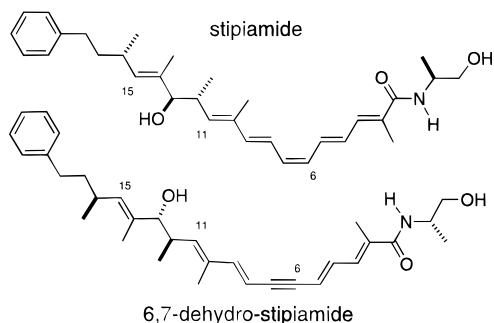# Synthesis of Stipiamide and a New Multidrug Resistance Reversal Agent, 6,7-Dehydrostipiamide

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Stipiamide,[1] a new member of a growing class of insecticidal polyene antibiotics,[2] has recently been patented (under the name phenalamid A₁) for its anti-HIV and antifungal activity.[3] Most remarkably, this compound has also been shown to reverse P-glycoprotein-mediated multidrug resistance (MDR), a condition common to many cancer cell lines.[4] The ability to potentiate the cytotoxicity of antitumor drugs toward drug resistant cells has been demonstrated by verapamil, cyclosporin-A,[5] GF120918,[6] and more recently hapalosin.[7] However, the clinical performance of MDR reversal agents has not been adequate, making the identification of new agents of great importance.[8] To this end a flexible route to stipiamide was pursued to make possible the synthesis of new MDR reversal agents. In spite of their wide range of activity, a synthetic route to the polyene antibiotics has not been reported.[9] Herein, we report the first total synthesis of stipiamide and the design and synthesis of 6,7-dehydrostipiamide (a new non-natural compound), now shown to potently reverse MDR in human MCF-7 adriamycin resistant breast cancer cells (MCF-7adrR).[10] Also, 6,7-dehydrostipiamide is remarkably less toxic relative to stipiamide.

The challenging (*E,E,Z,E,E*)-olefin array common to all members of the family was envisioned to arise from a Stille coupling as the final step as shown.

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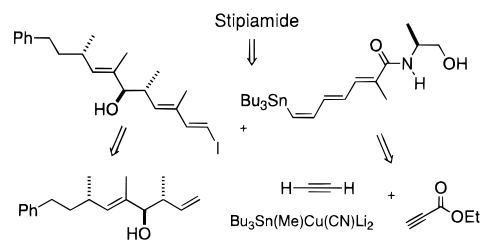(5) Ford, J. M.; Hait, W. N. *Pharacol. Rev.* **1990**, *42*, 155.
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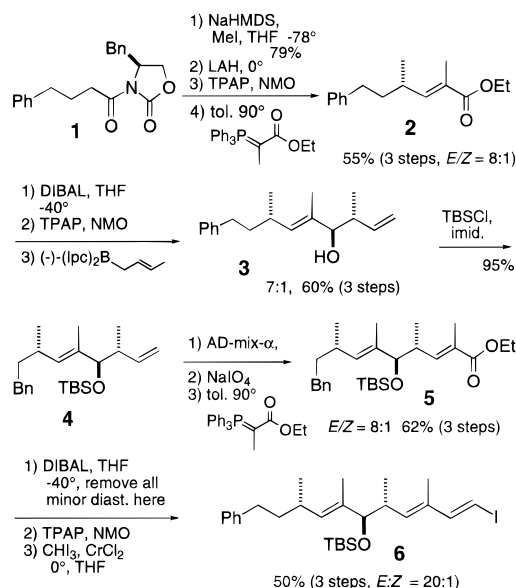(8) (a) Ford, J. M. *Eur. J. Cancer* **1996**, *32A*, 991. (b) Raderer, M.; Scheithauer, W. *Cancer* **1993**, *72*, 3553.
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The vinyl iodide was derived from an alcohol containing both a mono and trisubstituted olefin. A critical problem to be overcome then was the selective oxidative cleavage of the terminal olefin. The vinyltin fragment was planned to be assembled using a higher order tributylstannyl cuprate to acetylene to alkynyl ester tandem addition reaction, the first synthetic application of this kind.

The synthesis of the left side of stipiamide began with acyl oxazolidinone **1** methylated according to the procedure of Evans[11] with 25:1 selectivity. The mixture of diastereomers was reacted with LAH to remove the auxiliary, followed by oxidation to the aldehyde, and treatment with (carbethoxyethylidine) triphenylphosphorane to give **2** as an 8:1 diastereomeric mixture which was not separated. The unsaturated aldehyde was generated using DIBAL followed by tetrapropylammonium perruthenate (TPAP), 4-methylmorpholine *N*-oxide (NMO) oxidation.[12] Reaction with diisopinocampheyl *E*-crotylborane derived from (−)-(α)-pinene according to Brown[13] then gave the *anti*-homoallylic alcohol **3** in good overall yield. Attempted modification of the Evans aldol reaction using added aluminum chloride to give the *anti*-product[14] was explored without success. Use of the efficient crotylborane addition then required the development of a selective olefin oxidation. Differential olefin dihydroxylation proceeded very slowly and with little selectivity using catalytic OsO₄ and NMO.[15] The problem was overcome by simply reacting **4** with Sharpless' AD-mix-α reagent in *tert*-butyl alcohol and water.[16] The bulky cinchona alkaloid ligand·OsO₄ complex cleanly favored reactivity at the terminal olefin

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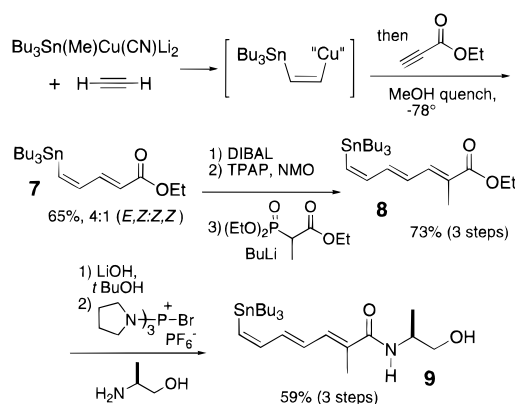(12) Griffith, W. P.; Ley, S. V. *Aldrichim. Acta* **1990**, *23*, 13.
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giving diol in 87% yield (~1:1 de) with no product resulting from oxidation of the internal trisubstituted olefin. The aldehyde was then generated using sodium periodate, followed by the Wittig reaction to provide unsaturated ester **5** in good yield and selectivity. The resulting alcohol product of DIBAL reduction of **5** was carefully purified by radial chromatography to remove the minor diastereomers resulting from the two Wittig and the crotyl boration reactions. This pure alcohol was then subjected to TPAP/NMO to access the intermediate enal. Takai reaction[16] using iodoform and flame dried chromous chloride was performed in THF to produce vinyl iodide **6** in 70% yield with high 20:1 *E:Z* selectivity.

Construction of the vinyltin portion of stipiamide began with tin-cuprate syn addition to acetylene following the precedence of Marino,[17] Fleming, and Pulido.[18] While conjugate additions of the intermediate derived from reaction of a higher order tributylstannylcuprate with acetylene to enones have been reported,[17,18] additions to alkynyl esters have not. Our route now highlights the utility of ethyl propiolate as a substrate for this reaction. The higher order tributylstannylcuprate reagent was generated from hexabutylditin, methyllithium, and copper(I) cyanide at −78 °C. Excess acetylene was then added directly to the cold solution, followed by addition of ethyl propiolate. After quenching with methanol,[20] ester **7** was obtained in 65% yield and 4:1 (*E,Z:Z,Z*) selectivity. DIBAL reduction gave dienyl alcohol from which the pure (2*E*, 4*Z*)-isomer was readily obtained using radial chromatography. Oxidation with TPAP/NMO followed by a Horner−Emmons reaction then gave triene ester **8** in good overall yield. Hydrolysis of **8** was achieved using lithium hydroxide in water added slowly to a *tert*-butyl alcohol solution of **8**.[19] The resultant acid was coupled with (*S*)-alaninol using bromotris-(pyrrolidino)phosphonium hexafluorophosphate (PyBrop)[20] to give **9**.

Completion of the synthesis of stipiamide involved deprotection of *E*-vinyliodide **6** with TBAF to access alcohol **10**. Stille coupling of **10** and **9** employed catalytic palladium(II) chloride bis-acetonitrile in *N*-methylpyrrolidinone[21] to give in 80% yield a 4:2:1 mixture of (−)-stipiamide, its all trans, and its 4*Z* isomer respectively as indicated by NMR, HRMS, and optical rotation.

A similar ratio of isomers was also observed in the authentic material suggesting that stipiamide readily isomerizes once formed.[22] As a first step toward improved MDR reversal, 6,7-dehydrostipiamide was designed and obtained from vinyl iodide **10** using Castro−Stephens coupling with acetylene **11**[23] in 50% yield.[24]

MCF-7adrR *in vitro* assays were then performed using synthetic stipiamide and 6,7-dehydrostipiamide. Minimal reversal of MDR in the presence of chemotherapeutic drugs except in the case of colchicine with stipiamide was found. This synergistic effect was earlier noted with natural stipiamide using colchicine resistant KB cells.[4] Synthetic stipiamide in the absence of the drug also proved to be highly toxic (ED$_{50}$ = 0.03 nM, MCF-7adrR cells) possibly arising from ATP-synthesis inhibition as noted previously with other members of the polyene class.[2] Conversely, we were gratified to find that 6,7-dehydrostipiamide showed excellent inhibition of MDR with near complete removal of toxicity (ED$_{50}$ = 1 μM)! With 6,7-dehydrostipiamide present (10 μM), the MCF-7adrR cell line ceased to exhibit MDR (ED$_{50}$ = 0.2 nM for adriamycin). Remarkably, this simple structural modification, from *Z*-olefin to alkyne, greatly lowers toxicity, presumably through abrogation of mitochondrial arrest, while promoting the ability to interact with P-glycoprotein reversing MDR. For comparison, in the same assay, a higher concentration of verapamil (22 μM), the clinical standard, was clearly less effective in MDR reversal (ED$_{50}$ = 10 nM).

To conclude, the synthesis of stipiamide was accomplished in 16 steps in 3.7% overall yield using the powerful combination of a Stille coupling and higher order tributylstannylcuprate chemistry. The synthesis of new structural variants based on this motif is currently underway.

Supporting Information Available: Complete experimental procedures and cytotoxicity data for stipiamide and 6,7-dehydrostipiamide (14 pages). See any current masthead page for ordering and Internet access instructions.

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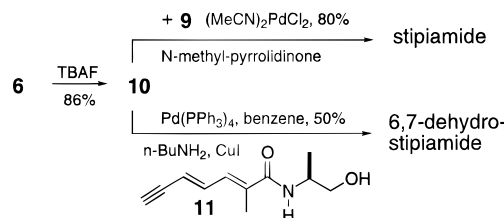(17) Takai, K.; Nitta, K.; Utimoto, K. *J. Am. Chem. Soc.* **1986**, *108*, 7408.

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