



Angiogenesis: Synthetic Emphasis on Fumagillin

Keith Korthals

Literature Presentation

July 1, 2004

- Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.
 - Picoul, W.; Bedel, O.; Haudrechy, A.; Langlois, Y. *Pure Appl Chem* 2003, 75 (2-3), 235-249.
 - Vosburg, D. A.; Weiler, S.; Sorensen, E. J. *Angewandte Chemie, International Edition* 1999, 38 (7), 971-974.
 - Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* 1990, 348 (6301), 555-7.
 - Corey, E. J.; Snider, B. B. *Journal of the American Chemical Society* 1972, 94 (7), 2549-&.
-



Overview

- Story of Angiogenesis
 - First Total Synthesis Fumagillin 1972
 - Recent Total Synthesis 1999
 - Synthesis of Analogs 2004
 - IC_{50} of the Analogs
-



What is Angiogenesis?

- Simply, this is the process of new blood vessel growth.
 - It is important in fetal development, cancer, diabetic retinopathy.
-



Current State of Drug Development

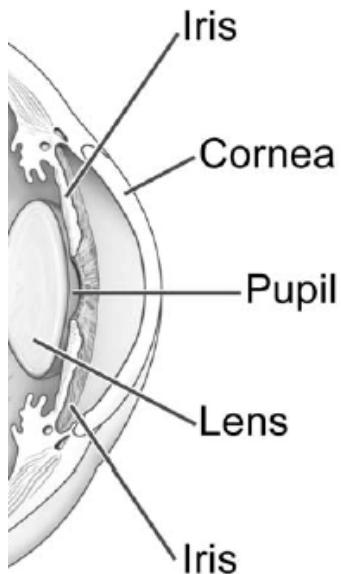
- The first angiogenesis-stimulating medicine is a prescription gel called Regranex (Ortho-McNeil Pharmaceuticals) that became FDA-approved to heal diabetic foot ulcers in December 1997.
 - Currently no FDA approved angiogenesis inhibitor
 - Many current clinical trials (~60).
-



How was the concept of Angiogenesis Inhibition Discovered?



Pioneer Work: Dr. Judah Folkman who when removing cancerous tissue made a simple observation that cancer tumors were “hot, bloody, and full of vessels.”



Attached a living tumor to the cornea in rabbits. Within two days blood vessels were observed growing into the cornea to the tumor. When the vessels reached the tumor, it grew 16,000 times its original size. When the tumor was removed, the blood vessels went away.



But what is the mechanism?

- Put your tumor in a blender.
 - Start your column.
 - Lose some hair, turn grey.
 - Ten years later you have a compound. Luckily, it sticks to heparin (anticoagant).
 - Absorbed onto a matrix (slow release pellet) when placed on rabbit eyes, blood vessels grow in.
 - When removed, blood vessels die off.
 - VEGF (Vascular endothelial growth factor-A , -C)
-



How to find an Angiogenesis Inhibitor?

- This was Bob Langer's job.
- Step one: Kill a few cows (1970's).
- Take over some space at Harvard.
- Hire an army of postdocs and scrape out some bone cartilage.
- Get your column and get a chair because you are going to be there a while.
- As luck would have it, a fungus starting growing in a cell culture of capillary cells. The cells withered away from this fungus.
- Breaking all the rules, Dr. Don Ingber cultured them.
- This was *Aspergillus fumigatus* which secreted fumagillin. Analog is in clinical trials (TNP-470).

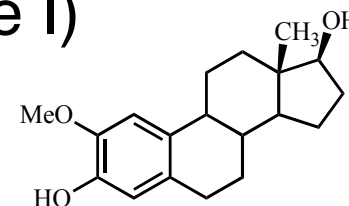
Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* 1990, 348 (6301), 555-7.

McCowen, M. C.; Callender, M.; Lawillis, J.F. *Science*, 1951, 113, 202.



Other Compounds

- Endostatin - cleaved product of the carboxyl-terminal domain of collagen (phase I)
- Angiostatin - large protein fragment (phase I)
- 2-Methoxyestradiol (phase II completed)
- Can be purchased from Aldrich





Diabetic Retinopathy

- Leading cause of blindness
 - Diabetes damages the blood vessels in the retina of the eye
 - **Mild Nonproliferative Retinopathy.** At this earliest stage, small areas of balloon-like swelling in the retina's tiny blood vessels occur.
 - **Moderate Nonproliferative Retinopathy.** As the disease progresses, some blood vessels that nourish the retina are blocked.
 - **Severe Nonproliferative Retinopathy.** Many more blood vessels are blocked, depriving several areas of the retina with their blood supply. These areas of the retina send signals to the body to grow new blood vessels for nourishment.
-



Diabetic Retinopathy

- **Proliferative Retinopathy.**
 - At this advanced stage, the signals sent by the retina for nourishment trigger the growth of new blood vessels. This condition is called proliferative retinopathy.
 - These new blood vessels are abnormal and fragile.
 - They grow along the retina and along the surface of the clear, vitreous gel that fills the inside of the eye.
 - These blood vessels do not cause symptoms or vision loss.
 - However, they have fragile walls. If they leak blood, vision loss and blindness can result.
-



Vision Effects



Normal vision

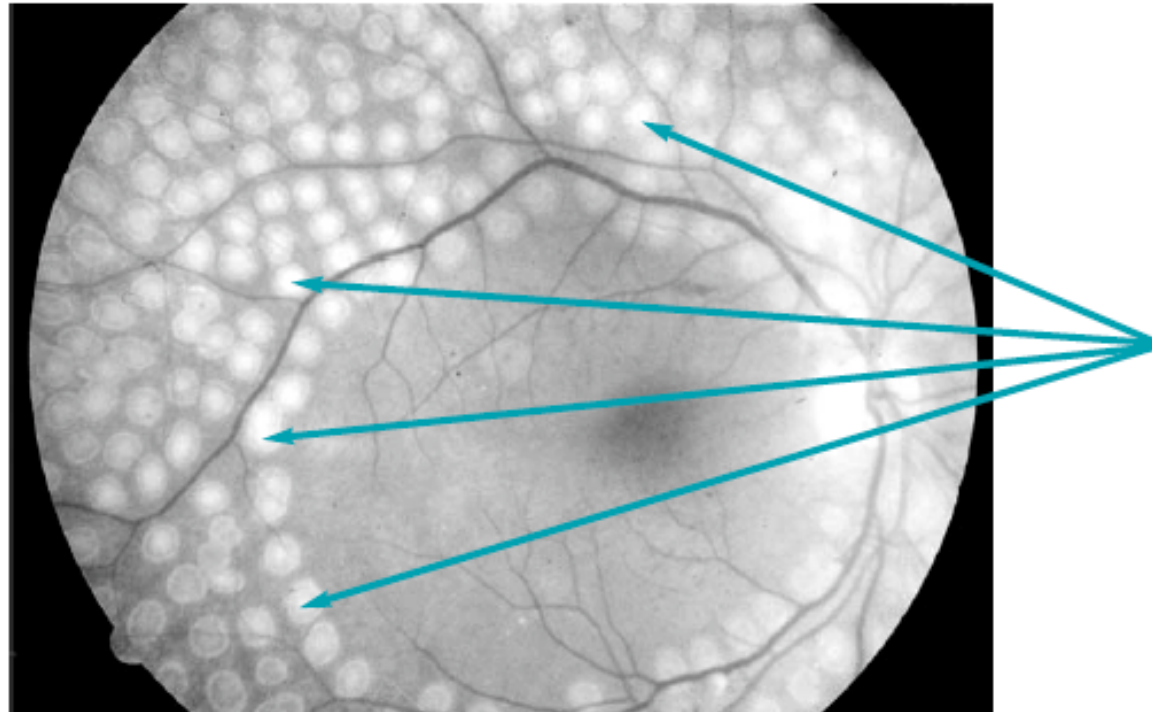


Same scene viewed by a person with diabetic retinopathy



Treatment Methods

- Laser surgery is currently the only treatment

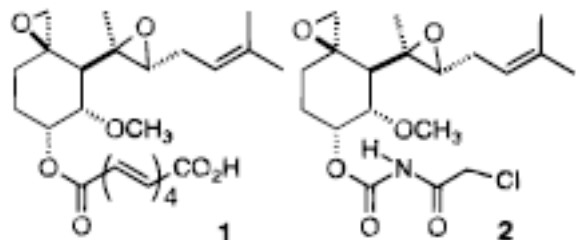


Scatter laser treatment



Fumagillin and Analogs

- Fumagillin first shown to inhibit angiogenesis in ~1990.
- It was known to have anti-cancer properties since 1958.
- First discovered in 1951.
- Used as an antibiotic.
- Analog TNP-470 is currently in clinical trials for cancer treatment as an angiogenesis inhibitor.
- The binding of this compound to an enzyme MetAP-2 (aminopeptidase-2) has an X-ray crystal structure.

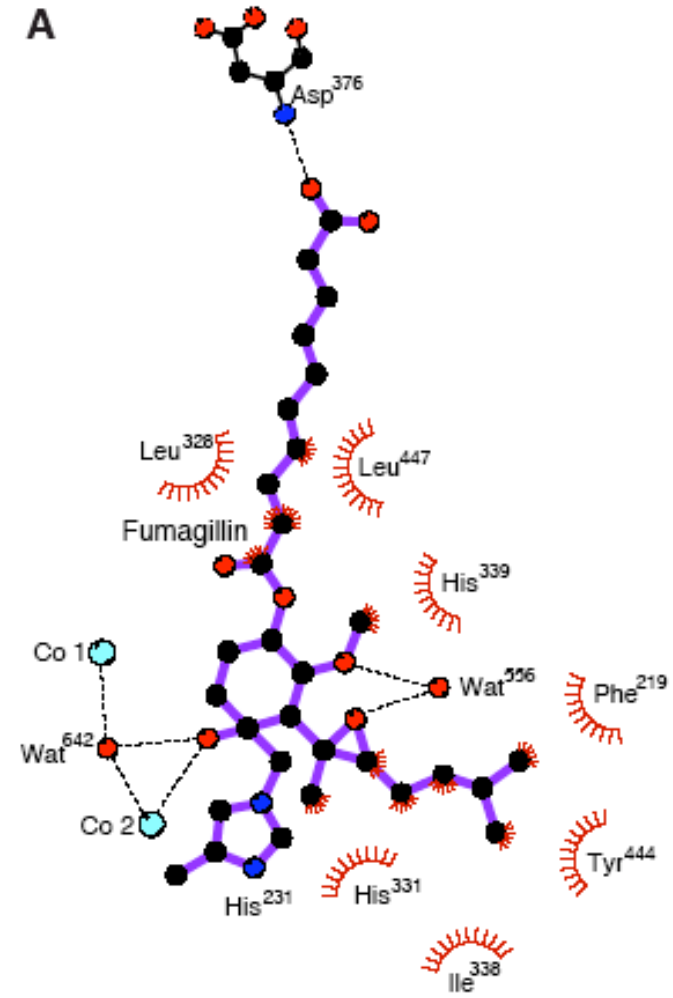
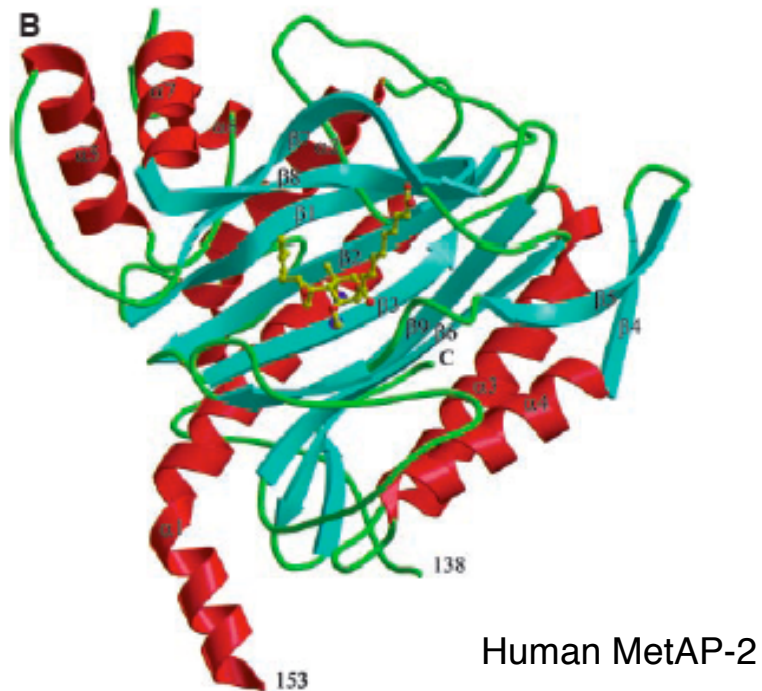


Ingber, D.; Fujita, T.; Kishimoto, S.; Sudo, K.; Kanamaru, T.; Brem, H.; Folkman, J. *Nature* 1990, 348 (6301), 555-7.



Fumagillin and Analogs

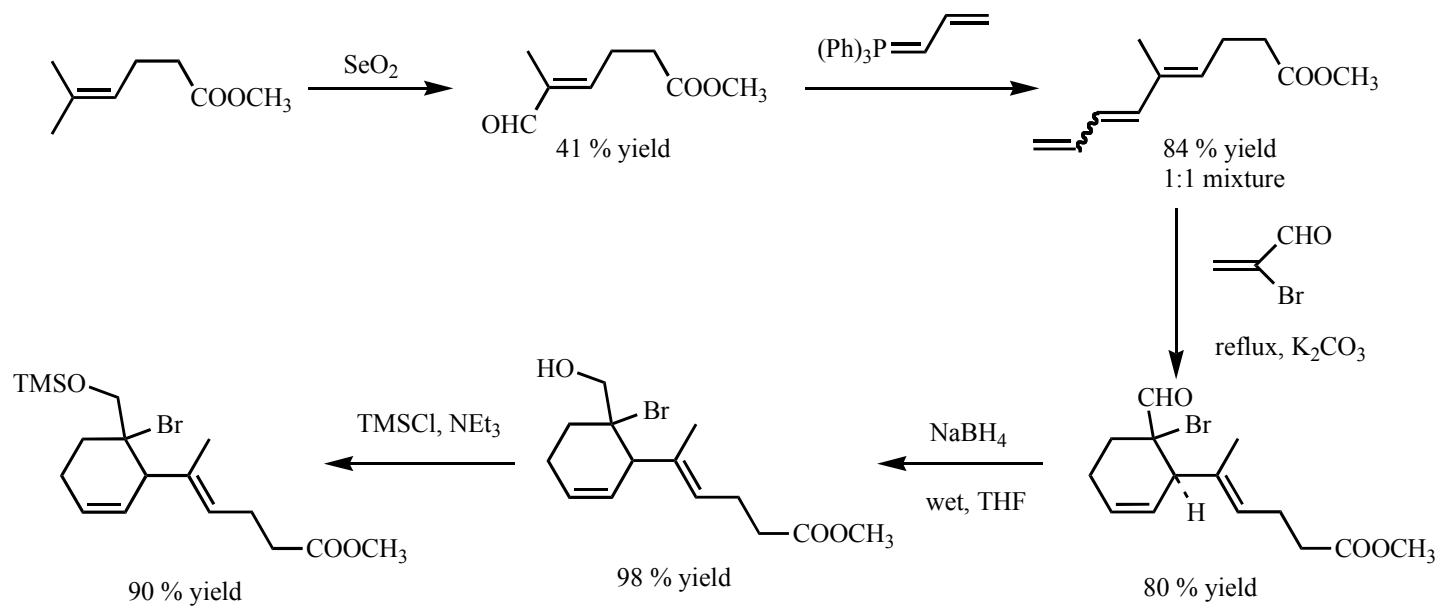
MetAP-2 removes NH₂-terminal methionines from proteins. This promotes the proliferation of endothelial cells (blood vessel cells)



Liu, S. P.; Widom, J.; Kemp, C. W.; Crews, C. M.; Clardy, J. *Science* 1998, 282 (5392), 1324-1327.



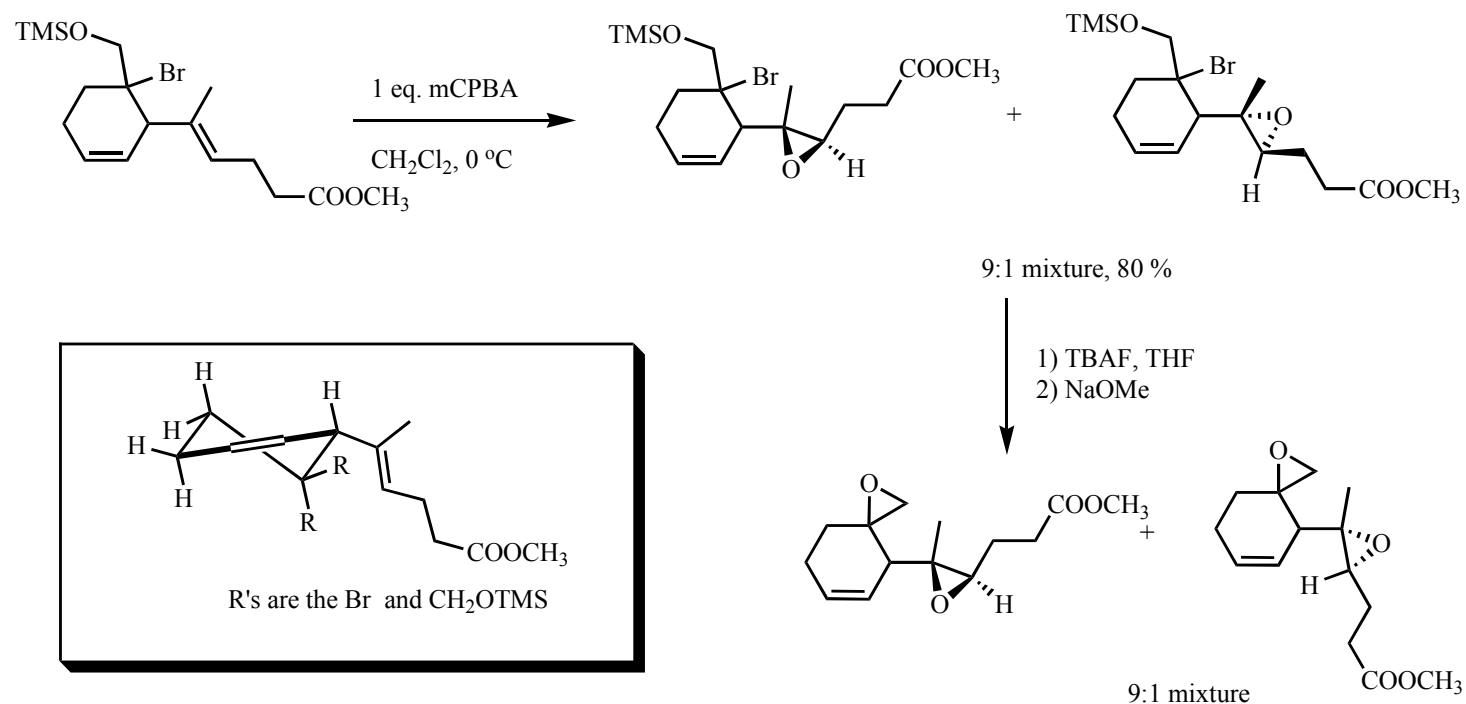
First Synthesis



Corey, E. J.; Snider, B. B. *Journal of the American Chemical Society* 1972, 94 (7), 2549-&.



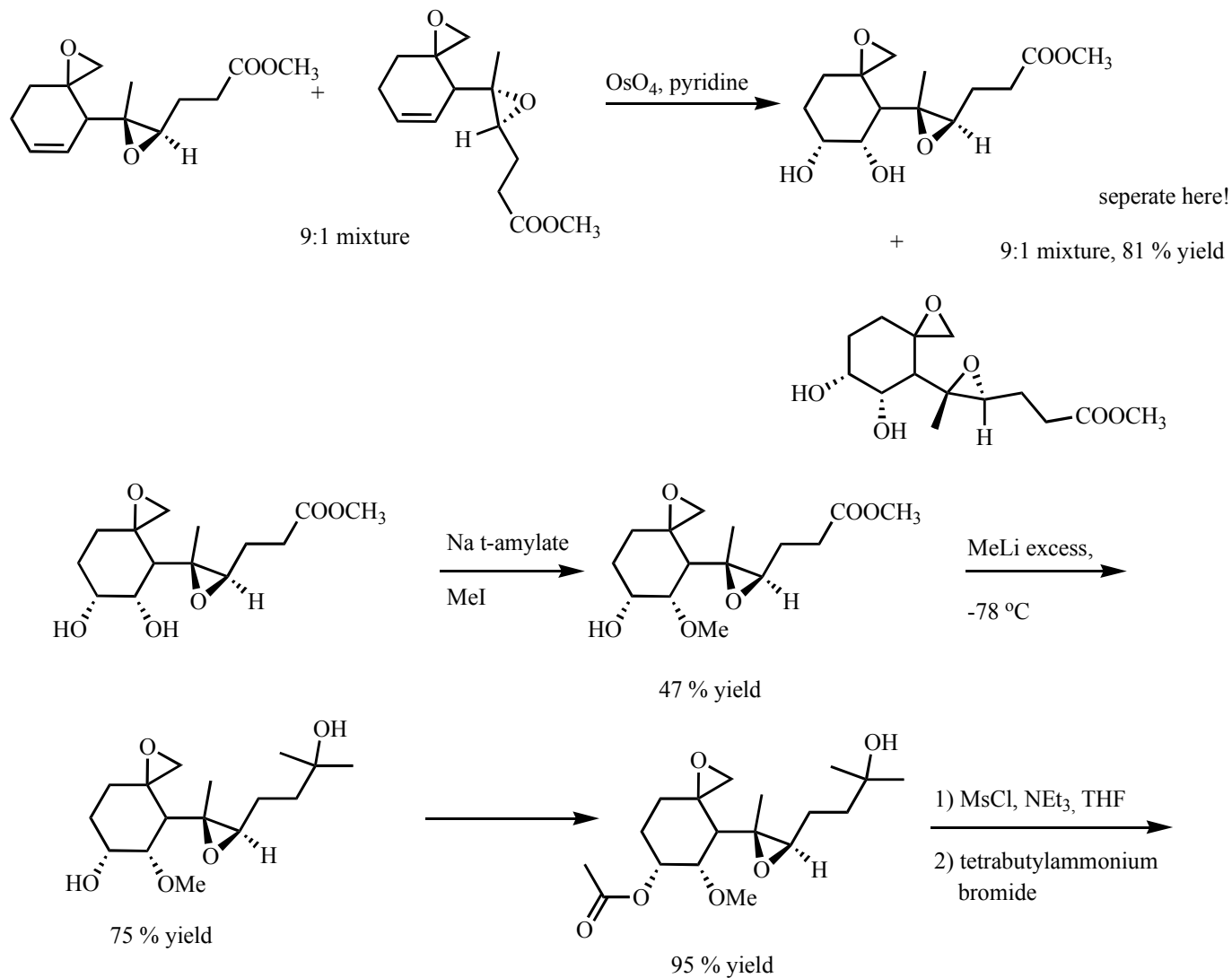
First Synthesis



Corey, E. J.; Snider, B. B. *Journal of the American Chemical Society* 1972, 94 (7), 2549-&.



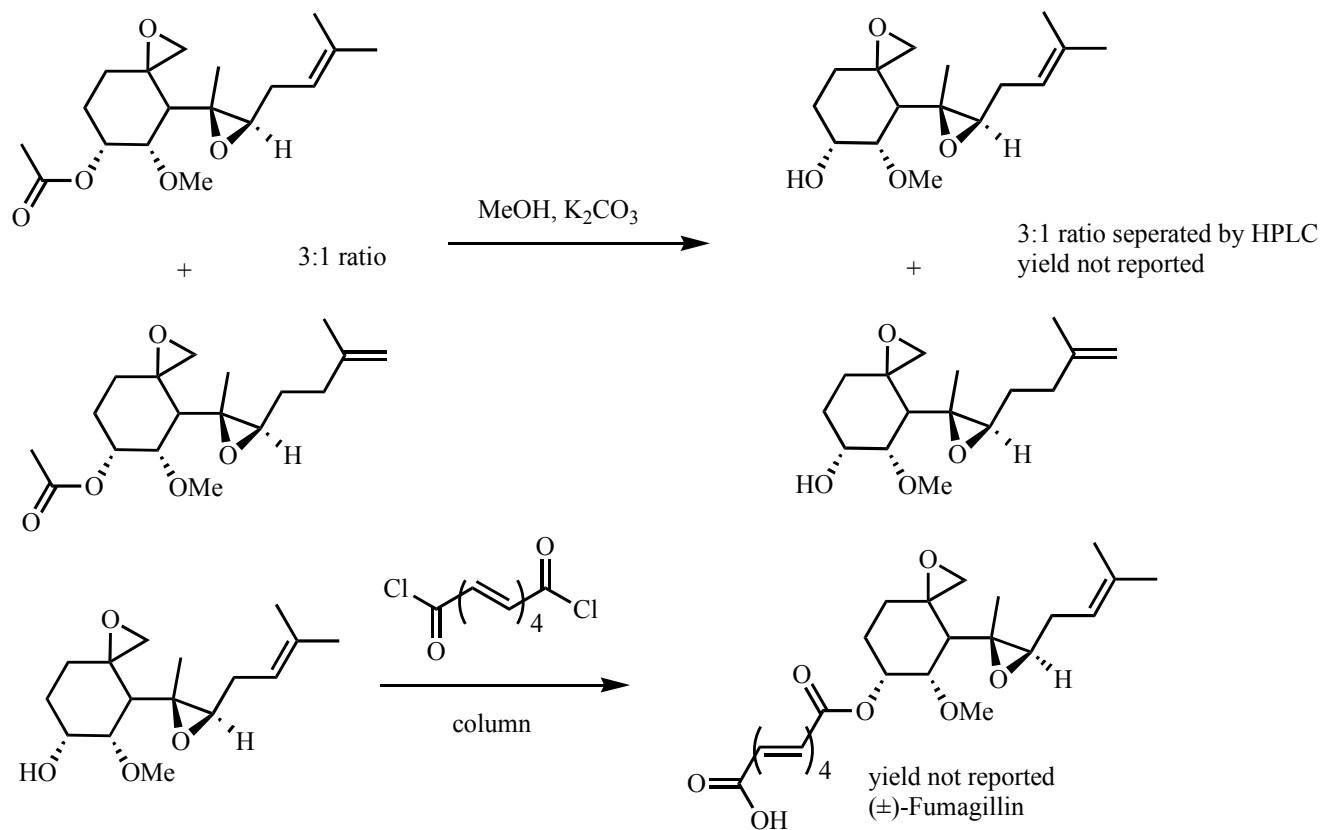
First Synthesis



Corey, E. J.; Snider, B. B. *Journal of the American Chemical Society* 1972, 94 (7), 2549-&.



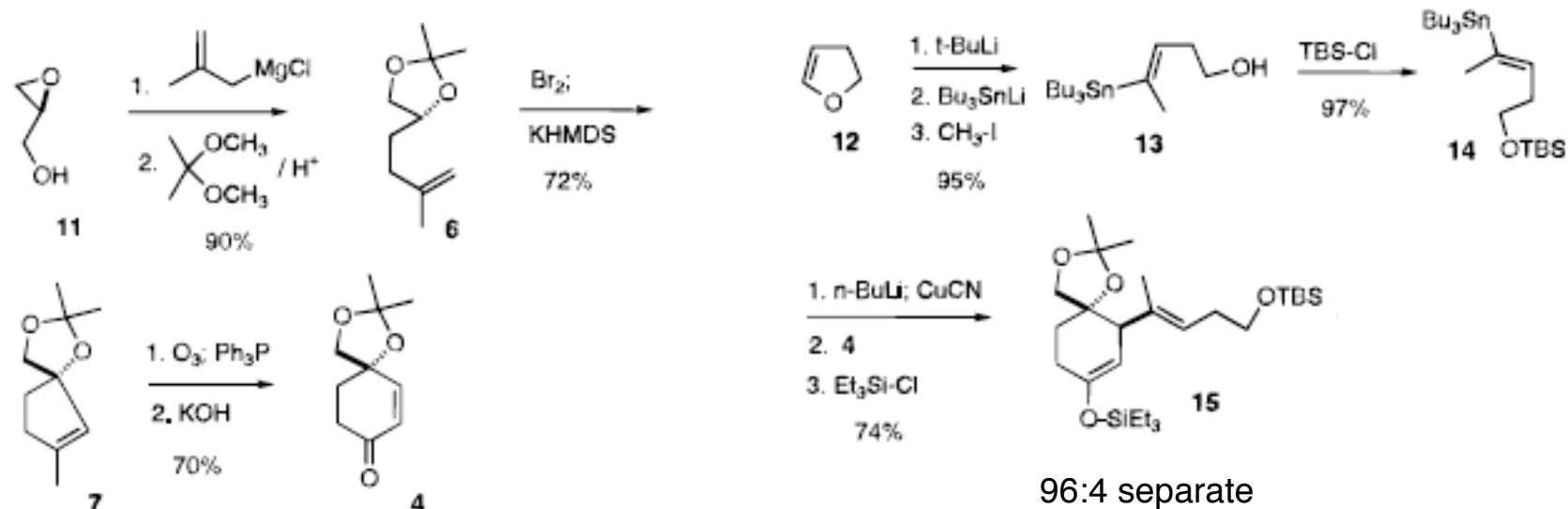
First Synthesis



Corey, E. J.; Snider, B. B. *Journal of the American Chemical Society* 1972, 94 (7), 2549-&.



Fumagillin Synthesis

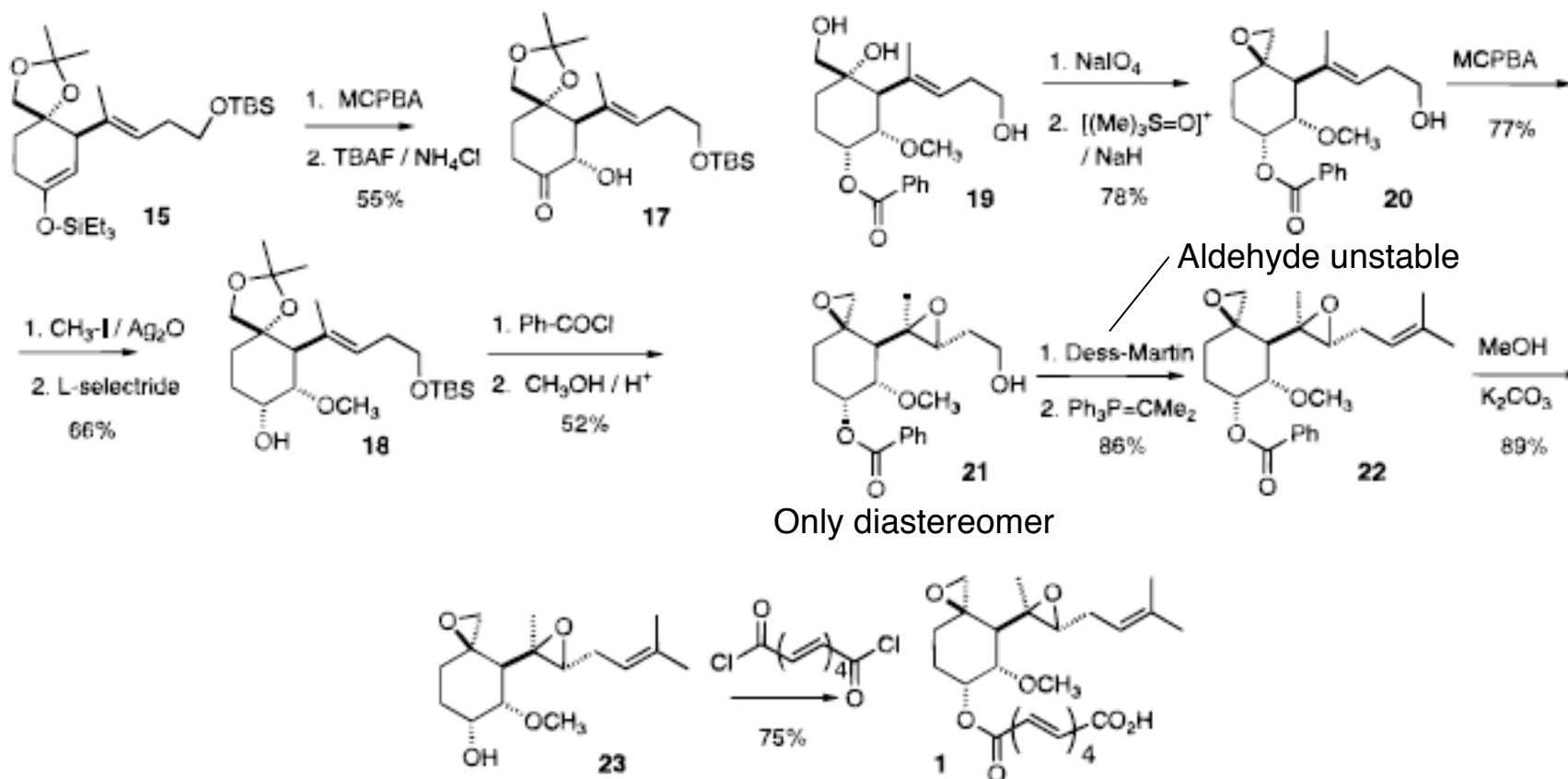


Taber, D. F.; Christos, T. E.; Rheingold, A. L.; Guzei, I. A. *Journal of the American Chemical Society* 1999, 121 (23), 5589-5590.

Lemenez, P.; Fargeas, V.; Poisson, J.; Ardisson, J.; Lallemand, J. Y.; Pancrazi, A. *Tetrahedron Letters* 1994, 35 (42), 7767-7770.



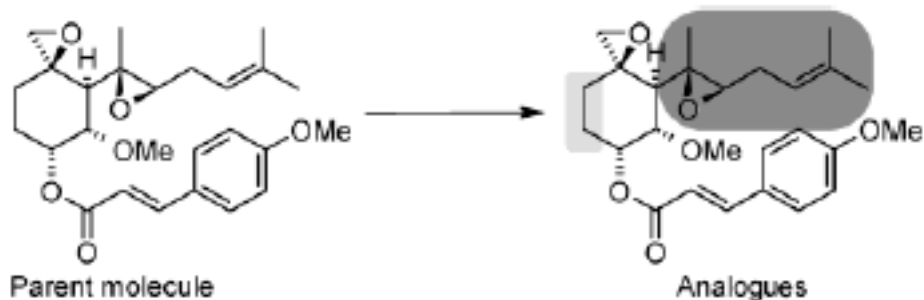
Fumagillin Synthesis



Taber, D. F.; Christos, T. E.; Rheingold, A. L.; Guzei, I. A. *Journal of the American Chemical Society* 1999, 121 (23), 5589-5590.

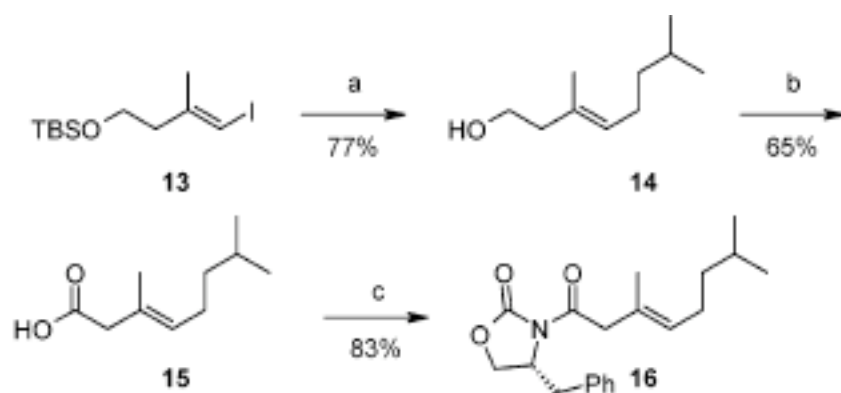


Fumagillin Synthesis



Areas to be modified: ■ 1. side chain modification ■ 2. ring modification

Modifications to the structure have been done. The epoxides can be removed and the side chain can be modified.

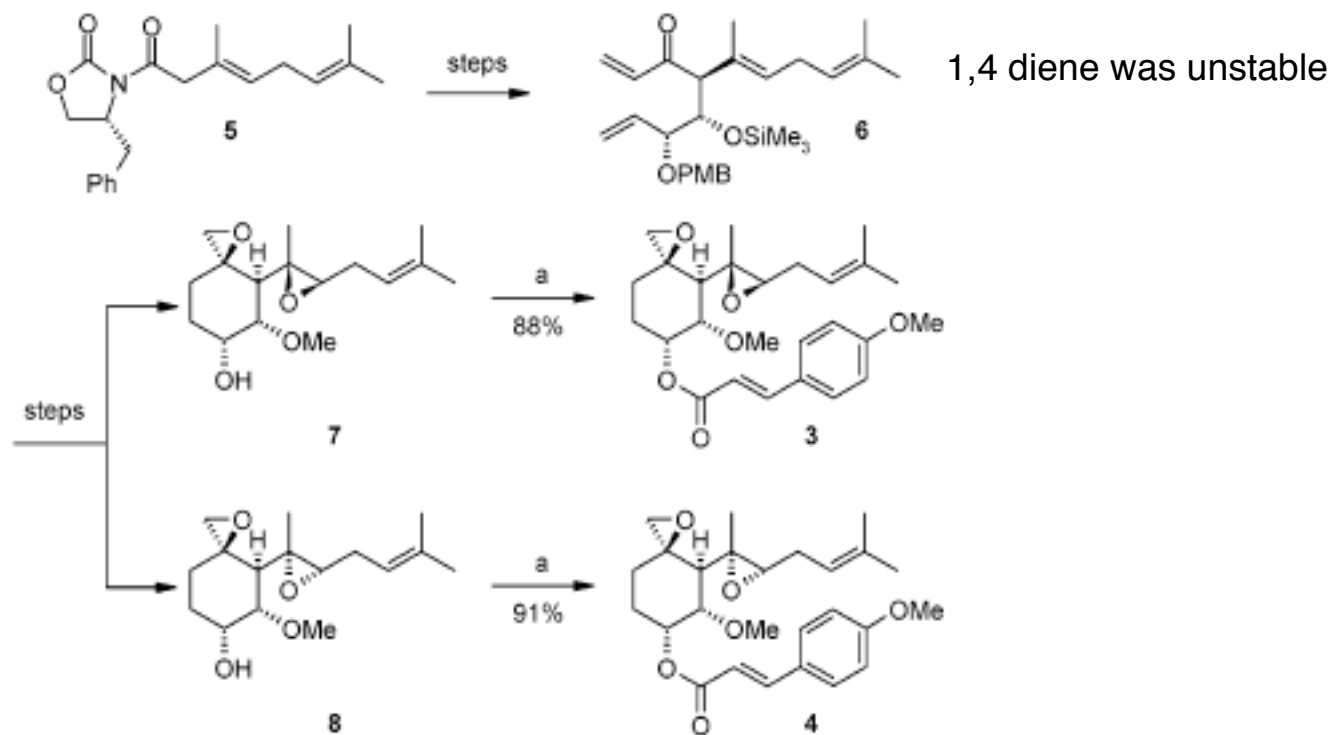


^a Key: (a) (i) isopentylZnCl (2.1 equiv), Pd(PPh₃)₄ (0.02 molar equiv), THF, 20 °C, 16 h, (ii) NBu₄F (1.1 equiv), THF, 20 °C, 2 h; (b) CrO₃ (3 equiv), H₂SO₄/H₂O/acetone, 0 °C, 15 min; (c) (i) (COCl)₂, DMF (cat.), CH₂Cl₂, 0 °C, 1 h → 20 °C, 3 h, (ii) (*R*)-(+)-4-benzyl-2-oxazolidinone (Li salt) (1 equiv), -78 °C, 20 min.

Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



Analog 4



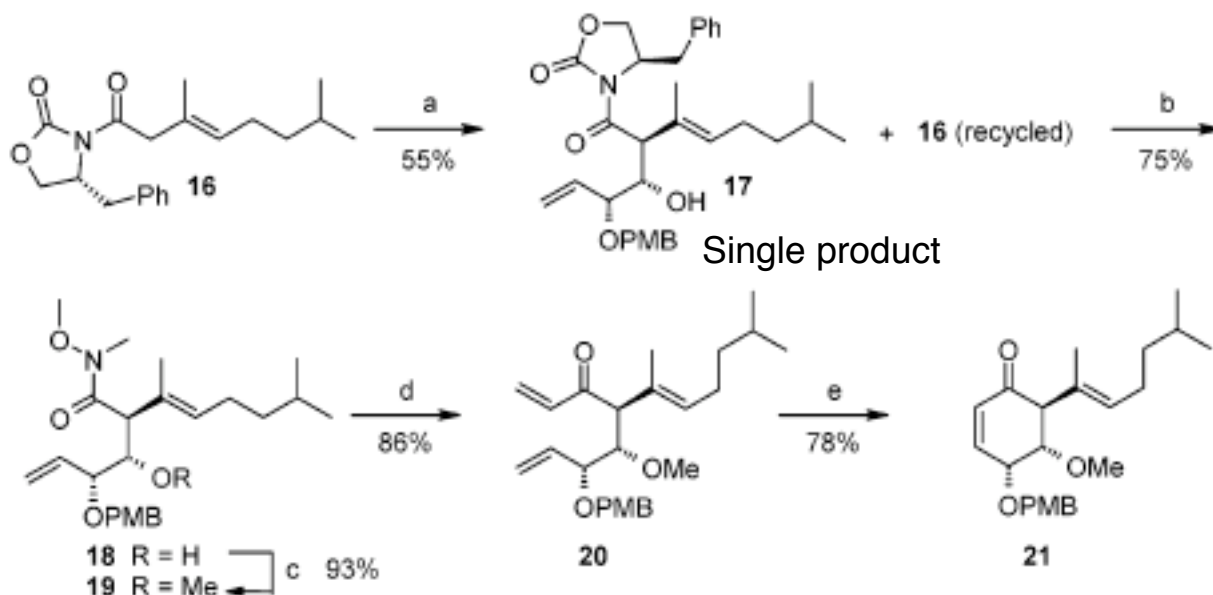
^a Key: (a) *p*-methoxycinnamic acid (8–10 equiv), DCC (8–10 equiv), DMAP (8–10 equiv), CH₂Cl₂, 20 °C, 5 h.

Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.

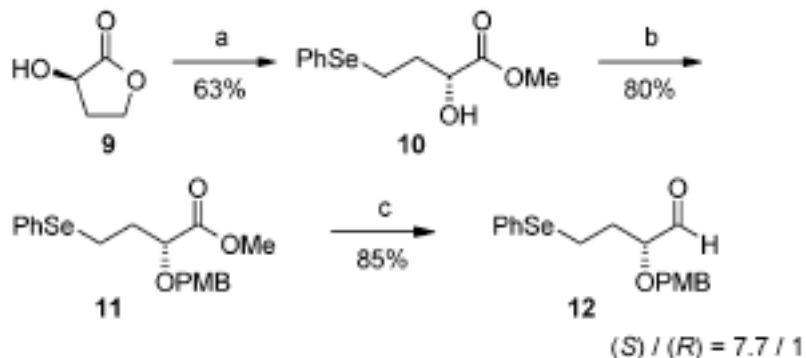
Boiteau, J. G.; Van de Weghe, P.; Eustache, J. *Organic Letters* 2001, 3 (17), 2737-2740.



Intermediate 21



^a Key: (a) (i) LDA (1 equiv), THF, $-78\text{ }^{\circ}\text{C}$, 30 min, (ii) 12 (1.1 equiv), $-78\text{ }^{\circ}\text{C}$, 1.5 h, (iii) evaporate, (iv) NBu_4IO_4 (2 equiv), CHCl_3 , $60\text{ }^{\circ}\text{C}$, 2 h; (b) AlMe_3 (3.5 equiv), *N,O*-dimethylhydroxylamine hydrochloride (3.5 equiv), THF, $20\text{ }^{\circ}\text{C}$, 24 h; (c) MeI (18 equiv), Ag_2O (5 equiv), Et_2O , reflux, 8 h; (d) $\text{CH}_2=\text{CHMgBr}$ (5 equiv), THF, $20\text{ }^{\circ}\text{C}$, 12 h; (e) Grubbs II (0.10 molar equiv), toluene, $70\text{ }^{\circ}\text{C}$, 45 min.



PhSeNa worked better than PhSe-SePh, NaBH_4

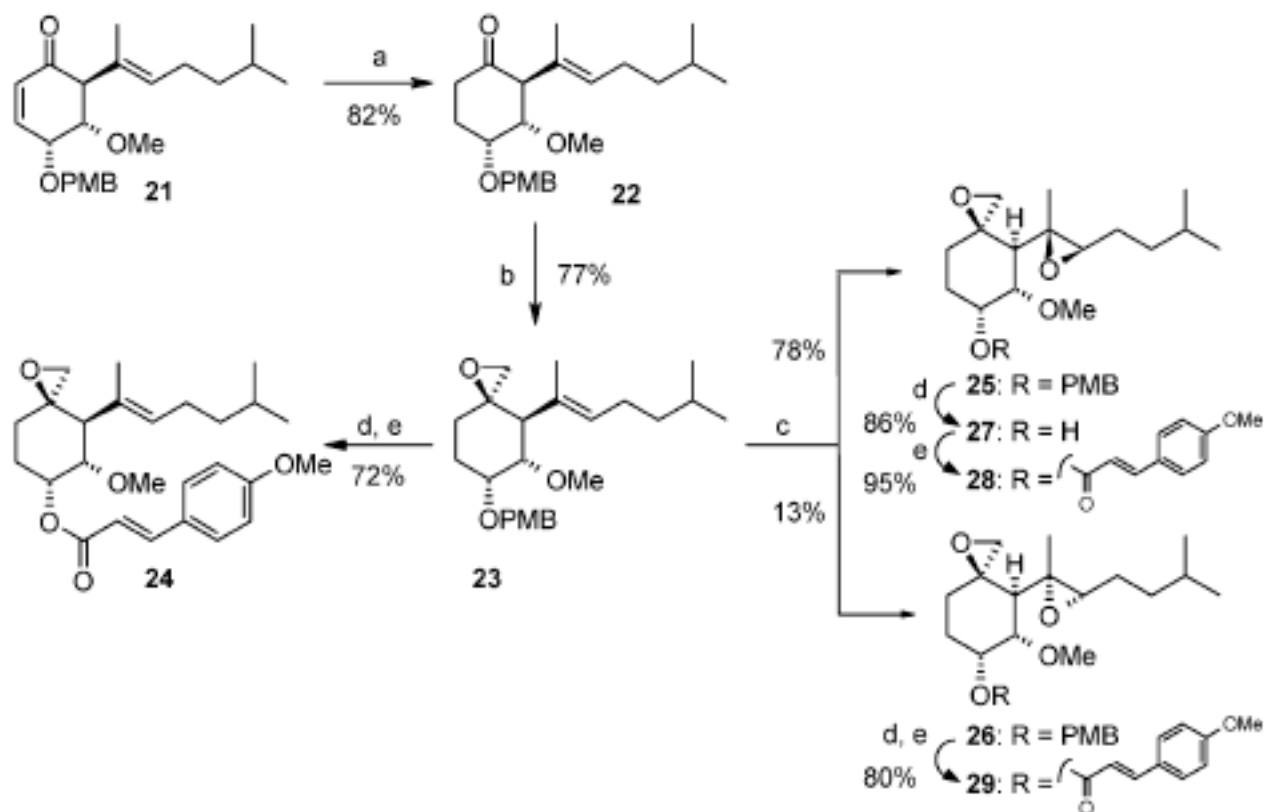
^a Key: (a) (i) PhSeNa (1.8 equiv), 18-crown-6 (0.05 equiv), THF, $20\text{ }^{\circ}\text{C}$, 2 h, (ii) HCl (1 M), then extract with AcOEt, (iii) CH_2N_2 (excess), Et_2O , $20\text{ }^{\circ}\text{C}$; (b) $\text{PMBOC}(\text{CCl}_3)=\text{NH}$, camphorsulfonic acid (0.2 equiv), $20\text{ }^{\circ}\text{C}$, 20 h; (c) DIBAL-H, toluene, $-78\text{ }^{\circ}\text{C}$, 20 min.

Silica gel racemized aldehyde

Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



Analogs 28 and 29

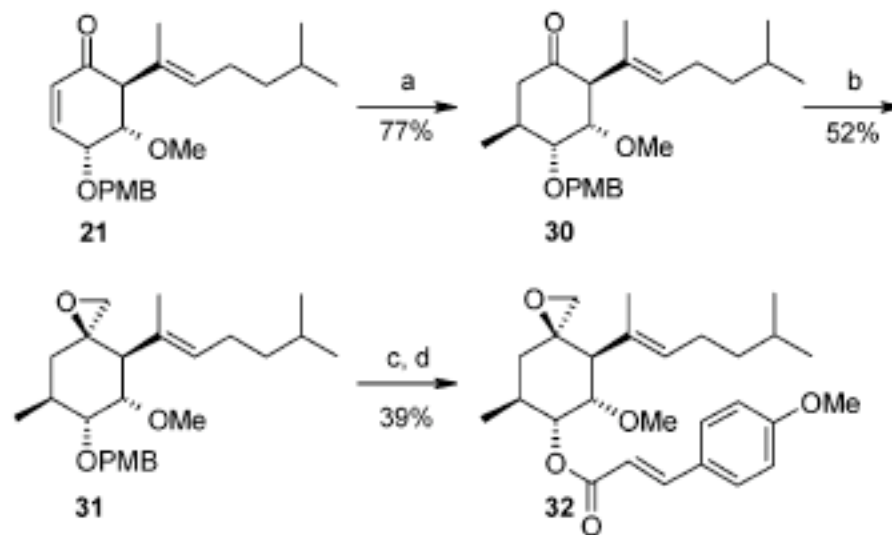


^a Key: (a) RaNi , THF, 0 °C, 20 min; (b) $\text{Me}_3\text{S}^+\text{OI}^-$ (15 equiv), NaH (10 equiv), LiI (12 equiv), DMSO/THF (1:1), 0 °C \rightarrow 20 °C, 30 min; (c) *m*-CPBA (1.5 equiv), NaHCO_3 (6 equiv), CH_2Cl_2 , 0 °C, 1 h, separate; (d) DDQ (1.1 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, 20 °C, 1 h; (e) *p*-methoxycinnamic acid (10 equiv), DCC (10 equiv), DMAP (10 equiv), CH_2Cl_2 , 20 °C, 48 h.

Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



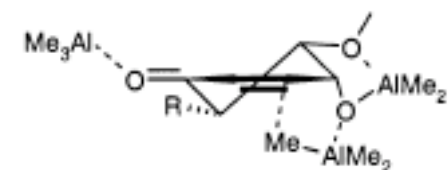
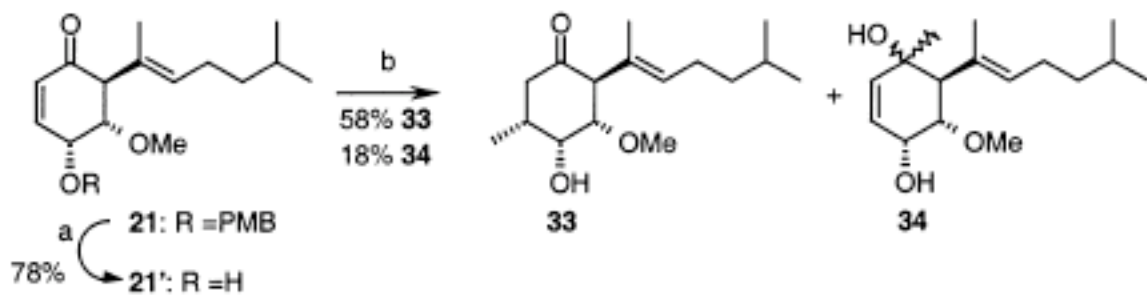
Analog 32



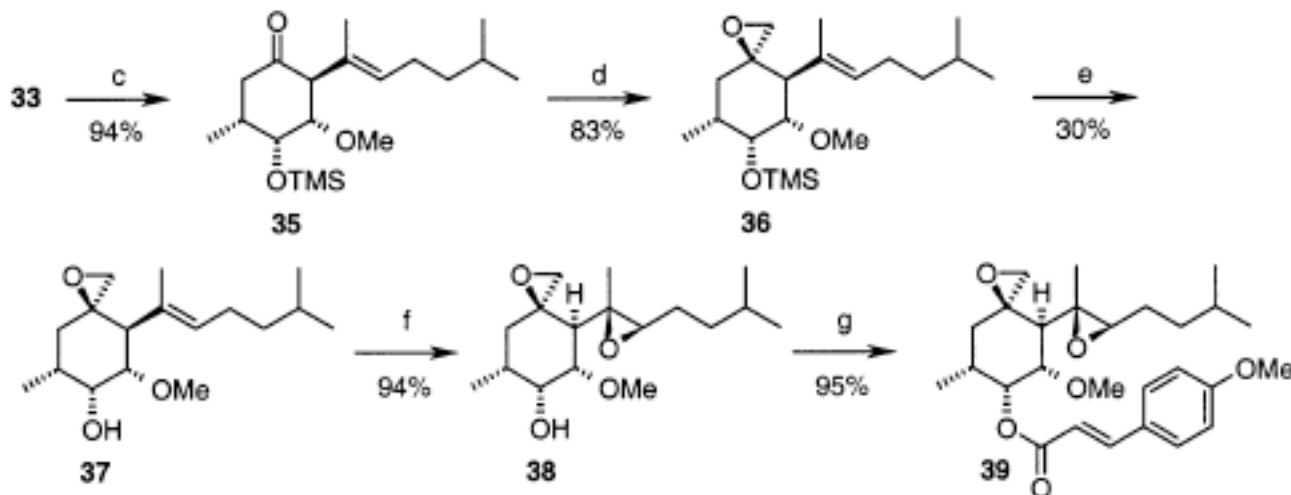
^a Key: (a) Me_2CuLi (1.1 equiv), Et_2O , 0 °C, 1 h; (b) CH_2I_2 (5 equiv), BuLi (5 equiv), THF, -78 °C, 1 h \rightarrow 20 °C, 1.5 h; (c) DDQ (1.1 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, 20 °C, 1 h; (d) *p*-methoxycinnamic acid (10 equiv), DCC (10 equiv), DMAP (10 equiv), CH_2Cl_2 , 20 °C, 24 h.



Analog 39



AlMe_3 coordination to explain selectivity.

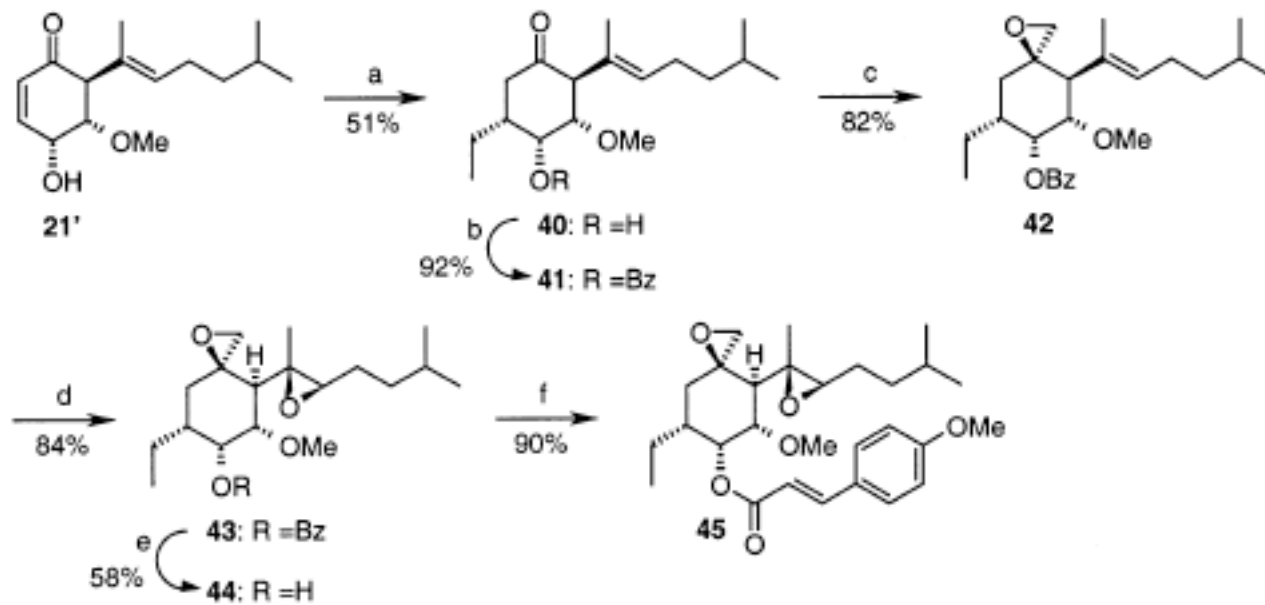


^a Key: (a) DDQ (1.1 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, 20 °C, 8 h; (b) AlMe_3 (10 equiv), CH_2Cl_2 , 20 °C, 4 h; (c) TMSCl (2.5 equiv), NEt_3 (10 equiv), DMAP (3 molar equiv), CH_2Cl_2 , 20 °C, 1 h; (d) $\text{Me}_3\text{S}^+\text{OI}^-$ (15 equiv), NaH (10 equiv), LiI (12 equiv), DMSO/THF (1:1), 0 → 20 °C, 2 h; (e) PPTS (0.1 molar equiv), MeOH/ H_2O (20:1), 20 °C, 24 h; (f) *m*-CPBA (1.5 equiv), NaHCO_3 (6 equiv), CH_2Cl_2 , 0 °C, 1 h; (g) *p*-methoxycinnamic acid (10 equiv), DCC (10 equiv), DMAP (10 equiv), CH_2Cl_2 , 20 °C, 16 h.

Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



Analog 45

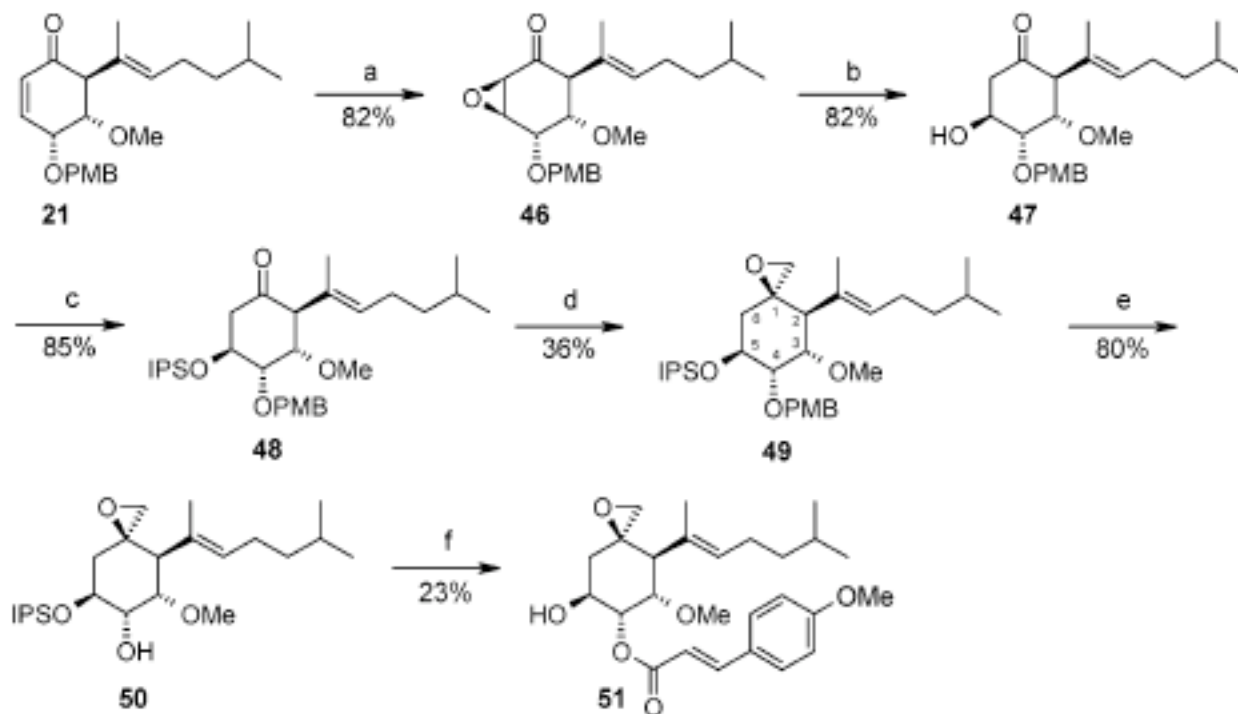


^a Key: (a) AlEt₃ (10 equiv), CH₂Cl₂, 20 °C, 4 h; (b) Benzoic acid (10 equiv), DCC (10 equiv), DMAP (10 equiv), CH₂Cl₂, 20 °C, 16 h; (c) Me₃S⁺OI⁻ (15 equiv), NaH (10 equiv), LiI (12 equiv), DMSO/THF (1:1), 0 → 20 °C, 2 h; (d) *m*-CPBA (1.5 equiv), NaHCO₃ (6 equiv), CH₂Cl₂, 20 °C, 1 h; (e) K₂CO₃, MeOH, 20 °C, 16 h; (f) *p*-methoxycinnamic acid (12 equiv), DCC (12 equiv), DMAP (12 equiv), CH₂Cl₂, 20 °C, 16 h.

Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



Analog 51

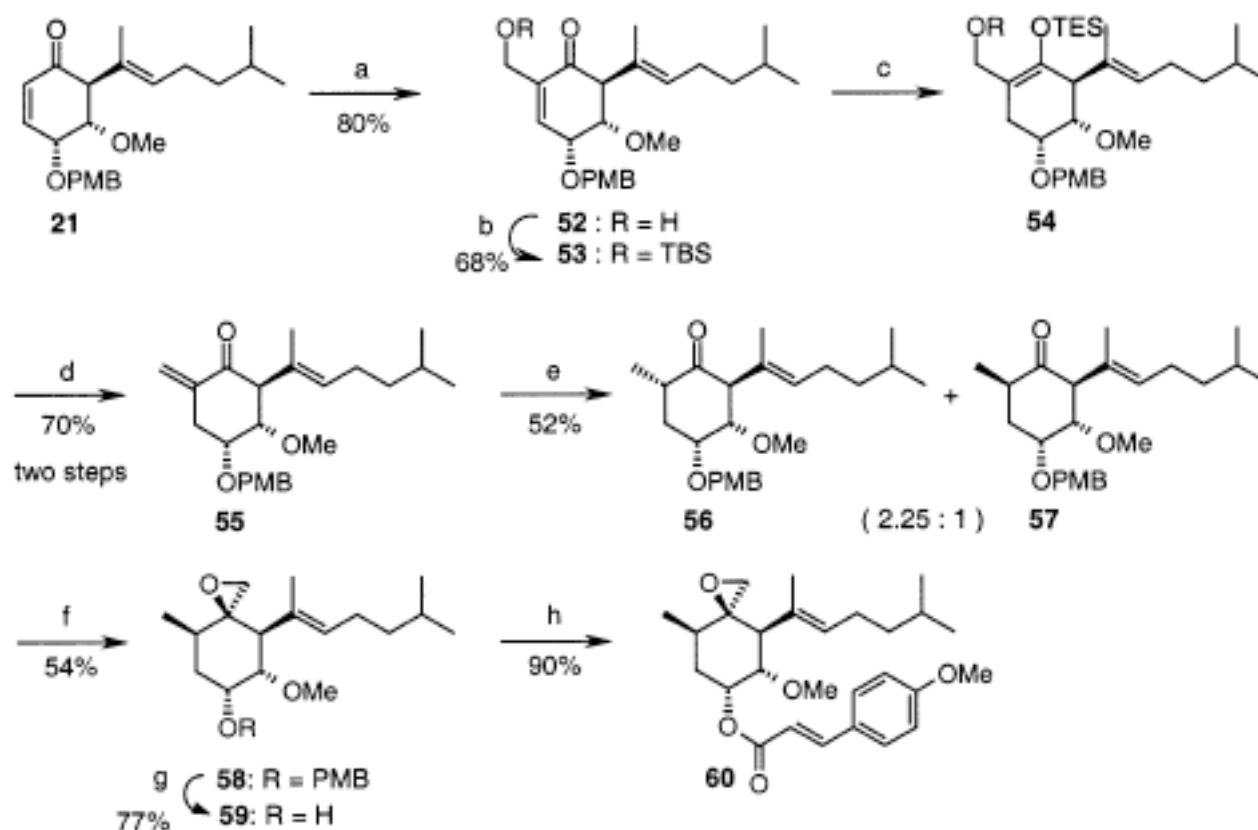


^a Key: (a) H₂O₂ (3 equiv), K₂CO₃, (0.1 equiv), MeOH, 0 °C, 30 min; (b) (PhSe)₂ (1.5 equiv), NaBH₄ (3 equiv), AcOH (0.5 equiv), 0 °C, 15 min; (c) ^tPr(Me)₂SiCl (1.5 equiv), DMAP (2 equiv), DMF/THF (1/1: v/v), 20 °C, 1 h; (d) CH₂I₂ (5 equiv), BuLi (5 equiv), -78 °C, 1 h → 20 °C, 1.5 h; (e) DDQ (1.2 equiv), CH₂Cl₂/H₂O, 20 °C, 2 h; (f) (i) *p*-methoxycinnamic acid (10 equiv), DCC (10 equiv), DMAP (10 equiv), CH₂Cl₂, 20 °C, 36 h, (ii) TBAF (1 equiv), 20 °C, 30 min.

Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



Analog 60



^a Key: (a) PBU_3 (0.85 equiv), HCHO , (40% in water, 2.6 equiv), THF, 20 °C, 16 h; (b) TBSCl (1.1 equiv), DMAP (1.5 equiv), THF, 20 °C, 4 h; (c) Et_3SiH (35 equiv), $\text{RhCl}(\text{PPh}_3)_3$ (0.03 molar equiv), toluene, 65 °C, 4 h; (d) TFA (1 M, 1 molar equiv), THF/ H_2O (1/1 v/v), 20 °C, 1.5 h; (e) RaNi , THF, 0 °C, 2 h; (f) CH_2I_2 (5 equiv), BuLi (5 equiv), -78 °C, 45 min \rightarrow 20 °C, 1.5 h; (g) DDQ (1.2 equiv), $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$, 20 °C, 1 h; (h) *p*-methoxycinnamic acid (10 equiv), DCC (10 equiv), DMAP (10 equiv), CH_2Cl_2 , 20 °C, 16 h.

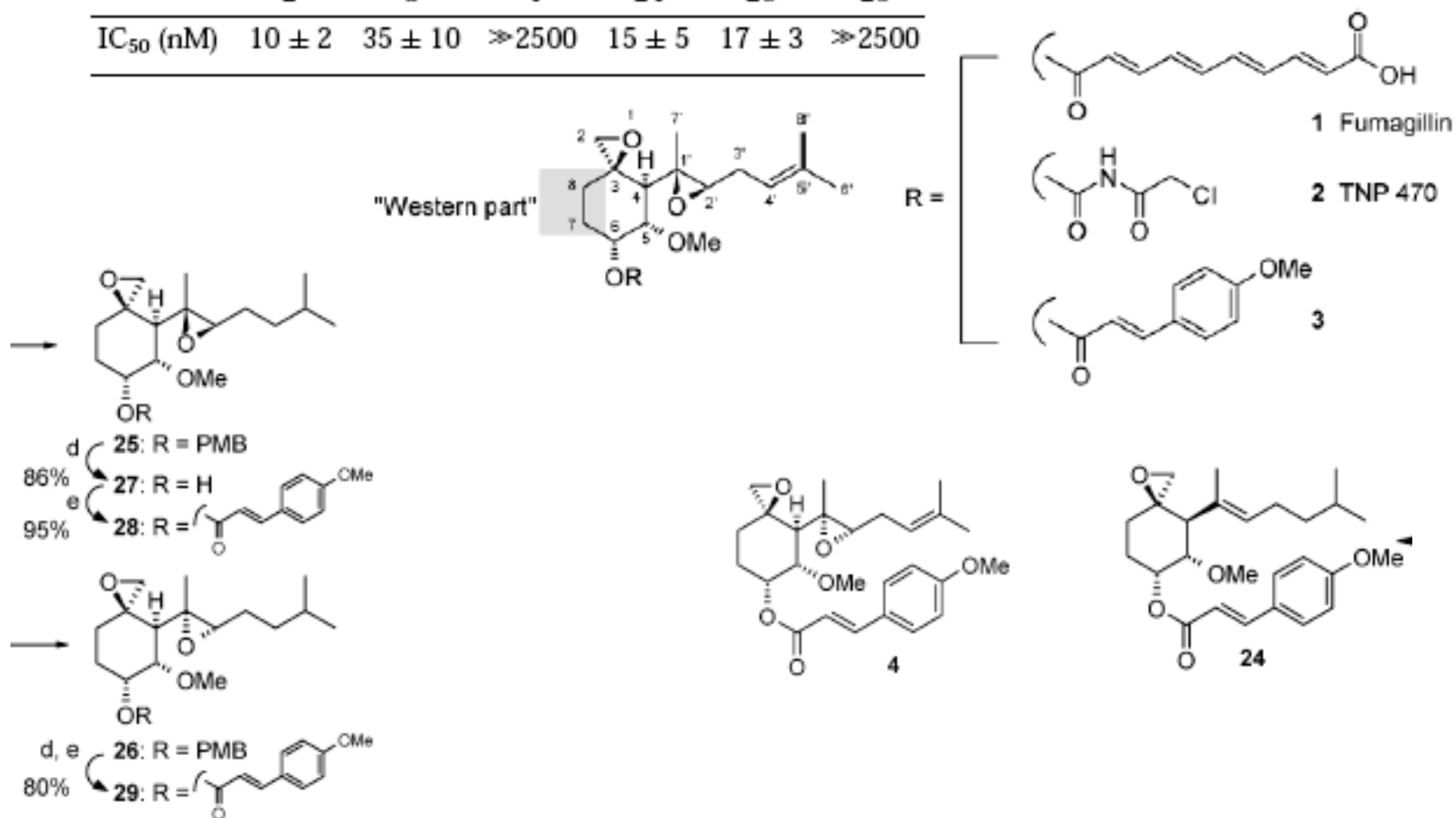
Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



Inhibition

TABLE 2. MetAP-2 Inhibition by Fumagillin and Side-Chain-Modified Analogues

	2	3	4	24	28	29
IC ₅₀ (nM)	10 ± 2	35 ± 10	≥2500	15 ± 5	17 ± 3	≥2500



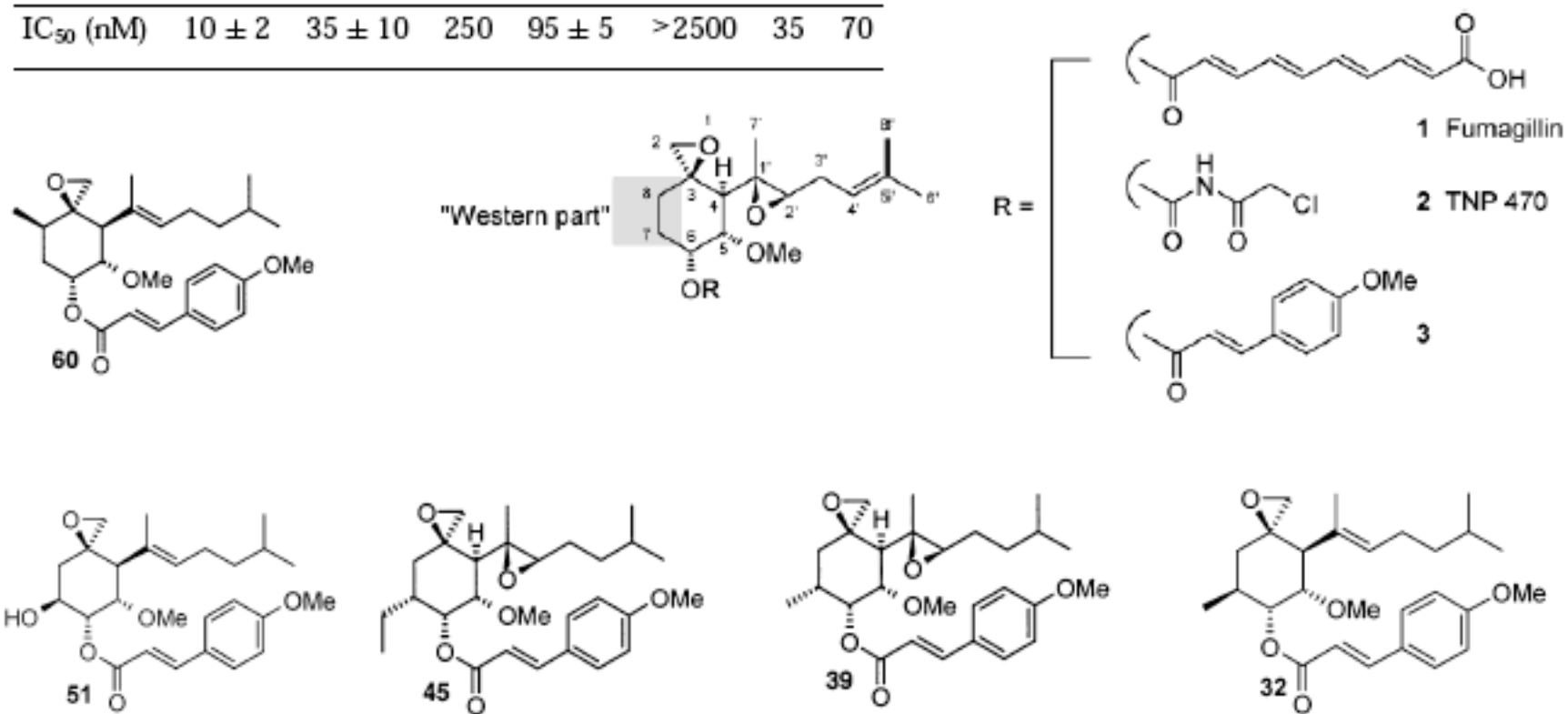
Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



Inhibition

TABLE 3. MetAP-2 Inhibition by Fumagillin and Ring-modified Analogues

	2	3	32	39	45	51	60
IC ₅₀ (nM)	10 ± 2	35 ± 10	250	95 ± 5	>2500	35	70



Rodeschini, V.; Boiteau, J.-G.; Van de Weghe, P.; Tarnus, C.; Eustache, J. *Journal of Organic Chemistry* 2004, 69 (2), 357-373.



The End
Thank you
