recycles. During the spirocyclization, a β-alkoxy enone was formed as a major byproduct. This enone appeared to be in equilibrium with spiroketal **13** and could be converted to the original mixture containing more **13** upon isolation and further treatment with acidic methanol.

Studies for the conversion of **13** to monensin were aided by an alternative, more direct prepartion of **13** from methyl monensin. Thus monensin methyl ester could be protected as the $C_{25}$,$C_{26}$ benzylidene derivative (PhCHO, $ZnCl_2$), oxidized to the $C_7$ ketone (PCC, $CH_2Cl_2$), and deprotected ($H_2$, Pd/C, MeOH) to **13** in approximately 40% overall yield.

The last strategic operation was reduction of the $C_7$ ketone to the axial hydroxyl of monensin. Previous work here had shown that certain 7-keto derivatives of monensin yielded the natural axial 7-hydroxyl configuration with very high stereoselection upon simple reduction with sodium borohydride in methanol, and we attributed at least part of the stereocontrol to the hindrance provided by the 1,3-axial 9-alkoxy group on the cyclohexanoid ring. The situation, however, would appear to be somewhat more complicated since the stereoselectivity of the borohydride reduction was lost upon addition of the terminal methanol side chain of **14**. It is likely that, as found in the X-ray crystal structure of monensin itself, the right-hand half of **14** and related molecules folds back toward the spiroketal ring system and thus places structurally remote features close to the $C_7$ carbonyl. While various borohydride reagents failed to provide **14** cleanly, borane–dimethyl sulfide complex provided the desired axial alcohol with 8:1 stereoselection (82% yield), and K-Selectride[3,9] (Aldrich) gave it exclusively (91% yield). Practically speaking, however, exceptionally high stereoselection is not critical to the synthesis since the minor epimer resolves from **14** with an $R_f$ difference of more than 0.2 on silica gel.

Final conversion to monensin followed previous work[1] and lead to sodium monensin by saponification, methyl ketal hydrolysis, and salt formation in 81% yield overall. It was necessary to conduct the ketal hydrolysis after saponification of the methyl ester to avoid extensive epimerization at $C_{24}$. Overall, the construction was a relatively efficient one leading to monensin A from the tetracyclic dilactone **3** in 19% overall yield.[10]

**Supplementary Material Available:** Complete experimental section (6 pages). Ordering information is given on any current masthead page.

(9) Brown, H. C.; Krishnamurthy, S. *J. Am. Chem. Soc.* **1972**, *94*, 7159.
(10) This work was supported by Grant HL25634 from the National Institutes of Health.

**Dongwei Cai, W. Clark Still***
*Department of Chemistry*
*Columbia University*
*New York, New York 10027*
*Received May 4, 1988*

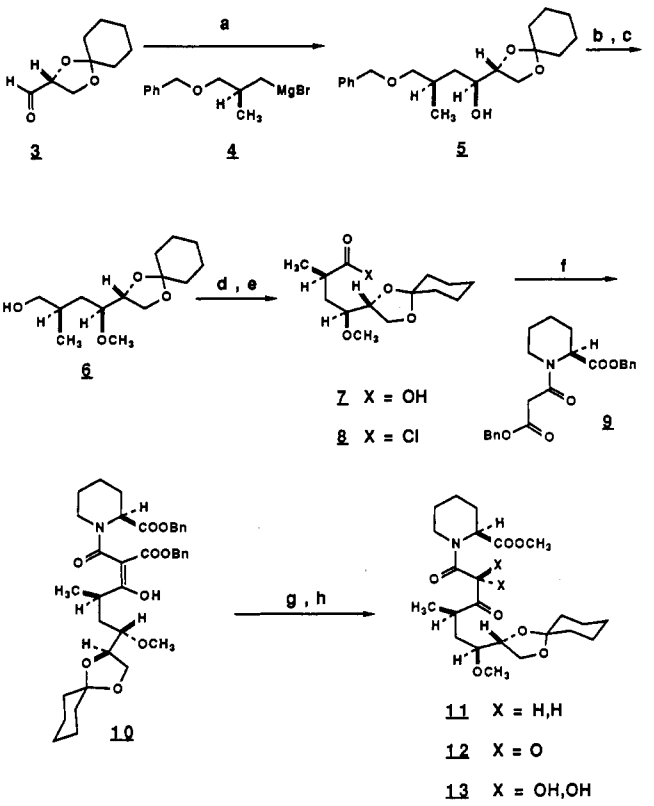
## Synthesis of the α,β-Diketo Amide Segment of the Novel Immunosuppressive FK506

*Summary:* A convenient synthesis of the optically active subunit **2**, incorporating the novel α,β,γ-tricarbonyl system of the potent immunosuppressive macrolide FK506, is described.

*Sir:* Recently the structure of FK506 was assigned as the novel macrocyclic lactone **1** isolated from *Streptomyces tsukubaensis* (no. 9993).[1] This unique 23-membered macrolide is an important new lead in the search for effective immunosuppressive agents. The exceptional activity of FK506 is reportedly considerably greater, in several assays, than cyclosporin A itself, which is currently the drug of choice in bone marrow and organ transplantations. The natural product contains a peculiar tricarbonyl system as incorporated in the hemiketal, α,β-diketo amide segment ($C_1 \rightarrow C_{15}$). Only two additional antifungal antibiotics, rapamycin[2] and 29-demethoxyrapamycin,[3] have been reported to possess similar functionalization. Rapamycin is currently under investigation by the National Cancer Institute as a potent antitumor agent. Recent efforts of Merck scientists have recorded diastereoselective pathways for preparation of two subunits of FK506 (**1**).[4] We illustrate our studies, providing the first synthetic route to the optically active α,β-diketo amide segment **2** ($C_1 \rightarrow C_{15}$) of the macrolide **1**.

From the onset of our interest, we have assumed that the sequential α,β,γ-tricarbonyl system of **1**, and rapamycin, is essential for biological activity as a site for selective nucleophilic additions. Significant structural fea-

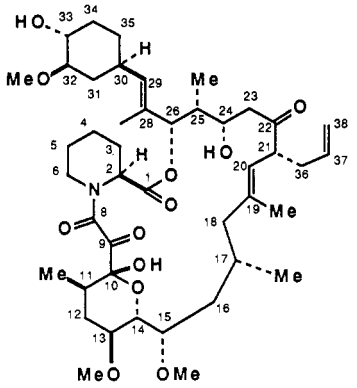
**Scheme I**[a]



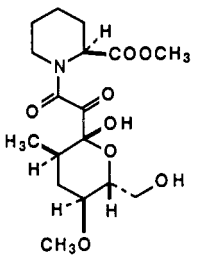

[a] (a) $Et_2O$, −78 °C, add **4** (75%); (b) dimsyl sodium, DMSO, $CH_3I$ (5 equiv), room temperature (84%); (c) Na (3 equiv), THF liquid $NH_3$ (90%); (d) THF, Celite, 0 °C, added dropwise 3 M Jones' reagent, 1 h (78%); (e) ClCOCOCl (20 equiv), benzene, +5 → 22 °C, 1 h, (98%); (f) THF, amide **9** (3 equiv), isopropylmagnesium chloride (2 equiv), −10 → 0 °C, 40 min; then **8** in THF (65%); (g) $H_2$ (1 atm), 10% Pd–C, EtOAc; then $CH_2N_2$, $Et_2O$ (100%); (h) $SeO_2$ (1.2 equiv), dioxane, reflux (76%).

(1) Tanaka, H.; Kuroda, A.; Marusawa, H.; Hatanaka, H.; Kino, T.; Goto, T.; Hashimoto, M. *J. Am. Chem. Soc.* **1987**, *109*, 5031.
(2) Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579. Swindells, D. C. N.; White, P. S.; Findlay, J. A. *Can. J. Chem.* **1978**, *56*, 2491.
(3) Findlay, J. A.; Liu, J.-S.; Burnell, D. J.; Nakashima, T. T. *Can. J. Chem.* **1982**, *60*, 2046.
(4) Askin, D.; Volante, R. P.; Reamer, R. A.; Ryan, K. M.; Shinkai, I. *Tetrahedron Lett.* **1988**, *29*, 277. Mills, S.; Desmond, R.; Reamer, R. A.; Volante, R. P.; Shinkai, I. *Ibid.* **1988**, *29*, 281.

0022-3263/88/1953-4643$01.50/0 



1



2

tures of 1 are notable as effective modulators of the electron deficiency of the tricarbonyl system.[5] Crystallographic studies demonstrate that the central carbonyl at $C_9$ is skewed out of plane with respect to the amide linkage, but it is internally hydrogen bonded to its adjacent hydrated carbonyl at $C_{10}$. No doubt these stabilizing factors have also served to reinforce the overall efficiency of our synthesis of the $\alpha,\beta$-diketo amide segment **2**, as summarized in Scheme I. Diastereoselective nucleophilic addition to the freshly prepared aldehyde **3** using the optically pure Grignard reagent **4** in anhydrous ether at −78 °C gave the secondary alcohol **5** as the predominant isomer (ratio of 8.4 to 1 as measured by high-field $^1$H NMR integration).[6] After O-methylation and sodium in liquid ammonia cleavage of the benzyl ether, preparative column chromatography on silica gel (gradient separation using 0 → 6% EtOAc in hexanes) conveniently afforded the purified primary alcohol **6** ($[\alpha]^{25}_D$ +1.13° (*c* 0.74, $CHCl_3$)), free of the $C_4$ diastereoisomer.[7] Oxidation of **6** conducted in tetrahydrofuran with added Celite at 0 °C, and dropwise addition of a 3 M solution of Jones' reagent led to the carboxylic acid **7** in 75–80% yields. No problems associated with $\alpha$-epimerization or ketal hydrolysis were observed under these conditions. Conversion to the acid chloride **8** was efficient with excess oxalyl chloride in benzene. Acylation of the pipecolic amide derivative **9**[8] was accomplished by preforming the magnesium enolate of **9** upon treatment with isopropylmagnesium chloride in tetrahydrofuran at 0 °C. Subsequent dropwise addition of **8** afforded 65% yield of the C-acylation adduct **10**, having slightly less polar chromatographic character than starting **9** and intense ultraviolet absorbance ($\lambda_{max}$ 204 nm, $\epsilon$ 25 500; and $\lambda_{max}$ 256 nm, $\epsilon$ 10 000). Proton magnetic resonance studies reveal that **10** ($[\alpha]^{25}_D$ −22.6° (*c* 1.08, $CHCl_3$)) exists as a complex mixture of mostly the *E*/*Z* enolic forms, as represented by **10**, together with the corresponding keto diastereomers (10–20%), and approximately a 3:1 ratio of amide conformers. Our characterizations were greatly simplified by catalytic hydrogenolysis of **10** with concomitant decarboxylation, quantitatively providing the $\beta$-keto amide **11** ($[\alpha]^{25}_D$ −44.5° (*c* 1.22, $CHCl_3$)) after workup with ethereal diazomethane.[9] Finally the central $C_9$ carbonyl was introduced via treatment with selenium dioxide in refluxing dioxane,[10] yielding a separable mixture of the desired $\alpha,\beta$-diketo amide **12** ($[\alpha]^{22}_D$ −19.0° (*c* 1.03, $CHCl_3$)) and its corresponding hydrate **13** ($[\alpha]^{22}_D$ −40.3° (*c* 0.92, $CHCl_3$)) in a ratio of approximately 2:1.[11] Rapid conversion to the tricarbonyl FK506 segment **2** ($[\alpha]^{22}_D$ +9.30° (*c* 1.48, $CHCl_3$)) was observed upon hydrolysis of the cyclohexylidene ketal of **12** with aqueous methanolic hydrogen chloride at room temperature (98% yield). As expected, our studies of the proton magnetic resonance spectra of **2** distinctly reveal two hydrogen-bonded hydroxyl groups at $\delta$ 3.75 (s, OH) and 3.16 (dd, OH, $J$ = 11.4 Hz and $J$ = 2.4 Hz). All substituents on the tetrahydropyranyl ring are equatorial, with the exception of the $C_{10}$ hydroxyl (anomeric effect). Proton and carbon spectral assignments of **2** correlated closely with data available from the natural product.[12]

In conclusion, we have identified an efficient route for the preparation of the optically active and pharmacologically interesting tricarbonyl component as found in antibiotic FK506 and rapamycin. Further studies will probe the biological significance of this novel functional array.

**Acknowledgment.** We acknowledge the National Science Foundation and the National Institutes of Health for their support. We also thank the NSF and the NIH for funds for the purchase of a Bruker AM 500 MHz spectrometer.

(5) Potent electrophiles often require structural or conformational modifications, which may mask (at least temporarily) or otherwise moderate the underlying electron deficiency in order to gain biochemical site specificity. This notion was first keenly developed for the $\beta$-lactam antibiotics.

(6) The aldehyde **3** was prepared in accord with: Sugiyama, T.; Sugawara, H.; Watanabe, M.; Yamashita, K. *Agric. Biol. Chem.* **1984**, *48*, 1841. Grignard reagent **4** was readily available from (*R*)-(−)-methyl-3-hydroxy-2-methylpropionate by using a variation of published procedures; Branca, von Q.; Fischli, A. *Helv. Chim. Acta* **1977**, *60*, 925. Carbon-bond formation with predominantly anti diastereoselection was expected based upon results of numerous nucleophilic additions to 2,3-*O*-isopropylidene-*D* (or *L*)-glyceraldehyde. For a review: McGarvey, G. J.; Kimura, M.; Oh, T.; Williams, J. M. *J. Carbohydr. Chem.* **1984**, *3*, 125. Malzer, J.; Angermann, A. *Tetrahedron Lett.* **1983**, *24*, 2843.

(7) For partial characterization of **6**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.95 (d, 3 H, $J$ = 7.5 Hz), 1.31–1.50 (m, 2 H), 1.50–1.70 (m, 10 H), 1.96 (m, 1 H), 2.59 (dd, 1 H, $J$ = 7.4 Hz and $J$ = 5.1 Hz), 3.34–3.45 (m, 2 H), 3.45 (s, 3 H), 3.45–3.57 (m, 1 H), 3.93 (AB of ABX, 2 H, $J_{AB}$ = 7.8 Hz, $J_{AX}$ = 6.3 Hz, $J_{BX}$ = 5.9 Hz, $\Delta\nu$ = 61.43), 4.09 (m, 1 H).

(8) Standard esterification of (−)-pipecolic acid (Aldrich) and N-acylation with benzyl malonyl chloride gave **9**.

(9) For partial characterization of **11**: $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 1.13 (d, 3 H, $J$ = 7.2 Hz), 1.20–1.49 (m, 4 H), 1.50–1.79 (m, 12 H), 1.98 (ddd, 1 H, $J$ = 15.2 Hz, $J$ = 8.2 Hz, $J$ = 2.7 Hz), 2.60 (br d, 1 H, $J$ = 15.0 Hz), 2.95 (m, 1 H), 3.21 (m, 2 H), 3.28 (s, 3 H), 3.65 (m, 1 H), 3.65–3.77 (m, 2 H), 3.72 (s, 3 H), 3.77–3.83 (m, 1 H), 3.95–4.11 (m, 2 H), 5.39 (m, 1 H). Additionally a small amount of enolic isomers and a 4:1 ratio of amide conformers were observed in the proton spectrum.

(10) For a review of the use of selenium dioxide with 1,3-dicarbonyl compounds: Rubin, M. B. *Chem. Rev.* **1975**, *75*, 177.

(11) For identification of **12**: $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.18 (d, 3 H, $J$ = 6.9 Hz), 1.28–1.85 (m, 15 H), 2.03 (doublet of AB of ABX, 2 H, $J$ = 3.9 Hz, $J_{AB}$ = 14.9 Hz, $J_{AX}$ = 7.95 Hz, $J_{BX}$ = 5.8 Hz, $\Delta\nu$ = 41.9 Hz), 2.32 (m, 1 H), 3.18 (s, 3 H), 3.20 (m, 1 H), 3.34 (m, 1 H), 3.45 (m, 1 H), 3.53 (m, 1 H), 3.78 (s, 3 H), 3.78 (m, 1 H), 4.09 (m, 2 H), 5.28 (br d, 1 H, $J$ = 5.1 Hz). The $\alpha,\beta$-diketo amide existed as a 4.6 to 1 ratio of amide conformers.

(12) For partial characterization of **2** (use FK506 numbering): $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.90 (d, 3 H, $J$ = 6.3 Hz, $C_{11}$-Me), 1.30–1.79 (m, 6 H, $C_3H$, $C_4HH$, $C_5HH$, $C_{12}H$), 2.07 (dt, 1 H, $J$ = 12.2 Hz, $J$ = 4.3 Hz, $C_{12}H$), 2.32 (br d, 1 H, $J$ = 13.4 Hz, $C_3H$), 2.38 (m, 1 H, $C_{11}H$), 3.16 (dd, 1 H, $J$ = 11.4 Hz, $J$ = 2.4 Hz, $C_{15}OH$), 3.26 (td, 1 H, $J$ = 13.5 Hz, $J$ = 3.2 Hz, $C_6H$), 3.40 (m, 1 H, $C_6H$), 3.41 (s, 3 H, $C_{13}OMe$), 3.53 (m, 1 H, $C_{13}H$), 3.69 (dt, 1 H, $J$ = 9.7 Hz, $J$ = 2.2 Hz, $C_{14}H$), 3.75 (br s, 1 H, $C_{10}OH$), 3.78 (m, 1 H, $C_{15}H$), 3.80 (s, 3 H, $C_1OMe$), 3.87 (td, 1 H, $J$ = 11.5 Hz, $J$ = 2.4 Hz, $C_{15}H$), 5.24 (br d, 1 H, $J$ = 5.5 Hz, $C_2H$); $^{13}$C NMR (125.8 MHz, $CDCl_3$) $\delta$ 15.27 ($C_{11}Me$), 21.20 ($C_4$), 24.72 ($C_5$), 26.40 ($C_3$), 31.53 ($C_{12}$), 35.15 ($C_{11}$), 44.37 ($C_6$), 51.50 ($C_2$), 52.91 ($C_1OMe$), 56.66 ($C_{13}OMe$), 61.29 ($C_{15}$), 72.85 ($C_{13}$), 74.12 ($C_{14}$), 98.89 ($C_{10}$), 166.51 ($C_8$), 171.15 ($C_1$), 197.38 ($C_9$). Extensive proton decoupling experiments and heteronuclear 2D-COSY were used to unambiguously correlate and assign proton and carbon data. All compounds of our synthetic route gave satisfactory infrared and high-resolution mass spectral data.

**D. R. Williams,* J. W. Benbow**
*Department of Chemistry*
*Indiana University*
*Bloomington, Indiana 47405*
*Received May 23, 1988*