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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.

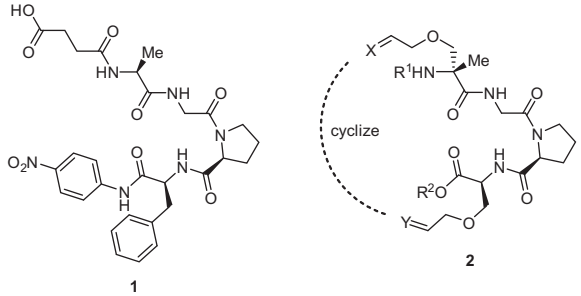

Figure 1. The design of macrocyclic peptides for Cyp inhibition.

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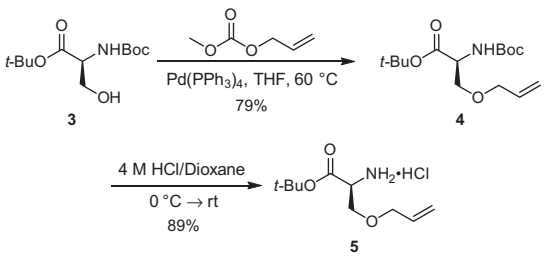
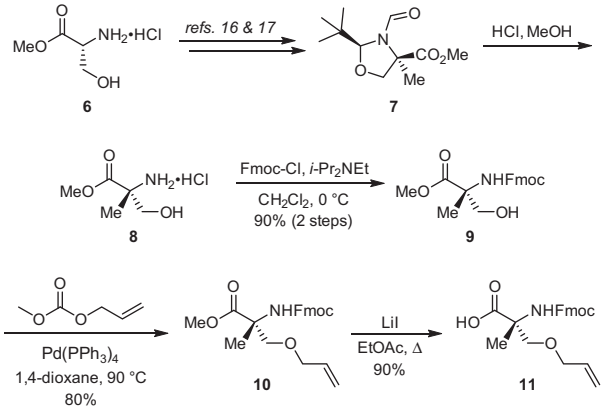
carbamate in **4** could be selectively cleaved over the *tert*-butyl ester utilizing the procedure of Han and co-workers to give amine salt **5** in 89% yield.[15]



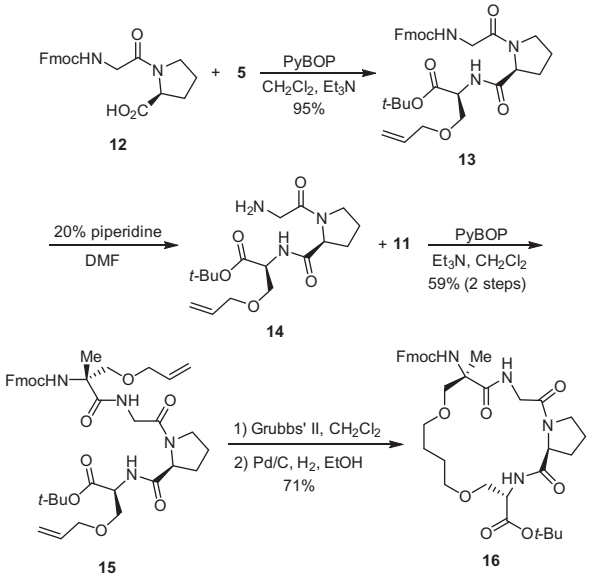
Scheme 1. Synthesis of allyl serine **5**.

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, ^1H 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]



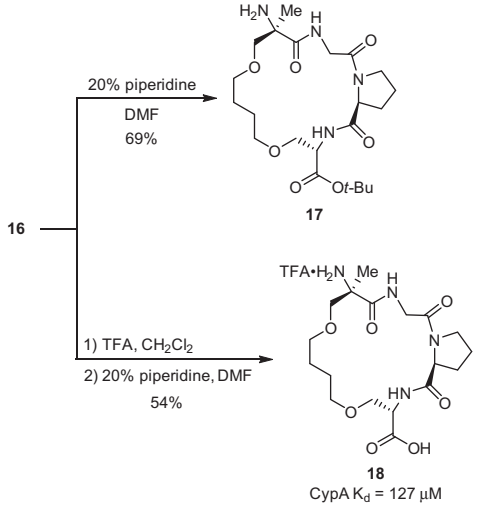
Scheme 2. Synthesis of tetrasubstituted allyl serine **11**.

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**.

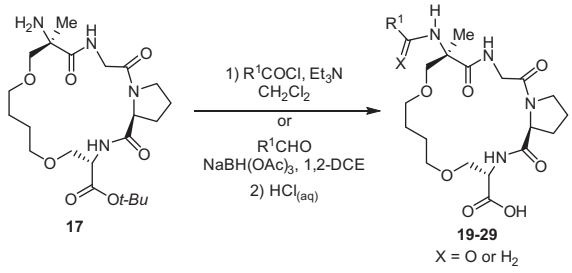
With facile access to macrocycle **16** we began to unmask the orthogonal protecting groups in order to further derivatize. Accordingly, treatment of **16** with piperidine in DMF delivered the amine **17** in 69% yield (Scheme 4). Alternatively, removing the *tert*-butyl ester first by reaction of **16** 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

using standard coupling conditions, however these amides did not exhibit appreciable CypA inhibition.

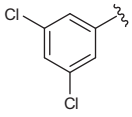
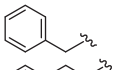
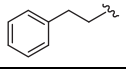


Scheme 5. Synthesis of macrocycles **19**–**29**.

Compounds were tested for CypA activity in a time-resolved FRET assay, and the results are summarized in Table 1. The positive control in this assay was cyclosporine A (CsA) which displayed a K_d = 10 nM. With the exception of the compact hydrophobic cyclobutyl analog **21**, amides did not show any appreciable CypA binding in this assay. As exemplified by compounds **19**, **20**, and **22** short or long chain alkyl amides and substituted aromatic amides were not active.

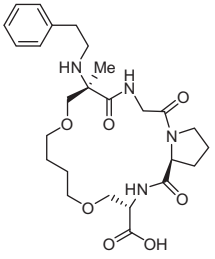
Amines that were formed through reductive amination generally displayed greater affinity towards CypA. With a one carbon linker, the K_d value increased significantly from cyclopentyl (compound **23**) to cyclohexyl (compound **24**) and finally cyclooctyl (compound **25**). Interestingly the pyran **26** and benzyl substituted compound **27** were completely inactive in this assay. Furthermore, the phenethyl analog **28** showed a K_d = 10.5 μM, while having a three carbon linker as in **29** was detrimental to activity. Taken together, this data supports that CypA binding is driven by hydrophobic interactions, and even small structural variations can disrupt this relationship.[21]

Table 1
Inhibition of CypA by macrocyclic peptides

Compound	X	R^1	CypA K_d (μM)[a]
CsA			0.010
18	N/A	H	127
19	O	Me	>600
20	O		>600
21	O		139
22	O		>600
23	H_2		>600
24	H_2		32
25	H_2		5.4
26	H_2		>600
27	H_2		>600
28	H_2		10.5
29	H_2		>600

[a] Values are the average of n = 2.

The physiochemical properties of this focused library of compounds were evaluated, and were generally favorable, however these macrocycles invariably suffered from poor permeability as exemplified by analog **28** (Fig. 2). Compound **28** has a log*D* value of −1.8 and excellent aqueous solubility. Notably, compound **28** does not bind to human plasma proteins, and has a very low rate of hepatic clearance. Finally, although these compounds are relatively weak CypA binders, they show good selectivity across a wide array of targets (CEREP panel 100+ targets greater than 100 μM).



28
CypA K_d = 10.5 μM
logD = -1.8
Sol(aq) > 1000 μM
Caco2 (A to B Papp $1x10^{-6}$ cm/s) = 0.10
Caco2 (B to A Papp $1x10^{-6}$ cm/s) = 0.17
hppb (% free) = 100%
hClint mic. (μL/min/mg) < 3
Secondary pharm > 100 μM @ 100+ targets

Figure 2. Physiochemical properties of **28**.

We have discovered a novel series of peptide-based macrocyclic CypA inhibitors which were synthesized by a straightforward and convergent route utilizing RCM of the corresponding linear tetrapeptide **15** as the key transformation. A focused library of compounds was prepared which demonstrated that small to medium hydrophobic groups increase affinity for CypA. These compounds generally possess favorable physiochemical properties, and display an excellent selectivity profile, but tend to suffer from poor permeability.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.09.039.

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