# Total synthesis of (+)-stachyflin: a potential *anti*-influenza A virus agent†

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**The first enantioselective total synthesis of (+)-stachyflin, a potential *anti*-influenza A virus agent, was achieved; the method features a $BF_3{\cdot}Et_2O$-induced domino epoxide-opening/rearrangement/cyclization reaction to stereoselectively form the requisite pentacyclic ring system in one step.**

Influenza has always remained a major health problem. The emergence of the fatal H5N1 avian influenza virus and the recent outbreak of the new H1N1 human influenza virus have raised worldwide concerns regarding the development of alternative and more efficacious *anti*-influenza agents.[1]

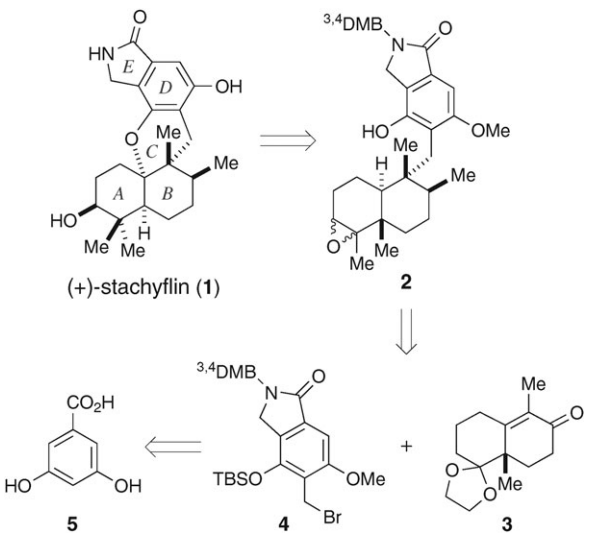
Stachyflin (**1**, Scheme 1) is a novel sesquiterpenoidal alkaloid isolated from a culture broth of *Stachybotrys* sp. RF-7260 by the Shionogi research group.[2] This substance was found to exhibit extremely potent antiviral activity against influenza A/WSN/33 (H1N1) virus ($IC_{50}$ = 0.003 μM) with a unique mode of action.[3,4] The antiviral action of **1** has been shown to inhibit the hemagglutinin (HA)-mediated 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.[5] This mechanism is quite different from that of the approved *anti*-influenza virus agents such as amantadine (Symmetrel®), zanamivir (Relenza®) and oseltamivir (Tamiflu®).[6] Stachyflin, therefore, is anticipated to be a promising candidate or a new lead for novel molecular-targeted *anti*-influenza A virus agents.[6]

The gross structure and relative stereochemistry of **1** has been determined by extensive spectroscopic studies (COSY, HMBC and NOESY spectra) and X-ray crystallographic analysis.[2a,b] This natural product possesses a novel pentacyclic 3*H*-naphtho[1′,8′a:5,6]pyrano[2,3-*e*]isoindol-3(2*H*)-one skeleton (ABCDE ring system) involving five asymmetric carbons, with the special characteristic features of *cis*-fused AB and BC rings, and an ether bond at the bridgehead of the AB ring junction.[2a,b] The absolute configuration of **1** was determined by circular dichroism (CD) spectrum as depicted in Scheme 1.[2b]

These desirable biological properties and unique structural features prompted us to undertake a project directed towards the total synthesis of (+)-**1** in an enantiomerically pure form.[7] To date, only one total synthesis of racemic (±)-**1** has been reported by the Shionogi research group,[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 have recently demonstrated the feasibility of our synthetic strategy towards (+)-**1** through construction of the tetracyclic model compound (ABCD ring system).[9] Herein, we have described the realization of the first enantioselective total synthesis of (+)-**1**.

Scheme 1 outlines our synthetic plan for (+)-**1** based on a novel and expeditious approach: synthesizing the target molecule **1** from epoxide **2** through an acid-induced domino epoxide-opening/rearrangement/cyclization reaction (see **2** → [**I** → **II** → **III**] → **19a,b** in Scheme 4) to stereoselectively construct the requisite ABCDE ring system with *cis*-fused AB- and BC-ring junctions in one step. Epoxide **2** possessing the *trans*-fused decalin system, in turn, could be prepared through stereocontrolled reductive alkylation of the known enone **3**[10] with bromide **4** accessible from 3,5-dihydroxybenzoic acid (**5**).

We first pursued the synthesis of bromide **4** starting from commercially available **5** (Scheme 2). The Kolbe–Schmitt reaction of **5** followed by methyl esterification produced dimethyl ester **6**, which was then converted to acetonide **7** *via* a three-step sequence involving selective reduction of the C4 methyl ester function, acetonide formation and methyl etherification. Subsequent regioselective bromination at C2 in **7** followed by reaction with cuprous cyanide provided nitrile **8**. Isoindolinone **9** was elaborated by hydrogenation of **8** and subsequent lactamization with a base. After protection of the lactam amide moiety in **9**,[11] the resulting $^{3,4}$DMB amide **10**[12]




**Scheme 1** Synthetic plan for (+)-stachyflin (**1**). $^{3,4}$DMB = 3,4-dimethoxybenzyl, TBS = *tert*-butyldimethylsilyl.

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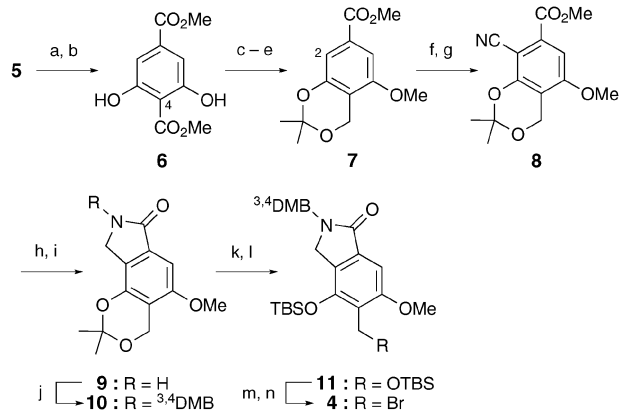
† Electronic supplementary information (ESI) available: Experimental procedures and characterization data for all new compounds along with copies of $^1$H and $^{13}$C NMR spectra. See DOI: 10.1039/c000193g

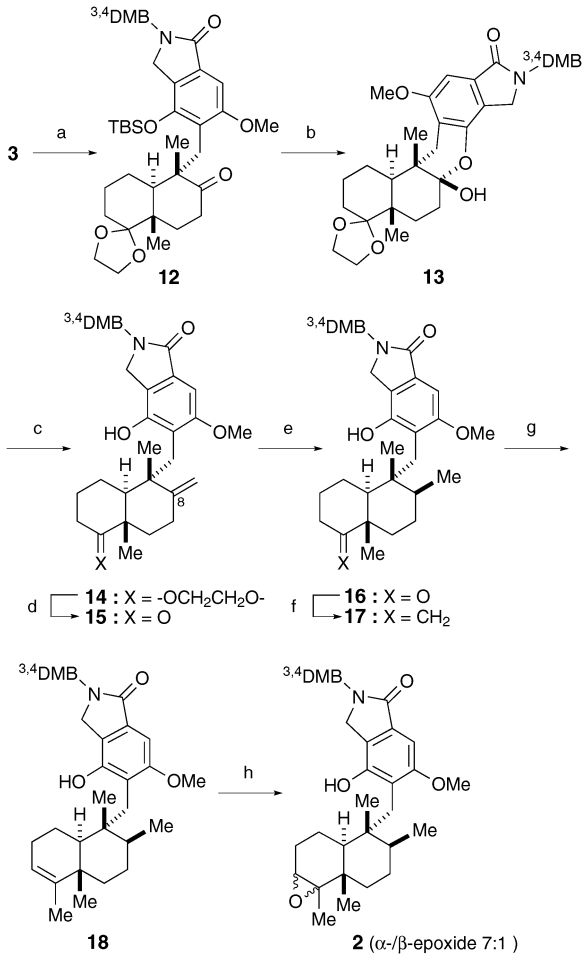


**Scheme 2** Synthesis of bromide **4**. *Reagents and conditions*: (a) $CO_2$, $KHCO_3$, glycerol, 180 °C; (b) $Me_2SO_4$, $KHCO_3$, acetone, reflux, 64% (2 steps); (c) $NaBH_4$, THF–$H_2O$, rt; (d) 2,2-dimethoxypropane, *p*-TsOH, rt; (e) MeI, $K_2CO_3$, acetone, rt, 79% (3 steps); (f) NBS, MeCN, 0 °C; (g) CuCN, DMF, 120 °C, 66% (2 steps); (h) $H_2$, $PtO_2$, EtOH–$CHCl_3$, rt; (i) NaOMe, MeOH, rt, 98% (2 steps); (j) 3,4-dimethoxybenzyl chloride, $NaN(SiMe_3)_2$, $Bu_4NI$, THF, 0 °C to rt, 78%; (k) 2 M HCl, THF, rt; (l) TBSCl, imidazole, DMF, rt, 75% (2 steps); (m) 48% aq HF, MeCN, 0 °C; (n) $PPh_3$, $CBr_4$, $CH_2Cl_2$, rt, 86% (2 steps). *p*-TsOH = *p*-toluenesulfonic acid, NBS = *N*-bromosuccinimide, DMF = *N*,*N*-dimethylformamide.

was transformed to bis-TBS ether **11** by acetonide cleavage and silylation of the two liberated hydroxy groups. Subsequent regioselective desilylation of **11** followed by bromination of the resulting benzylic alcohol provided the requisite bromide **4**.

After synthesizing bromide **4**, we performed the synthesis of epoxide **2**, the substrate for the key acid-induced domino reaction (Scheme 3). The critical reductive alkylation of enone **3**[10] (>99% *ee*) with **4** under Birch conditions proceeded smoothly and cleanly.[13] The expected coupling product **12** was obtained as the single diastereomer in 76% yield. Deprotection of the TBS group in **12** followed by Wittig methylenation of the resulting hemiacetal **13** produced *exo*-olefin **14**. To establish the C8 stereogenic center, the ethylene acetal moiety in **14** was first removed by acid treatment, and the resulting ketone **15** was subjected to hydrogenation [$H_2$ (1 atm), 10% Pd/C, $Et_3N$/MeOH 50 : 1, rt], which afforded the desired product **16** in 96% yield as the single diastereomer. Subsequent Wittig methylenation of **16** provided *exo*-olefin **17**, which was then converted to *endo*-olefin **18** by isomerization of the olefinic double bond. Finally, epoxidation of **18** with *m*CPBA afforded the desired epoxide **2** in 86% yield as an inseparable mixture of α- and β-epoxides (7 : 1 by 400 MHz $^1$H NMR).

With epoxide **2** in hand, the stage was set for the most crucial acid-induced domino epoxide-opening/rearrangement/cyclization event (Scheme 4). This domino reaction was successfully performed by treating **2** (α-/β-epoxide 7 : 1) with $BF_3{\cdot}Et_2O$[14] (10 equiv) in $CH_2Cl_2$ at −40 °C to room temperature for 2 h, which resulted in the formation of the desired products—**19a** (C3 α–OH) and **19b** (C3 β–OH), which were readily separated by silica gel column chromatography (66% yield for **19a**, 9% yield for **19b**). We believe that this domino sequence proceeds in a stepwise manner through the

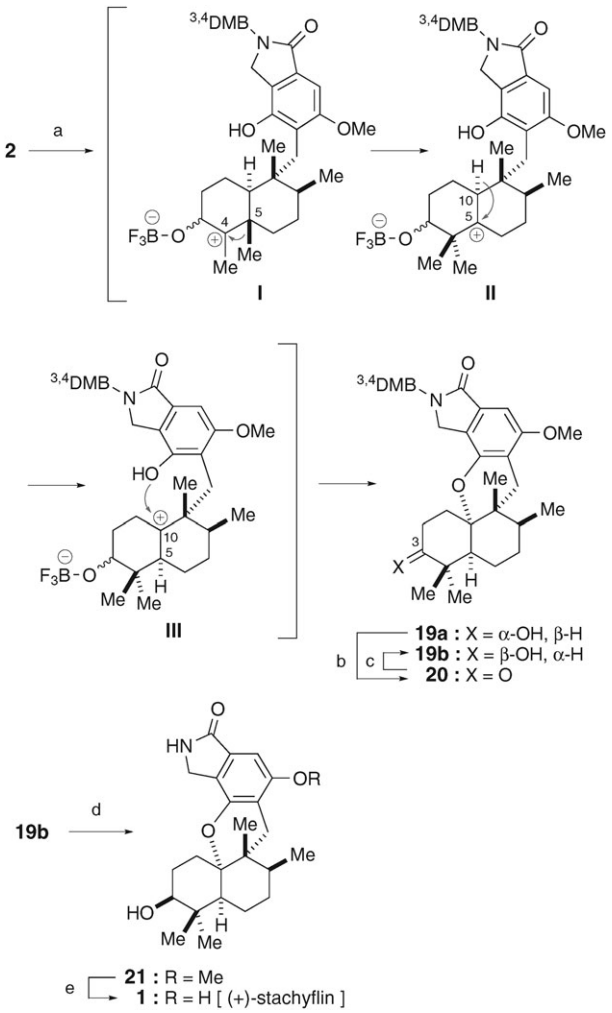


**Scheme 3** Synthesis of epoxide **2**. *Reagents and conditions*: (a) Li, liq. $NH_3$/THF, −78 to −30 °C; isoprene, $H_2O$, **4**, −30 °C to rt, 76%; (b) TBAF, THF, 0 °C to rt, 88%; (c) $Ph_3P^+CH_3Br^-$, *t*-BuOK, benzene, reflux, 86%; (d) 4 M HCl, THF, rt, 98%; (e) $H_2$ (1 atm), 10% Pd/C, $Et_3N$/MeOH 50 : 1, rt, 96%; (f) $Ph_3P^+CH_3Br^-$, *t*-BuOK, benzene, reflux, 74%; (g) $RhCl_3{\cdot}3H_2O$, EtOH, reflux, 100%; (h) *m*CPBA, $NaHCO_3$, $CH_2Cl_2$, −40 to −5 °C, 86% (α-/β-epoxide 7 : 1). TBAF = tetrabutylammonium fluoride, *m*CPBA = *m*-chloroperbenzoic acid.

carbocation intermediates such as **I**, **II** and **III**.[15] Thus, the first coordination-activation between the Lewis acid and the epoxide moiety of **2** would lead to an epoxide ring-opening and the formation of intermediate **I**, which would further produce intermediate **II** *via* migration of the C5 methyl group to the C4 carbocation centre. Intermediate **II** would undergo a 1,2-hydride shift from the C10 position to the C5 carbocation centre on the α-face of the molecule to provide intermediate **III**, wherein the C10 carbocation centre would be trapped by the inner phenolic hydroxy group to deliver, after the elimination of the Lewis acid, the desired cyclized products **19a,b**.

To continue the synthesis, inversion of the configuration at the C3 hydroxy group in **19a** was performed by Dess–Martin oxidation (94% yield) followed by lithium tri-*tert*-butoxyaluminium hydride reduction of the resulting ketone **20** with complete stereoselectivity, providing the desired **19b** (96% yield). Finally, compound **19b** was converted to (+)-stachyflin (**1**)[16] *via* a two-step operation involving deprotection of the $^{3,4}$DMB group using a hypervalent iodine(III) reagent[17]




**Scheme 4** Synthesis of (+)-stachyflin (**1**) through the key $BF_3{\cdot}Et_2O$-induced domino epoxide-opening/rearrangement/cyclization reaction of **2**. *Reagents and conditions*: (a) $BF_3{\cdot}Et_2O$, $CH_2Cl_2$, −40 °C to rt, 66% for **19a**, 9% for **19b**; (b) Dess–Martin periodinane, $CH_2Cl_2$, rt, 94%; (c) $LiAlH(t\text{-}BuO)_3$, THF, −20 °C, 96%; (d) PIFA, $CH_2Cl_2$, rt, 54%; (e) *n*-BuSLi, HMPA, 110 °C, 80%. PIFA = [bis(trifluoroacetoxy)iodo]benzene, HMPA = hexamethylphosphorous triamide.

(54% yield) followed by cleavage of the *O*-methyl moiety in the resulting lactam **21** (80% yield).

In conclusion, we accomplished the enantioselective total synthesis of (+)-stachyflin (**1**) in a highly efficient and convergent manner. The key transformation involved a novel acid-induced domino epoxide-opening/rearrangement/cyclization reaction (**2** → [**I** → **II** → **III**] → **19a,b**). Importantly, the synthesis has the potential for producing stachyflin analogues for the development of novel *anti*-influenza virus agents.

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## Notes and references


1 For a review on influenza virus infections, see: G. Neumann, T. Noda and Y. Kawaoka, *Nature*, 2009, **459**, 931–939.

2 (*a*) T. Kamigauchi, T. Fujiwara, H. Tani, Y. Kawamura and I. Horibe, Shionogi & Co., Ltd., Japan, PCT WO 9711947 A1, April 3, 1997; (*b*) K. Minagawa, S. Kouzuki, J. Yoshimoto, Y. Kawamura, H. Tani, T. Iwata, Y. Terui, H. Nakai, S. Yagi, N. Hattori, T. Fujiwara and T. Kamigauchi, *J. Antibiot*., 2002, **55**, 155–164; (*c*) K. Minagawa, S. Kouzuki and T. Kamigauchi, *J. Antibiot*., 2002, **55**, 165–171.

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

4 The *in vivo anti*-influenza virus activity of **1** has been also reported; see: (*a*) J. Yoshimoto, S. Yagi, J. Ono, K. Sugita, N. Hattori, T. Fujioka, T. Fujiwara, H. Sugimoto and N. Hashimoto, *J. Pharm. Pharmacol*., 2000, **52**, 1247–1255; (*b*) S. Yagi, J. Ono, J. Yoshimoto, K. Sugita, N. Hattori, T. Fujioka, T. Fujiwara, H. Sugimoto, K. Hirano and N. Hashimoto, *Pharm. Res*., 1999, **16**, 1041–1046.

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7 It has been reported that the *anti*-influenza A virus activity of racemic (±)-**1** is about half of that of natural (+)-**1** (see ref. 8), implying that unnatural (–)-**1** shows no potent *anti*-influenza A virus activity.

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11 Protection of the lactam amide was indispensable due to the low solubility of **9** in organic solvents.

12 After screening several protecting groups, the $^{3,4}$DMB group was found to be suitable for a later deprotection step (see **19b** → **21** in Scheme 4).

13 This type of reductive alkylation has been successfully utilized as a crucial step in our total synthesis of marine sesquiterpene quinones and hydroquinones; see: J. Sakurai, T. Oguchi, K. Watanabe, H. Abe, S. Kanno, M. Ishikawa and T. Katoh, *Chem.–Eur. J*., 2008, **14**, 829–837.

14 In our preliminary model studies (see ref. 9), $BF_3{\cdot}Et_2O$ gave the best result among several Lewis and Brønsted acids examined. Detailed results and discussion will be reported in a full account.

15 For a recent review on cationic domino reactions, see: L. F. Tietze, G. Brasche and K. M. Gericke, in *Domino Reactions in Organic Synthesis*, Wiley-VCH, Weinheim, 2006, pp. 11–42.

16 The optical rotation was $[\alpha]_D^{24}$ + 133.3 (*c* 0.46 in MeOH), and that ofnatural **1** was $[\alpha]_D^{24}$ + 138.7 (*c* 1.00 in MeOH). The spectroscopic properties (IR, $^1$H and $^{13}$C NMR, and HRMS) of synthetic sample **1** were identical to those of the natural product, which was kindly provided by Dr Kazuyuki Minagawa, Shionogi & Co., Ltd. (see ESI†).

17 The use of standard reagents such as ammonium cerium(IV) nitrate (CAN) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) resulted in failure. In the case of CAN, decomposition products were generated, while as per DDQ the starting material was recovered.