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Synthesis of 12-membered macrocyclic templates and library analogs for PPI

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ABSTRACT

We report novel syntheses of 12-membered macrocyclic templates and a library of 4000 macrocyclic analogs. The key macrocyclization step was performed at up to 100 g scale without resorting to syringe pumps, flow reactors or large volumes of solvent. An interesting observation of considerably different permeability properties was made on diastereomeric analogs due to differences in intramolecular hydrogen bond interactions.

Introduction

Protein–protein and peptide–protein interactions (PPI collectively) are the basis for the majority of regulatory pathways in biological systems. As such they represent important targets of intervention for the discovery of novel therapeutics. However, PPIs are inherently complex, multivariate, and poorly understood.[1] The surface interactions tend to be large and uncontoured in relation to small ligand–protein interactions; the binding partner can be anything from large globular and/or intrinsically unstructured proteins to a well-defined extruding peptide loop, tail, or small peptide; and the binding affinity for the endogenous interaction can range from femto- to millimolar. High-throughput screening has generally been unsuccessful in identifying lead matter for PPIs due to the rule-of-five centric existing screening collection.[2] Mutational studies however suggest that 'hotspots' exist at the binding interface, and small molecules with a more extended reach than those typically found in a screening file are more likely to bind productively.[3]

As part of our initial attempt to bridge the gap between what is currently available in the screening compound collection and that which is required to be successful in generating leads for PPI inhibition, we embarked on an effort to generate libraries of macrocyclic analogs. Herein we report the syntheses of 12-membered macrocylic templates (**1**) and a library of macrocyclic analogs (**2**) as shown in Scheme 1.[4] Overall 16 templates (**1**) were synthesized incorporating different amino acids into the ring and 3987 final analogs (**2**) were elaborated using amide, sulfonamide, heteroaryl or simple amine capping groups (R).

This type of 12-membered macrocylic skeleton has been reported in the literature. The synthesis of four apicidin analogs (**3**) in mg scale was reported by Ladlow and coworkers as shown in Figure 1.[5] These analogs all have a cyclic amino acid in the ring skeleton, proline or piperidine carboxylic acid, which led to an entropic factor favoring the ring formation. The cyclization at milligram scale was reported to be boosted by incorporating a (2-nitrophenyl)sulfonyl group (nosyl) as a 'back bone hinge' on the nitrogen atom opposite to the ring closing amidation point. This key step was carried out at a concentration as high as 20 mg/mL (~20 mM), as opposed to the less than 1 mg/mL typical for solution phase macrocyclization.[6] Detailed experimental procedures of this effort are not available in the two peer reviewed publications.[5a,b] The macrocyclization was performed at no more than 100 mg scale according to the published patent application. [5c]

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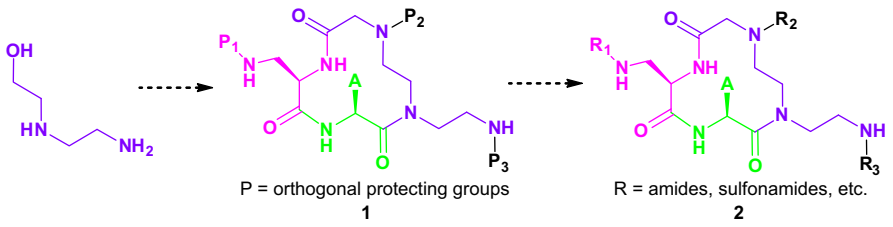


Scheme 1. Templates and library analogs of 12-membered macrocycles.

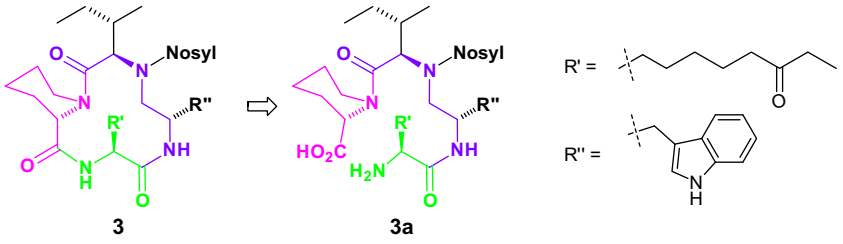
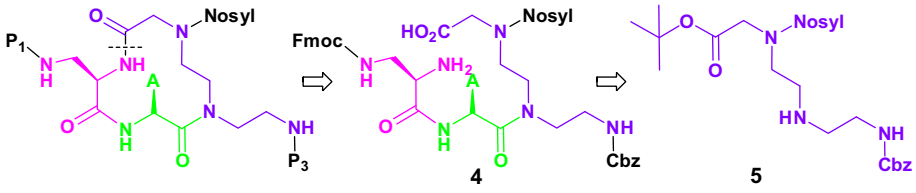


Figure 1. Macrocyclization for apicidin analogs.



Scheme 2. Retrosynthesis of macrocyclic templates.

We decided to adopt the same nosyl protecting group for P2 in our templates (**1**, P2 = nosyl). Our cyclization would not have the entropy advantage of a cyclic amino acid residue in the ring system. In addition, we would have to form the ring via activation of the carboxylic acid of glycine, instead of the diamino propionic acid (DAP) which corresponds to the cyclization point used by Ladlow and coworkers. This represented an unprecedented cyclization approach but necessary due to concerns of potential racemization associated with activation of chiral amino acids.[7] At our scale, racemization could represent particularly troublesome purification consequences. Furthermore, even at the high 20 mg/mL literature concentration, we would require ~80 L of solvent for the cyclization step of each of the 16 templates at 100 g scale. For practical considerations, we would have to develop conditions to further reduce the amount of solvents required for the cyclization.

Once settled on activation of the glycine residue for the key cyclization step and nosyl protecting group for P2, retrosynthetic analysis then led us to the cyclization substrate **4** and a triamine carboxylate intermediate **5** as indicated in Scheme 2. Intermediate **5** would serve as a common precursor from which standard amino acid chemistry would be applied to generate the macrocyclization substrates of all 16 templates, thus maximizing synthesis efficiency via synergy.

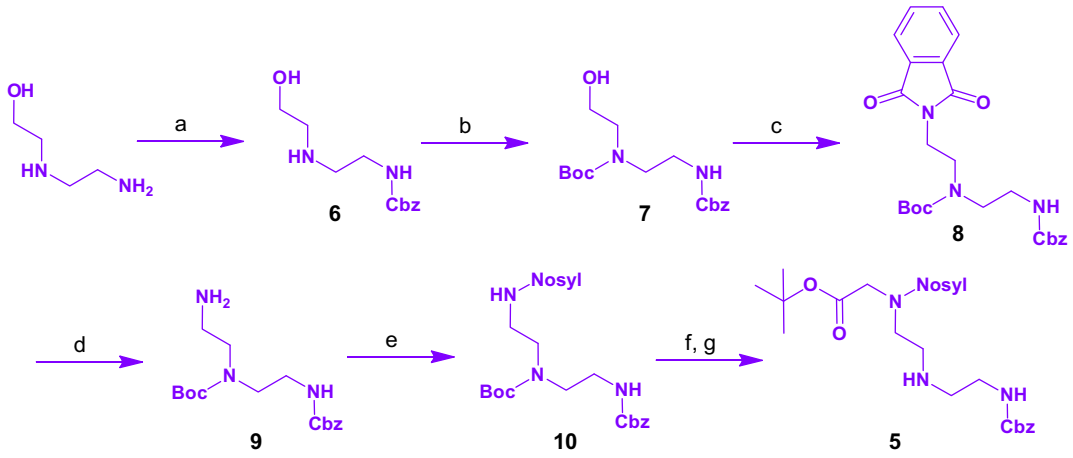
Results and discussion

Common intermediate **5** was synthesized using solution phase chemistry following Scheme 3.[8] Cbz protection of hydroxyethyl ethylene diamine[9] at the terminal amine followed by Boc protection and Mitsunobu reaction led to compound **8**. Hydrazinolysis followed by the installation of the nosyl group, selective alkylation of the nosyl nitrogen atom and selective removal of Boc in the presence of a *t*-butyl ester using a 1:5 mixture of TFA/DCM[10] led to intermediate **5**. This seven-step sequence required only one column chromatography purification (step f) and furnished about 8 kg of intermediate **5** in ~20% overall yield, using roughly 8 kg of hydroxyethyl ethylene diamine. Compounds **8**, **10** and **5** in this scheme are novel entities and have never been reported in the literature to the best of our knowledge.

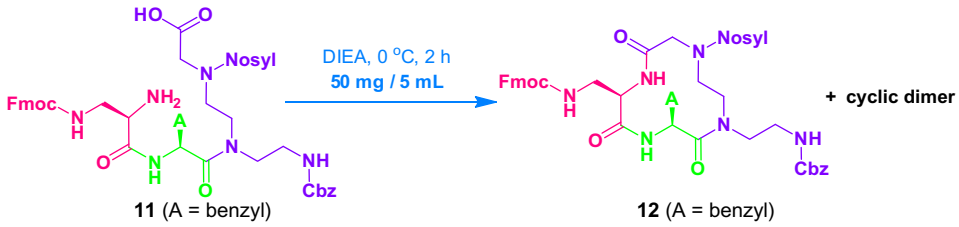
This was followed with sequential incorporation of Boc-amino acids and again selective Boc removal in the presence of the *t*-butyl ester using TFA in DCM. Eventual deprotection of both *t*-butyl carboxylate and Boc-amino groups at the penultimate step was accomplished with HCl/THF. This sequence enabled us to reliably generate ~100 g of each of the 16 cyclization substrates.

Initial cyclization attempts were made using 50 mg of substrate **11** in 5 mL solvent volumes (10 mg/mL, ~10 mM, Scheme 4, *A* = benzyl). The desired product **12** in our case was indeed formed but only in low yield, with significant dimer formation observed even at this concentration under various coupling agent/solvent conditions. For example, with PyBop/DCM the ratio of desired product versus dimer was 0.8 to 1.0. This improved somewhat when HATU was used, with HATU/DMF giving a better ratio of 1.8 to 1.0. This result is significantly different from the reported cyclization of apicidin analogs where cyclization of **3a** was achieved at 2 and 20 mg/mL concentrations with 80–90% isolated yields.[5a] Our result may be attributed to an unfavorable entropy factor derived from the lack of a cyclic amino acid residue in the ring skeleton as noted earlier.

Efforts were then made to alter the sequence of reagent addition, resulting in substantial dimer suppression when the coupling substrate was slowly introduced into a solution of the coupling reagent and base. For example, when 50 mg substrate **11** was introduced into a 2.5 mL DMF solution of HATU and DIEA

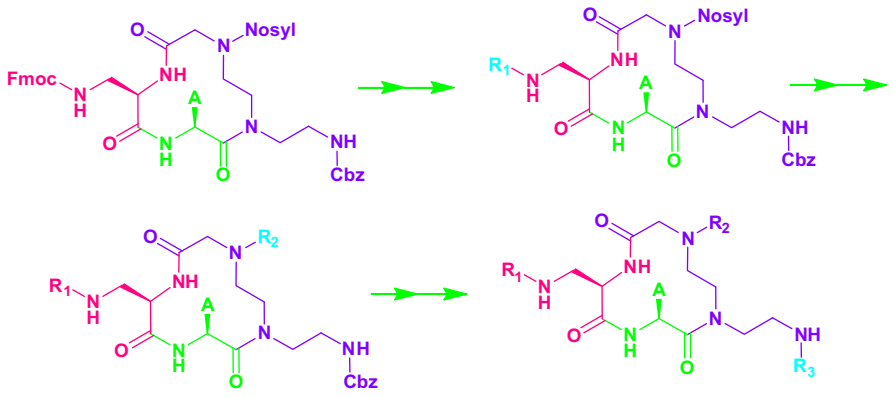


Scheme 3. Synthesis of common intermediate **5**. Reagents and conditions: (a) CbzOSu, DCM, 0 °C; (b) $(Boc)_2O$, DCM; (c) Ph_3P, DIAD, phthalimide, THF; (d) NH_2NH_2, EtOH; (e) nosyl-Cl; (f) $BrCH_2CO_2t$-Bu, ACN, K_2CO_3; (g) TFA/DCM (1:5).



scale (mg)	concentration (mg/mL)	condition	product : dimer	isolated yield
50	10	PyBop/DCM	0.8 : 1	NA
50	10	HATU/DMF	1.8 :1	NA
50	20	HATU/DMF, slow addn	3:1	NA
1,000	50	HATU/DMF, slow addn	>10:1	70%
10,000	125	HATU/DMF/ACN, slow addn	>10:1	78%

Scheme 4. Macrocyclization for template synthesis.



Scheme 5. Final analog synthesis from macrocyclic templates.

(20 mg/mL), the ratio of desired product to dimer increased to 3:1. On larger scale, the control of substrate addition rate became more practical and formation of dimer was further suppressed. Thus at 1 g scale in 20 mL DMF (50 mg/mL), slow substrate addition led to further improved product dimer ratio of >10:1 and an isolated yield of ~70%. Further optimization resulted in a 10 g scale reaction using only 80 mL solvent (125 mg/mL) with isolated product in ~80% yield after simple purification procedures. Ultimately, these procedures were performed on 16 substrates at scales up to 100 g with isolated desired product in high yield.[11]

From the templates, the final analogs were elaborated as shown in Scheme 5. Fmoc was first removed followed with elaboration of the R1 diversification. The nosyl group was then removed (PhSNa, K_2CO_3, DMF) with the introduction of the R2 diversification. The

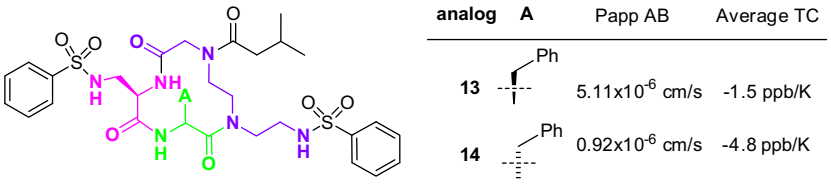


Figure 2. Permeability and intramolecular hydrogen bond interaction of diastereomers **13** and **14**.

Cbz group was finally removed (TMSI, DCM) and the last diversification R3 was incorporated. When R1 is a simple amine cap from aldehyde reductive amination, Boc was introduced to protect the newly formed secondary amine. Diversification was performed using standard chemistry, i.e. amide coupling, sulfonamide formation, heteroaryl S_NAr and reductive amination. Overall 3987 final analogs were generated in this effort out of a target of 5488 analog library ($16 \times 7 \times 7 \times 7$).

A subset of these analogs was evaluated in permeability assays. Interestingly, a pair of diastereomers, analogs **13** and **14** exhibited considerably different permeability (Fig. 2), with MDCKII-LE Papp AB 5.11×10^{-6} cm/s for analog **13** and 0.92×10^{-6} cm/s for analog **14**.[12] This correlation of permeability and stereochemistry, although not universal for all the library macrocyclic analogs we made, was observed on a number of other pairs of diastereomers. There are four amide or sulfonamide hydrogen atoms that may serve as hydrogen bond donors in these molecules. NMR studies revealed there is a higher degree of intramolecular hydrogen bonding (IMHB) in analog **13** compared with compound **14**, as measured by temperature coefficients (TC) of these individual hydrogen atoms. In $CDCl_3$ a TC higher than −3.0 ppb/K is typically associated with strong IMHB.[13,14] The average TCs for the four hydrogen atoms in **13** is −1.5, with three of the four higher than −3.0 ppb/K. On the other hand, the average TCs for the four hydrogen atoms of analog **14** is −4.8, with only one of the four higher than −3.0. This appears to indicate a substantially higher level of IMHB interaction for **13** compared with **14** which is consistent with the observed permeability difference.[14]

Summary and conclusions

We described here a novel synthesis pathway for the 12-membered macrocyclic templates (**1**) and the elaboration of a library of macrocyclic analogs (**2**) thereof. The key macrocyclization step was performed at up to 100 g scale without resorting to syringe pumps, flow reactors, large volume of solvent or solid phase chemistry. An interesting observation of considerably different permeability properties was made on diastereomeric analogs apparently due to intramolecular hydrogen bond interactions.

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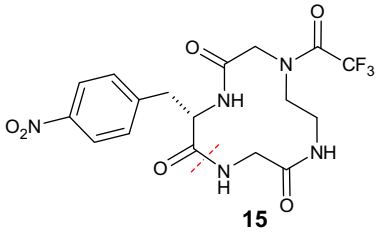

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11. *Detailed optimized macrocyclization of 11 at 10 g scale:* to a stirred solution of HATU (8.1 g, 21 mmol) and DIEA (2.7 g, 21 mmol) in 20 mL DMF was added a solution of the cyclization substrate 11 (10 g, 11 mmol) in 60 mL MeCN dropwise over a period of 6 h at 0 °C under argon. The resulting mixture was stirred at 0 °C for 30 min. and then poured into 200 mL ice-water with stirring. A precipitate formed and was collected via filtration. The solid was dried in a lyophilizer to furnish the desired cyclized product 12 as a pale powder, 7.7 g (78% yield).
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