ORGANIC LETTERS

2008
Vol. 10, No. 7
1369−1372

Synthesis of 7-*Epi* (+)-FR900482: An Epimer of Comparable Anti-Cancer Activity

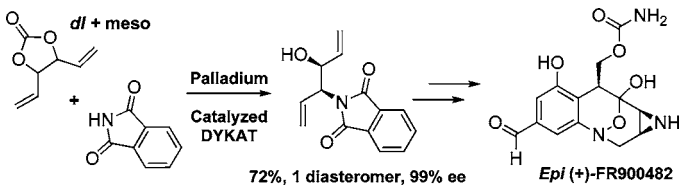
Barry M. Trost* and Brendan M. O'Boyle

Department of Chemistry, Stanford University, Stanford, California 94305-5080

bmtrost@stanford.edu

Received January 18, 2008

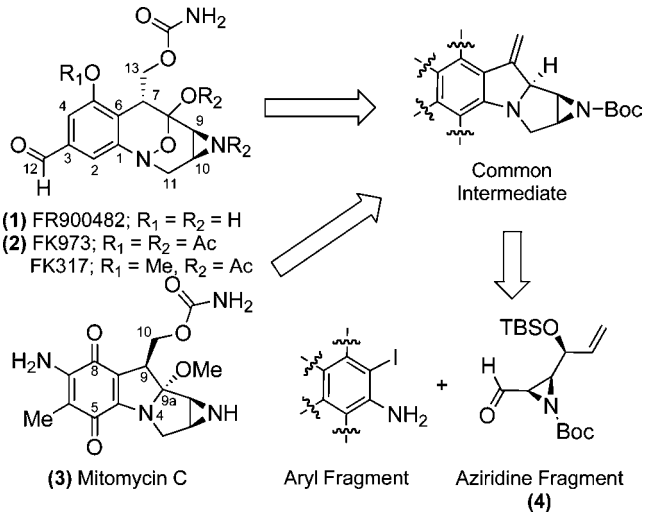
ABSTRACT



FR900482 is a potent anti-tumor therapeutic that has been investigated as a replacement candidate for the clinically useful Mitomycin C. Herein, we report synthesis and biological testing of 7-*Epi* (+)-FR900482, which demonstrates equal potency relative to the natural product against several cancer cell lines. Highlights of this work include utilization of our palladium-catalyzed DYKAT methodology and development of a Polonovski oxidative ring expansion strategy to yield this equipotent epimer in 23 linear steps.

FR900482 (**1**)[1] and mitomycin C (**3**)[2] possess structural similarities that suggest a common biosynthesis. These compounds also share similar modes of action[3,4] and medicinal properties. Specifically, mitomycin C is administered to patients suffering from a diverse range of cancers,[5] whereas **1** and its triacetate FK973 (**2**) are replacement candidates exhibiting superior activity and decreased toxicity.[6] We envisioned both natural products to be accessible via a common synthetic route (Scheme 1). In practice, preparation of the mitomycin skeleton en route to FR900482 would allow aziridine intermediate **4** to be a common precursor for the preparation of both natural products. Naturally occurring mitomycins of both C-9 stereochemistries are active (mitomycin numbering), but no data regarding the analogous FR900482 derivative was available prior to our work. This report describes our synthesis and biological testing of 7-*epi* (+)-FR900482.

Scheme 1. General Approach to FR900482 and the Mitomycins



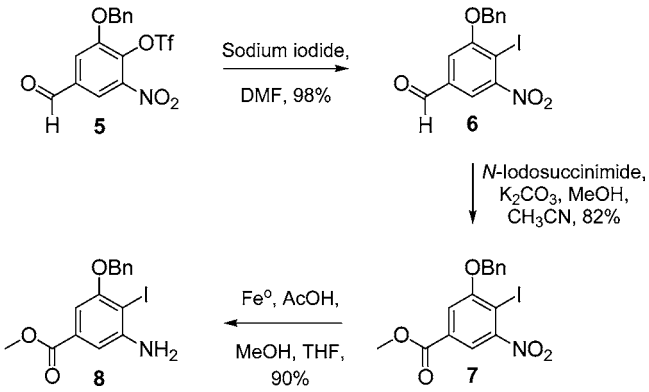
(1) Uchida, I.; Takase, S.; Kayakiri, H.; Kiyoto, S.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 4108.
(2) Gruschow, S.; Chang, L. C.; Mao, Y.; Sherman, D. H. *J. Am. Chem. Soc.* **2007**, *129*, 6470 and references therein.
(3) Mitomycin, C.; Tomasz, M. *Chem. Biol.* **1995**, *2*, 575.
(4) FR900482: Fukuyama, T.; Goto, S. *Tetrahedron Lett.* **1989**, *30*, 6491. See refs 25 and 26 for later studies on the mode of action.
(5) Bradner, W. T. *Cancer Treat. Rev.* **2001**, *27*, 35.
(6) Shimomura, K.; Manda, T.; Mukumoto, S.; Masuda, K.; Nakamura, T.; Mizota, T.; Matsumoto, S.; Nishigaki, F.; Oku, T.; Mori, J.; Shibayama, F. *Cancer Res.* **1988**, *48*, 1166.

10.1021/ol800127a CCC: $40.75
Published on Web 03/05/2008

FR900482 has been studied extensively, resulting in five total,[7−11] and several formal, syntheses,[12−14] the shortest of which was reported by Williams and requires 29 linear steps (33 total). We reasoned that uniting a suitable aromatic fragment with aziridine **4** would allow for convergent access to both series, leaving only changes in oxidation state to complete the synthesis (Scheme 1). Beginning with chemistry reported in this group,[15] we envisioned that aziridine fragment **4** could be assembled in an efficient manner. Finally, this approach introduces several polar functional groups late in the synthesis and avoids multiple protecting group manipulations.

Preparation of the aryl fragment **8** (Scheme 2) began with the known triflate **5**,[12] which is prepared in three steps from

Scheme 2. Preparation of the Aryl Fragment

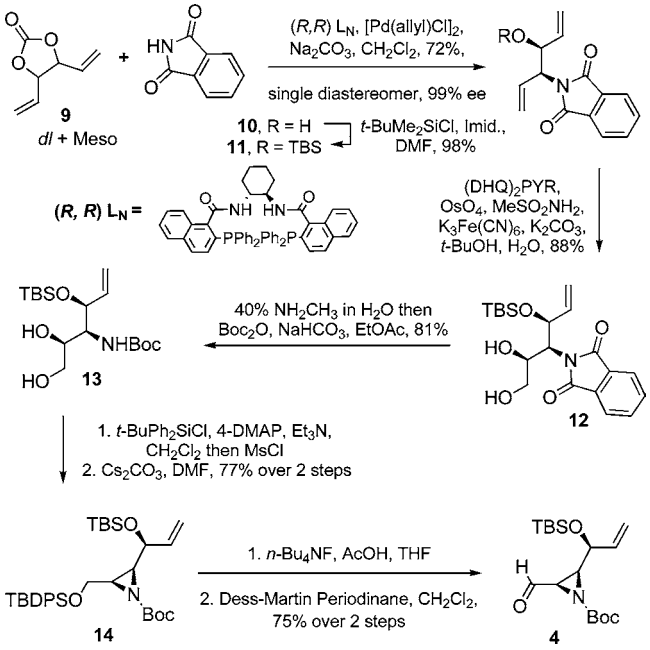


commercially available 5-nitrovanillin as reported previously. The aryl triflate **5** is converted to an aryl iodide **(6)** via nucleophilic aromatic substitution with sodium iodide in DMF. Oxidation of aldehyde **6** with NIS and K_2CO_3 in methanol affords the methyl benzoate **7**. This substrate is subjected to iron mediated reduction yielding aniline **8** without undesirable reduction of the aryl iodide.

The requisite aziridine **4** is prepared in eight steps from divinyl carbonate **9** (Scheme 3). First, a palladium-catalyzed DYKAT (dynamic catalytic asymmetric transformation) reaction[15] yields amino-alcohol **10** as a single stereoisomer. Following TBS protection, diene **11** is submitted to Sharpless dihydroxylation,[16] yielding the diol **12** with excellent chemo- and diastereoselectivity. The phthalimide group contained in amino-alcohol **12** is exchanged for a *tert*-butyl carbamate yielding diol **13**. Selective silylation and mesylation, followed by exposure of the crude mesylate to cesium carbonate in warm DMF affords the aziridine **14**. Finally, removal of the TBDPS group and Dess-Martin oxidation yields the completed aziridine fragment **4**.

Reductive amination between the aziridine **4** and aniline **8** affords the coupled product **15** (Scheme 4). Removal of the allylic TBS group and activation of the resulting alcohol with triflic anhydride yields the pyrrolidine **16**. Simple inversion at C-8 was initially expected; however, NOE analysis of intermediate **18** and its epimer reveals strong correlations between the C-8 and C-9 protons (Scheme 4

Scheme 3. Preparation of the Aziridine Fragment



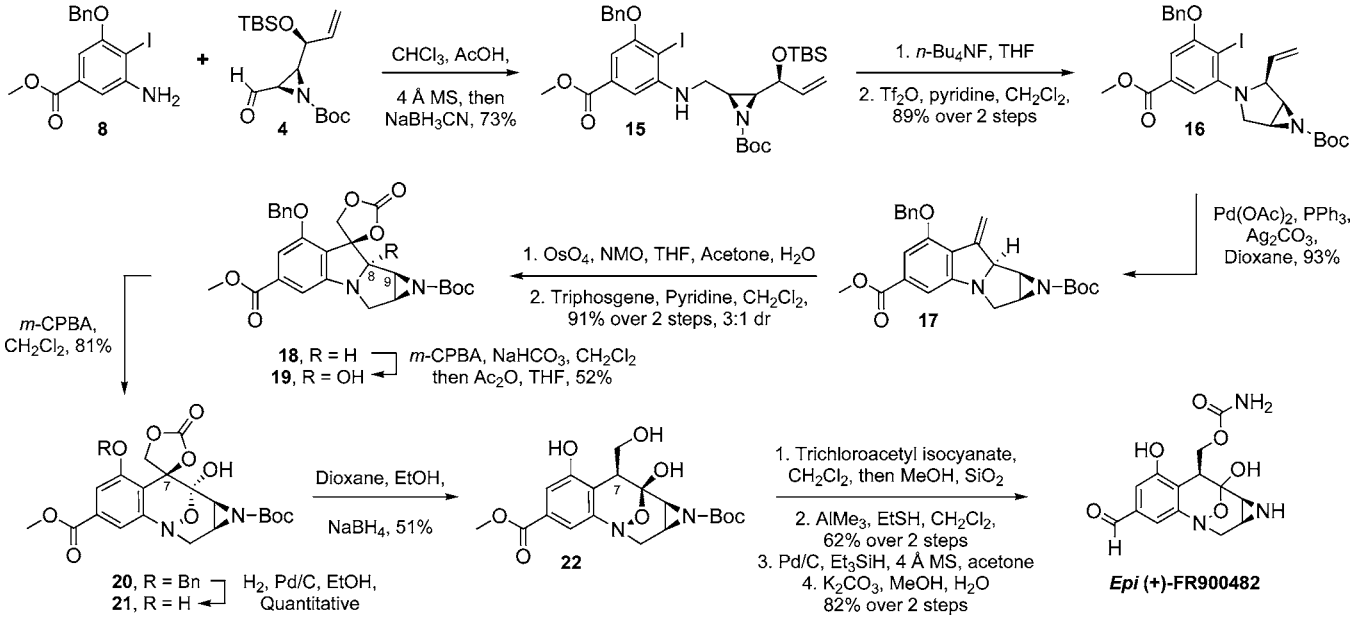
and Supporting Information). Thus, it appears that double inversion occurs via neighboring group participation of the *tert*-butyl carbamate placing the vinyl group *cis* to the aziridine.

The single carbon−carbon bond formation of our synthesis proceeds via Heck coupling[17] to produce the exocyclic olefin **17** and construct the "mitomycin" skeleton of the natural product. No detectable double bond isomerization is observed in this step, even though such a process would lead to aromatization, via formation of an indole.

Inspired by the pioneering works of Dmitrienko[18] and Ziegler,[19] we envisioned that a Polonovski reaction, followed by a subsequent oxidative ring expansion, might afford the

(7) Fukuyama, T.; Xu, L.; Goto, S. *J. Am. Chem. Soc.* **1992**, *114*, 383.
(8) Schkeryantz, J. M.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1995**, *117*, 4722.
(9) (a) Katoh, T.; Itoh, E.; Yoshino, T.; Terashima, S. *Tetrahedron* **1997**, *53*, 10229. (b) Yoshino, T.; Nagata, Y.; Itoh, E.; Hashimoto, M.; Katoh, T.; Terashima, S. *Tetrahedron* **1997**, *53*, 10239. (c) Katoh, T.; Nagata, Y.; Yoshino, T.; Nakatani, S.; Terashima, S. *Tetrahedron* **1997**, *53*, 10253.
(10) (a) Judd, T. C.; Williams, R. M. *Angew. Chem., Int. Ed.* **2002**, *41*, 4683. (b) Judd, T. C.; Williams, R. M. *J. Org. Chem.* **2004**, *69*, 2825.
(11) (a) Suzuki, M.; Kambe, M.; Tokuyama, H.; Fukuyama, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 4686. (b) Suzuki, M.; Kambe, M.; Tokuyama, H.; Fukuyama, T. *J. Org. Chem.* **2004**, *69*, 2831.
(12) Fellows, I. M.; Kaelin, D. E.; Martin, S. F. *J. Am. Chem. Soc.* **2000**, *122*, 10781.
(13) Paleo, M. R.; Aurrecoechea, N.; Jung, K. Y.; Rapoport, H. *J. Org. Chem.* **2003**, *68*, 130.
(14) Ducray, R.; Ciufolini, M. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 4688.
(15) Trost, B. M.; Aponick, A. *J. Am. Chem. Soc.* **2006**, *128*, 3931.
(16) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483.
(17) Abelman, M. M.; Overman, L. E.; Tran, V. D. *J. Am. Chem. Soc.* **1990**, *112*, 6959.
(18) (a) Dmitrienko, G. I.; Denhart, D.; Mithani, S.; Prasad, G. K. B.; Taylor, N. J. *Tetrahedron Lett.* **1992**, *33*, 5705. (b) Mithani, S.; Drew, D. M.; Rydberg, E. H.; Taylor, N. J.; Mooibroek, S.; Dmitrienko, G. I. *J. Am. Chem. Soc.* **1997**, *119*, 1159.
(19) (a) Ziegler, F. E. Belema, M. *J. Org. Chem.* **1994**, *59*, 7962. (b) Ziegler, F. E.; Belema, M. *J. Org. Chem.* **1997**, *62*, 1083.

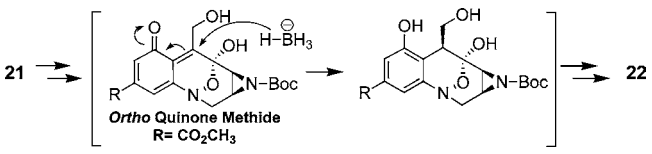
Scheme 4. Completion of the Synthesis



desired [3.3.1] bicycle.[20] To prevent aromatization during Polonovski conditions, the benzylic position is blocked by first dihydroxylating the *exo*-olefin and then protecting the resulting diol as a cyclic carbonate. To our delight, Polonovski oxidation of **18** affords **19** in good yield. Exposure of the aminal to a second equivalent of *m*-CPBA affords the hydroxylamine hemiketal **20**. Here initial formation of an amine oxide leads to spontaneous ring-opening, followed by recombination to yield the [3.3.1] bicycle, via the open eight-membered ketone.[21]

At this time, our attention turned to the crucial C-7 deoxygenation. Unfortunately, hydrolysis of the carbonate **20** proved difficult due to unexpected sensitivity of the *tert*-butyl carbamate toward base. This caused us to explore sequential hydrogenolysis to remove the C-7 oxygen substituent. Initial hydrogenolysis of the benzyl ether yields the phenol **21**, which contains a handle for directed reduction of the carbonate. Subsequent exposure of this phenol to sodium borohydride in methanol results in removal of the carbonate, but instead of the anticipated tetraol, a triol is formed containing a methoxy group at C-7 (not shown). Speculating that an *ortho* quinone methide intermediate is responsible for this transformation, optimization focused on suppressing the undesired solvolysis to facilitate capture of the reactive intermediate (Scheme 5). Remarkably, replacing methanol with ethanol inhibits solvolysis to such an extent that reduction of the *o*-quinone methide dominates to yield the triol **22**.

Scheme 5. Mechanism for *o*-Quinone Methide Reduction



The connectivity of **22** is confirmed by NMR analysis (COSY, HMBC, HSQC), but the configuration at C-7 remained uncertain. Completion of the total synthesis is accomplished in four additional steps. The primary alcohol of **22** is converted to a primary carbamate via treatment with trichloroacetyl isocyanate followed by *in situ* hydroylsis of the trichloroacetyl group. Exchange of the methyl ester for the ethyl thioester is followed by Fukuyama[22] reduction with palladium on carbon. Finally, the *tert*-butyl carbamate is removed via hydrolysis with saturated aqueous potassium carbonate in methanol, liberating the free aziridine. The product is isolated as a 3:2 mixture of separable hemiketal isomers, however, comparison to an authentic sample (NMR, TLC) shows that neither isomer matches the natural product, clearly demonstrating our product to be epimeric at C-7.[23]

Although we were somewhat disappointed not to have the natural product in hand, we were quite excited by the likelihood that this epimer might have comparable activity. Both Hopkins[24] and Williams[25] have independently demonstrated that Fe^{2+} mediated reductive activation of FR900482

(20) For a related oxidative ring expansion, see: Colandrea, V. J.; Rajaraman, S.; Jimenez, L. S. *Org. Lett.* **2003**, *5*, 785.

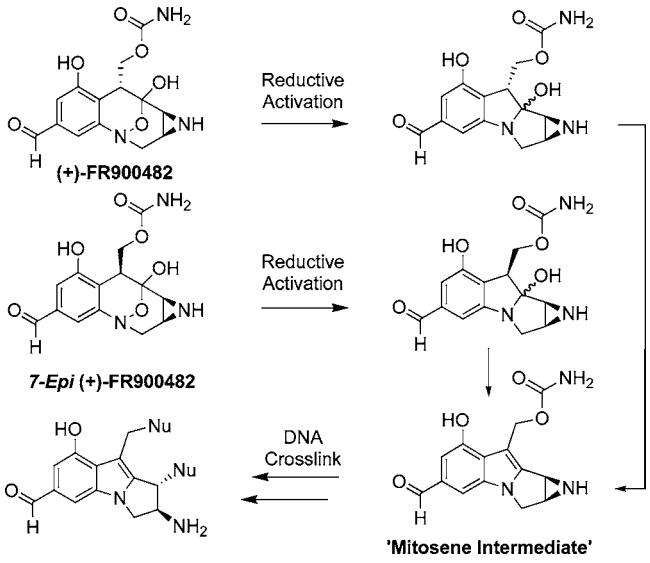
(21) The stereochemistry obtained in hemiketal **20** is assigned by comparison to its epimer, which could be obtained under slightly modified conditions (see Supporting Information for details).

(22) Fukuyama, T.; Tokuyama, H. *Aldrichim. Acta* **2004**, *37*, 87.

(23) This stereochemical result is in contrast to that observed by Danishefsky,[8] who observed formation of an aldehyde leading to the C-7 hydroxymethyl group *cis* to the hydroxylamine bridge. In our case, this pathway would be expected to give the natural stereochemistry. Formation of 7-*epi*-FR900482 suggests the *o*-quinone methide is reduced directly by sodium borohydride without the intermediacy of an aldehyde. Danishefsky was also able to obtain the opposite stereochemistry at C-7 via a samerium iodide mediated reduction of a benzylic epoxide. Unfortunately, reproduction of these condidtions failed on carbonate **20** presumably due to the lower reactivity for C-O homolytic cleavage of a cyclic carbonate compared to an epoxide.

in the presence of a thiol reducing agent affords the reactive "mitosene" intermediate capable of cross-linking DNA (Scheme 6). Obviously, the stereochemistry at C-7 has been

Scheme 6. Mechanism of Activation



lost and both 7-*epi*-FR900482 and the natural product must reach a common intermediate prior to alkylation of DNA. However, it remained unclear if reductive activation would function *in vivo* to yield the same "mitosene" intermediate, since, if it is an enzyme mediated process, it should, *a priori*, be sensitive to stereochemistry.

Naoe[26] has shown a connection between the metalloenzyme DT-diaphorase and cytotoxicity for FK317 (Scheme 1). In this case, exposure of cells to dicumarol, an inhibitor of this enzyme significantly lowers cytotoxicity; furthermore, FK317 is more active against cells expressing this enzyme. Given the selectivity of this family for inhibiting the growth of cancer cells, the reductive activation is expected to be enzyme dependent, as indiscriminate reduction would lead to a nonselective DNA alkylating reagent.

To our delight, comparison of our epimer to the natural product does demonstrate that the C-7 stereochemistry has only negligible effect on cytotoxicity against two human breast cancer cell lines (Table 1).

Table 1. Comparitive Cytotoxicity Data[a]

IC_{50} mM	SKBr3	MCF-7
FR900482	0.980	1.020
7-*Epi*-FR900482	1.000	1.100

[a] Preparation of the triacetate FK973 is known to increase cytotoxicity up to 50 fold.[6]

From a synthetic perspective, the fact that both epimers have equal activity also means that both are equally valuable synthetic targets. In this sense, our synthesis which requires 23 linear steps (29 total) and proceeds in 1.4% yield from commercially available 1,5-hexadiene-3,4-diol is the shortest route to this family yet disclosed.[27] Highlights of this synthesis include: novel formation of a chiral building block **4** via our DYKAT methodology, a chemo- and diastereoselective Sharpless dihydroxylation of **11**, effective utilization of the Polonovski reaction, as well as sequential hydrogenolysis via a putative *o*-quinone methide intermediate. As this synthesis efficiently delivers the mitomycin skeleton prior to oxidative expansion to the FR900482 series, this synthesis may also prove amenable to the preparation of mitomycins or analogues of either class. Efforts to expand upon this synthesis are underway and will be reported in due course.

Acknowledgment. We are grateful to the NIH (GM 33049) for their generous support of our program. Many thanks to David Barrett of Astellas for providing authentic material. We thank Dr. Yong Li and Dr. Hugo Menzella from Kosan Biosciences for the comparative biological testing of natural FR900482 against 7-*epi* FR900482. Mass spectra were provided by the Mass Spectrometry Regional Center of the UCSF, supported by the NIH Division of Research Resources.

Supporting Information Available: Experimental and spectroscopic data are provided for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL800127A

(24) (a) Woo, J.; Sigurdsson, S. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 1199. (b) Huang, H.; Pratum, T. K.; Hopkins, P. B. *J. Am. Chem. Soc.* **1994**, *116*, 2703. (c) Paz, M. M.; Hopkins, P. B. *J. Am. Chem. Soc.* **1997**, *119*, 5999. (d) Paz, M. M.; Hopkins, P. B. *Tetrahedron Lett.* **1997**, *38*, 343.

(25) (a) Williams, R. M.; Rajski, S. R. *Tetrahedron Lett.* **1992**, *33*, 2929. (b) Williams, R. M.; Rajski, S. R. *Tetrahedron Lett.* **1993**, *34*, 7023. (c) Williams, R. M.; Ducept, P. *Biochemistry* **2003**, *42*, 14696.

(26) Naoe, Y.; Inami, M.; Kawamura, I.; Nishigaki, F.; Tsujimoto, S.; Matsumoto, S.; Manda, T.; Shimomura, K. *Jpn. J. Cancer Res.* **1998**, *89*, 666.

(27) Fukuyama 1st synthesis:[7] 0.20% yield over 40 linear steps (40 total, racemic); Danishefsky:[8] 30 linear steps and 3.3% yield over the final 23 steps (yield of first 7 steps and number of off-line steps are not reported, racemic); Terashima:[9] 0.20% yield over 42 linear steps (57 total, +); Williams:[10] 0.08% over 29 linear steps (33 total, +); Fukuyama 2nd synthesis:[11] 1.7% over 31 linear steps (37 total, +).