Total Synthesis of Tryprostatin B: Generation of a Nucleophilic Prenylating Species from a Prenylstannane

Kristopher M. Depew,[1a] Samuel J. Danishefsky,*[,1a,b] Neal Rosen,[1c] and Laura Sepp-Lorenzino[1c]

Department of Chemistry, Columbia University Havemeyer Hall, New York, New York 10027 Laboratories for Bioorganic Chemistry and Molecular Oncogenesis The Sloan-Kettering Institute for Cancer Research 1275 York Avenue, New York, New York 10027

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Recently, Osada et al. reported on the isolation, structure proof, and biological activity of tryprostatins A (**1**) and B (**2**).[2] These compounds, isolated from a particular strain (BM 939) of *Aspergillus fumigatus*, were of interest to us on the basis of several considerations. First, tryprostatin B, as well as its less potent congener (A, **1**), were claimed to be cell cycle progression inhibitors of tsFT210 at the G_2/M phase barrier. Given our previous research[3] directed to other indole-containing structures involved in cell cycle modulation, access to these metabolites (particularly the more potent B compound, **2**) was much desired.

Our laboratory had already been concerned with synthesizing naturally occurring indolic-isoprene constructs. In earlier work,[4] we had developed a method for the introduction of a "reverse prenyl" group at the 3-position of a pyrroloindole (see structure **3**). In our recent synthesis of gypsetin,[5] we had also described the reaction of prenylborane (**4**) with an unstable 3-chloroindolenine (**5**), unsubstituted at C_2 or at N, to introduce a reverse prenyl group onto the 2-position of a tryptophan-derived indole.

Of course, the elegant method of Gribble allows for the metallation of C_2 of an indole when the indolic nitrogen is suitably protected.[6] Thus, in theory, a prenyl group could be introduced by alkylation of a 2-metallo derivative. However, we were skeptical that such methods could be applied to an L-tryptophan derivative with assured maintenance of its enantiomeric homogeneity. In contrast, our chloroindolenine strategy had been successfully conducted in the context of a tryptophan system without compromising its optical purity (see Figure 1, **5** → **6**). Therefore, we hoped to apply a conceptually related formalism for the tryprostatins, requiring access to a reverse prenylboron reagent, generalized as **7**. Such an entity might serve as a nucleophilic prenylating agent (via allylic transposition) to generate **8**. Our initial attempts along these lines involved reactions of tri-*n*-butylprenylstannane with 9-BBN-Br or 9-BBN-OTf (9-BBN = 9-borabicyclo[3.3.1]nonyl, OTf = triflate), which we hoped would generate *in situ* a reagent of type **7** prior to coupling with **5**. Unfortunately, these kinds of protocols were unsuccessful. Apparently, rearrangement of a presumed species (**7**) to the prenyl system (**4**), occurs competitively with its coupling to the chloroindolenine leading to mixtures of the previously encountered **6** along with the desired **8** (*vide infra*) in low yield.

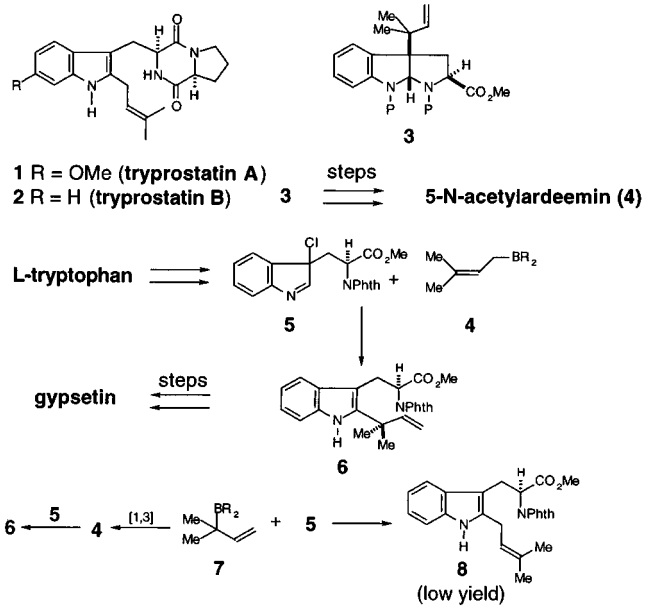

Figure 1.

We wondered about the possibility of generating a usable version of **7**, keeping in mind the key contributions of Keck,[7] Yamamoto,[8] Denmark,[9] Wardell,[10] and Thomas[11] which, read in the aggregate, established the possibility of nucleophilic allylation of Lewis acids by means of allylic and crotyltin reagents. In line with our recently developed procedure,[5] *N*-phthaloyl-L-tryptophan methyl ester (**9**) on treatment with *tert*-butyl hypochlorite cleanly generated **5** at 0 °C.[12] This CH_2Cl_2 solution was cooled to −78 °C and treated with stannane **10** followed by rapid addition of 2 equiv of BCl_3. *Upon workup, an 83% yield of the desired* ***8*** *was obtained.* Under these conditions only ca. 2−3% of compound **6** could be detected.

Perhaps, reaction of **10** with BCl_3 generates, transiently, **11** wherein reaction with chloroindolenine **5** would lead to the "ate"-like structure **12**. Intramolecular delivery of the prenyl function (Scheme 1, arrows) would culminate in the formation of **8**.[12]

Following the same protocol (Table 1), indoles **13** and **14**[13] were prenylated to afford **15** and **16**, respectively. Thus, a simple method to introduce a prenyl function at the 2-position of a 3-substituted indole is now available. We also note that the nucleophilic prenylation of ketones[14] (**17**, **18**, and **19** leading to **20**, **21** and **22**, respectively) by a related procedure has been accomplished.

Even as the full scope of this method for nucleophilic prenylation awaits definition, we focused on completion of the

(1) (a) Columbia University. (b) Laboratory for Bioorganic Chemistry, The Sloan-Kettering Institute for Cancer Research. (c) Laboratory for Molecular Oncogenesis, The Sloan-Kettering Institute for Cancer Research.

(2) (a) Cui, C.-B.; Kakeya, H.; Okada, G.; Onose, R.; Ubukata, M.; Takahashi, I.; Isono, K.; Osasa, H. *J. Antibiot.* **1995**, *48*, 1382. (b) Cui, C.-B.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. *J. Antibiot.* **1996**, *49*, 527. (c) Cui, C.-B.; Kakeya, H.; Osada, H. *J. Antibiot.* **1996**, *49*, 534.

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(6) (a) Saulnier, M. G.; Gribble, G. W. *J. Org. Chem.* **1982**, *47*, 757. (b) For another preparation of 2-substituted indoles, see: Fukuyama, T.; Chen, X.; Peng, G. *J. Am. Chem. Soc.* **1994**, *116*, 3127.

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(13) For example, see: Bennasar, M. L.; Zulaica, E.; Jimenez, J. M.; Bosch, J. *J. Org. Chem.* **1993**, *58*, 7756.

(14) We note recent examples of prenylation via an apparent prenylbarium species: (a) Yanagisawa, A.; Ogasawara, K.; Yasue, K.; Yamamoto, H. *J. Chem. Soc., Chem. Commun.* **1996**, 367. (b) Yanagisawa, A.; Habaue, S.; Yasue, K.; Yamamoto, H. *J. Am. Chem. Soc.* **1994**, *116*, 6130.

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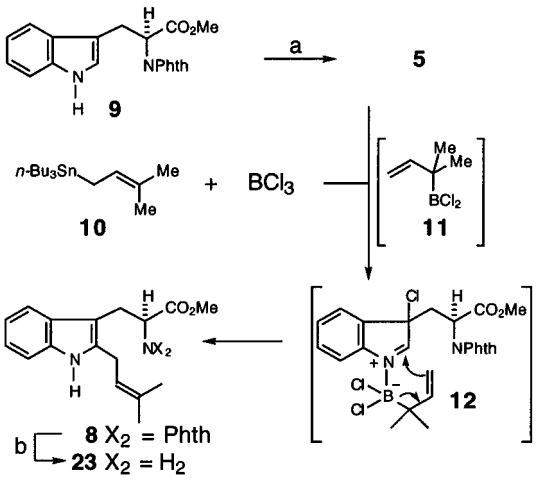
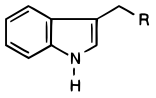
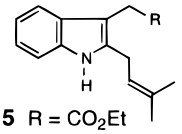
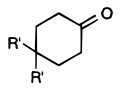
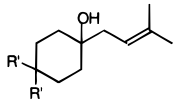
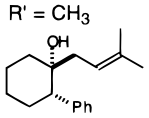
Scheme 1[a]



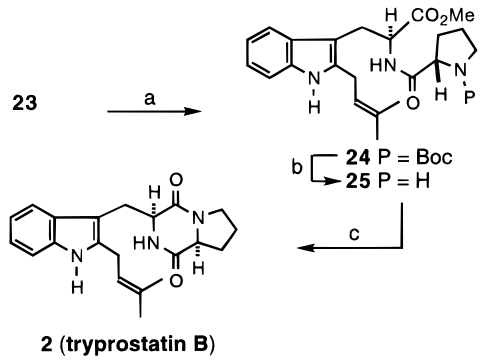
[a] Reagents and conditions: (a) *tert*-butyl hypochlorite (1.2 equiv, 0.5 M in CCl_4), Et_3N (1 equiv), CH_2Cl_2, 0 °C; then at −78 °C, **10** (4 equiv) and BCl_3 (2 equiv, 1.0 M in CH_2Cl_2, rapid addition) 83%. (b) Hydrazine hydrate (3.5 equiv), 3:1 MeOH/CH_2Cl_2 (0.1 M), 24 h, 82%.

Table 1. Prenylation of Indoles and Ketones

substrate	product	yield (%)
 **13** R = CO_2Et **14** R = $CH_2OTBDPS$	 **15** R = CO_2Et **16** R = $CH_2OTBDPS$	65[a] 81[a]
 **17** R' = H **18** R' = CH_3	 **20** R' = H **21** R' = CH_3	78[b] 88[b]
 **19**	 **22**	80[b]

[a] The chloroindolenine was formed first at −78 °C as discussed in the text. [b] BCl_3 (1.1 equiv, 1.0 M in CH_2Cl_2 from Aldrich) was added rapidly to a −78 °C CH_2Cl_2 solution (0.1 M) of the ketone (1.0 equiv) and the stannane (2.0 equiv) under argon.

Scheme 2[a]



2 (**tryprostatin B**)

[a] Reagents and conditions: (a) *N*-Boc-L-Pro-F (1.5 equiv), CH_2Cl_2, $NaHCO_3$, H_2O, 94%. (b) TMSI (1.2 equiv), MeCN, 0 °C. (c) NH_3/MeOH, 20 h, 67% from **24**.

total synthesis of tryprostatin B starting with **8**. This goal was smoothly accomplished by cleavage of the *N*-phthaloyl group, followed by coupling of the resultant amino ester **23** (Scheme 2) with *N*-Boc-L-proline acid fluoride[15] to afford **24**. Deprotection[16] led to **25** and then, by diketopiperazine formation, to **2**. Tryprostatin B (**2**) (67% from **24**; 46% from *N*-phthaloyltryptophan methyl ester) exhibited a high-field ^1H NMR spectrum identical to that provided by Osada.[2c] Its optical rotation was substantially the same ($[\alpha]^{27.5}_D = -74.6°$ (c = 0.64, $CHCl_3$) vs lit. $[\alpha]^{27}_D = -71.1°$ (c = 0.63, $CHCl_3$)) as that reported.

With tryprostatin B available to us through total synthesis, we were able to initiate investigations as to its biological properties. Given the earlier reports[2] that tryprostatin B inhibits progression of tsFT210 cells through the G_2/M phase, we studied the effectiveness of this drug in inhibiting the proliferation of logarithmically growing MCF-7 and MDA MB-468 human breast[17] cancer cells and Colo-205 human colon carcinoma cells. We found, in analogy with Cui et al.,[2] that in logarithmically growing cultures[17] tryprostatin B inhibits cell proliferation only at high concentrations (50 μg/mL). The growth of breast cell lines was inhibited but, curiously, not the colon cancer cell line. Furthermore, flow cytometric analysis of tryprostatin B-treated cells showed no cell cycle dependent arrest since the distribution of these cells in the G1, S, and G_2/M phases was the same as DMSO-treated cells (control). Given the earlier report of complete inhibition of cell cycle progression at 12.5 μg/mL, we then analyzed the effect of **2** on cell cycle progression in cells arrested in G_2/M by nocodazole[18] treatment. We found that, even at 50 μg/mL, **2** did not affect progression into G1. One clue to some of these discrepencies is that a DMSO solution of tryprostatin B, upon standing in air, undergoes slow conversion to a mixture of products. Solutions in which detectable byproducts have been produced are considerably more cytotoxic (ca. 50-fold) than those containing apparently homogeneous tryprostatin. Thus, while we have confirmed the activity starting with homogeneous tryprostatin, we cannot rule out the possibility that **2** may also be, in effect, a prodrug for a much more active entity.

Further investigations directed to the chemical transformations of tryprostatin B are in progress. We will examine the biological activity of these transformation products in the hope of identifying the particularly potent principle suggested by the data above. Also, the scope and generality of this new method for nucleophilic prenylation, which was the key to the highly concise total synthesis, are under study.

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Supporting Information Available: Experimental procedures for **8**, **15**, **16** and **20**−**22** and graphs explaining the effect of fully synthetic tryprostatin B on the (a) anchorage dependent growth and (b) cell cycle distribution of logarithmically growing cells in the presence or absence of **2** (6 pages). See any current masthead page for ordering and Internet access instructions.

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