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Design and synthesis of peptide-based macrocyclic cyclophilin inhibitors

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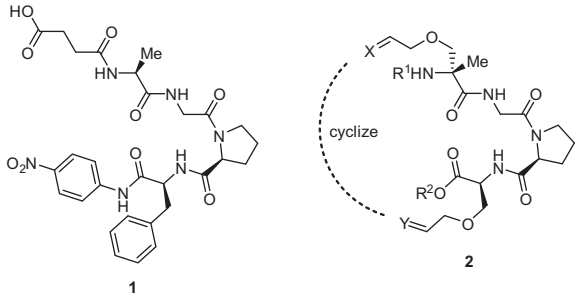
ABSTRACT

The efficient assembly of an 18-membered macrocyclic peptide core was realized by a straightforward and convergent approach utilizing ring-closing metathesis of the corresponding linear tetrapeptides as the key transformation. This approach allowed for the facile preparation of a focused library of novel macrocycles that culminated in the discovery of a cyclophilin A inhibitor with a K_d = 5.4 μM.

cis,trans-Peptidyl-prolyl isomerases (PPIases) are ubiquitous enzymes that catalyze the isomerization of prolyl peptide bonds from the thermodynamically unfavorable *cis*-conformation to the preferred *trans*-orientation.[1] The PPIases are subdivided into three families including the cyclophilins (Cyps), FK506-binding proteins (FKBPs), and parvulins.[2] The Cyps and FKBPs are collectively termed immunophilins owing to their affinity towards the immunosuppressant agents cyclosporine A (CsA)[3] and FK506,[4] respectively. Although macrocyclic ligands of the Cyps which possess potent immunosuppressive activities are known, these enzymes have also been proven as essential host factors for the replication of HIV[5] and HCV.[6] This has triggered the development of various non-immunosuppressive Cyp inhibitors for the treatment of HCV infection, however there are currently no drugs approved for this indication.[6b]

The linear tetrapeptide suc-Ala-Gly-Pro-Phe-*p*NA (**1**) is a well-known cyclophilin A (CypA) inhibitor with a K_d = 135 ± 20 μM[7] that is commonly used as a substrate in the chymotrypsin-coupled PPIase activity assay (Fig. 1).[8] Additionally, the structure of **1** bound to human CypA has been reported (PDB code: 1ZKF).[9] We thus sought to utilize **1** as a starting point for the design of macrocyclic tetrapeptide Cyp inhibitors. Notably, macrocyclic compounds often display more favorable physical properties, such as permeability, when compared to their linear counterparts.[10] Furthermore, macrocyclization can result in a more entropically favorable binding event due to ligand pre-organization.[11] To this end we designed compound **2** with functionalized serine residues containing reactive groups X and Y. We hypothesized that ring-closing metathesis would enable the synthesis of 18-membered macrocyclic peptides related to **1** and thus decided to target an analog of linear tetrapeptide **2** containing pendant alkene functionality (X and Y = CH_2) and orthogonal protecting groups at R^1 and R^2. Notably, among macrocyclic compounds produced in nature, 18-membered rings are particularly frequent.[12] Synthetically, we planned to first prepare two functionalized serine residues and stitch the peptide together via standard coupling techniques.



1 2

Figure 1. The design of macrocyclic peptides for Cyp inhibition.

The synthesis of the tetrapeptide began with the construction of the substituted serine derivatives. Accordingly, the known protected L-serine **3**[13] was alkylated under Tsuji–Trost conditions to deliver allyl serine **4** in 79% yield (Scheme 1).[14] The *tert*-butyl

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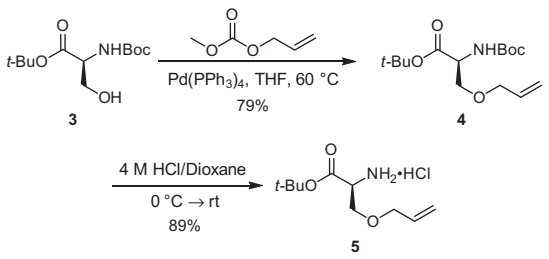
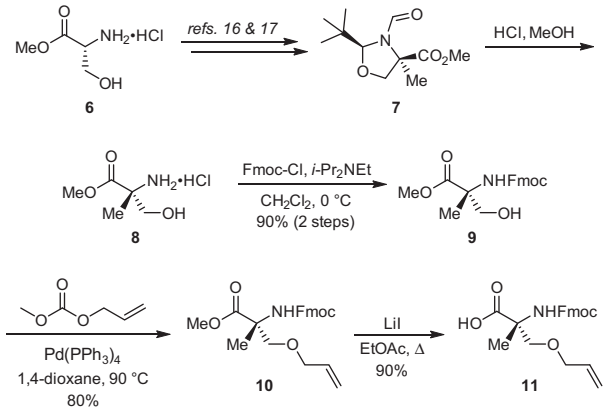
carbamate in **4** could be selectively cleaved over the *tert*-butyl ester utilizing the procedure of Han and co-workers to give amine salt **5** in 89% yield.[15]



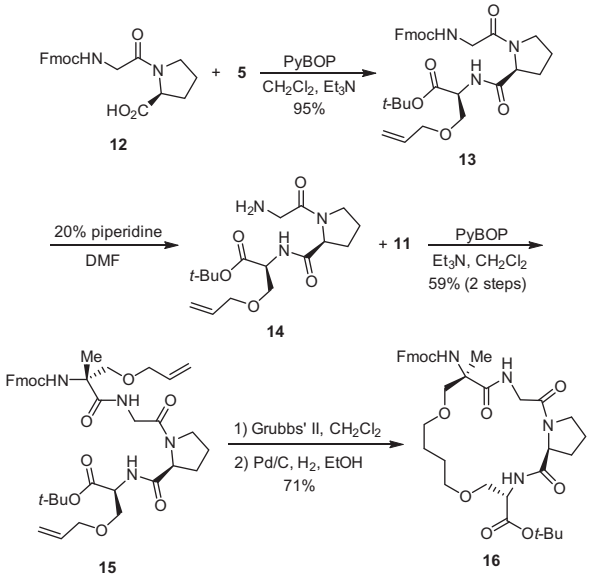
Scheme 1. Synthesis of allyl serine **5**.

The tetrasubstituted allyl serine **11** was prepared in 6 steps from D-serine methyl ester hydrochloride (**6**) (Scheme 2). Condensation of **6** with pivaldehyde, followed by formylation with acetic formic anhydride provided an intermediate ester that was subsequently methylated in a diastereoselective fashion to give the known oxazolidine **7**.[16,17] Treatment of **7** with anhydrous hydrogen chloride in methanol delivered the tetrasubstituted D-serine hydrochloride **8**, which was then protected as its 9-fluorenylmethyl carbamate **9** in 90% yield over the two steps. Allylation of **9** with the π–allyl cation complex formed from the reaction of allyl methyl carbonate with $Pd(PPh_3)_4$ gave allyl serine **10** in 80% yield. Notably, this transformation required an increased reaction temperature as compared to that of serine **3**, presumably due to the relatively hindered nature of the primary hydroxyl group in **9**. Finally, cleavage of the methyl ester in **10** with lithium iodide in refluxing ethyl acetate generated the desired tetrasubstituted allyl serine **11** in 90% yield. Attempts to hydrolyze the methyl ester in **10** with lithium hydroxide resulted in concomitant cleavage of the Fmoc carbamate, while reactions of **10** with boron trichloride resulted in allyl group cleavage to give predominately alcohol **9**. Importantly, ^{1}H and ^{19}F NMR analysis of the Mosher's amides derived from amines **5** and **8** revealed that each amine was >95% enantiomerically pure.[18]



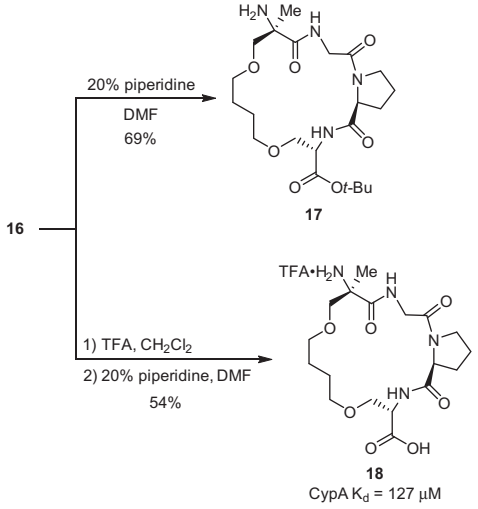
Scheme 2. Synthesis of tetrasubstituted allyl serine **11**.

With fragments **5** and **11** in hand, we sought to complete the synthesis of the requisite linear tetrapeptide. Accordingly, the known Gly-Pro fragment **12**[19] was coupled with amine **5** under standard conditions to deliver the tripeptide **13** in 95% yield (Scheme 3). Cleavage of the Fmoc carbamate in **13**, followed by coupling of the resultant amine **14** with acid **11** afforded the key linear tetrapeptide **15** in 59% overall yield from **13**. We were pleased to find that treatment of **15** with Grubbs' 2nd generation catalyst proceeded smoothly to give a mixture of *E*/*Z* olefin isomers that upon hydrogenation afforded the 18-membered macrocycle **16** in 71% yield.



Scheme 3. Macrocyclization of linear tetrapeptide **15**.

With facile access to macrocycle **16** we began to unmask the orthogonal protecting groups in order to further derivatize. Accordingly, treatment of **16** with piperidine in DMF delivered the amine **17** in 69% yield (Scheme 4). Alternatively, removing the *tert*-butyl ester first by reaction of **16** with TFA gave an intermediate acid, that could be further deprotected to give amino acid **18** in 54% overall yield. Notably, in a time resolved FRET CypA competition assay compound **18** had a K_d = 127 μM.[20] At this juncture, we designed and synthesized a focused library of macrocycles for testing in this assay.



Scheme 4. Synthesis of amine **17** and amino acid **18**.

Acylation or reductive alkylation of amine **17** with acid chlorides or aldehydes, respectively, followed by cleavage of the *tert*-butyl ester led to the formation of amides **19**–**22** and amines **23**–**29** (Scheme 5, Table 1). Amines **23**–**29** were most conveniently isolated as their hydrochloride salts. Our library also included amide derivatives of the acid moiety in **18**, which were constructed