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

Brian J. Albert, Ananthapadmanabhan Sivaramakrishnan, Tadaatsu Naka, Nancy L. Czaicki, and Kazunori Koide*

Contribution from the Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, Pennsylvania 15260

Received November 3, 2006; E-mail: koide@pitt.edu

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 rationally designed and synthesized. This analogue was 12 times more stable than the right fragment of the natural product. Using this more stable right fragment analogue, an FR901464 analogue, meayamycin, was 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.

Introduction

Controlled cellular processes such as signal transduction and gene expression are essential for homeostasis. When these processes become irregular, tumors may 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,[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. 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 analogues have the potential for clinical application.[13]

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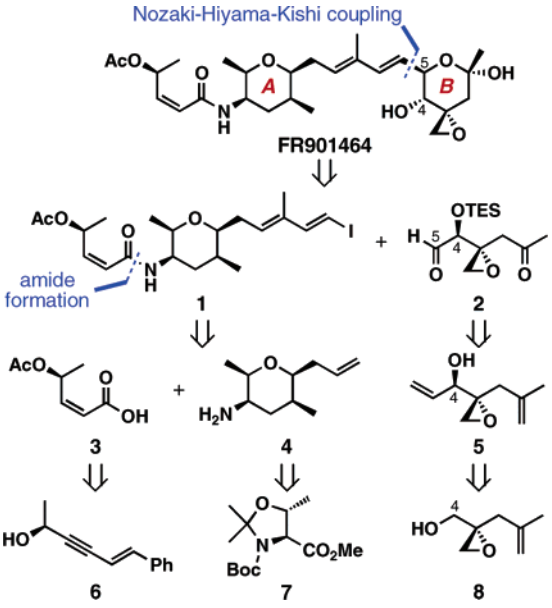
FR901464 induced both G_1 and G_2/M phase arrest in MCF-7 cells.[13,14] In contrast, adriamycin and camptothecin, both DNA synthesis inhibitors, induced S-phase arrest in the cell cycle, and Taxol, a microtubule modulator, induced G_2/M-phase arrest.[13] Since FR901464 was originally discovered as an 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 (aka. GEX1)[24−27] and the new natural product TMC-205[28] exhibited closely related biological activities as FR901464, although these compounds were less potent.

The unique profile of FR901464 has intrigued many synthetic chemists,[29] which culminated in 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 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 22 steps in the longest linear sequence and 41 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 analogues of FR901464 for biological studies. Moreover, 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 analogue via earlier and potentially risky installation of the spiroepoxide and ultimate convergency, namely the cross-coupling at the very end of the synthesis.[36]

Scheme 1. First Generation Retrosynthetic Analysis of FR901464



Results and Discussion

First Generation Synthetic Studies. Scheme 1 shows our first generation retrosynthetic analysis of FR901464. We envisioned a Nozaki−Hiyama−Kishi (NHK) 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 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 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

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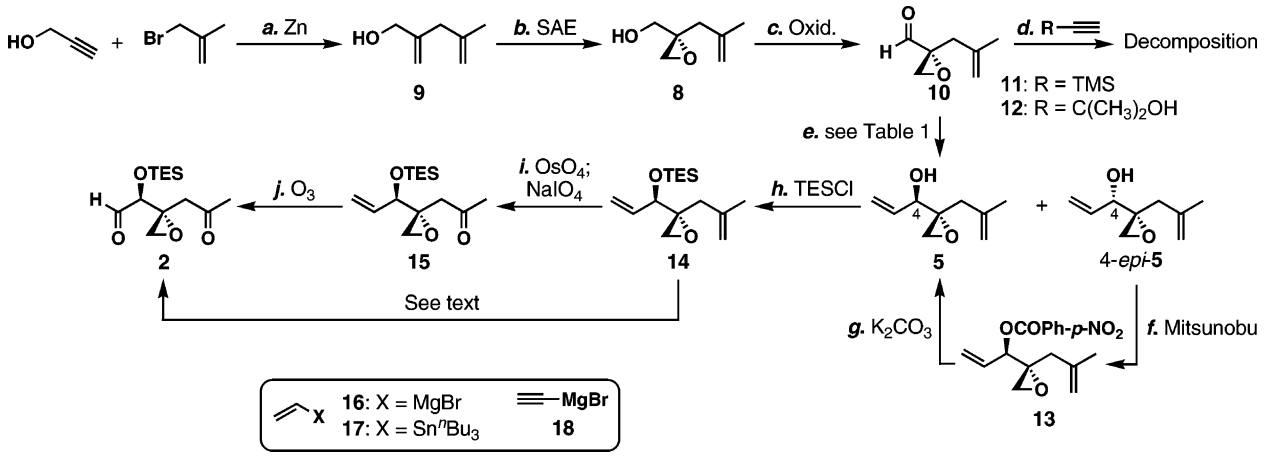
Scheme 2. Preparation of Ketoaldehyde **2**[a]



[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 1; (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%.

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

oxidation[41] of this alcohol in 81% yield. All of these steps could be executed in large scales (>20 g) without compromising yields.

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 above failure, we turned to substrate control (step e; Table 1). Vinylation of aldehyde **10** using **16** 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 $ZnCl_2$ with various stoichiometry did not improve the selectivity for the desired alcohol **5** (entries 2−4). Addition of hexanes finally improved the stereoselectivity for the desired alcohol (2:1) in a 55% combined yield (entry 5). Treatment of **10** with vinyllithium, prepared in situ from **17** (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 **18** (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 attempts, high levels of stereocontrol in this transformation remained elusive.

At this point, since our priority was to examine the validity of the final coupling, we proceeded to prepare ketoaldehyde **2** with the result shown in entry 5, Table 1. 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 alcohol **5** in 75% yield for the two steps. The resulting hydroxy group was protected as its TES ether, **14**, in quantitative yield. This compound was subjected to oxidative cleavage conditions (OsO_4, NMO; then $NaIO_4$) to afford enone **15** in 58% yield. Subsequent ozonolysis produced the *unstable* ketoaldehyde **2** in 54−82% yield. It should be mentioned that the ozonolysis of diene **14** to ketoaldehyde **2** was low yielding (<10%). Although this synthetic route allowed us to prepare **2** in a concise manner, we soon realized that chromatographic separation of alcohols **5** and 4-*epi*-**5** in a large scale was 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 a 50% combined yield but with no diastereoselectivity (dr = 1:1). In contrast, 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! The partial hydrogenations of this alcohol and its TES ether **20** using Lindlar's catalyst failed,

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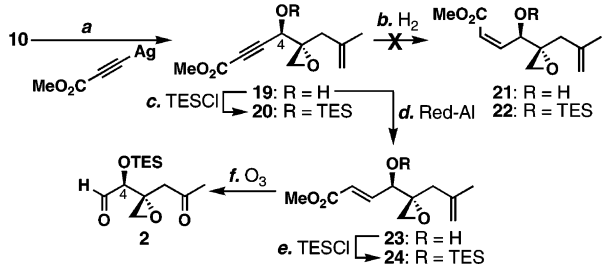
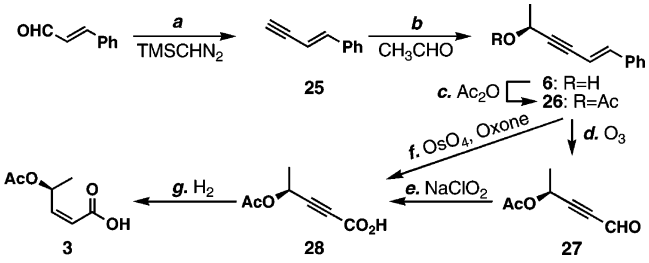
Scheme 3. Formation of C4 Stereocenter with Higher Stereocontrol[a]



[a] Conditions: (a) Ag−C≡C−CO_2Me (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) TRSCl (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]

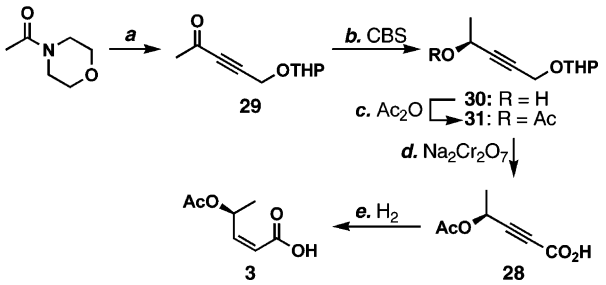


[a] Conditions: (a) cinnamaldehyde (1.1 equiv), $TMSCHN_2$ (1.0 equiv), LDA (1.0 equiv), THF, −78→0 °C, 84%; (b) CH_3CHO (2.3 equiv), $Zn(OTf)_2$ (1.0 equiv), (−)-*N*-methylephedrine (1.0 equiv), Et_3N (1.0 equiv), toluene, 23 °C, 41% (86:14 er); (c) Ac_2O (5.0 equiv), pyridine, 23 °C, quant.; (d) O_3, 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), ^{t}BuOH/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).

but the reduction with Red-Al produced **23** in 81% yield chemo- and stereoselectively.[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 seven 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. 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] 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 two steps and in six total steps from cinnamaldehyde. Later, we converted enyne **26** to acid **28** in one step via OsO_4 mediated oxidative cleavage, and subsequent partial reduction afforded **3** in 60% yield for the two steps. Therefore, acid **3** can now be prepared in five steps from cinnamaldehyde.

Scheme 5. Alternative Methods to Prepare Acid **3**[a]



[a] Conditions: (a) HC≡CCH_2OTHP (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_2O (4.6 equiv), pyridine (excess), 23 °C, 97% (two steps); (d) $Na_2Cr_2O_7$ (3.5 equiv), H_2SO_4, H_2O, acetone, 0→23 °C, 74%; (e) see Scheme 4.

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 two 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 five 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 efforts toward this intermediate is shown in Scheme 6. Treatment of the commercially available L-threonine derivative **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**.

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.

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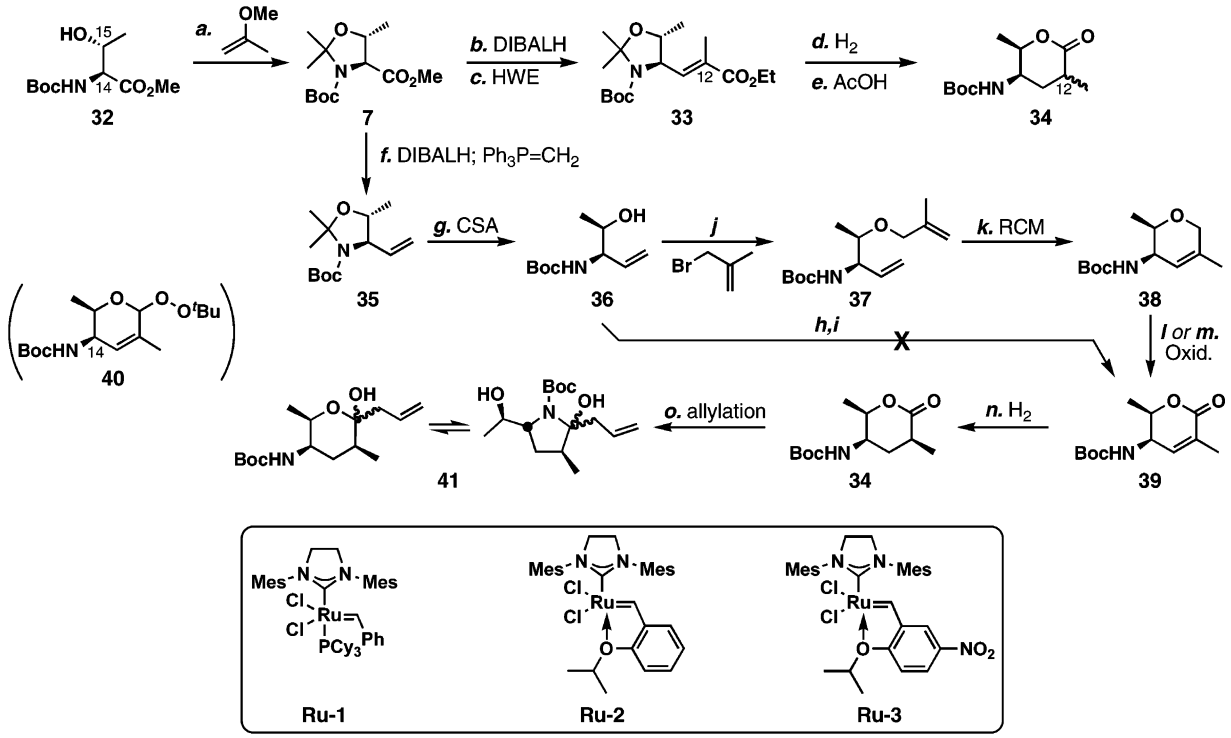
Scheme 6. Synthetic Approach A-Ring Formation[a]

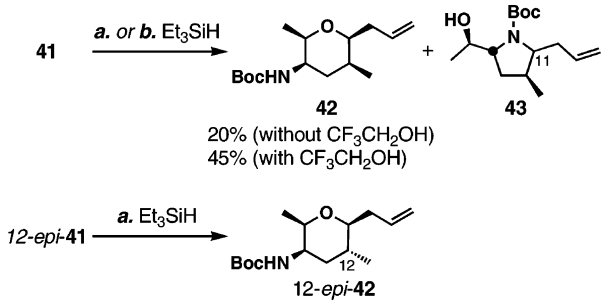


[a] Conditions: (a) 2-methoxypropene (2.0 equiv), CSA (1 mol %), CH_2Cl_2, 0 °C, quant.; (b) DIBALH (2.0 equiv), CH_2Cl_2, −78 °C; (c) $(EtO)_2P(O)CH(CH_3)CO_2Et$ (3.3 equiv), NaH (3.0 equiv), EtOH, 23 °C, 84% (two steps); (d) H_2 (1 atm), PtO_2 (1 mol %), EtOH, 23 °C, quant. (dr = 2:1); (e) AcOH, 58%; (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), ^{t}BuOOH (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%.

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 %. 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 ($Et_3SiH-BF_3{\cdot}OEt_2$) 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

Scheme 7. Preparation of **42**[a]



[a] Conditions: (a) Et_3SiH (4.0 equiv), $BF_3{\cdot}OEt_2$ (4.0 equiv), CH_2Cl_2, −78 °C, **42** (dr = 10:1); (b) Et_3SiH (10 equiv), $BF_3{\cdot}OEt_2$ (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).

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 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 $Et_3SiH-BF_3{\cdot}OEt_2$ afforded **50** in 44% yield

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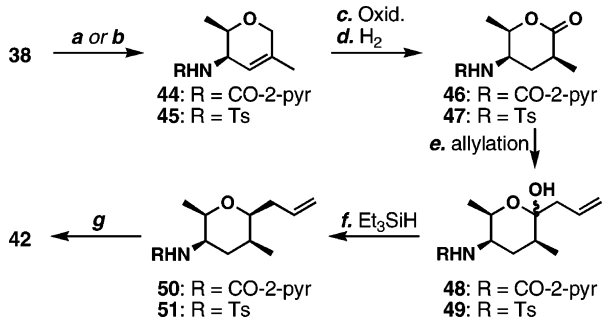
Scheme 8. Preparation of **41** Using Different Amine Protecting Groups[a]



[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) 6 N 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**, two steps).

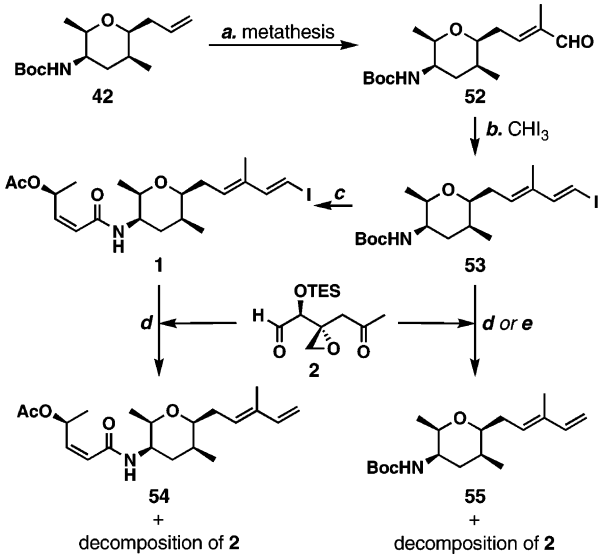
(61% BORSM; dr = 8:1). Disappointingly, we failed to find deprotection conditions to give **50** after vigorous screening of reaction conditions including the literature procedure.[59] Therefore, we turned our attention to other protecting groups.

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 ($Et_3SiH-BF_3$·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**.

As shown in Scheme 9, compound **42** was converted to unsaturated aldehyde **52** using cross-olefin metathesis conditions employing catalytic **Ru-1**. 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 steps in the longest linear sequence.

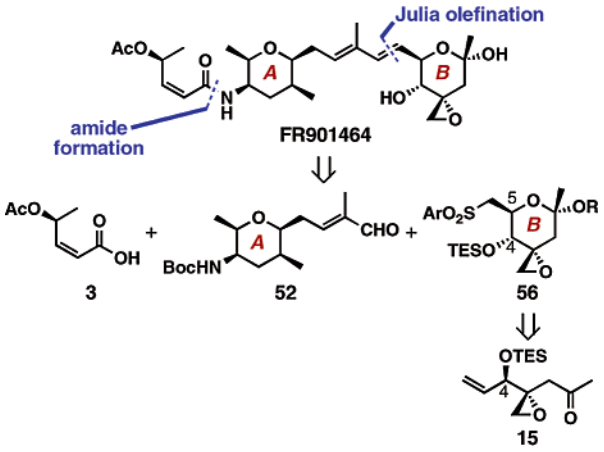
With both coupling partners in hand, the stage was set to test the NHK coupling. To our disappointment, treatment of vinyl iodide **1** and ketoaldehyde **2** under NHK conditions[37,38] caused proto-deiodination of **1** and decomposition of the ketoaldehyde. Similarly, treatment of vinyl iodide **53** and ketoaldehyde **2** to NHK 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.

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



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 toward the B-ring fragment **56**. Typically, hemiketals are formed by the intramo-

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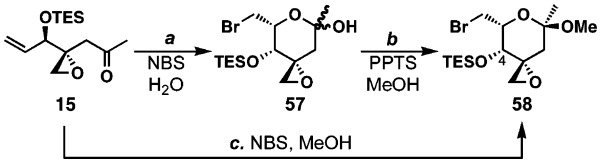
Scheme 11. Preparation of Methyl Glycoside **58**[a]



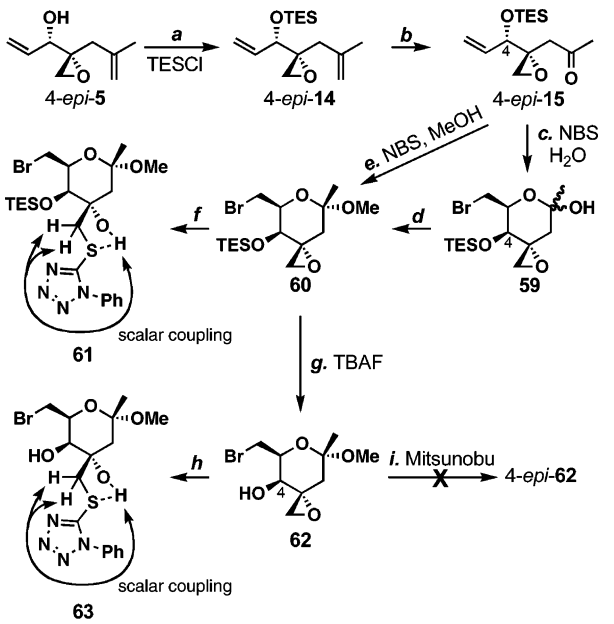
[a] Conditions: (a) NBS (1.1 equiv), THF, H_2O, 0→23 °C, 75%; (b) PPTS (10 mol %), MeOH, CH_2Cl_2, 23 °C, 40%; (c) NBS (1.5 equiv), 3 Å MS, MeOH, MeCN, 0→23 °C, 59%.

lecular attack of a hydroxy group onto a ketone, thus the hydroxy group is the origin of the ring oxygen atom. Here 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 NBS 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.

We thought that using 4-*epi*-**15** for the cyclization could reverse the observed stereocontrol for C5 (Scheme 12). Toward this end, 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. 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 needed to be 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 because of steric reasons. Due to the difficulty in inverting the C4-hyroxy group and sensitivity of the epoxide toward 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 the ^1H NMR

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), 3 Å 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.

experiment in $CDCl_3$, which supports the notion of a "strong" hydrogen bond.[66,67]

Third Generation Synthetic Studies. At this point, we had no choice but to employ a bolder approach to unite 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 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** rather than **66**. 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

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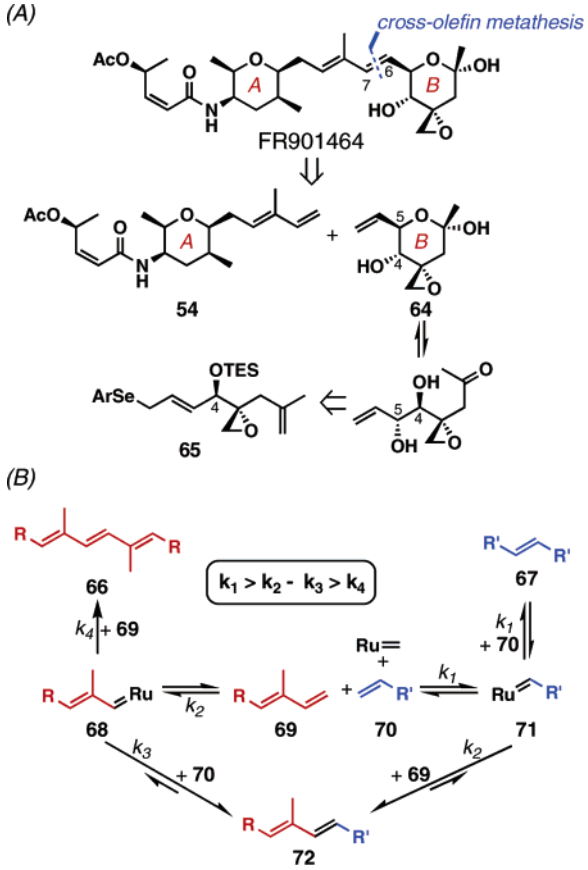
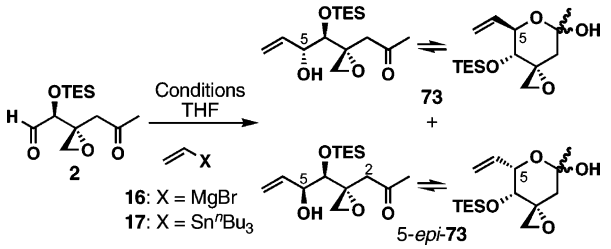
Scheme 13. Diene-ene Cross Metathesis Coupling Strategy



(Table 2). Treatment of the fragile ketoaldehyde **2** with **16** 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 **17** 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%) (entry 5). Neither switching the solvent to CH_2Cl_2 or toluene nor making the organocerium reagent from $CeCl_3$ and **16**[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 an 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 mixture. 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

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

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 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

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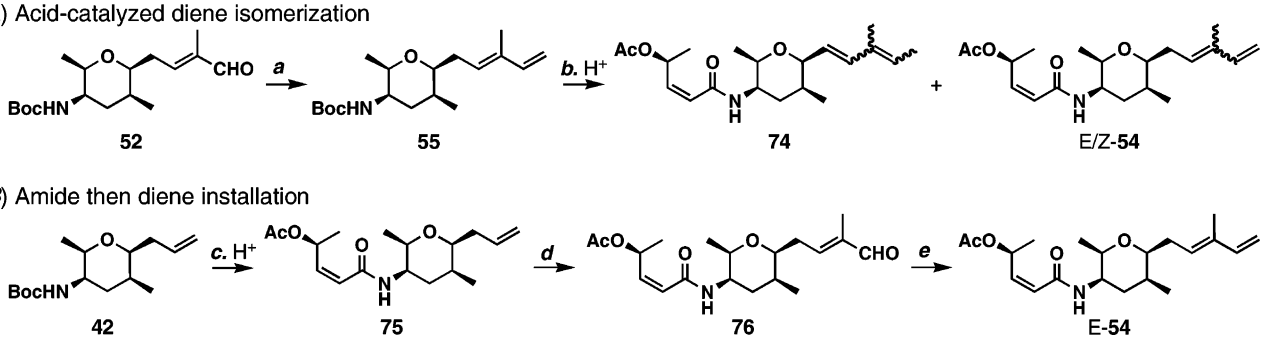
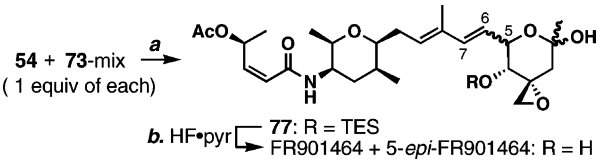
(72) Wirth, T. *Angew. Chem., Int. Ed.* **2000**, *39*, 4189−4189.

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.

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-pyrrolidino-pyridine (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 ^1H NMR analysis.

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). 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 equiv eroded selectivity presumably due to background hydrolysis reactions of the putative selenate. Finally, the nitro group on the aromatic ring was unnecessary for the high diastereoselectivity (entry 11).

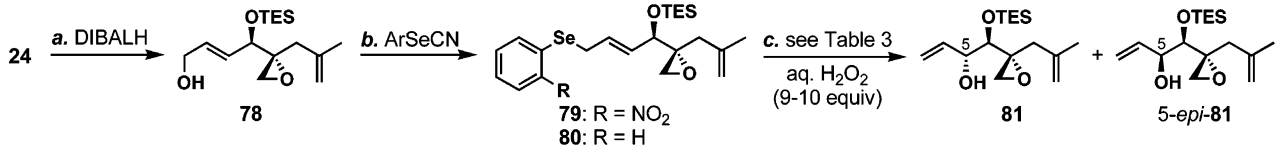
With the stereoselective [2,3]-sigmatropic rearrangement in hand, we proceeded to prepare right fragment **64** (Scheme 17). Primary alcohol **78** was converted to the desired secondary alcohol **81** this time in a one-pot procedure utilizing the selenide formation, [2,3]-rearrangement strategy in 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** 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 $J_{H4-H5} = 9.8$ Hz, similar to that found in the natural product ($J = 10$ Hz).[12] We now stereoselectively and efficiently formed **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

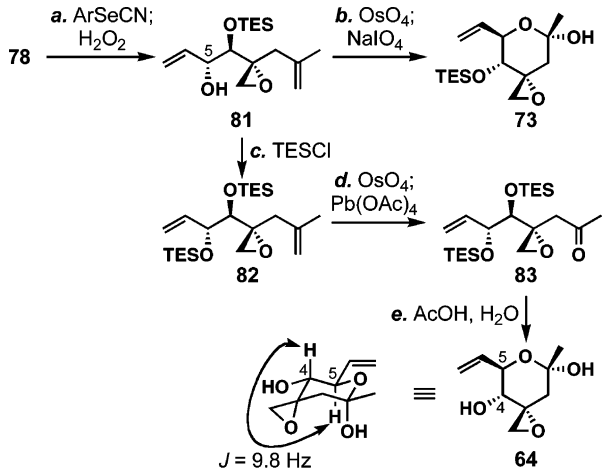
(73) Hassner, A.; Krepski, L. R.; Alexanian, V. *Tetrahedron* **1978**, *34*, 2069−2076.

Scheme 16. Preparation of Alcohols **81** and 5-*epi*-**81**[a]



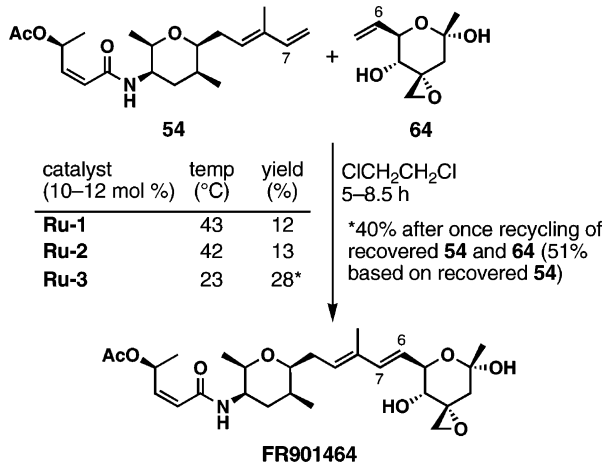
[a] Conditions: (a) DIBALH (3.0 equiv), THF, −78 °C, 95%; (b) ArSeCN (1.2 equiv), ^{n}Bu$_3$P (1.4 equiv), THF, 0 °C, quant.

Scheme 17. Preparation of **64**[a]



[a] Conditions: (a) 2-O_2NPhSeCN (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



Carreira alkynylation, formation of an unsaturated lactone by a ring-closing metathesis−regioselective allylic oxidation sequence, a mild, diastereoselective alkynylation reaction, and an E-selective Red-Al reduction. This total synthesis is the most concise to date, allowing for facile analogue 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** 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 h at pH 7 and 7.4, respectively. 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 ^{1}H 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** was formed 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.

Scheme 19. Summary of the Total Synthesis of FR901464

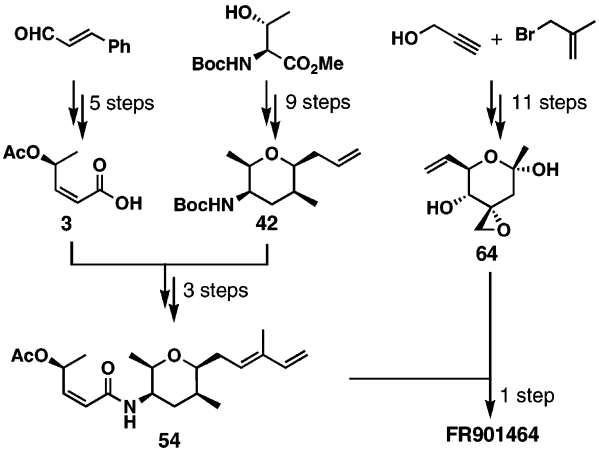
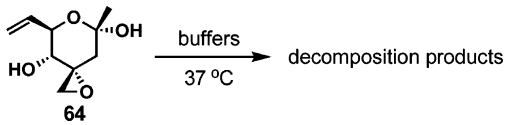


Table 4. Instability of **64** in Buffers

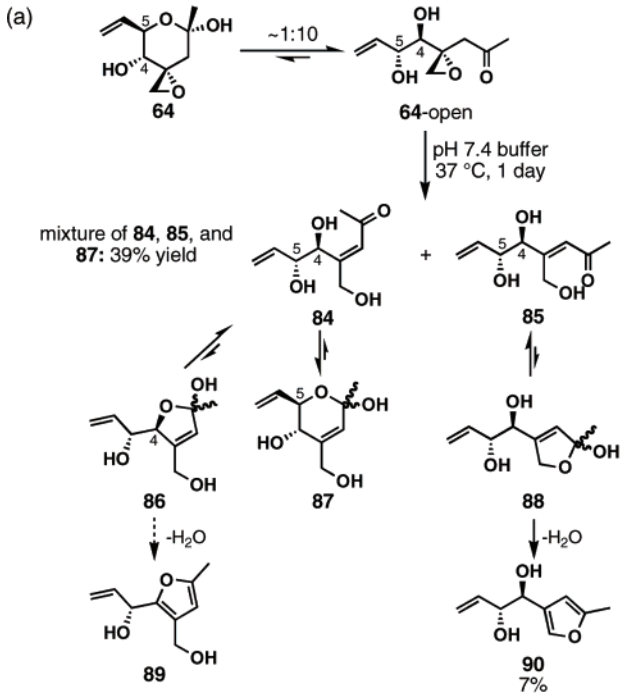


pH	$t_{1/2}$ (h)
6	12
7	8
7.4	4

Tumor Specificity with pH Sensitivity. It is intriguing to contemplate on why nature produces FR901464 with such subtle

(74) Motoyoshi, H.; Horigome, M.; Ishigami, K.; Yoshida, T.; Horinouchi, S.; Yoshida, M.; Watanabe, H.; Kitahara, T. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2178−2182.
(75) Grob, C. A. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 535−546.
(76) Grob, C. A.; Schiess, P. W. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 1−15.

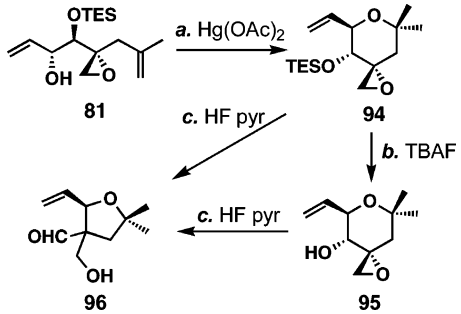
Scheme 20. Decomposition of **64**



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. 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 an 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 analogue. 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. Treatment of alcohol **81** with $Hg(OAc)_2$ followed by $NaBH_4$ and Et_3B[79] afforded 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**. Treatment of **95** with HF·pyridine also caused this rearrangement to occur, indicating that the TES group is not essential for the rearrangement.

Scheme 21. Preparation of the Right Fragment Analogue **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%.

The stability of **95** was then tested in various phosphate buffers at 37 °C (Table 5). The $t_{1/2}$ of **95** at pH 7 and 7.4 was now 48 h, which is a dramatic increase from **64** ($t_{1/2}$ = 4−8 h). Additionally, **95** did not appear to be significantly labile until 0.1 N 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 Analogue. Encouraged by the chemical stability of **95**, we proceeded to synthesize the more stable FR901464 analogue 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

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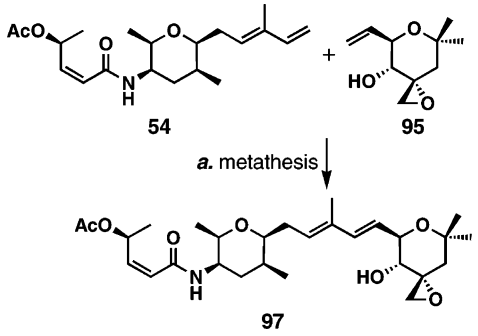
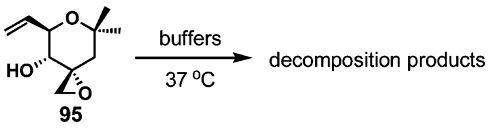
(78) Simon, S. M.; Schindler, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3497−3504.
(79) Kang, S. H.; Lee, J. H.; Lee, S. B. *Tetrahedron Lett.* **1998**, *39*, 59−62.
(80) Buttke, T. M.; Mccubrey, J. A.; Owen, T. C. *J. Immunol. Methods* **1993**, *157*, 233−240.
(81) Pommier, Y.; Vogt, A.; McPherson, P.; Koide, K. Unpublished results.

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 one recycling of recovered **54** and **95**.

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

97 in 28 total steps with the longest linear sequence consisting of 13 steps. This synthetic scheme enabled us to produce 30 mg of this analogue and should be scalable.

Biological Assay. We tested synthetic FR901464 and its analogue **97** against MCF-7 breast cancer cells and measured the cell viability using the MTS assay after 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 1.8 nM.[11] The potency of meayamycin was better and exceeded our expectation: the GI_{50} value of this analogue 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 analogue. Enabled by our total synthesis, we completed the synthesis of an FR901464 analogue (meayamycin) using the more stable right fragment in a total of 28 steps. The GI_{50} value of this analogue 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 analogue on a larger scale. The potency and stability of this analogue should facilitate our efforts to isolate cellular targets of FR901464. We are also studying how the stability of meayamycin would affect the tumor specificity.

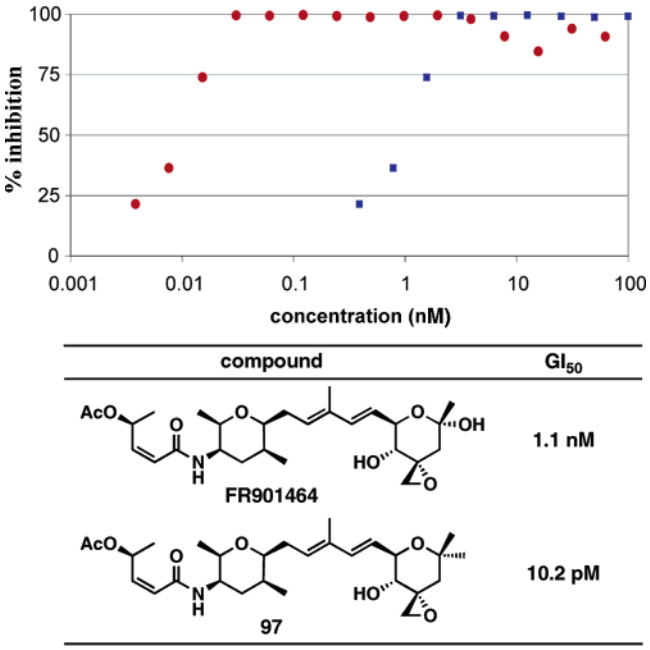


Figure 1. Growth inhibition of MCF-7 cells by FR901464 (blue squares) and meayamycin (**97**; red circles).

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Supporting Information Available: Experimental procedures and spectroscopic data for all the new compounds and FR901464. This material is available free of charge via the Internet at http://pubs.acs.org.

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