Convergent Synthesis of (−)-Mirabazole C Using a Chloroimidazolidium Coupling Reagent, CIP

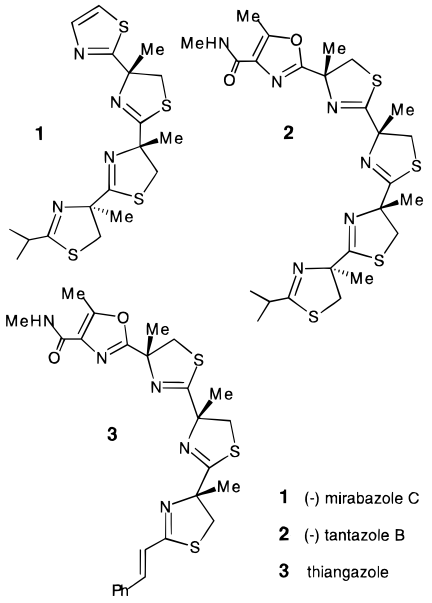
Kenichi Akaji, Naohiro Kuriyama, and Yoshiaki Kiso*

Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan

Received December 28, 1995[⊗]

Convergent synthesis of (−)-mirabazole C (**1**), a tetra thiazoline/thiazole alkaloid isolated from blue-green alga, has been described. The successive thiazoline rings of (−)-mirabazole C were formed by a single-step cyclization mediated by $TiCl_4$ treatment of tripeptide amide **4**. Convergent synthesis of the key intermediate **33** derived from three 2-methylcysteine residues was first achieved using a newly developed coupling reagent, 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate (CIP). The effectiveness of CIP for the coupling of α,α-dialkyl amino acids and the reaction pathway of the activation were clarified by the syntheses of model peptides containing an α,α-dimethylamino acid. A practical method of asymmetric synthesis of 2-methylcysteine by alkylation of 2,4-*cis*-oxazolidinone **23** has also been described.

(−)-Mirabazole C (**1**) was isolated by Moore and co-workers[1] from the terrestrial cyanophyte *Scytonemea mirabile* (Dillwyn) Bornet (strain BY-8-1) following the structure elucidation of tantazole[2] **2** from the same blue-green alga. Mirabazole C and structurally related mirabazoles, tantazoles, and thiangazole[3] (**3**) are a unique group of thiazoline/thiazole-containing alkaloids. Several members of this family of alkaloids have been shown to exhibit selective cytotoxicity against murine solid tumors or to exhibit unusual high inhibitory activity against HIV-1 protease in vitro.[4]



Originally, the three asymmetric carbons of mirabazole C had been elucidated to be in an *S*-configuration, because of the strong structural resemblance of mirabazole C to the tantazoles. The tantazoles had been degraded to (*R*)-2-methylcysteine, and the natural products were considered to be biosynthesized from this unusual amino acid. However, subsequent to the structural revision of tantazole B **2** by Fukuyama and Xu,[5] the structure of mirabazole C was re-examined synthetically by Parsons and Heathcock[6] and revised to **1**, in which the ring-A stereocenter has the *R*- rather than the *S*-configuration.

Because of the novel structural features as well as interesting biological activities, these alkaloids have stimulated considerable interest. In the synthetic works regarding these alkaloids, basically two different strategies have been adopted to construct the successive thiazoline rings: one is the sequential formation of thiazoline rings by cyclocondensation of 2-methylcysteine with imino ether[7] or by transformation of ammonium thioester into thiazoline[5] and the other is a simultaneous formation of thiazoline rings by Lewis acid mediated cyclocondensation of an appropriate precursor peptide consisting of 2-methylcysteine.[6,8] The latter procedure is more efficient than the former regarding the formation of successive thiazoline rings, but efficient preparation of the precursor peptide is often difficult because of the extremely low reactivity of 2-methylcysteine.[9] To overcome this difficulty, we have developed an efficient coupling agent, chloroimidazolidium agent 2-chloro-1,3-dimethylimidazolidium hexafluorophosphate (CIP), suitable for the condensation of an α,α-dialkylamino acid. Here we describe a method of convergent synthesis of (−)-mirabazole C using a newly developed coupling agent as well as the efficiency and reaction pathway of this new coupling agent.

Results and Discussion

Scheme 1 shows our retrosynthetic route of (−)-mirabazole C. The four successive thiazoline rings are simultaneously formed at the final step of the synthesis from the appropriately derivatized tripeptide amide **4** containing three 2-methylcysteine residues. The simultaneous formation by $TiCl_4$-mediated cyclodehydration was shown to be effective by Heathcock and co-workers[6,8] in the syntheses of mirabazoles and thiangazole. The

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1996.

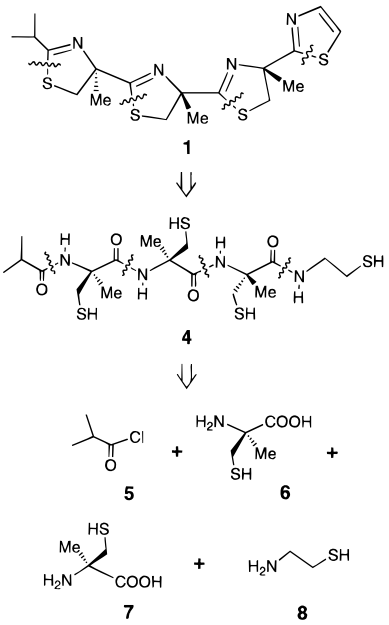
(1) Carmeli, S.; Moore, R. E.; Patterson, G. M. L. *Tetrahedron Lett.* **1991**, *32*, 2593.
(2) Carmeli, S.; Moore, R. E., Patterson, G. M. L.; Corbett, T. H.; Valeriote, F. A. *J. Am. Chem. Soc.* **1990**, *112*, 8195.
(3) Jansen, R.; Kunze, B.; Reichenbach, H.; Jurkiewicz, E.; Hunsmann, G.; Höfle, G. *Liebigs Ann. Chem.* **1992**, 357.
(4) Jurkiewicz, E.; Jansen, R.; Kunze, B.; Trowitzsch-Kienast, W.; Forche, E.; Reichenbach, H.; Höfle, G.; Hunsmann, G. *Antiviral Chem. Chemother.* **1992**, *3*, 189.
(5) Fukuyama, T.; Xu, L. *J. Am. Chem. Soc.* **1993**, *115*, 8449.
(6) Parsons, R. L., Jr.; Heathcock, C. H. *Tetrahedron Lett.* **1994**, *35*, 1379.
(7) Boyce, R. J.; Pattenden, G. *Synlett* **1994**, 587.
(8) Parsons,R. L., Jr; Heathcock, C. H. *J. Org. Chem.* **1994**, *59*, 4733.
(9) Walker, M. A.; Heathcock, C. H. *J. Org. Chem.* **1992**, *57*, 5566.

S0022-3263(95)02285-7 CCC: $12.00

Table 1. Yields (%) of dipeptides obtained by the different coupling methods

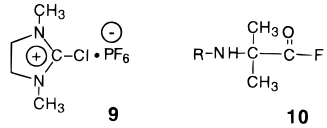
	R = Boc			R = Cbz			R = Fmoc		
	PyBroP	CIP	CIP/HOAt	PyBroP	CIP	CIP/HOAt	PyBroP	CIP	CIP/HOAt
R-Aib-Val-OMe	37	43	82	60	59	92	57	59	93
R-Aib-Aib-OMe	4	6	80	17	11	82	12	10	90
R-Val-Aib-OMe	41	44	80	60	60	85	50	51	87

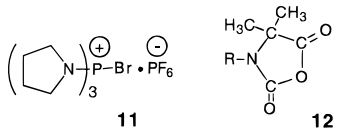
Scheme 1



most difficult aspect in this scheme is an efficient preparation of the precursor tripeptide amide **4**, since the coupling of an α,α-dialkylamino acid such as 2-methylcysteine is one of the most difficult reactions in peptide synthesis.[9,10] The convergent routes for preparation of the tripeptide amide were unsuccessful even with PyBroP which had been the best one among the coupling reagents available in the previous synthesis of mirabazole C.[9] Thus, prior to the synthesis of (−)-mirabazole C according to the scheme, we developed an efficient coupling agent and examined its efficiency as well as the reaction pathway using model peptides.

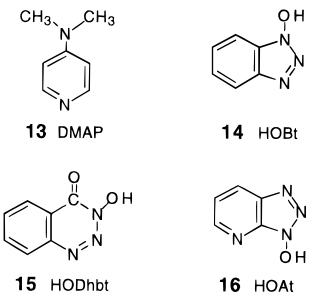
Coupling Agent. For the coupling of an α,α-dialkyl amino acid such as α,α-dimethyl amino acid (Aib, α-aminoisobutyric acid), a preactivated form of N^{α}-protected Aib (fluoride[11] **10** or *N*-carboxy anhydride[12] **12**) and a coupling reagent (PyBroP,[13] **11**) have been developed and applied for synthesis of Aib-containing peptaibols. Considering that a coupling reagent would be more convenient and applicable to the coupling of general α,α-dialkylamino acids than the preactivated form, we examined the use of CIP (**9**), originally developed by us for an efficient esterification reaction, as a coupling reagent.[14] We had demonstrated that the esterification of the Fmoc amino acid derivative to 4-alkoxybenzyl alcohol resin using CIP proceeded rapidly with the same or lower racemization level than the anchoring reaction using the conventional esterification reagents.





To evaluate the coupling efficiency, three types of model dipeptides containing Aib, the most simple α,α-dialkylamino acid, were synthesized using CIP: R-Aib-Val-OMe, R-Val-Aib-OMe, and R-Aib-Aib-OMe (R = Boc, Cbz, or Fmoc) (Table 1). Coupling proceeded at 25 °C for 60 min without an additive. For comparison, the same model peptide was prepared using PyBroP under the same conditions. For the Aib-Val and Val-Aib sequences, the yields were moderate when Cbz or Fmoc was employed as the N^{α}-protecting group, whereas N^{α}-Boc amino acids gave lower yields than the Cbz and Fmoc dipeptides. These low yields can be explained by the easy formation of the corresponding *N*-carboxy anhydride (NCA) of Boc amino acid as described by Frérot et al.[15]

In the preparation of the Aib-Aib sequence, extremely low yields were obtained for all three N^{α}-protecting groups. To improve the yield of CIP coupling, we then added DMAP[16] (**13**), HOBt[17] (**14**), HODhbt[18] (**15**), or HOAt[19] (**16**) to the reaction mixture. DMAP and HOBt are common additives used to enhance the reactivity of an active ester of an amino acid in peptide synthesis. The HODhbt ester of Fmoc amino acid has been reported to be an effective acylating agent in solid-phase peptide synthesis. HOAt, developed recently by Carpino, has also been reported to show pronounced reactivity enhancement in the acylation reaction, probably because of the neighboring group effect caused by the pyridine ring.



(10) Slomczynska, U.; Beusen, D. D.; Zabrocki, J.; Kociolek, K.; Redlinski, A.; Reusser, F.; Hutton, W. C.; Leplawy, M. T.; Marshall, G. R. *J. Am. Chem. Soc.* **1992**, *114*, 4095.

(11) Wenschuh, H.; Beyermann, M.; Krause, E.; Carpino, L. A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 3733.

(12) Spencer, J. R.; Antonenko, V. V.; Delaet, N. G. J.; Goodman, M. *Int. J. Pept. Protein Res.* **1992**, *40*, 282.

(13) Frérot, E.; Coste, J.; Pantaloni, A.; Defour, M-N.; Jouin P. *Tetrahedron* **1991**, *47*, 259 and references cited therein.

(14) (a) Akaji, K.; Kuriyama, N.; Kimura, T.; Fujiwara, Y.; Kiso, Y. *Tetrahedron Lett.* **1992**, *33*, 3177. (b) Akaji, K.; Kuriyama, N.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315.

(15) Frérot, E.; Coste, J.; Poncet, J.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1992**, *33*, 2815.

(16) Hassner, A.; Krepski, R.; Alexanian, V. *Tetrahedron* **1978**, *34*, 2069.

(17) König, W.; Geiger, R. *Chem. Ber.* **1973**, *106*, 3626.

(18) Atherton, E.; Cameron, L.; Meldal, M.; Sheppard, R. *J. Chem. Soc., Chem. Commun.* **1986**, 1763.

(19) Carpino, L A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

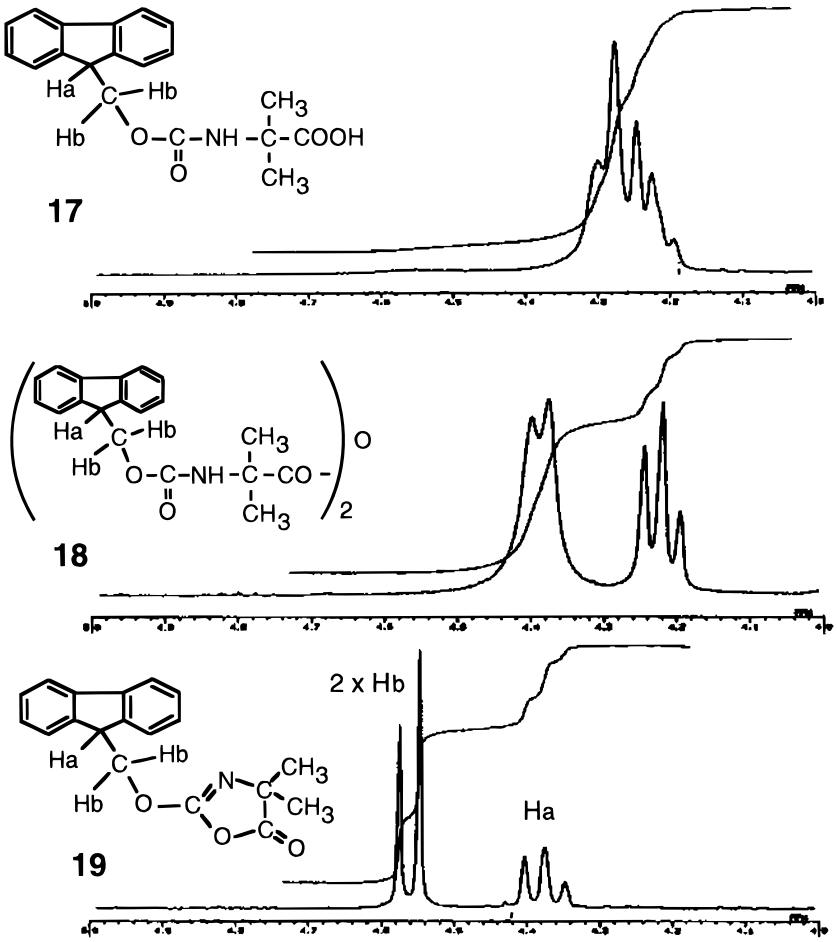


Figure 1. ^{1}H-NMR spectra (4−5 ppm) of Fmoc-Aib-OH (**17**), its anhydride (**18**), and oxazolone **19**.

All additives examined markedly enhanced the reactivity in catalytic amount. Table 1 shows the yields obtained by addition of HOAt in the coupling using CIP. The dipeptides including N^{α}-Boc peptides were obtained in excellent isolation yields. The amount of D-Val in the dipeptides prepared by the CIP/HOAt procedure was less than 0.5% when the derivatized hydrolysate of R-Val-Aib-OMe was examined by chiral GC analysis.[20] This shows that the coupling can be conducted without detectable racemization.

To estimate the reaction mechanism of the CIP coupling, two expected intermediates, Fmoc-Aib anhydride (**18**) and 2-[(9-fluorenylmethyl)oxy]-4-dimethyl-5-oxazolone (**19**), were prepared by the treatment of Fmoc-Aib-OH with DIPCDI. Both compounds were isolated and purified by silica gel column chromatography and characterized. From the comparison of ^{1}H-NMR chemical shifts of the two compounds (Figure 1), we could identify the upfield shift in δ of the methyleneoxy protons of the oxazolone **19** as described earlier for an oxazolone derived from Fmoc-Val-OH.[21] This upfield shift makes it possible to estimate each amount of Fmoc-Aib-OH, its anhydride **18**, and the oxazolone **19** in the reaction mixture using ^{1}H NMR.

Thus, Fmoc-Aib-OH in $CDCl_3$ was treated with CIP and the mixture was examined periodically by ^{1}H NMR.

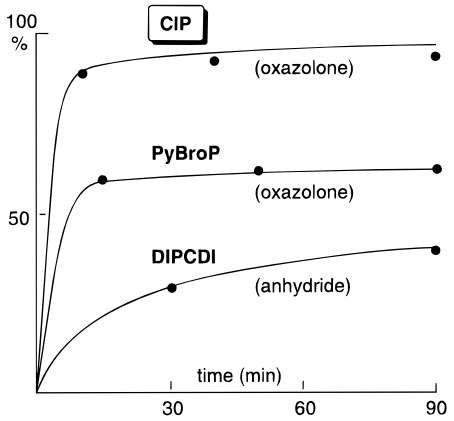


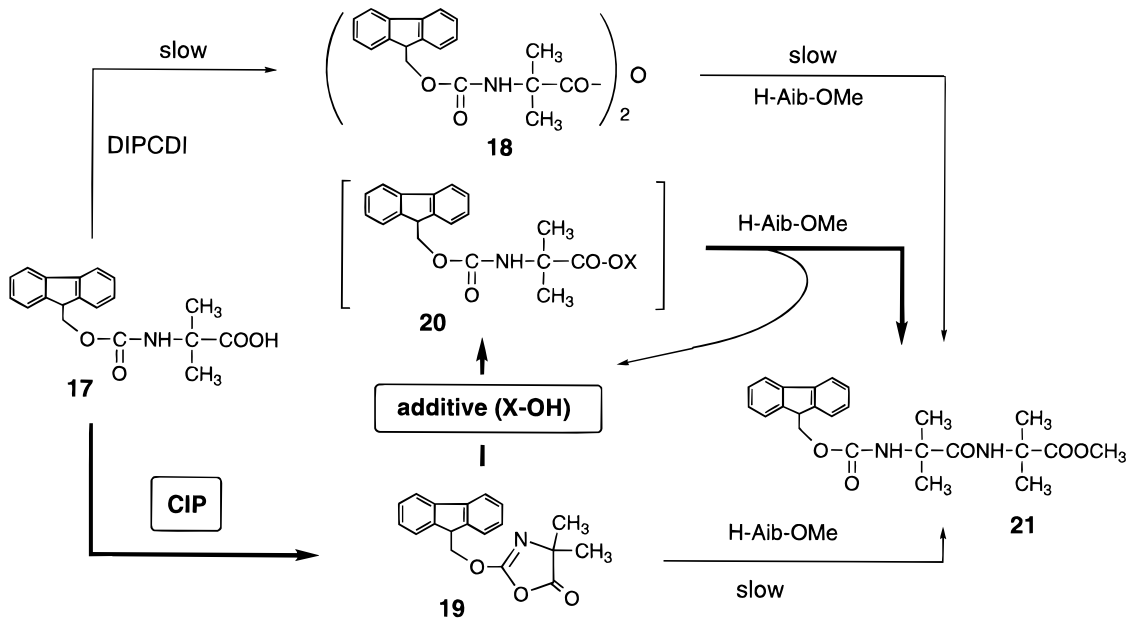
Figure 2. Time course of the activation. The reaction mixture was examined periodically by ^{1}H-NMR.

For comparison, activation with DIPCDI and PyBroP was also examined. In the activation with DIPCDI, slow formation of the anhydride (60% after 19 h) and no significant formation of the oxazolone were detected. By contrast, immediate transformation to the oxazolone (ca. 90% after 15 min) was detected by CIP activation; 60% transformation to the oxazolone was detected when PyBroP was used for activation. The kinds of intermediates and their formation rates were clearly different in these three coupling reagents (Figure 2). In the preparation of Fmoc-Aib-Val-OMe and Fmoc-Aib-Aib-OMe using CIP in $CDCl_3$, we could follow the gradual disappearance of the oxazolone methyleneoxy peaks on ^{1}H NMR. The

(20) Frank, H.; Woiwode, W.; Nicholson, G.; Bayer, E. *Liebigs Ann. Chem.* **1981**, 354.

(21) (a) Paquet, A.; Chen, F. M. F.; Benoiton, N. L.; *Can. J. Chem.* **1984**, *62*, 1335. (b) Perlow, D. S.; Erb, J. M.; Gould, N. P.; Tung, R. D.; Freidinger, R. M.; Williams, P. D.; Veber, D. F. *J. Org. Chem.* **1992**, *57*, 4394.

Scheme 2



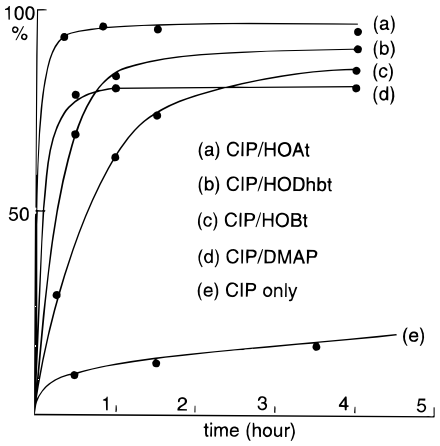


Figure 3. Time course of the coupling reaction. Fmoc-Aib-Aib-OMe was prepared by CIP coupling with or without an additive. The reaction mixture was examined periodically by ^{1}H-NMR.

rates of the peak disappearance corresponded well with the isolation yields shown in Table 1.

By the same monitoring method using ^{1}H NMR, the catalytic enhancement effects of the additives for the CIP coupling were also examined; the order of enhancement was HOAt > HODhbt > DMAP > HOBt (Figure 3). In the preparation of Fmoc-Aib-Aib-OMe, the coupling rate obtained by the addition of 0.5 equiv of HOBt was ca. nine times faster and that obtained by the addition of 0.25 equiv of HOAt was ca. 33 times faster than the rate without additives. The oxazolone-derived peaks disappeared completely within 20 min in CIP coupling in the presence of HOAt, HODhbt, or DMAP. In the CIP/HOBt coupling, fast formation of another intermediate and its gradual disappearance were detected on the ^{1}H-NMR spectrum. The intermediate would be the oxybenzotriazole ester of Fmoc-Aib.

These findings suggest that the CIP coupling proceeds according to the pathway shown in Scheme 2. The oxazolone of Fmoc-Aib formed through CIP activation is transformed to a highly active intermediate (**20**) by a catalytic amount of the additive; then the active intermediate ester quickly reacts with H-Aib-OMe to give Fmoc-Aib-Aib-OMe (**21**). The direct reaction of the oxazolone with an amine component is slow to moderate depending on the reactivity of the amine.

Synthesis of (−)-Mirabazole C. The model studies described above suggest that the coupling of 2-methylcysteine would be feasible using CIP/HOAt as a coupling agent. We then examined a practical route for stereoselective synthesis of (*R*)- or (*S*)-2-methylcysteine necessary for the preparation of the precursor tripeptide amide **33**.

Few routes have been successfully applied for the asymmetric synthesis of 2-methylcysteine because of the labile nature of the sulfhydryl group. Attempts to prepare 2-methylcysteine by asymmetric alkylation of chiral thiazolidine have been unsuccessful due to the predominant β-elimination under usual reaction conditions.[22] An extremely low reaction temperature (below −90 °C) and a unique starting thiazolidine derivative were neccesarry to avoid this side reaction.[23] A new asymmetric route based on chiral aziridine synthons[24] or an alternative route based on chiral bislactim synthons[25] contains multistep reactions to give the desired product with moderate yield. These reports prompt us to disclose an access for 2-methylcysteine from simple α-amino acids.

Figure 4 shows our synthetic route for (*R*)-2-methylcysteine. Application of the $BF_3{\cdot}OEt_2$-mediated condensation of Cbz-D-Ala-OH (**22**) with benzaldehyde dimethyl acetal was a modification of the method of Karady et al.[26] to give the oxazolidinone **23**. Under this condition, a major isomer crystallized easily had the 2,4-cis configuration as reported before.[26] The lower reaction temperature and fewer equivalents of $BF_3{\cdot}OEt_2$ caused increase of the 2,4-trans isomer **26** ratio. Alkylation of **23** with bromomethyl benzyl sulfide proceeded with a reasonable yield using lithium diethylamide (LDEA) as a base.

(22) Jeanguenat, A.; Seebach, D. *J. Chem. Soc., Perkin Trans. I* **1991**, 2291.
(23) Pattenden, G.; Thom, S. M.; Jones, M. F. *Tetrahedron* **1993**, *49*, 2131.
(24) Shao, H.; Zhu, Q.; Goodman, M. *J. Org. Chem.* **1995**, *60*, 790.
(25) Groth, U.; Schöllkopf, U. *Synthesis* **1983**, 37.
(26) Karady, S.; Amato, J. S.; Weinstock, L. M. *Tetrahedron Lett.* **1984**, *25*, 4337.

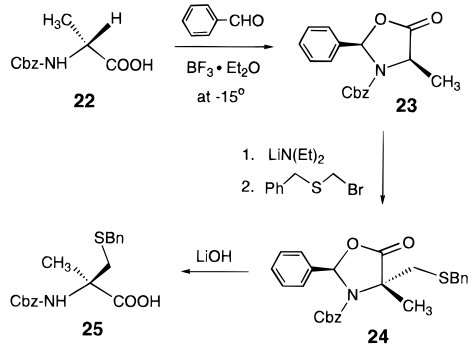


Figure 4. Synthetic route for (*R*)-2-methylcysteine.

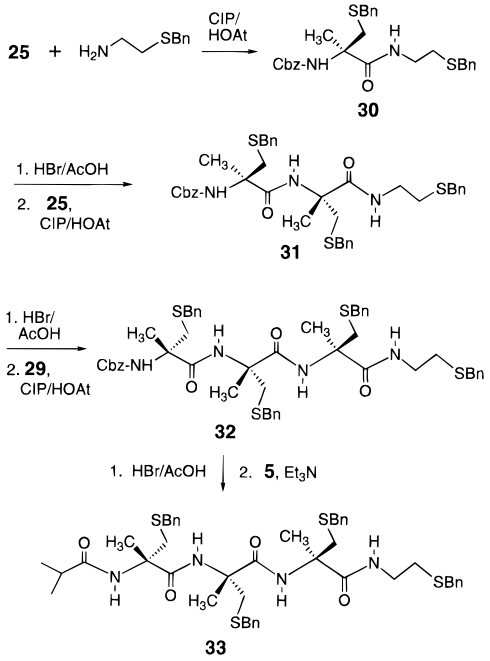


Figure 5. Synthetic route for the key intermediate tripeptide amide **33**.

Alkylated product **24** was obtained with an extremely poor yield when lithium diisopropylamide (LDA) or lithium hexamethyldisilazide (LHMDS) was employed as the base with or without dimethylpropyleneurea (DMPU). The same phenomena have been described in the related oxazolidinone by Seebach and Fadel.[27] Subsequent hydrolysis of **24** proceeded without difficulty. *N*-(Carbobenzyloxy)-*S*-benzyl-(*R*)-2-methylcysteine (**25**) was prepared by a three-step reaction from Cbz-D-Ala-OH in 28% overall yield; the same or superior yield than the reported values. Protected (*S*)-2-methylcysteine derivative **29** was similarly prepared starting from Cbz-L-Ala-OH.

Figure 5 shows the synthetic route for the key intermediate **33**. *N*-(Carbobenzyloxy)-*S*-benzyl-(*R*)-2-methylcysteine (**25**) was coupled with *S*-benzyl-2-aminoethanethiol by a 60 min reaction using CIP/HOAt to obtain *S*-protected-2-methylcysteine amide **30** quantitatively. The Cbz group of **30** was removed by treatment with HBr−AcOH, and the product was coupled with **25** using CIP/HOAt. The same deprotection by HBr−AcOH and the coupling by CIP/HOAt were repeated for the condensation of **31** and *N*-(carbobenzyloxy)-*S*-benzyl-(*S*)-2-methylcysteine (**29**) to give tripeptide amide **32**. The desired di- and tripeptide amides (**31** and **32**) were obtained in

(27) Seebach, D.; Fadel, A. *Helv. Chim. Acta* **1985**, *68*, 1243.

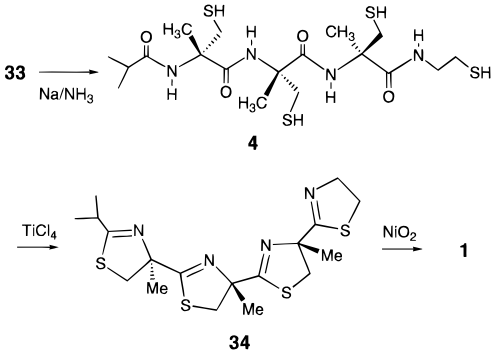
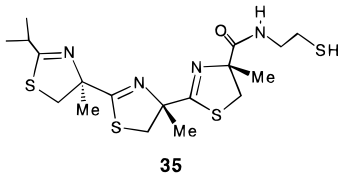


Figure 6. Synthetic route for (−)-mirabazole C (**1**).

reasonable yields (60% and 55%, respectively) with CIP/HOAt being the only coupling reagent, although the reaction rates were somewhat lower than those between the simple α,α-dimethylamino acids. The Cbz group of **32** was then removed with HBr−AcOH, and the resulting amine was acylated with isobutyryl chloride to obtain **33**. Our synthetic route shown in Figure 5 is one of the convergent routes which were unsuccessful with PyBroP in the previous synthetic work.[9]

Finally, the key intermediate **33** was converted to (−)-mirabazole C according to the route shown in Figure 6. Four benzyl groups, which were thiol protecting groups, were removed by treatment of **33** with sodium in ammonia. Without further purification, the isolated crude tetrathiol product **4** is immediately treated with titanium tetrachloride in CH_2Cl_2 to obtain dihydromirabazole C (**34**). During this cyclization reaction, a side product containing three but not the terminal unsubstituted thiazoline ring **35** was also formed. This side product was easily removed by flash chromatography. The terminal thiazoline ring of the purified dihydromirabazole C (**34**) was then oxidized by nickel peroxide to (−)-mirabazole C without difficulty. The synthetic material had the same spectroscopic properties as those reported for the natural product.



Conclusion

CIP in the presence of additive was found to be a suitable agent for the coupling of α,α-dialkylamino acid. All model dipeptides containing Aib have been obtained with quantitative yields using CIP/HOAt as a coupling agent. In the model studies, we found that the first intermediate, 2-[(9-fluorenylmethyl)oxy]-4-dimethyl-5-oxazolone formed by CIP activation, is transformed to the highly active ester by the catalytic additive to give the desired peptide without detectable racemization.

Convergent synthesis of (−)-mirabazole C was first achieved using CIP/HOAt. For the simultaneous formation of successive thiazoline rings of (−)-mirabazole C, the $TiCl_4$-mediated cyclization of a tripeptide amide (**4**) derived from 2-methylcysteine was successfully achieved. A convergent route for the key intermediate **33** by the successive coupling of 2-methylcysteine was first achieved using CIP/HOAt as a condensation agent. We consider that the combination of the $TiCl_4$-mediated single-step

cyclization and the efficient coupling of 2-methylcysteine using CIP/HOAt should be generally applicable for the syntheses of other members of the tantazole−mirabazole family of alkaloids.

Experimental Section

General. All reactions involving air or moisture sensitive reagents were conducted under an atmosphere of N_2 in septum-stoppered flasks. Solvents were reagent grade and dried prior to use. All flash chromatographic separations were carried out on Wakogel FC-40 obtained from WAKO Pure Chemical Ind. Ltd. Organometallic reagents and DIPCDI were obtained from Aldrich Chemical Co. D- and L-alanine were purchased from Peptide Institute (Osaka). Fmoc amino acid derivatives and PyBroP were obtained from CALBIOCHEM NOVABIOCHEM and used without further purification.

Melting points were uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a 270 or 300 MHz spectrometer with TMS as an internal standard. Optical rotations were measured at ambient temperature using a 1 mL cell. Analytical HPLC was carried out on a reverse phase column (4.6 × 150 mm), which was eluted with a linear gradient of CH_3CN (40−90%, 30 min) in 0.1% aqueous TFA at a flow rate of 1.0 mL/min.

Syntheses of Aib-Containing Model Dipeptides (Table 1). To the suspension of N^{α}-protected amino acid (0.5 mmol) with or without additive (0.25 equiv) in CH_2Cl_2 (4 mL) was added DIEA (4 equiv) at an ice-bath temperature. Then, the addition of a coupling agent (1 equiv) to the mixture was followed by that of 1.1 equiv of HCl salt of amino acid methyl ester. The mixture was stirred for 60 min at 25 °C and then taken into AcOEt (20 mL). The solution was washed with 5% citric acid and H_2O and then dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography which was eluted with *n*-hexane:AcOEt = 6:4. The purified product was crystallized from *n*-hexane. Table 1 summarizes each isolated yield. All known dipeptides, N^{α}-Boc and N^{α}-Cbz dipeptide methyl esters, had the same characterization data as the published values.[13] The characterization data of newly synthesized N^{α}-Fmoc dipeptide methyl esters are as follows. Fmoc-Aib-Val-OMe: mp 99−101 °C, ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.86 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.9 Hz, 3H), 1.52 (s, 3H), 1.55 (s, 3H), 2.17 (d of heptet, J = 8.6 Hz, 6.9 Hz, 1H), 3.70 (s, 3H), 4.20 (t, J = 6.8 Hz, 1H), 4.41 (d, J = 6.9 Hz, 2H), 4.52 (dd, J = 8.6 Hz, 4.6 Hz, 1H), 5.45 (s, 1H), 6.82 (d, J = 4.6 Hz, 1H), 7.26−7.78 (m, 8H). Anal. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39. Found: C, 68.06; H, 6.91; N, 6.36. Fmoc-Aib-Aib-OMe: mp 123−125 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.51 (s, 6H), 1.54 (s, 6H), 3.72 (s, 3H), 4.21 (t, J = 6.9 Hz, 1H), 4.41 (d, J = 6.9 Hz, 2H), 5.40 (s, 1H), 6.87 (s, 1H), 7.26−7.78 (m, 8H). Anal. Calcd for $C_{24}H_{28}N_2O_5$: C, 67.90; H, 6.65; N, 6.60. Found: C, 67.76; H, 6.62; N, 6.64. Fmoc-Val-Aib-OMe: mp 139−141 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.94 (d, J = 6.6 Hz, 3H), 0.97 (d, J = 6.6 Hz, 3H), 1.53 (s, 6H), 2.10 (m, 1H), 3.71 (s, 3H), 3.97 (m, 1H), 4.22 (t, J = 6.9 Hz, 1H), 4.39 (m, 2H), 5.47 (br d, 1H), 6.54 (br s, 1H), 7.26−7.77 (m, 8H). Anal. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39. Found: C, 68.44; H, 6.97; N, 6.38.

Estimation of D-Val Content. R-Val-Aib-OMe (ca. 2 mg) obtained above was hydrolyzed with 6 N HCl at 110 °C in a sealed tube. After 24 h, the solvent was removed in vacuo. To the residue was added 10% HCl in *n*-BuOH (500 μL), and the mixture was heated at 110 °C for 2 h. The solvent was removed in vacuo, and the resulting residue was taken into pentafluoropropionic anhydride (50 μL). The mixture was heated at 110 °C for 30 min, and then the excess reagent was removed under an N_2 stream. The residue was dissolved in CH_2Cl_2 (100 μL), and an aliquot (1 μL) of the solution was applied to a GC capillary Chirasil-Val-L column (0.26 mm × 25 m; elution condition, 95 °C for 2 min and then temperature gradient 4 °C/min): t_R; Aib 3.785 min, D-Val 6.045 min, L-Val 6.585 min. The D-Val content of all dipeptides examined was less than 0.5%, the value due to hydrolysis.

Fmoc-Aib Anhydride (18) and 2-[(9-Fluorenylmethyl)oxy]-4-dimethyl-5-oxazolone (19). To a stirred solution of Fmoc-Aib-OH (0.2 g, 0.61 mmol) in dry CH_2Cl_2 (5 mL) was added DIPCDI (97 μL, 0.61 mmol). After being stirred for 3 h at 25 °C, the mixture was directly applied to a silica gel column which was eluted with $CHCl_3$:MeOH = 40:1. The fast eluting fraction (R_f = 0.91 $CHCl_3$:MeOH = 40:1, oxazolone **19**) and the slow eluting fraction (R_f = 0.62 $CHCl_3$:MeOH = 40:1, anhydride **18**) were separated. Oxazolone: yield 30 mg; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.43 (s, 6H), 4.38 (t, J = 7.3 Hz, 1H), 4.56 (d, J = 7.3 Hz, 2H), 7.26−7.46 (m, 4H), 7.64 (d, J = 7.6 Hz, 2H), 7.78 (d, J = 7.6 Hz, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 25.3, 46.4, 66.8, 71.9, 120.2, 125.3, 127.2, 128.1, 141.4, 142.8, 157.0, 178.8. Anhydride: yield 40 mg; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.54 (s, 6H), 4.22 (t, J = 6.8 Hz, 1H), 4.39 (d, J = 6.8 Hz, 2H), 7.26−7.43 (m, 4H), 7.60 (d, J = 7.3 Hz, 2H), 7.76 (d, J = 7.6 Hz, 2H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 25.1, 47.2, 56.5, 66.5, 120.0, 125.0, 127.1, 127.7, 141.3, 144.0, 154.9, 175.1.

Monitoring of the Activation Step Using ^{1}H NMR (Figure 2). To a solution of 25 mg of Fmoc-Aib-OH in 0.8 mL of $CDCl_3$ were added DIEA (27 μL) and a coupling reagent (1 equiv). The mixture was examined immediately on a ^{1}H-NMR instrument. The methyleneoxy doublet peaks at 4.29 ppm due to the starting Fmoc-Aib-OH were gradually replaced by doublet peaks at 4.39 ppm due to the anhydride **18** or by those at 4.56 ppm due to the oxazolone **19**. Figure 2 shows the time course of the activation.

Monitoring of the Coupling Reaction Using ^{1}H NMR (Figure 3). To a mixture of Fmoc-Aib-OH (25 mg) and DIEA (74 μL) with or without an additive (0.25 equiv) in $CDCl_3$ (0.8 mL) were added a coupling reagent (1 equiv) and HCl· H-Aib-OMe (3 equiv). The mixture was similarly examined as above. The methyleneoxy doublet peaks at 4.56 ppm due to the oxazolone **19** were gradually replaced by those at 4.41 ppm due to Fmoc-Aib-Aib-OMe (**21**), the desired product. Figure 3 shows the time course of the coupling reaction. The rate constant of CIP coupling without additive (first 90 min), 1.87 × 10^{-3} min^{-1}. The rate constant of CIP/HOBt (0.5 equiv) (first 90 min), 17 × 10^{-3} min^{-1}. The rate constant of CIP/HOAt (0.1 equiv) (first 90 min), 17 × 10^{-3} min^{-1}. The rate constant of CIP/HOAt (0.25 equiv) (first 40 min), 61 × 10^{-3} min^{-1}.

(2*R*,4*R*)-2-Phenyl-3-(carbobenzyloxy)-4-methyloxazolidin-5-one (23). To a stirred solution of *N*-Cbz-D-alanine (10.0 g, 45 mmol) and benzaldehyde dimethyl acetal (6.8 mL, 45 mmol) in Et_2O (90 mL) was added 33 mL (270 mmol) of BF_3·Et_2O at −78 °C. The mixture was stirred at −15 °C for 4 days. The reaction mixture was slowly added to ice-cooled, saturated aqueous $NaHCO_3$ (200 mL), and the mixture was stirred for 30 min. After settling, the separated organic layer was washed with 5% $NaHCO_3$ and H_2O and then dried ($MgSO_4$). The solvent was removed *in vacuo*. The residue was dissolved in 140 mL of Et_2O/hexane (3:4), and the solution was kept standing at 4 °C for 2 h to precipitate white crystals with a yield of 10.1 g (72%): mp 60−61 °C; HPLC, t_R 15.92 min; $[\alpha]^{20}_D$ = +27.02 (c = 0.9, $CHCl_3$); IR (KBr) 1794, 1709, 1697, 1460, 1452, 1417 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.57 (d, J = 6.9 Hz, 3H), 4.48 (q, J = 6.9 Hz, 1H), 5.16 (br s, 2H), 6.64 (s,1H), 7.25−7.40 (m, 10H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 18.22, 52.15, 67.98, 89.06, 126.25, 128.12, 128.52, 128.64, 128.77, 129.79, 135.38, 136.94, 153.40, 172.52; EI-MS, 311.117 for M^+ (calcd 311.116 for $C_{18}H_{17}NO_4$).

A small amount of (2*S*,4*R*)-oxazolidinone **26** was recovered from the mother liquor when the reaction was performed at −20 °C with 2 equiv of BF_3·Et_2O for 1 day: mp 77−78 °C; HPLC, t_R 15.25 min; $[\alpha]^{24}_D$ = −95.91 (c = 0.7, $CHCl_3$); IR (KBr) 1801, 1713, 1460, 1448, 1416 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.69 (br d, 3H), 4.52 (br q, 1H), 5.00 (br m, 2H), 6.48 (s, 1H), 7.26−7.41 (m, 10H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 16.41, 16.69, 52.04, 67.65, 89.43, 126.56, 128.10, 128.48, 128.88, 130.10, 135.18, 136.42, 152.00, 172.27; EI-MS, 311.115 for M^+ (calcd 311.116 for $C_{18}H_{17}NO_4$).

(2*S*,4*S*)-Oxazolidinone **27** was prepared from Cbz-L-Ala-OH using the same procedure described for (2*R*,4*R*)-oxazolidinone

23: $[\alpha]^{21}_D = -27.11$ ($c = 1.1$, $CHCl_3$); EI-MS, 311.117 for M^+ (calcd 311.116 for $C_{18}H_{17}NO_4$).

(2*R*,4*R*)-2-Phenyl-3-(carbobenzyloxy)-4-methyl-4-(((phenylmethyl)thio)methyl)-oxazolidin-5-one (24). To a stirred solution of diethylamine (1.2 mL, 11.6 mmol) in dry THF (15 mL) at −78 °C was added 7.2 mL (11.6 mmol) of *n*-BuLi (1.6 M in hexane), and the mixture was stirred at −5 °C for 10 min. The solution was cooled to −78 °C, and a solution of 3.0 g (9.6 mmol) of **23** in THF (14.5 mL) was added at −78 °C. After the solution was stirred for 40 min, a solution of 3.1 g (14.3 mmol) of bromomethyl benzyl sulfide[9] in 2 mL of THF was added at −78 °C and the mixture was stirred overnight at 2 °C. The reaction was quenched with saturated aqueous NH_4Cl, and the product was extracted with AcOEt (150 mL). The organic layer was washed with 5% $NaHCO_3$ and H_2O, dried ($MgSO_4$), and rotary evaporated. The crude product was purified by silica gel column chromatography using $CHCl_3$ followed by flash chromatography using hexane:AcOEt (10:1) to yield 1.67 g (39%) of **24** as an oil: $[\alpha]^{23}_D = +129.11$ ($c = 2.8$, $CHCl_3$); IR ($CHCl_3$) 1794, 1713, 1497, 1456, 1408, 1371, 1348 cm^{-1}; ^1H NMR (300 MHz, 80 °C, DMSO-d_6) δ 1.72 (s, 3H), 2.95 (d, $J = 16.2$ Hz, 1H), 3.51 (d, $J = 16.2$ Hz, 1H), 3.69 (d, $J = 12.3$ Hz, 1H), 3.76 (d, $J = 12.3$ Hz, 1H), 5.02 (s, 2H), 6.62 (s, 1H), 7.21−7.50 (m, 15H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 23.54, 36.69, 36.98, 63.95, 67.38, 90.49, 126.97, 127.28, 127.69, 128.32, 128.50, 128.60, 128.73, 129.02, 135.18, 136.87, 137.64, 151.82, 173.87; FAB-MS, 448.158 for $[M + H]^+$ (calcd 448.158 for $C_{26}H_{26}NO_4S$).

(2*S*,4*S*)-Oxazolidinone **28** was similarly prepared from **27**. **28**: $[\alpha]^{26}_D = -128.93$ ($c = 0.6$, $CHCl_3$); FAB-MS, 448.159 for $[M + H]^+$ (calcd 448.158 for $C_{26}H_{26}NO_4S$).

***N*-(Carbobenzyloxy)-*S*-benzyl-(*R*)-2-methylcysteine [Cbz-MeCys(Bn)-OH] (25).** To a stirred solution of **24** (1.24 g, 2.77 mmol) in THF/H_2O (3:1, 40 mL) was added $LiOH\cdot H_2O$ (0.23 g, 5.49 mmol) at 4 °C. The mixture was stirred overnight at 25 °C, and then 5% $NaHCO_3$ (100 mL) was added to the reaction mixture. The aqueous layer was washed with *n*-hexane and then acidified to pH 2 using 2 N HCl. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and dried ($MgSO_4$). The solvent was removed in vacuo to yield 1.0 g (quantitative) of **25** as an oil. The product was used in subsequent reactions without further purification: $[\alpha]^{24}_D = +28.44$ ($c = 0.5$, EtOH); IR ($CHCl_3$) 1720, 1713, 1502, 1454 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.60 (s, 3H), 3.06 (d, $J = 13.9$ Hz, 1H), 3.23 (d, $J = 13.9$ Hz, 1H), 3.64 (s, 2H), 5.09 (s, 2H), 5.78 (br s, 1H), 7.20−7.32 (m, 10H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 23.28, 37.43, 37.86, 60.32, 66.88, 127.15, 128.10, 128.21, 128.53, 128.89, 136.12, 137.93, 154.97, 177.54; FAB-MS, 360.129 for $[M + H]^+$ (calcd 360.127 for $C_{19}H_{22}NO_4S$).

N-(Carbobenzyloxy)-*S*-benzyl-(*S*)-2-methylcysteine (**29**) was similarly prepared from **28**. **29**: $[\alpha]^{23}_D = -34.08$ ($c = 0.5$, EtOH); FAB-MS, 360.131 for $[M + H]^+$ (calcd 360.127 for $C_{19}H_{22}NO_4S$).

***N*-(Carbobenzyloxy)-*S*-benzyl-(*R*)-2-methylcysteine *N*-((Benzylthio)ethyl)amide [Cbz-MeCys(Bn)-NHCH$_2$CH$_2$SBn] (30).** To a stirred solution of Cbz-MeCys(Bn)-OH (0.60 g, 1.67 mmol) and diisopropylethylamine (DIEA, 1.45 mL) in CH_2Cl_2 (8 mL) at 4 °C were added HOAt (0.16 g, 1.17 mmol) and CIP (0.70 g, 2.51 mmol). Then, *S*-benzyl-2-aminoethanethiol hydrochloride[9] (0.51 g, 2.51 mM) was added and the resulting solution was stirred for 60 min at 25 °C. The solvent was removed in vacuo, and the residue was extracted with AcOEt. The organic layer was washed with 5% citric acid and H_2O and dried ($MgSO_4$). The solvent was removed in vacuo, and the resulting oil was purified by silica gel column chromatography using $CHCl_3$ to yield 0.84 g (99%) of the product as an oil: $[\alpha]^{24}_D = +4.76$ ($c = 0.2$, $CHCl_3$); IR ($CHCl_3$) 1720, 1672, 1495, 1454 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.52 (s, 3H), 2.51 (t, $J = 6.6$ Hz, 2H), 2.95 (d, $J = 13.9$ Hz, 1H), 3.14 (d, $J = 13.5$ Hz, 1H), 3.34 (q, $J = 6.3$ Hz, 2H), 3.65 (s, 2H), 3.67 (s, 2H), 5.07 (s, 2H), 5.58 (br s, 1H), 6.63 (br t, 1H), 7.21−7.35 (m, 15H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 28.36, 30.89, 35.63, 37.59, 38.13, 38.98, 59.87, 66.86, 127.13, 127.24, 128.14, 128.23, 128.53, 128.61, 128.82, 128.88, 136.10, 137.91, 138.02, 154.88, 172.77; FAB-MS, 509.196 for $[M + H]^+$ (calcd 509.193 for $C_{28}H_{33}N_2O_3S_2$).

***N*-(Carbobenzyloxy)-*S*-benzyl-(*R*)-methylcysteinyl-*S*-benzyl-(*R*)-methylcysteine *N*-((Benzylthio)ethyl)amide [Cbz-MeCys(Bn)-MeCys(Bn)-NHCH$_2$CH$_2$SBn] (31).** To a stirred solution of Cbz-MeCys(Bn)-NHCH$_2$CH$_2$SBn (**30**, 0.85 g, 1.67 mmol) in CH_2Cl_2 (1 mL) was added HBr/AcOH (3 mL) at 4 °C, and the mixture was stirred for 60 min at 25 °C. Ice-cooled CH_2Cl_2 (20 mL) and 5% $NaHCO_3$ (20 mL) were added to the mixture at 4 °C. The organic layer was washed with 5% $NaHCO_3$ and H_2O, and dried ($MgSO_4$). The solution was rotary evaporated at 4 °C to give HBr·H-MeCys(Bn)-NHCH$_2$CH$_2$SBn as an oil.

To the solution of Cbz-MeCys(Bn)-OH (**25**, 0.81 g, 2.26 mmol) in CH_2Cl_2 (5 mL) were added DIEA (1.16 mL, 6.68 mmol) and HOAt (0.16 g, 1.17 mmol). CIP (0.70 g, 2.50 mmol) and the above HBr·H-MeCys(Bn)-NHCH$_2$CH$_2$SBn in CH_2Cl_2 (3 mL) were then added to the mixture. The reaction mixture was stirred for 72 h at 25 °C. The solvent was removed in vacuo, and the residue was extracted with AcOEt (50 mL). The organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and H_2O and dried ($MgSO_4$). The crude product was purified by silica gel column chromatography using $CHCl_3$ followed by flash chromatography using hexane/AcOEt (5:2) to yield 0.72 g (60 %) of the desired dipeptide amide **31** as an oil: $[\alpha]^{23}_D = +24.05$ ($c = 0.4$, EtOH); IR ($CHCl_3$) 1714, 1685, 1664, 1535, 1495, 1454 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.46 (s, 6H), 2.55 (t, $J = 7.1$ Hz, 2H), 2.73 (d, $J = 13.5$ Hz, 1H), 2.89 (d, $J = 13.5$ Hz, 1H), 2.96 (d, $J = 13.5$ Hz, 1H), 3.33 (d, $J = 13.5$ Hz, 1H), 3.38 (q, $J = 6.7$ Hz, 2H), 3.59 (s, 2H), 3.66 (s, 2H), 3.70 (s, 2H), 5.06 (s, 2H), 5.42 (s, 1H), 6.62 (s, 1H), 7.18−7.31 (m, 21H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 22.79, 23.59, 30.39, 35.81, 37.27, 37.77, 39.01, 39.10, 59.53, 60.16, 67.38, 126.92, 127.22, 127.44, 128.23, 128.46, 128.61, 128.73, 128.84, 128.88, 155.68, 171.48, 172.36; FAB-MS, 716.269 for $[M + H]^+$ (calcd 716.265 for $C_{39}H_{46}N_3O_4S_3$).

***N*-(Carbobenzyloxy)-*S*-benzyl-(*S*)-methylcysteinyl-*S*-benzyl-(*R*)-methylcysteinyl-*S*-benzyl-(*R*)-methylcysteine *N*-((Benzylthio)ethyl)amide [Cbz-MeCys(Bn)-MeCys(Bn)-MeCys(Bn)-NHCH$_2$CH$_2$SBn] (32).** To a stirred solution of Cbz-MeCys(Bn)-MeCys(Bn)-NHCH$_2$CH$_2$SBn (**31**, 0.67 g, 0.94 mmol) in CH_2Cl_2 (1.5 mL) was added HBr/AcOH (4 mL) at 4 °C, and the mixture was stirred for 90 min at 25 °C. The deprotected HBr salt of the dipeptide amide HBr·H-MeCys(Bn)-MeCys(Bn)-NHCH$_2$CH$_2$SBn was isolated as described above for **31**.

To the solution of Cbz-MeCys(Bn)-OH (**29**, 0.47 g, 1.31 mmol) in CH_2Cl_2 (5 mL) were added DIEA (0.65 mL, 3.74 mmol) and HOAt (0.089g, 0.66 mmol). CIP (0.39 g, 1.40 mmol) and the HBr·H-MeCys(Bn)-MeCys(Bn)-NHCH$_2$CH$_2$SBn in CH_2Cl_2 (3 mL) were then added to the mixture. The reaction mixture was stirred for 72 h at 25 °C. The product was isolated as described for above dipeptide amide **31** and purified by silica gel column chromatography using $CHCl_3$ followed by flash chromatography using hexane/AcOEt (5:2) to give the Cbz tripeptide amide **32** (0.48 g, 55%) as an oil: $[\alpha]^{25}_D = -16.39$ ($c = 1.3$, EtOH); IR ($CHCl_3$) 1713, 1678, 1524, 1495, 1454 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.24 (s, 3H), 1.35 (s, 3H), 1.46 (s, 3H), 2.53 (m, 2H), 2.76 (d, $J = 13.9$ Hz, 1H), 2.85 (d, $J = 12.5$ Hz, 1H), 2.89 (d, $J = 13.2$ Hz, 1H), 2.97 (d, $J = 12.9$ Hz, 1H), 3.39 (m, 2H), 3.42 (d, $J = 13.2$ Hz, 1H), 3.69 (overlapping m, 9H), 4.89 (d, $J = 11.9$ Hz, 1H), 5.22 (d, $J = 12.2$ Hz, 1H), 5.40 (s, 1H), 6.22 (s, 1H), 7.14−7.35 (m, 22H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 21.08, 23.77, 24.08, 30.39, 35.72, 37.30, 37.77, 37.83, 38.92, 39.93, 59.33, 59.66, 60.16, 67.40, 126.83, 126.93, 127.24, 127.62, 128.43, 128.57, 128.61, 128.79, 128.88, 128.93, 129.00, 135.74, 137.64, 138.06, 138.43, 138.52, 155.61, 171.64, 172.85, 173.69; FAB-MS, 923.343 for $[M + H]^+$ (calcd 923.337 for $C_{50}H_{59}N_4O_5S_4$).

***N*-(2-Methylpropionyl)-*S*-benzyl-(*S*)-methylcysteinyl-*S*-benzyl-(*R*)-methylcysteinyl-*S*-benzyl-(*R*)-methylcysteine *N*-((Benzylthio)ethyl)amide [iPrCO-MeCys(Bn)-MeCys(Bn)-MeCys(Bn)-NHCH$_2$CH$_2$SBn] (33).** To a stirred solution of Cbz tripeptide amide **32** (0.38 g, 0.41 mmol) in CH_2Cl_2 (1 mL) was added HBr/AcOH (3 mL) at 4 °C, and the mixture was stirred for 90 min at 25 °C. The deprotected HBr salt of the tripeptide amide HBr·H-MeCys(Bn)-MeCys(Bn)-MeCys(Bn)-NHCH$_2$CH$_2$SBn was isolated as described for **32**,

and the product was dissolved in CH_2Cl_2 (3 mL). To this solution were added triethylamine (0.23 mL, 1.64 mmol) and $(CH_3)_2CHCOCl$ (0.13 mL, 1.24 mmol) at 4 °C, and the mixture was stirred for 120 min at 25 °C. CH_2Cl_2 (20 mL) was added to the reaction mixture. The organic layer was washed with H_2O, dried ($MgSO_4$),and rotary evaporated. The residue was purified by silica gel column chromatography using $CHCl_3$ to yield 0.28 g (80%) of the tripeptide amide **33** as a solid: mp 106−108 °C; $[\alpha]^{25}_D = +3.30$ ($c = 0.5$, EtOH); IR (KBr) 1676, 1649, 1519, 1495, 1452 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.03 (d, $J = 6.6$ Hz, 3H), 1.05 (d, $J = 6.9$ Hz, 3H), 1.32 (s, 3H), 1.34 (s, 3H), 1.47 (s, 3H), 2.12 (heptet, $J = 6.9$ Hz, 1H), 2.54 (m, 2H), 2.84 (d, $J = 13.5$ Hz, 1H), 2.88 (d, $J = 13.2$ Hz, 1H), 2.96 (d, $J = 13.2$ Hz, 2H), 3.38 (m, 2H), 3.45 (d, $J = 13.5$ Hz, 1H), 3.69 (overlapping m, 9H), 6.00 (s, 1H), 6.11 (s, 1H), 7.19−7.35 (m, 22H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 19.30, 19.39, 21.11, 24.01, 30.48, 35.31, 35.85, 37.52, 37.71, 37.79, 39.08, 39.80, 59.05, 59.30, 60.20, 126.84, 126.90, 127.29, 127.76, 128.43, 128.64, 128.84, 128.89, 128.95, 129.05, 129.16, 138.11, 138.24, 138.49, 138.65, 171.70, 172.65, 173.87, 177.82; FAB-MS, 859.345 for $[M + H]^+$ (calcd 859.342 for $C_{46}H_{59}N_4O_4S_4$).

Dihydromirabazole C (34). To the solution of iPrCO-MeCys(Bn)-MeCys(Bn)-MeCys(Bn)-$NHCH_2CH_2SBn$ (**33**, 0.145 g, 0.169 mmol) in dry THF (5 mL) was added 20 mL of liquid NH_3 at −78 °C. Na (0.3 g) was added to the solution, and the resulting dark blue solution was stirred for 60 min at −78 °C. NH_4Cl was added in portions until the blue color disappeared. The NH_3 of the mixture was removed under a stream of N_2, and the residue was dried in vacuo. To the dried solid was added CH_2Cl_2 (10 mL), and the mixture was filtered. The solvent was rotary evaporated at 4 °C, and the resulting oily residue was dried in vacuo to give the deprotected tripeptide amide **4** as an oil.

The deprotected product was dissolved in CH_2Cl_2 (4 mL). To this solution was added 2.7 mL of $TiCl_4$ (1.0 M solution in CH_2Cl_2) at −78 °C, and the mixture was stirred overnight at 25 °C. Saturated aqueous Na_2CO_3 (10 mL) was added, and the mixture was filtered. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 3). The combined organic fractions were washed with H_2O and dried ($MgSO_4$), and the solvent was rotary evaporated. The residue was purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (100:1) followed by flash chromatography using hexane/AcOEt (2:1) to provide 25 mg (35%) of dihydromirabazole C as an oil: $[\alpha]^{24}_D = -68.79$ ($c = 0.5$, $CHCl_3$); R_f 0.43 $CHCl_3$:MeOH = 100:1; ^1H NMR (270 MHz, $CDCl_3$) δ 1.230 (d, $J = 6.9$ Hz, 3H), 1.234 (d, $J = 6.9$ Hz, 3H), 1.57 (s, 3H), 1.59 (s, 3H), 1.66 (s, 3H), 2.83 (heptet, $J = 6.9$ Hz, 1H), 3.21 (d, $J = 11.2$ Hz, 1H), 3.24 (d, $J = 11.2$Hz, 1H), 3.26 (dd, $J = 8.2$ Hz, 2H), 3.28 (d, $J = 11.2$ Hz, 1H), 3.68 (d, $J = 11.6$ Hz, 1H), 3.71 (d, $J = 11.5$ Hz, 1H), 3.79 (d, $J = 11.2$ Hz, 1H), 4.29 (t, $J = 8.6$ Hz, 1H), 4.30 (t, $J = 8.6$ Hz, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 21.00, 21.19, 25.73, 26.11, 26.16, 33.10, 34.02, 42.80, 42.88, 64.33, 83.40, 83.70, 177.88, 179.12; FAB-MS, 427.113 for $[M + H]^+$ (calcd 427.112 for $C_{18}H_{27}N_4S_4$).

By flash chromatography 10 mg (14%) of a side product (**35**) was separated: $[\alpha]^{24}_D = -32.70$ ($c = 0.6$, $CHCl_3$); R_f 0.35 $CHCl_3$:MeOH = 100:1; ^1H NMR (270 MHz, $CDCl_3$) δ 1.24 (d, $J = 6.9$ Hz, 6H), 1.47 (s, 3H), 1.60 (s, 3H), 1.66 (s, 3H), 2.69 (m, 2H), 2.84 (heptet, $J = 6.9$ Hz, 1H), 3.18 (d, $J = 10.9$ Hz, 1H), 3.23 (d, $J = 11.2$ Hz, 1H), 3.28 (d, $J = 11.5$ Hz, 1H), 3.47 (m, 2H), 3.60 (d, $J = 11.9$ Hz, 1H), 3.66 (d, $J = 11.6$ Hz, 1H), 3.78 (d, $J = 11.2$ Hz, 1H), 7.12 (br t, 1H); ^{13}C NMR (68 MHz, $CDCl_3$) δ 21.01, 21.19, 24.64, 24.91, 26.09, 34.00, 41.24, 42.03, 42.84, 42.95, 83.31, 83.57, 84.51, 174.82, 178.47, 179.46; FAB-MS, 445.123 for $[M + H]^+$ (calcd 445.122 for $C_{18}H_{29}N_4OS_4$).

(−)-Mirabazole C (1). To the solution of dihydromirabazole C (6 mg, 0.01 mmol) in benzene (4 mL) was added 200 mg of crushed NiO_2 (ca. 2.0 mequiv of O_2/g), and the mixture was refluxed for 3 h. The mixture was filtered, and the solvent was rotary evaporated. The residue was purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (100:1) to yield 4 mg (67%) of **1** as an oil: $[\alpha]^{24}_D = -110.77$ ($c = 0.065$, $CHCl_3$) [lit.[1] $[\alpha]_D = -112$, $c = 0.03$, $CHCl_3$]; ^1H NMR (270 MHz, $CDCl_3$) δ 1.25 (d, $J = 6.9$ Hz, 3H), 1.26 (d, $J = 6.9$ Hz, 3H), 1.64 (s, 3H), 1.70 (s, 3H), 1.75 (s, 3H), 2.91 (heptet, $J = 6.9$ Hz, 1H), 3.31 (d, $J = 11.6$ Hz, 2H), 3.45 (d, $J = 11.6$ Hz, 1H), 3.74 (d, $J = 11.5$ Hz, 1H), 3.78 (d, $J = 11.2$ Hz, 1H), 3.84 (d, $J = 11.2$ Hz, 1H), 7.24 (d, $J = 3.3$ Hz, 1H), 7.77 (d, $J = 3.3$ Hz, 1H) [these data are essentially the same as those reported for the natural product[1]: ^1H NMR (300 MHz, $CDCl_3$) δ 1.23 (d, $J = 6.7$ Hz, 3H), 1.23 (d, $J = 6.7$ Hz, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 1.74 (s, 3H), 2.82 (qq, $J = 6.7$ Hz, 1H), 3.28 (d, $J = 11.3$ Hz, 1H), 3.28 (d, $J = 11.4$ Hz, 1H), 3.43 (d, $J = 11.3$ Hz, 1H), 3.73 (d, $J = 11.3$ Hz, 1H), 3.75 (d, $J = 11.3$ Hz, 1H), 3.80 (d, $J = 11.3$ Hz, 1H), 7.23 (d, $J = 3.3$ Hz, 1H), 7.76 (d, $J = 3.3$ Hz, 1H)]; FAB-MS, 425.096 for $[M + H]^+$ (calcd 425.096 for $C_{18}H_{25}N_4S_4$).

Supporting Information Available: GC analysis for the estimation of D-Val content, ^1H NMR data for the monitoring of the activation and coupling, HPLC data of cis and trans isomers of **23**, and ^1H and ^{13}C NMR spectra for compounds **1** and **30**−**35** (17 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.

JO952285H