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Synthetic FKBP12 Ligands. Design And Synthesis Of Pyranose Replacements.

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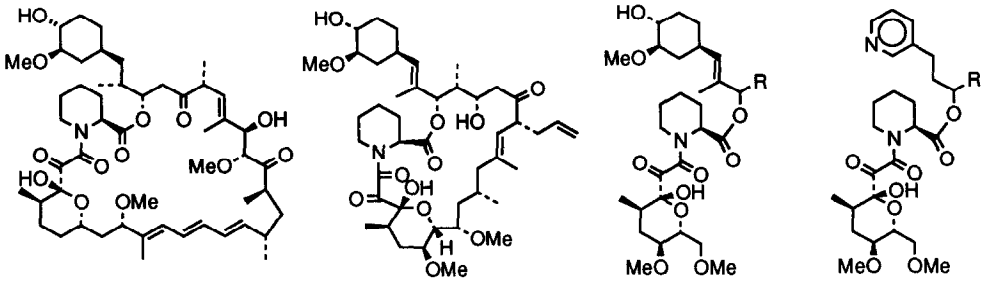
Abstract: A number of FKBP12 ligands were designed and synthesised and their affinity for FKBP12 assessed. In these ligands the pyranose ring of FK506 was replaced by other more synthetically accessible groups. The preparation of suitable intermediates for the synthesis of "dual domain" inhibitors (compounds 6a-c, 15 and 22) is also described.

FK506 is a potent immunosuppressive, macrocyclic natural product.[1] Once bound to the ubiquitous cytosolic protein FKBP12 it inhibits the protein phosphatase PP2B (calcineurin).[2] This enzyme is crucial in transcriptional control of the proliferative agent IL-2 and thus to the immune response.[3] The same phosphatase is inhibited by the structurally unrelated cyclic undecapeptide cyclosporin once it has bound to its cytosolic presenting protein, cyclophilin.[4] Rapamycin, which is also immunosuppressive, shows great structural similarity with FK506 in approximately half of its macrocyclic structure and binds tightly to FKBP12. However, the complex between rapamycin and FKBP12 does not inhibit calcineurin and the origin of rapamycin's immunosuppressive activity remains elusive.[5] X-ray crystallographic structure determinations of the complexes of FK506[6] and rapamycin[7] with FKBP12 have revealed that the common domains of these two natural products bind in nearly identical fashion within the protein. This forces the dissimilar and rather hydrophobic regions outwards into the aqueous environment. These then form composite drug-protein surfaces which mediate interaction with downstream targets, which ultimately leads to the observed biological activities.

Scheme 1.

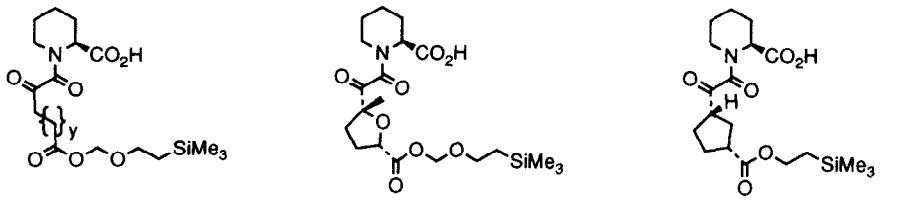


The objective of our program was to discover improved, structurally simplified, synthetic inhibitors of calcineurin based upon the structure and QSAR of FK506. This ambitious goal was to be achieved in a number of steps. Firstly we sought to simplify the region of FK506 that is bound within FKBP12. The affinity of these synthetic FKBP12 ligands was compared to that of compound (1) (scheme 1, R=pent-4-enyl) which is the excised binding domain of FK506.[8] This was intended to allow comparison of the relative affinities in the absence of constraints

imposed upon the binding domains by the remainder of the macrocyclic framework, which lies outside the binding protein. Secondly, we intended to fuse onto our simplified binding domains a suitable synthetic "effector domain".[9]

The key determinants of binding affinity were examined by sequential study of each of the components of the binding domain. The central pipecolinate dione core was retained, but the synthetically more challenging cyclohexyl ethenyl substituent was replaced by the 3-pyridylethyl substituent with little drop in affinity.[10] Thus compound (2) had a $K_d = 5x10^{-8}$ M which is very similar to that observed for compound (1) ($K_d = 1x10^{-8}$ M). With these results in hand we turned our attention to the replacement of the pyranose region.

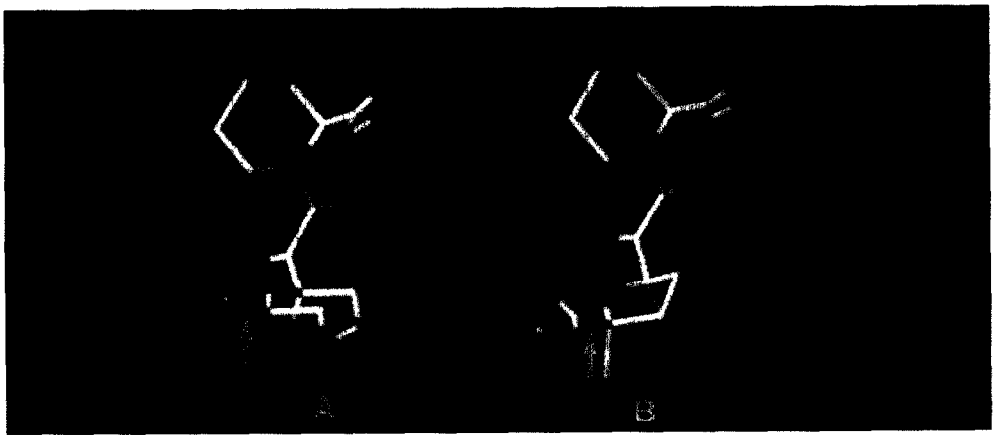
Scheme 2.



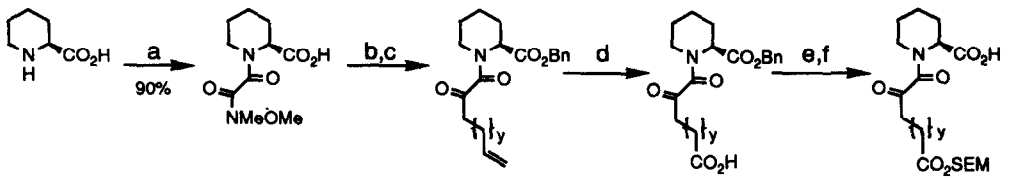
6,a-c 15 22

Our first replacements were with simple alkyl chains (6, a-c) (y = 1-3). Although these are flexible pyranose replacements the possibility exists that some conformational restriction will be induced upon this region once it is bound to FKBP12. The Weinreb amide[11] (3) (scheme 3) was allowed to react with excess Grignard reagents and the carboxylates protected as their benzyl esters (4, a-c). Cleavage of the terminal olefins proceeded in high yield without isolation of intermediates to give the carboxylates (5, a-c). Protection as SEM esters ($R=CH_2OCH_2CH_2SiMe_3$) and hydrogenolysis of the pipecolinate benzyl esters gave the required mono protected dicarboxylates (6, a-c), which served as intermediates for our "dual domain" inhibitors.[12] We also chose to replace the pyranose ring with cis-1,3 disubstituted furan or cyclopentane units, for which compounds 15 and 22 respectively served as intermediates. These were carefully selected to ensure that the ends of the effector loop is held rigidly and in a very similar orientation to that found in FK506. Diagram 1 shows a comparision between the shapes of the pyranose region in the bound conformation of FK506 (A) and our tetrahydrofuran replacement (B). Our aim was to ensure that the position and direction of the bond emanating from the pyanose replacement (indicated by ⇑) could overlay the corresponding bond from the pyranose ring in FK506.

Diagram 1.



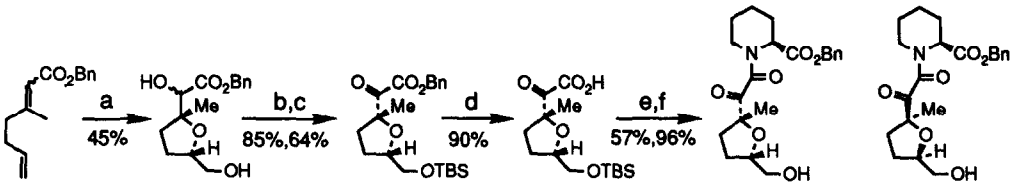
Scheme 3.



3 4a-c 5a-c 6a-c

Reagents and conditions:
a) ClCOCONMeOMe , BSTFA, CH_3CN b) $BrMgCH_2(CH_2)_yCHCH_2$, THF c) BnOH, DCC, DMAP, CH_2Cl_2 d) OsO_4, NMO, THF, H_2O then $Pb(OAc)_4$, toluene then $NaClO_2$, NaH_2PO_4, 1-Methylcyclohexene, t-BuOH, H_2O e) $ClCH_2OCH_2CH_2SiMe_3$, $(i\text{-}Pr)_2EtN$, CH_2Cl_2 f) H_2, Pd/C, EtOAc.

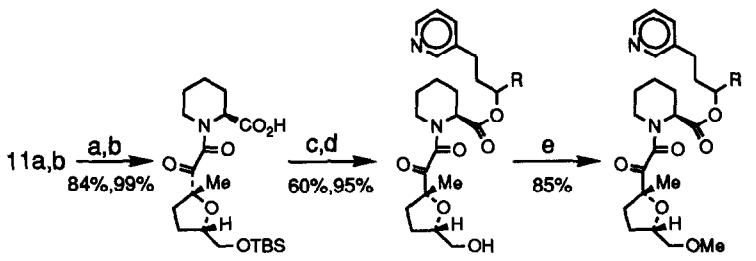
Scheme 4.



7 (±)8 (±)9 (±)10 11a 11b

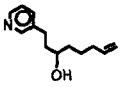
Reagents and conditions:
a) $KMnO_4$, CO_2, H_2O, Me_2CO, -20°C b) TBSCl, Et_3N, CH_2Cl_2, DMAP c) DMSO, ClCOCOCl, Et_3N, CH_2Cl_2, -78°C d) H_2 Pd/C, EtOAc e) Benzyl pipecolinate, DCC, DMAP, CH_2Cl_2 f) HF, CH_3CN.

Scheme 5.

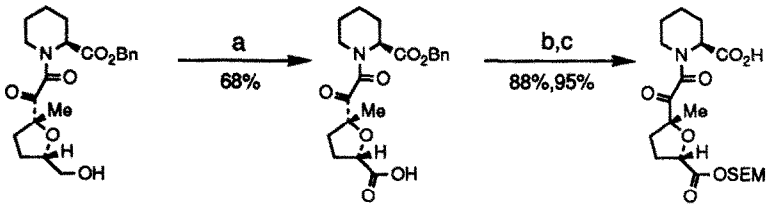


12 13

Binding Affinities[15] (K_d, M)	
12a	$2x10^{-6}$
12b	$1x10^{-5}$
13a	$2x10^{-6}$
13b	$1x10^{-5}$

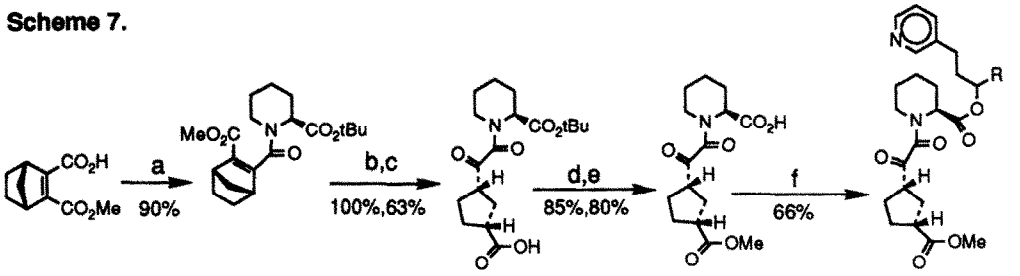
Reagents and conditions:
a) TBSCl, Et_3N, CH_2Cl_2, DMAP b) H_2, Pd/C, EtOAc c)  ,DCC, DMAP, CH_2Cl_2 d) HF CH_3CN e) Methyl triflate, 4-Methyl-2,6-di-t-butyl pyridine.

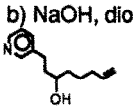
Scheme 6.



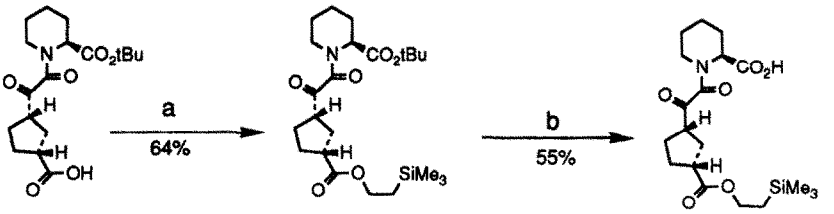
Reagents and conditions:
a) $RuCl_3.3H_2O$, $NaIO_4$, H_2O, MeCN, CCl_4 b) $ClCH_2OCH_2CH_2SiMe_3$, $(i\text{-}Pr)_2EtN$, CH_2Cl_2
c) H_2, Pd/C, EtOAc.

Scheme 7.



Reagents and conditions:
a) t-Butyl pipecolinate, DCC, DMAP, CH_2Cl_2 b) NaOH, dioxane-water c) O_3, -78°C, CH_2Cl_2
d) Cs_2CO_3, DMF, MeI e) TFA, CH_2Cl_2 f)  ,DCC, DMAP, CH_2Cl_2.

Scheme 8



Reagents and conditions:
a) $HOCH_2CH_2SiMe_3$, DCC, DMAP, CH_2Cl_2 b) HCO_2H, CH_2Cl_2.

Our synthesis was based upon the oxidative cyclisation of 1,5 dienes developed by Walba.[13] Thus treatment of diene (7)[14] (scheme 4) with potassium permanganate gave the diol (±8). Selective protection of the primary hydroxyl and oxidation proceeded smoothly to the α–ketoester (±9) as a single diastereomer. Hydrogenolysis of the benzyl ester gave the corresponding carboxylic acid (±10), which was used immediately since it showed some propensity to decarbonylate. It was coupled in good yield with (S)-benzyl pipecolinate, which proved to be an excellent resolving agent, since once the silyl protecting group had been removed, the resulting diastereomeric alcohols (11a and b) could be separated by column chromatography. The effectiveness of the tetrahydrofuran unit as a pyranose replacement was demonstrated by conversion of the separated isomers of (11) to compounds (12 a,b) and (13 a,b) (scheme 5) and measurement of their affinities for the binding protein, FKBP12.[15] The compounds showed good affinities when compared to the much more complex and synthetically demanding excised binding domain (2) (scheme 1). Compounds derived from (11a) consistently showed better levels of binding affinity when compared to those derived from the unwanted isomer (11b). Compound (11a) was oxidised to the acid (14) (scheme 6) and the protecting groups manipulated in good yield to give the required mono protected dicarboxylate (15), which served as the required intermediate for our "dual domain" inhibitors.[12]
Our third replacement for the pyranose region was a cyclopentane derivative. This could be obtained as a single enantiomer on a large scale based upon the route described by Kobayashi[16] (scheme 7). The chiral monoester (16) was acylated with t-butyl pipecolinate to give (17). Selective hydrolysis and ozonolysis resulted in the cleavage of the bicyclic system and *in situ* decarbonylation to the required carboxylate (18). The effectiveness of the cyclopentane as a pyranose replacement was assessed by preparation of (20). Thus alkylation of the cyclopentane carboxylate followed by deprotection of the pipecolinate carboxylate gave (19) which was used to acylate the substituted pyridine propanol which constituted our standard cyclohexenyl replacement. Compound (20) possessed good binding affinity ($K_d = 8x10^{-7}$ M) when compared to the synthetically more demanding excised binding domain compound (2) ($K_d = 5x10^{-8}$ M). The intermediate required for our "dual domain" inhibitors (22) (scheme 8) was obtained by esterification with trimethylsilyl ethanol followed by careful selective deprotection of the t-butyl ester (21). Our preferred SEM ester was not used in this series since this ester is required to pass intact through the deprotection of the t-butyl carboxylate, which in turn was required in order to effect the selective hydrolysis of (17) to (18). Nevertheless the silyl ethyl group proved adequate for use in the preparation of a limited number of "dual domain" macrocycles.
Having assessed the use of our pyranose replacements using compounds 12a,b 13a,b and 20 along with many others and with supplies of intermediates 6a-c, 15 and 22 in hand, the stage was set for our attempts to prepare biologically active "dual domain" inhibitors. These are the subject of the following publication.

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References and Notes:

1. Sigal, N. H. and Dumont, F. J. *Ann. Rev. Immunol.* **1992**, 10, 519.
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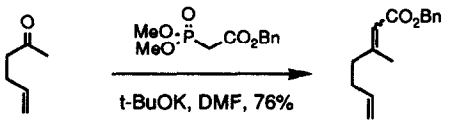
9. For similar work see Holt, D. A.; Konialian-Beck, A. L.; Oh, H-J.; Yen, H-K.; Rozamus, L. W.; Krog, A. J.; Erhard, K. F.; Ortiz, E.; Levy, M. A.; Brandt, M.; Bossard, M. J. and Luengo, J. I. *Bioorg. Med. Chem Letts.* **1994**, 4, 315. Luengo, J. I.; Konialian-Beck, A.; Levy, M.A.; Brandt, M.; Eggleston, D. S. and Holt, D. A. *Bioorg. Med. Chem. Letts.* **1994**, 4, 321.
10. Compound 2 (R = pent-4-enyl) was prepared by a route exactly analogous to that used to prepare (1) and described by our group in reference 8.
11. Prepared by refluxing dimethylhydroxylamine hydrochloride in excess oxalyl chloride for 16h. followed by evaporation of the excess oxalyl chloride.
12. See subsequent paper in this issue.
13. Walba, D. M.; Przybyla, C. A. and Walker, C. B. Jr. *J. Am. Chem. Soc.* **1990**, 112, 5624.
14. Diene (7) was prepared from 5-hexene-2-one by Horner-Emmons reaction with benzyl phosphonoacetate. The ambiguity of double bond isomers is eliminated in the subsequent Swern oxidation step.



15. The figures quoted for binding affinities are the average of duplicate determinations measured by displacement of a radiolabelled FK506 analogue from isolated purified FKBP12.[8] Other groups use the more indirect measurement of rotamase activity and express their ligand binding affinities as apparent inhibition constants (Ki,app.).[9]
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