CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200500852

Total Synthesis of (+)-Batzelladine A and (−)-Batzelladine D, and Identification of Their Target Protein

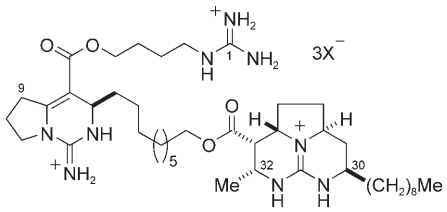
Jun Shimokawa,[b] Takanori Ishiwata,[c] Koji Shirai,[a] Hiroyuki Koshino,[c] Aya Tanatani,[b] Tadashi Nakata,[c, d] Yuichi Hashimoto,[b] and Kazuo Nagasawa*[a]

Abstract: Asymmetric total synthesis of batzelladine A (**1**) and batzelladine D (**2**) has been achieved. Our synthesis of batzelladines features 1) stereoselective construction of the cyclic guanidine system by means of successive 1,3-dipolar cycloaddition reaction and subsequent cyclization, 2) direct esterification of the bicyclic carboxylic acid **35** with the guanidine alcohol **8** or **59** to construct the whole carbon skeleton of batzelladines, and 3) one-step formation of the α,β-unsaturated aldehyde **53** from the primary alcohol **47** with tetra-*n*-propylammoniumperruthenate (TPAP), providing an efficient route to the left-hand bicyclic guanidine alcohol of batzelladine A (**1**). With the synthetic compounds **1** and **2** in hand, their target protein was examined by using immobilized CD4 and gp120 affinity gels. The results indicated that batzelladines A (**1**) and D (**2**) bind specifically to CD4.

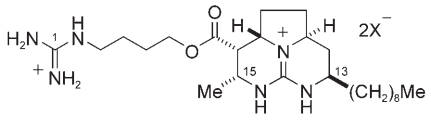
Keywords: batzelladine · guanidine · inhibitors · natural products · total synthesis

Introduction

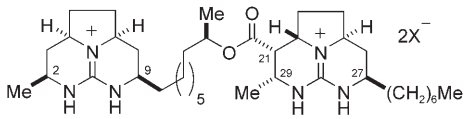
Batzelladines A–I are a novel class of polycyclic guanidine alkaloids isolated from Bahamian (batzelladines A (**1**), B, C, D (**2**), E) and Jamaican sponge (batzelladines F (**3**), G, H, I) by a SmithKline Beecham group in 1995 and 1997, respectively.[1,2] The structural feature of this family is a bicyclic and/or tricyclic guanidine structure, which is tethered by an alkyl ester unit. The unique structures of batzelladines have inspired considerable synthetic attention. Murphy et al. and Snider et al. independently reported the synthesis of a tricyclic guanidine moiety corresponding to the left-hand tricyclic guanidine portion of batzelladine F (**3**) by a biomimetic route.[3,4] In 1996 Snider et al. reported a total synthesis of batzelladine E through a biomimetic synthetic route.[3b] Subsequently, Overman et al. reported the total synthesis of bat-



Batzelladine A (**1**)



Batzelladine D (**2**)



Batzelladine F (**3**)

[a] K. Shirai, Prof. K. Nagasawa
Department of Biotechnology and Life Science
Faculty of Technology
Tokyo University of Agriculture and Technology
2-24-16 Naka-cho, Koganei, Tokyo 184-8588 (Japan)
Fax: (+81) 42-388-7295
E-mail: knaga@cc.tuat.ac.jp

[b] J. Shimokawa, Dr. A. Tanatani, Prof. Y. Hashimoto
Institute of Molecular and Cellular Biosciences
The University of Tokyo
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032 (Japan)

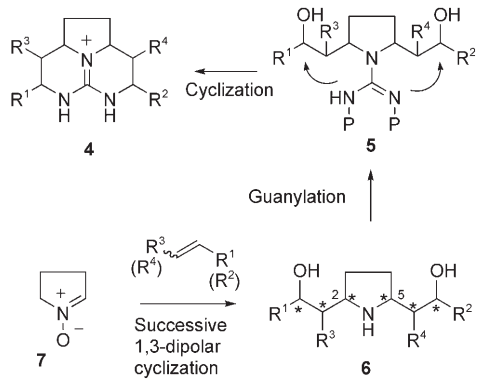
[c] T. Ishiwata, Dr. H. Koshino, Prof. T. Nakata
RIKEN (The Institute of Physical and Chemical Research)
2-1 Hirosawa, Wako-shi, Saitama 351-0198 (Japan)

[d] Prof. T. Nakata
Department of Chemistry, Faculty of Science
Tokyo University of Science
1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601 (Japan)

zelladines D (**2**)[5] and F (**3**)[6] by applying a tethered Biginelli reaction. Quite recently, Gin et al. reported the total synthesis of **2** by using [4+2] annulation of vinyl carbodiimides with *N*-alkylimines.[7] These synthetic successes, together with synthetic studies from other groups,[8–13] greatly contributed to structure revisions of batzelladines.

Batzelladines have been reported to disrupt protein–protein interactions. For example, batzelladines A–E block interaction between the surface of the HIV envelope glycoprotein gp120 and the extracellular domains of human CD4 receptor protein.[1a] Batzelladines F (**3**) and G induce dissociation of the complex between the protein kinase $p56^{lck}$ and CD4,[1b] and synthetic derivatives of batzelladines were reported to disrupt Nef–p53, Nef–actin, and Nef–$p56^{lck}$ interactions.[14] Elucidation of the mechanism by which protein–protein interactions are modulated by these small molecules is of great interest, since protein–protein associations are important in all aspects of cell biochemistry. Moreover, small molecules that influence protein–protein association would be new biological tools and potential therapeutic agents.[15] In particular, batzelladines or their derivatives may prove to be applicable for AIDS treatment. Because of our interest in the control of protein–protein interactions with small molecules, we have started the synthetic studies on batzelladines, and we recently have accomplished the total synthesis of (±)-batzelladine D (**2**)[16] and (+)-batzelladine A (**1**).[17] In this article, we describe the asymmetric total synthesis of (−)-batzelladine D (**2**) and, full detail of our synthetic studies on (+)-batzelladine A (**1**). We also describe the identification of target protein of batzelladines as the CD4 cell-surface receptor protein on T-cells by using the synthetic batzelladines.

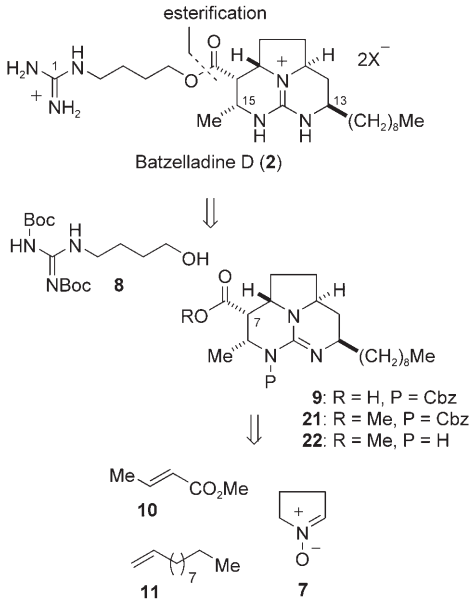
Synthetic plan: Stereoselective construction of the characteristic tricyclic guanidine structure in batzelladines is a key issue for the total synthesis of these natural products. Our synthetic concept of the tricyclic guanidine **4** is illustrated in Scheme 1. The tricyclic guanidine **4** could be constructed from the guanidine-substituted, 2,5-disubstituted pyrrolidine **5**, which has hydroxyl groups on its side chain at the β-positions. The 2,5-disubstituted pyrrolidine **6** could be prepared by successive 1,3-dipolar cycloaddition reaction[13] between the nitrone **7** and olefins. In this sequential process, hydroxyl groups on the side chains of pyrrolidine are introduced, and the newly generated stereochemistry at the C2- and C5-positions of pyrrolidine and at the hydroxyl groups on the side chains of **6** can be controlled simultaneously (Scheme 1). Moreover various substituents can be installed simply by changing the olefins. So it should be possible to synthesize stereoselectively various types of cyclic guanidines, as seen in batzelladines.



Scheme 1. Synthetic plan for the tricyclic guanidine **4**.

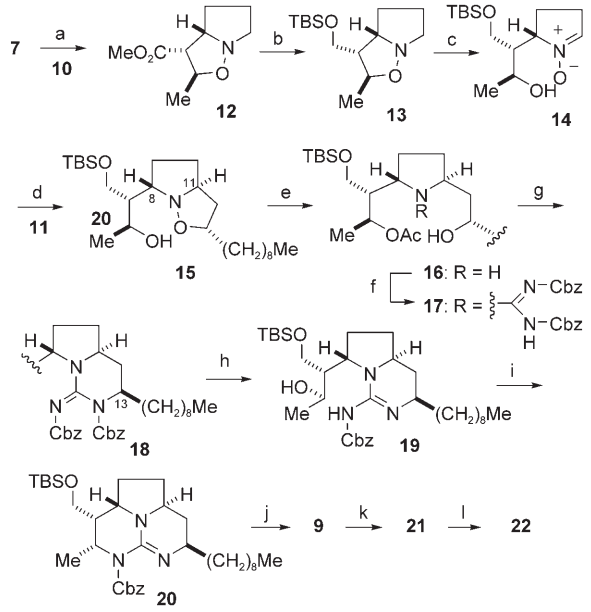
Results and Discussion

Total synthesis of (−)-batzelladine D (2): Batzelladine D (**2**) is a structurally relatively simple member of the batzelladine alkaloids, with five asymmetric carbon centers in the tricyclic guanidine moiety. As depicted in Scheme 2, batzelladine D (**2**) can be synthesized by esterification with the alcohol **8** and cyclic guanidine carboxylic acid **9**, which can in turn be prepared based upon the strategy illustrated in Scheme 1, specifically by using the nitrone **7** and two olefins, methyl crotonate (**10**) and 1-undecene (**11**). At the beginning of our synthetic studies on **2**, we examined the synthesis of the cyclic guanidine **9** as a racemic form.



Scheme 2. Retrosynthetic analysis of **2**.

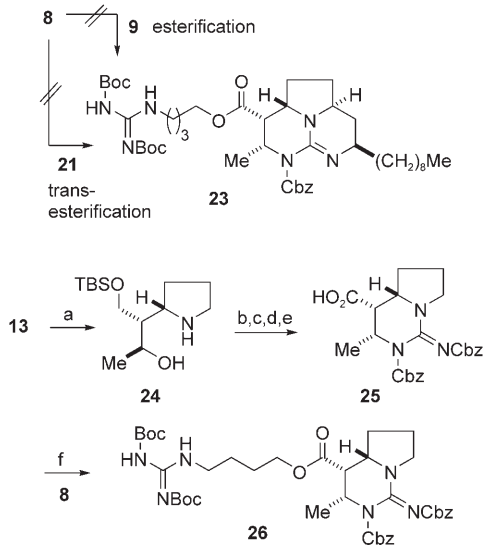
1,3-Dipolar cycloaddition of the nitrone **7** and methyl crotonate (**10**) in toluene gave the isoxazolidine **12** in 82% yield (Scheme 3). The ester group of **12** was reduced with $LiAlH_4$ and subsequent protection of the hydroxyl group as the *tert*-butyldimethylsilyl (TBS) ether gave **13** in 95% yield. The oxidation of the isoxazolidine **13** with *m*-chloroperoxybenzoic acid (*m*CPBA)[18] effected regioselective ring cleavage to give the nitrone **14**, which was subsequently sub-



Scheme 3. Synthesis of the tricyclic guanidine carboxylic acid **9**. Reagents and conditions: a) methyl crotonate (**10**), toluene, 100 °C, 82 %; b) 1) $LiAlH_4$, Et_2O, 0 °C; 2) TBSCl, imidazole, CH_2Cl_2, RT, 95 % (2 steps); c) mCPBA, CH_2Cl_2, 0 °C; d) 1-undecene (**11**), toluene, 110 °C, 76 % (2 steps); e) 1) Ac_2O, pyridine, RT; 2) Pd/C, H_2, EtOH, RT, 98 % (2 steps); f) bis-Cbz-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 0 °C to RT, 52 %; g) DEAD, PPh_3, THF, RT, 58 %; h) NaH, THF/MeOH (1:1), RT, 80 %; i) MsCl, Et_3N, CH_2Cl_2, 0 °C, 82 %; j) Jones reagent, acetone, 0 °C; k) $TMSCHN_2$, PhH/MeOH (7:2), RT, 47 % (2 steps); l) Pd/C, H_2, EtOH, RT, 85 %.

jected to a second 1,3-dipolar cycloaddition with 1-undecene (**11**) to give **15** in 76 % yield (2 steps). In this cycloaddition, 1-undecene (**11**) approached from the less-hindered side of **14** (β-face) in the *exo*-mode, and the newly generated stereochemistries of **15** at C8 and C11 were well controlled.[13] After protection of the hydroxyl group of **15** with acetic anhydride in pyridine, the N–O bond was reduced with hydrogen in the presence of 10 % Pd/C to give the *trans*-2,5-disubstituted-β-hydroxypyrrolidine **16** in 98 % yield. Treatment of **16** with bis-Cbz-2-methyl-2-thiopseudourea (Cbz = benzyloxycarbonyl) in the presence of mercury(II) chloride[19] generated the guanylated pyrrolidine **17** in 52 % yield. Treatment of **17** under the Mitsunobu reaction conditions[20] effected cyclization with inversion of the stereochemistry at C13 to give the bicyclic guanidine **18** in 58 % yield. Deprotection of one of the Cbz groups and the acetyl group of **18** took place simultaneously with sodium hydride in MeOH/THF (1:1)[21] to give **19** in 80 % yield. The tricyclic guanidine was formed on treatment of **19** with methanesulfonyl chloride in the presence of triethylamine to give **20** in 82 % yield. The TBS group of **20** was removed and oxidation of the resulting primary alcohol was performed with Jones reagent to give the carboxylic acid **9**.[22] The structure of **9** was confirmed by further conversion into the known ester **22**, reported by Overman,[23] through treatment of **9** with (trimethylsilyl)diazomethane and subsequent deprotection of the Cbz group with hydrogen over 10 % Pd/C.

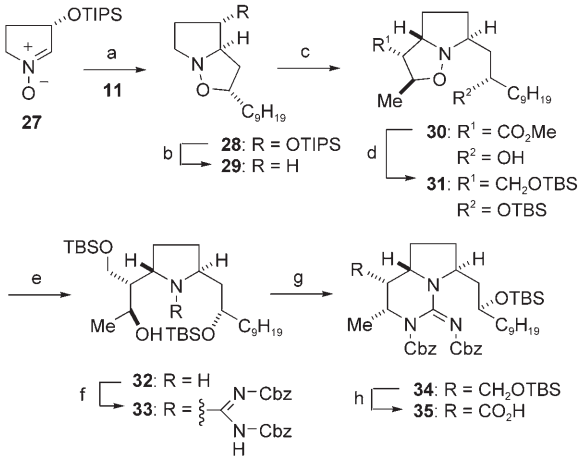
With the tricyclic guanidine carboxylic acid **9** in hand, esterification with the side chain alcohol **8** was examined (Scheme 4). Although various conditions, such as 1-ethyl-3-



Scheme 4. Esterification of **9**, **21**, and **25** with **8**. Reagents and conditions: a) $Pd(OH)_2$/C, H_2, EtOH, RT, 97 %; b) bis-Cbz-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 0 °C to RT, 60 %; c) DEAD, PPh_3, toluene, RT, 88 %; d) TBAF, THF, RT, 70 %; e) Jones reagent, acetone, 0 °C; f) **8**, EDCI, DMAP, CH_2Cl_2, RT, 40 % (2 steps).

(3-dimethylaminopropyl)carbodiimide (EDCI)/4-dimethylamino pyridine (DMAP), bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl)/triethylamine, or tosylchloride (TsCl)/pyridine, were examined, the ester **23** was not produced at all and the carboxylic acid **9** was decomposed. Thus, we next examined *trans*-esterification using the ester **21** and alcohol **8** in the presence of the Otera reagent,[24] but we failed to obtain the desired product **23**. These results might have been due to the low reactivity of the axially orientated carboxylic acid or ester group of **9** or **21** at C7, as Snider and Chen had suggested.[3b] To relax the rigidity of the axial orientation of the carboxylic acid of **9**, bicyclic guanidine carboxylic acid **25** was prepared from **13** (Scheme 4) and its esterification with **8** was examined. In this reaction, we found that the coupling reaction proceeded in the presence of EDCI and DMAP, giving the ester **26** in 40 % yield. Thus, we decided to introduce the side chain **8** prior to the tricyclic guanidine formation in the total synthesis of natural product.

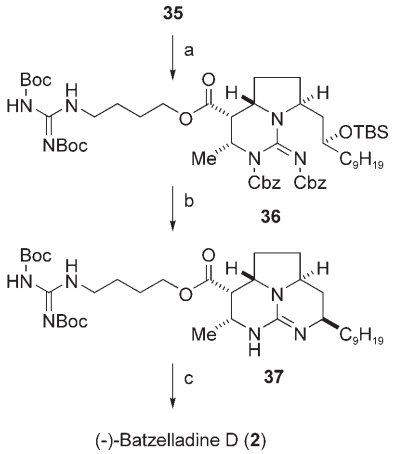
The optically active bicyclic guanidine carboxylic acid **35** was synthesized from the nitrone **27**[25] derived from malic acid, as shown in Scheme 5. In short, 1,3-dipolar cycloaddition reaction of the nitrone **27** and 1-undecene (**11**) gave the isoxazolidine **28**, and deoxygenation using the Barton–McCombie method[26] gave **29**. Treatment of **29** with mCPBA effected regioselective regeneration of the nitrone, and a second 1,3-dipolar reaction with methyl crotonate (**10**) gave the isoxazolidine **30**, the ester group of which was re-



Scheme 5. Synthesis of the bicyclic guanidine carboxylic acid **35**. Reagents and conditions: a) 1-undecene (**11**), toluene, 90°C, 75%; b) 1) CsF, EtOH, 90°C, 98%; 2) PhOC(S)Cl, DMAP, pyridine, RT,; 3) nBu$_3$SnH, AIBN, toluene, 100°C, 51% (2 steps); c) 1) mCPBA, CH_2Cl_2, 0°C; 2) methyl crotonate (**10**), toluene, 110°C, 75% (2 steps); d) 1) $LiAlH_4$, Et_2O, 0°C; 2) TBSCl, imidazole, CH_2Cl_2, RT, 88% (2 steps); e) Pd/C, H_2, EtOH, RT; f) bis-Cbz-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 0°C to RT, 84% (2 steps); g) DEAD, PPh_3, toluene, RT, 64%; h) 1) TBAF, THF, RT, 97%; 2) Jones reagent, acetone, 0°C.

duced with $LiAlH_4$. Subsequent protection of the two hydroxyl groups with TBSCl furnished **31**. Reduction of the N–O bond of **31** afforded the 2,5-disubstituted pyrrolidine **32**, which was treated with bis-Cbz-2-methyl-2-thiopseudourea to give **33**. The formation of the bicyclic guanidine was performed under the Mitsunobu reaction conditions and the bicyclic guanidine **34** was obtained. Selective deprotection of the primary silyl ether of **34** followed by Jones oxidation gave the bicyclic guanidine carboxylic acid **35**.

Then, esterification of the carboxylic acid **35** with the side chain alcohol **8** was conducted in the presence of EDCI and DMAP at 0°C, and the ester **36** was obtained in 66% yield from **34** (Scheme 6). After sequential deprotection of the

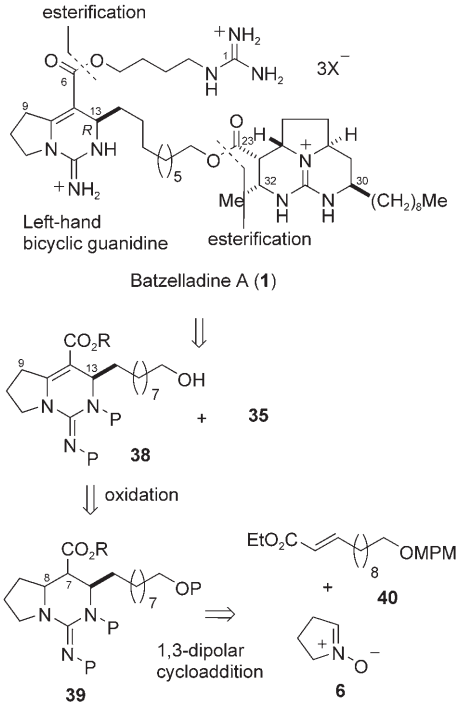


Scheme 6. Total synthesis of (–)-batzelladine D (**2**). Reagents and conditions: a) **8**, EDCI, DMAP, CH_2Cl_2, 0°C, 66% (from **34**); b) 1) HF/pyridine, THF, 0°C, 81%; 2) Pd/C, H_2, EtOH, RT; 3) DEAD, PPh_3, toluene, RT; c) TFA, CH_2Cl_2, RT, 66% (3 steps).

TBS and Cbz groups of **36** with HF/pyridine and hydrogen in the presence of 10% Pd/C, respectively, the tricyclic guanidine was formed under the Mitsunobu reaction conditions to give fully stereocontrolled **37**. Finally, deprotection of Boc groups was conducted with trifluoroacetic acid (TFA), and (–)-batzelladine D (**2**) was obtained in 66% yield. The spectral data (^{1}H NMR, ^{13}C NMR, and high-resolution mass spectra and optical rotation data) of the synthetic **2** were consistent with those of natural batzelladine D (**2**) reported by Patil et al.[1a]

Total synthesis of (+)-batzelladine A (1): Batzelladine A (**1**) has been reported to show the most potent biological activity among the batzelladine alkaloids.[1a] It has a characteristic left-hand bicyclic guanidine in addition to the right-hand tricyclic guanidine, a common structural features of these alkaloids. In the left-hand bicyclic guanidine, a chiral center is present at C13. In 2002, Duron and Gin synthetically determined this stereochemistry to be *R*.[10] We started our synthesis of (+)-**1** from this particular left-hand bicyclic guanidine.

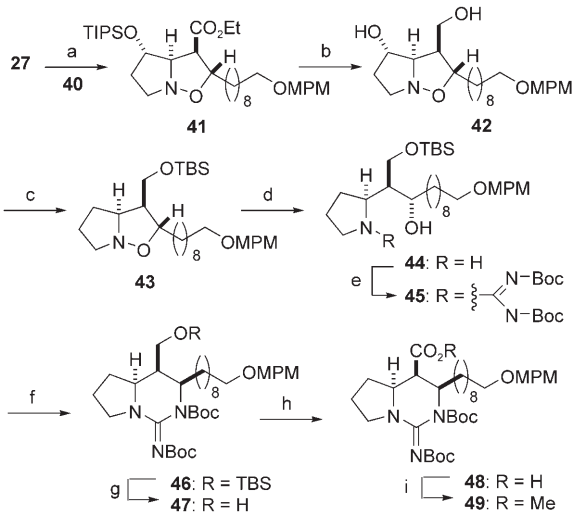
Application of the 1,3-dipolar cycloaddition reaction was planned for the synthesis of the bicyclic guanidine alcohol **38**, which corresponds to the left-hand bicyclic guanidine of **1** (Scheme 7). In this strategy, introduction of the double



Scheme 7. Retrosynthetic analysis of **1**.

bond at C7=C8 of **38** is addressed by means of oxidation of **39**.

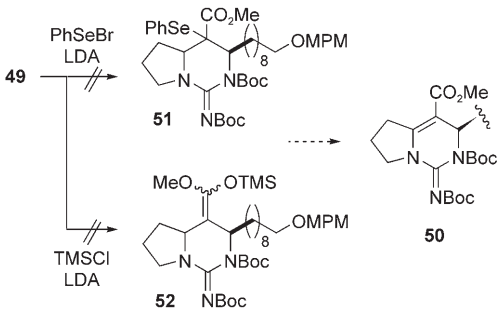
The synthesis of the bicyclic guanidine **49** is illustrated in Scheme 8; it involves reaction of the optically active nitrone



Scheme 8. Synthesis of the bicyclic guanidine **49**. Reagents and conditions: a) **40**, toluene, 90 °C; b) 1) $LiAlH_4$, Et_2O, 0 °C; 2) CsF, EtOH, 90 °C (59 % from **27**); c) 1) TBSCl, pyridine, RT; 2) PhOC(S)Cl, DMAP, pyridine, RT; 3) $n$$Bu_3SnH$, AIBN, toluene, 100 °C, 44 % (3 steps); d) $Pd(OH)_2$/C, H_2, EtOH, RT; e) bis-Boc-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, RT, 71 % (2 steps); f) DEAD, PPh_3, toluene, RT; g) TBAF, THF, RT, 81 % (from **45**); h) 1) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to RT; 2) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, tBuOH/H_2O, RT; i) $TMSCHN_2$, benzene/MeOH, RT, 71 % (from **47**).

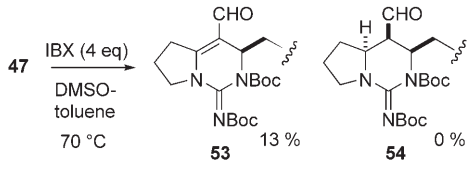
27 and olefin **40** through 1,3-dipolar cycloaddition (**27** into **41**), guanylation with bis-Boc-2-methyl-2-thiopseudourea (**44** into **45**; Boc = *tert*-butoxycarbonyl), and bicyclic guanidine construction under the Mitsunobu conditions (**45** into **46**).

Then, conversion of **49** into the α,β-unsaturated carbonyl compound **50** was investigated (Scheme 9). We firstly examined the oxidation of **49** by Sharpless′ method utilizing organoselenium reagents[27] or the Saegusa–Tsuji method,[28] but neither the reaction with phenylselenyl bromide in the presence of lithium diisopropylamide (LDA) nor the ketenesilyl acetal formation proceeded. Thus, we tried direct formation of the α,β-unsaturated aldehyde **53** from the primary alcohol **47** with *o*-iodoxybenzoic acid (IBX), as developed by Nicolaou et al.,[29] and obtained **53** in 13 % yield. Several oxidation reagents for this conversion were further investigated, and the results are summarized in Table 1. Among them, tetra-*n*-propylammoniumperruthenate (TPAP)[30] gave **53** in



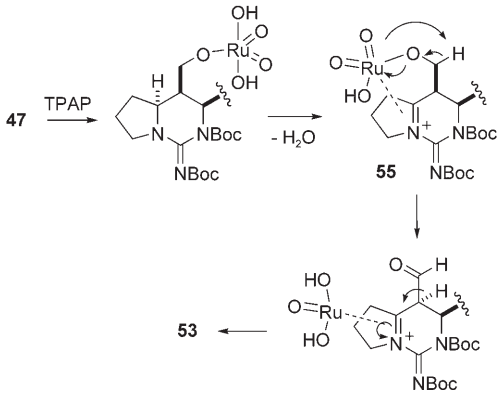
Scheme 9. Synthetic approaches of **50** from **49**.

Table 1. Investigation of oxidation of **47** to **53**.



Reagents	Solvent	T [°C]	**53** [%]	**54** [%]
TPAP(5 mol %)–NMO (4 equiv)	CH_2Cl_2	RT	47	0
PCC (4 equiv)	CH_2Cl_2	RT	6	0
PDC (4 equiv)	CH_2Cl_2	RT	36	0
Swern oxidation	CH_2Cl_2	−78	0	94

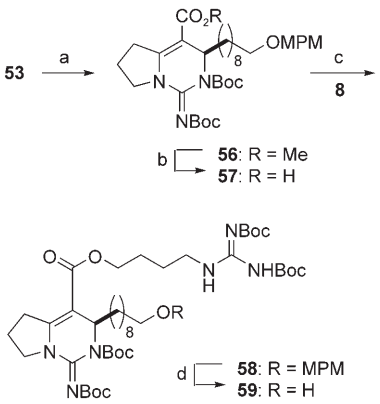
47 % yield with complete regioselectivity. Interestingly, oxidation of the aldehyde **54**, obtained through Swern oxidation, with TPAP did not proceed. This TPAP direct oxidation of **47** presumably occurs via the iminium–ruthenium–alkoxide complex **55** as an intermediate, as proposed by Murahashi et al.[31] (Scheme 10).



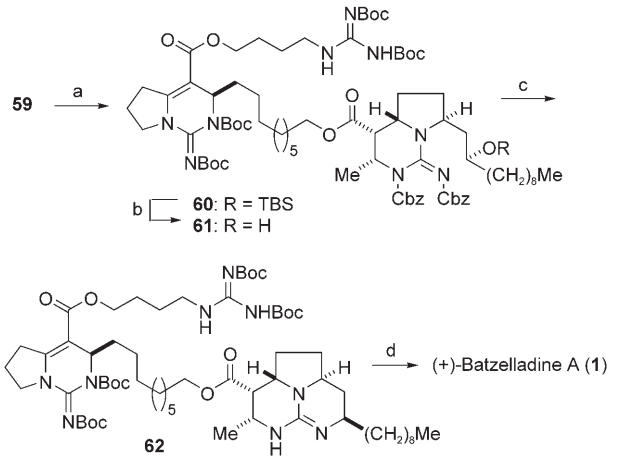
Scheme 10. Proposed mechanism of TPAP oxidation of **47**.

The resulting aldehyde **53** was further oxidized with sodium chlorite[32] to the carboxylic acid, and then treatment with (trimethylsilyl)diazomethane gave the methyl ester **56** in 86 % yield. Demethylation of the methyl ester **56** with *n*PrSLi and subsequent condensation with the guanidine alcohol **8** provided the ester in 54 % yield from **58**. Finally, the *p*-methoxybenzyl (MPM) group was deprotected with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give the alcohol **59** in 66 % yield (Scheme 11).

Total synthesis of batzelladine A (**1**) was achieved with the alcohol **59** and bicyclic guanidine carboxylic acid **35** (Scheme 12). The reaction of the carboxylic acid **35** and bicyclic guanidine alcohol **59** in the presence of EDCI and DMAP at 0 °C gave the ester **60** in 60 % yield. The TBS group of **60** was deprotected with HF/pyridine to give **61** in 89 % yield. After deprotection of the Cbz group with hydrogen in the presence of 10 % Pd/C, cyclization was performed under the Mitsunobu conditions to give tricyclic guanidine **62**. Finally, deprotection of the four Boc groups with TFA and subsequent purification with HPLC (PEGASIL-ODS,



Scheme 11. Synthesis of bisguanidine alcohol **59**. Reagents and conditions: a) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*BuOH/H_2O, RT; $TMSCHN_2$, RT, 65% (23% SM recovery); b) *n*PrSLi, HMPA, RT, c) **8**, BOPCl, Et_3N, CH_2Cl_2, 54% (from **56**); d) DDQ, CH_2Cl_2/H_2O, RT, 66%.



Scheme 12. Total synthesis of (+)-batzelladine A (**1**). Reagents and conditions: a) **35**, EDCI, DMAP, CH_2Cl_2, 0°C, 60%; b) HF/pyridine, THF, 0°C, 89%; c) 1) Pd/C, H_2, EtOAc, RT; 2) DEAD, PPh_3, toluene, RT; d) TFA/CH_2Cl_2, RT, 24% (from **61**).

eluted with 50% MeCN/H_2O 0.1% TFA) gave (+)-batzelladine A (**1**) in 24% yield from **61**.[17] All of the data for synthetic (+)-batzelladine A (**1**) were in good agreement with reported values.[1a] Thus, the structure of **1**, including the absolute stereochemistry, was confirmed.

Evaluation of the target protein of batzelladine A (1) and D (2): Batzelladines inhibit the binding of the HIV envelope glycoprotein gp120 and cellular CD4 receptor, which is the initial step of HIV infection into T-cells. To establish the mechanism of this interesting action of small molecules, identification of the target protein is fundamental. Recently, Bewley et al. conducted computer-assisted docking studies of gp120 with batzelladine F-type polycyclic guanidine compounds,[33] and suggested that the target protein of batzelladines is gp120. Intrigued with these results, we decided to test their hypothesis using synthetic batzelladines A (**1**) and D (**2**) and an affinity gel bearing gp120 and CD4.

The proteins gp120 and CD4, and ethanolamine (as a control) were treated with agarose gel activated with hydroxysuccinimide to obtain affinity gels bearing gp120, CD4, and ethanolamine, respectively. These affinity gels were each mixed with various concentrations of synthetic batzelladine D (**2**) from 0.1 to 0.5 $\mu g\,mL^{-1}$. After one hour at 4°C, the mixture was centrifuged, and the amount of free batzelladine D (**2**) in the supernatant was quantified by HPLC. With the gp120- or ethanolamine-immobilized affinity gel, **2** was recovered almost quantitatively at every concentration tested. In contrast, the amount of free **2** in the supernatant decreased when the CD4 affinity gel was used. These results indicate that the target protein of **2** is CD4 rather than gp120; this result is inconsistent with the result of the computer-assisted docking studies performed by Bewley et al. Using the above-mentioned affinity gel method, we conducted a Scatchard plot analysis of the binding of CD4 with synthetic batzelladines A (**1**) and D (**2**). As shown in Figure 1,

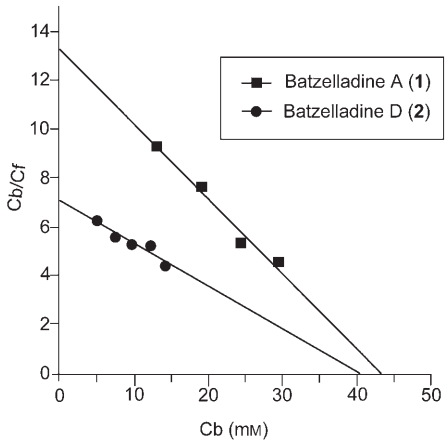


Figure 1. Scatchard plot analysis of batzelladine A (**1**) and batzelladine D (**2**) with CD4 affinity gel. (Cb: Concentration of CD4 bounded batzelladines of **1** and **2**. Cf: Concentration of free batzelladines of **1** and **2**.).

the plots showed good linearity ($r=0.97$ (**1**) and 0.91 (**2**)), suggesting that CD4 protein has a specific binding site for **1** and **2**. The association constants (K_a values) of **1** and **2** were calculated from the slope of the lines to be $3.0\times10^5\,M^{-1}$ and $1.8\times10^5\,M^{-1}$, respectively. This order of the values is consistent with the order of inhibitory activities of **1** and **2** against gp120–CD4 binding (IC_{50} values of 26 μM and 72 μM, respectively).[1a] Moreover, the Scatchard plot analysis suggested that the binding of **1** and **2** is specific and that their binding sites on CD4 are the same, since the Scatchard plot lines of **1** and **2** crossed the *x* axis at almost the same positions. These results indicate that the target protein of batzelladines A (**1**) and D (**2**) is CD4.

Conclusion

In summary, asymmetric total synthesis of the guanidine alkaloids (+)-batzelladine A (**1**) and (−)-batzelladine D (**2**) has been accomplished, by using a successive 1,3-dipolar cy-

cloaddition reaction protocol for the stereoselective construction of the tricyclic guanidine skeleton. A novel oxidation reaction from the primary alcohol **47** to α,β-unsaturated aldehyde **53** under TPAP–NMO (NMO = 4-methylmorpholine *N*-oxide) conditions was also developed and applied to the synthesis of the left-hand bicyclic guanidine of **1**. This also established the absolute stereochemistry. Furthermore, the target protein of **1** and **2** was examined using immobilized CD4 and/or gp120 affinity gel. The results indicated that batzelladines A (**1**) and D (**2**) bind to CD4, in contrast to the results of computer-assisted docking studies obtained using batzelladine F type polycyclic guanidine compounds. Further experiments to identify the binding site of batzelladines on CD4 are planned.

Experimental Section

General: Flash chromatography was performed on Silica gel 60 (spherical, particle size 0.040–0.100 mm; Kanto). Optical rotations were measured on a JASCO DIP polarimeter 370, using the sodium D line. IR spectra were measured with a JASCO VALOR-III FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on JEOL JNM-ECP500 instrument. Mass spectra were recorded on JEOL JMS-HX110 spectrometer with *m*-nitrobenzyl alcohol.

Isoxazolidine 28: A mixture of nitrone **27** (9.0 g, 35.0 mmol) and **11** (27.0 g, 175.0 mmol) in toluene (200 mL) was heated at 90 °C for 9 h. After cooling, the reaction mixture was concentrated in vacuo and purified by silica gel column chromatography (hexane/EtOAc; 16: 1 to 8:1) to give isoxazolidine **28** (10.8 g, 75 %) as a clear oil. $[\alpha]_D^{22} = 3.6$ ($c = 0.9$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.12$ (ddd, $J = 5.6$, 3.0, 3.0 Hz, 1H), 3.90 (m, 1H), 3.60 (ddd, $J = 8.1$, 3.0, 3.0 Hz, 1H), 3.37 (ddd, $J = 12.4$, 8.1, 6.4 Hz, 1H), 3.18 (ddd, $J = 12.4$, 6.4, 4.7 Hz, 1H), 2.15–2.02 (m, 3H), 1.72 (m, 1H), 1.62 (m, 1H), 1.43–1.20 (m, 15H), 1.13–0.95 (m, 21H), 0.87 ppm (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 78.7$, 76.6, 73.9, 55.2, 40.1, 34.2, 33.7, 31.5, 29.2, 29.14, 29.1, 28.9, 26.0, 22.3, 17.5, 13.7, 11.6 ppm; IR (neat): $\tilde{\nu} = 2927$, 2865, 1465, 1381, 1368 cm^{-1}; HRMS (FAB): calcd for $C_{24}H_{50}NO_2Si$ [M^++H]: 412.3611; found: 412.3622.

Isoxazolidine 29: A mixture of isoxazolidine **28** (10.7 g, 10.0 mmol) and CsF (11.9 g) in EtOH (20 mL) was refluxed at 90 °C for 9 h. After cooling, the solution was poured into H_2O and extracted with ethyl acetate. The solution was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH; 9:1) to give an alcohol (6.71 g, q.y.) as a clear oil. $[\alpha]_D^{22} = -18.7$ ($c = 0.69$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.08$ (m, 1H), 3.91 (m, 1H), 3.56 (m, 1H), 3.39 (m, 1H), 3.12 (m, 1H), 2.15–2.00 (m, 3H), 1.72 (m, 1H), 1.60 (m, 1H), 1.47–1.20 (m, 15H), 0.86 ppm (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 76.7$, 72.7, 55.0, 40.0, 33.3, 33.26, 31.5, 29.3, 29.2, 29.17, 29.0, 26.0, 22.3, 13.7 ppm; IR (neat): $\tilde{\nu} = 3362$, 2926, 2855, 1466 cm^{-1}; HRMS (FAB): calcd for $C_{15}H_{30}NO_2$ [M^++H]: 256.2277; found: 256.2288. Phenyl chlorothionoformate (5.8 mL) was added to a solution of the alcohol (5.40 g, 21.1 mmol) and DMAP (180 mg) in pyridine (100 mL) at room temperature. After stirring for 5 h, the reaction mixture was concentrated in vacuo and diluted with ethyl acetate. The solution was washed with H_2O twice, and the organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane-EtOAc; 12:1) to give xanthate as a yellow oil. To a solution of xanthate in toluene (100 mL), nBu$_3$SnH (17 mL) and azobisisobutyronitrile (AIBN; 345 mg) was added and refluxed at 110 °C for 20 min. The solution was concentrated in vacuo and the residue was purified by silica gel column chromatography (hexane/EtOAc 6:1 to 1:2) to give isoxazolidine **29** (2.56 g, 51 % overall). $[\alpha]_D^{23} = -34.1$ ($c = 1.19$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.01$ (m, 1H), 3.73 (m, 1H), 3.13 (m, 2H), 2.08–1.82 (m, 4H), 1.72–1.20 (m, 18H), 0.88 ppm (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 76.4$, 64.8, 57.0, 42.5, 33.9, 31.8, 31.7, 29.62, 29.57, 29.5, 29.2, 26.4, 24.3, 22.6, 14.0 ppm; IR (neat): $\tilde{\nu} = 2926$, 2855, 1465 cm^{-1}; HRMS (FAB): calcd for $C_{15}H_{30}NO$ [M^++H]: 240.2327; found: 240.2383.

Ester 30: *m*CPBA (1.81 g, 7.6 mmol) was added to a solution of isoxazolidine **29** (1.52 g, 6.35 mmol) in CH_2Cl_2 (30 mL) at 0 °C. After stirring for 5 min, a large excess of $Ca(OH)_2$ was added, the resulting mixture was filtered through a pad of Celite, and the filtrates were concentrated in vacuo to give nitrone as a clear oil. The mixture of the nitrone and **10** (3.4 mL, 32 mmol) in toluene (60 mL) was stirred at 100 °C for 1 h. After cooling, the reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (hexane/EtOAc; 2:1) to give methyl ester **30** (1.68 g, 75 % overall) as a clear oil. $[\alpha]_D^{23} = -86.2$ ($c = 1.01$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 4.30$ (m, 1H), 4.08 (q, $J = 6.8$ Hz, 1H), 3.92 (m, 1H), 3.70 (s, 3H), 3.37 (m, 1H), 3.04 (t, $J = 9.8$ Hz, 1H), 1.96–1.84 (m, 2H), 1.74 (ddd, $J = 4.6$, 10.1, 10.1 Hz, 1H), 1.59–1.24 (m, 19H), 1.32 (d, $J = 6.8$ Hz, 3H), 0.86 ppm (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 170.3$, 72.1, 69.1, 65.43, 65.35, 57.3, 51.8, 39.2, 37.6, 31.9, 29.7, 29.6, 29.5, 29.3, 28.9, 27.3, 25.7, 22.6, 16.6, 14.1 ppm; IR (neat): $\tilde{\nu} = 3361$, 2925, 2853, 1736, 1437 cm^{-1}; HRMS (FAB): calcd for $C_{20}H_{38}NO_4$ [M^++H]: 355.2801; found: 355.2840.

Silyl ether 31: A solution of methyl ester **30** (2.00 g, 5.63 mmol) in Et_2O (30 mL) was added slowly to a suspension of $LiAlH_4$ (320 mg, 8.43 mmol) in Et_2O (40 mL) at 0 °C. After stirring for 2 h at this temperature, the reaction was quenched by sequential addition of H_2O (500 μL), 2.0 M NaOH aq. (500 μL), and H_2O (1 mL). $MgSO_4$ was added, the resulting mixture was stirred for 20 min and filtered through a pad of Celite, and the filtrates were concentrated in vacuo to give an alcohol (2.26 g) which was used without further purification. TBSCl (3.12 g) was added to a solution of the alcohol and imidazole (2.82 g) in CH_2Cl_2 (70 mL) and the mixture was stirred at room temperature for 20 min. The reaction was quenched by the addition of H_2O and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc; 15:1) to give bis-TBS ether **31** (3.24 g, 85 %) as a colorless oil. $[\alpha]_D^{24} = -57.2$ ($c = 1.40$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 3.91$ (ddd, $J = 12.4$, 5.1, 4.7 Hz, 1H), 3.83–3.63 (m, 4H), 3.13 (ddd, $J = 16.2$, 6.0, 6.0 Hz, 1H), 2.33 (m, 1H), 1.95 (m, 1H), 1.76–1.61 (m, 4H), 1.53 (ddd, $J = 13.7$, 7.3, 6.4 Hz, 1H), 1.46–1.20 (m, 19H), 0.883 (s, 9H), 0.88 (s, 9H), 0.86 (t, $J = 6.8$ Hz, 3H), 0.06 (s, 6H), 0.05 ppm (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 73.0$, 70.6, 66.8, 65.2, 60.5, 54.3, 43.0, 38.1, 31.9, 31.1, 29.9, 29.6, 29.5, 29.3, 26.0, 25.8, 25.5, 24.7, 22.7, 18.1, 17.7, 14.1, −4.3, −4.4, −5.5, −5.6 ppm; IR (neat): $\tilde{\nu} = 2955$, 2928, 2856, 1471, 1463, 1387, 1361, 1254 cm^{-1}; HRMS (FAB): calcd for $C_{31}H_{66}NO_3Si_2$ [M^++H]: 556.4581; found: 556.4576.

Guanidine **33**: Pd/C (400 mg) was added to a solution of bis-TBS ether **31** (992 mg, 1.78 mmol) in EtOH (6.0 mL), and the reaction mixture was stirred at room temperature under a hydrogen atmosphere (balloon). After 20 h, the reaction mixture was filtered through a pad of Celite, and the filtrates were concentrated in vacuo to give pyrrolidine **32**. $HgCl_2$ (570 mg, 2.1 mmol) was added to a solution of **32** (978 mg, 1.75 mmol), 1,3-bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea (753 mg, 2.1 mmol), and triethylamine (850 μL, 6.1 mmol) in DMF (18 mL), and the resulting mixture was stirred for 30 min. The reaction mixture was diluted with EtOAc, and filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane/EtOAc; 8:1) to give the bis-Cbz-protected guanidine **33** (902 mg, 58 % overall).

Guanidine 34: Diethylazodicarboxylate (DEAD; 995 μL, 6.32 mmol) was added to a solution of guanidine **33** (3.66 g, 4.21 mmol) and PPh_3 (1.66 g, 6.32 mmol) in toluene (40 mL). After stirring for 5 min, the reaction was quenched with H_2O (10 μL) and concentrated in vacuo. Purification of the residue by flash chromatography (hexane/EtOAc; 9:1) gave bicyclic guanidine **34** (2.31 g, 65 %) as a clear oil. $[\alpha]_D^{24} = -160.0$ ($c = 1.68$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 7.36$–7.20 (m, 10H), 5.17 (d, $J = 12.4$ Hz, 1H), 4.97 (d, $J = 12.4$ Hz, 1H), 4.92 (d, $J = 12.4$ Hz, 1H), 4.90 (d, $J = 12.4$ Hz, 1H), 4.63 (m, 1H), 4.05 (m, 1H), 3.82 (m, 1H), 3.65 (m, 3H), 2.65–2.52 (m, 2H), 2.17 (m, 1H), 1.97 (m, 1H), 1.73–1.53 (m, 2H),

1.52–1.15 (m, 17H), 1.18 (d, $J=7.3$ Hz, 3H), 0.87 (t, $J=0.9$ Hz, 3H), 0.87 (s, 18H), 0.04 (s, 6H), 0.04 ppm (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=160.3$, 153.1, 150.5, 137.3, 135.6, 128.3, 128.2, 128.0, 128.0, 127.3, 70.6, 68.2, 66.7, 60.7, 57.7, 56.3, 49.8, 43.0, 41.8, 37.0, 31.9, 29.8, 29.6, 29.3, 28.4, 25.9, 25.7, 24.4, 22.6, 18.0, 14.4, 14.0, −4.4, −4.43, −5.6 ppm; IR (neat): $\tilde{\nu}=2953$, 2928, 2856, 1727, 1677, 1581, 1470, 1387, 1287, 1244 cm^{-1}; HRMS (FAB): calcd for $C_{48}H_{80}N_3O_6Si_2$ [M^++H]: 850.5586; found: 850.5580.

Ester 36: TBAF hydrate (44 mg) was added to a solution of bicyclic guanidine **34** (44 mg, 0.052 mmol) in THF (1.0 mL) at 0 °C and stirred at the temperature for 100 min. The reaction mixture was poured into saturated $NaHCO_3$ aq. and extracted with ethyl acetate. The combined organic layers were washed with saturated NH_4Cl aq., H_2O, and brine. The solution was concentrated in vacuo and purified by silica gel column chromatography (hexane/EtOAc; 2:1) to give mono-TBS ether protected alcohol (37.7 mg, 0.051 mmol, 99%) as a clear oil. $[\alpha]_D^{29}=-186.0$ ($c=1.16$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.36$–7.20 (m, 10H), 5.19 (d, $J=12.2$ Hz, 1H), 4.97 (d, $J=12.5$ Hz, 1H), 4.92 (d, $J=12.5$ Hz, 1H), 4.87 (d, $J=12.2$ Hz, 1H), 4.64 (m, 1H), 4.06 (m, 1H), 3.84–3.67 (m, 4H), 2.62 (m, 1H), 2.55 (ddd, $J=12.8$, 7.3, 3.1 Hz, 1H), 2.19 (m, 1H), 2.05 (m, 1H), 1.73–1.19 (m, 19H), 1.21 (d, $J=7.3$ Hz, 3H), 0.88 (t, $J=7.0$ Hz, 3H), 0.87 (s, 9H), 0.04 (s, 3H), 0.04 ppm (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=160.4$, 153.2, 150.6, 137.2, 135.6, 128.4, 128.2, 128.11, 128.1, 128.0, 127.4, 70.6, 68.3, 66.9, 60.3, 57.8, 56.3, 50.0, 42.8, 41.8, 37.0, 31.9, 29.9, 29.7, 29.6, 29.3, 28.5, 25.9, 24.5, 22.7, 18.0, 14.5, 14.1, −4.3, −4.4 ppm; IR (neat): $\tilde{\nu}=3430$, 2953, 2927, 2855, 1725, 1574, 1471 cm^{-1}; HRMS (FAB): calcd for $C_{42}H_{66}N_3O_6Si$ [M^++H]: 735.4721; found: 735.4724. Jones reagent (10 drops) was added to a solution of the alcohol (80.3 mg, 0.109 mmol) in acetone (2.0 mL) and stirred at 0 °C for 30 min. The mixture was added excess of 2-propanol and poured into ethyl acetate. The solution was washed with brine and the aqueous layer was re-extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the carboxylic acid **35**. EDCI (62.7 mg) and DMAP (6.7 mg) were added to a mixture of the carboxylic acid **35** and alcohol **8** (72.2 mg, 0.218 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C. After stirring at the temperature for 3 h, the reaction mixture was poured into H_2O and extracted with diethyl ether. The extracts were washed with saturated $NaHCO_3$ aq., brine, and H_2O and dried over $MgSO_4$. The solution was evaporated and the residue was purified by silica gel column chromatography (CH_2Cl_2/hexane/EtOAc; 16:8:3) to give ester **36** (78.5 mg, 0.074 mmol, 68%) as a clear oil. $[\alpha]_D^{25}=-137.3$ ($c=0.88$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=11.49$ (br, 1H), 8.31 (br, 1H), 7.25–7.12 (m, 10H), 5.15 (d, $J=12.4$ Hz, 1H), 4.97 (d, $J=12.4$ Hz, 1H), 4.90 (d, $J=12.7$ Hz, 1H), 4.87 (d, $J=12.7$ Hz, 1H), 4.49 (m, 1H), 4.09 (m, 3H), 3.86 (m, 2H), 3.41 (d, $J=12.4$, 6.8 Hz, 2H), 3.22 (dd, $J=7.7$, 4.7 Hz, 1H), 2.27 (m, 2H), 1.90–1.19 (m, 24H), 1.50 (s, 9H), 1.49 (s, 9H), 1.37 (d, $J=6.7$ Hz, 3H), 0.88 (t, $J=7.0$ Hz, 3H), 0.87 (s, 9H), 0.04 (s, 3H), 0.04 ppm (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=169.8$, 163.6, 160.0, 156.1, 153.3, 152.9, 150.0, 137.2, 135.6, 128.3–128.0 (12 carbons), 127.4, 83.1, 79.2, 71.1, 68.1, 66.8, 64.8, 58.5, 56.2, 52.2, 50.1, 40.7, 40.5, 40.4, 40.2, 37.4, 31.9, 29.9, 29.6, 29.3, 28.8, 28.3, 28.02, 27.98, 27.5, 25.9, 25.8, 25.6, 24.4, 22.7, 18.0, 16.4, 14.1, −4.32, −4.40 ppm; IR (neat): $\tilde{\nu}=3332$, 2929, 2856, 1724, 1614, 1455, 1416 cm^{-1}; HRMS (FAB): calcd for $C_{57}H_{91}N_6O_{11}Si$ [M^++H]: 1063.6515; found: 1063.6475.

(−)-Batzelladine D (2): HF/pyridine (200 μL) was added to a solution of **36** (78.5 mg 0.074 mmol) in THF (2.0 mL) in a polypropylene tube at 0 °C. After stirring for 4 h, saturated $NaHCO_3$ aq. (500 μL) and solid $NaHCO_3$ (100 mg) was added. The mixture was dried over $MgSO_4$, filtered through a pad of Celite, and washed with ethyl acetate. The filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane/EtOAc; 2:1) to give an alcohol (57.0 mg, 0.060 mmol, 81%) as a clear oil. $[\alpha]_D^{25}=-147.0$ ($c=1.17$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=11.47$ (br, 1H), 8.30 (br, 1H), 7.30–7.17 (m, 10H), 6.22 (d, $J=3.8$ Hz, 1H), 5.16 (d, $J=12.4$ Hz, 1H), 5.01 (d, $J=12.8$ Hz, 1H), 4.84 (d, $J=12.4$ Hz, 1H), 4.78 (d, $J=12.4$ Hz, 1H), 4.58 (m, 1H), 4.40 (m, 1H), 4.12 (dt, $J=11.1$, 5.1 Hz, 2H), 3.95 (m, 1H), 3.52 (br, 1H), 3.39 (dt, $J=6.6$, 6.0 Hz, 2H), 3.21 (dd, $J=7.7$, 3.8 Hz, 1H), 2.33 (m, 1H), 1.97 (m, 1H), 1.86 (m, 1H), 1.76–1.10 (m, 23H), 1.49 (s, 18H), 1.47 (d, $J=6.4$ Hz, 3H), 0.88 ppm (t, $J=6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=169.8$, 163.5, 159.3, 156.1, 153.6, 153.3, 152.6, 136.9, 135.1, 128.4, 128.1 ,127.9, 127.6, 127.4, 83.1, 79.2, 68.2, 66.4, 65.0, 56.9, 56.4, 53.1, 50.7, 44.4, 40.1, 36.9, 31.9, 29.9, 29.7, 29.6, 29.3, 28.7, 28.3, 28.0, 27.0, 26.3, 25.7, 25.6, 22.7, 16.5, 14.1 ppm; IR (neat): $\tilde{\nu}=3333$, 2927, 2855, 1728, 1690, 1639, 1616, 1576, 1497, 1456, 1416, 1367, 1328 cm^{-1}; HRMS (FAB): calcd for $C_{51}H_{77}N_6O_{11}$ [M^++H]: 949.5650; found: 949.5657. A catalytic amount of Pd/C was added to a solution of the alcohol (57.0 mg, 0.060 mmol) in EtOH (1.0 mL). The reaction mixture was stirred at room temperature under a hydrogen atmosphere (balloon) for 3 h, and the reaction mixture was filtered though a pad of Celite. The filtrates were concentrated in vacuo to give guanidine alcohol. DEAD (132 μL, 40% in toluene) was added to a solution of guanidine alcohol and PPh_3 (76.0 mg) in toluene (100 μL). After stirring for 6 h, the reaction was quenched with one drop of H_2O and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH; 9:1) to give tricyclic guanidine **37**. The tricyclic guanidine **37** was dissolved in 50% TFA in CH_2Cl_2 (500 μL). After 30 min, the solution was concentrated under reduced pressure and the residue was purified by HPLC (PEGASIL-ODS 45% MeCN aq. 0.1% TFA) to give (−)-batzelladine D (**2**; 27.4 mg, 0.040 mmol, 66% overall) as a viscous oil. $[\alpha]_D^{23}=-19.4$ ($c=1.04$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta=4.17$ (t, $J=6.4$ Hz, 2H), 3.95 (m, 1H), 3.85 (m, 1H), 3.53 (m, 2H), 3.21 (t, $J=7.0$ Hz, 2H), 3.13 (dd, $J=4.0$, 3.7 Hz, 1H), 2.34 (m, 1H), 2.22 (m, 2H), 1.76–1.50 (m, 7H), 1.50–1.20 (m, 19H), 0.89 ppm (t, $J=6.7$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): $\delta=170.6$, 158.7, 151.5, 65.4, 57.8, 57.3, 53.2, 49.9, 42.0, 36.9, 34.2, 33.0, 31.4, 30.7, 30.6, 30.4, 29.3, 26.9, 26.6, 26.2, 23.7, 18.4, 14.4 ppm; IR (neat): $\tilde{\nu}=3353$, 3203, 2928, 2857, 1730, 1651, 1644, 1326, 1205 cm^{-1}; HRMS (FAB): calcd for $C_{25}H_{47}N_6O_2$ [M^++H]: 463.3761; found: 463.3800.

Diol 42: A mixture of nitrone **27** (15.2 g, 59.0 mmol) and α,β-unsaturated ester **40** (25.9 g, 68.9 mmol) in toluene (100 mL) was heated at 90 °C for 19 h. After cooling, the reaction mixture was concentrated in vacuo to give isoxazolidine (42.0 g). A solution of the crude isoxazolidine (16.6 g, 40% of the above product) in Et_2O (30 mL) was added slowly to a suspension of $LiAlH_4$ (1.53 g, 40.3 mmol) in Et_2O (220 mL) at 0 °C. After stirring for 3 h, the reaction was quenched by sequential addition of H_2O (500 μL), 2.0 M NaOH aq. (500 μL), and H_2O (1 mL). $MgSO_4$ was added, the resulting mixture was stirred for 20 min and filtered through a pad of Celite, and the filtrates were concentrated in vacuo to afford a residue (16.4 g), which was used without further purification. A solution of the crude alcohol and CsF (11.4 g, 75.0 mmol) in EtOH (90.0 mL) was refluxed at 90 °C for 13 h. The reaction was concentrated and the residue was filtered through a Florisil column. The filtrates were concentrated and purified on silica gel ($CHCl_3$/MeOH; 20:1) to give diol **42** (6.63 g, 59% overall) as a clear viscous oil. $[\alpha]_D^{23}=-65.3$ ($c=0.63$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.25$ (d, $J=8.6$ Hz, 2H), 6.87 (d, $J=8.6$ Hz, 2H), 4.44–4.38 (m, 1H), 4.42 (s, 2H), 3.85 (d, $J=8.1$ Hz, 2H), 3.80 (s, 3H), 3.58–3.51 (m, 2H), 3.48 (ddd, $J=12.4$, 7.7, 4.3 Hz, 1H), 3.42 (t, $J=6.6$ Hz, 2H), 3.09 (ddd, $J=12.4$, 9.0, 6.8 Hz, 1H), 2.73 (m, 1H), 2.27 (m, 1H), 1.78 (m, 1H), 1.62–1.45 (m, 4H), 1.45–1.20 ppm (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=158.8$, 130.3, 129.0, 113.4, 77.8, 74.7, 72.2, 70.5, 69.9, 59.8, 54.93, 54.9, 52.4, 33.1, 29.4, 29.35, 29.2, 29.15, 26.0, 25.9 ppm; IR (neat): $\tilde{\nu}=3392$, 2929, 2854, 1613, 1586, 1513, 1464, 1361, 1302, 1248 cm^{-1}; HRMS (FAB): calcd for $C_{24}H_{40}NO_5$ [M^++H]: 422.2906; found: 422.2928.

Isoxazolidine 43: TBSCl (361 mg, 2.40 mmol) was added to the solution of diol **42** (774 mg, 1.84 mmol) in pyridine (20 mL) at room temperature. After 19 h, the mixture was concentrated and purified on silica gel ($CHCl_3$/MeOH; 30:1) to give the mono-TBS ether (799.4 mg, 81%) as a colorless oil. $[\alpha]_D^{22}=-16.4$ ($c=1.18$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.25$ (d, $J=8.6$ Hz, 2H), 6.86 (d, $J=8.6$ Hz, 2H), 4.72 (br, 1H), 4.67 (d, $J=9.4$ Hz, 1H), 4.41 (s, 2H), 4.30 (dt, $J=8.1$, 4.3 Hz, 1H), 4.07 (m, 1H), 3.93 (dd, $J=11.1$, 4.7 Hz, 1H), 3.83 (dd, $J=11.1$, 4.7 Hz, 1H), 3.79 (s, 3H), 3.55–3.48 (m, 1H), 3.42 (t, $J=6.8$ Hz, 2H), 2.73 (m, 1H), 2.28 (m, 2H), 1.69 (m, 2H), 1.57 (tt, $J=6.8$, 6.8 Hz, 2H), 1.48–1.16 (m, 12H), 0.87 (s, 9H), 0.09 (s, 3H), 0.07 ppm (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=158.9$, 130.6, 129.0, 113.5, 85.9, 77.9, 77.2, 72.3, 70.0, 58.8, 55.3, 55.1, 49.6, 32.9, 32.6, 29.6, 29.2, 29.1, 29.0, 26.0, 17.9, −5.7, −5.8 ppm; IR (neat): $\tilde{\nu}=3288$, 2929, 2856, 2416, 2349, 2285, 1613, 1586,

1513, 1464, 1361, 1302, 1249 cm^{-1}; HRMS (FAB): calcd for $C_{48}H_{81}N_2O_{10}Si$ [M^++H]: 536.3771; found: 536.3767. Phenyl chlorothionoformate (3.3 mL) was added to a solution of the mono-TBS alcohol (6.35 g, 11.9 mmol) and DMAP (145 mg) in pyridine (120 mL) at 0 °C. After stirring for 20 h at room temperature, the reaction mixture was diluted with Et_2O and washed with H_2O and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexane/EtOAc; 10:1) to give a xanthate (4.64 g, 58%) as a yellow oil. The xanthate (4.64 g) was dissolved in toluene (35 mL), and $n$$Bu_3SnH$ (3.7 mL) and AIBN (113 mg) were added. The resulting mixture was heated at 110 °C for 30 min. After cooling, the reaction mixture was concentrated in vacuo and purified on silica gel (hexane/EtOAc; 3:1) to afford isoxazolidine **43** (3.36 g, 94%) as a clear oil. $[\alpha]_D^{22}=-46.3$ ($c=1.21$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.24$ (d, $J=8.6$ Hz, 2H), 6.86 (d, $J=8.6$ Hz, 2H), 4.41 (s, 2H), 3.78 (s, 3H), 3.72–3.62 (m, 4H), 3.42 (t, $J=13.8$ Hz, 2H), 3.20 (m, 1H), 3.10 (m, 1H), 2.63 (m, 1H), 1.94 (m, 1H), 1.71 (m, 2H), 1.61–1.52 (m, 5H), 1.45 (m, 1H), 1.35–1.20 (m, 11H), 0.88 (s, 9H), 0.04 ppm (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=158.9$, 130.6, 129.0, 113.5, 78.6, 72.3, 70.0, 68.3, 61.5, 56.7, 55.0, 53.6, 34.5, 29.6, 29.5, 29.4, 29.3, 26.2, 26.0, 25.7, 24.9, 24.6, 18.0, −5.6, −5.7 ppm; IR (neat): $\tilde{\nu}=2929$, 2855, 1613, 1513, 1464, 1361, 1302, 1249, cm^{-1}; HRMS (FAB): calcd for $C_{48}H_{81}N_2O_9Si$ [M^++H]: 520.3822; found: 520.3820.

Guanidine 45: $Pd(OH)_2$/C (400 mg) was added to a solution of isoxazolidine **43** (797 mg, 1.53 mmol) in EtOH (5.0 mL), and the reaction mixture was stirred at room temperature under a hydrogen (balloon). After 3 h, the reaction mixture was filtered through a pad of Celite. The filtrates were concentrated in vacuo to give pyrrolidine **44**. $HgCl_2$ (500 mg, 1.85 mmol) was added to a solution of pyrrolidine **44**, 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (537 mg, 1.85 mmol) and triethylamine (640 μL, 4.62 mmol) in DMF (10.0 mL), and the resulting mixture was stirred for 30 min. The reaction mixture was diluted with ethyl acetate, and filtered through a pad of Celite. The filtrates were concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane/EtOAc; 5:1) to give the bis-Boc-protected guanidine **45** (830 mg, 71% overall).

Bicyclic guanidine 46: DEAD (750 μL, 1.65 mmol, 40% in toluene) was added to a solution of guanidine **45** (830 mg, 1.09 mmol) and PPh_3 (433 mg, 1.65 mmol) in toluene (10.0 mL). After stirring for 30 min, the reaction mixture was quenched with H_2O (50 μL) and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexane/EtOAc; 3:2) to give bicyclic guanidine **46** (860 mg, q.y.) as a clear oil. $[\alpha]_D^{24}=-128.9$ ($c=1.68$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.25$ (d, $J=8.6$ Hz, 2H), 6.87 (d, $J=8.6$ Hz, 2H), 4.45 (m, 1H), 4.42 (s, 2H), 3.79 (s, 3H), 3.70–3.47 (m, 5H), 3.42 (t, $J=6.8$ Hz, 2H), 2.60 (m, 1H), 2.00–1.85 (m, 2H), 1.81–1.54 (m, 6H), 1.54–1.18 (m, 12H), 1.48 (s, 9H), 1.47 (s, 9H), 0.89 (s, 9H), 0.06 (s, 3H), 0.06 ppm (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=159.2$, 159.1, 151.9, 150.0, 130.8, 129.2, 113.7, 81.8, 77.8, 72.5, 70.3, 60.5, 56.7, 55.2, 54.0, 46.7, 43.8, 29.8, 29.6, 29.5, 29.4, 29.3, 28.5, 28.4, 28.2, 28.16, 26.3, 26.2, 25.8, 22.1, 18.0, −5.5, −5.6 ppm; IR (neat): $\tilde{\nu}=2929$, 2856, 1732, 1678, 1586, 1513, 1471, 1390, 1365, 1326, 1301, 1249 cm^{-1}; HRMS (FAB): calcd for $C_{41}H_{72}N_3O_7$ [M^++H]: 746.5140; found: 746.5143.

Alcohol 47: TBAF (590 mg, 2.26 mmol) was added to a solution of TBS ether **46** (860 mg, 1.13 mmol) in THF (15 mL). After 2 h, the solution was diluted with ethyl acetate and washed with H_2O and saturated NH_4Cl aq. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified on silica gel (hexane/EtOAc; 1:2) to give alcohol **47** (580 mg, 81%) as a colorless oil. $[\alpha]_D^{24}=-133.4$ ($c=1.30$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.26$ (d, $J=8.6$ Hz, 2H), 6.87 (d, $J=8.6$ Hz, 2H), 4.42 (s, 2H), 4.41 (m, 1H), 3.80 (s, 3H), 3.73 (m, 2H), 3.63 (m, 2H), 3.52 (m, 1H), 3.43 (t, $J=6.6$ Hz, 2H), 2.62 (m, 1H), 1.98 (m, 2H), 1.78 (m, 1H), 1.70–1.53 (m, 3H), 1.48 (s, 9H), 1.48 (s, 9H), 1.42–1.20 ppm (m, 14H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=158.9$, 158.8, 151.9, 150.5, 130.5, 129.0, 113.5, 81.8, 77.6, 72.3, 70.0, 59.9, 57.2, 55.0, 54.2, 46.8, 43.4, 29.5, 29.3, 29.2, 29.0, 28.4, 28.3, 27.9, 26.1, 25.9, 21.9 ppm; IR (neat): $\tilde{\nu}=3369$, 2975, 2929, 2855, 1729, 1682, 1574, 1514, 1473, 1389, 1366, 1325, 1301, 1249 cm^{-1}; HRMS (FAB): calcd for $C_{35}H_{58}N_3O_7$ [M^++H]: 632.4275; found: 632.4274.

Aldehyde 53: TPAP (8.4 mg) was added in one portion to a stirred mixture of alcohol **47** (301 mg, 0.48 mmmol), NMO (225 mg, 1.92 mmol) and powdered 4 Å molecular sieves (240 mg) in CH_2Cl_2 (2.0 mL) at room temperature. After stirring for 30 min, the reaction mixture was concentrated in vacuo and the resulting black residue was purified on silica gel (hexane/EtOAc; 3:1) to give α,β-unsaturated aldehyde **53** (143 mg, 47%) as a yellow oil. $[\alpha]_D^{24}=-34.3$ ($c=1.01$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=9.51$ (s, 1H), 7.26 (d, $J=8.5$ Hz, 2H), 6.87 (d, $J=8.5$ Hz, 2H), 5.26 (dd, $J=8.5$, 4.5 Hz, 1H), 4.42 (s, 2H), 3.97 (ddd, $J=12.1$, 7.7, 7.7 Hz, 1H), 3.85 (ddd, $J=12.1$, 8.5, 4.5 Hz, 1H), 3.80 (s, 3H), 3.42 (t, $J=6.8$ Hz, 2H), 3.15 (ddd, $J=16.5$, 8.5, 4.5 Hz, 1H), 2.98 (ddd, $J=16.5$, 8.5, 8.5 Hz, 1H), 2.22–2.13 (m, 1H), 2.13–2.03 (m, 1H), 1.63–1.55 (m, 2H), 1.52 (s, 9H), 1.49 (s, 9H), 1.40–1.20 ppm (m, 14H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=158.9$, 158.8, 151.9, 150.5, 130.5, 129.0, 113.5, 81.8, 77.6, 72.3, 70.0, 59.9, 57.2, 55.0, 54.2, 46.8, 43.4, 29.5, 29.3, 29.2, 29.0, 28.4, 28.3, 27.9, 26.1, 25.9, 21.9 ppm; IR (neat): $\tilde{\nu}=2977$, 2930, 2855, 1739, 1695, 1651, 1613, 1514, 1457, 1416, 1392, 1368, 1335, 1304 cm^{-1}; HRMS (FAB): calcd for $C_{35}H_{54}N_3O_7$ [M^++H]: 628.3962; found: 628.3959.

Methyl ester 56: $NaClO_2$ (63.0 mg) and $NaH_2PO_4{\cdot}2H_2O$ (63.0 mg) in water (190 μL) were added to a mixture of aldehyde **53** (36.1 mg, 0.058 mmol) and 2-methyl-2-butene (300 μL) in *t*BuOH (520 μL) at room temperature, and the mixture was stirred vigorously for 14 h. An excess of $TMSCHN_2$ (40% in hexane) was added to the reaction mixture and the mixture was diluted with EtOAc. The mixture was extracted with ethyl acetate and the extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexane/EtOAc; 4:1) to give methyl ester **56** (24.6 mg, 0.037 mmol, 65%, 86% from recovered aldehyde) and the starting aldehyde **53** (8.3 mg, 0.013 mmol, 23%). $[\alpha]_D^{24}=18.7$ ($c=1.08$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=7.26$ (d, $J=8.5$ Hz, 2H), 6.87 (d, $J=8.5$ Hz, 2H), 5.21 (dd, $J=8.5$, 4.5 Hz, 1H), 4.43 (s, 2H), 3.80 (s, 3H), 3.76–3.69 (m, 1H), 3.74 (s, 3H), 3.48 (ddd, $J=11.5$, 8.0, 8.0 Hz, 1H), 3.42 (t, $J=6.8$ Hz, 2H), 3.23 (ddd, $J=17.8$, 8.2, 4.2 Hz, 1H), 2.92 (ddd, $J=17.8$, 9.0, 9.0 Hz, 1H), 2.10–1.90 (m, 2H), 1.60–1.00 (m, 16H), 1.51 (s, 9H), 1.49 ppm (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=165.7$, 158.8, 152.0, 150.8, 144.8, 131.5, 130.7, 129.2, 113.7, 102.9, 83.1, 79.6, 72.4, 70.2, 55.2, 53.0, 51.2, 48.8, 33.6, 31.2, 29.7, 29.6, 29.4, 29.37, 29.1, 28.7, 28.3, 28.2, 28.1, 26.2, 24.7, 21.3 ppm; IR (neat): $\tilde{\nu}=2979$, 2931, 2855, 1740, 1698, 1655, 1612, 1513, 1457, 1438, 1416, 1390, 1368, 1346 cm^{-1}; HRMS (FAB): calcd for $C_{36}H_{56}N_3O_8$ [M^++H]: 658.4067; found: 658.4064.

Ester 58: nPrSLi (71.9 mg) was added to a solution of methyl ester **56** (57.6 mg, 0.088 mmol) in HMPA (2.1 mL) at room temperature, and the mixture was stirred for 80 min. The reaction mixture was poured into ice/Et_2O, and washed with water. The aqueous layer was acidified to pH 3 with 0.1 M HCl aq. and extracted with Et_2O. After washing with HCl aq. (pH 3), the combined organic layer was dried over $MgSO_4$ and concentrated in vacuo to give carboxylic acid **57**. Triethylamine (37 μL) was added to a mixture of the crude carboxylic acid **57**, guanidine alcohol **8** (117 mg, 0.35 mmol), and BOPCl (56.0 mg) in CH_2Cl_2 (2 mL) at room temperature. After stirring for 12 h, the reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexane/EtOAc; 4:1) to give ester **58** (45.3 mg, 0.047 mmol, 54% overall) as a yellow oil. $[\alpha]_D^{24}=28.3$ ($c=0.88$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=11.50$ (br, 1H), 8.34 (br, 1H), 7.27 (d, $J=8.6$ Hz, 2H), 6.87 (d, $J=8.6$ Hz, 2H), 5.21 (dd, $J=8.6$, 4.3 Hz, 1H), 4.42 (s, 2H), 4.15 (m, 2H), 3.95 (ddd, $J=11.5$, 8.1, 7.7 Hz, 1H), 3.80 (s, 3H), 3.73 (ddd, $J=12.4$, 8.6, 3.3 Hz, 1H), 3.46 (dd, $J=12.4$, 6.8 Hz, 2H), 3.42 (t, $J=6.8$ Hz, 2H), 3.22 (ddd, $J=17.5$, 8.6, 4.3 Hz, 1H), 2.15–1.93 (m, 2H), 1.78–1.64 (m, 4H), 1.62–1.53 (m, 2H), 1.51 (s, 9H), 1.50 (s, 9H), 1.49 (s, 9H), 1.48 (s, 9H), 1.41–1.19 ppm (m, 14H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=165.1$, 163.6, 159.0, 158.8, 156.1, 153.3, 152.0, 150.8, 144.7, 130.8, 129.1, 113.7, 103.0, 83.1, 83.0, 79.6, 79.3, 72.5, 70.2, 63.6, 55.2, 53.0, 48.8, 40.4, 33.7, 31.2, 29.8, 29.6, 29.5, 29.46, 29.2, 28.3, 28.2, 28.1, 28.0, 26.3 26.2, 25.8, 24.8, 21.4 ppm; IR (neat): $\tilde{\nu}=$ 3332, 2979, 2931, 2856, 1739, 1731, 1723, 1715, 1704, 1695, 1643, 1633,

1622, 1614, 1514, 1455, 1415, 1391, 1368, 1331 cm^{-1}; HRMS (FAB): calcd for $C_{50}H_{81}N_6O_{12}$ [M^++H]: 957.5912; found: 957.5919.

Alcohol 59: DDQ (77 mg) was added to a solution of ester **58** (108.0 mg, 0.113 mmol) in CH_2Cl_2 (1.6 mL) and H_2O (180 μL) at room temperature and stirred for 1 h. The reaction mixture was poured into saturated $NaHCO_3$ aq. and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified on silica gel (hexane/EtOAc; 2:1) to give alcohol **59** (62.2 mg, 66%) as a clear oil. $[\alpha]_D^{24}=23.2$ ($c=1.52$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=11.49$ (br, 1H), 8.34 (br, 1H), 5.21 (dd, $J=8.5$, 4.7 Hz, 1H), 4.16 (dt, $J=12.4$, 5.1 Hz, 2H), 3.95 (ddd, $J=11.5$, 8.1, 7.7 Hz, 1H), 3.73 (ddd, $J=12.4$, 8.6, 4.3 Hz, 1H), 3.62 (t, $J=6.6$ Hz, 2H), 3.46 (dd, $J=12.4$, 6.9 Hz, 2H), 3.22 (ddd, $J=18.0$, 8.6, 4.3 Hz, 1H), 2.92 (ddd, $J=18.0$, 9.0, 9.0 Hz, 1H), 2.12–1.96 (m, 2H), 1.78–1.41 (m, 6H), 1.51 (s, 9H), 1.49 (s, 9H), 1.48 (s, 9H), 1.48 (s, 9H), 1.41–1.18 ppm (m, 14H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=165.2$, 163.6, 158.9, 156.2, 153.3, 152.1, 150.8, 144.8, 103.0, 83.2, 83.1, 79.7, 79.4, 63.6, 63.0, 53.0, 48.8, 40.5, 33.6, 32.8, 31.2, 29.7, 29.4, 29.3, 29.26, 29.0, 28.3, 28.1, 28.0, 26.3, 25.9, 25.7, 24.7, 21.4 ppm; IR (neat): $\tilde{\nu}=3329$, 2979, 2930, 2856, 1739, 1722, 1698, 1641, 1614, 1456, 1416, 1392, 1368, 1332 cm^{-1}; HRMS (FAB): calcd for $C_{42}H_{73}N_6O_{11}$ [M^++H]: 837.5337; found: 837.5337.

Ester 60: EDCI (11.0 mg) and DMAP (1.0 mg) was added to a mixture of crude carboxylic acid **35**—obtained by Jones oxidation of corresponding alcohol (12.8 mg, 0.017 mmol), alcohol **59** (13.2 mg, 0.016 mmol) in CH_2Cl_2 (400 μL)—at 0 °C. After stirring at 0 °C for 3 h, the reaction mixture was poured into H_2O and extracted with Et_2O. The extracts were washed with saturated $NaHCO_3$ aq. and brine, and dried over $MgSO_4$. The filtrates were concentrated in vacuo and the residue was purified by column chromatography on silica gel (CH_2Cl_2/hexane/EtOAc; 16:8:3) to give ester **60** (14.8 mg, 0.0094 mmol, 60%) as a clear oil. $[\alpha]_D^{24}=-52.4$ ($c=0.57$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=11.50$ (br, 1H), 8.34 (br, 1H), 7.36–7.20 (m, 10H), 5.21 (dd, $J=8.6$, 4.7 Hz, 1H), 5.15 (d, $J=12.4$, 1H), 4.97 (d, $J=12.8$, 1H), 4.90 (d, $J=12.4$, 1H), 4.87 (d, $J=12.4$, 1H), 4.48 (m, 1H), 4.25–4.10 (m, 3H), 4.10–4.02 (m, 1H), 4.05 (t, $J=6.8$ Hz, 2H), 3.96 (ddd, $J=11.5$, 8.1, 7.7 Hz, 1H), 3.86 (m, 1H), 3.77–3.70 (m, 1H), 3.46 (dd, $J=12.4$, 6.4 Hz, 2H), 3.27–3.17 (m, 2H), 2.92 (ddd, $J=18.0$, 9.0, 9.0 Hz, 1H), 2.26 (m, 2H), 2.18–1.92 (m, 2H), 1.90–1.45 (m, 14H), 1.51 (s, 9H), 1.50 (s, 9H), 1.49 (s, 9H), 1.48 (s, 9H), 1.45–1.20 (m, 29H), 0.95–0.80 (m, 12H), 0.04 (s, 3H), 0.03 ppm (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=169.9$, 165.1, 163.6, 160.0, 158.9, 156.2, 153.3, 152.9, 152.0, 150.8, 150.2, 144.8, 137.2, 135.6, 128.3, 128.1, 128.06, 128.0, 127.4, 103.0, 83.1, 83.0, 79.6, 79.3, 71.1, 68.1, 66.8, 65.5, 63.6, 58.5, 56.3, 53.0, 52.4, 50.3, 48.8, 40.6, 40.4, 37.4, 33.7, 31.9, 31.3, 29.9, 29.7, 29.6, 29.5, 29.46, 29.3, 29.2, 28.8, 28.3, 28.27, 28.2, 28.1, 28.0, 27.4, 26.3, 25.9, 25.8, 24.8, 24.4, 22.7, 21.4, 16.4, 14.1, −4.3, −4.4 ppm; IR (neat): $\tilde{\nu}=3334$, 2928, 2855, 1736, 1725, 1696, 1639, 1612, 1456, 1415, 1390, 1368, 1330, 1287, 1248 cm^{-1}; HRMS (FAB): calcd for $C_{84}H_{134}N_9O_{17}Si$ [M^++H]: 1569.9697; found: 1569.9678.

Alcohol 61: HF/pyridine (100 μL) was added to a solution of **60** (41.7 mg 0.027 mmol) in THF (300 μL) in a polypropylene tube at 0 °C. After stirring for 4 h, saturated $NaHCO_3$ aq. (500 μL) and solid $NaHCO_3$ (100 mg) were added. The mixture was dried over $MgSO_4$, filtered through a pad of Celite, and washed with ethyl acetate. The filtrates were concentrated in vacuo and the residue was purified by column chromatography on silica gel (hexane/EtOAc; 2:1) to give alcohol **61** (31.0 mg, 0.021 mmol, 80%). $[\alpha]_D^{24}=-66.3$ ($c=1.11$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta=$ 11.50 (br, 1H), 8.54 (br, 1H), 7.36–7.16 (m, 10H), 5.20 (dd, $J=8.5$, 4.7 Hz, 1H), 5.14 (d, $J=12.4$ Hz, 1H), 5.00 (d, $J=12.8$ Hz, 1H), 4.82 (d, $J=12.4$ Hz, 1H), 4,78 (d, $J=12.4$ Hz, 1H), 4.58 (m, 1H), 4.38 (m, 1H), 4.23–4.09 (m, 2H), 4.06 (dt, $J=6.8$, 3.4 Hz, 2H), 3.94 (m, 2H), 3.73 (m, 1H), 3.51 (br, 1H), 3.45 (dd, $J=12.4$, 6.8 Hz, 2H), 3.27–3.16 (m, 2H), 2.91 (ddd, $J=18.0$, 9.0, 9.0 Hz, 1H), 2.42–2.23 (m, 2H), 2.12–1.91 (m, 2H), 1.91–1.38 (m, 14H), 1.50 (s, 9H), 1.49 (s, 9H), 1.48 (s, 9H), 1.48 (s, 9H), 1.38–1.07 (m, 29H), 0.87 ppm (t, $J=6.8$ Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta=169.9$, 165.1, 163.6, 159.3, 158.9, 156.2, 153.7, 153.3, 152.7, 152.0, 150.8, 144.8, 136.9, 135.1, 128.4, 128.1, 128.0, 127.9, 127.5, 127.47, 127.4, 103.0, 83.2, 83.1, 79.6, 79.3, 68.2, 66.4, 66.3, 65.8, 63.6, 56.9, 56.5, 53.2, 53.0, 51.0, 48.8, 44.4, 40.4, 37.0, 33.7, 31.9, 31.3, 30.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 28.7, 28.5, 28.3, 28.2, 28.1, 28.0, 27.0, 26.3, 26.25, 25.8, 25.79, 24.8, 22.7, 21.4, 16.5, 14.1 ppm; IR (neat): $\tilde{\nu}=3331$, 2978, 2929, 2855, 1738, 1732, 1695, 1641, 1614, 1576, 1456, 1416, 1390, 1368, 1329 cm^{-1}; HRMS (FAB): calcd for $C_{78}H_{120}N_9O_{17}$ [M^++H]: 1454.8802; found: 1454.8811.

(+)-Batzelladine A (1): A catalytic amount of Pd/C was added to a solution of **61** (14.4 mg, 0.0099 mmol) in ethyl acetate (200 μL). The reaction mixture was stirred at room temperature under hydrogen (balloon) for 3 h, and was filtered. The filtrates were concentrated in vacuo to give a guanidine alcohol. DEAD (44 μL, 40% in toluene) was added to a solution of the guanidine alcohol (11.4 mg, 0.0096 mmol) and PPh_3 (25.2 mg) in toluene (300 μL). After stirring for 5 h, the reaction mixture was quenched with H_2O (10 μL) and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH; 1:1) to give tricyclic guanidine **62**. The tricyclic guanidine **62** (6.9 mg, 0.0059 mmol) was dissolved in 50% TFA in CH_2Cl_2 (500 μL) and stirred for 30 min. The reaction mixture was concentrated in vacuo, and the residue was purified by HPLC (50% MeCN aq. 0.1% TFA) to give (+)-batzelladine A (**1**) (2.52 mg, 0.0023 mmol, 24% overall) as a viscous oil. $[\alpha]_D^{25}=4.29$ ($c=0.25$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta=4.39$ (t, $J=6.1$ Hz, 1H), 4.21 (t, $J=6.4$ Hz, 2H), 4.13 (t, $J=6.7$ Hz, 2H), 3.93 (m, 1H), 3.83 (m, 2H), 3.66 (m, 1H), 3.52 (m, 2H), 3.32 (m, 1H), 3.22 (t, $J=7.3$ Hz, 2H), 3.12 (dd, $J=4.6$, 3.5 Hz, 1H), 2.98 (m, 1H), 2.35 (m, 1H), 2.28–2.17 (m, 3H), 2.10 (m, 1H), 1.76 (m, 2H), 1.72–1.52 (m, 9H), 1.48–1.23 (m, 29H), 1.27 (d, $J=6.7$ Hz, 3H), 0.89 ppm (t, $J=6.7$ Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): $\delta=170.7$, 166.2, 158.7, 153.1, 152.7, 151.5, 103.3, 66.0, 65.1, 57.7, 57.3, 53.2, 51.2, 49.9, 48.8, 45.6, 42.0, 37.5, 36.9, 34.2, 33.0, 31.9, 31.4, 29.7, 29.3, 27.0, 26.6, 26.2, 25.2, 23.7, 22.9, 18.4, 14.4 ppm; IR (neat): $\tilde{\nu}=2925$, 2854, 1732, 1697, 1683, 1648, 1637, 1558, 1347, 1092 cm^{-1}; HRMS (FAB): calcd for $C_{42}H_{74}N_9O_4$ [M^++H]: 768.5864; found: 768.5866.

Acknowledgements

We thank Dr. Masami Suganuma for helpful discussion. We acknowledge the fund from the Uehara Memorial Foundation. This work was also supported by Grant-in-Aid for Scientific Research on Priority Areas 17035025 from The Ministry of Education, Culture, Sports, Science, and Technology (MEXT).

[1] a) A. D. Patil, N. V. Kumar, W. C. Kokke, M. F. Bean, A. J. Freyer, J. C. De Brosse, S. Mai, A. Truneh, D. J. Faulkner, B. Carté, A. L. Breen, R. P. Hertzberg, R. K. Johnson, J. W. Westley, B. C. M. Potts, *J. Org. Chem.* **1995**, *60*, 1182–1188; b) A. D. Patil, A. J. Freyer, P. B. Taylor, B. Carté, G. Zuber, R. K. Johnson, D. J. Faulkner, *J. Org. Chem.* **1997**, *62*, 1814–1819.

[2] The identification of the source of these compounds was revised: a) R. W. M. Van Soest, J. C. Braekman, D. J. Faulkner, E. Hajdu, M. K. Harper, J. Vacelet, *Bull. Inst. R. Sci. Nat. Belg.* **1996**. *66*, 89–102; b) J. C. Braekman, D. Daloze, R. Tavares, E. Hajdu, R. W. M. Van Soest, *J. Nat. Prod.* **2000**, *63*, 193–196.

[3] a) B. B. Snider, J. Chen, A. D. Patil, A. J. Freyer, *Tetrahedron Lett.* **1996**, *37*, 6977–6980; b) B. B. Snider, J. Chen, *Tetrahedron Lett.* **1998**, *39*, 5697–5700.

[4] a) G. P. Black, P. J. Murphy, N. D. A. Walshe, D. E. Hibbs, M. B. Hursthouse, K. M. A. Malik, *Tetrahedron Lett.* **1996**, *37*, 6943–6946; b) G. P. Black, P. J. Murphy, N. D. A. Walshe, *Tetrahedron* **1998**, *54*, 9481–9488; c) G. P. Black, P. J. Murphy, A. J. Thornhill, N. D. A. Walshe, C. Zanetti, *Tetrahedron* **1999**, *55*, 6547–6554.

[5] F. Cohen, L. E. Overman, S. K. L. Sakata, *Org. Lett.* **1999**, *1*, 2169–2172.

[6] F. Cohen, L. E. Overman, *J. Am. Chem. Soc.* **2001**, *123*, 10782–10783.

[7] M. A. Arnold, S. G. Duron, D. Y. Gin, *J. Am. Chem. Soc.* **2005**, *127*, 6924–6925.

[8] A. V. R. Rao, M. K. Gurjar, J. Vasudevan, *J. Chem. Soc. Chem. Commun.* **1995**, 1369–1370.
[9] S. Louwrier, M. Ostendorf, A. Tuynman, H. Hiemstra, *Tetrahedron Lett.* **1996**, *37*, 905–908.
[10] S. G. Duron, D. Y. Gin, *Org. Lett.* **2001**, *3*, 1551–1554.
[11] P. A. Evans, T. Manangan, *Tetrahedron Lett.* **2001**. *42*, 6637–6640.
[12] a) M. C. Elliott, M. S. Long, *Tetrahedron Lett.* **2002**. *43*, 9191–9194; b) M. C. Elliott, M. S. Long, *Org. Biomol. Chem.* **2004**, *2*, 2003–2011.
[13] K. Nagasawa, H. Koshino, T. Nakata, *Tetrahedron Lett.* **2001**, *42*, 4155–4158.
[14] A. Olszewski, K. Sato, Z. D. Aron, F. Cohen, A. Harris, B. R. McDougall, W. E. Jr Robinson, L. E. Overman, G. A. Weiss, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 14079–14084.
[15] P. L. Toogood, *J. Med. Chem.* **2002**, *45*, 1543–1558.
[16] T. Ishiwata, T. Hino, H. Koshino, Y. Hashimoto, T. Nakata, K. Nagasawa, *Org. Lett.* **2002**, *4*, 2921–2924.
[17] J. Shimokawa, K. Shirai, A. Tanatani, Y. Hashimoto, K. Nagasawa, *Angew. Chem.* **2004**, *116*, 1585–1588; *Angew. Chem. Int. Ed.* **2004**, *43*, 1559–1562.
[18] a) J. J. Tufariello, G. B. Mullen, J. J. Tegeler, E. J. Trybulski, S. C. Wong, Sk. A. Ali, *J. Am. Chem. Soc.* **1979**, *101*, 2435–2442; b) J. J. Tufariello, J. M. Puglis, *Tetrahedron Lett.* **1986**, *27*, 1489–1492; c) Sk. A. Ali, M. I. M. Wazeer, *Tetrahedron Lett.* **1993**, *34*, 137–144.
[19] a) K. S. Kim, L. Qian, *Tetrahedron Lett.* **1993**, *34*, 7677–7680; b) E. I. Iwanowicz, M. A. Poss, J. Lin, *Synth. Commun.* **1993**, *23*, 1443–1445.
[20] D. S. Dodd, A. P. Kozikowski, *Tetrahedron Lett.* **1994**, *35*, 977–980.
[21] I. J. McAlpine, R. W. Armstrong, *Tetrahedron Lett.* **2000**, *41*, 1849–1853.
[22] K. Nagasawa, T. Ishiwata, Y. Hashimoto, T. Nakata, *Tetrahedron Lett.* **2002**, *43*, 6383–6385.
[23] A. S. Franklin, S. K. Ly, G. H. Mackin, L. E. Overman, A. J. Shaka, *J. Org. Chem.* **1999**, *64*, 1512–1519.
[24] J. Xiang, S. Toyoshima, A. Orita, J. Otera, *Angew. Chem.* **2001**, *113*, 3782–3784; *Angew. Chem. Int. Ed.* **2001**, *40*, 3670–3672.
[25] A. Goti, M. Cacciarini, F. Cardona, A. Brandi, *Tetrahedron Lett.* **1999**, *40*, 2853–2856.
[26] D. H. R. Barton, S. W. McCombie, *J. Chem. Soc. Perkin Trans. 1* **1975**, 1574.
[27] K. B. Sharpless, R. F. Lauer, A. Y. Teranishi, *J. Am. Chem. Soc.* **1973**, *95*, 6137–6139.
[28] a) Y. Ito, T. Hirao, T. Saegusa, *J. Org. Chem.* **1978**, *43*, 1011–1013; b) J. Tsuji, I. Minani, I. Shimizu, *Tetrahedron Lett.* **1983**, *24*, 5635–5638; c) I. Minani, K. Takahashi, I. Shimizu, T. Kimura, J. Tsuji, *Tetrahedron* **1986**, *42*, 2971–2977.
[29] K. C. Nicolaou, Y.-L. Zhong, P. S. Baran, *J. Am. Chem. Soc.* **2000**, *122*, 7596–7597.
[30] S. V. Ley, J. Norman, W. P. Griffith, S. P. Marsden, *Synthesis*, **1994**, 639–666.
[31] S. Murahashi, N. Komiya, H. Terai, T. Nakae, *J. Am. Chem. Soc.* **2003**, *125*, 15312–15313.
[32] a) G. A. Kraus, M. J. Taschner, *J. Org. Chem.* **1980**, *45*, 1175–1176; b) G. A. Kraus, B. Roth, *J. Org. Chem.* **1980**, *45*, 4825–4830.
[33] C. A. Bewley, S. Ray, F. Cohen, S. K. Collins, L. E. Overman, *J. Nat. Prod.* **2004**, *67*, 1319–1324.

Received: July 20, 2005
Published online: September 14, 2005

