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# Total Syntheses, Fragmentation Studies, and Antitumor/Antiproliferative Activities of FR901464 and Its Low Picomolar Analog

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## Abstract

FR901464 is a potent anticancer natural product that lowers the mRNA levels of oncogenes and tumor suppressor genes. In this article, we report a convergent enantioselective synthesis of FR901464, which was accomplished in 13 linear steps. Central to the synthetic approach was the diene-ene cross olefin metathesis reaction to generate the C6-C7 olefin without the use of protecting groups as the final step. Additional key reactions include a Zr/Ag-promoted alkynylation to set the C4 stereocenter, a mild and chemoselective Red-Al reduction, a reagent-controlled stereoselective Mislow-Evans-type [2,3]-sigmatropic rearrangement to install the C5 stereocenter, a Carreira asymmetric alkynylation to generate the C4′ stereocenter, and a highly efficient ring-closing metathesis-allylic oxidation sequence to form an unsaturated lactone. The decomposition pathways of FR901464's right fragment were studied under physiologically relevant conditions. Facile epoxide opening by β-elimination gave two enones, one of which could undergo dehydration via its hemiketal to form a furan. To prevent this decomposition pathway, a right fragment was then rationally designed and synthesized. This analog was 12 times more stable than the right fragment of the natural product. Using this more stable right fragment analog, an FR901464 analog, meayamycin, was then prepared in 13 linear steps. The inhibitions of human breast cancer MCF-7 cell proliferation by synthetic FR901464 and meayamycin were studied, and the $GI_{50}$ values for these compounds were determined to be 1.1 nM and 10 pM, respectively. Thus, meayamycin is among the most potent anticancer small molecules that do not bind to either DNA or microtubule.

### Keywords

FR901464; fragmentation; Meayamycin; hemiketal; NBS; Mislow-Evans rearrangement; olefin cross-metathesis; anticancer/antiproliferative activities; zirconium; alkynylation

## Introduction

Controlled cellular processes such as signal transduction and gene expression are essential for homeostasis. When these processes become irregular, tumors can develop. Tumorigenesis can be accounted for by approximately 200 molecular mechanisms,[1] many of which can in principle be targeted by drugs. However, currently available chemotherapeutic methods are limited to targeting DNA,[2,3] nuclear hormone receptors,[4] a tyrosine kinase,[5] a proteasome,

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[6,7] and the microtubule.[8,9] Clearly, there is an urgent need to fill the gap between the molecular mechanisms of tumorigenesis and available pharmacological approaches.

In the quest for anticancer natural products possessing new modes of action, the Nakajima group at the Fujisawa Pharmaceutical Company employed a reporter assay in human breast adenocarcinoma MCF-7 cells using the simian virus 40 (SV40) promoter[10] upstream of a reporter gene, which was stably transfected into the MCF-7 cell genome. Through their screening efforts, structurally unique FR901464 was isolated from the culture broth of a bacterium of *Pseudomonas* sp. No.2663, which activated the aforementioned reporter gene by 30-fold at 20 nM concentration in MCF-7 cells.[11-13] The same group revealed that FR901464 possessed antitumor activity against MCF-7 cells, human lung adenocarcinoma A549 cells, colon cancer SW480 and HCT116 cells, and murine leukemia P388 cells with an $IC_{50}$ of 0.6–3.4 nM.[11] This natural product also exhibited a prominent effect at 0.056–0.18 mg/kg against human adenocarcinoma A549 and 0.18–1 mg/kg against murine Colon 38 and Meth A cells implanted in mice, indicating that FR901464 or its structurally related analogs have the potential for clinical application.[13] FR901464 induced both $G_1$ and $G_2$/M phase arrest in MCF-7 cells.[13,14] In contrast, adriamycin and camptothecin, both DNA synthesis inhibitors, induced S-phase arrest in the cell cycle, and Taxol®, a microtubule modulator, induced $G_2$/M-phase arrest.[13] Since FR901464 was originally discovered as an activator of SV40 promoter, the Nakajima group sought endogenous genes that were upregulated by the natural product. Their focused approach, prior to the invention of DNA microarray,[15] showed that the mRNA levels of *p53*,[16,17] *p21 Cip-1*,[18,19] *E2F-1*,[20,21] and *c-Myc*[22,23] were downregulated while a housekeeping gene was intact. This result is both exciting and perplexing since *p53* is a well-known tumor suppressor gene and *c-Myc* is an oncogene. These findings reported by the Nakajima group and our subsequent findings strongly suggest that the mode of action of FR901464 is different from that of clinically used anticancer drugs. After the discovery of FR901464, other pharmaceutical companies used the same SV40 promoter in reporter gene systems and found that the previously known natural product herboxidiene (a.k.a. GEX1) [24-27] and the new natural product TMC-205[28] exhibited closely related biological activity as FR901464, although these compounds were less potent.

Not surprisingly, the unique profile of FR901464 has intrigued many synthetic chemists,[29] which culminated the first total synthesis of this natural product by the Jacobsen group[30,31] and the second and third by the Kitahara group.[32,33] The Jacobsen synthesis elegantly demonstrated the power of their asymmetric hetero-Diels-Alder reaction[34] in their total synthesis, which consisted of 19 steps in the longest linear sequence and a total of 40 steps.

The subsequent total synthesis from the Kitahara group took advantage of the chiral pool to assemble their fragments. This total synthesis required 42 total steps with the longest linear sequence being 24 steps.[35] The third total synthesis by the same group was the improvement of their earlier version with 41 steps in the longest linear sequence and 22 total steps.[33] These syntheses revealed inefficient installation of the spiroepoxide at late stages and the latent instability of FR901464. Clearly, a more versatile and concise approach was desired for synthetic accessibility to analogs of FR901464 for biological studies. Moreover, more quantitative analysis of the instability of FR901464 was needed as part of our efforts to understand the mode of action of this natural product. This full account describes our synthetic efforts that eventually allowed us to develop a remarkably potent FR901464 analog via earlier and potentially risky installation of the spiroepoxide and ultimate convergency, namely the cross-coupling at the very end of the synthesis.[36]

## Results and Discussion

### First Generation Synthetic Studies

Scheme 1 shows our first generation retrosynthetic analysis of FR901464. We envisioned a Nozaki-Hiyama-Kishi reaction[37,38] of **1** and **2** as the final coupling reaction because the reaction conditions are mild and it is generally in accordance with Felkin selectivity for additions into to α-chiral aldehydes. Vinyl iodide **1** would be prepared from acid **3** and amine **4** that were expected to be derived from propargylic alcohol **6** and the commercially available L-threonine derivative **7**, respectively. Ketoaldehyde **2** could be prepared from allylic alcohol **5** by oxidative cleavages of both olefins. Finally, this alcohol would arise from epoxyalcohol **8**.

We envisioned that the first milestone of our synthetic studies would be the preparation of ketoaldehyde **2** (Scheme 2). The first step toward this end, a zinc-mediated coupling of propargyl alcohol and methallyl bromide[39] afforded alcohol **9** in 93% yield. The subsequent Sharpless asymmetric epoxidation[40] proceeded smoothly to generate epoxyalcohol **8** in 90% yield with >97:3 er. The volatile aldehyde **10** was obtained by the Dess-Martin oxidation[41] of this alcohol in 81% yield. All of these steps could be executed in large scales (>20 g) without compromising yields.

With a large quantity of aldehyde **10** in hand, our next objective was to develop a stereoselective carbon-nucleophile addition to this aldehyde. Step d illustrates the two failed diastereomeric alkynylation reactions of aldehyde **10** using **11** and **12** as latent nucleophiles. We presumed that Carreira's asymmetric alkynylation conditions[42] could be sufficiently mild to be compatible with aldehyde **10**. To our disappointment, this aldehyde did not react at ambient temperature and decomposed at 40 °C.

In light of the poor selectivity in forming the C4 stereocenter described above, we turned our attention to substrate control (step e; Table 1). Vinylation of aldehyde **10** using $CH_2$=CHMgBr in THF (entry 1) gave a 1:1 mixture of the desired alcohol **5** and the undesired alcohol 4-*epi*-**5** in a combined 51% yield. The absolute configuration of these allylic alcohols was determined after further transformations as shown in Scheme 11. The addition of $ZnCl_2$ to the reaction mixture with various stoichiometry did not improve the selectivity for the desired alcohol **5** (entries 2–4). Addition of hexanes to the reaction mixture finally improved the stereoselectivity for the desired alcohol (2:1) in 55% combined yield (entry 5). Treatment of **10** with vinyllithium, prepared in situ from $CH_2$=CHSn$Bu_3$ (1.4 equiv) and $^n$BuLi (1.3 equiv),[43] gave a 1.2:1 mixture of **5** to 4-*epi*-**5** in a combined 46% yield (entry 6). Alkynylation of **10** with HC≡CMgBr (3.5 equiv) at -78 °C (entry 7) gave an inseparable mixture of diastereomers in 32% yield and low stereoselectivity (dr = 1.7 :1), and higher reaction temperatures caused Payne rearrangements to occur. Partial hydrogenation of these propargylic alcohols gave **5** and 4-*epi*-**5** in a combined yield of 93%, which enabled us to confirm the structures. After these and other even less fruitful attempts, high levels of stereocontrol in this transformation remained elusive.

At this point, our priority was to examine the validity of the final coupling. With this in mind, we carried on with the result shown in entry 5, Table 1, and proceeded to prepare ketoaldehyde **2**. To improve the overall yield for the formation of **5**, the undesired 4-*epi*-**5** was subjected to Mitsunobu conditions using 4-nitrobenzoic acid to invert the C4 stereocenter, and subsequent methanolysis of the resulting ester **13** with $K_2CO_3$ afforded the desired allylic alcohol **5** in 75% yield for the 2 steps. The resulting hydroxy group was protected as its triethylsilyl (TES) ether, **14**, in quantitative yield. This compound was then subjected to oxidative cleavage conditions ($OsO_4$, NMO; then $NaIO_4$) to afford enone **15** in 58% yield, and its subsequent ozonolysis produced the *unstable* ketoaldehyde **2** in 54–82% yield. It should be mentioned that the direct

ozonolysis of diene **14** to ketoaldehyde **2** was reproducibly low yielding (<10%). Although this synthetic route allowed us to prepare the target intermediate in a concise manner, we soon realized that chromatographic separation of alcohols **5** and 4-*epi*-**5** in a large scale would be a daunting task.

With an established synthetic route to ketoaldehyde **2**, we still desired to improve the C4 stereoselectivity (Scheme 3). Treatment of epoxyaldehyde **10** with methyl propiolate and $^{n}$BuLi formed alcohol **19** and its C4-epimer in 50% combined yield, but with no diastereoselectivity (dr = 1:1). In contrast, it was gratifying to find that the alkynylation method developed in our laboratory afforded alcohol **19** in 84% with good stereocontrol (dr = 6:1),[44] and the C4 stereochemistry was determined as described in our previous communication.[36] After all the struggles, the solution was right under our noses! Unexpectedly, the partial hydrogenations of this alcohol and its TES ether **20** using Lindlar's catalyst failed. However, the reduction of **19** with Red-Al successfully produced **23** in 81% yield in a chemo- and stereoselective.[45] Subsequent TES ether formation produced **24** in quantitative yield, and ozonolyses of both of the olefins of this compound produced ketoaldehyde **2** cleanly by crude $^{1}$H NMR analysis. Thus, access to this ketoaldehyde was attainable in a total of 7 steps. It is noteworthy that the ozonolysis of **24** was far more effective than that of **14**.

With the B-ring fragment **2** in hand, we embarked on the preparation of the A-ring fragment **1** as shown in Scheme 4. We chose the styryl unit to mask an aldehyde that would effectively suppress the volatility of otherwise low molecular weight intermediates. With this in mind, the preparation of acid **3** began by the homologation of cinnamaldehyde by the action of $TMSCHN_2$ to give enyne **25** in 84% yield.[46] The Corey-Fuchs method[47] was less efficient, and the Seyforth-Ohira-Bestmann method[48,49] gave 1-(1-methoxybut-3-ynyl)benzene. Subsequent Carreira asymmetric alkynylation[42] of **25** with acetaldehyde generated propargylic alcohol **6** in 41% yield with 86:14 er. In this reaction, slow addition of acetaldehyde to the reaction mixture proved to be crucial since rapid addition resulted in the aldol condensation of acetaldehyde. Recrystallization of **6** further improved the er to 98:2. Unfortunately the catalytic version of the asymmetric alkynylation reaction[50] was unsuccessful. After acetylation of propargylic alcohol **6**, the resulting ester, **26**, was converted to aldehyde **27** by ozonolysis in 89% yield. Ensuing oxidation of aldehyde **27** and partial reduction of the resulting alkyne using Lindlar's catalyst afforded acid **3** in 75% yield for the 2 steps, and in 6 total steps from cinnamaldehyde. More recently, we converted enyne **26** to acid **28** in 1 step via osmium tetroxide-mediated oxidative cleavage and subsequent partial reduction afforded **3** in 60% yield for the 2 steps. Therefore, acid **3** can now be prepared in 5 steps from cinnamaldehyde.

Despite the successful preparation of acid **3**, we wished to further improve the synthetic efficiency and scalability. More specifically, we deemed the use of $TMSCHN_2$ less attractive in large scales due to potential safety concerns. Because other homologation methods to convert cinnamaldehyde to enyne **25** failed as described above, we decided to pursue a different synthetic route; tetrahydro-2-(2-propynoxy)-2*H*-pyran was coupled with *N*-acetylmorpholine to form ynone **29** in 78–93% yields (Scheme 5). This ynone was reduced using the CBS catalyst[51] in $EtNO_2$[52] to afford **30**, which was acetylated to provide **31** in 97% yield for the 2 steps. Concurrent THP removal and oxidation using the Jones reagent afforded ynoic acid **28** in 74% yield and 86:14 er. Subsequent partial hydrogenation again gave acid **3**. This scheme could provide acid **3** in 5 steps and should be more scalable than Scheme 4.

The last building block needed to test our final coupling strategy was intermediate **4**, and our initial toward this intermediate is shown in Scheme 6 We chose to utilize the chiral pool as a source of material, since the commercially available L-threonine derivative **32** contained the C14 and C15 stereocenters identical to FR901464. Treatment of **32** with 2-methoxypropene

and CSA afforded oxazolidine **7** in quantitative yield. Subsequent reduction with DIBALH, and immediate exposure of Garner aldehyde[53] to Horner-Wadsworth-Emmons olefination conditions generated **33** in 84% yield. Hydrogenation of this unsaturated ester and lactonization afforded lactone **34**, but revealed the poor diastereoselectivity (dr = 2:1) in the hydrogenation of **33**. As it turned out later, the Kitahara group found similar results.[32] Since we were unable to achieve stereoselectivity in forming the C12 stereocenter, we needed to alter our strategy.

Since the diastereoselectivity in the hydrogenation of **33** was poor, we planned to obtain better stereocontrol in a more rigid cyclic substrate. Oxazolidine **7** was transformed into **35** in a one-pot procedure (DIBALH; $Ph_3P{=}CH_2$)[54] in 77% yield. Subsequent removal of the acetonide using catalytic CSA in MeOH afforded **36** in 95% yield. Methallylation of **36** by the action of $Ag_2O$ and methallyl bromide generated diene **37** in 86% yield, which was then subjected to ring-closing olefin metathesis conditions using 1 mol % of **Ru-1**[55] to form dihydropyran **38** in quantitative yield. Catalyst **Ru-2**[56] was also able to catalyze this reaction at 1 mol %, but we were unable to recover this catalyst. To prepare unsaturated lactone **39**, we found PDC to be the most efficient and selective allylic oxidant for **38** (72%). PCC–TBHP gave similar results for the oxidation of **38**, but gave peroxy-acetal **40** as a byproduct. Unfortunately, we were unable to effectively convert peroxy-acetal **40** to lactone **39** primarily due to epimerization at C14. A more direct approach to form the unsaturated lactone **39** was attempted by methacryl ester formation (step h) then ring-closing metathesis using catalyst **Ru-3** (step i),[57,58] but the second step was unsuccessful. Stereoselective hydrogenation of the unsaturated lactone **39** set the C-12 stereocenter of **34** in a 10:1 dr in quantitative yield. Allylation of this lactone afforded **41** as a mixture of hemiketal anomers and aminal anomers.

As shown in Scheme 7, subjection of **41** to reducing conditions ($Et_3SiH$-$BF_3{\bullet}OEt_2$) provided tetrahydropyran **42** in 20% as well as pyrrolidine **43** in 50% yield. The addition of $CF_3CH_2OH$ to the reduction conditions improved the yield of tetrahydropyran **42** to 45% yield, possibly by establishing an equilibrium between the putative oxocarbenium and *N*-acyliminium ions. 12-*epi*-**41** was subjected to the same reaction sequence, which gave tetrahydropyran 12-*epi*-**42** stereo- and chemoselectively in 85% yield.

Prior to the discovery of the reduction of **41** in the presence of $CF_3CH_2OH$, we explored other methods to improve the hemiketal reduction. To thwart the production of pyrrolidine **43**-type compounds, we also tested other amine-protecting groups (Scheme 8). For example, we prepared picolinamide **44** from **38** in 82% yield. Subsequent oxidation and stereoselective reduction were comparable to using the Boc protecting group. Allylation of **46** was accomplished in 58% yield (not optimized), and reduction with $Et_3SiH$-$BF_3{\bullet}OEt_2$ afforded **50** in 44% yield (61% BORSM; dr = 8:1). Disappointingly, we failed to find deprotection conditions to give **50** after vigorous screening of reaction conditions including the literature procedure.[59] Therefore, we turned our attention to other protecting groups.

The undesired pyrrolidine formation proceeds via the putative highly electrophilic acyliminium ion, and the corresponding sulfonyliminium ion would be less electrophilic,[60] suppressing the pyrrolidine formation. On the basis of this consideration, we decided to test a tosyl protecting group. Deprotection of the Boc group of **38** and subsequent protection of the resulting amine afforded sulfonamide **45** in 86% yield. Allylic oxidation and stereoselective hydrogenation were accomplished in 65 and 99% yields, respectively to afford lactone **47**. Allylation of **47** gave **49** in 63% yield (not optimized), which was then subjected to reduction conditions ($Et_3SiH$-$BF_3{\bullet}OEt_2$) to afford **51** in 68% yield (dr = 12:1). Deprotection proceeded smoothly using sodium naphthalenide,[61] which provided an alternative route to prepare amine **4** or carbamate **42**.

The synthesis of vinyl iodide **1** is shown in Scheme 9. Compound **42** was converted to unsaturated aldehyde **52** using cross-olefin metathesis conditions employing a catalytic amount of **Ru-3**. Takai vinyl iodide formation[62] generated iodoalkene **53** in 63% yield. Removal of the Boc group of this compound was accomplished in 1:9 TFA:$CH_2Cl_2$, and subsequent amide bond formation with acid **3** was accomplished by the action of HATU in 45% yield. Thus, access to vinyl iodide **1** was obtained in 10 longest linear steps.

With both coupling partners in hand, the stage was set to test the Nozaki-Hiyama-Kishi coupling. To our disappointment, treatment of vinyl iodide **1** and ketoaldehyde **2** under Nozaki-Hiyama-Kishi conditions[37,38] caused proto-deiodination of **1** and decomposition of the ketoaldehyde. Similarly, treatment of vinyl iodide **53** and ketoaldehyde **2** to Nozaki-Hiyama-Kishi conditions gave diene **55** and decomposition of the fragile ketoaldehyde. Therefore, we opted to modify our strategy and attempted a lithium-halogen exchange between $^n$BuLi and **53** to generate the corresponding alkenyllithium, but did not find any appreciable coupling to ketoaldehyde **2**. Having realized the fragile nature of ketoaldehyde **2** and the difficulty of forming the C5–C6 bond in the late stages of a synthesis, we began to explore other coupling possibilities.

## Second Generation Synthetic Studies

We envisioned the second retrosynthetic analysis featuring modified Julia coupling to form the C6–C7 bond as shown in Scheme 10. Julia olefinations[63,64] are typically highly E-selective and compatible with various functional groups. Therefore, we decided to pursue this strategy, for which acid **3** and aldehyde **52** were already prepared, but the preparation of the B-ring fragment **56** from ketone **15** required new methodology.

Displayed in Scheme 11 are our efforts focused on preparing the B-ring fragment **56**. Typically, hemiketals are formed by the intramolecular attack of a hydroxy group onto a ketone, thus the hydroxy group is the origin of the ring oxygen atom. We questioned whether the ketone oxygen atom could be used as an alternative source for the ring oxygen atom. This would allow for the exciting possibility of trapping the putative intermediate oxocarbenium ion with water or other nucleophiles to obtain hemiketals and ketals, among others. To test this idea, enone **15** was treated with NBS in $H_2O$/THF (1:8) to give **57** in 75% yield as a single diastereomer.[65] Unambiguous structural determination was determined after conversion of this hemiketal to methyl glycoside **58** in 40% yield. Similarly, we could treat enone **15** with *N*-bromosuccinimide in the presence of MeOH and obtain methyl glycoside **58** in 59% in one step. However, the stereochemistry at C5 was incorrect thereby making preparation of sulfone **56** too difficult from this intermediate.

Since the stereochemistry in **57** and **58** was wrong, we thought that using 4-*epi*-**15** for the cyclization could reverse the observed stereocontrol for C5. Towards this end, alcohol 4-*epi*-**5** was protected as its TES ether, and subsequent regioselective oxidative cleavage ($OsO_4$, NMO; $NaIO_4$) gave enone 4-*epi*-**15** in 60% yield (Scheme 12). Treatment of 4-*epi*-**15** with NBS in $H_2O$/acetone (1:8) gave hemiketal **59** in 95% yield as a single diastereomer. Methyl glycoside **60** could be prepared by the treatment of **59** with catalytic PPTS in MeOH/$CH_2Cl_2$ (1:3) in 75% yield. Alternatively, the same methyl glycoside could be formed in one step by treating 4-*epi*-**15** with NBS in MeOH/MeCN (1:10) in 47% yield. The absolute stereochemistry of **60** was determined by X-ray crystallographic analysis, also revealing the diastereoselectivities for the vinylations of epoxyaldehyde **10** shown in Table 1 and the alkynylation of epoxyaldehyde **10** shown in Scheme 3.

To arrive at sulfone **56**, the C-6 bromide needed to be substituted with a thiol, and the C4 stereocenter inverted. Treatment of methyl glycoside **60** with 4-phenyltetrazole thiol under various conditions only gave the undesired thioether **61**. At this point, we were unsure whether

the axial TES ether group was shielding the C6-Br group from $S_N2$ reactions. To make the C6 position more sterically accessible, we removed the TES group from **60** to give **62** in 80% yield. We then treated this compound with 4-phenyltetrazole thiol under basic conditions but again only found epoxide opening with the thiol to form **63**. A more severe problem is that Mitsunobu conditions failed to invert the C4 stereocenter presumably due to steric reasons. Due to the difficulty in inverting the C4-hyroxy group and sensitivity of the epoxide towards thiols, we abandoned the Julia olefination strategy. It is noteworthy that despite these synthetic problems, in **61** and **63** we observed couplings between the 3-hydroxy group and methylene protons adjacent to the sulfur atom in $^1$H NMR experiment in $CDCl_3$, which supports the notion of a "strong" hydrogen bond.[66,67]

## Third Generation Synthetic Studies

Disappointed by our two previous failed attempts, we had no choice but to employ a bolder approach to unite the two fully functionalized fragments. Such retrosynthetic analysis is shown in Scheme 13 (A), in which we continued to envisage forming the C6–C7 olefin as the final coupling. At the onset of this study, 1,3-diene–ene cross metatheses were poorly explored transformations[68] and had not been used in natural product synthesis (Scheme 13, B). However, we believed that this would be a viable approach because: (1) a ruthenium catalyst would preferentially react with the olefin of monoene **70** rather than the conjugated olefins of diene **69**; (2) the resulting ruthenium alkylidene **71** would preferentially react with **70** to form **67**, but this is reversible at elevated temperature and could be minimized by adding **70** slowly to the reaction mixture; (3) eventually, alkylidene **71** would react with the electronically and sterically most accessible terminal olefin of **69** to form the thermodynamically favored **72**; and (4) when ruthenium alkylidene **68** does form, this ruthenium species will react with **70** faster than **69** to form **72** and **66** respectively. This hypothesis was also corroborated by the Crimmins group in the crucial cross-coupling step.[69]

To pursue this strategy, the first task was to prepare the right fragment **64**. With ketoaldehyde **2** already in hand, a direct approach would be a chemoselective vinylation of this aldehyde (Table 2). Treatment of the fragile ketoaldehyde **2** with $CH_2$=CHMgBr gave an inseparable mixture of **73** and 5-*epi*-**73** with no diastereoselectivity (entry 1). Addition of HMPA or TMEDA (entries 2 and 3) slightly improved the Felkin selectivity and the overall yield of **73**. Generation of vinyllithium *in situ* from $^n$Bu$_3$SnCH=CH$_2$ and $^n$BuLi, and subsequent addition of **2** afforded **73** and 5-*epi*-**73** in 33% yield but again with no stereoselectivity (entry 4). Addition of HMPA gave better diastereoselectivity (dr = 2:1), but lower combined yield (16%) in producing **73** and 5-*epi*-**73** (entry 5). Neither switching the solvent to $CH_2Cl_2$ or toluene nor making the organocerium reagent from $CeCl_3$ and $CH_2$=CHMgBr[70,71] improved this difficult transformation. Additionally, ethynylation of ketoaldehyde **2** required higher temperatures (≥ -20 °C), leading to decomposition. We speculate that the low yields for these reactions are due to an intramolecular deprotonation-elimination pathway; the alkoxide generated after addition to the aldehyde can intramolecularly deprotonate a hydrogen atom at C2 and induce E1cb reaction. Although low yielding, we obtained the right coupling fragment **73** along with its inseparable diastereomer 5-*epi*-**73** and decided to pursue preliminary coupling studies after preparing diene **54**.

Our attempts to prepare diene **54** are shown in Scheme 14. Aldehyde **52** was treated with ylide $Ph_3P$=$CH_2$ to form diene **55**. Deprotection of the Boc protecting group in TFA/$CH_2Cl_2$ (1:9) and coupling with acid **3** gave **54** and **74** as an isomeric mixtures. Therefore, the Boc group removal should precede diene formation to avoid this acid-catalyzed isomerization. Removal of the Boc group in **42** (1:9 TFA/$CH_2Cl_2$) followed by coupling with acid **3** in one pot afforded **75** in 86% yield. Stereo- and regioselective cross metathesis of **75** with methacrolein using

**Ru-3** gave aldehyde **76** in 57% yield (67% BORSM), and subsequent Wittig reaction with ylide $Ph_3P{=}CH_2$ gave diene **54** in 86% yield.

With the fully functionalized intermediates **54** and **73** in hand, the stage was set to test our coupling strategy (Scheme 15). Treatment of diene **54** and the C6 epimeric mixture **73** gave the metathesis adduct **77** in 22% yield. Subsequent removal of the TES group was accomplished with HF•pyridine to furnish FR901464 (confirmed by HPLC analysis using the authentic FR901464) and presumably its C5-epimer, thereby supporting the viability of this strategy. We were very much surprised to find that when the reaction was neutralized with a pH 7.0 phosphate buffer, the natural product decomposed. This interesting observation prompted us to investigate the stability of FR901464 under physiological conditions (see Scheme 20).

Having realized that the final steps could potentially produce many byproducts due to instability, we strongly desired to prepare a right fragment in higher efficiency and with better stereocontrol of the C5 stereocenter. To test whether we could improve the C5 stereoselectivity, we switched to the TBS ether that might potentially favor the desired Felkin selectivity while preventing unwanted chelation of the α-alkoxy group (see Supporting Information). Preparation of this ketoaldehyde followed similarly as shown with the TES derivative (Scheme 2). However, subjection of the C4-OTBS-protected ketoaldehyde to various vinylation and alkynylation conditions formed the desired adduct in <5% yield and lacked the desired chemoselectivity. Therefore, we decided to develop a different approach to obtain the right fragment.

We turned our attention to the previously prepared enoate **24** (Scheme 3) since it contained all of the necessary carbons and the desired C4 stereochemistry. Up to this point, we were reluctant to pursue the following strategy because we could not predict the stereoselectivity of the [2,3]-sigmatropic rearrangement (Scheme 16).[72] Regardless, we began to explore methods to prepare **81** from intermediate **24** (Table 3), hoping that we could somehow control the stereoselectivity in question. The DIBALH reduction of **24** afforded allylic alcohol **78** in 95% yield, which was subsequently transformed to selenide **79** or **80** in quantitative yields, setting us up to explore a Mislow-Evans–type [2,3]-sigmatropic rearrangement despite no closely related reported reactions.

Table 3 shows the optimization of the [2,3]-sigmatropic rearrangements of **79** and **80** to form the desired alcohol **81**, whose stereochemistry was determined as shown in Scheme 17.[36] While the transformation occurred rapidly in polar solvents (entries 1–3), it was sluggish in $CH_2Cl_2$ (entry 4). Surprisingly, the base employed in this reaction played a critical role as shown in entries 5–7. When 2,6-lutidine was used, the reaction was slower as compared to pyridine presumably due to the added steric bulk of the nucleophile, leading to no improved diastereoselectivity. When DMAP was used, the reaction was very slow at low temperatures and needed to be warmed to ambient temperature to proceed at an adequate rate. Gratifyingly, the diastereoselectivity of this reaction was improved to 7.5:1 in favor of alcohol **81**. The more nucleophilic base, 4-pyrrolidinopyridine, gave the highest diastereoselectivity for this alcohol (dr = 8.1:1).[73] Although DMAP showed slightly lower stereoselectivity, we were attracted by its lower cost. The effect of the DMAP stoichiometry are shown in entries 6, 8, 9, and 10; decreasing the amount of DMAP below 3 equivalents eroded selectivity presumably due to background hydrolysis reactions of the putative selenate. Finally, the nitro group on the aromatic ring was shown to be unnecessary for the high diastereoselectivity (entry 11).

With the sufficiently stereoselective [2,3]-sigmatropic rearrangement in hand, we proceeded to prepare right fragment **64** (Scheme 17). Primary alcohol **78** was converted to the desired secondary alcohol **81** this time in one-pot procedure utilizing the selenide formation, [2,3]-rearrangement strategy in 91% combined yield (dr = 7:1). Oxidative cleavage of the 1,1-

disubstituted olefin of **81** gave the previously prepared compound **73** in a diastereomerically pure form, but in only 27% yield. To improve the regioselectivity of the oxidative cleavage sequence, we prepared bis-TES ether **82** from **81** in 95% yield. Subsequent dihydroxylation and $Pb(OAc)_4$-promoted diol cleavage afforded enone **83** in 71% yield. The removal of the TES ethers was found to occur best using $AcOH/H_2O/THF$ (3:1:3) to afford **64** in 91% yield. The diaxial nature of the C4 and C5 hydrogen atoms was confirmed by the $^1H$ NMR analysis showing $J_{H4\text{-}H5}$ = 9.8 Hz, similar to that found in the natural product ($J$ = 10 Hz),[12] thereby proving the relative stereochemistry of **64**. We were now stereoselectively and efficiently form **64** in 11 linear steps. Thus, the only remaining question was the cross metathesis in the absence of any protecting groups.

As shown in Scheme 18, the catalyst employed in the cross-metathesis of **54** and **64** influenced the efficiency of the reaction. The desirable conditions found were to employ a 1:1.8 ratio of **54** to **64** in the presence of 12 mol % of **Ru-3** in 1,2-dichloroethane to afford FR901464 in 40% yield after one recycle of unreacted starting materials (51% based on recovered **54** after one recycle). The fragile nature of **64** prevented more forcing reaction conditions, since facile fragmentation occurred at ≥47 °C in 1,2-dichloroethane. Additionally, right fragment **64** formed an unreactive homodimer under the reaction conditions, as determined by preparation of the right fragment homodimer and subjection of it to left fragment **54** and **Ru-3**.

To recapitulate our total synthesis of FR901464, we completed the synthesis in 29 total steps with the longest linear sequence of 13 steps (Scheme 19). Highlights of the synthesis include a diene-ene cross metathesis as the final synthetic step in the absence of protecting groups, a Mislow-Evans type [2,3]-sigmatropic rearrangement of a selenoxide, an asymmetric Carreira alkynylation, formation of an unsaturated lactone by a ring-closing metathesis-regioselective allylic oxidation sequence, a mild, diastereoselective electron deficient alkynylation reaction, and a trans-selective Red-Al reduction. This total synthesis is the most concise to date, allowing for facile analog preparation and access to each fully functionalized fragment for biological and chemical studies.[29]

## Stability of FR901464

Similar to the Jacobsen and Kitahara groups, we also noticed the instability of FR901464 toward acids, even as mild as silica gel.[30,74] However, the sensitivity of FR901464 under physiologically relevant conditions remained unreported, and therefore we decided to determine the half-life of **64** as a means to examine its stability in various phosphate buffers at 37 °C as shown in Table 4. Alarmingly, the half-lives of **64** are only 8 and 4 hours at pH 7 and 7.4 respectively, possibly limiting FR901464's potential as a biological probe. Consequently, we became intrigued by the decomposition pathways and subjected **64** to pH 7.4 buffer at 37 °C for 1 day and observed >3 products by TLC and HPLC analyses, some of which were minor and not isolated (Scheme 20). Among the products that we were able to partially characterize were furan **90** and enones **84** and **85** (*a*). We did not observe acrolein (by $^1H$ NMR or HPLC), **91**, or **92** via Kitahara's decomposition pathway (*b*).[74] We also looked for acetate **93** that would be formed by a Grob fragmentation (*c*),[75,76] but have insufficient evidence to confirm this product formation. Hemiketal **64** exists as a 10:1 equilibrium mixture with linear ketoalcohol **64-open** in both $CD_2Cl_2$ and $D_2O$, in accordance with Jacobsen's observation.[30] Therefore, enones **84** and **85** were presumably formed by the β-elimination via ketone **64-open**, and furan **90** by the dehydration of hemiketal **88**. Enone **84** can exist as its hemiketals **86** or **87**, but **86** should be thermodynamically disfavored due to its ring strain, and therefore furan **89** is not a major byproduct of this reaction over the time period studied.

### Tumor-specificity with pH sensitivity

It is intriguing to contemplate on why nature produces FR901464 with such subtle pH sensitivity. We speculate that under pressure for survival, the FR901464-producing organism may have had to find a way to inhibit the growth of enemies in acidic environments. Unfortunately this idea is difficult to test because the exact nature of the FR901464-producing organism has not yet been characterized. In the context of cancer medicine, we propose that the stability of the β-epoxy hemiketal under acidic environment can be exploited for the development of cancer-specific drugs because the pH within tumor cells is significantly lower than that of normal cells[77,78] and therefore β-epoxy hemiketal-containing anticancer agents would decompose more rapidly in normal cells while remaining active in tumor cells longer. Although the acidity difference between cancer and normal cells is well-known, this difference has not yet been fully exploited. Thus this study may open the door for such an approach.

Although the pH-sensitivity could be exploited in therapeutic areas, the instability of FR901464 may limit its potential as a probe for biological study, requiring a more stable analog. The replacement of the C1-hydroxy group with either a hydrogen atom[30] or a methoxy group[74] only marginally improved the potency of FR901464. We hypothesized that such increased stability and more desirable van der Waals interaction with target proteins might improve the potency of FR901464. To test this hypothesis, we prepared right fragment **95** (Scheme 21) which should be more stable based on the fragmentation studies (Table 5). Treatment of alcohol **81** with $Hg(OAc)_2$ followed by $NaBH_4$ and $Et_3B$[79] to afford ether **94** in 76% yield. Deprotection of this tetrahydropyran was accomplished by the action of TBAF to give **95** in 97% yield. Interestingly, we found that deprotection of **94** with HF•pyridine caused a pinacol-like rearrangement to yield the ring-contracted aldehyde **96**. We later found that treatment of **95** with HF•pyridine also caused this rearrangement to occur, indicating that the TES group is not essential for the rearrangement.

The stability of **95** was then tested in various phosphate buffers at 37 °C as shown in Table 5. The $t_{1/2}$ of **95** at pH 7 and 7.4 was now 48 hours, which is a dramatic increase from **64** ($t_{1/2}$ = 4–8 h). Additionally, **95** did not appear to be significantly labile until 0.1N $H_2SO_4$ was employed as the solvent. It should also be mentioned that aldehyde **96** (Scheme 19) was not observed even in the presence of $H_2SO_4$, showing the pinacol-like rearrangement should not be biologically relevant. Replacement of the C1-OH with a methyl group enhanced the stability of the right fragment of FR901464, and therefore could provide a suitable biological probe to study the unique biology of FR901464.

### Synthesis of a Stable FR901464 Analog

Encouraged by the chemical stability of **95**, we proceeded to synthesize the more stable FR901464 analog meayamycin (**97**) in 59% yield after one recycling of recovered starting materials using the same diene-ene metathesis strategy (Scheme 22). By having a more stable right fragment, we were able to more efficiently produce the desired product. Therefore, we completed the synthesis of **97** in 28 total steps with the longest linear sequence of 13 steps. This synthetic scheme enabled us to produce 30 mg of this analog and should be scalable.

### Biological Assay

With synthetic FR901464 and its analog **97** in hand, we tested these compounds against MCF-7 breast cancer cells and measured the cell viability by using the MTS assay after the 7- and 10-day incubation periods.[80] As Figure 1 shows, we observed the inhibition of cell growth in a concentration-dependent manner and determined the $GI_{50}$ value of FR901464 to be 1.1 nM, which is in good agreement with the literature value of 1.8 nM.[11] As we expected, the potency of meayamycin was better and exceeded our expectation: the $GI_{50}$ value of this analog was 10 pM, giving approximately a 100-fold improvement in the antiproliferative activity. We also

found that the mode of action of FR901464 did not involve either DNA binding or antimitotic activity.[81]

## Conclusion

We completed the most concise total synthesis of FR901464 to date, in a total of 29 steps. We then performed degradation studies on the fully functionalized right fragment of FR901464, and used this insight to rationally design a more stable right fragment analog. Enabled by our total synthesis, we completed the synthesis of a FR901464 analog (meayamycin) using the more stable right fragment in a total of 28 steps. The $GI_{50}$ value of this analog was a remarkable 10 pM in MCF-7 cells, making it one of the most potent anticancer agents known to date. With the successes of FR901464 in the xenograft models[13] and impressive potency of meayamycin, we are currently working toward preparing this analog on a larger scale. The potency and stability of this analog should facilitate our efforts to isolate cellular targets of FR901464. We are also studying how the stability of meayamycin would affect the tumor specificity.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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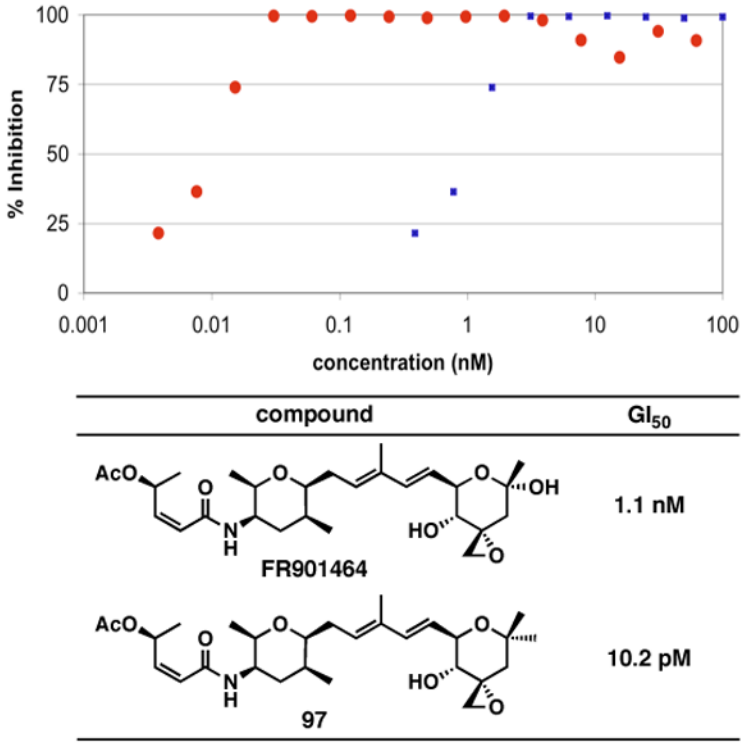
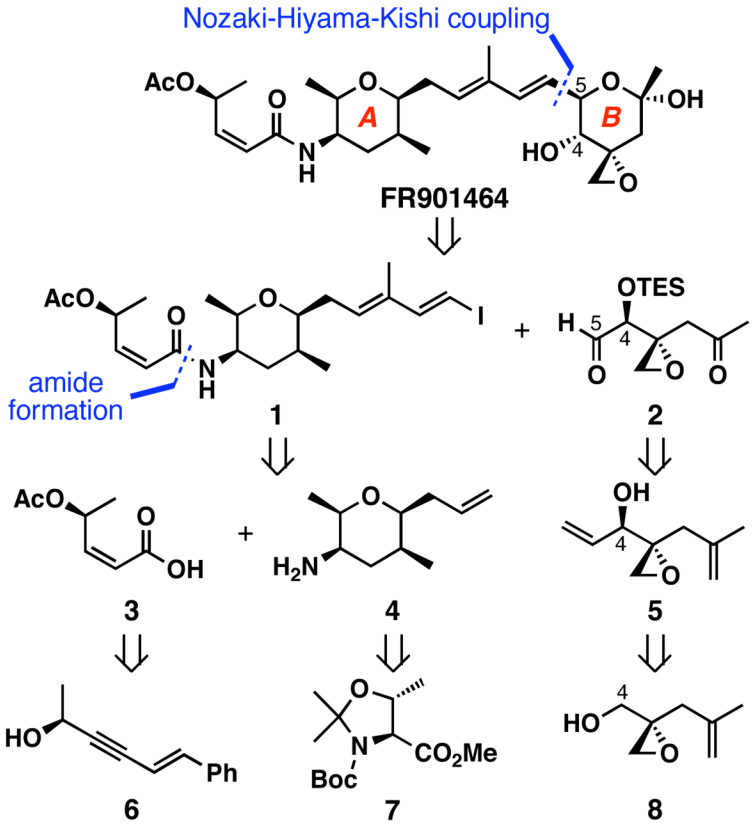
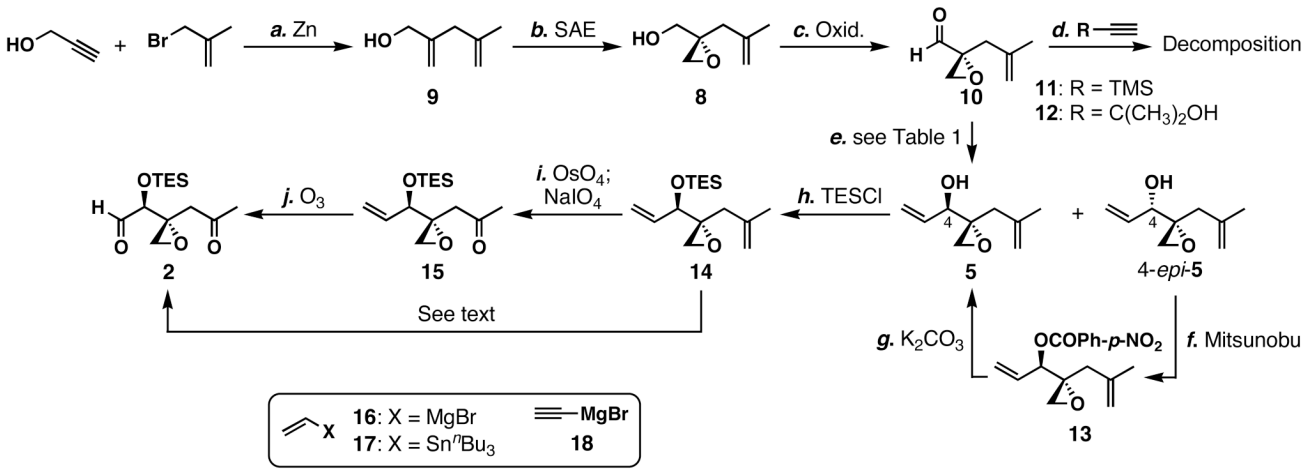



**Figure 1.**
Growth inhibition of MCF-7 cells by FR901464 (blue) and meayamycin (**97**; red)



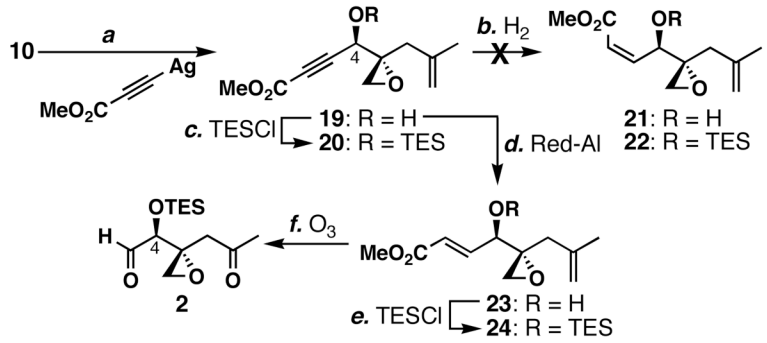

**Scheme 1.**
First generation retrosynthetic analysis of FR901464




**Scheme 2.**

Preparation of ketoaldehyde **2** [a]

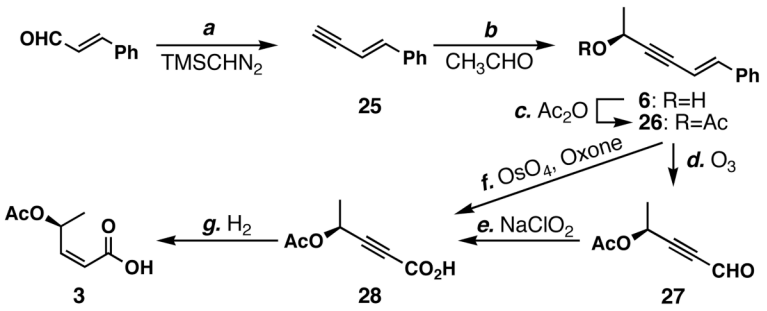
[a]Conditions: (a) propargyl alcohol (1.1 equiv), allyl bromide (1.1 equiv), zinc dust (3.1 equiv); then methallyl bromide (1.0 equiv), THF, 23 °C, 93%; (b) (+)-DIPT (9 mol %), $Ti(O^iPr)_4$ (8 mol %), $^t$BuOOH (1.9–2.2 equiv), $CH_2Cl_2$, -20 °C, 90%, er >97:3; (c) Dess-Martin periodinane (1.5 equiv), $CH_2Cl_2$, 0→23 °C, 81%; (d) alkyne **11** or **12** (1.3 equiv), $Zn(OTf)_2$ (1.2 equiv), (−)-*N*-methylephedrine (1.3 equiv), $Et_3N$ (1.3 equiv), toluene, 23→40 °C, 22 h; (e) see table below; (f) 4-$O_2NPhCO_2H$ (3.0 equiv), diisopropyl azodicarboxylate (2.9 equiv), $Ph_3P$ (3.0 equiv), THF, 0→23 °C, 85%; (g) $K_2CO_3$ (2.5 equiv), MeOH, 0 °C, 88%; (h) TESCl (1.1 equiv), imidazole (1.4 equiv), THF, 0 °C, quant.; (i) $OsO_4$ (0.05 equiv), NMO (1.0 equiv), THF, 0→23 °C, 58%; then $NaIO_4$ (1.0), THF/$H_2O$ (1:1), 23 °C, quant.; (j) $O_3$, $CH_2Cl_2$/MeOH (1:1), -78 °C; then $Me_2S$ (10 equiv), -78→23 °C, 54–82%.




**Scheme 3.**

Formation of C4 stereocenter with higher stereocontrol [a]

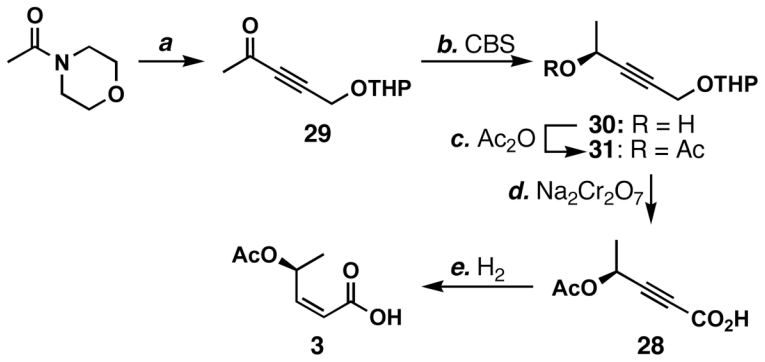
[a] Conditions: (a) Ag-C≡C-$CO_2$Me (1.7 equiv), $Cp_2ZrCl_2$ (1.3 equiv), AgOTf (0.2 equiv), $CH_2Cl_2$, 23 °C, 84% (dr = 6:1); (b) $H_2$ (1 atm), Lindlar's catalyst (1.2 –2.0 mol %), quinoline (0.94–1.5 equiv), EtOH, 23 °C, 7–17 h; (c) TESCl (1.1 equiv), imidazole (1.4 equiv), THF, 0 °C, 74%; (d) Red-Al (2.0 equiv), THF, -72 °C, 81%; (e) TESCl (1.4 equiv), imidazole (1.5 equiv), THF, 0 °C, quant.; (f) $O_3$, $CH_2Cl_2$/MeOH (1:1), -78 °C; then $Me_2S$ (17 equiv), -78→23 °C, quant. (based on $^1$H NMR).




**Scheme 4.**

Preparation of acid **3**

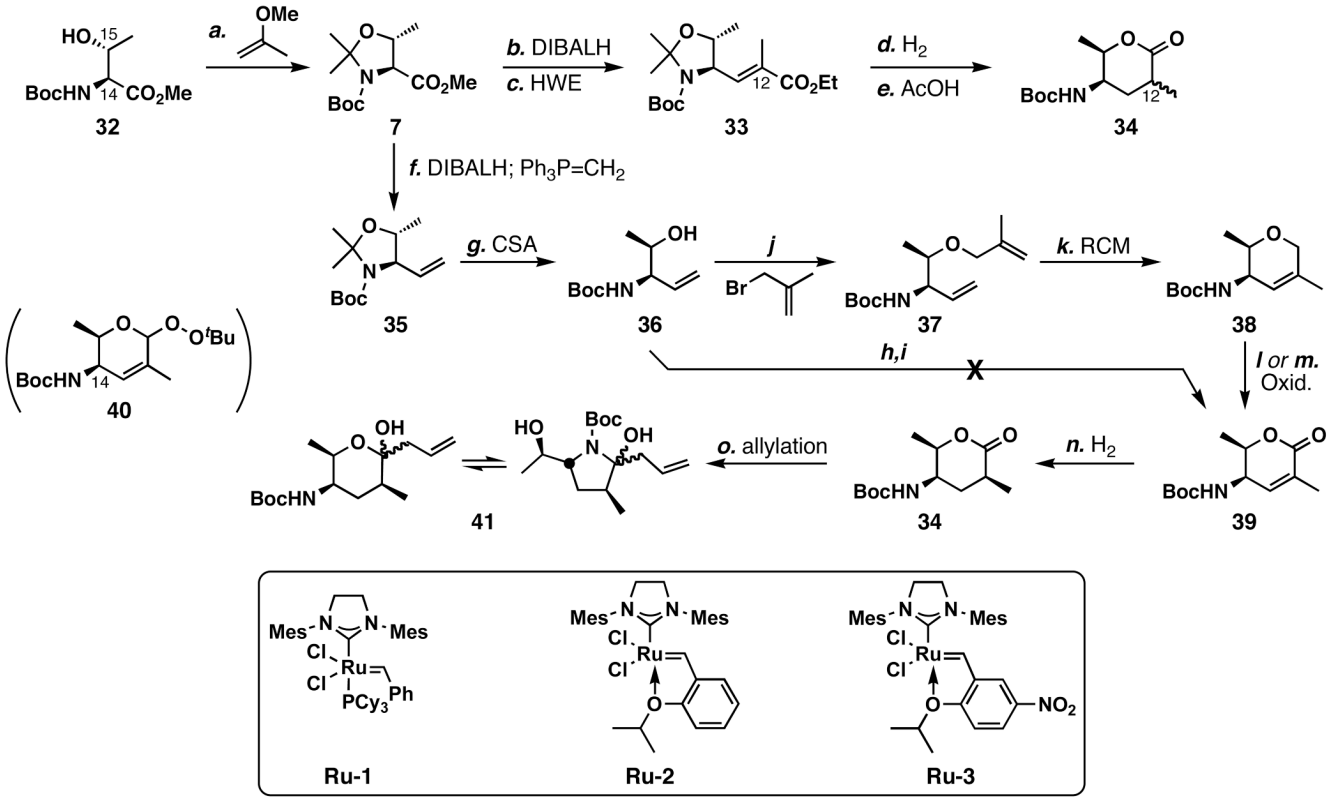
[a] Conditions: (a) cinnamaldehyde (1.1 equiv), $TMSCHN_2$ (1.0 equiv), LDA (1.0 equiv), THF, -78→0 °C, 84%; (b) $CH_3CHO$ (2.3 equiv), $Zn(OTf)_2$ (1.0 equiv), (−)-*N*-methylephedrine (1.0 equiv), $Et_3N$ (1.0 equiv), toluene, 23 °C, 41% (86:14 er); (c) $Ac_2O$ (5.0 equiv), pyridine, 23 °C, quant.; (d) $O_3$, $CH_2Cl_2$, -78 °C; then $Me_2S$ (10 equiv), -78→23 °C, 89%; (e) $NaClO_2$ (3.0 equiv), $NaH_2PO_4$ (2.0 equiv), 2-methyl-2-butene (15 equiv), $^tBuOH/H_2O$ (1:1), 23 °C; (f) $OsO_4$ (0.7 mol %), Oxone (4.0 equiv), DMF, 23 °C; (g) $H_2$, Lindlar's catalyst (1 mol %), quinoline (10 mol %), EtOH, 23 °C, 75% (for steps e and g), 60% (for steps f and g).




**Scheme 5.**

Alternative methods to prepare acid **3** [a]

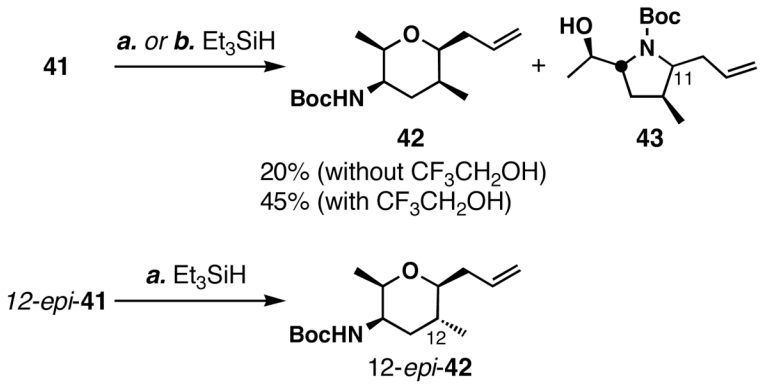
[a] Conditions: (a) HC≡CCH$_2$OTHP (3.0 equiv), $^n$BuLi (3.0 equiv), THF, -78→0 °C, 78–93%; (b) (*S*)-2-methyl-CBS-oxazaborolidine (0.2 equiv), catecholborane (1.6 equiv), EtNO$_2$, -78 °C; (c) Ac$_2$O (4.6 equiv), pyridine (excess), 23 °C, 97% (2 steps); (d) Na$_2$Cr$_2$O$_7$ (3.5 equiv), H$_2$SO$_4$, H$_2$O, acetone, 0→23 °C, 74%; (e) see Scheme 4.




**Scheme 6.**

Synthetic approach A-ring formation [a]

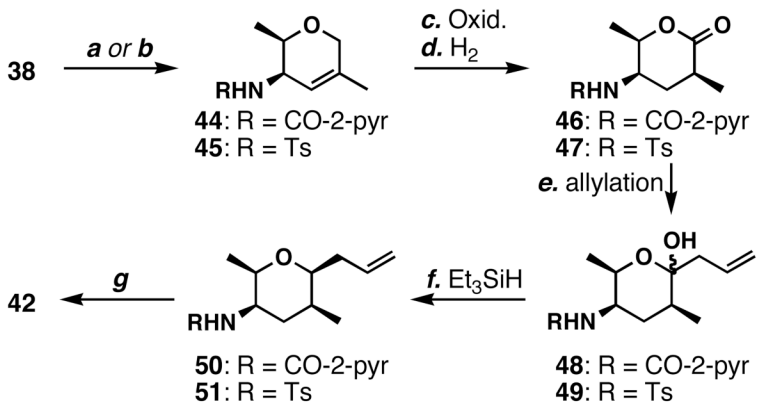
[a] Conditions: (a) 2-methoxypropene (2.0 equiv), CSA (1 mol %), $CH_2Cl_2$, 0 °C, quant.; (b) DIBALH (2.0 equiv), $CH_2Cl_2$, -78 °C; (c) $(EtO)_2P(O)CH(CH_3)CO_2Et$ (3.3 equiv), NaH (3.0 equiv), solvent, 23 °C, 84% (2 steps); (d) $H_2$ (1 atm), $PtO_2$ (1 mol %), EtOH, 23 °C, quant. (dr = 2:1); (e) AcOH, 58%; (f) DIBALH (2.0 equiv), $CH_2Cl_2$, -78 °C; then $Ph_3PCH_3Br$ (2.1 equiv), KO$^t$Bu (2.0 equiv), THF, -78→48 °C, 77%; (g) CSA (10 mol %), MeOH, 23 °C, 95%; (h) methacrylic acid (1.2 equiv), DCC (1.2 equiv), DMAP (0.1 equiv), $CH_2Cl_2$, 0 °C, 95%; (i) **Ru-3** (10 mol %), $ClCH_2CH_2Cl$, 83 °C; (j) methallyl bromide (4.0 equiv), $Ag_2O$ (1.5 equiv), DMF, 23 °C, 86%; (k) **Ru-1** (1 mol %), benzene, reflux, quant.; (l) PDC (6.0 equiv), $ClCH_2CH_2Cl$, reflux, 72%; (m) PCC (2.0 equiv), TBHP (4.0 equiv), Celite, benzene, 23 °C, 67% (n) $H_2$, $PtO_2$ (1 mol %), EtOH, 23 °C, quant. (dr = 10:1); (o) allyl-MgCl (2.0 equiv), THF, -78 °C, 96%.




**Scheme 7.**

Preparation of **42** [a]

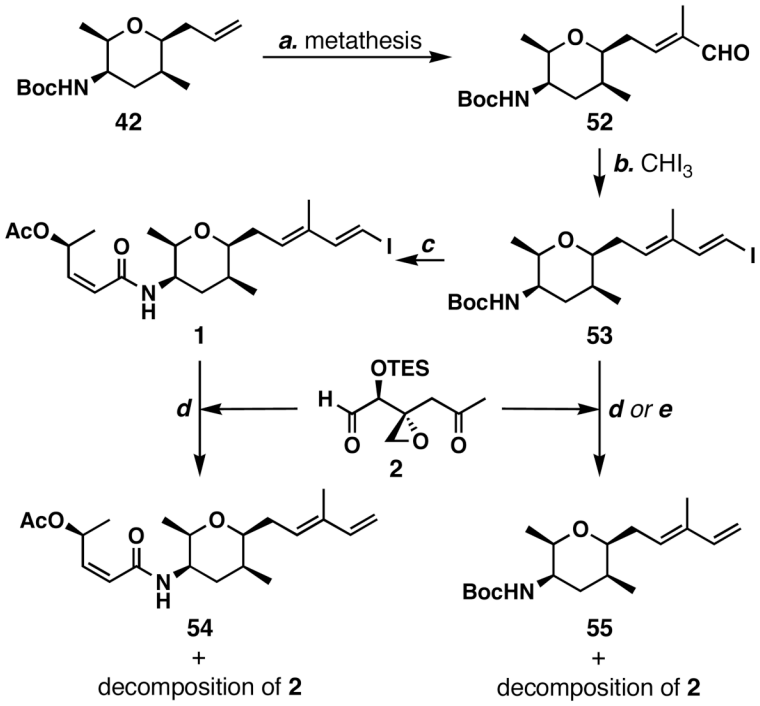
[a] Conditions: (a) $Et_3SiH$ (4.0 equiv), $BF_3$•$OEt_2$ (4.0 equiv), $CH_2Cl_2$, -78 °C, **42** (dr = 10:1); (b), $Et_3SiH$ (10 equiv), $BF_3$•$OEt_2$ (4.0 equiv), $CF_3CH_2OH$ (8.0 equiv), $CH_2Cl_2$, -78 °C; then $Boc_2O$ (0.5 equiv), -78→23 °C, **42** (dr = 10:1).




**Scheme 8.**

Preparation of **41** using different amine protecting groups [a]

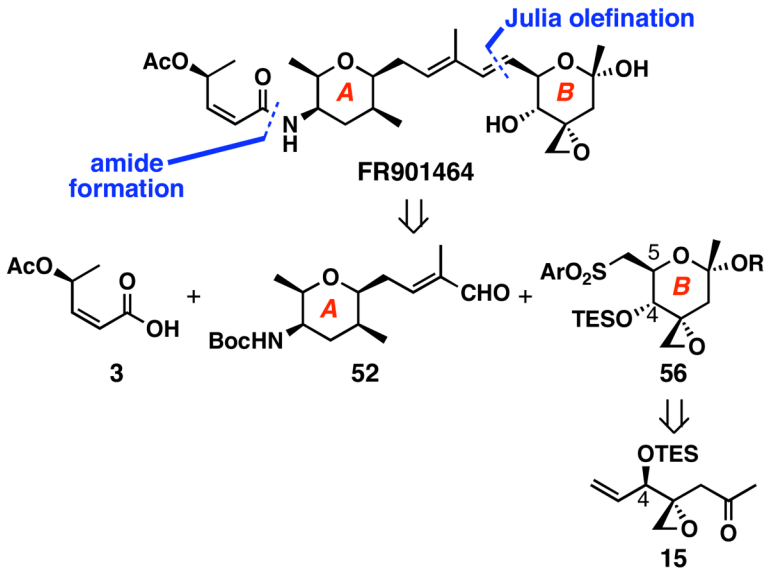
[a] Conditions: (a) TFA/$CH_2Cl_2$ (1:9), 23 °C; then pivaloyl chloride (3.0 equiv), DMAP (0.01 equiv), pyridine, 0→23 °C, 82% (**38→44**); (b) 6N HCl/THF (2:1), 23 °C; then TsCl (1.3 equiv), $K_2CO_3$, 23 °C, 86% (**38→45**); (c) PDC (4.0 equiv), $^t$BuOOH (4.0 equiv), benzene, 23 °C, 70% (**25**) or 65% (**26**); (d) $H_2$, $PtO_2$ (3 mol %), EtOH, 23 °C, 97% (**44→46**) or 99% (**45→47**); (e) allyl-MgCl (3.0 equiv), THF, -98 °C, 58% (**46→48**), or -78 °C, 63% (**47→49**); (f) $Et_3SiH$ (10 equiv), $BF_3$•$OEt_2$ (4.0 equiv), $CH_2Cl_2$/MeCN (1:1), -20 °C, 44% (**48→50**, 61% BORSM; dr = 8:1), or THF, -20 °C, 68% (**49→51**, dr = 12:1); (g) (from **51**) sodium naphthalenide, THF, -78 °C; then $Boc_2O$ (2.3 equiv), $Et_3N$ (4.0 equiv), DMAP (0.08 equiv), MeCN, 23 °C, 87% (**51→42**, 2 steps).



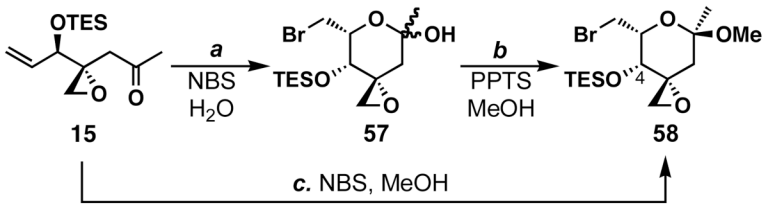

**Scheme 9.**

Failed synthetic attempts using the NHK strategy [a]

[a] Conditions: (a) **Ru-1** (5 mol %), methacrolein (10 equiv), $ClCH_2CH_2Cl$, 40 °C, 67%; (b) $CHI_3$ (2 equiv), $CrCl_2$ (6 equiv), THF, 0 °C, 63%; (c) TFA/$CH_2Cl_2$ (1:9), 23 °C; then **3** (1.7 equiv), HATU (1.7 equiv), $^{i}Pr_2NEt$ (3.6 equiv), MeCN, 23 °C, 45%; (d) $CrCl_2$ (7 equiv), $NiCl_2$ (~5 mol %), DMSO, 23 °C; (e) $^{n}BuLi$ (1–2 equiv), THF, -100→-78 °C.



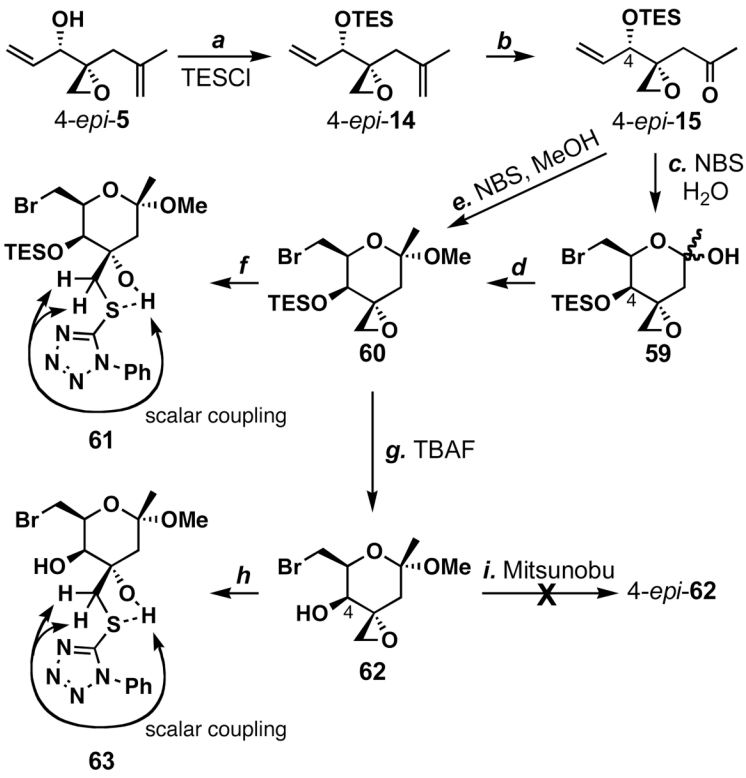

**Scheme 10.**
Julia olefination coupling strategy




**Scheme 11.**

Preparation of methyl glycoside **58** [a]

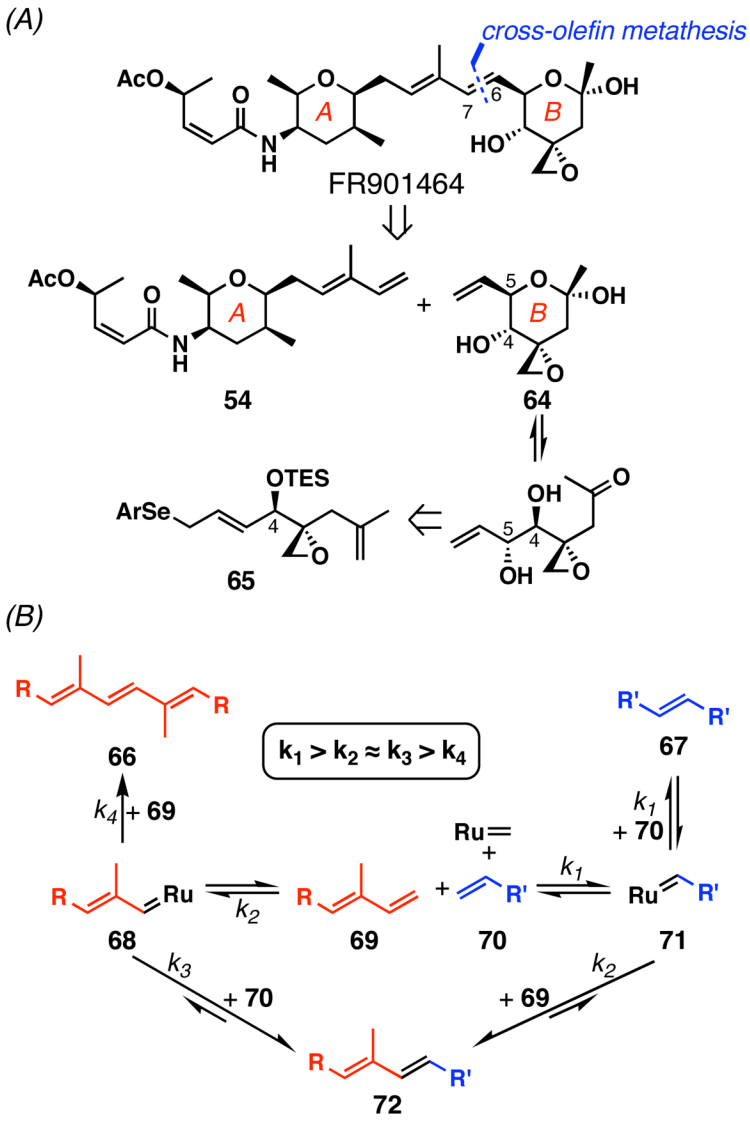
[a] Conditions: (a) NBS (1.1 equiv), THF, $H_2O$, 0→23 °C, 75%; (b) PPTS (10 mol %), MeOH, $CH_2Cl_2$, 23 °C, 40%; (c) NBS (1.5 equiv), 3Å MS, MeOH, MeCN, 0→23 °C, 59%.



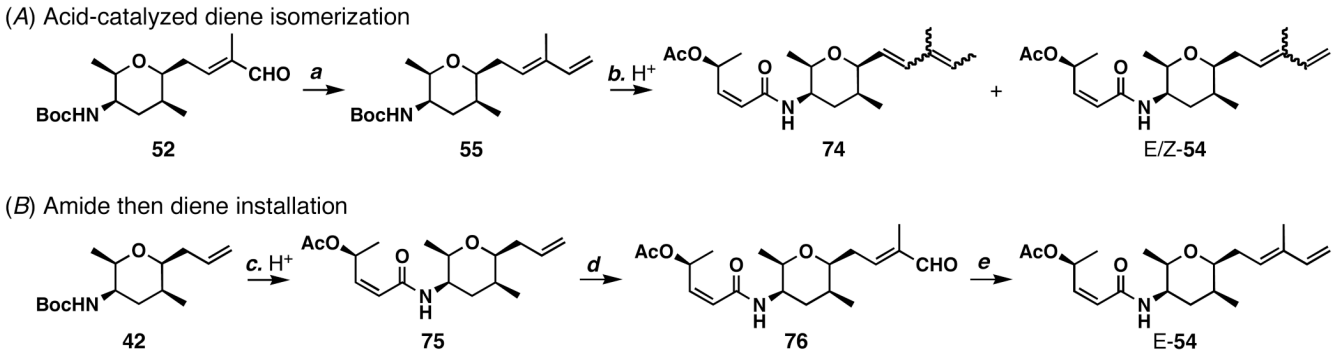

**Scheme 12.**

Preparation of thioethers **61** and **63** [a]

[a] Conditions: (a) TESCl (1.2 equiv), imidazole (1.4 equiv), THF, 0 °C, quant.; (b) $OsO_4$ (4.5 mol %), NMO (1.0 equiv), THF, $H_2O$, 23 °C; then $NaIO_4$ (1.0 equiv), $Et_2O$, $H_2O$, 23 °C, 60%; (c) NBS (2.0 equiv), acetone, $H_2O$, 23 °C, 95%; (d) PPTS (10 mol %), MeOH, $CH_2Cl_2$, 23 °C, 75%; (e) NBS (1.5 equiv), 3Å MS, MeOH, MeCN, 0→23 °C, 47%; (f) 4-phenyltetrazole thiol (1.2 equiv), $Et_3N$ (2.0 equiv), MeCN, reflux, 60%; (g) TBAF (1.1 equiv), THF, 0 °C, 80%; (h) 4-phenyltetrazole thiol (1.0 equiv), $Et_3N$ (2.0 equiv), MeCN, 50 °C, 74%; (i) 4-$O_2NPhCO_2H$ (2.1 equiv), DIAD (2.7 equiv), $Ph_3P$ (2.2 equiv), THF, 0→23 °C; then $K_2CO_3$ (1.0 equiv), MeOH, 23 °C.



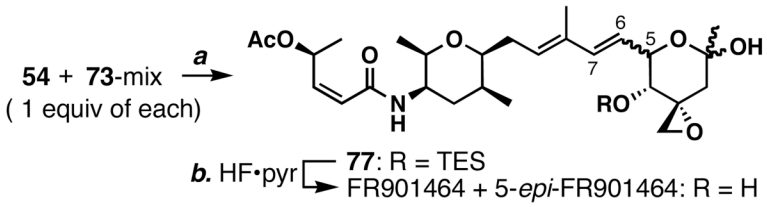

**Scheme 13.**
Diene-ene Cross Metathesis Coupling Strategy




**Scheme 14.**

Preparation of diene **54** [a]

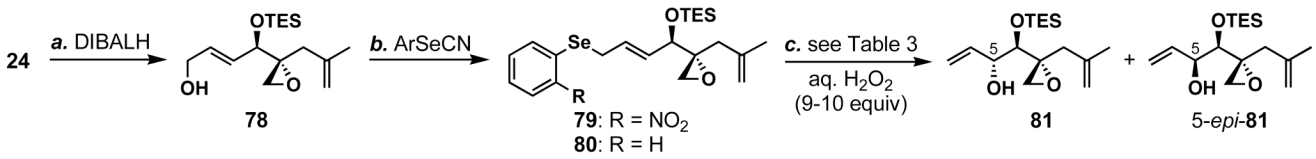
[a] Conditions: (a) $Ph_3PCH_3Br$ (1.4 equiv), $^tBuOK$ (1.3 equiv), THF, 0 °C, 80%; (b) TFA/$CH_2Cl_2$ (1:9), 23 °C; then **3** (1.7 equiv), HATU (1.7 equiv), $^iPr_2NEt$ (4.6 equiv), MeCN, 0 °C; (c) TFA/$CH_2Cl_2$ (1:9), 23 °C; then **3** (1.2 equiv), HATU (1.2 equiv), $^iPr_2NEt$ (4.0 equiv), MeCN, 23 °C, 86%; (d) **Ru-3** (0.05 equiv), methacrolein (20 equiv), $CH_2Cl_2$, 23 °C, 57% (67% based on recovered **75**); (e) $Ph_3PCH_3Br$ (1.4 equiv), $KO^tBu$ (1.2 equiv), THF, 0 °C, 86%.




**Scheme 15.**

Our first synthesis of FR901464 [a]

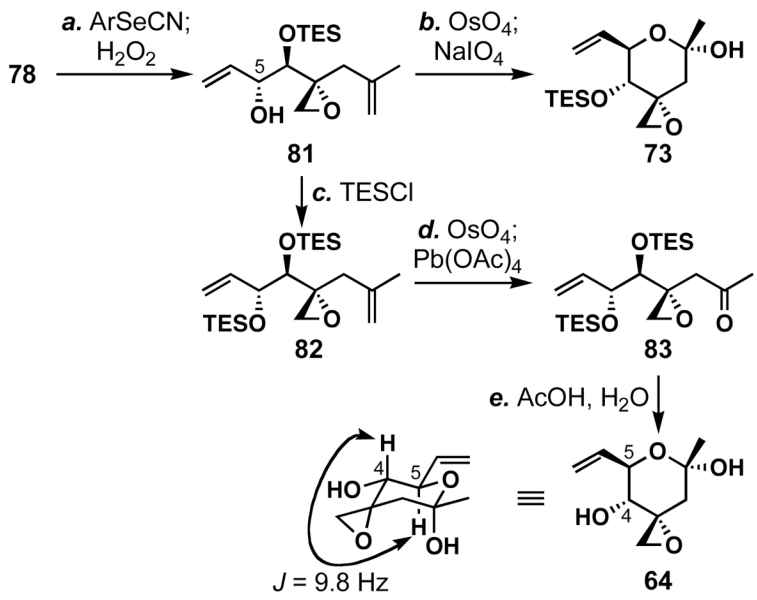
[a] Conditions: (a) **Ru-1** (0.2 equiv), THF, 40 °C, 22%; (b) HF•pyridine, THF, 0 °C.




**Scheme 16.**

Preparation of alcohols **81** and 5-*epi*-**81** [a]

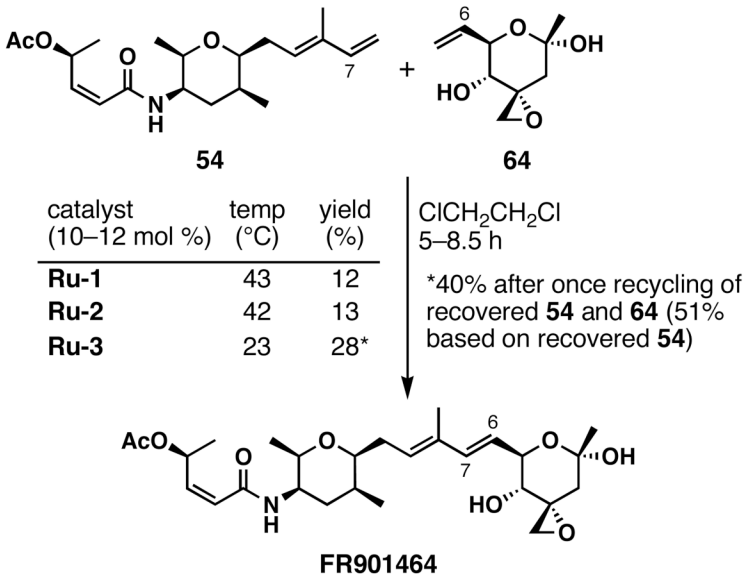
[a] Conditions: (a) DIBALH (3.0 equiv), THF, -78 °C, 95%; (b) ArSeCN (1.2 equiv), $^{n}Bu_3P$ (1.4 equiv), THF, 0 °C, quant.




**Scheme 17.**

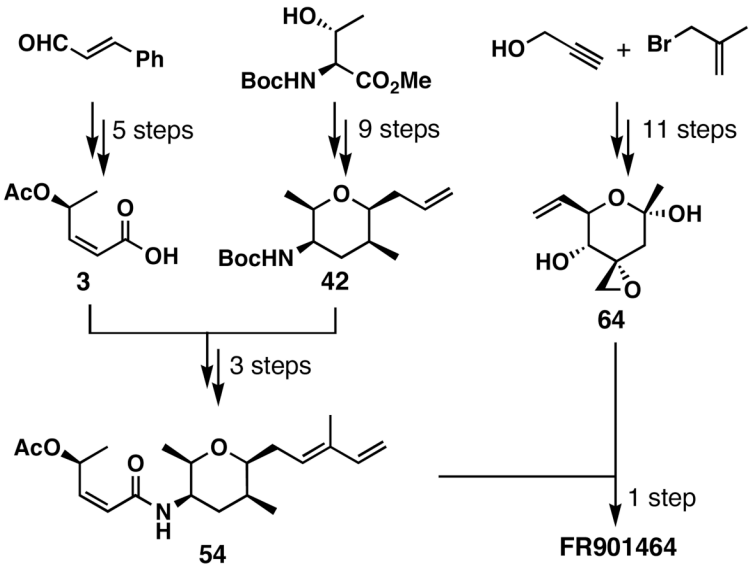
Preparation of **64** [a]

[a] Conditions: (a) 2-$O_2$NPhSeCN (1.2 equiv), $^n$Bu$_3$P (1.4 equiv), THF, 0 °C; then $H_2O_2$ (10 equiv), DMAP (4.0 equiv), -44→23 °C, 91% (dr = 7:1); (b) $OsO_4$ (0.02 equiv), $NaIO_4$ (2.5 equiv), 2,6-lutidine (1.6 equiv), dioxane/$H_2O$ (3:1), 0→23 °C, 27%; (c) TESCl (1.4 equiv), imidazole (1.6 equiv), THF, 0 °C, 95%; (d) $OsO_4$ (0.01 equiv), NMO (0.96 equiv), THF/$H_2O$ (10:1), 0 to 23 °C; then $Pb(OAc)_4$ (1.2 equiv), benzene, 0→23 °C, 71% (86% based on recovered **82**); (e) AcOH/$H_2O$/THF (3:1:3), 0→23 °C, 91%.

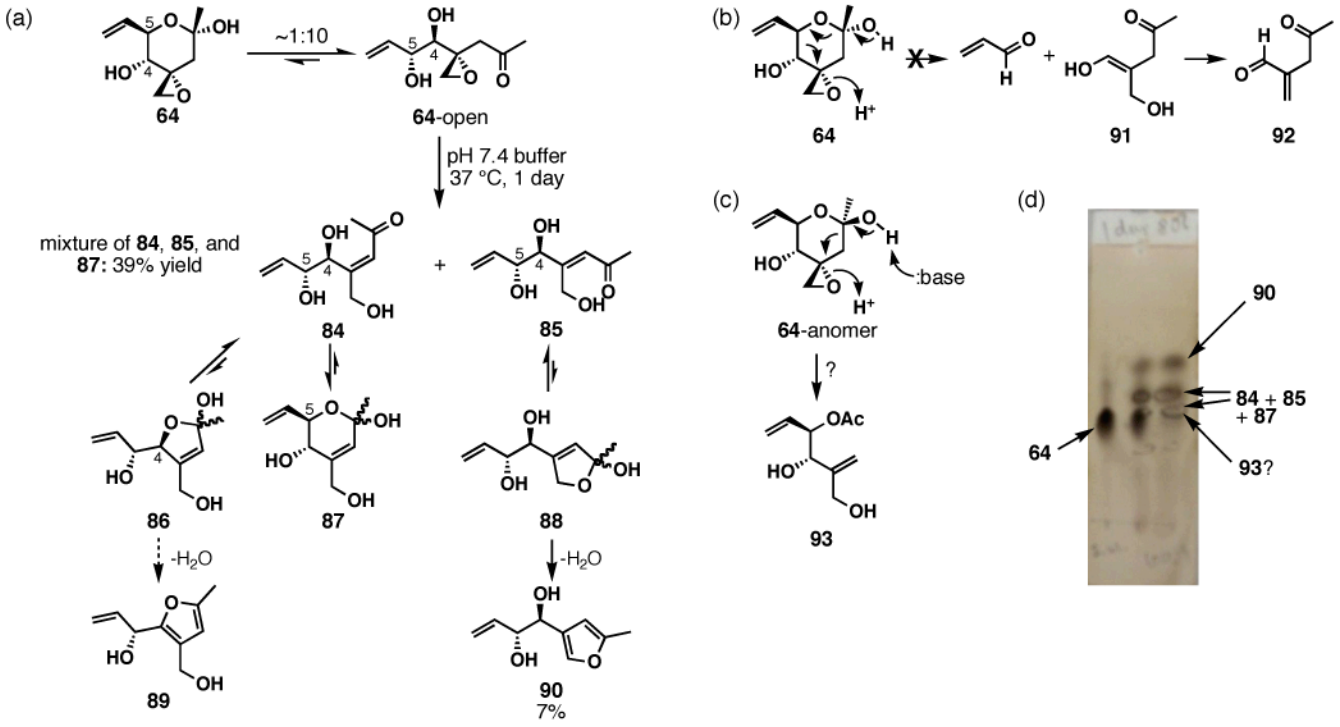



**Scheme 18.**

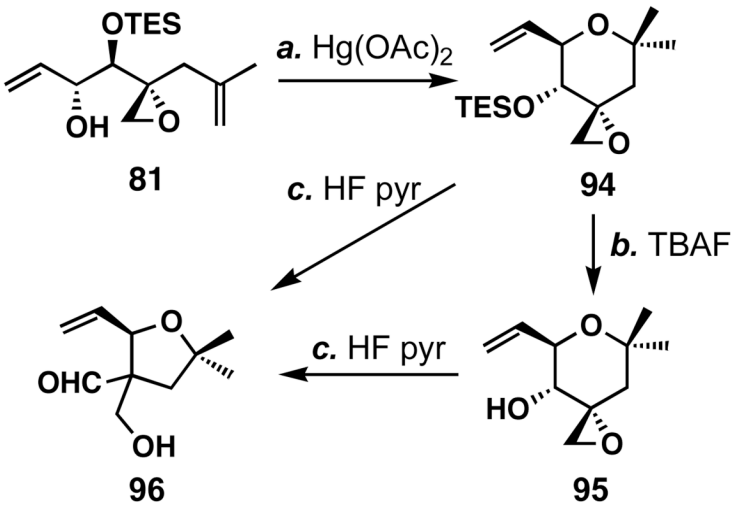
Final stage of the total synthesis of FR901464 [a]




**Scheme 19.**
Summary of the total synthesis of FR901464



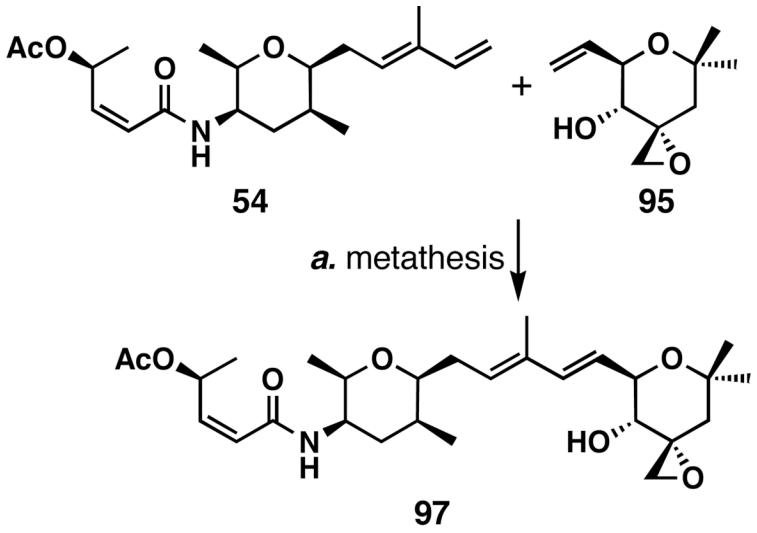

**Scheme 20.**
Decomposition of **64**




**Scheme 21.**

Preparation of the right fragment analog **95** [a]

[a] Conditions: (a) $Hg(OAc)_2$ (1.1 equiv), THF, 0→23 °C; then $NaBH_4$ (2.0 equiv), $Et_3B$ (1.0 equiv), -78→-44 °C, 76%; (b) TBAF (1.2 equiv), THF, 0 °C, 97%; (c) HF•pyr, THF, 0 °C, 73 %.




**Scheme 22.**

Cross metathesis of **54** and **95** [a]

[a] Conditions: (a) **54** (1.0 equiv), **95** (1.5 equiv), **Ru-3** (0.10 equiv), $ClCH_2CH_2Cl$, 40 °C, 59% after 1 recycling of recovered **54** and **95**.

**Table 1**

Step e in Scheme 2 Optimization for the preparation of allylic alcohols **5** and 4-*epi*-**5**

| entry | Conditions | solvents | isolated yield (%)[a] | 5:4-*epi*-5[b] |
|---|---|---|---|---|
| 1 | **16** | THF | 51 | 1.0:1 |
| 2 | **16**:$ZnCl_2$ (2:1) | THF | 71 | 0.3:1 |
| 3 | **16**:$ZnCl_2$ (1:1) | THF | 42 | 1.2:1 |
| 4 | **16**:$ZnCl_2$ (1:2) | THF | 42 | 0.8:1 |
| 5 | **16**:$ZnCl_2$ (1:1) | THF:hexane (1:1) | 55 | 2.0:1 |
| 6 | **17** + $^{n}$BuLi | THF | 46 | 1.2:1 |
| 7 [a,c] | **18** | THF | 32 | 1.7:1 |

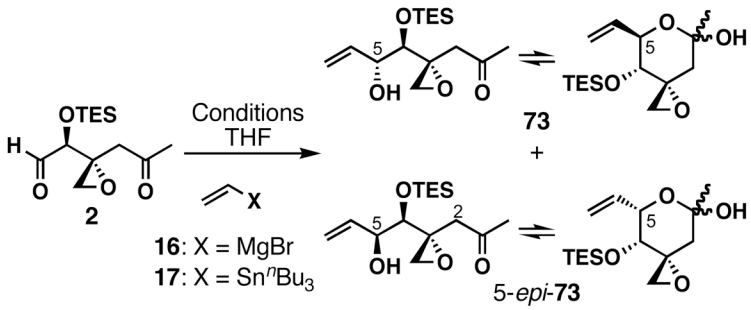
[a] Combined yield.

[b] Determined by $^{1}$H NMR analysis

[c] Result after partial hydrogenation with $H_2$ and Lindlar's catalyst

**Table 2**

Optimization for the preparation of right fragment **73** [a]




| entry | reagent (2 equiv) | additives (equiv) | % yield[a] | 73:5-*epi*-73 [b] |
|---|---|---|---|---|
| 1 | **16** | --- | 40 | 1.0:1 |
| 2 | **16** | HMPA (2.5) | 46 | 1.3:1 |
| 3 | **16** | TMEDA (2.5) | 31 | 2.0:1 |
| 4 | **17** | $^n$BuLi (1.9) | 33 | 1.0:1 |
| 5 | **17** | $^n$BuLi (1.9), HMPA (2.5) | 16 | 2.0:1 |

[a] Combined yield

[a] Determined by $^1$H NMR analysis

**Table 3**

Step c in Scheme 16 Optimization of the [2,3]-sigmatropic rearrangements of **79** and **80**

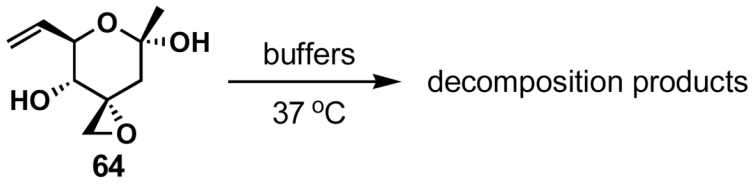
| entry | R | base (equiv) | solvent | temp. (°C) | % yield[a] | 81:5-*epi*-81 [b] |
|---|---|---|---|---|---|---|
| 1 | $NO_2$ | pyridine (5) | THF | -20 | 94 | 4.0:1 |
| 2 | $NO_2$ | pyridine (5) | EtOH | -44→-20 | 87 | 4.0:1 |
| 3 | $NO_2$ | pyridine (5) | acetone | -20 | 96 | 3.5:1 |
| 4 | $NO_2$ | pyridine (5) | $CH_2Cl_2$ | 0→20 | 67 | 3.5:1 |
| 5 | $NO_2$ | 2,6-lutidine (5) | THF | -20→0 | 73 | 4.0:1 |
| 6 | $NO_2$ | DMAP (5) | THF | -44→23 | 95 | 7.5:1 |
| 7 | $NO_2$ | 4-pyrrolidinopyridine (5) | THF | -44→23 | 84 | 8.1:1 |
| 8 | $NO_2$ | DMAP (3) | THF | -44→23 | 80 | 7.8:1 |
| 9 | $NO_2$ | DMAP (1.5) | THF | -44→23 | 87 | 5.3:1 |
| 10 | $NO_2$ | DMAP (0.5) | THF | -44→23 | 34 | 3.3:1 |
| 11 | H | DMAP (5) | THF | -44→23 | 91 | 7.0:1 |

[a] combined yield

[b] determined by $^1$H NMR analysis

**Table 4**

Instability of **64** in buffers

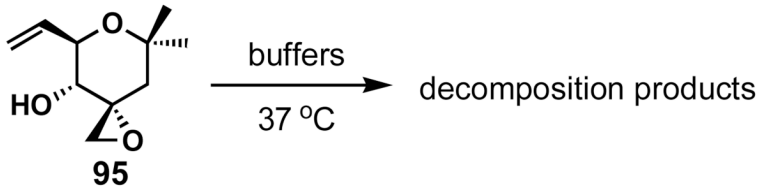



**64**

| pH | $t_{1/2}$ (h) |
|---|---|
| 6 | 12 |
| 7 | 8 |
| 7.4 | 4 |

**Table 5**

Decomposition of **95**




| pH | $t_{1/2}$ (h) |
|---|---|
| 0.1 N $H_2SO_4$ | 3.5 |
| 3 | 24 |
| 4 | 40 |
| 5 | 48 |
| 6 | 40 |
| 7 | 48 |
| 7.4 | 48 |