Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 20, pp. 2341-2346, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00404-1

Synthesis of FK506-Cyclosporin Hybrid Macrocycles.

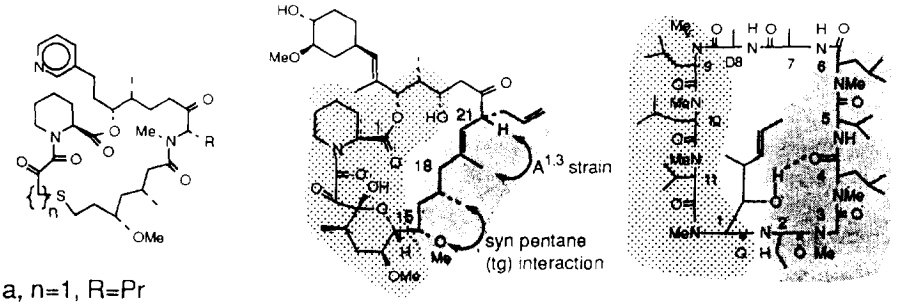
Simon J. Teague,* Martin E. Cooper and Michael J. Stocks.

Fisons plc, Pharmaceuticals Division, Bakewell Road, Loughborough, LE11 ORH, U.K.

Abstract: An attempt was made to synthesise calcineurin inhibitors using dual domain macrocyclic compounds that incorporated a FKBP12 binding domain together with a calcineurin recognition domain which had been designed by consideration of the relevant features of both FK506 and Cyclosporin A.

Investigation of the immunosuppressive agents FK506 and Cyclosporin A (CsA) (figure 1) has revealed that they bind to their respective immunophilins, FKBP12 and Cyclophilin (CyP) and that the resulting complexes both exert their biological effects through inhibition of the protein phosphatase, calcineurin (CN). The available information concerning the three dimensional structures of these complexes has recently been reviewed.[1] It is not yet proven that the two composite surfaces produced by FK506-FKBP12 and CsA-CyP are recognised in exactly the same location upon CN, however, the binding of the two complexes is mutually exclusive and recent experiments have narrowed the binding site(s) upon CN to a small area at the interface between the A and B subunits of this heterodimeric protein.[2]

Figure 1



1a, n=1, R=Pr
1b, n=2, R=iBu

FK506

Cyclosporin A

Structures of compounds **1a,b**, FK506 and Cyclosporin. Those parts of their structures which are bound within the binding proteins (FKBP12 or Cyclophilin) are indicated by the dotted areas. Those parts of each molecule which contribute to the composite surface required for inhibition of the biologically important enzyme calcineurin, are indicated by the shaded areas. Some of the interactions which limit the conformational freedom of the section of FK506 which is recognised by calcineurin, are indicated by the arrows.

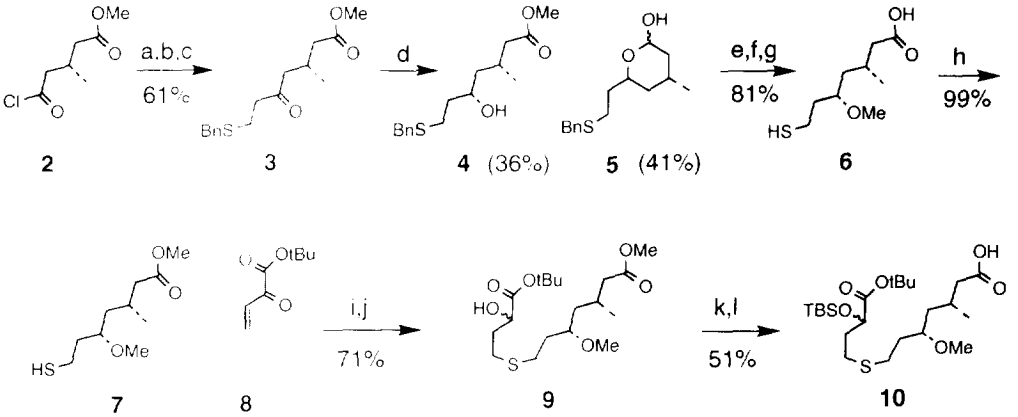
We have designed and synthesised macrocyclic compounds of the type of **1a,b** based upon consideration of the structures of FK506 and CsA together with an analysis of the principles which interrelate the structures of these compounds with their ability to facilitate specific protein-protein interactions.[3] Based upon these considerations we chose to synthesise

macrocyclic compounds incorporating an FKBP12 binding region together with a suitable rigid, lipophilic CN binding section, which is a hybrid of the relevant sections presented by FK506 or CsA. Thus **1a,b** have an intact FKBP12 binding domain together with section C15-18 of FK506 fused with residue 6 of CsA. The amide bond is used as an isosteric replacement of the C19-20 trisubstituted olefin found in FK506. Central to our design was the belief that in order to achieve calcineurin binding compounds **1a,b** must incorporate some of the rigidifying features (syn pentane $A^{1,3}$ strain interactions[4]) which are present in the CN binding section of the FK506 macrocycle.

Our initial efforts focused upon construction of the key 'effector domain' (**Scheme 1**). Friedel-Crafts acylation of ethylene with the readily available,[5] chiral acid chloride (**2**) followed by dehydrochlorination and addition of benzyl thiol gave (**3**). Addition of sodium borohydride to a methanolic soution of (**3**) at 25 °C resulted in isolation of a single diastereomeric alcohol (**4**).[6] The alternative alcohol epimer spontaneously lactonised and was reduced to a mixture of lactols (**5**) under the reaction conditions. This was a gratifying example of the effect of syn-pentane interactions upon reactivity. Hydrolysis of the carboxyl group in (**4**) allowed the alcohol to be methylated without significant lactonisation and also served to suppress unwanted reduction and intermolecular Claisen reactions of the ester during the subsequent removal of the benzyl group to give the thiol (**6**). The carboxyl group was selectively remethylated using ethereal diazomethane to give (**7**) which underwent smooth Michael addition to the highly reactive[7] acceptor (**8**). Reduction of the α-ketoester group proceeded in high yield at 25°C, however, a considerable amount of retro-Michael reaction was observed when the same reaction was carried out at 0°C. Protection of the alcohol and selective hydrolysis of the methyl ester[8] completed our synthesis of the required acid (**10**). A highly convergent route to the other macrocycle moiety (**14**) was required which delivered the two amino-acid nitrogens orthogonally protected (**Scheme 2**). Thus acylation of the chiral alcohol (**11**)[9] with BOC-pipecolinate followed by cleavage of the terminal olefin with ozone gave the aldehyde (**12**). Reaction between (**12**) and keto-phosphonate (**13**)[10] utilizing the mild lithium chloride/amine conditions developed by Masamune and Roush[11] gave the required enone without elimination of the pipecolinoxy group. Catalytic hydrogenation served to reduce the resulting enone and concomitantly removed the amine protecting group, giving the required amine (**14**). The sub-sections of the macrocycle were assembled as shown in **Scheme 3**. Acylation of amine (**14**) with acid (**10**) gave (**15**) in good yield using BOP reagent. Stepwise deprotection and macrocyclisation proceeded satisfactorily to give (**16**). Selective oxidation of the alcohol to the required α-ketoamide then proved extremely difficult using a wide range of standard reagents. Eventually conversion to the sulfoxide (**17**) followed by a novel intramolecular varient of the Swern reaction gave **1a** albeit in low yield.

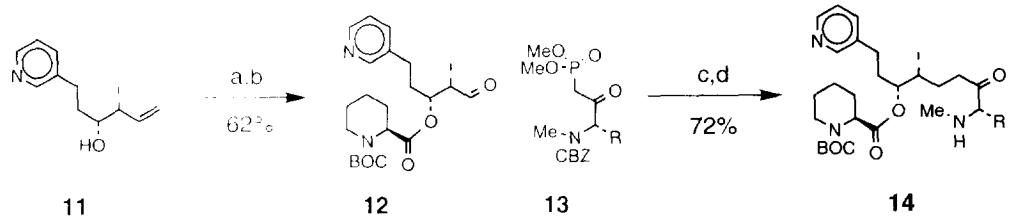
The route to **1b** was chosen to avoid the difficulty in selective oxidation by altering the order of bond forming steps which lead to the required macrocyclic product. Thus acylation of (**18**)[12]

Scheme 1.



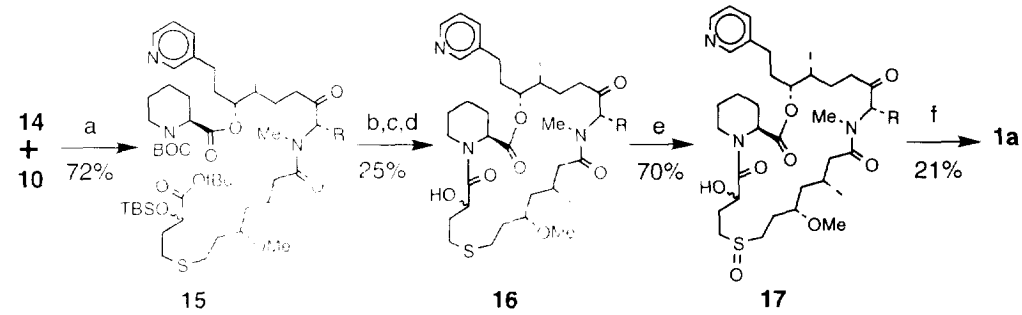
Reagents and conditions
a) CH_2CH_2, $AlCl_3$, CCl_4 b) Et_3N, distil c) BnSH, Et_2O d) $NaBH_4$, MeOH, 25°C e) NaOH, H_2O, EtOH f) NaH, MeI, DMF g) Na, NH_3, THF h) CH_2N_2, Et_2O i) Et_2O, 25°C j) $NaBH_4$, MeOH, 25°C k) TBSCl, Imidazole, CH_2Cl_2 l) NaOH, MeOH, H_2O

Scheme 2.



Reagents and conditions
a) BOC-(S)-Pipecolinic acid, DCC, DMAP, CH_2Cl_2 b) O_3, CH_2Cl_2, Ph_3P c) LiCl, $(i\text{-}Pr)_2NEt$, MeCN d) H_2, EtOH, Pd/C

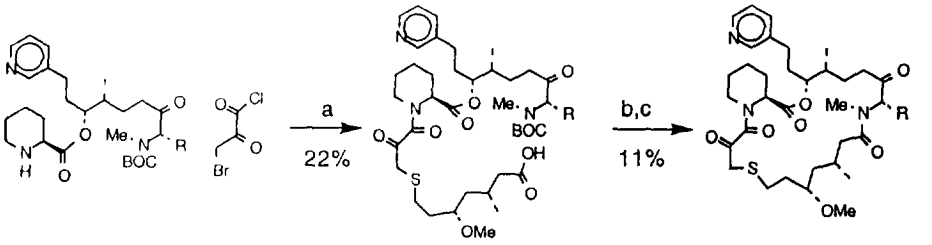
Scheme 3.



Reagents and conditions
a) BOP reagent, DMAP, CH_2Cl_2 b) HF, CH_3CN c) TFA, CH_2Cl_2 d) DCC, DMAP, DMAP.HCl, $CHCl_3$ reflux e) Dess-Martin reagent, CH_2Cl_2 f) ClCOCOCl, CH_2Cl_2, -78 , then NEt_3 warm to 25°C

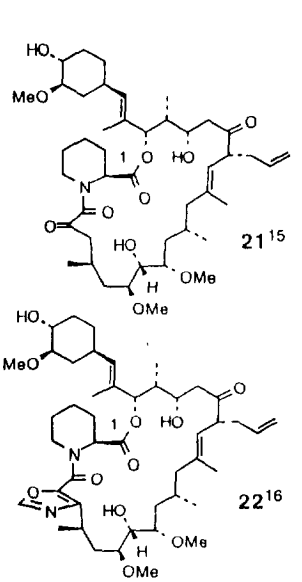
with the unusual acid chloride (**19**)[13] followed by *in situ* reaction with the thiol acid (**6**) gave (**20**) in a highly convergent manner but modest yield.[14] N-BOC deprotection and macrocyclisation gave **1b**.

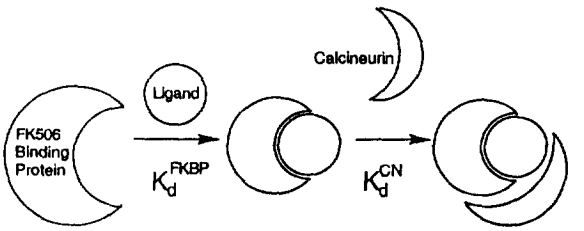
Scheme 4



Reagents and conditions
a) $NaHCO_3$, H_2O, then add **8** b) TFA, CH_2Cl_2 c) BOP reagent, DMAP, DMAP.HCl, CH_2Cl_2

Figure 2.





Ligand	K_d^{FKBP} (nM)	K_d^{CN} (nM)
FK506	0.2	20
Cyclosporin	*	4
17	>1000	>1000
1a	644	>1000
1b	90	>1000
21	320	350
22	344	300

* Cyclosporin binds to cyclophilin then the resulting complex binds and inhibits calcineurin with the K_d described

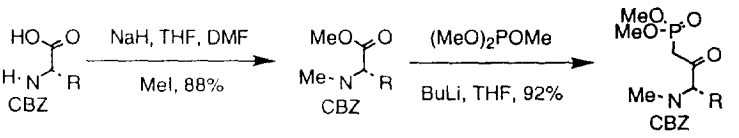
Examination of the binding affinities of compounds **1a** and **1b** showed that they bound to FKBP12 with moderate affinity. The intermediate hydroxysulfoxide **17** showed no affinity for the binding protein. Oxidation of this compound at C9 (FK506 numbering) without concomitant reduction of the sulfoxide to the sulfide also produced a compound with no affinity for FKBP12, thus the affinity which is observed for **1a** provides biological verification of the effectiveness of the intramolecular Swern reaction whose structure is also confirmed by careful examination of its spectral properties. The second equilibrium involving the ligand/binding-protein complex and calcineurin can be examined by adding a large molar excess of binding-protein to the assay thus eliminating the first equilibrium. Under these conditions the complexes from **1a** and **1b** showed no affinity for CN. A number of reasons may account for these observations. One possibility is that the increased ring size in **1a,b** may be detrimental to the second equilibrium. In this respect compounds **21** and **22** are instructive since despite their modest affinities for FKBP12 they do generate effective binding complexes for CN. This however, may be partly due to the residual hydroxyl group at C14 controling the local conformation either by hydrogen bonding to the binding protein or in a manner similar to that suggested in the macrocycle Swinholide A.[17] Another possibility is that the N-methylated amide used in **1a,b** both to facilitate their convergent synthesis and mimic the C19-20 trisubstituted olefin present in FK506, is unacceptable when presented to CN in the context of FKBP12 despite being acceptable when presented as part of CsA/Cyclophilin complex. Nonetheless, compounds of this type still present fascinating opportunities for the investigation of molecular recognition phenomena using synthetic ligands.

References and notes:

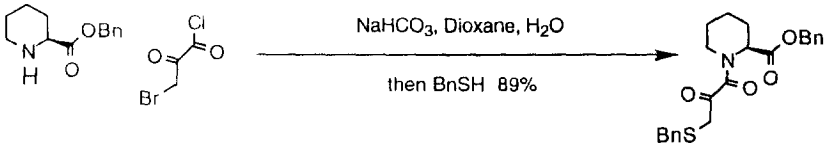
1 Braun, W.; Kallen, J.; Mikol, V.; Walkinshaw, M. D. and Wuthrich, K. *FASEB* **1995**, 9, 63

2. Husi, H.; Luyten, M. A. and Zurini M. G. M. *J. Biol. Chem.* **1994,** 269, 14199.
3. Teague, S. J. *Nature Structural Biology* **1995**, 2, 360.
4. Hoffmann, R. W. *Angew. Chem. Int. Ed. Engl.* **1992**, 31, 1124.
5. Prepared by treatment of the acid (Fluka) at 25℃ with two equivalents of oxalyl chloride in dichloromethane containing a catalytic amount of DMF.
6. The stereochemistry was assigned by lactonisation and analysis by NMR. Carroll, F. I.; Mitchell, G. N.; Blackwell, J. T.; Sobti, A. and Meck, R. *J Org. Chem.* **1974**, 39, 3890.
7. The enone **8** was prepared by a modification of the method of Nimitz, J. S. and Mosher, H. S. *J. Org. Chem.* **1981**, 46, 211. Thus t-butyl -oxo-1H-imidazole-1-acetate in THF was allowed to react with vinyl magnesium bromide at -78℃ for 10 minutes befor being quenched with saturated aqueous ammonium chloride at -40℃. The reaction was diluted with ether and solid sodium chloride added. Separation of the organic layer and evaporation at 25℃ gave (**8**) as a yellow oil which was used immediately.
8. Protection of the alcohol (**9**) greatly improved the selectivity of the hydrolyis step. The unprotected α-hydroxy t-butyl ester showed a suprisingly increased level of base sensitivity.

9. Caffrey, M.V.; Cladingboel, D. E.; Cooper, M. E.; Donald, D. K.; Furber, M.; Hardern, D. N.; Harrison, R. P.; Stocks, M. J. and Teague, S. J. *Bioorg. Med. Chem. Letts.* **1994**, 4, 2507
10. Phosphonate **13** was synthsised by methylation of the N-protected amino acid followed by treatment with 2.2 equivalents of phosphinate anion at -78℃.



11. Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R. and Sakai, T. *Tetrahedron Lett.* **1984**, 25, 2183.
12. Amine **18** was synthesised by a route exactly analogous to that descibed for **14**.
13. Acid chloride **19** was prepared by heating 3-bromopyruvic acid hydrate (Aldrich) in excess αα-dichloromethyl methyl ether at 50℃ for two hours. Distillation (~60℃ 1mmHg) gave a yellow oil which solidified upon storage at -20℃. Hashimoto, M.; Hemmi, K.; Kamiya, T.; Komori, T.; Nakaguti, O.; Saito, Y.; Shiokawa, Y.; Takasugi, H.; Takaya, T. and Teraji, T. *US 4207234 (CA 94:654610).*
14. The low yield was largely the result of the difficulties encountered in isolating the acid **20**. In other reactions acid chloride **19** was converted in high yield to α-keto β-thioalkyl amides eg.



Yields were very poor if the same reaction was carried out employing triethylamine as base.
15. This compound was obtained as a minor component when FK506 in pyridine was treated with hydrogen sulfide gas. We acknowledge D. N. Hardern, D. K. Donald and M. Furber of our laboratories for this observation.
16. Nussbaumer, P.; Grassberger, M. A.; Hiestand, P; Schuler W.; Schulz, G. and Zenke, G. *XIIth International Symposium on Medicinal Chemistry'* Basel **1992**.
17. Doi, M.; Ishida, T.; Kobayashi, M. and Kitagawa, I. *J. Org. Chem.* **1991**, 56, 3629.

(*Received in Belgium* 30 *May* 1995; *accepted* 4 *September* 1995)