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Stereoselective Synthesis of Rapamycin Fragment To Build a Macrocyclic Toolbox

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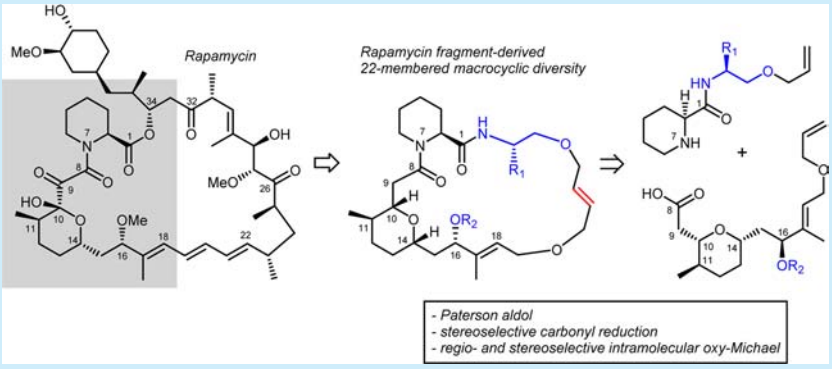
Supporting Information



ABSTRACT: A stereoselective synthesis of a rapamycin fragment is developed and further utilized toward building a macrocyclic chemical toolbox. The amino alcohol moiety embedded in the 22-membered macrocyclic ring allowed for the addition of a variation in the chiral side chain. The key reactions leading to the synthesis of the rapamycin-derived pyran fragment include the following: (i) Paterson aldol, (ii) stereoselective β-OH carbonyl reduction, and (iii) regio- and stereoselective intramolecular oxy-Michael reaction. The other piece needed for building the macrocyclic diversity was obtained from the coupling of various amino alcohol moieties with *S*-pipecolic acid.

Rapamycin (also known as sirolimus, **1**, Figure 1), a secondary metabolite, was first isolated from soil bacterium *Streptomyces hygroscopicus* at Easter Island (Rapa Nui) in the 1970s.[1] The pharmaceutical potential of rapamycin was originally discovered in a screen for novel antifungal agents. Later, it was found to be an antibiotic and immunosuppressant and has been used for several years to prevent rejection in organ transplantation.[2] Currently, it is approved for the treatment of cardiovascular diseases by the US Food and Drug Administration (FDA).[3] Rapamycin inhibits the activity of the mammalian target of rapamycin (mTOR) and interacts in the mammalian cells with the immunophilin FKBP12, and the FKBP12−rapamycin complex allosterically inhibits mTORC1 kinase activity by an unknown mechanism.[4] mTOR is an intracellular serine/threonine protein kinase that has a central role in various cellular processes, including cell growth and proliferation, protein synthesis, and autophagy.[5] In addition to this, the proliferative properties of rapamycin and its analogues are being extensively studied to search for promising anticancer agents.[6] Multiple studies have also shown that rapamycin can provide therapeutic benefits in experimental models of several age-related neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease.[7] Several studies have demonstrated that rapamycin is able to extend the lifespan in various species, including mice.[8] Because of the multiplicity of the mTOR downstream signaling pathway, different molecular mechanisms have been proposed for obtaining a better understanding of various biological functions of rapamycin.[9]

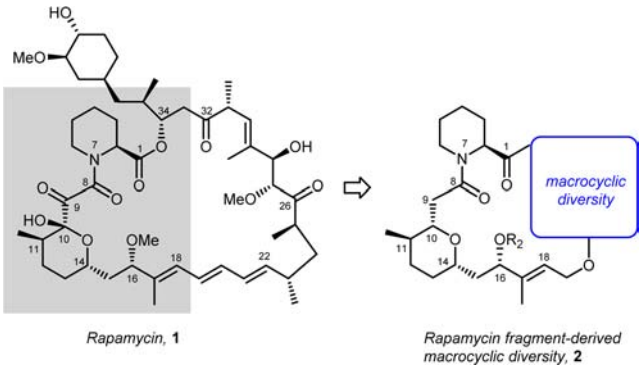


Figure 1. Our plan to build macrocyclic diversity based on the rapamycin fragment.

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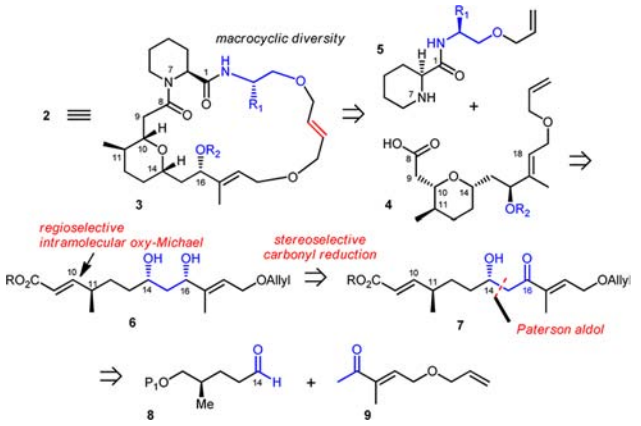
Because of these extensive biological properties, serious attention of the scientific community has been captured toward developing synthesis methods to obtain various rapamycin analogues and rapamycin-derived hybrid molecules.[10] In many cases, these analogues as well as hybrid compounds have shown promising results toward the binding to FKBP12 and its impact on cancer and neurodegenerative diseases.[3a,11] The remarkable biological properties of rapamycin and rapamycin-derived hybrid compounds also attracted our attention in developing the diversity-based synthesis of a new family of hybrid macrocyclic compounds. Shown in Figure 1 is our generic macrocyclic structure **2** that utilizes a rapamycin fragment having a stereodefined pyran moiety. In our diversity-based design strategy, we decided to keep the simplified version of the pyran ring of rapamycin lacking the −OH group at C-10 (replaced by C10−H) and the carbonyl moiety at the C-10 side chain. These two changes were planned to simplify our synthetic approach reaching the target. Our first goal was to develop a short and practical synthesis of this subunit shown in **2** and then to utilize this to building the macrocyclic diversity.

Our precise synthesis target (**3**) is shown in Scheme 1. In our design and synthesis approach, we divided **3** into two sections,

Scheme 1. Our Plan to Build Macrocycles Based on the Rapamycin Fragment

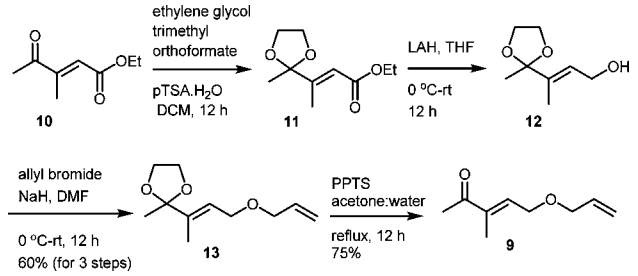


4 and **5**. Fragment **5** has a diversity site (R_1), and it can be easily obtained from *S*-pipecolic acid coupled to various amino alcohols having a terminal olefin. Following the coupling of **5** and **4** through an amide bond, we rely upon the ring-closing metathesis as the key 22-membered macrocyclic stitching reaction. The crucial pyran fragment **4** can be obtained from **6** by a regio- and stereocontrolled intramolecular oxy-Michael reaction. The 1,3-*syn*-dihydroxyl groups can be accessed by a stereoselective reduction of the *β*-OH keto derivative **7**. Finally, we envisioned the synthesis of **7** using Paterson's facial-selective aldol reaction giving us the starting materials **8** and **9**.

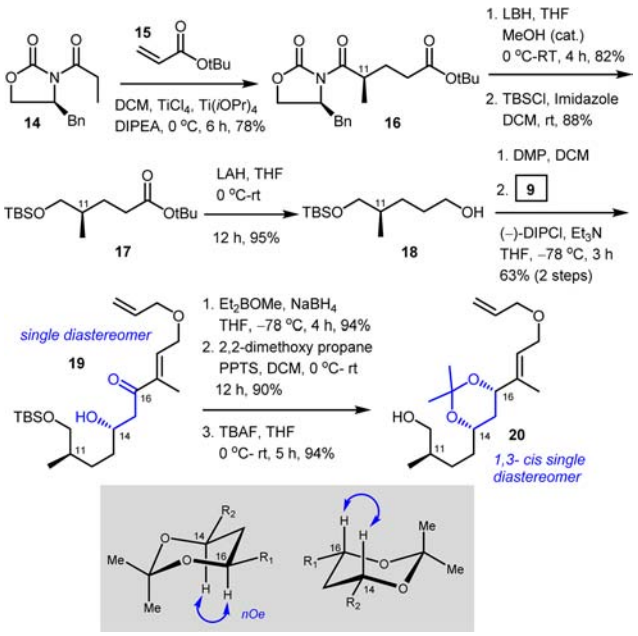
Our synthesis plan to obtain the keto fragment **9** is shown in Scheme 2. (*E*)-Ethyl 3-methyl-4-oxopent-2-enoate[12] **10** was subjected to keto protection (**11**) and ethylene glycol and then subjected to carboxyl ester reduction to give **12**. This upon allylation followed by the keto deprotection produced **9** in good yields.

The chiral aldehyde moiety **8** (P_1 = TBS) needed for the aldol reaction was obtained using Evans' Michael addition-based approach using *tert*-butyl acrylate **15** with oxazolidinone, **14** (Scheme 3).[13] The removal of an auxiliary led the synthesis of a primary alcohol which was then protected with TBS to

Scheme 2. Synthesis of the Keto Fragment 9



Scheme 3. Synthesis of the Key Intermediate 20



provide **17**. The *tert*-butyl ester was subjected to reduction with LAH to give **18**, which was then oxidized with DMP to yield aldehyde **8** (P_1 = TBS).[14] With freshly prepared aldehyde, we then subjected this to a *diastereoselective* aldol reaction with the keto fragment **9** using (−)-DIPCl-based Paterson aldol protocol.[15] This reaction worked very well and provided the *β*-hydroxyl carbonyl compound **19** in good yield as the *single diastereomer* (HPLC purity >94%). The *β*-hydroxyl carbonyl compound **19** was then treated with $NaBH_4$ in the presence of Et_2BOMe for a 1,3-*syn*-stereoselective *β*-hydroxyl carbonyl reduction[16] yielding *syn*-1,3-diol which was then protected with 2,2-dimethoxypropane. Finally, it was then treated with TBAF to yield the primary alcohol as the key intermediate **20**. At this stage, the relative stereochemistry of *cis*-1,3-diol (**20**) was assigned by 2D-NOESY experiments (see Scheme 3).

Our approach to regio- and stereocontrolled intramolecular oxy-Michael reaction is shown in Scheme 4.[17] Compound **20** was oxidized to aldehyde with DMP and then subjected to Horner−Wittig reaction to obtain *α*,*β*-unsaturated ethyl ester **21** in good yield.[18] The 1,3-diol was deprotected with PPTS and further treated with potassium *tert*-butoxide, which afforded a smooth intramolecular oxy-Michael cyclization giving **22** in 72% yield as a *single diastereomer*. The stereochemistry of the 2,6-*cis*-tetrahydropyran moiety (**22**) was assigned through the coupling constant of proton at C-10 and 2D-NOESY experiments. The details of the synthesis and the structural assignments are provided in the Supporting Information.

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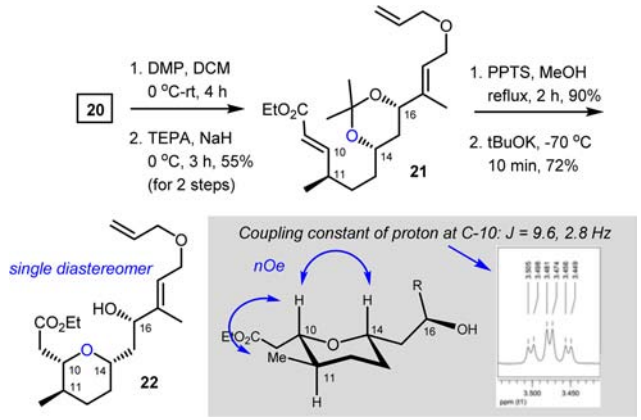
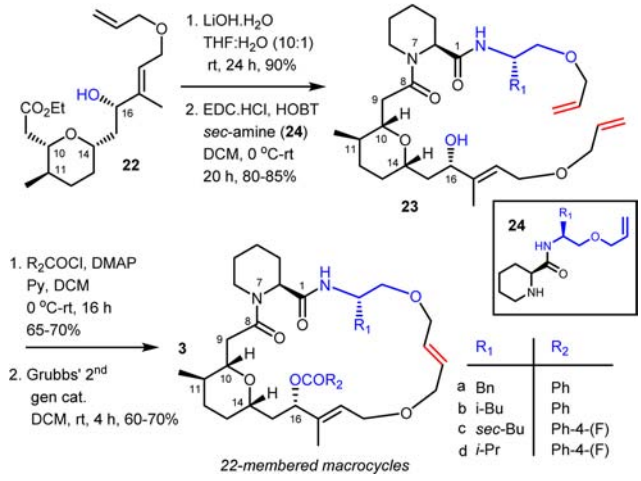
Scheme 4. Synthesis of 2,6-*cis*-Tetrahydropyran Scaffold 22



The *enantiopure* 2,6-*cis*-tetrahydropyran moiety (**22**) was hydrolyzed with LiOH to obtain the carboxylic acid, which was then coupled with four secondary amines **24** (Scheme 5) using

Scheme 5. Synthesis of Rapamycin Fragment-Derived Macrocycles 3a−d



EDC·HCl and HOBT as the coupling reagents to obtain **23**.[19] The synthesis details (with R_1 as the first diversity site) are provided in the Supporting Information. This was then treated with acid chloride (as the second diversity site) to obtain the ring-closing metathesis precursor. Finally, the stage was set to try a crucial ring-closing metathesis stitching reaction for obtaining 22-membered ring macrolides. To our delight, the RCM approach gave the macrocyclic product (**3**) in all four cases in good yields and with only *E*-olefin geometry across the double bond.[20] The synthesis details and full characterization are provided in the Supporting Information.

As a test study, the molecular docking studies were performed to predict the key binding interactions of our rapamycin fragment-derived hybrid macrocycles with human immunophilin FKBP-12 and FKBP12-rapamycin associated protein complexed with human immunophilin. The docking studies predicted a good binding interactions of rapamycin analogues with both the target proteins (see Figure 2). The detailed information on this study is provided in the Supporting Information.

All our macrocyclic compounds occupy the binding pockets of proteins (see Figure 2A,B). Compound **3d** showed good

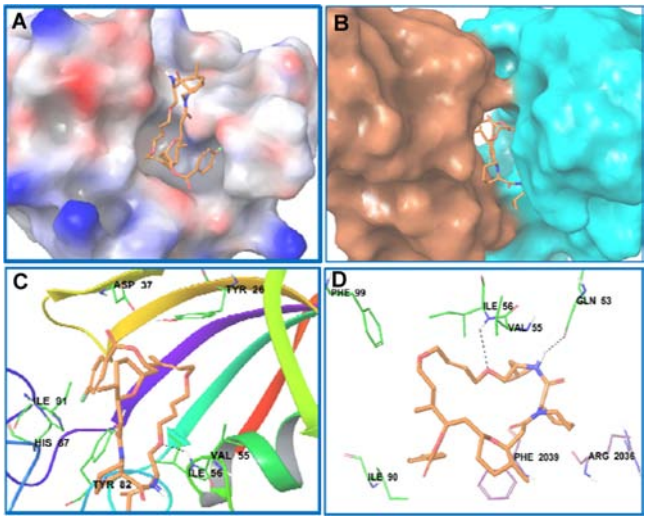


Figure 2. (A) **3d** at the binding pocket of human immunophilin FKBP-12. (B) Binding of **3d** with human immunophilin FKBP-12 and FKBP12-rapamycin associated protein complex. (C) Interactions of **3d** with the rapamycin binding site of human immunophilin FKBP-12. (D) Interactions of **3d** with human immunophilin FKBP-12 and FKBP12-rapamycin associated protein complex.

binding with both protein targets. It interacts with Ile-56 (H-Bond), His-87 ($\pi-\pi$ interaction), and A:Ile-56, A:Gln-53 (H-bond) with human immunophilin FKBP-12 and FRAP-human immunophilin complex, respectively (Figure 2C,D). In the case of human immunophilin FKBP-12 and FKBP12-rapamycin associated protein complex, Ile-56 is a common hydrogen-bonding residue except in the case of **3b**. Detailed information on the binding interactions, glide score, and contributing XP parameters of macrocycles (**3a−d**) docking with the human immunophilin FKBP-12 and human immunophilin FKBP12-FRAP complexes is provided in the Supporting Information.

In summary, we developed a practical, stereoselective synthesis of rapamycin fragment-derived pyran moiety which was further utilized in building 22-membered macrocycles. The biological studies with these compounds are in progress and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental section and spectral data are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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