DEGRADATION AND MANIPULATIONS OF THE IMMUNOSUPPRESSANT FK506: PREPARATION OF POTENTIAL SYNTHETIC INTERMEDIATES

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Abstract - *Degradation of the immunosuppressant FK506 is described.*

The fermentation product FK506 (**1**), isolated from *Streptomyces tsukubaensis* no. 9993 by a group of Fujisawa scientists, has stimulated a great deal of research.[2-4] Not the least source of interest in FK506 arises from its potent immunosuppressive powers. From a chemical viewpoint, the novel 23-membered macrolide structure with its varied menu of appendages and stereogenic centers provides a great deal of challenge to any program in total synthesis. Of course, the ultimate goal in studying FK506 is to identify with precision the minimum structural domains that are required for its immunosuppressive activity. Such information might provide a better insight into the mode of action of this molecule, and, by inference, begin to clarify the steric and electronic character of its receptor(s).

Our laboratory is investigating several facets of the FK506 problem. An effort in total synthesis is being conducted in parallel with a degradative program. In this paper we describe some of our initial accomplishments in the latter area.

We first addressed one of the sources of the chemical instability of FK506, *i.e.*, the ß-hydroxyketone sector (carbons 22-24).[5] Reduction of **1** with Li(*s*-Bu)$_3$BH (THF, -78°C, 1.5 h) occurred selectively at C_{22} to afford a single alcohol (**2**, Scheme 1, ~90% yield). There was little or no interference from competing reduction of either the C_9 or the C_{10} masked ketone. A rigorous assignment of the stereochemistry at C_{22} of this product is not offered.[6] However, examination of molecular models based on the molecular geometry provided in the previously published crystal structure[2] suggested that attack of hydride would occur from the ß-face of the molecule (as drawn), to provide a product with the 22*S* stereochemistry. Treatment of **2** with lead tetraacetate (1.5-2.0 equiv) in methanol (0-25°C) afforded the amide diester **3** (52%, 33% recovered **2**). The

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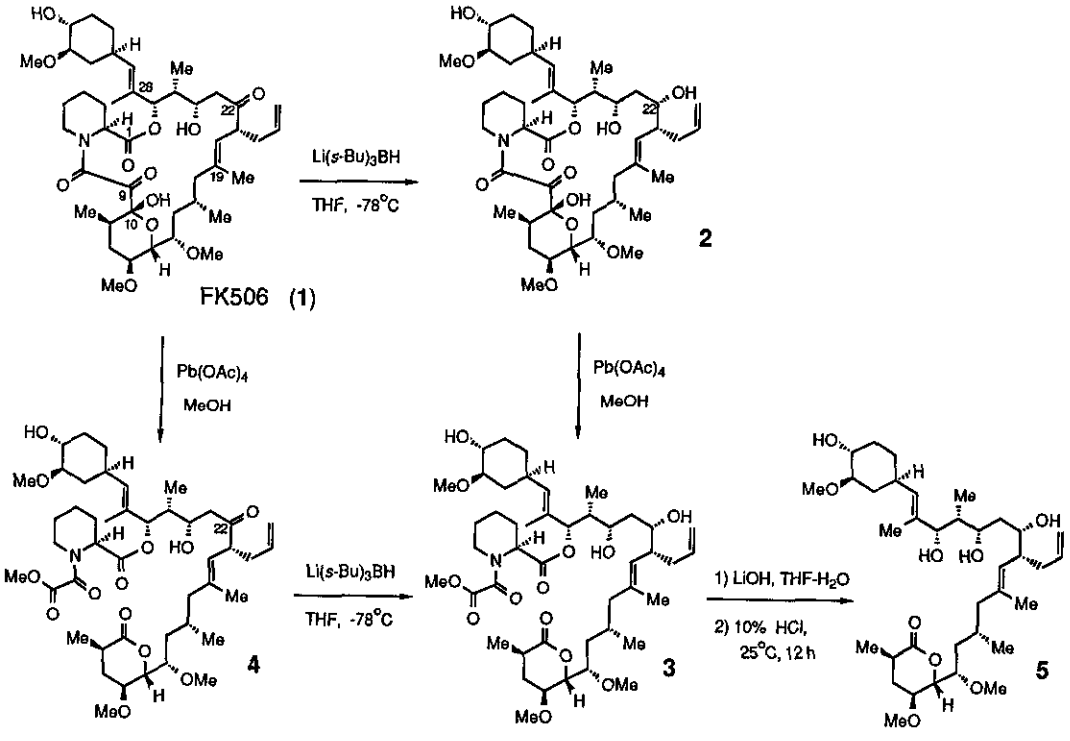
We dedicate this paper to Professor Derek H. R. Barton on the occasion of his 70th birthday. We are thankful for his continuing leadership in organic chemistry .

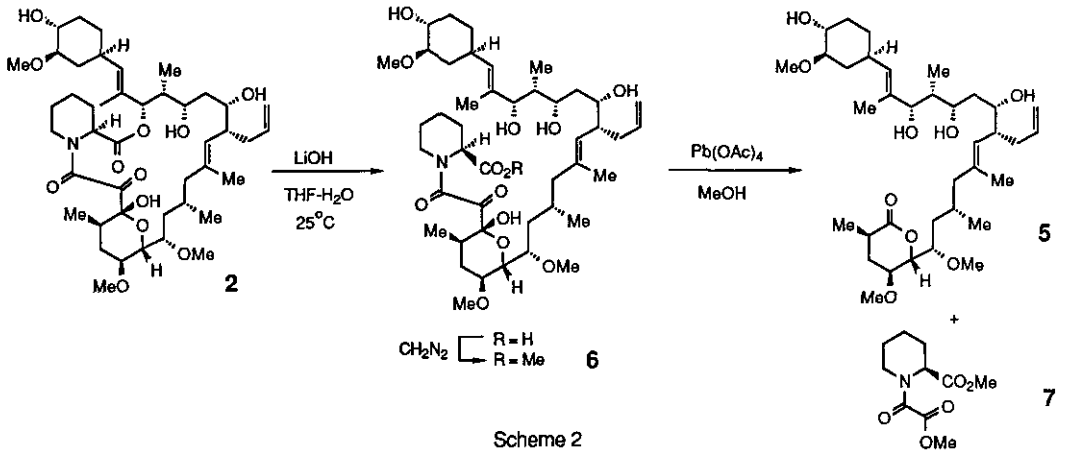
pipecolinate residue of **3** could be removed by treatment with lithium hydroxide (10 equiv, THF-H_2O, 25°C) followed by acidification (10% HCl, pH 1, 25°C, 12 h), thereby providing the lactone tetraol **5**[7] (65%).



Scheme 1

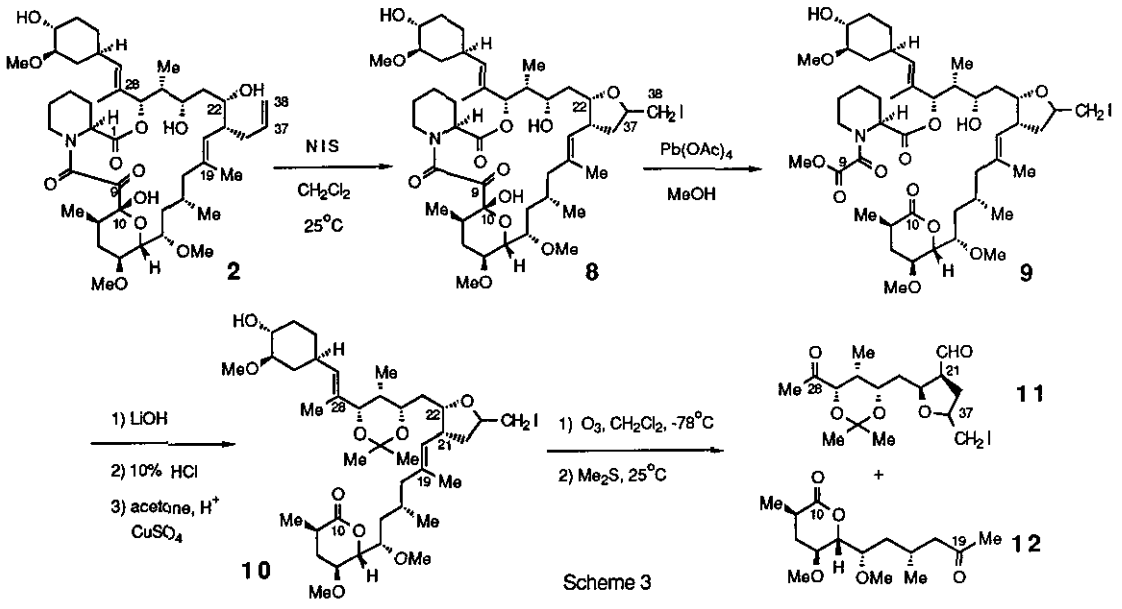
It was of interest to determine the stereochemical outcome when reduction of the C_{22} ketone was carried out on a *seco* variation of FK506. Treatment of **1** with lead tetraacetate in methanol afforded **4**, (81%), which upon reduction with Li(*s*-Bu)$_3$B H (THF, -78°C) gave rise to **3** as the only product (Scheme 1). The fact that the two routes provided the same reduction product suggests, but does not prove, that the local environment in the area of the C_{22} ketone is similar in **1** and **4**.

The oxalylpipecolinate residue could be excised and retrieved from the molecule. This degradative sequence (Scheme 2) began with treatment of **2** with lithium hydroxide (1.1 equiv, THF-H_2O, 25°C) followed by acidification (Dowex 15W, H^+ form). Esterification of the resulting carboxylic acid with diazomethane (ether, 0°C) led, in low yield (25%), to a substance formulated as **6**, the methyl ester of the 22-dihydro-*seco*-acid corresponding to FK506. Treatment of **6** with lead tetraacetate in methanol afforded **5** and 7.[8]



Scheme 2

We further investigated selective differentiation of the C_{22} oxygen by exploiting its proximal allyl group (Scheme 3). Toward this end, compound **2** was treated with *N*-iodosuccinimide in CH_2Cl_2 (1.5-2.0 equiv, 25°C, 2-4 days). A very slow reaction was noted. We were unable to drive the process to completion under more forcing conditions. Under the conditions described above, 37% of a single iodoether **8** was obtained (36% recovered **2**; stereochemistry at C_{37} not rigorously assigned). When this compound was subjected to the sequence: *(i)* $Pb(OAc)_4$, *MeOH*, *52%*; *(ii)* *LiOH*, *THF-MeOH-*H_2O (3:2:1), 25°C, 89%; (iii) acetone, $CuSO_4$, CSA, 76%; the lactone acetonide **10**[9] was produced. Ozonolysis of **10** (-78°C, CH_2Cl_2; Me_2S, 25°C) afforded **11**[10] and **12**.[11] The results of additional degradative and synthetic studies will be disclosed in due course.



Scheme 3

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5. Treatment of FK506 with 1 *N* NaOH is reported to cause cleavage of the C_{23}-C_{24} and C_{25}-C_{26} carbon-carbon bonds via a retroaldol process: see reference 2. We were unable to selectively hydrolyze the macrolactone ester linkage of FK506 using stoichiometric LiOH (THF-MeOH-H_2O, 0 °C).
6. Analysis and assignment of the ^1H nmr spectra of FK506 and related cyclic intermediates are severely complicated by the presence of rotamers involving the pipecolinic acid residue: see also reference 2.
7. Compound **5** was characterized: ^1H Nmr ($CDCl_3$, 500 MHz) δ 5.78 (dd, 1H, J = 9.2, 1.3 Hz), 5.09 (d, 1H, J = 9.9 Hz), 5.04 (dd, 1H, J = 17.0, 2.0 Hz), 4.97 (dd, 1H, J = 10.1, 2.1 Hz), 4.39 (m, 2H), 4.08 (dd, 1H, J = 7.9, 1.6 Hz), 3.86 (br m, 1H), 3.70 (ddd, 1H, J = 11.1, 7.8, 4.5 Hz), 3.50 (ddd, 1H, J = 7.2, 5.5, 1.6 Hz), 3.42 (s, 3H), 3.41 (s, 3H), 3.39 (s, 3H), 3.02 (ddd, 1H, J = 11.2, 8.7, 4.1 Hz), 2.70 (br s, 1H), 2.48 (m, 1H), 2.41 (m, 1H), 2.31 (m, 3H), 2.13 (m, 2H), 2.01 (m, 2H), 1.94 (m, 1H), 1.84 (m, 1H), 1.75-0.95 (m, 14H), 1.61 (d, 3H, J = 1.2 Hz), 1.55 (d, 3H, J = 0.3 Hz), 1.29 (d, 3H, J = 7.0 Hz), 1.89 (m, 3H), 0.60 (d, 3H, J = 7.1 Hz).

8. Compound **7**, which consisted of major and minor rotameric forms (*ca.* 2:1), was identical (by ^{1}H nmr and SiO_2 TLC comparison) with synthetic material prepared from L-pipecolinic acid. Compound **7** was characterized: ^{1}H Nmr ($CDCl_3$, 490 MHz) δ 5.25 (d, 1H, J = 5.7 Hz, major), 4.54 (d, 1H, J = 5.2 Hz, minor), 4.46 (br d, 1H, J = 12.7 Hz, minor), 3.89 (s, 3H, major), 3.85 (s, 3H, minor), 3.78 (s, 3H, minor), 3.77 (s, 3H, major), 3.58 (br d, 1H, J = 11.6 Hz, major), 3.34 (td, 1H, J = 13.2, 3.1 Hz, major), 2.91 (td, 2H, J = 13.0, 3.5 Hz, minor), 2.31 (br d, 1H, J = 14.1 Hz, major), 2.28 (br d, 1H, J = 13.7 Hz, minor), 1.8-1.3 (m, 5H); EIMS, *m/e* (relative intensity) 229 (M^+, 16), 170 (base), 142 (60), 110 (7), 82 (13), 70 (23).
9. Compound **10** was characterized: ^{1}H Nmr ($CDCl_3$, 490 MHz) δ 5.27 (d, 1H, J = 9.0 Hz), 5.09 (d, 1H, J = 10.0 Hz), 4.25-4.15 (m, 4H), 4.07 (dd, 1H, J = 7.9, 1.7 Hz), 3.70 (ddd, 1H, J = 11.1, 7.9, 4.5 Hz), 3.5 (ddd, 1H, J = 8.0, 6.1, 1.7 Hz), 3.43 (m, 1H), 3.41 (s, 3H), 3.40 (s, 3H), 3.39 (s, 3H), 3.27 (dd, 1H, J = 9.8, 5.1 Hz), 3.22 (dd, 1H, J = 9.8, 6.7 Hz), 3.07 (m, 1H), 3.01 (ddd, 1H, J = 11.3, 8.8, 4.2 Hz), 2.66 (br s, 1H), 2.49 (m, 1H), 2.31 (m, 2H), 2.12 (dd, 1H, J = 12.7, 3.8 Hz), 2.01 (dt, 2H, J = 12.7, 4.3 Hz), 1.94 (dd, 2H, J = 7.0, 5.3 Hz), 1.80-0.85 (m, 12H), 1.60 (d, 3H, J = 1.2 Hz), 1.54 (d, 3H, J = 1.0 Hz), 1.46 (s, 3H), 1.41 (s, 3H), 1.29 (d, 3H, J = 7.0 Hz), 0.84 (d, 3H, J = 6.2 Hz), 0.65 (d, 3H, J = 6.8 Hz).
10. Compound **11** was characterized: ^{1}H Nmr ($CDCl_3$, 490 MHz) δ 9.81 (d, 1H, J = 2.4 Hz), 4.46 (ddd, 1H, J = 10.3, 5.8, 4.5 Hz), 4.32 (d, 1H, J = 2.7 Hz), 4.19 (m, 2H), 3.27 (d, 2H, J = 5.6 Hz), 3.13 (m, 1H), 2.54 (ddd, 1H, J = 13.3, 7.2, 2.8 Hz), 2.17 (s, 3H), 1.94-1.81 (m, 3H), 1.46 (s, 3H), 1.47 (s, 3H), 0.80 (d, 3H, J = 6.8 Hz).
11. Compound **12** was characterized: ^{1}H Nmr ($CDCl_3$, 490 MHz) δ 4.11 (dd, 1H, J = 8.0, 1.7 Hz), 3.68 (ddd, 1H, J = 11.1, 8.0, 4.5 Hz), 3.46 (ddd, 1H, J = 7.8, 6.1, 1.7 Hz), 3.41 (s, 3H), 3.40 (s, 3H), 2.50 (m, 2H), 2.32 (m, 2H), 2.14 (s, 3H), 2.11 (m, 1H), 1.7-1.5 (m, 3H), 1.30 (d, 3H, J = 7.0 Hz), 0.95 (d, 3H, J = 6.6 Hz).

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