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First Generation Design, Synthesis, and Evaluation of Azepine-Based Cryptophycin Analogues

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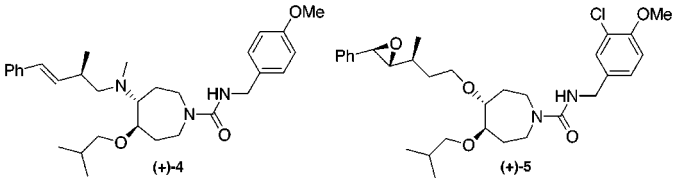
ABSTRACT



Azepine-based cryptophycin mimics (+)-4 and (+)-5 have been designed and synthesized. Biological evaluation revealed modest in vitro activity against several human tumor cell lines, thereby supporting the utility of novel scaffolds for the design and synthesis of cryptophycin analogues.

Cryptophycin-1 (**1**, Figure 1), parent member of a family of now more than 20 depsipeptides isolated from the blue-green alga (cyanobacterium) *Nostoc* sp., represents an important lead for the design of new drugs for the treatment of cancer. Initially observed to have modest antifungal activity,[1] cryptophycin-1 (**1**) was subsequently found to be an extraordinarily potent antimitotic agent, displaying strong cytotoxicity toward human tumor cells in culture, and anticancer activity against both human tumor xenographs and murine solid tumor models.[2] The cryptophycins act via inhibition of tubulin polymerization, resulting in tubulin aggregation and depolymerization to featureless linear fragments,[2,3] which in turn induce cell apoptosis and thereby the antiproliferative effect.[2c]

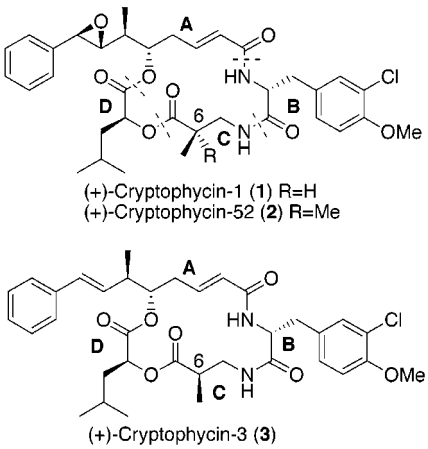


Figure 1. Cryptophycin antitumor agents.

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Given the potential of the cryptophycins as lead structures for new antitumor agents, interest in their synthesis and that of analogues has grown significantly over the past 10 years. The first reports on the total synthesis appeared in the mid-1990s from the research groups of Kitagawa[4a] and Moore and Titus.[4b] These reports were soon followed by numerous approaches comprising both formal and total syntheses.[5] Many of these studies have focused on the synthesis of (+)-cryptophycin-3 (**3**, Figure 1), the deoxy counterpart of (+)-cryptophycin-1 (**1**), displaying diminished cytotoxicity by 100-fold.[2a] Efforts to discover and develop cryptophycin analogues possessing improved pharmacokinetic properties led to (+)-cryptophycin-52 (**2**, Figure 1),[6] a semisynthetic, designed to enhance hydrolytic stability relative to (+)-**1** by incorporation of a second methyl substituent at C(6) (Figure 1). Cryptophycin-52 (**2**), currently in phase II clinical trials, is the most potent suppresser of microtubule dynamics discovered to date.[7] Moreover, in contrast to other antimitotic agents, such as Taxol, vinblastine, and vincristine, (+)-cryptophycin-52 (**2**) was shown to be minimally affected by multidrug resistance.[7a]

In view of the significant potential of the cryptophycins for cancer therapy, we initiated a synthetic program with the specific aim to develop a new class of analogues, exploiting the concept of nonpeptide peptidomimetics.[8] This design strategy entails replacement of the 16-membered macrolide ring of the cryptophycins with a suitable nonpeptide scaffold and attachment of the appropriate cryptophycin side chains with the required spatial orientation to mimic the conformation of the natural product. A premise of this strategy is that modification of the macrolide ring, which cause conformational change of side chains, significantly diminishes cytotoxicity.[2a]

At the outset of this program, little was known about the bioactive conformation of the cryptophycins bound to tubulin. We therefore took as a working hypothesis that the available X-ray structure of cryptophycin-3 (**3**, Figure 2) would comprise a reasonable representation of the solution conformation for scaffold design. Importantly, NOE studies by Moore et al. revealed that the preferred conformation of the side chains in DMSO solution appears to be nearly identical with that observed in the crystal structure.[2a] Extensive computer modeling studies suggested that a seven-membered ring could serve as a viable macrolide ring surrogate. The efficacy of the seven-membered ring in a variety of drugs

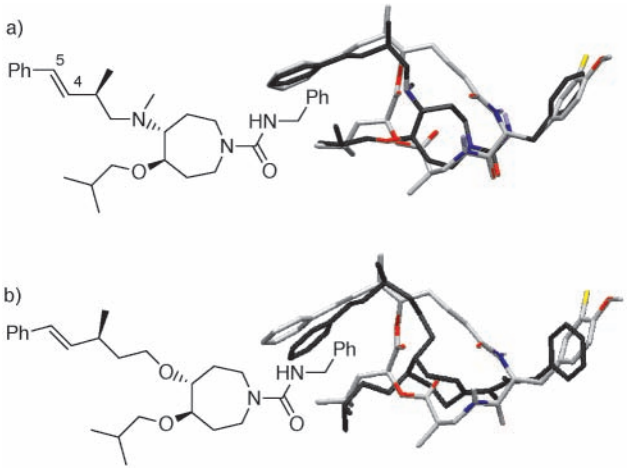

Figure 2. (a) Overlay of X-ray structure of cryptophycin-3 (**3**) and a simplified model of **4** and (b) overlay of X-ray structure of cryptophycin-3 (**3**) and a simplified model of **5**.

(e.g., benzodiazapenes) is believed to be related to the modest degree of flexibility that permits receptor-induced fit. Also important from the design perspective, the overall size of the seven-membered ring is smaller than that of the cryptophycin macrocycle (Figure 2). Thus, potential cryptophycin mimics having the putative biologically important substituents will not significantly occupy a volume larger than that of the cryptophycin and thereby avoid deleterious steric interactions during receptor binding.

Reasoning that an aryl substituent attached to the azepine ring nitrogen would nicely overlay the substituted tyrosine moiety in unit B (Figure 2a), and to alleviate the problem of possible hydrophobic collapse induced by van der Waals interactions between the two phenyl groups (as seen in our initial modeling studies), we incorporated a urea functionality in the side chain. An additional advantage of the urea moiety is the potential to mimic the corresponding carbonyl and NH groups in the BC peptide linkage of the cryptophycins. Upon incorporation of the remaining cryptophycin side chains on the azepine scaffold, the Monte Carlo conformational analysis revealed that the model structures (Figure 2) reproduced well the geometry of the side chains of cryptophycin-3 (**3**) at comparatively small energy costs.[9] As further illustrated in Figure 2a and 2b, we selected two different linkages for the side chain of unit A. A tertiary amine permitted excellent overlap with cryptophycin-3 (**3**), when unit A possessed an olefin (Figure 2a). Unfortunately, incorporation of a similar side chain possessing the β-epoxide (4*R*,5*R*) led to decomposition during the synthesis. On the other hand, use of an ether linkage (Figure 2b) did enable construction of the epoxide. However, to obtain best congruency in the side chain conformation, one additional methylene moiety was required. With these structural constraints in mind, we turned to the synthesis of **4** and **5** from epoxide **6** as outlined retrosynthetically in Scheme 1.

(3) (a) Mooberry, S. L.; Busquets, L.; Tien, G. *Int. J. Cancer* **1997**, 73, 440 and references therein. (b) Bai, R.; Durso, N. A.; Sackett, D. L.; Hamel, E. *Biochemistry* **1999**, *38*, 14302.

(4) (a) Kobayashi, M.; Kurosu, M.; Wang, W.; Kitagawa, I. *Chem. Pharm. Bull*. **1994**, *42*, 2394. (b) Barrow, R. A.; Hemscheidt, T.; Liang, J.; Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc*. **1995**, *117*, 2479.

(5) Eggen, M.; Nair, S. K.; Georg, G. I. *Org. Lett*. **2001**, *3*, 1813 and references therein.

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(7) (a) Wagner, M. M.; Paul, D. C.; Shih, C.; Jordan, M. A.; Wilson, L.; Williams, D. C. *Cancer Chemother. Pharmacol.* **1999**, *43*, 115 and references therein. (b) Panda, D.; Ananthnarayan, V.; Larson, G.; Shih, C.; Jordan, M. A.; Wilson, L. *Biochemistry* **2000**, *39*, 14121 (c) Kessel, D.; Luo, Y. *Cancer Lett.* **2000**, *151*, 25.

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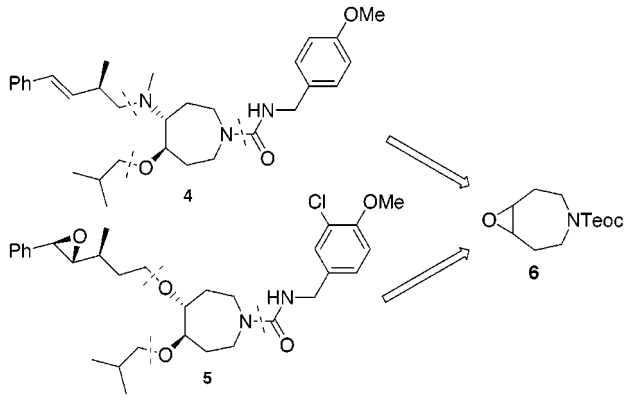
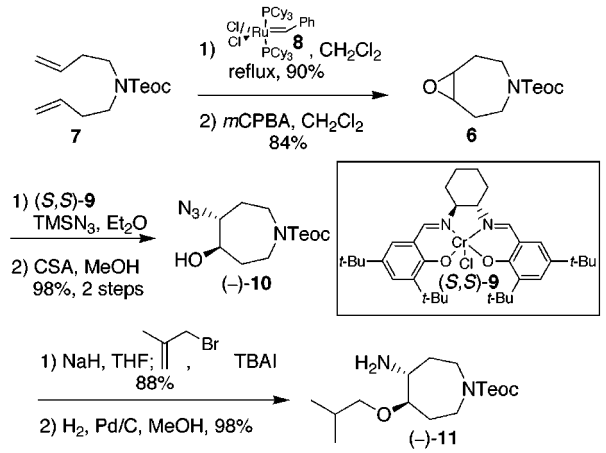
(9) Specific details of these studies are outlined in Supporting Information.

Scheme 1



The synthesis of (+)-**4** began with the olefin metathesis substrate **7**, which was obtained by reaction of known *N*-benzyl-*N*,*N*-bishomoallylamine with 2-(trimethyl)ethoxycarbonyl chloride [TeocCl] (Scheme 2).[10] Ring-closing

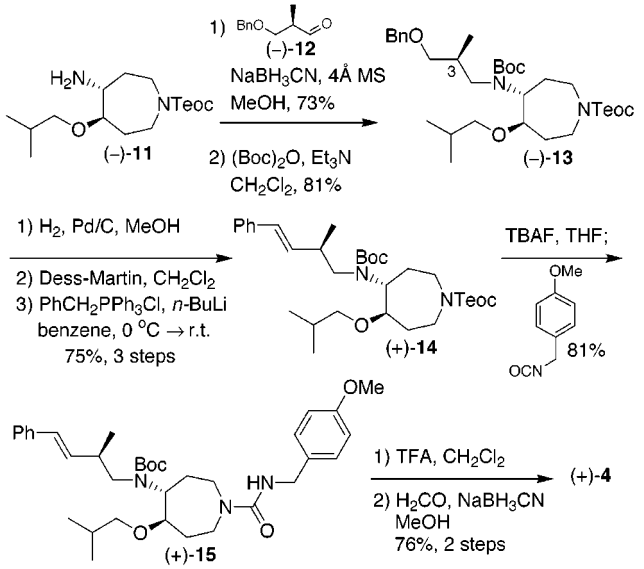
Scheme 2



metathesis with the Grubbs catalyst (**8**),[11] followed by oxidation of the olefin led to meso epoxide **6** in 76% yield (two steps). To introduce functionalities on the azepine ring, epoxide **6** was treated with trimethylsilyl azide in the presence of the Jacobsen Cr(salen) catalyst (*S*,*S*)-**9**; reaction of the resultant azido silyl ether with CSA then furnished hydroxy azide (−)-**10** in 98% yield with 87% ee (two steps).[12a−c] Subsequent alkylation of the hydroxyl followed by simultaneous reduction of the azide and alkene provided amine (−)-**11**, setting the stage for side chain incorporation.

Reductive amination[13] of aldehyde (−)-**12**[14] with amine (−)-**11** (Scheme 3) afforded the corresponding secondary

Scheme 3



amine, which was protected as the *tert*-butyl carbamate to give (−)-**13** as the major product [8:1 at C(3)] in an overall yield of 59% for the two steps. Protection of the amine with the bulky *t*-Boc group enabled HPLC purification later in the synthesis (vide infra). Hydrogenolysis of the benzyl ether followed in turn by Dess−Martin oxidation[15] and Wittig condensation[16] led to (+)-**14** in 75% (three steps; 10:1 *trans*/*cis*). The Teoc group was next removed with TBAF,[17] and the resultant secondary amine was coupled with commercially available 4-methoxybenzyl isocyanate to furnish (+)-**15**.[18] Purification by reverse-phase HPLC gave (+)-**15** (≥98% purity) as the *trans* isomer. Completion of the synthesis was achieved via removal of the *t*-Boc group (TFA) and reductive N-methylation (HCHO, $NaBH_4$).[13,19]

The synthesis of (+)-**5** began via epoxide opening with *i*-butanol, again in the presence of the Jacobsen Cr(salen) catalyst (*S*,*S*)-**9** (Scheme 4).[12d] Although this operation proceeded with only modest enantioselectivity (25% ee), alkylation of the resulting alcohol (−)-**16** with (−)-**17**[20]

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(16) for a review, see: Maryanoff, B. E.; Reitz, A. B. *Chem Rev*. **1989**, *89*, 863.

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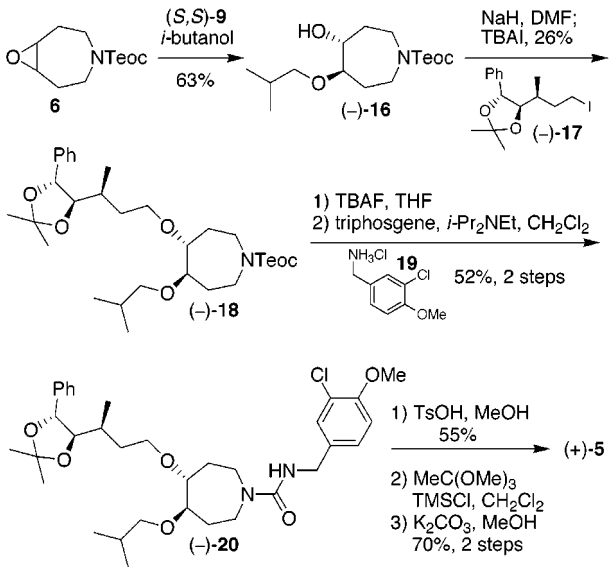
(19) To permit epoxidation of the olefin in the presence of a tertiary amine of compound (+)-**4**, the tertiary amine was complexed with $BF_3{\cdot}OEt_2$ prior to epoxidation [Ferrer, M.; Sánchez-Baeza, F.; Messeguer, A.; Diez, A.; Rubiralta, M. *J. Chem. Soc., Chem. Commun*. **1995**, 293]. After purification of the mixture of epoxides (1:1) by preperative TLC, only the α-epoxide was recovered, in 20% yield.

Scheme 4



furnished (−)-**18** in 26% yield as the major diastereomer [20:1].[21] We speculate that a kinetic resolution worked in our favor. Removal of the Teoc group with TBAF[17] and reaction with 3-chloro-4-methoxybenzyl isocyanate generated in situ from **19**[22] then provided (−)-**20**.[23] Final elaboration of (+)-**5** was achieved via hydrolysis of the acetonide, followed by a one-pot conversion of the 1,2-diol to the epoxide.[24]

Cryptophycin analogues (+)-**4** and (+)-**5** were tested for cytotoxicity in vitro against six human cancer cell lines. Mimic (+)-**4** displayed modest activity against four human tumor cell lines (Table 1), suggesting that the side chains indeed occupied reasonable spatial dispositions to achieve activity. In retrospect, however, we suspect that the low potency of (+)-**4** results from facile protonation of the tertiary amine, which in turn would significantly change the orientation of the side chain, as well as the ability of (+)-**4** to cross the cellular membrane. Ironically, compound (+)-**5**, which was expected to be more active as a result of the presence of the β-epoxide in unit A and the chloride in unit B,[2a] also displayed poor levels of activity, presumably because of the deleterious effects of possible conformational changes in the A side chain compared to compound (+)-**4**. The poor activity of our first generation mimics may also result from *increased* conformational mobility inherent to the long side chains required to position the requisite substituents, and/or to the lower rigidity of the azepine core compared to the cryptophycins.

Table 1. Biological Evaluation of Cryptophycin Mimics **4** and **5**[7a,25]

		GI_{50} (μg/mL)	
cell type	cell line	**4**	**5**
pancreas-a	BXPC-3	4.8	3.2
breast adn	MCF-7	>10	5.4
CNS gliobl	SF268	6.8	>10
lung-NSC	NCI−H460	3.7	>10
colon	KM20L2	2.1	7.8
prostate	DU-145	>10	>10

In summary, we have designed and synthesized first generation azepine-based cryptophycin analogues. Although only modest activities were observed, these results suggest that a new series of cryptophycin analogues with potential for improved cytotoxicity and pharmacokinetic properties may be available by attaching cryptophycin side chains to appropriately designed scaffolds.

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Supporting Information Available: Spectroscopic and analytical data for all intermediates and selected experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Preparation of (−)-**17** is outlined in Supporting Information.
(21) The major side product (60%) was an elimination product of (−)-**17**; the recovered starting material **16** proved to be a racemic mixture.
(22) Horning, E. C. *Organic Syntheses*; Wiley: New York, 1943; Collect. Vol. III, p 267.
(23) Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937.
(24) Kolb, H. C.; Sharpless, K. B. *Tetrahedron* **1992**, *48*, 10515.
(25) For comparison, cryptophycin-52 (**2**) displays an IC_{50} value of 0.037 nM (0.000025 μg/mL) against the MCF-7 mammary carcinoma (see ref 7a).