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Synthesis of Cryptophycin Affinity Labels and Tubulin Labeling

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### 13. ABSTRACT (Maximum 200 Words)

Cryptophyins 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 cryptophyins 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.
# Table of Contents

- Cover ................................................................. 1
- SF 298 ............................................................... 2
- Table of Contents ................................................ 3
- Introduction ....................................................... 4
- Body ................................................................. 5
- Key Research Accomplishments ............................. 6
- Reportable Outcomes ............................................. 6
- Conclusions ......................................................... 6
- References .......................................................... 7
- Appendices ........................................................... 9
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. 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 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 for the synthesis of octadienoate ester 5, a major component of the cryptophycins. 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 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 diisopinocampherylborane (prepared from (+)-β-methoxydiisopinocampherylborane) to generate the desired stereocchemistry 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 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 (Scheme 3), 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 synths, octadienoate ester 5 and depsipeptide fragment 6 were subjected to the Yamaguchi coupling reaction 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)\textsuperscript{30} furnished a diastereomeric mixture of epoxides 3 in the ratios of $\alpha:\beta = 2:1$ and $1:2$, respectively (Scheme 4). The mixture was separated by HPLC.

The biological testing was carried out individually for the $\alpha$ & $\beta$-isomers of 3. In the tubulin assembly assay, the benzophenone analogue 3 ($\beta$) of cryptophycin-24 (IC\textsubscript{50} = 7.4 $\mu$M) was half as active as cryptophycin-1 (IC\textsubscript{50} = 3.7 $\mu$M) and twice as active as cryptophycin-24 (IC\textsubscript{50} = 15 $\mu$M) (Table 1).\textsuperscript{31,32} In the cytotoxicity studies, analogue 3 ($\beta$) 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 ($\beta$) 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 $\alpha$-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**


**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 ($\beta$) 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.

References

Appendices

Figure 1. Structure of cryptophycins.

Scheme 1
Scheme 2
Scheme 3
Scheme 4
**Table 1. Biological Results**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tubulin Assembly IC$_{50}$, μM$^a$</th>
<th>Cytotoxicity IC$_{50}$, nM$^b$</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td>MCF7</td>
</tr>
<tr>
<td>1</td>
<td>3.7</td>
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<tr>
<td>2</td>
<td>15</td>
<td>0.13</td>
</tr>
<tr>
<td>4 (β)</td>
<td>7.4</td>
<td>0.078</td>
</tr>
<tr>
<td>4 (α)</td>
<td>&gt;100</td>
<td>6.0</td>
</tr>
</tbody>
</table>

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

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Abstract—An efficient synthesis of a Cl6 side chain benzophenone analogue of cryptophycin-24 using a crotylboronation reaction and Heck coupling as key steps is described. In an in vitro tubulin assembly assay, the benzophenone analogue of the β isomer (IC50 = 7.4 μM) is twice as active as cryptophycin-24 (IC50 = 15 μM).

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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 IC50 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 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 arenarid a and later from Nostoc sp. strain GSV 224,7 is also a potent inhibitor of tubulin polymerization.8 The IC50 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

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

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Figure 1. Structures of cryptophycins.
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 photoactive functionality to study the cryptophycin binding site on tubulin.

Formal and total syntheses of the cryptophycins have been published by several groups.\(^3,4,6,8\) Also, SAR studies based on naturally occurring cryptophycins and various synthetic analogues have been reported.\(^2,9-32\) Cryptophycin 1 (1) and the structurally less complex arenastatins A (2, cryptophycin-24) are close structural analogues (Fig. 1) and have very similar properties with regard to tubulin binding.\(^3,4\) 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\(^1,3\) 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 octadenoate 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 octadenoate ester 12 using a crotlyboronation approach to set both stereocenters in a single step.\(^3,4\) 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 crotlyboronation of 8 (Scheme 2) with crotyl disopino(6)amphiyborane (prepared from (+)-B-methoxydisopinoamphiyborane) 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 z,\(\beta\)-unsaturated tert-butyl ester 12 in 95% yield. Heck coupling\(^3,7,38\) on 12 with 4-bromobenzenophenone 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 octadenoate ester 6 in 71% yield.

The second key synthon 7 was readily synthesized starting from a N-Boc protected p-tyrosine derivative.\(^30,37\) Key synthons, octadenoate 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 Hüning’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 diasteromeric mixture of epoxides 4 in the ratios of $\alpha:\beta=2:1$ and 1:2, respectively (Scheme 3).\(^32,43\) The mixture was separated by HPLC.

The biological testing was carried out individually for the z & \(\beta\)-isomers of 4. In the tubulin assembly assay, the benzophenone analogue 4 (z) of cryptophycin-24 (IC\(_{50}=7.4\) \(\mu\)M) was half as active as cryptophycin-1 (IC\(_{50}=3.7\) \(\mu\)M) and twice as active as cryptophycin-24 (IC\(_{50}=15\) \(\mu\)M) (Table 1).\(^32,43\) In the cytotoxicity studies, analogue 4 (z) had reduced activity against the MCF7
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.

Acknowledgements

We thank the National Institutes of Health (NCI) for financial support (CA 70369). The Department of the Army is acknowledged for post-doctoral fellowships from the Breast Cancer Research Program to M. E. and R. V. This work was supported in part by the Kansas Technology Enterprise Corporation through the Centers of Excellence Program.

References and Notes

42. All new compounds were characterized by 1H NMR, 13C NMR, and HRMS data.
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 (5S,6R,2E,7E)-5-[(tert-butylidimethylsilyl)oxy]-6-methyl-8-phenyl-2,7-octadienoate, a major component of the cryptophycins, are reported. The first utilized the Noyori reduction and Frater alklylation of methyl 5-benzyloxy-3-oxopentanoate to set two stereogenic centers, which became the C16 hydroxy and C1' methyl of the cryptophycins. The second approach started from 3-p-methoxybenzylpropanol and a crotyl borane reagent derived from (-)-a-pinene to set both stereocenters in a single step and provided the dephenyl analogue, tert-butyl (5S,6R,2E)-5-[(tert-butylidimethylsilyl)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 depsyoxarenastatin 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 cytotoxicity2,4 and was not an effective substrate for the P-glycoprotein efflux mechanism in multiple-drug resistant cells.5 When administered intravenously, depsipeptide 1 was also very effective against mammary, colon, and pancreatic adenocarcinomas in mouse xenografts.6 Concurrently, Kobayashi and Kitagawa isolated a related cytotoxic agent from the Oki-nawan marine sponge Dysidea arenaria and named it arenastatin A (2).6,7 It was found that cryptophycin-24 was identical to this compound. Thus far, 25 compounds of the cryptophycin family have been reported through isolation.6,7

Figure 1. Structures of the cryptophycins.

Cryptophycin-1 R = Me, R' = H, X = Cl (1)
Cryptophycin-24 (arenastatin A) R, R' = H (2)
Cryptophycin-22 R, R' = Me, X = O (3)

Cryptophycin-1 (1) blocks the cell cycle at the G2/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.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 not net 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.16 In particular, the chlorohydrin derived from cryptophycin-1 (1)

Total Synthesis of Cryptophycin-24 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. In pursuit of stable analogues, synthetic approaches were developed probing the substituents and stereochemistry of the tyrosine and β-amino acid subunits,21–23 the stereochemical and electronic effects of the octadecenoate 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 demonstrated similar or improved bioactivity. Cryptophycin-52 (3) has been shown to stabilize microtubule dynamics28 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),37–39 cryptophycin-2,44,45 and cryptophycin-52 (3),40,44 as well as the synthesis of the octadecenoate 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).

Results and Discussion

Our interest in structurally flexible fragment syntheses was primarily for structure–activity relationship (SAR) studies, biochemical studies,33 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.30,31 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-leucine 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 Fraker alkylation to provide intermediate 7. This alkylation strategy allowed potential incorporation of alternative substitution at C1' through

the use of electrophiles other than iodomethane. A second route, in which a dephenyl ester 9 was the targeted synthone, allowed the probing of the C3' aromatic region of the side chain. Ester 9 was derived from a crotyl borination 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 octadentate ester 5 targeted flexibility in the C1' position in the target compounds. The asymmetric Noyori catalytic hydrogenation49 of β-keto ester 69 with the Ru(II)(BINAP)Br2 complex50 provided (S)-hydroxy ester 1251 in 97% yield and 97% ee (enantioemic excess was determined through chiral HPLC: Daicel Chiralcel OD-H). Frater alkylation52,53 of the diamin of β-hydroxyester 12 with iodomethane gave anti product 7 in 75% yield and 97% diastereometric 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 oxidation54 was experimentally more convenient and provided a comparable overall yield. Selective cleavage of the primary TBS-ether of 16 (HOAc, H2O, THF, 1:1:2)55 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 99% yield by Horner-Emmons homologation with the corresponding phosphonate and tetramethylguanidine or DBU and LiCl56, respectively. A comparison of the optical rotation of the methyl ester thus obtained (αD0 = +66.8º), with the published data for 19 (αD0 = +68.2º)57 verified the high optical purity and correct absolute stereochemistry of our product.

This approach provided the octadentate 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 octadentate 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 borination of 11 with crotyl disopinocampherylborane 10 (prepared from (+)-B-methoxydisopinocampherylborane) 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-butylidimethylsilyl trifluoromethanesulfonate

(58) This aldehyde was conveniently prepared in multigram quantities from 1,3-propanediol through the following sequence: (a) for monoprotection see: Urbaneck, R. A.; Sabes, S. F.; Forsyth, C. J. J. Am. Chem. Soc. 1998, 120, 2523. (b) NaClO (aq), TEMPO, NaHCO3, KBr, CH3Cl2, 95%.
Total Synthesis of Cryptophycin-24

Scheme 4

(TBSOT) 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 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. The terminal olefin of compound 9 is the key moiety necessary for modification at what becomes the C3'-aromatic position. Heck coupling of the phenyl triflate 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 TBAP deprotection of silyl ethers 20 and 9 with a polymer resin workup as described by Parlow and co-workers 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 (HOsI) followed by addition of β-alanine provided acid 27. EDCI activation and subsequent addition of L-leucine acid provided acid 6 in two steps without the necessity of extensive protecting group chemistry.

With the key synths, 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 with 2,4,6-trichlorobenzyl 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 dimethylsulfoxide64 using reported conditions66 completed the total synthesis of cryptophycin-24, arenastatin A (2). Through our methodology, we also synthesized the dephenyl desoxyprenastatin 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 C5 positions of the C16 aromatic side chain were achieved. Introduction of thearyl moiety at the C9 position was necessary to late stage in the synthesis by utilizing dephenyl desoxyprenastatin 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. H and 13C NMR spectra were obtained in CDCl3 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 benzenephone ketyl. CH3OH was freshly distilled from CaH2. DCE, TEA, and 2,6-lutidine were distilled from CaH2 prior to use. 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 (3R)-5-Benzoylxy-3-hydroxypentanoate (12).

Under a N2 atmosphere, a mixture of degassed methyl 5-benzyloxy-3-pentanoate (0.5 g, 2.12 mmol) and methanol (10 mL) was placed in a Parr hydrogenation bottle. To this was added RuBINAP/B2 (prepared from bis-(2-methylthio)ethyl-diazene-1,5-diene ruthenium (II) (4 mg, 0.015 mmol) and (S)-BINAP (8 mg, 0.030 mmol). Hydrogenation was carried out at 50 psi and 50 °C for 6 h. The catalyst was precipitated with Et2O (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%).


min. The reaction mixture was again cooled to –78 °C, and a solution of iodomethane (1.73 mL, 27.8 mmol) in HMFA (9.16 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 NH4Cl and extraction with EtO, the organic layer was washed with brine, water, and dried (MgSO4). The crude product was purified by flash column chromatography (hexane:EtOAc 3:1) to give product 7 as an oil (3.40 g, 77%): 92% diastereomeric excess was determined by integration of CHOCH (3H, NH) [αD]25 +14.9° (c 1.49, CHCl3); 1H NMR (500 MHz, CDCl3) δ 7.35–7.26 (m, 9H), 4.15 (s, 2H), 3.54–3.53 (m, 1H), 3.59 (s, 3H), 3.74–3.34 (m, 2H), 3.81–3.33 (d, J = 4.7 Hz, 1H), 2.68–2.56 (m, 1H), 1.83–1.72 (m, 2H), 1.20 (d, J = 6 Hz, 3H); 13C NMR (125 MHz, CDCl3) δ 175.8, 137.8, 128.3 (2C), 127.53, 127.49 (2C), 127.2, 72.0, 68.2, 51.5, 45.4, 38.6, 16.3; IR (film) 3500, 1725 cm–1; HRMS (FAB, NBM) calculated for C12H14O3S Li (M + H) 253.0784, found 253.1446.

Methyl (2S,3S)-3,5-Bis[(tert-butyl(dimethyl)siloxy)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 (15 psi) for 3 h. After filtration, solvent was removed under reduced pressure to give pure (by H NMR) diol as an oil. The oil was dissolved in DMP (13 mL) and treated with TBSOTri (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 NH4Cl (1 mL) and partitioned between EtO and H2O. Organic extracts were washed with NaHCO3, H2O, and dried (Na2SO4). The crude product was purified by flash column chromatography (hexane/EtOAc 90:10) to give 13 as a clear oil (4.93 g, 91%).


de25 +14.1° (c 1.85, CHCl3); [α]25 +20.6 (c 1.07, CHCl3); 1H NMR (500 MHz, CDCl3) δ 7.32–7.26 (m, 9H), 4.15 (s, 2H), 3.47–3.45 (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); 13C NMR (75 MHz, CDCl3) 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, NBM) calculated for C21H23O3Si (M + H) 359.1293, found 359.1307.

Methyl (2S,3S)-5-Benzoylxy-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 (4.45 g, 15.5 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

then the reaction was gradually warmed to room temperature over 6 h. The reaction was quenched with NH₄Cl solution and extracted with EtOAc. The combined organic layers were washed with water and brine and dried (MgSO₄). Column chromatography (hexane/EtOAc 99:1) gave styrene 16 as a colorless oil (155 mg, 74%): [α]D₂₀ +23° (c 0.77, CHCl₃); [α]NMR (300 MHz, CDCl₃) δ 7.4 (m, 5H), 6.41~6.36 (d, J = 16 Hz, 1H), 5.23~5.24 (d, J = 7, 7.7 Hz, 1H), 3.84 (m, 4H, 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), 0.90 (s, 6H), 0.056 (s, 6H); [α]NMR (75 MHz, CDCl₃) δ 137.8, 132.8, 129.8, 128.6, 126.0, 72.7, 60.1, 42.7, 36.7, 25.9 (C); 13C NMR (75 MHz, CDCl₃) δ 185.1, 181.0, 75.5 (C), 45.4 (C).

For (3S,4R,6E)-3-(tert-Butyldimethylsilyloxy)-4-methyl-6-phenyl-5-hexenal-1 (17). A solution of 16 (0.122 g, 6 mmol) in 1,2,4-trichlorobenzene (2 mL) and phosphorus pentasulphide (50 mg) was stirred at room temperature for 1 h. The reaction mixture was filtered through Celite and subjected directly to flash column chromatography (hexane: EtOAc 95:5 to 90:10) which gave aldehyde 18 as an oil (51 mg, 78%).

18 was used as obtained in the next reaction.

(3S,4R,6E)-3-(tert-Butyldimethylsilyloxy)-4-methyl-6-phenyl-5-hexenal-1 (17). A solution of 16 (0.122 g, 6 mmol) in DCM (2 mL) was treated with POCl₃ (6.3 mmol, 0.5 mL) and NMO (35 mg, 0.3 mmol), and 4 A molecular sieves (50 mg) was added. 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: EtOAc 95:5 to 90:10) which gave aldehyde 18 as an oil (51 mg, 78%).

18 was used as obtained in the next reaction.

(3S,4R,6E)-3-(tert-Butyldimethylsilyloxy)-4-methyl-6-phenyl-2,7,10-octadecadienoate (10). Procedure as previously reported: [α]D₂₀ +67° (c 0.63, CHCl₃); [α]NMR (500 MHz, CDCl₃) δ 7.2~7.4 (m, 5H), 6.96 (dd, J = 15.6, 7.5 Hz, 1H), 6.37 (d, J = 16 Hz, 1H), 6.20 (d, J = 15.6, 6.1 Hz, 1H), 5.95 (m, 1H), 5.75 (sept, J = 6.7 Hz, 1H), 3.72 (m, 4H, 2H), 2.44 (m, 1H), 1.36 (m, 2H), 1.10 (d, J = 6.9 Hz, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); [α]NMR (125 MHz, CDCl₃) δ 166.5, 146.4, 137.6, 131.9, 130.4, 128.7, 125.0, 126.0, 122.9, 75.0, 50.1, 45.2, 37.6, 23.8, 18.1, 16.2, 4.4~4.5, 4.5 (im). IR (film) 3050, 2920 cm⁻¹; HRMS (FAB, NBOAc) calecd for (M + Li) C₃₅H₅₃O₂SNa: 577.3751, found 577.3753.

(3S,4R,6E)-3-(tert-Butyldimethylsilyloxy)-4-methyl-6-phenyl-2,7,10-octadecadienoate (10). A solution of 16 (0.122 g, 6 mmol) in CH₂Cl₂ (2 mL) and Na₂CO₃ (0.2 mmol) was treated with TFA (3.5 mg, 0.05 mmol) and NMO (35 mg, 0.3 mmol), and 4 A molecular sieves (50 mg) was added. 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: EtOAc 95:5 to 90:10) which gave aldehyde 18 as an oil (51 mg, 78%).

18 was used as obtained in the next reaction.

(3S,4R,6E)-3-(tert-Butyldimethylsilyloxy)-4-methyl-6-phenyl-2,7,10-octadecadienoate (10). Procedure as previously reported: [α]D₂₀ +67° (c 0.63, CHCl₃); [α]NMR (500 MHz, CDCl₃) δ 7.2~7.4 (m, 5H), 6.96 (dd, J = 15.6, 7.5 Hz, 1H), 6.37 (d, J = 16 Hz, 1H), 6.20 (d, J = 15.6, 6.1 Hz, 1H), 5.95 (m, 1H), 5.75 (sept, J = 6.7 Hz, 1H), 3.72 (m, 4H, 2H), 2.44 (m, 1H), 1.36 (m, 2H), 1.10 (d, J = 6.9 Hz, 3H), 0.91 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); [α]NMR (125 MHz, CDCl₃) δ 166.5, 146.4, 137.6, 131.9, 130.4, 128.7, 125.0, 126.0, 122.9, 75.0, 50.1, 45.2, 37.6, 23.8, 18.1, 16.2, 4.4~4.5, 4.5 (im). IR (film) 3050, 2920 cm⁻¹; HRMS (FAB, NBOAc) calecd for (M + Li) C₃₅H₅₃O₂SNa: 577.3751, found 577.3753.
The page contains a segment of a scientific text discussing the purification and analysis of compounds. The text includes chemical reactions, experimental procedures, and results. The specific content is related to the purification of CH_3CHO (formaldehyde) and the analysis of its solution in CH_3CN (acetonitrile). The text mentions the use of flash chromatography and high-performance liquid chromatography (HPLC) for the purification and analysis of the compounds. The text also includes a procedure for the preparation of a solution and the analysis of its properties. The text is structured in a logical flow, with clear descriptions of the experimental setup, conditions, and results. The page also includes references to other scientific works, indicating a comprehensive approach to the research.
Total Synthesis of Cryptophycin-24

MS (FAB, TG/G) z 481.3 (M + H); HRMS (FAB, TG/G) calculated for C24H29N6O4S + 481.2500, found 481.2573.

Tert-Butyl (5S,6R,2E)-5-(2S,3S)-2-tert-Butyloxy-carbonyl-3-(4-methoxyphenyl)propanoylamino)-4-phenoxybutyronitrile (28).

The N-Boc amino acid 6 (95 mg, 0.198 mmol) was dissolved in THF (3.8 mL), and then DIA (43 μL, 0.248 mmol), 2,4,6-trichlorobenzoyl chloride (54 μL, 0.218 mmol) and DMAP (5 mg) were added. After 1 h, saturated aqueous NaHCO3 solution was added. The mixture was extracted with CH2Cl2. The combined organics were dried (MgSO4), filtered, and concentrated. Flash chromatography (80:20 to 70:30 hexanes/EtOAc) provided the desired ester 28 as a colorless oil (85 mg, 75%). [α]D20 +24.6° (c 1.00, CHCl3); [α]H NMR (400 MHz, CDC13) δ 7.12–7.10 (d, J = 8.5 Hz, 1H), 6.80–6.78 (d, J = 8.5 Hz, 2H), 6.73 (buried, 1H), 5.61–5.77 (br d, J = 15.6 Hz, 1H), 5.60–5.63 (d, J = 8.5 Hz, 1H), 4.44–4.48 (br d, d, J = 8.5 Hz, 2H), 1.55 (s, 3H), 1.25–1.30 (c, 11H), 1.14–1.19 (br s, 3H), 0.80 (s, 6H).

Desoxyxynastatin A (4) from 29. Ester 29 (50 mg, 0.065 mmol) was dissolved in CH2Cl2 (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 acidic mixture was dissolved in CH2Cl2 (6 mL), and DIA (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 NaHCO3 solution was added, and the aqueous phase was extracted with CH2Cl2. The combined organics were dried (MgSO4) filtered, and concentrated. Column chromatography (7:1 to 4:1 CH2Cl2/acetone) provided 4 as an oil (24 mg, 65%): [α]D20 +270° (c 0.80, CHCl3); [α]H NMR (400 MHz, CDC13) δ 7.73–7.15 (m, 5H), 7.11–6.99 (d, J = 8.6 Hz, 2H), 7.05–7.02 (d, J = 5.5 Hz, 1H), 6.81–6.78 (m, J = 8.6 Hz, 2H), 6.73–6.66 (d, J = 4.7, 10.5, 15 Hz, 1H), 6.41–6.37 (d, J = 15.6 Hz, 1H), 6.03–5.95 (d, J = 8.5 Hz, 2H), 5.93–5.85 (d, J = 8.5 Hz, 2H), 5.65–5.48 (d, J = 8.5 Hz, 2H), 5.08–5.00 (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 (d, 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, 3H), 1.34 (s, 3H), 1.32 (buried, 1H), 1.19–1.08 (d, J = 6.9 Hz, 3H), 0.85–0.81 (d, J = 6.5 Hz, 3H), 0.78–0.76 (d, J = 6.5 Hz, 3H). Other data as previously reported.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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