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Synthesis And Study Of A Non Macrocyclic FK506 Derivative.

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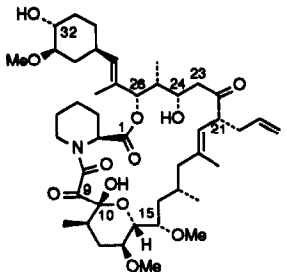
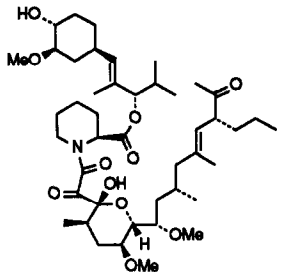
Abstract: The affinity of a non macrocyclic analogue of FK506 for the immunophilin FKBP12 was determined. The affinity of this analogue/FKBP12 complex for calcineurin was studied.

FK506, a macrocyclic natural product, has received intensive investigation because of its powerful immunosuppressive properties and the insights it provides into cytoplasmic signal transduction.[1] FK506 binds tightly to the ubiquitous cytosolic protein FKBP12,[2] and the resulting bimolecular complex then inhibits the calmodulin-dependent protein phosphatase PP2B (calcineurin).[3] This phosphatase is also inhibited by the complex formed between the structurally unrelated cyclic undecapeptide cyclosporin and cyclophilin.[4] Inhibition of calcineurin results in failure to dephosphorylate the nuclear transcription factor of activated T-cells (NFAT) and hence to disruption of IL-2 gene transcription and ultimately to down regulation of the T-cell component of the immune response.

In a previous paper[5] we examined the affinity of the excised binding domain of FK506 for the immunophilin FKBP12. This study allowed us to determine the affinity of the binding region in the absence of the constraints imposed upon it by the macrocyclic framework. Thus when those parts of the macrocycle which are not in contact with the immunophilin were removed, a 50-fold reduction in affinity was observed. In a continuation of this study we wished to determine the contribution of macrocyclic constraints to the affinity of the FK506/FKBP12 complex for calcineurin.[6]



FK-506



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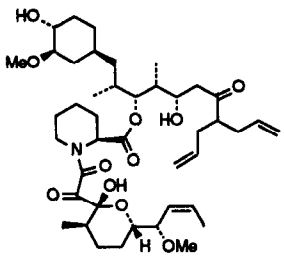
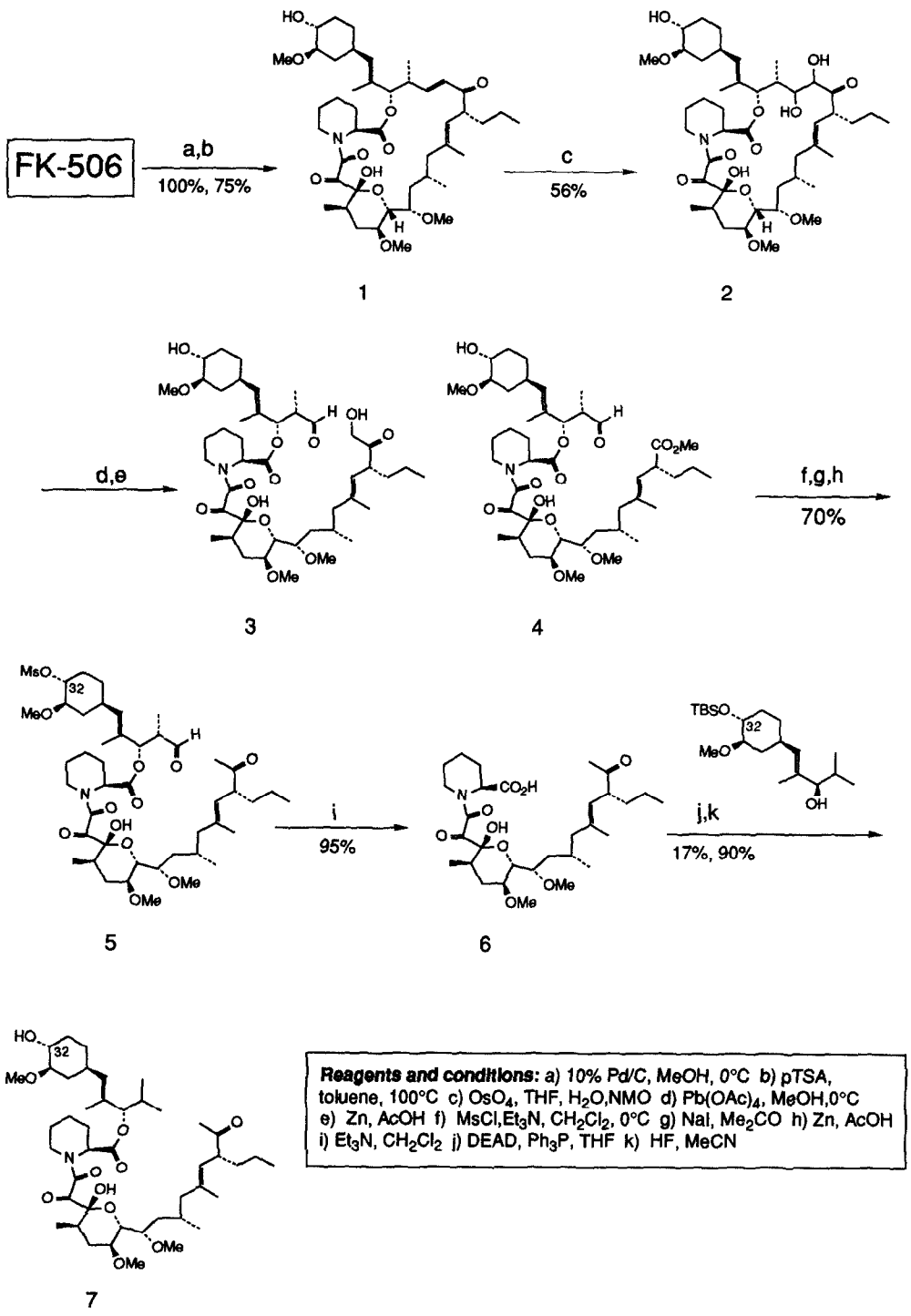


SLB506

Present understanding of the binding of the FK506/FKBP12 complex to calcineurin suggests that a composite surface is recognised. This surface consists of key residues of FKBP12 (Gly ^{89}, Ile ^{90} and Arg ^{42})[7] combined with lipophilic interactions contributed by the C15-C23 region of FK506.[8] These considerations led us to choose to cleave FK506 across the C23-C24 bond. It was anticipated that this would leave the key section of FK506


FK-506
a,b
100%, 75%
c
56%
1
2
d,e
f,g,h
70%
3
4
i
95%
j,k
17%, 90%
5
6
7
Reagents and conditions: a) 10% Pd/C, MeOH, 0°C b) pTSA, toluene, 100°C c) OsO_4, THF, H_2O,NMO d) $Pb(OAc)_4$, MeOH,0°C e) Zn, AcOH f) MsCl,Et_3N, CH_2Cl_2, 0°C g) NaI, Me_2CO h) Zn, AcOH i) Et_3N, CH_2Cl_2 j) DEAD, Ph_3P, THF k) HF, MeCN

intact and allow us to observe the affinity of a seco FK506/FKBP12 complex for calcineurin. It was also anticipated, from our previous work, that a seco FK506 compound would possess only moderate affinity for the immunophilin. However this component can be eliminated from the measurement of calcineurin inhibition by addition of a large excess of recombinant FKBP12 to the calcineurin affinity assay. This ensures that even for compounds of moderate binding affinity the inhibitory complex is fully formed and thus a true calcineurin affinity is measured.

FK506 was hydrogenated to the C21 propyl derivative using 10% palladium on carbon in methanol at 0℃. Hydrogenolysis of the unsaturated ester function was avoided by careful monitoring of the reaction. Dehydration to give the required C23-C24 enone (1) was accomplished by addition of catalytic p-toluene sulfonic acid to a preheated (100℃) solution of the the propyl derivative in toluene. Dihydroxylation was effected by addition of osmium tetroxide (0.1 equivalents, 4% in water) to a solution of enone (1) and N-methyl morpholine N-oxide (4 equivalents) in tetrahydrofuran. The two diastereomeric cis-diol addition products (2) were visible by t.l.c. but were passed through to the subsequent reaction as a mixture. Cleavage of the diol, in the presence of the labile C9-C10 α-hydroxy ketone function, can be achieved by addition of solid lead tetraacetate (1.4 equivalents) to a methanolic solution of (2) at 0℃. After 2hr at 0℃ the crude reaction mixture was evaporated to dryness, dissolved in glacial acetic acid and treated at room temperature with excess zinc dust for 15 min. The two cleaved products (3) and (4)[9] were isolated by flash chromatography in 31% and 22% yield respectively. The hydroxy ketone (3) from the fragmentation was converted to the required methyl ketone (5) by mesylation (2 equivalents mesyl chloride, excess triethylamine in dichloromethane at 0℃), displacement of the mesylate with iodide ion and subsequent reduction of the α-iodoketone by brief treatment with zinc dust in acetic acid (5 equivalents, 1 min). The β-acyloxy aldehyde group, which had proven suprisingly stable throughout these transformations, was finally removed by treatment with triethylamine giving the required acid (6). Esterification of acid (6) was problematical[10] but was eventually effected, albeit in low yield, by alkylation under Mitsunobu conditions with alcohol (7)[5] to afford the target compound (8).

Compound (8) has a Ki = 400 ±200 nM for FKBP12 but the resulting complex shows no affinity for calcineurin (Ki >10μM). The rigidifying effect of binding to the immunophilin[11] may not be sufficient to overcome the effects of hydrophobic collapse.[12] In a similar study Schreiber et al. synthesised SBL506[13] which likewise shows modest affinity for FKBP12 (Ki = 207 nM). Once formed, however the complex displays excellent affinity for calcineurin (Ki = 330 ± 80 nM). This is only a 13-fold decrease on the value for the FK506/FKBP12 complex (Ki = 25nM). The moderate affinities observed for FKBP12 in both compounds may reflect greater entropic cost on binding non macrocyclic ligands. The interesting level of calcineurin inhibition observed in SBL506 may be the result of those features which cause the "arms" of this seco variant to protrude outside the binding protein, in positions sufficiently similar to those found in the native complex. Although the dual requirements of restricting the binding region and allowing suitable groups to protrude may not be insurmountable in some other framework, a macrocycle certainly provides a simple and extremely efficient means of achieving this. It is anticipated that this work, like that of the SmithKleine Beecham group [14],will contribute to the design of 'dual domain' compounds with constrained FKBP12 binding domains and suitable, protruding calcineurin binding units.

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2.The presenting proteins, FKBP12 and cyclophilin, are the best characterised examples of a large group of proteins collectively referred to as the immunophilins.

3. a) For discovery and details of phosphatase assay see a) Liu, J.; Farmer, J. D. Jr.; Lane, W. S.; Friedman, J.; Weissman, I. and Schreiber, S. L. *Cell* **1991**, 66, 807. b) Fruman, D. A.; Klee, C. B.; Bierer, B.E. and Burakoff, S. J. *Proc. Natl. Acad. Sci. USA* **1992**, 89, 3686. c) Liu, J.; Albers, M. W.; Wandless, T. J.; Luan S.; Alberg, D. G.; Belshaw, P. J.; Cohen, P.; MacKintosh, C.; Klee, C. B. and Schreiber, S. L. *Biochemistry* **1992**, 31, 3896.

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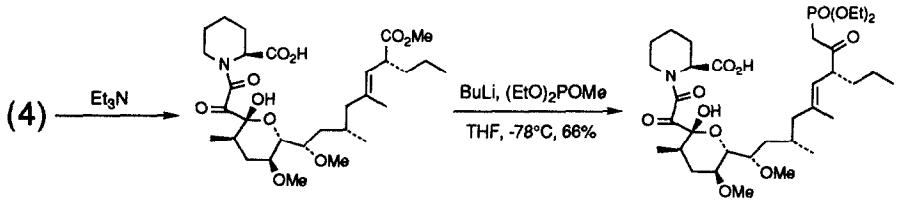
9. It proved possible to eliminate the top section from (4) and then to effect further selective transformations e.g.



10. For similar low yielding esterifications see. ref 6. Also see: a) Yohannes, D.; Myers, C. D. and Danishefsky, S. J. *Tetrahedron Lett.* **1993**, 34, 2075. b) Skotnicki, J. S. and Kearney, R. M. *Tetrahedron Lett.* **1994**, 35(2), 201. c) Goulet, M. T. and Hodkey, D. W. *Tetrahedron Lett.* **1991**, 32, 4627. d) Goulet, M. T and Boger, J. *Tetrahedron Lett.* **1990**, 31, 4845. For protection-deprotection strategy see Hayward, C. M.; Yohannes, D.; and Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, 115, 9345.

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