Total Synthesis of Cryptophycin Analogues. Isosteric Replacement of the C−D Ester

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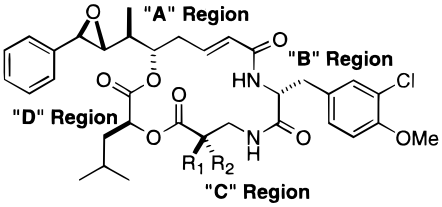
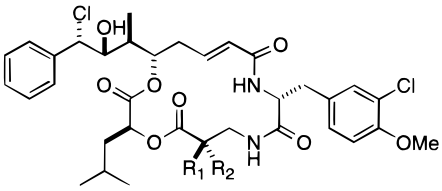
Introduction

The cryptophycins are an exciting new class of antitumor agents recently discovered from the terrestrial blue-green algae *Nostoc* sp. GSV 224.[1] These compounds have generated significant synthetic interest, due to their interesting and unique structural and biochemical properties.[2] The cytotoxic activity of cryptophycins is derived from their ability to inhibit microtubule polymerization,[3] and at the molecular level, cryptophycin 1 suppresses microtubule dynamics in a way that seems to distinguish it from other antimitotic drugs.[4] Additionally, many cryptophycin analogues show potent, broad-spectrum antitumor activity in human tumor xenograft models.[1,5] Cryptophycin 1 was identified as the most potent of the analogues derived from the algae, and its structure was proven via total synthesis.[6] The semisynthetic chlorohydrin, cryptophycin 8, showed similar in vitro cytotoxic activity but was found to have an improved therapeutic index relative to cryptophycin 1 in several animal models.[5] Furthermore, two synthetic analogues, LY355703 (cryptophycin 52) and cryptophycin 55, which differ from cryptophycins 1 and 8 by the addition of a methyl group in the C region, also show potent antitumor activity in in vitro and in vivo tumor models.[7,8,9] On the basis of the chemical stability studies of Moore and co-workers,[5] it may be useful to enhance the hydrolytic stability of the C−D ester bond. We have proposed that the replacement of the C−D ester bond with various ester isosteres could result in cryptophycin analogues with improved stability characteristics. In this paper, we describe our synthetic efforts to prepare several cryptophycin C−D isostere analogues and report their in vitro cytotoxicity properties.



R_1 = Me, R_2 = H **Cryptophycin 1**
R_1 = R_2 = Me **LY 355703 (Cryptophycin 52)**



R_1 = Me, R_2 = H **Cryptophycin 8**
R_1 = R_2 = Me **Cryptophycin 55**

Results and Discussion

In an effort to prepare cryptophycin analogues with enhanced C−D stability, we were interested in preparing several specific analogues. We sought to prepare epoxide and chlorohydrin derivatives of the C−D methylene ether isosteres (**1** and **3**) and both dimethyl (**2a**, **4a**) and monomethyl (**2b**, **4b**) C−D amides. We believed that these compounds would offer added stability to the molecule, while retaining many of the features necessary for maintaining the active conformation.

The Moore/Tius route to cryptophycin analogues[6] described the preparation of **11**, which was used in the synthesis of C−D isosteres.

Methylene ether dipeptide isosteres have been studied recently, and several approaches have been described.[10−12] The critical carbon−oxygen bond is most easily formed intramolecularly, according to the procedure of Ten Brink.[10] Although seven-membered ring intermediates have never been described using this chemistry, we felt that the methodology should be applicable to these analogues as well. The required C−D portion of the molecule (**10**) was prepared using a modified Ten Brink procedure, beginning with 2,2-dimethyl-3-amino-1-propanol (Scheme 1). This starting material was efficiently

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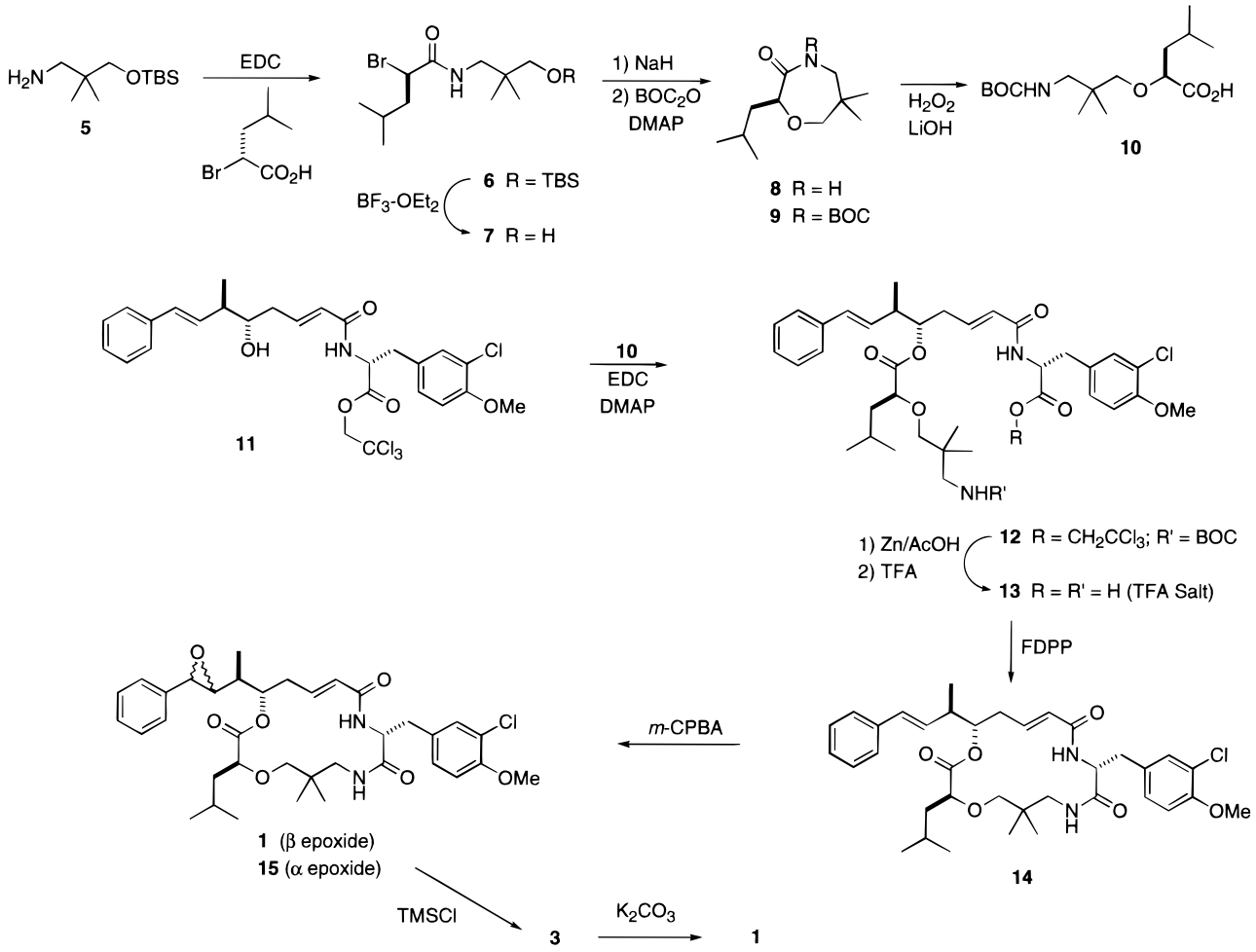
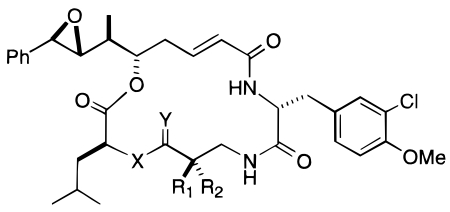
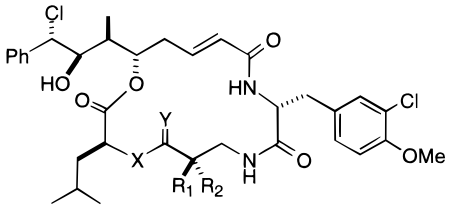
Scheme 1





Epoxide Series

C-D Ψ[CH_2O] (**1**) X=O, Y=HH, R_1=R_2=Me
C-D Ψ[CONH] (**2a**) X=NH, Y=O, R_1=R_2=Me
C-D Ψ[CONH] (**2b**) X=NH, Y=O, R_1=Me, R_2=H



Chlorohydrin Series

C-D Ψ[CH_2O] (**3**) X=O, Y=HH, R_1=R_2=Me
C-D Ψ[CONH] (**4a**) X=NH, Y=O, R_1=R_2=Me
C-D Ψ[CONH] (**4b**) X=NH, Y=O, R_1=Me, R_2=H

protected as the TBDMS ether (**5**) using standard conditions. This material was coupled to (*R*)-2-bromo-4-methylpentanoic acid with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) to give amide **6**. Silyl deprotection was effected using BF_3-OEt_2 to give the free alcohol **7**. Base-induced cyclization yielded the expected lactam **8**, which was converted to the BOC imide **9** using BOC_2O and DMAP. The lactam was opened with LiOOH and, following a reductive workup, yielded the *N*-BOC-protected dipeptide isostere **10**. Coupling of the A−B piece (**11**) to the C−D piece (**10**) was accomplished using EDC with catalytic DMAP to give the protected acyclic analogue **12**.[6] Reduction of the trichloroethyl ester with Zn in acetic acid, followed by amine deprotection using TFA, gave the acyclic amino acid **13** as the TFA salt. Macrocyclization was accomplished by treating **13** with pentafluorophenyl diphenylphosphinate (FDPP) in the presence of diisopropylethylamine to give **14**. Epoxidation of cryptophycins with *m*-CPBA typically yields about a 2:1 mixture of epoxides,[6] favoring the *β*-isomer. We observed a similar result for the epoxidation of **14**, giving **1** as the major product (ratio of **1**:**15** was 2:1). Since the epoxide mixture was only separable by reversed-phase HPLC, we chose to convert the mixture to their corresponding chlorohydrins. Previous studies indicate that the chlorohydrin mixtures are often separable by flash chromatography. Conversion to the chlorohydrins was accomplished via chlorotrimethylsilane, and the major isomer **3** was easily purified by flash chromatography. The pure chlorohydrin **3** could be converted to the pure epoxide **1** by simply treating with potassium carbonate in aqueous acetonitrile.

Our approach to the C−D amide analogues also utilized the A−B intermediate **11** (Scheme 2). This

Scheme 2

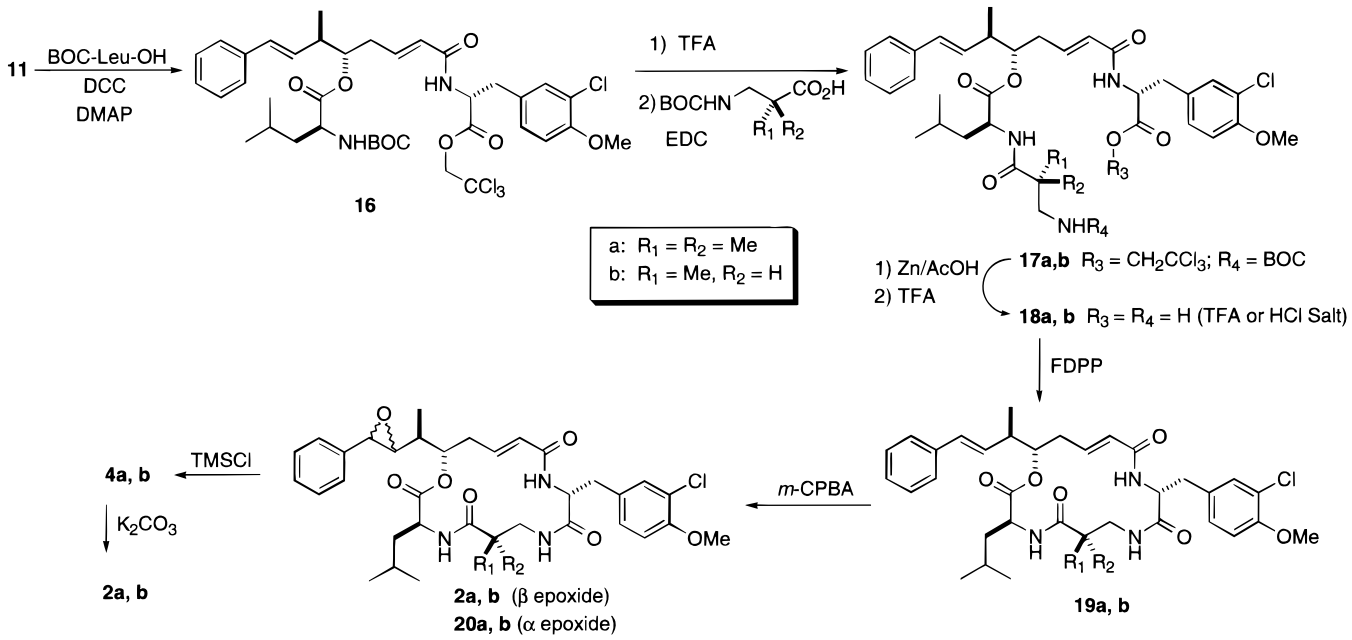


Table 1. In Vitro Cytotoxicity Data for Cryptophycin Analogues

compd	CCRF−CEM IC_{50} (nM)	KB IC_{50} (nM)
1	1.1	
2a	0.014	
2b		0.027
3	2.7	
4a	0.016	
4b		0.020
cryptophycin 1		0.0092
cryptophycin 8		0.019
LY355703 (cryptophycin 52)	0.022	0.038
cryptophycin 55	0.027	

material was coupled to BOC-leucine using DCC to yield the expected amide **16**. Deprotection of the N-terminal amine using TFA, followed by coupling to either *N*-BOC-2,2-dimethyl-*β*-alanine (**a** series) or (*R*)-*N*-BOC-2-methyl-*β*-alanine[6] (**b** series), produced the protected acyclic derivatives **17a** and **17b**, respectively. Using conditions similar to those described above, **17a** and **17b** were deprotected and cyclized to give the expected styrene macrocycles **19a** and **19b**. Epoxidation gave the expected 2:1 ratio of epoxides **2a,b** and **20a**,**b**. In the case of the **2a**:**20a** mixture, these were first converted to the corresponding chlorohydrins with TMSCl. Once again, the major chlorohydrin **4a** could be purified via flash chromatography and the pure *β*-epoxide **2a** prepared by treating **4a** with potassium carbonate in aqueous acetonitrile. For the **2b**:**20b** mixture, the epoxides were separated by reversed-phase HPLC (C-18, CH_3CN/water 65:35). The pure *β*-epoxide **2b** was similarly converted to the chlorohydrin **4b** by the action of TMSCl.

The six cryptophycin C−D isosteres were tested in an in vitro cytotoxicity assay, and the results are summarized in Table 1. The dimethylamides **2a** and **4a** showed in vitro activity similar to that of the esters LY355703 (cryptophycin 52) and cryptophycin 55 in the human leukemia CCRF−CEM cell line. Similarly, the monomethyl amides **2b** and **4b**, which were tested in the human nasopharyngeal carcinoma KB cell line, showed in vitro potency similar to cryptophycins 1 and 8. The activity of the methylene ether isosteres **1** and **3** was diminished ∼50-fold in CCRF−CEM relative to the esters. We have postulated that the carbonyl in the esters and amides may be participating in an intramolecular hydrogen bond or interacting directly with the receptor. This could explain the potency loss in the methylene ether series. Further studies are required to elucidate the in vivo stability of these molecules relative to the corresponding esters, and this work is ongoing.

Experimental Section

Melting points are uncorrected. NMR spectra were performed at the indicated field strengths in the indicated solvents. Anhydrous solvents were purchased from Aldrich, stored over 3A molecular sieves, and transferred via syringe or cannula. E. Merck silica gel 60 was used for flash chromatography. All reactions were performed under a nitrogen atmosphere. All yields are isolated yields of diastereomerically pure products unless otherwise noted. Selectivities were determined using reversed-phase HPLC and NMR integration techniques.

3-Amino-1-[(*tert*-butyldimethylsilyl)oxy]-2,2-dimethylpropane (5). A solution containing 10.0 g (96.9 mmol) of 3-amino-2,2-dimethyl-1-propanol and 16.2 mL (116 mmol) of triethylamine in 250 mL of CH_2Cl_2 was stirred at 0 °C. To this solution was added 474 mg (4 mol %) of (dimethylamino)pyridine (DMAP) and 16.1 g (107 mmol) of *tert*-butyldimethylsilyl chloride (TBSCl). The reaction mixture was allowed to warm to 25 °C over 1 h and stirred at that temperature for an additional 18 h. The reaction mixture was concentrated in vacuo and dissolved in 150 mL of ethyl acetate. The organic solution was washed three times with aqueous sodium bicarbonate solution, dried over sodium sulfate, and concentrated in vacuo to give 15.0 g of **5** as a light yellow oil: ^{1}H NMR δ ($CDCl_3$, 300 MHz) 0.05 (s, 6H), 0.81 (s, 6H), 0.86 (s, 9H), 1.39 (bs, 2H), 2.48 (s, 2H), 3.29 (s, 2H); IR ($CHCl_3$) 3672, 3387, 2858, 1472 cm^{-1}; MS (FD$^+$) *m*/*z* 218 (M + 1$^+$).

(*R*)-2-Bromo-4-(methylpentanoyl)-3-[(*tert*-butylsilyl)oxy]-2,2-dimethyl-1-amino amide (6). A solution of 3.25 g (16.7 mmol) of (*R*)-2-bromo-4-methylpentanoic acid[1] in 200 mL of CH_2Cl_2 was stirred at 0 °C as 2.25 g (16.7 mmol) of hydroxybenzotriazole (HOBt) was added, followed by the addition of 3.19 g (16.7 mmol) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC). The reaction mixture was stirred for 1.5 h at 0 °C, after which time 3.62 g (16.7 mmol) of the protected amino alcohol **5** in 25 mL of CH_2Cl_2 was added dropwise over about 5 min. The reaction mixture was stirred for an additional 2 h at 0 °C and poured into 200 mL of a saturated aqueous sodium

bicarbonate solution. The organic phase was washed twice with saturated aqueous sodium bicarbonate, twice with 10% aqueous citric acid, and twice with brine. The organic solution was dried over sodium sulfate and concentrated in vacuo to give 4.90 g (74%) of **6** as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.05 (s, 6H), 0.85 (s, 6H), 0.91 (s, 9H), 0.91−0.98 (m, 6H), 1.80−1.95 (m, 3H), 3.18 (dd, 1H, J = 14.7, 5.7 Hz), 3.22 (dd, 1H, J = 14.7, 3.0 Hz), 3.39 (s, 2H), 4.24 (dd, 1H, J = 5.7, 3.0 Hz), 7.00 (bs, 1H); IR ($CHCl_3$) 3674, 3421, 2931, 1670 cm^{-1}; MS (FD$^+$) m/z 394, 396 (M + 1$^+$). Anal. Calcd. For $C_{17}H_{36}NO_2Br$: C, 51.76; H, 9.20; N, 3.55. Found: C, 52.03; H, 9.17; N, 3.72.

(*R*)-2-Bromo-4-(methylpentanoyl)-3-hydroxy-2,2-dimethyl-1-aminoamide (7). To a solution containing 1.00 g (2.54 mmol) of the protected amide **6** in 20 mL of CH_2Cl_2 at 0 °C was added 0.34 mL (2.79 mmol) of boron trifluoride etherate (BF_3·OEt_2), and the reaction mixture was warmed to 25 °C. After the mixture was stirred for 1 h at 25 °C, an additional 0.34 mL of boron trifluoride etherate was added, and the reaction was stirred for an additional 1 h at 25 °C. The reaction was poured into 100 mL of water and diluted with an additional 50 mL of CH_2Cl_2. The organic solution was washed twice with saturated sodium bicarbonate solution, dried over sodium sulfate, and concentrated in vacuo to give 560 mg (79%) of the bromo alcohol **7** as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (s, 3H), 0.91 (s, 3H), 0.95 (d, 3H, J = 7.0 Hz), 0.98 (d, 3H, J = 7.1 Hz), 1.80−1.97 (m, 3H), 3.10 (dd, 1H, J = 14.7, 8.5 Hz), 3.18 (dd, 1H, J = 14.7, 4.5 Hz), 3.23 (s, 2H), 4.35 (dd, 1H, J = 8.5, 4.5 Hz), 6.84 (bs, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.1, 22.6, 26.4, 36.9, 44.6, 47.1, 49.8, 68.6, 171.0; IR ($CHCl_3$) 3421, 2961, 1654 cm^{-1}; MS (FD$^+$) m/z 280 (M$^+$).

6-*sec*-Butyl-3,3-dimethyl-5-oxocaprolactam (8). A solution containing 500 mg (1.78 mmol) of bromo alcohol **7** in 10 mL of anhydrous THF was added to a suspension of 86 mg (2.14 mmol) of sodium hydride (60% in mineral oil) at −78 °C. The reaction mixture was allowed to slowly warm to 25 °C over 1 h and quenched with 1 N HCl. The organic phase was separated, dried over sodium sulfate, and concentrated in vacuo. This crude material was purified by flash chromatography on silica gel, using ethyl acetate as the eluent to give 185 mg (52%) of **8** as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81 (s, 3H), 0.87 (d, 3H, J = 6.5 Hz), 0.95 (d, 3H, J = 6.5 Hz), 1.00 (s, 3H), 1.58 (m, 1H), 1.68 (m, 1H), 1.80 (m, 1H), 3.03 (m, 2H), 3.29 (d, 1H, J = 12.2 Hz), 3.66 (d, 1H, J = 12.2 Hz), 3.96 (dd, 1H, J = 10.0, 3.6 Hz), 6.28 (bs, 1H); IR ($CHCl_3$) 2961, 1668 cm^{-1}; MS (FD$^+$) m/z 199 (M$^+$).

***N*-BOC-6-*sec*-butyl-3,3-dimethyl-5-oxocaprolactam (9).** To a solution containing 2.00 g (10.0 mmol) of **8** in 50 mL methylene chloride was added 2.44 g (20,0 mmol) of 4-(*N*,*N*-dimethylamino)pyridine (DMAP), followed by 4.38 g (20.0 mmol) of di-*tert*-butyl dicarbonate. The reaction mixture was stirred at 25 °C for 2 h and then concentrated in vacuo. The crude mixture was diluted with 100 mL of ethyl acetate, and the organic solution was washed twice with 10% aqueous citric acid and twice with brine, dried over sodium sulfate, and concentrated in vacuo. This crude material was purified by flash chromatography on silica gel, using 25% ethyl acetate−hexane as the eluent, to give 2.10 g (70%) of **9** as a clear oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (s, 3H), 0.89 (d, 3H, J = 6.5 Hz), 0.93 (d, 3H, J = 6.6 Hz), 1.01 (s, 9H), 1.50−1.75 (m, 3H), 3.28 (d, 1H, J = 11.9 Hz), 3.35 (d, 1H, J = 15.0 Hz), 3.67 (d, 1H, J = 11.9 Hz), 3.91 (d, 1H, J = 15.0 Hz), 4.01 (dd, 1H, J = 8.4, 4.5 Hz); IR ($CHCl_3$) 2960, 1766, 1716 cm^{-1}.

***N*-BOC-3-amino-2,2-dimethylpropyl 2-(4-Methylpentanoic acid) Ether (10).** To a solution containing 125 mg (0.417 mmol) of **9** in 20 mL of THF was added 7 mL of water, and the reaction mixture was cooled to 5 °C. To the stirred reaction mixture was added 0.50 mL (4.17 mmol) of 30% hydrogen peroxide solution, followed by 87 mg (2.08 mmol) of LiOH hydrate. The reaction mixture was allowed to warm to 25 °C over 30 min and was quenched by the addition of concentrated sodium sulfite solution. The THF was removed in vacuo, and the resulting aqueous solution was adjusted to pH = 4 with 10% aqueous citric acid and extracted with ethyl acetate (three times). The combined organic extracts were dried over sodium sulfate and concentrated in vacuo to give 105 mg (79%) of **10** as a white amorphous solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (s, 3H), 0.92 (d, 3H, J = 6.2 Hz), 0.94 (d, 3H, J = 6.3 Hz), 0.96 (s, 3H), 1.44 (s, 9H), 1.58 (m, 1H), 1.70 (m, 1H), 1.86 (m, 1H), 2.85 (m, 1H), 2.93 (d, 1H, J = 8.5 Hz), 3.40 (d, 1H, J = 8.5 Hz), 3.82 (dd, 1H, J = 9.6, 3.9 Hz); IR (CHCl3) 2961, 1740, 1705 cm^{-1}; MS (FD$^+$) m/z 318 (M + 1$^+$).

Crypto-*N*-BOC−C−[CH_2O]−D−A−B−OCH_2CCl_3 (12). To a solution containing 323 mg (1.02 mmol) of **10** in 20 mL of CH_2Cl_2 was added 195 mg (1.02 mmol) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), followed by 10 mg of (dimethylamino)pyridine (DMAP). The reaction mixture was stirred at 25 °C for 30 min, after which time 500 mg (0.848 mmol) of cryptophycin A−B trichloroethyl ester (**11**) was added. The reaction was stirred for an additional 15 min, washed with 1 N HCl, dried over sodium sulfate, and concentrated in vacuo. The crude material was purified by flash chromatography using 25% ethyl acetate−hexane as the eluent. The major fraction was concentrated in vacuo to give 451 mg (67%) of **12** as a white amorphous solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81 (d, 3H, J = 6.3 Hz), 0.83 (d, 3H, J = 6.3 Hz), 0.89 (s, 6H), 0.95 (m 1H), 1.11 (d, 3H, J = 5.5 Hz), 1.44 (s, 9H), 1.45−1.62 (m, 2H), 1.71 (m, 1H), 2.48 (m, 2H), 2.60 (m, 1H), 2.85−3.30 (m, 5H), 3.74 (m, 1H), 3.86 (s, 3H), 4.70 (d, 1H, J = 11.9 Hz), 4.79 (d, 1H, J = 11.9 Hz), 5.04 (m, 2H), 5.43 (t, 1H, J = 6.0 Hz), 5.89 (d, 1H, J = 15.3 Hz), 6.04 (dd, 1H, J = 15.3, 8.6 Hz), 6.40 (d, 1H, J = 15.8 Hz), 8.81 (m, 1H), 6.85 (d, 1H, J = 8.5 Hz), 7.03 (d, 1H, J = 8.6 Hz), 7.18−7.36 (m, 7H); IR ($CHCl_3$) 2960, 1733, 1712, 1675 cm^{-1}; MS (FD$^+$) m/z 887 (M + 2$^+$).

Crypto C−D Ψ[CH_2O]styrene (14). To a solution of 50 mg (0.0631 mmol) of **12** in 8 mL of glacial acetic acid was added 200 mg of activated zinc dust. The suspension was sonicated for 45 min and stirred at room temperature for an additional 30 min. The mixture was filtered over a pad of Celite and the filtrate concentrated in vacuo. The resulting oily solid was treated with 5 mL of TFA and stirred at room temperature for 2 h. The solution was concentrated and used without any further purification. To a solution containing 45 mg (0.0583 mmol) of the crude amino acid **13** in 3 mL of DMF was added 55 μL (0.316 mmol) of diisopropylethylamine, followed by the addition of 32 mg (0.0833 mmol) of pentafluorophenyl diphenylphosphinate. The reaction was stirred at room temperature for 4 h and diluted with 50 mL of ethyl acetate and the organic phase washed with 1 N HCl (2×), saturated sodium bicarbonate solution (2×), and brine (2×). The solution was dried and concentrated in vacuo. The crude material was purified by flash chromatography on a silica gel comumn using 50% ethyl acetate−hexane as the eluent. Two fractions were collected, which correspond to the epimers at the isobutyl substitution site adjacent to the ether moiety. The major fraction was concentrated in vacuo to give 19 mg (47%) of **14** as a white amorphous solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.70 (d, 3H, J = 6.7 Hz), 0.78 (d, 3H, J = 6.5 Hz), 0.89 (s, 3H), 1.02 (s, 3H), 1.12 (d, 3H, J = 6.9 Hz), 1.18 (m, 1H), 1.45 (m, 1H), 1.73 (m, 1H), 2.28 (m, 1H), 2.54 (m, 2H), 2.90 (m, 2H), 3.19 (dd, 1H, J = 14.2, 7.0 Hz), 3.59 (m, 2H), 3.85 (s, 3H), 4.60 (dd, 1H, J = 15.7, 7.7 Hz), 5.15 (m, 1H), 5.70 (d, 1H, J = 15.0 Hz), 5.81 (d, 1H, J = 8.6 Hz), 5.99 (dd, 1H, J = 15.9, 8.8 Hz), 6.38 (d, 1H, J = 15.9 Hz), 6.57 (d, 1H, J = 8.6 Hz), 6.81 (d, 2H, J = 8.6 Hz), 6.85 (m, 1H), 7.06 (dd, 1H, J = 8.3, 1.8 Hz), 7.20−7.40 (m, 7H); IR ($CHCl_3$) 2957, 1745, 1671 cm^{-1}; MS (FD$^+$) m/z 638 (M$^+$).

Epoxide Mixture 1, 15. To a solution containing 47 mg (0.074 mmol) of **14** in 10 mL of CH_2Cl_2 was added 22.3 mg (0.11 mmol) of 85% *m*-CPBA. The reaction mixture was strirred at 25 °C and monitored by HPLC (reversed phase, C_{18}, 70% acetonitrile−water). After about 5 h, the reaction slowed, and an additional 44 mg of *m*-CPBA was added. The reaction mixture was stirred at 25 °C, and after another 12 h, the reaction was complete. The reaction mixture was diluted with 25 mL of CH_2Cl_2 and washed twice with a saturated sodium meta-bisulfite solution and twice with a saturated sodium bicarbonate solution. The organic layer was dried and concentrated in vacuo to give 38 mg (79%) of a white amorphous solid, which was charaterized as a 2:1 mixture of diastereomeric epoxides **1** and **15** (2:1 ratio). HPLC (reversed phase, C18, 70% acetonitrile−water, isocratic, 1.0 mL/min). Retention times: major isomer, 9.58 min; minor isomer, 10.28 min.

Crypto C−D Ψ[CH_2O]chlorohydrin 3. A solution containing 35 mg (0.53 mmol) of the epoxide mixture (**1** and **13**) in 3 mL of chloroform was cooled to −60 °C with stirring. To this

solution was added 34 μL (0.26 mmol) of chlorotrimethysilane all at once. The reaction mixture was stirred for 15 min at −60 °C and allowed to warm to 25 °C. The reaction mixture was quenched by the addition of 10 mL of water. An additional 20 mL of chloroform was added, and the organic solution was separated, dried over sodium sulfate, and concentrated in vacuo. The chlorohydrins were separated by by flash chromatography on silica gel using 50% ethyl acetate−hexane as the eluent. The minor chlorohydrin eluted first to give 8.6 mg (23%). This was followed by the elution of the major isomer, which was concentrated in vacuo to give 17 mg (46%) of **3** as a white amorphous solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93−0.98 (m, 9H), 1.05 (s, 3H), 1.07 (d, 3H, J = 7.7 Hz), 1.45 (m, 1H), 1.69 (m, 1H), 1.84 (m, 1H), 2.03 (d, 1H, J = 3.5 Hz), 2.35 (m, 1H), 2.56 (m, 2H), 2.72 (d, 1H, J = 13.3 Hz), 2.96 (m, 2H), 3.21 (dd, 1H, J = 14.3, 7.1 Hz), 3.69 (m, 2H), 3.89 (s, 3H), 3.99 (dd, 1H, J = 9.8, 2.2 Hz), 4.62 (m, 1H), 4.68 (d, 1H, J = 9.9 Hz), 5.28 (m, 1H), 5.80 (d, 1H, J = 15.0 Hz), 6.09 (d, 1H, J = 8.7 Hz), 6.63 (d, 1H, J = 7.7 Hz), 6.86 (d, 1H, J = 8.4 Hz), 6.89 (m, 1H), 7.11 (dd, 1H, J = 8.4, 1.8 Hz), 7.26 (d, 1H, J = 1.8 Hz), 7.30−7.40 (m, 6H); IR ($CHCl_3$) 2959, 1745, 1677, 1639 cm^{-1}; MS (FD$^+$) m/z 690 (M$^+$).

Pure Epoxide 1. To a solution of 10.0 mg (0.0145 mmol) of **3** in 5 mL of 50% acetonitrile/water was added 5 mg of sodium carbonate. The reaction was stirred at 25 °C for 1 h and then diluted with 10 mL of ethyl acetate. The organic solution was washed once with water, dried over sodium sulfate and concentrated in vacuo to give 8.0 mg (84%) of a white amorphous solid, which was characterized as pure epoxide **1**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (d, 3H, J = 6.4 Hz), 0.89 (d, 3H, J = 6.4 Hz), 0.93 (s, 3H), 1.07 (s, 3H), 1.18 (d, 3H, J = 6.7 Hz), 1.60 (m, 2H), 1.82 (m, 2H), 2.41 (m, 1H), 2.64 (m, 2H), 2.95 (m, 3H), 3.22 (dd, 1H, J = 14.2, 7.2 Hz), 3.61−3.69 (m, 2H), 3.72 (s, 1H), 3.91 (s, 3H), 4.65 (m, 1H), 5.35 (m, 1H), 5.67 (d, 1H, J = 8.5 Hz), 5.72 (d, 1H, J = 14.9 Hz), 6.57 (d, 1H, J = 9.1 Hz), 6.87 (d, 1H, J = 8.5 Hz), 6.90 (m, 1H), 7.11 (d, 1H, J = 6.7 Hz), 7.23−7.42 (m, 7H); IR (CHCl3) 2962, 1745, 1675, 1643 cm^{-1}; MS (FD$^+$) m/z 654 (M$^+$).

***N*-BOC-Leu-Crypto A−B (16).** To a solution containing 100 mg (0.221 mmol) of cryptophycin A−B trichloroethyl ester (**11**) and 82 mg (0.332 mmol) of *N*-BOC-leucine in 5 mL of methylene chloride was added 68 mg (0.332 mmol) of dicyclohexylcarbodiimide (DCC) and 5 mg of 4-(*N*,*N*-dimethylamino)pyridine (DMAP). The reaction mixture was strirred at 25 °C for 30 min and then diluted with 50 mL of ethyl acetate. The organic solution was washed with 0.5 N HCl and saturated sodium bicarbonate solution, dried over sodium sulfate, and concentrated in vacuo. The crude material was purified by flash chromatography using 25% ethyl acetate−hexane as the eluent. The major fraction was concentrated in vacuo to give 110 mg (62%) of a white amorphous solid, which was characterized as **16**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (d, 3H, J = 6.5 Hz), 0.88 (d, 3H, J = 6.5 Hz), 1.09 (d, 3H, J = 6.6 Hz), 1.42 (s, 9H), 1.46 (m, 1H), 1.63 (m, 2H), 2.49 (m, 2H), 2.58 (m, 1H), 3.04 (dd, 1H, J = 14.1, 6.9 Hz), 3.18 (dd, 1H, J = 14.1, 5.6 Hz), 3.83 (s, 3H), 4.17 (m, 1H), 4.65 (d, 1H, J = 11.9 Hz), 4.77 (d, 1H, J = 11.9 Hz), 4.95 (m, 2H), 5.86 (d, 1H, J = 15.7 Hz), 6.01 (dd, 1H, J = 15.7, 8.6 Hz), 6.37 (d, 1H, J = 15.7 Hz), 6.53 (d, 1H, J = 7.8 Hz), 6.74 (m, 1H), 6.80 (d, 1H, J = 8.3 Hz), 7.03 (d, 1H, J = 8.3 Hz), 7.19−7.35 (m, 7H); IR (KBr) 2959, 1739, 1707, 1678 cm^{-1}; MS (FD$^+$) m/z 802 (M + 2$^+$). Anal. Calcd For $C_{38}H_{48}N_2O_8Cl_4$: C, 56.87; H, 6.03; N, 3.49. Found: C, 57.07; H, 6.02; N, 3.39.

***N*-BOC-C(dimethyl)−Ψ[CONH]−D−A−B−OCH_2CCl_3 (17a).** To a solution containing 92 mg (0.115 mmol) of **16** in 2 mL of methylene chloride was added 2 mL of trifluoroacetic acid (TFA). The reaction mixture was stirred at 25 °C for 1 h and concentrated in vacuo to give 100 mg of a white amorphous solid, which was characterized as the corresponding TFA salt on the basis of its ^{1}H NMR spectrum: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.77 (d, 3H, J = 5.4 Hz), 0.85 (d, 3H, J = 5.2 Hz), 1.13 (d, 3H, J = 6.7 Hz), 1.72 (m, 3H), 2.41 (m, 1H), 2.60 (m, 2H), 2.99 (dd, 1H, J = 14.4, 7.1 Hz), 3.18 (dd, 1H, J = 14.4, 5,8 Hz), 3.85 (s, 3H), 4.07 (m, 1H), 4.65 (d, 1H, J = 11.9 Hz), 4.81 (d, 1H, J = 11.9 Hz), 4.93 (m, 1H), 5.08 (m, 1H), 5.90 (d, 1H, J = 15.4 Hz), 5.99 (dd, 1H, J = 15.8, 8.5 Hz), 6.42 (d, 1H, J = 15.8 Hz), 6.73 (m, 2H), 6.83 (d, 1H, J = 8.5 Hz), 7.01 (d, 1H, J = 8.0 Hz), 7.15−7.40 (m, 7H), 8.15 (bs, 3H). This material was used without further purification. To a solution containing 53 mg (0.244 mmol) of *N*-BOC-2,2-dimethyl-β-alanine in 10 mL of 50% THF−DMF were added 41 mg (0.305 mmol) of *N*-hydroxybenzotriazole (HOBt) and 52 mg (0.244 mmol) of 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide (EDC). The reaction mixture was stirred at 25 °C for 15 min, after which time a solution containing the TFA salt and 20 μL (0.183 mmol) of *N*-methylmorpholine (NMM) in 5 mL of DMF was added. The reaction was stirred at 25 °C for 15 h and diluted with 100 mL of ethyl acetate, and the organic solution was washed twice with 0.5 N HCl, twice with saturated sodium bicarbonate solution, and twice with brine. The organic layer was dried over sodium sulfate and concentrated in vacuo to give 105 mg (95%) of a white amorphous solid, which was characterized as **17a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (d, 3H, J = 6.2 Hz), 0.83 (d, 3H, J = 6.1 Hz), 1.08 (d, 3H, J = 6.8 Hz), 1.11 (s, 3H), 1.14 (s, 3H), 1.45 (s, 9H), 2.48 (t, 1H, J = 5.7 Hz), 2.55 (m, 1H), 3.01 (dd, 1H, J = 14.0, 7.8 Hz), 3.18 (m, 2H), 3.25 (m, 1H), 3.48 (d, 2H, J = 6.8 Hz), 3.81 (s, 3H), 4.33 (m, 1H), 4.63 (d, 1H, J = 11.8 Hz), 4.78 (d, 1H, J = 11.8 Hz), 5.00 (m, 2H), 5.22 (m, 1H), 5.91 (d, 1H, J = 15.8 Hz), 5.99 (dd, 1H, J = 15.8, 8.5 Hz), 6.27 (d, 1H, J = 6.5 Hz), 6.35 (d, 1H, J = 15.8 Hz), 6.71 (dt, 1H, J = 15.8, 6.0 Hz), 6.78 (d, 1H, J = 8.5 Hz), 7.04 (dd, 1H, J = 8.5, 1.6 Hz), 7.15−7.30 (m, 3H), 7.42 (m, 1H), 7.53 (m, 1H), 8.03 (d, 1H, J = 8.4 Hz); IR ($CHCl_3$) 2960, 1710, 1679, 1649 cm^{-1}; MS (FD$^+$) m/z 901 (M + 2$^+$).

***N*-BOC-C(monomethyl)−Ψ[CONH]−D−A−B−OCH_2CCl_3 (17b).** **17b** was prepared in an analogous fashion using (*R*)-*N*-BOC-2-methyl-β-alanine. The coupling reaction was carried out by making the mixed anydride of (*R*)-*N*-BOC-2-methyl-β-alanine (46 μL (0.27 mmol) of diisopropylamine and 35 μL (0.27 mmol) isobutyl chloroformate to (*R*)-*N*-BOC-2-methyl-β-alanine in 5 mL of dry THF at −15 °C). **17b** was isolated as a white amorphous solid (83% yield): $[\alpha]^{25}_D$ = −8.3 (c = 0.88, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.81 (d, 3H, J = 6.8 Hz), 0.86 (d, 3H, J = 6.8 Hz), 1.1 (d, 3H, J = 7.1 Hz), 1.1 (d, 3H, J = 6.8 Hz), 1.4 (m, 1H), 1.55 (m, 1H), 1.65 (m, 1H), 2.55 (m, 1H), 2.6 (m, 2H), 3.1 (dd, 1H, J = 14.2, 7.2 Hz), 3.2 (m, 2H), 3.86 (s, 3H), 4.4 (m, 1H), 4.68 (d, 1H, J = 12.0 Hz), 4.80 (d, 1H, J = 12 Hz), 5.0 (m, 1H), 5.0 (m, 1H), 5.0 (m, 1H), 5.95 (d, 1H, J = 15.4), 6.04 (dd, 1H, J = 15.9, 8.6 Hz), 6.12 (m, 1H), 6.4 (d, 1H J = 15.9 Hz), 6.75 (dt, 1H, J = 15.4, 6.4 Hz), 6.85 (d, 1H J = 8.2), 7.05 (dd, 1H, J = 8.2, 1.5 Hz), 7.15 (d, 1H, J = 7.6 Hz), 7.22(m, 1H), 7.22 (d, 1H, J = 1.5), 7.28−7.35 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz), 16.6, 21.5, 22.7, 24.5, 33.6, 36.6, 40.5, 41.3, 43.6, 51.3, 53.4, 56.1, 74.5, 76.3, 79.5, 94.4, 112.2, 122.3, 125.8, 126.2, 127.4, 128.6, 130.4, 131.2, 131.5, 136.9, 138.9, 154.1, 156.3, 165.8, 170.2, 172.6, 175.4.

Crypto Dimethyl C−D Amide Styrene 19a. To a solution of 100 mg (0.111 mmol) of **17a** in 15 mL of glacial acetic acid was added 400 mg of activated zinc dust. The suspension was sonicated for 45 min and stirred at room temperature for an additional 30 min. The mixture was filtered over a pad of Celite and the filtrate concentrated in vacuo. The resulting oily solid was treated with 10 mL of TFA and stirred at room temperature for 2 h. The solution was concentrated, and the resulting yellow foam was used without further purification. To a solution containing 85 mg (0.108 mmol) of the crude amino acid **18a** in 5 mL of DMF was added 113 μL (0.648 mmol) of diisopropylethylamine, followed by the addition of 54 mg (0.140 mmol) of pentafluorophenyl diphenylphosphinate. The reaction mixture was stirred at room temperature for 4 h and diluted with 100 mL of ethyl acetate and the organic phase washed with 1 N HCl (2×), saturated sodium bicarbonate solution (2×), and brine (2×). The solution was dried and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel using 50% ethyl acetate−hexane as the eluent to give 58 mg (83%) of a white amorphous solid, which was characterized as **19a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.74 (d, 3H, J = 5.9 Hz), 0.75 (d, 3H, J = 5.6 Hz), 1.15−1.22 (m, 9H), 1.38 (m, 2H), 1.49 (m, 1H), 2.38 (m, 1H), 2.55 (m, 2H), 2.98 (dd, 1H, J = 14.4, 7.7 Hz), 3.15 (m, 2H), 3.46 (dd, 1H, J = 12.6, 10.4 Hz), 3.87 (s, 3H), 4.43 (m, 1H), 4.65 (m, 1H), 5.12 (m, 1H), 5.61 (d, 1H, J = 6.4 Hz), 5.73 (d, 1H, J = 14.9 Hz), 5.90 (d, 1H, J = 8.3 Hz), 6.00 (dd, 1H, J = 15.8, 8.6 Hz), 6.39 (d, 1H, J = 15.8 Hz), 6.75 (m, 1H), 6.84 (d, 1H, J = 8.3 Hz), 6.94 (d, 1H, J = 8.2 Hz), 7.03 (d, 1H, J = 8.2 Hz), 7.18−7.40 (m, 6H); IR (KBr) 3415, 3319, 2961, 1740, 1653 cm^{-1}; MS (FD$^+$) m/z 651 (M$^+$).

Crypto Monomethyl C−D Amide Styrene 19b. 19b was prepared according to the procedure for the preparation of **19a** and was isolated as a white amorphous solid (50% yield): $[\alpha]^{25}_D$ = +46.5 (*c* = 0.81, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.74 (d, 3H, *J* = 6.6 Hz), 0.76 (d, 3H, *J* = 6.6 Hz), 1.2 (d, 3H, *J* = 7.2 Hz), 1.38 (dd, 2H, *J* = 7.7, 7.2 Hz), 1.55 (m, 1H), 2.35 (m, 1H), 2.55 (m, 3H), 3.0 (dd, 1H, *J* = 14.4, 7.7 Hz), 3.1 (dd, 1H, *J* = 14.4, 4.9 Hz), 3.4 (m, 1H), 3.5 (m, 1H), 3.86 (s, 3H), 4.4 (m, 1H), 4.7 (ddd, 1H, *J* = 7.7, 7.2, 4.9 Hz), 5.1 (m, 1H), 5.7 (d, 1H, *J* = 7.2 Hz), 5.75 (d, 1H, *J* = 16), 5.8 (d, 1H, *J* = 7.2), 6.04 (dd, 1H, *J* = 16.0, 8.8 Hz), 6.4 (d, 1H, *J* = 16.0 Hz), 6.75 (ddd, 1H, *J* = 16.0, 10.8, 4.2 Hz), 6.85 (d, 1H, *J* = 8.3 Hz), 7.05 (dd, 1H, *J* = 8.3, 2.0 Hz), 7.18 (d, 1H, *J* = 2.0 Hz), 7.21−7.35 (m, 5H), 7.25 (m, 1H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.8, 17.2, 21.5, 23.4, 24.7, 35.4, 36.3, 38.6, 41.0, 42.3, 51.1, 54.4, 56.2, 40.9, 76.5, 112.4, 122.5, 125.1, 126.1, 127.3, 128.4, 128.5, 129.6, 130.2, 130.8, 131.7, 136.8, 141.9, 154.1, 165.1, 171.0, 173.2, 175.8; EIMS *m*/*z* 637/639 (M^+); HREIMS *m*/*z* 637.2864 ($C_{35}H_{44}N_3O_6$35Cl, *D* = +5.5 mmu).

Crypto Dimethyl C−D Amide Epoxide Mixture 2a, 20a. To a solution containing 20 mg (0.031 mmol) of **19a** in 5 mL of CH_2Cl_2 was added 6.3 mg (0.037 mmol) of *m*-chloroperbenzoic acid (mCPBA). The reaction mixture was stirred at 25 °C and monitored by HPLC (reversed phase, C_{18}, 70% acetonitrile−water). After ∼5 h, the reaction slowed, and an additional 5 mg of *m*-CPBA was added. The reaction mixture was stirred at 25 °C, and after another 12 h, the reaction was complete. The reaction mixture was diluted with 25 mL of CH_2Cl_2 and washed twice with a saturated sodium metabisulfite solution and twice with a saturated sodium bicarbonate solution. The organic layer was dried and concentrated in vacuo to give 18 mg (85%) of a white amorphous solid, which was characterized as a 2:1 mixture of diastereomeric epoxides **2a** and **20a**: MS (FD^+) *m*/*e* 667 (M^+).

Crypto Monomethyl C−D Amide Epoxide 2b. The 2:1 mixture of epoxides **2b** and **20b** was prepared as described above. However, this mixture was separated by reversed-phase HPLC on C-18 silica (Econosil R C-18, 22 × 250 mm) eluting with CH_3CN/water 65:35 at 6 mL/min. **2b** (9 mg, 44%) eluted at 37.5 min, while **20b** (5 mg, 23%) eluted at 40 min. **2a**: $[\alpha]^{25}_D$ = +35.0 (*c* = 1, $CHCl_3$); ^1H NMR (acetone-d_6, 500 MHz) δ 0.8 (d, 6H, *J* = 6.5 Hz), 1.1 (d, 3H, *J* = 6.9 Hz), 1.35 (ddd, 1H, *J* = 13.7, 9.0, 4.8 Hz), 1.45 (ddd, 1H, *J* = 13.7, 10.8, 5.1 Hz), 1.65 (m, 1H), 1.85 (ddd, 1H, *J* = 7.7, 7.4, 4.9 Hz), 2.4 (ddd, 1H, *J* = 14.5, 11.5, 11.0 Hz), 2.55 (m, 1H), 2.65 (ddt, 1H, *J* = 14.5, 3.8, 2.0 Hz), 2.75 (dd, 1H, *J* = 14.5, 11.2 Hz), 3.0 (dd, 1H, *J* = 7.7, 2.0 Hz), 3.1 (ddd, 1H, *J* = 13.2, 2.1, 2.1 Hz), 3.2 (dd, 1H, *J* = 14.5, 3.6 Hz), 3.65 (ddd, 1H, *J* = 13.2, 8.8, 3.3 Hz), 3.82 (d, 1H, *J* = 2.0 Hz), 3.84 (s, 3H), 4.25 (ddd, 2H, *J* = 10.8, 8.1, 4.8 Hz), 4.45 (ddd, 1H, *J* = 11.2, 7.9, 3.6 Hz), 5.25 (ddd, 1H, *J* = 11.5. 4.9, 1.9 Hz), 5.9 (dd, 1H, *J* = 15.0, 1.9 Hz), 6.65 (ddd, 1H, *J* = 15.0, 11.0, 3.8 Hz), 7.0 (d, 1H, *J* = 8.4 Hz), 7.22 (dd, 1H, *J* = 8.4, 2.0 Hz), 7.35 (d, 1H, *J* = 8.1 Hz), 7.45 (d, 1H, *J* = 7.9 Hz), 7.8 (d, 1H, *J* = 8.8 Hz); ^{13}C NMR (acetone-d_6, 125 MHz) δ 13.9, 15.7, 21.5, 23.1, 25.3, 36.4, 37.7, 38.9, 40.6, 41.2, 41.3, 51.7, 56.4, 56.9, 59.3, 63.9, 76.0, 113.3, 122.4, 126.6, 126.7, 129.0, 129.2, 129.4, 131.4, 132.5, 138.6, 140.8, 154.6, 165.9, 171.9, 174.2, 177.2; EIMS *m*/*z* 653 (M^+); HREIMS *m*/*z* 653.2906 ($C_{35}H_{44}N_3O_7$35Cl, *D* = −3.8 mmu).

Crypto Dimethyl C−D Amide Chlorohydrin 4a. A solution containing 50 mg (0.075 mmol) of the epoxide mixture (**2a** and **20a**) in 2 mL of chloroform was cooled to −60 °C, and 47 μL (0.375 mmol) of chlorotrimethylsilane (TMSCl) was added. The reaction mixture was warmed to 25 °C and poured into 20 mL of water. An additional 20 mL of chloroform was added, and the organic layer was separated, dried over sodium sulfate, and concentrated in vacuo to give a mixture of chlorohydrins. The major chlorohydrin was purified by flash chromatography on silica gel using ethyl acetate as the eluent. The major fraction was concentrated in vacuo to give 19 mg (54%) of a white amorphous solid, which was characterized as **4a**: ^1H NMR ($CDCl_3$, 300 MHz) δ 0.92−0.96 (m, 6H), 1.03 (d, 3H, *J* = 6.9 Hz), 1.13 (s, 3H), 1.14 (s, 3H), 1.40−1.65 (m, 3H), 2.08 (m, 1H), 2.35 (m, 1H), 2.48 (m, 1H), 2.67 (d, 1H, *J* = 13.4 Hz), 2.92 (dd, 1H, *J* = 14.4, 8.6 Hz), 3.13 (m, 2H), 3.43 (dd, 1H, *J* = 12.7, 9.2 Hz), 3.88 (s, 3H), 3.99 (d, 1H, *J* = 9.6 Hz), 4.53 (m, 1H), 4.64 (d, 2H, *J* = 9.5 Hz), 5.23 (t, 1H, *J* = 9.5 Hz), 5.75 (m, 2H), 6.06 (d, 1H, *J* = 8.6 Hz), 6.76 (ddd, 1H, *J* = 14.9, 11.3, 3.4 Hz), 6.86 (d, 1H, *J* = 8.4 Hz), 6.93 (bd, 1H, *J* = 7.9 Hz), 7.06 (d, 1H, *J* = 8.1 Hz), 7.19 (s, 1H), 7.25−7.35 (m, 5H); IR (KBr) 3393, 3329, 2960, 1751, 1663 cm^{-1}; MS (FD^+) *m*/*e* 704 (M^+). Anal. Calcd for $C_{36}H_{47}N_3O_7Cl_2$: C, 61.36; H, 6.72; N, 5.96. Found: C, 61.63; H, 6.89; N, 5.67.

Crypto Monomethyl C−D Amide Chlorohydrin 4b. 4b was prepared according to the procedure for the preparation **4a** as described above. **4b**: 77% yield; $[\alpha]^{25}_D$ = +28.6 (*c* = 1.16, $CHCl_3$); ^1H NMR (500 MHz, $CHCl_3$) δ 0.92 (d, 3H, *J* = 6.8 Hz), 0.93 (d, 3H, *J* = 6.8 Hz), 1.02 (d, 3H, *J* = 7.0 Hz), 1.15 (d, 3H, *J* = 7.5 Hz), 1.55 (m, 2H), 1.65 (m, 1H), 2.35 (ddd, 1H, *J* = 14.5, 11.2, 10.9 Hz), 2.48 (m, 1H), 2.55 (m, 1H), 2.65 (m, 1H), 2.9 (dd, 1H, *J* = 14.6, 8.6 Hz), 3.15 (dd, 1H, *J* = 14.6, 4.7 Hz), 3.4 (m, 2H), 3.86 (s, 3H), 4.0 (brd, 1H, *J* = 9.6 Hz), 4.45 (m, 1H), 4.65 (d, 1H, *J* = 9.6 Hz), 4.65 (ddd, 1H, *J* = 8.6, 7.7, 4.7 Hz), 5.2 (ddd, 1H, *J* = 9.9, 9.9, 1.9 Hz), 5.8 (dd, 1H, *J* = 15.0, 1.5 Hz), 6.05 (d, 1H, *J* = 7.7 Hz), 6.2 (d, 1H, *J* = 8.2 Hz), 6.75 (ddd, 1H, *J* = 15.0, 10.9, 4.2 Hz), 6.85 (d, 1H, *J* = 8.6 Hz), 7.05 (dd, 1H, *J* = 8.6, 2.2 Hz), 7.18 (d, 1H, *J* = 2.2 Hz), 7.25 (brdd, 1H, *J* = 5.7, 4.1 Hz), 7.3−7.4 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 8.5, 14.8, 21.8, 22.8, 25.0, 35.4, 36.4, 38.6, 38.8, 40.9, 41.1, 51.1, 54.5, 56.1, 62.1, 73.9, 75.6, 112.4, 122.4, 124.0, 128.0, 128.2, 128.8, 129.8, 130.8, 138.8, 142.4, 154.0, 165.4, 171.1, 173.2, 175.8; EIMS *m*/*z* 653 (M^+ − HCl); HREIMS *m*/*z* 653.2841 ($C_{35}H_{44}N_3O_7$35Cl, *D* = +2.6 mmu).

Crypto Dimethyl C−D Amide Epoxide 2a. To a solution of 5.0 mg (0.0071 mmol) of **4a** in 2 mL of 50% acetonitrile/water was added 2 mg of sodium carbonate. The reaction was stirred at 25 °C for 1 h and then diluted with 10 mL of ethyl acetate. The organic solution was washed once with water, dried over sodium sulfate, and concentrated in vacuo to give 4.0 mg (85%) of a white amorphous solid, which was characterized as pure epoxide **2a**: ^1H NMR ($CDCl_3$, 300 MHz) δ 0.85 (m, 6H), 1.05−1.22 (m, 9H), 1.40 (m, 2H), 1.55 (m, 1H), 1.79 (dd, 1H, *J* = 12.6, 6.4 Hz), 2.41 (t, 1H, *J* = 12.5 Hz), 2.53 (t, 1H, *J* = 13.3 Hz), 2.92 (d, 1H, *J* = 7.4 Hz), 2.95−3.20 (m, 2H), 3.48 (dd, 1H, *J* = 12.6, 9.8 Hz), 3.67 (s, 1H), 3.87 (s, 3H), 4.42 (m, 1H), 4.64 (m, 1H), 5.25 (m, 1H), 5.51 (d, 1H, *J* = 6.6 Hz), 5.70 (d, 1H, *J* = 14.9 Hz), 5.87 (d, 1H, *J* = 8.4 Hz), 6.74 (m, 1H), 6.84 (d, 1H, *J* = 8.4 Hz), 6.96 (d, 1H, *J* = 9.0 Hz), 7.02 (d, 1H, *J* = 8.4 Hz), 7.17 (s, 1H), 7.22 (m, 2H), 7.30−7.42 (m, 6H); IR (KBr) 3414, 2960, 1744, 1654 cm^{-1}; MS (FD^+) *m*/*e* 667 (M^+). Anal. Calcd for $C_{36}H_{46}N_3O_7Cl$: C, 64.71; H, 6.94; N, 6.29. Found: C, 64.70; H, 7.11; N, 6.21.

In Vitro Cytotoxicity Assays. 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 the Biomek Automated Workstation (Beckman, Fullerton, CA). Micropipet tips were changed with each dilution. We have determined that cryptophycins need to be serially diluted in DMSO to reduce drug adsorption onto plastic and glass surfaces. Log-phase human tumor 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 UltraCHO serum-free medium or 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 per 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 adherent cell lines were treated with trypsin/EDTA to prepare cell suspensions. The cells in each well were quantitated using a ZBI Coulter counter, and an IC_{50} was determined.

Acknowledgment. We thank the Physical Chemistry Department at Eli Lilly and Co. for their help in the characterization of products. We also acknowledge our co-workers in Process Research at Eli Lilly and Co. for providing to us many of the cryptophycin intermediates used in this work. Finally, we would like to thank our co-workers at the University of Hawaii for their important contributions to our collaborative research effort.

Supporting Information Available: 300 or 500 MHz ^{1}H NMR spectra for compounds lacking combusion data (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

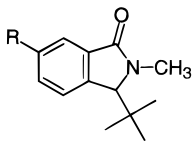
JO980536R

Additions and Corrections

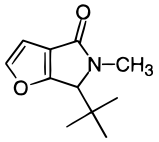
Vol. 59, 1994

Akihiro Orita, Masato Fukudome, Kouichi Ohe, and Shinji Murai*. Reactions via Carbonyl Anions. [4 + 1] Cyclocoupling of the Azadienyllithium with Carbon Monoxide.

Page 479. The structures of **27** and **29** should be replaced as shown below.



27a: R = H
b: R = CH_3
c: R = OCH_3



29

Pages 480 and 481. The assignment of the spectral data for compounds **27** and **29** should read as follows:

2,3-Dihydro-3-(1,1-dimethylethyl)-2-methyl-1*H*-isoindol-1-one (27a): ^{1}H NMR ($CDCl_3$) δ 3.24 (s, 3H, CH_3N); ^{13}C NMR ($CDCl_3$) δ 33.0 (CH_3N), 170.1 (C═O); IR (KBr) 1684 (C═O) cm^{-1}.

2,3-Dihydro-2,6-dimethyl-3-(1,1-dimethylethyl)-1*H*-isoindol-1-one (27b): ^{1}H NMR ($CDCl_3$) δ 3.23 (s, 3H, CH_3N); ^{13}C NMR ($CDCl_3$) δ 32.8 (CH_3N), 170.1 (C═O); IR (KBr) 1678 (C═O) cm^{-1}.

2,3-Dihydro-3-(1,1-dimethylethyl)-2-methyl-6-methoxy-1*H*-isoindol-1-one (27c): ^{1}H NMR ($CDCl_3$) δ 3.23 (s, 3H, CH_3N); ^{13}C NMR ($CDCl_3$) δ 32.9 (CH_3N), 169.9 (C═O); IR (KBr) 1680 (C═O) cm^{-1}.

6-(1,1-Dimethylethyl)-5-methyl-4*H*-furo[2,3-*c*]pyrrol-4(5*H*)-one (29): ^{1}H NMR ($CDCl_3$) δ 3.14 (s, 3H, CH_3N); ^{13}C NMR ($CDCl_3$) δ 32.0 (CH_3N), 169.5 (C═O); IR (KBr) 1682 (C═O) cm^{-1}.

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Richard K. Haynes,* William W.-L. Lam, Lam-Lung Yeung, Ian D. Williams, Andrew C. Ridley, Scott M. Starling, Simone C. Vonwiller, Trevor W. Hambley, Charles W. Jefford, and Patrick Lelandais . Highly Diastereoselective Conjugate Addition of Lithiated γ-Crotonolactone (But-2-en-4-olide) to Cyclic Enones To Give Syn-Adducts: Application to a Brefeldin Synthesis.

Page 4552. The co-author name of Charles W. Jefford, Département de Chimie Organique, Université de Genève 30, Quai Ernest-Ansermet, CH-1211 Genève 4, Switzerland, should be inserted before that of Patrick Lelandais and the address of Patrick Lelandais as given in the original paper changed to the foregoing address, such that the authorship and addresses read as follows:

Richard K. Haynes,[*,‡] William W.-L. Lam,[*,‡] Lam-Lung Yeung,[‡] Ian D. Williams,[‡] Andrew C. Ridley,[§] Scott M. Starling,[§] Simone C. Vonwiller,[§] Trevor W. Hambley,[§] Charles W. Jefford,[⊥] and Patrick Lelandais[⊥]

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Ahmed Abouabdellah, Jean-Pierre Bégué,* Danièle Bonnet-Delpon, and Truong Thi Thanh Nga. Diastereo- and Enantioselective Synthesis of Syn 1-Fluoroalkyl Isoserinates.

Page 8826, Schemes 5 and 6. The $[\alpha]_D$ of compound **19** is +17° (not −17° and −15°).

Page 8832 (first column, at the bottom). The (*S*)-*N*-(2,2,2-trifluoroethylidene)phenethylamine **18** was obtained in 90% yield (not in 55%).

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S0022-3263(98)04007-9
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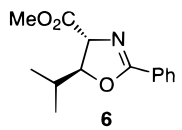
Vol. 63, 1998

James S. Panek* and Craig E. Masse. An Improved Synthesis of (4*S*,5*S*)-2-Phenyl-4-methoxycarbonyl-5-isopropyloxazoline from (*S*)-Phenylglycinol.

Page 2382. Structure **6** in eq 1 and Scheme 1 was incorrectly assigned as the (4*S*,5*S*)-isomer. Recent NMR investigations on this compound have revealed that this compound is actually the (4*R*,5*S*)-isomer which presumably forms via equilibration during the oxazoline formation (**5** → **6**).



6

Page 2384. The experimental heading for compound **6** should read **(4*R*,5*S*)-2-Phenyl-4-methoxycarbonyl-5-isopropyloxazoline (6)**. All of the characterization data are correctly listed.

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S0022-3263(98)04004-3
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