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Synthesis and evaluation of novel α-amino cyclic boronates as inhibitors of HCV NS3 protease

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ABSTRACT

We have designed and synthesized a novel series of α-amino cyclic boronates and incorporated them successfully in several acyclic templates at the P1 position. These compounds are inhibitors of the HCV NS3 serine protease, and structural studies show that they inhibit the NS3 protease by trapping the Ser-139 hydroxyl group in the active site. Synthetic methodologies and SARs of this series of compounds are described.

Hepatitis C Virus (HCV) infection affects more than 200 million people worldwide, leading to chronic liver disease such as cirrhosis, carcinoma and liver failure.[1] The current standard of care for HCV infection is a combination of injectable pegylated interferon-α (PEG IFN-α) plus oral ribavirin, which is effective in only 50% of genotype-1 patients achieving sustained viral response.[2] Also, this treatment has been associated with serious side effects including neuropsychiatric events, flu-like symptoms and hematological toxicities.[3] Therefore, there has been tremendous interest in the development of more effective therapeutics in treating HCV infection.

One of the clinically validated targets is HCV NS3/4A serine protease. HCV NS3/4A serine protease plays a critical role in the HCV replication, and inhibition of this enzyme results in viral suppression in the clinic.[4] The NS3 enzyme with the assistance of the co-factor NS4A is responsible for cleaving several downstream sites along the HCV viral polyprotein to produce functional proteins. The X-ray structures, both as an isolated domain and in the full-length NS3 protein have been determined.[5] The NS3 protease is a typical serine protease with a classical Asp-His-Ser catalytic triad, and it cleaves the scissile Cys-Ser amide bond by the nucleophilic attack of Ser-139. The NS3 protease has a flat, shallow and solvent-exposed substrate binding region. High throughput screening efforts have been ineffective in generating leads. Rational design of lead inhibitors typically originate from a peptide substrate via structure-based design and often results in compounds with significant peptide characteristics.

Extensive efforts in the discovery of HCV NS3 protease inhibitors have resulted in a number of candidates in various stages of clinical development.[6] The two most advanced compounds are VX-950 (telaprevir, Fig. 1)[6b] and SCH-503034 (boceprevir, Fig. 1)[6c] currently undergoing Phase III clinical trials, which has established proof of concept in suppression of virus counts. A common feature of these two compounds is that they both contain α-ketoamide moiety at the P1 position, which acts as an active-site

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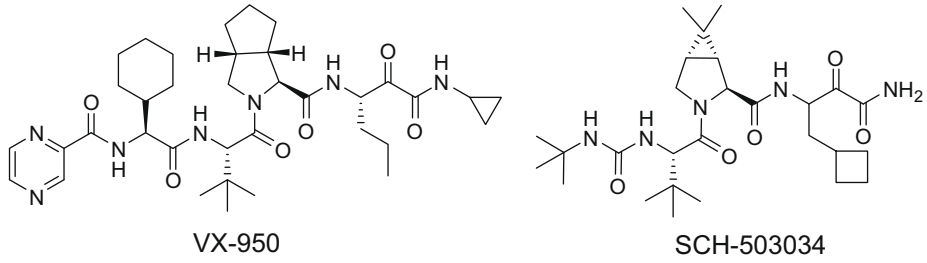


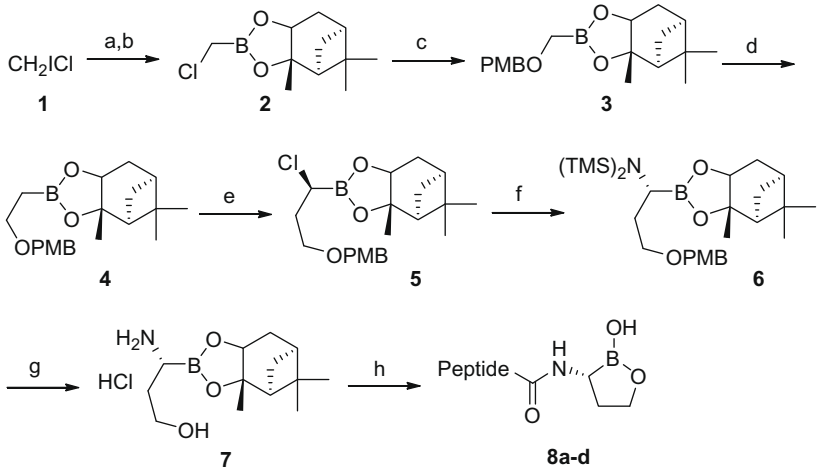
Figure 1. Known α-ketoamides as NS3 serine protease inhibitors.

serine trap and is essential for the inhibitory activity. After they bind to the enzyme, nucleophilic attack of α-ketoamide by the hydroxyl group of Ser-139 leads to a covalent tetrahedral intermediate stabilized by the oxyanion hole in the enzyme active site.

Classical α-amino boronic acids have been studied extensively as inhibitors of various proteases.[7] Velcade® is the first peptidomimetic boronic acid that has been successfully developed into a human therapeutic agent for treating relapsed multiple myeloma.[8] Also, boronic acid compounds have been reported as inhibitors of HCV NS3 serine protease by several research groups in pharmaceutical companies including Schering-Plough,[9a] Phenomix,[9b] Dupont,[9c] and BMS.[9d,e] In our ongoing effort to explore and use novel boron compounds such as benzoxaboroles as therapeutics,[10] we have envisioned that α-amino oxaborole (five-membered cyclic boronate) can serve as an active-site serine trap for HCV NS3 protease.[11] Our docking analysis suggests that through a covalent bond between the boron and Ser-139 hydroxyl group, α-amino oxaborole can be used to replace the P1 α-ketoamide moiety in VX-950 or SCH-503034 while maintaining the P2-P4 moieties. Our approach was to begin with the synthesis of α-amino oxaborole, followed by its incorporation into several NS3 protease inhibitor templates, including VX-950 and SCH-503034.

The synthetic route outlined in Scheme 1 has been developed to prepare the five-membered α-amino oxaborole inhibitors. The transmetalation of CH_2ICl **1** with BuLi in the presence of triisopropyl borate gave diisopropyl chloromethylboronate.[12] This intermediate was transesterified with (+)-pinanediol to give pinanediol ester **2**. The reaction of **2** with lithium *p*-methoxybenzyloxide gave the PMB-protected alcohol **3**.[13] The chain extension of **3** using the transmetalation conditions of the first step afforded boronate **4**. The methylene chloride insertion of **4** using dichloromethyllithium by the method of Matteson[14] gave (*R*) α-chloroboronate **5**. Nucleophilic displacement of **5** by lithium bis(trimethylsilyl)amide gave the TMS-protected α-amino boronate **6**, followed by treating with anhydrous HCl to produce α-amino boronate **7** as HCl salt. Coupling of **7** with peptide acid **a**–**d**[15] (Fig. 2) using HATU/DIEA yielded the carboxamide. Subsequently, treatment of the amide with isobutyl boronic acid in the presence of HCl afforded the desired product **8a**–**d**, with (*R*) α-amino oxaborole incorporated at the P1 position.[16] Using (−)-pinanediol ester of **2** via the same route, the corresponding (*S*) α-amino oxaborole inhibitors (**9c**,**d**) were also synthesized.

These novel α-amino oxaborole inhibitors were evaluated using FRET assay with HCV NS3/4A 1a protease domain.[17] The IC_{50} value of VX-950 was found to be 0.10 μM in this assay, which is in consistent with the reported value (K_i = 0.044 μM).[6b] When the α-amino oxaborole was incorporated into the VX-950 template, resulting compound **8a** exhibited good potency (IC_{50} = 0.12 μM). When the SCH-503034 template was used, the resulting compound **8b** was threefold less potent than **8a** (IC_{50} = 0.34 μM). On the other hand, incorporation of this α-amino oxaborole into the P2* isoindoline-containing template **c** resulted in a very potent compound **8c** (IC_{50} = 0.023 μM). Replacement of the isoindoline with an isoquinoline at the P2* site led to compound **8d** with an IC_{50} of 0.12 μM. From these studies, it appeared that template **c** worked best among the four peptide templates in combination with the α-amino oxaborole warhead. Based on these results, the two related templates **c** and **d** were selected for SAR exploration of cyclic boronate inhibitors with different ring size and substitution patterns.



Scheme 1. Reagents and conditions: (a) $B(OiPr)_3$, *n*-BuLi, THF, −78 °C, 50%; (b) (+)-pinanediol, Et_2O, 98%; (c) PMB–OH, *n*-BuLi, DMSO, 58%; (d) CH_2ICl, *n*-BuLi, THF, −78 °C, 47%; (e) $LiCHCl_2$, THF, −100 °C, then $ZnCl_2$, 75%; (f) $LiN(TMS)_2$, THF, −78 °C, 90%, (g) 4 N HCl in dioxane, hexane, 98%; (h) peptide acid **a**–**d**, HATU, DIEA, DMF, then *i*-$BuB(OH)_2$, MeOH/hexane, HCl, 20–32%.

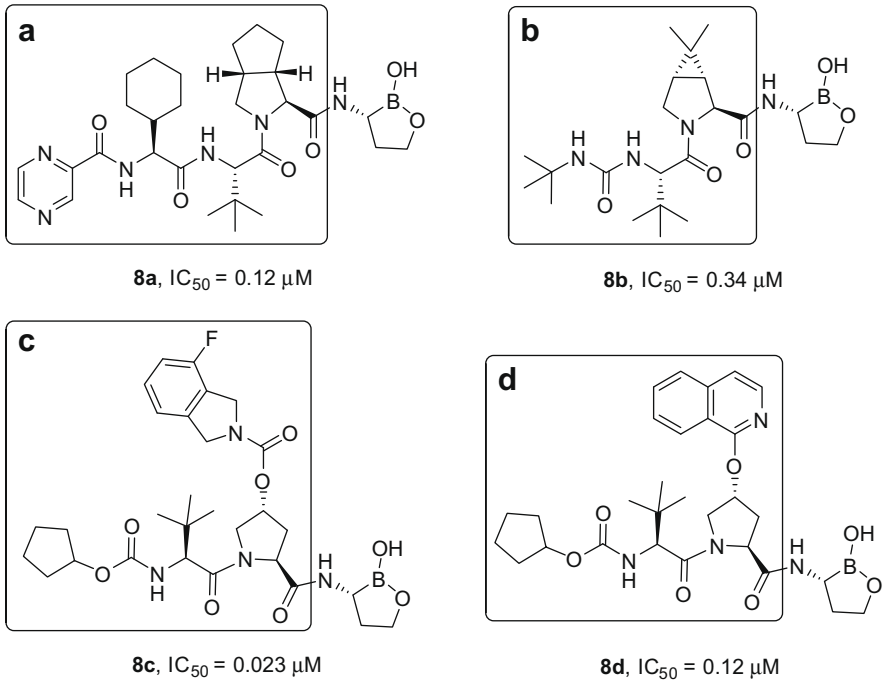
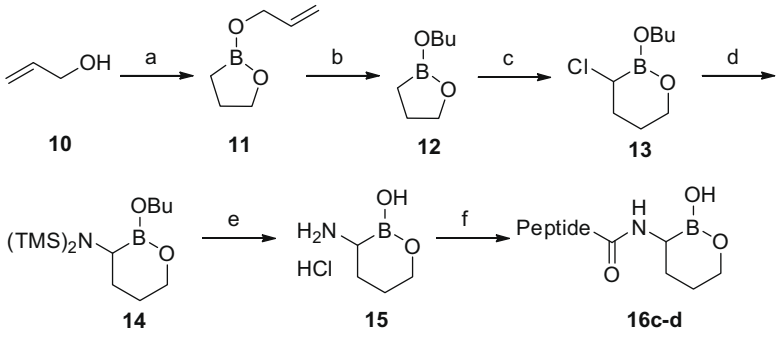
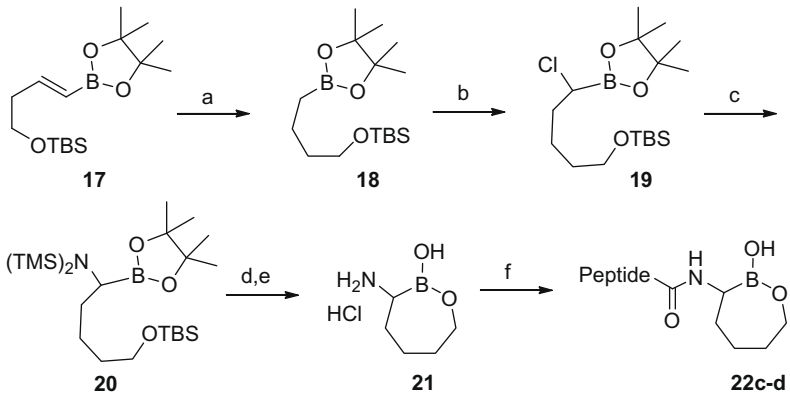


Figure 2. Four NS3 protease inhibitor templates evaluated.



Scheme 2. Reagents and conditions: (a) $NaBH_4$, AcOH, 120–130 °C, 40%; (b) *n*-BuOH, 71%; (c) $LiCHCl_2$, THF, −100 °C, 80%; (d) $LiN(TMS)_2$, THF, −78 °C, 73%; (e) 4 N HCl in dioxane, hexane, 91%; (f) peptide acid **c**–**d**, iBuOCOCl, TEA, NMM, 27–33%.



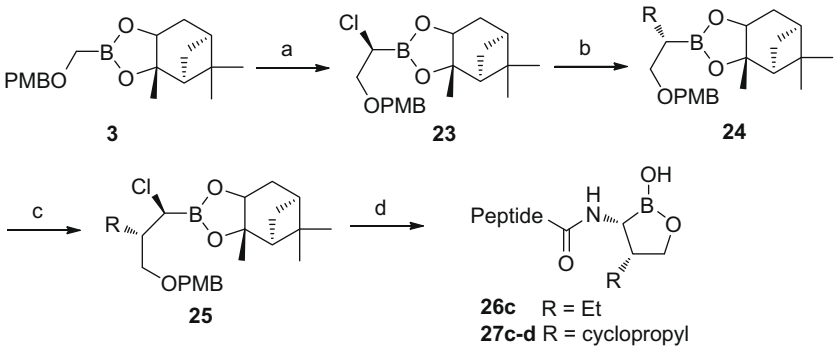
Scheme 3. Reagents and conditions: (a) Pd/C, H_2, MeOH, 99%; (b) $LiCHCl_2$, THF, −100 °C, 66%; (c) $LiN(TMS)_2$, THF, −78 °C, 73%; (d) 4 N HCl in dioxane, hexane, 80%; (e) PS-$PhB(OH)_2$, CH_3CN, HCl, 94%; (f) peptide acid **c**–**d**, iBuOCOCl, TEA, NMM, 17–20%.

Scheme 2 shows the synthetic route for the preparation of the six-membered α-amino cyclic boronates. Allyl alcohol **10** was first treated with $NaBH_4$–HOAc to give allyl boronate ester **11**.[18] After the transesterification of **11** with *n*-butyl alcohol, methy-

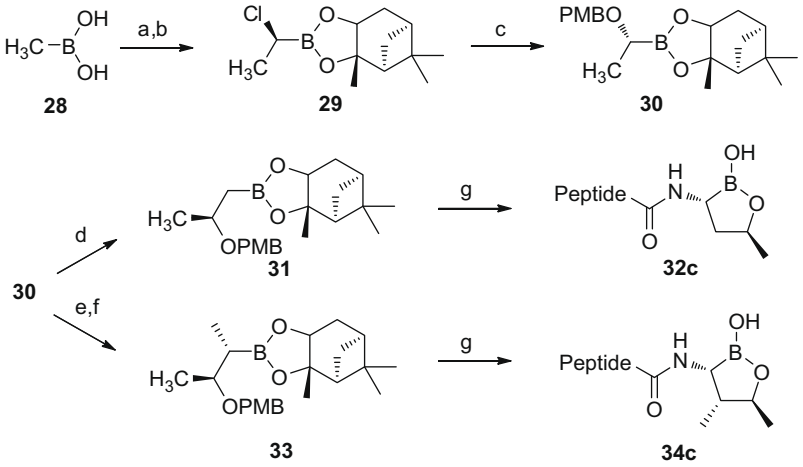
lene chloride insertion of boronate **14** gave chloroboronate **13**. Subsequently, treatment of **13** with lithium bis(trimethylsilyl)amide produced the TMS-protected amino boronate **14**. After treatment with anhydrous HCl, α-amino boronate **15** was isolated as a white solid as HCl salt. Coupling of **15** with peptide acid **c**–**d** was carried out using the mixed anhydride method[9c] to give compound **16c**–**d**.

Scheme 3 shows the synthetic route for the preparation of the seven-membered α-amino cyclic boronates. Commercially available TBS-protected hydroxyl containing boronate **17** was hydrogenated to give the saturated boronate **18**. Methylene chloride insertion of boronate **18** gave α-chloroboronate **19**. Treatment of **19** with lithium bis(trimethylsilyl)amide gave the TMS-protected amino boronate **20**. The treatment of **20** with anhydrous HCl led to the formation of α-amino boronate **21**. Coupling of **21** with peptide acid **c**–**d** was carried out similarly using the mixed anhydride method[9c] to give compound **22c**–**d**.

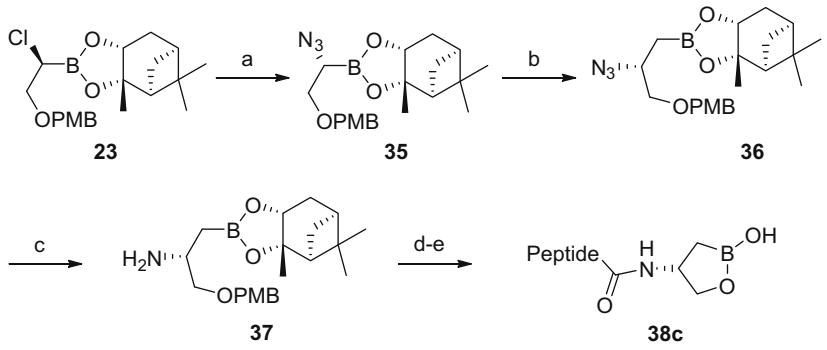
The preparation of the β-substituted oxaborole inhibitors is shown in Scheme 4. Methylene chloride insertion in compound **3** gave (*R*)-chloroboronate **23.** Reaction of **23** with Grignard reagent



Scheme 4. Reagents and conditions: (a) $LiCHCl_2$, THF, −100 °C, then $ZnCl_2$, 43%; (b) RMgBr, THF, −78 °C, 70–90%; (c) $LiCHCl_2$, THF, −100 °C, then $ZnCl_2$, 17–50%; (d) same as steps (f–h) in Scheme 1.



Scheme 5. Reagents and conditions: (a) (+)-pinanediol, Et_2O, 93%; (b) $LiCHCl_2$, THF, −100 °C, then $ZnCl_2$, 91%; (c) *n*-BuLi, PMB–OH, 70%; (d) CH_2ICl, *n*-BuLi, −78 °C, 52%; (e) $LiCHCl_2$, THF, −100 °C, then $ZnCl_2$, 63%; (f) CH_3MgBr, THF, −78 °C, 72%; (g) same as steps (e–h) in Scheme 1.

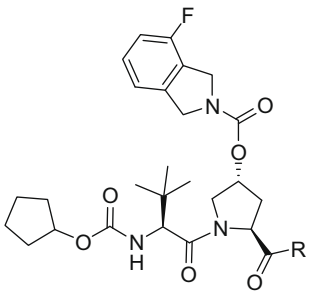


Scheme 6. Reagents and conditions: (a) NaN_3, DMF, 91%; (b) CH_2ICl, *n*-BuLi, −78 °C, 59%; (c) Pd/C, H_2, MeOH, 91%; (d) peptide acid **c**, HATU, DIEA, DMF, 32%; (e) *i*-$BuB(OH)_2$, MeOH/hexane, HCl, then DDQ, DCM/water, 27%.

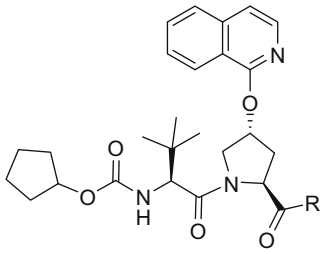
afforded alkyl substituted boronate **24b**, with inversion of stereochemistry. Methylene chloride insertion of **24** again using the same conditions from the previous step gave (*R*)-chloroboronate **25**, with a substitution at the β-position to the boron. Subsequently, compound **25** was taken on to final target **26c** and **27c–d** according to the procedures as shown in Scheme 1.

The γ-substituted oxaboroles were prepared using methylboronic acid **28** according to Scheme 5. After the esterification of **28** with (+)-pinanediol, methylene chloride insertion of pinanediol ester yielded (*R*)-chloroboronate **29**. The reaction of **29** with lithium *p*-methoxybenzyloxide afforded the PMB-protected alcohol **30**. The chain extension of α-methyl boronate **30** gave β-methyl

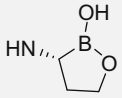
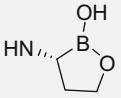
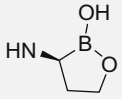
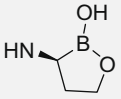
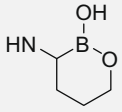
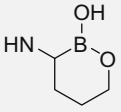
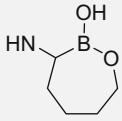
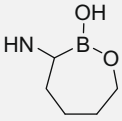
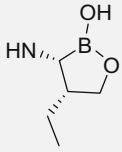
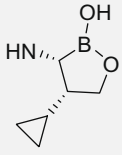
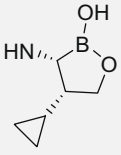
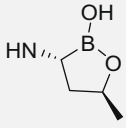
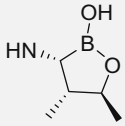
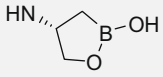
Table 1
In vitro activity of cyclic boronates against HCV NS3/4A 1a.[a]



8c-38c



8d-27d

Compounds	Cyclic boronate R	NS3/4A 1a IC_{50} (μM)	Compounds	Cyclic boronate R	NS3/4A 1a IC_{50} (μM)
8c		0.023	**8d**		0.12
9c		5.31	**9d**		2.75
16c[b]		0.32	**16d**[b]		0.73
22c[b]		0.024	**22d**[b]		0.17
26c		0.33	[c]		
27c		0.20	**27d**		1.93
32c		0.34	[c]		
34c		4.47	[c]		
38c		8.50	[c]		

[a] FRET assay with HCV NS3 1a protease domain in the buffer containing 20% glycerol, as described in Ref. 17. Values are means of duplicate or more experiments.
[b] C-α epimeric pair.
[c] Not synthesized.

boronate **31**, which was taken on to compound **32c** by the reactions similar to the chemistry as shown in Scheme 1. Alternatively, methylene chloride insertion of **30** followed by the reaction with methyl magnesium bromide gave di-methyl substituted boronate **33**, which was carried forward to prepare compound **34c** according to the procedures outlined in Scheme 1.

In addition to α-amino boronate inhibitors, β-amino cyclic oxaboroles were also synthesized to explore optimal position of boron in relation to the active site serine residue. As shown in Scheme 6, compound **23** was treated with sodium azide to give α-azido derivative **35**. Chain extension of **35** gave β-azido boronate **36**. Hydrogenation of **36** afforded β-amino boronate **37**. After compound **37** was incorporated in peptide template **c**, the coupling product was treated with isobutyl boronic acid in the presence of HCl and followed by DDQ treatment to afford product **38c**.

The data shown in Table 1 summarizes the IC_{50} values for the compounds prepared. In general, template **c** series exhibited better inhibitory activities than template **d** series, suggesting that the isoindoline moiety at the P2* site contribute more significantly on inhibitor binding. For both series, (*R*) α-amino oxaborole inhibitors (**8c**,**d**) were significantly more potent than the corresponding (*S*) isomers (**9c**,**d**), which validated the specificity of these inhibitors for HCV NS3 protease. The five-membered oxaboroles were replaced with racemic six- and seven-membered cyclic boronates to further probe the influence of ring size. Interestingly, the seven-membered boronate inhibitors (**22c**,**d**) showed comparable activities as the five-membered boronates (**8c**,**d**). The six-membered cyclic boronate inhibitors (**16c**,**d**) were several fold less active. The substitution at the β-position of these inhibitors (**26c**, **27c**,**d**) resulted in significant decrease in potency, indicating that the small substituents are not well tolerated. The substitution at the γ-position of these boronates (**32c**, **34c**) was not well-tolerated either. For β-amino oxaborole inhibitor **38c**, it was over 300 times less potent compared to the α-amino inhibitor **8c**, suggesting that positioning of the boron plays a critical role in the inhibition of NS3 protease by these α-amino cyclic boronate inhibitors.

X-ray structures were obtained by soaking preformed crystals of NS3 protease domain/synthetic 4A co-factor with compounds in 10–20% DMSO.[19] The X-ray crystal structure of inhibitor **8d** bound to NS3 protease is shown in Figure 3.[20a] The general binding mode of **8d** is very similar to that of an α-ketoamide such as SCH-503034.[6c] The peptide backbone from the inhibitor adopts the extended β-strand conformation and forms three pairs of H-bonds with the protein, while the P1-P4 side chains are properly oriented to fill in S1–S4 subsites respectively. As expected, the boron warhead is covalently linked to the hydroxyl group of Ser-139, and is locked in the negatively-charged tetrahedral form mimicking the transition state. The formation of such a tetrahedral boronate by trapping the Ser-139 with a boronic acid inhibitor of NS3 protease has been reported recently.[9a] It is also interesting to note that the oxygen-boron bond inside the five-membered oxaborole ring of inhibitor **8d** is cleaved, resulting in the hydroxyethyl side chain in the S1 pocket.

In order to understand the binding modes of this series of inhibitors, and also to investigate if the catalytic Ser-139 residue plays a role in the ring opening event, X-ray crystal structure of inhibitor **8d** bound to NS3 protease S139A mutant has been solved.[20b] At the binding site of the mutant as shown in Figure 4, inhibitor **8d** maintained the extended conformation with P2–P4 in respective subsites. However, with Ser-139 absent in this mutant enzyme, electron density clearly shows an intact five-membered oxaborole ring at the S1 subsite. Interestingly, additional electron density is present around the S1′ site which maps well to a spirocyclic glycerol adduct of compound **8d**. Glycerol is present in both the crystallography conditions and assay conditions. Since boron has an empty p orbital, it exists in equilibrium in aqueous solution between trigonal and tetrahedral forms. In the presence of glycerol, a similar equilibrium can also be formed between free oxaborole and the spirocyclic oxaborole glycerol adduct. Crystallography data with the mutant suggests that all boron trigonal and tetrahedral forms can access the enzyme active site. It is likely that the catalytic Ser-139 residue plays an important role in the ring opening of the oxaborole in the inhibition process.

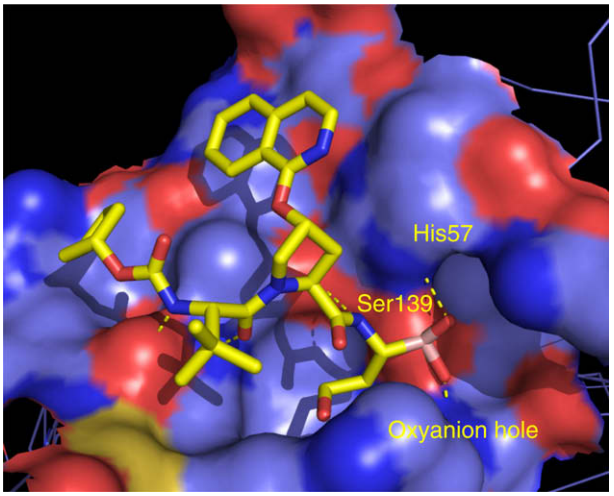


Figure 3. X-ray crystal structure of boronate inhibitor **8d** complexed with HCV NS3 serine protease

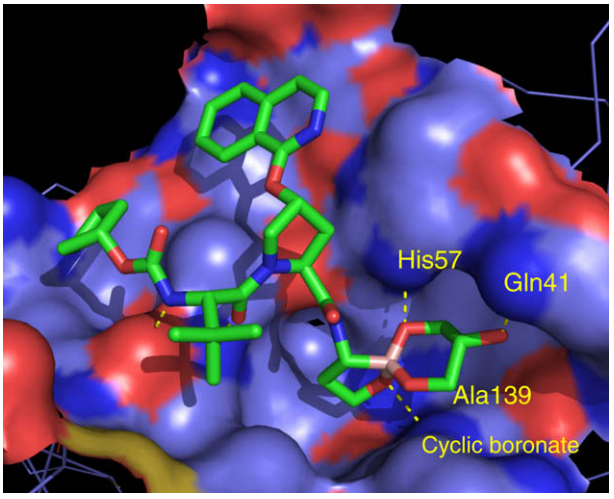


Figure 4. X-ray crystal structure of boronate inhibitor **8d** complexed with HCV NS3 mutant S139A

These cyclic boronate inhibitors have been evaluated in the replicon cellular assay.[21] In replicon assays using genotype 1a and 1b subgenomic HCV viruses, these inhibitors demonstrated weak potency with EC_{50} of >1.0 μM, presumably due to their poor cell permeability. Further studies to improve cellular activities are underway and will be reported in due course.

In conclusion, we have designed and synthesized a new series of HCV NS3 serine protease inhibitors in which novel α-amino cyclic boronate has been incorporated at the P1 position of HCV inhibitor scaffolds. To the best of our knowledge, this is the first report on the synthesis of α-amino cyclic boronates and their application as protease inhibitors. The SARs which include the influence of ring size, chirality, and substitution patterns of these compounds were established. Furthermore, X-ray structure of boronate inhibitor **8d**

complexed with HCV NS3 protease clearly demonstrated that these boronates inhibited the enzyme by trapping Ser-139 in the enzyme active site. These results provide a solid foundation for further optimizing these boronate inhibitors to improve enzymatic and cellular activities.

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15. Peptide acids **a**–**b** were purchased from Acme Bioscience, Inc. Peptide acids **c**–**d** were synthesized according to the procedures described in WO2009046098.
16. Typical experimental procedure for the coupling of α-amino oxaborole to peptide acid: To a solution of peptide acid **c** (81.9 mg, 0.158 mmol) and boronate **7** (137 mg, 0.473 mmol) in CH_2Cl_2 (8 mL) and DMF (4 mL) was added HATU (90.1 mg, 0.237 mmol) followed by DIEA (91 μL, 0.553 mmol) dropwise. The reaction was stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2 and washed with brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated. The residue was purified on a reverse phase column (C-18, CH_3CN/H_2O 1:1), affording the coupling product as a white solid (64.5 mg). A solution of this product (64.5 mg, 0.085 mmol) in hexanes (5 mL) and MeOH (5 mL) was treated with isobutyl boronic acid (17.4 mg, 0.171 mmol) and HCl (0.18 mL, 37% aqueous), respectively. The reaction was stirred at room temperature for 3 hr. The mixture was diluted with CH_2Cl_2 and washed with brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated. The residue was purified on a reverse phase column (C-18 coated silica gel, CH_3CN/H_2O 1:1) to afford 30.0 mg of **8c** as a white solid (yield 32%). MS *m*/*z* 603.3 $[M+1]^+$ (Calcd MS 602.3). ^1H NMR (300 MHz, CD_3OD) δ 7.32 (m, 1H), 7.15 (d, 1H), 7.16–6.98 (m, 1H), 6.78 (d, 1H), 5.37 (s, 1H), 4.71–4.61 (m, 3H), 4.50–4.40 (m, 2H), 4.13 (d, 1H), 3.88–3.84 (m, 2H), 3.62–3.48 (br, 1H), 2.96 (d, 1H), 2.62–2.55 (m, 1H), 2.34–2.30 (m, 1H), 1.90–1.84 (m, 1H), 1.77–1.71 (m, 1H), 1.55–1.28 (m, 8H), 1.21–1.17 (m, 2H), 1.04 (s, 9H). For experimental details described the preparation of other α-amino cyclic boronates, see WO2009046098. All final compounds were found to be stable after over 1 year of storage in air.
17. Compounds were assayed in the fluorescence enzymatic assay. Conditions: 100 nM enzyme (1a domain), 30 μM NS4A, 5 μM 5-FAM-EDVVP-Pra-SMSE-K(5-TAMRA) enzyme substrate in 50 mM Hepes, 300 mM NaCl, 20% glycerol, 2 mM DTT and 0.1% NP40. Wavelengths of 485 ex and 535 em were used on a Molecular Devices plate reader to measure initial rates.
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20. (a) The X-ray coordinates of compound **8d** with HCV NS3 protease have been deposited in the Protein Data Bank (PDB code 2xcn); (b) the X-ray coordinates of compound **8d** with HCV NS3 mutant S139A have been deposited in the Protein Data Bank (PDB code 2xcf).
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