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STUDIES RELATING TO THE IMMUNOSUPPRESSIVE ACTIVITY OF FK506

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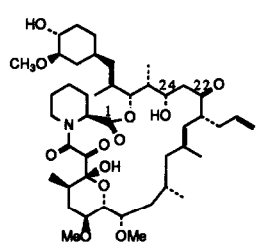
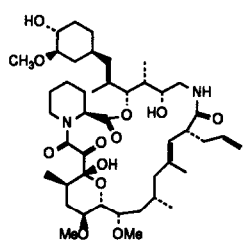
Abstract : Beckmann rearrangement of FK506 E-oxime gave amide **2** and the fragmentation product **4**. Compound **4** was also prepared by total synthesis.

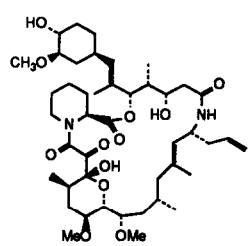
The immunosuppressant FK506 has been the subject of considerable synthetic[1] and degradative[2] studies. As part of our programme in this area we investigated the structural requirements for immunosuppressive activity through chemical manipulation of FK506, in the expectation that such information would support our synthetic programme.



FK506



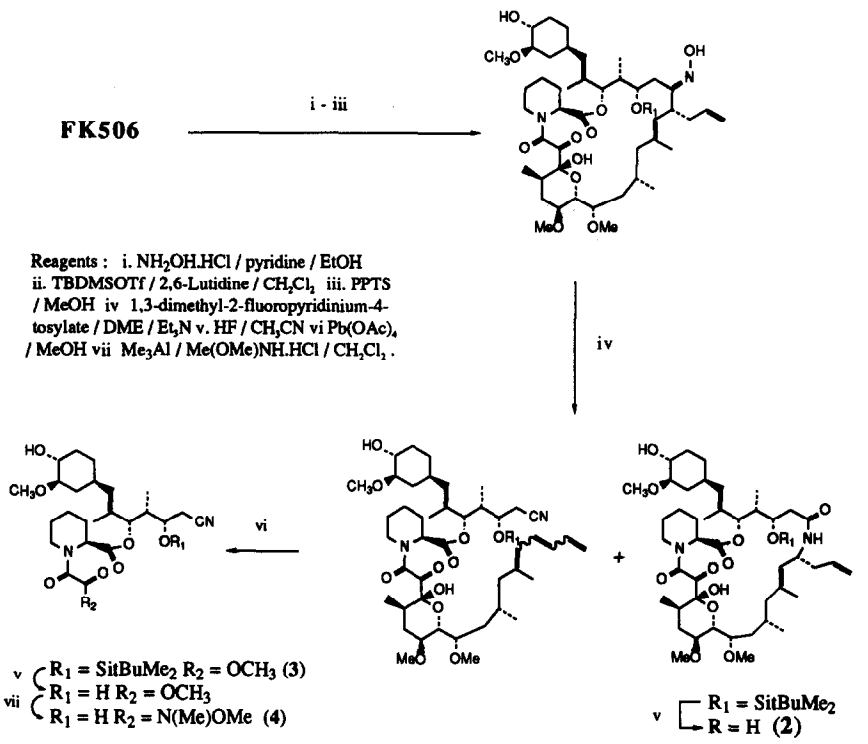
(1)



(2)

During the process of effecting modifications at C22 of FK506 we attempted to prepare the amides (**1**) and (**2**) by Beckmann rearrangement of the respective C22 Z- and E-oximes . In the case of amide (**1**) this could be achieved, albeit in low yield, by treatment of FK506 with O-mesitylsulphonylhydroxylamine followed by basic alumina.[3] Unfortunately amide (**2**) could not be isolated from this reaction and an alternative procedure was required (Scheme 1). To this end FK506 was converted to a separable mixture of C22 E / Z-oximes and the major E- isomer was protected as its C-24 *tert*-butyldimethylsilyl ether through a silylation / selective desilylation sequence. Beckmann rearrangement was accomplished with 1,3-dimethyl-2-fluoropyridinium 4-tosylate in DME / Et_3N to give, after deprotection with HF in acetonitrile, the amide (**2**). However, the yield of (**2**) was very low (6%) due to the fact that most of the oxime had

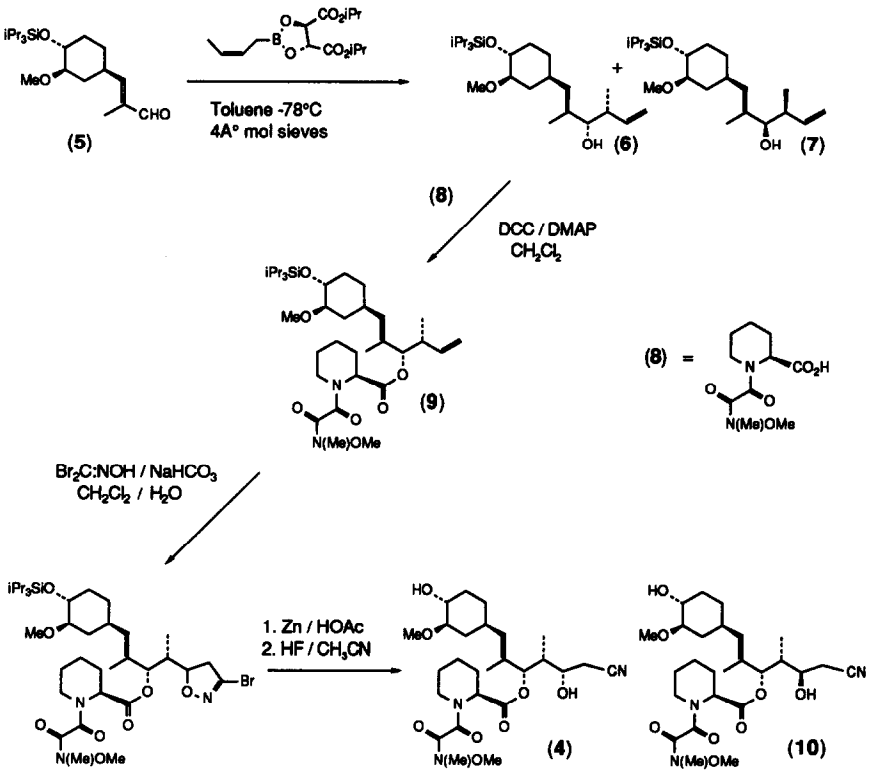
Scheme 1



undergone a fragmentation reaction, a process well known to occur when a stable carbonium ion can be formed. This gave a mixture of products resulting from proton loss / nucleophilic capture of a C21 cationic intermediate. These products were not separated but were treated directly with lead tetraacetate in MeOH to give, after chromatography, the fragmentation product (**3**) (49%). Desilylation of compound (**3**) followed by treatment with N,O-dimethylhydroxylamine hydrochloride / trimethylaluminium[4] in CH_2Cl_2 gave the amide (**4**) (33%).

Amides (**1**) and (**2**) showed greatly reduced activity as assessed by their ability to displace ^3H- labelled dihydro-FK506 from FKBP12[5] and to inhibit IL2 production in stimulated Jurkat cells.[5] Interestingly, the small fragments (**3**) and (**4**) showed low levels of activity in our screens for immunosuppression. Such low activities have, however, to be treated

Scheme 2[13]



with caution and in general we have prepared the relevant materials by total synthesis where this activity has been noted. The total synthesis of (**4**) was therefore undertaken (Scheme 2).

Treatment of the homochiral enal (**5**)[6] with the Z-crotylboronate derived from (R,R)-diisopropyltartrate[7] gave the alcohols (**6**) (53%) and (**7**) (21%). Alcohol (**6**) was coupled to the acylated (S)-pipecolinic acid (**8**)[8] with DCC and stoichiometric DMAP to give the ester (**9**) (85%). Cycloaddition with the nitrile oxide generated from dibromoformaldoxime[9] gave, as expected,[10] a 1:1 mixture of diastereomeric isoxazolines (82%) which underwent reductive elimination on treatment with zinc dust in glacial acetic acid.[11] Deprotection and chromatographic separation gave the β-hydroxynitriles (**4**) (25%) and (**10**) (27%).[12]

Synthetic (**4**) and its isomers[12] failed to show activity in the screen for IL2 inhibition, confirming the need for caution when drawing conclusions from the activity of derivatives of compounds possessing high biological activity.

References and Notes

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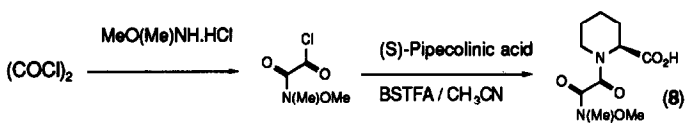
8. Preparation of (**8**)



(**8**)

BSTFA = N,O-bis(trimethylsilyl)trifluoroacetamide.

9. a) Vyas, D. M.; Chiang, Y.; Doyle, T. W.; *Tetrahedron Lett.* **1984**, 25, 487. b) Wade, P. A.; Pillay, M. K.; Singh, S.M; *Tetrahedron Lett.* **1982**, 23, 4563. c) Hagedorn, A. A.; Miller, B. J.; Nagy, J. O.; *Tetrahedron Lett.* **1980**, 21, 229. d) Caldirola, P.; Ciancaglione, M; De Amici, M.; De Micheli, C.; *Tetrahedron Lett.* **1986**, 27, 4647.
10. The levels of asymmetric induction in the addition of nitrile oxides to chiral olefins do not, in general, approach those attained in the aldol strategy. Little or no stereoselectivity has been observed with similar substrates: Houk, K. N.; Duh, H.; Wu, Y.; Moses, S. R.; *J. Am. Chem. Soc.* **1986**, 108, 2754.
11. Reductive cleavage of the N - OMe bond is slow in comparison with cleavage of the bromoisoxazoline.
12. In a similar manner compound (**7**) was converted to the isomeric pair of β-hydroxynitriles.
13. All new compounds were fully characterised by ^{1}H and ^{13}C NMR spectra, low resolution mass spectra, and microanalysis and / or HRMS. Yields refer to isolated, chromatographically homogeneous compounds.

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