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SYNTHESIS AND BIOLOGICAL EVALUATION OF CRYPTOPHYCIN ANALOGS WITH SUBSTITUTION AT C-6 (FRAGMENT C REGION)

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Abstract: Analogs of the antitumor agents cryptophycins 1 and 8 with dialkyl substitution at C-6 (fragment C) were synthesized and evaluated for in vitro cytotoxicity against human leukemia cells (CCRF-CEM). The activity of these analogs decreased as the size of the substituents at C-6 increased. The C-6 spirocylopropyl compound (**2g**) was highly potent in vitro and showed excellent antitumor activity in animal models.

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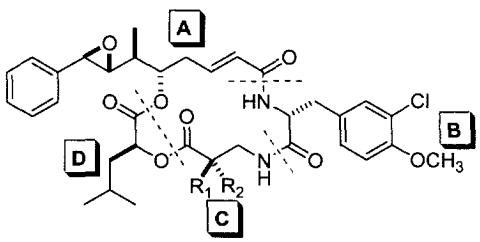
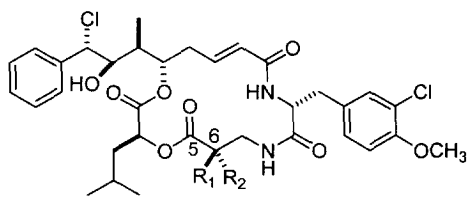
The depsipeptide cryptophycins derived from terrestrial blue-green algae exhibit high activity against a broad spectrum of solid tumors. The major cytotoxic component of the algal extracts, cryptophycin 1 (**1a**), was first isolated from a *Nostoc* cyanobacterium by Schwartz and coworkers in 1990.[1] Moore and coworkers later isolated several cryptophycins, including **1a**, from *Nostoc* sp GSV 224.[2] Following the report of the total synthesis of cryptophycin 1 by Tius et al.,[3] several syntheses of cryptophycins were reported.[4] A number of synthetic cryptophycins with promising antitumor activity, including the C-6 dimethyl substituted cryptophycins 52 (**1b**) and 55 (**2b**),[5] have subsequently been prepared.



1a: R_1 = Me, R_2 = H, Cryptophycin 1
1b: R_1, R_2 = Me, Cryptophycin 52



2a: R_1 = Me, R_2 = H, Cryptophycin 8
2b: R_1, R_2 = Me, Cryptophycin 55

Previous reports have shown that acyclic analogs of the cryptophycins (e.g., cryptophycin 5, **3**) in which the C-5 ester bond has been cleaved, are 3–4 orders of magnitude less active against human tumor cell lines in vitro.[6] We hypothesized that hydrolysis of this ester bond may be one process that would decrease the cytotoxicity of cryptophycins. If so, increased steric bulk at C-6 (geminal disubstitution, for example) may result in compounds with improved stability and antitumor activity. This report describes the preparation and

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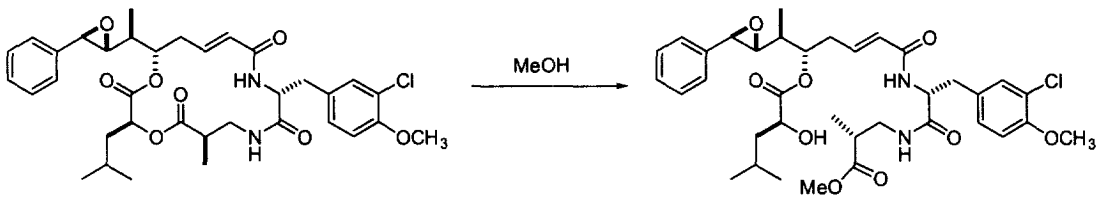
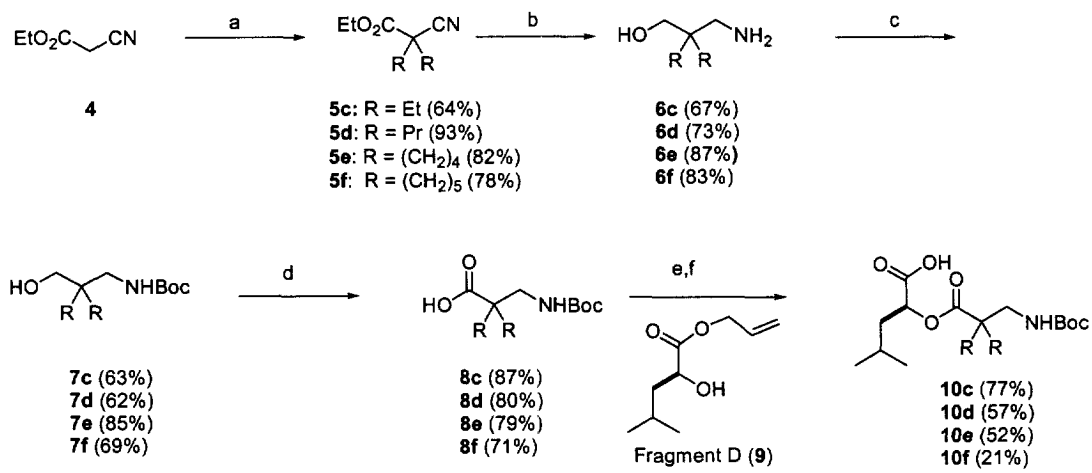
biological evaluation of eleven novel cryptophycin epoxide (**1**) and chlorohydrin (**2**) analogs having dialkyl substituents at C-6.



1a, Cryptophycin 1 **3**, Cryptophycin 5

Retrosynthetic analysis of the cryptophycins provides four fragments (A–D), which can be independently synthesized and coupled to form the macrocycle. Fragments A–B (**12**) and D (**9**) were prepared as previously described by Tius.[3] The new fragment C–D derivatives (**10**) were prepared as shown in Schemes 1 and 2. Ethyl cyanoacetate (**4**) was treated with Cs_2CO_3 and the appropriate alkyl halide in DMF to provide the dialkyl nitriles **5c–f**. The ester and nitrile groups were reduced with $LiAlH_4$ to give amino alcohols **6c–f**. Protection of the primary amine as the *t*-butyloxycarbonyl (Boc) derivative and oxidation of the primary alcohol with catalytic ruthenium tetroxide[7] yielded the desired fragment C acids **8c–f**.

Scheme 1. Preparation of Fragment C–D Analogs **10c–f**.



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5c: R = Et (64%)
5d: R = Pr (93%)
5e: R = $(CH_2)_4$ (82%)
5f: R = $(CH_2)_5$ (78%)

6c (67%)
6d (73%)
6e (87%)
6f (83%)

7c (63%)
7d (62%)
7e (85%)
7f (69%)

8c (87%)
8d (80%)
8e (79%)
8f (71%)

Fragment D (**9**)

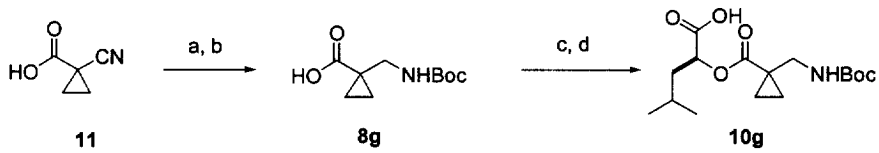
10c (77%)
10d (57%)
10e (52%)
10f (21%)

Reagents. (a) Cs_2CO_3/DMF/alkyl halide (EtI, PrI, $Br(CH_2)_3Br$, $Br(CH_2)_4Br$)/0-25 °C. (b) $LiAlH_4$/THF. (c) Boc_2O/NaOH/THF. (d) cat. $RuCl_3/NaIO_4/CH_3CN/CCl_4/H_2O$. (e) i. CDI; ii. **9**/THF or $PhCH_3$/reflux. (f) 0.2 mol% Pd $(Ph_3P)_4$/morpholine/THF.

Acids **8** were reacted with 1,1-carbonyldiimidazole (CDI) and treated with the allyl ester of (*S*)-leucic acid (Fragment D, **9**) in refluxing THF or toluene to give the fragment C–D esters.[8] The allyl protecting group was

removed with catalytic $Pd(PPh_3)_4$ in the presence of morpholine.[9] The cyclopropyl substituted fragment C–D analog **10g** was prepared from commercially available 2-cyclopropyl cyanoacetic acid (**11**, Scheme 2).

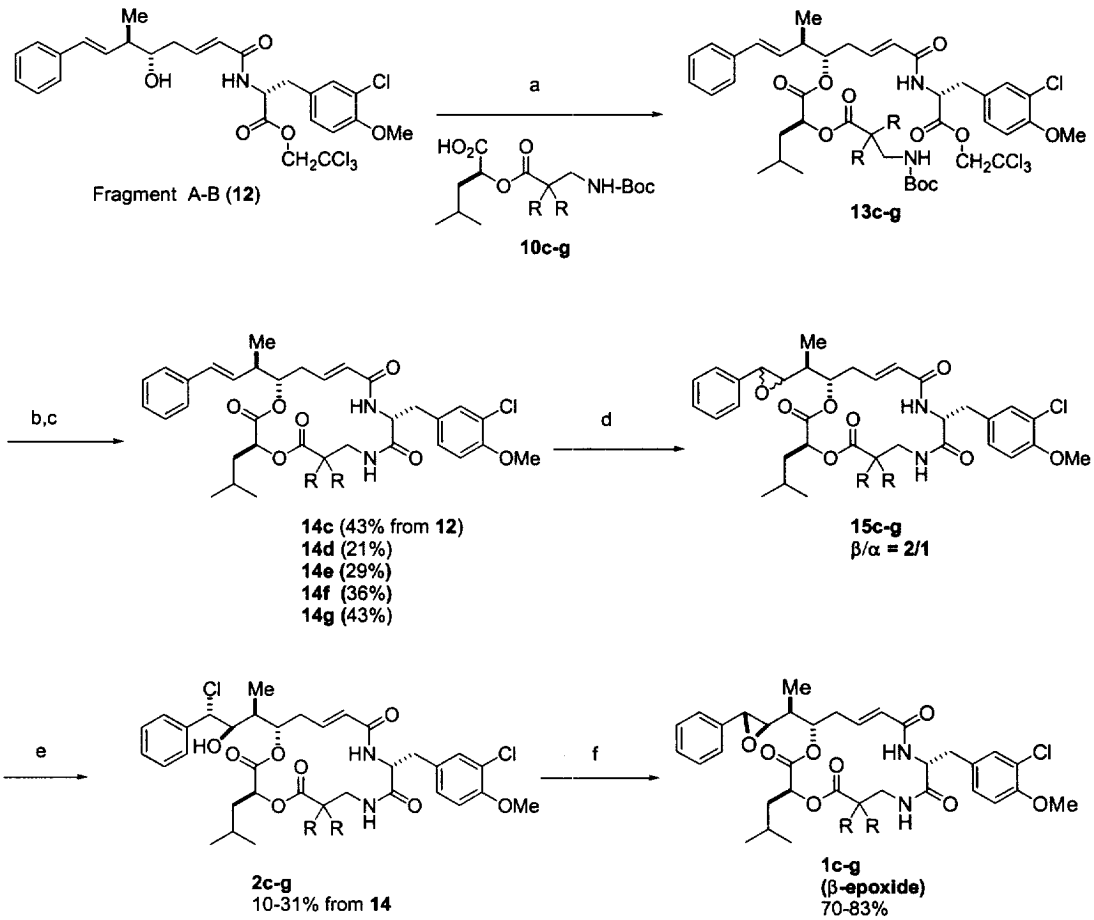
Scheme 2. Preparation Cyclopropyl Fragment C–D Acid **10g**.



11 **8g** **10g**

Reagents. (a) H_2/Pt_2O/HOAc (86%). (b) Boc_2O/NaOH/dioxane (93%). (c) DCC/**9**/CH_2Cl_2 (70%). (d) cat. $Pd(PPh_3)_4$/morpholine/THF (95%).

Scheme 3. Preparation of Cryptophycin Analogs **1** and **2**.



Reagents. (a) DCC/DMAP/CH_2Cl_2. (b) TFA. (c) 1 equiv 2-hydroxypyridine/$PhCH_3$. d) *m*-CPBA/$CHCl_3$/rt. (e) i. TMSCl/$CHCl_3$/-60 °C; ii. MeOH; iii. chromatography. (f) K_2CO_3/MeCN/H_2O.

Fragment C–D acids **10** were then coupled to fragment A–B (**12**) using 1,3-dicyclohexylcarbodiimide (DCC) and DMAP (Scheme 3). Removal of the Boc group with TFA followed by macrolactamization promoted by 2-hydroxypyridine[10] gave the cryptophycin macrocycles **14c–g**. The styrene double bond was epoxidized with *m*-CPBA to provide a 2:1 β:α mixture of diastereomeric epoxides **15**, which were not separated. The epoxide mixtures were converted to the chlorohydrins with trimethylsilyl chloride (TMSCl) at -60 °C and the desired chlorohydrins **2c–g** were isolated by silica gel or reverse phase chromatography in 10–31% overall yield from **14**.[11] Pure β-epoxides **1c–g** were obtained from the chlorohydrins by reaction with K_2CO_3 in acetonitrile/water.

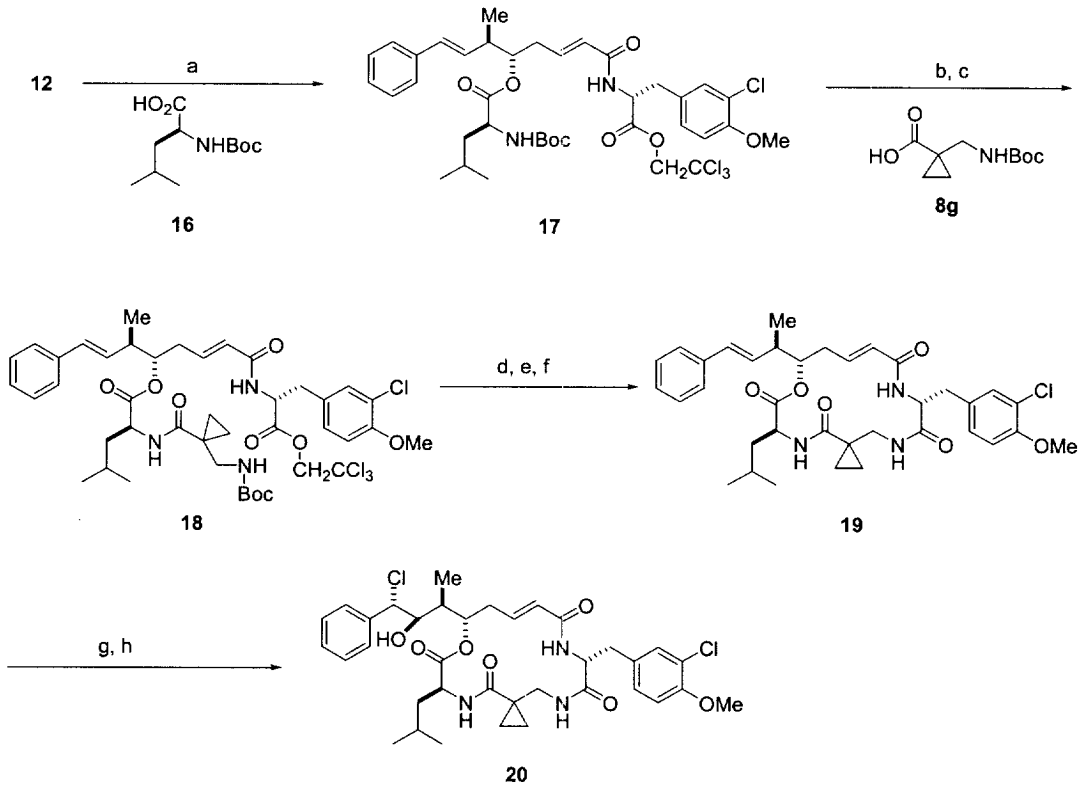
Compounds **1** and **2** were tested for in vitro cytotoxicity against the CCRF-CEM human leukemia cell line.[12] As can be seen in Table 1, as the size of the alkyl substituents at C-6 increases, the IC_{50} value markedly decreases for both the epoxide and chlorohydrin compounds. In the epoxide series, the spirocyclopropyl compound (**1g**), which is tenfold less active than cryptophycin 52 (**1b**), is the most active of the new analogs. In the chlorohydrin series, the cyclopropyl analog **2g** is approximately three times more active than cryptophycin 55 (**2b**); all other analogs are at least tenfold less active than cryptophycin 55.

Table 1. Cytotoxicity of Cryptophycin Fragment C Analogs **1** and **2** (CCRF-CEM Cell Line).

Compound	R	IC_{50} (nM) 1 (epoxide)	IC_{50} (nM) 2 (chlorohydrin)
b	Me	0.02	0.05
c	Et	1.1	1.6
d	Pr	8.5	8.7
e	$(CH_2)_4$	0.3	0.4
f	$(CH_2)_5$	63	110
g	$(CH_2)_2$	0.16	0.014

Seeking to improve the hydrolytic stability further, the spirocyclopropyl amide derivative (**20**) was prepared using *N*-Boc L-leucine (**16**) as the fragment D building block (Scheme 4). Thus fragment A–B (**12**) was coupled with **16** using DCC to provide ester **17** in 80% yield. The Boc group of **17** was removed with TFA, and the resulting amine was reacted with acid **8g** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBT) to give compound **18** in 77% yield. The macrolactam **19** was formed by sequential removal of the trichloroethyl ester (Zn/HOAc) and Boc protecting groups of **18** followed by ring closure using pentafluorophenyl diphenylphosphinate (FDPP) as described by Tius.[3] Epoxidation of **19** with *m*-CPBA gave a 2:1 mixture of β:α epoxides which were converted directly to the chlorohydrins with TMSCl. The desired chlorohydrin diastereomer (**20**) was isolated by chromatography on silica gel.

Scheme 4. Preparation of Cyclopropyl Amide **20**.



Reagents. (a) **16**, DCC/DMAP/CH_2Cl_2 (80%). (b) TFA. (c) EDCI/HOBt/**8g** (77%). (d) Zn/HOAc. (e) TFA (81%). (f) Et_2Ni-Pr/FDPP/DMF (82%). (g) *m*-CPBA/CH_2Cl_2 (90%). (h) i. TMSCl/$CHCl_3$/-60 °C; ii. chromatography (35%).

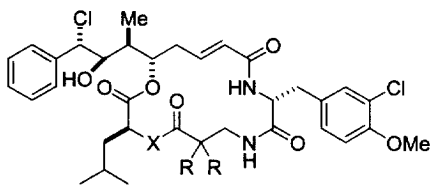
Compound **20** was tested for cytotoxicity against the CCRF-CEM cell line. The results (Table 2) indicate that replacing the fragment C–D ester with an amide has minimal effect on the cytotoxicity. The IC_{50} value for amide **20** is nearly identical to that for the ester **2g** and the previously reported 6,6-dimethyl amide **21**.[13]

Compounds **2d**, **2g**, and **20** were evaluated for in vivo antitumor activity (murine pancreatic adenocarcinoma PO3 model).[6] As might be expected, the C-6 dipropyl analog (**2d**) was essentially inactive at all doses tested (T/C >90% vs. T/C <5% for **2b**). This result is consistent with the cell-based cytotoxicity assay results. However, the C-6 cyclopropyl compounds **2g** and **20**, which were highly potent in vitro, showed excellent anti–tumor activity in vivo (T/C of 3–7% and 36%, respectively). It is interesting to note that the presumably more stable amide **20** is slightly less active than the corresponding ester analog **2g**.

These studies show that shielding substituents larger than methyl or cyclopropyl at C-6 markedly decrease the biological activity of cryptophycin analogs. While stability of the C-5 ester bond in cryptophycins may be essential for useful antitumor activity, small changes in the steric (and possibly

conformational) features of the fragment C region of the molecule significantly affect the biological activity of these compounds as well.

Table 2. Comparison of Cytotoxicity of Ester and Amide Cryptophycin Analogs.



Compound	X	R	IC_{50} (nM)
2b	O	Me	0.05
2g	O	$(CH_2)_2$	0.014
20	NH	$(CH_2)_2$	0.010
21[13]	NH	Me	0.016

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