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1-Aminocyclopropaneboronic Acid: Synthesis and Incorporation into an Inhibitor of Hepatitis C Virus NS3 Protease

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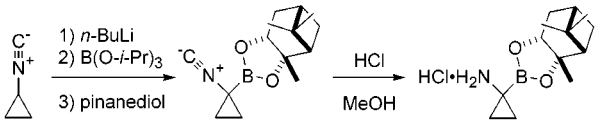
ABSTRACT

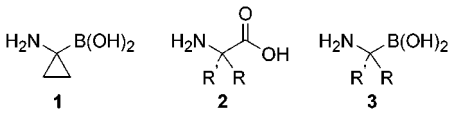


The previously unreported α,α-disubstituted 1-aminoboronate esters have potential utility in peptidomimetic design, particularly against serine protease targets. A concise synthesis of 1-aminocyclopropaneboronate pinanediol ester is reported, and a peptidyl derivative is shown to have modest affinity (K_i = 1.6 μM) for hepatitis C NS3 protease.

Hepatitis C virus chronically infects 1−2% of the world population and causes serious progressive liver disease, including cirrhosis and hepatocellular carcinoma, in a substantial fraction of affected individuals.[1] The best currently approved treatment, combination therapy with α-interferon and ribavirin, results in sustained loss of viral RNA in less than 50% of patients.[2] The development of effective hepatitis C therapeutics is therefore highly important.

Nonstructural protein 3 (NS3), an essential viral enzyme, is a serine protease responsible for cleavage of four sites on the hepatitis C viral polyprotein. In analogy with human immunodefficiency virus, inhibitors of the viral protease should prove to be potent antiviral therapeutics.[3] NS3 protease has several unique features, including a shallow, solvent exposed active site, a preference for cysteine as the P_1 amino acid in peptide substrates,[4] and inhibition by the C-terminal carboxylic acid cleavage product.[5] We and others have found that 1-aminocyclopropanecarboxylic acid is a potent, non-sulfur-containing replacement for the P_1 cysteine in peptide carboxylate inhibitors.[6] In addition, we have found that peptide boronic acids[7] are substantially more potent inhibitors of NS3 protease than the peptide carboxylates.

We therefore sought to capitalize on the combination of these observations by preparing 1-aminocyclopropaneboronic acid (**1**), an α,α-disubstituted amino boronic acid. Although α,α-disubstituted amino acids (**2**) are often important components of natural and synthetic enzyme inhibitors, the corresponding α,α-disubstituted amino boronic acids (**3**) were previously unknown. A concise synthesis of **1** and several related α,α-disubstituted amino boronic acids, together with their incorporation into peptides and determination of NS3 protease inhibitory activity, are reported below.



The established synthetic route to α-amino boronic acids developed by Matteson involves nucleophilic displacement

(1) (a) Liang, T. J.; Rehermann, B. Seeff, L. B.; Hoofnagle, J. H. *Ann. Intern. Med.* **2000**, *132*, 296−305. (b) Cohen, J. *Science* (*Washington, D.C.*) **1999**, *285*, 26−30.

(2) (a) Mchutchison, J. G.; et al. *N. Engl. J. Med.* **1998**, *339*, 1485−1492. (b) Poynard, T.; et al. *Lancet* **1998**, 352, 1426−1432. (c) Davis, G. L.; et al. *N. Engl. J. Med.* **1998**, *339*, 1493−1499.

(3) Redshaw, S.; Thomas, G. J. *Emerging Drugs* **1997**, *2*, 127−154.

(4) For a description of the protease nomenclature, see: Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157−162.

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of an α-chloroboronic ester by a nitrogen nucleophile such as bis(trimethylsilyl)amide or azide.[8] Retrosynthetic analysis of **1** suggests that this approach (Scheme 1, disconnection

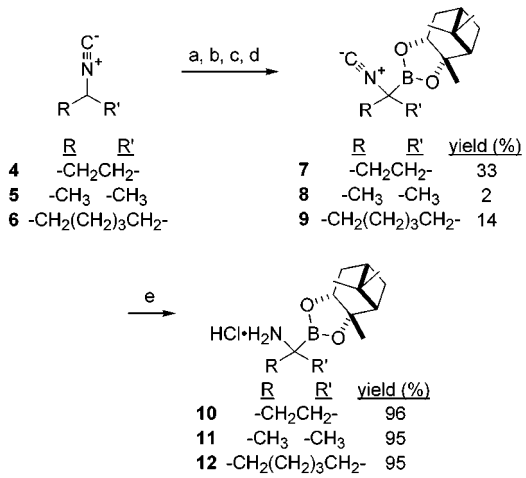
Scheme 1. Retrosynthetic Analysis of **1**

A) may prove troublesome, since it requires a nucleophilic displacement to occur at a tertiary cyclopropyl center. Initial experiments using this approach proved unsuccessful.[9] An alternative approach (Scheme 1, disconnection B) involves reaction of an α-aminocyclopropyl anion with a boron electrophile.

Schollkopf has demonstrated that metalated isocyanides are excellent α-amino anion equivalents.[10] They react with a variety of electrophiles in good yield, and the products can be readily converted to amines by acidic hydrolysis. In particular, amino acids have been prepared by carboxylation of metalated isocyanides, followed by hydrolysis.[11] The metalation of cyclopropyl isocyanide, **4**, with *n*-butyllithium at −70 °C has been reported.[10b] On the basis of these precedents, the synthesis of **1** was attempted by approach B (Scheme 1).

The synthesis of the (+)-pinanediol ester of **1** is shown in Scheme 2. Cyclopropyl isocyanide,[12] **4**, prepared by dehydration of cyclopropyl formamide with *p*-toluenesulfonyl chloride and tributylamine,[13] was metalated with *n*-butyllithium at −78 °C and treated with triisopropyl borate. After neutralization and conversion to the pinanediol ester, the desired α-isocyanoboronic ester was obtained in 33% yield.

Scheme 2. Synthesis of α,α-Disubstituted Amino Boronic Acids[a]

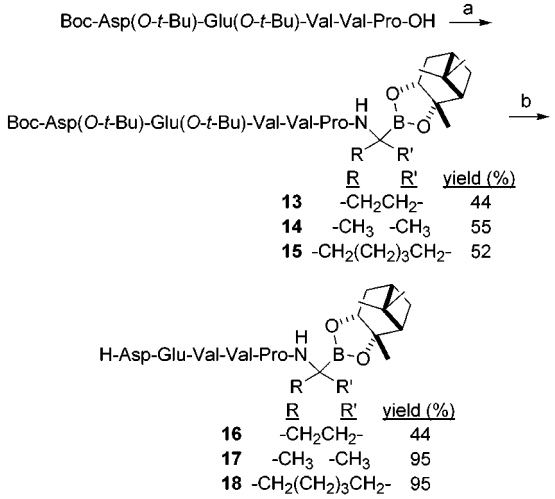


[a] Conditions: (a) *n*-BuLi or LTMP, THF, −78 °C, 30 min; (b) triisopropylborate, −78 °C to rt, 14 h; (c) $NaHSO_4$; (d) (+)-pinanediol, ether; (e) HCl, MeOH.

The pinanediol ester was chosen to protect the boronic acid for its excellent stability. Although there is a single previous report[14] describing preparation of unsubstituted and monosubstituted 1-isocyanoboronic esters by dehydration of α-formamidoboronic esters, this is the first example of the preparation of an α,α-disubstituted 1-isocyanoboronic ester. **7** was converted in high yield to the amine hydrochloride **10** by treatment with aqueous hydrochloric acid in methanol. The related dimethyl and cyclohexyl derivatives were also prepared by this route (using lithium 2,2,6,6-tetramethylpiperidide for the metalation step) from the commercially available isocyanides. The low yields in the preparation of **7**−**9** suggest that the product α-isocyanoboronic esters are not stable to the basic reaction conditions, since the isonitriles **4**−**6** are known to give high yields in reactions with other electrophiles.[10] Further attempts to optimize the reaction conditions are in progress. We note that extension of this chemistry to monosubstituted isocyanides would provide a general approach to the synthesis of α-amino boronic acids using readily available amines as starting materials.

The α-amino boronic acids **10**−**12** were each coupled to the pentapeptide Boc-Asp(*O*-*t*-Bu)-Glu(*O*-*t*-Bu)-Val-Val-Pro-OH and subsequently deprotected as shown in Scheme 3.

Scheme 3. Synthesis of Peptide Boronic Acids[a]



[a] Conditions: (a) **10**, **11**, or **12**, PyAOP, DIEA, DMF, rt, 2 h; (b) 90% TFA, 5% triisopropylsilane, CH_2Cl_2, rt, 3 h, or 4 N HCl/dioxane, rt, 3 h.

The peptide sequence was chosen on the basis of previous work elucidating the substrate specificity of HCV NS3

(5) De Francesco, R.; Pessi, A.; Steinkuhler, C. *Antiviral Ther.* **1998**, *3* (Supplement 3), 99−109.
(6) (a) Llinas-Brunet, M.; et al. WO 99/07733 (b) Graciani, N. R.; Combs, A. P. DuPont Pharmaceuticals, unpublished data.
(7) Kettner, C. A.; Shenvi, A. B. *J. Biol. Chem.* **1984**, *259*, 15106−15114.
(8) (a) Matteson, D. S.; Sadhu, K. M. *Organometallics* **1984**, *3*, 614−618. (b) Matteson, D. S.; Jesthi, P. K.; Sadhu, K. M. *Organometallics* **1984**, *3*, 1284−1288.
(9) Jagannathan, S.; Kettner, C. A. DuPont Pharmaceuticals, unpublished data.

protease.[15] In the case of peptide **13**, containing a 1-aminocyclopropane boronic ester residue, deprotection in trifluoroacetic acid containing 5% triisopropylsilane afforded a substantial, unidentified byproduct. However, use of 4 N hydrogen chloride/dioxane was found to provide a homogeneous sample of the desired product **16**.

The peptide boronic acids **16**, **17**, and **18** were tested for inhibition of HCV NS3 protease.[16,17] Compound **16**, containing 1-aminocyclopropaneboronic acid as the P1 residue, was found to be a modestly potent, reversible inhibitor, with a K_i of 1.6 μM, while **17** and **18** each had $K_i > 100$ μM. For comparison, the peptide carboxylate Ac-Asp-Glu-Val-Val-Pro-Acc-OH[18] has a K_i of 4.0 μM. Although **16** is not highly potent against NS3 protease, inhibitors based on 1-aminocyclopropaneboronic acid and other α,α-disubstituted amino boronic acids made accessible by this synthetic route may be useful for inhibition of other therapeutically important serine proteases.

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Supporting Information Available: Experimental procedures and analytical data for compounds **7**, **10**, **13**, and **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) (a) Schollkopf, U.; Gerhart, F. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 805. (b) Hoppe, D. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 789−804 and references therein.

(11) Matsumoto, K.; Suzuki, M.; Miyoshi, M. *J. Org. Chem.* **1973**, *38*, 2094−2096.

(12) **Caution:** cyclopropyl isocyanide may be explosive. As noted by a reviewer, methyl isocyanide is known to be explosive under certain conditions. Comparison of calculated ΔH_f values (RHF/MNDO) for methyl isocyanide (+60.3 kcal/mol) and cyclopropyl isocyanide (+83.8 kcal/mol) indicates that proper precautions should be taken when handling this material.

(13) Schollkopf, U.; Gerhart, F.; Hoppe, I.; Harms, R.; Hantke, K.; Scheunemanne, K.-H.; Eilers, E.; Blume, E. *Liebigs Ann. Chem.* **1976**, 183−202.

(14) Versleijen, J. P. G.; Faber, P. M.; Bodewes, H. H.; Braker, A. H.; van Leusen, D.; van Leusen, A. M. *Tetrahedron Lett.* **1995**, *36*, 2109−2112.

(15) Zhang, R.; Durkin, J.; Windsor, W. T.; McNemar, C.; Ramanathan, L.; Le, H. V. *J. Virol.* **1997**, *71*, 6208−6213. (b) Urbani, A.; Bianchi, E.; Narjes, F.; Tramontano, A.; De Francesco, R.; Steinkuhler, C.; Pessi, A. *J. Biol. Chem.* **1997**, *272*, 9204−9209.

(16) Compounds **16**−**18** were tested for enzyme inhibition as their pinanediol esters, as it has been demonstrated that boronic acid pinanediol esters are rapidly hydrolyzed to the free boronic acid in dilute aqueous solution (see ref 7).

(17) Inhibition of protease activity was determined using a modification of the reported method (Taliani, M.; et al. *Anal. Biochem.* **1996**, *240*, 60−67). The fluorescence-based continuous assay contained 50 mM Tris pH 7.0, 5 mM DTT, 50% glycerol, 2% CHAPS, 10 μM NS4A cofactor peptide, and 4 nM NS3 protease. Inhibitors were serially diluted into the reaction mixture followed by a 15 min preincubation with the enzyme. Catalysis was initiated by the addition of the fluorogenic ester substrate Ac-DED(EDANS)EEAbuΨ[COO]ASK(DABCYL)-NH_2 (final concentration = 5 μM). Assays were conducted at ambient temperature. Enzymatic activity was monitored with a Perkin-Elmer LS 50B luminescence spectrometer (excitation = 360 nm, emission = 530 nm, both slits were set to 10 nm). Inhibition constants were determined from a nonlinear least-squares fit of the data to the equation $V_{[I]}/V_o = 1/(1 + [I]/K_{i,app})$. The thermodynamic K_i was determined from $K_{i,app}$ via the relationship for competative kinetics: $K_i = K_{i,app}/(1 + [S]/K_m)$ where [S] = 5 μM and K_m = 20 μM.

(18) Acc = 1-aminocyclopropanecarboxylic acid.