ORGANIC LETTERS

2002
Vol. 4, No. 25
4483−4486

A New Strategy toward the Total Synthesis of Stachyflin, A Potent Anti-Influenza A Virus Agent: Concise Route to the Tetracyclic Core Structure

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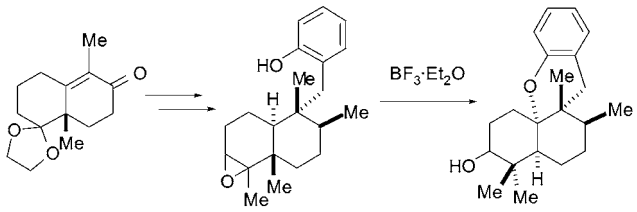
Received October 14, 2002

ABSTRACT



A new strategy directed toward the total synthesis of stachyflin, a potent and novel anti-influenza A virus agent isolated from a microorganism, has been presented through the enantioselective synthesis of the tetracyclic core structure. The synthetic method features a BF_3·Et_2O-induced domino epoxide-opening/rearrangement/cyclization reaction as the key step.

Stachyflin (**1**, Figure 1) is a novel sesquiterpenoidal alkaloid isolated from the culture broth of *Stachybotrys* sp. RF-7260 by Minagawa et al. at the Shionogi research group in 1997.[1] This secondary metabolite was found to exhibit potent antiviral activity against influenza A/WSN/33 (H1N1) virus in vitro with an IC_{50} value of 0.003 μM with a novel mechanism of action.[1−3] Stachyflin has been found to inhibit the fusion process between the viral envelop and the endosome constituting the cell membrane, which is an essential step in the entry of the influenza virus into host cells.[4] This mechanism is quite different from that of the known anti-influenza virus agents such as amantadine,[5] zanamivir,[6] and oseltamivir.[7] Therefore, **1** is anticipated to be a highly promising agent for novel influenza therapeutics.

The structure of stachyflin (**1**) was revealed by means of extensive spectroscopic studies and X-ray crystallographic

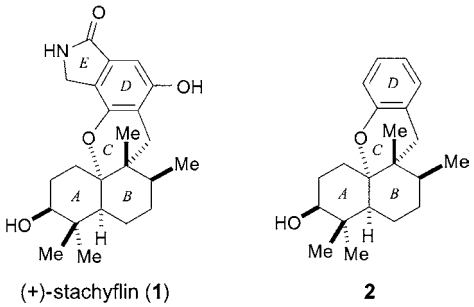


Figure 1. Structures of stachyflin (**1**) and the tetracyclic core **2**.

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(1) (a) Kamigauchi, T.; Fujiwara, T.; Tani, H.; Kawamura, Y.; Horibe, I (Shionogi & Co., Ltd, Japan) PCT WO 9711947 A1, April 3, 1997. (b) Minagawa, K.; Kouzuki, S.; Yoshimoto, J.; Kawamura, Y.; Tani, H.; Iwata, T.; Terui, Y.; Nakai, H.; Yagi, S.; Hattori, N.; Fujiwara, T.; Kamigauchi, T. *J. Antibiot*. **2002**, *55*, 155. (c) Minagawa, K.; Kouzuki, S.; Kamigauchi, T. *J. Antibiot*. **2002**, *55*, 165.

(2) It is reported that the anti-influenza A virus activity of stachyflin (**1**) is 1760 times more active than that of amantadine (IC_{50} = 5.3 μM) and is 250 times more active than that of zanamivir (IC_{50} = 0.75 μM); see ref 1b.

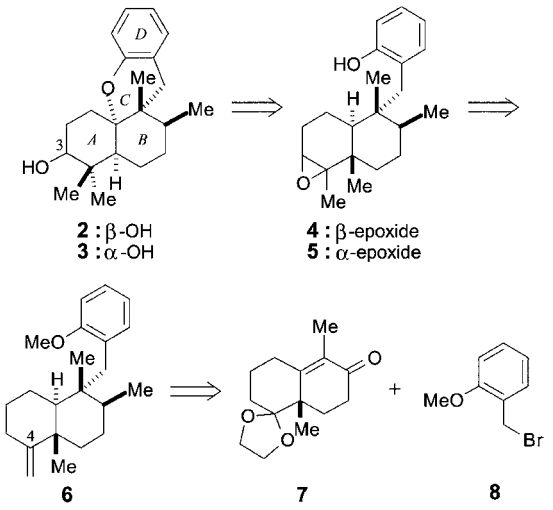
10.1021/ol0271032 CCC: $22.00
Published on Web 11/16/2002

analysis to have a novel pentacyclic 3*H*-naphtho[1′,8′a:5,6]-pyrano[2,3-*e*]isoindol-3-one skeleton (ABCDE ring system) with five asymmetric carbons,[1a,b] in which *cis*-fused AB rings and BC rings and an ether bond at the bridgehead of the AB ring junction are particularly characteristic features. The absolute configuration of **1** was determined by its circular dichroism (CD) spectrum as depicted in Figure 1.[1b]

Its remarkable biological properties as well as its unique structural features make **1** an exceptionally intriguing and timely target for total synthesis. To date, only one total synthesis of racemic (±)-**1** has been reported by Mori et al. at Shionogi & Co., Ltd., in 1998,[8] wherein the ABCDE ring system was built step-by-step starting from 2,3-dimethylcyclohexanone, which corresponds to the B ring of **1**. We embarked on a project directed at the total synthesis of optically active (+)-**1** and its analogues with the aim of exploring structure−activity relationships.[9] In this Letter, we report our preliminary results concerning a highly concise method for the synthesis of model compound **2**, which represents the tetracyclic core structure (ABCD ring system) having the requisite substituents and asymmetric carbons contained in **1**.

The synthetic plan for the tetracyclic core **2** is outlined in Scheme 1, which was designed on the basis of our previous

Scheme 1. Synthetic Plan for the Tetracyclic Core **2**



work.[10] The key feature in this plan is a biogenetic-type acid-induced domino epoxide-opening/rearrangement/cyclization reaction of epoxides **4** and **5** to construct the desired tetracyclic cores **2** and **3**, respectively, in one step, where we envisioned that this acid-induced domino reaction would proceed stereoselectively to install the requisite *cis*-fused AB ring junction (cf. **5**→**[I**→**II**→**III]**→**3**, Scheme 3). To the best of our knowledge, this domino sequence is hitherto unknown, and hence it involves an interesting possibility at a synthetic chemical level. The cyclized compound **3** having the epimeric C3 α-hydroxy group would be converted to target compound **2** through inversion of the C3 hydroxy group. Epoxides **4** and **5**, in turn, would be elaborated from intermediate **6** by sequential manipulation of the C4 *exo*-olefin moiety and deprotection of the phenolic *O*-methyl group, or vice versa. Intermediate **6**, which possesses the *trans*-fused decalin system, would be prepared through the stereocontrolled reductive alkylation of the known enone **7**[11] with the known bromide **8**[12] applying the related protocols previously described in the literature.[13]

At first, as shown in Scheme 2, we pursued the synthesis of epoxides **4** and **5**, precursors of the key acid-induced domino reaction. The synthesis commenced with the reductive alkylation of the known enantiomerically pure enone **7**[11] (>99% ee) with 2-methoxybenzyl bromide (**8**),[12] readily prepared from commercially available 2-methoxybenzyl alcohol. Thus, reduction of the enone **7** by treatment with lithium (3 equiv) in liquid ammonia, followed by trapping the intermediary lithium enolate through reaction with the bromide **8** (6 equiv), furnished the expected coupling product **9** as the sole diastereomer in 72% yield. Subsequent Wittig methylenation of the sterically hindered carbonyl group in **9** was best achieved by employing a combination of methyltriphenylphosphonium bromide and potassium *tert*-butoxide[14] in refluxing benzene, providing *exo*-olefin **10** in 86% yield.

For elaborating the C8 stereocenter, the ethylene acetal moiety in **10** was first removed by acid treatment (97%), and the resulting ketone derivative **11** was subjected to hydrogenation (1 atm) over 10% Pd/C in 50:1 triethylamine−methanol at ambient temperature, which afforded a mixture of diastereomers that were separated by column chromatog-

(3) In addition to the in vitro activity, the in vivo anti-influenza virus activity of stachyflin (**1**) and its derivatives was also extensively studied by the Shionogi research group; see: (a) Yoshimoto, J.; Yagi, S.; Ono, J.; Sugita, K.; Hattori, N.; Fujioka, T.; Fujiwara, T.; Sugimoto, H.; Hashimoto, N. *J. Pharm. Pharmacol*. **2000**, *52*, 1247. (b) Yagi, S.; Ono, J.; Yoshimoto, J.; Sugita, K.; Hattori, N.; Fujioka, T.; Fujiwara, T.; Sugimoto, H.; Hirano, K.; Hashimoto, N. *Pharm. Res*. **1999**, *16*, 1041.

(4) (a) Yoshimoto, J.; Kakui, M.; Iwasaki, H.; Sugimoto, H.; Fujiwara, T.; Hattori, N. *Microbiol. Immunol*. **2000**, *44*, 677. (b) Yoshimoto, J.; Kakui, M.; Iwasaki, H.; Fujiwara, T.; Sugimoto, H.; Hattori, N. *Arch. Virol*. **1999**, *144*, 865.

(5) Pinto, L. H.; Holsinger, L. J.; Lamb, R. A. *Cell* **1992**, *69*, 517.

(6) Hayden, F. G.; Osterhaus, A. D.; Treanor, J. J.; Fleming, D. M.; Aoki, F. Y.; Nicholson, K. G.; Bohnen, A. M.; Hirst, H. M.; Keene, O.; Wightman, K. *N. Engl. J. Med*. **1997**, *337*, 874.

(7) Lew, W.; Chen, X.; Kim, C. U. *Curr. Med. Chem*. **2000**, *7*, 663.

(8) Taishi, T.; Takechi, S.; Mori, S. *Tetrahedron Lett*. **1998**, *39*, 4347.

(9) Preliminary structure−activity studies of stachyflin derivatives have been performed by the Shionogi research group; see ref 1c.

(10) Nakamura, M., Suzuki, A.; Nakatani, M.; Fuchikami, T.; Inoue, M.; Katoh, T. *Tetrahedron Lett*. **2002**, *43*, 6929.

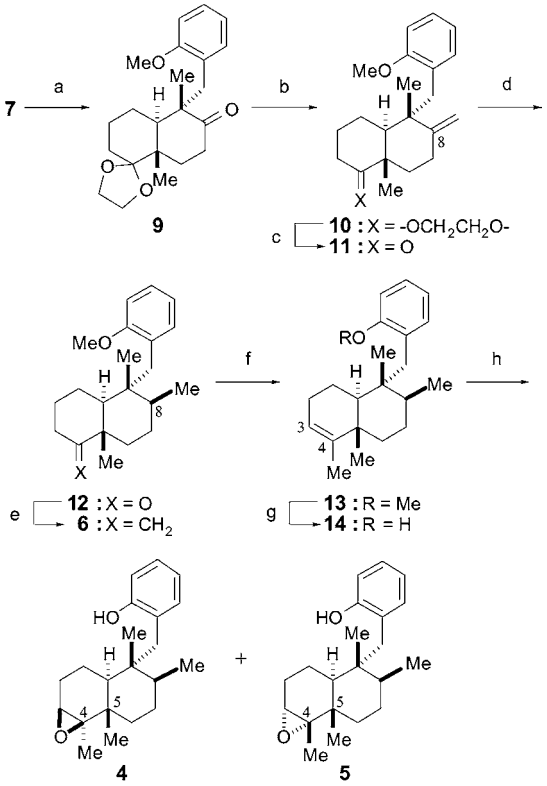
(11) Hagiwara, H.; Uda, H. *J. Org. Chem*. **1988**, *53*, 2308.

(12) Kelly, J. L.; Linn, J. A.; Selway, J. W. T. *J. Med. Chem*. **1989**, *32*, 1757.

(13) This type of reductive alkylation, originally developed by Stork et al. in the 1960s, has been widely utilized as a critical step in the total synthesis of marine sesquiterpene quinones and hydroquinones. For recent examples, see: (a) Ling, T.; Poupon, E.; Rueden, E. J.; Kim, S. H.; Theodorakis, E. A. *J. Am. Chem. Soc*. **2002**, *124*, 12261. (b) Stahl, P.; Kissau, L.; Mazitschek, R.; Huwe, A.; Furet, P.; Giannis, A.; Waldmann, H. *J. Am. Chem. Soc*. **2001**, *123*, 11586. (c) Poigny, S.; Guyot, M.; Samadi, M. *J. Org. Chem*. **1998**, *63*, 5890. (d) Locke, E. P.; Hecht, S. M. *Chem. Commun*. **1996**, 2717. (e) An, J.; Wiemer, D. F. *J. Org. Chem*. **1996**, *61*, 8775. (f) Bruner, S. D.; Radeke, H. S.; Tallarico, J. A.; Snapper, M. L. *J. Org. Chem*. **1995**, *60*, 1114. (g) Sarma, A. S.; Chattopadhyay, P. *J. Org. Chem*. **1982**, *47*, 1727.

(14) This reagent system is known to be effective for sterically hindered ketones; see: Fitjer, L.; Quabeck, U. *Synth. Commun*. **1985**, *15*, 855.

Scheme 2. Synthesis of Key Intermediates **4** and **5**[a]



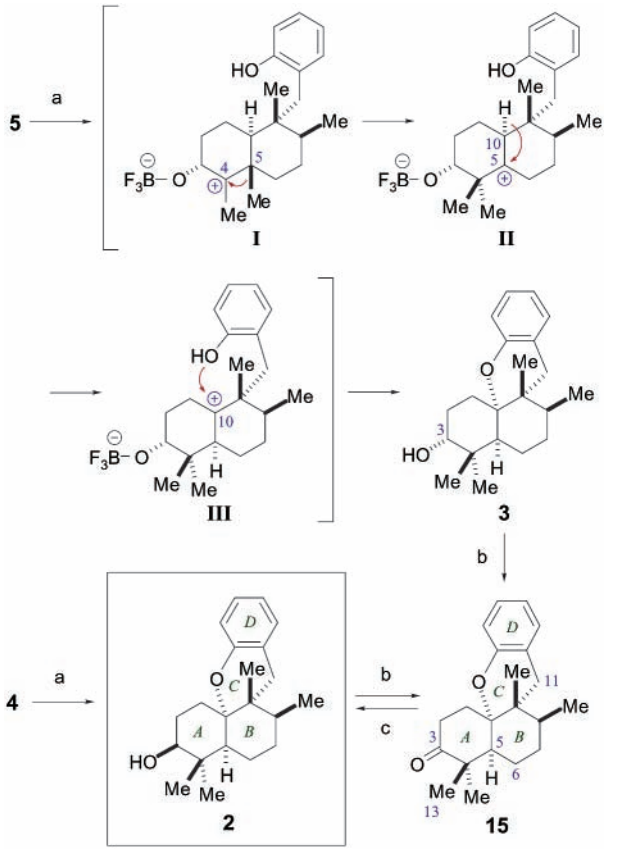
[a] Reagents and conditions: (a) Li, liquid NH_3, 2-methoxybenzyl bromide (**8**), THF, 72%. (b) $Ph_3P^+CH_3Br^-$, *t*-BuOK, benzene, reflux, 86%. (c) 4 M HCl, THF, rt, 97%. (d) H_2 (1 atm), 10% Pd−C, 50:1 Et_3N−MeOH, rt, 80%. (e) $Ph_3P^+CH_3Br^-$, *t*-BuOK, benzene, reflux, 100%. (f) $RhCl_3 \cdot 3H_2O$, EtOH, reflux, 100%. (g) *n*-BuSLi, HMPA, 100 °C, 84%. (h) MCPBA, $NaHCO_3$, CH_2Cl_2, 0 °C, 22% for **4**, 75% for **5**.

raphy on silica gel to give the desired product **12** (80%) and its C8 epimer (13%) in a ratio of ca. 6:1.[15]

Ketone **12** was further converted to the phenol derivative **14** having an olefinic bond at C3−C4 in 84% overall yield via a three-step sequence involving Wittig methylenation of the carbonyl group in **12**, rhodium-catalyzed isomerization[13a,b,d,e] of the resulting exocyclic olefin **6** to the more stable endocyclic olefin **13**, and demethylation of the phenolic *O*-methyl protecting group in **13** by reaction with lithium *n*-butylthiolate[10,16] in hexamethylphosphoramide (HMPA). Finally, treatment of *endo*-olefin **14** with *m*-chloroperbenzoic acid (MCPBA) in the presence of sodium hydrogen carbonate in dichloromethane at 0 °C led to the formation of a diastereomeric mixture of the desired epoxides **4** and **5**, which were separated by column chromatography on silica gel to produce *β*-epoxide **4** in 22% yield and α-epoxide **5** in 75% yield.[17] As mentioned earlier, we expected that α-epoxide **5** would be converted to the target compound **2** through the subsequent key acid-induced domino reaction followed by inversion of the C3 hydroxy group. Therefore, at this stage, we did not so much consider the stereoselectivity of this epoxidation reaction, and we proceeded with the projected synthesis to ascertain the feasibility of our devised synthetic strategy.

With epoxides **4** and **5** in hand, we next investigated the crucial acid-induced domino epoxide-opening/rearrangement/cyclization reaction as shown in Scheme 3. First, the major product, α-epoxide **5**, was subjected to the acid-induced domino reaction; thus, **5** was allowed to react with $BF_3 \cdot Et_2O$[18] (3 equiv) in dichloromethane at −30 °C for 30 min, which successfully led to the formation of the requisite cyclized compound **3** in a reasonable yield (41%).[19] To our knowledge, this is the first example of the direct construction of such a tetracyclic benzo[*d*]xanthene skeleton having a C3 hydroxy group. We believe that this domino transformation proceeds in a stepwise manner through carbocation intermediates such as **I**−**III**, transformation of which follows that described in our previous studies.[10] Thus, initial coordination−activation between the Lewis acid and the epoxide

Scheme 3. Synthesis of Tetracyclic Core **2** through the Key $BF_3 \cdot Et_2O$-Induced Domino Epoxide-Opening/Rearrangement/Cyclization Reaction of **4** and **5**.[a]



[a] Reagents and conditions: (a) $BF_3 \cdot Et_2O$, CH_2Cl_2, −30 °C, 41% for **5**→**3**, 15% for **4**→**2**. (b) Dess−Martin periodinane, CH_2Cl_2, rt, 87% for **3**→**15**, 85% for **2**→**15**. (c) H_2 (1 atm), PtO_2, 5:1 EtOH−$CHCl_3$, rt, 42% for **2**, 28% for **3**.

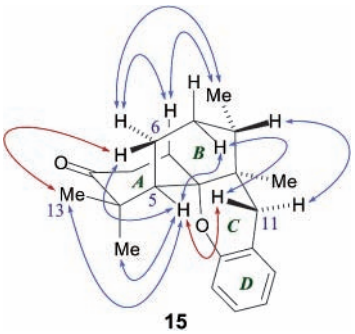
(15) When ethylene acetal **10** was used as a substrate for this hydrogenation under the same reaction conditions, a considerable decrease in the stereoselectivity at C8 was observed (*β*-Me:α-Me = ca. 2:1); this is probably due to an unfavorable conformation of the decalin ring. For similar examples of this phenomenon, see ref 13b,c,d,e.

(16) Katoh, T.; Nakatani, M.; Shikita, S.; Sampe, R.; Ishiwata, A.; Ohmori, O.; Nakamura. M.; Terashima, S. *Org. Lett.* **2001**, *3*, 2701.

moiety of **5** would lead to epoxide ring-opening and the formation of intermediate **I**, which then would provide intermediate **II** via migration of the C5 methyl group to the C4 carbocation center. Intermediate **II** would undergo a 1,2-hydride shift from the C10 position to the C5 cationic carbon center to furnish intermediate **III**, wherein the C10 carbocation center would be trapped by the inner phenolic hydroxy group to deliver, after elimination of the Lewis acid, the requisite cyclized product **3**.

As we expected, compound **3** could be converted to the target compound **2** via inversion of the C3 hydroxy group.[20] Thus, Dess−Martin oxidation of **3** provided the corresponding ketone **15** in 87% yield, which upon hydrogenation (1 atm) over Pt_2O[8] in 5:1 ethanol−chloroform at ambient temperature furnished, after separation by silica gel column chromatography, the desired **2** in 42% yield along with its C3 epimer **3** (28%).[21] On the other hand, the acid-induced domino reaction of the minor product, β-epoxide **4**, under the same reaction conditions as that described for the conversion of **5** to **3** resulted in production of cyclized product **2** in poor yield (15%).[19,22]

The structure and stereochemistry of cyclized products **2** and **3** were unambiguously confirmed by extensive spectroscopic analysis including NOESY experiment in the 500 MHz ^{1}H NMR spectrum of the ketone **15** derived from both **2** and **3** via Dess−Martin oxidation.[23] The selected NOESY correlation of **15** is depicted in Figure 2, wherein two key NOEs between Me-13 and H-6 and between H-5 and H-11



15

Figure 2. Selected NOESY correlation of the ketone **15**.

(indicated with red-colored arrows) established the *cis*-fused connectivity of the decalin ring (AB ring system).[24]

In summary, we have demonstrated the feasibility of our novel strategy toward (+)-stachyflin (**1**) through an enantioselective synthesis of the tetracyclic core structure **2**. The synthetic method features a BF_3·Et_2O-induced domino epoxide-opening/rearrangement/cyclization reaction as the key step. On the basis of the explored strategy, total synthesis of optically active stachyflin and its analogues is currently under investigation in our laboratory.

Acknowledgment. We are grateful to Prof. H. Miyaoka and Dr. H. Mitome, Tokyo University of Pharmacy and Life Science, for valuable suggestions. We also thank Dr. N. Sugimoto and Dr. N. Kawahara, National Institute of Health Science, for measurements of the high-resolution mass spectra and assistance with NMR experiments, respectively.

Note Added after ASAP Posting. In Scheme 3, formulation II, a bond was missing in the version posted ASAP on November 16, 2002; the corrected version was posted November 20, 2002.

Supporting Information Available: Detailed experimental procedures and full characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) The stereochemistry of epoxides **4** and **5** was proven by NOESY experiment in their 500 MHz ^{1}H NMR spectra. Thus, significant NOE interactions between the signals due to the C4 methyl group and the C5 methyl group were observed in α-epoxide **5**. On the other hand, no NOE correlation between the C4 methyl group and the C5 methyl group was detected in β-epoxide **4**.

(18) We preliminarily examined this acid-induced domino reaction using several Lewis acids or Brønsted acids such as BF_3·Et_2O, $AlCl_3$, $MeAlCl_2$, $TiCl_4$, TMSOTf, $Sc(OTf)_3$, *p*-TsOH·H_2O, camphorsulfonic acid, and CF_3SO_3H; among them, BF_3·Et_2O was found to give the best result.

(19) In this reaction, disappearance of the starting material could be ascertained by TLC analysis, while unidentified byproducts were produced along with the desired rearrangement/cyclization product.

(20) Attempts to invert the C3 hydroxy group by employing the Mitsunobu protocol were unsuccessful, presumably due to steric factors.

(21) When ketone **15** was reduced with $NaBH_4$, the undesired C3 epimer **3** was predominantly produced (**3**:**2** = ca. 4:1).

(22) Further investigation is required to optimize the yield of the cyclized product **2**; this is the subject of our current attention.

(23) Structural elucidation of cyclized products **2** and **3** was made at the stage of ketone **15** because some crucial signals were unfortunately overlapped in the 500 MHz ^{1}H NMR spectra of **2** and **3**.

(24) Similar NOESY correlation has been reported for the structural determination of stachyflin (**1**); see ref 1b.