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Isocomplestatin: Total Synthesis and Stereochemical Revision

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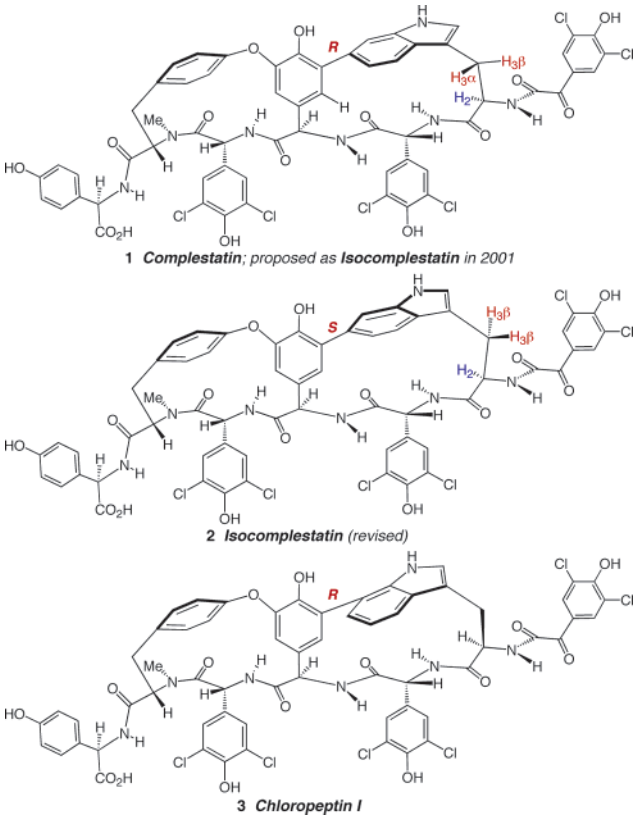
Complestatin was isolated in 1980 from the mycelium of *Streptomyces lavendulae* SANK 60477.[1] The structure of complestatin (**1**, Scheme 1), except for the stereochemical identity of the biaryl atropisomer, was elucidated through NMR spectroscopy by Seto.[2a] Chloropeptin I (**3**), an isomer of complestatin, was obtained in 1994 from *Streptomyces* sp. WK-3419.[3] Chloropeptin I and complestatin (also known as chloropeptin II) have significant biological activity; they inhibit HIV replication by disruption of gp-120 binding to the CD4 receptor of T-lymphocytes.[1,3]

The structure and stereochemistry of chloropeptin I were proposed in 1996 based on spectroscopic data[3b] and were later endorsed by a total synthesis carried out in our laboratories.[4] In 1998, Singh and Patane demonstrated that complestatin, bearing a more strained macrocyclic biaryl moiety, is converted to chloropeptin I when subjected to acidic conditions;[2b] a plausible pathway for the interconversion was put forth, as well. According to the proposed mechanism, the rearrangement would likely proceed with retention of stereochemistry. Thus, it would be plausible to suggest that complestatin bears the (*R*)-biaryl atropisomer (i.e., **1**). In 2001, Singh and co-workers described the isolation and biological activity of isocomplestatin, the atropisomer of complestatin. It was reported that isocomplestatin possesses physical, chemical, and spectral attributes nearly identical to those of complestatin.[5] The new natural product, however, was proposed to possess the *R* atropisomer[6] (even though complestatin was shown to rearrange to chloropeptin I). Isocomplestatin was believed to be distinct from complestatin due to notable differences in optical rotation (details below) and variations in the chemical shift of H_3 protons of the tryptophan residue (two signals at δ 2.87 and 3.44 ppm versus one at δ 2.89 ppm claimed to have been reported for complestatin[2a]).

Due to the above discrepancies, and as a preliminary step in a program aimed at total synthesis of complestatins **1** and **2**, we carefully examined various disclosures regarding structural and spectral characteristics of complestatins. These investigations led us to identify several noteworthy points. (1) The chemical shift for the H_2 proton of the tryptophan residues of both complestatin[2a] and isocomplestatin have been reported to be at δ 4.2 ppm.[5] However, examination of molecular models suggested that this proton resides at a different electronic environment (directly under the aromatic indole ring in the *R* atropisomer) and should be noticeably affected by ring-induced anisotropy. (2) In contrast to the claim by Singh and co-workers, our review of the disclosure by Seto et al.[2a] established that two signals were initially indicated at δ 2.9 and 3.5 ppm for the H_3 protons of complestatin's tryptophan unit. (3) It was reported[5] that *both* complestatin and isocomplestatin (one containing an *R* and the other an *S* atropisomer), when subjected to trifluoroacetic acid (TFA), rearrange to chloropeptin I. This would imply the unusual scenario that with one atropisomer the 1,2-shift proceeds with >98% retention, whereas the same rearrangement occurs with complete inversion in the case of the other diastereomer.[2b]

To address the above complications, and to establish conditions required for efficient formation of the biaryl bond of the strained ring, we decided to synthesize model macrocyclic peptides bearing the tryptophan moiety. On the basis of the findings of these studies, we would proceed toward the preparation of a member of the complestatin family. The results of these investigations, leading to the total synthesis of the *S* atropisomer of complestatin and clarification of the stereochemical identity of complestatin and isocomplestatin, are described herein.[7]

Scheme 1



1 ***Complestatin****; proposed as* ***Isocomplestatin*** *in 2001*

2 ***Isocomplestatin*** *(revised)*

3 ***Chloropeptin I***

Tripeptide **12**, required for our model studies, was first prepared (Scheme 2).[8] Treatment of D-tryptophan-based **4**[9] with sulfuric acid, conversion to the phenolic triflate, followed by protection of the indole (as NBoc amide) delivered **5** in 72% yield (for three steps). Pd-catalyzed coupling of **5** with bis(pinacolato)diboron was carried out in the presence of 10 mol % $PdCl_2(dppf)\cdot CH_2Cl_2$ and 12 mol % dppf[10] to afford **6** (86% yield).[11] Removal of the Boc and methyl carbonate groups under neutral conditions (TMSI),[12] coupling with α-ketoacid **7**, and hydrolysis of the methyl ester afforded **8** in 71% overall yield.

On a separate front, installation of an *ortho* iodide in D-4-hydroxyphenyl glycine **9** was effected in the presence of chloramine-T (94% yield). Removal of the Boc group with methanolic HCl[13] and coupling of the resulting free amine with protected amino acid **10**[14] in the presence of DEPBT[15] delivered dipeptide **11** (77% yield for two steps). Deprotection of the Boc amide and coupling with carboxylic acid **8** afforded **12** in 69% yield for two steps.

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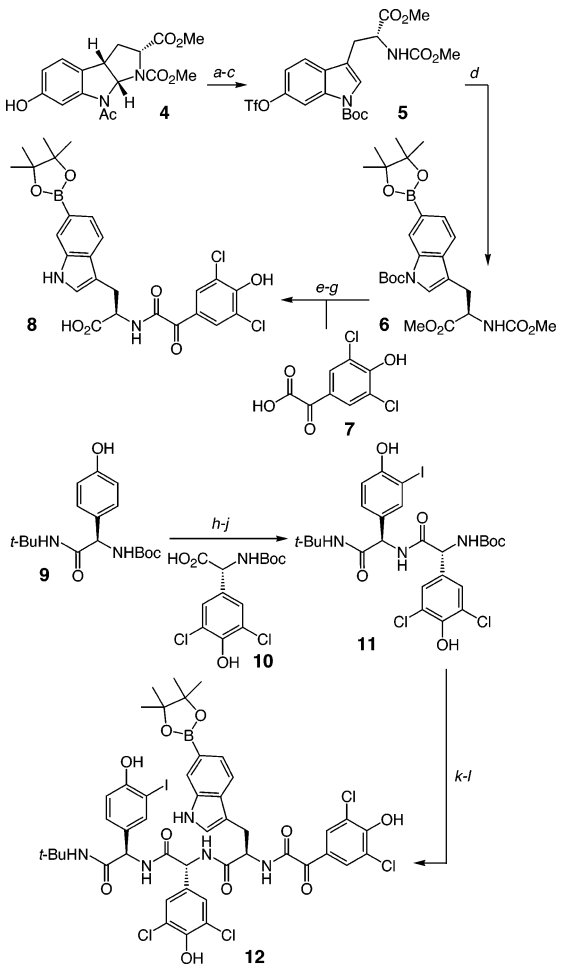
Scheme 2[a]

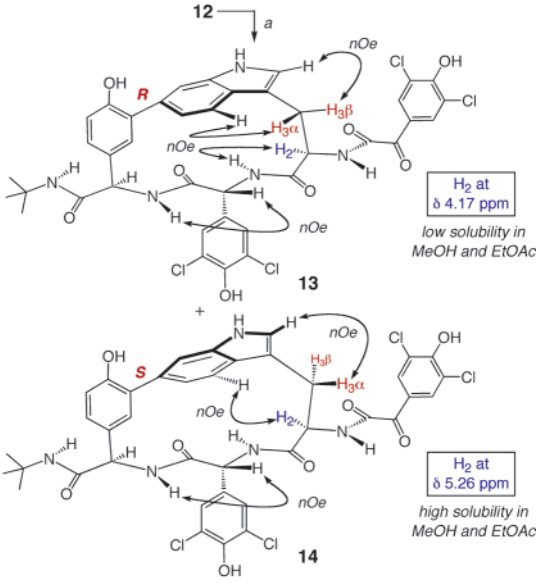


[a] Reaction conditions: (a) 10 equiv of H_2SO_4, MeOH, 22 °C, 1 h; (b) 1.1 equiv of Tf_2O, 1.2 equiv of TEA, CH_2Cl_2, −78 °C, 3 h; (c) 1.2 equiv of Boc_2O, cat DMAP, 1.2 equiv of Et_3N, CH_2Cl_2, 22 °C, 10 h, 72% from **4**; (d) 10 mol % $PdCl_2(dppf)\cdot CH_2Cl_2$, 12 mol % dppf, 1.2 equiv of bis(pinacolato)diboron, 3 equiv of KOAc, dioxane, 80 °C, 3 h, 86%; (e) 4 equiv of TMSI, $CHCl_3$, 50 °C, 2 h; (f) 1.1 equiv of **7**, 1.2 equiv of EDC, 1.2 equiv of HOAt, 1 equiv of $NaHCO_3$, THF, 0−22 °C, 14 h, 73% from **6**; (g) 10 equiv of LiOH, THF:H_2O (7:1), 0−22 °C, 2 h, 98%; (h) 1.1 equiv of chloramine-T, 1.1 equiv of NaI, DMF, 22 °C, 1 h, 94%; (i) HCl (g), MeOH, 0−22 °C, 2 h; (j) 1.1 equiv of **10**, 1.2 equiv of DEPBT, 1 equiv of $NaHCO_3$, THF, 0−22 °C, 11 h, 77% (two steps); (k) HCl (g), MeOH, 0−22 °C, 2 h; (l) 1.1 equiv of **8**, 1.2 equiv of HATU, THF, 0−22 °C, 4 h, 69% from **11**.

With tripeptide **12** in hand, we examined conditions for the formation of the strained macrocyclic structure by an intramolecular Suzuki−Miyaura process.[16] Representative data are illustrated in Table 1. With 0.5 equiv of $PdCl_2(dppf)\cdot CH_2Cl_2$ in dioxane at 50 °C, <2% conversion was observed (entry 1). When water was added as a cosolvent (7:1 dioxane:H_2O),[17] **14** was obtained but only in 10% yield (entry 2). When the reaction temperature was increased to 80 °C (entry 3, Table 1), an equal mixture of **13** and **14** was isolated in 52% combined yield (26% of each atropisomer).

Diastereomeric cyclic peptides **13** and **14** exhibit distinct physical and spectral properties (Table 1). Selected results of nOe experiments that support the stereochemical assignments are depicted in Table 1.[18] Consistent with the stereochemical assignments, the tryptophan H_2 proton in **13** appears at δ 4.2 ppm (the chemical shift reported for H_2 in the tryptophan residue of complestatin *and* isocomplestatin). The chemical shift for tryptophan H_2 of **14** is at δ 5.26 ppm. As mentioned earlier, molecular models indicate that H_2 of the *R* atropisomer of **1** or **13** is situated such that it would be

Table 1. Model Pd-Mediated Macrocyclizations[a]

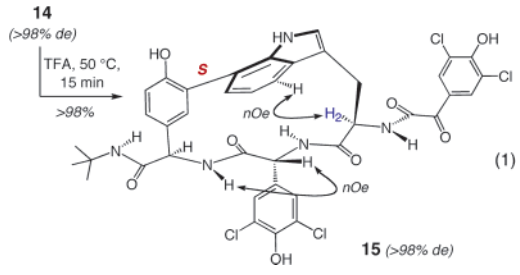


entry	equiv of Pd salt	solvent	temp (°C)	time (h)	yield (%)[b] **13**; **14**
1	0.5	dioxane	50	16	<2; <2
2	0.5	dioxane:H_2O (7:1)	50	4.5	<2; 10
3	1.0	dioxane:H_2O(7:1)	80	1	26; 26

[a] Conditions: 0.5−1 equiv of $PdCl_2(dppf)\cdot CH_2Cl_2$ in dioxane with 10 equiv of K_2CO_3. [b] Isolated yields.

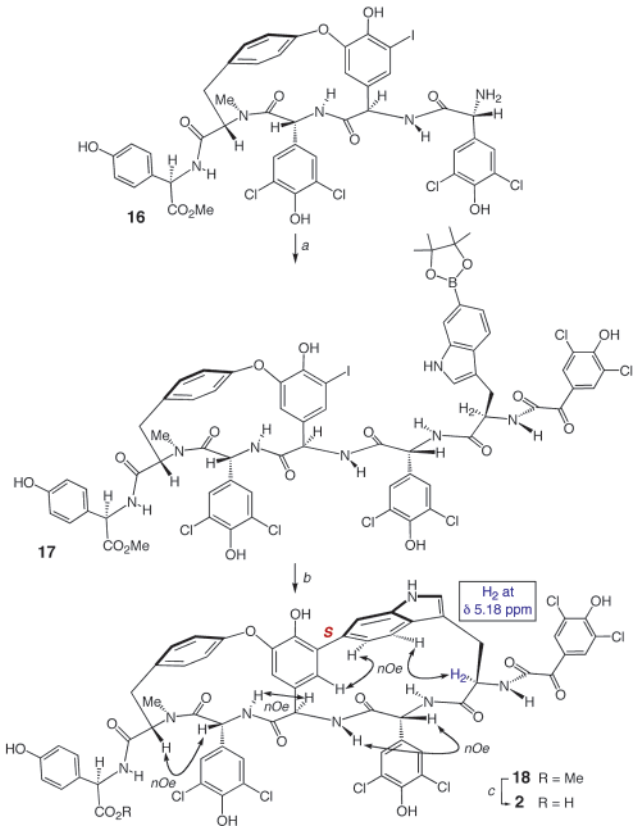
strongly influenced by the indole ring π cloud. These studies thus support the idea that it is unlikely for both complestatin atropisomers to have tryptophan H_2 proton signals appear at an identical chemical shift (δ 4.17 ppm). It should be noted that the tryptophan H_3 protons of **13** appear at similar chemical shifts reported for complestatin *and* isocomplestatin (δ 3.44 and 2.85 ppm);[2b] the corresponding signals for the *S* isomer **14** are at δ 3.42 and 3.23 ppm.

Heating pure samples of **13** and **14** (DMSO, 150 °C, 2 h) led to ~10% decomposition and <2% conversion to the alternative atropisomer; thus, the difference in the outcomes shown in entries 2−3 of Table 1 is likely not due to equilibration of the two atropisomers. When **14** was treated with neat TFA (eq 1), the 1,2-shift proceeded smoothly to afford **15** in >98% isolated yield and diastereomeric excess (de).[18] In a similar fashion, **13** isomerized to its corresponding *R* atropisomer (>98% yield and de).[18] The latter transformations provide support for the contention that the strain-releasing shift takes place with retention of stereochemistry, regardless of substrate stereochemistry.



Next, we focused on the total synthesis of a complestatin. Toward this end, as shown in Scheme 3, stereoisomerically pure pentapeptide **16**, synthesized according to our previously reported procedures,[4] was coupled with amino acid **8** (Scheme 2) to afford hexapeptide **17** (85% yield). Subjection of **17** to the macrocycliza-

Scheme 3. Total Synthesis of the *S* Atropisomer of Complestatin[a]



[a] Reaction conditions: (a) 1.1 equiv of **8**, 1.2 equiv of HATU, THF 0−22 °C, 4 h, 85%; (b) 1 equiv of $PdCl_2(dppf)\cdot CH_2Cl_2$, 10 equiv of K_2CO_3, 10:1 dioxane:H_2O, 80 °C, 1.5 h, 63%; (c) 10 equiv of LiOH, 7:1 THF:H_2O, 0 °C, 2 h, >98%.

tion conditions and methyl ester hydrolysis delivered **2** (via **18**) as a single stereoisomer in 63% yield (for two steps).[18]

The stereochemical identity of the atropisomer of the tryptophan moiety of **2** is based on the signature chemical shift of the H_2 proton (δ 5.18 ppm) and was ascertained by extensive nOe experiments (Scheme 3). We measured the optical rotation to be $[\alpha]^{26}_D = +36.0$ (c = 0.15, 2:1 MeOH:0.01 N NaOH). Moreover, through nOe studies and comparison of the chemical shift values with those for complestatin (**1**) and chloropeptin I (**3**),[18] we rigorously established that the integrity of the remaining stereogenic centers has been preserved.[19]

Analysis of the related spectral and physical data[18] clearly indicates that the material synthesized according to the route shown in Scheme 3 (**2**) is not complestatin; **2** is also different from the compound reported to be isocomplestatin.[5] Collectively, the above observations and comparison of ^{1}H NMR spectra of **13**, **14**, **1**, **2**, and an authentic sample of isocomplestatin[18] indicate that *the compound labeled as isocomplestatin is* the same as complestatin, and that the correct atropisomer of complestatin, yet to be isolated as a natural product, is **2**. These studies offer strong evidence that **1** (Scheme 1) is complestatin.

The above findings explain why the chemical shifts for H_2 of the tryptophan moiety for complestatin and isocomplestatin were reported to appear at δ 4.2 ppm.[5] Furthermore, observations presented herein address the claim that complestatin and isocomplestatin rearrange to chloropeptin I (TFA). That isocomplestatin is complestatin provides a rationale for why their tryptophan H_3 protons are at the same chemical shifts (complicated by the apparent, but nonexistent, discrepancy between the 2001 paper[5] and the 1989 disclosure[2a]). The remaining issue, the distinctly different optical rotation between complestatin and isocomplestatin, was resolved by re-measurement of the rotation of an authentic sample of isocomplestatin, generously provided by Dr. Singh. The value obtained by us, $[\alpha]^{26}_D = +25.9$ (c = 0.14, 2:1 MeOH:0.01 N NaOH versus −60° recorded previously),[5] is nearly identical to that documented for natural complestatin (+24.5 (c = 0.13, 2:1 MeOH: 0.01 N NaOH)).[20]

Stereoselective synthesis of complestatin and examination of biological activity of isocomplestatin are in progress.

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Supporting Information Available: Experimental procedures and spectral, analytical data for all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kaneko, I.; Fearon, D. T.; Austen, K. F. *J. Immunol.* **1980**, *124*, 1194−1198. (b) Kaneko, I.; Kamoshida, K.; Takahashi, S. *J. Antibiot.* **1989**, *42*, 236−241.

(2) (a) Seto, H.; Fujioka, T.; Furihata, K.; Kaneko, I.; Takahashi, S. *Tetrahedron Lett.* **1989**, *30*, 4987−4990. (b) Jayasuriya, H.; Salituro, G. M.; Smith, S. K.; Heck, J. V.; Gould, S. J.; Singh, S. B.; Homnick, C. F.; Holloway, M. K.; Pitzenberger, S. M.; Patane, M. A. *Tetrahedron Lett.* **1998**, *39*, 2247−2248.

(3) (a) Matsuzaki, K.; Ikeda, H.; Ogino, T.; Matsumoto, A.; Woodruff, H. B.; Tanaka, H.; Omura, S. *J. Antibiot.* **1994**, *47*, 1173−1174. (b) Gouda, H.; Matsuzaki, K.; Tanaka, H.; Hirono, S.; Omura, S.; McCauley, J. A.; Sprengeler, P. A.; Furst, G. T.; Smith, A. B. *J. Am. Chem. Soc.* **1996**, *118*, 13087−13088. (c) Tanaka, H.; Matsuzaki, K.; Nakashima, H.; Ogino, T.; Matsumoto, A.; Ikeda, H.; Woodruff, H. B.; Omura, S. *J. Antibiot.* **1997**, *50*, 58−65. (d) Matsuzaki, K.; Ogino, T.; Sunazuka, T.; Tanaka, H.; Omura, S. *J. Antibiot.* **1997**, *50*, 66−69.

(4) Deng, H.; Jung, J.-K.; Liu, T.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2003**, *125*, 9032−9034.

(5) Singh, S. B.; Jayasuriya, H.; Salituro, G. M.; Zink, D. L.; Shafiee, A.; Heimbuch, B.; Silverman, K. C.; Lingham, R. B.; Genilloud, O.; Teran, A.; Vilella, D.; Felock, P.; Hazuda, D. *J. Nat. Prod.* **2001**, *64*, 874−882.

(6) Although not explicitly stated nor structurally illustrated, this finding implies that complestatin bears the (*S*)-biaryl atropisomer.

(7) For related synthetic studies, see: (a) Gurjar, M. K.; Tripathy, N. K. *Tetrahedron Lett.* **1997**, *38*, 2163−2166. (b) Carbonnelle, A.-C.; Zamora, E. G.; Beugelmans, R.; Roussi, G. *Tetrahedron Lett.* **1998**, *39*, 4471−4472. (c) Elder, A. M.; Rich, D. H. *Org. Lett.* **1999**, *1*, 1443−1446. (d) Kai, T.; Kajimoto, N.; Konda, Y.; Harigaya, Y.; Takayanagi, H. *Tetrahedron Lett.* **1999**, *40*, 6289−6292. (e) Smith, A. B.; Chruma, J. J.; Han, Q.; Barbosa, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1697−1702.

(8) The α-ketoamide terminus must be present prior to macrocyclization; otherwise, removal of amine protecting groups (e.g., NBoc), as effected in the total synthesis of chloropeptin I (ref 4), could cause rearrangement to the less strained macrocycle.

(9) Taniguchi, M.; Anjiki, T.; Nakagawa, M.; Hino, T. *Chem. Pharm. Bull.* **1984**, *32*, 2544−2554.

(10) Abbreviations: dppf = 1,1′-bis(diphenylphosphino)ferrocene; EDC = 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOAt = 1-hydroxy-7-azabenzotriazole; HATU = 2-(1-*H*-7-azabenzotriazol)-1,1,3,3-methyluronium hexafluorophosphate; DEPBT = 3-(diethyloxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one.

(11) (a) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508−7510. (b) Ishiyama, T.; Itoh, Y.; Kitano, T.; Miyaura, N. *Tetrahedron Lett.* **1997**, *38*, 3447−3450.

(12) Olah, G. A.; Narang, S. C. *Tetrahedron* **1982**, *38*, 2225−2277.

(13) Pearson, A. J.; Chelliah, M. V.; Bignan, G. C. *Synthesis* **1997**, 536−541.

(14) (a) Kometani, T.; Watt, D. S.; Ji, T. *Tetrahedron Lett.* **1985**, *26*, 2043−2046. (b) Kometani, T.; Watt, D. S.; Ji, T.; Fitz, T. *J. Org. Chem.* **1985**, *50*, 5384−5387.

(15) Li, H.; Jiang, X.; Ye, Y.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91−93.

(16) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457−2483.

(17) Bois-Choussy, M.; Cristau, P.; Zhu, J. *Angew. Chem., Int. Ed.* **2003**, *42*, 4238−4241.

(18) See the Supporting Information for details of all spectral and physical data.

(19) In contrast to **13** and **14** (Table 1), repeated attempts to isomerize **2** led to decomposition (perhaps due to higher ring strain versus **1**).

(20) Subsequent re-examination of optical rotation of different batches of isocomplestatin by Dr. Singh supports the validity of this measurement.

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