Novel Cryptophycin Antitumor Agents: Synthesis and Cytotoxicity of Fragment "B" Analogues

Vinod F. Patel,*,† Sherri L. Andis,§ Joseph H. Kennedy,‡ James E. Ray,† and Richard M. Schultz§

Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285

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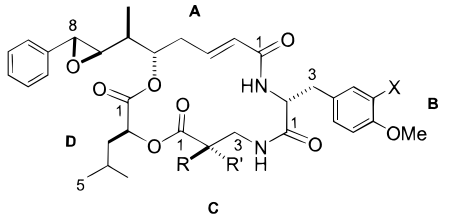
A general synthetic approach to novel cryptophycin analogues **6** is described. *N*-Hydroxysuccinimide active ester **15**, a key common intermediate, was converted to *β*-epoxide **6** in three steps, via initial coupling with unprotected amino acid **9**, followed by deprotection/macrolactamization of acyclic precursor **16**, and final oxidation of styrene **7** to install the C7−C8 *β*-epoxide. Cryptophycin styrenes **7** and *β*-epoxides **6**, bearing diverse side chains in fragment "B", were evaluated for cytotoxic activity. *β*-Epoxides **6**, in general, were significantly more potent than the corresponding α-epoxides **17** and styrenes **7**. A benzyl side chain was required for potent activity, with *β*-epoxide **6u**, possessing a 3-Cl,4-(dimethylamino)benzyl moiety, as the most potent cytotoxic agent prepared, with an IC_{50} = 54 pM, only 2-fold less than that of Cryptophycin-52 (**3**).

Introduction

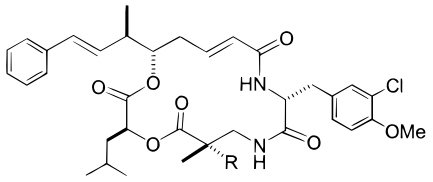
The first cryptophycin, Cryptophycin A (Chart 1, **1**), was isolated from terrestrial *Nostoc* sp. ATCC 53789 by Schwartz[1] at Merck and found to be a potent antifungal agent. Subsequently, in 1994, Moore and colleagues[2] discovered the potent and selective antitumor properties of the cryptophycin class of agents which revived interest in these novel depsipeptides. In addition to **1**, designated Cryptophycin A by Moore, several other cytotoxic analogues were isolated from blue-green algae (cyanobacteria) belonging to Nostocaceae.[1,3] Subsequently, studies were conducted to determine structure, chemical stability, and antitumor activity of these new analogues and provided a structure−activity relationship (SAR).[3] Cryptophycin A (**1**) displayed highly potent in vitro cytotoxicity in several tumor cell lines (IC_{50} = 9−20 pM); however, analogues lacking the epoxide in unit A (Chart 1),[4] the chlorine or the *O*-methyl group in unit B, and the intact macrolide ring showed diminished cytotoxicity which was consistent with marginal to negative activities in vivo. Information on the molecular target of **1** was first provided by Smith[5] who revealed that it disrupted microtubule structure. In further studies the binding site of **1** was identified to be the same as or overlapping with that of the Vinca alkaloids.[6] Cellular studies, however, comparing clinically used antimicrotubule agents, Paclitaxel, Vinblastine, and Colchicine, with **1** demonstrated that the cryptophycins were poor substrates for the drug efflux pump P-glycoprotein, a novel and potentially important property.[5]

Also in search of bioactive substances, Kobayashi and Kitagawa[7] isolated a related depsipeptide, Arenastatin A (Chart 1, **2**), from an Okinawan marine sponge, also exhibiting potent in vitro cytotoxicity against KB cells (IC_{50} = 5 pg/mL). In vivo, however, **2** showed nil to marginal antitumor activity which was not surprising based on the reported SAR for cryptophycin **1**.[8]

Chart 1



1: R = Me; R' = H; X = Cl **(Cryptophycin A)**
2: R = R' = X = H **(Arenastatin A)**
3: R = R' = Me; X = Cl **(Cryptophycin-52; LY355703)**



4: R = H **(Cryptophycin C)**
5: R = Me **(Cryptophycin-51)**

A synthetic analogue, Cryptophycin-52 (**3**) (Chart 1), was designed to enhance the hydrolytic stability of the C−D ester linkage in **1**.[3] In addition to meeting this criterion, the introduction of a methyl group at C2 in unit C of **1** also eliminated an asymmetric center. Cryptophycin-52 (**3**) is an exceptionally potent, broad-spectrum antitumor agent,[9,10] which retains activity against cell lines resistant to clinically used oncolytics.[10,11] It displayed impressive tumor growth inhibition of "wild-type"[12] and, more importantly, "resistant"[13,14] human tumor xenografts.[13] Recently, detailed investigations were performed to elucidate the effects of **3** on microtubule organization and mass. Interestingly, in addition to mitotic inhibition similar to that observed with other antimitotic agents, it exhibited some unique

* To whom correspondence should be addressed at: Kinetix Pharmaceuticals Inc., 200 Boston Ave, Suite 4700, Medford, MA 02155. E-mail: patel@kinetixpharm.com.
† Discovery Chemistry Research Division.
‡ Chemical Process Research and Development Division.
§ Cancer Research Division.

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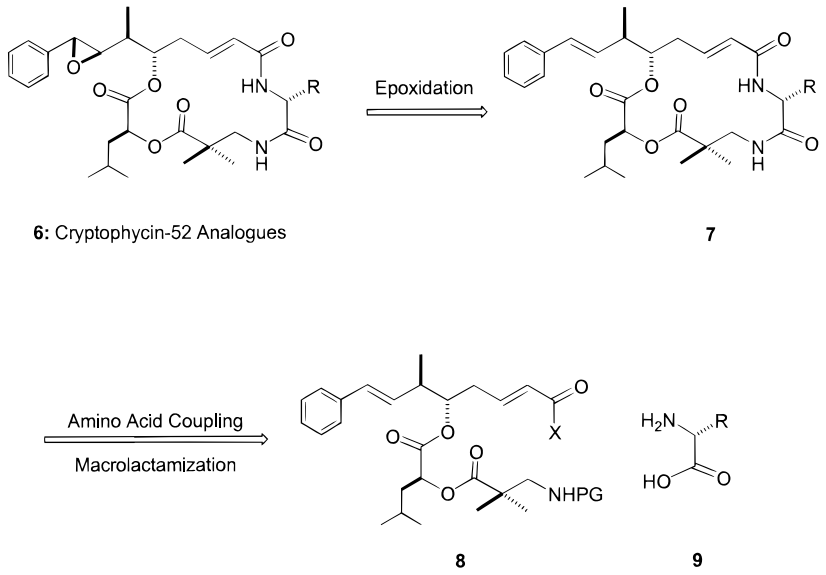
Scheme 1. Retrosynthetic Analysis



characteristics including the formation of microtubule bundles in cells with reduced microtubule polymer mass and the induction of asters particularly in prophase cells.[14] Encouraging preclinical data, including its potency and unique mechanistic profile, has supported the advancement of **3** (LY355703) to clinical trials for treatment of human cancers.

The formidable challenges associated with the synthesis of 16-membered cyclic depsipeptide **1** bearing multiple stereogenic centers were elegantly solved first by Tius and Moore,[15] who employed a disconnection at the amide and ester linkages to give units A−D. Synthesis and assembly of each unit, followed by macrolactamization to form the B−C amide as the final bond, led to the cryptophycins. Subsequently, several formal[16−18] and total[19−24] syntheses of members in this class of agents have appeared in the literature. In a collaborative effort,[25] a program at Lilly was initiated to expand the initial SAR[3] by preparing analogues with structural variations in fragments A,[26] B, C,[27] and D.[28] In this report, we describe the synthesis and cytotoxic activity of novel fragment "B" analogues **6** (Scheme 1).

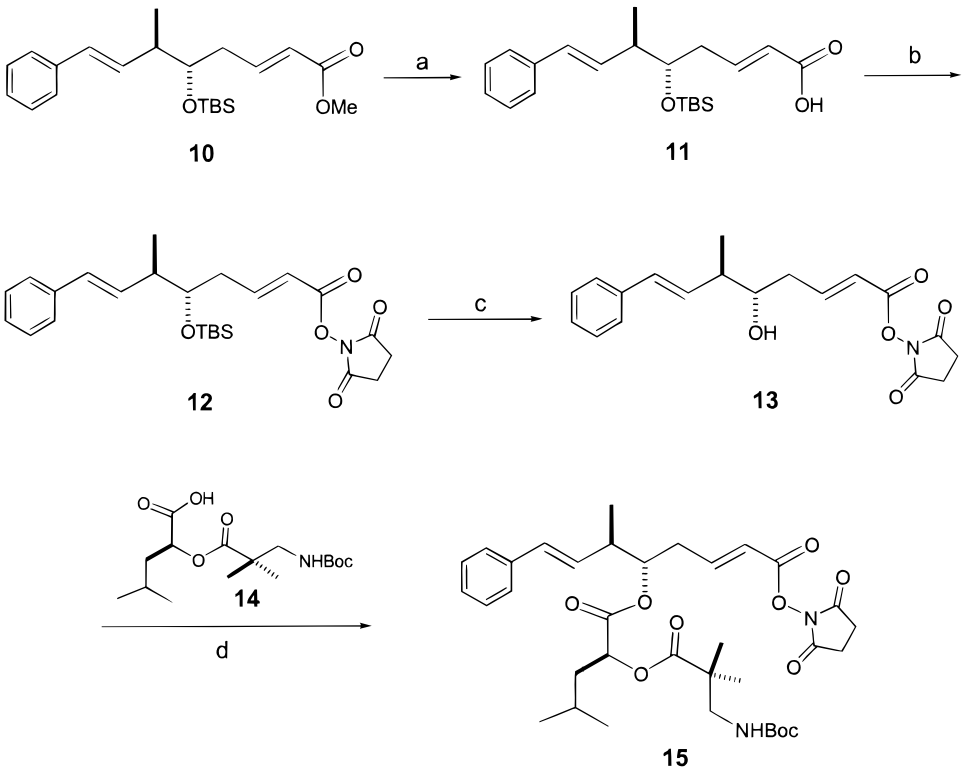
Chemistry

Our general approach to the synthesis of cryptophycin fragment "B" analogues **6** is described by the retrosynthetic analysis outlined in Scheme 1. Introduction of the C7−C8 β-epoxide was planned as the final step in the synthesis, a strategy commonly utilized for the preparation of cryptophycins,[15−24,26−28] presumably to avoid limitations imparted by the enhanced reactivity of the epoxide of a styryl system. Oxidation of Cryptophycin C (**4**) reveals that epoxidation occurs with excellent regioselectivity to afford the desired C7−C8 epoxide; however, the transformation is nonstereoselective to provide a mixture of 1:2 α:β-isomers, which could be separated to give β-isomer **1**.[15] To date, the efficient incorporation of the β-epoxide functionality remains a major challenge in the total synthesis of the cryptophycins.[29] Nevertheless, disconnection of styrene **7** at the two amide linkages (A−B and B−C) leads to key intermediate **8** and unnatural D-amino acid **9**. To avoid protecting groups, we elected to utilize a novel methodology, developed in our laboratory,[30] that would allow acylation of unprotected amino acid **9** with intermediate **8** to first form the A−B amide linkage. Subsequent deprotection of the amine and macrolactamization, under conditions previously described by Tius,[15] should lead to cryptophycin **7**.

Methyl ester **10**[15,31] was hydrolyzed to carboxylic acid **11**[15] which, without extensive purification, was converted to *N*-hydroxysuccinimide ester **12** in excellent overall yield (Scheme 2). Fluoride-mediated desilylation of **12**, under acidic conditions,[15] cleanly furnished alcohol **13** as white crystals. We next turned our attention to the nontrivial esterification of fragment A alcohol **13** with Boc-protected amino acid **14**.[24a,31] Preactivation of acid **14** with EDC in the presence of catalytic DMAP followed by addition of alcohol **13** at a final reaction concentration of 0.2 M provided key intermediate **15.** Under these specific conditions the coupling was reproducible and scalable on multigram quantities.[32]

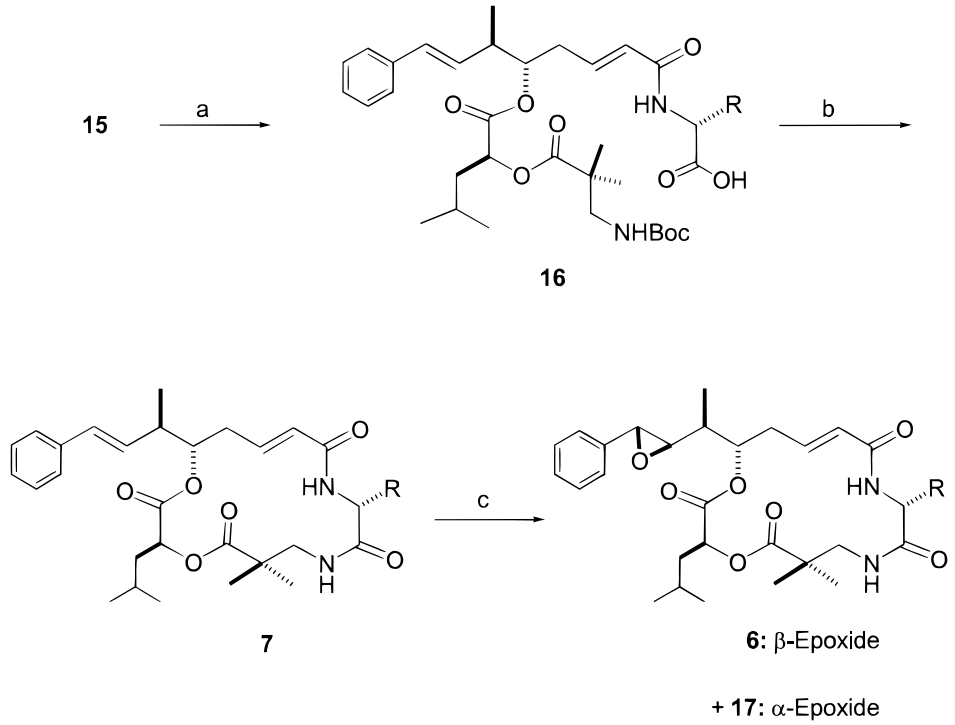
In previous work,[29,30] we discovered that *N*-hydroxysuccinimide esters, in the presence of *N*,*O*-bis(trimethylsilyl)acetamide (BSA), exclusively acylate aliphatic amines over other nucleophiles such as alcohols and carboxylic acids. Complete chemoselectivity is observed presumably because in situ silylation of oxygen provides protection, while silylation of amines serves to increase nucleophilicity.[33] Typically, couplings were performed by reacting intermediate **15** with 1.5 equiv of D-amino acid **9** in the presence of excess BSA and at elevated temperatures to afford acyclic precursor **16** (Scheme 3). A particularly noteworthy aspect of this reaction was the ease with which product **16** was isolated in high purity and yields (61−93%, see Table 1) by a simple aqueous acid wash to remove excess amine and byproducts. The commercial availability of amino acids **9**, except **9r,s**, was an advantage of this methodology and allowed very rapid access to precursor **16**. 3-Cl,4-OH-D-phenylalanine (**9r**) was obtained in one step by demethylation of known amino acid **9q**[15] using conditions described by Nakamoto[34] (Scheme 4). The remaining aniline **9s** was prepared from commercially available *p*-amino-D-phenylalanine (**9k**) (Scheme 5). Esterification of **9k** gave methyl ester **18** which was

Scheme 2[a]



[a] Reagents: (a) 1 N aq LiOH, acetone, quantitative; (b) EDC, *N*-hydroxysuccinimide, DMF, quantitative; (c) 48% aq HF, 0−25 °C, CH_3CN, 78%; (d) EDC, cat. DMAP, **14**, 0.2 M CH_2Cl_2, 80%.

Scheme 3[a]



[a] Reagents: (a) general method A: D-amino acid, **9**, *N*,*O*-bis(trimethylsilyl)acetamide, DMF, 55 °C, see Table 1; (b) general method B: (i) neat trifluoroacetic acid, 25 °C, (ii) pentafluorophenyl diphenylphosphinate, DIEA, 25 °C, DMF, see Table 3; (c) general method C: *m*CPBA (1.1 equiv), 0.2 M CH_2Cl_2, 25 °C; RP-HPLC separation, see Table 5.

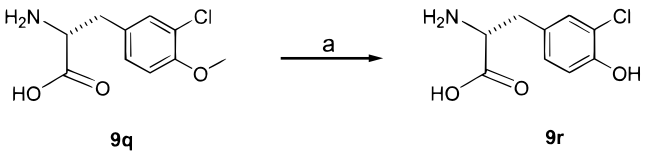
converted to diacetate **19**[35] in good overall yield. Fully protected amino acid **19** was chlorinated using 1.4 equiv of *N*-chlorosuccinimide[36] to deliver desired chloride **20**, with no evidence of dichlorination. Finally, a one-pot global deprotection of **20**, first using basic conditions to hydrolyze the methyl ester and one of the acetate groups followed by treatment with strong acid to remove the remaining acetate, furnished 3-Cl,4-NH_2-D-phenylalanine (**9s**). Overall the methodology for formation of amide **16** (Scheme 3) was remarkably general as indi-

Table 1. Elemental Analyses, Optical Rotations, and Yields for Carboxylic Acids **16**

compd	R	formula[1]	$[\alpha]_D$[2]	yield (%)
a	H	$C_{33}H_{48}N_2O_9 \cdot 0.5H_2O$	+32 ($c = 0.50$)	93
b	$CH(Me)_2$	$C_{36}H_{54}N_2O_9 \cdot 0.3H_2O$	+21.7 ($c = 1.01$)	96
c	CH(OH)Me*	$C_{35}H_{52}N_2O_{10} \cdot 0.5H_2O$	+16.9 ($c = 1.07$)	89
d	pyrrolidine	$C_{36}H_{52}N_2O_9 \cdot 0.5H_2O$	+66 ($c = 0.67$)	92
e	CH_2cyclohexyl	$C_{40}H_{60}N_2O_9 \cdot 0.75H_2O$	+25.3 ($c = 1.03$)	88
f	CH_2CH_2Ph	$C_{41}H_{56}N_2O_9$	+20.8 ($c = 1.06$)	72
g	CH_2OCH_2Ph	$C_{41}H_{56}N_2O_{10} \cdot 0.6H_2O$	+2.1 ($c = 0.97$)	79
h	(*p*-OH)Ph	$C_{39}H_{52}N_2O_{10} \cdot 1.0H_2O$	−20 ($c = 0.65$)	75
i	CH_2Ph	$C_{40}H_{54}N_2O_9 \cdot 0.5H_2O$	+9.5 ($c = 1.06$)	88
j	CH_2(*p*-OH)Ph	$C_{40}H_{54}N_2O_{10} \cdot 0.5H_2O$	+12 ($c = 0.60$)	87
k	CH_2(*p*-NH_2)Ph	$C_{40}H_{55}N_3O_9$	+5.0 ($c = 0.82$)	72
p	CH_2(*p*-I)Ph	$C_{40}H_{53}IN_2O_9 \cdot 0.5H_2O$	−4.1 ($c = 0.99$)	86
r	CH_2(*m*-Cl,*p*-OH)Ph	$C_{40}H_{53}N_2O_{10} \cdot 1.0H_2O$	+6.3 ($c = 0.96$)	67
s	CH_2(*m*-Cl,*p*-NH_2)Ph	$C_{40}H_{54}ClN_3O_9 \cdot 1.0H_2O$	−18 ($c = 0.57$)	61
v	CH_2(*m*,*p*-diOH)Ph	$C_{40}H_{54}N_2O_{11} \cdot 0.5H_2O$	+12 ($c = 0.84$)	75
w	CH_2(α-naphthyl)	$C_{44}H_{56}N_2O_9$	+49 ($c = 0.99$)	77
x	CH_2(*β*-naphthyl)	$C_{44}H_{56}N_2O_9 \cdot 1.0H_2O$	−9.8 ($c = 1.02$)	78
y	CH_2(3-pyridyl)	$C_{39}H_{53}N_3O_9 \cdot 1.0H_2O$	+7.7 ($c = 0.52$)	65
z	CH_2(3-indolyl)	$C_{424}H_{55}N_3O_9 \cdot 1.0H_2O$	−4.8 ($c = 0.84$)	85

[1] Compounds were analyzed for C,H,N; the results agreed to within ±0.4% of the theoretical values. [2] All optical rotations were performed in methanol at 589 nm. *(*S*)-Configuration at C3, derived from D-threonine.

Scheme 4[a]



9q **9r**

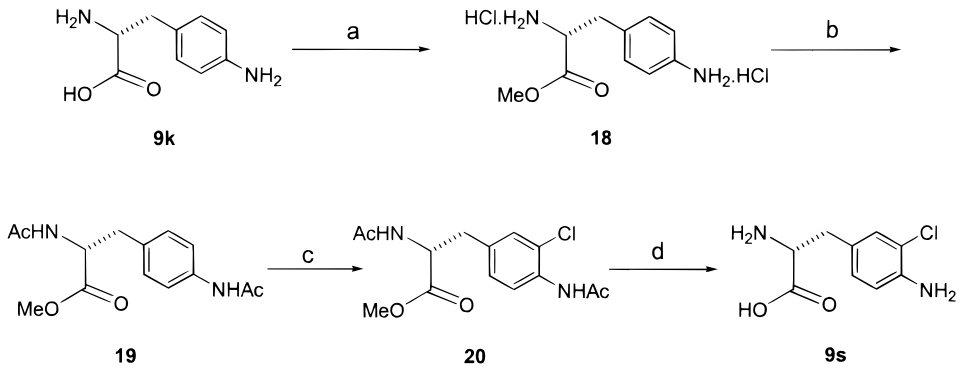
[a] Reagents: (a) 48% HBr, PhOH, reflux, 69%.

cated by the diverse amino acid side chains that were tolerated in the coupling reaction, including alcohols **9c**, phenols **9h,j,r,v**, anilines **9k,s**, and nitrogen heterocycles **9y**,**z** (see Table 1). Epimerization at C2 in unit B, a possible problem, was not evident under these conditions. Physical and chemical data for acid **16**, including proton NMR data (see representative examples in Table 2), was consistent with the structural assignments.

Conversion of precursor **16** to cyclic depsipeptide **7** was accomplished employing the procedure developed by Moore and Tius[15] in the synthesis of Cryptophycin C (**4**) (Chart 1). Accordingly, removal of the Boc group with neat trifluoroacetic acid followed by macrolactamization of the resulting amino acid under high-dilution conditions and using pentafluorophenyl diphenylphosphinate, as an activating agent, gave styrene **7** (Scheme 3). To our delight, the two-step cyclization sequence proved quite general, tolerating side chain hydroxyl groups, **16c**; phenols, **16h**,**j**,**r**,**v**; benzyl ethers, **16g**; anilines, **16k,s**; and pyridines, **16y** (see Table 3). Unfortunately, indolyl derivative **16z**, for reasons undetermined, failed to provide the desired cyclized product under these conditions. Proton NMR data for cyclic depsipeptide **7** indicated it to be a single diastereomer for which the 2-H in unit B was shifted downfield compared to acyclic precursor **16** (see representative examples in Table 4) and in agreement with the assignments for Cryptophycin C (**4**).[15]

The final step in the sequence to target agent **6** involved an oxidation to introduce the *β*-epoxide functionality.[15−22,24,26−28] We elected to employ conditions previously reported for the regioselective, but nonstereoselective, C7−C8 epoxidation of Cryptophycin C (**4**).[15] Treatment of styrene **7** with *m*-chloroperbenzoic acid (*m*CPBA) led to the expected mixture of α:*β*-isomers which were separated by reverse-phase HPLC to afford *β*-epoxide **6** (Scheme 3). Interestingly, regardless of the side chain on fragment B, the α:*β*-epoxide ratio remained at 1:2 (see Table 5). Assignment of the epoxide isomers was based on the shift of 8-H in unit A which resonates at ∼3.7 ppm for the *β*-isomer **6** (see representative examples in Table 6) and at ∼3.6 ppm for the corresponding α-isomer **17**, both appearing as doublets ($J = \sim 1.6$ Hz). It was found, however, that use of excess oxidant led to significant levels of bis-epoxidation, particularly in the case of sytrene **7d** where the C2−C3 olefin is more nucleophilic due to the presence of the

Scheme 5[a]



9k **18** **19** **20** **9s**

[a] Reagents: (a) AcCl, MeOH, reflux, 100%; (b) Ac_2O, pyridine, 25 °C, 81%; (c) NCS, CH_3CN, reflux, 50%; (d) (i) 1 N aq NaOH, dioxane, reflux, (ii) 5 N aq HCl, 95 °C, (iii) SCX cation exchange, 72%.

Table 2. Proton NMR Data for Representative Carboxylic Acids **16***

	16a: glycine	**16b**: valine	**16f**: homoPhe	**16h**: ([*p*-OH]Ph)glycine	**16j**: tyrosine
			Unit A		
PhH_5	7.36−7.15 (m)	7.36−7.10 (m)	7.39−7.07 (m)	7.35−7.09 (m)	7.35−7.16 (m)
3-H	6.65−6.48 (m)	6.59−6.49 (m)	6.61−6.51 (m)	6.57−6.52 (m)	6.52−6.56 (m)
8-H	6.41 (d, *J* 15.8)	6.41 (d, *J* 15.9)	6.41 (d, *J* 15.9)	6.40 (d, *J* 15.9)	6.39 (d, *J* 16.0)
7-H	6.08 (dd, *J* 16.1/8.9)	6.08 (dd, *J* 16.4/8.5)	6.08 (dd)[1]	6.07 (dd, *J* 16.2/8.3)	6.06 (dd, *J* 16.1/8.12)
2-H	6.01 (d, *J* 15.4)	6.15 (d, *J* 16.5)	6.07 (d, *J*, 15.7)	6.14 (d, *J* 15.7)	6.01 (d, *J* 15.2)
5-H	4.79−4.77 (m)	4.80−4.76 (m)	4.81−4.77 (m)	4.79−4.75 (m)	4.81−4.75 (m)
6-H	2.57−2.47 (m)	2.57−2.46 (m)	2.63−2.52 (m)	2.57−2.37 (m)	2.55−2.35 (m)
4-H_2	2.47−2.37 (m)	2.46−2.36 (m)	2.63−2.52 (m)	2.57−2.37 (m)	2.55−2.35 (m)
6-Me	1.10 (d, *J* 5.9)	1.02 (d)[1]	1.04 (d)[1]	1.02 (d)[1]	1.00 (d)[1]
			Unit B		
CO_2H	12.5 (br s)	12.6 (br s)	12.5 (br s)	12.6 (br s)	12.6 (br s)
NH	8.22 (t, *J* 5.4)	8.05 (d, *J* 8.5)	8.26 (t, *J* 7.7)	8.49 (d, *J* 7.5)	8.15 (d, *J* 7.9)
2-H		4.19 (dd, *J* 8.2/6.1)	4.22−4.15 (m)	5.21 (d, *J* 7.2)	4.39−4.32 (m)
R	3.76 (d, *J* 5.64, 2-H_2)	0.84 (d, *J* 6.7, 5H_3/4-Me)	7.39−7.07 (m, PhH_5)	9.45 (s, OH)	9.15 (s, OH)
			2.63−2.52 (m, 4-H_2)	7.70 (d, *J* 8.2, PhH_2)	6.96 (d, *J* 8.2, PhH_2)
			2.46−2.40 (m, 3-H_2)	7.14 (d, *J* 8.0, PhH_2)	6.59 (d, *J* 8.4, PhH_2)
					2.90 (dd, *J* 13.7/4.7, 3-H)
					2.71 (dd, *J* 13.8/4.7, 3′-H)
			Unit C		
NH	6.69 (t, *J* 6.1)	6.69 (t, *J* 6.1)	6.69 (t, *J* 5.6)	∼6.7 (t)[1]	6.48 (t, *J* 7.8)
3-H	3.24−3.08 (m)	3.18−3.12 (m)	3.18−3.12 (m)	3.24−3.05 (m)	3.14 (dd, *J* 13.4/6.9)
3-H′	3.08−2.97 (m)	3.04−2.97 (m)	3.08−3.05 (m)	3.03−2.97 (m)	3.00 (dd, *J* 13.4/6.5)
Boc	1.32 (s)	1.32 (s)	1.32 (s)	1.32 (s)	1.32 (s)
2-Me	1.05 (s)	1.05 (s)	1.05 (s)	1.05 (s)	1.05 (s)
2-Me	1.02 (s)	1.02 (s)	1.03 (s)	1.01 (s)	1.01 (s)
			Unit D		
2-H	4.91−4.89 (m)	4.93 (app q, *J* 5.4)[4]	4.92 (app q, *J* 5.4)[d]	4.90−4.88 (m)	4.88 (app q, *J* 5.7)[4]
3-H_2	1.63−1.44 (m)	1.62−1.50 (m)	1.61−1.50 (m)	1.66−1.56 (m)	1.64−1.42 (m)
4-H	1.63−1.44 (m)	1.50−1.45 (m)	1.50−1.45 (m)	1.50−1.38 (m)	1.64−1.42 (m)
5-H_3	0.75 (d, *J* 6.7)[2]	0.75 (d, *J* 6.4)[2]	0.75 (d, *J* 6.5)[2]	0.75 (d, *J* 6.4)[2]	0.75 (d, *J* 7.0)[2]
4-Me	0.73 (d, *J* 6.5)[2]	0.73 (d, *J* 6.4)[2]	0.73 (d, *J* 6.6)[2]	0.73 (d, *J* 6.4)[2]	0.72 (d, *J* 6.7)[2]

*In DMSO-d_6 at 300 MHz. [1] Partially buried. [2] Overlapping. [4] Buried. [3] app, apparent.

tertiary amide. It was essential, therefore, to use only a slight excess of oxidant which, in turn, required the reactions to be conducted at high concentrations to ensure complete conversions. Although dichloromethane was the solvent of choice, in cases where solubility of substrate was an issue (e.g., phenol **7j**), a polar solvent, such as tetrahydrofuran, was added without affecting regio- and stereoselectivity or yield. In cases where the substrate contained a free amino group (e.g., **7k**,**s**), it was necessary to apply a protecting group prior to epoxidation. Acid sensitivity of the epoxide functionality and the presence of labile ester linkages were factors considered in selecting Fmoc, a protecting group which is readily removed under mild basic conditions.[29] Treatment of anilines **7k**,**s** with Fmoc chloride under standard conditions[37] gave **7m**,**t**, which on epoxidation led to the expected mixture of epoxides **21** and **22**, respectively (Scheme 6). Deprotection of **21** and **22** with piperidine was rapid and clean to give the corresponding aniline which was separated to furnish the desired *β*-epoxides **6k**,**s**, respectively. Access to dimethylaniline analogue **6u** was gained through simple methylation of the corresponding aniline **6s** (Scheme 6). Although data in Table 4 reflect low yields for the formation of *β*-epoxide **6**, partly attributable to the difficult separations of the α:*β*-mixtures, this method was generally applicable for a broad range of substrates **7** and satisfactory. Attempts to epoxidize **7v**, however, led to significant decomposition, possibly due to oxidation of the 3,4-dihydroxyphenyl system to the corresponding *o*-quinone. Although protection of the hydroxyls would overcome this problem, it was not attempted. In the case

Table 3. Elemental Analyses, Optical Rotations, and Cyclization Yields for Styrenes **7**

compd	R	formula[1]	$[\alpha]_D$[2]	yield (%)
a	H	$C_{28}H_{38}N_2O_6$	−40 (c = 0.50)	76
b	$CH(Me)_2$	$C_{31}H_{44}N_2O_6 \cdot 0.5H_2O$	+21 (c = 0.24)	61
c	CH(OH)Me*	$C_{30}H_{42}N_2O_7 \cdot 0.5H_2O$	+54 (c = 0.24)	51
d	pyrrolidine	$C_{31}H_{42}N_2O_6 \cdot 0.75H_2O$	+41 (c = 0.52)	74
e	CH_2cyclohexyl	$C_{35}H_{50}N_2O_6$	+25 (c = 0.85)	56
f	CH_2CH_2Ph	$C_{36}H_{46}N_2O_6$	+43 (c = 0.90)	62
g	CH_2OCH_2Ph	$C_{36}H_{46}N_2O_7 \cdot 0.4H_2O$	+29 (c = 0.28)	57
h	(*p*-OH)Ph	$C_{34}H_{42}N_2O_7 \cdot 0.3H_2O$	+15 (c = 0.39)	59
i	CH_2Ph	$C_{35}H_{44}N_2O_6 \cdot 0.2H_2O$	+31 (c = 0.97)	55
j	CH_2(*p*-OH)Ph	$C_{35}H_{44}N_2O_7 \cdot 0.3H_2O$	+30 (c = 0.53)	80
k	CH_2(*p*-NH_2)Ph	$C_{35}H_{45}N_3O_6 \cdot 0.25H_2O$	+33 (c = 0.73, MeOH)	58
p	CH_2(*p*-I)Ph	$C_{35}H_{43}IN_3O_6$	+25 (c = 0.28)	22
r	CH_2(*m*-Cl,*p*-OH)Ph	$C_{35}H_{43}ClN_2O_7$	+29 (c = 0.84)	42
s	CH_2(*m*-Cl,*p*-NH_2)Ph	$C_{35}H_{44}ClN_3O_6$	+32 (c = 0.94)	62
v	CH_2(*m*,*p*-diOH)Ph	$C_{35}H_{44}N_2O_8 \cdot 1.3H_2O$	+34 (c = 0.59, MeOH)	52
w	CH_2(α-naphthyl)	$C_{39}H_{46}N_2O_6 \cdot 0.3H_2O$	+48 (c = 0.86)	56
x	CH_2(*β*-naphthyl)	$C_{39}H_{46}N_2O_6 \cdot 0.3H_2O$	+15 (c = 0.60)	65
y	CH_2(3-pyridyl)	$C_{34}H_{43}N_3O_6 \cdot 0.5H_2O$	+30 (c = 0.67, MeOH)	71

[1] Compounds were analyzed for C,H,N; the results agreed to within ±0.4% of the theoretical values. [2] All optical rotations were performed in chloroform, unless stated otherwise, at 589 nm. *(*S*)-Configuration at C3, derived from D-threonine.

of pyridyl **7y**, treatment with 1 equiv of *m*CPBA acid initially gave the *N*-oxide, which with further oxidant led to the corresponding epoxide. However, the reaction was not clean, and attempts to isolate the desired *β*-isomer **4w** were unsuccessful.

The synthetic approach described above proved remarkably general for accessing a variety of structurally complex cryptophycin analogues, styrene **7** and *β*-epoxide **6**. Consequently, we were able to probe the SAR in this region of the molecule for these two series.

Structure−Activity Relationships

Biological activity was measured in an in vitro cellular cytotoxicity assay using a human leukemia CCRF-CEM tumor cell line (see Experimental Section for the assay protocol).[38] Cryptophycin-52 (**3**), with an IC_{50} of 22 pM (see Table 7), is one of the most potent cytotoxic agents in this class of agents. The corresponding styrene, Cryptophycin-51 (**5**) (Chart 1), was approximately 630-fold less potent (IC_{50} = 13.9 nM) than **3**. It should be noted that during the total synthesis of Cryptophycin C (**4**), Tius and Moore[15] also prepared the epimer at C2 in unit B. This analogue, which corresponds to incorporation of the natural L-amino acid in fragment B, was 14−24-fold less active than Cryptophycin C (**4**).

In general, styrene analogues **7** were 20- to >140-fold less potent than Cryptophycin-51 (**5**). Chlorophenol **7r**, a styrene analogue lacking a methyl group, resulted in a 20-fold loss in cellular activity, while the corresponding chloroaniline **7s** displayed 36-fold less potency. These results are consistent with the Cryptophycin A (**1**) series, where the chlorophenol analogue (Cryptophycin-17) was 2.4−5-fold less potent than the parent (Cryptophycin-3).[39] Removal of the chlorine in **7r**,**s**, to give tyrosine derivative **7j** and aniline **7k**, respectively, led to a further 3-fold loss in potency. Conversely, the same alteration in the Cryptophycin A (**1**) series was reported to produce a 6−9-fold increase in activity.[39] This exemplifies the caution that must be taken when comparing data derived from different tumor cell lines. Interestingly, the cytotoxicity of unsubstituted aromatic **7i** is comparable to that of disubstituted aromatic **7r**,**s**. This result may be explained by considering cellular transport, a critical factor for cellular activity. Under passive conditions, it is reasonable to assume that the more hydrophobic **7i** diffuses across the cell membrane more easily than polar derivatives **7r**,**s**. Further increasing hydrophobicity in region B, e.g., naphthyl derivatives **7w**,**x**, however, led to total loss of activity. The bulky Fmoc moiety at C4 in the phenyl ring of **7m**,**t** was not well-tolerated. Replacing the phenyl moiety in **7i** with a cyclohexyl (**7e**), a group with similar hydrophobicity, abolishes activity suggesting that the phenyl ring is probably important for interaction at the molecular target. Substitution of the 4-hydroxyl group in **7j** with 4-iodide (**7p**) did not change the potency implying that H-bonding at this position is not required. In fact, losses in activity were observed on introduction of polar functionality, e.g., dihydroxy analogue **7v** and pyridine derivative **7y**. The methylene linkage between the core

Table 4. Proton NMR Data for Representative Styrenes **7***

	7a: glycine	**7b**: valine	**7f**: homoPhe	**7h**: ([*p*-OH]Ph)glycine[⊗]	**16j**: tyrosine[⊗]
			Unit A		
PhH_5	7.36−7.20 (m)	7.36−7.16 (m)	7.36−7.18 (m)	7.36−7.15 (m)	7.42−7.26 (m)
3-H	6.67 (ddd, *J* 15.2/8.8/5.8)	6.81 (ddd, *J* 15.1/10.3/4.7)	6.79−6.70 (m)	6.79−6.69 (m)	6.73−6.65 (m)[3]
8-H	6.44 (d, *J* 15.9)	6.43 (d, *J* 15.8)	6.42 (d, *J* 15.8)	6.54 (d, *J* 15.8)	6.40 (d, *J* 15.8)
7-H	6.02 (dd, *J* 15.8/8.8)	6.03 (dd, *J* 15.8/8.8)	6.03 (dd, *J* 15.8/8.8)	5.98 (dd, *J* 15.8/8.8)	6.00 (dd, *J* 16.1/8.8)
2-H	5.92 (d, *J* 15.8)	5.88 (d, *J* 15.7)	5.70 (d, *J* 15.6)	5.85 (d, *J* 14.8/1.0)	5.82 (d, *J* 15.1)
5-H	5.07−5.00 (m)	5.06 (ddd, *J* 8.3/6.8/1.72)	5.07−5.04 (m)	5.06−5.00 (m)	5.02 (m)
4-H/6-H	2.63−2.53 (m)	2.62−2.54 (m)	2.61−2.55 (m)	2.61−2.49 (m)	2.59−2.52 (m)
4′-H	2.49−2.38 (m)	2.47−2.35 (m)	2.40−2.31 (m)	2.39−2.30 (m)	2.33 (app q, J 11.2)
6-Me	1.15 (d)[1]	1.15 (d, *J* 6.9)	1.15 (d, *J* 6.9)	1.10 (d, *J* 6.8)	1.13 (d, *J* 6.8)
			Unit B		
NH	6.07−5.98 (d)[3]	5.59 (d, *J* 8.2)	5.50 (d, *J* 7.60)	7.65 (d, *J* 6.3)	not observed
2-H	3.92 (dd, *J* 16.9/5.8)	4.40 (dd, *J* 8.0/4.3)	4.53−4.45 (m)	5.28 (d, *J* 6.5)	4.57 (dd, *J* 9.6/3.8)
R	4.05 (dd, *J* 16.9/7.0, 2-H′)	2.47−2.35 (m, 3-H)	7.36−7.18 (m, PhH_5)	7.90 (dd, J 10/1.68, OH)	7.56 (d, *J* 7.6, OH)
		1.00 (d, *J* 6.9, 5-H_3)	2.73 (t, *J* 7.6, 4-H_2)	7.10 (d, *J* 8.5, PhH_2)	7.03 (d, *J* 8.4, PhH_2)
		0.90 (d, *J* 7.0, 3-Me)	2.40−2.31 (m, 3-H)	6.71 (d, *J* 8.4, PhH_2)	6.72 (d, *J* 8.3, PhH_2)
			1.99−1.86 (m, 3-H′)		3.02 (dd, *J* 13.3/1.6, 3-H)
					2.80 (dd, J 14.2/10.1, 3′-H)
			Unit C		
NH	7.08−7.04 (m)	7.36−7.16 (m)	7.36−7.18 (m)	7.36−7.15 (m)	7.22−7.20 (m)
3-H	3.44 (dd, *J* 13.5/8.4)	3.40 (dd, *J* 13.4/8.4)	3.37 (dd, *J* 13.5/8.3)	3.51 (dd, *J* 13.3/10.1)	3.44 (dd, *J* 13.3/9.7)
3-H′	3.18 (dd, *J* 13.4/4.3)	3.17 (dd, *J* 13.5/3.8)	3.18 (dd, *J* 13.4/3.8)	3.00 (d, *J* 13.4)	3.14 (dd, *J* 14.4/3.6)
2-Me	1.24 (s)	1.23 (s)	1.23 (s)	1.19 (s)	1.18 (s)
2-Me	1.18 (s)	1.18 (s)	1.18 (s)	1.16 (s)	1.17 (s)
			Unit D		
2-H	4.91 (dd, *J* 10.0/3.2)	4.91 (dd, *J*, 10.1/3.5)	4.91 (dd, *J* 9.9/3.4)	4.90 (dd, *J* 10/3.5)	4.99−4.86 (m)
3-H/4-H	1.76−1.62 (m)	1.74−1.48 (m)	1.71−1.63 (m)	1.66−1.54 (m)	1.65−1.55 (m)
3′-H	1.52−1.28 (m)	1.40−1.33 (m)	1.39−1.25 (m)	1.32−1.25 (m)	1.34−1.24 (m)
5-H_3	0.78 (d, *J* 6.4)	0.76 (d, *J* 6.8)	0.76 (d, *J* 6.7)	0.69 (d, *J* 7.1)[2]	0.71 (d, *J* 6.6)
4-Me	0.73 (d, *J* 6.4)	0.74 (d, *J* 6.7)	0.73 (d, *J* 6.6)	0.66 (d, *J* 7.1)[2]	0.69 (d, *J* 6.5)

*In $CDCl_3$ at 300 MHz. [⊗] In $CDCl_3/CD_3OD$ (1:1) at 300 MHz. [1] Partially buried. [2] Overlapping. [3] Buried. [4] app, apparent.

nucleus and the phenyl group was shown to be important for activity. A one-carbon increase in chain length (e.g., **7i** vs **7f**) produced a 4-fold loss in activity, while a one-carbon decrease (e.g., **7j** vs **7h**) led to a >2.3-fold loss. More drastic alterations in region B, including removal of the side chain (i.e., **7a**), replacement with aliphatic side chains (e.g., **7b**,**c**), and introducing a tertiary amide (e.g., **7d**) all produced inactive cryptophycin styrene analogues.

In the epoxide series, cryptophycins **6**, bearing a C7−C8 epoxide in the β-configuration, were 4−60-fold more potent than the corresponding α-isomers **17** (Table 7) and generally more potent than the corresponding styrenes **7**. Similar trends were found in the SAR of fragment "A" in the Cryptophycin A (**1**)[3] and Cryptophycin-52 (**3**)[26] series. Our data showed, for example, that for derivatives **i,j,k,m,r,s,t,w,x** possessing a benzyl side chain, epoxides **6** were 18−2000-fold more potent than styrenes **7**. In the β-epoxide family **6**, desmethyl agent **6r** was 24-fold less potent than parent **3**, which correlated well with the 27−40-fold decrease observed in the Cryptophycin A (**1**) series.[3] Replacing the oxygen in **6r** for nitrogen in **6s** maintained the potency at the subnanomolar level. Since the methylated phenols were more active, we prepared dimethylaniline **6u** and, indeed, realized a 10-fold increase in activity compared to aniline **6s**. With an IC_{50} of 54 pM, **6u** represented the most potent fragment "B" analogue, only 2-fold less potent than parent **3**. To our surprise, phenyl derivative **6i** was only ~3-fold less active than **6u**, while monosubstituted analogues **6j,k** were significantly less cytotoxic. Unexpectedly, intermediates **21** and **22**, bearing a bulky C4 Fmoc group, were relatively active considering they were a mixture of isomers. It is conceivable that the Fmoc derivatives act as prodrugs which cleave in vitro to provide the more potent **6k**,**s**, respectively. In the epoxide series, insertion of a methylene linkage (i.e., **6i** to **6f**) led to a dramatic 3200-fold decrease in activity. Adding an oxygen atom in ethyl-bridged **6f** to produce ether-linked derivative **6g** returned some activity, as observed in the styrene series. Eliminating the linkage between the aromatic moiety and the core nucleus (i.e., **6j** to **6h**) led to ~70-fold loss in activity. It is noteworthy that activity in the styrene series **7** was not necessarily a good predictor for activity in the epoxide series **6**. For example, styrenes **7e**,**w**,**x** showed no activity, while their counterpart epoxides **6e**,**w**,**x**, respectively, were all moderately active. Cyclohexyl analogue **6e** was the only nonaromatic side chain analogue that showed any reasonable potency. All others (**6a**−**d**) were completely inactive.

Conclusions

Our synthesis of fragment "B" analogues **6** featured a novel and efficient amide bond-forming reaction between an *N*-hydroxysuccinimide ester of key intermediate **15** and an unprotected amino acid **9**. This approach provided access to analogues bearing a diverse range of side chains in fragment B. Biological evaluation of these analogues in an in vitro cytotoxicity assay revealed a structure−activity relationship. In general, β-epoxides **6** were more potent than the corresponding α-epoxides **17** and styrenes **7**. Furthermore, a benzyl side chain was required for potent activity with 3-chloro-4-dimethylamino substitution in the phenyl ring, **6u**, leading to an exquisitely, potent cytotoxic agent (IC_{50} = 54 pM). The results clearly demonstrate that cytotoxic activity is sensitive to substitution in region "B";

Table 5. Physical−Chemical Data and Yields for β-Epoxides **6**

compd	R	formula[1]	$[\alpha]_D$[2]	RP-HPLC t_R (min)[3]	yield (%)[4]	β/α-ratio[5]
a	H	$C_{28}H_{38}N_2O_7 \cdot 0.75H_2O$	−22.7 ($c = 0.275$)	10.1[a]	48	1.7
b	$CH(Me)_2$	$C_{31}H_{44}N_2O_7 \cdot 0.5H_2O$	+23.6 ($c = 0.445$)	19.2[a]	30	1.9
c	CH(OH)Me*	$C_{30}H_{42}N_2O_8 \cdot 1.0H_2O$	+57.3 ($c = 0.345$)	8.80[a]	22	1.6
d	pyrrolidine	$C_{31}H_{42}N_2O_7$	+50.0 ($c = 0.435$)	18.0[a]	30	2.2
e	CH_2cyclohexyl	$C_{35}H_{50}N_2O_7 \cdot 0.3H_2O$	+16 ($c = 0.99$)	29.0[b]	18	1.9
f	CH_2CH_2Ph	$C_{36}H_{46}N_2O_7 \cdot 1.0H_2O$	+11.1 ($c = 1.14$)	26.4[b]	25	2.0
g	CH_2OCH_2Ph	HRMS[a]	+19 ($c = 0.38$)	26.3[b]	30	1.7
h	(*p*-OH)Ph	$C_{34}H_{42}N_2O_8 \cdot 1.4H_2O$	−22.0 ($c = 0.185$, MeOH)	13.3[a]	22	2.0
i	CH_2Ph	$C_{35}H_{44}N_2O_6 \cdot 0.2H_2O$	+31 ($c = 0.47$)	27.7[a]	25	2.0
j	CH_2(*p*-OH)Ph	$C_{35}H_{44}N_2O_8 \cdot 0.5H_2O$	+34.7 ($c = 0.215$)	4.67[a]	34[b]	2.2
k	CH_2(*p*-NH_2)Ph	$C_{35}H_{45}N_3O_7$	+31 ($c = 0.42$)	22.5[a]	23	2.1[c]
r	CH_2(*m*-Cl,*p*-OH)Ph	$C_{35}H_{43}ClN_2O_8 \cdot 0.5H_2O$	+16 ($c = 0.97$, MeOH)	19.8[b]	35	2.0
s	CH_2(*m*-Cl,*p*-NH_2)Ph	$C_{35}H_{44}ClN_3O_7$	+28 ($c = 0.88$)	19.4[a]	32	2.0[a]
w	CH_2(α-naphthyl)	$C_{39}H_{46}N_2O_7 \cdot 0.25H_2O$	+32 ($c = 0.91$)	28.8[b]	27	1.8
x	CH_2(β-naphthyl)	$C_{39}H_{46}N_2O_7$[b]	+13.3 ($c = 1.02$)	28.3[b]	18	1.9

[1] Compounds were analyzed for C,H,N; the results agreed to within ±0.4% of the theoretical values. [a] HRMS calcd for $C_{36}H_{47}N_2O_8$ *m/z* requires 635.3332, found 635.3324 (Δ −0.8 mmu). [b] Elemental analysis requires C, 71.54; found C, 72.10. HRMS calcd for $C_{39}H_{47}N_2O_7$ *m/z* requires 655.3383, found 655.3380 (Δ −0.3 mmu). [2] All optical rotations were performed in chloroform, unless stated otherwise, at 589 nm. [3] Epoxide (1 mg in 2 mL CH_3CN/H_2O (50/50)) was analyzed by RP-HPLC ($\lambda_{max} = 220$ nm, flow rate = 1.5 mL/min). [a] Nova-Pak C18 (3.9 × 150 mm) column: isocratic elution 50/50 CH_3CN/H_2O. [b] Kromasil C18 (4.6 × 250 mm) column: gradient elution 50/50−60/40 CH_3CN/H_2O (0−30 min). [4] Isolated yield of β-epoxide after preparative RP-HPLC separation. [a] 25% starting material was recovered. [5] Determined from the crude reaction mixture. [a] Ratio of epoxides after Fmoc deprotection, see Scheme 5. *(*S*)-Configuration at C3, derived from D-threonine.

however, additional studies are required to understand the effects of these agents at the molecular target. The availability of these analogues will allow studies to further probe the mechanism of action of this exciting class of novel antitumor agents. In addition, work is planned to further evaluate **6u**, with special emphasis on the effect of resistance factors on activity, solubility properties, and in vivo efficacy.

Experimental Section

Spectra were routinely obtained on a General Electric QE-300 instrument operating at 300 MHz for ^1H and a Bruker AC-250 instrument operating at 62.9 MHz for ^{13}C. Bruker AMX-500 and Varian 400 spectrometers were used, where stated, operating at 500 and 400 MHz, respectively, for ^1H and 125 and 100 MHz, respectively, for ^{13}C spectra. Spectral data, in various solvents (specified), are reported as parts per million (δ) values downfield from tetramethylsilane. Multiplicities of resonances are described as apparent (app), broad (br), singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). ^1H and ^{13}C chemical shift assignments are based on comparison with previously reported data[4] and detailed analysis of two-dimensional NMR spectra (HMQC, HMBC, COSY, ROESY) for Cryptophycin-52 (**3**).[29] IR spectra were run as KBr pellets or $CHCl_3$ solutions on a Nicolet 510P FT-IR spectrometer. Optical rotations were measured on an Autopol III automatic polarimeter or a Perkin-Elmer 341 instrument. UV spectra were obtained on a Shimadzu 2101PC instrument. Mass spectral data were obtained on either a VG-70SE (for FD), a SCIEX API-100 (for ESI), or a VG-ZAB2SE (for FAB and accurate mass measurements) spectrometer. Elemental analyses were performed using a Control Equipment Corp. 440 elemental analyzer for C, H, and N and a Lilly/Brinkman setup, employing a combustion titration method, for other elements.

Thin-layer chromatography (TLC) was performed on E. Merck silica gel 60 F_{254} plates. Spots were detected using a combination of UV and chemical detection [plates were dipped in a ceric ammonium molybdate solution (150 g of ammonium molybdate and 8 g of ceric(IV) sulfate in 1000 mL of 10% (v/v) aqueous sulfuric acid) and then heated on a hot plate]. "Silica gel flash column chromatography" was performed on E. Merck silica gel 60 (230−400 mesh, 40−63 μm). Analytical HPLC separations were performed on a LDC system with a CM 4000 multiple solvent delivery system, a spectromonitor 5000 photodiode array detector set to record at 254 or 220 nm, a Hitachi D-2500 chromato-integrator, and a Gilson FC204 fraction collector. Analytical reverse-phase (RP) HPLC was performed on a Kromasil C18 6-mm column (3.9 × 150 mm) using acetonitrile:water at a flow rate of 1.0 mL/min. Preparative RP-HPLC was performed on a 2.0-cm × 25-cm Kromasil C18 column using acetonitrile:water as the mobile phase and a flow rate of 28 mL/min. All HPLC solvents were filtered through a 0.22-mm membrane and then thoroughly degassed under vacuum. All solvents and reagents were obtained commercially and used as supplied unless otherwise stated. In those cases where solvents and reagents were dried and purified, literature procedures were followed. Where necessary, reactions were performed under a flow of dry nitrogen in a flame-dried flask.

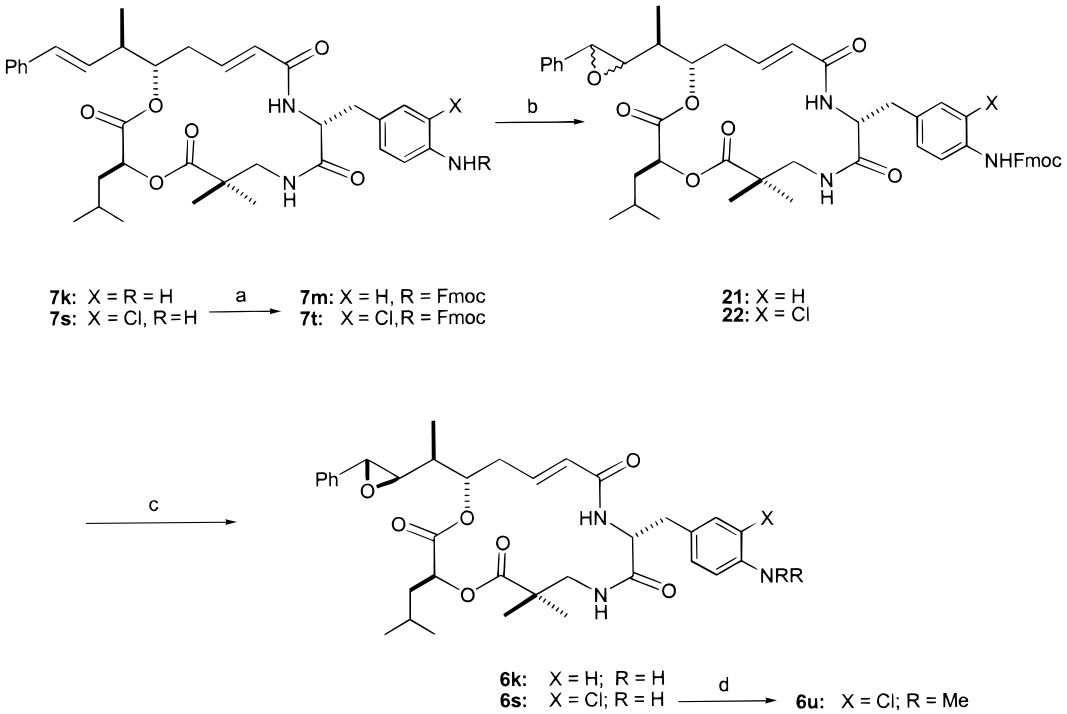
(5*S*)-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-(6*R*)-methyl-8-phenyl-(2*E*,7*E*)-octadienoic Acid, 11.[15] To a stirred solution of methyl ester **10**[31] (1.00 g, 2.673 mmol) in acetone (44 mL) was added 1 N aqueous LiOH (26 mL) at room temperature. The cloudy mixture was further diluted with

Table 6. Proton NMR Data for Representative β-Epoxides **6***

	6a: glycine	**6b**: valine	**6f**: homoPhe	**6h**: ([*p*-OH]Ph)glycine[⊗]	**16j**: tyrosine[⊗]
			Unit A		
PhH_3	7.41−7.32 (m)	7.40−7.31 (m)	7.46−7.35 (m)	7.36−7.27 (m)	7.36−7.31 (m)
PhH_2	7.31−7.25 (m)	7.28−7.13 (m)	7.33−7.25 (m)	7.26−7.16 (m)	7.21−7.30 (m)
3-H	6.66 (ddd, *J* 15.1/8.4/6.4)	6.80 (ddd, *J* 15.1/10.4/4.6)	6.83−6.76 (m)	6.79−6.70 (m)	6.78−6.70 (m)
2-H	5.90 (d, *J* 15.6)	5.85 (d, *J* 15.0)	5.75 (d, *J* 14.5)	5.91 (dd, *J* 15.5/1.9)	5.67 (d, *J* 15.6)
5-H	5.20−5.15 (m)	5.23 (dd, *J* 10.9/4.8)	5.29−5.23 (m)	5.23−5.18 (m)	5.18 (dd, J 10/4.6)
8-H	3.71 (d, *J* 1.6)	3.71 (d, *J* 1.1)	3.75 (d, *J* 1.6)	3.75 (d, *J* 1.7)	3.66 (s)
7-H	2.94 (dd, *J* 7.3/1.9)	2.94 (dd, *J* 7.5/1.4)	2.99 (dd, *J* 7.3/1.7)	2.96 (dd, *J* 7.4/2.0)	2.90 (dd, *J* 1.9/1.6)
4-H	2.58−2.51 (m)	2.69−2.36 (m)[4]	2.56−2.47 (m)	2.72−2.67 (m)	2.57−2.53 (m)
6-H	2.58−2.51 (m)	2.66−2.36 (m)	2.69−2.63 (m)	2.44−2.39 (m)	2.46−2.37 (m)
4-H′	1.87−1.81 (m)	1.84−1.74 (m)	1.85−1.61 (m)	1.88−1.81 (m)	1.78−1.69 (m)
6-Me	1.16 (d, *J* 7.3)[2]	1.17 (d, *J* 5.9)[2]	1.18 (d, *J* 6.8)	1.13 (d, *J* 6.9)	1.12 (d, *J* 7.0)
			Unit B		
NH	5.98 (t, *J* 6.3)	5.53 (d, *J* 8.0)	5.60 (d, *J* 7.8)	not observed	5.55 (br d, *J* 9)
2-H	4.01 (dd, *J* 16.8/6.7)	4.36 (dd, *J* 8.2/4.5)	4.67−4.51 (m)	5.27 (s)	4.71 (app q, *J* 7.5)[4]
R	3.94 (dd, *J* 16.7/5.9, 2-H′)	2.52−2.36 (m, 3-H)[4]	7.33−7.21 (m, PhH_5)	OH not observed	OH not observed
		0.99 (d, *J* 6.9, 4-H_3)	2.77 (t, *J* 7.7, 4-H_2)	7.13 (d, *J* 8.5, PhH_2)	6.98 (d, *J* 8.3, PhH_2)
		0.89 (d, *J* 7.3, 3-Me)	2.42−2.35 (m, 3-H)	6.74 (d, *J* 8.5, PhH_2)	6.71 (d, *J* 8.1, PhH_2)
			1.98−1.86 (m, 3-H′)		3.04−2.99 (m, 3-H_2)
			Unit C		
NH	7.08−7.04 (m)	7.23−7.13 (m)[4]	7.46−7.21 (m)[4]	not observed	7.36−7.21 (m)[3]
3-H	3.45 (dd, *J* 13.5/8.4)	3.41 (dd, *J* 13.4/8.6)	3.41 (dd, *J* 13.4/8.4)	3.53 (d, *J* 13.6)	3.44 (dd, *J* 13.7/9.1)
3-H′	3.16 (dd, *J* 13.5/4.0)	4.14 (dd, *J* 13.5/3.2)	3.22 (dd, *J* 13.4/3.5)	3.46 (d, *J* 7.2)	3.08 (dd, *J* 14.2/4.9)
2-Me	1.24 (s)	1.23 (s)	1.28 (s)	1.20 (s)	1.20 (s)
2-Me	1.18 (s)	1.18 (s)	1.22 (s)	1.18 (s)	1.14 (s)
			Unit D		
2-H	4.90 (dd, *J* 10/3.2)	4.89 (dd, *J*, 9.9/3.1)	4.94 (dd, *J* 10.4/3.5)	4.93 (dd, *J* 10/3.2)	4.81 (dd, *J* 10.1/3.4)
3-H/4-H	1.78−1.66 (m)	1.84−1.60 (m)	1.85−1.61 (m)	1.69−1.59 (m)	1.70−1.65 (m)
3-H′	1.42−1.33 (m)	1.38−1.25 (m)	1.40−1.29 (m)	1.30−1.22 (m)	1.31−1.24 (m)
5-H_3	0.87 (d, *J* 6.3)	0.87 (d, *J* 6.4)[2]	0.89 (d, *J* 6.4)	0.79 (d, *J* 6.2)[2]	0.82 (d, *J* 6.7)
4-Me	0.86 (d, *J* 6.3)	0.84 (d, *J* 7.3)[2]	0.88 (d, *J* 6.3)	0.78 (d, *J* 6.3)[2]	0.80 (d, *J* 6.4)

*In $CDCl_3$ at 300 MHz. [⊗] In $CDCl_3/CD_3OD$ (1:1) at 300 MHz. [†]In $CDCl_3$ at 400 MHz. [1] Partially buried. [2] Overlapping. [3] Buried. [4] app, apparent.

Scheme 6[a]



[a] Reagents: (a) Fmoc-Cl, $NaHCO_3$, dioxane−H_2O, 88% (**7m**) and 65% (**7t**); (b) *m*CPBA, 0.2 M CH_2Cl_2, 25 °C, quantitative (**21** and **22**); (c) piperidine, DMF, 25 °C, then RP-HPLC separation, 23% (**6k**) and 32% (**6s**); (d) MeI, K_2CO_3, CH_3CN, 70 °C, 47%.

acetone (20 mL), and the resulting yellow mixture stirred at room temperature for 23.5 h. The reaction was diluted with diethyl ether (400 mL), and the organics were washed with 1 N HCl (120 mL), brine (200 mL), and H_2O (160 mL). The organics were dried ($MgSO_4$) and concentrated in vacuo to leave a yellow oil which was purified by column chromatography (gradient: 5% AcOH in 20−40% EtOAc/*n*-hexanes) to give carboxylic acid **11** as a yellow oil (960 mg, 100%): $[\alpha]_D$ +87.6° (*c* = 1.05, $CHCl_3$); [lit.[15] $[\alpha]_D$ +87.0° (*c* = 1.4, $CHCl_3$)]; ^1H NMR ($CDCl_3$) δ 7.38−7.19 (m, PhH_5), 7.09 (ddd, *J* = 15.2,

Table 7. In Vitro Cytotoxicity of Styrenes **7**, α-Epoxides **17**, and *β*-Epoxides **6** in a CCRF-CEM Tumor Cell Line

		IC_{50} (nM)[1]		
compd	R	styrene **7**	α-epoxide **17**	*β*-epoxide **6**
	CH_2 (*m*-Cl,*p*-OMe)Ph	C-51 (**5**): 13.85		C-52 (**3**):[2] 0.022
a	H	>2000		>1940
b	$CH(Me)_2$	>1850		>1800
c	CH(OH)Me*	>1840		1060
d	pyrrolidine	>1860		1350
e	CH_2cyclohexyl	>1680		8.8
f	CH_2CH_2Ph	1660	>1620	598
g	CH_2OCH_2Ph	856	975	249
h	(*p*-OH)Ph	>1690		274
i	CH_2Ph	412		0.183
j	CH_2(*p*-OH)Ph	734		4.108
k	CH_2(*p*-NH_2)Ph	1540		10.11
m	CH_2(*p*-NHFmoc)Ph	>1200	α:*β* = 1:2 (**21**); 115	
p	CH_2(*p*-I)Ph	607		
r	CH_2(*m*-Cl,*p*-OH)Ph	269	31	0.52
s	CH_2(*m*-Cl,*p*-NH_2)Ph	500		0.58
t	CH_2(*m*-Cl,*p*-NHFmoc)Ph	>1250	α:*β* = 1:2 (**22**); 15.3	
u	CH_2(*m*-Cl,*p*-NMe_2)Ph			0.054
v	CH_2(*m*,*p*-diOH)Ph	1301		
w	CH_2(α-naphthyl)	>1570	327	35
x	CH_2(*β*-naphthyl)	>1570	866	48
y	CH_2(3-pyridyl)	997		

[1] See Experimental Section for assay protocol. [2] C-52 (**3**) was run as a positive control in each assay: SD = 0.0062 nM ($n = 6$); coefficient of variation = SD/mean = 28%. *(*S*)-Configuration at C3, derived from D-threonine.

7.6 and 7.9 Hz, 3-H), 6.38 (d, $J = 16$ Hz, 8-H), 6.16 (dd, $J = 16$ and 8 Hz, 7-H), 5.85 (d, $J = 15.8$ Hz, 2-H), 3.81−3.75 (m, 5-H), 2.49−2.37 (m, 6-H, 4-CH_2), 1.12 (d, $J = 6.7$ Hz, 6-Me), 0.91 (s, $SiCMe_3$), 0.065 (s, SiMe), 0.068 (s, SiMe); ^{13}C NMR ($CDCl_3$) *δ* 171.5 (C), 149.1 (CH), 137.5 (CH), 131.7 (CH), 130.4 (CH), 128.4 (CH), 127.0 (CH), 126.0 (CH), 122.5 (CH), 74.8 (CH), 42.8 (CH), 37.5 (CH_2), 25.8 (CH_3), 18.0 (C), 16.0 (CH_3), −4.5 (CH_3), −4.7 (CH_3); IR ($CHCl_3$) υ_{max} 2957, 2930, 2858, 1697, 1258, 1098, 838 cm^{-1}; UV (EtOH) λ_{max} 251 ($\epsilon = 17428$), 292 ($\epsilon = 1571$) nm; FDMS *m*/*z* 360.2 (M^+, 100).

1-[[(5*S*)-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-(6*R*)-methyl-1-oxo-8-phenyl-(2*E*,7*E*)-octadienyl]oxy]-2,5-pyrrolidinedione, 12. To a stirred solution of carboxylic acid **11** (720 mg, 2.0 mmol) in dry dimethylformamide (5.50 mL) were added 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) (459 mg, 2.4 mmol) and *N*-hydroxysuccinimide (299 mg, 2.6 mmol) at room temperature. The mixture was stirred for 28 h and diluted with EtOAc (100 mL), and the organics were washed with 1 N aqueous HCl (2 × 50 mL) and H_2O (75 mL), dried ($MgSO_4$), and concentrated in vacuo to leave an oil. Crude product was purified by column chromatography (gradient: 5−30% EtOAc/*n*-hexanes) to give active ester **12** as a pale-yellow oil (724 mg, 80%): $[\alpha]_D^{589}$ +71.3° ($c = 1.01$, $CHCl_3$); ^{1}H NMR ($CDCl_3$) *δ* 7.36−7.20 (m, PhH_5, 3-H), 6.38 (d, $J = 16$ Hz, 8-H), 6.14 (dd, $J = 16.1$ and 8.0 Hz, 7-H). 6.03 (d, $J = 16$ Hz, 2-H), 3.79 (q, $J = 4.3$ Hz, 5-H), 2.94 (brs, CH_2CH_2), 2.58−2.42 (m, 6-H, 4-CH_2), 1.10 (d, $J = 6.8$ Hz, 6-Me), 0.90 (s, $SiCMe_3$), 0.05 (s, $SiMe_2$); ^{13}C NMR ($CDCl_3$) *δ* 169.2 (C), 161.1 (C), 152.9 (CH), 137.3 (C), 131.5 (CH), 130.6 (CH), 128.4 (CH), 127.1 (CH), 126.0 (CH), 117.2 (CH), 74.6 (CH), 42.9 (CH), 38.0 (CH_2), 25.7 (CH_3), 25.5 (CH_2), 18.0 (C), 15.8 (CH_3), −4.5(CH_3), −4.6 (CH_3); IR υ_{max} ($CHCl_3$) 2957, 2931,2858, 1772, 1741, 1648, 1364, 1254, 1092, 1069, 838 cm^{-1}; UV (EtOH) λ_{max} 249 ($\epsilon = 11184$), 292 ($\epsilon = 1185$) nm; FDMS *m*/*z* 457 (M^+, 100). Anal. Calcd for $C_{25}H_{35}NO_5$: C, 65.61; H, 7.71; N, 3.06. Found: C, 65.51; H, 7.56; N, 3.02.

1-[[(5*S*)-Hydroxy-(6*R*)-methyl-1-oxo-8-phenyl-(2*E*,7*E*)-octadienyl]oxy]-2,5-pyrrolidinedione, 13. To a stirred solution of silyl ether **12** (2.50 g, 5.47 mmol) in CH_3CN (130 mL) was added 48% aqueous HF (15 mL) at 0 °C, and the solution stirred at 0 °C for 0.75 h and at room temperature for 4 h. The reaction was diluted with diethyl ether (300 mL), and the organics were washed with H_2O until the aqueous extract was ~pH 7. Organics were dried ($MgSO_4$) and concentrated in vacuo to give a yellow residue which crystallized from Et_2O to give alcohol **13** as white crystals (1.46 g, 78%): $[\alpha]_D^{589}$ −57.8° ($c = 1.06$, $CHCl_3$); ^{1}H NMR ($CDCl_3$) *δ* 7.41−7.20 (m, PhH_5, 3-H), 6.48 (d, $J = 16$ Hz, 8-H), 6.15−6.07 (m, 7-H, 2-H), 3.71−3.65 (m, 5-H), 2.83 (br s, CH_2CH_2), 2.60−2.33 (m, 6-H,4-CH_2),1.95 (brs, 5-OH), 1.14 (d, $J = 6.8$ Hz, 6-Me); ^{13}C NMR ($CDCl_3$) *δ* 169.2 (C), 161.0 (C), 152.4 (CH), 136.8 (C), 132.1 (CH), 130.6 (CH), 128.5 (CH), 127.4 (CH), 126.1 (CH), 117.4 (CH), 73.4 (CH), 43.5 (CH), 37.7 (CH_2), 25.5 (CH_2), 16.7 (CH_3); IR (KBr) υ_{max} 3457, 1804, 1773, 1735, 1724, 1209, 1099, 1067, 1049, 975, 744, 694 cm^{-1}; UV (EtOH) λ_{max} 250 ($\epsilon = 20535$) nm; FDMS *m*/*z* 343.2 (M^+, 100). Anal. Calcd for $C_{19}H_{21}NO_5$: C, 66.46; H, 6.16; N, 4.08. Found: C, 66.49; H, 6.16; N, 4.07.

(2*S*)-[3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2,2-dimethyl-1-oxopropoxy]-4-methylpentanoic Acid, 14. Carboxylic acid **14** was prepared[31] according to the literature procedure:[24a] $[\alpha]_D^{589}$ −29.9° ($c = 1.1$, MeOH); ^{1}H NMR (DMSO-d_6) *δ* unit C′ 6.61 (t, $J = 6.2$ Hz, NH), 3.16 (dd, $J = 13.6$ and 7.15 Hz, 3-H), 3.04 (dd, $J = 13.6$ and 5.7 Hz, 3′H), 1.33 (br s, CMe_3), 1.04 (br s, 6H, Me_2); unit D 13.08 (br s, CO_2H), 4.78 (dd, $J = 8.78$ and 3.72 Hz, 2-H), 1.72−1.60 (m, 3-H_2), 1.59−1.54 (m, 4-H), 0.88 (d, $J = 6.7$ Hz, 4-Me), 0.85 (d, $J = 6.6$ Hz, 5-H_3); ^{13}C NMR (DMSO-d_6) *δ* 175.3 (C), 171.9 (C), 155.8 (C), 77.7 (C), 70.5 (CH), 47.9 (CH_2), 43.2 (C), 39.2 (CH_2), 28.1 (3 × CH_3), 24.3 (CH), 22.8 (CH_3), 22.4 (CH_3), 22.2 (CH_3), 21.5 (CH_3); IR ($CHCl_3$) υ_{max} 2980, 2964, 1711, 1512, 1368, 1270, 1254, 1151 cm^{-1}; ESMS (PI) *m*/*z* 232 ($[M - Boc + 1]^+$, 100); (NI) *m*/*z* 300 ($[M - 1]^-$, 100). Anal. Calcd for $C_{16}H_{29}NO_6$: C, 57.99; H, 8.82; N, 4.23. Found: C, 57.95; H, 8.93; N, 4.28.

(1*S*,3*E*)-5-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-1-[(1*R*,2*E*)-1-methyl-3-phenyl-2-propenyl]-5-oxo-3-pentenyl-(2*S*)-[3-[[(1,1-dimethylethoxy)carbonyl]amino]-2,2-dimethyl-1-oxopropoxy]-4-methylpentanoate, 15. To a suspension of carboxylic acid **14** (1.28 g, 3.87 mmol) in dry dichloromethane (6 mL) were added EDC (742 mg, 3.87 mmol) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) (73 mg, 0.60 mmol), and the mixture stirred at room temperature, under a nitrogen atmosphere, for 0.5 h. A solution of alcohol **13** (1.02 g, 2.97 mmol) in dichloromethane (5.5 mL) was then added, and the reaction mixture stirred for a further 0.3 h. The reaction was diluted with dichloromethane (200 mL), and the organics were washed consecutively with 1 N aqueous HCl (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and H_2O (50 mL). The organics were dried ($MgSO_4$) and concentrated in vacuo to leave an oily residue, which was purified by column chromatography (gradient: 10−30% EtOAc/*n*-hexanes) to provide desired ester **15** as a yellow solid (1.68 g,

79%): $[\alpha]_D^{589}$ +39.5° (c = 1.04, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ unit A 7.35−7.20 (m, PhH_5, 3-H), 6.43 (d, J = 15.8 Hz, 8-H), 6.12 (d, J = 15.9 Hz,2-H), 5.99 (dd, J = 8.5 and 15.8 Hz, 7-H), 5.06−5.08 (m, 5-H), 2.85 (br s, CH_2CH_2), 2.68−2.61 (m, 6-H, 4-CH_2), 1.13 (d, J = 6.8 Hz, 6-Me); unit C 5.32 (br t, J = 6.0 Hz NH), 3.28−3.25 (m, 3-CH_2),1.43 (s, CMe_3), 1.21 (s, 2-Me), 1.19 (s, 2-Me); unit D 4.95 (dd, J = 9.8 and 3.8 Hz, 2-H), 1.73−1.64 (m, 3-H, 4-H), 1.59−1.49 (m, 3-H′), 0.85 (d, J = 6.4 Hz, 5-Me), 0.82 (d, J = 6.4, 4-Me); ^{13}C NMR ($CDCl_3$) δ 176.2 (C), 170.6 (C), 169.0 (C), 160.7 (C), 156.3 (C), 149.4 (CH), 136.7 (C), 131.7 (CH), 129.6 (CH), 128.5 (CH), 127.5 (CH), 126.1 (CH), 118.5 (CH), 78.9 (C), 75.8 (CH), 71.1 (CH), 69.3 (CH), 48.4 (CH_2), 43.8 (C), 40.6 (CH), 39.5 (CH_2), 35.0 (CH_2), 28.3 (CH_3), 25.5 (CH_2), 25.4 (C), 24.7 (CH), 22.8 (CH_3), 22.7 (CH_3), 22.4 (CH_3), 22.3 (CH_3), 21.5 (CH_3), 21.2 (CH_3), 16.7 (CH_3); IR (KBr) υ_{max} 3400, 2975, 1743,1367, 1206, 1126, 1145, 1068 cm^{-1}; UV (EtOH) λ_{max} 251 (ϵ = 16086), 284 (ϵ = 1953), 293 (ϵ = 1389) nm; FDMS m/z 657 (M^+, 100). Anal. Calcd for $C_{35}H_{48}N_2O_{10}$: C, 64.01; H, 7.37; N, 4.27. Found: C, 64.19; H, 7.27; N, 4.52.

3-Chloro-D-tyrosine, 9r. A suspension of *O*-methyl-D-tyrosine (**9q**)[15] (1.20 g, 5 mmol) in 48% aqueous hydrobromic acid (4 mL) and phenol (1.6 g, 17 mmol) was heated under reflux for 6 h. The reaction solution was concentrated to a dry solid, diluted with a mixture of *n*-BuOAc/EtOAc (1:9, 50 mL), and extracted with H_2O (2 × 5 mL). The aqueous phase was adjusted to pH 7 with concentrated NH_4OH and the resulting precipitate filtered, washed with H_2O (10 mL), and dried under vacuum to give chlorotyrosine **9r** as a white solid (760 mg, 69%): $[\alpha]_D^{589}$ −12.0° (c = 3.05, MeOH); ^{1}H NMR (DMSO-d_6/D_2O) δ 7.16 (d, J = 1.6 Hz, PhH), 6.97 (dd, J = 8.11 and 1.7 Hz, PhH), 6.81 (d, J = 8.3 Hz, PhH), 3.37 (dd, J = 8.1 and 4.1 Hz, 2-H), 3.97 (dd, J = 14.5 and 4.2 Hz, 3-H), 2.73 (dd, J = 14.4 and 8.2 Hz, 3-H′); ^{13}C NMR (DMSO-d_6) δ 170.4 (C), 152.4 (C), 130.8 (CH), 129.2 (CH), 126.5 (C), 119.6 (C), 116.8 (CH), 53.4 (CH), 34.7 (CH_2); FDMS m/z 215 (M^+, 100). Anal. Calcd for $C_9H_{10}NClO_3$: C, 50.13; H, 4.67; N; 6.50. Found: C, 50.40; H, 4.57; N, 6.81.

4-Amino-D-phenylalanine Methyl Ester, Dihydrochloride, 18. Acetyl chloride (20 mL, 0.28 mmol) was added dropwise to dry methanol (300 mL) at 0 °C under a dry nitrogen atmosphere. The solution was stirred at room temperature for 0.5 h and *p*-amino-D-phenylalanine (**9k**) (10 g, 0.056 mol) added in one portion. The mixture was heated at reflux for 3.5 h and concentrated in vacuo to provide methyl ester **18** as a hygroscopic, white solid (15.0 g, 100%): $[\alpha]_D^{589}$−5.9° (c = 2.02, H_2O); ^{1}H NMR (DMSO-d_6) δ 7.30−7.24 (m, PhH_4), 4.19 (t, J = 6.1, 2-H), 3.61 (s, OMe), 3.10−3.07 (m, 3-H_2); IR (KBr) υ_{max} 3414 (br), 2877 (br), 2589, 1744, 1509, 1245 cm^{-1}; UV (EtOH) λ_{max} 243 (ϵ = 10037), 292 (ϵ = 1347) nm; ESMS (NI) m/z 265.1 ($[M - 1]^-$, 48). Anal. Calcd for $C_{10}H_{16}N_2Cl_2O_2 \cdot 0.5H_2O$: C, 43.49; H, 6.20; N, 10.14; Cl, 25.68. Found: C, 43.85; H, 7.09; N, 9.86; Cl, 25.28.

***N*-Acetyl-4-(acetylamino)-D-phenylalanine Methyl Ester, 19.** To a stirred solution of diamine **18** (14.5 g, 0.054 mmol) in dry pyridine (256 mL) was added acetic anhydride (77 mL, 0.815 mol), and the mixture stirred at room temperature for 17.5 h. The resulting purple solution was concentrated in vacuo, diluted with H_2O (250 mL), neutralized to pH 7 with 5 N aqueous HCl, and extracted with a mixture of THF/EtOAc (1:5; 5 × 200 mL). Combined, dried ($MgSO_4$) organics were concentrated in vacuo to furnish fully protected amino acid **19** as a yellow powder (12.2 g, 81%): $[\alpha]_D^{589}$−40.1° (c = 0.85, MeOH); ^{1}H NMR (DMSO-d_6) δ 9.88 (s, NH), 8.28 (d, J = 7.7 Hz, NH), 7.43 (d, J = 8.4 Hz, PhH_2), 7.07 (d, J = 8.4 Hz, PhH_2), 4.35 (app. q, J = 2.8 Hz, 2-H), 3.54 (s, OMe), 2.89 (dd, J = 13.8 and 5.7 Hz, 3-H), 2.76 (dd, J = 13.7 and 9.1 Hz, 3-H′), 1.98 (s, $CH_3C{=}O$), 1.75 (s, $CH_3C{=}O$); IR (KBr) υ_{max} 3255 (br), 3066, 2401, 2375, 1746, 1651, 1542, 1519, 1403, 1214 cm^{-1}; UV (EtOH) λ_{max} 247 (ϵ = 15859) nm; FDMS m/z 278 (M^+, 100). Anal. Calcd for $C_{14}H_{18}N_2O_4$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.48; H, 6.48; N, 9.95.

***N*-Acetyl-4-(acetylamino)-3-chloro-D-phenylalanine Methyl Ester, 20.** To a stirred suspension of protected amino acid **19** (2.78 g, 10 mmol) in dry acetonitrile (175 mL) was added *N*-chlorosuccinimide (1.86 g, 14 mmol), and the mixture was heated under reflux for 19.5 h. The solvent was removed in vacuo and the residue diluted with EtOAc (200 mL) and washed with H_2O (2 × 50 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo to leave an oily residue which was purified by column chromatography (SiO_2; gradient elution: 30−100% EtOAc/*n*-hexanes) to provide chloride **20** as a white powder (1.56 g, 50%): $[\alpha]_D^{589}$ −27.4° (c = 0.80, MeOH); ^{1}H NMR (DMSO-d_6) δ 9.42 (s, NH), 8.31 (d, J = 7.8 Hz, NH), 7.55 (d, J = 8.2 Hz, PhH), 7.30 (d, J = 1.3 Hz, PhH), 7.10 (dd, J = 8.3 and 1.4 Hz, PhH), 4.44−4.37 (m, 2-H), 3.57 (s, OMe), 2.96 (dd, J = 13.8 and 5.4 Hz, 3-H), 2.79 (dd, J = 13.9 and 9.5 Hz, 3-H′), 2.03 (s, $CH_3C{=}O$), 1.75 (s, $CH_3C{=}O$); IR (KBr) υ_{max} 3291 (br), 1754, 1745, 1663, 1653, 1555, 1528, 1404, 1377, 1302 cm^{-1}; UV (EtOH) λ_{max} 244 (ϵ = 10197) nm; FDMS m/z 312 (M^+, 100). Anal. Calcd for $C_{14}H_{17}ClN_2O_4$: C, 53.77; H, 5.48; N, 8.96; Cl, 11.34. Found: C, 54.01; H, 5.54; N, 8.89; Cl, 11.57.

4-Amino-3-chloro-D-phenylalanine, 9s. To a stirred solution of protected amino acid **20** (1.00 g, 3.21 mmol) in dioxane (20 mL) was added 1 N aqueous NaOH (12.8 mL, 12.8 mmol). The mixture was heated under reflux for 24 h and the solvent removed in vacuo to give a solid. Crude product was redissolved in 5 N aqueous hydrochloric acid (30 mL) and heated at 95 °C for 18 h. The reaction solution was concentrated in vacuo and the resulting solid residue triturated with methanol (6 × 50 mL) to remove the majority of the inorganic salts. The product was further purified using cation-exchange resin (SCX column; 10 g for 400 mg of crude product) and eluting, under gravity, first with CH_3CN:H_2O (1:1) until eluent was pH 7 to remove the remaining inorganics and then with 2 M NH_3−MeOH:CH_3CN (1:1) to provide amino acid **9s** as an off-white solid (493 mg, 72%): $[\alpha]_D^{589}$ +23.4° (c = 0.26, H_2O); ^{1}H NMR (CD_3OD) δ 7.20 (d, J = 8.1 and 2.0 Hz, PhH), 6.83 (d, J = 8.2 Hz, PhH), 3.71 (dd, J = 8.7 and 4.4 Hz, 2-H), 3.18 (dd, J = 14.7 and 4.3 Hz, 3-H), 2.90 (dd, J = 14.6 and 8.7 Hz, 3-H′); IR (KBr) υ_{max} 3400 (br), 2900 (br), 1598, 1508, 1402, 1313 cm^{-1}; UV (EtOH) λ_{max} 243 (ϵ = 10754), 298 (ϵ = 2277) nm; FABMS m/z 215 ($[M + 1]^+$, 28). Anal. Calcd for $C_9N_{11}ClN_2O_2 \cdot 0.1H_2O$: C, 49.94; H, 5.22; N, 12.95; Cl, 16.38. Found: C, 49.93; H, 4.97; N, 12.57; Cl, 16.40.

Preparation of Carboxylic Acid 16 (General Method A). To a stirred solution of active ester **15** (1.0 mmol) in dimethylformamide (10 mL) were added *N*,*O*-bis(trimethylsilyl)acetamide (BSA) (3.0 mmol) and amino acid **9** (1.5 mmol). The mixture was heated at 55−60 °C, under a nitrogen atmosphere, until all active ester was consumed as judged by TLC analysis (reaction time = 10−30 h). The reaction solution was poured into 1 N aqueous HCl (200 mL) and extracted with EtOAc (3 × 50 mL). Combined organics were washed with 1 N aqueous HCl, brine, and H_2O (100 mL each), dried ($MgSO_4$), and concentrated in vacuo to provide carboxylic acid **16** as a solid. Further purification, where necessary, was performed using column chromatography (SiO_2; gradient elution: 2−5% AcOH in CH_2Cl_2−15% MeOH:CH_2Cl_2). ^{1}H NMR data were consistent with the assigned structures (see Table 2 for representative examples).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoylglycine, 16a.** Active ester **15** was coupled with glycine (**9a**), according to general method A, to give **16a** as a pale-yellow foam (93%): FABMS m/z 617.5 ($[M + 1]^+$, 25), 517.4 ($[M - Boc + 1]^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-valine, 16b.** Active ester **15** was coupled with D-valine (**9b**), according to general method A, to give **16b** as a pale-yellow foam (96%): ESMS (PI) m/z 659.5 ($[M + 1]^+$, 48), 559.4 ($[M - Boc + 1]^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-threo-**

nine, 16c. Active ester **15** was coupled with D-threonine (**9c**), according to modified general method A using BSA (4 equiv), to give **16c** as a pale-yellow foam (89%): ESMS (PI) *m*/*z* 661.4 ([M + 1]$^+$, 33), 561.3 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-proline, 16d.** Active ester **15** was coupled with D-proline (**9d**), according to general method A, to give **16d** as a yellow foam (92%): FABMS *m*/*z* 657.5 ([M + 1]$^+$, 60), 557.5 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-cyclohexyl-D-alanine, 16e.** Active ester **15** was coupled with cyclohexyl-D-alanine (**9e**), according to general method A, to give **16e** as a pale-yellow foam (88%): ESMS (PI) *m*/*z* 713.7 ([M + 1]$^+$, 65), 613.4 ([M − Boc + 1]$^+$, 100).

1-(1,1-Dimethylethyl)-(7*S*,10*S*,12*E*,16*R*)-4,4-dimethyl-10-[(1*R*,2*E*)-1-methyl-3-phenyl-2-propenyl]-7-(2-methylpropyl)-5,8,14-trioxo-16-(2-phenylethyl)-6,9-dioxa-2,15-diazaheptadec-12-enedioate, 16f. Active ester **15** was coupled with D-homophenylalanine (**9f**), according to general method A, to give **16f** as a pale-yellow foam (72%): ESMS (PI) *m*/*z* 721.5 ([M + 1]$^+$, 55), 621.6 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-*O*-(phenylmethyl)-D-serine, 16g.** Active ester **15** was coupled with *O*-benzyl-D-serine (**9g**), according to general method A, to give **16g** as a pale-yellow foam (72%): ESMS (PI) *m*/*z* 737.5 ([M + 1]$^+$, 70), 637.3 ([M − Boc + 1]$^+$, 100).

(2*R*)-*N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,-7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-2-(4-hydroxyphenyl)glycine, 16h. Active ester **15** was coupled with d-(4-hydroxyphenyl)glycine (**9h**), according to modified general method A using BSA (4 equiv), to give a yellow solid. Crude product was dissolved in 10% AcOH/CH_2Cl_2, stirred for 1 h, and concentrated in vacuo to give a solid which was purified by column chromatography (SiO_2; gradient elution: 3% AcOH in CH_2Cl_2−15% MeOH:CH_2Cl_2) to give **16h** as an off-white foam (75%): ESMS (PI) *m*/*z* 709.5 ([M + 1]$^+$, 50), 609.3 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-phenylalanine, 16i.** Active ester **15** was coupled with D-phenylalanine (**9i**), according to general method A, to give **16i** as a pale-yellow foam (88%): ESMS (PI) *m*/*z* 707.5 ([M + 1]$^+$, 50), 607.4 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-tyrosine, 16j.** Active ester **15** was coupled with D-tyrosine (**9j**), according to modified general method A using BSA (4 equiv), to give **16j** as an off-white solid (87%): ESMS (PI) *m*/*z* 723.5 ([M + 1]$^+$, 4), 623.5 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-4-amino-D-phenylalanine, 16k.** Active ester **15** was coupled with *p*-amino-D-phenylalanine (**9k**), according to modified general method A using BSA (6 equiv), to give **16k** as an orange foam (72%): ESMS (PI) *m*/*z* 722.5 ([M + 1]$^+$, 54), 622.6 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-4-iodo-D-phenylalanine, 16p.** Active ester **15** was coupled with *p*-iodo-D-phenylalanine (**9p**), according to general method A, to give **16p** as a pale-yellow solid (86%): ESMS (PI) *m*/*z* 833.7 ([M + 1]$^+$, 57), 733.4 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-chloro-D-tyrosine, 16r.** Active ester **15** was coupled with 3-chloro-D-tyrosine (**9r**), according to modified general method A using BSA (4 equiv), to give **16r** as a pale-yellow solid (67%): ESMS (PI) *m*/*z* 757.5 ([M + 1]$^+$, 50), 657.4 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-4-amino-3-chloro-D-phenylalanine, 16s.** Active ester **15** was coupled with 3-chloro-4-amino-D-phenylalanine (**9s**), according to modified general method A using BSA (6 equiv), to give **16s** as a golden solid (61%): ESMS (PI) *m*/*z* 756.6 ([M + 1]$^+$, 41), 656.4 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-hydroxy-D-tyrosine, 16v.** Active ester **15** was coupled with 3,4-dihydroxy-D-phenylalanine (D-DOPA) (**9v**), according to modified general method A using BSA (5 equiv), to give **16v** as a pale-yellow solid (75%): ESMS (PI) *m*/*z* 739.6 ([M + 1]$^+$, 46), 639.3 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-(1-naphthalenyl)-D-alanine, 16w.** Active ester **15** was coupled with 3-(1-naphthyl)-D-alanine (**9w**), according to general method A, to give **16w** as a pale-yellow solid (77%): ESMS (PI) *m*/*z* 757.6 ([M + 1]$^+$, 39), 657.4 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-(2-naphthalenyl)-D-alanine, 16x.** Active ester **15** was coupled with 3-(2-naphthyl)-D-alanine (**9x**), according to general method A, to give **16x** as a pale-yellow solid (78%): ESMS (PI) *m*/*z* 757.6 ([M + 1]$^+$, 60), 657.4 ([M − Boc + 1]$^+$, 100).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-(3-pyridinyl)-D-alanine, 16y.** Active ester **15** was coupled with 3-(3-pyridyl)-D-alanine (**9y**), according to general method A, to give **16y** as a pale-yellow solid (65%): ESMS (PI) *m*/*z* 708.5 ([M + 1]$^+$, 100), 608.3 ([M − Boc + 1]$^+$, 57).

***N*-[(1,1-Dimethylethoxy)carbonyl]-2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-tryptophan, 16z.** Active ester **15** was coupled with D-tryptophan (**9z**), according to general method A, to give **16z** as an off-white solid (85%): ESMS (PI) *m*/*z* 746.4 ([M + 1]$^+$, 60), 646.7 ([M − Boc + 1]$^+$, 100).

Preparation of Styrene 7 (General Method B). A solution of Boc amine **16** (1 mmol) in trifluoroacetic acid (10−20 mL) was stirred at room temperature and under a nitrogen atmosphere for 2−3 h. The solvent was removed in vacuo and the crude residue azeotroped with toluene (3 × 50 mL) and dried under high vacuum (5−24 h) to provide the corresponding amine salt as a solid, which was used in the next step without further purification. To a stirred solution of amine salt (1 mmol) in dry dimethylformamide (200 mL, 5 mM) was added *N*,*N*-diisopropylethylamine (DIPA) (3.0 mmol) followed by pentafluorophenyl diphenylphosphinate (FDPP) (1.5 mmol). The mixture was stirred under a nitrogen atmosphere for 15−40 h. The solvents were removed in vacuo and the residue purified by column chromatography (SiO_2; gradient elution: 20−80% EtOAc/*n*-hexanes) to provide styrene **7** as a solid. ^{1}H NMR data were consistent with the assigned structures (see Table 4 for representative examples).

Cyclo[2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoylglycyl], 7a. Boc amine **16a** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7a** as a white foam (76%): FDMS *m*/*z* 498 (M$^+$, 100).

Cyclo[2,2-dimethyl-*β*-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-

2,7-octadienoyl-D-valyl], 7b. Boc amine **16b** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7b** as an off-white solid (61%): FDMS *m*/*z* 541 (M^+, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-threonyl], 7c. Boc amine **16c** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7c** as a white powder (51%): FDMS *m*/*z* 542 (M^+, 100), 498 ($[M - C_2H_4O]^+$, 65).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-prolyl], 7d. Boc amine **16d** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7d** as an off-white solid (61%): FDMS *m*/*z* 541 (M^+, 100).

Cyclo[3-cyclohexyl-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl], 7e. Boc amine **16e** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7e** as a white solid (56%): FDMS *m*/*z* 595 (M^+, 100).

(3*S*,10*R*,13*E*,16*S*)-6,6-Dimethyl-16-[(1*R*,2*E*)-1-methyl-3-phenyl-2-propenyl]-3-(2-methylpropyl)-10-(2-phenylethyl)-1,4-dioxa-8,11-diazacyclohexadec-13-ene-2,5,9,12-tetrone, 7f. Boc amine **16f** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7f** as an off-white solid (62%): FDMS *m*/*z* 602.3 (M^+, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-*O*-(phenylmethyl)-D-seryl], 7g. Boc amine **16g** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7g** as an off-white solid (57%): FDMS *m*/*z* 619 (M^+, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-(2*R*)-2-(4-hydroxyphenyl)glycyl], 7h. Boc amine **16h** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7h** as a white solid (59%): FDMS *m*/*z* 590 (M^+, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-phenylalanyl], 7i. Boc amine **16i** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7i** as a white solid (55%): ESMS (PI) *m*/*z* 589.5 ($[M + 1]^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-D-tyrosyl], 7j. Boc amine **16j** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7j** as a yellow solid (80%): FDMS *m*/*z* 604 (M^+, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-4-amino-D-phenylalanyl], 7k. Boc amine **16k** was deprotected and the resulting amino acid cyclized, according to modified general method B using DIPA (6 equiv) and FDPP (3 equiv), to provide styrene **7k** as an orange foam (58%): ESMS (PI) *m*/*z* 604.4 ($[M + 1]^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-4-[[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino]-D-phenylalanyl], 7m. To a stirred solution of aniline **7k** (205 mg, 0.340 mmol) in a mixture of dioxane−H_2O (5:1, 20 mL) was added $NaHCO_3$ (43 mg, 0.510 mmol) followed by Fmoc-Cl (106 mg, 0.408 mmol) at room temperature. The mixture was stirred for 31.5 h, diluted with EtOAc (100 mL), and washed with H_2O (100 mL). The dried ($MgSO_4$) organic phase was concentrated in vacuo and the resulting crude oil purified by column chromatography (SiO_2; gradient elution: 25−75% EtOAc/*n*-hexanes) to give aniline **7m** as an off-white solid (248 mg, 88%): $[\alpha]_D^{589}$ +16.4° (*c* = 0.37, MeOH); ^{1}H NMR ($CDCl_3$) δ unit A 7.36−7.11 (m, PhH_5), 6.82−6.72 (m, 3-H), 6.41 (d, *J* = 15.8 Hz, 8-H), 6.01 (dd, *J* = 15.9 and 8.8 Hz, 7-H), 5.75 (d, *J* = 15.3 Hz, 2-H), 5.07−5.02 (m), 2.57−2.50 (m, 4-H, 6-H), 2.43−2.31 (m, 4-H′), 1.14 (buried d, 6-Me); unit B 8.06−7.99 (br s, NH), 7.80 (d, *J* = 7.4 Hz, PhH_2), 7.62 (d, *J* = 7.4 Hz, PhH_2), 7.43 (t, *J* = 7.4 Hz, PhH_2), 7.36−7.11 (m, PhH_4), 7.08 (d, *J* = 8.4 Hz, PhH_2), 5.47 (d, *J* = 7.7 Hz, NH), 4.77 (app. q, *J* = 6.0 Hz, Fmoc-CH), 4.51 (d, *J* = 6.8 Hz, Fmoc-CH_2), 4.31 (app. q, *J* = 6.8 Hz, 2-H); unit C 7.36−7.11 (m), 3.39 (dd, *J* = 13.3 and 8.3 Hz, 3-H), 3.17−3.11 (m, 3-H′), 0.74 (overlapping d, *J* = 5.9 Hz, 2-Me), 0.72 (overlapping d, *J* = 6.0 Hz, 2-Me); unit D 4.84 (dd, *J* = 9.9 and 2.5 Hz, 2-H), 1.72−1.52 (m, 3-H, 4-H), 1.37−1.28 (m, 3-H′), 1.23 (s, 5-H_3), 1.16 (s, 4-Me); IR (KBr) υ_{max} 3450 (br), 2960, 1740, 1717, 1680, 1525, 1479, 1152 cm^{-1}; UV (EtOH) λ_{max} 227 (ε = 29075), 247 (ε = 41236); 288 (ε = 5785), 299 (ε = 5349) nm; ESMS (PI) *m*/*z* 860.7 (M^+, 80). Anal. Calcd for $C_{50}H_{54}ClN_3O_8$: C, 69.80; H, 6.33; N, 4.88; Cl, 4.12. Found: C, 70.04; H, 6.22; N, 4.78; Cl, 4.43.

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-4-iodo-D-phenylalanyl], 7p. Boc amine **16p** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7p** as a yellow solid (22%): FDMS *m*/*z* 715 (M^+, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-chloro-D-tyrosyl], 7r. Boc amine **16r** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7r** as a white solid (42%): FDMS *m*/*z* 638 (M^+, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-chloro-4-amino-D-phenylalanyl], 7s. Boc amine **16s** was deprotected and the resulting amino acid cyclized, according to modified general method B using DIPA (6 equiv) and FDPP (3 equiv), to provide styrene **7s** as a yellow solid (62%): ESMS (PI) *m*/*z* 638.3 ($[M + 1]^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-chloro-4-[[(9*H*-fluoren-9-ylmethoxy)-carbonyl]amino]-D-phenylalanyl], 7t. To a stirred solution of aniline **7s** (305 mg, 0.470 mmol) in a mixture of dioxane−H_2O (5:1, 20 mL) was added $NaHCO_3$ (60 mg, 0.0718 mmol) followed by Fmoc-Cl (120 mg, 0.575 mmol). The mixture was stirred at room temperature for 48 h, diluted with EtOAc (150 mL), and washed with H_2O (100 mL). The dried ($MgSO_4$) organic phase was concentrated in vacuo and the resulting crude oil purified by column chromatography (SiO_2; gradient elution: 50−70% EtOAc/*n*-hexanes) to provide desired aniline **7t** as a yellow foam (284 mg, 69%): $[\alpha]_D^{589}$ +29.2° (*c* = 0.96, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ unit A 7.42−7.31 (m, PhH_5), 6.86−6.77 (m, 3-H), 6.45 (d, *J* = 15.8 Hz, 8-H), 6.05 (dd, *J* = 16 and 8.9 Hz, 7-H), 5.77 (d, *J* = 15.3 Hz, 2-H), 5.12−5.06 (m), 2.62−2.55 (m, 4-H, 6-H), 2.46−2.31 (m, 4-H′), 1.17 (d, *J* = 6.9 Hz, 6-Me); unit B 7.83 (d, *J* = 7.5 Hz, PhH_2), 7.66 (d, *J* = 7.3 Hz, PhH_2), 7.46 (t, *J* = 7.4 Hz, PhH_2), 7.42−7.31 (m, PhH_4), 7.16 (d, *J* = 8.2 Hz, PhH_2), 5.62 (d, *J* = 7.8 Hz, NH), 4.80 (app. q, *J* = 6.7 Hz, Fmoc-CH), 4.57 (d, *J* = 6.7 Hz, Fmoc-CH_2), 4.32 (app. q, *J* = 6.7 Hz, 2-H); unit C 7.29−7.24 (m), 3.48 (dd, *J* = 13.6 and 9.0 Hz, 3-H), 3.21−3.08 (m, 3-H′), 0.78 (d, *J* = 4.7 Hz, 2-Me), 0.76 (d, *J* = 5.2 Hz, 2-Me); unit D 4.89 (dd, *J* = 9.9 and 3.4 Hz, 2-H), 1.76−1.60 (m, 3-H, 4-H), 1.41−1.30 (m, 3-H′), 1.26 (s, 5-H_3), 1.20 (s, 4-Me); IR (KBr) υ_{max} 3430 (br), 3010, 2964, 1736, 1717, 1680, 1525, 1486, 1451, 1317, 1304, 1187, 1152 cm^{-1}; UV (EtOH) λ_{max} 246 (ε = 47652), 288 (ε = 5650); 300 (ε = 5419) nm; ESMS (PI) *m*/*z* 826.7 ($[M + 1]^+$, 33). Anal. Calcd for $C_{50}H_{55}N_3O_8$: C, 72.71; H, 6.71; N, 5.09. Found: C, 72.40; H, 6.53; N, 4.98.

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*, 7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl-3-hydroxy-D-tyrosyl], 7v. Boc amine **16v** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7v** as a white solid (52%): ESMS (PI) *m*/*z* 621.6 ($[M + 1]^+$, 100).

Cyclo[3-(1-naphthalenyl)-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-

hydroxy-6-methyl-8-phenyl-2,7-octadienoyl], 7w. Boc amine **16w** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7w** as a tan foam (56%): FDMS *m/z* 638 (M^+, 100).

Cyclo[3-(2-naphthalenyl)-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl], 7x. Boc amine **16x** was deprotected and the resulting amino acid cyclized, according to general method B, to provide styrene **7x** as a tan foam (65%): FDMS *m/z* 638.2 (M^+, 100).

Cyclo[3-(3-pyridinyl)-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*R*,7*E*)-5-hydroxy-6-methyl-8-phenyl-2,7-octadienoyl], 7y. Boc amine **16y** was deprotected and the resulting amino acid cyclized, according to modified general method B using DIPA (4 equiv) and FDPP (2.0 equiv), to provide styrene **7y** as a white powder (71%): ESMS (PI) *m/z* 590.5 ([M + 1]$^+$, 100).

Preparation of β-Epoxide 6 (General Method C). To a solution of styrene **7** (1 mmol) in dichloromethane (5 mL, 0.2 M) was added purified *m*-chloroperbenzoic acid (*m*CPBA) (1.1 mmol), and the mixture was stirred, under a nitrogen atmosphere, until all starting material **7** was consumed as judged by TLC and HPLC analysis (24−48 h). The reaction was diluted with dichloromethane (100 mL) and washed with 10% aqueous sodium meta-bisulfite ($Na_2S_2O_5$) (100 mL), H_2O (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and H_2O (100 mL). The dried (Na_2SO_4) organic phase was concentrated in vacuo to give a mixture of α:β-epoxides, which were separated by RP-HPLC (Kromasil C18; gradient elution: CH_3CN-H_2O; flow rate = 28 mL/min; UV detection at λ_{max} = 220 nm) to provide the desired β-epoxide **6** (in a few examples α-epoxide **17** was also isolated). ^{1}H NMR data for β-epoxide **6** was consistent with assigned structures (see Table 6 for representative data).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoylglycyl], 6a. Styrene **7a** was epoxidized, according to general method C, to provide **6a** as a white solid (48%): ESMS (PI) *m/z* 515.5 ([M + 1]$^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-D-valyl], 6b. Styrene **7b** was epoxidized, according to general method C, to provide **6b** as a white solid (30%): ESMS (PI) *m/z* 557.1 ([M + 1]$^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-D-threonyl], 6c. Styrene **7c** was epoxidized, according to general method C, to provide **6c** as a white solid (22%): ESMS (PI) *m/z* 559.4 ([M + 1]$^+$, 100), 541.3 ([M − H_2O + 1]$^+$, 90).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-D-prolyl], 6d. Styrene **7d** was epoxidized, according to general method C, to provide **6d** as a white solid (30%): ESMS (PI) *m/z* 555.3 ([M + 1]$^+$, 100).

Cyclo[3-cyclohexyl-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl], 6e. Styrene **7e** was epoxidized, according to modified general method C using dichloromethane:toluene (2:1), to provide **6e** as a white solid (18%): FDMS *m/z* 610.1 (M^+, 100).

(3*S*,10*R*,13*E*,16*S*)-6,6-Dimethyl-3-(2-methylpropyl)-10-(2-phenylethyl)-16-[(1*S*)-1-[(2*R*,3*R*)-3-phenyloxiranyl]-ethyl]-1,4-dioxa-8,11-diazacyclohexadec-13-ene-2,5,9,12-tetrone, 6f, and (3*S*,10*R*,13*E*,16*S*)-6,6-Dimethyl-3-(2-methylpropyl)-10-(2-phenylethyl)-16-[(1*S*)-1-[(2*S*,3*S*)-3-phenyloxiranyl]ethyl]-1,4-dioxa-8,11-diazacyclohexadec-13-ene-2,5,9,12-tetrone, 17f. Styrene **7f** was epoxidized, according to modified general method C using dichloromethane:toluene (2:1), to provide (i) β-epoxide **6f** as a white solid (25%): FDMS *m/z* 618.2 (M^+, 100) and (ii) α-epoxide **17f** as a white solid (10%): t_R = 27.5 min[b] (refer to Table 5, footnote 3); $[\alpha]_D^{589}$ +3.8° (*c* = 0.59, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ unit A 7.46−7.21 (m, Ph_5), 6.88−6.78 (m, 3-H), 5.81 (d, *J* = 15.4 Hz, 2-H), 5.28−5.22 (m, 5-H), 3.66 (s, 8-H), 2.99 (d, *J* = 7.7 Hz, 7-H), 2.75−2.64 (m, 4-H, 6-H), 1.84−1.70 (m, 4-H′), 1.11 (d, *J* = 7 Hz, 6-Me); unit B 7.46−7.21 (m, PhH_5), 5.62 (d, *J* = 7.4 Hz, NH), 4.59−4.52 (m, 2-H), 2.79 (dd, *J* = 7.9 and 5.8 Hz, 4-H_2), 2.44−2.36 (m, 3-H), 2.08−1.86 (m, 3-H′); unit C 3.47−3.40 (m, 3-H), 3.25 (dd, *J* = 14 and 3.7 Hz, 3-H′), 1.30 (s, 2-Me), 1.25 (s, 2-Me); unit D 5.04 (dd, *J* = 9.7 and 2 Hz, 2-H), 1.84−1.70 (m, 3-H_2, 4-H), 0.96 (d, *J* = 6.6 Hz, 5-H_3), 0.91 (d, *J* = 6.6 Hz, 4-Me); FDMS *m/z* 618.2 (M^+, 100). Anal. Calcd for $C_{36}H_{46}N_2O_7$: C, 70.68; H, 8.47; N, 4.71. Found: C, 70.53; H, 8.16; N, 4.44.

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-*O*-(phenylmethyl)-D-seryl], 6g, and Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2S,3S)-3-phenyloxiranyl]-2-heptenoyl-*O*-(phenylmethyl)-D-seryl], 17g. Styrene **7g** was epoxidized, according to modified general method C using dichloromethane:toluene (2:1), to provide (i) β-epoxide **6g** as a white solid (30%): FDMS *m/z* 635.1 (M^+, 100) and (ii) α-epoxide **17g** as a white solid (10%): t_R = 27.4 min[b] (refer to Table 5, footnote 3); $[\alpha]_D^{589}$ +35.0°(*c* = 0.06, $CHCl_3$); HRMS (FAB) for $C_{36}H_{47}N_2O_8$ *m/z* requires 635.3332, found 635.3336 (Δ +0.4 mmu).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-(2*R*)-2-(4-hydroxyphenyl)glycyl], 6h. Styrene **7h** was epoxidized, according to general method C, to provide **6h** as a white solid (22%): ESMS (PI) *m/z* 607.3 ([M + 1]$^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-D-phenylalanyl], 6i. Styrene **7i** was epoxidized, according to general method C, to provide **6i** as a white amorphous solid (25%): ESMS (PI) *m/z* 605.4 ([M + 1]$^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-D-tyrosyl], 6j. Styrene **7j** was epoxidized, according to modified general method C using *m*CPBA (1.5 equiv) added over 4 days, to provide **6j** as a white solid (34%): ESMS (PI) *m/z* 621.5 ([M + 1]$^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-4-amino-D-phenylalanyl], 6k. Styrene **7m** was epoxidized, according to general method C, and the crude reaction product purified by column chromatography (SiO_2; gradient elution: 25−75% EtOAc/*n*-hexanes) to provide epoxide **21** as a 1:2 mixture of α (t_R = 44.5 min[a]):β (t_R = 41.6 min[a]) (refer to Table 5, footnote 3) epoxides as an off-white solid (100%): HRMS (FAB) for $C_{50}H_{56}N_3O_9$ *m/z* requires 842.3938, found 842.3954 (Δ +1.60 mmu). Anal. Calcd for $C_{50}H_{56}N_3O_9$: C, 71.72; H, 6.58; N, 4.99. Found: C, 71.52; H, 7.30; N, 4.62.

To a solution of Fmoc amine **21** (195 mg, 0.232 mmol) in dry DMF (10 mL) was added piperidine (70 μL, 0.696 mmol), and the mixture stirred at room temperature for 6 h. The solvent was removed in vacuo to leave a solid residue which was purified by column chromatography (SiO_2; 20−100% EtOAc/*n*-hexanes−5% MeOH/CH_2Cl_2), and the resulting α:β-epoxides were separated by RP-HPLC to provide β-epoxide **6k** as a white glass (23%): ESMS (PI) *m/z* 620.7 [M + 1]$^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-3-chloro-D-tyrosyl], 6r, and Cyclo-[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*S*,3*S*)-3-phenyloxiranyl]-2-heptenoyl-3-chloro-D-tyrosyl], 17r. Styrene **7r** was epoxidized, according to modified general method C using dichloromethane:toluene (2:1), to provide (i) β-epoxide **6r** as a white solid (35%): FDMS *m/z* 654.3 (M^+, 100) and (ii) α-epoxide **17r** as a white solid (19%): t_R = 21.0 min[b] (refer to Table 5, footnote 3); $[\alpha]_D^{589}$ +10.7° (*c* = 1.32, MeOH); FDMS *m/z* 654 (M^+, 100); Anal. Calcd for $C_{35}H_{43}N_2O_8 \cdot 0.5H_2O$: C, 63.29; H, 6.68; N, 4.22. Found: C, 63.30; H, 6.81; N, 4.24.

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-4-amino-3-chloro-D-phenylalanyl], 6s. Styrene **7t** was epoxidized, according to general method C, and the crude reaction product purified by column chromatography (SiO_2; gradient elution: 20−75% EtOAc:*n*-hexanes) to provide epoxide **22** as a 1:2 mixture of α:β-epoxides (determined by ^{1}H NMR) as a white solid (100%): HRMS (FAB) for $C_{50}H_{54}N_3ClNaN_3O_9$ *m*/*z* requires 898.3446, found 898.4438 (Δ −0.8 mmu). Anal. Calcd for $C_{50}H_{54}ClN_3O_9 \cdot 2H_2O$: C, 65.81; H, 6.41; N, 4.60. Found: C, 65.92; H, 5.73; N, 4.27.

To a solution of Fmoc amine **22** (195 mg, 0.223 mmol) in dry DMF (4.5 mL) was added piperidine (66 μL, 0.669 mmol), and the mixture stirred at room temperature for 3 h. The solvent was removed in vacuo to leave a solid residue which was purified by column chromatography (SiO_2; 20−100% EtOAc/*n*-hexanes) and the resulting α:β-epoxide mixture separated by RP-HPLC to provide β-epoxide **6s** as a white solid (32%): ESMS (PI) *m*/*z* 654.4 $[M + 1]^+$, 100).

Cyclo[2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl-3-chloro-4-(dimethylamino)-D-phenylalanyl], 6u. To a stirred solution of epoxide **6s** (37.5 mg, 57.4 umol) in acetonitrile (7 mL) were added crushed, anhydrous K_2CO_3 (980 mg, 7.2 mmol) and MeI (2.1 g, 14.4 mmol), and the mixture was heated at 70 °C in a sealed vessel for 6 h. The mixture was filtered and the filtrate concentrated in vacuo to give a solid residue, which was further purified by column chromatography (SiO_2; gradient elution: 20−90% EtOAc/*n*-hexanes) to give dimethylaniline **6u** as a white powder (18.2 mg, 47%): $[\alpha]_D^{589}$ +27.6° (*c* = 0.31, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ unit A 7.35−7.30 (m, PhH_3), 7.24−7.22 (m, PhH_2), 6.76 (ddd, *J* = 15.2, 10.8 and 4.2 Hz, 3-H), 5.71 (d, *J* = 15.9, 2-H), 5.19 (dd, *J* = 10.4 and 3.4 Hz, 5-H), 3.66 (d, *J* = 1.6 Hz, 8-H), 2.90 (dd, *J* = 7.8 and 1.9 Hz, 7-H), 2.57 (br d, *J* = 14.5 Hz, 4-H), 2.49−2.40 (m, 4-H′), 1.13 (buried d, *J* = 6.7 Hz); unit B 7.24−7.22 (m, PhH_2), 7.19−7.17 (m, PhH), 5.46 (d, *J* = 7 Hz, NH), 4.73 (app. q, *J* = 6.5 Hz, 2-H), 3.09−2.98 (m, 3-H_2), 2.83 (br s, NMe_2); unit C 7.03−7.00 (m, NH), 3.47−3.38 (m, 3-H), 3.09−2.98 (m, 3-H′), 1.21 (s, 2-Me), 1.14 (s, 2-Me); unit D 4.80 (dd, *J* = 10.2 and 3.2 Hz, 2-H), 1.79−1.65 (m, 3-H, 4-H), 1.33−1.27 (m, 3-H′), 0.83 (d, *J* = 6.4 Hz, 5-H_3), 0.81 (d, *J* = 6.4 Hz, 4-Me); ESMS (PI) *m*/*z* 682.4 (M^+, 100). Anal. Calcd for $C_{37}H_{48}ClN_3O_7$: C, 65.14; H, 7.09; N, 6.16. Found: C, 65.10; H, 7.20; N, 6.09.

Cyclo[3-(1-naphthalenyl)-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl], 6w, and Cyclo[3-(1-naphthalenyl)-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*S*,3*S*)-3-phenyloxiranyl]-2-heptenoyl], 17w. Styrene **7w** was epoxidized, according to modified general method C using dichloromethane:toluene (2:1), to provide (i) β-epoxide **6w** as a white solid (27%): FDMS *m*/*z* 654.3 (M^+, 100) and (ii) α-epoxide **17w** as a white solid (12%): t_R = 29.7 min[b] (refer to Table 5, footnote 3); $[\alpha]_D^{589}$ +23.1° (*c* = 0.53, $CHCl_3$); FDMS *m*/*z* 654 (M^+, 100); HRMS (FAB) for $C_{39}H_{47}N_2O_7$ *m*/*z* requires 655.3383, found 655.3392 (Δ +0.9 mmu).

Cyclo[3-(2-naphthalenyl)-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*R*,3*R*)-3-phenyloxiranyl]-2-heptenoyl], 6x, and Cyclo[3-(2-naphthalenyl)-D-alanyl-2,2-dimethyl-β-alanyl-(2*S*)-2-hydroxy-4-(methylpentanoyl)-(2*E*,5*S*,6*S*)-5-hydroxy-6-[(2*S*,3*S*)-3-phenyloxiranyl]-2-heptenoyl], 17x. Styrene **7x** was epoxidized, according to modified general method C using dichloromethane:toluene (2:1), to provide (i) β-epoxide **6x** as a white solid (18%): FDMS *m*/*z* 654.3 (M^+, 100) and (ii) α-epoxide **17x** as a yellow solid (9%): t_R = 29.2 min[b] (refer to Table 5, footnote 3); $[\alpha]_D^{589}$ +8.6° (*c* = 0.65, $CHCl_3$); FDMS *m*/*z* 654.2 (M^+, 100).

In Vitro Cytotoxicity Assay.[38] Dose−response curves were generated to determine the concentration required for 50% inhibition of growth (IC_{50}). Test compounds were dissolved initially in DMSO at a concentration of 0.2 mg/mL. Serial 1:3 dilutions were made in DMSO using a Biomek Automated workstation (Beckman, Fullerton, CA). Micropipet tips were changed with each dilution. We have previously shown that cryptophycins need to be serially diluted in DMSO to reduce drug adsorption onto plastic and glass surfaces. Log-phase human CCRF-CEM leukemia cells were added to wells on 24-well plates (Costar, Cambridge, MA) at 4.8×10^4 cells/2 mL of assay medium/well. Assay medium consisted of RPMI-1640 medium supplemented with 10% dialyzed fetal bovine serum and 25 mM HEPES buffer. The series of compound dilutions in DMSO were added to duplicate wells at 10 μL/well. Two wells on each individual plate received 10 μL of DMSO as controls. The final concentration of DMSO was 0.5%. Plates were incubated for 72 h at 37 °C in a humidified 5% CO_2-in-air atmosphere. After incubation, the leukemia cells growing in suspension were quantitated using a ZBI Coulter counter, and an IC_{50} was determined.

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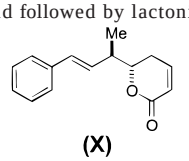
(31) Fragments A **10** and C−D **14** were kindly provided by the Chemical Process R&D Division at Eli Lilly and Company.

(32) Under nonoptimal conditions, alcohol **13** was converted to lactone **(X)** presumably via an initial isomerization of the α,β-unsaturated ester double bond followed by lactonization.



(X)

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