JOC Article

Total Synthesis and Structure Revision of *Stachybotrys* Spirolactams

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The spirolactam structure **1** reported in 1996 by Roggo et al. has been enantioselectively synthesized in 20 steps from (+)-Wieland−Miescher ketone. Spectra of this synthetic lactam differed from those of the natural spirolactam from the Roggo group, leading to the hypothesis that the natural lactam may be the regioisomer **27**, a structure previously ascribed by Jarvis et al. to the natural product stachybotrylactam. This hypothesis was confirmed by the first total synthesis of (−)-stachybotrylactam (**27**). Double reductive amination of two molecules of aldehyde **29** with one molecule of L-lysine led by analogous chemistry to the first synthesis of the pseudosymmetric "dimer" **30**, whose spectra corresponded to those of the natural "dimer" originally assigned structure **5** by the Roggo group.

Introduction

Bioactive fungal metabolites from diverse species of the genus *Stachybotrys*[1] have long attracted attention since pioneering work on the satratoxins (from *Stachybotrys atra*)[2] and the complement inhibitor K-76 (from *Stachybotrys complementi, nov. sp.* K-76).[3] More recently, a series of novel bioactive compounds have been isolated from *Stachybotrys* species (Figure 1) exemplified by stachybotrins (staplabins),[4] L-671,776,[5] stachyflins,[6] stachybocins (A−C),[7] stachybotrylactam,[8] and a group of spirodihydrobenzofuranlactams represented by structures **1**−**5**.[9] We were intrigued by the structures of spirodihydrobenzofuranlactams **1**−**5**, isolated from the cultures of two different *Stachybotrys* species by Roggo et al. in 1996, since these compounds were claimed to possess a D−E ring fusion which differed in regiochemistry from that of stachybotrylactam and the stachybocins. However, the ET-A receptor binding data reported for stachybocin A by Nakamura et al.[7a] were very similar to those of spirolactam **5** tested by Roggo.[9b] Moreover, these spirodihydrobenzofuranlactams **1**−**5** were reported to contain the same A−B−C−D ring core with the unique axial C(19) hydroxyl group as found in stachybocin **A** and stachybotrylactam.

The claimed pharmacological activities and undetermined absolute configuration of spirolactams **1**−**5** prompted us to examine synthetic access to these compounds. In our preliminary communication,[10] we reported the enantioselective total synthesis and structure revision of spirodihydrobenzofuranlactam **1**. In that work, we described that structure **1** does not correspond to the spirolactam isolated by Roggo, and we subsequently demonstrated by total synthesis of its regioisomer that the Roggo spirolactam **1** possessed D−E ring regiochemistry analogous to that assigned to stachybocin A and was identical to stachybotrylactam. This finding left open the question of the relationship between spirolactam **5**, reported by Roggo,[9] and the similar substance stachybo-

(1) For background about the fungus *Stachybotrys* species, see: Hinkley, S. F.; Mazzola, E. P.; Fettinger, J. C.; Lam, Y.-F.; Jarvis, B. B. *Phytochemistry* **2000**, *55*, 663 and cited references.

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(3) (a) Kaise, H.; Shinohara, M.; Miyazaki, W.; Izawa, T.; Nakano, Y.; Sugawara, M.; Sugiura, K.; Sasaki, K. *J. Chem. Soc., Chem. Commun.* **1979**, 726. (b) Hong, K.; Kinoshita, T.; Miyazaki, W.; Izawa, T.; Inoue, K. *J. Immunol.* **1979**, *122*, 2418. For total synthesis of racemic K-76, see: (c) Corey, E. J.; Das, J. *J. Am. Chem. Soc.* **1982**, *104*, 5551. (d) McMurry, J. E.; Erion, M. D. *J. Am. Chem. Soc.* **1985**, *107*, 2712.

(4) (a) Xu, X.; de Guzman, F. S.; Gloer, J. B. *J. Org. Chem.* **1992**, *57*, 6700. (b) Nozawa, Y.; Ito, M.; Sugawara, K.; Hanada, K.; Mizoue, K. *J. Antibiot.* **1997**, *50*, 641. (c) Kohyama, T.; Hasumi, K.; Hamanaka, A.; Endo, A. *J. Antibiot.* **1997**, *50*, 172. (d) Hu, W.; Ohyama, S.; Hasumi, K. *J. Antibiot.* **2000**, *53*, 241. (e) Hu, W.; Narasaki, R.; Ohyama, S.; Hasumi, K. *J. Antibiot.* **2001**, *54*, 962.

(5) (a) Pietrre, S. R.; Ganzhorn, A.; Hoflack, J.; Islam, K.; Hornsperger, J.-M. *J. Am. Chem. Soc.* **1997**, *119*, 3201 and cited references. (b) For synthesis and structure revision of L-671,776, see: (c) Falck, J. R.; Reddy, K. K.; Chandrasekhar, S. *Tetrahedron Lett.* **1997**, *38*, 5245.

(6) (a) Minagawa, K.; Kouzuki, S.; Tani, H.; Ishii, K.; Tanimoto, T.; Terui, Y.; Kamigauchi, T. *J. Antibiot.* **2002**, *55*, 239 and cited references. For first total synthesis of racemic stachyflin, see: (b) Taishi, T.; Takechi, S.; Mori, S. *Tetrahedron Lett.* **1998**, *39*, 4347.

(7) (a) Nakamura, M.; Ito, Y.; Ogawa, K.; Michisuj, Y.; Sato, S.-I.; Takada, M.; Hayashi, M.; Yaginuma, S.; Yamamoto, S. *J. Antibiot.* **1995**, *48*, 1389. (b) Ogawa, K.; Nakamura, M.; Hayashi, M.; Yaginuma, S.; Yamamoto, S.; Furihata, K.; Shin-ya, K.; Seto, H. *J. Antibiot.* **1995**, *48*, 1396.

(8) (a) Jarvis, B. B.; Salemme, J.; Morais, A. *Nat. Toxins* **1995**, *3*, 10. For other papers about fungal metabolites from *Stachybotrys*, see: (b) Ayer, W. A.; Miao, S. *Can. J. Chem.* **1993**, *71*, 487. (c) Sakai, K.; Watanabe, K.; Masuda, K.; Tsuji, M.; Hasumi, K.; Endo, A. *J. Antibiot.* **1995**, *48*, 447. (d) Kaneto, R.; Dobashi, K.; Kojima, I.; Sakai, K.; Shibamoto, N.; Yoshioka, T.; Nishida, H.; Okamoto, R.; Akagawa, H.; Mizuno, S. *J. Antibiot.* **1994**, *47*, 727. (e) Kamalov, L. S.; Aripova, S. F.; Isaev, M. I. *Chem. Nat. Compd.* **1999**, *35*, 82.

(9) (a) Roggo, B. E.; Petersen, F.; Sills, M.; Roesel, J. L.; Moerker, T.; Peter, H. H. *J. Antibiot.* **1996**, *49*, 13. (b) Roggo, B. E.; Hug, P.; Moss, S.; Stampfli, A.; Kriemler, H.-P.; Peter, H. H. *J. Antibiot.* **1996**, *49*, 374.

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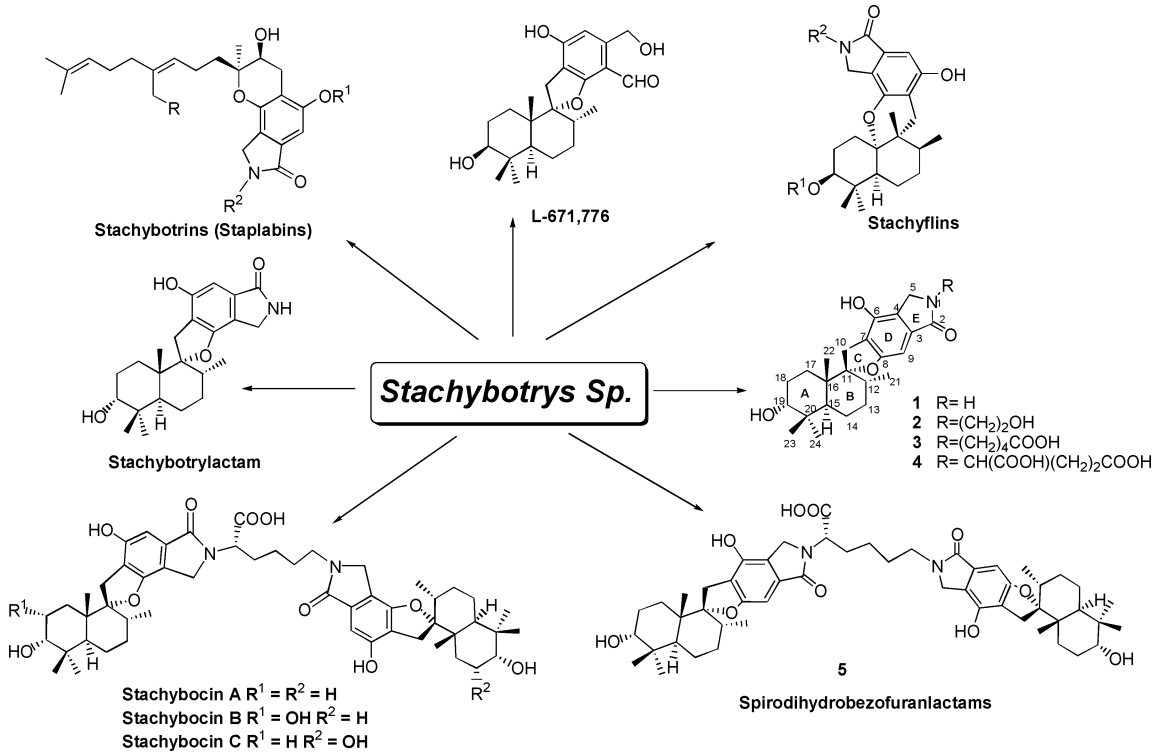
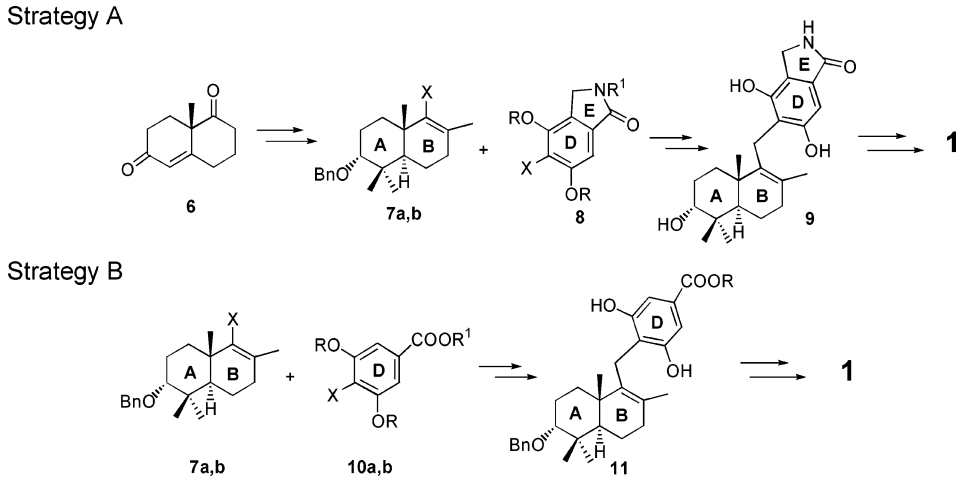


FIGURE 1.

SCHEME 1



cin A.[7] We now report our synthetic endeavors in this series. In our approach to the enantioselective total synthesis of these spirolactams **1**−**5**, we utilized the readily available (+)-Wieland−Miescher ketone **6**[11] to construct the AB bicyclic synthon **7a,b** (**a**, X = I; **b**, X = CHO). At this point, two independent strategies seemed feasible (Scheme 1). In the more convergent approach **A**, a bicyclic DE lactam equivalent such as **8** would be added to **7** to form an ABDE moiety such as **9**, which would be cyclized to form the required C ring. In strategy **B**, a resorcylic acid synthon **10a,b** (**a**, X = CHO; **b**, X = Br) would react with AB synthon **7** to create the tricylic ABD system **11**. Subsequent C-ring cyclization followed by lactam annulation would give the target, spirolactam **1**.

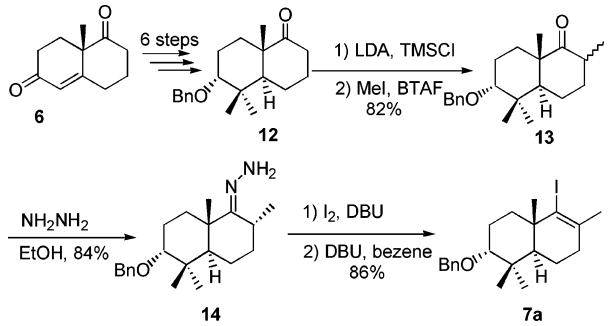
(11) Buchschacher, P.; Fürst, A.; Gutzwiller, J. *Organic Syntheses*; Wiley: New York, 1990; Collect. Vol. VII, p 368.

Results and Discussion

1. Synthesis of Spirolactam 1. Preparation of the AB bicyclic segment **7a** (Scheme 2) proceeded by conversion of enantiopure (+)-Wieland−Miescher ketone **6** (>98% ee) to the *trans*-decalone **12**.[12−14] Ketone **12** was then converted to the trimethylsilyl enol ether which was treated with benzyltrimethylammonium fluoride (BTAF) and methyl iodide[12] to give a chromatographically inseparable 9:1 mixture of the **13**α/**13**β methyl derivatives **13** in 82% yield. This mixture was reacted with hydrazine

(12) Smith, A. B., III; Mewshaw, R. *J. Org. Chem.* **1984**, *49*, 3685.
(13) (a) Kirk, D. N.; Petrow, V. *J. Chem. Soc.* **1962**, 1091. (b) Shimizu, T.; Hiranuma, S.; Hayashibe, S.; Yoshioka, H. *Synlett.* **1991**, 833.
(14) Yusui, K.; Kawada, K.; Kagawa, K.; Tokura, K.; Kitadokoro, K.; Ikenishi, Y. *Chem. Pharm. Bull.* **1993**, *41*, 1698.

SCHEME 2



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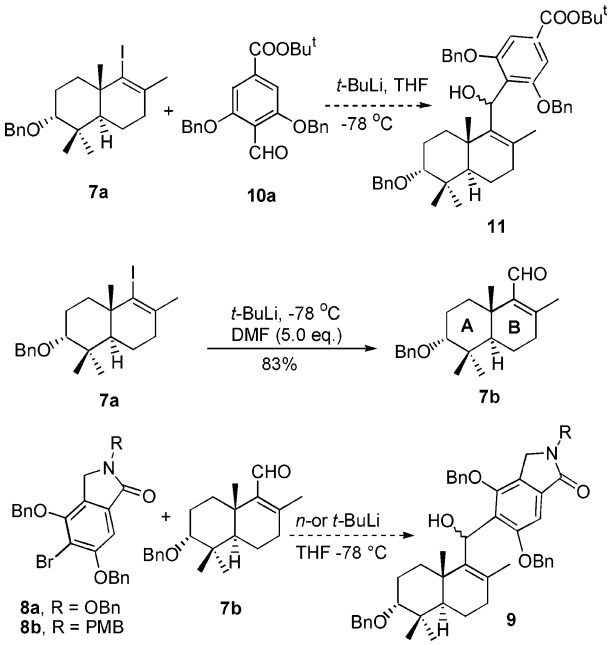
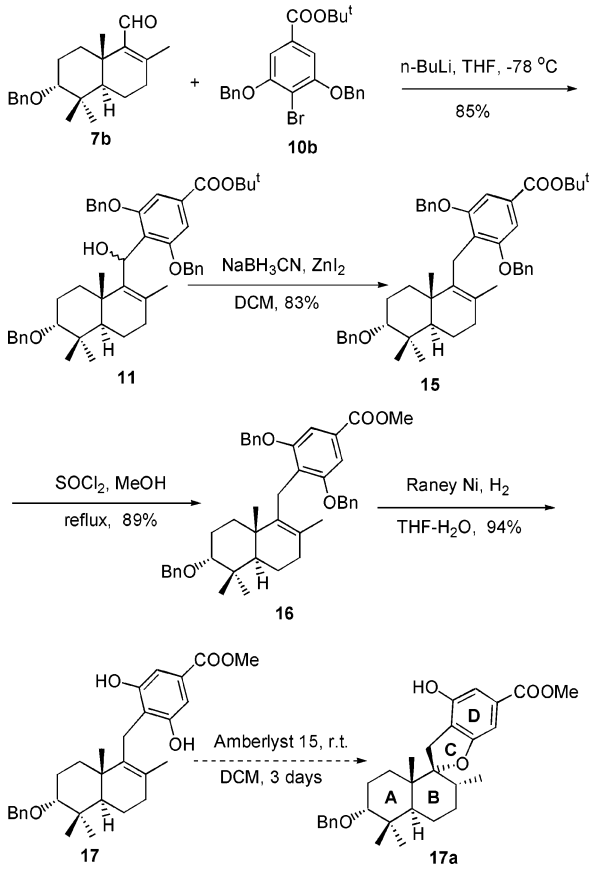


in refluxing ethanol to produce the thermodynamically stable hydrazone **14** in 84% yield. Treatment of the latter with iodine and DBU using the Danishefsky protocol gave the vinyl iodide **7a** in 86% yield, accompanied by 5−10% of chromatographically inseparable deiodinated alkene.[15]

At this point, iodide **7a** was lithiated using *t*-BuLi and the lithioalkene was allowed to react with methyl 4-formyl-3,5-dibenzyloxybenzoate **10a**.[16] No aldehyde addition to form **11** was observed, possibly due to the steric repulsion between the angular methyl and the *ortho* benzyloxy groups on the benzene ring (Scheme 3). Alternatively, a formyl group was installed on the AB ring system by reaction of the above lithioalkene with DMF, which gave the α,*β*-unsaturated aldehyde **7b** in 83% yield. When aldehyde **7b** was treated with the lithium derivatives of lactam **8a** or **8b**,[17] no evidence for addition to the aldehyde **7b** was observed.

In view of the above impasse, we turned to strategy **B** of Scheme 1. Low-temperature lithiation of *tert*-butyl-4-bromo-3,5-dibenzyloxybenzoate **10b**[18] followed by quenching with α, *β*-unsaturated aldehyde **7b** gave carbinol **11** in 85% yield (Scheme 4). The secondary benzylic alcohol was deoxygenated to **15** using the $NaBH_3CN/ZnI_2$ system,[19] and treatment of *tert*-butyl ester **15** with thionyl chloride in excess methanol produced the methyl ester **16**. At this point, attempts to hydrogenolyze the two phenolic benzyl ethers using 10% Pd/C or $Pd(OH)_2$ failed to give desired chemoselectivity. However, Raney nickel[19] selectively removed only the phenolic benzyl groups to give the dihydroxy ester **17**. Unfortunately, sustained efforts to cyclize the phenolic alkene system of **17** to generate the C-ring using Corey's method (THF/ethylene glycol, 2*N* HCl)[3c] or McMurry's method (Amberlyst 15/DCM),[3d] which were employed for synthesis of K-76, or other acidic conditions (TFA/$CHCl_3$; $Hg(OCOCF_3)_2$/THF; PTS/benzene), yielded only starting material or intractable mixtures.

SCHEME 4



To explore the reason for the striking failure to cyclize **17**, its X-ray single-crystal structure was determined (Scheme 5). We noted that the *O*-benzyl group at C(19) and the benzylic side chain at C(11) were both found to lie on the same side of the AB-bicyclic core, suggesting that their steric interaction could have interfered with the desired spiroannulation. Therefore, compound **18** was prepared in quantitative yield by hydrogenolysis of the *O*-benzyl group using 10% Pd/C. Spiroannulation now proceeded smoothly when the trihydroxy compound **18**

(15) (a) Di Grandi, M. J.; Jung, D. K.; Krol, W. J.; Danishefsky, S. J. *J. Org. Chem.* **1993**, *58*, 4989. (b) Barton, D. H. R.; Bashiardes, G.; Fourrey, J.-L. *Tetrahedron* **1988**, *44*, 147.

(16) Kende, A. S.; Zhong, M. *Synth. Commun.* **1999**, *29*, 3401.

(17) Syntheses of compounds **8a,b** are unpublished results from our laboratory.

(18) Lampe, J. W.; Hughes, P. F.; Biggers, C. K.; Smith, S. H.; Hu, H. *J. Org. Chem.* **1996**, *61*, 4572.

(19) Barrero, A. F.; Alvarez-Manzaneda, E. J.; Chahboun, R. *Tetrahedron* **1998**, *54*, 5635.

SCHEME 5

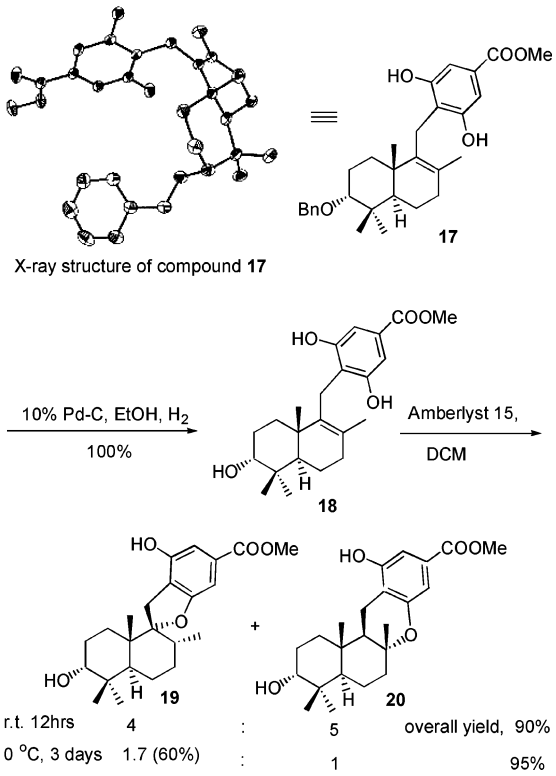


TABLE 1. Optimization of Spiroannulation of Compound 18 Using Amberlyst 15

entries	solvent	*T* (°C)	ratio (**19**:**20**)	time (day)	conversion[a] (%)
1	DCM	rt	0.9:1	1	100
2	THF	rt	2:1	2	10
3	ether	rt	1.5:1	2	20
4	methanol	rt	3:1	2	30
5	acetonitrile	rt	1.1:1	1	95
6	acetonitrile	0	1.1:1	2	65
7	benzene	rt	1.1:1	2	95
8	DCM	0	**1.7:1**	3	100
9	DCM	−20	3:1	3	10

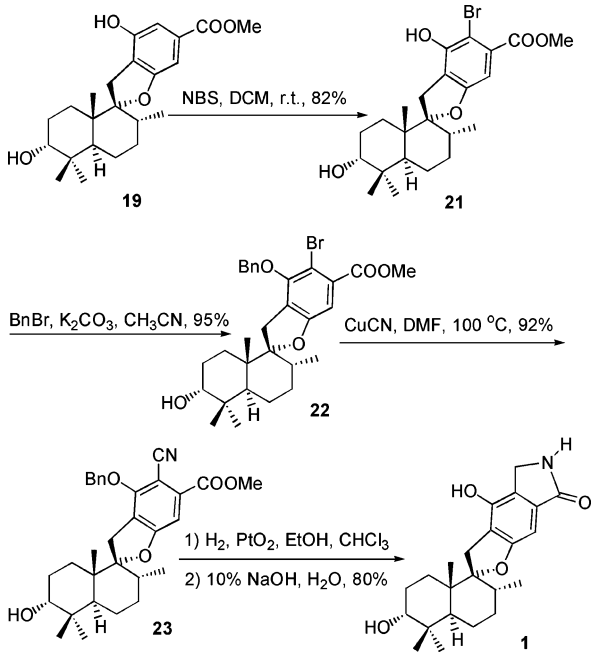
[a] The data of conversion are based on ^{1}H NMR spectra.

was treated with Amberlyst 15 in anhydrous dichloromethane at room temperature giving the chromatographically separable isomers benzofuran **19** and benzopyran **20** in a 9:10 ratio in 90% overall yield. However, only traces of cyclized compounds **19** and **20** were observed (by ^{1}H NMR) under Corey's reaction conditions.

A series of reaction conditions were then screened, including variation of solvents and temperatures, to optimize the reaction (Table 1). Finally, Amberlyst 15 in dichloromethane as solvent at 0 °C was found to afford benzofuran and benzopyran in a ratio of 1.7:1 (yield of benzofuran 60%), comparable to Corey's[3c] and McMurry's[3d] results in related systems. Protic solvents and dipolar aprotic solvents gave benzofuran as major product (up to 3:1, entry 4) but in low conversion (10−30%, entries 2−4); extension of the reaction time in these solvents led to gradual decomposition without increase of conversion. Control experiments showed the generation of **19** and **20** is irreversible under our optimum reaction conditions.

Regioselective aromatic bromination of benzofuran **19**

SCHEME 6

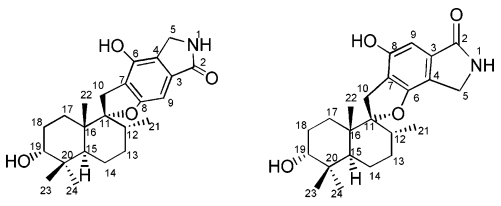


using NBS in dichloromethane provided the *ortho*-bromo derivative **21** in 82% yield, accompanied by 5−10% of chromatographically inseparable dibromo derivative. The structure of **21** was confirmed by single-crystal X-ray analysis of the derived benzyl ether **22**. Reaction of bromide **22** with 10 equiv of CuCN in DMF at 100 °C[3c] gave nitrile **23** in 92% yield. Hydrogenation of this nitrile using PtO_2 in EtOH/$CHCl_3$[20] and subsequent lactamization with 10% aqueous NaOH produced lactam **1**, whose structure was confirmed by single-crystal X-ray analysis (Scheme 6).

2. Synthesis of Regioisomer 27, Stachybotrylactam. Rather unexpectedly, the ^{1}H NMR and ^{13}C NMR spectra of our synthetic compound **1** did not match those measured for natural material reported by Roggo.[9b] Analysis of the evidence for this structure in Roggo's paper indicated that placement of the lone aromatic proton at C-9 was made only through the absence of an NOE for that proton to the methylene protons at C-5 or C-10. Yet the D−E regioisomer **27** would likewise lack an NOE from that aromatic proton to the methylene protons at C-5 or C-10. Structure **27** in fact corresponds to stachybotrylactam isolated in 1995 by Jarvis.[8a] We therefore embarked on the synthesis of **27** to test the possibility that the Roggo lactam was in fact stachybotrylactam (Figure 2). [21]

With intermediate **19** in hand, we could construct the regioisomer **27** by redirecting the ring bromination so as to form the *para*-bromo isomer, then following the same sequence as was employed earlier to make compound **1** (Scheme 7). Since the phenolic hydroxyl in **19** directed exclusively to *ortho*-bromination, we converted **19** to the bulky TBDPS ether **24** which was monobrominated using NBS and excess silica gel in DCM to give a 1:1 mixture of the two bromo regioisomers, easily separable by chromatography. In the absence of silica gel, or by use

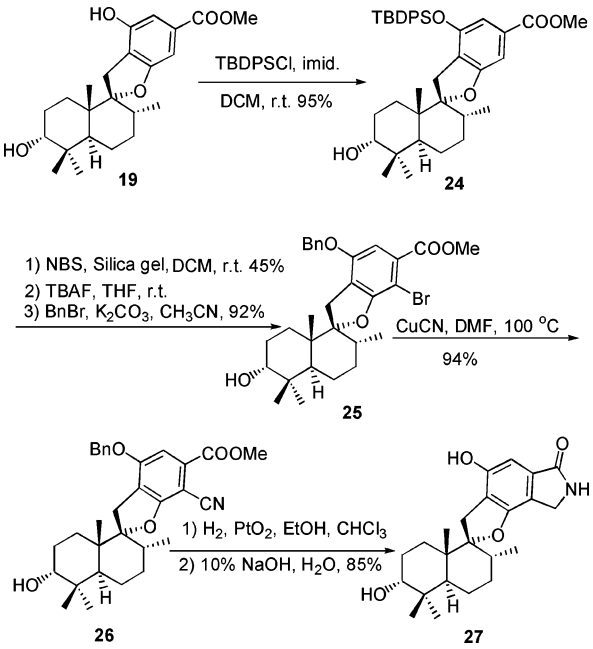
(20) Wrobel, J.; Dietrich, A.; Gorham, B. J.; Sestanj, K. *J. Org. Chem.* **1990**, *25*, 2694.



1 **27**

FIGURE 2.

SCHEME 7

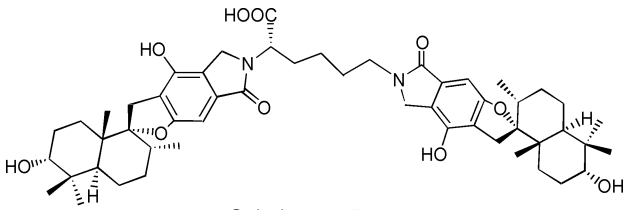


19 **24** **25** **26** **27**

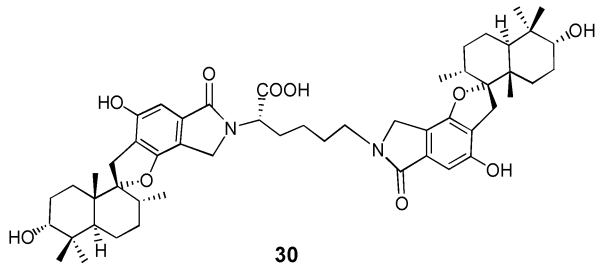
of other solvents or brominating reagents, inferior *para* regioselectivity was observed. Desilylation and *O*-benzylation of the *para* isomer gave **25** in 40% yield from **24**. Direct cyanation of **25** to **26** followed by nitrile reduction and subsequent treatment with base gave the desired D−E regioisomer **27**. The ^{1}H NMR and ^{13}C NMR spectra of our synthetic **27** were in agreement with the spectra provided us for the natural lactam "**1**" by Roggo and with those of natural stachybotrylactam sent by Jarvis. The absolute configuration of natural stachybotrylactam could not be established because neither the Jarvis nor the Roggo group had reported an optical rotation. However, Jarvis had measured the optical rotation of an *N*-hydroxyethyl derivative of stachybotrylactam ($[\alpha]_D^{20} = -16$, *c* 0.1, MeOH) which was very similar to that of our synthetic **27** ($[\alpha]_D^{20} = -21.3$, *c* 1.10, MeOH). Therefore, we conclude that the absolute configuration of natural stachybotrylactam is most likely correctly represented by stereoformula **27**.

3. Synthesis of Pseudosymmetric "Dimer". With the structure of Roggo's spirolactam **1** now revised to structure **27**, we anticipated that the structure of pseudosymmetric "dimer" **5** should be correspondingly revised to the regioisomer **30** shown in Figure 3. Structure **30** is in fact claimed to be that of stachybocin A, isolated by Nakamura et al. in 1995.[7,22]

The synthesis of pseudosymmetric "dimer" **30** was initiated starting from bromide **25** by Stille coupling with tributylvinyltin which gave the styrene derivative **28** in



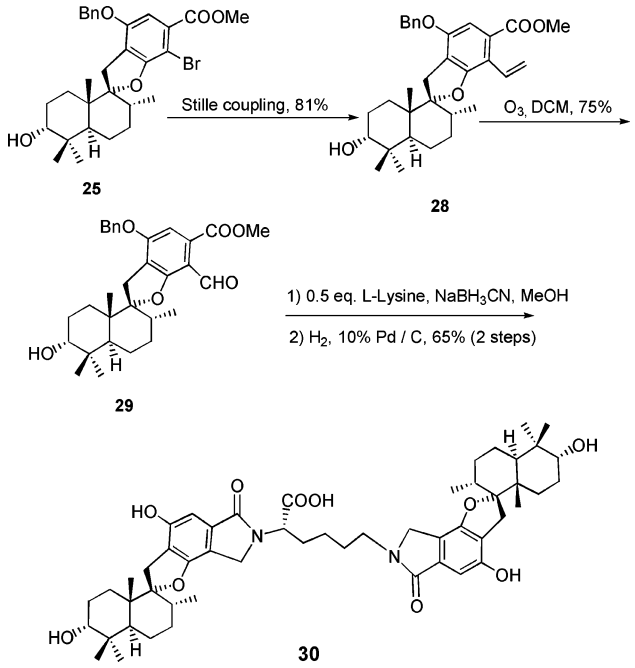
Spirolactam **5**



30

FIGURE 3.

SCHEME 8



25 **28** **29** **30**

81% yield. The styrene was ozonized to produce the aryl aldehyde **29** which was condensed with L-lysine using double reductive amination, spontaneous lactamization, and subsequent hydrogenolytic debenzylation. The pseudosymmetric "dimer" **30** was then obtained in 65% yield over the last two steps (Scheme 8). On the basis of HRMS, ^{1}H NMR, and ^{13}C NMR comparisons of our synthetic dimer **30** with the reference spectra provided by Roggo,

(21) Direct comparison of Jarvis' spectroscopic data with Roggo's data was impossible due to the different solvent systems used in measuring the NMR spectra. Roggo et al. have recently independently concluded that their lactam **1** is structurally identical to the stachybotrylactam reported by Jarvis.[8a] We have been unable to obtain actual reference samples of either the Roggo spirolactam or the Jarvis stachybotrylactam.

(22) Direct comparison of Nakamura's spectroscopic data with Roggo's data was not feasible due to the different solvent systems used in measuring the NMR spectra. Roggo et al. have recently independently concluded that their spirolactam **5** is structurally identical to our synthetic "dimer" **30** and its structure corresponds to that for stachybocin A depicted by Nakamura.[7]

we conclude that these are structurally identical compounds. Upon completion of our work, we received a sample of authentic stachybocin A from the Seto group.[7b] Careful measurement of the proton NMR of this natural material revealed significant differences between natural stachybocin A and our synthetic dimer **30**. The origin of this discrepancy remains unresolved at the present time.

Conclusions

We have completed the first enantioselective total syntheses of lactam **1**, its regioisomer **27**, and pseudosymmetric "dimer" **30** from (+)-Wieland−Miescher ketone in 5.1% (20 steps), 2.3% (22 steps), and 1.1% (24 steps) overall yields, respectively. As a result, the structure of natural spirolactam **1** was revised to its regioisomer **27**, stachybotrylactam. Correspondingly, the pseudosymmetric "dimer" **5** was also found to be structure **30**.

Acknowledgment. We thank Drs. John Huffman (Indiana University) and Rene Lachicotte (University of Rochester) for X-ray analyses and Prof. B. Jarvis (University of Maryland) and Dr. S. Roggo (Ciba-Geigy Limited) for kindly sending us copies of ^{1}H and ^{13}C NMR spectra of natural stachybotrylactam **1** and **5**. We also thank Prof. H. Seto (Tokyo University of Agriculture) and Dr. K. Ogawa (Asahi Chemical Industry Co., Ltd.) for generously providing us an authentic sample of stachybocin A.

Supporting Information Available: Data including full experimental details; ^{1}H NMR and ^{13}C NMR spectra for **1**, **7a**, **11**, **12**, **13**, **14**, **18**, **24**, **27**, **28**, **29**, **30**; the copies of proton and carbon NMR spectra of spirolactam **1** and **5** (provided by Dr. Roggo) and proton NMR spectrum of an authentic sample (provided by Dr. K. Ogawa) of stachybocin A; X-ray crystallographic data of compound **17**, **22**, and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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