Total Synthesis and Anti-Tubulin Activity of Epi-C3 Analogues of Cryptophycin-24

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Epi-C3-cryptophycin-24, epi-C3-*m*-chlorobenzyl-cryptophycin-24, and the corresponding styrenes were synthesized and tested in vitro against the MCF-7 and multidrug-resistant MCF-7/ADR breast cancer cell lines and in an in vitro tubulin assembly assay. The results demonstrate that the *S* configuration at the C3 stereocenter is not required to induce potent cytotoxicity and the *m*-Cl substituent present on the C10 side chain did not induce any large change in activity.

Introduction

The cryptophycins,[1] natural products isolated from the blue-green algae *Nostoc* sp. ATCC 53789 and *Nostoc* sp. GSV 224, possess antimitotic activity. Cryptophycin-1 (**1**, Figure 1)[2] exhibits potent cytotoxicity in breast cancer cell lines with IC_{50} values in the low picomolar range and maintains its potency in resistant cancer cell lines that overexpress P-glycoprotein.[3] Cryptophycin-24 (arenastatin A, **2**)[4] differs from cryptophycin-1 (**1**) in that it lacks both the chloro substituent on the C10 side chain and the methyl group at C6. Cryptophycin-24 (**2**) is also cytotoxic at picomolar concentrations.[4]

The cryptophycins have been shown to inhibit tubulin polymerization in vitro,[5−8] induce microtubule depolymerization in cells,[3] potently decrease microtubule dynamics,[9,10] and interact with tubulin to form ring structures.[11,12] The cryptophycins have also been found to activate the caspase (ICE/CED3) protein cascade[13] and induce the phosphorylation of the Bcl-2 family of proteins at picomolar concentrations.[14] Studies have revealed that the cryptophycins interact at or near the Vinca binding domain,[5−7,15] as well as at the rhizoxin/maytansine binding site.[16]

A recent review reported that all of the stereocenters of cryptophycin-1 (**1**) were required for optimal biological activity,[17] although to our knowledge, no synthesis of a C3 epimer of any cryptophycin has been reported. We are now detailing the synthesis and evaluation of C3-epi-cryptophycin-24 (**5**, Figure 2), C3-epi-*m*-chlorobenzyl-cryptophycin-24 (**6**), and the corresponding styrenes **3** and **4**. These were tested in vitro for their ability to (1) inhibit microtubule polymerization in a tubulin assembly assay and (2) induce cytotoxicity in the MCF-7 and the MCF-7/ADR breast cancer cell lines.

Chemistry

Analogues **5** and **6** were prepared by epoxidation of styrenes **3** and **4**, which were constructed from two key fragments: the "northern half" **15** and the "southern halves" **13** and **14**. The northern half **15** was obtained through asymmetric synthesis,[18] while southern halves **13** and **14** were formed by coupling easily accessed building blocks. The synthesis of the southern halves **13** and **14** began with the conversion of D-leucic acid (**7**)[19] to the corresponding benzyl ester (Scheme 1). The leucic acid benzyl ester was subsequently esterified with *N*-Boc-protected *β*-alanine to afford **8**. Intermediate **8** was deprotected using trifluoroacetic acid and then treated with DIEA, DCC, HOBT, and acid **9** or **10** to afford the protected southern half **11** or **12**. The benzyl esters were cleaved using palladium(II) hydroxide and hydrogen gas to reveal acids **13** and **14**. Acids **13** and **14** were activated using the Yamaguchi reagent, 2,4,6-trichlorobenzoyl chloride, and reacted with building

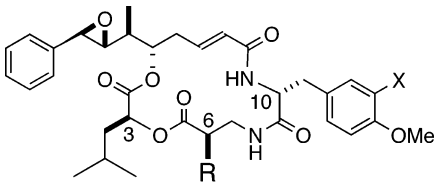

Figure 1. Structures of selected cryptophycins.

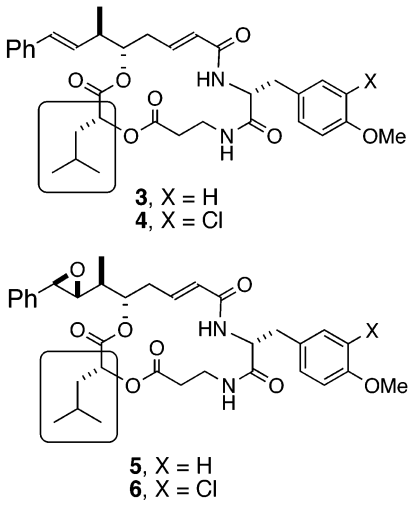

Figure 2. Structures of novel analogues.

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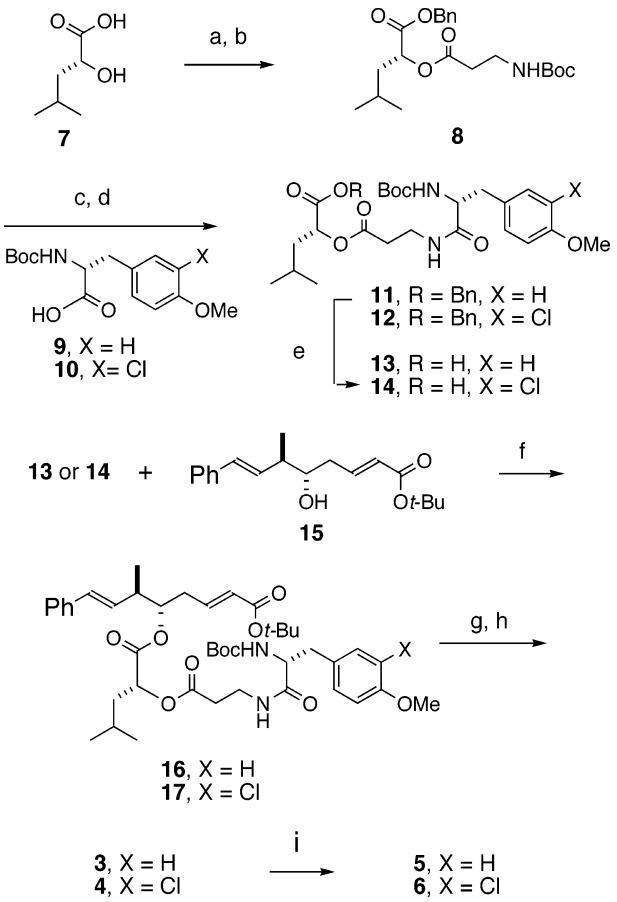
Scheme 1[a]



[a] (a) BnBr, Bu_4NI, K_2CO_3, 76%; (b) *N*-Boc-β-alanine, DCC, DMAP, 90%; (c) TFA; (d) DIEA, DCC, HOBT, **9** or **10**, 48−68%, two steps; (e) $Pd(OH)_2$, H_2, 95-98%; (f) DIEA, DMAP, 2,4,6-trichlorobenzoyl chloride, 82-85%; (g) TFA; (h) HBTU, DIEA, 60−74%, two steps; (i) DMD, 48−84%.

Table 1. Biological Results for in Vitro Tubulin Assembly Assay and Cytotoxicity Studies of C3-Epi Analogues

compd	tubulin assay IC_{50} (μM)	MCF-7 IC_{50} (nM)	MCF-7/ADR IC_{50} (nM)	resistance factor[a]
1	3.4 ± 0.80	0.009 ± 0.003	0.018 ± 0.007	2.0
2	15.8 ± 0.14	0.13 ± 0.06	0.25 ± 0.20	1.9
3	>100	>25	>25	
4	>100	>25	>25	
5	13.7	0.088	2.4	27.3
6	10.6	0.28	0.92	3.3

[a] The resistance factor is defined as the IC_{50} of the resistance cell line divided by the IC_{50} of the sensitive cell line.

block **15** to form esters **16** and **17**.[18] The *N*-Boc group and the *tert*-butyl ester were simultaneously cleaved from intermediates **16** and **17** using trifluoroacetic acid. The macrocycles were closed using HBTU (*O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) to form styrenes **3** and **4**.[18] Epoxidation using dimethyldioxirane (DMD)[20] provided mixtures of the α and β epoxides of **5** and **6**. The epoxides were separated using HPLC, and the β epoxides were tested in vitro.

Biological Testing

In the tubulin assembly assay,[5] styrene analogues **3** and **4** were inactive below 100 μM (Table 1). Epoxide analogues **5** and **6** were active in the low micromolar range with IC_{50} values of 13.7 and 10.6 μM, respectively, in the same range as cryptophycin-24 (**2**, IC_{50} = 15.8 μM). Cryptophycin-1 (**1**) was the most potent inhibitor of tubulin assembly, with an IC_{50} of 3.4 μM. Epoxide analogues **5** and **6** were active with IC_{50} values of 0.088 and 0.28 nM, respectively, in the MCF-7 breast cancer cell line compared to cryptophycin-1 (**1**, IC_{50} = 0.009 nM) and cryptophycin-24 (**2**, IC_{50} = 0.13 nM). With an IC_{50} of 0.92 nM, **6** was approximately one-third as active in the MCF-7/ADR MDR cell line as in the MCF-7 cell line. Compound **5** was 3% as active in the resistant cell line as in the MCF-7 cell line, with an IC_{50} of 2.4 nM. The activities of cryptophycin-1 (**1**) and cryptophycin-24 (**2**) were reduced by 50% in the MDR MCF-7/ADR cell line compared to the MCF-7 cell line. In vitro testing of styrenes **3** and **4** revealed that the compounds had IC_{50} values greater than 25 nM in both the MCF-7 and the MCF-7/ADR cell lines.

Conclusions

The results demonstrate that the C3 stereocenter is not required to be the *S* configuration for cryptophycin-24 (**2**) to induce cytotoxicity in the MCF-7 cell lines. Both epoxide analogues **5** and **6**, which possess the *R* configuration at C3, had activities comparable to that of cryptophycin-24 (**2**) in the MCF-7 cytotoxicity and tubulin assays. Overall, the general trend in activity of the epimeric C3 analogues tested was consistent with reports of other analogues in vitro; the styrene analogues were inactive in the tubulin assembly assay and had much reduced activity in the cytotoxicity assays, while the epoxide analogues possessed good activities.[17,21] The presence of the *m*-Cl substituent on the C10 side chain of epoxide **6** provided a slight increase in activity in the MCF-7/ADR cell line, but no general effect was noted in the MCF-7 cell line. Inversion of the C3 center did not reduce the ability of the cryptophycins to evade the P-glycoprotein drug efflux pump, which is overexpressed in the MCF-7/ADR breast cancer cell line.

Experimental Section. Syntheses

Preparation of Intermediates 3−6, 8, 11−14, 16, and 17. These compounds were synthesized in a manner similar to that described in our earlier work[22] except that D-leucic acid[19] was substituted for L-leucic acid in the synthesis.

Preparation of Epoxides 5 and 6. To a solution of styrene **3** (27.7 mg, 0.047 mmol) in acetone (2.0 mL) was added a solution of DMD[20] in acetone (2.0 mL), and the mixture was stirred at room temperature for 5 h. After the mixture was concentrated, the residue was purified using column chromatography on silica gel (40% EtOAc/hexanes). The diastereomeric mixture of epoxides was separated using HPLC (Vydac C18, internal diameter of 8 mm, eluent (isocratic), MeOH/H_2O 65:35, flow rate of 3 mL/min). The total yield of a mixture of **5** and α-**5** (β/α, 2:1), a white solid, was 13.6 mg, 48%. The synthesis and isolation of **6** was carried out in the same fashion and provided 22.8 mg (84%) of a mixture of **6** and α-**6** (β/α, 2:1) as a white solid.

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Supporting Information Available: Experimental protocols and characterization of compounds **3**−**6**, **8**, **11**−**14**, **16**, and **17**, including proton and carbon nuclear magnetic resonance spectra, and experimental protocols for the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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