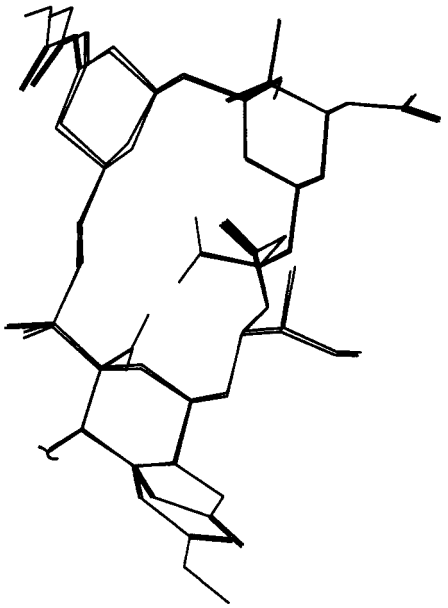

Figure 4. Superimposed energy minimized models (MM2) of bryostatin 1 and bryostatin 3 (C-20 ester side chain omitted for clarity).

on silica gel (21.4 mm i.d. column, hexane–acetone (7:3) as mobile phase), affording pure **4** (10 mg): IR (KBr) 3450, 2970–2920, 1785, 1735, 1715, 1650–1640, 1365, 1305, 1275, 1245, 1165, 1145, 1095, 1070–1040, 980 cm^{-1}; HR FABMS m/z 895.4331 $[M + Li]^+$, calcd for $C_{46}H_{64}O_{17}$ Li 895.4304, Δ = 3 ppm; UV (70% aqueous CH_3CN) λ_{max} (nm) 230, 266.

A second aliquot (25 g) of the EtOAc fraction was purified on Florisil (see above; solvent, hexane–acetone 90:10, 85:15, 80:20, and 70:30, 2.0 L each mixture). Fractions containing **5** (elution volume 5.0–7.0 L; 2.17 g) were combined and further separated on reversed-phase material (sample was coated on 40 g of packing obtained from a Waters PrepPAK 500/C18 cartridge, 55–100 μm, column 6 × 8 cm i.d.; MeOH–H_2O 90:10, elution volume 750–1050 mL), affording 418 mg. This fraction was purified by preparative HPLC (C-8, 21.4 mm i.d., MeCN–H_2O 88:12, sample load 40–80 mg) and 48 mg of crude **5** was obtained. The compound was finally purified by HSCCC in the solvent system hexane–EtOAc–MeOH–H_2O (14:6:10:7) (sample in 1:1 mixture (7 mL) of upper phase and lower phase; mobile phase = upper phase; flow of 5 mL/min; fractions 5 mL each; retention of stationary phase was ca. 90%). Pure **5** was obtained in fractions 28–42 (9 mg): IR (KBr) 3440, 2935–2920, 1780, 1740, 1720, 1650–1640, 1365, 1305, 1275, 1245, 1165, 1145, 1130, 1095, 1075, 1045, 1025, 1000, 980 cm^{-1}; HR FABMS m/z 893.4156 $[M + Li]^+$, calcd for $C_{46}H_{62}O_{17}$ Li 893.4147, Δ = 0.1 ppm; UV (70% aqueous CH_3CN) λ_{max} (nm) 228, 266.

Acknowledgment. We thank P. Andrews, T. McCloud, and A. Vatakis for their assistance in the isolation, Dr. B. Hilton and J. Klose for running NMR spectra, and Dr. C. Metral for providing high resolution mass spectra. We would also like to thank S. Forenza and K. Snader for support of this project. This project has been funded at least in part with Federal funds from the Department of Health and Human Services under contract number NO1-CO-74102. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Registry No. 4, 87370-86-3; 5, 132564-78-4; phorbol dibutyrate, 61557-88-8.

On the Remarkable Propensity for Carbon–Carbon Bond Cleavage Reactions in the C_8–C_{10} Region of FK-506

Matthew J. Fisher, Ken Chow, Anabella Villalobos, and Samuel J. Danishefsky*

Department of Chemistry, Yale University, New Haven, Connecticut 06511-8118

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It has been deduced from a series of transformations that formation of a tetrahedral intermediate at C_9 in FK-506 occasions fragmentation of the C_9–C_{10} bond by a retro-Claisen-like pathway or the C_9–C_8 bond by a benzilic acid type rearrangement. Reduction of FK-506 with L-Selectride leads to the formation of a boronate ester **18** rather than to the corresponding diol **17**, which had previously been formulated. Direct reduction of FK-506 with sodium triacetoxyborohydride (or hydrolysis of **18**) does provide access to (22*S*)-dihydro FK-506 **17**. The former reduction also leads to some 22*R* epimer, which is an intermediate in the total synthesis of FK-506.

Background

The enormous immunosuppressive activity of FK-506 (**1**)[1] has served to foster renewed interest in the use of organic molecules, of a nonpeptidyl nature, as modulators of the human immune system.[2] Particularly exciting is the fact that the potency of FK-506 is ca. 100 times greater than cyclosporin, which is the benchmark compound in the field.[3] While there have been some dramatic claims on behalf of FK-506 in suppressing rejection of various human organ transplants,[4] its generality as an adjutant for transplantation surgery has not yet been conclusively demonstrated.

Although a number of synthesis-based investigations in the FK-506 area have been described,[5] very few studies

(1) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hahimoto, M.; Taga, T. *J. Am. Chem. Soc.* **1987**, *109*, 5031.

(2) (a) FK-506: Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiotics* **1987**, *40*, 1249. (b) FR-900525: Hatanaka, H.; Kino, T.; Asano, M.; Goto, T.; Tanaka, H.; Okuhara, M. *J. Antibiotics* **1989**, *42*, 620. (c) FR-900520 and FR-900523: Hatanaka, H.; Kino, T.; Miyata, S.; Inamura, N.; Kuroda, A.; Goto, T.; Tanaka, H.; Okuhara, M. *J. Antibiotics* **1988**, *41*, 1592. (d) Rapamycin: Swindelly, S. D. C. N.; White, P. S.; Findlay, J. *Can. J. Chem.* **1978**, *56*, 2491. Martel, R. R.; Klicius, J.; Galet, S. *Can. J. Physiol. Pharmacol.* **1977**, *55*, 48.

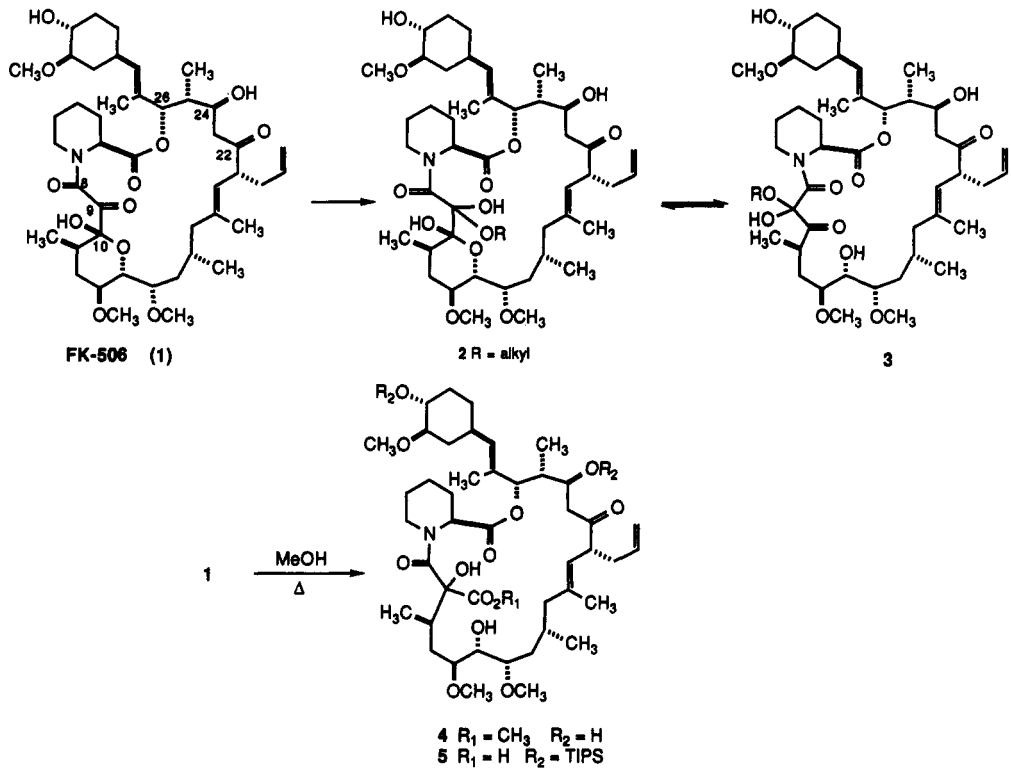
(3) Wenger, R. M. *Proc. Chem. Org. Nat. Prod.* **1986**, *50*, 123.

(4) Altman, L. K. *New York Times* October 18, 1989, 1. Starzl, T. E.; Fung, J.; Venkataramman, R.; Todo, S.; Demetris, A. J.; Jain, A. *The Lancet* **1989**, 1000.

(5) (a) Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5583. (b) Jones, T. K.; Reamer, R. A.; Desmond, R.; Mills, S. G. *J. Am. Chem. Soc.* **1990**, *112*, 2998. (c) Jones, A. B.; Villalobos, A.; Linde, R. G., III; Danishefsky, S. J. *J. Org. Chem.* **1990**, *55*, 2786.

0022-3263/91/1956-2900$02.50/0

Scheme I



dealing with the chemistry of the native material have been recorded.[1,6] We reasoned that a clearer insight into the chemical personality of FK-506 would be helpful in formulating hypotheses as to its predispositions for interaction with potential receptors. Clearly, the most provocative segment of the molecule is that containing carbons 8–10. This area is referred to as the "tricarbonyl region" although, in fact, C_{10} is engaged as a hemiketal with the hydroxyl at C_{14}. The investigation described herein began with an attempt to probe the reactivity of this tricarbonyl region with some representative nucleophiles.

Discussion of Results

Early efforts explored the possibility, however remote, that in the presence of an alcoholic solvent C_9 might exist as a stable alkoxy hemiketal (cf. 2), perhaps thus inducing ring–chain tautomerism of the C_{10} hemiketal (cf. 3). In the event, examination of the TLC chromatographic properties of a solution of 1 in methanol and the ^1H NMR spectrum of the residue left after evaporation of the volatiles failed to reveal any discernable reaction. However, a transformation did occur when a methanolic solution of FK-506 was maintained at reflux. There was obtained an 82% yield of hydroxy ester 4. The spectroscopic properties of the methanolysis products were in accord with its proposed structure.[7] Moreover, the assignment was corroborated by chemical means. A Merck group had reported that a C_{24}, C_{34} di-TIPS derivative of FK-506 underwent a benzilic acid rearrangement upon treatment with aqueous LiOH.[6b,c] Repetition of this sequence did indeed lead to the Merck hydroxy acid 5 which, upon esterification with diazomethane and desilylation, gave rise to a product whose ^1H NMR spectrum was identical with that of 4 (Scheme I). Presumably, this rearrangement reaction occurs through the intermediacy of the proposed target system 3.[8] While it seems likely by analogy with previous work[6b,c] that the formation of 4 involves migration of C_8 to the keto form of C_{10}, the possibility of an alternative route wherein 4 is derived from a C_{11} to a C_9 migration has not been excluded. Some competition from such a pathway was elegantly demonstrated by use of a C_9-labeled FK-506.

Given this result, the behavior of other potential C_9 tetrahedral intermediates was explored. We examined the possibility of forming of a C_9 cyanohydrin. Thus, FK-506 was treated with trimethylsilyl cyanide (TMSCN).[9] Surprisingly, there was isolated a 76% yield of the seco-cyanohydrin 6. That cleavage of the C_9–C_{10} bond had occurred was indicated by ^1H and ^{13}C NMR analysis. Most decisive in this regard was the appearance of a doublet (J = 7.0 Hz) centered at δ 1.31 that is assigned to the hydrogens of the methyl group at C_{11}, which is adjacent to the lactonic carbonyl group.[10] Additionally, a signal at δ 4.57 is assigned to the methine hydrogen of the cyanohydrin at C_9 of 6. The structure is also supported by the appearance of resonances in the ^{13}C NMR spectrum at δ 115.6 and 173.0 assigned to the cyano and lactonic carbons, respectively.

(6) (a) Coleman, R. S.; Danishefsky, S. J. *Heterocycles* **1989**, *28*, 157. (b) Askin, D.; Reamer, R. A.; Jones, T. K.; Volante, R. P.; Shinkai, I. *Tetrahedron Lett.* **1989**, *30*, 671. (c) Askin, D.; Reamer, R. A.; Joe, D.; Volante, R. P.; Shinkai, I. *Tetrahedron Lett.* **1989**, *30*, 6121. (d) Askin, D.; Reamer, R. A.; Joe, D.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1990**, *55*, 5451. (e) Askin, D.; Reamer, R. A.; Joe, D.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1990**, *55*, 5448. (f) Ok, H.; Arison, B. H.; Ball, R. G.; Beattie, T. R.; Fisher, M. H.; Wyvratt, M. J. *Tetrahedron Lett.* **1990**, *31*, 6477.

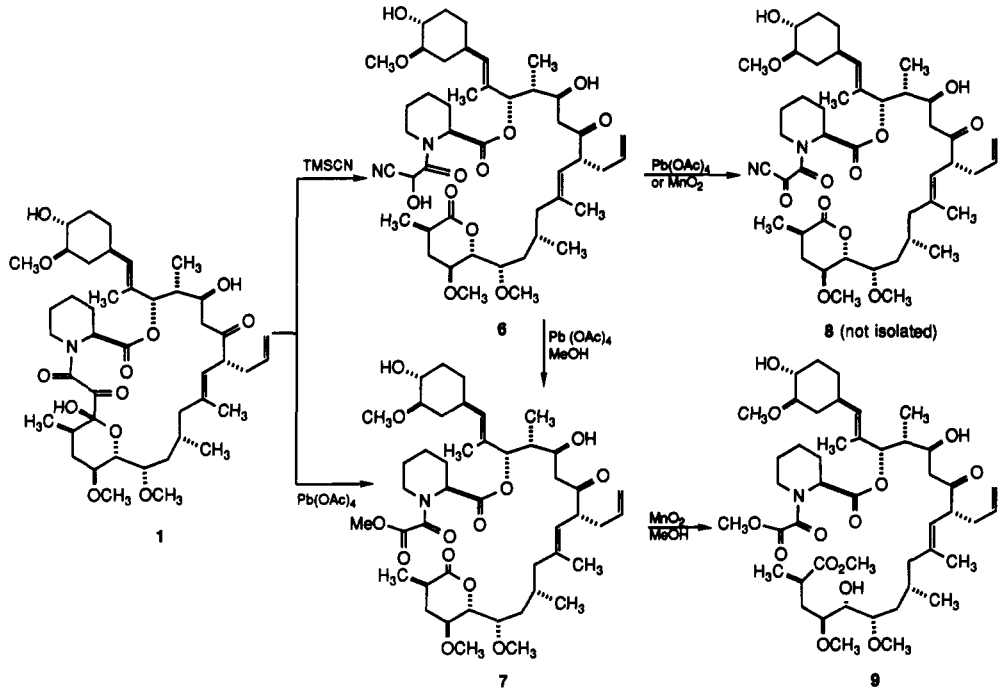
(7) The stereochemistry of the α-hydroxy ester has not been determined. More importantly, the presence of amide rotamers increases the spectral complexity to the point where homogeneity at this center is not certain.

(8) For a general review of vicinal polyketones, see: Rubin, M. B. *Chem. Rev.* **1975**, *75*, 177.

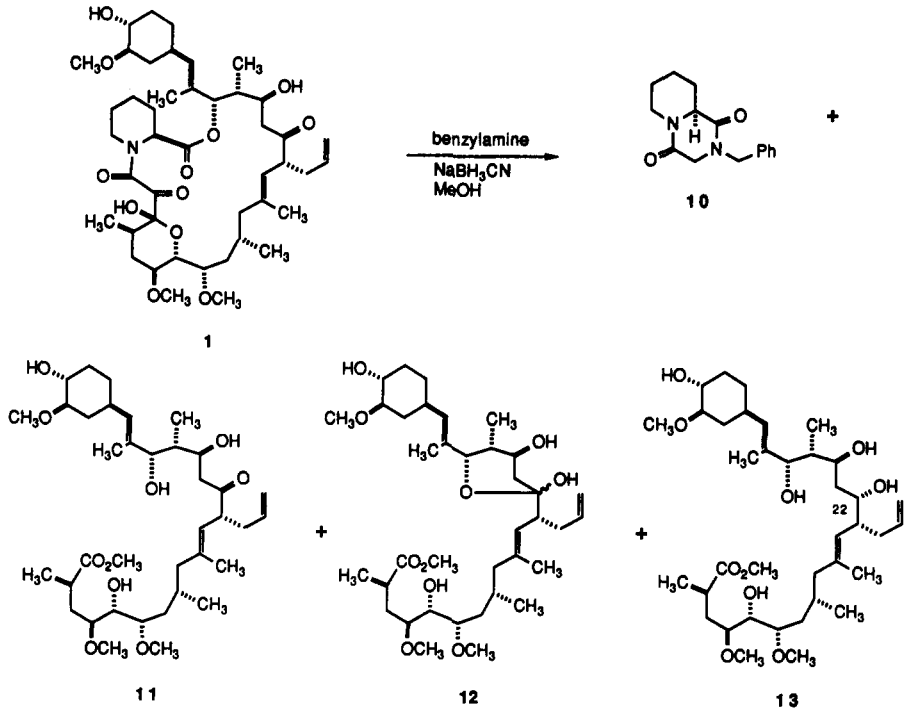
(9) Evans, D. A.; Truesdale, L.; Carroll, G. *Chem. Commun.* **1973**, 55. Gassman, P. G.; Talley, J. J. *Tetrahedron Lett.* **1978**, 3773.

(10) This methyl group resonated at ca. 0.2 ppm downfield from its counterpart in FK-506. The downfield shift of this methyl group was characteristic for compounds in which C_{10} was sp^2-hybridized (see Experimental Section).

Scheme II



Scheme III

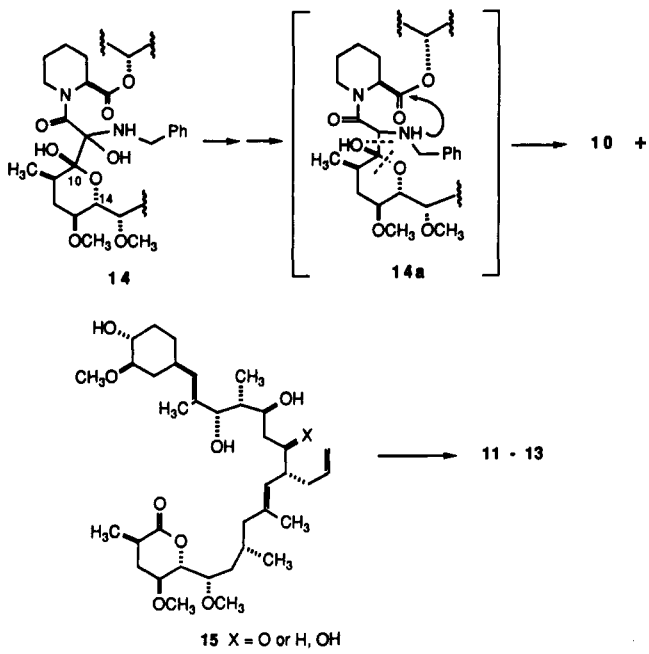


The assignment of structure **6** as the open chain cyanohydrin was also corroborated by chemical means. Treatment of FK-506 (**1**) with lead tetraacetate in methanol occasioned oxidative cleavage at C_9–C_{10}, providing compound **7**.[6a] Compound **7** was also obtained (79%) by reaction of **6** under the sme conditions (Scheme II). It is assumed that in this transformation the cyanohydrin moiety of **6** suffers oxidation to an acyl cyanide (cf. **8**), which then undergoes methanolysis to provide **7**. This hypothesis was further supported by the reaction of **6** with activated manganese dioxide in methanol.[11] These conditions led to the oxalyl ester **9**. Here, methanolysis of the acyl cyanide is accompanied by methanolysis of the lactone in unspecified sequence. That the MnO_2–methanol system can effect the methanolysis of a C_{10} lactone was further established by the conversion of **7** to **9** using the same protocol. The lability of seco FK-506 lactones such as **7** was an interesting finding that was to occur in several other transformations.

In both cases described previously, formation of a tetrahedral intermediate at C_9 tiggered fragmentation of either the C_8–C_9 bond (cf. formation of **4**) or the C_9–C_{10} bond (cf. formation of **6**). In an effort to further define the unique properties of this region of the molecule, we examined the reaction of FK-506 with benzylamine. At

(11) Corey, E. J.; Gilman, N. W.; Ganem, B. E. *J. Am. Chem. Soc.* **1968**, *90*, 5616.

Scheme IV



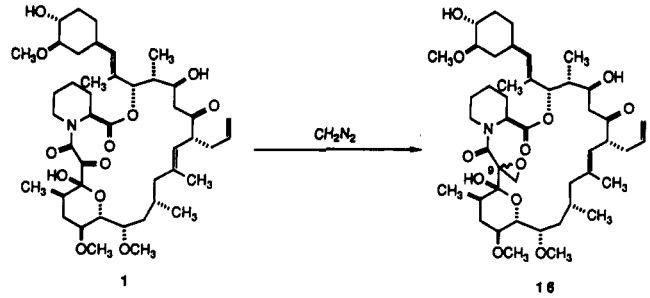
room temperature no reaction was noted. With a view to trapping any small amounts of Schiff base that might arise from a C_9 carbinolamine, FK-506 was allowed to react with benzylamine and sodium cyanoborohydride in methanol.[12] A most interesting series of reactions was thus set in motion.

There were isolated four products (Scheme III). The pipecolyloxalyl moiety had been sheared from the molecule and now appeared as the mixed diketopiperazine **10**, while the bulk of the erstwhile FK-506 structure was retrieved in the form of methyl esters **11–13**. The ring-chain tautomers **11** and **12**, obtained in 33% combined yield, were not separable. Compound **13**, the C_{22}-dihydro version of **11**, was obtained as a homogeneous entity in 35% yield. This compound must arise from slow reduction of **11**, or an earlier intermediate, with sodium cyanoborohydride. Treatment of **11–12** with sodium borohydride also generates **13**. The arguments concerning the stereochemistry at C_{22} in **13** will be discussed as the sequence of transformations is developed further. It seems reasonable that the route to compounds **10–13** begins with formation of carbinolamine **14**. Reduction, acyl transfer, and cleavage of the C_9–C_{10} bond in an unspecified order would produce **10** and the main fragments.

The precise stage where the C_9–C_{10} bond is severed is not known. One possibility involves retroaldol cleavage leading to a lactone (cf. **15**), which upon methanolysis (vide supra) affords the observed methyl esters (Scheme IV). Alternatively, at some stage after reductive amination has occurred (cf. **14a**), the C_{10} hemiacetal might revert to C_{10}-keto C_{14}-hydroxy tautomer. Cleavage of the C_9–C_{10} bond would correspond to a regiodirected retro-Claisen condensation process.

The permissibility of lactone methanolysis as the step that produces the methyl ester was suggested by the action of methanolic sodium cyanoborohydride on the previously described **7**. There was thus obtained compound **9**. Therefore, the methanolysis of a lactone arising from a retroaldol cleavage is kinetically viable. However, the retro-Claisen pathway is certainly not ruled out. *By either formulation, the C_9–C_{10} bond is cleaving under remarkably mild conditions.*

Scheme V



Each of the three processes described previously is suggestive of the ease of forming a tetrahedral intermediate at C_9 (either on FK-506 itself or on the C_{10}-keto form thereof). Another transformation that is similarly suggestive involves the reaction of FK-506 with diazomethane. This afforded a 74% yield of a 4.7:1 mixture of spiroepoxide diastereomers **16**.[8,13] The structure of the major product is in accord with its spectroscopic properties. Most persuasive in this regard was an AB quartet (J = 5.11 Hz) centered at δ 2.88 in the ^{1}H NMR spectrum, which is characteristic for the diastereotopic hydrogens of the newly formed epoxide. That the spiroepoxide products resulted from attack of diazomethane at C_9 was clearly evident by the absence of a signal for this carbon in the ^{13}C NMR spectrum of **16**. The proposed empirical formula was also verified by combustion analysis. That the major and minor products differ only in their configuration at C_9 is suggested by their nearly identical spectroscopic properties (see Experimental Section). These data do not serve to assign the stereochemistry of the individual compounds shown as **16** (Scheme V).

It will be recalled that Schreiber and colleagues have elegantly demonstrated that the binding of FK-506 with FKBP does not entail the formation of a tetrahedral intermediate at C_9 or C_{10}.[14] This result is a rigorous one in that the C_9 and C_{10} carbonyl carbons were uniquely labeled with ^{13}C by total synthesis.[5a] Our findings, albeit with very different nucleophiles and under quite different solvent conditions, do identify a high vulnerability of C_9 for nucleophilic attack. They further suggest that had a nucleophilic center of FKBP in fact formed a covalent bond with C_9 there would have been triggered subsequent reactions whose products would have been detected in the ^{13}C NMR method of observation. Thus, the chemistry described here is not mirrored in the primary FK-506–FKBP binding interaction at least in vitro.

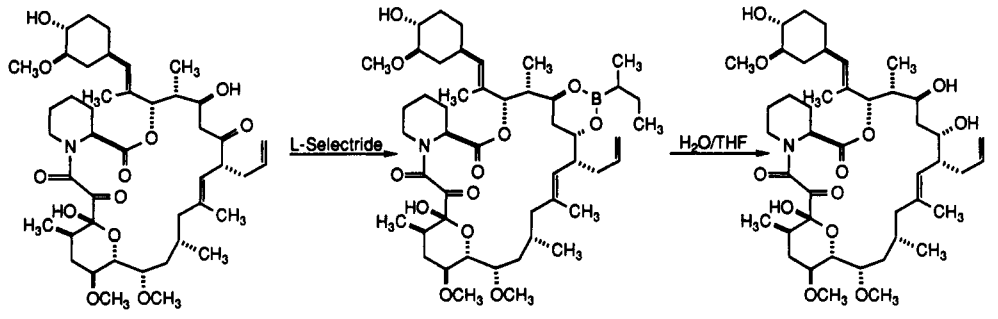
We have also investigated derivatives in which the C_{22} ketone is reduced. In a previous publication, we reported on the reduction of FK-506 with L-Selectride (Aldrich).[6a] The product thus obtained had the C_8–C_{10} tricarbonyl moiety intact and it was concluded that Selectride reduction had occurred at C_{22}. Upon consideration of the likely trajectory for this reduction, the assignment of structure **17** (i.e., 22*S*) for the presumed dihydro product was favored. Upon closer examination of all of the data, it is now clear that the immediate product of Selectride reduction is not simply a 22-dihydro derivative but is actually a boronate ester engaging the C_{24} and C_{22} hydroxyl groups.[15] Subsequent studies (vide infra) will reveal that

(12) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.

(13) During the course of our investigation, this transformation was reported. Cooper, M. E.; Donald, D. K.; Hardern, D. N. Chem. Abstr. **1990**, 113, 58793.

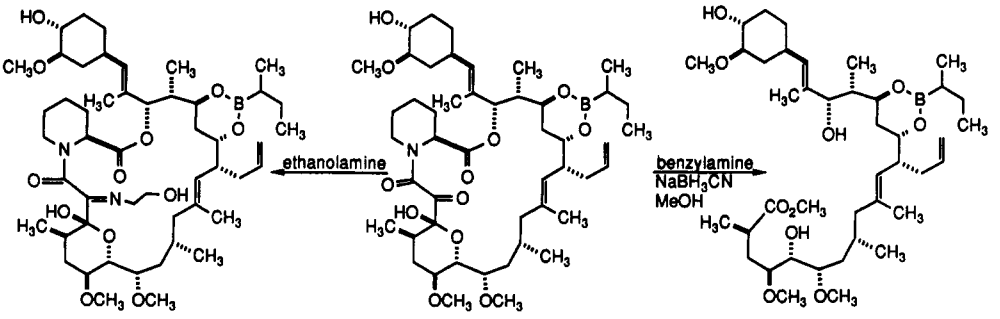
(14) Rosen, M. K.; Standaert, R. F.; Galat, A.; Nakatsuka, M.; Schreiber, S. L. *Science* **1990**, *248*, 863.

Scheme VI



1 18 17

Scheme VII



20 18 19

the configuration of C_{22} in this compound is indeed *S* and that the compound is properly represented as shown in 18 (Scheme VI).

Eventually it was found that the boronate function in 18 could be cleaved by long-term exposure to aqueous THF. Typically this reaction, when conducted over 2 days, affords a 45% (59% based on consumed 18) yield of (22*S*)-dihydro FK-506 (17). The ^1H NMR, ^{13}C NMR, and mass spectra of the compound prepared in this manner are in accord with the proposed structure, though they do not per se define the stereochemistry at C_{22}. This matter was to be clarified soon (vide infra).

Before the stability of the boronate linkage had been recognized, compound 18 (then presumed to be 17) was also subjected to the action of benzylamine and sodium cyanoborohydride in methanol. A 59% yield of the cleavage product 19, containing the *sec*-butyl boronate, was isolated. Support for this structure was derived from spectral data. Most decisive in this regard was a singlet in the ^1H NMR at δ 3.67 that is assigned to the methyl ester and a doublet (J = 7.2 Hz) at δ 1.19 that is assigned to the methyl group α to the ester moiety. That 19 still incorporated the boronate was further confirmed by the appearance of a molecular ion in the FAB mass spectrum spectrum at m/e 737.

Before we discovered that long-term exposure of 18 to aqueous THF would hydrolyze the boronate ester, a variety of more conventional treatments were attempted.[16] Most interesting of these was the reaction of 18 with ethanolamine (Scheme VI). *The principal product obtained was a C_9 imine with the boronate still intact.* The structure 20 proposed for this compound is supported by the absence of a signal at δ 198 in the ^{13}C NMR spectrum that is characteristic of a C_9 ketone, as well as by the presence of a signal at δ 171 that is readily assignable to the C_9 imine carbon. Additionally, the presence of the molecular ion m/e 915 in the FAB mass spectrum strongly corroborates the proposed structural formulation.

It was subsequently found that reduction of FK-506 with sodium triacetoxyborohydride in a mixture of HOAc and THF produces the two C_{22} epimeric alcohols. The major product, 17 (69%), was slightly contaminated by an unknown impurity, while the minor product (8%) is the pure C_{22} epimer 21.

The elegant studies of the Evans school[17] on the reduction of β-hydroxy ketones with sodium triacetoxyborohydride served to provide an initial basis for formulating the major compound as 17. Accordingly, the minor product from this reduction is assigned as the 22*R* compound 21. That this proposal is correct was convincingly demonstrated by comparison of the ^1H and ^{13}C NMR spectra of the minor reduction product with those of an authentic sample of 21. The "authentic" compound had been an intermediate in the Merck total synthesis of FK-506.[5b] Although, in the total synthesis, C_{22} ultimately emerges as a ketone, the assignment of this stereogenic center in compound 21 as *R* was secure. At an earlier stage, C_{22} had been fashioned by an erythro-selective aldol addition using a *N*-pentenoyloxazolidinone derived from L-valinol.[18] In the event, the ^1H NMR and ^{13}C NMR spectra of the minor reduction product were identical with those of authentic 21, while the corresponding spectra of 17 were clearly different. Most notable in this regard was the fact that 17 had an amide rotamer population of ca. 1:1, while the corresponding rotamer population for 21 was ca. 9:1.

With the configuration at C_{22} firmly assigned for the two 22-dihydro FK-506 epimers, we could determine the

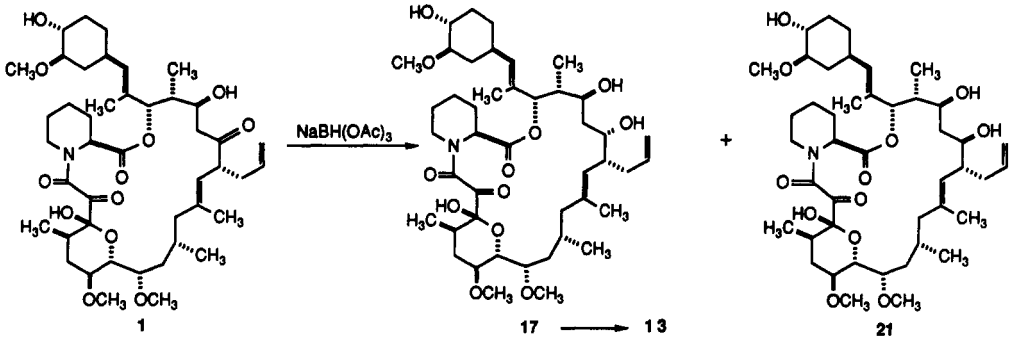
(15) The configuration of the *sec*-butyl group contained in compound 18 is not known. While ^1H and ^{13}C NMR analysis indicated that 18 was a single diastereomer, we are mindful that this apparent homogeneity may be the result of spectral coincidence.

(16) Of the methods employed, methanol, acetone–H_2O, diethanolamine, triethanolamine, and HOAc–H_2O gave either no reaction or extensive decomposition.

(17) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* 1988, *110*, 3560.

(18) Mills, S. G.; Desmond, R.; Reamer, R. A.; Volante, R. S.; Shinkai, I. *Tetrahedron Lett.* 1988, *29*, 281.

Scheme VIII



stereochemistry at the corresponding carbon in compound **13**. This compound is obtained from sodium borohydride reduction of the ring chain tautomers **11** and **12**, as well as by treatment of FK-506 with benzylamine and sodium cyanoborohydride. In the latter transformation, the precise stage at which the C_{22} ketone undergoes reduction has not been determined. When compound **17** was treated with sodium cyanoborohydride and benzylamine in methanol, the now familiar cascade was triggered leading to compound **13** and thus establishing it to have the 22*S* stereochemistry (Scheme VIII).

It is interesting to note that the reduction of the C_{22} ketone provides primarily the *S* alcohol for both the macrocycle **1** and, after the larger ring has been severed, cf. **11** and **12**. The selectivity observed in the reduction of the former compound may be the result of steric constraints imposed by the conformation of the macrocycle. That reduction of **11–12** gave the same result might be taken to suggest that the C_{10}–C_{34} sector does not suffer significant conformational change upon excision of the pipecolinate moiety. Alternatively, each case might simply reflect the local directivity of the C_{24} hydroxyl group.

Summary

It has been found that FK-506 can be converted by concise degradation to a compound in which the pipecolinyloxalyl fragment has been excised. This resultant product, incorporating C_{10}–C_{34}, could be valuable for the installation of alternate C_1–C_9 spacers by partial synthesis.[6c]

The issue of the stereochemistry in the C_{22} reduction products has been settled. The stereochemistry of compound **17**, shown to be *S* at C_{22}, is also fully assigned. The (22*R*)-dihydro FK-506 (compound **21**) is available as a minor product in one step from FK-506. It is interesting to note that ^{1}H NMR analysis reveals a large difference between the proclivity of both compounds for rotameric inhomogeneity. In the case of the 22*R* compound there seems to be a single preferred rotamer, while in the 22*S* compound, a 1:1 mixture predominates.

The availability of rather stable boronates (see compounds **18–20**) is another potentially important outcome. Compounds **18** and **20**, though containing the amide function, seem to exhibit high rotameric homogeneity. It will be of interest to evaluate the biological activity of these boronates and the effect of the resulting conformational rigidification in the C_{24}–C_{22} region.

The proclivity of nonhydric nucleophiles to attack C_9 has been demonstrated. An unanticipated tendency for fragmentation of the C_9–C_{10} or C_8–C_9 bond upon generation of a C_9 tetrahedral intermediate has been identified. *There is a clear implication that formation of tetrahedral character at C_9 of FK-506 brings with it major chemical lability leading to cleavage reactions.* Attempts to define the factors that are responsible for this effect are planned. It will be of considerable interest to learn whether the chemical characteristics of FK-506 identified in this work are operative in its interaction with any of its cellular receptors.

Experimental Section

Treatment of FK-506 with Methanol. Preparation of 4.[19] A solution of FK-506 (1; 55 mg, 69 μmol) and dry methanol (5.0 mL) was maintained at reflux for 3h, allowed to cool to 23 °C, and concentrated. The crude material was purified by chromatography (silica gel, 240–400 mesh, 5:1 EtOAc–hexane) to give 47 mg (82%) of 4 as a sticky white semisolid: ^{1}H NMR ($CDCl_3$, 490 MHz) δ 5.69 (m, 1 H), 5.32 (m, 1 H), 5.20–4.80 (m, 5 H), 4.60 (br s, 1 H), 4.10 (br d, *J* = 7.2 Hz, 1 H), 4.0 (br s, 1 H), 3.82 (s, OCH_3) 3.84–3.65 (m, 1 H), 3.60–3.10 (m, 6 H), 3.41, 3.40, 3.39, 3.31 (3 × OCH_3 including rotamers), 3.0 (m, 2 H), 2.80–2.55 (m, 1 H), 2.53–2.35 (m, 2 H), 2.33–2.15 (m, 3 H), 2.15–1.90 (m, 3 H), 1.90–1.20 (m, 14 H), 1.73, 1.63, 1.60 (s, CH=$CHCH_3$ including rotamers)) 1.20–0.80 (m, 12 H); IR (film) 3450, 1737, 1731, 1707, 1641 cm^{-1}; MS (FAB) *m/e* 836.5206 (836.5157 calcd for $C_{45}H_{74}NO_{13}$), 818, 626, 576. Anal. Calcd for $C_{45}H_{73}NO_{13}\cdot H_2O$: C, 63.31; H, 8.56; N, 1.64. Found: C, 63.43; H, 8.84; N, 1.59.

Treatment of FK-506 with TMSCN. Preparation of 6. A solution of FK-506 (1; 76 mg, 95 μmol) and dry benzene (1.8 mL) was treated with a catalytic amount of potassium cyanide/18-crown-6 complex followed by trimethylsilyl cyanide (13 μL, 95 μmol). The reaction mixture was maintained at 23 °C for 20 h before being concentrated. The residue was subjected to chromatography (silica gel, 240–400 mesh, EtOAc) to afford 60 mg (76%) of 6: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.69 (m, 1 H), 5.22–5.36 (m, 3 H), 4.93–5.05 (m, 4 H), 4.57 (br d, *J* = 3.3 Hz, 1 H), 4.08 (br d, *J* = 7.8 Hz, 1 H), 3.98 (m, 1 H), 3.70 (m, 1 H), 3.01–3.50 (m, 14 H, including 3.43, 3.41, 3.37, 3 × OCH_3), 3.01 (m, 1 H), 2.61–2.68 (m, 2 H), 2.30–2.48 (m, 5 H), 0.83–2.18 (m, 33 H), 1.31 (d, 7.1 Hz, 3 H); ^{13}C NMR (63 MHz, C_6D_6) δ 210.2, 173.0, 169.1, 164.7, 138.7, 136.3, 134.0, 131.6, 124.6, 116.5, 115.6, 84.7, 82.6, 82.5, 77.8, 73.9, 73.8, 68.0, 57.1, 56.3, 56.1, 58.7, 53.0, 54.6, 49.0, 46.5, 43.1, 39.6, 35.6, 35.5, 35.1, 33.5, 32.4, 32.0, 30.7, 27.3, 26.2, 24.7, 20.7, 20.2, 17.1, 16.3, 12.8, 9.6; IR (solution) 2933, 1732, 1667, 1450, 1376 cm^{-1}; MS (FAB) *m/e* 853.4864 (853.4827 calcd for $C_{45}H_{70}N_2O_{12}$). Anal. Calcd for $C_{45}H_{70}N_2O_{12}$: C, 65.06; H, 8.43; N, 3.37. Found: C, 64.9; H, 8.00; N, 2.97.

Oxidation of 6 with $Pb(OAc)_4$. Formation of 7. A solution of 6 (20 mg, 24 μmol) and dry methanol (1.5 mL) was treated with $Pb(OAc)_4$ (11 mg, 24 μmol) at 0 °C for 30 min after which time the reaction mixture was diluted with water and extracted with EtOAc (2×). The organic phases were combined and dried over $MgSO_4$, filtered, and concentrated. The residue was subjected to chromatography (silica gel, 240–400 mesh, 5:1 EtOAc/hexanes) to afford 16 mg (79%) of 7: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.70 (m, 1 H), 5.35 (br d, *J* = 8.5 Hz, 1 H), 5.20–5.25 (m, 2 H), 4.94–5.06 (m, 3 H), 4.44–4.47 (m, 1 H), 4.07 (d, *J* = 7.5 Hz, 1 H), 3.98 (m, 1 H), 3.88 (s, 3 H), 3.69 (m, 1 H), 3.34–3.57 (m, 13 H, including 3.42, 3.40, 3.37, 3 × OCH_3), 3.35 (m, 1 H), 2.65–2.67 (m, 2 H), 2.44–2.49 (m, 2 H), 2.28–2.33 (m, 3 H), 2.18 (m, 1 H), 1.99–2.06

(19) A copy of the ^{1}H NMR spectra for this compound is given in the supplementary material.

(m, 3 H), 1.95 (m, 1 H), 0.85–1.81 (m, 16 H), 1.70 (s, 3 H), 1.58 (s, 3 H), 1.29 (d, J = 7 Hz, 3 H), 0.90 (d, J = 6.2 Hz, 3 H), 0.88 (d, J = 6.1 Hz, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 211.0, 173.5, 169.1, 163.1, 161.3, 138.6, 135.5, 133.6, 130.9, 123.7, 116.5, 84.2, 82.6, 82.3, 77.4, 73.7, 73.5, 67.4, 57.5, 56.7, 56.3, 52.6, 52.4, 51.8, 48.4, 46.0, 44.3, 39.5, 39.1, 35.3, 35.1, 34.5, 33.3, 32.4, 31.3, 30.3, 27.0, 26.2, 25.0, 20.8, 19.6, 17.0, 16.5, 12.4, 8.8; IR (solution) 3570, 3480, 2940, 1740, 1660, 1445 cm^{-1}; MS (FAB) m/e 856.4769 (856.4825 calcd for $C_{45}H_{71}NO_{13}Na$).

Reaction of 6 with MnO_2. Preparation of 9. A mixture of 6 (10 mg, 12 μmol), MnO_2 (ca 20 mg), and methanol was stirred at 23 °C for 5 h. The reaction mixture was then filtered, and the filtrate was concentrated. The crude isolate was purified by chromatography (silica gel, 240–400 mesh, EtOAc–hexanes (5:1)) to provide 10 mg of 9 (99%) as a clear oil: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.68 (m, 1 H), 5.31 (m, 1 H), 5.20 (m, 2 H), 5.00 (m, 3 H), 4.45 (m, 1 H), 3.98 (m, 1 H), 3.87, 3.82 (s × 2, OMe for both major and minor rotamers), 3.66 (s, OCH_3), 3.39 (s, 3 H), 3.38 (s, 3 H), 3.32 (s, 3 H), 3.35–3.25 (m, 5 H), 3.17 (m, 1 H), 3.0 (m, 1 H), 2.8–2.55 (m, 5 H), 2.45 (m, 1 H), 2.35–1.95 (m, 7 H), 1.85–1.1 (m, 13 H), 1.67 (s, 3 H), 1.60 (s, 3 H), 1.19 (d, J = 7.0 Hz, 3 H), 1.10–0.8 (m, 2 H), 0.91 (d, J = 6.8 Hz, 3 H), 0.84 (d, J = 6.4, 3 Hz); ^{13}C NMR (63 MHz, $CDCl_3$) δ 211.2, 177.23, 169.3, 163.2, 161.5, 138.9, 135.6, 133.9, 133.5, 131.3, 131.1, 123.7, 116.6, 84.5, 81.9, 80.2, 78.1, 73.7, 73.5, 67.96, 57.9, 57.7, 56.5, 53.0, 52.4, 52.0, 51.4, 48.2, 45.9, 44.4, 39.8, 37.5, 35.8, 35.3, 24.8, 31.6, 27.5, 26.3, 25.2, 21.0, 19.8, 18.5, 16.8, 12.8, 9.2; IR (film) 3480, 2940, 1728, 1710, 1669, 1220 cm^{-1}; MS (FAB) m/e 888.5047 (888.5088 calcd for $C_{46}H_{75}NO_{14}Na$), 633.

Reaction of FK-506 and Diazomethane. Preparation of Spiroepoxides 16. A solution of FK-506 (1; 86 mg, 0.11 mmol) and Et_2O (5 mL) was treated with an excess of diazomethane (in Et_2O). The resulting solution was maintained at 23 °C for 12 h and then concentrated. The residue was subjected to chromatography (silica gel, 240–400 mesh, 5:1 EtOAc–hexanes), which afforded 53 mg (61%) of 16 major and 11 mg (13%) of 16 minor. 16 major: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.71 (m, 1 H), 5.68 (br s, 1 H), 5.00–5.29 (m, 5 H), 4.46 (br d, J = 13.3 Hz, 1 H), 4.00 (m, 1 H), 3.54 (br d, J = 9.7 Hz, 1 H), 3.31–3.51 (m, including 3.42, 3.38, 3.31, 3 × OCH_3, 13 H), 2.99–3.04 (m, 2 H), 2.95 (d, J = 5.1 Hz, 1 H), 2.81 (d, J = 5.1 Hz, 1 H), 2.13–2.52 (m, 7 H), 1.92–2.06 (m, 4 H), 0.85–1.80 (m, 18 H), 1.65 (s, 3 H), 1.58 (s, 3 H), 1.04 (d, J = 6.6 Hz, 3 H), 0.99 (d, J = 6.4 Hz, 3 H), 0.92 (d, J = 7.4 Hz, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 212.7, 170.4, 165.7, 138.9, 135.4, 132.4, 129.5, 122.1, 116.7, 96.7, 84.2, 76.9, 75.7, 74.1, 73.6, 73.4, 70.0, 65.8, 61.6, 56.7, 56.5, 56.3, 53.5, 50.7, 48.2, 42.9, 39.8, 39.7, 35.5, 34.9, 34.0, 33.7, 32.7, 26.9, 26.7, 24.5, 21.4, 20.7, 16.5, 16.3, 15.2, 14.2, 10.0. Anal. Calcd for $C_{45}H_{71}NO_{12}$: C, 65.88; H, 8.18; N, 1.36. Found: C, 65.74; H, 8.32; N, 1.45. 16 minor: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.70 (ddt, J = 6.9, 10.0, 16.9 Hz, 1 H), 5.62 (br d, J = 4.0 Hz, 1 H), 5.53 (s, 1 H), 5.13–5.00 (m, 5 H), 4.55 (br d, J = 11.7 Hz, 1 H), 4.2 (m, 1 H), 3.64 (dd, J = 3.6, 11.9 Hz, 1 H), 3.58 (d, J = 9.6 Hz, 1 H), 3.55 (m, 3 H), 3.42 (s, 3 H), 3.38 (s, 3 H), 3.34 (s, 3 H), 3.01 (ddd, J = 4.3, 8.8, 11.2 Hz, 1 H), 2.97 (d, J = 4.9 Hz, 1 H), 2.91 (d, J = 4.9 Hz, 1 H), 2.73 (m, 1 H), 2.65 (br s, 1 H), 2.50–2.35 (m, 2 H), 2.35–2.15 (m, 6 H), 2.15–2.0 (m, 3 H), 1.95–1.50 (m, 10 H), 1.65 (s, 3 H), 1.57 (s, 3 H), 1.45–1.25 (m, 44), 1.20–0.80 (m, 4 H), 1.06 (d, J = 6.5 Hz, 3 H), 0.89 (d, J = 6 Hz, 3 H), 0.86 (d, J = 7.2 Hz, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 209.4, 170.3, 166.1, 140.3, 135.2, 132.2, 129.1, 122.8, 116.8, 96.0, 84.3, 77.2, 75.3, 73.8, 73.7, 72.5, 70.6, 62.0, 57.8, 56.7, 56.5, 56.1, 53.5, 49.7, 48.2, 43.2, 39.8, 39.5, 36.6, 35.9, 35.1, 35.0, 33.4, 32.7, 31.4, 30.7, 28.3, 26.5, 25.0, 21.9, 20.1, 16.2, 15.4, 14.4, 6.8. Characteristic data for the product mixture: IR (film) 3472, 2932, 1736, 1704, 1640, 1093 cm^{-1}; MS (FAB) m/e 840.4965 (840.4876 calcd for $C_{45}H_{71}NO_{12}Na$).

Reductive Amination of FK-506. Preparation of 10–13. A solution of FK-506 (1; 0.10 g, 0.12 mmol) and dry methanol (1.5 mL) was treated with benzylamine (20 μL, 0.18 mmol) and sodium cyanoborohydride (ca. 50 mg). The reaction mixture was stirred at 23 °C for 14 h before being diluted with EtOAc. The resulting mixture was washed with water (2×) followed by brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The residue was subjected to chromatography (silica gel, 240–400 mesh, EtOAc) to afford 28 mg (35%) of 13, 14 mg (45%) of 10, and 26 mg (33%) of an inseparable mixture of 11 and 12. 10: mp 149–150 °C; ^{1}H NMR (490 MHz, $CDCl_3$) δ 7.26–7.37 (m, 5 H), 4.66 (br d, J = 13.0 Hz, 1 H), 4.59 (s, 2 H), 3.92 (br d, J = 12.0 Hz, 1 H), 3.86 (s, 2 H), 2.52 (m, 1 H), 2.45 (br d, J = 13.1 Hz, 1 H), 2.02 (br d, J = 12.5 Hz, 1 H), 1.73 (br d, J = 12.0 Hz, 1 H), 1.42–1.70 (m, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 165.5, 161.8, 135.6, 129.0, 128.5, 128.1, 59.5, 49.5, 48.8, 42.6, 31.4, 24.6 24.5; IR (solution) 2992, 2941, 2860, 1653, 1471 cm^{-1}; MS (CI) m/e 258.1363 (258.1369 calcd for $C_{15}H_{18}N_2O_2$). Characteristic data for the product mixture 11, 12: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.73 (m, 1 H), 5.30 (app dd, J = 9, 16 Hz, 1 H), 5.10–4.89 (m, 3 H), 4.57 (br s, 1 H), 4.23 (m, 1 H), 3.91 (m, 1 H), 3.67 (s, OCH_3), 3.66 (s, OCH_3), 3.50–3.25 (m, 4 H), 3.67, 3.67, 3.66, 3.65, 3.62 (3 × OCH_3 for both compounds), 3.17 (m, 1 H), 3.04 (m, 1 H), 2.80–2.60 (m, 3 H), 2.55–2.40 (m, 2 H), 2.40–2.00 (m, 4 H), 1.95–1.85 (m, 2 H), 1.80–1.45 (m, 12 H), 1.45–1.10 (m, 4 H),), 1.19 (app dd, J = 1.4, 7.1 Hz, 3 H), 1.10–0.80 (m, 7 H), 0.71 (d, J = 6.5 Hz, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 211.8, 177.3, 138.8, 138.3, 137.6, 135.5, 134.5, 132.3, 128.7, 128.3, 127.9, 125.1, 123.6, 116.6, 115.5, 99.1, 84.4, 84.4, 79.6, 78.5, 77.6, 77.2, 73, 6, 73.6, 73.2, 72.9, 71.5, 71.2, 68.5, 57.9, 57.5, 56.4, 52.7, 51.4, 48.9, 48.3, 48.2, 45.7, 39.0, 37.1, 36.5, 36.2, 35.4, 35.1, 35.0, 34.9, 33.9, 32.4, 31.4, 30.8, 30.7, 27.3, 27.1, 19.9, 19.8, 18.5, 18.5, 16.7, 16.5, 13.9, 13.8, 10.7, 5.7; IR (film) 3438, 2910, 1739, 1732, 1716, 1095 cm^{-1}; MS (FAB) m/e 691,4437 (691.4400 calcd for $C_{37}H_{64}O_{10}Na$) 659, 633, 409. 13: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.76 (m, 1 H), 5.33 (br d, J = 9.0 Hz), 4.97–5.07 (m, 3 H), 4.23 (br s, 1 H), 4.18 (br d, J = 9.8 Hz, 1 H), 3.78 (m, 1 H), 3.68 (s, 3 H), 3.34–3.46 (m, 15 H including 3.42, 3.41, 3.34, 3 × OCH_3), 3.16 (m, 1 H), 2.73 (m, 1 H), 2.50 (m, 1 H), 2.24–2.36 (m, 3 H), 1.92–2.11 (m, 5 H), 1.91 (dd, J = 9.8, 6.6 Hz, 1 H), 1.81 (m, 1 H), 1.50–1.69 (m, 7 H), 1.64 (s, 3 H), 1.59 (s, 3 H), 1.18–1.40 (m, 3 H), 1.20 (d, J = 7.1 Hz), 1.04 (ddd, app q, J = 11.7, 1 H), 0.90 (d, J = 6.5 Hz, 3 H), 0.84 (d, J = 7.0 Hz, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 177.4, 137.6, 136.8, 134.6, 128.3, 126.5, 116.0, 84.3, 77.6, 73.6, 72.8, 72.6, 71.3, 58.0, 57.5, 56.5, 51.6, 48.6, 44.0, 39.6, 38.8, 36.3, 36.2, 35.4, 34.9, 35.4, 34.5, 31.3, 30.7, 27.0, 20.2, 18.6, 16.7, 14.1, 5.1; IR (solution) 3400, 2900, 1732, 1640, 1455, 1380 cm^{-1}; MS (FAB) m/e 693, 4537 (693.4556 calcd for $C_{37}H_{66}O_{10}Na$). Anal. Calcd for $C_{37}H_{66}O_{10}$: C, 66.27; H, 9.85. Found: C, 65.67, H, 9.92).

Reduction of the Mixture 11–12 with $NaBH_4$. The compound mixture 11–12 (18 mg, 27 μmol) was dissolved dry methanol (1 mL) and treated with sodium borohydride (ca. 50 mg) at 0 °C. After 1.5 h, the reaction mixture was diluted with water and then extracted with EtOAc. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The residue was subjected to chromatography (silica gel, 240–400 mesh, EtOAc) to afford 8 mg (44%) of 13.

Treatment of 7 with Sodium Cyanoborohydride and Methanol. Formation of 9. A solution of 7 (24 mg, 29 μmol) and dry methanol (1 mL) was treated with sodium cyanoborohydride (2 mg, 30 μmol) at 23 °C. After 12 h, this solution was diluted with water and extracted with EtOAc. The organic material was dried ($MgSO_4$) and concentrated. The crude material was purified by chromatography (silica gel, 240–400 mesh, EtOAc–hexanes (5:1)) to give 19 mg (79%) of 9 as a clear oil.

Reductive Amination of 18. Preparation of 19. A solution of 18 (84 mg, 96 μmol) and dry methanol (2 mL) was treated with benzylamine (16 μL, 0.15 mmol) and sodium cyanoborohydride (ca. 50 mg). The reaction mixture was maintained at 23 °C for 22 h before being diluted with water and then extracted with EtOAc (2×). The organic phases were combined and washed with water followed by brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was subjected to chromatography (silica gel, 240–400 mesh, 5:1 EtOAc–hexanes) to afford 42 mg (59%) of 19 and 8 mg (32%) of 10. 19: ^{1}H NMR (490 MHz, $CDCl_3$) δ 5.77 (m, 1 H), 5.39 (br d, J = 8.0 Hz, 1 H), 4.97–5.08 (m, 3 H), 4.37–4.41 (m, 2 H), 3.84 (m, 1 H), 3.67 (s, 3 H), 3.33–3.46 (m, 12 H, including 3.41, 3.40, 3.33 (3 × OCH_3)), 3.16 (m, 1 H), 3.02 (m, 1 H), 2.70–2.75 (m, 2 H), 2.41 (m, 1 H), 1.19–2.34 (m, 22 H), 162 (s, 3 H), 1.55 (s, 3 H), 1.19 (d, J = 6.0 Hz, 3 H), 0.83–1.06 (m, 12 H), 0.60 (d, J = 7.0 Hz, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 177.3, 137.1, 137.0, 132.1, 128.5, 126.4, 115.8, 84.5, 79.9, 77.9, 76.6, 73.7, 72.3, 70.7, 57.9, 57.6, 56.5, 51.4, 48.4, 44.8, 39.7, 37.2, 36.8, 35.6, 35.3, 35.2, 35.0, 34.7, 31.6, 30.8, 27.3, 26.1, 19.9, 18.5, 16.8, 16.5, 15.3, 13.5, 13.5, 4.1; IR (film) 3439, 2925, 1736, 1453, 1693 cm^{-1}; MS (FAB) m/e 759.5232 (759.5197 calcd

for $C_{14}H_{73}BO_{10}Na$). Anal. Calcd for $C_{14}H_{73}BO_{10}$: C, 66.85; H, 9.92. Found: C, 66.19; H, 10.15.

Reaction of 18 with Ethanolamine. Formation of 20. A solution of 18 (38 mg, 44 μmol) in CH_2Cl_2 (2 mL) was treated with excess ethanolamine (ca. 0.1 mL), and the resulting solution was maintained at 23 °C for 24 h. The solution was then diluted with ether and washed with water and brine. The organic material was dried ($MgSO_4$) and concentrated. The crude product was purified by chromatography (silica gel, 240–400 mesh, EtOAc–hexane (5:1)), giving 32 mg (80%) of **20** as a clear oil: ^1H NMR ($CDCl_3$, 490 MHz) δ 5.73 (m, 1 H), 5.40 (br s, 1 H), 5.14–4.92 (m, 6 H), 4.56 (br d, J = 13.5, Hz, 1 H), 4.03 (dd, J = 9.1, 4.9 Hz, 1 H), 3.83–3.70 (m, 4 H), 3.65–3.50 (m, 2 H), 3.45–3.20 (m, 3 H), 3.42 (s, 3 H), 3.39 (s, 3 H), 3.30 (s, 3 H), 3.02 (ddd, J = 4.2, 8.8, 11.3 Hz, 1 H), 2.71–2.63 (m, 2 H), 2.55 (m, 1 H), 2.40–1.95 (m, 8 H), 1.95–1.15 (m, 17 H), 1.68 (s, 3 H), 1.55 (s, 3 H), 1.15–0.80 (m, 20 H); ^{13}C NMR (63 MHz, $CDCl_3$) 171.1, 167.6, 165.5, 137.3, 136.7, 129.1, 125.5, 115.6, 95.8, 84.3, 76.7, 74.9, 74.0, 73.7, 72.6, 70.8, 69.5, 61.8, 57.6, 56.8, 56.7, 56.2, 55.8, 50.1, 42.9, 41.0, 39.6, 36.2, 35., 35.1, 33.7, 33.0, 31.4, 31.2, 30.8, 28.1, 26.3, 26.2, 26.2, 25.0, 22.1, 20.2, 16.4, 15.7, 15.3, 15.3, 14.6, 13.5, 9.8; IR (film) 3403, 2936, 1734, 1638, 1444, 1196 cm^{-1}; MS (FAB) m/e 915.6202 (915.6120 calcd for $C_{50}H_{84}N_2BO_{12}$), 897, 687, 309.

(22*S*)-Dihydro FK-506 (17).[19] A solution of 18 (9.5 mg, 11 μmol) in THF (2.0 mL) was treated with H_2O (3.0 mL), and the resulting solution was maintained at 23 °C for 2 days. This solution was then diluted with saturated aqueous sodium bicarbonate and extracted with EtOAc. The organic material was dried (K_2CO_3) and concentrated. The crude isolate was purified by chromatography (silica gel, 240–400 mesh, THF–hexanes (40:60)), giving 3.7 mg of 17 (42%) and 2.7 mg of recovered 18: ^1H NMR (490 MHz, $CDCl_3$) δ 5.78 (m, 1 H), 5.30 (br s, 0.5 H), 5.22 (br s, 0.5 H), 5.20–4.92 (m, 5 H), 4.65 (br s, 1 H), 4.43 (br d, J = 7.6 Hz, 1 H), 3.95–3.80 (m, 13 H), 3.78–3.65 (m, 1 H), 3.65–3.52 (m, 2 H), 3.50–3.20 (m, 5 H), 3.41, 3.38, 3.37, 3.32, 3.30 (s, 3 × OCH_3 for both major and minor rotamers), 3.01 (m, 1 H), 2.84 (m, 0.7 H), 2.70–1.95 (m, 9 H), 1.95–1.20 (m, 14 H), 1.66, 1.64 (s, CH=$CHCH_3$ for both major and minor rotamers), 1.54, 1.48 (s, CH=CH CH_3 for both major and minor rotamers) 1.20–0.80 (m, 13 H); ^{13}C NMR (63 MHz, $CDCl_3$; data given for both major and minor rotamers) δ 195.97, 195.82, 169.32, 169.21, 165.65, 165.06, 137.48, 136.42, 135.86, 132.72, 131.66, 128.95, 126.69, 126.22, 115.72, 90.49, 97.11, 84.31, 78.17, 76.81, 75.52, 73.96, 73.84, 73.69, 73.40, 72.90, 71.81, 71.02, 70.06, 70.55, 57.02, 56.64, 56.58, 56.26, 56.11, 52.61, 49.40, 48.97, 44.58, 44.11, 44.00, 40.85, 39.61, 39.50, 37.05, 36.85, 35.91, 35.11, 35.05, 34.82, 34.08, 33.97, 33.02, 32.71, 32.61, 34.41, 30.82, 29.70, 27.35, 26.85, 26.71, 26.05, 24.71, 24.53, 21.47, 20.67, 16.47, 16.29, 15.77, 15.38, 14.62, 14.30, 10.50, 9.44; IR (film) 3455, 2928, 1733, 1642, 1453, 1089 cm^{-1}; MS (FAB) m/e 828.4909 (828.4876 calcd for $C_{44}H_{71}O_{12}NNa$), 578.

Reductive Amination of (22*S*)-Dihydro FK-506. A solution of 18 (20 mg, 25 μmol) and dry methanol (0.5 mL) was treated with benzylamine (3.20 μL, 29 μmol), and $NaBH_3CN$ (ca 50 mg) at 23 °C for 24 h. At this time, the solution was diluted with water (10 mL) and extracted with EtOAc. The extracts were dried ($MgSO_4$) and concentrated. The crude material was purified by chromatography (silica gel, 240–400 mesh, EtOAc–hexanes (5:1)), giving 6.6 mg (40%) of **13** as a clear oil.

Reduction of FK-506 with $NaBH(OAc)_3$. A solution of FK-506 (0.117 g, 0.146 mmol) and THF (1.75 mL) was treated with $NaBH(OAc)_3$ (0.154 g, 0.730 mmol) and HOAc (0.35 mL) at 23 °C for 2 h. At this time, the solution was diluted with H_2O and extracted with EtOAc. The combined extracts were washed with saturated aqueous sodium bicarbonate and brine. The organic material was dried ($MgSO_4$) and concentrated. The crude material was purified by chromatography (silica gel, 240–400 mesh, 60:40 hexanes–THF), giving 9.0 mg of **20**[19] (7.7%) and 81 mg of 17 (69%). Prepared in this fashion, 17 contains ca. 10% (as evidenced by the appearance of unassignable signals at δ 5.95 (br s) and 5.50 (br s)) of an unknown impurity. Nevertheless, this material was used successfully in the transformations outlined in this paper.

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Supplementary Material Available: NMR spectra for compounds **4**, **9**, **17**, and **19–21** (6 pages). Ordering information is given on any current masthead page.

Notes

Prostaglandin 1,15-Lactones of the F Series from the Nudibranch Mollusc *Tethys fimbria*

Guido Cimino, Antonio Crispino, Vincenzo Di Marzo, Aldo Spinella,* and Guido Sodano[‡]

Istituto per la Chimica di Molecole di Interesse Biologico del CNR, Via Toiano, 6 Arco Felice (NA), Italy

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We have recently reported for the first time the natural occurrence of prostaglandin 1,15-lactones of the E series (**1–3**) in the nudibranch *Tethys fimbria*.[1] More recently,[2] we have found that these lactones are biosynthesized from free prostaglandins in the mantle of the mollusc and are converted back into the prostaglandins upon detachment of the cerata (body appendices) during the behavioral defense mechanism known as autotomy. We describe now the isolation and structure characterization of prostaglandin 1,15-lactones of the F series from the same mollusc and from its egg masses. In addition, **4**, which was not detected previously,[1] has also been isolated by HPLC and its structure established by comparison with standard PGE_2-1,15-lactone 11-acetate.

$PGF_{2\alpha}$-1,15-lactone 11-acetate (**5**) and $PGF_{3\alpha}$-1,15-lactone 11-acetate (**6**) were isolated from the mantles and cerata of the mollusc in the relative amounts reported in Table I. Comparison of their ^1H NMR spectra (Experimental Section) with those of **1–3**[1] and with the published spectrum of synthetic $PGF_{2\alpha}$-1,15-lactone[3] (**7**) suggested

[‡] Current address: Dipartimento di Fisica, Università di Salerno, 84081 Baronissi (SA), Italy.

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