# Structural Revision of Natural Products

DENMARK LABORATORY GROUP MEETING

ANDREW FENG

10 MAY 2022

# OUTLINE

- Introduction
- Structural Revision Case Studies
- Stereochemical Revision Case Studies
- Conclusion & Outlook

# Methods of structural elucidation

| | | |
|---|---|---|
| Chemical degradation and derivatization | Mass spectrometry | IR spectroscopy |
| NMR spectroscopy | X-ray crystallography | Circular dichroism |

Nicolaou, K. C.; Snyder, S. A. *Angew. Chemie Int. Ed.* **2005**, *44* (7), 1012–1044.

# Some perspectives on structural elucidation

*We have now reached the stage where often we have insufficient material for a retention sample; where crystallization is not worth attempting; where determination of a melting point may be a prohibitive waste of material;* ***and yet, where we have learned more about the structure of that molecule than we did years ago with grams of substance.***

*-C. Djerassi (1980)*

*Until the mid-1960s, structure determination was an art that could be likened to solving a complicated detective case, but with the spectacular advancement in spectroscopy it has become* ***less inspiring****, and since the mid-1980s, in most cases,* ***structure determination has become rather "routine".***

*-K. Nakanishi (1991)*

C. Djerassi, *Steroids Made It Possible* (Ed.: J. I. Seeman), American Chemical Society, Washington, DC, 1990, p. 205 (Profiles, Pathways and Dreams Series).
K. Nakanishi, *A Wandering Natural Products Chemist* (Ed.: J. I. Seeman), American Chemical Society, Washington, DC, 1991, p. 230 (Profiles, Pathways and Dreams Series).

# Discovery of incorrect structures

Total synthesis of nominal structure

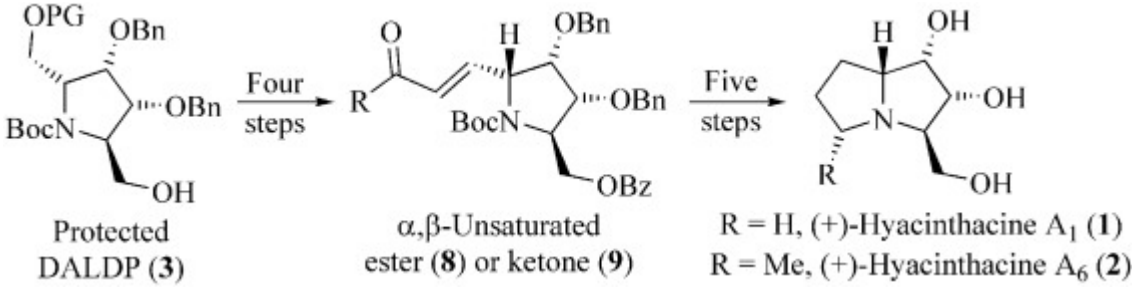
Biosynthetic plausibility

Computational predictions/calculations

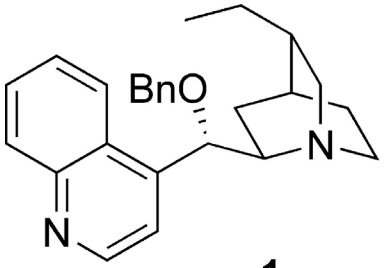
Nicolaou, K. C.; Snyder, S. A. *Angew. Chemie Int. Ed.* **2005**, *44* (7), 1012–1044. Suyama, T. L.; Gerwick, W. H.; McPhail, K. L. *Bioorg. Med. Chem.* **2011**, *19* (22), 6675–6701. Grauso, L.; Teta, R.; Esposito, G.; Menna, M.; Mangoni, A. *Nat. Prod. Rep.* **2019**, *36* (7), 1005–1030. Chhetri, B. K.; Lavoie, S.; Sweeney-Jones, A. M.; Kubanek, J. *Nat. Prod. Rep.* **2018**, *35* (6), 514–531.

# Retractions due to structural misassignment

## Total synthesis with mischaracterized intermediates/products




## Methods with mischaracterized catalyst



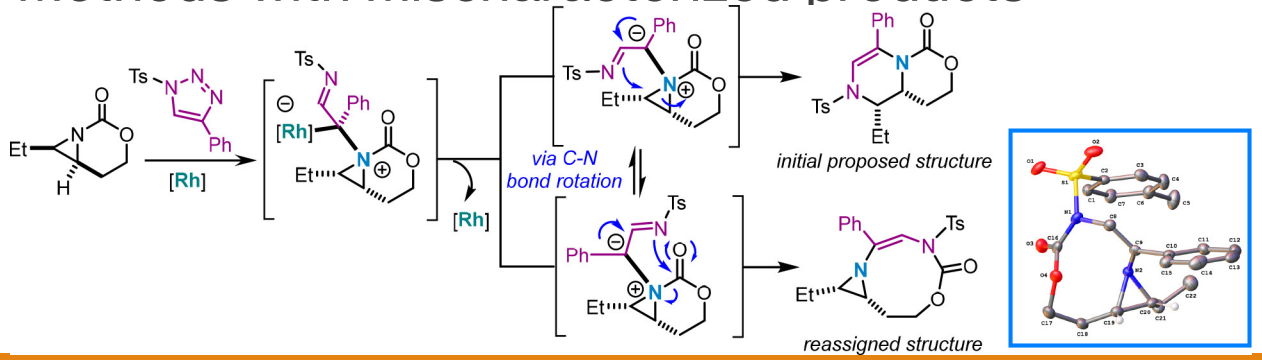

**1**

**1a:** **1**·$HPF_6$; **1b:** **1**·$HBF_4$;

**1c:** **1**·HOTs; **1d:** **1**·HOTf;

**1e:** **1**·camphorsulfonic acid

## Methods with mischaracterized products

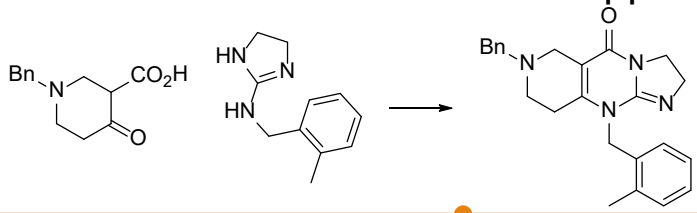



Izquierdo, I.; Plaza, M. T.; Tamayo, J. A.; Sanchez-Cantalejo, F. *Eur. J. Org. Chem.* **2007**, *36*, 6078-6083. Jang, D. O.; Kim, S. Y.; *J. Am. Chem. Soc.* **2008**, *130*, 16152-16153. Dequina, H. J.; Eshon, J.; Raskopf, W. T.; Fernandez, I.; Schomaker, J. M. *Org. Lett.* **2020**, *22*, 3637-3641.

# Legal issues from incorrect structures

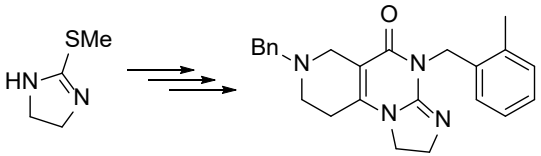
## 1973

Initially reported in patent with paltry characterization for antiseizure applications




## 2014

Branched structure discovered to be active, and competing patent filed



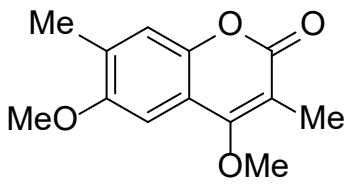

## 2013

Incorporated into NCI database, and potent cytotoxic activity discovered in 2013

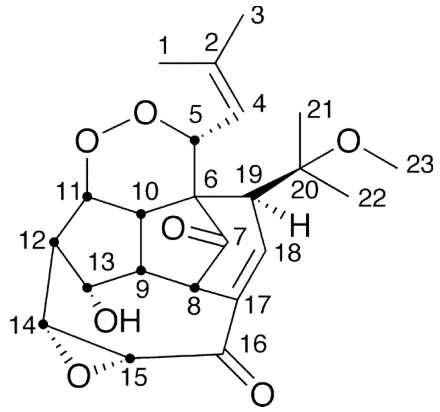
- “Confirmed” structure by MS only
- Patent granted and licensed to Oncoceutics
- Commercially available following original synthetic procedure

Staehle, H.; Koeppe, H.; Kummer, W. Imidazo(1,2-a)Pyrido(4,3-d)Pyrimidines with CNS Activity. DE2150062A1, 1973. Allen, J. E.; Krigsfeld, G.; Mayes, P. A.; Patel, L.; Dicker, D. T.; Patel, A. S.; Dolloff, N. G.; Messaris, E.; Scata, K. A.; Wang, W.; Zhou, J.-Y.; Wu, G. S.; El-Deiry, W. S. *Sci. Transl. Med.* **2013**, *5* (171), 171ra17. Jacob, N. T.; Lockner, J. W.; Kravchenko, V. V; Janda, K. D. *Angew. Chemie Int. Ed.* **2014**, *53* (26), 6628–6631.

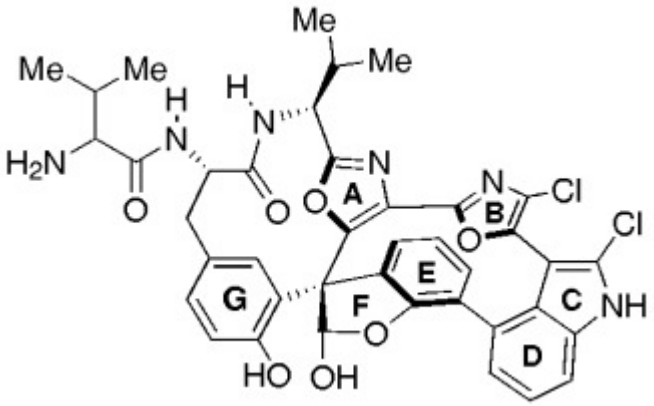
# STRUCTURAL REVISIONS




"Unnamed coumarin"




Hexacyclinol




Diazonamide A

# An unnamed coumarin

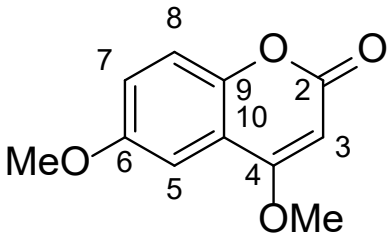
Isolated 80 mg from Jordanian tubers *C. decaisnei* in 1991

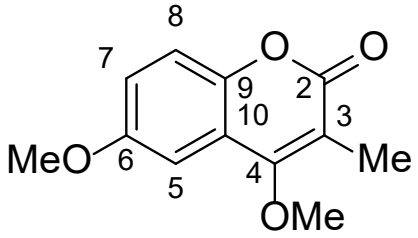
Molecular formula (MS): $C_{13}H_{14}O_4$; IR: 1650, 1600, 1320

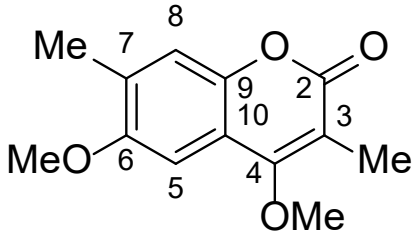
UV absorptions “typical of coumarins having 4-OMe and 6-OMe substituents”

$^1$H NMR (400 MHz, $CDCl_3$): 6.45 (s, 1H), 5.96 (s, 1H), 3.97 (s, 3H), 3.90 (s, 3H), 2.27 (s, 3H), 2.15 (s, 3H)

NOE at 6.45 with both OMe groups (36% and 32%)




Al-Tel, T. H.; Abu Zarga, M. H.; Sabri, S. S.; Feroz, M.; Fatima, N.; Shah, Z.; Atta-ur-Rahman. *Phytochemistry* **1991**, *30* (9), 3081–3085.

# Original structural assignment

| $^{13}C$ δ | $^{1}H$ δ (mult, int) | Assignment |
|---|---|---|
| **178.0** | | 2 |
| 159.1 | | 3 |
| 162.1 | | 4* |
| **91.8** | 6.45 (s, 1H) | 5 |
| 161.1 | | 6* |
| **158.5** | | 7 |
| 111.3 | **5.96 (s, 1H)** | 8 |
| 7.7 | 2.15 (s, 3H) | 3-Me |
| 56.5 | 3.93 (s, 3H) | 4-OMe |
| 55.8 | 3.90 (s, 3H) | 6-OMe |
| 19.7 | 2.27 (s, 3H) | 7-Me |
| 158.5 (typo) | | 9 |
| 108.7 | | 10 |

## $^{13}C$ NMR inconsistencies

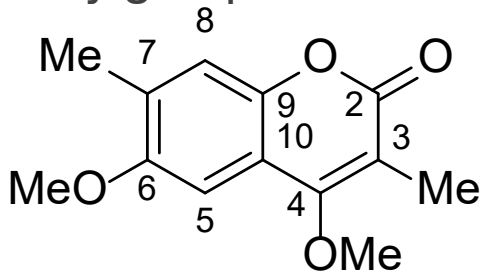
- Carbonyl downfield of typical coumarins (~150 ppm)
- C5 far upfield for only 1 OMe
- C7 resembles Ar-O
- Structure only accounts for 4 C bonded to O (δ $^{13}C$ > 140)

## $^{1}H$ NMR inconsistencies:

- 5.96 singlet assigned to aromatic H(C8)

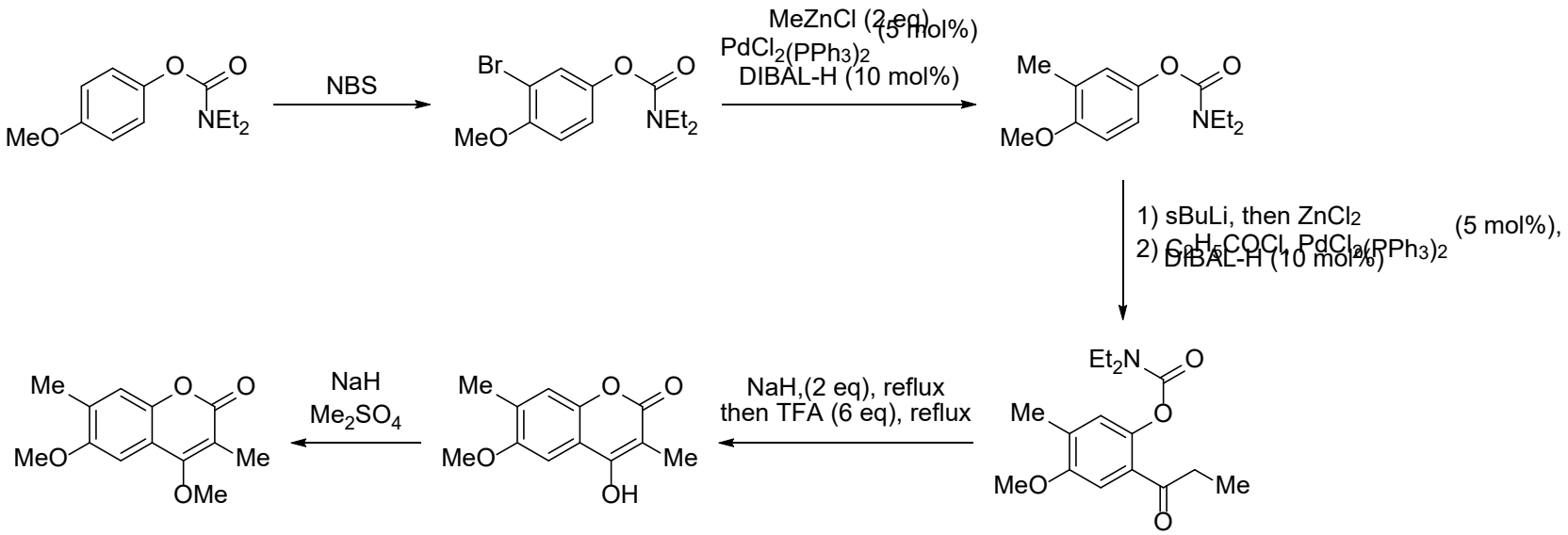
## NOE inconsistencies

- Similar NOE's observed from H(C5) to both methoxy groups

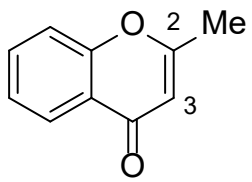



Al-Tel, T. H.; Abu Zarga, M. H.; Sabri, S. S.; Feroz, M.; Fatima, N.; Shah, Z.; Atta-ur-Rahman. *Phytochemistry* **1991**, *30* (9), 3081–3085.

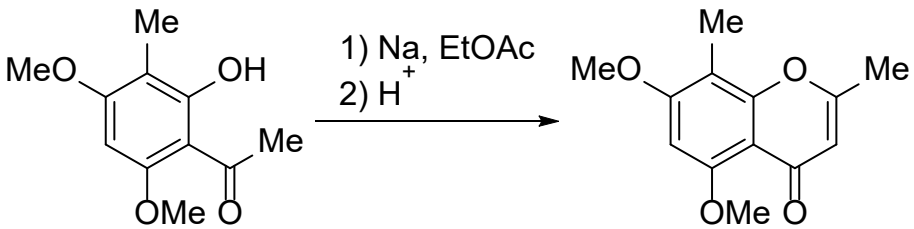
# Total synthesis and reassignment




| Isolated | Synthetic |
|---|---|
| 6.45 (s, 1H) | **7.13 (s, 1H)** |
| 5.96 (s, 1H) | **7.00 (s, 1H)** |
| 178.0 | **164.6** |
| 162.1 | 163.7 |
| 161.1 | 154.5 |
| 159.1 | **146.6** |
| 156.5 | **132.1** |



H(C3) reported at 6.14 ppm

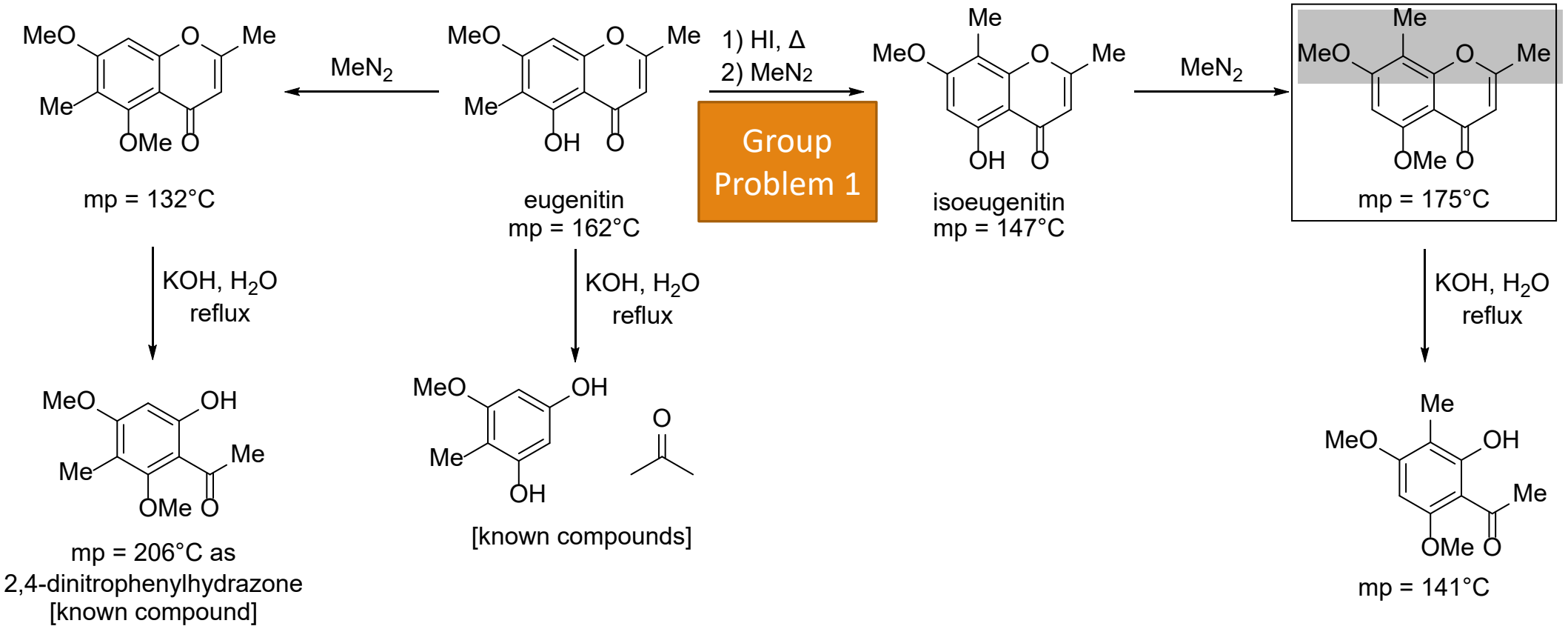



NMR and IR match isolated material. Mp = 173-174°C

Kalinin, A. V; Snieckus, V. *Tetrahedron Lett.* **1998**, *39* (28), 4999–5002. Eguchi, S. *Org. Mass Spectrom.* **1979**, *14* (7), 345–349.

# Pre-spectroscopic characterization

First isolated in 1949, obtained molecular formula $C_{12}H_{12}O_4$ by microanalysis




Schmid, H. *Helv. Chim. Acta* **1949**, *32* (3), 813–820.

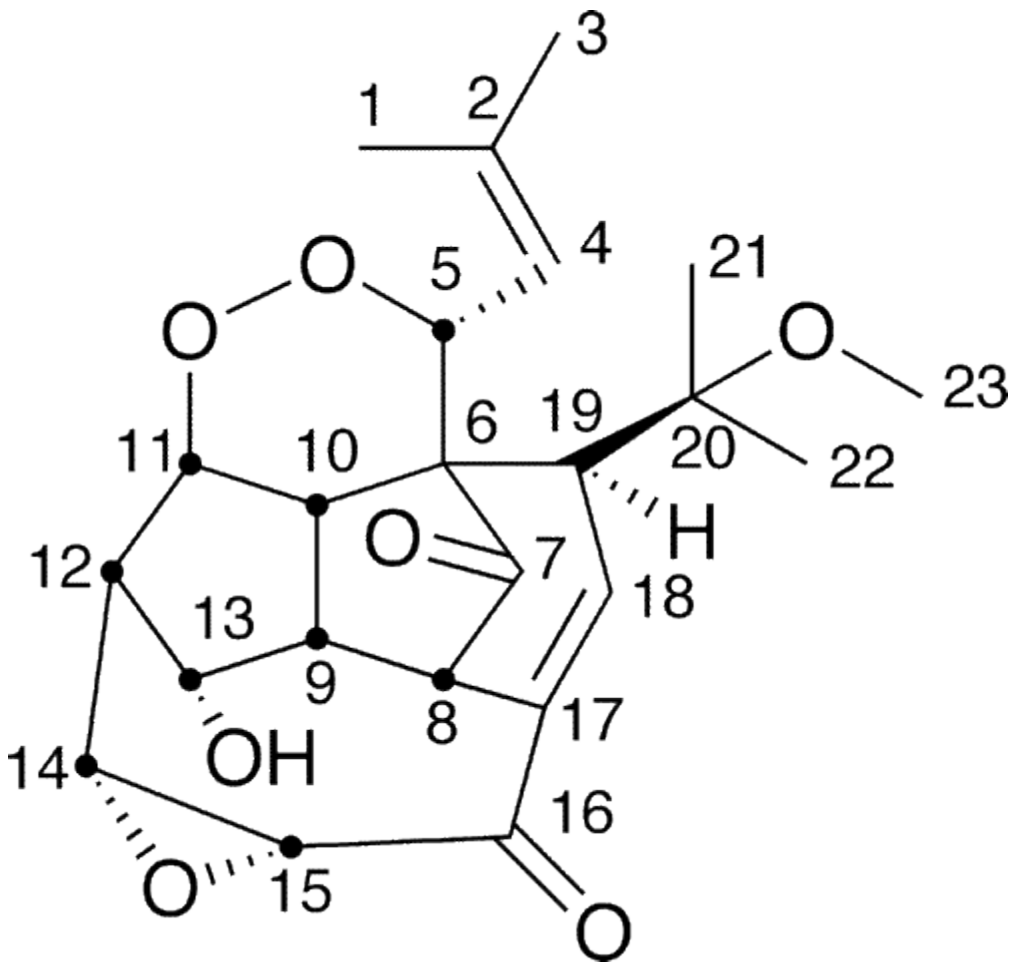
# Hexacyclinol

Isolated 25 mg (0.4%) from Siberian mushrooms *P. rudis*

Formula: $C_{23}H_{28}O_7$; IR: 3415, 1700, 1698, 1625

Skeleton assigned by COSY and HMBC, relative stereochemistry assigned by NOESY.

Highly strained endoperoxide assigned on the basis of $^1H$ chemical shift (5.46 d, 1H, H(C5) and 4.99 dd, 1H, H(C11))




Schlegel, B.; Härtl, A.; Dahse, H.-M.; Gollmick, F. A.; Gräfe, U.; Dörfelt, H.; Kappes, B. *J. Antibiot. (Tokyo).* **2002**, *55* (9), 814–817.

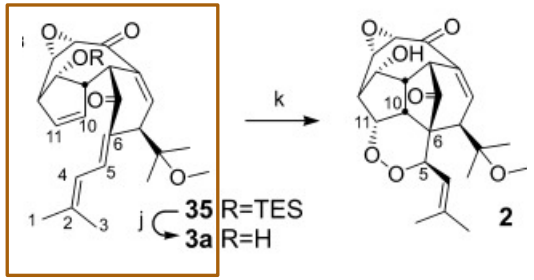
# A controversial total synthesis

**Total Syntheses of Hexacyclinol, 5-*epi*-Hexacyclinol, and Desoxohexacyclinol Unveil an Antimalarial Prodrug Motif****

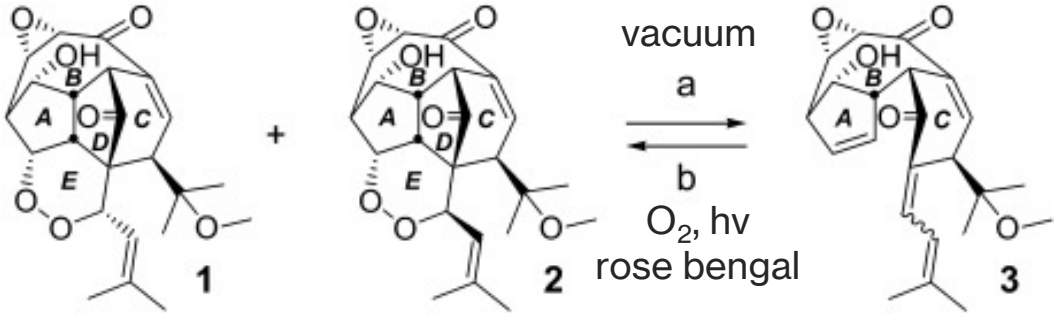
*James J. La Clair**

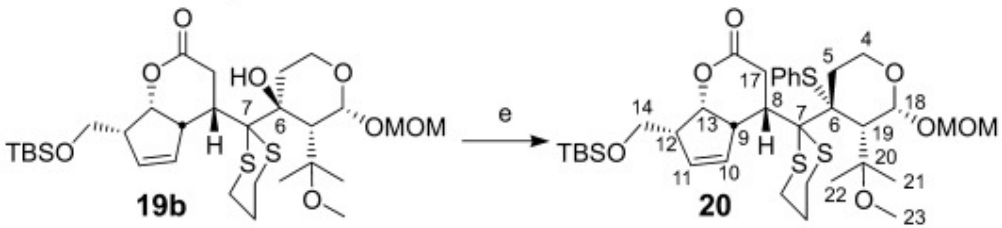
**Single author, 39 steps**

**3.6 g of penultimate 3a isolated in one run**
**0.8-1% overall**
**(average 89% per step)**




**Unusual chemistry**



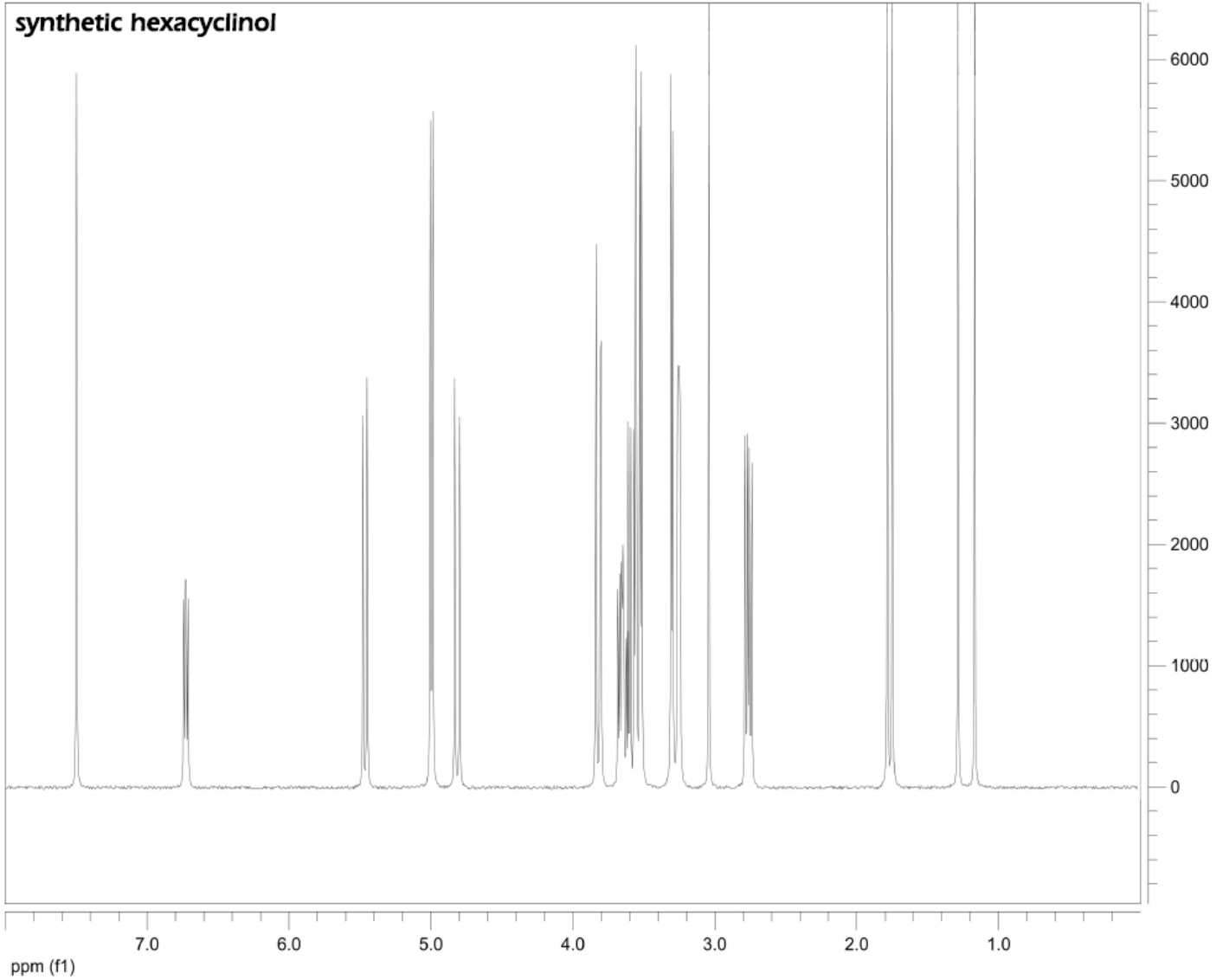




Missing characterization data/protocols for all intermediates, as well as $^{13}C$ NMR data for all 3 products.

- *"The spectra provided in the Supporting Information were collected by N. Voss (Berlin, Germany). The operator added the peak for $CDCl_3$ to the spectrum of synthetic hexacyclinol (**1**), however, this was done incorrectly at δ≈7.5 ppm and against the request of the author."*

La Clair, J. J. *Angew. Chemie Int. Ed.* **2006**, *45* (17), 2769–2773.

# How clean is too clean?

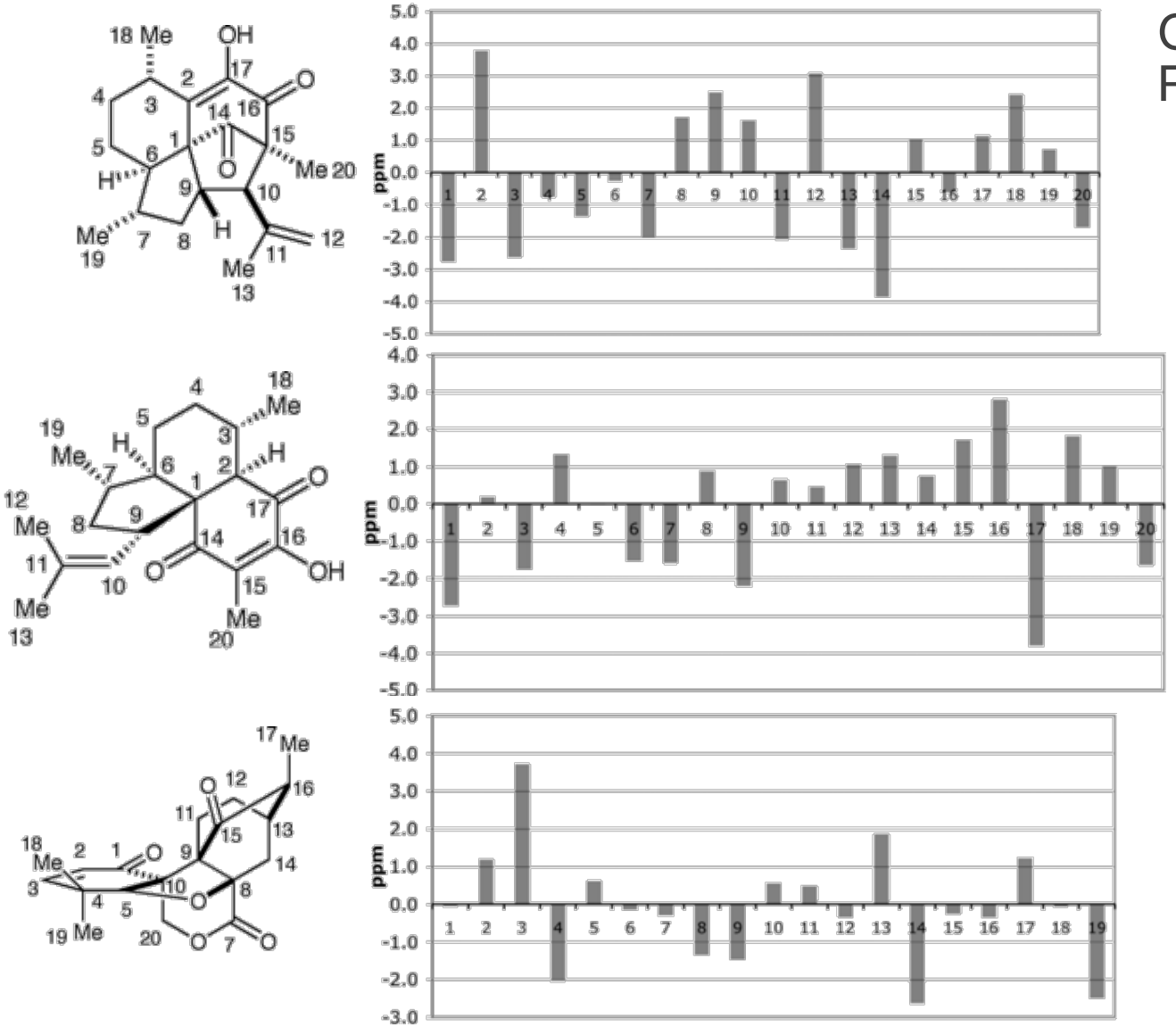



Spectral features:

- $CDCl_3$ resonance at 7.5 ppm
- Perfect lineshapes
- No $^{13}C$ satellites

La Clair, J. J. *Angew. Chemie Int. Ed.* **2006**, *45* (17), 2769–2773.

# $^{13}$C NMR calculation validation



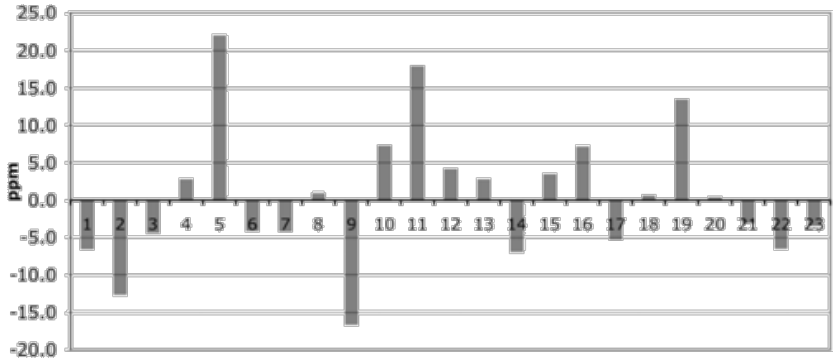

Calculation of $^{13}$C NMR shifts by Rychnovsky

- Validated by calculation of shifts for highly oxidized diterpenes
  - Elisapterosin B, elisabethin A, maoecrystal V
  - Structures minimized at HF/3-21G level
  - NMR shifts calculated with Mpw1pw91/6-31(d,p) GIAO
- All validation compounds returned average error of 1.5-2 ppm and max error of ~3.8 ppm

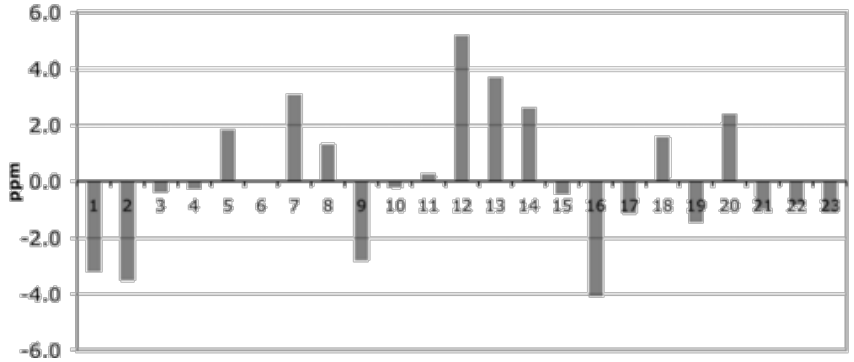
Rychnovsky, S. D. *Org. Lett.* **2006**, *8* (13), 2895–2898.

# Leads on a new structure

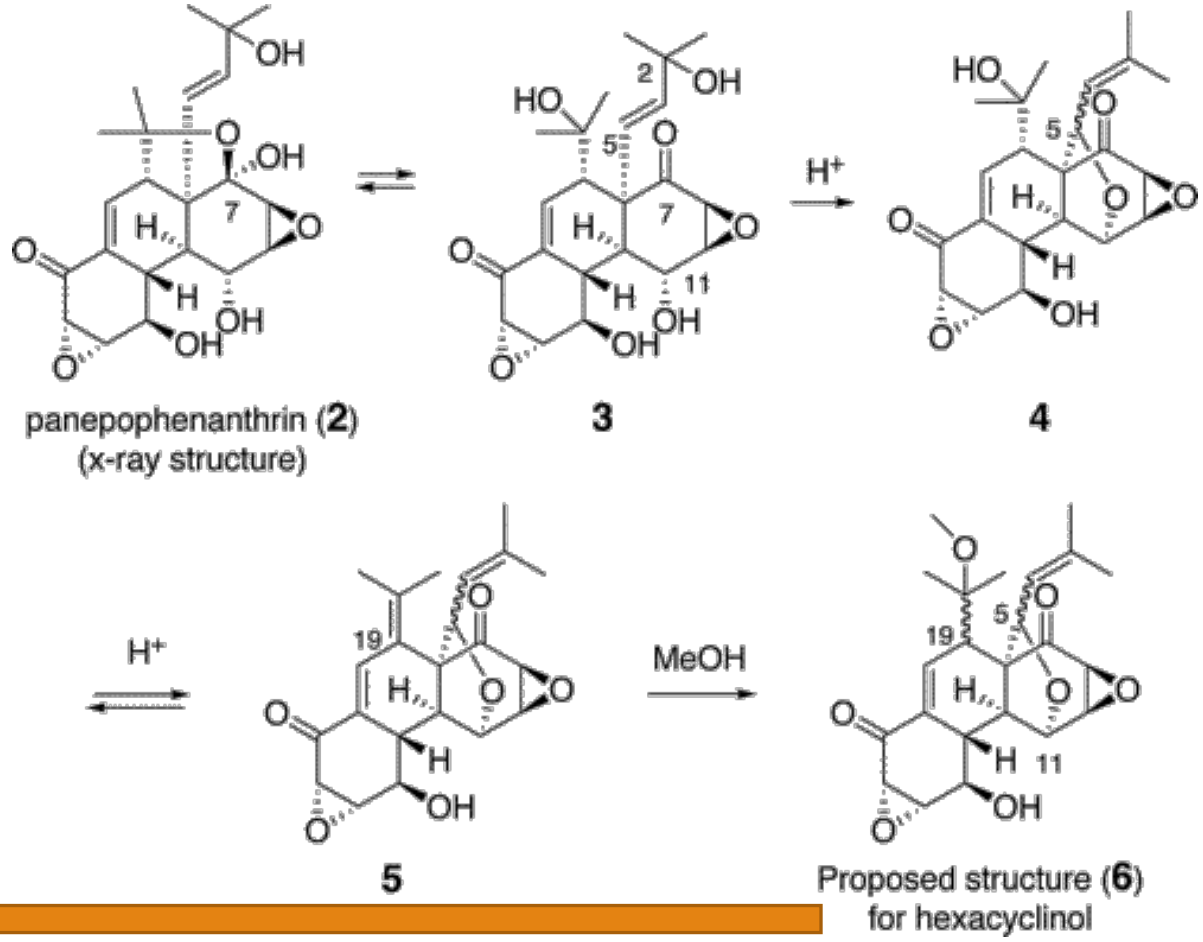
Proposed structure for hexacyclinol has average error of 6.8 ppm, 5 C >10 ppm off, max 22 ppm




Average: 1.8 ppm, max 5.8 ppm
Potential 2D miassignments due to close crosspeaks in $^{1}$H dimension




New proposal: isolation artifact from separate natural product also isolated from *P. Rudis*

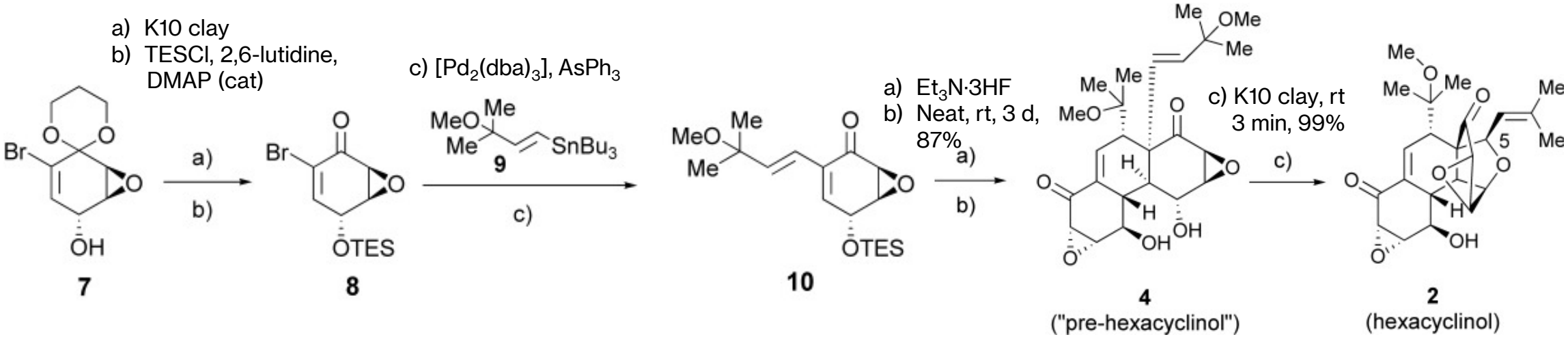



Rychnovsky, S. D. *Org. Lett.* **2006**, *8* (13), 2895–2898.

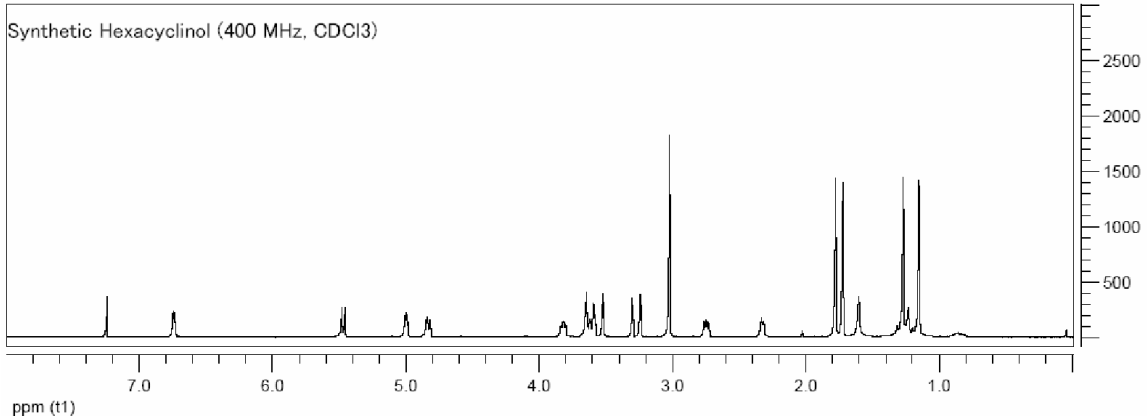
# Confirmation by total synthesis

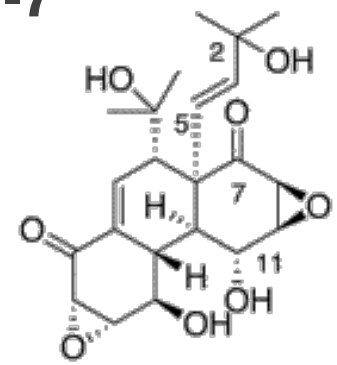
Retrosynthesis designed around Rychnovsky's hypothesis of isolation artifact

Exposure of panepophenanthrin to mildly acidic methanolic conditions unproductive




Established absolute configuration by synthesis from (+)-**7**



Porco Jr., J. A.; Su, S.; Lei, X.; Bardhan, S.; Rychnovsky, S. D. *Angew. Chemie Int. Ed.* **2006**, *45* (35), 5790–5792.

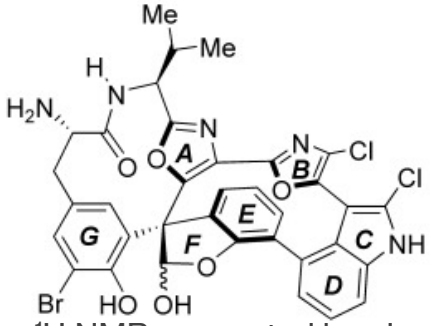
# Diazonamide A

Isolated 54 mg (0.021%) of diazonamide A and 132 mg (0.052%) of diazonamide B from ascidians in the Philippines

Formula, A (MS): $C_{40}H_{36}N_6O_7Cl_2$, $[M+H-H_2O]^+$ = 765.1999, found: 765.1998 (= 25 DBE!)

Formula, B (MS): $C_{35}H_{25}N_5O_6Cl_2Br$, $[M+H-H_2O]^+$ = 743.0340, found: 743.0590

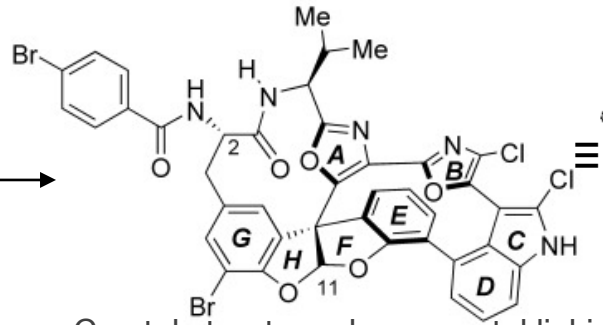
2D NMR provided fragments, but X-ray crystallography required to link fragments




$^1$H NMR suggested hemiacetal on ring F

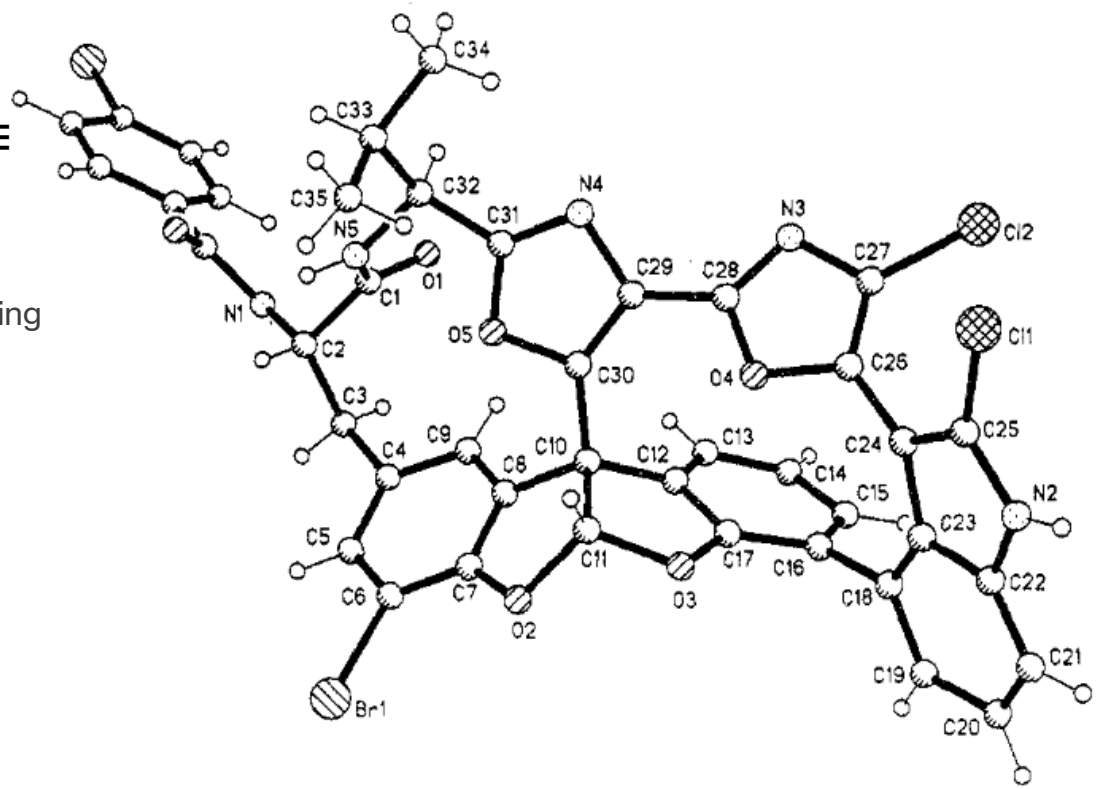
3.5 Hz coupling from H(C11) to $D_2O$ exchangeable proton

?




Crystal structure shows acetal linking F/G rings

≡



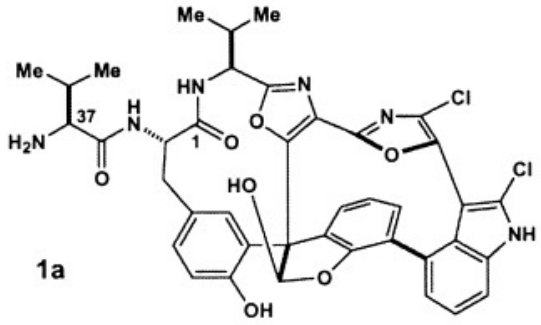

Concluded that acetal must have formed during derivatization.

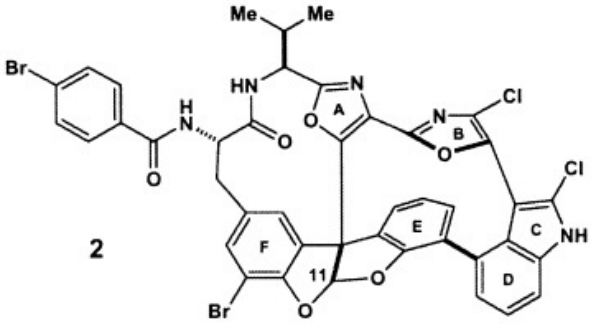
Diazonamide A and B NMR largely conserved, so heteratomic skeleton of A assigned analogously to B, with Val residue to make up mass.

Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113* (6), 2303–2304.

# Discovery of the error

Harran completed first synthesis of nominal diazonamide A and nominal p-bromobenzamide derivative of diazonamide B




Qualitative differences:

- **1a** undergoes net deformylation at C10 in HPLC conditions used to isolate diazonamide A
- Diketopiperazine formation from $NH_2$(C37) to O(C1) observed for **1a**
- Hemiacetal ionizes intact on MS
- NMR data of **2** is slightly different than reported

Li, J.; Jeong, S.; Esser, L.; Harran, P. G. *Angew. Chemie Int. Ed.* **2001**, *40* (24), 4765–4769.

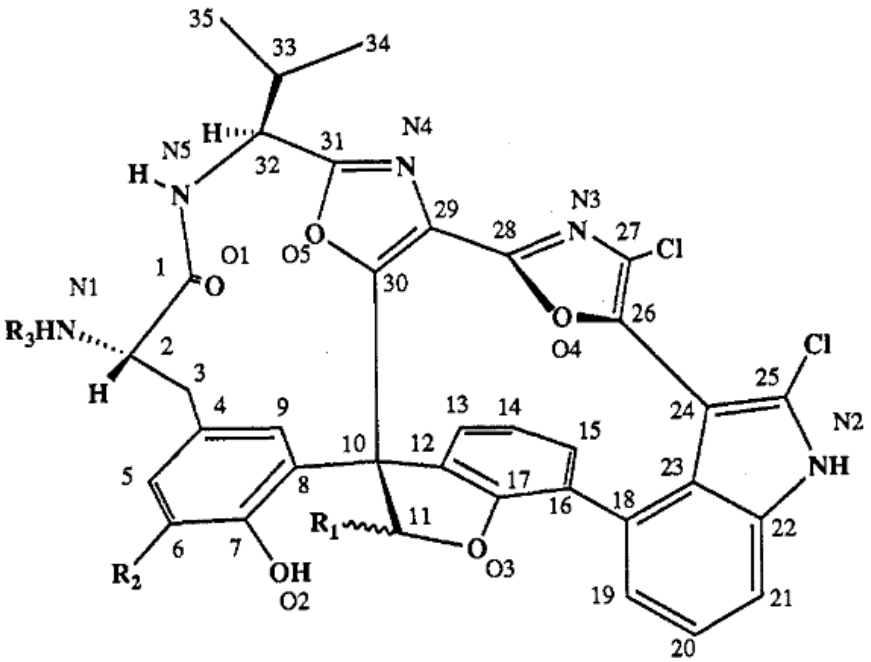
# Elucidating inconsistencies

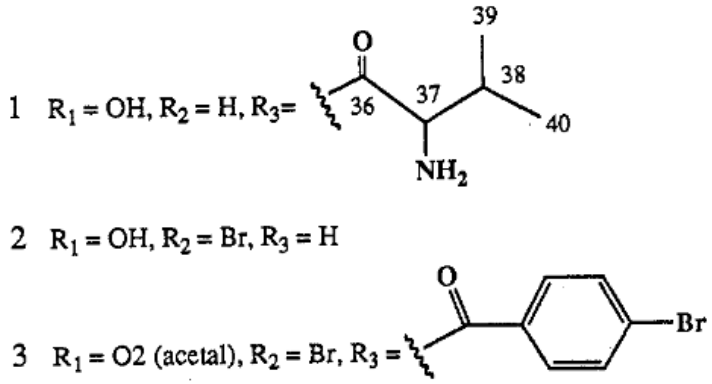
NMR inconsistencies in Val residue ($R^3$)

- N7 protons reported as sharp 1H doublet
- Acetylation turns C37 methine into a clean doublet
- C37 chemical shift (76.9 ppm) downfield of typical Val
- Suggest OH at C37, **requiring NH elsewhere to account for mass**

O → NH supported by MS

- Diazonamide B has exact mass 743.0340 amu
- Proposed hemiacetal structure ($C_{35}H_{26}N_5O_6Cl_2Br$) mass $[M+H-H_2O]^+$ 744.0416 amu (1400 ppm)
- Replacing O for NH ($C_{35}H_{25}N_6O_4Cl_2Br$) has mass $[M+H]^+$ 743.0576 amu (32 ppm)



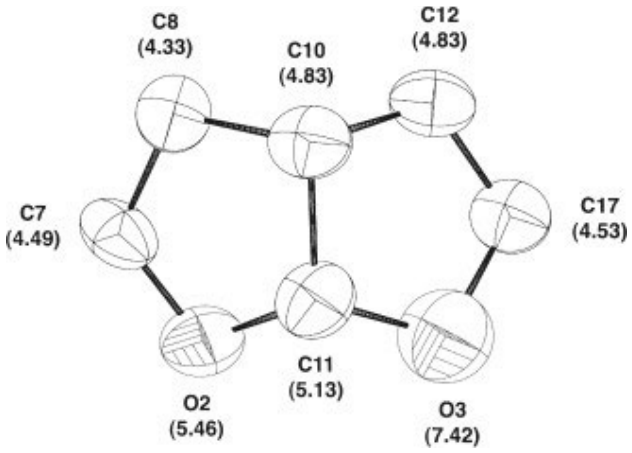




Li, J.; Burgett, A. W. G.; Esser, L.; Amezcua, C.; Harran, P. G. *Angew. Chemie Int. Ed.* **2001**, *40* (24), 4770–4773.

# Determining the O→NH location

## Acetal→Aminal

- Synthetic hemiacetal ionizes without loss of water
- Unusually long aryl C17-O3 bond at 1.433 Å, >3σ and larger than longest observed 1.409 Å
- High temperature factor for O3: average for acetal core is 4.8 $Å^2$, while O3 has $B_{eq}$ 7.4 $Å^2$
- Therefore, suggest NH instead of O3

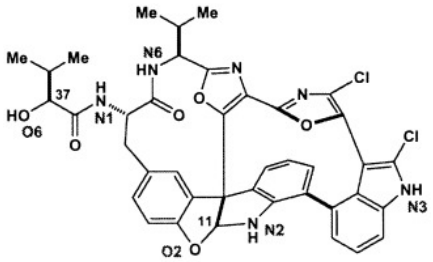



## $^1H$-$^{15}N$ HSQC

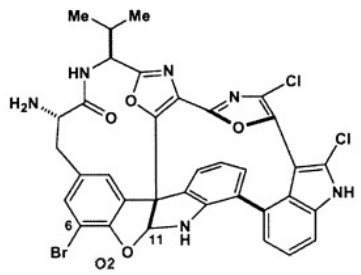
- Doublet originally assigned as valine C37-N7H not coupled to $^{15}N$, supporting C37-OH
- Four unique NH at 12.82 (N3H), 8.66 (N6H), 7.68 (N1H), **7.16 (N2H)**

## DQF-COSY

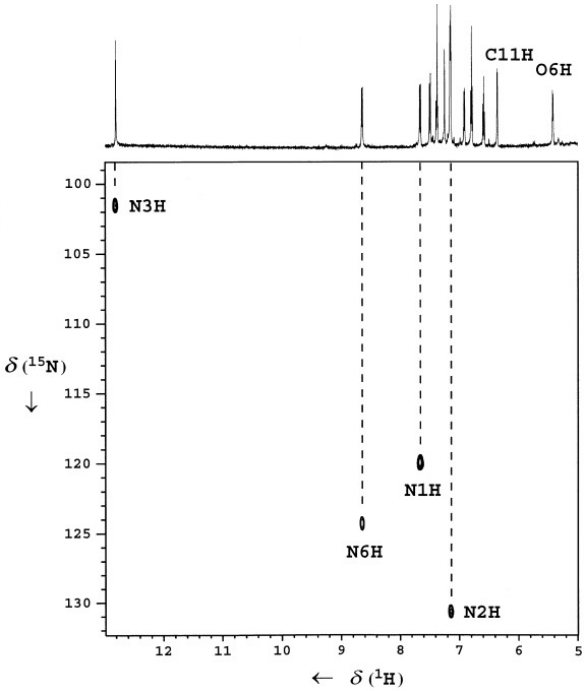
- 7.16 coupled to C11H



4 (–)-diazonamide A (revised Structure)



5 (–)-diazonamide B (revised Structure)

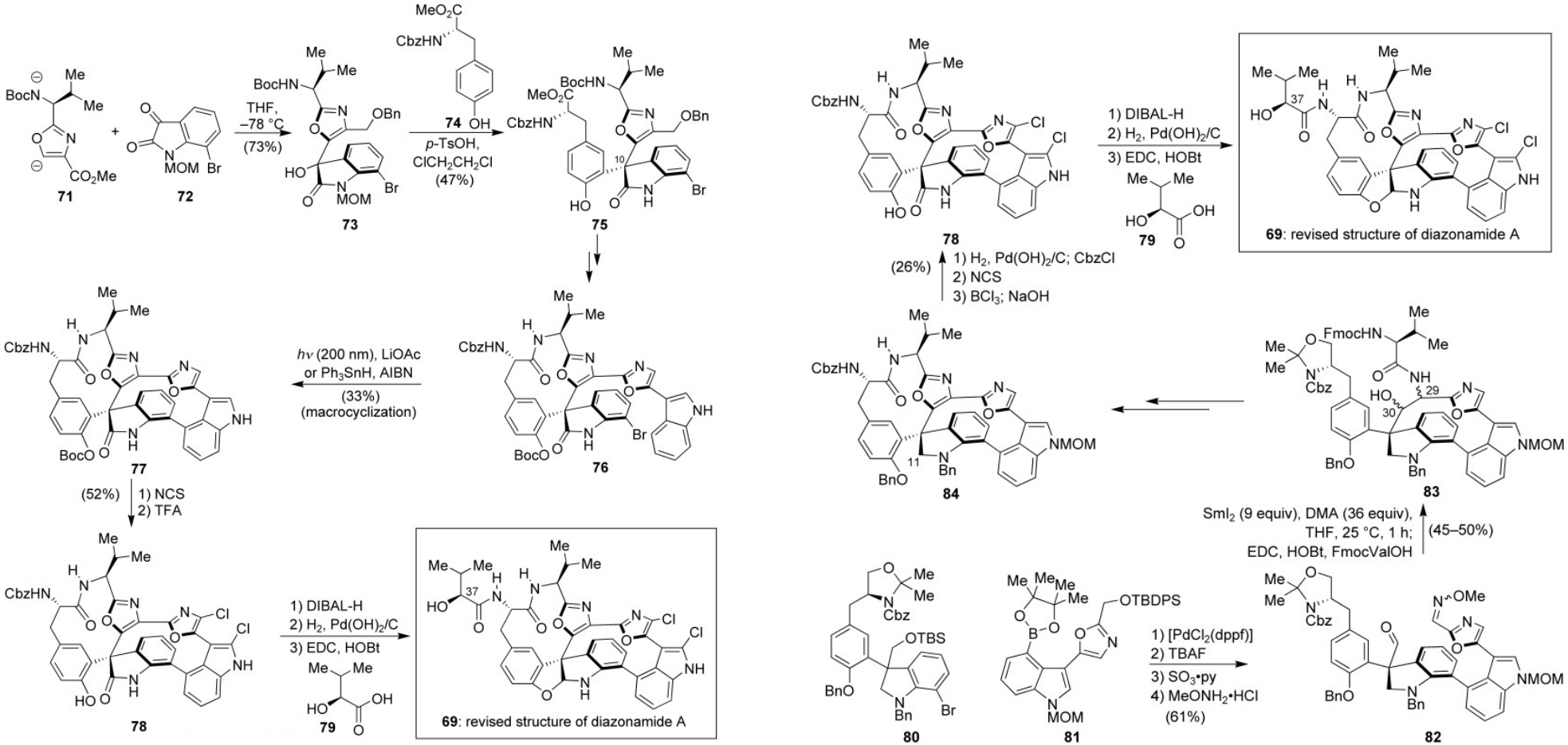



Li, J.; Burgett, A. W. G.; Esser, L.; Amezcua, C.; Harran, P. G. *Angew. Chemie Int. Ed.* **2001**, *40* (24), 4770–4773.

# Confirmation by total synthesis

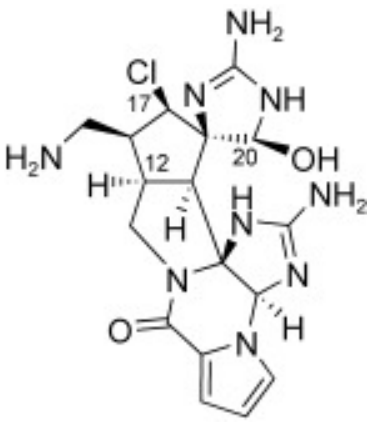
Completed by two strategies (both by Nicoloau)

Confirmed structure and configuration of isovaleric acid by comparison of OH resonance

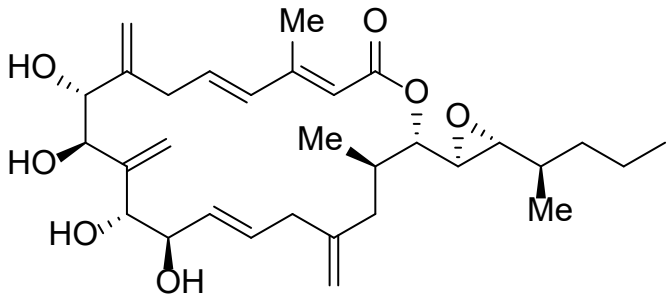



Nicolaou, K. C.; Bella, M.; Chen, D. Y.-K.; Huang, X.; Ling, T.; Snyder, S. A. *Angew. Chemie Int. Ed.* **2002**, *41* (18), 3495–3499. Nicolaou, K. C.; Bheema Rao, P.; Hao, J.; Reddy, M. V.; Rassias, G.; Huang, X.; Chen, D. Y.-K.; Snyder, S. A. *Angew. Chemie Int. Ed.* **2003**, *42* (15), 1753–1758. Nicolaou, K. C.; Snyder, S. A. *Angew. Chemie Int. Ed.* **2005**, *44* (7), 1012–1044.

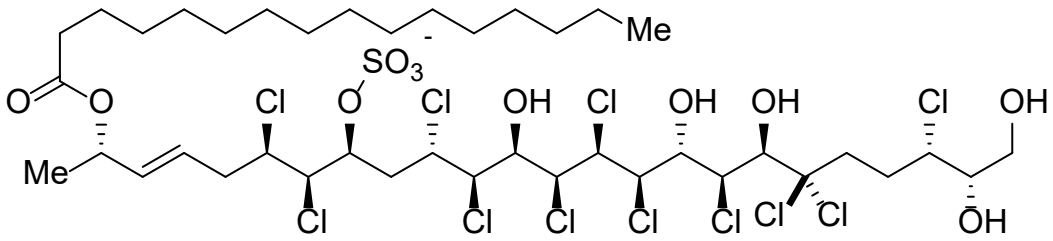
# STEREOCHEMICAL REVISIONS




Palau'amine




Amphidinolide A




Mytilipin B

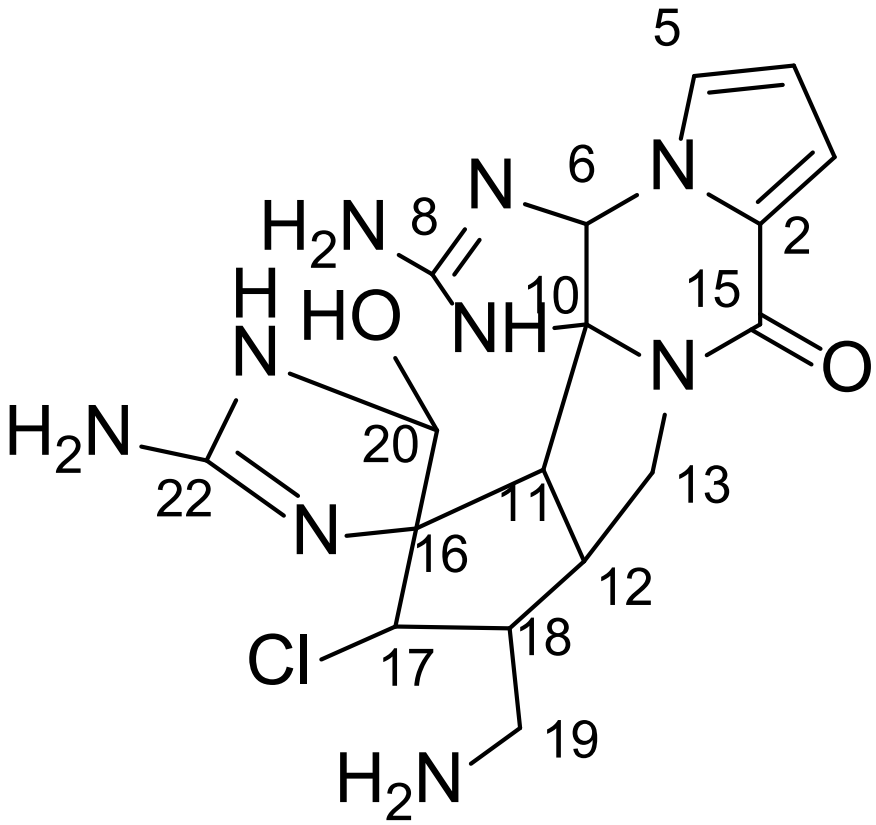
# Palau'amine: Skeletal structure

Isolated 14 mg (0.01%) from sponges *S. agiminata* in the Western Caroline Islands

Determined formula (MS) $C_{17}H_{22}N_9O_2Cl$ (M·HCl)

IR bands for OH + NH (3350 $cm^{-1}$ br), amide (1658 $cm^{-1}$), and guanidine hydrochloride (1700 $cm^{-1}$).

Planar skeleton assigned by acetylation and COSY, HMQC, HMBC

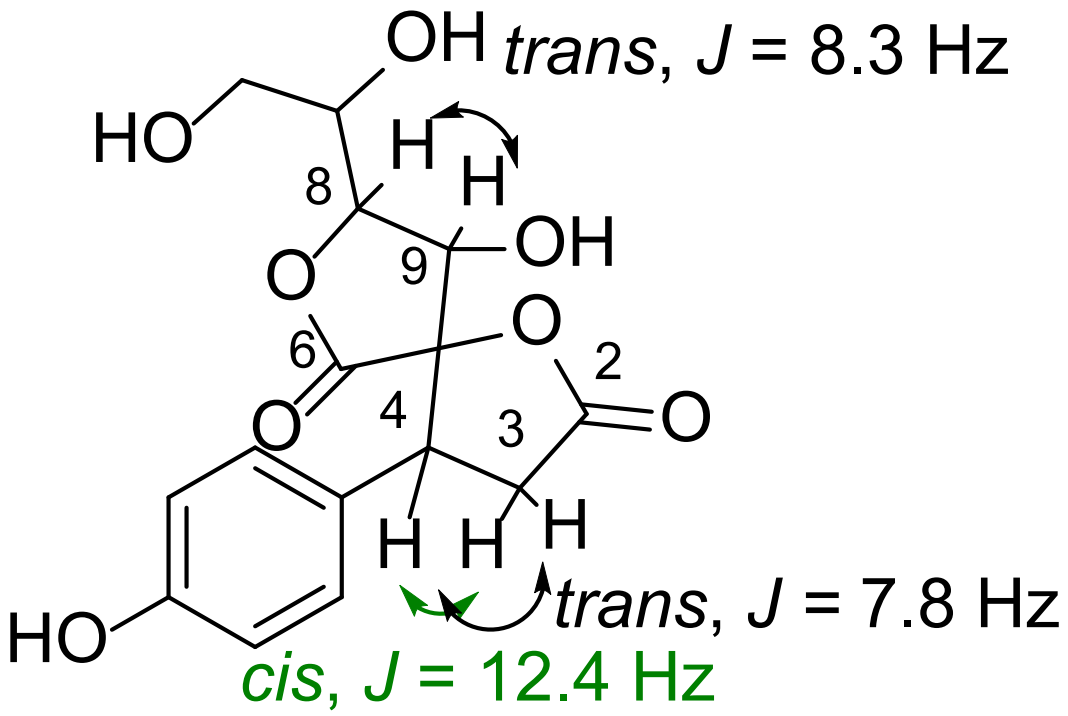


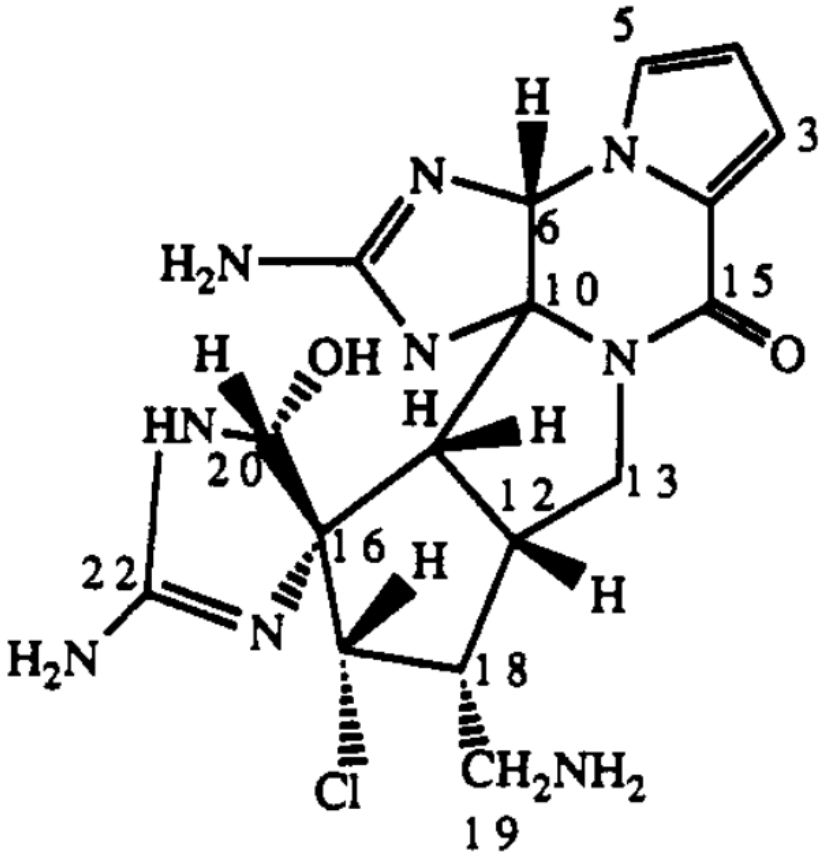

Kinnel, R. B.; Gehrken, H. P.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115* (8), 3376–3377.

# Relative configuration at C11/C12

Configuration of bridgehead carbons assigned by analogy to spiroannulated bislactone

- In model: assigned *cis* coupling of 12.4 and *trans* coupling of 7.9 Hz
- Since H11 had $J$ = 14.1 Hz, assigned H11-H12 as *cis*




Kinnel, R. B.; Gehrken, H. P.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115* (8), 3376–3377. Lowry, J. B.; Riggs, N. V. *Tetrahedron Lett.* **1964**, *5* (39), 2911–2917.

# Assignment of congeners

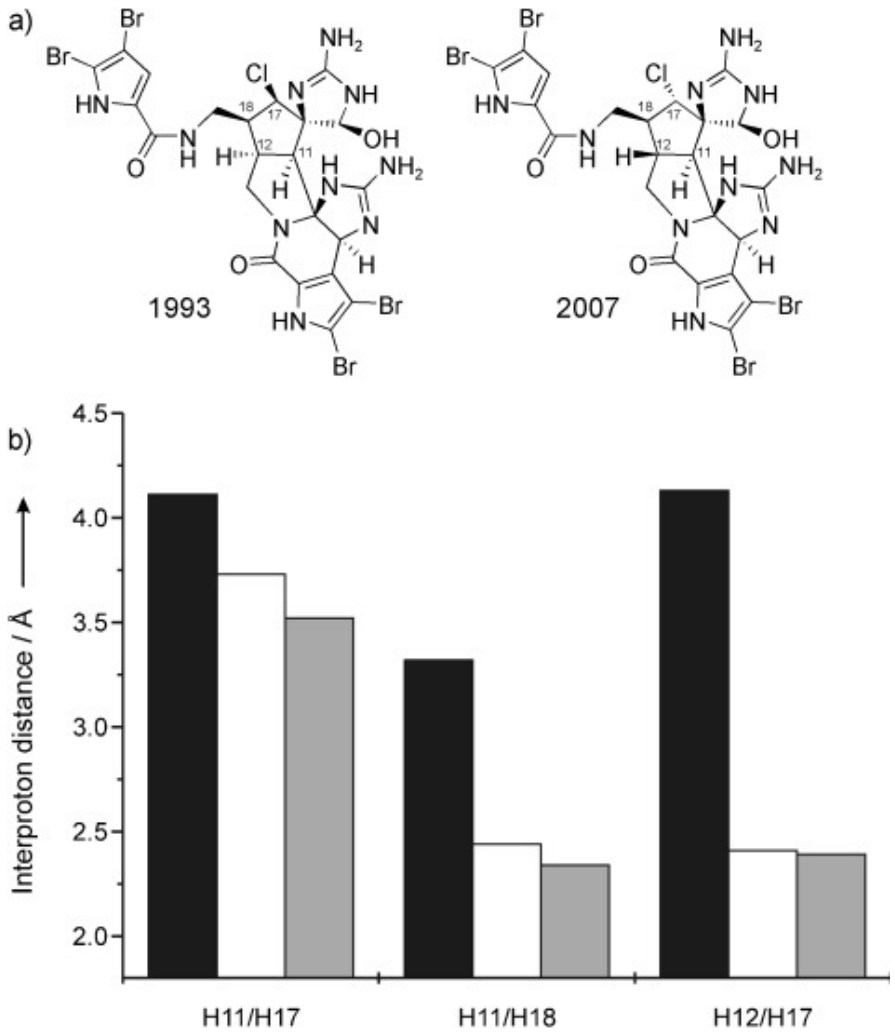
Series of congeners identified with *trans*-fusion

- Carteramine A (Jan 2007) by NOESY, $J$ = 14.4 Hz
- Konbu'acidin B (Feb 2007) by NOESY, $J$ = 13.8 Hz
- Tetrabromostyloguanidine (Mar 2007) by quantitative ROESY, $J$ = 14.4 Hz

Quantitative ROESY: crosspeak volume integral scales with mixing time and correlated to interproton distances calibrated using reliable distance

Comparison to computation:

- Black bars = *cis*-fusion
- White bars = *trans*-fusion
- Gray bars = experimental distance



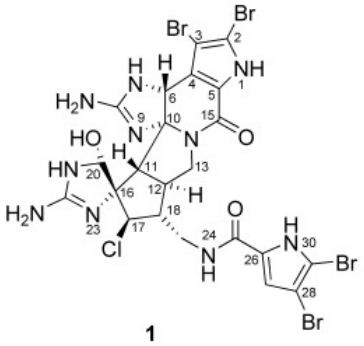

Köck, M.; Grube, A.; Seiple, I. B.; Baran, P. S. *Angew. Chemie Int. Ed.* **2007**, *46* (35), 6586–6594. Grube, A.; Köck, M. *Angew. Chemie Int. Ed.* **2007**, *46* (13), 2320–2324. Kobayashi, H.; Kitamura, K.; Nagai, K.; Nakao, Y.; Fusetani, N.; van Soest, R. W. M.; Matsunaga, S. *Tetrahedron Lett.* **2007**, *48* (12), 2127–2129. Buchanan, M. S.; Carroll, A. R.; Addepalli, R.; Avery, V. M.; Hooper, J. N. A.; Quinn, R. J. *J. Org. Chem.* **2007**, *72* (7), 2309–2317.

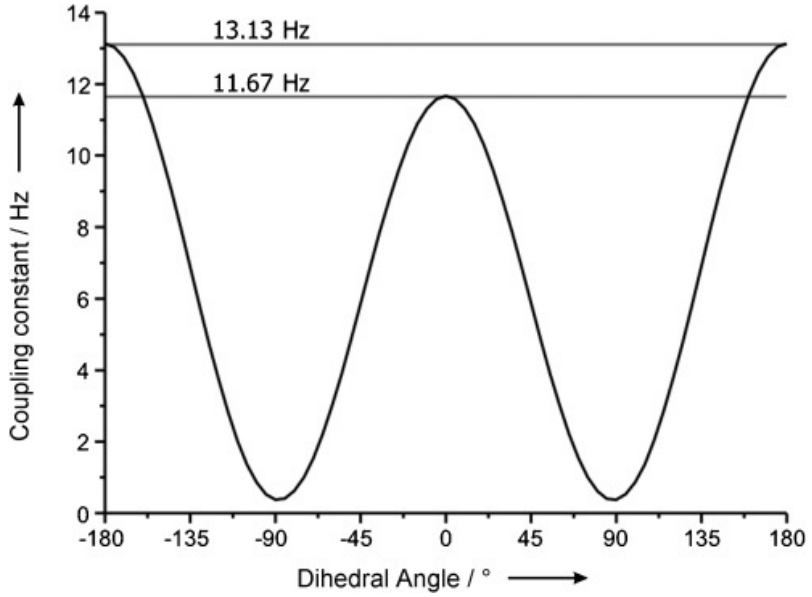
# A tenuous comparison

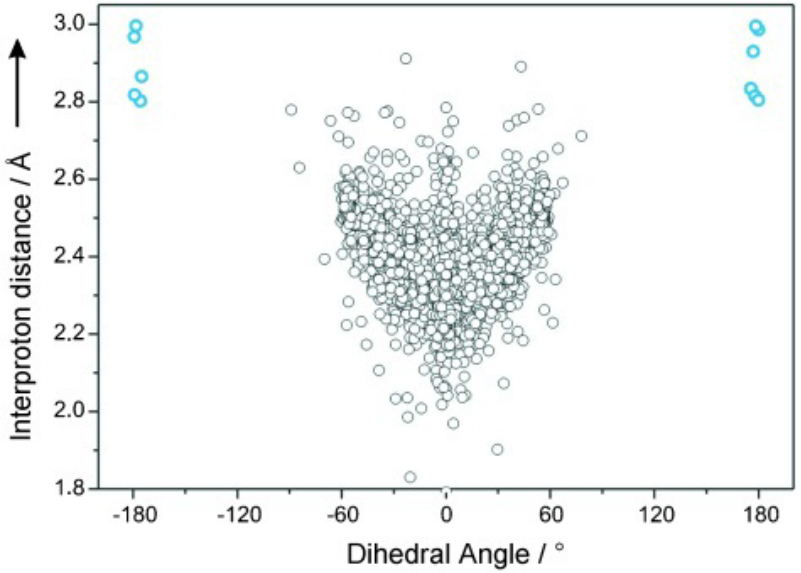
*Cis*-fused 5,5-bicycle intuitively more stable, calculated 6.5 kcal/mol more stable

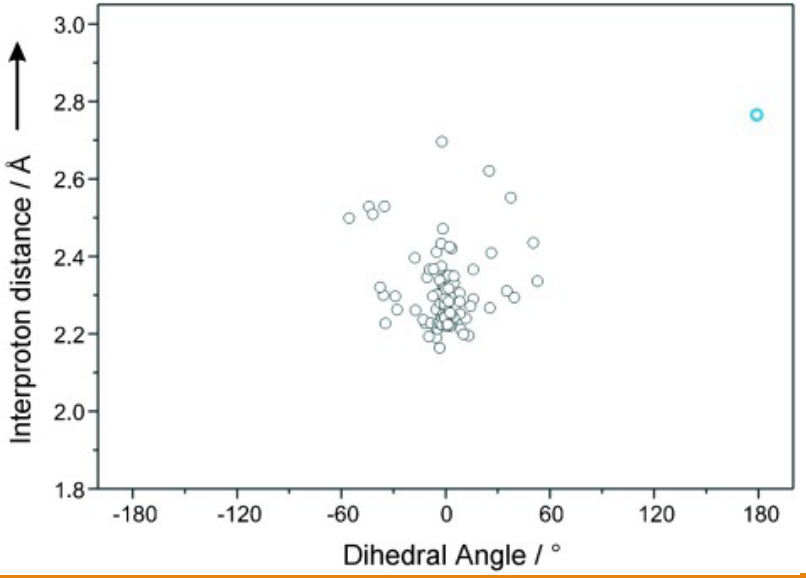
*Trans*-fused 5,5-bicycles rare, exceedingly so for 3-aza systems

Calculated coupling constants for tetrabromostyloguanidine indicate maximum possible *J* for *cis*-fusion is only 11.7 Hz



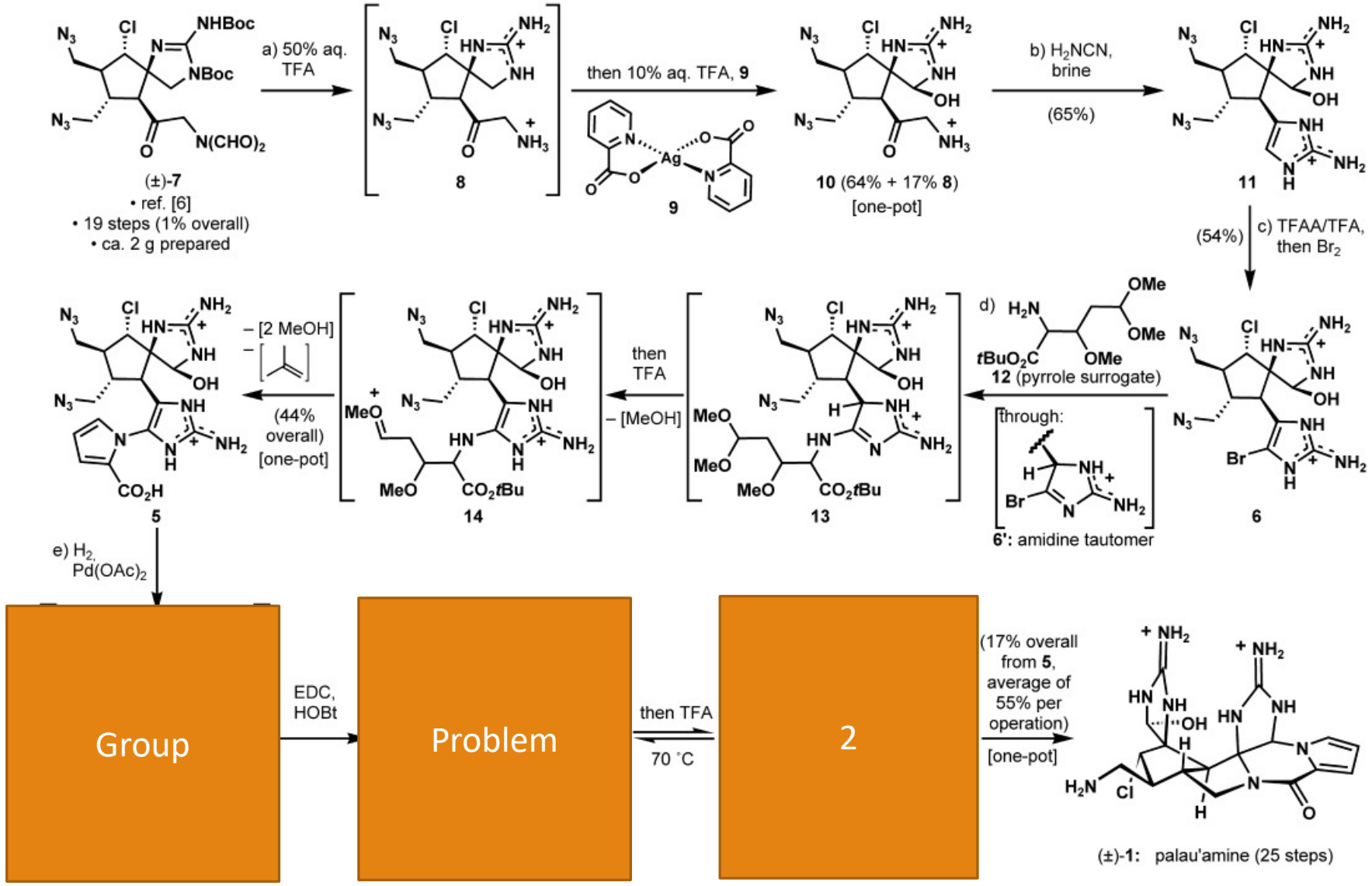




Köck, M.; Grube, A.; Seiple, I. B.; Baran, P. S. *Angew. Chemie Int. Ed.* **2007**, *46* (35), 6586–6594. Grube, A.; Köck, M. *Angew. Chemie Int. Ed.* **2007**, *46* (13), 2320–2324.

# Confirmation by total synthesis

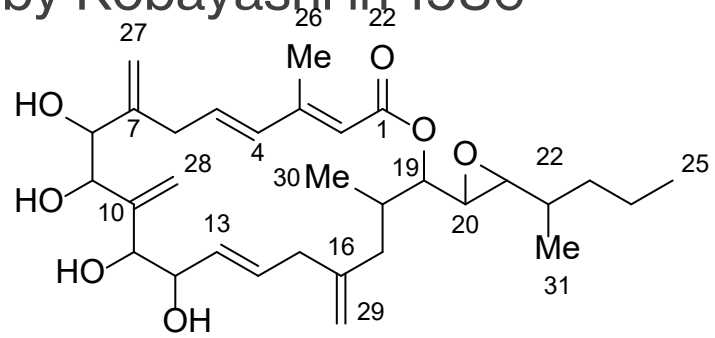



Seiple, I. B.; Su, S.; Young, I. S.; Lewis, C. A.; Yamaguchi, J.; Baran, P. S. *Angew. Chemie Int. Ed.* **2009**, *49* (6), 1095–1098.

# Amphidinolide A

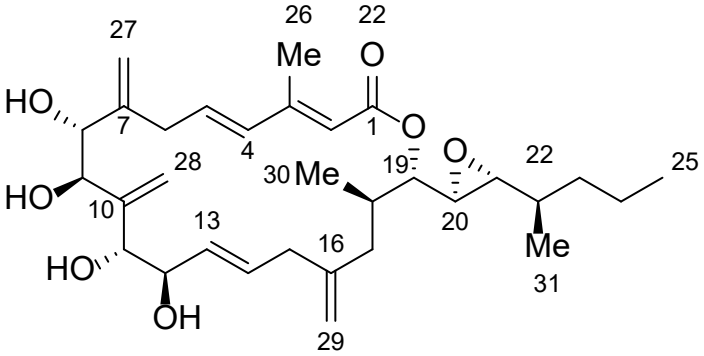
Isolated 7.5 mg (0.0002% yield) from 1000 L cultures of dinoflagellate *Amphidinium sp.*

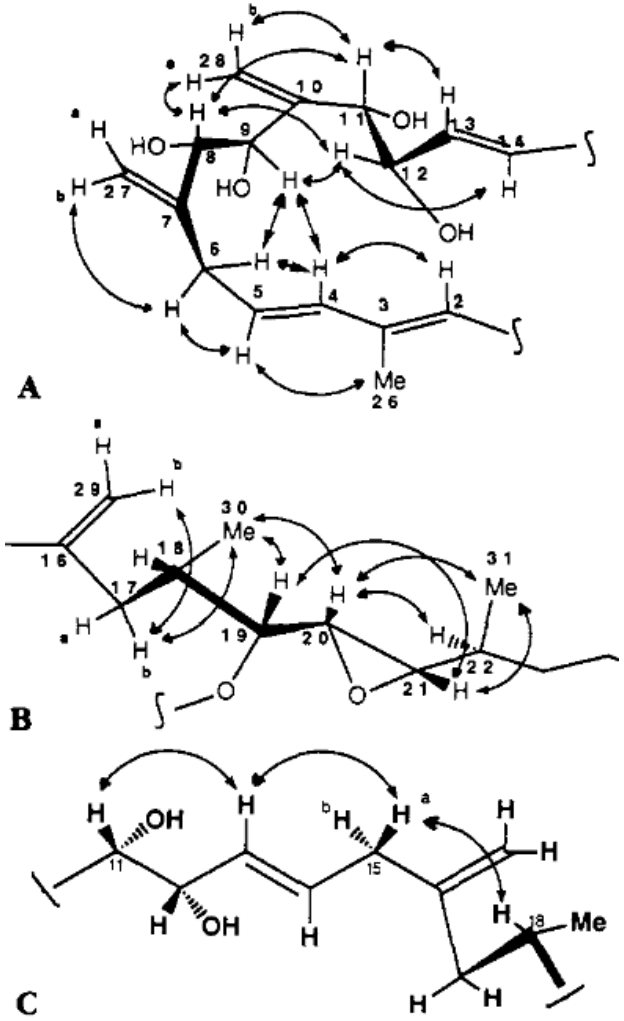
Planar structure assigned by COSY, HMQC, HMBC by Kobayashi in 1986




Relative configuration proposed by NOESY in 1991

- Complicated by flexibility of macrocyclic structure

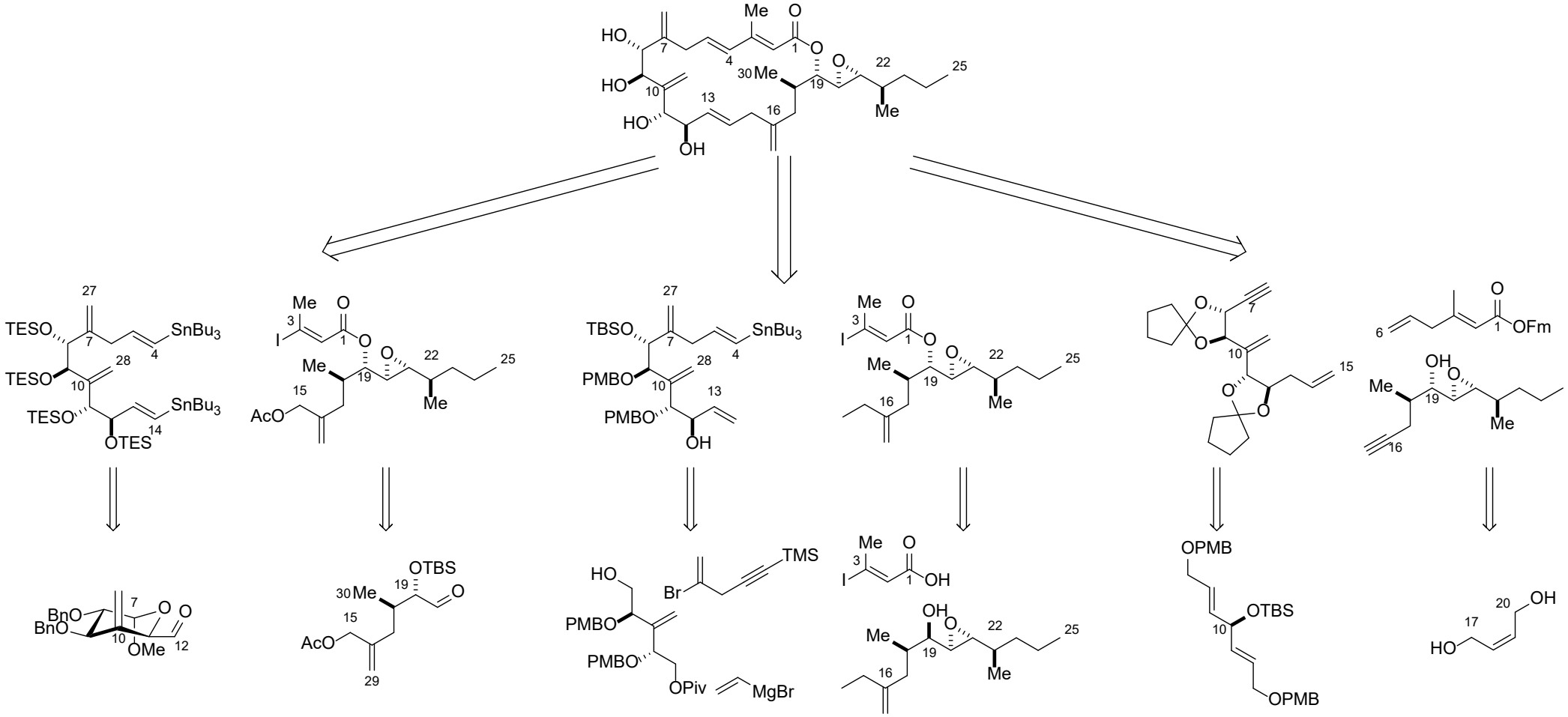



Kobayashi, J.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Yamasu, T.; Sasaki, T.; Hirata, Y. *Tetrahedron Lett.* **1986**, *27* (47), 5755–5758. Kobayashi, J.; Ishibashi, M.; Hirota, H. *J. Nat. Prod.* **1991**, *54* (5), 1435–1439.

# Discovery of misassignment

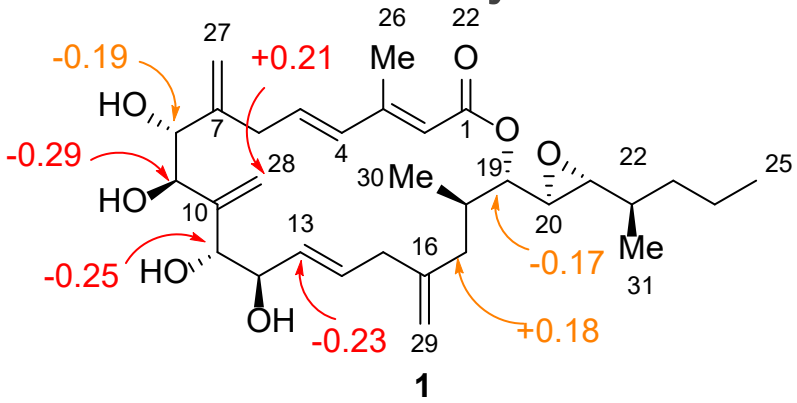
Attempts by Pattenden, Maleczka, and Trost all found that spectra did not match!




Lam, H. W.; Pattenden, G. *Angew. Chemie Int. Ed.* **2002**, *41* (3), 508–511. Maleczka Robert E.; Terrell, L. R.; Geng, F.; Ward, J. S. *Org. Lett.* **2002**, *4* (17), 2841–2844. Trost, B. M.; Chisholm, J. D.; Wrobleski, S. T.; Jung, M. *J. Am. Chem. Soc.* **2002**, *124* (42), 12420–12421.

# Identifying the problems

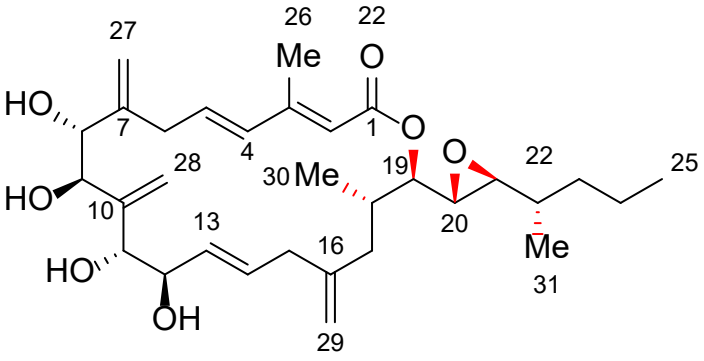
2D correlations of synthetic material verify planar structure.



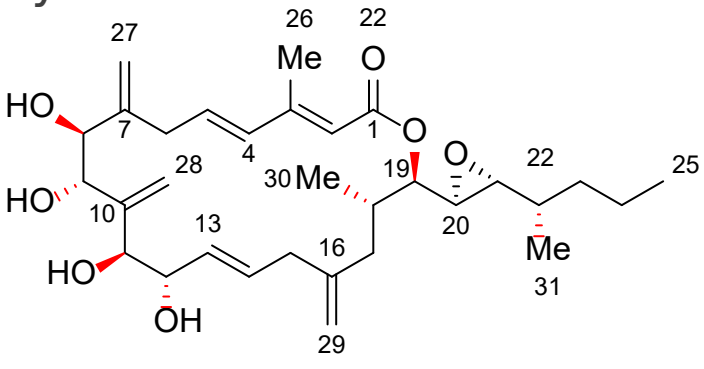

**1**

Suspect regions

- Near epoxide: acyclic NOE's
- Between tetraol and epoxide region: sequential NOE's
- Configuration of tetraol: largest and systematic chemical shift differences




**2**

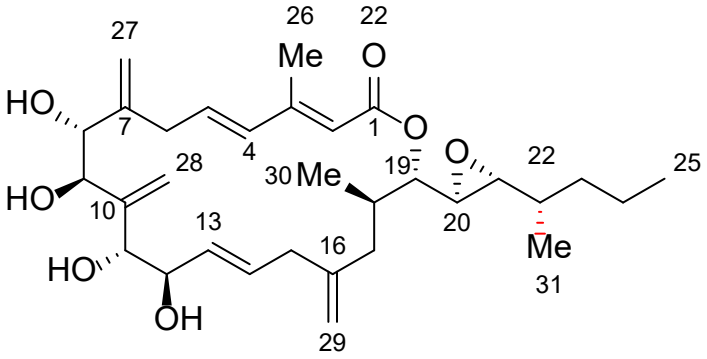



**3**

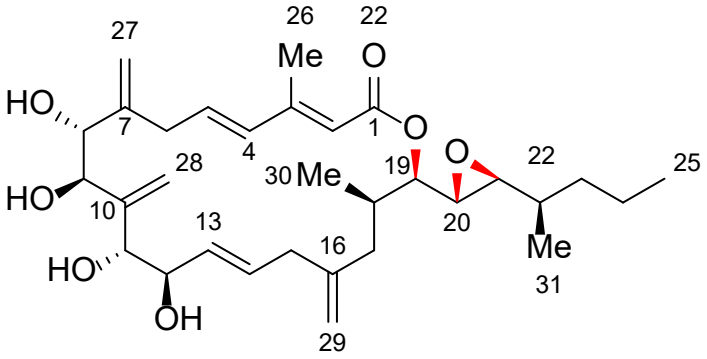
Trost, B. M.; Harrington, P. E. *J. Am. Chem. Soc.* **2004**, *126* (16), 5028–5029.

# Deconvoluting the epoxide region

Synthesized C22 epimer (**4**) and C19-C21 epimer (**5**)




**4**




**5**

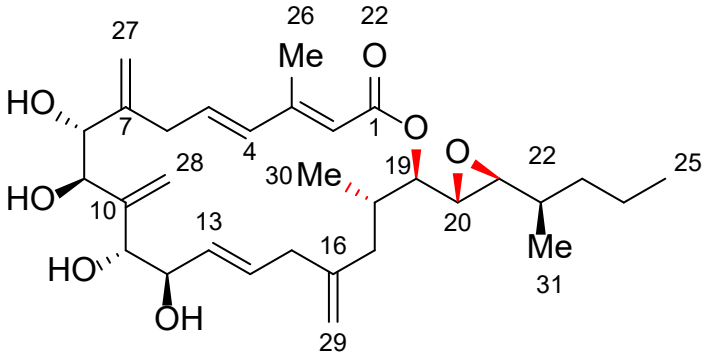
| Isomer | $J_{H18\text{-}H19}$ |
|---|---|
| Isolated material | 3.4 Hz |
| **4** | 3.8 Hz |
| **5** | 10.3 Hz |

Conclude that H18 and H19 are *trans*

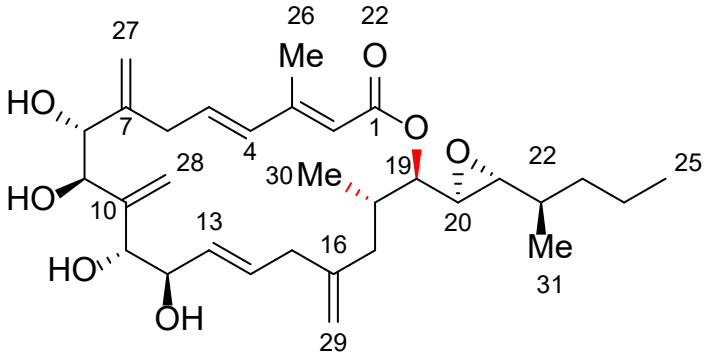
Trost, B. M.; Harrington, P. E. *J. Am. Chem. Soc.* **2004**, *126* (16), 5028–5029.

# Discovery of tetraol misassignment

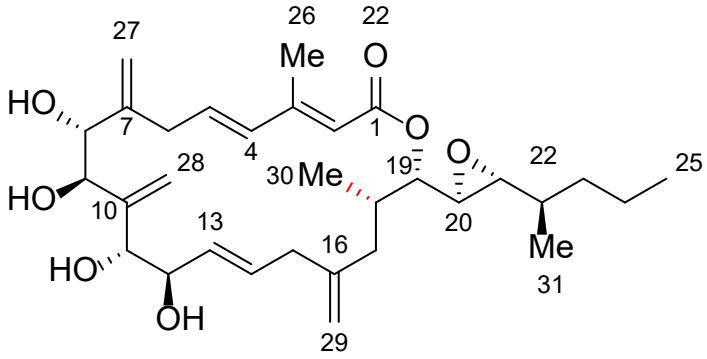
Synthesized C18-21 epimer (**6**), C18-C19 epimer (**7**), and C18 epimer (**8**)




**6**




**7**




**8**

Recapitulated *trans*-H18-H19 ($J_{H18\text{-}H19}$ = 10.5 Hz in **8**)

Found largest and systematic deviations in H9 and H11

| Proton | $^1$H δ synthetic – δ isolated | | | | | | |
|---|---|---|---|---|---|---|---|
| | **1** | **2** | **4** | **5** | **6** | **7** | **8** |
| 9 | -0.29 | -0.49 | -0.30 | -0.42 | -0.48 | -0.46 | -0.28 |
| 11 | -0.25 | -0.21 | -0.26 | -0.14 | -0.20 | -0.18 | -0.10 |

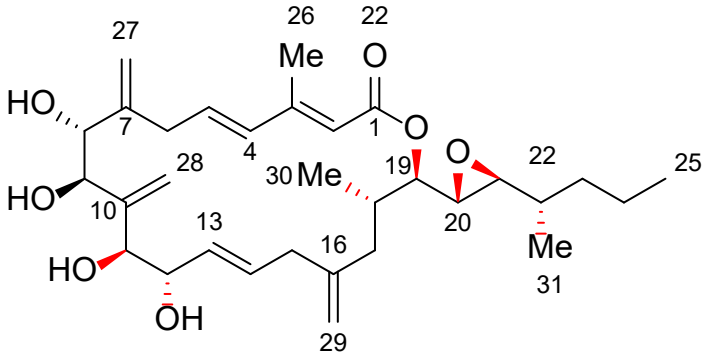
Trost, B. M.; Harrington, P. E. *J. Am. Chem. Soc.* **2004**, *126* (16), 5028–5029.

# Relative configuration between regions

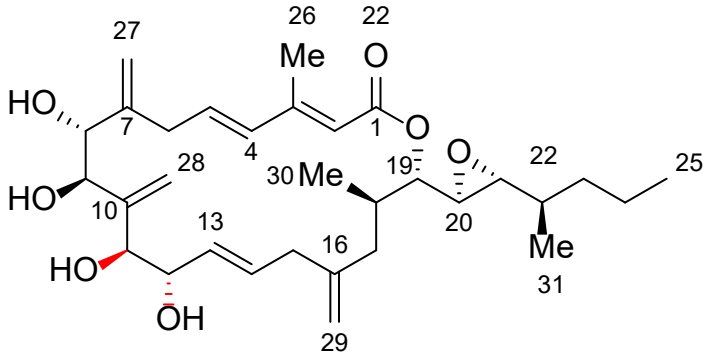
$J_{H8\text{-}H9}$ (<1 Hz) and $J_{H11\text{-}H12}$ (<1 Hz) recapitulated in isomers **1, 2, 4-8**.

- Conclude that relative configuration *within pairs* (H8/H9, H11/H12) are correct but *between pairs* (H9/H11) is incorrect

Synthesized **9** and **10**: inverted C11/C12, testing relative configuration between epoxide region and tetraol (same relative configuration in epoxide region as **1**)




**9**




**10**

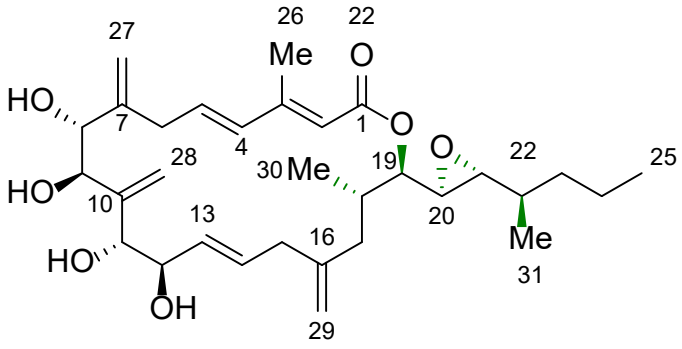
| Proton | $^1$H δ synthetic – δ isolated | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| 9 | -0.29 | -0.49 | -0.30 | -0.42 | -0.48 | -0.46 | -0.28 | **-0.30** | **+0.04** |
| 11 | -0.25 | -0.21 | -0.26 | -0.14 | -0.20 | -0.18 | -0.10 | **-0.07** | **-0.05** |

Trost, B. M.; Harrington, P. E. *J. Am. Chem. Soc.* **2004**, *126* (16), 5028–5029.

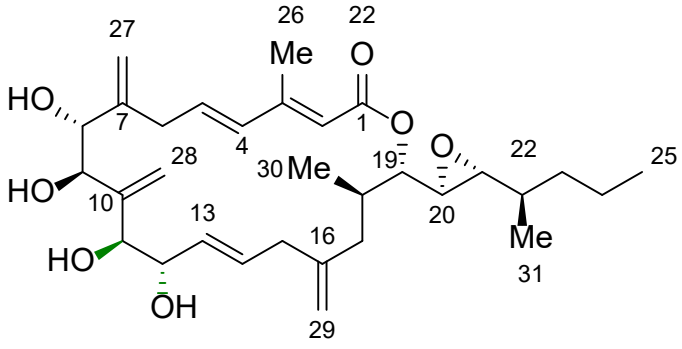
# Last hurdle and confirmation

**10** is close, but deviations in epoxide region (C18-C22) observed.

| Proton | $^1$H δ synthetic – δ isolated | | | |
|---|---|---|---|---|
| | **7** | **8** | **9** | **10** |
| 18 | **-0.03** | -0.16 | -0.07 | **-0.05** |
| 19 | **-0.05** | -0.16 | -0.10 | **-0.07** |
| 20 | **-0.01** | +0.06 | +0.07 | **+0.07** |
| 21 | **+0.01** | -0.06 | -0.08 | **-0.08** |

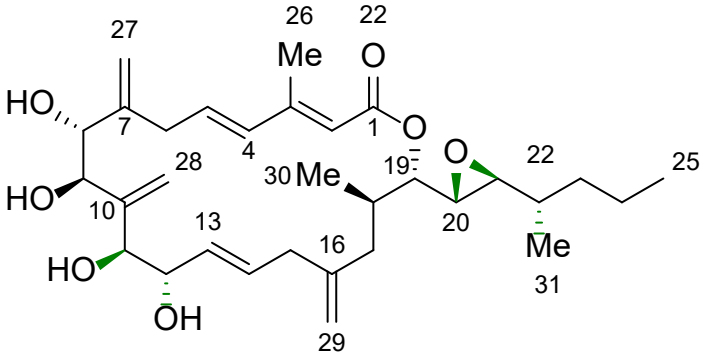


**7**



**10**

Synthesized **11**: epoxide configuration of **7**, tetraol configuration of **10**, and inter-region configuration of **10**



**11**

NMR consistency:

- $^1$H: <0.01 ppm for all but 2 protons (0.02, 0.03 ppm)
- $^{13}$C: <0.1 ppm for all carbons

Optical rotation:

- Synthetic **11**: $[\alpha]^{24}_D$ +56° (*c* 0.05, $CHCl_3$)
- Isolated: $[\alpha]^{24}_D$ +46° (*c* 1.0, $CHCl_3$)

Trost, B. M.; Harrington, P. E. *J. Am. Chem. Soc.* **2004**, *126* (16), 5028–5029. Trost, B. M.; Wrobleski, S. T.; Chisholm, J. D.; Harrington, P. E.; Jung, M. *J. Am. Chem. Soc.* **2005**, *127* (39), 13589–13597. Trost, B. M.; Harrington, P. E.; Chisholm, J. D.; Wrobleski, S. T. *J. Am. Chem. Soc.* **2005**, *127* (39), 13598–13610.

# Mytilipin B: Isolation and connectivity

Isolated 6.2 mg from 1.2 kg digestive glands of Italian mussels flagged as toxic

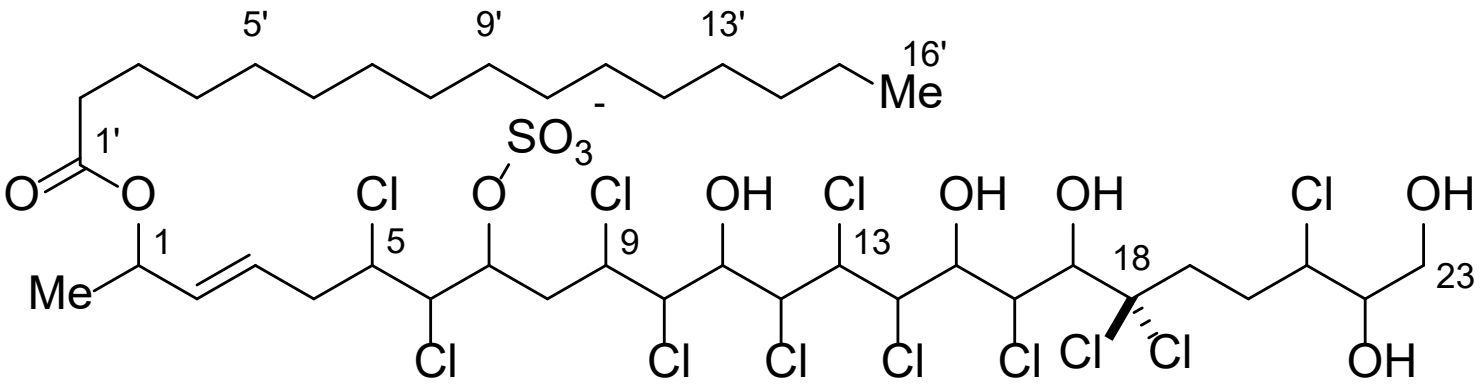
From ESIMS: $C_{40}H_{66}O_{11}SCl_{11}$ = 2 DBE

IR shows sulfate group ($\nu_{max}$ = 1240, 1220, 820 $cm^{-1}$), ester ($^{13}C$, 174.1 ppm), 5 hydroxyl groups by peracetylation

Carbon skeleton assembled by HMBC starting at C1', C18

Hydroxyl groups placed by HMBC correlations to acetoxy groups

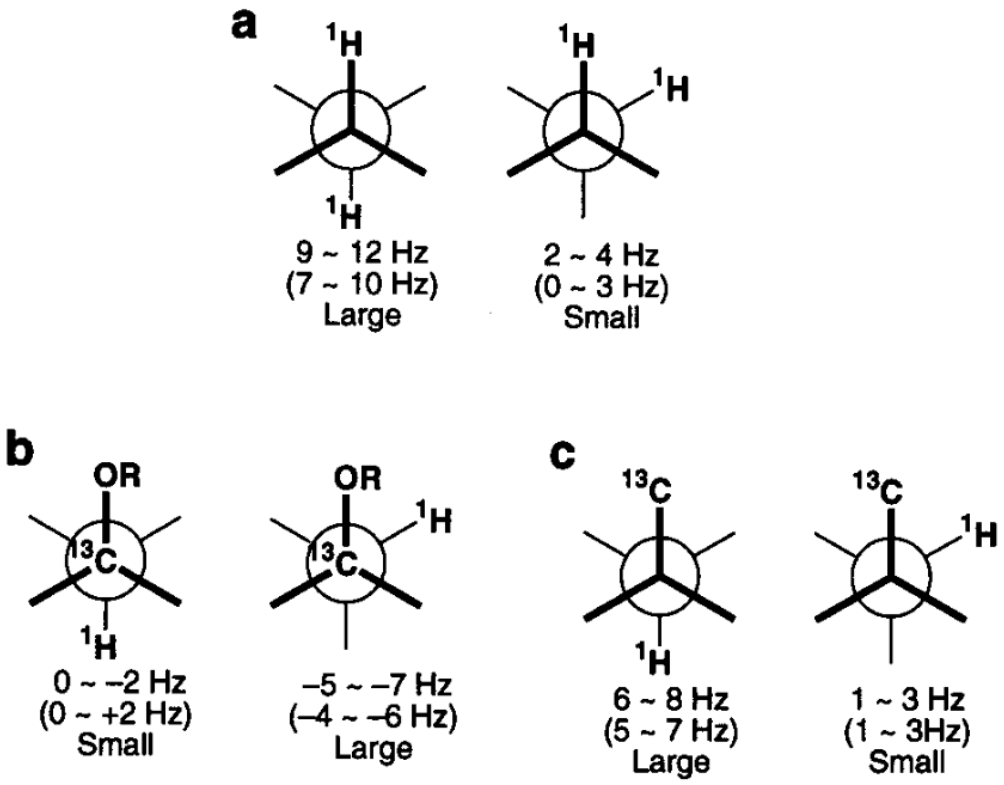
Sulfate more deshielding than single chlorine atom and placed according to chemical shift and remaining Cl filled in.

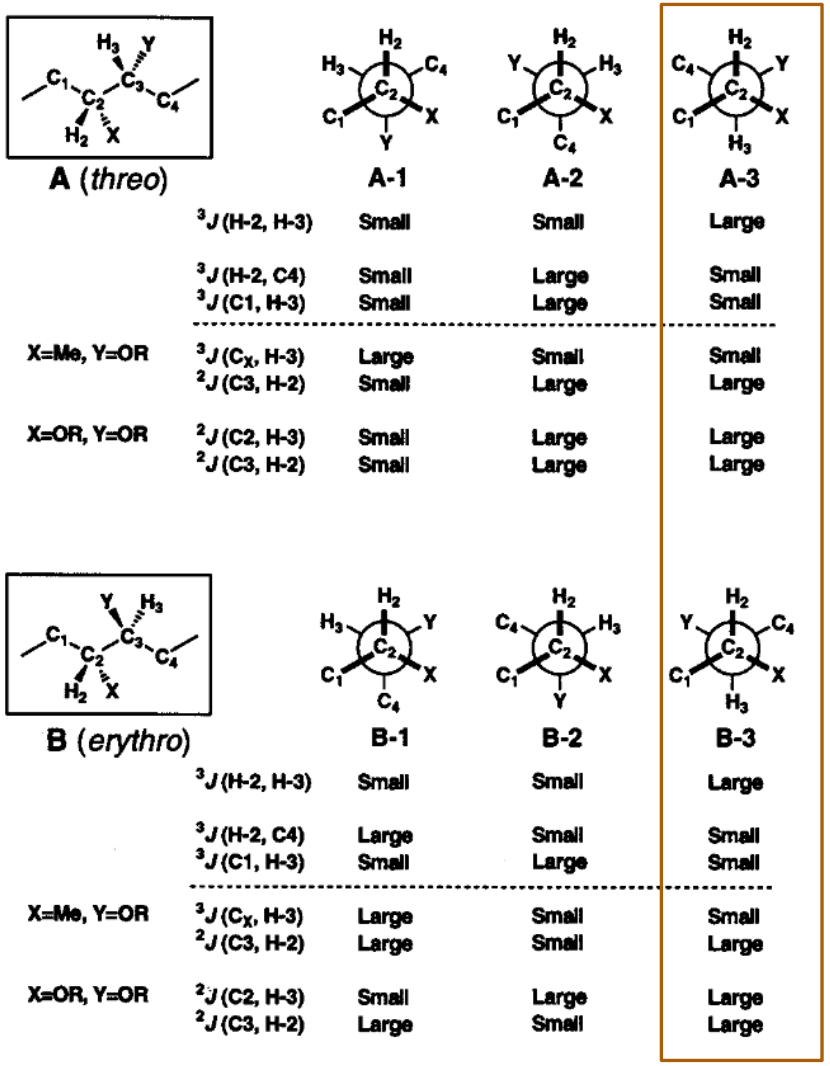



Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Rosa, M.; Ianaro, A.; Poletti, R. *J. Am. Chem. Soc.* **2002**, *124* (44), 13114–13120.

# *J*-based configuration analysis (JBCA)

Method for determining relative stereochemistry in acyclic structures, particularly with methyl/hydroxy/alkoxy-bearing stereocenters



| | | A-1 | A-2 | A-3 |
|---|---|---|---|---|
| A (*threo*) | $^3J$ (H-2, H-3) | Small | Small | Large |
| | $^3J$ (H-2, C4) | Small | Large | Small |
| | $^3J$ (C1, H-3) | Small | Large | Small |
| X=Me, Y=OR | $^3J$ ($C_X$, H-3) | Large | Small | Small |
| | $^2J$ (C3, H-2) | Small | Large | Large |
| X=OR, Y=OR | $^2J$ (C2, H-3) | Small | Large | Large |
| | $^2J$ (C3, H-2) | Small | Large | Large |

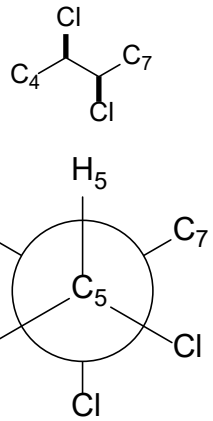
| | | B-1 | B-2 | B-3 |
|---|---|---|---|---|
| B (*erythro*) | $^3J$ (H-2, H-3) | Small | Small | Large |
| | $^3J$ (H-2, C4) | Large | Small | Small |
| | $^3J$ (C1, H-3) | Small | Large | Small |
| X=Me, Y=OR | $^3J$ ($C_X$, H-3) | Large | Small | Small |
| | $^2J$ (C3, H-2) | Large | Small | Large |
| X=OR, Y=OR | $^2J$ (C2, H-3) | Small | Large | Large |
| | $^2J$ (C3, H-2) | Large | Small | Large |

Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64* (3), 866–876.

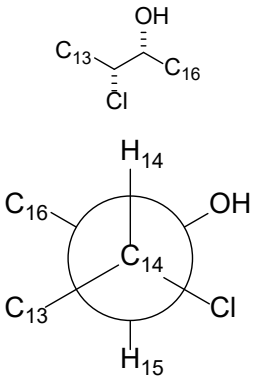
# Application to mytilipin B

Measured $^2J_{CH}$ and $^3J_{CH}$ by phase-sensitive HMBC or HETLOC NMR

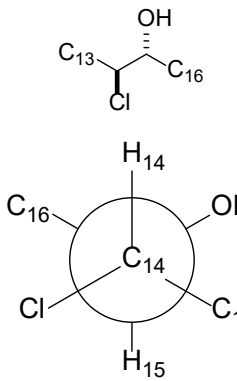
| C5-C6 axis (A1) | |
|---|---|
| Coupling | Magnitude, classification |
| $^3J_{H5-H6}$ | 2.8 Hz, small |
| $^3J_{H5-C7}$ | 0 Hz, small |
| $^3J_{C4-H6}$ | 0 Hz, small |
| $^3J_{C5-H-6}$ | -1.2 Hz, small |
| $^3J_{C6-H5}$ | -0.8 Hz, small |




| C14-C15 axis (A3 or B3) | |
|---|---|
| Coupling | Magnitude, classification |
| $^3J_{H14-H15}$ | 9.5 Hz, large |
| $^3J_{H14-C16}$ | 2.4 Hz, small |
| $^3J_{C13-H15}$ | 2.7 Hz, small |
| $^3J_{C14-H-15}$ | -4.6 Hz, small |
| $^3J_{C15-H14}$ | -5.4 Hz, small |

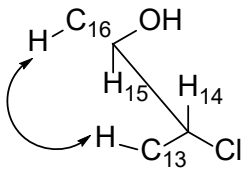



A3, *threo*

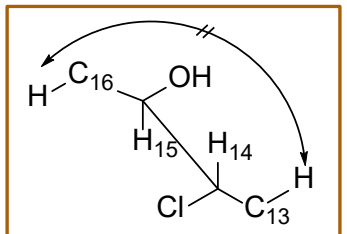



B3, *erythro*

A3 vs B3 requires ROESY



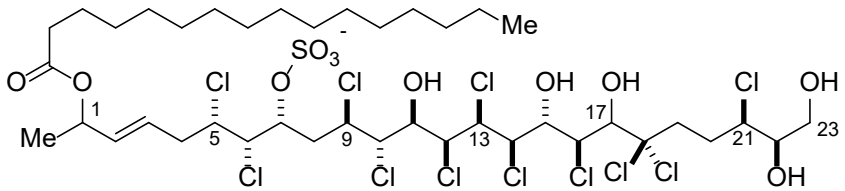

A3, *threo*




B3, *erythro*

- No ROE between H(C13) and H(C16), so C14-C15 is B3 = *anti*

By analogy, C5-C16 and C21-C22 relative configurations established



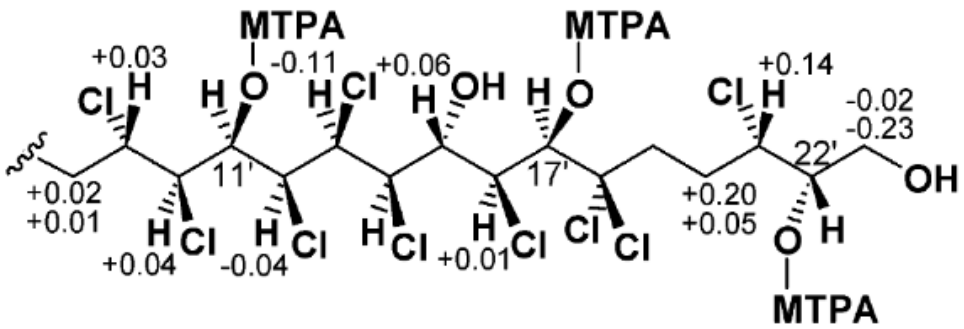

Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Rosa, M.; Ianaro, A.; Poletti, R. *J. Am. Chem. Soc.* **2002**, *124* (44), 13114–13120.

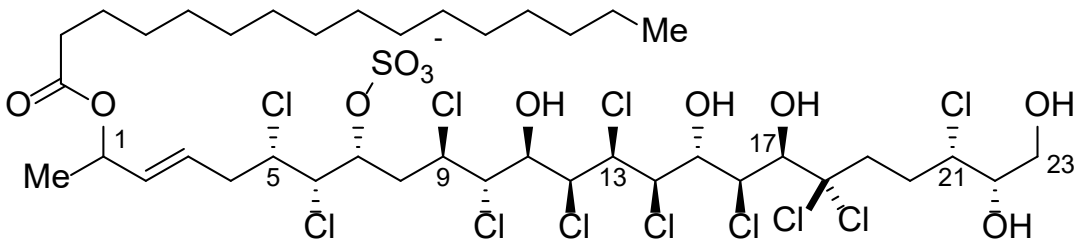
# Absolute configuration of C5-C23

C16-C17 unable to be assigned by JBCA due to nonstaggered conformation of $CCl_2$

- Can be deduced by from absolute configurations of C11 and C17 OH

Mosher ester (MTPA) analysis performed at C11, C17, C22 and assigned as (11*R*,17*R*,22*S*)

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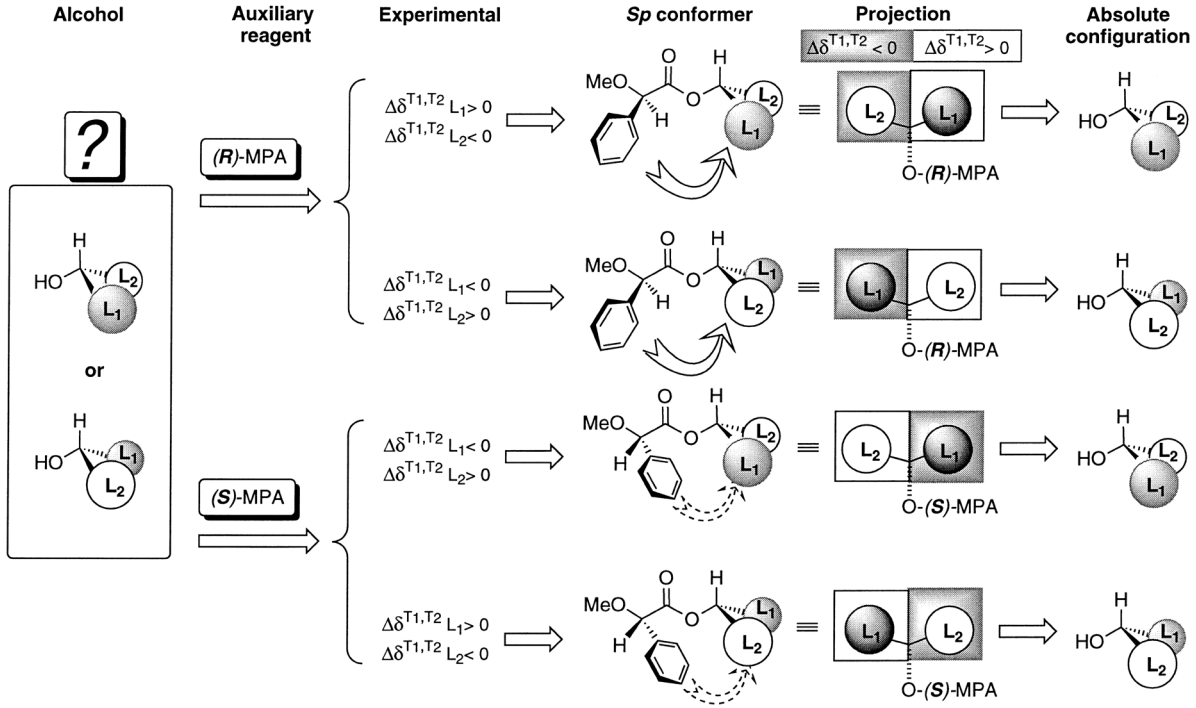
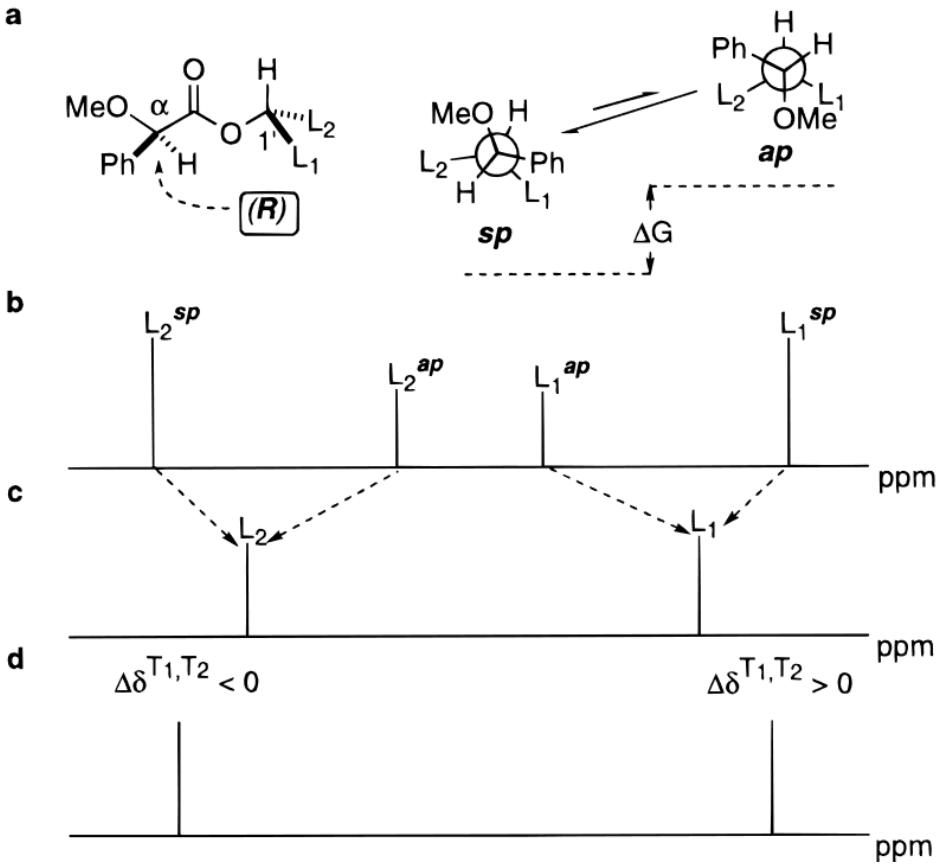
# Absolute configuration of C1

Insufficient material to analyze both (*R*)- and (*S*)-Mosher ester derivatives

Adapted Riguera modification

- Developed for methoxyphenylacetate (MPA) esters
- Theoretically requires analysis of only one ester
- $L_1$ and $L_2$ shift in opposite directions

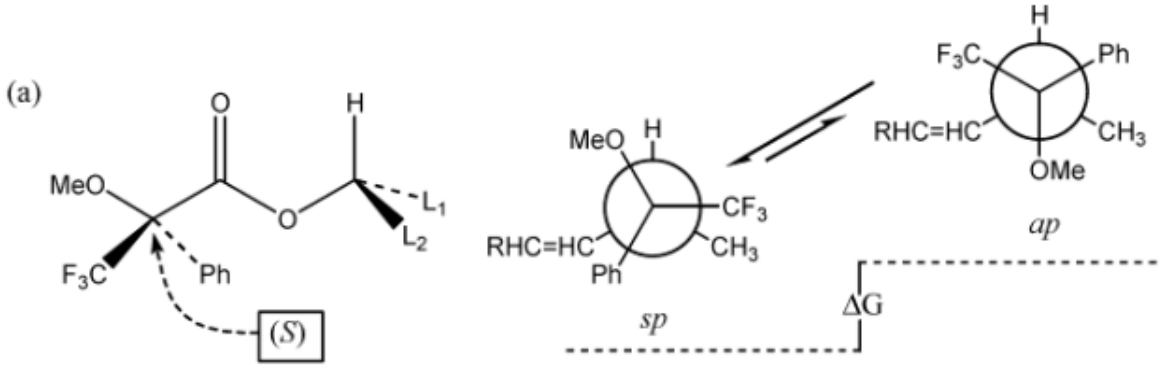


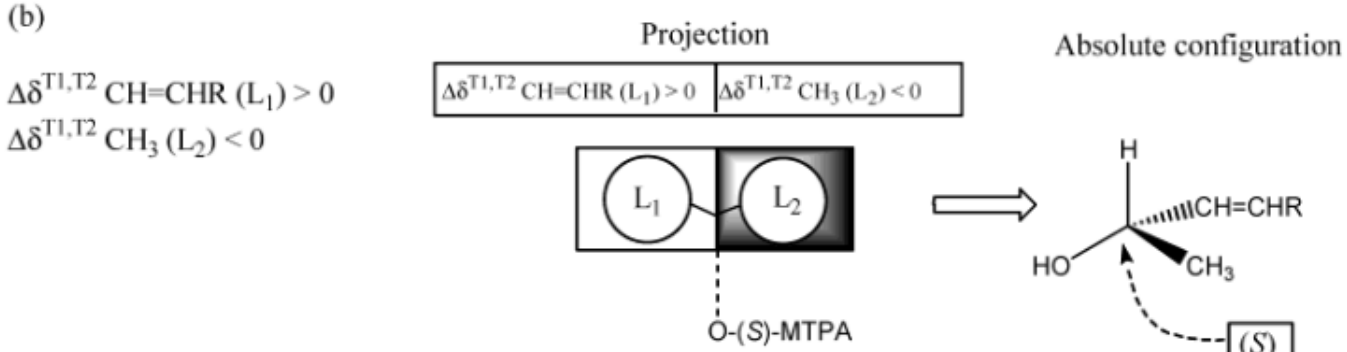



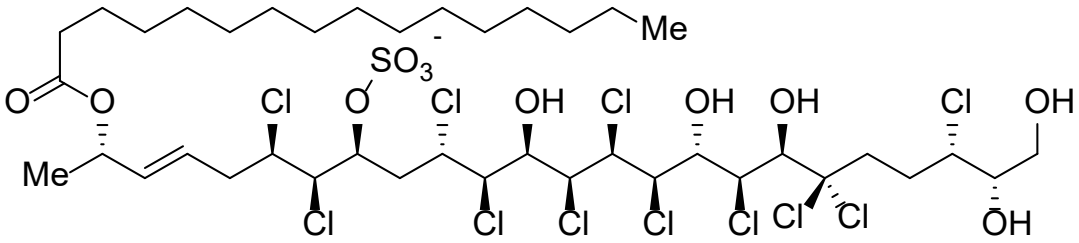

Latypov, S. K.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Am. Chem. Soc.* **1998**, *120* (5), 877–882. Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Rosa, M.; Ianaro, A.; Poletti, R. *J. Am. Chem. Soc.* **2002**, *124* (44), 13114–13120.

# Application to mytilipin B

## $LiAlH_4$ reduction and esterification with (*S*)-MTPA

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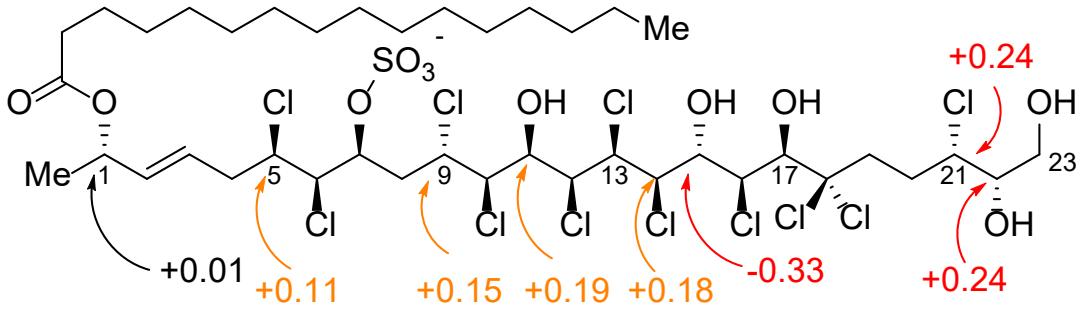
# Discovery of misassignment

Total synthesis of nominal structure by Carreira in 2008

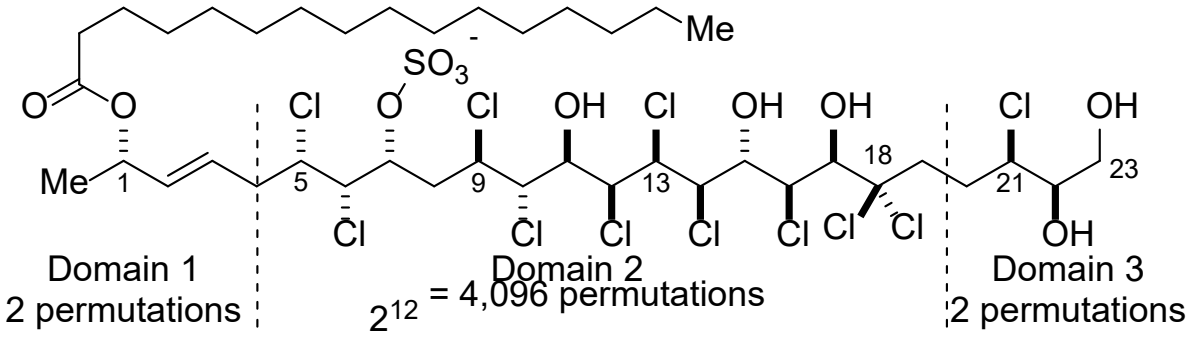



15 stereocenters = 32,768 possible stereoisomers!

No high-resolution spectra or FID available; $^{13}C$ NMR of isolated material and NMR from Mosher ester analyses missing

Reexamination of available peracetylated NMR confirmed planar structure

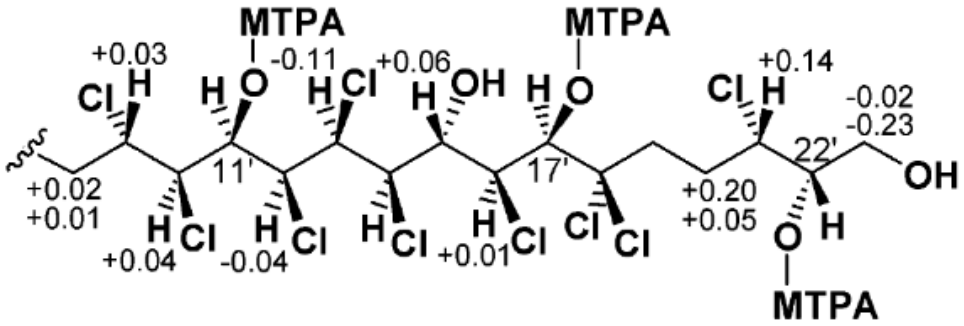
Decomposed problem into three domains




Nilewski, C.; Deprez, N. R.; Fessard, T. C.; Li, D. B.; Geisser, R. W.; Carreira, E. M. *Angew. Chemie Int. Ed.* **2011**, *50* (34), 7940–7943. Sondermann, P.; Carreira, E. M. *J. Am. Chem. Soc.* **2019**, *141* (26), 10510–10519.

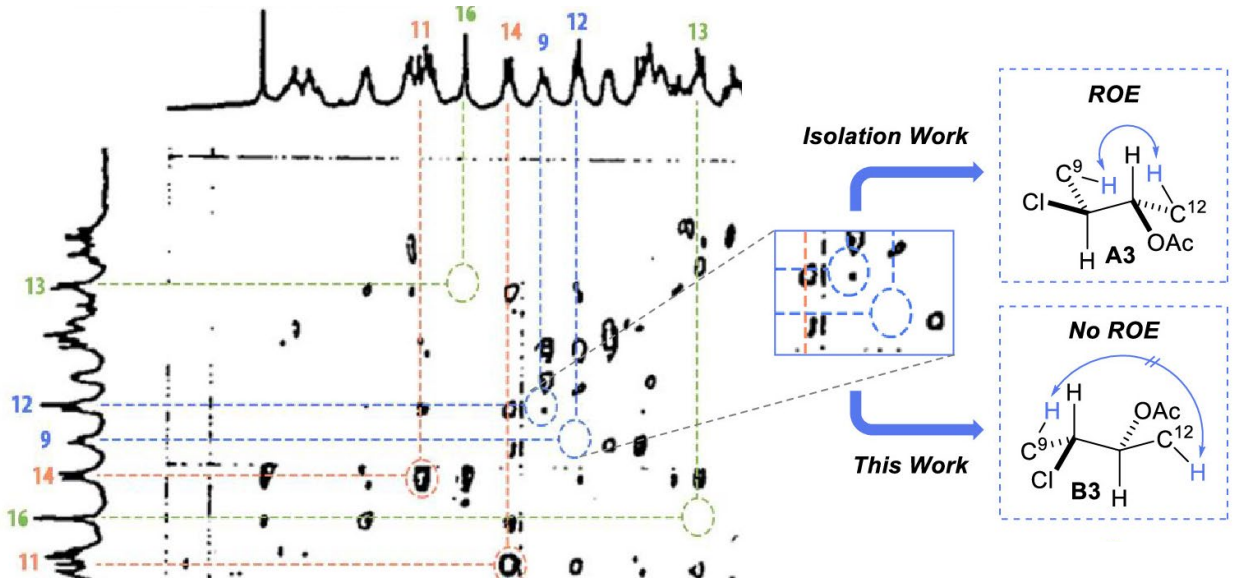
# Revision of C5-C18

## Reexamination of Mosher analysis



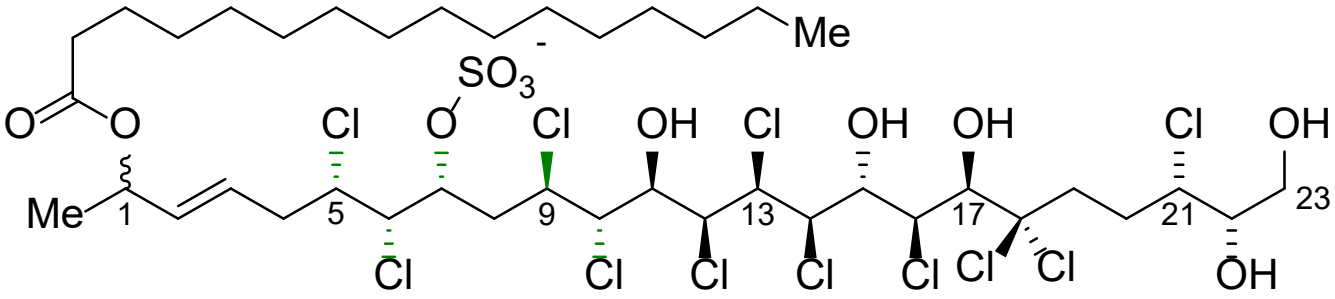

## Reexamination of JBCA and ROESY

- Confirmed C12-C13 and C14-C15 assignments
- Missing twinned crosspeak for C9-C12 ROESY
- C10-C11 should be B3 = *anti*



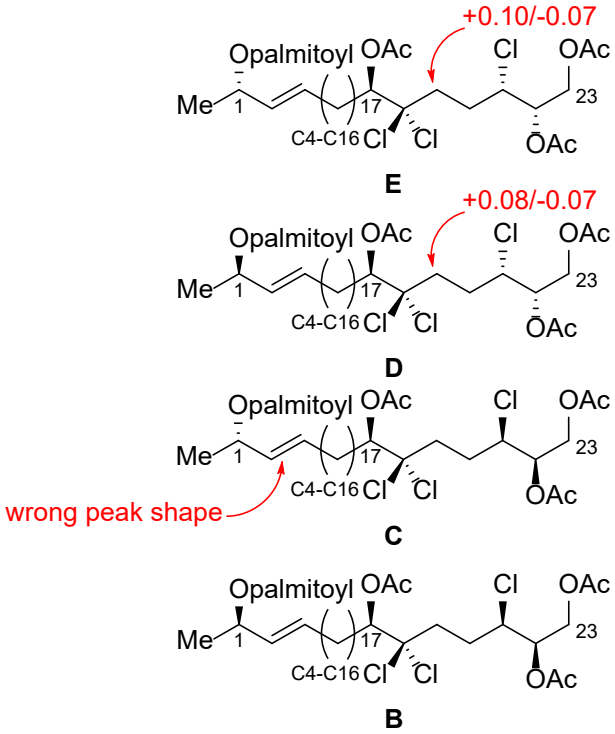

## Set out to synthesize C1 epimers

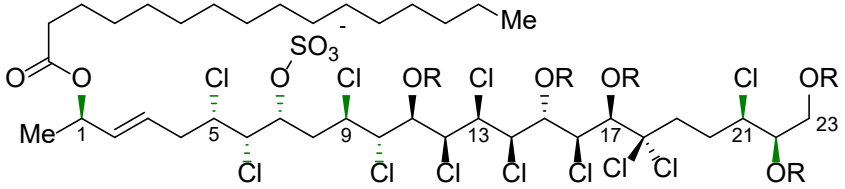
- Adaptation of Riguera method deemed suspect due to conformational differences between Mosher ester and MPA



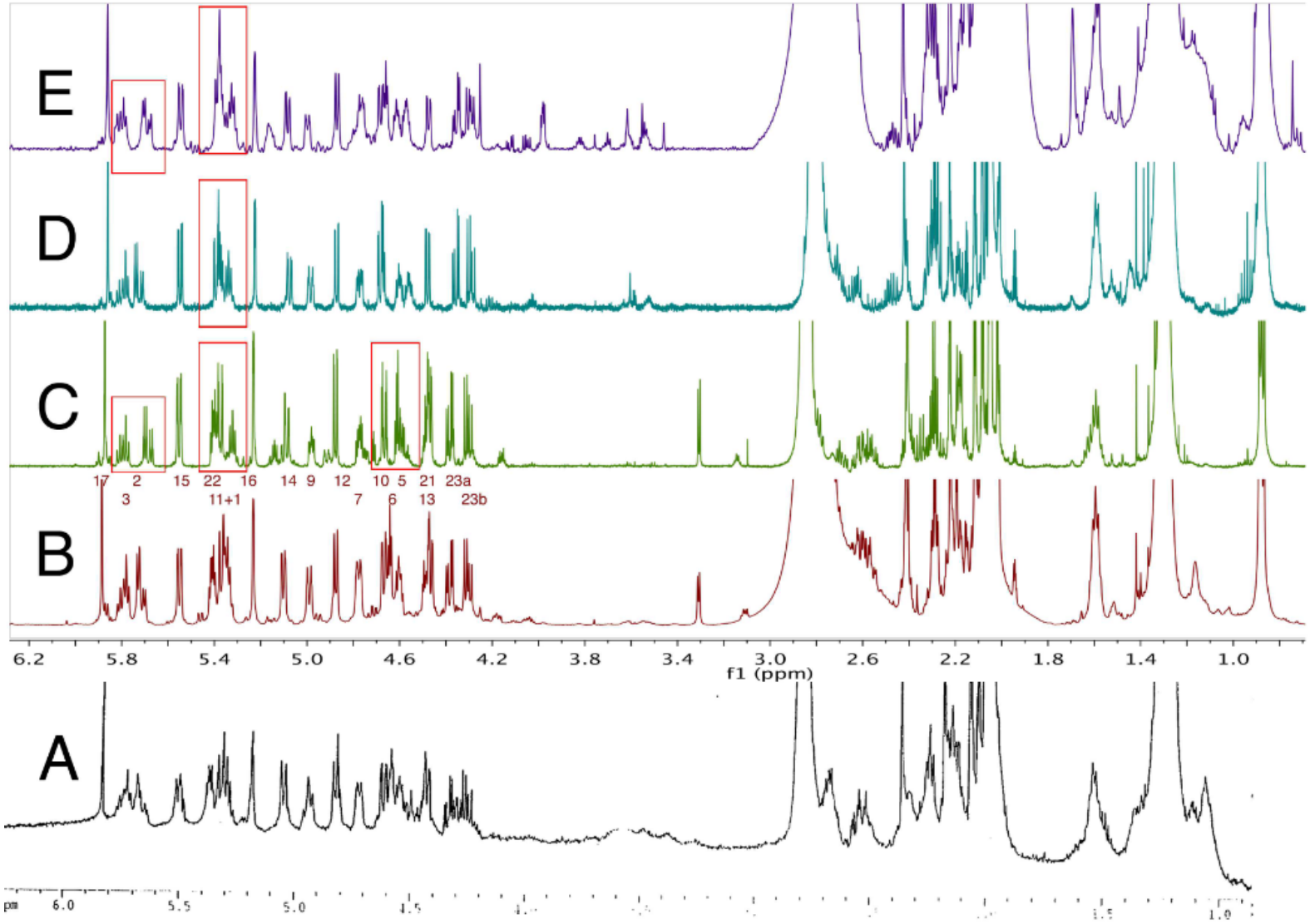

Sondermann, P.; Carreira, E. M. *J. Am. Chem. Soc.* **2019**, *141* (26), 10510–10519. Latypov, S. K.; Seco, J. M.; Quiñoá, E.; Riguera, R. MTPA vs MPA in the Determination of the Absolute Configuration of Chiral Alcohols by 1H NMR. *J. Org. Chem.* **1996**, *61* (24), 8569–8577.

# Interdomain relative configurations

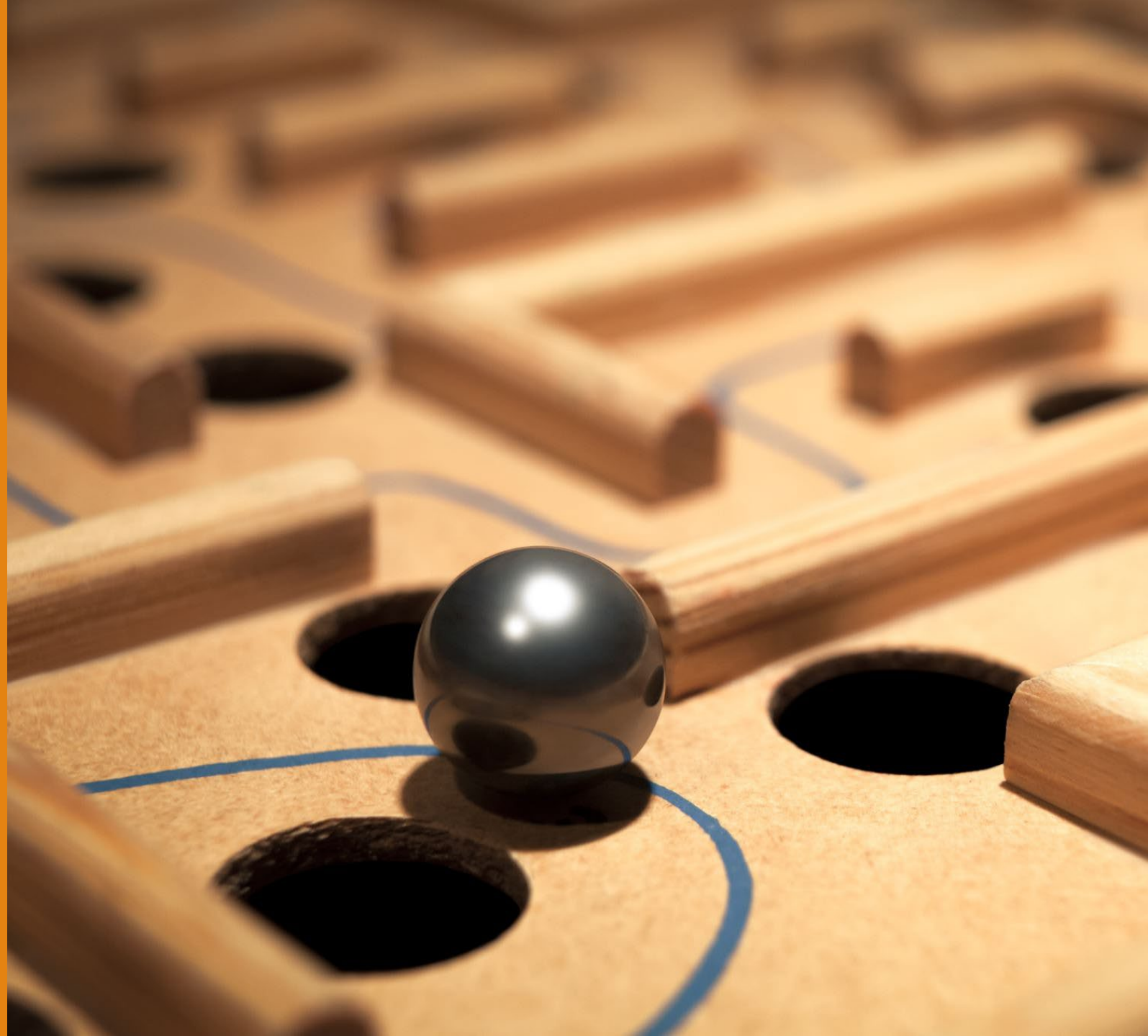



**A**, R = Ac (from natural sample)
**Mytilipin B**, R = H



# CONCLUSION & OUTLOOK



# Key takeaways

Pitfalls of spectroscopy/spectrometry

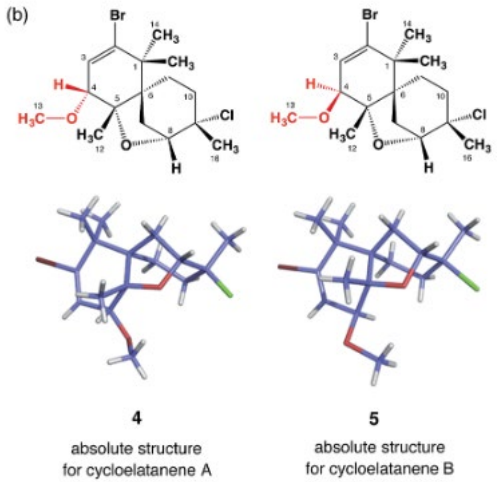
- Mass spectrometry: spontaneous oxidations, interpreting fragments
- X-ray crystallography: ambiguity among heteroatoms due to lack of H-scattering
- UV/IR spectroscopy: failure to consider alternative hypotheses (more) consistent with data
- NMR spectroscopy:
  - Poor signal dispersion and extensive unsaturation can lead to confusion in 2D-NMR assignments
  - Calculations of $^{13}C$ NMR shifts can help evaluate structures
  - Beware of spurious analogies/adaptations

Reassignment influences:

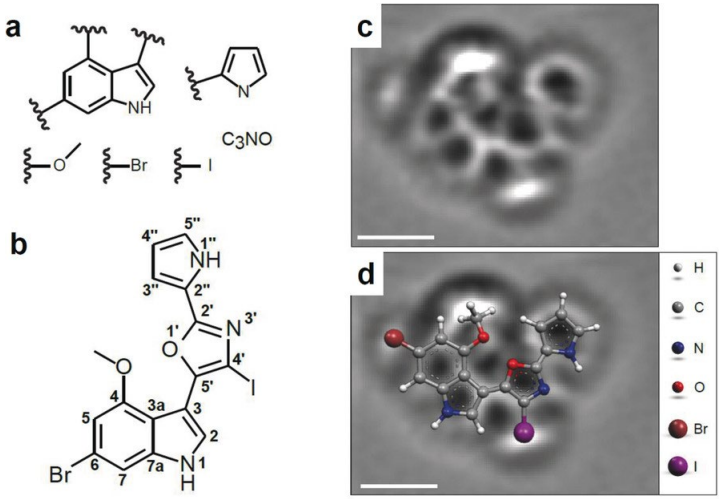
- Synthetic approach
- Biosynthetic hypotheses
- Structure-activity relationship studies

# Outlook

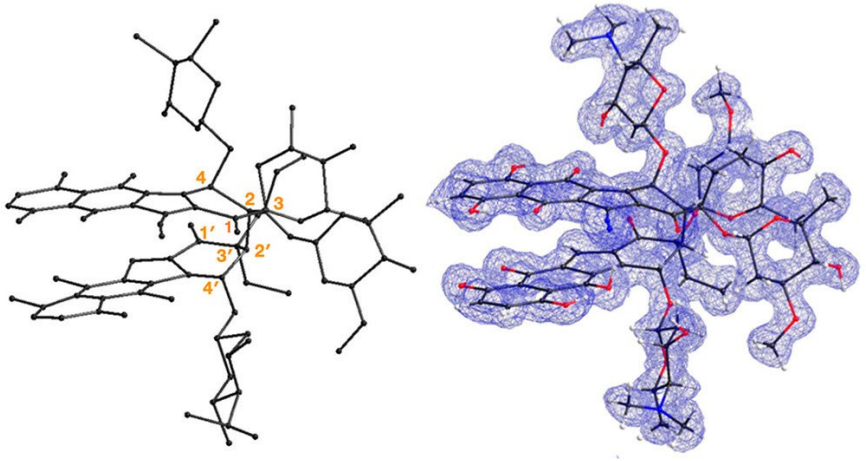
## Crystalline sponge x-ray (~5 µg)




## Atomic force microscopy (2 µg)

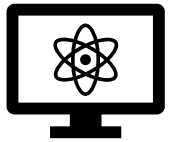



## MicroED (~200 ng)



## Computational Techniques

- Computer-assisted Structure Elucidation (CASE)
- Machine learning



Gross, L.; Schuler, B.; Pavliček, N.; Fatayer, S.; Majzik, Z.; Moll, N.; Peña, D.; Meyer, G. *Angew. Chemie Int. Ed.* **2018**, *57* (15), 3888–3908. Kim, L. J.; Xue, M.; Li, X.; Xu, Z.; Paulson, E.; Mercado, B.; Nelson, H. M.; Herzon, S. B. *J. Am. Chem. Soc.* **2021**, *143* (17), 6578–6585. Lee, S.; Hoshino, M.; Fujita, M.; Urban, S. *Chem. Sci.* **2017**, *8* (2), 1547–1550. Burns, D. C.; Mazzola, E. P.; Reynolds, W. F. *Nat. Prod. Rep.* **2019**, *36* (6), 919–933. Cobas, C. *Magn. Reson. Chem.* **2020**, *58* (6), 512–519.

# Relevant Literature

Helpful reviews:


- Nicolaou, K. C.; Snyder, S. A. Chasing Molecules That Were Never There: Misassigned Natural Products and the Role of Chemical Synthesis in Modern Structure Elucidation. *Angew. Chemie Int. Ed.* **2005**, *44* (7), 1012–1044. https://doi.org/10.1002/anie.200460864.
- Suyama, T. L.; Gerwick, W. H.; McPhail, K. L. Survey of Marine Natural Product Structure Revisions: A Synergy of Spectroscopy and Chemical Synthesis. *Bioorg. Med. Chem.* **2011**, *19* (22), 6675–6701. https://doi.org/10.1016/j.bmc.2011.06.011.
- Chhetri, B. K.; Lavoie, S.; Sweeney-Jones, A. M.; Kubanek, J. Recent Trends in the Structural Revision of Natural Products. *Nat. Prod. Rep.* **2018**, *35* (6), 514–531. https://doi.org/10.1039/C8NP00011E.

# Thank you!

QUESTIONS?