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Synthesis of BILN 2061, an HCV NS3 Protease Inhibitor with Proven Antiviral Effect in Humans

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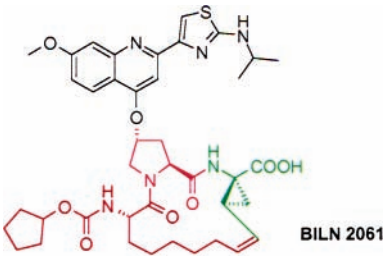
ABSTRACT



The synthesis of BILN 2061, an NS3 protease inhibitor with proven antiviral effect in humans, was accomplished in a convergent manner from four building blocks. The procedure described here was suitable for the preparation of multigram quantities of BILN 2061 for preclinical pharmacological evaluation.

Hepatitis C virus (HCV) is the leading cause of chronic liver disease worldwide.[1] It is estimated that 3% of the world population is infected with the virus. The latest and most effective therapy consists of PEGylated α-interferon in combination with the nucleoside analogue ribavirin, giving a sustained virological response of ∼50% in genotype 1 infected patients.[2] The existing therapies are associated with considerable side effects requiring discontinuation of treatment in certain patient populations. The high prevalence of infection together with the side effects and the limited efficacy of current therapies emphasize the need for new therapeutics.

HCV is a small enveloped, positive strand RNA virus that encodes a polyprotein of approximately 3000 amino acids containing two proteases within the nonstructural (NS) region, NS2/3 and NS3, which mediate the maturation of the NS region.[3] NS3 protease, one of the most intensively studied targets for anti-HCV therapy, is involved in four of the five cleavage events of the maturation process and is required for viral replication in the chimpanzee.[4,5]

The initial observation that hexameric *N*-terminal cleavage products of dodecapeptide substrates have an inhibitory effect on NS3[6] triggered a product-based inhibitor research project culminating in the discovery of BILN 2061.[7a] This protease

(1) (a) Choo, Q.-L.; Kuo, G.; Weiner, A. J.; Overby, L. R.; Bradley, D. W.; Houghton, M. *Science* **1989**, *244*, 359. (b) *The Hepatits C Viruses*; Hagedorn, C. H., Rice, C. M., Eds.; Springer: Berlin, 2000; *Curr. Top. Microbiol. Immunol. 242*.

(2) Cornberg, M.; Wedemeyer, H.; Manns, M. P. *Curr. Gastroenterol. Rep*. **2002**, *4*, 23.

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(4) Beaulieu, P. L.; Llinàs-Brunet, M. *Curr. Med. Chem.: Anti-Infect. Agents* **2002**, *1*, 163.

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(6) (a) Llinàs-Brunet, M.; Bailey, M.; Fazal, G.; Goulet, S.; Halmos, T.; LaPlante, S.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1713. (b) Steinküler, C.; Biasol, G.; Brunette, M.; Urbani, A.; Koch, U.; Cortese, R.; Pessi, A.; De Francesco, R. *Biochemistry* **1998**, *37*, 8899.

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inhibitor was developed in our laboratories via a rational approach involving the use of traditional medicinal chemistry, parallel synthesis, and structural data for the optimization of binding properties and downstream biopharmaceutical properties.[7] BILN 2061 is the first compound of its class to have reached clinical trials. It has shown oral bioavailability and antiviral effect in humans infected with HCV.[8] In the optimization phase that led to the discovery of BILN 2061, we developed a synthetic approach to support SAR studies and to produce several grams of drug substance for preclinical pharmacological studies. The core of BILN 2061 was built in a convergent manner using four building blocks: three nonnatural amino acids (**P1**, **P2**, and **P3**, Figure 1)[9] and a 4-quinolinol moiety. The preparation and ultimate assembly of these building blocks are disclosed in this paper.

A retrosynthetic analysis of BILN 2061 suggests that its 15-membered ring can be formed by ring-closing olefin metathesis of an acyclic tripeptide precursor (Figure 1).[10] This acyclic tripeptide precursor can, in turn, be assembled using standard solution peptide coupling procedures. Introduction of the quinoline moiety onto **P2** can be achieved early or late in the synthetic sequence by an S_N2 reaction with a hydroxyquinoline and an "activated" hydroxyproline as the electrophile. However, we recognized that a late introduction renders the sequence more convergent and was likely better suited for a quinoline bearing potentially chemically reactive substituents. So, for BILN 2061 we favored an S_N2 coupling strategy between a 4-hydroxyquinoline fragment and a preassembled macrocyclic ring containing a *cis*-(4*S*)-hydroxyproline residue.

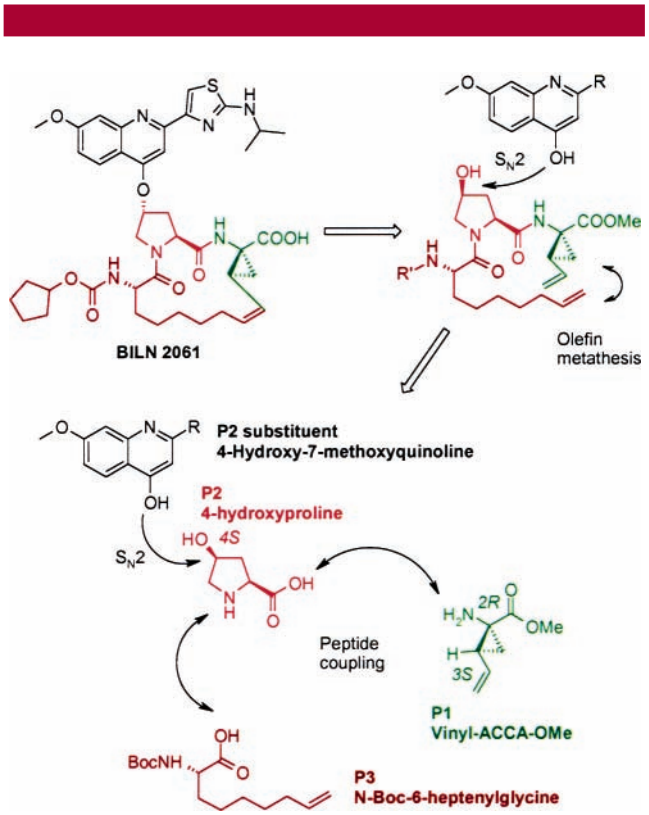

Figure 1. Retrosynthesis of BILN 2061.

The prohibitive cost of *cis*-hydroxyproline and its propensity to lactonize during peptide coupling procedures led us to envisage a double inversion sequence making use of the more readily available *trans* isomer. We therefore coupled (2*R*,3*S*)-3-vinyl-2-amino-2-cyclopropylcarboxylic acid (ACCA) methyl ester **1** (prepared in a racemic form and then resolved via an enzymatic hydrolysis of the methyl ester[11]) to commercially available *trans*-(2*S*,4*R*)-Boc-hydroxyproline **2** using standard peptide coupling reaction procedures with *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, Scheme 1).[12] The resulting suitably protected **P1**−**P2** dipeptide **3** was then epimerized at the 4-position of proline via a Mitsunobu reaction using *p*-nitrobenzoic acid (PNBA).[13] Regioselective hydrolysis of the *p*-nitrobenzoate group afforded Boc-*cis*-(2*S*,4*S*)-4-hydroxyproline-(2*R*,3*S*)-vinyl-ACCA methyl ester **4**.

For the **P3** building block, we sought a method suitable for the preparation of large quantities of material. We favored a sequence where the key step involves the catalytic enantioselective hydrogenation of an enamine using the DUPHOS system developed by Burk et al.[14] Commercially available 7-octene-1,2-diol **5** was cleaved by sodium perio-

(7) (a) Llinàs-Brunet, M.; Bailey, M.; Bolger, G.; Brochu, C.; Faucher, A.-M.; Ferland, J.-M.; Garneau, M.; Ghiro, E.; Gorys, V.; Grand-Maître, C.; Halmos, T.; Lapeyre-Paquette, N.; Liard, F.; Poirier, M.; Rhéaume, M.; Tsantrisos, Y.; Lamarre, D. *J. Med. Chem.* **2004**, *47*, 1605. (b) Rancourt, J.; Cameron, D.; Gorys, V.; Lamarre, D.; Poirier, M.; Llinàs-Brunet, M. *J. Med. Chem.* **2004**, *47*, 2511. (c) Goudreau, N.; Cameron, D. R.; Bonneau, P.; Gorys, V.; Plouffe, C.; Poirier, M.; Lamarre, D.; Llinàs-Brunet, M. *J. Med. Chem.* **2004**, *47*, 123. (d) Bailey, M. D.; Halmos, T.; Goudreua, N.; Lescop, E.; Llinàs-Brunet, M. *J. Med. Chem.* **2004**, ASAP. (e) Tsantrizos, Y. S.; Bolger, G.; Bonneau, P.; Cameron, D. R.; Goudreau, N.; Kukolj, G.; LaPlante, S. R.; Llinàs-Brunet, M.; Nar, H.; Lamarre, D. *Angew. Chem., Int. Ed.* **2003**, *42*, 1355. (f) Poupart, M. A.; Cameron, D. R.; Chabot, C.; Ghiro, E.; Goudreau, N.; Goulet, S.; Poirier, M.; Tsantrizos, Y. S. *J. Org. Chem.* **2001**, *66*, 4743. (g) Llinàs-Brunet, M.; Bailey, M.; Fazal, G.; Ghiro, E.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2267. (h) LaPlante, S. R.; Aubry, N.; Bonneau, P. R.; Kukolj, G.; Lamarre, D.; Lefebvre, S.; Li, H.; Llinàs-Brunet, M.; Plouffe, C.; Cameron, D. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2271.

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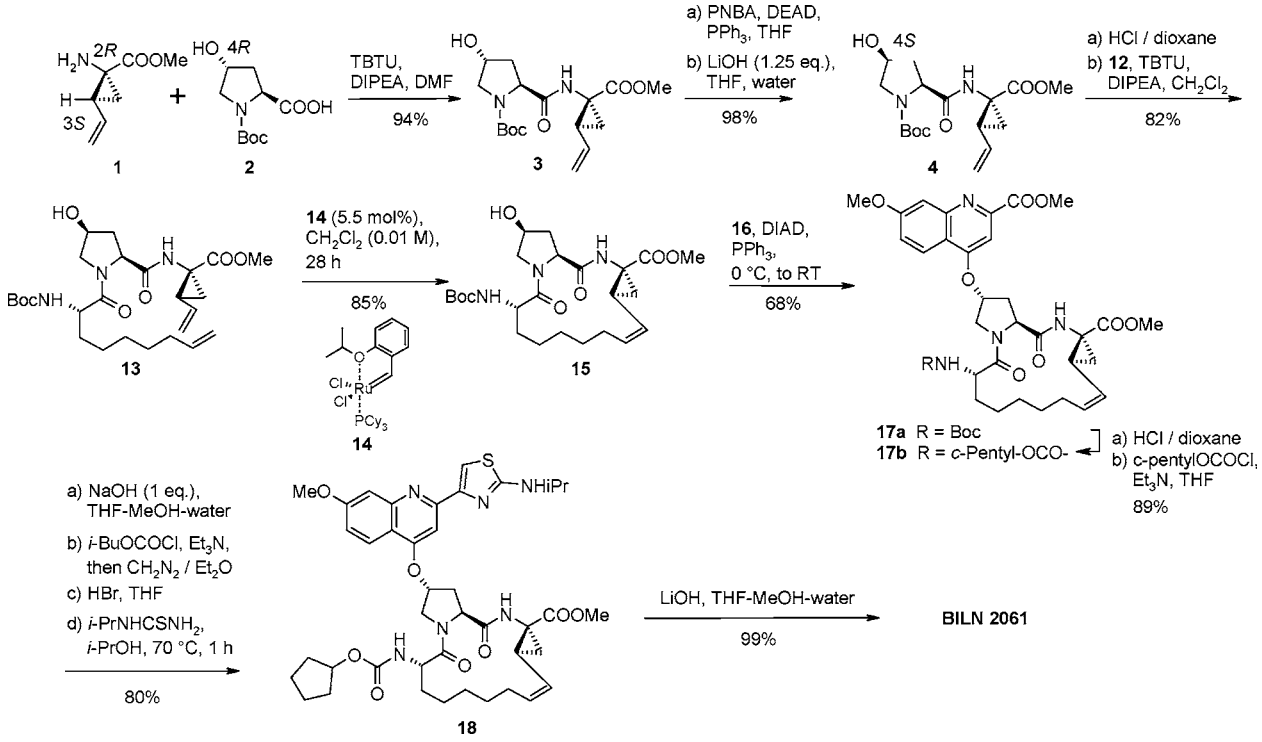
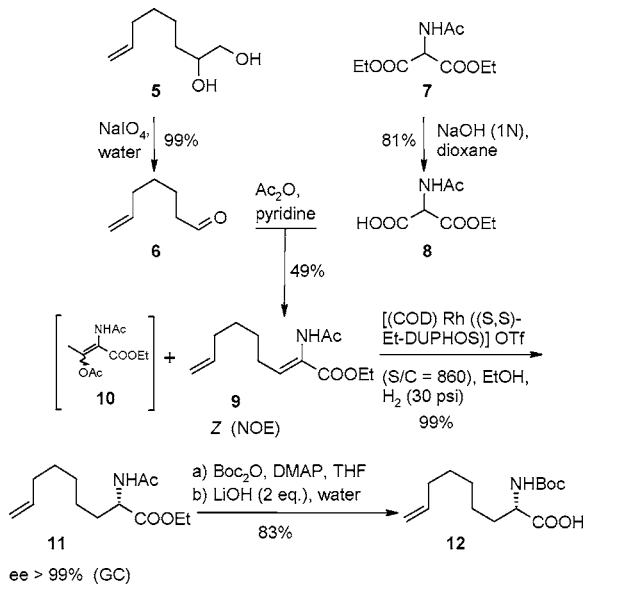
(13) Mitsunobu, O. *Synthesis* **1981**, 1.

Scheme 1



date to afford 6-heptenal **6** (Scheme 2). Diethyl 2-acetamidomalonate **7** was monohydrolyzed to **8** using 1 equiv of sodium hydroxide. Fragments **6** and **8** were subjected to a Perkin reaction[15] under conditions taken from Hengartner et al.,[16] producing selectively *Z*-ethyl 2-acetamido-2,8-nonadienoate **9** along with a small amount of ethyl 2-acetamido-3-acetoxy-2-propenoate **10** as a byproduct.[17] Alternative methods for the preparation of **9** such as the Wadsworth−Horner−Emmons olefination using a phosphonylglycinate and aldehyde **6** were also considered.[18] However, the simplicity of the so-called "Perkin route" appeared more advantageous. *Z*-2-Acetamido-2,8-nonadienoate **9** was then regio- and enantioselectively hydrogenated using [(COD) Rh ((*S*,*S*)-Et-DUPHOS)] OTf[14] to afford ethyl (*S*)-2-acetamido-8-nonenoate **11** in quantitative yield. Acetamide **11** was converted to the **P3** fragment **12** following the amide-to-carbamate transformation procedure described by Burk et al.[19]

Scheme 2



The 15-member macrocyclic ring was elaborated from fragments **4** (**P1**−**P2**) and **12** (**P3**) via a standard peptide coupling procedure with TBTU[12] to afford the acyclic tripeptide **13** (Scheme 1). The latter was then subjected to ring-closing olefin metathesis. Several ruthenium catalysts[20]

(14) Burk, M. J.; Allen, J. G.; Kiesman, W. F. *J. Am. Chem. Soc.* **1998**, *120*, 657 and references therein.

(15) For a discussion on the Perkin related reactions, see: Johnson, J. R. The Perkin Reaction and Related Reactions. In *Organic Reactions*; Adams, R., Bachmann, E. W., Fieser, L. F., Johnson, J. R., Snyder, H. R., Eds.; John Wiley & Sons: New York, 1942; Vol, 1, Chapter 8, pp 210−255 and references therein.

(16) Hengartner, U.; Valentine, D. Jr.; Johnson, K. K.; Larscheid, M. E.; Pigott, F.; Scheidl, F.; Scott, J. W.; Sun, R. C.; Townsend, J. M.; Williams, T. H. *J. Org. Chem.* **1979**, *44*, 3741.

(17) Byproduct **10** was likely formed by reaction of an enolate derived from **8** with acetic anhydride. When acetic anhydride was replaced by bulkier anhydrides (e.g., isobutyric anhydride) to try to minimize the side reaction leading to **10**, the rate of the Perkin reaction was considerably decreased and the reaction profile was less clean.

(18) Pham, T.; Lubell, W. D. *J. Org. Chem.* **1994**, *59*, 3676.

(19) Burk, M. J.; Allen, J. G. *J. Org. Chem.* **1997**, *62*, 7054.

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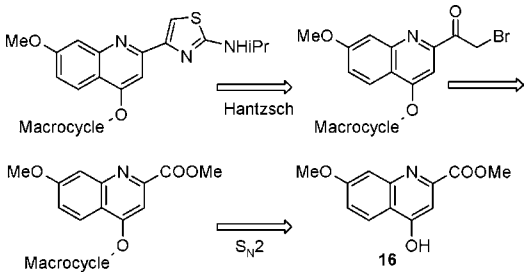

Figure 2. Strategy for the elaboration of the thiazolyl.

could be used to perform this reaction with similar conversion efficiency. However, Hoveyda's modified catalyst **14**[20b] afforded product **15** with less byproducts.[21] Furthermore, this catalyst could be recovered from the crude reaction isolate and recycled.

For the elaboration of the thiazolylquinoline building block, the synthetic sequence was primarily designed to facilitate SAR studies of the thiazolyl ring. For this reason, we favored a two-stage method where 2-carbomethoxyquinoline **16** is added to a (4*S*)-hydroxyproline-containing macrocyclic substrate via a S_N2 reaction (Figure 2). The thiazolyl ring is in turn elaborated using the classical Hantzsch synthesis from the α-bromoketone, which is easily prepared from the corresponding methyl ester.[22] Fragment **16** was prepared via a known two-step sequence from *o*-anisidine and dimethyl acetylenedicarboxylate.[23]

Coupling of the macrocyclic fragment **15** to hydroxyquinoline **16** via a Mitsunobu reaction[13] afforded the desired coupled product **17a** cleanly with no olefinic elimination byproducts (Scheme 1).[24] After conversion of the Boc group of **17a** into a cyclopentylcarbamate in two steps, the resulting product **17b** was subjected to a regioselective hydrolysis of the least hindered methyl ester. The resulting free carboxylate was converted into a bromomethyl ketone via a diazoketone intermediate. To complete the elaboration of the thiazolyl ring, the bromomethyl ketone was subsequently treated with isopropylthiourea to afford **18**. Hydrolysis of the remaining methyl ester under standard conditions completed the synthesis of BILN 2061.

In short, we describe a highly convergent route for the synthesis of BILN 2061, the first reported HCV NS3 protease inhibitor to have shown an antiviral effect in infected humans. The synthesis is based on the preparation and assembly of four fragments with an overall yield of 10−15% and a longest linear sequence of 24 steps. This route allowed for expedient SAR studies and optimization leading to BILN 2061 and was safely used to produce the first batches of active ingredient in multigram scale for preclinical pharmacological evaluation.

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Supporting Information Available: Experimental procedures, characterization data for all new compounds, and complete NMR characterization of BILN 2061 with fully assigned spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Poirier, M.; Ferland, J.-M.; Tsantrizos, Y. S. Manuscript in preparation.

(22) Alternatively, a fully assembled 4-hydroxy-7-methoxy-2-thiazolylquinoline can be coupled to the macrocyclic substrate as described in: Tsantrisos, Y. S.; Cameron, D,; Faucher, A.-M.; Ghiro, E.; Goudreau, N.; Halmos, T.; Llinàs-Brunet, M. Boehringer Ingelheim (Canada) Ltd. U.S. Patent 6,608,027, 2003; *Chem. Abstr*. 139:197768.

(23) Liu, K.-C.; Lu, W.-C.; Lee, L.-C. *Taiwan Yaoxue Zazhi* **1979**, *31*, 80; *Chem. Abstr.* 94:174832.

(24) By contrast, the S_N2 reaction of mesylated 4-hydroxyproline by 4-hydroxyquinoline derivatives produces a small amount of elimination byproduct that is difficult to separate from the desired substitution product (data not shown).