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HIGHLY SELECTIVE REACTIONS OF FK506 WITH DIAZOMETHANE

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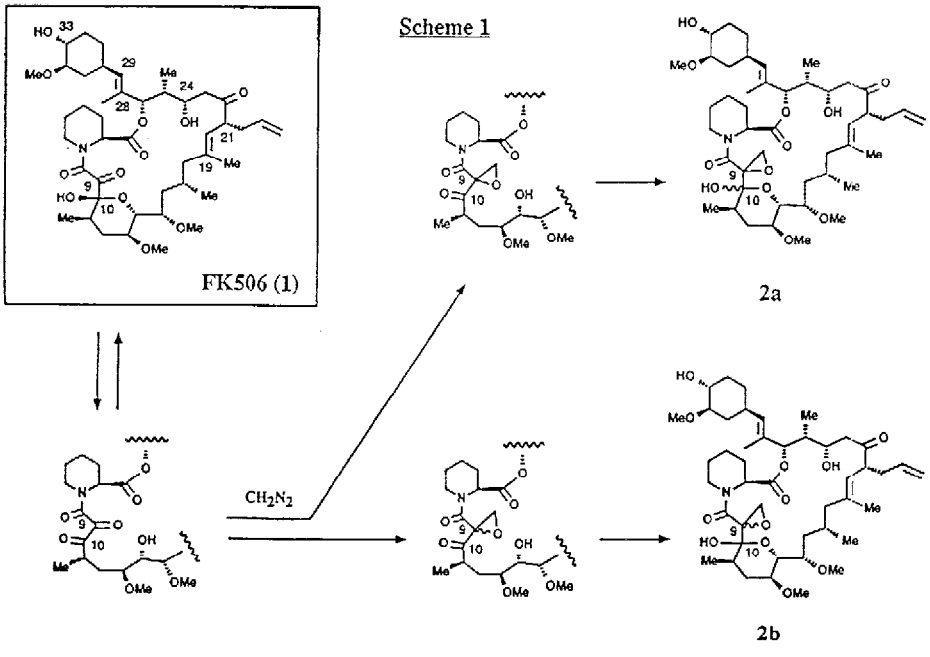
Summary: Three modes of diazomethane reactivity, namely oxirane formation, O-methylation and cyclopropanation, can be accomplished with excellent selectivity on the multifunctional molecule FK506.

The novel macrolide immunosuppressant FK506 (1) has recently been the subject of intensive chemical research. Although syntheses of various portions of the molecule have been reported,[1] cumulating in the total synthesis,[2] derivatisation studies (important for probing the essential requirements for immunosuppressive activity) have largely[3] remained buried in the patent literature.[4] Herein, we report some of our results in this field in which we show that a single, highly reactive species -diazomethane- can be used to effect extremely selective transformations of FK506.

Inspection of the structure of FK506 reveals the presence of a hemi-ketal masked tricarbonyl functionality. It is known that tricarbonyl functionalities are highly reactive toward CH_2N_2, undergoing methylene insertion or central carbon spiro-oxirane formation.[5] We have treated solutions of FK506 in Et_2O or CH_2Cl_2 with CH_2N_2 and observed rapid formation of two products which upon isolation were shown to be the spiro-oxirane diastereoisomers[6,7] 2 (Scheme 1, Scheme 2, and Table 1, entry 1).

Scheme 1



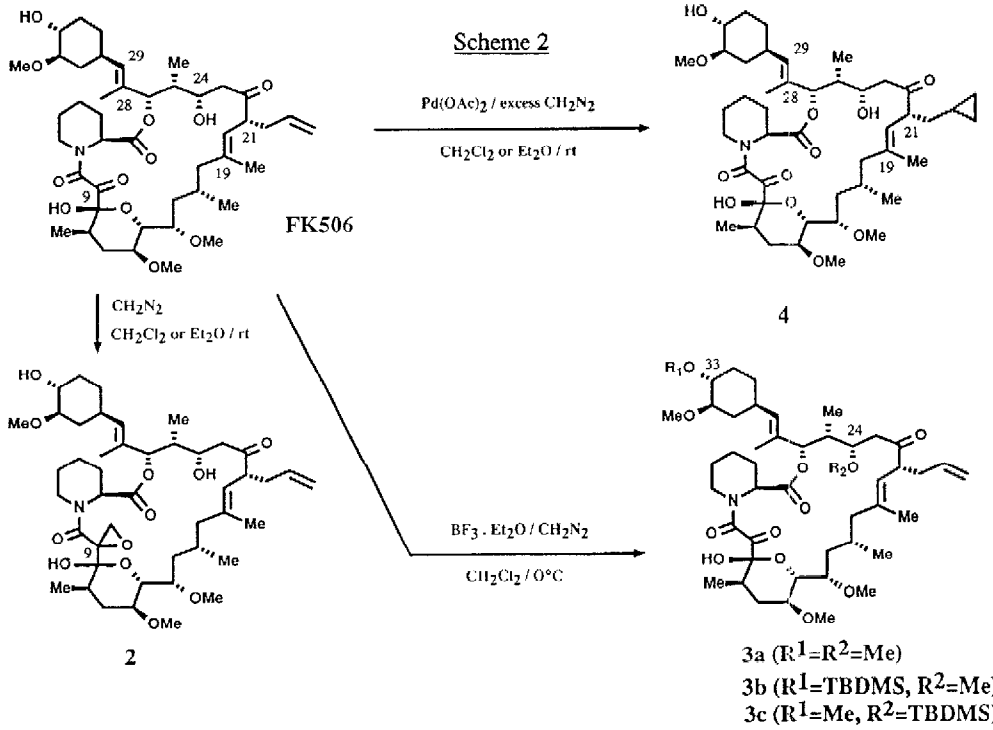
By analogy with other tricarbonyl systems, it is presumably the open tricarbonyl form of FK506 that reacts with CH_2N_2 so that formation of two diastereoisomers (absolute configurations not known) is most likely due to non-selective CH_2N_2 addition followed by selective hemi-ketalisation (leading to **2b**) but selective CH_2N_2 addition followed by non-selective hemi-ketalisation (leading to **2a**) is also possible (Scheme 1).

With the knowledge that CH_2N_2 readily attacks the tricarbonyl functionality of FK506 we were interested to discover whether further selective transformations could be achieved with this reagent, *without* concomitant spiro-oxirane formation. Focussing our attention initially on Lewis acid promoted alkylation of hydroxy groups,[8] we found that careful portionwise addition of CH_2N_2 to a solution of FK506 in CH_2Cl_2 in the presence of $BF_3.Et_2O$ enabled methylation of the C-24 and C-33 hydroxy functions[9] yielding the dialkylated derivative **3a** (Scheme 2 and Table 1, entry 2), spiro-oxirane products only being formed if insufficient amounts of $BF_3.Et_2O$ were present.[10] Appropriate protection of the 33- or 24-hydroxy functionalities of FK506 as their TBDMS ethers thus enabled preparation of the 24- and 33- mono-methylated derivatives of FK506 (**3b** and **3c**, respectively) after CH_2N_2 treatment and subsequent deprotection[11] (Scheme 2

and Table 1, entries 3 and 4).

Having accomplished selective alkylation, we investigated a third mode of CH_2N_2 reactivity, namely cyclopropanation of double bonds in the presence of a transition metal catalyst.[12] Suprisingly, we observed that addition of CH_2N_2 to a solution of FK506 in Et_2O or CH_2Cl_2 in the presence of *catalytic* $Pd(OAc)_2$ (10% wt) led to a single product (identified as the 21-cyclopropylmethyl derivative, **4**[13]- Scheme 2 and Table 1, entry 5) in 87% isolated yield *with no 9-oxirane formation*, even when a *large excess* of the azoalkane was used.[14]

Scheme 2



Mechanistic studies[12a] suggest that such cyclopropanation occurs *via* initial formation of a π-allyl palladium complex, formation of a π-allyl palladium carbenoid upon treatment with the azoalkane, and subsequent carbene insertion. Thus, the failure to cyclopropanate the other two available double bonds in the molecule (C28-C29 and C19-C20) is probably a consequence of their sterically hindered nature. Indeed the 21-n-propyl FK506 derivative [15] did not react with diazomethane in the presence of $Pd(OAc)_2$ under the same conditions (Table 1, entry 6). More suprising is the complete absence of spiro-oxirane products although excess CH_2N_2 was used. This was shown not to be a conformation effect as isolated **4** was smoothly transformed to the corresponding 9-spiro-oxirane diastereoisomers upon treatment with diazomethane under the conditions used for preparation of **2**. The explanation for this unusual selectivity is not clear and we are currently studying other relevant FK506 derivatives in order to obtain an insight for this peculiar observation.

Table 1: Treatment of FK506 and Derivatives with Diazomethane and Selected Additives.

Entry	Starting Material	Method[a]	Yield[b]	Product	
1	FK506	A	57/7[c]	**2**	
2	FK506	B	80	**3a**	(R^1 = R^2 = Me)
3	33-OTBDMS-FK506[d]	B	65	**3b**	(R^1 = TBDMS, R^2 = Me)[d]
4	24-OTBDMS-FK506[d]	B	80	**3c**	(R^1 = Me, R^2 = TBDMS)[d]
5	FK506	C	87	**4**	
6	21-n-Propyl-FK506[e]	C		-	Recovered starting material

[a] For experimental details of methods A,B and C see Ref. 16-18
[b] Yields (not optimized) of chromatographically isolated products: All compounds characterized by ^1H and ^{13}C NMR.
[c] Yields of isolated diastereoisomers from reaction in CH_2Cl_2 (mixed fractions not included in yield calculation).
[d] See reference 11 for the preparation of the silyl ethers, and their subsequent deprotection to the mono-alkylated FK506 derivatives.
[e] Prepared by hydrogenation of FK506 according to reference 15

References and Notes

1. For some recent synthetic studies see;
   a) Ireland R. E.; Wipf P.; Roper T. D. *J. Org. Chem.*, **1990**, *55*, 2284. b) Kocieński P.; Stocks M.; Donald D. K. *Synlett*, **1990**, 38 and *Tetrahedron Lett.*, **1990**, *31*, 1637. c) Linde R. G.; Egberston M.; Coleman R. S.; Jones A. B.; Danishefsky S. J. *J. Org. Chem.*, **1990**, *55*, 2771. d) Villalobos A.; Danishefsky S. J. *J. Org. Chem.*, **1990**, *55*, 2776. e) Jones A. B.; Villalobos A.; Linde R. G.; Danishefsky S. J. *J. Org. Chem.* **1990**, *55*, 2786 and references cited therein.
2. Jones T. K.; Reamer R. A.; Desmond R.; Mills S. G. *J. Am. Chem. Soc.*, **1990**, *112*, 2998.
3. Coleman R. S.; Danishefsky S. J. *Heterocycles*, **1989**, *28*, 157.
4. Patent Application PCT WO89/053044
5. Rubin M. *Chem. Rev.*, **1975**, *75*, 177. Formation of spiro-oxiranes has also been observed by Fisons chemists (see ref. 4).
6. Characteristic ^1H NMR data for oxiranes (250MHz, $CDCl_3$):
   Major diastereoisomer: 5.285 (d; J=2.5Hz; H-26); 3.54 (d; J=10Hz; H-14); 3.49 (dd; J=1 and 11Hz; H-15); 2.95 and 2.81ppm (AB; J_{AB}=5Hz; oxirane-CH_2)
   Minor diastereoisomer: 5.54 (br s; H-26); 3.63 (dd; J=3 and 11Hz; H-15); 3.58 (d; J=10Hz; H-14); 2.97 and 2.905ppm (AB; J_{AB}=5Hz; oxirane-CH_2).
7. Diastereoselectivities were estimated from ^1H NMR analysis of crude reaction mixtures by comparing oxirane-CH_2 AB-quartet signals. The ratio of isomers was *ca* 5:1 in CH_2Cl_2 and *ca* 3:2 in Et_2O.

8. Mastronardi I. O.; Flematti S. M.; Deferrari J. O.; Gros E. G. *Carbohydrate Research*, **1966**, *3*, 177.

9. Characteristic NMR data for methoxy compounds.
3a (ca 4:1 mixture of rotamers): Main rotamer: 5.26 (d; J=8Hz; H-29); 5.175 (d; J=7Hz; H-26); 4.78 (d; J=10Hz; H-20); 3.82 (dd; J=10 and 2Hz; H-14) 3.23, 3.325, 3.395, 3.445, and 3.45 (5 x s; 5 x OCH_3). Minor roatamer: 3.23, 3.325, 3.395, 3.445 and 3.45ppm (5 x s; 5 x OCH_3).
3b (ca 3:1 mixture of rotamers): Main rotamer: 5.25 (d; J=8Hz; H-29); 5.17 (d; J=7Hz; H-26); 4.79 (d; J=10Hz; H-20); 3.82 (dd; J=9 and 1.5Hz; H-14); 3.42, 3.40, 3.33 and 3.24 (4 X s; 4 x OCH_3); 0.086ppm (t-butyl). Minor rotamer: 3.90 (dd; J=9 and 2.5Hz; H-14).
3c (ca 2:1 mixture of rotamers): Main rotamer: 5.22 (d; J=7Hz; H-26); 4.84 (d; J=10Hz; H-20); 4.07 (m; H-24); 3.80 (dd; J=9 and 1.5Hz; H-14); 3.45, 3.44, 3.40 and 3.32 (4 X s; 4 x OCH_3); 2.78ppm (dd; J=15 and 7.5Hz); 0.86ppm (t-butyl). Minor rotamer: 4.26 (m; H-24) 3.94 (dd; J=9 and 2.5Hz; H-14); 0.086ppm (t-butyl).

10. Sufficient $BF_3.Et_2O$ must be present to remove the yellow colour of the added diazomethane immediately. In cases where the yellow colour was allowed to remain, spiro-oxirane formation occurred.
11. Prepared by treatment of FK506 with TBDMSCl/imidazole/DMF at room temperature. The 33-hydroxy function is selectively protected with 1.2 equivalents of TBDMSCl, the 33,24-*bis*-OTBDMS derivative is obtained using 2.4 equivalents of TBDMSCl and longer reaction times. Deprotection of TBDMS ethers was accomplished with 2% HF(40% aqueous solution) in acetonitrile at room temperature. The 33-OTBDMS group is rapidly hydrolysed while the 24-OTBDMS group requires several hours under these conditions thus allowing easy access to 24-OTBDMS-FK506.
12. a) Anciaux A. J.; Hubert A. J.; Noels A. F.; Petiniot N.; Teyssié P. *J. Org. Chem.*, **1980**, *45*, 695.
b) Suda M. *Synthesis*, **1981**, 714.
13. 250MHz ^1H NMR spectra in $CDCl_3$ showed a 5:2 amide rotamer mixture. The characteristic cyclopropyl signals were observed at 0.57-0.71 (m; 1-proton); 0.35-0.46 (m; 2-protons); and 0.0-0.12ppm (m; 2-protons).
14. As determined by TLC and HPLC analysis.
15. Available *via* selective reduction of the allylic side-chain; see European patent application 0 184 162 A2.
16. **Method A:** A solution of the substrate (1.0mmol) in 50ml of CH_2Cl_2 (or Et_2O) was stirred at room temperature and treated with 10ml of a CH_2N_2 solution in CH_2Cl_2 or Et_2O (CH_2N_2 solutions (approx. 1M) were freshly prepared from N-nitrosomethyl urea as described in Organic Synthesis, 40, **1960**). Spiro-oxirane formation began immediately and the reaction was shown to be complete by TLC analysis generally after 45min. The solvent was concentrated *in vacuo* and the residue purified by flash chromatography (EtOAc:hexanes 2:1).
17. **Method B:** 1 mmol of the substrate was treated at 0^0-5^0C with 2 ml of a stock-solution of $BF_3.Et_2O$ in CH_2Cl_2 (0.1ml of $BF_3.Et_2O$ in 50ml of CH_2Cl_2) and then an approximately 1M solution of CH_2N_2 in CH_2Cl_2 was added at such a rate that the CH_2N_2 yellow colour disappeared immediately. After addition of approximately 5ml of the CH_2N_2 solution, a further 2ml of the $BF_3.Et_2O$ stock-solution were added and CH_2N_2 addition was continued as above until TLC showed completion of the reaction. The solution was washed successively with aqueous $NaHCO_3$ and brine, concentrated *in vacuo* and the residue purified by flash chromatography.
18. **Method C:** A solution of FK506 (200mg, 0.249mmol) in 10ml of Et_2O or CH_2Cl_2 containing 10 wt% (20mg) of $Pd(OAc)_2$ was treated with 5ml portions of an approximateley 1M solution of CH_2N_2 in CH_2Cl_2 or Et_2O. After a total of 20ml of the CH_2N_2 solution had been addded, the mixture was filtered and concentrated *in vacuo* and the residue purified by flash chromatography (3:1 EtOAc:toluene). Use of much larger quantities of the CH_2N_2 has no effect on the reaction outcome.

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