

# Structural Requirements for the Antiproliferative Activity of Pre-mRNA Splicing Inhibitor FR901464

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**Abstract:** FR901464, a natural product isolated from a bacterium source, activates a reporter gene, inhibits pre-mRNA splicing, and shows antitumor activity. We previously reported the development of a more potent analogue, meayamycin, through the total synthesis of FR901464. Herein, we report detailed structure–activity relationships of FR901464 that revealed the significance of the epoxide, carbon atoms in

the tetrahydropyran ring, the *Z* geometry of the side chain, the 1,3-diene moiety, the C4-hydroxy group, and the C2'-carbonyl group. Importantly, the methyl group of the acetyl substituent was found to be inessential, leading to

**Keywords:** cancer • FR901464 • meayamycin • natural products • structure–activity relationships

a new potent analogue. Additionally, partially based on in vivo data, we synthesized and evaluated potentially more metabolically stable analogues for their antiproliferative activity. These structural insights into FR901464 may contribute to the simplification of the natural product for further drug development.

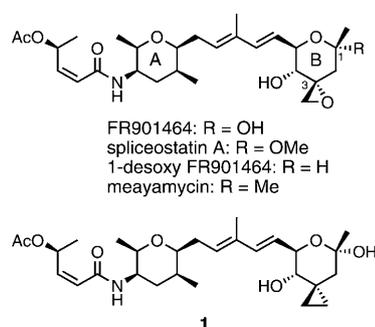
## Introduction

### Development of natural product-based anticancer agents:

Development of small molecule ligands for specific protein targets is a contemporary approach in drug discovery processes and chemical biology (reverse chemical genetics). The success of imatinib (Gleevec) exemplifies this approach,<sup>[1]</sup> but the generality of this rational approach remains to be seen,<sup>[2]</sup> as small molecules often bind to off-targets. Historically, especially in cancer medicine, major breakthroughs occurred through the target identifications of previously known anticancer natural products (forward chemical genetics). These targets were usually not obvious prior to these studies. For example, paclitaxel was first identified as an anticancer compound;<sup>[3]</sup> however, until Horwitz reported that this natural product bound to microtubule, this protein was presumably not viewed as a viable target.<sup>[4]</sup> Similarly, although lactacystin was discovered earlier,<sup>[5]</sup> the

proteasome was not widely accepted as a viable target to fight cancer until the target of lactacystin was found to be a proteasome unit.<sup>[5]</sup> Consequently, Bortezomib has been approved by the Food and Drug Administration (FDA) as the first proteasome inhibitor for cancer treatment.<sup>[6]</sup>

FR901464 (Scheme 1) may contribute to cancer therapy in a similar fashion. The isolation and antitumor activity of FR901464 was reported in 1996 by Nakajima and coworkers.<sup>[7]</sup> Although its biological profile was unique and indicative of a new mode of action, the molecular mechanisms of this antitumor agent were not known for a long time. In 2007, the Yoshida–Kitahara group reported that FR901464 inhibited pre-mRNA splicing and that its target was splicing



Scheme 1. Structures of FR901464 and previously prepared analogues.

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factor 3b (SF3b).<sup>[8]</sup> SF3b, consisting of seven proteins,<sup>[9]</sup> is required for pre-mRNA splicing and is part of the spliceosome, a complex of approximately 200 proteins and small nuclear RNAs (snRNAs) that catalyzes RNA splicing.<sup>[10]</sup> Although the specific molecular target of FR901464 has not yet been determined, it is likely that it is either spliceosomal associated protein (SAP)135 or SAP155.<sup>[8]</sup> The same research group also showed that the splicing inhibition of *p27* by FR901464 was linked to cell cycle arrest.<sup>[8]</sup> These studies indicate that the anticancer activity of FR901464 is directly linked to pre-mRNA splicing inhibition. This is potentially a breakthrough because splicing processes have never been exploited as therapeutic targets or biomarkers in cancer medicine. Moreover, post-transcriptional RNA modifications are an increasingly important theme in biology,<sup>[11]</sup> for which FR901464 or its analogues may be used as a chemical tool. Very recently, the Webb group reported the promising *in vivo* antitumor activity of an FR901464 analogue, which further supports the idea that FR901464 analogues could be antitumor drugs.<sup>[12]</sup>

Not surprisingly, several pharmaceutical companies recently used reporter assays related to those that the Nakajima group employed, and discovered a series of new natural products with biological profiles similar to that of FR901464.<sup>[13,14]</sup> The most notable natural products are the pladienolides,<sup>[14]</sup> a derivative of which is currently in phase I trials as the first drug candidate with splicing inhibitory activity.<sup>[15]</sup>

In addition to the significance of using splicing inhibitors as antitumor agents, there is a great need to develop chemical probes for RNA splicing because the process is not very tractable with currently available biological methods. As the first natural product that inhibits pre-mRNA splicing, FR901464 is now considered a prototype compound for splicing inhibitors. Given the unique mode of action in conjunction with its antitumor activity, we envision that FR901464 or its analogues will be widely used in oncology and RNA biology. Thus, it is important to understand the structure–activity relationships (SAR) of FR901464, which would enable the rational design of more potent analogues that are compatible with *in vivo* experiments.

**Synthetic studies of FR901464:** The Jacobsen group accomplished the first total synthesis of FR901464<sup>[16]</sup> and systematically studied the structure–activity relationship of this natural product.<sup>[17]</sup> The results of their SAR studies are described throughout this article where they are directly related to our studies. The second total synthesis was accomplished by the Kitahara group,<sup>[18]</sup> who later improved their synthetic scheme.<sup>[19]</sup> Our group reported the third total synthesis of FR901464 in 2006,<sup>[20,21]</sup> and later disclosed how the combination of the epoxide at the C3 position and the hydroxy group at the C1 position caused the decomposition of FR901464.<sup>[22]</sup>

**The C1-hydroxy group of FR901464:** Spliceostatin A (Scheme 1), the 1-methoxy analogue of FR901464, prepared

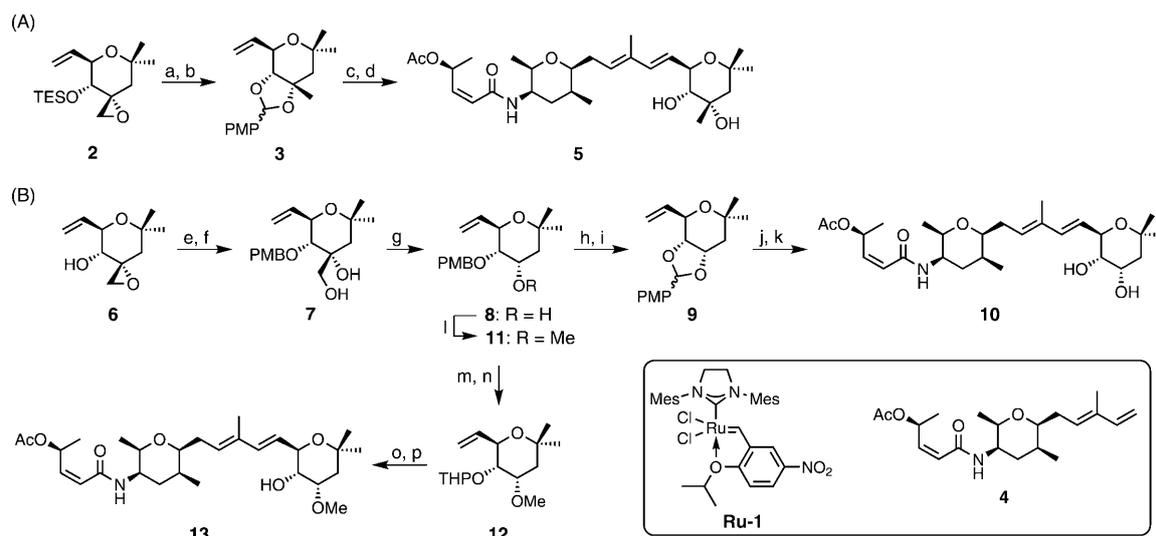
by the Kitahara group, is more active than FR901464 in enhancing gene expression of a reporter gene.<sup>[23]</sup> Unfortunately, the semi-quantitative description of the activity does not allow for complete quantitative assessment. Moreover, the methoxy group at the anomeric center without neighboring electron-withdrawing groups is acid-sensitive,<sup>[24]</sup> which raises the question of whether spliceostatin A is simply an FR901464-prodrug with enhanced cell permeability. Alternatively, the improved activity could be a result of the improved stability of spliceostatin A as compared to FR901464.<sup>[23]</sup> The 1-desoxy FR901464, prepared by the Jacobsen group, is slightly more active against Jurkat cells than FR901464.<sup>[17]</sup> This analogue shows an important feature about FR901464: its active form contains a cyclic B-ring. It is not clear whether the 1-hydroxy group participates in molecular recognition, because the improved stability of 1-desoxy FR901464 and loss of the hydroxy group may compromise each other, resulting in slightly better anticancer activity. We recently substituted the 1-hydroxy group with a methyl group and found that this analogue, meayamycin, was 100 times more potent against human breast cancer MCF-7 cells than FR901464.<sup>[22]</sup> Moreover, it is more potent than 1-desoxy FR901464 and should be more stable than spliceostatin A. Therefore, in this work, all of the analogues contain the geminal dimethyl group at the C1 position.

## Results and Discussion<sup>[25]</sup>

**The epoxide moiety:** The C3-cyclopropyl analogue **1** (Scheme 1) was prepared by the Jacobsen group and showed to be inactive even at a concentration of 4  $\mu\text{M}$  (i.e., more than three orders of magnitude less active than FR901464).<sup>[17]</sup> This result implies that the epoxide may be crucial for the biological activity of FR901464, but it was not clear whether the oxygen atom or the electrophilicity of the epoxide was important. If the electrophilicity is important, such a notion would be contradictory to the Yoshida–Kitahara team’s statement that FR901464 does not form a covalent bond with its target protein.<sup>[8]</sup> In light of this potential discrepancy, we set out to prepare non-epoxide analogues that still contain the oxygen atom at the C3 position (Scheme 2).

Compound **2**<sup>[22]</sup> was reduced with  $\text{LiAlH}_4$  to a diol, which was protected as an acetal to give **3** in 67% yield for the two steps (Scheme 2A). This acetal was subjected to olefin cross metathesis conditions with the previously prepared diene **4** and **Ru-1**<sup>[26]</sup> to give the cross-coupled compound in 41% yield. The acetal of this compound was hydrolyzed under acidic conditions to give analogue **5** in 66% yield.

To arrive at two similar analogues, alcohol **6**<sup>[22]</sup> was used as the starting material. The alcohol was protected as its PMB ether with 74% yield, and its epoxide was subsequently opened with KOH to give diol **7** in 77% yield (Scheme 2B). Diol **7** was cleaved by the action of  $\text{NaIO}_4$ , and the resulting ketone was immediately reduced with L-selectride to give alcohol **8** as a single diastereomer. Alcohol **8**



Scheme 2. Preparation of the C3 analogues **5**, **10**, and **13**. a)  $\text{LiAlH}_4$  (5.0 equiv),  $\text{Et}_2\text{O}$ , reflux, 80%; b) PMPCH(OMe) $_2$  (PMP = *para*-methoxyphenyl, 2.9 equiv), camphorsulfonic acid (CSA, 0.10 equiv),  $\text{CH}_2\text{Cl}_2$ , 23 °C, 84%; c) **3** (1.3 equiv), **4** (1.0 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.22 equiv), 1,2-dichloroethane (DCE), 42 °C, 41%; d)  $\text{AcOH}/\text{THF}/\text{H}_2\text{O}$  (3:1:1), 23 °C, 66%; e) PMBCl (PMB = *para*-methoxybenzyl, 2.9 equiv),  $\text{Ag}_2\text{O}$  (1.5 equiv), DMF, 60 °C, 74%; f) KOH (5.0 equiv),  $\text{DMSO}/\text{H}_2\text{O}$  (1.5:1), 55 °C, 77%; g)  $\text{NaIO}_4$  (1.2 equiv),  $\text{H}_2\text{O}/\text{THF}$  (2:1), 23 °C;  $\text{LiSBu}_3\text{BH}$  (3.1 equiv), THF, -78 °C, 69% (2 steps); h) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 3.0 equiv),  $\text{CH}_2\text{Cl}_2$ /saturated aqueous  $\text{NaHCO}_3$  (9:1), 23 °C; CSA (0.05 equiv), MeOH, 23 °C; 5% KOH/ $\text{H}_2\text{O}$ , 23 °C, 79% (3 steps); i) PMPCH(OMe) $_2$  (2.9 equiv), CSA (0.10 equiv),  $\text{CH}_2\text{Cl}_2$ , 23 °C, quantitative; j) **4** (1.0 equiv), **9** (1.3 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.22 equiv), DCE, 40 °C, 48%; k)  $\text{AcOH}/\text{THF}/\text{H}_2\text{O}$  (3:1:1), 23 °C, 68%; l) MeI (2.0 equiv), NaH (2.0 equiv), THF, 23 °C, 94%; m) DDQ (2.0 equiv),  $\text{CH}_2\text{Cl}_2$ /saturated aqueous  $\text{NaHCO}_3$  (9:1), 23 °C, 89%; n) 3,4-dihydro-2*H*-pyran (1.5 equiv), CSA (0.05 equiv),  $\text{CH}_2\text{Cl}_2$ , 23 °C, quantitative; o) **4** (1.2 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.20 equiv), DCE, 45 °C, 40%; p)  $\text{AcOH}/\text{THF}/\text{H}_2\text{O}$  (3:1:1), 23 °C, 63%.

was treated with DDQ to give a mixture of acetals and esters (see the Supporting Information), which was treated with acidic methanol and subsequently with KOH to give a diol. This diol was converted to acetal **9** in quantitative yield. Acetal **9** was then subjected to diene **4** and **Ru-1** to give a metathesis adduct in 48% yield, which was deprotected under acidic conditions to give **10** in 68% yield.

Alcohol **8** was also methylated by the action of MeI and NaH to give **11** in 94% yield. The PMB ether **11** was exposed to DDQ to give an alcohol in 89% yield, which was then protected as its tetrahydropyran (THP) ether **12** in quantitative yield. Compound **12** was treated with diene **4** and **Ru-1** to give the desired cross-coupled product in 40% yield. Finally, the THP ether of this product was hydrolyzed to give analogue **13** in 63% yield.

None of these non-epoxy analogues (**5**, **10**, and **13**) exhibited antiproliferative activity against MCF-7 cells even at a concentration of 10  $\mu\text{M}$ , meaning that they are at least six orders of magnitude less active than meayamycin (Table 1). These data, together with those reported by the Jacobsen group,<sup>[17]</sup> suggest that the epoxide of FR901464 plays a crucial role as an electrophile and presumably forms a covalent bond. Because non-specific epoxide opening by thiols *in vivo* may not be a major concern,<sup>[27]</sup> the potential covalent bond formation may be specific for the putative target(s).

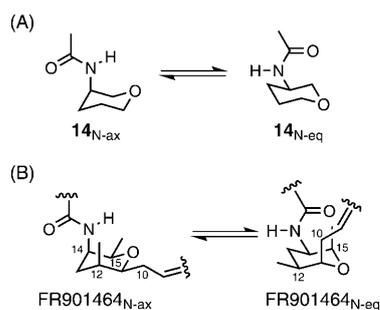
**The 12 position:** Vasella and co-workers studied the conformations of compound **14** in water and found that a signifi-

Table 1. Antiproliferative activities against MCF-7 cells.<sup>[a]</sup>

FR901464 analogue	GI <sub>50</sub> [nM]
meayamycin	0.02
<b>5</b>	> 10000
<b>10</b>	> 10000
<b>13</b>	> 10000
<b>22</b>	0.30
<b>28</b>	2.5
<b>29</b>	85
<b>30</b>	19
meayamycin B	0.008
4'-desacetyl meayamycin	0.48
4'- <i>epi</i> -desacetyl meayamycin	4.7
<b>41</b>	5
<b>48</b>	19
<b>50</b>	0.38
<b>59</b>	15
<b>60</b>	10

[a] Each experiment consists of at least twelve data points (twelve different concentrations).

cant population (1:3) of this compound existed as conformer **14**<sub>N-ax</sub> despite the bulk of the acetamide group (Scheme 3 A).<sup>[28]</sup> The somewhat surprising stability of the axial conformer prompted us to speculate that the more stable chair conformer of the A-ring of FR901464 might be FR901464<sub>N-ax</sub>, provided that the A-ring is indeed in a chair form (Scheme 3 B). This preference can be justified by arguing that in this conformer, if the amide NH group is hydro-



Scheme 3. Chair conformers of A) **14** and B) **FR901464**.

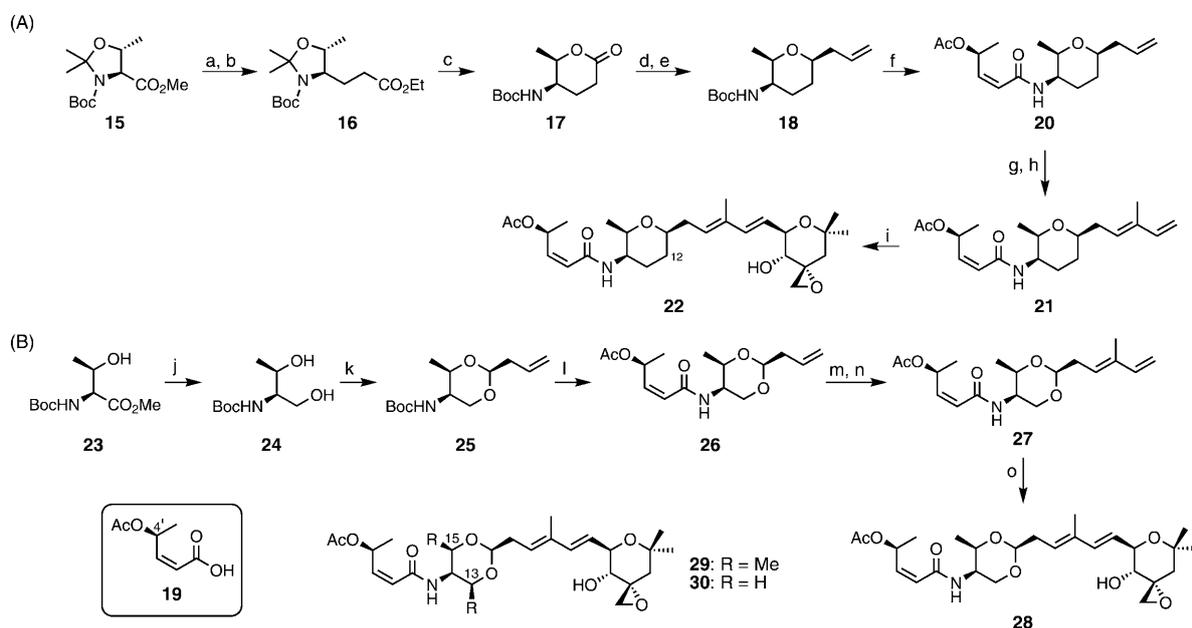
gen bonded to the oxygen atom of the A-ring, only the nitrogen lone pair would point toward the methyl group at the C12 position, minimizing the 1,3-diaxial interaction. On the other hand, in conformer **FR901464<sub>N-eq</sub>**, there is a substantial 1,3-diaxial interaction between the methyl group at the C15 position and the C10-allylic methylene group. Therefore, we hypothesized that **FR901464** might bind to its target(s) when it assumed the chair form **FR901464<sub>N-ax</sub>** in the A-ring.

With this hypothesis, it appeared to us that the 12-methyl group in the axial position destabilized this putative binding conformer. Therefore, removal of this methyl group might indeed increase the population of the bound conformer, thereby enhancing the potency of meayamycin. Moreover, inspired by the work of Wender et al. on a series of bryostatin analogues,<sup>[29]</sup> which was followed by the Smith group on

phorbazole analogues,<sup>[30]</sup> the substitution of the carbon at position 12 with an oxygen atom looked very appealing from a synthetic standpoint.

Our synthetic efforts toward analogue **22** began with the one pot DIBALH reduction–Wittig condensation reaction sequence of the L-threonine derivative **15** (Scheme 4 A).<sup>[31]</sup> The resulting enoate was hydrogenated to form the corresponding saturated compound **16** in 87% yield for the two steps. Treatment of this compound with acetic acid promoted the acetonide cleavage and intramolecular transesterification, giving lactone **17** in 73% yield. The allylation of this lactone followed by the  $\text{BF}_3 \cdot \text{OEt}_2$ – $\text{Et}_3\text{SiH}$ -mediated reduction of the resulting hemiketal provided tetrahydropyran **18** in 43% yield for the two steps. The same three-step sequence as used for our previous synthesis (1. Boc-deprotection–amidation with acid **19**; 2. olefin cross metathesis with methacrolein; 3. Wittig reaction with  $\text{Ph}_3\text{P}=\text{CH}_2$ )<sup>[21]</sup> afforded the conjugated diene **21** in 43% yield over the three steps. This fragment was then coupled with the compound **6** to give the designed analogue **22** in 50% yield. The total number of steps is 25 for this analogue with 11 steps in the longest linear sequence, which compares favorably to the synthesis of meayamycin (28 total steps and 13 steps in the longest linear sequence).

The acetal analogue synthesis was even more concise; treatment of the commercially available compound **23** with  $\text{NaBH}_4$  gave the known diol **24** in 87% yield (Scheme 4 B).<sup>[32]</sup> This diol was treated with 1,2-diethoxy-3-

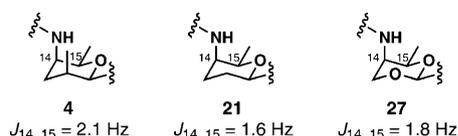


Scheme 4. Preparation of C12 analogues. a) Diisobutylaluminum hydride (DIBALH, 1.8 equiv),  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ ;  $\text{Ph}_3\text{P}=\text{CHCO}_2\text{Me}$  (1.5 equiv),  $45^\circ\text{C}$ , 87%; b)  $\text{H}_2$ , Pd/C (1 mol%), EtOAc,  $23^\circ\text{C}$ , quantitative; c) AcOH,  $80^\circ\text{C}$ , 73%; d)  $\text{CH}_2=\text{CHCH}_2\text{MgCl}$  (1.9 equiv), THF,  $-78^\circ\text{C}$ ; e)  $\text{Et}_3\text{SiH}$  (10 equiv),  $\text{BF}_3 \cdot \text{OEt}_2$  (4.0 equiv),  $\text{CF}_3\text{CH}_2\text{OH}$  (8.0 equiv),  $-78^\circ\text{C}$ , 43% (2 steps); f)  $\text{CH}_2\text{Cl}_2$ /trifluoroacetic acid (TFA, 9:1),  $23^\circ\text{C}$ ; **19** (1.2 equiv), *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU, 1.2 equiv), *iPr}\_2\text{NEt} (4.0 equiv),  $23^\circ\text{C}$ , 84%; g) methacrolein (10 equiv), **Ru-1** (2 mol%),  $23^\circ\text{C}$ , 60%; h)  $\text{Ph}_3\text{PCH}_2\text{Br}$  (2.2 equiv), KOtBu (2.2 equiv), THF,  $0^\circ\text{C}$ , 86%; i) **6** (1.6 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.21 equiv), DCE,  $35^\circ\text{C}$ , 50%; j)  $\text{NaBH}_4$  (2.0 equiv), EtOH,  $23^\circ\text{C}$ , 87%; k) 1,1-diethoxy-3-butene (1.0 equiv), CSA (1 mol%),  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$ , 67%; l)  $\text{CH}_2\text{Cl}_2$ /TFA (9:1),  $23^\circ\text{C}$ ; **19** (1.2 equiv), HATU (1.2 equiv), *iPr}\_2\text{NEt} (4.0 equiv),  $23^\circ\text{C}$ , 51%; m) methacrolein (10 equiv), **Ru-1** (2 mol%),  $23^\circ\text{C}$ , 62%; n)  $\text{Ph}_3\text{PCH}_2\text{Br}$  (2.2 equiv), KOtBu (2.2 equiv), THF,  $0^\circ\text{C}$ , 87%; o) **6** (1.6 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.22 equiv), DCE,  $35^\circ\text{C}$ , 53%.**

butene under acidic conditions to form the cyclic acetal **25** in 67% yield. After the same synthetic procedures described before, we were able to prepare the target analogue **28** in 15% yield for the 4 steps. The total number of steps is 21 for this analogue with only 11 steps in the longest linear sequence.

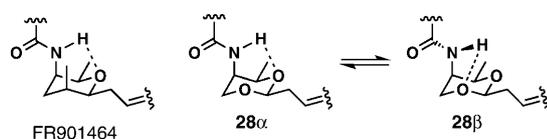
The antiproliferative activity of these two analogues was evaluated against MCF-7 cells (Table 1). The 12-desmethyl meayamycin **22** was less potent than meayamycin, but the  $GI_{50}$  is still in the respectable sub-nanomolar range (0.3 nM). The acetal analogue **28** was far less active, with the  $GI_{50}$  value at 2.5 nM. These data indicate the significance of hydrophobicity at the 12 position. The replacement of the A-ring with a cyclic acetal provided a means to probe the impact of substituents at positions 13 and 15. Thus, through similar synthetic schemes (see the Supporting Information), analogues **29** and **30** were prepared and evaluated. The additional methyl group at the 13 position in analogue **29** was detrimental to the biological activity. The replacement of the 15-methyl group with a hydrogen atom in analogue **30** also had a negative impact with respect to the biological activity. All together, the substituents at positions 12, 13, and 15 are intricately responsible for the biological activity of FR901464. Although these analogues are more synthetically accessible, we tentatively decided to exclude these simplifications because a sufficient quantity of meayamycin could be produced for the purpose of this study by using tissue-cultured cells.

To understand if the loss in activity of these A-ring analogues was due to a conformational change or a loss of hydrophobicity, NMR experiments were undertaken for dienes **4**, **21**, and **27** in  $CD_3OD$ . The  $J_{14,15}$  values were 2.1, 1.6, and



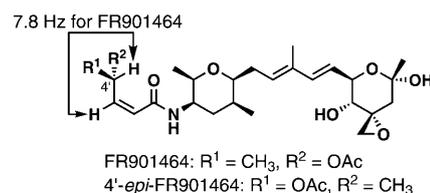
1.8 Hz for **4**, **21**, and **27**, respectively, indicating similar chair-like conformers. Therefore, the loss in activities for 12-desmethyl meayamycin, **22**, and **28** is mainly attributed to the loss of favorable hydrophobic interactions, and, to a lesser degree, a conformational change in the A-ring.

Why is compound **28** less potent than compound **22**? The lower potency of **28** can be attributed to the additional conformer **28 $\beta$** . From  $^1H$  NMR spectroscopic experiments, the secondary NH signal was very prominent in  $CDCl_3$ , which did not diminish upon the addition of  $CD_3OD$ . This indicates that there is strong hydrogen bonding between the NH



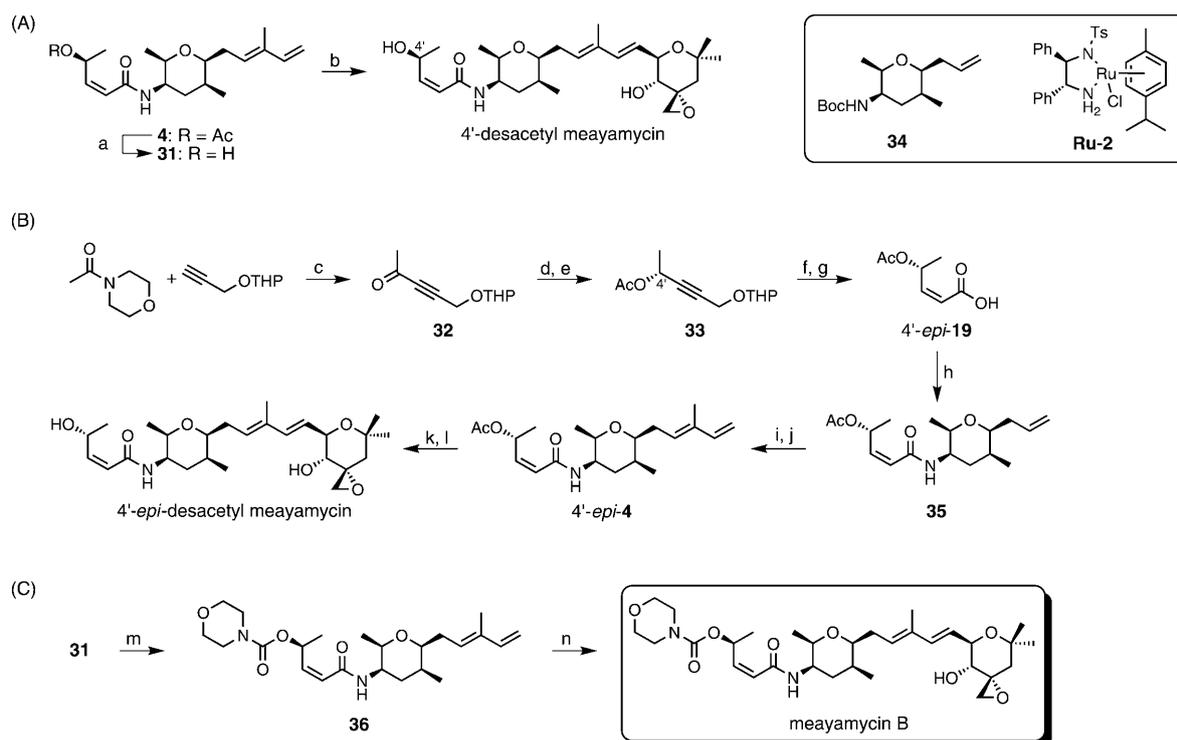
group and the oxygen atom in the A-ring (pyran or acetal). An additional reason for the lower potency of **28** may be attributed to the possibility that the additional oxygen is bound to a water molecule, which may need to be dissociated to enter the putative hydrophobic binding pocket.

**The 4' position:** Due to a 1,3-allylic strain, the proton at the 4' position is nearly in the same plane as the conjugated amide, as suggested by the  $^1H$  NMR spectrum of FR901464 ( $J_{3,4'} = 7.8 \text{ Hz}$ ). Inversion of the stereocenter at the 4' posi-



tion places its methyl and acetoxy substituents in opposite faces of the plane defined by the conjugated amide. This analogue, 4'-*epi*-FR901464, was synthesized by the Jacobsen group and found to be 15 times less active than FR901464.<sup>[17]</sup> The reduced activity could be because either the acetoxy or the 5'-methyl group is in the wrong orientation (or both groups). To probe this idea, we prepared 4'-desacetyl meayamycin, which began with the methanolysis of the previously prepared **4**<sup>[21,22]</sup> to give alcohol **31** in 82% yield (Scheme 5A). This alcohol was then subjected to olefin cross metathesis conditions with **6** and **Ru-1** to give 4'-desacetyl meayamycin in 81% yield. The potency of 4'-desacetyl meayamycin as compared to meayamycin dropped by 24-fold ( $GI_{50} = 0.48 \text{ nM}$ ; Table 1), further indicating the significance of the 4' position.

From this result, it was not clear whether this reduced activity was due to the loss of the acetyl group itself or possibly placing the methyl group in the  $\alpha$  face by virtue of the intramolecular hydrogen bonding between the 4'-hydroxy group and the amide group. Therefore, we also prepared 4'-*epi*-desacetyl meayamycin according to Scheme 5B. The enantiomer of acid **19** (i.e., *epi-19*) was prepared by the following route: *N*-acetyl morpholine was coupled to an in situ prepared lithium acetylide to give **32** in 90% yield.<sup>[33]</sup> This ynone was reduced with  $HCO_2Na$  and Noyori's catalyst,<sup>[34]</sup> **Ru-2**, to give an alcohol, which was acetylated to give **33** in 73% yield for the two steps. This material was then exposed to acidic methanol followed by the Jones' reagent to give an ynoic acid in 72%. The ynoic acid was hydrogenated in the presence of Lindlar's catalyst to give 4'-*epi-19* in 68% yield. Acid 4'-*epi-19* was then used to prepare 4'-*epi*-desacetyl meayamycin by the same synthetic route previously outlined.<sup>[21,22]</sup> This analogue was found to be one order of magnitude less active ( $GI_{50} = 4.7 \text{ nM}$ , Table 1) than 4'-desacetyl meayamycin, indicating that the acetyl group is important for the biological activity.



Scheme 5. Preparation of 4'-analogues. a)  $K_2CO_3$  (5.0 equiv), MeOH, 0°C, 82%; b)  $6$  (1.6 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.17 equiv), DCE, 32°C, 81%; c)  $nBuLi$  (2.8 equiv),  $HC\equiv CCH_2OTHP$  (3.0 equiv), THF, -78 to 0°C, 90%; d) **Ru-2** (2 mol %),  $HCO_2Na$  (10 equiv), cetyltrimethylammonium bromide (CTAB, 0.2 equiv),  $H_2O$ , 23°C, 78%; e)  $Ac_2O$  (5.0 equiv), pyridine, 23°C, 93%; f) pyridinium *para*-toluenesulfonate (PPTS, 0.2 equiv), MeOH, 23°C, 84%;  $Na_2Cr_2O_7$  (1.5 equiv), acetone,  $H_2SO_4$ , 0 to 23°C, 86%; g)  $H_2$ , Lindlar's catalyst (1 mol %), quinoline (0.10 equiv), EtOH, 23°C, 68%; h)  $34$  (1 equiv),  $CH_2Cl_2/TFA$  (9:1), 23°C; then 4'-*epi*- $19$  (1.2 equiv), HATU (1.2 equiv),  $iPr_2NEt$  (4.0 equiv), MeCN, 23°C, 71%; i) methacrolein (10 equiv), **Ru-1** (2 mol %), 23°C, 62%; j)  $Ph_3PCH_3Br$  (2.2 equiv),  $KOtBu$  (2.2 equiv), THF, 0°C, 84%; k)  $K_2CO_3$  (2.0 equiv), MeOH, 0°C, 86%; l)  $6$  (1.5 equiv), **Ru-1** (0.10 equiv), DCE, 35°C, 34%; m) 1,1'-carbonyldiimidazole (CDI, 1.5 equiv),  $CH_2Cl_2$ , 23°C; morpholine (4.1 equiv), 23°C, 80%; n)  $6$  (1.6 equiv), **Ru-1** (0.07 equiv), DCE, 42°C, 44%.

The correlation between the loss of the acetyl group and activity was a concern to us because esterases might hydrolyze the ester group in tissue culture and in vivo. Consequently, we measured the half-lives of meayamycin in mouse and human sera at a concentration of 10  $\mu M$ . As we expected, meayamycin was hydrolyzed to its desacetyl analogue ( $t_{1/2}$  = 2 h in mouse serum, 9 h in human serum), which was then converted to uncharacterized products. We hypothesized that if we prolong the half-life of meayamycin in sera, effective concentrations of this compound might be higher during the assays, thus improving the potency. To test this hypothesis, we replaced the acetyl group with a morpholine-based carbamate (meayamycin B) to enhance the stability under biological conditions as carbamates are known to be more stable than esters under biological conditions.<sup>[35]</sup>

The synthesis of this analogue was accomplished by the reaction of  $31$  with CDI followed by morpholine to give  $36$  in 80% yield (Scheme 5C). This carbamate was then coupled to  $6$  in the presence of **Ru-1** to give meayamycin B in 44% yield. The half-life of this carbamate analogue was 13 h in mouse serum (Figure 1), significantly longer than that of meayamycin, presumably due to the esterase-resistant nature of this compound. Encouraged by this result, the antiproliferative activity of this new analogue was measured;

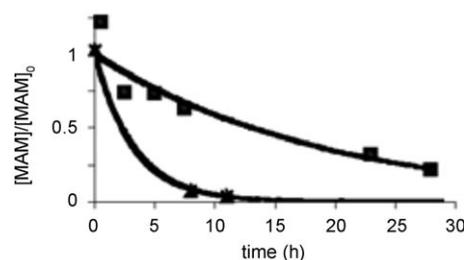


Figure 1. Degradation of meayamycin (triangles) and meayamycin B (squares) in mouse serum at 37°C (y axis: relative quantity of meayamycin (or meayamycin B) compared to the initial meayamycin (or meayamycin B) quantity). The initial meayamycin concentration was 10  $\mu M$ . Meayamycin:  $y = e^{-0.310x}$  ( $R^2 = 0.997$ ). Meayamycin B:  $y = e^{-0.053x}$  ( $R^2 = 0.954$ ). MAM: meayamycin or meayamycin B.

it was gratifying to find that meayamycin B was even more potent than the extremely potent meayamycin in MCF-7 cells (Table 2). With this result in hand, we tested meayamycin<sup>[36]</sup> and meayamycin B with various cancer cell lines. MCF-7 and MDA-MB-231, both human breast cancer cell lines, were the most responsive. PC3, HCT116 and H1299 were also very responsive, exhibiting  $GI_{50}$  values in a low picomolar range. A549 and DU145 cells were less responsive,

Table 2. GI<sub>50</sub> values of meayamycin and meayamycin B.<sup>[a]</sup>

Cell line	Meayamycin [pM]	Meayamycin B [pM]
MCF-7	20 ± 8.9	8 ± 2
MDA-MB-231	71 ± 55	15 ± 1.4
A549	258 ± 162	175 ± 77
DU145	1230 ± 697	211 ± 23
HCT116	157 ± 33	4 ± 2
H1299	841 ± 271	146 ± 30
PC3	196 ± 42	101 ± 4.0

[a] Each experiment consists of at least twelve data points (twelve different concentrations).

but the GI<sub>50</sub> values were still sub-nanomolar. On the average, meayamycin B is 3.1 ± 1.9 times more potent than meayamycin in these seven cancer cell lines.

After the synthesis and biological evaluation of meayamycin B were completed in our laboratory, the Webb group published similar carbamate analogues with great loss of activity.<sup>[12]</sup> In retrospect, we were fortunate that we chose morpholine for water solubility, the size of which appeared to be adequately small to fit in an unknown binding pocket unlike Webb's larger carbamate groups.

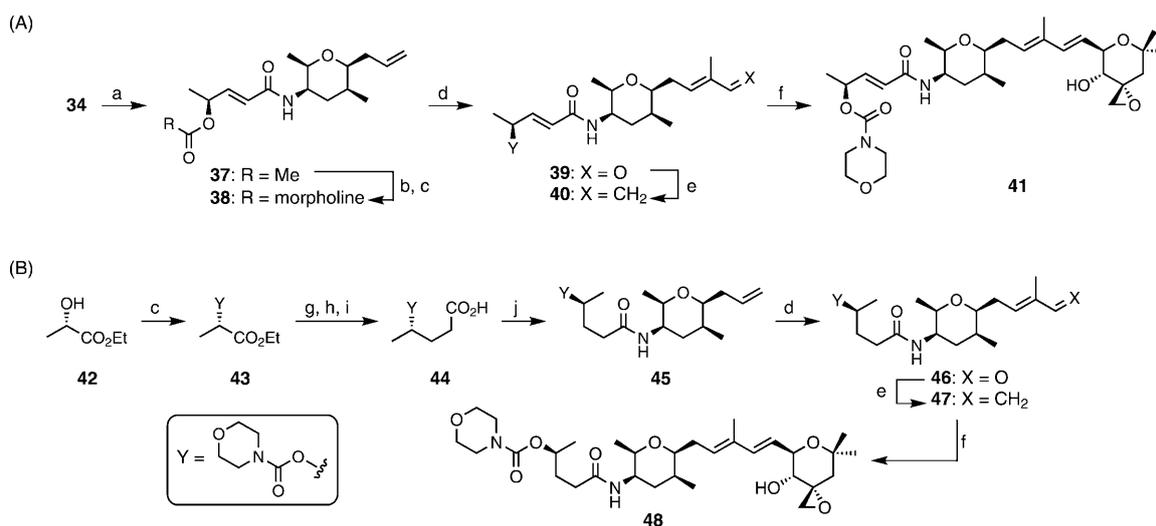
For in vivo studies, the most ideal vehicle is saline; therefore, the solubility of drugs in saline is a crucial parameter. Although meayamycin was not entirely soluble in saline at a concentration of 0.1 mg mL<sup>-1</sup>, meayamycin B was soluble at this concentration (presumably soluble at higher concentrations), showing an additional benefit for meayamycin B.<sup>[37]</sup>

**C2'–C3' Olefin on the side chain:** Next, we asked whether the C2'–C3' *trans* isomer was the actual active species after the potential isomerization of the *cis* enamide to its *trans*

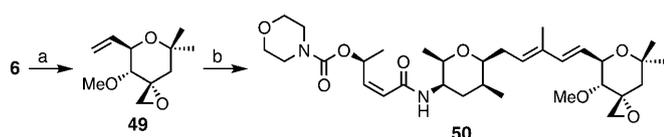
form. To address this question, we synthesized analogue **41** according to Scheme 6. A key step is the deliberate isomerization of the *cis* acid **19** to the activated *trans* acid derivative during the amide bond forming reaction. Specifically, we exploited the facile *cis*–*trans* isomerization of the HATU-activated acid **19** prior to the addition of amine **34** to yield a ≈ 1:1 *cis/trans* mixture as illustrated in Scheme 6A. Analogue **41** was assembled in three additional steps as illustrated. The GI<sub>50</sub> of this analogue was three orders of magnitude higher than that of the corresponding *cis* compound, indicating that the *cis* enamide is the active species.

Considering that an α,β-unsaturated amide could serve as an electrophile,<sup>[38]</sup> we asked whether the side chain was an electrophile. To answer this question, analogue **48** was designed and synthesized according to Scheme 6B. The GI<sub>50</sub> of this side chain analogue was found to be 19 nM with MCF-7 cells (Table 1; 93 nM with HCT116, data not shown). The antiproliferative activity of the two analogues **41** and **48** implies that the *cis* C2'–C3' enamide is not an electrophile but rather a structural requirement.

**The 4-hydroxy group:** 4-*O*-Alkyl FR901464 analogues were prepared and evaluated by others.<sup>[39]</sup> However, it was not clear to us whether the loss of activity was due to the large alkyl groups or the loss of a hydrogen bond donor. To minimize the additional sterics, we prepared the 4-*O*-methyl analogue **50** (Scheme 7). This analogue, meayamycin C, showed respectable potency with a GI<sub>50</sub> of 0.38 nM. The diminished activity may be explained by the loss of a hydrogen bond donor or the additional steric bulk. Nonetheless, this analogue may prove to be an important one because in a separate study we observed glucuronidation of meayamycin B as a major metabolite in mice, for which this hydroxy group is



Scheme 6. Preparation of C2'–C3' analogues **41** and **48**. a) CH<sub>2</sub>Cl<sub>2</sub>/TFA (9:1), 23 °C; **19** (1.7 equiv), HATU (1.3 equiv), *i*Pr<sub>2</sub>NEt (5.0 equiv), MeCN, 23 °C, 43%; b) K<sub>2</sub>CO<sub>3</sub> (2.5 equiv), MeOH, 0 °C to 23 °C, 75%; c) CDI (1.2 equiv), CH<sub>2</sub>Cl<sub>2</sub>, 23 °C; morpholine (2–5 equiv), 23 °C, 97% (**38**) or 94% (**43**); d) methacrolein (13–20 equiv), **Ru-1** (2 mol %), CH<sub>2</sub>Cl<sub>2</sub> (**39**) or PhH (**46**), 23 °C or 50 °C, 74% (**39**) or 63% (**46**); e) Ph<sub>3</sub>PCH<sub>2</sub>Br (2.4 equiv), KO<sup>t</sup>Bu (2.3 equiv), THF, 0 °C, 50% (**40**) or 76% (**47**); f) **6** (1.3 equiv), **Ru-1** (0.07–0.2 equiv), benzoquinone (0.2–0.5 equiv), DCE, 42–50 °C, 46% (**41**) or 36% (**48**); g) DIBALH (1.1 equiv), CH<sub>2</sub>Cl<sub>2</sub>, –78 °C; Ph<sub>3</sub>P=CHCO<sub>2</sub>Me (1.5 equiv), 23 °C; h) H<sub>2</sub>, Pd/C (2 mol %), EtOAc, 23 °C; i) NaOH (2.1 equiv), MeOH/H<sub>2</sub>O (1:1.1), 23 °C, 46% (over 3 steps); j) **34**, CH<sub>2</sub>Cl<sub>2</sub>/TFA (9:1), 23 °C; **44** (1.3 equiv), HATU (1.2 equiv), *i*Pr<sub>2</sub>NEt (3.4 equiv), 23 °C, 66%.



Scheme 7. Preparation of the C4-OMe analogue **50**. a) MeI (5.0 equiv), Ag<sub>2</sub>O (2.0 equiv), DMF, 23°C, 87%; b) **36** (1.0 equiv), **49** (1.5 equiv), **Ru-1** (7 mol%), benzoquinone (0.2 equiv), DCE, 42°C, 57%.

the only site. Detailed pharmacokinetic studies of meayamycin B and meayamycin C will be reported by our laboratory in due course. It is noteworthy that the olefin cross metathesis of **36** with **49** is reproducibly higher yielding than that with **6** (Scheme 5C). This difference proved to be crucial in the preparation of cyclopropyl analogues (see below).

**The 1,3-diene moiety:** We previously observed relatively facile isomerization of the 1,3-diene moiety under acidic conditions.<sup>[22]</sup> The same might occur in live cancer cells, raising a question of whether the *trans,trans*-diene moiety is actually the biologically active species. To examine the significance of the diene moiety, we synthesized the cyclopropyl analogues **59** and **60** (Scheme 8).

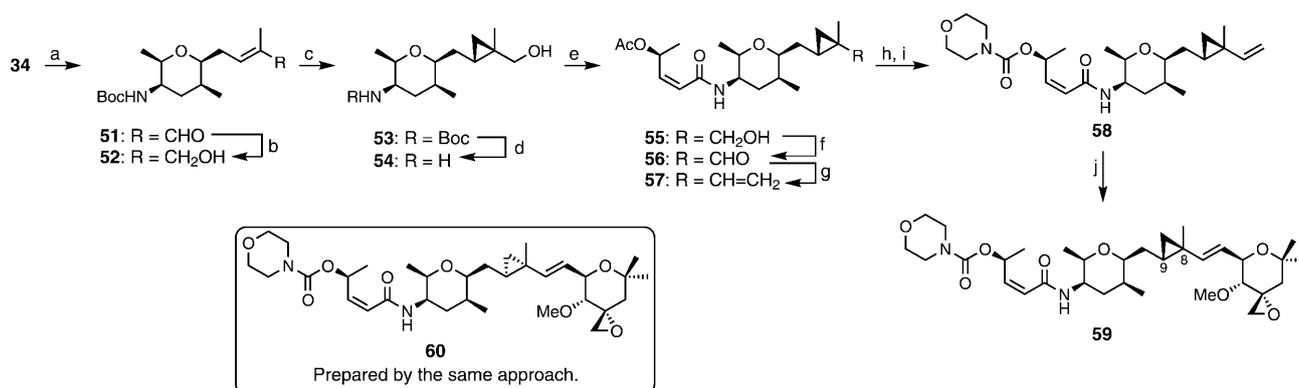
Compound **34** was first cross-coupled with methacrolein in the presence of **Ru-1** to give **51** in 84% yield. This aldehyde was converted to alcohol **52**, which was stereoselectively cyclopropanated by Charette's method<sup>[40]</sup> to form **53** in 70% yield with a diastereoselective ratio of 89:11. We also prepared the epimer at the C8 and C9 position in 88% with a diastereoselective ratio 87:13 (see the Supporting Information). The allylic cyclopropyl group, after the conversion of the hydroxy group of **53** to an olefin, was not compatible with acidic conditions for the Boc removal (data not shown). These failures prompted us to remove the Boc group from **53** with TFA before the derivatization of the hydroxy group. The resulting amine **54** was condensed with carboxylic acid **19** to form **55** in 48% yield for the two steps. The oxidation–olefination sequence gave alkene **57** in 56%

yield over two steps. Further transformations yielded alkene **58**. Interestingly, this alkene did not undergo cross olefin metathesis with alkene **6** but did so with alkene **49** to form **59** in 30% yield. By a similar sequence, diastereomer **60** was prepared (see the Supporting Information).

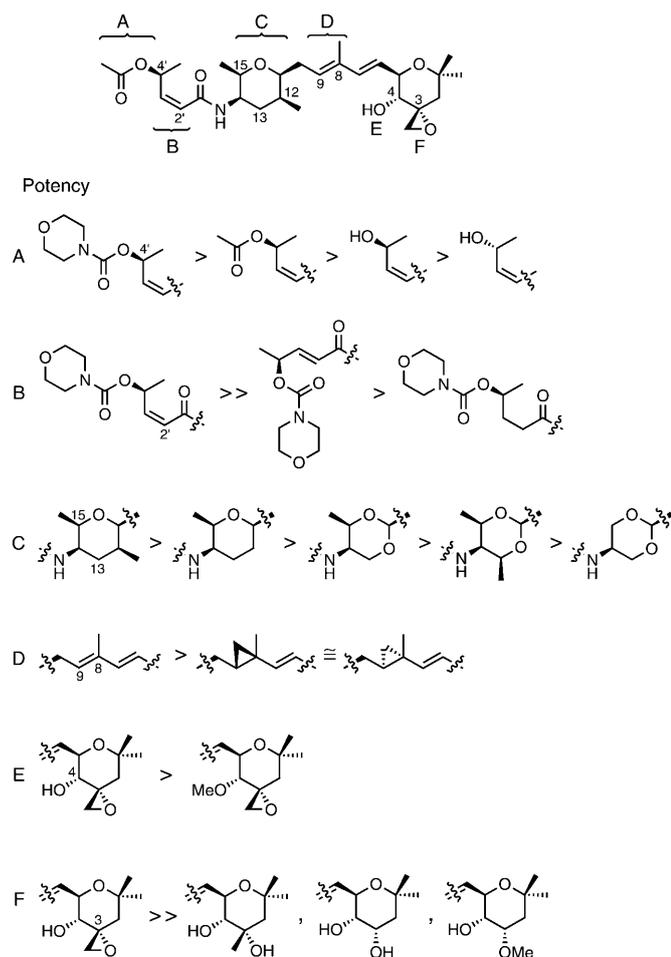
The GI<sub>50</sub> values of analogues **59** and **60** with MCF-7 cells were found to be 15 and 10 nM, respectively (Table 1). The potencies of these analogues were approximately one order of magnitude lower than that of meayamycin C but were still respectable. These results imply that the C8–C9 olefin of FR901464 remains *trans* as an active species in live cells. In order to fully understand the significance of the C8–C9 olefin, cyclopropyl analogues that would mimic the C8–C9 *cis* geometry must be prepared and evaluated. Nonetheless, the similar activity of **59** and **60** compared to meayamycin C implies that the C8–C9 olefin would remain *trans* as the active FR901464 species in cells.

## Conclusion

The C3-spiroepoxide (Scheme 9) was shown to be crucial for the retention of the antiproliferative activity, indicating that a covalent bond is presumably formed between target protein(s) and FR901464. The C12 position prefers to be hydrophobic, and the C4'-acetate group is important but the ester is labile, forming a less potent alcohol (4'-desacetyl meayamycin). The C2'–C3' side chain olefin needs to be in its *cis* form to be active. The replacement of one of the protons at position 13 with a methyl group diminished biological activity. Replacement of the 15-methyl group with a hydrogen atom was also somewhat detrimental. The C8–C9 olefin remains in its *trans* form as the active species of FR901464. Masking the 4-hydroxy group with a methyl group reduced the biological activity. Finally, the development of the more biologically stable and more saline-soluble C4'-carbamate analogue, meayamycin B, was shown to be significantly more potent than meayamycin in seven cancer



Scheme 8. Preparation of the cyclopropyl analogues. a) methacrolein (20 equiv), **Ru-1** (3 mol%), 23°C, 84%; b) DIBALH (2.0 equiv), CH<sub>2</sub>Cl<sub>2</sub>, –78°C, 98%; c) (*S,S*)-dioxaborolane (2.0 equiv), ZnEt<sub>2</sub> (5.0 equiv), CH<sub>2</sub>I<sub>2</sub> (7.0 equiv), CH<sub>2</sub>Cl<sub>2</sub>, 23°C, 70%, d.r. = 89:11; d) CH<sub>2</sub>Cl<sub>2</sub>/TFA (8:2), 0 to 23°C, quantitative; e) **19** (1.2 equiv), HATU (1.0 equiv), *i*Pr<sub>3</sub>NEt (4.0 equiv), MeCN, 23°C, 48%; f) Dess–Martin periodinane (2.0 equiv), CH<sub>2</sub>Cl<sub>2</sub>, 0°C, 70%; g) Ph<sub>3</sub>PCH<sub>3</sub>Br (6.0 equiv), KO<sup>t</sup>Bu (5.0 equiv), THF, 0°C, 80%; h) 1. K<sub>2</sub>CO<sub>3</sub> (2.5 equiv), THF, 0°C; i) CDI (2.5 equiv), CH<sub>2</sub>Cl<sub>2</sub>, 23°C; morpholine (5.0 equiv), 23°C, 94% (over 2 steps); j) **49** (1.7 equiv), **Ru-1** (0.1 equiv), benzoquinone (0.2 equiv), toluene, 70°C, 30%.



Scheme 9. Summary of this work.

cell lines. As FR901464 binds SF3b and inhibits pre-mRNA splicing, this observation could be very exciting because the meayamycins should also inhibit the same process and exhibit anticancer activity. Because there is no approved antitumor drug that acts by inhibiting pre-mRNA splicing, the mode of action, potency, and water solubility of meayamycin B might further trigger interest in both oncology and RNA biology.

## Experimental Section

### Materials and methods for the growth inhibition assays

**Materials:** FR901464 and its analogues were dissolved in DMSO as 10 mM stocks and stored at  $-20^{\circ}\text{C}$ . For the experiments, aliquots were thawed at room temperature and dilutions were prepared in RPMI 1640 medium containing 2% DMSO at  $2\times$  the desired concentration prior to addition to the cells.

**Cell culture:** MCF-7, A549, DU145, HCT116, MDA-MB-231, H1299, and PC3 cells were grown at  $37^{\circ}\text{C}$  in an atmosphere containing 5% carbon dioxide in Corning cell culture dishes (150 mm) in RPMI-1640 cell culture medium containing 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin–streptomycin solution (Invitrogen).

**Growth inhibition assay:** Cells were plated in 96-well plates at an initial density of 2000 or 4000 cells per well in 100  $\mu\text{L}$  of medium and were incubated for 24–48 h (depending on cell line) prior to compound addition (plating either 2000 or 4000 cells per well led to similar  $\text{GI}_{50}$  values). Serial two-fold dilutions were used in this experiment for the indicated ranges. The compounds were added to the cells at  $2\times$  the desired concentration in 100  $\mu\text{L}$  cell culture medium (containing 2% DMSO). The cells were then incubated for an additional 5–10 days. Cell proliferation was measured by using the commercial 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) dye reduction assay with phenazine methosulfate (PMS) as the electron acceptor (20  $\mu\text{L}$  per well). The absorbance (at 490 nm minus that at 630 nm) was measured by a Spectromax M5 plate reader (Molecular Devices) or Modulus II Microplate Multimode Reader (Turner BioSystem). Evaluation of FR901464 analogues was performed in quadruplicate at each concentration, and the final numbers were averaged. Microsoft Excel and GraphPad Prism 5 were used to construct dose–response curves and calculating the  $\text{GI}_{50}$  values. Growth inhibition is calculated as defined by the National Cancer Institute [ $\text{GI}_{50} = 100 \times (T - T_0) / (C - T_0)$ ];  $T_0$  = cell density at time zero;  $T$  = cell density of the test well after period of exposure to test compound;  $C$  = cell density of the vehicle treated.<sup>[41]</sup> Vincristine or cisplatin were used as controls.

**Procedure for the half-life determination for the decomposition of meayamycin and meayamycin B in mouse serum:** To a 1 dram glass vial was added mouse serum (1.0 mL), rhodamine B (10 mM in DMSO, 2.0  $\mu\text{L}$ ), and DMSO (7.0  $\mu\text{L}$ ) under an open atmosphere and warmed to  $37^{\circ}\text{C}$ . To the mixture was added meayamycin or meayamycin B (10 mM in DMSO, 1.0  $\mu\text{L}$ ), and the resulting solution was sealed with a cap, vortexed for 15 s, and then placed in a  $37^{\circ}\text{C}$  incubator. The decomposition was monitored by HPLC at the indicated times. The resulting data were normalized by dividing the ratios of meayamycin/rhodamine B or meayamycin B/rhodamine B by the ratio of the first data point. HPLC monitoring was performed on a Varian Pursuit XRs 5  $\text{C}_{18}$  column,  $250 \times 10.0$  cm,  $2.5 \text{ mL min}^{-1}$ , 30% MeOH in  $\text{H}_2\text{O}$  (containing 1%  $\text{HCO}_2\text{H}$ ) to 100% MeOH linear gradient elution from 0.5 to 22 min, monitored at 230 nm (for meayamycins) and 550 nm (for rhodamine B).

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- [1] R. Capdeville, E. Buchdunger, J. Zimmermann, A. Matter, *Nat. Rev. Drug Discovery* **2002**, *1*, 493–502.
- [2] A. Kamb, S. Wee, C. Lengauer, *Nat. Rev. Drug Discovery* **2007**, *6*, 115–120.
- [3] M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon, A. T. McPhail, *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.
- [4] a) P. B. Schiff, J. Fant, S. B. Horwitz, *Nature* **1979**, *277*, 665–667; b) P. B. Schiff, S. B. Horwitz, *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 1561–1565.
- [5] G. Fenteany, R. F. Standaert, W. S. Lane, S. Choi, E. J. Corey, S. L. Schreiber, *Science* **1995**, *268*, 726–731.
- [6] a) J. Adams, *Cancer Cell* **2004**, *5*, 417–421; b) S. V. Rajkumar, P. G. Richardson, T. Hideshima, K. C. Anderson, *J. Clin. Oncol.* **2004**, *23*, 630–639.
- [7] a) H. Nakajima, Y. Hori, H. Terano, M. Okuhara, T. Manda, S. Matsumoto, K. Shimomura, *J. Antibiot.* **1996**, *49*, 1204–1211; b) H. Na-

- kajima, B. Sato, T. Fujita, S. Takase, H. Terano, M. Okuhara, *J. Antibiot.* **1996**, *49*, 1196–1203; c) H. Nakajima, S. Takase, H. Terano, H. Tanaka, *J. Antibiot.* **1997**, *50*, 96–99.
- [8] D. Kaida, H. Motoyoshi, E. Tashiro, T. Nojima, M. Hagiwara, K. Ishigami, H. Watanabe, T. Kitahara, T. Yoshida, H. Nakajima, T. Tani, S. Horinouchi, M. Yoshida, *Nat. Chem. Biol.* **2007**, *3*, 576–583.
- [9] a) B. K. Das, L. Xia, L. Palandjian, O. Gozani, Y. Chyung, R. Reed, *Mol. Cell. Biol.* **1999**, *19*, 6796–6802; b) A. Krämer, P. Grüter, K. Gröning, B. Kastner, *J. Cell Biol.* **1999**, *145*, 1355–1368; c) C. L. Will, C. Schneider, A. M. MacMillan, N. F. Katopodis, G. Neubauer, M. Wilm, R. Lührmann, C. C. Query, *EMBO J.* **2001**, *20*, 4536–4546; d) C. L. Will, H. Urlaub, T. Achsel, M. Gentzel, M. Wilm, R. Lührmann, *EMBO J.* **2002**, *21*, 4978–4988.
- [10] Z. L. Zhou, L. J. Licklider, S. P. Gygi, R. Reed, *Nature* **2002**, *419*, 182–185.
- [11] D. D. Licatalosi, R. B. Darnell, *Nat. Rev. Genet.* **2010**, *11*, 75–87.
- [12] C. Lagiseti, A. Pourpak, T. Goronga, Q. Jiang, X. Cui, J. Hyle, J. M. Lahti, S. W. Morris, T. R. Webb, *J. Med. Chem.* **2009**, *52*, 6979–6990.
- [13] a) M. Sakurai, J. Kohno, M. Nishio, K. Yamamoto, T. Okuda, K. Kawano, N. Nakanishi, *J. Antibiot.* **2001**, *54*, 628–634; b) Y. Sakai, T. Tsujita, T. Akiyama, T. Yoshida, T. Mizukami, S. Akinaga, S. Horinouchi, M. Yoshida, T. Yoshida, *J. Antibiot.* **2002**, *55*, 863–872; c) Y. Sakai, T. Yoshida, K. Ochiai, Y. Uosaki, Y. Saitoh, F. Tanaka, T. Akiyama, S. Akinaga, T. Mizukami, *J. Antibiot.* **2002**, *55*, 855–860.
- [14] a) T. Sakai, N. Asai, A. Okuda, N. Kawamura, Y. Mizui, *J. Antibiot.* **2004**, *57*, 180–187; b) T. Sakai, T. Sameshima, M. Matsufuji, N. Kawamura, K. Dobashi, Y. Mizui, *J. Antibiot.* **2004**, *57*, 173–179.
- [15] Y. Kotake, J. Nijijima, M. Nagai, K. Okano, T. Sakai, M. Yoshida, T. Tsuchida, T. Nakashima, K. Dobashi, Y. Mizui, H. Shimizu, T. Uenaka, M. Iwata, M. Asada, K. Yoshimatsu, *Clin. Cancer Res.* **2003**, *9*, 6208S.
- [16] C. F. Thompson, T. F. Jamison, E. N. Jacobsen, *J. Am. Chem. Soc.* **2000**, *122*, 10482–10483.
- [17] C. F. Thompson, T. F. Jamison, E. N. Jacobsen, *J. Am. Chem. Soc.* **2001**, *123*, 9974–9983.
- [18] M. Horigome, H. Motoyoshi, H. Watanabe, T. Kitahara, *Tetrahedron Lett.* **2001**, *42*, 8207–8210.
- [19] H. Motoyoshi, M. Horigome, H. Watanabe, T. Kitahara, *Tetrahedron* **2006**, *62*, 1378–1389.
- [20] a) B. J. Albert, K. Koide, *Org. Lett.* **2004**, *6*, 3655–3658; b) K. Koide, B. J. Albert, *Yuki Gosei Kagaku Kyokaiishi (J. Synth. Org. Chem. Jpn.)* **2007**, *65*, 119–126.
- [21] B. J. Albert, A. Sivaramakrishnan, T. Naka, K. Koide, *J. Am. Chem. Soc.* **2006**, *128*, 2792–2793.
- [22] B. J. Albert, A. Sivaramakrishnan, T. Naka, N. L. Czaicki, K. Koide, *J. Am. Chem. Soc.* **2007**, *129*, 2648–2659.
- [23] H. Motoyoshi, M. Horigome, K. Ishigami, T. Yoshida, S. Horinouchi, M. Yoshida, H. Watanabe, T. Kitahara, *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2178–2182.
- [24] H. Furukawa, K. Koide, K. Takao, S. Kobayashi, *Chem. Pharm. Bull.* **1998**, *46*, 1244–1247.
- [25] A part of these results was also included in patents, see: K. Koide, B. J. Albert, A. Sivaramakrishnan, US 2008096879A1 20080424, **2008** and K. Koide, B. J. Albert, A. Sivaramakrishnan, WO 2009031999A1 20090312, **2009**.
- [26] A. Michrowska, R. Bujok, S. Harutyunyan, V. Sashuk, G. Dolgonos, K. Grell, *J. Am. Chem. Soc.* **2004**, *126*, 9318–9325.
- [27] a) B. J. Albert, K. Koide, *ChemBioChem* **2007**, *8*, 1912–1915; b) E. Weerapana, G. M. Simon, B. F. Cravatt, *Nat. Chem. Biol.* **2008**, *4*, 405–407.
- [28] B. Bernet, U. Piantini, A. Vasella, *Carbohydr. Res.* **1990**, *204*, 11–25.
- [29] a) P. A. Wender, J. De Brabander, P. G. Harran, J. M. Jimenez, M. F. T. Koehler, B. Lippa, C. M. Park, M. Shiozaki, *J. Am. Chem. Soc.* **1998**, *120*, 4534–4535; b) P. A. Wender, J. L. Baryza, *Org. Lett.* **2005**, *7*, 1177–1180; c) P. A. Wender, J. C. Horan, *Org. Lett.* **2006**, *8*, 4581–4584; d) A. B. Smith, T. M. Razler, R. M. Meis, G. R. Pettit, *Org. Lett.* **2006**, *8*, 797–799.
- [30] A. B. Smith, Z. Q. Liu, A. M. L. Hogan, D. S. Dalisay, T. F. Molinski, *Org. Lett.* **2009**, *11*, 3766–3769.
- [31] P. Garner, J. M. Park, *J. Org. Chem.* **1987**, *52*, 2361–2364.
- [32] a) K. Saito, S. Saijo, K. Kotera, T. Date, *Chem. Pharm. Bull.* **1985**, *33*, 1342–1350; b) Y. Nakamura, M. Hirai, K. Tamotsu, Y. Yonezawa, C. G. Shin, *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1369–1376.
- [33] M. M. Jackson, C. Leverett, J. F. Tozcko, J. C. Roberts, *J. Org. Chem.* **2002**, *67*, 5032–5035.
- [34] K. Matsumura, S. Hashiguchi, T. Ikariya, R. Noyori, *J. Am. Chem. Soc.* **1997**, *119*, 8738–8739.
- [35] a) T. L. Huang, A. Székács, T. Uematsu, E. Kuwano, A. Parkinson, B. D. Hammock, *Pharm. Res.* **1993**, *10*, 639–648; b) A. K. Ghosh, J. F. Kincaid, W. H. Cho, D. E. Walters, K. Krishnan, K. A. Hussain, Y. Koo, H. Cho, C. Rudall, L. Holland, J. Buthod, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 687–690.
- [36] B. J. Albert, P. A. McPherson, K. O'Brien, N. L. Czaicki, V. DeStefino, S. Osman, M. S. Li, B. W. Day, P. J. Grabowski, M. J. Moore, A. Vogt, K. Koide, *Mol. Cancer Ther.* **2009**, *8*, 2308–2318.
- [37] With this solubility at 0.1 mg mL<sup>-1</sup> in saline, it is possible to reach the maximum tolerated dosage of FR901464 (1 mg kg<sup>-1</sup>).
- [38] a) M. Groll, B. Schellenberg, A. S. Bachmann, C. R. Archer, R. Huber, T. K. Powell, S. Lindow, M. Kaiser, R. Dudler, *Nature* **2008**, *452*, 755; b) J. Clerc, M. Groll, D. J. Illich, A. S. Bachmann, R. Huber, B. Schellenberg, R. Dudler, M. Kaiser, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 6507–6512.
- [39] Y. Imamura, Y. Ohtsu, H. Tanaka, M. Hatakeyama, T. Manabe, H. Kawaguchi, H. Handa, T. Takahashi, *Heterocycles* **2004**, *64*, 51–56.
- [40] A. B. Charette, J. F. Marcoux, *Synlett* **1995**, 1197–1207.
- [41] <http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html>.

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