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Synthesis of a Promising Immunosuppressant: FR252921

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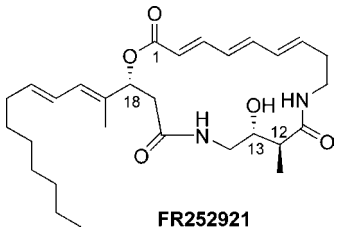
ABSTRACT



FR252921

A concise and highly convergent synthesis of the promising immunosuppressant FR252921 was achieved by using optically active titanium complexes to control the configuration of the three stereogenic centers.

In recent years, several new immunosuppressive agents have entered clinical trials, while others such as calcinuerin, cyclosporine A, and FK506 have already been developed for clinical use in organ transplantation. In 2003, a novel immunosuppressive agent, FR252921, was isolated from the culture broth of *Pseudomonas fluorescens* N° 408813.[1] This compound displays immunosuppressive activity against murine splenocyte proliferation stimulated with lipopolysaccharide (LPS) or anti-CD3 mAb in vitro without blocking T-cell activation. Further studies indicated that FR252921 inhibits protein-1 (AP-1) transcription activity and acts dominantly against antigen-presenting cells (APC) comparing to T-cell. It is also worth noting that FR252921 strongly synergizes the effects of FK506 in vitro and in vivo. When we started the synthesis of FR252921, the overall structure was established, but the absolute configuration of the three stereogenic centers was unknown. Interestingly, Pohanka et al. recently isolated from a strain of *Pseudomonas* a new antimicrobial agent, named pseudotrienic acid B, with a molecular architecture very similar to that of FR252921.[2] It was shown that this bioactive metabolite, possessing the well established *S* configuration at C12 and the *R* configuration at C13, was prone to macrolactonization in an acidic medium and, therefore, could be transformed into the macrolactone FR252921. On the basis of this nonstereoselective biomimetic transformation, it seemed reasonable to assume that FR252921 possesses the *S* configuration at C12 and the *R* configuration at C13, but until this year the configuration of the stereogenic center at C18 remained unknown. In 2006, Ma et al. disclosed an approach to an isomer of FR252921 that turned out to be the (*Z*,*E*,*E*) (12*R*,13*R*,18*R*) stereoisomer by examination of the ^{1}H NMR data and not the (*E*,*E*,*E*) (12*R*,13*R*,18*R*) isomer as reported (vide infra).[3] Very recently, Falck et al. unequivocally established, by total synthesis, the absolute configuration of FR252921, which was revealed to be (12*S*,13*R*,18*R*).[4]

In this context, we wish to report our results initially directed toward the elucidation of the absolute configuration of FR252921, and we disclose herein a convergent and versatile synthetic route to this novel immunosuppressant. Assuming that FR252921 possesses the *S* configuration at C12 and the *R* configuration at C13, we embarked in the synthesis of both *S* and *R* isomers at C18. The overall strategy employed to synthesize FR252921 needed to be flexible enough to allow access to both stereoisomers at C18, as well as to offer the potential to provide a wide range of structural derivatives for SAR studies. As outlined retrosynthetically

(1) (a) Fujine, K.; Tanaka, M.; Ohsumi, K.; Hashimoto, M.; Takase, S.; Ueda, H.; Hino, M.; Fujii, T. *J. Antibiot.* **2003**, *56*, 55−61. (b) Fujine, K.; Abe, F.; Seki, N.; Ueda, H.; Hino, M.; Fujii, T. *J. Antibiot.* **2003**, *56*, 62−67. (c) Fujine, K.; Ueda, H.; Hino, M.; Fujii, T. *J. Antibiot.* **2003**, *56*, 68−71.

(2) Pohanka, A.; Broberg, A.; Johansson, M.; Kenne, L.; Levenfors, J. *J. Nat. Prod.* **2005**, *68*, 1380−1385.

(3) Yu, S.; Liu, F.; Ma, D. *Tetrahedron Lett.* **2006**, *47*, 9155−9157.

(4) Falck, J. R.; He, A.; Fukui, H.; Tsutsui, H.; Radha, A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4527−4529.

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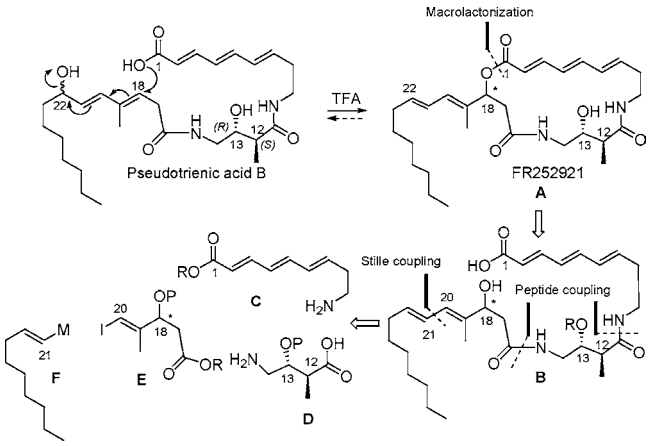
Scheme 1. Formation of FR252921 from Pseudotrienic Acid B and Retrosynthetic Analysis of FR252921

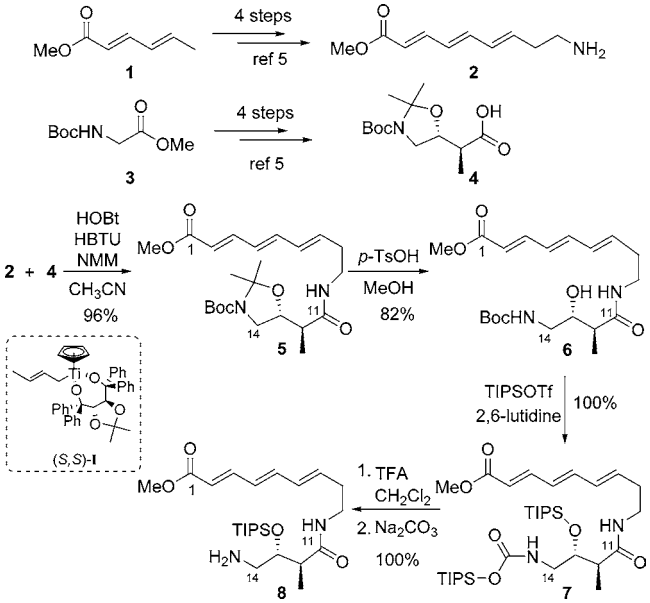


in Scheme 1, the construction of FR252921 relied on a macrolactonization of the seco-acid **B**, which could be dissected into the four key fragments **C**−**F**. The first amide bond would be formed by combining aminotrienic ester **C** and carboxylic acid **D**, while another peptide coupling between amine **D** and fragment **E** would install the second amide bond. The (*E*,*E*)-dienic side chain was planned to arise from a Pd-catalyzed cross-coupling between vinyl iodide **E** and alkenyl metal **F**.

As reported for the synthesis of pseudotrienic acid B, methyl sorbate **1** was transformed into aminotrienic ester **2** in four steps with an overall yield of 25% by using cross-metathesis and Horner−Wadsworth−Emmons olefination as the key steps.[5] The carboxylic acid **4**, in which the stereogenic centers were controlled by using the highly face-selective crotyltitanium complex (*S*,*S*)-**I**,[6] was prepared in four steps from the commercially available *N*-Boc glycine methyl ester **3** (43% overall yield). Subsequent coupling with aminotrienic ester **2** by using HOBt, HBTU, and NMM in acetonitrile produced amide **5** (96% yield), which represents the C1−C14 fragment of FR252921.[5] The *N*,*O*-dimethyloxazolidine **5** was cleaved by using *p*-TsOH in MeOH, which afforded the *N*-Boc-protected aminoalcohol **6** in 82% yield. Subsequent protection of the hydroxyl group at C13 as a TIPS ether (2 equiv of TIPSOTf, 2,6-lutidine) furnished aminoalcohol **7** quantitatively. It is worth mentioning that under these conditions the *tert*-butoxycarbamate was replaced by a TIPS carbamate,[7] which was merely cleaved upon treatment with TFA in CH_2Cl_2 to give the desired aminotrienic ester **8** (Scheme 2).

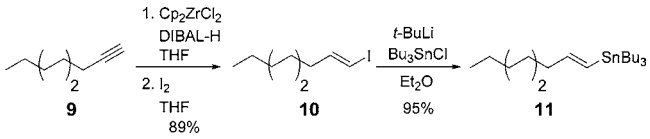
As a first approach, a Stille coupling was planned for the construction of the C20−C21 bond. The synthesis of the required alkenyl metal **F**, vinyl stannane **11**, was achieved from the commercially available non-1-yne **9**. A regio- and stereoselective hydrozirconation applied to alkyne **9** by using

Scheme 2. Synthesis of Aminotrienic Ester **8**



the Schwartz reagent Cp_2ZrHCl generated in situ (Cp_2ZrCl_2, DIBAL-H, THF) followed by iodolysis (I_2, THF) afforded the *E*-vinyl iodide **10** as a single stereoisomer (89% yield).[8] Its transformation to the corresponding vinyl stannane **11** was realized by performing a halogen−metal exchange upon treatment with *t*-BuLi (2.1 equiv, THF), followed by trapping of the resulting vinyllithium species with tributyltin chloride in THF (95% yield) (Scheme 3).

Scheme 3. Synthesis of Vinyl Stannane **11**



Next we turned our attention toward the synthesis of the required *E*-vinyl iodide of type **E** with the *S* configuration, compound **15**, which was prepared from the known allylic alcohol **12**.[9] Oxidation with manganese oxide furnished the corresponding aldehyde **13**, which was directly treated with allyltitanium complex (*R*,*R*)-**II** to produce, after TBS protection of the resulting homoallylic alcohol (TBSOTf, 2,6-lutidine), compound **14** with high enantioselectivity (>95% ee, 55% yield from **12**).[10] After regioselective oxidative cleavage of the terminal double bond (OsO_4, NMO then $NaIO_4$), the resulting aldehyde was further oxidized smoothly

(5) (a) Amans, D.; Bellosta, V.; Cossy, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 5870−5874 and references therein. (b) Amans, D.; Le Flohic, A.; Bellosta, V.; Meyer, C.; Cossy, J. *Pure Appl. Chem.* **2007**, *79*, 677−684.

(6) Hafner, A.; Duthaler, R. O.; Marti, R.; Rihs, J.; Rhote-Streit, P.; Schwarzenbach, F. *J. Am. Chem. Soc.* **1992**, *114*, 2321−2336.

(7) Sakaitani, M.; Ohfune, Y. *Tetrahedron Lett.* **1985**, *26*, 5543−5546.

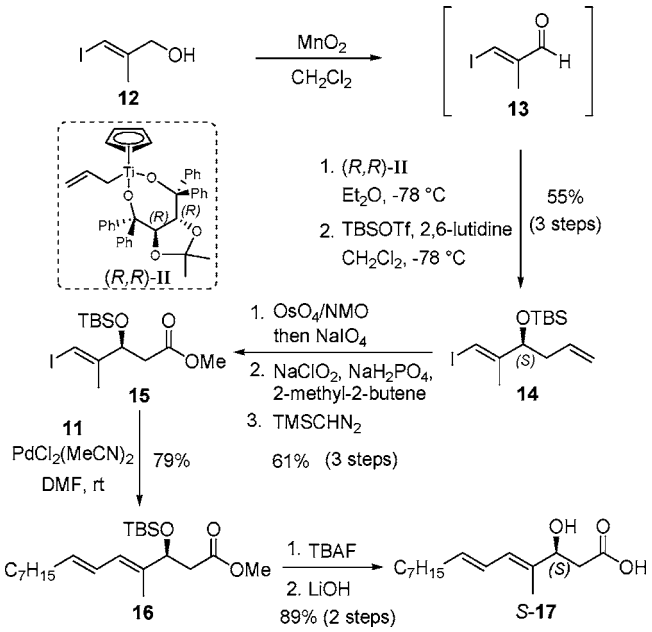
(8) Huang, Z.; Negishi, E. *Org. Lett.* **2006**, *8*, 3675−3678.

(9) Vinyl iodide **12** was obtained by performing a zirconium-assisted carboalumination of propargyl alcohol according to: Negishi, E.; Van Horn, D. E.; King, A. O.; Okukado, N. *Synthesis* **1979**, 501−502.

(10) The absolute configuration of **14** was confirmed by the ^{1}H NMR spectra of the two corresponding mandelates, following the procedure described by: Seco, J. M.; Quiñoa, E.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915−2925.

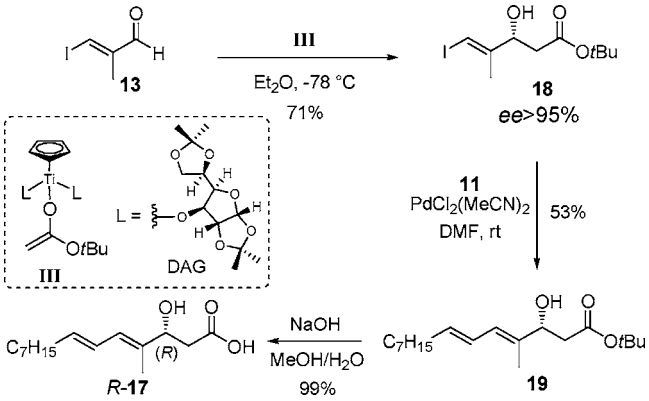
to the corresponding carboxylic acid ($NaClO_2$, NaH_2PO_4, 2-methyl-2-butene), which was then esterified upon treatment with trimethylsilyldiazomethane, thus giving rise to methyl ester **15** in 61% overall yield from **14** (3 steps). Coupling of vinyl stannane **11** and alkenyl iodide **15** was then achieved by performing a $PdCl_2(MeCN)_2$-catalyzed Stille cross-coupling in DMF,[11] which successfully led to the required (*E*,*E*)-diene **16**, corresponding to the C16−C29 fragment of FR252921 (79% yield). After deprotection (TBAF) and saponification (LiOH), the hydroxycarboxylic acid *S*-**17** was isolated in an overall yield of 89% (Scheme 4).

Scheme 4. Synthesis of Carboxylic Acid *S*-**17**



In parallel, the β-hydroxy dienic acid **17** possessing the *R* configuration was also synthesized. The versatile strategy disclosed above for the synthesis of its *S*-enantiomer can also be used by simply taking the (*S*,*S*)-**II** allyltitanium complex, but to shorten the preparation of this chiral carboxylic acid, we decided to investigate a more efficient synthetic route. Thus, aldehyde **13** was treated with the optically active titanium enolate **III**,[12] generated in situ from *tert*-butylacetate lithium enolate and $CpTi(ODAG)_2Cl$ titanium complex, which yielded the β-hydroxyester **18** in 71% yield with high optical purity (ee > 95%).[13] A [$PdCl_2(MeCN)_2$]-catalyzed Stille cross-coupling between vinyl stannane **11** and alkenyl iodide **18** afforded the (*E*,*E*)-diene **19**, which was subsequently saponified by NaOH to give the desired dienic carboxylic acid *R*-**17** almost quantitatively (99%). Thus, the chiral β-hydroxy acid *R*-**17** was synthesized efficiently in 3 steps from vinyl iodide **13** in 37% overall yield (Scheme 5).

Scheme 5. Synthesis of Carboxylic Acid *R*-**17**



At this stage, the core structure of FR252921 could be produced by assembling amine **8** and carboxylic acid *S*-**17** under classical peptide coupling conditions (HOBt, HBTU, NMM, CH_3CN), which allowed the isolation of dipeptide *S*-**20** (76% yield). Saponification of methyl ester by LiOH proceeded smoothly and set the stage for the crucial macrolactonization step. After screening a large number of unrewarding procedures,[14] we were pleased to note that the seco-acid *S*-**21** underwent macrocyclization by using Yamaguchi conditions (2,4,6-trichlorobenzoyl chloride, Et_3N, 4-DMAP, toluene, $c = 0.0015$ M, 65 °C),[15] which, however, produced the unexpected macrolactone *S*-**22** (48% yield for the 2 steps). Indeed, after close examination of the COSY $^1H-^1H$ correlation, we noticed that the most deshielded proton, which was usually attributed to H3 since it was localized in the deshielding plane of the carbonyl group, turned out to be H4. Therefore, this allowed us to assume a potential isomerization of the C2−C3 double bond, initially of *E* configuration, into a *Z*-olefin during the macrolactonization step under Yamaguchi conditions. Furthermore, examination of the ^1H spectrum also showed that the value of the coupling constant between H2 and H3 was 11.5 Hz, which was abnormally small for a *E* double bond. These observations allowed us to attribute the *Z* configuration for the C2−C3 double bond.[16] Treatment of silyl ether *S*-**22** with TBAF led to *S*-**23** in 84% yield, which is the (2*Z*,4*E*,6*E*,12*R*,13*S*,18*S*) stereoisomer of FR252921 (Scheme 7). Considering these results, we wondered about the influence of the absolute configuration of the C18 stereogenic center during the cyclization process. Indeed, some conformational restrictions could be the result of the relative orientation of

(11) (a) Stille, J. K. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 508−524. (b) Betzer, J.-F.; Lallemand, J.-L.; Pancrazi, A. *Synthesis* **1998**, 522−534. (c) Nicolaou, K. C.; Li, Y.; Sugita, K.; Monenschein, H.; Guntupalli, P.; Mitchell, H. J.; Fylaktakidou, K. C.; Vourloumis, D.; Giannakakou, P.; O'Brate, A. *J. Am. Chem. Soc.* **2003**, *125*, 15443−15454.

(12) (a) Riediker, M.; Duthaler, R. O. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 494−495. (b) Duthaler, R. O.; Herold, P.; Lottenbach, W.; Oertle, K.; Riediker, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 495−497. (c) Duthaler, R. O.; Hafner, A. *Chem. Rev.* **1992**, *92*, 807−832. (d) Duthaler, R. O.; Herold, P.; Wyler-Helfer, S.; Riediker, M. *Helv. Chim. Acta* **1990**, *73*, 659−673.

(13) The absolute configuration of **19** was confirmed by the ^1H NMR spectra of the two corresponding mandelates; see ref 10.

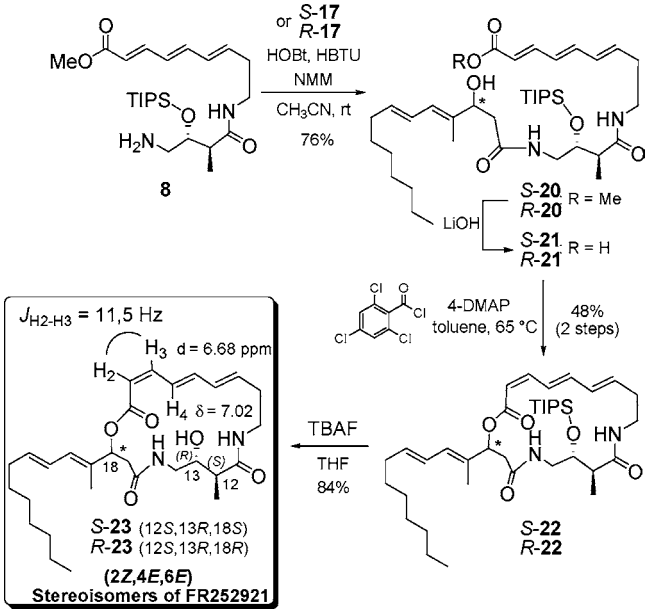
(14) Many unsuccessful procedures were attempted according to: (a) Trost, B. M.; Chisholm, J. D. *Org. Lett.* **2002**, *4*, 3743−3745. (b) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 636−6370. (c) Mitsunobu, O. *Synthesis* **1981**, 1−28. For a review on macrolactonizations, see: Parenty, A.; Moreau, X.; Campagne, J.-M. *Chem. Rev.* **2006**, *106*, 911−939.

(15) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989−1993.

the substituents located on the three stereogenic centers, and therefore the *R* stereoisomer at C18 needed to be synthesized.

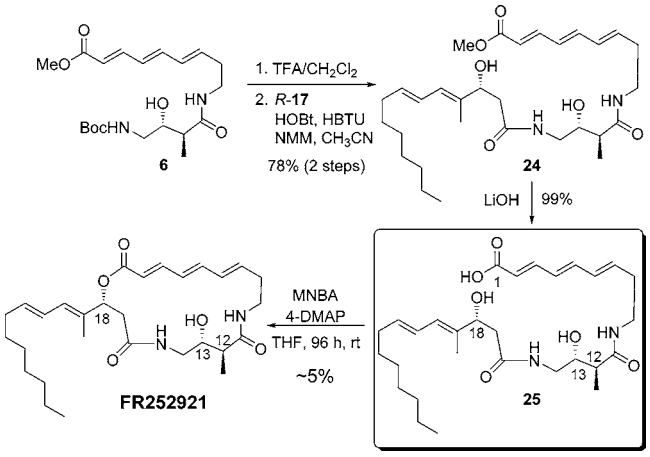
As previously, the carboxylic acid *R*-**17** was coupled with amine **8** to produce the dipeptide *R*-**21**, which after saponification and Yamaguchi macrolactonization furnished the protected macrolactone *R*-**22**, subsequently transformed to *R*-**23** upon treatment with TBAF (Scheme 6). Unfortunately, the presence of the *Z* configuration at the C2−C3 double bond was also observed in this case. Therefore, the isomerization of the C2−C3 double bond is not due to the configuration of the stereogenic center at C18 but probably to the conditions used in the Yamaguchi macrolactonization (Scheme 6).

Scheme 6. Synthesis of (2*Z*,4*E*,6*E*) Isomers *S*-**23** and *R*-**23**



These results allowed us to conclude that the presence of the TIPS groups could be indirectly responsible for the isomerization of the C2−C3 double bond and consequently would prevent the formation of FR252921. Therefore, treatment of the *N*-Boc aminoalcohol **6** with TFA in CH_2Cl_2, followed by a peptide coupling with *R*-**17** (HOBt, HBTU, NMM, CH_3CN), gratifyingly furnished dipeptide **24** with the free hydroxyl group at C13 (78% for 2 steps). After saponification of methyl ester **24** with LiOH, the resulting seco-acid **25** was isolated almost quantitatively (99% yield). This constitutes a formal synthesis of FR252921. The spectral and analytical data of **25** were in agreement with those previously reported by Falck et al.[4,17] After a macrolactonization using Shiina 2-methyl-6-nitrobenzoic anhydride (MNBA),[18] in the presence of 4-DMAP in a highly diluted THF solution (c = 0.0006 M), FR252921 was obtained in poor yield (~5%) accompanied by significant impurities, which could only be separated from the natural product by reverse-phase chromatography (Scheme 7).[19]

Scheme 7. Completion of the Synthesis of FR252921



In summary, a concise and highly convergent synthesis of the promising immunosuppressant FR252921 has been achieved. Notable features include highly enantioselective allyltitanations as well as an enantioselective aldolisation to control the configuration of the stereogenic centers, a Pd-catalyzed Stille cross-coupling to construct the (*E*,*E*)-diene and a macrolactonization to form the 19-membered macrolactone-dilactam. If isomers of FR252921 were obtained at first, the highly convergent strategy allowed us to synthesize the natural product by only minor modifications of the building blocks. However, the macrolactonization strategy is not suitable to prepare FR252921 in satisfying yields. Other strategies are currently underway to obtain appreciable amount of FR252921 to pursue biological testing. This modular and highly convergent synthesis should also be amenable to design analogues for biological evaluation. Our investigations along these lines will be reported in due course.

Acknowledgment. We wish to thank the Société de Chimie Thérapeutique/SERVIER for financial support of this work. We also wish to acknowledge Prof. J. R. Falck and Dr. A. He (University of Texas Southwestern Medical Center) for helpful discussions and for providing NMR spectra of some intermediates and Dr. K. Fujine (Fujisawa Pharmaceutical, Inc.) for providing NMR data of the natural product.

Supporting Information Available: Experimental procedures, characterization data of the described compounds, and copies of the ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) The NMR spectroscopic data published by Ma et al.[2] are also consistent with the (2*Z*,4*E*,6*E*,12*R*,13*R*,18*R*) isomer and not the (2*E*,4*E*,6*E*,12*R*,13*R*,18*R*) stereoisomer as reported.

(17) Significant variations of chemical shifts for the seco-acid **25** in both ^1H and ^{13}C NMR spectroscopy were observed from one sample to another. This is probably due to inter- or intramolecular hydrogen bonds, which are dependent on concentration. Our NMR data were at first inconsistent with those reported by Falck,[4] but in total agreement with a ^1H spectrum that they provided to us made at a different concentration. However, the optical rotation for our seco-acid was absolutely identical to the one reported by Falck. See experimental section for spectra.

(18) (a) Shiina, I.; Kubota, M.; Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535−7539. (b) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *69*, 1822−1830.

(19) Under the same conditions, Falck et al. have isolated FR252921 in 10% yield by reverse phase chromatography.