Biomimetic Alkaloid Synthesis

DOI: 10.1002/anie.200604377

A Concise Total Synthesis of the Notoamides C and D**

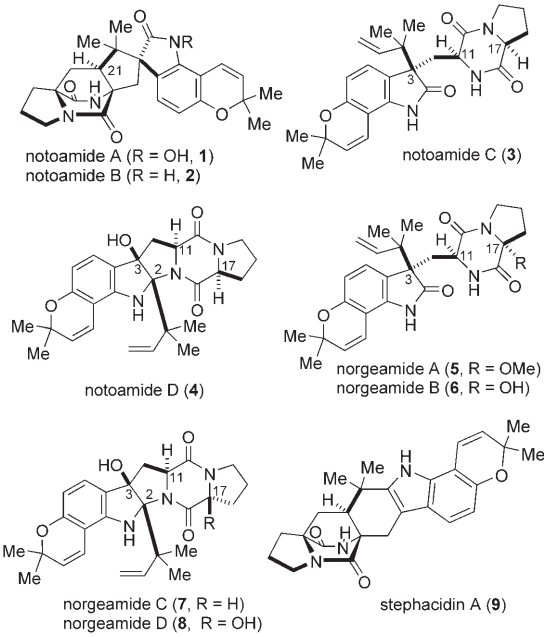
*Alan W. Grubbs, Gerald D. Artman, III, Sachiko Tsukamoto, and Robert M. Williams**

Dedicated to Professor David A. Evans on the occasion of his 65th birthday

The past several years have seen an explosion of new metabolites that are isolated from fungi and have interesting biological activities. Our research group has a rich history in the synthesis[1] and elucidation of the biosynthesis[2] of fungal metabolites derived from tryptophan, isoprene, proline, and proline derivatives.[3] Recently, two research groups have reported structurally related alkaloids that were isolated from two different strains of fungi, cultivated from marine environments. Tsukamoto and co-workers recently isolated four new prenylated indole alkaloids named the notoamides A–D (**1**–**4**, Scheme 1), along with the known alkaloids sclerotiamide and stephacidin A (**9**), from a marine strain of *Aspergillus* sp. cultivated from the common mussel, *Mytilus edulis*.[4] The structures of the notoamides A–D contain a pyranoindole ring system similar to those found in the stephacidins[5,6] and several paraherquamides.[7] In addition, notoamide A (**1**) and B (**2**) possess the bridged [2.2.2]diazaoctane ring system commonly found in the paraherquamide and stephacidin family. Interestingly, the nitrogen atom of the 2-oxindole moiety of notoamide A is oxidized to the *N*-hydroxy group. The notoamides C (**3**) and D (**4**) lack the bridged bicycle of **1** and **2**, and **4** contains the pyrrroloindole ring system.[8] The notoamides A–C exhibit moderate cytotoxicity against a panel of cancer cell lines but notoamide D shows no such activity.

In 2005, a related alkaloid family, the norgeamides (**5**–**8**, Scheme 1), was reported by researchers at the Hans–Knöll Institute.[9] These compounds were isolated from a strain of cold water *Aspergillius* fungi growing in the North Sea[9] and bear a striking resemblance to **3** and **4**. However, the distinguishing feature of the norgeamides is that the α position of the proline ring (at C17) has a methoxy substituent for norgeamide A (**5**) and a hydroxy group for norgeamide B (**6**) and D (**8**). As for the biological activity of the norgeamides, all have been found to inhibit the growth of a variety of cancer cell lines with different efficacies. Norgeamide A (**5**) was reported to be the most potent inhibitor of cell growth with observed values of 77–98% inhibition. Norgeamide B (**6**), which only differs from **5** in the substitution at the C17 position, was 20–30% less effective at inhibiting cell growth. The norgeamides C (**7**) and D (**8**) were the poorest inhibitors of this family of alkaloids with only 34–41% inhibition being observed. In comparison with the notoamides, it is reasonable to assume that the oxidation state of the proline ring system is vital for biological activity.

From a biosynthetic standpoint, one could envision a single biosynthetic pathway encompassing the notoamides and the norgeamides. To this end, a key starting substrate for this family, as well as for the stephacidins and several paraherquamide derivatives, would be substance **11** (Scheme 2). A single oxidation of **11** would generate the pyrroloindole ring system of **4**. It is particularly intriguing that

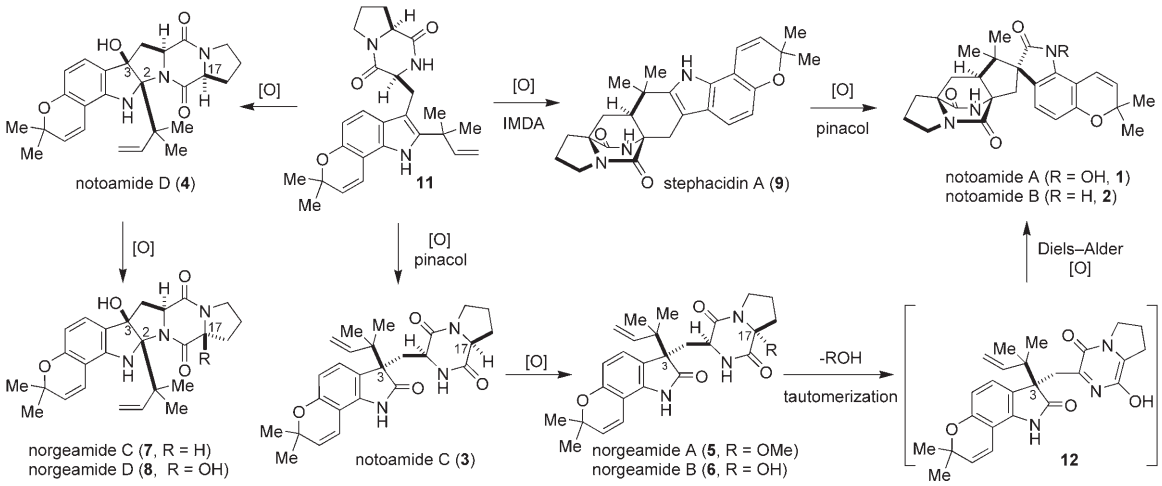


Scheme 1. Structures of the notoamides, norgeamides, and stephacidin A.

[*] A. W. Grubbs, Dr. G. D. Artman, III, Prof. R. M. Williams
Department of Chemistry, Colorado State University
Fort Collins, CO 80523 (USA)
E-mail: rmw@chem.colostate.edu
Prof. R. M. Williams
The University of Colorado Cancer Center
Aurora, CO 80045 (USA)
Fax: (+1) 970-491-3944
Homepage: http://rwindigo1.chm.colostate.edu/
Prof. S. Tsukamoto
Graduate School of Natural Science and Technology
Kanazawa University, Kakuma-machi, Kanazawa 920-1192 (Japan)
Fax: (+81) 76-264-6241
E-mail: sachiko@p.kanazawa-u.ac.jp

[**] We thank Dr. C. Rithner for assistance with the spectroscopic analyses. We are grateful to the National Institutes of Health for financial support to R.M.W (CA70375) and the generous NSRA postdoctoral fellowship for G.D.A. (GM72296–01). Mass spectra were obtained on instruments supported by the NIH Shared Instrument Grant GM49631. We are also grateful to Dr. I. Sattler of the Hans–Knöll Institute for kindly providing NMR spectra of the norgeamides.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

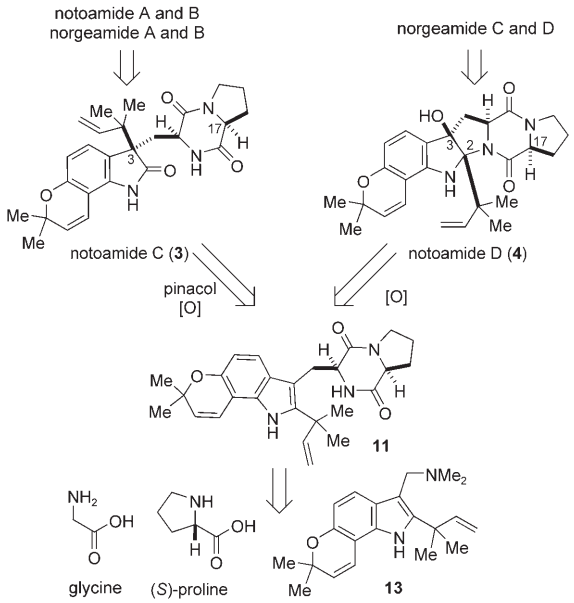


Scheme 2. Postulated biosynthetic relationships between the alkaloids.

4 is structurally identical to **7** except for the reported stereochemistry at the C17 position. A second oxidation of **11** would introduce the hydroxy group at the C17 position of **8**. As for the remaining alkaloids, oxidation and pinacol rearrangement of **11** would afford **3**, which, after oxidation at the C17 position, would give **5** and **6**. Interestingly, alkaloids **5** and **6** exist in the correct oxidation state to serve as substrates for a biosynthetic intramolecular Diels–Alder (IMDA) reaction, in which the elimination of MeOH or water from **5** and **6**, respectively, followed by tautomerization, would generate the putative azadiene **12**. Cycloaddition of the isoprenyl moiety of **12** could generate the bridged bicyclic core of **1** and **2**. Subsequent oxidation of the nitrogen atom of **2** would complete the biosynthesis of **1**. Alternatively, an oxidation and IMDA sequence from the key precursor **11** would directly generate stephacidin A (**9**), which, upon indole oxidation and Pinacol-type rearrangement, would generate **2**.

The possible biosynthetic relationship between the notoamides and the norgeamides with respect to the stephacidins and notoamides A/B, along with their biological activity, has stimulated us to explore their total synthesis. Retrosynthetically, we envisioned a convergent biomimetic synthesis of the notoamides C and D, and the norgeamides from **11** (Scheme 3). Oxidation and pinacol rearrangement of **11** would generate the 2-oxindole observed in notoamides A–C and the norgeamides A and B. Alternatively, oxidation of **11** and trapping with the tryptophyl amide would assemble the pyrroloindole system of **4** and provide a synthetic intermediate to explore conditions for epimerization to **7**, as well as for the oxidation at the C17 position to **8**. We anticipated that the key common intermediate **11** would be readily available from (*S*)-proline, glycine, and the gramine derivative **13**, which was recently synthesized by our group on a gram scale.[10]

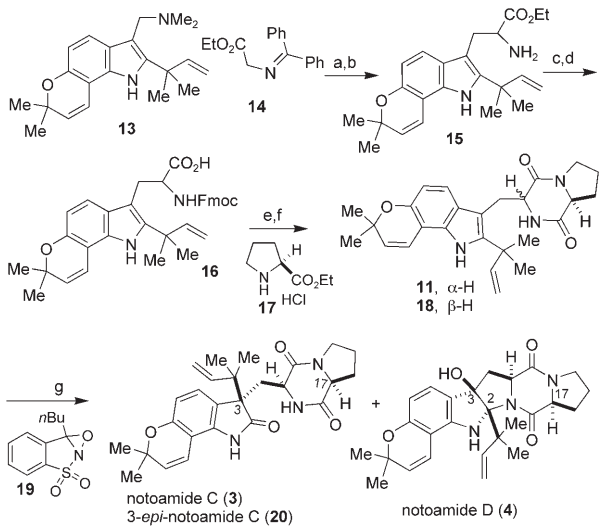
Our synthesis began with a coupling of the gramine **13** with the benzophenone imine of glycine **14** by using a method reported by the research groups of Somei and Kametani,[11] which after hydrolysis afforded the amino ester **15** (75%, Scheme 4). Introduction of the Fmoc group onto the amine of **15** followed by saponification of the ethyl ester with trimethyltin hydroxide according to conditions reported by



Scheme 3. Retrosynthetic plan.

Nicolaou et al.[12] afforded the acid **16** (74% yield from **15**). Coupling of the commercially available (*S*)-proline ethyl ester hydrochloride (**17**) with the acid **16** in the presence of BOPCl proceeded without problems, and the intermediate *N*-Fmoc peptide was deprotected and cyclized in the presence of morpholine to afford **11** and the 11-*epi* diastereomer which were readily separable by chromatography. Oxidation of the C2=C3 bond of the indole proved to be troublesome under numerous conditions, but was finally achieved using the oxaziridine[13] **19** to afford notoamide C (**3**, 28%) and 3-*epi*-notoamide C (**20**, 48%) as the major products (76% combined), along with a minor amount of notoamide D (**4**) and 2,3-*epi*-notoamide D (10% combined).

To the best of our knowledge, this oxidation is a new and direct method for the conversion of 2,3-disubstituted indoles into oxindoles, a well-known conversion, but one that is classically performed stepwise by treatment of the indole with

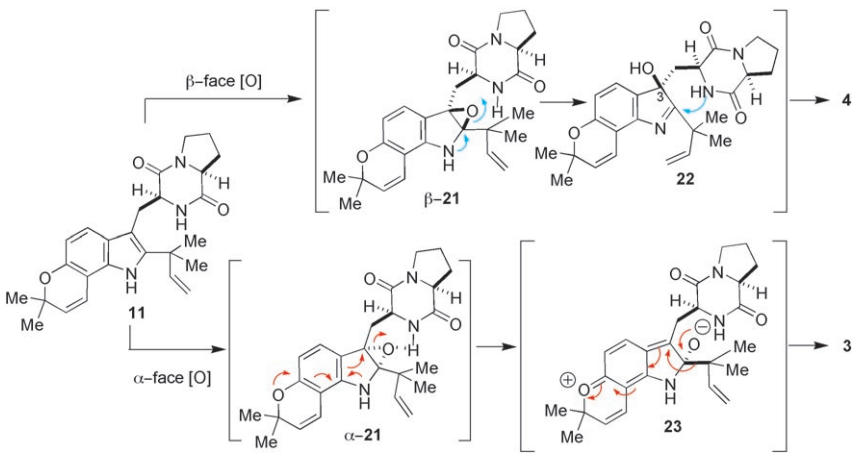


Scheme 4. Total synthesis of notoamide C (**3**), 3-*epi*-notoamide (**20**), and notoamide D (**4**). Reagents and conditions: a) nBu$_3$P, CH_3CN, reflux; b) 1 N HCl, CH_2Cl_2 (75% over 2 steps); c) FmocCl, 10% Na_2CO_3, 1,4-dioxane; d) Me_3SnOH, $ClCH_2CH_2Cl$ (74% over 2 steps); e) BOPCl, DIPEA (61%); f) morpholine, THF; **11** (40%) and **18** (35%); g) **19**, CH_2Cl_2; **3** (28%), **20** (48%), **4**, and 2,3-*epi*-notoamide (10% combined). BOPCl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride, DIPEA = *N*,*N*-diisopropylethylamine, Fmoc = 9-fluorenylmethyloxycarbonyl.

tert-butylhypochlorite to yield an unstable 3-chloroindolenine, followed by hydration to the corresponding *cis*-chlorohydrin, and pinacol-type rearrangement.[14,15] Interestingly, we had anticipated that direct oxidation of the C2=C3 bond of the indole would greatly favor formation of notoamide D. In this context, it is significant that notoamides C and D are isolated in roughly equal amounts from the fungi.[4] To rationalize why the oxindole species are formed as the dominant products, consideration of the mechanism of this oxidation/rearrangement proved insightful. Oxidation of the C2=C3 bond of 2,3-disubstituted indole derivatives is a well-precedented transformation.[14] We had anticipated that opening of the incipient β-2,3-epoxyindole β-**21** (Scheme 5) would occur through participation of the indole nitrogen atom to generate the usual 3-hydroxyindolenine species **22**, which would be trapped by the tryptophyl amide nitrogen atom and thus would generate the pyrroloindole of **4**. However, consideration of the electron-rich nature of the indolopyranyl ring system of **11** revealed that the alternative rupture of the putative epoxide at C3 (shown for α-**21**, Scheme 5), facilitated by participation of the pyranyl oxygen atom and the indole nitrogen atom, could generate the pseudo-*para*-quinone methide species **23**. Subsequent β-face migration of the reverse-prenyl group from C2 to C3 (from **21-α**) would quench the quinone methide and the 2-oxindole of **3** would be produced as the major product. The dominance of the latter pathway reveals an unexpectedly large electronic influence of the pyranyl oxygen atom.

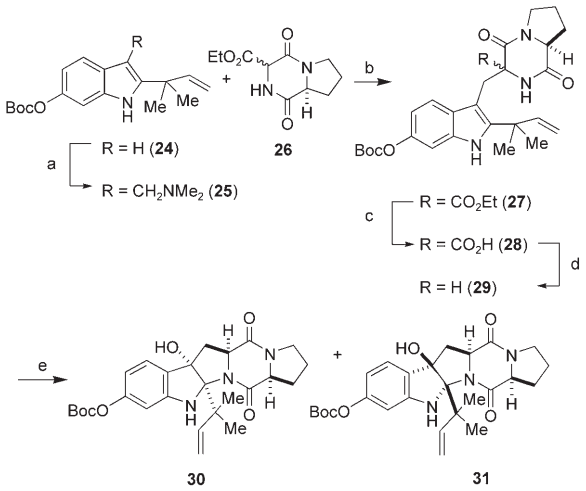
From a biosynthetic perspective, the oxidation of **11** must occur from both the α and β faces of the C2=C3 indole bond to accommodate the relative stereochemical differences between notoamide D (which must arise through β-face oxidation) against notoamides A, B, and C (which all arise from α-face oxidation of the same putative precursor **11**). Our attempts to model the competing conformations and transition states for this oxidation reaction have failed to provide a convincing rationale for the minor facial selectivity observed in the synthetic oxidation of **11** to **3** and its epimer **20**. Further experimental and computational data will be required to gain more understanding of this interesting stereochemical bias.

To obtain additional insight into the delicate electronic control of these two competing pathways, we decided to introduce an electron-withdrawing group onto the oxygen substituent at C6 with the expectation that this would attenuate the ring-opening at the C3 position and shunt ring-opening of the epoxide predominantly at the C2 position via the 3-hydroxyindolenine species, thereby favoring the formation of the pyrroloindole ring system of notoamide D (**4**). To this end, the *O*-Boc-protected prenylated indole **24** was converted into the gramine **25** in high yield (Scheme 6). Coupling of **25** with the known diketopiperazine **26** under the conditions reported by the research groups of Somei and Kametani afforded the ethyl ester **27**. Hydrolysis of **27** to the acid **28** followed by decarboxylation afforded **29** as a single diastereomer. Exposure of **29** to three equivalents of **19** in CH_2Cl_2 for three days at room temperature gave no detectable reaction. On the other hand, exposure of **29** to molecular oxygen in the presence of methylene blue afforded the pentacycles **30** and **31** as a mixture of diastereomers with no detectable oxindole products corresponding to **3**. These results thus demonstrate the vital role the electronic nature of the indole ring system plays in controlling these two distinct pathways. Further investigations into the mechanistic aspects of this unique transformation are underway.



Scheme 5. Proposed biosynthetic conversion of **11** into **3** and **4**.

In conclusion, we have achieved a concise, biomimetic synthesis of notoamides C (**3**) and D (**4**) through an



Scheme 6. Reagents and conditions: a) CH_2O, Me_2NH, AcOH, RT (85%); b) **26**, nBu_3P, CH_3CN, reflux (72%); c) LiOH, THF, H_2O, RT (98%); d) *hν*, $BrCCl_3$, 105 °C (75%); e) 1. *hν*, O_2, MeOH, methylene blue, 0 °C; 2. Me_2S, 62% over 2 steps.

oxidation of the proposed biosynthetic precursor **11**. The synthesis is readily adaptable to the incorporation of both stable and radioisotopic labels through the formaldehyde-based construction of the relevant gramine derivatives which will prove useful in future biosynthetic studies. In addition, we have found that the electronic properties of the indole ring greatly influences the regiochemistry of oxidation of the C2=C3 indole bond, thus resulting in either the pyrroloindole or the oxindole species.

The co-occurrence of the notoamides A–D along with stephacidin A and sclerotiamide[16] as metabolites from a strain of *Aspergillus* sp. cultivated from the common mussel, *Mytilus edulis* suggests two distinct biosynthetic pathways that interrelates these species; either the sequence from **11** through notoamide C to notoamide B and finally to notoamide A, or alternatively, oxidative conversion of **11** into stephacidin A, through notoamide B and then into notoamide A appears equally plausible. The oxidation of the piperazinedione nucleus of notoamide C to a putative azadiene species, necessary for an IMDA cyclization that would construct the bicyclo[2.2.2]diazaoctane ring system, remains an interesting, but as yet untested biogenesis. Some insight into these two distinct pathways can be gleaned from ab initio calculations reported by Domingo et al. on the IMDA reactions of azadiene species such as **12**.[17] These studies indicate a 4–7 kcal mol^{-1} energy difference in the transition states greatly favoring formation of the 21-*epi*-notoamide B Diels–Alder adduct. These calculations are supported thus far by experiments using simpler, yet structurally related systems.[18] Based on these data, we currently favor the latter path to notoamides A and B through stephacidin A. It might be further anticipated that the metabolites corresponding to the norgeamides A and B can arise from the interception (hydration/methanolysis) of such azadiene species, they could be biosynthetically equivalent or actual precursors to such azadiene species, and might further be expected to be co-metabolites (yet to be detected) with the notoamides. Further studies are currently being conducted to address these biogenetic interrelationships.

Received: October 25, 2006
Revised: November 29, 2006
Published online: February 16, 2007

Keywords: biomimetic synthesis · indoles · natural products · oxidation · pinacols

[1] For reviews of our previous synthetic and biosynthetic work concerning this class of alkaloids, see: a) R. M. Williams, R. J. Cox, *Acc. Chem. Res.* **2003**, *36*, 127–139; b) R. M. Williams, *Chem. Pharm. Bull.* **2002**, *50*, 711–740.

[2] For representative studies on the biosynthesis of these types of alkaloids, see: a) E. M. Stocking, J. F. Sanz-Cervera, C. J. Unkefer, R. M. Williams, *Tetrahedron* **2001**, *57*, 5303–5320; b) E. M. Stocking, J. F. Sanz-Cevera, R. M. Williams, *Angew. Chem.* **2001**, *113*, 1336–1338; *Angew. Chem. Int. Ed.* **2001**, *40*, 1296–1298; c) E. M. Stocking, R. M. Williams, J. F. Sanz-Cevera, *J. Am. Chem. Soc.* **2000**, *122*, 4489–4493.

[3] For a review on the biosynthesis of prenylated tryptophan alkaloids, see: R. M. Williams, E. M. Stocking, J. F. Sanz-Cevera, *Top. Curr. Chem.* **2000**, *209*, 98–173.

[4] See the preceding Communication: H. Kato, T. Yoshida, T. Tokue, Y. Nojiri, H. Hirota, T. Ohta, R. M. Williams, S. Tsukamoto, *Angew. Chem.* **2007**, *119*, 2304–2306; *Angew. Chem. Int. Ed.* **2007**, *46*, 2254–2256.

[5] a) J. Qian-Cutrone, S. Haung, Y-Z. Shu, D. Vyas, C. Fairchild, Z. Menendez, K. Krampitz, R. Dalterio, S. E. Klohr, Q. Gao, *J. Am. Chem. Soc.* **2002**, *124*, 14556–14557; b) J. Qian-Cutrone, K. D. Krampitz, Y.-Z. Shu, L. P. Chang, US Patent 6,291,461, **2001**.

[6] For total syntheses of the stephacidins, see: a) S. B. Herzon, A. G. Myers, *J. Am. Chem. Soc.* **2005**, *127*, 5342–5344; b) P. S. Baran, C. A. Guerrero, N. B. Ambhaikar, B. J. Hafensteiner, *Angew. Chem.* **2005**, *117*, 612–615; *Angew. Chem. Int. Ed.* **2005**, *44*, 606–609; c) P. S. Baran, C. A. Guerrero, N. B. Ambhaikar, B. J. Hafensteiner, *Angew. Chem.* **2005**, *117*, 3960–3963; *Angew. Chem. Int. Ed.* **2005**, *44*, 3892–3895; d) P. S. Baran, B. D. Hafensteiner, N. B. Ambhaikar, C. A. Guerrero, J. D. Gallagher, *J. Am. Chem. Soc.* **2006**, *128*, 8678–8693; e) see also the following Communication: T. J. Greshock, A. W. Grubbs, S. Tsukamoto, R. M. Williams, *Angew. Chem.* **2007**, *119*, 2312–2315; *Angew. Chem. Int. Ed.* **2007**, *46*, 2262–2265.

[7] a) J. G. Ondeyka, R. T. Goegelman, J. M. Schaeffer, L. Kelemen, L. Zitano, *J. Antibiot.* **1990**, *43*, 1375–1379; b) J. M. Liesch, C. F. Wichmann, *J. Antibiot.* **1990**, *43*, 1380–1386; c) S. E. Blanchflower, R. M. Banks, J. R. Everett, B. R. Manger, C. Reading, *J. Antibiot.* **1991**, *44*, 492–497.

[8] For syntheses of other indole alkaloids containing a pyrroloindole ring system, see: a) S. P. Marsden, K. M. Depew, S. J. Danishefsky, *J. Am. Chem. Soc.* **1994**, *116*, 11143–11144; b) T. Kawaski, M. Shinada, D. Kamimura, M. Ohzono, A. Ogawa, *Chem. Commun.* **2006**, 420–422; c) J. R. Fuchs, R. L. Funk, *Org. Lett.* **2005**, *7*, 677–680.

[9] The structures of the norgeamides were published on the internet detailing some aspects of the research performed at the Hans-Knöll Institute. See: http://www.hki-jena.de/ The pdf file describing the norgeamides has been removed from the website of the Hans–Knöll Institute, but is still accessible through internet searches with the key word "norgeamide".

[10] A. W. Grubbs, G. D. Artman, R. M. Williams, *Tetrahedron Lett.* **2005**, *46*, 9013–9016.

[11] a) M. Somei, Y. Karasawa, C. Kaneko, *Heterocycles* **1981**, *16*, 941 – 949; b) T. Kametani, N. Kanaya, M. Ihara, *J. Chem. Soc. Perkin Trans. 1* **1981**, 959 – 963.

[12] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew. Chem.* **2005**, *117*, 1402 – 1406; *Angew. Chem. Int. Ed.* **2005**, *44*, 1378 – 1382.

[13] a) F. A. Davis, J. C. Townson, D. B. Vashi, R. Thimma Reddy, J. P. McCauley, M. E. Harakal, D. Gosciniak, *J. Org. Chem.* **1990**, *55*, 1254 – 1261; b) B. B. Snider, H. Zeng, *J. Org. Chem.* **2003**, *68*, 545 – 563.

[14] For previous successful oxidations of the C2=C3 indole bond using *meta*-chloroperoxybenzoic acid or *t*BuOCl, see: a) R. M. Williams, T. Glinka, E. Kwast, H. Coffman, *J. Am. Chem. Soc.* **1990**, *112*, 808 – 821; b) T. D. Cushing, J. F. Sanz-Cevera, R. M. Williams, *J. Am. Chem. Soc.* **1996**, *118*, 557 – 579; c) R. M. Williams, T. Glinka, E. Kwast, *Tetrahedron Lett.* **1989**, *30*, 5575 – 5578.

[15] For previous reports of one-pot procedures generating oxindoles from indoles, see: a) N. Finch, W. I. Taylor, *J. Am. Chem. Soc.* **1962**, *84*, 1318 – 1320; b) S. P. Hollinshead, D. S. Grubisha, D. W. Bennett, J. M. Cook, *Heterocycles* **1989**, *29*, 529 – 537; c) S. Edmondson, S. J. Danishefsky, L. Sepp-Lorenzino, N. Rosen, *J. Am. Chem. Soc.* **1999**, *121*, 2147 – 2155; d) H. Wang, A. Ganesan, *J. Org. Chem.* **2000**, *65*, 4685 – 4693.

[16] A. C. Whyte, J. B. Gloer, D. T. Wicklow, P. F. Dowd, *J. Nat. Prod.* **1996**, *59*, 1093 – 1095.

[17] L. R. Domingo, R. J. Zaragozá, R. M. Williams, *J. Org. Chem.* **2003**, *68*, 2895 – 2902.

[18] a) L. A. Adams, C. R. Gray, R. M. Williams, *Tetrahedron Lett.* **2004**, *45*, 4489 – 4493; b) L. A. Adams, M. W. N. Valente, R. M. Williams, *Tetrahedron* **2006**, *62*, 5195 – 5200; c) J. F. Sanz-Cervera, R. M. Williams, J. A. Marco, J. M. López-Sánchez, F. González, M. E. Martínez, F. Sancenón, *Tetrahedron* **2000**, *56*, 6345 – 6358.

