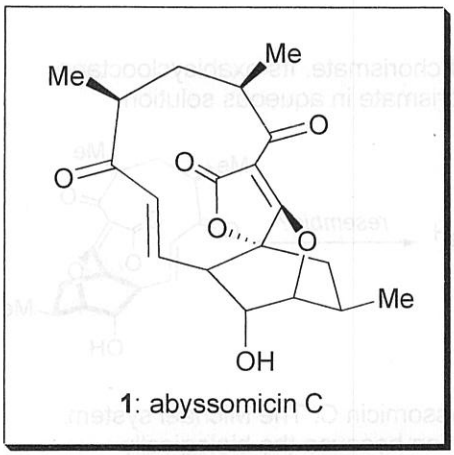
Total Synthesis of Abyssomicin C and its related works



1: abyssomicin C

review : K. C. Nicolaou *et al.*
Angew. Chem. Int. Ed., **2009**, 660
"Recent Advances in the Chemistry and Biology of Naturally Occurring Antibiotics"

Contents

---

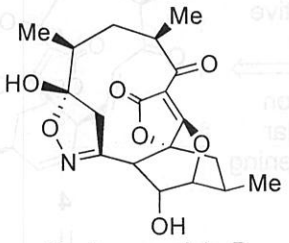
1. Introduction

Süssmuth *et al.*
Angew. Chem. Int. Ed., **2004**, 2574

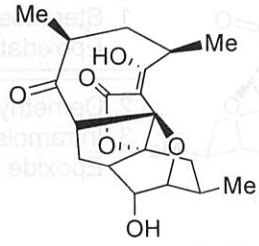
1-1. isolation

— Abyssomicin C was isolated from the actinomycete *Verrucosispora* strain AB 18-032, which was isolated from a sediment sample collected in the Japanese Sea.

— Two similar compounds, abyssomicin B and D, were also isolated, but they have no antibiotic activity. Only abyssomicin C has the activity againt Gram-positive bacteria.



2: abyssomicin B



3: abyssomicin D

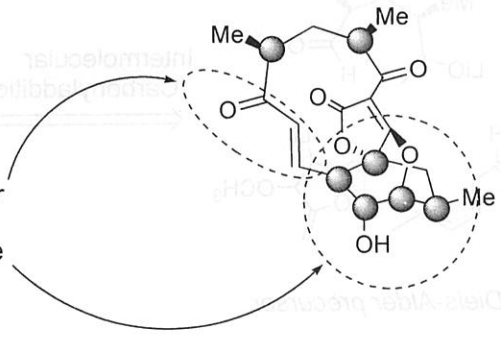
1-2. structural features

11 membered macrocyclic ring

7 strereogenic centers

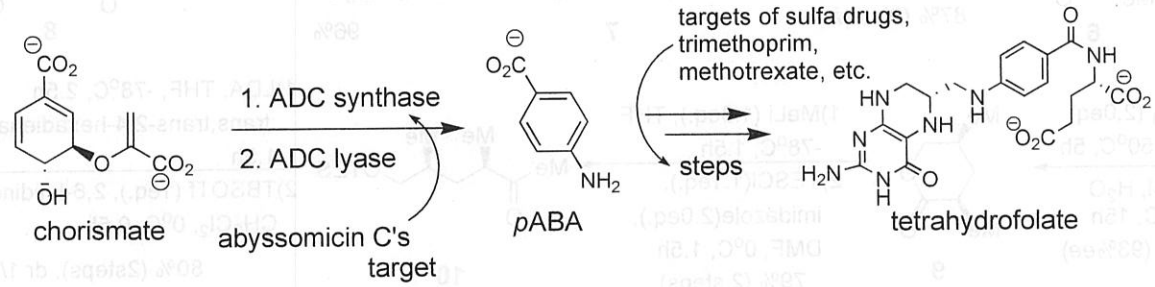
a potentially reactive Michael acceptor

tetronate oxabicyclo[2.2.2]octane core



1-3. biological activity

— Abyssomicin C inhibits ADC synthase, which is in charge of the conversion from chrismate into *p*-aminobenzoic acid (*p*ABA). *p*-ABA is indispensable for the biosynthesis of tetrahydrofolate.



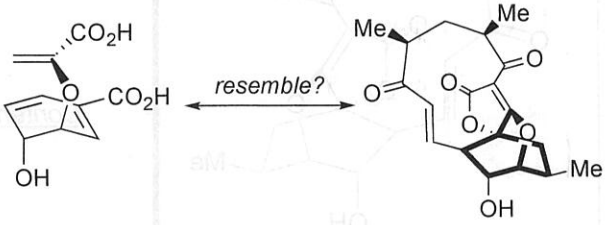
— *p*ABA is made in many microorganisms but not in human. That's why it's a promising lead compound of antibacterial drug.

— Abyssomicin C is effective against methicilin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). MIC is 4μg/mL against MRSA and 13μg/mL against VRSA.

— Abyssomicin C is considered a substrate-mimetic inhibitor of chorismate. Its oxabicyclooctane system seems to have similarities to the conformation of chorismate in aqueous solution.

NMR study about chorismate's conformation showed that 10~40% takes this pseudo-axial form in aqueous solution.

J. R. Knowles *et al.*
J. Am. Chem. Soc., **1987**, 5008



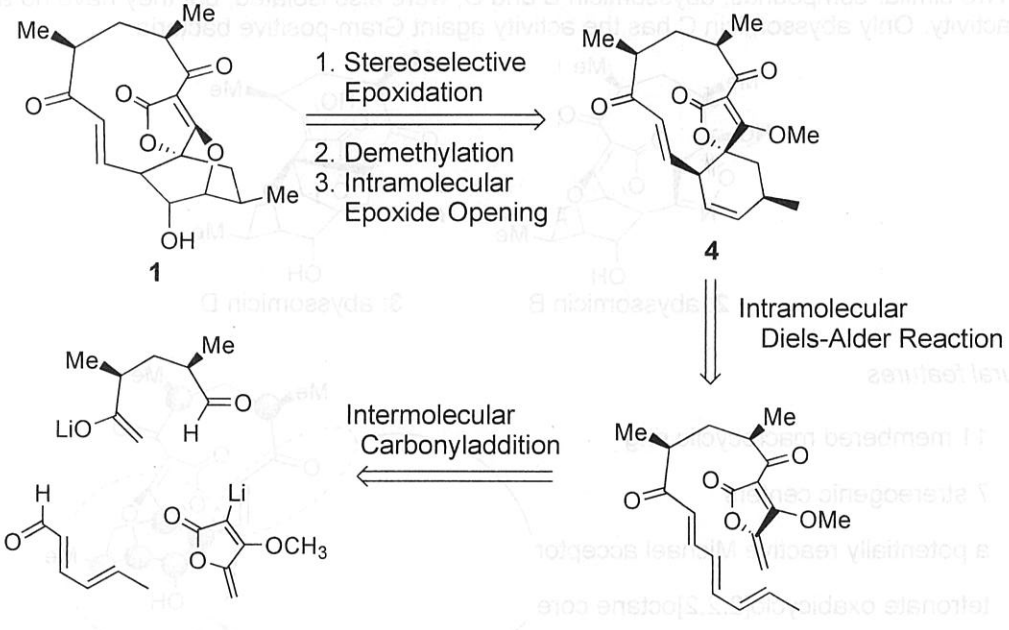
— And they supeculated about the inhibition mechanism of abyssomicin C. The Michael system adjacent to the oxabicyclooctane core is significant for inhibition because the biologically inactiveness of abyssomicin B and D, which doesn't have a Michael system. This system traps nucleophilic amino acid side chain of the target enzyme?

---

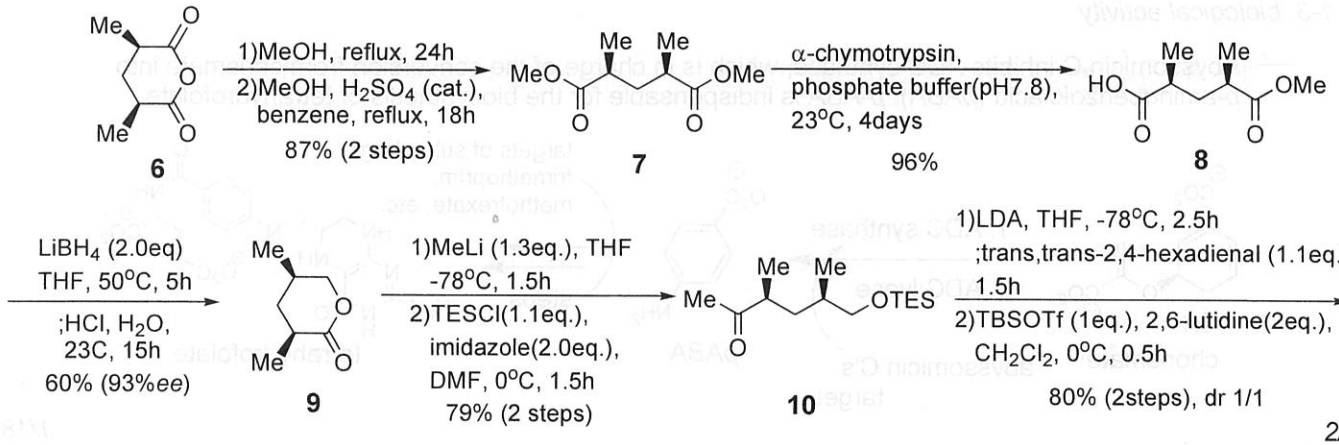
2. Sorensen's total synthesis

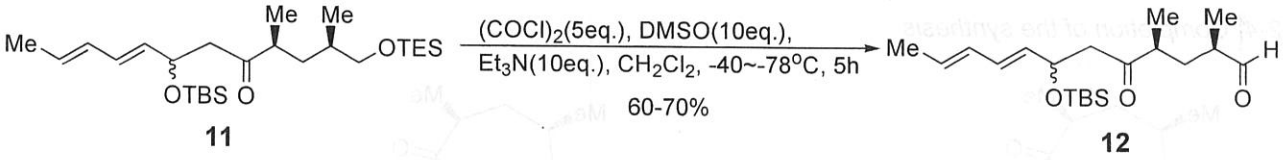
E. J. Sorensen *et al.*
Angew. Chem. Int. Ed., **2005**, 6533

2-1. Retrosynthetic Analysis and Strategy

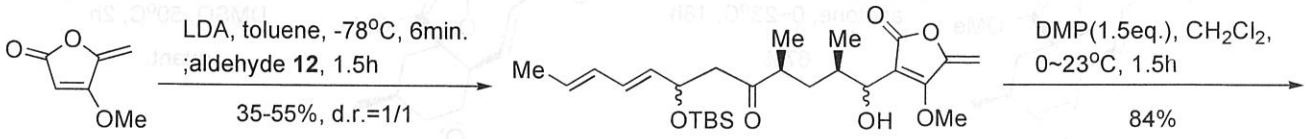


2-2. Synthesis of Diels-Alder precursor

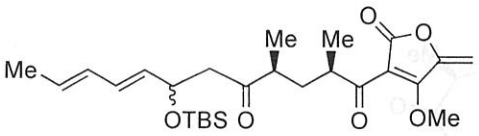




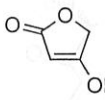
11 **12**



13 **14**

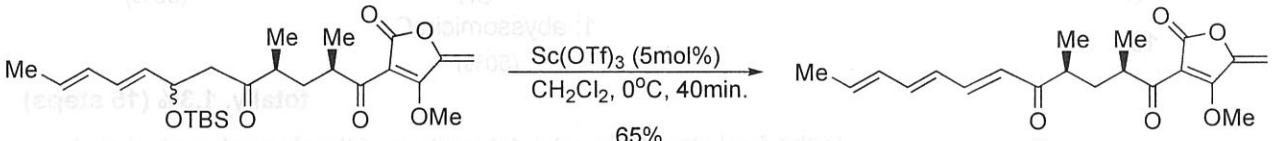


15

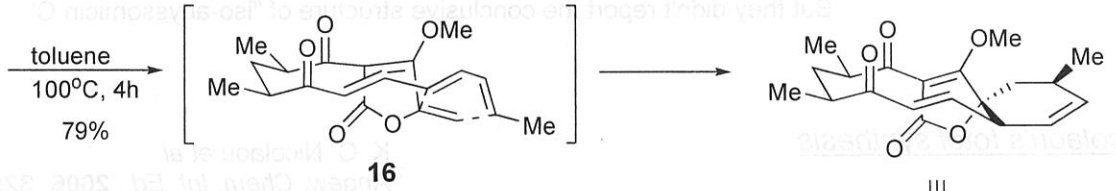
*__13__ can be prepared from  in 3 steps.

E. Yoshii *et al.*
J. Org. Chem., **1987**, 4135

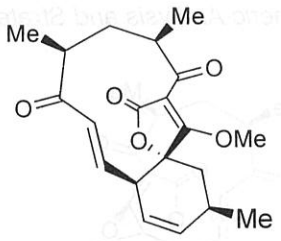
2-3. Diastereo-selective Diels-Alder reaction



15 **5**

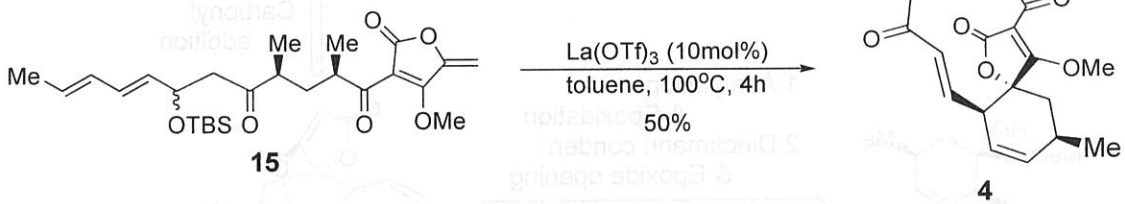


16



4

This intramolecular D.A. is completely regio- and diastereo-selective.
(They say no other isomer was observed.)
But trienone **5** is sensitive and difficult to handle with.
So they took a one-pot process. (shown below)



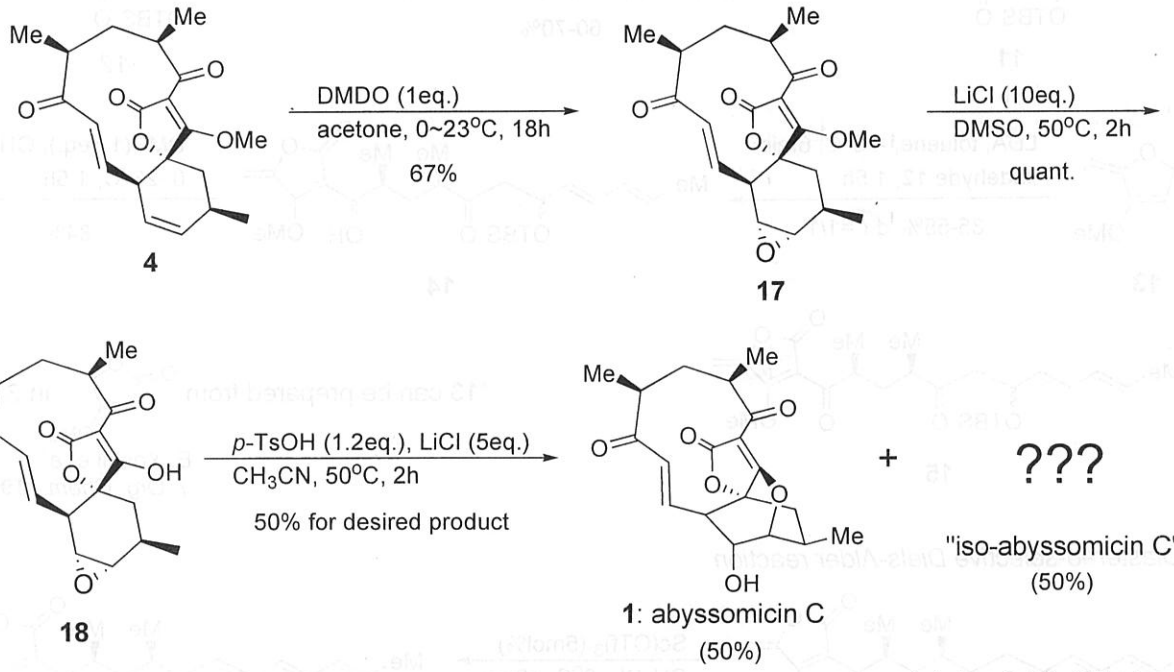
15 **4**

After trying some Lewis acids, they find heating with lanthanum triflate as Lewis acid gave the desired D.A. product, also regio and diastereoselectively in this case.

Why is this D.A. reaction completely diastereoselective ??

⟹ They conducted energetic calculation of the transition state.
This study proved the lowest-energy TS comformation resembled **16**.
It is 5.7kcal/mol lower than the next lowest TS in energy.

2-4. Completion of the synthesis



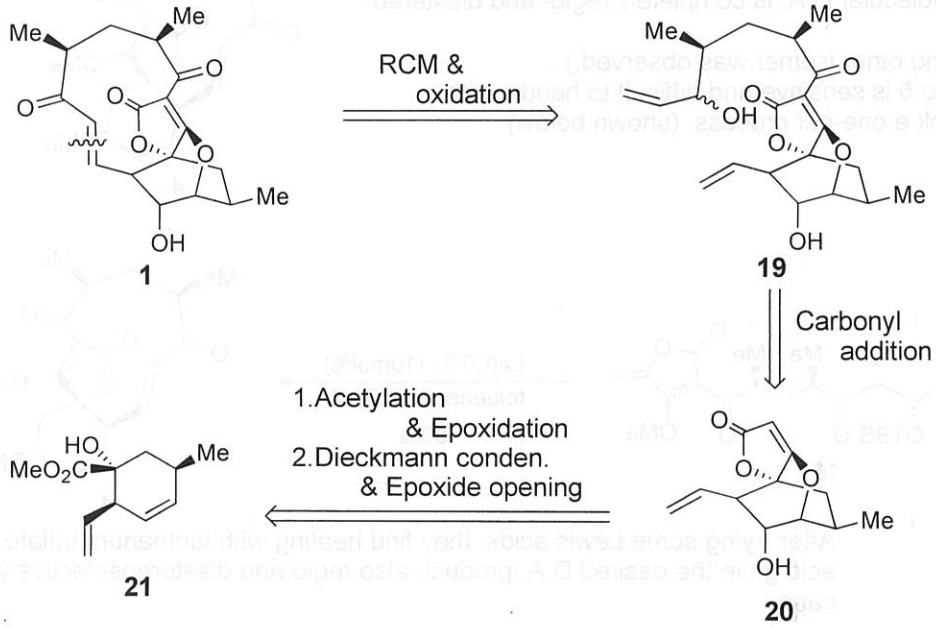
total y. 1.3% (15 steps)

In the final step, they got a 1:1 mixture of the desired product and undesired one.
But they didn't report the conclusive structure of "iso-abyssomicin C"...

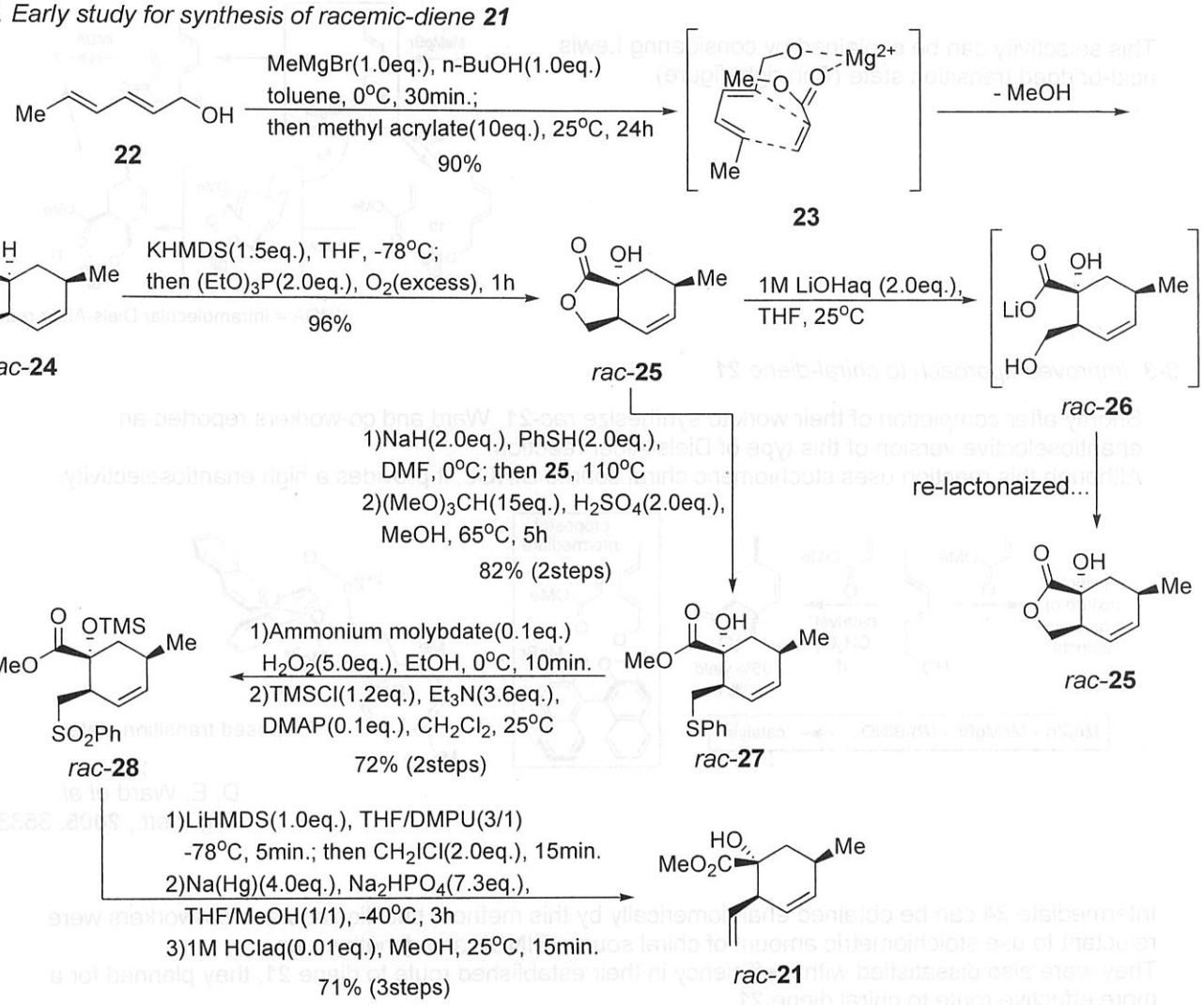
3. K. C. Nicolaou's total synthesis

K. C. Nicolaou *et al.*
Angew. Chem. Int. Ed., **2006**, 3256
J. Am. Chem. Soc., **2007**, 429

3-1. Retrosynthetic Analysis and Strategy

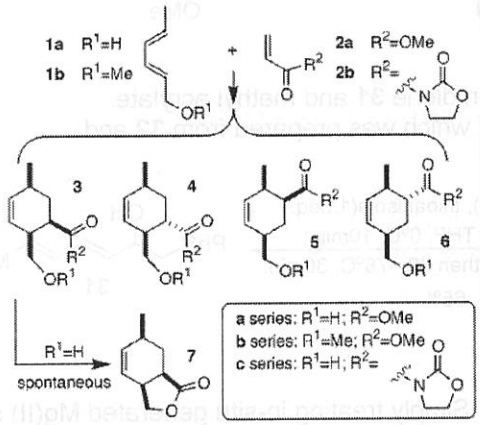


*3-2. Early study for synthesis of racemic-diene **21***



In the first step, they employed the Lewis acid-templated Diels-Alder reaction developed by Ward *et al.* D.A. reaction between 2,4-hexadienol and acrylate derivatives has low selectivity (four possible adducts, **3**-**6** in the figure below) by simply heating the reaction mixture. But in the presense of Mg(II) or Al(III) Lewis acid, high selectivity was obtained, they reported.

Table 1. Diels–Alder Reactions of 1 with 2



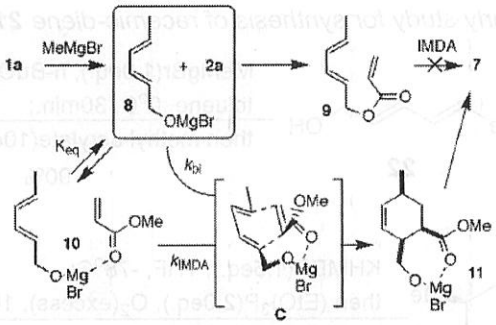
entry	diene/ dienophile	conditions[a]	adduct series	selectivity 7(or 3):4:5:6[b]	yield[c] (%)
1	1a/2a	120 °C	a	1.3:1:1.3:1.2	90
2	1a/2a	$TiCl_4$		*d*	
3	1a/2a	$TiCl_2(O^iPr)_2$		*d*	
4	1a/2a	$SnCl_4$		*d*	
5	1a/2a	$EtAlCl_2$		*d*	
6	1a/2a	Et_2AlCl	a	7 only	75
7	1a/2a	$MgBr_2{\cdot}OEt_2$	a	7 only	70
8	1a/2a	$MgBr_2{\cdot}OEt_2$, Et_3N[e]	a	7 only	75
9	1a/2a	MeMgBr[f]	a	7 only	35
10	1a/2a	MeMgBr[f,g]	a	7 only	75
11	1a/2a	MeMgBr, pentanol[f,h]	a	7 only	95
12	1b/2a	120 °C	b	1:1:1.8:1.4	90
13	1b/2a	Et_2AlCl	b	1:-:2:-	20
14	1b/2a	$MgBr_2{\cdot}OEt_2$		*d*	
15	1b/2a	$MgBr_2{\cdot}OEt_2$, Et_3N[e]		*d*	
16	1b/2a	MeMgBr, pentanol[f,h]		*d*	
17	1a/2b	120 °C	c	1.4:1:2:1	60
18	1a/2b	$MgBr_2{\cdot}OEt_2$	c	3.1:1:6.3:1	65
19	1a/2b	MeMgBr[f,g]	c	7.3:1.4:4.3:1	25

[a] Thermal reactions: a solution of the diene (0.5−2 M) and **2a** (2 equiv) or **2b** (1 equiv) in C_6D_6 was heated for 20−36 h.LA-mediated reactions: LA (1 equiv) and **2a** (2 equiv) or **2b** (1 equiv) were sequentially added to a solution of diene (0.1−0.3 M) in CH_2Cl_2 at 0 °C and then stirred at ambient temperature for 10−48 h. [b] Ratios measured by ^1H NMR of the crude reaction mixture (relative error estimated as ±10%). [c] Isolated. [d] DA adducts not detected. [e] 2 equiv of Et_3N. [f] MeMgBr (3 M in ether) was used; reaction in toluene. [g] 0.5 equiv of MeMgBr, reaction for 7 days. [h] 1 equiv of pentanol added (cf. note 15); reaction for 2 days.

get selectivity with Mg(II) or Al(III) Lewis acid

D. E. Ward *et al.*
Org. Lett., **2000**, 3937

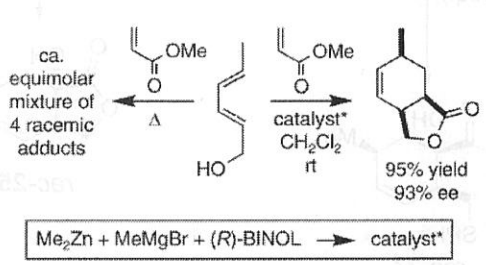
This selectivity can be explained by considering Lewis acid-bridged transition state (**C** in right figure).

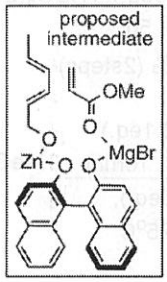


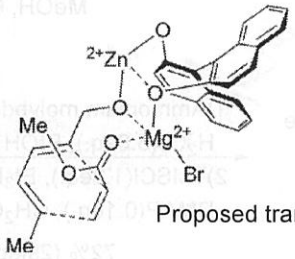
*IMDA = intramolecular Diels-Alder reaction

*3-3. Improved approach to chiral-diene **21***

Shortly after completion of their work to synthesize *rac*-**21**, Ward and co-workers reported an enantioselective version of this type of Diels-Alder reaction.
Although this reaction uses stochiomeric chiral source BINOL, it provides a high enantioselectivity.



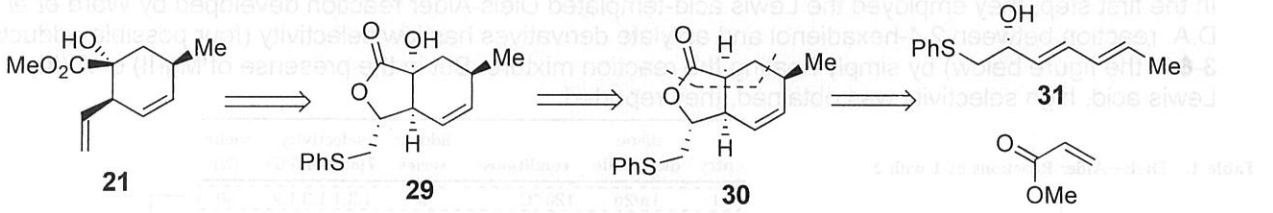




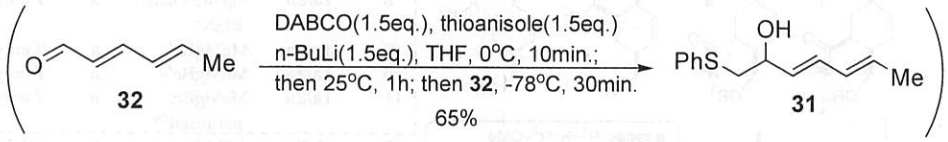
Proposed transition state

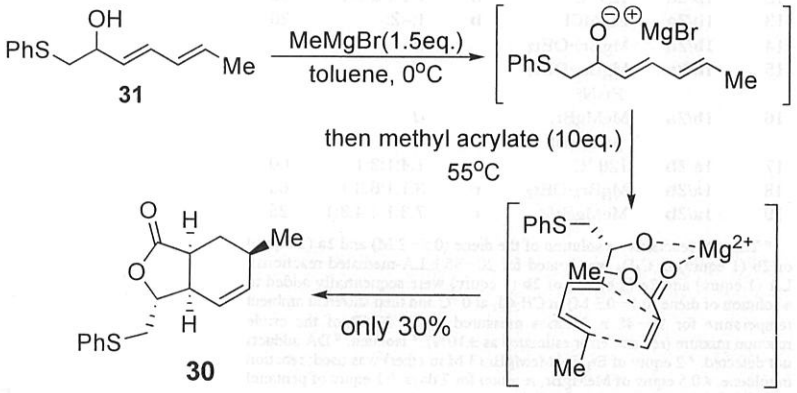
D. E. Ward *et al.*
Org. Lett., **2005**, 3533

Intermediate **24** can be obtained enantiomerically by this method, but Nicolaou and co-workers were reluctant to use stoichiometric amount of chiral source BINOL and dimethyl zinc.
They were also dissatisfied with inefficiency in their established route to diene **21**, they planned for a more effective route to chiral diene **21**.



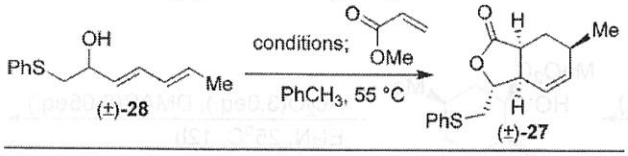
At First, they developed diastereoselective D.A. reaction between diene **31** and methyl acrylate.
Optimization of reaction conditions were examined using rac-**31**, which was prepared from **32** and lithiothioanisole (as shown below).

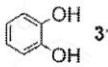
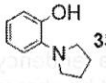




Simply treating in-situ generated Mg(II) salt of **31** with methyl acrylate gave only 30% desired product (ent.1 in the table in the next page).
That seems to be because of the bulkiness of the thiophenylmethylene unit.

Table 1. Optimization of the Lewis-Acid Templated Diels–Alder Reaction between (±)-28 and Methyl Acrylate

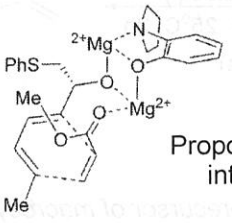


entry	auxiliary	base[a]	time[b]	yield[c]
1	none	MeMgBr (1.0 equiv)	24 h	30%
2	(±)-BINOL	Me_2Zn (1.0 equiv), MeMgBr (1.0 equiv)[d]	24 h[e]	< 5%[f]
3	 **31**	Me_2Zn (1.0 equiv), MeMgBr (1.0 equiv)[d]	36 h	35%
4	 **32**	MeMgBr (2.0 equiv)	24 h	49%
5	 **33**	MeMgBr (2.0 equiv)	48 h	55%
6	**33** (2.0 equiv)	MeMgBr (3.0 equiv)	48 h	70%
7	**33** (3.0 equiv)	MeMgBr (4.0 equiv)	12 h	80%

They tried to add some sacrificial alcohol and other Lewis acids, but these attempt was not effective...

Though they employed Ward's procedure, only trace TM was obtained. But decomp. of diene was reduced. (= prevented by auxiliary ??)

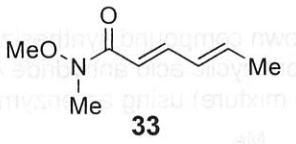
Using 3 eq. of auxiliary is not admirable, but this condition gave a good yield of cycloadduct **27**.



Proposed intermediate

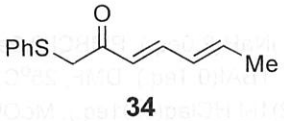
[a] Addition of base was performed at 0 °C before addition of methyl acrylate (10 equiv) and warming to 55 °C. [b] Reaction times reflect the time at which consumption of the diene was complete as measured by ^{1}H NMR spectroscopy on the crude reaction mixture unless stated otherwise. [c] Isolated yield unless stated otherwise. [d] Reaction performed according to the conditions summarized in Figure 5. [e] Reaction stopped before completion affording 80% of recovered diene. [f] Trace product detected in the ^{1}H NMR spectrum of recovered diene.

D. E. Ward *et al.*
Org. Lett., **2005**, 3533



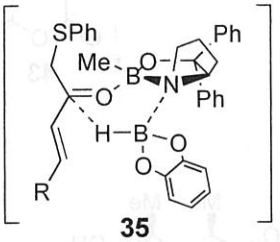
33

thioanisol (1.3eq.), DABCO(1.3eq.), *n*BuLi(1.3eq.)
THF, 0°C, 1h; then -78°C
;then alcohol amide **25**, 45min.
81%

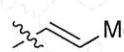


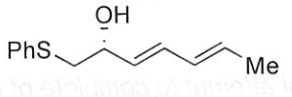
34

Me-(*R*)-CBS (10mol%),
catecholborane (1.25eq.),
CH_2Cl_2, -78°C, 24h
95%yield, 90%*ee*



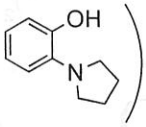
35

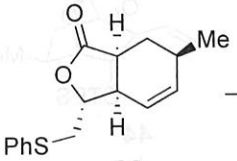
(R = )



36

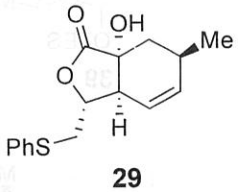
MeMgBr(4.0eq.),
aminophenol(3.0eq.)
toluene, 0°C;
then methyl acrylate (10eq.)
55°C, 12h
80%, perfectly diastereoselective

(*aminophenol = )



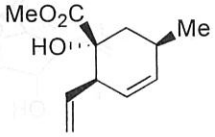
30

LiHMDS(1.5eq.), THF, -78°C, 30min.;
then $(EtO)_3P$(2.0eq.), O_2(excess), 1h
74%



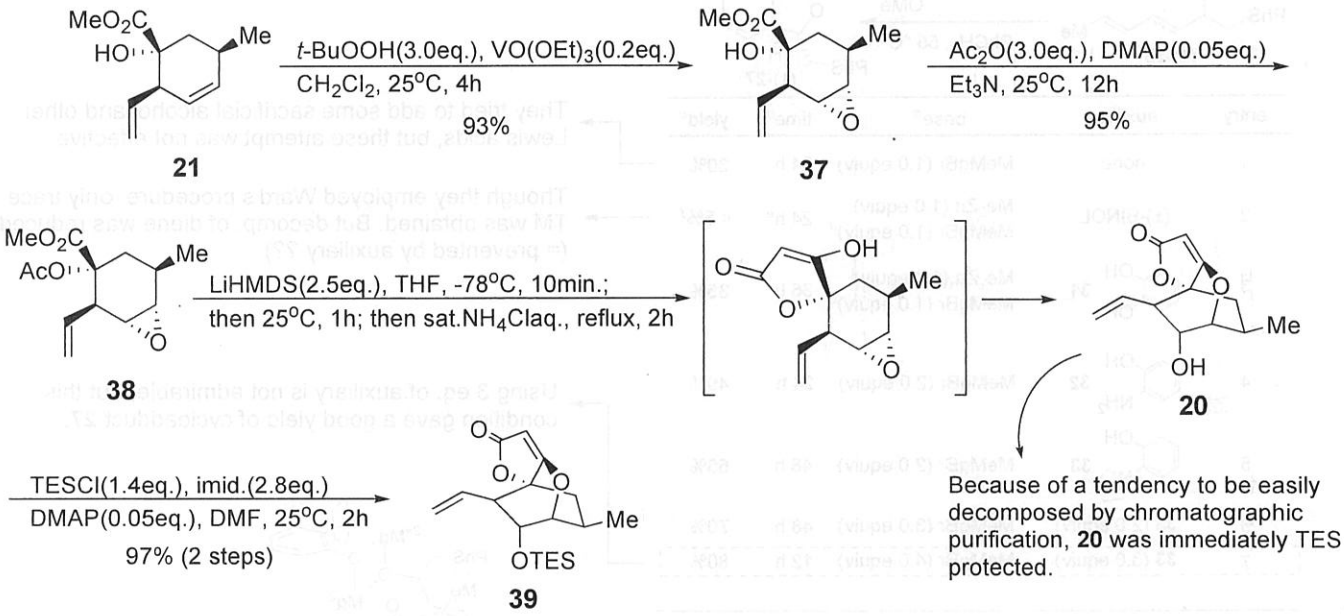
29

Li(9.6eq.), 4,4'-di-*t*-butylbiphenyl(0.5eq.)
THF, 0°C, 1h; then **29**, -48°C, 3h;
quench with MeOH to remove excess Li;
then K_2CO_3(1.0eq.), MeI(10eq.)
DMF, 60°C, 10min.
99%

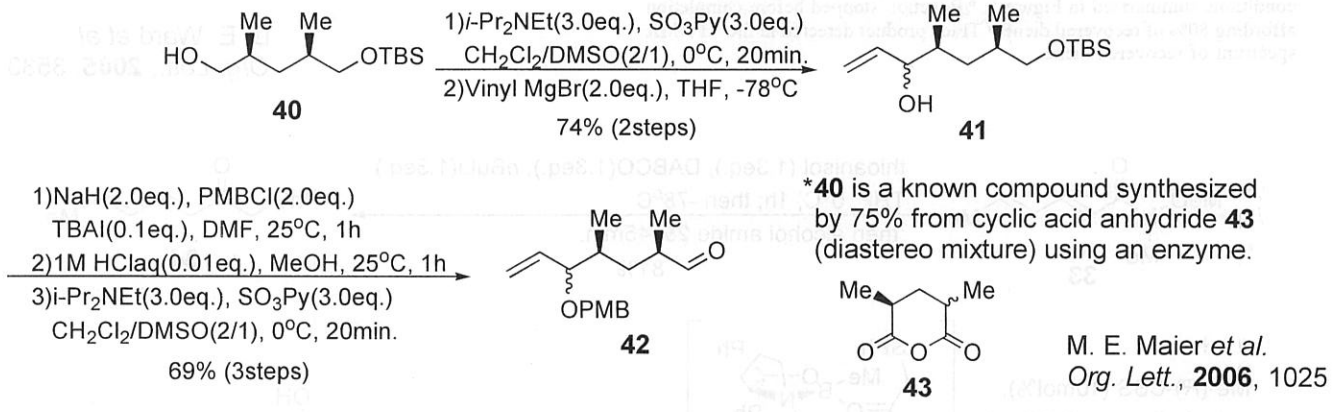


21

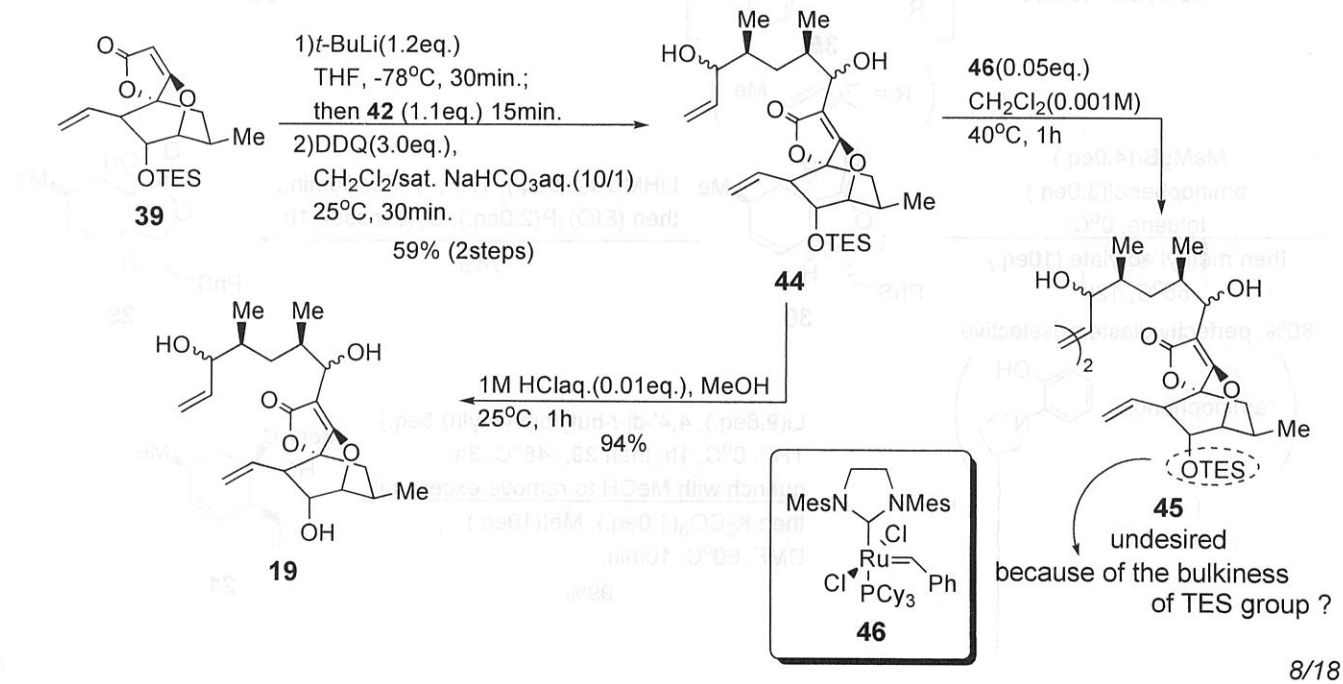
3-4. Synthesis of TES protected oxabicyclooctane core

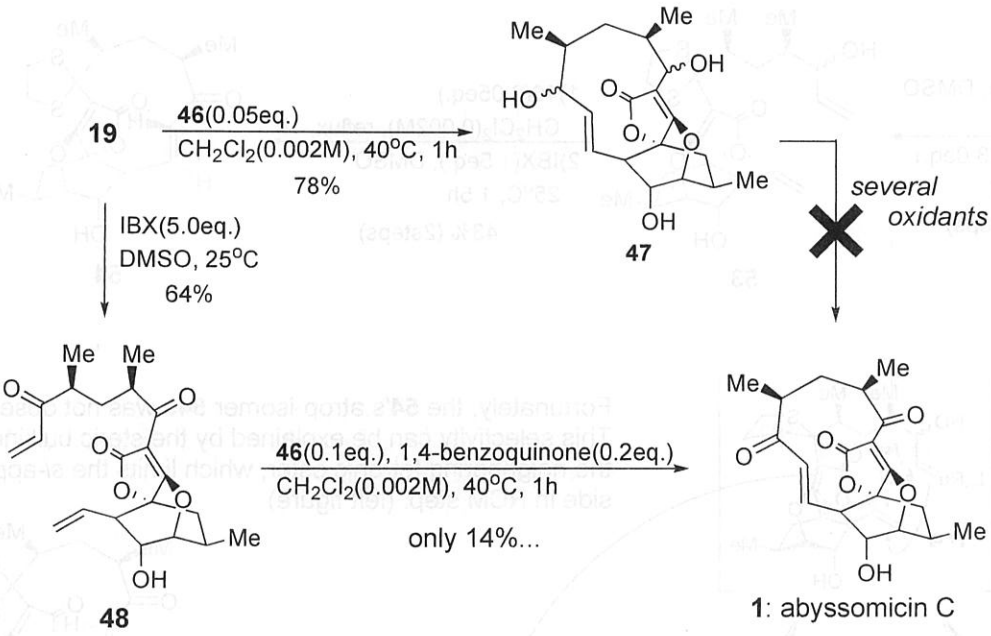


3-5. Synthesis of a precursor of macrocyclic ring



3-6. Initial attempt to complete of the synthesis



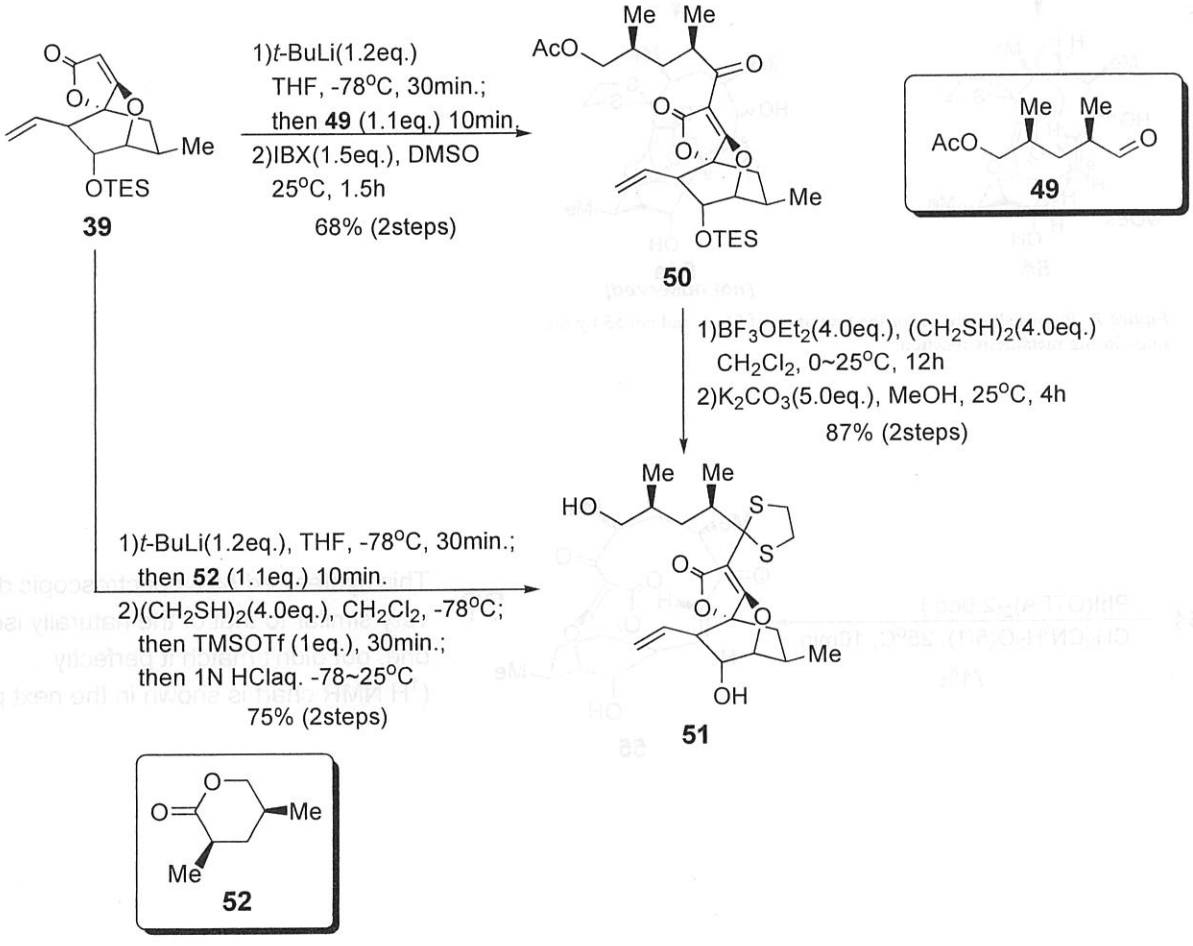


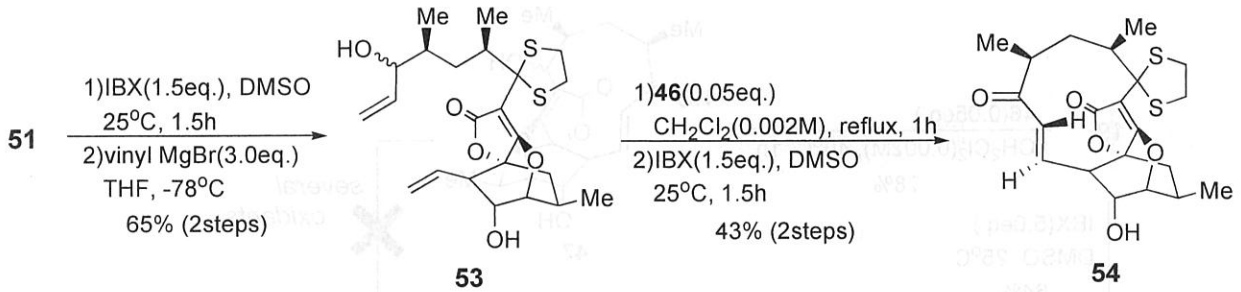
But problems appeared in the last step...

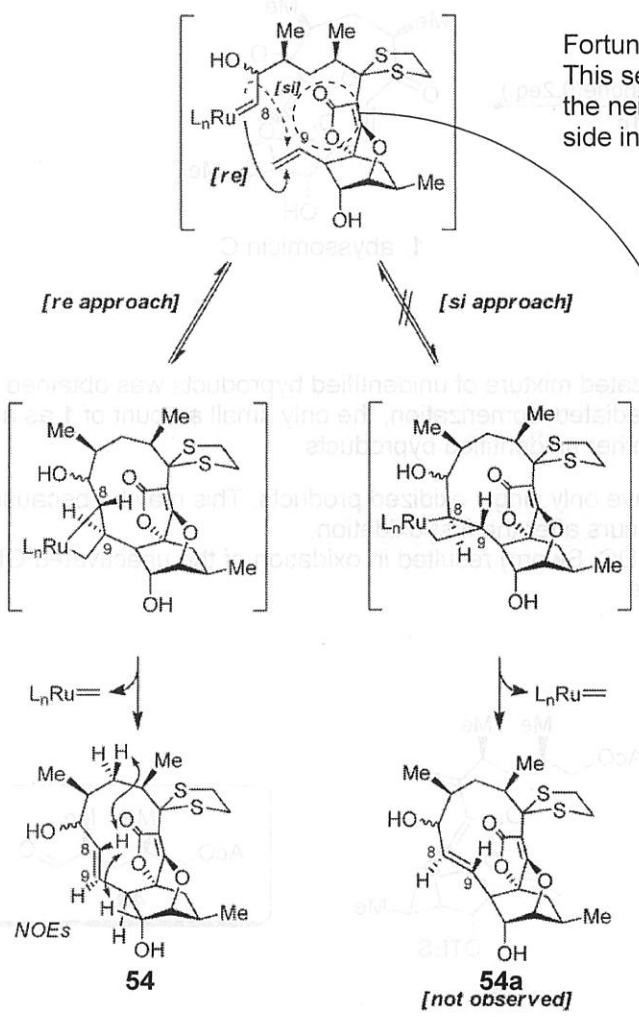
48 ⟶ **1** : In case without quinone, a complicated mixture of unidentified byproducts was obtained. With quinone to supress Ru(III) mediated isomerization, the only small amount of **1** as an inseparable mixture with **48**, and other unidentified byproducts.

47 ⟶ **1** : Selective oxidants (IBX, MnO_2) gave only singly oxidized products. This may be because intramolecular hemiketalization occurs after the first oxidation.
Stronger oxidants (Dess-Martin, PDC, Swern) resulted in oxidation of the unactivated OH group in the oxabicyclooctane core.

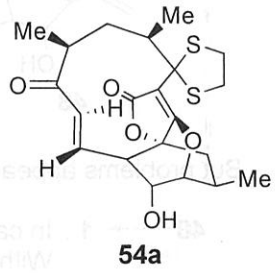
3-7. Successful route to complete the syntheis





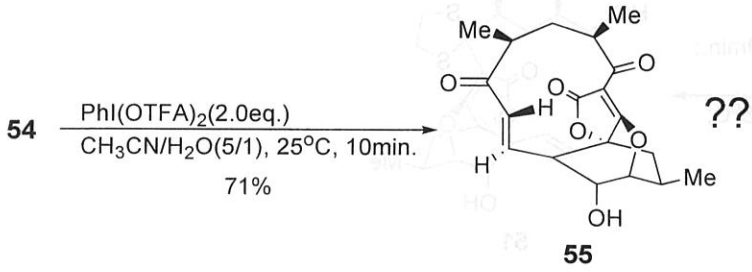


Fortunately, the **54**'s atrop-isomer **54a** was not observed. This selectivity can be explained by the steric bulkiness of the neighboring tetronic ester, which limits the *si*-approach side in RCM step. (left figure)



This ester moiety prevents *si*-face approach by its steric hindrance ??

Figure 7. Proposed rationale for the formation of **51a,b** and not **55** by the ring-closing metathesis reaction.



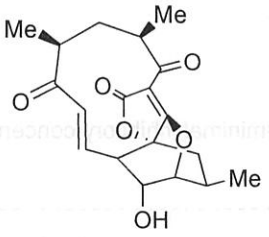
??

This syntesized **55**'s spectroscopic data was very similar to that of the naturally isolated one, but didn't match it perfectly...
(^{1}H NMR chart is shown in the next page)

But surprisingly, in unstabilized $CDCl_3$ for NMR measurement, **55** was slowly isomerized naturally isolated abyssomicin C (**1**), to give a mixture of **55** and **1** (1:2) after 24h.

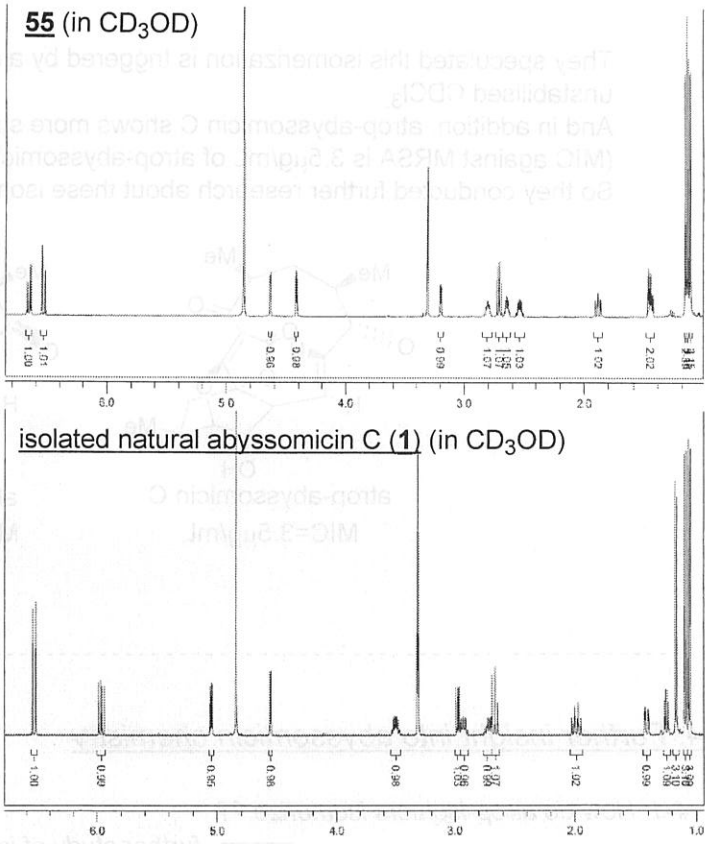
55

⇌ Unstabilized $CDCl_3$
24h, 25°C
67% based on starting **55**

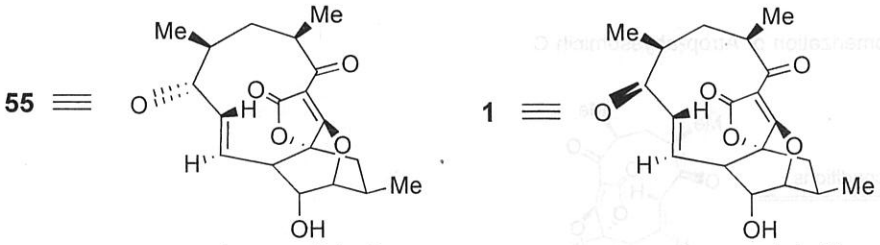


1: abyssomicin C

total y. 3.9% (17 steps)



So they isolated these two isomers and conducted X-ray analysis of the two.
The result showed that these are **atrop-isomer**. And **55** was named "atrop-abyssomicin C".



atrop-abyssomicin C　　abyssomicin C

In abyssomicin C, carbonyl group and its α,β-olefin in the Michael acceptor takes transoid conformation, whereas in atrop-abyssomicin C cisoid comformation.

X-ray structure of abyssomicin C and atrop-abyssomicin C

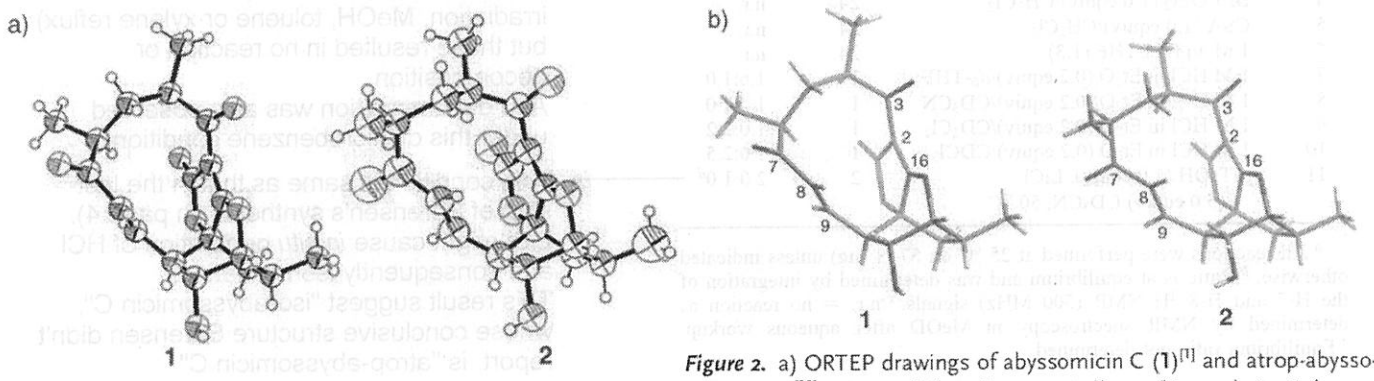
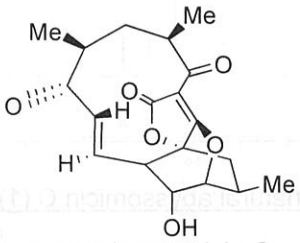
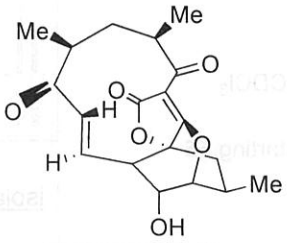


Figure 2. a) ORTEP drawings of abyssomicin C (**1**)[1] and atrop-abyssomicin C (**2**)[15] generated from X-ray crystallographic analysis. Spheres are drawn at a 50% probability level. b) Computer-generated stick models of **1** and **2** based on X-ray crystallographic data.

They speculated this isomerization is triggered by a catalytic amount of HCl present in unstabilised $CDCl_3$.
And in addition, atrop-abyssomicin C shows more strong antibacterial activity.
(MIC against MRSA is 3.5μg/mL of atrop-abyssomicin C, though 5.2μg/mL of abyssomicin C.)
So they conducted further research about these isomer to get more insight.



atrop-abyssomicin C
MIC=3.5μg/mL



abyssomicin C
MIC=5.2μg/mL

*MIC=minimal inhibitory concentration

---

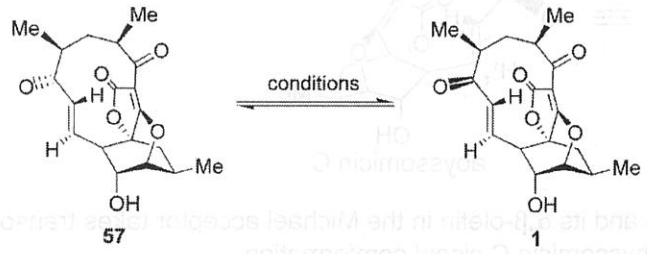
4. Further insight into abyssomicin chemistry

4-1. How do atrop-isomers isomerize ??

——— *further study of isomerization mechanism*

Nicolaou focused on this acid-catalyzed interconversion.
To get more information about it, they tried some thermal or acidic condition.

Table 2. Acid-Catalyzed Isomerization of Atrop-abyssomicin C (**57**) and Abyssomicin C (**1**)



57 ⇌ **1**

entry	conditions[a]	time (h)	ratio (57:1)[b]
1	unstabilized $CDCl_3$	24	1.0:2.0
2	d_4-1,2-dichlorobenzene, 180 °C	12	1.0:1.0
3	TFA/CH_2Cl_2 (1:1)	24	n.r.[c]
4	BF_3·OEt_2 (1.0 equiv)/CH_2Cl_2	24	n.r.
5	CSA (1.0 equiv)/CH_2Cl_2	24	n.r.
6	1 M aq HCl/THF (1:3)	24	n.r.
7	1 M HCl in Et_2O (0.2 equiv)/d_6-THF	1	1.6:1.0
8	1 M HCl in Et_2O (0.2 equiv)/CD_3CN	1	1.4:1.0
9	1 M HCl in Et_2O (0.2 equiv)/CD_2Cl_2	1	1.0:2.2
10	1 M HCl in Et_2O (0.2 equiv)/$CDCl_3$	1	1.0:2.5
11	*p*-TsOH (1.0 equiv), LiCl (5.0 equiv)/CD_3CN, 50 °C	2	2.0:1.0[d]

[a] All reactions were performed at 25 °C on **57** (1 mg) unless indicated otherwise. [b] Ratio is at equilibrium and was determined by integration of the H-7 and H-8 ^1H NMR (500 MHz) signals. [c] n.r. = no reaction as determined by NMR spectroscopy in MeOD after aqueous workup. [d] Equilibrium ratio not determined.

→ Although not noted in this table, they tried other thermal conditions (microwave irradiation, MeOH, toluene or xylene reflux) but these resulted in no reaction or decomposition.
And decomposition was also observed under this dichlorobenzene condition.

→ This condition is same as that in the last step of Sorensen's synthesis (in page 4).
LiCl might cause *in situ* generation of HCl and consequently isomerization.
This result suggest "iso-abyssomicin C", whose conclusive structure Sorensen didn't report, is "atrop-abyssomicin C".

The result showed that the high thermal barrier prevent abyssomicin C from interconversion because of a strained transition state and a catalytic amount of acid relieves the strain.
They proposed three possible mechanisms of this interconversion judging from these hypothesis.

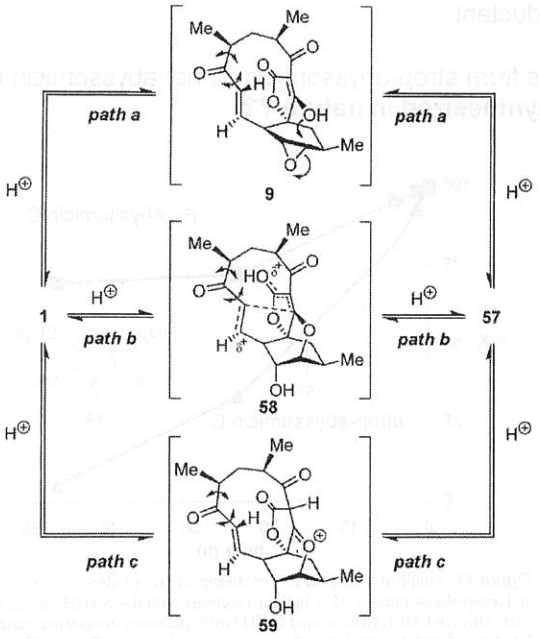


Figure 10. Proposed mechanisms for the acid-catalyzed interconversion of abyssomicin C (**1**) and atrop-abyssomicin C (**57**).

path a)

Acid makes tetronate a good leaving group and this causes closing of epoxide group. The carbonyl group rotate in this form, then the epoxide re-opens by attack of O-anion of tetronate.

path b)

Acid activates C-C double bond in tetronate core and intensify the electron defficiency. This causes bridging interaction and consequencely relieves the torsional strain of the macrocycle.

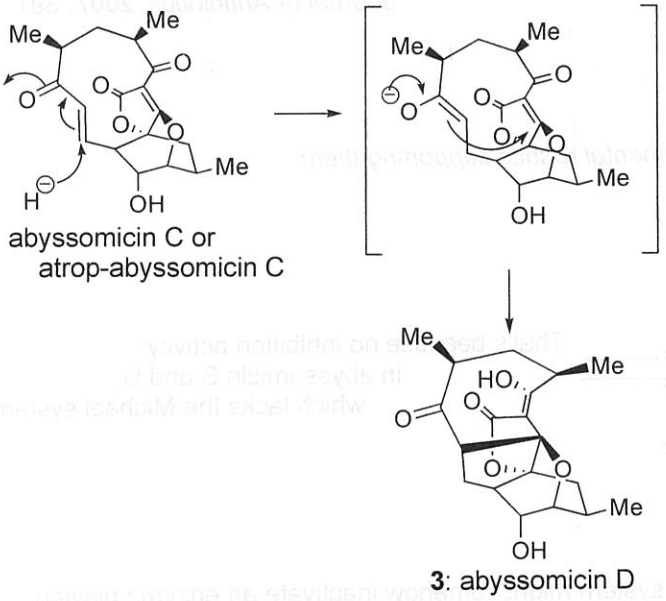
path c)

The core carbon of the tetronate structure was protonated by strong acid, affording oxacarbenium ion. This state relieves the macrocycle ring strain.

4-2. About abyssomicin D

—— Is it formed simply by reduction of abyssomicin C ??

It's reasonable to consider, by comparison of these structures, that abyssomicin D might be derived from abyssomicin C by Michael addition of hydride and successive enolate addition to tetronate core.

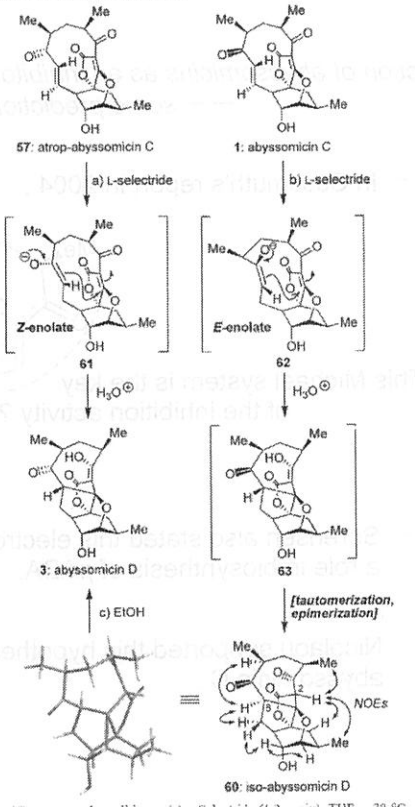


abyssomicin C or
atrop-abyssomicin C

3: abyssomicin D

Nicolaou tried to synthesize abyssomicin D from abyssomicin C and atrop-abyssomicin C using L-selectride as reductant (right scheme).

Scheme 8. 1,4-Reduction of Abyssomicin C (**1**) and Atrop-abyssomicin C (**57**) Leading to Iso-abyssomicin D (**60**) and Abyssomicin D (**3**), Respectively[a]



[a] Reagents and conditions: (a) L-Selectride (1.2 equiv), THF, −78 °C, 30 min, 60%. (b) L-Selectride (1.2 equiv), THF, −78 °C, 30 min, 44%. (c) EtOH, 25 °C, 3 h, 100%. Abbreviations: L-selectride, lithium tri-*sec*-butylborohydride.

As shown in the scheme, abyssomicin D was formed from atrop-abyssomicin C.
Reduction of abyssomicin C gave not abyssomicin C but its isomer "iso-abyssomicin D", and it isomerizes to abyssomicin D in ethanol at room temperature, maybe via proton transfer and bond rotations.
In addition, atrop-abyssomicin C is more reactive against reductant.

⟹ This result suggests nature-derived abyssomicin D is from atrop-abyssomicin C, not abyssomicin C.
= **Atrop-abyssomicin C is also synthesized in nature ??**

They conducted another experiment about the reduction of abyssomicin C and its atrop-isomer.
This experiment was using NADH analog as a biomimetic reductant, and the result showed atrop-abyssomicin C is clearly more reactive.
It supports the hyposesis that abyssomicin D is from atrop-abyssomicin C.

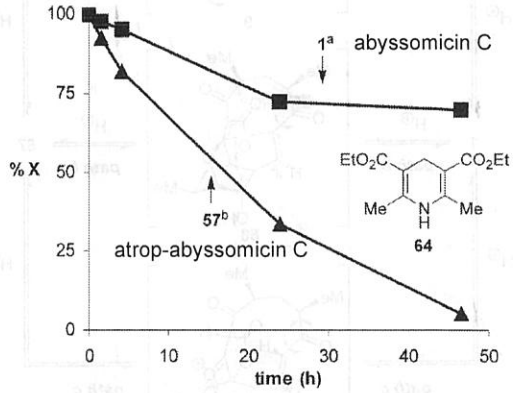


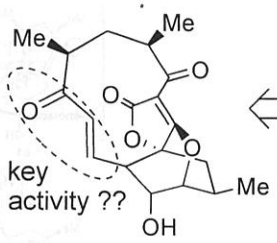
Figure 11. Graph showing the rate of disappearance of abyssomicin C (**1**) and atrop-abyssomicin C (**57**) in their reactions with the NADH analog **64** (5.0 equiv) in $CDCl_3$ (determined by ^1H NMR spectrum integration against 2,6-di-*tert*-butyl-4-methylphenol as an internal standard). (a) Neither abyssomicin D (**3**) nor iso-abyssomicin D (**60**) were detected in the crude ^1H NMR spectrum of the product. (b) Abyssomicin D (**3**) was the only product detected in the crude ^1H NMR spectrum.

Soon after the publication of this Nicolaou's *JACS* paper, Sussmuth *et al.* reported the isolation of atrop-abyssomicin C also from natural *Verrucosispora* strain AB 18-032.
Furthermore, **the main abyssomicin product synthesized by *Verrucosispora* is atrop-abyssomicin C** and abyssomicin C, which is initially isolated, is a minor product being formed from atrop-abyssomicin C under acidic consitions.

R. D. Süssmuth *et al.*
Journal of Antibiotics, **2007**, 391

4-3. Action of abyssomicins as an inhibitor
—— *some predictions and experimental results supporting them*

—— In Süssmuth's report in 2004...



This Michael system is the key of the inhibition activity ??

⟸ That's because no inhibition activity in abyssomicin B and D, which lacks the Michael system.

—— Sorensen also stated this electrophilic enone system might somehow inactivate an enzyme playing a role in biosynthesis of *p*ABA.

—— Nicolaou supported this hypothesis by refering the X-ray structure of abyssomicin C and atrop-abyssomicin C...

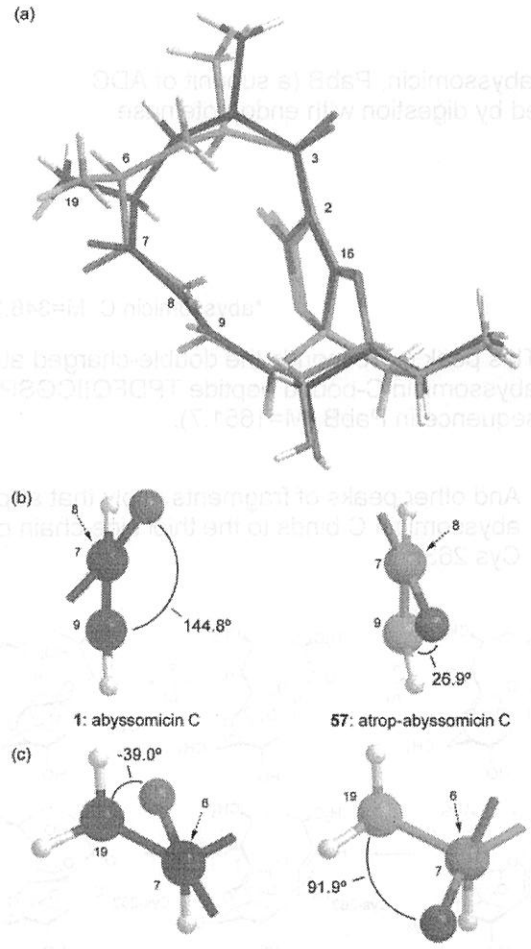


Figure 9. (a) Overlay of the X-ray derived structures of abyssomicin C (**1**, blue) and atrop-abyssomicin C (**57**, yellow). (b) View down the C7–C8 bond for **1** and **57**. (c) View down the C7–C6 bond for **1** and **57**.

By X-ray analysis, they are directing their attention to the Michael acceptor in these two isomers.
This analysis showed that carbonyl and olefin in the enone system in atrop-isomer take more planar structure. ((b) in left figure)
It may result in a higher degree of conjugation in atrop-abyssomicin, consequently a more reactive Michael acceptor.

⇩

Considering both this theory and the experimental fact that atrop-abyssomicin C shows more strong inhibition activity, it's reasonable that this Michael acceptor serves as the essential part in inhibition.

Süssmuth *et al.* reported their reserch about action of atrop-abyssomicin C as an inhibitor.
They prepared ADC synthase, which is the target enzyme of abyssomicin C.
Preincubation of this enzyme with abyssomicin C and atrop-abyssomicin C gave inactivation of the enzyme.

R. D. Süssmuth *et al.*
Angew. Chem. Int. Ed., **2007**, 8284

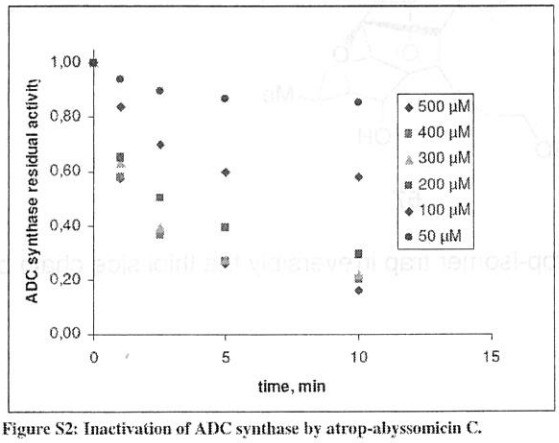


Figure S2: Inactivation of ADC synthase by atrop-abyssomicin C.

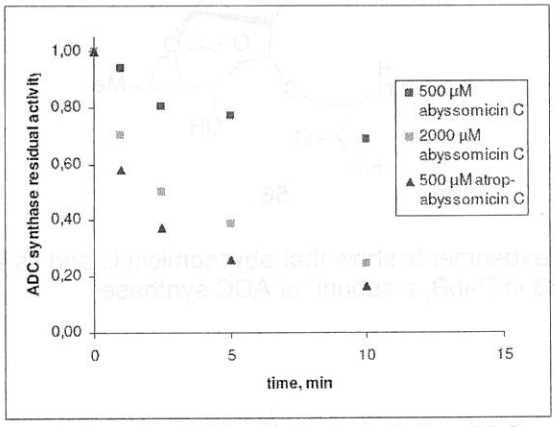
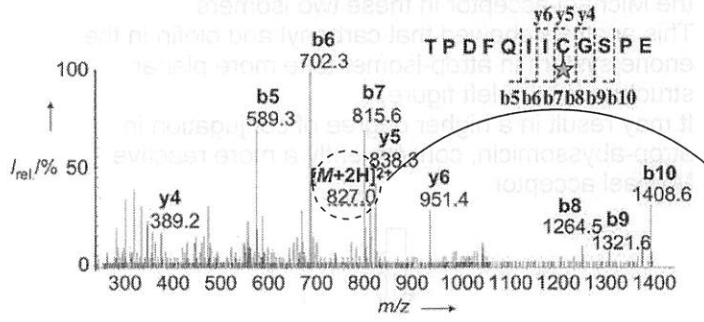


Figure S3: Comparison of ADC synthase inactivation by abyssomicin C and atrop-abyssomicin C.

left) Decrease of ADC synthase activity by addition of several concentration of atrop-abyssomicin C

right) Comparison of degree of ADC synthase inhibition between abyssomicin C and atrop-abyssomicin C. This means atrop-abyssomicin C is more potent inhibitor against ADC synthase.

To identify the location of the protein-binding site of atrop-abyssomicin, PabB (a subunit of ADC synthase) was incubated with atrop-abyssomicin C followed by digestion with endoproteinase GluC, then they analysed digestion mixture by MS/MS.



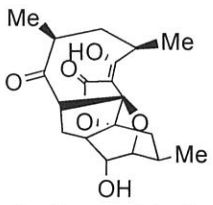
*abyssomicin C: M=346.37

This peak corresponds the double-charged atrop-abyssomicin C-bound peptide TPDFQIICGSPE sequence in PabB (M=1651.7).

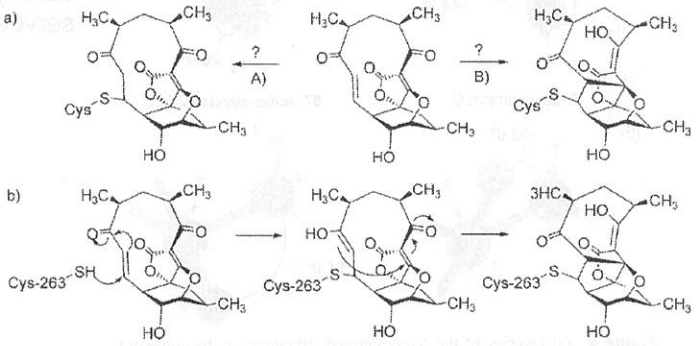
Figure 2. MS/MS sequencing of the abyssomicin-bound PabB target peptide (*m/z* 827.0) after GluC digest. Cys263 has been covalently modified by atrop-abyssomicin C.

And other peaks of fragments imply that atrp-abyssomicin C binds to the thiol side chain of Cys 263.

So they became sure that Cys 263's side chain bounds to atrop-abyssomicin, but another question was weather thiol side chain's attack gave a simple Michael addition adduct or a abyssomicin D-like skelton.

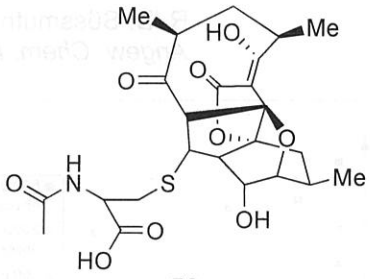


3: abyssomicin D

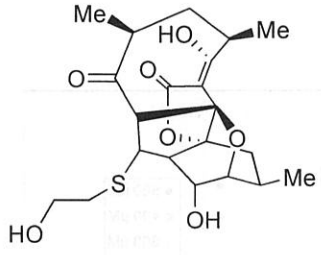


Scheme 2. Function of atrop-abyssomicin C as a Michael acceptor. a) Michael addition (pathway A) and dual Michael addition with subsequent rearrangement to an abyssomicin D derivative (pathway B). b) Proposed reaction with Cys263 based on conversion with S nucleophiles 2-sulfanylethanol and *N*-acetylcysteine, respectively.

They tested this hypothesis by using model thiol reactants, 2-sulfaethanol and *N*-acetylcysteine. Reaction of atrop-abyssomicin with each thiol in THF gave products **56** and **57** shown below.



56



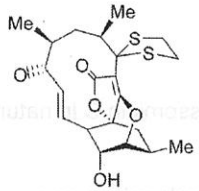
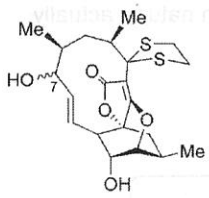
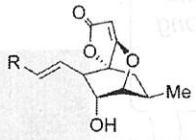
57

These experiments show that abyssomicin C and its atrop-isomer trap irreversibly the thiol side chain of Cys 263 in PabB, a subunit of ADC synthase.

4-4. Biological evaluation of synthesized analogues

Nicolaou also synthesized some analogues of abyssomicin C and evaluated the biological activity of these compounds.
They tested designed compounds containing Michael acceptor and intermediates of their total synthesis.

Table 3. Minimal Inhibitory Concentrations (MICs) of Abyssomicin C Analogues against Methicillin-Resistant *S. aureus* (MRSA)[a]

entry	compound		MIC (μM)
1	abyssomicin C		20
2	atrop-abyssomicin C		15
3	Ac-abyssomicin C		20
4			70
5		C7-(*R*)	>500
6		C7-(*S*)	>500
7		(R = H)	>500
8		(R = CO_2Me)	>500
9		(R = $C(O)CH_3$)	>500

[a] MIC values were determined with serial dilutions in 96-well plates against a 1:10 000 dilution in nutrient broth (DIFCO) of a 24 h culture of methicillin-resistant *S. aureus* (ATCC 33591) at 35 °C. Reported MIC values reflect the concentration at which no growth could be visually detected after 24 h.

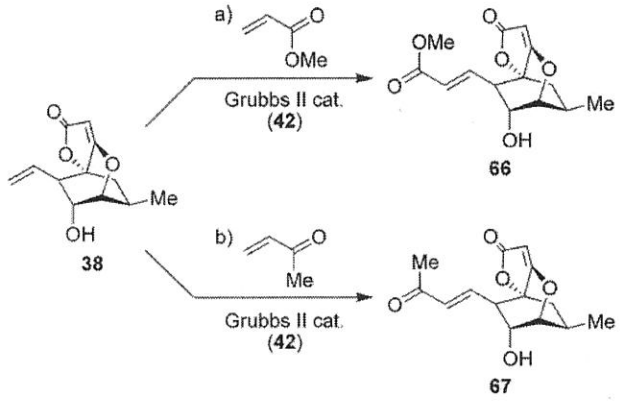
→ No decrease of inhibitory activity by acetylating the hydroxy group implies this -OH has nothing with the action of inhibition. (Unless it's subject to enzymatic acetate hydrolysis.)

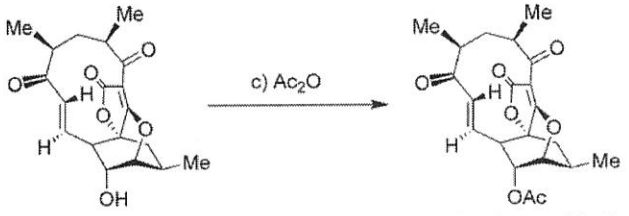
No activity of these compounds supports the hypothesis that the Michael acceptor is involved in the action of inhibition.

These results implies that a properly oriented Michael acceptor is necessary for inhibition activity.

*These designed compounds (ent.3, 8 and 9) were synthesized in schemes below.

Scheme 9. Synthesis of Simplified Analogues **66** and **67** via Cross-Metathesis and Ac-abyssomicin C (**68**)[a]





[a] Reagents and conditions: (a) **42** (0.05 equiv), methyl acrylate (10 equiv), CH_2Cl_2 (0.1 M), 40 °C, 1 h, 84%. (b) **42** (0.05 equiv), methyl vinyl ketone (10 equiv), CH_2Cl_2 (0.1 M), 40 °C, 1 h, 89%. (c) Ac_2O (15 equiv), Et_3N, 25 °C, 63%.

⟹ These synthetic analogues are not so successful.
But the hypothesis that the Michael acceptor plays a key role became more reliable, and other simplyfied compounds without decrease of activity are anticipated.

5. Summary

2004	First example of isolation of abyssomicins by Süssmuth *et al.*
2005	Sorensen *et al.* accomplished total synthesis of abyssomicin C But unknown isomer "iso-abyssomicin" was formed in the last step...
2006	Nicolaou *et al.* reported total synthesis via conceptually different way from Sorensen's one. They identified the atrop-isomer.
2007 Jan.	Nicolaou reported a further insight about abyssomicin chemistry. Implication for the exsistence of atrop-abyssomicin C in nature.
2007 Jun.	Süssmuth et al. reported the isolation of atrop-abyssomicin from natural source. atrop-abyssomicin C is the main atrop-isomer in nature, actually.

This fascinating tale and the still ongoing research on atropabyssomicin C demonstrate the power of total synthesis; and the studies derived from unexpected discoveries along the way provide insight into the structure, biosynthesis, and mechanism of action of bioactive molecules.

from
K. C. Nicolaou *et al.*
Angew. Chem. Int. Ed., **2009**, 660