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

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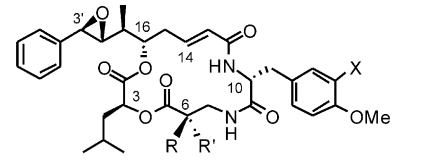
Abstract—An efficient synthesis of a C16 side chain benzophenone analogue of cryptophycin-24 using a crotylboration reaction and Heck coupling as key steps is described. In an in vitro tubulin assembly assay, the benzophenone analogue of the β isomer (IC_{50} = 7.4 μM) is twice as active as cryptophycin-24 (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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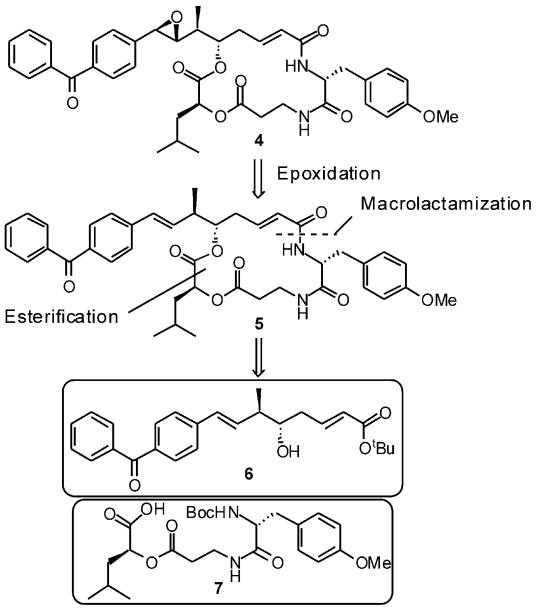
photoaffinity label for tubulin labeling studies. Because structural changes at the C16 side chain aromatic group are tolerated without loss of activity,[1,2] we targeted this position for the placement of a photoreactive functionality to study the cryptophycin binding site on tubulin.

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).

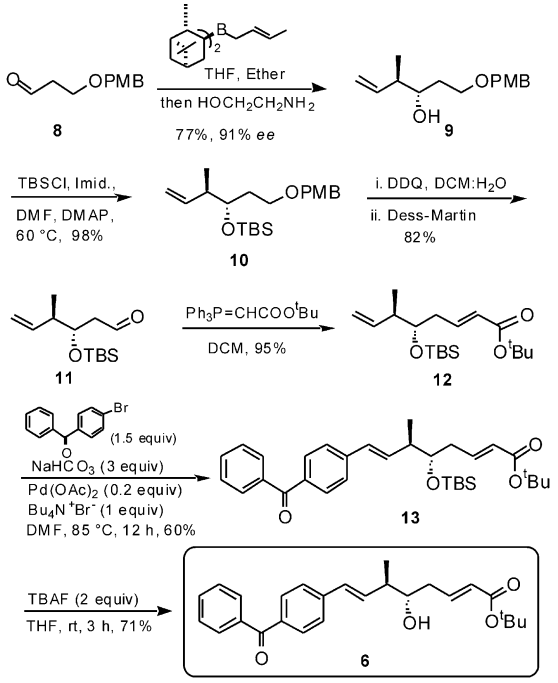
We and others have reported an efficient protocol[26,27,34,35] for the synthesis of octadienoate ester **12** using a crotylboration approach to set both stereocenters in a single step.[36] This method allows for the convergent synthesis of analogues modified at the phenyl group of the C16 side chain 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.

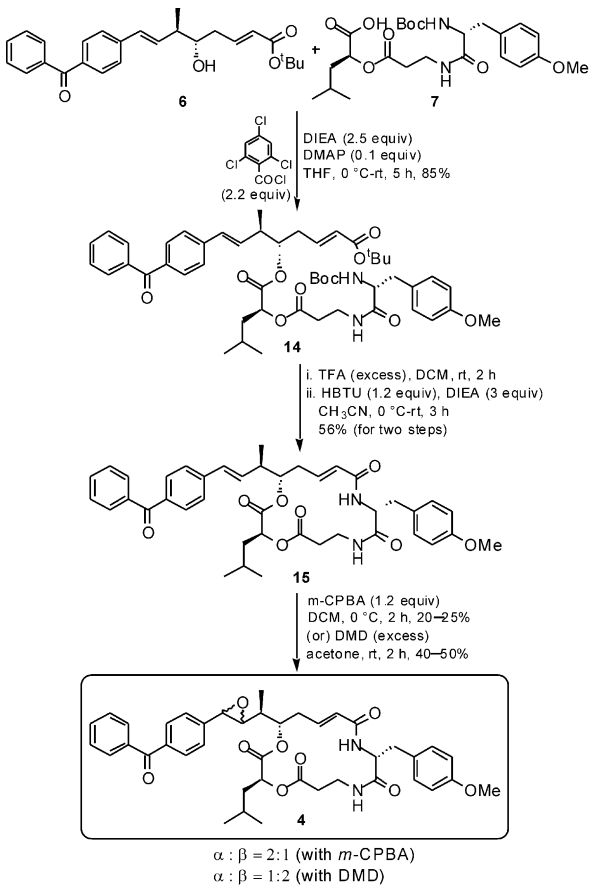
The biological testing was carried out individually for the α & β-isomers of **4**. In the tubulin assembly assay, the benzophenone analogue **4** (β) of cryptophycin-24 (IC_{50} = 7.4 μM) was half as active as cryptophycin-1 (IC_{50} = 3.7 μM) and twice as active as cryptophycin-24 (IC_{50} = 15 μM) (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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