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Application of Ring-Closing Metathesis for the Synthesis of Macrocyclic Peptidomimetics as Inhibitors of HCV NS3 Protease

Francisco Velázquez,* Srikanth Venkatraman,* Wanli Wu, Melissa Blackman, Andrew Prongay, Viyyoor Girijavallabhan, Neng-Yang Shih, and F. George Njoroge

Schering-Plough Research Institute, 2015 Galloping Hill Road, K-15-3 3545, Kenilworth, New Jersey 07033-1300

francisco.velazquez@spcorp.com; srikanth.venkatraman@spcorp.com

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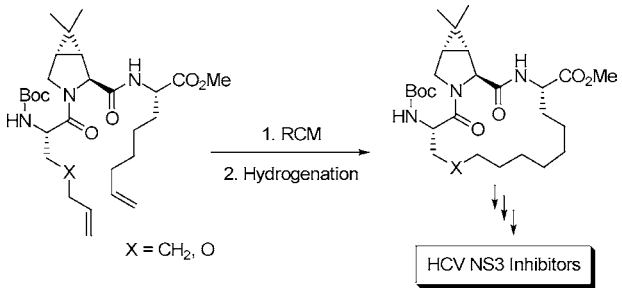
ABSTRACT



An efficient synthetic approach for the preparation of macrocyclic peptidomimetics for inhibition of HCV NS3 is presented. The macrocyclic core is built using ring-closing metathesis (RCM) of a tripeptidic diene. The presented approach allows the introduction of heteroatoms in strategic places along the macrocyclic ring. The methyl ester moiety in the RCM products was synthetically manipulated to install a keto-amide moiety via a Passerini reaction.

Hepatitis C (HCV) is a viral infection afflicting more than 3% of the world population. It is the leading cause of liver transplants in the United States and if left untreated could result in liver failure and hepatocellular carcinoma.[1] HCV NS3 protease is an enzyme which plays a pivotal role in replication of the HCV virus.[2] Inhibition of this enzyme has proven effective in reducing viral loads in humans, and considerable efforts by different research groups have been directed toward development of HCV NS3 protease inhibitors.[3] We recently reported the development of SCH 503034, a novel, orally bioavailable HCV NS3 protease inhibitor that is currently undergoing clinical trials.[4] In an effort to modify the highly peptidic nature of SCH 503034, we designed a new class of macrocyclic peptidomimetics as HCV NS3 protease inhibitors. These compounds were expected to be more potent and possess improved pharmacokinetic properties.

In this paper, we describe a synthetic methodology for preparation of these macrocyclic inhibitors of the HCV NS3 protease.[5] This approach is efficient in the construction of

(1) (a) Cohen, J. *Science* **1999**, *285*, 26. (b) Brown, R. S., Jr.; Gaglio, P. J. *Liver Transplant.* **2003**, *9*, S10.

(2) (a) Lindenbach, B. D.; Rice, C. M. *Nature* **2005**, *436*, 933. (b) Bartenschlager, R.; Ahlborn-Laake, L.; Mous, J.; Jacobsen, H. *J. Virol.* **1993**, *67*, 3835.

(3) See the following reviews and references therein: (a) Chen, S. H.; Tan, S. L. *Curr. Med. Chem* **2005**, *12*, 2317. (b) Goudreau, N.; Montse, L. B. *Expert Opin. Invest Drugs* **2005**, *14*, 1129.

(4) Venkatraman, S. et al. *J. Med. Chem.* **2006**, *49*, 6074.

(5) BILN 2061 was the first compound to undergo clinical trials. It is also a macrocyclic inhibitor of HCV NS3 protease. See: (a) Faucher, A. M. et al. *Org. Lett.* **2004**, *6*, 2901. (b) Llinas-Brunet, M. et al. *J. Med. Chem.* **2004**, *47*, 1605.

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macrocylic compounds with different ring sizes. Moreover, the versatility of this methodology allows thorough exploration of structure−activity relationships in different regions of the molecules. Our goal was to investigate different classes of compounds including macrocycles containing all-carbon ring systems and to introduce heteroatoms in strategic places along the macrocylic core as well. Our approach was based on the application of ring-closing metathesis (RCM) for construction of the key macrocyclic cores.[6] This approach has been employed for the preparation of other bioactive macrocyclic peptidomimetics such as apicidin A and related analogues for treatment of parasite-mediated infections[7] and inhibitors of BACE-1 for treatment of Alzheimer's disease.[8] Burk's catalyst for asymmetric hydrogenation was employed for the synthesis of the required amino acids, and the Passerini reaction was used for the construction of a keto-amide moiety required in HCV NS3 inhibitors.[9]

Our retrosynthetic analysis is shown in Figure 1. We

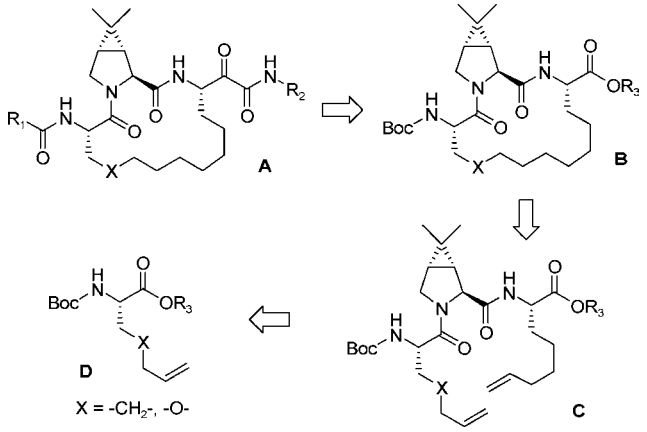
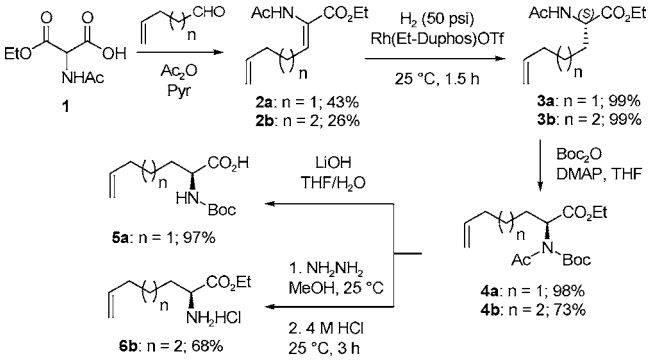


Figure 1. Retrosynthetic analysis for HCV NS3 macrocyclic inhibitors.

envisioned making inhibitors such as compound A, which are 16-membered macrocycles (15- and 17-membered macrocycles were also investigated), that contained an all-carbon aliphatic chain or had an oxygen atom incorporated into the macocyclic core. The keto-amide moiety, which acts as an electrophilic serine trap, was installed via Passerini reaction of the corresponding aldehyde derived from compound B.[10] The macrocyclic core, which is the main feature of these compounds, was obtained by hydrogenation of the ring-closing metathesis product of diene C. The RCM precursor could be obtained from peptide couplings using ω-unsaturated amino acids such as compound D, which in turn can be obtained through asymmetric hydrogenation using Burk's catalyst.[11]

A. Synthesis of 15-, 16-, and 17-Membered Macrocycles Containing an All-Carbon Aliphatic Chain.[12] For the preparation of macrocycles containing an all-carbon aliphatic chain, we first synthesized ω-unsaturated *N*-Boc-protected amino acid **5a** and amine hydrochloride salt **6b** (Scheme 1).[13]

Scheme 1. Synthesis of ω-Unsaturated Amino Acids



As mentioned above, synthesis of these intermediates involved a highly efficient asymmetric hydrogenation of α,β-unsaturated esters using Burk's catalyst.[14] The presence of the ω-unsaturation in **2a** and **2b** presented an additional challenge to the synthetic transformation since hydrogenation had to proceed chemoselectively to avoid overreduction of the substrates. Thus, Knoevenagel condensation of malonate derived monoester **1** with pent-4-enal gave α,β-unsaturated ester **2a** in moderate yield (43%). Likewise, the reaction of ester **1** with hex-5-enal gave α,β-unsaturated ester **2b** (26%). The chemo- and stereoselective asymmetric hydrogenation of the conjugated olefinic bond in the presence of the terminal olefin for compounds **2a** and **2b** proceeded with excellent efficiency. Thus, hydrogenation using rhodium (Et-DuPhos)OTf delivered the desired amino acids **3a** and **3b** in nearly quantitative yields and high enantiomeric excess (>98% ee). The amino acids **3a** and **3b** had the required *S*-configuration at the newly created stereogenic center, and their terminal olefin remained intact in the process. It is important to mention that chemoselectivity in the hydrogenation step was directed by the *N*-acetyl group in compounds **2a** and **2b**. The *N*-Boc-protected amino acids **4a** and **4b** were obtained from **3a** and **3b** in 98 and 73% yields, respectively.

Finally, hydrolysis of the ethyl ester and *N*-acetyl functionalities in **4a** using lithium hydroxide gave the desired

(6) (a) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199. (b) Tsantrizos, Y. S. *J. Organomet. Chem.* **2006**, *691*, 5163.

(7) Deshmukh, P. H.; Schulz-Fademrecht, C.; Procopiou, P. A.; Vigushin, D. A.; Coombes, R. C.; Barrett, A. G. M. *Adv. Synth. Catal.* **2007**, *349*, 175.

(8) Ghosh, A. K. et al. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 15.

(9) See the following examples and references therein for SAR of electrophilic serine traps in HCV NS3 inhibitors: (a) Bennett, J. M., et al. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 355. (b) Han, W.; Hu, Z.; Jiang, X.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 711.

(10) (a) Passerini, M. *Gazz. Chim. Ital*. **1921**, *51*, 126. (b) Ugi, I.; Meyr, R. *Chem. Ber*. **1961**, *94*, 2229.

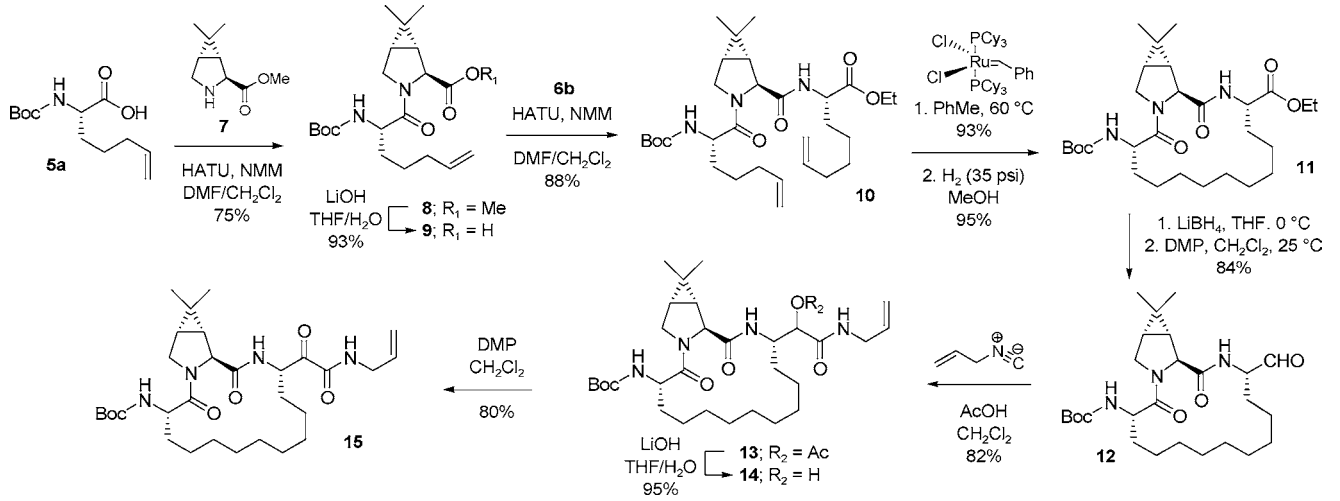
(11) (a) Burk, M. J. *J. Am. Chem. Soc.* **1991**, *113*, 8518. (b) Burk, M. J. *Acc. Chem. Res.* **2000**, *33*, 363.

(12) The synthesis of 16-membered macrocycles is shown in the manuscript. See the Supporting Information for synthesis and characterization of 15- and 17-membered macrocycles.

(13) For preparation of **5a** using an alternative approach, see: (a) Ripka, A. S.; Bohacek, R. S.; Rich, D. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 357. (b) Goudreau, N. et al. *J. Org. Chem.* **2004**, *69*, 6185.

(14) For examples of rhodium-catalyzed asymmetric hydrogenations, see: Chi, Y.; Tang, W.; Zhang, X. *Modern Rhodium-Catalyzed Organic Reactions*; Evans, P. A., Ed.; Wiley-VCH: Weinheim, 2005; pp 1−31.

Scheme 2. Synthesis of 16-Membered Macrocylic Compounds Containing an All-Carbon Aliphatic Chain



N-Boc-protected amino acid **5a** in nearly quantitative yield. On the other hand, *N*-acetyl cleavage followed by *N*-Boc deprotection in compound **4b** gave the corresponding amine hydrochloride salt **6b** in 68% yield.

Having on hand the required ω-unsaturated amino acids, we began construction of the macrocyclic core of our inhibitors. Synthesis of the macrocyclic core started with the HATU coupling of ω-unsaturated acid **5a** with dimethylcyclopropyl proline **7**[4,15] to give dipeptide **8** in 75% yield. Hydrolysis of the methyl ester **8** gave acid **9** in nearly quantitave yield. At this point, the ω-unsaturated amino acid **6b** was introduced to install the required terminal double bond for RCM giving tripeptide **10** in 88% yield. The crucial RCM reaction of diene **10** was carried out using Grubbs' first-generation catalyst[16] to deliver the corresponding alkene in 93% yield as a mixture of cis/trans isomers which were hydrogenated to afford the desired 16-membered macrocycle **11** (95%). This approach proved to be remarkably efficient in the preparation of other macrocycles, including 15- and 17-membered macrocycles, giving similar results.

After the successful synthesis of the macrocyclic core, we proceeded to the construction of the keto-amide moiety which serves as a serine trap in the class of inhibitors that were of interest. Ethyl ester **11** was converted to aldehyde **12** in a two-step sequence. Then, a Passerini reaction of aldehyde **12** and commercially available allyl isocyanide[17] gave the corresponding α-acetoxy amide **13** as an inconsequential mixture of diastereomers. Hydrolysis of the acetate in **13** proceeded in almost quantitative yield to give allyl hydroxyamide **14**. Finally, Dess−Martin[18] oxidation of **14** delivered the desired 16-membered macrocyle **15** which contains the required keto-amide serine trap.

B. Synthesis of 16-Membered Oxygen-Containing Macrocycles. As mentioned above, it was in our interest to investigate the introduction of heteroatoms in specific places along the macrocyclic system. Introduction of heteroatoms was aimed at changing properties such as solubility and pharmacokinetics in our inhibitors. We envisioned applying the RCM approach described above for preparation of these oxygen-containing macrocyclic systems. Thus, Boc-L-serine (**16**) was used as starting material for the synthesis of macrocycle **22**. Palladium-catalyzed *O*-allylation of **16** was required to avoid erosion of enantiomeric purity. Thus, treatment of **16** with methyl allyl carbonate and a catalytic amount of tetrakis(triphenylphosphine) palladium afforded **17** in 64% yield. Saponification of the ester functionality followed by HATU coupling with proline derivative **7** gave dipeptide **19**. The required diene **20** for ring-closing metathesis was obtained in a two-step process similar to that described above for the all-carbon macrocyclic analogues.[19] Thus, the crucial macrocyclization of diene **20** was carried out via RCM using Grubbs' first-generation catalyst to give the metathesis product as an inconsecuential mixture of cis/trans isomers. This reaction represents a new example of olefin metathesis for the construction of large heterocyclic systems.

Hydrogenation of the metathesis product gave macrocycle **21** in 63% yield along with a small amount of a side product arising from *O*-deallylation (ca. 15%). The methyl ester functionality in **21** was transformed into the required keto-amide moiety using the conditions previously described in the all-carbon analogues. Thus, the oxygen-containing macrocycle **22** was obtained in five steps from ester **21**.

The inhibition activity against HCV NS3 of these macrocycles was measured and found to be in the nanomolar range.[20] The all-carbon macrocycle **15** (Scheme 2) had a K_i* = 36 nM, whereas the oxygen-containing analogue **22**

(15) Zhang, R.; Mamai, A.; Madalengoitia, J. S. *J. Org. Chem.* **1999**, *64*, 547.

(16) (a) Schwab, P.; France, M. B.; Ziller, J. W.; Grubbs, R. H. *Angew. Chem. Int. Ed.* **1995**, *34*, 2039. (b) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18.

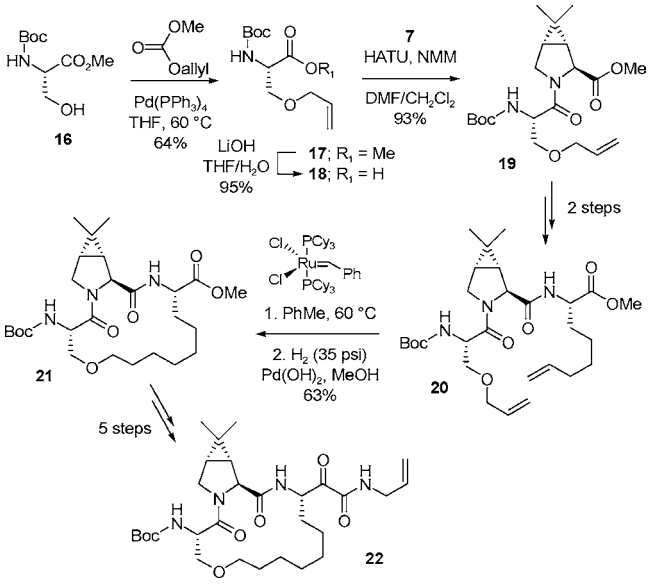
(17) Allyl isocyanide can be prepared by dehydration of *N*-allylformamide following the procedure described for the preparation of cyclopropyl isocyanide in: Schöllkopf, U. et al. *Liebigs Ann. Chem.* **1976**, *1*, 183.

(18) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.

(19) The methyl ester analogue of **6b** was used in the synthesis of oxygen-containing macrocycles.

(20) A manuscript describing the synthesis and structure−activity relationship (potency and pharmacokinetic profiles) of macrocyclic inhibitors using this approach is in preparation.

Scheme 3. Synthesis of 16-Membered Oxygen-Containing Macrocycles



(Scheme 3) had a $K_i^* = 150$ nM.[21] Moreover, experimental data suggests that a 16-membered macrocycle might be the optimum ring size for activity although 15-membered compounds were also well accommodated. A thorough study of the potency and pharmacokinetic profiles for these macrocylic inhibitors was carried out and will be disclosed in a separate publication. The synthetic strategy described herein proved to be efficient and flexible for SAR investigations at both ends of the macrocyclic core.

Macrocyclic keto-amide **23** was synthesized using the RMC approach described herein.[20] This compound is a 17-membered macrocycle with $K_i^* = 6$ nM. Figure 2 shows compound **23** bound to HCV NS3 protease, and several key interactions can be observed. From the X-ray, it was clear that the aliphatic chain of the macrocylic core made excellent contact in the lipophilic region of the active site. Moreover, the keto-amide moiety adopts a conformation which facilitates nuclephilic attack by Ser-139 leading to enzyme inhibition.

In conclusion, a highly efficient approach for the synthesis of macrocyclic peptidomimetics for the inhibition of HCV NS3 was developed using RCM. The flexibility of this approach allowed the synthesis of 15-, 16-, and 17-membered macrocycles from easily accessible intermediates. A simple modification of this methodology allowed introduction of heteroatoms in strategic places of the macrocylic system. Also, required ω-unsaturated amino acid starting materials were efficiently synthesized using a chemoselective asymmetric hydrogenation. The Passerini reaction allowed installation of the crucial keto-amide moiety present in this class of inhibitors.

(21) The HCV NS3 serine protease inhibitory value is provided as K_i^*. For a definition of K_i^* and discussion, see: Morrison, J. F.; Walsh, C. T. *Advances in Enzymology and Related Areas of Molecular Biology*; Meister, A., Ed.; John Wiley & Sons: Hoboken, NJ, 1988; Vol. 61, pp 201.

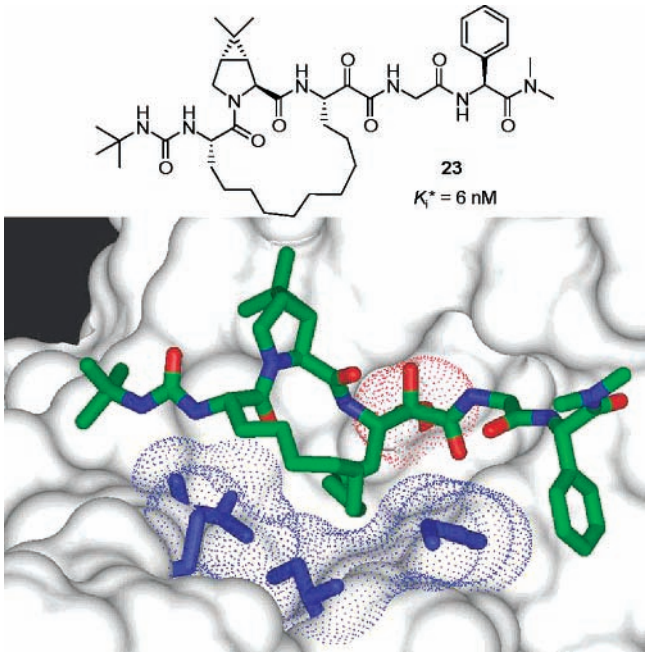


Figure 2. Macrocyclic keto-amide **23**. A 17-membered all-carbon macrocycle bound to the active site of HCV NS3 protease.

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Supporting Information Available: Experimental procedures, spectroscopical data, and copies of spectra for selected compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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