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Synthesis and biological activity of macrocyclic inhibitors of hepatitis C virus (HCV) NS3 protease

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Abstract—The 17-membered phenylalanine-based macrocycle **6** was prepared starting from 3-iodo-phenylalanine. Macrocyclization of alkene phenyl iodide **5** was effected through a palladium-catalyzed Heck reaction. The macrocyclic α-ketoamides were active inhibitors of the HCV NS3 protease, with the C-terminal acids and amides being more potent than *tert*-butyl esters.
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Hepatitis C virus (HCV) infection is the principal cause of chronic liver disease, leading to cirrhosis, hepatocellular carcinoma, or liver failure in humans.[1] It has infected more than 170 million people worldwide and has emerged as a major global health problem. Currently available therapy is α-interferon, either alone or in combination with ribavirin. Even the latest pegylated α-interferon-ribavirin combination therapy can generate sustained virological response in only 50% of infected patients.[2] Existing therapies are also fraught with several considerable side effects. The limited efficacy and adverse side effects of the current therapies have clearly raised the need to develop even more effective antiviral agents, stimulating intensive research in finding potent and orally bioavailable small molecule drug candidates.[3]

The HCV viral RNA genome encodes a polyprotein that consists of structural and nonstructural (NS) proteins. The chymotrypsin-like serine protease, located at N-terminal of NS3 nonstructural protein, is essential for viral replication in vivo.[4] It has been a valuable target for which a number of inhibitors have been described in the literature.[5]

A large number of protease inhibitors have been developed in recent years for the treatment of a variety of diseases, many of which are peptidic in nature.[6] Their designs are normally based on the cleavage of native substrates. It is often difficult to extract desirable pharmacokinetic properties from these peptidic compounds.[7] Lot of effort has been made to design peptidomimetics with less peptidic character. One of the strategies developed was macrocyclization of a peptide chain.[8] Many biologically active macrocyclic molecules, such as vancomycin family of antibiotics[9] and chloropeptins,[10] have been observed in nature. A number of potent and selective macrocyclic inhibitors with novel structures have also been designed for HIV protease,[11] ACE,[12] matrix metalloproteases (MMP),[13] and TACE.[14] Macrocycles are designed to pre-organize the conformation for enzyme binding, reducing entropy cost in the process.[15] They are potentially more stable to proteolytic degradation than their acyclic counterparts.[16]

Herein, we report a short and efficient synthesis of a series of phenylalanine-based macrocyclic HCV inhibitors. The 17-membered macrocycles were designed as a peptidomimetic of a P2–P3 dipeptide moiety. We presumed that incorporation of a macrocyclic ring in these inhibitors could enhance binding potency and improve pharmacokinetic profile. The size of the macrocycle was determined, based on the analysis of X-ray crystal structure of the enzyme binding site. Clearly, the major challenge in the synthesis of this type of compounds was macrocyclization step. We envisaged that the alkyl aryl linkage could be effected through a Heck reaction,[17] that is, palladium-catalyzed carbon–carbon bond formation between an aryl iodide and terminal alkene (Scheme 1).

Synthesis of the macrocycle commenced with the coupling of commercially available *N*-Boc-cyclohexylglycine **1** and

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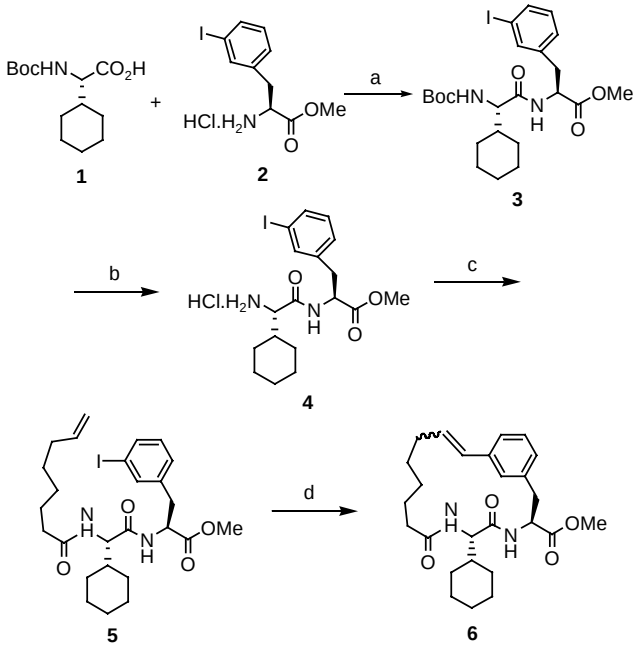
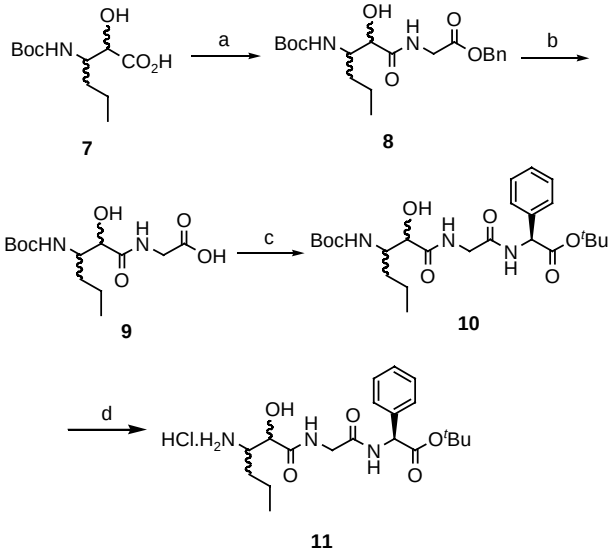


Scheme 1. Reagents and conditions: (a) HOOBt, EDC, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 84%; (b) 4 M HCl, dioxane, rt; (c) oct-7-enoic acid, HOOBt, EDC, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 68% (two steps); (d) $Pd(PPh_3)_4$, Et_3N, CH_3CN/DMF, 80 °C, 37%.

(3-iodo)phenylalanine methyl ester **2** (Scheme 1) using 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBt), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride, and 4-methylmorpholine (NMM) at −20 °C to give dipeptide **3**. The Boc-protecting group was removed and the resulting amine hydrochloride **4** was reacted with oct-7-enoic acid under similar coupling conditions (HOOBt, EDC, and NMM) to provide **5**, the precursor to the macrocycle. The key macrocyclization step came up next, wherein the terminal alkene and phenyl iodide in **5** were cyclized to a 17-membered macrocycle through a Heck reaction.[17] The reaction was performed in a sealed tube at 80 °C in anhydrous acetonitrile–dimethylformamide media in the presence of 10% palladium tetrakistriphenylphosphine and 10 equiv of triethylamine. The desired macrocycle **6** was obtained in 37% isolated yield. The product was a mixture of *cis*- and *trans*-isomers at the newly formed double bond.

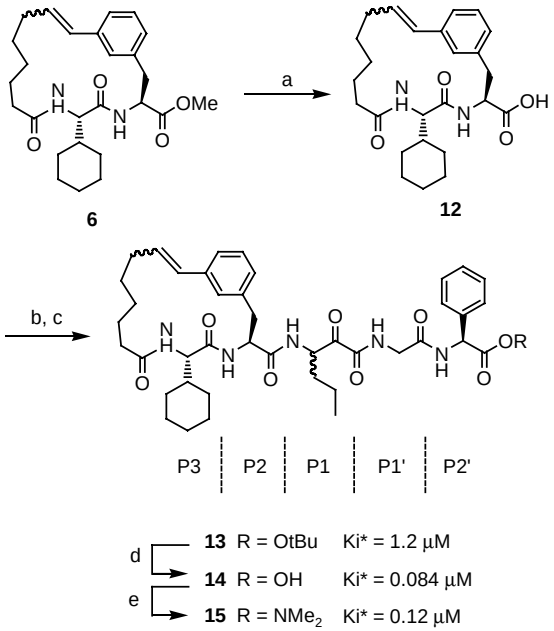
The right-hand tripeptide fragment of the molecule was prepared in a series of peptide couplings and protecting group manipulations (Scheme 2). First, norvaline hydroxyacid[18] **7** was coupled to glycine benzyl ester under standard conditions (HOOBt, EDC, and NMM) to give compound **8**, which was hydrogenated to give acid **9**. Coupling of **9** to commercially available phenylglycine *tert*-butyl ester provided tripeptide **10**. The Boc-protecting group was then selectively removed in the presence of *tert*-butyl ester using 2 M hydrogen chloride in 1:1 dioxane/ethyl acetate to give the desired amine **11** as a hydrochloride salt.

With both the left-hand macrocycle and the right-hand α-hydroxyamide fragment in hand, the stage was set to



Scheme 2. Reagents and conditions: (a) glycine benzyl ester, HOOBt, EDC, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 75%; (b) H_2, Pd–C, EtOH, rt, 98%; (c) phenylglycine *t*-butyl ester hydrochloride, HOOBt, EDC, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 81%; (d) 2 M HCl, dioxane/EtOAc (1:1), rt, quant.

assemble target molecules (Scheme 3). Thus, methyl ester **6** was hydrolyzed to macrocyclic acid **12**, which was coupled to amine **11** under standard conditions. The resulting α-hydroxyamide was then oxidized using Dess–Martin periodinane[19] to afford α-ketoamide **13**. This compound was an inseparable diastereomeric mixture of (*R*)- and (*S*)-isomers at P1 norvaline α-center. The corresponding C-terminal carboxylic acid **14**, also

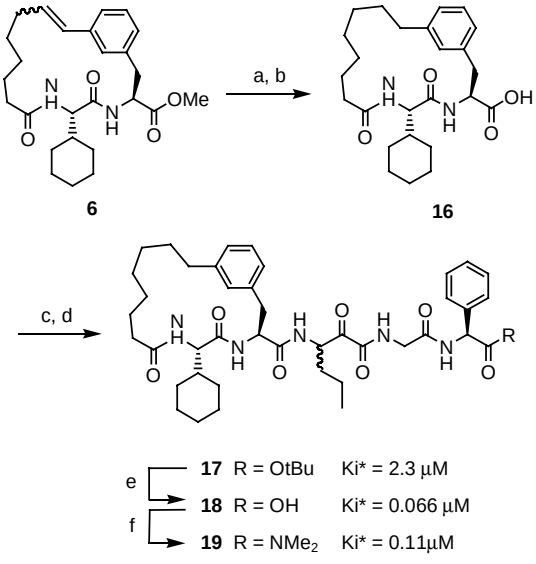


Scheme 3. Reagents and conditions: (a) LiOH, MeOH/THF/H_2O, rt, quant.; (b) **11**, HOOBt, EDCI, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 77%; (c) Dess–Martin periodinane, CH_2Cl_2, rt, 55%; (d) trifluoroacetic acid, CH_2Cl_2, quant.; (e) Me_2NH·HCl, HOOBt, EDC, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 65%.

as a diastereomeric mixture, was obtained from compound **13** by treatment with trifluoroacetic acid. The dimethyl amide **15** was prepared via coupling of **14** with dimethylamine.

To evaluate the effect of the double-bond on the potency of inhibitors, saturated analogs of compounds **13**–**15** were also synthesized, as outlined in Scheme 4. Thus, macrocycle **6** was hydrogenated to give the saturated macrocycle, which was hydrolyzed to its corresponding carboxylic acid **16**. The coupling between **16** and amine **11** provided an α-hydroxyamide, which on Dess–Martin periodinane oxidation afforded the desired α-ketoamide **17**. The *tert*-butyl ester was treated with trifluoroacetic acid to give acid **18**. The dimethyl amide analog **19** was prepared from **18** in a similar manner as the preparation of **15**.

The six macrocyclic compounds **13**–**15** and **17**–**19** were tested as hepatitis C virus NS3 serine protease inhibitors in a continuous assay.[20] The two *tert*-butyl esters were modestly active toward the enzyme (**13**: $K_i^* = 1.2$ μM; **17**: $K_i^* = 2.3$ μM). The carboxylic acids (**14**, **18**), however, were potent inhibitors of HCV NS3 protease, with K_i^* values of 0.084 and 0.066 μM, respectively. The dimethyl amides (**15**, **19**) were also very active HCV inhibitors with K_i^* at 0.12 and 0.11 μM, respectively. The *tert*-butyl esters (**13/17**) were more than ten times less potent than their corresponding acids (**14/18**) and amides (**15/19**). Presumably, the bulky *tert*-butyl group must have undergone unfavorable steric interactions with the enzyme surface. The smaller acid and amide groups were more tolerated. The potency was very close for any pair of inhibitors (**13/17**, **14/18**, and **15/19**) with or without the presence of a double bond in the macrocyclic ring. Although the double bond in compounds **13**–**15** existed as a *cis*/*trans* mixture, unsaturation in the macrocycle did not seem to affect the inhibition activity of these compounds.



Scheme 4. Reagents and conditions: (a) H_2, Pd–C, EtOH, rt, quant.; (b) LiOH, MeOH/THF/H_2O, rt, quant.; (c) **11**, HOOBt, EDC, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 73%; (d) Dess–Martin periodinane, CH_2Cl_2, rt, 46%; (e) trifluoroacetic acid, CH_2Cl_2, quant.; (f) Me_2NH·HCl, HOOBt, EDC, 4-methylmorpholine, DMF/CH_2Cl_2, −20 °C, 71%.

The X-ray crystal structure of inhibitor **18** bound to HCV NS3 protease was obtained (Fig. 1, PDB ID code 2A4Q). The diastereomer with (*S*)-norvaline at P1 was observed in the X-ray structure as it was more potent than (*R*)-isomer. A reversible covalent bond was formed between enzyme active site serine (Ser139) hydroxyl and the ketone carbonyl of the inhibitor. The macrocycle encircled the methyl group of alanine 156, which established a 'donut-shaped' conformation that provided extra interaction between the inhibitor and the protease. The *n*-propyl side chain of P1 norvaline fit very well into the S1 pocket. In addition, the benzene ring of P2′ phenylglycine reached the other side of the lysine136 side chain such that it and the P1 propyl group formed a 'C-shaped' clamp around the lysine residue. The compound also entered into multiple hydrogen bonding with the protease through its amide chain.

In summary, a short and concise synthesis of 17-membered macrocycles **12** and **16** has been carried out. The key macrocyclization step was achieved through a Heck reaction between the aryl iodide and terminal alkene in the presence of a palladium catalyst. Macrocyclic intermediates were coupled to a tripeptide right-hand fragment to provide α-ketoamides **13**–**15** and **17**–**19** after oxidation. The C-terminal carboxylic acids and amides **14**–**15** and **18**–**19** were potent HCV protease inhibitors with K_i^* values between 0.066 and 0.12 μM, while the bulky *tert*-butyl esters **13** and **17** were less active. X-ray structure of compound **18** bound to protease revealed that the macrocycle ring adopted a 'donut-shaped' conformation around the methyl group of Ala156. These macrocyclic inhibitors were at least a few fold more potent than similar noncyclic analogs. The investigation of pharmacokinetic properties is underway.

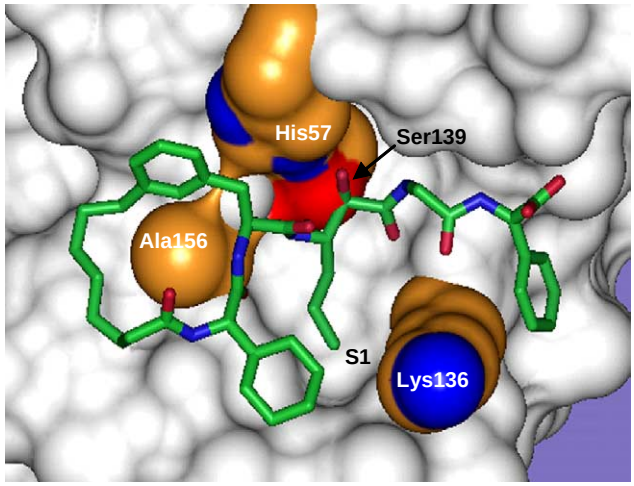


Figure 1. X-ray structure of compound **18** bound to HCV NS3 protease.

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