University of Pittsburgh

Chemistry Department

Total synthesis of Marinomycins A-C and of Their Monomeric Counterparts Monomarinomycin A and *iso*-Monomarinomycin A

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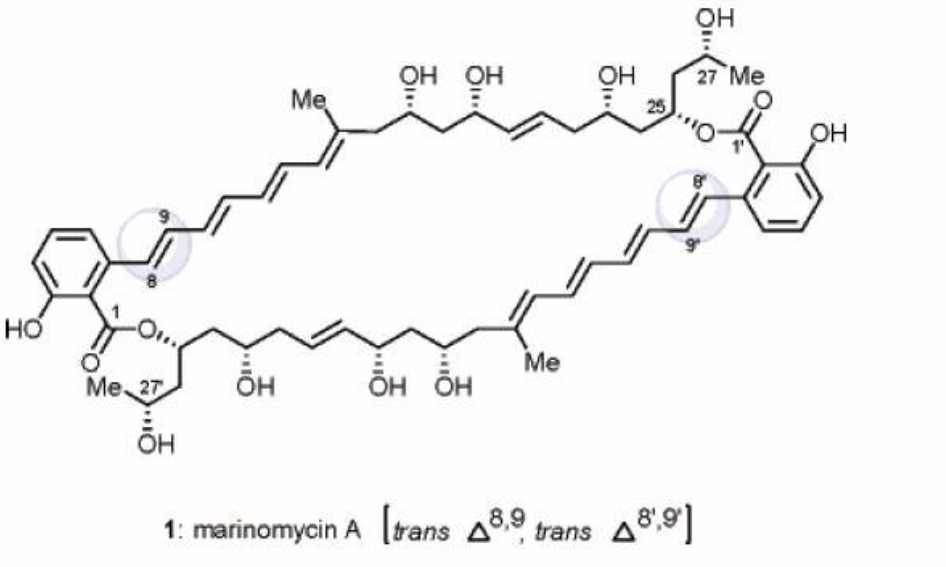
Filip Petronijevic
March, 12th 2007

Marinomycins-isolation and activity

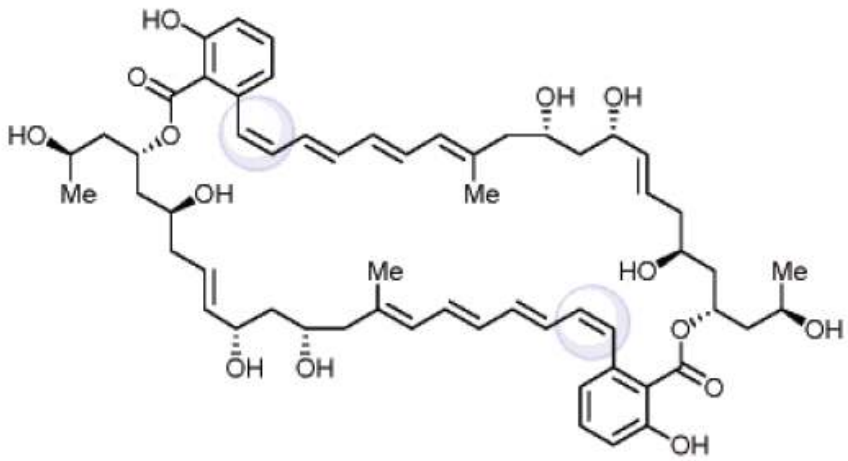
- Marinomycins A-C are new class of antibiotics
- Antibiotics are usually isolated from *Actinomycetes* (more than 10,000 bioactive compounds)
- The Fenical group isolated marinomycins A-C from a novel marine actinomycete *Marinospora* CNQ-140
- Marinomycins show potent antibiotic activity against methicillin-resistant *Staphilococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VREF)
- Marinomycin A has MIC_{90} 0.1-0.6 μM
- Marinomycins show potent cytotoxicities against some cancers (LC_{50} 2.7 μM) and melanomas (for SK-MEL-5, LC_{50} 5.0 nM)
- probably a novel mechanism of action
- only marinomycin A shows activity against *Candida albicans* (MIC_{90} 10 μM)

J.Amer.Chem.Soc. **2006**, *128*, 1622.

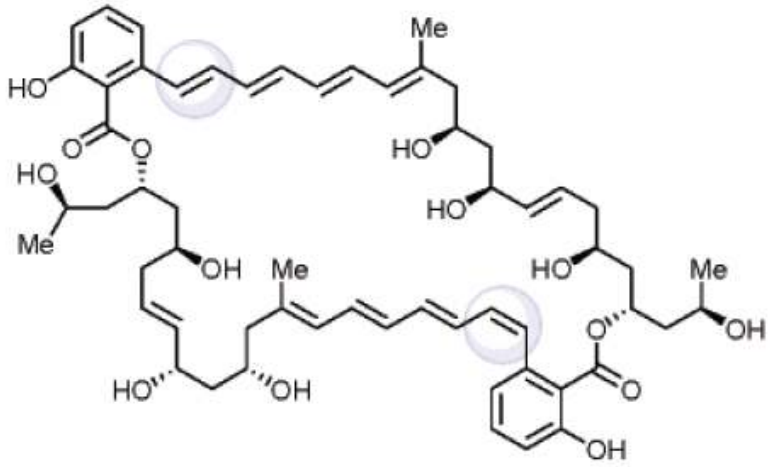
Structures of marinomycins A-C



1: marinomycin A [*trans* $\Delta^{8,9}$, *trans* $\Delta^{8',9'}$]



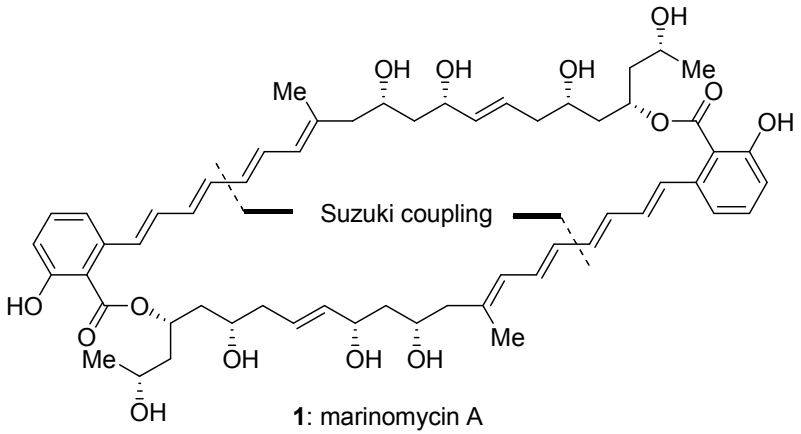
2: marinomycin B [*cis* $\Delta^{8,9}$, *cis* $\Delta^{8',9'}$]



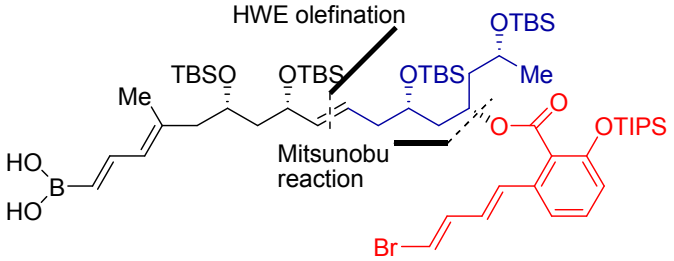
3: marinomycin C [*trans* $\Delta^{8,9}$, *cis* $\Delta^{8',9'}$]

- different geometries of double bonds
- 44-membered ring
- possible photoisomerisation of marinomycin A to B and C analogue
- note the symmetry of marinomycin A

Retrosynthetic analysis



1: marinomycin A



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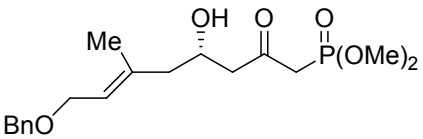

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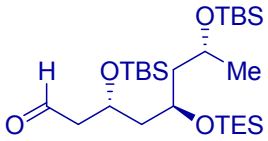

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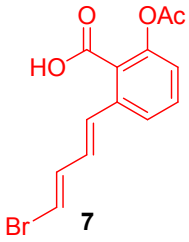

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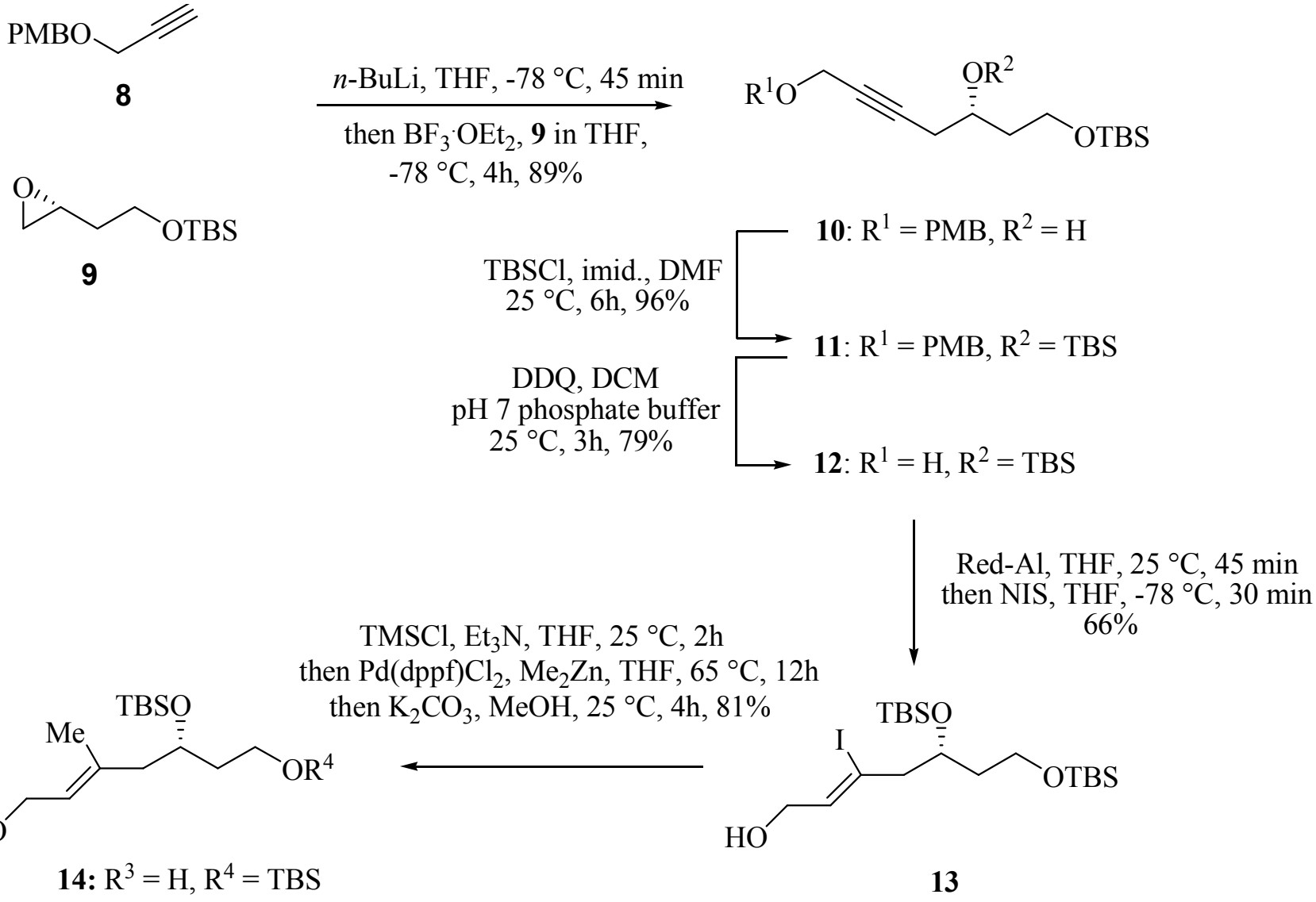
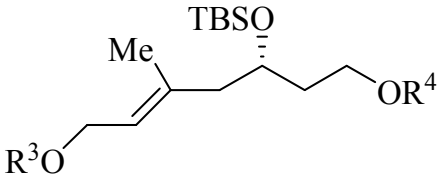
Preparation of ketophosphonate 5



Preparation of ketophosphonate 5



14: R^3 = H, R^4 = TBS

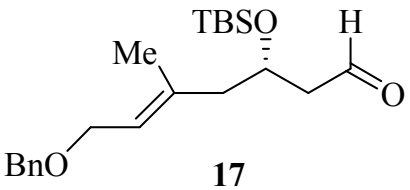
15: R^3 = Bn, R^4 = TBS

16: R^3 = Bn, R^4 = H

NaH, THF, 0 to 25 °C, 30 min
then BnBr, *n*-Bu_4NI, 6h, 88%

HF·py, THF, 0 °C, 3h, 77%

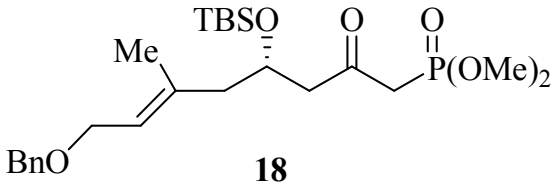
PCC, NaHCO3, 25 °C, 3h, 73%



17

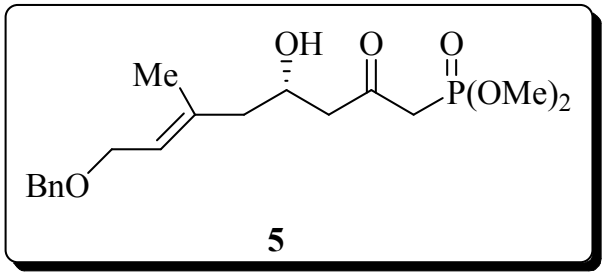
1. MeP(O)(OMe)2, n-BuLi, THF, -78 °C, 2h
then **17** in THF, -78 °C, 2h

2. PDC, 4 A MS, DMF, 25 °C, 12h
64 % over two steps



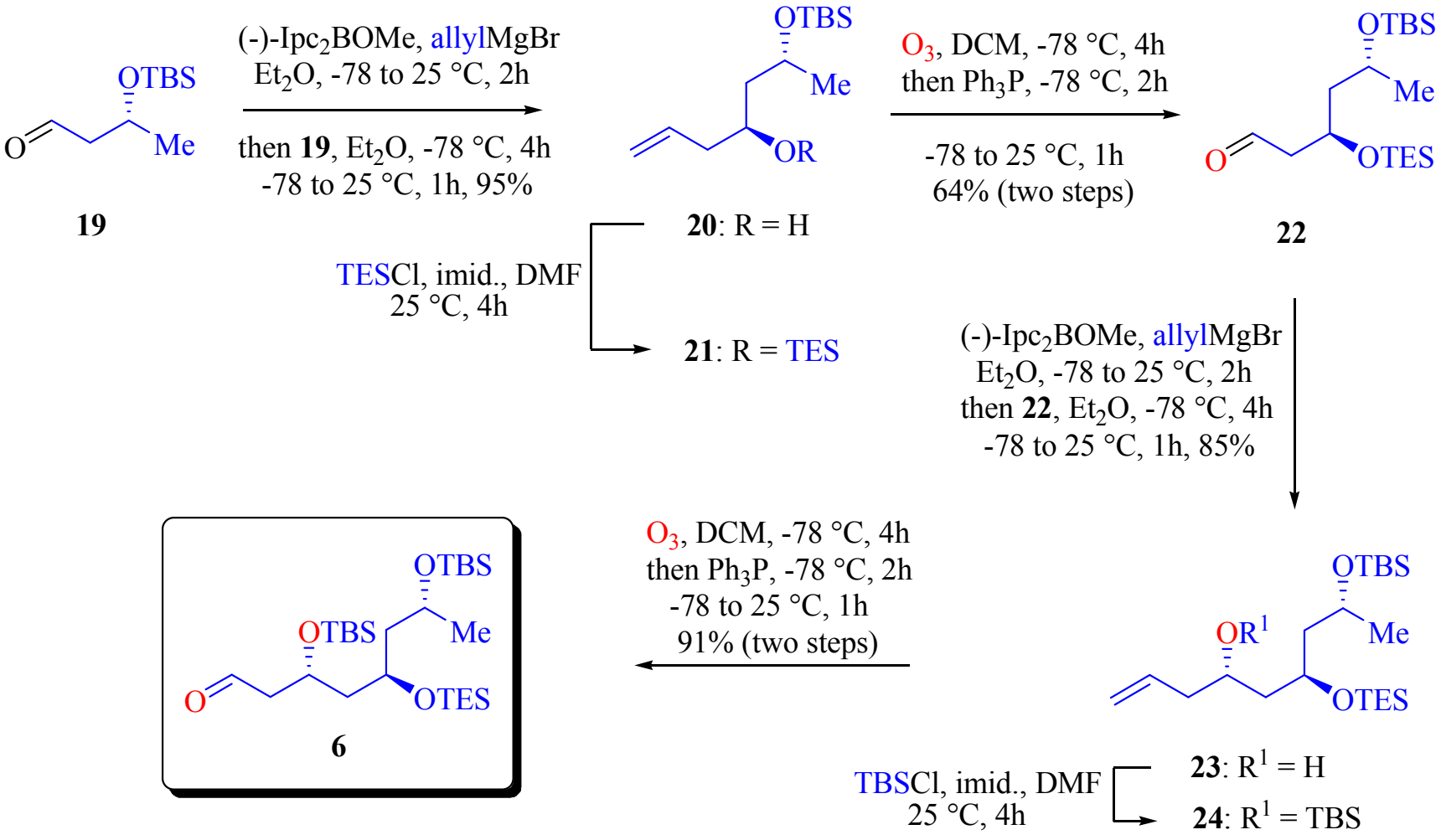
18

HF, MeCN
25 °C, 3h, 92%

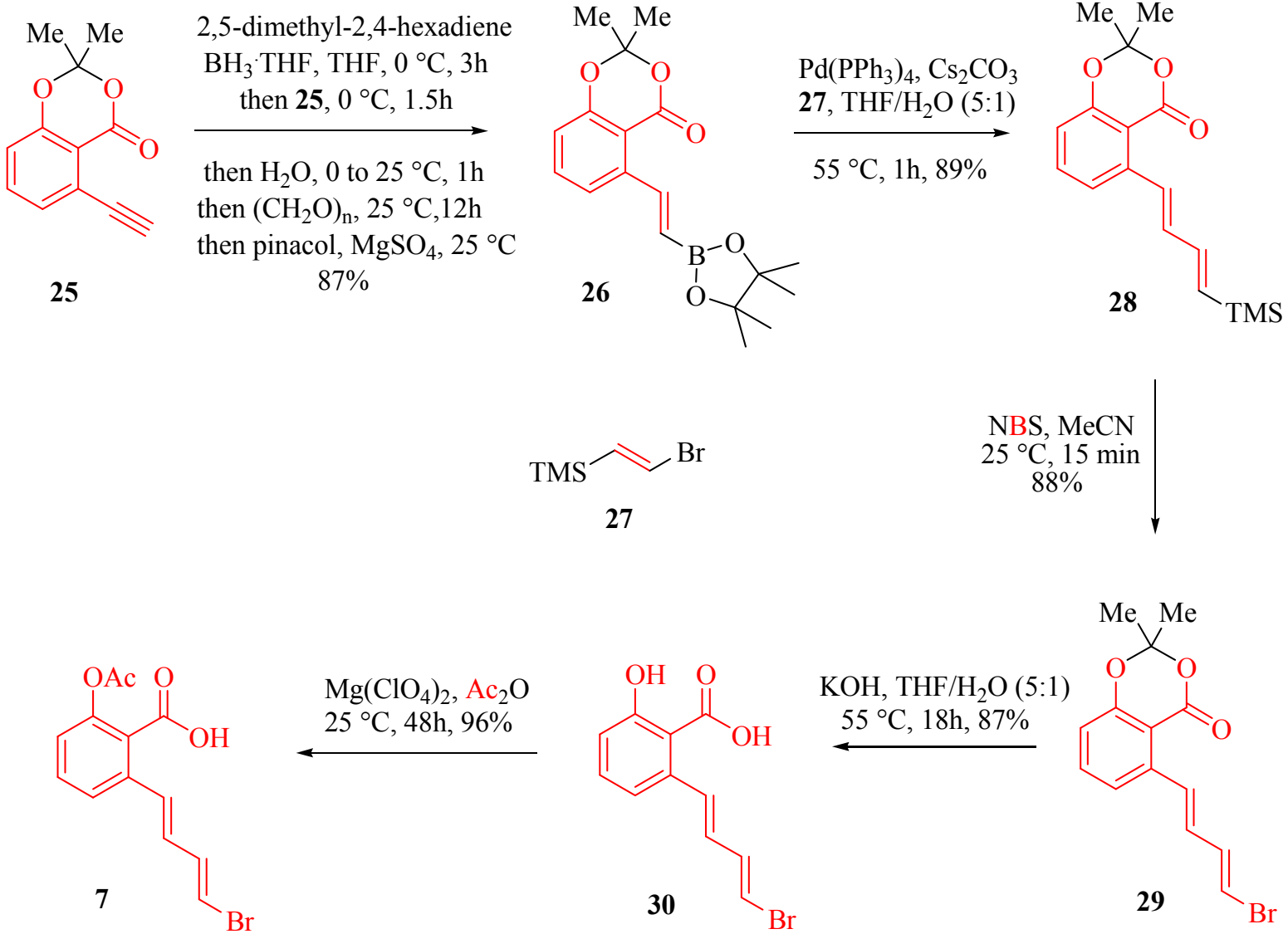


5

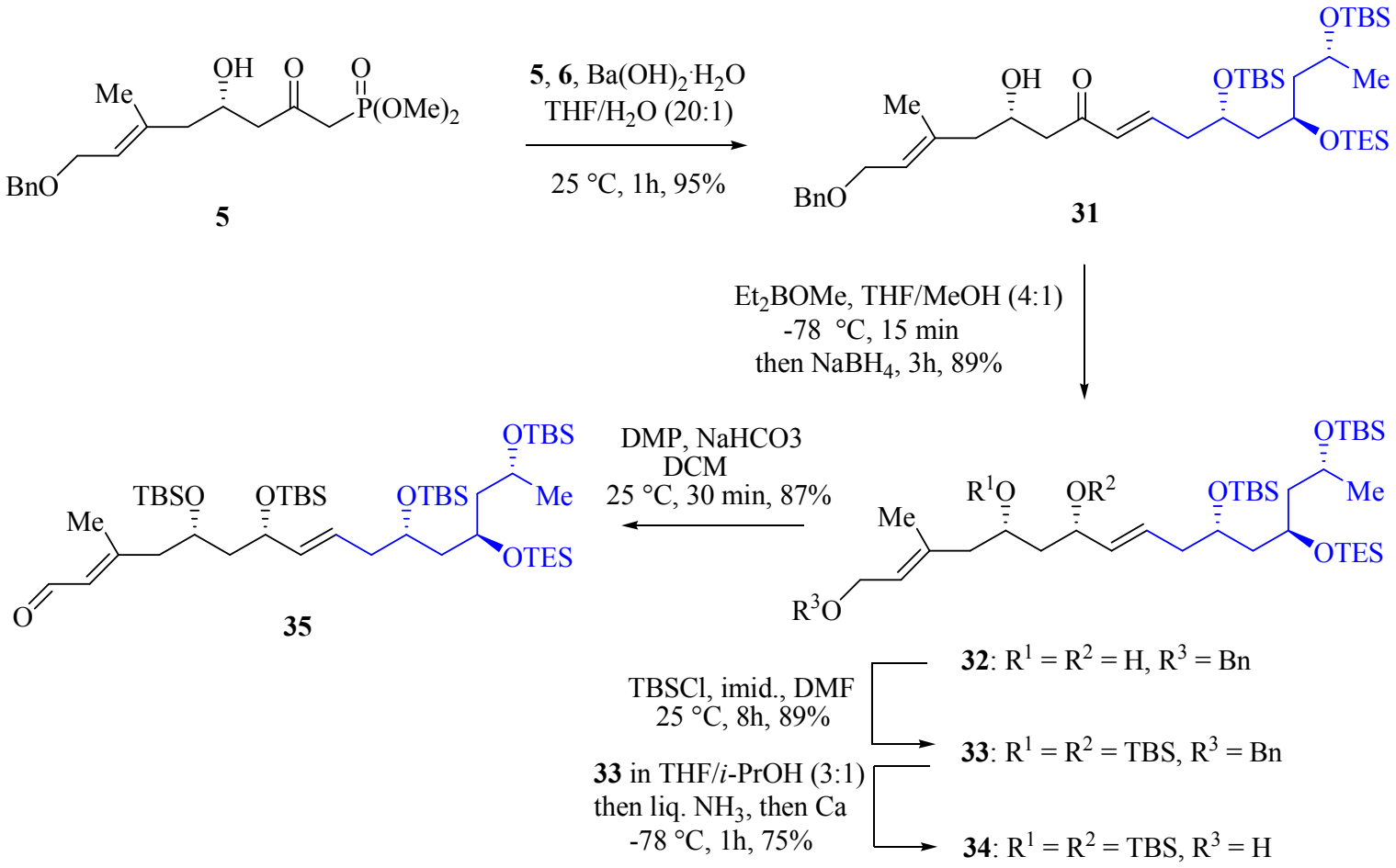
Preparation of Aldehyde 6



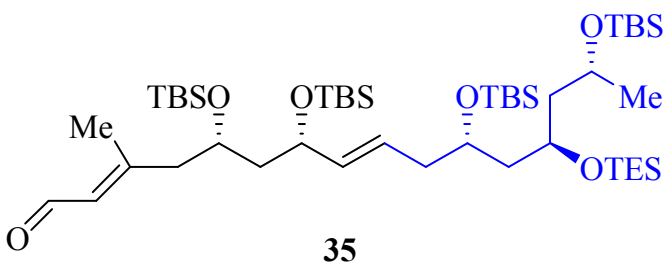
Preparation of Fragment 7



Preparation of Suzuki Coupling Precursor 39



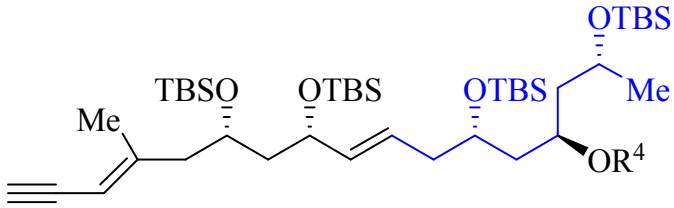
Preparation of Suzuki Coupling Precursor 39



35

i-PrNH$_2$, *n*-BuLi, THF
-78 to 0 °C, 30 min

then TMSCHN$_2$, THF
-78 °C, 30 min; then **35**
-78 to 25 °C, 2h, 85%

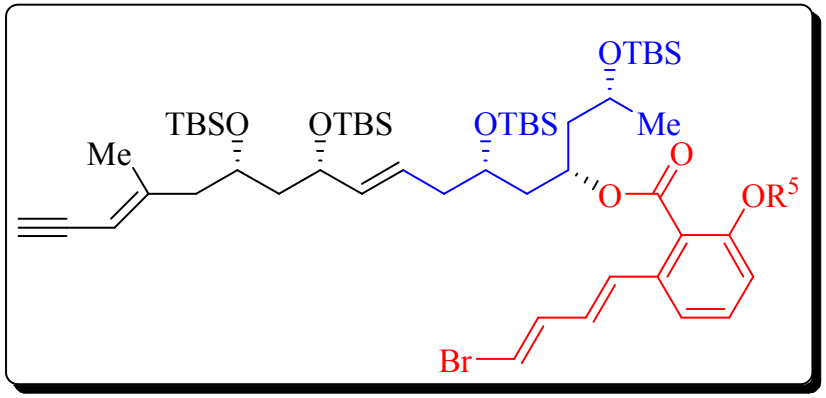


PPTS, EtOH
25 °C, 3h, 75%

36: R^4 = TES

37: R^4 = H

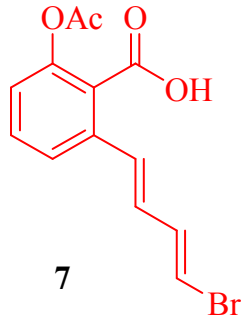
DEAD, PPh3, **7**
THF, 25 °C, 1h, 93%



38: R^5 = Ac

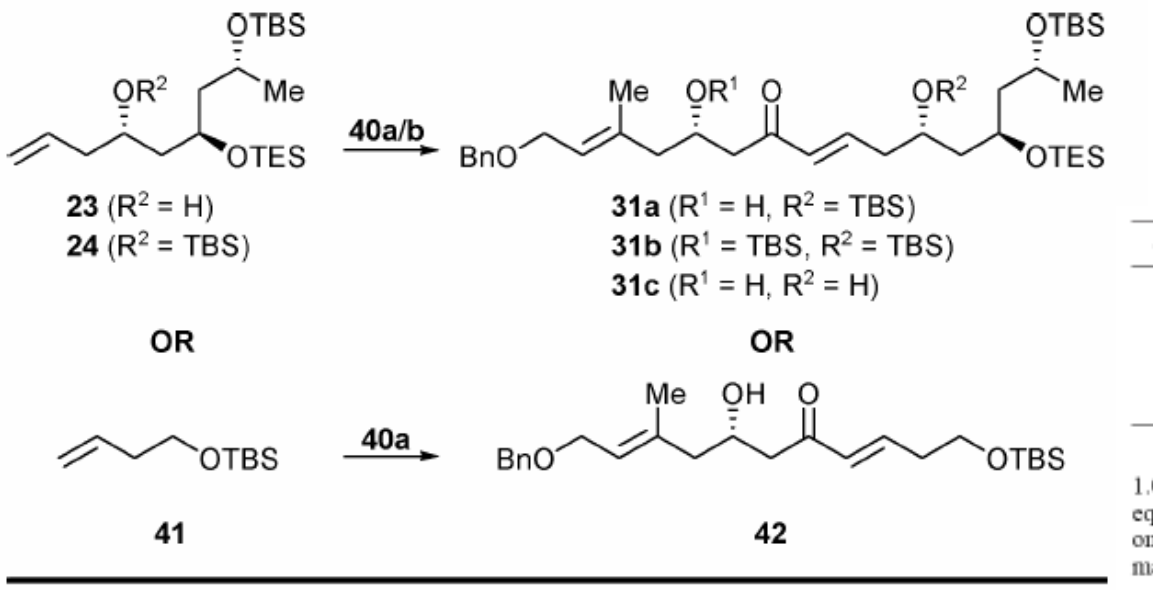
39: R^5 = TIPS

1. K_2CO_3, MeOH, 25 °C, 15 min
2. TIPSOTf, 2,6-lut., DCM, 25 °C, 18h
92% over two steps



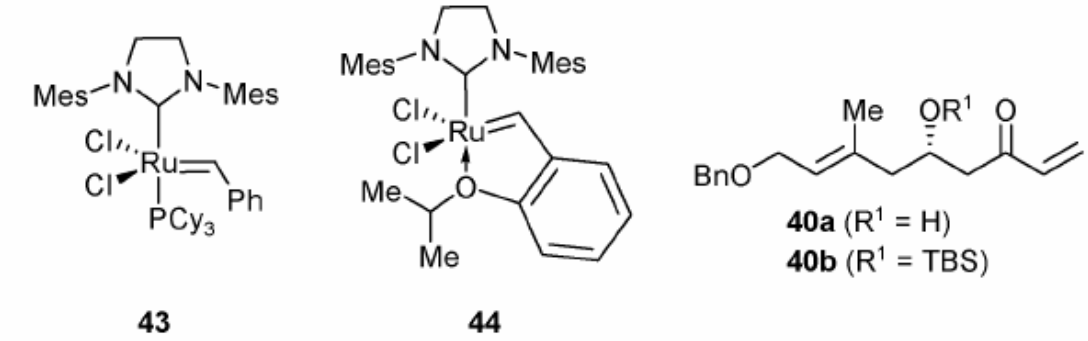
7

Olefin Cross-Metathesis toward Enone 31

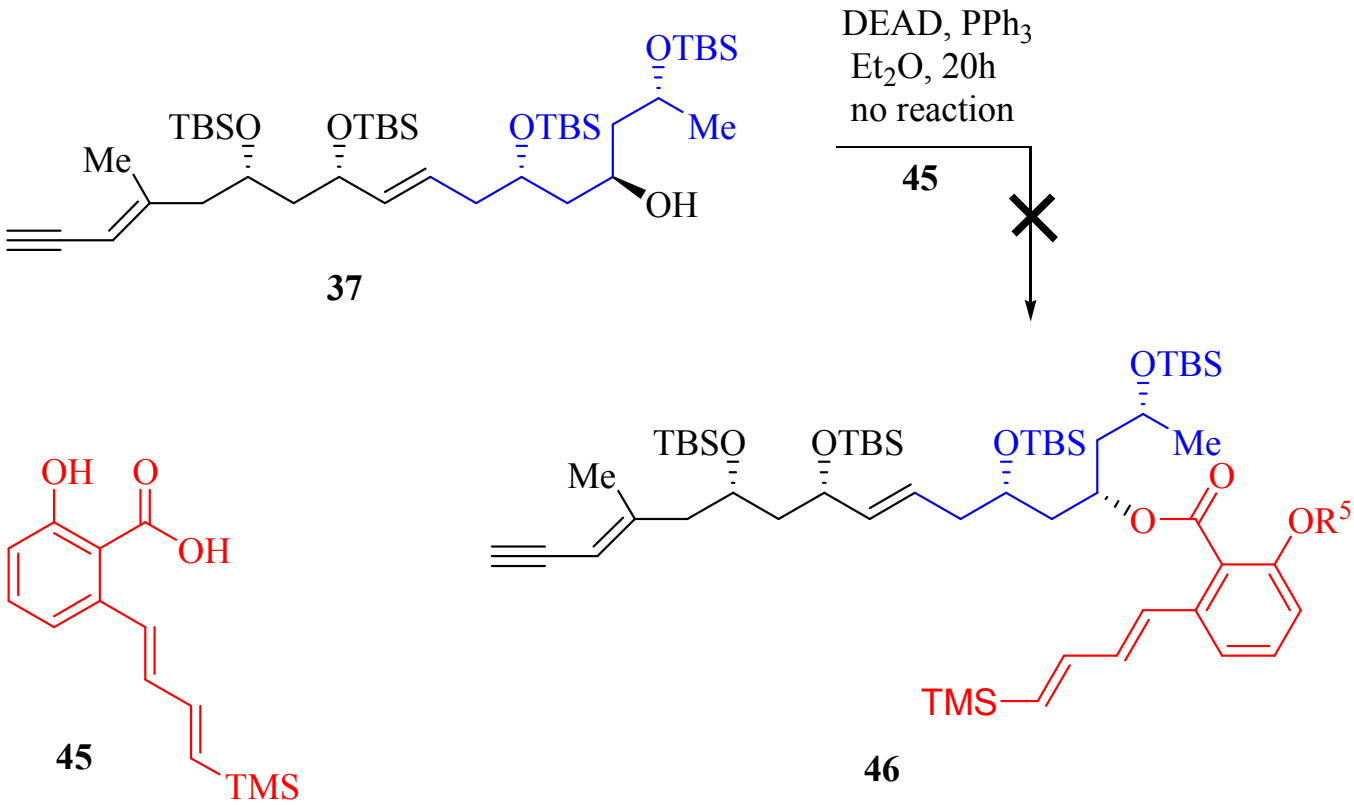


entry[a]	substrate (R^2)	substrate (R)	catalyst	product (% yield)[b]
1	**41**	**40a** (H)	**43**	**42** (80)
2	**24** (TBS)	**40a** (H)	**43**	**31a** (36, 72[c])
3	**24** (TBS)	**40b** (TBS)	**43**	**31b** (20, 65[d])
4	**23** (H)	**40a** (H)	**43**	**31c** (42)
5	**24** (TBS)	**40a** (H)	**44**	**31a** (44, 80[e])

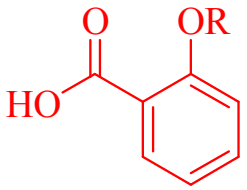
[a] All reactions carried out on 0.07 mmol scale at 25 °C for 18 h with 1.0 equiv of enone and 2.0 equiv of alkene, except in entry 5, where 3.0 equiv of enone and 1.0 equiv of alkene were used. [b] Isolated yield. [c] Based on 50% recovered starting material. [d] Based on 69% recovered starting material. [e] Based on 45% recovered starting material.



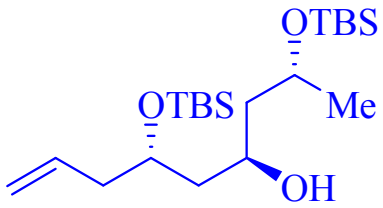
Attempted Mitsunobu Reaction between 37 and 45



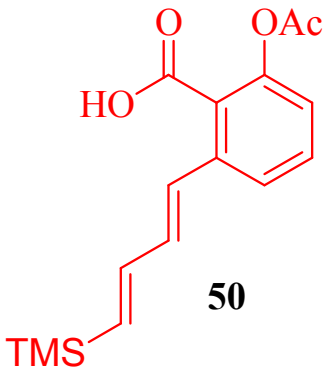
Model Studies toward Mitsunobu Reaction



48a: R = H
48b: R = Me
48c: R = Ac



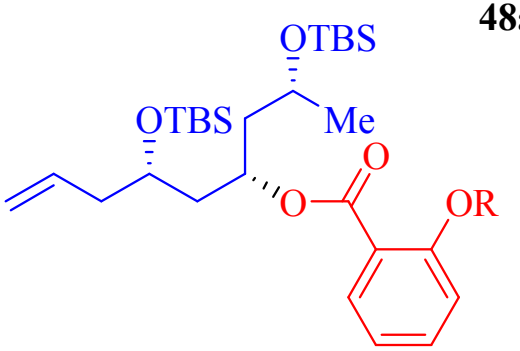
47



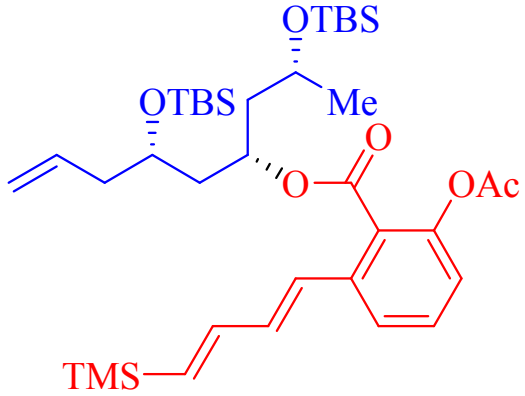
50

DEAD, PPh_3, THF
48a-c

DEAD, PPh_3, THF
50, 1h, 81%

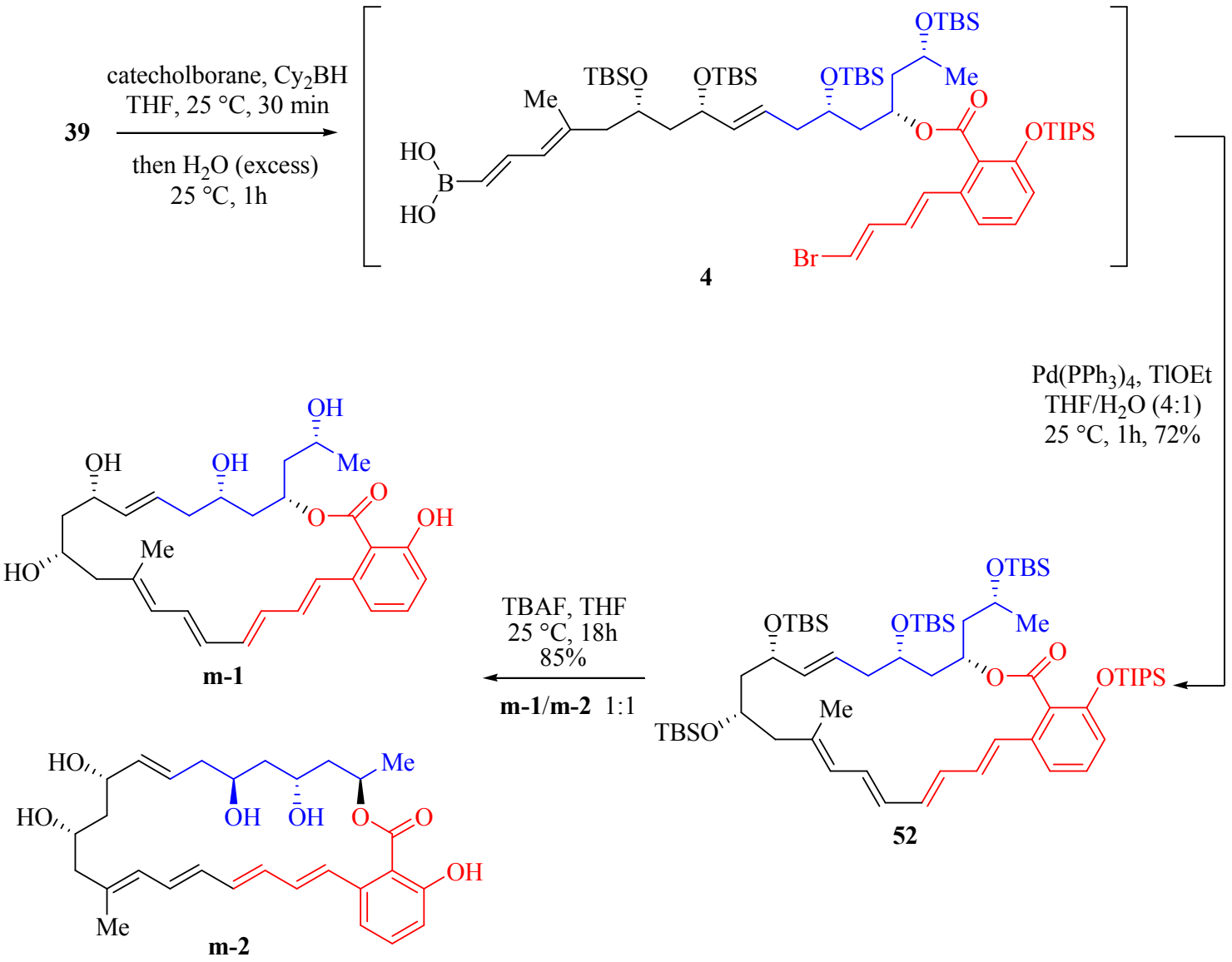


49a: R = H, 24h, trace product
49b: R = Me, 3h, 65%
49c: R = Ac, 1h, 72%

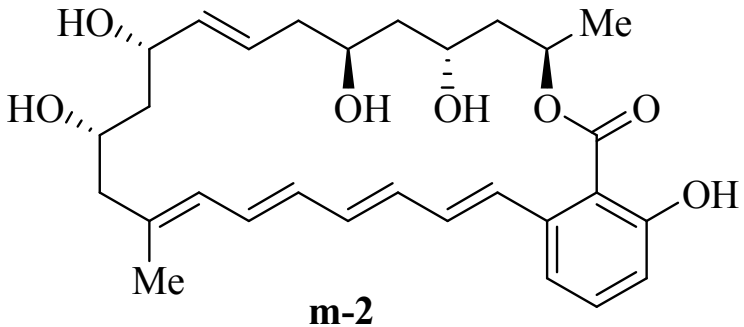


51

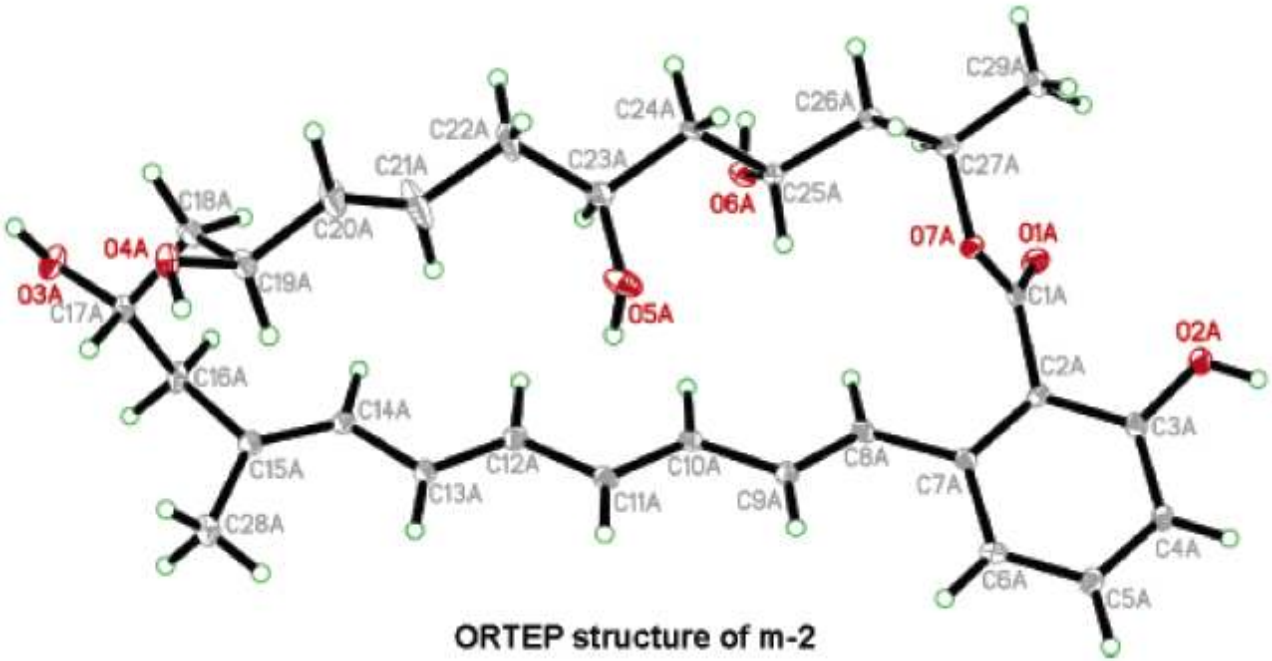
Formation of Monomarinomycins m-1 and m-2



X-ray crystal structure of m-2

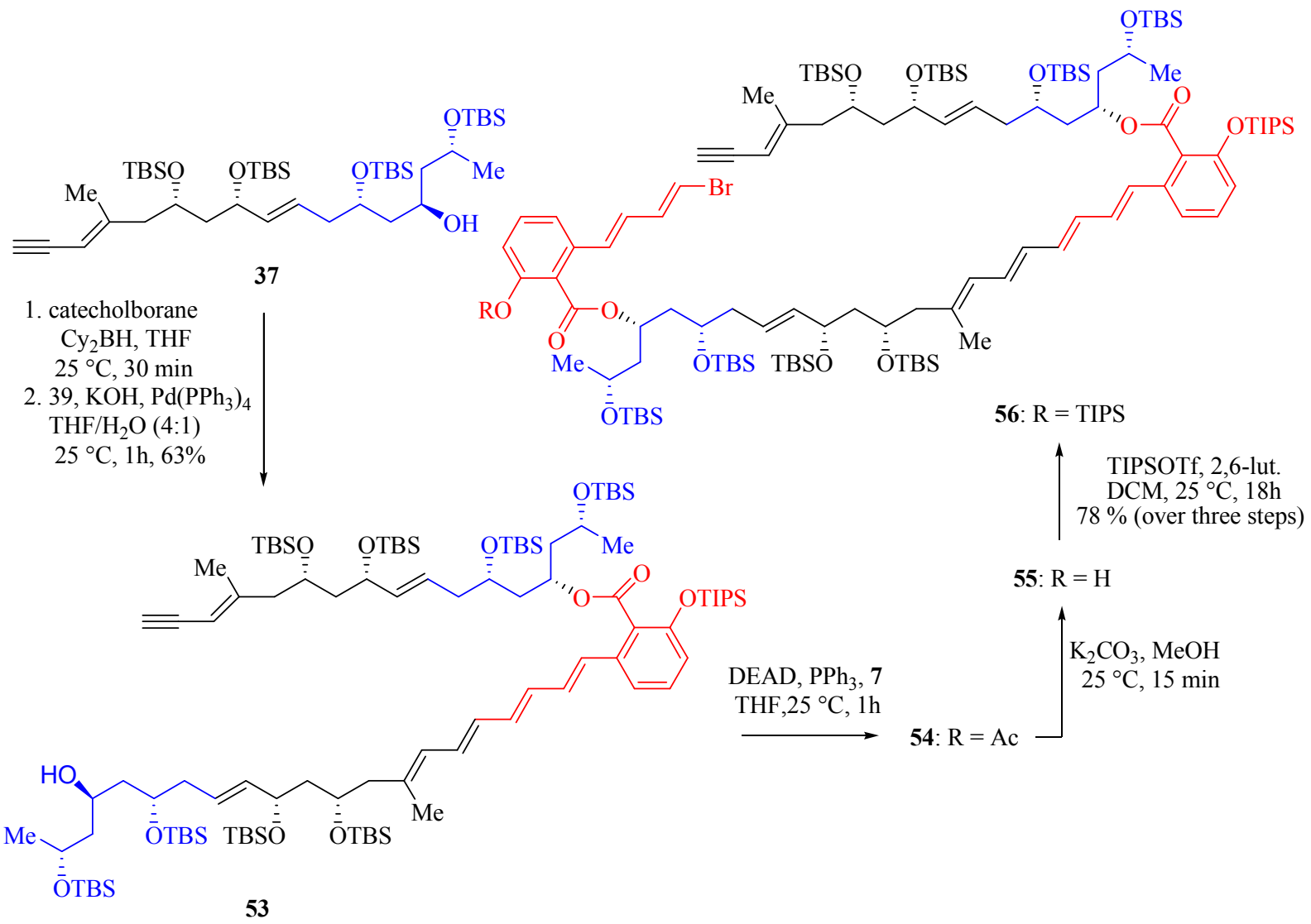


m-2

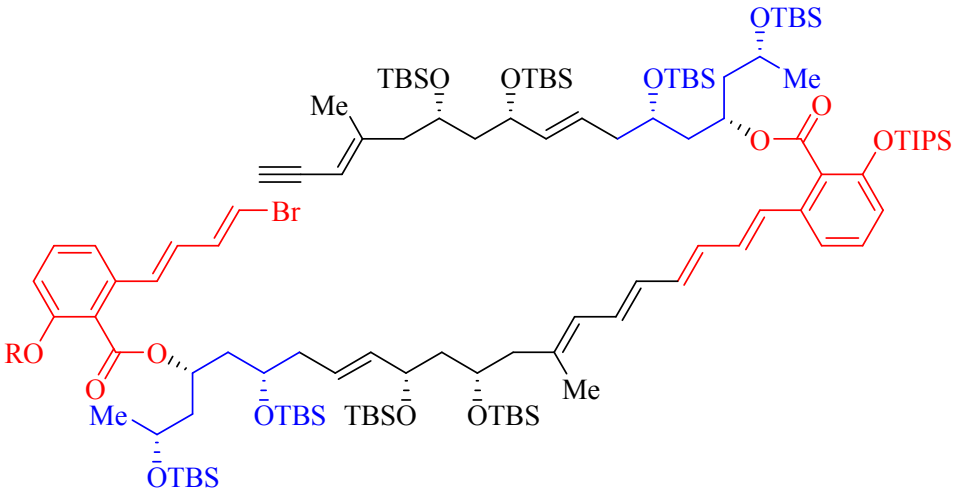


ORTEP structure of m-2

Toward the Completion of the Total Synthesis of Marinomycin A (1)



Completion of the Total Synthesis of Marinomycin A (1)

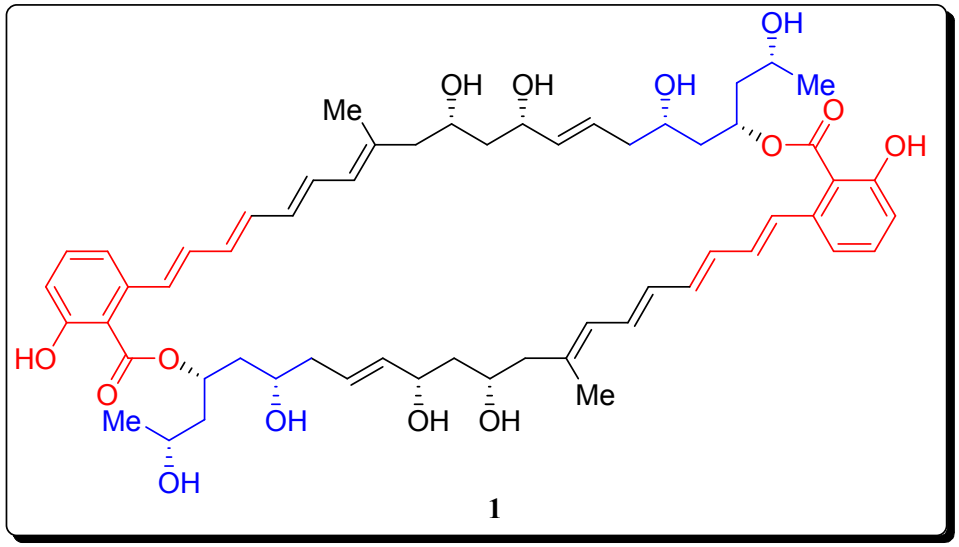


56: R = TIPS

1. catecholborane, Cy_2BH
THF, 25 °C, 30 min
2. TlOEt, $Pd(PPh_3)_4$
THF/H_2O (10:1)
25 °C, 4h

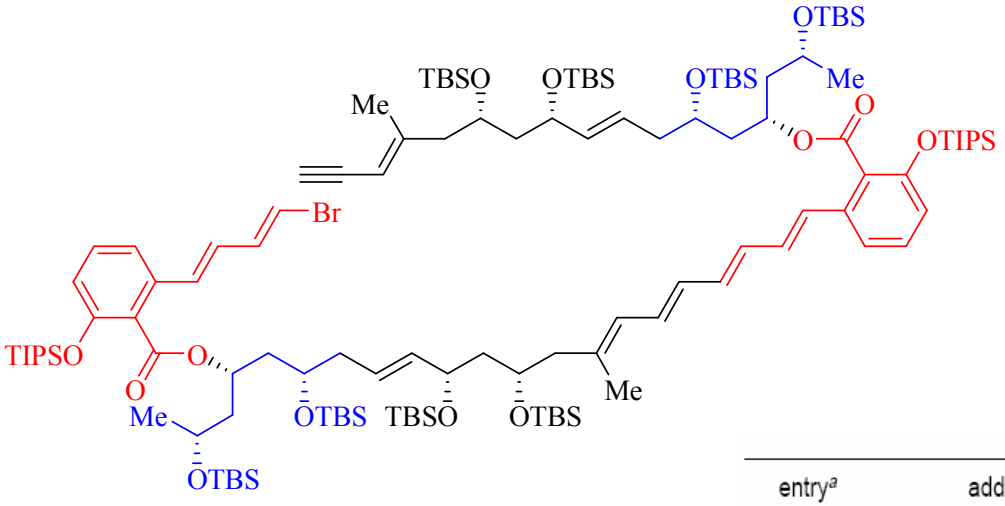
57: R = TIPS

TBAF, THF
25 °C, 18h
23% (over three steps)



1

Attempts To Improve the Yield of Marinomycin (1)

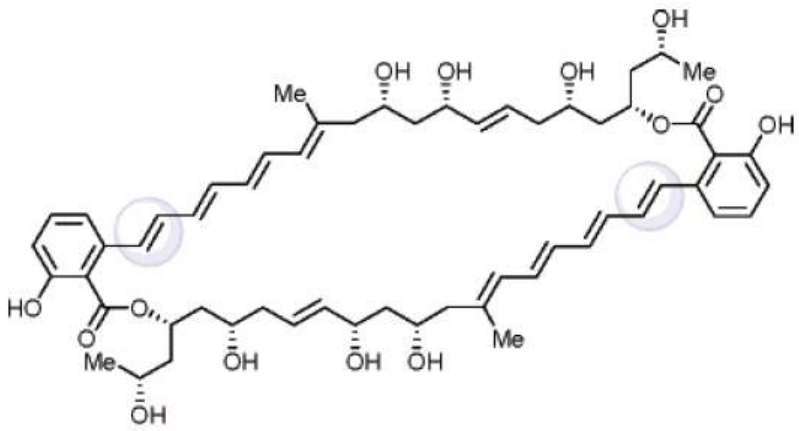


1. Cy_2BH, catecholborane then H_2O
2. conditions (see table)
3. TBAF

→ **1**

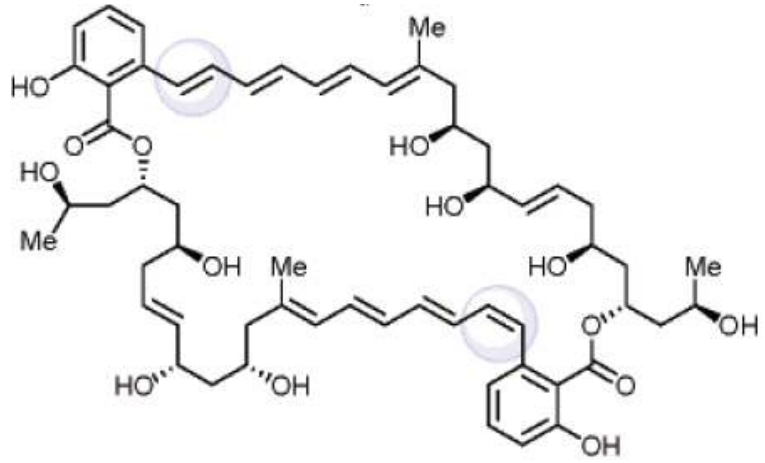
entry[a]	additive(s)	solvent(s)	yield (%)[b]
1	TlOEt	THF/H_2O (10:1)	23
2	Cs_2CO_3	THF/H_2O (2:1)	trace[c]
3	Me_3SnOH	THF/H_2O (2:1)	trace[c]
4	KOH	THF/H_2O (2:1)	trace[c]
5	KOAc	toluene	NR[d]
6	K_3PO_4	DME	5
7	*t*-BuOK	DME/*t*-BuOH (20:1)	17
8	Ag_2O, *t*-BuOK	DME/*t*-BuOH (20:1)	15
9	Ag_2O	THF	4
10	Ag_2O	DME	9
11	Ag_2O, K_2CO_3	DME	20

[a] All reactions were carried out on 1.0 mmol scale and under $Pd(PPh_3)_4$ catalysis, except for entry 6, in which $Pd_2(dba)_3$ was used instead. [b] Yields of **1** are over three steps, from **56**. [c] Trace product was observed by TLC analysis. [d] No reaction.



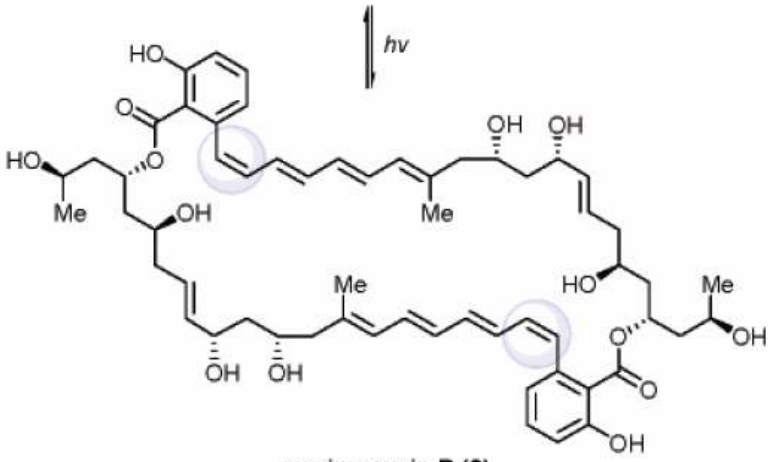
marinomycin A (1)

hv



marinomycin C (3)

hv



marinomycin B (2)

entry	time (min)	Ratio 1/2/3[a]
1	0	19:0:1
2	30	12.3:1:5.8
3	60	3.6:1:3.0
4	90	1.7:1:2.0
5	120	1.1:1:1.5

[a] Marinomycin A (**1**, 1.0 mg), after an optical rotation, was dissolved in MeOH (2.0 mL) and placed in an open vial under ambient light for 2 h. Small samples were removed every 30 min and analyzed by HPLC using the following conditions: C18-Kromasil 5μ column, 4.6 mm × 150 mm, 100 Å, 10−100% MeCN in H_2O over 20 min, 1.0 mL/min.

Biological Evaluation of the Synthesized Marinomycins

compd	*Staphylococcus aureus* methicillin-resistant (MRSA) MIC_{90} (μM)[a]	*Enterococcus faecium* vancomycin-resistant (VREF) MIC_{90} (μM)[a]	wild type *Candida albicans* MIC_{90} (μM)[b]	human colon carcinoma (HCT-116) IC_{50} (μg/mL)[c]
1	0.13	0.13	10	0.18
m-1	50	NSA[d]	NSA[d]	NSA[d]
m-2	NSA[d]	NSA[d]	NSA[d]	NSA[d]

[a] The optical density (OD) was measured at 600 nm using a Molecular Devices Emax microplate reader, and the MIC_{90} was determined by the analysis program SOFTmax PRO. The MIC_{90} of vancomycin is 0.195−0.391 mg/mL, and that of penicillin G is 6.25−12.5 mg/mL in this test. [b] Alamar blue was used as an indicator to measure cell proliferation. The dye yields a colorimetric change that enables the MIC to be confidently estimated by visual means; the MIC of amphotericin B in this test is 1.56−0.78 mg/mL. [c] The OD was measured at 492 nm using a Molecular Devices Emax microplate reader, and the IC_{50} was determined in a MTS colormetric assay; the IC_{50} of etoposide is 1−2 μg/mL in this test. [d] NSA = not significantly active (MIC_{90} values above 50.0 mM or IC_{50} values above 50 μg/mL).

Conclusion:

- The synthesis of the Marinomycins A-C presents an interesting challenge in synthetic organic chemistry

- The synthetic marinomycin A shows the same biological activity as the naturally occurring one

- The polyunsaturated nature of these molecules makes them particularly sensitive to both chemical and light activation

- The Suzuki reaction was used for macrocyclization in the last step of the synthesis as well as in the polyunsaturated chain synthesis

- Monomeric macrocyclization gave the monomarinomycins A and the structure of one of them is proved by X-ray crystallography

- Two monomarinomycins A show no activity

- Synthetic monomarinomycin A undergoes photoisomerisation to its analogues


That's all Folks!