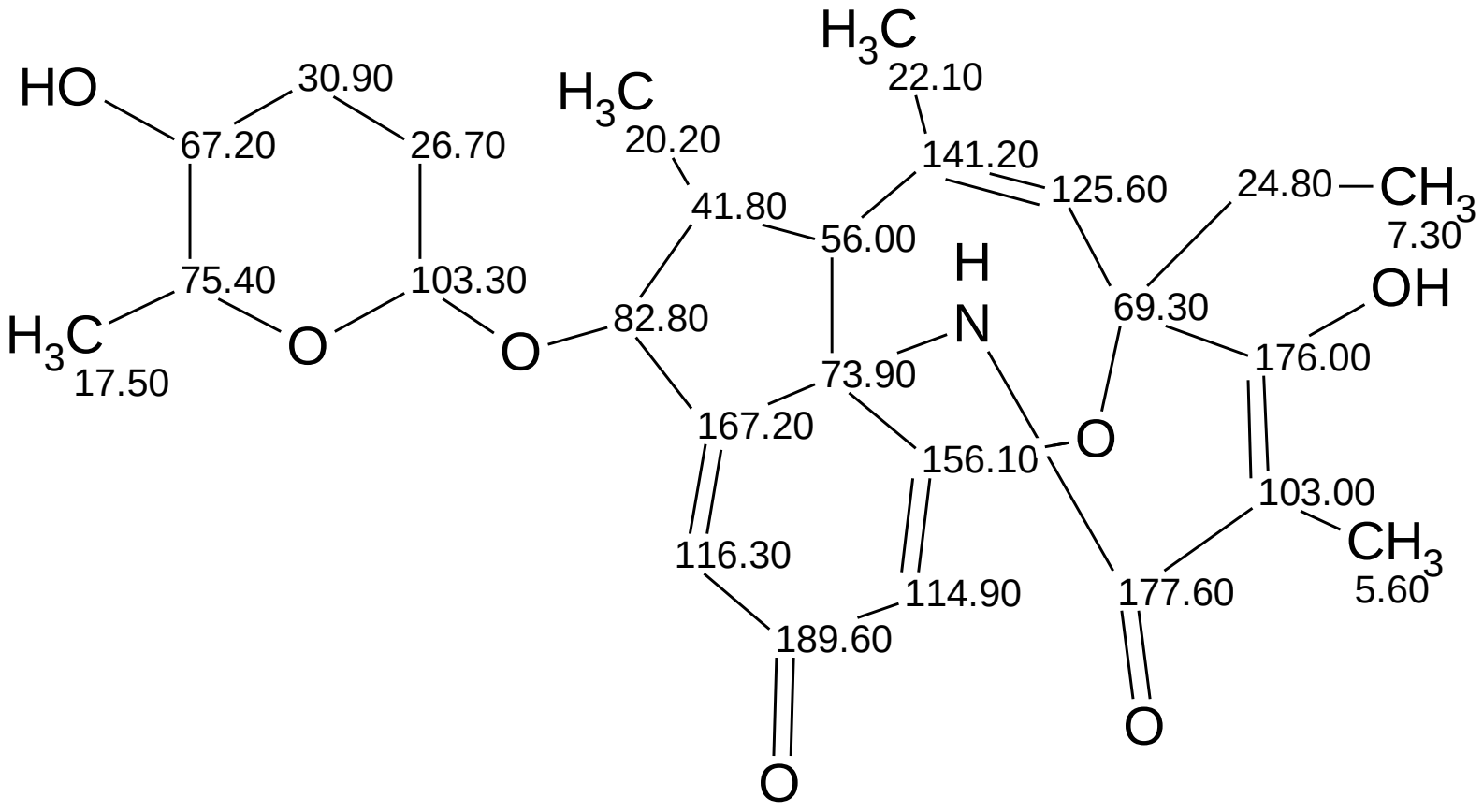


***Revised: d* = 1.26 , 2.55 ppm;**

***Original: d* = 4.70, 5.29 ppm**

Komoda, T.; Sugiyama, Y.; Abe, N.; Imachi, et al.
Tetrapetalone A, a novel lipoxygenase inhibitor from *Streptomyces sp*.

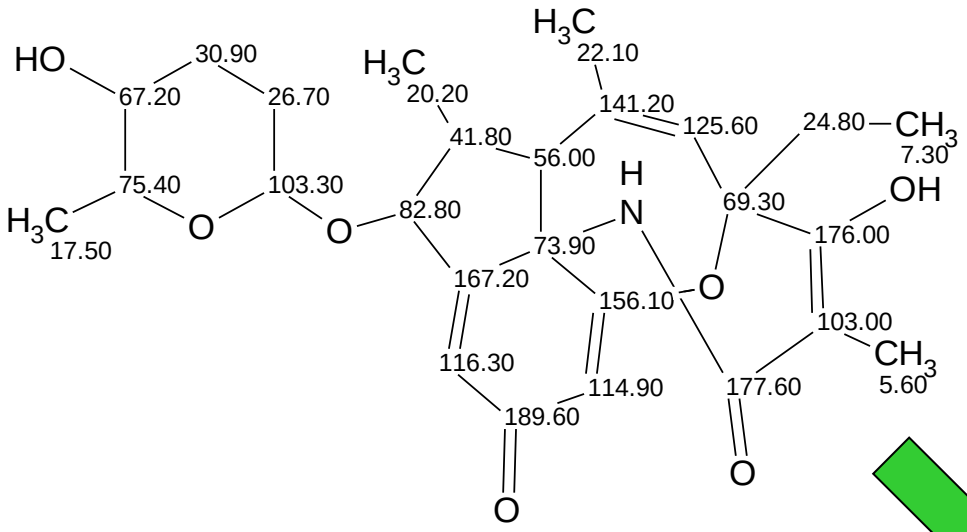
***Tetrahedron Letters* 44 (2003) 1659-1661**



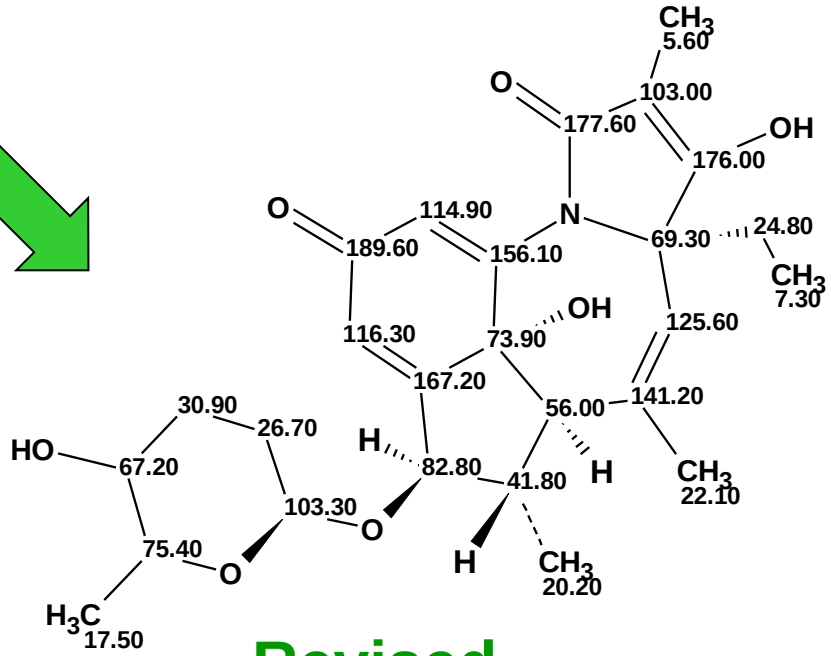
Komoda, T.; Sugiyama, Y.; Abe, N.; Imachi, M. et al

Revised structure of tetrapetalone A and its absolute stereochemistry.

Tetrahedron Letters 44 (2003) 7417-7419

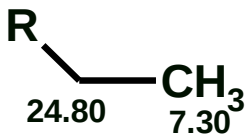


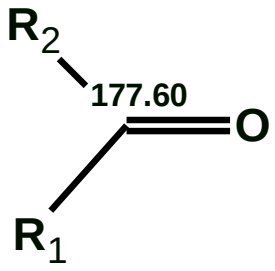
Original

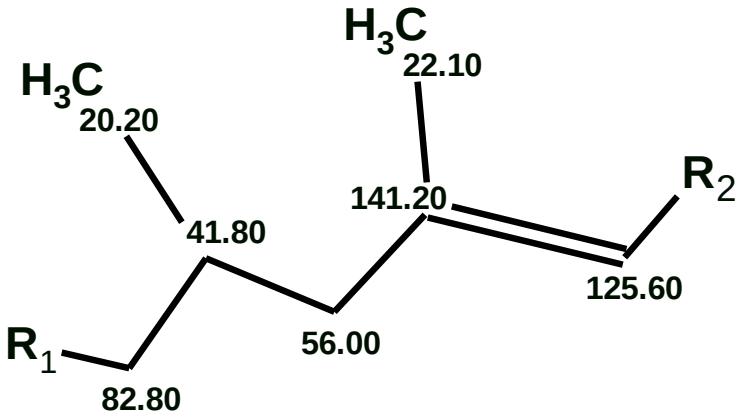


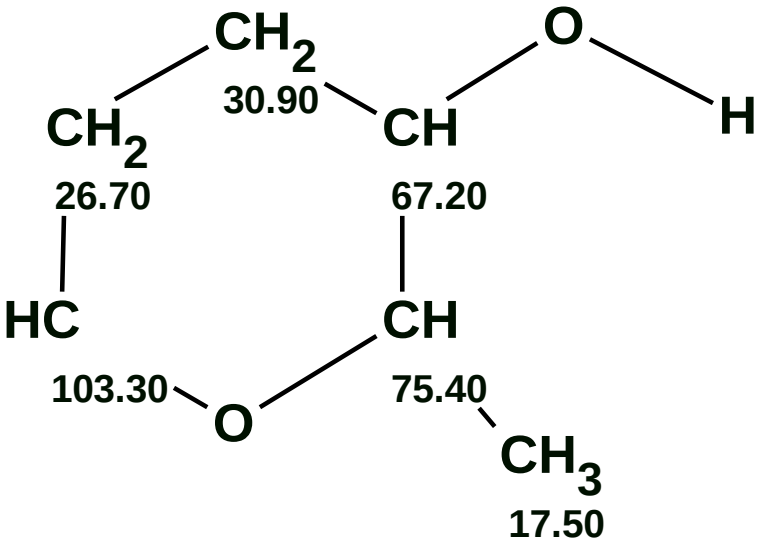
Revised

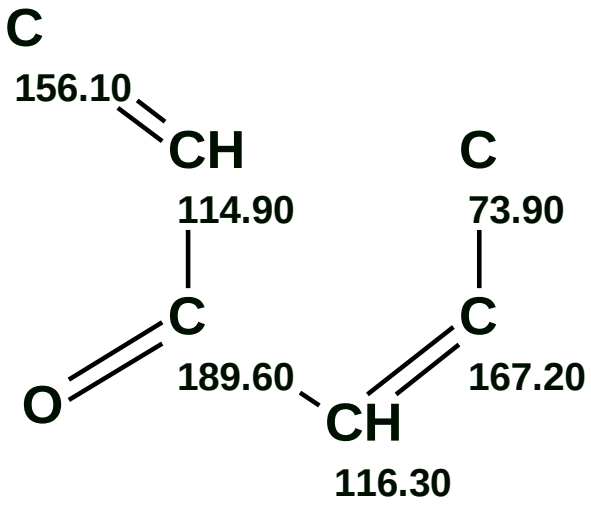
Fragments deduced by authors of ORIGINAL structure



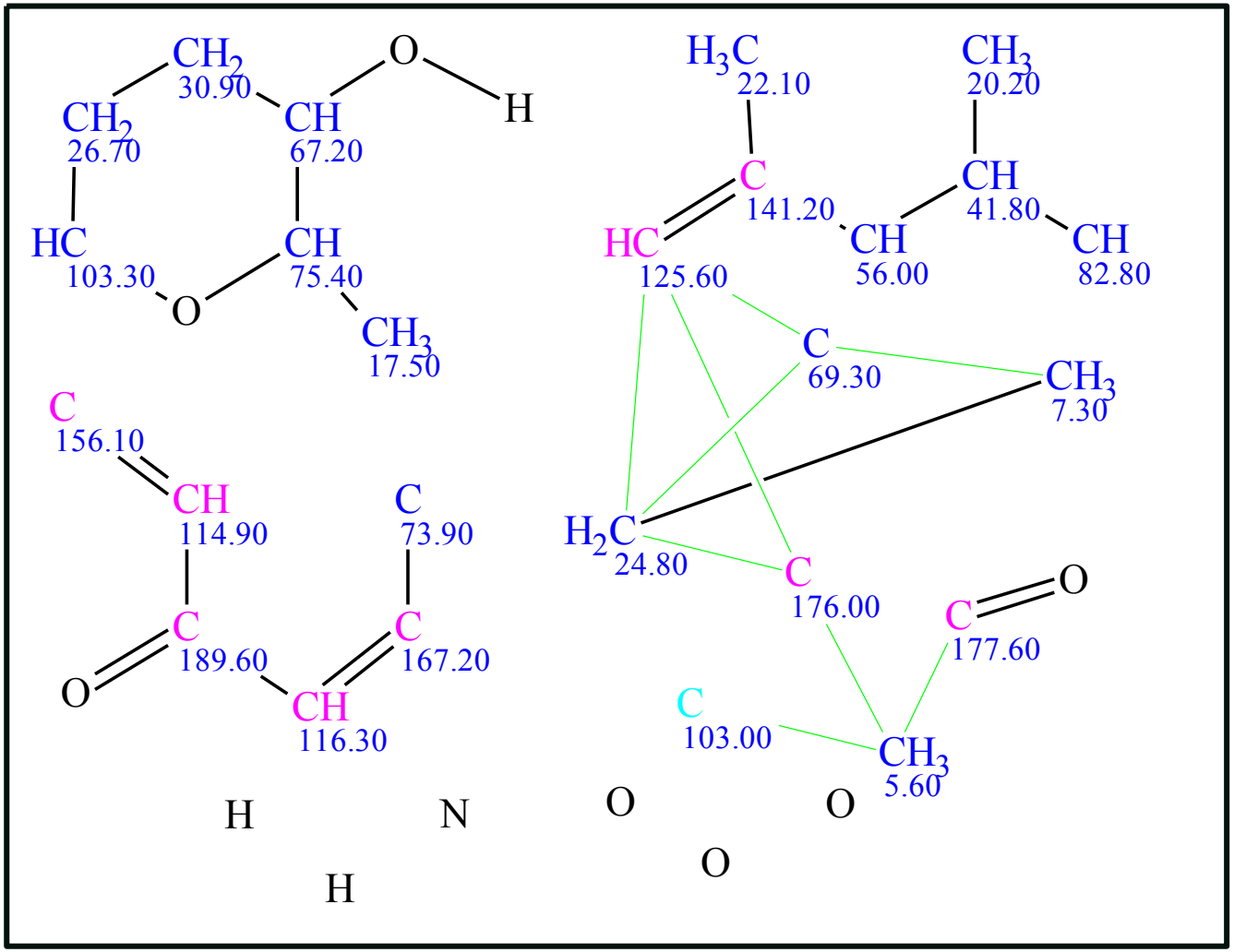






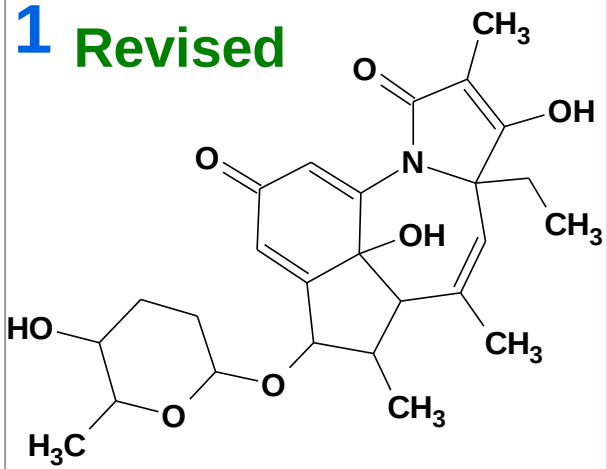
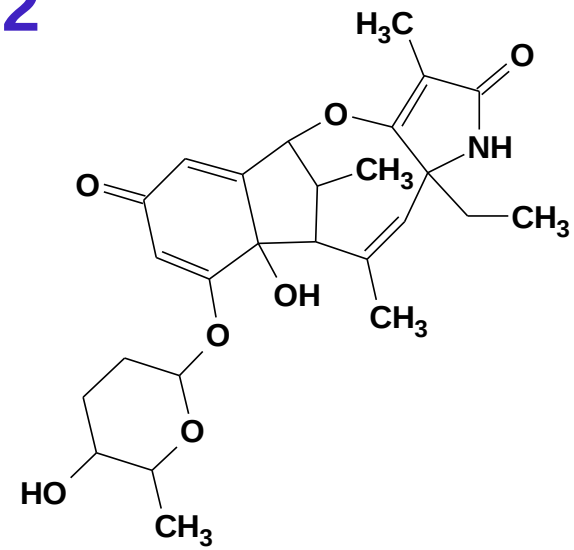
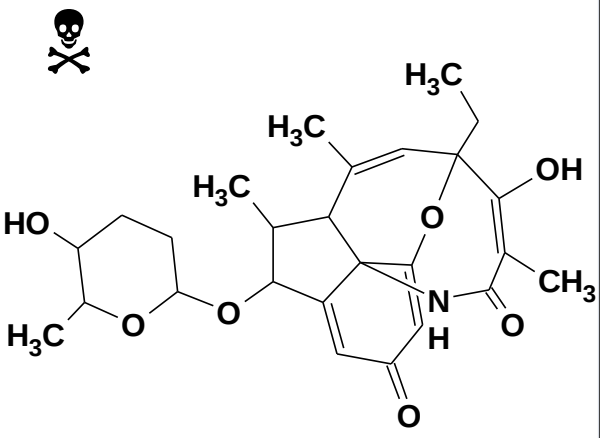


Hypothesis: 5 fragments inferred by authors from 2D NMR data are present.
The task: to complete the structure



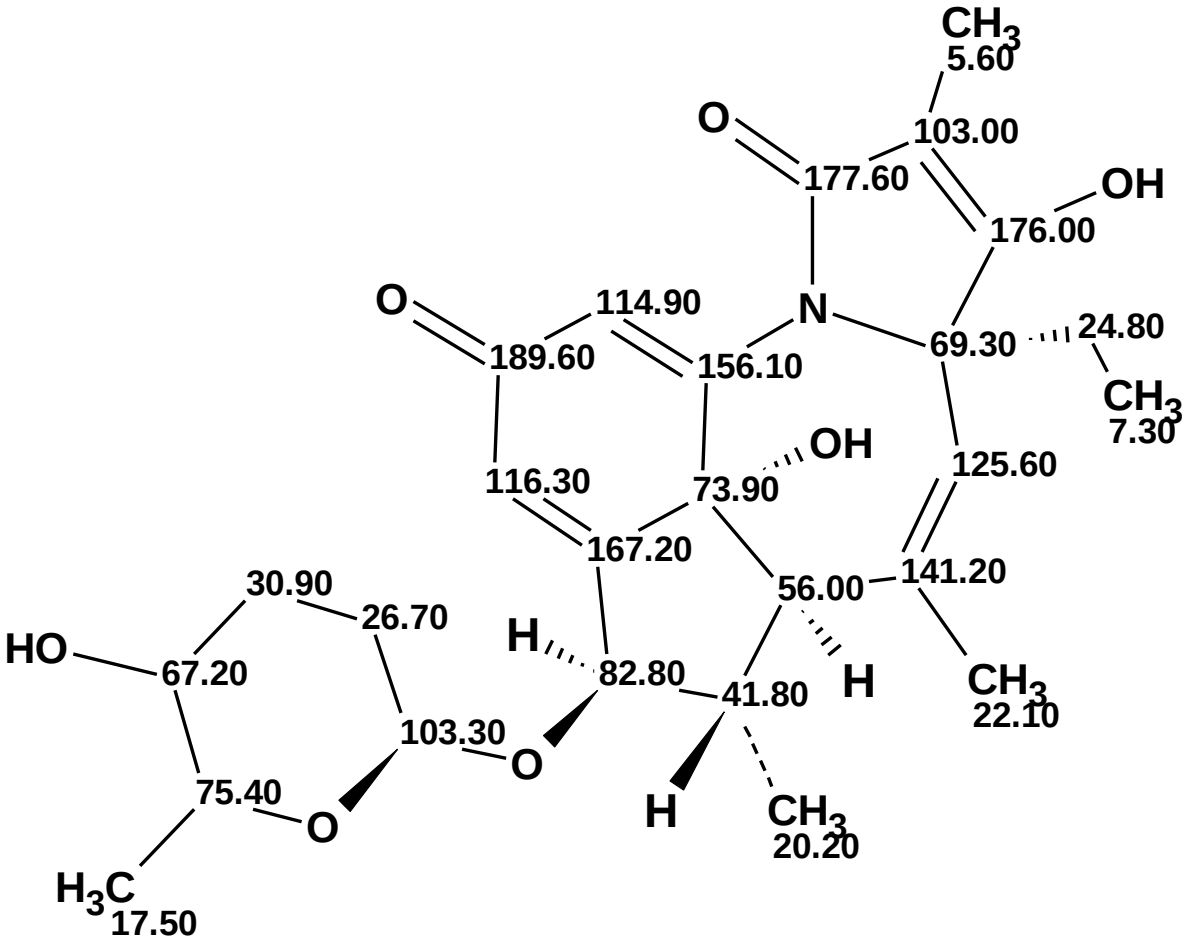
Strict structure generation

k= 16,465 → 13,672 → 9,203, t_g =61 s

1 **Revised** 	**2** 	**111** **Original** 
d_A(^{13}C) 2.160 d_N(^{13}C) 2.836	d_A(^{13}C) 2.922 d_N(^{13}C) 3.022	d_A(^{13}C) 3.370 d_N(^{13}C) 4.424

Relative stereochemistry determination

All 256 stereoisomers have been generated by StucEluc and right stereochemistry was determined by ^{13}C chemical shift prediction.

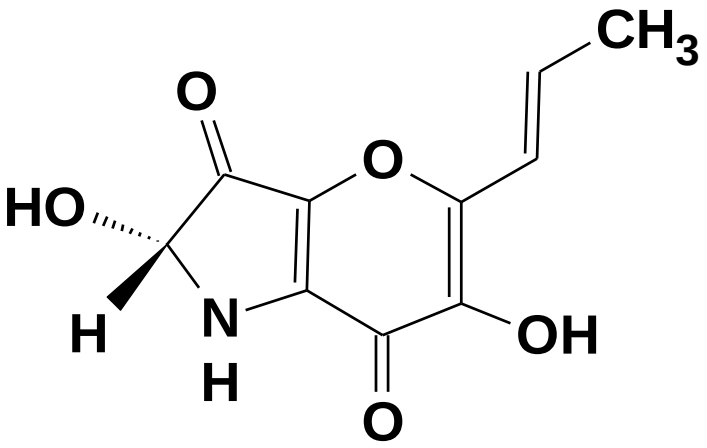


Hiort, J.; Maksimenka, K.; Reichert, M.; Perović-Ottstadt, S.; Lin, W. H.; Wray, V.; Steube, K.; Schaumann, K.; Weber, H.; Proksch, P.; Ebel, R.; Müller, W. E. G.; Bringmann, G.

New Natural Products from the Sponge-Derived Fungus Aspergillus niger

J. Nat. Prod. 2004, 67, 1532-1543

Structure was inferred from ^{1}H, ^{13}C, COSY, HMQC, HMBC, NOESY data.

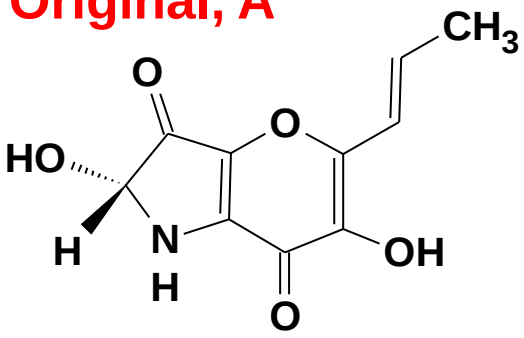


***Pyranonigrin A*, $C_9H_{10}NO_5$, EBD = 7**

The absolute configuration was established by Quantum Mechanical calculations of circular dichroism (CD) spectra.

Schlingmann, G.; Taniguchi, T.; He, H.; Bigelis, R.; Yang, H. Y.; Koehn, F.E.; Carter, G.T.; Berova, N.
Reassessing the Structure of *Pyranonigrin*.
***J. Nat. Prod*., 2007, 70, 1180 -1187**

Original, A



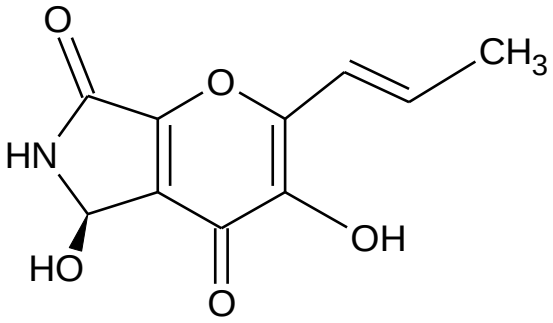
The substance was reisolated.

New 2D NMR data:

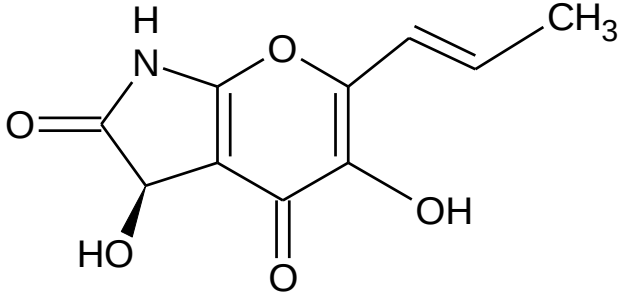
COSY, ROESY, ^{1}H-^{13}C HMBC + ^{1}H-^{15}N HMBC.

3 Proposed Structures

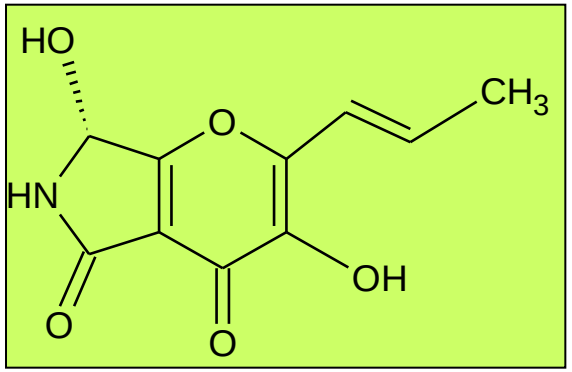
UV/CD QM calculations



B



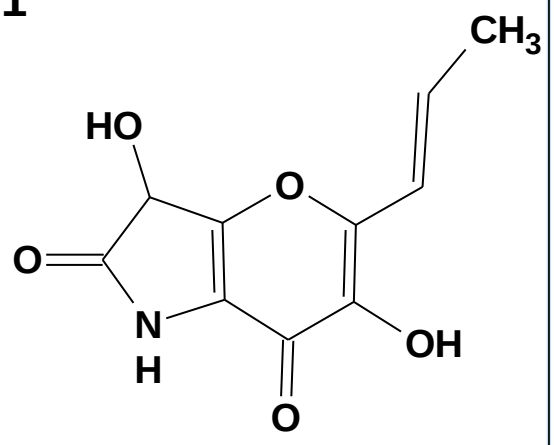
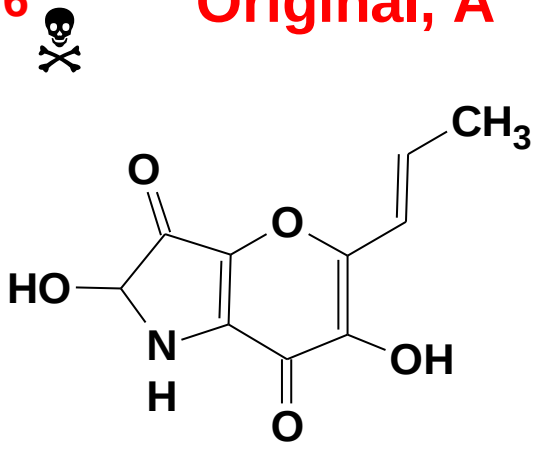
C



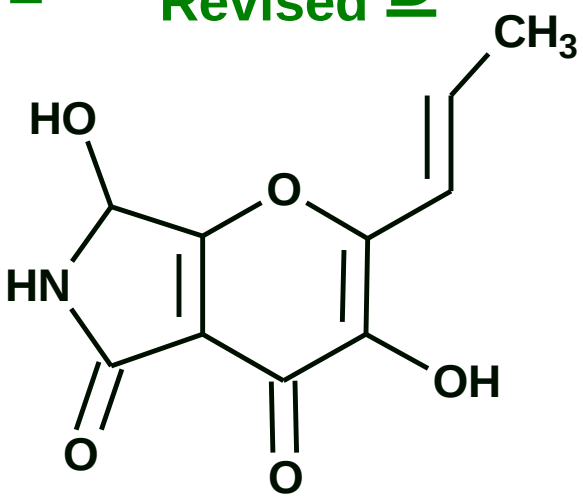
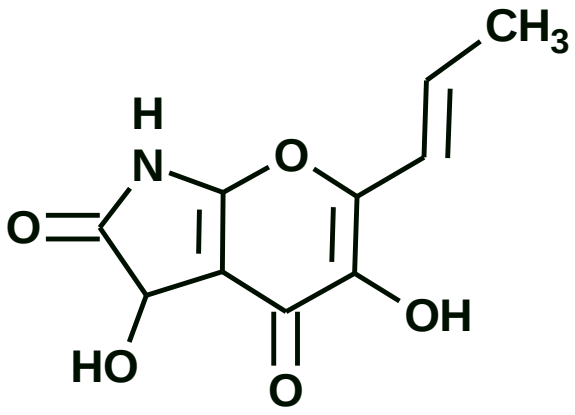
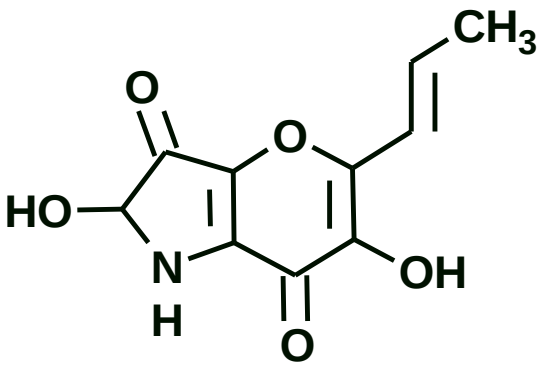
D

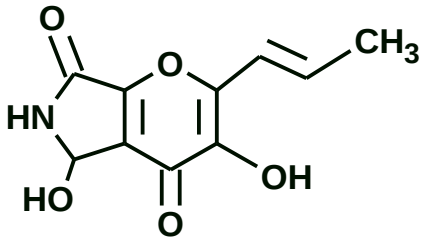
StrucEluc application to the initial NMR data used for inferring the original structure

Step 1. Hypothesis: there is no NSC in 2D NMR data.
Strict Structure Generation: k=109 → 81 → 72, t_g = 0.3 s

1	6 Original, A
	
$d_A(^{13}C)$ 5.603 $d_N(^{13}C)$ 5.311 $d_l(^{13}C)$ 7.695	$d_A(^{13}C)$ 10.664 $d_N(^{13}C)$ 7.689 $d_l(^{13}C)$ 10.029

Step 2. Fuzzy structure Generation, m=1, a=x
Result: k = 3024 → 2130 → 1144, t_g = 14 s.

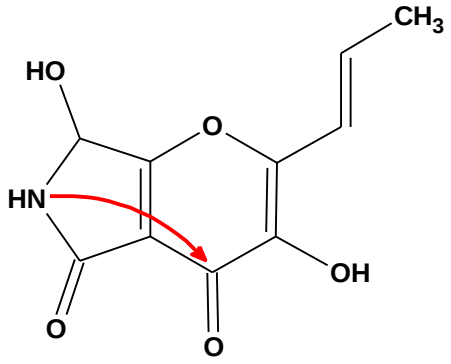
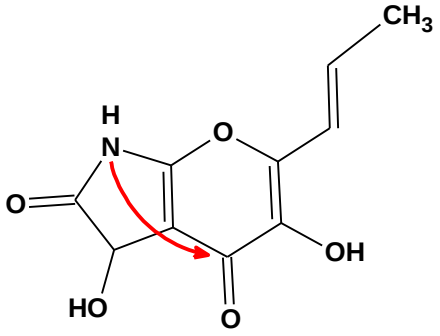
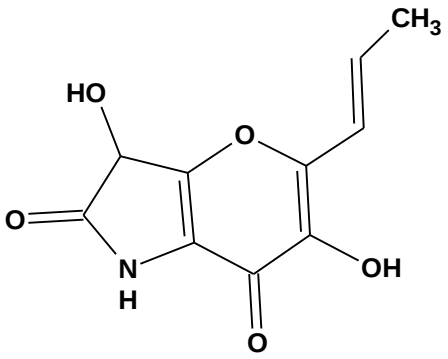
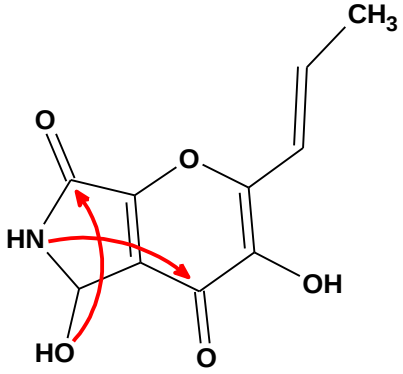
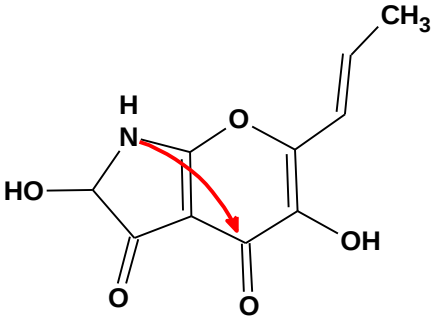
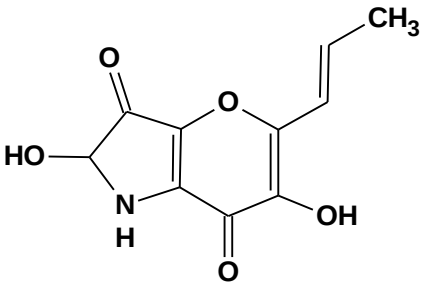
1 Revised D	5 C	31 A
		
$d_A(^{13}C)$: 2.027	$d_A(^{13}C)$: 5.264	$d_A(^{13}C)$: 9.003



B

Structure B was not generated

Step 3. Fuzzy structure Generation, m=2, a=x
Result: k = 18275 → 10725 → 3506, t_g=2m 23 s

1 D 	6 C 	7 
d_A(13C) : 2.027	d_A(13C) : 5.264	d_A(13C) : 5.603
8 B 	57 	95 A 
d_A(13C) : 5.633	d_A(13C) : 8.094	d_A(13C) : 10.664

Conclusions

Application of StrucEluc allows one:

- **Automatically generate all structural hypotheses from the set of axioms adopted by the chemist.**
- **Automatically choose the most probable structure.**
- **Automatically establish influence of any axiom on the final result.**
- **Avoid "unavoidable" pitfalls!**

Structure Elucidator Team

Sergey Molodtsov, Mikhail Elyashberg, Tatiyana Churanova, Kirill Blinov