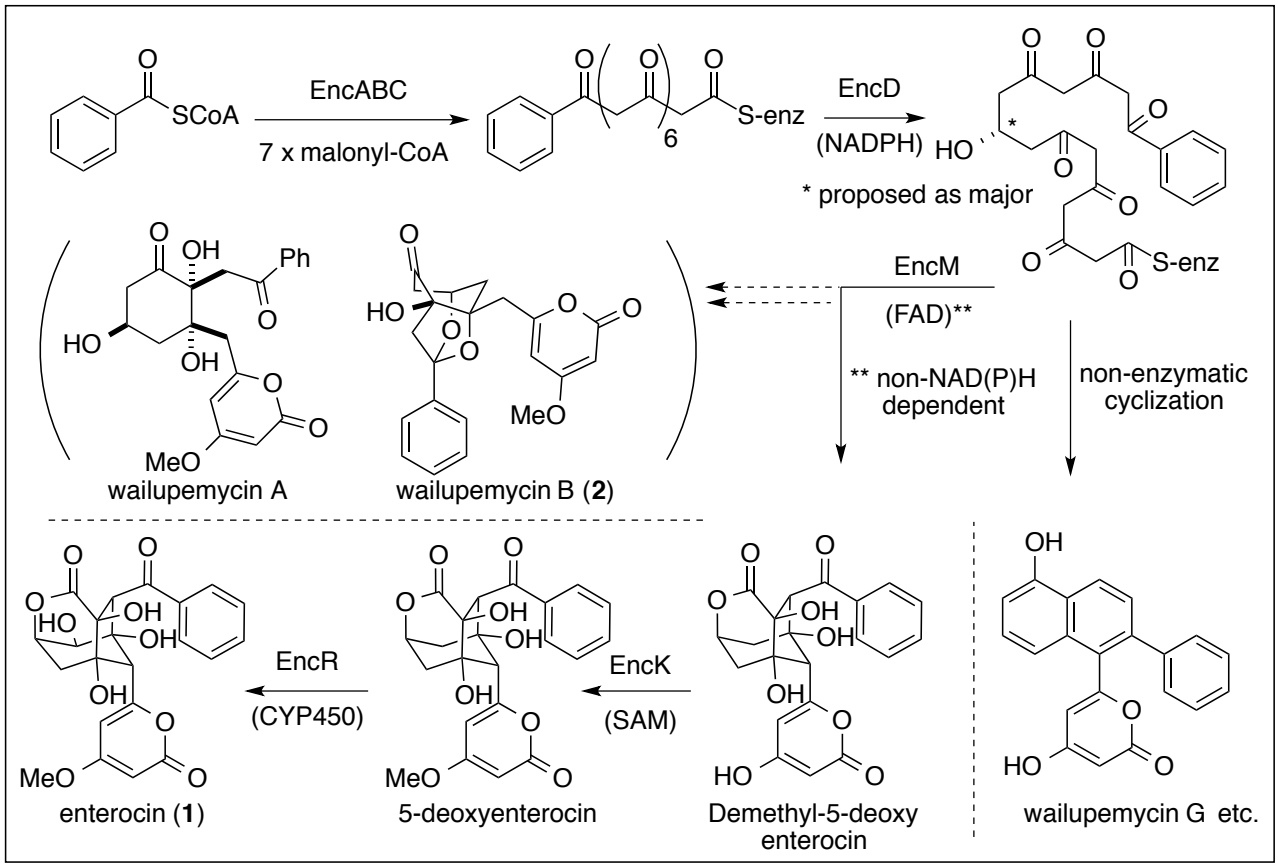
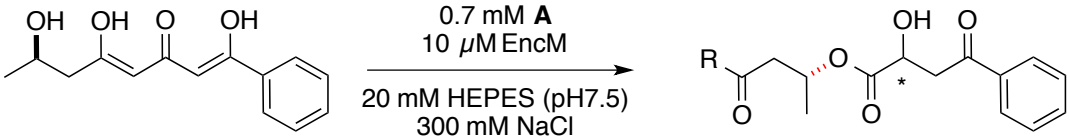
Problem Session (4)

15/04/24 Takuya Kaji

1. Enterocins and wailupemycins are polyketide natural products isolated from *S. maritimus*. Scheme shown below is the enzymatic synthesis of enterocin (**1**) and related polyketides. Consider reaction mechanism for cyclization step mediated by FAD-dependent oxygenase EncM.



cf. Model experiment using synthesized substrate **A**

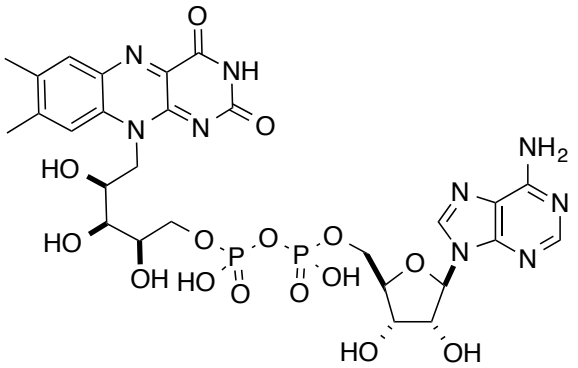


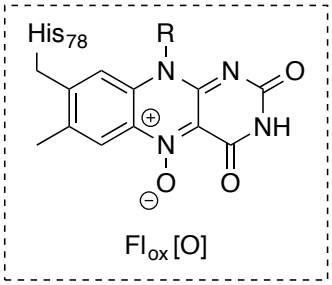
A

B
(R = OH or glycerol)

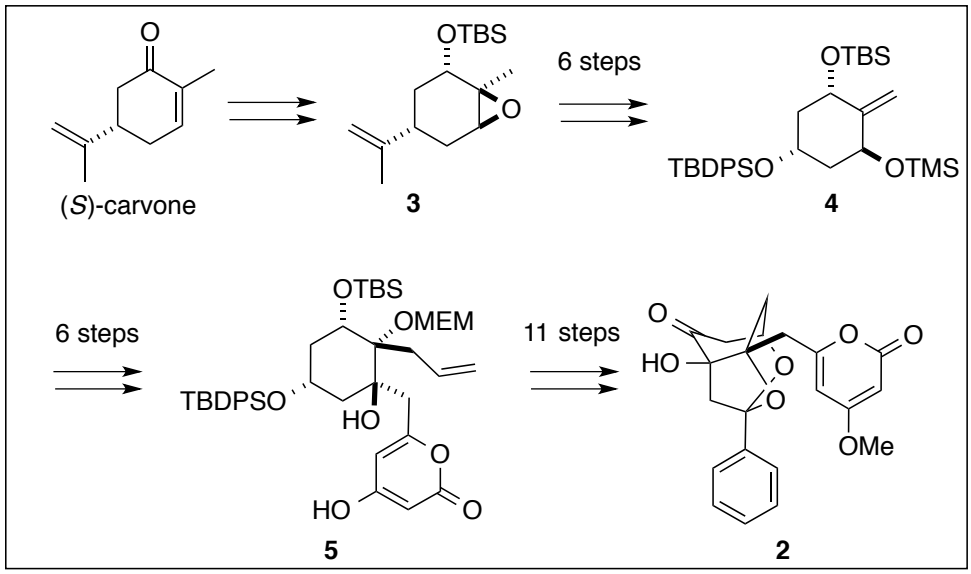
As for EncM, active species of FAD is considered as flavin-N5-oxoammonium shown in the dotted box.

FAD:



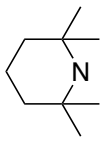


2. Scheme shown below is the total synthesis of (+)-wailupemycin B (**2**).
Rearrange reaction conditions (**a-f**, **g-l**, **m-w**) in appropriate order for 1) **3** to **4**, 2) **4** to **5**, and 3) **5** to **2**.



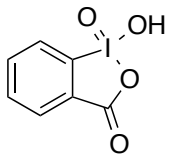
1)
- **a:** TBDPSCl, imidazole, DMF
- **b:** K_2CO_3, MeOH
- **c:** O_3, $NaHCO_3$, CH_2Cl_2-MeOH (5:1 v/v)
- **d:** Et_2AlCl, LiTMP, benzene
- **e:** TMSCl, imidazole, DMF
- **f:** Ac_2O, Et_3N, DMAP, CH_2Cl_2

TMP:



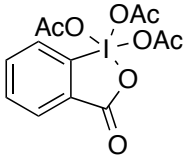
2)
- **g:** MEMCl, EtN*i*-Pr_2, DCE
- **h:** K_2CO_3, MeOH
- **i:** O_3, CH_2Cl_2-MeOH (5:1 v/v); Me_2S
- **j:** IBX, DMSO
- **k:** CH_2CHCH_2MgBr, THF
- **l:** 4-hydroxy-6-methylpyrane-2-one, *t*-BuLi, THF

IBX:

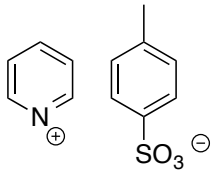


3)
- **m:** Me_2SO_4, K_2CO_3, acetone
- **n:** IBX, EtOAc, 77 ºC
- **o:** $NaIO_4$, OsO_4, THF-H_2O
- **p:** HF•py, THF
- **q:** PhMgBr, THF
- **r:** TFA, HOAc, THF-H_2O
- **s:** DMP, $NaHCO_3$, CH_2Cl_2
- **t:** IBX, DMSO, rt
- **u:** L-selectride, THF
- **v:** TBAF, THF
- **w:** 2-methoxypropene, PPTS, DCE

DMP:



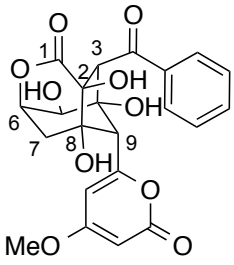
PPTS:



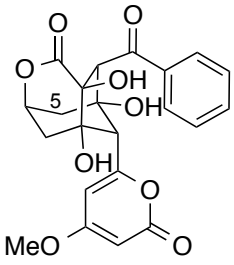
Problem Session (4) Answer

15/04/24 Takuya Kaji

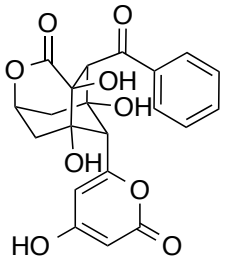
Enterocins: Unique polyketide natural products isolated from *S. maritimus*.



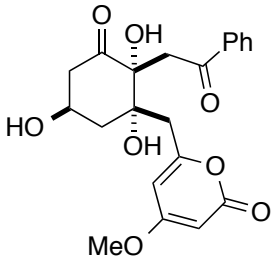
enterocin (**1**)



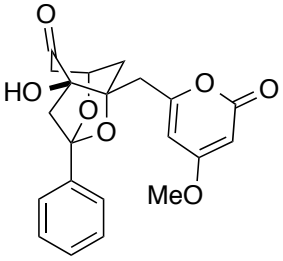
5-deoxyenterocin



Demethyl-5-deoxy enterocin



wailupemycin A



wailupemycin B (**2**)

43 mg	19 mg		4 mg	7 mg / 10 L
460 mg	685 mg		7 mg	- / 40 L

antibacterial
gram positive: 10 μg/mL (*S. aureus*)
gram negative: 4 μg/mL (*E. coli*)

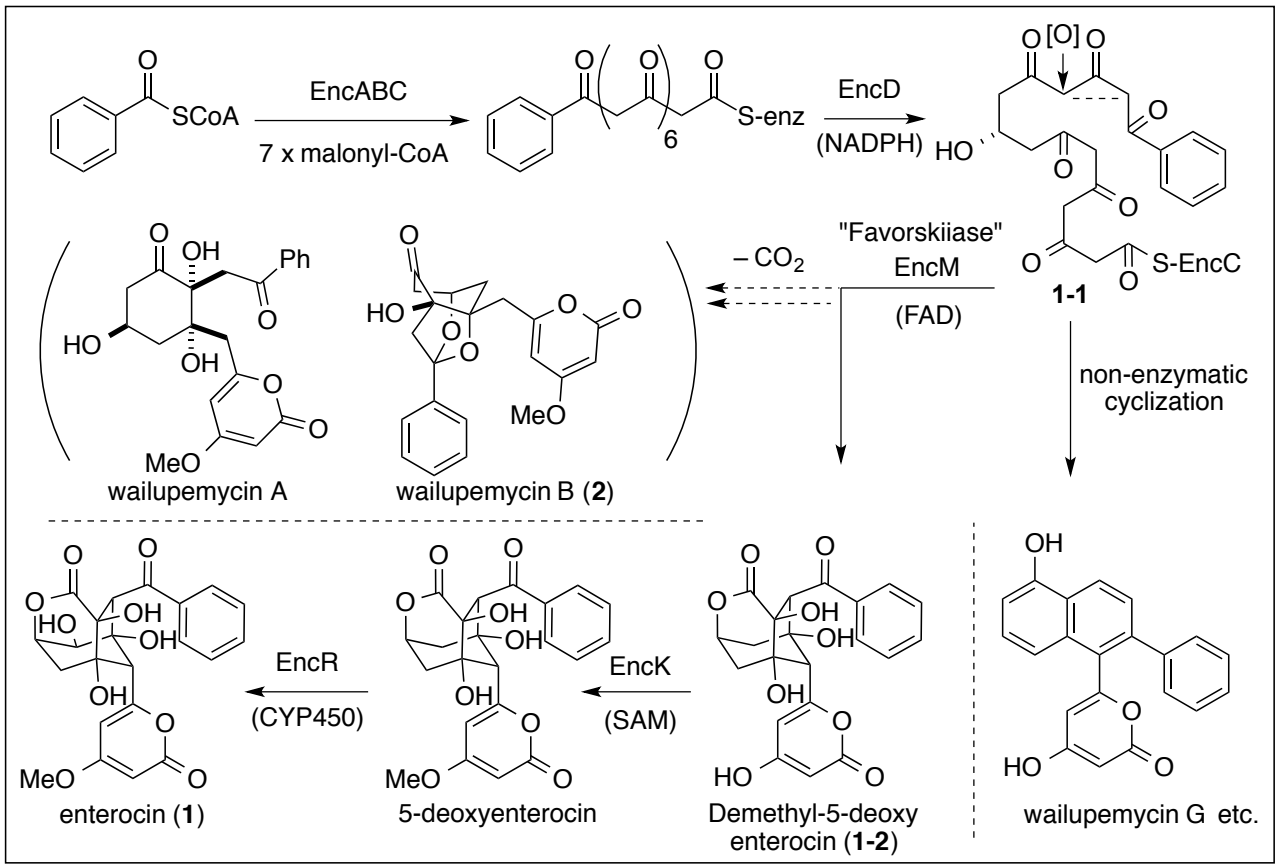
No antibacterial activity

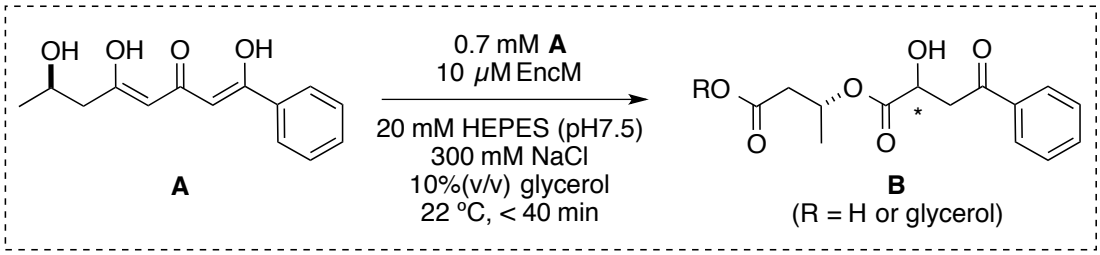
Sitachitta, N.; Gadepalli, M.; Davidson, B. S. *Tetrahedron* **1996**, *52*, 8073.

Biosynthesis of enterocin (1) (problem 1)

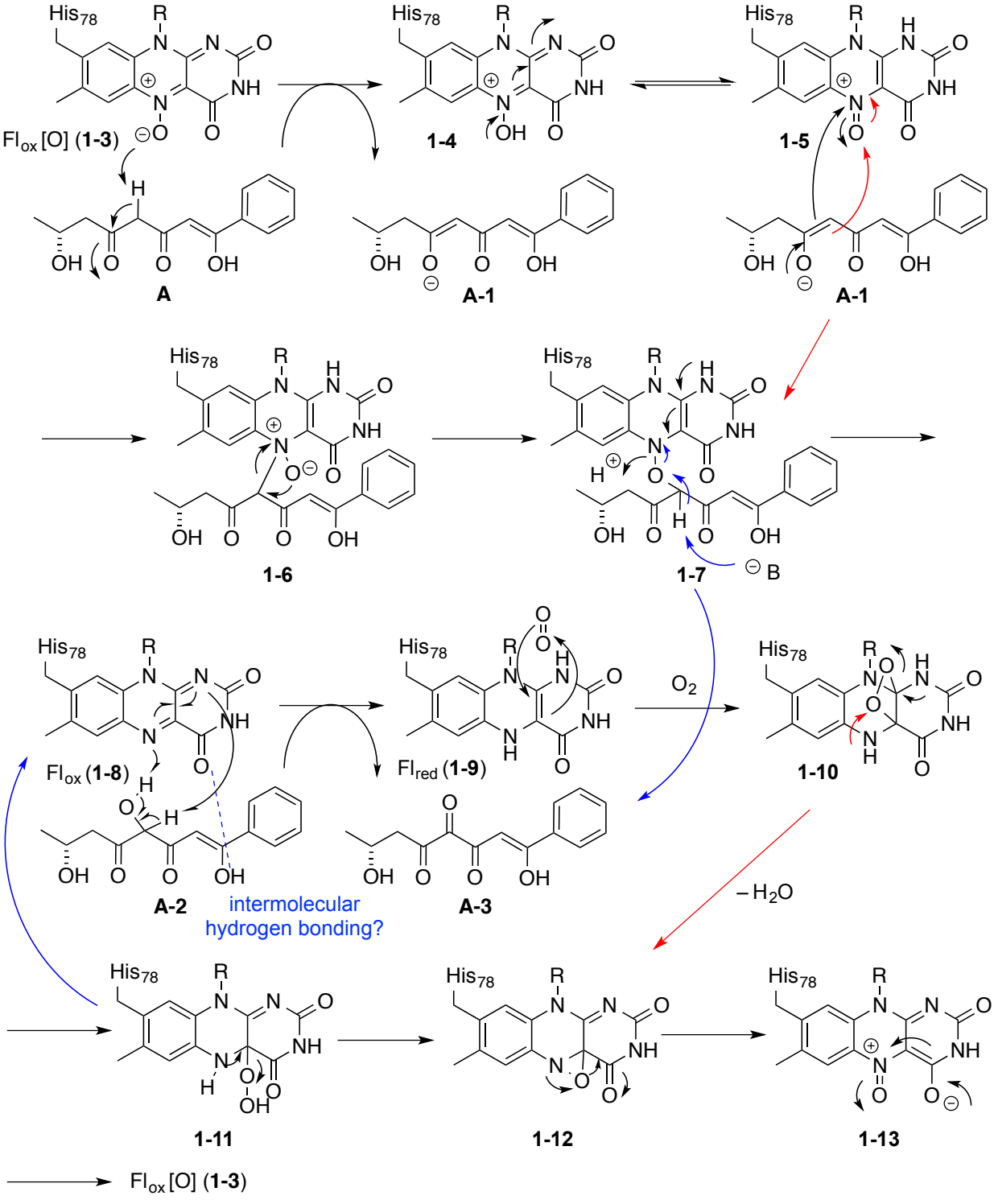
Piel, J.; Hoang, K.; Moore, B. S. *J. Am. Chem. Soc.* **2000**, *122*, 5415. "Discovery of gene cluster"
Xiang, L.; Kalaitzis, J. A.; Moore, B. S. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15609.
Cheng, Q.; Xiang, L.; Izumikawa, M.; Meluzzi, D.; Moore, B. S. *Nat. Chem. Biol.* **2007**, *3*, 557. "Total biosynthesis"
Teufel, R.; Miyanaga, A.; Michaudel, Q.; Stull, F.; Louie, G.; Noel, J. P.; Baran, P. S.; Palfey, B.; Moore, B. S. *Nature* **2013**, *503*, 552. "In vitro mechanistic analysis of EncM"

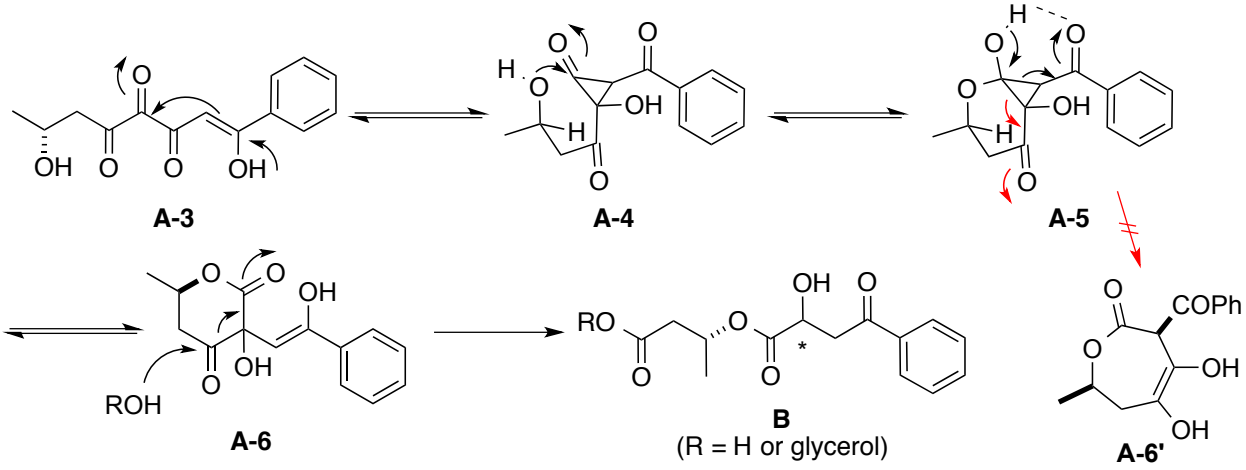
Paradime of modern biosynthetic study: Unique enzyme makes unique structure

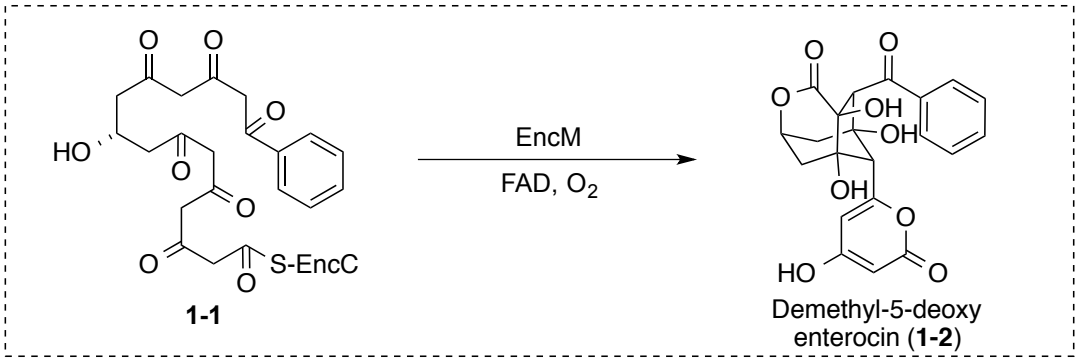


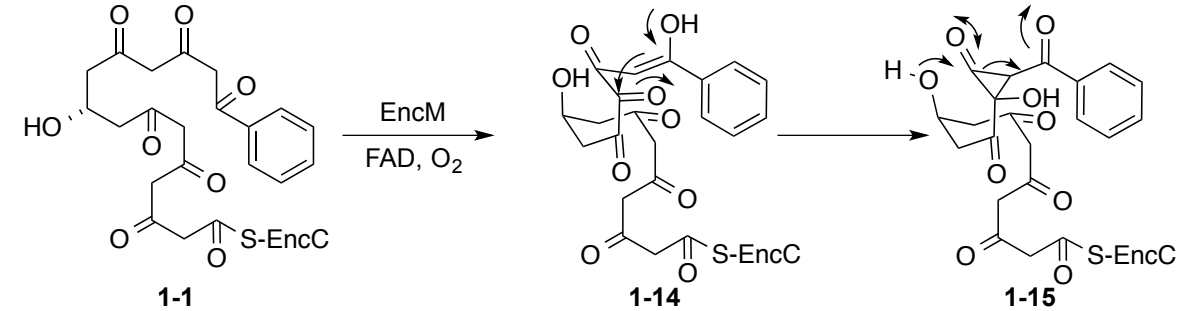


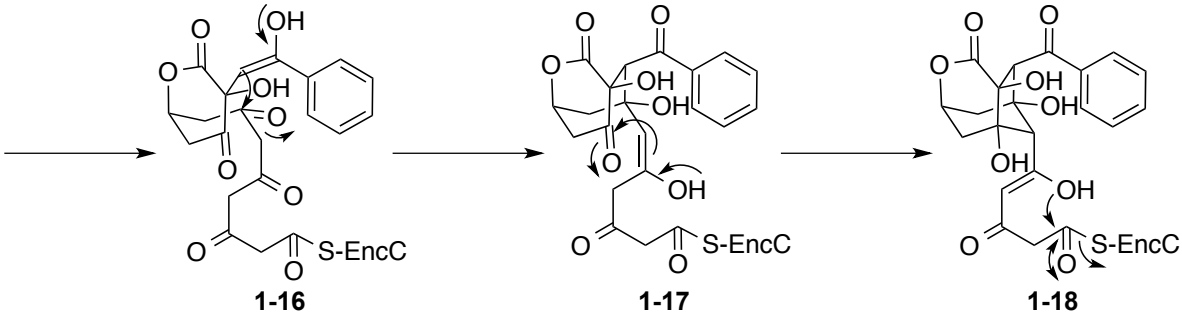
Proposed mechanism:

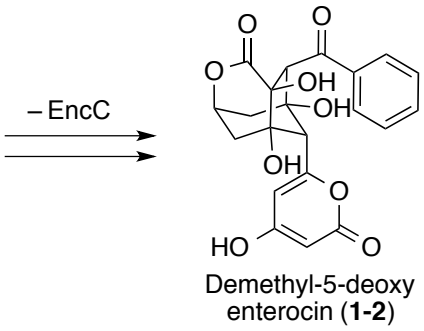



A-3
A-4
A-5
A-6
ROH
B
(R = H or glycerol)
A-6'
COPh


EncM
FAD, O2
S-EncC
1-1
Demethyl-5-deoxy
enterocin (1-2)

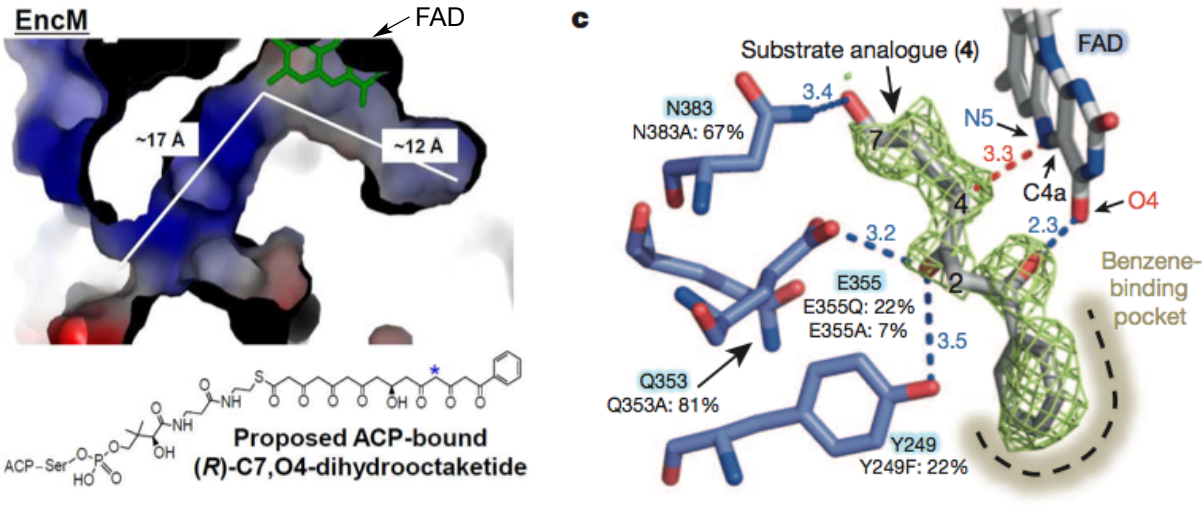

EncM
FAD, O2
S-EncC
1-1
1-14
1-15


S-EncC
1-16
1-17
1-18

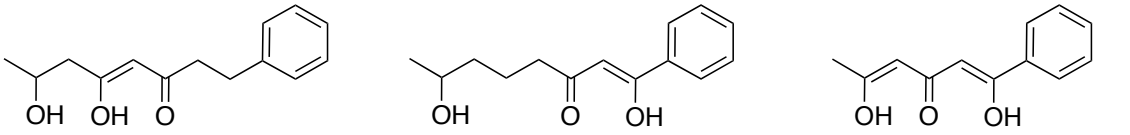

– EncC
Demethyl-5-deoxy
enterocin (1-2)

Discussion:

1. Substrate binding pocket of EncM and binding of model substrate **A**

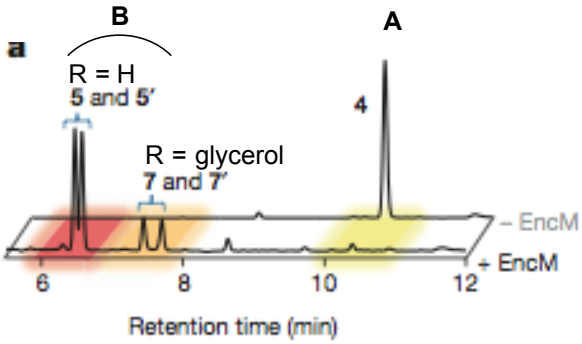


SAR of model substrate for EncM

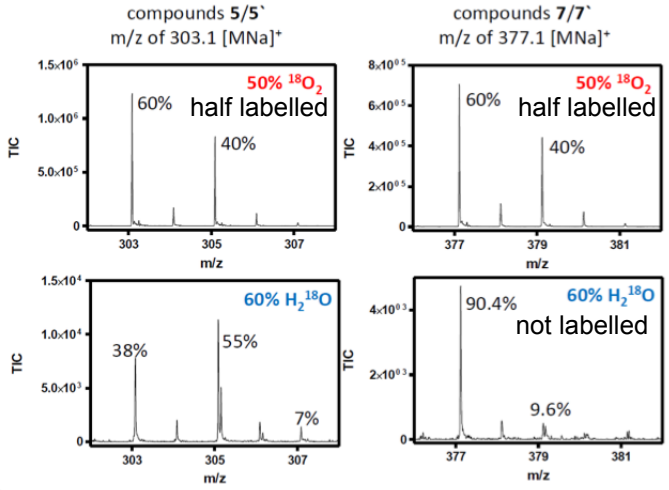


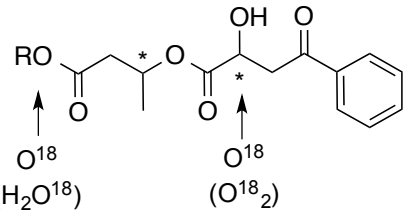
2. Supporting data for mechanism of model experiment

HPLC analysis of model experiment

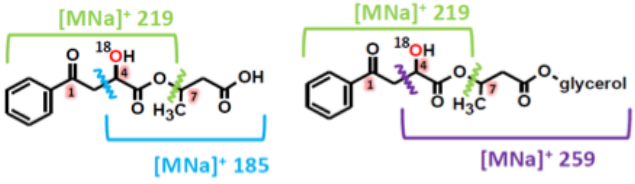


Isotope labelling study for conversion of **A** to **B**

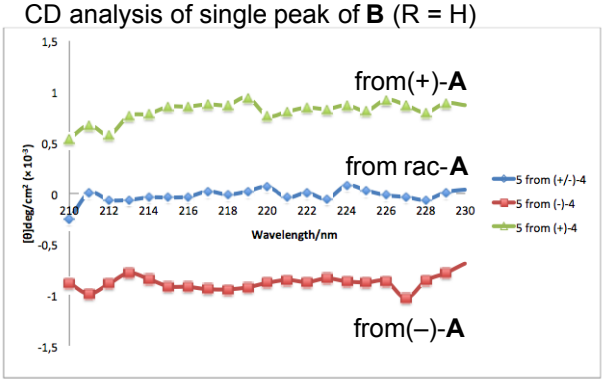




B (R = H or glycerol)



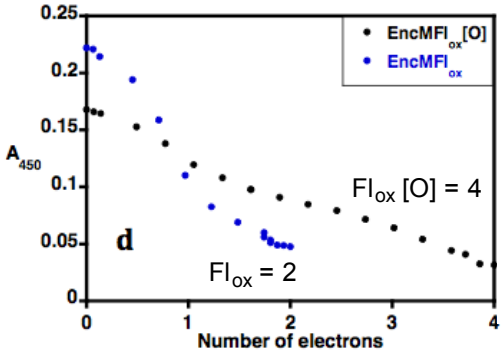
CD analysis of single peak of **B** (R = H)



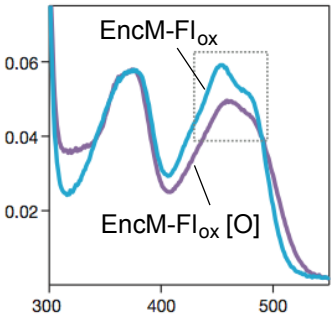
Supplementary Figure 25. Normalized circular dichroism spectrum for **5**, enzymatically prepared from (+/–), (–) and (+)-**4**.

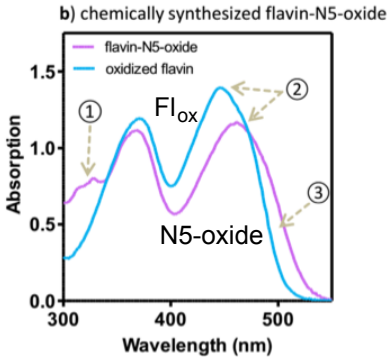
3. Supporting data for FAD_{ox} [O] (**1-3**)

Overlay of Abs_{450nm} against N of electron added relative for EncM concentration.

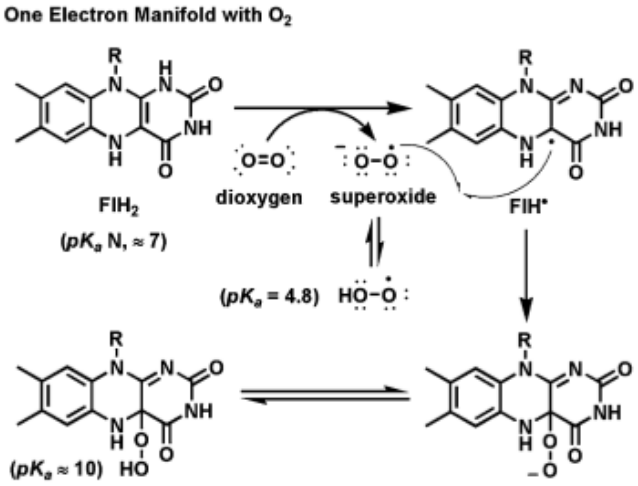


UV-Vis spectra of EncM





C4a-peroxide mechanism of normal Fl_{red}



HOMO electron density distribution of Fl_{ox}

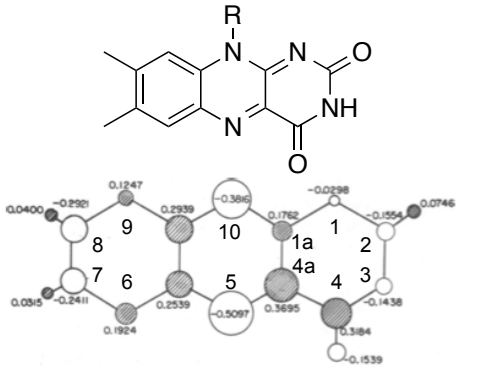
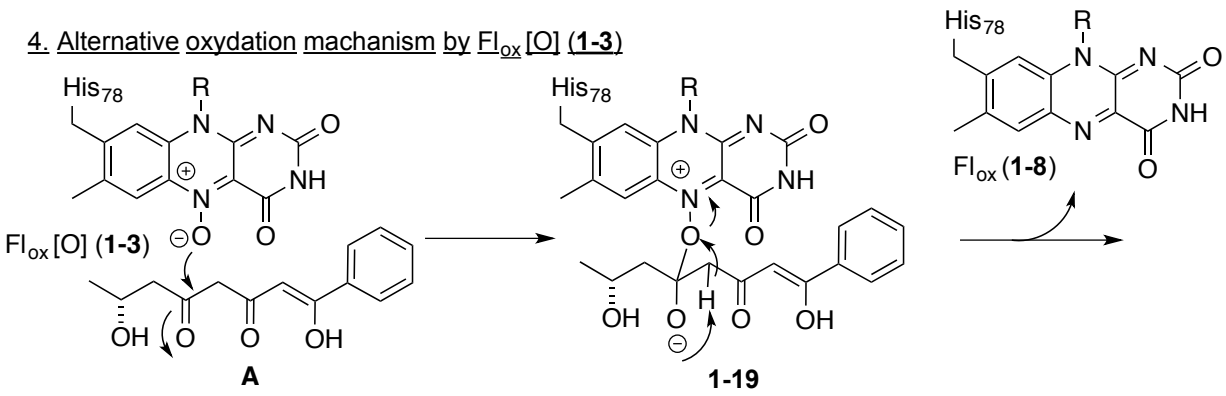


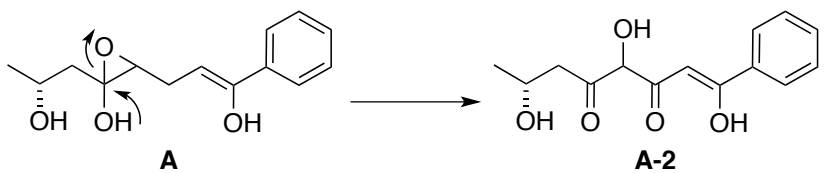
Fig. 2. Results of extended Hückel calculations on reduced isoalloxazine.

Orf, H.W.; Dolphin, D.
Proc. Nat. Acad. Sci. USA **1974**, *71*, 2646.

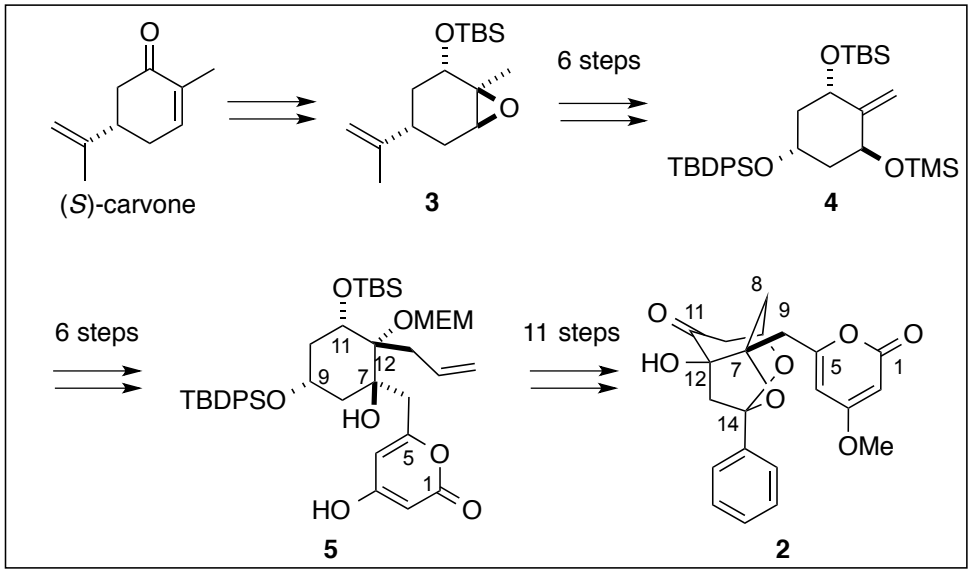
Review of flavoenzyme:
Walsh, C. T.; Wencewicz, T. A. *Nat. Prod. Rep.* **2013**, *30*, 175.

4. Alternative oxydation machanism by Fl_{ox} [O] (**1-3**)





Total synthesis of wailupemycin B (2) (problem 2)
Kirsch, S.; Bach, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 4685.
Kirsch, S. F.; Bach, T. *Chem. Eur. J.* **2005**, *11*, 7007.
Ref. of synthesis of **4**: Hatakeyama, S.; Numata, H.; Osanai, K.; Takano, S. *J. Org. Chem.* **1989**, *54*, 3515.

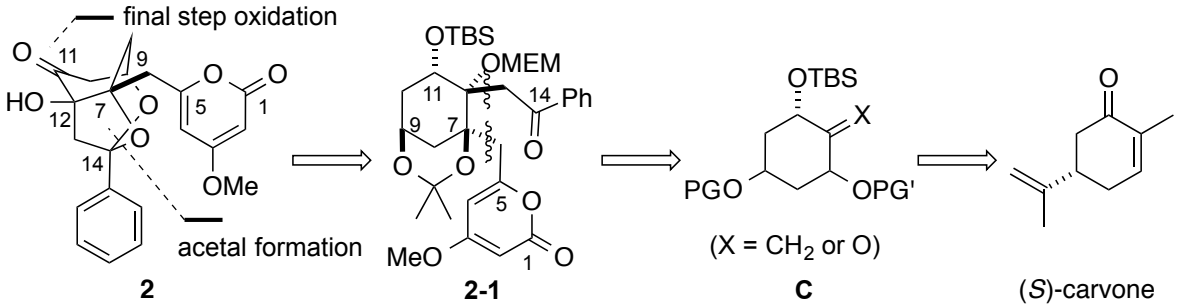


1) **c:** O_3, $NaHCO_3$, CH_2Cl_2-MeOH (5:1 v/v), –68 ºC
f: Ac_2O, Et_3N, DMAP, CH_2Cl_2, reflux
b: K_2CO_3, MeOH
a: TBDPSCl, imidazole, DMF 66% (4 steps)
d: Et_2AlCl, LiTMP, benzene, 0 ºC, 96%
e: TMSCl, imidazole, DMF

2) **i:** O_3, CH_2Cl_2-MeOH (5:1 v/v), –78 ºC; Me_2S, –78 ºC to rt, quant.
k: CH_2CHCH_2MgBr, THF, –20 ºC, quant. (d.r. = 90:10)
g: MEMCl, EtN*i*-Pr_2, DCE, 70 ºC, 80% (d.r. = > 95:5)
h: K_2CO_3, MeOH, 95%
j: IBX, DMSO, 98%
l: 4-hydroxy-6-methylpyrane-2-one (2.5 eq), *t*-BuLi (5.3 eq), THF, –85 ºC to 0 ºC; then **2-24**, –85 ºC, 82%

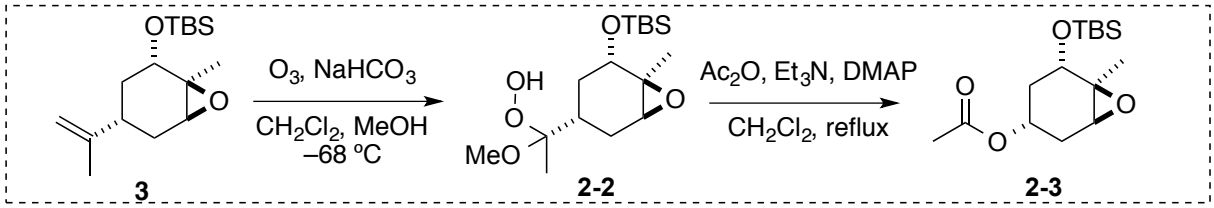
3) **m:** Me_2SO_4, K_2CO_3, acetone, 92% (d.r. = > 95:5)
p: HF•py, THF, 0 ºC to rt, 94%
t: IBX, DMSO, rt, 96%
u: L-selectride, THF, –78 ºC, 97%
w: 2-methoxypropene, PPTS, DCE, 90%
o: $NaIO_4$ (2.4 eq), OsO_4 (0.2 eq), NaOAc, THF-H_2O, 83%
q: PhMgBr, THF, –78 ºC, 89%
s: DMP, $NaHCO_3$, CH_2Cl_2, 91%
r: TFA, HOAc, THF-H_2O (1:2:2:4 v/v), 69%
v: TBAF, THF, 0 ºC
n: IBX, EtOAc, 77 ºC, 70% (2 steps)

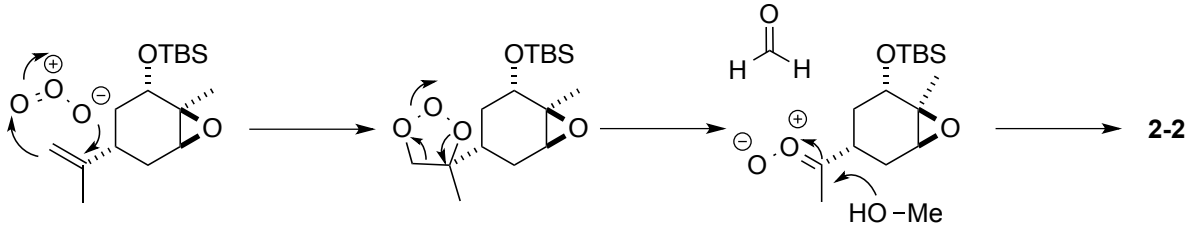
Retrosynthetic analysis of 2:



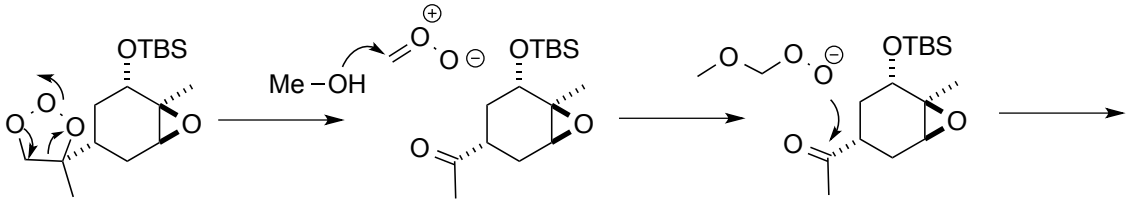
Proposed reaction mechanism:

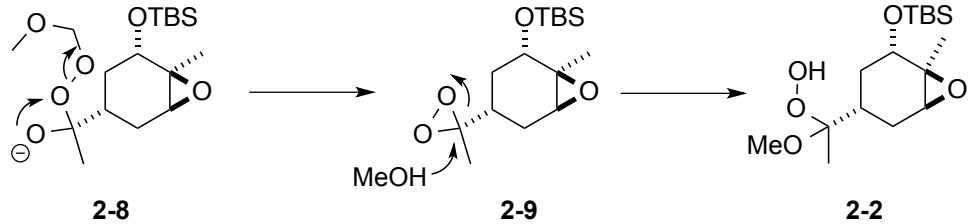
(1) **3** to **4:** Criegee rearrangement ± allyl alcohol formation

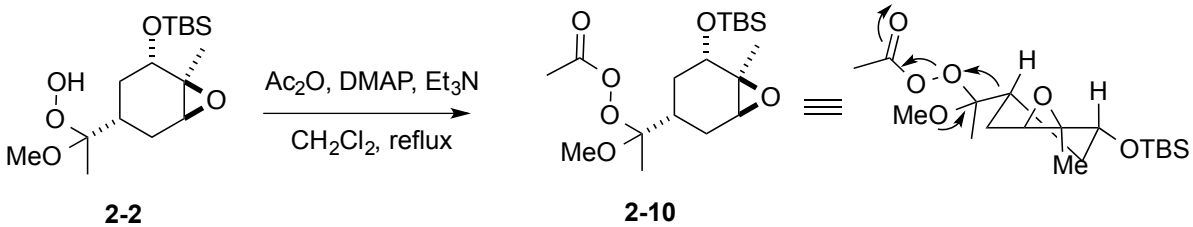


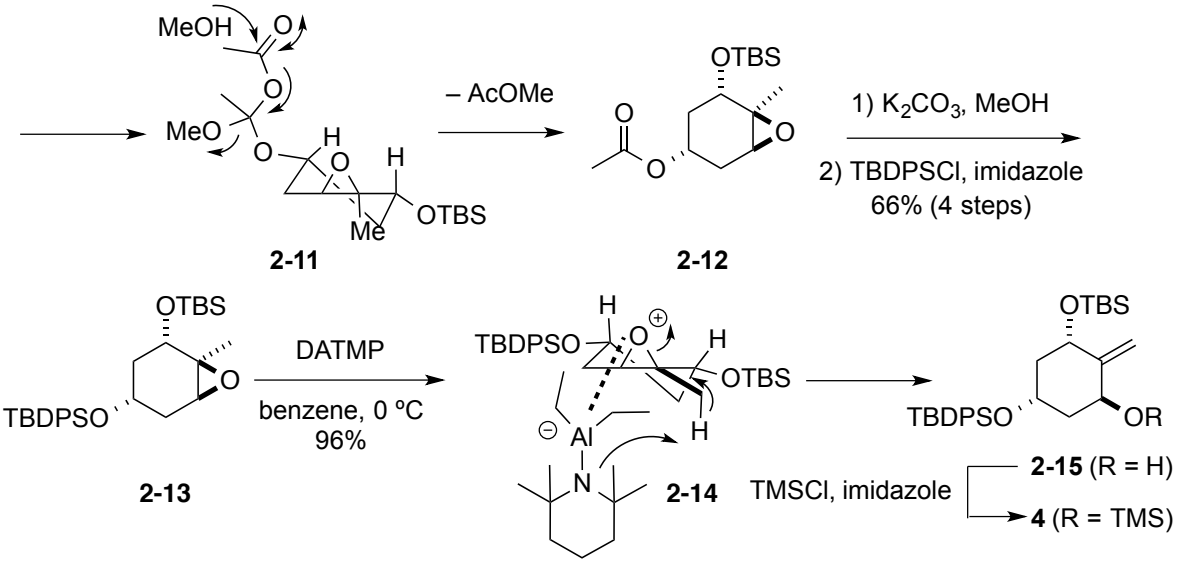


or

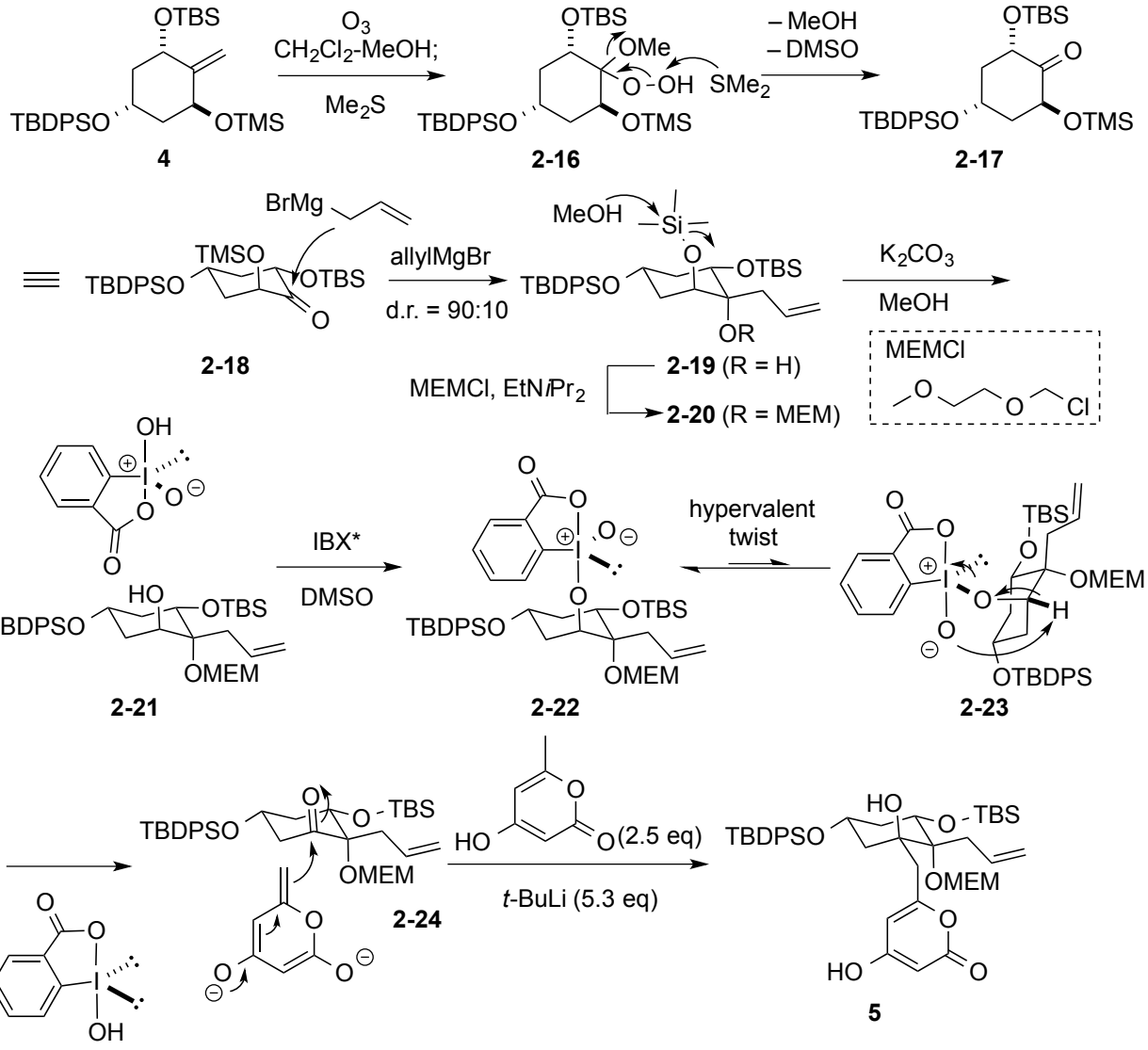






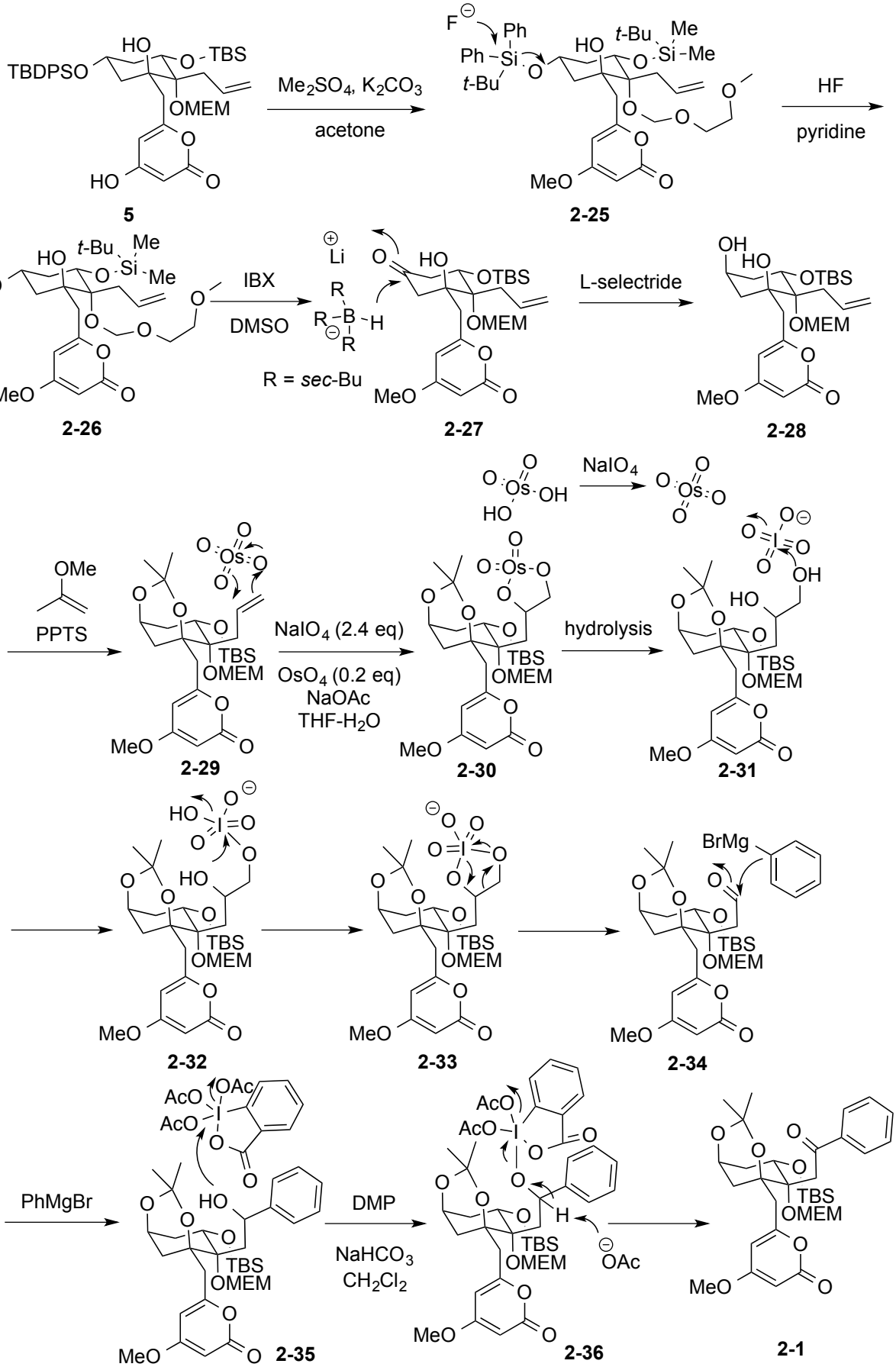


(2) **4** to **5**: Sequential stereoselective introduction of the peripheral substituents

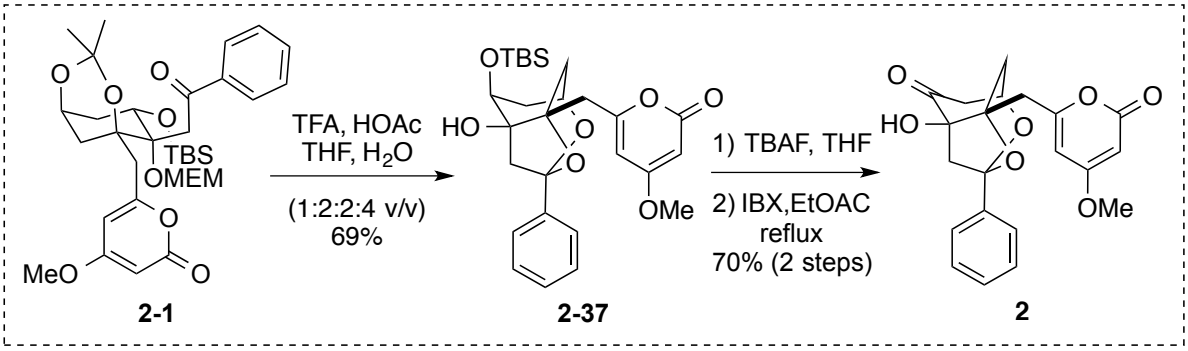


* For more about IBX, see also Todoroki-san's PS on 130216

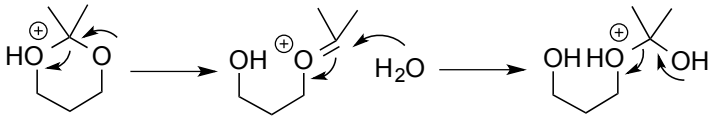
(3) **5** to **2**: Inversion of C9-OH + formation of PhCO + acetal formation + last step oxidation



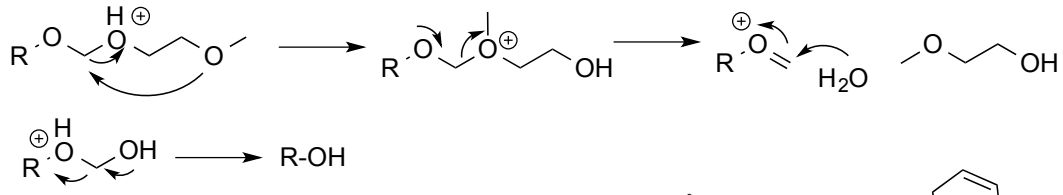
5 **2-25** **2-26** **2-27** **2-28** **2-29** **2-30** **2-31** **2-32** **2-33** **2-34** **2-35** **2-36** **2-1**

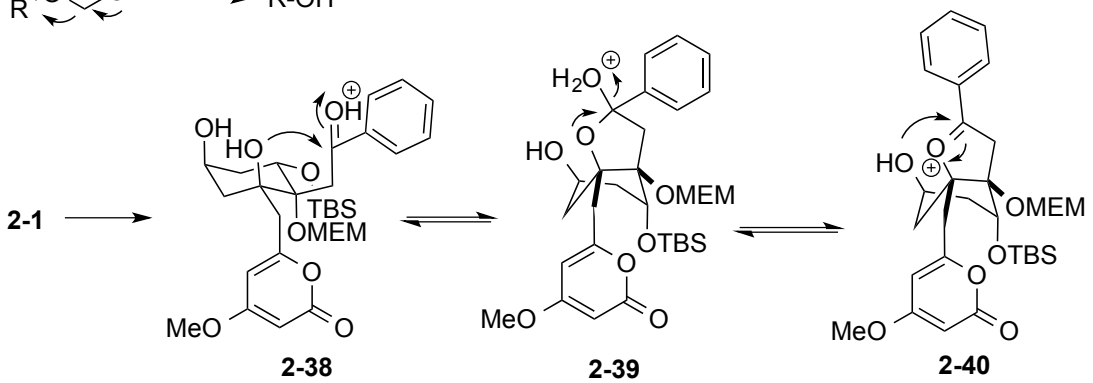


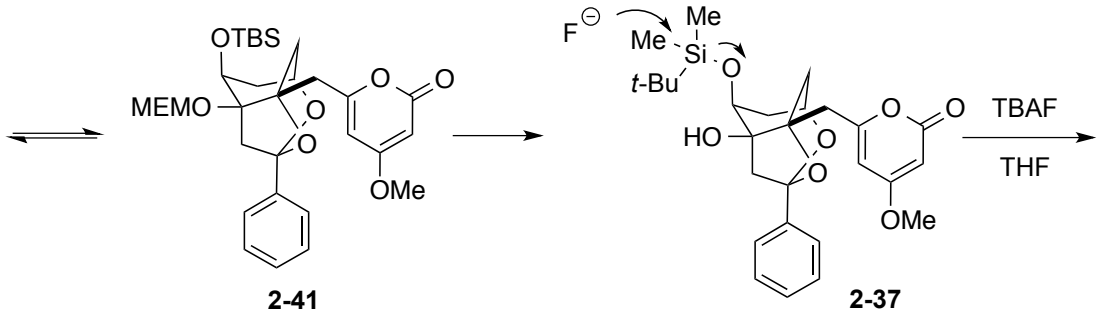
acetal deprotection

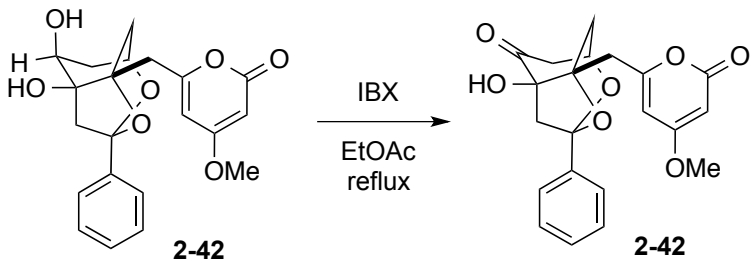


MEM deprotection



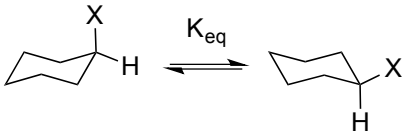






Discussion:

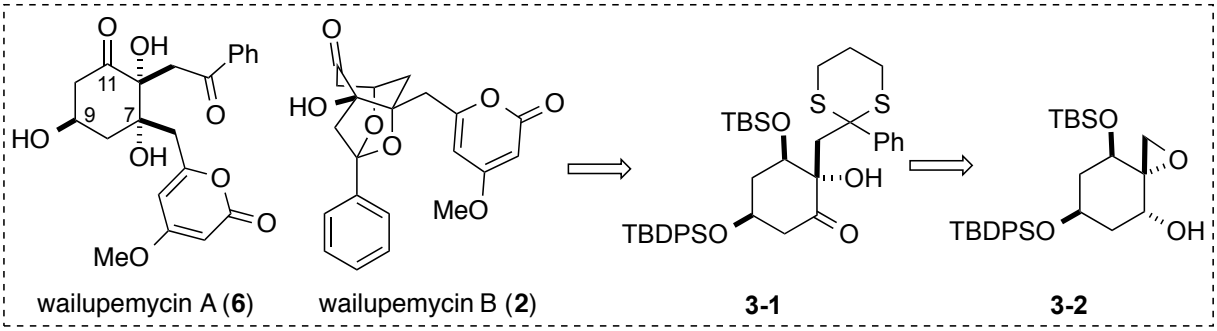
1. A value (–RT log(K_{eq})) of $OSiR_3$ in CH_2Cl_2

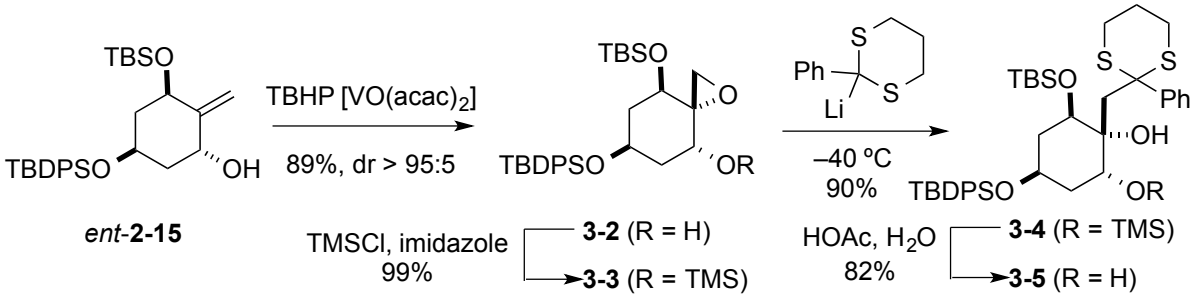


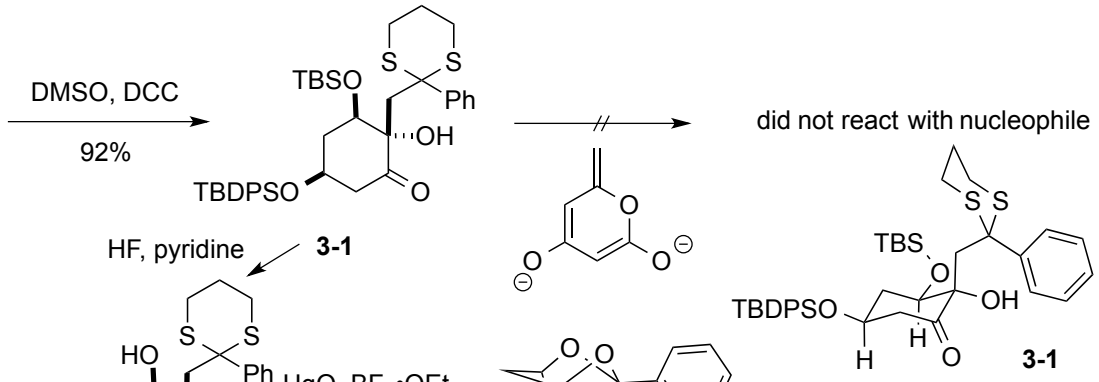
			cf.	
X =	$OSiMe_3$	1.31	Me	1.70
	$OSiMe_2$*t*-Bu	1.06	*t*-Bu	> 4.5
	$OSiPh_2$*t*-Bu	0.56	OMe	0.75

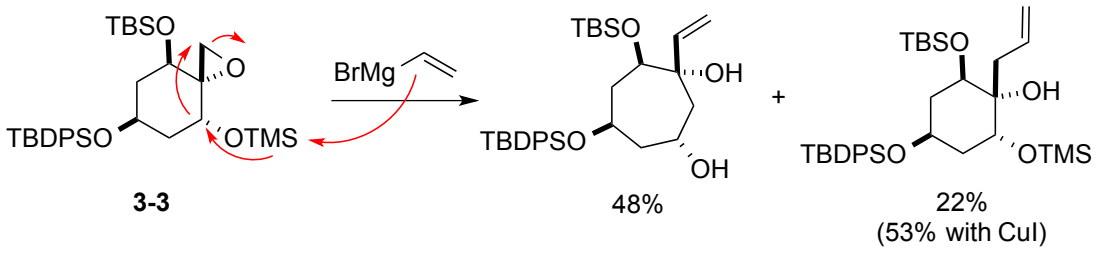
Marzabadi, C. H. et al. *J. Am. Chem. Soc.* **2003**, *125*, 15163.

2. Prior synthetic effort toward **6** / **1**

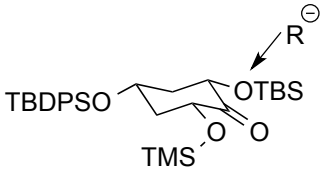


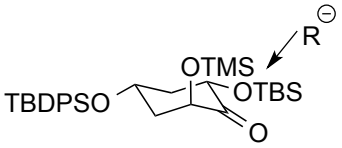


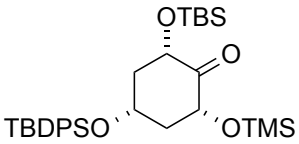
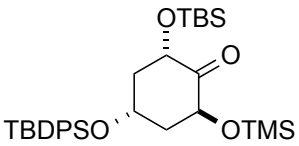




3. Facial diastereoselectivity of a nucleophilic approach to cyclehexanone





	R⊖	yield, d.r.
 11-*epi*-**2-18**	BrMg –20 ºC, THF	75%, 3:1
	Li—≡—Ph –75 ºC, THF	71%, 1:1
 **2-18**	BrMg –20 ºC, THF	quant., 9:1
	Li—≡—Ph –75 ºC, THF	89%, 1:1