Natural Products with Maleic Anhydride Structure: Nonadrides, Tautomycin, Chaetomellic Anhydride, and Other Compounds

Xiaolong Chen, Yuguo Zheng,* and Yinchu Shen

Institute of Bioengineering, Zhejiang University of Technology, Hangzhou 310032, P. R. China

Received January 6, 2006

Contents

* Corresponding author. Tel: +86-571-88320379. Fax: +85-571-88320630. E-mail: zhengyg@zjut.edu.cn.

1. Introduction and Background

Research on the chemical modification and biological activity of tautomycin (**1**)[1,2] and tautomycetin (**2**),[3,4] which were isolated and identified by researchers from China and Japan in the 1980s at RIKEN, Japan, found that compounds with maleic anhydride structure have special biological activities, such as antibiotic activities and enzymatic inhibition. Later, many natural products with maleic anhydride structure, such as phomoidride A (CP-225,917) (**3**), phomoidride B (CP-263,114) (**4**),[5−7] rubratoxin A (**5**),[8,9] rubratoxin B (**6**),[8,10−12] cornexistin (**7**),[13−16] hydroxycornexistin (**8**),[17,18] zopfiellin (**9**),[19,20] heveadride (**10**),[21] dihydroepiheveadride (**11**),[22,23] glauconic acid (**12**),[24−27] glaucanic acid (**13**),[24−27] scytalidin (**14**),[28−30] byssochlamic acid (**15**),[31,32] chaetomellic anhydride A (**16**),[33−35] puberulonic acid (**17**),[36−38] stipitotanic acid (**18**),[39−41] cordyanhydride A (**19**),[42] cordyanhydride B (**20**),[42] 2-carboxymethyl-3-*N*-hexyl-maleic acid anhydride (**21**),[43] tyromycin A (**22**),[44] 2-(*β*-carboxyethyl)-3-hexylmaleic anhydride (**23**), 2-(*β*-carboxyethyl)-3-octylmaleic anhydride (**24**), (*E*)-2-but-1-enyl-3-(*β*-carboxyethyl) maleic anhydride (**25**), telfairic anhydride (**26**),[45,46] itaconitin (**27**)[47,48] (See Figure 1), and so on, also proved to have similar properties. Of them, **3**−**15** have the core structure of nonadrides. Some are strong inhibitors of protein phosphatase (**1** and **2**), Ras farnesyl transferase and squalene synthase (**3** and **4**), and inositol monophosphatase (**17**). Some are herbicides (especially **6**−**8**) or antifungal agents (**9**−**11** and **14**), and others have antifungal or antibacterial activities.

The central role of protein phosphorylation/dephosphorylation in cellular regulation suggests that many disease states involve perturbation in the balance between protein kinase and protein phosphatase activities. Given that many of the

10.1021/cr050029r CCC: $65.00
Published on Web 04/18/2007

Xiaolong Chen was born in Xianju, Zhejiang, China, in 1970. After graduation, he worked as a researcher and teacher in Zhejiang University of Technology. He started the doctorate degree in 2004 at Zhejiang University of Technology. Since 1999, he has studied natural products such as valienamine, valiolamine, validamine, and tautomycin. His research interests are the production of natural products and their structural modification.

Yuguo Zheng was born in Xiangshan, Zhejiang, China, in 1961. He obtained a doctorate degree in biochemical engineering. Presently, he is Professor of Biochemical Engineering at Zhejiang University of Technology. His research interests are biochemical engineering and biotransformation and biocatalysis.

Yinchu Shen was born in Shengzhou, Zhejiang, China, in 1938. In 1962, he obtained his undergraduate degree from Fudan University. In the same year, he began graduate studies. He worked at Shanghai Institute of Pesticide and Shanghai Research Center of Biochemical Engineering in the China Chemical Engineering Department in 1964. He was voted as an academician in the Chinese Academy of Engineering in 1998. Then he moved to Zhejiang University of Technology as Professor of Biochemical Engineering. Since 2000, he has acted as the president of the university. His major research fields are the products of biochemical engineering.

natural inhibitors of protein phosphatases lead to elevated protein phosphorylation levels within cells and in doing so alter associated processes, protein phosphatases should be considered attractive targets for novel drug design. Presently, protein phosphatase inhibitors have the following therapeutic uses: (i) neurological disorders; (ii) metabolic disorders; (iii) respiratory diseases and allied disorders; (iv) immunosuppression; and (v) cancer therapy.[49,50] Therefore, to find efficient protein phosphatase inhibitors is a current hot issue. **1** and **2** exhibit strong inhibitory activities against protein phosphatases.

The currently understood function for Ras in signal transduction is in mediating the transmission of signals from external growth factors to the cell nucleus. Mutated forms of this GTP-binding protein are found in 30% of human cancers with particularly high prevalence in colon and pancreatic carcinomas. These mutations destroy the GTPase activity of Ras and cause the protein to be locked in its active, GTP-bound form. As a result, the signaling pathways are activated, leading to uncontrolled tumor growth. Ras function in signaling requires its association with the plasma membrane. This is achieved by posttranslational farnesylation of a cysteine residue present as part of the CA_1A_2X carboxyl terminal tetrapeptide of all Ras proteins. The enzyme that recognizes and farnesylates the CA_1A_2X sequence, Ras farnesyltransferase (PFTase), has become an important target for the design of inhibitors that might be interesting as antitumor agents. Several approaches have been taken in the search for in vivo active inhibitors of farnesyltransferase. These include the identification of natural products such as **3**, **4**, and **16**.

Because of the good biological activities of this sort of compound, many research groups have contributed to synthesis studies, especially for phomoidrides,[5] tautomycin,[51,52] tautomycetin,[52] and chaetomellic anhydride A.[53,54] Herein will be reviewed the classification, isolation, characterization, biological activity, and total syntheses of these natural products with maleic anhydride structure, as well as the structure modifications.

2. Classification of Compounds

Natural products with the maleic anhydride structure can be classified as nonadrides, tautomycin and tautomycetin, chaetomellic anhydrides, and other compounds according to whether the products contain the core structure of nonadrides, the presence of a nine-membered ring with an affixed maleic anhydride.

2.1. Nonadrides

The word "nonadride", which first occurred in 1962,[55] meant the substances, such as glaucanic acid, glauconic acid, and byssochlamic acid, were biosynthesized by two C_9 units. Later, the name nonadride has evolved to mean the compounds that own the core structure of nonadrides, a C_9 ring with an affixed anhydride. In this paper, the latter definition is employed for the classification that has been generally accepted.[56−59] At first, the nonadrides included **12**, **13**, and **15**. Later, other nonadrides, such as **3**−**11**, **14**, and **28**−**31** (see Figure 2), were discovered and became nonadrides members.

2.2. Tautomycin and Tautomycetin

The producers of tautomycin (**1**) and tautomycetin (**2**), *Streptomyces spiroverticillatus* and *S. griseochromogenes*

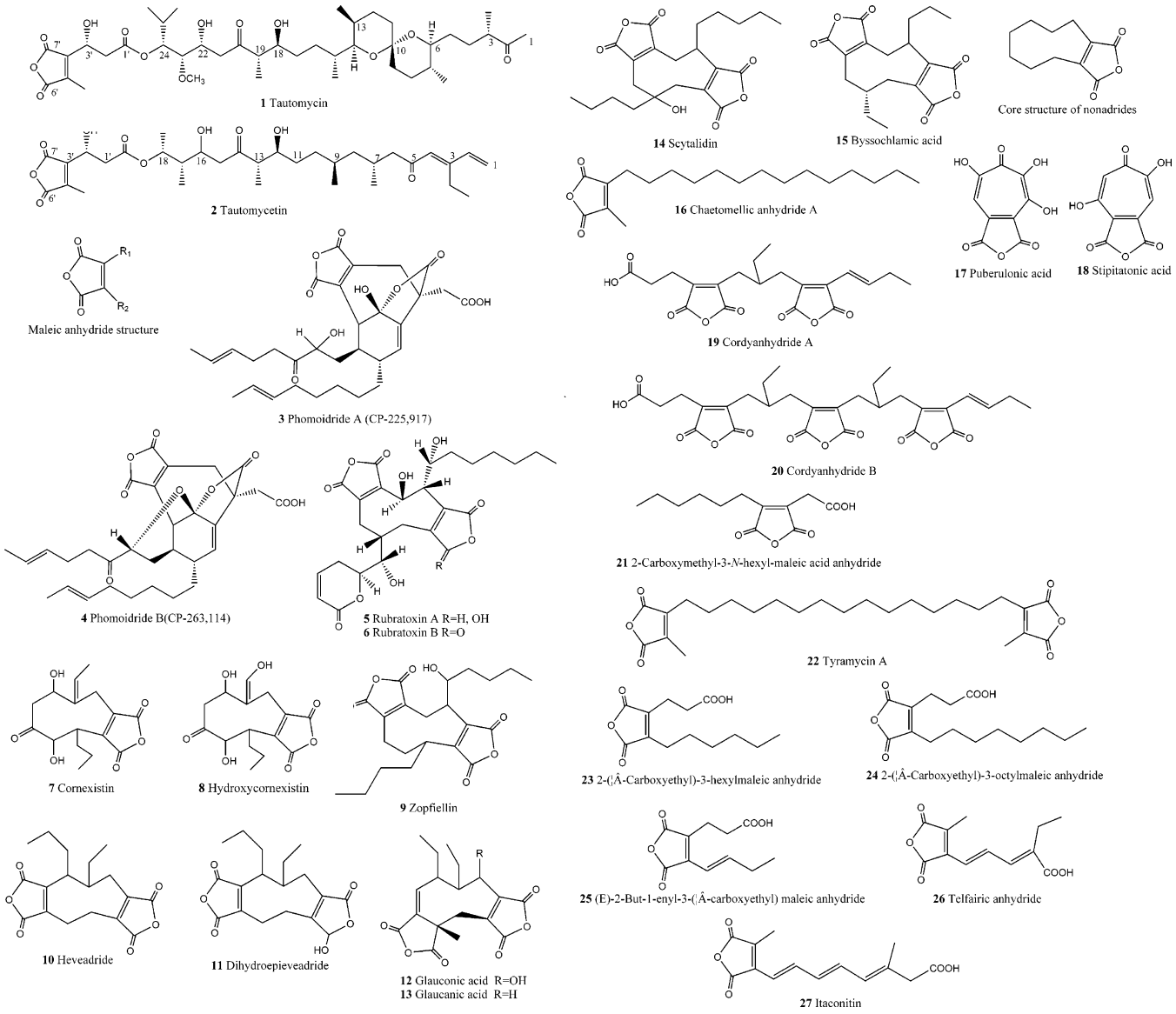


Figure 1. Chemical structures of natural compounds with maleic anhydride structure.

were screened in the isolation of natural products against *Sclerotinia sclerotiorum* in 1960s at Shanghai Institute of Pesticide, China. Because of the maleic anhydride structure in **1** and **2**, their isolation of them had become very difficult. Later, with the cooperation of Isono's Research Group of Antibiotics Laboratory, RIKEN, Japan, **1** and **2** were isolated and identified at RIKEN.[2−4,60] **1** and **2** are similar in structure. They both contain a 3-(1-hydroxy-2-caroxy) ethyl-4-methyl-2,5-furandione moiety, which is an anhydride. **1** is a well-known specific protein phosphatase inhibitor and a useful agent to study intracellular signal transductions.[52] Structurally related to **1**, **2** was also discovered to possess protein phosphatase inhibition activity. Furthermore, **2** possesses immunosuppressive activity. It can suppress interleukin-2 production, CD69, and interleukin-2 receptor (IL-2R) expression on the cell surface and graft rejection in organ transplantation.[61−64]

2.3. Other Compounds

This group includes **16**−**27** and **32** (Figure 2). **17** is an analogue of **18**. **19** and **22** possess two anhydrides, and **20** possesses three anhydrides. **21** is one of plant vigors, which is an important factor in resistance of crop to several diseases.[43]

3. Isolation and Identification

From the present reports, natural products with maleic anhydride structure were all produced by microorganisms (Table 1). Isolation of the compounds was usually performed as follows: the broth was extracted with organic solvents at low pH; the sample was subjected to chromatography on silica gel or LH-20; and finally the sample was further subjected to HPLC several times to obtain pure samples for structure elucidation.

4. Biological Activity of Compounds

4.1. Nonadrides

4.1.1. Phomoidrides

Phomoidrides have good biological activities. They have been shown in vitro to inhibit the enzymes SQS and Ras farnesyl transferase.[5−7] Therefore, **3** and **4** are thought to be

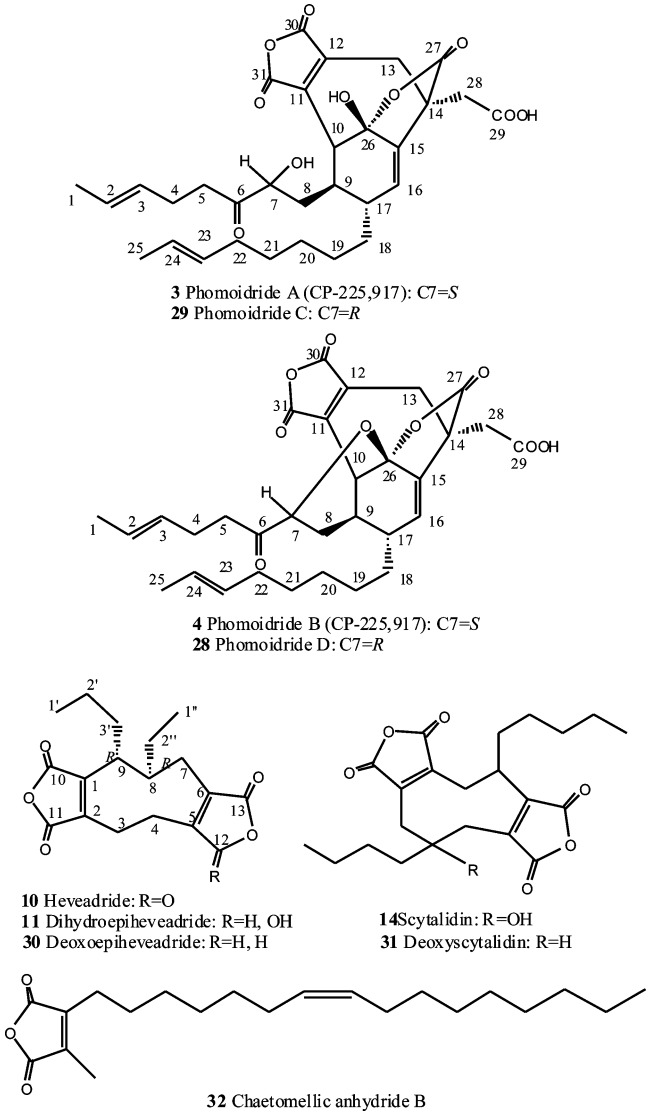


Figure 2. Chemical structures of phomoidride B, phomoidride D, deoxoepiheveadride, deoxyscytalidin, and chaetomellic anhydride B.

attractive lead structures for the development of cholesterol-lowering or anticancer agents.

4.1.1.1. Antihypercholesterolemic Properties. Cardiovascular disease (CVD) has been the leading cause of death in the world, especially in the United States every year since 1919; in the year 2000 alone, over 878 000 people died from diseases of cardiac or vascular origin, a number greater than the next four leading causes combined. Because numerous studies have established an unequivocal link between levels of serum cholesterol and CVD and, in humans, upward of 70% of serum cholesterol is derived from de novo biosynthesis, inhibitors of enzymes in the sterol biosynthetic pathway have served as useful therapies in preventing such diseases. To this end, research efforts in industrial settings have focused on designing pharmaceutical agents, referred to as statins, that inhibit the enzyme HMG-CoA reductase (See Figure 3; HMG-CoA → mevalonic acid). The sales of such agents have turned statins into multibillion dollar therapeutics.

While HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis, SQS promotes the first committed step of this process. It therefore represents a potentially useful target in treating hypercholesterolemia. However, to date, no commercially available therapeutics targeting this enzyme have been developed. For this reason, large-scale industrial programs have been initiated to discover SQS inhibitors. Phomoidrides and zaragozic acids represent such discoveries, and they will hopefully serve as lead compounds toward the development of clinically useful agents.

The mechanism by which SQS catalyzes the production of squalene (**35**) has been thoroughly studied and is depicted in Figure 3. In the first step, two molecules of farnesyl pyrophosphate (FPP, **33**) are condensed to form presqualene diphosphate (**34**), which is then directed by SQS to undergo a reductive rearrangement to **35**. Initial experiments have demonstrated that **3** and **4** exert their effects on the first half-reaction catalyzed by SQS (**33** → **34**). In addition, these studies have determined that SQS inhibition by the phomoidrides is reversible and of the mixed noncompetitive type with respect to the substrate **33**. Taken together, these data are consistent with a model in which **3** and **4** are capable of reversibly occupying FPP binding sites in the free enzyme

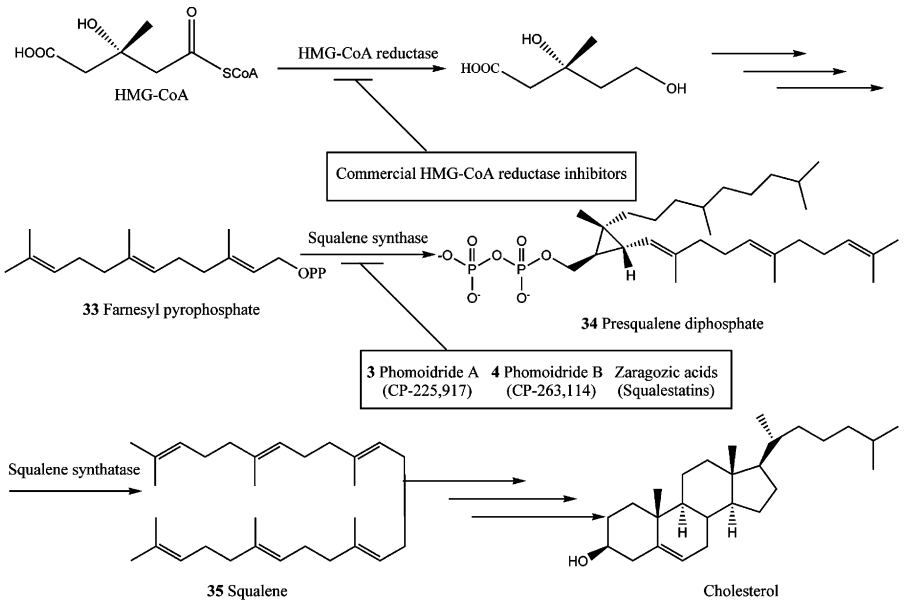


Figure 3. Simplified depiction of the cholesterol biosynthetic pathway and the sites of action of its inhibitors.

Table 1. Microorganisms Employed for the Production of Natural Products with Maleic Anhydride Structure

compounds	microorganisms	refs
phomoidrides	*Phoma* species	5−7, 65−67
rubratoxin A	*Pencillium rubrum*	8, 10, 11, 68−80
rubratoxin B		
cornexistin	*Paecilomyces variotii* SANK 21086	14, 18, 81
hydroxycornexistin		
zopfiellin	*Zopfiella curvata* No. 37−3	82, 83
heveadride	*Helminthosporium heveae* and *Bipolaris heveae* CBS 241.93	21−23, 84
dihydroepiheveadride		
scytalidin	*Scytalidium* species	28, 30, 85, 86
glauconic acid	*Penicillium glaucum* and *Penicillium purpurogenum*	57, 58, 87, 88
glaucanic acid		
byssochlamic acid	*Byssochlamys fulva*	31, 32
tautomycin	*Streptomyces spiroverticillatus*	1, 2, 89−91
tautomycetin	*Streptomyces griseochromogenes*	3, 4
chaetomellic anhydride a	*Chaetomella acutiseta*	35
puberulonic acid	*Penicillium puberulum* and *P. aurantio-virens*	92−96
stipitotanic acid	*Penicillium stipitatum* Thom	39−41, 97, 98
cordyanhydride A	*Cordyceps pseudomilitaris* BCC 1620	42
cordyanhydride B		
2-carboxymethyl-3-*N*-hexyl-maleic acid anhydride	*Aspergillus* FH−X-213	43
tyromycin A	*Tyromyces lacteus* (Fr.) Murr	44, 99, 100

or in the enzyme bound to a single FPP molecule. Further mechanistic studies are reported to be in progress.

4.1.1.2. Ras Biology and Anticancer Activity. Mutations in the Ras oncogene have been found in up to 30% of all human cancers. For this reason, the Ras oncoprotein is considered to be of great importance as a target in the search for anticancer agents. Common oncogenic mutations in Ras produce a constitutively active protein. It is fully capable of transducing intracellular signals but does so in an unregulated fashion because it is unable to hydrolyze guanosine triphosphate (GTP). Because all forms of Ras must be processed extensively by a series of enzymes to transduce their mitogenic signals, interfering with Ras processing is an effective method for curtailing the function of aberrant forms of the protein.

The Ras processing and activation pathway has been studied in great detail and is depicted in simplified form in Figure 4. The first step in the sequence involves the transfer of a farnesyl group by the enzyme Ras farnesyl transferase to a terminal cysteine in the Ras protein. This cysteine must be contained within a stretch of four C-terminal amino acid residues possessing the sequence CAAX (called a "CAAX box"). Peptidase cleavage of the tripeptide (AAX), which neighbors the newly modified cysteine, followed by methylation of the farnesyl-cysteine carboxylate by a methyltransferase enzyme, initiates translocation of the protein to the cell membrane. Further lipidation with palmitoyl groups on additional C-terminal cysteine residues allows membrane localization to take place. Once attached to the membrane, the inactive guanosine diphosphate (GDP)-bound Ras protein becomes active via the exchange of protein-bound GDP for GTP with the help of guanine-nucleotide exchange factors (GEFs). Subsequent GTP hydrolysis, induced by GTPase-activating proteins (GAPs), shuts off Ras-mediated signaling and returns the protein to its inactive state. Activating mutations in Ras found in human cancers all prevent this GAP-induced GTP hydrolysis and serve to keep the Ras oncoprotein permanently in the "on" state, allowing it to stimulate cellular proliferation continuously.

If Ras is not lapidated, and therefore not tethered to the plasma membrane, it cannot perform its intracellular functions. Therefore, by interrupting any of the steps by which the Ras protein is posttranslationally modified, therapeutics can prevent the growth of cancers containing the Ras oncoprotein. As inhibitors of Ras farnesyl transferase, the phomoidrides could play a vital role as lead structures for the design of novel anticancer agents.

Table 2. Rubratoxin B-Sensitive Microorganisms

microorganism	no. of species inhibited	minimal inhibitory conc, μg/mL
Bacillus sp.	4	1000
Micrococcus sp.	2	1000
Staphylococcus sp.	1	1000
Tetrahymena pyriformis	1	25
Volvox aureus	1	50

4.1.2. Rubratoxin B

Rubratoxin B **6** is a potent hepatotoxic mycotoxin produced by certain *Penicillium* fungi as described above. Antimicrobial[101,102] and antitumor[103] activities were investigated. Human rubratoxicosis was reported in 1996, indicating that **6** can be a threat to human health.

4.1.2.1. Antimicrobial Activity[101,102]. **6** had no effect at 100−1000 μg/mL on the algae, fungi, or Gram-negative bacteria investigated. However, there was a decrease in pigment in *Pseudomonas fluorescens* growth adjacent to the disc containing **6** (1000 μg/mL). There was no difference in cell density when measured turbidimetrically in concentrations as high as 3000 μg/mL, but there was a marked decrease in pigment in **6**-treated (1000 μg/mL) liquid cultures of *P. fluorescens*. Four species of *Bacillus* including *B. subtilis*, two species of *Micrococcus*, and *Staphylococcus aureus* were inhibited by 1000 μg of **6** per disc (Table 2). Colonial characteristics of the *Bacillus* species were markedly changed on nutrient-agar plates (1000 μg). The colonial growth was thin, spreading, and mycoides-like, whereas solvent-control colonies were thicker and softer in appearance. The inhibitory effect of **6** on *Micrococcus lysodeikticus* growth was noted in broth cultures after 18−24 h. The particular organism requires 48 h to reach the stationary

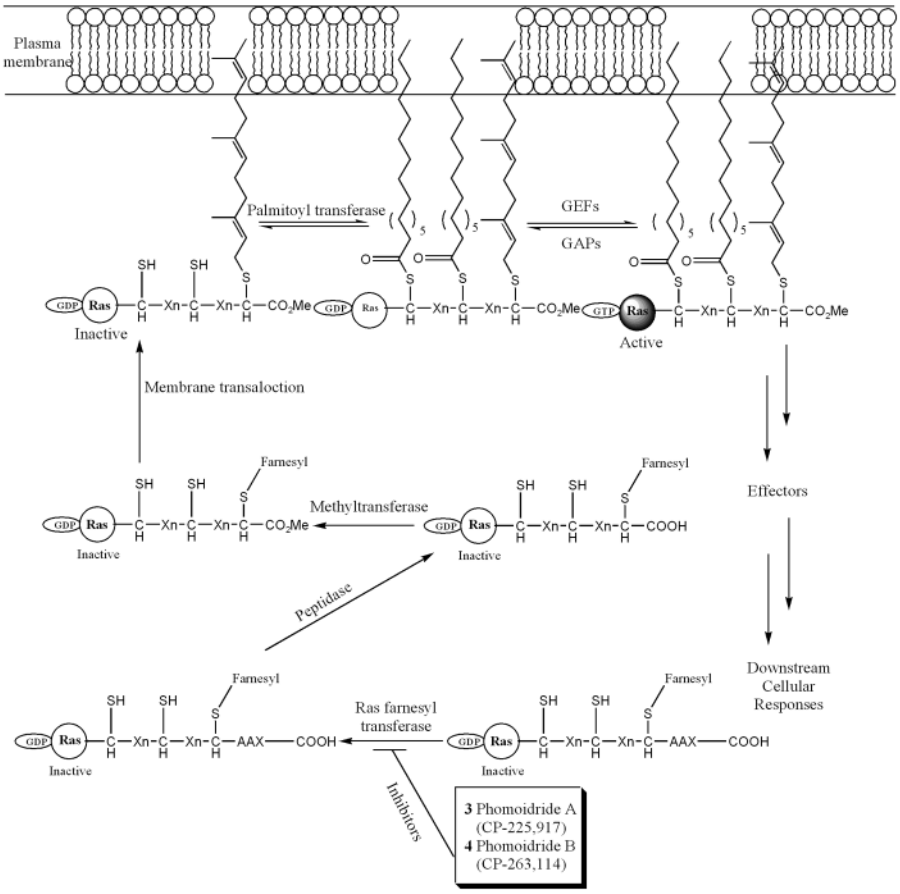


Figure 4. Simplified depiction of the post-translational processing and activation of H-Ras and its inhibition by phomoidrides (**3** and **4**). GEFs, guanine nucleotide exchange factors; GAPs, guanine triphosphatase activating proteins; GDP, guanosine diphosphate; GTP, guanosine triphosphate.

Table 3. Oncogenicity of Yoshida Ascites Sarcoma Cells in Vitro Contacted with Rubratoxin B[a]

no. of tumor cells	drug contact 30 min	average life span days ± SD	median survival time		D/T	LTS > 60 days
			T/C, days	ILS, %		
10^6	saline	11.1 ± 1.38			10/10	
10^6	DMSO (0.02 mL)	11.2 ± 1.2	11.2/11.1	0.9	10/10	
10^6	rubratoxin B (0.02 mg)	22.5 ± 0.7	22.5/11.1	102.7	2/10	8

[a] SD = standard deviation; T/C = treated/control; ILS = increased life span; D/T = dead/total; LTS = long-term survivors; DMSO = dimethylsulfoxide.

Table 4. Effect of Different Doses of Rubratoxin B Injected Intraperitoneally on the Yoshida Ascites Tumor Cells in Vivo[a]

no. of tumor cells (day 0)	drug (day + 1), mg/kg	average life span days ± SD	median survival time		D/T
			T/C, days	ILS, %	
10^6		11.1 ± 0.8			10/10
10^6	0.1	11.4 ± 0.55	11.4/11.1	2.7	10/10
10^6	0.2	13.8 ± 0.84	13.8/11.1	24.3	10/10
10^6	0.3	14.6 ± 2.3	14.6/11.1	31.5	10/10
10^6	0.4	15.6 ± 1.34	15.6/11.1	40.5	10/10

[a] SD = standard deviation; T/C = treated/control; ILS = increased life span; D/T = dead/total.

growth phase. No change in growth of *S. aureus* was observed for 6 h when tested against 2000 μg of **6** in Trypticase Soy broth; however, by the sixth hour, the *S. aureus* growing in the presence of **6** was very flocculant, and turbidity could not be determined. Growth of *T. pyriformis* and *V. aureus* was inhibited by less than 25 and 50 μg/mL, respectively.

4.1.2.2. Antitumor Activity[103]. 6 both in vitro (Table 3) and in vivo (Table 4) shows antitumor activity against Yoshida ascites sarcoma of the rat. The experiments in vivo demonstrated antitumor activity of rubratoxin B, but intraperitoneal administration of the drug, even at the highest dose, showed only an extension of the life span; however,

Table 5. Percentage Growth Inhibition of the Treated Weeds to That of the Untreated Weeds

percent inhibition (%)	rating	percent inhibition (%)	rating
0−10	0	51−70	3
11−30	1	71−90	4
31−50	2	91−100	5

Table 6. Herbicidal Activity of Cornexistin by Preemergence Soil Treatment

sort	weeds	dosage (active ingredient), ppm 500	100
gramineous weed	giant foxtail (*Setaria faberi* HERRM)	5	5
	large crabgrass (*Digitaria sanguinalis* (L.) SCOP.)	5	4
	Johnson grass (*Sorghum halepense* (L.) PERS.)	5	5
	barnyard grass	5	3
broadleaf weed	tail morning glory (*Ipomoea purpurea* (L.) ROTH)	5	4
	black nightshade (*Solanum nigrum* L.)	5	5
	velvetleaf (*Abutilon theophrasti* MEDIK)	5	5
	common cocklebur (*Xanthium pennsylvanicum* MALLR)	5	5

Table 7. Herbicidal Activity of Cornexistin by Foliar Treatment

sort	weeds	dosage (active ingredient), kg/ha 4	2
gramineous weed	giant foxtail	5	4
	large crabgrass	4	3
	Johnson grass	4	2
	barnyard grass	4	3
broadleaf weed	tail morning glory	4	3
	black nightshade	3	3
	velvetleaf	5	5
	common cocklebur	3	2

the survival of the animals was not influenced. The discrepancy between in vitro and in vivo data can be explained by the fact that **6** is a substance of biological origin that immediately after its administration can be metabolized in vivo by enzymes existing in the organism or can be bound to other cells or molecules and subtracted from the antitumor activity.

4.1.2.3. Rubratoxicosis. It was reported that **6** hinders cell proliferation[104,105] and induces apoptosis.[106−108] In addition, secretion of hepatic injury-related cytokines into the serum was detected in **6**-treated mice.[109] Also, cytokines appeared in the media of HL60, HepG2, and HuH-7 cells after **6** induced secretion of interleukin (IL)-8, macrophage colony stimulating factor (M-CSF), and granulocyte-macrophage (GM)-CSF.[110] Recently, Nagashima et al.[111] reported that an exogenous stimulus could induce secretion of M-CSF and GM-CSF in hepatocyte-derived cells, indicating that **6** ought to be an excellent model compound for studying the mechanisms of secretion of these cytokines.

4.1.3. Cornexistin and Hydroxycornexistin

In the course of new compound screening of microbial products for herbicidal efficacy a new compound named cornexistin (**7**) demonstrated promising activity as a postemergence herbicide with selectivity to corn.[14,112,113] The herbicidal activity of **7** against some species of common annual weeds by foliar treatment and by preemergence soil treatment is shown in Tables 5−7.[14,113] Plastic pots each 7.5 cm long, 20 cm wide, and 7 cm high were packed with soil, on which eight kinds of plants including four kinds of gramineous weeds and four kinds of broadleaved weeds were seeded, and covered with soil each to a depth of about 1 cm. The pot was buried in vermiculite in a box, which was placed on a bench in a greenhouse. Supplying water indirectly through vermiculite, the weeds were allowed to grow for about 2 weeks. At the end of this time, 5 cm^3 per pot of sample solutions of **7** or its sodium salt at various concentrations (previously prepared in the form of wet-table powders) was applied directly to the foliage of the weeds. All changes taking place after application of the herbicide were observed, and after 20 days the plants were examined and the effects were judged. However, corn seedings exhibited tolerance to **7**. This herbicidal spectrum suggests that the chemical may be useful for postemergence weed control with selective protection of corn.

7 also inhibited aspartate amino transferase (AAT, E.C. 2.6.1.1)[16] in vitro by only 20−30% at high concentration, only after incubation in a cell extract. In vitro incubation of duckweed (*Lemna pausicostata Hegelm.* 6746) protein extracts caused changes in AAT activity in patterns on polyacrylamide gels. **7** may be metabolized to an inhibitor of one or more AAT isozymes in vivo.

Later, hydroxycornexistin **8** was isolated from the broth of *Paecilomyces variotii* SANK 21086.[18,81] The herbicidal activities of **7** and **8** were compared in postemergence applications (Table 8). Although the level of inhibitory activity varied by species for **7** and **8**, the activity of the latter was particularly strong on broadleaf weeds. The species

Table 8. Herbicidal Activity of Cornexistin and Hydroxycornexistin[a]

sort	test species	cornexistin, ppm 31.2/5	15.6/3	7.81	hydroxycornexistin, ppm 31.2/5	15.6/3	7.81
broadleaf weeds	*Xanthium strumarium*	20	10	0	100	80	80
	Chenopodium album	30	30	30	90	80	20
	Ipomoea hederaceae	0	0	0	85	0	0
	Amaranthus sp.	0	0	0	80	60	0
	Abutilon theophrasti	30	60	0	70	40	40
	Polygonum convolvulus	90	40	20	100	100	40
crops	*Zea mays*	30	20	0	40	40	0
	Triticum aestevium	85	0	0	40	40	0
grass weeds	*Echinochloa crus-galli*	95	80	0	40	40	0
	Setaria faberi	20	20	20	70	0	0
	Sorghum bicolor	30	0	0	0	0	0
	Avena sativa	100	0	0	45	25	30
	Digitaria sanguinalis	20	20	0	80	20	20

[a] Injury was visually assessed on a scale of 0−100 where 100 represents complete plant death and 0 represents no effect.

Table 9. Activities of Zopfiellin for Inhibiting the Growth against Mold Fungi

pathogenic bacterium	minimum growth inhibition concentration (μg/mL)
Sclerotinia sclerotiorum	1.56
Sclerotinia cinerea	3.13
Botrytis cinerea	0.78
Rhynchosporium secalis	1.56
Aspergillus niger	3.13

Table 10. Inhibitory Activities of Zopfiellin against Microbes at pH 5.0

microbe	LD_{50}, μM
Botrytis cinerea	10
Collectotrichum. gloeosporioides	10
Collectotrichum. fragaria	10
Saccharomyce. cerevisiae	30
Collectotrichum. acutatum	30
Escherichia coli	>100
Fusarium oxysporum	>100

Xanthium stumarium (cocklebur), *Chenopodium album* (lambsquarter), *Ipornoea hederaceae* (morning glory), and *Polygonum convolvulus* (wild buckwheat) are very aggressive weeds yet appear quite sensitive to low concentrations of **8**.

4.1.4. Zopfiellin

Zopfiellin **9** is active against several fungi, bacterial, and yeast species and was especially effective against the plant pathogen *Botrytis cinerea*.[82,83,114] It was isolated in a discovery program searching for new types of fungicidal agents at Nissan Chemical. The antimicrobial activity is summarized in Table 9.

Later, pH modulation of zopfiellin antifungal to *Collectotrichum* and *Botrytis* was investigated by Futagawa et al.[114,115] **9** had strong inhibitory activity against *Collectotrichum* and *Botrytis* between pH 5.0 and 5.5, but the activity weakened dramatically at higher pH because of intramolecular ring closure of **9** from a tetracarboxylate to an anhydride form occurring at pH below 6.0. The inhibitory activities of **9** against microbes at pH 5.0 is shown in Table 10.

4.1.5. Heveadride and Dihydroepiheveadride

The incidence of life-threatening fugal infections has steadily increased in immunocompromised hosts such as HIV infected persons and cancer and transplant patients. Invasive pulmonary aspergillosis and *Pneumocystis carinii* pneumonia are a leading cause of deaths in bone marrow transplant

Table 11. Antifungal Spectra of Dihydroepiheveadride and Heveadride

		diameter of inhibition zone, mm			
		dihydroepiheveadride		heveadride	
sort	microorganisms	5 μg	100 μg	5 μg	100 μg
filamentous fungi	*Arthroderma benhamiae* IFM 41160	20		10	
	Aspergillus flavus IFM 41935	26		[a]	
	Aspergillus fumigatus IFM 41243	20			
	Aspergillus fumigatus IFM 41362	20			
	Aspergillus fumigatus IFM 47078	23			
	Aspergillus niger IFM 41398	21			
	Cladophialophora carrionii IFM 4808		26		
	Emericella nidulans IFM 46997	24			
	Epidermophyton floccosum IFM 46637	13		9	
	Fonsecaea pedrosoi IFM 4887				
	Fusarium oxysporum IFM 53787	10			
	Fusarium solani IFM 52712	12		9	
	Microsporum canis IFM 45108	25		10	
	Penicillium marneffei IFM 52703	21			
	Penicillium marneffei IFM 52697	11			
	Phialophora verrucosa IFM 4928				
	Scedosporium apiospermum IFM 52028	17		11	
	Trichophyton mentagrophytes IFM 40951	30		12	
	Trichophyton raubitschekii IFM 45579	20		12	
	Trichophyton rubrum IFM 45802	17		13	
	Trichophyton tonsurans IFM 5275	28		12	
	Trichophyton verrucosum IFM 46798	10			
	Trichophyton violaceum IFM 46913	25		16	
yeasts	*Candida albicans* ATCC 90028		16		15
	Candida albicans ATCC 90029		19		19
	Candida glabrata IFM 40217				
	Candida guilliermondii IFM 46823		8		
	Candida kefyr IFM 46921		12		8
	Candida krusei IFM 46834		15		
	Candida parapsilosis IFM 46863				
	Candida tropicalis IFM 46816				
	Cryptococcus neoformans ATCC 90112		14		11
	Saccharomyces cerevisiae IFM 40210		20		
	Pichia anomala IFM 53788				
	Trichosporon asahii IFM 48429	15		10	
	Trichosporon asteroides IFM 48608	10			
bacteria	*Bacillus subtilis* ATCC 6633				
	Eschrichia coli B				

[a] Means no inhibition.

Table 12. Antifungal Spectra of Scytalidin

sort	test microorganism	stock culture number (FSC)	width of inhibition zone, mm: scytalidin 100 μg	scytalidin 50 μg
Basidiomycetes	*Amylostereum chailletii* (Pers. Ex Fr.) Biodin	373	3	1
	Coniophora puteana[a] (Schum, Ex Fr.) Karst.	4	10	10
	Corticium laeve Pers. Ex Fr.	513	1	0
	Daedalea confragosa Bolt. Ex Fr.	140	9	8
	Daedalea unicolor Bull. Ex Fr.	141	8	5
	Fomes annosus[a] (Fr.) Karst.	142	4	2
	Fomes fomentarius (L. Ex Fr.) Kickx	125	5	4
	Fomes igniarius (L .Ex Fr.) Kickx	19	3	1
	Fomes pini (Thore Ex Pers.) Lloyd	413	11	10
	Fomes pinicola (Swartz Ex Fr.) Cke.	410	6	4
	Ganoderma applanatum (Pers. Ex Wallr.) Pat.	155	7	7
	Haematostereum sanguinolentum (Fr.) Pouzar	257	4	3
	Hirschioporus abietinus (Dicks. Ex Fr.) Donk	539	2	1
	Hirschioporus pargamenus (Fr.) Bond & Singer	463	2	1
	Lenzites saepiaria (Wulf. Ex Fr.) Fr.	424	7	4
	Odontia bicolor[a,b] (Alb. & Schw. Ex Fr.) Quel.	32	8	5
	Peniophora gigantea[a,b] (Fr.) Massee	526	16	15
	Polyporus adustus Willd. Ex Fr.	158	7	6
	Polyporus balsameus[a] Peck	442	15	12
	Polyporus schweinitzii[a] Fr.	179	14	11
	Polyporus tomentosus[a] Fr.	462	10	6
	Polyporus versicolor L. ex Fr.	182	5	4
	Poria carbonica Overh.	188	14	11
	Poria subacida[a] (Peck) Sacc.	202	5	4
	Scytinostrome galactina[a] (Fr.) Donk	425	8	4
Ascomycetes	*Ascocoryne sarcoides* (Jacq.) Tul.	474	6	4
	Cephaloascus fragrans Hanawa	501	21	18
	Ceratocystis[b] sp.	497	4	4
	Ceratocystis ulmi (Buism) C. Moreau	92	2	1
	Ceratocystis ulmi (Buism) C. Moreau	112	9	9
	Ceratocystis ulmi (Buism) C. Moreau	435	8	8
	Kirschsteiniella thujina (Peck) Pomerleau & Etheridge	454	1	0
	Nectria coccinea var. *faginata* Lohm., Wats. & Ayers	260	12	9
	Potebniamyces balsamicola Smerlis	535	8	6
	Scleroderris lagerbergii Gremmen	506	2	0
	Thyronectria balsamea (Cke. & Peck) Seeler	117	2	0
	Valsa friesii (Duby) Fckl.	274	6	5
Phycomycetes	*Phytophthora cinnamomi*[a] Rands	545	7	5
	Phytophthora infestans (Mont.) de Bary	544	11	10
	Phytophthora lateralis[a] Tucker & J.A. Milb.	471	15	12
	Pythium debaryanum Hesse	546	4	2
	Pythium irregulare Buis.	547	3	2
	Pythium mamillatum Meurs	548	4	3
	Pythium rostratum Butler	554	7	4
	Pythium salpingophorum Drechsler	549	4	2
imperfect fungi	*Aspergillus fumigatus*[b] Fres.	522	10	5
	Aspergillus niger[b] van Teigh	500	5	3
	Aureobasidium pullulens[b] (de Bary) Arnaud	499	5	2
	Candida albicans (Robin) Berk	507	2	0
	Cephalosporium[b] sp.	515	2	0
	Chrysosporium pruinosum[b] (Gilman & Abbot) Carmichel	511	2	0
	Chrysosporium[b] sp.	509	12	9
	Chrysosporium[b] sp.	460	4	3
	Cytospora decipiens Sacc.	429	7	5
	Graphium[b] sp.	525	6	5
	Libertella betulina Desm.	473	10	10
	Paecilomyces varioti[b] Bain	510	2	0
	Penicillium[b] sp.	520	8	7
	Phialophora melinii[b] (Nannf.) Conant	518	1	0
	Ptychogaster[b] sp.	461	5	5
	Trichoderma viride[b] Pers. ex Fr.	523	14	4

[a] Fungi associated with butt decay of coniferous trees. [b] Fungi associated with stein and deterioration of wood products.

recipients and in HIV-infected patients, respectively. Moreover, resistance to the azoles, which are the most widely used antifungals today, is attracting much attention. Therefore, there is a continuing need for new antifungal agents to overcome these fungal diseases. In the screening for new antifungal substances from fungal sources against pathogenic filamentous fungi, dihydroepiheveadride (**11**) was discovered as strong antifungal agents.[22,23] The antifungal activities of **11** and heveadride **10** against various fungi are listed in Table 11. **11** showed strong antifungal activity against various filamentous fungi including the human pathogens *A. fumigatus*, *P. marneffei* and dermatophytes *T. rubrum* and *T.*

Table 13. Antimicrobial Spectra of Tautomycin and Tautomycetin[a]

test organism	MIC, μg/mL tautomycin	tautomycetin
Sclerotinia sclerotirum	0.5	0.5
Botrytis cinerea IFO 5365	125	12.5
Alternaria mali IFO 8984	500	50
Colletotrichum lagenarlum IFO 7513	125	25
Glomerella cingulata IFO 9767	500	50
Rhizoctonia solani IFO 6258	125	200
Aspergillus niger	125	50
Pyricularia oryzae IFO 5994	125	50
Xanthomonas oryzae IFO 3312	32	
X. Citri IFO 3781	32	
Bacillus subtilis IFO 3513	>500	
Cochliobolus miyabeanus		25
Fusarium oxysporum		200
Penicillium chrysogenum		50

[a] The convention agar dilution method was used. Medium: PDA for yeasts and fungi; Bouillon agar for bacteria.

mentagrophytes at a concentration of 5 μg/paper disc (6 mm in diameter). However, the antifungal activity of deoxoepiheveadride **30** was almost not found against various filamentous fungi and yeasts, the same as that of **10**.

4.1.6. Scytalidin

Scytalidin **14** inhibited the growth of stain and decay fungi associated with deterioration of pulpwood chips in outside storage. The antifungal activity of **14** is shown in Table 12.[28−30,116]

4.2. Tautomycin and Tautomycetin

Tautomycin **1** and tautomycetin **2** both have good biological activities, including antimicrobial activity[2−4,60,89] and Ser/Thr protein phosphatase inhibitory activity.[49,52,117−143] Besides antifungal activities, they were found to induce morphological changes (bleb formation) of human leukemia cells K562 at a concentration of 0.1−1 μg/mL (tautomycin) and 3−100 μg/mL (tautomycetin), respectively.[1,3,89,130,131,144−146] Besides the above activities, **2** has immunosuppressive activity.[61−64,147]

4.2.1. Antimicrobial Activities[2—4,60,89]

In the course of screening of soil microorganisms for new antibiotics for agricultural use, strains of *S. spiroverticillatus* and *S. griseochromogenes* were found to produce new antibiotics, **1** and **2**, respectively, which have high activities against *Sclerotinia sclerotiorum* (both 0.5 μg/mL). **1** showed a good preventive effect to cucumber gray mold in pot tests as low as 6 μg/mL, but for **2**, the concentration was 25 μg/mL. The antimicrobial activities of **1** and **2** are summarized in Table 13. **2** showed no inhibitory activity against Gram-positive and Gram-negative bacteria tested. **1** and **2** are highly toxic to mice: LD_{50} are about 7.5 mg/kg and 35 mg/kg, respectively, when administered orally.

4.2.2. Inhibitory Activities for Ser/thr Protein Phosphatase[49,52,117—142,148]

Reversible phosphorylation is a key mechanism for regulating the biological activity of many human proteins that affect a diverse array of cellular processes, including protein−protein interactions, gene transcription, cell-cycle progression, and apoptosis. Once viewed as simple house keeping enzymes, recent studies have made it eminently clear that, like their kinase counterparts, protein phosphatases are dynamic and highly regulated enzymes. Therefore, the development of compounds that alter the activity of specific phosphatases is rapidly emerging as an important area in drug discovery. Because >98% of protein phosphorylation occurs on serine and threonine residues, the identification of agents that alter the activity of specific serine/threonine phosphatases seems especially promising for drug development in the future. Some inhibitors of serine/threonine phosphatases (PP1−PP6) have been discovered and identified, including **1** and **2**. Their inhibitory activities are listed in Table 14 with other inhibitors. Okadaic acid, a polyether fatty acid for the marine black sponge *Halichondria okakai* was first identified as a small molecular weight inhibitor of PP1 and PP2A and extensively studied.[149] Since then, about 40 compounds, which inhibit PP1 as well as PP2A, have been identified and purified. Using natural compounds, numerous experiments were performed to analyze the roles of PPs in various cellular events.[149] IC_{50}'s of these phosphatase inhibitors were almost the same for PP1 and PP2A, with exceptions such as Okadaic acid,[150,151] **1** and **2**. On the other hand, **1** and **2** are rather unique among the compounds in that they binds to and inhibit PP1 with higher affinity than they do PP2A. The ratio of the IC_{50} of **1** and **2** for PP2A to that for PP1 (the PP2A/PP1 ratio of IC_{50}) was reported to be within the range 2−50[52,152−154] and 39,[141] respectively. The structural basis for this is not yet clear. However, studies with structural analogues suggest that the higher affinity for PP1 may originate from the hydrophobic segment (C-1−C-16).[155] The results of Table 14 also demonstrate that **2** is the most specific PP1 inhibitor out of over 40 species of natural phosphatase inhibitors reported.

4.2.3. Immunosuppressive Activity of Tautomycetin

Tautomycetin **2** was identified recently as an immunosuppressor of activated T cells in organ transplantation.[61−64,147,165−167] Organ transplantation to replace diseased organs has become the standard treatment in terminal organ

Table 14. Inhibitory Activities of Tautomycin, Tautomycetin, and Other Inhibitors for Serine/Threonine Protein Phosphatases

compound	inhibition of ser/thr protein phosphatase activity (IC_{50}), nM PP1	PP2A	PP2B (calcineurin)	PP4	PP5*	PP7
tautomycin	0.21	0.94	>1 μM	0.2	10	ND
tautomycetin	1.6	62	ND	ND	ND	ND
okadaic acid	20−50	0.1−0.3	~4 μM	0.1	3.5	>1 μM
microcystin-LR microcystin-RR [156,157]	0.3−1	<0.1−1	~1 μM	0.15	1.0	>1 μM
nodularin [156,158]	2.4	0.3	>1 μM	ND	ND	>1 μM
calyculin A [159,160]	0.4	0.25	>1 μM	0.4	3	>1 μM
cantharidin [161,162]	1.1 μM	194	>10 μM	50	3.5 μM	ND
fostriecin [163,164]	45−48 μM	1.5−5.5	>100 μM	3.0	70 μM	ND

Table 15. In Vivo Immunosuppressive Effect of Tautomycetin on Graft Survival after Heterocardiac Transplantation in Rat

drug	conc, mg/kg	injection	survival days	no. of rats
cremophor-EL only	5	i.p.	9	4
	5	i.p.	10	3
CsA/cremophor-EL	5	i.p.	>100	2
tautomycin/PBS	0.05	i.p.	10	3
tautomycetin/PBS	0.05	i.p.	>160	12
tautomycetin/ME	0.05	i.v.	>160	8

Table 16. Suppressive Activity of Tautomycetin for Mouse Mixed Lymphocyte Reactions

	MLR (IC_{50}, nM)
tautomycetin	43.0
cyclosporine A	25.0

Table 17. Suppressive Activity of Tautomycetin against IL-2 Expression Stimulated by PMA and Ionomycin

	conc, μg/mL	suppressive activity	IC_{50}, μg/mL
tautomycetin	1	98	0.003
	0.1	88	
	0.01	83.9	
	0.001	12.8	
cyclosporine A	1	98.7	0.02
	0.1	87.6	
	0.01	24.8	
	0.001	7.4	

Table 18. Suppressive Activity of Tautomycetin against IL-2 Expression Stimulated by T Cell Receptor-Mediated Signaling

	concentration, μg/mL	suppressive activity	IC_{50}, μg/mL
tautomycetin	10^{-7}	74.8	6.6×10^{-9}
	10^{-8}	46.8	
	10^{-9}	37.5	
cyclosporine A	10^{-6}	96.2	4.0×10^{-7}
	10^{-7}	0.2	

Table 19. Suppressive Effect of Tautomycetin on Graft Rejection

	dose, mg/kg	duration of heart beat, day
cremophor RH-40 (control)		7.8
tautomycetin injection	0.01	>50
	0.03	>50
	0.1	>50
cyclosporine A injection	5	>50

Table 20. Inhibitory Activities of Chaetomellic Acids for PFTase, GGPTase-I, and GGPTase-II

	IC_{50}, nM			
compounds	human PFTase	bovine PFTase	GGPTase-I	GGPTase-I
chaetomellic acid A	55	35	92000	34000
chaetomellic acid B	185	100	54000	ND

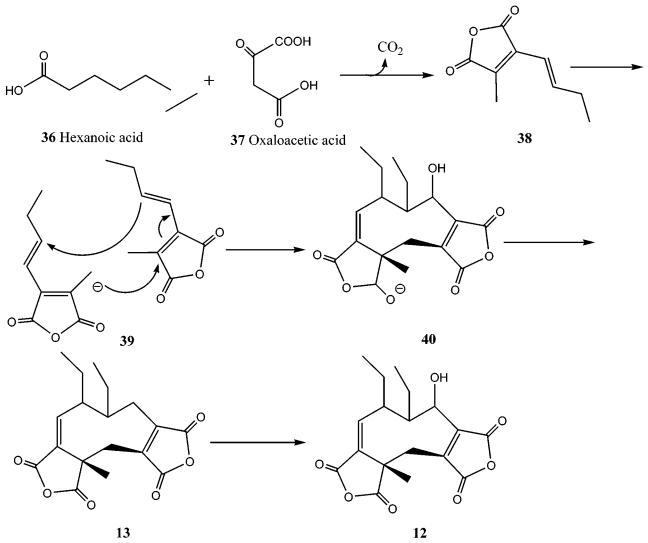
failure, such as in renal, hepatic, and cardiac diseases.[165] In most cases, induction of T cell-mediated immune responses to the highly polymorphic MHC molecules on nucleated cells in the grafted organ is the major barrier to successful transplantation. Improvement in graft survival is accomplished by precise HLA typing capability, greater surgical experience and skill, and the potential of cloned animals as organ donors. But genetic differences at loci other than MHC still trigger rejection. Therefore, continuous efforts to

Table 21. Inhibition of Cytosolic and Microsomal Leucine Aminopeptidases (Leu-APc, E.C. 3.4.11.1 and Leu-APm, E.C. 3.4.11.2) and Carboxypeptidase A (CP-A, E.C. 3.4.17.1) by Tyromycin and Comparison with Aminopeptidases Bound to the Cell Surface of HeLa Cells (HeLa-AP)

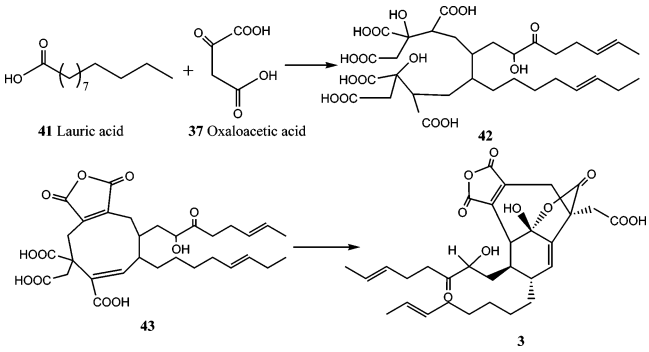
enzyme	substrate (50 μM)	IC_{50} (μg/mL)
Leu-APc (1mU/mL)	Leu-AMC	31
	Phe-AMC	25
Leu-APm (1mU/mL)	Leu-AMC	41
	Phe-AMC	27
CP-A (5mU/mL)	Hip-L-Phe [a]	60
HeLa-AP [b]	Leu-AMC	20
	Bzl-Cys-AMC	7

[a] 2 mM. [b] Cells corresponding to 300 μg of protein/mL.

Scheme 1. Proposed Biosynthetic Mechanisms of Glaucanic Acid and Glauconic Acid

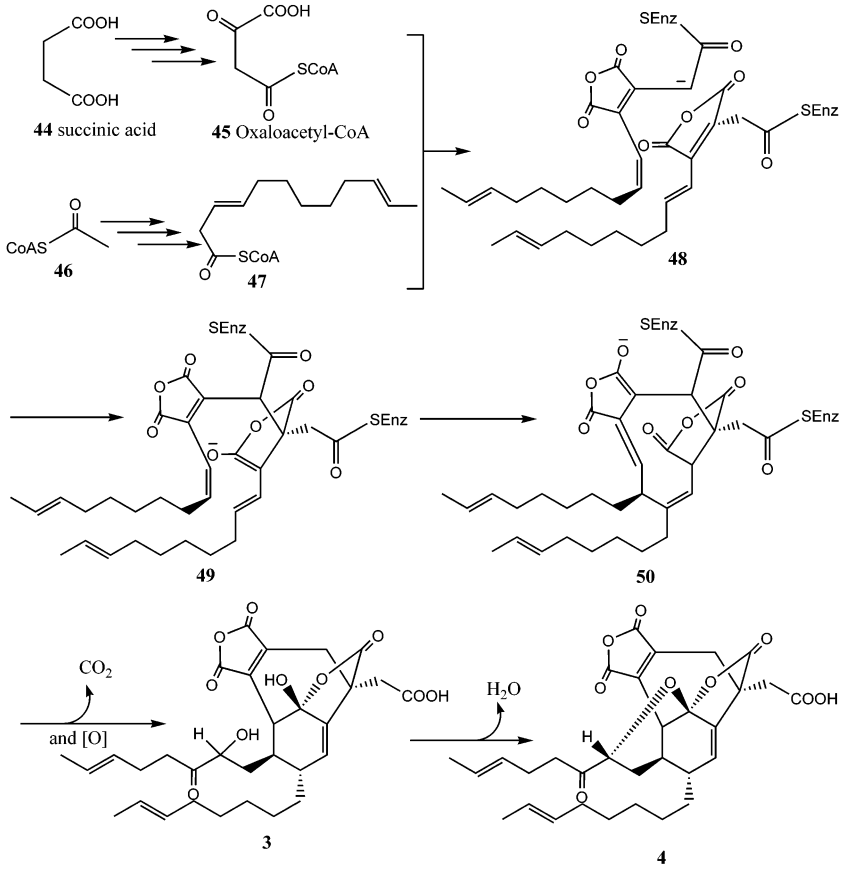


Scheme 2. Proposed Biosynthetic Pathway of Phomoidrides



discover effective and specific immunosuppressive agents have been intense. The systematic study of products from bacteria and fungi has led to the development of immunosuppressive drugs such as cyclosporine A, FK506 (tacrolimus), and rapamycin. Cyclosporine A and FK506 block T cell activation by preventing the induction of IL-2 gene expression, whereas rapamycin blocks the signaling pathway triggered by IL-2 receptor. They exert their pharmacological effects by binding to the immunophilins, and the immunophilin and drug complex binds and inhibits the Ser/Thr phosphatase calcineurin, which is activated when the intracellular calcium ion level rises on T cell activation. These drugs are effective immunosuppressive agents, but they are not free of problems. Because calcineurins are found in many cells, these drugs are expected to be deleterious in many other

Scheme 3. Sulikowski's Proposed Biosynthesis of Phomoidrides



tissues, such as kidney and liver. Therefore, it is important to develop new immunosuppressors with minimal toxicity that target molecules specifically involved in immune responses. It would also be helpful to have more than two different immunosuppressive drugs with different pharmacological effects. **2** is an activated T cell-specific immunosuppressor. It inhibited the induction of tyrosine phosphorylation of T cell-specific signaling mediators in T cell receptor (TcR) proximal signal transduction pathway, leading to induction of apoptosis. The in vivo study results are summarized in Table 15.

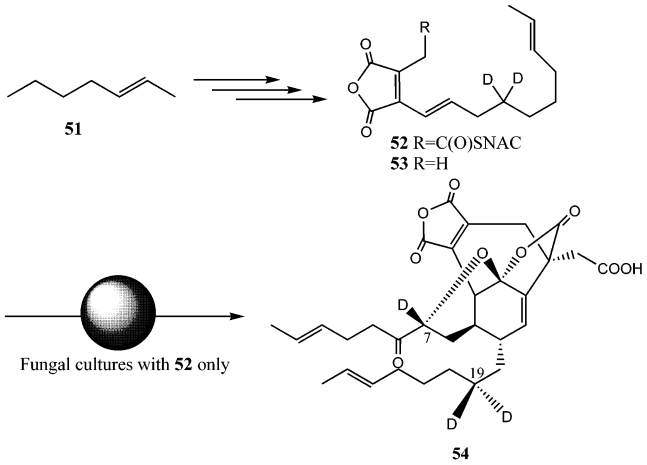
The suppressive activity against mouse mixed lymphocyte reaction, the expression of inerleukin-2 (IL-2) stimulated by PMA and ionomycin, the expression of inerleukin-2 (IL-2) stimulated by T cell receptor-mediated signaling, the tyrosine phosphorylation of intracellular proteins, expression of cell surface antigen CD90, and graft rejection in organ transplantation are listed in Tables 16−19.

From the above results, **2** was more effective than cyclosporine A. Therefore, **2** may be used as an active ingredient of an immunosuppressant in the near future.

4.3. Chaetomellic Anhydrides

Chaetomellic anhydride A (**16**) and chaetomellic anhydride B (**32**) are both strong inhibitors of farnesyl protein transferase (PFTase), geranylgeranyl-protein transferase type I (GGPTase-I), and geranylgeranyl-protein transferase type II (GGPTase-II). Ras genes with point mutations are associated with unregulated cellular growth.[33,35,168−173] They are found in approximately 30% of all human tumors and are the most common oncogenes associated with human carcinogenesis. More than 30% of lung, 50% of colon, and 90% of pancreatic

Scheme 4. Sulikowski's Deuterium Labeling Experiments



carcinomas present with mutated oncogenic forms of Ras. This high prevalence makes Ras an attractive target for antitumorigenic therapy. Polyisoprenylation of Ras, a modification necessary sufficient and required for its correct cellular localization and biological activity as well as for cell transformation, is catalyzed by PFTase (See Figure 4). This is the first step in a series of processing events required for the stable association of $p21^{ras}$ with the cell membrane. When isoprenylation is blocked, loss of transforming activity is observed. Inhibitors of PFTase are able to block Ras-dependent tumorigenesis and are potentially useful anticancer agents. Farnyl diphosphate preferentially serves as a substrate for PFTase versus other mammalian prenyl-protein tranferases including GGPTase-I and GGPTase-II. Similarly, some CAAX sequences preferentially serve as substrates for

PFTase, while others function best as substrates of GGPTase-I or GGPTase-II. Therefore, the activity regulation of GGPTase-I and GGPTase-II is also very important. The inhibitory activity of chaetomellic acids for PFTase, GGPTase-I, and GGPTase-II are summarized in Table 20.

4.4. Other Compounds

4.4.1. Puberulonic Acid and Stipitotanic Acid

Puberulonic acid (**17**) and stipitotanic acid (**18**) were first discovered as the antimicrobial agents.[39,41,93,174,175] Recently, **17** was also identified as the inhibitor for inositol monophosphatase (IMPase, E.C. 3.1.3.25). IMPase hydrolyzes all *myo*-inositol monophosphates arising from the second messenger *myo*-inositol 1,4,5-trisphophate (Ins(1,4,5)P3) in the phosphoinositide cycle, as well as from the de novo synthesis of L-*myo*-insitol 1-phosphate (L-Ins(1)P) from glucose 6-phosphate. The uncompetitive inhibition of IMPase by lithium ion has led to the hypothesis that the enzyme might be the target of lithium therapy. However, the narrow therapeutic window and the side effects often associated with lithium treatment have somewhat detracted from the value of the drug. In addition, other antipsychotic drugs are often required for patients suffering from acute mania, during the 7−10 days that it takes for lithium to exert its antimanic effect. Thus, several laboratories have initiated the search for other inhibitors of the enzyme. The inhibitory activity of **17** for IMPase was IC_{50} of 10 μM.

4.4.2. Tyromycin A

No antimicrobial (*Acinetobacter calcoaceticus*, *Bacillus brevis*, *B. subtilis*, *Micrococcus luteus*, *Mucor miehei*, *Naematospora coryli*, *Paecilomyces varioti*, *Penicillium notatum*) or cytotoxic (KB, ATCC CCL 17; BHK, ATCC CCL 10; HeLa S3 ATCC CCL 2.2 cells) activities could be detected for **22**. **22** strongly inhibits both the Leu-AMC and Bzl-Cys-AMC hydrolyzing activities of HeLa S3 cells.[44,100,176] The K_i values were determined to 4×10^{-5} M and 1.3×10^{-5} M. The inhibitory action of **22** on the cytosolic and microsomal leucine aminopeptidases of porcine kidney and on carboxypeptidase A of bovine kidney are shown in Table 21. All three enzyme inhibiting activity of **22** is dependent on the two maleic acid anhydrie moieties.

Other compounds with maleic anhydride not listed above were all antibiotics. From the description above, conclusion could be drawn that the natural products with maleic anhydride structure have strong biological activities. They are potent to be developed as drugs, antifungal agents, biopesticides, and herbicides.

5. Biosynthesis Studies

5.1. Nonadrides

Various research groups have studied the biosyntheses of members of the nonadrides family. Studies performed in the Barton and Sutherland research groups on the biosyntheses of **12** and **13** have proved that these nonadride natural products are produced in vivo by the head-to-head dimerization of a nine-carbon anhydride (Scheme 1).[58,177−181] This nine-carbon anhydride, in turn, is thought to arise biosynthetically from the condensation of hexanoic acid (**36**) and oxaloacetic acid (**37**). A key intermediate in their proposal was unsaturated anhydride (**38**) derived from the condensation of hexanoic acid and oxaloacetic acid. The head-to-head dimerization of this C-9 unit (**38**) was proposed to give glaucanic acid **13**. The dimerization itself was proposed to occur by addition of confugate base to **38** leading to a formal $6\pi + 4\pi$ cycloaddition affording *cis*,*cis*-cyclononadienolate (**40**).

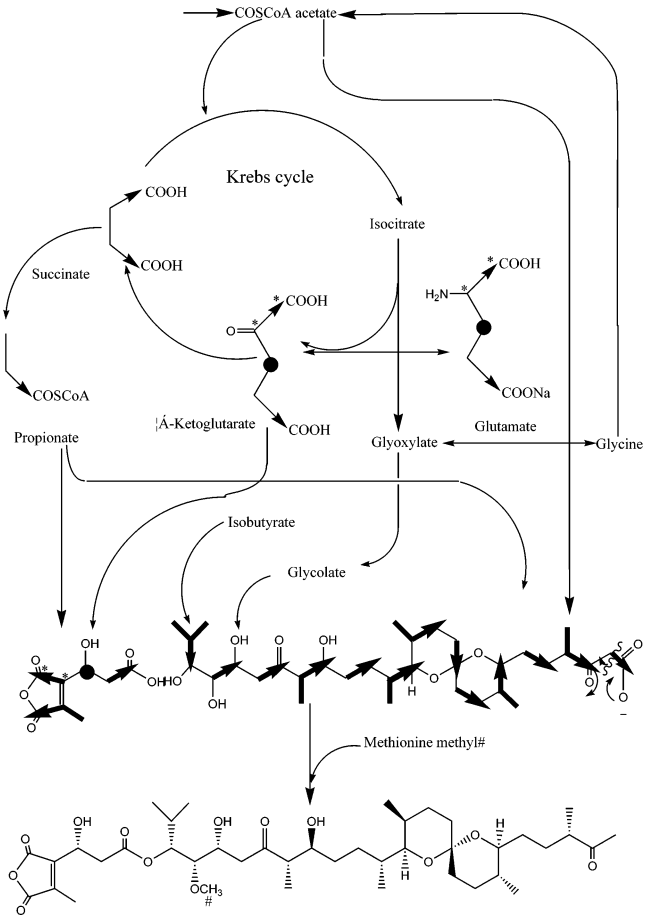


Figure 5. Biosynthesis of tautomycin. ●: derived from the C-2 methyl carbon of [2-^{13}C]acetate. ^{13}C Enrichment ratios at C-1′ and C-2′ are higher than those at C-4′ and C-7′.

Current biosynthetic proposals toward the phomoidrides arise directly from some of above work. In the second isolation report of the phomoidrides,[5,7] Kaneko and coworkers draw a direct analogy to this early work of Barton and Sutherland by proposing a series of biosynthetic disconnections tracing the phomoidrides back to lauric acid **41** and **37** (Scheme 2). These researchers speculate a head-to-head dimerization of two 16-carbon units to provide a maleic anhydride-containing nine-membered ring **43** via a stepwise mechanism proceeding through intermediate **42**. Homoenolate attack of the C26 caroxylate by C10 in **43** and tautomerization to form a pseudoacid leads to **3**.

In the initial investigations into phomoidride biosynthesis, Sulikowski and co-workers point out that two questions remain unanswered by Kaneko's model. First, it is not predictive of the (*Z*)-geometry in the phomoidride C15−C16 double bond. Unlike in **3** and **4**, the C4−C5 double bond in **12** is of the (*E*)-geometry, as is that between C15 and C16 in Kaneko's proposed intermediate 43. Second, the relative stereochemistry between C14 and C17 in **3** is opposite to that between the analogous centers in **12** (C6 and C3).

To clarify this apparent discrepancy, the Sulikowski group proposed a modification of Kaneko's ideas, drawing an

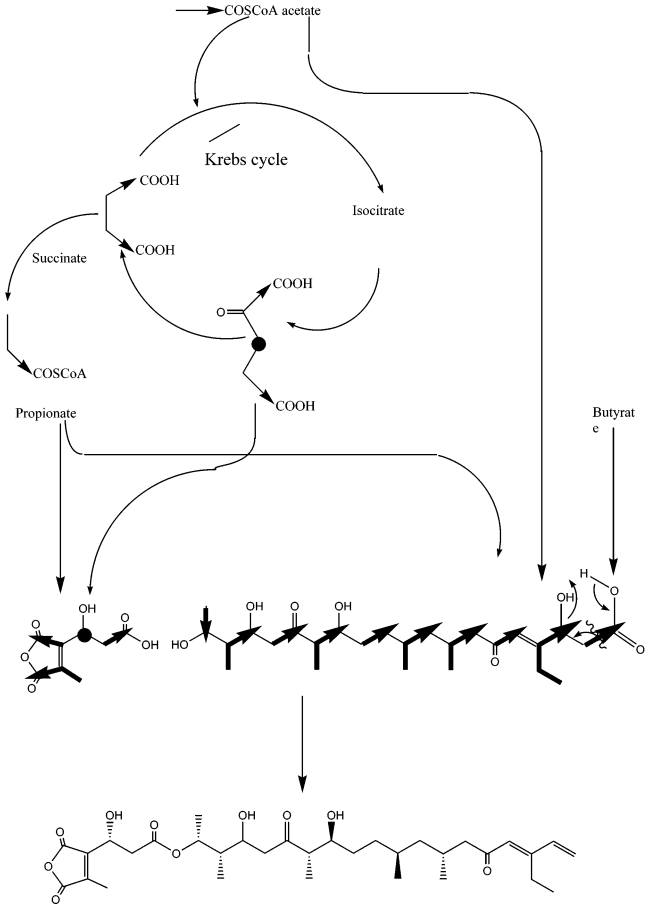
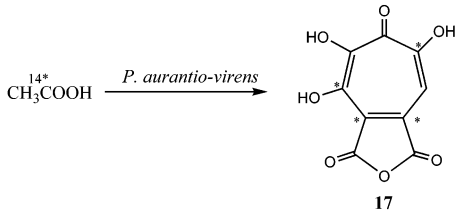


Figure 6. Biosynthesis of tautomycetin. ●: derived from the C-2 methyl carbon of [2-^{13}C]acetate.

analogy to polyketide and fatty acid biosynthesis (Scheme 3).[5,182] They suggest that two 16-carbon anhydrides are covalently attached to an enzyme via a thioester linkage **48** and are thus preorganized to undergo a stepwise dimerization process to yield the phomoidride skeleton. This hypothesis was investigated using ^{13}C-labeling studies. By incubating fungal cultures with ^{13}C-labeled succinic acid and acetyl-CoA derivatives, Sulikowski demonstrated that carbons C12−C14 and C27−C30 in **4** all originated from carbons found in succinic acid, and the remaining carbons could be traced back to acetyl-CoA **46**. These results supported their biosynthesis route, in which anhydrides **48** are formed from the condensation of a 12-carbon piece derived from **47** and the 4-carbon-containing oxaloacetyl-CoA **45**. In turn, **45** is known to be derived biosynthetically from succinic acid **44**, and **47** is postulated to originate from **46**. The proposed sequential Michael additions (**48** → **49** and **49** → **50**) followed by Dieckmann type cyclization, loss of CO_2, and oxidation to give **3** provide a reasonable mechanism for the formation of the phomoidride carbocyclic core but do not provide direct evidence for any of the individual steps in the pathway.

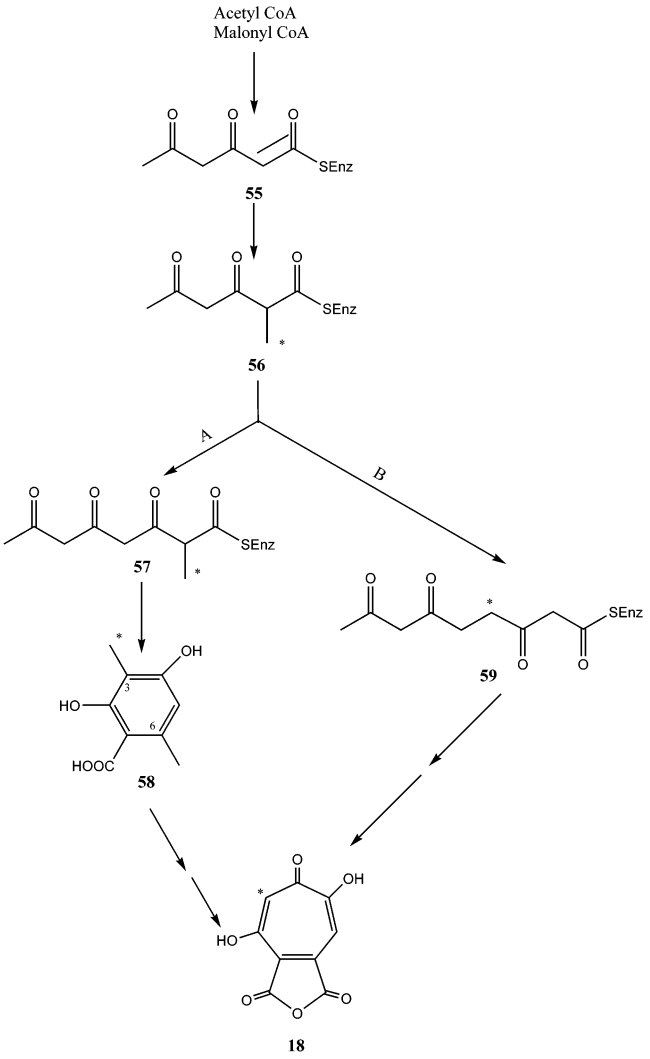
In a later paper, Sulikowski went on to test the proposed decarboxylative homodimerization mechanism through deuterium-labeling studies with maleic anhydride **52** (Scheme 4).[5,183] In these experiments, **52** was synthesized starting with hexenyl iodide **51** in 15 steps and fed to cultures of ATCC 74256, the producing fungus of the phomoidrides. Isolates of **4** from these fungal broth **54** revealed deuterium incorporation at C7 and C19 of the natural product as detected by ^{2}H NMR spectroscopy and confirmed by electrospray

Scheme 5. Biosynthesis of Puberrulonic Acid



17

Scheme 6. Biosynthesis Pathways of Stipitatonic Acid

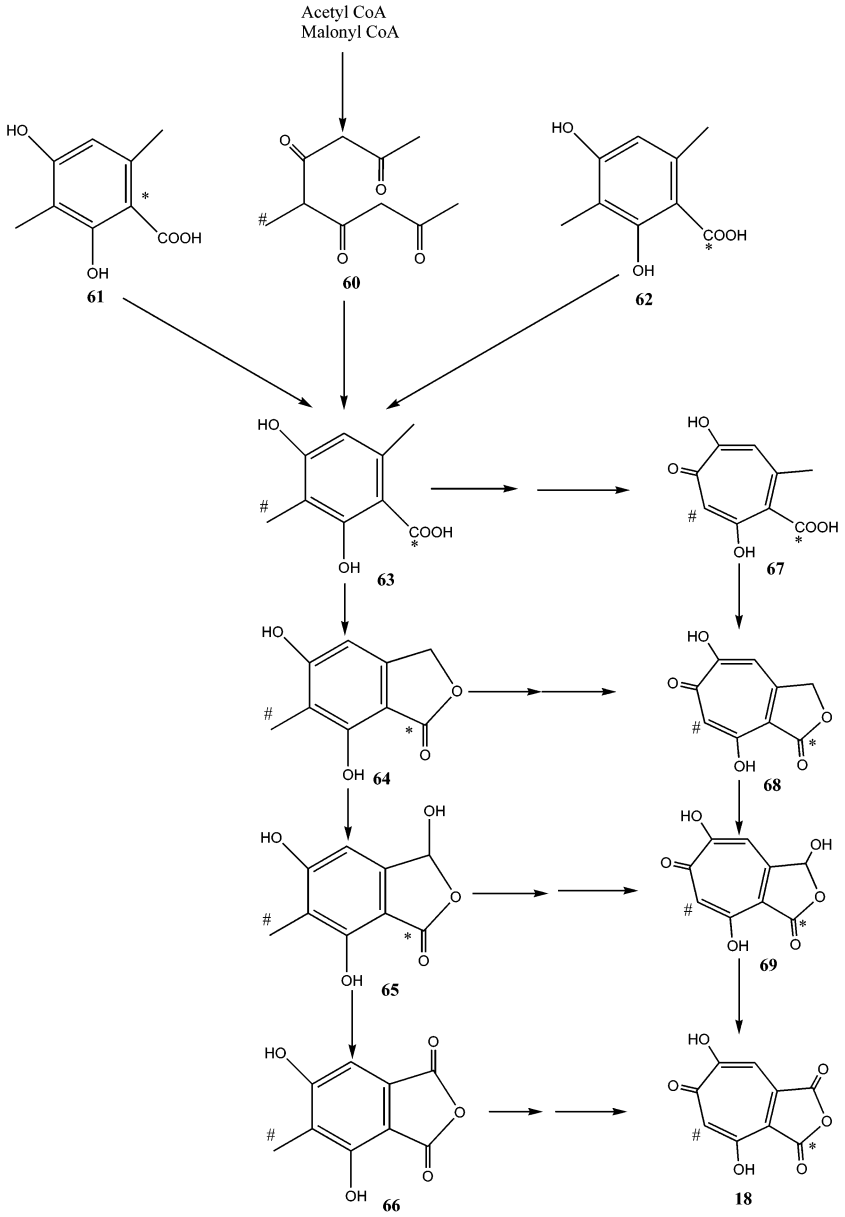


mass spectrometry. Feeding decarboxylated anhydride **53** to ATCC 74256 cultures failed to yield any deuterium incorporation into phomoidride isolates. These results are consistent with the proposed biosynthetic pathway depicted in Scheme 4.

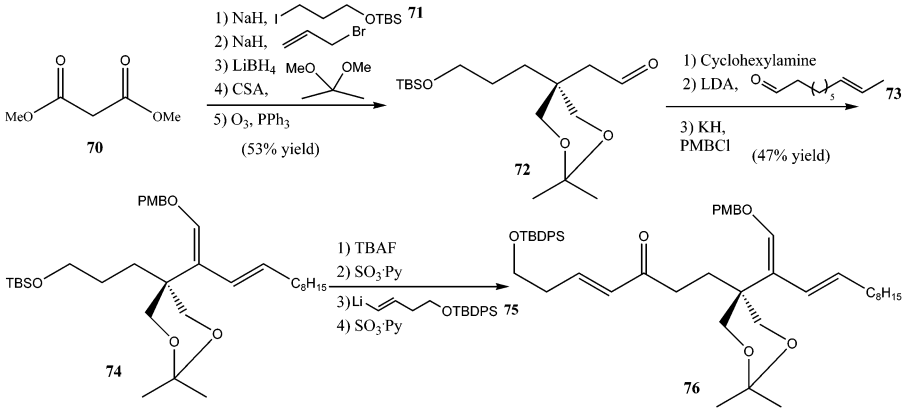
5.2. Tautomycin and Tautomycetin

Isono research group investigated the biosynthesis of the dialkylmaleic anhydride-containing antibiotics tautomycin and tautomycetin in 1995 with feeding experiments with ^{13}C labeled precursors.[184] In the feeding experiments, a tautomycin-producing strain *S. spiroverticillatus* sp. JC-84−44 and a tautomycetin-producing strain *S. griseochromogenus* sp. JC-84−1233 were employed for the investigation of biosynthesis pathways for tautomycin and tautomycetin. The biosyntheses of tautomycin and tautomycetin are depicted in Figures 7 and 8, respectively.

Scheme 7. Biosynthesis Pathways of Stipitatonic Acid

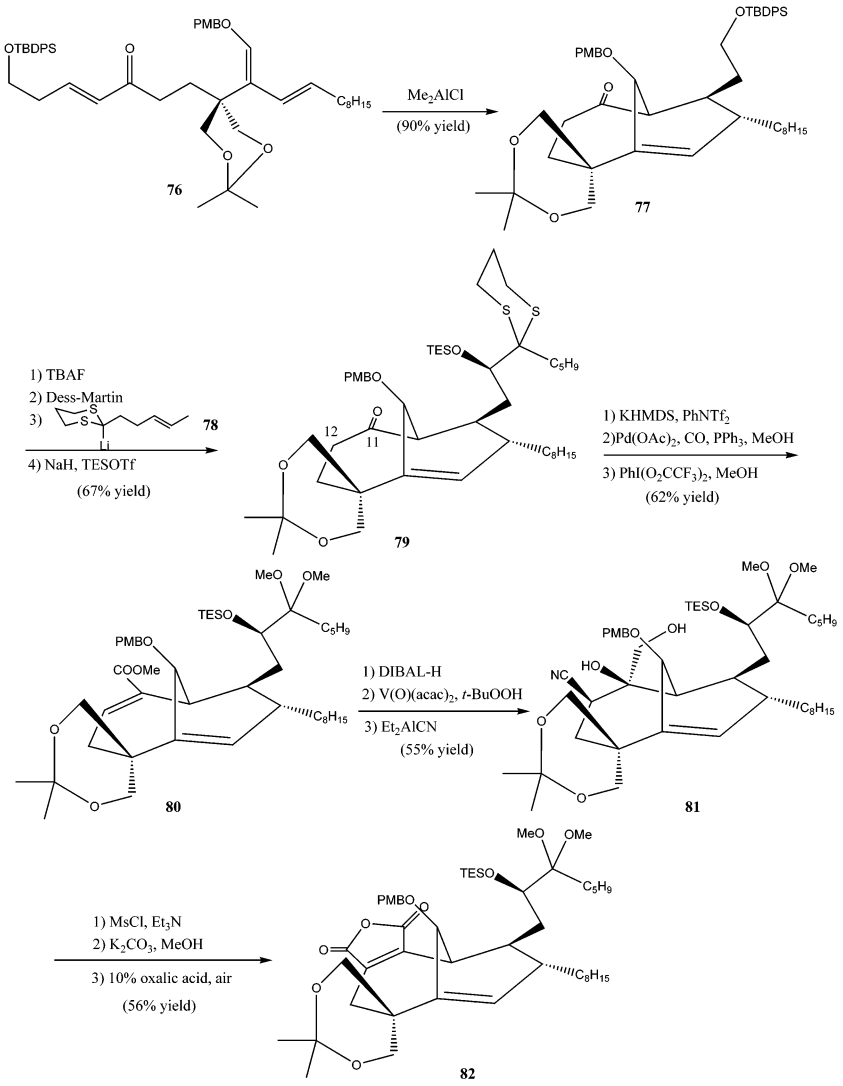


Scheme 8. Construction of the Diels−Alder Precursor



From the results of the Isono research group, the biosynthesis of tautomycin was supposed as follows. The right half chain of the tautomycin molecule is synthesized by a polyketide pathway that starts with isobutyrate followed by the introduction of a glycolate unit, and then five acetate and five propionate units. The terminal methyl carbon (C-1) was derived from [2-^{13}C] acetate, which may be metabolized to β-keto carboxylic acid, after which the terminal methyl detone is formed by decarboxylation. The left half of the tautomycin, the dialkylmaleic anhydride moiety, is

Scheme 9. Cycloaddition and Anhydride Formation



synthesized from one propionate and C-5 unit. C-1′ and -7′ were derived from sodium [1-^{13}C] acetate, and carbons 2′, 3′, and 4′ were derived from sodium [2-^{13}C] acetate. This labeling pattern suggests strongly that the C-5 unit may come from α-keto glutarate which is formed from acetate through Krebs cycle (Figure 5).

The right half chain of the tautomycetin molecule is formed via the polyketide pathway which starts with acetate followed by introduction of three acetate and four propionate units and one butyrate unit. The terminal methyl carbon (C-1) was derived from [2-^{13}C] acetate. The left half dialkylmaleic anhydride moiety has the same structure as that of tautomycin and gave a similar enrichment pattern. As described above, C-1′ and -7′ were derived from sodium [1-^{13}C] acetate and carbons 2′, 3′, and 4′ were derived from sodium [2-^{13}C] acetate. This labeling pattern suggested that the anhydride moiety was C-5 unit derived from α-keto glutarate as in the case of tautomycin (Figure 6).

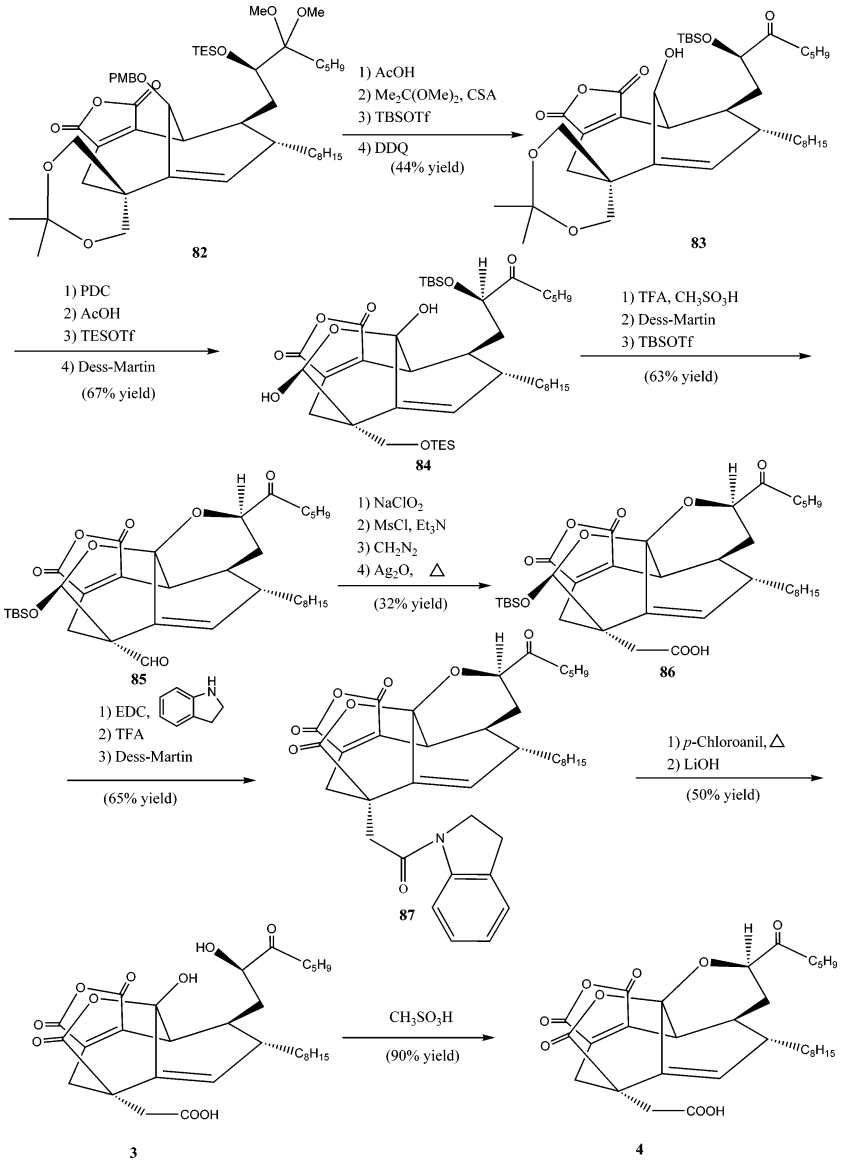
Since the absolute configuration of the dialkylmaleic anhydride moiety of tautomycetin is identical with that of tautomycin, both moieties may be biosynthesized by similar enzyme systems.

5.3. Puberulonic Acid and Stipitotanic Acid

Puberulonic acid (**17**) and stipitotanic acid (**18**) are the fungal tropolones. The biosynthesis of **17** was studied by Scott et al. using tracer experiments with *Penicillium aurantio-virens*.[185] [1-^{14}C]-Sodium acetate was fed to growing cultures of *P. aurantio-virens* NRRL 2138 and the radioactive **17** was isolated (Scheme 5). The ratio of specific activities that C-9 of **17** originates from C-1 of actetate. The corrected labeling pattern of **17** and the structural analogy suggest that **17** are biosynthesized from the precursors such as one acetate, three malonates, and one methionine.

It has been recognized for over decades that the tropolone system of the *Penicillium stipitatum* metabolites is formed from a combination of acetate and malonate units with the insertion of a "C1" unit at a hitherto undetermined stage.[186−197] Two principal pathways have been suggested for the biogenesis of stipitotanic acid **18** in *P. stipitatum* (Scheme 6). In the first of these (Scheme 6, path A),[97,195,196] 3-methylorsellinic acid **58** (or a closely related species) is postulated to undergo an oxidative ring expansion reaction leading to stipitatonic acid, biochemical oxidation of the original C6 substituent ($CH_3 \rightarrow COOH$) taking place at an undetermined point in the pathway. In a second postulate (Scheme 6, path B),[189] the seven-membered ring is formed directly from a modified C9 precursor **59**, by way of a methylene insertion reaction on **56** reminiscent of the vitamin B12 coenzyme mediated methylmalonyl → succinyl isomerization. Later, the proposed biosynthesis pathway of **18** was investigated

Scheme 10. Completion of the Total Synthesis of Phomoidrides



as path A, but more in detail (Scheme 7), employing the ^{13}C labeled precursors.[187]

To date, the biosyntheses of other natural products with maleic anhydride structure have not reported.

6. Chemical Syntheses

Because this group of compounds has strong biological activities, many research groups contributed their efforts to synthesize them, especially for **1**−**4** and **16**.

6.1. Phomoidrides

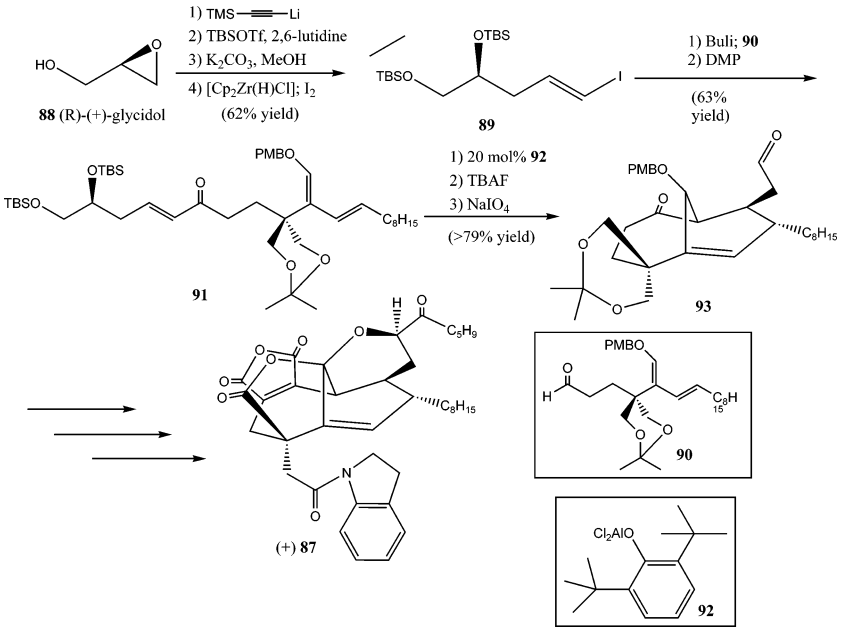
Since initial reports of the isolation of phomoidrides A and B, many synthetic chemists have traveled the arduous road toward their total syntheses.[5,66,198−223] Because of their biological activities and wide array of intriguing structural features, five total syntheses have been developed in recent years. Furthermore, a review was reported about the synthesis of phomoidrides.[5]

6.1.1. Nicolaou Research Group

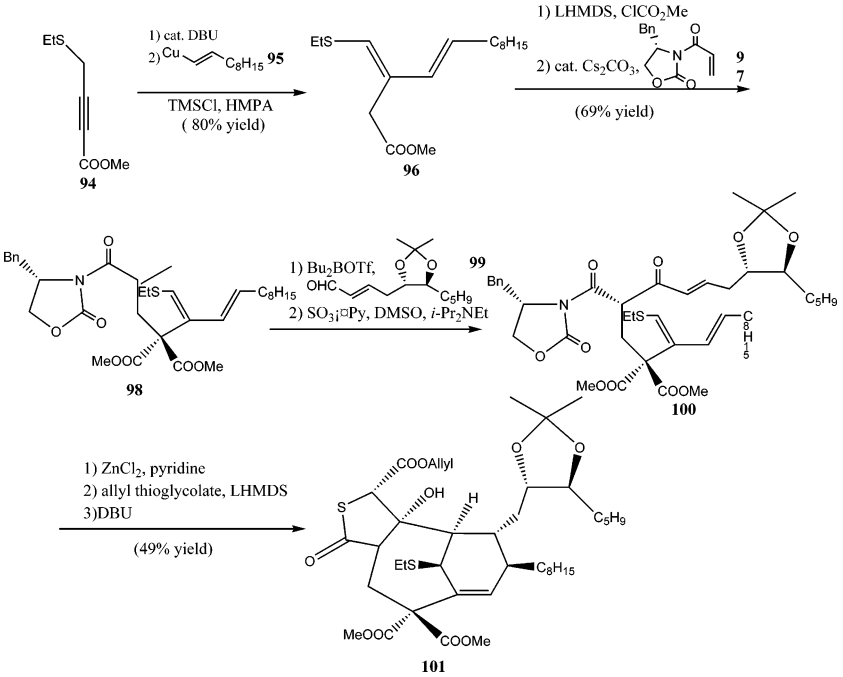
The first total synthesis was reported by the Nicolaou research group at Scripps[5,207−215,224−226] and incorporates as its key step a type II IMDA reaction to form the phomoidrides' bicyclo[4.3.1]decene core. Other noteworthy aspects of the approach include a unique tandem sequence to form the maleic anhydride, as well as an Arndt-Eistert homologation to elaborate the C14 quaternary center. Nicolaou's synthesis also gave rise to a number of novel chemical methods. These include two new one-carbon homologation methods and a range of new applications for iodine (V)-mediated oxidation chemistry.

Nicolaou's total synthesis (Scheme 8) commenced with the alkylation of commercially available dimethyl malonate **70** with iodide **71** and allyl bromide. The two esters were fully reduced, and the resulting diol was protected as its acetonide. Subsequent ozonolysis of the allyl group revealed aldehyde **72**. Next, the requisite diene for the IMDA reaction was assembled. Reaction of the cyclohexyl enamine of **72** with aldehyde **73** provided the corresponding enone, which was further reacted with potassium hydride and *p*-methoxybenzyl chloride to give diene **74** as the major product. Finally, deprotection of the primary TBS ether, oxidation to the aldehyde, addition of vinyllithium reagent **75**, and reoxidation to the α,β-unsaturated ketone completed the assembly of Diels−Alder precursor **76**.

Scheme 11. Nicolaou's Asymmetric Total Synthesis



Scheme 12. Fukuyama's Construction of the Bridgehead Olefin

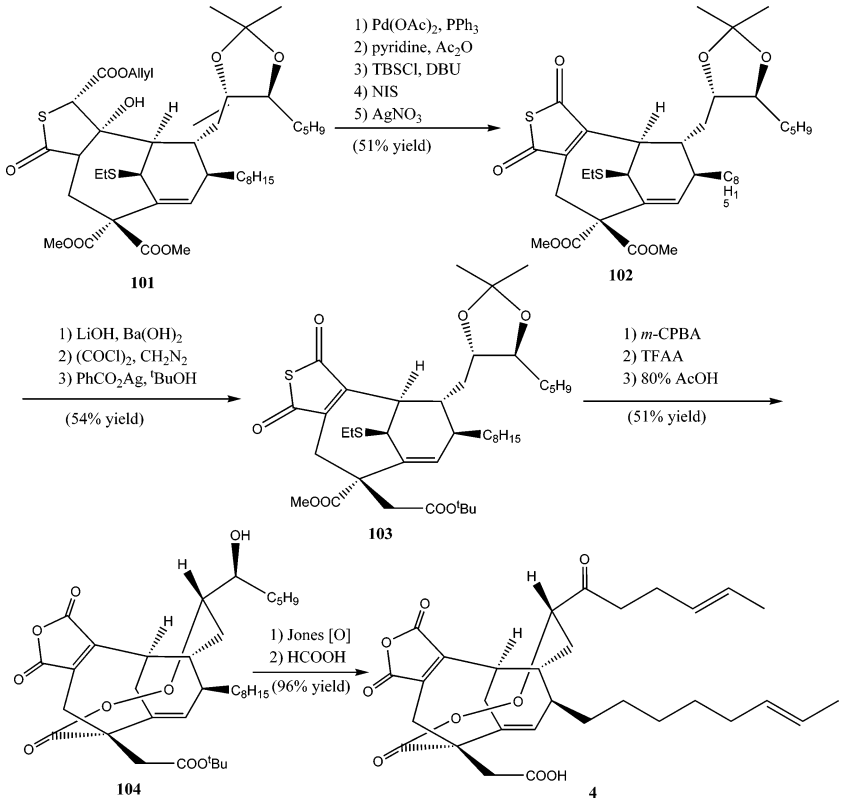


With **76** in hand, Nicolaou was ready to attempt the crucial Lewis acid-catalyzed type II IMDA reaction. As anticipated, triene **76** readily underwent the cycloaddition (Scheme 9) in high yield to give the biocyclo[4.3.1]decene core **77**. The primary silyl ether was then deprotected, oxidized to the corresponding aldehyde, reacted under umpolung conditions with lithio dithiane **78**, and protected to provide compound **79**. The incorporates the C1 to C6 side chains found in the phomoidrides methylene ketone in **79** into the desired maleic anhydride unit.

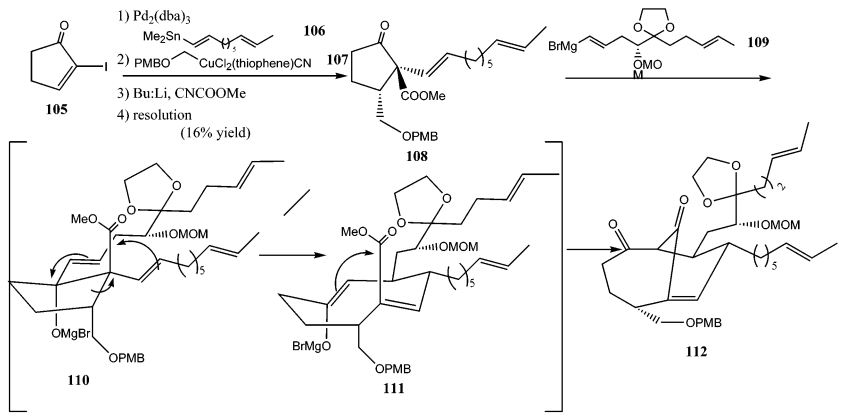
The Nicolaou group's next task was to transform the sterically hindered C11−C12 α-methylene ketone in **79** into the desired maleic anhydride unit. This problem required that the investigators develop an innovative solution. The sequence began with conversion of the detone function in **79** into its corresponding enol triflate, followed by carboxylation to yield an enoate. Subsequent interconversion of the dithiane protecting group to a methyl acetal using Stork's procedure gave enoate **80**. The carboxylate ester was then fully reduced and converted regioselectively into diol **81**. Completion of the anhydride function was accomplished in an ingenious one-pot sequence. This involved conversion of the primary alcohol function in **81** to a mesylate, epoxide formation, β-elimination, cyclization, tautomerization, autoxidation, and ammonia extrusion to yield maleic anhydride **82**.[5,209,213,214]

The greatest challenge remaining was to convert the locally symmetric acetonide in **82** into the quaternary γ-hydroxy-lactone found in the phomoidrides (Scheme 10). Thus, a series of protecting group manipulations (**82** → **83**), oxidation of the bridgehead alcohol to the corresponding detone, acetonide removal with concurrent lactol formation, and masking of the remaining primary alcohol as its TES ether

Scheme 13. Completion of Fukuyama's Total Synthesis



Scheme 14. Shair's Assembly of the Carbocyclic Core



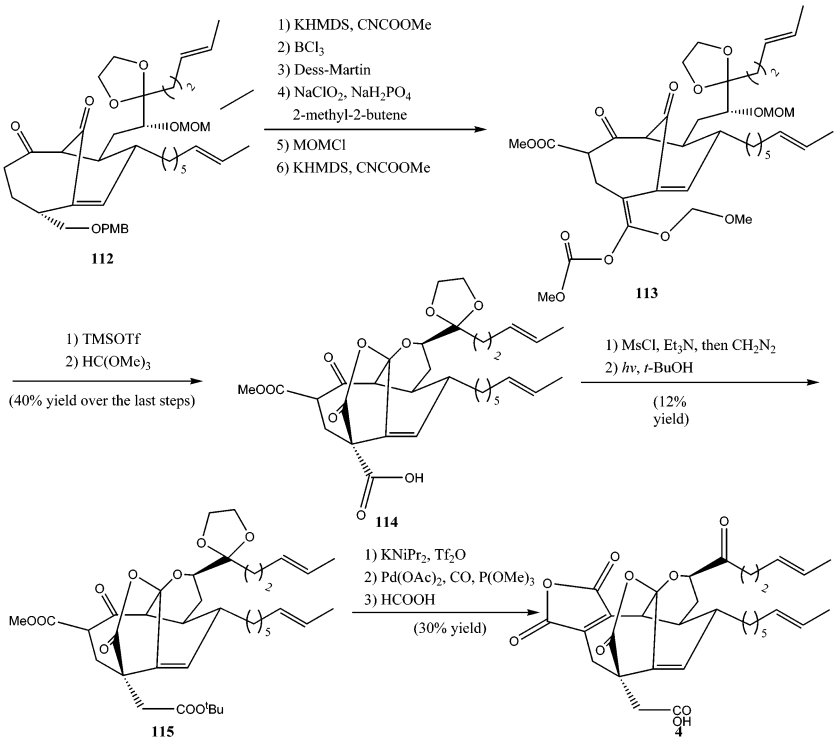
furnished hemiacetal **84**. This was then converted to aldehyde **85** and subjected to an Arndt-Eistert sequence to give rise to acid **86**. Coupling of the resulting free acid with indoline, deprotection of the TBS acetal, oxidation to the lactone, and a two-step deprotection sequence of the indoline amide gave **3**. Interconversion studies elegantly illustrated that **3** could be readily cyclized to **4** using methanesulfonic acid in chloroform, while **4** could be converted back into **3** by using LiOH in a THF/water mixture.

Nicolaou research group subsequently published the first asymmetric total synthesis of the phomoidrides, which established the absolute stereochemistry of the natural products.[5,209,212] Their approach relied upon intercepting their racemic route at intermediate aldehyde **90** (Scheme 11). They began with (*R*)-(+)-glycidol **88** and, in a four-step sequence involving alkylation and hydroiodination, arrived at vinyl iodide **89**. This intermediate was converted to the corresponding vinyllithium reagent, treated with aldehyde **90** (derived from racemic intermediate **74**; see Scheme 8), and oxidized to provide triene **91**. After considerable experimentation, it was found that Lewis acid **92** catalyzed the Diels−Alder reaction to produce a 5.7:1 mixture (70% d.e.) of diastereomeric cycloadducts. These were deprotected, chromatographically separated, and oxidatively cleaved with sodium periodate to provide enantiomerically enriched aldehyde **93**. This intermediate was carried through the previously published racemic synthesis to provide indoline (+)-**94**. The Nicolaou research group determined the absolute configuration of the natural product by comparing **94** to the analogous indoline derived from natural **4**. The synthetic compound possessed the opposite optical rotation to fungal material.

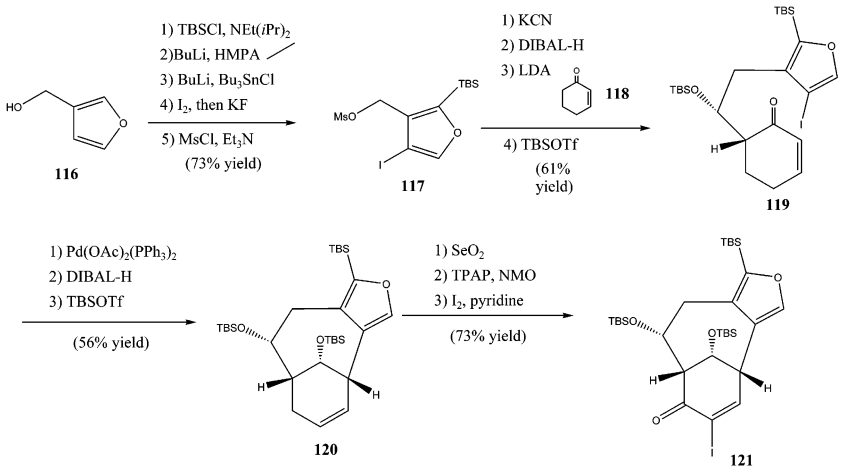
6.1.2. Fukuyama Research Group

Fukuyama and co-workers at the University of Tokyo completed the second total synthesis of the phomoidrides, which also represented the second asymmetric route to these molecules.[5,201] Like Nicolaou, Fukuyama chose to employ a

Scheme 15. Completion of Shair's Total Synthesis



Scheme 16. Assembly of the Carbocyclic Core of the Phomoidrides



type II IMA strategy for the construction of the phomoidrides' bicyclo[4.3.1]decene core (Scheme 12). He was then able to explore some exquisitely chemoselective chemistry for the facile completion of the total synthesis.

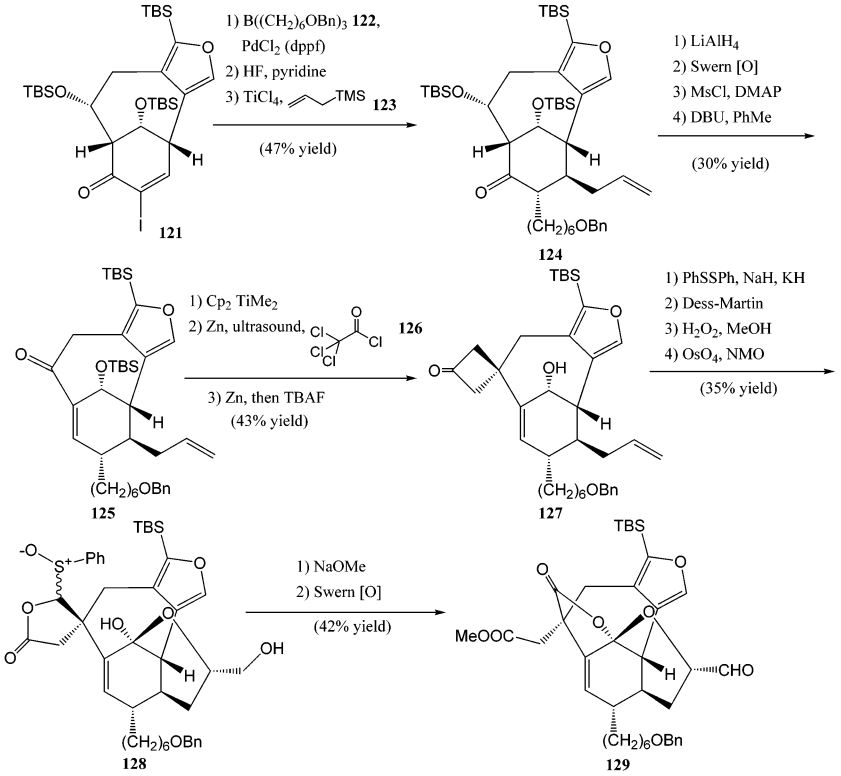
The Fukuyama research group began their efforts with methyl 4-ethylthio-2-butynoate **94**. This was treated with catalytic DBU to effect isomerization to the corresponding allene and then was reacted with organocopper reagent **95** to provide the 1,4-addition product **96**. Alkylation with methyl chloroformate and Michael addition of the resulting malonate into chiral acrylamide **97** gave diester **98**. Diastereoselective aldol reaction of **98** with aldehyde **99** followed by oxidation provided triene **100** in good yield, and a Diels–Alder cycloaddition catalyzed by $ZnCl_2$ furnished the desired bridgehead olefin. The chiral Evans oxazolidinone was replaced with lithium allyl thioglycolate, and an intramolecular aldol type cyclization gave γ-hydroxy thiolactone **101**.

The completion of Fukuyama's synthesis is depicted in Scheme 13. Deprotection of the allyl group in **101** followed by a one-pot dehydration and decarboxylation sequence gave a thiobutenolide. This was converted to thiomaleic anhydride **102** in three steps proceeding via the oxidation of a 2-silyloxythiophene derivative. Subsequent treatment with $LiOH/Ba(OH)_2$ gave rise both to maleic anhydride formation and to diastereoselective methyl ester saponification to yield a carboxylic acid. An Arndt-Eistert procedure then provided homologated ester **103**. Pummerer rearrangement and acetonide deprotection yielded **104**, and Jones oxidation and *tert*-butyl ester deprotection completed the total synthesis of **4**.

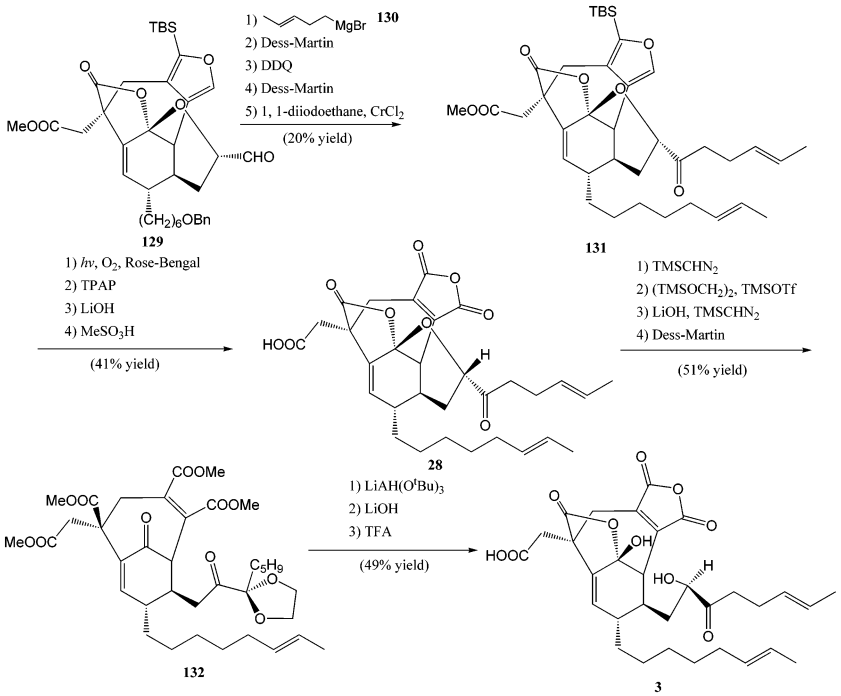
6.1.3. Shair Research Group

The third phomoidride total synthesis to be published was accomplished by Shair and co-workers at Harvard University (Scheme 14).[5,222,227] This route involves as its key step a "triple-domino" cyclization reaction, which proceeds in a single convergent operation to provide a highly functionalized bicyclo[4.3.1]decene core structure. Thus, synthetic

Scheme 17. Introduction of the Side Chains and the Quaternary Center



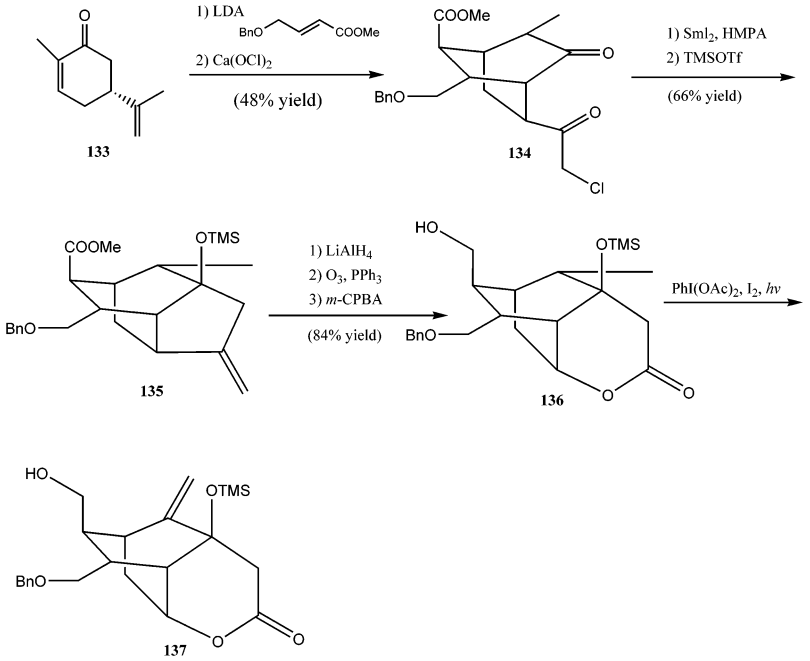
Scheme 18. Completion of the Total Synthesis



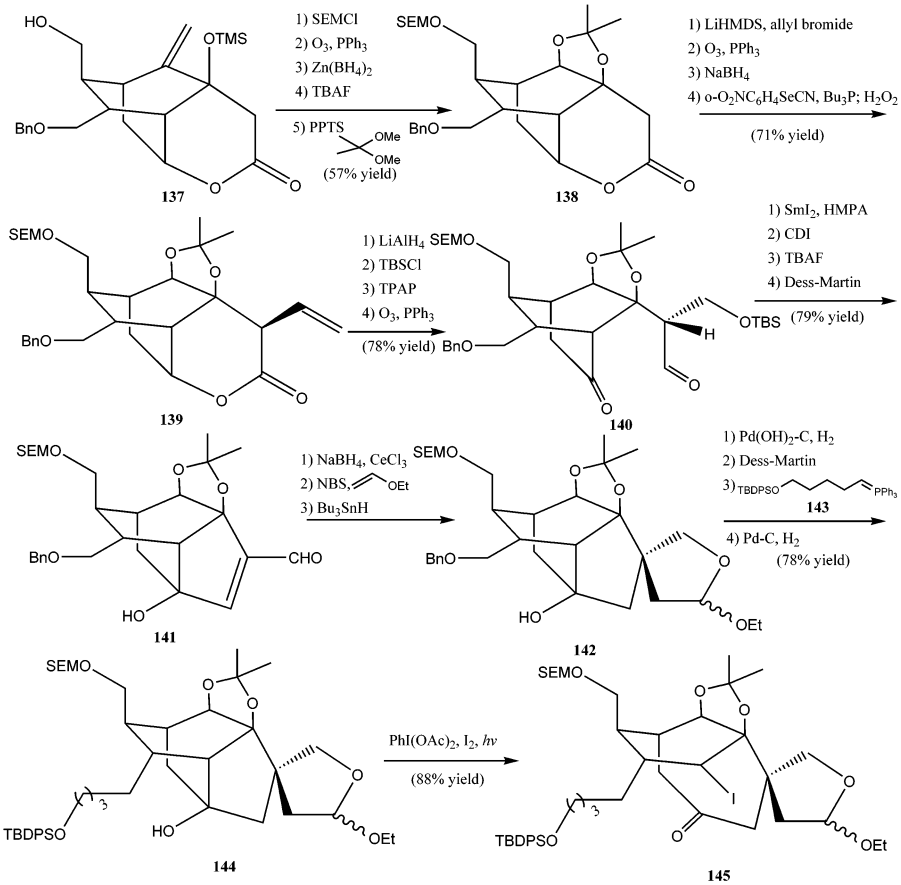
efforts in the Shair research group were initiated with a Stille cross-coupling reaction between 2-cyclopentenyl iodide **105** and stannane **106**. Conjugate addition with cuprate **107**, acylation with Mander's reagent, and kinetic resolution furnished ketone **108**, a substrate for the tandem phomoidride core-forming reaction. In the event, addition of Grignard reagent **109** to ketone **108** furnished **112** in good yield. This triple domino cyclization sequence (**108** → **112**) consists of chelation-controlled vinyl Grignard addition (**108** → **110**), anion-accelerated oxy-Cope rearrangement (**110** → **111**), and transannular Dieckmann-like cyclization (**111** → **112**).

Having accessed the core structure of the phomoidrides efficiently, Shair was faced with installing the C14 quaternary center and the maleic anhydride moieties to complete the total synthesis (Scheme 15). Following alkylation of **112** with Mander's reagent, the primary PMB ether was converted to enol carbonate **113** in a five-step protocol. Subjection of this compound to TMSOTf and trimethyl orthoformate gave pseudoester **114** directly. This unusual sequence is thought to involve a TMSOTf-promoted Fries-like rearrangement followed by MOM group removal and cyclization. To complete the quaternary center, Shair and co-workers

Scheme 19. Nagaoka's Total Synthesis to Phomoidrides, Part I



Scheme 20. Nagaoka's Total Synthesis to Phomoidrides, Part II

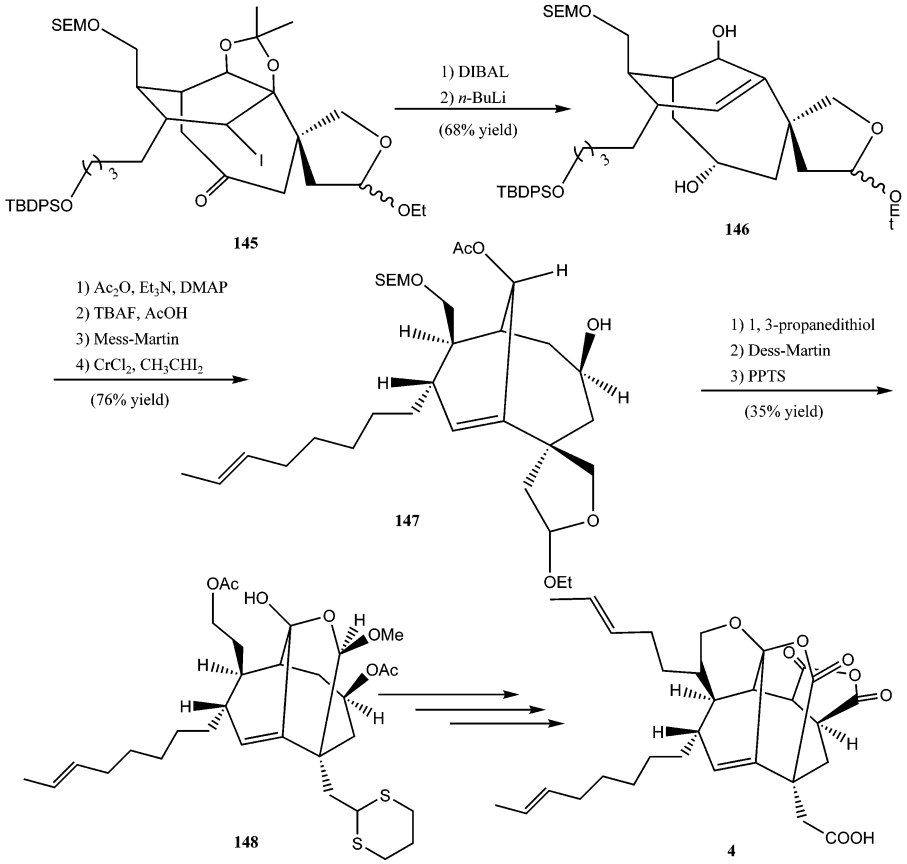


employed the Arndt-Eistert homologation also utilized by Nicolaou and Fukuyama. It provided **115** in low yield, a result thought to be a function of the instability of **114**, rather than an intrinsic deficit in the Arndt-Eistert sequence in general. The total synthesis was completed by preparing the maleic anhydride moiety utilizing a Pd(0)-$P(OMe)_3$-catalyzed carbonylation reaction of the corresponding enol triflate of **115**, followed by acidification to provide **4**. The use of a sterically unhindered palladium phosphite complex proved quite useful in this system given the hindered nature of the C11 enol triflate.

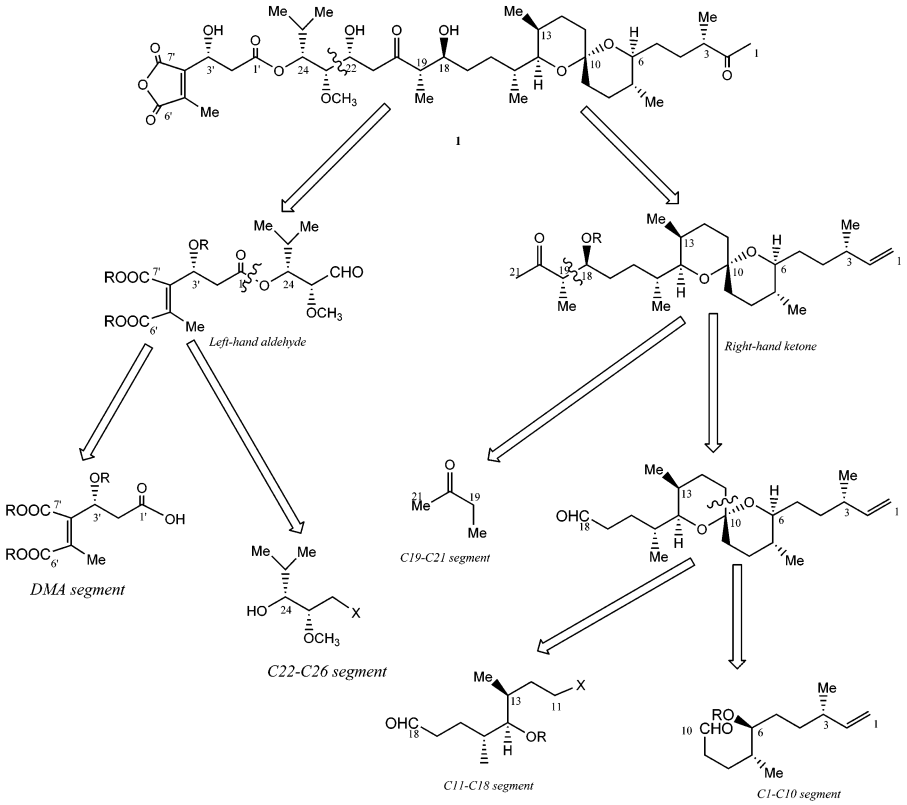
6.1.4. Danishefsky Research Group

The most recently completed total synthesis of the phomoidrides is that of Danishefsky and co-workers at the Sloan-Kettering Institute for Cancer Research.[5,66,202,203,216,228] Key

Scheme 21. Completion of Nagaoka's Total Synthesis

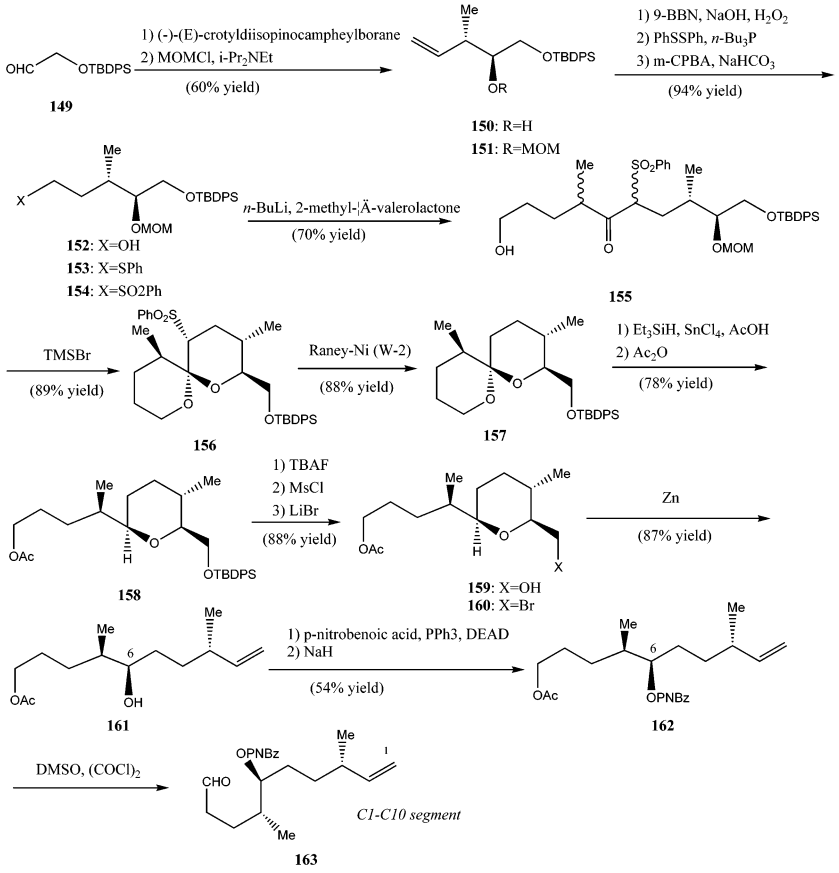


Scheme 22. Retrosynthesis of Tautomycin

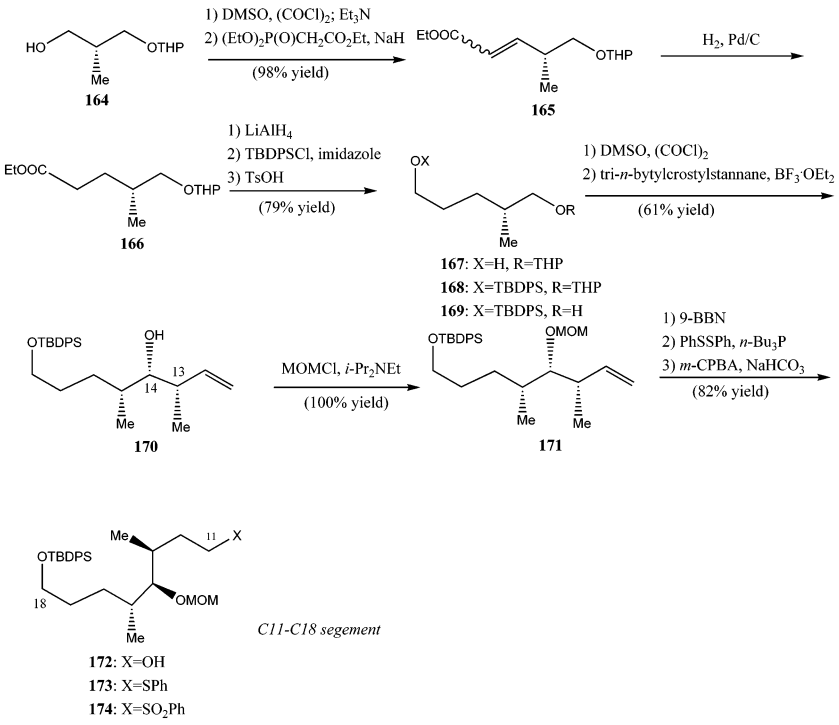


aspects of this effort include a sequential aldol reaction-intramolecular Heck ring closure sequence, a diastereoselective sulfur-mediated cleavage of a spirocyclobutanone, and a late stage C7 epimerization strategy to provide **3** and **4**. Danishefsky's route also provides an investigation of the stabilities of natural and unnatural C7 phomoidride epimers that led to the identification of the C7-R epimer of **4** (phomoidride D, **28**) in fermentation broths.

Scheme 23. Synthesis of the C1−C10 Segment



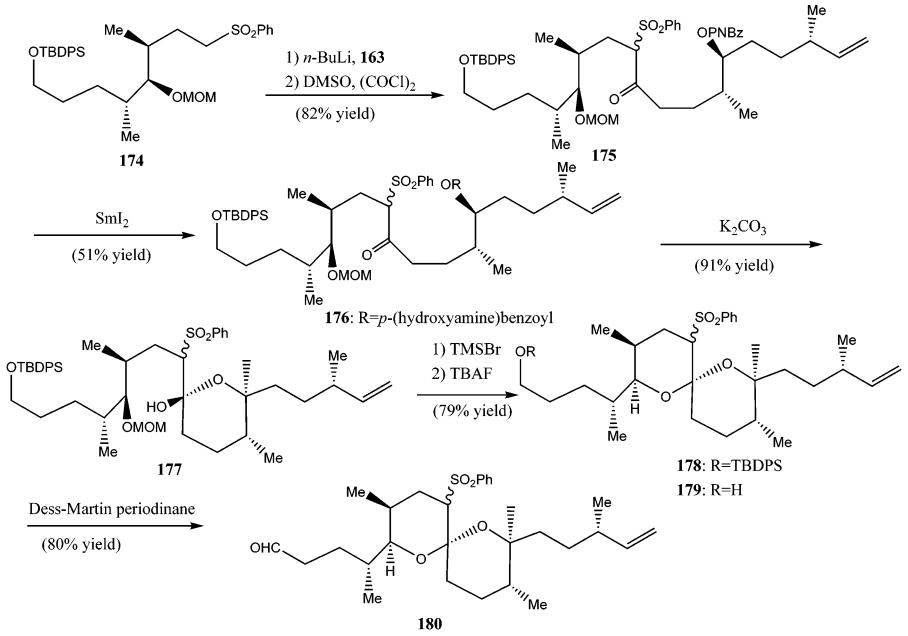
Scheme 24. Synthesis of the C11−C18 Segment



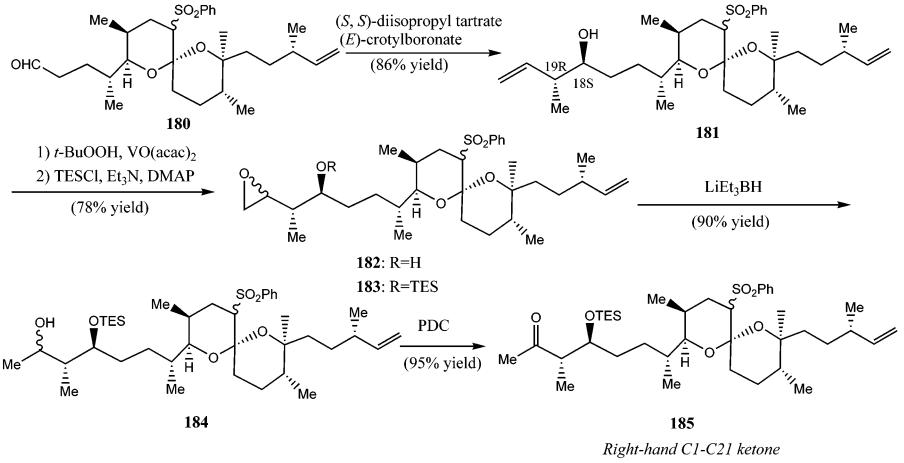
The total synthesis commenced with the five-step conversion of 3-furanmethanol **116** to mesylate **117**. This compound was then homologated with cyanide ion, converted to the requisite aldehyde, and reacted with the lithium enolate of cyclohexenone **118** to give Heck ring closure substrate **119** after TBS protection (Scheme 16).

The intramolecular Heck vinylation reaction proceeded smoothly to yield tricycle **120** after reduction and TBS protection. Allylic oxidation and iodination yielded vinyl iodide **121**, and intermediate poised for the incorporation of the alkyl side chains (Scheme 17). These were installed employing first a B-alkyl Suzuki-Miyaura cross-coupling

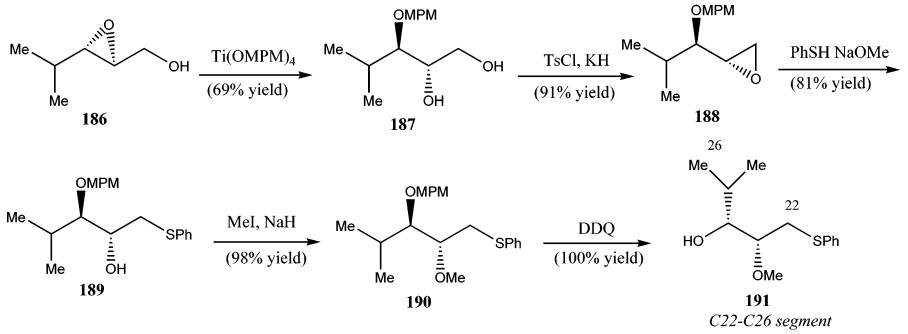
Scheme 25. Segment Coupling toward Right-Hand C1−C21 Ketone (Part I)



Scheme 26. Segment Coupling toward Right-Hand C1−C21 Letone (Part II)



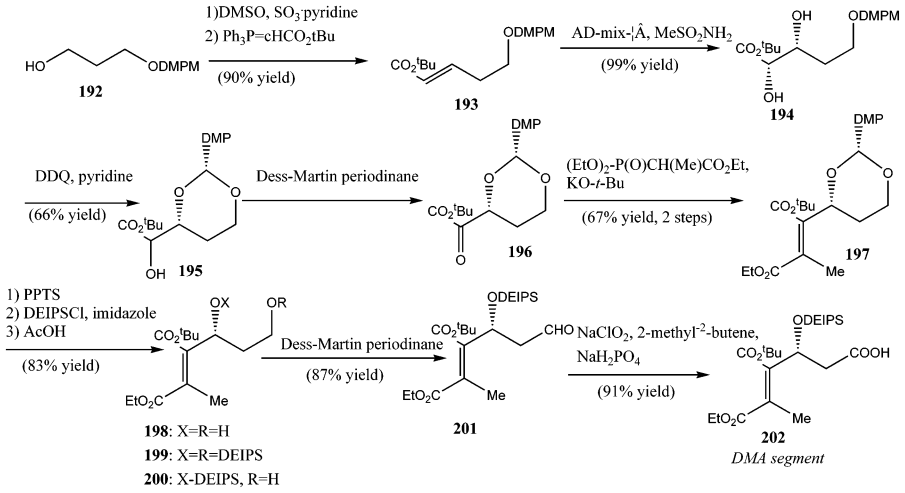
Scheme 27. Synthesis of the C22−C26 Segment



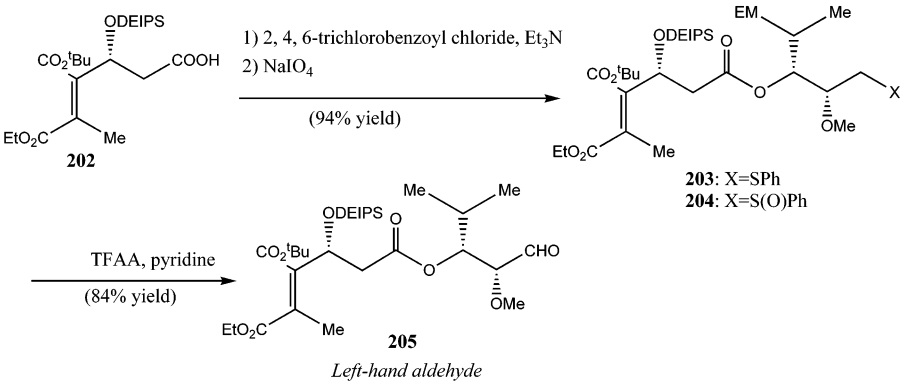
reaction between **121** and trialkylborane **122** and then a Sakurai type allylation with **122**. This sequence provided the desired trans side chain stereochemistry found in the natural product. After a series of chemoselective oxidation state manipulations, the bridgehead olefin was installed via a β-elimination of a secondary mesylate to give **125**.

To incorporate the succinic acid-derived quaternary center, Danishefsky employed a unique approach. He began with Tebbe olefination of **125** and affected on the resulting product a chemoselective [2+2] cycloaddition with dichloroketene generated from acid chloride **126**. Dechlorination with zinc yielded spirocyclobutanone **127**, and regioselective sulfenylation of **127** was then achieved using diphenyl disulfide. The molecule was then exhaustively oxidized starting with Dess-Martin oxidation of the secondary alcohol, and followed by regioselective Baeyer−Villiger oxidation of the cyclobutanone, conversion of the phenylsulfenyl lactone to the corresponding sulfoxide, and dihydroxylation of the allyl group to provide **128**. Saponification and oxidation gave lactone **129**, which has the quaternary center in place. This

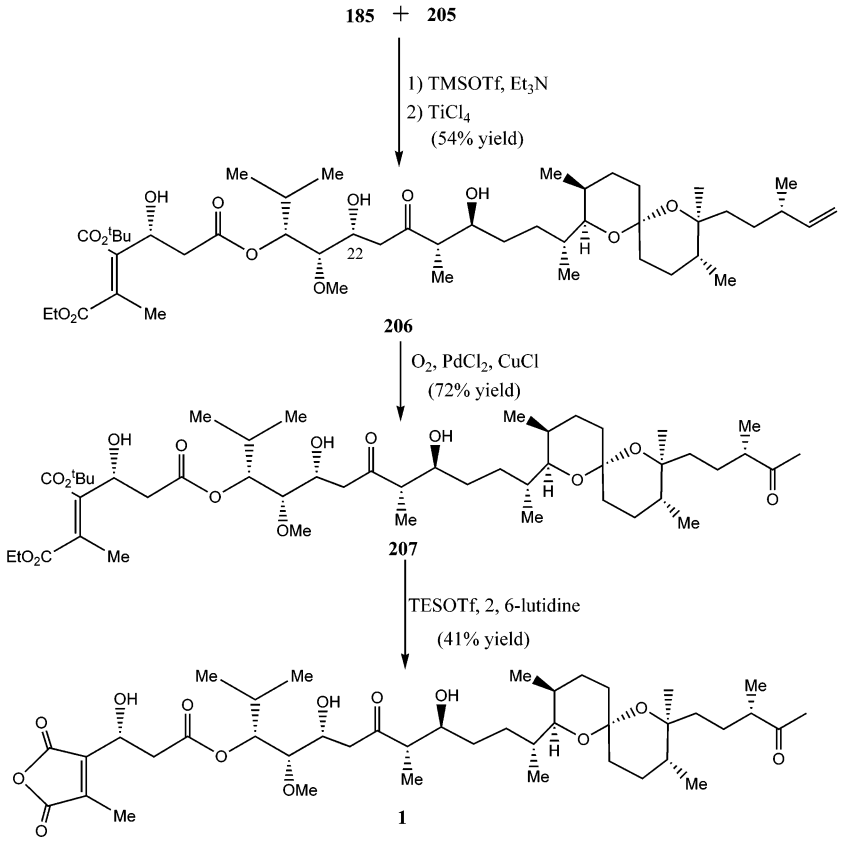
Scheme 28. Synthesis of the Dialkylmaleic Anhydride (DMA) Segment



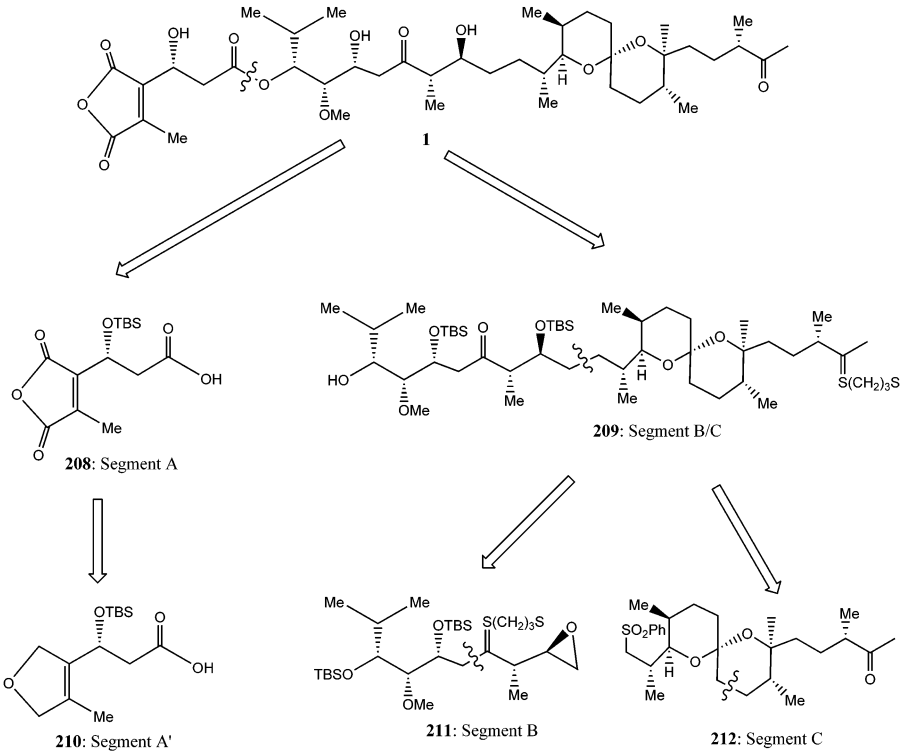
Scheme 29. Segment Coupling toward Left-Hand Aldehyde



Scheme 30. Aldol Coupling of Right-Hand Ketone and Left-Hand Aldehyde



Scheme 31. Retrosynthetic Analysis of Tautomycin by the Isobe Research Group



method for generating the quaternary center is quite distinct from methods employed by other groups.

Danishefsky's completion of the phomoidrides is detailed in Scheme 18. First, the two side chains were installed. The C1−C5 protion was incorporated via addition of Grignard reagent **130** to aldehyde **129**, and the C18−C25 piece was elaborated via debenzylation, oxidation, and reductive olefination with diiodoethane and CrC_{l2} to give **131**. Unmasking of the anhydride was accomplished in a straightforward fashion on treatment of **131** with singlet oxygen followed by TPAP oxidation. Subsequent hydrolysis and acidification afforded **28**. Because at the time the Danishefsky group was targeting **3** and **4** for synthesis, they extensively explored the use of early intermediates to arrive directly at compounds with the desired C7 stereochemistry without employing epimerization protocols. They met with limited success. Ultimately, they converted **28** to **3** in a seven-step sequence and thus completed their total synthesis.

6.1.5. Nagaoka Research Group

Recently, another total synthesis of **4** was carried out by Nagaoka and co-workers of Meiji Pharmaceutical University.[198,199,229] They employed alkoxyl radicals to selectively undergo dehydrogenation and β-scission to give rise to key structural elements of **4**. His route toward the phomoidrides utilized a sequential Michael addition strategy (Scheme 19). Following a sequential Michael reaction between (*S*)-carvone **133** and (*E*)-methyl 4-benzyloxycrotonate, allylic chlorination furnished bicycle **134**. Reductive cyclization with samarium diiodide followed by protection of the resulting tertiary alcohol gave **135**, which, after reduction, ozonolysis, and Baeyer−Villiger oxidation, resulted in lactone **136**. Photolytic olefination of **136** gave olefin **137**.

Advancement of olefin **137** is shown in Scheme 20. Oxidation, reduction, and protecting group manipulations furnished intermediate **138**, which was selectively alkylated, reduced, and eliminated to give lactone **139**. Further functional group manipulations then gave rise to pinacol precursor **140**. Pinacol coupling of **140**, followed by β-elimination and oxidation, provided intermediate **141**, thereby setting the stage for the introduction of the quaternary center. Then Nagaoka applied Stork's bromoacetal chemistry to build the quaternary center. After Luche reduction and application of an α-bromo ethyl acetal, a 5-exo-trig radical cyclization gave rise to intermediate spiroacetal **142**. Debenzylation of **142** followed by oxidation and hydrogenation gave alcohol **144**. Then **144** was treated with DIB and iodine to obtain keto-iodide **145**.

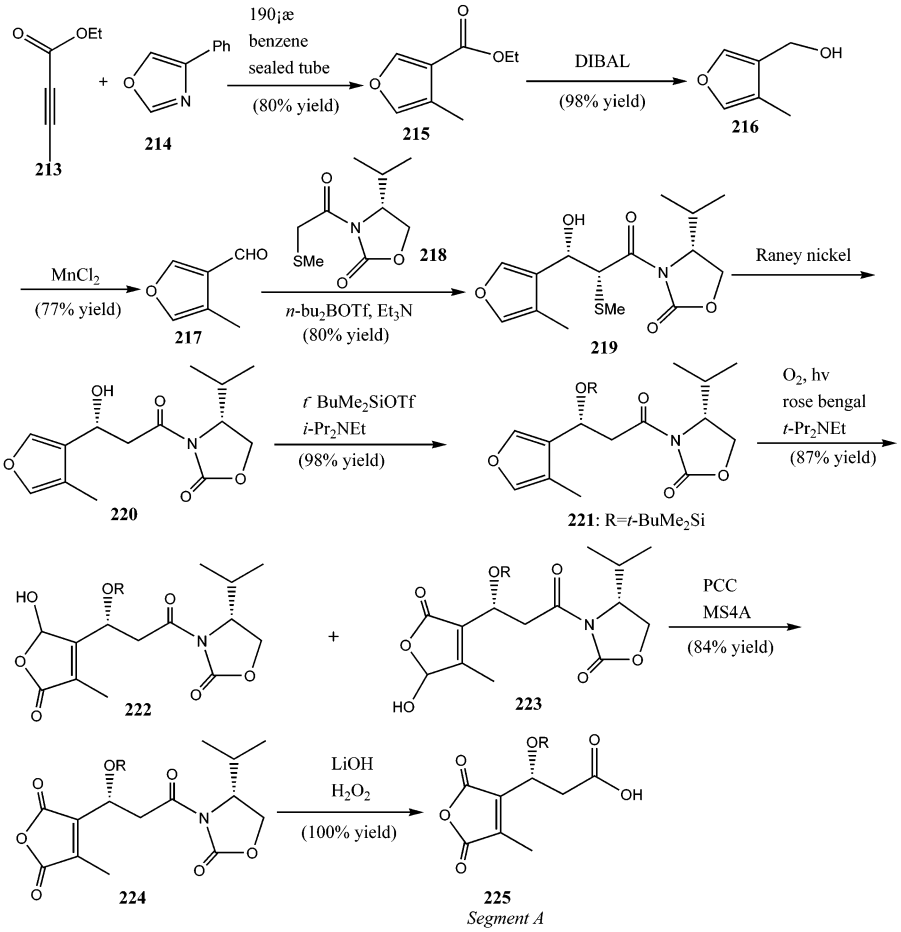
The completion of the synthesis was performed or supposed from **145** (Scheme 21). **145** was reduced with DIBAL, then the alcohol was treated with *n*-BuLi to provide the olefin **146**. Acetylation of **146** followed by deprotection of the *tert*-butyldiphenylsilyl (TBDPS) group with TBAF produced an alcohol, and oxidized to give **147**. Sequential transformation of **147** into the CP-precursor **148**, involving cyclic thioketal formation, SEM deprotection, and intramolecular transacetylation, Dess-Martin oxidation and methyl acetal formation with PPTS was found to proceed successfully. **4** was supposed to be achieved by the research group by the research group as shown in Scheme 21.

Other research groups have studied part of the synthesis process.[5−7,65,66,183,199,201−206,216−223,227−255]

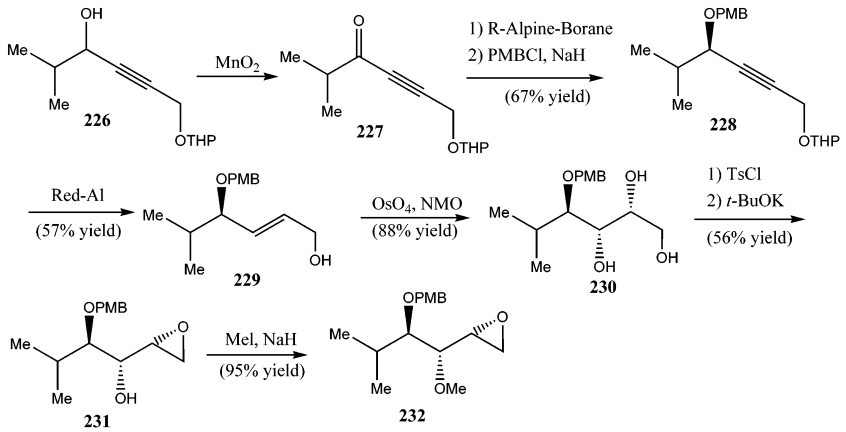
6.2. Tautomycin and Tautomycetin

Tautomycin (**1**) and tautomycetin (**2**) are antimicrobial agents, representative tumor promoters, and potent inhibitors for PP1 and PP2A. Recently, **2** was found to be immunosuppressive and potent as applied in transplantation. The structure of **1** contains spiroketal and maleic anhydride moieties and 13 chiral centers. In **2**, there is a maleic anhydride moiety, too. The structural complexity and unique biological activity stimulated many research groups to carry out synthetic studies of **1** and **2**.[51,52,91,256−278]

Scheme 32. Synthesis of Segment A



Scheme 33. Synthesis of Segment B (Part I)

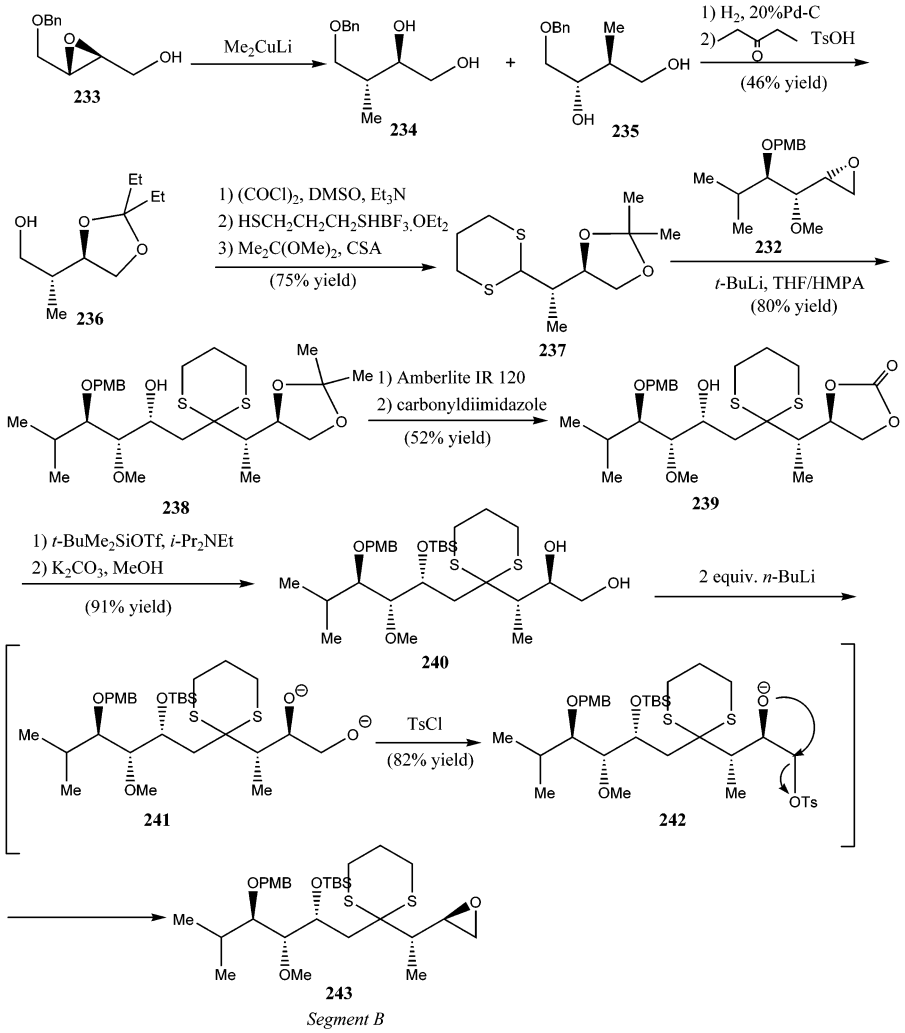


6.2.1. Total Synthesis of Tautomycin

6.2.1.1. Ichihara Research Group. The first total synthesis of **1** was carried out by Ichihara in Hokkaido University.[52,91,267,269,271,272,275−278] The retrosynthesis of **1** is shown in Scheme 22. Disconnection at the base sensitive C21−C22 bond divided the target into two large subunits, a right-hand C1−C21 ketone and a left-hand aldehyde (left from C22). The transform of this coupling is an aldol reaction controlled by chelation of the C23 methyl ether. The right-hand ketone could be synthesized by enantioselective addition of a C19−C21 segment to a C1−C18 segment in a reagent-controlled manner. The C1−C18 segment was further divided into two segments, C1−C10 and C11−C18, using a transform of the sulfone carbanion method.

Synthesis of the C1−C10 Segment. This process features two key points, the selective spiroketal formation (chiral transfer) and subsequent acetal reduction. As illustrated in Scheme 23, aldehyde **149** was subjected to Brown's asymmetric crotylboration protocol ((−)-(*E*)-OOH) to afford adduct **150** in high diastereo- and enantioselectivity (100% de and 92% ee). After the free hydroxyl was protected as a methoxymethyl (MOM) ether, the vinyl group was converted to phenyl sulfone in a three-step sequence: hydroboration, substitution by a phenylthio group, and oxidation to the sulfone (*m*-CPBA). Sulfone **154** was then lithiated by *n*-BuLi and coupled with 2-mehtyl-*δ*-valerolactone to yield **155** as a four-component mixture. Lewis acid-promoted MOM deprotection and spiroketalizaion were successfully achieved

Scheme 34. Synthesis of Segment B (Part II)



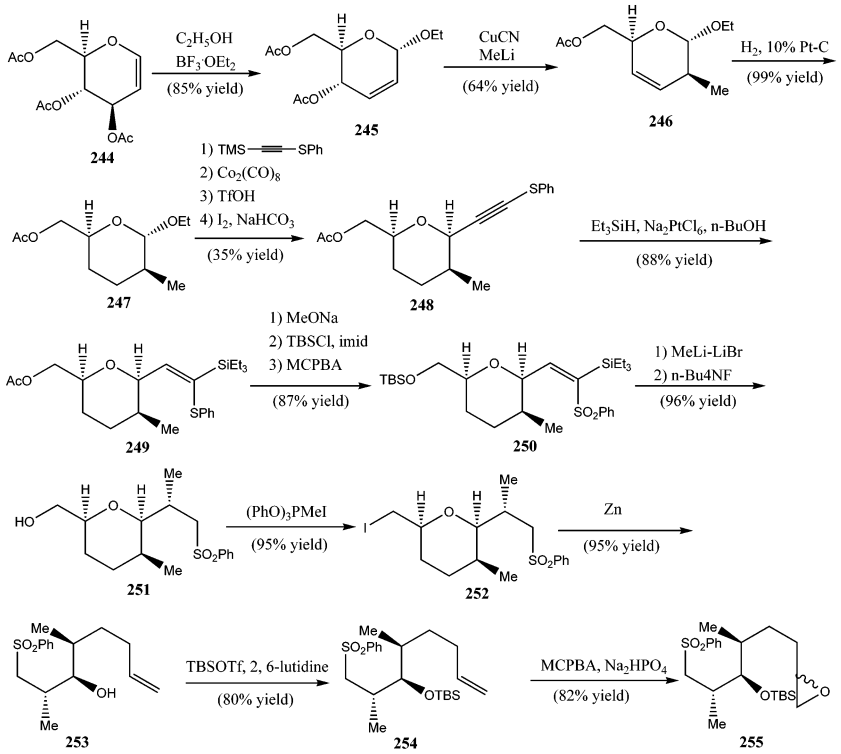
by bromotrimethylsilane (TMSBr) producing crystalline product **156**. Use of a large excess of Raney-Ni (W-2) cleanly converted **156** to the desired spiroketal **157**. Highly diastereoselective reduction of **157** with triethylsilane and tin(IV) chloride at −78 to −60 °C was followed by acidic workup to give **158**. Reductive ring-opening of bromide **160** derived from **158** with zinc and acetic acid furnished **161**, which was followed by Mitsunobu inversion to afford the diester **162**. Selective hydrolysis and Swern oxidation provided the aldehyde **163**.

Synthesis of C11−C18 Segment. The synthesis of C11−C18 was started with a known alcohol **164** (Scheme 24). The aldehyde derived from **164** by Swern oxidation was found to be easy to racemize, so it was immediately subjected to Horner-Wadsworth-Emmons reaction conditions, affording ene ester **165** as an isomeric mixture. Ene ester **165** was hydrogenated, reduced by lithium aluminum hydride, and submitted to subsequent protective group manipulations (*tert*-butylchlorodiphenylsilan; *p*-TsOH) to yield C14−C18 alcohol **169** in 79% overall yield. After **169** was oxidized, elongation of the four-carbon unit was attained by employing Yamamoto's crotylstannane addition to obtain the desired Cram *erythro* adduct **170**. The C14 hydroxyl was then protected by MOM ether for the purpose of smoothly spiroketal formation at C6−C14 (vide infra). A further routine sequence including hydroboration, phenylthio substitution, and oxidation afforded C11−C18 phenyl sulfone **174**.

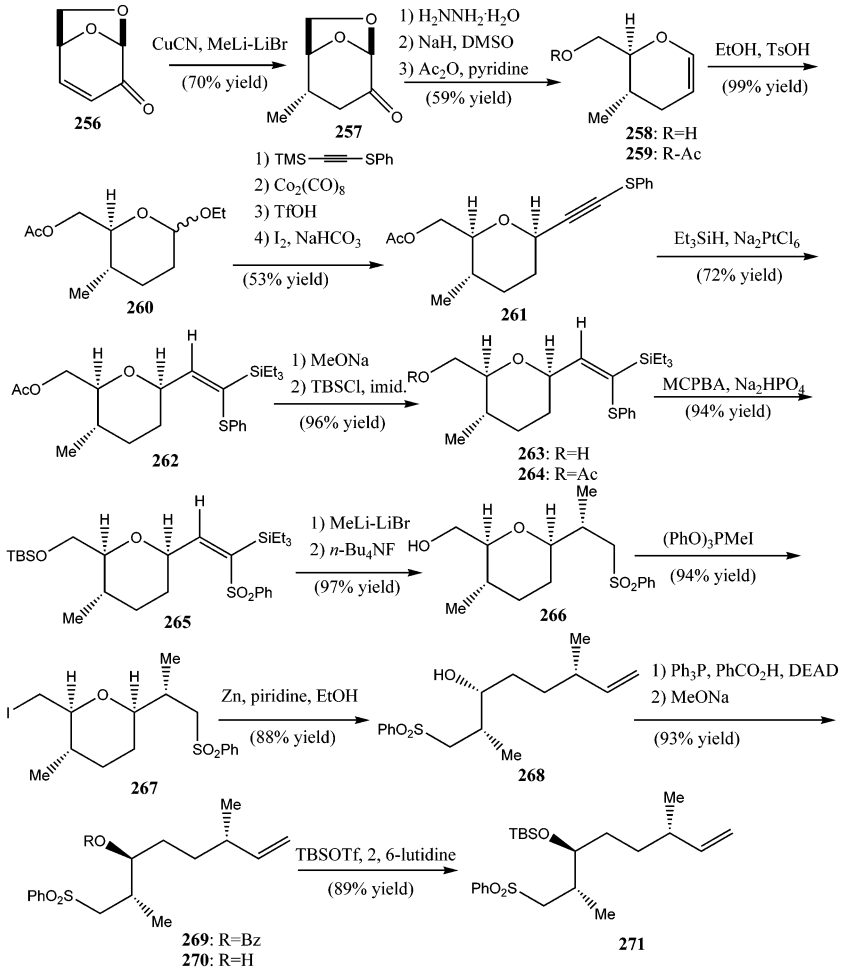
Segment Coupling Toward Right-Hand C1−C21 Ketone. The lithium salt of **174** generated by *n*-butyllithium was coupled with **163**, and the resultant alcohol was successively oxidized (Swern oxidation) to give two isomeric β-keto sulfone **175** (Scheme 25). Desulfurization and concomitantant reduction of the nitro group into the hydroxyamine afforded the desired product **176**. After hydrolysis of the benzoate group, cyclization to the spiroketal **177** was effected with bromotrimethylsilane. The silyl group of **177** was removed with TBAF to give the alcohol **178** and subsequent Dess-Martin oxidation afforded aldehyde **179**. Spirodetal **177** was then converted to the degradation product **180** to confirm the proposed structure. For the introduction of the C18 and C19 stereocenters, enantioselective crotylboration was examined. Condensation of **180** with Roush's (*E*)-crotylboronate afforded adducts **181** in satisfactory diastereoselection (9:1), Scheme 26. To differentiate the two vinyl groups of **181**, hydroxyl group-directed epoxidation of **181** with *t*-butyl hydroperoxide and vanadate afforded **183** in a 3:1 mixture of diastereomers. After protection of the hydroxy group, reduction of the epoxide **183** with lithium triethylborohydride proceeded smoothly to give the alcohol **184** which was then oxidized with pyridinium dichromate to the methyl detone **185**.

Synthesis of the C22−C26 Segment. Optically active epoxy alcohol **186** was employed as the starting material. Epoxy alcohol **186** was subjected to a titanium (IV)-mediated oxirane opening reaction (Ti-(OMPM)$_4$) to produce 1,2-diol

Scheme 35. Synthesis of Segment C (Part I)

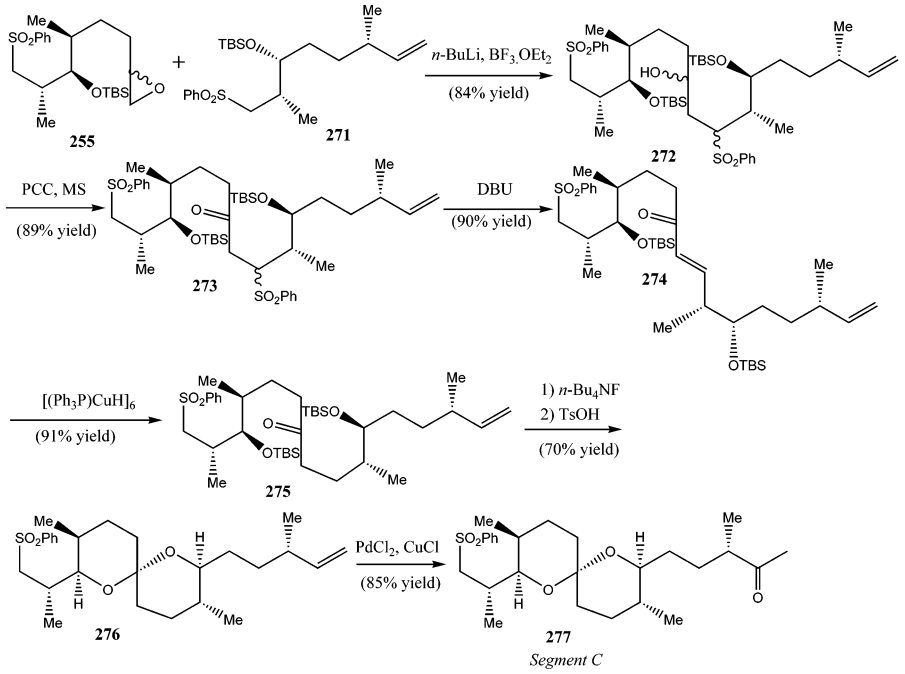


Scheme 36. Synthesis of Segment C (Part II)

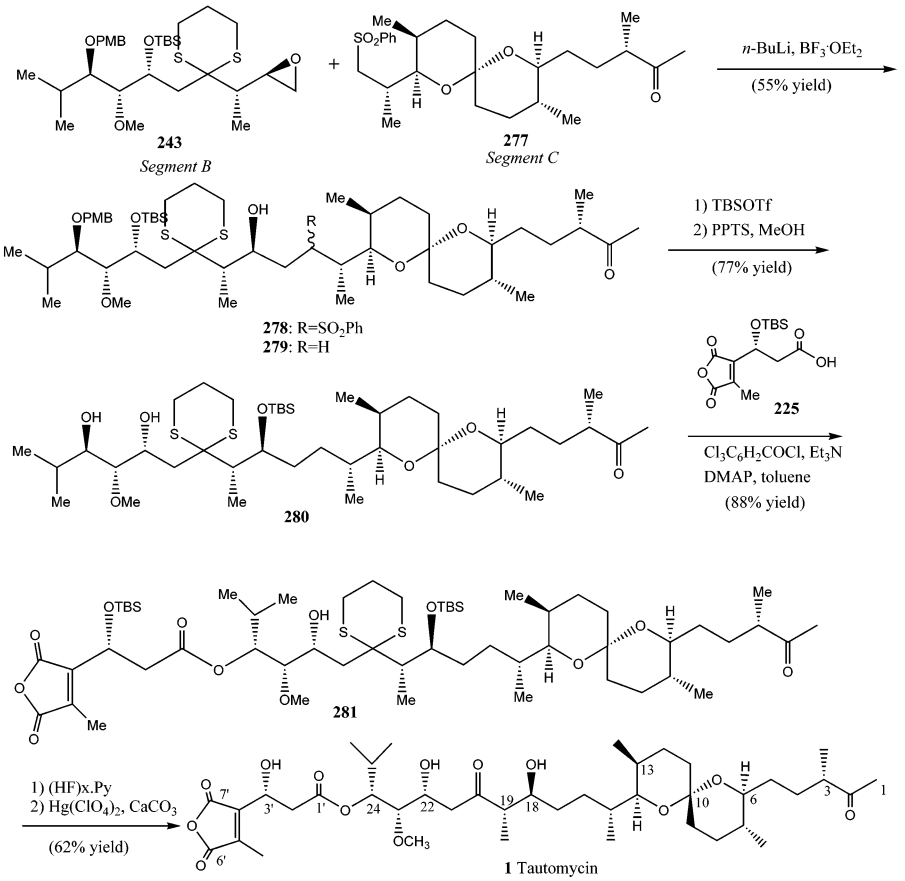


187 and 1,3-diol in 9.5:1 regioisomeric mixture (Scheme 27). Treatment of the mixture with *p*-toluenesulfonyl chloride and potassium hydride provided the epoxide **188**, which was reacted with thiophenoxide to give the sulfide **190**. Methy-

Scheme 37. Coupling Reaction to Obtain Segment C



Scheme 38. Coupling of Segment A, Segment B, and Segment C to Tautomycin



lation followed by deprotection with DDQ afforded the C22−C26 segment **191**.

Synthesis of Dialkylmaleic Anhydride (DMA) Segment. The synthesis began with mono(3,4-dimethoxy)benzyl (DMPM) ether **192** (Scheme 28). Alcohol **192** was oxidized under Parikh-Doering conditions and olefinated to afford *trans*-olefin **193**. Subsequent asymmetric dihydroxylation using AD-mix-β in the presence of methanesulfonamide successfully developed by Sharpless functioned with high enantioselectivity to provide diol **194.** Oxidative acetalization by DDQ in nonaqueous media achieved effective protection of the C1′ and C3′ hydroxyl groups as 3,4-dimethoxybenzylidene acetal region- and stereoselectively. The remaining free hydroxyl in **195** was then oxidized by Dess-Martin periodinane. Then, keto ester **196** was subjected to the Horner-Wadsworth-Emmons reaction producing the desired

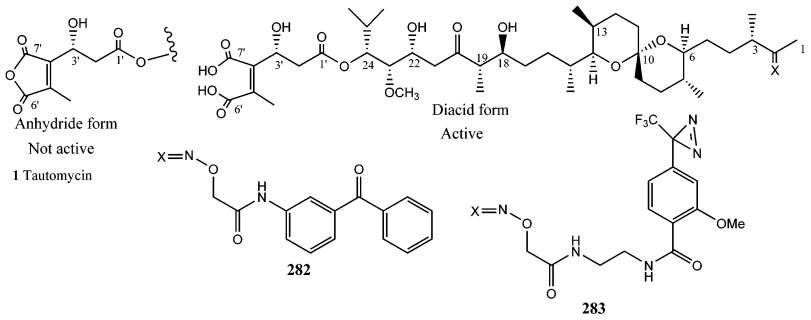


Figure 7. Tautomycin and photoaffinity probes.

dialkyl maleate **197**. Next, the acetal protecting group was removed by the action of pyridinium *p*-toluenesulfonate (PPTS) in methanol. The diethylisopropylsilyl (DEIPS) group, a slightly more acid-sensitive protecting group than TBDMS, was employed for final protection of the C3′ hydroxyl, and thus the diol **198** was bis-silylated. Selective primary silyl ether deprotection yielded C3′ DEIPS ether **200**, which was successively converted to DMS segment **202** via aldehyde **201**.

Segment Coupling Toward Left-Hand Aldehyde. Esterification of the C22−C26 segment and the DMA segment did not proceed via the acid chloride of **202** probably due to steric repulsion of both segments. Although dicyclohexylcarbodiimide-mediated esterification in the presence of DMAP proceeded to ca. 60% yield, the Yamaguchi method produced **203** in 94% yield (Scheme 29). Phenyl sulfide **203** was oxidized to sulfoxide **204** by $NaIO_4$, and **204** was further subjected to the Pummerer reaction promoted by trifluoroacetic acid anhydride (TFAA) and pyridine. The resultant α-(trifluoroacetoxy)sulfide was finally treated with sodium bicarbonate to furnish left-hand aldehyde **205** without any epimerization at the C23 position in 75% yield from sulfide **204**.

Aldol Coupling of Right-Hand Ketone and Left-Hand Aldehyde. Finally, aldol coupling of right-hand and left-hand aldehyde was performed to obtain **1**. In the presence of $TiCl_4$, the silyl enol ether derived from **205** was reacted with aldehyde **185**, followed by desilylation to afford the desired anti-Felkin product **206** as a single adduct in 54% yield (Scheme 30). On the basis of precedents for chelation-controlled Mukaiyama aldol reaction, the exceptional high selectivity in this reaction would be accounted for by chelation of $TiCl_4$ with the C23-methoxy group of the aldehyde **185**. Then, the coupling product **206** was converted to **1**. Pd-assisted selective oxidation of the terminal olefin afforded the methyl detone **207**. Contrary to the case of a simple model compound, deprotection of the *t*-butyl group under the common acidic conditions including Evans's conditions (trimethylsilyl triflate and 2,6-lutidine) caused extensive decomposition. Eventually, this problem was solved by use of TESOTf. Thus, deprotection of the *t*-butyl group with TESOTf and 2,6-lutidine and concomitant ring closure gave **1**.

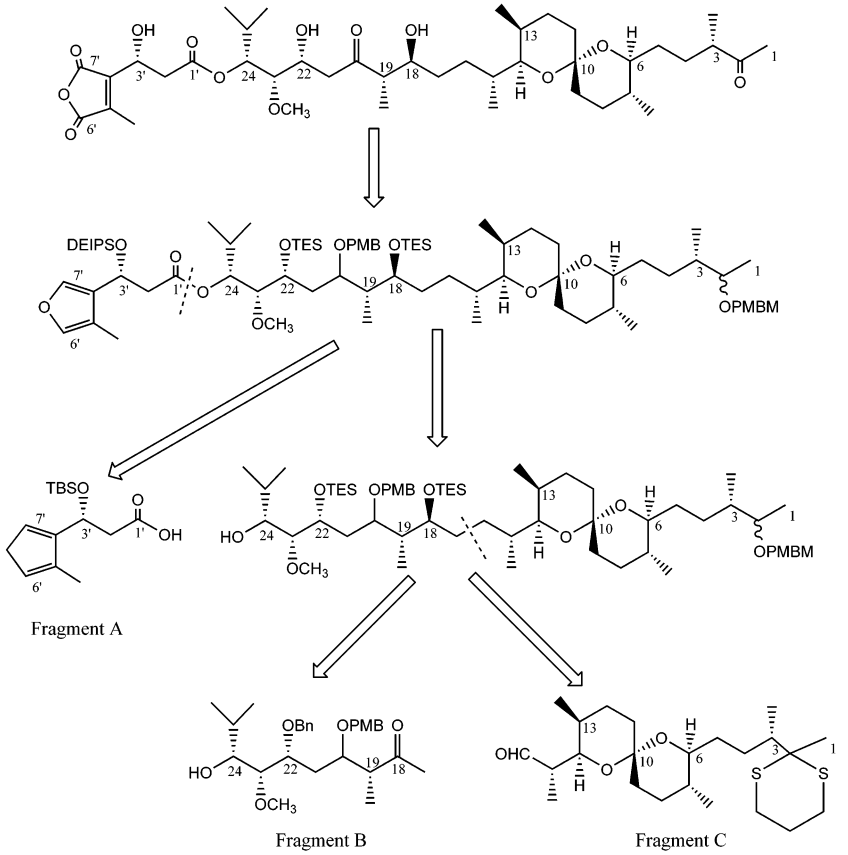
6.2.1.2. Isobe Research Group. Isobe research group was the second to report the total synthesis of **1**.[51,256,257,262−264,270,272,273,279] The retrosynthetic analysis is shown in Scheme 31. Disconnection of the C1′ ester bond in 1 afforded Segment A **208** or **210** and Segment B/C **209**, which was further disconnected into Segment B **211** and Segment C **212**. The most interesting feature is based on the construction of carbon backbone employing the epoxide opening reaction with carbanions.

Synthesis of Segment A. Diels−Alder addition of ethyl tetrolate **213** with 4-phenyloxazole **214** and spontaneous retro-Diels−Alder reaction with elimination of benzonitrile proceeded to furnish the 3,4-disubstituted furan **215** (Scheme 32). Reduction of the ester **215** with diisobutylaluminum hydride and subsequent oxidation with activated manganese(IV) oxide gave the aldehyde **217**. Symmetric aldol condensation involving chiral oxazolidinone boron enolate was chosen in the synthesis. Accordingly, the aldol reduction between boron enolate of chiral *N*-acetyloxazolidinone **218** and the aldehyde **217** exclusively provided the aldol adduct **219**. Desulfurization of **219** using Raney nickel resulted in affording a mixture of **220** and β-elimination product. This side reaction was avoided by employing a mixture of acetone and pH 7 phosphate buffer as the reaction media, in which only **220** was obtained in 75% yield. Protection of the hydroxy group as *t*-butyldimethylsilyl ether gave **221** quantitatively. Photosensitized oxidation of the furan **221** by a 500 W tungsten incandescent lamp under oxygen atmosphere in the presence of rose bengal and diisopropylethylamine gave a regioisomeric mixture of 2,3-disubstituted-4-hydroxybutenolides **222** and **223** in the presence of powdered and activated molecular sieves 4 Å furnished the maleic anhydride **224**. Removal of the auxiliary with lithium hydroperoxide gave the acid **225**.

Synthesis of Segment B. Activated manganese(IV) oxide oxidation of the racemic allyl alcohol **226** furnished the ynone **227**, which was treated with R-Alpine-Borane (Scheme 33). Hydrolysis of **228** with pyridinium *p*-toluenesulfonate in methanol followed by reduction using sodium bis(2-methoxyethoxy)aluminum hydride furnished **229**. Introduction of the two hydroxy groups to the allyl alcohol **229** at the C22 was achieved by osmium-catalyzed dihydroxylation which established both the stereogenic centers in a single step. Thus, diastereoselective dihydroxylation of the allyl alcohol **229** using a catalytic amount of osmium tetroxide with *N*-methylmorpholine *N*-oxide (NMO) as oxidants proceeded to provide a 12:1 mixture of the triol **230** and its diastereoisomer in 88% yield. Selective tosylation of the primary hydroxy group of **230** with *p*-toluenesulfonyl chloride provided the corresponding β-hydroxy sulfonate which was treated with potassium *t*-butoxide in tetrahydrofuran to yield the epoxy alcohol **231**. Then, the epoxy alcohol **231** was converted with sodium hydride and methyl iodide into part of Segment B **232** without Payne rearrangement.

The synthesis was continued with chiral epoxide **233** (Scheme 34). Treatment of **233** with dimethyl cuprate in ether afforded a mixture of **234** and **235**. Removal of the benzyl group of this mixture by hydrogenolysis and selective 1,2-diol protection with 3-pentanone provided **236**. The resulting alcohol **236** was oxidized by Swern oxidation to afford the aldehyde, which was subsequently treated with 1,3-pro-

Scheme 39. Retrosynthesis Plan for Tautomycin by the Shibasaki Research Group

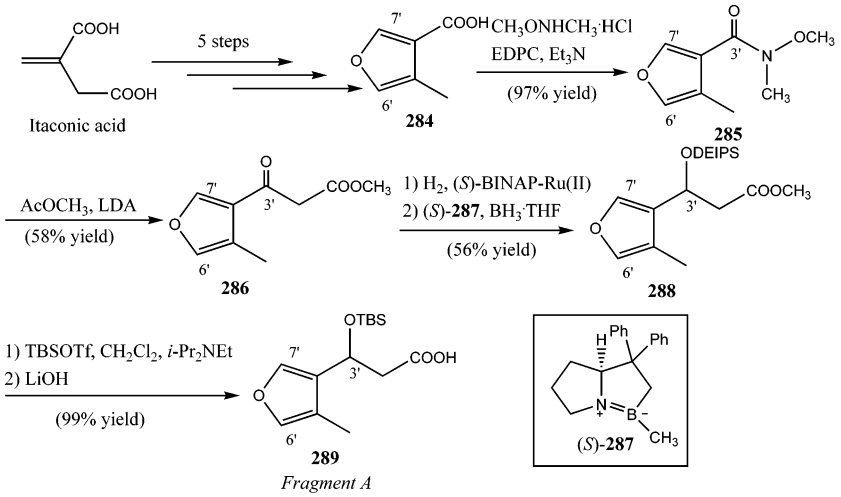


panedithiol and 2,2-dimethoxypropane in the presence of acid catalysts to yield another part of Segment B **237**. Treatment of **237** with *t*-butyllithium in a mixture of tetrahydrofuran and HMPA provided the corresponding dithiane anion, which was coupled with **232** to furnish the coupling product **238**. The final stage for the synthesis of Segment was carried out in Scheme 34. Protecting group manipulation of **238** provided the diol **240** in 47% overall yield. They speculated that the reaction of the alkoxide such as **241** with *p*-toluenesulfonyl chloride afforded the tosylate **242**, which would effect more rapid epoxide formation than sulfonium ion formation due to the high nucleophilicity of the alkoxide at the β-position of the sulfonate. In fact, the alkoxide **241** prepared by the reaction of **240** with 2 equiv of *t*-butyllithium was treated with *p*-toluenesulfonyl chloride to furnish Segment B **243**.

Synthesis of Segment C. The introduction of the C13 methyl group of tautomycin was achieved by S_N2' reaction of the allyl acetate **245**, which was prepared by Ferrier-glycosidation of tri-*O*-acetyl-D-glucal **244** with ethanol (Scheme 35). Thus, treatment of **245** with lithium methylcyanocuprate gave the methyl adduct **246**. Hydrogenation of the double bond of **246** in the presence of platinum on charcoal afforded **247**. *C*-Glycosidation of **247** with phenylthiotrimethylsilylacetylene and boron trifluoride etherate in acetonitrile, epimerization of phenylthioacetylene, and decomplexation with iodine in the presence of sodium hydrogen carbonate provided **248**. The hydrosilylation of the phenylthioacetylene **248** using 1 mol % of sodium hexachloroplatinate(IV) in a mixture of triethylamine and *n*-butanol at 110 °C gave **249**. Protecting group manipulation of **249** and oxidation with *m*-chloroperbenzoic acid gave the heteroolefin **250**. Treatment of **250** with methyllithium-lithium bromide complex in a mixture of hexane and ether (1:1) followed by desilylation with tetrabutylammonium fluoride afforded **251**. Then, treatment **251** with methyltriphenoxyphosphonium iodide and reductive ring opening of the resulting **252** by zinc furnished the open chain compound **253**. Protection of the alcohol **253** as *t*-butyldimethylsilyl ether and epoxidation of the olefin **254** with *m*-chloroperbenzoic acid furnished part of Segment C **255**.

Synthesis of the other part of Segment C began with induction of the C3 stereogenic center of tautomycin starting from levoglucosenone **256** (Scheme 36), which received conjugate addition of lithium methylcyanocuprate to give α-axial methyl adduct **257**. Treatment of the ketone **257** with hydrazine in ethanol and subsequent eliminative Wolff−Kishner reduction in DMSO afforded the vinyl ether **258**, which was further protected as acetate **259**. Addition of ethanol to **259** in the presence of *p*-toluenesulfonic acid gave a mixture of ethyl glycosides **260**. *C*-Glycodidatin of **260** in the presence of boron trifluoride etherate, epimerization of phenylthioacetylene, and decomplexation with iodine in the presence of sodium hydrogen carbonate, furnished phenylthioacetylene **261**. The hydrosilylation of phenylthioacetylene **261** provided the vinyl sulfide **262**. The acetate **263** was deprotected by sodium methoxide, and the resulting alcohol 266 was reprotected as *t*-butyldimethylsilyl ether **264**. Oxidation of the vinyl sulfide **264** with *m*-chloroperbenzoic acid afforded the heteroolefin **265**. Treatment of **265** with methyllithium-lithium bromide complex in a mixture of hexane and ether followed by desilylation with tetrabutylammonium fluoride afforded the *syn*-methyl adduct **266**. Conversion of **266** to the iodide **267** and subsequent ring opening of **267** with zinc gave **268**. The construction of the stereogenic center corresponding to C6 of tautomycin was achieved by inverting C6 hydroxy group of **268** by Mit-

Scheme 40. Synthesis of Fragment A by the Shibasaki Research Group



sunobu reaction. Hydrolysis of the benzoate **29** by sodium methoxide and protection of the alcohol **270** as *t*-butyldimethylsilyl ether furnished the other part of Segment C **271**.

Finally, coupling reaction of **255** with **271** was achieved by the reaction of sulfone carbanion with epoxide in the presence of Lewis acid (Scheme 37). Thus, treatment of **271** with *n*-butyllithium gave the corresponding sulfone carbanion, which was successively treated with boron trifluoride etherate and then **255** to produce the coupling product **272**. The coupling product **272** has two phenylsulfenyl groups, one of which should be removed selectively. This was accomplished by β-elimination of the keto-sulfone **273**, which was prepared by pyridinium chlorochromate oxidation **272** in the presence of molecular sieve 4 Å. Elimination of the β-phenylsulfonyl ketone **273** with 1,8-diazabicyclo-[5,4,0]-7-undecene (DBU) gave the enone **274**. Conjugate reduction of the enone **274** by (triphenylphosphine)copper hydride hexamer gave **275**. Removal of the silyl protecting groups with tetrabutylammonium fluoride and acid-catalyzed spiroketalization of the resulting keto-diol with *p*-toluenesulfonic acid afforded the spiroketal **276**. The Wacker oxidation of the terminal olefin of **276** led us to finish the synthesis of Segment C **277**, which was identical with authentic sample of Segment C derived form natural tautomycin.

Coupling of Segment A, Segment B, and Segment C to Tautomycin. Smooth coupling between the Segment B **243** and Segment C **277** proceeded to give **278** (Scheme 38). Desulfonylation was followed by protecting group manipulation involving (i) silylation at C18 hydroxy group with *t*-butyldimethysilyl trifluoromethanesulfonate (TBSOTf), and (ii) hydrolysis of the *p*-methoxybenzylidene group with pyridinium *p*-toluenesulfonate (PPTS) in methanol to give the diol **280**. Selective esterification of **280** with Segment A **225** under Yamaguchi conditions proceeded expectedly to afford the desired product **281**. Two-step deprotection **280** involving removal of *t*-butyldimethylsilyl groups with poly-(hydrogen fluoride)pyridine complex and cleavage of the two dithioketals using mercury perchlorate in aqueous acetonitrile furnished the synthetic **1**. The synthetic material proved to be identical in all respects with natural **1**.

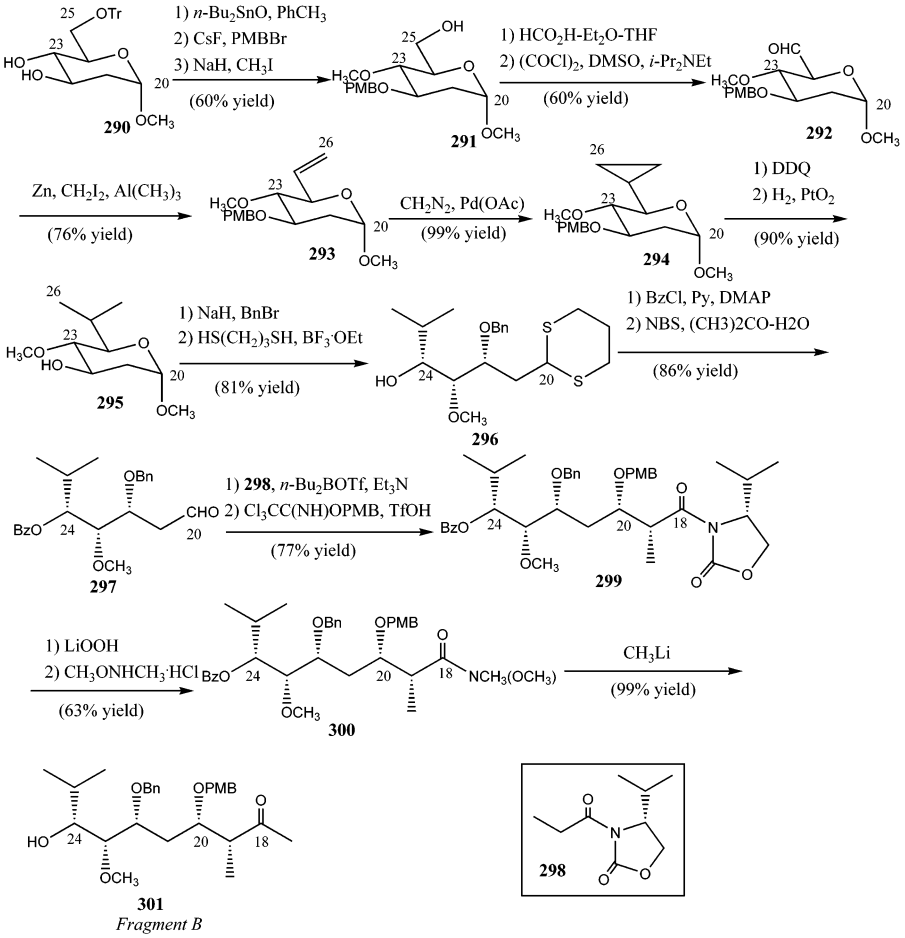
Later, Isobe research group studied a practical semisynthesis of 1 with C1−C20 segment, which was derived from the degradation of natural tautomycin. Recently, they synthesized photoaffinity probes of **1** (Figure 7). Two types of the photoaffinity probe, **282** and **283**, which possesses a benzophenone or a diazirine photophore on the *C*2 of **1**, have been accomplished though the selective reaction of pholabeling units with **1** diacid. The parent compound dramatically showed fluorescence quenching before the photoreaction due to the exciplex formation on the basis of the folded conformation. These probes could be employed for the photoaffinity labeling studies.

6.2.1.3. Shibasaki Research Group. The total synthesis of **1** by Shibasaki research group was similar to that by Isobe's.[266] The retrosynthesis plan is shown in Scheme 39.

Construction of Fragment A. The Fragment A **289** was synthesized using an asymmetric reduction of β-keto ester as a key step (Scheme 40). By using the literature procedure itaconic acid, a commercially available starting material, was transformed to the carboxylic acid **284** in a five-step sequence of reactions. Using DEPC as a condensing reagent the carboxylic acid **284** was converted to the amide **285**, which was treated with the lithium enolate of methyl acetate to yield the β-keto ester **286**. Asymmetric reduction was carried out using BH_3·THF and the oxazaboroliding catalyst **287** developed by Corey. Protection of the product as a diethylisopropylsilyl (DEIPS) ether furnished **288**, which was transformed to the benzyl ester via the carboxylic acid. The furan part of benzyl ester was first treated with singlet oxygen, and a subsequent PCC oxidation then furnished the maleic anhydride **289**.

Synthesis of Fragment B. As shown in Scheme 41, the 2-deoxyglucose derivative **290** was selected as a starting material for the synthesis of Fragment B, because the six carbon atoms on **290** corresponded to the C20−C25 positions of Fragment B. First the diol **290** was selectively monoprotected via the stannylene acetal, followed by *O*-methylatin and then cleavage of the trityl ether to give the alcohol **291**. The terminal isopropyl group was synthesized by a formation-opening of a cyclopropane ring. Namely, aldehyde **292**, given by Swern oxidation, was transformed to the olefin **293** using Nozaki reagent. Using the conditions of Suda, cyclopropanation proceeded smoothly to yield quanitatively and after an oxidative cleavage of PMB group with DDQ, the cyclopropane ring in the resulting alcohol **294** was found to be regioselectively opened by hydrogenolysis with PtO_2 to give **295**. Next **295** was protected as a Bn ether temporarily. The Bn group proved to be the only proecting group which was stable under the conditions for transformation to **296**

Scheme 41. Synthesis of Fragment B by the Shibasaki Research Group



using 1,3-propanedithiol and $BF_3{\cdot}Et_2O$. Protection of the resulting alcohol **296** as a Bz ester and cleavage of dithioacetal group with NBS yielded the aldehyde **297**. Construction of the C19 and C20 stereogenic centers was achieved by Evans' aldol reaction, using the aldehyde **297** and the known oxazolidinone **298**, then protected as a PMB ether to give **299**. After removal of the chiral auxiliary without cleavage of the Bz ester by selective hydrolysis using LiOOH, the resulting carboxylic acid was converted to the amide **300** using DEPC. The amide **300** was treated with an excess of CH_3Li to complete the synthesis of the hydroxyketone **301**, Fragment B.

Construction of Fragment C. As shown in the retrosynthetic analysis outlined in Scheme 39, it was planned to construct Fragment C using a Horner-Emmons reaction and a Julia olefination as the key steps. When the research group started synthetic studies on tautomycin, the absolute configuration of the seven stereogenic centers which are present in Fragment C was unknown. The research group therefore selected chiral building groups of which the both enantiomers were commercially available as starting materials. Thus, the C12−C16 unit 304, the C5−C11 unit 307, and the C1−C4 unit **309** were expected to be synthesizable from (−)-**302**, (+)-DET, and (+)-**302**, respectively (Scheme 42: i, ii, iii). With the three units **304**, **307**, and **309**, the research group attemped the crucial condensation reactions to obtain Fragment C (Scheme 42: iv).

Coupling of Fragment B and Fragment C to Fragment B/C. As shown in Scheme 43, the coupling of Fragment B and Fragment C was achieved by means of an aldol reaction.

Esterification with Fragment A and Fragment B/C. Finally, with Fragment A and Fragment B/C available, the coupling of them was attempted to give **1** (Scheme 44).

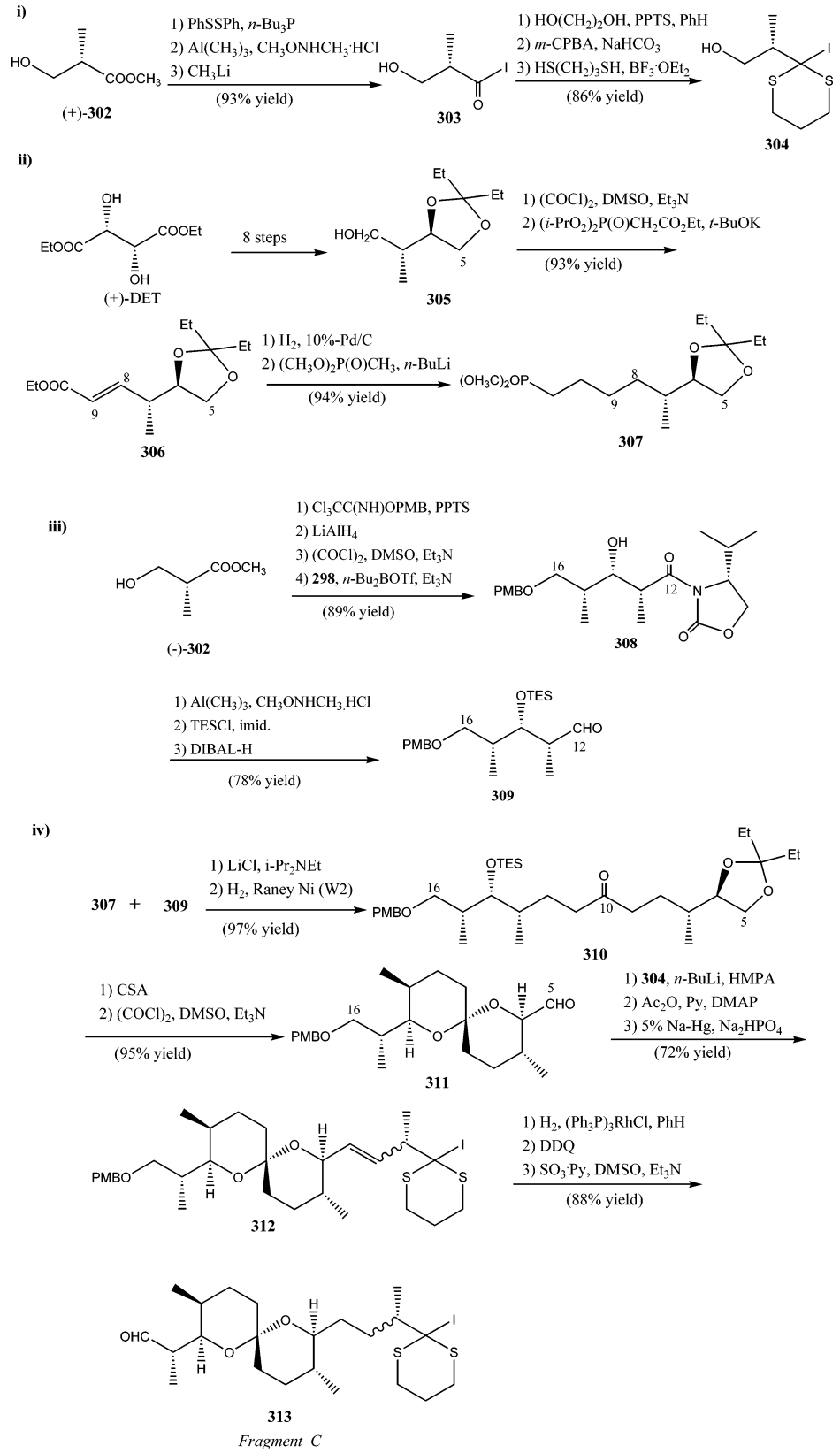
6.2.1.4. Chamberlin Research Group. The Chamberlin research group envisioned a highly convergent synthesis that would rely upon efficient coupling reactions of readily prepared subunits.[265] Their retrosynthesis plan is shown in Scheme 45.

Synthesis of the C1−C8 Subunit. The synthesis of the C1−C8 primary iodide (Scheme 46) begins with the selective oxidative cleavage of *S*-citronellene via a two-step procedure published by Ireland. Addition to the aldehyde **321** consistently gave the desired anti-aldol product **322**. Silylation of the newly formed hydroxyl substituent, reduction of the aryl ester with DIBAL, and conversion of the resultant primary alcohol into an iodide leaving group completed the C1−C8 subunit **325**.

Synthesis of the C12−C18 Subunit. Synthesis started with a geraniol epoxide (Scheme 47). The epoxide was opened with $NaBH_3CN$ to give the desired diol **326**. Next, the diol **326** was cleaved with $NaIO_4$ to give the corresponding aldehyde, which reacted with the boron enolate of Evan's norephedrine-derived chiral auxiliary in a "matched", double diastereoselective aldol reaction. Oxazolidinone **327** was protected as TBS ether and then reduced with the standard lithium benzyloxide transesterification method followed by DIBAL reduction to give primary alcohol **330**, which was transformed into the C12−C18 iodide **331**.

Construction of the C1−C18 Subunit via in Situ Double Alkylation. Treatment of lithiated acetone *N*,*N*-dimethyl-

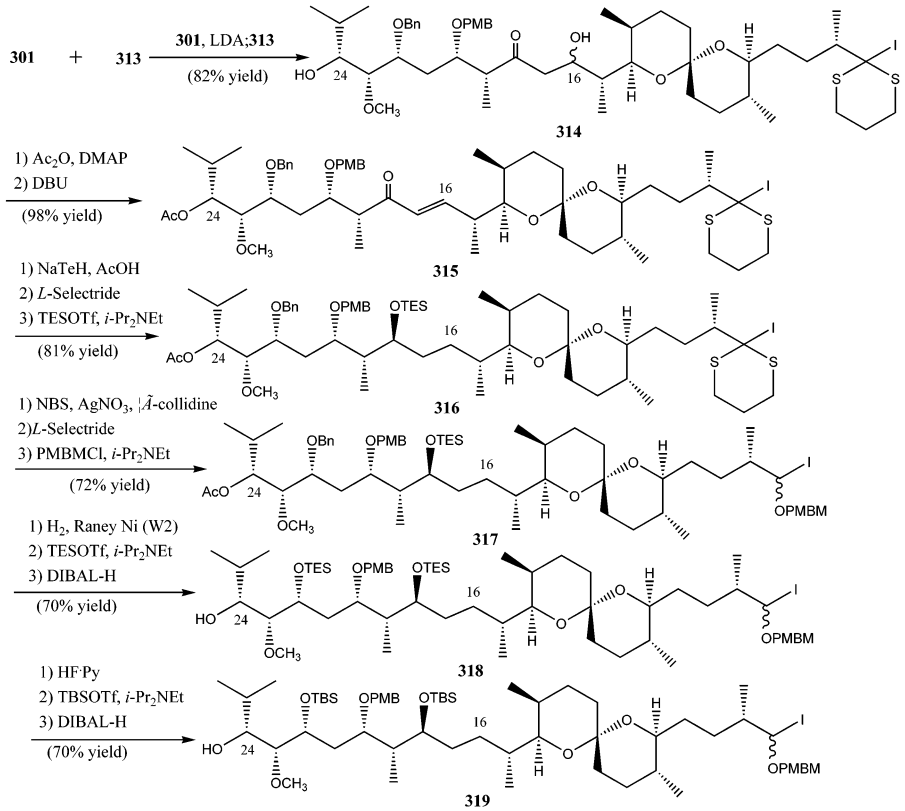
Scheme 42. Synthesis of Fragment C by the Shibasaki Research Group



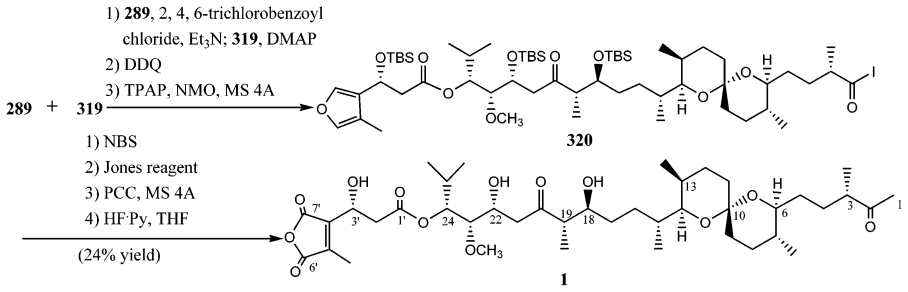
Fragment C

hydrazone with the iodide **325**, followed by a second lithiation (*n*-BuLi) and subsequent treatment with an equimolar amount of the iodide **331**, cleanly afforded the bis(silyloxy)hydrazone. Aqueous workup and treatment of the crude reaction product with HF in a mixture of CH_3CN and *i*-PrOH removed both TBS groups and induced spiroketalization to give **332** as a single isomer (Scheme 48). The completion of the synthesis of **334** required selective oxidative cleavage of the trisubstituted olefin of **332**, which was achieved by reapplying the Ireland conditions at lower temperature to give a 98% yield of the desired monoepoxide **333**. Oxidative cleavage of this productwas large scale. **335** was first saponified using the original conditions of Heathcock and Pirrung and then coupled to the Weinreb amine

Scheme 43. Coupling of Fragment B and Fragment C to Fragment B/C



Scheme 44. Coupling of Fragment A and Fragment B/C to Tautomycin



using DCC and HOAt. Finally, treatment of the anti-isomer with MeLi afforded the β-hydroxy ketone **337** in good overall yield without the need for hydroxyl protection.

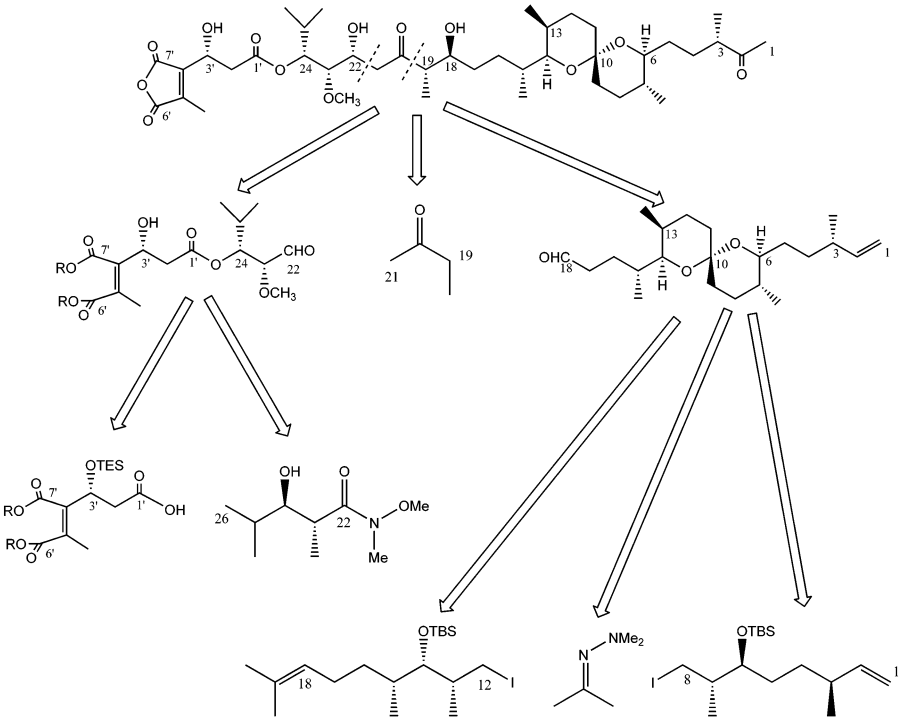
Synthesis of the C22−C26 Subunit. This simple subunit was constructed from the methoxyacetyl-substituted oxazolidinone **338**. Following Evan's precedent, the tin enolate of **338** was allowed to react with isobutyraldehyde in the presence of TMEDA to give all four possible diastereomers favoring the desired stereoisomer **339**. Oxazolidinone **339** was easily separated and then transaminated in standard fashion to give quantitative conversion to **340** (Scheme 49).

Synthesis of the C1−C7 Anhydride Subunit: Coupling to 340. The synthesis was performed with the addition of a mixed methyl cuprate to a symmetrical acetylenedicarboxylic ester **341**, followed by trapping of the intermediate with an electrophile (Scheme 50). The use of malonic acid equivalent 3-pentenoyl chloride ultimately gave the unstable enone **342** as a mixture of geometrical isomers. Reducing enone **342** with $NaBH_4$ afforded the alcohol (±)-**343**. Protection of the hydroxyl substituent as a TES ether **344**, ozonolytic cleavage of the disubstituted alkene, and subsequent oxidation of the aldehyde to a carboxylic acid gave (±)-**346**. With **340** and (±)-**346** in hand, the kinetic resolution was attempted using a mixed phosphonic anhydride esterification method to give **347** (Scheme 51). **347** was independently reduced by DIBAL to afford aldehydes **348**.

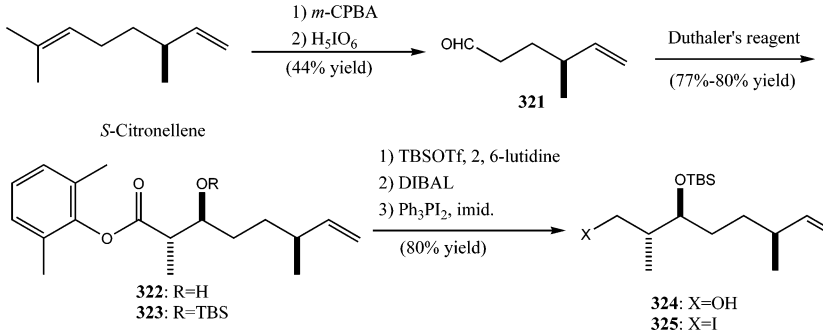
Coupling of Subunits 348 and 337: Completion of the Synthesis. As depicted in Scheme 52, treatment of **337** with TMSOTf and TEA concomitantly protected the hydroxy substituent and formed the silyl enol ether, which was used without purification. Reaction with **348** occurred cleanly, and the mixture of silyl ethers was directly subjected to deprotection conditions to afford a single detectable diastereomer, **349** for three steps. Wacker oxidation of the terminal alkene group in **349** gave the methyl ketone **350** without detectable epimerization. Finally, deprotection of the bis-benzyl ester **350** was performed to give **1**.

6.2.1.5. Other Research Groups. Several other research groups have synthesized the intermediate of **1**. The Nagumo research group studied the synthesis of C5−C16 fragment or C1−C18 fragment employing the regioselective enzymatic acetylation of the spiroketal diol.[260,280] The Armstrong[268] and Marshall[259] research groups synthesized the C1−C21 fragment of **1**; the later employed enantioenriched allenylstannane and zinc reagents derived from (*S*)-3-butyn-2-ol methanesulfonate. The Argade research group in India[258] utilized

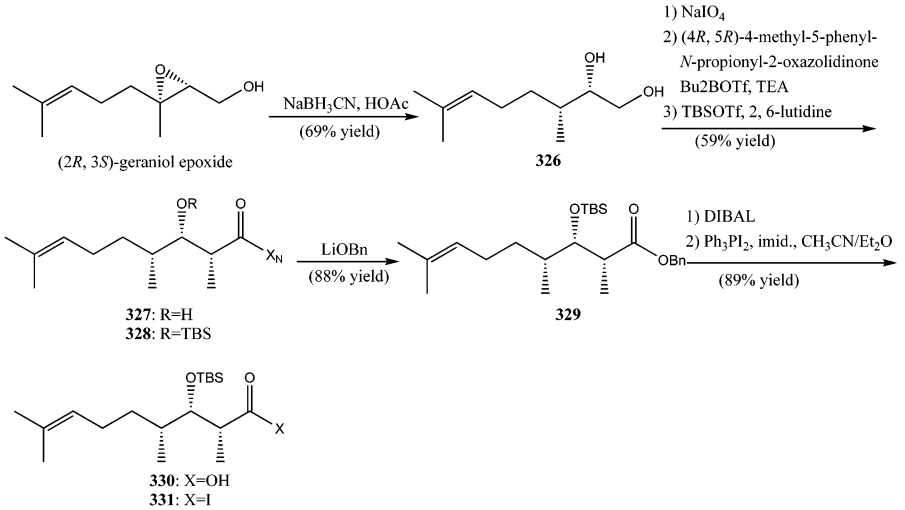
Scheme 45. Retrosynthesis Plan by the Chamberlin Research Group



Scheme 46. Synthesis of the C1−C8 Subunit



Scheme 47. Synthesis of the C12−C18 Subunit

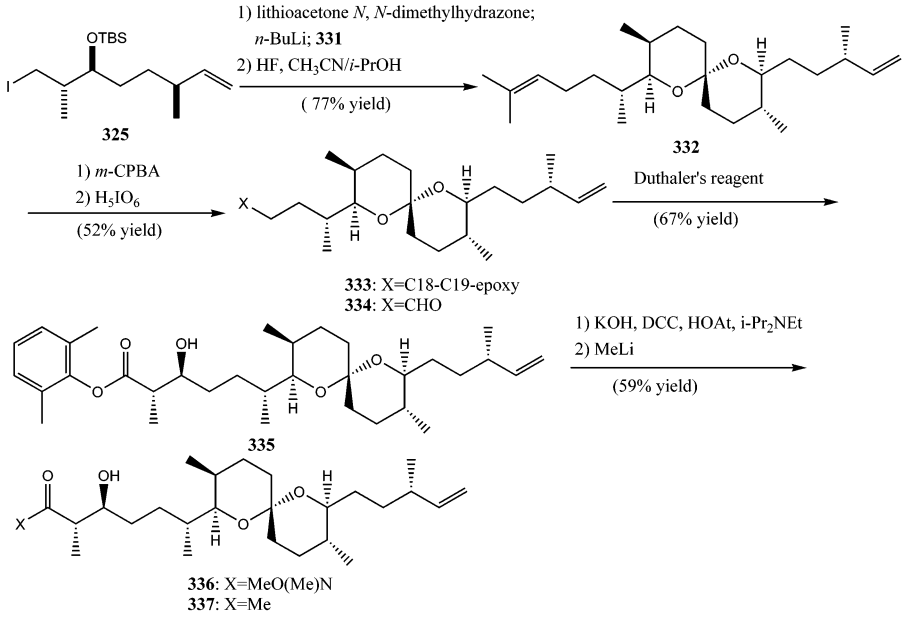


a convenient method for the construction of a (±)-2,3-disubstituted maleic anhydride segment of **1**.

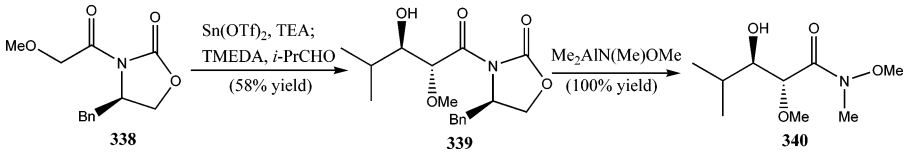
6.2.2. Total Synthesis of Tautomycetin

Selective PP1 inhibition activity of **2** attracted several research groups to synthesize it.[52,281,282] However, only the Ichihara research group synthesized **2** totally. The absolute and relative stereochemistries except C3′ of **2** were unknown until Shibasaki and co-workers determined the absolute stereochemistry of **2** except that of C16 by comparison of the spectral data between the dehydration product of **2** and

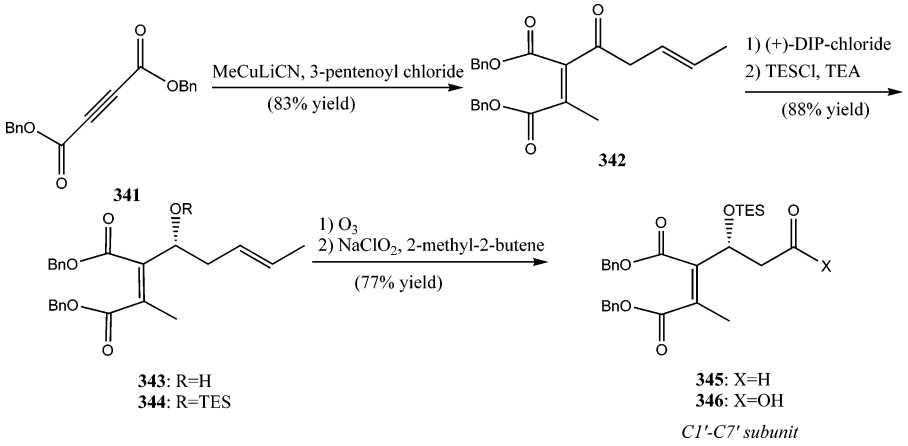
Scheme 48. Construction of the C1−C18 Subunit via in Situ Double Alkylation



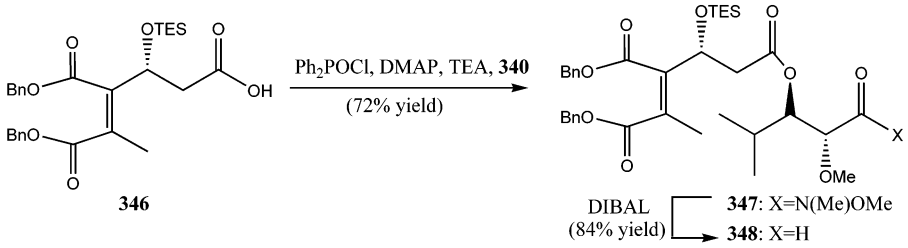
Scheme 49. Synthesis of the C22−C26 Subunit



Scheme 50. Synthesis of the C1−C7 Anhydride Subunit



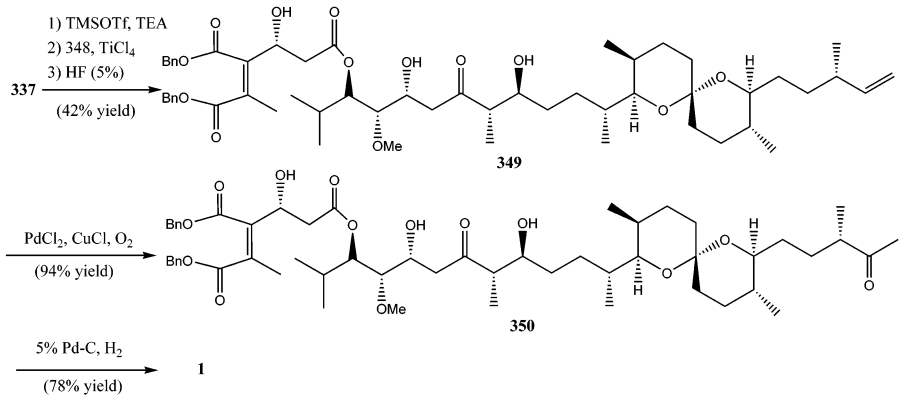
Scheme 51. Coupling of 340 and 346 to Give 348



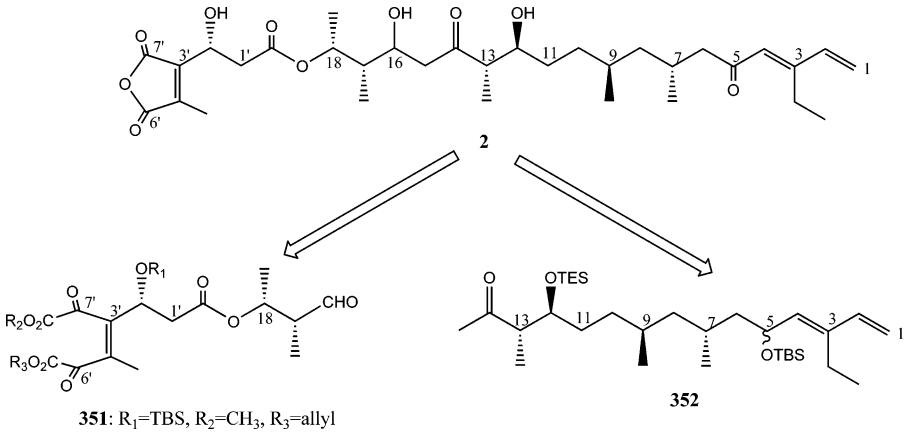
the synthetic diastereomers. Thus, it is necessary to synthesize both C16 diastereomers for securing the C16 stereochemistry in the synthesis of **2**. The retrosynthesis analysis is shown in Scheme 53. **2** is retrosynthetically disconnected into two segments named the C7′−C17 **351** and the C1−C16 segment **352**. The key issue for the synthesis of **352** is an efficient construction of the dienone moiety.

Synthesis of C7′−C17 Segment. The synthesis of the C7′−C17 segment **351** began with trimester **353**, Scheme 54. From trimester **353** derived from natural tautomycin, silylation followed by hydrolysis provided acid **354**. Condensation of **354** with **355** using DCC in the presence of DMAP proceeded smoothly to give **356**. The resultant anhydride **356** was treated with allyl alcohol in the presence

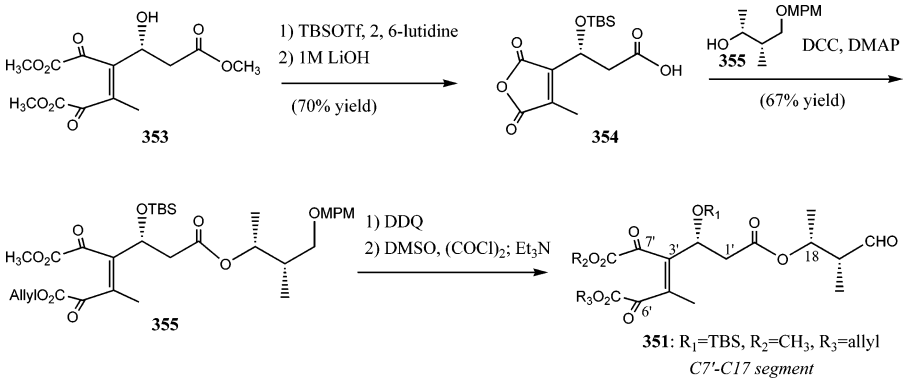
Scheme 52. Coupling of Subunits 348 and 337: Completion of the Synthesis



Scheme 53. The Retrosynthetic Analysis of Tautomycetin



Scheme 54. Synthesis of the C7′-C17 Segment



of triethylamine, and the subsequent methylation with diazomethane afforded a 1:1 mixture of differentially protected anhydride **357**. In this reaction, a prolonged reaction time caused migration of the allyl group. Deprotection and Swern oxidation furnished **351**.

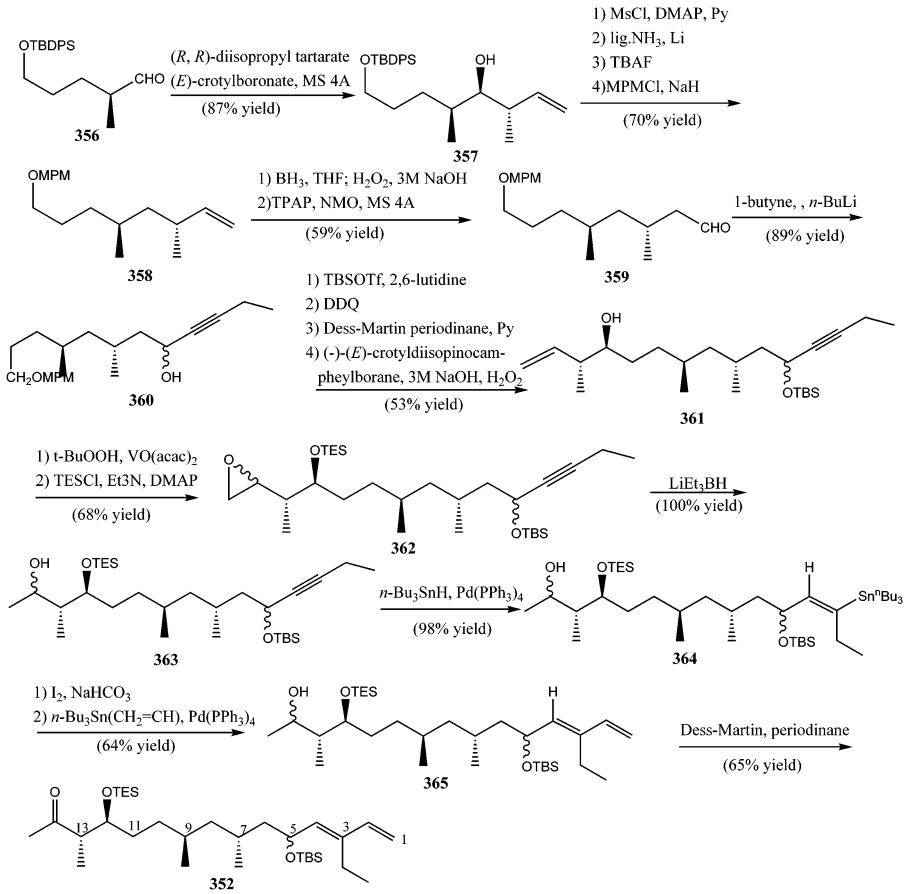
Synthesis of C1−C16 Segment. First, C5−C12 subsegment was synthesized. The synthesis of C5−C12 subsegment **359** started with an (*S*)-enantiomer of the aldehyde **356**, Scheme 55. Crotylboration with (*R*, *R*)-(*E*)-crotylboronate gave the desired adduct **357**. After mesylation of **357**, removal of the C8 oxygen atom was achieved by dissolving metal reduction followed by treatment with TBAF and MPM chloride gave **358**, which was then converted to aldehyde **359** by hydroboration and TPAP oxidation. Next, the C5−C12 segment **359** was converted to the C1−C16 segment **352** as shown in Scheme 55. Lithium acetylide derived from 1-butyne was coupled with aldehyde **359** to afford alcohol **360**. Then, **360** was converted to alcohol **363**. Treatment of alkyne **363** with a large excess tri-*n*-butyltin hydride in the presence of a palladium catalyst proceeded smoothly to give the desired adduct **364** in a highly regioselective manner. Coupling between **364** and vinylstannane furnished **365**. Finally, Dess-Martin oxidation of **365** afforded the C1−C16 segment **352**.

Coupling 351 and 352 to Produce Tautomycetin. As shown in Scheme 56, **2** was coupled with **351** and **352**.

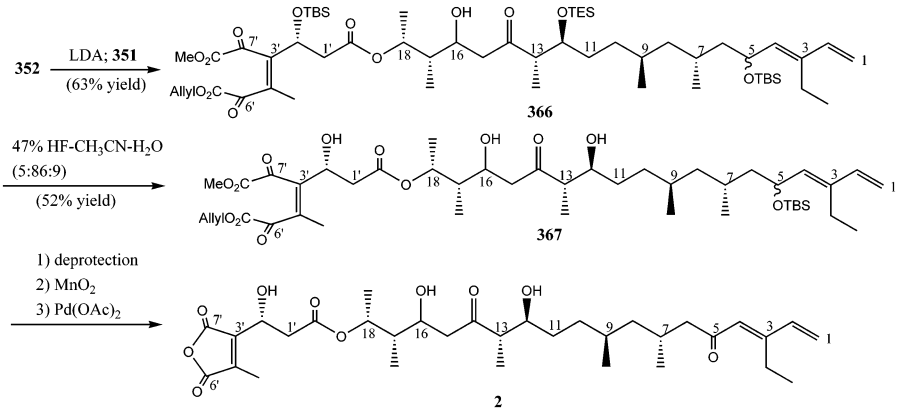
6.3. Chaetomellic Anhydrides

The potent and specific inhibition of Ras farnesyl-protein transferase (PFTase) by chaetomellic acids has attracted the attention of many research groups involved in the development of novel anticancer therapeutics. Studies of structure−activity relationships (SAR) and pharmacological tests have stimulated the development of chemical syntheses for the production of larger quantities of chaetomellic acids than

Scheme 55. Synthesis of the C1−C16 Segment



Scheme 56. Coupling of 351 and 352 to Produce Tautomycetin

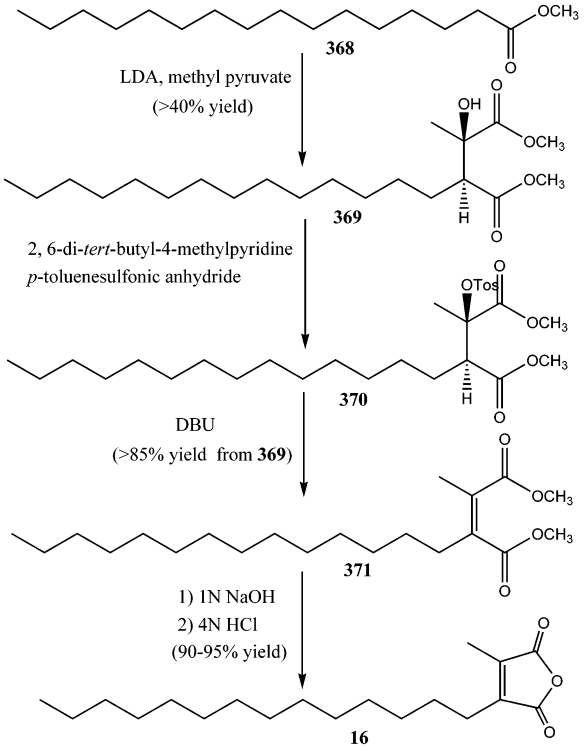


that available from natural sources. So far, 10 routes have been devised for the preparation of this interesting product.[34,53,54,283−296] The synthetic strategies investigated can be grouped into two general strategies: (i) alkylation of maleic precursors[54,285,286,290,291,297] and (ii) assembly of the pivotal 1,4-dicarnonyl group.[283,295] In spite of the variety of methods employed, most of the reported procedures suffer from one or more of the following disadvantages: (i) low yields, (ii) costly reagents, (iii) unstable precursors and/or reactants, (iv) harmful solvents, and (v) unwieldy protocols. As a result, most of procedures are not well-suited for large-scale production, as evidenced by the high price of **16**, which is sold by ICN and Calbio Chem at ca. 8000/g. Recently, Buyck et al. reported a new and efficient approach to produce **16** starting from 2,2-dichloropalmitic acid. Compared to the synthesis of **1**−**4**, the synthesis of **16** is much easier.

6.3.1. Singh Research Group

The first total synthesis of **16** was carried out by the Singh research group in Merck Research Laboratories.[34] The synthetic strategy is based on a biogenetic-type approach that involves an aldol reaction and subsequent double bond generation. Greater than 80% yield of the diastereomeric mixture (1:1) of the aldol products **369** from the methyl palmitate enolate **368** was isolated when **368** (LDA, −78 °C to −10 °C) was added dropwise to a cooled (−78 °C) solution of methyl pyruvate (Scheme 57). Tosylation of **369** using tosic anhydride 2,6-di-*tert*-butyl-4-methylpyridine gave tosylate **370**, which was used in the next reaction without purification. The elimination reaction of tosylate **370** in situ gave predominantly the citraconate diester **371** (*Z*-isomer) with a combined two-step (**369**−**371**) yield of >85%.

Scheme 57. Synthesis of Chaetomellic Anhydride A by the Singh Research Group (I)



Hydrolysis of methyl ester **371** by refluxing 1 N sodium hydroxide in a methanol−THF mixture gave a 90−95% yield of corresponding **16**.

Later, the research group studied the syntheses of chaetomellic anhydride B **32** and chaetomellic anhydride C **380** and modified the syntheses.[283] Their synthetic strategy of chaetomellic anhydrides involved a biomimetic-type aldol condensation of the appropriate fatty acid ester with pyruvate followed by elimination of an equivalent of water and hydrolysis of the ester groups. Scheme 58 shows the syntheses of chaetomellic anhydrides.

6.3.2. Branchaud Research Group

The second total synthesis of **16** was completed by the Branchaud research group at University of Oregon.[294,297] They reported a short and efficient synthesis of **16** utilizing a doubly chemoselective cross coupling of myristyl cobaloxicitraconic anhydride and diphenyl disulfide as the key step (Scheme 59). Sulfide oxidation followed by syn elimination provides **16** in 64% overall yield starting from myristyl bromide.

6.3.3. Schauble Research Group

Chaetomellic anhydride A (**16**) and chaetomellic anhydride B (**32**) were synthesized by the Schauble research group at Villanova University.[296] Malonic ester-type syntheses were used to construct the carbon skeletons of both **16** and **32**. Thus, the reaction of 1-bromotetradecane or 1-bromo-7-hexadecyne with dimethyl malonate and sodium hydride afforded only the monoalkyl malonates **383** and **384** in essentially quantitative yields, relative to the starting bromides (Scheme 60). The ensuing reaction of monoalkyl malonates **383** and **384** with excess methyl 2-bromopropionate and sodium hydride gave trimesters **385** and **386** in yields of 98−99%. Hydrolysis and decarboxylation of the trimesters **385** and **386**, effected by treatment with 3 M ethanolic potassium hydroxide/water, followed by acidification to pH 2, gave mixtures of the diastereomeric erythro and threo succinic acids (**387** and **388**, respectively), isolated in yields >90%, relative to the trimesters. Treatment **387** and **388** with *N*-methylmorpholine and methyl chloroformate afforded the corresponding anhydrides **389** and **390**. The oxidative sequence for conversion of succinic-type anhydrides **389** and **390** to maleic anhydrides **16** and **32** was carried out by reaction of the anhydrides with Et_3N and TMSOTf to give the corresponding 3-methyl-4-tetradecyl-2, 5-bis((trimethylsilyl) oxy)furan (**391**) and 3-(7-hexadecynyl)-4-methyl-2, 5-bis((trimethylsilyl)oxy)furan (**392**), respectively. Subsequent treatment of intermediate **391** with 1 mol % pure tetra-*n*-butylammonium bromide in dry methylene chloride, followed by addition of pure bromine gave **16** in 91% isolated yield. Similarly, oxidation of **392**, using pure NBS rather than bromine as the oxidant, afforded **32** in 88% yield.

6.3.4. Vederas Research Group

The Vederas research group reported a convenient two-step stereospecific preparation of **16**.[285] The synthesis that began with Michael addition of the organocopper reagent **393** to DMAD in the presence of HMPA, followed by capture of the resulting enolate with methyl iodide, generated **16** methyl ester **394** in 78% yield. Careful hydrolysis with lithium hydroxide affords **16** in quantitative yield (Scheme 61).

6.3.5. Argade Research Group

The Argade research group at National Chemical Laboratory of India have contributed studies of anhydrides.[54,290,291,295,298−303] They constructed three total synthetic routes to **16**. The first route utilized condensation of tetradecylimidazopyridinium bromide and maleic anhydride with three steps and 62% overall yield (Scheme 62, i). The second one employed a Witting reaction of a ylide adduct with tetradecyl aldehyde with two steps and 43% overall yield (Scheme 62, ii). The third one was via copper iodide (CuI) induced to synthesize **16** with two steps and 41% overall yield (Scheme 62, iii).

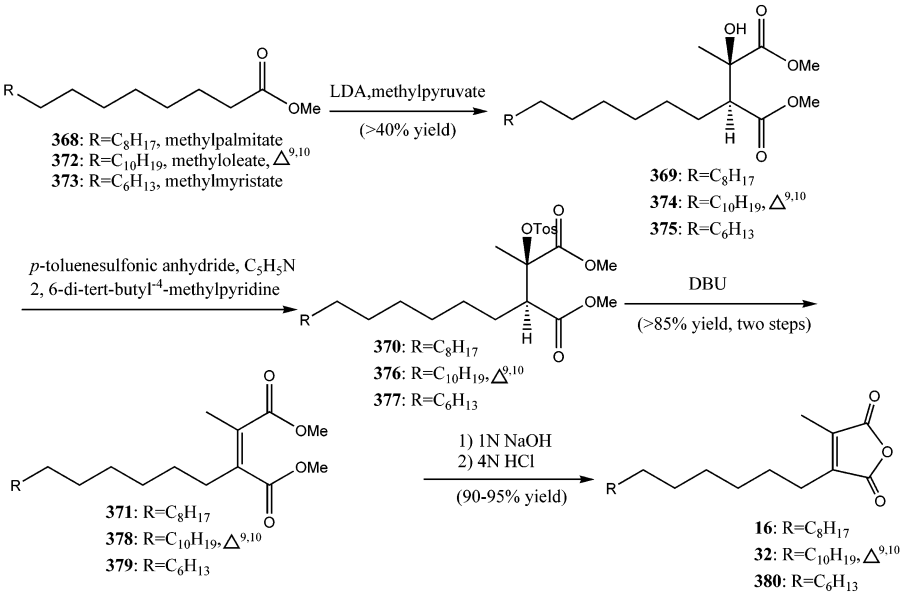
6.3.6. Samadi Research Group

A one-step synthesis of chaetomellic anhydrides was reported by the Samadi research group in France via Barton radical decarboxylation with 70 and 60% overall yields for **16** and **32**, respectively (as shown in Scheme 63).[286]

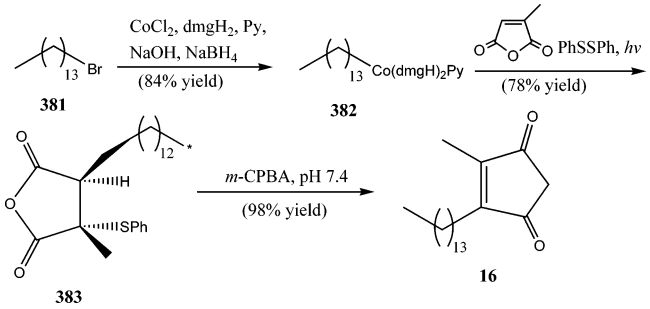
6.3.7. Buyck Research Group

An efficient synthesis of **16** was developed from inexpensive precursors by the Buyck research group. The starting material, 2,2-dichloropalmitic acid (**411**), was easily prepared on a large scale from 1-hexadecanol (**409**), following a two-step protocol with 83% overall yield (Scheme 64, i). The acid **411** was converted into the corresponding acyl chloride using oxalyl chloride and then immediately treated with allyl amine **412** to furnish the amide **413**. Subsequent cyclization of **413** afforded the expected γ-lactam **414**. Then, **414** was treated with CH_3ONa/MeOH to form the maleimide intermediate **415**. Finally, the hydrolysis of **415** afforded **16** (Scheme 64, ii) in 46% overall yield. Recently, they utilized a 2-pyridyl group as a cyclization auxiliary in the ATRC

Scheme 58. Syntheses of Chaetomellic Anhydrides by the Singh Research Group (II)



Scheme 59. Total Synthesis of Chaetomellic Anhydride A by the Branchaud Research Group



step, which allowed the final sequence of hydrolyses to be condensed to just a single operation that is linked to the FR in a one-pot reaction in 68% overall yield.[304]

6.4. Other Compounds

6.4.1. Byssochlamic Acid

The first synthesis of a member of the nonadride family was that of (±)-byssochlamic acid **15** by Stork.[305] This pioneering accomplishment, which created the nine-membered ring of **15** through Beckmann fragmentation of oxime **416**, provided the initial indication that a cis orientation of ethyl and *n*-propyl substituents was more stable. Reduction of **418** under thermodynamic conditions afforded **419** in high yield (Scheme 65).

The second synthesis of (±)-**15** was completed by the White research group at Oregon State University. This experiment hinges on a photoaddition−cycloreversion metathesis to construct the core cyclononadiene system.[306] Sensitized irradiation through Pyrex of bromomaleic anhydride in the presence of 1-pretene afforded the corresponding diacids after basic hydrolysis and characterization as dimethyl esters **420** (Scheme 66, i). Dehydrobromination of **420** gave cyclobutene **421**. The second component required for the synthesis, diol **424**, was prepared by the sequence shown in Scheme 66, ii). 4-Ethylcyclohexanone **422**, obtained by Jones' oxidation of the corresponding alcohol, was carboxylated and brominated to give **423**. Favorskii rearrangement of **423** and subsequent reduction with the complex of diisobutylaluminum hydride afforded **424**. The dipotassium alkoxide from **424** reacted with **421** to give diolide **425**. Irradiation of **425** in dilute solution afforded the intramolecular photoadduct **426**. Exposure **426** to refluxing toluene led to quantitative cycloreversion by opening of the central cyclobutane ring in the direction opposite to that by which it was formed to yield **427**. Basic hydrolysis of **427**, followed by oxidation of the carboxylates **428** with permanganate and acidification, afforded **15** exclusively.

Recently, the White research group reported the synthesis of (+)-15 by a subtle element of stereocontrol.[87]

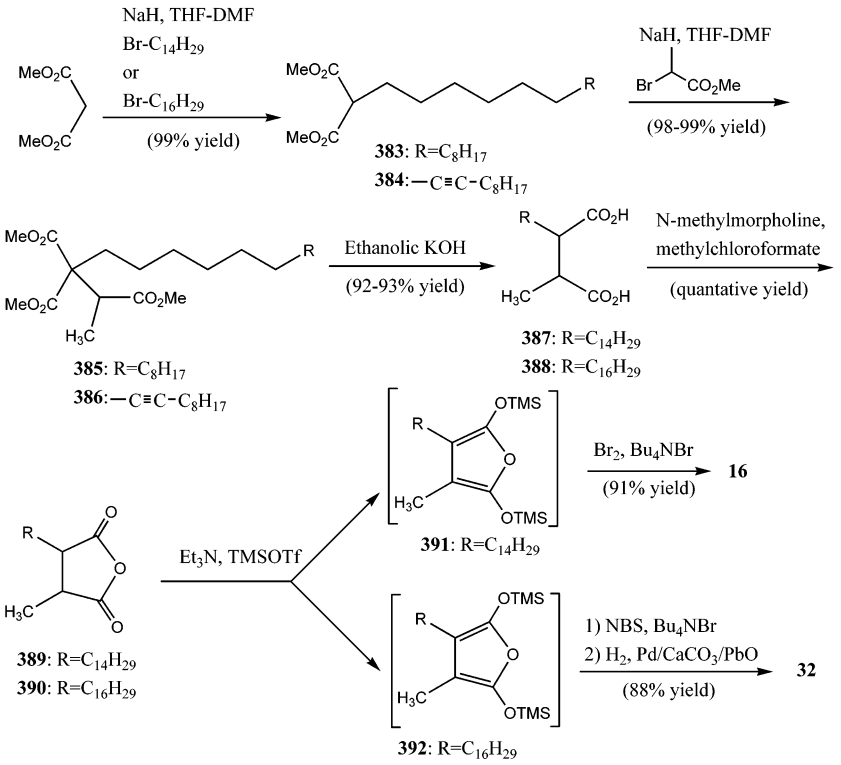
6.4.2. Tyromycin A

Because of the specific inhibition of tyromycin A **22** against the leucine and cysteine aminopeptidases bound to the outer surface of HeLa S3 cells and its cytostatic activity, development of chemical synthesis for **22** is a task of current interest.[44,100,307] The first synthesis has been completed by the Samadi research group in France by using the well-known decarboxylative Barton-radical coupling reaction (Scheme 67).[100] The synthesis began with a diacid **429**. **429** was converted to thiohydroxamic diesters **431**, using the Ph_3P/2,2′-dithiobis-(pyridine *N*-oxide) **430** coupling method. Irradiation in situ of the thiohydroxamic diesters **431**, in the presence of 10 equiv of citraconic anhydride, with a tungsten light, gave the intermediate addition product **432**, which upon purification on silica gel afforded the elimination product **22**.

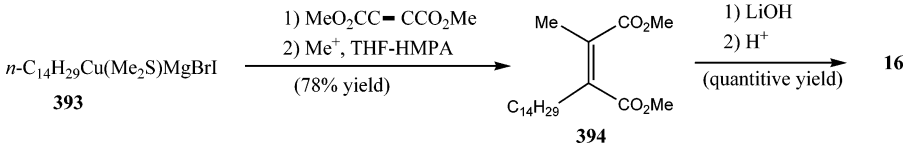
Later, the Argade research group developed a new facile synthetic route to this bioactive natural product **22** via a coupling reaction of citraconimide **398** and triphenylphosphine (TPP) adduct with aliphatic aldehyde (Scheme 68).[308] **22** was synthesized with a practical two-step synthesis in 71% overall yield.

The circuitous road toward the syntheses of the natural products with maleic anhydride structure is paved with discovery, invention, and adventure. In them, phomoidrides are some of the most complex natural products. Tautomycin contains spiroketal and maleic anhydride moieties and 13 chiral centers. Endeavoring to synthesize them provides an

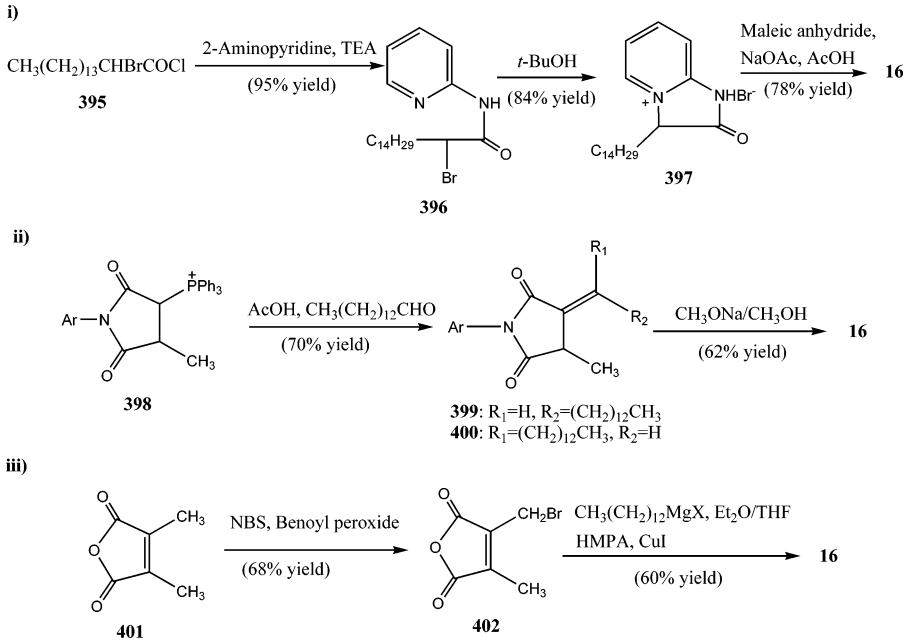
Scheme 60. Synthesis of Chaetomellic Anhydride A and Chaetomellic Anhydride B by the Schauble Research Group



Scheme 61. Synthesis of Chaetomellic Anhydride A by the Vederas Research Group



Scheme 62. Synthesis of Chaetomellic Anhydride A by the Argade Research Group

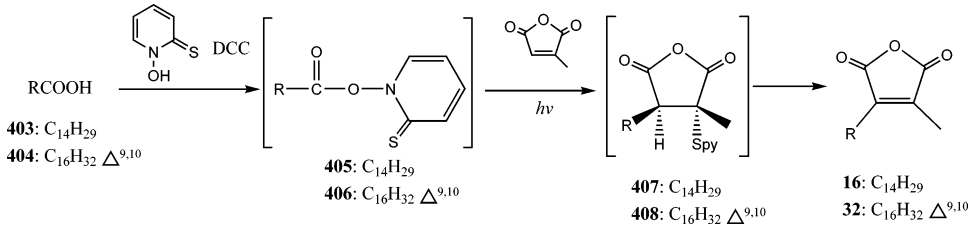


impetus for the discovery of novel chemical techniques and mechanistic proposals, which in turn deliver an increased understanding of the behavior of chemical systems. It is therefore here, in the labyrinth of total syntheses, that the field of chemical science is advanced.

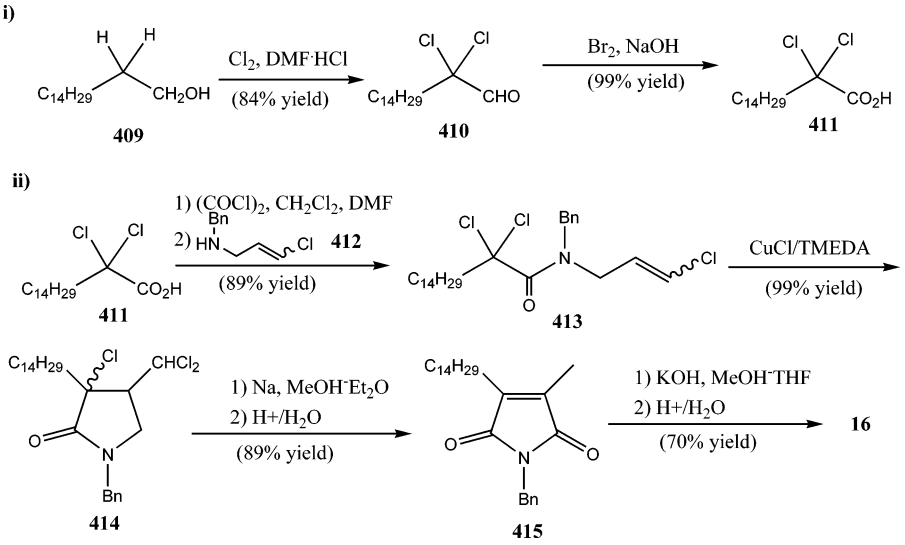
7. Structure Modification and Structure–Activity Relationship (SAR) Studies

There have been limited SAR studies conducted on this group of compounds, largely due to the protracted and

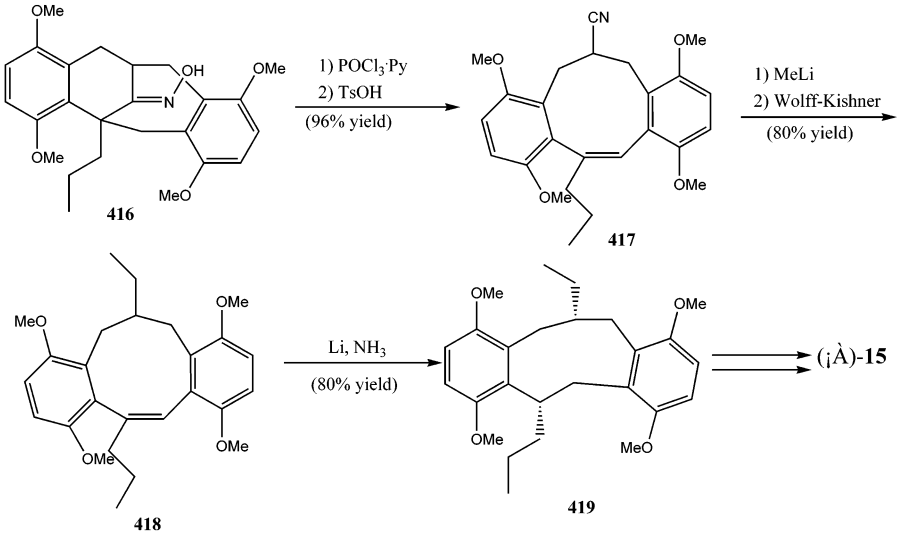
Scheme 63. Synthesis of Chaetomellic Anhydrides by the Samadi Research Group



Scheme 64. Synthesis of Chaetomellic Anhydride A by the Buyck Research Group

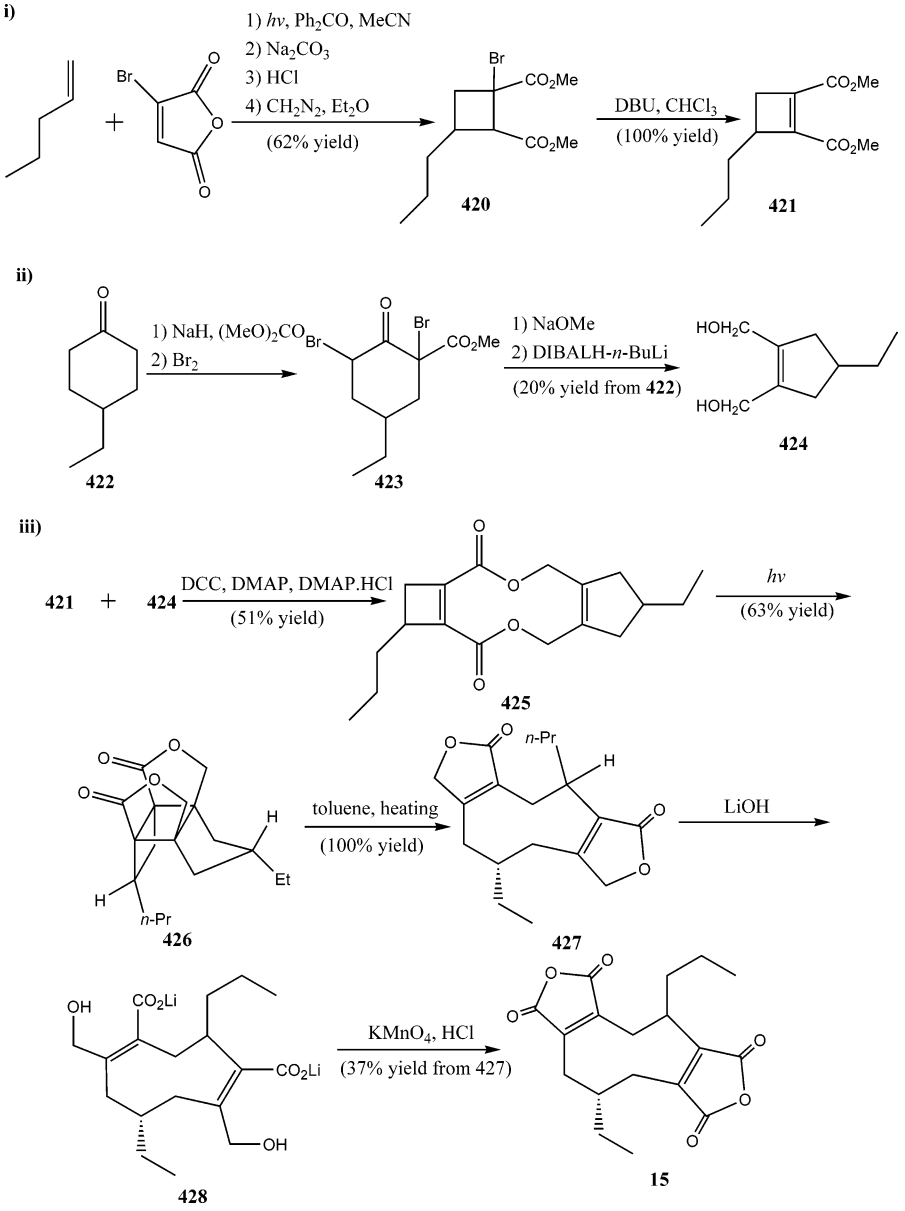


Scheme 65. Synthesis of (±)-Byssochlamic Acid by Stork

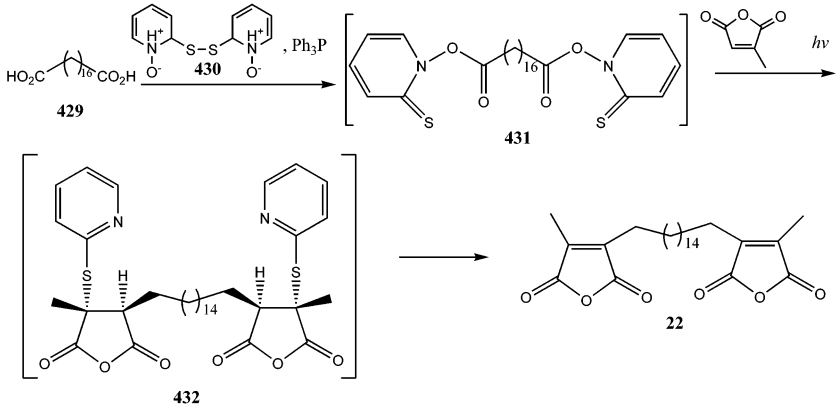


complex nature of their synthesis. Several research groups investigated the SAR of tautomycin **1** and chaetomellic anhydride **16**.[52,54,283−286,288,292,301,302,309] The Singh research group found that chaetomellic dicarboxylic acids have a high propensity to cyclize, and **16a** was, in fact, isolated as chaetomellic anhydride **16**. The cyclic form, however, is unstable under mild basic conditions (pH = 7.5) and is readily hydrolyzed to the dicarboxylate anion **16b**, which, apparently, is the biologically active component (Scheme 69).[35] PFTase activity of the diacid anion of **16a** is noncompetitive toward the acceptor peptide Ras but is highly competitive with respect to farnesyl pyrophosphate (FPP). This may be explained by the structural similarity between dicaroxylate anion **16b** and FPP, since both possess a hydrophilic head group bound to a hydrophobic tail (Figure 8). The maleate unit aligns well with the corresponding diphophate moiety, since the negatively charged oxygen atoms can achieve a space within 0.1 Å, while the flexible nature of the aliphatic chain permits it to fill the same space as the hydrophobic end of FPP upon binding to the enzyme. The Isobe research group proposed that an active form of **1** is the dicaroxylate, too.[310] The dialkylanhydride moiety in **16** mimics the pyrophosphate group in the substrate of farnesyl-protein transferase, and the anhydride moiety of **1** might be regarded as the phosphate group of the substrate. Considering this proposal, the anhydride moiety of **1** might directly interact with the phosphatase molecule.

Scheme 66. Synthesis of (±)-Byssochlamic Acid by the White Research Group



Scheme 67. Synthesis of Tyromycin A by the Samadi Research Group

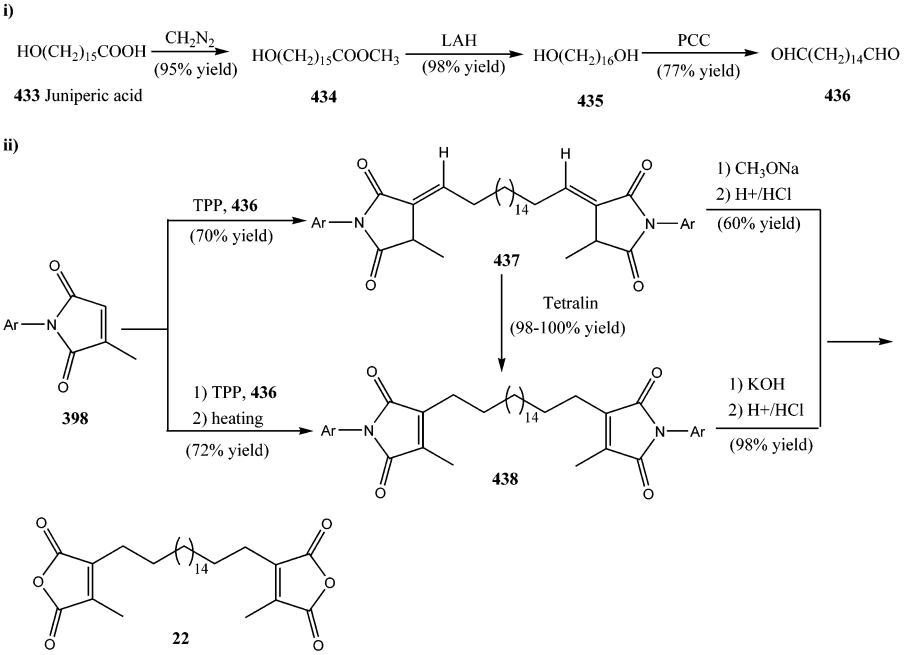


7.1. Tautomycin

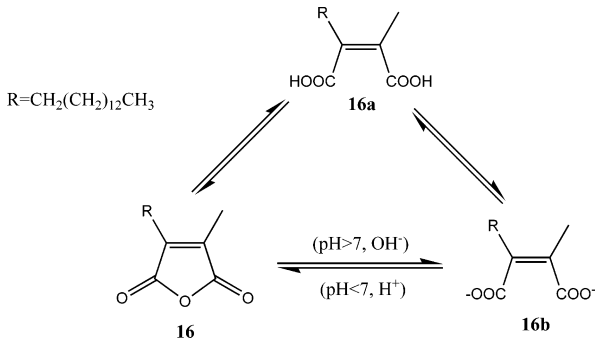
In the synthesis of **1**, a number of synthetic intermediates and **1** derivatives were synthesized. With these compounds, an SAR study of **1** was employed to identify the partial structure essential for protein phosphatase inhibition and apoptosis-inducing activity toward human leukemia Jurkat cells.[52,309]

The compounds are listed in Figure 9. The SAR studies are shown in Table 22. Among the compounds, **1** was the

Scheme 68. Synthesis of Tyromycin A by Argade



Scheme 69. Formation of Chaetomellic Anhydride A



most potent inhibitor and the most effective inducer of apoptosis. It inhibited PP1 and PP2A enzymatic activity concentration dependently with IC_{50} values of 20 and 75 pM, respectively, in the presence of 0.01% Brij-35, and an LC_{50} value of 1 μM. Esterification of the anhydride moiety of **1** markedly increased the IC_{50} for the protein phosphatases. The C1′−C7′ fragment of **1** had no inhibitory effect, but the fragment containing the C22−C26 moiety was inhibitory. These results suggest that the C22−C26 moiety is essential for inhibition of protein phosphatase activity and that the anhydride moiety enhances the inhibition. However, the esterification of the anhydride did not decrease, nor did the inclusion of the C22−C26 moiety increase the apoptosis-inducing activity. On the other hand, the C1−C18 moiety of **1** was essential for induction of apoptosis, and the conformation and the arrangement of functionalities of the C18−C26 carbon chain affected the apoptosis activity. However, modification of C1−C18, C1−C21, or C1−C26 compounds had little effect on phosphatase inhibitory activity.

7.2. Chaetomellic Anhydride A

Because of the potent inhibitory activity of **16** against Ras PFTase, several research groups investigated the structure modification of **16** and its SAR.[283,285] The first study of **16** SAR was studied by the Vederas research group.[285] Three dicarboxylate anion analogues of **16b** were synthesized and used as shown in Figure 10. And **16b** and its analogues **452**−**454** were evaluated for the inhibition of yeast PFTase and yeast protein geranylgeranyl-transferase (PGGTase-I) using the continuous fluorescence assay. The results are summarized in Table 23. **16b** inhibited PFTase with an IC_{50} of 17 μM but did not inhibit PGGTase-I at all (>300 μM). Compound **454**, containing a farnesyl side chain, was the most potent inhibitor of PFTase and exhibited a good selectivity for PFTase over PGGTase-I (100:1). In contrast, analogue **453**, containing a geranylgeranyl side chain, was a fairly good inhibitor of PGGTase-I (IC_{50} = 11.5 μM), although the level of selectivity for PGGTase-I over PFTase was lower (~10:1). Compound **454** was also shown to be a

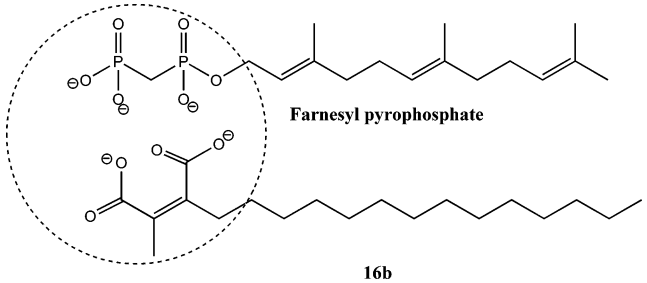


Figure 8. Structure similarity between the dianionic form of chaetomellic anhydride and farnesyl pyrophosphate.

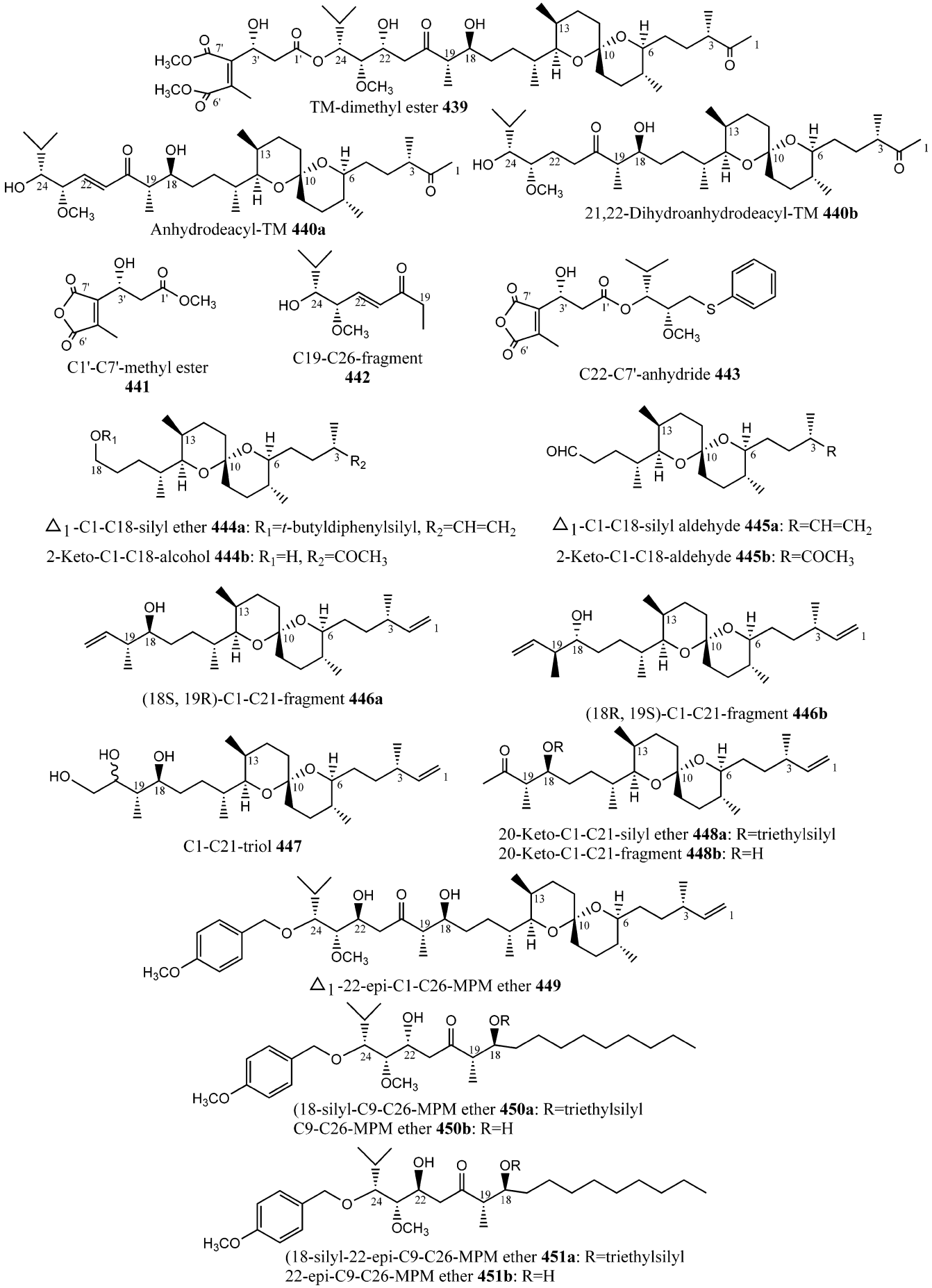


Figure 9. Chemical structures of tautomycin derivatives and related compounds.

Table 22. IC_{50} and LC_{50} Values of Tautomycin and Its Related Compounds for PP1, PP2A, and Jurkat Cells

	IC_{50} (μM)[a]		
compounds	PP1	PP2A	LC_{50} (μM)[b]
1	20×10^{-6}	75×10^{-6}	1
22-epi-**1**	50×10^{-3}	100×10^{-3}	20
439	0.2	0.5	1.3
440a	75	100	2
440b	>100	>100	>50
441	>100	>100	>50
442	>100	>100	50
443	40	40	>50
444a	>100	>100	>50
444b	>100	>100	>50
445a	>100	>100	15
445b	>100	>100	15
446a	>100	>100	10
446b	>100	>100	10
447	50	>100	20
448a	45	100	>50
448b	5	20	50
449	>100	>100	10
450a	>100	>100	ND[c]
450b	>100	>100	ND
451a	>100	>100	ND
451b	>100	>100	ND

[a] The phosphatase activities of PP1 (0.1 mU/mL) and PP2A (0.08 mU/mL) were assayed after preincubation for 15 min with 0.01% Brij-35 and tautomycin or its related compounds. [b] Cell viability was determined by the MTT assay. [c] ND, not determined.

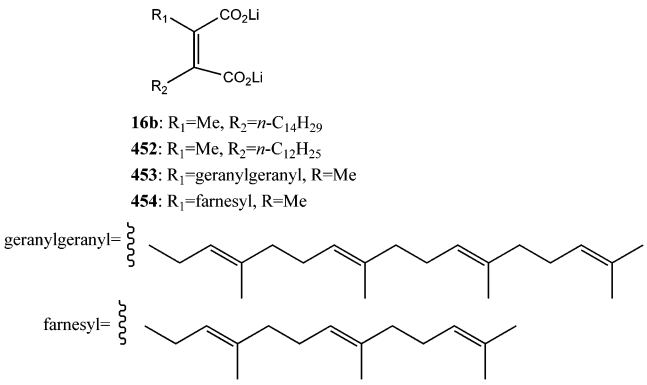


Figure 10. Analogues of chaetomellic anhydride A synthesized by the Verderas research group.

Table 23. Inhibition of Protein Prenyltransferases from Yeast with Chaetomellic Acid A and Its Analogues

	IC50 (μM)	
compound	PFTase	PGGTase-I
16b	17 ± 3	>300
452	4 ± 0.1	112 ± 3
453	96 ± 16	11.5 ± 0.6
454	2.4 ± 0.08	277 ± 21

competitive inhibitor of PFTase against FPP with a $K_i = 1.1 \pm 0.1$ μM.

The second study of **16** SAR was investigated by the Singh research group.[283] They proposed that the molecular modeling experiments with **16** and FPP indicated that a *cis*-diacid and a C-12 chain would be optimal for binding assuming an extended conformation of the side chain. To evaluate this prediction, the isomeric diacids of **16** and **32** and C-12 chain compound chaetomellic acid C **455** and its isomers (Figure 11) were synthesized, and the results are summarized in Table 24.

The vinyl diacid analogue of **458a** is 36 times less active when compared to **16**, and its trans isomer **459a** is completely inactive up to 100 μM. Both vinyl **458b** and *trans*-**459b** isomers of **32** were completely inactive up to 100 μM. Surprisingly, the prediction of chain length by molecular modeling did not hold up, and **455**, the acid that was predicted to be the most active, exhibited an IC_{50} value of 500 nM and was approximately 10-fold less active than **16**. However, the activity pattern of the vinyl **458c** and *trans* isomers **459c** of **455** was significantly different from the corresponding isomers of acids A and B. Compounds **458c** and **459c** showed IC_{50} values of 4 and 5 μM, respectively. They showed only 8- and 10-fold lower activity when compared to the corresponding *cis*-isomer **455**. Chaetomellic acids, with longer or shorter chains, forces the dicarboxylate away from the zinc register and leads to poor inhibitor binding and, hence, inhibitory activity. These results are consistent with the molecular ruler hypothesis of substrate binding and specificity. Substitutions of the olefinic methyl group with a smaller (for example, H) or larger group (for example, Ph) in the head unit of **16** resulted in a significant reduction in inhibitory activity. However, the hydroxymethyl group substitution did not have any significant effect on the potency of these compounds. For example, **456** and **457** displayed IC_{50} values of 250 and 270 nM, respectively. There was no significant difference in the inhibitory activities of chaetomellic acids when tested against other mammalian prenyltransferases such as bovine brain PFTase. The esterification of the carboxyl groups of chaetomellic acids caused the complete loss of inhibitory activities. The C-12 chain acid is 10 times less active against human PFTase when compared to the C-14 chain acid **16** vide supra. However, it is more active ($IC_{50} = 3-4$ μM) than **16** ($IC_{50} = 17$ or 225 μM) when tested against yeast PFTase. The observation of differences in the potency of chaetomellic acids between mammalian and fungal (or yeast) PFTase is noteworthy. **16** is more active against both recombinant human and bovine brain PFTase ($IC_{50} = 55$ nM) and is less active against yeast PFTase ($IC_{50} = 17-225$ μM), depending on the assay used. These differences in the IC_{50} values are similar to the differences in the affinity of FPP for the two enzymes. FPP has a higher affinity for mammalian PFTase ($K_D = 12$ nM) than for yeast PFTase ($K_D = 75$ nM). Inhibitors that compete with FPP for binding for their inhibitory activity may be expected to reflect this difference of the K_D values in their IC_{50} values.

Natural products play a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases and agents for other biological activities.[311,312] However, the combinatorial chemistry plays a major role in the drug development process. Most present drugs are biological, natural products derived from a natural product and are usually a semisynthetic modification or made by total synthesis with the pharmacophore from a natural product. Concerning the strong biological activities of the natural products with maleic anhydride structure, we can employ the maleic anhydride moiety of these natural products as the pharmacophore or as the core to synthesize other compounds with maleic anhydride structure and then to determine the biological activities. Then, the structure and activity relationship can be investigated.

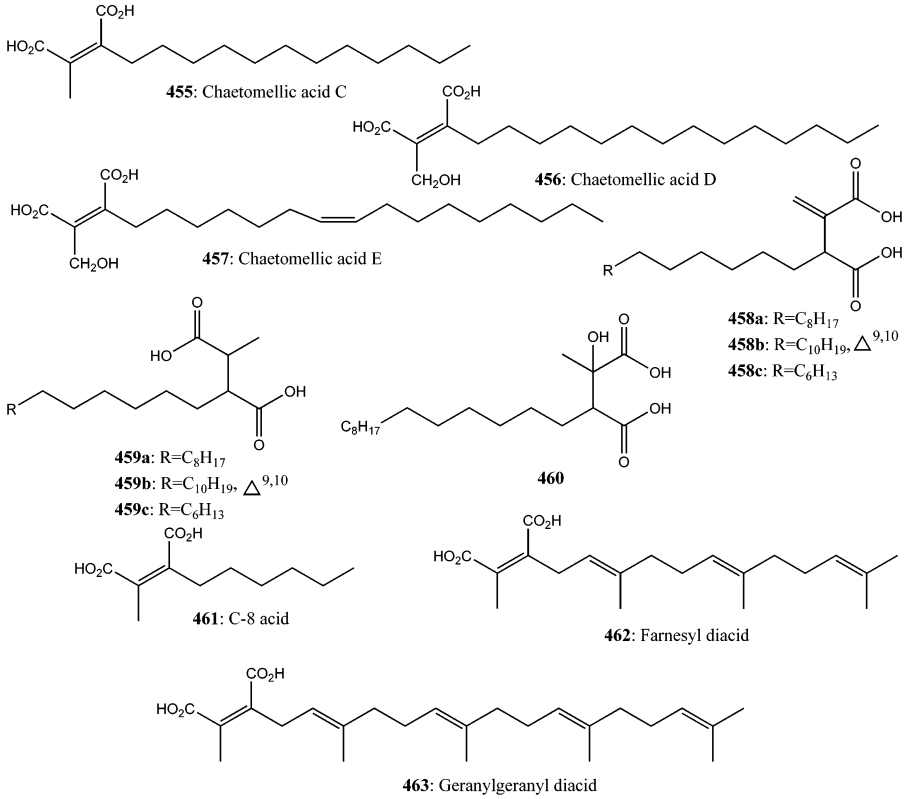


Figure 11. Analogues of chaetomellic acid A synthesized by the Singh research group.

Table 24. Prenyltranserase Activities (IC_{50}) of Chaetomellic Acids and Analogues

compds	rHPFTase[a]	GGPTase-I[b]	ScPFTase[c]	ScGGPTase[d]
16	55 nM	92 nM	17 μM	>300 μM
32	185 nM	54 nM	300 μM	
455	500 nM		4 μM	112 μM
456	250 nM			
457	270 nM			
458a	2 μM			
458b	>100 μM			
458c	4 μM			
459a	>100 μM			
459b	>100 μM			
459c	5 μM			
460	>100 μM			
461	>100 μM			
462			24 μM	277 μM
463			96 μM	11.5 μM

[a] Recombinant human PFTase. [b] Bovine brain GGPTase-I. [c] *Saccharomyces cerevisiae* PFTase. [d] *Saccharomyces cerevisiae* GGPTase.

8. Concluding Remarks

Natural products with maleic anhydride mentioned above have distinctive activities in biology, such as antibiotic, enzymatic inhibitors. Some of them are potent to be developed as pharmaceuticals and biopesticides, such as **1**−**4**, **6**−**9**, **11**, **16**−**18**, **22**, **30**; **7**−**9** have been produced in commercial scale as herbicides and antifungal agents. From SAR research, the maleic anhydride moiety has the important function in their structures. In recent years, Ras genes are found to be activated in about 30% of all human neoplasms. The Ras proteins (H, N, K-4A, and K-4B) are synthesized as cytosolic precursors and localized on the plasma membrane after post-translational modifications. The critical modification required for attachment to the inner membrane and for cell-transforming activity is the farnesylation of Ras. This prenylation is catalyzed by the enzyme PFTase. Therefore, inhibitors of PFTase could act as anticancer agents blocking an essential step for Ras activation. PFTase has become an important target for the design of inhibitors that might be interesting as antitumor agents. In natural products with maleic anhydride, **3**, **4**, and **16** are strong inhibitors against PFTase and potent to be used anticancer drug. As the special inhibitors against PP, **1** and **2** are also potent to be developed as antitumor agents and immunosuppressors. Furthermore, with the modification of their structures, new inhibitors related to them could be found. In the synthesis of new pharmaceuticals and biopesticides, the maleic anhydride structure may be important to be coupled into the compounds to study their inhibitory activities.

9. Acknowledgment

We thank National Major Basic Research Program of China (2003CB114402 and 2003CB716005) for the financial support.

10. References

(1) Ubukata, M.; Cheng, X. C.; Isono, K. *J. Chem. Soc. Chem. Commun.* **1990**, 244.
(2) Cheng, X.; Kihara, T.; Kusakabe, H.; Magae, J.; Kobayashi, Y.; Fang, R.; Ni, Z.; Shen, Y.; Ko, K. *J. Antibiot.* **1987**, *40*, 907.
(3) Cheng, X. C.; Ubukata, M.; Isono, K. *J. Antibiot.* **1990**, *43*, 890.
(4) Cheng, X. C.; Kihara, T.; Ying, X.; Uramoto, M.; Osada, H.; Kusakabe, H.; Wang, B. N.; Kobayashi, Y.; Ko, K. *J. Antibiot.* **1989**, *42*, 141.
(5) Spiegel, D. A.; Njardarson, J. T.; McDonald, I. M.; Wood, J. L. *Chem. Rev.* **2003**, *103*, 2691.
(6) Dabrah, T. T.; Harwood, H. J., Jr.; Huang, L. H.; Jankovich, N. D.; Kaneko, T.; Li, J. C.; Lindsey, S.; Moshier, P. M.; Subashi, T. A.; Therrien, M.; Watts, P. C. *J. Antibiot.* **1997**, *50*, 1.
(7) Dabrah, T. T.; Kaneko, T.; Massefski, W., Jr.; Whipple, E. B. *J. Am. Chem. Soc.* **1997**, *119*, 1594.
(8) Moss, M. O. *Microb. Toxins* **1971**, *6*, 381.

(9) Moss, M. O.; Wood, A. B.; Robinson, F. V. *Tetrahedron Lett.* **1969**, 367.
(10) Moss, M. O.; Robinson, F. V.; Wood, A. B. *Chem. Ind.* **1968**, 587.
(11) Moss, M. O.; Hill, I. W. *Mycopathol. Mycol. Appl.* **1970**, *40*, 81.
(12) Moss, M. O.; Robinson, F. V.; Wood, A. B.; Paisley, H. M.; Feeney, J. *Nature* **1968**, *220*, 767.
(13) Takahashi, S.; Nakajima, M.; Kinoshita, T.; Haruyama, H.; Sugai, S.; Homma, T.; Sato, S.; Haneishi, T. *ACS Symp. Ser.* **1994**, *551*, 74.
(14) Nakajima, M.; Itoi, K.; Takamatsu, Y.; Sato, S.; Furukawa, Y.; Furuya, K.; Honma, T.; Kadotani, J.; Kozasa, M.; Haneishi, T. *J. Antibiot.* **1991**, *44*, 1065.
(15) Haneishi, T.; Nakajima, M.; Koi, K.; Furuya, K.; Iwado, S.; Sato, S. Manufacture of cornexistin herbicide with Paecilomyces. Europe Patent 290193, 1988.
(16) Amagasa, T.; Paul, R. N.; Heitholt, J. J.; Duke, S. O. *Pestic. Biochem. Physiol.* **1994**, *49*, 37.
(17) Fields, S. C. Hydroxycornexistin herbicide. U.S. Patent 5424278, 1995.
(18) Fields, S. C.; Mireles-Lo, L.; Gerwick, B. C. *J. Nat. Prod.* **1996**, *59*, 698.
(19) Nakajima, Y.; Watanabe, H.; Adachi, T.; Tagawa, M.; Futagawa, M.; Nishino, Y. Zopfiellin isolation from culture broth. Japan Patent 08107792, 1996.
(20) Nagamura, A.; Aoki, H.; Nishimura, T.; Kinoshita, I. Purification of zopfiellin. Japan Patent 09087268, 1997.
(21) Crane, R. I.; Hedden, P.; MacMillan, J.; Turner, W. B. *J. Chem. Soc. Perkin Trans. 1* **1973**, 194.
(22) Hosoe, T.; Fukushima, K.; Itabashi, T.; Nozawa, K.; Takizawa, K.; Kawai, K.i. *Heterocycles* **2004**, *63*, 2581.
(23) Hosoe, T.; Fukushima, K.; Itabashi, T.; Nozawa, K.; Takizawa, K.; Okada, K.; Takaki, G. M. d. C.; Kawai, K. i. *J. Antibiot.* **2004**, *57*, 573.
(24) Barton, D. H. R.; Godinho, L. D. S.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1779.
(25) Barton, D. H. R.; Jackman, L. M.; Rodriguez-Hahn, L.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1772.
(26) Barton, D. H. R.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1769.
(27) Baldwin, J. E.; Barton, D. H. B.; Bloomer, J. L.; Jackman, L. M.; Rodriguez-Hahn, L.; Sutherland, J. K. *Experientia* **1962**, *18*, 345.
(28) Strunz, G. M.; Kakushima, M.; Stillwell, M. A. *J. Chem. Soc. Perkin Trans. 1* **1972**, 2280.
(29) Stillwell, M. A.; Magasi, L. P.; Strunz, G. M. *Can. J. Microbiol.* **1974**, *20*, 759.
(30) Stillwell, M. A.; Wall, R. E.; Strunz, G. M. *Can. J. Microbiol.* **1973**, *19*, 597.
(31) Schmidt, I.; Rehm, H. J. *Z. Lebensm.-Unters. Forsch.* **1969**, *141*, 313.
(32) Paul, I. C.; Sim, G. A.; Hamor, T. A.; Robertson, J. M. *J. Chem. Soc.* **1963**, 5502.
(33) Lingham, R. B.; Silverman, K. C.; Bills, G. F.; Cascales, C.; Sanchez, M.; Jenkins, R. G.; Gartner, S. E.; Martin, I.; Diez, M. T. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 370.
(34) Singh, S. B. *Tetrahedron Lett.* **1993**, *34*, 6521.
(35) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Goetz, M. A.; Jenkins, R. G.; Nallin-Omstead, M.; Silverman, K. C.; Bills, G. F.; Misley, R. T. *Tetrahedron* **1993**, *49*, 5917.
(36) Nozoe, T.; Doi, K.; Hashimoto, T. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 1071.
(37) Islam, K.; Stefanelli, S.; Sponga, F.; Denaro, M. Use of tropolone derivatives as inhibitors of the enzyme inositol monophosphatase. World Organization Patent 9637197, 1996.
(38) Bentley, R.; Thiessen, C. P. *Nature* **1959**, *184*, 552.
(39) Segal, W. *J. Chem. Soc.* **1959**, 2847.
(40) Segal, W. *Chem. Ind.* **1958**, 1726.
(41) Segal, W. *Chem. Ind.* **1957**, 1040.
(42) Isaka, M.; Tanticharoen, M.; Thebtaranonth, Y. *Tetrahedron Lett.* **2000**, *41*, 1657.
(43) Weidenmuller, H. L.; Cavagna, F.; Fehlhaber, H. W.; Prave, P. *Tetrahedron Lett.* **1972**, *33*, 3519.
(44) Weber, W.; Semar, M.; Anke, T.; Bross, M.; Steglich, W. *Planta Med.* **1992**, *58*, 56.
(45) Adeboya, M. O.; Edwards, R. L.; Laessoee, T.; Maitland, D. J.; Whalley, A. J. S. *Liebigs Ann.* **1996**, 1437.
(46) Goodall, G. D.; Haworth, R. D. *J. Chem. Soc.* **1936**, 399.
(47) Nakajima, S.; Koike, Y.; Kato, S. *Chem. Pharm. Bull.* **1972**, *20*, 751.
(48) Nakajima, S.; Kinoshita, K.; Shibata, S. *Chem. Ind.* **1964**, 805.
(49) Honkanen, R. E.; Golden, T. *Curr. Med. Chem.* **2002**, *9*, 2055.
(50) McCluskey, A.; Sim, A. T. R.; Sakoff, J. A. *J. Med. Chem.* **2002**, *45*, 1151.
(51) Kurono, M.; Isobe, M. *Tetrahedron* **2003**, *59*, 9609.
(52) Oikawa, H. *Curr. Med. Chem.* **2002**, *9*, 2033.
(53) Panditrao, A. N.; Hiralal, N. R. A process for the preparation of chaetomellic acid A anhydride. India Patent 184694, 2000.
(54) Kar, A.; Argade, N. P. *J. Org. Chem.* **2002**, *67*, 7131.
(55) Baldwin, J. E.; Barton, D. H. B.; Bloomer, J. L.; Jackman, L. M.; Rodriguez-Hahn, L.; Sutherland, J. K. *Experientia* **1962**, *18*, 345.
(56) Barton, D. H. R.; Godinho, L. D. S.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1779.
(57) Barton, D. H. R.; Jackman, L. M.; Rodriguez-Hahn, L.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1772.
(58) Barton, D. H. R.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1769.
(59) Baldwin, J. E.; Barton, D. H. R.; Sutherland, J. K. *J. Chem. Soc.* **1965**, 1787.
(60) Cheng, X. C.; Ubukata, M.; Isono, K. *J. Antibiot.* **1990**, *43*, 809.
(61) Han, D. J.; Jeong, Y. L.; Wee, Y. M.; Lee, A. Y.; Lee, H. K.; Ha, J. C.; Lee, S. K.; Kim, S. C. *Transplant. Proc.* **2003**, *35*, 547.
(62) Cooke, N. G.; Heusser, C. Tolerance induction by tautomycetin to immune reactions. U.S. Patent 2003073737, 2003.
(63) Chun, H. S.; Kim, J. G.; Chang, H. B.; Moon, S. K.; Son, H. J.; Hong, C. I.; Kim, J. W.; Lyu, N. H. Streptomyces sp. producing tautomycetin and immunosuppressant comprising tautomycetin as active ingredient. World Organization Patent 9950388, 1999.
(64) Chae, W. J.; Choi, J. M.; Yang, J. J.; Lee, S. K. *Yonsei Med. J.* **2004**, *45*, 978.
(65) Hepworth, D. *Chem. Ind.* **2000**, 59.
(66) Meng, D.; Tan, Q.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 3197.
(67) Spencer, P.; Agnelli, F.; Sulikowski, G. A. *Org. Lett.* **2001**, *3*, 1443.
(68) Emeh, C. O.; Marth, E. H. *Mycopathology* **1977**, *62*, 103.
(69) Emeh, C. O.; Marth, E. H. *Dev. Ind. Microbiol.* **1977**, *18*, 517.
(70) Emeh, C. O.; Marth, E. H. *J. Milk Food Tech.* **1976**, *39*, 184.
(71) Emeh, C. O.; Marth, E. H. *Mycopathology* **1976**, *59*, 137.
(72) Hayes, A. W.; Wyatt, E. P.; King, P. A. *Appl. Microbiol.* **1970**, *20*, 469.
(73) Hayes, A. W.; Wilson, B. J. *Appl. Microbiol.* **1968**, *16*, 1163.
(74) Moss, M. O.; Robinson, F. V.; Wood, A. B. *J. Chem. Soc.* **1971**, 619.
(75) Natori, S.; Sakaki, S.; Kurata, H.; Udagawa, S.; Ichinoe, M.; Saito, M.; Umeda, M.; Ohtsubo, K. *Appl. Microbiol.* **1970**, *19*, 613.
(76) Newberne, P. M. *Mycotoxins* **1974**, 163.
(77) Wilson, B. J.; Harbison, R. D. *J. Am. Vet. Med. Assoc.* **1973**, *163*, 1274.
(78) Wogan, G. N.; Edwards, G. S.; Newberne, P. M. *Toxicol. Appl. Pharmacol.* **1971**, *19*, 712.
(79) Townsend, R. J.; Moss, M. O.; Peck, H. M. *J. Pharm. Pharmacol.* **1966**, *18*, 471.
(80) Buechi, G.; Snader, K. M.; White, J. D.; Gougoutas, J. Z.; Singh, S. *J. Am. Chem. Soc.* **1970**, *92*, 6638.
(81) Fields, S. C. Hydroxycornexistin herbicide. U.S. Patent 5424278, 1995.
(82) Nakajima, Y.; Watanabe, H.; Adachi, T.; Tagawa, M.; Futagawa, M.; Nishino, Y. Zopfiellin isolation from culture broth. Japan Patent 08107792, 1996.
(83) Nagamura, A.; Aoki, H.; Nishimura, T.; Kinoshita, I. Purification of zopfiellin. Japan Patent 09087268, 1997.
(84) Nieminen, S.; Tamm, C. *Helv. Chim. Acta* **1981**, *64*, 2791.
(85) Nieminen, S.; Tamm, C. *Helv. Chim. Acta* **1981**, *64*, 2791.
(86) Ayer, W. A.; Lu, P. P.; Orszanska, H.; Sigler, L. *J. Nat. Prod.* **1993**, *56*, 1835.
(87) White, J. D.; Kim, J.; Drapela, N. E. *J. Am. Chem. Soc.* **2000**, *122*, 8665.
(88) Raistrick, H.; Smith, G. *Biochem. J.* **1933**, *27*, 1814.
(89) Magae, J.; Watanabe, C.; Osada, H.; Cheng, X. C.; Isono, K. *J. Antibiot.* **1988**, *41*, 932.
(90) Magae, J.; Watanabe, C.; Osada, H.; Cheng, X. C.; Isono, K. *J. Antibiot.* **1988**, *41*, 932.
(91) Oikawa, H.; Oikawa, M.; Ichihara, A.; Ubukata, M.; Isono, K. *Biosci. Biotechnol. Biochem.* **1994**, *58*, 1933.
(92) Erdtman, G. *Acta Chem. Scand.* **1950**, *4*, 1325.
(93) Oxford, A. E.; Raistrick, H.; Smith, G. *Chem. Ind.* **1942**, 485.
(94) Kiser, J. S.; Zellat, J. S. *Trans. N. Y. Acad. Sci.* **1945**, *7*, 210.
(95) Corbett, R. E.; Johnson, A. W.; Todd, A. R. *J. Chem. Soc.* **1950**, 6.
(96) Corbett, R. E.; Hassall, C. H.; Johnson, A. W.; Todd, A. R. *J. Chem. Soc.* **1950**, 1.
(97) Tanenbaum, S. W.; Bassett, E. W.; Kaplan, M. *Arch. Biochem. Biophys.* **1959**, *81*, 169.
(98) Doi, K.; Kitahara, Y. *Bull. Chem. Soc. Jpn.* **1958**, *31*, 788.
(99) Weber, W.; Semar, M.; Anke, T.; Bross, M.; Steglich, W. *Planta Med.* **1992**, *58*, 56.
(100) Poigny, S.; Guyot, M.; Samadi, M. *J. Org. Chem.* **1998**, *63*, 1342.
(101) Hayes, A. W.; Wyatt, E. P. *Appl. Microbiol.* **1970**, *20*, 164.
(102) Hayes, A. W. *Antimicrob. Agents Chemother.* **1973**, *4*, 80.
(103) Fimiani, V.; Richetti, A. *Chemotherapy* **1993**, *39*, 59.
(104) Nagashima, H.; Goto, T. *Toxicol. Lett.* **2000**, *118*, 47.

(105) Nagashima, H. *Maikotokishin* **1996**, *42*, 57.
(106) Nagashima, H.; Nakamura, K.; Goto, T. *Mycotoxins* **2004**, *54*, 21.
(107) Nagashima, H.; Ishizaki, Y.; Nishida, M.; Morita, I.; Murota, S.i.; Goto, T. *Maikotokishin* **1998**, *46*, 35.
(108) Nagashima, H.; Goto, T. *Maikotokishin* **1998**, *46*, 17.
(109) Nagashima, H.; Nakamura, K.; Goto, T. *Mycotoxins* **2001**, *51*, 7.
(110) Nagashima, H.; Nakamura, K.; Goto, T. *Toxicol. Lett.* **2003**, *145*, 153.
(111) Nagashima, H.; Nakamura, K.; Goto, T. *Toxicol. Lett.* **2005**, *155*, 259.
(112) Takeshiba, H.; Hizuka, J.; Sano, H.; Ozasa, M.; Nakajima, M. Preparation of cornexistin derivatives as herbicides, plant growth regulators, and germination inhibitors. Japan Patent 02209844, 1990.
(113) Haneishi, T.; Nakajima, M.; Koi, K.; Furuya, K.; Iwado, S.; Sato, S. Manufacture of cornexistin herbicide with Paecilomyces. Europe Patent 290193, 1988.
(114) Futagawa, M.; Wedge, D. E.; Dayan, F. E. *Pestic. Biochem. Physiol.* **2002**, *73*, 87.
(115) Futagawa, M.; Rimando, A. M.; Tellez, M. R.; Wedge, D. E. *J. Agric. Food Chem.* **2002**, *50*, 7007.
(116) Unligil, H. H. *Wood Sci.* **1978**, *11*, 30.
(117) Goeckeler, Z. M.; Wysolmerski, R. B. *J. Biol. Chem.* **2005**, *280*, 33083.
(118) Morimoto, H. *J. Oral Biosci.* **2004**, *46*, 270.
(119) Kim, S. S.; Kim, J. H.; Lee, S. H.; Chung, S. S.; Bang, O. S.; Park, D.; Chung, C. H. *J. Cell Sci.* **2002**, *115*, 2465.
(120) Resjo, S.; Goransson, O.; Harndahl, L.; Zolnierowicz, S.; Manganiello, V.; Degerman, E. *Cell. Signal.* **2002**, *14*, 231.
(121) Doskeland, S. O.; Serres, M. H.; Fladmark, K. E. Assay for phosphatase-targeting toxins. WO Patent 2000028325, 2000.
(122) cantara-Hernandez, R.; Vazquez-Prado, J.; Garcia-Sainz, J. A. *Br. J. Pharmacol.* **2000**, *129*, 724.
(123) Serres, M. H.; Fladmark, K. E.; Doskeland, S. O. *Toxicon* **1999**, *38*, 347.
(124) Resjo, S.; Oknianska, A.; Zolnierowicz, S.; Manganiello, V.; Degerman, E. *Biochem. J.* **1999**, *341*, 839.
(125) Piwien-Pilipuk, G.; Galigniana, M. D. *Mol. Cell. Endocrinol.* **1998**, *144*, 119.
(126) Ubukata, M.; Koshino, H.; Yamasaki, C.; Fujita, K. I.; Isono, K. *J. Antibiot.* **1997**, *50*, 801.
(127) Brockdorff, J.; Nielsen, M.; Svejgaard, A.; Dobson, P.; Ropke, C.; Geisler, C.; Odum, N. *Cytokine* **1997**, *9*, 333.
(128) Ford, S. L.; Abayasekara, D. R. E.; Persaud, S. J.; Jones, P. M. *J. Endocrinol.* **1996**, *150*, 205.
(129) Nishiyama, U.; Ubukata, M.; Magae, J.; Kataoka, T.; Erdoedi, F.; Hartshorne, D. J.; Isono, K.; Nagai, K.; Osada, H. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 103.
(130) Suganuma, M.; Okabe, S.; Sueoka, E.; Nishiwaki, R.; Komori, A.; Uda, N.; Isono, K.; Fujiki, H. *J. Cancer Res. Clin. Oncol.* **1995**, *121*, 621.
(131) Troll, W.; Sueoka, N.; Sueoka, E.; Laskin, J. D.; Heck, D. E. *Biol. Bull.* **1995**, *189*, 201.
(132) Takai, A.; Sasaki, K.; Nagai, H.; Mieskes, G.; Isobe, M.; Isono, K.; Yasumoto, T. *Biochem. J.* **1995**, *308*, 1039.
(133) Kovacs, C. S.; Chik, C. L.; Li, B.; Karpinski, E.; Ho, A. K. *Mol. Cell. Endocrinol.* **1995**, *110*, 9.
(134) Ho, A. K.; Chik, C. L. *Am. J. Physiol.* **1995**, *268*, E458.
(135) Takai, A.; Sasaki, K.; Nagai, H.; Mieskes, G.; Isobe, M.; Isono, K.; Yasumoto, T. *Biochem. J.* **1995**, *306*, 657.
(136) Fujiki, H.; Suganuma, M. *Adv. Cancer Res.* **1993**, *61*, 143.
(137) Ubukata, M.; Cheng, X. C.; Isobe, M.; Isono, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 617.
(138) Mermoud, J. E.; Cohen, P.; Lamond, A. I. *Nucleic Acids Res.* **1992**, *20*, 5263.
(139) Hori, M.; Magae, J.; Han, Y. G.; Hartshorne, D. J.; Karaki, H. *FEBS Lett.* **1991**, *285*, 145.
(140) Mitsuhashi, S.; Shima, H.; Tanuma, N.; Sasa, S.; Onoe, K.; Ubukata, M.; Kikuchi, K. *Mol. Cell. Biochem.* **2005**, *269*, 183.
(141) Mitsuhashi, S.; Matsuura, N.; Ubukata, M.; Oikawa, H.; Shima, H.; Kikuchi, K. *Biochem. Biophys. Res. Commun.* **2001**, *287*, 328.
(142) Mitsuhashi, S.; Shima, H.; Tanuma, N.; Matsuura, N.; Takekawa, M.; Urano, T.; Kataoka, T.; Ubukata, M.; Kikuchi, K. *J. Biol. Chem.* **2003**, *278*, 82.
(143) Mitsuhashi, S.; Shima, H.; Tanuma, N.; Matsuura, N.; Takekawa, M.; Urano, T.; Kataoka, T.; Ubukata, M.; Kikuchi, K. *J. Biol. Chem.* **2003**, *278*, 82.
(144) Isono, K.; Umakae, J.; Watanabe, C.; Kihara, T.; Osada, H.; Shu, D.; Chin, I.; Sei, K.; Ho, J. Antitumor pharmaceuticals containing antibiotic RS-44. Japan Patent 01003124, 1989.
(145) Magae, J.; Osada, H.; Nagai, K.; Yamasaki, M.; Isono, K. *J. Antibiot.* **1989**, *42*, 1290.
(146) Osada, H.; Magae, J.; Watanabe, C.; Isono, K. *J. Antibiot.* **1988**, *41*, 925.
(147) Kim, J. H.; Lee, T. Y.; Park, J.; Ha, H.; Kang, S. W.; Kim, Y. S. *Transplant. Proc.* **2005**, *37*, 1959.
(148) Mitsuhashi, S.; Shima, H.; Tanuma, N.; Matsuura, N.; Takekawa, M.; Urano, T.; Kataoka, T.; Ubukata, M.; Kikuchi, K. *J. Biol. Chem.* **2003**, *278*, 82.
(149) Fujiki, H.; Suganuma, M. *J. Cancer Res. Clin. Oncol.* **1999**, *125*, 150.
(150) Tachibana, K.; Scheuer, P. J.; Tsukitani, Y.; Kikuchi, H.; Vanengen, D.; Clardy, J.; Gopichand, Y.; Schmitz, F. J. *J. Am. Chem. Soc.* **1981**, *103*, 2469.
(151) Ishihara, H.; Martin, B. L.; Brautigan, D. L.; Karaki, H.; Ozaki, H.; Kato, Y.; Fusetani, N.; Watabe, S.; Hashimoto, K.; Uemura, D.; Hartshorne, D. J. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 871.
(152) Gupta, V.; Ogawa, A. K.; Du, X.; Houk, K. N.; Armstrong, R. W. *J. Med. Chem.* **1997**, *40*, 3199.
(153) Suganuma, M.; Fujiki, H.; Okabe, S.; Nishiwaki, S.; Brautigan, D.; Ingebritsen, T. S.; Rosner, M. R. *Toxicon* **1992**, *30*, 873.
(154) MacKintosh, C.; Klumpp, S. *FEBS Lett.* **1990**, *277*, 137.
(155) Takai, A.; Tsuboi, K.; Koyasu, M.; Isobe, M. *Biochem. J.* **2000**, *350*, 81.
(156) Matsushima, R.; Yoshizawa, S.; Watanabe, M. F.; Harada, K.; Furusawa, M.; Carmichael, W. W.; Fujiki, H. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 867.
(157) Eriksson, J. E.; Paatero, G. I. L.; Meriluoto, J. A. O.; Codd, G. A.; Kass, G. E. N.; Nicotera, P.; Orrenius, S. *Exp. Cell Res.* **1989**, *185*, 86.
(158) Yoshizawa, S.; Matsushima, R.; Watanabe, M. F.; Harada, K.; Ichihara, A.; Carmichael, W. W.; Fujiki, H. *J. Cancer Res. Clin. Oncol.* **1990**, *116*, 609.
(159) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Fujita, S.; Furuya, T. *J. Am. Chem. Soc.* **1986**, *108*, 2780.
(160) Kato, Y.; Fusetani, N.; Matsunaga, S.; Hashimoto, K.; Koseki, K. *J. Org. Chem.* **1988**, *53*, 3930.
(161) Dauben, W. G.; Gerdes, J. M.; Smith, D. B. *J. Org. Chem.* **1985**, *50*, 2576.
(162) Sierra, J. R.; Woggon, W. D.; Schmid, H. *Experientia* **1976**, *32*, 142.
(163) Mamber, S. W.; Okasinski, W. G.; Pinter, C. D.; Tunac, J. B. *J. Antibiot.* **1986**, *39*, 1467.
(164) Scheithauer, W.; Vonhoff, D. D.; Clark, G. M.; Shillis, J. L.; Elslager, E. F. *Eur. J. Cancer Clin. Oncol.* **1986**, *22*, 921.
(165) Shim, J. H.; Lee, H. K.; Chang, E. J.; Chae, W. J.; Han, J. H.; Han, D. J.; Morio, T.; Yang, J. J.; Bothwell, A.; Lee, S. K. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10617.
(166) Myles, D. C. *Curr. Opin. Biotechnol.* **2003**, *14*, 627.
(167) Myles, D. C. *Curr. Opin. Biotechnol.* **2003**, *14*, 627.
(168) Vilella, D.; Sanchez, M.; Platas, G.; Salazar, O.; Genilloud, O.; Royo, I.; Cascales, C.; Martin, I.; Diez, T.; Silverman, K. C.; Lingham, R. B.; Singh, S. B.; Jayasuriya, H.; Pelaez, F. *J. Ind. Microbiol. Biotechnol.* **2000**, *25*, 315.
(169) Qian, Y.; Sebti, S. M.; Hamilton, A. D. *Biopolymers* **1997**, *43*, 25.
(170) Marson, C. M.; Rioja, A. S.; Brooke, G.; Coombes, R. C.; Vigushin, D. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 255.
(171) Huang, L.; Lingham, R. B.; Harris, G. H.; Singh, S. B.; Dufresne, C.; Nallin-Omstead, M.; Bills, G. F.; Mojena, M.; Sanchez, M. *Can. J. Bot.* **1995**, *73*, S898.
(172) Goalstone, M. L.; Sadler, S. E. *J. Exp. Zool.* **2000**, *286*, 193.
(173) Gibbs, J. B.; Pompliano, D. L.; Mosser, S. D.; Rands, E.; Lingham, R. B.; Singh, S. B.; Scolnick, E. M.; Kohl, N. E.; Oliff, A. *J. Biol. Chem.* **1993**, *268*, 7617.
(174) Huber, G. *Angew. Chem.* **1951**, *63*, 501.
(175) Fuska, J.; Slavikova, E.; Adamkova, M. *Biologia (Bratislava)* **1975**, *30*, 669.
(176) Poigny, S.; Guyot, M.; Samadi, M. *J. Org. Chem.* **1998**, *63*, 1342.
(177) Moppett, C. E.; Sutherland, J. K. *J. Chem. Soc. Chem. Commun.* **1966**, 772.
(178) Huff, R. K.; Moppett, C. E.; Sutherland, J. K. *J. Chem. Soc. Perkin Trans. 1* **1972**, 2584.
(179) Cox, R. E.; Holker, J. S. E. *J. Chem. Soc. Chem. Commun.* **1976**, 583.
(180) Bloomer, J. L.; Moppett, C. E.; Sutherland, J. K. *J. Chem. Soc. Chem. Commun.* **1965**, 619.
(181) Barton, D. H. R. *Pure Appl. Chem.* **1963**, *6*, 663.
(182) Spencer, P.; Agnelli, F.; Williams, H. J.; Keller, N. P.; Sulikowski, G. A. *J. Am. Chem. Soc.* **2000**, *122*, 420.
(183) Sulikowski, G. A.; Agnelli, F.; Spencer, P.; Koomen, J. M.; Russell, D. H. *Org. Lett.* **2002**, *4*, 1447.
(184) Ubukata, M.; Cheng, X. C.; Uzawa, J.; Isono, K. *J. Chem. Soc. Perkin Trans. 1* **1995**, 2399.
(185) Scott, A. I.; Lee, E. *J. Chem. Soc. Chem. Commun.* **1972**, 655.
(186) Scott, A. I.; Wiesner, K. J. *J. Chem. Soc. Chem. Commun.* **1972**, 1075.
(187) Bryant, R. W., Jr.; Light, R. *Biochemistry (Moscow)* **1974**, *13*, 1516.
(188) Bryant, R. W., Jr.; Light, R. *Biochemistry (Moscow)* **1974**, *13*, 1516.

(189) Bentley, R.; Zwitkowits, P. M. *J. Am. Chem. Soc.* **1967**, *89*, 681.
(190) Bentley, R.; Ghaphery, J. A.; Keil, J. G. *Arch. Biochem. Biophys.* **1965**, *111*, 80.
(191) Bentley, R.; Theissen, C. P. *J. Biol. Chem.* **1963**, *238*, 3811.
(192) Bentley, R.; Keil, J. G. *J. Biol. Chem.* **1963**, *238*, 3806.
(193) Bentley, R. *J. Biol. Chem.* **1963**, *238*, 1895.
(194) Bentley, R. *J. Biol. Chem.* **1963**, *238*, 1889.
(195) Bentley, R.; Thiessen, C. P. *J. Biol. Chem.* **1963**, *238*, 1880.
(196) Bentley, R. *Biochem. Biophys. Res. Commun.* **1960**, *3*, 215.
(197) Bentley, R.; Thiessen, C. P. *Nature* **1959**, *184*, 552.
(198) Yoshimitsu, T.; Sasaki, S.; Arano, Y.; Nagaoka, H. *J. Org. Chem.* **2004**, *69*, 9262.
(199) Yoshimitsu, T.; Yanagiya, M.; Nagaoka, H. *Tetrahedron Lett.* **1999**, *40*, 5215.
(200) Yoshimitsu, T.; Yanagiya, M.; Nagaoka, H. *Tetrahedron Lett.* **1999**, *40*, 5215.
(201) Waizumi, N.; Itoh, T.; Fukuyama, T. *Tetrahedron Lett.* **1998**, *39*, 6015.
(202) Tan, Q.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 647.
(203) Tan, Q.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2000**, *39*, 4509.
(204) Spiegel, D. A.; Njardarson, J. T.; Wood, J. L. *Tetrahedron* **2002**, *58*, 6545.
(205) Njardarson, J. T.; McDonald, I. M.; Spiegel, D. A.; Inoue, M.; Wood, J. L. *Org. Lett.* **2001**, *3*, 2435.
(206) Njardarson, J. T.; Wood, J. L. *Org. Lett.* **2001**, *3*, 2431.
(207) Nicolaou, K. C.; Jung, J.; Yoon, W. H.; Fong, K. C.; Choi, H. S.; He, Y.; Zhong, Y. L.; Baran, P. S. *J. Am. Chem. Soc.* **2002**, *124*, 2183.
(208) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L.; Fong, K. C.; Choi, H. S. *J. Am. Chem. Soc.* **2002**, *124*, 2190.
(209) Nicolaou, K. C.; Zhong, Y. L.; Baran, P. S.; Jung, J.; Choi, H. S.; Yoon, W. H. *J. Am. Chem. Soc.* **2002**, *124*, 2202.
(210) Nicolaou, K. C.; Jung, J.; Yoon, W. H.; Fong, K. C.; Choi, H. S.; He, Y.; Zhong, Y. L.; Baran, P. S. *J. Am. Chem. Soc.* **2002**, *124*, 2183.
(211) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L.; Fong, K. C.; Choi, H. S. *J. Am. Chem. Soc.* **2002**, *124*, 2190.
(212) Nicolaou, K. C.; Jung, J. K.; Yoon, W. H.; He, Y.; Zhong, Y. L.; Baran, P. S. *Angew. Chem. Int. Ed.* **2000**, *39*, 1829.
(213) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L.; Fong, K. C.; He, Y.; Yoon, W. H.; Choi, H. S. *Angew. Chem. Int. Ed.* **1999**, *38*, 1676.
(214) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L.; Choi, H. S.; Yoon, W. H.; He, Y.; Fong, K. C. *Angew. Chem. Int. Ed.* **1999**, *38*, 1669.
(215) Nicolaou, K. C.; He, Y.; Fong, K. C.; Yoon, W. H.; Choi, H. S.; Zhong, Y. L.; Baran, P. S. *Org. Lett.* **1999**, *1*, 63.
(216) Kwon, O.; Su, D. S.; Meng, D.; Deng, W.; D'Amico, D. C.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **1998**, *37*, 1877.
(217) Diederichsen, U.; Lorenz, K.B. *Org. Synth.* **2003**, 326.
(218) Diederichsen, U. *Nachr. Chem. Tech. Lab.* **1999**, *47*, 1423.
(219) Devaux, J. F.; O'Neil, S. V.; Guillo, N.; Paquette, L. A. *Collect. Czech. Chem. Commun.* **2000**, *65*, 490.
(220) Clive, D. L. J.; Sgarbi, P. W. M.; He, X.; Sun, S.; Zhang, J.; Ou, L. *Can. J. Chem.* **2003**, *81*, 811.
(221) Clive, D. L. J.; Ou, L. *Tetrahedron Lett.* **2002**, *43*, 4559.
(222) Chen, C.; Layton, M. E.; Sheehan, S. M.; Shair, M. D. *J. Am. Chem. Soc.* **2000**, *122*, 7424.
(223) Bio, M. M.; Leighton, J. L. *J. Org. Chem.* **2003**, *68*, 1693.
(224) Nicolaou, K. C.; Baran, P. S. *Angew. Chem. Int. Ed.* **2002**, *41*, 2678.
(225) Nicolaou, K. C.; Baran, P. S.; Zhong, Y. L.; Fong, K. C.; Choi, H. S. *J. Am. Chem. Soc.* **2002**, *124*, 2190.
(226) Nicolaou, K. C.; Harter, M. W.; Boulton, L.; Jandeleit, B. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1194.
(227) Chen, C.; Layton, M. E.; Shair, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 10784.
(228) Kwon, O.; Su, D. S.; Meng, D.; Deng, W.; D'Amico, D. C.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **1998**, *37*, 1880.
(229) Yoshimitsu, T.; Yanagisawa, S.; Nagaoka, H. *Org. Lett.* **2000**, *2*, 3751.
(230) Sulikowski, G. A.; Liu, W.; Agnelli, F.; Corbett, R. M.; Luo, Z.; Hershberger, S. J. *Org. Lett.* **2002**, *4*, 1451.
(231) Sulikowski, G. A.; Agnelli, F.; Corbett, R. M. *J. Org. Chem.* **2000**, *65*, 337.
(232) Starr, J. T.; Carreira, E. M. *Angew. Chem. Int. Ed.* **2000**, *39*, 1415.
(233) Sgarbi, P.W.M.; Clive, D.L.J. *J. Chem. Soc. Chem. Commun.* **1997**, 2157.
(234) Ohmori, N. *J. Chem. Soc. Perkin Trans. 1* **2002**, 755.
(235) Ohmori, N. *J. Chem. Soc. Chem. Commun.* **2001**, 1552.
(236) Matsushita, T.; Ashida, H.; Kimachi, T.; Takemoto, Y. *J. Chem. Soc. Chem. Commun.* **2002**, 814.
(237) King, A. D., Jr.; Booth, A. N.; Stafford, A. E.; Waiss, A. C., Jr. *J. Food Sci.* **1972**, *37*, 86.
(238) Isakovic, L.; Ashenhurst, J. A.; Gleason, J. L. *Org. Lett.* **2001**, *3*, 4189.
(239) Hayashi, Y.; Itoh, T.; Fukuyama, T. *Org. Lett.* **2003**, *5*, 2235.
(240) Davies, H. M. L.; Ren, P. *Tetrahedron Lett.* **2000**, *41*, 9021.
(241) Dabrah, T. T.; Kaneko, T.; Massefski, W., Jr.; Whipple, E. B. *J. Am. Chem. Soc.* **1997**, *119*, 1594.
(242) Cuniere, N.; Paquette, L.A. *Annu. Rev. Immunol.* **2000**, *1*, No.
(243) Clive, D. L. J.; Cheng, H.; Gangopadhyay, P.; Huang, X.; Prabhudas, B. *Tetrahedron* **2004**, *60*, 4205.
(244) Clive, D. L. J.; Zhang, J. *Tetrahedron* **1999**, *55*, 12059.
(245) Clive, D. L. J.; Sun, S.; He, X.; Zhang, J.; Gagliardini, V. *Tetrahedron Lett.* **1999**, *40*, 4605.
(246) Clive, D. L. J.; Sun, S. *Tetrahedron Lett.* **2001**, *42*, 6267.
(247) Clive, D. L. J.; Sun, S.; Gagliardini, V.; Sano, M. K. *Tetrahedron Lett.* **2000**, *41*, 6259.
(248) Bio, M. M.; Leighton, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 890.
(249) Banwell, M. G.; Coster, M. J.; Edwards, A. J.; Voegtle, M. *Aust. J. Chem.* **2003**, *56*, 577.
(250) Banwell, M. G.; McRae, K. J.; Willis, A. C. *J. Chem. Soc. Perkin Trans. 1* **2001**, 2194.
(251) Baldwin, J. E.; Adlington, R. M.; Roussi, F.; Bulger, P. G.; Marquez, R.; Mayweg, A. V. W. *Tetrahedron* **2001**, *57*, 7409.
(252) Armstrong, A.; Davies, N. G. M.; Martin, N. G.; Rutherford, A. P. *Tetrahedron Lett.* **2003**, *44*, 3915.
(253) Armstrong, A.; Critchley, T. J.; Gourdel-Martin, M. E.; Kelsey, R. D.; Mortlock, A. A. *Tetrahedron Lett.* **2002**, *43*, 6027.
(254) Armstrong, A.; Critchley, T. J.; Gourdel-Martin, M. E.; Kelsey, R. D.; Mortlock, A. A. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1344.
(255) Armstrong, A.; Critchley, T. J.; Mortlock, A. A. *Synlett* **1998**, 552.
(256) Kurono, M.; Isobe, M. *Chem. Lett.* **2004**, *33*, 452.
(257) Kurono, M.; Shimomura, A.; Isobe, M. *Tetrahedron* **2004**, *60*, 1773.
(258) Deshpande, A. M.; Natu, A. A.; Argade, N. P. *Synthesis* **2001**, 702.
(259) Marshall, J. A.; Yanik, M. M. *J. Org. Chem.* **2001**, *66*, 1373.
(260) Nagumo, S.; Arai, T.; Akita, H. *Tetrahedron Lett.* **1997**, *38*, 6957.
(261) Nagumo, S.; Arai, T.; Akita, H. *Tetrahedron Lett.* **1997**, *38*, 5165.
(262) Tsuboi, K.; Ichikawa, Y.; Jiang, Y.; Naganawa, A.; Isobe, M. *Tetrahedron* **1997**, *53*, 5123.
(263) Jiang, Y.; Ichikawa, Y.; Isobe, M. *Tetrahedron* **1997**, *53*, 5103.
(264) Tsuboi, K.; Ichikawa, Y.; Naganawa, A.; Isobe, M.; Ubukata, M.; Isono, K. *Tetrahedron* **1997**, *53*, 5083.
(265) Sheppeck, J. E., II; Liu, W.; Chamberlin, A. R. *J. Org. Chem.* **1997**, *62*, 387.
(266) Shimizu, S.; Nakamura, S. i.; Nakada, M.; Shibasaki, M. *Tetrahedron* **1996**, *52*, 13363.
(267) Ichihara, A.; Oikawa, H.; Toshima, H. *Stud. Nat. Prod. Chem.* **1996**, *18*, 269.
(268) Maurer, K. W.; Armstrong, R. W. *J. Org. Chem.* **1996**, *61*, 3106.
(269) Ueno, T.; Oikawa, M.; Oikawa, H.; Ichihara, A. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 2104.
(270) Ichikawa, Y.; Tsuboi, K.; Jiang, Y.; Naganawa, A.; Isobe, M. *Tetrahedron Lett.* **1995**, *36*, 7101.
(271) Oikawa, M.; Ueno, T.; Oikawa, H.; Ichihara, A. *J. Org. Chem.* **1995**, *60*, 5048.
(272) Jiang, Y.; Ichikawa, Y.; Isobe, M. *Synlett* **1995**, 285.
(273) Naganawa, A.; Ichikawa, Y.; Isobe, M. *Tetrahedron* **1994**, *50*, 8969.
(274) Nakamura, S.; Shibasaki, M. *Tetrahedron Lett.* **1994**, *35*, 4145.
(275) Oikawa, H.; Oikawa, M.; Ueno, T.; Ichihara, A. *Tetrahedron Lett.* **1994**, *35*, 4809.
(276) Ichikawa, Y.; Tsuboi, K.; Naganawa, A.; Isobe, M. *Synlett* **1993**, 907.
(277) Ichikawa, Y.; Naganawa, A.; Isobe, M. *Synlett* **1993**, 737.
(278) Oikawa, M.; Oikawa, H.; Ichihara, A. *Tetrahedron Lett.* **1993**, *34*, 4797.
(279) Tsuboi, K.; Ichikawa, Y.; Isobe, M. *Synlett* **1997**, 713.
(280) Ishii, Y.; Nagumo, S.; Arai, T.; Akuzawa, M.; Kawahara, N.; Akita, H. *Tetrahedron* **2006**, *62*, 716.
(281) Oikawa, H.; Yoneta, Y.; Ueno, T.; Oikawa, M.; Wakayama, T.; Ichihara, A. *Tetrahedron Lett.* **1997**, *38*, 7897.
(282) Dai, J. P.; Sodeoka, M.; Shibasaki, M. *Tetrahedron Lett.* **1996**, *37*, 491.
(283) Singh, S. B.; Jayasuriya, H.; Silverman, K. C.; Bonfiglio, C. A.; Williamson, J. M.; Lingham, R. B. *Bioorg. Med. Chem.* **2000**, *8*, 571.
(284) Scholte, A. A.; Eubanks, L. M.; Poulter, C. D.; Vederas, J. C. *Bioorg. Med. Chem.* **2004**, *12*, 763.
(285) Ratemi, E. S.; Dolence, J. M.; Poulter, C. D.; Vederas, J. C. *J. Org. Chem.* **1996**, *61*, 6296.
(286) Poigny, S.; Guyot, M.; Samadi, M. *J. Chem. Soc. Perkin Trans. 1* **1997**, 2175.
(287) Panditrao, A. N.; Hiralal, N. R. A process for the preparation of 3-tetradecyl-2-oxo-3H-imidazo[1,2-a]pyridinium bromide India Patent 184878, 2000.
(288) Mechelke, M. F.; Webb, J. C.; Johnson, J. L. *J. Undergrad. Chem. Res.* **2005**, *4*, 117.

(289) Marriott, J.; Jarman, M.; Neidle, S. Preparation of nucleoside phosphate mimics as enzyme inhibitors World Organization Patent 9740006, 1997.
(290) Deshpande, A. M.; Natu, A. A.; Argade, N. P. *J. Org. Chem.* **1998**, *63*, 9557.
(291) Desai, S. B.; Argade, N. P. *J. Org. Chem.* **1997**, *62*, 4862.
(292) De Buyck, L.; Danieli, C.; Ghelfi, F.; Pagnoni, U. M.; Parsons, A. F.; Pattarozzi, M.; Roncaglia, F. *Tetrahedron* **2005**, *61*, 2871.
(293) De Buyck, L.; Cagnoli, R.; Ghelfi, F.; Merighi, G.; Mucci, A.; Pagnoni, U. M.; Parsons, A. F. *Synthesis* **2004**, 1680.
(294) Branchaud, B. P.; Slade, R. M. *Tetrahedron Lett.* **1994**, *35*, 4071.
(295) Argade, N. P.; Naik, R. H. *Bioorg. Med. Chem.* **1996**, *4*, 881.
(296) Kates, M. J.; Schauble, J. H. *J. Org. Chem.* **1996**, *61*, 4164.
(297) Slade, R. M.; Branchaud, B. P. *J. Org. Chem.* **1998**, *63*, 3544.
(298) Mondal, M.; Argade, N. P. *Tetrahedron Lett.* **2004**, *45*, 5693.
(299) Mhaske, S. B.; Argade, N. P. *Tetrahedron* **2004**, *60*, 3417.
(300) Kar, A.; Gogoi, S.; Argade, N. P. *Tetrahedron* **2005**, *61*, 5297.
(301) Kar, A.; Argade, N. P. *Tetrahedron* **2003**, *59*, 2991.
(302) Kar, A.; Argade, N. P. *Tetrahedron Lett.* **2002**, *43*, 6563.
(303) Gogoi, S.; Argade, N. P. *Tetrahedron* **2004**, *60*, 9093.
(304) Bellesia, F.; Danieli, C.; Buyck, L. D.; Galeazzi, R.; Ghelfi, F.; Mucci, A.; Orena, M.; Pagnoni, U. M.; Parson, A. F.; Roncaglia, F. *Tetrahedron* **2006**, *62*, 746.
(305) Stork, G.; Tabak, J. M.; Blount, J. F. *J. Am. Chem. Soc.* **1972**, *94*, 4735.
(306) White, J. D.; Dillon, M. P.; Butlin, R. J. *J. Am. Chem. Soc.* **1992**, *114*, 9673.
(307) Poigny, S.; Guyot, M.; Samadi, M. *J. Org. Chem.* **1998**, *63*, 1342.
(308) Poigny, S.; Guyot, M.; Samadi, M. *J. Org. Chem.* **1998**, *63*, 1342.
(309) Kawamura, T.; Matsuzawa, S. i.; Mizuno, Y.; Kikuchi, K.; Oikawa, H.; Oikawa, M.; Ubukata, M.; Ichihara, A. *Biochem. Pharmacol.* **1998**, *55*, 995.
(310) Sugiyama, Y.; Ohtani, I. I.; Isobe, M.; Takai, A.; Ubukata, M.; Isono, K. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3.
(311) Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022.
(312) Koehn, F. E.; Carter, G. T. *Nat. Rev. Drug Discovery* **2005**, *4*, 206.

CR050029R