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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF MACROCYCLIC FKBP LIGANDS

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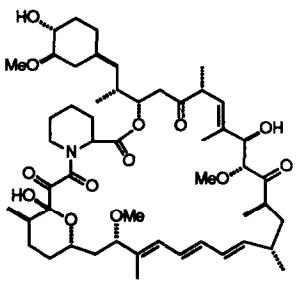
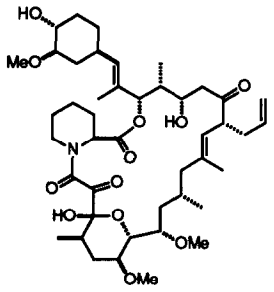
Abstract: A number of pipecolinate dilactones have been synthesized as simplified macrocyclic mimics of the binding domains in rapamycin (**1**) and FK506 (**2**). Crystallographic studies of these compounds indicate that the conformation of the pipecolinyl α-ketoamide region is preorganized for binding to FKBP. This is confirmed by the ability of these analogs to inhibit the FKBP *cis-trans* peptidyl-prolyl isomerase activity.

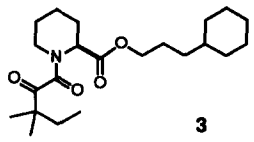
Rapamycin (**1**) and FK506 (**2**) are immunosuppressive natural products which show very promising profiles as therapeutic agents for preventing transplant rejection.[1] These macrolides interact with the cytosolic immunophilin FKBP12, potently inhibiting its *cis-trans* peptidyl-prolyl isomerase (PPIase) enzymatic activity.[2] The immunosuppressive activity of **1** and **2** originates from the resulting drug-FKBP bimolecular complexes, which block T-cell activation by interfering with two distinct cell signaling transduction pathways.[1]



1 Rapamycin



2 FK506



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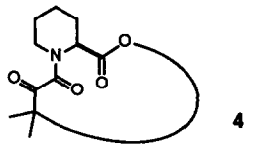

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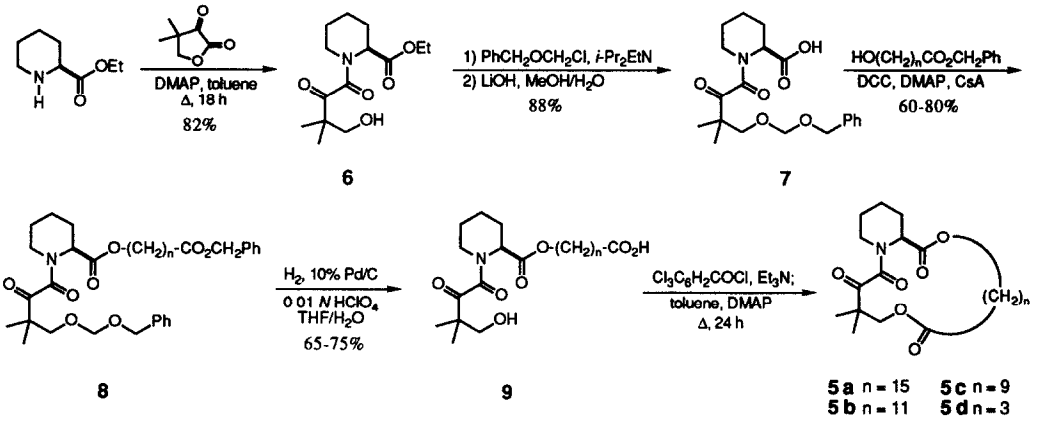
In the preceding Letter[3] we report the design of simplified pipecolinyl α-ketoamides, based on structural similarities of the binding domains from **1** and **2**, which are effective PPIase inhibitors of FKBP; the more potent analogs from the study contained a quaternary center adjacent to the ketone carbonyl, as illustrated in the *tert*-pentyl ketoamide **3**. To extend the scope of the series, we explored the incorporation of these binding domain elements into macrocyclic structures of the type shown in **4**. The objective was to use the macrocycle as a framework into which synthetic effector domains could be integrated to achieve biological activity. In this Letter, we describe some of our work on the synthesis and SAR of simplified macrocyclic FKBP ligands.

Dilactones **5** were selected as target compounds since they incorporate structural features required for FKBP affinity and are readily accessible via macrolactonization of hydroxy acids **9**. A number of different precursors **9** can be readily prepared from the common intermediate **6** by the route shown in Scheme 1.

Keto amide **6** was obtained in good yield by the DMAP-catalyzed condensation of ethyl pipecolinate with dihydro-4,4-dimethyl-2,3-furandione in refluxing toluene.[4] The primary hydroxy group in **6** was protected as a benzyloxymethyl ether and the ethyl ester hydrolyzed to **7**. The resulting carboxylic acid was then esterified with the benzyl esters of different ω-hydroxyalkanoates to provide **8**. Removal of both benzyl protecting

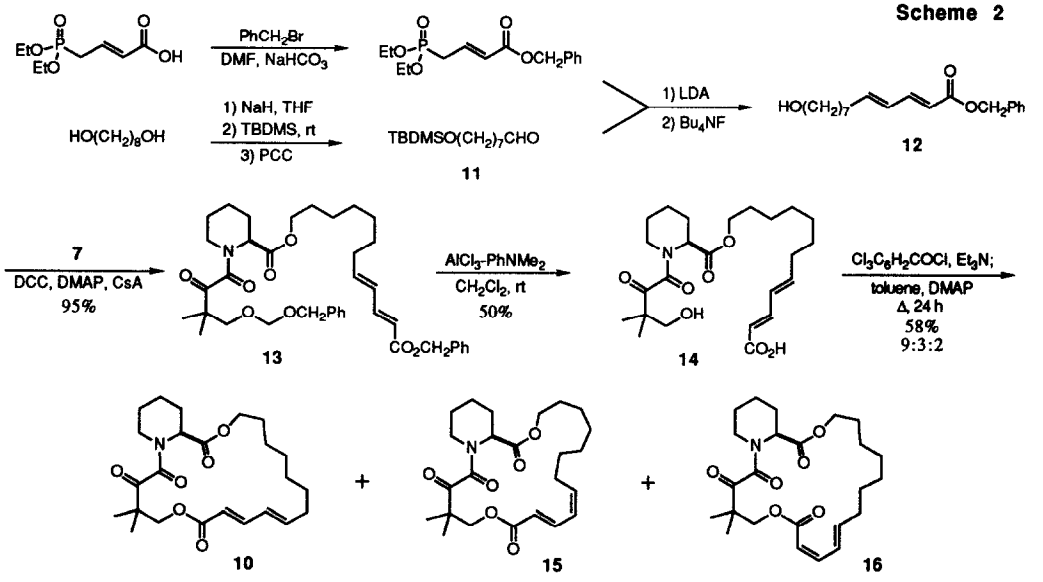
groups by catalytic hydrogenolysis[5,6] afforded the hydroxy acids **9**, which were converted to the target dilactones **5** using Yamaguchi's mixed anhydride macrolactonization conditions (2,4,6-$Cl_3C_6H_2COCl$, Et_3N, then DMAP in refluxing toluene under high dilution);[7] the yield of this reaction ranged from 50 to 55% for **5a-c**, but was low for the 13-membered lactone **5d** (only ~10%).[8]

Scheme 1



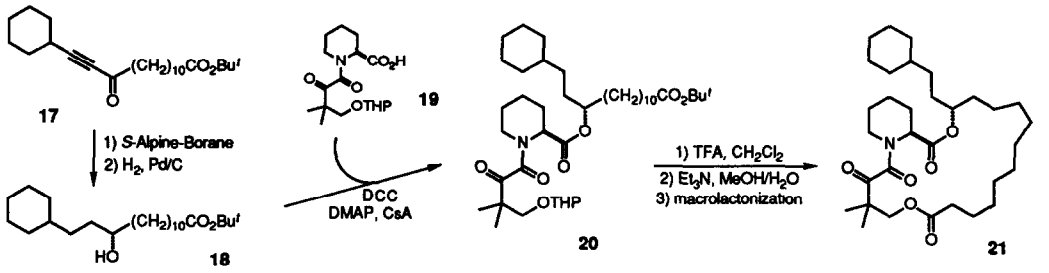
A similar reaction sequence was used for the synthesis of **10**, a dilactone containing a conjugated 1,3-diene, which was expected to mimic the conjugated trienyl function of rapamycin and impart some rigidity to the macrocyclic tether (Scheme 2). Thus, alcohol **12**, obtained from the condensation of the lithium salt of benzyl 4-(dimethylphosphono)crotonate[9] with aldehyde **11** and subsequent desilylation, was esterified with the carboxylic acid **7** to provide **13**. Removal of the benzyl protecting groups with the $AlCl_3$/*N*,*N*-dimethylaniline system[10] followed by macrolactonization of the resulting hydroxy acid **14** gave the desired dilactone **10**, along with the isomeric **15** and **16** in 58% overall yield (9:3:2 ratio of **10**:**15**:**16**). The last two compounds arise from *trans-cis* isomerization of the double bonds under the cyclization conditions (reflux in toluene for 24 h).

Scheme 2

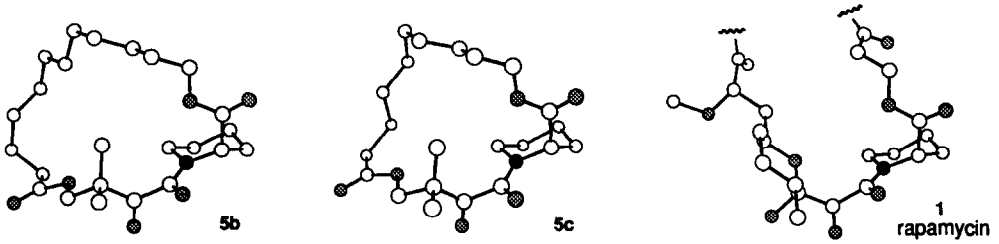


Dilactone **21**, containing a cyclohexylethyl chain with the same stereochemical arrangement as rapamycin, was also synthesized by the macrolactonization route (Scheme 3). Asymmetric reduction of the propargyl ketone **17** with (*S*)-(-)-Alpine-Borane[11] gave the *S*-alcohol in 70% yield (87% ee). Catalytic hydrogenation of this alcohol provided **18**, which was esterified with the acid **19**. Deprotection of the resulting **20** with TFA afforded the corresponding hydroxy acid, which was lactonized to **21** in 53% yield using Yamaguchi's conditions as above.

Scheme 3



The structures of dilactones **5b** and **5c** were confirmed from single crystal X-ray diffraction studies.[12] As shown below, the pipecolinate regions in both molecules have virtually identical conformational topologies, with a striking similarity to the one found in the crystal structures of rapamycin, either free[13] or bound to FKBP.[14] All the structures show the trans amide rotamer with orthogonal O=C-C=O dihedral angles and very similar torsions around the pipecolinate esters.



*Computer-generated structures from X-ray diffraction data of **5b** and **5c**, compared with the one of rapamycin[13]*

The macrocyclic dilactones synthesized in this study were found to inhibit the PPIase activity of FKBP.[15] Comparison of the potency of compounds **5** (Table) show that **5b** and **5c** (21 and 19-membered ring, respectively) show higher affinity for FKBP than the smaller or larger ring analogs; the L-configuration of the pipecolinate ring is required for PPIase inhibitory activity. The presence of the conjugated diene in **10** and **15** resulted in a loss of inhibitory potency when compared with **5b**. Finally, the cyclohexylethyl side chain in **21** did not promote an increase in inhibitory potency of the parent compound **5b**, as found for the nonmacrocyclic analogs described in the preceding Letter.

Table. Inhibition of the PPIase activity of FKBP by synthetic macrocyclic dilactones

compound	$K_{i,app}$ (μM)	compound	$K_{i,app}$ (μM)	compound	$K_{i,app}$ (μM)
DL-**5a**	11000	DL-**5c**	90	L-**10**	840
DL-**5b**	290	L-**5c**	30	L-**15**	340
L-**5b**	100	DL-**5d**	2900	L-**21**	190

In summary, a number of simplified macrocyclic FKBP ligands, based on the binding domains from rapamycin and FK506, have been synthesized and found to exhibit a range of PPIase inhibitory activity. X-ray studies of two of these compounds revealed that the pipecolinyl α-ketoamide subunits are prearranged in a conformation which should exhibit optimum interaction with FKBP. Structural information derived from these compounds provides valuable insight for the incorporation of synthetic effector domains that should ultimately result in immunosuppressive activity.

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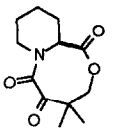
6. Hydrogenolysis of **8** with Pd/C in EtOH resulted in rapid deprotection of the benzyl ester. Hydrogenolysis of the BOM group only took place in the presence of diluted $HClO_4$.
7. a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989-1993. b) Tone, H.; Nishi, T.; Oikawa, Y.; Hikota, M.; Yonemitsu, O. *Chem.Pharm. Bull.* **1989**, *27*, 1167-1172.
8. Generation of medium-sized rings is usually more difficult than that of larger rings. In the case of **9** (n = 3), the lactonization to **5d** was sluggish and a small amount of eight-membered lactone **i**, product of attack of the primary alcohol to the pipecolinate ester, was also produced.



i

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12. The crystal structure of **5b** has been reported in reference 3b. Crystal data for **5c**: Ambient temperature data (295°K) on a crystal (0.80 x 0.65 x 0.20 mm) grown from acetone/acetonitrile and the structure solved in $P2_1$, monoclinic #4 (a= 11.572(5) Å, b=10.141(7) Å, c=19.529(6) Å, β=99.11(3)°, V=2262.6(10) $Å^3$, Z=4, d_{calc}=1.202 g/cm^3). A quadrant of data ($2\theta \leq 56^o$) were collected on an Enraf Nonius CAD4 diffractometer using variable speed ω-2θ scans and graphite monochromated molybdenum radiation yielding 5757 unique reflections (R_{int} = 0.026) which were corrected for Lorentz and polarization factors as well as for an isotropic decay. Full matrix least-squares refinement with 2235 observations ($I \geq 3\sigma(I)$) led to conventional crystallographic residuals of R = 0.062 and Rw = 0.081 with S = 1.648. Both crystallographically independent molecules display trans amide bonds. They differ somewhat in conformational detail (up to 15° in torsion angles) about the diketone moiety and the pipecolate ester.
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15. Apparent inhibition constants of the FKBP PPIase activity ($K_{i,app}$) were employed as estimates of ligand binding potentials. These values were determined as described in ref 3b.

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