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The Affinity Of The Excised Binding Domain Of FK-506 For The Immunophilin FKBP12.

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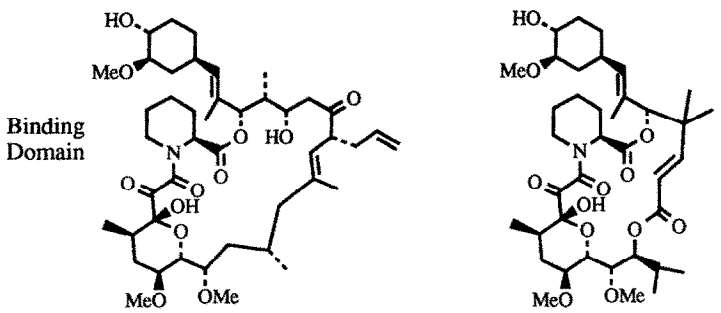
Abstract: The affinity of the binding domain of FK-506 was determined, in the absence of the constraints imposed upon it by the macrocyclic framework. Removal of those parts of FK-506 not involved in the binding of FK-506 to FKBP12 results in a 50-fold drop in affinity.

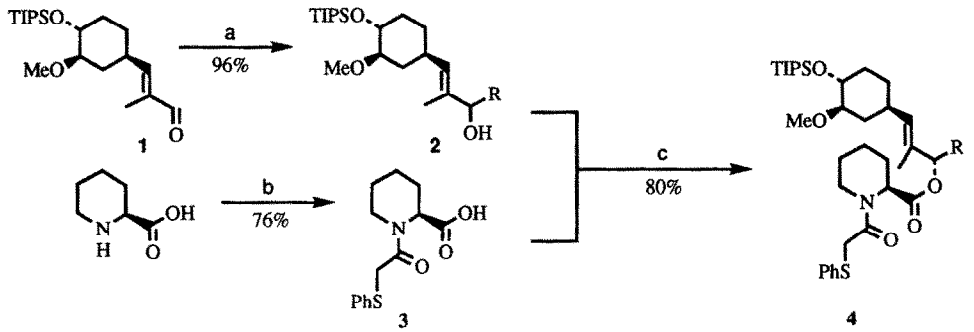
Investigations into the mechanism of action of the immunosuppressant FK-506 have provided new insights into cytoplasmic signal transduction[1]. FK-506 is bound by the cytosolic protein FKBP12 (FK binding protein, molecular weight 12,000)[2] and the structure of this complex has been determined by X-ray[3] and NMR methods[4]. These studies have shown that approximately half of the FK-506 macrocycle is deeply buried within the binding protein with the other half protruding outside and exposed to solvent[5]. An analogy has been drawn between this complex and an ice cream sitting upon an ice cream cone[6]. FKBP12 plays an analogous role to that of cyclophilin, the binding protein for the immunosuppressant cyclosporin[7]. These two presenting proteins are, at present the best characterised examples of the large group of proteins collectively known as immunophilins[8].

A composite surface formed from FK-506 and the immunophilin FKBP12 binds to and inhibits a protein phosphatase, calcineurin[9]. This inhibition results in interruption of cytoplasmic signal transduction, and ultimately leads to the observed immunosuppressive action of FK-506.

In the course of our investigations into this novel agent[10] we have separated the immunophilin binding region from the constraints imposed upon it by those parts of the macrocyclic framework which lie outside the binding protein. This was achieved by total synthesis of compound **1**, the immunophilin binding region, and determination of its ability to displace a radiolabelled FK-506 derivative from the isolated, homogeneous binding protein[11].

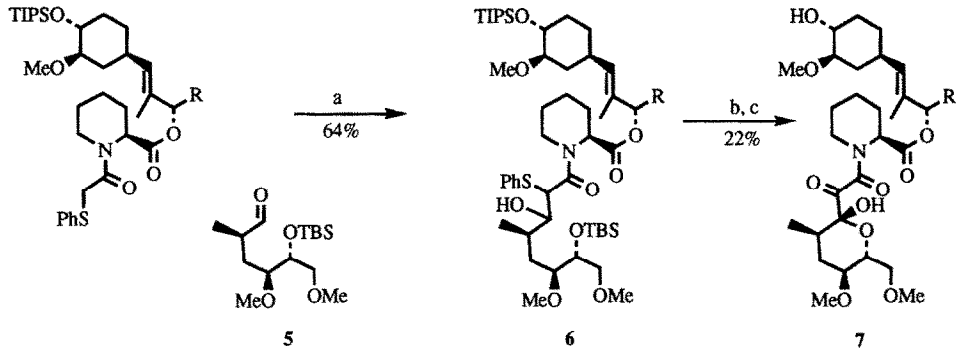


Scheme I



Reagents and Conditions a) 4-Pentenyl magnesium bromide, THF, 0°C b) N,O-*Bis*(trimethylsilyl)acetamide 4 equiv., CH_3CN, 25°C then thiophenyl acetyl chloride, 0°C. c) DCC, DMAP, CH_2Cl_2, 25°C.

Scheme II



Reagents and Conditions a) LDA, THF, -78°C b) Dess-Martin periodinane, CH_2Cl_2 c) HF, CH_3CN

4-Pentenylmagnesium bromide (approx. 1M in tetrahydrofuran (THF)) was added to an ice-cooled THF solution of the known[12] aldehyde **1** (scheme I). The resulting diastereomeric alcohols **2** were easily separated and the absolute stereochemistry assigned[13]. Pipecolinic acid was silylated and acylated *in situ* to give the required thiophenylacetyl derivative **3**. Esterification of the required, more polar, isomer, was then effected to give compound **4** in acceptable overall yield. Condensation of **4** with the known[14] aldehyde **5** (Scheme II) led to formation of the required adduct **6** as a mixture of diastereomers. Elaboration of **6** through to the target compound **7** was achieved by the method outlined by Danishefsky[15]. Thus the α-hydroxy sulphide was oxidised to the bright yellow and rather unstable *vic*-1,2,3-trione system that was treated with HF in acetonitrile to afford **7**.

The synthesis of compound **7** allows the determination of the affinity of the binding domain for the immunophilin FKBP12 in the absence of the constraints imposed upon it by the macrocyclic framework. It was reasoned that the macrocyclic framework, which protrudes out of the presenting protein, may serve to restrict the relative positions of individual elements within the binding domain and thus affect the affinity for the immunophilin. In a similar investigation Schreiber *et al.*[16] attempted to replace the non binding portion of FK-506 with an alternative 'scaffolding domain'. This led to the design and synthesis of 506BD. Here, an attempt was made to impose similar constraints using a simplified scaffolding element. A 20-fold drop in binding affinity between FK-506 and 506BD was observed. The affinity of FK-506 for the immunophilin FKBP12 was $K_d=2x10^{-10}$ M ($n=8, \pm 2x10^{-10}$ M) and that for compound **7** was $K_d = 1x10^{-8}$ M.($n=4, \pm 2.4x10^{-8}$). This result suggests a 50-fold drop in affinity when the constraints of the macrocyclic ring are removed. It is anticipated that compound **7** would also be a transition state inhibitor of FKBP rotamase activity[17]. The moderate affinity remaining in compound **7** may be the result of several features within the binding region, serving to constrain the conformation, even in the absence of the macrocyclic framework. Stereoelectronic control of the anomeric hydroxyl group and the consequences of $A^{1,3}$ strain interactions, impose considerable constraints upon the region and imply that the major effect of removing the macrocyclic framework may simply be to allow motion of the substituted cyclohexyl moiety relative to the rest of the binding region. The process of simplifying the binding domain and designing alternative, biologically active scaffolding frameworks is ongoing in our laboratories.

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