Total Synthesis of Cryptophycins via a Chemoenzymatic Approach

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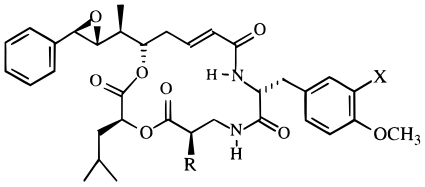
A highly convergent synthesis of cryptophycins in their enantiomerically-pure forms was achieved. Our strategy consists of the synthesis of the two units **3** and **4** and linking them together to form the macrocyclic ring. The upper unit **3** was prepared from **10** in four steps, and the lower unit **4** was prepared from **20** in three steps. Enantioselective biocatalytic methodology was used to prepare the requisite chiral building blocks, (*R*)-**11** and (*R*)-**19**. The stereochemical versatility of this synthetic approach is demonstrated by the synthesis of cryptophycin A and the four diastereomers of cryptophycin C.

Introduction

A wide array of novel bioactive cyclic peptides and depsipeptides have been isolated from Cyanobacteria.[1] The cryptophycins comprise the largest class of cyanobacterial depsipeptides with 25 members to date.[2] The most important member of this family is cryptophycin A (**1**), which was first isolated from *Nostoc* sp. ATCC 53789 by scientists at Merck.[3] It was found to be a potent antifungal agent, especially toward strains of *Cryptococcus*, which is a frequent opportunistic pathogen in immunodeficient persons suffering from AIDS and cancer, but unfortunately, it was too toxic to be of therapeutic use. In 1994, Moore's group in Hawaii described the isolation of **1** and six additional cytotoxic analogs from a terrestrial *Nostoc* sp. GSV 224.[4] One of the minor analogs was cryptophycin-24, which proved to be identical to arenastatin A (**2**) from an Okinawan sponge.[5]

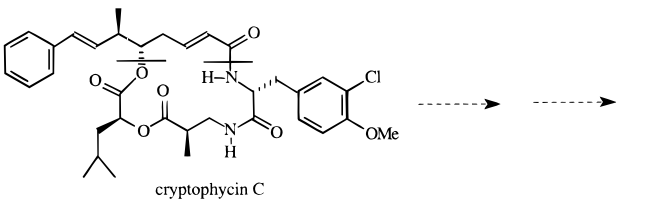


cryptophycin A **1** X = Cl; R = CH_3
arenastatin A **2** X = H; R = H

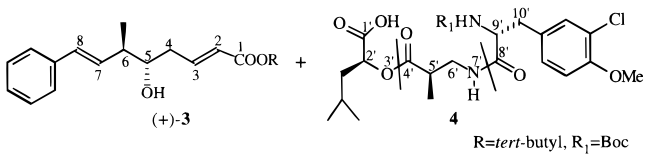
Cryptophycin A was discovered to inhibit tubulin polymerization[6] and was found to be effective against a broad spectrum of solid tumors, most notably, the drug resistant and multiple drug-resistant ones.[4] Preliminary animal data suggest that cryptophycin A may be useful against breast, pancreatic, and colon tumors. The gross chemical structures of cryptophycins were established using a combination of chemical and spectral techniques.[2] The absolute stereochemistry of **1** was deduced from the structures of cryptophycin C and D, which were obtained from total synthesis. Since large scale propagation of *Nostoc* sp. afforded **1** in very low yields (about 2 mg/L),[4] we turned our attention to total synthesis for the preparation of cryptophycins. Although Moore and coworkers have recently completed an elegant total synthesis of cryptophycin C and D,[7] their synthetic route is relatively lengthy and is not easily adaptable for the synthesis of cryptophycins in gram quantities. The objective of our synthetic explorations is to develop a more concise synthesis to alleviate the scarcity of cryptophycins and to prepare analogs for examining the relationship of chemical structure to biological activity. We herein report the total synthesis of cryptophycin A and the four diastereomers of cryptophycin C.

Results and Discussion

Retrosynthetic analysis of the cryptophycin carbon skeleton reveals that the macrocyclic ring may be most conveniently constructed by the assembly of the two fragments **3** and **4**, derived from the disconnection of the bonds between O(5)−C(1′) and C(1)−N(9′). The upper fragment **3** consists of a suitably protected derivative of (2*E*,7*E*,5*S*,6*R*)-5-hydroxy-6-methyl-8-phenylocta-2,7-dienoic acid and the lower fragment **4** consists of three subunits which are composed of (*S*)-2-hydroxy-4-methylvaleric acid, (*R*)-3-amino-2-methylpropanoic acid, and 3-chloro-*O*-methyl-D-tyrosine.



cryptophycin C



(+)-**3** **4**

R=*tert*-butyl, R_1=Boc

In the initial phases of our study, racemic compounds were used to examine the feasibility and efficiency of the important carbon−carbon bond-forming reaction for the construction of (±)-**3** (Scheme 1).

⊗ Abstract published in *Advance ACS Abstracts*, September 15, 1996.

(1) Moore, R. E. *J. Ind. Microbiol.* **1996**, *16*, 134−143.
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(4) Golakoti, T.; Ogino, J.; Heltzel, C. E.; Husrebo, T. L.; Jensen, C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Moobery, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1995**, *117*, 12030−12049.
(5) Kobayashi, M.; Aoki, S.; Ohyabu, N.; Kurosu, M.; Wang, W.; Kitagawa, I. *Tetrahedron Lett.* **1994**, *35*, 7969−7972.
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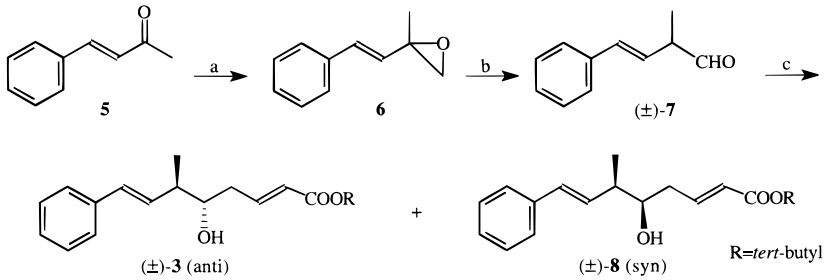
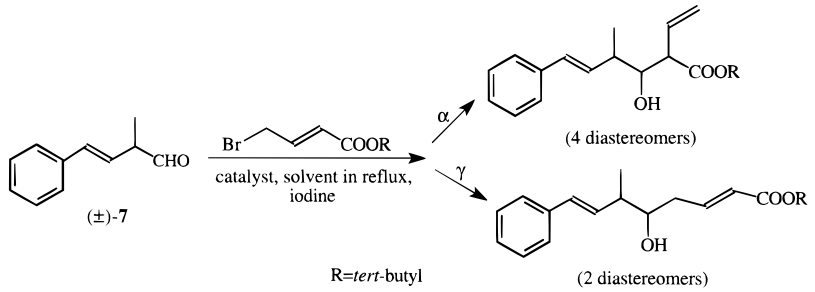
Scheme 1[a]



[a] (a) $(CH_3)_3S^+CH_3OSO_3^-$, CH_2Cl_2, NaOH, H_2O, 50 °C, >95%; (b) $MgBr_2$, ether, −25 °C, >95%; (c) *tert*-butyl 4-bromocrotonate (**9**), Zn−Pb, ether−benzene 1:1, reflux 60%.

Scheme 2



Our synthetic route begins with the commercially available *trans*-4-phenyl-3-buten-2-one (**5**) as the starting material. Reaction of **5** with trimethylsulfonium methyl sulfate under phase transfer catalysis conditions led to the unsaturated oxirane, **6** in excellent yield.[8] The epoxide **6** was then rearranged to the β,γ-unsaturated aldehyde (±)-**7** by its exposure to anhydrous $MgBr_2$ (generated in situ). The yield of this rearrangement was quantitative and no impurities were detected by means of NMR spectroscopy. The final and crucial step of the synthesis was the Reformatsky reaction of the aldehyde (±)-**7** with *tert*-butyl 4-bromocrotonate (**9**).[9] It is well known that the regioselectivity of addition of organometallic reagents, derived from substituted allyl halides, to carbonyl compounds is strongly dependent on solvents and metal catalysts. Hence, the four-carbon homologation, as depicted in Scheme 2, is expected to generate regiochemical problems arising from the behavior of the delocalized reagents of this type. As was succinctly pointed out by Hudlicky,[10] the regioselectivity of these additions depended heavily on the nature of the catalyst and the solvent used. As a rule, the γ-mode of addition increases with softness of the catalyst and with decreasing polarity of the solvent.

Therefore, a series of experiments were conducted with a view to defining the most suitable conditions favoring the yield of the desired γ-addition product. Indeed, after much experimentation we found that the best yield of the γ-addition products, **3** and **8** (60%), was attained using "dry" Zn−Pb couple in a gently boiling mixture of ether−benzene (1:1) (Table 1). However, the use of Zn−Pb catalyst in neat benzene was found to be unsatisfactory. In this case, the rate of C−C coupling was slow and other competitive processes such as the isomerization of the double bond in **7**, the cleavage of the *tert*-butyl ester of **9**, and the metal-induced coupling of the halo ester became dominant reactions. The diastereomeric esters (±)-**3** and (±)-**8** were easily separated by means of silica gel column chromatography and were later used for the synthesis of the four diastereomers of cryptophycin C. Compound (±)-**3**, eluted from the column as the less polar isomer, was transformed into cryptophycin C[2,7a] (**26**) and **30** (Scheme 8), thereby establishing that the stereochemistry of (±)-**3** is *anti* as shown in Scheme 1. However, as these compounds (±)-**3** and (±)-**8** are highly susceptible to dehydration on silica gel to give the corresponding triene derivative (**14** in Scheme 4), it was necessary to include triethylamine in the eluting solvent to avoid this problem.

Table 1. Addition of 9 to (±)-7

catalyst	solvent	yield (%)	products γ/α[a]
Zn	ether	low (<40)	10:90
Zn−Cu	ether	low (<40)	40:60
Zn−Cu	benzene	0	cleavage of *t*-butyl ester
Zn	benzene	0	cleavage of *t*-butyl ester
Zn−Cu	THF	0	condensation of aldehyde
Zn−Cu	benzene−ether	low (<20)	~50:50
Zn	benzene−ether	low (<30)	~40:60
Mg	ether	good (~80)	0:100
Mg	THF	0	condensation of aldehyde
Zn−Pb wet	ether	high (>80)	30:70
Zn−Pb dry	ether	high (>80)	50:50
Zn−Pb dry	benzene−ether	high (>80)	70:30

[a] The ratios of α- and γ-adducts were estimated from the NMR signals of the allylic methylenes (~2.4 ppm, 2H; γ-adducts) and/or the vinylic methylenes (~5.4 ppm, 2H; α-adducts).

Having established the applicability of the Reformatsky reaction, we then turned our attention to the synthesis of the upper fragment **3** in its enantiomerically pure form using a chemoenzymatic approach. We envisaged that the racemic ester (±)-**11**, which was prepared by the alkylation of methyl *trans*-styrylacetate (**10**) with dimethyl sulfate and LDA[11] in high yield, could be conveniently resolved using a lipase-catalyzed enanti-

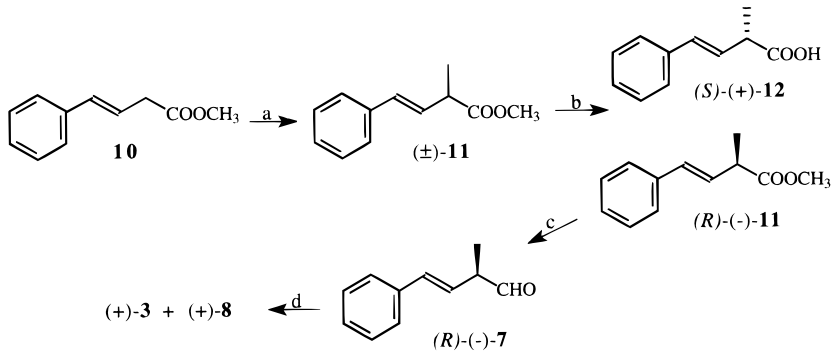
(7) (a) Barrow, R. A.; Hemscheidt, T.; Liang, J.; Paik, S.; Moore, R. E.; Tius, M. A. *J. Am. Chem. Soc.* **1995**, *117*, 2479−2490. (b) See also Kobayashi, M.; Kurosu, M.; Wang, W.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2394−2396, for the synthesis of arenastatin A.

(8) (a) Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353−1364. (b) Merz, A.; Märkl, G. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 845−846.

(9) Pinza, M.; Pifferi, G. *J. Pharm. Sci.* **1978**, *67*, 120−121.

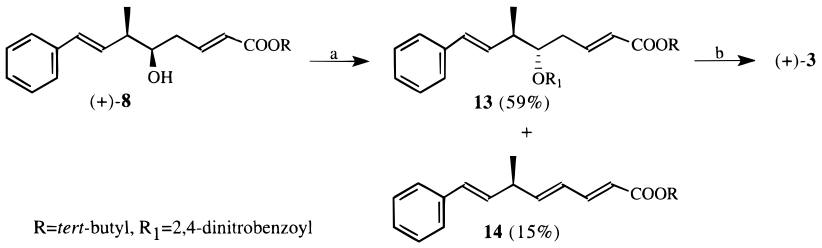
(10) Rice, L. E.; Boston, M. C.; Finklea, H. O.; Suder, B. J.; Frazier, J. O.; Hudlicky, T. *J. Org. Chem.* **1984**, *49*, 1845−1848.

Scheme 3[a]



[a] (a) $(CH_3O)_2SO_2$, LDA, THF, −60 to 5 °C, 92%; (b) 2-propanol-treated *Candida rugosa* lipase, 0.2 M phosphate buffer, rt, 48%; (c) DIBALH, ether, −70 °C, 95%; (d) *tert*-butyl 4-bromocrotonate, Zn−Pb.

Scheme 4[a]



[a] (a) 2,4-Dinitrobenzoic acid, Ph_3P, DEAD, THF; (b) K_2CO_3, methanol, (93%).

oselective hydrolysis. The requisite aldehyde (*R*)-**7** for the Reformatsky reaction could be easily obtained by the reduction of (*R*)-**11**.

Since *Candida* lipases have been widely used for the kinetic resolution of α-substituted carboxylic esters,[12] we examined three commercially available preparations for their suitability in catalyzing this transformation. Unfortunately, the lipases of *Candida cylindracea* OF-360 (Meito Sangyo), immobilized *Candida antartica* (Novo Nordisk), and *Candida rugosa* (CRL) (Sigma) all displayed poor enantioselectivity toward this substrate with *E*[13] values ranging from 1.5 to 5.0. However, it is well known that the enantioselectivity of CRL could often be enhanced by organic solvent treatment.[14] Kazlauskas[15] reported the conversion of CRL to a high enantioselectivity form by a simple 2-propanol treatment. Recent X-ray structures identified two conformational forms of CRL-open and -closed.[16] It was suggested that the 2-propanol treatment increased the activity and enantioselectivity by converting the closed form of CRL to the open form. Indeed, we found that the 2-propanol-treated CRL catalyzed the hydrolysis of (±)-**11** with high degree of enantioselectivity with an enantiomeric ratio, *E* of >100, to yield (*S*)-**12** in 45% yield and (*R*)-**11** in 48% yield. The enantiomeric purity of both the acid and ester was estimated to be greater than 96% ee from the analysis of their respective ^{1}H NMR spectra in the presence of a chiral shift reagent. In general, CRL has shown a consistent (*S*)-stereochemical preference for the hydrolysis of arylpropionic ester derivatives.[12] Moreover, the empirical rule, advanced by Kazlauskas,[15] for the 2-propanol-treated CRL also predicts that this enzyme preferentially cleaves the (*S*)-ester. Consequently, the remaining ester (*R*)-**11** was reduced with DIBALH at −70 °C to give the aldehyde (*R*)-**7** in 95% yield, which was stable for about 6 days at −20 °C under argon. The fourth and last step of the synthesis of **3** entails the Reformatsky reaction of (*R*)-**7** with the halo ester, **9**, using Zn−Pb couple as catalyst under the same reaction conditions as described for the homologation of the racemate. For no apparent reason, in this case the yield of the diastereomeric mixture, (+)-**3** and (+)-**8**, was found to be only 40% in contrast to the 60% that was repeatedly obtained using (±)-**7**, prepared by an alternate route. Since the homologation of **9** to (*R*)-**7** was not stereoselective and two diastereomers, (+)-**3** and (+)-**8**, were obtained in almost equal amounts, we decided to transform the *syn*-isomer **8** into the desired *anti*-isomer **3** by means of the Mitsunobu reaction.[17] After much experimentation, we found that the best yield of (+)-**3** was obtained with 2,4-dinitrobenzoic acid in the presence of triphenylphosphine and diethyl azodicarboxylate. Under these conditions the diester **13** was obtained in 59% yield accompanied by a slight quantity of the triene **14**. When 4-nitrobenzoic or phenoxyacetic acid was used instead, the predominant product formed was **14**. The activated ester **13** was conveniently hydrolyzed with K_2CO_3 in methanol to furnish the desired alcohol, (+)-**3**.

Having completed the synthesis of the upper fragment **3**, we then focused our attention on the synthesis of the lower fragment **4**, which is composed of three subunits. A chemoenzymatic approach was chosen for the synthesis of the β-amino acid subunit (*R*)-**19**. Reaction of methyl

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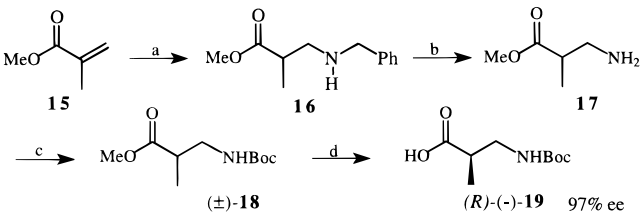
Scheme 5[a]



[a] (a) Benzylamine, methanol, (90%); (b) H_2, Pd/C, AcOH, methanol; (c) di-*tert*-butyl dicarbonate, dioxane, H_2O, (90% from **16**); (d) immobilized *Candida antarctica* lipase (31%).

methacrylate, **15**, with benzylamine at 70 °C for 4 days afforded the Michael addition product **16**, in 90% yield. Catalytic hydrogenation followed by amino group protection furnished (±)-**18** in 90% yield. A variety of lipases and proteases were examined for their abilities to catalyze the kinetic resolution of (±)-**18**, but most of them exhibited low enantioselectivity with *E* values less than 5. The only exception was the immobilized lipase of *Candida antartica* which showed moderate enantioselctivity with a *E* value of around 10. Therefore, it was necessary to deploy a multistep kinetic resolution[13,18] procedure to secure (*R*)-**19** of high optical purity. Thus, (±)-**18** was first exposed to enantioselective hydrolysis in aqueous medium catalyzed by the immobilized *Candida* lipase to yield (*R*)-**19** of moderate optical purity (48% ee), which in turn was subjected to enzymatic esterification with methanol in CCl_4 to yield the ester, (*R*)-(−)-**18**, in 89% ee. This enriched ester was again incubated with the same lipase to furnish the acid (*R*)-**19**, with an optical purity of 97% ee. The overall yield for the three-step procedure was 31% (Scheme 5).

The diester **20**, prepared following a known procedure,[7a] was converted into the trifluoroacetate **21** by treatment with TFA. The 3-chloro-*O*-methyl-D-tyrosine was prepared by the monochlorination of *N*-acetyl-*O*-methyl-D-tyrosine with sulfuryl chloride in acetic acid.[19] After protection of the amino group, the t-Boc derivative **22** was obtained in 71% overall yield. The coupling reaction of **21** with **22** was accomplished in 86% yield in tetrahydrofuran with a small excess of DCC, 1-hydroxybenzotriazole (HOBT), and diisopropylethylamine (DIEA) at 23 °C. Allyl ester cleavage was effected in THF containing dry morpholine and a catalytic amount of tetrakis(triphenylphosphine)palladium to furnish **4** in 90% yield[7a] (Scheme 6).

The final stages of synthesis entails the assembly of fragments **3** and **4** (Scheme 7). This was achieved by using DCC/ DMAP in dichloromethane to give the adduct **24** in 74% yield. After deprotection with TFA, macrolactamization of **25** with pentafluorophenyl diphenylphosphinate (FDPP) produced cryptophycin C, **26** (71% yield), whose physical constants were found to be identical to reported values.[7a] Epoxidation of **26** with *m*-CPBA[2,7] gave cryptophycin A, **1**, and the corresponding α-epoxide **27** in a ratio of 2:1 (78% yield), which were separated by HPLC (t_R of **1** = 5.65 min; t_R of **27** = 6.61 min). The analytical data of cryptophycin A, obtained from this total synthesis, were found to be in full agreement with those reported for cryptophycin A isolated from natural source.[2,7a]

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Reaction of **4** with (±)-**8** (*syn*) using the same reaction conditions furnished the analogs **28** and **29** in similar yields whereas compounds **26** and **30** were obtained when (±)-**3** (*anti*) was used instead for the condensation (Scheme 8). To confirm the structural assignments of **28** and **29**, we conducted the macrocyclization of (−)-**8** (derived from (*S*)-(+)-**11**), with the lower fragment **4**. The chromatographic behavior and spectral properties of this cyclized product were identical to **29**, thus validating our structural assignments of **28** and **29** as shown.

Conclusion

We have developed a highly convergent synthesis of the cryptophycins in their enantiomerically-pure forms. Our strategy entails the construction of the two fragments **3** and **4** and joining them together to form a macrocyclic ring. The upper fragment **3** was synthesized from **10** in four steps, and the lower fragment **4** was prepared from **20** in three steps. The requisite chiral building blocks (*R*)-**11** and (*R*)-**19** were prepared using enantioselective biocatalytic methodology. The stereochemical flexibility of this synthetic route is illustrated by the synthesis of cryptophycin A and the four diastereomers of cryptophycin C, in a relatively few steps.

Experimental Section

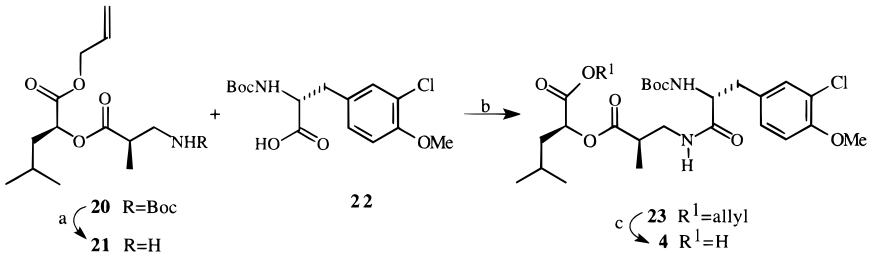
NMR spectra were obtained in $CDCl_3$ on a Bruker AM-300 instrument operating at 300 MHz for ^1H and 75 MHz for ^{13}C. Coupling constants are reported in hertz (Hz). EI and FAB mass spectra and high-resolution mass measurements were performed on a Kratos MS-80RFA DS55/DS90 mass spectrometer, and IR spectra were recorded on a Perkin Elmer 599 B infrared spectrophotometer. Optical rotations were measured on a Perkin Elmer 241 polarimeter at 23 °C in chloroform unless noted otherwise. Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} plates. Flash chromatography was carried out on Baker 40 μm silica gel. Anhydrous solvents were obtained using standard procedures.

(3*E*)-1,2-Epoxy-2-methyl-4-phenylbutene (6). To a solution of (3*E*)-4-phenylbuten-2-one (**5**) (0.1 mol, 14.6 g) in CH_2Cl_2 (100 mL) were added trimethylsulfonium methyl sulfate (0.12 mol, 22.6 g), 50% aqueous NaOH (50 mL), and benzyltriethylammonium chloride (500 mg). The reaction mixture was stirred at 50 °C for 12 h. Ether (200 mL) and water (200 mL) were added, organic layer was separated, washed with water (2 × 100 mL), dried ($MgSO_4$), and evaporated under reduced pressure to give product **6** (15.4 g, 96%) as an orange oil. NMR δ ^1H: 1.58 (s, 3H), 2.87 (AB, 5.2, 2H), 6.00 (d, 16.2, 1H), 6.65 (d, 16.2, 1H), 7.09−7.21 (m, 5H) ppm; δ ^{13}C: 19.81, 55.84, 56.21, 126.4 (2C), 127.9, 128.7 (2C), 130.7, 131.7, 136.4 ppm.

(3*E*)-2-Methyl-4-phenylbutenal [(±)-7]. Magnesium (300 mg) was covered with a solution of 1,2-dibromoethane (17.4 mmol, 1.5 mL) in 10 mL of anhyd ether under an argon atmosphere. After the reaction ceased, the heterogeneous mixture was cooled to −25 °C, and the solution of epoxide **6** (0.42 mol, 6.7 g) in ether (20 mL) was added. The reaction mixture was stirred at that temp for 0.5 h, quenched with 5 mL of 3 M NH_4Cl, and diluted with ether (50 mL). The ethereal layer was washed with 0.5 M citric acid, dried (Na_2SO_4), and concentrated to give aldehyde (±)-**7** (6.5 g, 97%) as yellow oil. No impurities in (±)-**7** were detected by NMR. This compound is stable in the freezer for few days. NMR δ ^1H: 1.32 (d, 7.0, 3H), 3.25 (dq, 7.0, 7.5, 1H), 6.15 (dd, 7.5, 16.0, 1H), 6.51 (d, 16.0, 1H), 7.19−7.42 (m, 5H), 9.64 (s, 1H) ppm; δ ^{13}C: 13.53, 50.25, 125.7, 126.3 (2C), 127.8, 128.6 (2C), 133.4, 137.1, 201.3 ppm.

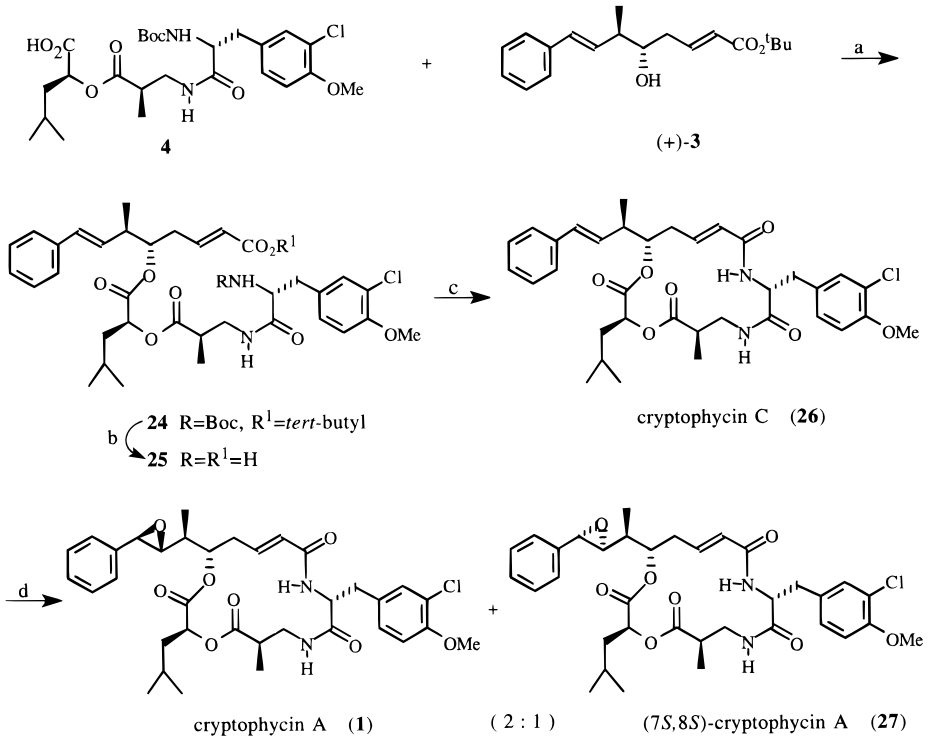
***tert*-Butyl 4-Bromocrotonate (9).** Crude 4-bromocrotonic acid[9] (17.2 g obtained from 0.116 mol of crotonic acid) was suspended in 25 mL of ether containing 1.8 g of sulfuric acid. The mixture was cooled to −40 °C, and isobutylene (about 30 mL) was added. The resulting reaction was stirred at 23 °C

Scheme 6[a]



[a] (a) TFA; (b) 1-hydroxybenzotriazole, DCC, THF, DIEA, (86%); (c) $Pd(PPh_3)_4$, morpholine (90%).

Scheme 7[a]



[a] (a) DCC, DMAP, CH_2Cl_2 (74%); (b) TFA; (c) FDPP, DMF (71% from **24**); (d) *m*-CPBA, CH_2Cl_2 (78%).

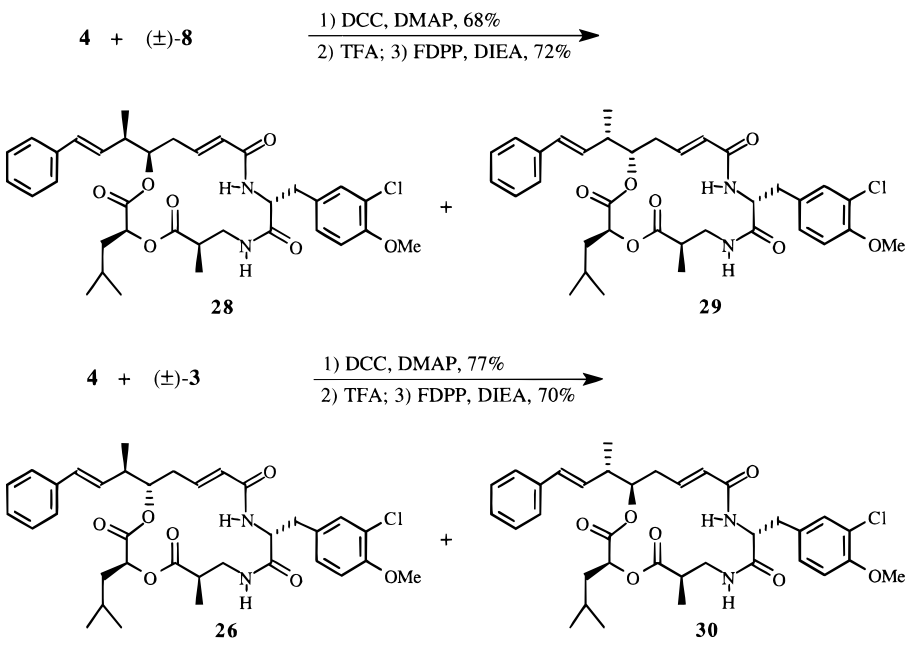
for 3 h and diluted with 50 mL of hexane containing 2 mL of triethylamine. After washing (2 × 20 mL of 1 M Na_2CO_3), drying ($MgSO_4$), and concentration, the crude product was passed through a short pad of silica gel (hexane−ether 50:1) to give 16.1 g (overall yield 64%) of **9** as an oil. R_f = 0.50 (hexane−ether 10:1); NMR δ ^{1}H: 1.49 (s, 9H), 4.00 (dd, 7.4, 1.0, 2H), 5.95 (dt, 15.3, 1.0, 1H), 6.90 (dt, 15.3, 7.4, 1H) ppm; δ ^{13}C: 28.61 (3C), 29.89, 81.44, 127.0, 141.1, 165.2 ppm.

Preparation of Zinc−Metal Couples. Zinc (500 mg, 20 mesh) was covered with either 3 M copper sulfate or 3 M lead acetate (3 mL). The mixture was stirred vigorously for 5 min. The plated couple was then rinsed with methanol and with solvent used in the reaction (for "wet" conditions it was used immediately) and dried at ~120 °C (0.2 mmHg) for 4 h (for "dry" conditions).

Additions to (3*E*)-2-Methyl-4-phenylbutenal [(±)-7]. The appropriate metal catalyst (~4 fold excess by weight) was covered with 4 mL of solvent of choice, under argon, and a crystal of iodine was added. A mixture of aldehyde (±)-**7** (1 mmol) and halo ester **9** (1.2 mmol) was dissolved in the same solvent, and a few drops of this mixture were added to the stirred suspension of catalyst. The reaction was then heated to the gentle reflux while the rest of reagents were added (30 min), and reflux was continued for the next 30 min. After cooling, the reaction was diluted with ether (20 mL), the catalyst and other precipitates were filtered off, and after concentration the residue was passed through short pad of silica gel (CH_2Cl_2−acetone 40:1). The ratio of regioisomers was measured by ^{1}H NMR.

***tert*-Butyl (2*E*,7*E*)-5-Hydroxy-6-methyl-8-phenylocta-2,7-dienoate [(±)-3] and [(±)-8].** "Dry" zinc−lead couple was used as a catalyst. The mixture of anhyd ether−benzene 1:1 (5 mL) was used as a solvent, and the above procedure was followed exactly until the concentration step. The residue was chromatographed on silica gel using CH_2Cl_2−hexane−Et_3N−acetone 200:60:3:2 as an eluent. The following compounds were obtained: α-isomers (75 mg, 25%) not separated (R_f = 0.64−0.66, CH_2Cl_2−acetone 20:1) colorless oil; (±)-**3**: (80 mg, 27%) colorless oil, R_f = 0.52 (CH_2Cl_2−acetone 20:1); HRMS *m*/*z* (M − 1) calcd (for $C_{19}H_{26}O_3$ − 1): 301.1804, found 301.1810; NMR δ ^{1}H: 1.11 (d, 6.9, 3H), 1.49 (s, 9H), 1.77 (brd, 3.6, 1H), 2.27−2.51 (m, 3H), 3.65 (m, 1H), 5.84 (dt, 15.6, 1.3, 1H), 6.13 (dd, 15.9, 8.6, 1H), 6.48 (d, 15.9, 1H), 6.91 (dt, 15.6, 7.0, 1H), 7.10−7.40 (m, 5H) ppm; δ ^{13}C: 17.51, 28.85 (3C), 37.90, 43.93, 74.58, 80.95, 126.2, 126.9 (2C), 128.1, 129.3 (2C), 131.7, 132.6, 137.7, 144.7, 166.5 ppm; (±)-**8**: (100 mg, 33%) colorless oil, R_f =0.45; HRMS *m*/*z* calcd (for $C_{19}H_{26}O_3$): 302.1837, found 302.1831; NMR δ ^{1}H: 1.15 (d, 6.8, 3H), 1.48 (s, 9H), 1.82 (brd, 3.9, 1H), 2.29 (ddd, 6.8, 7.2, 8.0, 1H), 2.39−2.51 (m, 2H), 3.67 (m, 1H), 5.82 (d, 15.6, 1H), 6.13 (dd, 15.9, 8.0, 1H), 6.46 (d, 16.0, 1H), 6.89 (dt, 15.6, 7.2, 1H), 7.12−7.38 (m, 5H) ppm; δ ^{13}C: 16.04, 28.80 (3C), 37.96, 43.72, 74.62, 80.94, 126.3, 126.8 (2C), 128.0, 129.2 (2C), 131.6, 132.6, 137.9, 144.9, 166.4 ppm.

Scheme 8



(±)-Methyl 2-Methyl-*trans*-styrylacetate [(±)-11]. To a stirred solution of diisopropylamine (0.9 mL, 6.4 mmol) in 4 mL of anhyd THF under argon was added at −4 °C the solution of butyllithium (2.7 mL, 2.5 M) in hexanes. After 5 min the mixture was cooled to −60 °C and a solution of ester **10** (1.06 g, 6 mmol) in 2 mL of ether was added. The reaction mixture was stirred at that temperature for 10 min, and then dimethyl sulfate (11 mmol, 1 mL) was added. The temperature of the reaction was quickly increased to 5 °C, and the resulting mixture was stirred for 15 min. The reaction was quenched with 1 M citric acid (10 mL) and diluted with 50 mL of hexane. The organic layer was washed with water, dried ($MgSO_4$), and concentrated. The residue was chromatographed over a short column of silica gel using hexane−ether 40:1 as an eluent to give (±)-**11** (1.05 g, 92%) as a colorless liquid. R_f = 0.35 (hexane−ether 10:1); NMR δ ^{1}H: 1.41 (d, 7.4, 3H), 3.36 (dq, 7.5, 1H), 3.72 (s, 3H), 6.32 (dd, 7.6, 15.9, 1H), 6.53 (d, 15.9, 1H), 7.14−7.40 (m, 5H) ppm; δ ^{13}C: 17.45, 43.17, 51.95, 126.4 (2C), 127.6, 128.1 (2C), 128.6, 131.5, 136.9, 174.9 ppm.

2-Propanol Treatment of *C. rugosa* Lipase. Crude *C. rugosa* lipase (10 g, Sigma) was dissolved in MES buffer (50 mL, 50 mM, pH 6.0, 4 °C, methanesulfonic acid was used to adjust pH) by stirring at 4 °C for 30 min. 2-propanol (50 mL) was added dropwise over 1 h at 4 °C. The cloudy solution was stirred at 4 °C for 46 h. The precipitate was removed by centrifuging at 2000*g* for 35 min at 2 °C. The supernatant was dialyzed against deionized distilled water (3 × 2 L) for 24 h at 4 °C. This lipase solution (120 mL) can be stored at 4 °C with 0.02% wt/vol sodium azide.

Hydrolysis of (±)-Methyl 2-Methyl-*trans*-styrylacetate [(±)-11] with 2-Propanol Treated *C. rugosa* Lipase. To a solution of 4 mL of CRL in 10 mL of phosphate buffer (0.2M, pH 7.0) was added 750 mg of ester (±)-**11**. The cloudy mixture was stirred at 25 °C for 120 h and then the pH of the reaction was adjusted to 11 by the addition of 2 M Na_2CO_3. Extraction with hexane−ether 5:1 (3 × 15 mL), drying ($MgSO_4$), and concentration of organic layer yielded ester (*R*)-(−)-**11** (373 mg, ee 93%). The water layer was diluted with 25 mL of 2 M citric acid, extracted with ether (3 × 15 mL), dried ($MgSO_4$), and concentrated to give acid (*S*)-(+)-**12** (310 mg, 45%). $[\alpha]_D$ +37.6° (c = 2.1); mp 80−81 °C; NMR δ ^{1}H: 1.40 (d, 7.1, 3H), 3.34 (dq, 7.0, 7.8, 1H), 6.27 (dd, 7.8, 15.9, 1H), 6.51 (d, 15.9, 1H), 7.16−7.45 (m, 5H) ppm; δ ^{13}C: 17.24, 40.17, 126.4 (2C), 127.7, 127.9 (2C), 128.6, 131.7, 136.7, 181.3 ppm. The ester (*R*)-(−)-**11** (373 mg, ee 93%) was stirred with 2 mL of CRL solution and 4 mL of phosphate buffer (0.2 M, pH 7.5) at 37 °C for 16 h. The pH of this reaction mixture was adjusted to 11, extracted with hexane−ether 5:1 (3 × 15 mL), dried ($MgSO_4$), and concentrated to give (*R*)-(−)-**11** (360 mg, 48%, ee > 96%). $[\alpha]_D$ −52.5° (c = 5.2); HRMS m/z calcd (for $C_{12}H_{14}O_2$): 190.0994, found 190.0991; NMR data was identical to that of (±)-**11**.

(2*S*)-Methyl 2-Methyl-*trans*-styrylacetate [(*S*)-(+)-11]. The acid (*S*)-(+)-**12** (300 mg) was stirred with 5 mL of standard diazomethane solution in ether at 25 °C for 5 min. Concentration gave 323 mg of (*S*)-(+)-**11** (100%); $[\alpha]_D$ +52° (c = 2.2); ee > 96%.

Standard Procedure for Determination of Optical Purity for Compounds. To a solution of 1.6 μmol of the appropriate compound in $CDCl_3$ was added 18 mg of tris[3-[(heptafluoropropyl)hydroxymethylene]-(+)-camphorato]-europium(III). The ^{1}H NMR spectra (useful spectral parameters: O1 = 3900, SW = 3800, NS = 160, LB = 0, GB = 0) were measured both for scalemic (+)-**3**, (+)-**8**, (*R*)-(−)-**11**, (*S*)-(+)-**11**, and corresponding racemic compounds.

(2*R*,3*E*)-2-Methyl-4-phenylbutenal [(*R*)-(−)-7]. The solution of ester (*R*)-(−)-**11** (300 mg, 1.57 mmol) in 3 mL of anhyd ether was cooled to −75 °C, and 1.75 mL of DIBALH (1.1 equiv, 1 M solution in hexane) was added carefully. The reaction mixture was stirred at −75 °C to −70 °C for 3 h and then quenched with 2 mL of 1 M NH_4Cl. Ether (30 mL) was added and then the organic phase was washed successively with 1M citric acid (2 × 10 mL), water (15 mL), dried (Na_2SO_4), and concentrated to give (*R*)-(−)-**7** (245 mg, 95%) as a colorless oil. $[\alpha]_D$ −118° (c = 2.3); NMR data were identical to that of (±)-**7**.

***tert*-Butyl (2*E*,7*E*,5*S*,6*R*)-5-Hydroxy-6-methyl-8-phenyl-octa-2,7-dienoate [(+)-3] and *tert*-Butyl (2*E*,7*E*,5*R*,6*R*)-5-Hydroxy-6-methyl-8-phenylocta-2,7-dienoate [(+)-8].** Zinc (500 mg, 20 mesh) was covered with 3 M lead acetate (3 mL). The mixture was stirred vigorously for 5 min. The plated couple was then rinsed successively with methanol (2 × 4 mL) and ether (2 × 4 mL) and dried at ~120 °C (0.2 mmHg) for 4 h. The catalyst was then covered with 5 mL of anhyd ether−benzene 1:1, under argon, and a crystal of iodine was added. A mixture of aldehyde (*R*)-(−)-**7** (224 mg, 1.4 mmol) and halo ester **9** (1.2 equiv, 370 mg, 1.7 mmol) were dissolved in the same solvent (1 mL), and a few drops of this mixture were added to the stirred suspension of catalyst. The reaction was then heated to the gentle reflux while the rest of reagents was added (30 min), and reflux was continued for the next 30 min. After cooling, the reaction was diluted with ether (20 mL), the catalyst and other precipitates were filtered off, and after concentration of the residue was chromatographed on silica gel using CH_2Cl_2−hexane−Et_3N−acetone 200:60:3:2 as an eluent. Compound (+)-**3** eluted as less polar (78 mg, 18%, ee > 96%); $[\alpha]_D$ +69° (c = 2.3); the other analytical data were

identical to that of (±)-**3**. Compound (+)-**8** - eluted as more polar (91 mg, 22%, ee > 96%); $[\alpha]_D$ +50° (c = 3.1); the other analytical data were identical to that of (±)-**8**.

Inversion of *tert*-Butyl (2*E*,7*E*,5*R*,6*R*)-5-Hydroxy-6-methyl-8-phenylocta-2,7-dienoate, (+)-8, to *tert*-Butyl (2*E*,7*E*,5*S*,6*R*)-5-Hydroxy-6-methyl-8-phenylocta-2,7-dienoate, (+)-3.

(a) *tert*-Butyl (2*E*,7*E*,5*S*,6*R*)-5-(2,4-Dinitrobenzoyloxy)-6-methyl-8-phenylocta-2,7-dienoate (13). To a solution of alcohol (+)-**8** (80 mg, 0.265 mmol), triphenylphosphine (3.9 equiv, 263 mg, 1 mmol), and 2,4-dinitrobenzoic acid (4 equiv, 233 mg, 1.1 mmol) in 3 mL of anhyd THF was added diethyl azodicarboxylate (3.9 equiv, 160 mL, 1 mmol) at 0 °C under argon. The reaction mixture was stirred at 40 °C for 50 h and then was diluted with 30 mL of ether, washed with 0.5 M solution of $NaHCO_3$ (10 mL), dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography to give **13** (78 mg, 59%) as a yellow oil. R_f = 0.19 (hexane−ether 4:1); $[\alpha]_D$ +59° (c = 3.1); HRMS m/z calcd (for $C_{26}H_{28}N_2O_8 - C_4H_9$): 439.1141 found, 439.1120; NMR δ ^{1}H: 1.19 (d, 7.0, 3H), 1.48 (s, 9H), 2.57−2.84 (m, 3H), 5.29 (q, 6.1, 1H), 5.84 (d, 15.6, 1H), 6.06 (dd, 15.9, 8.7, 1H), 6.43 (d, 15.9, 1H), 6.81 (dt, 15.6, 7.5, 1H), 7.17−7.38 (m, 5H), 7.75 (d, 8.4, 1H), 8.42 (dd, 8.4, 2.2, 1H), 8.78 (d, 2.2, 1H) ppm; δ ^{13}C: 17.58, 28.12 (3C), 34.27, 42.96, 79.86, 81.51, 119.6, 126.2 (2C), 126.7, 127.4, 127.8, 128.7 (2C), 129.6, 131.2, 132.1, 133.1, 133.6, 141.3, 148.9 (2C), 163.8, 165.3 ppm.

(b) *tert*-Butyl (2*E*,7*E*,5*S*,6*R*)-5-Hydroxy-6-methyl-8-phenylocta-2,7-dienoate, (+)-3. To a solution of diester **13** (70 mg, 0.141 mmol) in 4 mL of methanol was added 100 mg of K_2CO_3. The reaction mixture was stirred for 0.5 h, diluted with ether (30 mL), washed with 10 mL of water, dried ($MgSO_4$), and then concentrated. The residual oil was passed through short pad of silica with CH_2Cl_2−hexane−Et_3N−acetone 200:60:3:3 to give compound (+)-**3** (39 mg, 92%) possessing analytical data ($[\alpha]_D$ +70° (c = 2.2)) as previously described.

Methyl 3-(Benzylamino)-2-methylpropanoate (16). A mixture of 70 mL (0.67 mol) of methyl methacrylate (**15**), benzylamine (60 mL, 0.55 mol), and methanol (50 mL) was stirred at 70 °C for 4 days. After evaporation of the volatiles, the crude product was purified by flash chromatography (CH_2Cl_2−hexane−MeOH, 7:2:1) to give compound **16** (53g, 90%) as a colorless oil. NMR δ ^{1}H: 1.17(d, 6.9, 3H), 1.63 (brs, NH), 2.6 (m, 2H), 2.88 (dd, 10.1, 3.7, 1H), 3.68 (s, 3H), 3.78 (s, 2H), 7.20−7.35 (m, 5H) ppm; δ ^{13}C: 15.33, 40.12, 51.64, 52.13, 53.71, 126.9, 128.0 (2C), 128.4 (2C), 140.3, 176.3 ppm; EIMS m/z (rel intensity): 207 (M^+, 4), 120 (84), 106 (71), 91 (100); HRMS calcd (for $C_{12}H_{17}NO_2$) 207.1259, found: 207.1255.

Methyl 3-Amino-2-methylpropanoate (17). To a solution of ester **16** (2.0 g, 9.66 mmol) and acetic acid (1 mL) in 35 mL of ethanol was added Pd/C (10%, 0.3 g). The resulting mixture was stirred under hydrogen at 25 °C for 16 h. The catalyst was filtered off, and the filtrate was concentrated to give **17** as an acetate (2.3 g). This crude compound was used directly for next step without purification.

Methyl 3-(*tert*-Butoxycarbonylamino)-2-methylpropanoate (18). To a stirred mixture of compound **17** (2.3 g, 12.9 mmol), dioxane (7.5 mL), and H_2O (7.5 mL) at 0 °C was added Et_3N (3.2 mL) followed by di-*tert*-butyl dicarbonate (3.0 g, 13.6 mmol). The reaction was stirred at 0 °C for 1 h and then at 25 °C for 7 h. The mixture was concentrated, and the residue was extracted with EtOAc (3 × 20 mL). The combined EtOAc solution was washed with brine (30 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by silica gel column chromatography (hexane−EtOAc 3:2) to give compound **18** as a colorless oil (1.9 g, 90% overall). NMR δ ^{1}H: 1.17 (d, 7.2, 3H), 1.44 (s, 9H), 2.67 (m, 1H), 3.26 (m, 2H), 3.70 (s, 3H), 4.95 (m, 1H) ppm; δ ^{13}C: 14.73, 28.32 (3C), 39.91, 42.91, 51.84, 79.33, 155.91, 176.2 ppm; EIMS m/z: 218 (M^+ + 1, 4), 202 (1), 161 (54), 144 (55), 130 (42), 112 (60), 101 (48), 88 (80), 57 (100); HRMS calcd (for $C_{10}H_{19}NO_4$ + H) 218.1392, found 218.1389.

(2*R*)-3-(*tert*-Butoxycarbonylamino)-2-methylpropanoic Acid [(*R*)-(−)-19]. A suspension of ester **18** (10 g, dissolved in 10 mL of acetonitrile) in phosphate buffer (0.2M, pH 7. 95 mL) was stirred with immobilized *Candida antartica* (Novo Nordisk) lipase (1.5 g) at 25 °C for 42 h. The reaction was acidified to pH 3 with 5% $KHSO_4$, and EtOAc (40 mL) was added. The enzyme was filtered off, and the two layers were separated. The aqueous phase was extracted with EtOAc (2 × 10 mL). The combined organic solutions were dried ($MgSO_4$) and concentrated, and the residue was passed through short plug of silica gel (hexane−EtOAc−HOAc 60:40:1) to give the acid (*R*)-(−)-**19** (5.44 g, 48% ee) and the ester (*S*)-(+)-**18** (3.83 g, 74% ee). The acid (*R*)-(−)-**19** (5.16 g, 48% ee) was then esterified with MeOH (10 mL) in the presence of immobilized *C. antarctica* lipase (1.29 g) in CCl_4 (200 mL) at 50 °C for 29 h. The enzyme was removed, and the filtrate was concentrated. Separation of the products on silica gel (as above) gave ester (*R*)-(−)-**18** (3.63 g, 89% ee) and acid (*R*)-(−)-**19** (1.66 g, 34% ee). The (−)-ester (3.6 g, 89% ee, dissolved in 3.4 mL of acetonitrile) was hydrolyzed again with immobilized *C. antarctica* lipase (0.7 g) in phosphate buffer (0.2 M, pH 8, 34 mL) at 25 °C for 48 h. The reaction was worked up, and the products were separated as described before to give acid (*R*)-(−)-**19** as colorless crystals (2.863 g, 97% ee). $[\alpha]_D$ −17.9° (c = 2.3, MeOH), lit.[7a] $[\alpha]_D$ −18.4° (c = 2.0, MeOH). The overall yield of (*R*)-(−)-**19** was 31%. Other physical data are identical with those reported in the literature.[7a]

Allyl (2*S*,2′*R*)-2-[(3′-Amino-2′-methylpropanoyl)oxy]-4-methylpentanoate (21). The solution of allyl (2*S*,2′*R*)-2-[[3′-[(*tert*-butoxycarbonyl)amino]-2′-methylpropanoyl]oxy]-4-methylpentanoate[7a] (**20**) (300 mg, 0.84 mmol) in 3 mL of CH_2Cl_2 was treated with TFA (7 mL) at 0 °C then stirred at rt for 2 h. The resulting mixture was concentrated and dried in vacuo. The crude product **21** (312 mg, TFA salt) was used directly in the next reaction.

Compound 23. To a stirred solution of crude ester **21** (312 mg, 0.84 mmol), *N*-(*tert*-butoxycarbonyl)-3-(3-chloro-4-methoxyphenyl)-D-alanine (**22**) (277 mg, 0.84 mmol), 1-hydroxybenzotriazole (129 mg, 0.84 mmol), and diisopropylethylamine (350 μL) in anhyd THF (10 mL) at 0 °C was added DCC (190 mg, 1.1 equiv, 0.92 mmol). The mixture was stirred at 0 °C for 30 min and then at 23 °C for 16 h. All the precipitate was filtered off, and the filtrate was concentrated. The residue was dissolved in EtOAc (150 mL) and washed with 5% $KHSO_4$ (20 mL) and 5% $NaHCO_3$ (20 mL), dried ($MgSO_4$), and concentrated. Silica gel column chromatographic purification (hexane−acetone 7:3) gave ester **23** as a colorless gum (410 mg, 86%). $[\alpha]_D$ −34.2° (c = 2.44); NMR δ ^{1}H: 0.93 (d, 6.3, 3H), 0.96 (d, 6.3, 3H), 1.18 (d, 7.0, 3H), 1.39 (s, 9H), 1.66−1.83 (m, 3H), 2.76 (m, 1H), 2.97 (m, 2H), 3.16 (ddd, 13.9, 9.5, 4.8, 1H), 3.69 (m, 1H), 3.86 (s, 3H), 4.33 (m, 1H), 4.65 (brd, 5.2, 2H), 5.11 (brd, 9.1, 1H), 5.19 (m, NH), 5.28 (d, 10.4, 1H), 5.36 (d, 17.2, 1H), 5.90 (m, 1H), 6.83 (d, 8.4, 1H), 6.86 (m, NH), 7.03 (d, 8.4, 1H), 7.19 (brs, 1H) ppm; δ ^{13}C: 14.72, 21.55, 23.03, 24.83, 28.32 (3C), 37.80, 39.51, 40.40, 41.83, 55.74, 56.11, 66.43, 70.73, 79.70, 112.2, 119.2, 122.1, 128.6, 130.0, 131.0, 131.2, 153.8, 155.1, 171.1 (2C), 173.8 ppm; MS m/z: 569 (M^+ + 1, 52), 515 (13), 513 (33), 497 (20), 495 (42), 453 (82), 451 (100), 417 (50), 341 (56), 313 (65), 252 (53), 195 (85), 184 (74), 155 (63), 141 (83); HRMS calcd (for $C_{28}H_{41}{}^{35}ClN_2O_8$ + H): 569.2630, found: 569.2640.

Compound 4. A solution of allyl ester **22** (727 mg, 1.28 mmol), $Pd(PPh_3)_4$ (149 mg, 0.1 equiv), and morpholine (1.1 mL, 10 equiv) in anhyd THF (16 mL) was stirred at 23 °C for 1 h. The solvent was removed, and the residue was dissolved in EtOAc (150 mL), washed with 5% $KHSO_4$ (30 mL), dried ($MgSO_4$), and concentrated. Silica gel column chromatographic purification (hexane−acetone−HOAc 120:80:1) afforded acid **4** as a white solid (608 mg, 90%). $[\alpha]_D$ −20.9° (c = 1.46); NMR δ ^{1}H: 0.93 (d, 6.1, 3H), 0.97 (d, 6.1, 3H), 1.17 (d, 6.5, 3H), 1.38 (s, 9H), 1.77 (m, 3H), 2.78 (m, 1H), 2.85−3.06 (m, 2H), 3.21 (m, 1H), 3.63 (m, 1H), 3.86 (s, 3H), 4.25 (dd, 14.7, 7.8, 1H), 4.88 (brs, OH), 5.13 (m, 1H), 5.31 (m, NH), 6.83 (brd, 8.3, 1H), 6.91 (m, NH), 7.02 (brd, 8.3, 1H), 7.19 (brs, 1H) ppm; δ ^{13}C ($CDCl_3$ + trace MeOH): 14.52, 21.55, 23.03, 24.83, 28.20 (3C), 37.84, 39.45, 40.14, 41.83, 55.79, 56.11, 70.93, 80.32, 112.1, 122.1, 128.6, 129.8, 131.0, 153.8, 155.6, 171.8, 173.3, 174.0 ppm.

Compound 24. To a stirred solution of acid **4** (129 mg, 0.244 mmol), alcohol **3** (70 mg, 0.232 mmol) and DMAP (7.1 mg, 0.06 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added DCC (53 mg, 0.255 mmol). The resulting mixture was stirred at 0 °C for 1 h and then at 23 °C for 16 h. The precipitate was filtered off, and the filtrate was concentrated. The residue was dissolved in EtOAc (50 mL), washed with brine−5% $KHSO_4$ (pH ∼ 3, 10 mL) and 5% $NaHCO_3$ (10 mL), dried ($MgSO_4$), and concentrated. Chromatography on silica gel (hexane−EtOAc 3:2) gave the pure product **24** as a white solid (140 mg, 74%). $[\alpha]_D$ −12.9° (c = 4.3); NMR δ ^{1}H: 0.79 (d, 6.4, 3H), 0.84 (d, 6.4, 3H), 1.13 (d, 6.9, 3H), 1.16 (d, 7.3, 3H), 1.35 (s, 9H), 1.48 (s, 9H), 1.51−1.71 (m, 3H), 2.38−2.71 (m, 4H), 2.87 (m, 1H), 3.16 (m, 1H), 3.23 (dd, 14.0, 5.1, 1H), 3.71 (m, 1H), 3.84 (s, 3H), 4.38 (m, 1H), 4.98 (dd, 9.7, 3.7, 1H), 5.06 (m, 1H), 5.74 (m, NH), 5.83 (d, 15.6, 1H), 6.01 (dd, 15.8, 8.7, 1H), 6.42 (d, 15.8, 1H), 6.84 [2H: (d, 8.4, 1H), (m, 1H)], 7.02 (m, NH), 7.11 (dd, 8.4, 2.1 1H), 7.17−7.36 (m, 6H) ppm; δ ^{13}C: 14.60, 17.04, 21.33, 22.91, 24.74, 28.23 (3C), 28.33 (3C), 35.13, 37.65, 39.63, 40.92, 41.12, 42.24, 56.14 (2C), 70.74, 76.61, 79.50, 80.53, 112.1, 122.1, 126.2 (2C), 126.4, 127.5, 128.6 (3C), 130.0, 130.8, 131.2, 131.8, 136.9, 141.7, 153.7, 155.6, 165.8, 171.0, 171.6, 173.6 ppm; FAB-MS m/z: 813 (M^+ + 1), 713, 657, 412, 228; FAB-HRMS calcd (for $C_{44}H_{61}{}^{35}ClN_2O_8$ + H): 813.4093, found: 813.3093.

Compound 25. To a solution of compound **24** (57 mg, 0.07 mmol) in 1 mL of CH_2Cl_2 at 0 °C was slowly added TFA (4 mL), and resulting solution was stirred at 23 °C for 2 h. The reaction mixture was concentrated and dried in vacuo. The crude product (56 mg) was used directly in the next reaction.

Cryptophycin C (26). To a solution of compound **25** (56 mg, 0.07 mmol) in anhyd DMF (9.5 mL) at rt were added FDPP (41 mg, 1.5 equiv, 0.1 mmol) and DIEA (37 μL). The reaction mixture was stirred under argon at 23 °C for 16 h, diluted with 20 mL of EtOAc, and then washed successively with 5 mL portions of 5% $KHSO_4$, 5% $NaHCO_3$, and water. The organic layer was dried ($MgSO_4$) and concentrated. The residue was subjected to chromatography on silica gel (CH_2Cl_2−acetone 4:1) to give the desired product as a white solid (32 mg, 71%). $[\alpha]_D$ +36.4° (c = 2.1), {lit.[7a] $[\alpha]_D$ +28.8° (c = 0.65)}. Other analytical data were in full agreement with literature.[7a]

Cryptophycin A (1) and (7*S*,8*S*)-Cryptophycin A (27). A solution of cryptophycin C (**26**) (50 mg, 78.3 μmol) in 10 mL of CH_2Cl_2 was stirred at 23 °C for 45 h, while *m*-CPBA (52 mg, 300 μmol) was added portionwise. The mixture was then diluted with 50 mL of EtOAc, washed successively with 10 mL portions of 5% $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated to give colorless residue, which was passed through a short pad of silica gel with hexane−acetone 3:2 to give **1** and **27** as a mixture (40.7 mg, 78%). Epoxides **1** and **27** were separated by HPLC (Waters 4 μm C_{18} cartridge, internal diameter 8 mm, eluent (isocratic) MeOH−H_2O 65:35, flow 3 mL/min) to give cryptophycin A (**1**) (26 mg): t_R=5.65 min; $[\alpha]_D$ +32.5° (c = 2.0, MeOH), {lit.[2] $[\alpha]_D$ +33.8° (c = 1.8, MeOH)]}, with NMR and MS data in agreement with lit.,[2] and (7*S*,8*S*)-cryptophycin A (**27**) (13 mg). **27**: t_R = 6.61 min; $[\alpha]_D$ +23.6° (c = 1.1, MeOH); NMR δ ^{1}H: 0.89 (d, 6.4, 3H), 0.91 (d, 6.4, 3H), 1.05 (d, 7.0, 3H), 1.25 (d, 7.2, 3H), 1.51 (m, 1H), 1.74 (m, 3H), 2.50−2.81 (m, 3H), 2.91 (brd, 7.4, 1H), 3.04 (dd, 14.4, 7.2, 1H), 3.16 (dd, 14.4, 5.4, 1H), 3.32 (m, 1H), 3.51 (brddd, 13.5, 4.5, 4.5, 1H), 3.60 (brs, 1H), 3.88 (s, 3H), 4.82 (brdd, 13.7, 7.2, 1H), 4.92 (m, 1H), 5.14 (brdd, 10.0, 4.0, 1H), 5.73−5.87 (m, 2H), 6.71 (ddd, 15.3, 9.6, 5.3, 1H), 6.85 (d, 8.4, 1H), 7.00 (m, NH), 7.09 (brd, 8.4, 1H), 7.24 (m, 3H), 7.34 (m, 3H) ppm; δ ^{13}C: 13.50, 14.11, 21.41, 23.14, 24.70, 35.15, 36.73, 38.33, 39.32, 41.06, 41.21, 53.73, 56.23, 56.43, 63.21, 71.50, 77.34, 112.2, 122.5, 125.2, 125.4 (2C), 128.3, 128.4, 128.6 (2C), 129.8, 131.0, 137.1, 141.4, 154.0, 165.5, 170.8, 171.0, 175.7 ppm; MS m/z: 656 (M^+ with ^{37}Cl, 10), 654 (M^+ with ^{35}Cl, 27), 412 (18), 280 (18), 227 (47), 195 (58), 91 (100); HRMS calcd (for $C_{35}H_{43}{}^{35}ClN_2O_8$): 654.2708, found: 654.2715.

(5*R*)-Cryptophycin C (28) and (6*S*)-Cryptophycin C (29). Compounds **28** and **29** were prepared starting from alcohol (±)-**8** (49 mg, 0.162 mmol) and acid **4** (90 mg, 0.168 mmol). The synthetic procedures for compounds **24**−**26** were followed. Separation of the final products **28** and **29** [49% overall yield starting from (±)-**8**] by means of chromatography on silica gel with CH_2Cl_2−acetone 4:1 afforded **28** (less polar, R_f = 0.24, 23 mg) and **29** (more polar, R_f = 0.18, 27 mg) as white solids. (5*R*)-cryptophycin C (**28**): $[\alpha]_D$ +59.9° (c = 1.8); NMR δ ^{1}H: 0.90 (brd, 4.8, 3H), 0.94 (brd, 4.8, 3H), 1.14 (d, 6.7, 3H), 1.24 (d, 7.4, 3H), 1.69 (m, 2H), 2.39 (m, 1H), 2.70 (m, 3H), 3.06 (dd, 14.4, 7.4, 1H), 3.15 (dd, 14.4, 5.2, 1H), 3.41 (m, 1H), 3.56 (m, 1H), 3.86 (s, 3H), 4.83 (m, 2H), 5.15 (m, 1H), 5.75 (d, 15.7, 1H), 5.92 (brd, 8.0, 1H), 6.00 (dd, 15.9, 8.6, 1H), 6.47 (m, 1H), 6.51 (d, 15.9, 1H), 6.86 (d, 8.4, 1H), 6.95 (m, NH), 7.08 (dd, 8.4, 2.0, 1H), 7.21 (d, 2.0, 1H), 7.21−7.36 (m, 5H) ppm; δ ^{13}C: 14.42, 17.31, 21.31, 23.14, 24.72, 34.94, 35.81, 39.10 (2C), 40.32, 40.56, 53.90, 56.24, 71.02, 78.63, 112.4, 122.4, 126.2 (2C), 126.6, 127.7, 128.3, 128.7 (2C), 129.6, 129.7, 131.0, 132.2, 136.7, 139.0, 154.0, 165.8, 170.8 (2C), 174.4 ppm; MS m/z: 640 (M^+ with ^{37}Cl, 4), 638 (M^+ with ^{35}Cl, 6), 414 (16), 412 (45), 368 (26), 280 (6), 227 (50), 195 (26), 91 (65); HRMS calcd (for $C_{35}H_{43}{}^{35}ClN_2O_7$): 638.2759, found: 638.2759. (6*S*)-cryptophycin C (**29**): $[\alpha]_D$ +2.6° (c = 2.3); NMR δ ^{1}H: 0.86 (d, 6.2, 3H), 0.88 (d, 6.2, 3H), 1.15 (d, 6.8, 3H), 1.23 (d, 7.2, 1H), 1.46 (m, 1H), 1.72 (m, 2H), 2.40 (m, 1H), 2.56 (m, 2H), 2.71 (m, 1H), 3.03 (dd, 14.5, 7.3, 1H), 3.14 (dd, 14.5, 5.6, 1H), 3.30 (m, 1H), 3.49 (brddd, 13.5, 4.3, 4.3, 1H), 3.86 (s, 3H), 4.83 (m, 1H), 4.88 (dd, 9.6, 3.8, 1H), 5.05 (brdd, 9.2, 6.3, 1H), 5.77 (m, 2H), 6.06 (dd, 15.9, 7.6, 1H), 6.43 (d, 15.9 1H), 6.69 (ddd, 15.3, 9.8, 5.4, 1H), 6.83 (d, 8.4 1H), 6.97 (brdd, 5.5, 5.5, 1H), 7.08 (dd, 8.4, 2.0, 1H), 7.22 (d, 2.0, 1H), 7.22−7.35 (m, 5H) ppm; δ ^{13}C: 14.13, 15.75, 21.50, 22.91, 24.62, 35.13, 36.54, 38.32, 39.72, 41.10, 41.56, 53.73, 56.14, 71.51, 77.34, 112.2, 122.3, 125.0, 126.2 (2C), 127.6, 128.4, 128.6 (2C), 129.9, 130.5, 131.0, 131.4, 136.8, 141.7, 153.9, 165.5, 170.8, 171.1, 175.6 ppm; MS m/z: 640 (M^+ with 37Cl, 6), 638 (M^+ with ^{35}Cl, 15), 414 (34), 412 (91), 368 (28), 280 (16), 227 (100), 195 (47), 91 (56); HRMS calcd (for $C_{35}H_{43}{}^{35}ClN_2O_7$): 638.2759, found: 638.2743.

(5*R*,6*S*)-Cryptophycin C (30). Compound **30** was prepared starting from alcohol (±)-**3** (60 mg, 0.2 mmol) and acid **4** (111 mg, 0.21 mmol). The synthetic procedures for compounds **24**−**26** were followed. Separation of the mixture of products **26** and **30** [52% overall yield starting from (±)-**3**] by means of column chromatography on silica gel with CH_2Cl_2−acetone 4:1 afforded **26** (less polar, R_f = 0.27, 35 mg) and **30** (more polar, R_f = 0.19, 31 mg) as white solids (compound **26** possessed exactly the same analytical data as given above). (5*R*, 6*S*)-cryptophycin C (**30**): $[\alpha]_D$ +11.3° (c = 2.2); NMR δ ^{1}H: 0.81 (brs, 6H), 1.16 (d, 6.7, 3H), 1.24 (d, 8.4, 3H), 1.59 (m, 3H), 2.42 (m, 1H), 2.68 (m, 3H), 3.06 (dd, 14.4, 7.4, 1H), 3.15 (dd, 14.4, 5.1, 1H), 3.40 (m, 1H), 3.54 (m, 1H), 3.87 (s, 3H), 4.86 (m, 2H), 5.08 (m, 1H), 5.78 (d, 15.9 1H), 5.97 (m, NH), 6.01 (dd, 15.9, 8.3, 1H), 6.43 (d, 15.9, 1Hz), 6.46 (m, 1H), 6.86 (d, 8.5, 1H), 6.93 (m, NH), 7.08 (brd, 8.5, 1H), 7.22 (d, 2.0, 1H), 7.23−7.36 (m, 5H) ppm; δ ^{13}C: 14.41, 16.62, 21.20, 22.92, 24.65, 34.57, 35.81, 39.10, 39.20, 40.33 (2C), 54.05, 56.23, 71.02, 78.24, 112.4, 122.5, 126.2 (2C), 126.6, 127.6, 128.3, 128.6 (2C), 129.6, 130.0, 130.9, 131.9, 136.8, 139.0, 154.2, 165.9, 170.7, 170.8, 174.4 ppm; MS m/z: 640 (M^+ with ^{37}Cl, 10), 638 (M^+ with ^{35}Cl, 21), 414 (33), 412 (100), 368 (26), 280 (13), 227 (64), 195 (31), 91 (53); HRMS calcd (for $C_{35}H_{43}{}^{35}ClN_2O_7$): 638.2759, found: 638.2772.

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Supporting Information Available: NMR (^{1}H, ^{13}C, ^{1}H−^{1}H COSY) spectra of compounds **1**, (+)-**3**, **4**, (−)-**7**, (+)-**8**, **23**, **24**, **26**−**30**; MS and IR spectra of compounds **1**, **26**−**30** (39 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.

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