# Total Synthesis of the Antitumor Depsipeptide FR-901,228

Khan W. Li, Jerry Wu, Wenning Xing, and Julian A. Simon*,†

*Department of Chemistry and Chemical Biology*
*Harvard University*
*Cambridge, Massachusetts 02138*

*Received April 26, 1996*

The identification in 1982 of a mutation in the Ha-*ras* gene provided the first indication of a link between specific molecular changes in regulatory proteins and cancer.[1−3] The mutant *ras* protein, in which valine replaces glycine-12, was shown capable of inducing morphological changes in mouse fibroblast (NIH-3T3) cells. In these cells the transformed phenotype is indicative of oncogenic activation and correlates with increased tumorigenicity. Recently, a diverse class of natural products and synthetic drugs has been identified that reverses this phenotype and hence "de-transforms" tumorigenic cell lines. Among these are radicicol[4,5] and the tyrphostins,[6] leptomycin B,[7,8] L-739,749,[9] and trapoxin A.[10,11] A new member of this class, FR-901,228 (**1**), was isolated from the culture broth of the terrestrial bacterium *Chromobacterium violaceum* using a phenotypic reversion assay of Ha-*ras* transformed NIH-3T3 cells. FR-901,228 was also shown to be highly active in animal-based assays.[12−14] This finding is not surprising since the ability of a drug to reverse the morphological effect of oncogenic transformation is often accompanied by in vivo antitumor activity. The molecular basis for either activity of FR-901,228 has yet to be identified.[15]

FR-901,228 (**1**) (Figure 1) is a bicyclic depsipeptide structurally unrelated to known classes of cyclic peptides.[16] In addition to a dehydro amino acid, *Z*-butyrine, the depsipeptide incorporates an unusual building block, (3*S*,4*E*)-3-hydroxy-7-mercapto-4-heptenoic acid **5a**. A disulfide bond between this thiol and

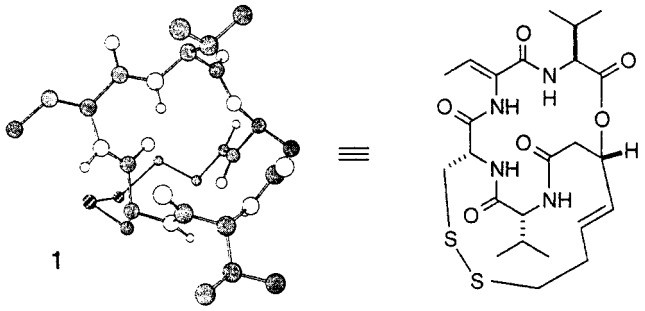
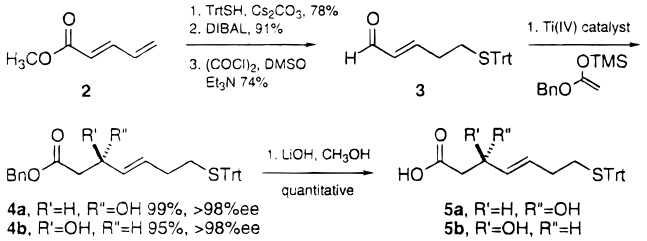



**Figure 1.**

**Scheme 1**




D-cysteine suggests the possibility of a redox-controlled conformational switch.[17] The reducing environment inside the cell is expected to convert FR-901,228 to a monocyclic dithiol. The effect of disulfide reduction on the detransforming activity is unknown. In an effort to address issues raised by the striking antitumor activity of FR-901,228, we have undertaken its total synthesis.

There are three principal challenges associated with the synthesis of FR-901,228: (1) the asymmetric construction of the hydroxy mercapto heptenoic acid, (2) the formation of a 16-membered cyclic depsipeptide, and (3) an intramolecular oxidative coupling of the thiols to form a 15-membered disulfide-containing ring. First, we planned to use an asymmetric aldol reaction to construct a protected mercapto $\beta$-hydroxy acid. Secondly, we anticipated that acylation of the allylic secondary alcohol would render it susceptible to elimination and therefore designed a route in which the ester bond is formed late in the synthesis. Finally, molecular modeling of a monocyclic depsipeptide intermediate suggested that the conformation required for intramolecular disulfide formation would be accessible, being within 2 kcal/mol of the global minimum. In this communication we describe the successful application of this approach.

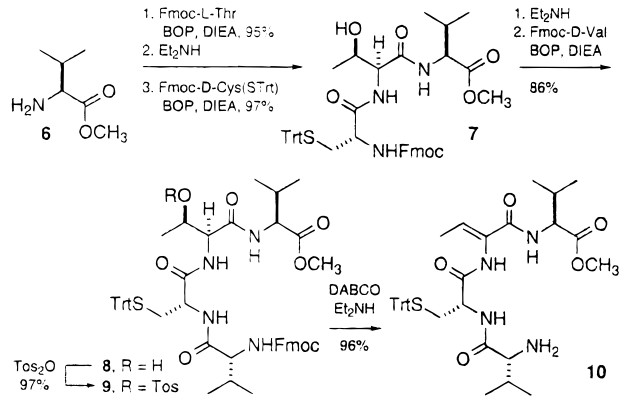
The Carreira catalytic asymmetric aldol reaction was used to synthesize the thiol-containing $\beta$-hydroxy acid.[18] The aldol substrate **3** was prepared by the three-step sequence shown in Scheme 1. Conjugate addition of cesium triphenylmethyl thiolate anion to methyl 2,4-pentadienoate **2** afforded a $\beta,\gamma$-unsaturated methyl ester which, upon further exposure to $Cs_2CO_3$, yielded the $\alpha,\beta$-unsaturated ester. DIBAL reduction to the primary alcohol followed by Swern oxidation gave the $\alpha,\beta$-unsaturated aldehyde **3**. On the basis of the sense of asymmetric induction observed by Carreira et al., we anticipated that Ti(IV)-catalyzed addition of *O*-benzyl, *O*-TMS ketene acetal to aldehyde **3**, in the presence of the ligand derived from (*S*)-(−) binaphthyl amino alcohol, would afford an aldol with the naturally occurring (*S*) configuration. The aldol product **4a** was formed in 99% yield with >98% enantiomeric excess as judged by $^{19}F$ NMR of the corresponding MTPA ester. The correct absolute stereochemistry was confirmed by the subsequent

† Present address: Molecular Pharmacology Program, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104 (e-mail, jsimon@fred.fhcrc.org).

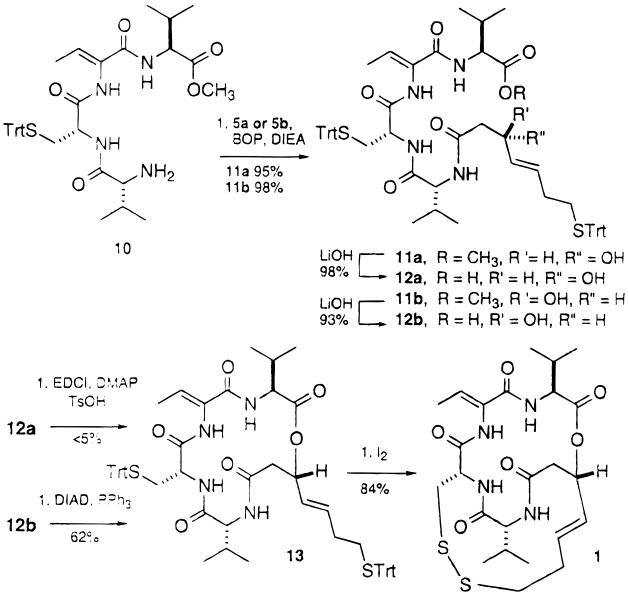
(1) Tabin, C. J.; Bradley, S. M.; Bargmann, C. I.; Weinberg, R. A.; Papageorge, A. G.; Scolnick, E. M.; Dhar, R.; Lowry, D. R.; Chang, E. H. *Nature (London)* **1982**, *300*, 143−149.
(2) Reddy, E. P.; Reynolds, R. K.; Santos, E.; Barbacid, M. *Nature (London)* **1982**, *300*, 149−152.
(3) Taparowsky, E.; Suard, Y.; Fasano, O.; Shimizu, K.; Goldfarb, M.; Wigler, M. *Nature (London)* **1982**, *300*, 762−765.
(4) Evans, G.; White, N. H. *Trans. Br. Mycol. Soc.* **1966**, *49*, 563−576.
(5) Zhao, J. F.; Nakano, H.; Sharma, S. *Oncogene* **1995**, *11*, 161−173.
(6) Levitzki, A.; Gazit, A. *Science* **1995**, *267*, 1782−1788.
(7) Hamamoto, T.; Gunji, S.; Tsuji, H.; Beppu, T. *J. Antibiot.* **1983**, *36*, 639−645.
(8) Nishi, K.; Yoshida, M.; Fujiwara, D.; Nishikawa, M.; Horinouchi, S.; Beppu, T. *J. Biol. Chem.* **1994**, *269*, 6320−6324.
(9) Kohl, N. E.; Wilson, F. R.; Mosser, S. D.; Giuliani, E.; DeSolms, S. J.; Conner, M. W.; Anthony, N. J.; Holtz, W. J.; Gomez, R. P.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9141−9145.
(10) Itazaki, H.; Nagashima, K.; Sugita, K.; Yoshida, H.; Kawamura, Y.; Yasuda, Y.; Matsumoto, K.; Ishii, K.; Uotani, N.; et al. *J. Antibiot.* **1990**, *43*, 1524−1532.
(11) Taunton, J.; Hassig, C. A.; Schreiber, S. L. *Science* **1996**, *272*, 408−411.
(12) Ueda, H.; Nakajima, H.; Hori, Y.; Fujita, T.; Nishimura, M.; Goto, T.; Okuhara, M. *J. Antibiot.* **1994**, *47*, 301−310.
(13) Shigematsu, N.; Ueda, H.; Takase, S.; Tanaka, H.; Yamamoto, K.; Tada, T. *J. Antibiot.* **1994**, *47*, 311−314.
(14) Ueda, H.; Manda, T.; Matsumoto, S.; Mukumoto, S.; Nishigaki, F.; Kawamura, I.; Shimomura, K. *J. Antibiot.* **1994**, *47*, 315−323.
(15) Ueda, H.; Nakajima, H.; Goto, T.; Okuhara, M. *Biosci., Biotechnol., Biochem.*, **1994**, *58*, 1579−1583.
(16) Triostin A is a bicyclic depsipeptide with a disulfide bridge. Olsen, R. K. *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, Chapter 1, pp 1−33. Ulithiacyclamide is a thiazole and oxazoline containing cyclopeptide with a disulfide bridge. Ireland, C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688−5691.
(17) Shioiri, T.; Hamada, Y.; Kato, S. *Biochem. Pharmacol.* **1987**, *36*, 4181−4185.
(18) Carreira, E. M.; Singer, R. A.; Lee, W. *J. Am. Chem. Soc.* **1994**, *116*, 8837−8838.

S0002-7863(96)01372-8 CCC: $12.00 

**Scheme 2**




**Scheme 3**




conversion to natural FR-901,228 (see below). Hydrolysis of the benzyl ester with LiOH in MeOH/$H_2O$ gave the hydroxy acid **5a** in five steps with an overall 52% yield.

The peptide portion was assembled by standard peptide synthesis methods as shown in Scheme 2. L-valine methyl ester was coupled to *N*-Fmoc-L-threonine using the BOP reagent (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate).[19] Removal of the *N*-Fmoc group with $Et_2NH$, followed by coupling to *N*-Fmoc-D-cysteine-(*S*-triphenylmethyl), provided the tripeptide **7** in a 92% yield. The tetrapeptide **8** was prepared by deprotection of **7** and BOP-mediated peptide coupling of the resulting amine with *N*-Fmoc-D-valine. At this stage the L-threonine residue was converted into *Z*-dehydrobutyrine. The secondary hydroxyl group was activated as the tosylate **9** which underwent elimination upon treatment with DABCO. Addition of $Et_2NH$ to the reaction mixture effected the removal of the Fmoc protecting group to give **10** in 74% yield from L-valine.

Coupling of **5a** and **10** with BOP, DIEA proceeded smoothly (Scheme 3) to give the hydroxy methyl ester **11a**, and LiOH-mediated hydrolysis afforded **12a** in 93% yield. At this juncture we turned our attention to forming the crucial ester linkage. A survey of the cyclic depsipeptide literature suggested that macrolactonization of peptide-containing hydroxy acids is a more difficult task than similar amide bond-forming cyclizations.[20] Macrolactonization of **12a** under a variety of conditions yielded only recovered starting material or undesired side products. The Keck modification[21] of the Steglich esterification (carbodiimide, DMAP·HOTs, THF) yielded the desired lactone **13** but in a disappointingly low yield. The ready availability of the enantiomeric aldol product **5b** led us to consider an esterification strategy involving hydroxyl activation (e.g. Mitsunobu reaction[22] ). Thus, the peptide **11b** containing the unnatural (*R*)-(+) hydroxy acid **5b** was prepared by an analogous route using the (*R*)-(+) binaphthyl amino alcohol in the aldol reaction. Cyclization of the hydroxy acid **12b** with DEAD and $PPh_3$ afforded a lactone identical to **13** as well as several side products. Extensive optimization of the Mitsunobu reaction led to conditions that afforded the lactone **13** in 62% yield.[23] Addition of TsOH was critical for suppressing elimination of the activated allylic alcohol.[24,25] Oxidation of the bis-(*S*-triphenylmethyl)lactone with iodine in dilute MeOH solution[26] provided an 84% yield of FR-901,228 (**1**). The synthetic material gave $^1H$ and $^{13}C$ NMR spectral data as well as optical rotation identical to those published for the natural product. The synthetic sequence presented in this communication gives the depsipeptide FR-901,228 in 14 steps and an overall 18% yield. The Mitsunobu reaction, previously used in the several syntheses of nonpeptidic lactones,[27−29] was shown to be effective in depsipeptide construction as well.

Chemical synthesis provides the most effective means to assess the functional role of the various elements found in FR-901,228. For example, a depsipeptide analog lacking the disulfide bond but retaining the bicyclic structure will address the role of the putative redox conformational switch. Conversely, a monocyclic depsipeptide will test the need for a bicyclic framework. Derivatized analogs will also aid in the isolation of the FR-901,228 receptor. This molecular target of FR-901,228 mediates its antitumor activity and therefore is a regulator of cellular growth and proliferation in the absence of the drug. Identification of this target will aid in our understanding of the complex regulatory machinery that is subverted in cellular transformation.

**Acknowledgment.** We would like to thank Professor Erick Carreira (California Institute of Technology), Professor Stuart Schreiber, Professor David Evans, and Jack Taunton (Harvard University) for helpful discussions. Mass spectral data were obtained by Dr. Andrew Tyler and Ms. Jennifer Lynch at the Harvard Mass Spectroscopy Facility. Financial support by the Department of Chemistry and Chemical Biology, Harvard University, Glaxo-Wellcome, and Merck is gratefully acknowledged. The NIH BRS Shared Instrumentation Grant Program 1-S10-RR04870 and the NSF (CHE 88-14019) are acknowledged for providing NMR facilities.

**Supporting Information Available:** Experimental procedures and spectral data for all compounds (18 pages). See any current masthead page for ordering and Internet access instructions.

JA9613724

(19) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *14*, 1219−1222.
(20) Ueda, K.; Waki, M.; Izumiya, N. *Int. J. Pept. Protein Res.* **1987**, *30*, 33−39.
(21) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394−2395.
(22) Mitsunobu, O. *Synthesis* **1981**, *1981*, 1−28.
(23) The optimum conditions for the lactonization are shown below: To a solution of $PPh_3$ (25 equiv) and TsOH·$H_2O$ (5 equiv) in THF was added DIAD (20 equiv), and the resulting mixture was stirred at 20 °C (30 min). The heterogenous mixture was cooled to 0 °C, and a solution of the hydroxy acid **12b** in THF was added by syringe pump (2 h). The resulting mixture was allowed to stir for an additional 60 min after the addition was completed. The solvents were removed under reduced pressure, and the mixture was purified by flash chromatography to give **13** (62% yield).
(24) Hughes, D. L. *Org. React. (N.Y.)* **1992**, *42*, 335−656.
(25) Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1988**, *110*, 6487−6491.
(26) Kamber, B.; Hartmann, A.; Eisler, K.; Riniker, B.; Rink, H.; Sieber, P.; Rittel, W. *Helv. Chim. Acta* **1980**, *63*, 899−915.
(27) Still, W. C.; Ohmizu, H. *J. Org. Chem.* **1981**, *46*, 5242−5244.
(28) Barrett, A. G. M.; Carr, R. A. E.; Attwood, S. V.; Richardson, G.; Walshe, N. D. A. *J. Org. Chem.* **1986**, *51*, 4840−4856.
(29) Adam, G.; Zibuck, R.; Seebach, D. *J. Am. Chem. Soc.* **1987**, *109*, 6176−6177.