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13. ABSTRACT (Maximum 200 Words)

Cryptophycins are a potent, tumor-selective class of tubulin-binding antimitotic anti-cancer agents with excellent activity against MDR cancers. For the development of these promising compounds into useful chemotherapeutic agents, detailed information about the binding domain of the cryptophycins is essential. We plan to map the cryptophycin binding through photoaffinity labeling studies. Toward this goal, we have synthesized the C16 side chain benzophenone analogue of cryptophycin-24. The synthesis is convergent and allows for convenient modification to produce various analogues having photoaffinity labels attached at the C16 side chain. The C16-benzophenone photolabeled analogue was active in the tubulin assembly assay and is therefore a suitable candidate for further studies to explore the tubulin binding domain of cryptophycin. Presently, these compounds are being used for photoaffinity labeling studies with tubulin in collaboration with Professor Richard Himes' laboratory.

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Introduction

Tubulin-binding anticancer agents are valuable chemotherapeutic agent. Antimitotic agents that interact with tubulin, the major protein component of microtubules, fall into different groups. The Vinca alkaloids, colchicine, podophyllotoxin, nocodazole and the recently discovered cryptophycins and arenastatins prevent tubulin from forming microtubules by inducing the disassembly of microtubules. Taxol on the other hand preferentially binds to the microtubules and causes the formation of hyperstable forms.[1] By interfering with the microtubule dynamics, these antimitotic compounds stop mitosis and cell proliferation. As a result, they are potential antitumor agents. Many such compounds, however, are too cytotoxic to be clinically useful. It is important, therefore, to continue to develop new compounds which could be useful as chemotherapeutic agents.

The cryptophycins, isolated from blue-green algae (*Nostoc* sp.), are a potent tumor-selective class of tubulin-binding antimitotic agents that show excellent activity against multi-drug resistant (MDR) cancer cell lines and against mammary derived tumors.[2,3] Cryptophycin-1 (**1**, Fig. 1) is the major cytotoxin in *Nostoc* sp.[4,5] and displays IC_{50} values in the pM range. Of special importance is the reduced susceptibility of the cryptophycins to P-glycoprotein-mediated multiple drug resistance in comparison to vinblastine, colchicine, and taxol.[6] *In vivo* studies (human tumor xenografts) with cryptophycin 1, demonstrated a remarkable reduction of tumor burden.[5] A structurally related compound cryptophycin-24, (**2**, Fig. 1, also named arenastatin A), isolated from the Okinawan marine sponge *Dysidea arenaria*[7] and later from *Nostoc* sp. strain GSV 224[8] is also a potent inhibitor of tubulin polymerization.[9] The IC_{50} for arenastatin A cytotoxicity against KB cells was 5 pg/mL.[7,10]

The interaction of cryptophycin-1 with tubulin and microtubules *in vitro* showed that cryptophycin is an effective inhibitor of tubulin polymerization at substoichiometric concentrations.[11] Cryptophycin-1 causes tubulin to aggregate and depolymerizes microtubules into linear polymers as seen by electron microscopy.[11,12] It has been demonstrated that cryptophycin-1 inhibits vinblastine binding to tubulin.[11,13-15] Thus, cryptophycin-1 belongs to a growing group of compounds that bind to the vinca binding domain on tubulin. However, due to the structural differences between vinca alkaloids and cryptophycins it may be that the binding domains simply overlap.[15,16] The possibility of covalent binding of cryptophycin-1 to tubulin has also been studied and the results demonstrate that a covalent addition of cryptophycin to tubulin does not occur.[12]

Cryptophycins are one of the best recent lead in the search for anticancer therapies. Although relatively little is known about the interactions of cryptophycins with tubulin, it is believed that the cryptophycins may interact in a manner different from those of other tubulin-binding antimitotic agents. For the development of these promising compounds into useful chemotherapeutic agents, detailed information about the binding domain of the cryptophycins is essential. Therefore, we planned to prepare a cryptophycin analogue bearing a photoaffinity label for tubulin labeling studies. Because structural changes at the C16 side chain aromatic group are tolerated without loss of activity,[2,3] we targeted this position for the placement of a photoreactive functionality to study the cryptophycin binding site on tubulin. The information obtained will be used to search for more bioactive candidates for *in vivo* and *in vitro* testing.

Body

Cryptophycin-1 (**1**) and the structurally less complex arenastatin A (**2**, cryptophycin-24) are close structural analogues (Fig. 1.) and have very similar properties with regard to tubulin binding.[9] Therefore, we prepared an affinity label of cryptophycin-24 for our labeling studies, because it can be prepared in fewer synthetic steps than the cryptophycin-1 analogues. The enzymatic/hydrolytic instability[17] of cryptophycin-24 should not pose a problem during the *in vitro* tubulin investigations. The retrosynthetic analysis of a benzophenone analogue of cryptophycin-24 (**3**) reveals that the desepoxy analogue **4** can be assembled from two main building blocks, the octadienoate ester **5**, and the depsipeptide unit **6** (Scheme 1).

Our group has reported two efficient protocols[18-20] for the synthesis of octadienoate ester **5**, a major component of the cryptophycins.[2,21-24] The first approach utilized an asymmetric Noyori reduction followed by a Frater alkylation to set the two stereocenters of the octadienoate ester **5**. The second approach started from 3-*p*-methoxybenzyl oxypropanal **7** and a crotylborane reagent derived from (-)-α-pinene to set both the stereocenters in a single step[25] and provided ester **11** in five steps. The latter method allows for the convergent synthesis of analogues modified at the phenyl group of the C16 side chain using Heck chemistry. Since we proposed to introduce the labels at the C16 aromatic ring, we decided to follow the crotylboration approach to prepare the octadienoate ester and subject it to Heck coupling with the desired aromatic moiety to get the required backbone analogue. The key step utilized the crotylboration of **7** (Scheme 2) with crotyl diisopinocampheylborane (prepared from (+)-*B*-methoxydiisopinocampheylborane) to generate the desired stereochemistry at the two chiral centers of **8** in 77% yield (91% *ee*). Silyl protection of the secondary alcohol **8** with *tert*-butyldimethylsilyl chloride and imidazole afforded the silyl ether **9** in 98% yield. Deprotection of the *p*-methoxybenzyl ether with DDQ followed by DMP-oxidation of the resulting alcohol furnished the aldehyde **10** in 82% yield over two steps. Wittig olefination of **10** provided the α,β-unsaturated *tert*-butyl ester **11** in 95% yield. Heck coupling[26,27] on **11** with 4-bromobenzophenone in the presence of palladium acetate and sodium bicarbonate furnished the required ester **12** in 60% yield. Deprotection of the silyl ether with tetrabutylammonium fluoride led to the isolation of the desired octadienoate ester **5** in 71% yield.

The second key synthon **6** was readily synthesized starting from a *N*-Boc protected D-tyrosine derivative **13** (Scheme 3),[20,28] which in turn was prepared from commercially available D-tyrosine methyl ester through *N*-Boc protection followed by methylation of the phenolic hydroxyl group and hydrolysis of the ester with sodium hydroxide. Activation of **13** with DCC and *N*-hydroxysuccinimide followed by addition of β-alanine **14** provided acid **15**. EDCI activation and subsequent addition of L-leucic acid **16** provided acid **6** in two steps without the necessity of extensive protecting group chemistry (Scheme 3).

Key synthons, octadienoate ester **5** and depsipeptide fragment **6** were subjected to the Yamaguchi coupling reaction[29] with 2,4,6-trichloro benzoylchloride in the presence of diisopropylethylamine to afford intermediate **17** in 85% yield (Scheme 4). Simultaneous deprotection of the *tert*-butyl ester and the *N*-Boc with trifluoroacetic acid produced the cyclization precursor and HBTU activation provided the desired macrocycle **4** in 56%

yield. Epoxidation of **4** with *m*-CPBA or dimethyl dioxirane (DMD)[30] furnished a diastereomeric mixture of epoxides **3** in the ratios of α:β = 2:1 and 1:2, respectively (Scheme 4). The mixture was separated by HPLC.

The biological testing was carried out individually for the α & β-isomers of **3**. In the tubulin assembly assay, the benzophenone analogue **3** (β) of cryptophycin-24 (IC_{50} = 7.4 μM) was half as active as cryptophycin-1 (IC_{50} = 3.7 μM) and twice as active as cryptophycin-24 (IC_{50} = 15 μM) (Table 1).[31,32] In the cytotoxicity studies, analogue **3** (β) had reduced activity against the MCF7 and HCT-116 compared to **1** and **2**, but was still active in the pM or low nM range. In comparison to **1** and **2**, analogue **3** (β) had much reduced activity against MCF7-ADR cells compared to the MCF7 cell line. This suggests that the addition of the benzophenone moiety makes the compound a better substrate for the p-glycoprotein multi-drug transporter. The α-analogue of **3** had poor activity in all of the biological tests (Table 1).

Key Research Accomplishments

- Synthesis of octadienoate ester having the C16 benzophenone moiety in sufficient quantity
- Total synthesis of C16 benzophenone photoaffinity analogue of cryptophycin-24
- C16 Benzophenone photoaffinity labeled cryptophycin-24 inhibits the tubulin polymerization at half concentration as cryptophycin-24
- C16 Benzophenone photoaffinity labeled cryptophycin-24 retains submicromolar cytotoxicity in breast cancer cell line MCF-7
- Photoaffinity labeling studies of tubulin are being carried out in collaboration with Professor Richard Himes' laboratory in the Department of Molecular Biosciences

Reportable Outcomes

Cryptophycin Affinity Labels: Synthesis and Biological Activity of a Benzophenone Analogue of Cryptophycin-24, R. Vidya, M. J. Eggen, G. I. Georg, R. H. Himes, *Bioorg. Med. Chem. Lett.* **2003**, 13, 757-760.

Cryptophycin Affinity Labels: Synthesis of a Benzophenone Analogue of Cryptophycin-24, R. Vidya, M. J. Eggen, G. I. Georg, *Poster presented, 223rd ACS National Meeting*, Orlando, FL, United States, April 7-11, **2002**.

Conclusions

We have completed an efficient synthesis of a C16 benzophenone photoaffinity analogue of cryptophycin-24. The synthesis is convergent and allows for convenient modification to produce various analogues having photoaffinity labels attached at C16 side chain. Also, the synthesis of the key fragment, octadienoate ester has been well optimized and used to synthesize azido photoaffinity labeled cryptophycin-24. The C16-benzophenone photolabeled analogue **3** (β) was active in the tubulin assembly assay and

is therefore a suitable candidate for further studies to explore the tubulin binding domain of cryptophycin. Presently, these compounds are being used for photoaffinity labeling studies with tubulin in collaboration with Professor Richard Himes' laboratory. Our plans are to make this derivative in a radioactive form for photolabeling studies.

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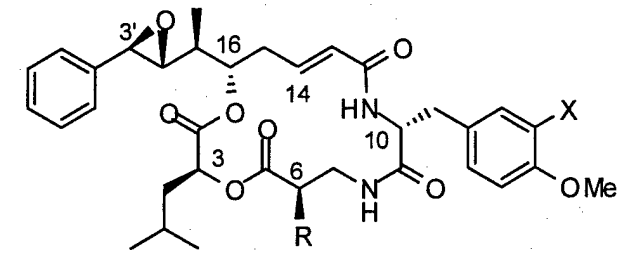
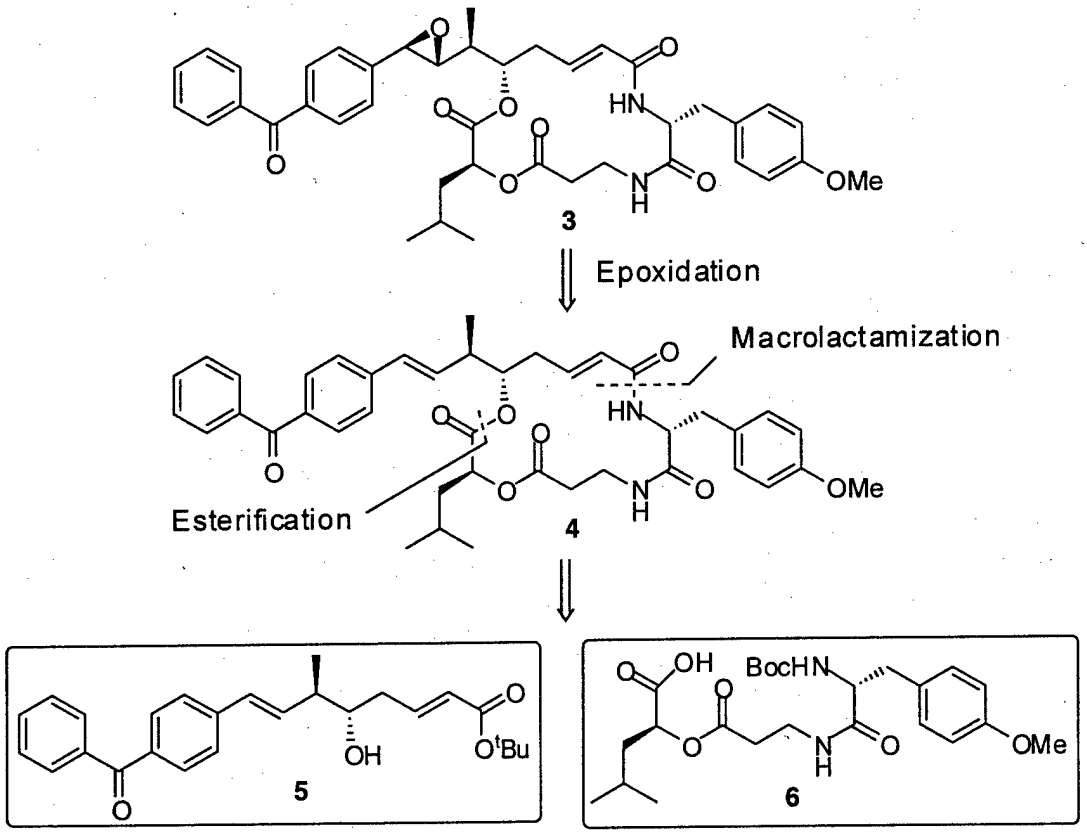
Appendices

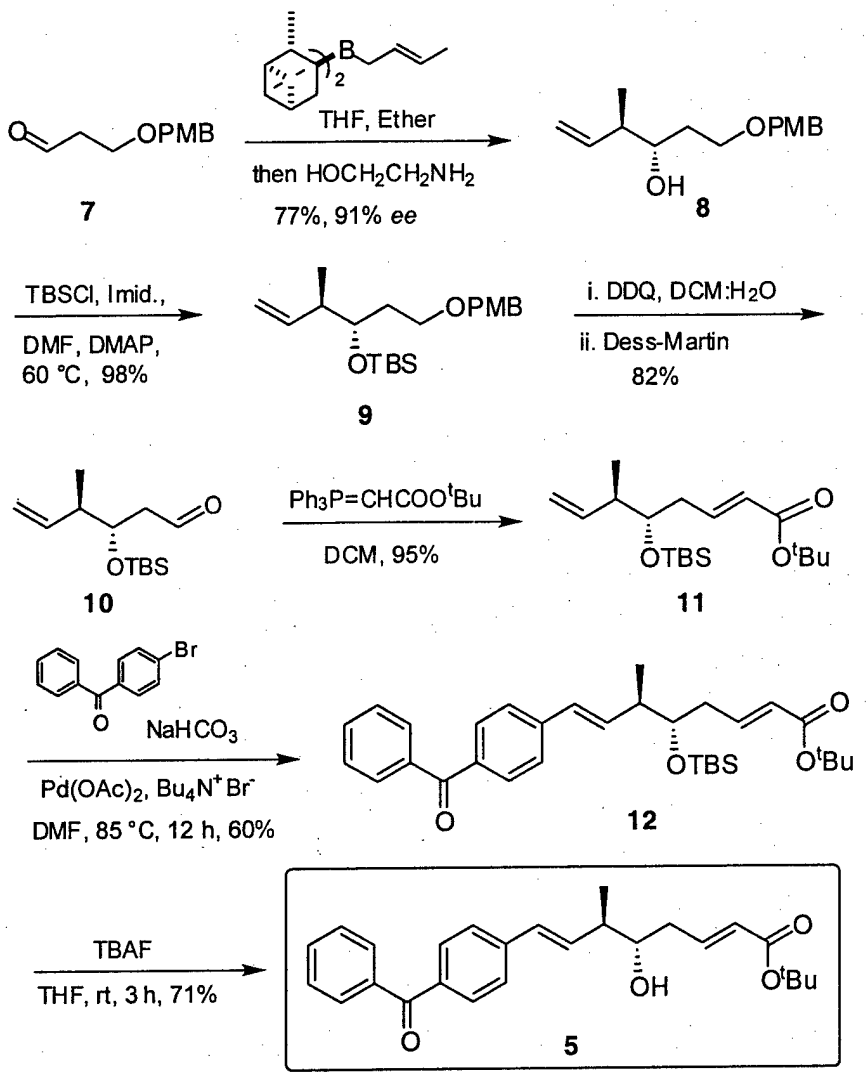


R	X	
Me	Cl	Cryptophycin-1 (**1**)
H	H	Cryptophycin-24 (Arenastatin A) (**2**)

Figure 1. Structure of cryptophycins.



Scheme 1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OPMB
OTBS
9
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ii. Dess-Martin
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OTBS
10
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DCM, 95%
OTBS
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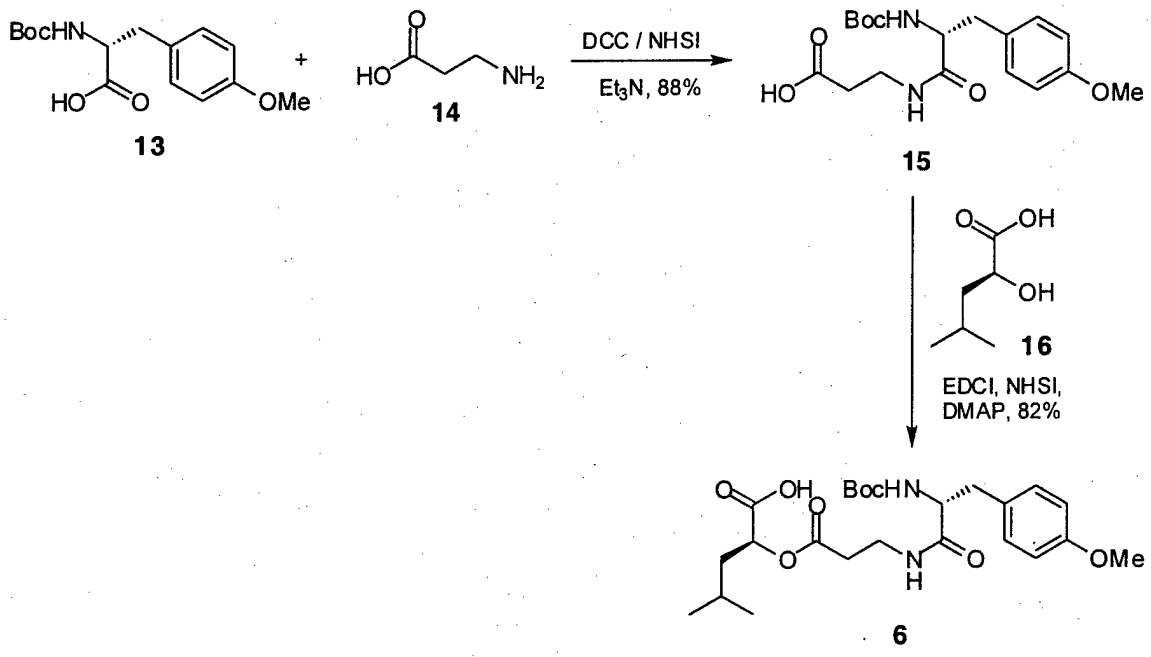
Scheme 2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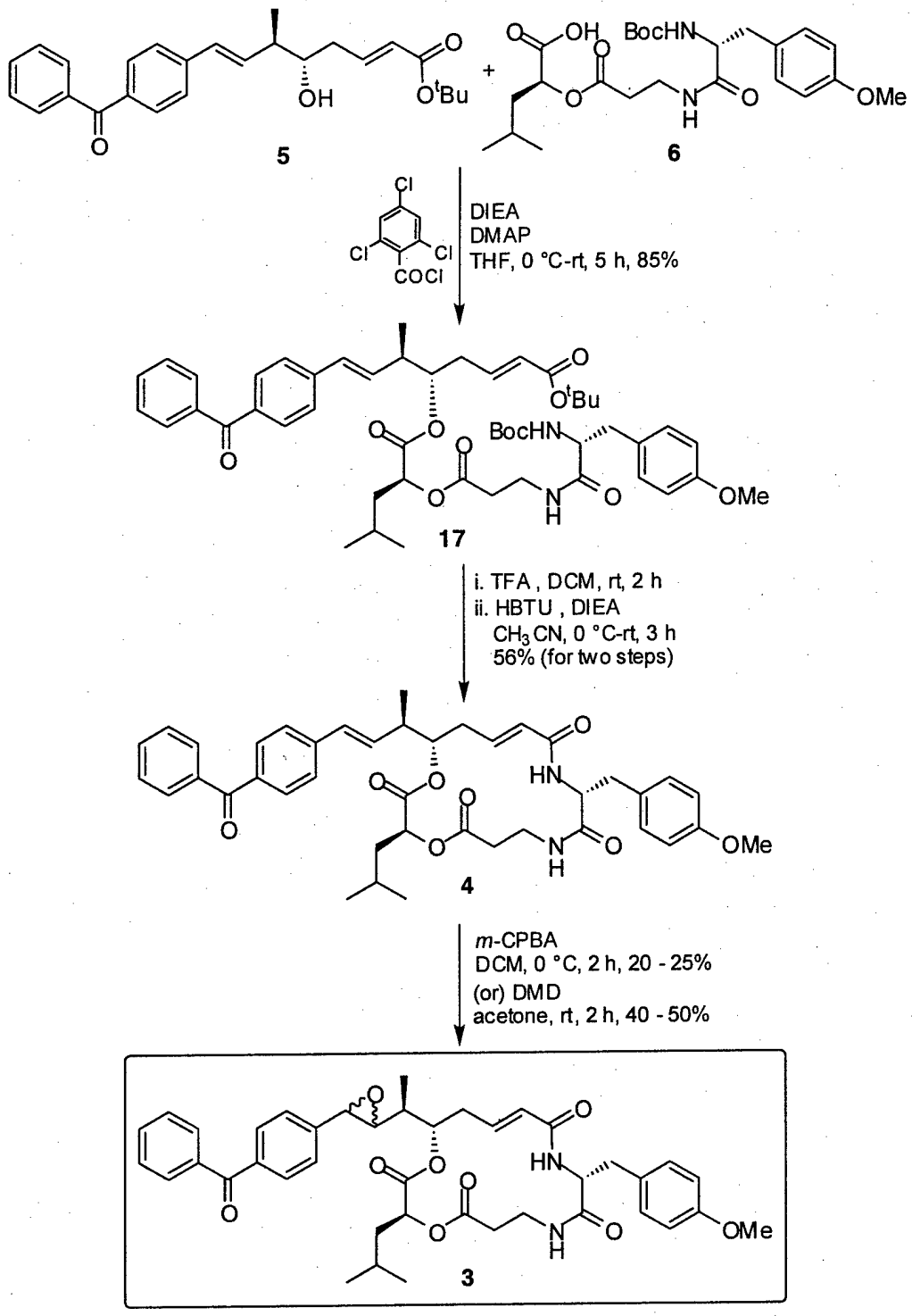
Scheme 3



α : β = 2:1 (with *m*-CPBA)

α : β = 1:2 (with DMD)

Scheme 4

Table 1. Biological Results

Compound	Tubulin Assembly IC_{50}, μM[a]	Cytotoxicity IC_{50}, nM[b]		
		MCF7	MCF7-ADR	HCT-116
1	3.7	0.003	0.013	0.027
2	15	0.13	0.164	0.285
4 (β)	7.4	0.078	70	1.1
4 (α)	>100	6.0	447	25.3

[a]Tubulin at 1.5 mg/mL was assembled at 37 °C for 15 min in the presence of PEM buffer, 0.5 mM GTP and 8% DMSO. Microtubules were pelleted and the protein remaining in the supernatant determined. The IC_{50} value is the concentration that reduces the amount of pelleted protein by 50%.

[b]The IC_{50} value is the concentration that inhibits the proliferation by 50% after 72h (MCF-7 and MCF7-ADR) or 24h (HCT-116) of cell growth.

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Cryptophycin Affinity Labels: Synthesis and Biological Activity of a Benzophenone Analogue of Cryptophycin-24

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Received 8 May 2002; accepted 7 October 2002

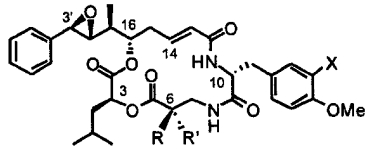
Abstract—An efficient synthesis of a C16 side chain benzophenone analogue of cryptophycin-24 using a crotylboration reaction and Heck coupling as key steps is described. In an in vitro tubulin assembly assay, the benzophenone analogue of the β isomer (IC_{50} = 7.4 μM) is twice as active as cryptophycin-24 (IC_{50} = 15 μM).

The cryptophycins, isolated from blue-green algae (*Nostoc* sp.), are a potent tumor-selective class of tubulin-binding antimitotic agents that show excellent activity against multi-drug resistant (MDR) cancer cell lines and against mammary derived tumors.[1,2] Cryptophycin-1 (**1**, Fig. 1) is the major cytotoxin in *Nostoc* sp.[3,4] and displays IC_{50} values in the pM range. Of special importance is the reduced susceptibility of the cryptophycins to P-glycoprotein-mediated multiple drug resistance in comparison to vinblastine, colchicine, and paclitaxel.[5] In vivo studies (human tumor xenografts) with cryptophycin-1 demonstrated a remarkable reduction of tumor burden.[4] A structurally related compound cryptophycin-24, (**2**, Fig. 1, also named arenastatin A), isolated from the Okinawan marine sponge *Dysidea arenaria*[6] and later from *Nostoc* sp. strain GSV 224,[7] is also a potent inhibitor of tubulin polymerization.[8] The IC_{50} for arenastatin A cytotoxicity against KB cells was 5 pg/mL.[6,9] A hydrolytically more stable synthetic analogue, cryptophycin-52 (**3**), was selected for clinical trials.[10–12]

The interaction of cryptophycin-1 with tubulin and microtubules in vitro showed that cryptophycin is an effective inhibitor of tubulin polymerization at substoichiometric concentrations.[13] Cryptophycin-1 causes tubulin to aggregate and depolymerizes microtubules into linear polymers as seen by electron microscopy.[13,14]

It has been demonstrated that cryptophycin-1 inhibits vinblastine binding to tubulin.[13,15–17] Thus, cryptophycin-1 belongs to a growing group of compounds that bind to the vinca binding domain on tubulin. However, due to the structural differences between vinca alkaloids and cryptophycins it may be that the binding domains simply overlap.[11,17] The possibility of covalent binding of cryptophycin-1 to tubulin has also been studied and the results demonstrate that a covalent addition of cryptophycin to tubulin does not occur.[14]

Cryptophycins are one of the best recent lead in the search for anticancer therapies. Although relatively little is known about the interactions of cryptophycins with tubulin, it is believed that the cryptophycins may interact in a manner different from those of other tubulin-binding antimitotic agents. For the development of these promising compounds into useful chemotherapeutic agents, detailed information about the binding domain of the cryptophycins is essential. Therefore, we planned to prepare a cryptophycin analogue bearing a



R	R'	X	
Me	H	Cl	Cryptophycin-1 (**1**)
H	H	H	Cryptophycin-24 (Arenastatin A) (**2**)
Me	Me	Cl	Cryptophycin-52 (**3**)

Figure 1. Structures of cryptophycins.

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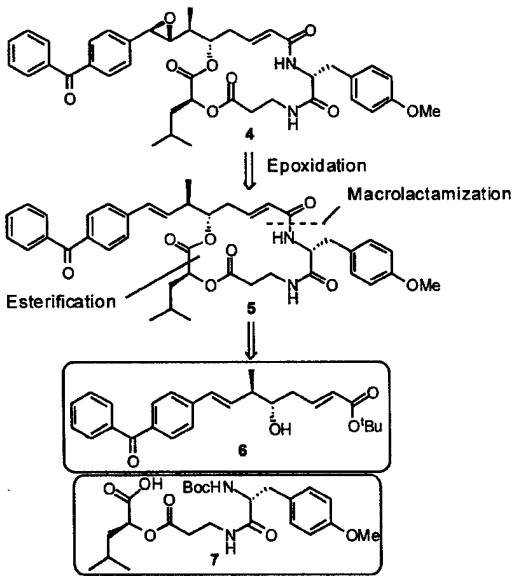
photoaffinity label for tubulin labeling studies. Because structural changes at the C16 side chain aromatic group are tolerated without loss of activity,[1,2] we targeted this position for the placement of a photoreactive functionality to study the cryptophycin binding site on tubulin.

Formal and total syntheses of the cryptophycins have been published by several groups.[1,18–28] Also, SAR studies based on naturally occurring cryptophycins and various synthetic analogues have been reported.[2,29–32] Cryptophycin 1 (**1**) and the structurally less complex arenastatin A (**2**, cryptophycin-24) are close structural analogues (Fig. 1) and have very similar properties with regard to tubulin binding.[8] Therefore, we prepared an affinity label of cryptophycin-24 for our labeling studies, because it can be prepared in fewer synthetic steps than the cryptophycin-1 analogues. The enzymatic/hydrolytic instability[33] of cryptophycin-24 should not pose a problem during the in vitro tubulin investigations. The retrosynthetic analysis of a benzophenone analogue of cryptophycin-24 (**4**) reveals that the desepoxy analogue **5** can be derived from two main building blocks, the octadienoate ester **6**, and the peptide unit **7** (Scheme 1).

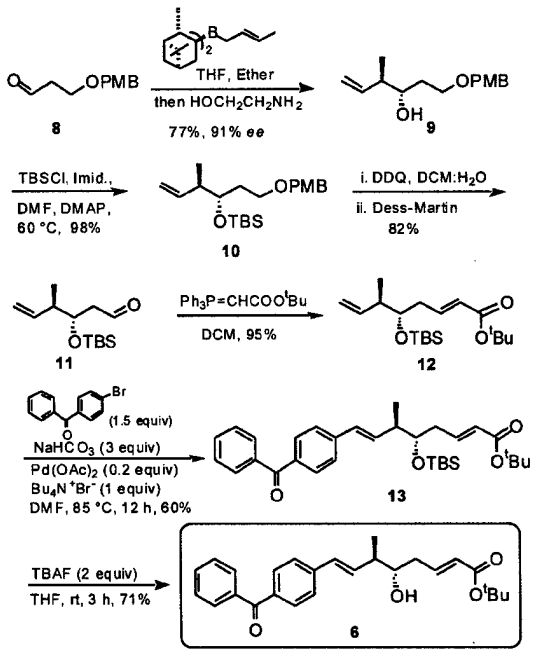
We and others have reported an efficient protocol[26,27,34,35] for the synthesis of octadienoate ester **12** using a crotylboration approach to set both stereocenters in a single step.[36] This method allows for the convergent synthesis of analogues modified at the phenyl group of the C16 side chain using Heck chemistry. The key step utilized the crotylboration of **8** (Scheme 2) with crotyl diisopinocampheylborane (prepared from (+)-*B*-methoxydiisopinocampheyl-borane) to generate the desired stereochemistry at the two chiral centers of **9** in 77% yield (91% ee). Silyl protection of the secondary alcohol **9** with *tert*-butyldimethylsilyl chloride and imidazole afforded the silyl ether **10** in 98% yield. Deprotection of the *p*-methoxybenzyl ether with DDQ followed by DMP-oxidation of the resulting alcohol furnished the aldehyde **11** in 82% yield over two steps. Wittig–Horner olefination of **11** provided the α,β-unsaturated *tert*-butyl ester **12** in 95% yield. Heck coupling[37,38] on **12** with 4-bromobenzophenone in the presence of palladium acetate and sodium bicarbonate furnished the required ester **13** in 60% yield. Deprotection of the silyl ether with tetrabutylammonium fluoride led to the isolation of the desired octadienoate ester **6** in 71% yield.

The second key synthon **7** was readily synthesized starting from a *N*-Boc protected D-tyrosine derivative.[39,27] Key synthons, octadienoate ester **6** and peptide fragment **7** were subjected to the Yamaguchi coupling reaction.[40] The acid **7** was activated with 2,4,6-trichlorobenzoyl chloride in the presence of Hünig's base and a catalytic amount of DMAP. Addition of the alcohol **6** to the mixed anhydride afforded the intermediate **14** in 85% yield (Scheme 3). Simultaneous deprotection of the *tert*-butyl ester and the *N*-Boc with trifluoroacetic acid produced the cyclization precursor and HBTU activation provided the desired macrocycle **15** in 56% yield. Epoxidation of **15** with *m*-CPBA or dimethyl dioxirane (DMD)[41] furnished a diastereomeric mixture of epoxides **4** in the ratios of α:β = 2:1 and 1:2, respectively (Scheme 3).[42] The mixture was separated by HPLC.

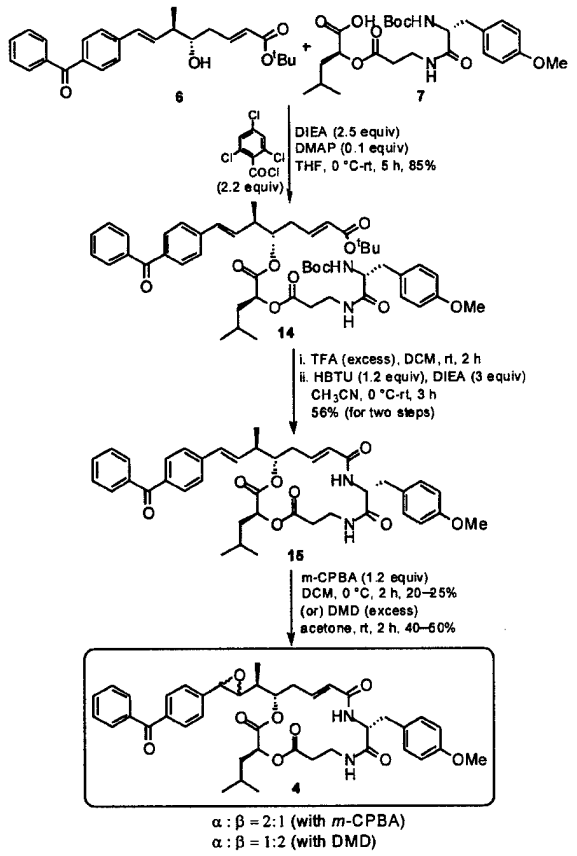
The biological testing was carried out individually for the α & β-isomers of **4**. In the tubulin assembly assay, the benzophenone analogue **4** (β) of cryptophycin-24 (IC_{50} = 7.4 μM) was half as active as cryptophycin-1 (IC_{50} = 3.7 μM) and twice as active as cryptophycin-24 (IC_{50} = 15 μM) (Table 1).[32,43] In the cytotoxicity studies, analogue **4** (β) had reduced activity against the MCF7



Scheme 1.



Scheme 2.



Scheme 3.

Table 1. Biological results

Compd	Tubulin assembly IC_{50}, μM[a]	Cytotoxicity IC_{50}, nM[b]		
		MCF7	MCF7-ADR	HCT-116
1	3.7	0.003	0.013	0.027
2	15	0.13	0.164	0.285
4 (β)	7.4	0.078	70	1.1
4 (α)	>100	6.0	447	25.3

[a]Tubulin at 1.5 mg/mL was assembled at 37 °C for 15 min in the presence of PEM buffer, 0.5 mM GTP and 8% DMSO. Microtubules were pelleted and the protein remaining in the supernatant determined. The IC_{50} value is the concentration that reduces the amount of pelleted protein by 50%.
[b]The IC_{50} value is the concentration that inhibits the proliferation by 50% after 72 h (MCF-7 and MCF7-ADR) or 24 h (HCT-116) of cell growth.

and HCT-116 cell lines compared to **1** and **2**, but was still active in the pM or low nM range. In comparison to **1** and **2**, analogue **4** (β) had much reduced activity against MCF7-ADR cells compared to the MCF7 cell line. This suggests that the addition of the benzophenone moiety makes the compound a better substrate for the p-glycoprotein multi-drug transporter. The α-analogue of **4** had poor activity in all of the biological tests (Table 1).

In summary, an efficient synthesis of a benzophenone photoaffinity analogue of cryptophycin-24 has been achieved. The photolabeled analogue **4** (β) was active in the tubulin assembly assay and is therefore a suitable candidate for further studies to explore the tubulin binding domain of cryptophycin. Our plans are to make this derivative in a radioactive form for photolabeling studies.

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Total Synthesis of Cryptophycin-24 (Arenastatin A) Amenable to Structural Modifications in the C16 Side Chain

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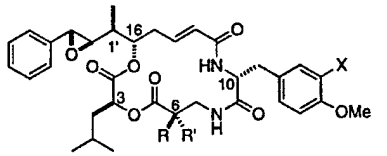
Two efficient protocols for the synthesis of *tert*-butyl (5*S*,6*R*,2*E*,7*E*)-5-[(*tert*-butyldimethylsilyl)-oxy]-6-methyl-8-phenyl-2,7-octadienoate, a major component of the cryptophycins, are reported. The first utilized the Noyori reduction and Frater alkylation of methyl 5-benzyloxy-3-oxopentanoate to set two stereogenic centers, which became the C16 hydroxyl and C1′ methyl of the cryptophycins. The second approach started from 3-*p*-methoxybenzyloxypropanal and a crotyl borane reagent derived from (−)-α-pinene to set both stereocenters in a single step and provided the dephenyl analogue, *tert*-butyl (5*S*,6*R*,2*E*)-5-[(*tert*-butyldimethylsilyl)oxy]-6-methyl-2,7-octadienoate, in five steps. This compound was readily converted to the 8-phenyl compound via Heck coupling. The silanyloxy esters were efficiently deprotected and coupled to the C2−C10 amino acid fragment to provide desepoxyarenastatin A and its dephenyl analogue. The terminal olefin of the latter was further elaborated via Heck coupling. Epoxidation provided cryptophycin-24 (arenastatin A).

Introduction

A novel, cyclic depsipeptide isolated from the blue-green algae (cyanobacterium) *Nostoc* sp. ATCC 53789 was reported by Schwartz and co-workers in 1990.[1] This compound demonstrated extremely potent activity against filamentous fungi of the genus *Cryptococcus* and thus was named cryptophycin A (**1**) (also known as cryptophycin-1, Figure 1).[1] Subsequently, Moore and co-workers isolated the same compound and several structural relatives from *Nostoc* sp. GSV 224.[2,3] Cryptophycin-1 (**1**), the most abundant of the macrolides, was found to have significant tumor-selective cytotoxicity[3−5] and was not an effective substrate for the P-glycoprotein efflux mechanism in multiple-drug resistant cells.[6] When administered intravenously, depsipeptide **1** was also very effective against mammary, colon, and pancreatic adenocarcinomas in mouse xenographs.[7] Concurrently, Kobayashi and Kitagawa isolated a related cytotoxic agent from the Okinawan marine sponge *Dysidea arenaria* and named it arenastatin A (**2**).[8−10] It was found that cryptophycin-24 was identical to this compound. Thus far, 25 compounds of the cryptophycin family have been reported through isolation.[2,7]



Cryptophycin-1 R = Me, R' = H, X = Cl (1)
Cryptophycin-24 (Arenastatin A) R, R', X= H (2)
Cryptophycin-52 R, R' = Me, X = Cl (3)

Figure 1. Structures of the cryptophycins.

Cryptophycin-1 (**1**) blocks the cell cycle at the G_2/M phase apparently through inhibition of tubulin polymerization into microtubules.[11,12] This compound binds to a tubulin site distinct from the colchicine site, but one that may overlap with the vinblastine site.[11,13−15] Its extreme potency (100−1000-fold greater than paclitaxel and vinblastine) has led to additional studies investigating other possible modes of action.[12,16,17] It was found that cryptophycin-1 is a highly potent stabilizer of microtubule dynamics at concentrations (≤100 nM) that have no effect on net microtubule polymerization.[16]

Semisynthetic analogues of cryptophycin-1 (**1**) primarily focusing on the reactive epoxide moiety have resulted in loss of activity except for the halohydrins.[18] In particular, the chlorohydrin derived from cryptophycin-1 (**1**)

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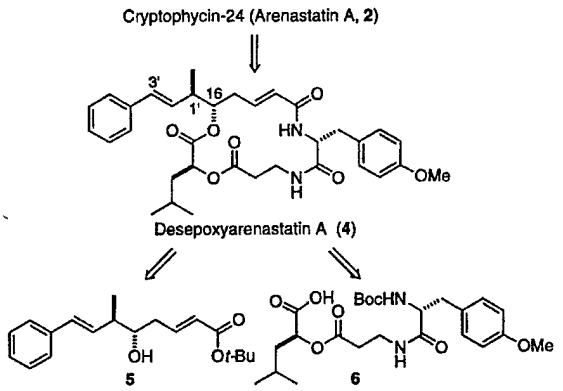
demonstrated higher activity in vivo than the parent compound. However, comparisons of tubulin assembly and cell toxicity data indicated that the halohydrins have reduced or no biological activity and that the observed activity resulted from the regeneration of the parent compound **1**.[19] In pursuit of stable analogues, synthetic approaches were developed probing the substituents and stereochemistry of the tyrosine[20] and β-amino acid subunits,[21-23] the stereochemical and electronic effects of the octadienoate ester subunit,[24-26] and replacement of the α-hydroxy acid subunit with an α-amino acid.[27] These investigations gave rise to cryptophycin-52 (**3**), a synthetic analogue currently in phase II clinical trials.[28] This analogue is hydrolytically more stable than **1** due to the presence of *gem*-dimethyl substituents on the β-amino acid moiety and demonstrates similar or improved bioactivity. Cryptophycin-52 (**3**) has been shown to stabilize microtubule dynamics[28] and be very effective against numerous human tumor cell lines.[29] This compound also accumulated within cells to a concentration consistent with mitotic arrest without altering microtubule polymer concentration.[30] Paclitaxel, which has been shown to have a second mechanism of action by the hyperphosphorylation of Bcl2, renders cancer cells susceptible to apoptosis.[31] Cryptophycin-52 (**3**), likewise, was recently reported to also have this mechanism of action and to be the most potent agent known in this respect.[32]

The outstanding activity, extreme potency, and arrival of a clinical candidate of the cryptophycins has generated a large amount of interest in the total synthesis of arenastatin A (**2**),[33-36] cryptophycin-1 (**1**),[26,37-40] cryptophycin-2,[41,42] and cryptophycin-52 (**3**),[40,43] as well as the synthesis of the octadienoate ester fragment.[44-48] The following discussion fully discloses our previously reported fragment syntheses,[44,45] as well as the completion of the synthesis of cryptophycin-24 (arenastatin A, **2**).

Scheme 1



Results and Discussion

Our interest in structurally flexible fragment syntheses was primarily for structure–activity relationship (SAR) studies, biochemical studies,[11] and photolabeling experiments with tubulin protein. To achieve this goal, we targeted cryptophycin-24 (**2**), which lacks a chiral center at C6 and the chlorine substituent in the tyrosine moiety. The activity of **2**, though slightly diminished in certain cancer cell lines in comparison to cryptophycin-1 (**1**), is similar with respect to microtubule depolymerization.[10,13] The retrosynthetic analysis of cryptophycin-24 (**2**) is shown in Scheme 1. The synthesis of **2** can be simplified by utilizing desepoxyarenastatin **4** as the final precursor with epoxidation being the last step in the total synthesis. Further retrosynthetic analysis of desepoxyarenastatin reveals that **4** can be assembled from ester **5** and amino acid **6**, synthesized from L-leucic acid, β-alanine and D-*O*-methyltyrosine.

The structurally more complex fragment, ester **5**, was targeted via two approaches (Scheme 2). The first approach, which provided flexibility at the C1′ position, utilized an asymmetric reduction of **8** to set the C16 hydroxyl group and then incorporated the second stereocenter anti using a Frater alkylation to provide intermediate **7**. This alkylation strategy allowed potential incorporation of alternative substitution at C1′ through

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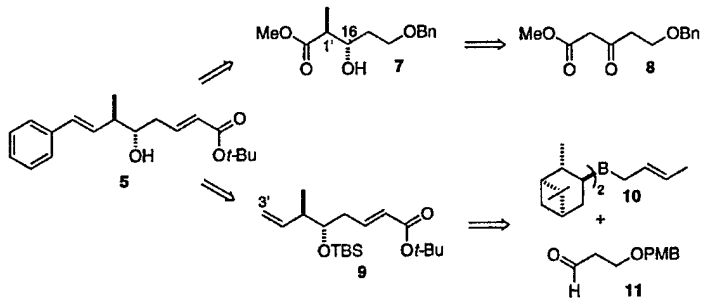
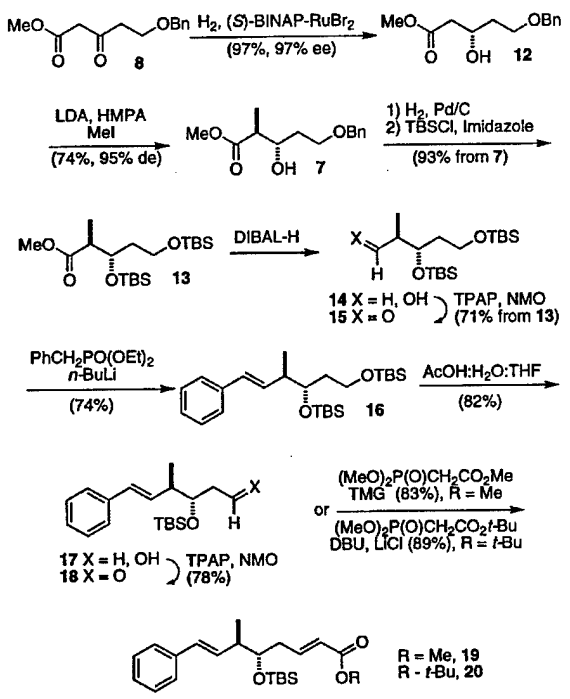
Scheme 2



Scheme 3



the use of electrophiles other than iodomethane. A second route, in which a dephenyl ester **9** was the targeted synthon, allowed the probing of the C3′ aromatic region of the side chain. Ester **9** was derived from a crotyl boration of aldehyde **11** with borane **10**, which incorporated both backbone stereocenters in a single step. With **9** in hand, we could vary the aryl substituents using a Heck coupling strategy in the final step of the fragment synthesis or potentially after the 16-membered cryptophycin macrocycle was intact for thorough SAR studies.

Our first approach (Scheme 3) to the octadienate ester **5** targeted flexibility in the C1′ position in the target compounds. The asymmetric Noyori catalytic hydrogenation[49] of β-keto ester **8**[50] with the (*R*)-Ru(BINAP)Br_2 complex[51] provided (*S*)-hydroxy ester **12**[52] in 97% yield and 97% ee (enantiomeric excess was determined through chiral HPLC: Daicel Chiralcel OD-H). Frater alkylation[53,54] of the dianion of β-hydroxyester **12** with iodomethane gave anti product **7** in 75% yield and 95% diastereomeric excess. The minor isomer was easily removed by flash chromatography. Debenzylation of **7** (95%), followed by silylation of the resulting diol, provided compound **13** in 98% yield. DIBAL-H reduction of **13** to the corresponding aldehyde **15** and subsequent Horner-Emmons reaction (diethyl benzylphosphonate) yielded styrene **16**. It was found, however, that complete reduction to the primary alcohol **14** with DIBAL-H, followed by TPAP oxidation[55] was experimentally more convenient and provided a comparable overall yield. Selective cleavage of the primary TBS-ether of **16** (HOAc, H_2O, THF; 1:1:2)[56] was followed by TPAP oxidation to yield aldehyde **18**. The aldehyde **18** was converted directly to the methyl ester **19** in 83% yield or the *tert*-butyl ester **20** in 89% yield by Horner-Emmons homologation with the corresponding phosphonate and tetramethylguanidine or DBU and LiCl,[57] respectively. A comparison of the optical rotation of the methyl ester thus obtained ($[\alpha]_D = +66.8$), with the published data for **19** ($[\alpha]_D = +68.2$)[37] verified the high optical purity and correct absolute stereochemistry of our product.

This approach provided the octadienoate ester backbone **20** in 10 steps (overall yield of 20%) from readily available starting materials. The synthesis can be carried out on a relatively large scale. Although this route provided an effective method for modification at C1′, we required a method to more efficiently modify the C3′ aromatic substituent.

A second approach (Scheme 4) to octadienoate ester **5**, amenable to SAR studies at the C3′ phenyl group of the C16 side chain, was achieved starting from aldehyde **11**.[58] The key step utilized the crotyl boration of **11** with crotyl diisopinocampheylborane **10** (prepared from (+)-*B*-methoxydiisopinocampheylborane) to generate the desired stereochemistry at the two chiral centers of **21** in 76% yield (91% ee).[59] Silyl protection of the secondary alcohol with *tert*-butyldimethylsilyl trifluoromethanesulfonate

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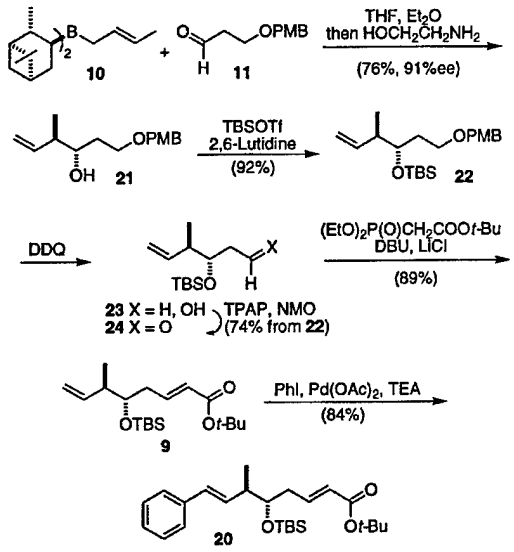
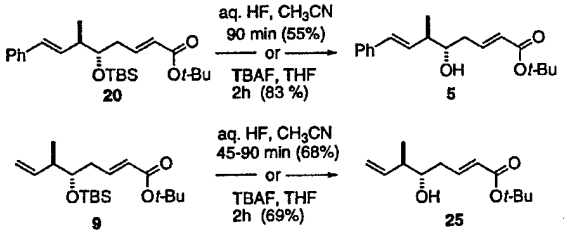
(58) This aldehyde was conveniently prepared in multigram quantities from 1,3-propanediol through the following sequence: (a) for monoprotection see: Urbanek, R. A.; Sabes, S. F.; Forsyth, C. J. *J. Am. Chem. Soc.* **1998**, *120*, 2523. (b) NaOCl (aq), TEMPO, $NaHCO_3$, KBr, CH_2Cl_2, 99%.

Scheme 4



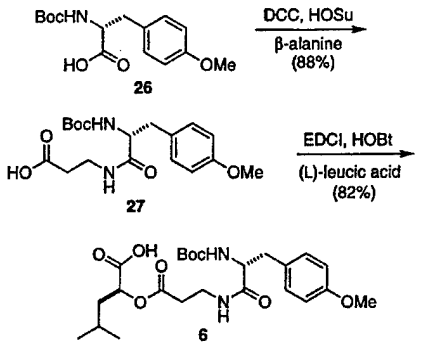
Scheme 5



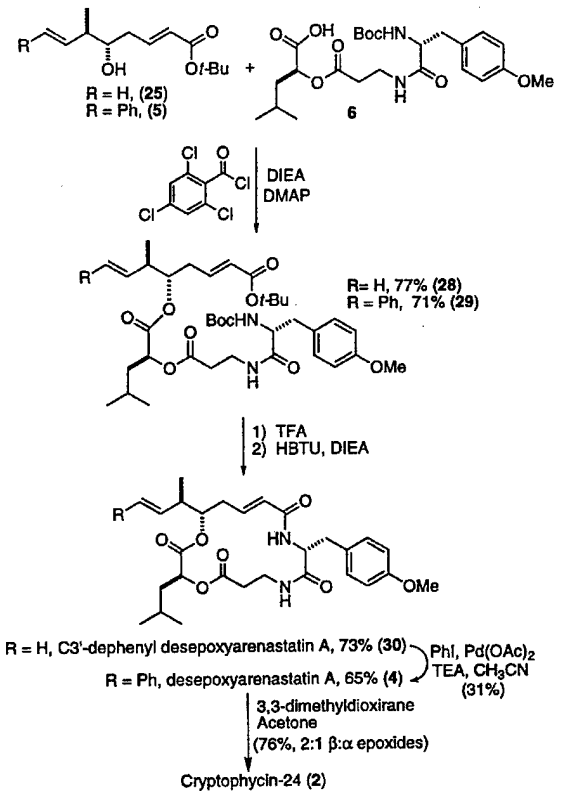
(TBSOTf) in the presence of 2,6-lutidine proceeded at low temperature to silyl ether **22** in 92% yield. Short reaction times were essential for optimal outcome. Rapid deprotection of the *p*-methoxybenzyl ether with DDQ provided a separable mixture of *p*-methoxybenzaldehyde and the desired primary alcohol. However, carrying forward a mixture of *p*-methoxybenzaldehyde and the alcohol **23** was experimentally more convenient. This mixture was subjected to catalytic TPAP oxidation[55] conditions in the presence of NMO producing the easily separable aldehyde **24** after chromatography in 74% yield over two steps. The Horner-Emmons homologation to form the α,β-unsaturated *tert*-butyl ester **9** proceeded cleanly using *tert*-butyl diethylphosphonoacetate, DBU and LiCl.[57] The terminal olefin of compound **9** is the key moiety necessary for modification at what becomes the C3′-aromatic position. Heck coupling[60,61] utilizing $Pd(OAc)_2$, PhI and triethylamine provided the aryl synthon **20** in 84% yield (39% overall in 6 steps from aldehyde **11**). Comparison of the optical rotations of this **20** with the rotation of **20** from the Noyori reduction/Frater alkylation route confirmed its optical purity and correct absolute stereochemistry.

Deprotection (Scheme 5) of aryl synthon **20** with 49% HF in acetonitrile provided hydroxy ester **5** in reasonable yield. These conditions were also used for the deprotection of **9**, providing hydroxy ester **25**. Alternatively, it was demonstrated that TBAF deprotection of silyl ethers

Scheme 6



Scheme 7



20 and **9** with a polymer resin workup as described by Parlow and co-workers[62] provided the desired hydroxy esters **5** and **25** cleanly.

The second key synthon **6** was readily synthesized starting from *N*-Boc amino acid **26** (Scheme 6). Activation of **26** with DCC and *N*-hydroxysuccinimide (HOSu) followed by addition of β-alanine provided acid **27**. EDCI activation and subsequent addition of L-leucic acid provided acid **6** in two steps without the necessity of extensive protecting group chemistry.

With the key synthons, hydroxy esters **5** and **25** and acid **6**, in hand, we proceeded with macrocycle formation. Acid **6** was activated (Scheme 7) in a Yamaguchi coupling reaction[63] with 2,4,6-trichlorobenzoyl chloride in the presence of diisopropylethylamine (DIEA) and catalytic

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DMAP. Addition of the alcohols **25** or **5** to the mixed anhydride provided advanced intermediates **28** and **29**, respectively. Deprotection of the *tert*-butyl ester and the *N*-Boc with trifluoroacetic acid (TFA) produced the cyclization precursors. Under dilute conditions, HBTU activation provided the desired macrocycles **30** and **4**. Epoxidation of **4** with dimethyldioxirane[64] using reported conditions[36] completed the total synthesis of cryptophycin-24, arenastatin A (**2**). Through our methodology, we also synthesized the dephenyl desepoxyarenastatin A (**30**) for modification studies on the C16 aromatic side chain. Heck coupling conditions directly converted compound **30** to **4**, albeit in low yield.

In summary, convergent total syntheses of cryptophycin-24 (**2**) amenable to modifications at the C1′ and C3′ positions of the C16 aromatic side chain were achieved. Introduction of the aryl moiety at the C3′ position can be made at a late stage in the synthesis by utilizing dephenyl desepoxyarenastatin A (**30**) or dephenyl synthon **9**. Application of this methodology toward analogues for SAR and biochemical studies exploring the cryptophycin binding site on tubulin are currently in progress.

Experimental Section

General Methods. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ with a 300, 400 or 500 MHz spectrometer. High-resolution mass spectra were obtained at the University of Kansas mass spectrometry support laboratory. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. Chiral HPLC analysis was performed using a Chiralcel OD-H column. Column chromatography was carried out on silica gel (230–400 mesh, Merck). THF and diethyl ether were distilled from sodium benzophenone ketyl, CH_2Cl_2 was freshly distilled from CaH_2. DIEA, TEA and 2,6-lutidine were distilled from CaH_2 prior to use. All other reagents were commercially available and used without further purification. All moisture-sensitive reactions were carried out under inert atmosphere in oven dried glassware.

Methyl (3*R*)-5-Benzyloxy-3-hydroxypentanoate (12). Under a N_2 atmosphere, a mixture of degassed methyl 5-benzyloxy-3-oxopentanoate (0.5 g, 2.12 mmol) and methanol (10 mL) was placed in a Parr hydrogenation bottle. To this was added Ru(BINAP)Br_2 (prepared from bis-(2-methylallyl)cycloocta-1,5-diene ruthenium (II) (4 mg, 0.013 mmol) and (*S*)-BINAP (8 mg, 0.013 mmol). Hydrogenation was carried out at 50 psi and 50 °C for 5 h. The catalyst was precipitated with Et_2O (100 mL) and filtered through a plug of Celite. The filtrate was concentrated, and the residue was purified by flash column chromatography (hexane/ether 65:35) to give pure alcohol **12** as an oil (0.49 g, 97%): *97% enantiomeric excess was determined by chiral HPLC (Daicel Chiralcel OD-H, 254 nm, 25:1 hexane/2-propanol, 254 nm, 1 mL/min, retention time **12** = 17.2 min, ent-**12** = 21.6 min*); $[\alpha]^{20}_D$ +11.8° (*c* 1.07, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.52 (s, 2H), 4.27–4.23 (m, 1H), 3.70 (s, 3H), 3.74–3.62 (m, 2H), 3.41–3.40 (d, J = 5.6 Hz, 1H), 2.51–2.50 (d, J = 10.5 Hz, 2H), 1.86–1.76 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 138.0, 128.5, 128.4, 127.73, 127.68, 73.3, 68.0, 67.0, 51.7, 41.4, 36.0; IR (film) 3500 (br), 1725 cm^{-1}; HRMS (FAB, NBA) calcd for $C_{13}H_{19}O_4$ (M + H) 239.1283, found 239.1307.

Methyl (2*S*,3*S*)-5-Benzyloxy-3-hydroxy-2-methylpentanoate (7). To a solution of diisopropylamine (6.8 mL, 49 mmol) in THF (75 mL) at −78 °C was added *n*-BuLi (2.5 M in hexanes, 17.4 mL, 43.5 mmol). After the mixture was stirred at −78 °C for 20 min, **12** (4.40 g, 18.5 mmol) was added. The reaction mixture was stirred at −78 °C for 20 min and slowly warmed to −20 °C and maintained at this temperature for 90 min. The reaction mixture was again cooled to −78 °C, and a solution of iodomethane (1.73 mL, 27.8 mmol) in HMPA (8.16 mL, 47.0 mmol) was added. The reaction mixture was stirred at −78 °C for 15 min, warmed slowly to −15 °C over 1.5 h, and maintained at this temperature overnight. Following treatment with saturated aqueous NH_4Cl and extraction with Et_2O, the organic layer was washed with brine, water and dried ($MgSO_4$). The crude product was purified by flash column chromatography (hexane:Et_2O 3:1) to give product **7** as an oil (3.40 g, 77%): *92% diastereomeric excess was determined by integration of CHOH in ^{1}H NMR*; $[\alpha]^{20}_D$ +14.0° (*c* 1.49, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.26 (m, 5H), 4.52 (s, 2H), 3.94–3.93 (m, 1H), 3.69 (s, 3H), 3.74–3.64 (m, 2H), 3.31–3.30 (d, J = 4.7 Hz, 1H), 2.58–2.56 (m, 1H), 1.83–1.72 (m, 2H), 1.20 (d, J = 6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.8, 137.8, 128.3 (2C), 127.53, 127.49 (2C), 73.1, 72.0, 68.2, 51.5, 45.4, 33.6, 13.6; IR (film) 3500,1725 cm^{-1}; HRMS (FAB, NBA) calcd for $C_{14}H_{21}O_4$ (M + H) 253.1440, found 253.1446.

Methyl (2*S*,3*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methylpentanoate (13). To a solution of **7** (3.70 g, 14.68 mmol) in THF (35 mL) was added 10% Pd/C (0.940 g). The solution was flushed with argon and hydrogenated (50 psi) for 3 h. After filtration, solvent was removed under reduced pressure to give pure (by ^{1}H NMR) diol as an oil. The oil was dissolved in DMF (13 mL) and treated with TBSCl (5.83 g, 38.9 mmol) and imidazole (5.89 g, 77.8 mmol). After 16 h at room temperature, the reaction was treated with saturated aqueous NH_4Cl (1 mL) and partitioned between Et_2O and H_2O. Organic extracts were washed with $NaHCO_3$, H_2O, and brine and dried (Na_2SO_4). The crude product was purified by flash column chromatography (hexane/EtOAc 90:10) to give **13** as a clear oil (4.95 g, 93%): $[\alpha]^{20}_D$ + 14.11° (*c* 1.885, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.14–4.10 (m, 1H), 3.72–3.61 (m, 2H), 3.64 (s, 3H), 2.70–2.66 (m, 1H), 1.69–1.63 (m, 1H), 1.60–1.54 (m, 1H), 1.11–1.09 (d, J = 7 Hz, 3H), 0.87 (s, 9H), 0.86 (s, 9H), 0.05 (s, 3H), 0.035 (s, 3H), 0.025 (s, 3H), 0.022 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 70.3, 59.2, 51.4, 45.6, 36.0, 25.9 (3C), 25.7 (3C), 18.2, 18.0, 11.4, −4.6, −4.9, −5.4 (2C); IR (film) 1725 cm^{-1}; MS (EI) *m/z* 375(M^+ − Me, 1); HRMS (FAB, TG/G[3:1 thioglycerol/glycerol]) calcd for (M + H) $C_{19}H_{43}O_4Si_2$ 391.2700, found 391.2680.

(2*R*,3*S*)-3,5-Bis[(*tert*-butyldimethylsilyl)oxy]-2-methylpentan-1-ol (14). DIBALH (13.35 mL, 1.0 M in hexanes) was slowly added over a period of 15 min to a solution of ester **13** (2.07 g, 5.31 mmol) at −78 °C in THF (50 mL). The reaction was allowed to warm to −10 °C over 3 h. The reaction was quenched with MeOH (5 mL) and warmed to room temperature after gas evolution ceased. The reaction was partitioned between EtOAc (150 mL) and H_2O (50 mL). The aqueous layer was extracted with EtOAc. Combined organics were dried (Na_2SO_4), concentrated, and subjected to column chromatography (2:1 hexane:Et_2O) to provide the primary alcohol **14** as a colorless oil (1.74 g, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.90–3.86 (m, 1H), 3.78–3.75 (dd, J = 4, 11 Hz, 1H), 3.66–3.63 (t, J = 6.4 Hz, 2H), 3.53–3.49 (dd, J = 5.3, 11 Hz, 1H), 1.79–1.74 (m, 3H), 1.01–1.00 (d, J = 7 Hz, 3H), 0.88 (s, 9H), 0.87 (s, 9H), 0.075 (s, 3H), 0.070 (s, 3H), 0.03 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 74.1, 65.2, 59.7, 38.6, 37.6, 25.88, 25.86, 18.2, 18.0, 14.3, −4.4, −4.8, −5.3; IR (film) 3440 (br) cm^{-1}; HRMS (FAB, NBA/LiOAc) calcd for $C_{18}H_{42}O_3Si_2Li$ (M + Li) 369.2832, found 369.2842.

(3*R*,4*S*,1*E*)-4,6-Bis[(*tert*-butyldimethylsilyl)oxy]-3-methyl-6-phenylhexene (16). Alcohol **14** (200 mg, 0.552 mmol) was dissolved in CH_2Cl_2 (4 mL) and CH_3CN (0.4 mL). TPAP (9 mg, 5 mol %), NMO (97 mg, 0.83 mmol), and 4 Å molecular sieves (115 mg) were added, and the mixture was vigorously stirred for 1 h. The mixture was filtered through Celite. The filtrate was concentrated and purified by flash column chromatography (hexane/EtOAc 95:5) to provide aldehyde **15** (175 mg, 88%). A solution of diethyl benzylphosphonate (219 mg, 0.96 mmol) in THF (4 mL) was cooled to −78 °C. To this was added *n*-BuLi (2.5 M in hexanes, 0.37 mL, 0.91 mmol). The reaction mixture was stirred at − 78 °C for 30 min and treated with a solution of aldehyde **15** (175 mg, 0.49 mmol) in THF (1 mL). Stirring was continued at −78 °C for another 1 h, and

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then the reaction was gradually warmed to room temperature over 6 h. The reaction was quenched with NH_4Cl solution and extracted with Et_2O. The combined organic layers were washed with water and brine and dried ($MgSO_4$). Column chromatography (hexane/EtOAc 99:1) gave styrene **16** as a colorless oil (155 mg, 74%): $[\alpha]^{20}_D$ +23° (*c* 0.77, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.4 (m, 5H), 6.41–6.36 (d, J = 16 Hz, 1H), 6.23–6.15 (dd, J = 16, 7.7 Hz, 1H), 3.84 (m, 1H), 3.73–3.65 (m, 2H), 2.49 (m, 1H), 1.70–1.63 (m, 2H), 1.12 (d, J = 6.9 Hz, 3H), 0.93 (s, 9H), 0.90 (s, 9H), 0.091 (s, 6H), 0.056 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.8, 132.8, 129.8, 128.4, 126.9, 126.0, 72.7, 60.1, 42.7, 36.7, 25.9 (6C), 18.3, 18.1, 15.5, −4.5 (2C), −5.3 (2C); IR (film) 3020 cm^{-1}; MS (EI) *m/z* 303 (M^+ − OTBS, 12); HRMS (FAB, NBA) calcd for $C_{25}H_{46}O_2Si_2$ (M^+) 377.2332, found 377.2303.

(3*S*,4*R*,5*E*)-3-[(*tert*-Butyldimethylsilyl)oxy]-4-methyl-6-phenyl-5-hexen-1-ol (17). A solution of **16** (0.123 g, mmol) in AcOH and aqueous THF (AcOH/H_2O/THF 1:1:2, 6 mL) was stirred at room temperature. After 72 h, the reaction mixture was neutralized with saturated $NaHCO_3$ solution to pH = 7 and extracted with EtOAc. The combined organic layers were washed with water and dried ($MgSO_4$). Flash column chromatography (hexane/EtOAc 90:10) gave pure product as colorless oil (0.071 g, 82%): $[\alpha]^{20}_D$ +28.3° (*c* 0.675, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.3 (m, 5H), 6.42–6.37 (d, J = 16 Hz, 1H), 6.18–6.10 (dd, J = 16, 7.8), 3.91–3.87 (m, 1H), 3.77–3.73 (m, 2H), 2.62–2.51 (m, 1H), 2.05 (br s, 1H), 1.76–1.72 (m, 2H), 1.12–1.10 (d, J = 6.8 Hz, 3H), 0.91 (s, 9H), 0.12 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.5, 132.5, 130.0, 128.5, 128.4, 127.0, 126.0, 74.6, 60.1, 42.7, 35.0, 25.9, 18.0, 14.8, −4.3, −4.6; IR (film) 3350 (br), 3020 cm^{-1}; HRMS (FAB, NBA/LiOAc) calcd for (M + Li) $C_{19}H_{32}O_2SiLi$ 327.2332, found 327.2323.

(3*S*,4*R*,5*E*)-3-[(*tert*-Butyldimethylsilyl)oxy]-4-methyl-6-phenyl-5-hexenal (18). To alcohol **17** (65 mg, 0.2 mmol) in CH_2Cl_2 (2 mL) and CH_3CN (0.2 mL) were added TPAP (3.5 mg, 5 mol %), NMO (35 mg, 0.3 mmol), and 4 Å molecular sieves (50 mg). The mixture was stirred at room temperature for 1 h. The reaction mixture was filtered through Celite and subjected directly to flash column chromatography (hexane: Et_2O 95:5 to 90:10) which gave aldehyde **18** as an oil (51 mg, 78%). Aldehyde **18** was used as obtained in the next reaction.

Methyl (5*S*,6*R*,2*E*,7*E*)-5-[(*tert*-Butyldimethylsilyl)oxy]-6-methyl-8-phenyl-2,7-octadienoate (19). Procedure as previously reported:[37] $[\alpha]^{20}_D$ +67° (*c* 0.63, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.2–7.4 (m, 5H), 6.96 (ddd, J = 15.6, 7.8, 7.5 Hz, 1H), 6.37 (d, J = 16 Hz, 1H), 6.16 (dd, J = 15.9, 8.1 Hz, 1H), 5.84 (d, J = 15.7 Hz, 1H), 3.75 (m, 1H), 3.72 (s, 3H), 2.44 (m, 1H), 2.36 (m, 2H), 1.10 (d, J = 6.9 Hz, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.8, 146.4, 137.6, 131.9, 130.4, 128.5, 127.0, 126.0, 122.9, 75.0, 51.4, 42.8, 37.6, 25.8, 18.1, 16.2, − 4.4, − 4.5; IR (film) 2950, 1720, 1650 cm^{-1}; MS (EI) *m/z* 374 (M^+, 1); HRMS (FAB, NBA) calcd for $C_{22}H_{35}O_3Si$ (M + H) 375.2355, found 375.2367.

***tert*-Butyl (5*S*,6*R*,2*E*,7*E*)-5-[(*tert*-Butyldimethylsilyl)oxy]-6-methyl-8-phenyl-2,7-octadienoate (20).** A solution of *tert*-butyldiethylphosphonoacetate (0.864 mL, 3.68 mmol), DBU (0.330 mL, 2.21 mmol), and LiCl (0.109 g, 2.58 mmol) was stirred vigorously at room temperature for 30 min in CH_3CN (35 mL) and then added dropwise to a solution of aldehyde **18** (0.586 g, 1.84 mmol) in CH_3CN (4.4 mL). After 75 min, the reaction mixture was washed with saturated aqueous NH_4Cl solution, H_2O, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated. Flash column chromatography (hexane/EtOA, 95:5) provided *tert*-butyl ester **20** as an oil (0.582 g, 76%): $[\alpha]^{20}_D$ +69° (*c* 0.73, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–7.17 (m, 6H), 6.86–6.76 (dt, J = 8, 16 Hz, 1H), 6.38–6.33 (d, J = 16 Hz, 1H), 6.18–6.10 (dd, J = 8, 16 Hz, 1H), 5.74–5.69 (d, J = 16 Hz, 1H), 3.74–3.69 (app q, J = 6 Hz, 1H), 2.47–2.40 (m, 1H), 2.32–2.27 (m, 2H), 1.45 (s, 9H), 1.09–1.06 (d, J = 7 Hz, 3H), 0.88 (s, 9H), 0.033 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 144.8, 137.6, 132.0, 130.4, 128.5 (2 C), 127.0, 126.0 (2 C), 125.1, 80.0, 75.1, 42.8, 37.3, 28.1 (3 C), 18.1, 16.1, −4.4, −4.5; IR (film) 2910, 1695, 1645 cm^{-1}; MS (CI) *m/z* 417 (M + 1); HRMS (FAB, NBA) calcd for $C_{25}H_{41}O_3Si$ (M + H) 417.2825, found 417.2848.

(3*S*,4*R*)-1-(4-Methoxybenzyloxy)-4-methyl-5-hexen-3-ol (21). To a stirred mixture of potassium *tert*-butoxide (7.75 g, 69.06 mmol), THF (72 mL), and *trans*-2-butene (25.7 mL, 89.8 mmol) was added *n*-BuLi (1.6 M in hexanes, 43.2 mL, 69.1 mmol) at −78 °C. After the addition was complete, the mixture was stirred at −45 °C for 10 min, producing a bright canary yellow/orange solution. This solution was recooled to −78 °C, and to it was added dropwise a solution of (+)-*B*-methoxydiisopinocampheylborane (26.23 g, 82.87 mmol) in Et_2O (89 mL). After the reaction mixture was stirred at −78 °C for 30 min, BF_3·Et_2O (11.74 mL, 92.54 mmol) was added dropwise followed immediately by aldehyde **11**[58] (16.11 g, 82.87 mmol). The reaction was stirred for an additional 3 h at −78 °C. The mixture was then concentrated, redissolved in dry Et_2O (150 mL), and cooled to 0 °C, and ethanolamine (4.2 mL) was added with vigorous stirring. The reaction was warmed to room temperature and stirred for 72 h. The mixture was then filtered and the filtrate concentrated. Column chromatography of the filtrate (95:5 to 80:20 hexane/EtOAc) provided the pure alcohol **21** (13.20 g, 76%): *91% enantiomeric excess was determined by chiral HPLC comparing alcohol **21** with alcohol ent-**21** derived from reaction with the* (−)-*borane (Daicel Chiralcel OD-H, 254 nm, 99:1 hexanes/2-propanol, 0.5 mL/min, retention time*: *ent-**21** = 32.2 min, **21** = 34.6 min*); $[\alpha]^{20}_D$ +2.7° (*c* 1.5, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.28–7.26 (d, J = 8.7 Hz, 2H), 6.91–6.88 (d, J = 8.7 Hz, 2H), 5.87–5.78 (m, 1H), 5.11 (br s, 1H), 5.08–5.06 (br d, J = 5 Hz, 2H), 4.47 (s, 2H), 3.82 (s, 3H), 3.74–3.61 (m, 3H), 2.82–2.81 (d, J = 3 Hz, 1H), 2.28–2.22 (m, 1H), 1.76–1.69 (m, 2H), 1.07–1.05 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 140.5, 130.1, 129.3 (2C), 115.4, 113.8 (2C), 74.3, 73.0, 68.9, 55.3, 44.0, 33.5, 15.8; IR (film) 3420, 3040 cm^{-1}; HRMS (FAB, NBA) calcd for $C_{15}H_{22}O_3$ (M^+) 250.1569, found 250.1577.

(3*S*,4*R*)-3-[(*tert*-Butyldimethylsilyl)oxy]-1-(4-methoxybenzyloxy)-4-methyl-5-hexene (22). Alcohol **21** (0.150 g, 0.599 mmol) was dissolved in CH_2Cl_2 (6 mL) and cooled to −78 °C. 2,6-Lutidine (0.14 mL, 1.20 mmol) was added, followed by TBSOTf (0.165 mL, 0.719 mmol) dropwise via syringe. After 10 min, the reaction was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and concentrated. Flash column chromatography (90:10 hexane/EtOAc) provided a colorless oil (0.201 g, 92%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.25–7.23 (d, J = 8.6 Hz, 2H), 6.88–6.86 (d, J = 8.6 Hz, 2H), 5.80–5.71 (ddd, J = 7, 11, 16 Hz, 1H), 5.00 (br s, 1H), 4.97–4.95 (br d, J = 7 Hz, 1H), 4.44–4.41 (d, J = 11 Hz, 1H), 4.39–4.36 (d, J = 11 Hz, 1H), 3.80 (s, 3H), 3.77–3.73 (m, 1H), 3.48–3.45 (dt, J = 2, 7 Hz, 2H), 2.29–2.26 (m, 1H), 1.70–1.63 (m, 2H), 1.00–0.98 (d, J = 6.9 Hz, 3H), 0.88 (s, 9H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 140.7, 130.7, 129.2 (2C), 114.5, 113.7 (2 C), 72.5, 67.1, 55.3, 43.4, 33.2, 25.9, 18.1, 14.5, −4.4, −4.5; IR (film) 3080 cm^{-1}; HRMS (FAB, NBA) calcd for $C_{21}H_{36}O_3Si$ (M^+) 364.2434, found 364.2442.

(3*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]-4-methyl-5-hexen-1-ol (23). The *p*-methoxybenzyl ether **22** (1.600 g, 4.388 mmol) was dissolved in CH_2Cl_2 (20.7 mL). Water (1.3 mL) and solid DDQ (0.996 g, 4.388 mmol) were added. After 1 h, CH_2Cl_2 (200 mL) and saturated aqueous $NaHCO_3$ (40 mL) were added. The organic layer was further washed with $NaHCO_3$ and brine until the organics were pale yellow in color. The organics were dried ($MgSO_4$), filtered and concentrated. Flash column chromatography (80:20 hexane/EtOAc) provided a mixture of desired primary alcohol **23** and *p*-methoxybenzaldehyde which was used without further purification in the next reaction: ^1H NMR (400 MHz, $CDCl_3$) δ 5.78–5.70 (ddd, J = 7, 10.6, 17 Hz, 1H), 5.04–5.02 (br d, J = 7 Hz, 1H), 4.99 (br s, 1H), 3.83–3.77 (m, 1H), 3.73–3.69 (m, 2H), 2.39–2.35 (m, 1H), 1.68–1.63 (m, 2H), 1.00–0.99, (d, J = 6.9 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 132.0, 114.3, 74.3, 60.5, 43.2, 34.6, 25.8, 18.0, 13.9, −4.4, −4.6.

(3*S*)-3-[(*tert*-Butyldimethylsilyl)oxy]-4-methyl-5-hexenal (24). To alcohol **23** was added CH_2Cl_2 (15 mL) and freshly activated crushed 4 Å molecular sieves (0.380 g), NMO (0.617 g, 5.27 mmol) and TPAP (0.077 g, 0.219 mmol). After the reaction was complete by TLC (15–30 min), the mixture was

concentrated and directly purified by flash chromatography (95:5 hexane/EtOAc) to provide aldehyde **24** as an oil (0.784 g, 74% over two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.78–9.72 (t, J = 2 Hz, 1H), 5.79–5.70 (ddd, J = 7, 10.5, 17 Hz, 1H), 5.06–5.04 (m, 1H), 5.05–4.99 (m, 1H), 4.20–4.17 (m, 1H), 2.50–2.43 (app dt, J = 2, 6 Hz, 2H), 2.39–2.33 (m, 1H), 1.03–1.01 (d, J = 6.9 Hz, 3H), 0.87 (s, 9H), 0.08 (s, 3H), 0.04 (s, 3H); IR (film) 3090, 1715 cm^{-1}.

***tert*-Butyl (5*S*,6*R*,2*E*)-5-[(*tert*-Butyldimethylsilyl)oxy]-6-methyl-2,7-octadienoate (9).** *tert*-Butyldiethylphosphonoacetate (1.16 mL, 4.95 mmol), DBU (0.44 mL, 2.97 mmol), and LiCl (0.147 g, 3.47 mmol) were stirred vigorously at room temperature for 30 min in CH_3CN (10.8 mL). The solution was added dropwise via syringe to a solution of aldehyde **24** (0.600 g, 2.48 mmol) in CH_3CN (5.7 mL). After 2 h, saturated aqueous NH_4Cl solution was added. The organics were further washed with brine. The aqueous layers were cross-extracted with EtOAc. The combined organics were dried ($MgSO_4$), filtered and concentrated. Flash chromatography (95:5 hexane/EtOAc) provided product as a colorless oil (0.750 g, 89%): $[\alpha]^{20}_D$ +0.7° (c 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82–6.75 (dt, J = 8, 16 Hz, 1H), 5.80–5.71 (buried, 1H), 5.74–5.70 (br d, J = 16 Hz, 1H), 5.03 (br s, 1H), 5.01–4.99 (br d, J = 9 Hz, 1H), 3.67–3.63 (m, 1H), 2.29–2.23 (m, 3H), 1.46 (s, 9H), 1.00–0.99 (d, J = 6.8 Hz, 3H), 0.88 (s, 9H), 0.033 (s, 3H), 0.027 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 145.1, 140.1, 125.0, 115.1, 80.0, 74.9, 43.3, 36.8, 28.1 (3 C), 25.8 (3 C), 18.1, 15.3, −4.4, −4.6; IR (film) 3060, 1695 cm^{-1}; HRMS (FAB, NBA) calcd for $C_{19}H_{37}O_3Si$ (M + H) 341.2512, found 341.2514.

***tert*-Butyl (5*S*,6*R*,2*E*,7*E*)-5-[(*tert*-Butyldimethylsilyl)oxy]-6-methyl-8-phenyl-2,7-octadienoate (20).**[36] Olefin **9** (75 mg, 0.22 mmol) was weighed into a dry seal tube and flushed with argon. CH_3CN (2 mL) was added, followed by iodobenzene (49 mg, 0.24 mmol), $Pd(OAc)_2$ (2.5 mg, 0.011 mmol), and TEA (0.31 mL, 2.2 mmol). The tube was sealed and heated at 80–85 °C for 24 h. The mixture was concentrated and subjected directly to column chromatography (90:10 hexane/EtOAc) to provide the product **20** as an oil (77 mg, 83%): $[\alpha]^{20}_D$ +64° (c = 0.73, $CHCl_3$); $[\alpha]^{20}_D$ +69° (c 0.73, $CHCl_3$) via the Noyori reduction/Frater alkylation route; spectral data consistent with other route.

***tert*-Butyl (5*S*,6*R*,2*E*,7*E*)-5-Hydroxy-6-methyl-8-phenyl-2,7-octadienoate (5).[36] HF Procedure.** HF (48% w/w, aq, 13 μL) was added to ester **20** (0.077 g, 0.19 mmol) dissolved in CH_3CN (1.9 mL) at 0 °C. After 1.5 h, CH_2Cl_2 (10 mL) and saturated aqueous $NaHCO_3$ solution were added until the pH was neutral. The aqueous layer was extracted with CH_2Cl_2. Combined organics were dried ($MgSO_4$), filtered, and concentrated. Flash chromatography (90:10 to 85:15 hexane/EtOAc) provided a pale yellow oil (30 mg, 54%).

TBAF Procedure. The ester **20** (25 mg, 0.060 mmol) was dissolved in THF (530 μL). TBAF solution (1 M in THF, 66 μL, 0.066 mmol) was added dropwise. After 2 h, Amberlyst-15 (180 mg), the calcium salt of Amberlyst-15 (180 mg),[62] and 1 mL of THF were added and the mixture stirred for an additional 2.5 h. The resin was removed by filtration and rinsed with CH_2Cl_2. The filtrate was concentrated and subjected to a short plug of silica (80:20 hexane/EtOAc), providing a pale yellow oil (14 mg, 78%): $[\alpha]^{20}_D$ +66° (c 0.80, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.36 (d, J = 7 Hz, 2H), 7.32–7.28 (app t, J = 7 Hz, 2H), 7.25–7.20 (t, J = 7 Hz, 1H), 6.95–6.88 (dt, J = 7.5, 15.6 Hz, 1H), 6.48–6.44 (d, J = 16 Hz, 1H), 6.17–6.11 (dd, J = 8.6, 16 Hz, 1H), 5.86–5.82 (br d, J = 15.6 Hz, 1H), 3.67–3.62 (m, 1H), 2.48–2.38 (m, 2H), 2.36–2.28 (m, 1H), 1.86 (br s, 1H), 1.47 (s, 9H), 1.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 144.0, 137.0, 131.8, 130.9, 128.5 (2C), 127.4, 126.1 (2C), 125.4, 80.2, 73.8, 43.2, 37.1, 28.1 (3C), 16.8; IR (film) 3420, 1690, 1635 cm^{-1}; MS (CI) m/z 320 (M + NH_3, 9), 303 (M + H, 3), 264 (100); HRMS (FAB, NBA) calcd for $C_{19}H_{27}O_3$ (M + H) 303.1960, found 303.1980.

***tert*-Butyl (5*S*,6*R*,2*E*)-5-Hydroxy-6-methyl-2,7-octadienoate (25). HF Procedure.** Ester **9** (100 mg, 0.294 mmol) was dissolved in CH_3CN (0.294 mL) and cooled to 0 °C. HF (48% w/w, aq, 24 μL) was added. After 25 min, saturated aqueous $NaHCO_3$ solution was added. The aqueous layer was extracted with CH_2Cl_2. Organics were dried ($MgSO_4$), filtered, and concentrated. Flash column chromatography (90:10 to 85:15 hexane/EtOAc) provided the desired alcohol as a colorless oil (45 mg, 68%).

TBAF Procedure. Ester **9** (25 mg, 0.073 mmol) was dissolved in THF (650 μL). TBAF solution (1 M in THF, 81 μL, 0.081 mmol) was added dropwise. After 2 h, Amberlyst-15 (220 mg), the calcium salt of Amberlyst-15 (220 mg),[62] and 1 mL of THF were added and the mixture stirred for an additional 2.5 h. The resin was removed by filtration and thoroughly rinsed with CH_2Cl_2. The filtrate was concentrated and subjected to a short plug of silica (80:20 hexane/EtOAc) to provide an oil (11 mg, 69%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93–6.85, 5.85–5.80, 5.78–5.69, 5.15–5.14 (m, 1H), 5.13–5.10 (m, 1H), m (1), dddd (1.5, J = 4, 6.9, 14 Hz, 1H), m (2H), 1.67–1.66 (d, J = 4 Hz, 1H), 1.47 (s, 9H), 1.05–1.04 (d, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 144.0, 139.6, 125.5, 116.9, 80.2, 73.4, 43.8, 36.9, 28.1 (3 C), 16.2; IR (film) 3460, 3100, 1655 cm^{-1}; MS (CI) m/z 244 (M + NH_3, 15), 227 (M + H, 8), 188 (100); HRMS (FAB, NBA) calcd for $C_{13}H_{23}O_3$ (M + H) 227.1647, found 227.1669.

3-[(2*R*)-(*tert*-Butoxycarbonylamino)-3-(4-methoxyphenyl)propanoylamino]propanoic Acid (27). DCC (4.37 g, 21.2 mmol) in DME (25 mL) was added dropwise to a solution of Boc-D-Tyr(Me)OH **26** (5.00 g, 16.9 mmol) and *N*-hydroxysuccinimide (2.92 g, 25.4 mmol) in DME (75 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 24 h. A solution of 3-aminopropionic acid (2.10 g, 20.3 mmol) and TEA (4.72 mL, 34.0 mmol) in water (50 mL) was slowly added in 10 portions. After 2 h, the reaction mixture was filtered and the residue washed with water. The combined filtrates were concentrated, and a K_2CO_3 (aq, 5%) solution was added to the residue until pH 9. The aqueous layer was washed with CH_2Cl_2, acidified to pH 2 with 10% aqueous HCl, and extracted with CH_2Cl_2. This organic phase was then washed with brine, dried (Na_2SO_4), and concentrated to an oily residue. The residue was triturated with pentane and the resulting precipitate was filtered. The product was recrystallized (ether/pentane) to give pure acid **27** as a crystalline solid (5.50 g, 88%): mp 103–105 °C; $[\alpha]^{20}_D$ −7.9° (c 1.5, $CHCl_3$); ^{1}H NMR (400 MHz MHz, $CDCl_3$) δ 7.06 (d, J = 8.6 Hz, 2H), 6.98 (br s, 1H), 6.83 (d, J = 8.6 Hz, 2H), 5.48 (br d, J = 8.5 Hz, 1H), 4.45 (br d, J = 7.6 Hz, 1H), 3.73 (s, 3H), 3.47 (br s, 1H), 3.45 (br s, 1H), 2.89 (d, J = 6.7 Hz, 2H), 2.44 (m, 2H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.3, 171.7, 158.5, 155.8, 130.2(2C), 128.4, 113.9(2C), 80.5, 55.6, 55.1, 38.2, 34.4, 33.5, 28.2(3C); IR (KBr) 3336, 1710, 1684, 1649 cm^{-1}; HRMS (FAB, TG/G) calcd for $C_{18}H_{27}N_2O_6$ (M + H) 367.1881, found 367.1875.

(2*S*)-2-[3-(2*R*)-2-*tert*-Butoxycarbonylamino-3-(4-methoxyphenyl)propanoylamino]propanoyloxy]-4-methylpentanoic Acid (6).[36] To a stirred solution of acid **27** (5.50 g, 15 mmol) and *N*-hydroxysuccinimide (3.40 g, 30 mmol) in DME (100 mL) and CH_2Cl_2 (100 mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.80 g, 25 mmol) at room temperature. After 24 h, the reaction mixture was diluted with EtOAc (200 mL) and washed with water, 1% HCl, 5% aqueous $NaHCO_3$ solution, H_2O, and brine. The organic phase was then dried (Na_2SO_4) and concentrated to give the activated ester. The intermediate was dissolved in CH_3CN (100 mL), and DMAP (9.16 g, 75 mmol) and L-leucic acid (4.95 g, 37.5 mmol) were added at room temperature. The reaction mixture was stirred for 18 h and then acidified with HCl (0.1 N) at 0 °C until pH 2. The solution was extracted with EtOAc, and the combined extracts were washed with H_2O and brine. The mixture was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (CH_2Cl_2/EtOAc 3:1) to give acid **6** (5.90 g, 82%) as a foam: $[\alpha]^{20}_D$ −21° (c 0.77, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, J = 8.5 Hz, 2H), 6.85 (br s, 1H), 6.77 (br d, J = 7.6 Hz, 2H), 5.36 (m, 1H), 5.12 (m, 1H), 4.28 (m, 1H), 3.74 (s, 3H), 3.45 (br s, 2H), 2.95 (br d, J = 6.2 Hz, 2H), 2.52 (m, 2H), 1.75 (m, 3H), 1.36 (s, 9H), 0.94 (d, J = 6.2 Hz, 3H), 0.91 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 171.7, 158.5, 155.8, 130.3, 128.4, 113.9, 80.5, 71.2, 55.2, 39.4, 37.9, 33.9, 28.2, 24.6, 23.0, 21.5, 14.1;

MS (FAB, TG/G) *m/z* 481.3 (M + H); HRMS (FAB, TG/G) calcd for $C_{24}H_{39}N_2O_8$ (M + H) 481.2550, found 481.2573.

***tert*-Butyl (5*S*,6*R*,2*E*)-5-[(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(4-methoxyphenyl)propanoylamino]propanoyloxy]-4-methylpentanoyloxy]-6-methylocta-2,7-dienoate (28).** *N*-Boc amino acid **6** (314 mg, 0.65 mmol) was dissolved in THF (12.5 mL). DIEA (142 μL, 0.82 mmol), 2,4,6-trichlorobenzoyl chloride (97 μL, 0.62 mmol), and DMAP (5 mg) were added. After 30 min, the alcohol **25** (74 mg, 0.33 mmol) in THF (300 μL) was added dropwise via syringe. After 45 min, saturated aqueous $NaHCO_3$ solution was added. The aqueous layer was then extracted with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and concentrated. Flash chromatography (80:20 to 70:30 hexanes/EtOAc) provided the desired ester **28** as a thick oil (173 mg, 77%): $[\alpha]^{20}_D$ −24.6° (*c* 1.00, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.12−7.10 (d, *J* = 8.5 Hz, 2H), 6.80−6.78 (d, *J* = 8.5 Hz, 2H), 6.73 (buried m, 1H), 5.81−5.77 (br d, *J* = 15.6 Hz, 1H), 5.69−5.60 (ddd, *J* = 8, 10.8, 16.6 Hz, 1H), 5.44−5.42 (br d, *J* = 8 Hz, 1H), 5.07 (s, 1H), 5.05−5.03 (d, *J* = 7 Hz, 1H), 4.98−4.94 (dd, *J* = 4, 10 Hz, 1H), 4.96−4.92 (buried, 1H), 4.38−4.32 (m, 1H), 3.74 (s, 3H), 3.61−3.55 (m, 1H), 3.48−3.43 (m, 1H), 3.14−3.09 (dd, *J* = 5.5, 14 Hz, 1H), 2.93−2.88 (m, 1H), 2.51−2.38 (m, 5H), 1.78−1.68 (m, 3H), 1.59−1.52 (m, 1H), 1.46 (s, 9H), 1.34 (s, 9H), 1.00−0.98 (d, *J* = 7 Hz, 3H), 0.92−0.91 (d, *J* = 6.5 Hz, 3H), 0.90−0.88 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.6, 165.7, 158.3, 155.4, 141.8, 138.3, 130.3 (2C), 129.1, 126.0, 116.6, 113.8, 80.4, 79.5, 76.2, 71.2, 55.9, 55.1, 41.2, 39.6, 37.7, 35.1, 34.6, 34.3, 28.2 (3C), 28.1 (3C), 24.6, 23.0, 21.4, 16.4; MS (FAB, TG/G) *m/z* 689.4 (M+H); HRMS (FAB, TG/G) calcd for $C_{37}H_{57}N_2O_{10}$ (M + H) 689.4013, found 689.4030.

Dephenyldesepoxyarenastatin A (30). The starting ester **28** (170 mg, 1.48 mmol) was dissolved in CH_2Cl_2 (5 mL), and TFA (300 μL) was added. After 1 h, toluene (5 mL) was added and the reaction mixture was concentrated in vacuo. The mixture was then redissolved in CH_3CN (23 mL), and DIEA (123 μL, 0.706 mmol) and HBTU (107 mg, 0.282 mmol) were added. After the mixture was stirred for 1 h, saturated aqueous $NaHCO_3$ and CH_2Cl_2 were added. The aqueous layer was extracted with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered,and concentrated. Flash chromatography (7:1 to 4:1 CH_2Cl_2/acetone) provided the desired macrocycle **30** as a colorless oil (88 mg, 73%): $[\alpha]^{20}_D$ +24° (*c* 0.80, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.11−7.09 (d, *J* = 8.6 Hz, 2H), 7.05−7.02 (t, *J* = 5.7 Hz, 1H), 6.81−6.79 (d, *J* = 8.6 Hz, 2H), 6.71−6.63 (ddd, *J* = 5, 10.3, 15 Hz, 1H), 5.84−5.82 (d, *J* = 8 Hz, 1H), 5.75−5.71 (d, *J* = 14 Hz, 1H), 5.69−5.65 (m, 1H), 5.08 (br s, 1H), 5.05−5.04 (d, *J* = 6 Hz, 1H), 5.02−4.97 (ddd, *J* = 2, 5, 11 Hz, 1H), 4.94−4.91 (dd, *J* = 4, 9.5 Hz, 1H), 4.72−4.66 (m, 1H), 3.76 (s, 3H), 3.54−3.47 (m, 1H), 3.47−3.40 (m, 1H), 3.16−3.11 (dd, *J* = 6, 14.4 Hz, 1H), 3.03−2.97 (dd, *J* = 7.6, 14.4 Hz, 1H), 2.44−2.28 (m, 2H), 1.76−1.66 (m, 1H), 1.48−1.40 (m, 1H), 1.04−1.02 (d, *J* = 7 Hz, 3H), 0.92−0.90 (d, *J* = 6.4 Hz, 3H), 0.88−0.87 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 170.82, 170.76, 165.7, 158.5, 141.8, 138.6, 130.2 (2C), 128.5, 124.9, 116.5, 114.1 (2C), 76.8, 71.4, 55.2, 54.3, 42.4, 39.8, 36.1, 35.2, 34.2, 32.5, 24.5, 22.9, 21.5, 16.6; IR (film) 3420, 3300, 2990, 1740, 1675, 1525 cm^{-1}; MS (FAB+, TG/G) 515.3 (M + H); HRMS (FAB, TG/G) calcd for $C_{28}H_{39}O_7N_2$ (M + H) 515.2757, found 515.2775.

***tert*-Butyl (5*S*,6*R*,2*E*,7*E*)-5-[(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(4-methoxyphenyl)propanoylamino]propanoyloxy]-4-methylpentanoyloxy]-6-methyl-8-phenyl-2*E*,7*E*-octadienoate (29).**[36] The *N*-Boc amino acid **6** (95 mg, 0. 198 mmol) was dissolved in THF (3.8 mL), and then DIEA (43 μL, 0.248 mmol), 2,4,6-trichlorobenzoyl chloride (34 μL, 0.218 mmol), and DMAP were added. After 1.5 h, the alcohol **5** (30 mg, 0. 099 mmol) dissolved in THF (200 μL) was added dropwise. After 1 h, saturated aqueous $NaHCO_3$ solution was added. The mixture was extracted with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and concentrated. Column chromatography (80:20 to 70:30 hexanes/EtOAc) provided the desired ester **29** as a colorless oil (54 mg, 71%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.32−7.18 (m, 5H), 7.13−7.10 (d, *J* = 8.5 Hz, 2H), 6.80−6.78 (d, *J* = 8.5 Hz, 2H), 6.76 (buried m, 1H), 6.41−6.37 (d, *J* = 15.9 Hz, 1H), 6.02−5.95 (dd, *J* = 8.6, 15.9 Hz, 1H), 5.83−5.80 (d, *J* = 15.6 Hz, 1H), 5.45−5.43 (d, *J* = 8 Hz, 1H), 5.03−5.00 (m, 1H), 4.96−4.93 (dd, *J* = 4, 10 Hz, 1H), 4.36−4.31 (m, 1H), 3.75 (s, 3H), 3.54−3.52 (m, 1H), 3.48−3.41 (m, 1H), 3.15−3.10 (dd, *J* = 5.5, 14 Hz, 1H), 2.93−2.88 (m, 1H), 2.60−2.40 (m, 5H), 1.71−1.57 (m, 3H), 1.47 (s, 9H), 1.34 (s, 9H), 1.33 (buried, 1H), 1.10−1.08 (d, *J* = 6.9 Hz, 3H), 0.83−0.81 (d, *J* = 6.5 Hz, 3H), 0.78−0.76 (d, *J* = 6.5 Hz, 3H); other data as previously reported.[36]

Desepoxyarenastatin A (4) from 29.[36] Ester **29** (50 mg, 0.065 mmol) was dissolved in CH_2Cl_2 (6 mL), and TFA (200 μL) was added. After 1 h, toluene (2 mL) was added, and the reaction mixture was concentrated in vacuo. The resulting acid was dissolved in CH_3CN (6 mL), and DIEA (33 μL, 0.19 mmol) and HBTU (28 mg, 0.075 mmol) were added. The reaction was stirred at room temperature for 45 min. Saturated aqueous $NaHCO_3$ solution was added, and the aqueous phase was extracted with CH_2Cl_2. The combined organics were dried ($MgSO_4$), filtered, and concentrated. Column chromatography (7:1 to 4:1 CH_2Cl_2/acetone) provided **4** as an oil (24 mg, 65%): $[\alpha]^{20}_D$ +27° (*c* 0.80, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.33−7.15 (m, 5H), 7.11−7.09 (d, *J* = 8.6 Hz, 2H), 7.05−7.02 (t, *J* = 5.5 Hz, 1H), 6.81−6.78 (d, *J* = 8.6 Hz, 2H), 6.73−6.66 (ddd, *J* = 4.7, 10.5, 15 Hz, 1H), 6.41−6.37 (d, *J* = 15.8 Hz, 1H), 6.03−5.96 (dd, *J* = 8.8, 15.8 Hz, 1H), 5.77−5.75 (d, *J* = 7.9 Hz, 1H), 5.75−5.71 (d, *J* = 15 Hz, 1H), 5.06−5.01 (ddd, *J* = 2, 6.6, 11 Hz, 1H), 4.91−4.88 (dd, *J* = 3.6, 10 Hz, 1H), 4.73−4.67 (m, 1H), 3.76 (s, 3H), 3.54−3.48 (m, 1H), 3.46−3.39 (m, 1H), 3.15−3.11 (dd, *J* = 6, 14.4, 1H), 3.04−2.98 (dd, *J* = 7.5, 14.4 Hz, 1H), 2.57−2.52 (m, 3H), 2.39−2.30 (m, 1H), 1.78−1.57 (m, 3H), 1.35−1.27 (m, 1H), 1.13−1.11 (d, *J* = 6.8 Hz, 3H), 0.73−0.72 (d, *J* = 6.4 Hz, 3H), 0.70−0.69 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 170.9, 170.8, 165.6, 158.5, 141.7, 136.7, 131.8, 130.2 (2C), 128.6 (2C), 128.5, 127.5, 126.1 (2C), 125.0, 114.1 (2C), 76.6, 71.5, 55.2, 54.3, 42.2, 39.7, 36.4, 35.2, 34.2, 32.4, 24.3, 22.6, 21.2, 17.2; IR (film) 3365, 3260, 2940, 1725, 1710, 1665 cm^{-1}; MS (FAB, TG/G) *m/z* 591.3 (M + H); HRMS (FAB, TG/G) calcd for $C_{34}H_{43}N_2O_4$ (M + H) 591.3070, found 591.3069.

Desepoxyarenastatin A (4) from 30. Olefin **30** (17 mg, 0.033 mmol) was dissolved in CH_3CN (0.33 mL) in a dry sealed tube. The solution was flushed with argon, and then iodobenzene (4 mL, 0.036 mmol), $Pd(OAc)_2$ (1.1 mg, 0.005 mmol), and TEA (46 mL, 0.330 mmol) were added. The tube was sealed and placed in a 80−85 °C oil bath with vigorous stirring overnight. After 20 h, the solution was filtered and purified by silica gel chromatography to obtain product **4** as a solid (3 mg, 31% based on recovered starting material) and unreacted starting material (6 mg).

Arenastatin A (2). Olefin **4** (5.0 mg, 8.5 μmol) was reacted as previously reported[36] with dimethyldioxirane[64] to obtain a 2:1 mixture (*de was determined by HPLC Phenomenex Hypersil 5 μm, C18, 150* × 3.2 mm, 254 nm, 3:2 CH_3CN/H_2O, *0.5 mL/min, retention time*: *β= 7.2 min*, α = *7.9 min*) of epoxide diastereomers (3.9 mg, 76%): $[\alpha]^{20}_D$ +37° (*c* 0.10, $CHCl_3$).[7,36]

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Supporting Information Available: ^1H NMR spectra for compounds **2**, **4**−**7**, **9**, **13**, **14**, **16**, **18**−**25**, and **27**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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