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Discovery of novel phosphonate derivatives as hepatitis C virus NS3 protease inhibitors

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ABSTRACT

A novel class of phosphonate derivatives was designed to mimic the interaction of product-like carboxylate based inhibitors of HCV NS3 protease. A phosphonic acid (compound **2**) was demonstrated to be a potent HCV NS3 protease inhibitor, and a potential candidate for treating HCV infection. The syntheses and preliminary biological evaluation of this phosphonate class of inhibitor are described.

Hepatitis C virus (HCV) infection remains a significant public health problem; an estimated 3% of the world population has been infected.[1] HCV infection is usually asymptomatic and frequently becomes chronic which leads to a significantly increased risk of developing hepatocellular carcinoma and liver cirrhosis. The current standard of therapy is based on α-interferon (Peg-Intron® and Pegasys®) in combination with ribavirin. This existing therapy is only partially effective with ~50% of patients with HCV genotype 1 demonstrating sustained virological responses. Furthermore, the current therapy is very poorly tolerated with ~75% of patients exhibiting systemic side-effects including flu-like symptoms, hematological abnormalities and neuropsychiatric symptoms such that treatment is deferred in the majority of patients due to mild disease, a low chance of response or contra-indications.

In recent years, direct anti-viral drugs targeting the inhibition of viral enzymes have shown promise in human clinical trials. One of these viral targets is the protein product of the non structural gene 3 (NS3) which possesses both protease and helicase activities. So far, there are several inhibitors targeting NS3 protease activity, such as ciluprevir **1** (BILN-2061), telaprevir (VX-950) and boceprevir (SCH 503034), that have shown impressive anti-viral activity in man.[2–4]

There are generally two classes of NS3 protease inhibitor. One is comprised of product based inhibitors which contain a P1 carboxylic acid such as **1** (BILN-2061), or the carboxylate isostere acylsulfonamide, first disclosed by Bristol-Myers Squibb scientists.[5] The second class of NS3 inhibitors are so called serine traps. This class contains an electrophilic carbonyl group, such as a α-ketoamide, represented by VX-950 and SCH 503034. This α-ketoamide forms a covalent bond with the active site serine residue in a reversible fashion.

A major challenge to developing small molecule NS3 inhibitors is the featureless and relatively solvent exposed active site. A potent inhibitor requires a series of weak lipophilic and electrostatic interactions distributed along its contact with the protease. In the case of product based inhibitors, the P1 carboxylate has been shown to be crucial for potency; it helps to anchor the inhibitor through H-bond interactions with the oxyanion hole.[6]

Phosphonates have been studied extensively in drug design as biologically active compounds and as isosteric replacements for carboxylates.[7] In our HCV NS3 protease inhibitor project, we envisioned that a pentavalent phosphonic acid would bind to the S1 oxyanion hole in a similar fashion as the carboxylate. Molecular modeling studies suggested that a phosphonic acid would be capable of making extensive interactions with the oxyanion hole of HCV protease. As shown in Figure 1, phosphonic acid **2** was expected to maintain similar interactions with the His 57 side chain and the Gly 137 backbone amide, as observed for BILN-2061. Furthermore, the phosphonic acid might make additional H-bonds with the side chains of Ser 139 and Lys 136, both of which are conserved active site residues. Because of the potential for more extensive interactions with the oxyanion hole, it was expected phosphonic acid **2**

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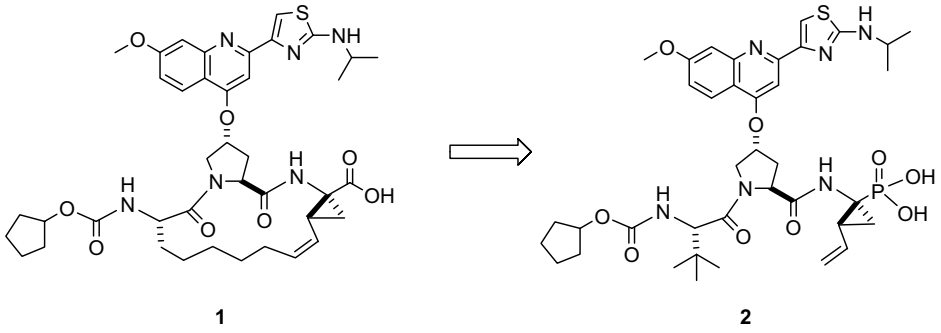


1 2

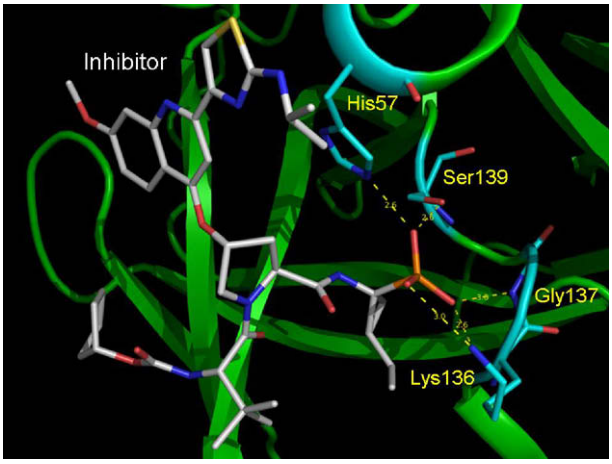


Figure 1. Proposed phosphonate NS3 protease inhibitor. Model of phosphonic inhibitor **2** bound to HCV protease. Atoms are colored according to atom type, except for carbons, which is colored white for inhibitor and cyan for the oxyanion hole residues of the protease, respectively. Yellow dashes indicate potential H-bonds, green cartoons represent HCV protease.

would bind the enzyme stronger than its carboxylic acid counterpart.

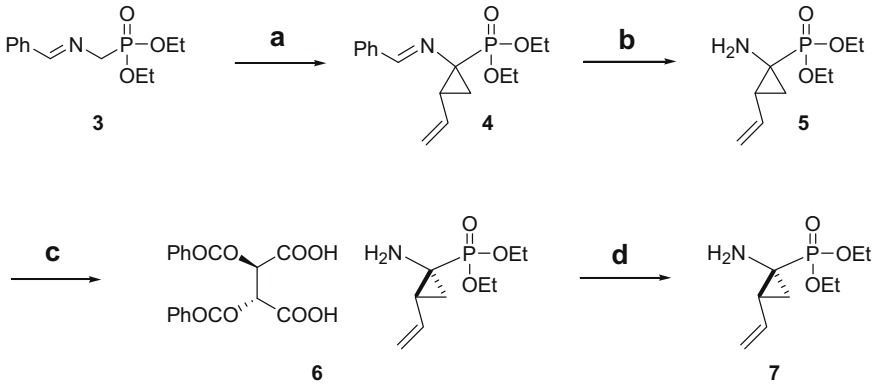
It was our concern that the strong acidity of phosphonic acids may interfere with membrane permeability. Further analysis of modeling results suggested that one of the hydroxyl groups of the phosphonic acid is solvent exposed and could accommodate a large ester moiety. It is therefore possible to reduce the polarity of phosphonic acid analogs by ester formation, without a loss in potency. In this Letter, we wish to report the results of our research.

The P1 amino phosphonate was prepared in good overall yield starting from the commercially available intermediate **3** (Scheme 1).

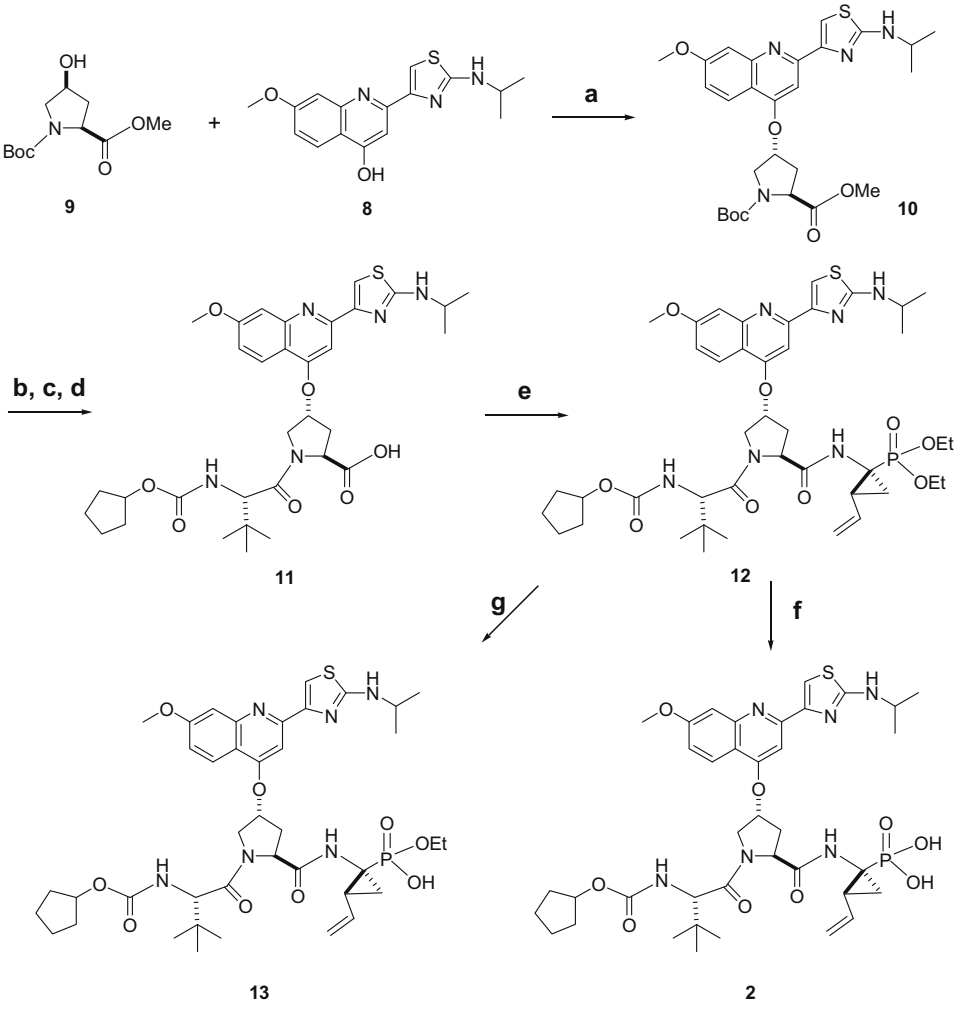
Condensation of the [(benzylidene-amino)-methyl]-phosphonic acid diethyl ester **3** with *trans*-1,4-dibromo-but-2-ene in the presence of cesium hydroxide and a phase transfer catalyst provided the corresponding phosphonate **4**. After the resulting imine was hydrolyzed, chiral resolution provided the optically pure amine salt **6** as the salt of dibenzoyl-L-tartaric acid.[8] A simple biphasic extraction was used to free the amine salt to yield the free amine **7** in quantitative yield.

The synthesis of phosphonate NS3 inhibitors utilizing amine **7** is outlined in Scheme 2.

The P2 aminothiazole **8** was reacted with hydroxyl proline **9** under Mitsunobu conditions to give ether **10**. Protecting group removal, followed by peptide coupling with *tert*-butyl leucine and subsequent saponification provided the carboxylic acid dipeptide **11**. Coupling with amino phosphonate **7** proceeded smoothly



Scheme 1. Synthesis of P1 amino phosphonate. Reagents and conditions: (a) 1,4-dibromo-but-2-ene, CsOH, $BnEt_3NCl$, CH_2Cl_2, reflux 24 h; (b) 1 N aq HCl, rt 3 h, 40% for two steps; (c) dibenzoyl-L-tartaric acid, CH_3CN, 40%; (d) $NaHCO_3$, CH_2Cl_2, 100%.



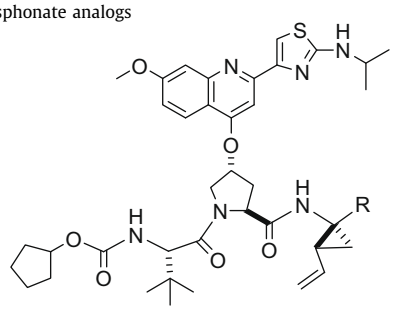
Scheme 2. Synthesis of phosphonic acids. Reagents and conditions: (a) DIAD, PPh_3, THF, 70%; (b) HCl/dioxane, methylene chloride, 2 h; (c) *tert*-butyl leucine, HATU, DMF, Hunig's base, 81% for steps b and c; (d) LiOH, THF:H_2O:MeOH, rt, 2 h, 100%; (e) aminophosphonate **7**, HATU, NMM, methylene chloride, 12 h, 70%; (f) TMSI, CH_3CN, 0 °C to rt, 4 h, 80%; (g) NaI, pyridine, reflux, 20 h, 85%.

to provide fully protected phosphonate tripeptide **12**. To obtain the phosphonic acid **2**, the phosphonate diethyl ester was hydrolyzed with TMSI in acetonitrile. The mono phosphonic acid **13** was obtained by a selective hydrolysis using NaI in pyridine.

The phosphonate inhibitors were evaluated in a biochemical assay using the catalytic domain of the NS3 protein and NS4A as a cofactor. Compound **2** showed very potent inhibition of NS3 with an IC_{50} = 0.9 nM. This IC_{50} is better than the corresponding acid **14** (IC_{50} 3 nM). The cellular activity of compound **2** in Huh-7 replicon cells was 92 nM, about 15-fold higher when compared to the carboxylic acid compound **14**. We suspected that the reduced activity in replicon cells was due to the highly polar phosphonic acid moiety. A Caco-2 permeability assay of compound **2** indeed showed low permeability.[9]

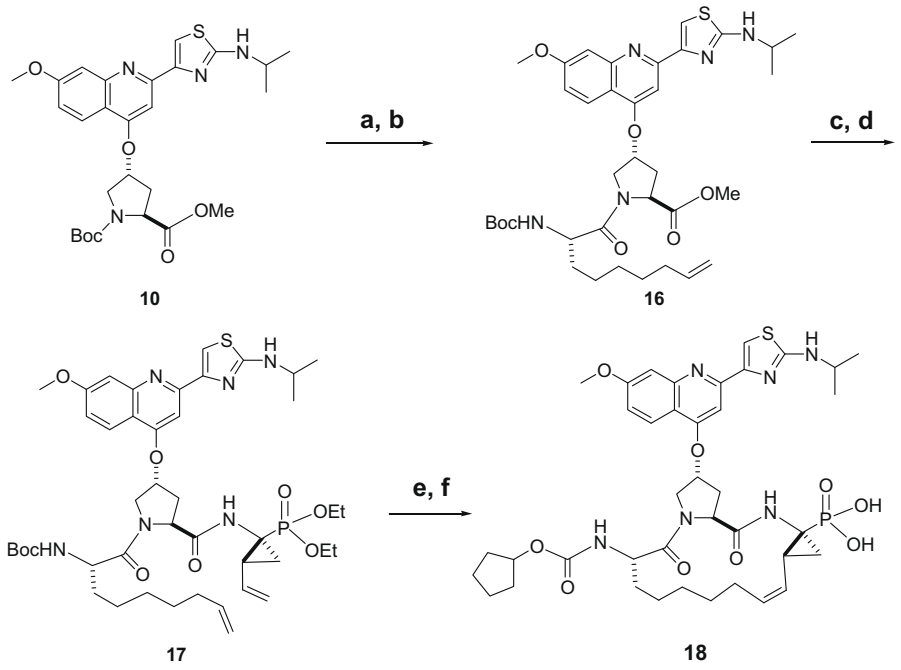
Hoping to reduce the hydrophilicity of the phosphonic acid functionality, we evaluated the mono acidic compound **13**. Unexpectedly, compound **13** showed compromised potency both in the biochemical assay and in the replicon assay compared to compound **2** (eightfold higher IC_{50} and fourfold higher EC_{50}, respectively). To elucidate whether the reduced activity of compound **13** was due to a steric or an electronic effect of the OEt moiety, we synthesized the trifluoroethyl (TFE) mono acid **15**. The IC_{50} of the TFE mono-ester remains almost unchanged as the OEt analog (6 nM), but the replicon activity was improved eightfold (Table 1). These results clearly demonstrated that the cellular activity of phosphonate mono-esters is strongly influenced by the nature of the alkyl group (in this case, an electron withdrawing trifluoroethyl group).

Table 1
Activities of phosphonate analogs



Compounds	R	IC_{50}[a]	EC_{50}[a]	CC_{50}[b]
14	CO_2H	3	6	>50,000
2	$PO(OH)_2$	0.9	92	>50,000
13	PO(OH)(OEt)	7	360	>50,000
15	PO(OH)(OTFE)	6	48	>50,000

[a] All values are given in nM.
[b] Cytotoxicity was measured in Huh-7 cells.

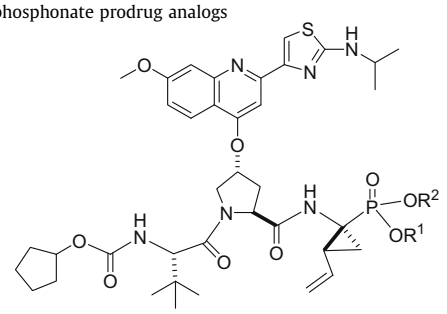


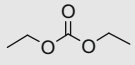
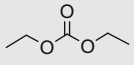
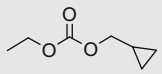
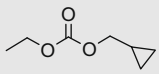
Scheme 3. Synthesis of macrocyclic phosphonic acid. Reagents and conditions: (a) HCl/dioxane, methylene chloride, 2 h; (b) 2-*tert*-butoxycarbonylamino-non-8-enoic acid, HATU, DMF, Hunig's base, 60% for two steps; (c) LiOH, THF:H_2O:MeOH, rt, 2 h, 100%; (d) aminophosphonate **7**, HATU, NMM, methylene chloride, 12 h, 60%; (e) Grubb's catalyst (G1), methylene chloride, reflux 14 h, 20%; (f) TMSI, CH_3CN, rt, 4 h, 80%.

Encouraged by these promising results, we then explored the macrocyclic phosphonate **18**. Scheme 3 depicts the synthesis of compound **18** starting from ether **10**. The Boc protection group of **10** was removed under acidic condition, condensation of resulting amine salt with 2-*tert*-butoxycarbonylamino-non-8-enoic acid yielded dipeptide **16**. Hydrolysis of methyl ester with aq LiOH provided acid which was coupled with amine **7** using HATU and NMM. The resulting intermediate **17** was cyclized with Grubb's G1 catalyst to give the macrocyclic phosphonate diethyl ester in 20% yield. Subsequent hydrolysis with TMSI as described before provided target phosphonic acid **18**.

Macrocyclic phosphonic acid **18** indeed exhibits very potent activity against HCV NS3 protease with an IC_{50} = 1 nM and an EC_{50} = 5 nM.

Table 2
Activities of phosphonate prodrug analogs



Compounds	R^1	R^2	IC_{50}[a]	EC_{50}[a]
19			280	39
20			85	41

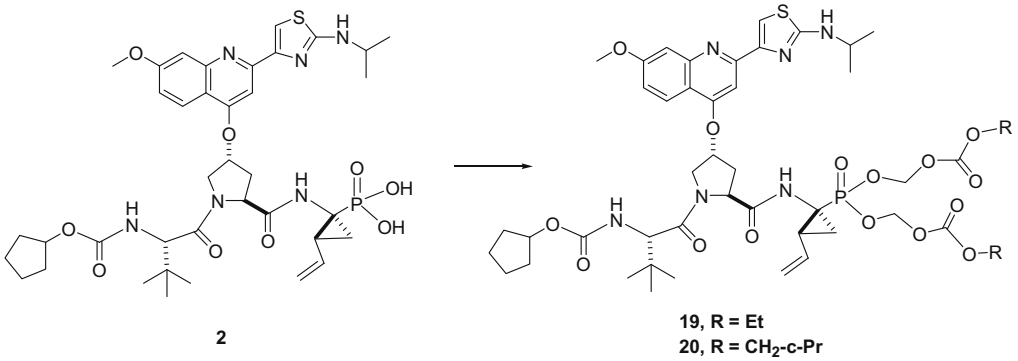
[a] All values are given in nM.

With the promising phosphonic acid NS3 inhibitor in hand, the pharmacokinetic properties of the phosphonate **2** were evaluated in both rat and dog. Although the intravenous plasma exposure (measured as AUC), half-lives and clearance in both species were generally good, the oral bioavailability was rather low. The poor oral bioavailability of the phosphonate **2** was attributed to the low permeability of the highly charged phosphonate anion under physiological conditions. One way to overcome the low membrane permeability of the phosphonate was a pro-drug approach. As seen in tenofovir and adefovir, ester formation with acyloxymethyl and alkoxy-carbonyloxymethyl delivered the active agents to systemic circulation by enhancing intestinal absorption.[10] A search of literature for possible alkoxy-carbonyloxymethyl ester prodrugs indicated that the most straight forward examples for phosphonates were the bis-(ethoxycarbonylmethoxy) ester and the bis-(cyclopropylmethoxycarbonylmethoxy) ester. The synthesis of these prodrugs is described in Scheme 4. The activities of these prodrugs are listed in Table 2.

The pharmacokinetic properties of phosphonate prodrug **19** were evaluated in dogs to determine if the prodrug approach improved the oral bioavailability. After intravenous administration of 1.1 mg/kg of compound **19** in dogs, the apparent systemic clearance was high relative to the liver blood flow (1.7 L/h/kg); a significant amount of the mono ester was detected as the major metabolite. The parent compound **2** was also observed. Oral administration at 2.7 mg/kg gave F% = 1 of the parent compound. Another prodrug, compound **20** showed a similar profile in dog PK studies.

Further optimization efforts to improve oral absorption of the phosphonate, as well as anti-viral activities, will be reported in the future.

In summary, a novel class of phosphonate derivatives as potent NS3 protease inhibitors was identified. These inhibitors have been shown to have nanomolar activities in biochemical and replicon assays. Phosphonate compound **2** was shown to be even more potent than the corresponding carboxylate **14** in the enzyme assay.



Scheme 4. Synthesis of phosphonate prodrugs. Et_3N, DMP, carbonic acid chloromethyl ester ethyl ester, 50 °C, 22 h, 22% for compound **19**. Et_3N, DMF, TBAI, carbonic acid chloromethyl ester cyclopropylmethyl ester, 70 °C, 5 h, 18% for compound **20**.

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