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Efficient Removal of Pipecolinate from Rapamycin and FK506 by Reaction with *n*-$Bu_4N^+CN^-$

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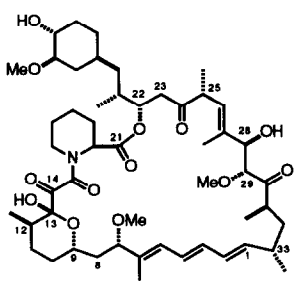
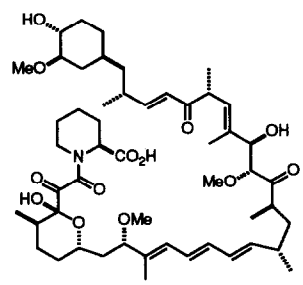
Abstract: The reaction of rapamycin (**1**) and FK506 (**11**) with *n*-$Bu_4N^+CN^-$ / aq. THF results in an efficient excision of their pipecolinate subunits, leading in good yields to compounds **3** and **12**, respectively. Two sequential ring cleavage reactions take place: a fast cyanide-promoted fragmentation of the tricarbonyl functionality followed by a slower cyanide-catalyzed ester cleavage. The cyanide reagent provides a mild, chemoselective system for intramolecular transesterifications in structurally complex substrates.

Rapamycin (**1**) is an immunosuppressive agent currently under evaluation for organ transplantation and autoimmune disease.[1] This macrolide binds tightly to the intracellular receptor FKBP,[2] and the resultant bimolecular complex blocks a signal transduction pathway that leads to T-cell activation. Rapamycin is currently viewed as a ligand with two functional domains: a) binding domain, centered on the pipecolinyl α-ketoamide subunit, which binds in a hydrophobic cavity of the protein,[3] and b) the rest of the macrocyclic tether (C_1 to C_8 and C_{22} to C_{33}), which, together with portions of FKBP, is responsible for the immunosuppressive activity by interacting with an as yet unknown downstream target in the T-cell. We have initiated studies on the chemistry of rapamycin in order to investigate the structural requirements for the biological activity of the molecule.[4] We describe herein a mild and efficient method to excise the pipecolinate subunit from **1** using *n*-$Bu_4N^+CN^-$ / aq. THF, a system that promotes intramolecular transesterification under extremely mild and chemoselective conditions.

1
rapamycin

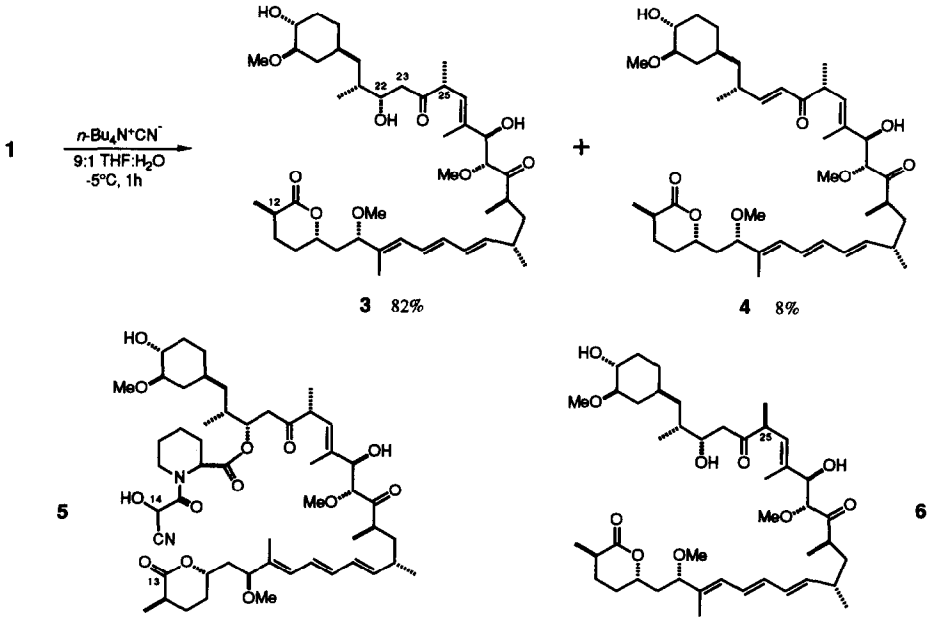




2

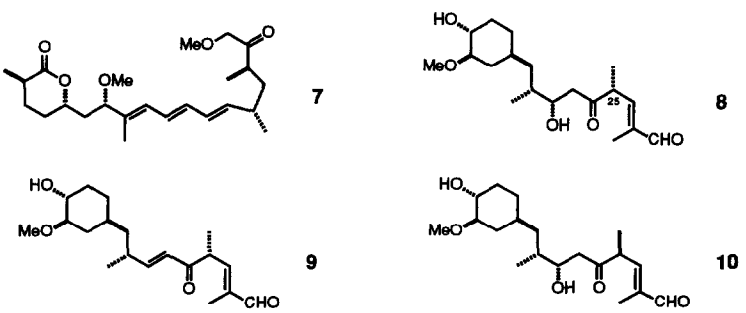
One of our primary goals with rapamycin was to hydrolyze the macrocyclic lactone and use the C_{22} hydroxyl group to incorporate synthetic binding domain subunits. However, standard hydrolytic conditions ($NaHCO_3$, NaOH) promote C_{22} carboxylate β-elimination,[4,5] leading to **2**. In the search for alternative reagents,[6] we explored the reaction of rapamycin with cyanide reagents. Thus when **1** was treated with *n*-$Bu_4N^+CN^-$ (2 equiv) in aqueous THF, a fast reaction took place at low temperatures (-5 °C) to provide **3**, a product of pipecolinate excision, in 82% yield, along with a small amount (~8%) of the β-elimination product, **4**.[7] Compound **3** arises from double disconnection of the macrocycle at C_{13}-C_{14} and O-C_{22} bonds. When the reaction was carried out at -78 °C, a surprisingly rapid C_{13}-C_{14} ring cleavage[8,9] took place to give the C_{14}

cyanohydrin **5**;[10,11] this intermediate undergoes subsequent cyanide-catalyzed ester hydrolysis to afford **3** as the temperature is raised to -5 °C.



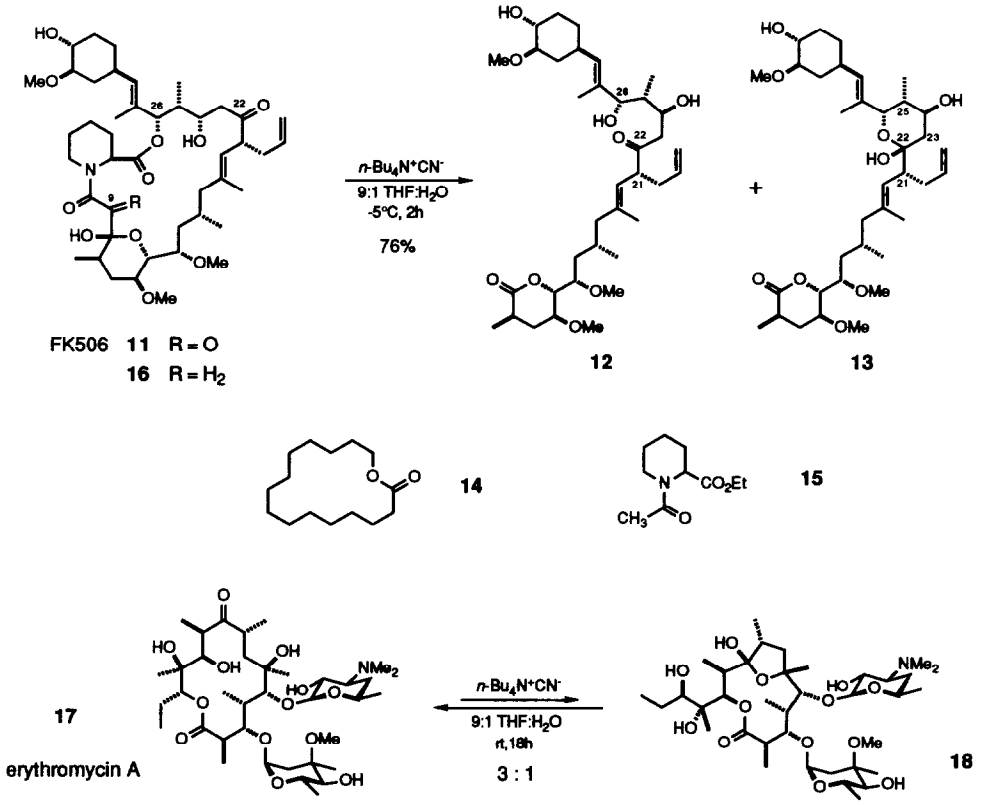
With anhydrous THF as solvent, a greater amount of **4** was obtained in the reaction (0 °C, 2:1 mixture of **3**:**4** in 74% yield); under these conditions, a single equivalent of n-$Bu_4N^+CN^-$ effects conversion to **3** – excess reagent promotes epimerization at C_{25}, eventually leading to a mixture of **6**:**3** in a 2.3:1 thermodynamic ratio.[12] This same mixture was reached when purified **3** was treated with an excess of n-$Bu_4N^+CN^-$ in anhydrous THF. The highest yield of **3** was obtained with 10% water in THF, which seems to moderate the reagent basicity, minimizing both the extent of C_{22}-C_{23} elimination and C_{25} epimerization. Excision of the pipecolinate in rapamycin also proceeds efficiently with KCN/MeOH at 0 °C to provide a mixture of the methyl esters derived from **3** and **4** by methanolysis of the δ-lactones in 85% yield.

The rapamycin fragments **3**, **4** and **6** can be subjected to C_{28}-C_{29} retroaldol cleavage using $ZnCl_2$/THF, as previously reported for rapamycin.[4] By this protocol the trienyl lactone **7** [13] was obtained, along with their respective aldehydes **8**-**10** in good yields.



When the n-$Bu_4N^+CN^-$ / aq. THF cleavage was applied to FK506 (**11**), an analogous reaction was observed and compound **12**, which exists in equilibrium with the hemiketal **13**, was obtained in 76%

yield.[14,15] In contrast, simple model systems such as pentadecanoic ω-lactone (**14**) or ethyl *N*-acetylpipecolinate (**15**) were inert to the reaction conditions and were recovered unchanged even after prolonged times at 23 °C. This suggests that the pipecolinate hydrolyses of **1** and **11** result from some type of neighboring group participation, such as intramolecular transesterification of the intermediate cyanohydrin hydroxyl. Indeed, when 9-desoxo-FK506[16] (**16**) was treated with *n*-$Bu_4N^+CN^-$/aq. THF no reaction could be observed under the same conditions that produce **12** from **11**. On the other hand, treatment of erythromycin A (**17**) under these conditions results in equilibration at room temperature to isomeric **18**,[17] supporting the proposed transesterification mechanism for cleavage of **1** and **11** and underscoring the mild nature of the conditions.[17,18]



In summary, an expedient method for the disconnection of the pipecolinate subunit from rapamycin and FK506 using the *n*-$Bu_4N^+CN^-$ / aq. THF transesterification system has been developed. The reaction conditions for this transformation are extremely mild, and intact nonmacrocyclic segments can be obtained efficiently in just one step. The fragments such as **3**, **6**, **8** and **10**, which retain the C_{22} hydroxyl group with the natural configuration, should prove valuable for mechanistic and synthetic investigations, as well as for the preparation of analogs for biological evaluation.

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References and Notes.

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2. a) Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber, S. L. *Nature* **1989**, *341*, 758-760. b) Siekierka, J. J.; Hung, S. H. Y.; Poe, M.; Lin, C. S.; Sigal, N. H. *Nature* **1989**, *341*, 755-757.
3. Van Duyne, G. D.; Standaert, R. F.; Schreiber, S. L.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 7433-7434.
4. Luengo, J. I.; Konialian, A. L.; Holt, D. A. *Tetrahedron Lett.* **1993**, *34*, 991-994.
5. Yohannes, D.; Myers, C. D.; Danishefsky, S. J. *Tetrahedron Lett.* **1993**, *34*, 2075-2078.
6. The "tricarbonyl" system in FK506 and rapamycin has a propensity to undergo benzilic acid rearrangement upon treatment with basic or nucleophilic reagents. See: a) Fisher, M. J.; Chow, K.; Villalobos, A.; Danishefsky, S. J. *J. Org. Chem.* **1991**, *56*, 2900-2907. b) Askin, D.; Reamer, R. A.; Joe, D.; Volante, R. P.; Shinkai, I. *Tetrahedron Lett.* **1989**, *30*, 6121-6124, and ref 4 above.
7. Structure assignment of **3** and **4** was based on ^{1}H and ^{13}C NMR analysis ($CDCl_3$). Thus, compared to rapamycin, H_9 (δ 4.27), H_{12} (δ 2.43) and Me_{12} (δ 1.29) in **3** show downfield shifts of 0.41, 0.45 and 0.34 ppm, respectively, as expected from a tetrahydropyran to valerolactone conversion; in addition, lactonic C_{13} appears at 174.3 ppm. ^{1}H NMR spectrum of **3** shows an ABX system for the CH_2-CH group at C_{23}-C_{22}: δ 2.46 (dd, J = 17.5, 9.8 Hz), 2.58 (dd, J = 17.5, 2.3 Hz), 3.83 (m). Spectrum of **4** shows H_{22} and H_{23} at δ 6.72 and 6.11 coupled to each other with a *trans*-olefin coupling of 15.6 Hz.
8. Cleavage of the corresponding C_9-C_{10} bond in FK506, promoted by the generation of tetrahedral intermediates at C_9, has been described in ref 6a above.
9. Disconnection of the C_{13}-C_{14} bond probably originates from cyanide attack at the C_{14} carbonyl followed by retro-Claisen-like fragmentation, as reported by Danishefsky *et al.* (ref. 6a above) on FK506 when subjected to Evans' cyanosilylation conditions (cat. KCN/18-crown-6, TMSCN, benzene, 23 °C, 20 h). When we applied these conditions to rapamycin, we only obtained a complex mixture of products.
10. Compound **5** can also be obtained by treatment of rapamycin with KCN in MeOH at -78 °C.
11. The 14-cyanohydrin in **5** was confirmed from the two doublets centered at δ 5.08 (H_{14}) and 4.61 (14-OH, exchangeable with D_2O), coupled to each other with a J = 6.6 Hz as well as the 14-CN carbon at δ 114.9.
12. Epimerization at C_{25} was demonstrated by deuterium incorporation experiments: in the presence of a trace of D_2O (THF, n-$Bu_4N^+CN^-$, r.t., 3 h), **6** showed 90% deuteration at the C_{25} along with a smaller extent, *ca.* 50%, at C_{23}.
13. Yohannes, D.; Danishefsky, S. J. *Tetrahedron Lett.* **1992**, *33*, 7469-7472.
14. The same equilibrium has been observed for the ring-opened methyl ester of **12** in ref 6a, above.
15. This equimolecular mixture was inseparable by chromatography. Structural assignment of both compounds relied on ^{13}C NMR (C_{22} at δ 212.0 and 99.0 for **12** and **13**, respectively) as well as ^{1}H NMR differential spectroscopy, which shows H_{21} and both H_{23}, the protons flanking C_{22}, shifted downfield in **12** (δ 3.46, 2.78, 2.64) with respect to **13** (δ 2.49, 1.81, 1.74) by 0.9-1.0 ppm. Hemiketal **13** exists as a single epimer at C_{22}; this was assigned as *R* based on the vicinal coupling constants of the tetrahydropyranyl ring protons: $J_{23a,24} = J_{23b,24} = J_{24,25} = ca.$ 3 Hz and $J_{25,26} = ca.$ 1 Hz, consistent with H_{24eq}, H_{25eq} and H_{26ax}.
16. Prepared by the reaction of FK506 with H_2S in methanol-pyridine. For an alternative route to **16**, see: Emmer, G.; Weber-Roth, S. *Tetrahedron* **1992**, *48*, 5861-5874.
17. This equilibration has been previously reported after heating erythromycin A at 45-50 °C for 3 days in 3:1 Et_2NH-MeOH. The use of Et_2NH is crucial, since no reaction took place with Et_3N. See: Kibwage, I. O.; Busson, R.; Janssen, G.; Hoogmartens, J.; Vanderhaeghe, H.; Bracke, J. *J. Org. Chem.* **1987**, *52*, 990-996.
18. Erythromycin A is sensitive to basic conditions, undergoing a translactonization-elimination ring opening when treated with diluted NaOH: Waddell, S. T.; Blizzard, T. A. *Tetrahedron Lett.* **1992**, *33*, 7827-7830.

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