Synthesis of Boronic Acid Analogues of α-Amino Acids by Introducing Side Chains as Electrophiles

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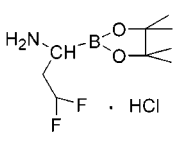
A synthetic route has been developed which has allowed us to prepare novel α-aminoboronic acids as inhibitors of serine proteases. These compounds were prepared to study the roles of proteases in biological systems. This methodology affords α-aminoboronic acids with the general formula R′-NHCH(R)BO_2-pinanediol, where R = $-CH_2CHF_2$, $-CH_2CO_2tBu$, and $-(CH_2)_2CO_2Me$ and R′ = either H or C(O)R″. The latter two compounds are the boronic acid analogues of the natural amino acids aspartic acid and glutamic acid with the side chain carboxylate protected as a *tert*-butyl or a methyl ester, respectively. Following acylation of the amino group, the side chain *tert*-butyl ester of boroaspartic acid was removed by treatment with TFA. Boroglutamic acid was obtained as the free boronic acid by hydrolysis with HCl. Prior syntheses of α-aminoboronic acids involve the initial addition of an organometallic reagent to a trialkyl borate ester. These conditions do not allow the preparation of compounds with functionalities that are not stable to the strongly basic reaction conditions. The methodology described here allows the preparation of α-aminoboronic acids by introducing side chains as electrophiles. This is particularly advantageous for side chains which are prone to elimination or unwanted enolate formation. Specifically, $BrCH_2CHF_2$, $BrCH_2$-COO^tBu, and CH_2═CHCOOMe were allowed to react with the stabilized anion of (phenylthio)-methane boronate, $PhSCH_2BO_2C_6H_{12}$, to give the substituted boronate. The substituted (phenylthio)-methane boronate was converted to the corresponding sulfonium ion by treatment with methyl iodide and subsequently displaced with iodide. The α-iodo derivative was converted to the amine by conventional methods.

Introduction

Serine proteases are a group of enzymes that have a common catalytic mechanism. They are involved in the mediation of a number of pathological conditions and, therefore, are the targets for inhibition by therapeutic agents.[1] Serine proteases that are pharmacological targets include elastase[2] involved in inflammation and emphysema, the blood coagulation enzymes (such as thrombin, factor Xa, and factor VIIa),[3] and, more recently, hepatitis C protease.[4] The latter is required for viral replication,[5] and consequently, the development of hepatitis C protease inhibitors is an important therapeutic objective.

Highly effective inhibitors of serine proteases can be obtained by preparing peptide analogues of substrates where the scissile bond is replaced by an electrophilic moiety such as trifluoromethyl ketones, aldehydes, α-keto acids and amides, and α-aminoboronic acids.[1] We have targeted hepatitis C protease for inhibition by aminoboronic acids due to our longstanding interest in these analogues[6,7] and their greater potencies determined from earlier comparisons (Kettner, unpublished data). Processing by the viral protease occurs almost exclusively at a cysteine residue.[8] On the basis of the observation that $-CHF_2$ is an isostere for a sulfhydryl group,[9] we have developed a synthesis for α-aminoboronic acids with a difluoroethyl side chain **1**. The synthesis of **1**, together with its incorporation into a peptide and its effectiveness in the inhibition of hepatitis C protease, is reported. In addition, the syntheses of new α-aminoboronic acids boroaspartate **2** and boroglutamate **3**[10] as their respective esters are also described.



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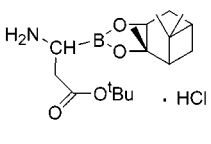

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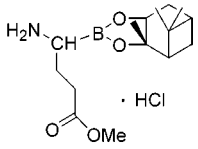

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The different synthetic approaches that have been used in the synthesis of α-aminoboronic acids containing a

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(1) Powers, J. C.; Harper, J. *Inhibitors of Serine Proteases*; Elsevier Science Publishing Co.: New York, 1986; Vol. 12.

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(4) Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Hostomska, Z. *Cell* **1996**, *87*, 331.

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(9) Narjes, F.; Brunetti, M.; Colarusso, S.; Gerlach, B.; Koch, U.; Biasol, G.; Fattori, D.; De Francesco, R.; Matassa, V.; Steinkuhler, C. *Biochemistry* **2000**, *39*, 1849.

(10) The prefix "boro" is used to designate the boronic acid analogue of the corresponding amino acid where the −COOH group is replaced by $B(OH)_2$. The suffixes $-C_{10}H_{16}$ and $-C_6H_{12}$ indicates the boronate pinanediol and pinacol ester, respectively. In the peptide Pz-CO-Val-Val-Hyp(Bzl)-OH, Pz is pyrazine and Hyp(Bzl) is 4-hydroxyproline with the hydroxyl group protected as a benzyl ether.

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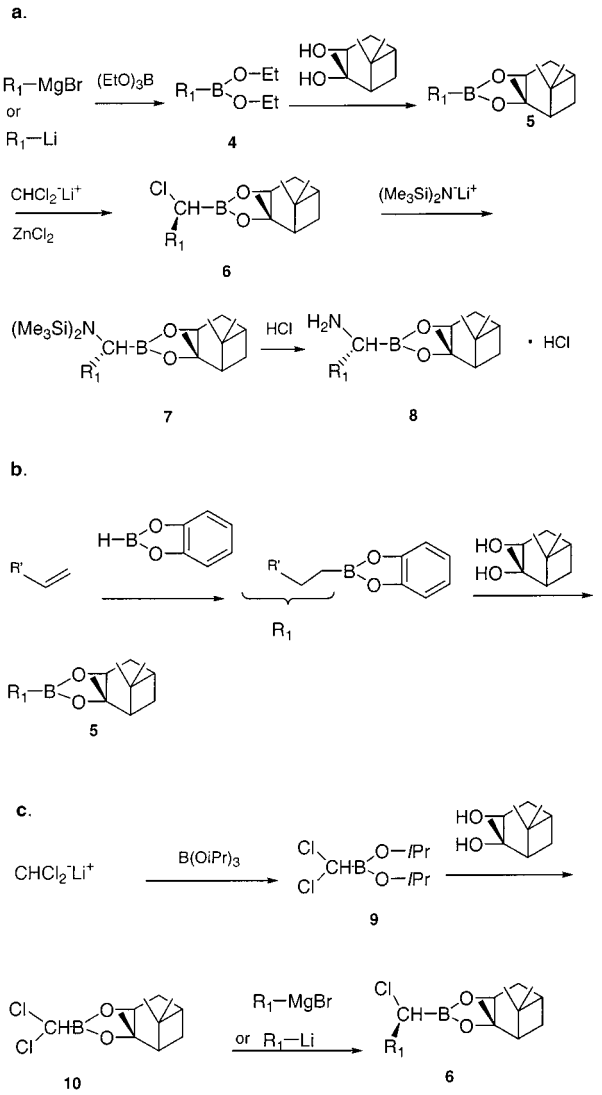
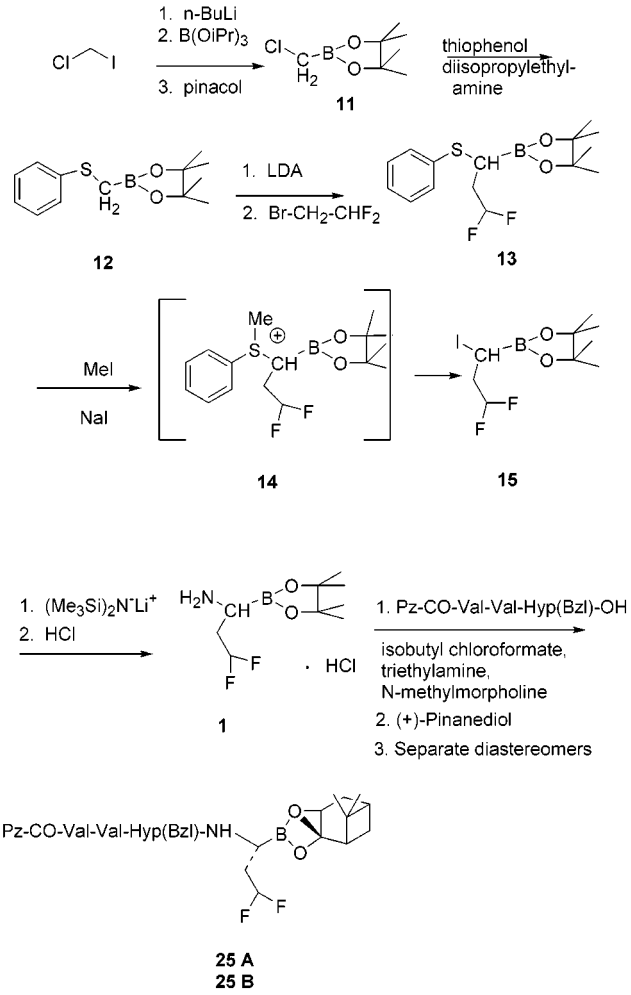
Scheme 1



variety of side chains are outlined (Scheme 1). In the first approach, a Grignard reagent or other suitable nucleophile is added to trialkyl borate to give a substituted dialkyl boronate **4** (Scheme 1a). Transesterification with a suitable diol protecting group like pinanediol gives the boronate ester **5**. The α-chloroalkyl intermediate **6** is obtained by the stereospecific addition of the anion of methylene chloride to the boronic pinanediol ester.[11] Nucleophilic displacement of the chloride of **6** by a nitrogen nucleophile such as lithium bis(trimethylsilyl)-amide gives the bissilane-protected amine **7**.[12] Subsequent treatment of **7** with anhydrous HCl gives the amine as the hydrochloride salt **8**. The alkyl side chain can also be introduced as an olefin[13] wherein hydroboration with catecholborane and transesterification with pinanediol gives the alkyl boronate **5** (Scheme 1b). The preparation of α,α-dichloromethyl ester **10** also allows the introduction of the side chain as a nucleophile to give **6** (Scheme 1c).[14]

The procedures outlined in Scheme 1 have been successfully used to synthesize a number of α-aminoboronic

Scheme 2



acids, but limitations exist for these approaches. Either a stable nucleophile, Grignard reagent, or olefin must be available for generation of the boronate ester **5**. Here, we describe a procedure for the synthesis of novel α-aminoboronic acids where the α-side-chain substituent is derived from the reaction of an electrophile with the stabilized $PhSCH_2BO_2$-pinacol methide anion. In addition to the synthesis of an α-aminoboronic acid with a 2,2-difluoroethyl side-chain **1**, we have also prepared compounds with a carboxymethyl and a carboxyethyl side chain (boroaspartate **2** and boroglutamate **3**, respectively). Analogues of aspartic acid and glutamic acid where the side-chain carboxylates were replaced with a boronic acid have been synthesized;[15−17] however, boroaspartate and boroglutamate, the analogues of natural amino acids, have not been previously reported. They are expected to be applicable to the inhibition of other serine proteases of general interest. It should be noted that we have drawn from the earlier chemistry developed by Matteson et al.[18](See Discussion).

Results

Scheme 2 shows the synthetic scheme for the preparation of an α-aminoboronic acid with a difluoroethyl side

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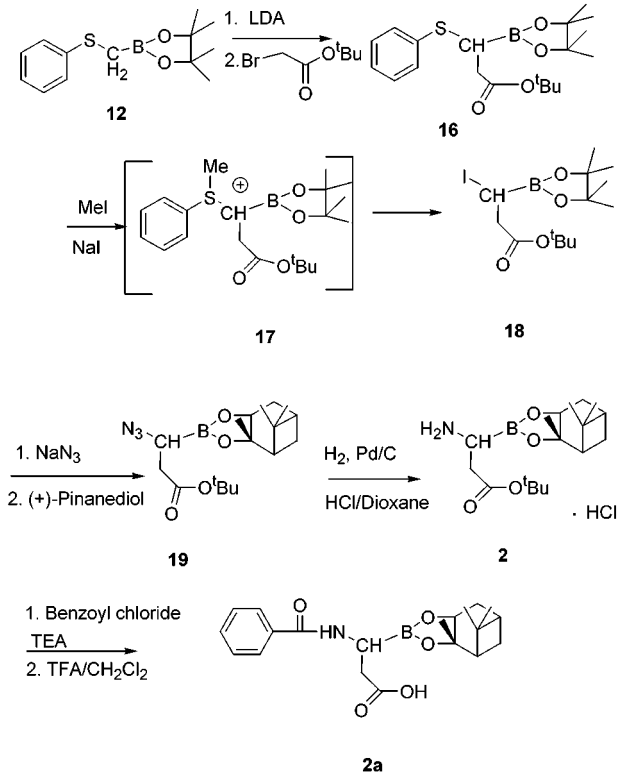
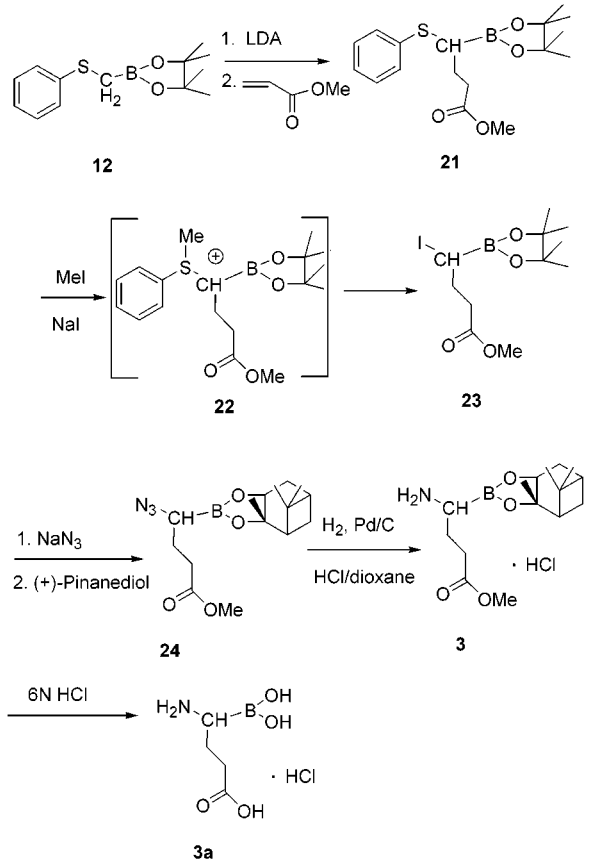
(17) Hangauer, D. G.; Hsiao, G. K. *Synthesis* **1998**, 1043.
(18) Matteson, D. S.; Arne, K. *Organometallics* **1982**, *1*, 280.

Scheme 3



Scheme 4



chain **1**, its incorporation into a peptide, and resolution of the peptide diastereomers. Pinacol chloromethyl boronate[19] **11** was allowed to react with 1 equiv of benzenethiol in the presence of diisopropylethylamine to give the boronate **12**. (Phenylthio)methane boronate[18] **12** was added to 1 equiv of LDA at 0 °C, and the precipitated anion was quenched with 2-bromo-1,1-difluoroethane to provide the substituted boronate **13** as a mixture of enantiomers (56% yield). The boronate **13** was alkylated with an excess of methyl iodide to give the sulfonium salt **14** which, in the presence of sodium iodide, was converted to the iodoboronic ester **15 (**49% yield) in situ. The iodide **15** was treated with LHMDS at −78 °C to form the disilazane boronate, which was readily hydrolyzed with 3 equiv of anhydrous HCl in dioxane to give **1** as the HCl salt (52% yield). The amine **1** was coupled to Pz-CO-Val-Val-Hyp(Bzl)-OH[10] using the mixed anhydride procedure[20] to provide the peptide boronic ester as a mixture of diastereomers. The pinacol ester was converted to the pinanediol ester by transesterification, due to its greater stability, and the diastereomers were separated by silica gel chromatography (67% yield).

The utility and versatility of the chemistry outlined is further demonstrated in the preparation of derivatives of boroaspartate **2** (Scheme 3) and boroglutamate **3** (Scheme 4). For the preparation of **2**, the anion of **12** was treated with an excess of *tert*-butyl bromoacetate at 0 °C to give the substituted boronic ester **16** (35% yield). Following the steps in Scheme 2, the iodide **18** was obtained (31% yield). Treatment of **18** with sodium azide under phase transfer catalysis conditions[21] followed by transesterification to the pinanediol ester provided the α-azido derivative **19** (59% yield).[22] Catalytic hydrogenation in the presence of 1 equiv of anhydrous HCl gave the amine **2** as the HCl salt (79% yield). **2** was further characterized as its benzoyl derivative **2a**. It was treated with benzoyl chloride, and the *tert*-butyl ester in the side chain was removed with TFA in methylene chloride to give the free acid (43%).

H-boroGlu(OMe)-$C_{10}H_{16}$ **3** was also prepared by a similar series of reactions (Scheme 4). After generation of the anion of **12**, a Michael acceptor (in this case methyl acrylate) was added to the anion at 0 °C to provide **21 (**21% yield). Following the steps in Scheme 2, the iodide **23** was obtained (38% yield). Treatment of **23** with sodium azide in DMF at 60 °C followed by transesterification to the pinanediol ester provided the α-azido derivative **24** (26% yield). Catalytic hydrogenation in the presence of 1 equiv of anhydrous HCl afforded the amine **3** as the HCl salt (55% yield). Amine **3** was refluxed in 6 N HCl to give **3a** as the free boronic acid (90%).

Discussion

In the conventional preparation of α-aminoboronic acids, side chains are introduced as nucleophiles[24] or they are introduced by hydroboration of the appropriately functionalized alkene[13] (Scheme 1). In the current synthetic approach, the side chain is introduced as an

(19) Matteson, D. S.; Majumdar, D. *J. Organomet. Chem.* **1979**, *170*, 259.
(20) Anderson, G. W.; Zimmerman, J.; Callahan, F. *J. Am. Chem. Soc.* **1967**, *89*, 5012.
(21) Matteson, D. S.; Sadhu, K. M.; Peterson, M. L. *J. Am. Chem. Soc.* **1986**, *108*, 810.
(22) The phase transfer catalysis conditions used in the preparation of **19** employed dichloromethane as one solvent component. Diazidomethane is a potential, hazardous byproduct of this reaction. It is recommended that alternative solvent systems described by Singh and Matteson[23] be used.
(23) Singh, R. P.; Matteson, D. S. *J. Org. Chem.* **2000**, *65*, 6650.
(24) Matteson, D. S. *Chem. Rev.* **1989**, *89*, 1535.

electrophile following the preparation of the (phenylthio)-methide boronate anion (Schemes 2−4). This anion was first reported by Matteson et al.[18] and was shown to react with simple, activated electrophiles. The sulfonium salt of $PhSCH(R)BO_2C_6H_{12}$ was prepared by alkylation with methyl iodide, and it was converted to the α-iodoboronic ester by treatment with sodium iodide using an approach described by Corey and Jautelat[25] for non-boronic acid derivatives. However, the usefulness of this chemistry was not fully realized due to the difficulty of converting the substituted (phenylthio)methane boronate to the α-iodoboronic ester. This reaction is reported to be slow at room temperature (3 days) and can be hampered by competing dehydrohalogenation. We have found the conversion of $PhSCH(R)BO_2C_6H_{12}$ (**13** for example) to the α-iodoboronic ester could be achieved in good yields by refluxing in anhydrous acetonitrile for 3−6 h. This key improvement allows easy access to a wide variety of α-iodoboronic esters. We recognized that these α-iodoboronic esters could be easily converted into the corresponding α-aminoboronic esters. Interestingly, the formation of the sulfonium ion and the conversion to the α-iodoboronate does not take place when a pinanediol ester is used in place of a pinacol ester, presumably due to steric hindrance. It should be noted that modifying existing reaction pathways for the preparation of α-aminoboronic acids previously had received little consideration due to the versatility of the homologation reaction and displacement of the α-chloro group with a nucleophile.[11,23] We found that the (phenylthio)methide anion chemistry and its reaction with electrophiles could allow us access α-aminoboronic esters with functionalities that would otherwise not survive the harsh basic conditions.

One example involved the attempted preparation of 1-amino-3,3-difluoropropyl boronic ester **1**. In attempts to prepare **1**, low-temperature transmetalation of 2-bromo-1,1-difluoroethane with *tert*-butyllithium[26] followed by treatment with triisopropyl borate and isolation as its pinanediol or pinacol ester did not give the desired product. Presumably this was due to the formation of the volatile *β*-elimination product, difluoroethylene. An attempt was made to prepare the Grignard reagent derived from 2-bromo-1,1-difluoroethane and to treat it with dichloromethyl boronate **10** (Scheme 1c). This reaction also failed probably due to a similar *β*-elimination. However, the possibility exists that the (difluoroethyl)-lithium anion and the Grignard reagent had formed and were not sufficiently reactive to provide the desired product. Other conventional methods of preparing α-aminoboronic acids also failed to give compound **1**. For example, hydroboration of 1,1′-difluoroethene gave an intractable mixture from which the desired product, difluoroethyl boronate, (compound **5** in Scheme 1b where R is $-CH_2CHF_2$) was present at levels not exceeding 4%. Another approach to prepare difluoro compounds involves treatment of an aldehyde with (diethylamino)sulfur trifluoride (DAST).[27] Attempts to prepare the key intermediate (the protected aldehyde side chain derived from 2-bromomethyl-1,3-dioxolane) in a manner analogous to that described by Mantri et al.[28] resulted in elimination of the acetal to the vinyl glycol ether.[29] Utilizing our present approach, we were able to prepare 1-amino-3,3-difluoropropyl boronate **1** in good yields and to incorporate it into a peptide to give Pz-CO-Val-Val-Hyp(Bzl)-NH-$CH(CH_2CHF_2)$-$BO_2C_{10}H_{16}$. The two diastereomers were separated to give enantiomerically pure isomers, **25A,B**. In general, serine proteases have a strong preferences for substrates containing L-amino acids and α-aminoboronic acids[6] in the *R* configuration in this position. **25A** was the most effective inhibitor of hepatitis C protease binding with a K_i of 12 nM. This compound is most likely in the *R* configuration.

In addition to the α-aminoboronic acid containing a difluoroethyl side chain, we were interested in α-aminoboronic acids with alkyl carboxylate side chains (either as an ester or a free carboxylate). Attempts to prepare the boronic acid analogue of aspartic acid using the chemistry outlined in Scheme 1 were unsuccessful. We felt that the methodology developed above would be useful in preparing these α-aminoboronic acids.

For example, in efforts to prepare boroaspartate **2**, treatment of both **9** and **10** (Scheme 1c) with *tert*-butyl lithioacetate with and without a Lewis acid catalyst did not provide the corresponding α-chloroboronic ester **6** (R is *tert*-butoxycarbonylethyl). The enolate was not nucleophilic enough to displace the chloride. In contrast, the preparation of boroaspartate (Scheme 3) was readily achieved with the current methodology. The anion of **12** was treated with an excess of *tert*-butyl bromoacetate at 0 °C to give the substituted boronic ester **16**. The key intermediate, α-iodoboronic ester **18**, was obtained and treated with sodium azide under phase transfer catalysis conditions[21,22] followed by transesterification to the pinanediol ester to provide the α-azido derivative **19**. Catalytic hydrogenation in the presence of 1 equiv of anhydrous HCl provided amine **2** as the hydrochloride salt. This compound was incorporated into peptides, and the *tert*-butyl ester was readily removed with TFA to generate the free acid.

We have prepared the boronic acid analogue of glutamic acid by introducing the side-chain carboxyethyl group as an electrophile (Scheme 4). In contrast to the difluoroethyl compound and boroaspartic acid, boroglutamic acid can probably be prepared by the traditional routes (Scheme 1). Hiscox and Matteson[30] have prepared the 1-chloro-*tert*-butoxycarbonylpropionyl boronate by reacting the anion of methylene chloride with *tert*-butoxycarbonylethyl boronate. This reagent was used as an intermediate in the preparation of the Japanese beetle pheromone, [*R*-(*Z*)]-5-(1-decenyl)dihydro-2(3*H*)-furanone. In our attempts to prepare the boroglutamate **3**, the thiol ether **21** (Scheme 4) was not obtained when the anion of **12** (Scheme 2) was allowed to react with 1 equiv of methyl 3-bromopropionate. The boronate **12** was recovered along with methyl acrylate, the latter arising from enolate formation followed by bromide elimination. The desired product **21** was obtained when the anion of **12** was allowed to react with an excess of methyl acrylate. This led to the successful synthesis of **3**, H-boroGlu(OMe)-$C_{10}H_{16}$. The amine **3** was incorporated into peptides, and the side-chain methyl ester was cleaved by treatment with potassium trimethylsilanolate.[31] This reaction gave

(25) Corey, E. J.; Jautelet, M. *Tetrahedron Lett.* **1968**, *55*, 5787.
(26) Bailey, W. F.; Patricia, J. J. *J. Organomet. Chem.* **1988**, *352*, 1.
(27) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574.
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(30) Hiscox, W. C.; Matteson, D. S. *J. Organomet. Chem.* **2000**, 614−615, 314.
(31) Laganis, E. D.; Chenard, B. *Tetrahedron Lett.* **1984**, *25*, 5831.

the desired product in low yields. The major products were the components resulting from the cleavage of the carbon−boron bond. However, treatment of **3** with 6 N HCl simultaneously hydrolyzed the side-chain methyl ester and the pinanediol ester to give the boronic acid **3a** in good yields.

In another case, *tert*-butyl acrylate was used in place of methyl acrylate in an attempt to prepare the *tert*-butyl ester analogue of **3**. The *tert*-butyl analogue of **21** was obtained, but further conversion to the corresponding α-iodo analogue failed due to cleavage of the *tert*-butyl ester. We speculate that the boronic ester moiety is acting as an intramolecular Lewis acid in the cleavage of the *tert*-butyl ester.

The sequence of reactions outlined in Schemes 2−4 has made it possible to prepare many structurally diverse α-aminoboronic acids. In addition to the specific compounds we have prepared, higher order acrylates or alkyl halides can be used to give more complex side chains. This is particularly valuable for the preparation of compounds with side chains that contain sensitive functionalities such as ketones, phosphonates, and sulfonamides.

Clearly, there is significant value in the present synthetic approach for preparing α-aminoboronic acids with versatile side chains containing sensitive functionalities. Similarly, the modification of the reaction (Scheme 2) allowing the introduction of side chains as Michael addition products is novel (Scheme 4). In conclusion, we have developed a method for the synthesis of α-aminoboronic acids with diverse substituents under conditions amenable to the introduction of side chains as electrophiles.

Experimental Section

Materials and Methods. All reactions were carried out under anhydrous conditions under a positive pressure of dry nitrogen unless otherwise stated. Sure-seal solvents and reagents were used as purchased from Aldrich and Lancaster Chemical Co. ^{1}H and ^{13}C NMR spectra were recorded at 300, 500, and 600 MHz, and ^{19}F NMR spectra were recorded at 300 MHZ. Pz-CO-Val-Val-Hyp(Bzl)-OH was synthesized using the mixed anhydride coupling conditions[20] employing isobutyl chloroformate, triethylamine, and *N*-methylmorpholine.

Pinacol 1-(Phenylthio)-3,3-difluoropropane-1-boronate (13). Butyllithium (50.6 mL, 126 mmol, 2.5 M in hexanes) was added dropwise to a solution of diisopropylamine (18.4 mL, 133 mmol) dissolved in THF (40 mL) at 0 °C in a 500 mL round-bottom flask. A solution of pinacol (phenylthio)methane boronate[18] (31.6 g, 126 mmol) in THF (40 mL) was added dropwise over a period of 10 min to yield a white precipitate. After the mixture was stirring for 1 h at 0 °C, 2-bromo-1,1-difluoroethane (51 mL, 630 mmol) was added dropwise. The precipitate slowly dissolved. The solution was allowed to warm to room temperature and stirred for 16 h. Excess cold 10% phosphoric acid was added, and the mixture was stirred for 5 min. Ether (100 mL) was added, and the phases were separated. The organic layer was dried over sodium sulfate and filtered. The filtrate was concentrated in vacuo and distilled (bp 119−122 °C, 0.4 mmHg) to give 22 g (56%) of **13** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.43−7.19 (m, 5H), 6.16−5.78 [tt, 1H, J = 4.6 Hz (inner triplet), J = 56.8 Hz (outer triplet)], 2.82 (m, 1H), 2.38−2.19 (m, 2H), 1.23 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 134.95, 130.70, 128.94, 126.84, 119.39−113.04 (t, J = 238.9 Hz), 84.39, 36.15−35.58 (t, J = 21.4 Hz), 24.63, 24.57, 23.98 (br);[32] ^{19}F NMR ($CDCl_3$) δ −116.97 (t, 1F, J = 16.8 Hz), −116.77 (t, 1F, J = 16.8 Hz); HRMS calcd for $C_{15}H_{21}BF_2O_2S$ (M^+) 314.1323, found 314.1328.

Pinacol 1-Iodo-3,3-difluoropropane-1-boronate (15). Boronate **13** (6.00 g, 19.1 mmol) was dissolved in anhydrous acetonitrile (60 mL). Anhydrous methyl iodide (24.0 mL, 380 mmol) and sodium iodide (5.76 g, 38.2 mmol) were added. The reaction mixture was vigorously refluxed for 5 h. The solvent was evaporated in vacuo. The residue was partitioned between water (40 mL) and ether (40 mL). The phases were separated, and the aqueous phase was washed with an equal volume of ether. The combined organic phases were dried over Na_2SO_4 and evaporated to give a brown oil. This material was distilled to give 3.1 g (48.8%) of the α-iodo boronate **15**: bp 63−65 °C, 0.4 mmHg; ^{1}H NMR ($CDCl_3$) δ 6.18−5.64 [tt, 1H, J = 4.4 Hz (inner triplet), J = 56.4 Hz (outer triplet)], 3.21 (t, 1H, J = 8.4 Hz), 2.43−2.36 (m, 2H), 1.25 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 120.49−114.13 (t, J = 238.4 Hz), 84.14, 39.38−38.79 (t, J = 21.8 Hz), 24.32, 24.16; HRMS calcd for $C_9H_{16}BF_2IO_2$ (M + H) 333.0334, found 333.0326.

Pinacol 1-Amino-3,3-difluoropropane-1-boronate−Hydrochloride (1). Iodide **15** (2.7 g, 8.1 mmol) was dissolved in anhydrous THF (10 mL) and was added dropwise to a cool (−78 °C) solution consisting of lithium bis(trimethylsilyl)amide (9.68 mL, 9.68 mmol, 1.0 M in THF) and THF (10 mL). The reaction mixture was allowed to warm to room temperature and stirred for 12 h. It was concentrated in vacuo, and hexane was added. The reaction mixture was cooled to −78 °C, and 4 N anhydrous hydrogen chloride in dioxane (6.05 mL, 24.2 mmol) was added dropwise. The mixture was warmed to room temperature and stirred for 5 h. Solvent was removed by evaporation, and chloroform was added. Insoluble material was removed by filtration. The filtrate was evaporated almost to dryness, and hexanes were added. The desired product crystallized. It was isolated and washed with cold hexane to yield 1.1 g (52%) of **1** as the amine hydrochloride: mp 138−141 °C; ^{1}H NMR ($CDCl_3$) δ 7.86 (br, 3H), 6.22−6.01 [(tt, 1H, J = 4.0 Hz (inner triplet), J = 55.7 (outer triplet)], 3.42 (m, 1H), 2.76−2.51 (m, 2H), 1.32 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 119.21−116.10 (t, J = 240.3 Hz), 86.28, 33.56−32.97 (t, J = 22.1 Hz), 24.92, 24.82; ^{19}F NMR −115.33 (t, 1F, J = 16.9 Hz), −115.51 (t, 1F, J = 16.9 Hz); HRMS calcd for $C_9H_{18}BO_2F_2N$ (M^+) 222.1399, found 222.1415.

Pz-CO-Val-Val-Hyp(Bzl)-NH-CH(CH$_2$CHF$_2$)-BO$_2$-C$_{10}$H$_{16}$ (25A).[10] Pz-CO-Val-Val-Hyp(Bzl)-OH (1.2 g, 2.28 mmol) was dissolved in THF (10 mL), and *N*-methylmorpholine (0.25 mL, 2.28 mmol) was added. The solution was cooled to −20 °C, and isobutyl chloroformate (0.30 mL, 2.28 mmol) was added. After 5 min, a cold (−20 °C) solution of **1** dissolved in chloroform (10 mL) was added followed by the addition of triethylamine (0.32 mL, 2.28 mmol). The reaction was allowed to warm to room temperature and stirred overnight. The mixture was filtered, and the filtrate was concentrated in vacuo. After the oily residue was dissolved in ethyl acetate (30 mL), it was washed with 0.2 N HCl, 5% $NaHCO_3$, and saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and concentrated. The material was redissolved in methanol (10 mL) and transesterified with (+)-pinanediol (0.38 g, 2.28 mmol). After 2 h, the methanol was evaporated and the crude reaction mixture was purified on a 4 × 90 cm Sephadex LH-20 column using methanol as a solvent. The desired product was obtained as an amorphous solid (1.19 g, 67%). TLC in 100% ethyl acetate indicated the two diastereomers with R_f of 0.31 and 0.25, respectively. The diastereomer with R_f of 0.31 was isolated by silica gel chromatography. The diastereomeric mixture (0.05 g) was loaded onto the column, and it was eluted with a stepwise gradient of ethyl acetate: hexane from a ratio of 20:80 to a ratio of 80:20. Fractions containing the product were concentrated in vacuo to give 0.019 g (36%) of the diastereomer **25A**: ^{1}H NMR ($CDCl_3$) δ 9.40 (d, 1H), 8.76 (d, 1H), 8.57 (m, 1H), 8.43 (d, 1H), 7.78 (m, 1H), 7.34 (m, 5H), 6.10−5.91 [(tt, 1H, J = 4.0 Hz (inner triplet), J = 56.6 Hz (outer triplet)], 4.78 (m, 2H), 4.61 (t, 1H), 4.42 (q, 2H), 4.22 (d, 1H), 4.08 (d, 1H), 3.62 (m, 1H), 3.18 (m, 1H), 2.31−1.85 (m, 10H), 1.32 (s, 3H), 1.27 (s, 3H), 0.98−0.81 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 173.19, 171.84, 170.66, 163.20, 147.46, 144.49,

(32) The carbon α to boron is broad and not always visible due to coupling to the boron atom.

144.17, 142.75, 137.57, 128.43, 127.82, 127.68, 118.70−117.12 (t, J = 238.7 Hz), 85.26, 71.15, 57.81, 57.44, 55.84, 52.75, 51.70, 39.73, 38.19, 35.89, 35.36, 34.39, 31.93, 31.67, 28.75, 27.19, 26.35, 24.08, 19.41, 19.33, 18.07, 17.58; HRMS calcd for $C_{40}H_{55}BF_2N_6O_7$ (M + H) 781.4271, found 781.4275.

Pz-CO-Val-Val-Hyp(Bzl)-NH-CH(CH$_2$CHF$_2$)-BO$_2$-C$_{10}$H$_{16}$ (25B). The peptide was synthesized from Pz-CO-Val-Val-Hyp(Bzl)-OH and α-aminoboronic acid **1** using the procedure described for peptide **25A**. The diastereomeric mixture (0.05 g) was loaded onto a silica gel column, and it was eluted with a stepwise gradient of ethyl acetate: hexane from a ratio of 50:50 to 90:10. TLC in 100% ethyl acetate indicated the product at R_f of 0.25. Fractions containing the product were concentrated in vacuo to give 0.016 g (32%) of the desired product **25B**: ^{1}H NMR ($CDCl_3$) δ 9.35 (d, 1H), 8.76 (d, 1H), 8.56 (m, 1H), 8.33 (d, 1H), 7.60 (m, 1H), 7.35 (m, 5H), 6.91 (d, 1H), 6.01−5.78 [(tt, 1H, J = 4.0 Hz (inner triplet), J = 56.8 Hz (outer triplet)], 4.65 (m, 1H), 4.54 (m, 3H), 4.42 (m, 3H), 4.28 (m, 3H), 3.98 (m, 1H), 3.58 (m, 1H), 2.95 (m, 1H), 2.44 (m, 1H), 2.36−1.80 (m, 10H), 1.33 (s, 3H), 1.27 (s, 3H), 1.05−0.97 (m, 12H), 0.82 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.63, 171.43, 171.15, 163.44, 147.49, 144.35, 144.03, 142.76, 137.49, 128.52, 127.94, 127.52, 118.92−117.01 (t, J = 238.5 Hz), 85.16, 71.71, 58.59, 57.51, 57.02, 52.31, 51.69, 39.74, 38.15, 35.96, 34.91 (br), 34.19, 30.65, 30.28, 28.74, 27.18, 26.34, 24.06, 19.53, 19.48, 18.26, 18.04; HRMS calcd for $C_{40}H_{55}BF_2N_6O_7$ (M + H) 781.4271, found 781.4260.

Pinacol 1-(Phenylthio)-2-(*tert*-butoxycarbonyl)ethane-1-boronate (16). Compounds **16** was prepared by the procedure described for the preparation of **13** (35%): ^{1}H NMR ($CDCl_3$) δ 7.43−7.19 (m, 5H), 2.96 (t, 1H), 2.66 (d, 2H), 1.42 (s, 9H), 1.24 (d, 12H); ^{13}C NMR ($CDCl_3$) δ 171.82, 135.62, 130.74, 128.72, 126.44, 84.01, 80.69, 37.57, 28.04, 24.05 (br), 24.71, 24.51; HRMS calcd for $C_{19}H_{29}BO_4S$ (M^+) 364.1880, found 364.1883.

(+)-Pinanediol 1-Azido-2-(*tert*-butoxycarbonyl)ethane-1-boronate (19). Boronate **16** was converted to the iodide **18** according to the procedure described for the preparation of **15**. Iodide **18** was in turn converted to the azide boronate under phase transfer catalysis conditions.[21,22] The azide was transesterified to the (+)-pinanediol ester derivative **19** and isolated (59%): ^{1}H NMR ($CDCl_3$) δ 4.32 (t, 1H), 3.32 (m, 1H), 2.58 (m, 2H), 2.28−1.85 (m, 6H), 1.39 (s, 9H), 1.35(d, 3H), 1.22 (s, 3H), 0.77 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.97, 86.94, 81.36, 78.56, 51.19, 44.20 (br), 39.41, 38.16, 35.21, 28.41, 28.02, 27.01, 26.29, 23.99; IR (film) 2092, 1724 cm^{-1}.

(+)-Pinanediol 1-(Benzoylamido)-2-(*tert*-butoxycarbonyl)ethane-1-boronate (2a). 1-Amino-2-(*tert*-butoxycarbonyl)ethane-1-boronate (**2**) was obtained by catalytic hydrogenation of the azide **19** (79%). The amine was treated with benzoyl chloride in the presence of triethylamine. Subsequent deprotection with TFA followed by HPLC purification yielded the benzoyl derivative **2a** (43%): ^{1}H NMR ($CDCl_3$) δ 8.62 (s, 1H), 7.82 (d, 2H), 7.55 (t, 1H), 7.39 (t, 2H), 4.32 (d, 1H), 3.21 (t, 1H), 2.78 (m, 2H), 2.42−1.81 (m, 5H), 1.51 (d, 1H), 1.45 (s, 3H), 1.27 (s, 3H), 0.87 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.88, 172.16, 134.16, 128.90, 128.27, 128.24, 84.23, 83.85, 52.38, 40.22, 38.34, 36.72, 29.27, 27.36, 26.82, 24.23; HRMS calcd for $C_{20}H_{26}BNO_5$ (M^+) 371.1904, found 371.1888.

Pinacol 1-(Phenylthio)-3-(methoxycarbonyl)propane-1-boronate (21). Boronate **21** was prepared by the procedure described for the preparation of **13** (21%): ^{1}H NMR ($CDCl_3$) δ 7.37−7.12 (m, 5H), 3.60 (s, 3H), 2.74 (t, 1H), 2.42 (m, 2H), 1.98 (m, 2H), 1.18 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 173.51, 135.96, 130.32, 128.71, 126.27, 84.08, 51.50, 32.54, 29.12 (br), 26.07, 24.71, 24.63; HRMS calcd for $C_{17}H_{25}BO_4S$ (M^+) 336.1567, found 336.1569.

Pinacol 1-Iodo-3-(methoxycarbonyl)propane-1-boronate (23). Iodide **23** was prepared by the procedure described for the preparation of **15** (38%): ^{1}H NMR ($CDCl_3$) δ 3.63 (s, 3H), 3.30 (t, 1H), 2.40 (m, 2H), 2.15 (m, 2H), 1.24 (s, 12H), ^{13}C NMR ($CDCl_3$) δ 172.95, 84.10, 51.63, 35.34, 29.65, 24.39, 24.23; HRMS calcd for $C_{11}H_{20}BIO_4$ (M^+) 354.0499, found 354.0512.

(+)-Pinanediol 1-Azido-3-(methoxycarbonyl)propane-1-boronate (24). The azide **24** was prepared by treating **23** with sodium azide in DMF followed by transesterification with (+)-pinanediol (26%): ^{1}H NMR ($CDCl_3$) δ 4.37 (d, 1H), 3.68 (s, 1H), 3.21(m, 1H), 2.51−1.82 (m, 9H), 1.43 (s, 3H), 1.29(s, 3H), 1.10 (d, 1H), 0.84 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.36, 86.99, 78.59, 51.65, 51.07. 39.44, 38.11, 35.22, 31.47, 28.49, 26.99, 26.49, 26.03, 25.95, 23.94; IR (film) 2094, 1738 cm^{-1}.

1-Amino-3-(hydroxycarbonyl)propane-1-boronate−Hydrochloride (3a). The azide **24** was converted to the amine **3** by catalytic hydrogenation. The amine was characterized as the free boronic acid **3a** following hydrolysis with aqueous HCl (90%): ^{1}H NMR (CD_3OD) δ 2.88 (d, 1H), 2.40 (m, 2H), 1.90 (m, 2H); ^{13}C NMR (CD_3OD) δ 174.61, 40.43 (br), 31.37, 25.44; HRMS calcd for $C_{14}H_{24}BNO_4$ (pinanediol ester adduct, M + H) 282.1876, found 282.1868.

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Supporting Information Available: Experimental procedures and characterization data for compounds **2a**, **3a**, **16**, **19**, **21**, **23,** and **24** are provided along with ^{1}H and ^{13}C NMR spectra of compounds **1**, **2a**, **3a**, **13**, **15**, **16**, **19**, **21**, **23**, **24**, and **25A,B.** This material is available free of charge via the Internet at http://pubs.acs.org.

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