Published in final edited form as:

Chemistry. 2011 July 4; 17(28): 7890–7903. doi:10.1002/chem.201003489.

Development of a Flexible Strategy Toward FR900482 and the Mitomycins

Prof. Barry M. Trost^[a], Dr. Brendan M. O'Boyle^[b], Dr. Wildeliz Torres^[c], and Dr. Michael K. Ameriks^[d]

Barry M. Trost: bmtrost@stanford.edu; Brendan M. O'Boyle: brendan_oboyle@merck.com; Wildeliz Torres: wildeliz@hotmail.com; Michael K. Ameriks: mameriks@its.jnj.com

^[a]Department of Chemistry, Stanford University, Stanford, CA 94305-5080 (USA), Fax: (+1) 650-725-0002

^[b]Merck Research Laboratories: BMB 3-128, 33 Avenue Louis Pasteur, Boston, MA 02115

^[c]Department of Chemistry, University of Puerto Rico, Río Piedras Campus, PO Box 23346, San Juan, PR 00931-3346

^[d]Johnson & Johnson Pharmaceutical Research & Development, L.L.C., 3210 Merryfield Row, San Diego, CA 92121

Abstract

FR900482 and the mitomycins are two intriguing classes of alkaloid natural products that have analogous biological mechanisms and obvious structural similarity. Both classes possess potent anti-cancer activity, a feature that has led to their investigation and implementation for the clinical treatment of human cancer. Given the structural similarity between these natural products, we envisioned a common synthetic strategy by which both classes could be targeted through assembling the mitomycin skeleton prior to further oxidative functionalization. Realization of this strategy with respect to FR900482 was accomplished through the synthesis of 7-*epi*-FR900482, which displayed equal potency relative to the natural product against two human cancer cell lines. With the challenging goal of a synthesis of either mitomycin or FR900482 in mind, several methodologies were explored. While not all of these methods ultimately proved useful for our synthetic goal, a number of them led to intriguing findings that provide a more complete understanding of several methodologies. In particular, amination *via* π -allyl palladium complexes for the synthesis of tetrahydroquinolines, 8-membered heterocycle formation *via* carbonylative lactamization, and amination through late-stage C-H insertion *via* rhodium catalysis all featured prominently in our synthetic studies.

Keywords

alkaloid; asymmetric catalysis; mitomycin; aziridine; DYKAT

FR900482 and the mitomycins are an important class of bioactive natural products that have been extensively investigated due to their intriguing molecular architecture and potential anti-cancer properties.¹ Indeed, numerous synthetic approaches have been reported that are based upon several unique strategies.^{2,3} While laudable, these methods are hampered by length and multiple exchanges of protecting groups limiting their overall synthetic efficiency.

We hoped to improve on previous efforts by reducing the number of synthetic steps to the desired target. It was also envisioned that a common intermediate could potentially access either the mitomycin or the FR900482 ring system with maximal flexibility for the variation of the aromatic core. Thus potentially either natural product or analogs of both classes could be prepared through a common synthetic strategy. Because no asymmetric route to the mitomycin family has yet been reported, the ability to access either enantiomer of the mitomycin system would represent a significant milestone in synthetic methods toward this family of natural products. Herein we disclose our venture toward that goal.

Results and Discussion

Pd-catalyzed amination

Our initial retrosynthesis of **1** envisioned that the [3.3.1] bicyclic core of FR900482 would be assembled via transannular hemiketalization of the 8-membered hydroxylamine derivative **8**. The key transformation involved an 8-*endo* cyclization of palladium π -allyl complex **11** to generate the benzazocine **10**. This intermediate could in turn be derived from allene **12** or the corresponding allylic carbonate **13**.

While this retrosynthetic analysis is unconventional, precedent existed for cyclization to yield an 8-membered carbocycle in preference to a possible 6-membered isomer through a palladium π -allyl alkylation.⁴ For example, both allylic carbonates and allenes were known to favor the formation of the larger 8 or 9 membered rings, with little or none of the smaller 6 or 7 membered ring systems (Eq. 1–3). Although an aniline would be the nucleophile in our case we were hopeful that regioselective formation of the benzazocine would be possible considering that the benzazocine core has minimal transannular interactions, which typically disfavor medium sized ring formation.⁵ This should allow for nucleophilic attack at the sterically more accessible terminal position of the π -allyl unit.

Equation 1

Equation 2

Equation 3

To examine whether this cyclization would be possible, anilines **20a-e** were prepared. Hydropalladation of the terminal allene was anticipated to precede 8-*endo* cyclization onto the palladium π -allyl complex. Under a variety of conditions (Table 1) formation of the desired benzazocine core was not observed and instead the tetrahydroquinoline ring system was formed (**21a-e**) with good to excellent diastereoselectivity in some cases. Both the yield and the diastereoselectivity of the cyclization varied with the electronic and steric environment of the arylamine nucleophile and allene electrophile.

Further experimentation focused on synthesis of the corresponding allylic carbonates (**22a** and **22b**). It was anticipated that some of the entropic barrier to 8-*endo* cyclization could be overcome by generation of the *anti* π -allyl complex, which must be the initial intermediate resulting from ionization of **22a** or **22b**. Furthermore, the 8-*endo* product must arise from attack on the *anti* π -allyl, as the *syn* intermediate would lead to a *trans* olefin within the newly formed ring. Again good yields and excellent diastereoselectivities could be obtained, but only for the undesired tetrahydroquinolines **23a** and **23b**. Thus, it appears that Pd-catalyzed cyclizations to form benzazocins cannot be achieved when the formation of a tetrahydroquinoline is a competing process.

While both of these amination methods were ultimately not productive for continuing on with the synthesis, it is important to note that the 1,2,3,4-tetrahydroquinolines **24** are a class of medically important compounds in their own right. Among these L-689,560⁶ (**25**) and vintamycin (**26**)⁷ are two examples of highly substituted tetrahydroquinolines that might be amenable to synthesis through this method (Figure 2).

Carbonylative Lactamization

Our initial retrosynthetic analysis was modified in favor of a carbonylative lactamization approach (Scheme 2). While carbonylative lactamization had not been reported for the formation of 8-membered heterocycles, this method appeared well suited for formation of the benzazocine core of FR-900482. Preparation of **27** appeared feasible with minimal difficulty and cyclization seemed likely particularly because the olefin and aromatic ring would preclude the transannular interactions and lessen the entropic barrier that causes 8-membered rings to be notoriously difficult to prepare. Synthesis of the requisite lactamization precursor **27** (with incorporation of a handle required for asymmetric synthesis) was accomplished through a Carreira alkynylation of aldehyde **29** with trimethylsilyl acetylene to yield alkyne **31** in good yield and excellent enantioselectivity. The success of this reaction with the highly epimerizable substrate is noteworthy and multiple other alkynylation methods were unsuccessful. While other protocols for this reaction (including Carreira's catalytic conditions) were attempted, none gave the desired product in acceptable yield. Thus, the synthesis of three lactamization precursors (**27a**, **27b**, and **27c**) was accomplished through incorporation of iodine, *cis*-reduction of the alkyne with diimide, and Sm or Zn reduction of the nitro group to the hydroxylamine oxidation state. It should be noted that samarium(II) iodide functioned with excellent chemoselectivity even in the presence of the vinyl iodide.

With cyclization substrates **27a-c** in hand, a thorough investigation of the palladium-catalyzed carbonylative lactamization was performed (Table 3). Upon refluxing a dilute solution of vinyl iodide **27a**, triethylamine, triphenylphosphine, and 5% tetrakis(triphenylphosphine)palladium(0) in THF (0.03M) under an atmosphere of carbon monoxide, a 36% yield of the desired 8-membered ring **28a** was obtained, along with some recovered starting material (49% brsm, entry 1). Changing the base (Entry 2) failed to increase the yield, while changing the palladium catalyst and reaction concentration significantly improved the yield (Entries 3 and 4). These optimized reaction conditions

proved applicable to modification of the substrate as well. Modification of the protecting group on the hydroxylamine to TBDPS was tolerated, as was employing benzyl ether **27c** (Entries 5 and 6).

At this stage multiple attempts to install the aziridine functionality on **28** failed. The lactams **28a-c** could be selectively reduced ($\text{BH}_3\cdot\text{SMe}_2$, THF, 0 °C, 55%), however, aziridination of either the unsaturated lactam or *cis*-olefin failed under various nitrene or epoxide based approaches.

In order to circumvent these difficulties, an alternative approach was devised in which the aziridine moiety would be introduced earlier in the synthesis.

Aziridine and Aromatic Fragment Approaches

The revised approach relied on an early and convergent union of an aziridine (**39**) and aromatic fragment (**40**) (Scheme 4) and a late stage intramolecular C-C bond formation to complete the 8-membered core (**37**) of the molecule. Several alternative reactions might accomplish this, but a Nozaki-Hiyama-Kishi or reductive ynal coupling seemed the most likely methods of success.

As shown in Scheme 5, the aromatic fragment **43** was prepared in three steps from known aldehyde **41**.⁸ A Sonogashira coupling⁹ of **41** and TMS acetylene was followed by protection of the aldehyde as an acetal. An iron metal reduction of the nitro group gave aniline **43** in a 34% overall yield from **41**.

Synthesis of the aziridine fragment began from the known DYKAT of butadiene monoepoxide (**44**) and phthalimide (**45**) outlined in equation 4.¹⁰ This reaction yields the protected amino-alcohol **47** in nearly quantitative yield and excellent enantioselectivity.

Equation 4

After conversion of the phthalimide to a Boc carbamate (**48**), the introduction of the second stereocenter was accomplished in diastereoselective fashion through a *syn* directed epoxidation¹¹ yielding **49** (Scheme 6). Furthermore, heating the reaction mixture results in selective decomposition of the minor *anti* epoxide through formation of an oxazolidinone. While the two epoxide diastereomers were chromatographically separable, it proved helpful to heat the reaction for a longer duration on larger scale to simplify the purification process.

It was anticipated at this stage that the desired aziridine (**39**) could be prepared via an aza-Payne rearrangement¹² of **50** if an appropriate base and oxaphilic electrophile is used. Unfortunately, attempts to accomplish this transformation under a variety of conditions proved fruitless and only the oxazolidinone was obtained. One alternative to epoxide opening would be a suitably activated diol, provided reasonable diastereoselectivity could be achieved. Fortunately, under the conditions of Sharpless, good diastereoselectivity and excellent yield were obtained for the formation of diol **52** from **51** (Table 4).

With this result in hand, the desired aziridine fragment was quickly completed (Scheme 7). The Sharpless dihydroxylation was sufficiently clean that the immediate product was never purified beyond separatory funnel workup to remove reagents employed in the dihydroxylation. Instead, cleavage of the phthalimide protecting group and *in situ* protection of the polar amino-diol as a *tert*-butyl carbamate was accomplished under the conditions described in Scheme 6 to prepare **53**.

Chemoselective protection of the primary alcohol with a *tert*-butyl-diphenylsilyl group and *in situ* mesylation, followed by warming the crude mesylate in dimethylformamide and cesium carbonate resulted in the formation of aziridine **54**. Aziridines **55** and **56** were prepared *via* desilylation of the TBDPS ether **56** and Dess-Martin oxidation. This entire synthetic sequence requires 8 steps from butadiene monoepoxide and gives **56** in 27% overall yield.

Alternatively, diol **53** could be bis-mesylated and analogous conditions resulted in selective three-membered ring formation providing mesylate **57**. Compound **57** could also be prepared by mesylation of **55** (MsCl, Et₃N, CH₂Cl₂, 90%).

While attempts at coupling the fragments **43** and **57** via nucleophilic addition failed, reductive amination of **43** and **56** proved to be a reliable method for accomplishing this transformation (Scheme 8). This reaction was limited to the aniline oxidation state and failed for the corresponding hydroxylamine. Because aldehyde **56** was quickly reduced even in the presence of triacetoxyborohydride, it was essential to drive the dehydration prior to addition of the reducing agent. As a result incorporation of the desired hydroxylamine oxidation state directly was complicated. Similar conditions to yield a coupling product through the intermediacy of a nitron were not successful.

The desired 8-membered benzazocine core (**60** or **61**) was anticipated to arise through a reductive cyclization under the conditions of Montgomery¹³ and Jamison,¹⁴ or via prior elaboration of the alkyne (**58** or **59**) to facilitate an intramolecular N.H.K. coupling reaction (Scheme 8). However, neither approach proved fruitful for the continuation of the synthesis and the synthetic approach needed to be modified prior to continuing forward.

We envisioned that a more established C-C bond forming reaction, such as the Heck coupling,¹⁵ would provide the sought after benzazocine core **41**. However, in order to prove this hypothesis the parent aromatic and aziridine fragments of the convergent approach in Scheme 4 would have to be modified accordingly (Scheme 9).

The preparation of a suitable aromatic fragment was relatively trivial. Although the Heck reaction could potentially function with the triflate that had been already installed on the aromatic ring **41**, it seemed more logical to approach the difficult Heck reaction with the most activated precursor possible. To accomplish this, the triflate was exchanged for an iodine atom *via* nucleophilic aromatic substitution yielding aryl iodide **65**. Furthermore, because the Songashira reaction (in the previous approach; Scheme 5) had proven quite sensitive to the electronic nature of the aromatic ring, it seemed prudent to employ an electron withdrawing group as a surrogate for the C₁₂ aldehyde. As such, the aldehyde was oxidized in a single step to methyl ester **66** by employing *N*-iodosuccinimide as an oxidizing reagent under basic conditions.¹⁶ Finally an iron mediated reduction¹⁷ proved to be chemoselective for the conversion of the nitro group to the amine in the presence of the iodide yielding **67** in only six linear steps from 5-nitrovanillin. Furthermore, this route proved to be extremely scalable and chromatography was only required in the final step to remove the excess iron from the product. In total 20–25 grams of **67** were prepared in the duration of this study.

Modification of the aziridine fragment might appear to be much more challenging. However, during the course of the first generation studies above, Trost and Aponick reported a DYKAT reaction employing divinylcarbonate (**68**) and phthalimide to produce amino-alcohol **69** as a single diastereomer in very high enantioselectivity.¹⁸ To employ this reaction in our synthesis, the subsequent dihydroxylation was required to be chemo and diastereoselective. It has been demonstrated that allylic groups play a pronounced role in the rate of dihydroxylation under Sharpless conditions,¹⁹ thus it was anticipated that the required selectivity might be accomplished with the proper choice of protecting group.

To test this possibility, the *tert*-butylsilyl ether **70** was prepared in three steps from hexa-1,5-diene-3,4-diol (available from TCI-America as a stereorandom mixture). The DYKAT reaction was initially performed under the conditions reported by Trost and Aponick to give the amino-alcohol **69** in 72% yield and 99% ee as a single diastereomer. It was subsequently determined that the catalyst loading could be lowered to 0.5 mol% of [Pd(allyl)Cl]₂ and 1.5 mol% of the (*R,R*) Trost naphthyl ligand without a decrease in yield. Lowering the catalyst loading further resulted in substantially lowered yield due to incomplete conversion, even when the concentration of the reaction medium was increased to maintain the same catalyst concentration. After silyl protection, **70** was submitted to the Sharpless dihydroxylation under conditions identical to those optimized on the first generation system. The reaction was remarkably selective for the desired product. While there was another isomer present in the reaction mixture (~10%), it could not be determined by NMR analysis whether this side-product was a diastereomer, or a product of dihydroxylation of the opposite olefin. Aziridine **74** was accessed in five additional steps via the same sequence that was used for the first generation aziridine synthesis (29% overall yield from **68** in 6 linear steps).

It should be noted that nucleophiles other than phthalimide were successful in the DYKAT reaction. Trost²⁰ recently reported that utilization of *N,N*-benzoyl-*tert*-butylcarbamate (**75**) results in nucleophilic addition of the imide to the π -allyl palladium species, followed by migration of the benzoyl group. With divinyl carbonate this reaction is successful (Equation 5) and results in the direct formation of amino-alcohol **76** in 52% yield (based on the imide or 40% yield based on divinylcarbonate). This reaction was not optimized further. Side-products were obtained indicating that subsequent ionization of the allylic benzoyl group is somewhat competitive with the initial ionization. Unfortunately, the Sharpless dihydroxylation described above was completely ineffective when **76** is employed as a substrate and several diol products were obtained as an inseparable mixture. This strategy would have saved two steps as neither removal of the phthalimide group nor TBS protection would be required. While not useful for the present purpose, **76** may prove to be a versatile synthetic intermediate at some later date and is prepared in only two steps from commercial sources.

Equation 5

With aziridine **74** and aniline **67** in hand reductive amination proved successful and provided **77** in good yield (Equation 6). Our efforts then turned to the investigation of the crucial C6–C7 bond formation that was planned to proceed via Heck coupling.

Equation 6

While conditions analogous to those reported by Danishefsky ($\text{Pd}[\text{PPh}_3]_4$, CH_3CN , Et_3N , reflux) gave almost no conversion, the conditions of Overman²¹ gave complete conversion to a single identifiable product (**78**) which was isolated in 87% yield (Equation 7). However, this product was clearly not the desired *exo*-cyclic olefin that would be expected from a Heck reaction. Olefin peaks in the δ 5–6 ppm region were not observed; however, mass spectroscopic analysis of the product revealed the anticipated molecular weight ($[\text{M}]^+ = 580.2974$ amu). After in depth NMR experiments (COSY, HMBC, HSQC and ROESY) the structure of this new product was determined to be the structure indicated below. Remarkably, transannular amination outcompetes the expected β -hydride elimination in this case, giving rise to **78**.²²

Equation 7

This reaction, while intriguing, presented a substantial obstacle to the synthesis of FR900482. Several changes were made to Heck precursor **77** in an attempt to favor formation of the expected Heck product (**37**). Removal of the TBS group (*n*- Bu_4NF , THF, quant.) followed by the same Heck reaction conditions led to the formation of two products (presumably diastereomers) neither of which contained olefin signals that would be indicative of an exocyclic double bond. In contrast, protection of the aniline with a trifluoroacetate or acetate gave a product which was unreactive under the same Heck reaction conditions, even after prolonged reflux (dioxane 101 °C). Microwave irradiation of the same reaction mixture resulted in eventual cleavage of the trifluoroacetate (presumably via nucleophilic action of the carbonate base) and immediate formation of the same tetracycle (**78**) as described above. At this point, the assembly of the 8-membered benzazocine core was abandoned.

It was then anticipated that incorporation of a pyrrolidine ring system prior to the Heck reaction would allow us to reach the desired [3.3.1] bicyclononane ring system present in FR900482. In addition, this strategy would allow us to target either the mitomycin ring system or the FR900482 system through modification of the aromatic ring fragment (Scheme 12).

For this strategy to be feasible, modification to the correct oxidation state at C-8 was essential. One method to accomplish this goal is to perform a C-H insertion reaction directed by the neighboring C-7 hydroxymethyl group in **83** or **84**. To set up the C-H insertion,

hydroboration of **82** in a diastereoselective manner was crucial. The high number of heteroatoms in such a compact structure makes this novel approach very challenging since there are several activated carbons that could hypothetically react; 1) the two positions adjacent to the aniline nitrogen (C-8 and C-11); 2) the benzylic position (C-7); 3) the aziridine carbons (C-9 and C-10); and 4) the benzylic carbons contiguous to the oxygen atom in the -OBn group (C-13). It was anticipated that a sulfamate ester, such as **83** might favor insertion at the desired C-8 position over C-7 as has been demonstrated by the studies of Du Bois.²³ However, **84** was also explored since the carbamate is present in the natural product and its implementation would lead to a streamlined synthetic approach. Either pyrrolidine diastereomer (**81a** and **81b**) was prepared through inversion (**86**, Tf₂O, pyridine, CH₂Cl₂) or double inversion ([Ir(COD)Cl]₂, **87**, DABCO) and the desired Heck reaction proceeded in excellent yield for both substrates (Scheme 13).

Hydroboration of **82a** with 9-BBN led to a 4:2:1 mixture of **90a**, **90b** along with the ring opened aziridine (**89**). Ideally for the C-H insertion the hydroxymethyl and C-8 hydrogen should be *cis*, thus **90b** appeared to be ideally substituted to access the mitomycin ring system. Alternatively, hydroboration of **82b** led to exclusive formation of the *trans* stereochemistry between the C-7 hydroxymethyl and C-8 hydrogen substituents (**91**).

In order to continue forward, the carbamate **92** and sulfamate ester **94** were prepared (Scheme 15). Although the *trans* substituted diastereomers (**92** and **94**) appeared to be a less compelling C-H insertion starting material, it was investigated first since the higher diastereoselectivity in the prior hydroboration allowed access to pure material without tedious separation of diastereomers. Exposure of **92** to the conditions of Du Bois, yielded the five-membered spirocyclic product **93**. The sulfamate ester **94** also favored the same regioselectivity rather than the desired C-8 insertion product.

Diastereomers **96** and **99** were also attempted in the C-H insertion reaction (Scheme 16). The 1:1 mixture of primary carbamates **96** led to formation of a 1:1 mixture of the undesired 5-membered C-H insertion product **97** (β diastereomer) and 10-membered C-H insertion product **98** (α diastereomer).

The 1:1 diastereomeric mixture of sulfonamides **99** also led to a 1:1 mixture of products. The α diastereomer favored insertion at C-11 to form the 10-membered ring **101** (10% NOE between -CH₂OR and H-9). The β diastereomer formed the very unexpected zwitterionic product **100** by attack of the aniline nitrogen (10% NOE between H7-H9 and 6% NOE between -CH₂OR and H-8). This intriguing compound proved stable since its NMR was identical after weeks at room temperature on the bench.

The isolation of these large rings was very surprising. Nevertheless, it has been reported by Selkti that structural parameters such as conformational factors play a strong influence and could favor the intramolecular formation of a seven-membered ring in piperidine substrates.²⁴

Due to these difficulties, a Polonovski/deoxygenation sequence more reminiscent of Ziegler²⁵ and Dmitrienko's²⁶ original work was examined. To this end, dihydroxylation of either **82a** or **82b** and protection of the resulting diol was anticipated to yield material that would serve in the desired C-8 oxidation. Again, **82a** was modestly selective for dihydroxylation from the alpha face leading to **102a** as the major stereoisomer, which could be readily separated from the minor epimer after protection of the diol as a cyclic carbonate. Olefin **82b** led exclusively to a single diastereomer, which was also protected as a cyclic carbonate (**102b**).

It must be noted that the stereochemistry arising at C-8 for the formation of **102a** was inaccurately assigned in our original communication.²⁷ The stereochemistry at C-7, which was assigned by comparison to C-8 was also reversed. This misassignment resulted from the rather large NOE enhancement that was observed in **102a** and *epi*-**102a** between C-8 and C-9. It was only after synthesis of the C-8 epimer **102b** and further NOE studies that the correct assignment of stereochemistry was realized (Figure 3). This finding reversed stereochemistry in several intermediates which had been previously reported, but did not affect the end result as these centers were ultimately irrelevant to the final outcome of the synthesis.

Carbonates **102a**, *epi*-**102a** and **102b** were all subjected to the Polonovski conditions optimized by Ziegler (*m*-CPBA, CH₂Cl₂ then Ac₂O, THF).²⁸ Compound **102b** successfully yielded the C-8 acetate **103**. In contrast to Ziegler's observation that hydrolysis of the acetate occurred readily, hydrolysis of **103** did not occur upon exposure to aqueous sodium bicarbonate. Hydrolysis under more forcing conditions (Cs₂CO₃, MeOH) led to hydrolysis of the carbonate and isolation of the corresponding triol. Hydrolysis with methoxide and methanol led to spontaneous rearrangement to the internal cyclic carbonate **104**. None of these products participated successfully in the desired oxidative ring expansion reaction.²⁹ It was anticipated that a benzylic epoxide might prove amenable to deoxygenation following the oxidative expansion reaction. Thus **105** was prepared, but its extreme instability to acid disqualified it as a potential precursor to the desired bicyclic system.

As an alternative, the Polonovski/oxidation sequence was attempted on *epi*-**102a** and **102a**. While the minor diastereomer failed to give any desired product, **102a** led to the anticipated aminor product (**106**) in moderate yield over two steps. Exposure of this product to a second oxidation led to a mixture of **107** and **108**. Surprisingly, the composition of this mixture was dependent on the quality and purification of **106**. Exposure of **106** to slightly acidic conditions (SiO₂ chromatography) led to intractable mixtures of diastereomers **107** and **108**. However, purification of **106** by reversed phase HPLC (NH₄OH buffer 0.1%/ACN) led to pure quantities of **107**. The most likely explanation for these observations is that the aminor is formed kinetically as a single diastereomer, but exposure to acid results in epimerization of the C₈ stereochemistry. Equilibration of these diastereomers after oxidative ring expansion was not observed under acidic or basic conditions. Thus, it is proposed that the oxidative ring expansion is stereospecific and different aminor diastereomers result in opposite diastereomers after the oxidative ring expansion.

Completion of the synthesis rested upon deoxygenation of **107**. After a variety of failed attempts to selectively cleave the cyclic carbonate, it was observed that hydrogenolysis of the benzyl ether followed by treatment with sodium borohydride in methanol resulted in not only hydrolysis of the cyclic carbonate, but also led to incorporation of a methoxy group at C-7 (Equation 9).

Equation 9

The remarkable transformation outlined in Equation 9 was attributed to facile formation of an *ortho*-quinone methide intermediate (Scheme 21), which rendered C-7 electrophilic in nature and resulted in the capture of methanol through solvolysis. It was immediately

recognized that this reaction might provide access to the desired deoxygenated product if conditions could be found to favor a net reductive termination of the reaction rather than a redox neutral solvolysis. Investigation focused on adjustment of the reaction medium, which might slow or render reversible the undesired solvolysis. While *i*-PrOH gave only very limited solubility of sodium borohydride, ethanol affected the same reaction yielding **110** as the sole identifiable product (Scheme 20).

With **110** in hand, but no evidence to confirm the stereochemistry at C-7, further effort focused on completion of the synthetic route.³⁰ The primary alcohol was converted to a primary carbamate via treatment with trichloroacetyl isocyanate followed by in situ hydrolysis of the trichloroacetyl group. Exchange of the methyl ester for an ethyl thioester (**111**) was followed by Fukuyama reduction with palladium on carbon to yield the corresponding aldehyde.³¹ The *tert*-butyl carbamate was 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.

The likely mechanism which led to the epimeric C-7 stereochemistry is outlined in Scheme 21. Previous experimentation by Danishefsky had generated an *ortho*-quinone methide intermediate in the absence of a reducing reagent.³² In our case deoxygenation of **107** most likely begins with exchange of the phenol for a phenoxyboronate complex such as **113**. Subsequent intramolecular reduction causes chemoselective cleavage of the cyclic carbonate to be possible in the presence of the *tert*-butyl carbamate protecting group. However, the resulting tetraol (**114**) is disposed for elimination of its tertiary benzylic alcohol to generate *ortho*-quinone methide **115**. This reactive intermediate is proposed to decompose through a hydride shift yielding aldehyde **116**, which is reduced from the less hindered face. Alternatively, the *ortho*-quinone methide could be directly reduced by sodium borohydride which would presumably lead to the opposite configuration at C-7. The observation that the hydroxymethyl group is formed on the convex face of the molecule seems more consistent with very fast decomposition of the *ortho*-quinone methide through a hydride shift and then a slower reduction of an aldehyde, which is thermodynamically favored to be on the less hindered face of the molecule.

A substantial amount of effort failed to introduce the C-7 hydroxymethyl substituent in the configuration required for the natural product. While it was disappointing not to have the natural product in hand after so much effort, we were excited by the possibility that the C-7 epimer of FR900482 might have comparable activity based on the established biological mechanism of this natural product.

It is known³³ that reduction of the hydroxylamine to the aniline oxidation state is the triggering event that precedes formation of the reactive mitosene intermediate capable of cross-linking DNA (Scheme 22). For FR900482 and *epi*-FR900482 the triggering event should be the same, generating intermediates **117** and **119** respectively. It is unknown what mechanism the reductive activation proceeds by and it seemed quite possible that the initial N-O bond reduction might be sensitive to the stereochemistry at C-7. Furthermore, dehydration to mitosene intermediate **118** is not instantaneous. Studies on the mitomycins have demonstrated the lifetime of an aminated intermediate such as **117** or **119** is strongly dependent on the substitution of the aromatic ring.^{[i]34} Thus it also seems likely that the lifetime of such an intermediate could be strongly dependent on the stereochemistry at C-7. However, if these two steps are comparable, the stereochemistry at C-7 would not be expected to have any influence on the biological activity since the eventual mitosene intermediate **118** is identical.

In fact, when FR900482 and *epi*-FR900482 were tested side by side comparable cytotoxicity was collected from each within the experimental margin of error (Table 5).

Conclusion

This work highlights several novel and challenging approaches toward FR900482 and mitomycin congeners. Each strategy allowed us to gain a better knowledge in terms of the scope and limitations of the synthetic methodology employed and in terms of the reactivity of the intermediate species involved. For example, we were able to synthesize a challenging 8-membered benzazacine heterocycle, through a carbonylative lactamization. Palladium catalyzed π -allyl alkylation instead afforded highly substituted tetrahydroquinolines which are important pharmacophores. Large *N*-heterocyclic rings were also produced under Rh-catalyzed C-H insertion conditions, highlighting the importance of proximity effects in these reactions. Our synthetic efforts ultimately led to 7-*epi*-FR900482 in 23 linear steps (29 total) and 1.4% overall yield which represents a route that is the shortest yet disclosed for the preparation of close FR900482 analogs. It also demonstrates that the stereochemistry at C-7 is irrelevant to the biological activity of FR900482.

Prior to this study, it was not possible to predict based on what was known about the biological mechanism that a C7 epimer should be equally active compared to the natural product. The question comes down to what is the mechanism by which the compound is activated since the C-7 stereocenter is obliterated. The fact that this stereocenter is irrelevant to activity suggests the activation mode may be non-enzymatic. Because 7-*epi*-FR900482 has analogous *in vitro* activity compared to the natural product, its synthesis is functionally equivalent to that of the natural product whose syntheses all are significantly longer. Furthermore, it is also possible that this epimer could have an improved biological profile compared to the natural product, particularly because it may not have all the deleterious side-effects that have caused FK973 to be withdrawn from clinical trials. This is especially true because the convergent synthetic route presented above allows for significant variation of functional groups on *epi*-FR900482 that would not be possible by semi-synthesis.

Thus, the synthesis of 7-*epi*-FR900482 demonstrates that a substantially shorter route to such structural types is possible and that an unnatural analogue can have equal activity relative to the natural product. We have learned much about the synthesis of such structures and about the biological mechanism that makes these structures valuable. Thus, the main objective of creating a more efficient synthesis of highly active FR analogs for further structure-activity studies in order to find a potent analog devoid of undesired side-effects has indeed been accomplished.

Experimental Section

Reactions were carried out in flame- or oven-dried glassware (overnight, 120 °C) equipped with a TeflonTM coated magnetic stirring bar under a positive stream of argon or nitrogen. All reagents and solvents obtained from commercial sources were used without further purification. Anhydrous toluene, benzene, diethyl ether, hexane, dichloromethane, dimethylformamide and tetrahydrofuran (THF) were obtained from a Solv-Tek solvent purification system by passing through a column of activated alumina. Acetone was distilled from calcium sulfate. Methanol was distilled from magnesium methoxide. Reactions were monitored by thin layer chromatography (TLC) using 0.25 mm pre-coated Silica Gel 60 F254 glass plates (EMD Chemicals Inc.). Normal and flash column chromatography were performed with 32–63 μ m standard grade silica gel (Sorben Technologies). Nuclear magnetic resonance (NMR) spectra were recorded on a Varian UI-600 (¹H at 600 MHz, ¹³C at 150 MHz), UI-500 (¹H at 500 MHz, ¹³C at 125 MHz) or Mercury 400 (¹H at 400

MHz, ^{13}C at 100 MHz) magnetic resonance spectrometers. Infrared spectra were obtained neat on NaCl plates using a Nicolet IR 100 FT-IR Spectrometer (Thermo Scientific) and reported in wavenumbers (cm^{-1}). High-resolution FAB mass spectrometry (HRMS) data were measured on a Micromass Q-ToF API US mass spectrometer (Waters Corporation, Milford MA) in positive electrospray ionization mode (+ESI). Melting points were determined on a Thomas-Hoover melting point apparatus in open capillaries and are uncorrected. Optical rotation data was obtained with a Jasco DIP-360 digital polarimeter at the sodium D line (589 nm) in the solvent and concentration indicated.

General procedure for carbonylative lactamization: Synthesis of lactame 28a

A solution of vinyl iodide **27a** (296 mg, 0.423 mmol) in DMA (4.1 mL) was added at once to a solution of dichlorobis(triphenylphosphine)palladium(II) (14.8 mg, 0.021 mmol), and triethylamine (90.0 mg, 0.124 mL, 0.889 mmol) in DMA (10.0 mL). Carbon monoxide was bubbled through the resulting red solution for 30 min. The reaction mixture was heated to 65°C and stirred at that temperature for 5.5 h. After cooling to room temperature, water was added and the reaction mixture was extracted with EtOAc (x3). The combined organic extracts were washed with brine, dried (MgSO_4), filtered, and concentrated under reduced pressure to give **28a** as a brown oil. Purification by column chromatography (SiO_2 , 30% ether/pet ether to 50% ether/pet ether) afforded 198 mg (78%) of the lactame as a white solid, mp = $53\text{--}55^\circ\text{C}$, R_f = 0.37 (50% ether/pet ether). $[\alpha]_D^{22} = -13.8^\circ$ (c 5.28, CH_2Cl_2). IR (CDCl_3): 1724, 1700, 1586, 1434, 1325, 1252, 1234 cm^{-1} . ^1H NMR (300 MHz, CDCl_3): δ 7.81 (s, 1H), 7.56 (d, J = 1.2 Hz, 1H), 7.33 (m, 5H), 6.25 (dd, J = 8.3, 11.7 Hz, 1H), 5.66 (d, J = 11.7 Hz, 1H), 5.55 (m, 1H), 5.09 (s, 2H), 3.89 (s, 3H), 2.88 (m, 2H), 1.01 (s, 9H), 0.77 (s, 9H), 0.15 (d, J = 1.7 Hz, 6H), -0.15 (s, 3H), -0.26 (s, 3H). ^{13}C NMR (75 MHz, CDCl_3): δ 169.3, 167.4, 156.3, 154.1, 151.4, 137.1, 129.4, 128.4, 127.8, 127.4, 117.4, 116.5, 108.1, 105.5, 70.1, 69.9, 52.1, 31.1, 30.3, 26.1, 25.7, 18.1, 17.9, -5.24 , -5.30 , -5.60 . HRMS Calc'd. for $\text{C}_{32}\text{H}_{47}\text{NO}_6\text{Si}_2$: 597.2942. Found: 597.2943.

DYKAT reaction of divinyl carbonate 68: Synthesis of alcohol 69

According to the known procedure of Trost,³⁵ a dry 250 mL flask was charged with sodium carbonate (0.1060 g, 1.0 mmol), phthalimide (2.2364 g, 15.2 mmoles), (*R,R*)-naphthyl ligand (0.2373 g, 0.30 mmoles) and $[(\eta^3\text{-C}_3\text{H}_5)\text{PdCl}]_2$ (0.0366 g, 0.10 mmoles). The reaction vessel was evacuated and backfilled with nitrogen several times and then degassed methylene chloride (120 mL) was injected. A separate flask was charged with divinylcarbonate **68**, which was dissolved in degassed methylene chloride (40 mL). After 15 minutes the divinylcarbonate solution (20 mmoles, 2.800 g) was transferred via canula into the reaction flask. The reaction was stirred at room temperature for 96h, after which time the solvent was removed under reduced pressure and the remaining crude material was purified by flash chromatography on silica gel (gradient, 10% to 30% EtOAc/PE) to afford the product **69** as a white solid (3.503g, 72%). The enantiomeric excess was determined via evolution on a Chiracell AS column with 5% i-PrOH/heptane at a flow rate of 1 mL/min. Monitoring at 230nm showed the (*S,S*) enantiomer with a retention of 19.8 min and the (*R,R*) enantiomer which eluted at 28.3 min. This method showed the enantiomeric excess to be greater than 99%. R_f = 0.35 (4:1 Hexane/EtOAc); $[\alpha]_D = -86.4$ (c = 1.00, CH_2Cl_2); IR (thin film): 3463, 1707, 1387 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3): δ 7.87-7.81 (m, 2H), 7.76-7.71 (m, 2H), 6.22 (ddd, J = 6.9, 10.4, 17.3 Hz, 1H), 5.83 (ddd, J = 4.7, 10.5, 15.3 Hz, 1H), 5.43-5.15 (m, 4H), 4.89 (t, J = 6.6 Hz, 1H), 4.72-4.65 (m, 1H), 3.52 (d, J = 8.7 Hz, 1H); ^{13}C NMR (100 MHz, CDCl_3): δ 168.8, 137.3, 134.2, 132.1, 131.6, 123.5, 118.8, 116.8, 72.7, 58.6 ppm. HRMS (EI+) calc'd for $\text{C}_{11}\text{H}_6\text{NO}_3$ (M)⁺, 243.0895, found 243.0892.

Heck Reaction: Synthesis of exocyclic olefin **82a**

The aryl iodide **81a** (45.8 mg, 0.080 mmol), palladium acetate (1.9 mg, 7.9 μ mol), triphenylphosphine (8.9 mg, 31.6 μ mol) and silver carbonate (43.8 mg, 0.16 mmol) were transferred to a round bottomed flask. The flask was evacuated and refilled with nitrogen three times. Dioxane (1.6 mL, freshly distilled over sodium) was injected and the reaction was heated to 80 °C in an oil bath. After 1 hour the reaction was complete and allowed to cool. The heterogeneous suspension was filtered through celite to remove palladium black and the filter cake was washed with ethyl acetate. The filtrate was concentrated under reduced pressure. Flash chromatography (20% EtOAc/hexane) gave compound **82a** as a yellow foam (33.0 mg, 93%). R_f = 0.30 (4:1 Hexane/EtOAc); $[\alpha]_D^{25}$ = +23 (c = 0.90, $CDCl_3$); IR (thin film): 2948, 1716 (sharp), 1566; 1H NMR (500 MHz, $CDCl_3$): δ 7.48–7.33 (m, 5H), 7.18 (d, J = 0.9 Hz, 1H), 7.00 (d, J = 1.0 Hz, 1H), 6.04 (d, J = 3.2 Hz, 1H), 5.38 (d, J = 2.5 Hz, 1H), 5.18 (s, 2H), 4.68 (t, J = 2.7 Hz, 1H), 3.90 (s, 3H), 3.69 (d, J = 11.8 Hz, 1H), 3.38 (dd, J = 4.3, 12.1 Hz, 1H), 3.34 (t, J = 4.4 Hz, 1H), 3.25 (dd, J = 0.4, 4.9 Hz, 1H), 1.49 (s, 9H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.1, 160.6, 159.4, 155.8, 144.0, 136.5, 132.8, 128.9, 128.4, 127.9, 121.5, 110.0, 108.1, 105.8, 82.2, 71.6, 70.6, 55.9, 52.6, 49.2, 45.6, 28.2, ppm; HRMS (EI+) calc'd for $C_{26}H_{28}N_2O_5$ (M)⁺, 448.1998, found 448.1998.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge D. Barrett and Astellas Pharmaceuticals for providing an authentic sample of FR900482 and Dr. Yong Li and Hugu Menzella for biological testing of FR900482 and its epimer. We also acknowledge S. Lynch for his invaluable help in the acquisition of 2-D NMR data. We thank the Stanford Graduate Fellowship Program and NIH (GM 33049) for their financial support of our program and Johnson Matthey for their supply of precious metal salts.

References

1. Bradner WT. Cancer Treatment Reviews. 2001; 27:35. [PubMed: 11237776] (b) Pazdur R, Ho DH, Daugherty K, Bradner WT, Krakoff IH, Rbner MN. Invest New Drugs. 1991; 9:337.(c) 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. [PubMed: 3342397]
2. For a review on the topic see: Coleman RS. Current Opinion in Drug Discovery and Development. 2001; 4:435. Successful approaches: Fukuyama T, Goto S. Tetrahedron Lett. 1989; 30:6491. Fukuyama T, Xu L, Goto S. J Am Chem Soc. 1992; 114:383. McClure KF, Danishefsky SJ, Schulte GK. J Org Chem. 1994; 59:355. Schkeryantz JM, Danishefsky SJ. J Am Chem Soc. 1995; 117:4722. Danishefsky SJ, Schkeryantz JM. Synlett. 1995:475. Katoh T, Itoh E, Yoshino T, Terashima S. Tetrahedron Lett. 1996; 37:3471. Yoshino T, Nagata Y, Itoh E, Hashimoto M, Katoh T, Terashima S. Tetrahedron Lett. 1996; 37:3475. Katoh T, Yoshino T, Nagata Y, Nakatani S, Terashima S. Tetrahedron Lett. 1996; 37:3479. Katoh T, Itoh E, Yoshino T, Terashima S. Tetrahedron. 1997; 53:10229. Yoshino T, Nagata T, Itoh E, Hashimoto M, Katoh T, Terashima S. Tetrahedron. 1997; 53:10239. Katoh T, Nagata Y, Yoshino T, Nakatani S, Terashima S. Tetrahedron. 1997; 53:10253. Suzuki M, Kambe M, Tokuyama H, Fukuyama T. Angew Chem, Int Ed. 2002; 41:4686. Suzuki M, Kambe M, Tokuyama H, Fukuyama T. J Org Chem. 2004; 69:2831. [PubMed: 15074935] Judd TC, Williams RM. Angew Chem, Int Ed. 2002; 41:4683. Judd TC, Williams RM. J Org Chem. 2004; 69:2825. [PubMed: 15074934]
3. Formal syntheses: Fellows IM, Kaelin DE, Martin SF. J Am Chem Soc. 2000; 122:10781. Paleo MR, Aurecochea N, Jung KY, Rapoport HJ. Org Chem. 2003; 68:130. Shaw KJ, Luly JR, Rapoport H. J Org Chem. 1985; 50:4515. Jones RJ, Rapoport H. J Org Chem. 1990; 55:1144. Synthesis of analogues or related natural products: Kambe M, Arai E, Suzuki M,

- Tokuyama H, Fukuyama T. *Org Lett.* 2001; 3:2575. [PubMed: 11483064] Trost BM, O'Boyle BM. *Org Lett.* 2008; 10:1369. [PubMed: 18318539] Incomplete studies: Dmitrienko GI, Denhart D, Mithani S, Prasad G, Taylor NJ. *Tetrahedron Lett.* 1992; 33:5705. Mithani S, Drew DM, Rydberg EH, Taylor NJ, Mooibroek S, Dmitrienko GI. *J Am Chem Soc.* 1997; 119:1159. Trost BM, Ameriks MK. *Org Lett.* 2004; 6:1745. [PubMed: 15151404] Ziegler FE, Belema M. *J Org Chem.* 1994; 59:7962. Ziegler FE, Belema M. *Org Chem.* 1997; 62:1083. Zhang W, Wang C, Jimenez LS. *Synth Commun.* 2000; 30:351. Greshock TJ, Funk RL. *J Am Chem Soc.* 2002; 124:754. [PubMed: 11817942] Young IS, Kerr MA. *Angew Chem Int Ed.* 2003; 42:3023. Lim HJ, Sulikowski GA. *Tetrahedron Lett.* 1996; 37:5243. Colandrea VJ, Rajaraman S, Jimenez LS. *Org Lett.* 2003; 5:785. [PubMed: 12633071]
4. Trost BM, Vos BA, Brzezowski CM, Martina DP. *Tetrahedron Lett.* 1992; 33:717.
 5. Transannular interactions are minimized by unsaturation in the forming ring.
 6. (a) Omura S, Nakagawa A. *Tetrahedron Lett.* 1981; 22:2199. (b) Steinhagen H, Corey EJ. *Org Lett.* 1999; 1:823. [PubMed: 10823211] (c) Hill ML, Raphael RA. *Tetrahedron Lett.* 1986; 27:1293. (d) Morimoto Y, Matsuda F, Shirahama H. *Tetrahedron.* 1996; 52:10609. (e) Morimoto Y, Shirahama H. *Tetrahedron.* 1996; 52:10631. (f) Morimoto Y, Matsuda F, Shirahama H. *Synlett.* 1991:202.
 7. (a) Leeson PD, Carling RW, Moore KW, Moseley AM, Smith JD, Stevenson G, Chan T, Baker R, Foster AC, Grimwood S, Kemp JA, Marshall GR, Hoogsteen KJ. *Med Chem.* 1992; 35:1954. (b) Carling RW, Leeson PD, Moseley AM, Smith JD, Saywell K, Tricklebank MD, Kemp JA, Marshall GR, Foster AC, Grimwood S. *Bioorg Med Chem Lett.* 1993; 3:65. (c) Takamura M, Funabashi K, Kanai M, Shibasaki MJ. *J Am Chem Soc.* 2001; 123:6801. [PubMed: 11448184]
 8. Fellows IM, Kaelin DE, Martin SF. *J Am Chem Soc.* 2000; 122:10781.
 9. Negishi E, Anastasia L. *Chem Rev.* 2003; 103:1979. [PubMed: 12744698]
 10. (a) Trost BM, Van Vranken DL, Bingel C. *J Am Chem Soc.* 1992; 114:9327. (b) Trost BM, Bunt RC, Lemoine RC, Calkins TL. *J Am Chem Soc.* 2000; 122:5986.
 11. Ohfuné Y, Kurokawa N. *Tetrahedron Lett.* 1984; 25:1587.
 12. Najime R, Pilard S, Vaultier M. *Tetrahedron Lett.* 1992; 33:5351.
 13. (a) Montgomery J. *Angew Chem Int Ed.* 2004; 43:3890. (b) Montgomery J, Tang XT. *J Am Chem Soc.* 1999; 121:6098. (c) Knapp-Reed B, Mahandru GM, Montgomery J. *J Am Chem Soc.* 2005; 127:13156. [PubMed: 16173738] (d) Tang X, Montgomery J. *J Am Chem Soc.* 2000; 122:6950.
 14. (a) Chan J, Jamison TF. *J Am Chem Soc.* 2003; 125:11514. [PubMed: 13129351] (b) Colby EA, O'Brien KC, Jamison TF. *J Am Chem Soc.* 2005; 127:4297. [PubMed: 15783211] (c) Colby EA, O'Brien KC, Jamison TF. *J Am Chem Soc.* 2004; 126:998. [PubMed: 14746449] (d) Chan J, Jamison TF. *J Am Chem Soc.* 2004; 126:10682. [PubMed: 15327327]
 15. Abelman MM, Overman LE, Tran VD. *J Am Chem Soc.* 1990; 112:6959.
 16. McDonald C, Holcomb H, Kennedy K, Kirkpatrick E, Leathers T, Vanemon P. *J Org Chem.* 1989; 54:1213.
 17. Fonseca T, Gigante B, Marques MM, Gilchrist TL, De Clercq E. *Bioorganic and Medicinal Chemistry.* 2004; 12:103. [PubMed: 14697775]
 18. Trost BM, Aponick A. *J Am Chem Soc.* 2006; 128:3931. [PubMed: 16551099]
 19. Corey EJ, Noe MC. *J Am Chem Soc.* 1996; 118:319.
 20. Trost BM, Fandrick DR, Brodmann T, Stiles DT. *Ang Chem Int Ed.* 2007; 46:6123.
 21. Abelman MM, Overman LE, Tran VD. *J Am Chem Soc.* 1990; 112:6959.
 22. For a more complete discussion see: Trost BM, O'Boyle BM, Hund D. *Chem Eur J.* 2010; 16:9772. [PubMed: 20669198]
 23. (a) Espino CG, Du Bois J. *Angew Chem.* 2001; 113:618. *Angew Chem Int Ed.* 2001; 40:598. (b) Espino CG, Wehn PM, Chow J, Du Bois J. *J Am Chem Soc.* 2001; 123:6935.
 24. Toumieux S, Compain P, Martin OR, Selkti M. *Org Lett.* 2006; 8:4493. [PubMed: 16986933]
 25. (a) Ziegler FE, Belema M. *J Org Chem.* 1994; 59:7962. (b) Ziegler FE, Belema M. *J Org Chem.* 1997; 62:1083.
 26. Dmitrienko GI, Denhart D, Mithani S, Prasad GKB, Taylor NJ. *Tetrahedron Lett.* 1992; 33:5705. (b) Mithani S, Drew DM, Rydberg EH, Taylor NJ, Mooibroek S, Dmitrienko GI. *J Am Chem Soc.* 1997; 119:1159.

27. See reference 3f.
28. It should be noted that the Polonovski oxidation was unsuccessful with other protecting groups for the 1,2 diol (acetone). An electron withdrawing protecting group appeared to be required for reasonable efficiency in the Polonovski reaction.
29. Only starting material was recovered when **103** and **104** were treated with *m*-CPBA and NaHCO₃ in CH₂Cl₂ followed by Ac₂O in THF.
30. The connectivity of **110** was confirmed by NMR analysis (*g*COSY, *g*HMBC, *g*HSQC), but the configuration at C7 remained uncertain.
31. Fukuyama T, Tokuyama H. Aldrichim Acta. 2004; 37:87.
32. See reference 2e.
33. (a) Woo J, Sigurdsson ST, Hopkins PB. J Am Chem Soc. 1993; 115:1199.(b) Huang H, Pratum TK, Hopkins PB. J Am Chem Soc. 1994; 116:2703.(c) Paz MM, Hopkins PB. Tetrahedron Lett. 1997; 38:343.(d) Paz MM, Hopkins PB. J Am Chem Soc. 1997; 119:5999.(e) Williams RM, Rajski SR, Rollins SB. Chemistry and Biology. 1997; 4:127. [PubMed: 9190287] (f) Beckerbauer L, Tepe JJ, Cullison J, Reeves R, Williams RM. Chemistry and Biology. 2000; 7:805. [PubMed: 11033083] (g) Williams RM, Ducept P. Biochemistry. 2003; 42:14696. [PubMed: 14661983]
34. Gruschow S, Chang LC, Mao Y, Sherman DH. J Am Chem Soc. 2007; 129:6470. [PubMed: 17461583]
35. Trost BM, Aponick A. J Am Chem Soc. 2006; 128:3931. [PubMed: 16551099]

FR900482 - (1)

FR66979 - (4)

FK973 - R₁=R₂=Ac - (2)FK317 - R₁=Me; R₂=Ac - (3) Mitomycin C - R = NH₂ - (5)Mitomycin A - R = OCH₃ - (6)

7-Demethylmitomycin A - R = OH - (7)

Figure 1.
FR00482 and mitomycin family of compounds

Figure 2.
Pharmaceutically important tetrahydroquinolines

Figure 3.
Reassignment of C-8 stereochemistry

Scheme 1.
Original retrosynthetic analysis of FR-900482.

Scheme 2.
Modified retrosynthetic analysis of FR-900482

a. cat AgNO_3 , NIS, acetone, 87%; **b.** DIBAL-H, THF; **c.** K_2CO_3 , MeOH; **d.** NaH, BnBr, Bu_4NI , THF; **e.** **35**, pyr., AcOH, MeOH; **f.** SmI_2 (4 eq), THF, MeOH; **g.** Zn (2.2 eq), THF, MeOH, H_2O ; **h.** TBDPSCI or TBSCl, imid., CH_2Cl_2

Scheme 3.

Synthesis of vinyl iodides **27a**, **27b**, and **27c**

a. cat AgNO_3 , NIS, acetone, 87%; **b.** DIBAL-H, THF; **c.** K_2CO_3 , MeOH; **d.** NaH, BnBr, Bu_4NI , THF; **e.** **35**, pyr., AcOH, MeOH; **f.** SmI_2 (4 eq), THF, MeOH; **g.** Zn (2.2 eq), THF, MeOH, H_2O ; **h.** TBDPSCI or TBSCl, imid., CH_2Cl_2

Scheme 4.

Revised retrosynthesis of FR900482; NHK coupling strategy

Scheme 5.
Synthesis of aromatic subunit **45**

Scheme 6.
Synthesis of amino-epoxide **50**

Scheme 7.
Completion of the aziridine fragment

Scheme 8.
Synthesis of reductive cyclization precursor **61**

Scheme 9.
Revised retrosynthesis of FR900482; Heck coupling strategy

Scheme 10.
Synthesis of a modified aromatic fragment

Scheme 11.
Synthesis of a modified aziridine fragment

Chemistry. Author manuscript; available in PMC 2013 July 09.

Scheme 13.
Successful Heck reaction

Scheme 14.
Hydroboration of **82a** and **82b**

Scheme 15.
Investigation of the C-H Insertion Reaction

Scheme 16.

Further investigation of the C-H insertion reaction

Scheme 17.
Dihydroxylation of **82a** and **82b**

Scheme 18.
Polonovski and oxidation/ring expansion of **102**

Scheme 19.
Successful Polonovski/oxidative ring expansion

Scheme 20.
Completion of the synthesis of *epi*-FR900482

Scheme 21.
Mechanism of the deoxygenation

Scheme 22.

Biological mechanism of action for *epi*-FR900482 and FR900482

Table 1

Selected optimization studies for Pd- π -allyl alkylation of **20**

Entry	Substrate	Pd Source ^a	Solvent	T (°C)	Yield ^b	d.r. ^c
1	20a	Pd ₂ dba ₃ ·CHCl ₃	THF	90	sm	-
2 ^d	20a	Pd ₂ dba ₃ ·CHCl ₃	THF	90	sm	-
3 ^d	20a	Pd ₂ dba ₃ ·CHCl ₃	dioxane	100	sm	-
4	20a	[(Pd(C ₃ H ₅ Cl) ₂)]	dioxane	100	94%	92:8
5 ^d	20a	[(Pd(C ₃ H ₅ Cl) ₂)]	dioxane	100	94%	76:24
6	20b	Pd ₂ dba ₃ ·CHCl ₃	THF	80	27% 10%	62:38
7	20c	[Pd(C ₃ H ₅ Cl) ₂]	dioxane	100	21a + 37% 20a	>95:5
8	20d	[Pd(C ₃ H ₅ Cl) ₂]	dioxane	100	88%	85:15
9	20e	[Pd(C ₃ H ₅ Cl) ₂]	dioxane	100	trace pdt	-

^a 5% of Pd source.^b Isolated yield^c Diastereomeric ratios were determined by NMR analysis of the crude reaction mixture.^d 4% TBAT was used as an additive.

Table 2

Selected optimization studies for Pd- π -allyl alkylation of **22**.

<p>27a - R₁=CO₂Me; R₂=OTBS</p> <p>27b - R₁=CO₂Me; R₂=OTBDPS</p> <p>27c - R₁=CH₂OBn; R₂=OTBDPS</p>		<p>28a - R₁=CO₂Me; R₂=OTBS</p> <p>28b - R₁=CO₂Me; R₂=OTBDPS</p> <p>28c - R₁=CH₂OBn; R₂=OTBDPS</p>	
Entry	Substrate	Pd Source ^a	Solvent	Ligand	Yield ^c
1	22a	Pd ₂ dba ₃ ·CHCl ₃	THF	PPh ₃	23%
2	22a	Pd ₂ dba ₃ ·CHCl ₃	THF	dppp	51%
3	22a	Pd ₂ dba ₃ ·CHCl ₃	THF	P(OPh) ₃	67%
4	22a	[(Pd(C ₃ H ₅)Cl) ₂]	THF	P(OPh) ₃	69%
5	22a	Pd ₂ dba ₃ ·CHCl ₃	THF	--	23%
6	22b	Pd ₂ dba ₃ ·CHCl ₃	THF	P(OPh) ₃	73%
7	22b	Pd ₂ dba ₃ ·CHCl ₃	DMSO	P(OPh) ₃	75%
8	22b	Pd ₂ dba ₃ ·CHCl ₃	PhH	P(OPh) ₃	23%
9	22b	Pd ₂ dba ₃ ·CHCl ₃	THF	P(O-iPr) ₃	41%
10	22b	Pd(OAc) ₂ ^b	THF	P(OPh) ₃	86%
11	22b	Pd(OAc) ₂	DMSO	dppf	85%
12	22b	Pd(dppe) ₂ ^b	DMSO	--	82%

^a 10% of Pd source unless stated otherwise.

^b 20% of Pd source.

^c All yields are isolated yields after silica gel chromatography.

Table 3

Selected optimization studies for Pd-catalyzed carbonylative lactamization

Entry	Substrate	Pd Source	Solvent	Ligand	Base	Yield ^a
1	27a	Pd[PPh ₃] ₄	THF	PPh ₃	Et ₃ N	36%
2	27a	Pd[PPh ₃] ₄	THF	PPh ₃	<i>i</i> -Pr ₂ NEt	36%
	27a	PdCl ₂ [PPh ₃] ₂	DMA ^b	none	Et ₃ N	41%
4	27a	PdCl ₂ [PPh ₃] ₂	DMA ^c	none	Et ₃ N	78%
5	27b	PdCl ₂ [PPh ₃] ₂	DMA ^c	none	Et ₃ N	66%
6	27c	PdCl ₂ [PPh ₃] ₂	DMA ^c	none	Et ₃ N	64%

^a Isolated yield.^b 0.01M^c 0.03M

Table 4**Stereoselective dihydroxylation of **51****

Entry	Catalyst and Ligand	Yield	<i>d.r.</i> ^a
1	OsO ₄ (1 mol%), NMO, THF	98%	1.25:1
2	K ₂ OsO ₂ (OH) ₄ (1 mol%), (DHQ) ₂ PHAL (2 mol%)	71%	5:1
3	K₂OsO₂(OH)₄ (1 mol%), (DHQ)₂PYR (2 mol%)	98%	8.5:1
4	K ₂ OsO ₂ (OH) ₄ (1 mol%), (DHQ) ₂ AQN (2 mol%)	90%	5:1
5	K ₂ OsO ₂ (OH) ₄ (1 mol%), (DHQD) ₂ PHAL (2 mol%)	85%	1:1.5

^aDiastereomeric ratio determined by NMR.

Table 5Comparative biological testing of FR900482 and *epi*-FR900482

IC50 μ M	SKBr3	MCF-7
FR900482	0.980	1.020
7- <i>epi</i> FR900482	1.000	1.100

Comparison of FR900482 and *epi*-FR900482 was performed side by side at Kosan Biosciences. Activity within the range of experimental error was observed.