



The Contribution To Binding Of The Pyranoside Substituents In The Excised Binding Domain Of FK-506

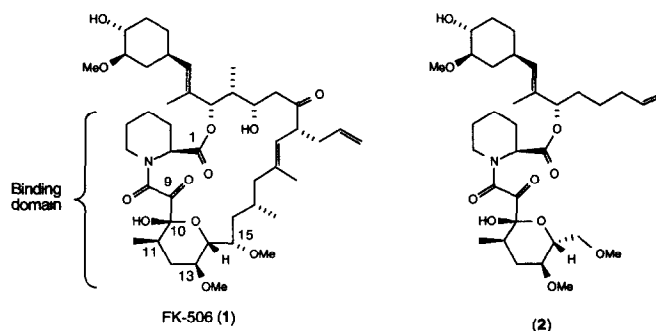
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Abstract: The contributions of the C-11 methyl and C-13 / C-15 methoxy substituents to the affinity of FK-506 for the immunophilin FKBP-12 were investigated. Subtraction of the C-11 methyl group led to a 400-fold drop in affinity. Removal of the C-13 and C-15 methoxy substituents led to a much smaller drop in affinity.

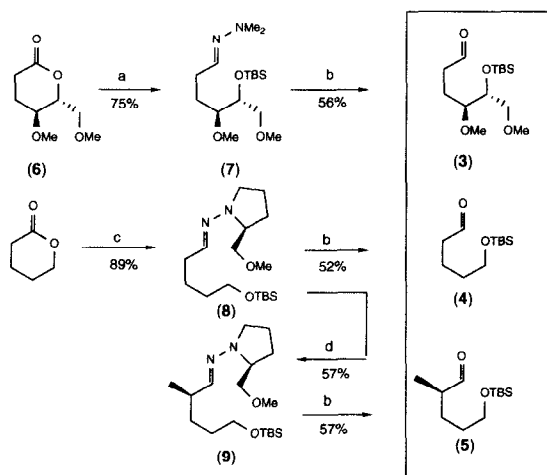
FK-506 (**1**) has been the focus of intense investigation in both the chemical¹ and biological² communities since its disclosure by Tanaka and co-workers³. Excitement was aroused by its potent immunosuppressant activity, and investigation of its mechanism of action has led to new insights into cytoplasmic signal transduction⁴. FK-506 is bound by the cytosolic protein FKBP-12 (FK Binding Protein, MWt 12,000) and the structure of this complex has been determined by both X-ray⁵ and NMR⁶ methods. These studies show that approximately half of FK-506 is deeply buried within the binding protein and that the other half is exposed to solvent⁷.

It has also been reported that the composite surface formed by the complex of FK-506 and FKBP-12 binds to, and inhibits a protein phosphatase, calcineurin⁸. It is this inhibition that results in the interruption of the cytoplasmic signal transduction, and ultimately leads to the observed immunosuppressive action of FK-506.



We recently reported⁹ the affinity of the excised binding domain of FK-506 for the immunophilin FKBP-12. Without the constraints imposed by the macrocyclic framework, the excised binding domain **2** has an affinity of $K_d = 1 \times 10^{-8} \text{M}$ ($n=4 \pm 2.4 \times 10^{-8} \text{M}$) as judged by its ability to displace a radiolabelled FK-506 derivative from the isolated, homogenous binding protein¹⁰.

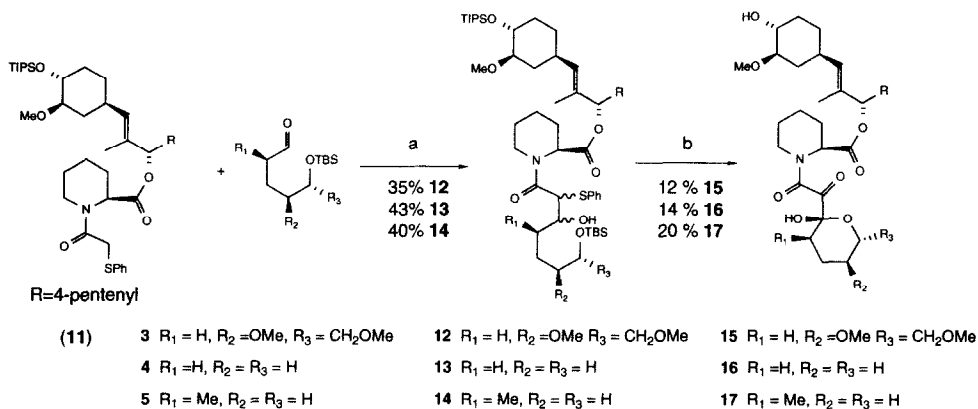
We now report further studies on the various contributions of the methyl and methoxy substituents to the overall binding in these alicyclic compounds.



Preparation of the required aldehydes (Scheme 1)

Reagents and conditions:

- a) i) DIBALH, CH_2Cl_2 , -70°C ii) N,N-dimethylhydrazine, CH_2Cl_2 iii) TBSCl / CH_2Cl_2 / imidazole
 b) Ozone, CH_2Cl_2 , -30°C
 c) i) DIBALH, CH_2Cl_2 , -70°C ii) SAMP, CH_2Cl_2 iii) TBSCl / CH_2Cl_2 / imidazole
 d) LDA, MeI, THF, -78°C



Preparation of binding domain analogues (Scheme 2)

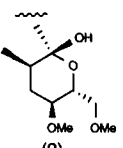
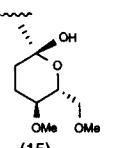
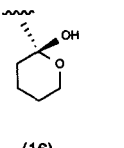
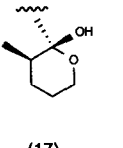
Reagents and conditions:

- a) LDA, THF, -78°C
 b) i) Dess-Martin periodinane (2 eq), pyridine (8eq) ii) HF / CH_3CN

The preparation of the various substituted aldehydes **3**, **4**, **5** is described in Scheme 1¹¹. The lactone **6**¹² was reduced and the resulting lactol condensed with N,N-dimethylhydrazine to afford the hydrazone **7** in 75% yield after silylation of the resulting secondary alcohol. Ozonolysis of the hydrazone afforded aldehyde **3** in high yield. In a similar fashion δ -valerolactone afforded the substituted SAMP¹³-hydrazone **8** in 89% overall yield. Ozonolysis of the resulting hydrazone afforded aldehyde **4**, whilst alkylation, in the usual manner¹⁴, afforded the α -methyl derivative **9** in 57% yield with a de of 94%¹⁵. Ozonolysis of the hydrazone **9** afforded aldehyde **5** without racemisation of the α -methyl substituent.

Condensation of the known thiophenyl derivative **11**⁹ with the aldehydes **3**, **4**, **5** was achieved by the procedure outlined by Danishefsky¹⁶. Oxidation of the condensation adducts **12**, **13**, **14** followed by *in situ* deprotection of the silylether with HF in acetonitrile effected ring closure to afford the requisite series of compounds **15**, **16**, **17** (Scheme 2).

The synthesis of the series of compounds **15**, **16**, **17**, allowed us to examine the role of the pyranoside substituents in the overall affinity of the binding domain of FK-506 for the immunophilin FKBP-12 (Table 1).

Table 1 The binding affinities of the various binding domain analogues				
Compound				
	(2)	(15)	(16)	(17)
Affinity	$K_d = 1 \times 10^{-8} \text{ M}$ ($n=4 \pm 2.4 \times 10^{-8} \text{ M}$)	$K_d = 4 \times 10^{-5} \text{ M}$ ($n=2 \pm 1 \times 10^{-5} \text{ M}$)	$K_d = 2.9 \times 10^{-5} \text{ M}$ ($n=4 \pm 1 \times 10^{-5} \text{ M}$)	$K_d = 2 \times 10^{-7} \text{ M}$ ($n=2 \pm 0.1 \times 10^{-7} \text{ M}$)

Comparison of the compounds **2** and **17** show that the 2 pendant methoxy groups of the pyranoside ring contribute little to the binding. Removal of the α -methyl group from **2** and **17** (compounds **15** and **16**) showed a more dramatic drop in binding.

The possibility that this reduction in affinity was the result of gross chemical differences, such as 7-membered hemi-ketal formation¹⁷ or C-9 carbonyl hydration¹⁸, was eliminated by NMR evidence. Examination of the X-ray crystal structure suggests that the C-11 methyl group lies in a shallow pocket composed of residues His⁸⁷ and Ile⁹¹. This may be critical for the correct orientation of the pyranoside ring to be achieved leading to the formation of the Asp³⁷ C-10 (OH) hydrogen bond¹⁹.

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