Total Synthesis and Antitubulin Activity of C10 Analogues of Cryptophycin-24

Suzanne B. Buck,[†] Jacquelyn K. Huff,[‡] Richard H. Himes,[‡] and Gunda I. Georg*[,†]

Department of Medicinal Chemistry and Department of Molecular Biosciences, University of Kansas, 1251 Wescoe Drive, Lawrence, Kansas 66045-7582

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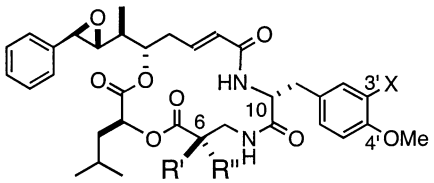
The unsubstituted, 3′-Cl, 4′-C1, and 3′,4′-diCl C10 analogues of cryptophycin-24 were prepared via total synthesis and tested in vitro for cytotoxicity against MCF-7 and multi-drug-resistant MCF-7/ADR breast cancer cell lines and in a tubulin assembly assay. The ED_{50} values ranged from 7.2 to 15.8 μM in the tubulin assay and from 0.05 to 3.4 nM in the cell assays. The presence of a 3′-C1 and/or 4′-C1 substituent on the C10 phenyl ring increased cytotoxicity in the MCF-7 cell line compared to the unsubstituted phenyl ring. The most potent compound in this series possessed a 3′-C1 substituent on the C10 phenyl ring. The 3′-C1 analogue had ED_{50} values of 50 and 580 pM in the MCF-7 and MCF-7/ADR cell lines, respectively. Its activity was very similar to the parent compound cryptophycin-24. Substitution of the 4′-MeO group in cryptophycin-24 with a 4′-C1 moiety did not significantly affect cytotoxicity against MCF-7 and MCF-7/ADR cells compared to the parent compound. These results demonstrated that the 4′-MeO group in cryptophycin-24 is not essential and can be replaced with 3′-C1 or 4′-C1 substituents.

Introduction

The cryptophycins[1−4] are antimitotic, cyanobacterial metabolites isolated from the blue-green algae *Nostoc sp.* ATCC 53789[5] and *Nostoc sp.* GSV 224.[6,7] When cryptophycin-1 (1, Figure 1) was first isolated,[5] Schwartz and co-workers chose the name cryptophycin because of the compound's highly potent fungicidal activity against filamentous fungi and yeast of the genus *Cryptococcus*. Because of the narrow breadth of antifungal activity of cryptophycin-1 (**1**) and its low therapeutic index,[8] the development of cryptophycin-1 as an antifungal compound was not pursued. Cryptophycin-1 was later isolated by Moore and co-workers who screened over 1000 extracts of blue-green algae in search of compounds that exhibited antitumor activity.[6] Cryptophycin-1, the most abundant cytotoxin of the cryptophycins,[9] has potent activity in the pM range in the multi-drug-resistant (MDR) cell lines SKVLB1 and MCF-7/ADR,[10] thereby surpassing vinblastine, paclitaxel, and colchicine in the ability to retain cytotoxic activity in MDR cell lines.

A structurally simpler cryptophycin, cryptophycin-24 (arenastatin A, **2**), was isolated from an Okinawan marine sponge,[11] *Dysidea arenaria*. Although cryptophycin-24 lacks the chlorine substituent on the aryl ring of the C10 side chain and the methyl group at C6, it was extremely potent against KB cells (ED_{50} = 5 pM).[11] Unfortunately, cryptophycin-24 has a half-life of approximately 10 min in mouse serum[12] due to ester hydrolysis. Therefore, cryptophycin-52 (**3**), the C6 *gem*-dimethyl analogue of cryptophycin-1, was prepared to decrease the rate of ester hydrolysis in vivo.[13] This synthetic analogue is the first cryptophycin to undergo clinical trials for the treatment of cancer.[14]



Cryptophycin-1 (**1**), R' = Me, R'' = H, X = Cl
Cryptophycin-24 (**2**), R' = R'' = X = H
Cryptophycin-52 (**3**), R' = R'' = Me, X = Cl

Figure 1. Structures of the cryptophycins.

The cryptophycins are microtubule-destabilizing agents that elicit cytotoxic effects by binding noncovalently to a site at, or overlapping, the *Vinca* site on tubulin and at the rhizoxin/maytansine site.[10,12,15−18] Cryptophycin-1 also induces the phosphorylation of Bcl-2[19] and activates the apoptosis process.[20,21] Similar to other known antimitotic agents,[22] cryptophycin-1 slows microtubule dynamics at low concentrations[23,24] while at subnanomolar concentrations it causes complete loss of microtubules within cells.[23] Through its action on microtubules and possibly other yet to be discovered targets, cryptophycin induces potent cytotoxicity by activating the apoptotic pathway.[20,21]

One of the strong attributes of cryptophycin is that it lacks cross resistance with paclitaxel, doxorubicin,[25] and other families of drugs such as DNA alkylators, toposiomerase II inhibitors, and antimetabolites[26] that are generally used in combination protocols for the treatment of cancer. Cryptophycin-1 has also been found to exhibit excellent antiproliferative activity in vivo against five solid tumors of murine origin (i.e. colon adenocarcinomas 38 and 51, taxol-sensitive and taxol-resistant mammary adenocarcinoma M16, and pancreatic ductal adenocarcinoma) that were subcutaneously transplanted into mice.[6,27] In vitro, cryptophycin-1 was shown

* To whom correspondence should be addressed. Tel: (785) 864-4498. Fax: (785) 864-5836. e-mail: georg@ku.edu.
[†] Department of Medicinal Chemistry.
[‡] Department of Molecular Biosciences.

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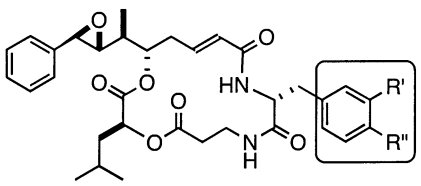
to be highly toxic to the human nasophyrngeal carcinoma and the human colorectal adenocarcinoma cell lines.[6]

Structure−activity relationship (SAR) studies of the C10 side chain of cryptophycin-1 demonstrated that introduction of a second chloro substituent at the C5′ position of the C10 side chain decreased activity by a factor of 120, while absence of the C3′-Cl decreased activity by a factor of 10.[27] A factor of 30 decrease in activity was observed when the C4′-*O*-methyl group was absent.[27] These data suggested that the 3′-C1 and 4′-MeO are required for optimal activity. More recently, it was revealed that the cytotoxicity of cryptophycin can be more dramatically affected by altering the C10 side chain. Patel and co-workers prepared analogues of cryptophycin-52 (**3**) that were tested in the human leukemia CCRF−CEM tumor cell line.[13] Substitutions for the benzyl moiety and alternative substitution patterns on the aromatic ring were made. Substitution of the C10 aryl ring with a naphthyl ring, altering the tether length between the macrocycle and the aryl ring, removing the aromatic ring, or replacing it with a cyclohexyl group, led to analogues with varying degrees of reduced activity. Variations in the substitution pattern of the benzyl ring of the cryptophycin-52 analogues revealed that the 3′-Cl substituent provides an increased in vitro activity, whereas compounds lacking the 3′-Cl substituent were active only in the 4 to 120 nM range with the exception of the analogue containing an unsubstituted benzyl ring at C10 which was active and had an ED_{50} value of 183 pM. The compound with the most activity was the 3′-C1, 4′-NMe_2 analogue, which had an IC_{50} value 2.5 times that of cryptophycin-52.

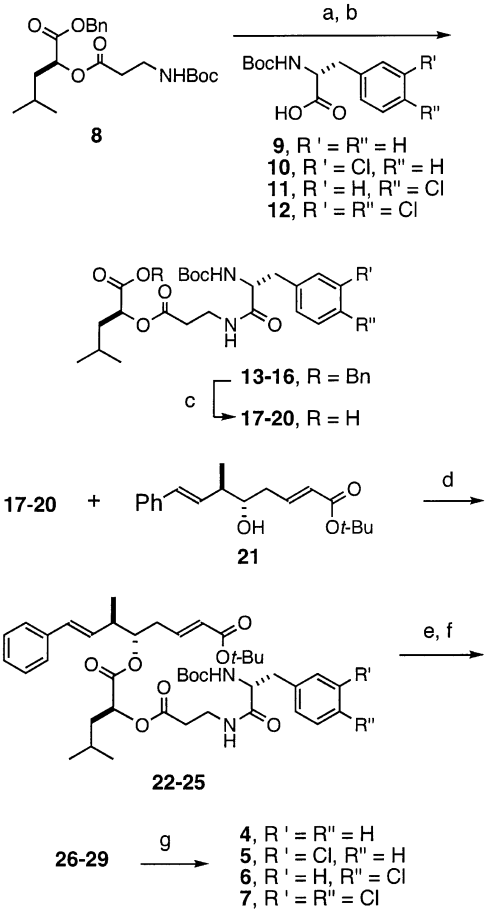
The SAR determined from isolated analogues of cryptophycin-1 and synthesized cryptophycin analogues of cryptophycin-52 suggested that any analogue synthesized should retain the following on the C10 side chain for optimal activity: (1) the R stereochemistry at C10, (2) a benzyl moiety, and (3) a chloro substitution at the 3′ (meta) position. We chose to conduct a study of the C10 substitution pattern of the benzyl ring using a series of chlorinated analogues of cryptophycin-24 that carry no other substituents (such as a methoxy or amino groups) to further define the importance of the 3′-CI substituent and evaluate the importance of the 4′-MeO substituent with regard to tubulin interaction and cytotoxicity. To this end, cryptophycin-24 analogues **4**−**7** (Figure 2) were synthesized and tested in an in vitro tubulin assembly assay and in the MCF-7 and the MCF-7/ADR breast cancer cell lines.



4, R′ = R″ = H
5, R′ = Cl, R″ = H
6, R′ = H, R″ = Cl
7, R′ = R″ = Cl

Figure 2. Structures of novel C10 analogues.

Scheme 1[a]



[a] Reagent and conditions: (a) TFA; (b) DCC, HOBT, DIEA, acid, 66−99%, two steps; (c) $Pd(OH)_2$, H_2, 81−96%; (d) DIEA, DMAP, 2,4,6-trichlorobenzoyl chloride, 21, 77−96%; (e) TFA; (f) HBTU, DIEA, 55−88%; (g) DMD, 59−74%.

Chemistry

Analogues **4**−**7** were synthesized from two key fragments: the "northern half" **21** and the "southern halves" **17**−**20** (Scheme 1). Northern half **21** was obtained through asymmetric synthesis,[28,29] while the southern halves[28] were formed through the coupling of three commercially available building blocks: (1) L-leucic acid, (2) *β*-alanine, and (3) a D-phenylalanine moiety.

Synthesis of the C10 analogues **4**−**7** began with benzyl-protected **8**.[30] The Boc group was cleaved using TFA and the resulting amine was coupled to the N-Boc-phenylalanine moieties **9**−**12** to provide **13**−**16** (Scheme 1).[30] The benzyl esters of **13**−**16** were cleaved using palladium(II) hydroxide and hydrogen gas to reveal acids **17**−**20**. Acids **17**−**20** were activated using the Yamaguchi reagent, 2,4,6-trichlorobenzoyl chloride, and coupled to **21**, to yield esters **22**−**25**.[28,29] The N-Boc group and the *tert*-butyl ester were simultaneously cleaved from intermediates **22**−**25** using TFA.[31] The macrocycles were subsequently closed using HBTU (*O*-benzotriazol-l-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) as the coupling reagent,[31] thus forming **26**−**29**. Epoxidation of styrenes **26**−**29** with dimethyldioxirane (DMD)[32] provided mixtures of the α and *β* epoxides **4**−**7**. The *β* epoxides were separated from the *ac* epoxides using HPLC and tested in vitro in a tubulin assembly assay and a cytotoxicity assay.

Table 1. Biological Results for in Vitro Tubulin Assembly Assay and Cytotoxicity Studies of C10 Analogues **4**−**7**

compound	tubulin assay: ED_{50} (μM)	MCF-7: ED_{50} (nM)	MCF-7/ADR: ED_{50} (nM)
1	3.4 ± 0.80	0.009 ± 0.003	0.018 ± 0.007
2	15.8 ± 0.14	0.13 ± 0.06	0.25 ± 0.20
4	13.1 ± 1.0	0.34 ± 0.13	1.57 ± 1.11
5	7.2	0.05 ± 0.03	0.58 ± 0.21
6	12.5 ± 0.7	0.12 ± 0.04	0.66 ± 0.40
7	12.6 ± 6.7	0.08 ± 0.03	3.4 ± 1.6

Biological Testing

Cryptophycin-24 (**2**) and its analogues were active in the tubulin assembly assay[33] with ED_{50} values in the range of 7.2−15.8 μM (Table 1). The 3′-C1 analogue **5** was the most active derivative (ED_{50} = 7.2 μM). The 4′-C1 analogue **6** (ED_{50} = 12.5 μM), unsubstituted **4** (ED_{50} = 13.1 μM), and 3′,4′-diCl derivative **7** (ED_{50} = 12.6 μM) were essentially as active as cryptophycin-24 (**2**, ED_{50} = 15.8 μM). Cryptophycin-1 (ED_{50} = 3.4 μM) was about 4 times as active as cryptophycin-24. Although the analogues **4**−**7** were all active in the MCF-7 breast cancer cell line with ED_{50} values ranging from 0.05 to 0.34 nM (Table 1), none were as cytotoxic as cryptophycin-1 (ED_{50} = 0.009 nM). The activity of cryptophycin-24, ED_{50} = 0.13 nM, was within the range of the synthesized analogues. The most active compounds were 3′-C1 analogue **5** (ED_{50} = 0.05 nM) and 3′,4′-diCl analogue **7** (ED_{50} = 0.08 nM), whereas 4′-Cl derivative **6** (ED_{50} = 0.12 nM) and unsubstituted **4** (ED_{50} = 0.34 nM) were slightly less active. In the MCF-7/ADR breast cancer cell line, no analogue was as cytotoxic as cryptophycin-1 with an ED_{50} value of 0.018 nM. The most active analogues, 3′-C1 **5** and 4′-C1 **6** had ED_{50} values of 0.58 and 0.66 nM, respectively. The two other analogues, unsubstituted **4** and 3′,4′-diC1 **7**, had activities about 40 to 80% less than **5** and **6**.

Discussion

The results showed that the unsubstituted phenyl analogue **4**, 3′-Cl **5**, 4′-Cl **6**, and the 3′,4′-diCl **7** analogues had similar activities in the tubulin assembly assay and the MCF-7 cell line assay compared to cryptophycin-24. It can therefore be concluded that the 4′-MeO group of cryptophycin-24 is not essential for tubulin interaction and cytotoxicity against MCF-7 cells and that the 4′-MeO can be replaced with 3′-Cl, 4′-Cl or 3′,4′-diCl moieties. Overall, the 2 to 3-fold decrease in activity for the analogues and cryptophycin-24 in these two assays, in comparison to cryptophycin-1, is not substantial.

In the MCF-7/ADR cell line, cryptophycin-1 displayed a 2-fold decrease in activity compared to the non-multi-drug-resistant cell line. The difference between the activities of cryptophycin-24 and analogues **4**−**7** is more pronounced, varying from a 5-fold to a 43-fold decrease in activity in the MCF-7/ADR cell line when compared to cryptophycin-1. This could be due to a change in the interaction of these analogues with the P-glycoprotein present in the multi-drug-resistant cell line.

As was seen for cryptophycin-1, cryptophycin-24 displayed a 2-fold decrease of cytotoxicity (ED_{50} = 0.25 nM) in the MCF-7/ADR cells when compared to its activity against MCF-7 cells (ED_{50} = 0.13 nM). The 2- to 3-fold decrease of activity of the 3′-C1 analogue **5** and the 4′-Cl analogue **6** against MCF-7/ADR cells compared to cryptophycin-24 is not substantial. The 6-fold and 14-fold decrease in activity of the unsubstituted analogue **4** and the 3′,4′-diCl analogue **7**, respectively, is more significant.

The results demonstrate that the 4′-MeO is not essential for cytotoxicity against MCF−7/ADR cells in the cryptophycin-24 series of compounds. The 4′-MeO moiety can be replaced by a 3′-C1 or a 4′-C1 group without significant effects on the cytotoxicity against MCF-7/ADR cells when compared to the parent compound cryptophycin-24.

It is interesting to note that the decrease in activities of analogues **4**−**7** in the MCF-7/ADR cell line compared to the MCF-7 cell line was significantly more (5 to 40-fold) when compared to cryptophycin-l and cryptophycin-24 which only displayed 2-fold decreases.

The most potent cryptophycin-24 analogue prepared was the 3′-C1 analogue **5**, which was very similar to the parent compound cryptophycin-24. Analogue **5** was twice as active as cryptophycin-24 in the tubulin assembly assay and in the cell cytotoxicity assay against MCF-7 cells and about 2-fold less active against the MCF-7/ADR cell line.

Experimental Section

Instruments and Analyses. Proton and carbon nuclear magnetic resonance spectra were recorded on a DRX400 MHz or a Bruker DRX500 MHz. All chemical shifts were recorded in parts per million (ppm), and $CDCl_3$ was used as the internal standard. Mass spectra were obtained from a ZAB HS mass spectrometer (VG Analytical Ltd., Manchester, U. K.) equipped with a 11/250 data system. Fast atom bombardment mass spectrometry (FABMS) experiments were performed with a Xenon gun operated at 8 keV energy and 0.8 mA emission at the MS laboratory at the University of Kansas. Fast atom bombardment high-resolution mass spectra (FAB HRMS) were recorded at 1:10 000 resolution using linear voltage scans under data system control and collected data in a multichannel analyzer mode (MCA). A Perkin-Elmer 1420 Ratio Recording Infrared Spectrophotometer or an Avatar 320 FTIR was used to record infrared spectra. All optical rotations were obtained using a Perkin-Elmer model 214 Polarimeter or an Autopol IV Automatic Polarimeter at room temperature. Melting points were obtained using a Fisher-Johns melting point apparatus and are uncorrected.

Materials and Methods. Tetrahydrofuran and diethyl ether were freshly distilled over sodium and benzophenone. Methylene chloride was freshly distilled over CaH_2. All silica gel (230−400 mesh) used for column chromatography was purchased from VWR Scientific Products. All moisture-sensitive reactions were carried out using oven-dried glassware and under a positive pressure of argon. Solvents and reagents that are commercially available were used without further purification unless otherwise noted.

The N-Boc-D-phenylalanines required for the C10 analogue syntheses were obtained from the PepTech Corporation. Cryptophycin-1 (**1**) was isolated from the *Nostoc sp.* ATCC 53789 algae.[5,6] Cryptophycin-24 (**2**) was prepared as described in ref 28.

Tubulin Assembly Assay.[33] Varying concentrations of the cryptophycin-24 analogues were incubated (total volume of 100 μL) at 37 °C for 15 min with tubulin (1.5 mg/mL) in PEM buffer (pH 6.9, consisting of 0.1 M PIPES, 1 mM EGTA, 1 mM $MgSO_4$) containing 0.5 mM GTP and 8% DMSO. Following polymerization, centrifugation for 4 min (37 °C at 50 000 rpm) sedimented the microtubules formed, leaving any unassembled tubulin in the supernatant. The Bradford method[34] was used to quantitate the protein concentration (unpolymerized tubulin concentration) in the supernatant from which the protein

concentration in the pellet was calculated. The concentration of the cryptophycin-24 analogue that reduced the amount of microtubules by 50% was defined as the ED_{50} value.

Cell Cytotoxicity Assay.[33] The cytotoxicity of the cryptophycin-24 analogues was determined using the MCF-7 and MCF-7/ADR breast cancer cell lines. Approximately 2000 cells per well were plated in 96-well culture dishes in Dulbecco's MEM/F12 containing 10% fetal calf serum. New medium, containing varying concentrations of the cryptophycin-24 analogue, was substituted for the original medium at 24 h. After incubation for 72 h, cell proliferation, using sulforhodamine B dye was measured.[35] The ED_{50} value was defined as the concentration of the cryptophycin-24 analogue that reduced cell proliferation by 50%.

Preparation of Benzyl-Protected Southern Halves 13−16 (Sample Procedure). To a solution of Boc-protected benzyl ester **8** (1.97 g, 5.02 mmol) in CH_2Cl_2 (17.5 mL) at 0 °C was added TFA (3.9 mL, 50.2 mmol) dropwise. The ice bath was removed, and the solution was stirred at room temperature for 3.5 h. Dry toluene (1.0 mL) was added, and the solution was concentrated. Quantitative yield was assumed and the crude salt was used directly in the next reaction. To a solution of the benzyl ester salt (5.02 mmol) in THF (62 mL) were added HOBT (0.68 g, 5.02 mmol), DIEA (2.00 mL, 11.5 mmol), and N−Boc-D-phenylalanine **(9**, 2.00 g, 7.54 mmol). After cooling the solution to 0 °C, DCC (1.14 g, 5.52 mmol) was added. The solution was stirred at 0 °C for 30 min, then allowed to warm to room temperature and stirred for 16 h. After removing the urea byproduct by filtration, the solution was concentrated and dissolved in EtOAc. Following extraction once each with a 5% $KHSO_4$ solution and a saturated $NaHCO_3$ solution, the organic layer was dried ($MgSO_4$). The product was filtered, concentrated, and purified by flash column chromatography on silica gel (10% acetone:hexanes).

Benzyl (*2S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(phenyl)propionylamino]propionyloxy]-4-methylpentanoate (13). The total yield of **13**, a white foam, was 2.70 g. 99%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.37 (m, 5H), 7.27−7.19 (m, 5H), 6.63 (br d, 1H), 5.23−5.12 (m, 4H), 4.39−4.37 (m, 1H), 3.59−3.58 (m, 1H), 3.34 (dd, 1H, J = 6.4 Hz, 12.6 Hz), 3.12−3.04 (m, 2H), 2.57−2.54 (m, 2H), 1.81−1.69 (m, 2H), 1.68−1.61 (m, 1H), 1.40 (s, 9H), 0.94 (d, 3H, J = 6.3 Hz), 0.92 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 171.3, 170.8, 155.2, 136.6, 135.0, 129.4, 129.3 (2C), 128.6 (2C), 128.5 (2C), 128.4, 128.2 (2C), 79.8, 71.1, 67.2, 55.7, 39.4, 38.8, 35.0, 34.0, 28.2 (3C), 24.5, 22.9, 21.5; IR ($CDCl_3$) 3450, 2980, 2960, 1740, 1710, 1680 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{30}H_{41}N_2O_7$ (M + H)$^+$: 541.2914, found 541.2924; $[\alpha]_D$ −27.3 (*c* 0.498, $CHCl_3$).

Benzyl (2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(3-chlorophenyl)propionylamino]propionyloxy]-4-methylpentanoate (14). The total yield of **14**, a white foam, was 2.09 g, 82%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43−7.36 (m, 5H), 7.29−7.21 (m, 3H), 7.10−7.08 (m, 1H), 6.75−6.73 (m, 1H), 5.25−5.16 (m, 4H), 4.38 (d, 1H, J = 6.4 Hz), 3.64−3.63 (m, 1H), 3.54−3.49 (m, 1H), 3.11 (dd, 1H, J = 6.3 Hz, 13.7 Hz), 3.01−3.00 (m, 1H), 2.58−2.57 (m, 2H), 1.81−1.71 (m, 2H), 1.69−1.64 (m, 1H), 1.42 (s, 9H), 0.95 (d, 3H, J = 6.4 Hz), 0.93 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 171.5, 171.2, 155.6, 139.3, 135.4, 134.6, 130.2, 130.0, 129.1 (2C), 129.0, 128.7 (2C), 128.0, 127.4, 80.4, 71.6, 67.9, 55.9, 39.9, 39.0, 35.6, 34.7, 28.7 (3C), 25.0, 23.4, 21.9; IR ($CDCl_3$) 3460, 2980, 2960, 1740, 1710, 1680 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{30}H_{40}N_2O_7Cl$ (M + H)$^+$: 575.2524, found 575.2500; $[\alpha]_D$ −21.8 (*c* 0.476, $CHCl_3$).

Benzyl (2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(4-chlorophenyl)propionylamino]propionyloxy]-4-methylpentanoate (15). The total yield of **15**, a white foam, was 1.70 g, 66%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46−7.33 (m, 5H), 7.29−7.23 (m, 2H), 7.16−7.12 (m, 2H), 6.78−6.76 (m, 1H), 5.23−5.14 (m, 4H), 4.39−4.37 (m, 1H), 3.62−3.60 (m, 1H), 3.50−3.46 (m, 1H), 3.08 (dd, 1H, J = 6.2 Hz, 13.6 Hz), 3.00−2.95 (m, 1H), 2.58−2.55 (m, 2H), 1.81−1.71 (m, 2H), 1.70−1.61 (m, 1H), 1.41 (s, 9H), 0.94 (d, 3H, J = 6.4 Hz), 0.92 (d, 3H, J = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 171.6, 171.5, 155.6, 135.6, 135.4, 133.1, 131.3, 131.1 (2C), 129.1 (2C), 129.0 (2C), 128.7 (2C), 80.4, 71.6, 67.8, 55.9, 39.9, 38.6, 35.6, 34.6, 28.7 (3C), 25.0, 23.4, 21.9; IR ($CDCl_3$) 3440, 2980, 2940, 1740, 1710, 1680, 1500 cm^{-1}; HRMS (FAB, PEG300) *m/e* calcd for $C_{30}H_{41}N_2O_7Cl$ (M + H)$^+$: 575.2524, found 575.2500; $[\alpha]_D$ −27.0 (*c* 0.512, $CHCl_3$).

Benzyl (2*S*)-2-[3-[(2R)-2-*tert*-Butoxycarbonylamino-3-(3,4-dichlorophenyl)propionylamino]propionyloxy]-4-methylpentanoate (16). The total yield of **16**, a white foam, was 1.00 g, 68%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44−7.32 (m, 7H), 7.04 (dd, 1H, J = 1.6 Hz, 8.6 Hz), 6.84−6.82 (m, 1H), 5.25−5.15 (m, 4H), 4.38−4.36 (m, 1H), 3.65 (dd, 1H, J = 6.0 Hz, 12.8 Hz), 3.55−3.47 (m, 1H), 3.09 (dd, 1H, J = 6.2 Hz, 13.8 Hz), 2.95 (dd, 1H, J = 6.6 Hz, 7.5 Hz), 2.58 (t, 2H, J = 5.8 Hz), 1.82−1.57 (m, 3H), 1.41 (s, 9H), 0.94 (d, 3H, J = 6.3 Hz), 0.92 (d, 3H, J = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8; 171.6, 171.2, 155.6, 137.7, 135.3, 132.6, 131.8, 131.2, 130.7, 129.3, 129.1 (2C), 129.0, 128.6 (2C), 80.4, 71.6, 67.9, 55.7, 39.8, 38.4, 35.6, 34.7, 28.7 (3C), 25.0, 23.4, 21.9; IR ($CDCl_3$) 3420, 3320, 2960, 2940, 1740, 1720, 1680, 1510 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{30}H_{39}N_2O_7Cl_2$ (M + H)$^+$: 609.2134, found 6019.2109; $[\alpha]_D$ −19.8 (*c* 0.466, $CHCl_3$).

Preparation of Acids 17−20 (Sample Procedure). After degassing a solution of the benzyl ester **13** (1.79 g, 3.13 mmol) in EtOAc (45 mL) for 5 min with argon, $Pd(OH)_2$ (0.22 g, 0.31 mmol) was added. The solution was stirred under an atmosphere of hydrogen gas using a hydrogen filled balloon. After 2 h, the solution was filtered and concentrated. The crude product obtained was 95% pure as determined by ^{1}H NMR and was used without further purification.

(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(phenyl)-propionylamino]propionyloxy]-4-methylpentanoic Acid (17). The total yield of **17**, a white foam, was 0.87 g, 81%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27−7.17 (m, 5H), 6.86 (m, 1H), 5.26−5.20 (m, 1H), 5.10−5.00 (m, 1H), 4.39−4.37 (m, 1H), 3.52−3.50 (m, 2H), 3.23−3.08 (m, 1H), 3.00−2.99 (min, 1H), 2.57−2.55 (m, 2H), 1.82−1.71 (m, 3H), 1.40 (s, 9H), 0.98 (d, 3H, J = 6.2 Hz), 0.95 (d, 3H, J = 6.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.7 (2C), 171.4, 155.8, 136.4, 129.3 (2C), 128.4 (2C), 126.8, 80.6, 71.3, 55.6, 39.4, 38.8, 35.2, 33.4, 28.2 (3C), 24.6, 23.0, 21.4; IR ($CDCl_3$) 3500−2500, 3440, 3380, 2980, 2900, 1720, 1680 cm^{-1}; HRMS (FAB, PEG300) *m/e* calcd for $C_{23}H_{35}N_2O_7$ (M + H)$^+$: 451.2444, found 451.2438; $[\alpha]_D$ −20.0 (*c* 0.49, $CHCl_3$).

(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(3-chlorophenyl)propionylamino]propionyloxy]-4-methylpentanoic Acid (18). The total yield of **18**, a white foam was 1.28 g, 85%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25−7.19 (m, 3H), 7.07 (d, 1H, J = 2.7 Hz), 6.93−6.90 (m, 1H), 5.31−5.28 (m, 1H), 5.13−5.10 (m, 1H), 4.40−4.38 (m, 1H), 3.58−3.56 (m, 2H), 3.15−3.10 (m, 1H), 2.95−2.92 (m, 1H), 2.62−2.60 (m, 2H), 1.84−1.82 (m, 2H), 1.74−1.73 (m, 1H), 1.41 (s, 9H), 0.99 (d, 3H, J = 6.4 Hz), 0.97 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 171.8, 171.3, 155.8, 138.6, 134.0, 129.6, 129.4, 127.5, 126.9, 80.6, 71.3, 55.2, 39.4, 38.3, 35.2, 33.9, 28.1 (3C), 24.6, 22.9, 21.4; IR ($CDCl_3$) 3500−2500, 3420, 3340, 2980, 2940, 2880, 1720, 1680 cm^{-1}; HRMS (FAB, PEG300, NBA) *m/e* calcd for $C_{23}H_{34}ClN_2O_7$ (M + H)$^+$: 485.2054, found 485.2051; $[\alpha]_D$ −20.8 (*c* 0.528, $CHCl_3$).

(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(4-chlorophenyl)propionylamino]propionyloxy]-4-methylpentanoic Acid (19). The total yield of **19**, a white foam, was 1.00 g, 68%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.9−10.7 (br s, 1H), 7.22 (d, 2H J = 7.6 Hz), 7.11 (d, 2H, J = 7.6 Hz), 6.29−6.27 (m, 1H), 5.55 (d, 1H, J = 7.6 Hz), 5.12−5.08 (m, 1H), 4.40−4.38 (m, 1H), 3.52−3.50 (m, 2H), 3.03−3.00 (m, 1H), 2.91−2.90 (m, 1H), 2.54−2.52 (m, 2H), 1.90−1.80 (m, 3H), 1.38 (s, 9H), 0.97 (d, 3H, J = 6.0 Hz), 0.95 (d, 3H, J = 6.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.1, 171.4 (2C), 155.7, 135.0, 132.6, 130.7 (2C), 128.5 (2C), 80.6, 71.5, 53.7, 39.5, 37.9, 35.2, 33.9, 28.1 (3C), 24.6, 23.0, 21.4; IR ($CDCl_3$) 3460, 3000, 2880, 1750, 1680 cm^{-1}; HRMS (FAB, PEG300) *m/e* calcd for $C_{23}H_{34}$-

ClN_2O_7 (M + H)$^+$: 485.2055, found 485.2035; $[\alpha]_D$ −20.0 (*c* 0.574, $CHCl_3$).

(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(3,4-dichlorophenyl)propionylamino]propionyloxy]-4-methylpentanoic Acid (20). The total yield of **20**, a white foam was 0.13 g, 95%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (d, 1H, *J* = 7.8 Hz), 7.02 (d, 2H, *J* = 8.2 Hz), 5.96−5.88 (m, 1H), 5.33−5.32 (m, 1H), 5.09−5.07 (m, 1H), 4.36−4.35 (m, 1H), 3.57−3.53 (m, 2H), 3.11−3.10 (m, 1H), 3.00−2.90 (m, 1H), 2.67−2.58 (m, 2H), 1.86−1.81 (m, 2H), 1.75−1.70 (m, 1H), 1.41 (s, 9H), 0.99 (d, 3H, *J* = 6.4 Hz), 0.97 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 171.8, 171.3, 155.7, 136.8, 132.1, 131.3, 130.8, 130.2, 128.8, 80.7, 71.4, 55.0, 39.4, 37.7, 35.3, 34.0, 28.1 (3C), 24.6, 22.9, 21.4; IR ($CDCl_3$) 3440, 3380, 2980, 2940, 2900, 3500−2500, 1730, 1720, 1580 cm^{-1}; HRMS (FAB, PEG300) *m/e* calcd for $C_{23}H_{33}N_2O_7Cl$ (M + H)$^+$: 519.1665, found 519.1658; $[\alpha]_D$ −22.4 (*c* 0.438, $CHCl_3$).

Preparation of *tert*-Butyl Esters 22−25 (Sample Procedure). After drying over $MgSO_4$ for 30 min, a solution of acid **17** (0.21 g, 0.46 mmol) in THF (0.46 mL) was removed via syringe and cooled to 0 °C. To this were added DIEA (0.10 mL, 0.58 mmol), 2,4,6- trichlorobenzoyl chloride (0.08 mL, 0.51 mmol), and DMAP (0.002 g, 0.02 mmol). After 2 h, a solution of alcohol **21** (0.07 g, 0.23 mmol) in THF (3.4 mL) was added and stirred at room temperature for 2 h. The solution was diluted with CH_2Cl_2 and rinsed with a saturated $NaHCO_3$ solution. The aqueous layer was extracted three times with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$). After filtering and concentrating, the crude product was purified using column chromatography on silica gel (100% hexanes to 70% EtOAc:hexanes).

***tert*-Butyl *(5S,6R)*-5-[(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-phenylpropionylamino]propionyloxy-4-methylpentanoyloxy]-6-methyl-8-phenylocta-(2*E*,7*E*)-dienoate (22).** The total yield of **22**, a pale yellow oil, was 0.14 g, 81%: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32−7.19 (m, 10H), 6.74−6.72 (m, 1H), 6.49−6.46 (m, 1H), 6.43 (d, 1H, *J* = 15.8 Hz), 6.02 (dd, 1H, *J* = 8.7 Hz, 15.8 Hz), 5.86 (d, 1H, *J* = 15.6 Hz), 5.50 (d, 1H, *J* = 6.9 Hz), 5.06−5.05 (m, 1H), 4.98 (dd, 1H, *J* = 3.7 Hz, 9.9 Hz), 4.36−4.35 (m, 1H), 3.58−3.56 (m, 1H), 3.48−3.39 (m, 1H), 3.25−3.22 (m, 1H), 3.05−3.00 (m, 1H), 2.64−2.49 (m, 5H), 1.74−1.61 (m, 1H), 1.50 (s, 9H), 1.37 (s, 9H), 1.13 (d, 3H, *J* = 6.8 Hz), 0.94−0.88 (m, 2H), 0.86 (d, 3H, *J* = 6.4 Hz), 0.81 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 171.8, 171.2, 166.2, 156.9, 142.2, 137.7, 137.2, 132.2, 130.4, 129.8 (2C), 129.0 (2C), 128.8 (2C), 127.9, 127.0, 126.8, 126.6 (2C), 80.6, 79.9, 76.8, 71.6, 56.2, 41.4, 40.0, 39.1, 35.6, 35.2, 34.7, 28.7 (3C), 28.6 (3C), 25.0, 23.3, 21.8, 14.6; IR ($CDCl_3$) 3480, 2980, 2960, 1740, 1710, 1680 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{42}H_{59}N_2O_9$ (M + H)$^+$: 735.4221, found 735.4220; $[\alpha]_D$ +1.52 (*c* 2.76, $CHCl_3$).

***tert*-Butyl (5*S*,6*R*)-5-[(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(3-chlorophenyl)propionylamino]propionyloxy-4-methylpentanoyloxy]-6-methyl-8- phenylocta-(2*E*,7*E*)-dienoate (23).** The total yield of **23**, a pale yellow oil, was 0.13 g, 77%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.13 (m, 9H), 7.03−7.00 (m, 1H), 6.84−6.82 (m, IH), 6.42 (d, 1H, *J* = 15.8 Hz), 6.02 (dd, 1H, *J* = 8.7 Hz, 15.8 Hz), 5.85 (d, 1H, *J* = 15.6 Hz), 5.65 (d, 1H, *J* = 7.5 Hz), 5.09−5.07 (m, 1H), 4.98 (dd, 1H, *J* = 3.6 Hz, 10.0 Hz), 4.45−4.44 (m, 1H), 3.59−3.57 (m, 1H), 3.52−3.50 (m, 1H), 3.26 (dd, 1H, *J* = 4.7 Hz, 13.6 Hz), 2.96−2.92 (m, 1H), 2.63−2.55 (m, 3H), 2.50−2.45 (m, 2H), 1.70−1.62 (m, 1H), 1.50 (s, 9H), 1.37 (s, 9H), 1.14 (d, 3H, *J* = 6.8 Hz), 0.94−0.89 (m, 2H), 0.85 (d, 3H, *J* = 6.5 Hz), 0.79 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 171.1, 170.8, 165.8, 155.4, 141.7, 139.5, 136.7, 133.9, 131.7, 130.0, 129.5, 129.3, 128.5 (2C), 127.5, 127.4, 126.9, 126.7, 126.1 (2C), 80.4, 79.5, 76.4, 71.2, 55.4, 41.1, 39.5, 38.3, 35.2, 34.9, 34.3, 28.2 (3C), 28.1 (3C), 24.5, 22.8, 21.2, 16.9; IR ($CDCl_3$) 3420, 3320, 1730, 1700, 1660 cm_; HRMS (FAB, PEG600) *m/e* calcd for $C_{42}H_{58}N_2O_9Cl$ (M + H)$^+$: 769.3831, found 769.3845; $[\alpha]_D$ +0.37 (*c* 2.70, $CHCl_3$).

***tert*-Butyl (5*S*,6*R*)-5-[(2*S*)-2-[3-[(2*R*)-2-*tert*-Butoxycarbonylamino-3-(4-chlorophenyl)propionylamino]propionyloxy-4-methylpentanoyloxy]-6-methyl-8-phenylocta-(2*E*,7*E*)-dienoate (24).** The total yield of **24**, pale yellow oil, was 0.19 g, 96%: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36−7.18 (m, 9H), 7.16−7.14 (m, 1H), 7.00−6.82 (m, 1H), 6.43 (d, 1H, *J* = 15.9 Hz), 6.03 (dd, 1H, *J* = 8.7 Hz, 15.9 Hz), 5.89 (d, 1H, *J* = 15.7 Hz), 5.63 (d, 1H, *J* = 8.5 Hz), 5.07−5.05 (m, 1H), 4.98 (dd, 1H, *J* = 3.6 Hz, 10.1 Hz), 4.46 (d, 1H, *J* = 5.6 Hz), 3.62−3.61 (m, 1H), 3.50−3.49 (m, 1H), 3.26 (dd, 1H, *J* = 5.0 Hz, 13.7 Hz), 2.96 (dd, 1H, *J* = 8.6 Hz, 13.2 Hz), 2.63−2.45 (m, 5H), 1.75−1.62 (m, 1H), 1.50 (s, 9H), 1.40 (s, 9H), 1.14 (d, 3H, *J* = 6.8 Hz), 0.96−0.89 (m, 2H), 0.86 (d, 3H, *J* = 6.5 Hz), 0.80 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 171.3, 170.8, 166.3, 155.9, 142.3, 137.2, 136.4, 132.8, 132.2, 131.2 (2C), 130.4, 129.0 (2C), 128.8 (2C), 128.0, 126.6 (2C), 126.5, 80.9, 80.0, 76.8, 71.6, 56.0, 41.6, 40.0, 38.4, 35.7, 35.5, 34.8, 28.7 (3C), 28.6 (3C), 25.0, 23.3, 21.7, 17.5; IR ($CDCl_3$) 3480, 3000, 2960, 1740, 1710, 1680 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{42}H_{58}N_2O_9Cl$ (M + H)$^+$: 769.3831, found 769.3859; $[\alpha]_D$ −1.3 (*c* 2.2, $CHCl_3$).

***tert*-Butyl *(5 S,6R)*-5-[(2S)-2-[3-[(2R)-2-*tert*-Butoxycarbonylamino-3-(3,4-dichlorophenyl)propionylamino]-propionyloxy-4-methylpentanoyloxy].-6-methyl-8-phenylocta-(2*E*,7*E*)-dienoate (25).** The total yield of **25**, a pale yellow oil, was 0.15 g, 82%: ^{1}H NMR (500 MHz, $CDCl_3$) 7.37−7.11 (m, 8H), 7.11−7.10 (m, 1H), 6.86−6.83 (m, 1H), 6.43 (d, 1H, *J* = 15.9 Hz), 6.02 (dd, 1H, *J* = 8.7 Hz, 15.9 Hz), 5.86 (d, 1H, *J* = 15.7 Hz), 5.72 (d, 1H, *J* = 7.9 Hz), 5.04−5.02 (m, 1H), 4.97 (dd, 1H, *J* = 3.5 Hz, 10.1 Hz), 4.46 (d, 1H, *J* = 4.8 Hz), 3.60−3.55 (m, 1H), 3.54−3.53 (m, 1H), 3.29−3.25 (m, 1H), 2.94−2.92 (m, 1H), 2.61−2.55 (m, 3H), 2.45−2.44 (m, 2H), 1.64−1.53 (m, 1H), 1.50 (s, 9H), 1.38 (s, 9H), 1.14 (d, 3H, *J* = 6.8 Hz), 0.92−0.86 (m, 2H), 0.85 (d, 3H, *J* = 6.5 Hz), 0.78 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3 (2C), 170.9, 165.9, 155.4, 141.9, 137.8, 136.7, 131.8, 131.4, 130.5, 130.1, 129.9, 128.8, 128.5 (2C), 128.3, 127.5, 126.1 (2C), 126.0, 80.4, 79.6, 76.3, 71.2, 55.2, 41.2, 39.5, 37.7, 35.3, 35.0, 34.3, 28.2 (3C), 28.1 (3C), 24.5, 22.8, 21.2, 17.0; IR ($CDCl_3$) 3440, 2980, 2960, 1740, 1710, 1680 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{42}H_{57}Cl_2N_2O_9$ (M + H)$^+$: 803.3441, found 803.3460; $[\alpha]_D$ −4.84 (*c* 3.00, $CHCl_3$).

Preparation of Styrenes 26−29 (Sample Procedure). To a solution of Boc-protected *tert*-butyl ester **22** (0.08 g, 0.11 mmol) in CH_2Cl_2 (5.0 mL) was added TFA (0.38 mL, 4.4 mnmol) dropwise. After stirring at room temperature for 2 h, dry toluene (0.2 mL) was added. The solution was concentrated in vacuo and pumped on overnight. The crude product was used directly without further purification in the next reaction. To a solution of the TFA salt (0.11 mmol) in CH_3CN (4.0 mL) were added DIEA (0.57 mL, 0.33 mmol) and HBTU (0.05 g, 0.13 mmol). After stirring at room temperature for 3 h, the solution was quenched with a solution of saturated $NaHCO_3$. The organic layer was extracted three times with CH_2Cl_2 and dried ($MgSO_4$). Following filtration and concentration, the crude product was purified using column chromatography on silica gel (10% acetone:CH_2Cl_2 containing 1% HOAc to 20% acetone:CH_2Cl_2 containing 1% HOAc).

Demethoxydesepoxycryptophycin-24 (26). The total yield of **26**, a white solid, was 0.033 g, *55%:* mp 242−243 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.21 (m, 10H), 7.04−7.02 (m, 1H), 6.73 (ddd, 1H, *J* = 4.9 Hz, 10.3 Hz, 15.2 Hz), 6.41 (d, 1H, *J* = 15.9 Hz), 6.04 (dd, 1H, *J* = 8.8 Hz, 15.9 Hz), 5.75 (dd, 1H, *J* = 1.1 Hz, 15.2 Hz), 5.65 (d, 1H, *J* = 8.3 Hz), 5.07 (ddd, 1H, *J* = 2.0 Hz, 4.5 Hz, 6.5 Hz), 4.92 (dd, 1H, *J* = 3.6 Hz, 9.9 Hz), 4.82 (dd, 1H, *J* = 2.2 Hz, 7.9 Hz), 3.54−3.46 (m, 2H), 3.33 (dd, 1H, *J* = 5.5 Hz, 14.4 Hz), 3.11 (dd, 1H, *J* = 7.7 Hz, 14.4 Hz), 2.58−2.53 (m, 4H), 2.39−2.36, (m, 1H), 1.68−1.60 (m, 2H), 1.37−1.30 (m, 1H), 1.15 (d, 3H, *J* = 6.9 Hz), 0.76 (d, 3H, *J* = 6.4 Hz), 0.73 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 171.2, 171.1, 165.9, 142.0, 137.1, 137.0, 132.2, 130.6, 129.6 (2C), 129.1 (2C), 129.0 (2C), 128.0, 127.3, 126.6 (2C), 125.5, 76.8, 71.9, 54.4, 42.7, 40.1, 36.8, 36.5, 34.7, 32.9, 24.7, 23.0, 21.6, 17.7; IR (CH_2Cl_2) 3440, 3060, 3000, 1740, 1680, 1650, 1430, 1220 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{33}H_{41}N_2O_6$ (M + H)$^+$: 561.2965, found 561.2960; $[\alpha]_D$ +31 (*c*

0.40, $CHCl_3$). 10-(3′-Chlorobenzyl)demethoxybenzyldesepoxycryptophycin-24 (27). The total yield of 27, a white solid, was 0.077 g, 88%: mp 213-215 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.22 (m, 9H), 7.13−7.11 (m, 1H), 6.72 (ddd, 1H, J = 4.8 Hz, 10.0 Hz, 15.2 Hz), 6.43 (d, 1H, J = 15.8 Hz), 6.03 (dd, 1H, J = 8.8 Hz, 15.8 Hz), 5.78 (d, 1H, J = 15.4 Hz), 5.67 (d, 1H, J = 8.6 Hz), 5.08−5.07 (m, 1H), 4.91 (dd, 1H, J = 3.5 Hz, 10.0 Hz), 4.83 (dd, 1H, J = 7.9 Hz, 14.0 Hz), 3.58−3.55 (m, 1H), 3.49−3.45 (m, 1H), 3.24 (dd, 1H, J = 5.6 Hz, 14.4 Hz), 3.05 (dd, 1H, J = 7.6 Hz, 14.4 Hz), 2.61−2.52 (m, SH), 1.32−1.23 (m, 1H), 1.15 (d, 3H, J = 6.8 Hz), 0.96−0.90 (m, 2H), 0.77 (d, 3H, J = 6.4 Hz), 0.73 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.7, 170.4, 165.6, 141.3, 138.9, 136.6, 134.3, 131.7, 130.1, 129.8, 129.3, 128.5 (2C), 128.4, 127.5, 127.3, 127.0, 126.1 (2C), 125.2, 71.5, 53.6, 42.2, 39.6, 36.3, 35.7, 34.5, 32.4, 24.3, 22.6, 21.1, 17.2; IR (CH_2Cl_2) 3420, 2980, 2950, 1750, 1680, 1630, 1510, 1390 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{33}H_{40}N_2O_6Cl$ (M + H)$^+$: 595.2575, found 595.2559; $[\alpha]_D$ +19 (*c* 0.28, $CHCl_3$).

10-(4′-Chlorobenzyl)demethoxybenzyldesepoxycryptophycin-24 (28). The total yield of **28**, a white solid, was 0.051 g, 83%: mp 225−227 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.17 (m, 9H), 6.90−6.88 (m, 1H), 6.72 (ddd, 1H, J = 5.3 Hz, 10.0 Hz, 15.3 Hz), 6.45 (d, 1H, J = 15.8 Hz), 6.04 (dd, 1H, J = 8.8, 15.8 Hz), 5.77 (d, 1H, J = 15.4 Hz), 5.66 (d, 1H, J = 8.5 Hz), 5.05 (dd, 1H, J = 6.6 Hz, 9.4 Hz), 4.92 (dd, 1H, J = 3.5 Hz, 9.4 Hz), 4.83 (dd, 1H, J = 7.7 Hz, 14.3 Hz), 3.61−3.55 (m, 1H), 3.48−3.41 (m, 1H), 3.23 (dd, 1H, J = 4.7 Hz, 14.4 Hz), 3.08 (dd, 1H, J = 7.6 Hz, 14.4 Hz), 2.60−2.53 (m, 4H), 2.42−2.35 (m, IH), 1.71−1.68 (m, 2H), 1.39−1.32 (m, 1H), 1.15 (d, 3H, J = 5.0 Hz), 0.78 (d, 3H, J = 6.4 Hz), 0.74 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.8, 170.4, 165.6, 141.4, 136.6, 135.2, 132.7, 131.8, 130.5 (2C), 130.0, 128.7 (2C), 128.5 (2C), 127.5, 126.1 (2C), 125.1, 71.5, 53.6, 42.2, 39.6, 36.3, 35.3, 34.4, 32.3, 24.3, 22.6, 21.1, 17.2; IR (CH_2Cl_2) 3000, 2960, 1750, 1720, 1650, 1380 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{33}H_{40}ClN_2O_6$ (M + H)$^+$: 595.2575, found 595.2570; $[\alpha]_D$ +33 (*c* 0.08, $CHCl_3$).

10-(3′,4′-Dichlorobenzyl)demethoxybenzyldesepoxycryptophycin-24 (29). The total yield of **29**, a white solid, was 0.049 g, 80%: mp 225−226 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35−7.24 (m, 7H), 7.10 (dd, 1H, J = 2.0 Hz, 8.2 Hz), 6.83−6.81 (m, 1H), 6.70 (ddd, 1H, J = 5.6 Hz, 9.7 Hz, 15.3 Hz), 6.44 (d, 1H, J = 15.8 Hz), 6.03 (dd, 1H, J = 8.8 Hz, 15.8 Hz), 5.80 (d, 1H, J = 15.6 Hz), 5.72 (d, 1H, J = 8.9 Hz), 5.03 (dd, 1H, J = 6.6 Hz, 9.2 Hz), 4.91 (dd, 1H, J = 3.5 Hz, 10.0 Hz), 4.83 (dd, 1H, J = 7.4 Hz, 15.0 Hz), 3.65−3.64 (m, 1H), 3.40−3.35 (m, 1H), 3.23 (dd, 1H, J = 6.0 Hz, 14.3 Hz), 3.04 (dd, 1H, J = 7.5 Hz, 14.3 Hz), 2.62−2.53 (m, 4H), 2.44−2.38 (m, 1H), 1.67−1.60 (m, 2H), 1.37−1.33 (m, 1H), 1.16 (d, 3H, J = 6.9 Hz), 0.78 (d, 3H, J = 6.4 Hz); 0.73 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 170.8, 170.3, 165.7, 141.3, 137.2, 136.6, 132.4, 131.8, 131.2, 130.9, 130.4, 130.0, 128.6 (2C), 128.5, 127.5, 126.1 (2C), 125.2, 71.4, 53.4, 42.2, 39.6, 36.3, 35.1, 34.6, 32.3, 24.3, 22.6, 21.1, 17.2; IR (CH_2Cl_2) 3420, 2980, 2950, 2900, 1740, 1680, 1630, 1510 cm^{-1}; HRMS (FAB, PEG600) *mle* calcd for $C_{33}H_{39}Cl_2N_2O_6$ (M + H)$^+$: 629.2185; $[\alpha]_D$ +18.3 (*c* 0.231, $CHCl_3$).

Preparation of Epoxides 4−7 (Sample Procedure). To a solution of styrene **26** (32.0 mag, 0.057 mmol) in acetone (2.0 mL) was added a solution of dimethyldioxirane[30] in acetone (2.0 mL). The solution was stirred at room temperature for 5 h. After concentrating, the residue was purified using column chromatography on silica gel (40% EtOAc:hexanes). The total yield of a mixture of 1:2 α:β-epoxides that were separated by HPLC (Vydac C18, internal diameter 8 mm, eluent (isocratic) MeOH:H_2O 65:35, flow 3 mL/min).

Demethoxycryptophycin-24 (4). The total yield of a mixture of **4** and α-**4** (β:α, 2:1), a white solid, was 21.9 mg, 66%. **4**, t_R = 10.4 min; mp 234−235 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.21 (m, 10H), 7.00 (br t, 1H, J = 5.1 Hz), 6.71 (ddd, 1H, J = 4.9 Hz, 10.4 Hz, 15.2 Hz), 5.72 (dd, 1H, J = 1.2 Hz, 15.2 Hz), 5.59 (d, 1H, J = 8.1 Hz), 5.24 (ddd, 1H, J = 2.0 Hz, 5.0 Hz, 11.0 Hz), 4.91 (dd, 1H, J = 3.5 Hz, 10.0 Hz), 4.82 (dd, 1H, J = 2.1 Hz, 7.9 Hz), 3.70 (d, 1H, J = 1.9 Hz), 3.58−3.53 (m, 1H), 3.48−3.44 (m, 1H), 3.25 (dd, 1H, J = 5.5 Hz, 14.5 Hz), 3.13−3.07 (m, 1H), 2.94 (dd, 1H, J = 1.9 Hz, 7.5 Hz), 2.61−2.54 (m, 3H), 2.49−2.43 (m, 1H), 1.75−1.65 (m, 3H), 1.34−1.30 (m, 1H), 1.16 (d, 3H, J = 6.7 Hz), 0.86 (d, 3H, J = 6.3 Hz), 0.85 (d, 3H, J = 6.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 170.6, 170.5, 165.3, 141.0, 136.6, 136.5, 129.1 (2C), 128.7 (2C), 128.6 (2C), 128.5, 127.0, 125.5 (2C), 125.1, 75.8, 71.1, 63.0, 59.0, 53.9, 40.6, 39.4, 36.3, 36.0, 34.2, 32.4, 24.3, 22.7, 21.2, 13.4; IR (film on KBr) 3286, 2958, 2925, 2853, 1743, 1678, 1536, 1453, 1371, 1261, 1173, 1012, 751, 698 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{33}H_{41}N_2O_7$ (M + H)$^+$: 577.2914, found 577.2903; $[\alpha]_D$ +41 (*c* 0.07, $CHCl_3$).

10-(3′-Chlorobenzyl)demethoxybenzylcryptophycin-24 (5). The total yield of a mixture of **5** and α-**5** (β:α, 2:1), a white solid, was 8.3 mg, 59%. **5**, t_R = 25.5 min; mp 213-214 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40−7.22 (m, 8H), 7.13−7.11 (m, 1H), 6.93 (br t, 1H, 5.3 Hz), 6.70 (ddd, 1H, J = 5.0 Hz, 10.0 Hz, 15.1 Hz), 5.75 (d, 1H, J = 15.6 Hz), 5.71 (d, 1H, J = 8.7 Hz), 5.21 (dd, 1H, J = 5.0 Hz, 11.0 Hz), 4.90 (dd, 1H, J = 3.3 Hz, 10.0 Hz), 4.81 (dd, 1H, J = 8.0 Hz, 14.0 Hz), 3.71 (d, 1H, J = 1.5 Hz), 3.60−3.52 (m, IH), 3.50−3.44 (m, 1H), 3.24 (dd, 1H, J = 5.6 Hz, 14.3 Hz), 3.05 (dd, 1H, J = 8.1 Hz, 14.4 Hz), 2.95 (dd, 1H, J = 1.8 Hz, 7.5 Hz), 2.59−2.53 (m, 3H), 2.51−2.41 (m, 1H), 1.73−1.66 (m, 2H), 1.35−1.31 (m, 2H), 1.17 (d, 3H, J = 6.8 Hz), 0.86 (d, 3H, J = 6.2 Hz), 0.85 (d, 3H, J = 6.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.6, 170.3, 165.4, 141.0, 138.8, 136.6, 134.3, 129.8, 129.3, 128.7 (2C), 128.5, 127.2, 127.1, 125.5 (2C), 125.2, 75.9, 71.1, 63.0, 59.0, 53.6, 40.5, 39.6, 36.6, 35.6, 34.4, 32.4, 24.3, 22.8, 21.2, 13.4; IR (film on KBr) 3404, 3277, 2959, 2927, 2854, 1745, 1731, 1679, 1673, 1536, 1467, 1371, 1243, 1173, 1077, 751, 698 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{33}H_{40}N_2O_7Cl$ (M + H)$^+$: 611.2524, found 611.2529; $[\alpha]_D$ +17 (*c* 0.36, $CHCl_3$).

10-(4′-Chlorobenzyl)demethoxybenzylcryptophycin-24 (6). The total yield of a mixture of **6** and α-**6** (β:α, 2:1), a white solid, was 13.4 mg, 74%. **6**, t_R = 24.1 min; mp 223−225 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41−7.35 (m, 5H), 7.28−7.25 (m, 2H), 7.17−7.15 (m, 2H), 6.90 (br t, 1H, J = 5.6 Hz), 6.70 (ddd, 1H, J = 5.0 Hz, 10.0 Hz, 15.2 Hz), 5.73 (dd, 1H, J = 1.0 Hz, 15.3 Hz), 5.64 (d, 1H, J = 8.5 Hz), 5.21 (ddd, 1H, J = 2.0 Hz, 5.0 Hz, 11.1 Hz), 4.90 (dd, 1H, J = 3.4 Hz, 10.0 Hz), 4.80 (dd, 1H, J = 2.0 Hz, 8.2 Hz), 3.71 (d, 1H, J = 2.0 Hz), 3.54−3.45 (m, 2H), 3.22 (dd, 1H, J = 5.9 Hz, 14.3 Hz), 3.07−3.05 (m, 1H), 2.94 (dd, 1H, J = 2.0 Hz, 7.6 Hz), 2.61−2.55 (m, 4H), 1.83−1.80 (m, 2H), 1.33−1.27 (m, 2H), 1.17 (d, 3H, J = 7.2 Hz), 0.86 (d, 3H, J = 6.4 Hz), 0.85 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 170.7, 170.4, 165.4, 141.0, 136.6, 135.1, 130.7, 130.5 (2C), 128.7 (2C), 128.6 (2C), 128.5, 125.5 (2C), 125.2, 75.8, 71.1, 63.0, 58.9, 53.6, 40.6, 39.4, 36.6, 35.3, 34.4, 32.3, 24.4, 22.8, 21.2, 13.4; IR (film on KBr) 3278, 2958, 2928, 2872, 1744, 1677, 1536, 1493, 1371, 1242, 1173, 1072, 1014, 752, 698 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{33}H_{40}N_2O_7Cl$ (M + H)$^+$: 611.2524, found 611.2517; $[\alpha]_D$ +35 (*c* 0.08, $CHCl_3$).

10-(3′,4′-Dichlorobenzyl)demethoxybenzylcryptophycin-24 (7). The total yield of a mixture of **7** and α-**7** (β:α, 2:1), a white solid, was 13.8 mg, 65%. **7**, t_R = 32.3 min; mp 192−194 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.34 (m, 6H), 7.26 (d, 1H, J = 1.4 Hz), 7.11 (dd, 1H, J = 2.0 Hz, 10.0 Hz), 6.82 (br t, 1H, J = 5.6 Hz), 6.70 (ddd, 1H, J = 5.3 Hz, 9.8 Hz, 15.2 Hz), 5.75 (d, 1H, J = 15.5 Hz), 5.71 (d, 1H, J = 8.7 Hz), 5.20 (dd, 1H, J = 5.0 Hz, 11.1 Hz), 4.90 (dd, 1H, J = 3.4 Hz, 10.2 Hz), 4.82 (dd, 1H, J = 7.8 Hz, 14.6 Hz), 3.71 (d, 1H, J = 1.9 Hz), 3.68−3.62 (m, 1H), 3.42−3.35 (m, 1H), 3.23 (dd, 1H, J = 5.9 Hz, 14.5 Hz), 3.03 (dd, 1H, J = 7.6 Hz, 14.4 Hz), 2.95 (dd, 1H, J = 1.9 Hz, 7.5 Hz), 2.65−2.55 (m, 3H), 251−2.42 (m, 1H), 1.85−1.81 (m, 1H), 1.74−1.67 (m, 2H), 1.37−1.32 (m, 1H), 1.18 (d, 3H, J = 6.9 Hz), 0.87 (d, 3H, J = 6.4 Hz), 0.86 (d, 3H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.3, 170.7, 170.2, 165.5, 140.9, 137.1, 137.1, 136.6, 132.4, 131.2, 130.9, 130.4, 128.7 (2C), 128.5, 128.4, 125.5 (2C), 75.9, 71.1, 62.9, 58.9, 53.4, 40.5, 39.4, 36.6, 35.1, 34.6, 32.3, 24.4, 22.8, 21.2, 13.4; IR (film on KBr) 3289, 2958, 2926, 2852, 1741, 1673, 1537, 1470, 1373,

1243, 1173, 1030, 751, 698 cm^{-1}; HRMS (FAB, PEG600) *m/e* calcd for $C_{33}H_{39}N_2O_7Cl_2$ $(M + H)^+$: 645.2134, found 645.2144; $[\alpha]_D$ +18 (*c* 0.20, $CHCl_3$).

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