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Synthesis And Evaluation Of Dual Domain Macrocyclic FKBP12 Ligands.

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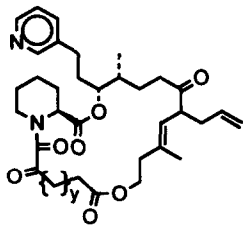
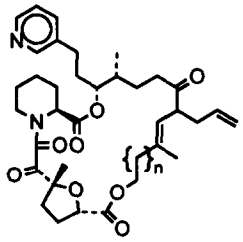
Abstract: A number of dual domain, macrocyclic FKBP12 ligands were synthesised in which the FK506 effector domain was fused to simplified FKBP12 binding domains. The resulting macrocyclic compounds possessed moderate binding affinities for FKBP12 but showed no activity in an assay for FKBP12 dependent calcineurin inhibition.

X-ray crystallographic structure determinations of the complexes of FK506[1] and rapamycin[2] with the immunophilin FKBP12 have revealed that the common domains of these two natural products bind in nearly identical fashion within FKBP12. The dissimilar domains of the macrocycles protrude outside FKBP12 to form composite drug-protein surfaces. FK506 and cyclosporin disrupt early T-cell activation,[3] however rapamycin has no effect on this but rather interrupts later events associated with a signal from the lymphokine receptor.[4] This has led to the dual domain concept[5] in which the macrocycles are divided into two separate molecular domains; one with high affinity for FKBP12 (binding domain) and the other with an "effector domain" which is part of a composite ligand-protein surface responsible for biological activity. Our approach was first to design simplified replacement binding domains and then to fuse on an appropriate effector domain.[6] First we synthesised macrocycles (10 a-c, y = 1-3) (Scheme 1) which have a flexible pyranose ring replacement. We then designed and synthesised rigid pyranose replacements (11 a,b n = 1,2) and (12 a,b n = 1,2) which were intended to hold the ends of the effector loop rigidly and in a very similar orientation to that found in FK506.

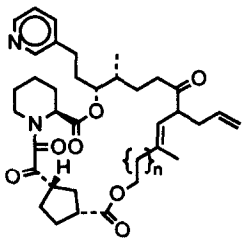
Scheme 1.



10a-c



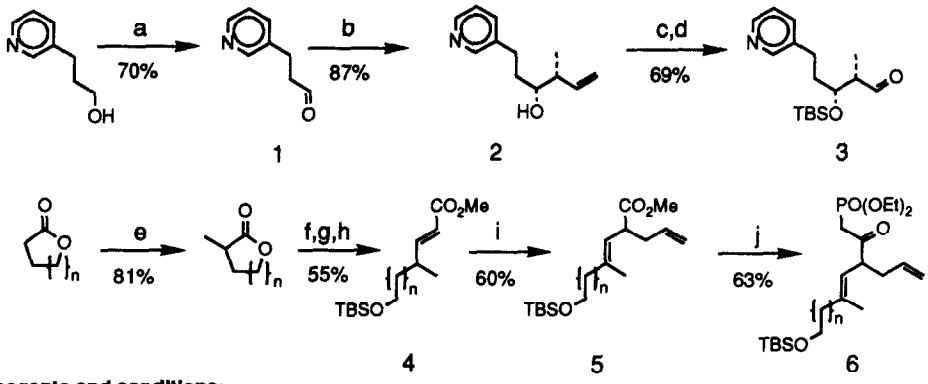
11a,b



12a,b

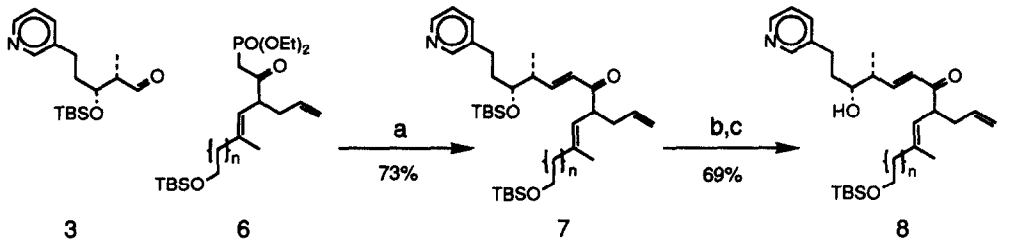
In order to achieve our goal of synthesising a range of macrocyclic dual domain compounds, we required a flexible and large scale synthesis of the "effector domain". This was achieved as shown in schemes 2 and 3. Aldehyde (1) (scheme 2) was reacted with Z-crotylborane[7] to give the alcohol (2) in 87% yield and 84%ee. Protection of the alcohol function and cleavage of the olefin gave aldehyde (3). The required αβ–unsaturated esters (4, n=1 and 2) were prepared by alkylation of the respective lactones followed by reduction and reaction with carbomethoxymethylene triphenylphosphorane. The crucial deconjugative alkylation proceeded cleanly in

Scheme 2.



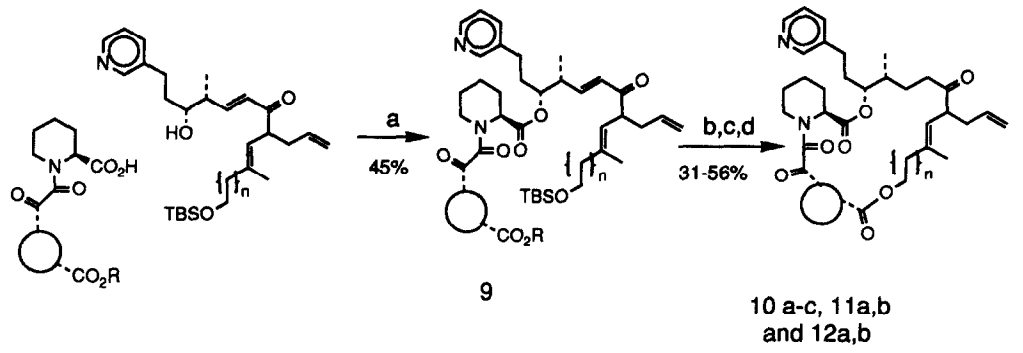
Reagents and conditions:
a) DMSO, ClCOCOCl, Et_3N, -78°C b) (-)-β-Methoxydiisopinocamphenylborane, Z-crotyl potassium, BF_3OEt_2, THF c) TBSCl, imidazole, DMF d) O_3, CH_2Cl_2, Ph_3P e) LDA / THF / MeI / HMPA f) DIBAlH / toluene g) Ph_3PCHCO_2Me, THF h) TBSCl, Imidazole, DMF i) LDA, excess HMPA, allyl bromide, -78°C j) $(EtO)_2POMe$, BuLi, THF.

Scheme 3.



Reagents and conditions:
a) LiCl, $(i\text{-}Pr)_2NEt$, MeCN b) HF, CH_3CN c) TBSCl, Et_3N, CH_2Cl_2, DMAP.

Scheme 4.



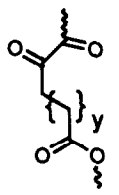
Reagents and conditions:
a) DCC, DMAP, CH_2Cl_2 b) For R=SEM: HF, MeCN For R= $Me_3SiCH_2CH_2$: HF, MeCN then TFA, CH_2Cl_2
c) EDCI, CH_2Cl_2 d) Ph_4Pd, Bu_3SnH, THF, H_2O.

the presence of four equivalents of hexamethylphosphoramide[8] to give a separable mixture of trisubstituted olefins (5) (E/Z =13/1). Reaction with the anion of diethyl methylphosphonate gave the required keto-phosphonates (6).

Reaction between aldehyde (3) and keto-phosphonates (6) (scheme 3) utilising the mild lithium chloride / amine conditions developed by Masamune and Roush[9] gave the required enones (7) in good yield. Removal of both protecting groups followed by selective reprotection of the primary hydroxyl gave the required "effector domains" (8). The ease and convergency of this route combined to make it possible to prepare multigram quantities of the required compounds for use in our program.

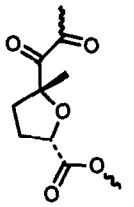
The required macrocycles were synthesised as shown in scheme 4. With the binding domain replacement protected as the SEM ester (R = $Me_3SiCH_2CH_2OCH_2$), acylation of the "effector domain" gave the required seco compounds (9), which were smoothly deprotected with hydrofluoric acid and cyclised to the required macrocycles. For the compounds in which the pyranose ring had been replaced by cyclopentane (12a and b) we were forced to use the more acid stable trimethylsilylethyl ester. This required sequential removal of the protecting groups in (9) by treatment with hydrofluoric acid then trifluoroacetic acid. The second step resulted in partial trifluoroacetylation of the alcohol group which had to be hydrolysed by treatment with aqueous bicarbonate before macrocyclization. The enone function was selectively reduced in all the compounds in excellent yield to give the required macrocyclic compounds (10,11 and 12). The binding affinities of these compounds for FKBP12 are shown in scheme 5.

Scheme 5. Binding affinity (K_d M.)[10] of the macrocycles 10, 11 and 12 for the immunophilin FKBP12.



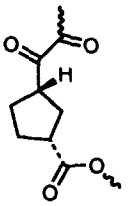
10

y = 1 5.5×10^{-6}
y = 2 2.0×10^{-6}
y = 3 4.5×10^{-6}



11

n = 1 8×10^{-6}
n = 2 4×10^{-5}



12

n = 1 4.0×10^{-7}
n = 2 2.0×10^{-7}

Surprisingly these compounds showed only micromolar affinity for FKBP12. This is very similar to that observed in similar alicyclic compounds.[11] Thus it seems that the rigidifying effects of the FK506 effector domain[12] does not result in any productive conformational preorganisation of the binding domain. The compounds also showed no activity in an assay of FKBP12 dependent calcineurin inhibition.[13] Whether these compounds lack biological activity because the "effector domain" is inappropriately orientated or because the ester group is a poor mimic of the surface presented by FK506 in the region of the key FKBP12 amino acid side chains,[14] is as yet an unanswered question.

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