Natural Products Synthesis

DOI: 10.1002/anie.200700321

Synthesis and Stereochemical Assignment of FR252921, a Promising Immunosuppressant**

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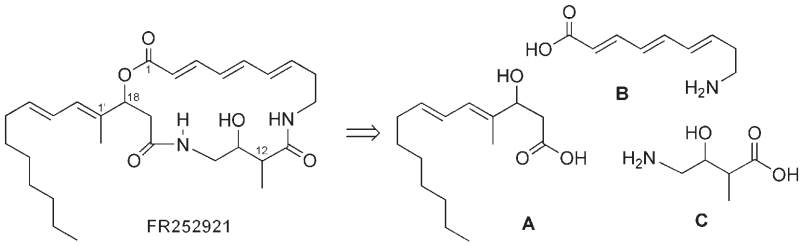
Dedicated to Professor Koichi Narasaka

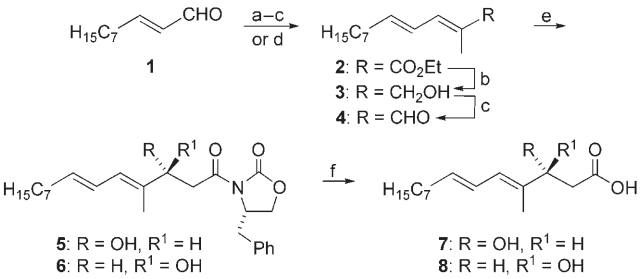
FR252921, an unusual 19-membered lactone–dilactam distinguished by a lipophilic C_{12} appendage, was isolated from the culture broth of *Pseudomonas fluorescens* by Fujine et al., who published its structure without characterization of its three stereogenic centers.[1] Interest in FR252921 from this[2] and other[3,4] laboratories stems in large part from its unique pharmacological profile. In contrast with other immunosuppressants, FR252921 potently inhibits both lipopolysaccharide-(LPS)-stimulated and anti-CD3 mAb-stimulated splenocyte proliferation in vitro without blocking T-cell activation.[5] It also decreases transcription regulated by activating protein 1 (AP-1) despite a null effect on the transcription factors NF-AT and NF-κB. Notably, FR252921 synergizes strongly the effects of FK506 in vitro and in vivo.[6] To expedite current studies into the site of action of FR252921 and conclude the complete assignment of its structure, we describe herein the asymmetric total synthesis of FR252921 and three diastereomers by a versatile, convergent strategy.



Scheme 1. Retrosynthetic analysis of FR252921.

Retrosynthetic analysis dissected FR252921 into segments **A**–**C** (Scheme 1), which were chosen to provide convenient access to the minimum stereoarray[7] needed to establish the relative and absolute configuration of the natural product as well as to support future structure–activity-relationship studies.[8,9] The segments would be assembled by following a sequence C→BC→ABC, which postponed the macrolactonization of the labile C18–OH group to the final stages of the synthesis to minimize opportunities for β elimination.

The route to both enantiomers of segment **A** (Scheme 2) commenced with (*E*)-2-decenal (**1**), which was homologated with commercial ethyl 2-(diethylphosphono)propionate to give a chromatographically separable 12:1 mixture of the 2*E*,4*E* diene ester **2** and its 2*Z*,4*E* isomer. A routine reduction–oxidation sequence from **2** with DIBAL-H and MnO_2 led to alcohol **3** and then dienal **4**[10] in excellent overall yield. Alternatively, **4** could be obtained in a single step by the $CrCl_2$-induced condensation of **1** with 2,2-dichloropropanal (OMM Scientific).[11] The samarium-mediated Reformatsky addition of (*S*)-4-benzyl-3-(2-bromoacetyl)oxazolidin-2-one[12] (**9**) to **4** at low temperature produced predominately the 3*R* alcohol **5** (81%), which was hydrolyzed smoothly to the free acid **7**. Hydrolysis of the minor 3*S* diastereomer **6**, isolated in 10% yield, gave **8**.



Scheme 2. Synthesis of carboxylic acids **7** and **8**: a) $(EtO)_2P(O)CH(CH_3)CO_2Et$, NaH, THF, 0°C, 2 h, 92% (*Z*,*E*/*E*,*E* 1:12); b) DIBAL-H, CH_2Cl_2, 0°C, 20 min; c) $MnO_2/MgSO_4$, CH_2Cl_2, 23°C, 2 days, 93% for two steps; d) H_3CCCl_2CHO, $CrCl_2$, $BF_3{\cdot}Et_2O$, THF/DMF (2:1), 23°C, 14 h, 74% (*Z*,*E*/*E*,*E* 1:10); e) **9**, SmI_2, THF, −86°C, 14 h, 91% combined; f) LiOH, H_2O_2, THF/H_2O, 4°C, 2.5 h, 97%. DIBAL-H = diisobutylaluminum hydride, DMF = *N*,*N*-dimethylformamide.

Segment **B** (Scheme 3) was quickly stitched together by hydrozirconation of homopropargyl tosylate[13] (**10**) and cross-coupling with the *E* vinylzirconocene derived from (*E*)-3-(*tert*-butyldimethylsilyloxy)-1-iodopropene[14] (**14**) at room temperature in the presence of $Pd(OAc)_2$ and 2′-(dicyclohexylphosphanyl)-*N*,*N*-dimethyl-(1,1′-biphenyl)-2-amine[15] (DPBA). The resulting *E*,*E* diene **11**, which was

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[**] Dr. Noriaki Inamura (Astellas Pharma, Inc.) and Dr. Kiyotaka Fujine (Fujisawa Pharmaceutical, Inc.) graciously provided a sample of the natural material and spectra. We thank Dr. Thomas G. LaCour and Dr. Suju Joseph for preliminary experiments. We are grateful for financial support from the Robert A. Welch Foundation and NIH (GM31278, DK38226).

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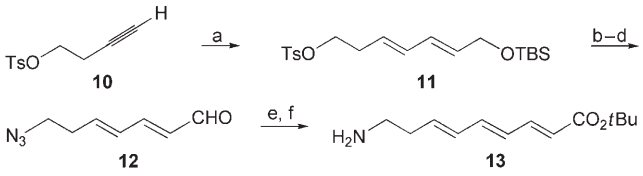
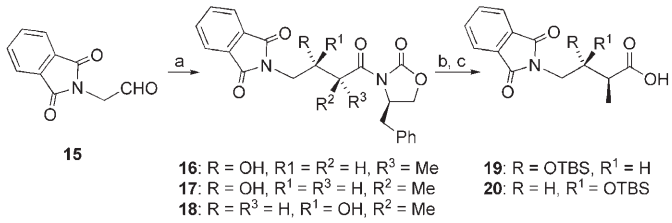


Scheme 3. Synthesis of amine **13**: a) 1. $[Cp_2ZrClH]$, THF, 23 °C, 1 h; 2. **14**, $Pd(OAc)_2$, DPBA, THF, 23 °C, 11 h, 98%; b) NaN_3, DMF, 23 °C, 14 h, 90%; c) nBu_4NF, THF, 0 °C, 1 h; d) MnO_2, CH_2Cl_2, 23 °C, 14 h, 92% for two steps; e) $(MeO)_2P(O)CH_2CO_2tBu$, NaH, THF, 0 °C, 1 h, 95%; f) Ph_3P, THF/H_2O (4:1), 23 °C, 20 h, 93%. TBS = *tert*-butyldimethylsilyl, Ts = *p*-toluenesulfonyl.

generated in excellent overall yield and with excellent stereoselectivity, was transformed into aldehyde **12** by treatment with sodium azide in DMF, followed by desilylation and oxidation with MnO_2. Horner–Wadsworth–Emmons (HWE) homologation of **12** and Staudinger reduction of the azide delivered *tert*-butyl ester **13** exclusively in the all-*E* configuration, as judged by $^1H/^{13}C$ NMR spectroscopy.

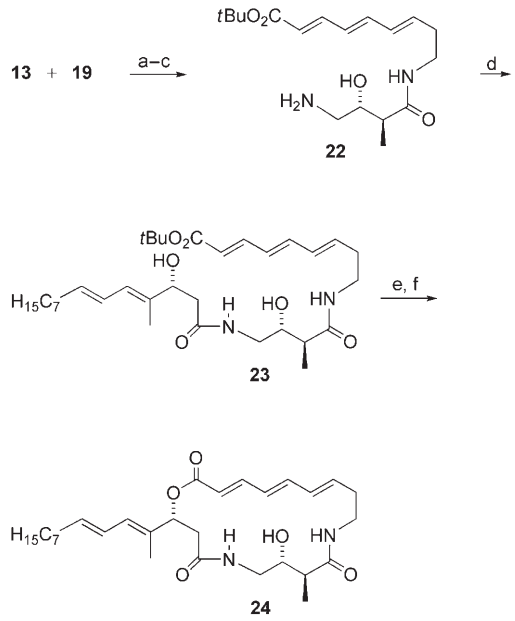
On the basis of a review of the spectral data for FR252921[8] and literature precedent for related substructures,[4,9] we opted to prepare the *anti* 2*S*,3*R* stereoisomer and either *syn* enantiomer of segment **C** to complete the stereoarray (Scheme 4). To this end, the Evans asymmetric aldol



Scheme 4. Synthesis of carboxylic acids **19** and **20**: a) 1) **21**, nBu_2BOTf (2.0 equiv), iPr_2NEt (1.05 equiv), CH_2Cl_2, 0 °C, 40 min; 2) **15**, −78 °C, 0.5 h, then 0 °C, 1 h, 90% (d.r. ≈ 1:1); b) TBSOTf (1.05 equiv), 2,6-lutidine, CH_2Cl_2, 0 °C, 13 h, 99%; c) LiOH, H_2O_2, THF/H_2O (4:1), 4 °C, 17 h, 89%. Tf = trifluoromethanesulfonyl.

reaction[16] emerged as the most effective of the many enantioselective procedures evaluated. In practice, the addition of (*R*)-4-benzyl-3-propionyloxazolidin-2-one[17] (**21**) to 2-phthalimidoacetaldehyde[18] (**15**) in the presence of equimolar amounts of nBu_2BOTf and iPr_2NEt gave the *syn* adduct **16** as a single diastereomer in 90% yield. With a two-fold excess of the boron triflate and the amine, the *anti* adduct **17** (absolute configuration confirmed by X-ray analysis, CCDC-634599) began to appear (**16**: 71%, **17**: 14%). As anticipated,[19] the diastereoselectivity was reversed as the reagent ratio was skewed toward excess boron triflate, and a mixture of **17** and the non-Evans *syn* adduct **18** was formed. As our immediate objective was the preparation of both an *anti* isomer and a *syn* isomer, the conditions were adjusted to give an approximately 1:1 mixture of **17** and **18** in good yield. The two diastereomers were separated conveniently by fractional crystallization after rough chromatography of the crude product mixture. Silylation of the secondary alcohol and removal of the chiral auxiliary then afforded the free acids **19** and **20** (Scheme 4).

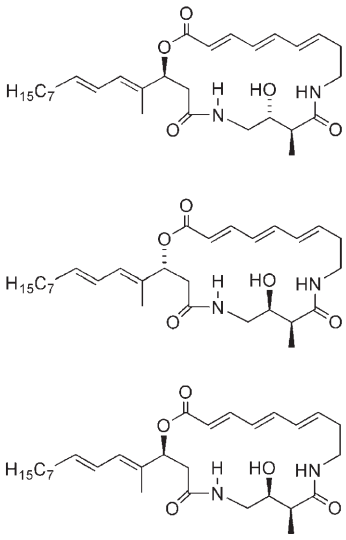
To assemble the macrocycle, amine **13** was united with the silyloxy acid **19** by using a suspension of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) in CH_2Cl_2 (Scheme 5). Excess hydrazine and tetrabutylammonium fluo-



Scheme 5. Synthesis of macrocycle **24**: a) EDC, HOBT, CH_2Cl_2, 23 °C, 2 h, 97%; b) nBu_4NF/AcOH (1.5:2), THF, 23 °C, 15 h, 91%; c) $H_2NNH_2 \cdot H_2O$ (50 equiv), EtOH, 45 °C, 5 min, ≈99% crude; d) **7**, EDC, HOBT, CH_2Cl_2, 23 °C, 2.5 h, 92%; e) 1) TMSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 2.5 h; 2) citric acid, MeOH, 4 °C, 14 h, 91%; f) MNBA, DMAP, THF, 23 °C, 96 h, 10–15%. DMAP = 4-dimethylaminopyridine, HOBT = 1-hydroxy-1*H*-benzotriazole, TMS = trimethylsilyl.

ride were employed to remove the phthalidyl and silyl protecting groups, respectively.[20] The product, aminoalcohol **22**, was then condensed with hydroxyacid **7** to afford the bisamide **23**. The transformation of **23** into the corresponding seco acid by cleavage of the *tert*-butyl ester according to the procedure described by Danishefsky and co-workers[21] proceeded well and set the stage for the final ring closure.[22] This final intermediate proved to be quite labile (perhaps because the two hydroxy groups are unprotected[20]) and, consequently, compatible with only the mildest macrolactonization procedures.[23] In particular, the procedure of Shiina et al.[24] with 2-methyl-6-nitrobenzoic anhydride (MNBA) proved uniquely suitable and rewarded us with a satisfactory yield of **24**, which was isolated by reversed-phase preparatory TLC.

The remaining diastereomers **25**, **26**, and **27** (Scheme 6) were acquired uneventfully by repetition of the series of reactions in Scheme 5 with the segment combinations **8**/**13**/**19**, **7**/**13**/**20**, and **8**/**13**/**20**, respectively. Of the four macrocycles, only **24** was fully consistent with the natural material by $^1H/^{13}C$ NMR spectroscopy and HPLC, and with respect to its



Scheme 6. Diastereomers of FR252921.

melting point and optical rotation. Thus, we established that FR252921 has the configuration 12*S*,13*R*,18*R*.[1]

Received: January 24, 2007
Published online: May 8, 2007

Keywords: configuration determination · immunosuppressants · macrocycles · natural products · total synthesis

[1] K. Fujine, M. Tanaka, K. Ohsumi, M. Hashimoto, S. Takase, H. Ueda, M. Hino, T. Fujii, *J. Antibiot.* **2003**, *56*, 55 – 61. The ^{1}H NMR spectrum of FR252921 appears to have been recorded in [D_6]dimethyl sulfoxide rather than in CD_2Cl_2.

[2] Presented in part at the 229th American Chemical Society National Meeting, San Diego, CA, March 13 – 17, **2005**, ORGN abstract 417.

[3] For the purported synthesis of the 12*R*,13*R*,18*R* diastereomer (numbering as in Scheme 1), see: S. Yu, F. Liu, D. Ma, *Tetrahedron Lett.* **2006**, *47*, 9155 – 9157. However, the NMR spectroscopic data are consistent with a *Z* isomer of the triene unit (see reference [20]).

[4] For a nonstereoselective, biogenic synthesis from pseudotrienic acid A, see: A. Pohanka, A. Broberg, M. Johansson, L. Kenne, J. Levenfors, *J. Nat. Prod.* **2005**, *68*, 1380 – 1385.

[5] K. Fujine, F. Abe, N. Seki, H. Ueda, M. Hino, T. Fujii, *J. Antibiot.* **2003**, *56*, 62 – 67.

[6] K. Fujine, H. Ueda, M. Hino, T. Fujii, *J. Antibiot.* **2003**, *56*, 68 – 71.

[7] There are four possible enantiomeric pairs that correspond to the structural formula of FR252921. Each pair is diastereomeric with respect to the other three pairs. Thus, when analyzed in conjunction with optical rotation or chiral-phase chromatography, one isomer from each of the diastereomeric pairs (that is, a total of four) contains the minimum stereoarray needed to establish the relative and absolute configuration of FR252921.

[8] Available NMR spectroscopic data were most consistent with an *anti* configuration of the methyl and hydroxy groups at C12 and C13.

[9] The 4-amino-(3*R*)-hydroxy-(2*S*)-methylbutanoyl subunit occurs in other bioactive natural products; see, for example: D. Amans, V. Bellosta, J. Cossy, *Angew. Chem.* **2006**, *118*, 6002 – 6004; *Angew. Chem. Int. Ed.* **2006**, *45*, 5870 – 5874.

[10] For an alternative synthesis of **2** and its *Z*,*E* isomer, see: B. B. Snider, T. Liu, *J. Org. Chem.* **2000**, *65*, 8490 – 8498.

[11] The $CrCl_2$ methodology proved impractical logistically on a large scale. Wittig olefination with commercial 2-(triphenylphosphoranylidene)propionaldehyde furnished an 8:1 mixture of *E* and *Z* isomers in moderate yield.

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[20] Retention of the silyl protecting group on the hydroxy group at C13 improved the yield of the macrolactonization significantly (50–55%). Regrettably, desilylation of the macrocycle (for example, with HF, HF/pyridine, *n*BuN_4F, *n*Bu_4NF/HOAc, pyridinium *p*-toluenesulfonate) after lactonization engendered a host of complications, including intra/intermolecular Michael addition, β elimination, *trans* lactonization, and, most notably, a facile *E*/*Z* isomerization of the triene.

[21] A. B. Jones, A. Villalobos, R. G. Linde, S. J. Danishefsky, *J. Org. Chem.* **1990**, *55*, 2786 – 2797.

[22] For a review on macrolactonization, see: A. Parenty, X. Moreau, J.-M. Campagne, *Chem. Rev.* **2006**, *106*, 911 – 939.

[23] Among the many procedures attempted, those described in the following articles also proved unsatisfactory: a) O. Mitsunobu, *Synthesis* **1981**, 1 – 28; b) E. J. Corey, K. C. Nicolaou, *J. Am. Chem. Soc.* **1974**, *96*, 5614 – 5616; c) Y. Oohashi, K. Fukumoto, T. Mukaiyama, *Bull. Chem. Soc. Jpn.* **2005**, *78*, 1508 – 1519; d) J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989 – 1993.

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