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FR901464: Total Synthesis, Proof of Structure, and Evaluation of Synthetic Analogues

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Abstract: The natural product FR901464 (**1**) was isolated by the Fujisawa Pharmaceutical Co. and shown to have intriguing biological properties including impressive antitumor activity. In this paper we describe the first total synthesis of **1** in full detail. A chiral building block synthetic strategy was used to assemble the target: optically active components were generated using asymmetric catalytic reactions, and these fragments were coupled together at a late stage in a convergent synthesis. In particular, a versatile, asymmetric hetero-Diels−Alder (HDA) reaction was developed in the context of this synthesis and used with great effectiveness for the preparation of the two densely functionalized pyran rings. The flexible nature of the synthetic route also allowed us to prepare a series of analogues of **1**. These compounds were used to prove the relative stereochemistry of the natural product as well as to probe the importance of certain structural features of FR901464 with regard to biological activity.

Background and Retrosynthetic Analysis

In 1996, the Fujisawa Pharmaceutical Co. reported the isolation of three novel compounds produced by a bacterium obtained from a Japanese soil sample.[1] The three were closely related structurally (Figure 1), differing only in the substitution pattern about the right-hand pyran ring, and were designated FR901463, FR901464, and FR901465.[1] These natural products were discovered as part of a program to identify small molecules that induce transcriptional regulation, following the hypothesis that such compounds might hold potential as antitumor agents. All three were found to enhance the activity of the promoter of the SV40 DNA tumor virus in M-8 cells.[1,2] Upon further investigation, the Fujisawa researchers found that these compounds displayed potent cytotoxicity against a number of different human solid tumor cell lines, with IC_{50} values on the order of 1 ng/mL.[1] A study evaluating the ability of these small molecules to prolong the life of tumor-bearing mice revealed that FR901464 (**1**) was most active, and as a result this compound was studied further in mechanistic experiments.[3] FR901464 was shown to effect G_1 and G_2/M cell cycle arrest and also to induce DNA fragmentation as well as cell shrinkage during the process of causing cell death.[3] Some of the phenotypic changes observed in M-8 cells treated with FR901464 were found to be similar to those of known histone deacetylase inhibitors such as trichostatin A.[3−5] However, analysis of the acetylation state of the histones isolated from M-8 cells indicated that **1** was in fact *not* an inhibitor of histone deacetylase activity, but instead appeared to have a distinct, unknown, and potentially very interesting mode of action.[4]

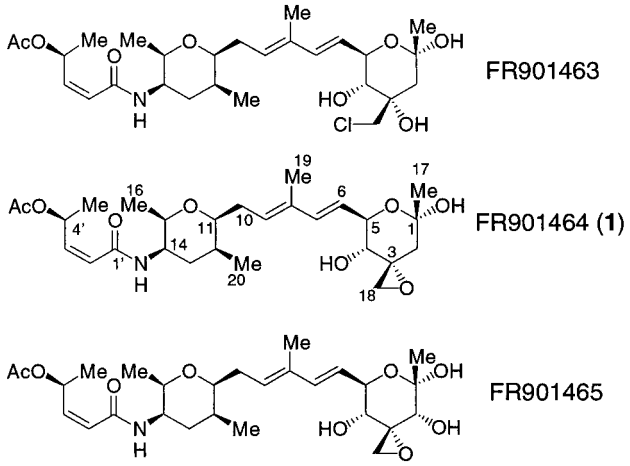

Figure 1. Structures of FR901463, FR901464, and FR901465.

We were inspired to pursue a total synthesis of FR901464 because of the interesting biological properties outlined above coupled with the limited supply of natural material,[6] and also as a result of the interesting structural characteristics of the molecule.[7] FR901464 is composed of three discrete chiral fragments joined by conjugated, unsaturated linkages. As such, we considered it a potential target for a chiral building block synthetic strategy, wherein the target is assembled in a convergent manner using effective and general fragment coupling strategies from optically active components generated independently by asymmetric catalysis.[8] Furthermore, the relative stereochemistry of **1** had been assigned only tentatively: the absolute stereochemistry at C4′ had been established by

(1) Nakajima, H.; Sato, B.; Fujita, T.; Takase, S.; Terano, H.; Okuhara, M. *J. Antibiot.* **1996**, *49*, 1196−1203.

(2) M-8 cells are a human mammary adenocarcinoma cell line stably transfected with an SV40 promoter-driven CAT reporter gene. See ref 1.

(3) Nakajima, H.; Hori, Y.; Terano, H.; Okuhara, M.; Manda, T.; Matsumoto, S.; Shimomura, K. *J. Antibiot.* **1996**, *49*, 1204−1211.

(4) Nakajima, H.; Kim, Y. B.; Terano, H.; Yoshida, M.; Horinouchi, S. *Exp. Cell Res.* **1998**, *241*, 126−133.

(5) Taunton, J.; Hassig, C. A.; Schreiber, S. L. *Science* **1996**, 272, 408−411.

(6) Samples of natural FR901464 were not available from the Fujisawa Pharmaceutical Co. nor from any other sources.

(7) For a preliminary communication of this work, see: Thompson, C. F.; Jamison, T. F.; Jacobsen, E. N. *J. Am. Chem. Soc.* **2000**, *122*, 10482−10483. For other synthetic efforts toward the synthesis of FR901464, see: Horigome, M.; Watanabe, K.; Kitahara, T. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1999**, *41*, 73−78; *Chem. Abstr.* **2000**, *132*, 676.

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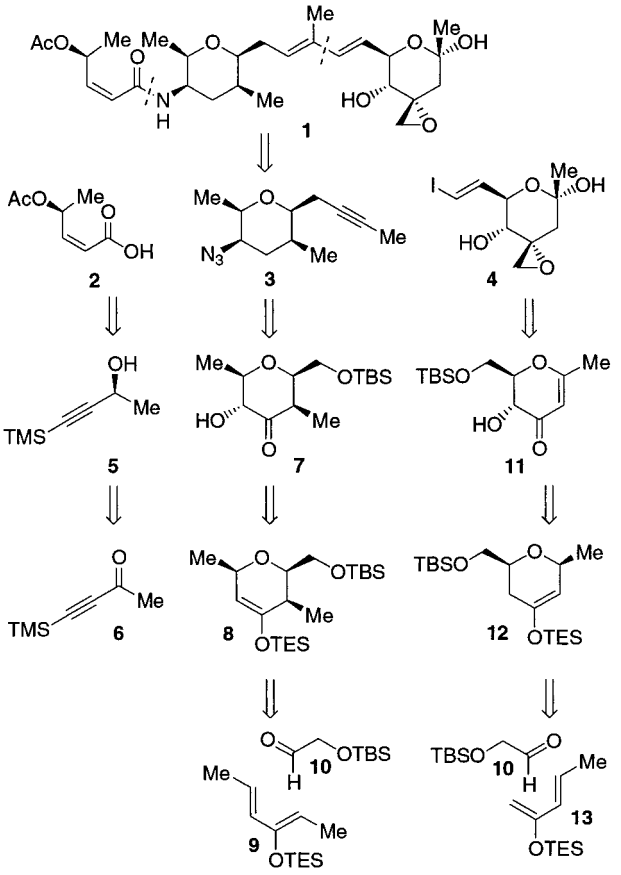
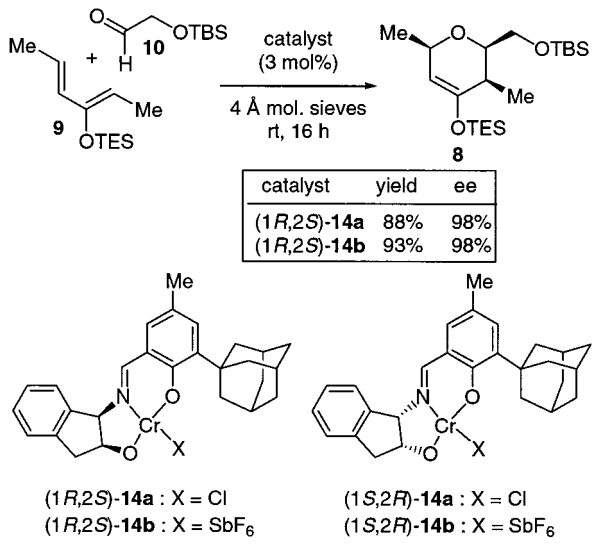
Scheme 1. Preliminary Retrosynthetic Analysis



degradation studies, but only the relative stereochemistry within each of the pyran rings had been ascertained by NMR.[9] Thus, the relative stereochemistry between the three fragments had not been established.[9a] In principle, the use of asymmetric catalytic methods would allow the construction of either enantiomer of each fragment in a straightforward manner using the same chemistry. Coupling these chiral fragments would provide access to diastereomers that differ only in the relative stereochemical relationship *between* the fragments, thereby allowing unambiguous assignment of the complete stereochemistry of FR901464. Finally, a convergent and flexible route to **1** would allow straightforward access to synthetic analogues of **1** of varying complexity. These may serve to help establish which elements of the complex structure are important for biological activity and ultimately may lead to the development of biological tools to help elucidate the mechanism of action of FR901464.

Our synthetic plan was devised taking all of the objectives outlined above into account. FR901464 contains two pyran rings joined via a diene moiety as well as an acyclic side chain attached to the central pyran ring by an α,β-unsaturated amide bond. Disconnection of the amide and the diene leads to fragments **2**−**4** (Scheme 1). We envisioned joining these components at the latest possible stage in the synthesis using a cross-coupling reaction to generate the diene and standard peptide coupling methods to form the amide. Our principal focus then became the highly efficient construction of the three chiral fragments of FR901464 using asymmetric catalytic methods. We anticipated taking advantage of recent advances in asymmetric reduction methodologies to introduce the lone stereocenter in fragment **2**. The two densely functionalized pyran rings, each of which contains four stereocenters, clearly presented a more substantial synthetic challenge. We recognized that an endo-selective asymmetric hetero-Diels−Alder (HDA) reaction between a hexadiene derivative such as **9** and a suitably functionalized aldehyde (e.g., **10**) would provide a most efficient construction of the pyran ring framework of the central fragment with simultaneous introduction of three out of the four stereocenters, as in **8**. A similar synthetic strategy might allow enantioselective access to dihydropyran derivative **12**, a possible intermediate in the preparation of the right-hand fragment.

However, at the outset of this project, no such enantioselective HDA reactions were known between unactivated aldehydes and dienes bearing a single electron-donating substituent.[10] Recognizing the potential utility of such methodology not only for the synthesis of **1** but also as a general synthetic method, we undertook a focused effort aimed toward the discovery of catalysts for the asymmetric HDA reaction between **9** and **10**. This led to the discovery of the novel tridentate chromium catalyst **14**, which provides the desired mono-oxygenated dihydropyran adduct **8** with excellent enantio- and diastereoselectivity (eq 1).[11] In addition, the new catalytic method

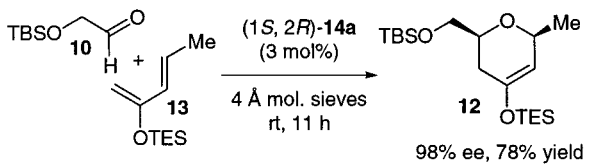


(1)

catalyst	yield	ee
(1*R*,2*S*)-**14a**	88%	98%
(1*R*,2*S*)-**14b**	93%	98%

(1*R*,2*S*)-**14a** : X = Cl
(1*R*,2*S*)-**14b** : X = SbF_6
(1*S*,2*R*)-**14a** : X = Cl
(1*S*,2*R*)-**14b** : X = SbF_6

exhibited promising scope with respect to both diene and dienophile, with a variety of reacting partners undergoing endo-selective cycloaddition with high ee. In particular, reaction of aldehyde **10** with pentadiene derivative **13** afforded dihydropyran **12** in 78% yield and 98% ee (>95% de) in the presence of catalyst (1*R*,2*S*)-**14a** (eq 2).[11]



(2)

98% ee, 78% yield

(8) For other work from this group illustrating this chiral building block approach, see: (a) Schaus, S. E.; Brånalt, J. E.; Jacobsen, E. N. *J. Org. Chem.* **1998**, *63*, 4876−4877. (b) Lebel, H.; Jacobsen, E. N. *J. Org. Chem.* **1998**, *63*, 9624−9625. (c) Chavez, D. E.; Jacobsen, E. N. *Angew. Chem., Int. Ed.*, in press.

(9) (a) Nakajima, H.; Takase, S.; Terano, H.; Tanaka, H. *J. Antibiot.* **1997**, *50*, 96−99. (b) Nakajima, H. Private communication.

(10) The only asymmetric HDA reactions able to employ a diene containing less than two oxygens required very reactive, electron-deficient aldehydes such as glyoxylate derivatives. For a recent review of the HDA reaction, see: Jorgensen, K. A. *Angew. Chem., Int. Ed.* **2000**, *39*, 3558−3588.

(11) Dossetter, A. G.; Jamison, T. F.; Jacobsen, E. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2398−2400.

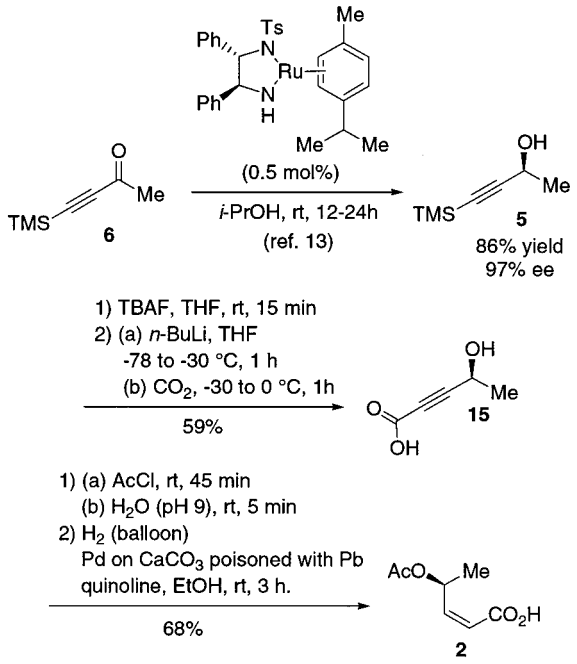
With the new asymmetric catalytic HDA reaction providing convenient access to the key building blocks **8** and **12**, the total synthesis of FR901464 required the development of effective routes to the fully elaborated tetrahydropyran units as well as a successful fragment coupling strategy. As described in this paper, both tasks presented significant challenges, ultimately requiring us to redesign our approach to the initial chiral building blocks. This exercise inspired the extension of the HDA methodology in interesting new directions, and it also led us to a substantially more efficient overall synthesis of **1** than that which was anticipated in the initial plan. The final route proved to be very flexible, providing straightforward access to diastereomers in order to confirm the absolute stereochemistry of **1**, as well as to novel analogues for preliminary biological studies.

Results and Discussion

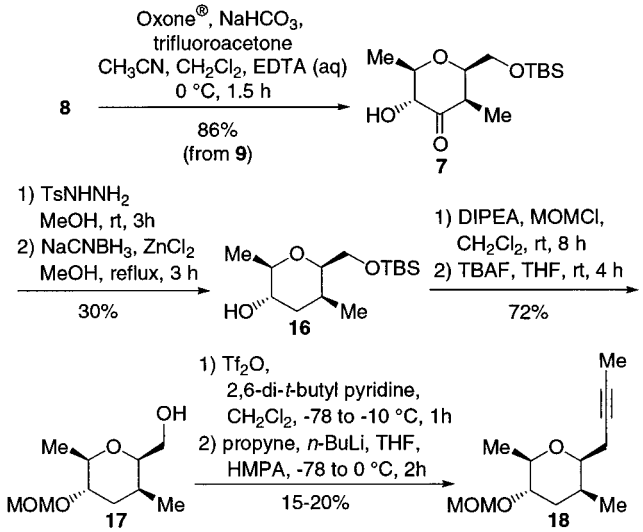
(I) Fragment Synthesis. Although the left-hand side chain **2** is a relatively simple, low-molecular-weight compound, its efficient synthesis presents interesting challenges nonetheless. We anticipated that the *Z*-substituted acrylic acid derivative might be sensitive to isomerization and other decomposition pathways, so we endeavored to introduce the alkene as late as possible in the synthesis by means of partial hydrogenation of the appropriate propargylic alcohol derivative. Of the various valuable and highly effective methods devised recently for the preparation of enantioenriched propargylic alcohols,[12] the Noyori Ru-catalyzed transfer hydrogenation of ynones[13] has proven to be particularly versatile.[8b,c] Its application to the reduction of commercially available 4-(trimethylsilyl)-3-butyn-2-one **6** afforded propargylic alcohol **5** in excellent yield and with high enantioselectivity (Scheme 2). Following desilylation, the terminal alkyne was carboxylated by sequential deprotonation and reaction with CO_2 to furnish hydroxy acid **15** upon workup.[14] Acetylation of the free hydroxyl group and Lindlar reduction provided the desired cis allylic acetate, thereby completing a short and efficient synthesis of fragment **2**.

The initial approach taken to the synthesis of the central fragment began with the asymmetric catalytic HDA reaction between commercially available aldehyde **10** and diene **9**, which afforded adduct **8** with outstanding enantio- and diastereoselectivity (eq 1).[11] Oxidation[15] of the silyl enol ether functionality provided α-hydroxy ketone **7** in good yield and with high diastereoselectivity (Scheme 3). Following tosylhydrazone formation, a one-pot reduction/fragmentation reaction led to the tetrasubstituted tetrahydropyran **16** in modest yield.[16] After a straightforward protection/deprotection sequence afforded **17**, installation of the alkyne was investigated. A wide variety of leaving groups, counterions, and reaction conditions were examined, yet this seemingly straightforward substitution proved extremely problematic. It was found that S_N2 displacement could be effected on the triflate derivative of **17** with propynyllithium

Scheme 2. Synthesis of the Left-Hand Side Chain



Scheme 3. Initial Approach to the Central Fragment



in THF/HMPA to furnish **18**, but this procedure was difficult to effect reproducibly and afforded low yields in the best of cases.

While the route outlined in Scheme 3 did provide access to the central pyran fragment, the late-stage incorporation of the alkyne unit in the conversion of **17** to **18** imposed a severe penalty with respect to yield and efficiency. In retrospect, the fact that the alkyne was being introduced in this manner was an artifact of the selection of silyloxyacetaldeyde **10** as the dienophile in the critical asymmetric HDA reaction. Indeed, it became evident that intermediate **8** was ill-suited as a precursor to the central fragment, a problem that was rendered particularly ironic by the fact that we had designed the asymmetric catalytic reaction producing **8** specifically for the purposes of this synthesis.

In reevaluating the key HDA reaction, we considered three approaches that would allow introduction of the alkyne moiety directly as a component of the dienophile in the HDA reaction. The first and most straightforward plan employed aldehyde **19**, wherein the triple bond is positioned correctly with respect to

(12) For leading recent examples, see: (a) Brown, H. C.; Ramachandran, V. P. *Acc. Chem. Res.* **1992**, *25*, 16−24. (b) Corey, E. J.; Cimprich, K. A. *J. Am. Chem. Soc.* **1994**, *116*, 3151. (c) Helal, C. J.; Magriotis, P. A.; Corey, E. J. *J. Am. Chem. Soc.* **1996**, *118*, 10938−10939. (d) Nakamura, M.; Hirai, A.; Sogi, M.; Nakamura, E. *J. Am. Chem. Soc.* **1998**, *120*, 5846−5847. (e) Tao, B.; Ruble, C.; Hoic, D.; Fu, G. C. *J. Am. Chem. Soc.* **1998**, *120*, 5091−5092. (f) Li, Z.; Upadhyay, V.; DeCamp, A. E.; DiMichele, L.; Reider, P. J. *Synthesis* **1999**, 1453−1458. (g) Frantz, D. E.; Fässler, R.; Carreira, E. M. *J. Am. Chem. Soc.* **2000**, *122*, 1806−1807.

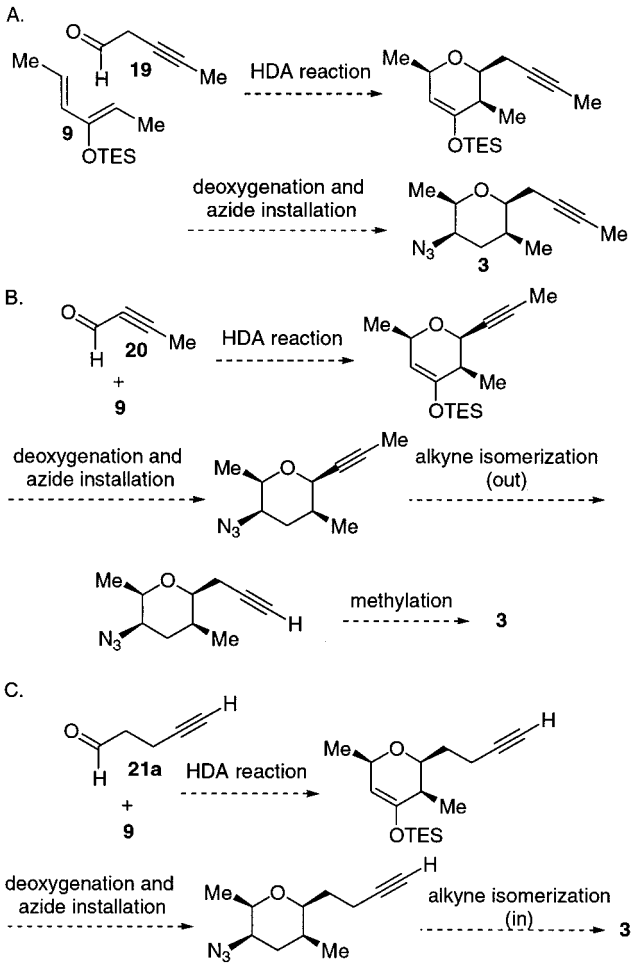
(13) Matsumura, K.; Hashiguchi, S.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1997**, *119*, 8378−8379.

(14) Getty, S.; Berson, J. *J. Am. Chem. Soc.* **1991**, *113*, 4607−4621.

(15) Yang, D.; Wong, M.; Yip, Y. *J. Org. Chem.* **1995**, *60*, 3887−3889.

(16) Kim, S.; Oh, C. H.; Ko, J. S.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927−1932.

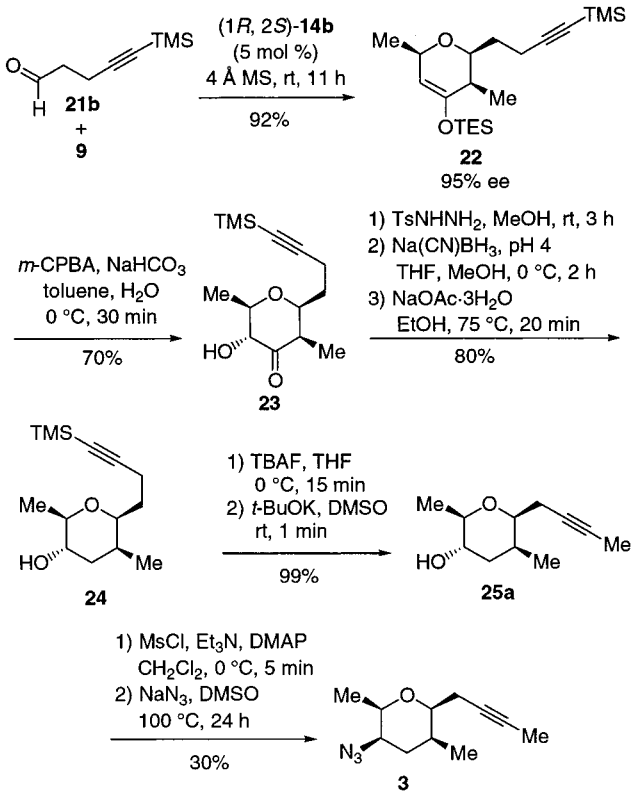
Scheme 4. New Synthetic Approaches to the Central Fragment



target fragment **3** (Scheme 4A). However, efforts to use aldehyde **19** in HDA reactions were unsuccessful, as this aldehyde proved to be highly unstable and decomposed under the reaction conditions, presumably through isomerization to highly reactive allenic intermediates. Attempts to effect HDA reactions of **19** protected as a dicobalt hexacarbonyl complex also led to complex product mixtures. The second strategy employed conjugated 4-carbon ynal **20** and relied on the well-precedented tendency of internal alkynes to migrate to a terminal position in the presence of strong base (Scheme 4B).[17] Completion of middle fragment **3** would then be attained by methylation of the terminal acetylide. The third approach relied on cycloaddition of the 5-carbon terminal ynal **21** and would require a far more unusual tactic involving selective isomerization of the terminal alkyne to an internal position[18] (Scheme 4C). This strategy was deemed the more attractive of the latter two, not only by virtue of it being more concise but also because it would involve establishing the entire carbon framework of **3** in the initial HDA reaction.

The asymmetric catalytic HDA reaction between **9** and aldehyde **21b** afforded adduct **22** with outstanding enantio- and diastereoselectivity (Scheme 5).[19,20] While both (1*R*,2*S*)-**14a** and

Scheme 5. Modified Route to the Central Fragment



(1*R*,2*S*)-**14b** catalyzed the reaction in good yield, the SbF_6 complex afforded substantially higher ee (95 vs 86%). As before, three of the four stereogenic centers found in the central pyran ring of **1** were thus established in a single reaction. Rubottom oxidation of **22** to **23** proceeded diastereoselectively[21] and in good yield using a biphasic system buffered with $NaHCO_3$ and toluene as the organic solvent. A two-step procedure was applied for reduction and fragmentation of the tosylhydrazone intermediate to provide **24** in good yield.[22] Following desilylation, the critical isomerization of the terminal alkyne to the desired internal position was effected with KOtBu in DMSO.[18] This precedented, yet rarely used methodology proved extraordinarily effective, with the reaction proceeding in virtually quantitative yield within 1 min.

Conversion of alcohol **25a** to the key intermediate **3** was accomplished in low yield by means of invertive displacement with NaN_3 on the corresponding mesylate. We had anticipated from the outset that such substitution processes on the central fragment would be challenging due to the sterically congested nature of the tetrasubstituted tetrahydropyran ring. Furthermore, we recognized that the azide functionality might prove incompatible with the conditions to be used in late-stage coupling reactions, and that therefore this substitution would have to be timed carefully in the overall synthetic sequence. As a result, we decided to postpone optimization of the nitrogen installation until the setting for coupling of the two pyran rings was more clearly defined.

Our first approach to the highly substituted right-hand pyran ring of **1** began with a HDA reaction between diene **13** and **10**

(17) Brown, C. A.; Yamashita, A. *J. Am. Chem. Soc.* **1975**, *97*, 891−892.

(18) Takano, S.; Shimazaki, Y.; Iwabuchi, Y.; Ogasawara, K. *Tetrahedron Lett.* **1990**, *31*, 3619−3622.

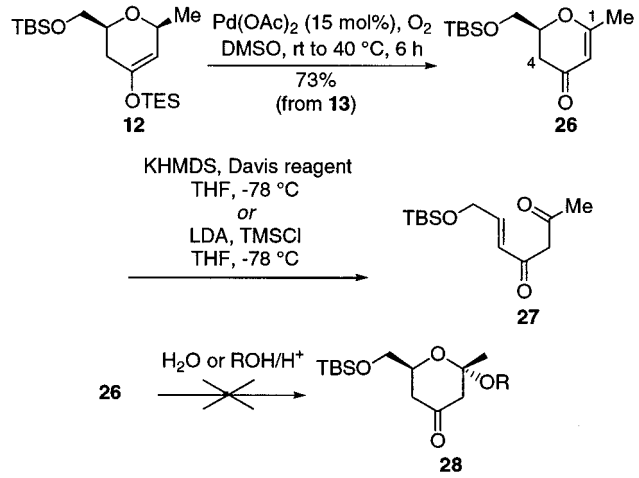
(19) For the method of synthesis of aldehyde **21a**, see: Cruciani, P.; Stammler, R.; Aubert, C.; Malacria, M. *J. Org. Chem.* **1996**, *61*, 2699−2708.

(20) Aldehyde **21a** underwent cycloaddition with similar ee, but its reactivity was greatly diminished. The absolute stereochemistry of HDA adducts was assigned by analogy to assignments made in ref 11.

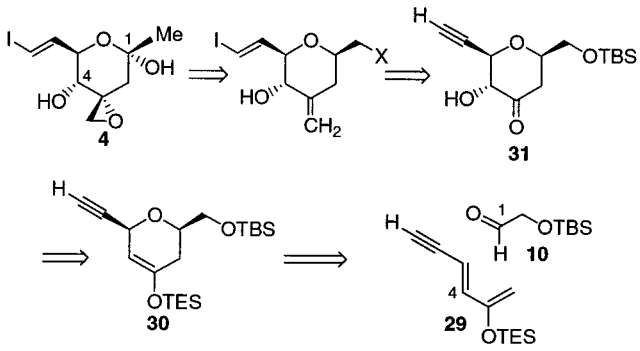
(21) The undesired epimer of the α-hydroxy ketone was generated in 10−15% yield but was removed readily by flash chromatography.

(22) Nair, V.; Sinhababu, A. *J. Org. Chem.* **1978**, *43*, 5013−5017.

Scheme 6. Initial Approaches to the Right-Hand Fragment



Scheme 7. New Retrosynthetic Analysis of the Right-Hand Fragment

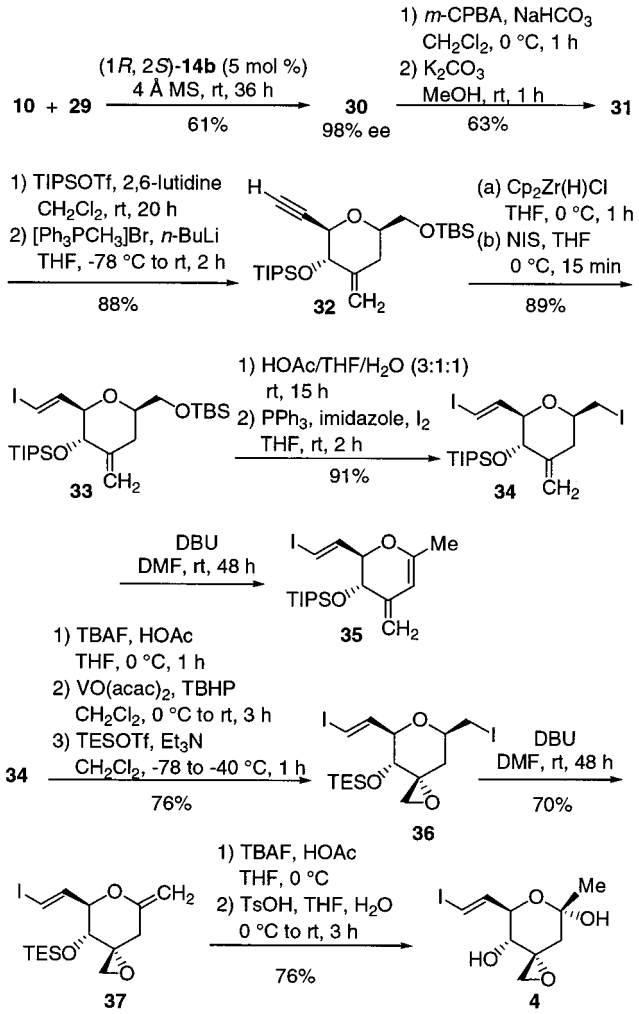


(eq 2). Cycloadduct **12** was subjected to Saegusa oxidation[23] to give dihydropyranone **26** in good yield (Scheme 6). However, efforts aimed toward elaboration of **26** to the fully functionalized right-hand fragment by enolization−oxidation of the ketone in **26** or by prior hydration to **28** were all unsuccessful. As in the case of the middle fragment, the problems encountered in the synthesis of the fully functionalized tetrahydropyran framework were tied to the original selection of reacting partners in the HDA reaction. Therefore, we sought an alternative route to the right-hand fragment that would utilize the key cyclization strategy to greater effect.

As illustrated in the retrosynthetic analysis in Scheme 7, installation of the C4 hydroxyl group as in **31** could be rendered straightforward if the enol ether generated in the HDA cyclization bore the regiochemistry shown in **30** rather than that of **12**. This would require reversal of the roles of the HDA reacting partners, such that the aldehyde dienophile would contribute the C1 carbon of the pyran ring. In this context, we were particularly intrigued by the possibility of using the novel dienyne **29** in a HDA reaction with **10** to begin the synthesis of the right-hand pyran (Scheme 7). If successful, the reaction would afford cycloadduct **30**, which bears the entire carbon framework of the right-hand fragment with the exception of the epoxide methylene unit. Also, the C4 hydroxyl could be installed directly from the silyl enol ether intermediate using a Rubottom oxidation, thereby avoiding the difficulties outlined above in manipulations of **12**. Finally, the C17 silyl ether would provide a functional handle to incorporate the hemiketal through an elimination/hydration sequence.

(23) (a) Larock, R. C.; Hightower, T. R.; Kraus, G. A.; Hahn, P.; Zheng, D. *Tetrahedron Lett.* **1995**, *36*, 2423−2426. (b) Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011−1013.

Scheme 8. Modified Route to the Right-Hand Fragment



Reaction between the dienyne **29**[24] and aldehyde **10** in the presence of catalyst (1*R*,2*S*)-**14a** led to formation of the highly functionalized pyran **30** in >99% ee (Scheme 8). However, this transformation proceeded slowly (>40 h), and **30** was isolated in <50% yield.[25] A switch to SbF_6 catalyst (1*R*,2*S*)-**14b** led to improved reactivity and product yield while maintaining high ee (98%). As anticipated, Rubottom oxidation of **30** afforded the α-hydroxy ketone directly. A mixture of epimers was obtained initially, but this could be subjected to equilibration with K_2CO_3 in MeOH to afford the desired isomer exclusively.[26] It was somewhat surprising and certainly fortunate that no transposition of the ketone and hydroxyl group was observed during this equilibration process. Following protection of the free hydroxyl group and methylenation of the ketone, selective reaction of the terminal alkyne with Schwartz's reagent and

(24) Dienyne **30** was synthesized from trimethylsilyl propynal in three steps with an overall yield of 50%. See Supporting Information.

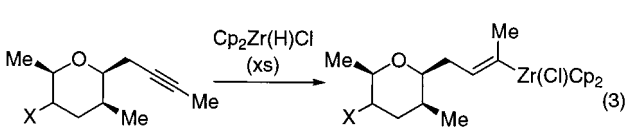
(25) For most cases examined, nearly quantitative yields are observed in HDA reactions with the tridentate Schiff base−chromium catalysts. However, both the dienyne **30** and HDA adduct **31** displayed only marginal stability, and this fact, combined with the sluggishness of this particular reaction, accounts for the modest yield in this reaction. No other products were observed. Results similar to those obtained with SbF_6 catalyst (1*R*,2*S*)-**14b** could be produced with the chloride catalyst (1*R*,2*S*)-**14a** at ∼50 °C (50−60% isolated yields, 96−98% ee).

(26) On a preparative scale (5−10 mmol), best results were obtained by subjecting the HDA reaction mixture to quick filtration through a pad of silica gel to remove sieves and catalyst. The crude HDA adduct was then subjected to Rubottom oxidation and equilibration with K_2CO_3. Using this procedure, a 30% yield for the three-step sequence could be obtained reproducibly.

trapping of the resulting vinylzirconium intermediate with NIS afforded vinyl iodide **33**.

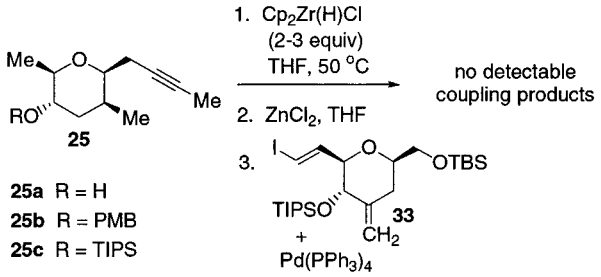
At this stage, an elimination/hydration sequence was attempted in order to install the hemiketal unit. The primary hydroxyl group was deprotected and converted to the iodide. However, prolonged treatment with DBU to effect elimination to the exocyclic enol led to spontaneous migration of the double bond to the endocyclic position to provide the undesired isomer **35**. Since enol ether isomerization was most likely driven by formation of the conjugated diene system, we evaluated whether this problem might be circumvented by installation of the epoxide functionality prior to the elimination event. This strategy would require that the potentially sensitive epoxide be carried through several steps of the synthesis, including the critical fragment-coupling events. On the other hand, if successful, this rather risky approach would allow a very direct route to the right-hand fragment. Deprotection of the TIPS group in **34** and directed epoxidation[27] of the alkene provided the epoxide as a single diastereomer. The free hydroxyl group was reprotected as a TES ether to provide **36**, and this compound was subjected to reaction with DBU in order to effect elimination of the alkyl iodide. The epoxide withstood the lengthy reaction time (48 h) that was required, and no isomerization of the enol ether to the endocyclic position was observed. Intermediate **37** was isolated in good yield and was readily purified by chromatography on silica gel and fully characterized. Deprotection of the epoxy alcohol and hydration of the enol ether with aqueous *p*-toluenesulfonic acid provided **4**, completing the synthesis of the fully functionalized right-hand pyran fragment. Although the compatibility of the epoxide with the conditions necessary for fragment coupling had not yet been addressed, this functionality had already displayed impressive robustness toward both basic and acidic reaction conditions, and its early installation had facilitated the challenging elimination/hydration sequence to introduce the hemiketal.

(II) Fragment Couplings and Completion of the Synthesis of FR901464. With synthetic access to all three fragments of FR901464 in hand, our efforts turned toward examination of coupling strategies. The relatively high degree of substitution in the conjugated dienyl unit of **1** would likely require highly reactive partners for the cross-coupling reaction. On the other hand, if the fragment assembly were to be executed at a late stage in the synthesis in order to maximize convergency, a high degree of functionalization in both pyran rings would have to be tolerated in the diene synthesis. Palladium-catalyzed Negishi coupling protocols generally display a balance of high reactivity and good functional group tolerance, and these were identified as most promising for linking the two pyran fragments. Hydrozirconation of the central fragment alkyne with Schwartz's reagent under equilibrating conditions was expected to proceed both stereospecifically and regioselectively to provide the desired vinylzirconium species (eq 3).[28] In initial studies it was found,

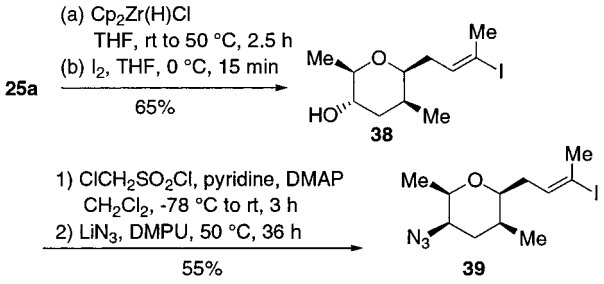


not unexpectely, that the azide did not survive treatment with excess Schwartz's reagent in THF at elevated temperatures. For this reason, we endeavored to link the two fragments prior to azide installation. However, hydrozirconation of **25** and attempted coupling to model system **33** under standard Negishi-type conditions did not yield any desired product, even with the hydroxyl group of **25** protected (Scheme 9). This was quite unexpected in light of the fact that Theodorakis et al. had carried out a hydrozirconation/Negishi coupling sequence successfully on a closely related system in the context of their synthesis of reveromycin B.[29]

Scheme 9. Unsuccessful Coupling Attempts



Scheme 10. Stereo- and Regioselective Synthesis of the Central Fragment as Vinyl Iodide Derivative **38**



These findings prompted us to redesign the cross-coupling reaction by introducing the central fragment as a vinyl iodide and the right-hand pyran as the vinylzinc component. This reversal of coupling partners was anticipated to have two significant advantages. First, hydrozirconation of the terminal alkyne in **32** (Scheme 8), a precursor to the right-hand pyran fragment, had been shown to proceed very cleanly under mild conditions. A high-yielding hydrometalation such as this was clearly desirable for the first step in an intricate coupling sequence involving multiple transformations. In contrast, treatment of the central fragment with Schwartz's reagent under more forcing conditions was found to generate several detectable byproducts. These impurities were possibly responsible for the failure of the coupling attempts outlined in Scheme 9. Formation of an isolable vinyl iodide intermediate (e.g., **38**, Scheme 10) would allow removal of these byproducts in a purification step. Second, because the $Cp_2Zr(H)Cl$ would be consumed by the point in the reaction at which the vinyl iodide was added, the central fragment could be elaborated to azide **39** prior to the cross-coupling event. This would entail a more convergent approach to the synthesis of FR901464.

Hydrozirconation of **25a** with excess $Cp_2Zr(H)Cl$ under equilibrating conditions followed by trapping the vinylzirconium intermediate with I_2 afforded the desired vinyl iodide **38** in 65% isolated yield (Scheme 10).[30] As anticipated, installation of the azide required careful optimization of the leaving group, nucleophile, and reaction conditions. It was found that the azide substitution product **39** could be accessed in 55% yield by conversion of alcohol **37** to the monochloromethanesulfonate

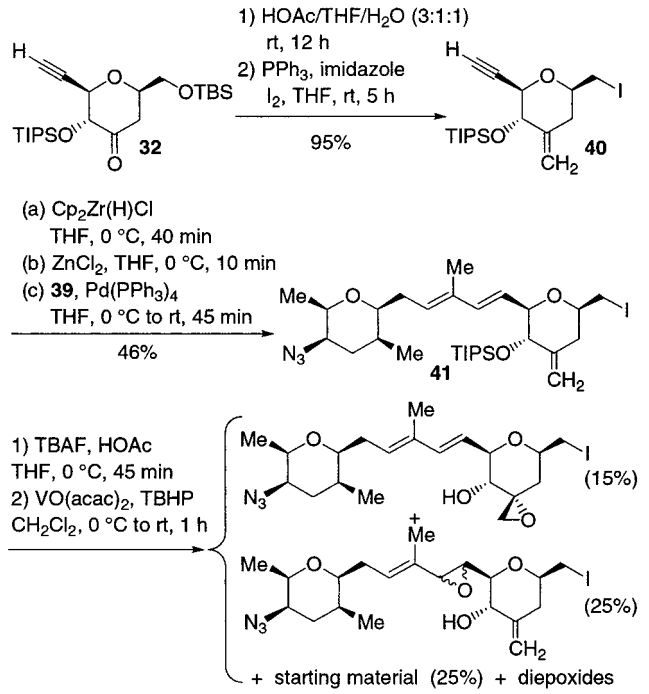
(27) Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev.* **1993**, *93*, 1307−1370.

(28) Hu, T.; Panek, J. S. *J. Org. Chem.* **1997**, *62*, 4912−4913.

(29) Drouet, K. E.; Theodorakis, E. A. *J. Am. Chem. Soc.* **1999**, *121*, 456−457.

(30) None of the undesired vinyl iodide regioisomer was obtained. Some more polar byproducts were isolated, but their structure was not completely determined.

Scheme 11. Attempted Fragment Coupling/Epoxidation Sequence



derivative,[31] followed by displacement with LiN_3 in DMPU.[32] Cross-coupling was then accomplished by hydrozirconation of **32**, transmetalation to Zn, and reaction with vinyl iodide **39** in the presence of $Pd(PPh_3)_4$ (Scheme 11). The reaction proceeded smoothly to produce **41** in an unoptimized yield of 46%. However, attempted installation of the spiro epoxide via directed epoxidation reaction proved unsuccessful. Competing oxidation of the homoallylic conjugated double bond led to side products, and the desired allylic epoxide was generated only in low yield.

Faced with this difficulty in installing the epoxide after fragment coupling, we ventured to attempt the coupling reaction with the epoxide already in place. Although we could find no precedent for selective hydrozirconation of an alkyne in the presence of an epoxide, we relied on the very high reactivity of Schwartz's reagent toward terminal alkynes. Indeed, hydrozirconation of **42**, followed by coupling to vinyl iodide **38** under standard Negishi conditions, provided the desired highly functionalized diene **43** in excellent yield (Scheme 12).

At this point, completion of the synthesis of FR901464 required only installation of the hemiketal and azide reduction followed by acylation of the resulting amine with side chain **2**. The ordering of these events was critical, and it proved preferable to introduce the side chain prior to hemiketal formation. The concern with such a sequence was that epimerization of the C4′ stereocenter or acetate elimination might occur during the lengthy reaction time required for iodide elimination. Fortunately, model system **44** was recovered in nearly quantitative yield with no deterioration of enantiopurity when subjected to the reaction conditions used for iodide elimination. With this information in hand, completion of the synthesis of FR901464

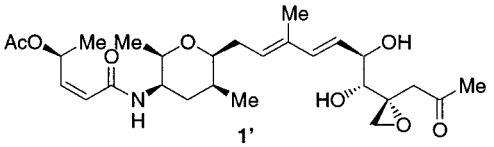
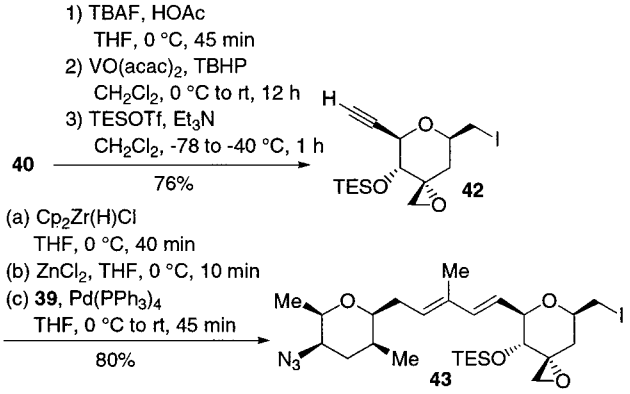
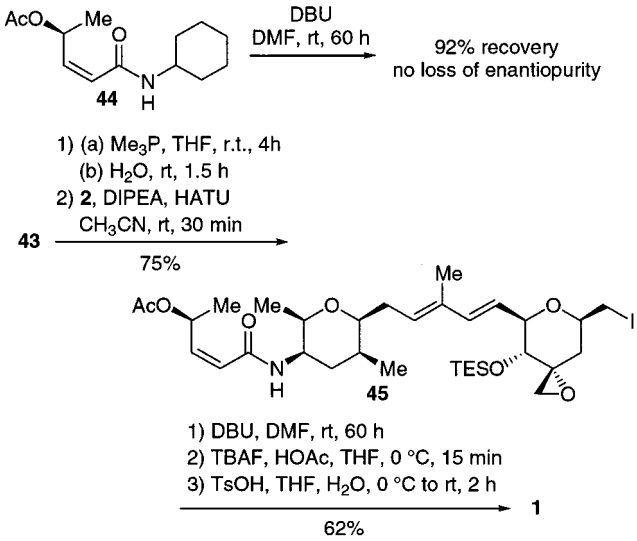


Figure 2. Open-chain form of FR901464.

Scheme 12. Cross-Coupling of the Fully Functionalized Pyran Units



Scheme 13. Completion of the Synthesis



proved straightforward (Scheme 13). Reduction of azide **43** with Me_3P[33] followed by amide bond formation using standard coupling conditions provided **45** in good yield. An elimination/deprotection/hydration sequence was then used to install the hemiketal and complete the synthesis of FR901464. ^1H and ^{13}C NMR spectra of this synthetic material matched those of the natural product provided by Dr. H. Nakajima (Fujisawa). HRMS and optical rotation data also corresponded with those of the natural product. It was interesting to note that the ^1H NMR spectra of both synthetic and natural FR901464 displayed a series of minor peaks (~10%), consistent with the presence of a minor isomer of the natural product.[34] Evidence was obtained subsequently (vide infra) that this minor isomer is the open-chain ketone form of the molecule (**1′**, Figure 2).

(III) Confirmation of the Structure of FR901464. Despite the fact that NMR and optical rotation data for synthetic **1**

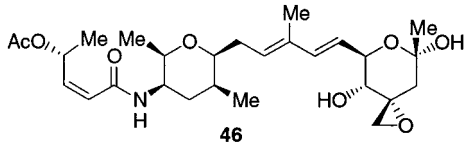
(31) Shimizu, T.; Ohzeki, T.; Hiramoto, K.; Hori, N.; Nakata, T. *Synthesis* **1999**, 1373−1385.

(32) The use of mesylate as a leaving group provided only traces of the desired product. The triflate was unstable and afforded 20−30% yield of the desired product. Low yields were obtained with the nosylate leaving group. LiN_3 was superior to NaN_3. DMPU was superior to move traditional solvents for azide displacements such as DMF and DMSO, which gave very low yields of the desired products. Elimination (35%) was a competing side reaction.

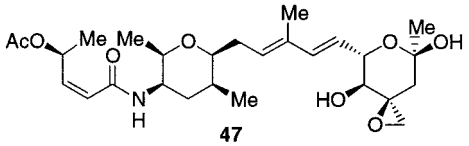
(33) Knapp, S.; Jaramillo, C.; Freeman, B. *J. Org. Chem.* **1994**, *59*, 4800−4804. Me_3P was used because reduction of the sterically hindered azide did not reach completion after several days in the presence of Ph_3P.

(34) The presence of ~10% of a minor isomer was also seen in the ^1H NMR of **4**.

Table 1. Proof of Structure



46



47

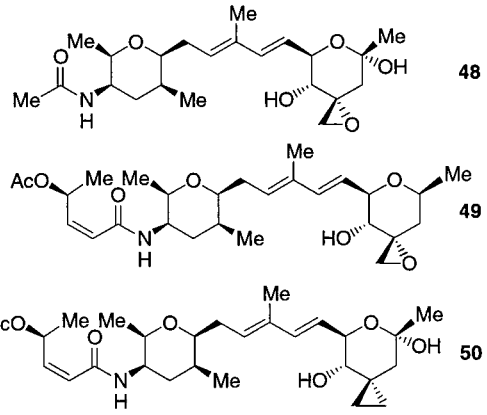
compound	^{1}H NMR spectrum	$[\alpha]^{23}_D$[a]
1	matches natural product	−13.0
46	differs from natural product	+12.7
47	matches natural product	- 69.4

[a] All measurements made in CH_2Cl_2. Lit.[1] $[\alpha]^{23}_D$ for FR901464 = −12 (*c* 0.5, CH_2Cl_2).

matched those of natural FR901464, the possibility remained that we had in fact prepared a diastereomer of natural product comprised of fragments with the same relative but different absolute stereochemistry. As noted in the Introduction, in the original structural elucidation of FR901464 the absolute stereochemistry at C4′ had been established with certainty by degradation of the natural product, but only the relative stereochemistry was reported for the pyran rings.[9a,b] Because the three chiral components of FR901464 are isolated each from the other by planar, conjugated linker elements, we could not rule out that diastereomers containing fragments of different absolute but identical relative stereochemistries might give rise to indistinguishable NMR spectra. To ascertain that we had indeed prepared FR901464 and with the goal of making a corresponding, unambiguous stereochemical assignment of the natural product, we prepared compounds **46** and **47** (Table 1).[35] Although compound **46** bears the unnatural stereochemistry at C4′, and therefore could not have been the correct stereoisomer, it was examined in order to assess whether FR901464 might have the structure of *ent*-**46**. Although the optical rotation of **46** matched that of the natural product (with opposite sign), the ^{1}H NMR of **46** displayed significant differences from that of the natural product. In particular, the NH proton was shifted downfield by 0.15 ppm while the C4′ proton was shifted upfield by 0.15 ppm relative to their positions in the spectra of natural FR901464. From this information, it was clear that synthetic **1** had the correct relative stereochemical relationship between the left-hand and central fragments. It remained to be shown that the right-hand pyran of synthetic **1** had the correct absolute stereochemistry.[36] The ^{1}H NMR spectrum of compound **47** matched that of FR901464, but the observed optical rotation differed significantly from that of the natural product. This established that **47** is not FR901464 and allowed us to verify that synthetic **1**, prepared as described, is the same compound as FR901464 reported by the Fujisawa Pharmaceutical Co. in 1996.

(35) These compounds were prepared using the chemistry described for the synthesis of FR901464 but employing enantiomeric catalysts for the HDA or Noyori transfer hydrogenation as needed.

(36) We did not evaluate the diastereomer of **1** bearing only a central fragment of opposite absolute stereochemistry (i.e., *ent*-**3**) as that assigned to FR901464, but it can be ruled out as a possibility, nonetheless. This is due to the fact that the *relative* stereochemistry between the left-hand and central fragments is established by evaluation of **47**. In order for the structure bearing *ent*-**3** to be correct, it would require that the chemical shift changes observed in **47** for the NH and C4′ protons relative to FR901464 be canceled out exactly by switching the configuration of the right-hand fragment.



48

49

50

Figure 3. Analogues of FR901464 synthesized and assayed.

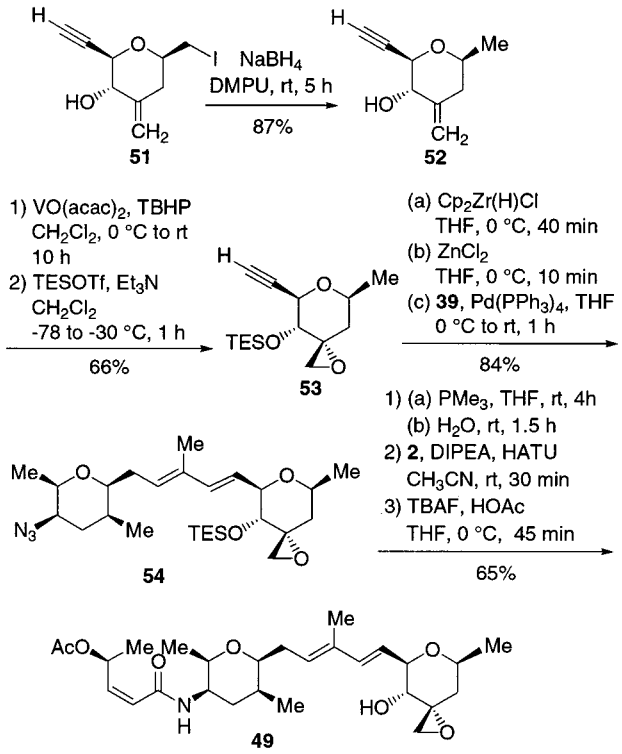
(IV) Synthesis and Evaluation of Analogues of FR901464. In light of the impressive biological activity and intriguing mode of action reported for **1**, we decided to synthesize several nonisomeric analogues of the natural product and evaluate them in a biological assay along with the natural and unnatural diastereomers of FR901464. In particular, compounds **48**−**50** (Figure 3) were chosen as targets for synthesis in order to probe the importance of certain structural features of FR901464. We hoped that the information gathered in studying analogues of **1** would lay the groundwork for preparation of simpler compounds that retain the biological activity of FR901464 and can be used as biological tools. Also, we envision future studies to discern the as yet unidentified cellular target of FR901464 using affinity chromatography, or by attachment of a fluorescent or photoaffinity label to the natural product. To attempt these types of experiments, it is important to find a region of FR901464 that can be modified without causing a deleterious effect on biological activity.

Compound **48** was synthesized in the same manner as FR901464, except that following azide reduction the amine was simply acetylated with Ac_2O.[37] This compound was designed to test the importance of the side chain for biological activity. Clearly, if the side chain proved unnecessary, the amine would provide a convenient functional handle for use in biological assays. Deshydroxy compound **49** was synthesized by the route depicted in Scheme 14, the main deviation from the synthesis of **1** being the reduction of the iodide in **51** to a methyl group using $NaBH_4$ in DMPU. Compound **49** was of interest to probe the importance of the hemiketal, which allows natural FR901464 to access minor isomers (e.g., open chain or furanose) that may be of biological importance. Also, **49** was expected to circumvent the instability observed in FR901464 that is attributable to the hemiketal.[38] Finally, compound **50** was synthesized to probe the role of the epoxide (Scheme 15). The cyclopropyl group was installed via a Simmons−Smith reaction, and the remainder of the synthesis paralleled that of FR901464. Upon completion of the synthesis of **50**, we were surprised to find that the ratio of **50** to the minor isomer with which it was in equilibrium was ∼1.5:1 in CD_2Cl_2 rather than the ∼10:1 ratio observed for FR901464 (vide supra). On the basis of spectroscopic evidence, it appears that the minor component is the open-chain ketone form of the molecule.[39] The difference in the ratio of isomers was surprising, as we had expected the cyclopropane to enforce a conformation in the right-hand pyran

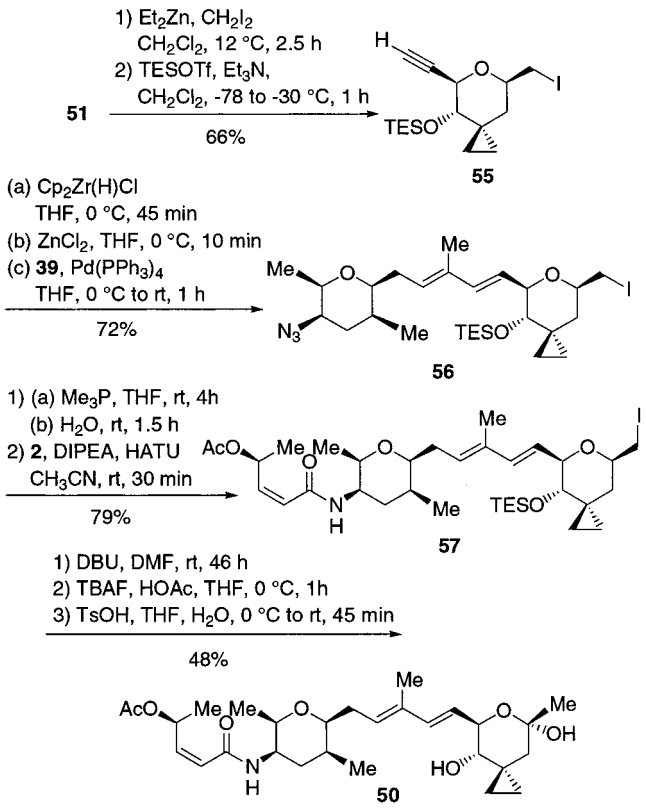
(37) See Supporting Information for details.

(38) FR901464 could be isolated by purification on preparative TLC, but significant decomposition was observed when purification by flash chromatography was attempted. FR901464 is unstable in MeOH and in $CHCl_3$.

Scheme 14. Synthesis of **49**



Scheme 15. Synthesis of **50**



similar to that seen with the epoxide. The shift in equilibrium could possibly be explained by an intramolecular H-bond between the hydroxyl group of the hemiketal and the epoxide, and this stabilization may help to favor the cyclic structure.

The results of a biological assay testing the effect of FR901464, its diastereomers, and the other synthetic analogues on Tag Jurkat cells are summarized in Table 2.[40] Tag Jurkat cells were treated with THF solutions of the compounds shown in Table 2, while control Tag Jurkat cells were treated with THF alone; cells treated with compounds and control cells were counted after 48 h. IC_{50} values were determined by treating the cells with solutions of the compounds being tested over a range of concentrations.[41] It is important to note that this assay involves the ability of these compounds to inhibit the growth of whole cells and that changes in biological activity may be unrelated to the ability of the compounds to interact with the target of FR901464. Other factors, such as solubility, ease of entry into the cells, stability, and even changes in the mechanism of action, may be responsible for observed gains or losses in activity with respect to the natural product. Such factors are hard to assess in such a general assay, and, although interesting, the biological data presented here must be interpreted with caution. The IC_{50} of FR901464 was determined to be 1 ng/mL, a value which corresponds closely to IC_{50} values reported by Fujisawa scientists for FR901464 against several cancer cell lines.[1] Diastereomers **46** and **47** are less active than **1** by 1 and 2 orders of magnitude, respectively. This drop in biological activity further serves to confirm the correct structure of FR901464. In fact, the biological activity observed for **47** is very possibly due to contamination with a small amount of the natural diastereomer **1**, because the right-hand ring used in the synthesis of this compound is only of 98−99% ee.

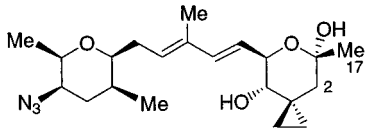
Table 2. Activity of **1** and Synthetic Analogues in Cell-Killing Experiments[a]

compound	IC_{50} (ng/mL)	IC_{50} (nM)
1	1 ± 0.2	2
46	15 ± 5	30
47	200 ± 100	400
48	700 ± 200	1700
49	0.8 ± 0.2	1.6
50	>2000	>4000

[a] Compounds were administered as THF solutions and assayed for their ability to inhibit the growth of Tag Jurkat cells. See text and Supporting Information for details.

The side chain of FR901464 does appear to be key for biological activity, as a nearly 3 orders of magnitude drop in the ability to inhibit cell growth was observed when the side chain was replaced by an acetate group (compound **48**). Also, replacement of the epoxide with a cyclopropyl moiety (compound **50**) led to a complete loss in biological activity at the highest concentrations tested, demonstrating the importance of the epoxide functionality in the biological activity of **1**.[42] In

(39) The model system depicted below was synthesized and also existed as a ~1.5:1 ratio of isomers, with the major one being the one drawn. The IR spectrum contained a band at 1705 cm^{-1}, indicating the presence of a carbonyl group. Also, the ^1H NMR of this compound displayed signals for the C-17 methyl group and both C-2 protons that were shifted downfield by 0.8 ppm in the minor isomer. These facts are consistent with the minor isomer being the open-chain ketone form.



(40) We were unable to obtain the M-8 cell line from Fujisawa and were unsuccessful in developing a more specific assay based on up-regulation of the SV40 gene using transient transfection (see ref 4). Tag Jurkat cells are a human T cell lymphoma expressing the T antigen (Tag). See: Northrop, J. P.; Ulman, K. S.; Crabtree, G. R. *J. Biol. Chem.* **1993**, *268*, 2917−2923.

(41) The IC_{50} is the minimum concentration of compound that causes cells to grow at ≤50% the rate of control cells.

(42) FR901463 bears a chlorohydrin in place of the epoxide (ref 1) and may be functionally equivalent.

contrast, desoxy analogue **49** retained fully the activity of the parent molecule. This result establishes that the closed tetrahydropyran ring is sufficient for activity, and it suggests that the minor, open-chain ketone isomer is not responsible for the cytotoxicity of FR901464. The fact that the hemiketal is not a critical feature of FR901464 points to this terminus of the molecule as a site for coupling to a resin or attachment of a fluorescent tag or photoaffinity label.

Conclusions

The total synthesis of FR901464 showcases the power of asymmetric catalytic reactions as a core strategy in natural products synthesis, and also illustrates how a target can give rise to an entirely new enantioselective reaction methodology. The discovery of novel tridentate chromium catalyst **14** was inspired and guided by the quest for an efficient route to the central fragment of **1**. In the end, asymmetric hetero-Diels−Alder reactions with highly functionalized, alkyne-containing reaction partners led to efficient syntheses of both the central and right-hand pyran fragments of FR901464. Finally, high-yielding coupling reactions allowed completion of the convergent synthesis.

The power of the chiral building block strategy and the flexibility of this particular synthetic route were further demonstrated in the preparation of analogues for proof of the FR901464 structure and for biological studies. Ready access to either enantiomer of each chiral fragment allowed us to synthesize a series of compounds that proved the relative and absolute stereochemistry of **1**. Removal of the hemiketal functionality of FR901464 resulted in a new compound (**49**) that is more stable than **1**, exists as a single isomeric form, and retains the biological activity of the natural product. Thus, the right-hand terminus of FR901464 has been identified as a site for the types of modifications that may prove useful in future biological studies. We hope this will enable the discovery of the cellular target of **1**, a worthwhile goal given the potent biological activity and striking phenotypic changes induced by FR901464.

Acknowledgment. This work was supported by the NIH (GM-59316) and by a postdoctoral fellowship to T.F.J. from the Cancer Research Fund of the Damon Runyon−Walter Winchell Foundation (DRG-1431). We thank Dr. H. Nakajima (Fujisawa) for generously providing spectra of **1** and D. Schmidt (Harvard) for help with biological assays.

Supporting Information Available: Complete experimental procedures and characterization data, and NMR spectra of synthetic and natural FR901464 (**1**) and **46**−**50** (PDF). This material is available via the Internet at http://pubs.acs.org.

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