Structure-Based Design of an Acyclic Ligand That Bridges FKBP12 and Calcineurin

Merritt B. Andrus and Stuart L. Schreiber*

Department of Chemistry
Harvard University, 12 Oxford Street
Cambridge, Massachusetts 02138

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Advances in structural biology have provided access to the atomic structures of many protein–ligand complexes. Protein structure-based ligand design, however, has proved to be a more significant challenge—the few successful examples have involved much trial and error. A particularly challenging problem is posed by the structures of immunophilin complexes of the natural products cyclosporin A (CsA), FK506, and rapamycin.[1] These compounds have two protein-binding surfaces and can bind two proteins simultaneously. When they do so, they disrupt specific signaling pathways that control the cell cycle.[2,3]

The structure-based design of a ligand that forms a calcineurin-binding cyclophilin complex with affinity greater than CsA was recently described.[4] We now report the structure-based design of several acyclic (seco) variants of FK506 (**1**), including SBL506 (**2**) (for seco bridging ligand related to FK506), which binds to the intracellular receptor of FK506 and rapamycin (FKBP12) and forms an FKBP12 complex that binds to calcineurin.[5]

Previous studies had revealed that even minor perturbations of the FKBP12–FK506 structure[6] can have a dramatic effect on calcineurin binding.[7] Nevertheless, our goal was to simplify the structure of FK506 while maintaining its ability to form a noncovalent bridge between FKBP12 and calcineurin. We chose to remove the C18 methylene in FK506's macrocyclic ring to allow the resulting "arms" of the seco variant to adopt a geometry similar to that found in the native complex. Although this methylene is not in contact with FKBP12, it is part of the FKBP12–FK506 composite surface[7b] and as such may contribute to the binding of the complex to calcineurin (Figure 1). Additional features of the SBL506 structure are highlighted in Figure 2. A *cis*-olefin was introduced to fix the orientation of the C17-methyl group relative to C15 (note Figure 1), the C13-methoxyl was removed since it sterically clashes with the C15-methoxyl in the bound conformation of FK506, and C27–C28 olefin was replaced with the corresponding saturated unit found in rapamycin with the intention of allowing the cyclohexyl unit to form the additional hydrogen bond found in the FKBP12–rapamycin complex,[8,9] and a diallyl ketone was introduced to emulate the geometry found in the C19–C22 region of FK506 (note Figure 1). In the course of our SBL506 synthesis, two simplified SBLs were also prepared

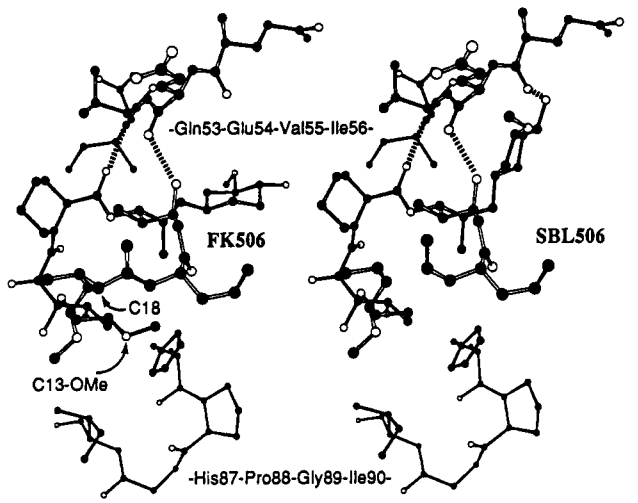

Figure 1. (Left) Portion of the FKBP12–FK506 composite surface that appears to contact calcineurin (refs 6c, 7b). Hypothetical model of the corresponding region of the FKBP12–SBL506 complex.

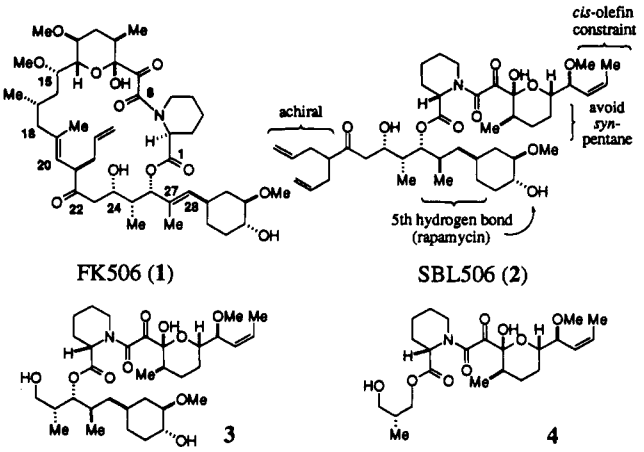

Figure 2. Structures of FK506, SBL506, truncated variants, and a summary of SBL506 design considerations.

that lacked either the diallylmethyl ketone (**3**) or this group and the cyclohexyl-containing side chain (**4**) (Figure 2).

Synthetic studies were initiated with an Evans aldol reaction[10] of **5**[11] that provided **6** in 96% yield with 15:1 selectivity (Scheme I). The aldol adduct was converted to the aldehyde **7** via the Weinreb amide.[12] A (*Z*)-olefination, selective deprotection, and oxidation provided the aldehyde **8**. An aldol reaction of **8** with methyl *O*-2,4-dimethoxybenzyl glycolate followed by an ester hydrolysis and an alcohol protection provided the carboxylic acid **9**.[13]

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(7) For example, replacing the methyl group of the C15 methoxyl of FK506 with a proton (a) or changing the Ile90 residue of FKBP12 to lysine (b) results in ca. 10^3-fold loss in affinity to calcineurin: (a) Liu, J.; Albers, M. W.; Wandless, T. J.; Luan, S.; Alberg, D. G.; Belshaw, P. J.; Cohen, P.; MacIntosh, C.; Klee, C. B.; Schreiber, S. L. *Biochemistry* **1992**, *31*, 3896–3901. (b) Yang, D.; Rosen, M. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 819–820.
(8) Van Duyne, G. D.; Standaert, R. F.; Schreiber, S. L.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 7433–7434.
(9) A referee has provided an important insight into this aspect of our design. There is a hydrogen bond between the cyclohexyl hydroxyl and the main-chain carbonyl of Gln53 in the FKBP12–rapamycin complex. If the reduced side chain of SBL506 were to adopt the conformation found in the complex, steric interactions would exist between carbons in SBL506 that correspond to C24 and C28 in FK506 (see Figure 1). We agree with this analysis and feel that it provides an avenue to further optimize the FKBP12-binding properties of designed ligands. It will be exciting to analyze this region of the ligand if attempts to determine the structure of the FKBP12–SBL506 complex are successful.
(10) Evans, D. A.; Bartroli, J.; Shih, T. L. *J. Am. Chem. Soc.* **1981**, *103*, 2127–2129. Ku, T. W.; Kondrad, K. H.; Gleason, J. G. *J. Org. Chem.* **1989**, *54*, 3487–3491.
(11) (*S*)-Methyl 3-hydroxy-2-methylpropionate (Aldrich) was protected with TBSCl, reduced with DIBAL-H (1 equiv) at –90 °C, and treated with carbomethoxymethylidene triphenylphosphorane and then magnesium metal in methanol and finally DIBAL-H to give aldehyde **5**.
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(13) Cf. Nakatsuka, M.; Ragan, J. A.; Sammakia, T.; Smith, D. B.; Uehling, D. E.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 5583–5601.

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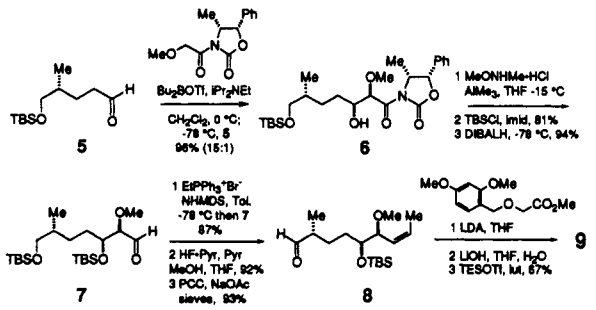
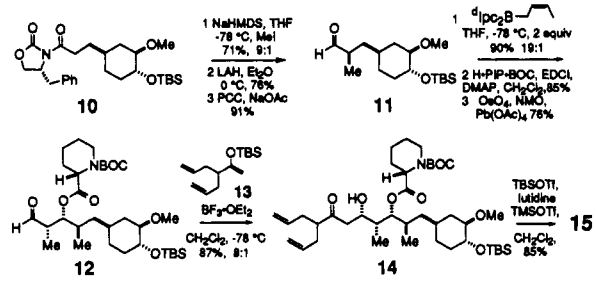
Scheme I



Scheme II



An Evans's asymmetric alkylation of the oxazolidone **10**[14] with methyl iodide (9:1, 71%)[15] was followed by a reduction–oxidation sequence to provide aldehyde **11** (Scheme II). Stereoselective addition of diisopinocampheyl (*Z*)-crotylborane (^{d}Ipc$_2$B-*Z*-crotyl) derived from (+)-α-pinene[16] to **11** provided a homoallylic alcohol (19:1, 90%) that was esterified[17] with BOC-(*S*)-pipecolinic acid and oxidatively cleaved to provide aldehyde **12**.

Both Heathcock and Evans have demonstrated that trialkylsilyl alkenyl ethers of methyl ketones add to chiral aldehydes in the presence of boron trifluoride etherate to give the *syn* diastereomer with good facial selectivity.[18] Thus, *tert*-butyldimethylsilyl alkenyl ether **13** was formed from the corresponding methyl ketone using TBS triflate and triethylamine.[19] Precomplexation of aldehyde **12** with 10 equiv of boron trifluoride etherate followed by the addition of 4 equiv of **13** yielded the anticipated β-hydroxy ketone **13** (8:1, 87%), which was converted in a one-pot protection–deprotection reaction to the amine **15**.

The coupling of **9** and **15** (1:1 stoichiometry) provided the amide **16** (Scheme III).[20] The final five steps paralleled those used in an earlier synthesis of FK506[12] and provided SBL506 (**2**) as a 1:1 mixture of rotamers and a 10:1 mixture of six-membered and seven-membered hemiketals.[21] The truncated compounds **3** and **4** were synthesized by an analogous sequence of reactions.

The binding properties of **1–4** were determined by enzyme inhibition assays[22] and are summarized in Table I. The results

Scheme III

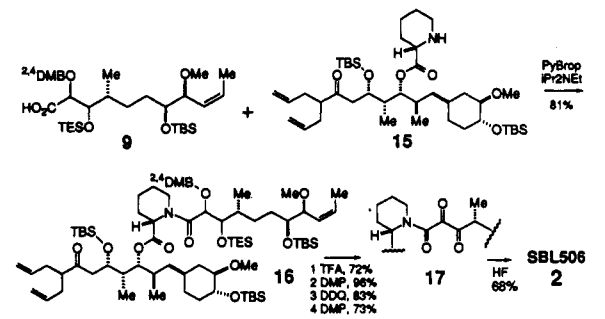


Table I. FKBP12 and Calcineurin Inhibition by SBL506, FK506, and Related Ligands

compd	K_i (nM) for FKBP12	K_i (nM) calcineurin (FKBP12 complex)
SBL506 (**2**)	207	330 ± 80
3	25	1.6×10^3
4	77	$>5.0 \times 10^4$
FK506 (**1**)	0.4	25

reveal several unanticipated properties of the synthetic ligands. First, the more complex structure SBL506 (**20** actually binds with lower affinity to FKBP12 (K_i = 207 nM) than either **3** (K_i = 25 nM) or **4** (K_i = 77 nM). This may reflect the greater entropic cost of binding SBL506 with its greater number of rotatable bonds. Presumably, several of these bonds suffer restricted rotation upon binding FKBP12. Second, the FKBP12–SBL506 complex shows appreciable binding to calcineurin (K_i = 330 nM), with an affinity that is only 13-fold lower than that of the FKBP12–FK506 complex. FKBP12–**3** and FKBP12–**4**, in contrast, show significantly diminished binding to calcineurin.

At the outset of these studies we felt that the greatest challenge would be to construct a molecule with structural elements necessary to contact calcineurin, since structures of immunophilin–ligand–calcineurin complexes have not yet been determined. The results presented here suggest that this aspect of the challenge may not be insurmountable. To create molecules that can rival the remarkable, "double-edged" molecular recognition properties of FK506 (as well as CsA and rapamycin), a better optimization of their immunophilin-binding surface will be required (for example, see discussion stimulated by a referee in footnote 9). The availability of high-resolution structures of cyclophilin A–CsA[23], FKBP12–FK506, and FKBP12–rapamycin provides a platform for pursuing this goal.

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Supplementary Material Available: Spectral data for all compounds including precursors to compounds **6** and **10** (19 pages). Ordering information is given on any current masthead page.

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