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On the Total Synthesis and Preliminary Biological Evaluations of 15(*R*) and 15(*S*) Aza-dEpoB: A Mitsunobu Inversion at C15 in Pre-Epothilone Fragments

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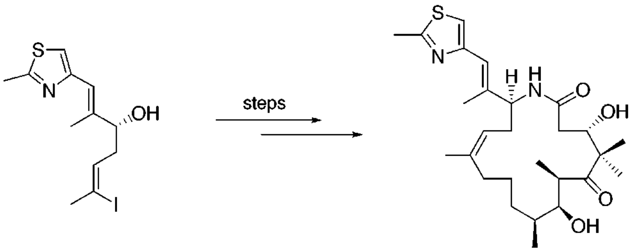
ABSTRACT



The syntheses of two epothilone analogues, 15(*S*)-aza-12,13-desoxyepothilone B and the epimeric 15(*R*)-aza-12,13-desoxyepothilone B, are described. A Mitsunobu inversion was utilized for elaboration of pre-epothilone fragments to the corresponding macrolactam. Tubulin binding and cytotoxicity profiles of these analogues are presented.

The previous Letter set forth the background of the epothilone project from our perspective. In particular, it described a key asymmetric dihydroxylation reaction. We took recourse to AD mix-α[1] (see **3** → **4**) to reach the 15(*S*) enantiomer (**5a**), and shortly thereafter dEpoB (**1**). Not surprisingly, recourse to AD mix-*β* leads to the antipodal 15(*R*) stereoseries (see compound **6**) en route to **7** and **7a** (Scheme 1).

With compounds **5a** and **7a** in hand, we wondered about the possibility of displacement of the C-15 hydroxyl centers, thereby enabling the introduction of nitrogen-based nucleophiles.[2] Assuming strict inversion of configuration could be realized, it was hoped that the 15(*R*) alcohol (**7a**) would lead eventually to a 15(*S*) aza analogue of dEpoB (**2**) while the 15(*S*) alcohol (**5a**) would pave the way for reaching the epimeric 15(*R*) aza series (vide infra **17**). In this way, we could ascertain the bioprofiles of such new dEpoB systems, in macrolactam rather than macrolactone settings.[3] We further hoped to evaluate the consequences of permuting the C-15 stereochemistry on the tubulin binding and cytotoxicity profiles.[4] Finally, we hoped to evaluate the capacity of these

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(1) (a) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L, Morikawa, K.; Wang, Z.-M.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768. (b) Aldrichimica Acta **1994**, *27*, 70.

(2) (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) Luo, Y.; Blaskovich, M. A.; Lajoie, G. A. *J. Org. Chem.* **1999**, *64*, 6106.

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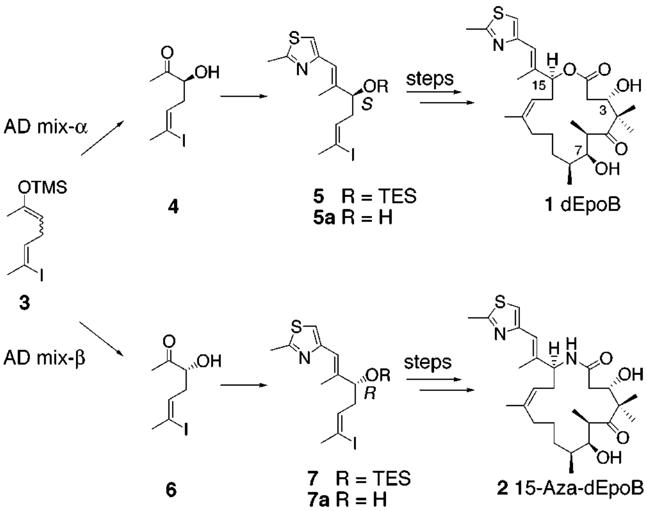
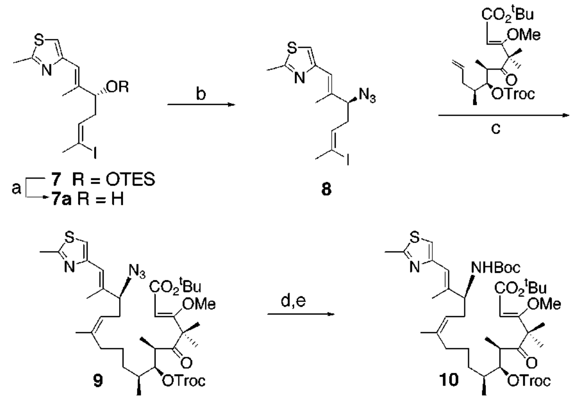
Scheme 1. Asymmetric Dihydroxylation



aza compounds to function in the context of otherwise resistant tumors. An account of the chemical synthesis of our targets and their preliminary evaluation is provided herein.

Deprotection of **7** as shown afforded alcohol **7a** (Scheme 2). The latter underwent Mitsunobu inversion,[2] as indicated, to provide azide **8**. While the reaction worked smoothly, a great deal of effort was necessary to enable the successful integration of this azido function into our synthetic schemes.[5] Eventually, a passage was navigated. It was found that a palladium-mediated *B*-alkyl Suzuki cross-coupling[6] could be conducted with the azide in place (see **8** → **9**). In the context of the C2−C3 enol ether, Staudinger reduction of **9** is possible, leading, after nitrogen protection, to **10**.[7]

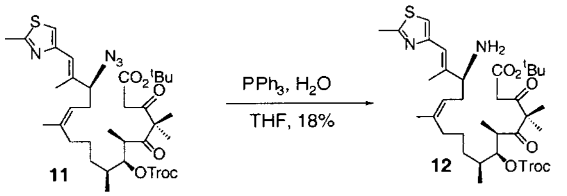
Scheme 2. Mitsunobu Inversion[a]



Conditions: a) HOAc:THF:H_2O, 98%; b) PPh_3, DIAD, HN_3, THF, 66%; 9-BBN, THF then Pd(dppf)Cl_2, $AsPh_3$, $CsCO_3$, DMF 70%; d) PPh_3, THF, H_2O, 92%; e) Boc_2, ACN, Et_3N, 65%.

By contrast, in a related azido substrate **11** containing the C3 ketone, Staudinger reduction en route to **12** could be conducted in our hands in only 18% yield (Scheme 3).

Scheme 3. Staudinger Reduction of Azido-*β*-keto Ester



Reduction of the azide to the amine is apparently complicated by Schiff base formation (inter- or intramolecular) between the in situ generated 15-amino functionality and the 3-keto group.[8]

With the N-^{t}Boc protective function in place at C15, we could conduct the ruthenium-mediated asymmetric Noyori-type reduction[9] at C3 (see transformation **10** → **14**, Scheme 4). By contrast, the corresponding reduction at the stage of **11** was not successful. Apparently the azide function is not compatible with the strongly acidic Noyori reduction protocol, though it does not seem to be converted to the amino group under these conditions.

With **14** in hand, the ^{t}Boc and *tert*-butyl ester groups were cleaved concurrently to afford amino acid **15**. This reaction could be conducted in the presence of a free C3 hydroxyl group. This group also did not interfere with HATU-mediated macrolactamization[10] to afford **16**, albeit in only 50% yield. Finally, the C7 Troc group was discharged through the action of zinc dust under the influence of sonication, affording the desired **2**.[11] In much the same way, the 15(*S*) precursor (**5**) was converted to 15(*R*)-epi-aza-dEpoB (**17**).

The fully synthetic 15-aza-12,13-desoxyepothilone B (**2**) and the epimeric 15-aza-12,13-desoxyepothilone B (**17**) analogues have been tested against a variety of tumor cells in order to evaluate their antitumor activity. Initially, the aza

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(3) (a) Compound **2** was first reported in the patent literature: Vite, G. D.; Borzilleri, R. M.; Kim, S.-H.; Johnson, J. A. Patent WO 99/102514, 1999. It was then reported at (b) the 219th National Meeting of the American Chemical Society, San Francisco, March 2000, by one of us (S.J.D.; ORGN 43) and then by (c) Vite, G.; Borzilleri, R.; Fooks, C.; Johnson, J.; Kim, S.-H.; Leavitt, K.; Mitt, T.; Regueiro-Ren, A.; Schmidt, R.; Zheng, P.; Lee, F. (ORGN 286).

(4) For permutations at C-15 in the lactone series, see: (a) Harris, C. R.; Danishefsky, S. J. *J. Org. Chem.* **1999**, *64*, 8434. (b) Meng, D. F.; Bertinato, P.; Balog, A.; Su, D. S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10073.

(5) Mitsunobu attempts on late-stage epothilone constructs were low yielding.

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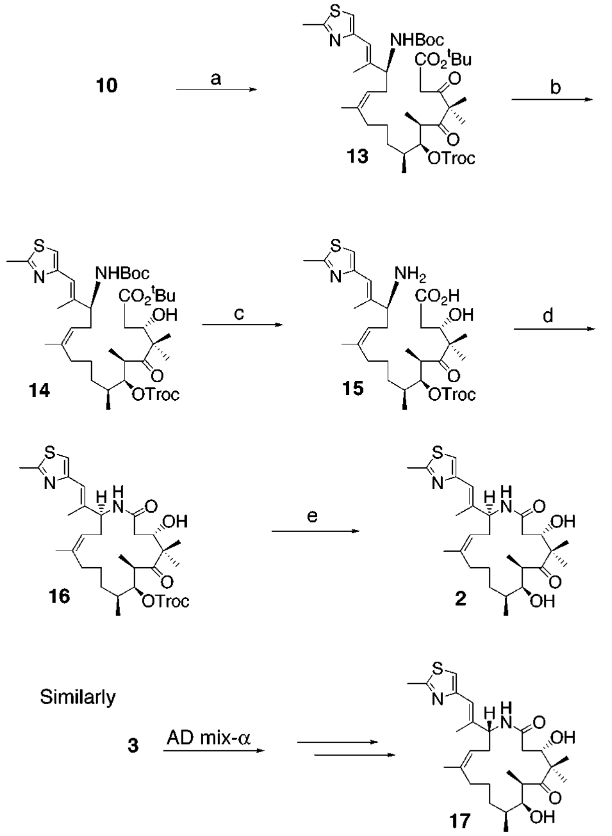
(11) Wasserman, H. H.; Robinson, R. P.; Carter, C. G. *J. Am. Chem. Soc.* **1983**, *105*, 1697.

Scheme 4. Synthesis of Aza-dEpoB (**2**)[a]



Conditions: a) p-TsOH, acetone, 82%; b) $[Ru(BINAP)Cl_2]_2$- Et_3N, 0.12M HCl/MeOH, H_2, 1200psi, 78%; c) TFA, CH_2Cl_2; d) HATU, Hoat, DIPEA, CH_2Cl_2, 50%; e) zinc dust, HOAc, sonication.

analogues (**2** and **17**) were assayed to determine their relative ability to bind tubulin in comparison to dEpoB. The results indicated that for 15-aza-dEpoB (**2**) microtubulin stabilizing activity was maintained, retaining 75% of the activity of dEpoB. However, the epimeric 15-aza-dEpoB analogue (**17**) did not appreciably stabilize microtubules within the detection limits of the assay. Hence, a major effect of C15 stereochemistry on the microtubule stabilization properties of the aza agents has been uncovered. Similarly, in cytotoxicity studies, a direct comparison of 15-aza-dEpoB (**2**) with dEpoB (**1**), using the CCRF-CEM cell line, showed that it was only slightly less potent than dEpoB (4.8× less active). By contrast, the corresponding 15-epi-aza-dEpoB (**17**) analogue displayed severely reduced antitumor activity (21× less active). We also noted that, both aza-dEpoB (**2**) and its corresponding epimer (**17**) were not active against several benchmark paclitaxel or vinblastine resistant tumor cells (CCRF-CEM/taxol and CCRF-CEM/VBL, respectively) used in earlier studies. By contrast, dEpoB itself is highly active against these resistant lines.[12] In addition, we have successfully epoxidized **2** to produce the corresponding aza-EpoB analogue. This compound has recently been advanced to phase 1 clinical trials by the Bristol-Myers Squibb company.[3c]

In summary, the feasibility of carrying out an oxygen to nitrogen Mitsunobu inversion in seco dEpoB systems portends access to various novel structural variants of the system. For the moment, however, aza systems **2** and **17** do not appear to be as clinically promising as dEpoB.

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